Supporting Information

Choi et al. 10.1073/pnas.1406123111

SI Materials and Methods

Immunoblotting. Western blotting was carried out according to standard protocols. Briefly, cells were lysed in radioimmunoprecipitation assay buffer containing a mixture of protease inhibitors (Roche) and Halt phosphatase inhibitor (Thermo Scientific). Lysates were separated on polyacrylamide gels and transferred to PVDF membranes. Antibodies used in this study are listed in Table S4.

Real-Time Quantitative PCR. RNA was isolated using the Nucleospin RNA II kit (Machery-Nagel). Superscript II (Invitrogen) was then used for first-strand cDNA synthesis, and target genes were quantified by SYBR GREEN quantitative PCR. Primers are described in Table S2. Values were normalized to the housekeeping gene *Ubc*.

β-Catenin Inhibitor Treatment. Calphostin C (Enzo Life Sciences), PNU 74654 (Tocris Bioscience), Quercetin (Sigma), and Indomethacin (Sigma) were dissolved in DMSO. MYC lymphoma cells were seeded in 96-well plates, and the percentage of viable cells after 72 h of drug treatment was assessed by staining with PrestoBlue (Life Technologies A-13261).

Retroviral and Lentiviral Infection. To generate retrovirus, the Phoenix-Ecotropic retroviral packaging line (Garry Nolan, Stanford University, Stanford, CA) was transfected using the calcium-phosphate method in media supplemented with 25 μM chloroquine. To generate lentivirus, HEK 293T cells were transfected also using the calcium-phosphate method in media supplemented with 25 μM chloroquine. Media were changed 12–24 h later to complete growth medium, and subsequently, retroviral or lentiviral supernatant was harvested 24 and 48 h later. Lymphoma cells were infected by resuspending in retroviral or lentiviral supernatant containing 5 μg/mL polybrene and centrifuging at 2,500 rpm for 90 min in a Jouan C3i Centrifuge with a T20 rotor. Infected cells were then allowed to recover in fresh growth medium for 24 h before beginning phenotypic assays or drug selection.

