Supplemental Data

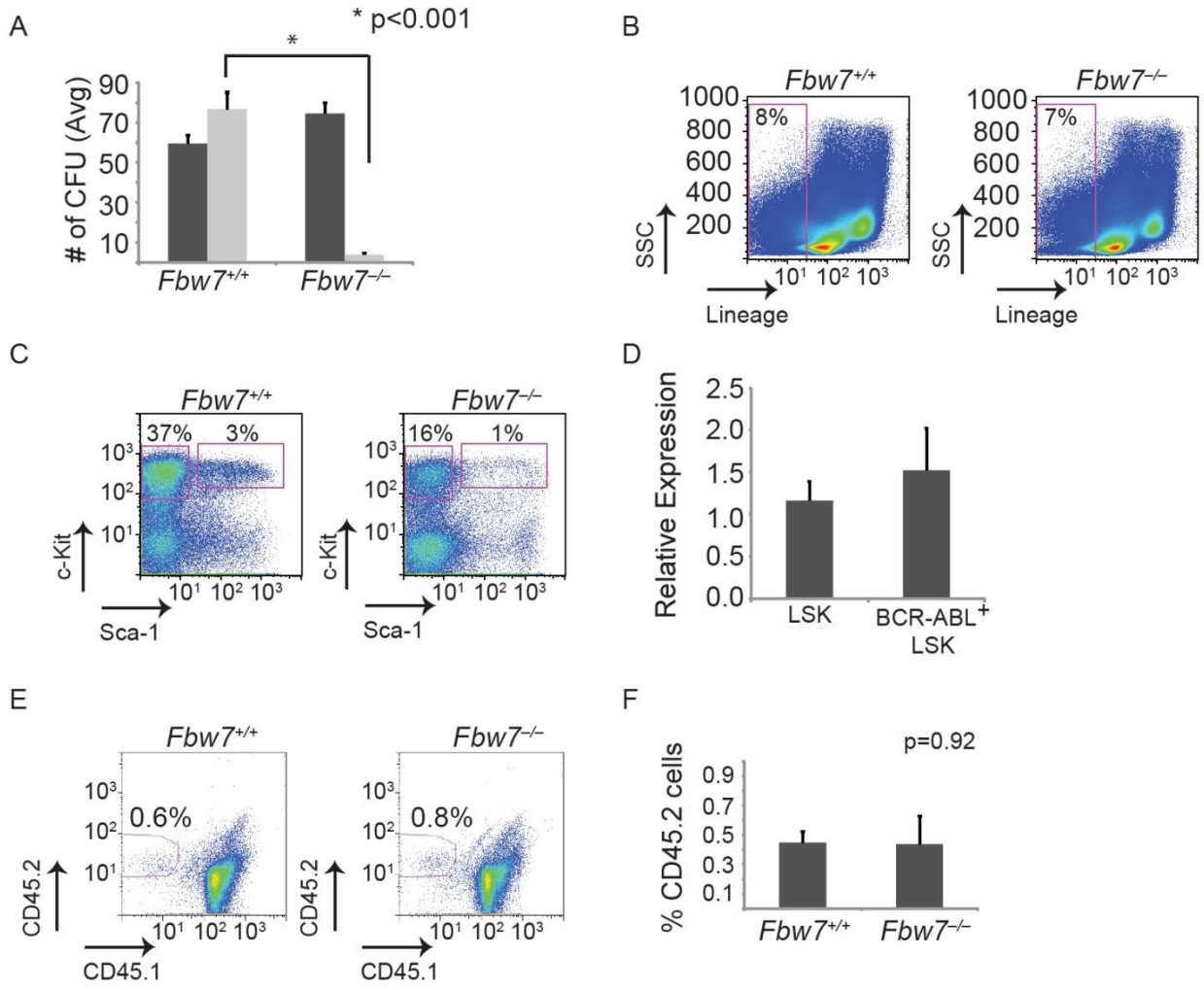

Figure S1, related to Figure 1. *Fbw7* deletion results in progressive loss of stem and progenitor cell re-populating capacity. (A) Average colony-forming units from sort purified CD150^{+} LSKs sorted from either *VavCre*$^{+}$;*Fbw7*$^{+/+}$ or *VavCre*$^{+}$;*Fbw7*$^{-/-}$ mice. (B) FACS analysis showing percentage of Lin^{-} cells from the bone marrow of *VavCre*$^{+}$;*Fbw7*$^{+/+}$ or *VavCre*$^{+}$;*Fbw7*$^{-/-}$ mice. (C) FACS analysis gated on Lin^{-} and stained with c-Kit and Sca-1 from 2 week old *VavCre*$^{+}$;*Fbw7*$^{+/+}$ or *VavCre*+;*Fbw7*$^{-/-}$ mice. n=3 mice for each genotype. (D) QRT-PCR analysis of *Fbw7* expression in sorted LSK from WT mice or Bcr-Abl^{+} LSK. (E-F) FACS plots (E) and graph (F) show percentage of CD45.2 (either *VavCre*$^{+}$;*Fbw7*$^{+/+}$ or *VavCre*$^{+}$;*Fbw7*$^{-/-}$) cells in the BM of irradiated CD45.1 mice 24 hours after transplantation. n=5 mice from each genotype. Error bars indicate +/-SD.

