

Checkpoint and inflammatory markers in chronic colitis and colorectal cancer

Ramya Ephraim

B. Sc (Microbiology, Genetics, Chemistry), M. Sc Biotechnology

**Thesis submitted for the fulfilment of the requirements for the degree of
Master of Applied Research**

**Victoria University, Australia
Institute for Health and Sport**

16 March 2023

Abstract

Cancer immunotherapy has evolved major breakthroughs in the last years and since then, it has subsequently been developed into a novel method for treating cancer through boosting the immune system, rather than using chemotherapeutic agents to directly attack the tumour cells. This treatment can be broadly classified either as cancer vaccines, adoptive cellular immunotherapy, or therapies using immune checkpoint blockades. These markers which are expressed on cancer cells to be used as an escape mechanism from the immune system, have revolutionised cancer treatment in recent years. Immune checkpoint inhibitors (ICI), in single agent and combination regimens, have produced durable and long-lasting clinical responses. The ICIs developed against cytotoxic T-lymphocyte-associated antigen 4 (CTLA4) and Programmed death 1 (PD1), block immune-inhibitory receptors on activated T-cells, amplifying immune responses.

. Inflammatory Bowel Disease (IBD) refers to a group of disorders that cause chronic gastrointestinal inflammation including Crohn's disease (McDaniel et al.) and ulcerative colitis (UC), not only the chronic colitis. The current available treatments for chronic colitis include corticosteroids, anti-inflammatories and antibiotics that reduce non-specific inflammation with the aim to decrease relapse rates, however, such treatments are ineffective in the long term. Patients with chronic colitis are at increased risk for developing colorectal cancer (CRC) with longer duration, extent of colitis and a familial history of CRC. Checkpoint markers are expressed on cancer cells as an escape mechanism from the immune system and checkpoint inhibitors have revolutionized cancer treatment in recent years. There is often a limit in using chemotherapy due to the side effects that leads to reduced efficacy, which leads to an urgent need in improving treatments, identifying new targets and the action mechanisms of those targets in cancer progression.

Winnie mouse and severely inflamed *Winnie* prolapse mice are a viable model of spontaneous chronic inflammation with immune responses similar to human chronic colitis. Determining the level of checkpoint markers in these mice tissues will provide insights into disease initiation and progression and to identify novel targets for new therapeutics design. In this study we were interested in checkpoint and inflammatory markers, as well as identification of early CRC gene markers in IBD by using Next Generation Sequencing (NGS) to search for different genetic alterations that originate in colon segments. The overall aim of this study is to analyse the up and

down regulated genes and study the pathways in Winnie mice and Winnie prolapse mice when compared with the C57BL/6 mice.

Based our overall study and analyses of genes and pathways it was identified that the cancer related genes and pathways that were highly enriched in Winnie prolapse mice when compared to Winnie mice which could lead to the fact that inflammation may be linked to cancer or pre-cancerous stages. Further study of these checkpoint markers could lead to the development of biomarkers for chronic colitis patients, as well as a better knowledge of inflammation and cancer, which could lead to the development of treatments that use biomarker expression inhibitors or vaccinations to prevent disease progression.

Master of Applied Research Student Declaration

“I, Ramya Ephraim, declare that the Master of Applied Research thesis entitled Checkpoint and inflammatory markers in chronic colitis and colorectal cancer is no more than 50,000 words in length including quotes and exclusive of tables, figures, appendices, bibliography, references, and footnotes. This thesis contains no material that has been submitted previously, in whole or in part, for the award of any other academic degree or diploma. Except where otherwise indicated, this thesis is my own work”.

“I have conducted my research in alignment with the Australian Code for the Responsible Conduct of Research and Victoria University’s Higher Degree by Research Policy and Procedures.

Signature

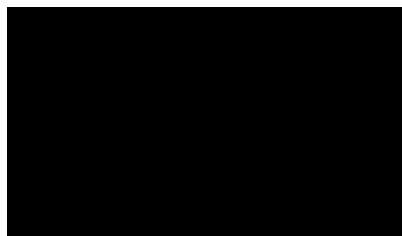
A black rectangular box redacting the signature.

Date: 16/03/2023

Ethics Declaration

“All research procedures reported in the thesis were approved by the Victoria University Animal Experimentation Ethics Committee (AEC18-016).”

Signature:

A black rectangular box redacting the signature.

Date: 16/03/2023

Acknowledgements

Firstly, I would like to begin by thanking God Almighty for His abundant grace throughout this journey until completion.

I would like to express my sincere gratitude to Prof. Vasso Apostolopoulos for giving me this opportunity and for believing in me, which has been the greatest source of my motivation to keep going. Thank you for all your help, I am falling short of words to express my utmost respect and gratitude towards you.

I also thank Prof. Kulmira Nurgali for the invaluable guidance throughout this research and for your honest feedback, always challenging me to do better.

I am extremely grateful to Dr. Sarah Fraser for her immense support, training with RNA extractions, bioanalyzer. I especially thank her for her patience and encouragement and always making sure that I was comfortable and happy, for all the phone calls and long conversations.

Thank you, Dr. Jack Feehan, for your valuable input and feedback. Thank you, Dr. Rhian Stavelly for your training with heatmaps and Majid Davidson for colon tissue collections. I also thank my fellow students Rhiannon Filippone and Lauren Sahakian for LCN2 assays and immunohistochemistry analysis. I thank Mr. Scott Coutts from Micromon Genomics for library preparation and RNA sequencing (Next Generation Sequencing) and Dr. Deanna Deveson from Monash Bioinformatics for RNA-Seq data exploration and Visualisation through Degust and QC metrics. I would like to thank the research staff at Victoria University for being so helpful, from enrolment to completion.

I would like to thank the Rotary Club of Wyndham for their scholarship support which contributed to the timely completion of this thesis.

I am thankful to my mother for her love and relentless prayers. I thank my loving brothers for being my pillars of strength and my sisters-in law for their immense support and encouragement, always lifting my spirits. I also thank my mother-in-law, brother-in-law and co-sister for their prayers and always checking on my progress. I thank my best friend for being my biggest cheer leader and backing me up along the way. I also thank all my friends and family for encouragement.

Lastly, I thank my beautiful children for their immense cooperation and understanding. I am extremely grateful and thankful to my loving husband for being my biggest support system, helping me in every step of this journey, especially with technical issues, encouraging me and making sure I fulfil my dream and reach my goal. This would not have been possible without you.

List of Abbreviations

CAC	Colitis Associated Cancer
CD	Crohn's Disease
CPM	Counts Per Million
CRC	Colorectal Cancer
DC	Dendritic Cells
DEGs	Differentially Expressed Genes
FDR	False Discovery Rate
IBD	Inflammatory Bowel Disease
IECs	Intestinal Epithelial Cells
JAK	Janus Kinase
KEGG	Kyoto Encyclopedia of Genes and Genomes
MAPK	Mitogen Activated Protein Kinases
MC	Mast Cells
MDS	Multidimensional Scaling
MHC	Major Histocompatibility
ORA	Over representation Analysis
RCTs	Randomised Controlled Trials
RIN	RNA Integrity Number
STAT	Signal Transducer and Activator of Transcription
TNF	Tumour Necrosis Factor
TNFi	TNF Inhibitors
TNFR	Tumour Necrosis Factor Receptor
UC	Ulcerative Colitis

Table of Contents

Chapter 1	10
Chapter 1, Literature Review	11
Chapter 1a: Checkpoint markers and tumour microenvironment: what do we know?	12
Introduction	12
Checkpoint markers/inhibitors	13
Role of Immune checkpoint inhibitors	13
PD-1/PD-L1	14
CTLA-4	14
T cell immunoglobulin and mucin domain-containing protein 3 (TIM-3):	14
Lymphocyte-activation gene 3 (LAG-3):	14
Indoleamine 2,3-dioxygenase (IDO):	15
Siglecs	15
Role of TME cells in relation to immune checkpoint blockade	16
Conclusion	17
References	18
Chapter 1b: Cancer Immunotherapy: The Checkpoint between Chronic colitis and colorectal cancer	21
Abstract	21
Introduction	22
Inflammatory Bowel Disease	23
Mouse Models	24
Inflammatory mediators in IBD	25
<i>Immune cells in IBD</i>	26
<i>Cytokines and chemokines</i>	27
<i>Toll-like receptors</i>	28
<i>The nuclear factor kappa B</i>	28
<i>Matrix metalloproteinases</i>	28
<i>Cyclooxygenase-2</i>	29
<i>Myeloid differentiation primary response 88</i>	29
Inflammation and Cancer	31

<i>Colitis associated cancer (CAC)/ CRC</i>	31
Cancer microenvironment	32
Checkpoint markers	33
<i>Role of checkpoint inhibitors</i>	34
<i>Stimulatory Checkpoint Molecules</i>	38
Challenges and Gaps	40
Conclusion and Future prospects	40
References	41
Chapter 2	52
Transcriptomic analysis of differentially expressed genes in mouse models of chronic colitis (Winnie mice)	54
Abstract	54
Introduction	54
Materials and Methods	56
Results	60
Overrepresentation Analysis (ORA)	68
Discussion	76
References	79
Chapter 3	84
Differential gene expression of checkpoint markers and cancer markers in mouse models of spontaneous chronic colitis	86
Abstract	86
Introduction	87
Materials and Methods	89
<i>Mice and tissues</i>	89
<i>RNA extraction</i>	90
<i>Library Preparation and RNA Sequencing</i>	90
<i>Data Analysis</i>	90
Results and Discussion	91
Pathway Analysis	92
Gene Analysis	105
Conclusion	107
Limitations and future directions:	108
References	109
Chapter 4	128
Appendix	139

Chapter 1c: Configuring therapeutic aspects of immune checkpoints in lung cancer.....	223
Abstract	224
Introduction.....	224
Significance of immune checkpoints inhibitors	225
<i>Immunoediting</i>	<i>226</i>
<i>Tumour mutational burden</i>	<i>226</i>
Mechanism of action of ICIs.....	227
<i>PD-1 pathway</i>	<i>228</i>
<i>CTLA-4 pathway.....</i>	<i>229</i>
<i>Other immune checkpoints</i>	<i>230</i>
<i>LAG-3</i>	<i>230</i>
<i>VISTA.....</i>	<i>231</i>
<i>Human endogenous retrovirus-H long terminal repeat-associating protein 2 (HHLA2)</i>	<i>232</i>
<i>TIM-3</i>	<i>233</i>
<i>TIGIT</i>	<i>233</i>
Role of ICIs in the treatment of lung cancer	236
<i>ICIs as monotherapy.....</i>	<i>236</i>
<i>ICIs in combination with chemotherapy.....</i>	<i>237</i>
<i>ICIs in combination with radiotherapy.....</i>	<i>239</i>
<i>Combination of ICIs with other therapies</i>	<i>241</i>
Clinical trials of drugs under development targeting immune checkpoints	243
Limitations of ICI therapy	244
<i>Patient harbouring EGFR mutation</i>	<i>245</i>
<i>Immune-related adverse events (irAEs).....</i>	<i>245</i>
<i>Co-administration of steroids</i>	<i>246</i>
Conclusion	246
References.....	247

Chapter 1

Chapter context and preface

Chapter 1a discusses checkpoint markers in the tumor microenvironment (TME) which includes non-cancerous cells like fibroblasts, immune cells, and blood vessel cells, along with proteins produced by all these cells, supporting cancer growth. The TME is a dynamic and complex environment where various immune cells with both pro- and anti-cancer effects are present. Interactions between cancer cells and the TME, which includes stromal cells and extracellular matrix components, play a vital role in promoting cancer cell diversity, evolution, drug resistance, and metastasis. As cancer cells transform and recruit other cells, the TME undergoes mechanical changes due to alterations in the extracellular matrix. Understanding these cellular and molecular mechanisms can help develop safer and more effective cancer treatments, potentially disrupting the relationship between cancer cells and their microenvironment. The role of checkpoint markers in cancer are discussed. In chapter 1b, the different mouse models of colitis and the inflammatory mediators are presented, and checkpoint markers in regard to chronic colitis leading to colorectal cancer are discussed. These two parts to chapter 1, identified the gaps in the literature and helped inform the subsequent project plan of this thesis.

Chapter 1, Literature Review

Chapter 1a: Checkpoint markers and tumour microenvironment: what do we know?

Introduction

The cancer microenvironment, or tumor microenvironment (TME), describes the non-cancerous cells present in the tumor such as, fibroblasts, immune cells and cells that comprise the blood vessels and proteins produced by all of the cells present in the tumor that support the growth of the cancer cells (Whiteside, 2008). A developing TME is a complicated, dynamic entity (Apostolopoulos, 2019, Barriga et al., 2019). Diverse innate and adaptive immune cells that are capable of both pro- and anti-tumorigenic actions infiltrate the site (Anderson and Simon, 2020). The dynamic interactions between cancer cells and their microenvironment, which consists of stromal cells (a cellular component) and extracellular matrix components (a non-cellular component), are crucial for promoting the heterogeneity of cancer cells, clonal evolution, and multidrug resistance, which leads to cancer cell progression and metastasis (Baghban et al., 2020). The nervous system has also been described to interact with cancer cells which is integral to regulate tumor growth, angiogenesis, and metastasis (Kuol et al., 2018c, Kuol et al., 2018b, Kuol et al., 2018a). Following cancer cell transformation and associated cell recruitment, the TME becomes a mechanically complex niche, owing to changes in the extracellular matrix architecture (Amos and Choi, 2021). The development of effective and secure therapeutic approaches to treat cancer can be aided by understanding the underlying cellular and molecular mechanisms driving these connections. This is a novel modality to indirectly disrupt the relationship between cancer cells.

Cancer immunotherapy progressed at fast pace in the last years. Immunotherapy for cancer was first described by William B. Coley, who noted tumor shrinkage and disappearance following treatment with a bacterial toxin in the 1890s (McCarthy, 2006, Říhová and Šťastný, 2015). Since then, it has subsequently been developed into a novel method for treating cancer through boosting the immune system, rather than using chemotherapeutic agents to directly attack the cancer cells (Apostolopoulos and McKenzie, 1994, Apostolopoulos and McKenzie, 2017). This treatment can be broadly classified either as cancer vaccines, adoptive cellular immunotherapy, or therapies using immune checkpoint blockades (checkpoint markers)

(Cebon, 2018, Korman et al., 2006). These markers which are expressed on cancer cells to be used as an escape mechanism from the immune system, have revolutionized cancer treatment in recent years (Gun et al., 2019, Zhang and Chen, 2018, Koury et al., 2018). The cells within the TME influence the response to immune checkpoint blockade that covers a range of monoclonal antibody-based therapies aiming to block the interaction of inhibitory receptors (immune checkpoints) expressed on the surface of immune cells, with their ligands (Russell et al., 2021). The main targets for these treatments are cytotoxic T-lymphocyte-associated antigen (CTLA-4) and programmed cell death (PD-1) or programmed cell death ligand 1 (PD-L1). The PD-1/PD-L1 axis is at the forefront of interactions between immune, stromal and tumor cells (Kuol et al., 2018d, Petitprez et al., 2020).

Checkpoint markers/inhibitors

Checkpoint markers are expressed on cancer cells as an escape mechanism from the immune system (Beatty and Gladney, 2015). One of the fundamental roles of the immune system is its ability to differentiate between self and non-self-antigens and attack the non-self-antigens using “checkpoints”. The monoclonal antibody checkpoint inhibitors, CTLA-4 inhibitors (ipilimumab, tremelimumab), PD1/PD-L1 inhibitors (pembrolizumab, nivolumab, durvalumab, atezolizumab), have been routinely used in clinical practice for the treatment of a number of cancers (Figure 1) (Lemiale et al., 2019). As such, checkpoint inhibitors have been used in patients with non-small cell lung cancer, renal cell carcinoma, melanoma, squamous cell carcinoma of the head and neck, urothelial cancer and Hodgkin lymphoma (Seidel et al., 2018a). In contrast to classical chemotherapy, checkpoint inhibitors do not target cancers cells; but rather they enhance activation of immune cells, particularly T cells (Gun et al., 2019). In comparison to chemotherapy, tolerance to checkpoint markers is higher with fewer reported side effects. Checkpoint marker inhibitors, improve the outcome of many cancer cases, which may lead to treatment of many patients in future.

Role of Immune checkpoint inhibitors

The aim of cancer immunotherapy is to promote the activity of CD8⁺ T cells within the TME to assist the initiation of tumor-specific T cells in lymphoid organs and establish efficient and durable antitumor immunity (Kuol et al., 2017). To reach optimal effectiveness, many studies

have combined treatments that use different targets such as nivolumab (targets PD-1), and ipilimumab (targets CTLA-4) (Rotte, 2019). PD-1 is a checkpoint protein expressed on T cells that act as a type of “off switch” for T cell function. Within last 3 years alone, five new checkpoint inhibitors (all targeting PD-1 or PD-L1), two new cell therapies (targeting CD19), and one new CD3-targeted bispecific antibody (also targeting CD19) have been approved (Shalabi et al., 2017) (Figure 1).

PD-1/PD-L1: PD-1/CD279 is expressed on activated T cells which binds to PD-L1 or Programmed death ligand 2 (PD-L2) on tumor cells, resulting in inactivation and death of T cells. The absence of PD-1 expression on T cells has shown to significantly delay in tumor growth and increase CD8⁺ T cells within the TME in mouse models. Nivolumab, is one of PD-1 inhibitors that has been approved by the FDA for use in metastatic melanoma (Figure 1).

CTLA-4: CTLA-4/CD152 is responsible for suppressing CD8⁺ T-cell activation. Ipilimumab and tremelimumab, are anti-CTLA-4 humanized monoclonal antibodies that have the potential to inhibit CTLA-4 ligand-driven immunosuppression (Kolar et al., 2009). CTLA-4 and PD-1 are negative regulators of T-cell immune function (Figure 1).

T cell immunoglobulin and mucin domain-containing protein 3 (TIM-3): TIM-3 is a negative regulator immune checkpoint. TIM-3 is expressed on interferon gamma (IFN)- γ producing CD4⁺ T helper 1 (Th1) and CD8⁺ T cells (Tc1) as well as on natural killer cells, mast cells, dendritic cells, B cells, macrophages (Figure 1) (He et al., 2018). TIM-3 is as a target for anticancer immunotherapy since it is expressed on dysfunctional CD8⁺ T cells as well as on regulatory T cells; two key immune cell populations that constitute immunosuppression within the TME. Blockage of the TIM-3/PD-1 combination is more effective than single blockade in regards to restoring IFN- γ secreting CD8⁺ T cells (Anderson, 2014).

Lymphocyte-activation gene 3 (LAG-3): LAG3 is an important immune checkpoint molecule with relevance to several diseases, including cancer (Graydon et al., 2021). LAG-3 binds to MHC class II in addition to other ligands including galectin-3 and LSECtin. Like PD-1, LAG3 (or CD223) is up-regulated on numerous cell types including, tumor-infiltrating lymphocytes (CD4, CD8) and regulatory T cells. LAG3 is important for optimal T cell regulation and homeostasis (Ruffo et al., 2019). Despite showing that blockade of LAG3 alone was effective

at reducing tumor growth this was only weakly effective but blockade of both LAG3 and PD-1 synergistically reduced tumor growth and increased survival of mice (Andrews et al., 2017, Woo et al., 2012). Tumor-infiltrating lymphocytes with high LAG3 expression have been noted in solid tumors including, ovarian cancer, melanoma, colorectal cancer (CRC), and haematological malignancies including Hodgkin and diffuse large B-cell lymphoma (Lythgoe et al., 2021). LAG3 reduces cytokine and granzyme production and proliferation whilst encouraging differentiation into regulatory T cells (Graydon et al., 2021)

Indoleamine 2,3-dioxygenase (IDO): IDO is an enzyme which degrades tryptophan, an essential amino acid (Manson et al., 2016) abundant in the lung, colon, and intestine. It catalyses the oxidative ring cleavage of the pyrrole moiety of not only tryptophan, but also serotonin, melatonin, and other indoleamine derivatives. IDO is expressed by cancer cells but also by endothelial cells, immune cells within the TEM, peripheral blood cells, fibroblasts and in blood (Meireson et al., 2020). The expression of IDO1 could be activated by the presence of IFN- γ , lipopolysaccharide, and tumor necrosis factor. Over-expression of IDO1 adjusts the poor prognosis in different types of cancers like melanoma, pancreatic, ovarian and CRC (Xu et al., 2019).

Siglecs: Sialic acid-binding immunoglobulin-like lectins (Siglecs) are transmembrane sialic acid-binding proteins of the immunoglobulin superfamily characterized by the presence of an N-terminal V-set Ig-like domain along with variable numbers of C2 set domains (Jiang et al., 2022). Siglecs are of interest in cancer since they have been shown to induce immunosuppression, and as such, attractive as targets for anti-cancer effects. Siglecs are primarily expressed by immune cells and thus far 14 siglecs (Siglec1-14) have been identified. Different immune cells express different Siglecs and interact with various ligands on cancer cells. For example, Siglec-7 interacts with salic acid, Siglet-9 with MUC1/MUC16, and Siglec-10 with CD24. Inhibitory Siglecs are Siglec-3, -5, -7, -9, and -10. Siglec-3/CD33 has been studied as a myeloid lymphoma marker in clinical studies. CD33 is associated with myelin-associated glycoprotein (Ahluwalia et al.)/Siglec-4 (Kelm and Ravindran, 2007). Siglec-8 is expressed on the surface of human eosinophils, mast cells, and basophils and promotes cytokine-dependent death. Siglec-9 is highly expressed on neutrophils, and induces cell death upon ligation by monoclonal antibodies (Graeter et al., 2019).

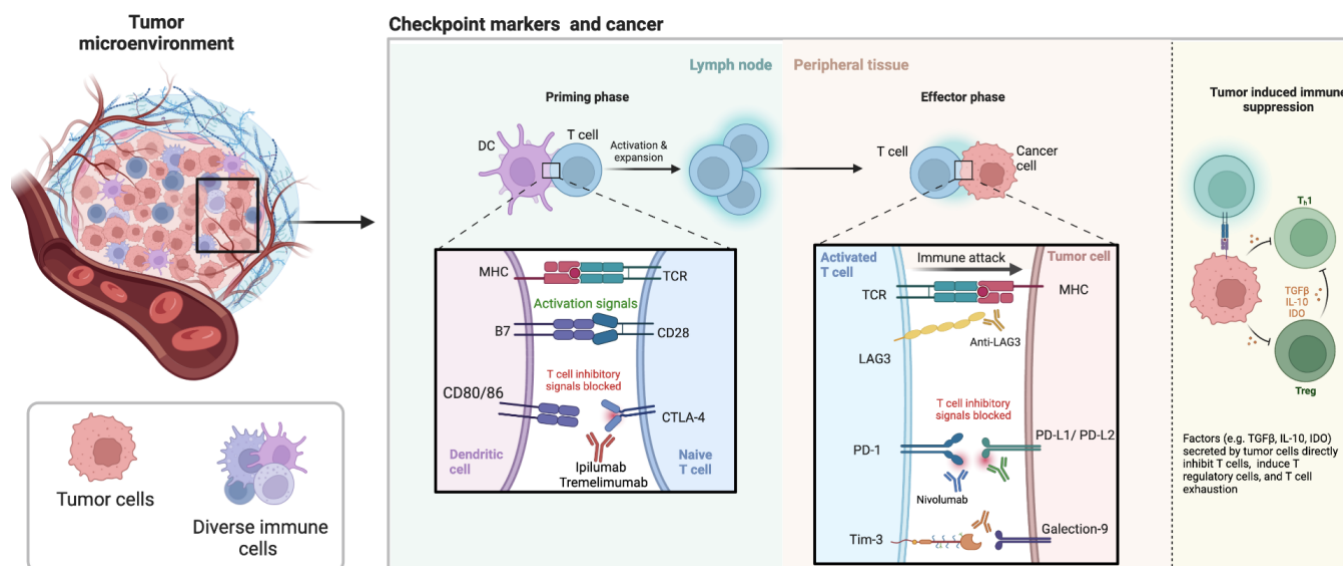


Figure 1. The TME is complex involving diverse immune cells and checkpoint markers.

Role of TME cells in relation to immune checkpoint blockade

Immuno-oncology has come to accept the idea of "hot" and "cold" tumors, where "hot" refers to tumors that contain more inflammatory cells than a specified threshold and "cold" refers to tumors that do not (Zemek et al., 2020). Immune checkpoint inhibitor medication is ineffective in patients with cold TME, which is characterized by minimal effector T cell infiltration, going from cold to hot TME is essential to enhancing the efficacy of immune checkpoint inhibitor therapy (Han et al., 2022a). Tumor-associated macrophages are highly immunosuppressive protecting cancer cells through eliminating the anti-tumor T cells by overexpressing PD-L1, PD-L2, CD80, and Siglec-15 (Zhou et al., 2022b). Immune checkpoint blockade therapies based on the TME have made many achievements like PD-1/PD-L1 and CTLA4 checkpoint inhibitors, which have revolutionized the treatments (Huang et al., 2022). Immune checkpoint molecules such as PD-1, IDO-1, LAG-3, and TIM-3 restrict immune function, and tumor cells use them to avoid host immune surveillance (Karihtala et al., 2022). The immune environment supports the invasion and spread of the cancer, and cancer cells have the power to alter the immune system. To engage part in the process of mutual adaptation, these cells also secrete cytokines, immunological checkpoint systems, and tertiary lymphoid structures. Tumor cells interact with the fibroblasts, vascular system, and microbiome (Xu et al., 2022). Other products within the TME, including, B cells, fibroblast cells, myeloid lineage cells, and the vasculature, also play a part (Mao et al., 2021). The other elements of the TME may positively or adversely

impact the production of effective antitumor immunity provided the primary objective of successful cancer therapy is to elicit long lasting memory T cell responses (Petitprez et al., 2020).

Conclusion

Determining the relationship between tumor cells and the TME is crucial to develop cancer treatments. Immune checkpoint inhibitors may cause immune-related adverse events, some of which are clinically serious to potentially life-threatening. The establishment of biomarkers that can identify patients who are more likely to benefit from immune checkpoint inhibitor therapy is essential to optimize the use of immunotherapy. The regulation of the TME based on multiple omics results can suggest innovative therapeutic strategies to prevent tumors from succeeding in immune escape and to support antitumoral effects. Bioinformatics approaches will also need to continue to develop, to improve our understanding of a responsive TME. It is well established that the TME affects immune checkpoint inhibitor responses. Predictive and prognostic biomarkers can be determined using cutting-edge technology, which can also be utilized to investigate the immunological background of malignancies.

References

1. Whiteside, T.L. The tumor microenvironment and its role in promoting tumor growth. *Oncogene* **2008**, *27*, 5904-5912, doi:10.1038/onc.2008.271.
2. Apostolopoulos, V. Cancer Vaccines: Research and Applications. *Cancers (Basel)* **2019**, *11*, doi:10.3390/cancers11081041.
3. Barriga, V.; Kuol, N.; Nurgali, K.; Apostolopoulos, V. The Complex Interaction between the Tumor Micro-Environment and Immune Checkpoints in Breast Cancer. *Cancers (Basel)* **2019**, *11*, doi:10.3390/cancers11081205.
4. Anderson, N.M.; Simon, M.C. The tumor microenvironment. *Curr Biol* **2020**, *30*, R921-r925, doi:10.1016/j.cub.2020.06.081.
5. Baghban, R.; Roshangar, L.; Jahanban-Esfahlan, R.; Seidi, K.; Ebrahimi-Kalan, A.; Jaymand, M.; Kolahian, S.; Javaheri, T.; Zare, P. Tumor microenvironment complexity and therapeutic implications at a glance. *Cell Communication and Signaling* **2020**, *18*, 59, doi:10.1186/s12964-020-0530-4.
6. Kuol, N.; Stojanovska, L.; Apostolopoulos, V.; Nurgali, K. Role of the Nervous System in Tumor Angiogenesis. *Cancer Microenviron* **2018**, *11*, 1-11, doi:10.1007/s12307-018-0207-3.
7. Kuol, N.; Stojanovska, L.; Apostolopoulos, V.; Nurgali, K. Role of the nervous system in cancer metastasis. *J Exp Clin Cancer Res* **2018**, *37*, 5, doi:10.1186/s13046-018-0674-x.
8. Kuol, N.; Stojanovska, L.; Apostolopoulos, V.; Nurgali, K. Crosstalk between cancer and the neuro-immune system. *J Neuroimmunol* **2018**, *315*, 15-23, doi:10.1016/j.jneuroim.2017.12.016.
9. Amos, S.E.; Choi, Y.S. The Cancer Microenvironment: Mechanical Challenges of the Metastatic Cascade. *Frontiers in Bioengineering and Biotechnology* **2021**, *9*, doi:10.3389/fbioe.2021.625859.
10. McCarthy, E.F. The toxins of William B. Coley and the treatment of bone and soft-tissue sarcomas. *Iowa Orthop J* **2006**, *26*, 154-158.
11. Říhová, B.; Šťastný, M. [History of Immuno-therapy - from Coley Toxins to Check-points of the Immune Reaction]. *Klinická onkologie : casopis Ceske a Slovenske onkologicke spolecnosti* **2015**, *28 Suppl 4*, 4s8-14.
12. Apostolopoulos, V.; McKenzie, I.F. Cellular mucins: targets for immunotherapy. *Crit Rev Immunol* **1994**, *14*, 293-309, doi:10.1615/critrevimmunol.v14.i3-4.40.
13. Apostolopoulos, V.; McKenzie, I.F.C. Cellular Mucins: Targets for Immunotherapy. *Crit Rev Immunol* **2017**, *37*, 421-437, doi:10.1615/CritRevImmunol.v37.i2-6.110.
14. Cebon, J. Perspective: cancer vaccines in the era of immune checkpoint blockade. *Mamm Genome* **2018**, *29*, 703-713, doi:10.1007/s00335-018-9786-z.
15. Korman, A.J.; Peggs, K.S.; Allison, J.P. Checkpoint blockade in cancer immunotherapy. *Adv Immunol* **2006**, *90*, 297-339, doi:10.1016/S0065-2776(06)90008-X.
16. Gun, S.Y.; Lee, S.W.L.; Sieow, J.L.; Wong, S.C. Targeting immune cells for cancer therapy. *Redox Biol* **2019**, *25*, 101174-101174, doi:10.1016/j.redox.2019.101174.
17. Zhang, H.; Chen, J. Current status and future directions of cancer immunotherapy. *J Cancer* **2018**, *9*, 1773-1781, doi:10.7150/jca.24577.
18. Koury, J.; Lucero, M.; Cato, C.; Chang, L.; Geiger, J.; Henry, D.; Hernandez, J.; Hung, F.; Kaur, P.; Teskey, G.; et al. Immunotherapies: Exploiting the Immune System for Cancer Treatment. *Journal of Immunology Research* **2018**, *2018*, 9585614, doi:10.1155/2018/9585614.
19. Russell, B.L.; Sooklal, S.A.; Malindisa, S.T.; Daka, L.J.; Ntwasa, M. The Tumor Microenvironment Factors That Promote Resistance to Immune Checkpoint Blockade Therapy. *Front Oncol* **2021**, *11*, doi:10.3389/fonc.2021.641428.
20. Kuol, N.; Stojanovska, L.; Nurgali, K.; Apostolopoulos, V. PD-1/PD-L1 in disease. *Immunotherapy* **2018**, *10*, 149-160, doi:10.2217/imt-2017-0120.
21. Petitprez, F.; Meylan, M.; de Reyniès, A.; Sautès-Fridman, C.; Fridman, W.H. The Tumor Microenvironment in the Response to Immune Checkpoint Blockade Therapies. *Frontiers in Immunology* **2020**, *11*, doi:10.3389/fimmu.2020.00784.

22. Beatty, G.L.; Gladney, W.L. Immune escape mechanisms as a guide for cancer immunotherapy. *Clin Cancer Res* **2015**, *21*, 687-692, doi:10.1158/1078-0432.Ccr-14-1860.
23. Lemiale, V.; Meert, A.-P.; Vincent, F.; Darmon, M.; Bauer, P.R.; Van de Louw, A.; Azoulay, E.; Groupe de Recherche en Reanimation Respiratoire du patient, d.O.-H. Severe toxicity from checkpoint protein inhibitors: What intensive care physicians need to know? *Annals of intensive care* **2019**, *9*, 25-25, doi:10.1186/s13613-019-0487-x.
24. Seidel, J.A.; Otsuka, A.; Kabashima, K. Anti-PD-1 and Anti-CTLA-4 Therapies in Cancer: Mechanisms of Action, Efficacy, and Limitations. *Front Oncol* **2018**, *8*, 86-86, doi:10.3389/fonc.2018.00086.
25. Kuol, N.; Stojanovska, L.; Nurgali, K.; Apostolopoulos, V. The mechanisms tumor cells utilize to evade the host's immune system. *Maturitas* **2017**, *105*, 8-15, doi:10.1016/j.maturitas.2017.04.014.
26. Rotte, A. Combination of CTLA-4 and PD-1 blockers for treatment of cancer. *Journal of Experimental & Clinical Cancer Research* **2019**, *38*, 255, doi:10.1186/s13046-019-1259-z.
27. Shalabi, A.; Tang, J.; Hubbard-Lucey, V.M. Comprehensive analysis of the clinical immuno-oncology landscape. *Annals of Oncology* **2017**, *29*, 84-91, doi:10.1093/annonc/mdx755.
28. Kolar, P.; Knieke, K.; Hegel, J.K.; Quandt, D.; Burmester, G.R.; Hoff, H.; Brunner-Weinzierl, M.C. CTLA-4 (CD152) controls homeostasis and suppressive capacity of regulatory T cells in mice. *Arthritis and rheumatism* **2009**, *60*, 123-132, doi:10.1002/art.24181.
29. He, Y.; Cao, J.; Zhao, C.; Li, X.; Zhou, C.; Hirsch, F.R. TIM-3, a promising target for cancer immunotherapy. *Onco Targets Ther* **2018**, *11*, 7005-7009, doi:10.2147/OTT.S170385.
30. Anderson, A.C. Tim-3: An Emerging Target in the Cancer Immunotherapy Landscape. *Cancer Immunology Research* **2014**, *2*, 393-398, doi:10.1158/2326-6066.Cir-14-0039.
31. Graydon, C.G.; Mohideen, S.; Fowke, K.R. LAG3's Enigmatic Mechanism of Action. *Frontiers in Immunology* **2021**, *11*, doi:10.3389/fimmu.2020.615317.
32. Ruffo, E.; Wu, R.C.; Bruno, T.C.; Workman, C.J.; Vignali, D.A.A. Lymphocyte-activation gene 3 (LAG3): The next immune checkpoint receptor. *Semin Immunol* **2019**, *42*, 101305, doi:10.1016/j.smim.2019.101305.
33. Andrews, L.P.; Marciscano, A.E.; Drake, C.G.; Vignali, D.A. LAG3 (CD223) as a cancer immunotherapy target. *Immunol Rev* **2017**, *276*, 80-96, doi:10.1111/imr.12519.
34. Woo, S.R.; Turnis, M.E.; Goldberg, M.V.; Bankoti, J.; Selby, M.; Nirschl, C.J.; Bettini, M.L.; Gravano, D.M.; Vogel, P.; Liu, C.L.; et al. Immune inhibitory molecules LAG-3 and PD-1 synergistically regulate T-cell function to promote tumoral immune escape. *Cancer Res* **2012**, *72*, 917-927, doi:10.1158/0008-5472.CAN-11-1620.
35. Lythgoe, M.P.; Liu, D.S.K.; Annels, N.E.; Krell, J.; Frampton, A.E. Gene of the month: lymphocyte-activation gene 3 (LAG-3). *J Clin Pathol* **2021**, *74*, 543-547, doi:10.1136/jclinpath-2021-207517.
36. Manson, G.; Norwood, J.; Marabelle, A.; Kohrt, H.; Houot, R. Biomarkers associated with checkpoint inhibitors. *Annals of Oncology* **2016**, *27*, 1199-1206, doi:10.1093/annonc/mdw181.
37. Meireson, A.; Devos, M.; Brochez, L. IDO Expression in Cancer: Different Compartment, Different Functionality? *Front Immunol* **2020**, *11*, 531491, doi:10.3389/fimmu.2020.531491.
38. Xu, X.; Ren, J.; Ma, Y.; Liu, H.; Rong, Q.; Feng, Y.; Wang, Y.; Cheng, Y.; Ge, R.; Li, Z.; et al. Discovery of cyanopyridine scaffold as novel indoleamine-2,3-dioxygenase 1 (IDO1) inhibitors through virtual screening and preliminary hit optimisation. *Journal of Enzyme Inhibition and Medicinal Chemistry* **2019**, *34*, 250-263, doi:10.1080/14756366.2018.1480614.
39. Jiang, K.Y.; Qi, L.L.; Kang, F.B.; Wang, L. The intriguing roles of Siglec family members in the tumor microenvironment. *Biomark Res* **2022**, *10*, 22, doi:10.1186/s40364-022-00369-1.
40. Kelm, S.; Ravindran, R. 3.24 - Siglecs. In *Comprehensive Glycoscience*, Kamerling, H., Ed.; Elsevier: Oxford, 2007; pp. 523-538.
41. Graeter, S.; Simon, H.-U.; von Gunten, S. Granulocyte death mediated by specific antibodies in intravenous immunoglobulin (IVIG). *Pharmacological Research* **2019**, doi:<https://doi.org/10.1016/j.phrs.2019.02.007>.
42. Zemek, R.M.; Chin, W.L.; Nowak, A.K.; Millward, M.J.; Lake, R.A.; Lesterhuis, W.J. Sensitizing the Tumor Microenvironment to Immune Checkpoint Therapy. *Frontiers in Immunology* **2020**, *11*, doi:10.3389/fimmu.2020.00223.

43. Han, X.; Wei, Q.; Lv, Y.; Weng, L.; Huang, H.; Wei, Q.; Li, M.; Mao, Y.; Hua, D.; Cai, X.; et al. Ginseng-derived nanoparticles potentiate immune checkpoint antibody efficacy by reprogramming the cold tumor microenvironment. *Molecular Therapy* **2022**, *30*, 327-340, doi:<https://doi.org/10.1016/j.ymthe.2021.08.028>.
44. Zhou, Q.; Liang, J.; Yang, T.; Liu, J.; Li, B.; Li, Y.; Fan, Z.; Wang, W.; chen, W.; Yuan, S.; et al. Carfilzomib modulates tumor microenvironment to potentiate immune checkpoint therapy for cancer. *EMBO Molecular Medicine* **2022**, *14*, e14502, doi:<https://doi.org/10.15252/emmm.202114502>.
45. Huang, P.; Xu, L.; Jin, M.; Li, L.; Ke, Y.; Zhang, M.; Zhang, K.; Lu, K.; Huang, G. Construction and Validation of a Tumor Microenvironment-Based Scoring System to Evaluate Prognosis and Response to Immune Checkpoint Inhibitor Therapy in Lung Adenocarcinoma Patients. *Genes* **2022**, *13*, 951.
46. Karihtala, K.; Leivonen, S.-K.; Karjalainen-Lindsberg, M.-L.; Chan, F.C.; Steidl, C.; Pellinen, T.; Leppä, S. Checkpoint protein expression in the tumor microenvironment defines the outcome of classical Hodgkin lymphoma patients. *Blood Advances* **2022**, *6*, 1919-1931, doi:10.1182/bloodadvances.2021006189.
47. Xu, S.; Ye, C.; Chen, R.; Li, Q.; Ruan, J. The Landscape and Clinical Application of the Tumor Microenvironment in Gastroenteropancreatic Neuroendocrine Neoplasms. *Cancers (Basel)* **2022**, *14*, 2911.
48. Mao, X.; Xu, J.; Wang, W.; Liang, C.; Hua, J.; Liu, J.; Zhang, B.; Meng, Q.; Yu, X.; Shi, S. Crosstalk between cancer-associated fibroblasts and immune cells in the tumor microenvironment: new findings and future perspectives. *Mol Cancer* **2021**, *20*, 131, doi:10.1186/s12943-021-01428-1.

Chapter 1, Literature Review

Chapter 1b: Cancer Immunotherapy: The Checkpoint between Chronic colitis and colorectal cancer

Abstract

IBD is a group of diseases that cause intestinal inflammation and lesions because of an abnormal immune response to host gut microflora. Alterations in the gut microbiota are a common feature in the pathology of IBD; however, it is not clear whether these alterations are the cause or consequence of gut inflammation and how these bacterial communities contribute to the pathogenesis of IBD. Corticosteroids, anti-inflammatories, and antibiotics are often used to reduce non-specific inflammation and relapse rates; however, such treatments are ineffective over time. Patients with chronic colitis are more susceptible to developing CRC, especially those with a longer duration of colitis. There is often a limit in using chemotherapy due to side effects, leading to reduced efficacy, leaving an urgent need to improve treatments and identify new therapeutic targets. Cancer immunotherapy has made significant advances in recent years and is mainly categorized as cancer vaccines, adoptive cellular immunotherapy, or immune checkpoint blockade therapies. Checkpoint markers are expressed on cancer cells to evade the immune system, and as a result checkpoint inhibitors have transformed cancer treatment in the last 5 -10 years. Immune checkpoint inhibitors have produced long-lasting clinical responses in both single and combination therapies. *Winnie* mice are a viable model of spontaneous chronic colitis with immune responses like human IBD. Determining the expression levels of checkpoint markers in tissues from these mice will provide insights into disease initiation, progression, and cancer. Such information will lead to identification of novel checkpoint markers and the development of treatments with or without immune checkpoint inhibitors or vaccines to slow or stop disease progression.

Introduction

IBD are multifactorial disorders. Alterations on host gut microflora is a contributing factor but not the solely. In fact, interactions between environmental and genetic factors, impaired regulation of immunity, gut barrier dysfunction, and alterations in the intestinal commensal microbiota form the predominant etiologic hypothesis associated with the pathogenesis and development of IBD. Approximately 80,000 individuals in Australia are living with IBD, with 5,500 new cases diagnosed annually; globally this number equates to 6.8 million individuals with females being more affected than males. Available treatments for IBD in surgery, corticosteroids, anti-inflammatories (aminosalicylates, balsalazide and olsalazine), antibiotics and immune suppressors (azathioprine, methotrexate, mercaptopurine), all of which aim to reduce non-specific inflammation to decrease relapse rates. Such treatments, however, are ineffective in the long term (Triantafyllidis et al., 2011). Gradual development of inflammation causes oedema in the intestinal wall, ulceration, and long term can lead to CRC (Axelrad et al., 2016b). There is often a limit in using chemotherapy due to side-effects leading to reduced efficacy, making evaluation of improved treatments, as well as new targets and mechanisms critical.

Inflammation is a core feature of several diseases including IBD. Chronic inflammatory diseases frequently result in the development of poorly regulated cellular processes that may lead to cancer. (Chen et al., 2017). Colitis-associated cancer (CAC) is a type of CRC that develops following a long period with IBD. The link between inflammation as a potential cause in CRC is being studied. Crohn's disease (McDaniel et al.) is associated with a greater risk of developing CAC by up to 8.3% and ulcerative colitis (UC) by up to 33.2% when compared to the non-IBD affected population's risk of developing CRC.

Cancer immunotherapy dates back to 1891, when William Coley, the father of immunotherapy, attempted to use the immune system to treat cancer after discovering that combinations of live and inactivated *Streptococcus pyogenes* and *Serratia marcescens* were able to induce tumour regression in patients with sarcoma (McCarthy, 2006, Říhová and Šťastný, 2015). Since then, it has evolved into a novel and effective method of treating cancer by enhancing the immune

system instead of directly targeting tumour cells with chemotherapeutics (Farkona et al., 2016, Agarwal, 2016). Immunotherapies can be broadly classified into cancer vaccines (Apostolopoulos et al., 1998, Apostolopoulos et al., 1996, Pietersz et al., 2006), adoptive cellular immunotherapy, or checkpoint inhibitor therapy (Cebon, 2018, Korman et al., 2006). Checkpoint markers are present on cancer cells to evade the immune system, and immune checkpoint inhibitors (ICIs) operate by inhibiting these checkpoint proteins/markers, allowing the immune system to destroy cancer cells. In recent years, the use of ICIs has improved cancer treatment. (Barriga et al., 2019, Gun et al., 2019, Koury et al., 2018, Kuol et al., 2017, Kuol et al., 2018d, Kuol et al., 2022, Zhang and Chen, 2018).

Inflammatory Bowel Disease

IBD is a chronic condition with 2 major pathologies, UC and CD. (Hendrickson et al., 2002). The typical clinical symptoms for IBD include gut hypersensitivity and abdominal pain which are associated with chronic diarrhea and rectal bleeding (Camilleri, 2011). CD is mainly characterised by severe chronic inflammation expressed as trans-mural skip lesions all across the intestinal tract and UC exhibits continuous mucosal and submucosal inflammation extending from the rectum to the colon (Baumgart and Carding, 2007). IBD's immune characteristics are a result of aberrant responses of the innate and adaptive immune systems (Silva et al., 2016). Approximately 95% of UC patients have inflammation in the rectum, with 25% having inflammation restricted to the rectum (Love, 2019). Long-term management of IBD is challenging due to toxic long term effects of therapies or unresponsiveness in patients. (Pithadia and Jain, 2011). IBD is most common between the second and third decades of life, with another high point between the ages of 60 and 70 (Silva et al., 2016). There is a significant increased risk of new mental illness postpartum among women with IBD, specifically in the presence of CD, (van der Woude and Shitrit, 2019) with the rate of depression and anxiety being highest during active disease states (Neuendorf et al., 2016). Of relevance, it was recently shown that inflammation in the intestines caused by an acute dose of methamphetamine causes leaky gut syndrome, systemic inflammation, inflammation in the brain and mood disorders such as anxiety (Davidson et al., 2022, Papageorgiou et al., 2019, Prakash et al., 2017). Gut inflammation and changes in behaviour are closely related. In recent years, a range of IBD therapeutic drugs have emerged that include sulfasalazine, azathioprine, corticosteroids,

classical immunosuppressive agents, and anti-tumour necrosis factor (TNF)- α antibodies (Gao et al., 2019)

Mouse Models

Mouse models of IBD and CRC are widely used to help in the understanding of how living tissues function and the biology of underlying gut diseases (Kiesler et al., 2015). Computer models and intestinal cell cultures are also used to study gut disorders; however, such models cannot replicate complicated interactions that exist in the whole digestive system, especially in the gut where the extremely important interactions between the mucus and the gut microbiome take place. Although cell lines are effective models for studies and are commonly used to understand factors released by cancer cells and their receptors, they cannot be used for studies on tumour growth and metastatic spread of cancer (Mirabelli et al., 2019). In mice models, acute and chronic colitis are mediated intrarectally by administering 2,4,6-trinitro benzene sulfonic acid (TNBS) - oxazolone, which induces a T-cell-mediated response against hapten modified autologous proteins/luminal antigens (Wirtz et al., 2007). In comparison to dinitrobenzene sulfonic acid (DNBS), TNBS is considered toxic due to its highly oxidative properties, which can lead to an explosion when in contact with bases like sodium and potassium hydroxide (Morampudi et al., 2014b). The most used chemically induced model of intestinal inflammation is dextran sulphate sodium (DSS). Mice are fed DSS-enriched water for several days, which appears to be especially harmful to colonic epithelial cells of the basal crypts (Wirtz et al., 2007). Colitis in these animal models result from injury repair similar to UC, but it must be controlled to prevent differences in DSS concentration and irregular water uptake by the animals; this would result in imbalanced exposure and fluctuation in the level, extent, and distribution of tissue injury in the colon between animals. All of these characteristics contribute to heterogeneity and restrict the ability to evaluate outcomes across studies conducted by various researchers (Morampudi et al., 2014b).

The inflammatory colitis inducing agents are diluted in different levels of ethanol concentrations and administered via rectal instillation in the DNBS colitis models (Morampudi et al., 2014a). The ethanol treatment is required to interrupt the colonic mucosal barrier, enabling DNBS or TNBS to enter into the lamina propria and haptenize the localised colonic and gut microbial proteins, allowing them to become immunogenic and stimulate host immune

responses (Eissa et al., 2017). DNBS leads to severe inflammation in the colon and rectum, eliciting a strong inflammatory response associated with a high levels of myeloperoxidase (Peritore et al., 2021), IL-1 β and TNF- α (Andrade et al., 2020). Care must be taken during DNBS administration to ensure that the concentration of DNBS does not exceed to avoid rapid death of animals due to bowel punctures and sepsis. Due to the heterogeneity and inability to accurately monitor the degree of colitis in these chemically induce animal models of IBD, other models are required to study colitis in a more homogenous environment. As such the *Winnie* mouse model has been used to study colitis in animal models.

Mucin 2 is highly expressed on epithelial cells especially that of the colon. A single missense mutation in the *Muc2* mucin gene causes endoplasmic reticulum stress in intestinal goblet cells, a depleted intestinal mucus barrier, and spontaneous distal colonic inflammation by 6 weeks of age in the *Winnie* mouse model of spontaneous chronic colitis. (Eri et al., 2011). *Winnie* mice exhibit symptoms of diarrhea, ulcerations and rectal bleeding and pain at different stages of colitis like human IBD (Rahman et al., 2015). Colitis in *Winnie* mice is initiated predominantly by an IL-23 mediated cytokine storm which can be ameliorated by administering anti-IL-23 monoclonal antibodies and dexamethasone (Wang et al., 2015c). As *Winnie* mice are created through a single nucleotide point mutation (which is not a gene deletion) causing clinical features resembling human colitis with an intact functional immune system, these mice are excellent pre-clinical models for studies in the pathophysiology of IBD. In addition colonic cultures from *Winnie* mice, secrete high levels of IL-1 β . Extensive studies undertaken in *Winnie*'s have rated them as the best murine model accessible for understanding human chronic colitis and its pathogenesis (Rahman et al., 2015).

Inflammatory mediators in IBD

Inflammation is induced because of tissue damage following an infection, chemical irritation, or shock (Chen et al., 2017). In response to signals produced by resident macrophages, mast cells, or epithelium, neutrophils migrate to the site of inflammation (Kim and Luster, 2015, Selders et al., 2017, Krystel-Whittemore et al., 2016). A network of signalling molecules, including growth factors, cytokines, and chemokines, then recruits other immune cells to the site of inflammation. (Turner et al., 2014, Morán et al., 2013). During chronic inflammation, inflammatory infiltrates mainly consist of lymphocytes and macrophages (Vatankhah et al., 2015). Many studies have reported that patients with IBD exhibit specific miRNA expression

profiles (Chapman and Pekow, 2015), which might be involved in the initiation/development of inflammation (Fisher and Lin, 2015, Schaefer et al., 2015, Olaru et al., 2013, Wang and Chen, 2019, Dalal and Kwon, 2010, Xu and Zhang, 2016). Despite many miRNAs which contribute to the pathogenesis of IBD, the exact role of most is still unclear (Moein et al., 2019).

Immune cells in IBD

Macrophages are the intermediary cells between the innate and adaptive immune systems, and are responsible for the secretion of growth factors, cytokines and reactive oxygen and nitrogen species (Mittal et al., 2014, Sheng et al., 2013, Sheng et al., 2010, Sheng et al., 2011). Although these factors usually promote the inflammatory responses that lead to healing, prolonged inflammation may result in continuous tissue damage and subsequent sustained cell proliferation, potentially leading to malignant transformation. (Coussens and Werb, 2002, Morgillo et al., 2018, Moss and Blaser, 2005, Morrison, 2012, Hanahan and Weinberg, 2011). Macrophages play an vital role in the pathogenesis of chronic inflammation and contribute to disease advancement and/or maintenance by secreting pro-inflammatory cytokines such as TNF- α (Xiao et al., 2013), and are therefore, commonly linked with inflammatory-related diseases, including IBD.

Eosinophils have also been associated with long-term intestinal inflammation and are abundantly present in IBD (Filippone et al., 2019). Eosinophils are not only involved in inflammation but also induce alterations to the enteric nervous system and are linked with disease severity (Stavelly et al., 2022a). The chemokine receptor, CCR3 which plays a role in the recruitment and activation of eosinophils has been shown that its blockade via a CCR3 antagonist attenuates disease severity and morphological damage to inflamed intestinal tissues in the spontaneous model of chronic colitis (*Winnie* mice) (Filippone et al., 2022b). Similarly, in guinea pigs, intestinal inflammation / colitis caused following TNBS treatment results in increase of eosinophils at the site of inflammation. Use of CCR3 antagonists alleviates enteric neuropathy and restores functional changes of the intestines (Filippone et al., 2018).

Other immune cells have also been described in IBD and include dendritic cells, neutrophils, natural killer cells, T cells, B cells, Th1/Th17 cells, Th2 cells, regulatory T cells, leading to a complicated interaction between the immune cells, epithelial cells and the intestinal microbiota. Activation of the immune system highlights the role of immune cells in the pathophysiology of IBD (Fig. 1) (Lu et al., 2022).

Cytokines and chemokines

Cytokines control host immune response in infection, inflammation, and trauma. Proinflammatory cytokines, such as interleukin (IL)-1 influences the TME, promotes cancer initiation and progression (Gelfo et al., 2020) and TNF- α stimulates cancer cell growth, proliferation, invasion, metastasis and tumour angiogenesis (Barriga et al., 2019, Kuol et al., 2018c, Kuol et al., 2018b, Kuol et al., 2018a, Wang and Lin, 2008). Chemokines, cytokines, and their downstream targets have received a lot of attention in the research on inflammation-induced cancer. (Chen et al., 2017). These inflammatory mediators promote tumour development, infiltration, metastasis and assist in angiogenesis (Aguilar-Cazares et al., 2019). The inflammatory cells, and their associated chemokines and cytokines affect the entire tumour organ and regulate the growth, migration and differentiation of all cell types including neoplastic cells, fibroblasts and endothelial cells in the TME (Coussens and Werb, 2002, Chow and Luster, 2014, Landskron et al., 2014). Toll-like receptors (TLR) and nucleotide oligomerization domain receptors (Inoue et al.) are pathogen-sensitive innate immune receptors that, when activated, cause the production of chemokines and cytokines that recruit immune cells. (Silva et al., 2016). TLR signalling pathways also stimulate production of other proinflammatory cytokines such as IL-12 and IL-6 (Silva et al., 2016). Due to these effects, the function of cytokines in IBD which may lead to cancer is important to identify novel treatment of IBD.

TNF- α is one of the main pro-inflammatory cytokines, secreted by macrophages in IBD and has become an significant target for IBD therapy due to dramatic reduction of inflammatory markers and structural harm to the mucosa following its inhibition (Fig. 1) (Sanchez-Munoz et al., 2008, Strober and Fuss, 2011). The study of endogenous biochemical signals that attribute to chronic intestinal inflammation might lead to the development of more successful treatment. (Carty and Rampton, 2003, Biasi et al., 2013). Since TNF- functions have been found to be a major target for the development of treatments, the TNF-inflammatory pathway in IBD has been extensively studied. Several anti-TNF monoclonal antibodies, including infliximab, adalimumab, certolizumab pegol, and golimumab (Fig. 1), have been developed because of this approach but these drugs are primarily ineffective in many patients or lose effectiveness over time (Argollo et al., 2017). However, the monoclonal antibody infliximab has been shown to effectively treat IBD (Xiao et al., 2013).

Toll-like receptors

Toll-like receptors (TLRs) are transmembrane receptors, also known as pattern recognition receptors and stimulate pro/anti-inflammatory gene functions and restrict adaptive immune responses (Mogensen, 2009, Silva et al., 2016, Jang et al., 2015). In CD, the regulation of TLR2 and TLR4 receptors are higher compared to healthy controls, which triggers faulty immune recognition (Szebeni et al., 2008, Oliveira-Nascimento et al., 2012), and is linked to chronic inflammation in IBD (Silva et al., 2016).

The nuclear factor kappa B

The nuclear factor kappa B (NF- κ B) pathway has been widely studied as a model of pro-inflammatory signalling pathway, due to its role in the expression of pro-inflammatory cytokines, chemokines, and adhesion molecules. (Lawrence, 2009). NF- κ B regulates the expression of a variety of genes that are involved in the innate immune response, such as IL-1, IL-2, IL-6, IL-12, IFN- γ and TNF- α (Silva et al., 2016, Liu et al., 2017a) and play key roles in many physiological and pathophysiological processes and mediating inflammatory signals (Prakash et al., 2016). In IBD these cytokines cause colonic tissue damage and NF- κ B has been found to be a key regulator in this immune setting. NF- κ B is over expressed in patients with IBD and influences mucosal inflammation (Atreya et al., 2008). Blockade of NF- κ B activation has been used as a strategy to treat IBD. MG-341 an inhibitor of 26S proteasome, a target for NF- κ B inhibition has been shown to attenuate colonic inflammation (Atreya et al., 2008).

Matrix metalloproteinases

Matrix metalloproteinases (MMPs), also known as matrixins, are very minimal or negligible in the usual tissues, however, their function is transcriptionally controlled by inflammatory cytokines, growth factors, hormones, as well as cell–cell and cell–matrix interactions (Nagase et al., 2006). MMPs are also regulated by precursor zymogen activation and prevented by endogenous inhibitors and tissue inhibitors of metalloproteinases (TIMPs) (Visse and Nagase, 2003). MMP-9 has been determined as a crucial pathogenic factor in IBD, being elevated in IBD patients exhibiting a malfunctioning intestinal tight-junction barrier with increased intestinal permeability (Bischoff et al., 2014). The loss of intestinal epithelial barrier function is a significant factor in the onset and persistence of intestinal inflammation. (Fig. 1). The

specific role of MMP-9 in intestinal barrier function is still uncertain (Al-Sadi et al., 2019). The inflammatory component of a developing neoplasm often includes a diverse leukocyte population — for example, neutrophils, dendritic cells, macrophages, eosinophils and mast cells, as well as lymphocytes — all of which secrete a wide array of cytokines and cytotoxic mediators including reactive oxygen species, serine and cysteine proteases, MMPs, membrane-perforating agents, as well as soluble mediators of cell killing, such as IFNs, TNF- α , and cytokines (Kuper et al., 2000, Wahl and Kleinman, 1998).

Cyclooxygenase-2

COX-2, a key enzyme in fatty acid metabolism, is activated during both inflammation and cancer. It is stimulated by pro-inflammatory cytokines at the site of inflammation, and increased COX-2-induced prostaglandin synthesis facilitates cancer cell proliferation, angiogenesis, inhibits apoptosis, and enhances metastatic potential, making it a hot topic in research. (Desai et al., 2018). In IBD, COX-2 is highly induced by intestinal epithelial cells and since COX-2 plays a role in the development of CRC, inhibition of COX-2 may reduce the incidence of CRC. In fact, non-steroidal anti-inflammatory drugs and COX-inhibitors used in a double-blind placebo-controlled human clinical trial in patients with IBD was beneficial in most IBD patients without any exacerbations of IBD (El Miedany et al., 2006). In addition, double targeting using an inhibitor of nitric oxide synthase as well as COX-2 inhibitor in IBD has the potential for the treatment of inflammation and colitis (Dudhgaonkar et al., 2007).

Myeloid differentiation primary response 88

Myeloid differentiation primary response 88 (MyD88) is the established adaptor for inflammatory signalling pathways following activation of TLRs and IL-1 receptor families (Deguine and Barton, 2014). MyD88 signalling is involved in the advancement of CAC in colonic myeloid cells. Intestinal myeloid cells are important for maintaining local homeostasis and have a major role in regulating the existence of colitis and CAC (Song et al., 2018). MyD88 deletion also causes an increase in mucosal expression of COX-2, p-STAT3, β -catenin, and cyclinD1; all of which are associated with further DNA damage and β -catenin mutation. The addition of dextran sulphate sodium to drinking water enables MyD88 knockout mice to develop severe colitis, macrophages and CD4⁺ T cell infiltration in the intestinal mucosa (Araki et al., 2005). In addition, MyD88 knockout mice infected with *Salmonella*

Typhimurium endured enhanced intestinal tissue loss and showed barrier disruption, compared to wild-type mice (Bhinder et al., 2014). Thus, myeloid MyD88 signalling protects the intestine from inflammation as well as tumourigenesis during the development of CAC (Song et al., 2018).

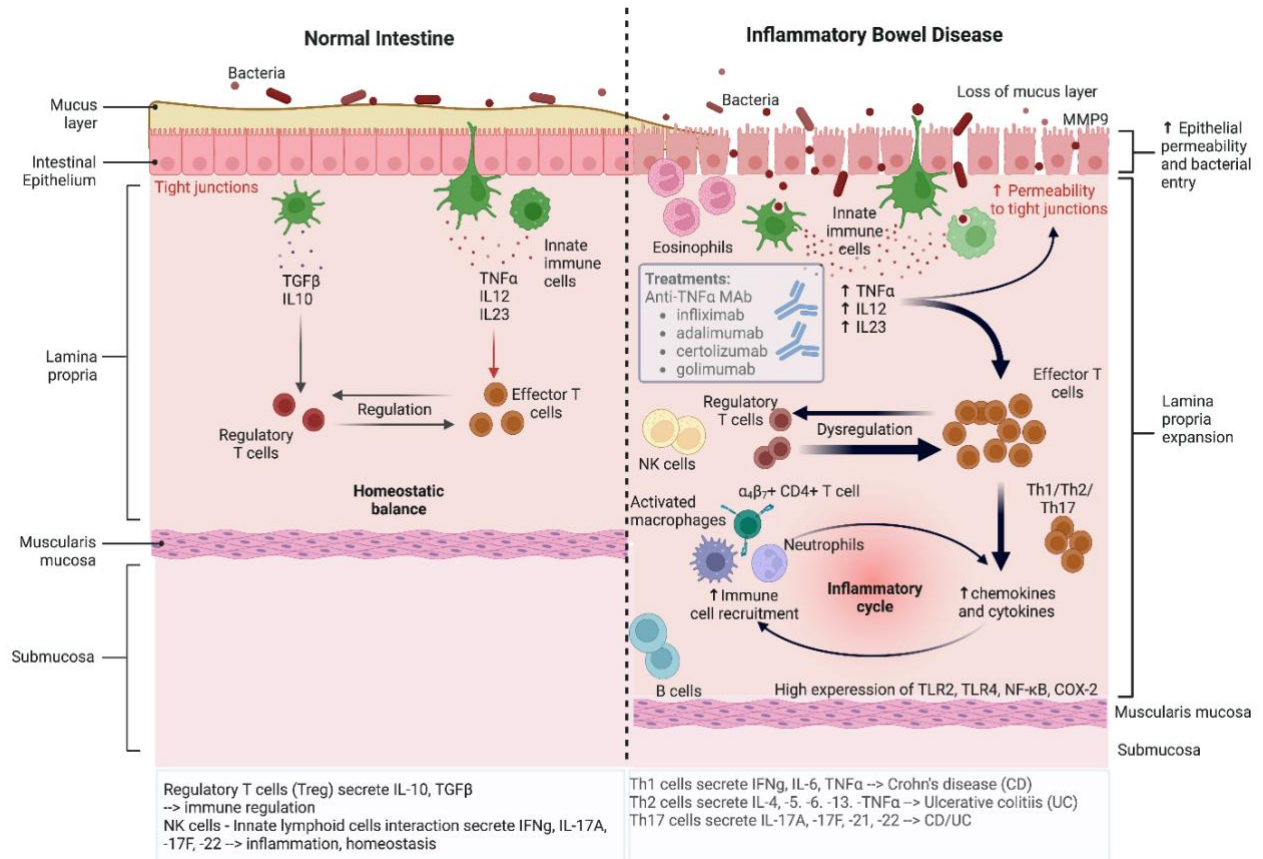


Figure 1. The immunological complexity of IBD.

Abbreviations: CD, Crohn's disease; COX-2, cyclooxygenase 2; IL, ; IFN-g, ; MAb, monoclonal antibody; MMP9, matrix metalloproteinase 9; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells; NK, natural killer; Th, helper T cells; TLR, toll-like receptor; TNF-a,; UC, ulcerative colitis. Created using Biorender.com

Inflammation and Cancer

There is a firm link between chronic inflammation and cancer, mediated by several inflammatory pathways including cytokines and mediators. In addition to exogenous mutagens, immune cell infiltrates in the colon develop a surrounding rich in reactive oxygen and nitrogen species, which can result in DNA damage, enabling the onset of oncogenesis. (Francescone et al., 2015). Dysplasia can be polypoid or plain, localised, distributed, or multifocal in IBD patients. When dysplasia is observed in patients, it exposes the entire colon at risk of neoplasia, requiring surgical removal of the entire colon and rectum. These morphological and biological differences pose major challenges in clinical cancer surveillance in IBD patients more than in the general population, raising critical questions over chronic inflammation's contribution to the develop CRC.

Colitis associated cancer (CAC)/ CRC.

CAC is a subset of CRC that can be developed in patients with long-standing IBD. The role of inflammation as a risk factor in CRC has been largely investigated, both in CAC and sporadic tumours (Grivennikov, 2013). CRC is the third most common malignancy in the world, with more than 1 million annual new cases worldwide (Song et al., 2019). It is one of the most diagnosed and causes of cancer deaths worldwide. CRC is linked to prolonged period of colitis and anatomic significance, along with the presence of other inflammatory symptoms such as primary sclerosing cholangitis (Dienstmann et al., 2017)

Colitis-associated CRC and random CRC are different in terms of presentation and molecular characteristics. These variations are brought on by variations in DNA methylation, which induces changes in gene expression (Khalil et al., 2019). The use of drugs for treating inflammation, such as 5-aminosalicylates and steroids, may inhibit the advancement of CRC in IBD (Itzkowitz and Yio, 2004a). CRC affects the caecum, colon, and rectum. Most CRC patients receive chemotherapy prior to or following surgery, however, diarrhea, constipation, oral mucositis, nausea, and vomiting are frequent gastrointestinal side effects occurring in up to 80-90% of patients. These side effects may also result in the development of malnutrition and dehydration in patients leading to rapid weight loss (cachexia) (McQuade et al., 2016). Early death rates of up to 4.8% associated with chemotherapy are due to gastrointestinal toxicity (O'Brien et al., 2006). Due to these complications, the administration of chemotherapy

is often restricted, leading to reduction in efficacy. Although animal models of IBD have given insights into the underlying cause of CAC, the molecular processes by which inflammation stimulates cancer remain is not clear (Currey et al.).

Cancer microenvironment

The cancer microenvironment refers to cells present around the cancer cells which include, fibroblasts, natural killer cells, macrophages, monocyte derived dendritic cells, CD4⁺ T cells, CD8⁺ T cells, regulatory T cells, eosinophils, neutrophils, blood vessels and proteins produced by all these cells and support the development of cancer cells (Whiteside, 2008). The cancer microenvironment is quite complex (Fig. 2) (Apostolopoulos, 2019, Barriga et al., 2019). The range of innate and adaptive immune cells present in the cancer microenvironment secrete both pro- and anti-tumourigenic mediators. Cancer cell interactions with its surrounding microenvironment (stromal cells, extracellular matrix, immune cells), are important for cancer cells clonal evolution, heterogeneity, as well as developing resistance against drugs, which all lead to the proliferation, growth and metastasis of cancer cells (Baghban et al., 2020). In addition, the nervous system interacts with cancer cells, adding to their regulation, growth, angiogenesis, and metastasis (Kuol et al., 2018c, Kuol et al., 2018b, Kuol et al., 2018a). Cancer immunotherapy involves boosting of immune CD4⁺, CD8⁺ T cells and B cells to kill or block cancer cells (Apostolopoulos and McKenzie, 1994, Apostolopoulos and McKenzie, 2017). However, cancer cells have evolved, and certain checkpoint makers are expressed which allows their escape from immune attack. These include CTLA-4 and PD-1 or PD-L1. The PD-1/PD-L1 pathway is the frontline of interactions between immune cells, stromal cells, and cancer cells (Ephraim et al., 2022b).

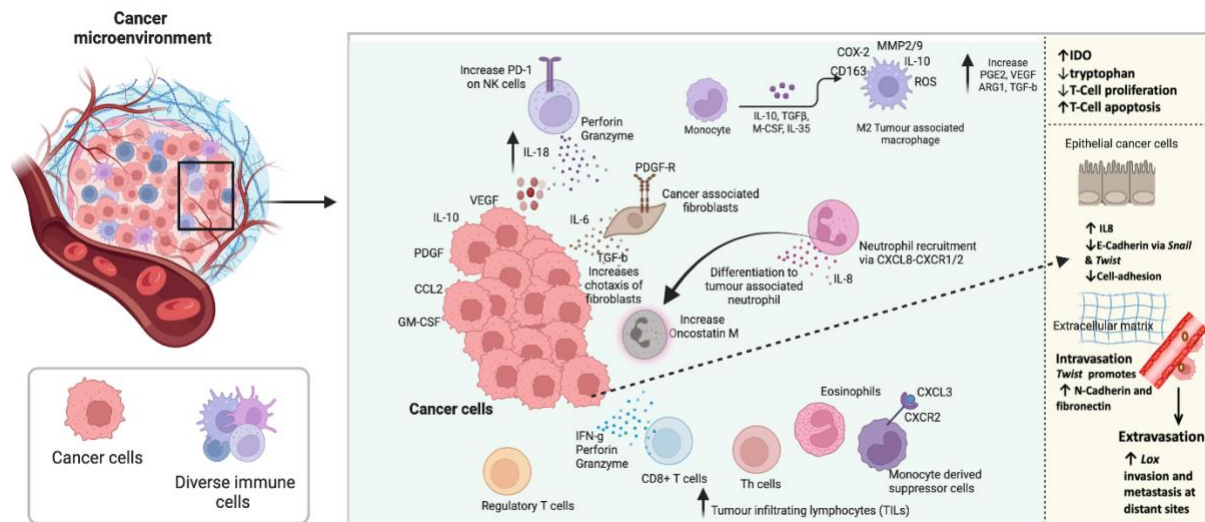


Figure 2. The immunological complexity of the cancer microenvironment. Created using Biorender.com
Abbreviations: ARG1, arginase 1; CCL2, C-C motif chemokine ligand 2; CD, cluster differentiation; COX-2, cyclooxygenase 2; CXCL3, chemokine (C-X-C motif) ligand 3; CXCR2, C-X-C motif chemokine receptor 2; GM-CSF, granulocyte macrophage colony stimulating factor; IDO, ; IL, ; Lox, lysyl oxidase; M-CSF, macrophage colony stimulating factor; MMP2/9, matrix metalloproteinase 2/9; NK, natural killer; PD-1, ; PDE2, phosphodiesterase 2; PDGF, platelet derived growth factor; PD-L1; ROS, reactive oxygen species; TGFβ, tumour growth factor beta; VEGF, vascular endothelial growth factor.

