Assessing the Role of MYSM1 Catalytic Activity in MYC Driven Carcinogenesis

Viktoria Plackoska

Department of Physiology
McGill University, Montréal, Canada

March 2023

A thesis submitted to McGill University in partial fulfillment of the requirements of the degree of Master of Science.

Table of Contents

Abstract

Myb-like, SWIRM and MPNs domain 1 (MYSM1) is a nuclear-chromatin binding protein with deubiquitinase (DUB) catalytic activity. In recently published studies, we demonstrated that MYSM1 maintains the expression of genes encoding ribosomal proteins in hematopoietic cells, and co-localizes to the promoters of these genes with the oncogenic transcription factor MYC. As the oncogenic activity of MYC is linked to induction of ribosome biogenesis and protein synthesis, we further tested the role of MYSM1 in the regulation of MYC oncogenic function, crossing the *Eu-Myc* mouse model of B cell lymphoma with the *Mysm1*$^{-/-}$ and *Mysm1*$^{FL/FL}$ *Cre*^{ERT2} lines to establish constitutive and inducible *Mysm1*-deletions in cancer cells. This demonstrated that the loss of MYSM1 protein expression can inhibit oncogenic activity of MYC in mouse models, making it a potential drug target for hematological malignancies.

In the current study, we aim to analyze whether the loss of MYSM1 DUB catalytic activity can also delay the onset and progression of *Eu-Myc* tumors, as well as gain insights into the host immune cell infiltration within the tumor microenvironment. A novel mouse strain *Mysm1*^{D660N}, expressing a catalytically inactive MYSM1, was crossed to the *Eu-Myc* mouse model of B cell lymphoma to generate mice of *Eu-Myc Mysm1*$^{DN/DN}$, *Eu-Myc Mysm1*$^{D660N/FL}$ *Cre*^{ERT2}, and control genotypes. Survival studies and adoptive tumor cell transfer studies demonstrated that the loss of MYSM1 catalytic activity in B-cell lymphoma is protective against disease onset and progression and is associated with increased immune cell presence and activation within the tumors. Our preliminary data also shows a co-localization of MYSM1 and MYC with the PRC1 complex at the promoters of genes encoding ribosomal proteins, suggesting their functional interactions in the regulation of ribosomal gene expression. These findings are an important step toward establishing MYSM1 as a potential drug target for MYC-driven hematological malignancies.

Résumé

Myb-like, SWIRM and MPNs domain 1 (MYSM1) est une protéine de liaison à la chromatine nucléaire dotée d'une activité catalytique de déubiquitinase (DUB). Dans des études récemment publiées, nous avons démontré que MYSM1 maintient l'expression des gènes codant pour les protéines ribosomales dans les cellules hématopoïétiques, et se colocalise aux promoteurs de ces gènes avec le facteur de transcription oncogène MYC. L'activité oncogène de MYC étant liée à l'induction de la biogenèse des ribosomes et de la synthèse des protéines, nous avons testé le rôle de MYSM1 dans la régulation de la fonction oncogène de MYC, en croisant le modèle de souris *Eu-Myc* de lymphome à cellules B avec les lignées *Mysm1*$^{-/-}$ et *Mysm1*$^{FL/FL}$ *Cre*^{ERT2} pour établir des délétions constitutives et inductibles de *Mysm1* dans les cellules cancéreuses. Cela a démontré que la perte d'expression de la protéine MYSM1 peut inhiber l'activité oncogène de MYC dans des modèles murins, ce qui en fait une cible médicamenteuse potentielle pour les hémopathies malignes.

Dans la présente étude, nous souhaitons analyser si la perte de l'activité catalytique DUB de MYSM1 peut également retarder l'apparition et la progression des tumeurs *Eu-Myc*, et obtenir des informations sur l'infiltration des cellules immunitaires de l'hôte dans le microenvironnement tumoral. Une nouvelle souche de souris *Mysm1*^{D660N} exprimant un MYSM1 catalytiquement inactif a été croisée avec le modèle de souris *Eu-Myc* de lymphome à cellules B afin de générer des souris de génotypes *Eu-Myc Mysm1*$^{DN/DN}$, *Eu-Myc Mysm1*$^{D660N/FL}$ *Cre*^{ERT2} et de contrôle. Des études de survie et de transfert adoptif de cellules tumorales ont démontré que la perte de l'activité catalytique de MYSM1 dans les lymphomes à cellules B est protectrice contre l'apparition et la progression de la maladie, et est associée à une présence et une activation accrues des cellules immunitaires dans les tumeurs. Nos données préliminaires montrent également une co-localisation

de MYSM1 et MYC avec le complexe PRC1 au niveau des promoteurs des gènes codant pour les protéines ribosomiques, ce qui suggère leurs interactions fonctionnelles dans la régulation de l'expression des gènes ribosomiques. Ces résultats constituent une étape importante vers l'établissement de MYSM1 comme une cible médicamenteuse potentielle pour les hémopathies malignes induites par MYC.

Acknowledgements

What do a master's thesis and a marathon have in common? They are exhausting, require copious amounts of training and the support of many people around you, but ultimately are incredibly rewarding. The journey to completing my master's degree has been simultaneously the longest and shortest time of my life (with an entire pandemic snuck in there too). It truly could not have been possible without the support of not only my lab mates but my friends and family as well.

A very special thanks to my supervisor Dr. Anastasia Nijnik who not only took a chance on me when agreeing to supervise me, but who has also been incredibly patient and always provided the best guidance and support (particularly when it came to writing this thesis). To June who has been on this journey with me since day one. Your friendship both in the lab (with long flow days) and outside (with walks and coffee chats) has been such a gift and I couldn't be more grateful for your support. To Dania, who has not only spent countless hours in the animal facility with me and the mice but has also always been there to have a great chat! And Marwah, whose presence fills the lab with such positive energy, working with you has been a pleasure. To Jacky, Yue and HanChen who have graciously mentored me on all the necessary techniques to complete my degree. Your time, patience and generosity have been integral to my success. To the rest of the Nijnik lab: Roselyn, Gabi, Emily and Selin each of you have made the lab a wonderful place to be and for that I am truly grateful. I would like to thank my committee members Dr. Daniela Quail, Dr. Ursula Stochaj and Dr. Kolja Eppert for providing me with feedback and support throughout my studies. I would also like to thank and acknowledge the funding support from The Leukemia and Lymphoma Society of Canada that made all of this research possible.

Aside from everyone in the lab, I would be remiss to not mention my roommate and friend McKenzie. Saddled together as strangers when I moved to Montreal, when the pandemic first

started, you have since become someone I can always count on for support. From reading abstracts for grammatical errors to sitting down over dinner and having amazing life chats you have been a big part of what has kept me sane throughout this entire degree.

This acknowledgement wouldn't be complete without mentioning my friends and family back home. To my friends who have been by my side when I first thought about starting this journey and who have stuck around until its completion. Coming home and visiting you all has always been something I've looked forward to. Lastly, and most importantly, a huge thank you to my family who has always been here supporting me in everything I've aspired to do. Whether it's listening to my long passionate rants about immunology or encouraging me when experiments failed, I truly would not have been able to get through this degree without you. Thank you.

Contribution of Authors

Chapter 1-5: were kindly reviewed by Dr. Anastasia Nijnik.

Chapter 4.1: The experimental work was carried out, data acquired and analyzed by Viktoria Plackoska. Dania Shaban assisted with tissue collection and mouse monitoring.

Chapter 4.2: The experimental work was carried out and analyzed by Viktoria Plackoska with assistance from Joo Eun (June) Kim and Yue Liang for data acquisition.

Chapter 4.3: The experimental work was carried out, data acquired and analyzed by Viktoria Plackoska with assistance from Dania Shaban for tissue collection and mouse monitoring.

Chapter 4.4: The experimental work was carried out, data acquired and analyzed by Viktoria Plackoska for Figure 8B. Figure 8A was analyzed and provided by HanChen Wang. Bigwig files for genomic snapshots were provided by HanChen Wang and the data generated by Viktoria Plackoska.

Mouse colony management was conducted by Viktoria Plackoska, under the supervision of Dr. Anastasia Nijnik, and with the assistance of Ms. Catherine Gagne for mouse weaning and Dania Shaban, Gabriela Blaszczyk, Andrew Yim, Emily Cha and Selin Birgen for genotyping.

List of Figures and Tables

Appendix I: Supplemental Figures

Abbreviated Terms

ABC-DLBCL – activated blood B cell/activated B cell-diffuse large B cell lymphoma
APC – Antigen presenting cells
BC – Body condition
B cell – B lymphocyte
BCR – B cell receptor
bHLH-LZ – basic helix-loop-helix leucine zipper
BL – Burkitt lymphoma
Breg – Regulatory B cell
CAR T cell – Chimeric antigen receptor T cell
cCD1 – Conventional dendritic cell type-1
cDC2 – Conventional dendritic cell type-2
CD4+ CTL – CD4+ T cell with cytotoxic activity
C/EBPβ – CCAAT/enhancer-binding protein β
CLL – Chronic lymphocytic leukemia
CNS – Central nervous system
CO – Vehicle corn oil
CODOX-M/IVAC – cyclophosphamide, doxorubicin, vincristine, ifosfamide, cytarabine and etoposide
COO – Cell-of-origin
CTLA-4 – Cytotoxic T-lymphocyte-associated antigen 4
DA-EPOCH-R – Dose-adjusted etoposide, doxorubicin, cyclophosphamide, vincristine, prednisone, and rituximab
DAMP – Danger associated molecular pattern
DC – Dendritic cells
DHL – Double hit lymphoma
DLBCL – Diffuse large B cell lymphoma
DLBCL-NOS – Diffuse large B cell lymphoma – not otherwise specified
DUB – Deubiquitinase
EBV – Epstein-Barr Virus
FL – Follicular lymphoma
FUSE – Far upstream sequence elements
GC – Germinal Center
GCB-DLBCL – Germinal center like- diffuse large B cell lymphoma
HL – Hodgkin lymphoma
HLA – Human leukocyte antigen
HSC – Hematopoietic stem cell
ICB – Immune checkpoint blockade
IFNγ – Interferon-gamma
IGH – Immunoglobulin heavy chain
JAMM – JAB1-MPN-MOV34
LFA-1 – lymphocyte function-associated antigen-1
MALT – Mucosa associated lymphoid tissue

MB – Myc homology box
MCL – mantle cell lymphoma
MHC – major histocompatibility complex
MoDC – Monocyte derived dendritic cells
MPN – Mpr1/Pad1 N-terminal
Myc – Myelocytomatosis oncogene
MYC – *c-Myc* protein
MYCL – *l-Myc* protein
MYCN – *n-Myc* protein
MYSM1 – Myb-like, SWIRM, MPNs Domain 1
$NHEIII_1$ – Nuclease hypersensitivity element III 1
NHL – Non-Hodgkin lymphoma
NK – Natural killer cell
NKT – Natural killer T cell
PAMP – Pathogen associated molecular pattern
PD-1 – Programmed cell death protein-1
pDCs – Plasmacytoid dendritic cell
PGCF – polycomb group RING finger
PHC – polyhomeotic subunit
PI3K – Phosphoinositide 3-kinase
PRC1 – polycomb repressive complex 1
R-CHOP – Rituximab cyclophosphamide, doxorubicin hydrochloride, vincristine and prednisone
Rituximab – Anti-CD20 monoclonal antibody
RP-genes – Ribosomal protein genes
RYBP – RING1 and YY1-binding protein
SLL – small lymphocytic lymphoma
TAA – Tumor associated antigen
TAD – Transactivating domain
TAM – Tumor associated macrophage
TC – Tissue culture
T cell – T lymphocyte
TCR – T cell receptor
TGF-β – Transforming growth factor β
THL – Triple hit lymphoma
TIL – Tumor infiltrating lymphocyte
TIM-3 – T cell immunoglobulin and mucin-domain containing-3
TIME – Tumor immune microenvironment
TLS – Tertiary lymphoid structure
TME – Tumor microenvironment
TMX – Tamoxifen
Treg – Regulatory T cells
VCAM-1 – Vascular cell adhesion molecule-1
WHO – World Health Organization

Chapter 1: Literature Review

B Cell Carcinogenesis, Immune System in Cancer, MYC and MYSM1

1.1 – B cell Carcinogenesis

In Canada alone it is expected that 233 900 new cancer cases will be diagnosed in 2022. Of these diagnoses 12 450 will be lymphomas, 11 400 and 1050 for Non-Hodgkin (NHL) and Hodgkin lymphoma (HL), respectively[1]. Currently NHL ranks 6th in terms of prevalence and 7th in terms of mortality while HL ranks 22nd in prevalence and 21st in mortality among all cancer types[1]. While clinically lymphomas are generalized as either Hodgkin or Non-Hodgkin, the diversity and classification of all possible lymphomas is extensive. The World Health Organization (WHO) currently classifies any haematolymphoid tumours into three groups based on cell type: B cell, T cell/NK cell and stroma-derived neoplasms[2]. B cell tumours specifically are classified into 4 groups containing 19 sub-types with multiple specific cancers in each group. Of particular interest are tumours that fall within the mature B cell neoplasms classification as many of the Hodgkin and Non-Hodgkin lymphoma subtypes fall within this group[2]. Some examples of B cell lymphomas are: diffuse large B cell lymphoma (DLBCL), follicular lymphoma (FL), chronic lymphocytic leukemia (CLL)/small lymphocytic lymphoma (SLL), mantle cell lymphoma (MCL), marginal zone lymphoma, Burkitt lymphoma (BL) and lymphoplasmacytic lymphoma[2]. DLBCL is the leading form of non-Hodgkin lymphoma diagnoses and will be focused on in this review[3]. Burkitt lymphoma is a rare form of non-Hodgkin lymphoma but due to its etiology and use in research it will also be discussed[3]. To better understand B cell carcinogenesis, it is critically important to understand the etiology, pathophysiology and underlying molecular mechanisms of each unique subtype to be able to develop effective treatments.

1.1.1 – Diffuse large B cell Lymphoma Etiology and Mechanisms of Disease

Diffuse large B cell lymphoma is the most common form of Non-Hodgkin B cell lymphoma comprising 30-40% of all diagnoses[3]. It is considered to be a heterogeneous disease indicating that is has several etiologies[4,5]. DLBCL can arise de novo (primary) or can result as progression or transformation from less aggressive non-Hodgkin lymphomas such as follicular lymphoma, mucosa associated lymphoid tissue (MALT) and small lymphocytic lymphoma (secondary)[2,4]. When attempting to classify DLBCL tumors genetic, immunophenotypic and morphological characteristics are considered and the cumulative presentation is what dictates the type and treatment of DLBCL[2,4,5]. DLBCL is classified into four groups: DLBCL not otherwise specified (DLBCL-NOS), DLBCL/ high grade B cell lymphoma with MYC and BCL2 rearrangements, EBV-positive diffuse B cell lymphoma and DLBCL associated with chronic inflammation[2]. DLBCL-NOS is the most common of the four forms of DLBCL. The cell-of-origin (COO) refers to the origin of the cell that gave rise to the DLBCL-NOS and is what is used to further categorize DLBCL-NOS into three primary groups[4,6]. These groups are referred to as germinal cell like-DLBCL (GCB-DLBCL), activated blood B cell/activated B cell-DLBCL (ABC-DLBCL) and unclassified-DLBCL[6].

Most DLBCL lymphomas arise from mature B cell neoplasms that have experienced the germinal center (GC) reaction and are described as large cells with round to ovoid nuclei, vesicular chromatin, mature B cell phenotype and lack of criteria defining specific large B cell lymphoma entities[2,4,6].

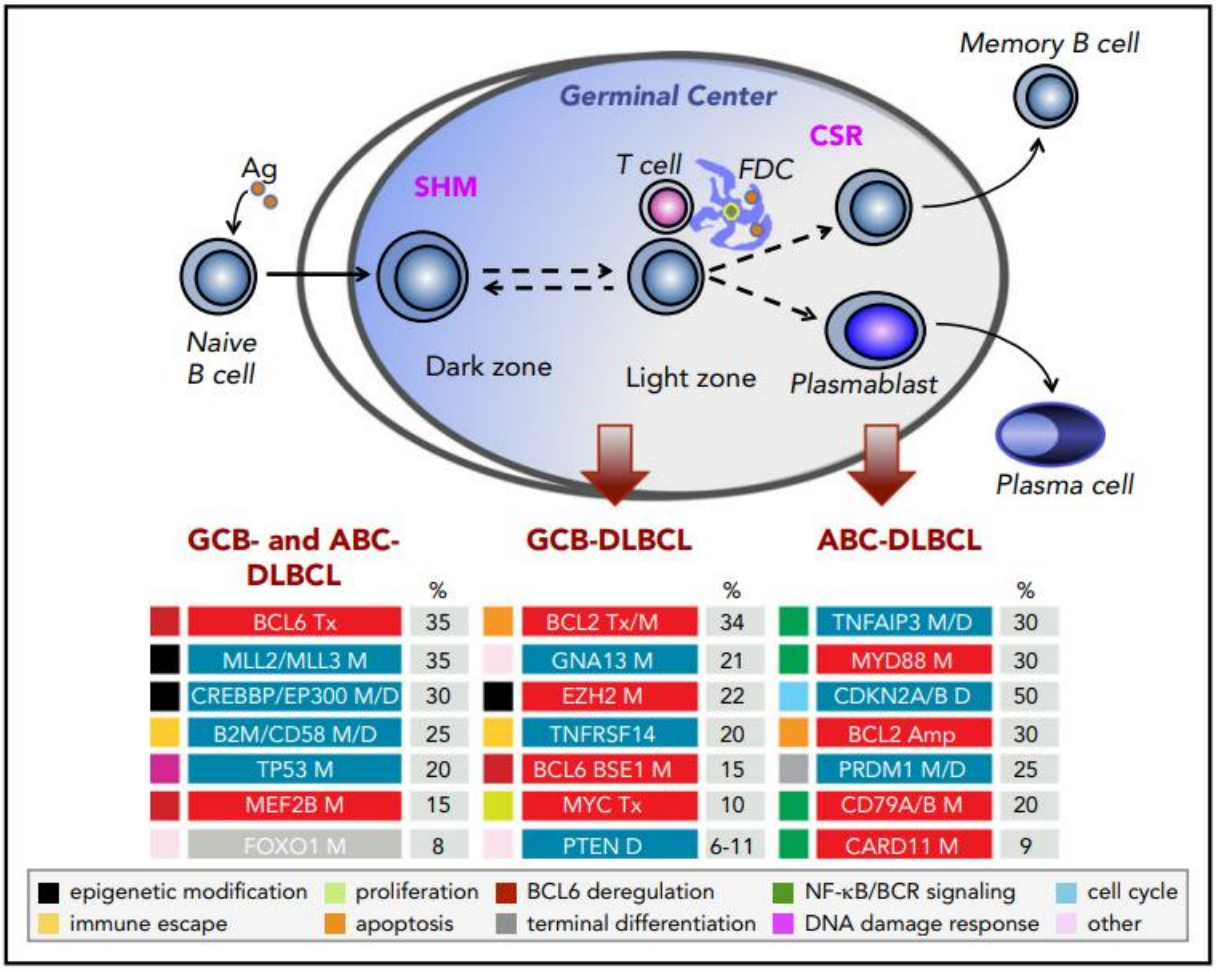

Figure 1: Genetic lesions associated with the DLBCL-NOS subtypes; blue indicates loss-of-function, red indicates gain-of-function. The coloured squares on the left indicate distinct categories that are impacted by the genetic lesion. The lesions are divided by DLBCL sub-types as well as lesions that are shared among the groups. Tx denotes chromosomal translocation, M denotes mutation, D denotes deletion and Amp denotes amplification. The percentages on the right refer to the percentage of DLBCL-NOSs that have these genetic lesions (image adapted from Laura Pasqualucci and Riccardo Dalla-Favera, *Blood*, 2018[4]).

Many genetic lesions can occur that eventually give rise to DLBCL. Highlighted in figure 1 are the breakdowns of the types of genetic lesions that occur within the DLBCL-NOS subtypes. It is evident that there are several etiologies that lead to the development of DLBCL, each of which has different mechanisms. More recent work done by Wright and colleagues aims to sort and classify

DLBCL into more specific sub-types based on their genetic profiles rather than the cell-of-origin method and argues that this form of classification has improvements for clinical treatment, as the genetic profile of each DLBCL drastically impacts its response to treatment[7]. This work along with many others solidifies the point that the heterogeneity of DLBCL contributes to its challenges for developing effective treatments.

Of particular interest are genetic alterations involving MYC as they represent more aggressive cases of DLBCL that have decreased response to treatment and poor clinical outcomes[8–10]. Roughly 5-15% of diagnosed DLBCL are linked to rearrangements in *c-Myc* [9,11–15]. *C-Myc* alterations can occur as a single hit, double hits with *BCL2* or *BCL6*, or triple hits with both *BCL2* and *BCL6* rearrangements[8,11–14,16]. Valera's study, which consisted of 219 cases of DLBCL, found that 3% of cases were MYC single-hit, 4% were double and/or triple-hit, 2% had MYC amplifications (greater than 4 gene copies) and 19% had MYC gains (3-4 gene copies). Cases that had MYC single, double or triple-hits and MYC amplifications but not MYC gains were associated with unfavourable outcomes for overall and progression-free survival[9]. These chromosomal rearrangements result in the over expression of MYC protein, which often leads to increased and uncontrolled proliferation[17,18]. DLBCL that have either double or triple hit gene rearrangements are referred to as double-hit lymphomas (DHL) and triple-hit lymphomas (THL) respectively and have been recently reclassified into a separate group of DLBCLs mentioned previously: diffuse large B-cell lymphoma/ high grade B-cell lymphoma with MYC and BCL2 rearrangements[2].

Many DHL and THL have intermediate phenotypes between DLBCL and Burkitt lymphoma[19,20]. To better understand the full spectrum of DLBCL it is important to also review the etiology and mechanisms of disease for Burkitt lymphoma.

1.1.2 – Burkitt Lymphoma Etiology and Mechanisms of Disease

Burkitt lymphoma is one of the more aggressive forms of B cell lymphoma, while rare in adults, it is commonly associated with children and highly prevalent in equatorial Africa[21–23]. In the US it also represents 20-30% of pediatric lymphoma cases but only about 1-2% of NHL cases in adults[22–24]. Overall however, it is still one of the rarer forms of B cell lymphoma relative to the rest[3]. It commonly presents with rapidly enlarging masses of the jaw or periorbital region and is most often associated with extranodal sites such as CNS, kidneys, adrenals, gonads, and gastrointestinal tract[22,23]. It is described as an aggressive, mature B cell neoplasm composed of CD10+, BCL6+, BCL2-/weak, high Ki67 index (>95%) and an IGH::MYC juxtaposition medium-sized B cells with a germinal center phenotype[2]. Up until recently the WHO classified BL into three subtypes: endemic, sporadic and immunodeficiency-associated Burkitt lymphoma; it has now since been changed to EBV-positive, EBV-negative and immunodeficiency associated Burkitt lymphoma[2]. This change was due to recent findings that have shown that the grouping of EBV-positive/EBV-negative BLs had more distinct molecular features, regardless of epidemiological context[2]. For instance, while both EBV-positive and negative BLs share mutations within coding regions impacting BCR and PI3K signaling and apoptotic pathways, EBV-positive BLs show significantly higher levels of somatic hypermutation particularly within noncoding regions[2,25,26].