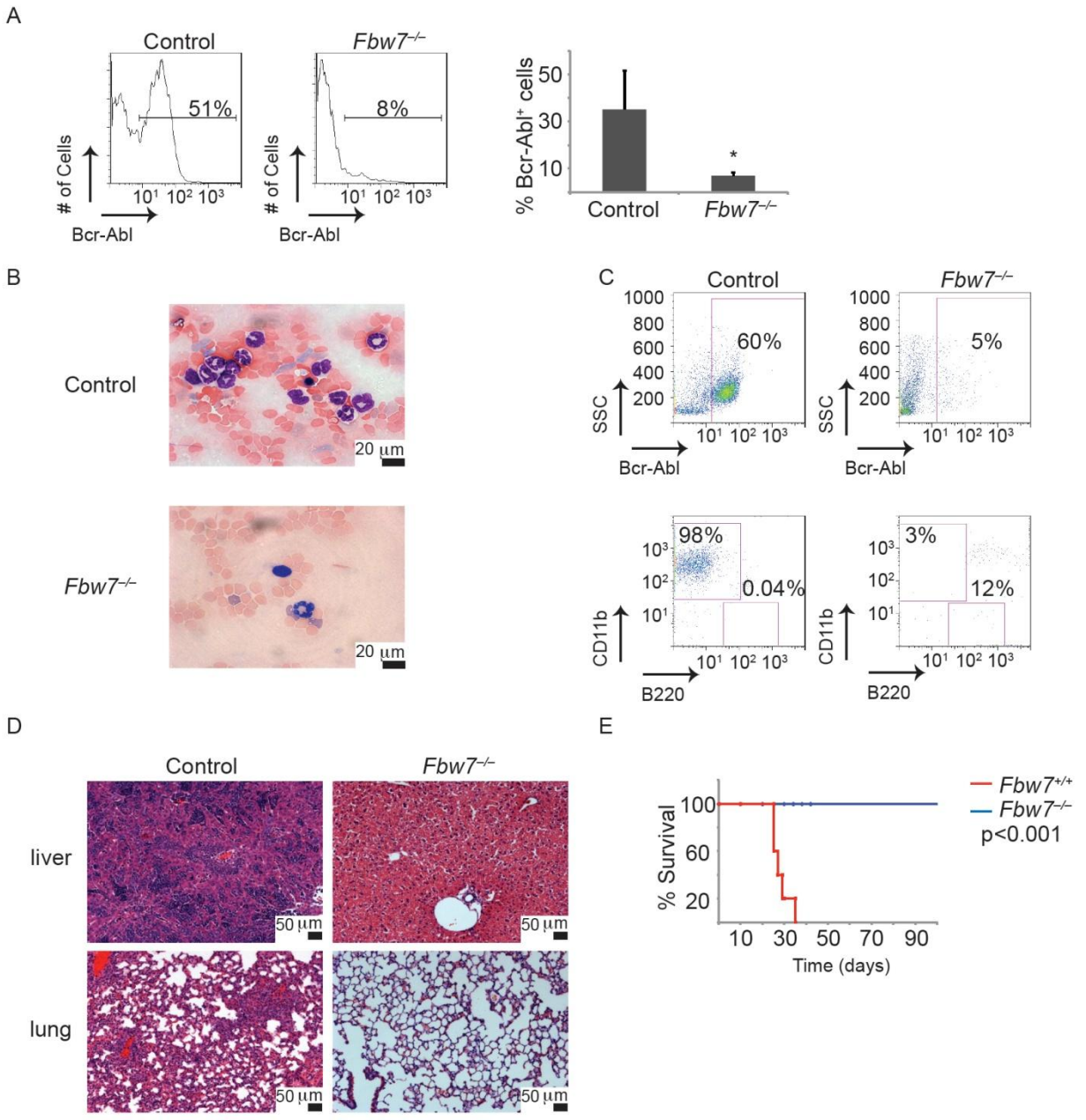

Figure S2, related to Figure 2. *Fbw7* deficiency affects LIC activity in a mouse model of CML. (A) FACS analysis and (B) peripheral blood smears taken from secondary recipient mice receiving equal numbers of Bcr-Abl$^+$ LSK cells from spleens of either *MxCre*$^+$*;Fbw7*$^{+/+}$ or *MxCre*$^+$*;Fbw7*$^{-/-}$ animals (n=4 for each genotype). (C) FACs analysis of peripheral blood from *MxCre*$^+$*;Fbw7*$^{+/+}$ and *MxCre*$^+$*;Fbw7*$^{-/-}$ secondary recipient mice showing the lineage markers CD11b$^+$ (myeloid) and B220$^+$ (B-lymphoid). (D) H&E staining of liver and lung in *MxCre*$^+$*;Fbw7*$^{+/+}$ and *MxCre*$^+$*;Fbw7*$^{-/-}$ secondary recipient animals. (E) Kaplan Meier survival curve of secondary recipient mice that received Bcr-Abl$^+$ *MxCre*$^+$*;Fbw7*$^{+/+}$ or Bcr-Abl$^+$ *MxCre*$^+$*;Fbw7*$^{-/-}$ (normalized to LSK number) cells from the spleen of primary recipient animals. Error bars indicate +/-SD.

