Supporting Information

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SI Materials and Methods

Immunoblotting. Protein was extracted using Radio-Immunoprecipitation Assay (RIPA) lysis buffer. Lysates were then separated by SDS/PAGE and transferred to PVDF membranes using standard protocols. Antibodies used are described in Table S5.

Real-Time Quantitative PCR (qPCR). RNA was isolated using the Rneasy Mini Kit (Qiagen). Superscript II (Invitrogen) was then used for first-strand cDNA synthesis, and target genes were quantified by SYBR GREEN qPCR. Primers are described in Table S4. Values were normalized to the housekeeping gene Ubc.

Microarray Analysis. For gene expression profiling, total RNA was extracted using the Qiagen RNeasy Kit including DNase treatment. Thirty micrograms of total RNA from each specimen and 30 μg of Stratagene mouse reference RNA were labeled with Cy5 and Cy3, respectively, and cohybridized to Stanford MEEBO oligonucleotide microarrays according to standard protocols. After overnight hybridization and washing, arrays were imaged using a GenePix 4000B scanner (Axon). Microarray scans were gridded and processed using The Institute for Genomic Research (TIGR) SpotFinder version 3.11. Threshold for spot inclusion was set at background +2 SDs. Channel integrated intensities were normalized using TIGR Midas version 2.19. Spots were filtered with background checking on both channels and signal/noise ratio of 2. Intensities were corrected using local block Lowess normalization, with smoothing parameter of 0.33. SD regularization was also performed within and across arrays. Finally, a low-intensity filter was applied (minimum integrated intensity of 20,000 in both channels). The resulting expression data were converted to log2 ratios using TIGR MEV, median centered by array, and annotated using SOURCE (1). Processed and raw microarray data have been deposited into the National Center for Biotechnology Information Gene Expression Omnibus database (accession no. GSE 32341). Differentially expressed genes between sample comparison groups were identified using the Rank Product method (2). Probes with false positive rate <10% were selected as significant. For comparison of individual samples, correlation coefficients, scatterplots, and 95% prediction intervals for linear regression were generated using Medcalc version 10.0.

1. Diehn M, et al. (2003) SOURCE: A unified genomic resource of functional annotations, ontologies, and gene expression data. *Nucleic Acids Res* 31:219–223.

2. Breitling R, Armengaud P, Amtmann A, Herzyk P (2004) Rank products: A simple, yet powerful, new method to detect differentially regulated genes in replicated microarray experiments. *FEBS Lett* 573:83–92.

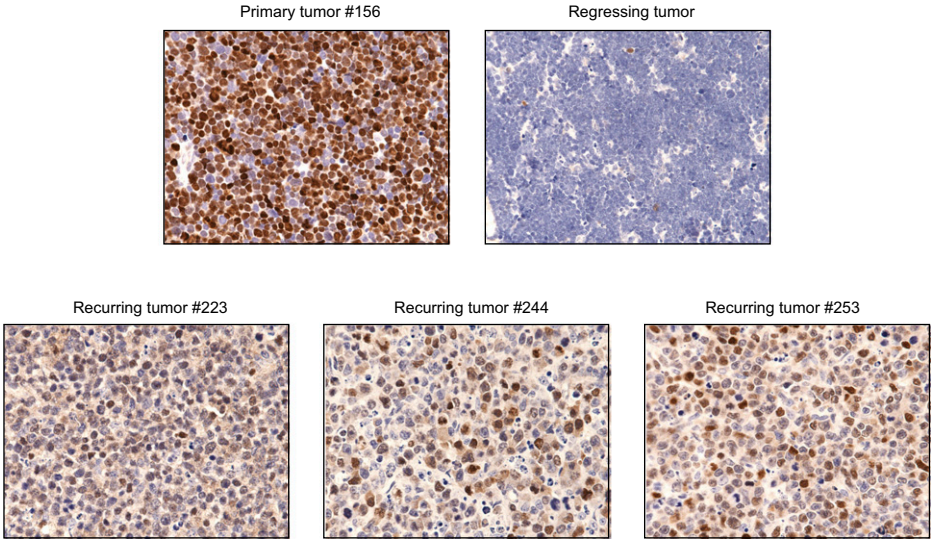

Fig. S1. Immunohistochemical staining for MYC in recurring lymphomas. Tissue sections from primary (#156), regressing, and recurring (#223, #244, and #253) lymphomas were stained for MYC (Epitomics) and counterstained with hematoxylin.

