Supplementary Materials and Methods

Reagents and DNA constructs

Imatinib was purchased from Novartis Pharmaceuticals. JSL-1 (purity > 95%, HPLC) was synthesized in our lab. Human and mouse γ-catenin shRNA were purchased from Sigma-Aldrich (Shanghai, China). The pCMV6-γ-catenin and FoxM1 constructs were purchased from OriGene (Rockville, MD). MSCV-BCR-ABL-IRES-EGFP construct was generously provided by Dr. Ruibao Ren (State Key Laboratory for Medical Genomics and Shanghai Institute of Hematology, RuiJin Hospital). Lentiviral-TOP-dGFP-reporter and lentiviral-FOP-dGFP-reporter constructs were purchased from Addgene (Cambridge, MA). The EZ-ChIP Chromatin Immunoprecipitation Kit was from EMD Millipore (Billerica, MA).

Cell lines

KBM5 and KBM5-T315I cells were cultured in Iscove's modified Dulbecco's medium (IMDM) (Invitrogen, Shanghai, China) supplemented with 10% heat-inactivated fetal bovine serum (FBS) [1]. K562 cells were cultured in RPMI 1640 medium supplemented with 10% FBS [1]. 293T and Plat-E cells were maintained in Dulbecco's modified Eagle's medium (DMEM) with 10% FBS. Cells were kept at 37°C in a humidified incubator with 5% CO_2. Cells were confirmed to be mycoplasma-free.

Primary cells

Mononuclear cells were isolated by histopaque gradient centrifugation (density 1.077; Sigma-Aldrich, Shanghai). $CD34^+$ cells were isolated using the CD34 MicroBead Kit (Miltenyi Biotech, Germany) and kept in IMDM medium supplemented with 10% FBS and the following growth factors: granulocyte-macrophage colony-stimulating factor (GM-CSF, 100 ng/ml), stem cell factor (SCF, 100 ng/ml), interleukin-3 (IL-3, 20 ng/ml) and interleukin-6 (IL-6, 20 ng/ml). The growth factors were purchased from PeproTech Inc (Rocky Hill, NJ).

Pull-down of JSL-1–bound proteins

KBM5 cells were harvested and lysed in RIPA buffer supplemented with protease and phosphatase inhibitors with or without brief sonication. After centrifugation at 12,000 g for 30 min, the resulting supernatant (2 mg/ml) was collected and equally divided into 2 parts, then incubated with 100 μl biotin or biotin-JSL-1 beads in RIPA buffer overnight at 4°C, then beads were washed 3 times with RIPA buffer, and the bead-bound proteins were eluted, separated by SDS-PAGE, and visualized by silver staining.

Establishment of KBM5 cells stably expressing γ-catenin

KBM5 cells were electrotransfected with pCMV6 (empty vector) or pCMV6-JUP (γ-catenin) constructs, then treated with G418 (400 μg/ml) for 2 weeks; cells stably expressing empty vector or γ-catenin (Vector, γ-catenin #1, and γ-catenin #2) were used for experiments. Overexpression of γ-catenin was confirmed by Western blot

analysis. The same number of KBM5 (Vector, γ-catenin #1 and γ-catenin #2) cells were seeded in 6-well plates and treated with JSL-1 (50 nM) for the indicated time periods. Cells were examined daily with a hemocytometer by trypan blue exclusive assay.

CML mouse model

BCR-ABL–expressing (GFP$^+$) leukemia cells and myeloid cells were labeled with anti-mouse Gr-1-APC and Mac-1-PE antibodies (BD Pharmingen). The antibodies anti-mouse lineage cocktail APC, -mouse Ly-6A/E (Sca-1) PE-CF594, -mouse CD48 APC-Cy7, -mouse CD117 APC-H7 and -mouse CD34-PE were from BD Biosciences and the antibodies anti-mouse CD117-PE, -mouse CD135 (Flt3) PE-Cy5, -mouse CD150 PE-Cy7, and -mouse CD16/CD32 (FcγRII/III) PE-Cy7 were from eBiosciences. Granulocyte-macrophage progenitors (GMPs, Lin$^-$Sca-1$^-$c-Kit$^+$CD34$^+$FcγRII/III^{hig}), LSK cells (Lin$^-$Sca-1$^-$c-Kit$^+$), LT-HSCs (LSK Flt3$^-$CD150$^+$ CD48$^-$) and ST-HSCs (LSK Flt3$^-$CD150$^-$CD48$^-$) were detected in BM and splenic cells [2-4]. The CML mice were excluded from the analysis if died during the experiments for LSK parameter analysis. Blinding was not applied.