BL was first linked to chromosomal translocations of MYC on chromosome 8 to immunoglobulin heavy chain (IGH) on chromosome 14 in humans in the late 1970's to early 1980's[27–29]. It was also linked less frequently to MYC translocations on the κ light chain on chromosome 2 or the λ light chain on chromosome 22. All of these translocations result in constitutive over-expression and activation of MYC, transforming it into and oncogene that promotes growth and

proliferation[27–29]. Since then many studies have been done to identify other genetic drivers of BL that work in conjunction with MYC to drive tumorigenesis[25,26].

While understanding the etiology of B cell lymphomas solves one part of the problem, understanding how DLBCL and BL can evade the immune system and how they respond to current treatments is critical in identifying areas of research to enhance the development of more effective therapies.

1.2 – Antitumor Immunity in B Cell Lymphoma

As established, MYC is a potent driver of multiple subtypes of B cell lymphoma. As such, it is important to understand how MYC aberrations impact antitumor immunity. It is also of interest to understand how DLBCL and BL are currently treated to potentially determine new avenues for therapeutic development.

1.2.1 – Role of Dendritic Cells in Antitumor Immunity

Dendritic cells (DC) are a type of immune cells that play a central role in the initiation of antigen-specific immunity and tolerance. Their main function is to serve as a connection between the adaptive and innate immune response as antigen presenting cells (APC). DCs are considered to be professional APCs that are responsible for sampling and presenting antigens to T cells, in addition to providing immunomodulatory signals through cytokines and cell-to-cell contacts. DCs are able to sample their environment through surface and intracellular receptors that recognize pathogen associated molecular patterns (PAMPs) and danger associated molecular patterns (DAMPs)[30–32].

There are several subsets of DCs that are characterized by their distinct expression of surface markers and cytokines which have been reviewed in-depth here[32]. Briefly, there are conventional type1 DCs (cDC1), conventional type 2 DC (cDC2), plasmacytoid DCs (pDCs) and monocyte

derived DCs (MoDCs). A favourable antitumor immune response is largely dependent on the ability of DCs to present tumor-associated antigens (TAA) to T cells (particularly CD8+ T cells). In mice cDC1s are associated with superior cross-presentation of antigens leading to stronger CD8+ T cell immune response, while also supporting Th1 polarization of CD4+ T cells. cDC2s responses are context dependent but have been found to induce CD4+ T cell immunity in cancer. pDCs are generally poor APCs but have been implicated to be involved in cancer cell killing indirectly through increased IFNγ production leading to NK cell activation. MoDCs are found to both enhance and inhibit antitumor immune responses[32].

The subtype of DCs that are present and the factors they secrete can largely affect the Immune response. Generally, the presence of DCs in the tumor microenvironment (TME) has been found to be a positive predictor of survival in DLBCL[33], however lymphoma-exposed DCs expressing CCAAT/enhancer-binding protein β (C/EBPβ) in *Eu-Myc* mice were found to promote tumor cell maintenance and survival[34]. Work done by Scheuerpflug and colleagues highlights the use of immune checkpoint blockade (ICB) and how it positively impacts the antitumor immune response to B cell lymphoma. This was achieved by enhancing DCs ability to activate T cells as well as increasing the expression of costimulatory molecules and cytokine secretion to favor Th1 polarization of immune response[35]. In summary, DCs play a vital role in orchestrating both pro- and antitumor immune responses.

1.2.2 – Role of Macrophages in Antitumor Immunity

Macrophages are a heterogeneous group of innate immune cells that arise from adult hematopoietic stem cells (HSC) or from embryonic precursors (fetal yolk-sac and fetal liver). Macrophages that arise from embryonic precursors contribute to tissue-resident macrophage populations while macrophages that arise from HSCs are either peripheral monocyte-derived macrophages or tissue-

resident macrophages. They are involved in various processes such as tissue remodeling, wound healing, antigen presentation and phagocytosis[36,37].

Macrophages that are involved in the immune response to tumors are known as tumor associated macrophages (TAM), and traditionally such macrophages have been categorized into a binary phenotype of M1 or M2 macrophages. TAMs have since been identified to fall within a spectrum between M1 and M2 phenotypes. TAMs have largely been shown to be pro-tumorigenic when expressing M2 polarization and antitumorigenic when expressing M1 polarization[36–39]. The polarization of macrophages is largely dependent on the TME and the tumor immune microenvironment (TIME). Macrophages that are exposed to hypoxic and highly fibrous microenvironments within tumors in conjunction with cytokines that are polarized in a Th2 response (IL-4, IL-13, IL-10) develop immunosuppressive phenotypes and are often marked by high expression of CD163 (M2). These macrophages then continue to promote a pro-tumorigenic environment through several mechanisms such as T cell exhaustion/suppression through immune checkpoint interactions, and recruitment of regulatory T cells (Tregs). In contrast, macrophages that are exposed to a Th1 polarized microenvironment express high levels of CD68 and continue to drive the immune response in an antitumorigenic fashion, which has been reviewed in more detail by various groups[36–40].

In DLBCL several studies have shown that TAMs with M2 polarization are a predictor of poor prognosis for progression-free and overall survival[41,42]. Work done by Shen and colleagues demonstrated that the number of CD163+ M2 macrophages correlated negatively with DLBCL prognosis and found that macrophage depletion led to suppressed tumor growth in mouse xenograft models of DLBCL[41]. This demonstrates how macrophages can drastically impact the outcome for patients with DLBCL. However, innate immune cells are only one part of the immune

response to cancer, and cells from the adaptive immune system are also critical and are discussed below.

1.2.3 – Role of T Cells in Antitumor Immunity

T cells have long been established as some of the main players in the immune response to cancer. Both CD4+ and CD8+ T cells are intimately involved in promoting antitumor immunity. CD8+ cytotoxic T cells are responsible for cell mediated killing of tumor cells through engagement of major histocompatibility complex (MHC) class I antigen presentation, and subsequent tumor cell lysis via granzyme and perforin. As a result, CD8+ T cells directly contribute to antitumor immunity[43,44]. CD4+ T cells are able to both directly and indirectly exert antitumor immune responses. Upon engagement of T cell receptor (TCR) through MHC class II restricted antigen presentation, CD4 + T helper cells are able to indirectly promote an antitumor immune response through secretion of various cytokines and chemokines (such as IFNγ) that promote the activation of CD8+ T cells, recruitment of natural killer (NK) cells and M1 macrophages (ultimately polarizing to a Th1 response)[45,46]. More recently, it has been shown that a subset of CD4+ T cells is able to directly exert cytotoxic functions, in an MHC class II restricted manner, through the release of granzyme B and perforin in an antigen specific fashion. They are referred to as CD4+ with cytotoxic activity (CD4+ CTL)[47]. The details of how CD4+ and CD8+ T cells are to promote antitumor immune response are beyond the scope of this review but have been covered in depth here[43–46]. However, CD4+ T cells are also able to promote pro-tumorigenic responses by promoting angiogenesis (Th17 response) and suppression of tumor infiltrating lymphocytes (TIL) through Foxp3+ Regulatory T cells (Tregs)[45].

T cell dysregulation also plays a role in unfavourable immune responses to cancer. T cell dysregulation can be caused by various factors that all lead to T cell exhaustion.

Immunosuppressive cells or factors from the TME are able to push T cells to exhaustion. For example, TGF-β is a cytokine that induces the expression of immune checkpoint markers such as TIM-3, PD-1 and CTLA-4, while also inhibiting the secretion of IFNγ and granzyme B in CD8+ T cells, to promote T cell exhaustion[48,49]. Prolonged TCR stimulation with lack of co-stimulatory molecules can also push T cells to exhaustion leading to dysfunction[43,48,49]. While T cells play a central role in antitumor immunity, they are heavily influenced by the TME and TIME. As such, determining how T cells respond in relation to other immune cells is of great importance to better understand the complexities of antitumor immunity in cancer.

1.2.4 – Role of B Cells in Antitumor Immunity

Several reviews have been published that detail the role of B cells in both pro and antitumorigenic responses[50,51]. Pro-tumorigenic functions of B cells are largely attributed to a subset of B cells known as regulatory B cells (Bregs). Bregs play a role in the suppression of many cell types such as Th1, Th17, CD4+ T effector cells, CD8+ T cells and monocytes, while contributing to the activation of others such as Foxp3+ Tregs and altering the cytokine production of DCs[52]. These combined effects lead to tumor maintenance and immune evasion[52,53]. Conversely, the review done by Kinker and colleagues highlights the importance of well-organized tertiary lymphoid structures (TLS) within tumors of varying cancer types, as they could promote the activation of B cells with antitumor effects[50]. These B cells within well-formed TLSs (AID+/BCL6+, differentiated memory and plasma cells) were found to impact antitumor immunity through tumor-antigen presentation, antibody production and tumor-killing potential. Interestingly, studies have also shown that the presence of B cells from TLSs is associated with an improved response to both immunotherapeutic and chemotherapeutic treatments in patients with melanoma (with metastatic lymph node involvement) and breast cancer[54,55].

Evidently, the role of DCs, macrophages, T cells and B cells within the antitumor immune response is multifaceted and complex and requires the formation of appropriate structures and cell-to-cell communication to illicit favourable responses. Therefore, understanding of the immune response specifically within the context of MYC-driven cancers becomes important in defining the landscape for B cell lymphoma.

1.2.5 – Impact of Aberrant MYC Expression on Antitumor Immunity in B Cell Lymphoma

MYC overexpression within lymphoid malignancies has a wide-ranged impact on the antitumor immune response. The effects can be seen within antigen presentation, expression of adhesion and costimulatory molecules, T cell tolerance, innate immunity, apoptosis, and metabolism. An in-depth review of these topics can be found here[56]. The findings are briefly summarized below.

MYC overexpression results in evasion of immune surveillance via impaired T cell recognition for both cytotoxic and helper T cells. This is due to MYC overexpression impacting HLA antigen presentation. It has an impact on both HLA class I and class II molecules[57,58]. MYC overexpression leads to downregulation of class I molecules[57] and reduced peptide presentation via class II molecules as a result of decreased expression of the class II editor HLA-DM[58]. A wide range of co-stimulatory and adhesion molecules such as lymphocyte function-associated antigen-1 (LFA-1), vascular cell adhesion molecule-1 (VCAM-1), CD40 and CD80 are all downregulated when MYC is overexpressed[59–61]. These all have impacts in T cell stimulation and activation[56,59–61]. In innate immunity, MYC overexpression is associated with high levels of CD47 expression on tumor cells, leading to decreased recruitment of macrophages and decreased tumor phagocytosis[62,63].

Aberrant MYC expression negatively impacts the antitumor immune response in B cell lymphoma through several avenues. This may provide some insight as to why patients experience poorer

prognosis when MYC aberrations are present and highlights the importance of MYC function within B cell lymphoma.

1.2.6 – Current Treatments for Diffuse Large B Cell Lymphoma and Burkitt Lymphoma

DLBCL is currently treated with regimen known as R-CHOP which consists of an anti-CD20 antibody (Rituximab) and four chemotherapeutic drugs cyclophosphamide, doxorubicin hydrochloride, vincristine and prednisone. This treatment is administered every 21 days for 6-8 cycles[64,65]. While this is effective for the majority of patients, roughly 30% relapse and of these relapsed patients the majority have DHL (MYC and BCL2)[64,65]. Standard intensive treatment for relapsed patients consists of high-dose polychemotherapy and autologous stem cell transplantation, which only cures a minority of patients[66]. Several reviews have been done to identify reasons for the failure of standard treatment such as tumor heterogeneity, tumor microenvironment and host variabilities[67], as well as identifying avenues for novel treatment development such as CAR T cell therapies, immune checkpoint inhibitors and molecular pathway inhibitors[67–69].

BL is highly susceptible to chemotherapy, however intensive treatments fare better in children as toxicity becomes of greater concern when BL is diagnosed in adults[70]. Treatment choice for BL is done based on risk stratifications that have been reviewed here[23,71,72]. The two standard approaches that are often chosen are either DA-EPOCH-R (dose-adjusted etoposide, doxorubicin, cyclophosphamide, vincristine, prednisone, and rituximab) and CODOX-M/IVAC (cyclophosphamide, doxorubicin, vincristine, ifosfamide, cytarabine and etoposide)[71]. BL has a high survival rate of 80-90% but cases that do not respond or relapse after primary treatment are also treated with autologous stem cell transplantation[70].

While current treatments for BL are largely effective, the treatment of DLBCL still remains an area where the current standard is not as effective with roughly 30% of patients experiencing

relapse, especially when MYC aberrations are involved. Therefore, targeting MYC as a therapeutic intervention is of great importance as it is correlated with poor prognosis in DLBCL.

1.3 – MYC

The *Myc* gene family is a group of regulatory genes that consists of *c-Myc* (MYC), *n-Myc* (MYCN) and *l-myc* (MYCL)[73–79]. The three genes are paralogs and function as pleiotropic transcription factors, that modulate global gene expression and play a role in various cellular processes such as proliferation, differentiation, cell cycle, metabolism, and apoptosis[80–83]. While all three genes have similar functions, they are differentiated regarding timing of expression and tissue specificity throughout development[76]. As MYC is ubiquitously expressed throughout development and is implicated to be dysregulated in up to 70% of all cancers[74,84,85], it is of critical importance to understand its function, and will be focused on here.

1.3.1 – *c-Myc* as an Oncogene

MYC, as mentioned previously, is encoded by the gene *c-Myc* and is located on chromosome 8 within locus 8q24.21 and is approximately 6kb in length[75,77]. It contains 3 exons and 4 promoters, with promotor 2 being responsible for the production of the majority of MYC transcripts[75]. MYC protein is estimated to be involved in the regulation of ~15% of all genes[86]. Unsurprisingly, as a result MYC is highly regulated at the transcriptional and post-translational modification stages[75,87]. At the chromosomal level, the MYC gene is regulated through multiple signalling pathways, cis-regulatory elements, chromatin remodeling, transcription factors and its own auto-suppression. An in-depth review of the mechanisms for *c-Myc* regulation can be found here[75,87] and some highlighted examples are as follows. Almost every major pathway that is involved in cell proliferation and quiescence impacts the MYC promoter and either directly or indirectly regulates MYC transcription, with WNT, Hedgehog and Notch being a few examples [75,87]. Far upstream

sequence elements (FUSE) and nuclease hypersensitivity element III 1 ($NHEIII_1$) are cis-regulatory elements within the MYC gene and can form non-canonical DNA structures, such as G-quadruplex configurations, thereby impacting transcription[75,87]. This along with various other forms of chromatin remodeling and MYC protein itself acting as its own suppressor and activator tightly control its expression[75,87].

However, there are many common genetic alterations that result in the transformation of MYC into an oncogene. The two most common errors that occur are gene amplification and translocation but point-mutations within enhancer and coding regions also occur[84,85,88]. Gene amplification, taking place through genome doubling or tandem duplications, is the most commonly observed marker of MYC dysregulation in various human cancers[74,89]. In the context of B cell lymphoma, MYC translocations with one of three immunoglobulin genes on chromosomes 2, 14 or 22 results in constitutive overexpression of MYC leading to the development of lymphomas[88,90]. Point mutations can also occur likely due to somatic hypermutation and also result in dysregulated expression of MYC protein. An example of this are mutations at the N-terminal region around the two major phosphorylation sites which impact protein stability leading to impaired proteasomal degradation as well as negative regulation of MYC[88,91,92]. *C-Myc* oncogenic activity requires additional mutagenic events for tumor formation as evidenced by the predictable delay in tumor onset in various models[93]. These tumorigenic effects of MYC are observed in both humans and mice[93].

Understanding the impacts of *c-Myc* alterations on MYC protein structure and function are of the utmost importance to better understand how MYC can potentially be targeted in cancer therapies.

1.3.2 – MYC Structure

When transcribed, MYC is a 439 amino acid protein which consists of an N-terminal transactivating domain (TAD), a central region and a C-terminal DNA binding domain. The N-terminal TAD is comprised of the MYC homology boxes (MB) o, I and II subdomains that are involved in the regulation of protein stability as well as transcriptional activity[76,94,95]. MBI in particular has a motif (canonical phosphodegron) which contains residues S62 and T58 that regulate the stability of MYC through sequential and hierarchal phosphorylation[76,91]. Residue S62 is phosphorylated by RAS/MEK/ERK/CDK_2, which primes residue T58 to be phosphorylated by GSK3β followed by dephosphorylation of S62 by PIN-I and PP2A, and results in MYC being targeted by SCF^{FBXW7} for Ub-proteosome-mediated degradation[76,91]. This phosphorylation is antagonised by phosphorylation of S67, which is proximal to residue S62, by Aurora B Kinase counteracting GSK3β phosphorylation and leading to MYC stability[76,96]. MBII is the most studied region of the MYC TAD, and is considered fundamental for MYC biological activity and indispensable for full MYC oncogenic activity in vivo, which has been reviewed in depth here [75,76,94] and demonstrated here[97]. The central region is comprised of MBIIIb and MBIV which are involved in MYC cell transformation[76,83,98]. The C-terminal domain contains a basic helix-loop-helix leucine zipper (bHLH-LZ) which plays a key role in DNA binding[99,100]. MYC preferentially binds to the canonical E box sequence 5'-CACGTG-3' of target gene promoters at physiological levels. When MYC is deregulated, it is also able to bind the far more abundant non-canonical E box 5-CANNTG-3' sequence[101,102]. The bHLH-LZ is also involved in the stabilization with its obligate heterodimer partner MAX, which is essential for its oncogenic activity[99,103]. As a result of its structure the MYC is an intrinsically disordered protein with various transient states and at steady-state levels has a short half-life of 20-30 minutes[104,105].

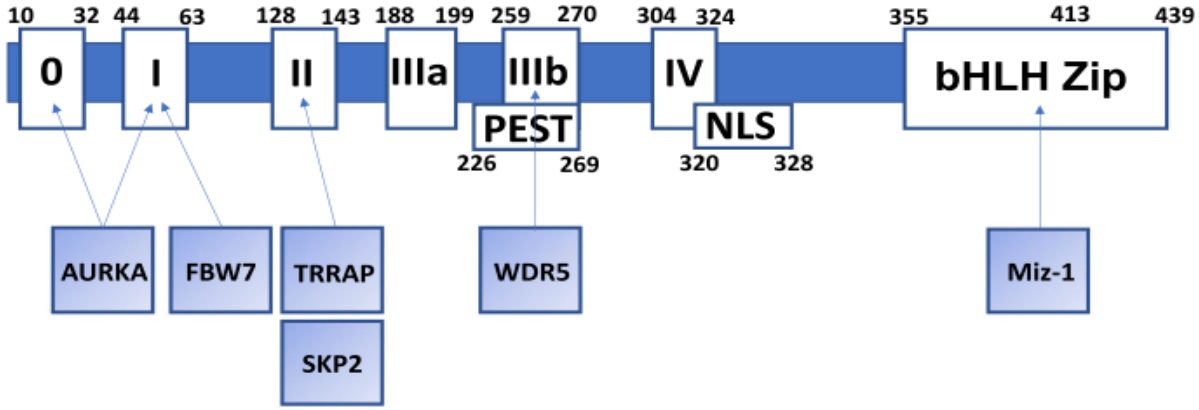

Figure 2: MYC structure and binding sites for a few major MYC-interacting proteins; the N-terminal TAD, central region and C-terminal DNA binding domain are shown from left to right respectively (image adapted from Madden. Et al, *Molecular Cancer*, 2021[106])

1.3.3 – The Role of MYC in Ribosomal Biogenesis

Functionally, as mentioned previously, MYC plays a role in various cellular processes such as proliferation, differentiation, cell cycle, metabolism, and apoptosis[80–83]. Of particular interest is the role of MYC in ribosome biogenesis. By regulating the expression of auxiliary factors that are required for rRNA processing, ribosome assembly and the export of mature ribosomal subunits from the nucleus into the cytoplasm, MYC is intricately involved in several steps of ribosomal biogenesis[107]. This has been reviewed extensively by several groups and can be found here[107–109]. Briefly, MYC has been shown to promote ribosome biogenesis by enhancing the RNA pol I-dependent transcription of rRNA through chromatin remodeling and co-factor recruitment, and also the RNA pol II-dependent transcription of genes encoding structural ribosomal proteins, factors for rRNA processing (such as fibrillarin, nucleolin and nucleophosmin), and factors involved in ribosomal subunit export and translation initiation [107,108,110–115].

1.3.4 – The Role of MYC in B Cell Development

In addition to non-cell specific functions, MYC is also involved in cell specific processes. In B cells MYC is required for germinal center formation (GC)[116]. MYC is initially expressed when mature B cells encounter antigen and are activated with the assistance of T cells triggering the germinal center formation. Subsequent up-regulation and expression of BCL6 leads to MYC suppression as it directly binds to its promoter[117,118]. The transition from MYC to BCL6 expression is associated with formation of the dark zone and leads to expansion of highly proliferative centroblasts[118]. Upon up-regulation of NF-κB and IRF4, in a select subpopulation of B cells within the light zone, MYC is re-expressed and BCL6 is down-regulated. This subset of light zone, MYC-positive cells is associated with high-affinity BCRs and are ready to enter the dark zone again for subsequent rounds of somatic hypermutation, which propels the GC reaction. Cells that are MYC-negative in the light zone are primed to exit the GC as memory cells or early plasmoblasts[118].

Given the critical role of MYC as a proto-oncogene in various forms of cancer it has become a target of great interest for therapeutic drug development.

1.3.5 – MYC as a Drug Target

For decades MYC has been dubbed the "undruggable drug target". This is due to its intrinsically disordered nature, lack of available ligand binding domains, as well as concerns for damaging off-target effects for normal proliferative cells[75,76]. The majority of the efforts for drug development has centered around indirectly targeting MYC through its various associated transcription factors such as its dimerization partner MAX. More recent studies have aimed to identify the conformationally stable transient states of MYC when it is bound to its co-factors. Work done by Macdonald and colleagues[119] identified the X-ray crystal structure for a component of MYC in complex with WDR5 and proposed that small molecules that bind in the "MYC site" of WDR5

could be developed to disrupt the MYC-WDR5 protein interaction. The same group had previously found that the interaction between MYC and WDR5 was essential for tumor maintenance in B cell lymphoma[120] indicating that interfering with these protein interactions is a promising avenue for inhibition of MYC driven cancers. The structure for MYCN-Aurora-A kinase-binding was also characterized, and the interruption of this protein interaction could be induced by altering Aurora-A kinase conformation through selective Aurora-A kinase inhibitors. Interruption of this interaction resulted in the destabilization of MYCN and subsequent degradation, which is of importance as MYCN's increased stability is one factor shown to drive neuroblastoma. This illustrated the role of MYCN-Aurora-A kinase binding in neuroblastoma and how targeting MYC co-factors could lead to promising cancer therapeutics[121]. Han and colleagues also found that small-molecule inhibitors for MYC could suppress tumor growth and enhance immunotherapy by increasing MYC degradation and synergizing with anti-PD1 immunotherapy[122].