Checkpoint markers

The ability of the immune system to distinguish between self and non-self-antigens using "checkpoints" is one of its most important functions. Checkpoint markers are present on cancer cells as a means of evading immune attack. Checkpoint inhibitors therapy differ from traditional chemotherapy by increasing the activation of immune cells, specifically T cells. (Fig. 3) (Gun et al., 2019). In contrast to chemotherapy, tolerance to checkpoint inhibitors appears to be higher, resulting in fewer side effects and a better outcome for cancer patients. (Deshpande et al., 2020). ICIs are monoclonal antibodies designed for an increasing number of malignant diseases. Checkpoint inhibitors include CTLA-4 inhibitors (ipilimumab, tremelimumab) and PD1/PD-L1 inhibitors (pembrolizumab, nivolumab, durvalumab, atezolizumab), are more frequently used in clinical trials for the treatment of several cancers) Lemiale et al., 2019(. Determining the role of PD-1/PD-L1, PD-L2, IDO, Siglecs, and CTLA-4 in animal models of IBD, patients with IBD, as well as in CRC can provide insights into disease initiation and progression that may assist in identification of novel targets. Furthermore, anti-PD-1/PD-L1 therapies are not relevant to all patients, implying the need to identify additional targetable immune checkpoints (Lim et al., 2021).

Role of checkpoint inhibitors

The purpose of cancer immunotherapy is to stimulate cytotoxic T lymphocytes / CD8⁺ T cells against tumour associated proteins/receptors and, help the initiation of tumour specific T cells in lymphoid organs to achieve efficient and long lasting anti-tumour immunity (Borst et al., 2018). However, the TME is complicated involving immune cells, cytokines, and checkpoint markers (Ephraim et al., 2022b). Using ICIs as a single agent or in combination treatments with chemotherapy, radiotherapy, or immunotherapeutic intervention, have produced efficient and long-term clinical outcomes (Kuol et al., 2018d).

PD-1 (CD279) is a checkpoint molecule on T cells that prevent T cells from damaging its own cells in the body. It is present on activated T cells and binds to PD-L1 or PD-L2 on tumour cells, causing deactivation and death of T cells. In mouse models, the loss of PD-1 expression on T cells has been observed to substantially prolong tumour growth and increase CD8⁺ T cells inside the TME (Fig. 3). Nivolumab, is a PD-1 inhibitor, approved by the FDA is in use for metastatic melanoma (Larkin et al., 2019). In mouse models of IBD and IBD patients, PD-1 is upregulated on T cells, macrophages, dendritic cells, B cells and in colon tissues that are inflamed. Some examples of drugs that target PD-1 include Pembrolizumab (Keytruda) and Nivolumab (Yousuf et al., 2020). In addition, ipilimumab (Yervoy) is a monoclonal antibody that inhibits CTLA-4 activity, providing a similar effect. Drugs that target the PD-1 and CTLA-4 pathways have specifically demonstrated considerable clinical efficiency and gained approval as single-agent or combination therapy for regular use (Rotte, 2019). Advances in immuno-oncology have started to revolutionize the standard of care for many types of cancer. Pembrolizumab and nivolumab are two PD1-blocking antibodies that have shown effectiveness in individuals with metastatic CRC (mismatch-repair-deficient) and microsatellite instability-high (dMMR-MSI-H) and have gained rapid FDA approval (Ganesh et al., 2019).

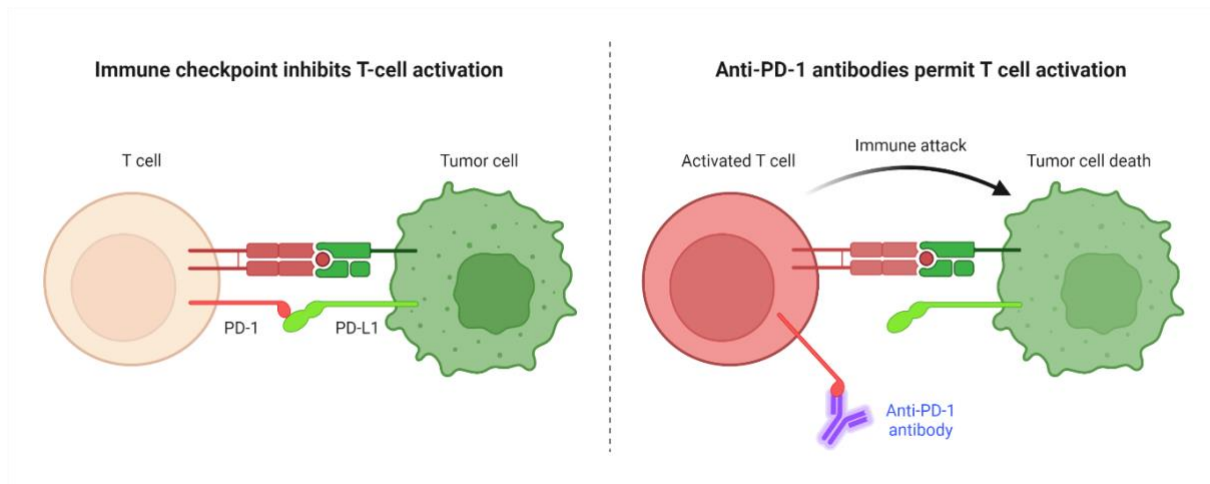


Figure 3. Immune checkpoints inhibit T cell activation in the cancer microenvironment, upon ligation of PD-1 expressed by T cells to its ligand PD-L1 on cancer cells. Anti-PD-1 monoclonal antibodies interfere with PD-1/PD-L1 interaction and permits T cell activation and lysis of cancer cells. Figure created using Biorender.com.

CTLA-4, also known as CD152, is an immune checkpoint receptor that inhibits CD8⁺ T-cell activation. It is present on regulatory T cells and facilitates their immunosuppressive effect (Fig. 4) (Rowshanravan et al., 2018). Anti-CTLA-4 monoclonal antibodies - ipilimumab and tremelimumab have the ability to prevent CTLA-4 ligand-driven immunosuppression (Kolar et al., 2009). CTLA-4 and PD-1 are inhibitors of T-cell immune function (Buchbinder and Desai, 2016). CTLA-4 is a CD28 homolog has a stronger bond with B7 (Buchbinder and Desai, 2016). CTLA4 is linked to the progression of CRC (Zou et al., 2018).

Siglecs are transmembrane sialic acid-binding proteins of the immunoglobulin superfamily that contain an N-terminal V-set Ig-like domain as well as a variable number of C2 set domains. (Table 1) (Läubli and Varki, 2020). Siglec-3 is also known as CD33 and has been determined as a myeloid lymphoma marker in clinical studies much before any other Siglec was discovered (Hernández-Caselles et al., 2006). Siglec-3 was mapped to chromosome 19 and previous studies on cDNA isolation and cloning suggested CD33 association with MAG (Siglec-4) (Kelm and Ravindran, 2007). Siglec-8 is present on the surface of human eosinophils, mast cells, and basophils, and its activation by specific glycan ligands or antibodies initiates loss of eosinophils and reduces mast cell degranulation (Wei et al., 2018b). Siglec-8 promotes cytokine-dependent death (Kano et al., 2013). Siglec-9 is upregulated on neutrophils, and induces cell death when associated with monoclonal antibodies (Graeter et al., 2019). Current

research on Siglecs have demonstrated major roles in tumour immunosurveillance including immunosuppression, that are appealing anti-cancer molecular targets (Lim et al., 2021).

Table 1. Siglecs and their function

Siglecs	Function	References
Siglec-1 (CD169)/ Sialoadhesin	Cell adhesion, cancer progression	(Delputte et al., 2011, O'Neill et al., 2013)
Siglec-2 (CD22)	Dampening B cell receptor activation	(Ren et al., 2018)
Siglec-3 (CD33)	Downstream signalling function	(Tsai et al., 2021)
Siglec-4 (Myelin Associated Glycoprotein, MAG)	Interaction between MAG and cancer-associated MUC1, stabilizes myelin-axon interactions	(Sun et al., 2004, Lim et al., 2021)
Siglec-5 (CD170)	Associates with leukocyte counter-receptor P-selectin glycoprotein ligand-1, prevent leukocyte recruitment to sites of inflammation and maintains a pro-cancer environment.	(Erickson-Miller et al., 2003)
Siglec-6 (CD327)	Immune-inhibitory, inhibitory receptor on mast cells in CRC	(Yu et al., 2018)
Siglec-7 (p75/AIRM1, CD328)	Natural killer (NK) cell-inhibitory receptor bearing immunoreceptor tyrosine-based inhibition (ITIM) motifs	(Hong et al., 2021)
Siglec-8	A target in allergen-induced inflammation	(Trebo et al., 2021)
Siglec-9 (CD329)	Binds to MUC1 with sialylated T-antigen (MUC1 ST)	(Choi et al., 2021)
Siglec-10	Repress DAMP-mediated innate inflammatory responses	(Barkal et al., 2019)
Siglec-11	Inhibitory function, microglial activities	(Fraschilla and Pillai, 2017, Lim et al., 2021)
Siglec-12 (Siglec-XII)	Recruit SHP2-related oncogenic pathways	(Mitra et al., 2011)
Siglec-13	Deleted in humans	(Lim et al., 2021)
Siglec-14	Suppresses myeloid inflammatory responses	(Huang et al., 2018)
Siglec-15	Upregulated on cancer cells and tumour-infiltrating myeloid cells	(Wang et al., 2019)
Siglec-16	Expressed on cancer cells	(Chen et al., 2020)
Siglec-17	Deleted in humans but pseudogene exists	(Lim et al., 2021)

IDO is an enzyme that reduces tryptophan, an essential amino acid (Manson et al., 2016), and is overexpressed in the colon, intestines as well as the lung. The mechanism by which IDO works is by catalysing the oxidative ring cleavage of pyrrole's in tryptophan, serotonin,

melatonin, and other indoleamine derivatives (Prendergast et al., 2014). In CRC, the regulation of IDO by tumours is associated with metastases and inversely linked with infiltration of T cells (Xu et al., 2018). The expression of IDO1 could be generated by IFN- γ , lipopolysaccharide, and TNF. Thus, in response to inflammatory signals under pathophysiological conditions IDO1 is highly up regulated by the immune system, and over-expression of IDO1 improves detection in different types of cancers, including melanoma, pancreatic, ovarian, and colorectal (Xu et al., 2019). The IDO1 paralog IDO2, despite having received far less research, may be a possible alternative as a therapeutic target in cancer immunotherapy. IDO2 is substantially less efficient than IDO1 at metabolising tryptophan, and its functions are instead the result of interactions with other, as-yet-unidentified proteins that may change in various inflammatory and neoplastic circumstances (Mondanelli et al., 2021).

Tim-3 is a marker selectively present on IFN- γ -producing CD4⁺ T helper 1 (Th1) and CD8⁺ T cytotoxic 1 (Tc1) T cells (Fig. 4) (Hou et al., 2017). Tim-3 is upregulated in CRC, compared to normal tissues (Yu et al., 2017). The Tim-3 pathway is a target for anticancer immunotherapy due to its expression on both non-functional CD8⁺ T cells and Tregs—two main immune cell groups that suppress the immune activity in tumour tissue (Das et al., 2017) and has exhibited remarkable results in preclinical cancer models. Tim-3/PD-1 pathway co-blockade is more successful than either Tim-3 or PD-1 pathway blockade only at rebuilding tumour antigen-specific IFN- γ production in CD8⁺ T cells in mice carrying tumours (Anderson, 2014).

LAG-3 is a critical immune checkpoint marker with implications for a variety of diseases, including cancer. (Fig. 4) (Graydon et al., 2021). LAG-3, along with other ligands such as galectin-3 and LSEC-tin, binds to MHC class II. LAG3 (or CD223), like PD-1, is expressed on a variety of cell types, such as tumor-infiltrating lymphocytes (CD4, CD8) and regulatory T cells. LAG3 is essential for effective T cell expression and homeostasis. (Ruffo et al., 2019). Suppression of LAG3 showed reduced tumour growth which was not very effective, however, suppression of LAG3 and PD-1 together, not only reduced tumour growth but also increased survival rate in mice (Andrews et al., 2017, Woo et al., 2012). Tumour-infiltrating lymphocytes expressing elevated levels of LAG3 have been found in solid tumours such as ovarian cancer, melanoma, and CRC, as well as Hodgkin's and diffuse large B-cell lymphoma (Lythgoe et al., 2021). LAG3 inhibits cytokine and granzyme production and proliferation while promoting regulatory T cell differentiation. (Graydon et al., 2021).

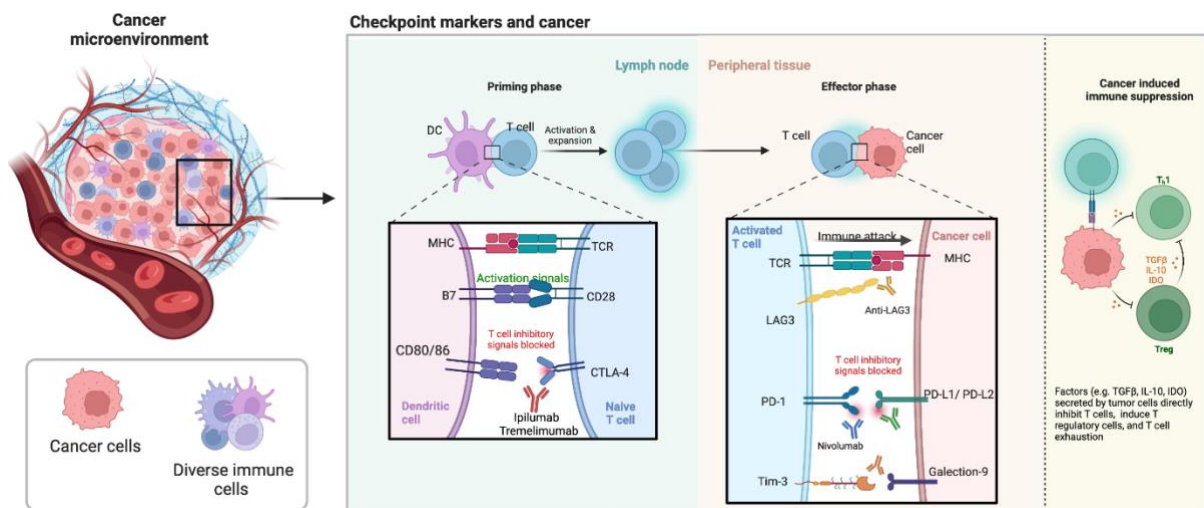


Figure 4. The cancer microenvironment is complex involving diverse immune cells and checkpoint markers. Figure reproduced from Ephraim *et al.*, (Ephraim *et al.*, 2022b). CD, cluster of differentiation; CTLA-4, ; DC, dendritic cells; IDO, ; IL, ; LAG3, ; MHC, major histocompatibility complex; PD-1, ; PDL-1, ; PD-L2, ; TCR, T cell receptor; TGF, TNF; Tim-3,; Treg, regulatory T cells.

Stimulatory Checkpoint Molecules

The CD70-CD27 axis promotes T-lymphocyte expansion and differentiation by stimulating the NF-κB pathway. (Inoue *et al.*, 2019). CD28 is present on almost all human CD4⁺ T cells and most of CD8⁺ T cells. Binding with its two ligands -CD80 and CD86, present on dendritic cells, initiates T cell expansion (Eastwood *et al.*, 2010). CD40 and its ligand CD40L may trigger antigen presenting cells to permit CD8⁺ T cell priming (Schoenberger *et al.*, 1998). CD40 is a key signalling pathway for the function of B cells, monocytes, and dendritic cells, and plays an important role in the inflammatory pathways of non-hemopoietic cells. CD40 is expressed by monocytes and dendritic cells, and it is overexpressed when dendritic cells migrate from the periphery to depleting lymph nodes in response to a microbial threat. (O'Sullivan and Thomas, 2003). CD40 protein expression is significantly higher in CRC compared with normal tissue (Pang *et al.*, 2017). The first proof-of-concept study that shows how using CD122 alone or in conjunction with a vaccination or a Glucocorticoid-induced TNFR-related protein (GITR) monoclonal antibody immunotherapy might boost and maintain anticancer responses. These findings support the use of CD122 as a monotherapy target or in combination with other immune-targeted treatments for CRC (Villarreal *et al.*, 2017). CD137 is an inducible T-cell costimulatory receptor and a member of the TNF receptor superfamily. CD137 and CD137L expression was upregulated in all investigated colon cancer tissues compared to normal colon

tissues. Targeted microenvironment imaging strategies may be used for early detection of tumours, and isotope labelled anti-CD-11b could be further evaluated as a potential probe (Cheng et al., 2014). Inflammatory responses closely regulate CD163 expression, with anti-inflammatory signals (e.g., IL-10, glucocorticoids) stimulating CD163 expression while proinflammatory signals (e.g., lipopolysaccharide, TNF- α , IFN- γ) suppressing CD163 synthesis (Yu et al., 2015).

The B7 ligand family comprises of 10 members -CD80 (B7-1), CD86 (B7-2), PD-L1 (B7-H1), PD-L2 (B7-DC or CD273), ICOSL (B7-H2), CD276 (B7-H3), B7S1 (B7-H4, B7x or Vtn1), V-domain immunoglobulin suppressor of T cell activation (VISTA) (B7-H5, GI24, or PD-1H), B7-H6, and B7-H7 (HHLA2) (Wang et al., 2020). B7-1 or CD80 is upregulated in dysplastic colonic mucosa of UC patients, with CD80 signalling between intestinal epithelial cells and T-cells representing a critical factor in the development of inflammatory colonic carcinogenesis from low to advanced dysplasia (Scarpa et al., 2015). Oncogenic insults, such as oxidative DNA damage linked to long-term intestinal inflammation, can induce CD80 expression. The stromal B7-2 or CD86/CD163 ratio could be used for personal risk assessment of relapse and mortality for stage II-III CRC (Xu et al., 2021). Together with tumour staging, this ratio may aid in personalized treatment. B7-H1, PD-L1, or CD274 are upregulated in colorectal carcinoma and have been linked to cell differentiation and tumor-node-metastasis placement. (Shi et al., 2013). B7-H2 or Inducible costimulatory ligand (ICOS-L) expressed on CD8⁺ T cells in the tumour micro-environment are closely associated with progression of CRC (Cao et al., 2018). B7-H3 or CD276 are potentially associated with CRC advancement and evasion of cancer immune surveillance (Zhou and Jin, 2021). B7-H4 is associated with CD133 and CD44 regulation in CRC tissues, and B7-H4 knockout mice prevents growth of tumour spheroids, cell migration, and infiltration in CRC cell lines which shows that it could be a potential prognostic biomarker for CRC (Zhou et al., 2022a). B7-H5 (also known as VISTA, C10orf54, PD-1H, Gi24, and Dies1) is expressed at higher levels in cancer sections compared to non-cancer tissues, promotes tumour immune escape, associated with lymph node participation, cancer stage, and survival (Wang et al., 2021b). As such, B7-H5 is an important marker for prognosis and a potential target for the immunotherapy of CRC. B7-H6 is activated at the surface of CD14⁺CD16⁺ proinflammatory monocytes and neutrophils in response to Toll-like receptors or proinflammatory cytokines such as IL-1 β and TNF α (Ni and Dong, 2017). The novel B7-H6/CD3 bispecific IgG-like T cell engaging antibody may be used for the treatment of CRC and haematologic malignant cells (Sun et al., 2021a). The level of

HHLA2 expression in CRC patients was discovered to be positively connected with a high death rate and strongly associated to the complexity of invasion and CD8+ T cell infiltration status, implying that it may operate as an independent prognostic factor related with overall survival; however, the thorough regulation of HHLA2 in CRC remains unclear. Human leukocyte antigen (HLA)-G is a member of HLA family and soluble HLA-G (sHLA-G) levels are shown to be elevated in pediatric patients with CD compared to UC, making it a potential biomarker for CD. (Cansever et al., 2022). In both haematological and solid tumours, there is a high rate of HLA-G surface activity and elevated sHLA-G serum levels. HLA-G and sHLA-G regulation is associated with poor treatment results in cancer patients, implying a role in cancer cells' immune escape mechanism. (Bortolotti et al., 2014).

Challenges and Gaps

Although the frequency of checkpoint inhibitors as causes of adverse events is much less than chemotherapy treatments, their use may lead to emergency ward if severe immune-related adverse events (irAEs). The occurrence of irAEs is also less frequent following immunotherapy compared to chemotherapy, however, its best that intensivists are familiar with the side effects of these medications, especially if in intensive care unit admission is required) Lemiale et al., 2019.(The immunosuppressive features of tumour lesions play a big part in the advancement of cancer and are a big challenge for effective immunotherapies. Blocking the pathways of PD-1/PD-L1 has demonstrated excellent therapeutic efficacy for cancer patients with a variety of diseases. However, the effectiveness of authorised ICIs in treating CRC is poor, and only a small proportion of individuals benefit from them. The current ICIs are not suitable for most CRC patients with microsatellite stable or mismatch repair proficiency or reduced levels of microsatellite instability. This inadequate treatment effectiveness highlights the critical need to identify more checkpoint markers in CRC.

Conclusion and identification of Gaps

Though there has been significant research and information on checkpoint markers, their roles in IBD and CRC development remain unclear and require further study. Studying checkpoint markers in the *Winnie* mouse models that closely resemble human models may lead to the discovery of biomarkers for screening IBD patients and for the advancement of understanding

inflammation and cancer that may lead to designing drugs that use inhibitors of biomarker expression or use vaccines to prevent disease progression. However, these treatments require significant improvement, one of the main aspects is to identify the baseline (pre-treatment) biomarkers to predict immune responses. In general, biomarkers are mainly divided into two functional categories: “prognostic” and “predictive” (Nalejska et al., 2014). A prognostic biomarker can be defined based on the way patient’s body or tumour biology influence the patient’s clinical outcome. This includes patients at high risk for disease relapse who may benefit from earlier treatments. On the other hand, a predictive biomarker is defined by the effects of treatment, including tumour response, and the improvements in overall survival of patients, disease-free survival, and progression-free survival (Kitano et al., 2018). Further research into these animal models and studying the function and effects of ICIs in these animal models will shed light on new targeted therapies for IBD as well as CRC.

This led to the aim of this thesis, of (i) identification of genes in spontaneous mouse model of chronic colitis (inflammation) / severe inflammation (chapter 2), and, (ii) identification of cancer pathways and genes (chapter 3).

References

1. Matricon, J.; Barnich, N.; Ardid, D. Immunopathogenesis of inflammatory bowel disease. *Self/nonself* **2010**, *1*, 299-309, doi:10.4161/self.1.4.13560.
2. Hendrickson, B.A.; Gokhale, R.; Cho, J.H. Clinical aspects and pathophysiology of inflammatory bowel disease. *Clinical microbiology reviews* **2002**, *15*, 79-94, doi:10.1128/cmr.15.1.79-94.2002.
3. Triantafillidis, J.K.; Merikas, E.; Georgopoulos, F. Current and emerging drugs for the treatment of inflammatory bowel disease. *Drug design, development and therapy* **2011**, *5*, 185-210, doi:10.2147/DDDT.S11290.
4. Axelrad, J.E.; Lichtiger, S.; Yajnik, V. Inflammatory bowel disease and cancer: The role of inflammation, immunosuppression, and cancer treatment. *World journal of gastroenterology* **2016**, *22*, 4794-4801, doi:10.3748/wjg.v22.i20.4794.
5. Chen, L.; Deng, H.; Cui, H.; Fang, J.; Zuo, Z.; Deng, J.; Li, Y.; Wang, X.; Zhao, L. Inflammatory responses and inflammation-associated diseases in organs. *Oncotarget* **2017**, *9*, 7204-7218, doi:10.18632/oncotarget.23208.
6. McCarthy, E.F. The toxins of William B. Coley and the treatment of bone and soft-tissue sarcomas. *The Iowa orthopaedic journal* **2006**, *26*, 154-158.
7. Říhová, B.; Šťastný, M. [History of Immuno-therapy - from Coley Toxins to Check-points of the Immune Reaction]. *Klin Onkol* **2015**, *28 Suppl 4*, 4s8-14.
8. Farkona, S.; Diamandis, E.P.; Blasutig, I.M. Cancer immunotherapy: the beginning of the end of cancer? *BMC medicine* **2016**, *14*, 73-73, doi:10.1186/s12916-016-0623-5.
9. Agarwal, M.B. Is cancer chemotherapy dying? *Asian journal of transfusion science* **2016**, *10*, S1-S7, doi:10.4103/0973-6247.182735.
10. Apostolopoulos, V.; Lofthouse, S.A.; Popovski, V.; Chelvanayagam, G.; Sandrin, M.S.; McKenzie, I.F. Peptide mimics of a tumor antigen induce functional cytotoxic T cells. *Nat Biotechnol* **1998**, *16*, 276-280, doi:10.1038/nbt0398-276.

11. Apostolopoulos, V.; McKenzie, I.F.; Pietersz, G.A. Breast cancer immunotherapy: current status and future prospects. *Immunol Cell Biol* **1996**, *74*, 457-464, doi:10.1038/icb.1996.76.
12. Pietersz, G.A.; Pouniotis, D.S.; Apostolopoulos, V. Design of peptide-based vaccines for cancer. *Curr Med Chem* **2006**, *13*, 1591-1607, doi:10.2174/092986706777441922.
13. Cebon, J. Perspective: cancer vaccines in the era of immune checkpoint blockade. *Mammalian genome : official journal of the International Mammalian Genome Society* **2018**, *29*, 703-713, doi:10.1007/s00335-018-9786-z.
14. Korman, A.J.; Peggs, K.S.; Allison, J.P. Checkpoint blockade in cancer immunotherapy. *Advances in immunology* **2006**, *90*, 297-339, doi:10.1016/S0065-2776(06)90008-X.
15. Barriga, V.; Kuol, N.; Nurgali, K.; Apostolopoulos, V. The Complex Interaction between the Tumor Micro-Environment and Immune Checkpoints in Breast Cancer. *Cancers (Basel)* **2019**, *11*, doi:10.3390/cancers11081205.
16. Gun, S.Y.; Lee, S.W.L.; Sieow, J.L.; Wong, S.C. Targeting immune cells for cancer therapy. *Redox biology* **2019**, *25*, 101174-101174, doi:10.1016/j.redox.2019.101174.
17. Koury, J.; Lucero, M.; Cato, C.; Chang, L.; Geiger, J.; Henry, D.; Hernandez, J.; Hung, F.; Kaur, P.; Teskey, G.; et al. Immunotherapies: Exploiting the Immune System for Cancer Treatment. *Journal of Immunology Research* **2018**, *2018*, 9585614, doi:10.1155/2018/9585614.
18. Kuol, N.; Stojanovska, L.; Nurgali, K.; Apostolopoulos, V. The mechanisms tumor cells utilize to evade the host's immune system. *Maturitas* **2017**, *105*, 8-15, doi:10.1016/j.maturitas.2017.04.014.
19. Kuol, N.; Stojanovska, L.; Nurgali, K.; Apostolopoulos, V. PD-1/PD-L1 in disease. *Immunotherapy* **2018**, *10*, 149-160, doi:10.2217/imt-2017-0120.
20. Kuol, N.; Yan, X.; Barriga, V.; Karakkat, J.; Vassilaros, S.; Fyssas, I.; Tsimpanis, A.; Fraser, S.; Nurgali, K.; Apostolopoulos, V. Pilot Study: Immune Checkpoints Polymorphisms in Greek Primary Breast Cancer Patients. *Biomedicines* **2022**, *10*, doi:10.3390/biomedicines10081827.
21. Zhang, H.; Chen, J. Current status and future directions of cancer immunotherapy. *Journal of Cancer* **2018**, *9*, 1773-1781, doi:10.7150/jca.24577.
22. Camilleri, M. Managing symptoms of irritable bowel syndrome in patients with inflammatory bowel disease. *Gut* **2011**, *60*, 425-428, doi:10.1136/gut.2010.234583.
23. Baumgart, D.C.; Carding, S.R. Inflammatory bowel disease: cause and immunobiology. *The Lancet* **2007**, *369*, 1627-1640, doi:[https://doi.org/10.1016/S0140-6736\(07\)60750-8](https://doi.org/10.1016/S0140-6736(07)60750-8).
24. Silva, F.A.R.; Rodrigues, B.L.; Ayrisono, M.d.L.S.; Leal, R.F. The Immunological Basis of Inflammatory Bowel Disease. *Gastroenterology research and practice* **2016**, *2016*, 2097274-2097274, doi:10.1155/2016/2097274.
25. Love, B.L. Management of patients with inflammatory bowel disease: current and future treatments. *Acute pain* **2019**, *10*, 00.
26. Pithadia, A.B.; Jain, S. Treatment of inflammatory bowel disease (IBD). *Pharmacological Reports* **2011**, *63*, 629-642.
27. van der Woude, C.J.; Shitrit, A.B.-G. Pregnancy, psychiatry and IBD: multidisciplinary care is crucial. *Nature Reviews Gastroenterology & Hepatology* **2019**, doi:10.1038/s41575-019-359.
28. Neuendorf, R.; Harding, A.; Stello, N.; Hanes, D.; Wahbeh, H. Depression and anxiety in patients with Inflammatory Bowel Disease: A systematic review. *Journal of Psychosomatic Research* **2016**, *87*, 70-80, doi:<https://doi.org/10.1016/j.jpsychores.2016.06.001>.
29. Davidson, M.; Rashidi, N.; Nurgali, K.; Apostolopoulos, V. The Role of Tryptophan Metabolites in Neuropsychiatric Disorders. *Int J Mol Sci* **2022**, *23*, doi:10.3390/ijms23179968.
30. Papageorgiou, M.; Raza, A.; Fraser, S.; Nurgali, K.; Apostolopoulos, V. Methamphetamine and its immune-modulating effects. *Maturitas* **2019**, *121*, 13-21, doi:10.1016/j.maturitas.2018.12.003.
31. Prakash, M.D.; Tangalakakis, K.; Antonipillai, J.; Stojanovska, L.; Nurgali, K.; Apostolopoulos, V. Methamphetamine: Effects on the brain, gut and immune system. *Pharmacol Res* **2017**, *120*, 60-67, doi:10.1016/j.phrs.2017.03.009.
32. Gao, Y.; Bai, D.; Zhao, Y.; Zhu, Q.; Zhou, Y.; Li, Z.; Lu, N. LL202 ameliorates colitis against oxidative stress of macrophage by activation of the Nrf2/HO-1 pathway. *Journal of Cellular Physiology* **2019**, *234*, 10625-10639, doi:10.1002/jcp.27739.

33. Kiesler, P.; Fuss, I.J.; Strober, W. Experimental Models of Inflammatory Bowel Diseases. *Cellular and molecular gastroenterology and hepatology* **2015**, *1*, 154-170, doi:10.1016/j.jcmgh.2015.01.006.
34. Mirabelli, P.; Coppola, L.; Salvatore, M. Cancer Cell Lines Are Useful Model Systems for Medical Research. *Cancers* **2019**, *11*, 1098, doi:10.3390/cancers11081098.
35. Wirtz, S.; Neufert, C.; Weigmann, B.; Neurath, M.F. Chemically induced mouse models of intestinal inflammation. *Nat Protoc* **2007**, *2*, 541-546, doi:10.1038/nprot.2007.41.
36. Morampudi, V.; Bhinder, G.; Wu, X.; Dai, C.; Sham, H.P.; Vallance, B.A.; Jacobson, K. DNBS/TNBS colitis models: providing insights into inflammatory bowel disease and effects of dietary fat. *Journal of visualized experiments : JoVE* **2014**, e51297-e51297, doi:10.3791/51297.
37. Morampudi, V.; Bhinder, G.; Wu, X.; Dai, C.; Sham, H.P.; Vallance, B.A.; Jacobson, K. DNBS/TNBS colitis models: providing insights into inflammatory bowel disease and effects of dietary fat. *J Vis Exp* **2014**, e51297, doi:10.3791/51297.
38. Eissa, N.; Kermarrec, L.; Hussein, H.; Bernstein, C.N.; Ghia, J.E. Appropriateness of reference genes for normalizing messenger RNA in mouse 2,4-dinitrobenzene sulfonic acid (DNBS)-induced colitis using quantitative real time PCR. *Sci Rep* **2017**, *7*, 42427, doi:10.1038/srep42427.
39. Peritore, A.F.; D'Amico, R.; Cordaro, M.; Siracusa, R.; Fusco, R.; Gugliandolo, E.; Genovese, T.; Crupi, R.; Di Paola, R.; Cuzzocrea, S.; et al. PEA/Polydatin: Anti-Inflammatory and Antioxidant Approach to Counteract DNBS-Induced Colitis. *Antioxidants (Basel)* **2021**, *10*, doi:10.3390/antiox10030464.
40. Andrade, A.W.L.; Guerra, G.C.B.; de Souza Araújo, D.F.; de Araújo Júnior, R.F.; de Araújo, A.A.; de Carvalho, T.G.; Fernandes, J.M.; Diez-Echave, P.; Hidalgo-García, L.; Rodriguez-Cabezas, M.E.; et al. Anti-Inflammatory and Chemopreventive Effects of Bryophyllum pinnatum (Lamarck) Leaf Extract in Experimental Colitis Models in Rodents. *Front Pharmacol* **2020**, *11*, 998, doi:10.3389/fphar.2020.00998.
41. Eri, R.D.; Adams, R.J.; Tran, T.V.; Tong, H.; Das, I.; Roche, D.K.; Oancea, I.; Png, C.W.; Jeffery, P.L.; Radford-Smith, G.L.; et al. An intestinal epithelial defect conferring ER stress results in inflammation involving both innate and adaptive immunity. *Mucosal Immunol* **2011**, *4*, 354-364, doi:10.1038/mi.2010.74.
42. Rahman, A.A.; Robinson, A.M.; Jovanovska, V.; Eri, R.; Nurgali, K. Alterations in the distal colon innervation in Winnie mouse model of spontaneous chronic colitis. *Cell and Tissue Research* **2015**, *362*, 497-512, doi:10.1007/s00441-015-2251-3.
43. Wang, R.; Hasnain, S.Z.; Tong, H.; Das, I.; Chen, A.C.-H.; Oancea, I.; Proctor, M.; Florin, T.H.; Eri, R.D.; McGuckin, M.A. Neutralizing IL-23 is superior to blocking IL-17 in suppressing intestinal inflammation in a spontaneous murine colitis model. *Inflammatory bowel diseases* **2015**, *21*, 973-984.
44. Kim, N.D.; Luster, A.D. The role of tissue resident cells in neutrophil recruitment. *Trends Immunol* **2015**, *36*, 547-555, doi:10.1016/j.it.2015.07.007.
45. Selders, G.S.; Fetz, A.E.; Radic, M.Z.; Bowlin, G.L. An overview of the role of neutrophils in innate immunity, inflammation and host-biomaterial integration. *Regen Biomater* **2017**, *4*, 55-68, doi:10.1093/rb/rbw041.
46. Krystel-Whittemore, M.; Dileepan, K.N.; Wood, J.G. Mast Cell: A Multi-Functional Master Cell. *Frontiers in Immunology* **2016**, *6*, doi:10.3389/fimmu.2015.00620.
47. Turner, M.D.; Nedjai, B.; Hurst, T.; Pennington, D.J. Cytokines and chemokines: At the crossroads of cell signalling and inflammatory disease. *Biochimica et Biophysica Acta (BBA) - Molecular Cell Research* **2014**, *1843*, 2563-2582, doi:<https://doi.org/10.1016/j.bbamcr.2014.05.014>.
48. Morán, G.A.G.; Parra-Medina, R.; Cardona, A.G.; Quintero-Ronderos, P.; Rodríguez, É.G. Cytokines, chemokines and growth factors. In *Autoimmunity: From Bench to Bedside [Internet]*; El Rosario University Press: 2013.
49. Vatankhah, A.; Halász, J.; Piurkó, V.; Barbai, T.; Rásó, E.; Tímár, J. Characterization of the inflammatory cell infiltrate and expression of costimulatory molecules in chronic echinococcus granulosus infection of the human liver. *BMC Infect Dis* **2015**, *15*, 530-530, doi:10.1186/s12879-015-1252-x.

50. Chapman, C.G.; Pekow, J. The emerging role of miRNAs in inflammatory bowel disease: a review. *Therapeutic advances in gastroenterology* **2015**, *8*, 4-22, doi:10.1177/1756283X14547360.
51. Fisher, K.; Lin, J. MicroRNA in inflammatory bowel disease: Translational research and clinical implication. *World journal of gastroenterology* **2015**, *21*, 12274-12282, doi:10.3748/wjg.v21.i43.12274.
52. Schaefer, J.S.; Attumi, T.; Opekun, A.R.; Abraham, B.; Hou, J.; Shelby, H.; Graham, D.Y.; Streckfus, C.; Klein, J.R. MicroRNA signatures differentiate Crohn's disease from ulcerative colitis. *BMC immunology* **2015**, *16*, 5-5, doi:10.1186/s12865-015-0069-0.
53. Olaru, A.V.; Yamanaka, S.; Vazquez, C.; Mori, Y.; Cheng, Y.; Abraham, J.M.; Bayless, T.M.; Harpaz, N.; Selaru, F.M.; Meltzer, S.J. MicroRNA-224 negatively regulates p21 expression during late neoplastic progression in inflammatory bowel disease. *Inflammatory bowel diseases* **2013**, *19*, 471-480, doi:10.1097/mib.0b013e31827e78eb.
54. Wang, C.; Chen, J. microRNAs as therapeutic targets in intestinal diseases. *ExRNA* **2019**, *1*, 23, doi:10.1186/s41544-019-0026-9.
55. Dalal, S.; Kwon, J. The Role of MicroRNA in Inflammatory Bowel Disease. *Gastroenterology & hepatology* **2010**, *6*, 714-722.
56. Xu, X.M.; Zhang, H.J. MiRNAs as new molecular insights into inflammatory bowel disease: Crucial regulators in autoimmunity and inflammation. *World Journal of Gastroenterology* **2016**, *22*, 2206-2218, doi:10.3748/wjg.v22.i7.2206.
57. Moein, S.; Vaghari-Tabari, M.; Qujeq, D.; Majidinia, M.; Nabavi, S.M.; Yousefi, B. MiRNAs and inflammatory bowel disease: An interesting new story. *Journal of Cellular Physiology* **2019**, *234*, 3277-3293, doi:10.1002/jcp.27173.
58. Mittal, M.; Siddiqui, M.R.; Tran, K.; Reddy, S.P.; Malik, A.B. Reactive oxygen species in inflammation and tissue injury. *Antioxidants & redox signaling* **2014**, *20*, 1126-1167, doi:10.1089/ars.2012.5149.
59. Sheng, K.C.; Day, S.; Wright, M.D.; Stojanovska, L.; Apostolopoulos, V. Enhanced Dendritic Cell-Mediated Antigen-Specific CD4+ T Cell Responses: IFN-Gamma Aids TLR Stimulation. *J Drug Deliv* **2013**, *2013*, 516749, doi:10.1155/2013/516749.
60. Sheng, K.C.; Pietersz, G.A.; Tang, C.K.; Ramsland, P.A.; Apostolopoulos, V. Reactive oxygen species level defines two functionally distinctive stages of inflammatory dendritic cell development from mouse bone marrow. *J Immunol* **2010**, *184*, 2863-2872, doi:10.4049/jimmunol.0903458.
61. Sheng, K.C.; Wright, M.D.; Apostolopoulos, V. Inflammatory mediators hold the key to dendritic cell suppression and tumor progression. *Curr Med Chem* **2011**, *18*, 5507-5518, doi:10.2174/092986711798347207.
62. Coussens, L.M.; Werb, Z. Inflammation and cancer. *Nature* **2002**, *420*, 860-867, doi:10.1038/nature01322.
63. Morgillo, F.; Dallio, M.; Della Corte, C.M.; Gravina, A.G.; Viscardi, G.; Loguercio, C.; Ciardiello, F.; Federico, A. Carcinogenesis as a Result of Multiple Inflammatory and Oxidative Hits: a Comprehensive Review from Tumor Microenvironment to Gut Microbiota. *Neoplasia (New York, N.Y.)* **2018**, *20*, 721-733, doi:10.1016/j.neo.2018.05.002.
64. Moss, S.F.; Blaser, M.J. Mechanisms of Disease: inflammation and the origins of cancer. *Nature Clinical Practice Oncology* **2005**, *2*, 90-97, doi:10.1038/ncponc0081.
65. Morrison, W.B. Inflammation and Cancer: A Comparative View. *Journal of Veterinary Internal Medicine* **2012**, *26*, 18-31, doi:10.1111/j.1939-1676.2011.00836.x.
66. Hanahan, D.; Weinberg, Robert A. Hallmarks of Cancer: The Next Generation. *Cell* **2011**, *144*, 646-674, doi:<https://doi.org/10.1016/j.cell.2011.02.013>.
67. Xiao, B.; Laroui, H.; Ayyadurai, S.; Viennois, E.; Charania, M.A.; Zhang, Y.; Merlin, D. Mannosylated bio-reducible nanoparticle-mediated macrophage-specific TNF- α RNA interference for IBD therapy. *Biomaterials* **2013**, *34*, 7471-7482, doi:<https://doi.org/10.1016/j.biomaterials.2013.06.008>.
68. Filippone, R.T.; Sahakian, L.; Apostolopoulos, V.; Nurgali, K. Eosinophils in Inflammatory Bowel Disease. *Inflamm Bowel Dis* **2019**, *25*, 1140-1151, doi:10.1093/ibd/izz024.

69. Stavely, R.; Rahman, A.A.; Sahakian, L.; Prakash, M.D.; Robinson, A.M.; Hassanzadeganroudsari, M.; Filippone, R.T.; Fraser, S.; Eri, R.; Bornstein, J.C.; et al. Divergent Adaptations in Autonomic Nerve Activity and Neuroimmune Signaling Associated With the Severity of Inflammation in Chronic Colitis. *Inflamm Bowel Dis* **2022**, *28*, 1229-1243, doi:10.1093/ibd/izac060.
70. Filippone, R.T.; Dargahi, N.; Eri, R.; Uranga, J.A.; Bornstein, J.C.; Apostolopoulos, V.; Nurgali, K. Potent CCR3 Receptor Antagonist, SB328437, Suppresses Colonic Eosinophil Chemotaxis and Inflammation in the Winnie Murine Model of Spontaneous Chronic Colitis. *Int J Mol Sci* **2022**, *23*, doi:10.3390/ijms23147780.
71. Filippone, R.T.; Robinson, A.M.; Jovanovska, V.; Stavely, R.; Apostolopoulos, V.; Bornstein, J.C.; Nurgali, K. Targeting eotaxin-1 and CCR3 receptor alleviates enteric neuropathy and colonic dysfunction in TNBS-induced colitis in guinea pigs. *Neurogastroenterol Motil* **2018**, *30*, e13391, doi:10.1111/nmo.13391.
72. Lu, Q.; Yang, M.F.; Liang, Y.J.; Xu, J.; Xu, H.M.; Nie, Y.Q.; Wang, L.S.; Yao, J.; Li, D.F. Immunology of Inflammatory Bowel Disease: Molecular Mechanisms and Therapeutics. *J Inflamm Res* **2022**, *15*, 1825-1844, doi:10.2147/JIR.S353038.
73. Gelfo, V.; Romaniello, D.; Mazzeschi, M.; Sgarzi, M.; Grilli, G.; Morselli, A.; Manzan, B.; Rihawi, K.; Lauriola, M. Roles of IL-1 in Cancer: From Tumor Progression to Resistance to Targeted Therapies. *Int J Mol Sci* **2020**, *21*, doi:10.3390/ijms21176009.
74. Kuol, N.; Stojanovska, L.; Apostolopoulos, V.; Nurgali, K. Role of the Nervous System in Tumor Angiogenesis. *Cancer Microenviron* **2018**, *11*, 1-11, doi:10.1007/s12307-018-0207-3.
75. Kuol, N.; Stojanovska, L.; Apostolopoulos, V.; Nurgali, K. Role of the nervous system in cancer metastasis. *J Exp Clin Cancer Res* **2018**, *37*, 5, doi:10.1186/s13046-018-0674-x.
76. Kuol, N.; Stojanovska, L.; Apostolopoulos, V.; Nurgali, K. Crosstalk between cancer and the neuro-immune system. *J Neuroimmunol* **2018**, *315*, 15-23, doi:10.1016/j.jneuroim.2017.12.016.
77. Wang, X.; Lin, Y. Tumor necrosis factor and cancer, buddies or foes? *Acta Pharmacol Sin* **2008**, *29*, 1275-1288, doi:10.1111/j.1745-7254.2008.00889.x.
78. Aguilar-Cazares, D.; Chavez-Dominguez, R.; Carlos-Reyes, A.; Lopez-Camarillo, C.; Hernandez de la Cruz, O.N.; Lopez-Gonzalez, J.S. Contribution of Angiogenesis to Inflammation and Cancer. *Frontiers in oncology* **2019**, *9*, 1399-1399, doi:10.3389/fonc.2019.01399.
79. Chow, M.T.; Luster, A.D. Chemokines in cancer. *Cancer immunology research* **2014**, *2*, 1125-1131, doi:10.1158/2326-6066.CIR-14-0160.
80. Landskron, G.; De la Fuente, M.; Thuwajit, P.; Thuwajit, C.; Hermoso, M.A. Chronic Inflammation and Cytokines in the Tumor Microenvironment. *Journal of Immunology Research* **2014**, *2014*, 149185, doi:10.1155/2014/149185.
81. Sanchez-Munoz, F.; Dominguez-Lopez, A.; Yamamoto-Furusho, J.-K. Role of cytokines in inflammatory bowel disease. *World journal of gastroenterology* **2008**, *14*, 4280-4288, doi:10.3748/wjg.14.4280.
82. Strober, W.; Fuss, I.J. Proinflammatory cytokines in the pathogenesis of inflammatory bowel diseases. *Gastroenterology* **2011**, *140*, 1756-1767, doi:10.1053/j.gastro.2011.02.016.
83. Carty, E.; Rampton, D.S. Evaluation of new therapies for inflammatory bowel disease. *British journal of clinical pharmacology* **2003**, *56*, 351-361, doi:10.1046/j.1365-2125.2003.01965.x.
84. Biasi, F.; Leonarduzzi, G.; Oteiza, P.I.; Poli, G. Inflammatory bowel disease: mechanisms, redox considerations, and therapeutic targets. *Antioxidants & redox signaling* **2013**, *19*, 1711-1747, doi:10.1089/ars.2012.4530.
85. Argollo, M.; Fiorino, G.; Hindryckx, P.; Peyrin-Biroulet, L.; Danese, S. Novel therapeutic targets for inflammatory bowel disease. *Journal of autoimmunity* **2017**, *85*, 103-116.
86. Mogensen, T.H. Pathogen recognition and inflammatory signaling in innate immune defenses. *Clinical microbiology reviews* **2009**, *22*, 240-273, doi:10.1128/CMR.00046-08.
87. Jang, J.-H.; Shin, H.W.; Lee, J.M.; Lee, H.-W.; Kim, E.-C.; Park, S.H. An Overview of Pathogen Recognition Receptors for Innate Immunity in Dental Pulp. *Mediators of inflammation* **2015**, *2015*, 794143-794143, doi:10.1155/2015/794143.

88. Szebeni, B.; Veres, G.; Dezsöfi, A.; Rusai, K.; Vannay, A.; Mraz, M.; Majorova, E.; Arató, A. Increased expression of Toll-like receptor (TLR) 2 and TLR4 in the colonic mucosa of children with inflammatory bowel disease. *Clinical and experimental immunology* **2008**, *151*, 34-41, doi:10.1111/j.1365-2249.2007.03531.x.
89. Oliveira-Nascimento, L.; Massari, P.; Wetzler, L.M. The Role of TLR2 in Infection and Immunity. *Frontiers in immunology* **2012**, *3*, 79-79, doi:10.3389/fimmu.2012.00079.
90. Lawrence, T. The nuclear factor NF-kappaB pathway in inflammation. *Cold Spring Harbor perspectives in biology* **2009**, *1*, a001651-a001651, doi:10.1101/cshperspect.a001651.
91. Liu, T.; Zhang, L.; Joo, D.; Sun, S.-C. NF-κB signaling in inflammation. *Signal transduction and targeted therapy* **2017**, *2*, 17023, doi:10.1038/sigtrans.2017.23.
92. Prakash, M.D.; Miller, S.; Randall-Demllo, S.; Nurgali, K. Mesenchymal Stem Cell Treatment of Inflammation-Induced Cancer. *Inflamm Bowel Dis* **2016**, *22*, 2694-2703, doi:10.1097/MIB.0000000000000900.
93. Atreya, I.; Atreya, R.; Neurath, M.F. NF-kappaB in inflammatory bowel disease. *J Intern Med* **2008**, *263*, 591-596, doi:10.1111/j.1365-2796.2008.01953.x.
94. Nagase, H.; Visse, R.; Murphy, G. Structure and function of matrix metalloproteinases and TIMPs. *Cardiovascular Research* **2006**, *69*, 562-573, doi:10.1016/j.cardiores.2005.12.002.
95. Visse, R.; Nagase, H. Matrix Metalloproteinases and Tissue Inhibitors of Metalloproteinases. *Circulation Research* **2003**, *92*, 827-839, doi:doi:10.1161/01.RES.0000070112.80711.3D.
96. Bischoff, S.C.; Barbara, G.; Buurman, W.; Ockhuizen, T.; Schulzke, J.-D.; Serino, M.; Tilg, H.; Watson, A.; Wells, J.M. Intestinal permeability--a new target for disease prevention and therapy. *BMC gastroenterology* **2014**, *14*, 189-189, doi:10.1186/s12876-014-0189-7.
97. Al-Sadi, R.; Youssef, M.; Rawat, M.; Guo, S.; Dokladny, K.; Haque, M.; Watterson, M.D.; Ma, T.Y. MMP-9-induced increase in intestinal epithelial tight permeability is mediated by p38 kinase signaling pathway activation of MLCK gene. *American journal of physiology. Gastrointestinal and liver physiology* **2019**, *316*, G278-G290, doi:10.1152/ajpgi.00126.2018.
98. Kuper, H.; Adami, H.O.; Trichopoulos, D. Infections as a major preventable cause of human cancer. *J Intern Med* **2000**, *248*, 171-183.
99. Wahl, L.M.; Kleinman, H.K. Tumor-associated macrophages as targets for cancer therapy. *J Natl Cancer Inst* **1998**, *90*, 1583-1584.
100. Desai, S.J.; Prickril, B.; Rasooly, A. Mechanisms of Phytonutrient Modulation of Cyclooxygenase-2 (COX-2) and Inflammation Related to Cancer. *Nutrition and Cancer* **2018**, *70*, 350-375, doi:10.1080/01635581.2018.1446091.
101. El Miedany, Y.; Youssef, S.; Ahmed, I.; El Gaafary, M. The gastrointestinal safety and effect on disease activity of etoricoxib, a selective cox-2 inhibitor in inflammatory bowel diseases. *Am J Gastroenterol* **2006**, *101*, 311-317, doi:10.1111/j.1572-0241.2006.00384.x.
102. Dudhgaonkar, S.P.; Tandan, S.K.; Kumar, D.; Raviprakash, V.; Kataria, M. Influence of simultaneous inhibition of cyclooxygenase-2 and inducible nitric oxide synthase in experimental colitis in rats. *Inflammopharmacology* **2007**, *15*, 188-195, doi:10.1007/s10787-007-1603-3.
103. Deguine, J.; Barton, G.M. MyD88: a central player in innate immune signaling. *F1000prime reports* **2014**, *6*, 97-97, doi:10.12703/P6-97.
104. Song, J.; Chen, Z.; Geng, T.; Wang, M.; Yi, S.; Liu, K.; Zhou, W.; Gao, J.; Song, W.; Tang, H. Deleting MyD88 signaling in myeloid cells promotes development of adenocarcinomas of the colon. *Cancer Lett* **2018**, *433*, 65-75, doi:10.1016/j.canlet.2018.06.036.
105. Araki, A.; Kanai, T.; Ishikura, T.; Makita, S.; Uraushihara, K.; Iiyama, R.; Totsuka, T.; Takeda, K.; Akira, S.; Watanabe, M. MyD88-deficient mice develop severe intestinal inflammation in dextran sodium sulfate colitis. *J Gastroenterol* **2005**, *40*, 16-23, doi:10.1007/s00535-004-1492-9.
106. Bhinder, G.; Stahl, M.; Sham, H.P.; Crowley, S.M.; Morampudi, V.; Dalwadi, U.; Ma, C.; Jacobson, K.; Vallance, B.A. Intestinal epithelium-specific MyD88 signaling impacts host susceptibility to infectious colitis by promoting protective goblet cell and antimicrobial responses. *Infect Immun* **2014**, *82*, 3753-3763, doi:10.1128/IAI.02045-14.
107. Francescone, R.; Hou, V.; Grivennikov, S.I. Cytokines, IBD, and Colitis-associated Cancer. *Inflammatory Bowel Diseases* **2015**, *21*, 409-418, doi:10.1097/mib.0000000000000236.

108. Grivennikov, S.I. Inflammation and colorectal cancer: colitis-associated neoplasia. *Seminars in immunopathology* **2013**, *35*, 229-244, doi:10.1007/s00281-012-0352-6.
109. Song, G.; Lu, Y.; Yu, Z.; Xu, L.; Liu, J.; Chen, K.; Zhang, P. The inhibitory effect of polysaccharide from *Rhizopus nigricans* on colitis-associated colorectal cancer. *Biomedicine & Pharmacotherapy* **2019**, *112*, 108593, doi:<https://doi.org/10.1016/j.biopha.2019.01.054>.
110. Dienstmann, R.; Vermeulen, L.; Guinney, J.; Kopetz, S.; Tejpar, S.; Tabernero, J. Consensus molecular subtypes and the evolution of precision medicine in colorectal cancer. *Nature Reviews Cancer* **2017**, *17*, 79-92.
111. Khalil, A.; Rubin, D.T.; Haider, H.I.; Meckel, K.; Bissonnette, M.; Talisila, N.; Siva, S.; Li, Y.C.; Deng, Z.; Pekow, J.; et al. IBD-associated colon cancers differ in DNA methylation and gene expression profiles compared to sporadic colon cancers. **2019**, doi:10.1093/ecco-jcc/jjz014.
112. Itzkowitz, S.H.; Yio, X. Inflammation and cancer IV. Colorectal cancer in inflammatory bowel disease: the role of inflammation. *Am J Physiol Gastrointest Liver Physiol* **2004**, *287*, G7-17, doi:10.1152/ajpgi.00079.2004.
113. McQuade, R.M.; Stojanovska, V.; Abalo, R.; Bornstein, J.C.; Nurgali, K. Chemotherapy-Induced Constipation and Diarrhea: Pathophysiology, Current and Emerging Treatments. *Frontiers in pharmacology* **2016**, *7*, 414-414, doi:10.3389/fphar.2016.00414.
114. O'Brien, M.E.R.; Borthwick, A.; Rigg, A.; Leary, A.; Assersohn, L.; Last, K.; Tan, S.; Milan, S.; Tait, D.; Smith, I.E. Mortality within 30 days of chemotherapy: a clinical governance benchmarking issue for oncology patients. *British journal of cancer* **2006**, *95*, 1632-1636, doi:10.1038/sj.bjc.6603498.
115. Currey, N.; Jahan, Z.; Caldon, C.E.; Tran, P.N.; Benthani, F.; De Lacavalerie, P.; Roden, D.L.; Gloss, B.S.; Campos, C.; Bean, E.G.; et al. Mouse Model of Mutated in Colorectal Cancer; Gene Deletion Reveals Novel Pathways in Inflammation and Cancer. *Cellular and Molecular Gastroenterology and Hepatology*, doi:10.1016/j.jcmgh.2019.01.009.
116. Whiteside, T.L. The tumor microenvironment and its role in promoting tumor growth. *Oncogene* **2008**, *27*, 5904-5912, doi:10.1038/onc.2008.271.
117. Apostolopoulos, V. Cancer Vaccines: Research and Applications. *Cancers (Basel)* **2019**, *11*, doi:10.3390/cancers11081041.
118. Baghban, R.; Roshangar, L.; Jahanban-Esfahlan, R.; Seidi, K.; Ebrahimi-Kalan, A.; Jaymand, M.; Kolahian, S.; Javaheri, T.; Zare, P. Tumor microenvironment complexity and therapeutic implications at a glance. *Cell Communication and Signaling* **2020**, *18*, 59, doi:10.1186/s12964-020-0530-4.
119. Apostolopoulos, V.; McKenzie, I.F. Cellular mucins: targets for immunotherapy. *Crit Rev Immunol* **1994**, *14*, 293-309, doi:10.1615/critrevimmunol.v14.i3-4.40.
120. Apostolopoulos, V.; McKenzie, I.F.C. Cellular Mucins: Targets for Immunotherapy. *Crit Rev Immunol* **2017**, *37*, 421-437, doi:10.1615/CritRevImmunol.v37.i2-6.110.
121. Ephraim, R.; Fraser, S.; Nurgali, K.; Apostolopoulos, V. Checkpoint Markers and Tumor Microenvironment: What Do We Know? *Cancers (Basel)* **2022**, *14*, doi:10.3390/cancers14153788.
122. Deshpande, R.P.; Sharma, S.; Watabe, K. The Confounders of Cancer Immunotherapy: Roles of Lifestyle, Metabolic Disorders and Sociological Factors. *Cancers* **2020**, *12*, 2983, doi:10.3390/cancers12102983.
123. Lemiale, V.; Meert, A.-P.; Vincent, F.; Darmon, M.; Bauer, P.R.; Van de Louw, A.; Azoulay, E.; Groupe de Recherche en Reanimation Respiratoire du patient, d.O.-H. Severe toxicity from checkpoint protein inhibitors: What intensive care physicians need to know? *Annals of intensive care* **2019**, *9*, 25-25, doi:10.1186/s13613-019-0487-x.
124. Lim, J.; Sari-Ak, D.; Bagga, T. Siglecs as Therapeutic Targets in Cancer. *Biology (Basel)* **2021**, *10*, doi:10.3390/biology10111178.
125. Borst, J.; Ahrends, T.; Bąbała, N.; Melief, C.J.M.; Kastenmüller, W. CD4⁺ T cell help in cancer immunology and immunotherapy. *Nature Reviews Immunology* **2018**, doi:10.1038/s41577-018-0044-0.
126. Larkin, J.; Chiarion-Sileni, V.; Gonzalez, R.; Grob, J.J.; Rutkowski, P.; Lao, C.D.; Cowey, C.L.; Schadendorf, D.; Wagstaff, J.; Dummer, R.; et al. Five-Year Survival with Combined

- Nivolumab and Ipilimumab in Advanced Melanoma. *N Engl J Med* **2019**, *381*, 1535-1546, doi:10.1056/NEJMoa1910836.
127. Yousuf, H.; Mekki, R.; Khan, K.; Hussain, A. Pembrolizumab-Induced Sarcoid-Like Reaction in a Patient With Lung Cancer. *Cureus* **2020**, *12*, e12395-e12395, doi:10.7759/cureus.12395.
 128. Rotte, A. Combination of CTLA-4 and PD-1 blockers for treatment of cancer. *Journal of Experimental & Clinical Cancer Research* **2019**, *38*, 255, doi:10.1186/s13046-019-1259-z.
 129. Ganesh, K.; Stadler, Z.K.; Cercek, A.; Mendelsohn, R.B.; Shia, J.; Segal, N.H.; Diaz, L.A. Immunotherapy in colorectal cancer: rationale, challenges and potential. *Nature Reviews Gastroenterology & Hepatology* **2019**, *16*, 361-375, doi:10.1038/s41575-019-0126-x.
 130. Rowshanravan, B.; Halliday, N.; Sansom, D.M. CTLA-4: a moving target in immunotherapy. *Blood* **2018**, *131*, 58-67, doi:10.1182/blood-2017-06-741033.
 131. Kolar, P.; Knieke, K.; Hegel, J.K.; Quandt, D.; Burmester, G.R.; Hoff, H.; Brunner-Weinzierl, M.C. CTLA-4 (CD152) controls homeostasis and suppressive capacity of regulatory T cells in mice. *Arthritis Rheum* **2009**, *60*, 123-132, doi:10.1002/art.24181.
 132. Buchbinder, E.I.; Desai, A. CTLA-4 and PD-1 Pathways: Similarities, Differences, and Implications of Their Inhibition. *Am J Clin Oncol* **2016**, *39*, 98-106, doi:10.1097/coc.0000000000000239.
 133. Zou, C.; Qiu, H.; Tang, W.; Wang, Y.; Lan, B.; Chen, Y. CTLA4 tagging polymorphisms and risk of colorectal cancer: a case-control study involving 2,306 subjects. *Onco Targets Ther* **2018**, *11*, 4609-4619, doi:10.2147/ott.S173421.
 134. Läubli, H.; Varki, A. Sialic acid-binding immunoglobulin-like lectins (Siglecs) detect self-associated molecular patterns to regulate immune responses. *Cell Mol Life Sci* **2020**, *77*, 593-605, doi:10.1007/s00018-019-03288-x.
 135. Hernández-Caselles, T.; Martínez-Esparza, M.; Pérez-Oliva, A.B.; Quintanilla-Cecconi, A.M.; García-Alonso, A.; Alvarez-López, D.M.R.; García-Peñarrubia, P. A study of CD33 (SIGLEC-3) antigen expression and function on activated human T and NK cells: two isoforms of CD33 are generated by alternative splicing. *Journal of Leukocyte Biology* **2006**, *79*, 46-58, doi:<https://doi.org/10.1189/jlb.0205096>.
 136. Kelm, S.; Ravindran, R. 3.24 - Siglecs. In *Comprehensive Glycoscience*, Kamerling, H., Ed.; Elsevier: Oxford, 2007; pp. 523-538.
 137. Wei, Y.; Chhibi, K.D.; Zhang, F.; Ye, X.; Wang, L.; Zhang, L.; Robida, P.A.; Moreno-Vinasco, L.; Schnaar, R.L.; Roers, A.; et al. Mast Cell-Specific Expression of Human Siglec-8 in Conditional Knock-in Mice. *International journal of molecular sciences* **2018**, *20*, 19, doi:10.3390/ijms20010019.
 138. Kano, G.; Almanan, M.; Bochner, B.S.; Zimmermann, N. Mechanism of Siglec-8-mediated cell death in IL-5-activated eosinophils: role for reactive oxygen species-enhanced MEK/ERK activation. *J Allergy Clin Immunol* **2013**, *132*, 437-445, doi:10.1016/j.jaci.2013.03.024.
 139. Graeter, S.; Simon, H.-U.; von Gunten, S. Granulocyte death mediated by specific antibodies in intravenous immunoglobulin (IVIG). *Pharmacological Research* **2019**, doi:<https://doi.org/10.1016/j.phrs.2019.02.007>.
 140. Delputte, P.L.; Van Gorp, H.; Favoreel, H.W.; Hoebeke, I.; Delrue, I.; Dewerchin, H.; Verdonck, F.; Verhasselt, B.; Cox, E.; Nauwynck, H.J. Porcine sialoadhesin (CD169/Siglec-1) is an endocytic receptor that allows targeted delivery of toxins and antigens to macrophages. *PLoS One* **2011**, *6*, e16827, doi:10.1371/journal.pone.0016827.
 141. O'Neill, A.S.; van den Berg, T.K.; Mullen, G.E. Sialoadhesin - a macrophage-restricted marker of immunoregulation and inflammation. *Immunology* **2013**, *138*, 198-207, doi:10.1111/imm.12042.
 142. Ren, X.; Ji, Y.; Jiang, X.; Qi, X. Down-regulation of siglec-2 (CD22) predicts worse overall survival from HBV-related early-stage hepatocellular carcinoma: a preliminary analysis from Gene Expression Omnibus. *Biosci Rep* **2018**, *38*, doi:10.1042/bsr20181423.
 143. Tsai, T.Y.; Huang, M.T.; Sung, P.S.; Peng, C.Y.; Tao, M.H.; Yang, H.I.; Chang, W.C.; Yang, A.S.; Yu, C.M.; Lin, Y.P.; et al. SIGLEC-3 (CD33) serves as an immune checkpoint receptor for HBV infection. *J Clin Invest* **2021**, *131*, doi:10.1172/jci141965.
 144. Sun, J.; Shaper, N.L.; Itonori, S.; Heffer-Laue, M.; Sheikh, K.A.; Schnaar, R.L. Myelin-associated glycoprotein (Siglec-4) expression is progressively and selectively decreased in the

- brains of mice lacking complex gangliosides. *Glycobiology* **2004**, *14*, 851-857, doi:10.1093/glycob/cwh107.
145. Erickson-Miller, C.L.; Freeman, S.D.; Hopson, C.B.; D'Alessio, K.J.; Fischer, E.I.; Kikly, K.K.; Abrahamson, J.A.; Holmes, S.D.; King, A.G. Characterization of Siglec-5 (CD170) expression and functional activity of anti-Siglec-5 antibodies on human phagocytes. *Exp Hematol* **2003**, *31*, 382-388, doi:10.1016/s0301-472x(03)00046-8.
146. Yu, Y.; Blokhuis, B.R.J.; Diks, M.A.P.; Keshavarzian, A.; Garssen, J.; Redegeld, F.A. Functional Inhibitory Siglec-6 Is Upregulated in Human Colorectal Cancer-Associated Mast Cells. *Front Immunol* **2018**, *9*, 2138, doi:10.3389/fimmu.2018.02138.
147. Hong, S.; Yu, C.; Rodrigues, E.; Shi, Y.; Chen, H.; Wang, P.; Chapla, D.G.; Gao, T.; Zhuang, R.; Moremen, K.W.; et al. Modulation of Siglec-7 Signaling Via In Situ-Created High-Affinity cis-Ligands. *ACS Cent Sci* **2021**, *7*, 1338-1346, doi:10.1021/acscentsci.1c00064.
148. Trebo, A.; Ditsch, N.; Degenhardt, T.; Kuhn, C.; Rahmeh, M.; Schmoeckel, E.; Mayr, D.; Czogalla, B.; Kolben, T.; Meister, S.; et al. First Evidence for a Role of Siglec-8 in Breast Cancer. *Int J Mol Sci* **2021**, *22*, doi:10.3390/ijms22042000.
149. Choi, H.; Ho, M.; Adeniji, O.S.; Giron, L.; Bordoloi, D.; Kulkarni, A.J.; Puchalt, A.P.; Abdel-Mohsen, M.; Muthumani, K. Development of Siglec-9 Blocking Antibody to Enhance Anti-Tumor Immunity. *Front Oncol* **2021**, *11*, 778989, doi:10.3389/fonc.2021.778989.
150. Barkal, A.A.; Brewer, R.E.; Markovic, M.; Kowarsky, M.; Barkal, S.A.; Zaro, B.W.; Krishnan, V.; Hatakeyama, J.; Dorigo, O.; Barkal, L.J.; et al. CD24 signalling through macrophage Siglec-10 is a target for cancer immunotherapy. *Nature* **2019**, *572*, 392-396, doi:10.1038/s41586-019-1456-0.
151. Fraschilla, I.; Pillai, S. Viewing Siglecs through the lens of tumor immunology. *Immunol Rev* **2017**, *276*, 178-191, doi:10.1111/imr.12526.
152. Mitra, N.; Banda, K.; Altheide, T.K.; Schaffer, L.; Johnson-Pais, T.L.; Beuten, J.; Leach, R.J.; Angata, T.; Varki, N.; Varki, A. SIGLEC12, a human-specific segregating (pseudo)gene, encodes a signaling molecule expressed in prostate carcinomas. *J Biol Chem* **2011**, *286*, 23003-23011, doi:10.1074/jbc.M111.244152.
153. Huang, P.J.; Low, P.Y.; Wang, I.; Hsu, S.D.; Angata, T. Soluble Siglec-14 glycan-recognition protein is generated by alternative splicing and suppresses myeloid inflammatory responses. *J Biol Chem* **2018**, *293*, 19645-19658, doi:10.1074/jbc.RA118.005676.
154. Wang, J.; Sun, J.; Liu, L.N.; Flies, D.B.; Nie, X.; Toki, M.; Zhang, J.; Song, C.; Zarr, M.; Zhou, X.; et al. Siglec-15 as an immune suppressor and potential target for normalization cancer immunotherapy. *Nat Med* **2019**, *25*, 656-666, doi:10.1038/s41591-019-0374-x.
155. Chen, Z.; Yu, M.; Guo, L.; Zhang, B.; Liu, S.; Zhang, W.; Zhou, B.; Yan, J.; Ma, Q.; Yang, Z.; et al. Tumor Derived SIGLEC Family Genes May Play Roles in Tumor Genesis, Progression, and Immune Microenvironment Regulation. *Front Oncol* **2020**, *10*, 586820, doi:10.3389/fonc.2020.586820.
156. Manson, G.; Norwood, J.; Marabelle, A.; Kohrt, H.; Houot, R. Biomarkers associated with checkpoint inhibitors. *Annals of Oncology* **2016**, *27*, 1199-1206, doi:10.1093/annonc/mdw181.
157. Prendergast, G.C.; Smith, C.; Thomas, S.; Mandik-Nayak, L.; Laury-Kleintop, L.; Metz, R.; Muller, A.J. Indoleamine 2,3-dioxygenase pathways of pathogenic inflammation and immune escape in cancer. *Cancer Immunol Immunother* **2014**, *63*, 721-735, doi:10.1007/s00262-014-1549-4.
158. Xu, G.; Wang, T.; Li, Y.; Huang, Z.; Wang, X.; Zheng, J.; Yang, S.; Fan, Y.; Xiang, R. A highly potent and selective inhibitor Roxyl-WL targeting IDO1 promotes immune response against melanoma. *Journal of Enzyme Inhibition and Medicinal Chemistry* **2018**, *33*, 1089-1094, doi:10.1080/14756366.2018.1471688.
159. Xu, X.; Ren, J.; Ma, Y.; Liu, H.; Rong, Q.; Feng, Y.; Wang, Y.; Cheng, Y.; Ge, R.; Li, Z.; et al. Discovery of cyanopyridine scaffold as novel indoleamine-2,3-dioxygenase 1 (IDO1) inhibitors through virtual screening and preliminary hit optimisation. *Journal of Enzyme Inhibition and Medicinal Chemistry* **2019**, *34*, 250-263, doi:10.1080/14756366.2018.1480614.
160. Mondanelli, G.; Mandarano, M.; Belladonna, M.L.; Suvieri, C.; Pelliccia, C.; Bellezza, G.; Sidoni, A.; Carvalho, A.; Grohmann, U.; Volpi, C. Current Challenges for IDO2 as Target in Cancer Immunotherapy. *Front Immunol* **2021**, *12*, 679953, doi:10.3389/fimmu.2021.679953.

