SUPPLEMENTAL MATERIALS AND METHODS

Mouse Lines
Mouse line B6.Cg-Tg(IghMyc)22Bri/J, also known as EuMyc, is a widely studied model of B cell lymphoma and overexpresses cMYC under the control of the immunoglobulin heavy chain locus enhancer\(^1\). Mouse lines Mysm1\(^{-/-}\) and Mysm1\(^{fl/fl}\) carry the loss-of-function and the conditional alleles of Mysm1 gene, respectively, and were previously described\(^2\)-\(^4\). Mysm1\(^{fl/fl}\)Cre\(\text{ERT2}\) mice were derived for tamoxifen-induced Mysm1-gene deletion by crossing the Mysm1\(^{fl/fl}\) and Gt(ROSA)26Sortm1(cre/\(\text{ERT2}\)) strains, as previously described\(^4\). All lines were on the C57BL/6 genetic background. The mice were maintained under specific pathogen-free conditions. All experiments were in accordance with the guidelines of the Canadian Council on Animal Care, and protocol AUP-7643 approved by the McGill University Animal Care Committee.

Tamoxifen Mouse Treatment
For tamoxifen-induced Mysm1-gene deletion, mice of Mysm1\(^{fl/fl}\)Cre\(\text{ERT2}\) and control genotypes were injected intraperitoneally with tamoxifen (Sigma-Aldrich, T5648) in sterilized corn oil at 0.15 mg/gram per injection, with 8 doses administered in total over 16 days. Successful deletion of Mysm1 exon 3 was validated by PCR analyses of the genomic DNA from hematopoietic and lymphoid organs of the mice, as described previously\(^4\),\(^5\). Control animals of the same genotypes injected with vehicle corn oil (Sigma-Aldrich) were also included in the analyses.

Adoptive Transfer of B cell Lymphoma Cells
Tumour cells harvested from mice of EuMyc Mysm1\(^{fl/fl}\)Cre\(\text{ERT2}\) and control EuMyc Mysm1\(^{fl/fl}\) genotypes were processed to single cell suspension, subjected to red blood cell lysis in ACK buffer (0.15M NH\(_4\)Cl, 10mM KHCO\(_3\), 0.1mM EDTA) and cryopreserved. Subsequently, the tumour cells were transferred via an intravenous injection at 10\(^6\) cells per recipient, into wild type C57BL/6 mice, previously subjected to 3.5 Gy whole body irradiation in a RS2000 irradiator (Rad Source). The recipients were administered with tamoxifen or vehicle corn oil, as described above\(^4\),\(^5\), and mouse health and survival monitored over subsequent 100 days. Survival was defined as the time to the terminal stage of disease, at which point the animals were euthanized as determined in the protocol AUP-7643 approved by the McGill University Animal Care Committee.

Culture of Cell Lines
Murine B cell line Ba/F3 was maintained at 0.5-2 x 10\(^6\) cells/mL in RPMI-1640 (Wisent) with 10% Fetal Calf Serum (FCS;Wisent), 2mM L-Glutamine, 100\(\mu\)g/mL streptomycin, 100U/mL penicillin (Wisent), and 5% WEHI conditioned media as the source of IL-3. Ba/F3 cell line stably expressing triple-FLAG-tagged murine MYSM1 was previously described\(^5\). EuMyc lymphomas cells\(^6\), expressing the same triple-FLAG-tagged murine MYSM1 construct, were derived through retroviral transduction with pMSCV vector (Addgene), as previously described\(^7\). The stable lines were maintained under 2\(\mu\)g/mL puromycin selection (Wisent).

Culture of Primary Cells
Tumour cells harvested from spleen and lymph nodes of EuMyc mice of different Mysm1 genotypes were cultured on a monolayer of irradiated Ink4a\(^{-/-}\) mouse embryonic fibroblasts (MEFs) in media containing 45% DMEM (Life Technologies), 45% IMEM (Life Technologies),
Loss of MYSM1 inhibits the oncogenic activity of cMYC in B cell lymphoma

10% FCS (Wisent), 100μg/mL streptomycin and 100U/mL penicillin (Wisent), and 5x10^{-5} M β-mercaptoethanol (Sigma-Aldrich)6.

**Flow Cytometry and Cell Sorting**
Cell suspensions of mouse spleen and lymph nodes were prepared in 45% DMEM (Life Technologies), 45% IMEM (Life Technologies), 10% FCS (Wisent), 100μg/ml streptomycin and 100U/ml penicillin (Wisent), and 5x10^{-5} M β-mercaptoethanol (Sigma-Aldrich). The cells were stained for surface-markers in PBS with 2% FCS for 20 minutes on ice with eFluor450-conjugated anti-CD45R/B220 (RA3–6B2, BioLegend). Fixable Viability Dye eFluor506 (eBioscience) was used to discriminate dead cells, and compensation done with BD™ CompBeads (BD Biosciences).