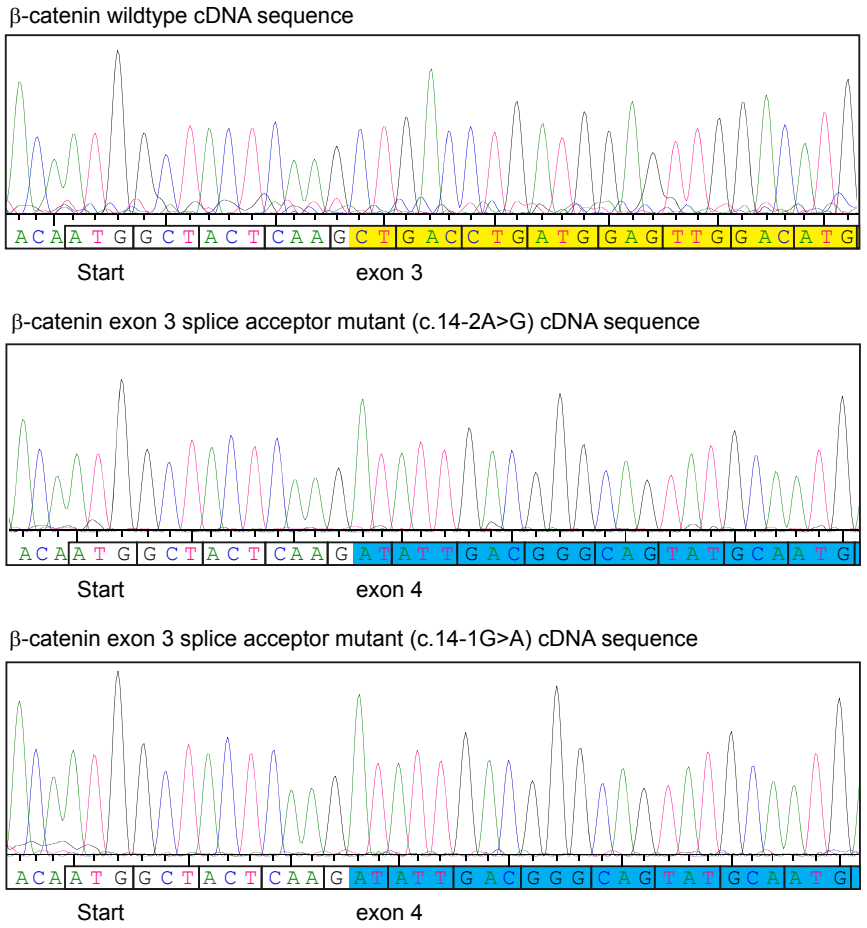

Fig. S1. Sequencing of β-catenin cDNA confirms deletion of exon 3 in MYC lymphoma cells with splice acceptor site mutations (related to Fig. 1). β-catenin cDNA was amplified from either (*A*) normal WT thymus or (*B* and *C*) MYC lymphoma cells with exon 3 splice acceptor site mutations. PCR products were directly sequenced using a primer upstream of the translational start site. Shown are sequence chromatograms with the start site and downstream included exon annotated.