161. Hou, N.; Ma, J.; Li, W.; Zhao, L.; Gao, Q.; Mai, L. T-cell immunoglobulin and mucin domain-containing protein-3 and galectin-9 protein expression: Potential prognostic significance in esophageal squamous cell carcinoma for Chinese patients. *Oncol Lett* **2017**, *14*, 8007-8013, doi:10.3892/ol.2017.7188.
162. Yu, M.; Lu, B.; Liu, Y.; Me, Y.; Wang, L.; Zhang, P. Tim-3 is upregulated in human colorectal carcinoma and associated with tumor progression. *Mol Med Rep* **2017**, *15*, 689-695, doi:10.3892/mmr.2016.6065.
163. Das, M.; Zhu, C.; Kuchroo, V.K. Tim-3 and its role in regulating anti-tumor immunity. *Immunol Rev* **2017**, *276*, 97-111, doi:10.1111/imr.12520.
164. Anderson, A.C. Tim-3: An Emerging Target in the Cancer Immunotherapy Landscape. *Cancer Immunology Research* **2014**, *2*, 393-398, doi:10.1158/2326-6066.Cir-14-0039.
165. Graydon, C.G.; Mohideen, S.; Fowke, K.R. LAG3's Enigmatic Mechanism of Action. *Frontiers in Immunology* **2021**, *11*, doi:10.3389/fimmu.2020.615317.
166. Ruffo, E.; Wu, R.C.; Bruno, T.C.; Workman, C.J.; Vignali, D.A.A. Lymphocyte-activation gene 3 (LAG3): The next immune checkpoint receptor. *Semin Immunol* **2019**, *42*, 101305, doi:10.1016/j.smim.2019.101305.
167. Andrews, L.P.; Marciscano, A.E.; Drake, C.G.; Vignali, D.A. LAG3 (CD223) as a cancer immunotherapy target. *Immunol Rev* **2017**, *276*, 80-96, doi:10.1111/imr.12519.
168. Woo, S.R.; Turnis, M.E.; Goldberg, M.V.; Bankoti, J.; Selby, M.; Nirschl, C.J.; Bettini, M.L.; Gravano, D.M.; Vogel, P.; Liu, C.L.; et al. Immune inhibitory molecules LAG-3 and PD-1 synergistically regulate T-cell function to promote tumoral immune escape. *Cancer Res* **2012**, *72*, 917-927, doi:10.1158/0008-5472.CAN-11-1620.
169. Lythgoe, M.P.; Liu, D.S.K.; Annels, N.E.; Krell, J.; Frampton, A.E. Gene of the month: lymphocyte-activation gene 3 (LAG-3). *J Clin Pathol* **2021**, *74*, 543-547, doi:10.1136/jclinpath-2021-207517.
170. Inoue, S.; Ito, H.; Tsunoda, T.; Murakami, H.; Ebi, M.; Ogasawara, N.; Kasugai, K.; Kasai, K.; Ikeda, H.; Inaguma, S. CD70 expression in tumor-associated fibroblasts predicts worse survival in colorectal cancer patients. *Virchows Archiv* **2019**, *475*, 425-434, doi:10.1007/s00428-019-02565-1.
171. Eastwood, D.; Findlay, L.; Poole, S.; Bird, C.; Wadhwa, M.; Moore, M.; Burns, C.; Thorpe, R.; Stebbings, R. Monoclonal antibody TGN1412 trial failure explained by species differences in CD28 expression on CD4⁺ effector memory T-cells. *Br J Pharmacol* **2010**, *161*, 512-526, doi:10.1111/j.1476-5381.2010.00922.x.
172. Schoenberger, S.P.; Toes, R.E.; van der Voort, E.I.; Offringa, R.; Melief, C.J. T-cell help for cytotoxic T lymphocytes is mediated by CD40-CD40L interactions. *Nature* **1998**, *393*, 480-483, doi:10.1038/31002.
173. O'Sullivan, B.; Thomas, R. CD40 and dendritic cell function. *Crit Rev Immunol* **2003**, *23*, 83-107.
174. Pang, X.; Zhang, L.; Wu, J.; Ma, C.; Mu, C.; Zhang, G.; Chen, W. Expression of CD40/CD40L in colon cancer, and its effect on proliferation and apoptosis of SW48 colon cancer cells. *J buon* **2017**, *22*, 894-899.
175. Villarreal, D.O.; Allegranza, M.J.; Smith, M.A.; Chin, D.; Luistro, L.L.; Snyder, L.A. Targeting of CD122 enhances antitumor immunity by altering the tumor immune environment. *Oncotarget* **2017**, *8*, 109151-109160, doi:10.18632/oncotarget.22642.
176. Cheng, D.; Zou, W.; Li, X.; Tan, H.; Yang, X.; Shi, H. Early diagnosis of colorectal cancer by CD11b⁺ myeloid-derived suppressor cells imaging. *Journal of Nuclear Medicine* **2014**, *55*, 1397-1397.
177. Yu, X.; Guo, C.; Fisher, P.B.; Subjeck, J.R.; Wang, X.-Y. Chapter Nine - Scavenger Receptors: Emerging Roles in Cancer Biology and Immunology. In *Advances in Cancer Research*, Wang, X.-Y., Fisher, P.B., Eds.; Academic Press: 2015; Volume 128, pp. 309-364.
178. Wang, C.; Feng, H.; Cheng, X.; Liu, K.; Cai, D.; Zhao, R. Potential Therapeutic Targets of B7 Family in Colorectal Cancer. *Front Immunol* **2020**, *11*, 681, doi:10.3389/fimmu.2020.00681.
179. Scarpa, M.; Brun, P.; Scarpa, M.; Morgan, S.; Porzionato, A.; Kotsafti, A.; Bortolami, M.; Buda, A.; D'Inca, R.; Macchi, V.; et al. CD80-CD28 signaling controls the progression of

- inflammatory colorectal carcinogenesis. *Oncotarget* **2015**, *6*, 20058-20069, doi:10.18632/oncotarget.2780.
180. Xu, G.; Jiang, L.; Ye, C.; Qin, G.; Luo, Z.; Mo, Y.; Chen, J. The Ratio of CD86+/CD163+ Macrophages Predicts Postoperative Recurrence in Stage II-III Colorectal Cancer. *Front Immunol* **2021**, *12*, 724429, doi:10.3389/fimmu.2021.724429.
 181. Shi, S.J.; Wang, L.J.; Wang, G.D.; Guo, Z.Y.; Wei, M.; Meng, Y.L.; Yang, A.G.; Wen, W.H. B7-H1 expression is associated with poor prognosis in colorectal carcinoma and regulates the proliferation and invasion of HCT116 colorectal cancer cells. *PLoS One* **2013**, *8*, e76012, doi:10.1371/journal.pone.0076012.
 182. Cao, Y.; Cao, T.; Zhao, W.; He, F.; Lu, Y.; Zhang, G.; Hu, H.; Wang, Z. Expression of B7-H2 on CD8(+) T cells in colorectal cancer microenvironment and its clinical significance. *Int Immunopharmacol* **2018**, *56*, 128-134, doi:10.1016/j.intimp.2018.01.018.
 183. Zhou, W.T.; Jin, W.L. B7-H3/CD276: An Emerging Cancer Immunotherapy. *Front Immunol* **2021**, *12*, 701006, doi:10.3389/fimmu.2021.701006.
 184. Zhou, B.; Lu, Y.; Zhao, Z.; Shi, T.; Wu, H.; Chen, W.; Zhang, L.; Zhang, X. B7-H4 expression is upregulated by PKCdelta activation and contributes to PKCdelta-induced cell motility in colorectal cancer. *Cancer Cell Int* **2022**, *22*, 147, doi:10.1186/s12935-022-02567-1.
 185. Wang, J.; Wu, H.; Chen, Y.; Zhu, J.; Sun, L.; Li, J.; Yao, Z.; Chen, Y.; Zhang, X.; Xia, S.; et al. B7-H5 blockade enhances CD8(+) T-cell-mediated antitumor immunity in colorectal cancer. *Cell Death Discov* **2021**, *7*, 248, doi:10.1038/s41420-021-00628-4.
 186. Ni, L.; Dong, C. New B7 Family Checkpoints in Human Cancers. *Mol Cancer Ther* **2017**, *16*, 1203-1211, doi:10.1158/1535-7163.Mct-16-0761.
 187. Sun, X.; Zhao, J.; Ma, L.; Sun, X.; Ge, J.; Yu, Y.; Ma, J.; Zhang, M. B7-H6 as an efficient target for T cell-induced cytotoxicity in haematologic malignant cells. *Invest New Drugs* **2021**, *39*, 24-33, doi:10.1007/s10637-020-00976-5.
 188. Cansever, M.; Göktaş, M.A.; Arslan, D.; Patiroğlu, T. Serum levels of soluble HLA-G correlate with disease activity in pediatric patients with Crohn's disease. *Saudi J Gastroenterol* **2022**, *28*, 233-238, doi:10.4103/sjg.sjg_327_21.
 189. Bortolotti, D.; Gentili, V.; Rotola, A.; Cassai, E.; Rizzo, R.; Di Luca, D. Impact of HLA-G analysis in prevention, diagnosis and treatment of pathological conditions. *World J Methodol* **2014**, *4*, 11-25, doi:10.5662/wjm.v4.i1.11.
 190. Nalejska, E.; Mączyńska, E.; Lewandowska, M.A. Prognostic and predictive biomarkers: tools in personalized oncology. *Molecular diagnosis & therapy* **2014**, *18*, 273-284, doi:10.1007/s40291-013-0077-9.
 191. Kitano, S.; Nakayama, T.; Yamashita, M. Biomarkers for Immune Checkpoint Inhibitors in Melanoma. *Frontiers in Oncology* **2018**, *8*, doi:10.3389/fonc.2018.00270.

Chapter 2

Chapter context and preface

Chapter 1a and 1b, described the important role of the tumour microenvironment, checkpoint markers in inflammation, cancer and in chronic colitis. In this chapter 2 the genes expressed in the colon of mice with spontaneous chronic colitis (inflammation, *Winnie* mice) and severe colitis mice (sever inflammation and colon prolapse, *Winnie-prolapse* mice) are evaluated. This analysis provides an understanding of the pathways and genes involved in inflammation, in the context of chronic colitis.

Chapter 2

Transcriptomic analysis of differentially expressed genes in mouse models of chronic colitis (*Winnie* mice)

Abstract

The *Winnie* mouse, carrying a missense mutation in the *Muc2* gene, is a model for chronic colitis closely demonstrating symptoms of IBD. Herein, transcriptomic analyses of the differentially expressed genes in the distal colon of *Winnie* mice with mild colitis and *Winnie-Prolapse* mice with severe colitis compared to control C57BL/6 mice are presented. The gene ontology analysis and KEGG pathway analysis demonstrated the upregulation of genes in immune and inflammation-related pathways, metabolic pathways, cancer-related pathways, and neurological processes. Further research into these pathways and individual genes may lead to the identification of new targets for the treatment of IBD. The overexpression of oncogenes, in particular, may serve as an indicator of inflammation progressing to cancer.

Introduction

IBD is a chronic, relapsing inflammatory disorder of the small and large intestines, consisting of two major pathologies, Crohn's disease and ulcerative colitis (Caruso et al., 2020). Common clinical symptoms of IBD include abdominal pain and gut hypersensitivity, which are associated with chronic diarrhoea and rectal bleeding (Ledergerber et al., 2021, Camilleri, 2011). Crohn's disease is distinguished by severe chronic inflammation which presents as transmural skip lesions throughout the intestinal tract, whilst in ulcerative colitis, mucosal and submucosal inflammation continuously ascends from the rectum to the colon (Kellermann and Riis, 2021, Baumgart and Carding, 2007). IBD is highly prevalent within 20-30-year aged group with another peak in the 60-70-year age groups. The immune characteristics of IBD arise from abnormal innate and adaptive immune responses (Silva et al., 2016). The current available and first line treatments for IBD are anti-inflammatory drugs such as aminosalicylates, corticosteroids, immunosuppressants, and targeted therapies like cytokine blockers, TNF- α blockers, integrin blockers, ILblockers, and Janus Kinase (Kumric et al.) inhibitors (molecular therapy). Despite these advances, managing IBD is difficult long term due to the toxic side

effects of current treatments, or refractory responses in patients (Cai et al., 2021). As such, there is an urgent need for new treatment strategies.

Mucin 2 (*Muc2*) is a gel-forming mucin acting as a mucus barrier in the intestinal tract. Silencing *Muc2* gene in mice is linked to high levels of *IL-6* and carcinogenesis. In human colon cancer cell lines, expression of *MUC2* suppresses migration of cells, whilst application of short hairpin RNA to suppress *MUC2* expression promotes cell metastasis through *IL-6* signalling (Hsu et al., 2017). In addition, *Muc2* has been shown to protect the intestinal tract against infectious colitis by inhibiting pathogenic and commensal bacteria attachment to the colonic mucosa (Bergstrom et al., 2010). As such, *Muc2* plays a key role in protecting host against bacterial infections, inflammation, and progression to carcinogenesis. The *Winnie* mouse model of spontaneous chronic colitis closely resembles human IBD. These mice contain a point mutation in the *Muc2* gene and develop chronic colitis under pathogen-free conditions within 6-7 weeks of age, which progress to severe colitis by the age of 12–16 weeks (Heazlewood et al., 2008). The mutation in the *Muc2* gene leads to a thinner and more fragile mucus layer and increased intestinal permeability (Eri et al., 2011). As a result, there is enhanced susceptibility to microbes, toxins and other antigens which are commonly present within the gut lumen (Robinson et al., 2017b). In *Winnie* mice, endoplasmic reticulum stress in the goblet cells causes intestinal barrier failure, resulting in greater interaction between the microbiota and the mucosa, diminished epithelial cell conditioning of dendritic cells and macrophages, and direct epithelial cell stress (McGuckin et al., 2011). Further, the predominance of IL-23-mediated cytokine storm causing colitis in *Winnie* mice can be ameliorated by the administering anti-IL-23 monoclonal antibodies and dexamethasone (Das et al., 2013, Wang et al., 2015b). With just a single nucleotide point (SNP) mutation in the *Muc2* gene, results in clinical features in *Winnie* mice, closely resembling human chronic colitis. As such, *Winnie* mice are an excellent pre-clinical model to study IBD pathogenesis. *Winnie* mice develop spontaneous inflammation in the colorectal area with symptoms of chronic diarrhoea, ulcerations, rectal bleeding, and pain, similar to human IBD (Heazlewood et al., 2008, Eri et al., 2011). Approximately 25% of *Winnie* mice develop severe colitis leading to a prolapsed rectum, which is caused by unknown mechanisms but is associated with extensive damage to the intrinsic and extrinsic intestinal innervation (Rahman et al., 2016b).

The subset of *Winnie* mice with rectal prolapse (*Winnie-Prolapse*), exhibit more pronounced diarrhoea and intestinal bleeding compared to *Winnie* mice. Rectal prolapse is associated with increased / severe inflammation, gross morphological damage, and rectum muscular hypertrophy (Rahman et al., 2016a). When compared to *Winnie* mice, *Winnie-Prolapse* mice have a more severe neuronal loss in the myenteric plexus. Heightened activity of the splanchnic nerve was noted in *Winnie* but not *Winnie-Prolapse* mice. Conversely, vagal nerve activity is greater in *Winnie-Prolapse* mice compared to *Winnie* mice (Stavely et al., 2022a). Eosinophils and their regulatory molecules have been associated with chronic intestinal inflammation and gastrointestinal dysfunctions; eosinophil accumulation in the gut is prominent in IBD. *Winnie* and *Winnie-Prolapse* mice exhibit different stages of colon inflammation and increased eosinophil infiltration (Filippone et al., 2022a).

Given the urgent need for new IBD treatments, whilst also recognising the potential link between chronic inflammation and the development of CRC, it is important to identify molecular mechanisms involved in disease progression. The current study focuses on understanding the different pathways involved in the up and down-regulated genes from mouse models closely resembling human IBD, which might further lead to novel therapeutic discoveries as well as give insights into the paths of inflammation that might lead to cancer.

Materials and Methods

Animals

Winnie mice (12-14 weeks of age, n=7: 3 males and 4 females) and *Winnie* mice exhibiting rectal prolapse (*Winnie-Prolapse*) (18-23 weeks old, n=7: n=4 males and n=3 females) and C57BL/6 control mice (14 weeks old, n=8: n=4 males and n=4 females) were obtained from the colony at the Victoria University Werribee Animal Facility (Melbourne, VIC Australia). All mice were acclimatised for 5 days and had free access to food and water. Mice were housed in a temperature-controlled environment with 12-hour day/night cycles and had limited access to food and water. All animal experiments in this study complied with guidelines of the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes and were approved by the Victoria University Animal Experimentation Ethics Committee (AEC18-016).

Tissue Collection

Mice were culled by an overdose of pentobarbital (100 mg/kg, intraperitoneally). For RNA sequencing, similar sections of distal colon segments were collected from each mouse, immediately placed into liquid nitrogen, and stored at -80 °C until RNA extraction was performed. For histological analysis, distal colon tissues were removed, cut along the mesenteric border, cleared of contents, fixed in Zamboni's fixative for 24 hours, and embedded in the optimum cutting temperature (OCT) compound (Tissue Tek, CA, USA) and frozen in liquid nitrogen-cooled isopentane (Stavely et al., 2022b, Sahakian et al., 2021)

Histology

Distal colon tissue sections (15 µm thickness) were cut using a cryostat (Leica, Germany) and processed for hematoxylin and eosin or Alcian blue staining. Sections were mounted on glass slides with distrene plasticiser xylene. Images of the whole cross-section were taken using a Metafer slide scanning system (MetaSystems, Germany) and stitched together using the Vslide software (MetaSystems, Germany). The ImageJ software (National Institute of Health, Bethesda, MD, USA) was used to analyse the histological images. Gross morphological damage of the colonic mucosa (hematoxylin and eosin [H&E] staining) was evaluated as previously described (Robinson et al., 2017b, Filippone et al., 2022a) using the following parameters: aberrant crypt architecture (score range, 0–3), changes to crypt length (0–3), crypt abscesses (0–3), leukocyte infiltration (0–3), epithelial damage (0–3), and ulceration (0–3). Analysis of the goblet cell density (Alcian Blue staining) was performed using image deconvolution and conversion to 8-bit grayscale by ImageJ software. All slides were coded, analysis was performed blindly, and reported as averages of three 500 µm² areas per section.

Assessment of inflammation

Faecal samples were collected and weighed from C57BL/6, Winnie and Winnie-Prolapse mice used in the study, snap frozen in liquid nitrogen, and stored at -80 °C. The level of inflammation was measured from faecal samples using a Lipocalin-2 mouse ELISA kit (Abcam, VIC Australia) according to the manufacturer's instructions. Faecal samples were reconstituted in phosphate buffered saline containing 0.1% Tween 20, vortexed for 20 minutes and centrifuged

for 10 minutes at 12,000 rpm at 4 °C. Supernatants were collected and analysed; all standards, controls and samples were assayed in triplicates.

RNA extraction

TRIzol™ (Invitrogen, VIC Australia) was used to extract total RNA, which was purified using the RNeasy® Mini kit (Qiagen, Hilden, Germany), including column DNase treatment. Tissue homogenisation was achieved with a TissueLyser bead homogeniser (Qiagen). The integrity of the total RNA samples was determined using the Agilent 2100 Bioanalyser's RNA 6000 Nano chip (Agilent Biotechnologies, Australia).

Library Preparation

The RNA-Seq library was formulated by starting with >100 ng of RIN 8+ total RNA, including RNA quality control (CE integrity analysis by AATI Fragment Analyser, Fluorimetric quantitation by Invitrogen Qubit). The Poly-A mRNA was chosen, and MGIEasy stranded mRNA chemistry was carried out. All samples were quantified and analysed using Qubit and Bioanalyser and passed the QNA QC standard.

RNA Sequencing

The sequencing was conducted externally at Micromon Genomics facility (Monash University, Melbourne Australia) using MGITech MGISEQ2000RS hardware (MGISEQ-2000RS High-throughput Sequencing Set). MGIEasy V2 chemistry set was used for generating a library using 4 sequencing lanes and at least 400 m raw reads per lane with paired-end 100b reads for accuracy and processing to compressed fastq files. Those files are delivered electronically.

Bioinformatics was performed by Monash Bioinformatics. Raw fastq files were analysed using the RNAsik pipeline version 1.5.4 (Tsyganov et al., 2018b) using STAR aligner (Dobin et al., 2013) and the *Mus musculus* reference, GRCm38 (Genbank accession GCA_000001635.2). Reads were quantified using featureCounts (Liao et al., 2014b) producing the raw genes count matrix and various quality control metrics. Raw counts were then analysed with Degust (Powell, 2019), a web tool that performs differential expression analysis using limma voom normalisation (Law et al., 2014), producing counts per

million library size normalisation and trimmed mean of M values normalisation (Robinson and Oshlack 2010) for RNA composition normalisation. Degust (Powell, 2019) also provides several quality plots including classical multidimensional scaling (MDS). The Database for Annotation, Visualisation, and Integrated Discovery software (DAVID), Gene Ontology enRIchment anaLysis and visualisation tool (GoRilla), Gene ontology for enrichment analysis, Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway were used to analyse the differential gene expression based on the overrepresentation analysis (ORA). The total number of genes were extracted from degust using edgeR with an absolute log₂-fold change of 1, (x2-fold change) and false discovery rate (FDR < 0.05).

In the current study, the biological processes (BP) in the gene ontology software under the Panther classification system (Panther GO-slim BP) were analysed. Only the overrepresented categories have been selected and the underrepresented categories were eliminated. The top 20 categories were selected based on the significance (FDR) by sorting them from the smallest to largest. The entire list of GO-slim BP is included in the Appendix (Table 1 and Table 2).

A collection of databases on genomes, biological pathways, diseases, medications, and chemicals makes up the KEGG pathway. KEGG is a database resource for analysing high-level biological systems, their functions, and their utilities in regard to their molecular-level information. KEGG is a large-scale molecular dataset produced by genome sequencing and other high-throughput experimental technologies. DAVID is a functional annotation tool commonly used to determine the biological significance of large datasets. DAVID tool was used to visualise genes on BioCarta and KEGG pathways based on the log₁₀ p-values corrected by the Benjamini-Hochberg procedure.

Data Analysis

Data are expressed as mean ± standard deviation unless stated otherwise. Statistical analysis was conducted with GraphPad Prism (version 8.1) (Graphpad Software Inc., San Diego, CA, USA). Parametric data were analysed by one-way ANOVA with the Tukey post hoc test for multiple comparisons. P values less than 0.05 were considered significant.

Comparison to human IBD

The relevant dataset mentioned in this chapter has been taken from <https://github.com/Humanitas-Danese-s-omics/ibd-meta-analysis-data> and in the Open Science Framework repository for the comparison of human IBD data to the *Winnie* mouse models in this thesis. The selected data is from colon tissues from ulcerative colitis patients vs colon tissues from controls https://github.com/Humanitas-Danese-s-omics/ibd-meta-analysis-data/blob/main/data/human/dge/Colon_UC-vs-Colon_Control.diffexp.tsv (This comparison is summarised in a Supplementary table, in the Appendix)

Statement

The Library preparation and RNA sequencing (Next generation sequencing) were conducted by Micromon at Monash as Victoria University does not have the required equipment for this particular sequencing. The raw counts were converted into normalised values by bioinformaticians at Monash Bioinformatics due to time constraints specially to perform the lengthy calculations for more than 15,000 genes and unavailability of the required software. All RNA extractions, NGS comprehensive data analysis and the figures presented in this chapter as well as chapter 3, were done by me.

Results

Gross morphological damage and the level of inflammation in the colon of *Winnie* and *Winnie-Prolapse* mice.

The average body weight of *Winnie* (25.2 ± 0.3 g, $P < 0.05$) and *Winnie-Prolapse* (23.5 ± 0.8 g, $P < 0.001$) mice ($n=8/\text{group}$) was lower than the body weight of control mice (28.0 ± 0.7 g), however no significant differences were observed between the *Winnie* and *Winnie-Prolapse* mice. Histology of the colon was performed to assess gross morphological damage using haematoxylin and eosin staining (Figure 1A-A’). Both *Winnie* and *Winnie-Prolapse* mice had severe colonic mucosal damage compared to controls (histological score: 1.2 ± 0.2) ($P < 0.0001$ for both). The most severe histological damage was observed in *Winnie-Prolapse* mice (histological score: 14.1 ± 1.7), which was higher than *Winnie* mice (histological score: 8.7 ± 1.9 ,

$P < 0.001$) (Figure 1D). The width of the colon mucosa was greater in both *Winnie* mice ($201.7 \pm 24.5 \mu\text{m}$, $P < 0.001$) and *Winnie-Prolapse* mice ($237.3 \pm 46.9 \mu\text{m}$, $P < 0.0001$) compared to C57BL/6 controls ($100.4 \pm 16.1 \mu\text{m}$) (Figure 1E). The average width of the colon muscle was greater in *Winnie-Prolapse* mice ($160.7 \pm 78.6 \mu\text{m}$) compared to *Winnie* mice ($73.4 \pm 8.0 \mu\text{m}$, $P < 0.05$) and controls ($34.4 \pm 7.2 \mu\text{m}$, $P < 0.01$) (Figure 1B-B'', F). Goblet cell density was quantified as the percentage of Alcian blue-staining within the average of 3 areas of $500 \mu\text{m}^2$ of colonic mucosa per section in the colon cross sections (Figure 1C-C''). A reduction in the goblet cell density was observed in both *Winnie* mice ($10.1 \pm 1.5 \%$, $P < 0.01$) and *Winnie-Prolapse* mice ($7.4 \pm 1.9 \%$, $P < 0.001$) compared to controls ($20.9 \pm 5.2 \%$) (Figure 1G).

Faecal lipocalin-2 (LCN-2), also known as neutrophil gelatinase-associated lipocalin, is a highly sensitive non-invasive marker of intestinal inflammation (Robinson et al., 2017b). Both *Winnie* mice ($667.6 \pm 38.4 \text{ pg/mL}$, $P < 0.001$) and *Winnie-Prolapse* mice ($752.9 \pm 51.1 \text{ pg/mL}$, $P < 0.0001$) had increased levels of faecal LCN-2 compared to controls ($518.6 \pm 52.8 \text{ pg/mL}$) (Figure 1H). The level of faecal LCN-2 in *Winnie-Prolapse* mice was higher compared to *Winnie* mice ($P < 0.05$).

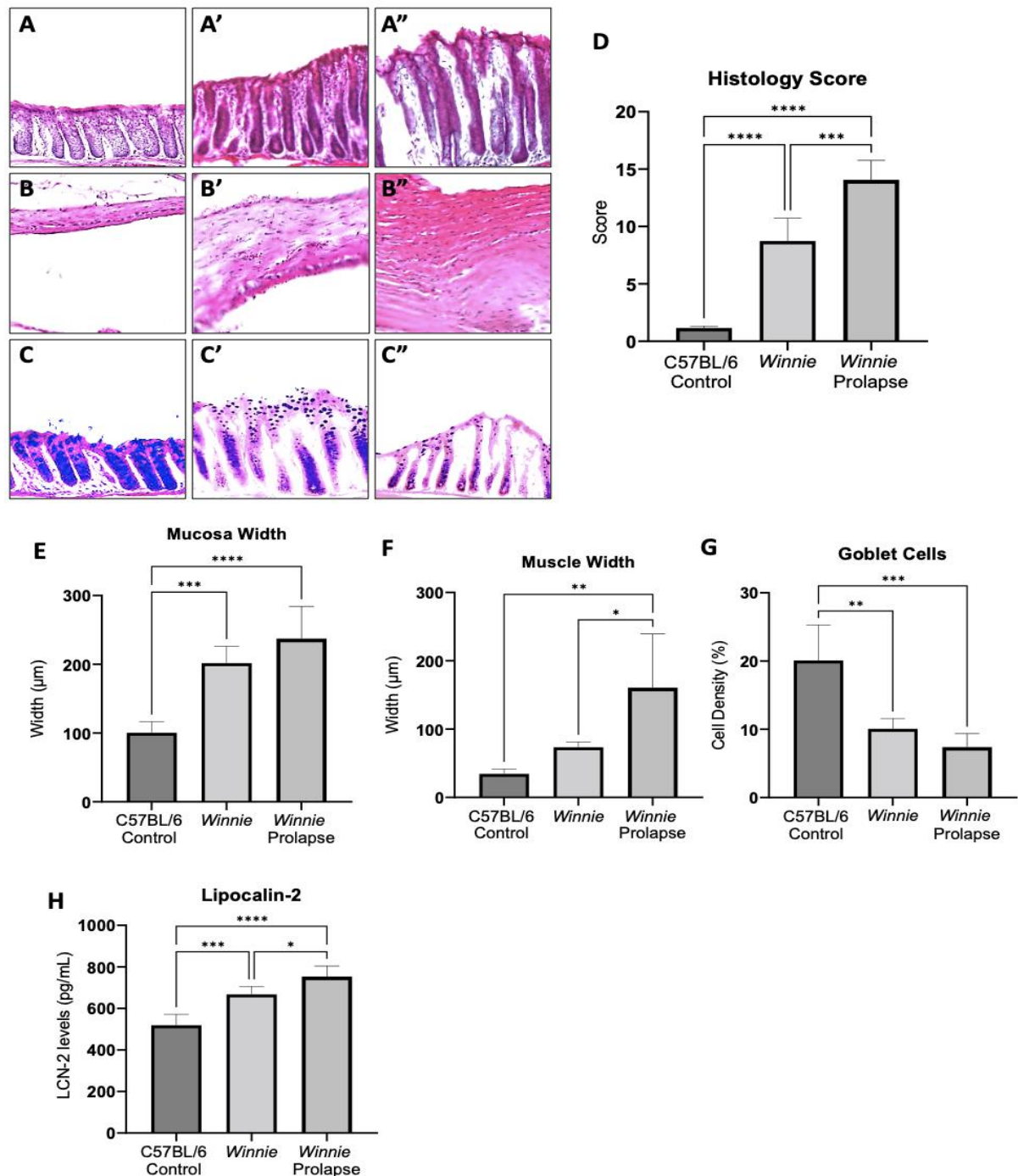
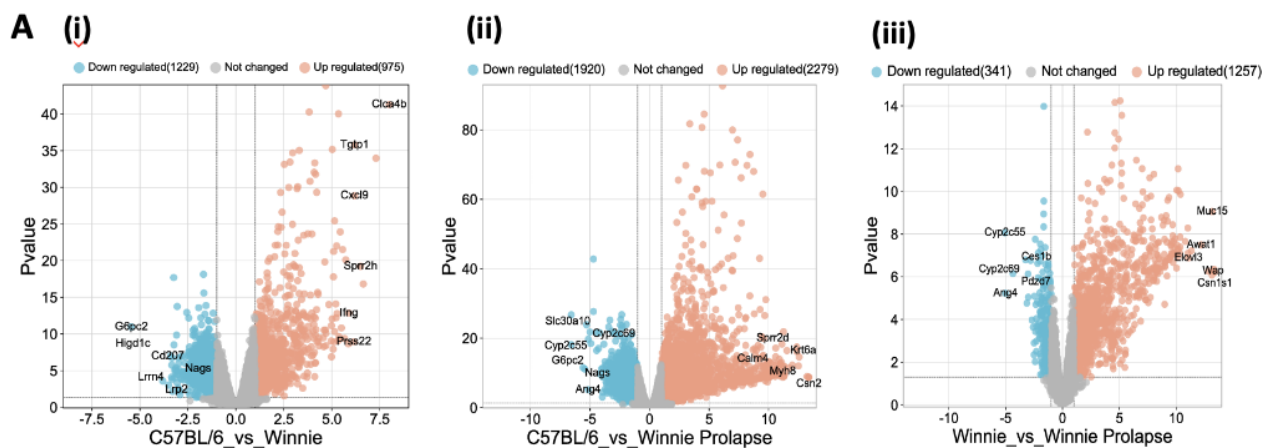


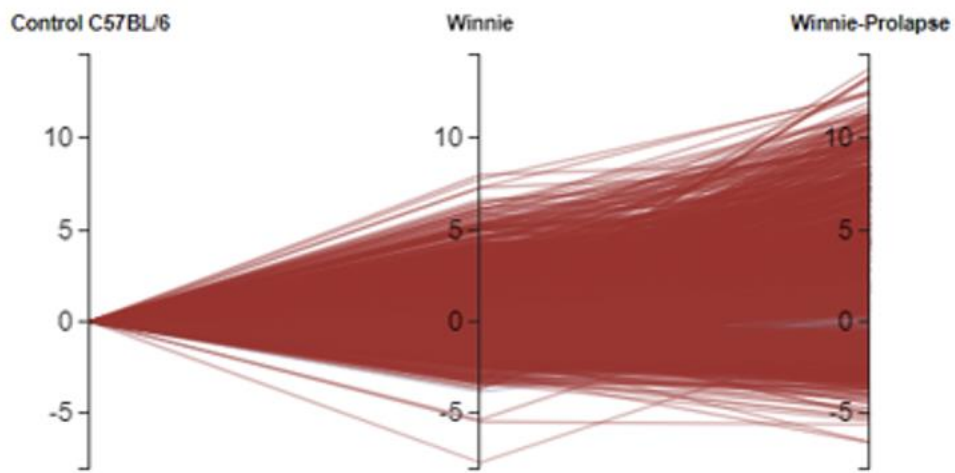
Figure 1. Histomorphological properties of colon tissues and the level of inflammation in control C57BL/6 mice, Winnie mice and Winnie-Prolapse mice. Representative histological images of haematoxylin and eosin stain of the mucosa (A-A''), muscle (B-B''), and Alcian Blue staining of goblet cells (C-C'') in cross-sections of the colon from C57BL/6, Winnie, and Winnie-Prolapse mice. Quantification of the total histological score (D), mucosal width (E), muscle width (F), and density of goblet cells (G) within an average of 3 areas of 500 μm² of colonic mucosa per section. (H) The level of inflammation was measured by assessing the inflammatory marker, faecal lipocalin-2 (LCN-2) in faeces from C57BL/6, Winnie, and Winnie-Prolapse mice. One-way ANOVA followed by the Tukey post hoc test for multiple group comparison was performed using Graph Pad Prism (Graph Pad Software Inc., San Diego, CA, USA). Data presented as mean ± standard error of the mean. n=5 mice/group. **P*<0.05, ***P*<0.01, ****P*<0.001, and *****P*<0.0001.

Transcriptomic analysis of C57BL/6, Winnie, and Winnie-Prolapse mice.

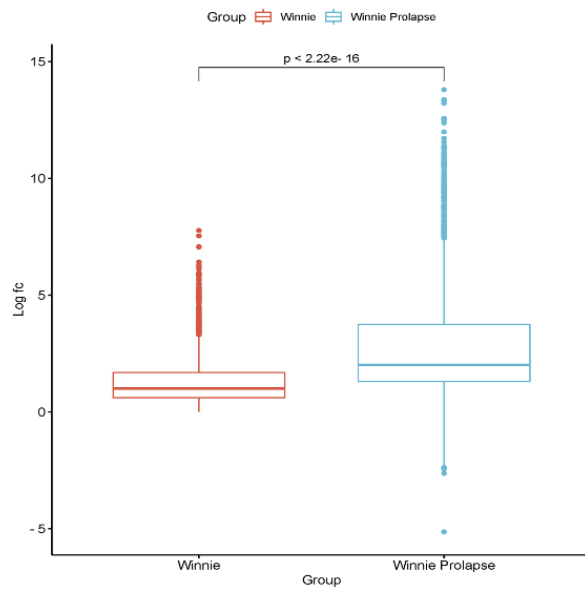
The three mice groups (C57BL/6, Winnie, Winnie-Prolapse) co-expressed 18,054 genes (Figure 2A). The RNA sequencing analysis identified many differentially expressed genes (DEGs) (Figure 2B) (adj. $P < 0.05$), with 4331 DEGs between C57BL/6, Winnie and Winnie-Prolapse mice. C57BL/6 and Winnie mice co-expressed 2,137 genes and C57BL/6 and Winnie-Prolapse mice co-expressed 4,146 genes. Winnie and Winnie-Prolapse mice co-expressed 1,263 genes (Figure 2C (i)). Winnie mice expressed 64 unique genes that were upregulated, and 233 downregulated, Winnie-Prolapse mice expressed 1,336 unique genes that were upregulated and 970 downregulated. Winnie mice co-expressed 892 upregulated and 926 downregulated genes with Winnie-Prolapse mice. The remaining 22 were null genes (Figure 2C (ii)). Analysis of genes based on the sex of Winnie and Winnie-Prolapse were conducted per group. Samples did not cluster according to animal sex on Principal component analysis suggesting minimal inter-sex variance (Figure 2D, E). A parallel coordinates plot to display the gene expression is shown in Figure 2F, 2G (ii). Several genes that were not expressed in Winnie mice were significantly overexpressed in Winnie-Prolapse mice (Table I).



F



G (i)



H

Gene Name	Winnie (LogFc)	Winnie Prolapse (LogFc)
Oas1f	0	7.213462376
Skint2	0	7.937053237
Krtap19-5	0	7.949352051
Sprr4	0	8.037884432
Lce1j	0	8.076562442
Krtap22-2	0	8.189172129
Lce1h	0	8.206451662
Krtap11-1	0	8.258912462
Krtap14	0	8.325135811
Klk5	0	8.327467582
Lypd5	0	8.442983684
Lce1k	0	8.448393674
Krtap15	0	8.475311637
Lce6a	0	8.482881637
Lce1l	0	8.728031273
Krtap1-5	0	8.771642932
Sptlc3	0	8.819854693
Aadacl3	0	9.029174729
Lce1i	0	9.048212635
Lce1m	0	9.20391574
Flg2	0	9.403937061
Klk6	0	9.439941966
Lce1e	0	9.505755369
Lce1g	0	9.521170001
Krtap7-1	0	9.631266346
Il1f5	0	9.662346514
Serpinb3d	0	9.703625525
Lce1f	0	9.778065791
Lce1a2	0	9.82998589
Krt77	0	9.890119848
Lce1d	0	10.09434585
Krt71	0	10.15099477
Idi2	0	10.32833585
Lce1a1	0	10.76727764
Defb6	0	10.99829189
Panx3	0	11.00380313

(ii)

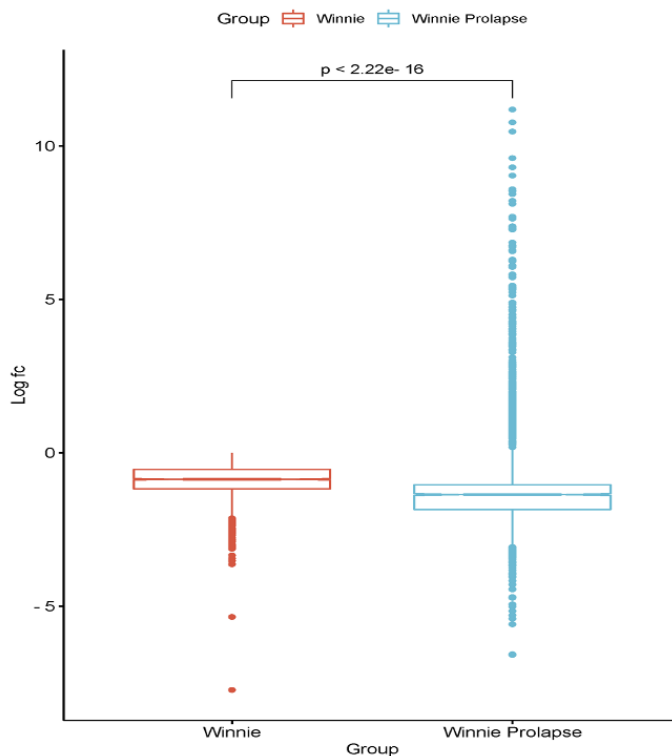


Figure 2. Gene Expression (A) Volcano plots where x-axis represents the log Fc and Y axis represents log₁₀ p value. Each dot represents one gene. Blue dot represents downregulated genes, red dot represents upregulated genes and grey dots represent no significant change in (i) C57BL/6 vs Winnie (ii) C57BL/6 vs Winnie-Prolapse (iii) Winnie vs Winnie-Prolapse mice. (B) Heatmap of genes identified across C57BL/6, Winnie mice and Winnie-Prolapse mice with > 2-fold changes. C57BL/6 mice (C1-C8), Winnie mice (W1-W7) and Winnie-Prolapse (WP1-7). (C) Venn diagram showing (i) number of genes with > 2-fold changes in C57BL/6 mice, Winnie mice and Winnie-Prolapse mice. (ii) Venn diagram showing gene expression (>

2-fold) in C57BL/6, *Winnie* and *Winnie-Prolapse*. **(D)** Multidimensional scaling (MDS) plot showing intersex variance between C57BL/6 (C1 to C4 are males in orange, C5 to C8 are females in blue), *Winnie* (W1 to W3 are males in purple, W4 to W7 are females in brown) and *Winnie-Prolapse* mice (WP1 to WP4 are males in red, WP5 to WP7 are females in green). **(E)** Clustering of individual groups. X-axis represents mice groups and Y-axis represents counts per million (CPM). **(F)** Parallel coordinates or parallel plot of C57BL/6, *Winnie* and *Winnie-Prolapse* mice, each line represents each gene. **(G)**. Box plots of **(i)** upregulated genes in *Winnie* and *Winnie-Prolapse* mice and, **(ii)** downregulated genes in *Winnie* and *Winnie-Prolapse* mice. **(H)** Differential gene expression in *Winnie-Prolapse* mice compared to *Winnie* mice.

Among the top-most variable genes (i.e., those with the largest fold change), *Clca4b* (chloride channel accessory 4B) was the most upregulated in *Winnie* mice with highest log fc of 7.76 (Figure 3A); this was associated with pancreatic and renin secretion pathways and *Wap* (Whey acidic protein) in *Winnie-Prolapse* was the most upregulated gene with a log fc 13.79 (Figure 3B) associated with immune system process gene ontology. Of the downregulated genes, in *Winnie* mice, *Abca12* (ATP-binding cassette sub-family A member 12) was downregulated with a log fc of -7.7 (Figure 3C) and, in *Winnie-Prolapse*, *Cyp2c55* (cytochrome P450, family 2, subfamily c, polypeptide 55) was downregulated with a log fc of -6.56 (Figure 3D).

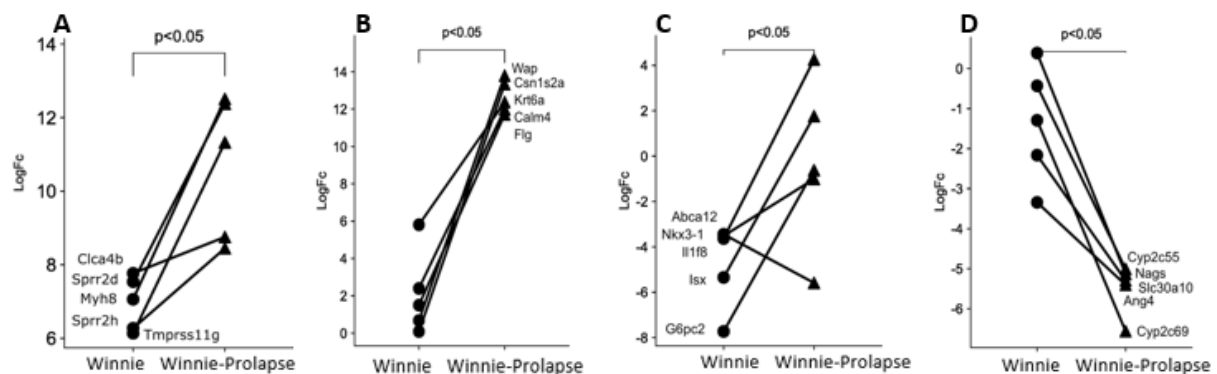


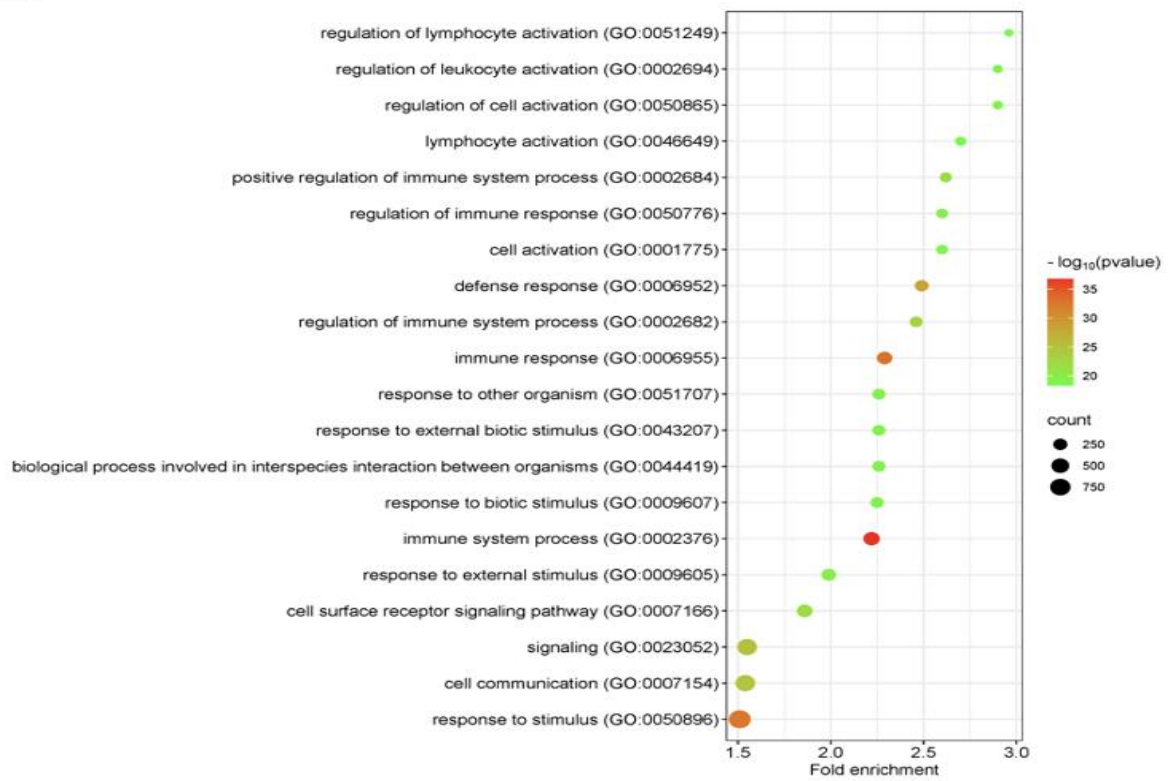
Figure 3. Gene expression of (A) Top 5 upregulated genes in *Winnie* mice, (B) top 5 upregulated genes in *Winnie-Prolapse* mice and (C) top 5 downregulated genes in *Winnie* mice, (D) top 5 downregulated genes in *Winnie-Prolapse* mice. X-axis represents *Winnie* and *Winnie-Prolapse* and Y-axis represents the log fold change.

Overrepresentation Analysis (ORA)

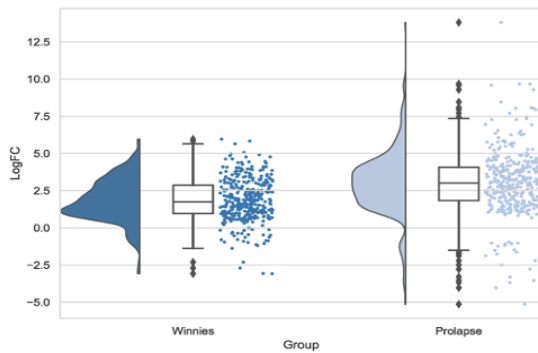
Winnie and Winnie-Prolapse mice are enriched in immune and inflammatory process functions.

The differentially expressed genes across C57BL/6, *Winnie* and *Winnie-Prolapse* mice showed that immune system process (GO:0002376) and immune response (GO:0006955) (Figure 4A) were significantly enriched which involved both up and downregulated genes based on the $\log_{10}$ p value. To enable comparisons between the differentiation capacity of the cells, genes from immune system process (GO:0002376) gene ontology were used to filter the dataset. Of the filtered genes, the three groups expressed 379 genes, of which 346 were upregulated in *Winnie* mice and 357 upregulated in *Winnie-Prolapse* and the remaining were downregulated (Figure 4B). *Cxcl9* (Log Fc 5.9) and *Cxcr2* (log fc 5.8) were significantly upregulated with highest log fold change in *Winnie* mice and *Wap* (log fc 13.7) and *LFT* (log fc 9.6) were upregulated with the greatest log fc in *Winnie-Prolapse* in the immune system process gene ontology. *Alas2* (log fc -3.0) and *H2-T3* (log fc -3.0) were significantly downregulated in *Winnie* mice and *Ang4* (-5.1) and *H2-T3* (-4.0) were downregulated in *Winnie-Prolapse* mice in the immune system process. There are several chemokines and cytokines that were differentially expressed in the immune system process (Figure 4C). There were 154 immunoglobulins that were differentially expressed in the immune system process ontology, of which *Ighv14-3* (log fc 5.6) was overexpressed in *Winnie* mice and *Ighv1-47* (log fc 7.4) in *Winnie-Prolapse* mice (Figure 4D).

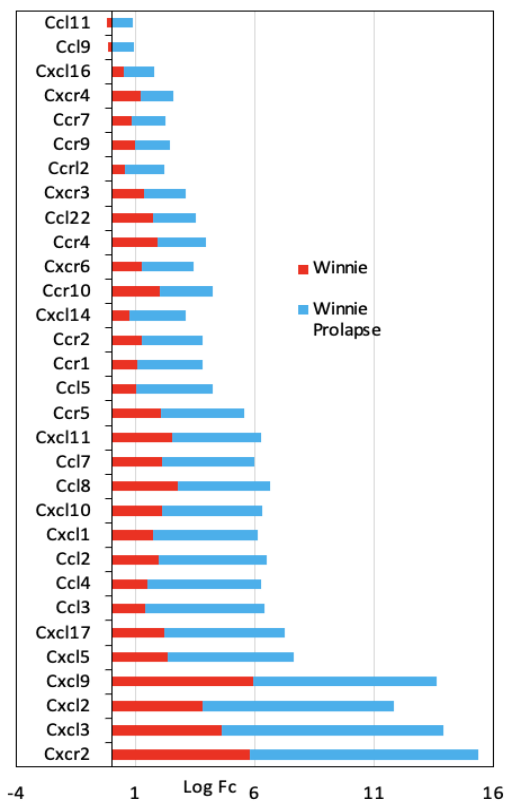
A



B Immune system process



C Chemokines and cytokines in Immune system process



D Immunoglobulins in Immune system process

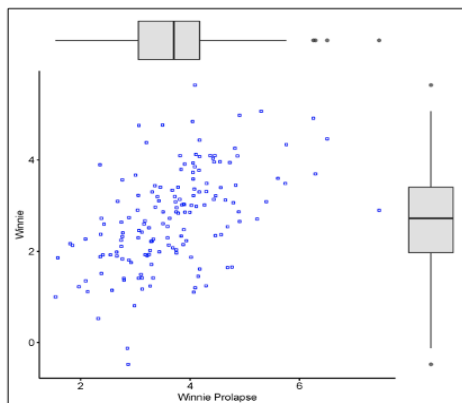


Figure 4. Pathway Analysis – Gene Ontologies (GO) (A) GO-Slim biological processes through Panther classification (overrepresentation test) on the Gene Ontology database, test type is Fisher's exact and correction by calculating the False Discovery rate (FDR). The colour of the bubbles represents the significance of FDR, and the size of the bubble represents the number of genes involved in each pathway. Top 20 significantly enriched Gene Ontology pathways across C57BL/6, *Winnie* mice and *Winnie-Prolapse* mice. (B) Raincloud plot (combination of violin plot, box plot and jitter plot). Number of differentially expressed genes associated with Immune system process, (C) differential gene expression of chemokines and cytokines associated with immune system process gene ontology in *Winnie* and *Winnie-Prolapse*, (D) scatter plot of immunoglobulins associated with immune system process gene ontology.

Enrichment in metabolic, cancer-related, nervous system and inflammatory pathways

The highly enriched KEGG pathways across the three groups based on the number of genes were metabolic pathways, pathways in cancer, neuroactive ligand-receptor interaction pathway, cytokine-cytokine interaction pathway and cell adhesion molecules (Figure 5A). There was a significant similarity in gene expression in *Winnie* mice and *Winnie-Prolapse*, however *Winnie-Prolapse* expressed a significantly greater number of genes in each category. Metabolic pathway consisted of 312 differentially expressed genes, of which *Ido1* (log fc 5.3) and *Nos2* (log fc 4.8) were significantly upregulated in *Winnie* mice; with *Elovl3* (log fc 11.0) and *Olah* (log fc 10.4) being highly upregulated in *Winnie-Prolapse* mice. *G6pc2* (log fc -3.4) and *Nags* (log fc -3.3) were downregulated in *Winnie* mice and *Cyp2c55* (log fc -6.5) and *G6pc2* (log fc -5.5) were significantly downregulated in *Winnie-Prolapse* mice (Figure 5B).

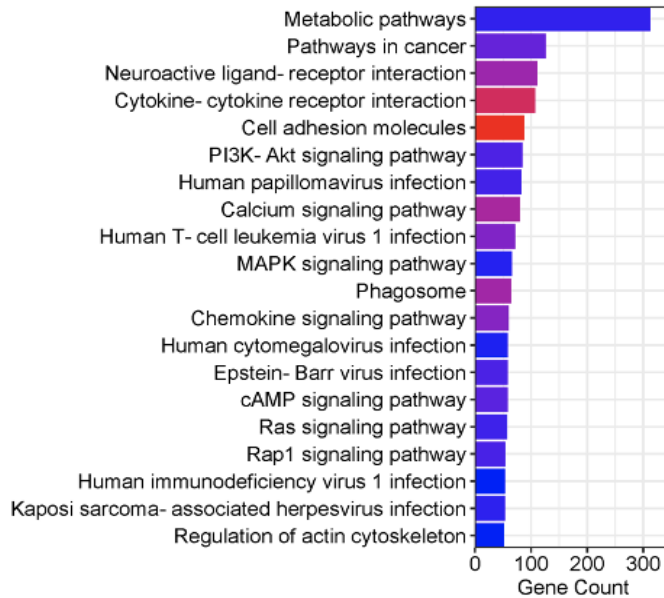
A total of 126 differentially expressed genes were present in pathways in cancers across three groups making it the second highest enriched category (Figure 5C). The most significantly upregulated genes in *Winnie* mice were *Ifn-γ* (log fc 5.6) and *Nos2* (log fc 4.8) and in *Winnie-Prolapse* mice, *Calm4* (log fc 11.9) and *Calml3* (log fc 10.8) were significantly upregulated in the cancer pathways. Among the downregulated genes, *Nkx3-1* (log fc -5.3) and *Kng2* (log fc -2.1) in *Winnie* mice and *Wnt8b* (log fc -4.0) and *Edn1* (log fc -2.6) in *Winnie-Prolapse* mice were the significantly downregulated in pathways in cancer.

Neuroactive ligand-receptor pathway has a total of 112 differentially expressed genes, of which *Fpr1* (log fc 3.5) and *Adm2* (log fc 3.4) were the most upregulated genes in *Winnie* mice and *Fpr1* (log fc 7.3) and *Fpr2* (log fc 6.3) were most upregulated in *Winnie-Prolapse* mice. This pathway consisted mostly of downregulated genes. *Tacr3* (log fc -2.3) and *Kng2* (log fc -2.1) were the most downregulated in *Winnie* mice and *Grin2b* (log fc -3.6) and *Galr2* (log fc -3.4) were highly downregulated in *Winnie-Prolapse* (Figure 5D).

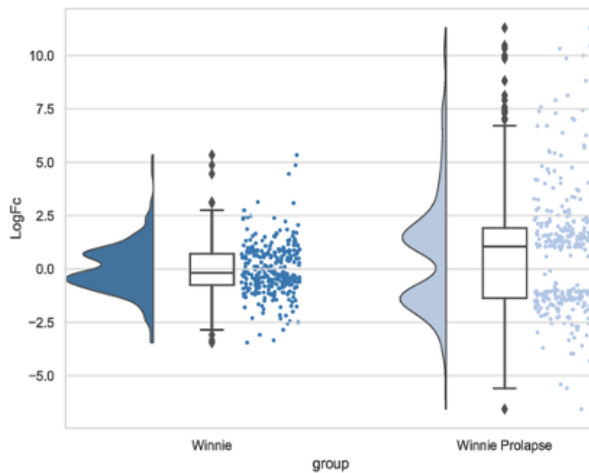
The cytokine-cytokine interaction pathway consisting of differentially expressed cytokine and chemokines has 107 genes in total, of which *Cxcl9* (log fc 5.9) and *Cxcr2* (log fc 5.8) were most upregulated in *Winnie* mice similar to immune system process and *Cxcr2* (log fc 9.5) and *Cxcl3* (log fc 9.2) were most upregulated in *Winnie-Prolapse* mice. *Gdf10* (log fc -1.7) and *Ackr4* (log fc -1.3) were most downregulated in *Winnie* mice whereas, *Klk1b4* (log fc -2.6) and *Il15* (log fc -2.2) were highly downregulated in *Winnie-Prolapse* mice in this pathway (Figure 5E). Several interleukins and interferon were differentially expressed in the cytokine-cytokine interaction pathway (Figure 5F).

When each mouse group were analysed separately (C57BL/6 vs *Winnie*, and C57BL/6 vs *Winnie-Prolapse*), a few unique pathways with differentially expressed genes were enriched in *Winnie* mice that were absent in *Winnie-Prolapse*, such as, the regulation of actin cytoskeleton and Influenza A were overexpressed in *Winnies* that were not present in *Winnie-Prolapse*. (Figure 6A). A number of unique pathways were noted to be enriched in *Winnie-Prolapse* but not in *Winnie* mice with PI3K-AKT signalling pathway was highly enriched in *Winnie-Prolapse* mice and absent in *Winnie* mice (Figure 6B).

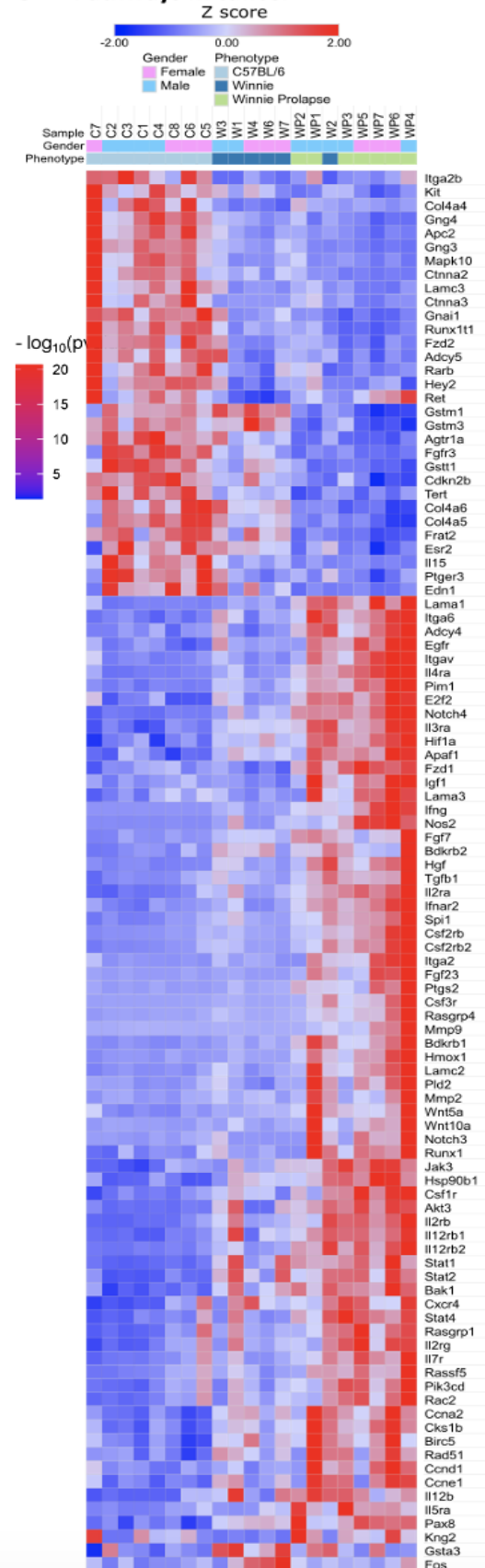
A



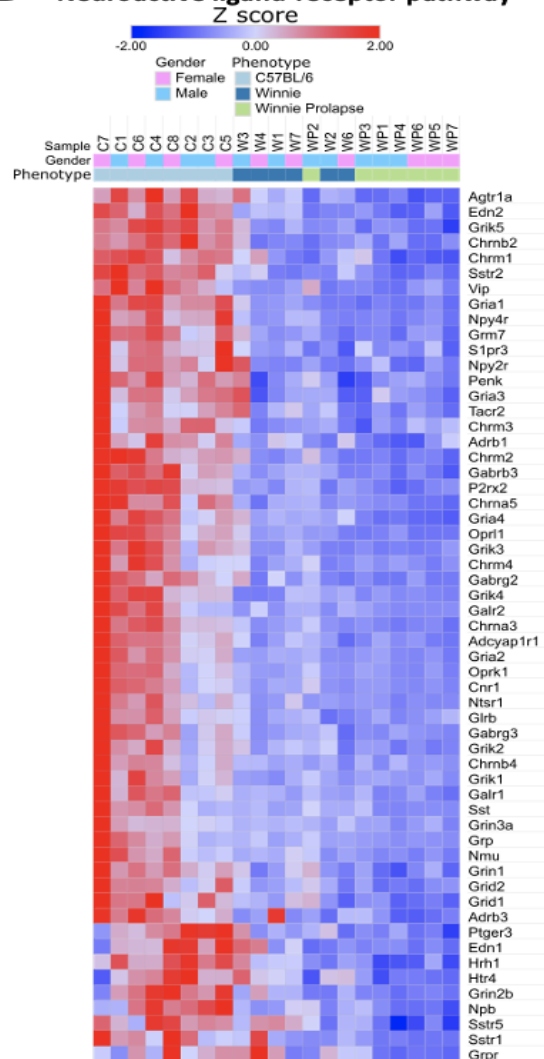
B. Metabolic pathways



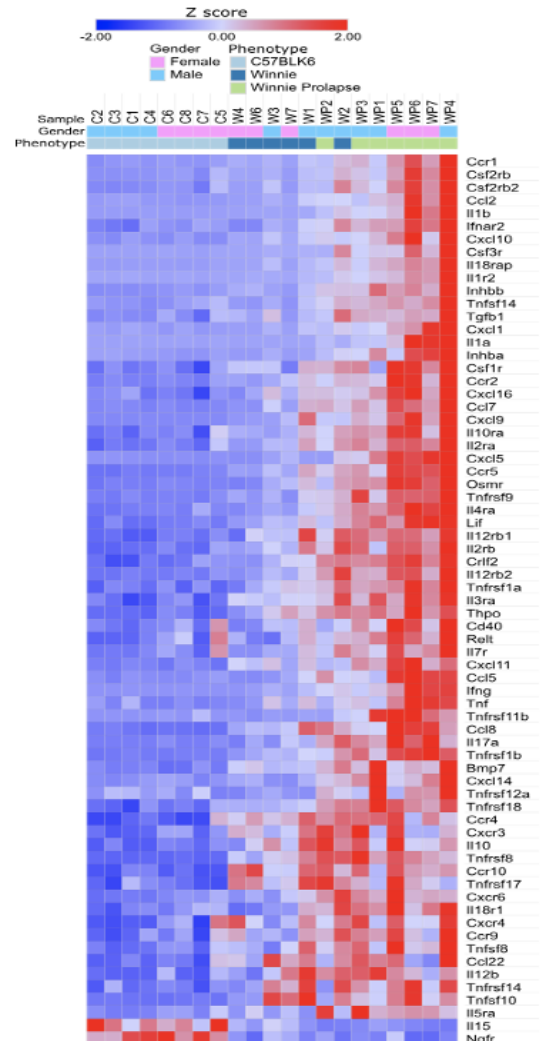
C Pathways in cancer



D Neuroactive ligand-receptor pathway



E Cytokine-Cytokine interaction pathway



F Interleukins and Interferon

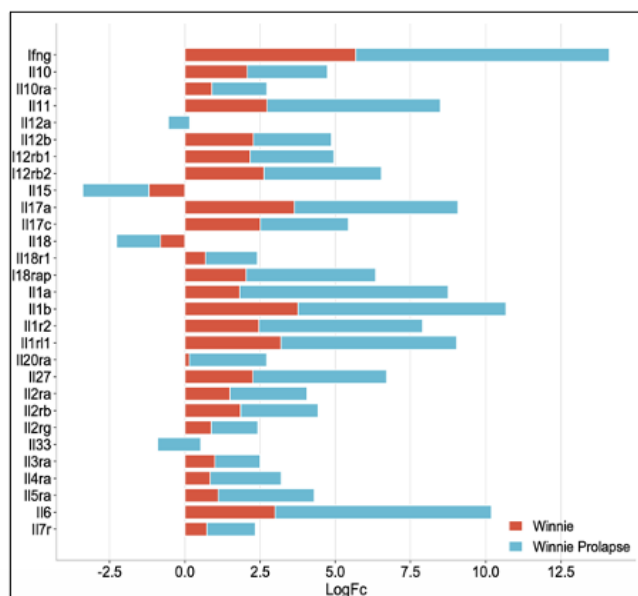


Figure 5. Pathway Analysis – KEGGs (A) Top 20 KEGG pathways across C57BL/6, *Winnie* mice and *Winnie-Prolapse* mice. The length of the bar represents number of genes, and the colour represents *P* values. **(B)** Raincloud plot (combination of violin plot, box plot and jitter plot). Number of differentially expressed genes in metabolic pathways, **(C)** heatmap of differential expression of genes associated with pathways in cancer across C57BL/6, *Winnie* mice and *Winnie-Prolapse* mice, **(D)** heatmap of differential expression of genes associated with neuroactive ligand-receptor pathway across C57BL/6, *Winnie* mice and *Winnie-Prolapse* mice. **(E)** Heatmap of differential expression of genes associated with cytokine-cytokine receptor interaction pathway across C57BL/6, *Winnie*, and *Winnie-Prolapse* mice, and **(F)** differential gene expression of interferon and interleukins in *Winnie* and *Winnie-Prolapse*. C57BL/6 mice (C1-C8), *Winnie* mice (W1-W7) and *Winnie-Prolapse* (WP1-7).

