Table S1

Inverse PCR primers for STAT5 site deletion	
STAT5A site	(F) 5'- pGTTCATAAACTTCACGTGTCAA-3'
	(R) 5'-pATGTGATCAATATTATTGTTAGTAT-3'
STAT5B site	(F) 5'- pGGTGCAGAATGTGTAGGTTTG-3'
	(R) 5'-pAAATTAAAAAAAAAATCTTTGAAGC-3'
Primers for ChIP PCR analysis	
STAT5A binding site	(F) 5'- GCATCTCTGACCTCTCAGCA-3'
	(R) 5'- CAGAAACAAAATTCCCAGCTTT-3'
STAT5B binding site	(F) 5'-GGGATTGGTATGAAGGAACG-3'
	(R) 5'-AGCGAAACTCCGTCTCAAAA-3'
GAPDH control	(F) 5'- TCTGGGGACTAGGGGAAGGA-3'
	(R) 5'- CCGCAAGGAGAGCTCAAGGT-3'
Oligo sequences for shRNA	
SCR (scrambled)	5'-GCGCGCTTTGTAGGATTCG-3'
shSIRT1-3	5'- GAAGTGCCTCAGATATTAA-3'
shKU70-1	5'- GGGAAAGTTACCAAGAGAA-3'
shKU70-2	5'- GAAGATGCCCTTTACTGAA-3'
shSTAT5-1	5'- GTACTTCATCATCCAGTAC-3'
shSTAT5-2	5'- GGAGAACCTCGTGTTCCTG-3'

Figure S1. SIRT1 gene expression in cell lines

Cells were transduced with BCR-ABL vector MIG210 (p210) or empty vector MIG R1 (R1) for 7 days, and harvested for protein analysis.

Figure S2. Tyrosine kinase activity in KCL-22 and KCL-22M cells after imatinib treatment

Cells were treated with indicated concentrations of imatinib for 48 hours, and analyzed for tyrosine phosphorylation by Western blot.

Figure S3. Regulation of SIRT1 gene expression

(A) Expression of exogenously introduced SIRT1 was not affected by BCR-ABL knockdown. KCL-22 and K562 cells were stably transduced with empty or SIRT1 expressing vector, followed by mock (SCR) or BCR-ABL knock down (shABL). **(B)** Analysis of SIRT1 mRNA stability. Twelve hours after imatinib treatment, actinomycin D was added and cells were harvested at times indicated following actinomycin D addition. RNA was analyzed by RT-PCR.

Figure S4. STAT5 binding sites on SIRT1 promoter

STAT5 binding sites on SIRT1 promoter between -1484 and -2852 bp. Putative STAT5 binding sites were identified by rVista program (Loots, G.G. & Ovcharenko, I. *Nucleic Acids Res* 2004, **32**: W217-221), and no other STAT members were found in this region. Relative positions for STAT5 and other known transcriptional factor binding sites are shown at the bottom panel.

Figure S5. Effects of BCR-ABL expression levels on SIRT1 activation in CD34$^+$ cells

(A) A representative plot showing flow cytometry sorting of p210 transduced CD34$^+$ cells. High and low GFP expressing cells were sorted co rresponding to high and lo w BCR-ABL expressing cells, respectively. **(B)** Effects of BCR-ABL ex pression levels on several known S TAT5 target genes were analyzed by RT-PCR.

Figure S6. Effects of sirtinol treatment on cell cycle of CML cell lines

KCL-22 (A) and K-562 (B) cells were treated with conditions as indicated for two days and cells were labeled with propidium iodine (PI) for cell cycle analysis.

Figure S7. Effect of graded SIRT1 knockdown on KCL22 cells

(A) SIRT1 expression level after graded SIRT1 knockdown for 3 days. KCL22 cells were transduced with scrambled shRNA (SCR) or shSIRT1-3 at different MOI of 0.5, 1, 2.5, 5, 10 and 20. **(B,C)** A substantial level of SIRT1 knockdown was needed to induce apoptosis. Three day s after transduction, the cells were m ock treated (B) or treated with 2.5 μM IM (C) f or another 3 days and apoptosis was measured by Annexin-V staining and FACS analysis. MOI of 5 was used in all transduction experiment in the main text unless specified otherwise.

Figure S8. SIRT1 over-expression protects CML cells from oxidative stress but does not rescue apoptosis induced by BCR-ABL inhibition

(A) SIRT1 over-expression and im atinib treatment. KCL-22 and K562 cells were stably transduced with SIRT1 (KCL-22SIRT1, K562 SIRT1) over-expressing or em pty (KCL-22puro,

K562puro) retroviral vectors. Levels of SIRT1 over-expression were shown in Supplem ental Figure 1b. Transduced cells were then treated with indicated concentrations of imatinib (IM) for 48h. Apoptosis was analyzed by flow cyto metry and the percentage of annexin V^+ cells was plotted. **(B)** SIRT1 over-expression and BCR-ABL knockdown. BCR-ABL was knocked down by shABL lentiviral vector in control (puro vect or) or SIRT1 over-expressing cells, shown in A. Apoptosis was analyzed at day 4 and 5 after shA BL transduction. **(C)** SIRT1 over-expression protected CML cells from oxidative stress. Control or SIRT1 ove r-expressing CML cells were treated with H_2O_2 for 22h, and analyzed for apoptosis by flow cytometry.

Figure S9. Effects of SIRT1 knockout on functions of mouse hematopoietic progenitor cells and lineage differentiation

(A) Colony for mation assay. Bone m arrow cells fr om three-month old wild type (WT) and SIRT1 knockout (KO) mice were plated with methylcellulose medium and colonies were scored after two weeks. **(B, C)** Analysis of hematopoietic stem cell frequency and cell cycle. Hematopoietic stem cells were iden tified by SLAM m arkers using $Lin^-CD150^+CD41^-CD48^-$ combination in BABL/c strain (B). Hematopoietic stem cells were further analyzed for cell cycle status using Hoechst 33342 and pyronin Y staining (C). n=3 each genotype. **(D)** Comparison of blood lineage cells in wild type and knockout mice. n=3 each genotype.

Figure S10. Effects of SIRT1 knockout in transplantation recipients

(A,B) Similar transduction and engraftm ent efficiency was observed for $SIRT1^{+/+}$ and $SIRT1^{-/-}$ bone marrow cells transduced by the MIG R1 vector. Peripher al blood mononuclear cells were analyzed for GFP expression 30 days (A) and 60 days (B) after transplantation. **(C)** Blood cell

counts of recipients mice two months after transplantation of wild type or knockout bone marrow cells (2.5 million each) without gene transduction.

Figure S11. Effects of SIRT1 knockout on CML progression

(A) Survival curves for mice receiving $1.0x10^5$ mononuclear cells transduced by BCR-ABL MIG210 vector (p210) or empty vector (R1). The experiment was performed as described in Figure 6A. **(B)** Comparison of blood smear of mice receiving BCR-ABL transduced wild type or SIRT1 knockout cells.