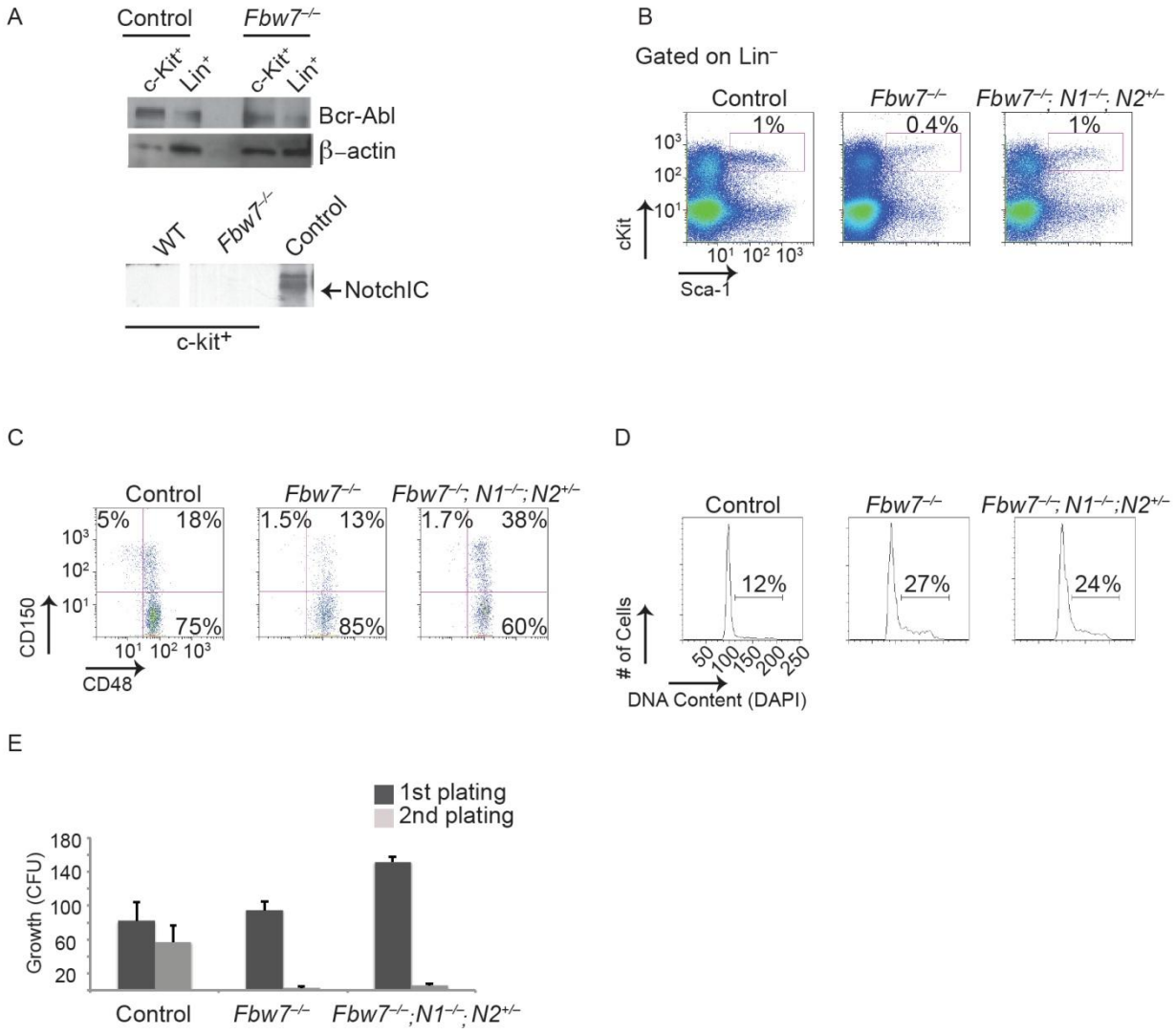

Figure S3, related to Figure 3. Notch stability does not contribute to Fbw7 induced HSC defects. (A) Western blot analysis using anti-Bcr, anti-cleaved-Notch-1 and anti-βactin antibodies in WT and *Fbw7*$^{-/-}$ Bcr-Abl^{+} total c-Kit cells. (B) FACS analysis of BM derived LSKs and (C) frequency of CD150 and CD48 populations in LSKs of Control, *MxCre*$^{+}$*;Fbw7*$^{-/-}$ and *MxCre*$^{+}$*;Fbw7*$^{-/-}$*;Notch1*$^{-/-}$*;Notch2*$^{+/-}$ mice two weeks after the last polyI-polyC treatment. (D) Cell cycle status of LSK cells in Control, *MxCre*$^{+}$*;Fbw7*$^{-/-}$ and *MxCre*$^{+}$*;Fbw7*$^{-/-}$*;Notch1*$^{-/-}$*;Notch2*$^{+/-}$ mice