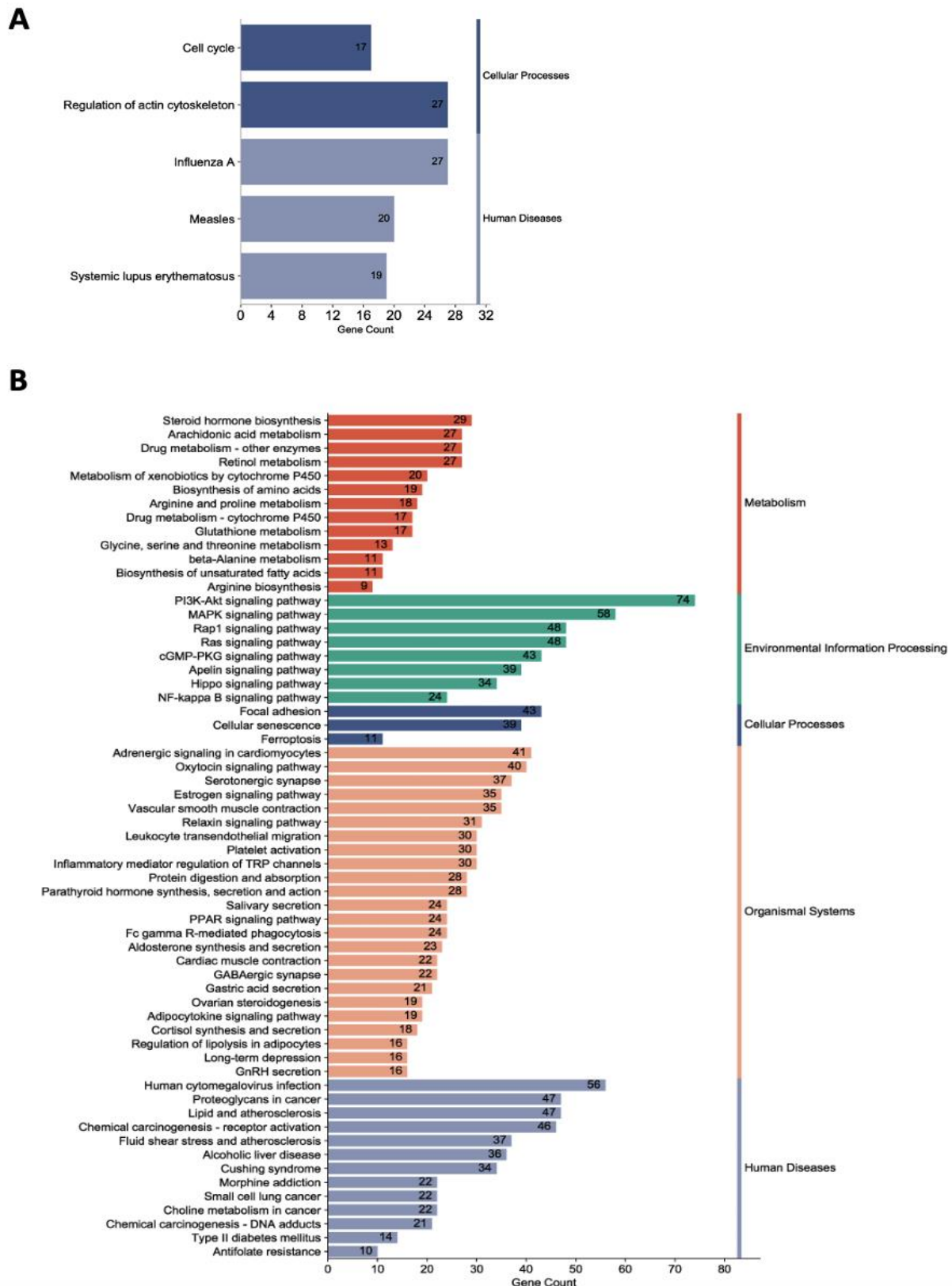


Figure 6. KEGG Pathways (A) Unique pathways found only in Winnie mice and absent in Winnie-Prolapse and, (B) unique pathways only present in Winnie-Prolapse and not in Winnie mice.

Discussion

The study herein demonstrated changes in gene expression in the well-established *Winnie* mouse model of spontaneous chronic colitis, with a focus on the transcriptional changes that occur with increasing disease severity in *Winnie-Prolapse* mice. This analysis enables to determine the genes expressed along the pathway of inflammation leading to severe colitis. The second most important issue addressed was the hypothesis that severe inflammation leads to CRC and as such, several novel genes were identified which could be translated as valuable targets for the early diagnosis and treatment of IBD-induced cancer as well as potentially being used to detect early stages of CRC. Three groups of mice were evaluated, *Winnie*, *Winnie-Prolapse* and control C57BL/6 mice, each showing distinct gene expression profiles, but with key similarities in physiologically relevant domains. The expression of genes in *Winnie* and *Winnie-Prolapse* related to immune system processes. Chemokines and cytokines play a pivotal role in the regulation of mucosal inflammation by promoting leukocyte migration to sites of inflammation ultimately leading to tissue damage and destruction (Singh et al., 2016); this drives intestinal inflammation and diarrhoea, intestinal stenosis, fistula formation and colitis-associated neoplasia. Indeed, *Winnie* and *Winnie-Prolapse* mice differentially expressed several genes associated with immune system function and cytokine-cytokine interaction, such as *Cxcl9* and *Cxcl10* which were significantly overexpressed in the transcriptome of *Winnie-Prolapse* distal colon, indicating a strong link between inflammation and cancer. This corresponds to previous findings which showed high expression levels of *Cxcl9* and *Cxcl10* in CRC by promoting lymphocyte infiltration into the cancer microenvironment to suppress cancer cell growth (Li et al., 2019). Given the extent of colitis in *Winnie-Prolapse* mice, this is an unsurprising finding. These genes were associated with most of the enriched pathways i.e., chemokine signaling pathway,

TNF signaling pathway and inflammatory response pathways. Interestingly, patients with IBD also show high levels of chemokines such as *Cxcl5*, *Cxcl1* that have cancer cell promoting functions (Li et al., 2022); (Friedrich et al., 2021). Additionally, pro-inflammatory cytokines (*Ifn- γ* , *Il-1 β* and *Tnf- α*) which were also overexpressed in *Winnie* and *Winnie-Prolapse* mice which are also prominent in patients with IBD (Singh et al., 2016). As such, there is close resemblance between the *Winnie* mouse model and humans with colitis regarding disease severity, as well as the relationship between inflammation and cancer. Furthermore, high levels

of chemokines and cytokines are known to increase intestinal inflammation and tissue damage in IBD patients. Blocking of cytokines, especially *Il-12* and *Il-23*, has always been in the pipeline as treatment for IBD. In this study, *Il-6* was significantly overexpressed in *Winnie* and *Winnie-Prolapse* mice and *Il-6* trans-signaling holds great promise for immunoregulatory therapy of IBD (Schreiber et al., 2021). *Il-6* is also elevated in patients with CRC and it is linked to poor prognosis and development of CRC (Lin et al., 2020). *Ifn- γ* gene was significantly overexpressed in both *Winnie* and *Winnie-Prolapse* mice. *Ifn- γ* is a key player in the pathogenesis of IBD, although in the context of the disease, its immune-modulating and epithelial cell-directed actions have received much attention (Langer et al., 2019, Ahluwalia et al., 2018). *Ifn- γ* increases the permeability of intestinal vessels by disrupting vascular-endothelial-cadherin junctions, associated with increased inflammation and progression of IBD. The pathogenic role of *Ifn- γ* -mediated intestinal vessel activation in IBD might pave the way for vascular-directed treatment of the disease (Langer et al., 2019).

Inflammation is mediated by three primary pathways: NF- κ B, MAPK, and JAK-STAT (Chen et al., 2018) all of which were enriched in both *Winnie* and *Winnie-Prolapse* mice. Dysregulation of one or more of these processes can result in inflammation-related disease (Seif et al., 2017). Non-canonical NF- κ B signalling and potential MAPK inhibitors could be a promising new direction for IBD therapeutic targets and biomarkers - some of which are in human clinical trials (McDaniel et al., 2016); (Coskun et al., 2011). The Food and Drug Administration has approved two JAK inhibitors to treat ulcerative colitis: tofacitinib (Xeljanz) and upadacitinib (Rinvoq) (Liu et al., 2022).

Differential expression of immunoglobulins, especially *Ighv* and *Igkv* associated with immune system processes were shown to be upregulated and further studies are required to ascertain their role in IBD. Nonetheless, several studies have shown that patients with mutated *Ighv* respond better to chemoimmunotherapy (Rozovski et al., 2018) and almost all acute myeloid leukemia-derived *Igkv* had somatic hypermutations compared to mature myeloid cell-derived *Igkv* that had no mutation implying IGKV somatic hypermutation may play a role in leukemogenesis (Wang et al., 2015a). However, its role in CRC is not clear. The gene *Tnfsf13b* also known as B-cell activating factor (*BAFF*), is a member of the TNF superfamily, was shown to be overexpressed in *Winnie-Prolapse* mice. Overproduction of *BAFF* has been linked to autoimmune diseases and is a potential candidate for IBD (Kumric et al., 2021). Aberrant B-

cell responses in patients with Crohn's disease, serves to be a new target for therapeutics regulating B-cell function (Timmermans et al., 2016), however, its role in ulcerative colitis is not clear.

Evidence shows that the interaction between the immune and the nervous systems are bidirectional and play a critical role in the regulation of intestinal inflammation (Bernardazzi et al., 2016). Understanding the relationship between the nervous and intestinal immune systems will provide new insights leading to the identification of innovative therapeutic approaches to treat IBD (Goverse et al., 2016). In fact, *Winnie* mice show decreased neuronal activity and reduced smooth muscle responses to cholinergic and nitrergic stimulation (Robinson et al., 2017b) which aligns with the data herein. Further, colitis affects the smooth muscle (Kataria et al., 2022) and ulcerative colitis causes reduction in motor activity in the distal colon (Bassotti et al., 2014). The fact that several of these alterations resemble abnormal motor capabilities in patients with IBD further exemplifies that the *Winnie* mouse model is remarkably similar to human IBD.

Due to its potential anti-inflammatory effect *in vivo*, activation of the cholinergic system has been proposed for the treatment of patients with IBD (Serafini et al., 2022). *GRIN2B*-related neurodevelopmental disorder and muscle tone abnormalities have been noted in less than 100 individuals (Sabo et al., 2023). This gene was the most downregulated in the neuroactive ligand-receptor pathway in *Winnie* and *Winnie-Prolapse* mice.

Conclusion

The main findings herein show that *Winnie* and *Winnie-Prolapse* mice are highly enriched (upregulated) in inflammatory and immune related pathways/processes and the downregulated genes were highly enriched in neurological pathways. Pathway analysis showed a similarity between *Winnie* and *Winnie-Prolapse* mice with *Winnie-Prolapse* mice having a significantly greater number of genes in every pathway with higher log fold change, which correlates with the severity of inflammation. A number of cancer-related genes were overexpressed in *Winnie-Prolapse* mice, which could be further studied to understand the mechanisms of inflammation-induced CRC. Differentially expressed genes in *Winnie* mice were similar to those expressed in patients with IBD, which shows the close resemblance between the *Winnie* model and human IBD. The findings open many opportunities to study these genes and pathways in detail in order

to identify novel therapeutic targets for IBD. Detailed studies of identified novel pathways and genes may provide insight into new therapeutic targets against IBD with a long-lasting response and reduced relapse rates.

Limitations and Future Directions:

Although these NGS data are descriptive at this point, they do point out key pathways and genes up or down regulated. It is important to validate some of these genes and pathways at the protein level and Western blots, immunohistochemistry and functional assays will be conducted to validate these findings. Based on the transcriptome and mechanism/pathway findings in this thesis, as well as data to be gathered from these future studies, novel diagnostic kits can be developed to identify IBD as well as cancer in early stages of disease. In addition, the development of novel interventions for the treatment of IBD will be developed. Prevention strategies for the progression of mild to severe inflammation to inflammation associated cancers, will be developed, and tested in vivo in the Winnie mice. This work will then lead to translation of novel therapeutics to human clinical trials for the treatment of IBD, and CRC.

References

- AGEMATSU, K. 2000. Memory B cells and CD27. *Histol Histopathol*, 15, 573-6.
- AHLUWALIA, B., MORAES, L., MAGNUSSON, M. K. & ÖHMAN, L. 2018. Immunopathogenesis of inflammatory bowel disease and mechanisms of biological therapies. *Scandinavian Journal of Gastroenterology*, 53, 379-389.
- BASSOTTI, G., ANTONELLI, E., VILLANACCI, V., BALDONI, M. & DORE, M. P. 2014. Colonic motility in ulcerative colitis. *United European Gastroenterol J*, 2, 457-62.
- BAUMGART, D. C. & CARDING, S. R. 2007. Inflammatory bowel disease: cause and immunobiology. *The Lancet*, 369, 1627-1640.
- BERGSTROM, K. S. B., KISSOON-SINGH, V., GIBSON, D. L., MA, C., MONTERO, M., SHAM, H. P., RYZ, N., HUANG, T., VELCICH, A., FINLAY, B. B., CHADEE, K. & VALLANCE, B. A. 2010. Muc2 Protects against Lethal Infectious Colitis by Disassociating Pathogenic and Commensal Bacteria from the Colonic Mucosa. *PLOS Pathogens*, 6, e1000902.
- BERNARDAZZI, C., PÊGO, B. & DE SOUZA, H. S. 2016. Neuroimmunomodulation in the Gut: Focus on Inflammatory Bowel Disease. *Mediators Inflamm*, 2016, 1363818.
- CAI, Z., WANG, S. & LI, J. 2021. Treatment of Inflammatory Bowel Disease: A Comprehensive Review. *Front Med (Lausanne)*, 8, 765474.
- CAMILLERI, M. 2011. Managing symptoms of irritable bowel syndrome in patients with inflammatory bowel disease. *Gut*, 60, 425-428.

- CARUSO, R., LO, B. C. & NUNEZ, G. 2020. Host-microbiota interactions in inflammatory bowel disease. *Nat Rev Immunol*, 20, 411-426.
- CENGİZ, D., GÜRÇAY, E., KARAAHMET, Ö. Z., KARAAHMET, F., UMay, E. & ÇAKCI, A. 2017. Inflammatory arthritis complicated by inflammatory bowel disease: two case reports. *Turkish Journal of Physical Medicine & Rehabilitation* (2587-0823), 63, 266-271.
- CHEN, L., DENG, H., CUI, H., FANG, J., ZUO, Z., DENG, J., LI, Y., WANG, X. & ZHAO, L. 2018. Inflammatory responses and inflammation-associated diseases in organs. *Oncotarget*, 9, 7204-7218.
- COLOMBEL, J.-F., PANACCIONE, R., BOSSUYT, P., LUKAS, M., BAERT, F., VAŇÁSEK, T., DANALIOGLU, A., NOVACEK, G., ARMUZZI, A., HÉBUTERNE, X., TRAVIS, S., DANESE, S., REINISCH, W., SANDBORN, W. J., RUTGEERTS, P., HOMMES, D., SCHREIBER, S., NEIMARK, E., HUANG, B., ZHOU, Q., MENDEZ, P., PETERSSON, J., WALLACE, K., ROBINSON, A. M., THAKKAR, R. B. & D'HAENS, G. 2017. Effect of tight control management on Crohn's disease (CALM): a multicentre, randomised, controlled phase 3 trial. *The Lancet*, 390, 2779-2789.
- COSKUN, M., OLSEN, J., SEIDELIN, J. B. & NIELSEN, O. H. 2011. MAP kinases in inflammatory bowel disease. *Clin Chim Acta*, 412, 513-20.
- DOBIN, A., DAVIS, C. A., SCHLESINGER, F., DRENKOW, J., ZALESKI, C., JHA, S., BATUT, P., CHAISSON, M. & GINGERAS, T. R. J. B. 2013. STAR: ultrafast universal RNA-seq aligner. 29, 15-21.
- ERI, R. D., ADAMS, R. J., TRAN, T. V., TONG, H., DAS, I., ROCHE, D. K., OANCEA, I., PNG, C. W., JEFFERY, P. L., RADFORD-SMITH, G. L., COOK, M. C., FLORIN, T. H. & MCGUCKIN, M. A. 2011. An intestinal epithelial defect conferring ER stress results in inflammation involving both innate and adaptive immunity. *Mucosal Immunol*, 4, 354-64.
- FILIPPONE, R. T., DARGAHI, N., ERI, R., URANGA, J. A., BORNSTEIN, J. C., APOSTOLOPOULOS, V. & NURGALI, K. 2022. Potent CCR3 Receptor Antagonist, SB328437, Suppresses Colonic Eosinophil Chemotaxis and Inflammation in the Winnie Murine Model of Spontaneous Chronic Colitis. *International Journal of Molecular Sciences*, 23, 7780.
- FILIPPONE, R. T., ROBINSON, A. M., JOVANOVSKA, V., STAVELY, R., APOSTOLOPOULOS, V., BORNSTEIN, J. C. & NURGALI, K. 2018. Targeting eotaxin-1 and CCR3 receptor alleviates enteric neuropathy and colonic dysfunction in TNBS-induced colitis in guinea pigs. *Neurogastroenterol Motil*, 30, e13391.
- FRIEDRICH, M., POHIN, M., JACKSON, M. A., KORSUNSKY, I., BULLERS, S. J., RUE-ALBRECHT, K., CHRISTOFORIDOU, Z., SATHANANTHAN, D., THOMAS, T., RAVINDRAN, R., TANDON, R., PERES, R. S., SHARPE, H., WEI, K., WATTS, G. F. M., MANN, E. H., GEREMIA, A., ATTAR, M., BARONE, F., BRENNER, M., BUCKLEY, C. D., COLES, M., FREI, A. P., LASSEN, K. G., POWRIE, F. M., MCCUAIG, S., THOMAS, L., COLLANTES, E., UHLIG, H. H., SANSOM, S. N., EASTON, A., RAYCHAUDHURI, S., TRAVIS, S. P., POWRIE, F. M., OXFORD, I. B. D. C. I. & ROCHE FIBROBLAST NETWORK, C. 2021. IL-1-driven stromal–neutrophil interactions define a subset of patients with inflammatory bowel disease that does not respond to therapies. *Nature Medicine*, 27, 1970-1981.
- GOVERSE, G., STAKENBORG, M. & MATTEOLI, G. 2016. The intestinal cholinergic anti-inflammatory pathway. *J Physiol*, 594, 5771-5780.
- HSU, H.-P., LAI, M.-D., LEE, J.-C., YEN, M.-C., WENG, T.-Y., CHEN, W.-C., FANG, J.-H. & CHEN, Y.-L. 2017. Mucin 2 silencing promotes colon cancer metastasis through interleukin-6 signaling. *Scientific Reports*, 7, 5823.

- KATARIA, J., KERR, J., LOURENSSEN, S. R. & BLENNERHASSETT, M. G. 2022. Nintedanib regulates intestinal smooth muscle hyperplasia and phenotype in vitro and in TNBS colitis in vivo. *Scientific Reports*, 12, 10275.
- KELLERMANN, L. & RIIS, L. B. 2021. A close view on histopathological changes in inflammatory bowel disease, a narrative review. *Digestive Medicine Research*, 4.
- KUMRIC, M., ZIVKOVIC, P. M., TICINOVIC KURIR, T., VRDOLJAK, J., VILOVIC, M., MARTINOVIC, D., BRATANIC, A., LIZATOVIC, I. K. & BOZIC, J. 2021. Role of B-Cell Activating Factor (BAFF) in Inflammatory Bowel Disease. *Diagnostics (Basel)*, 12.
- LANGER, V., VIVI, E., REGENSBURGER, D., WINKLER, T. H., WALDNER, M. J., RATH, T., SCHMID, B., SKOTTKE, L., LEE, S., JEON, N. L., WOHLFAHRT, T., KRAMER, V., TRIPAL, P., SCHUMANN, M., KERSTING, S., HANDTRACK, C., GEPPERT, C. I., SUCHOWSKI, K., ADAMS, R. H., BECKER, C., RAMMING, A., NASCHBERGER, E., BRITZEN-LAURENT, N. & STÜRZL, M. 2019. IFN- γ drives inflammatory bowel disease pathogenesis through VE-cadherin-directed vascular barrier disruption. *J Clin Invest*, 129, 4691-4707.
- LEDERGERBER, M., LANG, B. M., HEINRICH, H., BIEDERMANN, L., BEGRÉ, S., ZEITZ, J., KRUPKA, N., RICKENBACHER, A., TURINA, M., GREUTER, T., SCHREINER, P., ROTH, R., SIEBENHÜNER, A., VAVRICKA, S. R., ROGLER, G., BEERENWINKEL, N., MISSELWITZ, B., ANDEREGG, C., BAUERFEIND, P., BEGLINGER, C., BEGRÉ, S., BELLI, D., BENGGA, J. M., BIEDERMANN, L., BIGLER, B., BINEK, J., BLATTMANN, M., BOEHM, S., BOROVICKA, J., BRAEGGER, C. P., BRUNNER, N., BÜHR, P., BURNAND, B., BURRI, E., BUYSE, S., CREMER, M., CRIBLEZ, D. H., DE SAUSSURE, P., DEGEN, L., DELARIVE, J., DOERIG, C., DORA, B., DORTA, G., EGGER, M., EHMANN, T., EL-WAFA, A., ENGELMANN, M., EZRI, J., FELLE, C., FLIEGNER, M., FOURNIER, N., FRAGA, M., FREI, P., FREI, R., FRIED, M., FROEHLICH, F., FUNK, C., FURLANO, R. I., GALLOT-LAVALLÉE, S., GEYER, M., GIRARDIN, M., GOLAY, D., GRANDINETTI, T., GYSI, B., HAACK, H., HAARER, J., HELBLING, B., HENGSTLER, P., HERZOG, D., HESS, C., HEYLAND, K., HINTERLEITNER, T., HIROZ, P., HIRSCHI, C., HRUZ, P., IWATA, R., JOST, R., JUILLERAT, P., BRONDOLO, V. K., KNELLWOLF, C., KNOBLAUCH, C., KÖHLER, H., KOLLER, R., KRIEGER-GRÜBEL, C., KULLAK-UBLOCK, G., KÜNZLER, P., LANDOLT, M., LANGE, R., LEHMANN, F. S., MACPHERSON, A., MAERTEN, P., MAILLARD, M. H., MANSER, C., MANZ, M., MARBET, U., MARX, G., MATTER, C., MCLIN, V., MEIER, R., MENDANOVA, M., et al. 2021. Abdominal pain in patients with inflammatory bowel disease: association with single-nucleotide polymorphisms prevalent in irritable bowel syndrome and clinical management. *BMC Gastroenterology*, 21, 53.
- LI, H., WU, M. & ZHAO, X. 2022. Role of chemokine systems in cancer and inflammatory diseases. *MedComm*, 3, e147.
- LI, X., ZHONG, Q., LUO, D., DU, Q. & LIU, W. 2019. The prognostic value of CXC subfamily ligands in stage I-III patients with colorectal cancer. *PLoS One*, 14, e0214611.
- LIAO, Y., SMYTH, G. K. & SHI, W. J. B. 2014. featureCounts: an efficient general purpose program for assigning sequence reads to genomic features. 30, 923-930.
- LIN, Y., HE, Z., YE, J., LIU, Z., SHE, X., GAO, X. & LIANG, R. 2020. Progress in Understanding the IL-6/STAT3 Pathway in Colorectal Cancer. *Onco Targets Ther*, 13, 13023-13032.
- LISO, M., DE SANTIS, S., VERNA, G., DICARLO, M., CALASSO, M., SANTINO, A., GIGANTE, I., ERI, R., RAVEENTHIRARAJ, S., SOBOLEWSKI, A., PALMITESSA, V., LIPPOLIS, A., MASTRONARDI, M., ARMENTANO, R., SERINO, G., DE ANGELIS, M. & CHIEPPA, M. 2019. A Specific Mutation in Muc2 Determines Early Dysbiosis in Colitis-Prone Winnie Mice. *Inflammatory Bowel Diseases*, 26, 546-556.

- LIU, E., ASLAM, N., NIGAM, G. & LIMDI, J. K. 2022. Tofacitinib and newer JAK inhibitors in inflammatory bowel disease-where we are and where we are going. *Drugs Context*, 11.
- LOVE, B. L. 2019. Management of patients with inflammatory bowel disease: current and future treatments. *Acute pain*, 10, 00.
- MCDANIEL, D. K., EDEN, K., RINGEL, V. M. & ALLEN, I. C. 2016. Emerging Roles for Noncanonical NF- κ B Signaling in the Modulation of Inflammatory Bowel Disease Pathobiology. *Inflamm Bowel Dis*, 22, 2265-79.
- PROCTOR, D. T., PATEL, Z., LAMA, S., RESCH, L., VAN MARLE, G. & SUTHERLAND, G. R. 2019. Identification of PD-L2, B7-H3 and CTLA-4 immune checkpoint proteins in genetic subtypes of meningioma. *OncoImmunology*, 8, e1512943.
- RAHMAN, A. A., ROBINSON, A. M., BROOKES, S. J. H., ERI, R. & NURGALI, K. 2016. Rectal prolapse in Winnie mice with spontaneous chronic colitis: changes in intrinsic and extrinsic innervation of the rectum. *Cell and Tissue Research*, 366, 285-299.
- RAHMAN, A. A., ROBINSON, A. M., JOVANOVSKA, V., ERI, R. & NURGALI, K. 2015. Alterations in the distal colon innervation in Winnie mouse model of spontaneous chronic colitis. *Cell Tissue Res*, 362, 497-512.
- ROBINSON, A. M., GONDALIA, S. V., KARPE, A. V., ERI, R., BEALE, D. J., MORRISON, P. D., PALOMBO, E. A. & NURGALI, K. 2016. Fecal Microbiota and Metabolome in a Mouse Model of Spontaneous Chronic Colitis: Relevance to Human Inflammatory Bowel Disease. *Inflammatory Bowel Diseases*, 22, 2767-2787.
- ROBINSON, A. M., RAHMAN, A. A., CARBONE, S. E., RANDALL-DEMLLO, S., FILIPPONE, R., BORNSTEIN, J. C., ERI, R. & NURGALI, K. 2017. Alterations of colonic function in the Winnie mouse model of spontaneous chronic colitis. *Am J Physiol Gastrointest Liver Physiol*, 312, G85-g102.
- ROZOVSKI, U., KEATING, M. J. & ESTROV, Z. 2018. Why Is the Immunoglobulin Heavy Chain Gene Mutation Status a Prognostic Indicator in Chronic Lymphocytic Leukemia? *Acta Haematologica*, 140, 51-54.
- SABO, S. L., LAHR, J. M., OFFER, M., WEEKES, A. L. & SCENIAK, M. P. 2023. GRIN2B-related neurodevelopmental disorder: current understanding of pathophysiological mechanisms. *Frontiers in Synaptic Neuroscience*, 14.
- SCHREIBER, S., ADEN, K., BERNARDES, J. P., CONRAD, C., TRAN, F., HÖPER, H., VOLK, V., MISHRA, N., BLASE, J. I., NIKOLAUS, S., BETHGE, J., KÜHBACHER, T., RÖCKEN, C., CHEN, M., COTTINGHAM, I., PETRI, N., RASMUSSEN, B. B., LOKAU, J., LENK, L., GARBERS, C., FEUERHAKE, F., ROSE-JOHN, S., WAETZIG, G. H. & ROSENSTIEL, P. 2021. Therapeutic Interleukin-6 Trans-signaling Inhibition by Olamkicept (sgp130Fc) in Patients With Active Inflammatory Bowel Disease. *Gastroenterology*, 160, 2354-2366.e11.
- SEIF, F., KHOSHMIRSAFA, M., AAZAMI, H., MOHSENZADEGAN, M., SEDIGHI, G. & BAHAR, M. 2017. The role of JAK-STAT signaling pathway and its regulators in the fate of T helper cells. *Cell Communication and Signaling*, 15, 23.
- SERAFINI, M. A., PAZ, A. H. & NUNES, N. S. 2022. Cholinergic immunomodulation in inflammatory bowel diseases. *Brain Behav Immun Health*, 19, 100401.
- SILVA, F. A. R., RODRIGUES, B. L., AYRIZONO, M. D. L. S. & LEAL, R. F. 2016. The Immunological Basis of Inflammatory Bowel Disease. *Gastroenterology research and practice*, 2016, 2097274-2097274.
- SINGH, U. P., SINGH, N. P., MURPHY, E. A., PRICE, R. L., FAYAD, R., NAGARKATTI, M. & NAGARKATTI, P. S. 2016. Chemokine and cytokine levels in inflammatory bowel disease patients. *Cytokine*, 77, 44-9.

- STAVELY, R., RAHMAN, A. A., SAHAKIAN, L., PRAKASH, M. D., ROBINSON, A. M., HASSANZADEGANROUDSARI, M., FILIPPONE, R. T., FRASER, S., ERI, R., BORNSTEIN, J. C., APOSTOLOPOULOS, V. & NURGALI, K. 2022. Divergent Adaptations in Autonomic Nerve Activity and Neuroimmune Signaling Associated With the Severity of Inflammation in Chronic Colitis. *Inflamm Bowel Dis*, 28, 1229-1243.
- TIMMERMANS, W. M., VAN LAAR, J. A., VAN DER HOUWEN, T. B., KAMPHUIS, L. S., BARTOL, S. J., LAM, K. H., OUWENDIJK, R. J., SPARROW, M. P., GIBSON, P. R., VAN HAGEN, P. M. & VAN ZELM, M. C. 2016. B-Cell Dysregulation in Crohn's Disease Is Partially Restored with Infliximab Therapy. *PLoS One*, 11, e0160103.
- TSYGANOV, K., JAMES PERRY, A., KENNETH ARCHER, S. & POWELL, D. J. J. O. O. S. S. 2018. RNAsik: A Pipeline for complete and reproducible RNA-seq analysis that runs anywhere with speed and ease. 3, 583.
- VANCAMELBEKE, M. & VERMEIRE, S. 2017. The intestinal barrier: a fundamental role in health and disease. *Expert Rev Gastroenterol Hepatol*, 11, 821-834.
- WANG, C., XIA, M., SUN, X., HE, Z., HU, F., CHEN, L., BUESO-RAMOS, C. E., QIU, X. & YIN, C. C. 2015. IGK with conserved IGKV/IGKJ repertoire is expressed in acute myeloid leukemia and promotes leukemic cell migration. *Oncotarget*, 6, 39062-72.

Chapter 3

Chapter context and preface

In chapter 2, it was clear that several pathways and genes were up- or down- regulated in spontaneous chronic colitis mice, *Winnie* mice. These gene and pathway were further enhanced in severe chronic colitis mice (older mice; *Winnie prolapse* mice). Of interest several pathways of the cancer pathways were noted in chapter 2, which implies that cancer pathways are upregulated in inflammation, at a time when no cancer is present. As such, in this chapter 3, the cancer genes and checkpoint markers involved in cancer as well as cancer pathways were evaluated, which would give insights into pathways and genes involved in inflammation induced cancer.

Chapter 3

Differential gene expression of checkpoint markers and cancer markers in mouse models of spontaneous chronic colitis

Abstract

The presence of checkpoint markers on cancer cells work as an escape strategy. Here, the transcriptome of the distal colons of C57BL/6, *Winnie* (a mouse model of spontaneous colitis), and severely inflamed and prolapsed colons, the *Winnie-Prolapse* mice, were analysed. The identification of checkpoint markers and early cancer markers are of utmost importance to gain clarity regarding the relationship between colitis and progressive inflammation leading to cancer. Herein, the gene expression of checkpoint makers, cancer-related pathways, and cancer genes in colon tissues of mouse models of chronic colitis (*Winnie* and *Winnie-Prolapse* mice) using next generation sequencing are determined. Identification of such genes and their subsequent expression and role at the protein level would enable novel markers for the early diagnosis of cancer in IBD patients. The differentially expressed genes in the colonic transcriptome were analysed based on Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway.

Introduction

Immunotherapy refers to immune cells (antibody, T cell or dendritic cell therapies) or their activation *in vivo* to manage, treat or prevent disease. Immunotherapy includes, cellular therapies, monoclonal antibody therapies, immune checkpoint inhibitors, non-specific immunotherapies, oncolytic virus therapies, T-cell therapies, immune modulators, dendritic cell therapies and cancer vaccines (Cebon, 2018, Korman et al., 2006). Non-specific immunotherapy, also referred as non-specific immunomodulating agents, involve cytokines (interferon and interleukins) and adjuvants to stimulate immune cells to lyse cancer cells. Immune checkpoints are expressed by cancer cells which bind to their ligands on T cells which act as a switch-off signal for T cells from attacking cancer cells. As a result, cancer cells evade T-cell responses and host immunity through negative regulators of the immune system, such as PD-1, PD-L1, PD-L2, CTLA-4, TIM3, 2B4, the B and T lymphocyte attenuator (BTLA), and LAG3 (Barriga et al., 2019) (Kuol et al., 2018d, Kuol et al., 2017, Ephraim et al., 2022b, Ephraim et al., 2022a, Khadela et al., 2023); summarised in Chapter 1. Treatments that target these specific pathways to block or inhibit their actions are referred as immune checkpoint blockade therapies (Gun et al., 2019, Zhang and Chen, 2018, Koury et al., 2018). These immune checkpoint blockade therapies, developed against CTLA-4, PD1, PD-L1 inhibitors, and LAG3 inhibitors for several types of cancers have been approved by the US Food and Drug Administration (Johnson et al., 2022). Combination immunotherapy based on immune checkpoint blockade therapies has become a first-line treatment preference for several cancers, including hepatocellular carcinoma, renal cell carcinoma, lung cancer, cervical cancer, and gastric cancer. Immune checkpoint blockade therapies in combination regimens have improved objective response rates, disease control rates, progression-free survival, and overall survival time in patients with advanced cancer compared to those receiving immune checkpoint blockade monotherapy (Ribas and Wolchok, 2018, Kreamer, 2014).

IBD, namely ulcerative colitis and Crohn's disease, involving the gastrointestinal tract (Kaser et al., 2010, Wehkamp et al., 2016) is highly prevalent within the developed countries. Crohn's disease can affect any part of the gastrointestinal tract whereas ulcerative colitis affects the colon (Hedrick and Friel, 2013). IBD is an immune-mediated inflammatory disease involving a complex interplay of host genes and environmental influences (Dave et al., 2014). As noted in chapter 2, inflammatory and immune related pathways/processes are highly upregulated as well as certain cancer related genes, with those of the neurological pathways being highly

downregulated in animal models of ulcerative colitis. The search for new therapeutic strategies that target immunological pathways has become of great interest, such as, the IL-12/IL-23 axis, the IL-6 pathway, or Janus Kinase (Kumric et al.) inhibitors (Kim and Kim, 2021). These modulate anti-inflammatory signalling pathways such as transforming growth factor-1 (TGF-1) (Yun et al., 2019), leukocyte adhesion and migration into inflamed intestinal mucosa (Chen et al., 2018) by blocking different subunits of $\alpha 4\beta 7$ integrins or other adhesion molecules (Dotan et al., 2020). Infliximab, an anti-tumour TNF- α monoclonal antibody, is currently used as a first line treatment for patients with severe IBD (Lee and Fedorak, 2010); this results in adequate mucosal healing. However, such treatment increases susceptibility to infections and cancer (Lichtenstein, 2013). Therefore, new improved and more specific immune targets are required for IBD.

CRC affects the caecum, colon and rectum (Phipps et al., 2013, Kuipers et al., 2015) is the third most common malignancy world-wide and second most common cause of cancer-related deaths with more than 1 million new cases each year (Song et al., 2019, Morse et al., 2020). Colitis-associated cancer (CAC) is a subset of CRC that can develop with long-standing IBD (Hnatyszyn et al., 2019, Low et al., 2014, Sebastian et al., 2014, Wu et al., 2018, Kanneganti et al., 2011, Potack and Itzkowitz, 2008, Barkin et al., 2012). The role of inflammation as a risk factor in CRC is highly studied, both in CAC and in sporadic tumours (Grivennikov, 2013, Long et al., 2017, Itzkowitz and Yio, 2004b). Most patients receive chemotherapy before or after surgery; however, 80-90% of patients experience, diarrhoea, constipation, oral mucositis, nausea, and vomiting (Andreyev et al., 2005). As a result, patients develop malnutrition and dehydration leading to rapid weight loss (cachexia) (Murphy and Lynch, 2009). Currently, pembrolizumab (Keytruda) and nivolumab (Opdivo) are monoclonal antibody treatments that target PD-1 and, Ipilimumab (Yervoy) is a monoclonal antibody that blocks CTLA-4 (Buchbinder and Desai, 2016) ipilimumab can be used in combination with nivolumab (Opdivo) but not used alone for CRC (Kooshkaki et al., 2020). Novel testing methods and predictive markers are critical for enabling earlier detection and integrating rapid therapy to reduce CAC mortality and morbidity (Zhen et al., 2018). In addition, the effect of recent immunosuppressive IBD therapies on inflammation and carcinogenesis should be evaluated (Axelrad et al., 2016a).

Although there are several mouse models of IBD the molecular mechanisms of IBD and early events by which inflammation promotes cancer are still not clear (Currey et al., 2019, Shah and Itzkowitz, 2022). The *Winnie* mouse is a model of spontaneous chronic inflammation with immune activation similar to that seen in human IBD (and chapter 2). *Winnie* mice have a missense mutation in the *Muc2* gene, which results in aberrant biosynthesis of mucin, decreased mucin storage in goblet cells, and forms non-glycosylated *Muc2* precursor within goblet cells (Heazlewood et al., 2008). The *Muc2* gene expression is reduced or absent in patients with Crohn's disease, whereas *Muc2* production and secretion are decreased in active ulcerative colitis, resulting in a thin mucus layer, and increased intestinal absorption (Kang et al., 2022). The commonalities between *Winnie* mice and patients with ulcerative colitis include changes in the morphology and motility associated with the colon, transit time as well as microbial and metabolomic profiles of (Dalal and Chang, 2014, Stavely et al., 2018). Determining the gene expression levels of checkpoint markers like *Pd-1*, *Pd-L1*, *Pd-L2*, *Ido*, *Siglece* (ortholog of *SIGLEC 9* in human), *Ctla4*, *Tim3* (*Havcr2*) in the *Winnie* mouse model, as well as in severely inflamed *Winnie* mice (*Winnie-Prolapse* mice) will provide insights into disease initiation and progression. In addition, analysing the genes expressed in these mice using next generation sequencing, may lead to identification of new targets for IBD as well as progression from inflammation to cancer. Further, understanding the role of B7 proteins family of ligands (B7-H3/Cd276, B7-H4/Vtcn1, B7-H5/Vista, B7-H6/Ncr3lg1, B7-H7/Hhla2) may give rise to novel checkpoint inhibitor therapies for IBD and CRC.

Materials and Methods

Mice and tissues

Three groups of mice were used in this study, *Winnie* mice (13-14 weeks, n=3 male and n=4 female), *Winnie* mice with rectal prolapse (*Winnie-Prolapse*) (18-23 weeks, n=4 males and n=3 females) and control C57BL/6 mice (14 weeks, n=4 males and n=4 females). *Winnie* mice, *Winnie-Prolapse* mice and control C57BL/6 mice were obtained from Victoria University animal colony, Werribee Animal Facility (Melbourne, Australia). All mice were kept in a well-ventilated room at 22° C with a 12-hour light and dark cycles and *ad libitum* food and water access. The Victoria University Animal Experimentation Ethics Committee approved all animal experiments, which followed the guidelines of the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes (AEC18-016). All mice faecal samples were collected,

homogenised, and tested for lipocalin-2 (Lcn-2) levels as a measure of inflammation using the NGAL mouse ELISA kit (Abcam, Australia); described in chapter 2. The *Winnie* mice with the most inflammation (n=8; 4 males, 4 females) were chosen for RNA extraction from colon tissues (data in Chapter 2, Figure 1).

RNA extraction

Total RNA was extracted using TRIzol™ (Invitrogen, Australia) and purified using the RNeasy® Mini kit (Qiagen, Hilden, Germany), which included DNase treatment. TissueLyser EXPAND was used to homogenise colon tissues and the total RNA samples' integrity was determined using the Agilent 2100 Bioanalyser's RNA 6000 Nano chip (Agilent Biotechnologies). An RNA Integrity Number of > 8.5 were used for RNAseq analysis. The purity of the RNA was determined using a Denovix DS-11 spectrophotometer to measure the 260/280 and 260/230 ratios, and the concentration of individual RNA samples was quantified using an Invitrogen Qubit RNA BR assay (Gene Target Solutions, Sydney, Australia).

Library Preparation and RNA Sequencing

As described in chapter 2, the RNA-Seq library was constructed, with more than 100 ng of total RNA, including RNA quality control (CE integrity analysis by AATI Fragment Analyser, Fluorimetric quantitation by Invitrogen Qubit). Poly-A mRNA was selected, and MGIEasy stranded mRNA chemistry was performed. All of the samples were quantified and analysed with the Qubit and Bio-analyser, and they all passed the QNA QC standard. Micromon (Monash University, Australia) performed the sequencing on MGITech MGISEQ2000RS hardware (MGISEQ-2000RS High-throughput Sequencing Set). The MGIEasy V2 chemistry set was used to generate the library, with at least 400 m raw reads per lane and paired-end 100b reads for accuracy and processing to compressed fastq files. Files were then delivered digitally.

Data Analysis

Monash Bioinformatics conducted the bioinformatics (Monash University, Australia). The RNAsik pipeline version 1.5.4 (Tsyganov et al., 2018a) and the *Mus musculus* reference, GRCm38 (Genbank accession GCA 000001635.2) were used to analyse raw fastq files. Using featureCounts (Liao et al., 2014a), reads were quantified, yielding the raw gene count matrix and various quality control metrics. Raw counts were then analysed with Degust, a web tool that performs differential expression analysis using limma voom normalisation (Law et al., 2018), producing counts per million library size normalisation and trimmed mean of M values,

normalisation for RNA composition normalisation (Robinson and Oshlack, 2010). Degust also includes a number of quality plots, such as classic multidimensional scaling and MA plots. To analyse differential gene expression based on overrepresentation analysis, DAVID (The Database for Annotation, Visualisation, and Integrated Discovery) software for KEGG (Kyoto Encyclopedia of Genes and Genomes) pathways were used. EdgeR was preferred to obtain the total number of genes from Degust. Due to the large number of genes, an absolute log fold change (abs log fc) of 1 and a false discovery rate (FDR) of 0.05 were selected as the cut-off.

Results and Discussion

The *Winnie* mouse model, a model of spontaneous chronic colitis, has been used to study intestinal alterations, intrinsic and extrinsic innervations of the colon and the interactions with the enteric nervous system. However, the potential of *Winnie* mice to act as a pre-cancerous model, especially following its progression to severe inflammation which results in rectal prolapse (*Winnie-Prolapse* mice), has not been investigated thus far. By analysing the transcriptome of *Winnie* vs *Winnie-Prolapse* and comparing to wild-type C57BL/6 control mice, may provide novel insights in the relationship between inflammation and cancer and whether long-standing inflammation has a higher rate of progressing into cancer. Herein, the presence of checkpoint markers and cancer markers in *Winnie* and *Winnie-Prolapse* mice were determined and compared to age matched C57BL/6 mice for analysis (Figure 1). The presence of these markers will give new insights into new targets for the immunotherapy in chronic colitis/IBD as well their role in the progression to CAC/CRC. The current study compared the gene expression of checkpoint and cancer genes of C57BL/6 control mice, *Winnie* mice and severely inflamed *Winnie-Prolapse* mice.

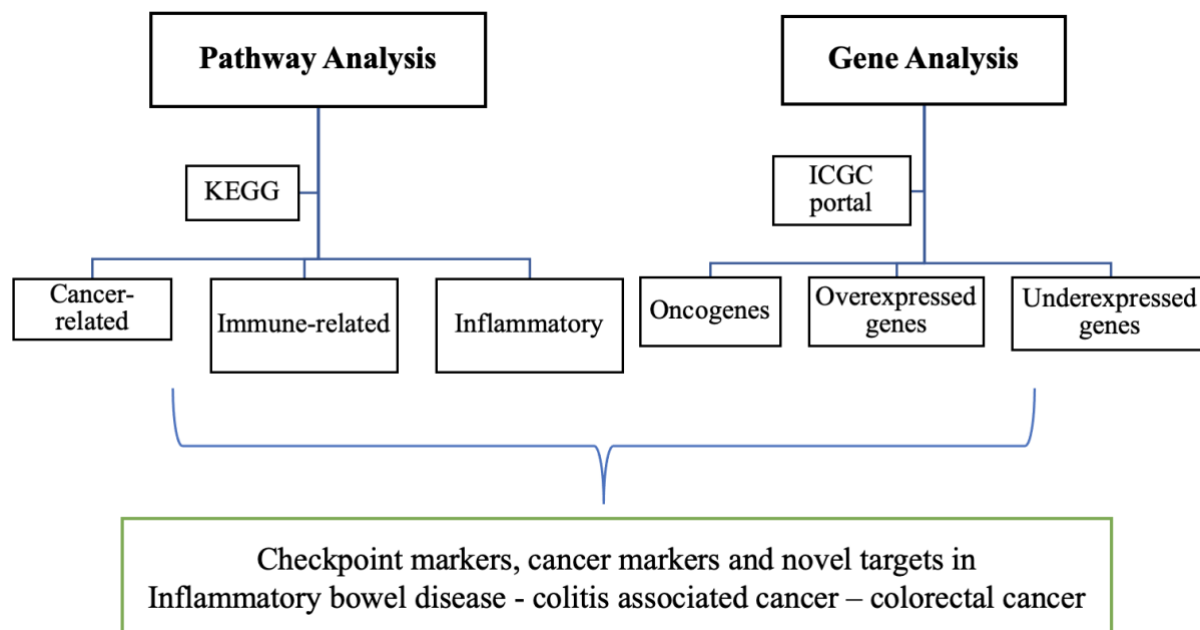


Figure 1. Flowchart – Overview of pathway and gene analysis

Pathway Analysis

Inflammation pathways, cell adhesion molecules, neuroactive-ligand receptor interaction pathways and pathways of viruses are upregulated in Winnie mice, with, metabolic and cancer pathways significantly expressed in Winnie-Prolapse mice: Insights into pathways involved in the progression of inflammation to cancer.

Pathway analysis of the transcriptome showed that C57BL/6 and *Winnie* mice co-expressed genes that were significantly enriched in neuroactive-ligand receptor interaction, cytokine-cytokine receptor interaction and cell adhesion molecules (Figure 2A). C57BL/6 and *Winnie-Prolapse* mice co-expressed genes that were significantly overexpressed in metabolic pathways, pathways in cancer and neuroactive-ligand receptor interaction (Figure 2B). Both *Winnie* and *Winnie-Prolapse* shown overlap in several pathways including, cell adhesion and cytokine-cytokine receptor interaction Figure 2A, B).

Further, neuroactive-ligand receptor interaction pathway is among the highly enriched categories in *Winnie* and *Winnie-Prolapse* mice. Oxidative stress and inflammation both of

which have been shown to be highly present in patients with chronic colitis were also significantly exhibited by *Winnie* mice (Wang et al., 2021c). Oxidative stress is a critical factor in the pathogenesis of CRC, which indicates that the functions and roles of neuroactive ligand-receptor in CRC are worthy of further investigation (Perše, 2013).

It is evident that the transcriptome of the distal colon contains greater number of genes associated with immune and inflammatory related pathways that involves cytokines and chemokines that stimulate cancer cell growth, disrupt differentiation, and aid in cancer cell survival. Thus, it is not only important for biomarker development but also for novel therapeutic targets.

Chronic colitis in *Winnie* mice show increased metabolite production and alterations of several metabolic pathways, primarily affecting amino acid synthesis and the breakdown of monosaccharides to short chain fatty acids (Robinson et al., 2016). This also leads to the fact that the severely inflamed *Winnie-Prolapse* mice were greatly enriched in metabolic pathways. These supply energy and vital metabolites to cancer cells which in turn promote biosynthesis, proliferation, and other important processes of tumorigenesis.

Interestingly, pathways of virus infections of, human T-cell leukemia virus 1, human papilloma virus, Epstein Barr virus, Kaposi sarcoma-associated herpesvirus, and human immunodeficiency virus were highly enriched in both *Winnie* and *Winnie-Prolapse* mice (Figure 2A, B), suggesting chronic inflammation in these mice follow the virus inflammation pathways, all of which are known to be involved in the development of cancer.

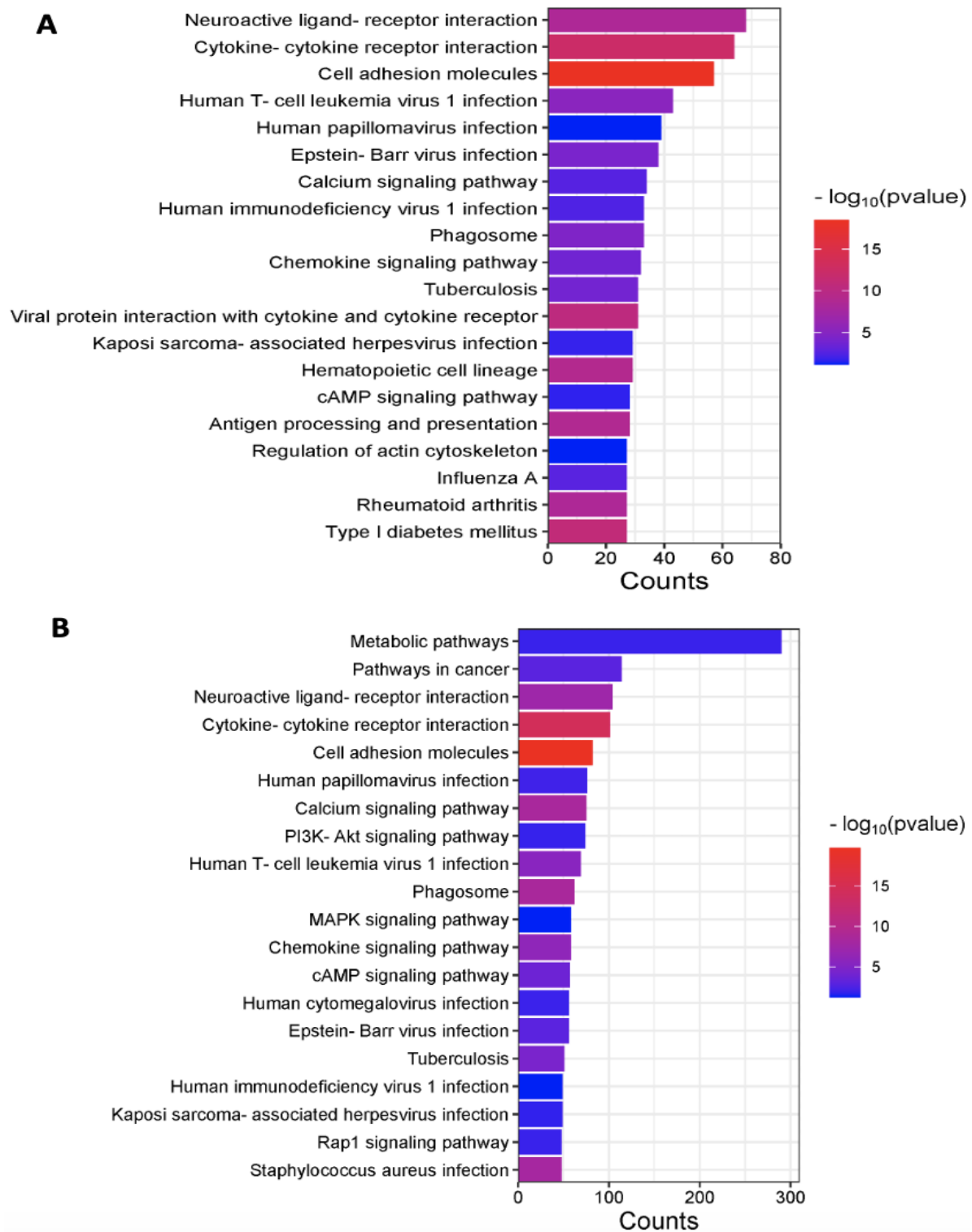


Figure 2. Pathway Analysis – KEGG -Bar chart where the number of genes are shown on the x-axis and the KEGG pathways are represented on the y-axis. **(A)** Top 20 KEGG pathways from genes co-expressed by C57BL/6 and Winnie mice and **(B)** top 20 KEGG pathways from genes co-expressed by C57BL/6 and Winnie-Prolapse mice. The length of the bar represents the number of genes, and the color represents the significance of $\log_{10}$ p value.

The pathways in the *Winnie* mice are predominantly those relating to inflammation and virus pathways. Even though these are also present in *Winnie-Prolapse* mice, the most highly expressed pathways are those of metabolic, and pathways involved in cancer (Figure 3), which align with the hypothesis of severe inflammation leading to cancer.

The cancer related pathways which are expressed by both *Winnie* and *Winnie-Prolapse* mice include *Pd-L1/Pd-1* checkpoint pathways, toll-like receptor pathways, C-type lectin receptor signalling pathways, pro-inflammatory *Il-17/Th17/Th1/Th2/Tnf* signalling pathways, JAK-STAT signalling pathways and cell adhesion molecules (Figure 3A). Of note, pathways which are only expressed in *Winnie-Prolapse* mice but not in *Winnie* mice, are those of chemical carcinogenesis, choline metabolism in cancer, as well as signalling pathways of RAS, Rap1, mitogen-activated protein kinase (MAPK) and PI3K-Akt (Figure 3B). RAS, Rap1 and JAK-STAT pathways have been shown to be the main drivers in the oncogenic circuitry which support cancer cell growth, migration, survival, invasion and metastasis (Gimple and Wang, 2019, Gutierrez-Hoya and Soto-Cruz, 2020, Zhang et al., 2017).

In addition, MAPK pathway is involved in the regulation of genes, uncontrolled cell proliferation, cancer promoting properties and in resistance of cells to apoptosis (Braicu et al., 2019). As such, MAPK inhibitors have been used for the management and treatment of cancer. In fact, MAPK is aberrantly activated in patients with CRC and inhibition of MAPK pathway enhances the cytotoxic effects of chemotherapeutic drugs in CRC cells (Han et al., 2022b). Further, the PI3K-Akt, TGF-beta and Wnt signalling pathways are linked to the development of CRC (Koveitypour et al., 2019).

Winnie mice express genes of inflammation and virus pathways which progresses to pathways of cancer in *Winnie-Prolapse* mice. Identifying and understanding genes in cancer pathways and their progression for inflammation to severe inflammation to cancer, may translate to improved treatments for patients with cancers which rely on these pathways.

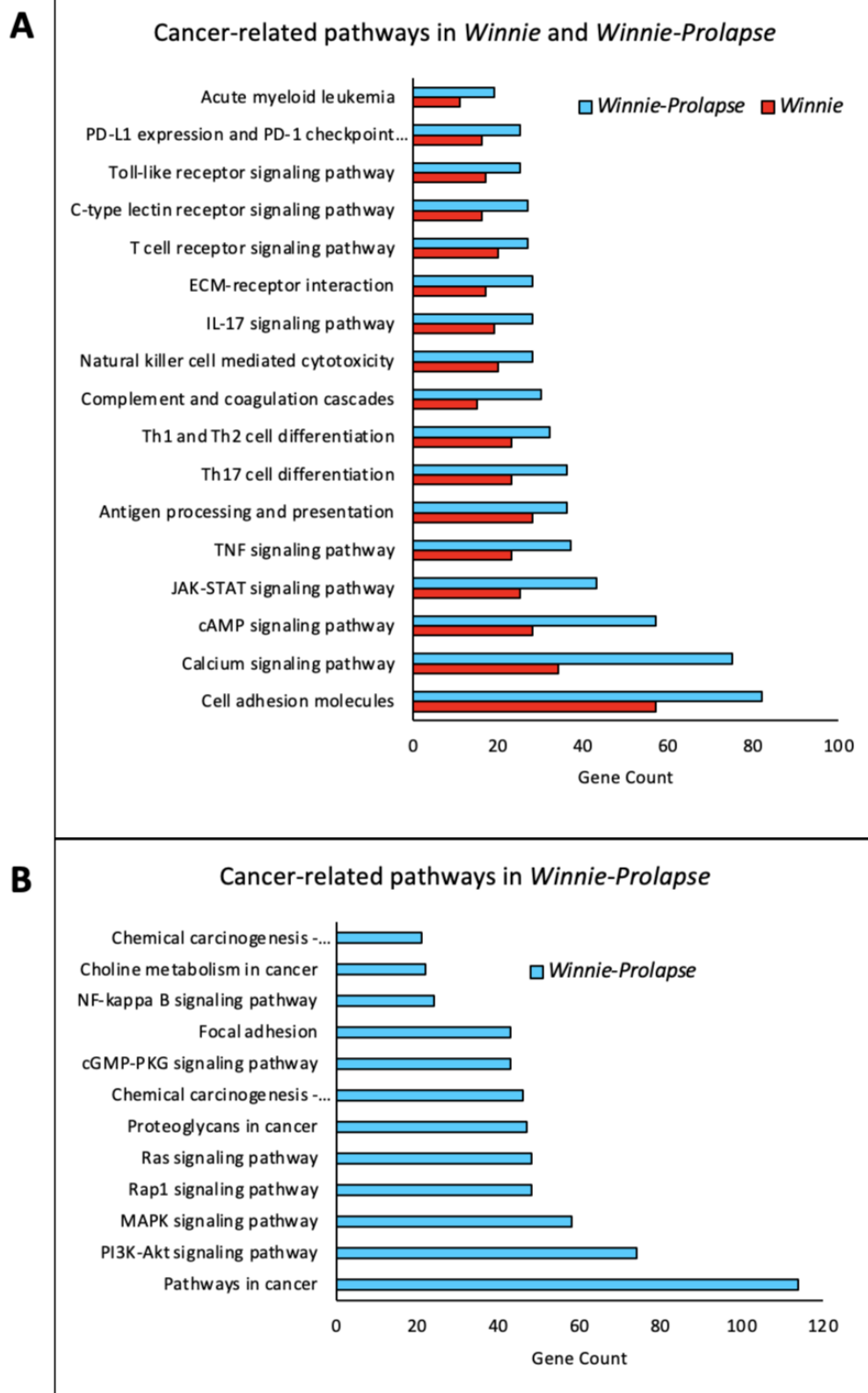
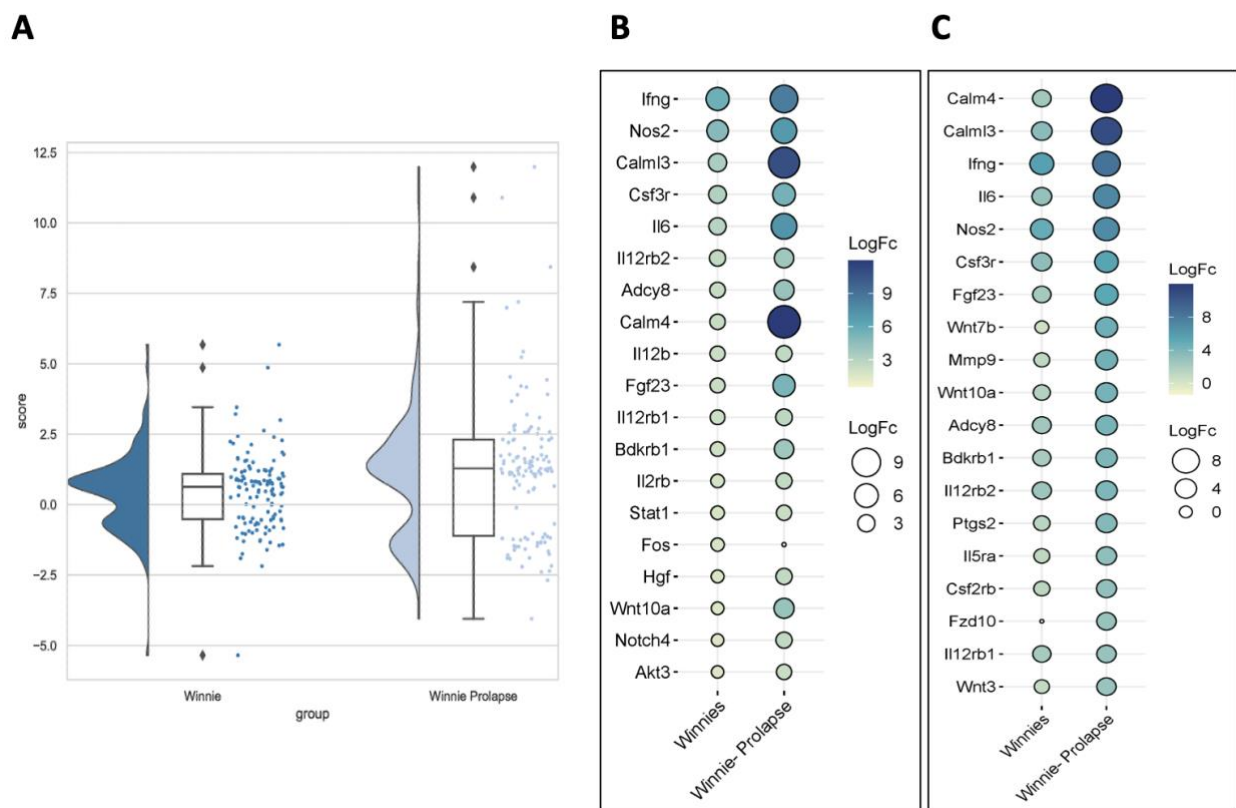


Figure 3. Bar chart where the number of genes are shown on the x-axis and the KEGG pathways are represented on the y-axis. **(A)** Cancer-related KEGG pathways in *Winnie* and *Winnie-Prolapse* mice. **(B)** Cancer-related pathways exhibited only in *Winnie-Prolapse* and absent in *Winnie* mice. The length of the bar represents the number of genes.

The combination of violin plots, box plots and jitter plots are shown in Figure 4A of differentially expressed genes associated with cancer. The most upregulated gene in *Winnie* mice was *Ifn- γ* (Figure 4B). Overexpression of *IFN- γ* has been linked with upregulation of *IDO1*, *CTLA-4*, *TIM3*, *PD1*, *PD-L1*, and *LAG3* (Seidel et al., 2018b). As shown herein these genes are significantly upregulated in *Winnie* mice. In addition, calmodulin-4 (*Calm-4*) (log fc 11.9) was the most upregulated in *Winnie-Prolapse* (Figure 4C). Overexpression of Calmodulin dependent serine kinase (CASK) in conjunction with heparan sulfate proteoglycan syndecan-2 has been linked with the increased metastatic capacity of CRC that affects lymph nodes, increased vascular invasiveness, and liver metastasis (Villalobo and Berchtold, 2020). The most down-regulated gene in *Winnie* mice was NK3 Homeobox 1 (*Nkx3-1*) (log fc -5.3) (Figure 4D), which has been identified as a prostatic tumour suppressor gene and is downregulated in metastatic prostate cancers (Gurel et al., 2010) but its role in CRC is not clear. The most downregulated gene in *Winnie-Prolapse* mice was shown to be Wnt family member 8B (*Wnt8b*) (log fc -4.0) (Figure 4E). Wnt signaling pathway is being investigated as a therapeutic target against CRC (Zhao et al., 2022, Nie et al., 2020).



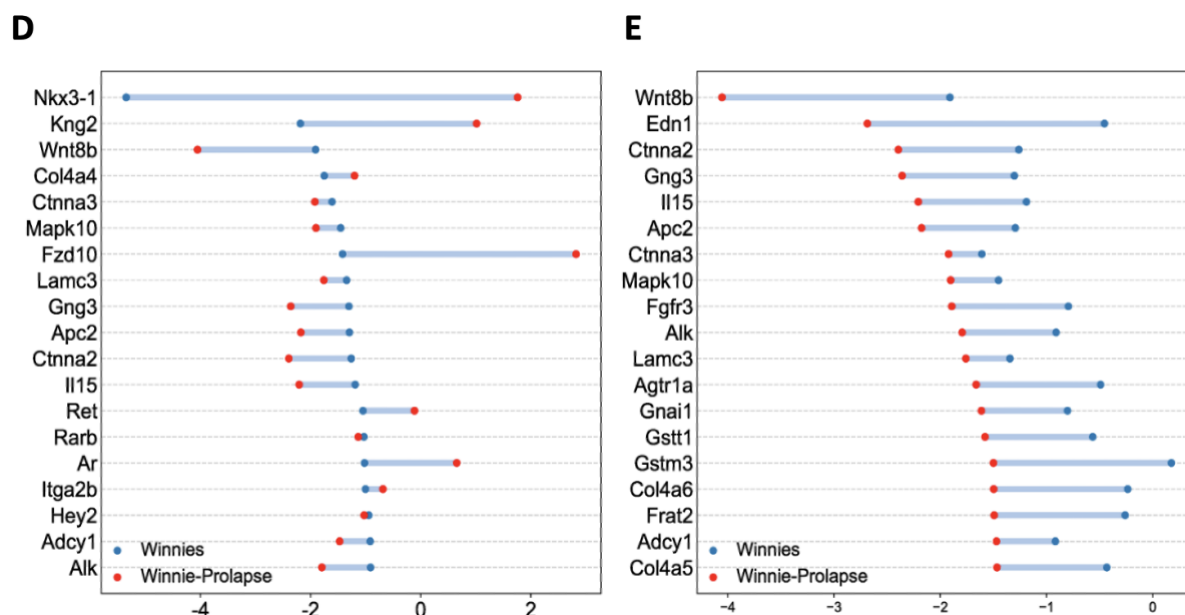


Figure 4. Gene Expression (DEGs in Pathways in Cancer) - Raincloud plot (combination of violin plot, box plot and jitter plot). **(A)** Number of differentially expressed genes associated with pathways in cancer in *Winnie* mice. **(B)** Top 20 upregulated genes associated with pathways in cancer. **(C)** Top 20 upregulated genes associated with pathways in cancer in *Winnie-Prolapse* mice. **(D)** Top 20 downregulated genes associated with pathways in cancer in *Winnie* mice. **(E)** Top 20 downregulated genes associated with pathways in cancer in *Winnie-Prolapse* mice

Pathway analysis shows differential expression of checkpoint markers that are associated with cell adhesion molecules (CAMs), PD-L1 expression and PD-1 pathway in Winnie and Winnie-Prolapse mice.

Intercellular and cell-extracellular matrix interactions in cancer involve a variety of cell adhesion molecules (Janiszewska et al., 2020). A STRING protein-protein interaction network of CAMs and *Pd-L1* expression and *Pd-1* pathway are shown in Figure 5A. Pathway analysis of the transcriptome showed that CAMs are overexpressed in *Winnie* and *Winnie-Prolapse* mice when compared with age-matched C57BL/6 control mice (Figure 5A-C). The most upregulated CAM in *Winnie* as well as *Winnie-Prolapse* is *Ctla-4* (log fc 3.8 in *Winnie* and 4.5 in *Winnie-Prolapse*) (Figure 5D, E). *Ctla-4* is an inhibitory immune checkpoint present on tumour-infiltrating lymphocytes and has been evaluated in several types of cancers. In CRC, *Ctla-4* promotes tumour growth and metastasis (Fu et al., 2020) as well as silencing CRC cell lysate-loaded dendritic cells for immunotherapy (Hirsch and Zafar, 2011, Xie et al., 2020). Inhibition of *Ctla-4* can reactivate T cells in patients with CRC boosting their immune cells against cancer cells (Derakhshani et al., 2021).

Highly expressed genes also included histocompatibility 2 (H2) antigens, *H2-Dmb1*, *H2-Dma*, *H2-Q6*, *H2-Q7*, *H2-Ab1*, *H2-Q10*, *H2-Aa* (Figure 5D, E). T cell immunoreceptor with Ig and ITIM domains (*Tigit*) (log fc 3.2 in *Winnie* and 3.6 in *Winnie-Prolapse*) and *Pd-L1/Cd274* (log fc 2.2 in *Winnie* and 3.2 in *Winnie-Prolapse*) were upregulated in *Winnie* and *Winnie-Prolapse* mice (Figure 5D, E). Based on previous studies, upregulation of *TIGIT* and *PD-1* in CRC with mismatch repair deficiency is linked to tumour nodes metastases (TNM) stage and disease-free survival (Zhou et al., 2021) which makes it a target of interest. The inducible T-cell co-stimulator (*Icos*) belongs to the B7-CD28 immunoglobulin superfamily was shown to be upregulated in *Winnie* (log fc 1.9) and *Winnie-Prolapse* (log fc 2.7) mice (Figure 5D, E). *ICOS* has gained attention due to its expression in several cancers and its expression in CRC is associated with improved survival, and may be an appropriate clinical biomarker for CRC (Zhang et al., 2016).

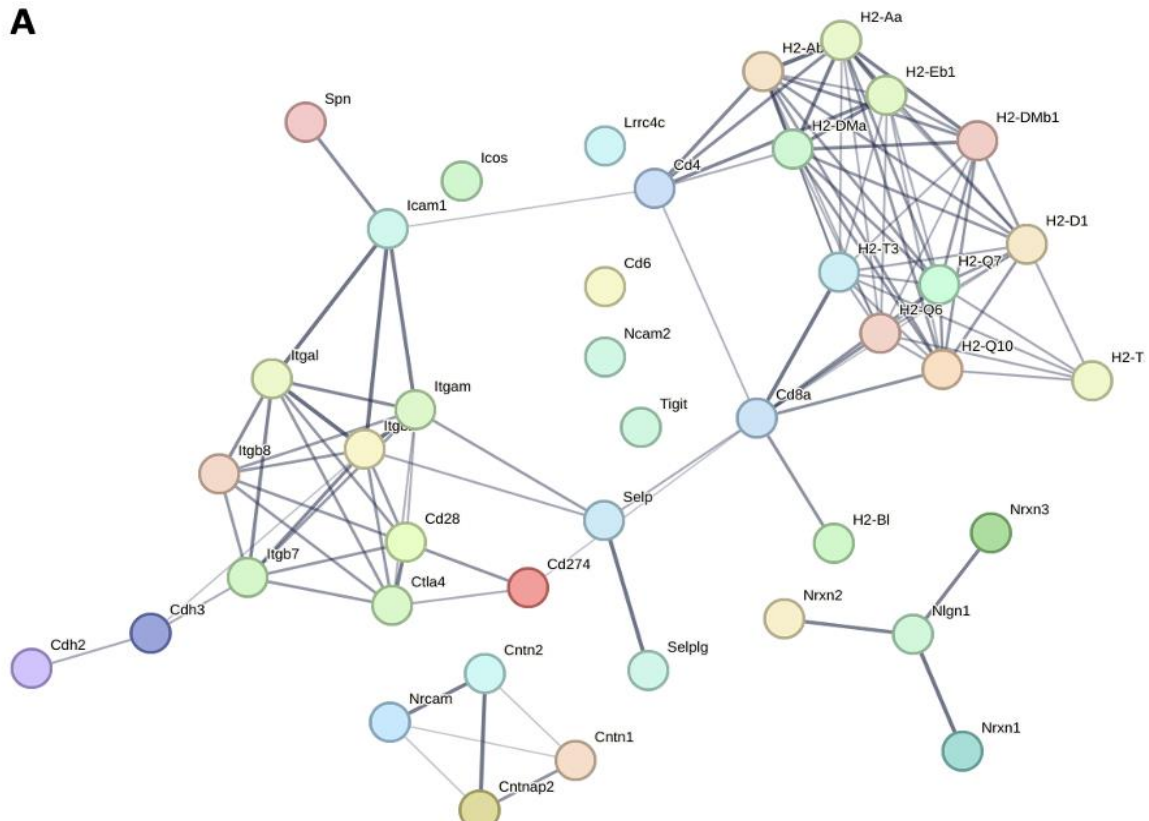
Selp/Cd62 (Selectin P) (log fc 1.8 in *Winnie* and 4.0 in *Winnie-Prolapse*) and *Itga2b* (Integrin alpha 2b) were upregulated in *Winnie* (log fc 1.1) mice and significantly upregulated in *Winnie-Prolapse* (log fc 2.5) mice. *Itga2b* (Sun et al., 2021b) and *Selp* (Korniluk et al., 2017) are cancer-related genes with a high value for cancer detection. In addition, *Selplg/Cd162* (P-selectin glycoprotein ligand-1) and *Itgal* (Integrin Subunit Alpha L) are upregulated in *Winnie* (log fc 1.4 and 1.4 respectively) and significantly overexpressed in *Winnie-Prolapse* mice (log fc 2.5 and 2.5). In CRC, lack of *Selplg* is linked to CRC growth and may be a good diagnostic or prognostic biomarker (Wang and Sun, 2022). *Itgal* plays a key role in cancer development, construction of the TME and in angiogenesis of cancers including CRC (Zhang et al., 2020).

