Extended Experimental Procedures

Generation of Conditional BCR/ABL Knockin Mice. The targeting vector was made to conditionally express BCR/ABL and eGFP (Figure 1A and Figure S1). The mouse chromosome 10 sequence (Bcr is located at nucleotides: 74,503,000~74,660,000) was retrieved from the Ensembl database and used as a reference. A mouse RP23-463H8 BAC DNA was used as a template for generating the homology arms and the southern probes for screening targeted events. The 5' homology arm (~5.1 kb) and the 3' homology arm (~4.1 kb) were generated by PCR using high-fidelity Taq DNA polymerase and the RP23-463H8 BAC DNA as the template. The Lox-Stop-Lox cassette (~1.5 kb) was amplified from a LSL-H/P knockin vector[1]. These fragments were cloned into the FtNwCD or pCR4.0 vector and were confirmed by restriction digestion and sequencing. The Bcr-Abl-IRES-eGFP cassette (~7.9 kb) was released from plasmid MSCVp210-IRES/eGFP (kind gift of Richard Van Etten, Tufts Medical Center) by ClaI and HpaI digestion. The final vector was obtained by standard molecular cloning. Aside from the homology arms, the final vector also contains a Lox-Stop-Lox cassette (~1.5 kb), Bcr-Abl-IRES-eGFP cassette (~7.9 kb), Frt sequences flanking the Neo expression cassette (the neo cassette was used for positive selection of the electroporated ES cells), and a DTA expression cassette (for negative selection of the ES cells). The final vector was confirmed by both restriction digestion and end sequencing analysis. NotI was used for linearizing the final vector prior to electroporation. Approximately 30 µg of NotI-linearized final gene targeting vector DNA was electroporated into ~10^7 C57BL/6 ES cells and selected with 200 µg /ml G418. Two plates of G418-resistant ES clones (~192) were selected for screening.

The primary ES screening was performed with 3' PCR. Approximately 20 potential targeted clones were identified from one plate. Six clones (A6, A8, B4, B6, C5, and C6) were expanded for further analysis. Upon completion of the ES clone expansion, additional Southern confirmation analysis was performed. Based on this analysis, five out of the six expanded clones (A6, A8, B4, B6, and C5) were confirmed for homologous recombination with a single neo integration (Figure 1B). Two clones (A6 and A8) were injected into blastocysts (B6Tyr). The following male chimeras were generated: 85%, 75%, 55%, 20%, and 10% (from clone A6) and 98%, 90%, 70%, 60%, 30%, and 15% (from clone A8). The male chimeras were bred with wild-type C57BL/6 female mice. Heterozygous progeny from both clones were generated and identified by PCR.

Southern Blot Analysis. Southern blot analysis to distinguish the targeted *Bcr*$^{LSL\text{-}BCR/ABL}$ allele and the wild-type allele was performed with 5', 3', and neo probes. The 5' and 3' external probes were generated by PCR and cloned into the pCR4.0 backbone, and correct cloning was confirmed by sequencing.

Sequence of the 5' probe:

GGCATAGAGCATCATTACTAATTCAGGAGTTTCTACAACCTGTTTCCCAAGTTCTTA
CAGTTATTAATCTCCAAATCCATAAGACACTCACTGAGGGACGTGACCGCACAAAC
GTGCAGGCTGGTGACAAGAACATAGTTCTAGGCCCCATGAGACAGTGGTGCGTTG
ACAAAGAATTCTAGGTCGAGAGTGGACCATAAAGAGCATATGGCAACAGGGAGCA
TGACAGTTGGGGCTGGGCTGGGATGTTTCAGAAGAATTACCCAACTTAGAGGGAG
CCTCTCCTCTTCTGAACAGGAAGTG

Sequence of the 3' probe:

GAATGGGAAGGATAGAGACTGCCCATCTCTCCTGTGCCTCCAAACTACTCCTCGG
TTATCAACATTCCCCCACTAAACGGGTACATTTGTGACAGTTCATAAACCTACATAG
ACACATCATTATCACCCACAGTATGCACTTATATTAGGATTCTCTCTTAGTGTTGCG
CCTGTATGTTTAGTTCTTTTTGGCCTTAACATGCCCAAGTGTTGCAGCCCAAAGAAC
ATGGGCTGAACATTCTTTTCTGTCTTGTATGGGAGGGGAAAGGGGCCCTGCCACTT
AGCCATCCGCCTCTGTCGGGGTTATGTATTGATGTTCATTAGACGGCGAAC