with DAPI assessed by FACS analysis. (E) Average colony number in CFU assay is shown in sorted CD150^{+} LSKs. Error bars indicate +/-SD of n=4 mice

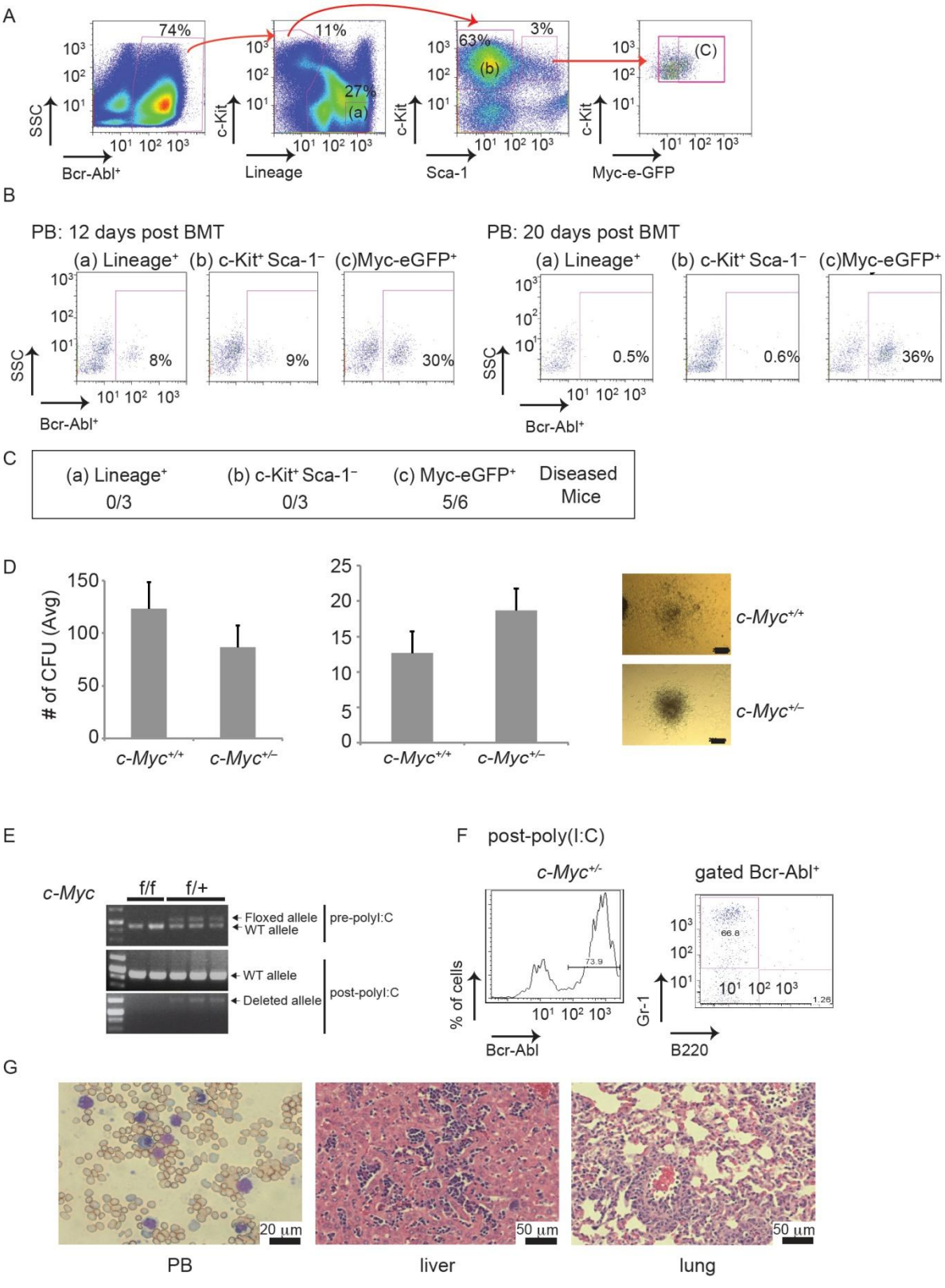
A
74%
11%
27%
(a)
63%
(b)
3%
(C)
SSC
c-Kit
Bcr-Abl+
Lineage
Sca-1
Myc-e-GFP
B
PB: 12 days post BMT
(a) Lineage+ (b) c-Kit+ Sca-1− (c)Myc-eGFP+
8%
9%
30%
PB: 20 days post BMT
0.5%
0.6%
36%
C
(a) Lineage+ 0/3
(b) c-Kit+ Sca-1− 0/3
(c) Myc-eGFP+ 5/6
Diseased Mice
D
# of CFU (Avg)
c-Myc+/+
c-Myc+/−
E
c-Myc
f/f
f/+
Floxed allele
WT allele
pre-polyI:C
WT allele
Deleted allele
post-polyI:C
F
post-poly(I:C)
c-Myc+/-
gated Bcr-Abl+
% of cells
Bcr-Abl
Gr-1
B220
66.8
1.26
G
20 μm
50 μm
50 μm
PB
liver
lung