Cd6 is upregulated in *Winnie* and highly overexpressed in *Winnie-Prolapse* mice, which is overexpressed in the inflamed mucosa of patients with IBD and accelerates intestinal mucosal immune responses via promoting CD4+ T cell proliferation and differentiation into Th1/Th17 cells (Ma et al., 2019). *Cd6* ligand is also associated with the aggressiveness and metastatic potential of human cancers, Thus, *Cd6* may serve as a novel therapeutic target for treatment of IBD and cancer immunotherapy in the future (Ruth et al., 2021).

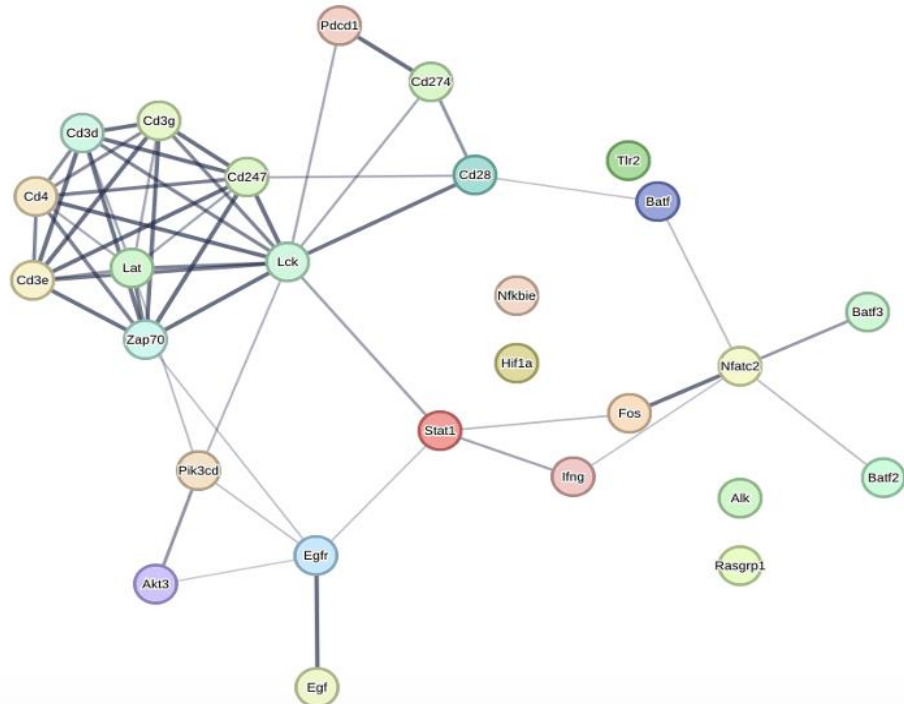
In addition, there were 15 downregulated CAMs (Figure 5D) of which the most downregulated CAM in *Winnie* (log fc -3.0) and *Winnie-Prolapse* (log fc -4.0) mice were histocompatibility 2, T region locus 3 (*H2-T3*), which is mainly involved in protein binding. Cadherin 3 or P-Cadherin (*Cdh3*) are downregulated in *Winnie* (log fc -1.3) but upregulated in *Winnie-Prolapse*

(log fc 1.0). It is abundant in the placenta and is overexpressed in several cancers but the expression levels in CRC are not clear (Kumara et al., 2017).

A



B



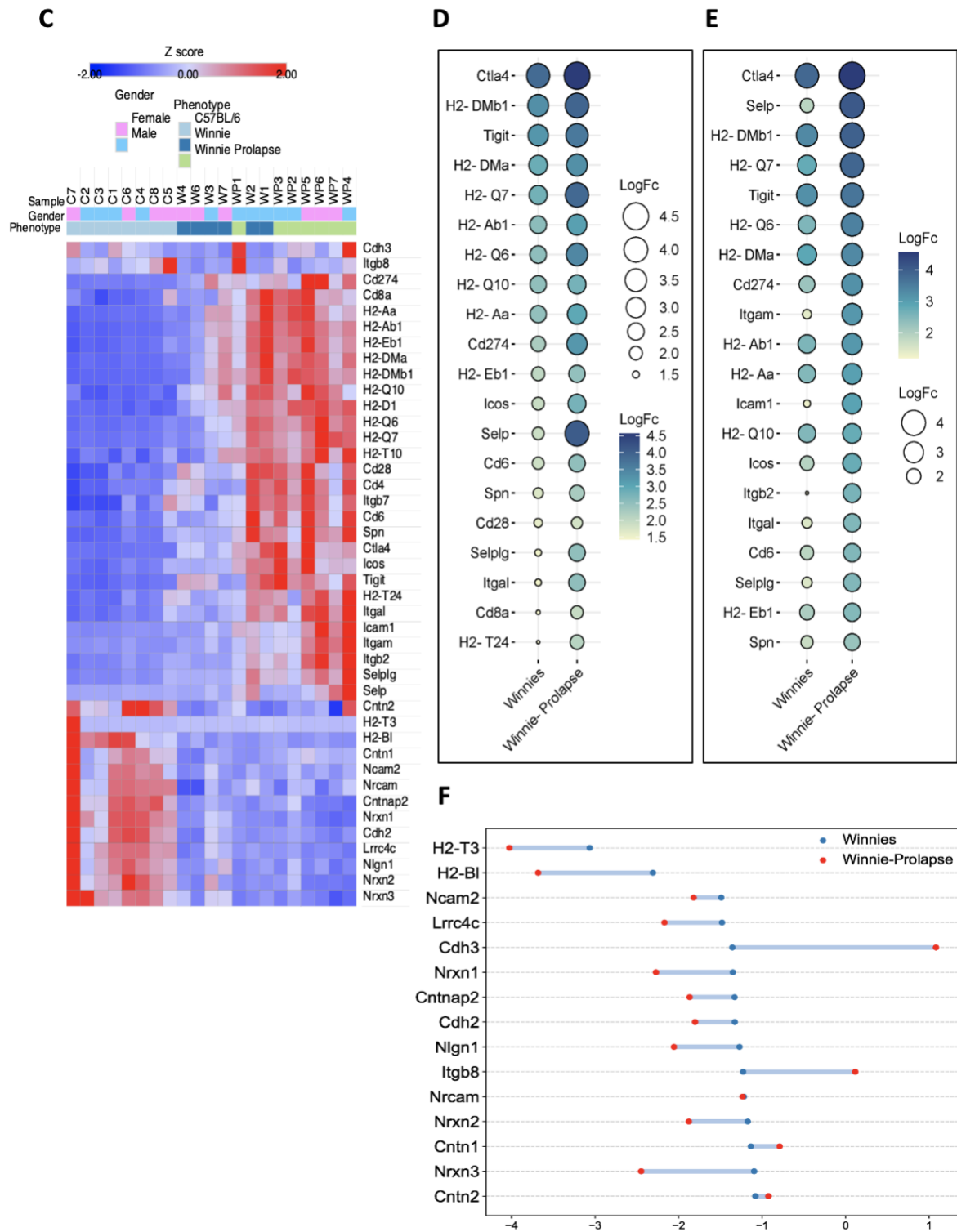


Figure 5. Gene Expression (CAMs). (A, B) STRING network – each circle represents one gene, and the length of the node represents the level of interaction. (A) Protein-protein interaction between genes associated with cell adhesion molecules and, (B) protein-protein interaction between genes associated with PD-L1 expression and PD-1 checkpoint pathways. (C) Heatmap of differential expression of genes associated with cell adhesion molecules across C57BL/6, Winnie and Winnie-Prolapse mice based on the Z score, hierarchically clustered based on Euclidean distance. Top 20 upregulated genes associated with cell adhesion molecules in (D) Winnie and (E) Winnie-Prolapse mice. (F) Downregulation of genes associated with cell adhesion molecules in Winnie and Winnie-Prolapse. C57BL/6 mice (C1-C8), Winnie mice (W1-W7) and Winnie-Prolapse (WP1-7).

The role of *Pd-1*, *Pd-L1*, *Ctla-4*, *Ido1*, *Tim3* and *Lag3* (Figure 6) and their upregulation have been studied extensively in various types of cancers including CRC in humans. These markers were shown to be upregulated in both *Winnie* and *Winnie-Prolapse* mice compared to C57BL/6 mice. In addition, high expression of *Cd45* (also known as *Ptprc*) (Figure 6) was noted in *Winnie* and *Winnie-Prolapse* mice. Increased *Cd45* expression in CRC cells in pre-treated primary cancers is responsible for poor regression and recurrence-free survival of patients (Park et al., 2021). Further, primary tumour volume and secondary metastases is reduced by targeting *Cd276* (Liu et al., 2021). The expression of *Cd276* was also noted to be upregulated in *Winnie* and *Winnie-Prolapse* mice compared to controls. Moreover, *Cd177* was previously shown to be highly expressed in CRC/CAC (Zhou et al., 2017) and was also shown to be highly upregulated in both *Winnie* (log fc 3.1) and *Winnie-Prolapse* (log fc 3.5) mice; certainly a target of interest in the treatment and prognosis of inflammation, IBD and cancer.

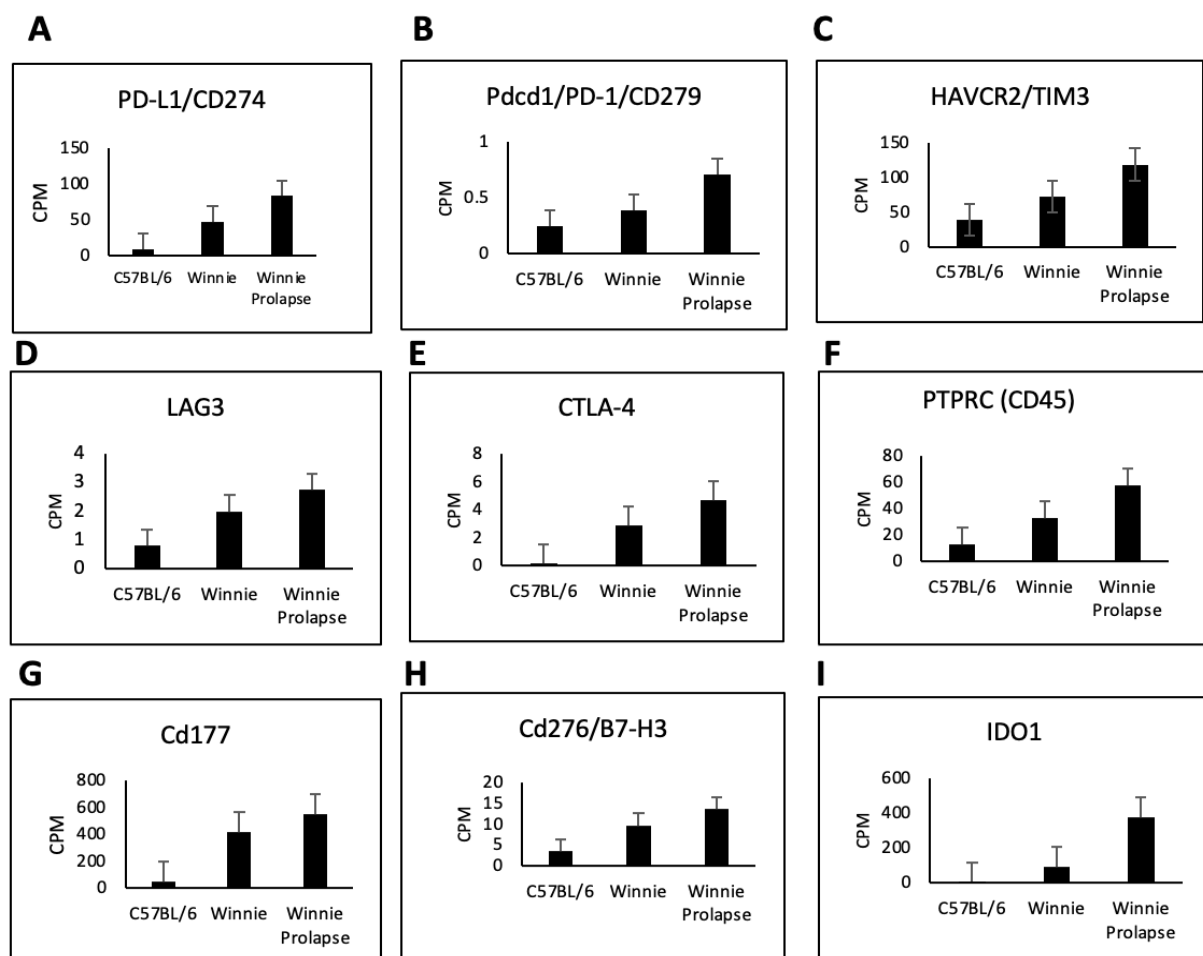


Figure 6. (Expression of checkpoint markers) Bar graphs showing differential gene expression in counts per million (CPM; y-axis) for checkpoint markers. Expression is shown for C57BL/6, *Winnie* and *Winnie-Prolapse* mice where checkpoint markers are enhanced in *Winnie*'s and even further enhanced in *Winnie-Prolapse*. Bars represent mean $\pm$ standard deviation.

Transcriptome of Winnie-Prolapse mice are enriched in cancer-related pathways that are absent in Winnie mice.

As noted in Figure 3, pathway analysis of *Winnie-Prolapse* and C57BL/6 mice showed great enrichment in cancer-related pathways especially, PI3K-AKT signalling pathway, MAPK signalling pathway, RAP1 signalling pathway, RAS signalling pathway, and NF- κ B signalling pathway. In particular, RAS protein operates in two main cellular pathways - the mitogen-activated protein kinases (MAPK) and phosphoinositide-3 kinase (PI3K) pathways (Zenonos and Kyprianou, 2013). These are vital in controlling various processes in a normal cell, including cell growth and survival. Activation of PI3K-Akt signalling pathway has been noted in 60–70% of patients with CRC (Hoxhaj and Manning, 2020), and as such, inhibitors of route components could be used as therapeutic drugs (Hayden and Ghosh, 2014). The PI3K-AKT signalling pathway regulates the cell cycle and involved in quiescence, proliferation, and development of cancer. Within the PI3-AKT pathway genes which are highly expressed in *Winnie-Prolapse* mice include *Ccnd1*, *Col1a1*, *Hsp90b1*, *Itga6*, *Lamc2* (Figure 7A). Genes involved in Ras signalling are shown in Figure 7B and those involved in NF- κ B signalling pathway are depicted in Figure 7C.

The Ras/Raf/MEK/ERK cascade is involved in the control of growth signals, cell survival, and invasion in cancer (Dillon et al., 2021). Proteoglycans in cancer are situated in the glycocalyx of cancer cells, proteoglycans provide a contact link between the cell membrane and the surrounding extracellular matrix, thereby playing a central role in regulating cancer cell adhesion and migration (Wei et al., 2020). The nuclear factor-kappa B (NF- κ B) signalling pathway has been linked to carcinogenesis as a regulator of immune response and inflammation (Liu et al., 2017b). NF- κ B may contribute to the progression of CRC (Patel et al., 2018). Therapeutic stimulation of cGMP/PKG pathways provide a promising avenue for CRC (Browning et al., 2010) prevention and treatment, however, further preclinical data are required to fully understand their role. Rap1 signalling regulates integrin or cadherin-mediated cell adhesion, protease levels (e.g., matrix metalloproteinase), and cytoskeletal alterations, all of which are linked to cancer cell proliferation, invasion, and metastasis (Zhang et al., 2017). Active Rap1 inhibits tumour invasion in several cancers, implying that Rap1 signalling has cancer-specific pleiotropic effects (Looi et al., 2020). Targeting Rap1 signalling and its

controllers may control carcinogenesis, metastasis, chemoresistance, and immunological evasion (Li et al., 2021a).

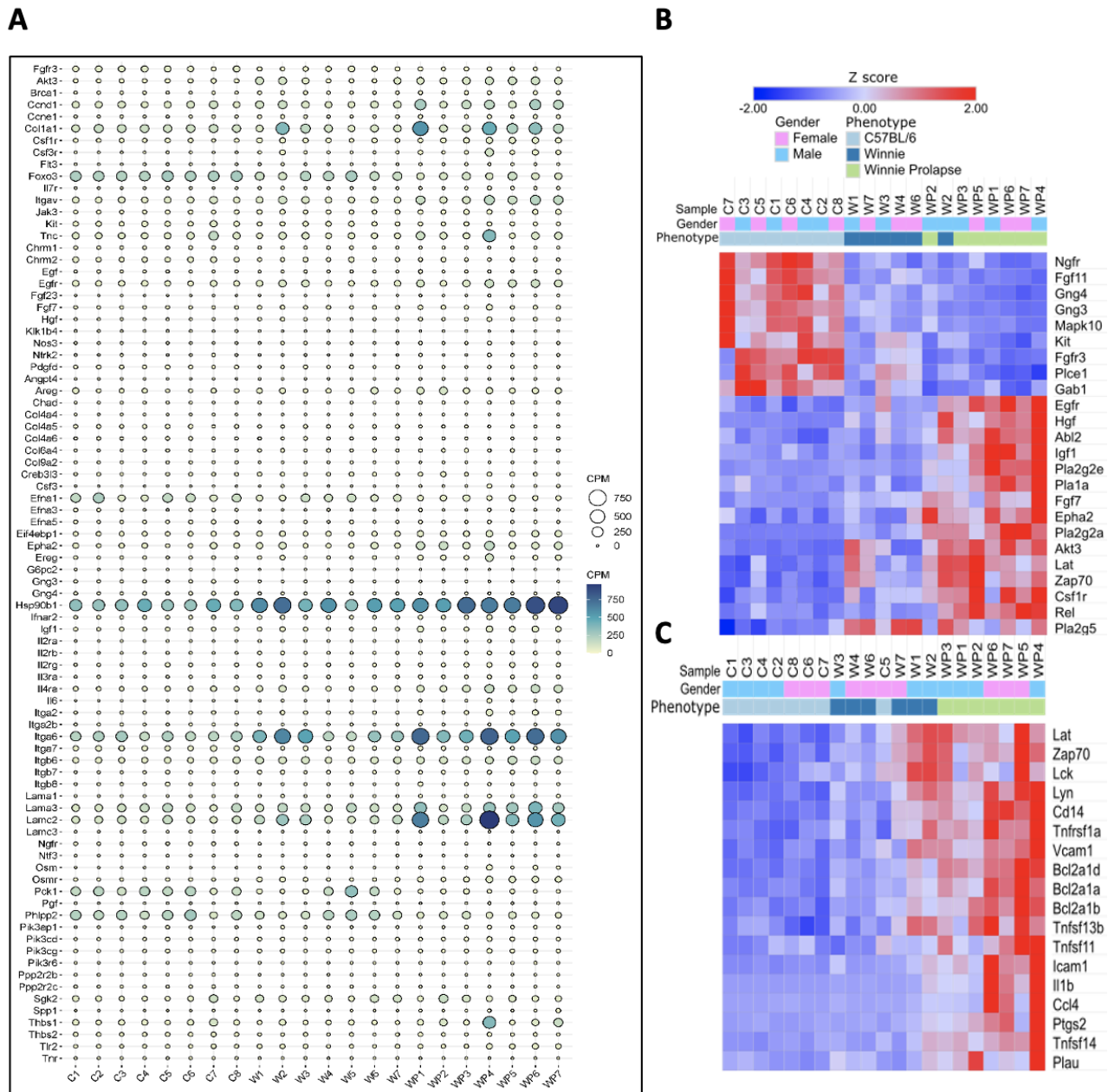


Figure 7. Gene Expression (A) Differential gene expression associated with PI3-AKT signaling pathway, size of the bubble represents counts per million (CPM). (B) Heatmap of genes involved in Ras signaling pathway and (C) heatmap of genes involved in NF-kB signaling pathway. C57BL/6 mice (C1-C8), Winnie mice (W1-W7) and Winnie-Prolapse (WP1-7).

Gene Analysis

Transcriptome of Winnie and Winnie-Prolapse mice are highly expressed in cancer genes (oncogenes)

Genes from C57BL/6, *Winnie* and *Winnie-Prolapse* mouse colon tissues were analysed on The International Cancer Genome Consortium Data Portal (ICGC Data Portal) (<http://www.icgc.org/>). There were 115 genes from our data that matched the cancer gene database. Out of which, the DEGs from C57BL/6 and *Winnie* mice showed 50 (32 upregulated and 18 downregulated) similar genes (Figure 8A) and 106 DEGs (75 upregulated and 31 downregulated) were found to be similar in C57BL/6 and *Winnie-Prolapse* (Figure 8B). The list of top 10 up- and down-regulated cancer genes in *Winnie* and *Winnie-Prolapse* mice are shown in Figures 8C-F.

Colony-stimulating factor 3 receptor (*Csf3r*) was the most upregulated gene in *Winnie* (log fc 3.2) and *Winnie-Prolapse* mice (log fc 5.4). (Figure 8C, D). *CSF3* and *CSF3R* are highly expressed in CRC which are important regulators which promote pro-tumour behaviour in cancer and immune cells in CRC. *Csf3r* correlates with many genes linked to poor prognosis in CRC (Saunders et al., 2021). When correlations of *CSF3* and *CSF3R* expression with patient demographics, tumour stage, and consensus molecular subtype were examined from the dataset obtained from The Cancer Genome Atlas Firehose Legacy, it was noted that *CSF3* and *CSF3R* expression is highest in consensus molecular subtype-1 and consensus molecular subtype 4. In addition, the intestine-specific homeobox (*Isx*) (log fc -3.5) (Figure 8E) gene was found to be highly downregulated in *Winnie* mice which correlates to previous findings that *Isx* is downregulated in colon adenomas (Pan et al., 2021). Insulin-like growth factor 2 mRNA-binding protein 2 (*Igf2bp2*) was the most downregulated in *Winnie-Prolapse* (log fc -3.4) (Figure 8F) and is known to promote CRC progression (Cui et al., 2021) and its dysregulation is associated with progression of cancers and cancer stem cells (Wang et al., 2021a). Several cancer genes show significant differential expression between *Winnie* and *Winnie-Prolapse* (Figure 8G) including Myeloid leukemia factor 1 (*Mlf1*) (log fc 0.06 in *Winnie* and 3.9 in *Winnie-Prolapse*) and GATA binding protein 3 (*Gata3*) (log fc 0.6 in *Winnie* and 3.9 in *Winnie-Prolapse*), but their roles and function in CRC are unclear.

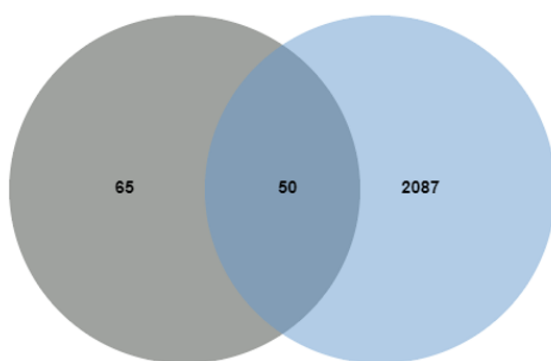
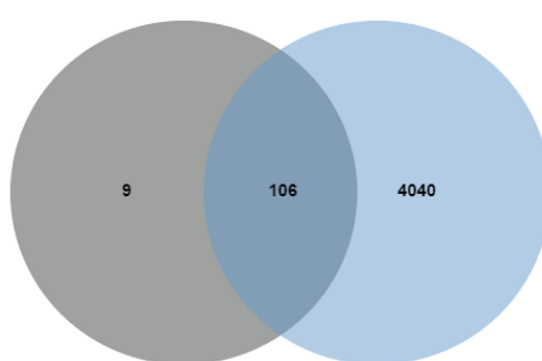
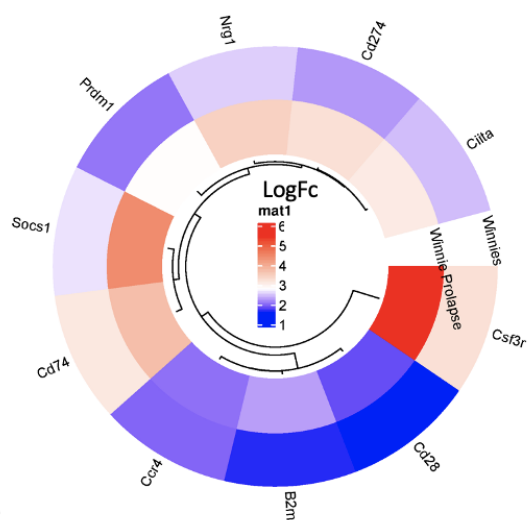
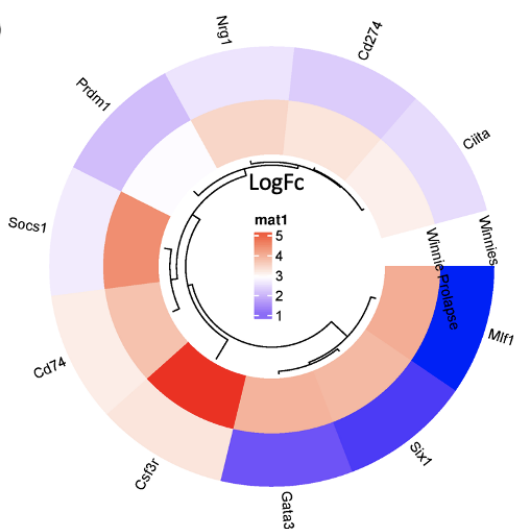
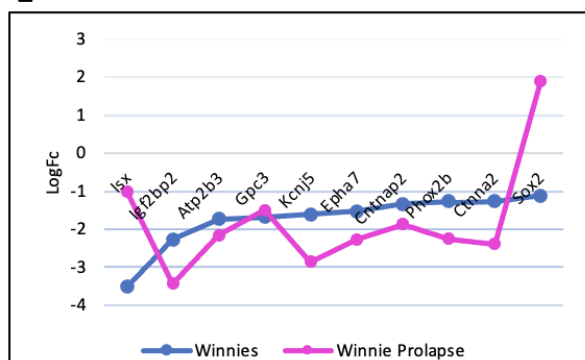
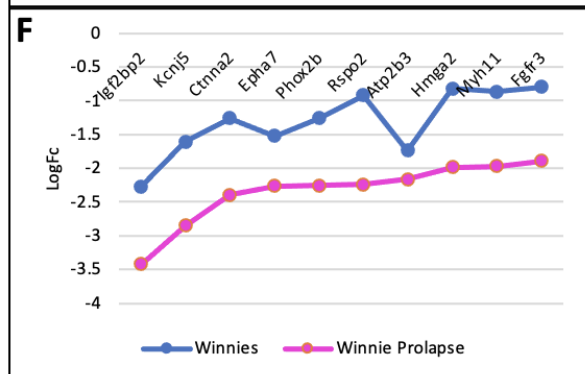
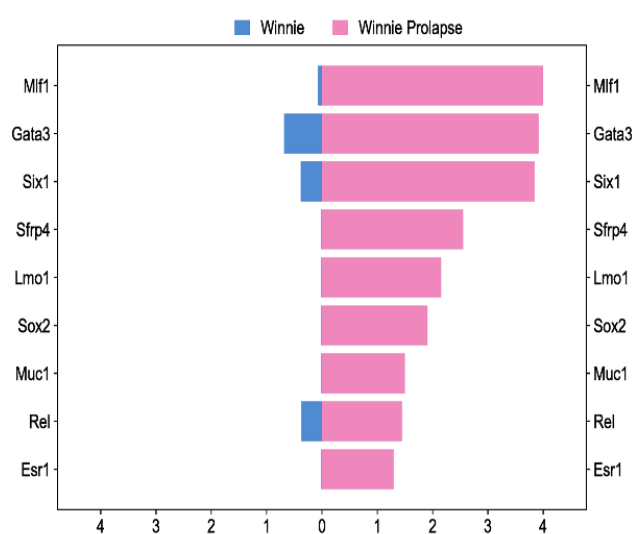
A**B****C****D****E****F****G**

Figure 7. Cancer Genes Analysis - Venn diagram of similar genes between the Cancer genes from ICGC portal (Cancer gene consortium) and **(A)** C57BL/6/*Winnie* mice, or **(B)** C57BL/6/*Winnie-Prolapse* mice. **(C)** Top 10 upregulated cancer genes in *Winnie* mice, **(D)** top 10 upregulated cancer genes in *Winnie-Prolapse* mice, **(E)** top 10 downregulated cancer genes in *Winnie* mice and **(F)** top 10 downregulated genes in *Winnie-Prolapse* mice. **(G)** Significant gene expression in *Winnie-Prolapse* when compared to *Winnie* mice

Other genes linked to CAC and CRC

Other genes overexpressed in the transcriptome of *Winnie* (log fc 7.7) and *Winnie-Prolapse* (log fc 8.7) mice include, Chloride channel accessory 4 (*CLCA4*) a human ortholog of *Clca4b*; its overexpression and function is to inhibit migration and invasion by suppressing the epithelial-to-mesenchymal transition (EMT) via PI3K/ATK signalling and predicts favourable prognosis of CRC (Chen et al., 2019). Previous studies have shown improved overall survival in patients with various types of cancer, including CRC by decreasing the expression of *CLCA4*. Transporter associated with antigen processing 1 (*Tap1*) was significantly overexpressed in both *Winnie* (log fc 2.7) and *Winnie-Prolapse* (log fc 3.3) mice, associated with antigen processing and presentation pathways. Reduced *Tap1* expression is linked to poor prognosis in patients with CRC and are linked to immune checkpoint genes (Ling et al., 2017), DNA methylation, tumour mutation burden, microsatellite instability, and neoantigens. *Tap1* could be a potential therapeutic target as well as a biomarker for early detection of cancer (Li et al., 2021b) (Ling et al., 2017).

Conclusion

The overall findings in this current analysis have shown overexpression of cell adhesion molecules and pathways in cancer especially in *Winnie-Prolapse* mice when compared to *Winnie* mice. The presence and overexpression of oncogenes and cancer-related pathways that were highly enriched in *Winnie-Prolapse* mice compared to *Winnie* mice, indicates that inflammation may be linked to cancer or pre-cancerous stages, leading to the hypothesis that chronic and progressive inflammation may lead to cancer. Even though the area of checkpoint markers has been a hot-topic in the last 5-years, their functions in IBD and inflammation that leads to CRC are still not clear and require further studies. Given the similarity between the transcriptome of human IBD patients and spontaneous models of IBD i.e., *Winnie* mice and the severely inflamed model of *Winnie-Prolapse*, it is worthwhile studying these checkpoint

markers further in order to understand their role in inflammation and their relationship to progression to cancer.

Although the clinical development of immune checkpoint inhibitors therapy has ushered in a new era of anti-cancer therapy, with sustained responses and significant survival advantages observed in multiple cancers, most patients do not benefit. Therefore, more attention has been paid to the identification and development of predictive biomarkers, as early as inflammation to severe inflammation to development of cancer (Bai et al., 2020). Chemotherapy and checkpoint inhibitor immunotherapies are increasingly used in combinations (Nikanjam et al., 2020). Immune checkpoint blockade is able to induce durable responses across multiple types of cancers, however, the remarkable responses to immunotherapies are currently limited to a minority of patients and indications, highlighting the need for more effective and novel approaches (Wei et al., 2018a). Overall, this study identified genes associated with colitis (inflammation) and progression to prolapse (severe inflammation), with several enriched genes associated with cancer pathways were shown in particular in *Winnie-Prolapse* mice.

Limitations and future directions:

The regulation of the TME based on multiple omics results can suggest innovative therapeutic strategies to prevent cancer cells from immune escape and to support anti-cancer effects. Bioinformatics approaches will also need to continue to develop, to improve our understanding of a responsive TME. It is well established that the TME affects immune checkpoint inhibitor responses. Predictive and prognostic biomarkers can be determined using cutting-edge technology, which can also be used to determine the immunological background of malignancies. Though there has been significant research and information on checkpoint markers, their roles in IBD and development of CRC / inflammation associated cancer, remains unclear and requires further study. Studying checkpoint markers in the *Winnie* mouse models that closely resemble human models may lead to the discovery of biomarkers for screening IBD patients and for the advancement of understanding inflammation and cancer that may lead to designing drugs that use inhibitors of biomarker expression or use vaccines to prevent disease progression. This includes patients at high risk for disease relapse who may benefit from earlier treatments. Although the clinical development of immune checkpoint inhibitors therapy has ushered in a new era of anti-cancer therapy, with sustained responses and significant survival advantages observed in multiple cancers, most patients do not benefit.

References

- AGARWAL, M. B. 2016. Is cancer chemotherapy dying? *Asian journal of transfusion science*, 10, S1-S7.
- AGUILAR-CAZARES, D., CHAVEZ-DOMINGUEZ, R., CARLOS-REYES, A., LOPEZ-CAMARILLO, C., HERNANDEZ DE LA CRUZ, O. N. & LOPEZ-GONZALEZ, J. S. 2019. Contribution of Angiogenesis to Inflammation and Cancer. *Frontiers in oncology*, 9, 1399-1399.
- AHLUWALIA, B., MORAES, L., MAGNUSSON, M. K. & ÖHMAN, L. 2018. Immunopathogenesis of inflammatory bowel disease and mechanisms of biological therapies. *Scandinavian Journal of Gastroenterology*, 53, 379-389.
- AL-SADI, R., YOUSSEF, M., RAWAT, M., GUO, S., DOKLADNY, K., HAQUE, M., WATTERSON, M. D. & MA, T. Y. 2019. MMP-9-induced increase in intestinal epithelial tight permeability is mediated by p38 kinase signaling pathway activation of MLCK gene. *American journal of physiology. Gastrointestinal and liver physiology*, 316, G278-G290.
- AMOS, S. E. & CHOI, Y. S. 2021. The Cancer Microenvironment: Mechanical Challenges of the Metastatic Cascade. *Frontiers in Bioengineering and Biotechnology*, 9.
- ANDERSON, A. C. 2014. Tim-3: An Emerging Target in the Cancer Immunotherapy Landscape. *Cancer Immunology Research*, 2, 393-398.
- ANDERSON, N. M. & SIMON, M. C. 2020. The tumor microenvironment. *Curr Biol*, 30, R921-r925.
- ANDRADE, A. W. L., GUERRA, G. C. B., DE SOUZA ARAÚJO, D. F., DE ARAÚJO JÚNIOR, R. F., DE ARAÚJO, A. A., DE CARVALHO, T. G., FERNANDES, J. M., DIEZ-ECHAVE, P., HIDALGO-GARCÍA, L., RODRIGUEZ-CABEZAS, M. E., GÁLVEZ, J. & ZUCOLOTTI, S. M. 2020. Anti-Inflammatory and Chemopreventive Effects of Bryophyllum pinnatum (Lamarck) Leaf Extract in Experimental Colitis Models in Rodents. *Front Pharmacol*, 11, 998.
- ANDREWS, L. P., MARCISCANO, A. E., DRAKE, C. G. & VIGNALI, D. A. 2017. LAG3 (CD223) as a cancer immunotherapy target. *Immunol Rev*, 276, 80-96.
- ANDREYEV, H. J. N., VLAVIANOS, P., BLAKE, P., DEARNALEY, D., NORMAN, A. R. & TAIT, D. 2005. Gastrointestinal symptoms after pelvic radiotherapy: Role for the gastroenterologist? *International Journal of Radiation Oncology*Biophysics*, 62, 1464-1471.
- APOSTOLOPOULOS, V. 2019. Cancer Vaccines: Research and Applications. *Cancers (Basel)*, 11.
- APOSTOLOPOULOS, V., LOFTHOUSE, S. A., POPOVSKI, V., CHELVANAYAGAM, G., SANDRIN, M. S. & MCKENZIE, I. F. 1998. Peptide mimics of a tumor antigen induce functional cytotoxic T cells. *Nat Biotechnol*, 16, 276-80.
- APOSTOLOPOULOS, V. & MCKENZIE, I. F. 1994. Cellular mucins: targets for immunotherapy. *Crit Rev Immunol*, 14, 293-309.
- APOSTOLOPOULOS, V., MCKENZIE, I. F. & PIETERSZ, G. A. 1996. Breast cancer immunotherapy: current status and future prospects. *Immunol Cell Biol*, 74, 457-64.
- APOSTOLOPOULOS, V. & MCKENZIE, I. F. C. 2017. Cellular Mucins: Targets for Immunotherapy. *Crit Rev Immunol*, 37, 421-437.
- ARAKI, A., KANAI, T., ISHIKURA, T., MAKITA, S., URAUSHIHARA, K., IIYAMA, R., TOTSUKA, T., TAKEDA, K., AKIRA, S. & WATANABE, M. 2005. MyD88-deficient mice develop severe intestinal inflammation in dextran sodium sulfate colitis. *J Gastroenterol*, 40, 16-23.
- ARGOLLO, M., FIORINO, G., HINDRYCKX, P., PEYRIN-BIROULET, L. & DANESE, S. 2017. Novel therapeutic targets for inflammatory bowel disease. *Journal of autoimmunity*, 85, 103-116.
- ATREYA, I., ATREYA, R. & NEURATH, M. F. 2008. NF-kappaB in inflammatory bowel disease. *J Intern Med*, 263, 591-6.

- AXELRAD, J. E., LICHTIGER, S. & YAJNIK, V. 2016a. Inflammatory bowel disease and cancer: The role of inflammation, immunosuppression, and cancer treatment. *World J Gastroenterol*, 22, 4794-801.
- AXELRAD, J. E., LICHTIGER, S. & YAJNIK, V. 2016b. Inflammatory bowel disease and cancer: The role of inflammation, immunosuppression, and cancer treatment. *World journal of gastroenterology*, 22, 4794-4801.
- BAGHBAN, R., ROSHANGAR, L., JAHANBAN-ESFAHLAN, R., SEIDI, K., EBRAHIMI-KALAN, A., JAYMAND, M., KOLAHIAN, S., JAVAHERI, T. & ZARE, P. 2020. Tumor microenvironment complexity and therapeutic implications at a glance. *Cell Communication and Signaling*, 18, 59.
- BAI, R., LV, Z., XU, D. & CUI, J. 2020. Predictive biomarkers for cancer immunotherapy with immune checkpoint inhibitors. *Biomarker Research*, 8, 34.
- BARKAL, A. A., BREWER, R. E., MARKOVIC, M., KOWARSKY, M., BARKAL, S. A., ZARO, B. W., KRISHNAN, V., HATAKEYAMA, J., DORIGO, O., BARKAL, L. J. & WEISSMAN, I. L. 2019. CD24 signalling through macrophage Siglec-10 is a target for cancer immunotherapy. *Nature*, 572, 392-396.
- BARKIN, J. A., SUSSMAN, D. A. & ABREU, M. T. 2012. Chromoendoscopy and advanced imaging technologies for surveillance of patients with IBD. *Gastroenterology & hepatology*, 8, 796.
- BARNESTEIN, R., GALLAND, L., KALFEIST, L., GHIRINGHELLI, F., LADOIRE, S. & LIMAGNE, E. 2022. Immunosuppressive tumor microenvironment modulation by chemotherapies and targeted therapies to enhance immunotherapy effectiveness. *Oncoimmunology*, 11, 2120676.
- BARRIGA, V., KUOL, N., NURGALI, K. & APOSTOLOPOULOS, V. 2019. The Complex Interaction between the Tumor Micro-Environment and Immune Checkpoints in Breast Cancer. *Cancers (Basel)*, 11.
- BASSOTTI, G., ANTONELLI, E., VILLANACCI, V., BALDONI, M. & DORE, M. P. 2014. Colonic motility in ulcerative colitis. *United European Gastroenterol J*, 2, 457-62.
- BAUMGART, D. C. & CARDING, S. R. 2007. Inflammatory bowel disease: cause and immunobiology. *The Lancet*, 369, 1627-1640.
- BEATTY, G. L. & GLADNEY, W. L. 2015. Immune escape mechanisms as a guide for cancer immunotherapy. *Clin Cancer Res*, 21, 687-92.
- BERGSTROM, K. S. B., KISSOON-SINGH, V., GIBSON, D. L., MA, C., MONTERO, M., SHAM, H. P., RYZ, N., HUANG, T., VELCICH, A., FINLAY, B. B., CHADEE, K. & VALLANCE, B. A. 2010. Muc2 Protects against Lethal Infectious Colitis by Disassociating Pathogenic and Commensal Bacteria from the Colonic Mucosa. *PLOS Pathogens*, 6, e1000902.
- BERNARDAZZI, C., PÊGO, B. & DE SOUZA, H. S. 2016. Neuroimmunomodulation in the Gut: Focus on Inflammatory Bowel Disease. *Mediators Inflamm*, 2016, 1363818.
- BHINDER, G., STAHL, M., SHAM, H. P., CROWLEY, S. M., MORAMPUDI, V., DALWADI, U., MA, C., JACOBSON, K. & VALLANCE, B. A. 2014. Intestinal epithelium-specific MyD88 signaling impacts host susceptibility to infectious colitis by promoting protective goblet cell and antimicrobial responses. *Infect Immun*, 82, 3753-63.
- BIASI, F., LEONARDUZZI, G., OTEIZA, P. I. & POLI, G. 2013. Inflammatory bowel disease: mechanisms, redox considerations, and therapeutic targets. *Antioxidants & redox signaling*, 19, 1711-1747.
- BISCHOFF, S. C., BARBARA, G., BUURMAN, W., OCKHUIZEN, T., SCHULZKE, J.-D., SERINO, M., TILG, H., WATSON, A. & WELLS, J. M. 2014. Intestinal permeability--a new target for disease prevention and therapy. *BMC gastroenterology*, 14, 189-189.
- BORST, J., AHREND, T., BAŁA, N., MELIEF, C. J. M. & KASTENMÜLLER, W. 2018. CD4+ T cell help in cancer immunology and immunotherapy. *Nature Reviews Immunology*.
- BORTOLOTTI, D., GENTILI, V., ROTOLA, A., CASSAI, E., RIZZO, R. & DI LUCA, D. 2014. Impact of HLA-G analysis in prevention, diagnosis and treatment of pathological conditions. *World J Methodol*, 4, 11-25.
- BRAICU, C., BUSE, M., BUSUIOC, C., DRULA, R., GULEI, D., RADULY, L., RUSU, A., IRIMIE, A., ATANASOV, A. G., SLABY, O., IONESCU, C. & BERINDAN-NEAGOE, I.

2019. A Comprehensive Review on MAPK: A Promising Therapeutic Target in Cancer. *Cancers (Basel)*, 11.
- BROWNING, D. D., KWON, I. K. & WANG, R. 2010. cGMP-dependent protein kinases as potential targets for colon cancer prevention and treatment. *Future Med Chem*, 2, 65-80.
- BUCHBINDER, E. I. & DESAI, A. 2016. CTLA-4 and PD-1 Pathways: Similarities, Differences, and Implications of Their Inhibition. *Am J Clin Oncol*, 39, 98-106.
- CAI, Z., WANG, S. & LI, J. 2021. Treatment of Inflammatory Bowel Disease: A Comprehensive Review. *Front Med (Lausanne)*, 8, 765474.
- CAMILLERI, M. 2011. Managing symptoms of irritable bowel syndrome in patients with inflammatory bowel disease. *Gut*, 60, 425-428.
- CANSEVER, M., GÖKTAŞ, M. A., ARSLAN, D. & PATIROĞLU, T. 2022. Serum levels of soluble HLA-G correlate with disease activity in pediatric patients with Crohn's disease. *Saudi J Gastroenterol*, 28, 233-238.
- CAO, Y., CAO, T., ZHAO, W., HE, F., LU, Y., ZHANG, G., HU, H. & WANG, Z. 2018. Expression of B7-H2 on CD8(+) T cells in colorectal cancer microenvironment and its clinical significance. *Int Immunopharmacol*, 56, 128-134.
- CARTY, E. & RAMPTON, D. S. 2003. Evaluation of new therapies for inflammatory bowel disease. *British journal of clinical pharmacology*, 56, 351-361.
- CARUSO, R., LO, B. C. & NUNEZ, G. 2020. Host-microbiota interactions in inflammatory bowel disease. *Nat Rev Immunol*, 20, 411-426.
- CEBON, J. 2018. Perspective: cancer vaccines in the era of immune checkpoint blockade. *Mammalian genome : official journal of the International Mammalian Genome Society*, 29, 703-713.
- CHAPMAN, C. G. & PEKOW, J. 2015. The emerging role of miRNAs in inflammatory bowel disease: a review. *Therapeutic advances in gastroenterology*, 8, 4-22.
- CHEN, H., LIU, Y., JIANG, C. J., CHEN, Y. M., LI, H. & LIU, Q. A. 2019. Calcium-Activated Chloride Channel A4 (CLCA4) Plays Inhibitory Roles in Invasion and Migration Through Suppressing Epithelial-Mesenchymal Transition via PI3K/AKT Signaling in Colorectal Cancer. *Med Sci Monit*, 25, 4176-4185.
- CHEN, L., DENG, H., CUI, H., FANG, J., ZUO, Z., DENG, J., LI, Y., WANG, X. & ZHAO, L. 2017. Inflammatory responses and inflammation-associated diseases in organs. *Oncotarget*, 9, 7204-7218.
- CHEN, L., DENG, H., CUI, H., FANG, J., ZUO, Z., DENG, J., LI, Y., WANG, X. & ZHAO, L. 2018. Inflammatory responses and inflammation-associated diseases in organs. *Oncotarget*, 9, 7204-7218.
- CHEN, Z., YU, M., GUO, L., ZHANG, B., LIU, S., ZHANG, W., ZHOU, B., YAN, J., MA, Q., YANG, Z., XIAO, Y., XU, Y., LI, H. & YE, Q. 2020. Tumor Derived SIGLEC Family Genes May Play Roles in Tumor Genesis, Progression, and Immune Microenvironment Regulation. *Front Oncol*, 10, 586820.
- CHENG, D., ZOU, W., LI, X., TAN, H., YANG, X. & SHI, H. 2014. Early diagnosis of colorectal cancer by CD11b+ myeloid-derived suppressor cells imaging. *Journal of Nuclear Medicine*, 55, 1397-1397.
- CHOI, H., HO, M., ADENIJI, O. S., GIRON, L., BORDOLOI, D., KULKARNI, A. J., PUCHALT, A. P., ABDEL-MOHSEN, M. & MUTHUMANI, K. 2021. Development of Siglec-9 Blocking Antibody to Enhance Anti-Tumor Immunity. *Front Oncol*, 11, 778989.
- CHOW, M. T. & LUSTER, A. D. 2014. Chemokines in cancer. *Cancer immunology research*, 2, 1125-1131.
- COSKUN, M., OLSEN, J., SEIDELIN, J. B. & NIELSEN, O. H. 2011. MAP kinases in inflammatory bowel disease. *Clin Chim Acta*, 412, 513-20.
- COUSSENS, L. M. & WERB, Z. 2002. Inflammation and cancer. *Nature*, 420, 860-867.
- CUI, J., TIAN, J., WANG, W., HE, T., LI, X., GU, C., WANG, L., WU, J. & SHANG, A. 2021. IGF2BP2 promotes the progression of colorectal cancer through a YAP-dependent mechanism. *Cancer Sci*, 112, 4087-4099.
- CURREY, N., JAHAN, Z., CALDON, C. E., TRAN, P. N., BENTHANI, F., DE LACAVALERIE, P., RODEN, D. L., GLOSS, B. S., CAMPOS, C., BEAN, E. G., BULLMAN, A., REIBE-

- PAL, S., DINGER, M. E., FEBBRAIO, M. A., CLARKE, S. J., DAHLSTROM, J. E. & KOHONEN-CORISH, M. R. J. Mouse Model of Mutated in Colorectal Cancer Gene Deletion Reveals Novel Pathways in Inflammation and Cancer. *Cellular and Molecular Gastroenterology and Hepatology*.
- CURREY, N., JAHAN, Z., CALDON, C. E., TRAN, P. N., BENTHANI, F., DE LACAVALERIE, P., RODEN, D. L., GLOSS, B. S., CAMPOS, C., BEAN, E. G., BULLMAN, A., REIBEPAL, S., DINGER, M. E., FEBBRAIO, M. A., CLARKE, S. J., DAHLSTROM, J. E. & KOHONEN-CORISH, M. R. J. 2019. Mouse Model of Mutated in Colorectal Cancer Gene Deletion Reveals Novel Pathways in Inflammation and Cancer. *Cellular and molecular gastroenterology and hepatology*, 7, 819-839.
- DALAL, S. & KWON, J. 2010. The Role of MicroRNA in Inflammatory Bowel Disease. *Gastroenterology & hepatology*, 6, 714-22.
- DALAL, S. R. & CHANG, E. B. 2014. The microbial basis of inflammatory bowel diseases. *The Journal of Clinical Investigation*, 124, 4190-4196.
- DAS, I., PNG, C. W., OANCEA, I., HASNAIN, S. Z., LOURIE, R., PROCTOR, M., ERI, R. D., SHENG, Y., CRANE, D. I., FLORIN, T. H. & MCGUCKIN, M. A. 2013. Glucocorticoids alleviate intestinal ER stress by enhancing protein folding and degradation of misfolded proteins. *J Exp Med*, 210, 1201-16.
- DAS, M., ZHU, C. & KUCHROO, V. K. 2017. Tim-3 and its role in regulating anti-tumor immunity. *Immunol Rev*, 276, 97-111.
- DAVE, M., PAPADAKIS, K. A. & FAUBION, W. A., JR. 2014. Immunology of inflammatory bowel disease and molecular targets for biologics. *Gastroenterology clinics of North America*, 43, 405-424.
- DAVIDSON, M., RASHIDI, N., NURGALI, K. & APOSTOLOPOULOS, V. 2022. The Role of Tryptophan Metabolites in Neuropsychiatric Disorders. *Int J Mol Sci*, 23.
- DEGUINE, J. & BARTON, G. M. 2014. MyD88: a central player in innate immune signaling. *F1000prime reports*, 6, 97-97.
- DELPUTTE, P. L., VAN GORP, H., FAVOREEL, H. W., HOEBEKE, I., DELRUE, I., DEWERCHIN, H., VERDONCK, F., VERHASSELT, B., COX, E. & NAUWYNCK, H. J. 2011. Porcine sialoadhesin (CD169/Siglec-1) is an endocytic receptor that allows targeted delivery of toxins and antigens to macrophages. *PLoS One*, 6, e16827.
- DERAKHSHANI, A., HASHEMZADEH, S., ASADZADEH, Z., SHADBAD, M. A., RASIBONAB, F., SAFARPOUR, H., JAFARLOU, V., SOLIMANDO, A. G., RACANELLI, V., SINGH, P. K., NAJAFI, S., JAVADRASHID, D., BRUNETTI, O., SILVESTRIS, N. & BARADARAN, B. 2021. Cytotoxic T-Lymphocyte Antigen-4 in Colorectal Cancer: Another Therapeutic Side of Capecitabine. *Cancers (Basel)*, 13.
- DESAI, S. J., PRICKRIL, B. & RASOOLY, A. 2018. Mechanisms of Phytonutrient Modulation of Cyclooxygenase-2 (COX-2) and Inflammation Related to Cancer. *Nutrition and Cancer*, 70, 350-375.
- DESHPANDE, R. P., SHARMA, S. & WATABE, K. 2020. The Confounders of Cancer Immunotherapy: Roles of Lifestyle, Metabolic Disorders and Sociological Factors. *Cancers*, 12, 2983.
- DIENSTMANN, R., VERMEULEN, L., GUINNEY, J., KOPETZ, S., TEJPAR, S. & TABERNERO, J. 2017. Consensus molecular subtypes and the evolution of precision medicine in colorectal cancer. *Nat Rev Cancer*, 17, 79-92.
- DILLON, M., LOPEZ, A., LIN, E., SALES, D., PERETS, R. & JAIN, P. 2021. Progress on Ras/MAPK Signaling Research and Targeting in Blood and Solid Cancers. *Cancers (Basel)*, 13.
- DOBIN, A., DAVIS, C. A., SCHLESINGER, F., DRENKOW, J., ZALESKI, C., JHA, S., BATUT, P., CHAISSON, M. & GINGERAS, T. R. J. B. 2013. STAR: ultrafast universal RNA-seq aligner. 29, 15-21.
- DOTAN, I., ALLEZ, M., DANESE, S., KEIR, M., TOLE, S. & MCBRIDE, J. 2020. The role of integrins in the pathogenesis of inflammatory bowel disease: Approved and investigational anti-integrin therapies. *Med Res Rev*, 40, 245-262.

- DUDHGAONKAR, S. P., TANDAN, S. K., KUMAR, D., RAVIPRAKASH, V. & KATARIA, M. 2007. Influence of simultaneous inhibition of cyclooxygenase-2 and inducible nitric oxide synthase in experimental colitis in rats. *Inflammopharmacology*, 15, 188-95.
- EASTWOOD, D., FINDLAY, L., POOLE, S., BIRD, C., WADHWA, M., MOORE, M., BURNS, C., THORPE, R. & STEBBINGS, R. 2010. Monoclonal antibody TGN1412 trial failure explained by species differences in CD28 expression on CD4⁺ effector memory T-cells. *Br J Pharmacol*, 161, 512-26.
- EISSA, N., KERMARREC, L., HUSSEIN, H., BERNSTEIN, C. N. & GHIA, J. E. 2017. Appropriateness of reference genes for normalizing messenger RNA in mouse 2,4-dinitrobenzene sulfonic acid (DNBS)-induced colitis using quantitative real time PCR. *Sci Rep*, 7, 42427.
- EL MIEDANY, Y., YOUSSEF, S., AHMED, I. & EL GAAFARY, M. 2006. The gastrointestinal safety and effect on disease activity of etoricoxib, a selective cox-2 inhibitor in inflammatory bowel diseases. *Am J Gastroenterol*, 101, 311-7.
- EPHRAIM, R., FEEHAN, J., FRASER, S., NURGALI, K. & APOSTOLOPOULOS, V. 2022a. Cancer Immunotherapy: The Checkpoint between Chronic Colitis and Colorectal Cancer. *Cancers (Basel)*, 14.
- EPHRAIM, R., FRASER, S., NURGALI, K. & APOSTOLOPOULOS, V. 2022b. Checkpoint Markers and Tumor Microenvironment: What Do We Know? *Cancers (Basel)*, 14.
- ERI, R. D., ADAMS, R. J., TRAN, T. V., TONG, H., DAS, I., ROCHE, D. K., OANCEA, I., PNG, C. W., JEFFERY, P. L., RADFORD-SMITH, G. L., COOK, M. C., FLORIN, T. H. & MCGUCKIN, M. A. 2011. An intestinal epithelial defect conferring ER stress results in inflammation involving both innate and adaptive immunity. *Mucosal Immunol*, 4, 354-64.
- ERICKSON-MILLER, C. L., FREEMAN, S. D., HOPSON, C. B., D'ALESSIO, K. J., FISCHER, E. I., KIKLY, K. K., ABRAHAMSON, J. A., HOLMES, S. D. & KING, A. G. 2003. Characterization of Siglec-5 (CD170) expression and functional activity of anti-Siglec-5 antibodies on human phagocytes. *Exp Hematol*, 31, 382-8.
- FARKONA, S., DIAMANDIS, E. P. & BLASUTIG, I. M. 2016. Cancer immunotherapy: the beginning of the end of cancer? *BMC medicine*, 14, 73-73.
- FILIPPONE, R. T., DARGAHI, N., ERI, R., URANGA, J. A., BORNSTEIN, J. C., APOSTOLOPOULOS, V. & NURGALI, K. 2022a. Potent CCR3 Receptor Antagonist, SB328437, Suppresses Colonic Eosinophil Chemotaxis and Inflammation in the Winnie Murine Model of Spontaneous Chronic Colitis. *International Journal of Molecular Sciences*, 23, 7780.
- FILIPPONE, R. T., DARGAHI, N., ERI, R., URANGA, J. A., BORNSTEIN, J. C., APOSTOLOPOULOS, V. & NURGALI, K. 2022b. Potent CCR3 Receptor Antagonist, SB328437, Suppresses Colonic Eosinophil Chemotaxis and Inflammation in the Winnie Murine Model of Spontaneous Chronic Colitis. *Int J Mol Sci*, 23.
- FILIPPONE, R. T., ROBINSON, A. M., JOVANOVSKA, V., STAVELY, R., APOSTOLOPOULOS, V., BORNSTEIN, J. C. & NURGALI, K. 2018. Targeting eotaxin-1 and CCR3 receptor alleviates enteric neuropathy and colonic dysfunction in TNBS-induced colitis in guinea pigs. *Neurogastroenterol Motil*, 30, e13391.
- FILIPPONE, R. T., SAHAKIAN, L., APOSTOLOPOULOS, V. & NURGALI, K. 2019. Eosinophils in Inflammatory Bowel Disease. *Inflamm Bowel Dis*, 25, 1140-1151.
- FISHER, K. & LIN, J. 2015. MicroRNA in inflammatory bowel disease: Translational research and clinical implication. *World journal of gastroenterology*, 21, 12274-12282.
- FRANCESCONI, R., HOU, V. & GRIVENNIKOV, S. I. 2015. Cytokines, IBD, and Colitis-associated Cancer. *Inflammatory Bowel Diseases*, 21, 409-418.
- FRASCHILLA, I. & PILLAI, S. 2017. Viewing Siglecs through the lens of tumor immunology. *Immunol Rev*, 276, 178-191.
- FRIEDRICH, M., POHIN, M., JACKSON, M. A., KORSUNSKY, I., BULLERS, S. J., RUE-ALBRECHT, K., CHRISTOFORIDOU, Z., SATHANANTHAN, D., THOMAS, T., RAVINDRAN, R., TANDON, R., PERES, R. S., SHARPE, H., WEI, K., WATTS, G. F. M., MANN, E. H., GEREMIA, A., ATTAR, M., BARONE, F., BRENNER, M., BUCKLEY, C. D., COLES, M., FREI, A. P., LASSEN, K. G., POWRIE, F. M., MCCUAIG, S., THOMAS,

- L., COLLANTES, E., UHLIG, H. H., SANSOM, S. N., EASTON, A., RAYCHAUDHURI, S., TRAVIS, S. P., POWRIE, F. M., OXFORD, I. B. D. C. I. & ROCHE FIBROBLAST NETWORK, C. 2021. IL-1-driven stromal–neutrophil interactions define a subset of patients with inflammatory bowel disease that does not respond to therapies. *Nature Medicine*, 27, 1970-1981.
- FU, X., LUO, H., ZHENG, Y., WANG, S., ZHONG, Z., WANG, Y. & YANG, Y. 2020. CTLA-4 immunotherapy exposes differences in immune response along with different tumor progression in colorectal cancer. *Aging (Albany NY)*, 12, 15656-15669.
- GANESH, K., STADLER, Z. K., CERCEK, A., MENDELSON, R. B., SHIA, J., SEGAL, N. H. & DIAZ, L. A. 2019. Immunotherapy in colorectal cancer: rationale, challenges and potential. *Nature Reviews Gastroenterology & Hepatology*, 16, 361-375.
- GAO, Y., BAI, D., ZHAO, Y., ZHU, Q., ZHOU, Y., LI, Z. & LU, N. 2019. LL202 ameliorates colitis against oxidative stress of macrophage by activation of the Nrf2/HO-1 pathway. *Journal of Cellular Physiology*, 234, 10625-10639.
- GELFO, V., ROMANIELLO, D., MAZZESCHI, M., SGARZI, M., GRILLI, G., MORSELLI, A., MANZAN, B., RIHAWI, K. & LAURIOLA, M. 2020. Roles of IL-1 in Cancer: From Tumor Progression to Resistance to Targeted Therapies. *Int J Mol Sci*, 21.
- GIMPLE, R. C. & WANG, X. 2019. RAS: Striking at the Core of the Oncogenic Circuitry. *Front Oncol*, 9, 965.
- GOVERSE, G., STAKENBORG, M. & MATTEOLI, G. 2016. The intestinal cholinergic anti-inflammatory pathway. *J Physiol*, 594, 5771-5780.
- GRAETER, S., SIMON, H.-U. & VON GUNTEN, S. 2019. Granulocyte death mediated by specific antibodies in intravenous immunoglobulin (IVIG). *Pharmacological Research*.
- GRAYDON, C. G., MOHIDEEN, S. & FOWKE, K. R. 2021. LAG3's Enigmatic Mechanism of Action. *Frontiers in Immunology*, 11.
- GRETEN, F. R. & GRIVENNIKOV, S. I. 2019. Inflammation and Cancer: Triggers, Mechanisms, and Consequences. *Immunity*, 51, 27-41.
- GRIVENNIKOV, S. I. 2013. Inflammation and colorectal cancer: colitis-associated neoplasia. *Seminars in immunopathology*, 35, 229-244.
- GUN, S. Y., LEE, S. W. L., SIEOW, J. L. & WONG, S. C. 2019. Targeting immune cells for cancer therapy. *Redox biology*, 25, 101174-101174.
- GUREL, B., ALI, T. Z., MONTGOMERY, E. A., BEGUM, S., HICKS, J., GOGGINS, M., EBERHART, C. G., CLARK, D. P., BIEBERICH, C. J., EPSTEIN, J. I. & DE MARZO, A. M. 2010. NKX3.1 as a marker of prostatic origin in metastatic tumors. *Am J Surg Pathol*, 34, 1097-105.
- GUTIERREZ-HOYA, A. & SOTO-CRUZ, I. 2020. Role of the JAK/STAT Pathway in Cervical Cancer: Its Relationship with HPV E6/E7 Oncoproteins. *Cells*, 9.
- HAN, X., WEI, Q., LV, Y., WENG, L., HUANG, H., WEI, Q., LI, M., MAO, Y., HUA, D., CAI, X., CAO, M. & CAO, P. 2022a. Ginseng-derived nanoparticles potentiate immune checkpoint antibody efficacy by reprogramming the cold tumor microenvironment. *Molecular Therapy*, 30, 327-340.
- HAN, Z., MENG, L., HUANG, X., TAN, J., LIU, W., CHEN, W., ZOU, Y., CAI, Y., HUANG, S., CHEN, A., ZHAN, T., HUANG, M., CHEN, X., TIAN, X. & ZHU, Q. 2022b. Inhibition of p38 MAPK increases the sensitivity of 5-fluorouracil-resistant SW480 human colon cancer cells to noscapine. *Oncol Lett*, 23, 52.
- HANAHAN, D. & WEINBERG, ROBERT A. 2011. Hallmarks of Cancer: The Next Generation. *Cell*, 144, 646-674.
- HAYDEN, M. S. & GHOSH, S. 2014. Regulation of NF- κ B by TNF family cytokines. *Seminars in immunology*, 26, 253-266.
- HE, Y., CAO, J., ZHAO, C., LI, X., ZHOU, C. & HIRSCH, F. R. 2018. TIM-3, a promising target for cancer immunotherapy. *Onco Targets Ther*, 11, 7005-7009.
- HEAZLEWOOD, C. K., COOK, M. C., ERI, R., PRICE, G. R., TAURO, S. B., TAUPIN, D., THORNTON, D. J., PNG, C. W., CROCKFORD, T. L., CORNALL, R. J., ADAMS, R., KATO, M., NELMS, K. A., HONG, N. A., FLORIN, T. H., GOODNOW, C. C. &

- MCGUCKIN, M. A. 2008. Aberrant mucin assembly in mice causes endoplasmic reticulum stress and spontaneous inflammation resembling ulcerative colitis. *PLoS Med*, 5, e54.
- HEDRICK, T. L. & FRIEL, C. M. 2013. Colonic crohn disease. *Clin Colon Rectal Surg*, 26, 84-9.
- HENDRICKSON, B. A., GOKHALE, R. & CHO, J. H. 2002. Clinical aspects and pathophysiology of inflammatory bowel disease. *Clinical microbiology reviews*, 15, 79-94.
- HERNÁNDEZ-CASELLES, T., MARTÍNEZ-ESPARZA, M., PÉREZ-OLIVA, A. B., QUINTANILLA-CECONI, A. M., GARCÍA-ALONSO, A., ALVAREZ-LÓPEZ, D. M. R. & GARCÍA-PENARRUBIA, P. 2006. A study of CD33 (SIGLEC-3) antigen expression and function on activated human T and NK cells: two isoforms of CD33 are generated by alternative splicing. *Journal of Leukocyte Biology*, 79, 46-58.
- HIRSCH, B. R. & ZAFAR, S. Y. 2011. Capecitabine in the management of colorectal cancer. *Cancer Manag Res*, 3, 79-89.
- HNATYSZYN, A., HRYHOROWICZ, S., KACZMAREK-RYŚ, M., LIS, E., SŁOMSKI, R., SCOTT, R. J. & PŁAWSKI, A. 2019. Colorectal carcinoma in the course of inflammatory bowel diseases. *Hereditary Cancer in Clinical Practice*, 17, 18.
- HONG, S., YU, C., RODRIGUES, E., SHI, Y., CHEN, H., WANG, P., CHAPLA, D. G., GAO, T., ZHUANG, R., MOREMEN, K. W., PAULSON, J. C., MACAULEY, M. S. & WU, P. 2021. Modulation of Siglec-7 Signaling Via In Situ-Created High-Affinity cis-Ligands. *ACS Cent Sci*, 7, 1338-1346.
- HOPKINS, A. M., ROWLAND, A., KICHENADASSE, G., WIESE, M. D., GURNEY, H., MCKINNON, R. A., KARAPETIS, C. S. & SORICH, M. J. 2017. Predicting response and toxicity to immune checkpoint inhibitors using routinely available blood and clinical markers. *Br J Cancer*, 117, 913-920.
- HOU, N., MA, J., LI, W., ZHAO, L., GAO, Q. & MAI, L. 2017. T-cell immunoglobulin and mucin domain-containing protein-3 and galectin-9 protein expression: Potential prognostic significance in esophageal squamous cell carcinoma for Chinese patients. *Oncol Lett*, 14, 8007-8013.
- HOXHAI, G. & MANNING, B. D. 2020. The PI3K-AKT network at the interface of oncogenic signalling and cancer metabolism. *Nat Rev Cancer*, 20, 74-88.
- HSU, H.-P., LAI, M.-D., LEE, J.-C., YEN, M.-C., WENG, T.-Y., CHEN, W.-C., FANG, J.-H. & CHEN, Y.-L. 2017. Mucin 2 silencing promotes colon cancer metastasis through interleukin-6 signaling. *Scientific Reports*, 7, 5823.
- HUANG, P., XU, L., JIN, M., LI, L., KE, Y., ZHANG, M., ZHANG, K., LU, K. & HUANG, G. 2022. Construction and Validation of a Tumor Microenvironment-Based Scoring System to Evaluate Prognosis and Response to Immune Checkpoint Inhibitor Therapy in Lung Adenocarcinoma Patients. *Genes*, 13, 951.
- HUANG, P. J., LOW, P. Y., WANG, I., HSU, S. D. & ANGATA, T. 2018. Soluble Siglec-14 glycan-recognition protein is generated by alternative splicing and suppresses myeloid inflammatory responses. *J Biol Chem*, 293, 19645-19658.
- INOUE, S., ITO, H., TSUNODA, T., MURAKAMI, H., EBI, M., OGASAWARA, N., KASUGAI, K., KASAI, K., IKEDA, H. & INAGUMA, S. 2019. CD70 expression in tumor-associated fibroblasts predicts worse survival in colorectal cancer patients. *Virchows Archiv*, 475, 425-434.
- ITZKOWITZ, S. H. & YIO, X. 2004a. Inflammation and cancer IV. Colorectal cancer in inflammatory bowel disease: the role of inflammation. *Am J Physiol Gastrointest Liver Physiol*, 287, G7-17.
- ITZKOWITZ, S. H. & YIO, X. 2004b. Inflammation and Cancer IV. Colorectal cancer in inflammatory bowel disease: the role of inflammation. *American Journal of Physiology-Gastrointestinal and Liver Physiology*, 287, G7-G17.
- JANG, J.-H., SHIN, H. W., LEE, J. M., LEE, H.-W., KIM, E.-C. & PARK, S. H. 2015. An Overview of Pathogen Recognition Receptors for Innate Immunity in Dental Pulp. *Mediators of inflammation*, 2015, 794143-794143.
- JANISZEWSKA, M., PRIMI, M. C. & IZARD, T. 2020. Cell adhesion in cancer: Beyond the migration of single cells. *The Journal of biological chemistry*, 295, 2495-2505.