Engraftment of human CD34$^+$ cells in immunodeficient mice

Human CML CD34$^+$ cells were treated with JSL-1 for 72 hr *in vitro*, then cells (1×10^6 cells/mouse) were transplanted into sublethally irradiated (300 cGy) 8-week-old NOD.Cg-*Prkdc*^{scid} Il2*rg*^{tm1Sug}/JicCrl mice (NOG mice, CIEA, Kawasaki,

Japan) by tail-vein injection. BM and spleen cells from NOG mice were harvested after 12 weeks; the engraftment of human cells was analyzed by BD FACS Aria II flow cytometer [3, 5].

ChIP assay

After JSL-1 or another HDACi treatment for 24 hr, K562 cells were crosslinked in 1% formaldehyde for 10 min at room temperature. Cells were washed with cold PBS, harvested, and resuspended in SDS lysis buffer containing protease inhibitor cocktail. Crosslinked DNA was sheared to 200-1,000 bp by sonication and centrifuged at 13,000 rpm at 4°C for 10 min. Protein A/G agarose (60 μl of a 50% slurry) was added and incubated at 4°C for 1 hr. The supernatants were immunoprecipitated with anti-FoxM1 antibody (1:500) or normal rabbit IgG at 4°C overnight. Pellets were washed and protein–DNA complexes were eluted and reversed by overnight incubation at 65°C with proteinase K. The purified DNA was used for PCR reactions as follows: 5 min at 94°C for initial denaturation, 30 s at 94°C for denaturation, 30 s at 60°C (γ-catenin promoter) for annealing, 30 s at 72°C for extension, for 35 cycles, then 72°C 5 min for final extension. The primers used were listed in Table S3.

Real-time quantitative RT-PCR

Total RNA was extracted by using the RNeasy Mini Kit (Qiagen, Valencia, CA). Reverse transcription was performed using the QuantiTect Reverse Transcription Kit

(Qiagen). qPCR assay was carried out on an iCycler iQ Real-Time Thermocycler (Bio-Rad Laboratories, Hercules, CA). The primers used were listed in Supplementary Table S3.

Luciferase assay

CML CD34$^+$ cells were transduced with TOP- or FOP-lentivirus for 48 hr, then treated with JSL-1 for another 24 hr. Luciferase activity was measured by use of the dual-luciferase assay kit (Promega, Shanghai, China) [6].

Subcutaneous xenograft experiments

Male *nu/nu* BALB/c mice (4~6-week-old) were purchased from Slac Laboratory Animal Co (Shanghai, China). KBM5 or KBM5-T315I cells (1×10^7 cells/mouse in FBS-free IMDM medium) was injected subcutaneously into the flanks of nude mice [1]. Tumors were measured every other day. When the tumors reached approximately 100 mm^3, mice were randomly divided into two (KBM5) or three (KBM5-T315I) groups to receive treatments. JSL-1 dissolved in DMSO, then diluted with vehicle [30% Cremophor EL/ethanol (4:1), 70% PBS] was administrated at 40 mg/kg/d by intraperitoneal injection, and imatinib dissolved in sterile double-distilled water was given at 50 mg/kg/d by gavage. The mice were euthanized after 2 or 3 weeks of treatments, and tumor xenografts were immediately removed, weighed, fixed and stored at -80°C. For animal studies, the exact sample size was given in the respective figure legend or labled in the figures.

Immunohistochemical staining

Formalin-fixed CML cell-derived xenografts were embedded in paraffin, sectioned (4-μm thick). Immunohistochemistry was performed with antibodies against c-ABL and Ki67 by using the MaxVision Kit (Maixin Biol, Fuzhou, China) [1, 7, 8]. Color was developed with diaminobenzidine and H_2O_2 with hematoxylin counterstained.

Study approval

This study was approved by the Sun Yat-sen University Ethics Committee according to institutional guidelines and the Declaration of Helsinki principles, and written informed consent was received from participants prior to inclusion in the study. All animal studies were conducted with the approval of the Sun Yat-sen University Institutional Animal Care and Use Committee.

Supplementary references

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myeloid leukemia cells by effective inhibition of a new AHI-1-BCR-ABL-JAK2 complex. J Natl Cancer Inst. 2013; 105: 405-23.

6. Jin Y, Lu Z, Ding K, Li J, Du X, Chen C, et al. Antineoplastic mechanisms of niclosamide in acute myelogenous leukemia stem cells: inactivation of the NF-kappaB pathway and generation of reactive oxygen species. Cancer Res. 2010; 70: 2516-27.
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Supplementary Tables