Figure S12. Effect of SIRT1 knockout on FOXO1 acetylation of mouse bone marrow progenitor cells

$Lin^{lo/-}$ bone marrow progenitor cells were enriched by depleting the lineage positive cells with lineage antibody cocktail-conjugated magnetic beads using EasySep. Cells were fixed by para-formaldehyde for 10 min, and stained with APC-labeled anti-CD150 antibody. After permeabilization with Triton X-100, cells were stained with acetylated-FOXO1 antibody followed by Alexa 680-conjugated second antibody, and analyzed by FACS. Individual labeling panels and overlay for comparison were shown in A and B respectively.

Figure S13. Representative sorting schemes for LSK cell from Taconic BALB/c bone marrow cells

The top four plots showed unlabeled cells, isotype control labelling, and single label of Sca-1 or c-Kit in bone marrow mononuclear cells. The bottom two plots showed Sca-1 and c-Kit labeling in Lin^- gated cells: normal (left) and BCR-ABL transduced GFP^+ cells (right). The rate of $Sca\text{-}1^+$

cells (about 2%) in Lin^- population in this study was higher than 0.29% previously reported for Jackson BALB/c strain (Spangrude, G.J. and Brooks D. Blood, 1993: 82:3327-3332). However, Taconic BALB/c strain is known to have higher level of $Sca\text{-}1^+$ cells (Jurecic, R. et al. Blood, 1993: 82: 2673-2683).

Figure S14. SIRT1 expression in KRAS transformation

(A) KRAS transduction of mouse bone marrow cells increased SIRT1 expression. Mouse bone marrow cells were transduced with two rounds of infection with KRAS retroviral vector. Mononuclear cells were then harvested for analysis. Background SIRT1 level was elevated in these cultured mouse bone marrow cells likely due to the use of growth factor cocktails including IL3 and SCF that have been shown to increase STAT5 phopsorylation (Yamada, Y., *et al. Blood* 2008; **112**, 2500-2507 and Jiang, X., et al. *Proc Natl Acad Sci U S A* 1999; **96**, 12804-12809). Constitutive KRAS expression further increased SIRT1 level. **(B)** KRAS transduction did not increase SIRT1 expression in NIH-3T3 cells. NIH- 3T3 cells were transduced with empty vector (neo) or KRAS expressing vector, and selected for neomycin resistance. After recovery in drug free medium, cells were then analyzed for SIRT1 expression (Left panel) and plated for soft agar colony formation (Right panel). SIRT1 level remained unchanged in spite of effective transformation.

Figure S15. Effects of tenovin-6 and imatinib treatment on physiological indexes

Normal BALB/c mice were subjected to the same 10 day treatment with vehicle, imatinib, tenovin-6 or combination as described in Figure 7A and B. Body weight (A) was monitored during drug treatment, and total leukocyte and erythrocyte counts (B) were analyzed at day 16.

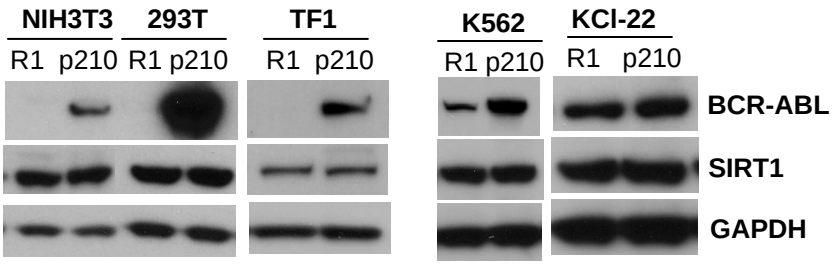
NIH3T3
293T
TF1
K562
KCl-22
R1 p210 R1 p210 R1 p210 R1 p210 R1 p210
BCR-ABL
SIRT1
GAPDH

Figure S1

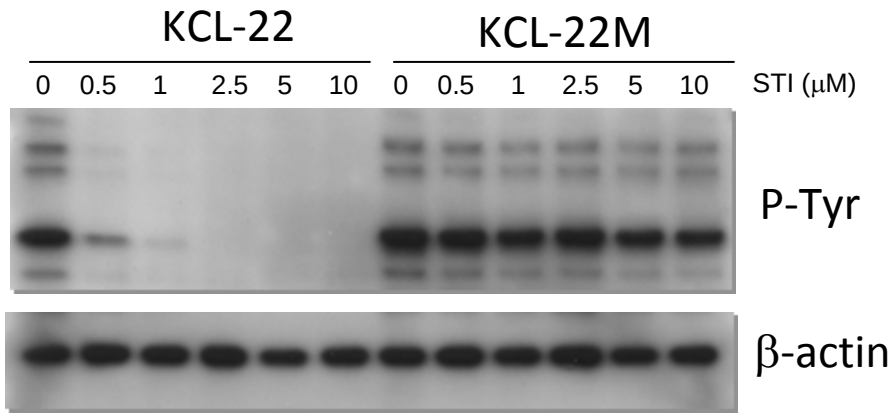

Figure S2

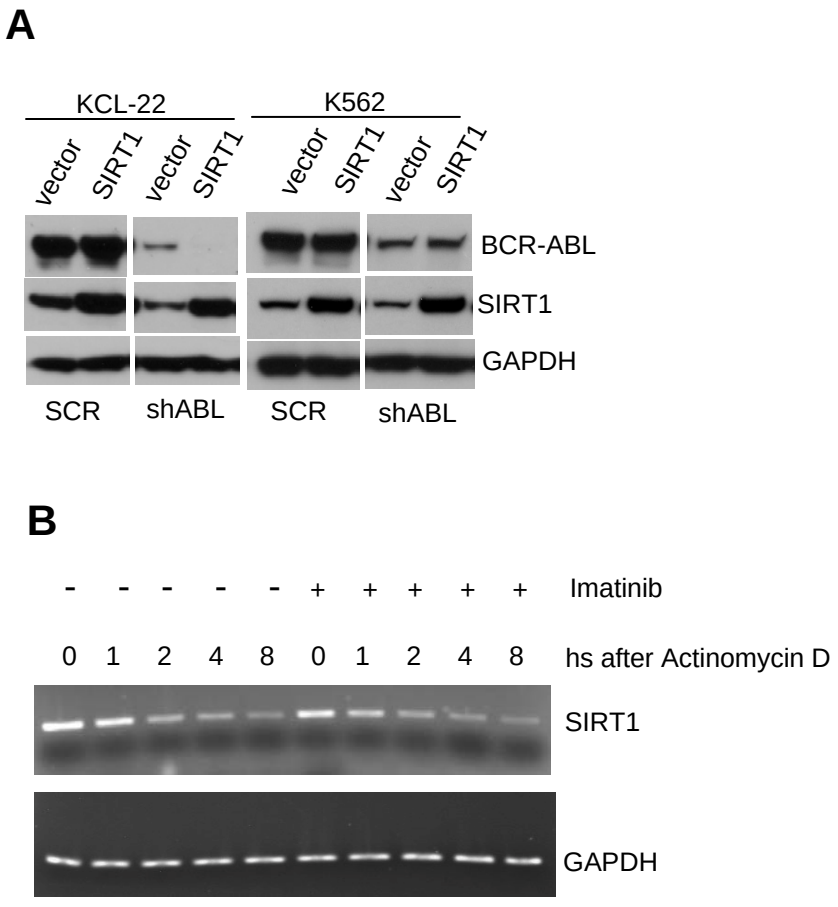
A
KCL-22
K562
vector
SIRT1
vector
SIRT1
vector
SIRT1
vector
SIRT1
BCR-ABL
SIRT1
GAPDH
SCR
shABL
SCR
shABL
B
- - - - - + + + + +
Imatinib
0 1 2 4 8 0 1 2 4 8
hs after Actinomycin D
SIRT1
GAPDH