Intracellular staining for flow cytometry was performed as previously described5, 8. Briefly, the cells were fixed in 2% paraformaldehyde (PFA) in PBS with 2% FCS at 37°C for 10 minutes, and permeabilized in 90% methanol for 30 minutes on ice. The cells were stained with intracellular antibodies: Alexa Fluor 488 anti-p53 (clone 1C12, Cell Signaling), or unconjugated anti-cMYC (clone D84C12, Cell Signaling) or anti-eEF1G (EPR7200, Abcam) with Alexa Fluor 488 anti-rabbit IgG highly cross-adsorbed secondary antibody (Life Technologies), or appropriate isotype controls. All data were acquired on FACS Canto II flow cytometer (BD Biosciences) and analyzed with FACS Diva (BD Biosciences) or FlowJo (Tree Star) software.

Cell sorting was performed on FACS Aria II (BD Biosciences), with cells pre-stained with PE anti-IgM (II/41, eBioscience), PerCP-Cy5.5 anti-CD45R/B220 (RA3–6B2, BioLegend), and DAPI to discriminate dead cells.

**Protein Synthesis Rate Measurements**
Analysis of protein synthesis rates was performed using the O-propargyl-puromycin (OPP) incorporation method. Briefly, cells were cultured in the presence of 20 μM OPP for 30 minutes, stained with Fixable Viability Dye eFluor506 (eBioscience), fixed in 2% paraformaldehyde (PFA) in PBS with 2% FCS at 37°C for 10 minutes, and permeabilized in 90% methanol for 30 minutes on ice. The cells were then washed with PBS, and staining for OPP incorporation using the Click-iT™ Plus OPP Alexa Fluor 488 Protein Synthesis Assay Kit (Life Technologies, Thermo Fisher Scientific) according to the manufacturer’s protocols. Samples were analyzed by flow cytometry on FACS Canto II with FACS Diva software (BD Biosciences).

**RNA Isolation and qPCR**
RNA isolation from cell lines was carried out using the MagMAX total RNA kit (Ambion, Life Technologies) according to the manufacturer’s protocol. RNA quality was assessed on Bioanalyzer RNA Pico chips (Agilent), and cDNA was prepared using the qScript XLT cDNA Supermix (Quanta Biosciences) with 2-5ng RNA input per reaction. qPCRs were performed on a StepOnePlus instrument with Power SYBR Mastermix (Applied Biosystems, Life Technologies). The primers were purchased from IDT Technologies, and the sequences are provided in Supplemental Table S2.

**Chromatin Immunoprecipitation**
ChIP was performed as described previously5, 9, with minor modifications. Briefly, cells were fixed with 1% formaldehyde in the culture media for 10 minutes at room temperature, followed by
addition of 0.125M of glycine to stop fixation. Nuclei were extracted with 5 minutes lysis in 0.25% Triton buffer (10mM Tris-HCl pH8, 10mM EDTA, 0.5mM EGTA), followed by 30 minutes in 200mM NaCl buffer (10mM Tris-HCl pH8, 1mM EDTA, 0.5mM EGTA). Nuclei were resuspended in sonication buffer (10mM Tris pH8, 140mM NaCl, 1mM EDTA, 0.5mM EGTA, 0.5% SDS, 0.5% Triton X-100, 0.05% NaDOC) and sonicated for twelve cycles of 30 seconds with a digital sonifier (Branson Ultrasonics at 80%, with 30 seconds rest in cooled circulating water.

Beads were prepared overnight with 40μL of Dynabeads Protein G (Invitrogen, Life Technologies) conjugated to 5μg of antibodies: anti-FLAG M2 (Sigma, F1804) or anti-cMYC (clone D84C12, Cell Signaling). Immunoprecipitation was performed by overnight incubation of antibody-bead matrices with sheared chromatin from the equivalent of 5x10^6 cells. Immune complexes were washed sequentially for 2 min at room temperature with 1 ml of the following buffers: wash B (1% Triton X-100, 0.1% SDS, 150-mM NaCl, 2-mM EDTA, and 20-mM Tris-HCl, pH 8), wash C (1% Triton X-100, 0.1% SDS, 500-mM NaCl, 2-mM EDTA, and 20-mM Tris-HCl, pH 8), wash D (1% NP-40, 250-mM LiCl, 1-mM EDTA, and 10-mM Tris-HCl, pH 8), and TEN buffer (50-mM NaCl, 10-mM Tris-HCl, pH 8, and 1-mM EDTA). The samples were de-crosslinked by overnight incubation at 65°C in 1% SDS buffer (50mM Tris-HCl pH8, 10mM EDTA). Following RNaseA and Proteinase K enzymatic treatments, ChIP DNA was purified using Qiaquick PCR Cleanup kit (Qiagen). ChIP enrichment was quantified using qPCR analysis, with primer sequences provided in Table S3. All CT values were normalized to those of the pro-opiomelanocortin (Pomc) gene, which serves as a negative binding region. Enrichment was calculated relative to input DNA.