Table S1. Characteristics of patients with chronic myelogenous leukemia

Patient No	Gender/ age (yr)	Stage of disease	Sample	Date of diagnosis	Prior therapy	WBC Count (10^9/L)	Blast cell (%)	BCR-ABL positive
1	F/49	CP	PB	11/2013	Imatinib	280.19	0.03%	+
2	M/16	CP	BM	5/2014	Initial	473.51	5.5%	+
3	F/42	CP	BM	6/2014	Initial	281.96	5.5%	+
4	F/15	CP	BM	7/2014	Initial	176.58	ND	+
5	F/38	CP	BM	6/2014	Initial	173.12	0.01%	+
6	F/34	CP	PB	1/2015	Initial	110.26	0.04%	+
7	F/42	CP	BM	12/2012	Hydroxyurea	2.13	ND	+
8	F52	CP	BM	3/2012	Hydroxyurea	44.84	0.24%	+
9	F/50	CP	BM	7/2014	Initial	23.93	0.01%	+
10	M/25	CP	BM	7/2014	Initial	284.90	9%	+
11	M/41	CP	BM	2/2013	Imatinib	213.6	ND	+
12	F/30	CP	BM	8/2014	Initial	361.08	3%	+
13	M/31	BP	PB	8/2014	Initial	809.49	54%	+ (T315I mutation)
14	M/29	AP	BM	8/2014	Initial	119.8	19.5%	+
15	F/38	CP	PB	9/2014	Initial	312.37	3.65%	+
16	M/23	AP	BM	11/2014	Initial	183.66	32%	+
17	F/18	AP	BM	9/2014	Initial	49.46	9%	+
18	M/29	CP	BM	9/2014	Initial	47.8	3%	+
19	M/26	CP	BM	9/2014	Initial	57	2%	+
20	M/43	CP	BM	9/2014	Initial	310.39	8.5%	+
21	M/40	CP	BM	10/2014	Initial	483.85	2%	+

CP: chronic phase; AP: accelerated phase; BP: blast crisis phase; PB: peripheral blood; BM: bone marrow; WBC: white blood cells; ND: not detected.

Table S2. Information of antibodies.

Antibody	Vendor	Catalog number
Acetyl-H3K9	Cell Signaling Technology	9671
Histone H3	Santa Cruz Biotechnology	sc-10809
Acetyl-H4K16	EMD Millipore	05-1232
Histone H4	EMD Millipore	05-858
γ-catenin	BD Biosciences	610253
β-catenin	BD Biosciences	610153
c-ABL	Santa Cruz Biotechnology	sc-887
FoxM1	Santa Cruz Biotechnology	Sc-500
Ki67	Maxim	RMA-0542
PCNA	Santa Cruz Biotechnology	sc-56
Actin	Sigma-Aldrich	A5228
Goat-Anti-Mouse 800CW	LICOR	926-32210
Goat-Anti-Rabbit 800CW	LICOR	926-32211
HRP-anti-Mouse	Pierce Biotechnology	31437
HRP-anti-Rabbit	Pierce Biotechnology	31463

Information of antibodies for CML mice model.

Antibody	Vendor	Catalog number
Lin-APC	BD Biosciences	51-9003632
c-Kit-PE	eBioscience	12-1171-8
c-Kit-APC-H7	BD Biosciences	560185
Sca-1-PE-CF594	BD Biosciences	562730
CD135-PE-cy5	eBioscience	15-1351-82
CD150-PE-cy7	eBioscience	25-1502-82
CD48-APC-cy7	BD Biosciences	561242
CD34-PE	BD Biosciences	551387
CD16/32-PE-Cy7	eBioscience	25-0161-82
Mac-1(CD11b)-PE	BD Biosciences	553311
Gr-1-APC	BD Biosciences	553129

Information of antibodies for NOG mice.

Antibody	Vendor	Catalog number
CD45-FITC	eBioscience	11-0459-42
CD34-FITC	eBioscience	11-0349-42
CD33-PE-Cy7	BD Biosciences	333952
CD11B-PE	BD Biosciences	555388
CD19-APC	BD Biosciences	555415
CD14-PerCP-Cy5.5	BD Biosciences	562692
CD3-Alexa Fluor 700	BD Biosciences	557943

Table S3. Primers for PCR

Genes	Sense primer	Antisense primer
c-Myc	5'-CAGCGACTCTGAGGAGGAAC-3'	5'-TCGGTTGTTGCTGATCTGTC-3'
CCND1	5'-GCTGTGCATCTACACCGACA-3'	5'-CCACTTGAGCTTGTTCACCA-3'
γ-catenin	5'-CAACCAGGAGAGCAAGCTGA-3'	5'-CCTCCACAATGGCAGGCTTA-3'
LEF1	5'-CGAATGTCGTTGCTGAGTGT-3'	5'-GCTGTCTTTCTTTCCGTGCT-3'
BCR-ABL	5'-TCCACTCAGCCACTGGATTTAA-3'	5'-TGAGGCTCAAAGTCAGATGCTACT-3'
18S	5'-AAACGGCTACCACATCCAAG-3'	5'-CCTCCAATGGATCCTCGTTA-3'