Figure S3


multiTF :: TFBS in the alignment
STAT5A -2235
hg19_xenoRefGen CATCTCTGACCTCTCAGCATACTAACAATAATATTGATCACATAGCATTTCTTGTAGTTC
hg19_refGene_NM CATCTCTGACCTCTCAGCATACTAACAATAATATTGATCACATAGCATTTCTTGTAGTTC 2760
hg19_xenoRefGen ATAAACTTCACGTGTCAATAATATACTATAAATATAAATGAATTAATGAACAACAGGATG
hg19_refGene_NM ATAAACTTCACGTGTCAATAATATACTATAAATATAAATGAATTAATGAACAACAGGATG 2820
hg19_xenoRefGen CTCATAAGCTTACAGACATCTTTTTCTCAAAAAAGCTGGGAATTTTGTTTCTGTTTTATT
hg19_refGene_NM CTCATAAGCTTACAGACATCTTTTTCTCAAAAAAGCTGGGAATTTTGTTTCTGTTTTATT 2880
hg19_xenoRefGen GGGATACTGACTCTCAACATTTCATATATTGCATTCCACCAACGTAGCTGAGAGTCAATT
hg19_refGene_NM GGGATACTGACTCTCAACATTTCATATATTGCATTCCACCAACGTAGCTGAGAGTCAATT 2940
hg19_xenoRefGen TATGAAATATTTTGTAGTGTAAGACAGAAAGTGGGGAGGACCAAGTATGTCAACCACTAG
hg19_refGene_NM TATGAAATATTTTGTAGTGTAAGACAGAAAGTGGGGAGGACCAAGTATGTCAACCACTAG 3000
hg19_xenoRefGen GAGTGTGGTGCCTAGTCAGGAATTGGGAGGAGTGTAGCAAGAAAGGAAGGACAACAGGAT
hg19_refGene_NM GAGTGTGGTGCCTAGTCAGGAATTGGGAGGAGTGTAGCAAGAAAGGAAGGACAACAGGAT 3060
hg19_xenoRefGen TTGGTCATTGATTGGTCAGATGGATTTCAGAGGGATTGGTATGAAGGAACGCTTCAAAGA
hg19_refGene_NM TTGGTCATTGATTGGTCAGATGGATTTCAGAGGGATTGGTATGAAGGAACGCTTCAAAGA 3120
STAT5B -1838
hg19_xenoRefGen TTTTTTTTTTAATTTAAGTTCCAGGATACAGGTGCAGAATGTGTAGGTTTGTTACATACT
hg19_refGene_NM TTTTTTTTTTAATTTAAGTTCCAGGATACAGGTGCAGAATGTGTAGGTTTGTTACATACT 3180
hg19_xenoRefGen TATAGGTGAGCCATGGTGGTTTGCTGCACCAATCAACCCCTCATCTAGGTTTTATTTATA
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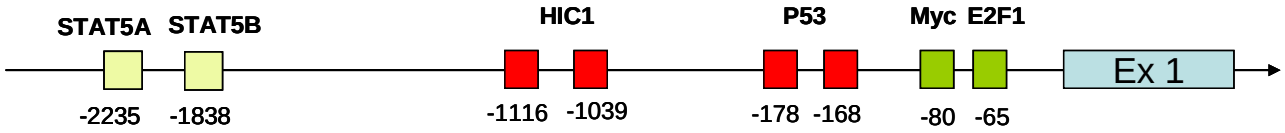
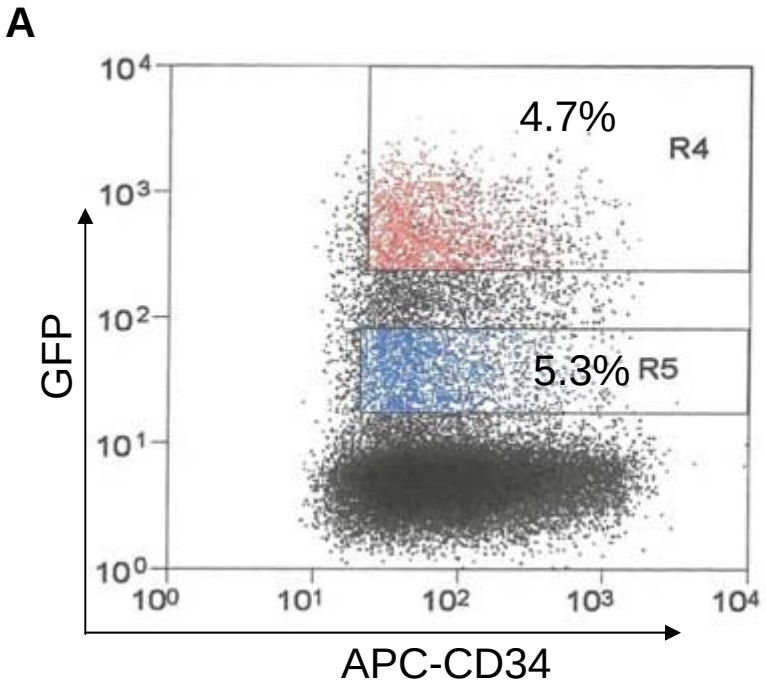