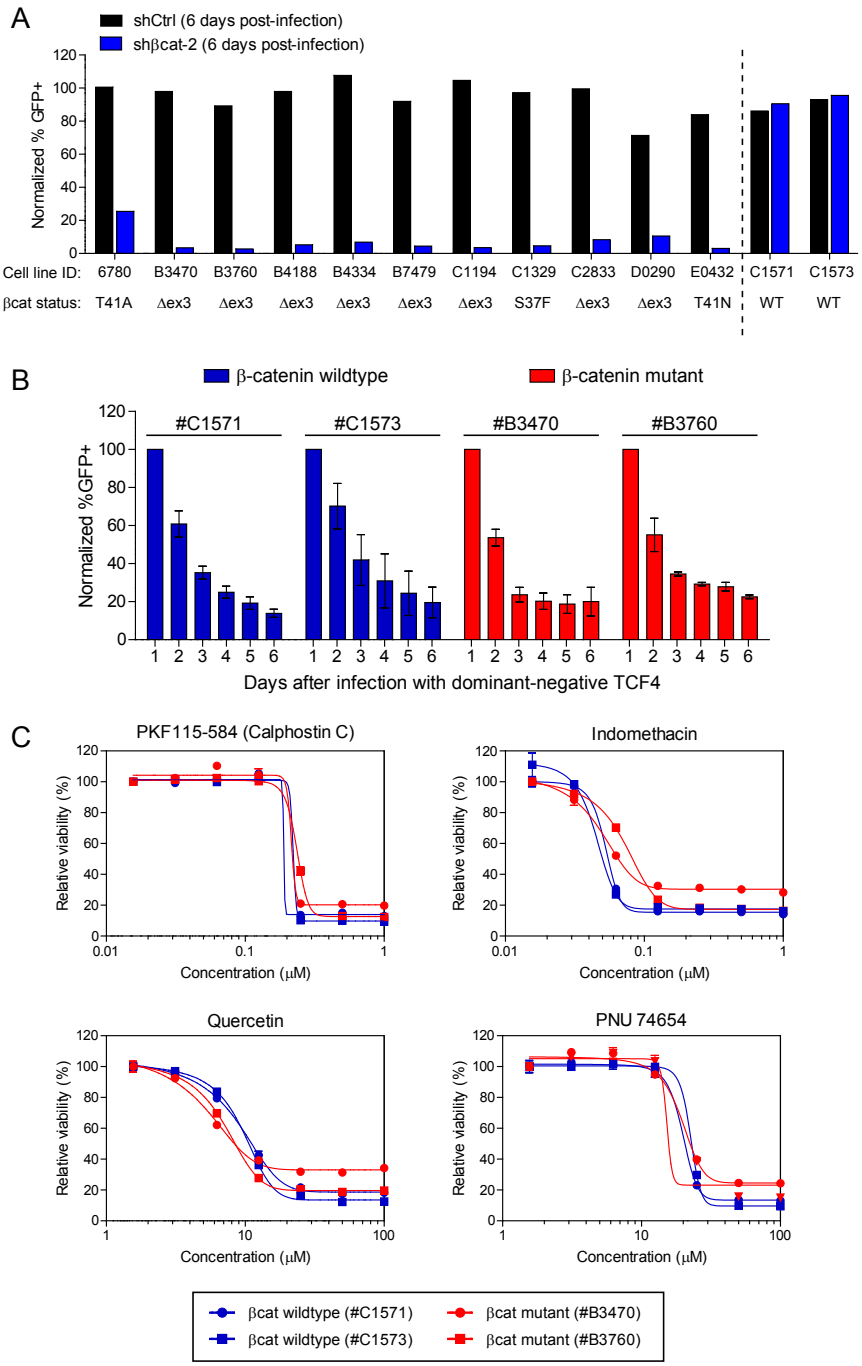

Fig. S2. β-catenin mutation status correlates with sensitivity to β-catenin knockdown, but not with sensitivity to dominant-negative T-cell factor 4 (TCF4)/transcription factor 7-like 2 or reported small-molecule inhibitors of β-catenin (related to Fig. 2). (*A*) Cell lines derived from Eμ-tTA/tetO-MYC lymphomas were infected with either a control shRNA (shCtrl) or a β-catenin specific shRNA (shßcat-2) (as in Fig. 2). Values represent the percentage of GFP^+-infected cells 6 d after infection, normalized to values at 1 d after infection. Also shown is the β-catenin mutation status of each cell line. (*B*) MYC lymphomas with either WT or mutant β-catenin were infected with a retroviral construct containing dominant-negative TCF4 and coexpressing GFP (MSCV-TCFΔN31-PIG). The percentage of GFP^+ cells was quantified over time by flow cytometry. Values are normalized to the percentage of GFP^+ cells at day 1 after infection and represent means ± SD of three independent experiments. (*C*) MYC lymphoma cells with WT or mutant β-catenin were treated for 72 h with varying concentrations of the indicated β-catenin inhibitors (PFK115-584/calphostin C, indomethacin, quercetin, or PNU-74654). Cell viability was assessed using Prestoblue reagent.

