SUPPLEMENTARY METHODS

Antibodies

Endogenous MYC was detected by Western blotting using the N-262 polyclonal antibody (*Santa Cruz)* or the 9E10 monoclonal antibody (*Vanderbilt Antibody and Protein Resource*). Exogenous MYC proteins expressed from the MSCV–IRES–GFP–MYC vector carried the HA-epitope tag, and were detected with the 12CA5 monoclonal antibody (*Cold Spring Harbor Laboratory Antibody Shared Resource*). BIM was detected using the Y36 antibody (*Epitomics*). Actin was monitored using the AC-15 antibody (*Sigma*). Flow cytometry (FACS) for immunophenotyping was performed using PE-Cy5 conjugated antibodies against B220 (103210; *Biolegend*), Gr-1 (108410; *Biolegend*), and CD11b (Mac-1α; 101210; *Biolegend*). Phosphorylation-specific antibodies (pT244) were generated by *Open Biosystems* in rabbits immunized with either a mKLH-conjugated synthetic phosphopeptide corresponding to residues 240–250 of MYC (LHEE[pT]PPTTSS) or a synthetic peptide corresponding to the unphosphorylated sequence. Phosphorylation-specific antibodies were affinity-purified (EpiMax Affinity Purification Kit; *Epitomics*) by negative selection against the non-phosphorylated peptide antigen, followed by positive selection against the phosphorylated peptide antigen. ELISA assays (*Pierce*) were used to characterize reactivity of purified and unpurified antibodies against the unphosphorylated peptide, the T244 phosphorylated peptide, or a peptide phosphorylated at T248.

Plasmid DNA constructions

WT and T58A MYC proteins were expressed using the MSCV-IRES-GFP MYC vectors described previously. [1] The P245A, T244A, and Δ244-248 mutants were generated in the MSCV–IRES–GFP–MYC backbone using the Quickchange XL Site-directed Mutagenesis Kit (*Stratagene*). Primer sequences available upon request. To generate MYC protein standards, the human c-MYC cDNA was cloned into the pSUMO vector (*Life Sensors*) for expression as a SUMO-fusion in *E. coli* (pSUMO–MYC).

Retroviral transductions

MSCV-IRES-GFP MYC vectors expressing WT or mutant MYC proteins, or an empty vector control, were packaged into retroviruses using calcium-phosphate-mediated transfection of the Ecotropic Phoenix packaging cell line. Media was changed 16 hours post transfection, and viral supernatants collected every 12 hours thereafter for 36 hours. Recipient cells were transduced with retroviruses for 16 hours in the presence of 4 μg/ml polybrene.

Cycloheximide chase assays

To measure the relative stability of WT and mutant MYC proteins, NIH3T3 cells expressing the indicated MYC proteins were treated for various times with 50 μg/ml cycloheximide (*Sigma*). Cellular proteins were then harvested via RIPA lysis, resolved by SDS-PAGE, and detected by Western blotting using the Odyssey Imaging System (*LiCor*). Half-lives were calculated by normalizing MYC signals at each time point to that of actin, expressing each as a fraction of the normalized MYC signal at time zero, and fitting the resulting datasets to an exponential decay.

Detection of MYC phosphorylation at T244

Protein extracts were prepared from transduced NIH3T3 cells expressing the indicated HA–MYC proteins by RIPA lysis. Immunoprecipitation reactions were performed with the anti-HA antibody, the products resolved by SDS-PAGE, and MYC proteins detected by Western blotting.

Anchorage-independent growth

The ability of MYC to induce anchorage-independent growth was studied in NIH3T3 cells stably expressing either WT- or mutant-MYC oncoproteins (or vector control). For each assay, 20,000 cells were resuspended in 2 ml of DMEM/FCS (*Gibco*) containing 0.4% Type VII-A agarose (*Sigma),* and layered onto a bed of otherwise identical media containing 0.8% agarose. After 21 days, plates were de-identified and colonies counted under bright-field microscopy.