STAT5A
STAT5B
HIC1
P53
Myc
E2F1
Ex 1
-2235
-1838
-1116
-1039
-178
-168
-80
-65

Figure S4


A
4.7%
R4
5.3%
R5
GFP
APC-CD34
10^0
10^1
10^2
10^3
10^4

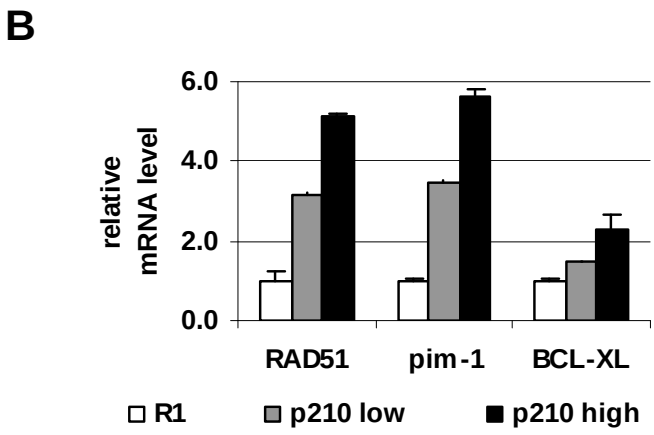

B
relative mRNA level
6.0
4.0
2.0
0.0
RAD51
pim-1
BCL-XL
R1
p210 low
p210 high

Figure S5

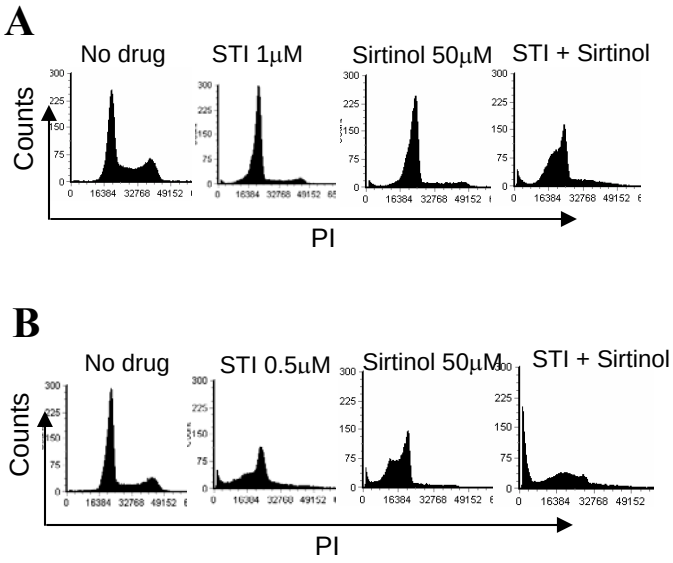

A
No drug
STI 1μM
Sirtinol 50μM
STI + Sirtinol
Counts
PI
B
No drug
STI 0.5μM
Sirtinol 50μM
STI + Sirtinol
Counts
PI

Figure S6

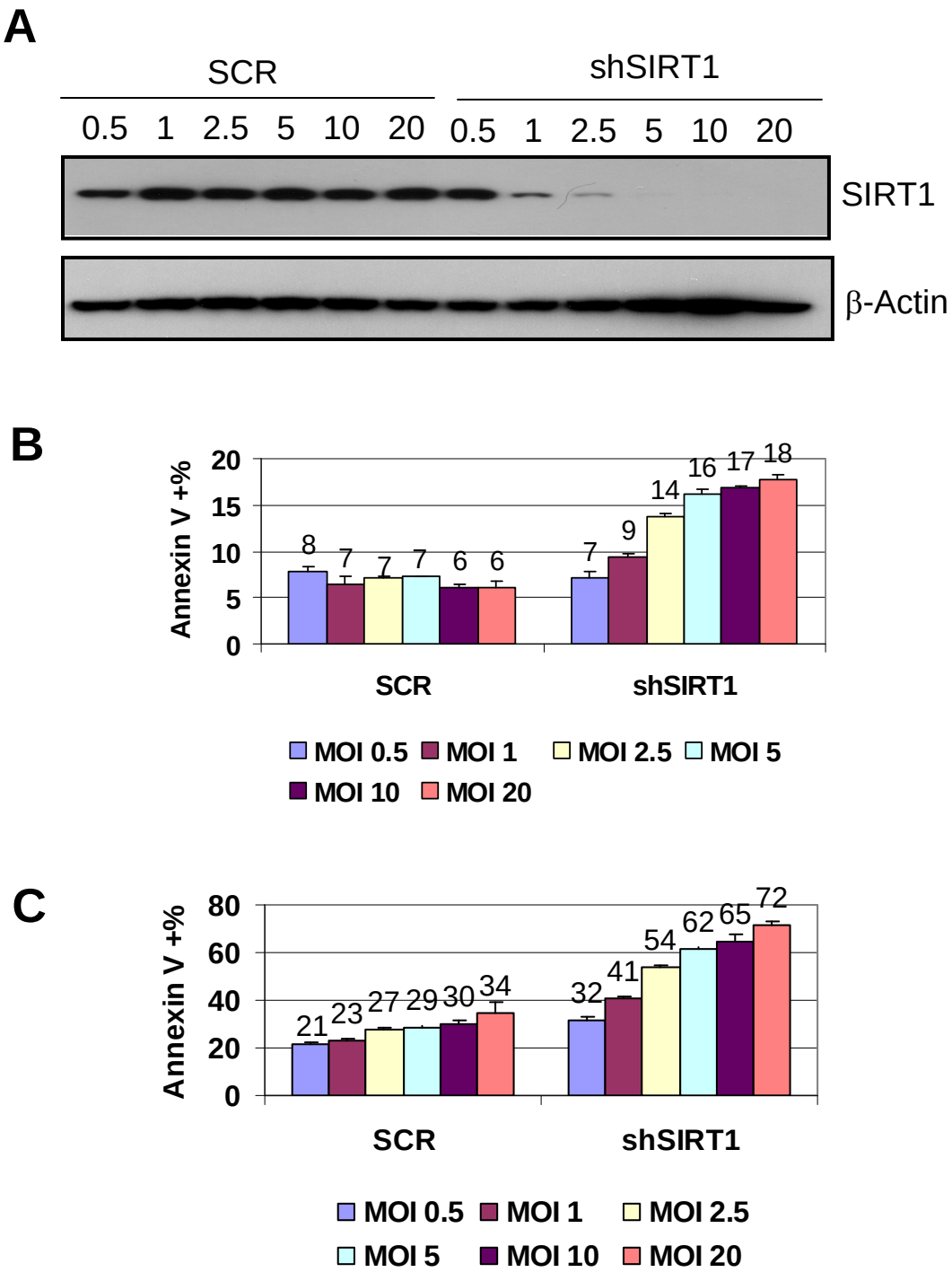
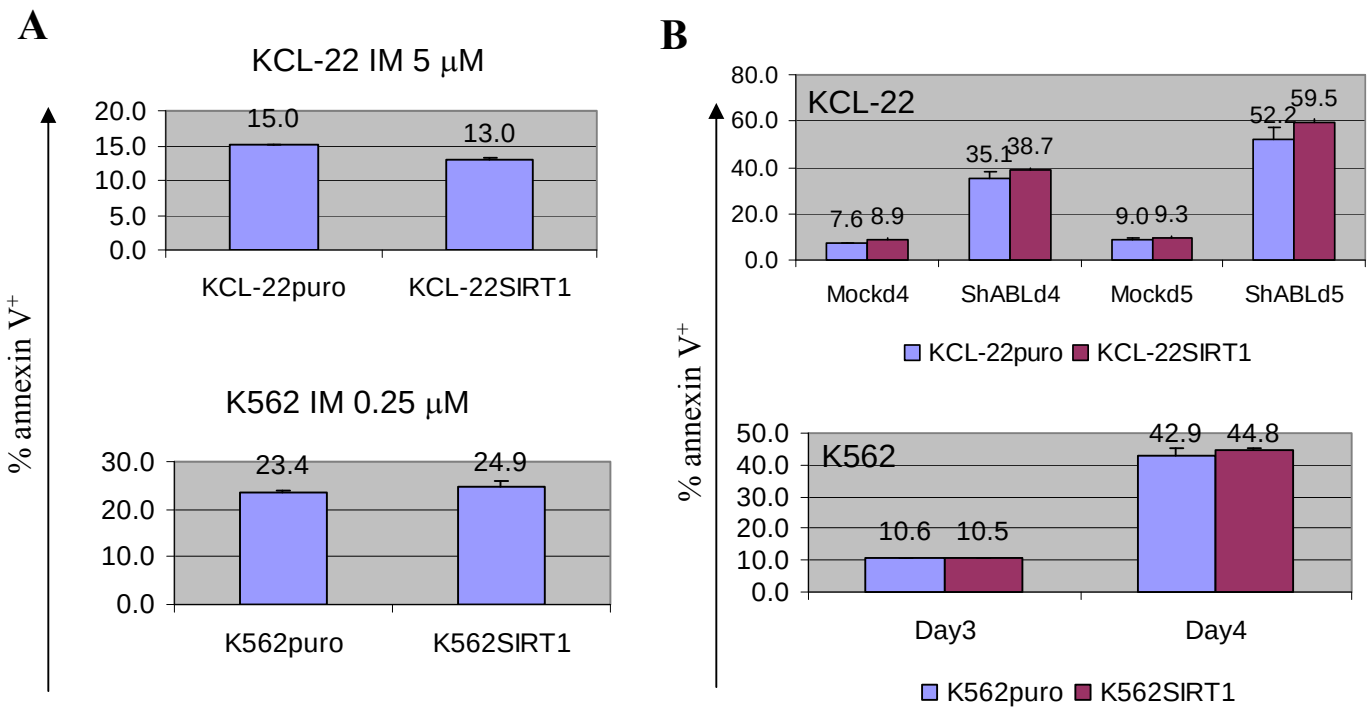