While significant progress has been made in recent years to identify potential drugs for MYC driven cancers, none have progressed beyond clinical trial phases I/II; a review of the current prospective drug targets for MYC can be found here[76]. Some other targets for inhibiting MYC oncogenic functions currently being studied in pre-clinical or phase I/II clinical trials are: MYC-MAX, MAX:MAX, Aurora- A kinase, PLK1, PP2A and USP7[76]. These findings open the door for research into new MYC interacting co-factors as potential drug targets.

1.4 – Myb-like, SWIRM, MPNs Domain 1 (MYSM1)

Our lab has recently demonstrated that loss of MYSM1 in hematopoietic stem cells results in downregulated ribosomal protein gene expression and increased activation of p53 stress response pathway[123]. The work done by Lin and colleagues from our lab further demonstrated that the loss of MYSM1 in mouse models of B cell lymphoma led to delay in tumor onset and progression, also

via the downregulation of ribosomal gene expression and protein synthesis in the tumor cells[124].This in conjunction with the work done by Jiang and colleagues[125], demonstrating that MYSM1 and MYC interact in B1a cells, prompts the idea that MYSM1 may be of importance in MYC regulation. As a result, MYSM1 will be discussed in-depth within this section of the literature review.

1.4.1 – MYSM1 Structure

Myb-like, SWIRM, MPNs Domain 1 (MYSM1) is an 828 amino acid protein within the JAMM metalloprotease family. It is a deubiquitinating (DUB) enzyme with histone H2AK119ub as one of its substrates[126]. The three distinct domains that make up MYSM1 are the SANT, SWIRM and MPN domains. The N-terminal SANT domain is structurally similar to the DNA-binding domain of the transcription factor MYB, is able to bind DNA in vitro, and is required for MYSM1 association with histones in vivo[126,127]. The SWIRM domain of MYSM1 most closely resembles the Swi3 subfamily of SWIRM domains and forms an HTH-related fold which has 5 α-helices. However, the MYSM1 SWIRM domain lacks DNA binding activity which more closely resembles the LSD1 subfamily of SWIRM domains [127]. Lastly, the C-terminal MPN (Mpr1/Pad1 N-terminal) metalloprotease domain is the catalytic domain of MYSM1. It is characterized by $Zn2^{+}$-binding and has a JAMM (JAB1-MPN-MOV34) motif with a canonical sequence of $EXnHSHX_7SX_2D$[126,128]. The MPN domain functions as an isopeptidase that selectively hydrolyzes linkages between ubiquitin/ubiquitin-like proteins and their target proteins or between ubiquitin monomers within a polymeric chain[128].

1.4.2 – Roles of MYSM1 and PRC1 as Epigenetic Regulators of Gene Expression

The role of MYSM1 in the regulation of hematopoiesis and immune cell development has been covered in detail in this review[126]. Of particular interest, is MYSM1's role in the epigenetic

regulation of gene expression. As stated previously, MYSM1 modifies histone tails by deubiquitinating histone H2AK119, which is a repressive epigenetic mark. As the result, MYSM1 is considered to function as an activator of gene expression[126–128].

The epigenetic landscape that controls gene expression in mammals is very complex with multiple protein complexes that each play a role in either activation or suppression of gene expression. In opposition to MYSM1, polycomb repressive complex 1 (PRC1) is involved in the silencing of gene expression through ubiquitination of histone H2AK119 [129]. PRC1 is an E3 ubiquitin ligase complex and is composed of several proteins: RING1A/RING1B catalytic subunits, one of 6 polycomb group RING finger (PCGF) proteins (PCGF1-6), and a host of auxiliary subunits that are dictated by the PCGF proteins.

PRC1 can form either canonical or variant complexes. The canonical complex involves PCGF2 or PCGF4 with one of 5 chromodomain-containing paralogues (CBX2, CBX4, CBX6, CBX7, CBX8), and a polyhomeotic (PHC) subunit (PHC1, PHC2 or PHC3). Conversely, variant PRC1 is composed of PGCF1-6, RING1 and YY1-binding protein (RYBP) or YAF2 and various additional subunits that are dictated by the respective PCGF components. An in-depth review of the proteins that make up the various PRC1 complexes can be found here[129].

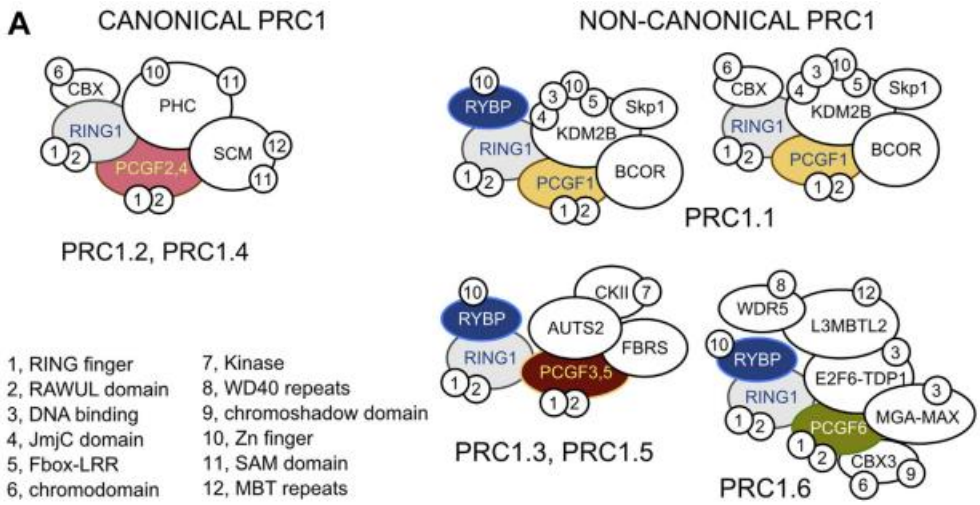

Figure 3: Overview of PRC1 composition for variants PRC1.1-1.6; all 6 variants of PRC1 have been shown above and their corresponding auxiliary proteins. The figure summarizes the current understanding of PRC1 complexes. The numbers surrounding the PCGF complexes correspond to type of domains present and are denoted in the legend on the left (image adapted from Vidal and Starowicz, *Experimental Haematology*, 2017[130]).

In the context of MYC driven lymphomas, variant PRC1.6 is of particular interest as it is a MYC-interacting complex[131]. Work done by Tanaskovic and colleagues demonstrated that loss of PCGF6 in *Eu-Myc* mice leads to accelerated lymphomagenesis in a manner that appears to be independent of PRC1.6. However, they did not fully rule out the contribution of the formation of PRC1.6 complex (containing MGA/MAX) as an antagonist to MYC/MAX-dependent transcription in other B cell lymphoma models[132]. As referenced previously, work done by Thomas and colleagues found that the loss of WDR5 was protective in MYC driven B cell lymphoma using the *Eu-Myc* mouse model[120]. While this study did not directly asses the role of PRC1 within the context of lymphoma, it is important to note that WDR5 is a component of variant PRC1.6, suggesting that this work should be further expanded to see if the essential MYC-WDR5 interactions observed could be due to the involvement of PRC1.6.[120,129,130] These findings contribute to our understanding of how MYSM1 and PRC1 are able to regulate gene expression, as well as providing

further insight into the potential interactions between PRC1 and MYC and how they might subsequently interact with MYSM1.

1.4.3 – Further Defining the Relationship Between MYSM1 and MYC in B Cell Lymphoma

To further address the relationship between MYSM1 and MYC in the context of B cell lymphoma, the *Eu-Myc* mouse model was used throughout my thesis and in previously published work from our lab. *Eu-Myc* mice were first established and described by Adams and colleagues in 1985 and are a model that closely resembles BL, with the *c-Myc* oncogene under the control of IGH enhancer resulting in development of lymph node tumors and death by 4 months of age[29]. Our lab further bred *Eu-Myc* mice with either constitutive or inducible loss of MYSM1 protein (*Eu-MYC Mysm1*$^{-/-}$, *Eu-MYC* Cre^{ERT2} *Mysm1*$^{fl/fl}$). These mice were used to assess the impacts of the loss of MYSM1 on the onset and progression of B cell lymphoma[124]. It was found that the loss of MYSM1 protein in B cell tumors resulted in repressed induction of ribosomal protein genes, reduced cellular protein synthesis rates, increased levels of p53 tumor suppressor, and ultimately delayed onset and progression of B cell lymphoma. This study also identified 45 shared genome-binding sites between MYC and MYSM1 that were within 1kb of the nearest gene transcription start site. Of these 45 binding sites, 28 were localized near the genes encoding ribosomal proteins, while 4 others were near the genes encoding translation factors. Together, this work suggests that MYSM1 and MYC may co-regulate ribosomal protein genes and that loss of MYSM1 inhibits MYC oncogenic function[124].

However, to further develop MYSM1 as a potential drug target for B cell lymphoma, understanding the impacts of MYSM1 catalytic activity on tumor progression has yet to be done and is the focus of my thesis. To establish *Eu-Myc* mice with a constitutive or inducible loss of MYSM1 catalytic activity, *Eu-Myc* mice were bred with a novel mouse strain that was developed

and characterized in our lab that expresses catalytically inactive MYSM1[133]. Using CRISPR/Cas-9 mediated genome editing, these mice were developed to express a catalytically inactive MYSM1 as a result of a point mutation within exon 16 of *Mysm1* (*Mysm1*^{D660N}). The point mutation resulted in a single amino acid substitution of aspartic acid 660 to an arginine within the catalytically active MPN domain. These mutant mice *Mysm1*$^{DN/DN}$ presented with the same phenotypes as the full *Mysm1*- knockouts and demonstrated that the deubiquitinase catalytic activity of MYSM1 is essential for its in vivo functions [133].

By using these novel mice, we aim to further address whether MYSM1 may be an effective drug target in treating MYC-driven B cell lymphomas, by assessing the impact of the loss of MYSM1 catalytic activity on lymphoma disease progression. The hypothesis and rationale for my thesis have been elaborated on in Chapter 2.

1.4.4 – DUBs as Drug Targets

While my thesis aims to further identify MYSM1 as a potential drug target for B cell lymphoma, it is important to highlight the legitimacy of using DUBs as drug targets. Several reviews have been done that aim to define DUBs as novel and promising targets for cancer therapy[134–136]. The appeal of DUBs as drug targets arises from their key role in many cellular processes that are often pathogenic (such as cancer), and they also contain a well-defined active site, ideal for small-molecule inhibitors. One such example is USP7 which is involved in the regulation of MYC and MYCN stability[137]. Work done by Schauer and colleagues using breast cancer cell line MCF7 found that selective inhibition of USP7 via XL177A leads to cancer cell killing through a p53-dependent mechanism[138]. This work and many others have led to the development of several inhibitors targeting USP7 that are currently in clinical trials in various cancers, as summarized here[76]. This demonstrates that DUBs are a viable option for cancer therapeutics and establishes the

groundwork to continue to investigate MYSM1 as a potential drug target for MYC-driven B cell lymphoma.

Chapter 2: Rationale and Objectives

2.1 – Overview

Given that non-Hodgkin lymphomas are the 6th most common form of cancer, ranking 7th in mortality in Canada, and will affect more than 12 000 new people within the year, it is imperative to find more specific and effective treatments[1]. The challenge in developing specific and effective treatments for B cell lymphoma arises due to the complex etiology and heterogeneity of the disease. Current standard of treatment varies slightly among the subtypes of B cell lymphoma but the most common practice for DLBCL is R-CHOP therapy, which comprises chemotherapy plus the rituximab anti-CD20 monoclonal antibody[64–66,70]. In the event of cancer relapse alternative immunotherapy, targeted drug therapies and autologous stem cell transplants are often the next step. Effective drug therapies require high specificity and minimal off target effects, and the development of more target-specific drugs is urgently needed.

MYSM1 is a nuclear deubiquitinase (DUB) that targets histone H2A-K119ub and acts as an epigenetic regulator of gene expression in hematopoiesis and immunity[126]. Previous work in our lab showed that an inducible deletion of *Mysm1* gene was strongly protective against B cell lymphoma onset and progression in the Eu-*Myc* mouse model, in which the disease is driven by MYC overexpression from the immunoglobulin heavy chain locus enhancer[29,124]. Adoptive mouse-to-mouse tumor cell transfers demonstrated that the loss of MYSM1 in the tumor cells was sufficient for this protective activity, but did not rule out additional MYSM1 functions in the tumor microenvironment or antitumor immunity. At the cellular and molecular level, the loss of MYSM1 in tumor cells was associated with downregulation of the MYSM1/MYC co-regulated genes encoding ribosomal proteins and translation factors, reduced protein synthesis rate, and increased levels of p53 tumor suppressor protein[124].

2.2 – Rationale and Hypotheses

I. To further develop MYSM1 into a potential drug target, testing the effects of the loss of MYSM1 DUB catalytic activity on lymphoma onset and progression is essential, as this more closely mimics the expected effects of a pharmacological inhibitor. To do this, our lab has established a *Mysm1*^{D660N} mouse strain (to be abbreviated *Mysm1*^{DN}) that expresses a catalytically inactive MYSM1[133]. In my research project, this mouse strain was crossed to the Eu-*Myc* and *Mysm1*^{FL} Cre^{ERT2} mouse strains[124,139]. We therefore derived cohorts of Eu-*Myc* B cell lymphoma mice with either constitutive or tamoxifen-induced loss of MYSM1 catalytic activity: Eu-*Myc* *Mysm1*$^{DN/DN}$ and Eu-*Myc* Cre^{ERT2} *Mysm1*$^{FL/DN}$, respectively, as well as the appropriate control mouse groups.

We hypothesize that the loss of MYSM1 catalytic activity in Eu-*Myc* tumors will result in protection against B-cell lymphoma progression.

II. Adoptive mouse-to-mouse tumor cell transfers demonstrated that the loss of MYSM1 in the tumor cells was sufficient for protection against the disease, but did not rule out additional MYSM1 functions in the tumor microenvironment or antitumor immunity[124]. Indeed, MYSM1 is expressed in many immune cell types and has complex effects in the regulation of immune cell development and activation[125,126,133,139]. The role of MYSM1 specifically in antitumor immunity however has not been previously investigated, and will be studied in the current research project.

We hypothesize that the loss of MYSM1 catalytic activity in Eu-*Myc* tumors will not only affect the tumor cell physiology but also the tumor infiltrating immune cells, with possible effects on antitumor immunity.

III. Our previous studies implicated MYSM1 as a co-regulator of the MYC-target genes encoding ribosomal proteins and translation factors (to be abbreviated as *RP*-genes)[123], and we proposed that this activity underlies the protective effect of MYSM1-loss against MYC-driven lymphoma. MYSM1 binds MYC[125], localizes at the known MYC-binding sites within *RP*-gene promoters[124], and the loss of MYSM1 results in reduced *RP*-gene expression and protein synthesis[123,124,126,139]. However, the mechanisms through which MYSM1 regulates *RP*-genes remains poorly understood.

MYSM1 is primarily known as a DUB for histone H2A-K119ub[126], which is a repressive epigenetic mark deposited on chromatin by the polycomb repressive complex 1 (PRC1)[129,130]. Traditionally, PRC1 and H2AK119ub were known to mediate stable gene repression during cellular differentiation[129–131], however recent studies implicated specifically the PRC1.6 variant of the complex in the regulation of housekeeping transcriptional programs[129,131].

We hypothesize that MYSM1 may regulate the expression of genes encoding ribosomal proteins and translation factors in cross-talk with PRC1.6. Here I will test for co-localization of PRC1.6 at the known MYSM1/MYC binding sites within such gene promoters.

2.3 – Objectives and Scope

Aim I: To test if the loss of MYSM1 catalytic activity protects from MYC driven lymphoma

To determine the protective effects of catalytically inactive MYSM1, Eu-*Myc* mice were bred with the novel mouse strain *Mysm1*^{DN} that expresses a catalytically inactive MYSM1^{D660N}[133].

a. Survival studies comparing *Eu-Myc Mysm1*$^{DN/DN}$ mice against mice of control genotypes were conducted, testing the effect of a constitutive loss of MYSM1 catalytic activity on B cell lymphoma.

b. A mouse model allowing a tamoxifen-induced loss of MYSM1 catalytic activity was established (*Eu-Myc* Cre^{ERT2} *Mysm1*$^{FL/DN}$), with the appropriate control groups. These mice were used in adoptive mouse-to-mouse tumor cell transfer experiments, testing the effect of a tumor-intrinsic loss of MYSM1 catalytic activity on lymphoma disease progression.

Aim II: To explore the effect of the loss of MYSM1 catalytic activity on antitumor immunity

a. Tumors with a loss of MYSM1 catalytic activity were compared against tumors of control genotypes for immune cell infiltration. Comprehensive flow cytometry protocols quantifying most major immune cell types and analyzing their activation and polarization states were used.

b. A pilot mouse-to-mouse tumor cell transfer study was conducted to test the effects of a selective loss of MYSM1 catalytic activity in the tumor microenvironment on lymphoma disease progression.

Aim III. To explore how MYSM1 and MYC co-regulate ribosomal protein gene expression

For this purpose, we investigated the co-localization of the MYSM1 and MYC binding sites at the promoters of genes encoding ribosomal proteins and translation factors (*RP*-genes) with the binding sites of PRC1.6. The studies encompassed: **a)** bioinformatics meta-analyses of published ChIP-seq datasets, followed by **b)** validation with ChIP-qPCR assays in murine B cell lymphoma cell lines.

Chapter 3: Materials and Methods

3.1 – Materials and Methods for Mouse Survival Studies

3.1.1 – Mouse Strains

Mice used for all experiments were maintained under specific pathogen-free conditions. Mouse line B6.Cg-Tg(IgHMyc)22Bri/J, conventionally referred to as *Eu-Myc*, is a widely used model to study B cell lymphoma. It develops tumors due to overexpression of MYC under the control of the immunoglobulin heavy chain locus enhancer[29,124]. Novel mouse line *Mysm1*^{D660N} (herein referred to as *Mysm1*^{DN}) was recently generated in our lab to study the effects of the loss of MYSM1 catalytic activity; aspartic acid 660 was mutated to an asparagine within the MPN catalytic domain resulting in a functionally inactive MYSM1 protein, as described here[133]. *Mysm1*^{DN} mice were bred with the *Eu-Myc* strain and the *Mysm1*$^{FL/FL}$*Cre*^{ERT2} strain to generate mice of *Eu-Myc Mysm1*$^{DN/DN}$, *Eu-Myc Mysm1*$^{FL/DN}$*Cre*^{ERT2}, *Eu-Myc Mysm1*$^{FL/FL}$*Cre*^{ERT2} and control *Eu-Myc Mysm1*$^{FL/+}$*Cre*^{ERT2} genotypes[124,139].

3.1.2 – Mouse-to-Mouse Tumor Adoptive Cell Transfer

To determine the effects of inducible loss of MYSM1 catalytic activity on onset and progression of B cell lymphoma, adoptive tumor cell transfer experiments were performed, as described here. Cryopreserved tumor cells from mice of *Eu-Myc Mysm1*$^{FL/DN}$*Cre*^{ERT2} and control *Eu-Myc Mysm1*$^{FL/+}$*Cre*^{ERT2} genotypes were thawed at 37ºC for 30-60 seconds. Cells were then resuspended in phosphate-buffered saline pH 7.4 (PBS, Thermo Fisher Scientific) and injected at 1.0x10^{6} cells per mouse intravenously into recipient wild type C57BL/6 mice that had been subjected to 3.5 Gy whole-body irradiation. The recipients then received intraperitoneal injections of tamoxifen or vehicle corn oil as described below[124,139]. Mouse health and survival were monitored over subsequent 100 days.

To determine the effects of a selective loss of MYSM1 catalytic activity in the tumor microenvironment on cancer disease progression, adoptive tumor cell transfer experiments were performed as follows. Cryopreserved tumor cells from mice of *Eu-Myc* genotype were thawed at 37ºC for 30-60 seconds. Cells were then resuspended in PBS and injected intravenously at $1.0x10^6$ cells per mouse into recipient mice of $Mysm1^{FL/DN}Cre^{ERT2}$, $Mysm1^{FL/FL}Cre^{ERT2}$ and $Mysm1^{FL/+}Cre^{ERT2}$ C57BL/6 genotypes that had been subjected to 3.5Gy whole-body irradiation. The recipients then received intraperitoneal injections of tamoxifen as described below[124,139]. Mouse health and survival were monitored over subsequent 100 days.

3.1.3 – Tamoxifen Mouse Treatment

To generate inducible loss of MYSM1 catalytic activity in tumor baring Cre^{ERT2} transgenic mice, the mice were injected intraperitoneally with tamoxifen (Sigma-Aldrich, T5648) in sterilized corn oil at 0.12mg/gram per injection, with 8 doses administered in total over 16 days. This was done after mice received intravenous injections of tumors. Control mice of the same genotypes were injected with vehicle corn oil (Sigma-Aldrich). Tamoxifen-induced $Mysm1^{FL}$ allele deletion was verified in the previous work of our research team, as described here[124,133,139].

3.1.4 – Ethics Approval

All mouse experiments were in accordance with the guidelines of the Canadian Council on Animal Care and approved by McGill Animal Care Committee under the animal use protocol AUP-7643.

3.1.5 – Statistics

Statistical analyses for mouse survival used Mantel-Cox and Gehan-Breslow-Wilcoxon tests in Prism version 9.0 (GraphPad Inc.); $p<0.05$ was considered significant.

3.2 – Materials and Methods for the Analysis of MYSM1-Catalytically Inactive Eu-*Myc* Tumors

3.2.1 – Harvesting of Lymph Node Tumors

Mice that reached clinical endpoint with a body condition (BC) score of less than two with swollen lymph nodes were euthanized humanely according to animal use protocol AUP-7643. Masseter, axillary and inguinal lymph node tumors were harvested. Tumors were then rinsed with PBS. Tumors were homogenized manually between two frosted glass slides (Thermo Fisher Scientific, 12-550-343) into 10mL of ice-cold PBS in a 10 cm tissue-culture (TC) dish (Corning, C353003 (08-772E)). The tumor suspension was filtered through 40µm sterile filter (VWR, 28145-477) with a 10mL syringe (BD Biosciences, B302995(361042472)) into a 15mL centrifuge tube (Thermo Fisher Scientific, 0553859B). Cells were then centrifuged at 700rpm for 7 minutes at 4ºC. Cells were resuspended into 10mL of B cell lymphoma freezing media, which consists of 10% dimethyl sulfoxide (DMSO, Sigma-Aldrich, D8418-100ML), 20% FBS and 70% B cell lymphoma culture media (BCM). BCM was prepared with 45% IMDM (Life Technologies, 12440053), 45% DMEM (Life Technologies, 11995073), 10% FBS, 100U/mL penicillin and 100µg/mL streptomycin. Cells were aliquoted at 1mL into 2mL cryovials (Corning, C431386(03-374-059)) and frozen at -80ºC in a Mr.Frosty^{TM} freezing container (Thermo Fisher Scientific, 5100-0001) containing isopropanol (Thermo Fisher Scientific, BP26181) for 24 hours. The frozen cell vials were subsequently moved to storage containers and kept at -80ºC for short-term storage or in liquid nitrogen for long-term storage.