Primers for chromatin immunoprecipitation

Primer	**Sequence**
γ-catenin-promoter Sense	5'-CGTAGTAGGCCCTCATGGGA-3'
γ-catenin-promoter Antisense	5'-AGGGCCGAACTTTGTACCAG-3'

Table S4. Long-term culture-initiating cell (LTC-IC) limiting dilution analysis

	Frequency of LTC-IC	Range defined by ±1 SE
Control	1/7710	(5516-10778)
Imatinib	1/7620	(5450-10652)
JSL-1	1/44542	(31620-62744)
Combination	1/94073	(63216-139992)

Supplementary Figure Legends

Figure S1. γ-Catenin does not alter β-catenin in primary CML CD34$^+$ cells. (A) Silencing γ-catenin did not affect the protein levels or cellular localization of β-catenin. CML CD34$^+$ cells were transduced with control shRNA (Scramble) and γ-catenin shRNA for 72 hr, the protein levels of γ-catenin and β-catenin in whole cell lysates (WCL), cytoplasma, and nuclear were detected by Western blot analysis. **(B)** Silencing β-catenin did not affect the protein levels or cellular localization of γ-catenin. K562 cells were transfected with control siRNA (Mock) and β-catenin siRNA for 72 hr, the protein levels of β-catenin and γ-catenin in WCL, cytoplasma, and nuclear were detected by Western blot analysis.

Figure S2. JSL-1 inhibited γ-catenin gene transcription through FoxM1 in CML cells. (A) MG132 did not rescue JSL-1-mediated decline of γ-catenin. K562 cells were pre-treated with MG132 (1.0 μM) for 2 hr, then with JSL-1 (1.0 μM) for 24 hr. Western blot analysis of γ-catenin and Dvl3 protein levels. **(B)** qRT-PCR analysis of γ-catenin mRNA level in JSL-1-treated K562 cells. **(C)** Western blot analysis of γ-catenin, β-catenin and FoxM1 in HDACis-treated K562 cells. **(D)** qRT-PCR analysis of γ-catenin in HDACis-treated K562 cells. **(E)** qRT-PCR analysis of γ-catenin in 293T cells after transfected with FoxM1 encoding plasmid (pCMV6-FoxM1). **(F)** HDACis inhibited binding of FoxM1 to the γ-catenin promoter in K562 cells assayed by ChIP. IgG was a negative control. ** $P<0.01$, *** $P<0.0001$.

Figure S3. Silencing γ-catenin has minimal effect on the proliferation, survival and colony formation in human NBM $CD34^+$ cells. Normal bone-marrow (NBM) $CD34^+$ cells were transduced with lentiviral shRNA against control or γ-catenin for 48 hr, then treated with imatinib (2.5 μM) for 24 hr. **(A)** mRNA evaluation of γ-catenin (*left*) and CTNNB1 (β-catenin) (*right*) was performed by qRT-PCR in NBM $CD34^+$ cells. **(B)** Representative flow cytometry histograms of apoptosis in NBM $CD34^+$ cells. **(C-D)** Apoptosis in NBM $CD34^+CD38^-$ stem/primitive progenitor cells and $CD34^+CD38^+$ committed progenitor cells analyzed by Annexin V-FITC and CD38-PE labeling. **(E)** Cell viability in human NBM $CD34^+$ cells determined by MTS assay. **(F)** Colony-formation assay of human CML $CD34^+$ cells was carried out in drug-free methylcellulose medium (H4434) for 14 days. ** $P<0.01$, *** $P<0.0001$. **(G)** KBM5 cells were electrotransfected with pCMV6 (empty vector) or pCMV6-JUP (γ-catenin) constructs, then treated with G418 (400 μg/ml) for 2 weeks; cells stably expressing empty vector or γ-catenin (Vector, γ-catenin #1, and γ-catenin #2) were used for experiments. Overexpression of γ-catenin was confirmed by Western blot analysis. **(H)** The same number of KBM5 (Vector, γ-catenin #1 and γ-catenin #2) cells were seeded in 6-well plate and treated with JSL-1 (50 nM) for the indicated time periods. Cells were examined daily with a hemocytometer by trypan blue exclusive assay.