A
SCR
shSIRT1
0.5 1 2.5 5 10 20 0.5 1 2.5 5 10 20
SIRT1
β-Actin
B
Annexin V +%
20
15
10
5
0
8 7 7 7 6 6
7 9 14 16 17 18
SCR
shSIRT1
MOI 0.5 MOI 1 MOI 2.5 MOI 5
MOI 10 MOI 20
C
Annexin V +%
80
60
40
20
0
21 23 27 29 30 34
32 41 54 62 65 72
SCR
shSIRT1
MOI 0.5 MOI 1 MOI 2.5
MOI 5 MOI 10 MOI 20

Figure S7


A
KCL-22 IM 5 μM
% annexin V+
20.0
15.0
10.0
5.0
0.0
15.0
13.0
KCL-22puro
KCL-22SIRT1
K562 IM 0.25 μM
30.0
20.0
10.0
0.0
23.4
24.9
K562puro
K562SIRT1
B
KCL-22
80.0
60.0
40.0
20.0
0.0
7.6
8.9
35.1
38.7
9.0
9.3
52.2
59.5
Mockd4
ShABLd4
Mockd5
ShABLd5
KCL-22puro
KCL-22SIRT1
K562
50.0
40.0
30.0
20.0
10.0
0.0
10.6
10.5
42.9
44.8
Day3
Day4
K562puro
K562SIRT1

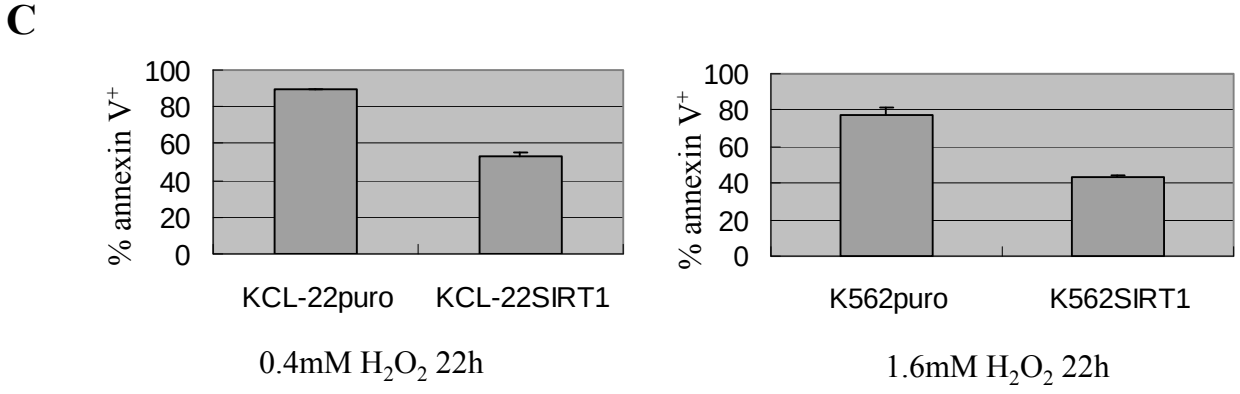

C
% annexin V+
100
80
60
40
20
0
KCL-22puro
KCL-22SIRT1
0.4mM H2O2 22h
K562puro
K562SIRT1
1.6mM H2O2 22h