- JIANG, K. Y., QI, L. L., KANG, F. B. & WANG, L. 2022. The intriguing roles of Siglec family members in the tumor microenvironment. *Biomark Res*, 10, 22.
- JOHNSON, D. B., NEBHAN, C. A., MOSLEHI, J. J. & BALKO, J. M. 2022. Immune-checkpoint inhibitors: long-term implications of toxicity. *Nature Reviews Clinical Oncology*, 19, 254-267.
- KANG, Y., PARK, H., CHOE, B. H. & KANG, B. 2022. The Role and Function of Mucins and Its Relationship to Inflammatory Bowel Disease. *Front Med (Lausanne)*, 9, 848344.
- KANNEGANTI, M., MINO-KENUDSON, M. & MIZOGUCHI, E. 2011. Animal Models of Colitis-Associated Carcinogenesis. *Journal of Biomedicine and Biotechnology*, 2011, 342637.
- KANO, G., ALMANAN, M., BOCHNER, B. S. & ZIMMERMANN, N. 2013. Mechanism of Siglec-8-mediated cell death in IL-5-activated eosinophils: role for reactive oxygen species-enhanced MEK/ERK activation. *J Allergy Clin Immunol*, 132, 437-45.
- KARIHTALA, K., LEIVONEN, S.-K., KARJALAINEN-LINDSBERG, M.-L., CHAN, F. C., STEIDL, C., PELLINEN, T. & LEPPÄ, S. 2022. Checkpoint protein expression in the tumor microenvironment defines the outcome of classical Hodgkin lymphoma patients. *Blood Advances*, 6, 1919-1931.
- KASER, A., ZEISSIG, S. & BLUMBERG, R. S. 2010. Inflammatory bowel disease. *Annu Rev Immunol*, 28, 573-621.
- KATARIA, J., KERR, J., LOURENSSEN, S. R. & BLENNERHASSETT, M. G. 2022. Nintedanib regulates intestinal smooth muscle hyperplasia and phenotype in vitro and in TNBS colitis in vivo. *Scientific Reports*, 12, 10275.
- KELLERMANN, L. & RIIS, L. B. 2021. A close view on histopathological changes in inflammatory bowel disease, a narrative review. *Digestive Medicine Research*, 4.
- KELM, S. & RAVINDRAN, R. 2007. 3.24 - Siglecs. In: KAMERLING, H. (ed.) *Comprehensive Glycoscience*. Oxford: Elsevier.
- KHADELA, A., CHAVDA, V. P., POSTWALA, H., EPHRAIM, R., APOSTOLOPOULOS, V. & SHAH, Y. 2023. Configuring Therapeutic Aspects of Immune Checkpoints in Lung Cancer. *Cancers (Basel)*, 15.
- KHALIL, A., RUBIN, D. T., HAIDER, H. I., MECKEL, K., BISSONNETTE, M., TALISILA, N., SIVA, S., LI, Y. C., DENG, Z., PEKOW, J., ZHANG, C., HERNANDEZ, K., JASMINE, F., KIBRIYA, M. G., HYMAN, N. & WEBER, C. 2019. IBD-associated colon cancers differ in DNA methylation and gene expression profiles compared to sporadic colon cancers.
- KIESLER, P., FUSS, I. J. & STROBER, W. 2015. Experimental Models of Inflammatory Bowel Diseases. *Cellular and molecular gastroenterology and hepatology*, 1, 154-170.
- KIM, J. W. & KIM, S. Y. 2021. The Era of Janus Kinase Inhibitors for Inflammatory Bowel Disease Treatment. *Int J Mol Sci*, 22.
- KIM, N. D. & LUSTER, A. D. 2015. The role of tissue resident cells in neutrophil recruitment. *Trends in immunology*, 36, 547-555.
- KITANO, S., NAKAYAMA, T. & YAMASHITA, M. 2018. Biomarkers for Immune Checkpoint Inhibitors in Melanoma. *Frontiers in Oncology*, 8.
- KOLAR, P., KNIEKE, K., HEGEL, J. K., QUANDT, D., BURMESTER, G. R., HOFF, H. & BRUNNER-WEINZIERL, M. C. 2009. CTLA-4 (CD152) controls homeostasis and suppressive capacity of regulatory T cells in mice. *Arthritis Rheum*, 60, 123-32.
- KOOSHKAKI, O., DERAKHSHANI, A., HOSSEINKHANI, N., TORABI, M., SAFAEI, S., BRUNETTI, O., RACANELLI, V., SILVESTRI, N. & BARADARAN, B. 2020. Combination of Ipilimumab and Nivolumab in Cancers: From Clinical Practice to Ongoing Clinical Trials. *Int J Mol Sci*, 21.
- KORMAN, A. J., PEGGS, K. S. & ALLISON, J. P. 2006. Checkpoint blockade in cancer immunotherapy. *Advances in immunology*, 90, 297-339.
- KORNILUK, A., KAMIŃSKA, J., KISZŁO, P., KEMONA, H. & DYMICKA-PIEKARSKA, V. 2017. Lectin adhesion proteins (P-, L- and E-selectins) as biomarkers in colorectal cancer. *Biomarkers*, 22, 629-634.
- KOURY, J., LUCERO, M., CATO, C., CHANG, L., GEIGER, J., HENRY, D., HERNANDEZ, J., HUNG, F., KAUR, P., TESKEY, G. & TRAN, A. 2018. Immunotherapies: Exploiting the Immune System for Cancer Treatment. *Journal of Immunology Research*, 2018, 9585614.

- KOVEITYPOUR, Z., PANAHI, F., VAKILIAN, M., PEYMANI, M., SEYED FOROOTAN, F., NASR ESFAHANI, M. H. & GHAEDI, K. 2019. Signaling pathways involved in colorectal cancer progression. *Cell Biosci*, 9, 97.
- KREAMER, K. M. 2014. Immune Checkpoint Blockade: A New Paradigm in Treating Advanced Cancer. *J Adv Pract Oncol*, 5, 418-31.
- KRYSTEL-WHITTEMORE, M., DILEEPAN, K. N. & WOOD, J. G. 2016. Mast Cell: A Multi-Functional Master Cell. *Frontiers in Immunology*, 6.
- KUIPERS, E. J., GRADY, W. M., LIEBERMAN, D., SEUFFERLEIN, T., SUNG, J. J., BOELEN, P. G., VAN DE VELDE, C. J. H. & WATANABE, T. 2015. Colorectal cancer. *Nature reviews. Disease primers*, 1, 15065-15065.
- KUMARA, H., BELLINI, G. A., CABALLERO, O. L., HERATH, S. A. C., SU, T., AHMED, A., NJOH, L., CEKIC, V. & WHELAN, R. L. 2017. P-Cadherin (CDH3) is overexpressed in colorectal tumors and has potential as a serum marker for colorectal cancer monitoring. *Oncoscience*, 4, 139-147.
- KUMRIC, M., ZIVKOVIC, P. M., TICINOVIC KURIR, T., VRDOLJAK, J., VILOVIC, M., MARTINOVIC, D., BRATANIC, A., LIZATOVIC, I. K. & BOZIC, J. 2021. Role of B-Cell Activating Factor (BAFF) in Inflammatory Bowel Disease. *Diagnostics (Basel)*, 12.
- KUOL, N., STOJANOVSKA, L., APOSTOLOPOULOS, V. & NURGALI, K. 2018a. Crosstalk between cancer and the neuro-immune system. *J Neuroimmunol*, 315, 15-23.
- KUOL, N., STOJANOVSKA, L., APOSTOLOPOULOS, V. & NURGALI, K. 2018b. Role of the nervous system in cancer metastasis. *J Exp Clin Cancer Res*, 37, 5.
- KUOL, N., STOJANOVSKA, L., APOSTOLOPOULOS, V. & NURGALI, K. 2018c. Role of the Nervous System in Tumor Angiogenesis. *Cancer Microenviron*, 11, 1-11.
- KUOL, N., STOJANOVSKA, L., NURGALI, K. & APOSTOLOPOULOS, V. 2017. The mechanisms tumor cells utilize to evade the host's immune system. *Maturitas*, 105, 8-15.
- KUOL, N., STOJANOVSKA, L., NURGALI, K. & APOSTOLOPOULOS, V. 2018d. PD-1/PD-L1 in disease. *Immunotherapy*, 10, 149-160.
- KUOL, N., YAN, X., BARRIGA, V., KARAKKAT, J., VASSILAROS, S., FYSSAS, I., TSIMPANIS, A., FRASER, S., NURGALI, K. & APOSTOLOPOULOS, V. 2022. Pilot Study: Immune Checkpoints Polymorphisms in Greek Primary Breast Cancer Patients. *Biomedicines*, 10.
- KUPER, H., ADAMI, H. O. & TRICHOPOULOS, D. 2000. Infections as a major preventable cause of human cancer. *J Intern Med*, 248, 171-83.
- LANDSKRON, G., DE LA FUENTE, M., THUWAJIT, P., THUWAJIT, C. & HERMOSO, M. A. 2014. Chronic Inflammation and Cytokines in the Tumor Microenvironment. *Journal of Immunology Research*, 2014, 149185.
- LANGER, V., VIVI, E., REGENSBURGER, D., WINKLER, T. H., WALDNER, M. J., RATH, T., SCHMID, B., SKOTTKE, L., LEE, S., JEON, N. L., WOHLFAHRT, T., KRAMER, V., TRIPAL, P., SCHUMANN, M., KERSTING, S., HANDTRACK, C., GEPPERT, C. I., SUCHOWSKI, K., ADAMS, R. H., BECKER, C., RAMMING, A., NASCHBERGER, E., BRITZEN-LAURENT, N. & STÜRZL, M. 2019. IFN- γ drives inflammatory bowel disease pathogenesis through VE-cadherin-directed vascular barrier disruption. *J Clin Invest*, 129, 4691-4707.
- LARKIN, J., CHIARION-SILENI, V., GONZALEZ, R., GROB, J. J., RUTKOWSKI, P., LAO, C. D., COWEY, C. L., SCHADENDORF, D., WAGSTAFF, J., DUMMER, R., FERRUCCI, P. F., SMYLYE, M., HOGG, D., HILL, A., MARQUEZ-RODAS, I., HAANEN, J., GUIDOBONI, M., MAIO, M., SCHOFFSKI, P., CARLINO, M. S., LEBBE, C., MCARTHUR, G., ASCIERTO, P. A., DANIELS, G. A., LONG, G. V., BASTHOLT, L., RIZZO, J. I., BALOGH, A., MOSHYK, A., HODI, F. S. & WOLCHOK, J. D. 2019. Five-Year Survival with Combined Nivolumab and Ipilimumab in Advanced Melanoma. *N Engl J Med*, 381, 1535-1546.
- LÄUBLI, H. & VARKI, A. 2020. Sialic acid-binding immunoglobulin-like lectins (Siglecs) detect self-associated molecular patterns to regulate immune responses. *Cell Mol Life Sci*, 77, 593-605.

- LAW, C., ALHAMDOOSH, M., SU, S., DONG, X., TIAN, L., SMYTH, G. & RITCHIE, M. 2018. RNA-seq analysis is easy as 1-2-3 with limma, Glimma and edgeR [version 3; peer review: 3 approved]. *F1000Research*, 5.
- LAWRENCE, T. 2009. The nuclear factor NF-kappaB pathway in inflammation. *Cold Spring Harbor perspectives in biology*, 1, a001651-a001651.
- LEDERGERBER, M., LANG, B. M., HEINRICH, H., BIEDERMANN, L., BEGRÉ, S., ZEITZ, J., KRUPKA, N., RICKENBACHER, A., TURINA, M., GREUTER, T., SCHREINER, P., ROTH, R., SIEBENHÜNER, A., VAVRICKA, S. R., ROGLER, G., BEERENWINKEL, N., MISSELWITZ, B., ANDEREGG, C., BAUERFEIND, P., BEGLINGER, C., BEGRÉ, S., BELLI, D., BENGUA, J. M., BIEDERMANN, L., BIGLER, B., BINEK, J., BLATTMANN, M., BOEHM, S., BOROVICKA, J., BRAEGGER, C. P., BRUNNER, N., BÜHR, P., BURNAND, B., BURRI, E., BUYSE, S., CREMER, M., CRIBLEZ, D. H., DE SAUSSURE, P., DEGEN, L., DELARIVE, J., DOERIG, C., DORA, B., DORTA, G., EGGER, M., EHMANN, T., EL-WAFA, A., ENGELMANN, M., EZRI, J., FELLE, C., FLIEGNER, M., FOURNIER, N., FRAGA, M., FREI, P., FREI, R., FRIED, M., FROELICH, F., FUNK, C., FURLANO, R. I., GALLOT-LAVALLÉE, S., GEYER, M., GIRARDIN, M., GOLAY, D., GRANDINETTI, T., GYSI, B., HAACK, H., HAARER, J., HELBLING, B., HENGSTLER, P., HERZOG, D., HESS, C., HEYLAND, K., HINTERLEITNER, T., HIROZ, P., HIRSCHI, C., HRUZ, P., IWATA, R., JOST, R., JUILLERAT, P., BRONDOLO, V. K., KNELLWOLF, C., KNOBLAUCH, C., KÖHLER, H., KOLLER, R., KRIEGER-GRÜBEL, C., KULLAK-UBLOCK, G., KÜNZLER, P., LANDOLT, M., LANGE, R., LEHMANN, F. S., MACPHERSON, A., MAERTEN, P., MAILLARD, M. H., MANSER, C., MANZ, M., MARBET, U., MARX, G., MATTER, C., MCLIN, V., MEIER, R., MENDANOVA, M., et al. 2021. Abdominal pain in patients with inflammatory bowel disease: association with single-nucleotide polymorphisms prevalent in irritable bowel syndrome and clinical management. *BMC Gastroenterology*, 21, 53.
- LEE, J. B., KIM, H. R. & HA, S.-J. 2022. Immune Checkpoint Inhibitors in 10 Years: Contribution of Basic Research and Clinical Application in Cancer Immunotherapy. *Immune Netw*, 22.
- LEE, T. W. & FEDORAK, R. N. 2010. Tumor necrosis factor- α monoclonal antibodies in the treatment of inflammatory bowel disease: clinical practice pharmacology. *Gastroenterol Clin North Am*, 39, 543-57.
- LEMIALE, V., MEERT, A.-P., VINCENT, F., DARMON, M., BAUER, P. R., VAN DE LOUW, A., AZOULAY, E. & GROUPE DE RECHERCHE EN REANIMATION RESPIRATOIRE DU PATIENT, D. O.-H. 2019. Severe toxicity from checkpoint protein inhibitors: What intensive care physicians need to know? *Annals of intensive care*, 9, 25-25.
- LI, H., HAN, G., LI, X., LI, B., WU, B., JIN, H., WU, L. & WANG, W. 2021a. MAPK-RAP1A Signaling Enriched in Hepatocellular Carcinoma Is Associated With Favorable Tumor-Infiltrating Immune Cells and Clinical Prognosis. *Frontiers in Oncology*, 11.
- LI, H., WU, M. & ZHAO, X. 2022. Role of chemokine systems in cancer and inflammatory diseases. *MedComm*, 3, e147.
- LI, X., ZENG, S., DING, Y., NIE, Y. & YANG, M. 2021b. Comprehensive Analysis of the Potential Immune-Related Biomarker Transporter Associated With Antigen Processing 1 That Inhibits Metastasis and Invasion of Ovarian Cancer Cells. *Frontiers in Molecular Biosciences*, 8.
- LI, X., ZHONG, Q., LUO, D., DU, Q. & LIU, W. 2019. The prognostic value of CXCL subfamily ligands in stage I-III patients with colorectal cancer. *PLoS One*, 14, e0214611.
- LIAO, Y., SMYTH, G. K. & SHI, W. 2014a. featureCounts: an efficient general purpose program for assigning sequence reads to genomic features. *Bioinformatics*, 30, 923-30.
- LIAO, Y., SMYTH, G. K. & SHI, W. J. B. 2014b. featureCounts: an efficient general purpose program for assigning sequence reads to genomic features. 30, 923-930.
- LICHTENSTEIN, G. R. 2013. Comprehensive review: antitumor necrosis factor agents in inflammatory bowel disease and factors implicated in treatment response. *Therapeutic advances in gastroenterology*, 6, 269-293.
- LIM, J., SARI-AK, D. & BAGGA, T. 2021. Siglecs as Therapeutic Targets in Cancer. *Biology (Basel)*, 10.

- LIN, Y., HE, Z., YE, J., LIU, Z., SHE, X., GAO, X. & LIANG, R. 2020. Progress in Understanding the IL-6/STAT3 Pathway in Colorectal Cancer. *Onco Targets Ther*, 13, 13023-13032.
- LING, A., LÖFGREN-BURSTRÖM, A., LARSSON, P., LI, X., WIKBERG, M. L., ÖBERG, Å., STENLING, R., EDIN, S. & PALMQVIST, R. 2017. TAP1 down-regulation elicits immune escape and poor prognosis in colorectal cancer. *Oncoimmunology*, 6, e1356143.
- LIU, E., ASLAM, N., NIGAM, G. & LIMDI, J. K. 2022. Tofacitinib and newer JAK inhibitors in inflammatory bowel disease-where we are and where we are going. *Drugs Context*, 11.
- LIU, S., LIANG, J., LIU, Z., ZHANG, C., WANG, Y., WATSON, A. H., ZHOU, C., ZHANG, F., WU, K., ZHANG, F., LU, Y. & WANG, X. 2021. The Role of CD276 in Cancers. *Front Oncol*, 11, 654684.
- LIU, T., ZHANG, L., JOO, D. & SUN, S.-C. 2017a. NF- κ B signaling in inflammation. *Signal transduction and targeted therapy*, 2, 17023.
- LIU, T., ZHANG, L., JOO, D. & SUN, S. C. 2017b. NF- κ B signaling in inflammation. *Signal Transduct Target Ther*, 2, 17023-.
- LONG, A. G., LUNDSMITH, E. T. & HAMILTON, K. E. 2017. Inflammation and Colorectal Cancer. *Current colorectal cancer reports*, 13, 341-351.
- LOOI, C. K., HIL, L. W., NGAI, S. C., LEONG, C. O. & MAI, C. W. 2020. The Role of Ras-Associated Protein 1 (Rap1) in Cancer: Bad Actor or Good Player? *Biomedicines*, 8.
- LOVE, B. L. 2019. Management of patients with inflammatory bowel disease: current and future treatments. *Acute pain*, 10, 00.
- LOW, D., MINO-KENUDSON, M. & MIZOGUCHI, E. 2014. Recent advancement in understanding colitis-associated tumorigenesis. *Inflammatory bowel diseases*, 20, 2115-2123.
- LU, Q., YANG, M. F., LIANG, Y. J., XU, J., XU, H. M., NIE, Y. Q., WANG, L. S., YAO, J. & LI, D. F. 2022. Immunology of Inflammatory Bowel Disease: Molecular Mechanisms and Therapeutics. *J Inflamm Res*, 15, 1825-1844.
- LYTHGOE, M. P., LIU, D. S. K., ANNELS, N. E., KRELL, J. & FRAMPTON, A. E. 2021. Gene of the month: lymphocyte-activation gene 3 (LAG-3). *J Clin Pathol*, 74, 543-547.
- MA, C., WU, W., LIN, R., GE, Y., ZHANG, C., SUN, S., CONG, Y., LI, X. & LIU, Z. 2019. Critical Role of CD6highCD4+ T Cells in Driving Th1/Th17 Cell Immune Responses and Mucosal Inflammation in IBD. *J Crohns Colitis*, 13, 510-524.
- MANSON, G., NORWOOD, J., MARABELLE, A., KOHRT, H. & HOUOT, R. 2016. Biomarkers associated with checkpoint inhibitors. *Annals of Oncology*, 27, 1199-1206.
- MAO, X., XU, J., WANG, W., LIANG, C., HUA, J., LIU, J., ZHANG, B., MENG, Q., YU, X. & SHI, S. 2021. Crosstalk between cancer-associated fibroblasts and immune cells in the tumor microenvironment: new findings and future perspectives. *Mol Cancer*, 20, 131.
- MCCARTHY, E. F. 2006. The toxins of William B. Coley and the treatment of bone and soft-tissue sarcomas. *The Iowa orthopaedic journal*, 26, 154-158.
- MCDANIEL, D. K., EDEN, K., RINGEL, V. M. & ALLEN, I. C. 2016. Emerging Roles for Noncanonical NF- κ B Signaling in the Modulation of Inflammatory Bowel Disease Pathobiology. *Inflamm Bowel Dis*, 22, 2265-79.
- MCGUCKIN, M. A., ERI, R. D., DAS, I., LOURIE, R. & FLORIN, T. H. 2011. Intestinal secretory cell ER stress and inflammation. *Biochem Soc Trans*, 39, 1081-5.
- MCQUADE, R. M., STOJANOVSKA, V., ABALO, R., BORNSTEIN, J. C. & NURGALI, K. 2016. Chemotherapy-Induced Constipation and Diarrhea: Pathophysiology, Current and Emerging Treatments. *Frontiers in pharmacology*, 7, 414-414.
- MEIRESON, A., DEVOS, M. & BROCHEZ, L. 2020. IDO Expression in Cancer: Different Compartment, Different Functionality? *Front Immunol*, 11, 531491.
- MIRABELLI, P., COPPOLA, L. & SALVATORE, M. 2019. Cancer Cell Lines Are Useful Model Systems for Medical Research. *Cancers*, 11, 1098.
- MITRA, N., BANDA, K., ALTHEIDE, T. K., SCHAFFER, L., JOHNSON-PAIS, T. L., BEUTEN, J., LEACH, R. J., ANGATA, T., VARKI, N. & VARKI, A. 2011. SIGLEC12, a human-specific segregating (pseudo)gene, encodes a signaling molecule expressed in prostate carcinomas. *J Biol Chem*, 286, 23003-11.
- MITTAL, M., SIDDIQUI, M. R., TRAN, K., REDDY, S. P. & MALIK, A. B. 2014. Reactive oxygen species in inflammation and tissue injury. *Antioxidants & redox signaling*, 20, 1126-1167.

- MOEIN, S., VAGHARI-TABARI, M., QUJEQ, D., MAJIDINIA, M., NABAVI, S. M. & YOUSEFI, B. 2019. MiRNAs and inflammatory bowel disease: An interesting new story. *Journal of Cellular Physiology*, 234, 3277-3293.
- MOGENSEN, T. H. 2009. Pathogen recognition and inflammatory signaling in innate immune defenses. *Clinical microbiology reviews*, 22, 240-273.
- MONDANELLI, G., MANDARANO, M., BELLADONNA, M. L., SUVIERI, C., PELLICCIA, C., BELLEZZA, G., SIDONI, A., CARVALHO, A., GROHMANN, U. & VOLPI, C. 2021. Current Challenges for IDO2 as Target in Cancer Immunotherapy. *Front Immunol*, 12, 679953.
- MORAMPUDI, V., BHINDER, G., WU, X., DAI, C., SHAM, H. P., VALLANCE, B. A. & JACOBSON, K. 2014a. DNBS/TNBS colitis models: providing insights into inflammatory bowel disease and effects of dietary fat. *J Vis Exp*, e51297.
- MORAMPUDI, V., BHINDER, G., WU, X., DAI, C., SHAM, H. P., VALLANCE, B. A. & JACOBSON, K. 2014b. DNBS/TNBS colitis models: providing insights into inflammatory bowel disease and effects of dietary fat. *Journal of visualized experiments : JoVE*, e51297-e51297.
- MORÁN, G. A. G., PARRA-MEDINA, R., CARDONA, A. G., QUINTERO-RONDEROS, P. & RODRÍGUEZ, É. G. 2013. Cytokines, chemokines and growth factors. *Autoimmunity: From Bench to Bedside [Internet]*. El Rosario University Press.
- MORGILLO, F., DALLIO, M., DELLA CORTE, C. M., GRAVINA, A. G., VISCARDI, G., LOGUERCIO, C., CIARDIELLO, F. & FEDERICO, A. 2018. Carcinogenesis as a Result of Multiple Inflammatory and Oxidative Hits: a Comprehensive Review from Tumor Microenvironment to Gut Microbiota. *Neoplasia (New York, N.Y.)*, 20, 721-733.
- MORRISON, W. B. 2012. Inflammation and Cancer: A Comparative View. *Journal of Veterinary Internal Medicine*, 26, 18-31.
- MORSE, M. A., HOCHSTER, H. & BENSON, A. 2020. Perspectives on Treatment of Metastatic Colorectal Cancer with Immune Checkpoint Inhibitor Therapy. *The oncologist*, 25, 33-45.
- MOSS, S. F. & BLASER, M. J. 2005. Mechanisms of Disease: inflammation and the origins of cancer. *Nature Clinical Practice Oncology*, 2, 90-97.
- MURPHY, K. T. & LYNCH, G. S. 2009. Update on emerging drugs for cancer cachexia. *Expert opinion on emerging drugs*, 14, 619-632.
- NAGASE, H., VISSE, R. & MURPHY, G. 2006. Structure and function of matrix metalloproteinases and TIMPs. *Cardiovascular Research*, 69, 562-573.
- NALEJSKA, E., MACZYŃSKA, E. & LEWANDOWSKA, M. A. 2014. Prognostic and predictive biomarkers: tools in personalized oncology. *Molecular diagnosis & therapy*, 18, 273-284.
- NEUENDORF, R., HARDING, A., STELLO, N., HANES, D. & WAHBEH, H. 2016. Depression and anxiety in patients with Inflammatory Bowel Disease: A systematic review. *Journal of Psychosomatic Research*, 87, 70-80.
- NI, L. & DONG, C. 2017. New B7 Family Checkpoints in Human Cancers. *Mol Cancer Ther*, 16, 1203-1211.
- NIE, X., LIU, H., LIU, L., WANG, Y. D. & CHEN, W. D. 2020. Emerging Roles of Wnt Ligands in Human Colorectal Cancer. *Front Oncol*, 10, 1341.
- NIKANJAM, M., ARGUELLO, D., GATALICA, Z., SWENSEN, J., BARKAUSKAS, D. A. & KURZROCK, R. 2020. Relationship between protein biomarkers of chemotherapy response and microsatellite status, tumor mutational burden and PD-L1 expression in cancer patients. *International Journal of Cancer*, 146, 3087-3097.
- O'BRIEN, M. E. R., BORTHWICK, A., RIGG, A., LEARY, A., ASSERSOHN, L., LAST, K., TAN, S., MILAN, S., TAIT, D. & SMITH, I. E. 2006. Mortality within 30 days of chemotherapy: a clinical governance benchmarking issue for oncology patients. *British journal of cancer*, 95, 1632-1636.
- O'NEILL, A. S., VAN DEN BERG, T. K. & MULLEN, G. E. 2013. Sialoadhesin - a macrophage-restricted marker of immunoregulation and inflammation. *Immunology*, 138, 198-207.
- O'SULLIVAN, B. & THOMAS, R. 2003. CD40 and dendritic cell function. *Crit Rev Immunol*, 23, 83-107.

- OLARU, A. V., YAMANAKA, S., VAZQUEZ, C., MORI, Y., CHENG, Y., ABRAHAM, J. M., BAYLESS, T. M., HARPAZ, N., SELARU, F. M. & MELTZER, S. J. 2013. MicroRNA-224 negatively regulates p21 expression during late neoplastic progression in inflammatory bowel disease. *Inflammatory bowel diseases*, 19, 471-480.
- OLIVEIRA-NASCIMENTO, L., MASSARI, P. & WETZLER, L. M. 2012. The Role of TLR2 in Infection and Immunity. *Frontiers in immunology*, 3, 79-79.
- PAN, Z., HE, Y., ZHU, W., XU, T., HU, X. & HUANG, P. 2021. A Dynamic Transcription Factor Signature Along the Colorectal Adenoma-Carcinoma Sequence in Patients With Co-Occurrent Adenoma and Carcinoma. *Front Oncol*, 11, 597447.
- PANG, X., ZHANG, L., WU, J., MA, C., MU, C., ZHANG, G. & CHEN, W. 2017. Expression of CD40/CD40L in colon cancer, and its effect on proliferation and apoptosis of SW48 colon cancer cells. *J buon*, 22, 894-899.
- PAPAGEORGIOU, M., RAZA, A., FRASER, S., NURGALI, K. & APOSTOLOPOULOS, V. 2019. Methamphetamine and its immune-modulating effects. *Maturitas*, 121, 13-21.
- PARK, S. Y., KIM, J. Y., JANG, G. B., CHOI, J. H., KIM, J. H., LEE, C. J., LEE, S., BAEK, J. H., PARK, K. K., KIM, J. M., CHANG, H. J., CHO, N. C. & NAM, J. S. 2021. Aberrant activation of the CD45-Wnt signaling axis promotes stemness and therapy resistance in colorectal cancer cells. *Theranostics*, 11, 8755-8770.
- PATEL, M., HORGAN, P. G., MCMILLAN, D. C. & EDWARDS, J. 2018. NF- κ B pathways in the development and progression of colorectal cancer. *Translational Research*, 197, 43-56.
- PERITORE, A. F., D'AMICO, R., CORDARO, M., SIRACUSA, R., FUSCO, R., GUGLIANDOLO, E., GENOVESE, T., CRUPI, R., DI PAOLA, R., CUZZOCREA, S. & IMPELLIZZERI, D. 2021. PEA/Polydatin: Anti-Inflammatory and Antioxidant Approach to Counteract DNBS-Induced Colitis. *Antioxidants (Basel)*, 10.
- PERŠE, M. 2013. Oxidative stress in the pathogenesis of colorectal cancer: cause or consequence? *Biomed Res Int*, 2013, 725710.
- PETITPREZ, F., MEYLAN, M., DE REYNIÈS, A., SAUTÈS-FRIDMAN, C. & FRIDMAN, W. H. 2020. The Tumor Microenvironment in the Response to Immune Checkpoint Blockade Therapies. *Frontiers in Immunology*, 11.
- PHIPPS, A. I., LINDOR, N. M., JENKINS, M. A., BARON, J. A., WIN, A. K., GALLINGER, S., GRYFE, R. & NEWCOMB, P. A. 2013. Colon and rectal cancer survival by tumor location and microsatellite instability: the Colon Cancer Family Registry. *Diseases of the colon and rectum*, 56, 937-944.
- PIETERSZ, G. A., POUNIOTIS, D. S. & APOSTOLOPOULOS, V. 2006. Design of peptide-based vaccines for cancer. *Curr Med Chem*, 13, 1591-607.
- PITHADIA, A. B. & JAIN, S. 2011. Treatment of inflammatory bowel disease (IBD). *Pharmacological Reports*, 63, 629-642.
- POTACK, J. & ITZKOWITZ, S. H. 2008. Colorectal cancer in inflammatory bowel disease. *Gut and liver*, 2, 61-73.
- PRAKASH, M. D., MILLER, S., RANDALL-DEMLLO, S. & NURGALI, K. 2016. Mesenchymal Stem Cell Treatment of Inflammation-Induced Cancer. *Inflamm Bowel Dis*, 22, 2694-2703.
- PRAKASH, M. D., TANGALAKIS, K., ANTONIPILLAI, J., STOJANOVSKA, L., NURGALI, K. & APOSTOLOPOULOS, V. 2017. Methamphetamine: Effects on the brain, gut and immune system. *Pharmacol Res*, 120, 60-67.
- PRENDERGAST, G. C., SMITH, C., THOMAS, S., MANDIK-NAYAK, L., LAURY-KLEINTOP, L., METZ, R. & MULLER, A. J. 2014. Indoleamine 2,3-dioxygenase pathways of pathogenic inflammation and immune escape in cancer. *Cancer Immunol Immunother*, 63, 721-35.
- RAHMAN, A. A., ROBINSON, A. M., BROOKES, S. J. H., ERI, R. & NURGALI, K. 2016a. Rectal prolapse in Winnie mice with spontaneous chronic colitis: changes in intrinsic and extrinsic innervation of the rectum. *Cell Tissue Res*, 366, 285-299.
- RAHMAN, A. A., ROBINSON, A. M., BROOKES, S. J. H., ERI, R. & NURGALI, K. 2016b. Rectal prolapse in Winnie mice with spontaneous chronic colitis: changes in intrinsic and extrinsic innervation of the rectum. *Cell and Tissue Research*, 366, 285-299.

- RAHMAN, A. A., ROBINSON, A. M., JOVANOVSKA, V., ERI, R. & NURGALI, K. 2015. Alterations in the distal colon innervation in Winnie mouse model of spontaneous chronic colitis. *Cell and Tissue Research*, 362, 497-512.
- REN, X., JI, Y., JIANG, X. & QI, X. 2018. Down-regulation of siglec-2 (CD22) predicts worse overall survival from HBV-related early-stage hepatocellular carcinoma: a preliminary analysis from Gene Expression Omnibus. *Biosci Rep*, 38.
- RIBAS, A. & WOLCHOK, J. D. 2018. Cancer immunotherapy using checkpoint blockade. *Science*, 359, 1350-1355.
- ŘÍHOVÁ, B. & ŠTASTNÝ, M. 2015. [History of Immuno-therapy - from Coley Toxins to Check-points of the Immune Reaction]. *Klin Onkol*, 28 Suppl 4, 4s8-14.
- ROBINSON, A. M., GONDALIA, S. V., KARPE, A. V., ERI, R., BEALE, D. J., MORRISON, P. D., PALOMBO, E. A. & NURGALI, K. 2016. Fecal Microbiota and Metabolome in a Mouse Model of Spontaneous Chronic Colitis: Relevance to Human Inflammatory Bowel Disease. *Inflamm Bowel Dis*, 22, 2767-2787.
- ROBINSON, A. M., RAHMAN, A. A., CARBONE, S. E., RANDALL-DEMLLO, S., FILIPPONE, R., BORNSTEIN, J. C., ERI, R. & NURGALI, K. 2017a. Alterations of colonic function in the Winnie mouse model of spontaneous chronic colitis. *Am J Physiol Gastrointest Liver Physiol*, 312, G85-g102.
- ROBINSON, A. M., RAHMAN, A. A., CARBONE, S. E., RANDALL-DEMLLO, S., FILIPPONE, R., BORNSTEIN, J. C., ERI, R. & NURGALI, K. 2017b. Alterations of colonic function in the Winnie mouse model of spontaneous chronic colitis. *American Journal of Physiology-Gastrointestinal and Liver Physiology*, 312, G85-G102.
- ROBINSON, M. D. & OSHLACK, A. 2010. A scaling normalization method for differential expression analysis of RNA-seq data. *Genome Biology*, 11, R25.
- ROTTE, A. 2019. Combination of CTLA-4 and PD-1 blockers for treatment of cancer. *Journal of Experimental & Clinical Cancer Research*, 38, 255.
- ROWSHANRAVAN, B., HALLIDAY, N. & SANSOM, D. M. 2018. CTLA-4: a moving target in immunotherapy. *Blood*, 131, 58-67.
- ROZOVSKI, U., KEATING, M. J. & ESTROV, Z. 2018. Why Is the Immunoglobulin Heavy Chain Gene Mutation Status a Prognostic Indicator in Chronic Lymphocytic Leukemia? *Acta Haematologica*, 140, 51-54.
- RUFFO, E., WU, R. C., BRUNO, T. C., WORKMAN, C. J. & VIGNALI, D. A. A. 2019. Lymphocyte-activation gene 3 (LAG3): The next immune checkpoint receptor. *Semin Immunol*, 42, 101305.
- RUSSELL, B. L., SOOKLAL, S. A., MALINDISA, S. T., DAKA, L. J. & NTWASA, M. 2021. The Tumor Microenvironment Factors That Promote Resistance to Immune Checkpoint Blockade Therapy. *Frontiers in Oncology*, 11.
- RUTH, J. H., GURREA-RUBIO, M., ATHUKORALA, K. S., RASMUSSEN, S. M., WEBER, D. P., RANDON, P. M., GEDERT, R. J., LIND, M. E., AMIN, M. A., CAMPBELL, P. L., TSOU, P. S., MAO-DRAAYER, Y., WU, Q., LANIGAN, T. M., KESHAMOUNI, V. G., SINGER, N. G., LIN, F. & FOX, D. A. 2021. CD6 is a target for cancer immunotherapy. *JCI Insight*, 6.
- SABO, S. L., LAHR, J. M., OFFER, M., WEEKES, A. L. & SCENIAK, M. P. 2023. GRIN2B-related neurodevelopmental disorder: current understanding of pathophysiological mechanisms. *Frontiers in Synaptic Neuroscience*, 14.
- SAHAKIAN, L., FILIPPONE, R. T., STAVELY, R., ROBINSON, A. M., YAN, X. S., ABALO, R., ERI, R., BORNSTEIN, J. C., KELLEY, M. R. & NURGALI, K. 2021. Inhibition of APE1/Ref-1 Redox Signaling Alleviates Intestinal Dysfunction and Damage to Myenteric Neurons in a Mouse Model of Spontaneous Chronic Colitis. *Inflamm Bowel Dis*, 27, 388-406.
- SANCHEZ-MUNOZ, F., DOMINGUEZ-LOPEZ, A. & YAMAMOTO-FURUSHO, J.-K. 2008. Role of cytokines in inflammatory bowel disease. *World journal of gastroenterology*, 14, 4280-4288.
- SAUNDERS, A. S., BENDER, D. E., RAY, A. L., WU, X. & MORRIS, K. T. 2021. Colony-stimulating factor 3 signaling in colon and rectal cancers: Immune response and CMS classification in TCGA data. *PLoS One*, 16, e0247233.

- SCARPA, M., BRUN, P., SCARPA, M., MORGAN, S., PORZIONATO, A., KOTSAFTI, A., BORTOLAMI, M., BUDA, A., D'INCA, R., MACCHI, V., STURNIOLO, G. C., RUGGE, M., BARDINI, R., CASTAGLIUOLO, I., ANGRIMAN, I. & CASTORO, C. 2015. CD80-CD28 signaling controls the progression of inflammatory colorectal carcinogenesis. *Oncotarget*, 6, 20058-69.
- SCHAEFER, J. S., ATTUMI, T., OPEKUN, A. R., ABRAHAM, B., HOU, J., SHELBY, H., GRAHAM, D. Y., STRECKFUS, C. & KLEIN, J. R. 2015. MicroRNA signatures differentiate Crohn's disease from ulcerative colitis. *BMC immunology*, 16, 5-5.
- SCHOENBERGER, S. P., TOES, R. E., VAN DER VOORT, E. I., OFFRINGA, R. & MELIEF, C. J. 1998. T-cell help for cytotoxic T lymphocytes is mediated by CD40-CD40L interactions. *Nature*, 393, 480-3.
- SCHREIBER, S., ADEN, K., BERNARDES, J. P., CONRAD, C., TRAN, F., HÖPER, H., VOLK, V., MISHRA, N., BLASE, J. I., NIKOLAUS, S., BETHGE, J., KÜHBACHER, T., RÖCKEN, C., CHEN, M., COTTINGHAM, I., PETRI, N., RASMUSSEN, B. B., LOKAU, J., LENK, L., GARBERS, C., FEUERHAKE, F., ROSE-JOHN, S., WAETZIG, G. H. & ROSENSTIEL, P. 2021. Therapeutic Interleukin-6 Trans-signaling Inhibition by Olamkicept (sgp130Fc) in Patients With Active Inflammatory Bowel Disease. *Gastroenterology*, 160, 2354-2366.e11.
- SEBASTIAN, S., HERNÁNDEZ, H. V., MYRELID, P., KARIV, R., TSIANOS, E., TORUNER, M., MARTI-GALLOSTRA, M., SPINELLI, A., VAN DER MEULEN-DE JONG, A. E. & YUKSEL, E. S. 2014. Colorectal cancer in inflammatory bowel disease: results of the 3rd ECCO pathogenesis scientific workshop (I). *Journal of Crohn's and Colitis*, 8, 5-18.
- SEIDEL, J. A., OTSUKA, A. & KABASHIMA, K. 2018a. Anti-PD-1 and Anti-CTLA-4 Therapies in Cancer: Mechanisms of Action, Efficacy, and Limitations. *Frontiers in oncology*, 8, 86-86.
- SEIDEL, J. A., OTSUKA, A. & KABASHIMA, K. 2018b. Anti-PD-1 and Anti-CTLA-4 Therapies in Cancer: Mechanisms of Action, Efficacy, and Limitations. *Front Oncol*, 8, 86.
- SEIF, F., KHOSHMIRSAFA, M., AAZAMI, H., MOHSENZADEGAN, M., SEDIGHI, G. & BAHAR, M. 2017. The role of JAK-STAT signaling pathway and its regulators in the fate of T helper cells. *Cell Communication and Signaling*, 15, 23.
- SELDERS, G. S., FETZ, A. E., RADIC, M. Z. & BOWLIN, G. L. 2017. An overview of the role of neutrophils in innate immunity, inflammation and host-biomaterial integration. *Regenerative biomaterials*, 4, 55-68.
- SERAFINI, M. A., PAZ, A. H. & NUNES, N. S. 2022. Cholinergic immunomodulation in inflammatory bowel diseases. *Brain Behav Immun Health*, 19, 100401.
- SHAH, S. C. & ITZKOWITZ, S. H. 2022. Colorectal Cancer in Inflammatory Bowel Disease: Mechanisms and Management. *Gastroenterology*, 162, 715-730.e3.
- SHALABI, A., TANG, J. & HUBBARD-LUCEY, V. M. 2017. Comprehensive analysis of the clinical immuno-oncology landscape. *Annals of Oncology*, 29, 84-91.
- SHENG, K. C., DAY, S., WRIGHT, M. D., STOJANOVSKA, L. & APOSTOLOPOULOS, V. 2013. Enhanced Dendritic Cell-Mediated Antigen-Specific CD4⁺ T Cell Responses: IFN-Gamma Aids TLR Stimulation. *J Drug Deliv*, 2013, 516749.
- SHENG, K. C., PIETERSZ, G. A., TANG, C. K., RAMSLAND, P. A. & APOSTOLOPOULOS, V. 2010. Reactive oxygen species level defines two functionally distinctive stages of inflammatory dendritic cell development from mouse bone marrow. *J Immunol*, 184, 2863-72.
- SHENG, K. C., WRIGHT, M. D. & APOSTOLOPOULOS, V. 2011. Inflammatory mediators hold the key to dendritic cell suppression and tumor progression. *Curr Med Chem*, 18, 5507-18.
- SHI, S. J., WANG, L. J., WANG, G. D., GUO, Z. Y., WEI, M., MENG, Y. L., YANG, A. G. & WEN, W. H. 2013. B7-H1 expression is associated with poor prognosis in colorectal carcinoma and regulates the proliferation and invasion of HCT116 colorectal cancer cells. *PLoS One*, 8, e76012.
- SHIRAVAND, Y., KHODADADI, F., KASHANI, S. M. A., HOSSEINI-FARD, S. R., HOSSEINI, S., SADEGHIRAD, H., LADWA, R., O'BYRNE, K. & KULASINGHE, A. 2022. Immune Checkpoint Inhibitors in Cancer Therapy. *Curr Oncol*, 29, 3044-3060.

- SILVA, F. A. R., RODRIGUES, B. L., AYRIZONO, M. D. L. S. & LEAL, R. F. 2016. The Immunological Basis of Inflammatory Bowel Disease. *Gastroenterology research and practice*, 2016, 2097274-2097274.
- SINGH, U. P., SINGH, N. P., MURPHY, E. A., PRICE, R. L., FAYAD, R., NAGARKATTI, M. & NAGARKATTI, P. S. 2016. Chemokine and cytokine levels in inflammatory bowel disease patients. *Cytokine*, 77, 44-9.
- SONG, G., LU, Y., YU, Z., XU, L., LIU, J., CHEN, K. & ZHANG, P. 2019. The inhibitory effect of polysaccharide from *Rhizopus nigricans* on colitis-associated colorectal cancer. *Biomedicine & Pharmacotherapy*, 112, 108593.
- SONG, J., CHEN, Z., GENG, T., WANG, M., YI, S., LIU, K., ZHOU, W., GAO, J., SONG, W. & TANG, H. 2018. Deleting MyD88 signaling in myeloid cells promotes development of adenocarcinomas of the colon. *Cancer Lett*, 433, 65-75.
- STAVELY, R., FRASER, S., SHARMA, S., RAHMAN, A. A., STOJANOVSKA, V., SAKKAL, S., APOSTOLOPOULOS, V., BERTRAND, P. & NURGALI, K. 2018. The Onset and Progression of Chronic Colitis Parallels Increased Mucosal Serotonin Release via Enterochromaffin Cell Hyperplasia and Downregulation of the Serotonin Reuptake Transporter. *Inflamm Bowel Dis*, 24, 1021-1034.
- STAVELY, R., RAHMAN, A. A., SAHAKIAN, L., PRAKASH, M. D., ROBINSON, A. M., HASSANZADEGANROUDSARI, M., FILIPPONE, R. T., FRASER, S., ERI, R., BORNSTEIN, J. C., APOSTOLOPOULOS, V. & NURGALI, K. 2022a. Divergent Adaptations in Autonomic Nerve Activity and Neuroimmune Signaling Associated With the Severity of Inflammation in Chronic Colitis. *Inflamm Bowel Dis*, 28, 1229-1243.
- STAVELY, R., SAHAKIAN, L., FILIPPONE, R. T., STOJANOVSKA, V., BORNSTEIN, J. C., SAKKAL, S. & NURGALI, K. 2022b. Oxidative Stress-Induced HMGB1 Translocation in Myenteric Neurons Contributes to Neuropathy in Colitis. *Biomolecules*, 12.
- STROBER, W. & FUSS, I. J. 2011. Proinflammatory cytokines in the pathogenesis of inflammatory bowel diseases. *Gastroenterology*, 140, 1756-1767.
- SUN, J., SHAPER, N. L., ITONORI, S., HEFFER-LAUC, M., SHEIKH, K. A. & SCHNAAR, R. L. 2004. Myelin-associated glycoprotein (Siglec-4) expression is progressively and selectively decreased in the brains of mice lacking complex gangliosides. *Glycobiology*, 14, 851-7.
- SUN, X., ZHAO, J., MA, L., SUN, X., GE, J., YU, Y., MA, J. & ZHANG, M. 2021a. B7-H6 as an efficient target for T cell-induced cytotoxicity in haematologic malignant cells. *Invest New Drugs*, 39, 24-33.
- SUN, Z., DANG, Q., LIU, Z., SHAO, B., CHEN, C., GUO, Y., CHEN, Z., ZHOU, Q., HU, S., LIU, J. & YUAN, W. 2021b. LINC01272/miR-876/ITGB2 axis facilitates the metastasis of colorectal cancer via epithelial-mesenchymal transition. *J Cancer*, 12, 3909-3919.
- SZEBENI, B., VERES, G., DEZSÖFI, A., RUSAI, K., VANNAY, A., MRAZ, M., MAJOROVA, E. & ARATÓ, A. 2008. Increased expression of Toll-like receptor (TLR) 2 and TLR4 in the colonic mucosa of children with inflammatory bowel disease. *Clinical and experimental immunology*, 151, 34-41.
- TIMMERMANS, W. M., VAN LAAR, J. A., VAN DER HOUWEN, T. B., KAMPHUIS, L. S., BARTOL, S. J., LAM, K. H., OUWENDIJK, R. J., SPARROW, M. P., GIBSON, P. R., VAN HAGEN, P. M. & VAN ZELM, M. C. 2016. B-Cell Dysregulation in Crohn's Disease Is Partially Restored with Infliximab Therapy. *PLoS One*, 11, e0160103.
- TREBO, A., DITSCH, N., DEGENHARDT, T., KUHN, C., RAHMEH, M., SCHMOECKEL, E., MAYR, D., CZOGALLA, B., KOLBEN, T., MEISTER, S., MAHNER, S., JESCHKE, U. & HESTER, A. 2021. First Evidence for a Role of Siglec-8 in Breast Cancer. *Int J Mol Sci*, 22.
- TRIANTAFILLIDIS, J. K., MERIKAS, E. & GEORGOPOULOS, F. 2011. Current and emerging drugs for the treatment of inflammatory bowel disease. *Drug design, development and therapy*, 5, 185-210.
- TSAI, T. Y., HUANG, M. T., SUNG, P. S., PENG, C. Y., TAO, M. H., YANG, H. I., CHANG, W. C., YANG, A. S., YU, C. M., LIN, Y. P., BAU, C. Y., HUANG, C. J., PAN, M. H., WU, C. Y., HSIAO, C. D., YEH, Y. H., DUAN, S., PAULSON, J. C. & HSIEH, S. L. 2021. SIGLEC-3 (CD33) serves as an immune checkpoint receptor for HBV infection. *J Clin Invest*, 131.

- TSYGANOV, K., JAMES PERRY, A., KENNETH ARCHER, S. & POWELL, D. 2018a. RNAsik: A Pipeline for complete and reproducible RNA-seq analysis that runs anywhere with speed and ease. *The Journal of Open Source Software*, 3, 583.
- TSYGANOV, K., JAMES PERRY, A., KENNETH ARCHER, S. & POWELL, D. J. J. O. O. S. S. 2018b. RNAsik: A Pipeline for complete and reproducible RNA-seq analysis that runs anywhere with speed and ease. 3, 583.
- TURNER, M. D., NEDJAI, B., HURST, T. & PENNINGTON, D. J. 2014. Cytokines and chemokines: At the crossroads of cell signalling and inflammatory disease. *Biochimica et Biophysica Acta (BBA) - Molecular Cell Research*, 1843, 2563-2582.
- VAN DER WOUDE, C. J. & SHITRIT, A. B.-G. 2019. Pregnancy, psychiatry and IBD: multidisciplinary care is crucial. *Nature Reviews Gastroenterology & Hepatology*.
- VATANKHAH, A., HALÁSZ, J., PIURKÓ, V., BARBAI, T., RÁSÓ, E. & TÍMÁR, J. 2015. Characterization of the inflammatory cell infiltrate and expression of costimulatory molecules in chronic echinococcus granulosus infection of the human liver. *BMC infectious diseases*, 15, 530-530.
- VILLALOBO, A. & BERCHTOLD, M. W. 2020. The Role of Calmodulin in Tumor Cell Migration, Invasiveness, and Metastasis. *Int J Mol Sci*, 21.
- VILLARREAL, D. O., ALLEGREZZA, M. J., SMITH, M. A., CHIN, D., LUISTRO, L. L. & SNYDER, L. A. 2017. Targeting of CD122 enhances antitumor immunity by altering the tumor immune environment. *Oncotarget*, 8, 109151-109160.
- VISSE, R. & NAGASE, H. 2003. Matrix Metalloproteinases and Tissue Inhibitors of Metalloproteinases. *Circulation Research*, 92, 827-839.
- WAHL, L. M. & KLEINMAN, H. K. 1998. Tumor-associated macrophages as targets for cancer therapy. *J Natl Cancer Inst*, 90, 1583-4.
- WANG, B. & SUN, Y. 2022. SELPLG Expression Was Potentially Correlated With Metastasis and Prognosis of Osteosarcoma. *Pathol Oncol Res*, 28, 1610047.
- WANG, C. & CHEN, J. 2019. microRNAs as therapeutic targets in intestinal diseases. *ExRNA*, 1, 23.
- WANG, C., FENG, H., CHENG, X., LIU, K., CAI, D. & ZHAO, R. 2020. Potential Therapeutic Targets of B7 Family in Colorectal Cancer. *Front Immunol*, 11, 681.
- WANG, C., XIA, M., SUN, X., HE, Z., HU, F., CHEN, L., BUESO-RAMOS, C. E., QIU, X. & YIN, C. C. 2015a. IGK with conserved IGKV/IGKJ repertoire is expressed in acute myeloid leukemia and promotes leukemic cell migration. *Oncotarget*, 6, 39062-72.
- WANG, J., CHEN, L. & QIANG, P. 2021a. The role of IGF2BP2, an m6A reader gene, in human metabolic diseases and cancers. *Cancer Cell Int*, 21, 99.
- WANG, J., SUN, J., LIU, L. N., FLIES, D. B., NIE, X., TOKI, M., ZHANG, J., SONG, C., ZARR, M., ZHOU, X., HAN, X., ARCHER, K. A., O'NEILL, T., HERBST, R. S., BOTO, A. N., SANMAMED, M. F., LANGERMANN, S., RIMM, D. L. & CHEN, L. 2019. Siglec-15 as an immune suppressor and potential target for normalization cancer immunotherapy. *Nat Med*, 25, 656-666.
- WANG, J., WU, H., CHEN, Y., ZHU, J., SUN, L., LI, J., YAO, Z., CHEN, Y., ZHANG, X., XIA, S., CHEN, W. & SHI, T. 2021b. B7-H5 blockade enhances CD8(+) T-cell-mediated antitumor immunity in colorectal cancer. *Cell Death Discov*, 7, 248.
- WANG, R., HASNAIN, S. Z., TONG, H., DAS, I., CHE-HAO CHEN, A., OANCEA, I., PROCTOR, M., FLORIN, T. H., ERI, R. D. & MCGUCKIN, M. A. 2015b. Neutralizing IL-23 is superior to blocking IL-17 in suppressing intestinal inflammation in a spontaneous murine colitis model. *Inflamm Bowel Dis*, 21, 973-84.
- WANG, R., HASNAIN, S. Z., TONG, H., DAS, I., CHEN, A. C.-H., OANCEA, I., PROCTOR, M., FLORIN, T. H., ERI, R. D. & MCGUCKIN, M. A. 2015c. Neutralizing IL-23 is superior to blocking IL-17 in suppressing intestinal inflammation in a spontaneous murine colitis model. *Inflammatory bowel diseases*, 21, 973-984.
- WANG, R., MONIRUZZAMAN, M., WONG, K. Y., WIID, P., HARDING, A., GIRI, R., TONG, W. H., CREAGH, J., BEGUN, J., MCGUCKIN, M. A. & HASNAIN, S. Z. 2021c. Gut microbiota shape the inflammatory response in mice with an epithelial defect. *Gut Microbes*, 13, 1-18.

- WANG, X. & LIN, Y. 2008. Tumor necrosis factor and cancer, buddies or foes? *Acta Pharmacol Sin*, 29, 1275-88.
- WEHKAMP, J., GÖTZ, M., HERRLINGER, K., STEURER, W. & STANGE, E. F. 2016. Inflammatory Bowel Disease. *Dtsch Arztebl Int*, 113, 72-82.
- WEI, J., HU, M., HUANG, K., LIN, S. & DU, H. 2020. Roles of Proteoglycans and Glycosaminoglycans in Cancer Development and Progression. *Int J Mol Sci*, 21.
- WEI, S. C., DUFFY, C. R. & ALLISON, J. P. 2018a. Fundamental Mechanisms of Immune Checkpoint Blockade Therapy. *Cancer Discovery*, 8, 1069-1086.
- WEI, Y., CHHIBA, K. D., ZHANG, F., YE, X., WANG, L., ZHANG, L., ROBIDA, P. A., MORENO-VINASCO, L., SCHNAAR, R. L., ROERS, A., HARTMANN, K., LEE, C.-M., DEMERS, D., ZHENG, T., BOCHNER, B. S. & ZHU, Z. 2018b. Mast Cell-Specific Expression of Human Siglec-8 in Conditional Knock-in Mice. *International journal of molecular sciences*, 20, 19.
- WHITESIDE, T. L. 2008. The tumor microenvironment and its role in promoting tumor growth. *Oncogene*, 27, 5904-5912.
- WIRTZ, S., NEUFERT, C., WEIGMANN, B. & NEURATH, M. F. 2007. Chemically induced mouse models of intestinal inflammation. *Nat Protoc*, 2, 541-6.
- WOO, S. R., TURNIS, M. E., GOLDBERG, M. V., BANKOTI, J., SELBY, M., NIRSCHL, C. J., BETTINI, M. L., GRAVANO, D. M., VOGEL, P., LIU, C. L., TANGSOMBATVISIT, S., GROSSO, J. F., NETTO, G., SMELTZER, M. P., CHAUX, A., UTZ, P. J., WORKMAN, C. J., PARDOLL, D. M., KORMAN, A. J., DRAKE, C. G. & VIGNALI, D. A. 2012. Immune inhibitory molecules LAG-3 and PD-1 synergistically regulate T-cell function to promote tumoral immune escape. *Cancer Res*, 72, 917-27.
- WU, Y., YAO, J., XIE, J., LIU, Z., ZHOU, Y., PAN, H. & HAN, W. 2018. The role of autophagy in colitis-associated colorectal cancer. *Signal Transduction and Targeted Therapy*, 3, 31.
- XIAO, B., LAROU, H., AYYADURAI, S., VIENNOIS, E., CHARANIA, M. A., ZHANG, Y. & MERLIN, D. 2013. Mannosylated bio-reducible nanoparticle-mediated macrophage-specific TNF- α RNA interference for IBD therapy. *Biomaterials*, 34, 7471-7482.
- XIE, Y.-H., CHEN, Y.-X. & FANG, J.-Y. 2020. Comprehensive review of targeted therapy for colorectal cancer. *Signal Transduction and Targeted Therapy*, 5, 22.
- XU, G., JIANG, L., YE, C., QIN, G., LUO, Z., MO, Y. & CHEN, J. 2021. The Ratio of CD86+/CD163+ Macrophages Predicts Postoperative Recurrence in Stage II-III Colorectal Cancer. *Front Immunol*, 12, 724429.
- XU, G., WANG, T., LI, Y., HUANG, Z., WANG, X., ZHENG, J., YANG, S., FAN, Y. & XIANG, R. 2018. A highly potent and selective inhibitor Roxyl-WL targeting IDO1 promotes immune response against melanoma. *Journal of Enzyme Inhibition and Medicinal Chemistry*, 33, 1089-1094.
- XU, S., YE, C., CHEN, R., LI, Q. & RUAN, J. 2022. The Landscape and Clinical Application of the Tumor Microenvironment in Gastroenteropancreatic Neuroendocrine Neoplasms. *Cancers*, 14, 2911.
- XU, X., REN, J., MA, Y., LIU, H., RONG, Q., FENG, Y., WANG, Y., CHENG, Y., GE, R., LI, Z. & BIAN, J. 2019. Discovery of cyanopyridine scaffold as novel indoleamine-2,3-dioxygenase 1 (IDO1) inhibitors through virtual screening and preliminary hit optimisation. *Journal of Enzyme Inhibition and Medicinal Chemistry*, 34, 250-263.
- XU, X. M. & ZHANG, H. J. 2016. MiRNAs as new molecular insights into inflammatory bowel disease: Crucial regulators in autoimmunity and inflammation. *World Journal of Gastroenterology*, 22, 2206-2218.
- YOUSUF, H., MEKKI, R., KHAN, K. & HUSSAIN, A. 2020. Pembrolizumab-Induced Sarcoid-Like Reaction in a Patient With Lung Cancer. *Cureus*, 12, e12395-e12395.
- YU, M., LU, B., LIU, Y., ME, Y., WANG, L. & ZHANG, P. 2017. Tim-3 is upregulated in human colorectal carcinoma and associated with tumor progression. *Mol Med Rep*, 15, 689-695.
- YU, X., GUO, C., FISHER, P. B., SUBJECK, J. R. & WANG, X.-Y. 2015. Chapter Nine - Scavenger Receptors: Emerging Roles in Cancer Biology and Immunology. In: WANG, X.-Y. & FISHER, P. B. (eds.) *Advances in Cancer Research*. Academic Press.

- YU, Y., BLOKHUIS, B. R. J., DIKS, M. A. P., KESHAVARZIAN, A., GARSSSEN, J. & REDEGELD, F. A. 2018. Functional Inhibitory Siglec-6 Is Upregulated in Human Colorectal Cancer-Associated Mast Cells. *Front Immunol*, 9, 2138.
- YUN, S. M., KIM, S. H. & KIM, E. H. 2019. The Molecular Mechanism of Transforming Growth Factor- β Signaling for Intestinal Fibrosis: A Mini-Review. *Front Pharmacol*, 10, 162.
- ZEMEK, R. M., CHIN, W. L., NOWAK, A. K., MILLWARD, M. J., LAKE, R. A. & LESTERHUIS, W. J. 2020. Sensitizing the Tumor Microenvironment to Immune Checkpoint Therapy. *Frontiers in Immunology*, 11.
- ZENONOS, K. & KYPRIANOU, K. 2013. RAS signaling pathways, mutations and their role in colorectal cancer. *World J Gastrointest Oncol*, 5, 97-101.
- ZHANG, H. & CHEN, J. 2018. Current status and future directions of cancer immunotherapy. *Journal of Cancer*, 9, 1773-1781.
- ZHANG, L., LI, Z., SKRZYPCZYNSKA, K. M., FANG, Q., ZHANG, W., O'BRIEN, S. A., HE, Y., WANG, L., ZHANG, Q., KIM, A., GAO, R., ORF, J., WANG, T., SAWANT, D., KANG, J., BHATT, D., LU, D., LI, C. M., RAPAPORT, A. S., PEREZ, K., YE, Y., WANG, S., HU, X., REN, X., OUYANG, W., SHEN, Z., EGEN, J. G., ZHANG, Z. & YU, X. 2020. Single-Cell Analyses Inform Mechanisms of Myeloid-Targeted Therapies in Colon Cancer. *Cell*, 181, 442-459.e29.
- ZHANG, Y., LUO, Y., QIN, S. L., MU, Y. F., QI, Y., YU, M. H. & ZHONG, M. 2016. The clinical impact of ICOS signal in colorectal cancer patients. *Oncoimmunology*, 5, e1141857.
- ZHANG, Y. L., WANG, R. C., CHENG, K., RING, B. Z. & SU, L. 2017. Roles of Rap1 signaling in tumor cell migration and invasion. *Cancer Biol Med*, 14, 90-99.
- ZHAO, H., MING, T., TANG, S., REN, S., YANG, H., LIU, M., TAO, Q. & XU, H. 2022. Wnt signaling in colorectal cancer: pathogenic role and therapeutic target. *Molecular Cancer*, 21, 144.
- ZHAO, H., WU, L., YAN, G., CHEN, Y., ZHOU, M., WU, Y. & LI, Y. 2021. Inflammation and tumor progression: signaling pathways and targeted intervention. *Signal Transduct Target Ther*, 6, 263.
- ZHEN, Y., LUO, C. & ZHANG, H. 2018. Early detection of ulcerative colitis-associated colorectal cancer. *Gastroenterol Rep (Oxf)*, 6, 83-92.
- ZHOU, B., LU, Y., ZHAO, Z., SHI, T., WU, H., CHEN, W., ZHANG, L. & ZHANG, X. 2022a. B7-H4 expression is upregulated by PKCdelta activation and contributes to PKCdelta-induced cell motility in colorectal cancer. *Cancer Cell Int*, 22, 147.
- ZHOU, G., PENG, K., SONG, Y., YANG, W., SHU, W., YU, T., YU, L., LIN, M., WEI, Q., CHEN, C., YIN, L., CONG, Y. & LIU, Z. 2017. CD177+ neutrophils suppress epithelial cell tumorigenesis in colitis-associated cancer and predict good prognosis in colorectal cancer. *Carcinogenesis*, 39, 272-282.
- ZHOU, Q., LIANG, J., YANG, T., LIU, J., LI, B., LI, Y., FAN, Z., WANG, W., CHEN, W., YUAN, S., XU, M., XU, Q., LUAN, Z., XIA, Z., ZHOU, P., HUANG, Y. & CHEN, L. 2022b. Carfilzomib modulates tumor microenvironment to potentiate immune checkpoint therapy for cancer. *EMBO Molecular Medicine*, 14, e14502.
- ZHOU, W. T. & JIN, W. L. 2021. B7-H3/CD276: An Emerging Cancer Immunotherapy. *Front Immunol*, 12, 701006.
- ZHOU, X., DING, X., LI, H., YANG, C., MA, Z., XU, G., YANG, S., ZHANG, D., XIE, X., XIN, L. & LUO, X. 2021. Upregulation of TIGIT and PD-1 in Colorectal Cancer with Mismatch-repair Deficiency. *Immunol Invest*, 50, 338-355.
- ZOU, C., QIU, H., TANG, W., WANG, Y., LAN, B. & CHEN, Y. 2018. CTLA4 tagging polymorphisms and risk of colorectal cancer: a case-control study involving 2,306 subjects. *Onco Targets Ther*, 11, 4609-4619.

Chapter 4

Chapter 4

Overall Discussion, Conclusion and Future Directions

Overall Discussion

This research project mainly highlights on the factor that cancer genes were found and upregulated as early as in inflammation and severe inflammation stages in *Winnie* and *Winnie-Prolapse* mice which are models of spontaneous chronic colitis and not cancer models. This gives rise to two important factors i.e the relationship between inflammation and cancer, is inflammation in IBD an early sign of CRC and if the early markers can detect the presence of CRC in IBD patients due to the similarity in the *Winnie* mice and human IBD patients. These early markers/genes can be used for novel therapies against IBD and early detection of CRC in IBD patients.

Inflammatory bowel disease refers to two gastrointestinal diseases: Crohn's disease and ulcerative colitis. Ulcerative colitis only affects the colon and always starts at the anus, with the disease progressing upward. In ulcerative colitis, inflammation only affects the inner mucosa, whereas in Crohn's disease, inflammation can extend all the way through the bowel wall, from the mucosa to the muscle and even to the thin outermost layer of digestive tract cells (serosa). The inflammatory process causes blood vessel dilation with increased warmth, fluid oozing into the tissue, inflammatory cell infiltration, and mucosal ulceration. Infectious agents such as bacteria, fungi, parasites, and even viruses can cause inflammation. It can also be caused by chemical exposure, burns, trauma, or a variety of other factors (Chen et al., 2018). The cause of IBD is unknown, but there is substantial research evidence to suggest that interactions between environmental factors, intestinal bacteria, immune dysregulation, and genetic factors may play a role. Inflammatory bowel disease can be diagnosed at any age, but it is most common in youth and again around the age of 40-50. An increasing number of studies have reported that patients with IBD exhibit specific miRNA expression profiles (Chapman and Pekow, 2015), which might be involved in the initiation/ progression of inflammation (Fisher and Lin, 2015, Schaefer et al., 2015, Olaru et al., 2013, Wang and Chen, 2019, Dalal and Kwon, 2010, Xu and Zhang, 2016). In spite of a long list of miRNAs contributed in the pathogenesis of the IBD, the precise role of most of miRNAs are still unknown (Moein et al.,

2019). TNF- α is one of the main pro-inflammatory cytokines, secreted by MQ in IBD and has become an important target for IBD therapy due to dramatic reduction of inflammatory markers and mucosa structural damage following its inhibition (Sanchez-Munoz et al., 2008, Strober and Fuss, 2011).

C-reactive protein (CRP) is an acute-phase protein of hepatic origin is increased after IL-6 is secreted by macrophages and T cells. CRP is the main indicator of inflammation in IBD. Inflammation promotes all stages of tumorigenesis and promotes the development of cancer (Greten and Grivennikov, 2019). The immune cell infiltrates in the colon create an environment rich in reactive oxygen species and reactive nitrogen species, which can cause DNA damage in addition to exogenous mutagens, facilitating the initiation of cancer (Francescone et al., 2015). Dysplasia (the abnormal growth or development of a tissue or organ) in patients with IBD can be polypoid or flat, localized, diffused, or multifocal. Cancer cells, as well as surrounding stromal and inflammatory cells, form an inflammatory tumour microenvironment (Greten and Grivennikov) through well-coordinated reciprocal interactions. Chronic inflammation promotes tumour progression and treatment resistance, whereas inducing acute inflammatory responses frequently stimulates dendritic cell (DC) maturation and antigen presentation, leading to anti-tumor immune responses. In addition, multiple signaling pathways, such as nuclear factor kappa B (NF- κ B), Janus kinase/signal transducers and activators of transcription (JAK-STAT), toll-like receptor (TLR) pathways, cGAS/STING, and mitogen-activated protein kinase (MAPK); inflammatory factors, including cytokines (e.g., interleukin (IL), interferon (IFN), and tumor necrosis factor (TNF)- α), chemokines (e.g., C-C motif chemokine ligands (CCLs) and C-X-C motif chemokine ligands (CXCLs)), growth factors (e.g., vascular endothelial growth factor (VEGF), transforming growth factor (TGF)- β), and inflammasome are important regulators of initiation and decrease of inflammation (Zhao et al., 2021).

The discovery of immune checkpoint proteins such as PD-1/PDL-1 and CTLA-4 have been a breakthrough in the field of cancer immunotherapy in metastatic melanoma, renal cell carcinoma, head and neck cancers and non-small lung cancer by creating humanized monoclonal antibodies targeting these immune checkpoint proteins (Shiravand et al., 2022). The US FDA has approved three different categories of immune checkpoint inhibitors (ICIs) such as PD-1 inhibitors (Nivolumab, Pembrolizumab, and Cemiplimab), PDL-1 inhibitors (Atezolimumab, Durvalumab and Avelumab), and CTLA-4 inhibitor (Ipilimumab) (Lee et al.,

2022). Unfortunately, not all patients respond to these drugs (Hopkins et al., 2017) which highlights the discovery of novel checkpoint markers and biomarkers. Recent trials associate ICIs with radiotherapy, standard chemotherapy (anthracyclines or platinum-based anticancer drugs), targeted therapies (HER-2 blocking agents, TKi and others), and combination therapies (i.e., PD-1 and CTLA-4 blocking agents) (Barnestein et al., 2022).

Mouse models of IBD and CRC are widely accepted to help in the understanding of how living tissues function and the biology underlying gut diseases (Kiesler et al., 2015). Computer models and intestinal cell cultures are also used to study gut disorders; however, such models cannot replicate complicated interactions between the whole digestive system, mainly in the gut where interactions between mucus and microorganisms takes place. In murine both acute and chronic colitis is induced by intrarectal administration of the covalently reactive reagents 2,4,6-trinitro benzene sulfonic acid (TNBS) which is considered to be a hazardous chemical (Morampudi et al., 2014b). Dextran Sulfate Sodium (DSS)-Induced mice are most widely used models for intestinal inflammation. Major drawbacks to the DSS model are the variations in the concentration of DSS needed for the induction of colitis in different animal facilities, as well as inconsistency in water uptake by mice, that leads to inconsistent exposure to DSS, resulting in variation in the degree, extent and distribution of mucosal injury and ulceration in the colon (Morampudi et al., 2014b).