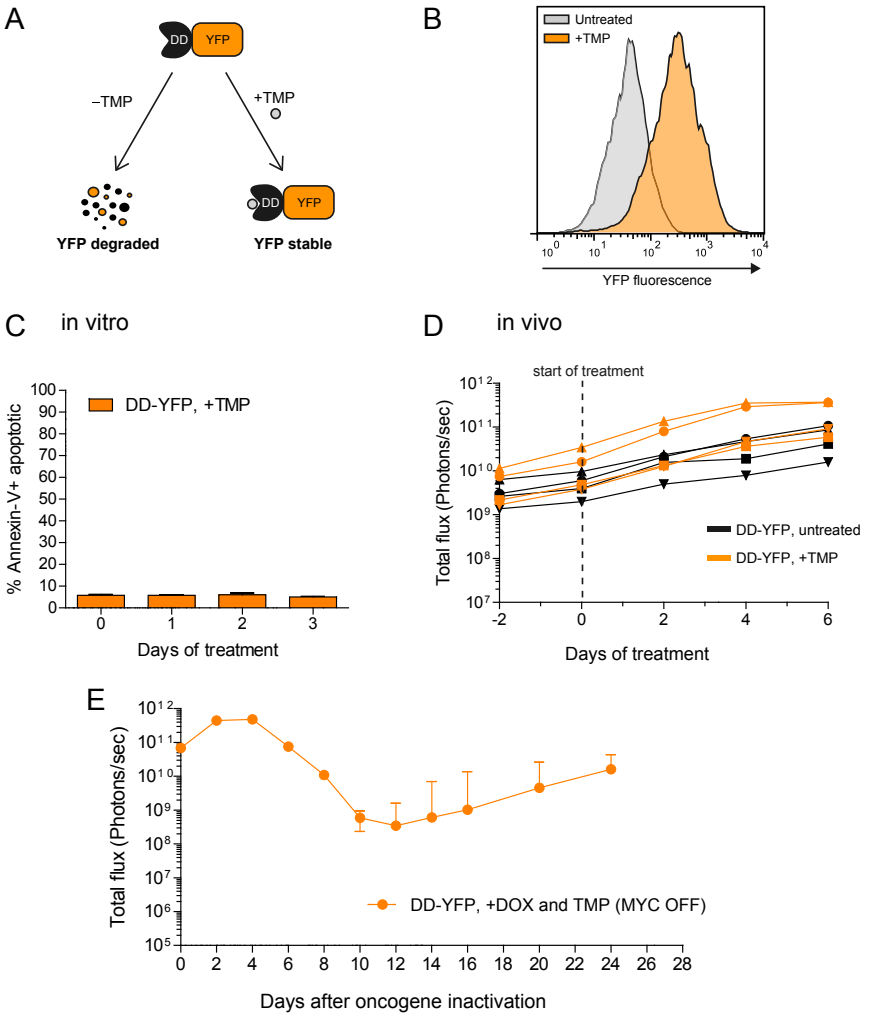

Fig. S3. Treatment with trimethoprim (TMP) and induced expression of a destabilizing *Escherichia coli* dihydrofolate reductase (DD)-YFP control does not affect growth of MYC lymphomas in vitro or in vivo (related to Fig. 4). (*A*) Schematic of the DD-YFP control. In the absence of the ligand TMP, the DD-YFP fusion protein is constitutively degraded, and on addition of TMP, DD-YFP is rapidly stabilized. (*B*) MYC lymphoma cells expressing DD-YFP were either treated with DMSO vehicle control (–TMP) or with 1 μM TMP (+TMP) for 24 h, and YFP fluorescence was measured by flow cytometry. (*C*) MYC lymphoma cells expressing DD-YFP were treated with 1 μM TMP for 0–3 d, and the percentage of Annexin-V$^+$ apoptotic cells was measured. (*D*) MYC lymphoma cells expressing DD-YFP and firefly luciferase (LUC2) were transplanted s.c. into the flanks of recipient SCID mice. Mice were either left untreated ($n = 4$) or treated with TMP ($n = 4$) beginning at day 0, and the bioluminescent signal was quantified over time. (*E*) A separate cohort of SCID mice ($n = 3$) was transplanted s.c. with MYC lymphoma cells expressing DD-YFP and LUC2. Mice were treated with both doxycycline (DOX) and TMP, and the bioluminescent signal was quantified over time.

Table S1. Summary of β-catenin mutations identified in a panel of 30 Eμ-tTA/tetO-MYC primary lymphomas

Number	Tumor ID	Mutation type	Nucleotide	Amino acid
1	B4282	–	–	–
2	B4283	Missense	c.133T > G	p.S45A
3	B4284	–	–	–
4	B4329	–	–	–
5	B4331	Splice site	c.14–1G > A	p.A5_A80del (Δexon3)
6	B6421	–	–	–
7	B6422	Missense	c.121A > G	p.T41A
8	B6678	–	–	–
9	B6680	–	–	–
10	B6867	–	–	–
11	B7647	Missense	c.100G > A	p.G34R
12	B8516	–	–	–
13	C0826	–	–	–
14	C0844	–	–	–
15	C0846	Splice site	c.14–2A > G	p.A5_A80del (Δexon3)
16	C1194	Splice site	c.14–1G > A	p.A5_A80del (Δexon3)
17	C1296	Missense	c.94G > A	p.D32N
18	C1369	–	–	–
19	C1497	–	–	–
20	C1571	–	–	–
21	C1573	–	–	–
22	C1630	–	–	–
23	C1951	Missense	c.121A > G	p.T41A
24	C2497	Missense	c.134C > T	p.S45F
25	C2498	–	–	–
26	C2978	–	–	–
27	C3598	–	–	–
28	D0289	–	–	–
29	D0290	Splice site	c.14–2A > G	p.A5_A80del (Δexon3)
30	E0432	Missense	c.122C > A	p.T41N