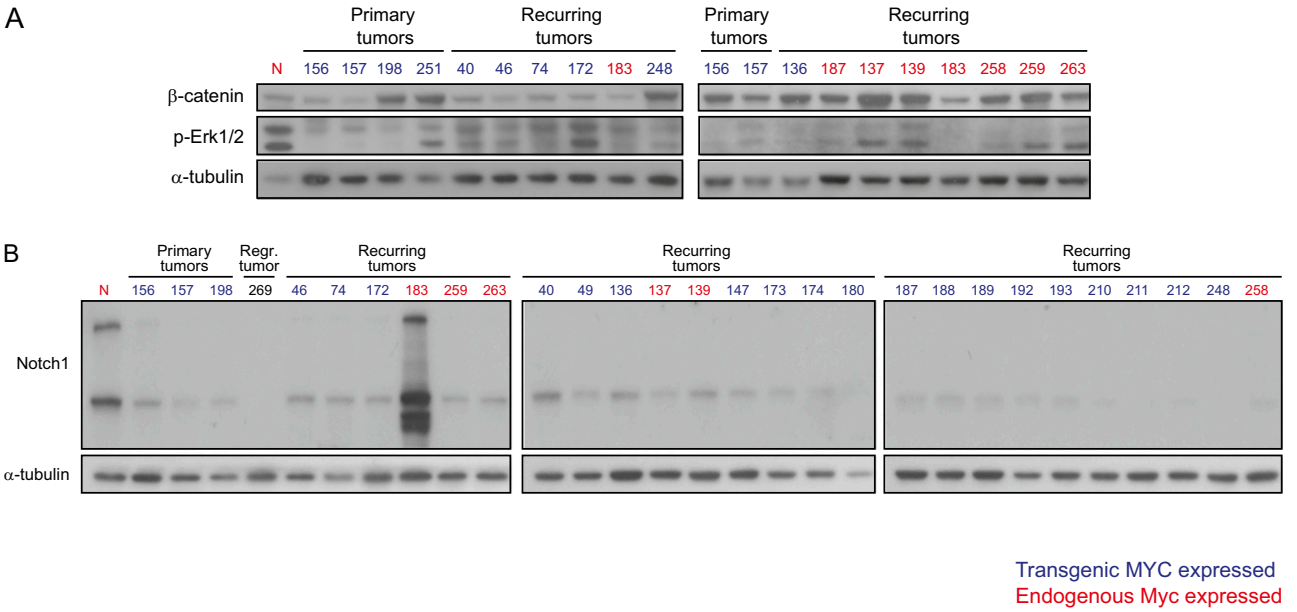

Fig. S2. Status of the Wnt/beta-catenin, RAS/MAPK, and Notch pathways in recurring lymphomas. (*A*) Western blot analysis for levels of beta-catenin and phospho-Erk1/2 in a panel of primary and recurring lymphomas. (*B*) Western blot analysis for levels of Notch1 in a panel of primary, regressing, and recurring lymphomas. N, normal thymus control.

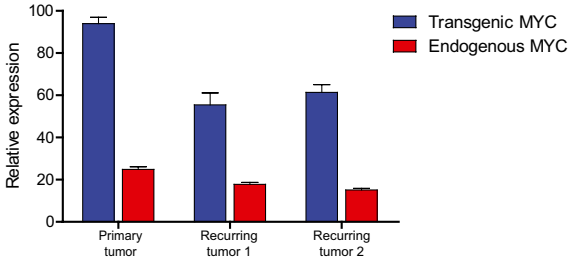

Fig. S3. Expression of transgenic and endogenous MYC in primary and recurring lymphoma lines used in MYC-knockdown experiments. Real-time qPCR analysis of transgenic and endogenous MYC expression in a primary lymphoma line and two independently derived recurring lymphoma lines.