Quantitative PCR analysis of BCR/ABL message. Splenic RNA was extracted by TRIzol (Invitrogen) and treated with DNase I according to the manufacturer's instructions. RNA concentrations were quantified in triplicate with the Nanodrop 2000 Spectrophotometer (Thermo Scientific). Two μg total RNA was used to synthesize the first cDNA strand with the SuperScript™ III First-Strand Synthesis System for RT-PCR (Invitrogen). RNA copy numbers were determined by TaqMan Absolute real-time PCR. The probe and primer set for BCR/ABL was designed to detect the breakpoint of this fusion gene using the Primer Express software (version 1.0, ABI). Forward primer: 5'-tccactcagccactggatttaa-3'; Reverse primer: 5'-gatgctactggccgctgaa-3'; Probe: 6-FAM-cagagttcaaaagcc-TGM. The primer and probe mixture was produced by ABI. The BCR/ABL standard was produced with a 500-bp fragment of BCR-ABL cDNA that contains the detection area. A human BCR/ABL-murine Bcr fusion standard curve was generated to quantify expression of BCR/ABL from the knockin allele and Bcr from the wild type *Bcr* allele using the same standard. The standard was made by overlap extension PCR with two primer sets. The human BCR/ABL forward primer was 5'-cagaagaagtgtttcagaagc-3' and the reverse primer was 5'-

gaagacccctgtctgtttgctgttatctccactggccaca-3’. The mouse Bcr forward primer was 5’-tgtggccagtggagataacagcaaacagacaggggtcttc-3’ and the reverse primer was 5’-tccaggaggaagaggaaggt-3’. PCR was performed using the ABI StepOnePlus. The mRNA expression of Bcr was quantified by TaqMan Comparative real-time PCR using the primer and probe set Mm01168911_m1 from ABI.

Figure S1. Construction and expression analysis of the BCR/ABL-ires-GFP knockin allele, related to Figure 1.

(A) Schematic of conditional BCR/ABL knockin vector construction. See details of construction in the methods section.
(B) Schematic of conditional BCR/ABL knockin screening strategy for correctly targeted ES cells.
(C) GFP distribution in peripheral blood leukocytes from Vav-Cre;BCR/ABL-ires-GFP knockin mouse. GFP positivity was highest in MAC1+GR1+ cells of peripheral blood. GFP fluorescence was measured by flow cytometry.
(D) GFP distribution in bone marrow progenitors from Vav-Cre;BCR/ABL-ires-GFP knockin mouse. GFP positivity was highest in HSCs compared to other bone marrow progenitors. HSCs were isolated as CD150+CD48-LSK cells.

Figure S2. Hematopoietic Characterization of BCR/ABL Mice, Related to Figure 2.

(A) WBCs (top) and spleen size (bottom) were normal in adult Mx1-Cre;B/A mice (age 10-14 months of age). Control = Mx1-Cre or Mx1-Cre;A/E mice.
(B) Bone marrow progenitor frequencies were not altered in adult Vav-Cre;B/A expressing mice compared to Cre^{neg};B/A mice (n=3 at each time point of 6, 9, 12 and 18 months of age). Displayed is the 6 month time point. White bars represent Cre^{neg};B/A mice and shaded bars represent Vav-Cre;B/A mice. HSC frequencies were measured by CD150+CD48-LSK cell counts; Bone marrow CMP, GMP and MEP frequencies were percent of the LK population; Lineage markers for T (CD3) and myeloid (Mac-GR1) cell frequencies (in whole bone marrow) were not altered in the presence of a recombined BCR/ABL knockin allele. B (B220) cell frequencies were slightly diminished in Vav-Cre;B/A mice. NS=not significant and *$p<0.05$. Error bars denote the standard deviation.
(C) Bone marrow progenitor frequencies were not altered in adult Mx1-Cre;B/A mice compared to control mice (wild type or Cre^{neg};B/A) except for a slight decrease in myeloid cell frequencies. 8-10 month old wild type, Cre^{neg};B/A or Mx1-Cre;B/A mice that had been induced with pIpC at 6 weeks of age and then again at 6 months of age were analyzed for frequencies of HSCs, CMP, GMP, MEP (all a percent of whole bone marrow numbers) and lineage as in part B. n=4-6 mice per genotype, *$P<0.05$; **$P<0.01$. All Y-axes are frequency of cell type in whole bone marrow.
(D) Vav-Cre;B/A homozygous mice or control mice (Cre^{neg};B/A) were treated with oral imatinib or vehicle (mock) twice a day for two weeks and then evaluated for peripheral blood GFP.
(E) Targeting of BCR/ABL to the Bcr locus reduces HSC numbers in young adult mice. HSC (left) and LSK (right) frequencies in the bone marrow of 10-12 week

old wild type (WT), Cre^{neg};B/A (B/A) and Vav-Cre;B/A mice were calculated from 3-6 mice per genotype, *P<0.05, ***P<0.001.