3.2.2 – Cell Preparation for Flow Cytometry Analysis

The flow cytometry analysis was performed on cryopreserved tumor cell samples of *Eu-Myc* *Mysm1*$^{\Delta/DN}$*Cre*^{ERT2} and control *Eu-Myc Mysm1*$^{FL/DN}$*Cre*^{ERT2} genotypes, previously harvested from

mice subjected to tamoxifen or corn-oil treatment, respectively. The vials of cells were thawed at 37ºC for 30-60 seconds. Cells were washed in pre-warmed B cell lymphoma culture media (BCM) and centrifuged at 700rpm for 7 minutes. Then the cells were resuspended in 1mL of BCM for counting. A 1:20 dilution using trypan blue (Life Technologies, 15250061) was done before loading 10µL onto a hemocytometer. The absolute cell number was then calculated, and cells plated for flow cytometry staining.

3.2.3 – Cell Staining for Flow Cytometry Analysis

Mouse tumor cells were plated at $4.0x10^6$cells/well in a 96-well u-bottom plate (Falcon, 351177(08-772-54)). Viability Dye eFluor® 506 (eBioscience, ThermoFisher Scientific) was used at 1:1000 dilution in PBS for 20 minutes on ice to discriminate dead cells. Following viability staining, the cells were stained for surface-markers in PBS with 2% FBS for 20 minutes on ice using the following antibodies: Panel 1, BUV395-conjugated anti-mouse CD45 (30-F11, BD Biosciences); PerCPCy5.5-conjugated anti-mouse CD3 (17A2, BioLegend); PerCPCy5.5-conjugated anti-mouse CD19 (6D5, BioLegend); PerCPCy5.5-conjugated anti-mouse NK-1.1 (PK136, BioLegend); PerCPCy5.5-conjugated anti-mouse Ly6G (1A8, BioLegend); PerCPCy5.5-conjugated anti-mouse TER119 (TER-119, BioLegend); BUV737-conjugated anti-mouse CD11c (N418, eBioscience); BV650-conjugated anti-mouse MHCII (M5/114.15.2, BioLegend); BV785-conjugated anti-mouse F4/80 (BM8, BioLegend); PE-Cy7-conjugated anti-mouse CD64 (X54-5/7.1, BioLegend); eFluor450®-conjugated anti-mouse CD45R/B220 (RA3-6B2, eBioscience); APC-Cy7-conjugated anti-mouse XCR1 (ZET, BioLegend); PE-conjugated anti-mouse CD172a/SIRPa (P84, BioLegend); APC-conjugated anti-mouse CD371/CLEC12A (5D3/CLECL12A, BioLegend); FITC-conjugated anti-mouse CD317/PDAC-1 (927, BioLegend); Panel 2, APC-Cy7-conjugated anti-mouse Ly6G (1A8, BioLegend); PerCPCy5.5-conjugated anti-

mouse Ly6C (HK1.4, BioLegend); PE-Cy7-conjugated anti-mouse CD274/PD-L1 (10F.9G2, BioLegend); PE-conjugated anti-mouse CD80 (16-10A1, eBioscience); APC-conjugated anti-mouse CD86 (GL-1, BioLegend); FITC-conjugated anti-mouse CD40 (HM40-3, BioLegend); Panel 3, BUV395-conjugated anti-mouse CD3 (145-2C11, BD Biosciences); BUV737-conjugated anti-mouse CD4 (RM4-5, BD Biosciences); BV650-conjugated anti-mouse CD8 (53-6.7, BioLegend); APC/e-Fluor® 780-conjugated anti-human/mouse CD45R/B220 (RA3-6B2, eBioscience); PE-conjugated, anti-mouse CD27 (LG.3A10, BioLegend); eFluor® 450-conjugated anti-mouse CD11b (M1/70, eBioscience); PE-Cy7-conjugated anti-mouse CD279/PD1 (29F.1A12, BioLegend); BV785-conjugated anti-mouse CD223/LAG-3 (C9B7W, BioLegend); Alex-Fluor® 488-conjugated anti-mouse CD366/TIM3 (8B.2C12, eBioscience); Panel 4, APC-Cy7-conjugated anti-mouse CD8a (53-6.7, BioLegend); PerCPCy5.5-conjugated anti-mouse/human CD44 (IM7, BioLegend); BV650-conjugated anti-mouse CD45R/B220 (RA3-6B2, BioLegend); BV785-conjugated anti-mouse CD62L (MEL-14, BioLegend); PE-conjugated anti-T-bet (4B10, BioLegend); BV421-conjugated anti-mouse RORγt (Q31-378, BD Biosciences); Alexa-Fluor® 647-conjugated anti-GATA3 (16E10A23, BioLegend); FITC-conjugated monoclonal anti-FoxP3 (FJK-16s, eBioscience); PE-Cy7-conjugated anti-mouse CD152/CTLA-4 (UC10-4B9, BioLegend); BV785-conjugated rat IgG1, $_{K}$ Isotype (RTK2071, BioLegend); Alexa-Fluor® 488-conjugated rat IgG1, $_{K}$ Isotype (RTK2071, BioLegend); PE-conjugated mouse IgG1 $_{K}$ Isotype (MOPC-21, BioLegend); BV421-conjugated mouse IgG2a $_{K}$ Isotype (MOPC-173, BioLegend) and Alexa-Fluor® 647 mouse IgG2b $_{K}$ Isotype (MPC-11, BioLegend); Panel 5, PE-conjugated anti-mouse IgM (RMM-1, BioLegend). Duplicate antibodies in each panel are not listed here but can be found in section 3.2.4. Isotype controls were performed for LAG-3, TIM3, T-bet, RORγt and GATA3, and each isotype control was run twice on one sample from each group. For the

intracellular staining of T cell lineage specific transcription factors, mouse tumor cells were fixed and permeabilized using the FoxP3 Transcription Factor Staining Buffer Set (00-5523-00, eBioscience), according to the manufacturer's protocol. Compensation was performed with BD™ CompBeads (BD Biosciences). The data was acquired on BD Fortessa and analyzed with FACS Diva (BD Biosciences) and FlowJo (TreeStar, BD Biosciences) software.

3.2.4 – Flow Cytometry Staining Panels

Panel 1 – Dendritic Cells

CD45	BUV395
CD3	PerCPCy5.5
CD19	PerCPCy5.5
NK1.1	PerCPCy5.5
Ly6G	PerCPCy5.5
TER119	PerCPCy5.5
CD11c	BUV737
MHCII	BV650
F4/80	BV785
CD64	PeCy7
B220	eFluor450
XCR1	APCCy7
CD172a/Sirpa	PE
CLEC12A	APC
PDCA1	FITC

Panel 2 – Myeloid Cells

CD45	BUV395
B220	eFluor450
Ly6G	APCCy7
Ly6C	PerCPCy5.5
CD11c	BUV737
F4/80	BV785
MHCII	BV650
PD-L1	PE-Cy7
CD80	PE
CD86	APC
CD40	FITC

Panel 3 – T Cells (cell-surface)

CD3	BUV395
CD4	BUV737
CD8	BV650
CD44	APC
NK1.1	PerCPCy5.5
B220	APC-eFluor780
CD27	PE
CD11b	eFluor450
PD1	PE-Cy7
LAG3	BV785
TIM3	AF488

Panel 4 – T Cells (cell-surface and intracellular)

CD3	BUV395
CD4	BUV737
CD8	APC-Cy7
CD44	PerCPCy5.5
B220	BV650
CD62L	BV785
Fixation & Permeabilization	
Tbet	PE
RORγt	BV421
GATA3	AF647
FoxP3	FITC
CTLA4	PE-Cy7

Stain 5 – Lymphoma cells

CD19	PerCPCy5.5
B220	eFluor450
IGM	PE
MHCII	BV650
PD-L1	PE-Cy7
CD86	APC
CD40	FITC

3.2.5 – Flow Cytometry Gating Strategies

The gating to identify and analyze different immune cell populations was done using FlowJo software (TreeStar, BD Biosciences) and the gating strategies are presented in the Supplemental Figures section (S1-S4).

3.2.6 – Statistics

Prism version 9.0 (GraphPad Inc.) was used for statistical analysis. Student's two-tailed t-test was performed for two datasets; $p<0.05$ was considered significant.

3.3 – Materials and Methods for the Analysis of MYSM1, MYC and WDR5 DNA binding

3.3.1 – Ba/F3 Cell Culture

Frozen stocks of murine pre-B cell line, Ba/F3 (DSMZ, ACC 300), were thawed at 37ºC for 30-60 seconds. Cells were then washed in Ba/F3 specific media, which consists of 84% RPMI-1640 (Gibco, 11875119), 10% fetal-bovine serum (FBS, Gibco, 12483-020), 6% WEHI media as a source of IL-3, 100U/mL penicillin and 100µg/mL streptomycin (Winset) and 2mM L-glutamine (Life Technologies, 609-065-EL). Cells were centrifuged at 1250rpm for 3 minutes, then counted and plated at $1.0x10^6$cells/mL. They were passaged every two days until a total of 20 million cells in exponential growth phase was reached. Cell culture was performed as described previously[123].

3.3.2 – ChIP-qPCR

ChIP and subsequent enrichment was quantified with qPCR as previously described with minor modifications[123]. The antibody targets were anti-WDR5 (D9E1I, 13105, Cell Signaling Technolgies) and anti-MYSM1 (ab193081, EPR18657, Abcam). The antibodies were washed 4 times with medium stringent washes and the chromatin sonicated as per the same protocol. All qPCRs were performed on a StepOnePlus instrument with Power SYBR Mastermix (Applied Biosystems, Life Technologies). The primers used targeted the following genes: *Rsp10*, *Rsp24*, *Rpl7*, *Rpl9*, *Rpl11*, *Rpl13*, *Eef1g* and *PomC*, were purchased from IDT Technologies, and their sequences are listed below.

Table 1: ChIP-qPCR Primer Sequences

Target Gene (mm9)	Forward Sequence	Reverse Sequence
Rps10 199 bp downstream (Chr17:27,771,920- 27,771,988)	gtggccttcaaactcctctc	actcagagtcgactgaagaaga
Rps24 0 bp upstream TSS (5'UTR) (Chr14:25,309,903- 25,310,020)	cttgcgcgttgatatgattgg	gataagcgacggatagtgctg
Rpl7 141 bp downstream (Chr1:16,094,250- 16,094,373)	ctcagtttgctcctggtactg	tgtatctgagtgtagcctgga
Rpl9 8 bp upstream (Chr5:65,782,562- 65,782,678)	caaacagaggatgggttcagatt	gccctgacggattacaagaac
Rpl11 70 bp upstream (Chr4:135,609,214- 135,609,356)	cggatggagacggatgaaag	ctcgtttgtctgcctagaagaa
Rpl13 18 bp upstream (Chr8:125,626,232- 125,626,358)	cacttccctttcgcctgattt	ggcagagactcacctcctatac

Eef1g 197 bp downstream (Chr19:9,041,728- 9,041,874)	gctccggtgattagggtcac	ctccaggccctagaaaccat
POMC 744 bp downstream (Chr12:3,953,603- 3,955,695)	aggcagatggacgcacataggtaa	tccacttagaactggacagaggct

3.3.3 – Genomic Snapshots

Genomic snapshots were generated using Bigwig files from various published ChIP-seq datasets[123,140–142]. Files were visualized using IGV[143]. The reads were mapped to the mm9 reference genome. Read enrichment was compared to input DNA from the same cells for all respective sequences. Enrichment of protein binding at the following gene targets *Rsp10, Rsp14, Rsp24, Rpl7, Rpl9, Rpl11, Rpl13* and *Eef1g* was assessed, and the target proteins with their respective cell types are summarized below.

Table 2: Summary of Public ChIP-seq Datasets Used to Analyze for Protein Binding at Ribosomal Gene Promoters, including Cell Types, Proteins, and Citations.

Protein	Cell Type	Citations
MYSM1	Ba/F3	Belle & Wang et. al, *JCI Insights*, 2016[123]
MYC	HPC7	Wilson et. al, *Blood*, 2016[142]
RING1B	Resting B Cells	Frangini et. al, *Molecular Cell*, 2013[141]
Cbx7	Resting B Cells	Frangini et. al, *Molecular Cell*, 2013[141]
WDR5	Resting B Cells	Kieffer-Kwon et. al, *Molecular Cell*, 2017[140]
HDAC2	Resting B Cells	Kieffer-Kwon et. al, *Molecular Cell*, 2017[140]
Ezh2	Resting B Cells	Frangini et. al, *Molecular Cell*, 2013[141]

Chapter 4: Results

4.1 – Loss of MYSM1 Catalytic Activity is Protective Against MYC Driven B cell Lymphoma

4.1.1 – Overview of the Study Design

To assess the effects of the loss of MYSM1 catalytic activity on lymphoma onset and progression, mice with a constitutive and inducible loss of MYSM1 activity were established, and their survival compared against mice of control genotypes. For this purpose, Eu-*Myc* B cell lymphoma mouse strain was crossed with a novel mouse strain *Mysm1*^{DN} that expresses a catalytically inactive MYSM1[133], and with the Cre^{ERT2} *Mysm1*^{FL} mouse strain that allows tamoxifen induced deletion of the *Mysm1*^{FL} allele, as validated in our recent studies[124,139].

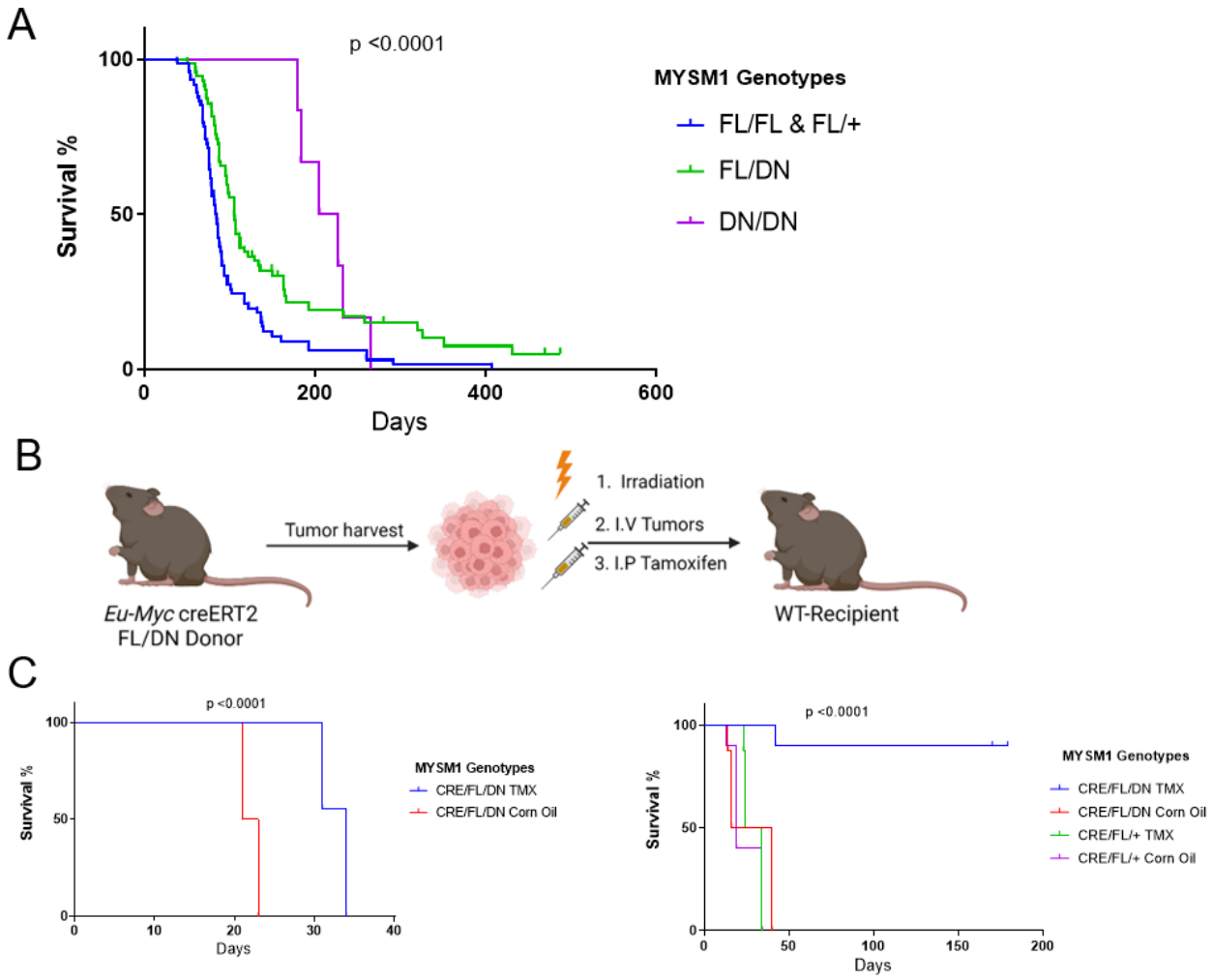

Figure 4. Loss of MYSM1 catalytic activity leads to an increased survival of *Eu-Myc* mice. (A) Survival curves

of *Eu-Myc* mice of *Mysm1*$^{FL/+}$, *Mysm1*$^{FL/FL}$ (n= 69 for both *Mysm1*$^{FL/+}$ and *Mysm1*$^{FL/FL}$ combined), *Mysm1*$^{FL/DN}$ (n=59), and *Mysm1*$^{DN/DN}$ (n=6) genotypes, where DN represents the D660N catalytically inactive allele of MYSM1. Mice homozygous for the *Mysm1*^{DN} allele show significant increase in survival compared to the other three groups. Mantel-Cox and Gehan-Breslow-Wilcoxon tests were used to evaluate the data; *p*-value <0.0001 **(B)** Schematic demonstrating the adoptive *Eu-Myc* tumor cell transfer experiments. Tumors were harvested from *Eu-Myc* Cre^{ERT2} mice of *Mysm1*$^{FL/DN}$ and *Mysm1*$^{FL/+}$ genotypes. Cohorts of wild type recipient mice were irradiated with 3.5Gy and received an intravenous injection of 10^6 tumor cells, followed by intraperitoneal injections of tamoxifen (TMX) or control corn oil (CO). Tamoxifen is known to activate Cre^{ERT2} and induce the *Mysm1*^{FL} allele deletion in the tumor cells. The survival of the recipient mice was monitored until the clinical endpoint. **(C)** Survival curves from two independent adoptive tumor cell transfer experiments conducted as shown in (B), with tumor cells of the same genotypes but from different donor mice. Trial 1 (left) and trial 2 (right) each used n= 8-10 recipient mice per group, sex- and age-matched between the groups. Although the exact time-course of *Eu-Myc* lymphoma disease progression varied between the two trials, a significant increase in survival with the TMX-induced loss of MYSM1 catalytic activity in the tumor (blue) was observed in both trials; curves were statistically evaluated using the Mantel-Cox and Gehan-Breslow-Wilcoxon tests, $p<0.0001$.

4.1.2 – Prolonged survival of Eu-*Myc* mice homozygous for catalytically inactive MYSM1

To assess the effect of a constitutive loss of MYSM1 catalytic activity, we compared the survival of Eu-*Myc Mysm1*$^{DN/DN}$ and Eu-*Myc Mysm1*$^{DN/FL}$ mice against mice of control genotypes. The control group included littermate mice of Eu-*Myc Mysm1*$^{FL/+}$ and Eu-*Myc Mysm1*$^{FL/FL}$ genotypes that retained normal MYSM1 activity (as no tamoxifen treatment was applied in the study). Such control mice showed no difference in survival between each other or when compared to previously published datasets from Eu-*Myc Mysm1*$^{+/+}$ mice[144].

We observed significantly prolonged survival of Eu-*Myc Mysm1*$^{DN/DN}$ mice homozygous for the catalytically inactive MYSM1 relative to control Eu-*Myc* mice (Figure 4A). Furthermore, there

was some trend toward prolonged survival of Eu-*Myc* *Mysm1*$^{DN/+}$ mice heterozygous for the catalytically inactive MYSM1 relative to the control group (Figure 4A). This demonstrated the protective effect of a constitutive loss of MYSM1 catalytic activity against B cell lymphoma.

4.1.3 – Prolonged survival of mice with an induced loss of MYSM1 catalytic activity in the tumors

To study the effects of an inducible loss of MYSM1 catalytic activity on lymphoma disease progression, tumor cells were harvested from Eu-*Myc* Cre^{ERT2} mice of test *Mysm1*$^{FL/DN}$ and control *Mysm1*$^{FL/+}$ genotypes. Such tumors were adoptively transferred via an intravenous injection into cohorts of wild type recipient mice at 10^6 cells per mouse. The recipient mice were then treated with tamoxifen to activate Cre^{ERT2} and induce the *Mysm1*^{FL} to *Mysm1*$^{\Delta}$ allele conversion, leaving either 'catalytically-dead' *Mysm1*$^{\Delta/DN}$ or control *Mysm1*$^{\Delta/+}$ tumors (Figure 4B). Additional cohorts of recipient mice were treated with vehicle corn oil, and these mice are expected to retain the expression of active wild type MYSM1 in the tumors from the *Mysm1*^{FL} allele. Mouse survival across the four recipient groups was tracked and the findings are presented here (Figure 4C).

A strong increase in the survival of recipient mice baring tumors that lost MYSM1 catalytic activity (*Mysm1*$^{\Delta/DN}$) was observed relative to the recipient mice baring control tumors (*Mysm1*$^{\Delta/+}$ or *Mysm1*$^{FL/DN}$). Two experiments were performed and the significant protective effect was seen in both experiments (Figure 4C). Importantly, a significant proportion of the mice with tumors that lost MYSM1 catalytic activity had full remission and survived past the trial endpoint (Figure 4C). This indicates that loss of MYSM1 catalytic activity within the tumor is strongly protective against lymphoma progression.

4.2 – Loss of MYSM1 Catalytic Activity in Eu-*Myc* Tumors Modulates Antitumor Immunity

4.2.1 – Overview of Study Design

In the adoptive tumor cell transfer study presented above, wild type recipient mice baring Eu-*Myc* tumors with an induced loss of MYSM1 catalytic activity (*Mysm1*$^{\Delta/DN}$) demonstrated significantly prolonged survival compared to mice baring control tumors (*Mysm1*$^{\Delta/+}$ or *Mysm1*$^{FL/DN}$) (Figure 4B-C). To assess if in addition to the effects on tumor cell physiology, the loss of MYSM1 catalytic activity within the tumor may also affect antitumor immunity, the Eu-*Myc* tumor cells of test and control *Mysm1*-genotypes from this experiment were harvested and analyzed by flow cytometry for immune cell infiltration. Many immune cell populations were quantified, measuring each cell type as a percentage of total live cells within the tumor, and immune cell activation was also assessed. Respective results are as follows.