Figure S4. Silencing γ-catenin reduces CML LSCs growth in CML mice. Analysis of LSCs in the splenic cells of CML mice received treatment with shγ-catenin ± imatinib. **(A)** Photomacrograph of representative spleens from each group. **(B)** GFP^+

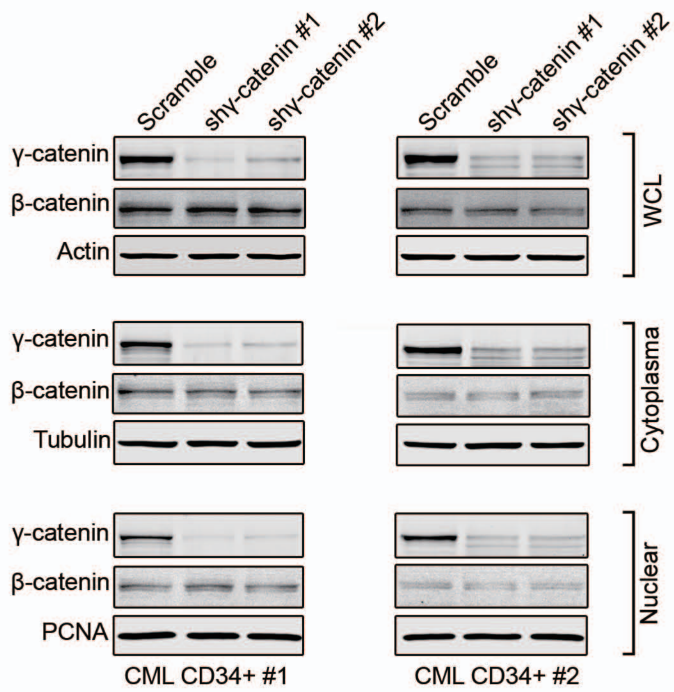
cell proportion in spleens. **(C)** Proportion of GFP^+ myeloid cells ($Gr\text{-}1^+$ $Mac\text{-}1^+$) in spleens. **(D)** Representative flow cytometry plots of GMPs in spleens. **(E)** GMP cell proportion in the spleen. **(F)** Representative flow cytometry plots of LSK cells, LT-HSCs, and ST-HSCs in the spleen. Results for the GFP^+ population in spleens were shown: LSK cells (**G**), LT-HSCs (**H**), and ST-HSCs (**I**). * P<0.05, ** P<0.01, *** P<0.0001.

Figure S5. Pharmacological inhibition of γ-catenin has minimal effect on the survival, colony formation and self-renewal capacity in human NBM $CD34^+$ cells. **(A)** Representative flow cytometry plots of apoptosis in NBM $CD34^+$ cells. **(B)** Quantitative analysis of apoptosis in NBM $CD34^+$ $CD38^-$ stem/primitive progenitor cells and $CD34^+$ $CD38^+$ committed progenitor cells. **(C)** JSL-1 did not induce apoptosis in NBM quiescent $CD34^+$ cells. NBM $CD34^+$ cells were labeled with carboxyfluorescein diacetate succinimidyl ester (CFSE), then cultured with JSL-1 for 96 hr; cells were labeled with Annexin V-PE and analyzed by flow cytometry; results for $CFSE_{max}$ and Annexin V^+ cells were shown. **(D)** JSL-1 had minimal effect on the long-term serial replating ability of human NBM HSCs. **(E)** JSL-1 did not affect long-term culture-initiating cell (LTC-IC) in human NBM cells. The number of LTC-IC in human NBM cells. *** P<0.0001.

Figure S6. Pharmacological inhibition of γ-catenin reduces growth of CML LSCs in mice. Analysis of LSCs in the splenic cells of CML mice received treatment with

imatinib ± JSL-1. **(A)** Representative photomacrograph of spleens from each group. **(B)** GFP^+ cells in the spleen. **(C)** GFP^+ myeloid cells (Gr-1^+ Mac-1^+) cells in the spleen. **(D)** Representative flow cytometry plots of GMP in the spleen of mice treated with imatinib and JSL-1. **(E)** GMP cells in the spleen. **(F)** Representative flow cytometry plots of LSK cells, LT-HSCs, and ST-HSCs in the spleen of mice treated with imatinib and JSL-1. Results for the GFP^+ population in the spleen: LSK cells (**G**), LT-HSCs (**H**), and ST-HSCs (**I**). * $P<0.05$, ** $P<0.01$, *** $P<0.0001$.

Figure S1



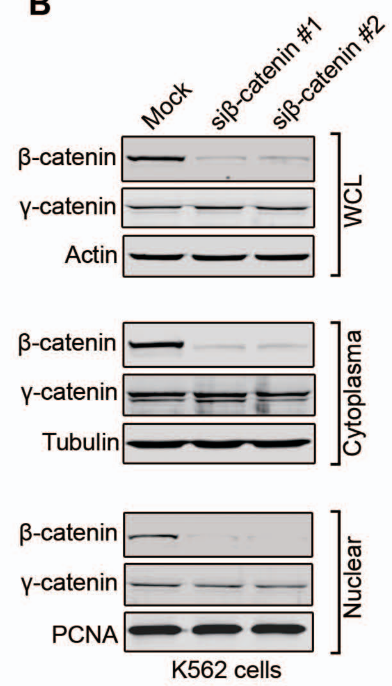


Figure S2

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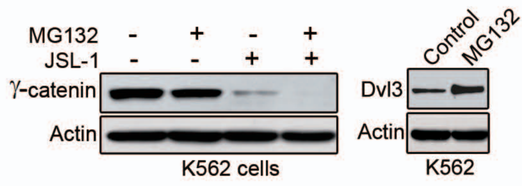