(F) Displayed are H&E stains of spleens, livers and kidneys from a Vav-Cre;B/A;A/E mouse (bottom panel) that succumbed to a myeloproliferative neoplasm at 6 months of age and a WT control mouse (top panels).

(G) Wild type (WT; n=6), Vav-Cre;B/A (n=6) or Vav-Cre;B/A;A/E (n=6) mice were evaluated for peripheral blood GFP (top panel). There was not a significant difference in GFP between the Vav-Cre;B/A and Vav-Cre;B/A;A/E mice. All mice were just over 12 months of age. By this age all of the Vav-Cre;B/A;A/E mice developed a monocytosis (bottom panel), which precedes a fatal myeloproliferation. ***P<0.001.

Supplemental figures

Figure S1

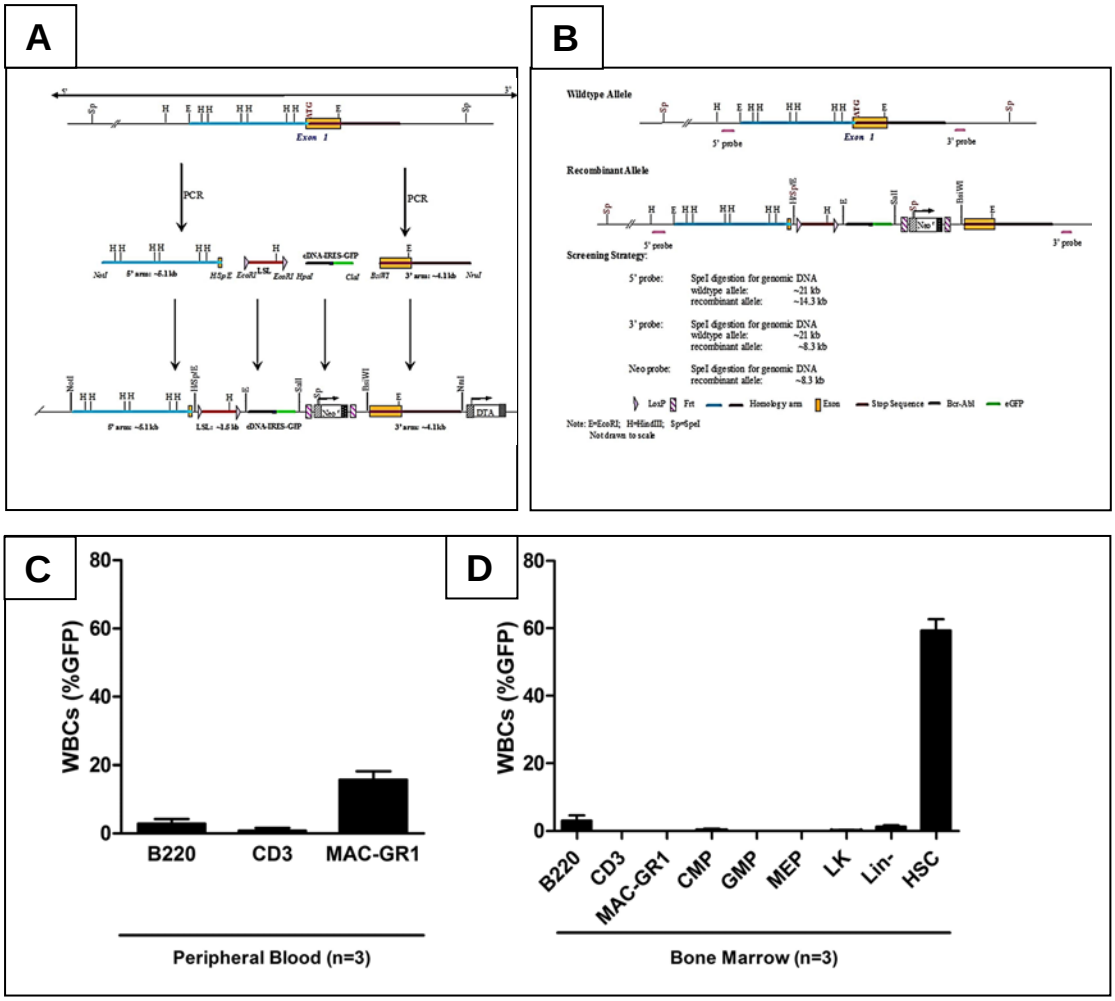
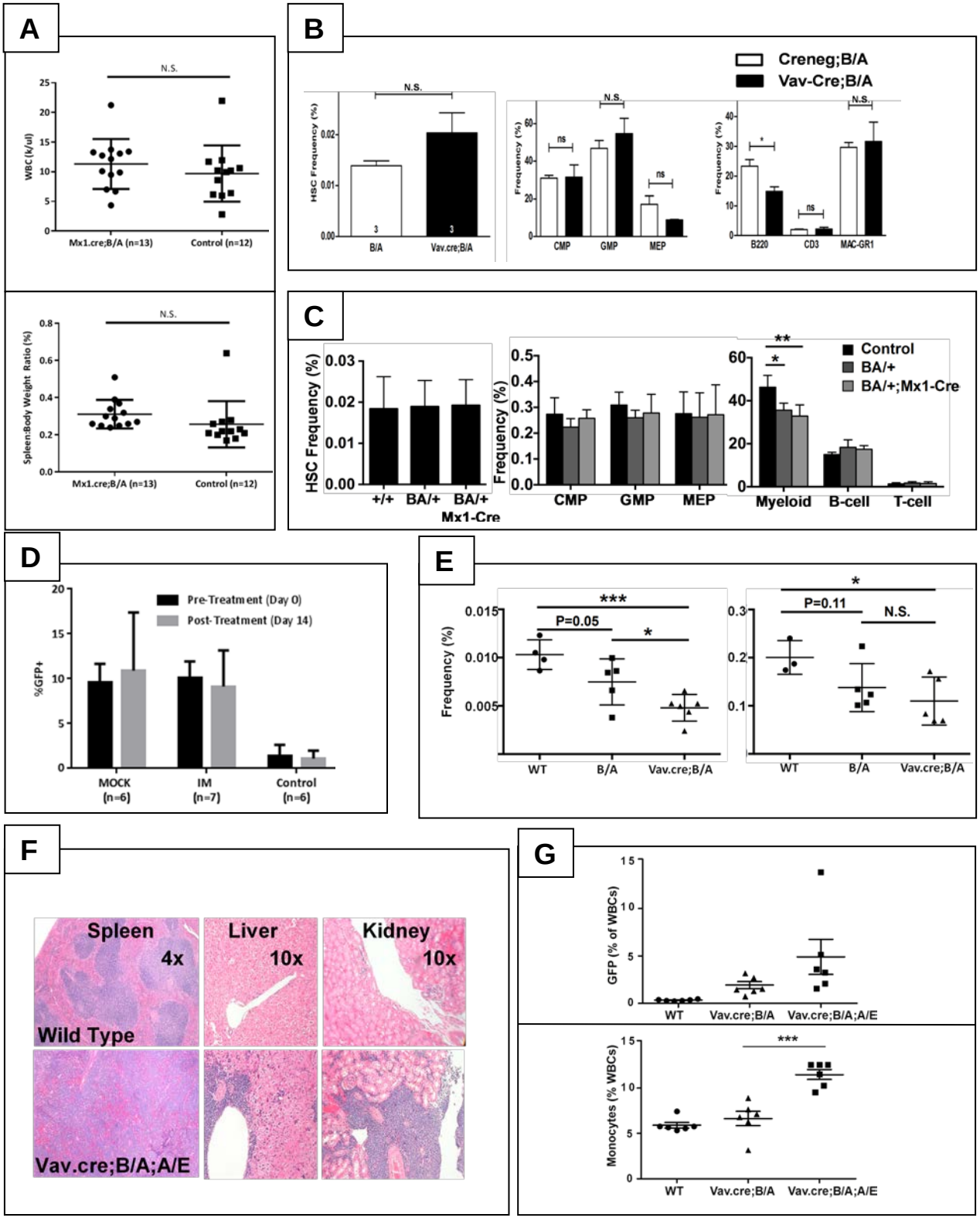


Figure S2


A
N.S.
WBC (k/ul)
Mx1.cre;B/A (n=13)
Control (n=12)
Spleen:Body Weight Ratio (%)
B
Creneg;B/A
Vav-Cre;B/A
HSC Frequency (%)
B/A
Vav.cre;B/A
Frequency (%)
CMP
GMP
MEP
B220
CD3
MAC-GR1
C
HSC Frequency (%)
+/+
BA/+
BA/+ Mx1-Cre
Control
BA/+;Mx1-Cre
Myeloid
B-cell
T-cell
D
Pre-Treatment (Day 0)
Post-Treatment (Day 14)
%GFP+
MOCK (n=6)
IM (n=7)
Control (n=6)
E
P=0.05
P=0.11
WT
B/A
Vav.cre;B/A
F
Spleen 4x
Liver 10x
Kidney 10x
Wild Type
Vav.cre;B/A;A/E
G
GFP (% of WBCs)
Monocytes (% WBCs)
Vav.cre;B/A;A/E