Figure S4, related to Figure 5. LIC potential is specific to c-Myc$^+$ LSK and loss of one allele of *c-Myc* does not alter CML progression. (A) FACS sort to isolate Bcr-Abl$^+$ Lineage$^+$, Bcr-Abl$^+$ c-Kit$^+$, and Bcr-Abl$^+$ cMyc-GFP$^+$ LSK. (B) The threes cell populations were then transplanted into lethally irradiated recipients, and PB was analyzed at day 12 and 20 to determine % of Bcr-Abl$^+$ cells. (C) Proportion of mice that developed lethal CML. (D) Average CFU from *MxCre*$^+$*;c-Myc*$^{+/+}$ and *MxCre*$^+$*;c-Myc*$^{+/-}$ cells infected with Bcr-Abl expressing retrovirus. Left bar graph: primary plating, Right bar graph: secondary plating. Image on right are representative colonies from control or *c-Myc*$^{+/-}$ plated Bcr-Abl$^+$ LSKs. Scale bar equals 200 μm. Error bars indicate +/-SD. (E) genotyping PCR from recipient BM depicting deleted allele. (F) FACS analysis of PB at day 14 post-transplantation of recipient mice. (G) Bood smears approximately 20 days post transplantation stained with Wright-Giemsa and H&E staining of liver and lung in *MxCre*$^+$*;c-Myc*$^{+/-}$ recipient animals.

