Retroviral transduction and transplantation assays

Bone marrow (BM) harvested from C57BL/6-Ly5.1 mice (7–8 weeks old) treated 3.5–4 days previously with 150 mg/kg 5-fluorouracil (5-FU; Sigma-Aldrich, St. Louis, MO) was pre-stimulated for 24 hours at 37°C in DMEM supplemented with 10% fetal bovine serum (FBS), 50 ng/mL stem cell factor (SCF), 5 ng/mL IL-6, and 1 ng/mL IL-3 (PeproTech, Rocky Hill, NJ). BM cells were transduced in the presence of 5 μg/mL polybrene (Sigma-Aldrich, St. Louis, MO) for 48 hours at 37°C by co-culture with BOSC23 retroviral producer cells co-transfected one day prior with MIB;MIV, MIB-RE;MIV–c-Kit^{D814V}, or MIB-RE;MIV–c-Kit$^{T417IΔ418–419}$. Retrovirally transduced BM cells were removed from the co-cultures, washed and resuspended in phosphate buffered saline (PBS). C57BL/6-Ly5.2 mice (~8–12 weeks old) were lethally irradiated with a split dose of 9.0 Gy spaced by 4 hours and then transplanted with transduced cells. Secondary recipient mice (C57BL/6-Ly5.2; ~8–12 weeks old) were lethally irradiated as described above and transplanted with 5×10^4 to 1×10^6 whole bone marrow cells from primary recipients. Recipient mice were monitored for leukemia development by observation of physical symptoms and by peripheral blood (PB) analysis using FACS to detect Bex or Vex single-positive or Bex;Vex double-positive cells. Primary recipient animals remaining disease-free were sacrificed at 50–60 weeks post-transplant and disease-free secondary recipients were sacrificed at 25 weeks post-transplant.

Peripheral blood and bone marrow harvest

Beginning at 3–4 weeks post-transplant, PB was collected from the lateral tail vein of recipient mice. Red blood cells (RBCs) were removed by sedimentation in 2% dextran/PBS for 30 minutes at 37°C followed by lysis with ACK (0.15 M NH_4Cl, 1 mM $KHCO_3$, 0.1 mM EDTA, pH 7.2 – 7.4). The remaining leukocytes were washed in PBS and resuspended in HBSS/2% FBS. BM cells were isolated from tibias and femurs by flushing in cold PBS and resuspended in HBSS/2% FBS.

Histology and cytology

Tissues (spleen, liver, lung, femur, sarcoma, lymph node, thymus) from moribund mice were fixed in 10% neutral buffered formalin and femur samples decalcified in 8% HCl/dH_2O for 12hr after fixation. Fixed tissues were dehydrated in ethanol, cleared in xylene, embedded in paraffin, and specimen sections stained with hematoxylin and eosin. For morphologic analysis, 3×10^4 Bex^+Vex^+ myeloid scatter-gated bone marrow cells were FACS-purified into PBS/12% FBS then cytospun onto glass slides and stained with Wright-Giemsa.

Table S1. Retroviral integration sites in RE;D814V and RE;T417IΔ418-419 mice with AML

Genotype Animal no.	Insertion site loci	Gene(s)* and respective location of insertion
RE;T417IΔ418-419 1	16qB3 4qE2 11qD	7 kb 5' of *Dtx3l* and 20 kb 5' of *Parp9* 300 bp 3' of *Pdg* and 8.8 kb 5' of *Kif1b* 23 kb 3' of *Spag9*
RE;T417IΔ418-419 2	17qE1.2 1qH3 5qE2	Intron 12 of *L3mbt14* 10 kb 5' of ***Fcgr2b*** and 22 kb 3' of *Fcrla* 55 kb 5' of *Rchy1*
RE;T417IΔ418-419 3	7qE3 13qD1 7qF1	8 kb 5' of *Trim30a* Intron 2-Exon 3-Intron 3 of *Mrps27* and 9 kb 5' of *Mtap1b* 29 kb 3' of *Pth* and 48 kb 3' of *Btbd10*
RE;T417IΔ418-419 4	7qF3	Intron 1 of *Ccdc101*; 18 kb 5' of *Sult1a1*; 29 kb 3' of *Nupr1*;and 45 kb 5' of ***Coro1a***
RE;T417IΔ418-419 5	5qB3 3qE1 5qF 7qB4 5qG2	58 kb 3' of *Clnk* Intron 10 of *Gfm1*; 6 kb 5' of *Lxn*; 27 kb 5' of *Rarres1*; and 52 kb 3' of *Mlf1* No known genes within 60 kb Exon 8 of *Ccdc155*; 11 kb 3' of *Pth2*; 14 kb 5' of *Dkkl1*; 17 kb 3' of *Slc17a7*; and 22 kb 5' of *Tead2* 16 kb 3' of *Cldn4*; 20 kb 3' of *Wbscr27*; 23 kb 5' of *Cldn3*
RE;D814V 1	1qC4	23 kb 5' of *Scg2*
RE;D814V 2	1qC4	Intron 2 of *Dock10*
RE;D814V 3	6qF1	23 kb 5' of *Cecr2*
RE;D814V 4	7qF4 14qD2	23 kb 5' of *Inpp5a* Intron 1 of *Pebp4*; 19 kb 5' of ***Egr3***; and 42 kb 5' of *Bin3*