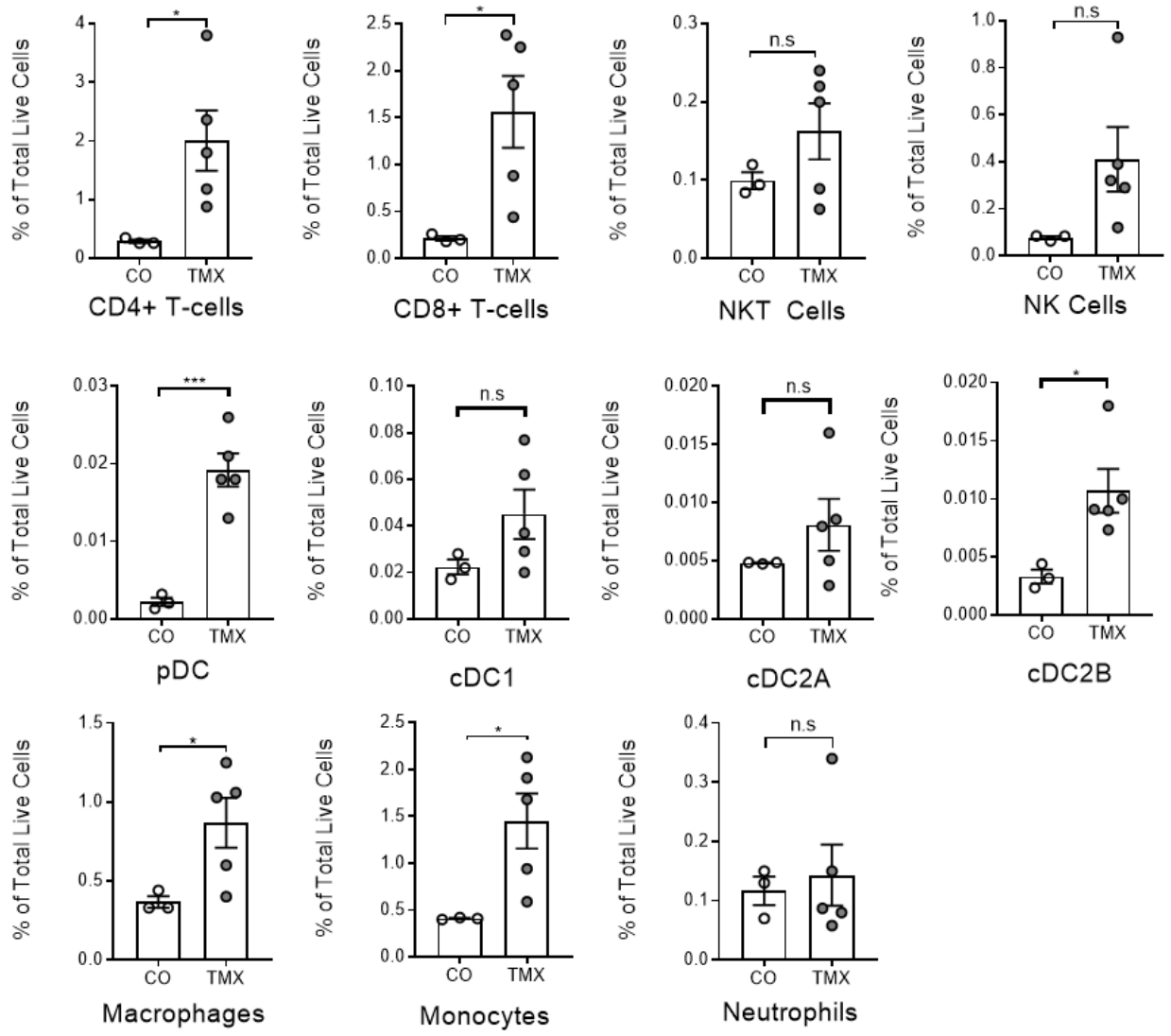

Figure 5. Increased infiltration of immune cells into the *Eu-Myc* tumors expressing a catalytically inactive MYSM1 (*Mysm1*$^{\Delta/DN}$) relative to control tumors (*Mysm1*$^{FL/DN}$). Data is from the wild type recipient mice that received *Eu-Myc* Cre^{ERT2} Mysm1$^{FL/D660N}$ tumor cells followed by either tamoxifen (n=5) or a control corn-oil treatment (n=3). Tamoxifen is known to activate Cre^{ERT2} and induce the *Mysm1*^{FL} allele deletion in the tumor cells. An increase in immune cell infiltration is demonstrated in tumors lacking MYSM1 catalytic activity, and unpaired *t*-test revealed significant increases in CD4+ T cells, CD8+ T-cells, plasmacytoid dendritic cells (pDC), conventional dendritic cell type 2B (cDC2B), macrophages, and monocytes; *p*-values are abbreviated as * $p<0.05$, ** $p<0.01$, *** $p<0.001$, and **** $p<0.0001$. Cells were gated as shown in the Supplemental Figures S1-S4.

4.2.2 – Increased immune cell infiltration in Eu-*Myc* tumors lacking MYSM1 catalytic activity

We observed that the loss of MYSM1 catalytic activity within the Eu-*Myc* tumors led to increased immune cell infiltration into the tumor. Immune cells the frequencies of which were significantly increased in *Mysm1*$^{\Delta/DN}$ relative to control *Mysm1*$^{FL/DN}$ tumors include: $CD4^+$ T-cells, $CD8^+$ T-cells, plasmacytoid dendritic cells (pDCs), conventional cDC2B dendritic cells, monocytes, and macrophages (Figure 5). While the frequency of NK cells, NKT cells, cDC1 and cDC2A conventional dendritic cells, and neutrophils did not show a statistically significant increase, an upward trend in the abundance of these cell types was also observed in the tumors lacking MYSM1 catalytic activity (*Mysm1*$^{\Delta/DN}$) relative to control tumors (*Mysm1*$^{FL/DN}$) (Figure 5).

4.2.3 – Increased immune cell activation in tumors lacking MYSM1 catalytic activity

Tumor infiltrating macrophages were compared for the expression of MHCII, co-stimulatory molecules CD80, CD86, and CD40, and checkpoint marker PD-L1, comparing tumors lacking MYSM1 catalytic activity (*Mysm1*$^{\Delta/DN}$) relative to control tumors (*Mysm1*$^{FL/DN}$). We observed a significant increase in the levels of CD40, CD86 and MHCII on macrophages in the tumors lacking MYSM1 catalytic activity, while PD-L1 levels showed an upward trend but were not significantly increased (Figure 6A-B).

Tumor infiltrating $CD8^+$ T cells were classified into naïve ($CD62L^+CD44^-$) and effector memory ($CD62L^-CD44^+$) subsets. In the tumors lacking MYSM1 catalytic activity (*Mysm1*$^{\Delta/DN}$), there was an increase in the proportion of effector memory $CD8^+$ T cells and corresponding reduction in the proportion of naïve $CD8^+$ T cells (Figure 6B-C). However, the proportion of $CD8^+$ T cells expressing the exhaustion marker CTLA4 was not elevated (Figure 6B). Overall, this indicates an increase in immune cell activation in tumors lacking MYSM1 catalytic activity.

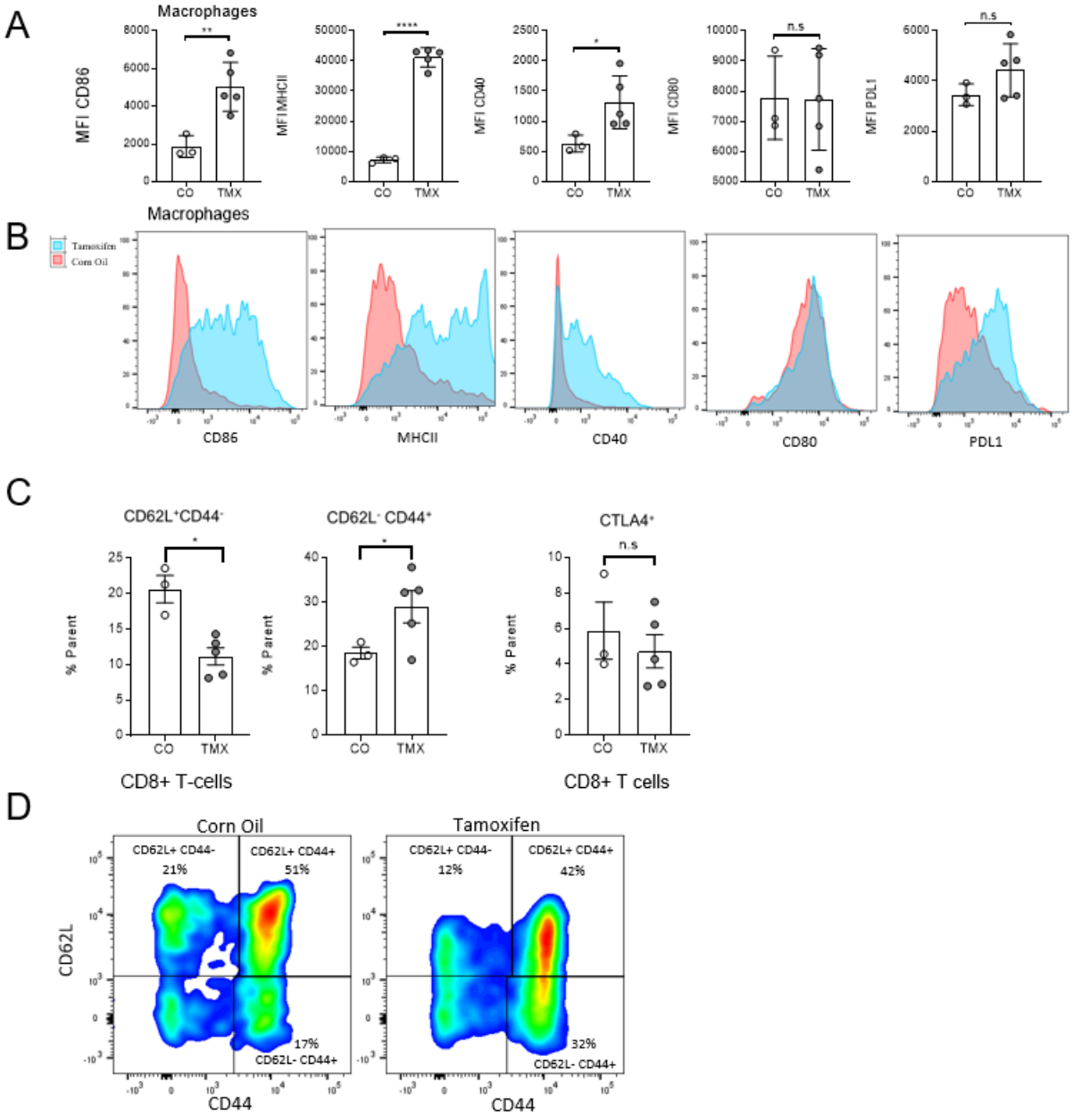

Figure 6. Increased activation of immune cells in the *Eu-Myc* tumors expressing a catalytically inactive MYSM1 (*Mysm1*$^{\Delta/DN}$) relative to control tumors (*Mysm1*$^{FL/DN}$). (A) Analysis of the expression of CD86, MHCII, CD40, CD80 and PDL1 markers on tumor associated macrophages, gated as live CD45+ B220- CD64^{+} Ly6C^{-} Ly6G^{-} cells; MFI – mean fluorescence intensity. Upregulation of CD86, MHCII, and CD40 on macrophages in the tumors expressing catalytically inactive MYSM1 is demonstrated. **(B)** Normalized histogram overlays for the respective markers in (A) the corn oil treated control *Mysm1*$^{FL/DN}$ (red) and tamoxifen treated *Mysm1*$^{\Delta/DN}$ (blue) cohorts. **(C)** Increase in the proportion of CD44^{+}CD62L^{-} effector memory CD8+ T cells, without upregulation of the CTLA4

exhaustion marker, in tumors expressing a catalytically inactive MYSM1 (*Mysm1*$^{\Delta/DN}$). **(D)** Representative flow plots for CD8+ T cells data in (C). Significance was measured via unpaired *t*-test; *p*-values are abbreviated as * $p<0.05$, ** $p<0.01$, *** $p<0.001$, and **** $p<0.0001$.

4.3 – Testing the Impact of the Loss of MYSM1 catalytic activity in tumor microenvironment

4.3.1 – Overview of Study Design

While a selective loss of MYSM1 or its catalytic activity in the Eu-*Myc* tumor cells is strongly protective against lymphoma disease progression (Figure 4C)[124], the effects of altered MYSM1 activity in the tumor microenvironment on lymphoma progression remain unknown. In the current pilot study Eu-*Myc* Cre^{ERT2} mice of *Mysm1*$^{FL/FL}$, *Mysm1*$^{FL/DN}$, and control *Mysm1*$^{FL/+}$ genotypes were engrafted with wild type Eu-*Myc* lymphoma cells. The mice were subsequently treated with tamoxifen, to activate Cre^{ERT2} and induce the *Mysm1*^{FL} to *Mysm1*$^{\Delta}$ allele conversion, resulting in *Mysm1*$^{\Delta/\Delta}$ 'deficient', *Mysm1*$^{\Delta/DN}$ 'catalytically-dead', or control *Mysm1*$^{\Delta/+}$ tumor microenvironments, without affecting the genotype of the wild type Eu-*Myc* tumors (Figure 7A). Mouse survival across the groups was monitored and the results are presented below.

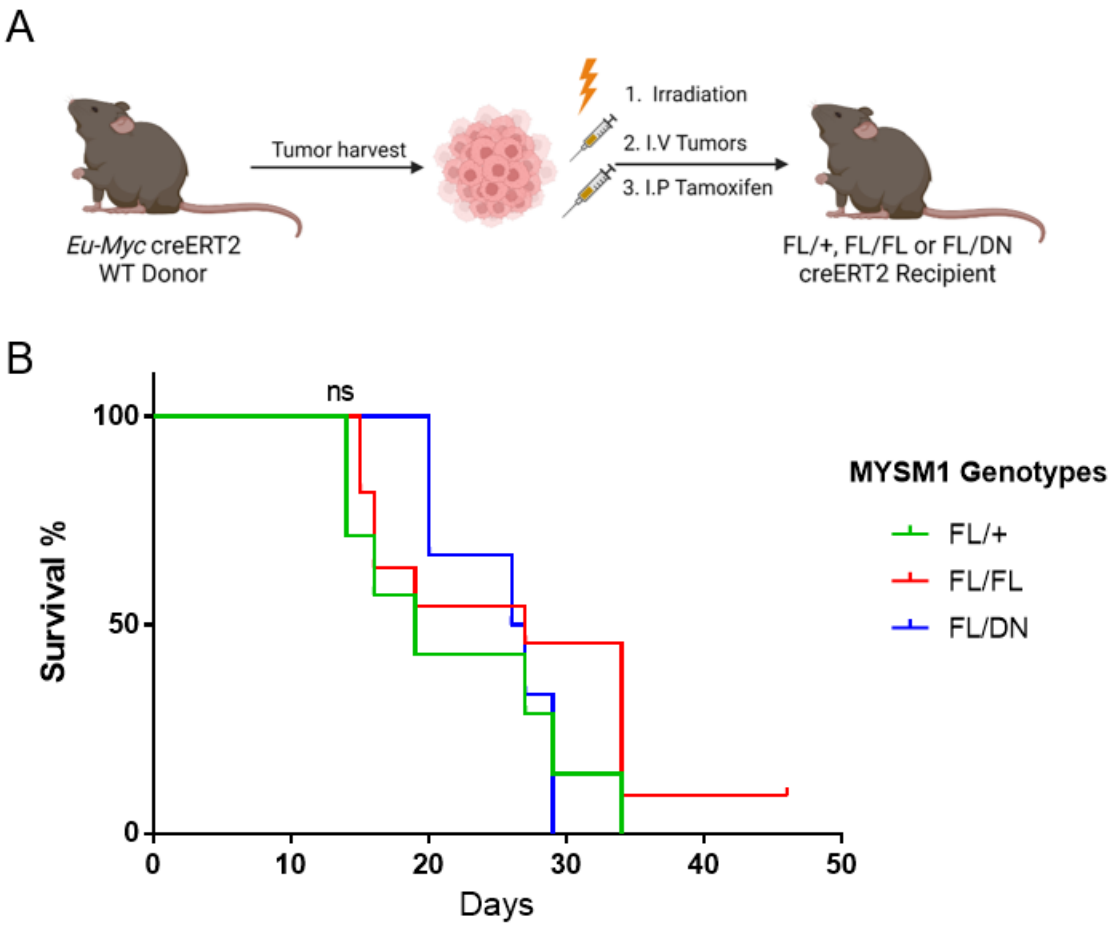

Figure 7: Loss of MYSM1 catalytic activity in the tumor microenvironment does not affect survival (A) Schematic of the experiment, demonstrating recipient mice of $Cre^{ERT2}Mysm1^{FL/+}$, $Cre^{ERT2}Mysm1^{FL/FL}$, and $Cre^{ERT2}Mysm1^{FL/DN}$ genotypes receiving wild type *Eu-Myc* tumors, followed by tamoxifen to activate Cre^{ERT2} and induce the $Mysm1^{FL}$ allele deletion. **(B)** Survival curves for recipient mice with an induced loss of MYSM1 (red) or loss of its DUB catalytic activity (blue) upon injection with wild type *Eu-Myc* tumors. The recipient mice were of $Cre^{ERT2}Mysm1^{FL/+}$ (control, n=7), $Cre^{ERT2}Mysm1^{FL/FL}$ (n=11), and $Cre^{ERT2}Mysm1^{FL/DN}$ (n=6) genotypes, and sex and aged matched between the experimental groups. Mantel-Cox and Gehan-Breslow-Wilcoxon tests revealed no significant differences in survival between the three groups.

4.3.2 – Loss of MYSM1 catalytic activity in the tumor microenvironment does not affect survival

No significant differences in the survival were observed between tumor baring mice of $Mysm1^{\Delta/\Delta}$ MYSM1-deficient, $Mysm1^{\Delta/DN}$ 'catalytically-dead', or control $Mysm1^{\Delta/+}$ genotypes (Figure 7B).

The experimental mice died within a time frame very consistent with tumor induced mortality in past Eu-*Myc* tumor cell transfer experiments (Figure 4C)[124], however unusually in this study many animals did not have obvious tumors at the time of euthanasia. Our experience argues against tamoxifen or *Mysm1*-ablation being the cause of death, as recent studies with tamoxifen-induced *Mysm1*-ablation in Cre^{ERT2} $Mysm1^{FL/FL}$ and Cre^{ERT2} $Mysm1^{FL/DN}$ mice, without tumor engraftment, did not cause significant mortality up to 30-weeks after the treatment[133]. Therefore, our preliminary data suggests that the loss of MYSM1 or its catalytic activity in the tumor microenvironment does not affect survival of tumor baring mice, however the current study should be repeated, including further control groups, and the cause of death thoroughly assessed.

4.4 – Co-localization of MYSM1, MYC, and PRC1.6 binding sites at ribosomal gene promoters

4.4.1 – Overview of Study Design

Previous studies by our research team indicated that MYSM1 promotes the expression of MYC-target genes encoding ribosomal proteins and translation factors (*RP*-genes)[123], however the mechanisms through which MYSM1 regulates such genes remain poorly understood. MYSM1 is a known DUB for histone H2AK119ub[123,125,126,133,139], and MYC was recently shown to interact with one of the polycomb complexes that deposit H2AK119ub on chromatin, namely PRC1.6[131]. Here we hypothesize that MYSM1 may regulate the expression of genes encoding ribosomal proteins and translation factors in cross-talk with PRC1.6. We therefore investigate the co-localization of the MYSM1 and MYC binding sites at such gene promoters with the binding sites of PRC1.6, using: a) bioinformatics meta-analyses of published ChIP-seq datasets, and b) validation with ChIP-qPCR assays in murine B cell lymphoma cell line Ba/F3.

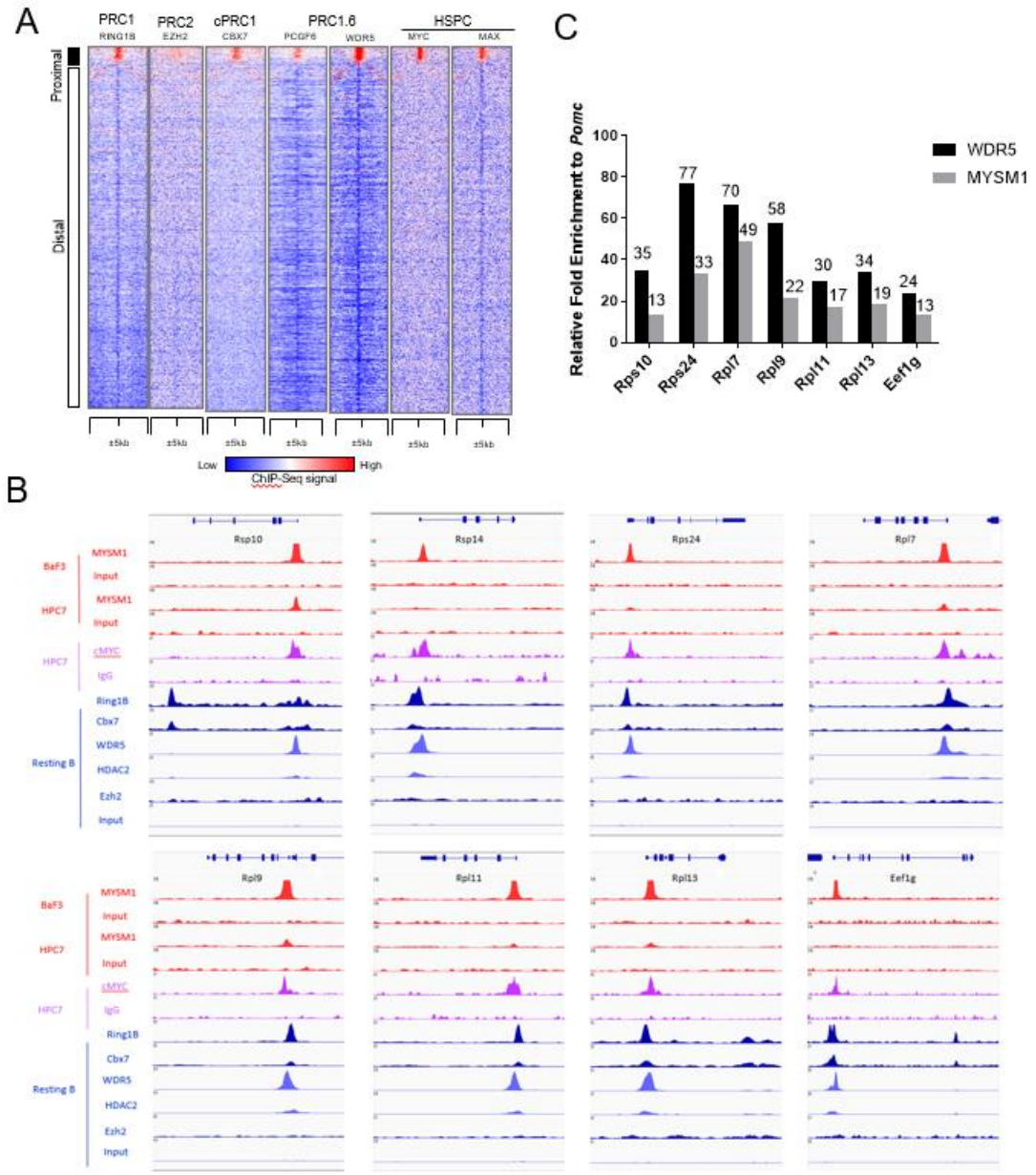

Figure 8. Exploring the mechanisms of gene regulation by MYSM1: co-localization of the MYSM1 and MYC binding at ribosomal gene promoters with the binding sites of PRC1. (A) Enrichment of PRC1 at the gene-proximal MYSM1 DNA binding sites, corresponding primarily to the promoters of genes encoding ribosomal proteins and translation factors. Data is from the following public ChIP-seq datasets: RING1B, EZH2, and CBX7 from resting B cells (Frangini et al., 2013), PCGF6 from B lymphoma (Tanaskovic et al., 2022), WDR5 from resting B cells (Kieffer-Kwon et al., 2017), and MYC and MAX from hematopoietic progenitor cells HPC7 (Wilson et al., 2016, further information and citations are provided in Table 2). **(B)** Genomic snapshots of select genes encoding ribosomal

proteins and translation factors, with the gene structure in the top panel and ChIP-Seq tracks from the datasets listed in (A) in the panels below. Co-localization of MYSM1, MYC, RING1B, WDR5, and to a lesser extent CBX7 binding at the genes is demonstrated. **(C)** ChIP-qPCR assay conducted in the Ba/F3 murine B cell lymphoma line, showing the binding of MYSM1 and WDR5 at the common sites proximal to the genes encoding ribosomal proteins and translation factors. Reproduced in two independent experiments.