Table S2. Primers used in this study

	Primers	
Target	Forward primer (5′ → 3′)	Reverse primer (5′ → 3′)
Primers for sequencing		
β-catenin genomic PCR and sequencing (intron2-exon3)	GGTGAACTGGCTTTTGGTGT	TCCTTCAACCCACTTGTGCT
β-catenin cDNA PCR	GTCAGCTCGTGTCCTGTGAA	CTTAAAGATGGCCAGCAAGC
β-catenin cDNA sequencing	GAGCCGTCAGTGCAGGAG	
Primers for qRT-PCR		
Axin2	AACCTATGCCCGTTTCCTCTA	GAGTGTAAAGACTTGGTCCACC
β-catenin	TCCCTGAGACGCTAGATGAGG	CGTTTAGCAGTTTTGTCAGCTC
Bcl-xL	GACAAGGAGATGCAGGTATTGG	TCCCGTAGAGATCCACAAAAGT
Bim	CACCTGCTGTGTGCTTCCTA	TTCAGTGAGCCATCTTGACG
Enc1	CTGTTTCATAAGTCCTCCTACGC	CACCACTGAACATGGCTTCG
Mecom	CTTTGAATCCAAGGCAGAGC	GACAGCATGTGCTTCTCCAA
MYC (transgenic)	GGTCACACCCTTCTCCCTTC	AGCAGCTCGGTCACCATC
Runx2	AGAGTCAGATTACAGATCCCAGG	TGGCTCTTCTTACTGAGAGAGG
Ubc	AGCCCAGTGTTACCACCAAG	ACCCAAGAACAAGCACAAGG

Table S3. shRNAs used in this study

shRNAs	shRNA oligos (for cloning into LMP)
shControl	TGCTGTTGACAGTGAGCGATCTCGCTTGGGCGAGAGTAAGTAGTGAAGCCACAGATGTACTTACTCTCGCCCAAGCGAGAGTGCCTACTGCCTCGGA
shßcat-2	TGCTGTTGACAGTGAGCGACCAGTGTGGGTGAATACTTTATAGTGAAGCCACAGATGTATAAAGTATTCACCCACACTGGCTGCCTACTGCCTCGGA
shßcat-3	TGCTGTTGACAGTGAGCGAATGCAGAATACAAATGATGTATAGTGAAGCCACAGATGTATACATCATTTGTATTCTGCATGTGCCTACTGCCTCGGA
shßcat-4	TGCTGTTGACAGTGAGCGACCAAGTCAGCGACTTGTTCAATAGTGAAGCCACAGATGTATTGAACAAGTCGCTGACTTGGGTGCCTACTGCCTCGGA

Table S4. Antibodies used in this study

Antibodies

Target	Clone/catalog no.	Company
α-tubulin	T9026	Sigma
β-actin	AM1829b	Abgent
β-catenin	610154	BD Biosciences
Bcl-xL	610209	BD Biosciences
Bim	2819	Cell Signaling Technology
Cleaved caspase-3	9661	Cell Signaling Technology
ICAT	FL-81/sc-99240	Santa Cruz Biotechnology, Inc.
*K*i-67	556003	BD Biosciences
MYC	9E10	Calbiochem
Vinculin	SAB4200080	Sigma