Figure S8

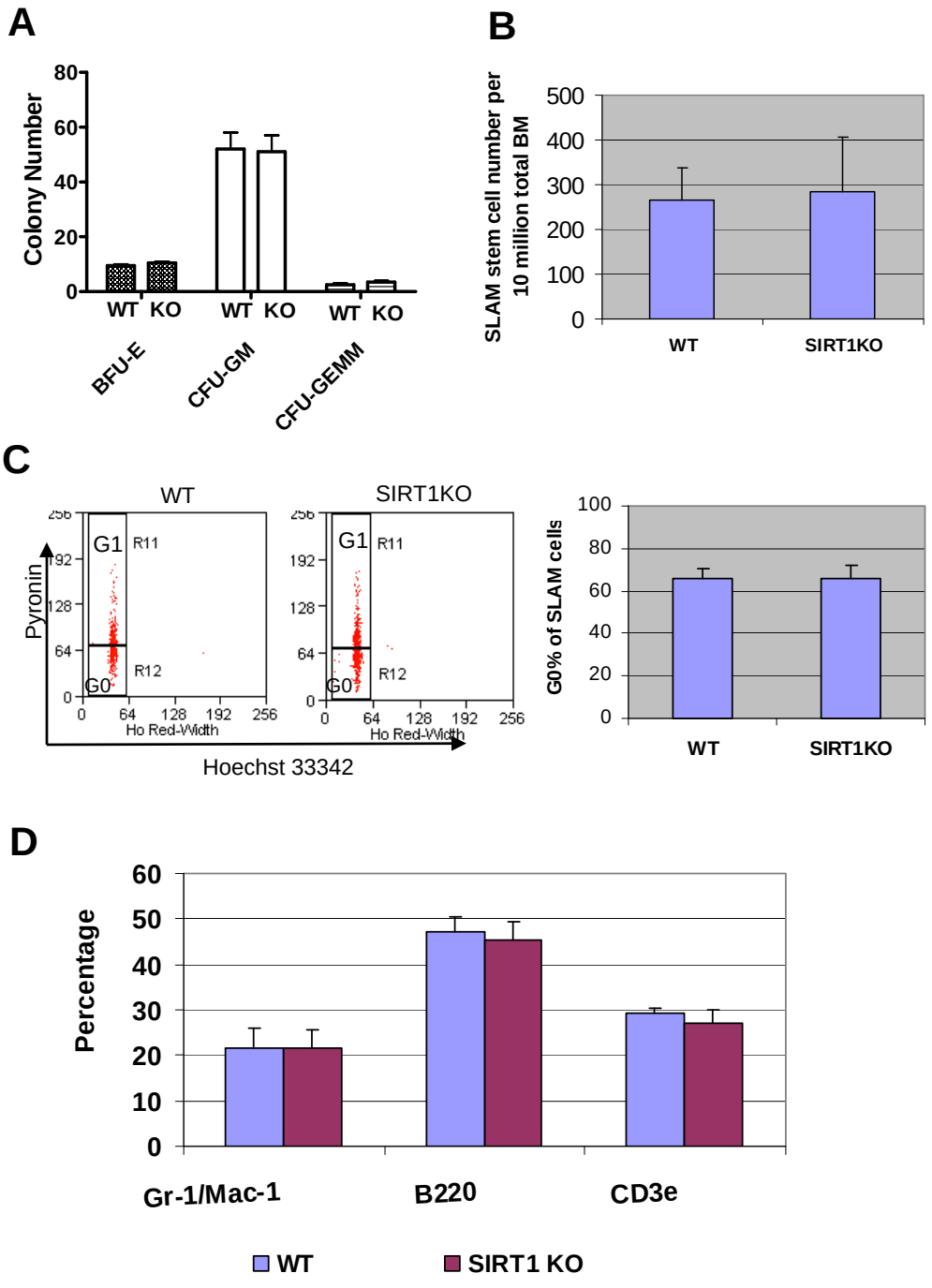

A
Colony Number
80
60
40
20
0
WT KO
WT KO
WT KO
BFU-E
CFU-GM
CFU-GEMM
B
SLAM stem cell number per 10 million total BM
500
400
300
200
100
0
WT
SIRT1KO
C
WT
SIRT1KO
Pyronin
G1
R11
R12
G0
256
192
128
64
0
0 64 128 192 256
Ho Red-Width
Hoechst 33342
G0% of SLAM cells
100
80
60
40
20
0
WT
SIRT1KO
D
Percentage
60
50
40
30
20
10
0
Gr-1/Mac-1
B220
CD3e
WT
SIRT1 KO

Figure S9

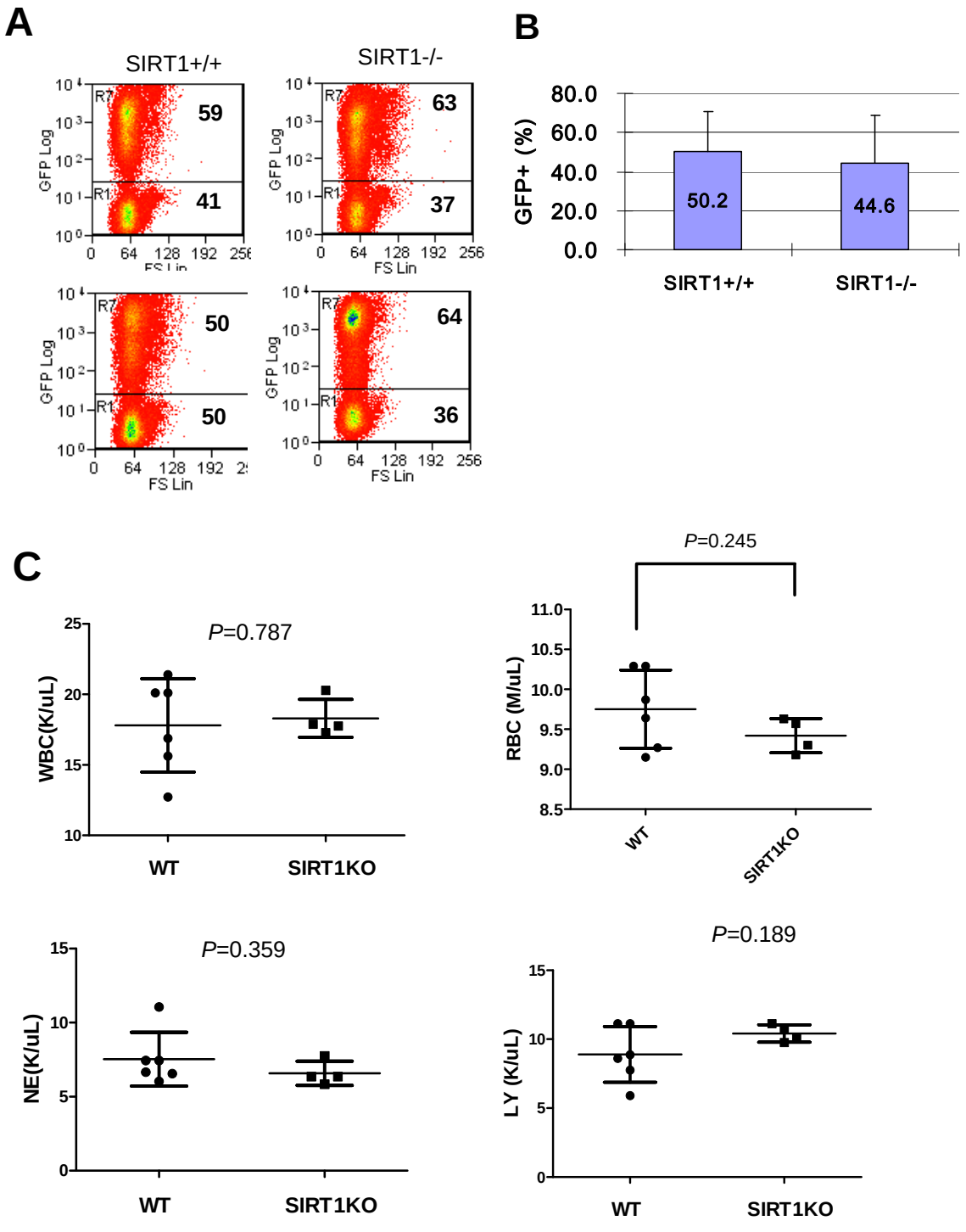

A
SIRT1+/+
SIRT1-/-
GFP Log
FS Lin
R7
R1
59
41
63
37
50
50
64
36
B
GFP+ (%)
80.0
60.0
40.0
20.0
0.0
50.2
44.6
SIRT1+/+
SIRT1-/-
C
P=0.787
WBC(K/uL)
WT
SIRT1KO
P=0.245
RBC (M/uL)
P=0.359
NE(K/uL)
P=0.189
LY (K/uL)