4.4.2 – Meta-analyses of public ChIP-Seq data: enrichment of PRC1.6 at MYSM1 binding sites

To test for the co-localization of MYSM1 genomic binding sites with the binding sites of polycomb repressor complexes, our previously published MYSM1 ChIP-seq datasets from pre-B Ba/F3 cells and multipotent progenitor HPC7 cells[123] were consolidated with public polycomb ChIP-seq data from B lymphocytes. This included ChIP-seq datasets for the PRC1 catalytic subunit E3 ubiquitin ligase RING1B, for PRC2 catalytic subunit EZH2, for the CBX7 subunit of canonical PRC1, and for PCGF6 and WDR5 subunits of variant PRC1.6[140,141]. Previously analyzed ChIP-seq datasets for MYC and its dimerization partner MAX from the HPC7 cells were also included into the analyses[142].

As in our previous studies, MYSM1 genomic binding sites were classified as gene-proximal or gene-distal, corresponding to <1kb and >1kb from the nearest transcription start site, respectively, and the gene-proximal sites localized to the promoters of genes encoding ribosomal proteins and translation factors (*RP*-genes)[123,124]. The binding of MYC and MAX at such MYSM1 binding sites was observed (Figure 8A), as in our previous studies[124]. Importantly, we also observed the binding of the PRC1 E3 ubiquitin ligase catalytic subunit RING1B, but not of the PRC2 catalytic subunit EZH2 at such sites, suggesting PRC1 but not PRC2 binding. Among other PRC1 complex proteins we observed the binding of the CBX7 subunit of canonical PRC1, and of the PCGF6 and WDR5

subunits of variant PRC1.6 (Figure 8A). Genomic snapshots of the ribosomal protein genes *Rsp10*, *Rsp14*, *Rsp24*, *Rpl7*, *Rpl9*, *Rpl11*, *Rpl13* and translation factor gene *Eef1g* further demonstrate the overlap in the genomic binding sites of MYSM1, MYC, RING1B, and WDR5 at these gene promoters (Figure 8B). We acknowledge the contribution and supervision of research assistant HanChen Wang for these bioinformatics analyses.

4.4.3 – ChIP-qPCR validation of WDR5 binding at the MYSM1 binding sites of ribosomal genes

ChIP-qPCR assays were conducted in the pre-B cell line Ba/F3 to test for the binding of WDR5 and MYSM1 at the previously characterized MYSM1 binding sites within the promoters of genes encoding ribosomal proteins and translation factors. Increased enrichment for both MYSM1 and WDR5 was observed at all *RP*-genes tested relative to an off-target site within control *PomC* gene (Figure 8C). PCGF6 was not included into the analyses due to a lack of ChIP-grade commercial antibodies. WDR5 is known to associate with other protein complexes apart from PRC1.6[145]. However, in addition to the ChIP-qPCR data shown above, the binding of RING1B at such sites has been shown by HanChen Wang in our research team (*unpublished*). Cumulatively these data support our hypothesis that the binding of WDR5 in this case indicates the recruitment of PRC1.6.

Chapter 5: Discussion

5 – Discussion

Our study explored MYSM1 as a potential drug target for MYC-driven B cell lymphoma. Specifically, we analyzed the effects of the loss of MYSM1 catalytic activity on lymphoma disease onset and progression and concluded that the loss of MYSM1 catalytic activity is protective against B cell lymphoma (Figure 4). As the next step, to better understand how the loss of MYSM1 catalytic activity affects disease progression, it will be important to assess its effects on the tumor cells. Previous work done by Lin et al. and colleagues from our lab demonstrated that a full loss of MYSM1 in lymphoma cells resulted in a reduced expression of the MYC/MYSM1 co-regulated genes encoding ribosomal proteins, reduced protein synthesis, elevated levels of p53 tumor suppressor, and increased tumor cell death[124]. These data were acquired using RT-qPCR to determine the expression of genes encoding ribosomal proteins, OPP-incorporation assays to measure protein synthesis rates, intracellular flow cytometry to measure p53 protein levels, and viability dye and Annexin V staining assays to measure tumor cell survival in culture. Going forward, it is of great interest to repeat these assays with tumors that lack MYSM1 catalytic activity and to determine if the same effects are observed as with the full loss of MYSM1 protein. We anticipate that a similar response will be observed in tumors that lack MYSM1 catalytic activity as seen in tumors with full loss of MYSM1.

All the data presented here and in our previous studies have been acquired exclusively in mice. While animal research is essential to develop proof of concept, the next milestone in our research program would require the use of human tissues to test if the mechanisms described here are also conserved in humans. To do this we could use human lymphoma cell lines, such as U-2946, which is an established human DLBCL line that contains the t(8;14) chromosomal translocation and strongly overexpresses MYC. This cell line has already been validated and used as a model to test

inhibitors of a BCL2 family protein MCL1 [146]. Using CRISPR-Cas-9 system the U-2946 cell line could be edited to express a catalytically inactive MYSM1 with the D669N mutation, which is equivalent to the D660N mutation in our mouse models. Upon validation of these cell lines, they could be analyzed against control cells for differences in cell viability and proliferation, expression of ribosomal genes, and protein synthesis rates, to test if the mechanisms seen with the loss of MYSM1 catalytic activity in murine tumors are also conserved in human.

As reviewed in Chapter 1, DUBs have become appealing drug targets to treat various diseases, including cancer, and one of the most common methods chosen is the development of small molecule inhibitors that target the active site of DUBs. For example, small molecule inhibitors of USP7 such as P22077, GNE-6640, GNE6776, FT671 and XL177A are currently in pre-clinical development to treat various forms of cancer[76]. To develop a small molecule inhibitor for MYSM1 the first step would be to perform high-throughput screening using compound libraries, followed by structure-activity relationship (SAR) studies of validated hits, as suggested by this review[147]. This would allow for the identification of potential compounds that inhibit MYSM1, either by targeting its catalytically active MPN domain or by acting as allosteric inhibitors. To perform these screens, protocols for large-scale purification of MYSM1 protein have already been established by our collaborators (Dr. Bhushan Nagar, McGill-Biochemistry)[148]. They have also established a fluorogenic assay with the Ub-rhodamine-110 substrate for MYSM1 catalytic activity, and such an assay can be used both in the high-throughput screen and for the follow-up evaluation of the inhibitory activity of select compounds identified by the screen.

We have also shown that loss of MYSM1 catalytic activity within tumor cells enhances immune infiltration and activation in the tumors (Figures 5-6). The mechanism by which MYSM1 affects tumor cell recognition by immune cells can now be analyzed. Tumor cells are known to modulate

immune responses in various ways, and for lymphoma cells in particular, several reviews have been done that summarize these mechanisms [149–151]. Such mechanisms can include production of various secreted immunosuppressive factors, disruption of antigen presentation via the downregulation of MHCI and MHCII molecules on the tumor cells, downregulation of the costimulatory B7 family proteins such as CD80 and CD86, and an upregulation of checkpoint markers such as PD-L1 to interfere with immune mediated killing of the tumor. To address how the loss of MYSM1 catalytic activity impacts immune modulation by tumor cells we could analyze and compare the 'secretome' of MYSM1-deficient and control tumors in culture, and measure their expression of MHC, co-stimulatory, and checkpoint markers. We could also perform an immune-mediated tumor killing assay, by transducing tumor cells to express ovalbumin as a model antigen, co-culturing them with OT-I CD8+ T cells specific for ovalbumin peptides, and measuring tumor cell killing using flow cytometry as done by Pimentel and colleagues[152], comparing control and *Mysm1*-mutant tumors. This would test how well the immune system is able to recognize tumors of varying MYSM1 genotypes and characterize how the tumors with and without MYSM1 catalytic activity modulate the immune response.

Our preliminary results also suggest that the loss of MYSM1 catalytic activity in the tumor microenvironment does not affect disease progression (Figure 7B). As the mice from this study did not develop obvious tumors, cancer could not be confirmed as the cause of death, however given our previous experience with this model, it is unlikely that their death was due to another cause. Nevertheless, this study needs to be repeated, to validate cancer as the cause of death, and to immunophenotype the mice with flow cytometry, assessing the effects of a systemic MYSM1-loss on immune cell development in the bone marrow and on immune cell numbers and activation states within the tumors. In the future, we can specifically interrogate the effects of MYSM1-loss

in the different immune cell lineages on lymphoma disease progression by engrafting wild type *Eu-Myc* tumor cells into mice with a lineage specific loss of MYSM1, for example in DCs (CD11c-cre *Mysm1*$^{FL/FL}$), myeloid cells (LysM-cre *Mysm1*$^{FL/FL}$), or T cells (Lck-Cre *Mysm1*$^{FL/FL}$), comparing against control *Mysm1*-wild-type CD11c-cre, LysM-cre, or Lck-Cre mouse cohorts. Most of the mouse strains for this study are already available. We hypothesize that the deleterious effects of the loss of MYSM1 on immune cell production balance against its positive effects on immune cell activation, resulting in an overall a neutral effect of MYSM1 loss on antitumor immunity.

Lastly, our study indicates that the binding sites of MYSM1 and MYC at the ribosomal gene promoters co-localize with the binding sites of WDR5 (Figure 8). WDR5 alone does not demonstrate the involvement of PRC1.6, as it is also a known component of other chromatin remodeling complexes[124]. However, in conjunction with work done by HanChen Wang (*unpublished*), which identified the co-localization of RING1B to the same sites at the promoters of ribosomal protein genes, this suggests the involvement of WDR5 in a PRC1.6 dependent manner. PRC1.6 has been shown to interact with MYC to regulate gene expression through histone H2A-K119ub[131]. In neuroblastoma, PRC1 and MYCN were found to co-repress the transcription of enzymes involved in fatty acid metabolism via modification of histone H2A-K119ub, resulting in decreased tumor suppression[153]. In contrast Tanaskovic's study revealed that the loss of the PRC1.6 subunit PCGF6 in *Eu-Myc* mice resulted in accelerated lymphomagenesis, which they suggest to be independent of PRC1.6[132]. However, they did not look into the involvement of RING1A/B in this context and therefore cannot fully rule out the possibility of regulation via PRC1.6. To further establish that MYSM1 cooperates with these transcriptional and epigenetic

regulators to modulate ribosomal gene expression, it will be of interest to assess the effects of MYSM1-loss on WDR5 and RING1B binding at these gene promoters.

Additionally, it is important to address that the ChIP-seq datasets for MYSM1 and other transcriptional and epigenetic regulators incorporated into the ChIP-data meta-analysis in our work (Figure 8) were not derived from primary Eu-*Myc* tumor cells, but instead from murine lymphoma cell lines, primary non-transformed B cells, or primary HSPCs. While our current work focussed on the analysis of MYSM1 interactions with MYC and PRC1 at ribosomal gene promoters, MYC and PRC1 are also known to be involved in the transcriptional regulation of other cellular processes. It would therefore be of interest to perform MYSM1 ChIP-seq in Eu-*Myc* tumor cells using our previously established protocol[123], to reveal its interactions with MYC and PRC1 at other gene promoters. It will also be important to perform ChIP-seq analyses for MYC, PRC1 proteins RING1B and WDR5, and the histone mark H2AK119ub, comparing Eu-*Myc* tumor cells from control and *Mysm1*-mutant mice, to test the effects of MYSM1 loss-of-function. In conjunction with ChIP-seq, performing ATAC-seq on these same cells would allow us to gain insight into the chromatin accessibility and epigenetic landscape of B cell tumors that lack MYSM1 catalytic activity relative to controls. This is of interest as MYSM1 and PRC1 are important antagonistic epigenetic regulators of histone-H2AK119. These data would provide novel insight into the genome-wide chromatin accessibility within B cell lymphoma tumors that lack MYSM1 catalytic activity to further understand how MYSM1 is able to modulate the progression of MYC driven tumors.

Transcriptional programs of ribosome biogenesis and protein synthesis play a central role in cellular transformation and cancer. Our work advances the understanding of the epigenetic

regulators of such transcriptional programs in cancer cells and suggests MYSM1 as a novel drug-target for their inhibition.

Chapter 6: Conclusion

6 – Conclusion

In summary, our work establishes the importance of MYSM1 catalytic activity in the onset and progression of B cell lymphoma. *Eu-Myc* mice that lack MYSM1 catalytic activity experience delayed onset of tumors and ultimately increased survival. These effects may be partially attributed to the impact of the loss of MYSM1 catalytic activity on antitumor immunity, whereby tumors that lack MYSM1 catalytic activity have increased immune cell infiltration and immune cell activation relative to control tumors. Our preliminary results also suggest that a selective loss of MYSM1 in the tumor microenvironment does not impact disease progression. Finally, we have begun to explore how MYSM1 may interact with MYC and PRC1.6 to co-regulate the expression of genes encoding ribosomal proteins and translation factors. However, to further develop MYSM1 as an effective drug target for B cell lymphoma, the protective effects of MYSM1 loss against the disease need to be validated in a human experimental system. Furthermore, the effects of the loss of MYSM1 on the tumor microenvironment need to be further investigated, and we are planning to repeat and expand the results shown in Figure 7B. This and the development of small-molecule inhibitors targeting MYSM1 are the critical next steps to establish MYSM1 as a viable drug target.

Appendix I: Supplemental Figures

Figure S1

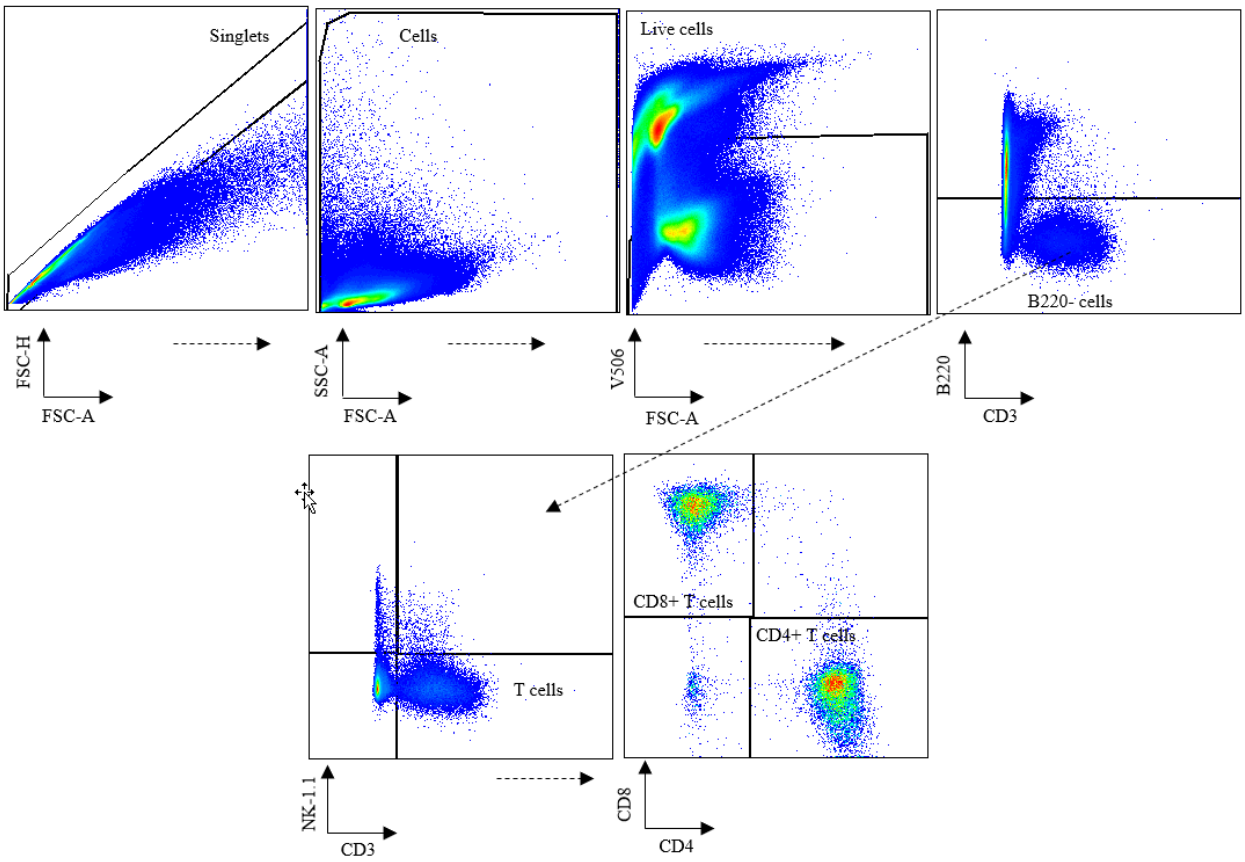

Figure S1. Flow cytometry gating strategy for CD4+ and C8+ T cells. FlowJo was used to analyze the data. All major subpopulations were gated on singlets and live B220- cells within the tumor. Arrows help to show the gating hierarchy.

Figure S2

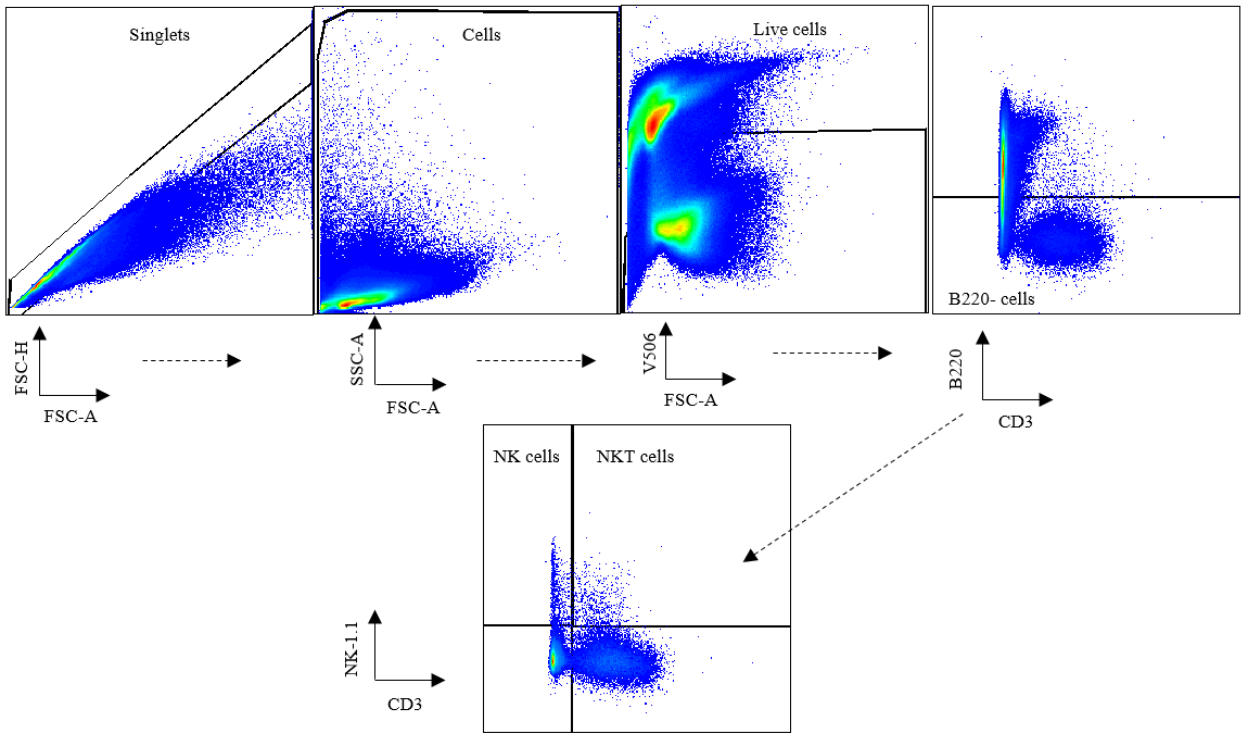

Figure S2. Flow cytometry gating strategy for NK and NKT cells. FlowJo was used to analyze the data. All major subpopulations were gated on singlets and live B220- cells within the tumor. Arrows help to show the gating hierarchy.