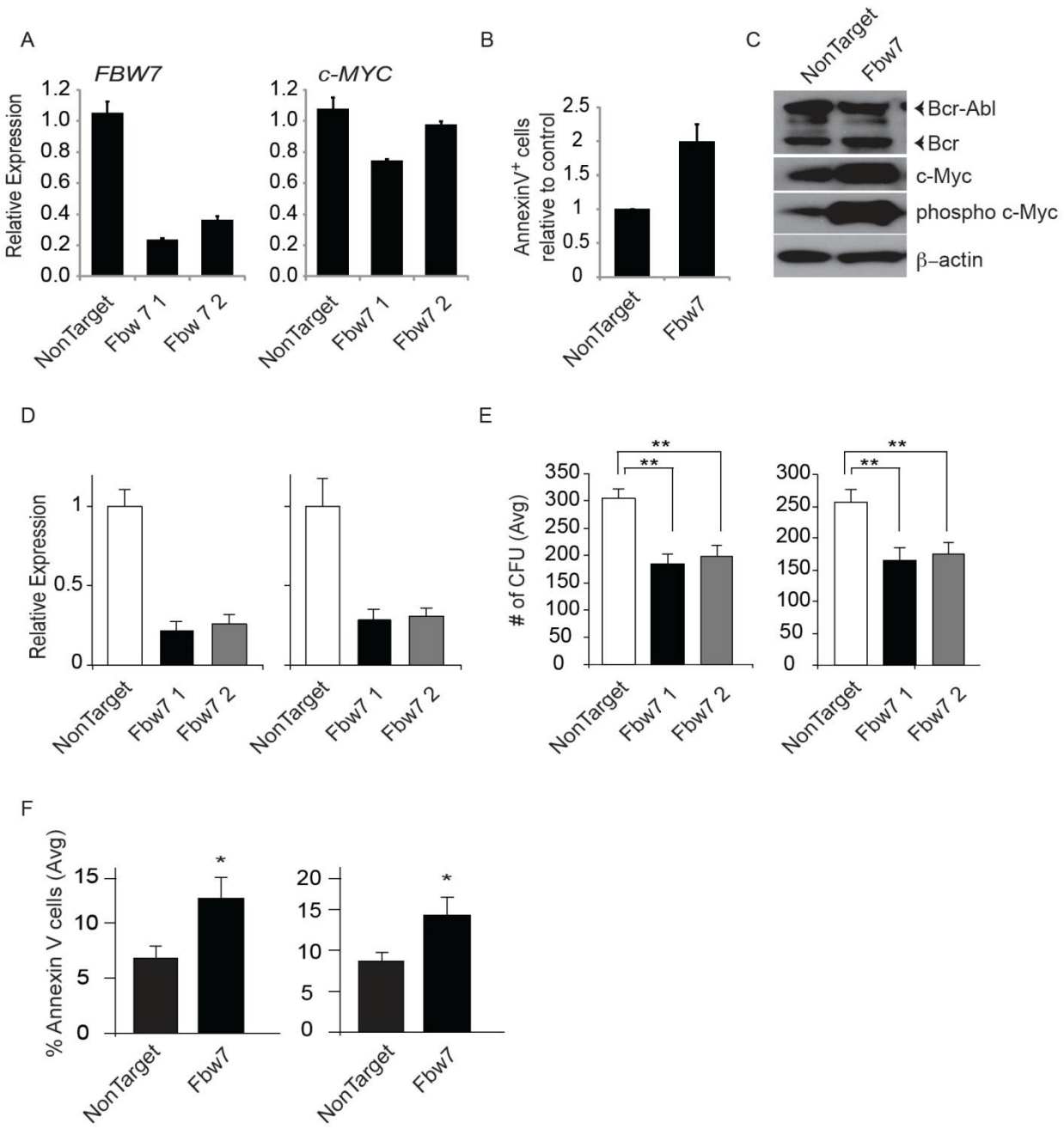

Figure S5, related to Figure 8. Loss of Fbw7 in human CML cell line and CML derived CD34$^+$. (A-C) KU812, a CML cell line, following silencing of Fbw7. (A) QRT-PCR analysis of *c-MYC* and *FBW7* expression. (B) Bar graph of % of Annexin V$^+$ cells relative to control. (C) Western blot for c-Myc, Phospho-c-Myc, Bcr-Abl, and β-actin. (D-F) Bone marrow cells from two CML patients were transfected with a control siRNA or one of two independent Fbw7 siRNAs by electroporation, and the CD34$^+$Lin$^-$ fraction was then sorted and cultured on OP-9 cells. (D) QRT-PCR analysis of *FBW7* mRNA level. (E) Colony formation by CD34$^+$Lin$^-$ cells transfected with Fbw7 siRNAs. (F) The proportion of apoptotic cells among CD34$^+$Lin$^-$ cells was determined by staining with annexin V. Error bars indicate +/-SD