Primary mouse B-cell analyses

Wild-type and mutant MYC proteins were compared for their ability to drive proliferation of B-cells by retroviral transduction of fetal liver cells, followed by management of cells under conditions that promote B-cell growth. [2] Briefly, fetal liver (E12.5–E13.5) suspension was plated on a feeder layer of INK4A$^{-/-}$ mouse embryonic fibroblasts (MEFs; irradiated at 5000 rads) in B-cell media (RPMI with 10%FBS, 100U/ml Penicillin-Streptomycin, 4mM L-Glutamine, and 55 μM β-mercaptoethanol) supplemented with IL-7 (1 ng/ml; *RD Bioscience*), and incubated for 24 hours. Cells were then infected with retroviruses co-expressing GFP and WT–, T58A–, or P245A–MYC (from the MSCV–IRES–GFP vectors), or a GFP-only-expressing control. Beginning 24 hours after infection, IL-7 was progressively removed from the media by serial passaging, yielding a predominantly B-cell population (~80%: B220+ IgM-/low), as determined by FACS. GFP-positive cell percentage was assessed by FACS every 2 days for 8-10 days by flow cytometry to determine the relative enrichment of MYC expressing populations.

Adoptive transfer assay of lymphomagenesis

Embryonic fetal livers derived from pregnant E14.5 C57BL/6 WT mice (or congenic p53$^{+/-}$ mice) provided the source of hematopoietic stem cells (HSCs) [1]. Stem cells were infected with retroviruses expressing either HA-tagged WT or mutant MYC using vectors (MSCV–IRES–GFP–MYC) which co-express IRES-driven GFP. Prior to reconstitution, the population of GFP-positive cells was measured by FACS (~10%), and 500,000 cells were injected via the tail vein into irradiated 4-6 week old C57BL/6 WT female recipient mice. Reconstituted animals were routinely monitored for signs of distress and disease by palpating the lymph nodes, and survival defined based on the time taken from stem cell reconstitution until the animal was moribund. At the end-point, animals were imaged for expression of GFP and sacrificed for tumor analyses.

Immunophenotyping

Tumor-cell-containing lymph nodes were dissected from reconstituted mice and single-cell suspensions obtained by passing them through a 100 µm Nylon mesh strainer (*BD Sciences*). Contaminating erythrocytes were lysed by treating cell suspensions with ACK buffer (0.15 M NH_4Cl, 10 mM $KHCO_3$, and 0.1 mM EDTA) for five minutes. Tumor cells were harvested by centrifugation, washed in PBS, and resuspended in FACS Buffer (2% FCS in PBS) for flow cytometry analysis using the indicated antibodies. Data were acquired using a Guava easyCyte Flow Cytometer (*Millipore*).

Quantification of MYC

To prepare MYC standards for quantitative Western blotting, the pSUMO–MYC vector was transformed into *E. coli* (Rosetta cells; *EMD Millipore*), and SUMO-MYC fusion proteins induced, isolated, and purified according to the manufacturer's instructions (*Life Sensors*). SUMO–MYC protein was quantified by comparison to known amounts of BSA via SDS–PAGE and Coomassie-blue staining. Lysates from MYC-expressing cells were prepared via RIPA lysis, resolved by SDS–PAGE in parallel with MYC standards, and Western blotting performed using the 9E10 anti-MYC antibody and the Odyssey Imaging System.

Determination of p53 status

Single-cell suspensions were made from lymphomas derived by expressing WT, T58A, and P245A MYC, in $p53^{+/-}$ recipient HSCs. Crude genomic DNA from the tumors was extracted by overnight lysis (0.45% NP40, 0.45% Tween 20, 0.01% gelatin, 50 mM KCl, 10 mM Tris [pH 8.5], 1.5 mM $MgCl_2$, and 50 µg Proteinase K), followed by inactivation of Proteinase K at 95°C for five minutes. To monitor p53 status, 2 µl of this crude preparation was analyzed by PCR, using primers [1] that give different size reaction products for the wild-type or null p53 allele.

SUPPLEMENTARY REFERENCES

1 Hemann MT, Bric A, Teruya-Feldstein J, Herbst A, Nilsson JA, Cordon-Cardo C *et al*. Evasion of the p53 tumour surveillance network by tumour-derived MYC mutants. Nature 2005; 436: 807-811.

2 McLaughlin J, Chianese E, Witte ON. In vitro transformation of immature hematopoietic cells by the P210 BCR/ABL oncogene product of the Philadelphia chromosome. Proceedings of the National Academy of Sciences of the United States of America 1987; 84: 6558-6562.