Figure S3

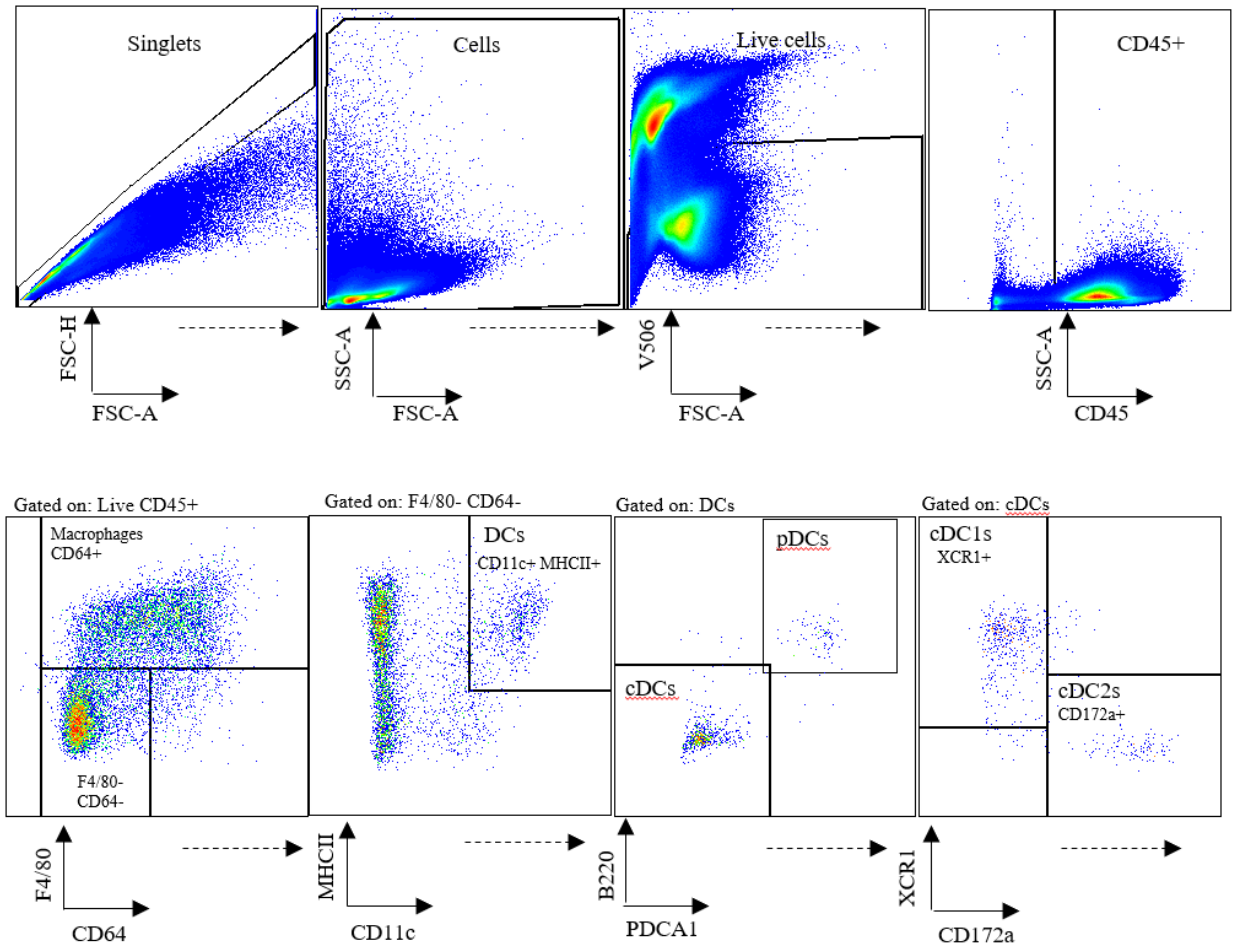

Figure S3. Flow cytometry gating strategy for DCs. FlowJo was used to analyze the data. All major subpopulations were gated on singlets and live CD45+ cells within the tumor. Arrows help to show the gating hierarchy.

Figure S4

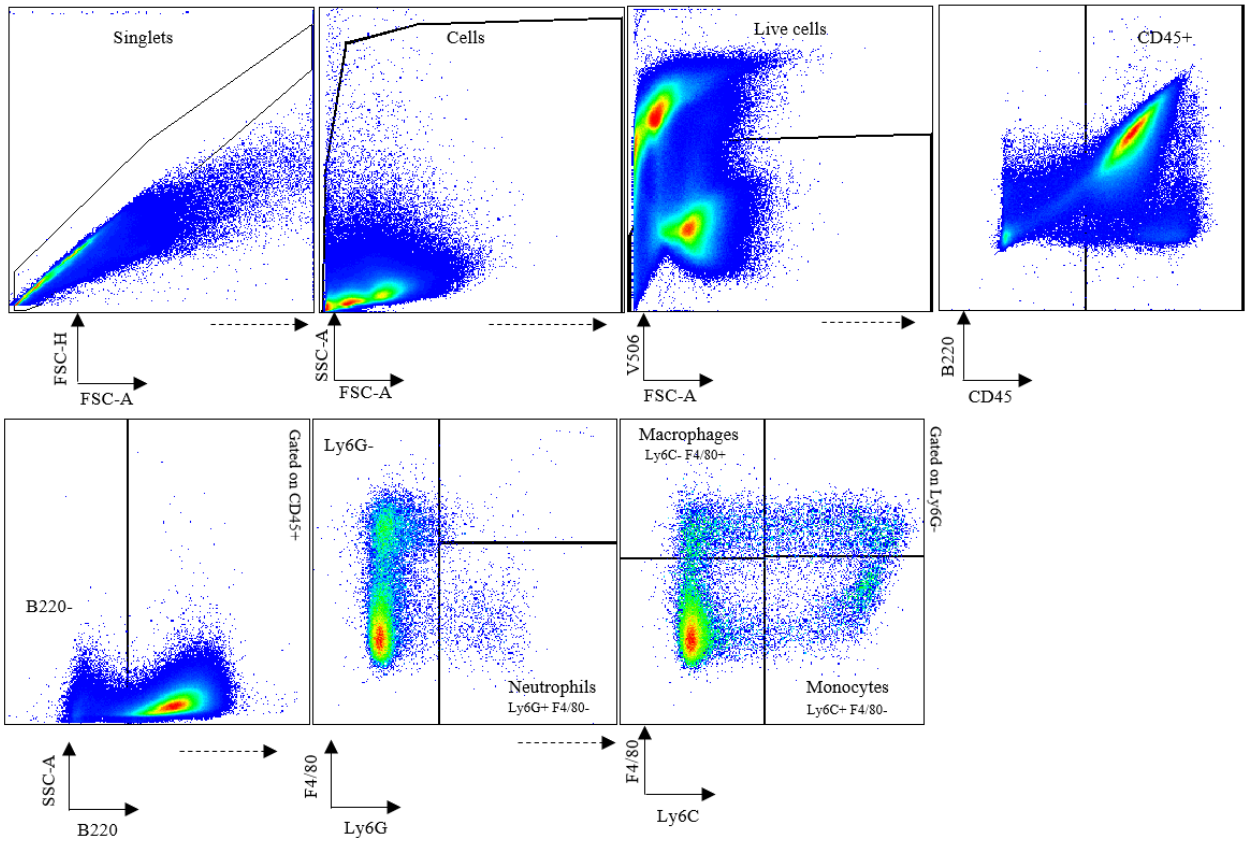

Figure S4. Flow cytometry gating strategy for monocytes, macrophages, and neutrophils. FlowJo was used to analyze the data. All major subpopulations were gated on singlets and live CD45+ and B220- cells within the tumor. Arrows help to show the gating hierarchy.

Appendix II: References

References

1. Brenner DR, Poirier A, Woods RR, et al. Projected estimates of cancer in Canada in 2022. *CMAJ*. 2022;194(17):E601-E607. doi:10.1503/CMAJ.212097/TAB-RELATED-CONTENT

2. Alaggio R, Amador C, Anagnostopoulos I, et al. The 5th edition of the World Health Organization Classification of Haematolymphoid Tumours: Lymphoid Neoplasms. *Leuk 2022 367*. 2022;36(7):1720-1748. doi:10.1038/s41375-022-01620-2

3. Perry AM, Diebold J, Nathwani BN, et al. Non-Hodgkin lymphoma in the developing world: review of 4539 cases from the International Non-Hodgkin Lymphoma Classification Project. *Haematologica*. 2016;101(10):1244. doi:10.3324/HAEMATOL.2016.148809

4. Pasqualucci L, Dalla-Favera R. Genetics of diffuse large B-cell lymphoma. *Blood*. 2018;131(21):2307-2319. doi:10.1182/BLOOD-2017-11-764332

5. Martelli M, Ferreri AJM, Agostinelli C, Di Rocco A, Pfreundschuh M, Pileri SA. Diffuse large B-cell lymphoma. *Crit Rev Oncol Hematol*. 2013;87(2):146-171. doi:10.1016/J.CRITREVONC.2012.12.009

6. Alizadeh AA, Elsen MB, Davis RE, et al. Distinct types of diffuse large B-cell lymphoma identified by gene expression profiling. *Nat 2000 4036769*. 2000;403(6769):503-511. doi:10.1038/35000501

7. Wright GW, Huang DW, Phelan JD, et al. A Probabilistic Classification Tool for Genetic Subtypes of Diffuse Large B Cell Lymphoma with Therapeutic Implications. *Cancer Cell*. 2020;37(4):551-568.e14. doi:10.1016/J.CCELL.2020.03.015/ATTACHMENT/C7078A98-A180-4859-BC87-F7704F45E442/MMC5.XLSX

8. Nguyen L, Papenhausen P, Shao H. The Role of c-MYC in B-Cell Lymphomas: Diagnostic and Molecular Aspects. *Genes 2017, Vol 8, Page 116*. 2017;8(4):116. doi:10.3390/GENES8040116

9. Valera A, López-Guillermo A, Cardesa-Salzmann T, et al. MYC protein expression and genetic alterations have prognostic impact in patients with diffuse large B-cell lymphoma treated with immunochemotherapy. *Haematologica*. 2013;98(10):1554-1562. doi:10.3324/HAEMATOL.2013.086173

10. Savage KJ, Johnson NA, Ben-Neriah S, et al. MYC gene rearrangements are associated with a poor prognosis in diffuse large B-cell lymphoma patients treated with R-CHOP chemotherapy. *Blood*. 2009;114(17):3533-3537. doi:10.1182/BLOOD-2009-05-220095

11. Kramer MHH, Hermans J, Wijburg E, et al. Clinical Relevance of BCL2, BCL6, and MYC Rearrangements in Diffuse Large B-Cell Lymphoma. *Blood*. 1998;92(9):3152-3162. doi:10.1182/BLOOD.V92.9.3152

12. Kawasaki C, Ohshima K, Suzumiya J, et al. Rearrangements of Bcl-1, Bcl-2, Bcl-6, and C-Myc in Diffuse Large B-Cell Lymphomas. *https://doi.org/103109/10428190109097730*. 2009;42(5):1099-1106.

doi:10.3109/10428190109097730

13. Ladanyi M, Offit K, Jhanwar S, Filippa D, Chaganti R. MYC Rearrangement and Translocations Involving Band 8q24 in Diffuse Large Cell Lymphomas. *Blood*. 1991;77(5):1057-1063. doi:10.1182/BLOOD.V77.5.1057.1057

14. Li S, Lin P, Fayad LE, et al. B-cell lymphomas with MYC/8q24 rearrangements and IGH@BCL2/t(14;18)(q32;q21): an aggressive disease with heterogeneous histology, germinal center B-cell immunophenotype and poor outcome. *Mod Pathol*. 2012;25(1):145-156. doi:10.1038/MODPATHOL.2011.147

15. Li S, Desai P, Lin P, et al. MYC/BCL6 double-hit lymphoma (DHL): a tumour associated with an aggressive clinical course and poor prognosis. *Histopathology*. 2016;68(7):1090-1098. doi:10.1111/HIS.12884

16. Karube K, Campo E. MYC Alterations in Diffuse Large B-Cell Lymphomas. *Semin Hematol*. 2015;52(2):97-106. doi:10.1053/J.SEMINHEMATOL.2015.01.009

17. Klapproth K, Wirth T. Advances in the understanding of MYC-induced lymphomagenesis. *Br J Haematol*. 2010;149(4):484-497. doi:10.1111/J.1365-2141.2010.08159.X

18. Adhikary S, Eilers M. Transcriptional regulation and transformation by Myc proteins. *Nat Rev Mol Cell Biol 2005 68*. 2005;6(8):635-645. doi:10.1038/nrm1703

19. Bellan C, Stefano L, Giulia DF, Rogena EA, Lorenzo L. Burkitt lymphoma versus diffuse large B-cell lymphoma: a practical approach. *Hematol Oncol*. 2010;28(2):53-56. doi:10.1002/HON.916

20. Olszewski AJ, Kurt H, Evens AM. Defining and treating high-grade B-cell lymphoma, NOS. *Blood*. 2022;140(9):943-954. doi:10.1182/BLOOD.2020008374

21. Gopal S, Gross TG. How I treat Burkitt lymphoma in children, adolescents, and young adults in sub-Saharan Africa. *Blood*. 2018;132(3):254-263. doi:10.1182/BLOOD-2018-04-844472

22. Roschewski M, Staudt LM, Wilson WH. Burkitt's Lymphoma. Longo DL, ed. *https://doi.org/101056/NEJMra2025746*. 2022;387(12):1111-1122. doi:10.1056/NEJMRA2025746

23. Crombie J, LaCasce A. The treatment of Burkitt lymphoma in adults. *Blood*. 2021;137(6):743-750. doi:10.1182/BLOOD.2019004099

24. Teras LR, Desantis CE, Cerhan JR, et al. 2016 US lymphoid malignancy statistics by World Health Organization subtypes. *CA Cancer J Clin*. 2016;66(6):443-459. doi:10.3322/CAAC.21357

25. Panea RI, Love CL, Shingleton JR, et al. The whole-genome landscape of Burkitt lymphoma subtypes. *Blood*. 2019;134(19):1598-1607. doi:10.1182/BLOOD.2019001880

26. Grande BM, Gerhard DS, Jiang A, et al. Genome-wide discovery of somatic coding and noncoding mutations in pediatric endemic and sporadic Burkitt lymphoma. *Blood*.

2019;133(12):1313-1324. doi:10.1182/BLOOD-2018-09-871418

27. Zech L, Haglund U, Nilsson K, Klein G. Characteristic chromosomal abnormalities in biopsies and lymphoid-cell lines from patients with burkitt and non-burkitt lymphomas. *Int J Cancer*. 1976;17(1):47-56. doi:10.1002/IJC.2910170108

28. Taub R, Kirsch I, Morton C, et al. Translocation of the c-myc gene into the immunoglobulin heavy chain locus in human Burkitt lymphoma and murine plasmacytoma cells. *Proc Natl Acad Sci*. 1982;79(24):7837-7841. doi:10.1073/PNAS.79.24.7837

29. Adams JM, Harris AW, Pinkert CA, et al. The c-myc oncogene driven by immunoglobulin enhancers induces lymphoid malignancy in transgenic mice. *Nature*. 1985;318(6046):533-538. doi:10.1038/318533A0

30. Patente TA, Pinho MP, Oliveira AA, Evangelista GCM, Bergami-Santos PC, Barbuto JAM. Human dendritic cells: Their heterogeneity and clinical application potential in cancer immunotherapy. *Front Immunol*. 2019;10(JAN):3176. doi:10.3389/FIMMU.2018.03176/BIBTEX

31. Gardner A, Ruffell B. Dendritic Cells and Cancer Immunity. *Trends Immunol*. 2016;37(12):855-865. doi:10.1016/J.IT.2016.09.006

32. Wculek SK, Cueto FJ, Mujal AM, Melero I, Krummel MF, Sancho D. Dendritic cells in cancer immunology and immunotherapy. *Nat Rev Immunol 2019 201*. 2019;20(1):7-24. doi:10.1038/s41577-019-0210-z

33. Yuan CT, Chuang SS, Cheng PY, et al. Decreased CD11c-positive dendritic cells in the tumor microenvironment predict double-hit/triple-hit genotype and survival in diffuse large B-cell lymphoma. *J Pathol Clin Res*. 2022;8(5):436-447. doi:10.1002/CJP2.283

34. Rehm A, Gätjen M, Gerlach K, et al. Dendritic cell-mediated survival signals in Eμ-Myc B-cell lymphoma depend on the transcription factor C/EBPβ. *Nat Commun 2014 51*. 2014;5(1):1-14. doi:10.1038/ncomms6057

35. Scheuerpflug A, Ahmetlić F, Bauer V, Riedel T, Röcken M, Mocikat R. The role of dendritic cells for therapy of B-cell lymphoma with immune checkpoint inhibitors. *Cancer Immunol Immunother*. 2021;70(5):1343-1350. doi:10.1007/S00262-020-02767-6/FIGURES/5

36. DeNardo DG, Ruffell B. Macrophages as regulators of tumour immunity and immunotherapy. *Nat Rev Immunol 2019 196*. 2019;19(6):369-382. doi:10.1038/s41577-019-0127-6

37. Mantovani A, Allavena P, Marchesi F, Garlanda C. Macrophages as tools and targets in cancer therapy. *Nat Rev Drug Discov 2022 2111*. 2022;21(11):799-820. doi:10.1038/s41573-022-00520-5

38. Kridel R, Steidl C, Gascoyne RD. Tumor-associated macrophages in diffuse large B-cell lymphoma. *Haematologica*. 2015;100(2):143-145. doi:10.3324/HAEMATOL.2015.124008

39. Pham L V., Pogue E, Ford RJ. The role of macrophage/B-cell interactions in the pathophysiology of B-cell lymphomas. *Front Oncol*. 2018;8(MAY):147. doi:10.3389/FONC.2018.00147/BIBTEX

40. Orecchioni M, Ghosheh Y, Pramod AB, Ley K. Macrophage polarization: Different gene signatures in M1(Lps+) vs. Classically and M2(LPS-) vs. Alternatively activated macrophages. *Front Immunol*. 2019;10(MAY):1084. doi:10.3389/FIMMU.2019.01084/BIBTEX

41. Shen L, Li H, Shi Y, et al. M2 tumour-associated macrophages contribute to tumour progression via legumain remodelling the extracellular matrix in diffuse large B cell lymphoma. *Sci Reports 2016 61*. 2016;6(1):1-10. doi:10.1038/srep30347

42. Li YL, Shi ZH, Wang X, Gu KS, Zhai ZM. Tumor-associated macrophages predict prognosis in diffuse large B-cell lymphoma and correlation with peripheral absolute monocyte count. *BMC Cancer*. 2019;19(1):1-10. doi:10.1186/S12885-019-6208-X/FIGURES/5

43. van der Leun AM, Thommen DS, Schumacher TN. CD8+ T cell states in human cancer: insights from single-cell analysis. *Nat Rev Cancer 2020 204*. 2020;20(4):218-232. doi:10.1038/s41568-019-0235-4

44. Han J, Khatwani N, Searles TG, Turk MJ, Angeles C V. Memory CD8+ T cell responses to cancer. *Semin Immunol*. 2020;49:101435. doi:10.1016/J.SMIM.2020.101435

45. Kim HJ, Cantor H. CD4 T-cell Subsets and Tumor Immunity: The Helpful and the Not-so-Helpful. *Cancer Immunol Res*. 2014;2(2):91-98. doi:10.1158/2326-6066.CIR-13-0216

46. Waldman AD, Fritz JM, Lenardo MJ. A guide to cancer immunotherapy: from T cell basic science to clinical practice. *Nat Rev Immunol 2020 2011*. 2020;20(11):651-668. doi:10.1038/s41577-020-0306-5

47. Takeuchi A, Saito T. CD4 CTL, a cytotoxic subset of CD4+ T cells, their differentiation and function. *Front Immunol*. 2017;8(FEB):194. doi:10.3389/FIMMU.2017.00194/BIBTEX

48. Zhang Z, Liu S, Zhang B, Qiao L, Zhang Y, Zhang Y. T Cell Dysfunction and Exhaustion in Cancer. *Front Cell Dev Biol*. 2020;8:17. doi:10.3389/FCELL.2020.00017/BIBTEX

49. Thommen DS, Schumacher TN. T Cell Dysfunction in Cancer. *Cancer Cell*. 2018;33(4):547-562. doi:10.1016/J.CCELL.2018.03.012

50. Kinker GS, Vitiello GAF, Ferreira WAS, Chaves AS, Cordeiro de Lima VC, Medina T da S. B Cell Orchestration of Anti-tumor Immune Responses: A Matter of Cell Localization and Communication. *Front Cell Dev Biol*. 2021;9:1282. doi:10.3389/FCELL.2021.678127/BIBTEX

51. Zhao K liang, Yang X jia, Jin H zhong, Zhao L, Hu J li, Qin W juan. Double-edge Role of B Cells in Tumor Immunity: Potential Molecular Mechanism. *Curr Med Sci*. 2019;39(5):685-689. doi:10.1007/S11596-019-2092-5/METRICS

52. Sarvaria A, Madrigal JA, Saudemont A. B cell regulation in cancer and anti-tumor

immunity. *Cell Mol Immunol 2017 148*. 2017;14(8):662-674. doi:10.1038/cmi.2017.35

53. Zhang Y, Gallastegui N, Rosenblatt JD. Regulatory B cells in anti-tumor immunity. *Int Immunol*. 2015;27(10):521-530. doi:10.1093/INTIMM/DXV034

54. Helmink BA, Reddy SM, Gao J, et al. B cells and tertiary lymphoid structures promote immunotherapy response. *Nat 2020 5777791*. 2020;577(7791):549-555. doi:10.1038/s41586-019-1922-8

55. Lu Y, Zhao Q, Liao JY, et al. Complement Signals Determine Opposite Effects of B Cells in Chemotherapy-Induced Immunity. *Cell*. 2020;180(6):1081-1097.e24. doi:10.1016/j.cell.2020.02.015

56. Vera de Jonge A, Mutis T, Roemer MGM, Scheijen B, Chamuleau MED. Impact of MYC on Anti-Tumor Immune Responses in Aggressive B Cell Non-Hodgkin Lymphomas: Consequences for Cancer Immunotherapy. *Cancers 2020, Vol 12, Page 3052*. 2020;12(10):3052. doi:10.3390/CANCERS12103052

57. Torsteinsdottir S, Masucci MG, Ehlin-Henriksson B, et al. Differentiation-dependent sensitivity of human B-cell-derived lines to major histocompatibility complex-restricted T-cell cytotoxicity. *Proc Natl Acad Sci*. 1986;83(15):5620-5624. doi:10.1073/PNAS.83.15.5620

58. Staege MS, Lee SP, Frisan T, et al. MYC overexpression imposes a nonimmunogenic phenotype on Epstein-Barr virus-infected B cells. *Proc Natl Acad Sci U S A*. 2002;99(7):4550-4555. doi:10.1073/PNAS.072495599/ASSET/23113B3D-E122-4AC7-9086-038931811445/ASSETS/GRAPHIC/PQ0724955005.JPEG

59. Inghirami G, Grignani F, Sternas L, Lombardi L, Knowles DM, Dalla-Favera R. Down-regulation of LFA-1 Adhesion Receptors by C-myc Oncogene in Human B Lymphoblastoid Cells. *Science (80-)*. 1990;250(4981):682-686. doi:10.1126/SCIENCE.2237417

60. David A, Arnaud N, Fradet M, et al. c-Myc dysregulation is a co-transforming event for nuclear factor-κB activated B cells. *Haematologica*. 2017;102(5):883-894. doi:10.3324/HAEMATOL.2016.156281

61. Pajic A, Staege MS, Dudziak D, et al. Antagonistic effects of c-myc and Epstein-Barr virus latent genes on the phenotype of human B cells. *Int J Cancer*. 2001;93(6):810-816. doi:10.1002/IJC.1404

62. Casey SC, Tong L, Li Y, et al. MYC regulates the antitumor immune response through CD47 and PD-L1. *Science (80-)*. 2016;352(6282):227-231. doi:10.1126/SCIENCE.AAC9935/SUPPL_FILE/PAPV2.PDF

63. Li W, Gupta SK, Han W, et al. Targeting MYC activity in double-hit lymphoma with MYC and BCL2 and/or BCL6 rearrangements with epigenetic bromodomain inhibitors. *J Hematol Oncol*. 2019;12(1):1-13. doi:10.1186/S13045-019-0761-2/FIGURES/8