Supplemental Experimental Procedures

Generation and analysis of disease mice for *in vivo* studies: Genotyping and analysis of deltetion for mouse strains was preformed as previously described (Reavie et al., 2010) and primers are listed in the Genotyping Primers section below. 4-6 weeks old mice were sacrificed and the bone marrow was flushed from femurs and tibias. Single cell suspensions were prepared, and red blood cells were lysed NH_4Cl buffer for 3 minutes on ice. Subsequently, Lin^- cells were obtained using the mouse hematopoietic progenitor enrichment kit (Stem Cell Technologies) per manufacturer's protocol and stained with a lineage cocktail, c-Kit, and Sca-1 antibodies. LSK cells mice were sort purified and cultured overnight at 37° C in 5% CO_2 in Opti-MEM medium (Gibco) supplemented with 10% FBS (Sigma Aldrich), 1% Penn/Strep (Gibco), 100 ng/mL of mouse recombinant SCF (Peprotech) and 20 ng/mL of mouse recombinant TPO (Peprotech) in 96 well U-bottom plates (cell density was ~20,000 cells/ well). Twelve hours post cell sort, cells were infected with Bcr-Abl-NGFR or Bcr-Abl-GFP retrovirus (Wertheim et al., 2002) and spun at 2500 rpm for 90 minutes at 30° C. The plates were incubated at 37° C in 5% CO_2 for four hours and then the media was carefully aspirated and replaced with fresh medium. The infection procedure was repeated the next day and the cells were allowed to rest for 24 hours following infection. Finally, the infection efficiency (~20-40%) was determined by flowcytometry and Bcr-Abl-$NGFR^+$ or Bcr-Abl-GFP^+ LSK cells were transplanted (cells were normalized to ~20,000-40,000 $Lineage^-$ Bcr-Abl-$NGFR^+$ LSKs for each genotype) into congenic lethally irradiated (2x 550 rads) recipient mice with 2-5x10^5 support bone marrow cells. Deletion of *Fbw7*, *p53*, and *c-Myc* was initiated on day 7 post transplantation 3 injections every other day with poly(I:C) (Amersham) at a concentration of 5 μg/g of body weight. Disease development was assessed by the presence of Bcr-Abl^+ cells in the peripheral blood by flowcytometry. Mice were sacrificed and analyzed upon exhibiting physical signs of disease, such as hunched posture, and limited movement.

For secondary transplantation experiments, mice were sacrificed approximately 20 days after the initial transplantation and whole spleen (normalized for ~10,000-20,000 Bcr-Abl$^+$ LSKs for each genotype) was transplanted into lethally irradiated (2x 550 rads) secondary recipient mice.

For B-ALL experiments, total BM was isolated and transduced with Bcr-Abl-GFP retrovirus followed by culture overnight at 37° C in 5% CO_2 in Opti-MEM medium supplemented with 10% FBS, 1% Penn/Strep, and 10 ng/ml mouse recombinant IL-7 (Peprotech) at a density of 5x10^6 cells per well in a 6-well plate (Corning). The following day transduction efficiency was determined by flowcytometry and 2x10^6 cells were transplanted into congenic lethally irradiated (2x 550 rads) recipient mice. Twelve post-transplant peripheral was obtained and monitored for Bcr-Abl-GFP$^+$ B220$^+$ cells. Day 13 post transplantation mice underwent 3 injections every other day with poly(I:C) (Amsersham) at a concentration of 10 μg/g of body weight. PB was once again monitored for Bcr-Abl-GFP$^+$ B220$^+$ cells at day 21 post-transplantation, and recipients were followed for disease development.

All mice were analyzed using FACS analysis and hematoxylin and eosin staining of formalin-fixed paraffin embedded tissues (i.e liver and lung). Peripheral blood was smeared on a slide and subsequently stained using the Giemsa-Wright staining method.

***In vitro* Colony Forming Unit Assays:** For the *in vitro* CFU experiments, mice received 5 intra-peritoneal (IP) injections of poly(I:C) at a dose of 5 μg/g, and LSK cells were purified 10 days post poly(I:C). LSK cells were purified and infected Bcr-Abl-NGFR or Bcr-Abl-GFP retrovirus with as described above. Bcr-Abl$^+$ lineage negative cells were sorted 72 hours after the first infection and

1000 cells were seeded into cytokine-supplemented methylcellulose medium (MethoCult 3434, Stem Cell Technologies). Colonies were counted 7 days after they were seeded and 4000 cells were re-plated and colonies were counted after another 7 days (14 days total). For human CFU assay, cells were collected after 7 days of culture, and 1×10^3 $CD34^+$ cells were transferred to Methocult medium (MethoCult GF H4435, StemCell Technologies). The number of colonies was counted 1 week after plating.

Immunoblot analysis: Immunoblotting was carried out as previously described (Thompson et al., 2008). LSKs or c-Kit^+ BM cells were lysed with RIPA lysis buffer (50 mM TrisHCl, 150 mM NaCl, 0.1% SDS, 0.5% NaDeoxycholate, 1% NP40) and cells were incubated on ice for 20 minutes and cellular debris were pelleted by ultra-centrifugation. Total cell lysates were separated by SDS-PAGE on a 10% Tris-HCl gel and were transferred to nitrocellulose membranes. Membranes were probed with anti-c-Myc (9402, Cell Signaling), anti-phospho-c-Myc (Cell Signaling), anti-cleaved Notch 1 (2421, Cell Signaling), anti-Bcr (3902, Cell Signaling), and anti-actin (Clone C4, Milipore) antibodies. Blots incubated with secondary horseradish peroxidase (HRP) conjugated anti-mouse IgG or anti-rabbit antibodies (Amersham, GE Healthcare Biosciences Piscataway, NJ) in TBS-T according the manufacturer's protocol. Bands were visualized using chemiluminescence (GE Healthcare).