Dinitrobenzene sulfonic acid (DNBS)-induced colitis mouse models are used to study the pathogenesis of IBD. DNBS causes severe inflammation in the colon and rectum, and induces a strong inflammatory response that is associated with a significant increase in myeloperoxidase (MPO) activity and overproduction of IL-1 β and TNF- α . During administration of DNBS careful precaution should be applied to ensure the concentration of DNBS is not too high to prevent the rapid death of animals resulting from bowel perforation and sepsis.

This study has comprehensively explored the transcriptome of the distal colons of a mouse model of spontaneous chronic colitis. A point mutation in the *Muc2* gene in *Winnie* mice, leads to loss of colonic epithelial integrity, reduced mucous secretion by goblet cells and inflammation which is very similar to active ulcerative colitis in humans. *Winnie* mice present with mild inflammation by 14-16 weeks of aged which progressed to severe inflammation in *Winnie-Prolapse* mice. *Winnie* colonic explant culture cytokine assays show large amounts of

IL-1 β being secreted, even without any stimulation. *Winnie* mice display symptoms of diarrhea, ulcerations and rectal bleeding and pain at different stages of colitis similar to those in IBD human disease (Rahman et al., 2015). Colitis in *Winnie* is caused predominantly by the predominantly IL-23 cytokine storm which can be ameliorated by administering anti-IL-23 antibodies and dexamethasone (Robinson et al., 2017a).

The main focus of this study was to determine the changes in gene expression levels in three mice models (*Winnie*, *Winnie-Prolapse* and C57BL/6 control mice). This enables to determine the genes expressed along the pathway of no-inflammation to mild inflammation/colitis to severe inflammation and prolapse. The second most important issue addressed was the hypothesis that severe inflammation leads to CRC and as such, several novel genes were identified which could potentially be translated as valuable targets for the early diagnosis and treatment of IBD as well as potentially being used to detect early stages of CRC.

Beginning with a detailed literature review (Chapter 1) of the relationship between the checkpoint markers and TME, followed by checkpoint markers in chronic colitis and CRC.

Chapter 2 explored and documented a number of biological pathways that were significantly enriched in C57BL/6, *Winnie*, *Winnie-Prolapse*. The main findings show that *Winnie* and *Winnie-Prolapse* mice are highly enriched (upregulated) in inflammatory and immune related pathways/processes which was an unsurprising finding based on the severity of inflammation but the significant overexpression of chemokines like *Cxcr2* and *Cxcl9* in *Winnie* as well as *Winnie-Prolapse* mice is of interest especially based on their higher regulation in tumour tissues in CRC patients as per recent studies. These chemokines play a crucial role in inflammation and cancer and the higher regulation of these in *Winnie* mice also shows the similarity with IBD patients. Novel bispecific antibodies have been targeted against cytokines and integrins in IBD according to previous research which leads to another interesting find in this project i.e the presence and differential expression of more than 100 immunoglobulins, were found in *Winnie* mouse models. Treatments targeting B cells in IBD patients has been undergoing a lot of research in many areas.

There were more than 300 genes altered that were involved in the metabolic pathways in the *Winnie* and *Winnie-Prolapse* mice, which have been a current hot topic of metabolomic studies in IBD patients. Further study into these genes involved in the metabolic pathway could provide

insights into IBD pathogenesis and discovery of novel indicators for the diagnosis and prognosis assessment of IBD.

The second most important find in this project were number of genes that were altered in the cancer pathways which will lead to many novel findings when studied in detail. The point to be highlighted is the fact that there more than 100 genes that were differentially expressed, majority of which were upregulated in *Winnie* in the initial stages of IBD and much more in *Winnie-Prolapse* mice due to the severity of inflammation. These mice have not yet progressed to cancer but the presence of cancer genes in IBD mice makes it an interesting topic of research. The checkpoint markers and cancer genes are discussed further in chapter 3.

The third most enriched pathway was the neuroactive-ligand receptor pathway where all the genes were downregulated. Along with the other symptoms of inflammation, loss of function resulting from a neurological response that fails motor nerve function which is evidently seen in our results. There were more than 100 downregulated genes with significant changes, these included the cholinergic receptors, genes encoding the ion-gated channels, genes encoding the gamma-aminobutyric acid (GABA) receptor which are major neurotransmitter inhibitors, glutamate receptors etc. majority of the downregulated genes were highly enriched in neurological pathways. There have been several studies on the gut-immune-brain axis and changes in the central nervous system in IBD patients. Many studies have also shown nervous system disorders in IBD patients which in turn is related to the gut and brain axis and IBD patients are at a higher risk of developing these disorders compared to general population. This also proves that *Winnie* mice show vast resemblance to human IBD. Detailed study of these altered genes might provide novel insight giving rise to novel therapies to control these disorders.

Winnie-Prolapse mice expressed a greater number of genes in every pathway when compared to *Winnie* mice and also significantly up or downregulated due to the disease severity. This shows that *Winnie-Prolapse* model could be used to study the pre-cancerous genes and check if it progresses to cancer.

Ultimately, this work provides insights to improve our understanding of the levels and progression of inflammation in *Winnie-Prolapse* compared to *Winnie* mice and lead to improvements in the management of IBD in both areas, predictive and prognostic. The

overexpression/ upregulation of chemokines, cytokines, interleukins, interferon, immunoglobulins show the severity of inflammation and immune-related pathways. The downregulation of genes was mainly associated with the nervous system processes and pathways, which aligns with the characteristics of *Winnie* and *Winnie-prolapse* mice based on previous papers.

Chapter 3 is mainly divided into pathway analysis and gene analysis. This is more focused on the immune checkpoint markers and cancer markers in the transcriptome of *Winnie* and *Winnie-Prolapse* mice. There has been very less information about checkpoint markers present in IBD patients, but this study has shown the presence of checkpoint markers in *Winnie* mice which are IBD mouse models and haven't yet progressed to cancer. As such checkpoint markers are present on cancer cells but their presence in the transcriptome of *Winnie* and *Winnie-Prolapse* mice might lead to novel insight into the relationship between inflammation and cancer. The PD1-PD-L1 pathway has been widely studied in many cancers and drugs targeting this pathway have been approved for several forms of cancer but not for IBD. The presence of these markers in IBD patients might lead to early diagnosis and prevention of progression into CRC. *Pd1*, *Pd-l1*, *Ctla4*, *Lag3*, *Ido1* are highly expressed in *Winnie* and *Winnie-Prolapse* mice when compared with controls. Testing for the presence of these markers in IBD patients and also treatments targeting these markers might give rise to novel therapeutics for IBD. Based on the severity of inflammation in *Winnie-Prolapse* mice, many of the cancer-related pathways were significantly enriched when compared to *Winnie* mice. The presence of several immune checkpoint markers and cancer markers were detected in *Winnie* mice which most were even more upregulated in *Winnie-Prolapse* mice. All the cancer markers and checkpoint markers were significantly overexpressed in *Winnie-Prolapse* compared to *Winnie* mice.

When genes were compared to the genes were cancer patients on the cancer gene consortium, there were several common genes. It is as interesting fact that there were several common genes between IBD mouse models and CRC patients.

Conclusion

Determining the relationship between tumor cells and the TME is crucial to develop cancer treatments. Immune checkpoint inhibitors may cause immune-related adverse events, some of which are clinically serious to potentially life-threatening. Pathway analysis showed similar pathways in both *Winnie* and *Winnie-Prolapse* with *Winnie-Prolapse* mice having significantly

greater number of genes in every pathway with higher log fold change given the severity of inflammation. A number of genes were overexpressed in cancer pathways which could be further studied as novel therapeutic approaches against IBD as well as inflammation induced CRC. Differentially expressed genes in *Winnie* mice were similar to those expressed in patients with IBD which shows the close resemblance between *Winnie* and human IBD. The findings open many opportunities to study these genes and pathways in detail in order to identify novel therapeutic targets for IBD. Understanding the importance of inflammatory pathways in IBD, as well as a lack of response to traditional therapy in a substantial percentage of patients, has led to the development of novel medications over the last two decades. Understanding the relationship between the nervous and intestinal immune systems will provide new insights leading to the identification of innovative therapeutic approaches to treat IBD (Goverse et al., 2016). The presence and overexpression of oncogenes and cancer-related pathways that were highly enriched in *Winnie-Prolapse* mice compared to *Winnie* mice, indicates that inflammation may be linked to cancer or pre-cancerous stages, leading to the hypothesis that chronic and progressive inflammation may lead to cancer. Even though the area of checkpoint markers has been a hot-topic in the last 5-years, their functions in IBD and inflammation that leads to CRC are still not clear and require further studies. Given the similarity between the transcriptome of human IBD patients and spontaneous models of IBD i.e., *Winnie* mice and the severely inflamed model of *Winnie-Prolapse*, it is worthwhile studying these checkpoint markers further in order to understand their role in inflammation and their relationship to progression to cancer. Overall, this study identified genes associated with colitis (inflammation) and progression to prolapse (severe inflammation), with several enriched genes associated with cancer pathways were shown in particular in *Winnie-Prolapse* mice.

Future Directions

The regulation of the TME based on multiple omics results can suggest innovative therapeutic strategies to prevent cancer cells from immune escape and to support anti-cancer effects. Bioinformatics approaches will also need to continue to develop, to improve our understanding of a responsive TME. It is well established that the TME affects immune checkpoint inhibitor responses. Predictive and prognostic biomarkers can be determined using cutting-edge technology, which can also be used to determine the immunological background of malignancies. Though there has been significant research and information on checkpoint markers, their roles in IBD and development of CRC / inflammation associated cancer, remains

unclear and requires further study. Studying checkpoint markers in the *Winnie* mouse models that closely resemble human models may lead to the discovery of biomarkers for screening IBD patients and for the advancement of understanding inflammation and cancer that may lead to designing drugs that use inhibitors of biomarker expression or use vaccines to prevent disease progression. This includes patients at high risk for disease relapse who may benefit from earlier treatments. Although the clinical development of immune checkpoint inhibitors therapy has ushered in a new era of anti-cancer therapy, with sustained responses and significant survival advantages observed in multiple cancers, most patients do not benefit. Therefore, more attention should be paid to the identification and development of predictive biomarkers, as early as inflammation to severe inflammation to development of cancer (Bai et al., 2020).

Findings from the current studies in this thesis have provided important information of the genes involved in mild and severe colitis in mouse models. The data provides key genes upregulated and downregulated, and the identification of checkpoints and cancer related markers.

Future studies will involve:

1. Polymerase chain reaction (PCR) experiments to confirm expression of some of the identified genes.
2. Bioplex cytokine assays to confirm the expression/presence of the cytokines in the circulation.
3. Flow cytometry analysis of intracellular cytokines as well as immune cells present in colon homogenates.
4. Immunohistochemistry and western blot analysis, to identify the protein expression of the key genes identified.

Based on the transcriptome and mechanism/pathway findings in this thesis, as well as data to be gathered from these future studies, novel diagnostic kits can be developed to identify IBD as well as cancer in early stages of disease. In addition, the development of novel interventions for the treatment of IBD will be developed. Prevention strategies for the progression of mild to severe inflammation to inflammation associated cancers, will be developed, and tested *in vivo* in the *Winnie* mice. This work will then lead to translation of novel therapeutics to human clinical trials for the treatment of IBD, and CRC.

References

- BAI, R., LV, Z., XU, D. & CUI, J. 2020. Predictive biomarkers for cancer immunotherapy with immune checkpoint inhibitors. *Biomarker Research*, 8, 34.
- BARNESTEIN, R., GALLAND, L., KALFEIST, L., GHIRINGHELLI, F., LADOIRE, S. & LIMAGNE, E. 2022. Immunosuppressive tumor microenvironment modulation by chemotherapies and targeted therapies to enhance immunotherapy effectiveness. *Oncoimmunology*, 11, 2120676.
- CHAPMAN, C. G. & PEKOW, J. 2015. The emerging role of miRNAs in inflammatory bowel disease: a review. *Therapeutic advances in gastroenterology*, 8, 4-22.
- CHEN, L., DENG, H., CUI, H., FANG, J., ZUO, Z., DENG, J., LI, Y., WANG, X. & ZHAO, L. 2018. Inflammatory responses and inflammation-associated diseases in organs. *Oncotarget*, 9, 7204-7218.
- DALAL, S. & KWON, J. 2010. The Role of MicroRNA in Inflammatory Bowel Disease. *Gastroenterology & hepatology*, 6, 714-22.
- FISHER, K. & LIN, J. 2015. MicroRNA in inflammatory bowel disease: Translational research and clinical implication. *World journal of gastroenterology*, 21, 12274-12282.
- FRANCESCONI, R., HOU, V. & GRIVENNIKOV, S. I. 2015. Cytokines, IBD, and Colitis-associated Cancer. *Inflammatory Bowel Diseases*, 21, 409-418.
- GOVERSE, G., STAKENBORG, M. & MATTEOLI, G. 2016. The intestinal cholinergic anti-inflammatory pathway. *J Physiol*, 594, 5771-5780.
- GRETEN, F. R. & GRIVENNIKOV, S. I. 2019. Inflammation and Cancer: Triggers, Mechanisms, and Consequences. *Immunity*, 51, 27-41.
- HOPKINS, A. M., ROWLAND, A., KICHENADASSE, G., WIESE, M. D., GURNEY, H., MCKINNON, R. A., KARAPETIS, C. S. & SORICH, M. J. 2017. Predicting response and toxicity to immune checkpoint inhibitors using routinely available blood and clinical markers. *Br J Cancer*, 117, 913-920.
- KIESLER, P., FUSS, I. J. & STROBER, W. 2015. Experimental Models of Inflammatory Bowel Diseases. *Cellular and molecular gastroenterology and hepatology*, 1, 154-170.
- LEE, J. B., KIM, H. R. & HA, S.-J. 2022. Immune Checkpoint Inhibitors in 10 Years: Contribution of Basic Research and Clinical Application in Cancer Immunotherapy. *Immune Netw*, 22.
- MOEIN, S., VAGHARI-TABARI, M., QUJEQ, D., MAJIDINIA, M., NABAVI, S. M. & YOUSEFI, B. 2019. MiRNAs and inflammatory bowel disease: An interesting new story. *Journal of Cellular Physiology*, 234, 3277-3293.
- MORAMPUDI, V., BHINDER, G., WU, X., DAI, C., SHAM, H. P., VALLANCE, B. A. & JACOBSON, K. 2014. DNBS/TNBS colitis models: providing insights into inflammatory bowel disease and effects of dietary fat. *Journal of visualized experiments : JoVE*, e51297-e51297.
- OLARU, A. V., YAMANAKA, S., VAZQUEZ, C., MORI, Y., CHENG, Y., ABRAHAM, J. M., BAYLESS, T. M., HARPAZ, N., SELARU, F. M. & MELTZER, S. J. 2013. MicroRNA-224 negatively regulates p21 expression during late neoplastic progression in inflammatory bowel disease. *Inflammatory bowel diseases*, 19, 471-480.
- RAHMAN, A. A., ROBINSON, A. M., JOVANOVSKA, V., ERI, R. & NURGALI, K. 2015. Alterations in the distal colon innervation in Winnie mouse model of spontaneous chronic colitis. *Cell and Tissue Research*, 362, 497-512.
- ROBINSON, A. M., RAHMAN, A. A., CARBONE, S. E., RANDALL-DEMLLO, S., FILIPPONE, R., BORNSTEIN, J. C., ERI, R. & NURGALI, K. 2017. Alterations of colonic function in the Winnie mouse model of spontaneous chronic colitis. *Am J Physiol Gastrointest Liver Physiol*, 312, G85-g102.
- SANCHEZ-MUNOZ, F., DOMINGUEZ-LOPEZ, A. & YAMAMOTO-FURUSHO, J.-K. 2008. Role of cytokines in inflammatory bowel disease. *World journal of gastroenterology*, 14, 4280-4288.

- SCHAEFER, J. S., ATTUMI, T., OPEKUN, A. R., ABRAHAM, B., HOU, J., SHELBY, H., GRAHAM, D. Y., STRECKFUS, C. & KLEIN, J. R. 2015. MicroRNA signatures differentiate Crohn's disease from ulcerative colitis. *BMC immunology*, 16, 5-5.
- SHIRAVAND, Y., KHODADADI, F., KASHANI, S. M. A., HOSSEINI-FARD, S. R., HOSSEINI, S., SADEGHIRAD, H., LADWA, R., O'BYRNE, K. & KULASINGHE, A. 2022. Immune Checkpoint Inhibitors in Cancer Therapy. *Curr Oncol*, 29, 3044-3060.
- STROBER, W. & FUSS, I. J. 2011. Proinflammatory cytokines in the pathogenesis of inflammatory bowel diseases. *Gastroenterology*, 140, 1756-1767.
- WANG, C. & CHEN, J. 2019. microRNAs as therapeutic targets in intestinal diseases. *ExRNA*, 1, 23.
- XU, X. M. & ZHANG, H. J. 2016. MiRNAs as new molecular insights into inflammatory bowel disease: Crucial regulators in autoimmunity and inflammation. *World Journal of Gastroenterology*, 22, 2206-2218.
- ZHAO, H., WU, L., YAN, G., CHEN, Y., ZHOU, M., WU, Y. & LI, Y. 2021. Inflammation and tumor progression: signaling pathways and targeted intervention. *Signal Transduct Target Ther*, 6, 263.

Appendix

Table 1. Gene Ontologies in Winnie mice upregulated genes (Panther GO Slim classification)

PANTHER GO-Slim Biological Process	Gene Count	fold Enrichment	raw P-value	FDR
immune system process (GO:0002376)	230	6.81	1.59E-113	3.54E-110
immune response (GO:0006955)	203	7.28	1.20E-103	1.33E-100
defence response (GO:0006952)	151	7.99	2.97E-80	2.20E-77
biological process involved in interspecies interaction between organisms (GO:0044419)	124	7.33	8.56E-62	2.72E-59
response to biotic stimulus (GO:0009607)	124	7.32	1.04E-61	2.88E-59
response to another organism (GO:0051707)	124	7.33	8.56E-62	3.17E-59
response to external biotic stimulus (GO:0043207)	124	7.33	8.56E-62	3.80E-59
regulation of immune system process (GO:0002682)	118	7.5	1.23E-59	3.04E-57
lymphocyte activation (GO:0046649)	96	9.72	1.01E-56	2.24E-54
defence response to another organism (GO:0098542)	111	7.63	1.17E-56	2.36E-54
positive regulation of immune system process (GO:0002684)	105	8.19	4.79E-56	8.87E-54
leukocyte activation (GO:0045321)	97	9.25	1.05E-55	1.80E-53
regulation of lymphocyte activation (GO:0051249)	87	10.99	5.82E-55	9.23E-53
cell activation (GO:0001775)	97	8.88	2.32E-54	3.44E-52
regulation of leukocyte activation (GO:0002694)	88	10.45	4.21E-54	5.84E-52
regulation of cell activation (GO:0050865)	88	10.37	7.25E-54	9.47E-52
immune effector process (GO:0002252)	91	8.88	5.64E-51	6.59E-49
positive regulation of lymphocyte activation (GO:0051251)	81	10.89	6.65E-51	7.38E-49
adaptive immune response based on somatic recombination of immune receptors built from immunoglobulin superfamily domains (GO:0002460)	85	9.86	1.38E-50	1.46E-48
positive regulation of cell activation (GO:0050867)	82	10.5	1.57E-50	1.52E-48
positive regulation of leukocyte activation (GO:0002696)	82	10.5	1.57E-50	1.59E-48
lymphocyte mediated immunity (GO:0002449)	85	9.63	6.39E-50	5.91E-48
response to bacterium (GO:0009617)	95	7.89	1.90E-49	1.69E-47
leukocyte mediated immunity (GO:0002443)	85	9.27	7.58E-49	6.47E-47
immunoglobulin mediated immune response (GO:0016064)	77	11.05	8.71E-49	6.67E-47

regulation of immune response (GO:0050776)	93	7.99	8.32E-49	6.84E-47
B cell mediated immunity (GO:0019724)	77	11.05	8.71E-49	6.90E-47
adaptive immune response (GO:0002250)	85	8.98	6.51E-48	4.82E-46
response to external stimulus (GO:0009605)	129	4.97	1.48E-47	1.06E-45
immune response-regulating signaling pathway (GO:0002764)	82	9.11	1.20E-46	8.33E-45
positive regulation of immune response (GO:0050778)	86	8.18	8.93E-46	6.01E-44
immune response-activating signal transduction (GO:0002757)	77	9.53	5.90E-45	3.74E-43
immune response-activating cell surface receptor signaling pathway (GO:0002429)	77	9.53	5.90E-45	3.85E-43
immune response-regulating cell surface receptor signaling pathway (GO:0002768)	77	9.37	1.58E-44	9.50E-43
activation of immune response (GO:0002253)	78	9.15	1.74E-44	1.01E-42
defence response to bacterium (GO:0042742)	83	8.26	1.78E-44	1.02E-42
antigen receptor-mediated signaling pathway (GO:0050851)	74	9.94	2.75E-44	1.53E-42
innate immune response (GO:0045087)	88	7.43	5.03E-44	2.73E-42
phagocytosis (GO:0006909)	72	10.04	2.47E-43	1.31E-41
B cell receptor signaling pathway (GO:0050853)	68	10.81	1.25E-42	6.46E-41
complement activation (GO:0006956)	67	10.94	2.74E-42	1.38E-40
protein activation cascade (GO:0072376)	67	10.82	4.83E-42	2.38E-40
cell recognition (GO:0008037)	69	10.15	8.52E-42	4.02E-40
phagocytosis, engulfment (GO:0006911)	67	10.71	8.45E-42	4.08E-40
humoral immune response (GO:0006959)	77	7.82	7.30E-40	3.38E-38
positive regulation of response to stimulus (GO:0048584)	105	5.1	2.08E-39	9.42E-38
B cell activation (GO:0042113)	67	9.62	2.17E-39	9.63E-38
production of molecular mediator of immune response (GO:0002440)	66	9.52	1.38E-38	6.00E-37
endocytosis (GO:0006897)	70	6.41	1.30E-31	5.54E-30
membrane invagination (GO:0010324)	70	6.37	1.80E-31	7.56E-30
vesicle budding from membrane (GO:0006900)	70	6	4.38E-30	1.80E-28
response to cytokine (GO:0034097)	48	7.71	6.17E-25	2.36E-23

cellular response to cytokine stimulus (GO:0071345)	46	7.91	2.51E-24	9.28E-23
vesicle organization (GO:0016050)	71	4.15	4.66E-22	1.62E-20
membrane organization (GO:0061024)	80	3.68	8.47E-22	2.89E-20
inflammatory response (GO:0006954)	35	7.14	1.30E-17	4.38E-16
cytokine-mediated signaling pathway (GO:0019221)	33	6.87	2.89E-16	9.30E-15
T cell activation (GO:0042110)	27	7.98	6.72E-15	2.13E-13
response to organic substance (GO:0010033)	62	2.94	4.77E-13	1.45E-11
leukocyte cell-cell adhesion (GO:0007159)	20	10.2	5.11E-13	1.53E-11
regulation of T cell activation (GO:0050863)	19	10.8	8.45E-13	2.50E-11
vesicle-mediated transport (GO:0016192)	76	2.51	1.85E-12	5.40E-11
cellular response to organic substance (GO:0071310)	54	3.12	1.99E-12	5.72E-11
regulation of cell-cell adhesion (GO:0022407)	19	10.03	2.47E-12	7.04E-11
regulation of leukocyte cell-cell adhesion (GO:1903037)	18	10.64	4.14E-12	1.16E-10
regulation of cell adhesion (GO:0030155)	20	7.58	4.77E-11	1.31E-09
cellular response to chemical stimulus (GO:0070887)	60	2.68	6.49E-11	1.76E-09
leukocyte migration (GO:0050900)	19	7.8	9.57E-11	2.56E-09
positive regulation of T cell activation (GO:0050870)	14	10.35	1.38E-09	3.59E-08
positive regulation of leukocyte cell-cell adhesion (GO:1903039)	14	10.35	1.38E-09	3.64E-08
positive regulation of cell-cell adhesion (GO:0022409)	14	10.1	1.79E-09	4.62E-08
positive regulation of cell adhesion (GO:0045785)	14	8.45	1.21E-08	3.08E-07
response to chemical (GO:0042221)	67	2.15	2.14E-08	5.40E-07
neutrophil migration (GO:1990266)	13	8.94	2.29E-08	5.64E-07
granulocyte chemotaxis (GO:0071621)	13	8.54	3.60E-08	8.68E-07
granulocyte migration (GO:0097530)	13	7.84	8.39E-08	2.00E-06
cell-cell adhesion (GO:0098609)	25	3.55	2.10E-07	4.92E-06
leukocyte chemotaxis (GO:0030595)	13	7.12	2.20E-07	5.10E-06
antigen processing and presentation (GO:0019882)	14	6.37	2.48E-07	5.67E-06
myeloid leukocyte migration (GO:0097529)	13	6.99	2.64E-07	5.93E-06
defence response to symbiont (GO:0140546)	11	8.34	4.98E-07	1.07E-05

defence response to virus (GO:0051607)	11	8.34	4.98E-07	1.08E-05
lymphocyte migration (GO:0072676)	10	9.54	5.92E-07	1.24E-05
response to virus (GO:0009615)	11	7.93	7.59E-07	1.57E-05
ERK1 and ERK2 cascade (GO:0070371)	11	7.39	1.37E-06	2.75E-05
regulation of ERK1 and ERK2 cascade (GO:0070372)	11	7.39	1.37E-06	2.77E-05
cell chemotaxis (GO:0060326)	13	5.57	2.47E-06	4.85E-05
mononuclear cell migration (GO:0071674)	10	7.78	2.81E-06	5.46E-05
cell migration (GO:0016477)	27	2.87	3.12E-06	6.02E-05
positive regulation of ERK1 and ERK2 cascade (GO:0070374)	10	7.04	6.03E-06	1.15E-04
response to lipopolysaccharide (GO:0032496)	13	4.99	7.16E-06	1.36E-04
cellular response to biotic stimulus (GO:0071216)	13	4.93	8.10E-06	1.52E-04
response to molecule of bacterial origin (GO:0002237)	13	4.87	9.16E-06	1.69E-04
regulation of leukocyte proliferation (GO:0070663)	8	8.76	1.36E-05	2.45E-04
regulation of mononuclear cell proliferation (GO:0032944)	8	8.76	1.36E-05	2.47E-04
regulation of lymphocyte proliferation (GO:0050670)	8	8.76	1.36E-05	2.49E-04
mitotic nuclear division (GO:0140014)	25	2.75	1.44E-05	2.53E-04
mitotic cell cycle process (GO:1903047)	25	2.75	1.44E-05	2.55E-04
mitotic cell cycle (GO:0000278)	25	2.75	1.44E-05	2.57E-04
cellular response to lipopolysaccharide (GO:0071222)	12	4.86	2.03E-05	3.44E-04
cellular response to molecule of bacterial origin (GO:0071219)	12	4.73	2.58E-05	4.34E-04
response to tumor necrosis factor (GO:0034612)	9	6.49	3.08E-05	5.14E-04
T cell proliferation (GO:0042098)	7	9.41	3.24E-05	5.30E-04
cell adhesion (GO:0007155)	32	2.31	3.21E-05	5.32E-04
regulation of T cell proliferation (GO:0042129)	7	9.41	3.24E-05	5.34E-04
biological adhesion (GO:0022610)	32	2.3	3.31E-05	5.36E-04
cell motility (GO:0048870)	27	2.46	4.72E-05	7.33E-04
localization of cell (GO:0051674)	27	2.46	4.72E-05	7.38E-04
sister chromatid segregation (GO:0000819)	11	4.58	7.22E-05	1.10E-03
mitotic sister chromatid segregation (GO:0000070)	10	5.01	7.86E-05	1.16E-03

positive regulation of cytokine production (GO:0001819)	7	7.67	9.70E-05	1.40E-03
cellular response to lipid (GO:0071396)	12	4.03	1.04E-04	1.48E-03
response to lipid (GO:0033993)	13	3.66	1.31E-04	1.83E-03
cellular response to tumor necrosis factor (GO:0071356)	8	5.91	1.50E-04	2.08E-03
nuclear division (GO:0000280)	28	2.21	1.79E-04	2.43E-03
regulation of cytokine production (GO:0001817)	11	4.07	1.87E-04	2.50E-03
response to interleukin-1 (GO:0070555)	7	6.68	2.03E-04	2.70E-03
regulation of immune effector process (GO:0002697)	11	3.97	2.27E-04	3.00E-03
positive regulation of intracellular signal transduction (GO:1902533)	16	2.94	2.43E-04	3.20E-03
response to oxygen-containing compound (GO:1901700)	19	2.61	2.70E-04	3.50E-03
regulation of MAPK cascade (GO:0043408)	14	3.18	2.78E-04	3.58E-03
MAPK cascade (GO:0000165)	15	3.02	2.91E-04	3.73E-03
T cell differentiation (GO:0030217)	5	10.56	2.93E-04	3.74E-03
positive regulation of MAPK cascade (GO:0043410)	11	3.83	3.01E-04	3.82E-03
cell-matrix adhesion (GO:0007160)	8	5.26	3.05E-04	3.84E-03
regulation of cytokine production involved in immune response (GO:0002718)	4	16.89	3.12E-04	3.89E-03
positive regulation of immune effector process (GO:0002699)	10	4.11	3.38E-04	4.19E-03
leukocyte proliferation (GO:0070661)	8	5.14	3.47E-04	4.21E-03
lymphocyte proliferation (GO:0046651)	8	5.14	3.47E-04	4.24E-03
mononuclear cell proliferation (GO:0032943)	8	5.14	3.47E-04	4.26E-03
microtubule cytoskeleton organization involved in mitosis (GO:1902850)	8	5.14	3.47E-04	4.29E-03
nuclear chromosome segregation (GO:0098813)	12	3.48	3.60E-04	4.32E-03
T cell mediated immunity (GO:0002456)	8	5.03	3.95E-04	4.72E-03
chromosome segregation (GO:0007059)	13	3.23	4.00E-04	4.75E-03
organelle fission (GO:0048285)	28	2.12	4.05E-04	4.79E-03
defence response to Gram-positive bacterium (GO:0050830)	10	4	4.11E-04	4.83E-03
cytokine production (GO:0001816)	11	3.65	4.29E-04	5.02E-03
mitotic spindle organization (GO:0007052)	7	5.59	5.20E-04	6.02E-03
locomotion (GO:0040011)	28	2.06	5.39E-04	6.20E-03
negative regulation of T cell activation (GO:0050868)	5	8.7	6.13E-04	6.97E-03

negative regulation of leukocyte cell-cell adhesion (GO:1903038)	5	8.7	6.13E-04	7.01E-03
defence response to fungus (GO:0050832)	4	13.14	6.42E-04	7.27E-03
negative regulation of immune system process (GO:0002683)	7	5.17	7.85E-04	8.84E-03
negative regulation of lymphocyte activation (GO:0051250)	5	7.78	9.37E-04	1.05E-02
integrin-mediated signaling pathway (GO:0007229)	7	4.93	1.01E-03	1.12E-02
negative regulation of cell-cell adhesion (GO:0022408)	5	7.39	1.14E-03	1.25E-02
negative regulation of cell adhesion (GO:0007162)	6	5.72	1.17E-03	1.27E-02
negative regulation of cell activation (GO:0050866)	5	7.04	1.37E-03	1.46E-02
negative regulation of leukocyte activation (GO:0002695)	5	7.04	1.37E-03	1.47E-02
negative regulation of T cell proliferation (GO:0042130)	4	9.86	1.51E-03	1.59E-02
antimicrobial humoral response (GO:0019730)	10	3.25	1.74E-03	1.82E-02
regulation of adaptive immune response (GO:0002819)	8	3.88	1.82E-03	1.89E-02
chemotaxis (GO:0006935)	15	2.48	1.87E-03	1.92E-02
taxis (GO:0042330)	15	2.48	1.87E-03	1.93E-02
toll-like receptor signaling pathway (GO:0002224)	5	6.43	1.94E-03	1.99E-02
pattern recognition receptor signaling pathway (GO:0002221)	5	6.16	2.29E-03	2.33E-02
cell-substrate adhesion (GO:0031589)	8	3.7	2.39E-03	2.42E-02
response to type I interferon (GO:0034340)	3	14.78	2.53E-03	2.53E-02
mitotic chromosome condensation (GO:0007076)	3	14.78	2.53E-03	2.54E-02
type I interferon signaling pathway (GO:0060337)	3	14.78	2.53E-03	2.55E-02
T cell receptor signaling pathway (GO:0050852)	6	4.79	2.61E-03	2.60E-02
positive regulation of molecular function (GO:0044093)	25	1.92	2.89E-03	2.87E-02
cell cycle process (GO:0022402)	29	1.83	2.98E-03	2.94E-02
positive regulation of adaptive immune response (GO:0002821)	7	3.98	3.04E-03	2.98E-02
movement of cell or subcellular component (GO:0006928)	37	1.68	3.07E-03	3.01E-02
regulation of lymphocyte mediated immunity (GO:0002706)	8	3.53	3.09E-03	3.01E-02

regulation of chromosome segregation (GO:0051983)	5	5.69	3.10E-03	3.01E-02
regulation of cell cycle process (GO:0010564)	12	2.65	3.12E-03	3.02E-02
regulation of hydrolase activity (GO:0051336)	22	2.01	3.16E-03	3.04E-02
cellular response to oxygen-containing compound (GO:1901701)	15	2.42	3.62E-03	3.43E-02
positive regulation of hydrolase activity (GO:0051345)	15	2.37	3.93E-03	3.70E-02
regulation of mitotic nuclear division (GO:0007088)	5	5.28	4.10E-03	3.84E-02
positive regulation of signal transduction (GO:0009967)	19	2.05	4.40E-03	4.09E-02
intracellular signal transduction (GO:0035556)	44	1.56	4.93E-03	4.53E-02
spindle organization (GO:0007051)	7	3.57	5.24E-03	4.63E-02
mitotic cell cycle phase transition (GO:0044772)	9	2.96	5.16E-03	4.64E-02
regulation of mitotic cell cycle phase transition (GO:1901990)	7	3.57	5.24E-03	4.65E-02
regulation of nuclear division (GO:0051783)	5	4.93	5.32E-03	4.69E-02
positive regulation of lymphocyte mediated immunity (GO:0002708)	7	3.51	5.70E-03	4.98E-02

Table 2. Gene Ontologies in Winnie-Prolapse mice (Panther GO-Slim classification)

PANTHER GO-Slim Biological Process	Gene Count	fold Enrichment	raw P-value	FDR
immune system process (GO:0002376)	346	3.72	9.38E-85	2.08E-81
immune response (GO:0006955)	298	3.89	6.39E-76	7.10E-73
defence response (GO:0006952)	216	4.15	2.19E-58	1.62E-55
regulation of immune system process (GO:0002682)	177	4.09	5.02E-47	2.23E-44
positive regulation of immune system process (GO:0002684)	155	4.39	2.99E-44	1.10E-41
biological process involved in interspecies interaction between organisms (GO:0044419)	176	3.78	5.08E-43	1.25E-40
response to biotic stimulus (GO:0009607)	176	3.78	6.32E-43	1.40E-40
response to another organism (GO:0051707)	176	3.78	5.08E-43	1.41E-40
response to external biotic stimulus (GO:0043207)	176	3.78	5.08E-43	1.61E-40
regulation of immune response (GO:0050776)	140	4.37	7.97E-40	1.61E-37
cell activation (GO:0001775)	134	4.46	7.49E-39	1.39E-36
leukocyte activation (GO:0045321)	131	4.54	1.16E-38	1.98E-36
lymphocyte activation (GO:0046649)	127	4.67	1.63E-38	2.59E-36
regulation of cell activation (GO:0050865)	118	5.05	2.20E-38	3.25E-36
regulation of leukocyte activation (GO:0002694)	117	5.05	4.75E-38	6.58E-36
defence response to other organism (GO:0098542)	153	3.82	7.04E-38	9.20E-36
immune response-regulating signaling pathway (GO:0002764)	119	4.81	4.57E-37	5.34E-35
regulation of lymphocyte activation (GO:0051249)	112	5.14	4.90E-37	5.44E-35
response to external stimulus (GO:0009605)	208	2.91	1.63E-36	1.73E-34
positive regulation of immune response (GO:0050778)	126	4.35	9.34E-36	9.01E-34
immune response-regulating cell surface receptor signaling pathway (GO:0002768)	110	4.86	1.09E-34	1.00E-32
immune response-activating signal transduction (GO:0002757)	109	4.9	1.31E-34	1.12E-32

immune response-activating cell surface receptor signaling pathway (GO:0002429)	109	4.9	1.31E-34	1.16E-32
positive regulation of lymphocyte activation (GO:0051251)	104	5.08	4.21E-34	3.46E-32
positive regulation of cell activation (GO:0050867)	106	4.93	7.27E-34	5.57E-32
positive regulation of leukocyte activation (GO:0002696)	106	4.93	7.27E-34	5.77E-32
activation of immune response (GO:0002253)	110	4.69	1.38E-33	1.02E-31
immune effector process (GO:0002252)	120	4.26	2.39E-33	1.71E-31
leukocyte mediated immunity (GO:0002443)	113	4.48	4.74E-33	3.29E-31
response to bacterium (GO:0009617)	129	3.89	1.13E-32	7.60E-31
adaptive immune response based on somatic recombination of immune receptors built from immunoglobulin superfamily domains (GO:0002460)	108	4.55	4.28E-32	2.79E-30
lymphocyte mediated immunity (GO:0002449)	109	4.49	5.81E-32	3.68E-30
antigen receptor-mediated signaling pathway (GO:0050851)	100	4.88	9.58E-32	5.91E-30
adaptive immune response (GO:0002250)	110	4.22	2.09E-30	1.25E-28
positive regulation of response to stimulus (GO:0048584)	168	2.96	2.57E-30	1.50E-28
innate immune response (GO:0045087)	123	3.78	3.50E-30	1.99E-28
immunoglobulin mediated immune response (GO:0016064)	93	4.85	1.99E-29	1.08E-27
B cell mediated immunity (GO:0019724)	93	4.85	1.99E-29	1.11E-27
B cell receptor signaling pathway (GO:0050853)	86	4.97	7.20E-28	3.81E-26
defence response to bacterium (GO:0042742)	108	3.91	1.30E-27	6.71E-26
phagocytosis (GO:0006909)	90	4.56	5.44E-27	2.74E-25
cell recognition (GO:0008037)	87	4.65	1.35E-26	6.67E-25
phagocytosis, engulfment (GO:0006911)	83	4.82	3.00E-26	1.45E-24
complement activation (GO:0006956)	81	4.81	1.37E-25	6.45E-24
production of molecular mediator of immune response (GO:0002440)	86	4.51	1.45E-25	6.70E-24
B cell activation (GO:0042113)	86	4.49	1.89E-25	8.55E-24
protein activation cascade (GO:0072376)	81	4.76	2.40E-25	1.07E-23
humoral immune response (GO:0006959)	100	3.69	3.87E-24	1.65E-22
response to cytokine (GO:0034097)	78	4.56	1.58E-23	6.60E-22
cellular response to cytokine stimulus (GO:0071345)	72	4.5	1.43E-21	5.86E-20
endocytosis (GO:0006897)	92	3.06	7.94E-18	2.80E-16

membrane invagination (GO:0010324)	92	3.04	1.10E-17	3.83E-16
vesicle budding from membrane (GO:0006900)	94	2.93	4.58E-17	1.56E-15
inflammatory response (GO:0006954)	58	4.3	6.30E-17	2.12E-15
cytokine-mediated signaling pathway (GO:0019221)	52	3.94	4.39E-14	1.41E-12
response to organic substance (GO:0010033)	125	2.16	2.62E-13	8.31E-12
response to chemical (GO:0042221)	158	1.85	9.10E-12	2.77E-10
T cell activation (GO:0042110)	39	4.19	1.39E-11	4.17E-10
leukocyte cell-cell adhesion (GO:0007159)	30	5.56	1.45E-11	4.28E-10
vesicle organization (GO:0016050)	103	2.19	1.58E-11	4.63E-10
leukocyte migration (GO:0050900)	33	4.93	1.77E-11	5.11E-10
cellular response to chemical stimulus (GO:0070887)	122	1.98	6.80E-11	1.93E-09
cellular response to organic substance (GO:0071310)	101	2.12	9.16E-11	2.57E-09
regulation of cell-cell adhesion (GO:0022407)	27	5.18	5.01E-10	1.37E-08
membrane organization (GO:0061024)	116	1.94	5.12E-10	1.39E-08
regulation of T cell activation (GO:0050863)	26	5.37	5.72E-10	1.53E-08
regulation of catalytic activity (GO:0050790)	109	1.98	6.22E-10	1.64E-08
cell-cell adhesion (GO:0098609)	54	2.79	9.16E-10	2.39E-08
regulation of cell adhesion (GO:0030155)	31	4.27	1.17E-09	3.01E-08
regulation of hydrolase activity (GO:0051336)	70	2.33	2.78E-09	6.79E-08
regulation of leukocyte cell-cell adhesion (GO:1903037)	24	5.16	4.87E-09	1.14E-07
regulation of molecular function (GO:0065009)	126	1.79	7.87E-09	1.80E-07
cell migration (GO:0016477)	62	2.4	1.08E-08	2.45E-07
granulocyte chemotaxis (GO:0071621)	22	5.25	1.63E-08	3.66E-07
cell adhesion (GO:0007155)	79	2.07	2.76E-08	6.06E-07
biological adhesion (GO:0022610)	79	2.07	2.94E-08	6.40E-07
granulocyte migration (GO:0097530)	22	4.82	5.37E-08	1.15E-06
leukocyte chemotaxis (GO:0030595)	23	4.58	5.78E-08	1.22E-06
negative regulation of hydrolase activity (GO:0051346)	31	3.43	7.39E-08	1.55E-06
regulation of peptidase activity (GO:0052547)	33	3.17	1.39E-07	2.84E-06
regulation of endopeptidase activity (GO:0052548)	32	3.18	1.93E-07	3.79E-06
locomotion (GO:0040011)	75	2	2.00E-07	3.86E-06

regulation of leukocyte proliferation (GO:0070663)	16	6.37	2.06E-07	3.87E-06
regulation of mononuclear cell proliferation (GO:0032944)	16	6.37	2.06E-07	3.91E-06
regulation of lymphocyte proliferation (GO:0050670)	16	6.37	2.06E-07	3.94E-06
positive regulation of T cell activation (GO:0050870)	19	5.1	2.18E-07	4.00E-06
positive regulation of leukocyte cell-cell adhesion (GO:1903039)	19	5.1	2.18E-07	4.03E-06
myeloid leukocyte migration (GO:0097529)	22	4.3	2.66E-07	4.84E-06
positive regulation of cell-cell adhesion (GO:0022409)	19	4.98	2.93E-07	5.25E-06
negative regulation of peptidase activity (GO:0010466)	28	3.42	3.35E-07	5.95E-06
skin development (GO:0043588)	18	5.23	3.44E-07	6.06E-06
negative regulation of endopeptidase activity (GO:0010951)	27	3.45	4.58E-07	8.00E-06
cell motility (GO:0048870)	63	2.09	5.24E-07	8.95E-06
localization of cell (GO:0051674)	63	2.09	5.24E-07	9.02E-06
neutrophil migration (GO:1990266)	19	4.75	5.21E-07	9.03E-06
cell chemotaxis (GO:0060326)	24	3.74	6.18E-07	1.04E-05
chemotaxis (GO:0006935)	42	2.52	6.90E-07	1.13E-05
taxis (GO:0042330)	42	2.52	6.90E-07	1.14E-05
negative regulation of proteolysis (GO:0045861)	28	3.27	7.04E-07	1.15E-05
positive regulation of cell adhesion (GO:0045785)	20	4.39	7.14E-07	1.16E-05
negative regulation of catalytic activity (GO:0043086)	37	2.69	8.66E-07	1.39E-05
tissue development (GO:0009888)	37	2.6	2.37E-06	3.63E-05
external encapsulating structure organization (GO:0045229)	24	3.35	3.04E-06	4.60E-05
extracellular matrix organization (GO:0030198)	24	3.35	3.04E-06	4.63E-05
positive regulation of immune effector process (GO:0002699)	23	3.43	3.46E-06	5.19E-05
epidermis development (GO:0008544)	17	4.46	4.06E-06	6.01E-05
intermediate filament organization (GO:0045109)	17	4.46	4.06E-06	6.05E-05
extracellular structure organization (GO:0043062)	24	3.26	4.39E-06	6.46E-05
regulation of proteolysis (GO:0030162)	38	2.42	6.93E-06	1.01E-04
regulation of immune effector process (GO:0002697)	24	3.15	7.46E-06	1.07E-04
intermediate filament-based process (GO:0045103)	18	3.95	8.31E-06	1.18E-04

intermediate filament cytoskeleton organization (GO:0045104)	18	3.95	8.31E-06	1.18E-04
defence response to symbiont (GO:0140546)	16	4.41	8.63E-06	1.20E-04
defence response to virus (GO:0051607)	16	4.41	8.63E-06	1.21E-04
cytokine production (GO:0001816)	25	3.02	8.84E-06	1.22E-04
negative regulation of cell activation (GO:0050866)	12	6.14	9.12E-06	1.24E-04
negative regulation of leukocyte activation (GO:0002695)	12	6.14	9.12E-06	1.25E-04
response to virus (GO:0009615)	16	4.19	1.43E-05	1.90E-04
negative regulation of lymphocyte activation (GO:0051250)	11	6.22	1.97E-05	2.57E-04
antigen processing and presentation (GO:0019882)	20	3.31	2.31E-05	2.96E-04
response to lipid (GO:0033993)	27	2.76	2.30E-05	2.97E-04
response to lipopolysaccharide (GO:0032496)	22	3.07	2.41E-05	3.07E-04
negative regulation of cellular protein metabolic process (GO:0032269)	41	2.17	2.47E-05	3.13E-04
negative regulation of molecular function (GO:0044092)	38	2.24	2.61E-05	3.29E-04
negative regulation of cell-cell adhesion (GO:0022408)	11	5.91	2.82E-05	3.54E-04
response to molecule of bacterial origin (GO:0002237)	22	2.99	3.34E-05	4.14E-04
negative regulation of immune system process (GO:0002683)	15	4.03	3.82E-05	4.71E-04
regulation of cytokine production (GO:0001817)	22	2.96	3.92E-05	4.81E-04
response to oxygen-containing compound (GO:1901700)	42	2.1	4.03E-05	4.92E-04
negative regulation of T cell activation (GO:0050868)	10	6.32	4.27E-05	5.15E-04
negative regulation of leukocyte cell-cell adhesion (GO:1903038)	10	6.32	4.27E-05	5.17E-04
leukocyte proliferation (GO:0070661)	16	3.74	4.48E-05	5.23E-04
lymphocyte proliferation (GO:0046651)	16	3.74	4.48E-05	5.26E-04
mitotic nuclear division (GO:0140014)	49	1.96	4.45E-05	5.28E-04
mononuclear cell proliferation (GO:0032943)	16	3.74	4.48E-05	5.29E-04
mitotic cell cycle process (GO:1903047)	49	1.96	4.45E-05	5.31E-04
mitotic cell cycle (GO:0000278)	49	1.96	4.45E-05	5.34E-04
negative regulation of protein metabolic process (GO:0051248)	41	2.12	4.79E-05	5.56E-04
epidermal cell differentiation (GO:0009913)	14	4.18	5.00E-05	5.75E-04

negative regulation of cell adhesion (GO:0007162)	13	4.51	4.98E-05	5.76E-04
T cell proliferation (GO:0042098)	11	5.37	5.49E-05	6.26E-04
regulation of T cell proliferation (GO:0042129)	11	5.37	5.49E-05	6.29E-04
positive regulation of cytokine production (GO:0001819)	12	4.78	6.21E-05	7.03E-04
T cell receptor signaling pathway (GO:0050852)	14	4.07	6.36E-05	7.16E-04
epithelial cell differentiation (GO:0030855)	17	3.38	7.33E-05	8.14E-04
mononuclear cell migration (GO:0071674)	14	3.96	8.02E-05	8.86E-04
regulation of MAPK cascade (GO:0043408)	29	2.4	8.19E-05	9.00E-04
epithelium development (GO:0060429)	21	2.86	8.78E-05	9.60E-04
regulation of cell population proliferation (GO:0042127)	36	2.16	1.05E-04	1.12E-03
supramolecular fibber organisation (GO:0097435)	54	1.81	1.16E-04	1.24E-03
MAPK cascade (GO:0000165)	31	2.27	1.20E-04	1.26E-03
regulation of leukocyte mediated immunity (GO:0002703)	20	2.87	1.23E-04	1.30E-03
regulation of cellular protein metabolic process (GO:0032268)	76	1.63	1.43E-04	1.50E-03
positive regulation of leukocyte mediated immunity (GO:0002705)	18	3.02	1.52E-04	1.57E-03
positive regulation of peptidyl-tyrosine phosphorylation (GO:0050731)	10	5.12	1.63E-04	1.66E-03
lymphocyte migration (GO:0072676)	12	4.16	1.77E-04	1.79E-03
positive regulation of lymphocyte mediated immunity (GO:0002708)	17	3.1	1.80E-04	1.81E-03
integrin-mediated signaling pathway (GO:0007229)	14	3.58	1.91E-04	1.91E-03
cellular response to biotic stimulus (GO:0071216)	20	2.76	1.93E-04	1.92E-03
positive regulation of molecular function (GO:0044093)	61	1.7	2.14E-04	2.12E-03
cellular response to lipopolysaccharide (GO:0071222)	19	2.8	2.35E-04	2.30E-03
regulation of lymphocyte mediated immunity (GO:0002706)	18	2.89	2.45E-04	2.38E-03
regulation of adaptive immune response (GO:0002819)	17	2.99	2.51E-04	2.42E-03
cell population proliferation (GO:0008283)	38	2	2.70E-04	2.57E-03
ERK1 and ERK2 cascade (GO:0070371)	14	3.42	2.83E-04	2.68E-03
regulation of ERK1 and ERK2 cascade (GO:0070372)	14	3.42	2.83E-04	2.69E-03

toll-like receptor signaling pathway (GO:0002224)	10	4.67	2.91E-04	2.74E-03
regulation of lipase activity (GO:0060191)	15	3.22	2.96E-04	2.75E-03
zymogen activation (GO:0031638)	11	4.22	2.95E-04	2.75E-03
T cell differentiation (GO:0030217)	8	6.14	2.93E-04	2.75E-03
cellular response to molecule of bacterial origin (GO:0071219)	19	2.72	3.14E-04	2.90E-03
cell-matrix adhesion (GO:0007160)	14	3.34	3.43E-04	3.16E-03
vesicle-mediated transport (GO:0016192)	119	1.43	3.49E-04	3.20E-03
cell adhesion mediated by integrin (GO:0033627)	12	3.79	3.53E-04	3.22E-03
pattern recognition receptor signaling pathway (GO:0002221)	10	4.48	3.82E-04	3.46E-03
peptidyl-tyrosine phosphorylation (GO:0018108)	13	3.49	3.91E-04	3.52E-03
regulation of protein metabolic process (GO:0051246)	78	1.56	4.13E-04	3.69E-03
positive regulation of adaptive immune response (GO:0002821)	15	3.1	4.19E-04	3.72E-03
regulation of cell cycle (GO:0051726)	37	1.95	4.33E-04	3.83E-03
cell differentiation (GO:0030154)	116	1.42	4.86E-04	4.26E-03
immune system development (GO:0002520)	20	2.59	4.88E-04	4.27E-03
multicellular organism development (GO:0007275)	127	1.39	4.96E-04	4.30E-03
T cell mediated immunity (GO:0002456)	14	3.2	4.94E-04	4.30E-03
intracellular signal transduction (GO:0035556)	111	1.43	5.45E-04	4.71E-03
peptidyl-tyrosine modification (GO:0018212)	13	3.33	5.75E-04	4.93E-03
regulation of cell cycle process (GO:0010564)	27	2.17	5.80E-04	4.95E-03
positive regulation of ERK1 and ERK2 cascade (GO:0070374)	13	3.33	5.75E-04	4.95E-03
anatomical structure formation involved in morphogenesis (GO:0048646)	24	2.28	5.99E-04	5.06E-03
response to interleukin-1 (GO:0070555)	11	3.81	5.95E-04	5.06E-03
animal organ development (GO:0048513)	65	1.62	5.98E-04	5.07E-03
cellular developmental process (GO:0048869)	116	1.41	6.08E-04	5.11E-03
positive regulation of cell cycle process (GO:0090068)	10	4.13	6.32E-04	5.29E-03
negative regulation of T cell proliferation (GO:0042130)	7	6.27	6.45E-04	5.37E-03
negative regulation of multicellular organismal process (GO:0051241)	16	2.82	6.61E-04	5.48E-03

regulation of phospholipase activity (GO:0010517)	14	3.07	6.98E-04	5.72E-03
positive regulation of catalytic activity (GO:0043085)	47	1.75	7.22E-04	5.89E-03
system development (GO:0048731)	115	1.41	7.37E-04	5.99E-03
chemical homeostasis (GO:0048878)	47	1.74	7.64E-04	6.19E-03
cellular response to lipid (GO:0071396)	20	2.44	7.94E-04	6.41E-03
movement of cell or subcellular component (GO:0006928)	89	1.47	7.99E-04	6.42E-03
carboxylic acid metabolic process (GO:0019752)	52	1.69	8.01E-04	6.42E-03
oxoacid metabolic process (GO:0043436)	52	1.67	8.89E-04	7.00E-03
positive regulation of T cell proliferation (GO:0042102)	7	5.79	9.20E-04	7.20E-03
positive regulation of cell migration (GO:0030335)	15	2.83	9.36E-04	7.24E-03
positive regulation of cell cycle (GO:0045787)	10	3.84	1.00E-03	7.69E-03
organic acid biosynthetic process (GO:0016053)	21	2.4	1.07E-03	8.11E-03
carboxylic acid biosynthetic process (GO:0046394)	21	2.4	1.07E-03	8.14E-03
regulation of peptidyl-tyrosine phosphorylation (GO:0050730)	10	3.71	1.25E-03	9.21E-03
hematopoietic or lymphoid organ development (GO:0048534)	18	2.48	1.32E-03	9.66E-03
hemopoiesis (GO:0030097)	18	2.48	1.32E-03	9.69E-03
unsaturated fatty acid metabolic process (GO:0033559)	11	3.38	1.35E-03	9.83E-03
positive regulation of hydrolase activity (GO:0051345)	33	1.9	1.41E-03	1.02E-02
regulation of mitotic cell cycle (GO:0007346)	21	2.28	1.44E-03	1.03E-02
unsaturated fatty acid biosynthetic process (GO:0006636)	6	6.45	1.43E-03	1.03E-02
blood circulation (GO:0008015)	17	2.57	1.48E-03	1.06E-02
muscle cell development (GO:0055001)	10	3.58	1.54E-03	1.09E-02
regulation of nuclear division (GO:0051783)	10	3.58	1.54E-03	1.10E-02
circulatory system process (GO:0003013)	17	2.54	1.60E-03	1.13E-02
positive regulation of cell motility (GO:2000147)	15	2.64	1.67E-03	1.17E-02
regulation of intracellular signal transduction (GO:1902531)	59	1.57	1.75E-03	1.19E-02
homeostatic process (GO:0042592)	51	1.63	1.71E-03	1.19E-02
regulation of B cell proliferation (GO:0030888)	5	7.68	2.12E-03	1.43E-02

regulation of cytokine production involved in immune response (GO:0002718)	5	7.68	2.12E-03	1.44E-02
nuclear division (GO:0000280)	55	1.58	2.17E-03	1.46E-02
vasculature development (GO:0001944)	16	2.53	2.31E-03	1.53E-02
phospholipase C-activating G protein-coupled receptor signaling pathway (GO:0007200)	12	2.93	2.29E-03	1.53E-02
regulation of leukocyte migration (GO:0002685)	8	4.09	2.30E-03	1.53E-02
regulation of blood pressure (GO:0008217)	11	3.11	2.33E-03	1.54E-02
negative regulation of MAPK cascade (GO:0043409)	9	3.58	2.62E-03	1.72E-02
regulation of phospholipase C activity (GO:1900274)	12	2.87	2.68E-03	1.74E-02
positive regulation of phospholipase C activity (GO:0010863)	12	2.87	2.68E-03	1.74E-02
activation of phospholipase C activity (GO:0007202)	12	2.87	2.68E-03	1.75E-02
antimicrobial humoral response (GO:0019730)	19	2.24	2.90E-03	1.87E-02
positive regulation of locomotion (GO:0040017)	15	2.6	2.96E-03	1.89E-02
positive regulation of cellular component movement (GO:0051272)	15	2.6	2.96E-03	1.89E-02
inositol phosphate-mediated signaling (GO:0048016)	13	2.69	2.93E-03	1.89E-02
positive regulation of lipase activity (GO:0060193)	12	2.8	3.12E-03	1.96E-02
positive regulation of phospholipase activity (GO:0010518)	12	2.8	3.12E-03	1.96E-02
monocarboxylic acid biosynthetic process (GO:0072330)	12	2.8	3.12E-03	1.97E-02
regulation of mitotic nuclear division (GO:0007088)	9	3.45	3.20E-03	2.00E-02
blood vessel morphogenesis (GO:0048514)	15	2.52	3.36E-03	2.09E-02
cell-substrate adhesion (GO:0031589)	15	2.52	3.36E-03	2.10E-02
organelle fission (GO:0048285)	56	1.54	3.50E-03	2.16E-02
regulation of response to external stimulus (GO:0032101)	19	2.17	3.60E-03	2.22E-02
blood vessel development (GO:0001568)	15	2.48	3.62E-03	2.23E-02
organic acid metabolic process (GO:0006082)	54	1.55	3.75E-03	2.30E-02
response to tumor necrosis factor (GO:0034612)	11	2.88	3.82E-03	2.32E-02
fatty acid biosynthetic process (GO:0006633)	11	2.88	3.82E-03	2.33E-02

negative regulation of cell population proliferation (GO:0008285)	10	3.07	3.92E-03	2.37E-02
protein processing (GO:0016485)	16	2.32	3.96E-03	2.38E-02
tube development (GO:0035295)	16	2.32	3.96E-03	2.38E-02
cellular lipid metabolic process (GO:0044255)	54	1.54	4.03E-03	2.41E-02
fatty acid metabolic process (GO:0006631)	23	1.99	4.27E-03	2.54E-02
monocarboxylic acid metabolic process (GO:0032787)	29	1.83	4.53E-03	2.67E-02
regulation of cell migration (GO:0030334)	21	2.03	4.55E-03	2.67E-02
cell cycle process (GO:0022402)	64	1.47	4.62E-03	2.70E-02
arginine catabolic process (GO:0006527)	4	8.6	4.65E-03	2.70E-02
tube morphogenesis (GO:0035239)	15	2.37	4.68E-03	2.71E-02
defense response to fungus (GO:0050832)	5	5.97	4.63E-03	2.71E-02
lipid metabolic process (GO:0006629)	62	1.47	5.16E-03	2.97E-02
inorganic ion homeostasis (GO:0098771)	36	1.7	5.18E-03	2.98E-02
cell cycle phase transition (GO:0044770)	22	2.02	5.24E-03	3.00E-02
actin cytoskeleton organization (GO:0030036)	43	1.59	5.54E-03	3.14E-02
positive regulation of signal transduction (GO:0009967)	41	1.61	5.93E-03	3.31E-02
ion homeostasis (GO:0050801)	37	1.68	6.07E-03	3.38E-02
actin filament-based process (GO:0030029)	44	1.58	6.13E-03	3.40E-02
myofibril assembly (GO:0030239)	7	3.76	6.22E-03	3.40E-02
muscle tissue development (GO:0060537)	7	3.76	6.22E-03	3.41E-02
cellular response to oxygen-containing compound (GO:1901701)	30	1.76	6.21E-03	3.42E-02
sister chromatid segregation (GO:0000819)	15	2.27	6.20E-03	3.42E-02
striated muscle cell development (GO:0055002)	7	3.76	6.22E-03	3.42E-02
circulatory system development (GO:0072359)	22	1.95	6.19E-03	3.43E-02
regulation of multicellular organismal process (GO:0051239)	44	1.57	6.41E-03	3.50E-02
regulation of cell cycle phase transition (GO:1901987)	17	2.15	6.67E-03	3.59E-02
regulation of systemic arterial blood pressure (GO:0003073)	9	3.02	6.63E-03	3.59E-02
positive regulation of MAPK cascade (GO:0043410)	17	2.15	6.67E-03	3.60E-02
cation homeostasis (GO:0055080)	35	1.66	7.09E-03	3.79E-02

response to type I interferon (GO:0034340)	4	7.16	7.24E-03	3.84E-02
type I interferon signaling pathway (GO:0060337)	4	7.16	7.24E-03	3.85E-02
muscle structure development (GO:0061061)	13	2.45	7.65E-03	4.05E-02
alpha-amino acid metabolic process (GO:1901605)	17	2.1	7.69E-03	4.06E-02
cellular chemical homeostasis (GO:0055082)	33	1.67	7.88E-03	4.16E-02
positive regulation of intracellular signal transduction (GO:1902533)	27	1.8	7.95E-03	4.18E-02
regulation of biological quality (GO:0065008)	107	1.31	8.53E-03	4.45E-02
positive regulation of cell population proliferation (GO:0008284)	21	1.93	8.52E-03	4.46E-02
cellular response to tumor necrosis factor (GO:0071356)	10	2.69	8.57E-03	4.46E-02
muscle cell differentiation (GO:0042692)	10	2.69	8.57E-03	4.47E-02
mitotic sister chromatid segregation (GO:0000070)	13	2.37	8.90E-03	4.58E-02
regulation of cell motility (GO:2000145)	21	1.91	8.94E-03	4.60E-02
regulation of protein kinase activity (GO:0045859)	18	2.08	9.08E-03	4.64E-02
cell cycle (GO:0007049)	66	1.42	9.43E-03	4.78E-02
regulation of locomotion (GO:0040012)	21	1.9	9.41E-03	4.79E-02

Table 3. Genes present in Immune system processes

Gene.Name	Winnie-Prolapse log2fc	Winnies log2fc	FDR	P value
<i>Wap</i>	13.79714	0.677091	1.41E-16	1.67E-18
<i>Ltf</i>	9.673253	4.626596	1.76E-22	4.08E-25
<i>Il1f5</i>	9.662347	0	5.97E-16	8.30E-18
<i>Cxcr2</i>	9.57748	5.82737	1.69E-12	4.71E-14
<i>Cxcl3</i>	9.285582	4.628674	1.70E-21	5.26E-24
<i>Clec4e</i>	8.075212	3.789273	2.16E-15	3.42E-17
<i>Wfdc21</i>	8.048884	1.692075	1.76E-20	7.66E-23
<i>Cxcl2</i>	8.04164	3.823978	7.21E-21	2.52E-23
<i>Skint2</i>	7.937053	0	3.79E-10	1.77E-11
<i>Cxcl9</i>	7.696753	5.958386	3.81E-25	3.37E-28
<i>Ighv1-47</i>	7.454192	2.898153	1.37E-11	4.56E-13
<i>Fpr1</i>	7.339883	3.575324	5.07E-19	3.32E-21
<i>Ngp</i>	7.285662	2.675519	2.48E-07	2.21E-08
<i>Btn1a1</i>	7.28434	0.956028	1.12E-09	5.86E-11
<i>Trem1</i>	6.947237	3.215434	2.40E-13	5.55E-15
<i>Il1a</i>	6.929227	1.825899	3.79E-27	1.05E-30
<i>Skint9</i>	6.526939	0.687965	9.36E-11	3.81E-12
<i>Ighv2-9-1</i>	6.505299	4.464995	1.35E-09	7.23E-11
<i>Wfdc5</i>	6.384617	0.974527	1.82E-10	8.02E-12
<i>Fpr2</i>	6.349307	2.460612	1.64E-21	4.81E-24
<i>Ighv1-58</i>	6.290089	3.696418	2.17E-11	7.60E-13
<i>Igkv8-18</i>	6.252258	4.913677	2.66E-05	4.11E-06
<i>Wfdc12</i>	6.247012	2.464644	5.66E-11	2.20E-12
<i>Wfdc3</i>	6.059594	-0.3789	5.11E-11	1.96E-12
<i>Sele</i>	5.861775	4.043303	5E-06	6.33E-07
<i>Clec4d</i>	5.836774	2.396235	7.00E-15	1.21E-16
<i>Ighv1-4</i>	5.758344	4.33982	3.42E-11	1.26E-12
<i>Ceacam12</i>	5.754638	3.901131	2.10E-07	1.83E-08
<i>Ighv9-1</i>	5.741213	3.484521	1.02E-07	8.37E-09
<i>Reg3g</i>	5.694923	1.38973	1.22E-08	8.10E-10
<i>Wfdc18</i>	5.68324	1.69169	1.85E-16	2.26E-18
<i>Ighv7-3</i>	5.605875	3.597133	2.71E-22	6.90E-25
<i>Trem3</i>	5.586044	2.166659	1.08E-12	2.82E-14
<i>Fcrlb</i>	5.484349	3.498345	1.84E-16	2.23E-18
<i>Gm9733</i>	5.479533	3.435461	5.34E-11	2.06E-12
<i>Csf3r</i>	5.431781	3.21324	2.85E-14	5.60E-16
<i>Ighv1-18</i>	5.393028	3.089698	9.86E-18	8.69E-20
<i>Ighv7-1</i>	5.297675	5.068151	5.29E-11	2.04E-12
<i>Sirpb1c</i>	5.2861	3.575203	1.50E-16	1.77E-18

<i>Cxcl5</i>	5.274331	2.378757	3.07E-12	8.87E-14
<i>Ighv5-4</i>	5.225168	2.70456	6.42E-11	2.54E-12
<i>Cxcl17</i>	5.050434	2.239488	6.83E-07	6.83E-08
<i>Ccl3</i>	5.01125	1.430124	2.97E-16	3.88E-18
<i>Ighv13-2</i>	4.904557	2.653231	3.37E-07	3.11E-08
<i>Ighv1-7</i>	4.903486	4.978743	1.23E-10	5.15E-12
<i>Ubd</i>	4.900364	3.986475	5.23E-13	1.29E-14
<i>Igkv8-28</i>	4.889251	2.865357	1.06E-14	1.88E-16
<i>Ighv1-80</i>	4.865691	4.087993	7.84E-14	1.65E-15
<i>Defb1</i>	4.851038	-0.95944	5.34E-10	2.59E-11
<i>Ighv5-6</i>	4.834454	3.449645	2.10E-10	9.36E-12
<i>Ighv2-3</i>	4.82183	4.256638	2.65E-11	9.39E-13
<i>Igkv17-127</i>	4.789131	3.063	1.41E-14	2.59E-16
<i>Ccl4</i>	4.769346	1.522419	1.67E-13	3.75E-15
<i>Igkv14-126</i>	4.764345	1.656057	2.36E-09	1.33E-10
<i>Igkv4-57</i>	4.724948	3.944651	1.83E-13	4.14E-15
<i>Slpi</i>	4.71789	3.64728	9.88E-23	2.03E-25
<i>Ighv7-4</i>	4.684632	1.643703	1.06E-10	4.38E-12
<i>Ighv1-81</i>	4.683924	2.537778	9.09E-09	5.87E-10
<i>Ighv2-2</i>	4.64371	3.122869	4.53E-17	4.47E-19
<i>Ighv1-59</i>	4.56572	2.364279	4.25E-08	3.19E-09
<i>Ctla4</i>	4.565291	3.855765	3.07E-25	2.03E-28
<i>Igkv6-32</i>	4.55941	3.397228	4.54E-12	1.36E-13
<i>Igkv4-50</i>	4.54687	3.961605	4.19E-06	5.22E-07
<i>Sirpb1b</i>	4.531157	2.812032	9.87E-16	1.43E-17
<i>Ccl2</i>	4.512716	1.994133	1.52E-15	2.31E-17
<i>Ifitm6</i>	4.486545	0.611311	8.50E-17	9.42E-19
<i>Igkv3-12</i>	4.469066	3.21681	7.76E-10	3.91E-11
<i>Igkv4-91</i>	4.464202	2.342969	3.90E-13	9.28E-15
<i>Igkv3-7</i>	4.451171	4.097348	7.96E-08	6.34E-09
<i>Igkv8-16</i>	4.437435	3.954778	0.000229	4.57E-05
<i>Ighv1-66</i>	4.432371	3.136441	6.38E-07	6.32E-08
<i>Ighv1-63</i>	4.416949	4.034501	2.13E-08	1.49E-09
<i>Cxcl1</i>	4.396016	1.755752	2.18E-11	7.65E-13
<i>Sema7a</i>	4.378492	1.845943	2.99E-20	1.41E-22
<i>Igkv4-80</i>	4.368332	4.09828	4.29E-08	3.22E-09
<i>Ighv1-20</i>	4.301311	3.487162	0.000243	4.86E-05
<i>Ighv10-1</i>	4.295259	3.307261	1.49E-12	4.03E-14
<i>Ighv1-75</i>	4.289454	1.245735	3.70E-16	4.88E-18
<i>Rnase2b</i>	4.285797	-0.16247	1.48E-10	6.35E-12
<i>Igkv3-2</i>	4.264848	3.012112	1.52E-09	8.21E-11
<i>Cxcl10</i>	4.201982	2.132884	1.66E-14	3.14E-16
<i>Igkv4-63</i>	4.175916	1.61337	9.83E-10	5.10E-11
<i>Igkv1-135</i>	4.173485	4.074774	1.09E-12	2.87E-14