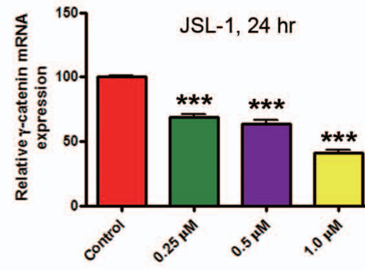

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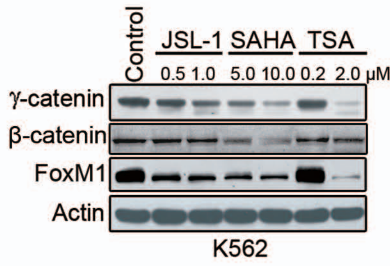

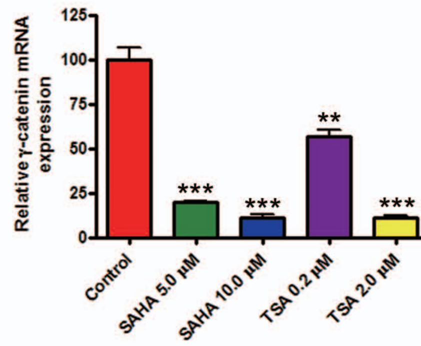

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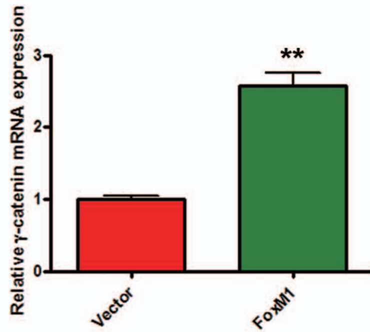