Figure S10

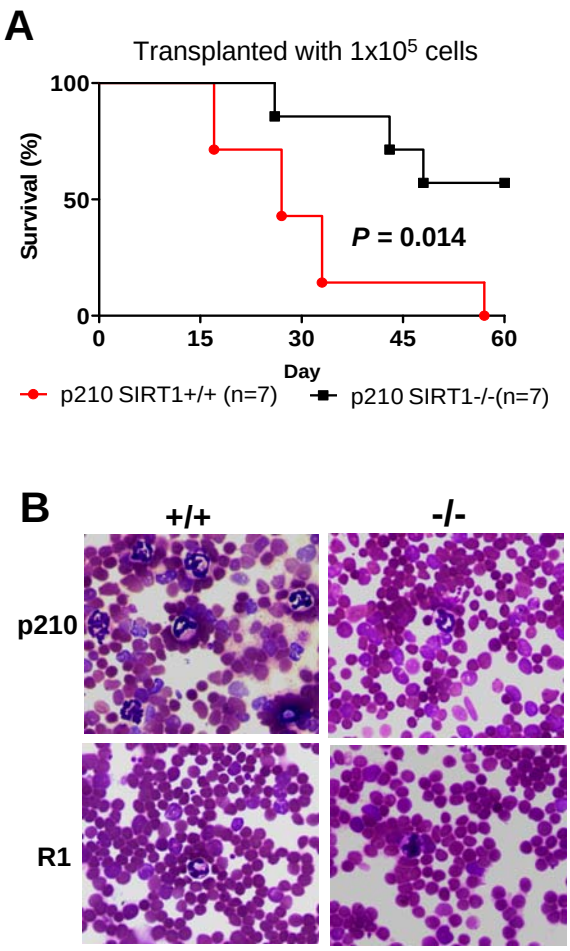
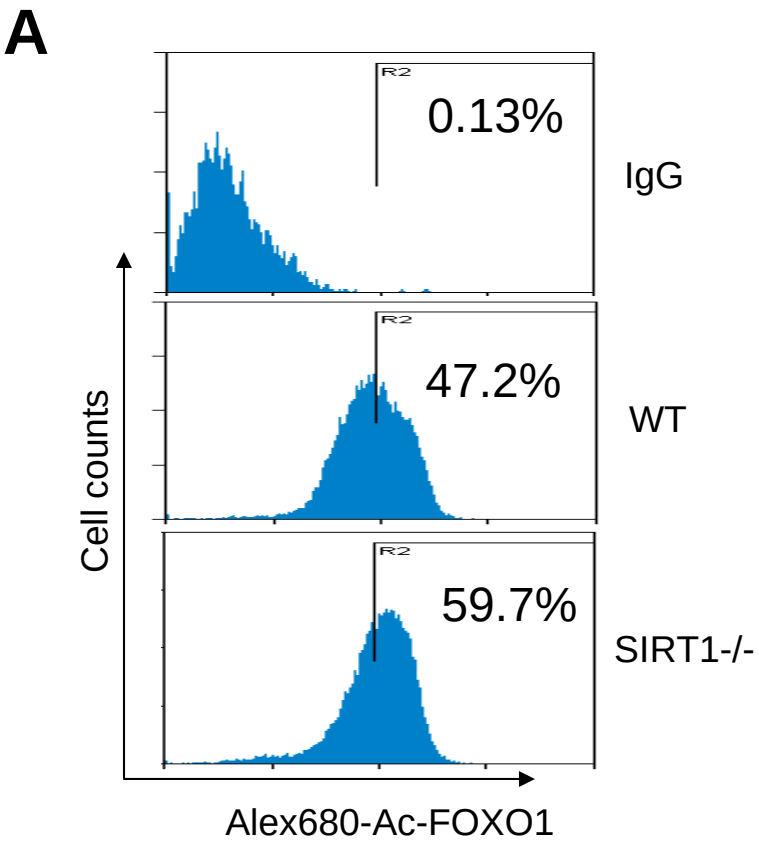

A
Transplanted with 1x10^5 cells
100
50
0
Survival (%)
P = 0.014
0
15
30
45
60
Day
p210 SIRT1+/+ (n=7)
p210 SIRT1-/-(n=7)
B
+/+
-/-
p210
R1