Isolation and depletion of Fbw7 in human cells: Bone marrow mononuclear cells from patients in different phases of CML were kindly provided by Dr. Abdel-Wahab (Leukemia Service, Memorial Sloan Kettering Cancer Center) or purchased from AllCells. For siRNA transfcetion, we transfected bone marrow mononuclear cells with 300 nM Fbw7 siRNAs (Stealth Select RNAi siRNA, Invitrogen) by electroporation with the use of an Amaxa Nucleofector II device (Lonza) according to the manufacture’s instructions. The transfected cells were then stained with antibodies to human CD34

(8G12), CD3 (SK7), CD16 (3G8), CD19 (SJ25C1), CD20 (L27), CD14 (MϕP9), and CD56 (NCAM16.2) (BD Biosciences). CD3, CD16, CD19, CD20, CD14, and CD56 were used as lineage markers. $CD34^+$ Lin^- cells were purified by FACS and cocultured with OP-9 cells for 7 days.

For lentiviral tranduction, UCB or patient BM $CD34^+$ cells were isolated using $CD34^+$ selection kit following manufacturer's instructions (Stem Cell Technologies). Cells were cultured in Stemspan (Stem Cell Technologies), supplemented with 50 ng/ml SCF, 50ng/ml Flt3L, and 100 ng/ml Tpo for 24 hours followed by two spinoculation with virus supernatant (2,500 rpm, 30°, 90 minutes). 24 hours post infection cells were selected with 2 μg/ml puromycin for 48hrs. KU812 cell line was cultured in RPMI +10% FCS, and was infected in similar manner, pLKO shRNA plasmids against Fbw7 were purchased from Sigma. $CD34^+CD38^+$ and $CD34^+CD38^{low/-}$ cells were purified by FACS using CD34 PE-conjugated and CD38 FITC conjugated antibodies (BD Pharmingen). For assays of apoptosis, the cells were collected after 7 days of culture, stained for cell surface markers, further incubated for 15 min with annexin V (BD Biosciences) and then analyzed by flow cytometry.

Human siRNA: Fbw7 siRNAs (Stealth Select siRNA, Invitrogen)

KD#1: 5'-GGGCAUACUAAUAGAGUCUAUUCAU-3'

KD#2: 5'-AGUUGGCACUCUAUGUGCUUUCAUU-3'

Human shRNAs: TRC2-pLKO lentiviral vector Fbw7 shRNAs (Sigma Aldrich)

shRNA1- CCGGACTTCCACTGTGCGTTGTATGCTCGAGCATACAACGCACAGTGGAAGTTTTTTG

shRNA 2-

CCGGAGTTTCAACGAGACTTCATTTCTCGAGAAATGAAGTCTCGTTGAAACTTTTTTG

Genotyping Primers:

FBW7-R recombined allele	ggcttagcatatcagctatgg
Fbw7 R	atagtaatcctcctgccttggc
Fbw7 F	attgatacaaactggagacgagg
MxCre F	gcctacaatatggatttccca
MxCre R	cttgcgaacctcatcactc
c-Myc R	ttttctttccgattgctgac
c-Myc F	taagaagttgctattttggc
c-Myc R recombined allele	tcgcgcccctgaattgctagga
C-Myc F	ccgaccgggtccgagtccctatt
p53-WT	acagcgtggtggtaccttat
p53-GT-Common	tatactcagagccggcct
p53-GT-MUT	ctatcaggacatagcgttgg

qRT-PCR Primers:

species	gene	sequence
mouse	Puma F	gcggcggagacaagaaga
mouse	Puma R	agtcccatgaagagattgtacatgac
mouse	p21F	ttccgcacaggagcaaagt
mouse	p21R	cggcgcaactgctcact
mouse	Bax F	tggagctgcagaggatgattg
mouse	Bax R	agctgccacccggaaga
mouse	Fbw7 F	gtgatagagccccagttcca
mouse	Fbw7 R	cctcagccaaaattctccag
mouse	c-Myc F	cttctctccttcctcggactc
mouse	c-Myc R	ggagatgagcccgactccgacctc
human	FBW7 F	gtgatagaaccccagtttca
human	FBW7 R	cttcagccaaaattctccag
human	c-MYC F	gctgcttagacgctggattt
human	c-MYC R	cgaggtcatagttcctgttgg
human	GAPDH-F	cttttgcgtcgccagccgag
human	GAPDH-R	ccaggcgcccaatacgacca