ChIP-Seq data analyses
The following ChIP-Seq datasets were included in the analyses: (I) cMYC ChIP-Seq from HPC7 murine hematopoietic progenitor cells from Wilson NK et. al. 10, 11; (II) MYSM1 ChIP-Seq from Ba/F3 murine B cell progenitors stably expressing 3xFLAG-tagged MYSM1 12. The reads were mapped to the UCSC mouse mm9 reference genome with Bowtie 1.0.013, and chromatin binding sites identified using peak detection algorithm MACS1.4.14, with comparisons for read enrichment against control input DNA from the same cells. Normalized sequence read density profiles (bigwig) were generated with Homer tool15 and visualized with IGV16.

Statistical analyses
Statistical comparisons were performed with Prism 7.01 (GraphPad), using Student’s t-test for two groups, ANOVA for multiple comparisons, and Log-rank (Mantel-Cox) test for survival data.
**Supplemental Table S2. RT-qPCR Primers Sequences.**

| Target Gene | Forward Sequence | Reverse Sequence |
|-------------|------------------|------------------|
| *Rps3*      | ctgaaggcagctagagcttt | tccaaggagtttgtagcgtaga |
| *Rps10*     | gtaggcgactgcaagacctc | cagccctagctttcttgtca |
| *Rps24*     | gcagtgacgcgtctcttttt | ggttcggatgtgtaacttgtt |
| *Rpl7*      | ccttgattgctcgtcttttt | agcctttttactcggttccc |
| *Rpl9*      | catccaggagaatggtccttttt | cagtcccttctcagacacatag |
| *Rpl11*     | aatgagaagattgtgctcactg | caactcataetccgcacct |
| *Rpl13*     | gaaacaagtccagggagta | tgctccgatgccaaga |
| *Eef1g*     | tcacgagaggagaacaagaac | cagggaccagccatctttatc |
| *Hprt*      | caggcagacgtttgtggt | ttgcgtcatcttagctttt |
| *Mysm1*     | gggatcggcacctactgtgct | tggaagggagagagatctttatg |
## Supplemental Table S3. ChIP-qPCR Primer Sequences.

| Target Region (mm9) | Forward Sequence | Reverse Sequence |
|---------------------|------------------|------------------|
| **Rps3** 98 bp downstream (Chr7:106,631,961-106,632,121)** | aatacacaatctacggccatcc | agatttccaagaagaggaggaagtaag |
| **Rps10** 199 bp downstream (Chr17:27,771,920-27,771,988) | gttgccttcaacctctctgc | actcagtagctgactgaagaaga |
| **Rps24** 0 bp upstream TSS (5'UTR) (Chr14:25,309,903-25,310,020) | cttgcgcttgatagctggg | gataaggcaggggtagttgctg |
| **Rpl7** 141 bp downstream (Chr1:16,094,250-16,094,373) | ctcagttgctctgtgactg | tgcctgtggcctcgggaa |
| **Rpl9** 8 bp upstream (Chr5:65,782,562-65,782,678) | ccaacagaggatgggttcagatt | gccctgacgggattacaagacc |
| **Rpl11** 70 bp upstream (Chr4:135,609,214-135,609,356) | cggatggagacggatgaaag | ctcgtttgtctgctggagaa |
| **Rpl13** 18 bp upstream (Chr8:125,626,232-125,626,358) | cttgcgcttgatagctggg | gataaggcaggggtagttgctg |
| **Eef1g** 197 bp downstream (Chr19:9,041,728-9,041,874) | gctccgctgattagggtcac | ctcacggtgctgaagaacat |
| **POMC** 744 bp downstream (Chr12:3,953,603-3,955,695) | aggcatggagcagcataggtaa | ttcacttgaactggcagagagct |
| **Ncl** 479bp downstream (Chr1:88,255,445-88,255,551) | ctaaggtggccctctcctc | gatacggggtccgggagtag |
| **Cdk7** 47bp upstream (Chr13:101,500,944-101,501,053) | gtcctacggaagctgtagg | gaatcactcagcatagagttg |
| **Npas4** 728bp downstream (Chr19:4,989,020-4,989,243) | ctatgctgtggctttcttct | gtaacactggactgactgc |
| **Mgmt** 267bp upstream (Chr7:144,085,843-144,086,027) | gattcctagtgggcttaactttc | ccagacctgaaactgggacttctt
SUPPLEMENTAL FIGURES

Supplemental Figure S1. Supplemental cMYC and MYSM1 ChIP-qPCR analysis. The data demonstrates the binding of cMYC at known MYC target genes Ncl and Cdk7, the binding of MYSM1 at MYSM1 target genes Npas4 and Mgmt, and the binding of both transcriptional regulators at Rpl11, consistent with previous data and confirming the specificity of the ChIP analyses. Data was acquired in Ba/F3 cells and is from one experiment. All Ct values were normalized to those of the pro-opiomelanocortin (Pomc) gene, which serves as a negative binding region. Enrichment was calculated relative to input DNA.
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