Figure S11


A
R2
0.13%
IgG
R2
47.2%
WT
R2
59.7%
SIRT1-/-
Cell counts
Alex680-Ac-FOXO1

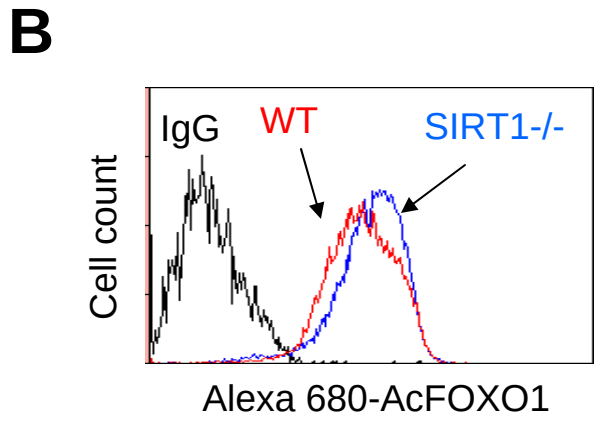

B
IgG
WT
SIRT1-/-
Cell count
Alexa 680-AcFOXO1

Figure S12

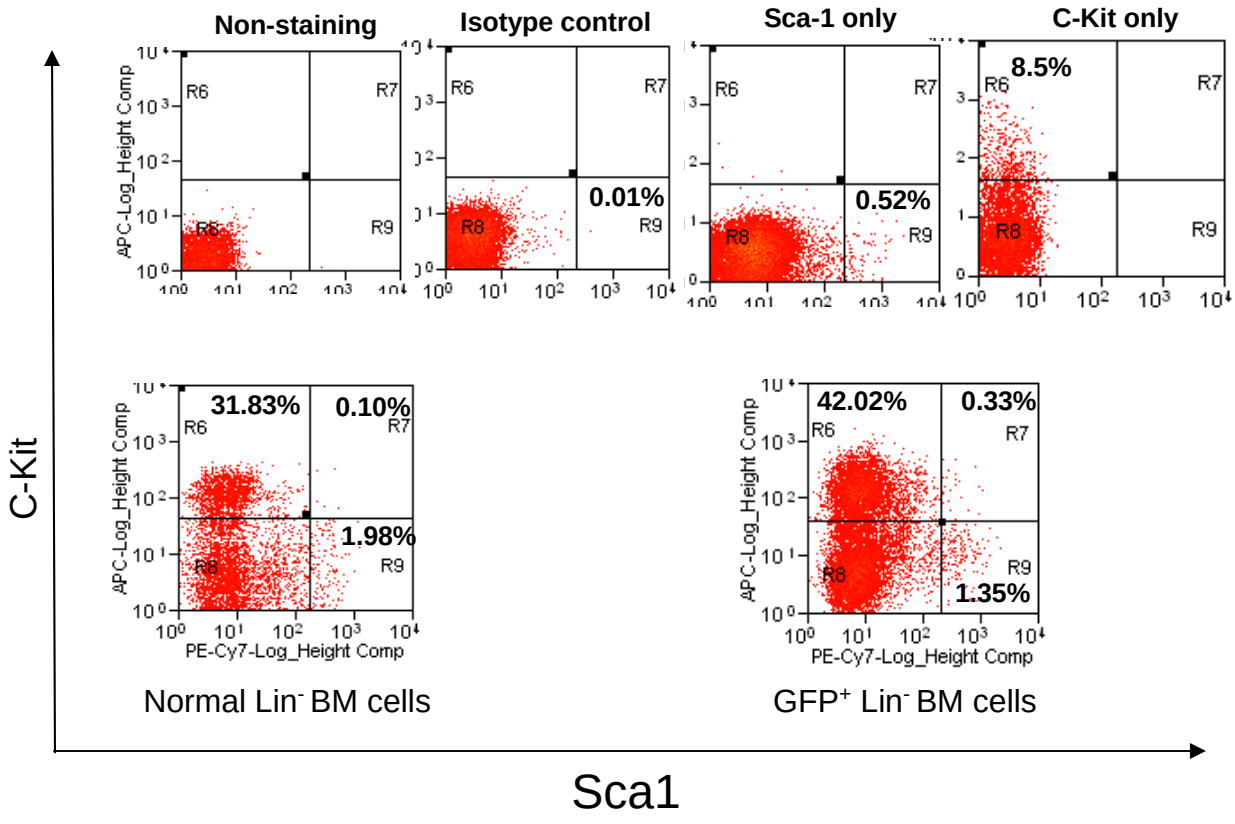

Non-staining
Isotype control
Sca-1 only
C-Kit only
R6
R7
R8
R9
APC-Log_Height Comp
PE-Cy7-Log_Height Comp
0.01%
0.52%
8.5%
31.83%
0.10%
1.98%
42.02%
0.33%
1.35%
C-Kit
Sca1
Normal Lin- BM cells
GFP+ Lin- BM cells

Figure S13

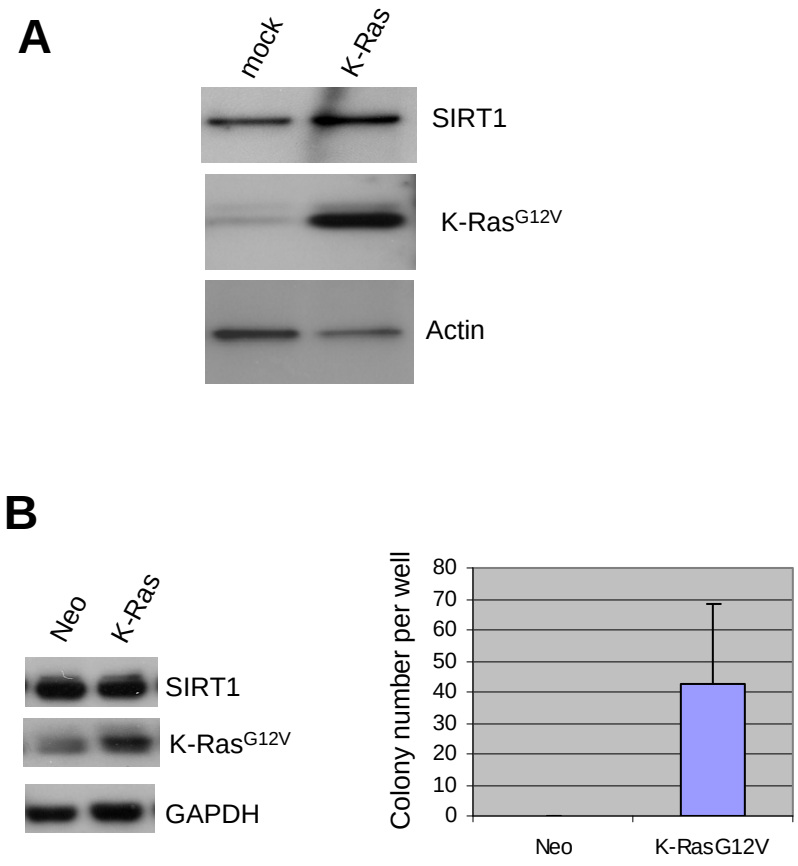

A
mock
K-Ras
SIRT1
K-RasG12V
Actin
B
Neo
K-Ras
SIRT1
K-RasG12V
GAPDH
Colony number per well
80
70
60
50
40
30
20
10
0
Neo
K-RasG12V

Figure S14

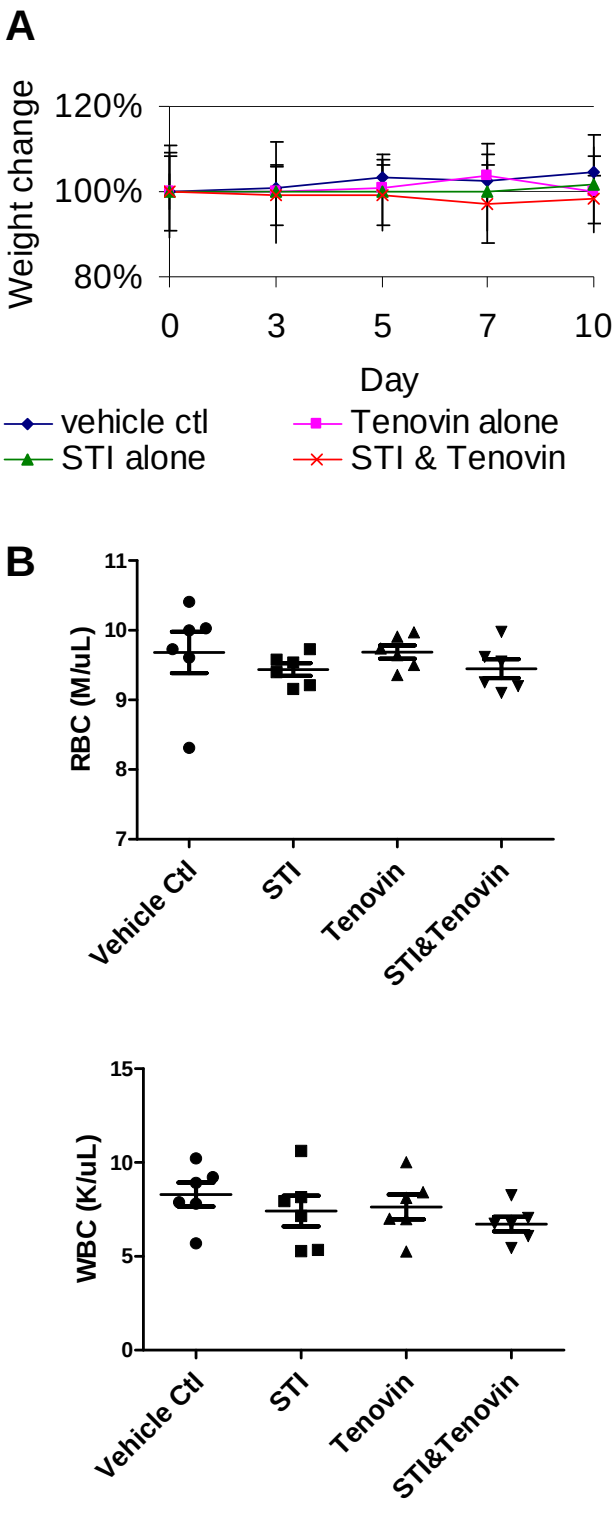

A
Weight change
120%
100%
80%
0
3
5
7
10
Day
vehicle ctl
Tenovin alone
STI alone
STI & Tenovin
B
RBC (M/uL)
11
10
9
8
7
Vehicle Ctl
STI
Tenovin
STI&Tenovin
WBC (K/uL)
15
10
5
0
Vehicle Ctl
STI
Tenovin
STI&Tenovin

Figure S15