<i>Ighv2-6</i>	4.17276	4.434282	6.15E-07	6.07E-08
<i>Ighv3-6</i>	4.168163	3.298321	7.96E-11	3.21E-12
<i>Ighv1-9</i>	4.162341	3.774761	1.34E-08	8.99E-10
<i>Ighv2-6-8</i>	4.14612	1.451429	0.000136	2.56E-05
<i>Igkv10-96</i>	4.114697	2.984275	8.75E-18	7.61E-20
<i>Ighv5-17</i>	4.106789	4.12219	7.99E-08	6.37E-09
<i>Igkv10-94</i>	4.093792	1.200963	6.03E-15	1.03E-16
<i>Ighv14-3</i>	4.088289	5.641494	1.56E-07	1.33E-08
<i>Igkv8-30</i>	4.084664	3.853889	2.14E-09	1.20E-10
<i>Selp</i>	4.078386	1.883535	3.03E-07	2.75E-08
<i>Igkv4-74</i>	4.07537	3.363564	4.12E-08	3.09E-09
<i>Igkv1-122</i>	4.06511	1.103854	9.74E-08	7.93E-09
<i>Igkv9-123</i>	4.061398	3.221544	3.33E-05	5.3E-06
<i>Ighv1-12</i>	4.056259	3.583702	2.76E-07	2.48E-08
<i>Ighv6-3</i>	4.055164	3.936229	9.33E-09	6.04E-10
<i>Igkv6-13</i>	4.051956	3.72783	1.69E-06	1.88E-07
<i>Ppbbp</i>	4.047959	0.310943	0.000377	8E-05
<i>Ighv1-77</i>	4.046407	4.843062	8.65E-06	1.17E-06
<i>Igkv13-84</i>	4.024928	3.006028	4.52E-10	2.16E-11
<i>Igkv1-117</i>	4.016164	1.868956	4.22E-15	7.04E-17
<i>Ighv8-8</i>	3.994826	2.856608	1.28E-08	8.54E-10
<i>Ighv2-9</i>	3.930835	2.230379	3.81E-11	1.42E-12
<i>Tnfrsf23</i>	3.92374	1.571318	1.76E-24	2.25E-27
<i>Gata3</i>	3.907902	0.67444	1.30E-22	2.95E-25
<i>Ighv5-16</i>	3.907073	2.858196	2.18E-05	3.29E-06
<i>Ighv1-54</i>	3.903135	3.910792	1.79E-05	2.64E-06
<i>Ighv3-8</i>	3.893687	3.024879	2.11E-05	3.17E-06
<i>Igkv1-110</i>	3.884964	2.474799	2.98E-21	9.74E-24
<i>H2-Q7</i>	3.883834	2.790798	1.23E-15	1.85E-17
<i>Igkv9-124</i>	3.879545	3.035411	1.14E-05	1.59E-06
<i>Igkv6-20</i>	3.874212	2.148574	1.60E-12	4.41E-14
<i>Ccl8</i>	3.863153	2.802701	5.91E-17	6.25E-19
<i>Ccl7</i>	3.860729	2.1223	1.15E-15	1.71E-17
<i>Gm5150</i>	3.851839	2.954474	1.07E-16	1.23E-18
<i>Ighv10-3</i>	3.850786	3.015776	7.15E-07	7.21E-08
<i>Igkv12-89</i>	3.837397	2.33404	5.92E-07	5.83E-08
<i>Igkv4-86</i>	3.836077	3.794624	1.96E-06	2.22E-07
<i>Igkv3-1</i>	3.818113	4.095785	7.05E-07	7.09E-08
<i>Igkv12-98</i>	3.810619	3.137083	3.87E-07	3.64E-08
<i>Ighv1-53</i>	3.782595	2.833715	1.69E-11	5.71E-13
<i>Igkv4-69</i>	3.779075	3.402445	0.001409	0.000353
<i>Lbp</i>	3.759162	1.787926	3.50E-12	1.02E-13
<i>Ighv1-52</i>	3.753901	2.955937	4.20E-07	3.99E-08
<i>Igkv12-38</i>	3.751735	1.965531	0.001029	0.000248

<i>Igkv4-57-1</i>	3.746872	2.864107	5.21E-08	3.99E-09
<i>Ighv9-2</i>	3.745123	2.034301	0.010115	0.003334
<i>Cxcl11</i>	3.744562	2.529739	3.63E-17	3.44E-19
<i>Ighv1-76</i>	3.732592	3.08539	7.36E-07	7.44E-08
<i>Igkv16-104</i>	3.719855	3.198995	7.07E-08	5.56E-09
<i>Cd74</i>	3.701444	3.102304	4.39E-14	8.94E-16
<i>Igkv4-58</i>	3.684726	2.077304	3.62E-08	2.69E-09
<i>Ighv5-9-1</i>	3.674367	3.339328	0.000538	0.000119
<i>Tigit</i>	3.637866	3.221232	2.16E-11	7.52E-13
<i>Igkv6-14</i>	3.627259	2.722995	4.33E-06	5.41E-07
<i>Igkv6-25</i>	3.610808	2.13673	9.67E-10	5.00E-11
<i>Ighv6-6</i>	3.588388	2.293068	9.22E-09	5.97E-10
<i>Ighv1-74</i>	3.587079	2.545224	3.37E-09	1.98E-10
<i>Sirpb1a</i>	3.545453	2.509834	6.20E-10	3.05E-11
<i>Igkv4-72</i>	3.505396	2.863931	7.14E-08	5.64E-09
<i>Ighv1-78</i>	3.504893	2.605147	5.23E-07	5.08E-08
<i>Ighv5-2</i>	3.497196	4.766351	2.17E-05	3.28E-06
<i>Skint3</i>	3.483994	0.373766	2.64E-10	1.21E-11
<i>Ccr5</i>	3.46797	2.082275	1.65E-18	1.20E-20
<i>Ighv8-5</i>	3.456636	3.404557	0.019865	0.007379
<i>H2-Q6</i>	3.444736	2.526538	2.91E-16	3.74E-18
<i>Ighv1-11</i>	3.437076	1.986672	0.003896	0.001118
<i>Ighv1-64</i>	3.427614	3.106616	1.08E-10	4.47E-12
<i>Igkv3-10</i>	3.404363	3.196333	1.79E-05	2.63E-06
<i>Pla2g2e</i>	3.384664	0.803024	5.48E-19	3.67E-21
<i>Ighv1-5</i>	3.362779	2.969061	0.00107	0.000259
<i>Igkv4-59</i>	3.356313	3.43859	7.61E-09	4.85E-10
<i>Adam8</i>	3.346181	1.019113	5.61E-16	7.65E-18
<i>Igkv14-111</i>	3.330055	2.266299	4.10E-12	1.21E-13
<i>H2-DMa</i>	3.327686	2.868565	2.33E-16	2.88E-18
<i>Igkv3-4</i>	3.325628	1.416204	8.49E-06	1.14E-06
<i>Ighv1-72</i>	3.306817	3.298585	1.38E-08	9.34E-10
<i>Igkv15-103</i>	3.300636	2.220284	1.10E-07	9.04E-09
<i>Igkv2-109</i>	3.292175	1.711297	4.39E-11	1.66E-12
<i>Ighv5-9</i>	3.285503	2.215233	5.12E-10	2.47E-11
<i>Igkv5-37</i>	3.27091	1.241354	0.005589	0.001688
<i>Ighv5-15</i>	3.270265	2.021182	0.025116	0.009716
<i>Clec7a</i>	3.267694	1.404431	9.75E-16	1.40E-17
<i>Igkv12-44</i>	3.250245	1.929714	5.88E-09	3.65E-10
<i>Igkv14-130</i>	3.248071	2.515726	0.0003	6.18E-05
<i>Ccl5</i>	3.230444	1.035688	3.88E-16	5.13E-18
<i>Cd274</i>	3.216665	2.227677	1.26E-19	7.35E-22
<i>Ighv4-1</i>	3.21502	1.902247	3.40E-07	3.14E-08
<i>Igkv4-68</i>	3.200131	4.381445	2.99E-07	2.71E-08

<i>Ighv14-4</i>	3.182277	1.923379	0.004917	0.001455
<i>Igkv5-43</i>	3.175787	2.669268	1.76E-11	5.99E-13
<i>Ighv1-55</i>	3.157112	2.599174	8.51E-07	8.78E-08
<i>Igkv4-53</i>	3.125431	1.172946	2.07E-08	1.44E-09
<i>Igkv3-5</i>	3.122438	1.417051	2.41E-05	3.69E-06
<i>Ighv1-22</i>	3.119828	2.419239	6.04E-07	5.95E-08
<i>Ighv1-62-2</i>	3.102961	1.494167	0.001331	0.000332
<i>H2-Ab1</i>	3.09412	2.557233	1.67E-15	2.56E-17
<i>Csf2rb</i>	3.091414	1.238764	1.60E-12	4.44E-14
<i>Ighv1-42</i>	3.066789	2.456276	0.000245	4.92E-05
<i>Igkv7-33</i>	3.064011	4.758728	2.52E-06	2.93E-07
<i>Ighv1-61</i>	3.059352	1.4114	0.003645	0.001035
<i>Ighv1-19</i>	3.053583	3.225965	1.27E-06	1.37E-07
<i>Igkv8-19</i>	3.053138	2.295177	0.000282	5.77E-05
<i>Igkv5-45</i>	3.04098	2.900281	0.003046	0.000844
<i>Ighv1-31</i>	3.002224	3.667607	0.002026	0.000532
<i>Fcgr1</i>	2.997466	1.780545	1.57E-16	1.88E-18
<i>Ighv2-5</i>	2.984613	0.807116	0.000706	0.000162
<i>H2-Aa</i>	2.980312	2.485972	5.80E-12	1.80E-13
<i>Wfdc17</i>	2.966429	1.239304	4.21E-15	7.00E-17
<i>Cd80</i>	2.932235	0.991531	2.58E-09	1.47E-10
<i>Igkv1-88</i>	2.921829	1.751977	3.89E-05	6.31E-06
<i>Klrc1</i>	2.902672	1.887854	5.22E-10	2.52E-11
<i>Igkv4-70</i>	2.882233	2.740291	0.000173	3.34E-05
<i>Iglv1</i>	2.881108	1.806552	5.76E-10	2.80E-11
<i>Tlr8</i>	2.867956	1.194974	9.10E-10	4.64E-11
<i>Tnfrsf1b</i>	2.867673	1.523169	2.17E-24	2.88E-27
<i>Ighv3-4</i>	2.866608	-0.47491	0.003145	0.000874
<i>Ighv3-1</i>	2.852793	-0.12232	0.000212	4.2E-05
<i>Igkv8-27</i>	2.79013	1.371782	0.000211	4.16E-05
<i>Ighv1-82</i>	2.787648	1.404936	3.73E-05	6.03E-06
<i>Ubash3a</i>	2.781517	2.045655	5.13E-06	6.53E-07
<i>Igkv4-62</i>	2.77523	0.90112	0.094964	0.047535
<i>Ighv1-39</i>	2.770153	2.404032	3.76E-05	6.06E-06
<i>H2-Q10</i>	2.765037	2.503657	3.09E-15	5.05E-17
<i>Ighv1-85</i>	2.764154	1.833778	0.01831	0.006704
<i>Cd5</i>	2.763109	1.883019	6.40E-07	6.36E-08
<i>Ighv9-3</i>	2.762541	2.325067	3.93E-06	4.85E-07
<i>Ighv1-15</i>	2.762502	3.567431	1.39E-05	2E-06
<i>Ighv1-26</i>	2.744358	2.637025	1.32E-08	8.89E-10
<i>Igkv4-61</i>	2.742572	2.105961	0.000669	0.000152
<i>Ighv1-69</i>	2.73931	2.242669	5.45E-07	5.31E-08
<i>Ccr1</i>	2.721018	1.091425	2.63E-14	5.13E-16
<i>Bst1</i>	2.696827	0.624975	2.65E-10	1.21E-11

<i>Csf2rb2</i>	2.691496	1.270224	1.73E-09	9.45E-11
<i>Btnl2</i>	2.684079	2.189292	0.000331	6.9E-05
<i>Igkv8-21</i>	2.677096	3.094952	6.16E-05	1.06E-05
<i>Ighv1-50</i>	2.65978	1.899614	5.5E-05	9.28E-06
<i>Ighv8-12</i>	2.657368	1.981467	0.002228	0.000591
<i>C4b</i>	2.61252	1.304062	1.00E-12	2.59E-14
<i>Il12b</i>	2.589713	2.280459	9.05E-06	1.23E-06
<i>Il2rb</i>	2.577465	1.851492	3.51E-10	1.63E-11
<i>Igkv12-46</i>	2.572529	1.147127	5.47E-06	7.03E-07
<i>Ccr2</i>	2.559526	1.267368	1.06E-14	1.90E-16
<i>Igkv8-24</i>	2.545743	1.922395	0.000347	7.25E-05
<i>Selplg</i>	2.504657	1.493983	1.67E-10	7.27E-12
<i>Nfam1</i>	2.490157	1.016396	2.93E-08	2.12E-09
<i>H2-Eb1</i>	2.477781	2.026399	4.43E-12	1.32E-13
<i>Cd14</i>	2.458806	1.432947	6.30E-23	1.19E-25
<i>Tlr13</i>	2.451441	1.356338	1.15E-08	7.60E-10
<i>Ighv9-4</i>	2.427529	2.59243	0.004502	0.001316
<i>Pdcd1lg2</i>	2.421059	1.123755	5.23E-06	6.69E-07
<i>Iglc3</i>	2.406047	1.921539	3.4E-06	4.12E-07
<i>Ighv14-1</i>	2.388436	2.473159	0.091558	0.04552
<i>Ifitm1</i>	2.385004	1.050305	2.58E-18	1.97E-20
<i>Igkv17-121</i>	2.379165	1.514193	0.00048	0.000105
<i>Themis2</i>	2.377117	1.051609	6.41E-07	6.37E-08
<i>Ighv1-34</i>	2.376582	2.722492	0.005595	0.00169
<i>Cxcl14</i>	2.371214	0.733975	2.67E-16	3.34E-18
<i>Mzb1</i>	2.366364	1.726074	1.36E-10	5.74E-12
<i>Iglc2</i>	2.364931	2.373568	4.10E-07	3.89E-08
<i>Igkv14-100</i>	2.357162	1.878266	0.00883	0.002863
<i>Igkv1-99</i>	2.352714	3.895495	0.000416	8.94E-05
<i>Stat1</i>	2.33041	1.85011	9.77E-16	1.41E-17
<i>Igkv4-79</i>	2.320778	0.529608	0.019127	0.007074
<i>C5ar1</i>	2.319264	0.879272	1.28E-08	8.60E-10
<i>Clec4a3</i>	2.316862	1.577794	2.46E-09	1.39E-10
<i>Clec4n</i>	2.307321	1.198697	4.71E-11	1.79E-12
<i>Fgr</i>	2.302908	1.210679	1.09E-08	7.13E-10
<i>H2-Q4</i>	2.296233	1.631105	3.72E-17	3.58E-19
<i>Trem14</i>	2.287072	0.865823	2.07E-08	1.44E-09
<i>Anxa1</i>	2.279359	0.949463	2.19E-21	7.05E-24
<i>B2m</i>	2.260328	1.631894	4.88E-12	1.48E-13
<i>Cd3d</i>	2.250553	1.56882	7.18E-08	5.67E-09
<i>Ccr10</i>	2.237246	2.016662	5.28E-09	3.25E-10
<i>Runx2</i>	2.2208	1.30771	6.33E-13	1.59E-14
<i>Cxcr6</i>	2.205486	1.263075	5.67E-07	5.55E-08
<i>5730507C01Rik</i>	2.202207	1.133896	3.31E-08	2.43E-09

<i>Tbx21</i>	2.197196	1.58869	1.45E-05	2.08E-06
<i>Klri2</i>	2.170348	1.903495	4.49E-05	7.43E-06
<i>Tnfsf8</i>	2.170246	1.438278	1.92E-05	2.84E-06
<i>Ighv5-12</i>	2.128907	1.116112	0.045818	0.019775
<i>H2-T24</i>	2.098547	1.42526	1.27E-05	1.79E-06
<i>Tyrobp</i>	2.097664	0.898426	6.88E-13	1.74E-14
<i>Iglv3</i>	2.091437	1.352057	1.86E-05	2.74E-06
<i>Igkv6-15</i>	2.085625	2.269885	0.000284	5.82E-05
<i>Cd3g</i>	2.077095	1.582839	1.21E-06	1.30E-07
<i>Clec4a1</i>	2.071037	1.411807	2.17E-11	7.59E-13
<i>Zap70</i>	2.058164	1.533009	6.86E-07	6.86E-08
<i>Slamf7</i>	2.019053	1.453695	5.79E-08	4.48E-09
<i>Ccr4</i>	2.011348	1.945212	1.01E-06	1.07E-07
<i>Sell</i>	1.983289	0.216197	0.002261	0.000601
<i>Alas2</i>	1.974945	-3.0842	1.14E-09	6.05E-11
<i>Iglc1</i>	1.969207	1.224005	0.000124	2.3E-05
<i>Pdcd1</i>	1.9055	1.073131	0.000104	1.88E-05
<i>Lgals9</i>	1.873652	1.426887	7.93E-12	2.53E-13
<i>Cd4</i>	1.860531	1.422509	1.57E-06	1.73E-07
<i>Ighv14-2</i>	1.852395	2.130663	0.036075	0.014922
<i>Ceacam10</i>	1.846384	1.212809	2.60E-12	7.43E-14
<i>Cd244</i>	1.833524	1.092385	1.29E-06	1.40E-07
<i>Sh2b2</i>	1.825263	0.593234	1.87E-06	2.10E-07
<i>Runx3</i>	1.825087	1.094022	7.71E-07	7.84E-08
<i>H2-T10</i>	1.824257	1.151124	3.38E-07	3.12E-08
<i>Slamf9</i>	1.817365	0.985155	7.67E-08	6.09E-09
<i>Cd28</i>	1.813217	1.588541	3.77E-08	2.81E-09
<i>Igkv19-93</i>	1.807926	2.16583	0.015016	0.005301
<i>Ackr2</i>	1.807599	0.780123	0.000332	6.91E-05
<i>Ccl22</i>	1.796093	1.730486	3.41E-05	5.44E-06
<i>Cd226</i>	1.785983	0.989028	0.000312	6.45E-05
<i>Cxcr3</i>	1.732298	1.381255	2.98E-05	4.66E-06
<i>H2-D1</i>	1.73026	1.145174	1.80E-10	7.90E-12
<i>Cd86</i>	1.716274	0.955498	1.19E-07	9.89E-09
<i>Themis</i>	1.676492	1.281286	0.005098	0.001519
<i>Ccrl2</i>	1.646559	0.57629	7.97E-06	1.07E-06
<i>Cd3e</i>	1.632908	1.200185	3.71E-05	5.99E-06
<i>Itk</i>	1.615027	0.855727	0.000983	0.000235
<i>Il7r</i>	1.610413	0.729023	0.000128	2.39E-05
<i>Fes</i>	1.598122	0.806132	4.18E-12	1.24E-13
<i>H60c</i>	1.589572	0.742969	0.043211	0.018463
<i>Lyn</i>	1.586874	0.949441	1.22E-12	3.25E-14
<i>Igkv5-39</i>	1.581532	1.859237	0.001224	0.000302
<i>C3ar1</i>	1.550234	1.004143	3.65E-06	4.46E-07

<i>Igkv6-23</i>	1.540964	1.002411	0.044592	0.019157
<i>C5ar2</i>	1.539469	0.650149	5.98E-05	1.02E-05
<i>Ptk6</i>	1.534677	1.139041	9.27E-10	4.74E-11
<i>Itga6</i>	1.521503	0.796344	8.12E-10	4.10E-11
<i>Bst2</i>	1.517413	0.820713	1.69E-10	7.40E-12
<i>Cmklr1</i>	1.500954	0.664361	4.59E-05	7.62E-06
<i>Cd40</i>	1.493073	0.446667	0.000104	1.88E-05
<i>Tlr6</i>	1.472375	0.463753	6.45E-06	8.42E-07
<i>Ccr9</i>	1.468011	1.006338	0.000216	4.28E-05
<i>Csf1r</i>	1.461359	0.739212	7.57E-09	4.82E-10
<i>Rel</i>	1.435933	0.358392	1.17E-10	4.84E-12
<i>Ccr7</i>	1.43008	0.850285	0.020835	0.007797
<i>Tlr12</i>	1.427116	1.424437	4.25E-05	6.95E-06
<i>Gcsam</i>	1.409994	1.098373	0.002558	0.000691
<i>Tlr2</i>	1.395267	0.406056	1.4E-05	2E-06
<i>Sit1</i>	1.389963	0.981122	0.039126	0.016414
<i>Cst7</i>	1.388098	0.837199	0.010876	0.003633
<i>Cxcr4</i>	1.381582	1.22641	0.001235	0.000305
<i>Clec4a2</i>	1.363601	0.963491	0.000244	4.89E-05
<i>Fyb</i>	1.333843	0.885339	5.04E-06	6.39E-07
<i>Hck</i>	1.327364	0.542736	0.001527	0.000386
<i>Cd84</i>	1.301694	0.565946	0.000219	4.36E-05
<i>Slamf6</i>	1.276249	0.802918	0.032835	0.013349
<i>Lax1</i>	1.260487	0.879769	0.002366	0.000632
<i>Cxcl16</i>	1.253721	0.535896	6.37E-11	2.51E-12
<i>Xcl1</i>	1.25139	1.522107	0.001625	0.000414
<i>Ubash3b</i>	1.24452	0.154402	2.48E-07	2.21E-08
<i>Txk</i>	1.219005	0.673682	0.001915	0.000499
<i>Flt3</i>	1.208163	0.751148	0.01796	0.006553
<i>Klrd1</i>	1.196203	0.959048	0.004307	0.001254
<i>Srm</i>	1.174136	0.606274	4.43E-07	4.22E-08
<i>Stat2</i>	1.172617	1.006932	3.79E-09	2.26E-10
<i>Jak3</i>	1.170432	0.667639	9.58E-08	7.78E-09
<i>Xcr1</i>	1.169164	1.036521	0.021548	0.008103
<i>H2-K1</i>	1.165588	0.650703	1.46E-05	2.1E-06
<i>Igkv1-133</i>	1.151724	1.09185	0.464071	0.344442
<i>Ulbp1</i>	1.147624	0.795288	0.006168	0.001895
<i>Tnfrsf14</i>	1.141578	1.020801	1.34E-05	1.92E-06
<i>Cd38</i>	1.116108	0.885064	8.49E-07	8.74E-08
<i>Tlr11</i>	1.086969	1.155627	0.014631	0.005147
<i>Tlr9</i>	1.083684	0.773694	0.215597	0.130667
<i>S100a14</i>	1.083622	0.590302	9.26E-06	1.26E-06
<i>Runx1</i>	1.077203	0.485152	1.27E-06	1.37E-07
<i>Vpreb3</i>	1.073817	0.297173	0.073622	0.034981

<i>Ly86</i>	1.05158	0.685487	0.004565	0.001337
<i>H2-T22</i>	1.030652	0.582791	1.89E-08	1.31E-09
<i>Lck</i>	1.030185	0.89497	0.003343	0.000939
<i>Slc39a10</i>	1.029553	0.445205	0.001196	0.000294
<i>H2-M3</i>	1.028898	0.632632	1.19E-05	1.68E-06
<i>H2-M2</i>	1.012231	0.55506	0.048635	0.021238
<i>Klri1</i>	1.010734	0.887676	0.115239	0.06009
<i>Plvap</i>	1.008619	0.551273	0.000981	0.000234

Table 4. Immunoglobulins present in Winnie and Winnie-Prolapse mice

Gene Name	Winnie - Prolapse log2fc	Winnie log2fc	P value	FDR
<i>Igha</i>	3.15331	2.47311	3.80E-13	9.02E-15
<i>Ighe</i>	2.360514	0.856446	0.030141	0.012049
<i>Ighg1</i>	4.817597	2.451552	4.65E-09	2.83E-10
<i>Ighg2b</i>	5.151567	3.123592	8.70E-23	1.74E-25
<i>Ighg2c</i>	7.714039	4.909786	2.95E-16	3.80E-18
<i>Ighg3</i>	4.293092	2.937319	3.50E-18	2.77E-20
<i>Ighj1</i>	3.258803	2.937614	3.83E-09	2.29E-10
<i>Ighj2</i>	3.087386	2.27292	3.67E-10	1.70E-11
<i>Ighj3</i>	3.13729	1.979591	1.22E-12	3.22E-14
<i>Ighj4</i>	3.026148	2.174063	4.81E-12	1.45E-13
<i>Ighv10-1</i>	4.295259	3.307261	1.49E-12	4.03E-14
<i>Ighv10-3</i>	3.850786	3.015776	7.15E-07	7.21E-08
<i>Ighv1-11</i>	3.437076	1.986672	0.003896	0.001118
<i>Ighv1-12</i>	4.056259	3.583702	2.76E-07	2.48E-08
<i>Ighv1-15</i>	2.762502	3.567431	1.39E-05	2E-06
<i>Ighv1-18</i>	5.393028	3.089698	9.86E-18	8.69E-20
<i>Ighv1-19</i>	3.053583	3.225965	1.27E-06	1.37E-07
<i>Ighv1-20</i>	4.301311	3.487162	0.000243	4.86E-05
<i>Ighv1-22</i>	3.119828	2.419239	6.04E-07	5.95E-08
<i>Ighv1-26</i>	2.744358	2.637025	1.32E-08	8.89E-10
<i>Ighv1-31</i>	3.002224	3.667607	0.002026	0.000532
<i>Ighv13-2</i>	4.904557	2.653231	3.37E-07	3.11E-08
<i>Ighv1-34</i>	2.376582	2.722492	0.005595	0.00169
<i>Ighv1-39</i>	2.770153	2.404032	3.76E-05	6.06E-06
<i>Ighv1-4</i>	5.758344	4.33982	3.42E-11	1.26E-12
<i>Ighv14-1</i>	2.388436	2.473159	0.091558	0.04552
<i>Ighv14-2</i>	1.852395	2.130663	0.036075	0.014922
<i>Ighv1-42</i>	3.066789	2.456276	0.000245	4.92E-05
<i>Ighv14-3</i>	4.088289	5.641494	1.56E-07	1.33E-08
<i>Ighv14-4</i>	3.182277	1.923379	0.004917	0.001455
<i>Ighv1-47</i>	7.454192	2.898153	1.37E-11	4.56E-13
<i>Ighv1-5</i>	3.362779	2.969061	0.00107	0.000259
<i>Ighv1-50</i>	2.65978	1.899614	5.5E-05	9.28E-06
<i>Ighv1-52</i>	3.753901	2.955937	4.20E-07	3.99E-08
<i>Ighv1-53</i>	3.782595	2.833715	1.69E-11	5.71E-13
<i>Ighv1-54</i>	3.903135	3.910792	1.79E-05	2.64E-06
<i>Ighv1-55</i>	3.157112	2.599174	8.51E-07	8.78E-08

<i>Ighv1-58</i>	6.290089	3.696418	2.17E-11	7.60E-13
<i>Ighv1-59</i>	4.56572	2.364279	4.25E-08	3.19E-09
<i>Ighv1-61</i>	3.059352	1.4114	0.003645	0.001035
<i>Ighv1-62-2</i>	3.102961	1.494167	0.001331	0.000332
<i>Ighv1-63</i>	4.416949	4.034501	2.13E-08	1.49E-09
<i>Ighv1-64</i>	3.427614	3.106616	1.08E-10	4.47E-12
<i>Ighv1-66</i>	4.432371	3.136441	6.38E-07	6.32E-08
<i>Ighv1-69</i>	2.73931	2.242669	5.45E-07	5.31E-08
<i>Ighv1-7</i>	4.903486	4.978743	1.23E-10	5.15E-12
<i>Ighv1-71</i>	4.168168	2.333197	7.57E-05	1.33E-05
<i>Ighv1-72</i>	3.306817	3.298585	1.38E-08	9.34E-10
<i>Ighv1-74</i>	3.587079	2.545224	3.37E-09	1.98E-10
<i>Ighv1-75</i>	4.289454	1.245735	3.70E-16	4.88E-18
<i>Ighv1-76</i>	3.732592	3.08539	7.36E-07	7.44E-08
<i>Ighv1-77</i>	4.046407	4.843062	8.65E-06	1.17E-06
<i>Ighv1-78</i>	3.504893	2.605147	5.23E-07	5.08E-08
<i>Ighv1-80</i>	4.865691	4.087993	7.84E-14	1.65E-15
<i>Ighv1-81</i>	4.683924	2.537778	9.09E-09	5.87E-10
<i>Ighv1-82</i>	2.787648	1.404936	3.73E-05	6.03E-06
<i>Ighv1-84</i>	3.867716	1.278426	1.07E-05	1.48E-06
<i>Ighv1-85</i>	2.764154	1.833778	0.01831	0.006704
<i>Ighv1-9</i>	4.162341	3.774761	1.34E-08	8.99E-10
<i>Ighv2-2</i>	4.64371	3.122869	4.53E-17	4.47E-19
<i>Ighv2-3</i>	4.82183	4.256638	2.65E-11	9.39E-13
<i>Ighv2-5</i>	2.984613	0.807116	0.000706	0.000162
<i>Ighv2-6</i>	4.17276	4.434282	6.15E-07	6.07E-08
<i>Ighv2-6-8</i>	4.14612	1.451429	0.000136	2.56E-05
<i>Ighv2-9</i>	3.930835	2.230379	3.81E-11	1.42E-12
<i>Ighv2-9-1</i>	6.505299	4.464995	1.35E-09	7.23E-11
<i>Ighv3-1</i>	2.852793	-0.12232	0.000212	4.2E-05
<i>Ighv3-4</i>	2.866608	-0.47491	0.003145	0.000874
<i>Ighv3-5</i>	6.442042	5.181769	3.33E-08	2.45E-09
<i>Ighv3-6</i>	4.168163	3.298321	7.96E-11	3.21E-12
<i>Ighv3-8</i>	3.893687	3.024879	2.11E-05	3.17E-06
<i>Ighv4-1</i>	3.21502	1.902247	3.40E-07	3.14E-08
<i>Ighv5-12</i>	2.128907	1.116112	0.045818	0.019775
<i>Ighv5-15</i>	3.270265	2.021182	0.025116	0.009716
<i>Ighv5-16</i>	3.907073	2.858196	2.18E-05	3.29E-06
<i>Ighv5-17</i>	4.106789	4.12219	7.99E-08	6.37E-09
<i>Ighv5-2</i>	3.497196	4.766351	2.17E-05	3.28E-06
<i>Ighv5-4</i>	5.225168	2.70456	6.42E-11	2.54E-12
<i>Ighv5-6</i>	4.834454	3.449645	2.10E-10	9.36E-12
<i>Ighv5-9</i>	3.285503	2.215233	5.12E-10	2.47E-11
<i>Ighv5-9-1</i>	3.674367	3.339328	0.000538	0.000119

<i>Ighv6-3</i>	4.055164	3.936229	9.33E-09	6.04E-10
<i>Ighv6-6</i>	3.588388	2.293068	9.22E-09	5.97E-10
<i>Ighv7-1</i>	5.297675	5.068151	5.29E-11	2.04E-12
<i>Ighv7-3</i>	5.605875	3.597133	2.71E-22	6.90E-25
<i>Ighv7-4</i>	4.684632	1.643703	1.06E-10	4.38E-12
<i>Ighv8-12</i>	2.657368	1.981467	0.002228	0.000591
<i>Ighv8-5</i>	3.456636	3.404557	0.019865	0.007379
<i>Ighv8-8</i>	3.994826	2.856608	1.28E-08	8.54E-10
<i>Ighv9-1</i>	5.741213	3.484521	1.02E-07	8.37E-09
<i>Ighv9-2</i>	3.745123	2.034301	0.010115	0.003334
<i>Ighv9-3</i>	2.762541	2.325067	3.93E-06	4.85E-07
<i>Ighv9-4</i>	2.427529	2.59243	0.004502	0.001316
<i>Igkc</i>	3.127404	2.517277	5.28E-13	1.30E-14
<i>Igkj1</i>	3.25261	2.384156	7.79E-14	1.63E-15
<i>Igkj2</i>	2.703988	1.9551	7.90E-11	3.19E-12
<i>Igkj3</i>	2.802235	2.042936	8.79E-08	7.09E-09
<i>Igkj4</i>	2.163621	1.441164	8.9E-06	1.2E-06
<i>Igkj5</i>	2.494659	2.028477	1.46E-09	7.82E-11
<i>Igkv10-94</i>	4.093792	1.200963	6.03E-15	1.03E-16
<i>Igkv10-96</i>	4.114697	2.984275	8.75E-18	7.61E-20
<i>Igkv1-110</i>	3.884964	2.474799	2.98E-21	9.74E-24
<i>Igkv11-125</i>	1.199394	1.596449	0.293955	0.191997
<i>Igkv1-117</i>	4.016164	1.868956	4.22E-15	7.04E-17
<i>Igkv1-122</i>	4.06511	1.103854	9.74E-08	7.93E-09
<i>Igkv1-133</i>	1.151724	1.09185	0.464071	0.344442
<i>Igkv1-135</i>	4.173485	4.074774	1.09E-12	2.87E-14
<i>Igkv12-38</i>	3.751735	1.965531	0.001029	0.000248
<i>Igkv12-41</i>	2.388202	2.515045	7.30E-10	3.66E-11
<i>Igkv12-44</i>	3.250245	1.929714	5.88E-09	3.65E-10
<i>Igkv12-46</i>	2.572529	1.147127	5.47E-06	7.03E-07
<i>Igkv12-47</i>	4.101788	2.23966	6.39E-06	8.33E-07
<i>Igkv12-89</i>	3.837397	2.33404	5.92E-07	5.83E-08
<i>Igkv12-98</i>	3.810619	3.137083	3.87E-07	3.64E-08
<i>Igkv13-84</i>	4.024928	3.006028	4.52E-10	2.16E-11
<i>Igkv14-100</i>	2.357162	1.878266	0.00883	0.002863
<i>Igkv14-111</i>	3.330055	2.266299	4.10E-12	1.21E-13
<i>Igkv14-126</i>	4.764345	1.656057	2.36E-09	1.33E-10
<i>Igkv14-130</i>	3.248071	2.515726	0.0003	6.18E-05
<i>Igkv15-103</i>	3.300636	2.220284	1.10E-07	9.04E-09
<i>Igkv16-104</i>	3.719855	3.198995	7.07E-08	5.56E-09
<i>Igkv17-121</i>	2.379165	1.514193	0.00048	0.000105
<i>Igkv17-127</i>	4.789131	3.063	1.41E-14	2.59E-16
<i>Igkv1-88</i>	2.921829	1.751977	3.89E-05	6.31E-06
<i>Igkv1-99</i>	2.352714	3.895495	0.000416	8.94E-05

<i>Igkv19-93</i>	1.807926	2.16583	0.015016	0.005301
<i>Igkv2-109</i>	3.292175	1.711297	4.39E-11	1.66E-12
<i>Igkv2-112</i>	5.058	2.509098	4.87E-07	4.71E-08
<i>Igkv2-116</i>	10.95544	4.089878	4.65E-13	1.13E-14
<i>Igkv3-1</i>	3.818113	4.095785	7.05E-07	7.09E-08
<i>Igkv3-10</i>	3.404363	3.196333	1.79E-05	2.63E-06
<i>Igkv3-12</i>	4.469066	3.21681	7.76E-10	3.91E-11
<i>Igkv3-2</i>	4.264848	3.012112	1.52E-09	8.21E-11
<i>Igkv3-4</i>	3.325628	1.416204	8.49E-06	1.14E-06
<i>Igkv3-5</i>	3.122438	1.417051	2.41E-05	3.69E-06
<i>Igkv3-7</i>	4.451171	4.097348	7.96E-08	6.34E-09
<i>Igkv4-50</i>	4.54687	3.961605	4.19E-06	5.22E-07
<i>Igkv4-53</i>	3.125431	1.172946	2.07E-08	1.44E-09
<i>Igkv4-55</i>	3.399217	2.262569	1.13E-08	7.43E-10
<i>Igkv4-57</i>	4.724948	3.944651	1.83E-13	4.14E-15
<i>Igkv4-57-1</i>	3.746872	2.864107	5.21E-08	3.99E-09
<i>Igkv4-58</i>	3.684726	2.077304	3.62E-08	2.69E-09
<i>Igkv4-59</i>	3.356313	3.43859	7.61E-09	4.85E-10
<i>Igkv4-61</i>	2.742572	2.105961	0.000669	0.000152
<i>Igkv4-62</i>	2.77523	0.90112	0.094964	0.047535
<i>Igkv4-63</i>	4.175916	1.61337	9.83E-10	5.10E-11
<i>Igkv4-68</i>	3.200131	4.381445	2.99E-07	2.71E-08
<i>Igkv4-69</i>	3.779075	3.402445	0.001409	0.000353
<i>Igkv4-70</i>	2.882233	2.740291	0.000173	3.34E-05
<i>Igkv4-72</i>	3.505396	2.863931	7.14E-08	5.64E-09
<i>Igkv4-74</i>	4.07537	3.363564	4.12E-08	3.09E-09
<i>Igkv4-79</i>	2.320778	0.529608	0.019127	0.007074
<i>Igkv4-80</i>	4.368332	4.09828	4.29E-08	3.22E-09
<i>Igkv4-86</i>	3.836077	3.794624	1.96E-06	2.22E-07
<i>Igkv4-91</i>	4.464202	2.342969	3.90E-13	9.28E-15
<i>Igkv5-37</i>	3.27091	1.241354	0.005589	0.001688
<i>Igkv5-39</i>	1.581532	1.859237	0.001224	0.000302
<i>Igkv5-43</i>	3.175787	2.669268	1.76E-11	5.99E-13
<i>Igkv5-45</i>	3.04098	2.900281	0.003046	0.000844
<i>Igkv5-48</i>	1.978701	2.559126	0.000133	2.5E-05
<i>Igkv6-13</i>	4.051956	3.72783	1.69E-06	1.88E-07
<i>Igkv6-14</i>	3.627259	2.722995	4.33E-06	5.41E-07
<i>Igkv6-15</i>	2.085625	2.269885	0.000284	5.82E-05
<i>Igkv6-17</i>	3.07287	1.567848	4.68E-09	2.86E-10
<i>Igkv6-20</i>	3.874212	2.148574	1.60E-12	4.41E-14
<i>Igkv6-23</i>	1.540964	1.002411	0.044592	0.019157
<i>Igkv6-25</i>	3.610808	2.13673	9.67E-10	5.00E-11
<i>Igkv6-32</i>	4.55941	3.397228	4.54E-12	1.36E-13
<i>Igkv7-33</i>	3.064011	4.758728	2.52E-06	2.93E-07

<i>Igkv8-16</i>	4.437435	3.954778	0.000229	4.57E-05
<i>Igkv8-18</i>	6.252258	4.913677	2.66E-05	4.11E-06
<i>Igkv8-19</i>	3.053138	2.295177	0.000282	5.77E-05
<i>Igkv8-21</i>	2.677096	3.094952	6.16E-05	1.06E-05
<i>Igkv8-24</i>	2.545743	1.922395	0.000347	7.25E-05
<i>Igkv8-27</i>	2.79013	1.371782	0.000211	4.16E-05
<i>Igkv8-28</i>	4.889251	2.865357	1.06E-14	1.88E-16
<i>Igkv8-30</i>	4.084664	3.853889	2.14E-09	1.20E-10
<i>Igkv9-120</i>	3.830871	4.810471	1.78E-09	9.74E-11
<i>Igkv9-123</i>	4.061398	3.221544	3.33E-05	5.3E-06
<i>Igkv9-124</i>	3.879545	3.035411	1.14E-05	1.59E-06
<i>Iglc1</i>	1.969207	1.224005	0.000124	2.3E-05
<i>Iglc2</i>	2.364931	2.373568	4.10E-07	3.89E-08
<i>Iglc3</i>	2.406047	1.921539	3.4E-06	4.12E-07
<i>Iglj1</i>	2.200318	1.487659	9.21E-05	1.65E-05
<i>Iglj3</i>	1.134715	1.00327	0.072147	0.034083
<i>Iglv1</i>	2.881108	1.806552	5.76E-10	2.80E-11
<i>Iglv2</i>	3.694267	3.887165	1.67E-09	9.10E-11
<i>Iglv3</i>	2.091437	1.352057	1.86E-05	2.74E-06
<i>Igsf21</i>	-1.35951	-0.81495	0.000519	0.000115
<i>Igsf6</i>	2.860783	1.215677	5.36E-13	1.32E-14

All the samples have been quantified with Qubit and analysed with Bioanalyser. They all passed our QNA QC standard.

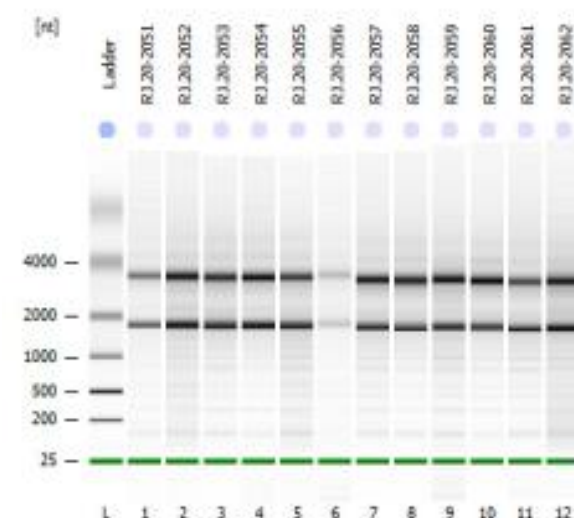
Sample ID	Sample Name	Qubit Conc. (ng/μL)	RIN	Report	Volume (μL)
20-2051	3C4_C57BLK6_Males	81	9.3	RamyaJyothi.RNA-1.27042020	>100
20-2052	3C5_C57BLK6_Males	190.5	8.8	RamyaJyothi.RNA-1.27042020	20
20-2053	4C6_C57BLK6_Males	255	9.2	RamyaJyothi.RNA-1.27042020	20
20-2054	4C8_C57BLK6_Males	187.5	9.6	RamyaJyothi.RNA-1.27042020	20
20-2055	3C6_C57BLK6_Males	209	8.4	RamyaJyothi.RNA-1.27042020	20
20-2056	4C7_C57BLK6_Males	44.1	8	RamyaJyothi.RNA-1.27042020	20
20-2057	4C1_C57BLK6_Females	243.5	8.5	RamyaJyothi.RNA-1.27042020	20
20-2058	4C2_C57BLK6_Females	315	9.3	RamyaJyothi.RNA-1.27042020	20
20-2059	4C5_C57BLK6_Females	164.5	8.6	RamyaJyothi.RNA-1.27042020	>100
20-2060	5C7_C57BLK6_Females	340	9.2	RamyaJyothi.RNA-1.27042020	20
20-2061	4C4_C57BLK6_Females	206	8.3	RamyaJyothi.RNA-1.27042020	20
20-2062	5C6_C57BLK6_Females	174.5	8.2	RamyaJyothi.RNA-1.27042020	20
20-2063	2034_Winnie_Males	495	9.9	RamyaJyothi.RNA-2.27042020	20
20-2064	2037_Winnie_Males	216.5	9.7	RamyaJyothi.RNA-2.27042020	20
20-2065	2036_Winnie_Males	415	9.2	RamyaJyothi.RNA-2.27042020	20

20-2066	W3_Winnie_Males	495	10	RamyaJyothi.RNA-2.27042020	15
20-2067	W4_Winnie_Males	450	10	RamyaJyothi.RNA-2.27042020	15
20-2068	386_Winnie_Females	345	9.5	RamyaJyothi.RNA-2.27042020	20
20-2069	387_Winnie_Females	415	9.5	RamyaJyothi.RNA-2.27042020	20
20-2070	388_Winnie_Females	325	9.4	RamyaJyothi.RNA-2.27042020	20
20-2071	389_Winnie_Females	435	9.6	RamyaJyothi.RNA-2.27042020	20
20-2072	2031_Winnie_Females	445	8.9	RamyaJyothi.RNA-2.27042020	20
20-2073	452_Prolapse_Males	740	9.5	RamyaJyothi.RNA-3.27042020	20
20-2074	17-7_B_Prolapse_Males	620	8.6	RamyaJyothi.RNA-4.27042020	20
20-2075	391_Prolapse_Males	740	8.8	RamyaJyothi.RNA-3.27042020	20
20-2076	554_Prolapse_Males	990	10	RamyaJyothi.RNA-3.27042020	20
20-2077	21st_Jun_19_Prolapse_Females	780	9.5	RamyaJyothi.RNA-3.27042020	20
20-2078	399_Prolapse_Females	2400	9.9	RamyaJyothi.RNA-3.27042020	20
20-2079	21-May_Prolapse_Females	465	9.3	RamyaJyothi.RNA-3.27042020	20
20-2080	408_Prolapse_Females	1000	8.6	RamyaJyothi.RNA-3.27042020	20
20-2081	2086_Prolapse_Females	720	9.2	RamyaJyothi.RNA-3.27042020	20

Assay Class: Eukaryote Total RNA Nano
 Data Path: C:\...2100 expert\data\2020-04-27\Ramya\yothi.RNA-1.27042020.xad

Created: 27/04/2020 3:13:26 PM
 Modified: 27/04/2020 3:37:19 PM

Electrophoresis File Run Summary



Instrument Information:

Instrument Name: MicromerNGS
 Serial#: DEL3804363
 Firmware: C.01.069
 Type: G2939A

Assay Information:

Assay Origin Path: C:\Program Files (x86)\Agilent\2100 bioanalyzer\2100 expert\assays\RNA\Eukaryote Total RNA Nano Series II.asy

Assay Class: Eukaryote Total RNA Nano

Version: 2.6

Assay Comments: Total RNA Analysis ng sensitivity (Eukaryote)

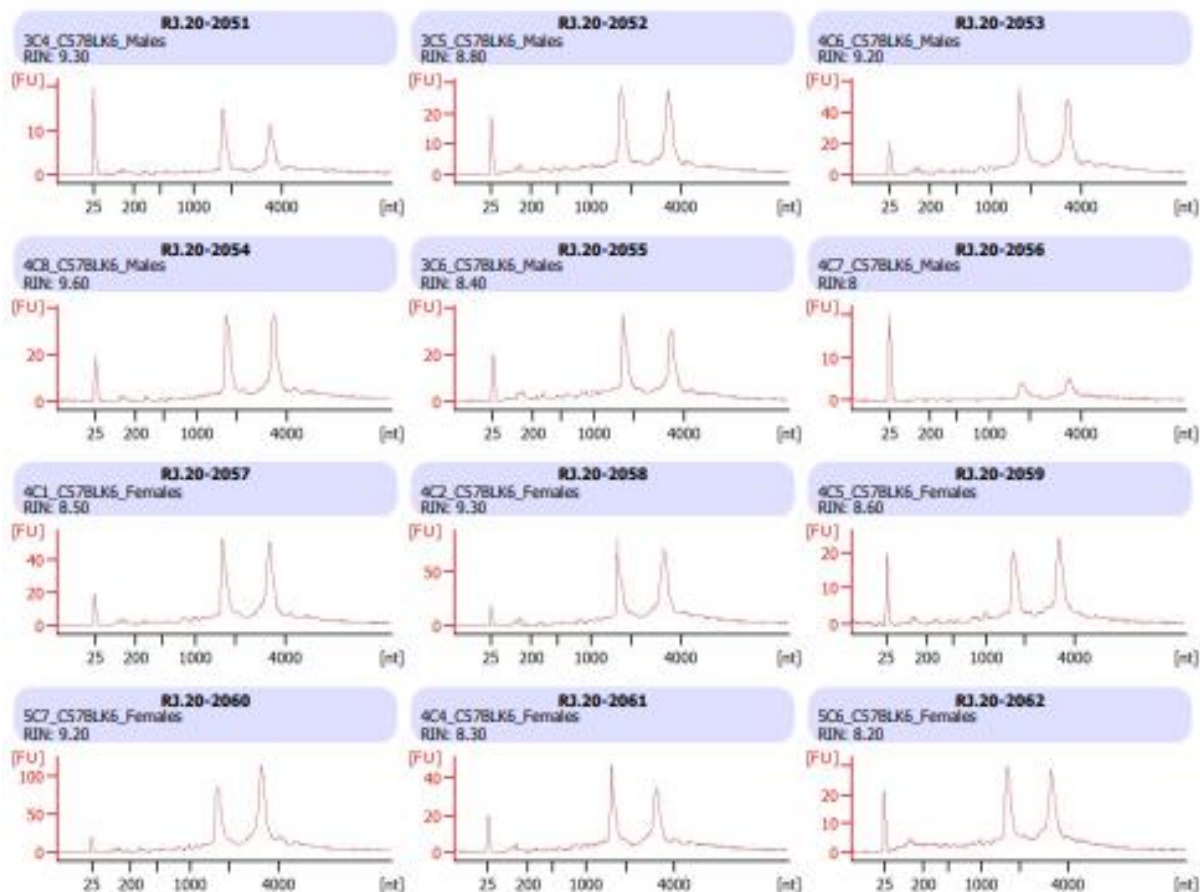
© Copyright 2003 - 2009 Agilent Technologies, Inc.

Chip Information:

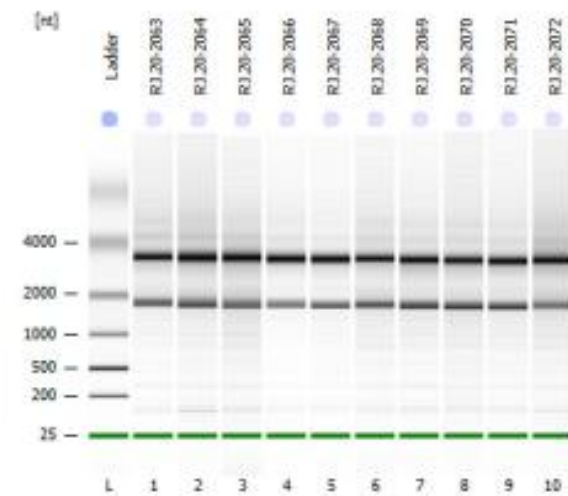
Chip Lot #:

Reagent Kit Lot #:

Chip Comments:



Electrophoresis File Run Summary



Instrument Information:

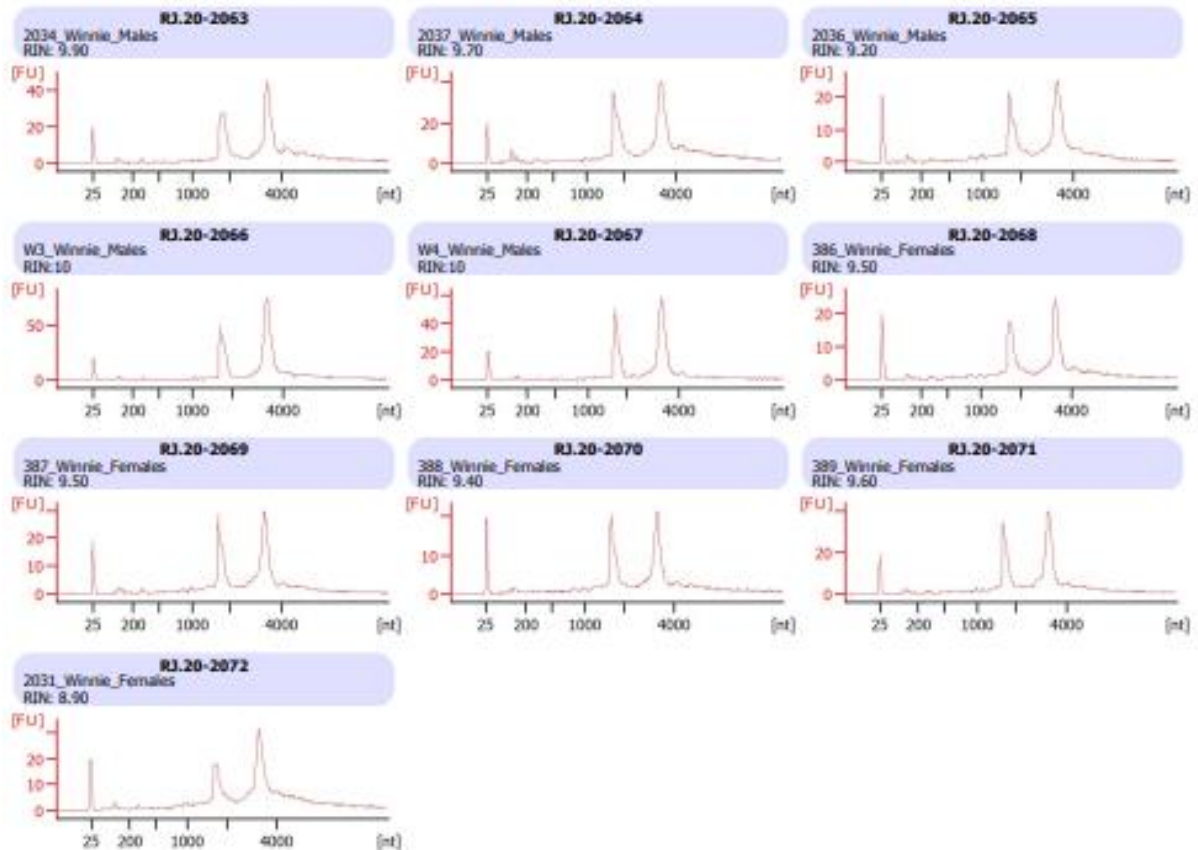
Instrument Name: MicromerNGS
Serial#: DE13804363
Firmware: C.01.069
Type: G2939A

Assay Information:

Assay Origin Path: C:\Program Files (x86)\Agilent\2100 bioanalyzer\2100 expert\assays\RNA\Eukaryote Total RNA Nano Series II.assay
Assay Class: Eukaryote Total RNA Nano
Version: 2.6
Assay Comments: Total RNA Analysis ng sensitivity (Eukaryote)
© Copyright 2003 - 2009 Agilent Technologies, Inc.

Chip Information:

Chip Lot #:
Reagent Kit Lot #:
Chip Comments:



Electrophoresis File Run Summary

Instrument Information:

Instrument Name: MicromerNGS
 Serial#: DE13804363
 Firmware: C.01.069
 Type: G2939A

Assay Information:

Assay Origin Path: C:\Program Files (x86)\Agilent\2100 bioanalyzer\2100 expert\assays\RNA\Eukaryote Total RNA Nano Series ILEsy
 Assay Class: Eukaryote Total RNA Nano
 Version: 2.6
 Assay Comments: Total RNA Analysis ng sensitivity (Eukaryote)
 © Copyright 2003 - 2009 Agilent Technologies, Inc.

Chip Information:

Chip Lot #:
 Reagent Kit Lot #:
 Chip Comments:

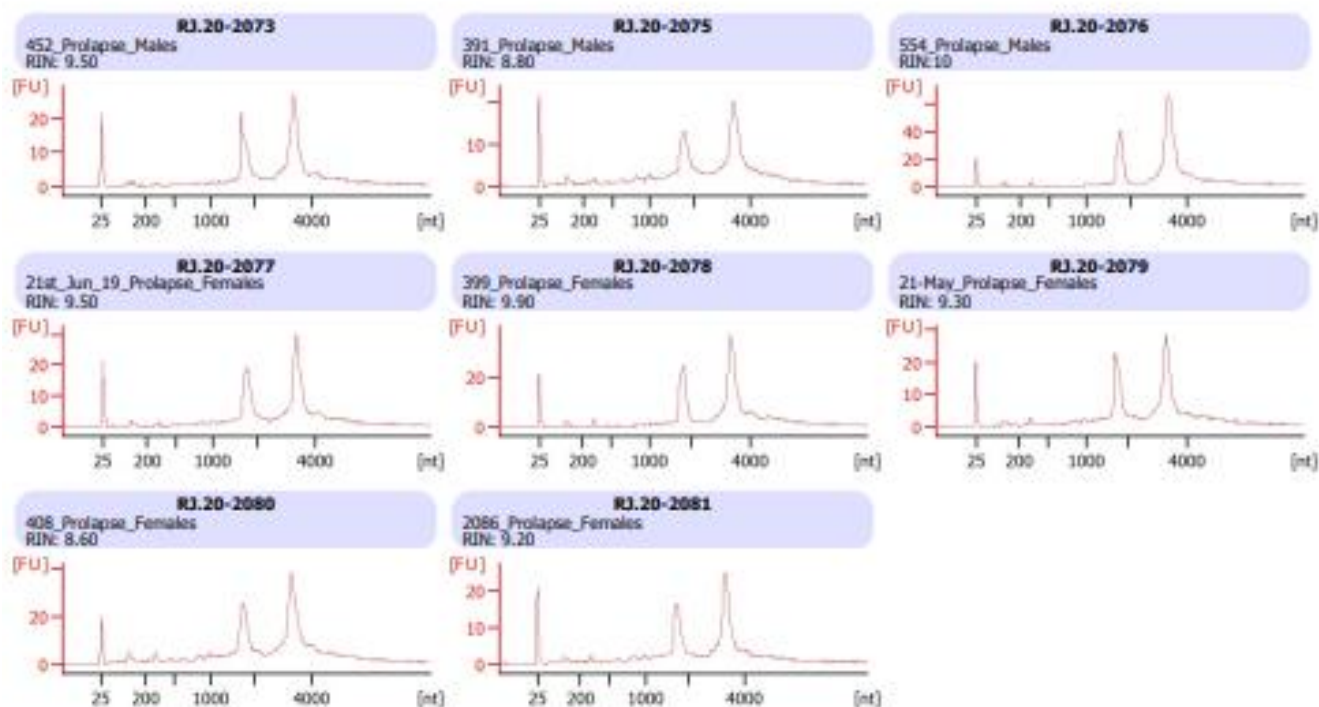


Table 5. Genes expressed in *Winnie* and *Winnie-Prolapse* mice as identified in this thesis, which are co-expressed in tissues from patients with IBD

Gene	Human IBD (log2fc)	Winnie (log2fc)	Winnie- Prolapse (log2fc)
ADCY1	0.806288	-1.06329	-1.47145
ADCY4	0.65426	0.666094	1.59062
ADCY5	-0.4137	-0.78992	-1.25549
ADCY8	-0.47502	2.631836	4.168722
AHCYL2	-0.49888	-0.58164	-1.33469
ATCAY	-0.2366	-1.34468	-1.6455
CALY	-0.3287	-1.01139	-1.6082
CHSY3	1.399478	0.815848	1.152281
CMYA5	0.07921	0.056679	4.3198
CNPY1	-0.25393	-1.13132	-1.72292
CRY2	0.164414	-0.34148	-1.27739
CRYAB	0.299761	-1.26117	-0.78446
CRYBB3	-1.6781	-0.84714	-2.01377
CRYM	-0.86061	-1.25804	-2.00501
CYBA	-0.02848	0.446389	1.0479
CYBB	1.405267	1.057927	2.213576
CYP11A1	-0.32337	-2.71405	-2.54654
CYP17A1	-0.65765	-0.74126	4.746828
CYP1A1	-0.08908	0.589933	3.260197
CYP1B1	0.73705	-0.17083	2.49991
CYP26B1	1.571305	-0.02187	1.208363
CYP27A1	-0.27004	-0.82443	-1.08147
Cyp2c44	-0.09642	-0.49673	-1.56457
CYP2E1	-0.16123	-0.37086	-2.31036
CYP2S1	-0.44363	-0.28283	-1.24464
CYP2U1	-0.16401	-2.12192	-2.61839
CYP2W1	-1.72549	-0.42427	-1.17515
CYP4B1	0.008921	-2.12326	-1.73011
CYP7B1	2.097491	0.42513	1.249021
CYR61	0.658049	0.608042	1.309264
CYS1	-0.70855	-0.69373	-1.47652
CYSRT1	-0.75565	0.342567	5.035469

CYTH4	0.601986	0.871662	1.567251
CYTIP	0.682198	0.02077	1.020732
CYTL1	0.710466	-0.46472	-1.09872
CYYR1	1.09921	0.932758	1.775197
DPYD	0.422904	-1.2433	-2.34271
DPYSL4	1.103661	-1.31857	-2.10554
DPYSL5	0.042555	-1.59847	-1.88575
DYRK3	0.935889	0.038726	1.520165
ESYT3	-0.2375	-1.48095	2.276136
EYA2	-2.03265	0.39075	2.94728
FXYD1	-0.7072	-0.95672	-1.41328
FXYD3	-1.70274	0.650712	1.016705
FXYD4	-1.11282	-2.24792	-1.57109
FXYD5	0.615595	0.906895	1.82882
FXYD6	0.631242	-1.61833	-1.63407
FXYD7	-0.1753	-1.13204	-2.04358
FYB	0.53725	0.94947	1.333373
GLYCAM1	-0.03474	-1.72272	4.753653
GUCY1A3	1.796091	-0.92529	-1.53343
GUCY1B3	1.718951	-1.11584	-1.30839
GYPA	-0.12904	-1.08912	1.044459
HEY2	0.556475	-1.1721	-1.02758
HYDIN	-0.73478	1.756496	2.299665
HYKK	-1.12402	-0.82742	-1.90737
HYOU1	0.471956	0.823575	1.240025
IYD	-0.1457	-0.42261	-1.56353
KY	0.325658	0.585081	3.779098
LEFTY1	-0.83165	-0.56719	-1.06008
LY6D	3.884868	1.471938	3.822084
LY6E	0.512826	1.314603	1.801705
LY6G6C	-1.91057	1.266808	2.775413
LY6H	-0.08697	-0.91841	-1.46873
LY6K	-0.10064	0.603776	2.17989
LY86	0.004555	0.773605	1.051128
LYN	0.963881	1.061229	1.586383
LYPD2	-0.52918	0.193591	4.233789
LYPD3	-0.1887	2.38434	9.342552
LYPD5	2.257099	0	8.439778

LYZL4	-0.41301	-0.71876	-1.39463
MYBL2	-0.15161	0.718657	1.03182
MYBPC1	-0.09365	1.511164	4.778383
MYBPC2	0.016887	0.764492	5.093293
MYCBPAP	-0.70467	-0.83292	-1.93037
MYF6	1.544198	2.635125	7.045805
MYH1	-0.55418	5.862213	10.31731
MYH11	0.698693	-0.96872	-1.97105
MYH2	-0.40458	0.104726	3.770381
MYH4	-1.16032	5.2604	10.72755
MYH8	-0.31297	7.27691	12.50626
MYL1	-0.42524	0.470146	4.835699
MYL3	-0.11599	4.508452	10.00549
MYL7	-0.27791	0.515044	2.034904
MYL9	1.172108	-1.07474	-1.89253
MYLK	0.273412	-0.96921	-1.69428
MYLK2	-0.29861	1.756729	6.239936
MYLK4	-0.02479	5.281899	9.696538
MYLPF	-0.50011	1.587256	5.827013
MYO18B	-0.64482	0.40088	2.268404
MYO1F	0.641012	1.079286	1.982843
MYO1G	0.680841	0.85628	1.36237
MYO3A	-0.58292	-0.24654	-1.03247
MYOCD	0.58149	-0.82851	-1.32445
MYOF	0.938232	0.912744	1.165853
MYOM2	-0.45432	-0.36852	4.050824
MYOT	-0.39872	5.316895	8.801333
MYOZ1	0.372761	3.992647	8.42007
MYOZ2	-0.28412	-0.67134	3.345364
MYPN	-0.33808	-0.49212	2.232929
MYPOP	-0.42476	-1.06131	-1.0896
MYRF	-0.3837	-2.31511	-0.82564
MYRIP	0.01937	-1.31458	-1.74973
MYT1	-0.32556	-0.61686	-1.25266
MYT1L	-1.06254	-2.01093	-2.17247
NPY2R	0.309547	-1.40469	-1.7793
NPY4R	-1.56001	-1.87915	-2.38606
NPY6R	-0.98343	-0.68618	-1.61241

NYAP1	-0.1806	-1.08311	-2.64954
P2RY13	0.441877	0.625254	1.096098
P2RY4	-3.03889	0.027443	-1.22938
PGLYRP1	1.022435	1.189683	1.676819
PGLYRP4	0.414902	3.087069	9.178568
PHYHIP	0.338882	-1.38411	2.958674
PHYHIPL	-2.0763	-0.95173	-1.15655
PINLYP	0.255042	1.877884	7.392354
PYCR1	0.064556	1.162392	1.390705
PYGM	0.169434	-0.70514	2.256584
PYHIN1	0.757198	0.260268	1.362719
PYROXD2	0.051417	-0.05855	-1.16646
RALYL	-1.01168	-1.61798	-1.70529
RIPPLY3	-0.53891	0.770225	1.70414
RUFY4	0.436317	0.262059	2.629262
RYR1	-0.05696	1.291221	4.817871
RYR2	0.635041	-1.31776	-1.83905
RYR3	-0.29732	-0.75635	-1.19737
SMYD1	2.742775	0.02961	4.000752
SPRY4	-0.04482	1.075922	1.839708
SYCE3	-0.3803	-1.44639	-2.93855
SYCN	-0.14281	-2.06014	-3.82829
SYCP2	-0.00207	1.260454	2.692705
SYN1	0.245889	-1.04067	-1.79667
SYN2	0.555231	-0.90575	-1.94887
SYNDIG1	0.042884	-1.19947	-2.06256
SYNDIG1L	-0.82595	0.931753	1.257014
SYNE2	-0.02646	-0.43705	-1.04989
SYNE4	-0.76893	0.656295	1.830785
SYNGR1	-0.20724	-1.44287	-1.48851
SYNGR3	0.028777	-1.04213	-1.96175
SYNM	1.504567	-1.13533	-1.88308
SYNPO2	0.15596	-0.96689	-1.71019
SYNPO2L	-0.29868	1.059316	4.877796
SYNPR	0.70208	-2.20226	-1.83088
SYP	-0.76125	-1.1582	-1.70409
SYPL2	0.622811	-1.46937	1.997488
SYT1	-0.05732	-1.60315	-2.10606

SYT11	0.853268	-1.07681	-1.16845
SYT14	-0.4358	-1.28661	-1.6006
SYT16	0.291363	-1.03597	2.214269
SYT17	-1.29987	-1.27937	-1.4395
SYT2	-0.76453	-1.59686	-2.29137
SYT3	0.284438	-1.34848	-1.08266
SYT4	0.324419	-2.04563	-2.12368
SYT6	-0.15134	-1.93523	-2.40434
SYT8	-1.57529	1.209612	1.842306
SYT9	-0.13195	-1.62957	-1.28049
SYTL1	0.2832	-0.28672	-1.17979
SYTL3	0.565732	0.806163	1.484504
TSPYL4	0.258355	-1.36147	-1.50175
TSPYL5	0.73023	-0.95434	-1.28462
TTYH1	-0.03807	-0.11576	1.710979
TYMP	0.840435	-1.61055	-2.29801
TYROBP	0.631436	1.030337	2.09716
WDFY1	0.275021	1.076322	1.218541
WDFY2	0.532878	0.742849	1.032507
YBX2	-2.04059	-0.94695	-1.23861
YJEFN3	-1.66324	-0.97518	-1.0655
YPEL3	0.352605	-1.08511	-1.47374
YPEL4	0.171015	-0.6083	-1.12989
ZFYVE21	0.167555	-0.54875	-1.01672
ZMYND15	0.551273	1.425378	1.848329

Publications from this thesis

1. **Ephraim, R.**, Fraser, S., Nurgali, K. and Apostolopoulos, V. Checkpoint markers and tumour microenvironment: what do we know? *Cancers*, 14:3788-3793, 2022. [IF=6.575, Q1]. doi: 10.3390/cancers 14153788.
2. **Ephraim, R.**, Feehan, J., Fraser, S., Nurgali, K. and Apostolopoulos, V. Cancer immunotherapy: the checkpoint between chronic colitis and colorectal cancer. *Cancers*, 14:6131-6151, 2022. [IF=6.575, Q1]. doi: 10.3390/cancers14246131.
3. **Ephraim, R.**, Fraser, S., Devereaux, J., Stavely, R., Feehan, J., Eri, R., Nurgali, K. and Apostolopoulos, V. Differential gene expression of checkpoint makers and cancer markers in mouse models of spontaneous chronic colitis. *Cancers*, 15:4793-4814, 2023. [IF=6.575, Q1] doi:10.3390/cancers15194793.

Submitted

4. **Ephraim, R.**, Fraser, S., Sahakian, L., Stavely, R., Devereaux, J., Davidson, M., Feehan, J., Nurgali, K. and Apostolopoulos, V Transcriptomic analysis of differentially expressed genes in mouse models of chronic colitis (*Winnie mice*). *Scientific Reports (Springer Nature)*, Submitted 4 October 2023. [IF=4.997, Q1]
5. Devereaux, J., Stavely, R., **Ephraim, R.**, Filippone, R.T., Davidson, M., Kiatos, D., Apostolopoulos, V. Nurgali, K. Changes in energy metabolism associated with chronic intestinal inflammation. *Inflammatory Bowel Diseases*, (Submitted, 3 October 2023). [IF=4.9, Q1].

Other Publications

6. Khadela, A. Chavda, V., Postwala, H., **Ephraim, R.**, Apostolopoulos, V. and Shah, Y. Configuring therapeutic aspects of immune checkpoints for lung cancer. *Cancers*, 15:543-552, 2023. [IF=6.575, Q1] doi: 10.3390/cancers15020543.
7. Valiukas, Z., **Ephraim, R.**, Tangalakakis, K., Davidson, M., Apostolopoulos, V. and Feehan, J. Immunotherapies for Alzheimer Disease: A Review. *Vaccines*, 10:1527-1541, 2022. [IF=4.961, Q1]. doi: 10.3390/vaccines10091527.

Seminars and Workshops

1. 5th Annual Immuno-Oncology Combinations 360° conference, Cancer Research Institute, 2020
2. Victorian Cancer Bioinformatics Symposium 2020
3. qPCR webinar, Merck Group
4. Introduction to R for Biologists (Melbourne Bioinformatics)