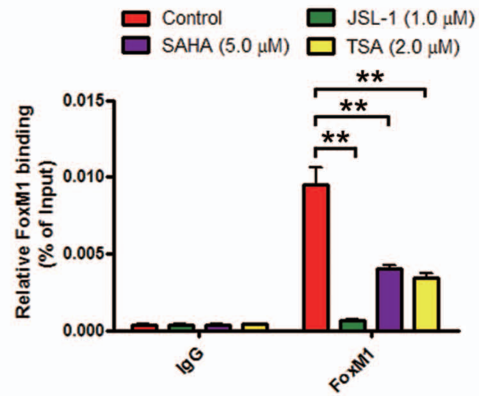

Figure S3

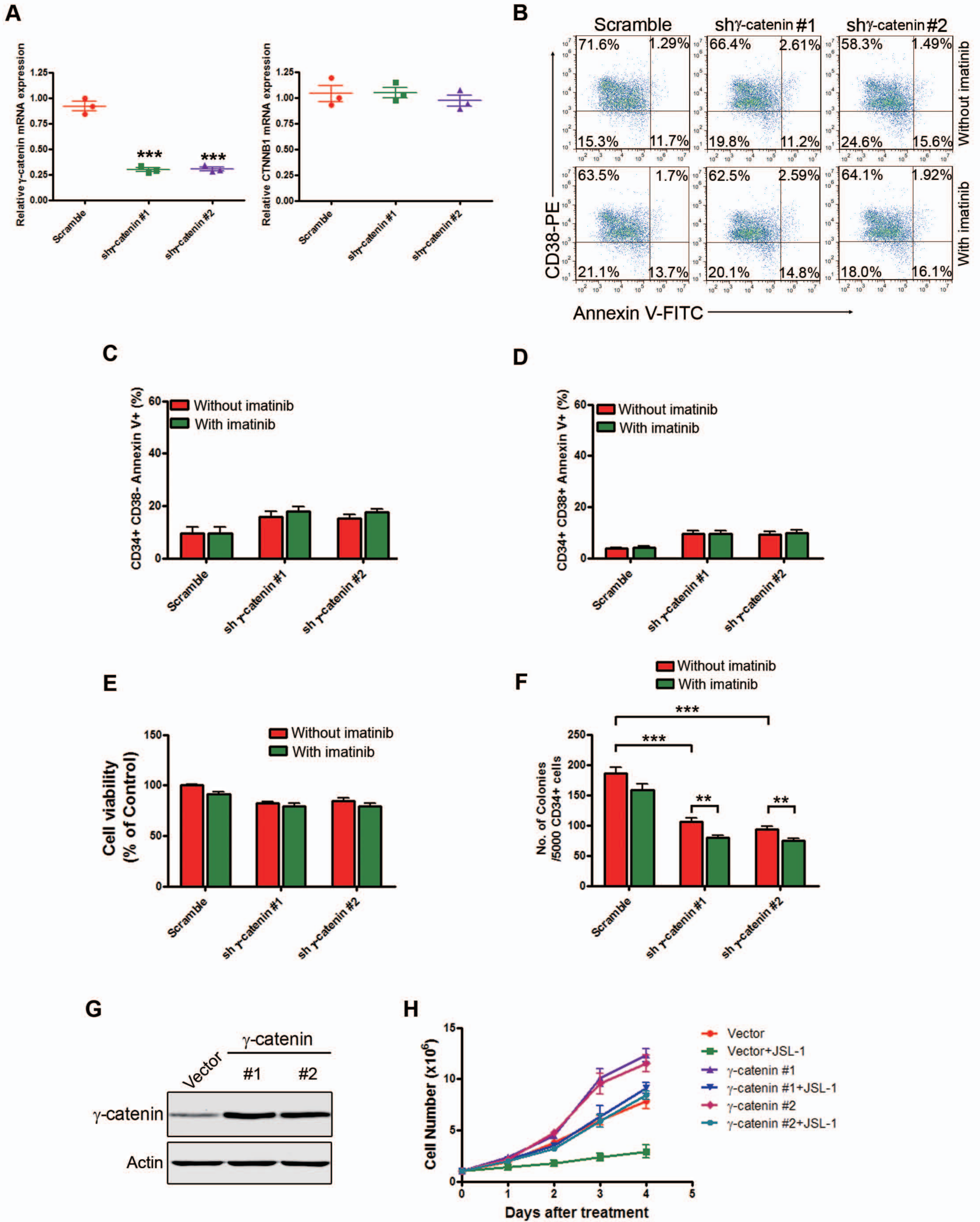

Figure S4