Table S1. Summary of MYC expression in all analyzed recurring lymphomas

Sample ID	MYC expressed	tTA mutation
40	Transgenic	H100Y
46	Transgenic	None
49	Transgenic	None
74	Transgenic	None
136	Transgenic	None
137	Endogenous	None
139	Endogenous	None
147	Transgenic	N82T
172	Transgenic	N82T
173	Transgenic	H100Y
174	Transgenic	E147K
180	Transgenic	E147K
183	Endogenous	None
187	Transgenic	E147K
188	Transgenic	H100Y
189	Transgenic	None
192	Transgenic	H100Y
193	Transgenic	E147K
210	Transgenic	E147K
211	Transgenic	H100Y
212	Transgenic	E147K
223	Endogenous	None
244	Endogenous	None
248	Transgenic	E147K
253	Endogenous	None
258	Endogenous	None
259	Endogenous	None
263	Endogenous	None

Table S2. Comparison of number of genes significantly differentially expressed between group I (primary tumors) vs. group II (recurring tumors with transgenic MYC) and group III (recurring tumors with endogenous Myc)

	Primary tumors vs. transgenic MYC			
Primary tumors vs. endogenous Myc	Up	Down	n.s.	Total
Up	2	0	83	85
Down	0	14	70	84
n.s.	0	18	—	
Total	2	32		

n.s., not significantly differentially expressed.

Table S3. Microarray comparison of group II (recurring tumors with transgenic MYC) vs. group III (recurring tumors with endogenous Myc)

Twenty-seven genes up-regulated	Five genes down-regulated
1700058J15Rik	Dmrtc1a
Arc	Map2k7
B2m	Plunc
BC016579	Schip1
Cnot1	Timm17a
Cox17	
Cx3cl1	
Edg1	
Eif3s12	
Furin	
Fzd3	
H2-Ob	
Hpcal1	
Idi2	
Kif26b	
Krtap6-1	
Lcp1	
Matr3	
Nit1	
Pcyt1a	
Pik3r1	
Rdh7	
Rxrip110	
Tmcc3	
Trfr2	
Ubc	
Zfp46	

Table S4. Primers used in this work

Gene	Species	Forward primer (5′ to 3′)	Reverse primer (5′ to 3′)
Primers for RT-qPCR			
Bax	M	AAGCTGAGCGAGTGTCTC	CCTTGAGCACCAGTTTGC
Deltex1	M	ATCAGTTCCGGCAAGACACAG	CGATGAGAGGTCGAGCCAC
Hes1	M	TCAACACGACACCGGACAAAC	ATGCCGGGAGCTATCTTTCTT
MYC	H	GGTCACACCCTTCTCCCTTC	AGCAGCTCGGTCACCATC
Myc	M	TCTCCATCCTATGTTGCGGTC	TCCAAGTAACTCGGTCATCATCT
Ncl	M	AGAACCCACATGGCAAAC	AAGGTCTCTTCAGTGGTATCC
Notch1	M	AACAGTGCCGAATGTGAGTGG	AAGTGACGCAAGAGCACCTAG
Odc1	M	TGATCCTGATACCTTCGTTC	GCTATGATTCTCACTCCAGAG
Ubc	M	AGCCCAGTGTTACCACCAAG	ACCCAAGAACAAGCACAAGG
Primers for PCR/sequencing			
tTA	—	CCTCAGTGGATGTTGCCTTT	CCTGCACCTGAGGAGTGAAT
Myc exon2	—	CTGAAAGGGGAGTGGTTCAG	AGGAGGTCCATCCAACCTCT
Myc exon3	—	GTATAGCGTCCGGGATTCAG	TGAGGAAAGGAAGTTTGAGGA
Notch1 exon26	—	ACGGGAGGACCTAACCAAAC	CAGCTTGGTCTCCAACACCT
Notch1 exon27	—	CGCTGAGTGCTAAACACTGG	GTTTTGCCTGCATGTACGTC
Notch1 exon34	—	TGAGACTGCCAAAGTGTTGC	CCTGAAGCACTGGAAAGGAC

Table S5. Antibodies used in this work

Target	Clone/catalog no.	Company
Bim	2819	Cell Signaling Technology
β-Catenin	610154	BD Biosciences
p-Erk1/2	4376	Cell Signaling Technology
Human MYC	9E10	Calbiochem
MYC	C-19	Santa Cruz Biotechnology
MYC	1472-1	Epitomics
Notch1	D1E11	Cell Signaling Technology
p27kip1	610241	BD Biosciences
α-Tubulin	T9026	Sigma