64. Mondello P, Mian M. Frontline treatment of diffuse large B-cell lymphoma: Beyond R-CHOP. *Hematol Oncol*. 2019;37(4):333-344. doi:10.1002/HON.2613

65. Coiffier B, Sarkozy C. Diffuse large B-cell lymphoma: R-CHOP failure—what to do? *Hematology*. 2016;2016(1):366-378. doi:10.1182/ASHEDUCATION-2016.1.366

66. Gisselbrecht C, Glass B, Mounier N, et al. Salvage regimens with autologous transplantation for relapsed large B-cell lymphoma in the rituximab era. *J Clin Oncol*. 2010;28(27):4184-4190. doi:10.1200/JCO.2010.28.1618

67. He MY, Kridel R. Treatment resistance in diffuse large B-cell lymphoma. *Leuk 2021 358*. 2021;35(8):2151-2165. doi:10.1038/s41375-021-01285-3

68. Miao Y, Medeiros LJ, Li Y, Li J, Young KH. Genetic alterations and their clinical implications in DLBCL. *Nat Rev Clin Oncol 2019 1610*. 2019;16(10):634-652. doi:10.1038/s41571-019-0225-1

69. Wang L, Li L rong, Young KH. New agents and regimens for diffuse large B cell lymphoma. *J Hematol Oncol 2020 131*. 2020;13(1):1-23. doi:10.1186/S13045-020-01011-Z

70. Roschewski M, Dunleavy K, Abramson JS, et al. Multicenter Study of Risk-Adapted Therapy with Dose-Adjusted EPOCH-R in Adults with Untreated Burkitt Lymphoma. *J Clin Oncol*. 2020;38(22):2519-2529. doi:10.1200/JCO.20.00303

71. Dunleavy K. Approach to the diagnosis and treatment of adult burkitt's lymphoma. *J Oncol Pract*. 2018;14(11):665-671. doi:10.1200/JOP.18.00148

72. Jacobson C, La Casce A. How I treat Burkitt lymphoma in adults. *Blood*. 2014;124(19):2913-2920. doi:10.1182/BLOOD-2014-06-538504

73. Meyer N, Penn LZ. Reflecting on 25 years with MYC. *Nat Rev Cancer 2008 812*. 2008;8(12):976-990. doi:10.1038/nrc2231

74. Kalkat M, De Melo J, Hickman KA, et al. MYC Deregulation in Primary Human Cancers. *Genes 2017, Vol 8, Page 151*. 2017;8(6):151. doi:10.3390/GENES8060151

75. Carabet LA, Rennie PS, Cherkasov A. Therapeutic Inhibition of Myc in Cancer. Structural Bases and Computer-Aided Drug Discovery Approaches. *Int J Mol Sci 2019, Vol 20, Page 120*. 2018;20(1):120. doi:10.3390/IJMS20010120

76. Llombart V, Mansour MR. Therapeutic targeting of "undruggable" MYC. *eBioMedicine*. 2022;75. doi:10.1016/J.EBIOM.2021.103756

77. Dalla-Favera R, Bregni M, Erikson J, Patterson D, Gallo RC, Croce CM. Human c-myc onc gene is located on the region of chromosome 8 that is translocated in Burkitt lymphoma cells. *Proc Natl Acad Sci*. 1982;79(24):7824-7827. doi:10.1073/PNAS.79.24.7824

78. Schwab M, Ellison J, Buschf M, et al. Enhanced expression of the human gene N-myc consequent to amplification of DNA may contribute to malignant progression of neuroblastoma. *Proc Natl Acad Sci*. 1984;81(15):4940-4944. doi:10.1073/PNAS.81.15.4940

79. Nau MM, Brooks BJ, Battey J, et al. L-myc, a new myc-related gene amplified and

expressed in human small cell lung cancer. *Nat 1985 3186041*. 1985;318(6041):69-73. doi:10.1038/318069a0

80. McMahon SB. MYC and the Control of Apoptosis. *Cold Spring Harb Perspect Med*. 2014;4(7):a014407. doi:10.1101/CSHPERSPECT.A014407

81. Stine ZE, Walton ZE, Altman BJ, Hsieh AL, Dang C V. MYC, metabolism, and cancer. *Cancer Discov*. 2015;5(10):1024-1039. doi:10.1158/2159-8290.CD-15-0507/43152/P/MYC-METABOLISM-AND-CANCERMYC-METABOLISM-AND-CANCER

82. Melnik S, Werth N, Boeuf S, et al. Impact of c-MYC expression on proliferation, differentiation, and risk of neoplastic transformation of human mesenchymal stromal cells. *Stem Cell Res Ther*. 2019;10(1):1-18. doi:10.1186/S13287-019-1187-Z/FIGURES/6

83. Herbst A, Hemann MT, Tworkowski KA, Salghetti SE, Lowe SW, Tansey WP. A conserved element in Myc that negatively regulates its proapoptotic activity. *EMBO Rep*. 2005;6(2):177-183. doi:10.1038/SJ.EMBOR.7400333

84. Beroukhim R, Mermel CH, Porter D, et al. The landscape of somatic copy-number alteration across human cancers. *Nat 2010 4637283*. 2010;463(7283):899-905. doi:10.1038/nature08822

85. Ciriello G, Miller ML, Aksoy BA, Senbabaoglu Y, Schultz N, Sander C. Emerging landscape of oncogenic signatures across human cancers. *Nat Genet 2013 4510*. 2013;45(10):1127-1133. doi:10.1038/ng.2762

86. Dang C V., O'Donnell KA, Zeller KI, Nguyen T, Osthus RC, Li F. The c-Myc target gene network. *Semin Cancer Biol*. 2006;16(4):253-264. doi:10.1016/J.SEMCANCER.2006.07.014

87. Wierstra I, Alves J. The c-myc Promoter: Still MysterY and Challenge. *Adv Cancer Res*. 2008;99:113-333. doi:10.1016/S0065-230X(07)99004-1

88. Dang C V., Resar LMS, Emison E, et al. Function of the c-Myc Oncogenic Transcription Factor. *Exp Cell Res*. 1999;253(1):63-77. doi:10.1006/EXCR.1999.4686

89. Burrell RA, McGranahan N, Bartek J, Swanton C. The causes and consequences of genetic heterogeneity in cancer evolution. *Nat 2013 5017467*. 2013;501(7467):338-345. doi:10.1038/nature12625

90. Ahmadi SE, Rahimi S, Zarandi B, Chegeni R, Safa M. MYC: a multipurpose oncogene with prognostic and therapeutic implications in blood malignancies. *J Hematol Oncol 2021 141*. 2021;14(1):1-49. doi:10.1186/S13045-021-01111-4

91. Yeh E, Cunningham M, Arnold H, et al. A signalling pathway controlling c-Myc degradation that impacts oncogenic transformation of human cells. *Nat Cell Biol 2004 64*. 2004;6(4):308-318. doi:10.1038/ncb1110

92. Salghetti SE, Kim SY, Tansey WP. Destruction of Myc by ubiquitin-mediated proteolysis: cancer-associated and transforming mutations stabilize Myc. *EMBO J*. 1999;18(3):717-726. doi:10.1093/EMBOJ/18.3.717

93. Dang C V. MYC on the Path to Cancer. *Cell*. 2012;149(1):22-35. doi:10.1016/J.CELL.2012.03.003

94. Cowling VH, Cole MD. Mechanism of transcriptional activation by the Myc oncoproteins. *Semin Cancer Biol*. 2006;16(4):242-252. doi:10.1016/J.SEMCANCER.2006.08.001

95. Kato GJ, Barrett J, Villa-Garcia M, Dang C V. An amino-terminal c-myc domain required for neoplastic transformation activates transcription. *Mol Cell Biol*. 1990;10(11):5914. doi:10.1128/MCB.10.11.5914

96. Jiang J, Wang J, Yue M, et al. Direct Phosphorylation and Stabilization of MYC by Aurora B Kinase Promote T-cell Leukemogenesis. *Cancer Cell*. 2020;37(2):200-215.e5. doi:10.1016/j.ccell.2020.01.001

97. Kalkat M, Resetca D, Lourenco C, et al. MYC Protein Interactome Profiling Reveals Functionally Distinct Regions that Cooperate to Drive Tumorigenesis. *Mol Cell*. 2018;72(5):836-848.e7. doi:10.1016/j.molcel.2018.09.031

98. Stone J, De Lange T, Ramsay G, et al. Definition of regions in human c-myc that are involved in transformation and nuclear localization. *Mol Cell Biol*. 1987;7(5):1697. doi:10.1128/MCB.7.5.1697

99. Sammak S, Hamdani N, Gorrec F, et al. Crystal Structures and Nuclear Magnetic Resonance Studies of the Apo Form of the c-MYC:MAX bHLHZip Complex Reveal a Helical Basic Region in the Absence of DNA. *Biochemistry*. 2019;58(29):3144-3154. doi:10.1021/ACS.BIOCHEM.9B00296/ASSET/IMAGES/LARGE/BI-2019-002966_0007.JPEG

100. Halazonetis TD, Kandil AN. Determination of the c-MYC DNA-binding site. *Proc Natl Acad Sci U S A*. 1991;88(14):6162. doi:10.1073/PNAS.88.14.6162

101. Nair SK, Burley SK. X-Ray Structures of Myc-Max and Mad-Max Recognizing DNA: Molecular Bases of Regulation by Proto-Oncogenic Transcription Factors. *Cell*. 2003;112(2):193-205. doi:10.1016/S0092-8674(02)01284-9

102. Blackwell TK, Huang J, Ma A, et al. Binding of myc proteins to canonical and noncanonical DNA sequences. *Mol Cell Biol*. 1993;13(9):5216-5224. doi:10.1128/MCB.13.9.5216-5224.1993

103. Amati B, Brooks MW, Levy N, Littlewood TD, Evan GI, Land H. Oncogenic activity of the c-Myc protein requires dimerization with Max. *Cell*. 1993;72(2):233-245. doi:10.1016/0092-8674(93)90663-B

104. Beaulieu ME, Castillo F, Soucek L. Structural and Biophysical Insights into the Function of the Intrinsically Disordered Myc Oncoprotein. *Cells 2020, Vol 9, Page 1038*. 2020;9(4):1038. doi:10.3390/CELLS9041038

105. Andresen C, Helander S, Lemak A, et al. Transient structure and dynamics in the disordered c-Myc transactivation domain affect Bin1 binding. *Nucleic Acids Res*. 2012;40(13):6353-6366. doi:10.1093/NAR/GKS263

106. Madden SK, de Araujo AD, Gerhardt M, Fairlie DP, Mason JM. Taking the Myc out of

cancer: toward therapeutic strategies to directly inhibit c-Myc. *Mol Cancer 2021 201*. 2021;20(1):1-18. doi:10.1186/S12943-020-01291-6

107. Van Riggelen J, Yetil A, Felsher DW. MYC as a regulator of ribosome biogenesis and protein synthesis. *Nat Rev Cancer 2010 104*. 2010;10(4):301-309. doi:10.1038/nrc2819

108. Destefanis F, Manara V, Bellosta P. Myc as a Regulator of Ribosome Biogenesis and Cell Competition: A Link to Cancer. *Int J Mol Sci 2020, Vol 21, Page 4037*. 2020;21(11):4037. doi:10.3390/IJMS21114037

109. Campbell KJ, White RJ. MYC Regulation of Cell Growth through Control of Transcription by RNA Polymerases I and III. *Cold Spring Harb Perspect Med*. 2014;4(5):a018408. doi:10.1101/CSHPERSPECT.A018408

110. Jones TR, Cole MD. Rapid cytoplasmic turnover of c-myc mRNA: requirement of the 3' untranslated sequences. *Mol Cell Biol*. 1987;7(12):4513-4521. doi:10.1128/MCB.7.12.4513-4521.1987

111. Eberhardy SR, Farnham PJ. c-Myc Mediates Activation of the cad Promoter via a Post-RNA Polymerase II Recruitment Mechanism. *J Biol Chem*. 2001;276(51):48562-48571. doi:10.1074/jbc.M109014200

112. Eberhardy SR, Farnham PJ. Myc recruits P-TEFb to mediate the final step in the transcriptional activation of the cad promoter. *J Biol Chem*. 2002;277(42):40156-40162. doi:10.1074/jbc.M207441200

113. Rahl PB, Lin CY, Seila AC, et al. C-Myc regulates transcriptional pause release. *Cell*. 2010;141(3):432-445. doi:10.1016/j.cell.2010.03.030

114. Grandori C, Gomez-Roman N, Felton-Edkins ZA, et al. c-Myc binds to human ribosomal DNA and stimulates transcription of rRNA genes by RNA polymerase I. *Nat Cell Biol 2005 73*. 2005;7(3):311-318. doi:10.1038/ncb1224

115. Patange S, Ball DA, Wan Y, et al. MYC amplifies gene expression through global changes in transcription factor dynamics. *Cell Rep*. 2022;38(4). doi:10.1016/j.celrep.2021.110292

116. Calado DP, Sasaki Y, Godinho SA, et al. The cell-cycle regulator c-Myc is essential for the formation and maintenance of germinal centers. *Nat Immunol 2012 1311*. 2012;13(11):1092-1100. doi:10.1038/ni.2418

117. Dominguez-Sola D, Victora GD, Ying CY, et al. The proto-oncogene MYC is required for selection in the germinal center and cyclic reentry. *Nat Immunol 2012 1311*. 2012;13(11):1083-1091. doi:10.1038/ni.2428

118. Ott G, Rosenwald A, Campo E. Understanding MYC-driven aggressive B-cell lymphomas: pathogenesis and classification. *Blood*. 2013;122(24):3884-3891. doi:10.1182/BLOOD-2013-05-498329

119. Macdonald JD, Chacón Simon S, Han C, et al. Discovery and Optimization of Salicylic Acid-Derived Sulfonamide Inhibitors of the WD Repeat-Containing Protein 5-MYC Protein-Protein Interaction. *J Med Chem*. 2019;62(24):11232-11259.

doi:10.1021/ACS.JMEDCHEM.9B01411/SUPPL_FILE/JM9B01411_SI_002.CSV

120. Thomas LR, Adams CM, Wang J, et al. Interaction of the oncoprotein transcription factor MYC with its chromatin cofactor WDR5 is essential for tumor maintenance. *Proc Natl Acad Sci U S A*. 2019;116(50):25260-25268. doi:10.1073/PNAS.1910391116/SUPPL_FILE/PNAS.1910391116.SD04.XLSX

121. Richards MW, Burgess SG, Poon E, et al. Structural basis of N-Myc binding by Aurora-A and its destabilization by kinase inhibitors. *Proc Natl Acad Sci U S A*. 2016;113(48):13726-13731. doi:10.1073/PNAS.1610626113/SUPPL_FILE/PNAS.201610626SI.PDF

122. Han H, Jain AD, Truica MI, et al. Small-Molecule MYC Inhibitors Suppress Tumor Growth and Enhance Immunotherapy. *Cancer Cell*. 2019;36(5):483-497.e15. doi:10.1016/j.ccell.2019.10.001

123. Belle JI, Langlais D, Nijnik A. MYSM1 maintains ribosomal protein gene expression in hematopoietic stem cells to prevent hematopoietic dysfunction Graphical abstract. 2020. doi:10.1172/jci.insight.125690

124. Lin YH, Wang HC, Fiore A, et al. Loss of MYSM1 inhibits the oncogenic activity of cMYC in B cell lymphoma. *J Cell Mol Med*. 2021;25(14):7089-7094. doi:10.1111/JCMM.16554

125. Jiang X-X, Liu Y, Li H, et al. MYSM1/miR-150/FLT3 inhibits B1a cell proliferation. *Oncotarget*. 2016;7(42):68086-68096. doi:10.18632/ONCOTARGET.11738

126. Fiore A, Liang Y, Lin YH, et al. Deubiquitinase MYSM1 in the Hematopoietic System and beyond: A Current Review. *Int J Mol Sci 2020, Vol 21, Page 3007*. 2020;21(8):3007. doi:10.3390/IJMS21083007

127. Yoneyama M, Tochio N, Umehara T, et al. Structural and Functional Differences of SWIRM Domain Subtypes. *J Mol Biol*. 2007;369(1):222-238. doi:10.1016/J.JMB.2007.03.027

128. Birol M, Echalier A. Structure and Function of MPN (Mpr1/Pad1 N-terminal) Domain-Containing Proteins. *Curr Protein Pept Sci*. 2014;15(5):504-517. doi:10.2174/1389203715666140221095109

129. Blackledge NP, Klose RJ. The molecular principles of gene regulation by Polycomb repressive complexes. *Nat Rev Mol Cell Biol 2021 2212*. 2021;22(12):815-833. doi:10.1038/s41580-021-00398-y

130. Vidal M, Starowicz K. Polycomb complexes PRC1 and their function in hematopoiesis. *Exp Hematol*. 2017;48:12-31. doi:10.1016/J.EXPHEM.2016.12.006

131. Scelfo A, Fernández-Pérez D, Tamburri S, et al. Functional Landscape of PCGF Proteins Reveals Both RING1A/B-Dependent-and RING1A/B-Independent-Specific Activities. *Mol Cell*. 2019;74(5):1037-1052.e7. doi:10.1016/j.molcel.2019.04.002

132. Tanaskovic N, Dalsass M, Filipuzzi M, et al. Polycomb group ring finger protein 6 suppresses Myc-induced lymphomagenesis. *Life Sci Alliance*. 2022;5(8).

doi:10.26508/LSA.202101344

133. Liang Y, Bhatt G, Tung LT, et al. Deubiquitinase catalytic activity of MYSM1 is essential in vivo for hematopoiesis and immune cell development. *Sci Reports 2023 131*. 2023;13(1):1-13. doi:10.1038/s41598-023-27486-7

134. Lim K-H, Baek K-H. Deubiquitinating Enzymes as Therapeutic Targets in Cancer. *Curr Pharm Des*. 2013;19(22):4039-4052. doi:10.2174/1381612811319220013

135. Harrigan JA, Jacq X, Martin NM, Jackson SP. Deubiquitylating enzymes and drug discovery: emerging opportunities. *Nat Rev Drug Discov 2017 171*. 2017;17(1):57-78. doi:10.1038/nrd.2017.152

136. Tu R, Ma J, Zhang P, et al. The emerging role of deubiquitylating enzymes as therapeutic targets in cancer metabolism. *Cancer Cell Int 2022 221*. 2022;22(1):1-14. doi:10.1186/S12935-022-02524-Y

137. Wang Z, Kang W, You Y, et al. USP7: Novel drug target in cancer therapy. *Front Pharmacol*. 2019;10(APR):427. doi:10.3389/FPHAR.2019.00427/BIBTEX

138. Schauer NJ, Liu X, Magin RS, et al. Selective USP7 inhibition elicits cancer cell killing through a p53-dependent mechanism. *Sci Reports 2020 101*. 2020;10(1):1-15. doi:10.1038/s41598-020-62076-x

139. Förster M, Belle JI, Petrov JC, Ryder EJ, Clare S, Nijnik A. Deubiquitinase MYSM1 Is Essential for Normal Fetal Liver Hematopoiesis and for the Maintenance of Hematopoietic Stem Cells in Adult Bone Marrow. *Stem Cells Dev*. 2015;24(16):1865-1877. doi:10.1089/SCD.2015.0058/ASSET/IMAGES/LARGE/FIGURE7.JPEG

140. Kieffer-Kwon KR, Nimura K, Rao SSP, et al. Myc Regulates Chromatin Decompaction and Nuclear Architecture during B Cell Activation. *Mol Cell*. 2017;67(4):566-578.e10. doi:10.1016/j.molcel.2017.07.013

141. Frangini A, Sjöberg M, Roman-Trufero M, et al. The Aurora B Kinase and the Polycomb Protein Ring1B Combine to Regulate Active Promoters in Quiescent Lymphocytes. *Mol Cell*. 2013;51(5):647-661. doi:10.1016/j.molcel.2013.08.022

142. Wilson NK, Schoenfelder S, Hannah R, et al. Integrated genome-scale analysis of the transcriptional regulatory landscape in a blood stem/progenitor cell model. *Blood*. 2016;127(13):e12-e23. doi:10.1182/BLOOD-2015-10-677393

143. Thorvaldsdóttir H, Robinson JT, Mesirov JP. Integrative Genomics Viewer (IGV): high-performance genomics data visualization and exploration. *Brief Bioinform*. 2013;14(2):178-192. doi:10.1093/BIB/BBS017

144. Lin YH, Forster M, Liang Y, et al. USP44 is dispensable for normal hematopoietic stem cell function, lymphocyte development, and B-cell-mediated immune response in a mouse model. *Exp Hematol*. 2019;72:1-8. doi:10.1016/J.EXPHEM.2019.01.001

145. Guarnaccia AD, Tansey WP. Moonlighting with WDR5: A Cellular Multitasker. *J Clin Med 2018, Vol 7, Page 21*. 2018;7(2):21. doi:10.3390/JCM7020021

146. Quentmeier H, Drexler HG, Hauer V, et al. Diffuse Large B Cell Lymphoma Cell Line U-2946: Model for MCL1 Inhibitor Testing. *PLoS One*. 2016;11(12):e0167599. doi:10.1371/JOURNAL.PONE.0167599

147. Lange SM, Armstrong LA, Kulathu Y. Deubiquitinases: From mechanisms to their inhibition by small molecules. *Mol Cell*. 2022;82(1):15-29. doi:10.1016/J.MOLCEL.2021.10.027

148. Hassiepen U, Eidhoff U, Meder G, et al. A sensitive fluorescence intensity assay for deubiquitinating proteases using ubiquitin-rhodamine110-glycine as substrate. *Anal Biochem*. 2007;371(2):201-207. doi:10.1016/J.AB.2007.07.034

149. Menter T, Tzankov A. Mechanisms of immune evasion and immune modulation by lymphoma cells. *Front Oncol*. 2018;8(MAR):54. doi:10.3389/FONC.2018.00054/BIBTEX

150. de Charette M, Houot R. Hide or defend, the two strategies of lymphoma immune evasion: potential implications for immunotherapy. *Haematologica*. 2018;103(8):1256-1268. doi:10.3324/HAEMATOL.2017.184192

151. Upadhyay R, Hammerich L, Peng P, Brown B, Merad M, Brody JD. Lymphoma: Immune Evasion Strategies. *Cancers 2015, Vol 7, Pages 736-762*. 2015;7(2):736-762. doi:10.3390/CANCERS7020736

152. Olivo Pimentel V, Yaromina A, Marcus D, Dubois LJ, Lambin P. A novel co-culture assay to assess anti-tumor CD8+ T cell cytotoxicity via luminescence and multicolor flow cytometry. *J Immunol Methods*. 2020;487:112899. doi:10.1016/J.JIM.2020.112899

153. Ding Y, Yang J, Ma Y, et al. MYCN and PRC1 cooperatively repress docosahexaenoic acid synthesis in neuroblastoma via ELOVL2. *J Exp Clin Cancer Res*. 2019;38(1):1-16. doi:10.1186/S13046-019-1492-5/FIGURES/6