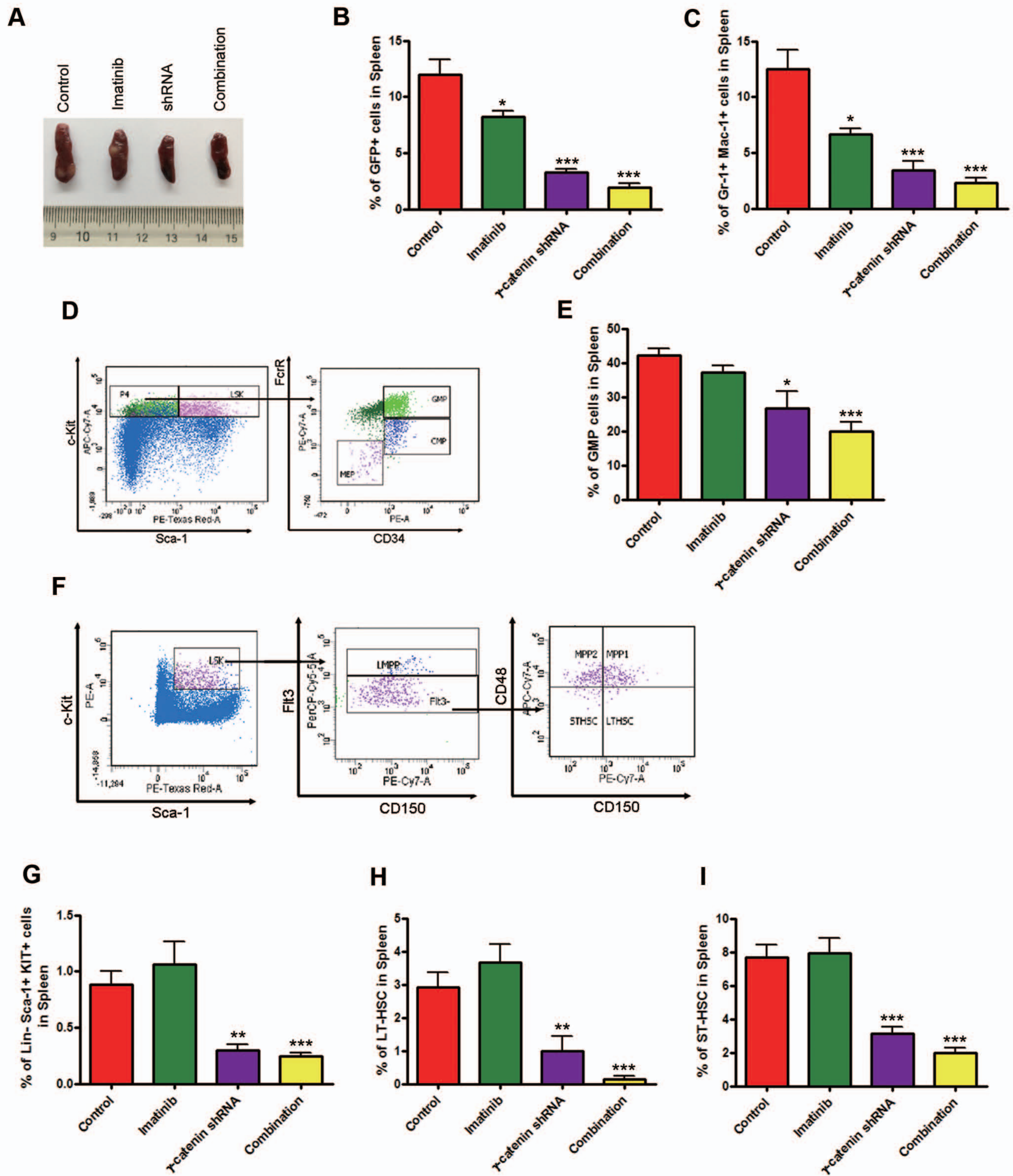

Figure S5

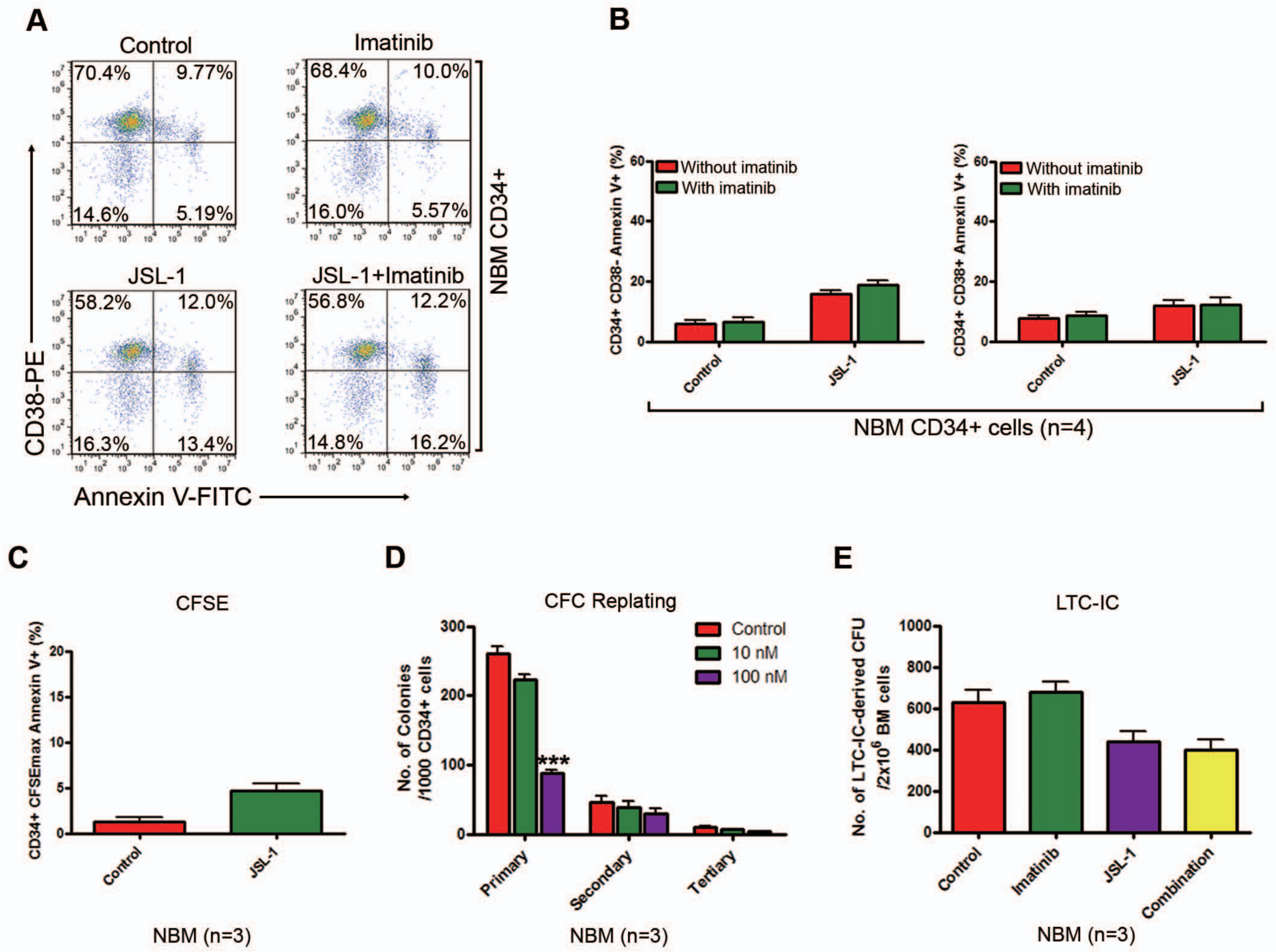

Figure S6