PCR products generated by inverse PCR or splinkerette-PCR methods were gel-purified and sequenced using MSCV-LTR specific primers. Resulting sequence data was mapped to the mouse genome using the Blat search program on the UCSC Genome Browser database. Blat hits of >95% identity to the mouse genome were considered real events and are reported in this table. RE;D814V samples 1 and 2 each contained an insertion mapping to Chr. 1qC4, but the individual integration sites were >108 kb apart and therefore classified as unique insertion sites, based on statistical analyses previously reported (Mikkers et al. Nat Genet. 2002; Slape et al. Cancer Res. 2007; Cattoglio et al. Blood. 2007).

*Known genes into which a retrovirus inserted directly and/or genes that flank the insertion site by an arbitrary cut-off of 60 kb.

Bold text: Found as a CIS in Suzuki et al. Nat Genet. 32:166-174, 2002, or Mouse Retrovirus Tagged Cancer Gene Database, http://rtcgd.ncifcrf.gov/.

Figure S1. Co-expression of c-Kit$^{T417IΔ418–419}$ and RUNX1-ETO promotes AML with significantly delayed kinetics
Peripheral blood analysis of RE;c-Kit$^{T417IΔ418–419}$ mice at various time points post-transplant. Left panels for representative Mouse A and Mouse B show persistence of chimerism in RE;c-Kit$^{T417IΔ418–419}$ mice over time without selective expansion, indicating that this mutational combination is not sufficient to promote clonal proliferation (n=18). Mouse A (left column) is representative of an animal that likely acquired additional genetic changes in a RE;c-Kit$^{T417IΔ418–419}$ progenitor cell which promoted selective expansion of this clone at 37 weeks post-transplant.

Figure S2. Bex and Vex mean fluorescence intensity levels in RE;c-Kit$^{T417IΔ418–419}$ and RE;c-Kit^{D814V} mice
Mean fluorescence intensities (MFI) of Bex or Vex were used as relative measures of RE, c-Kit^{D814V} or c-Kit$^{T417IΔ418–419}$ expression levels in peripheral blood leukocytes from RE;c-Kit^{D814V} and RE;c-Kit$^{T417IΔ418–419}$ mice. Each line represents Bex and Vex fluorescence levels in a given animal during a pre-leukemic phase (8–10 weeks post-transplant) and when the same animal became moribund.

Figure S3. Granulocytic sarcomas are commonly detected in RE;c-Kit^{D814V} mice with AML
(A) Flow cytometric profiles of granulocytic sarcoma cells from two representative moribund RE;c-Kit^{D814V} mice. (B) Representative Wright-Giemsa stained cytospin of FACS-purified Bex^{+}Vex^{+} myeloid scatter-gated sarcoma cells and H&E stained sarcoma tissue section obtained from moribund animals (original magnifications ×630).

Figure S4. Neoplastic cells associated with RE;c-Kit^{D814V} and c-Kit^{D814V} myeloproliferative neoplasia are disseminated to peripheral tissues
(A) Significant splenomegaly was observed in moribund RE;c-Kit^{D814V} and c-Kit^{D814V} mice with MPN. Spleen weight (grams) are means plus SD. *P <.01, when compared to Bex;Vex controls, unpaired t-test. (B) H&E-stained tissue sections demonstrate extensive infiltration of peripheral tissues by MPN cells. Data are representative of a minimum of 4 moribund RE;c-Kit^{D814V} and 3 moribund c-Kit^{D814V} mice with MPN. Original magnifications in the left and right columns of RE;c-Kit^{D814V} and c-Kit^{D814V} were ×100 and ×630, respectively.

Figure S1

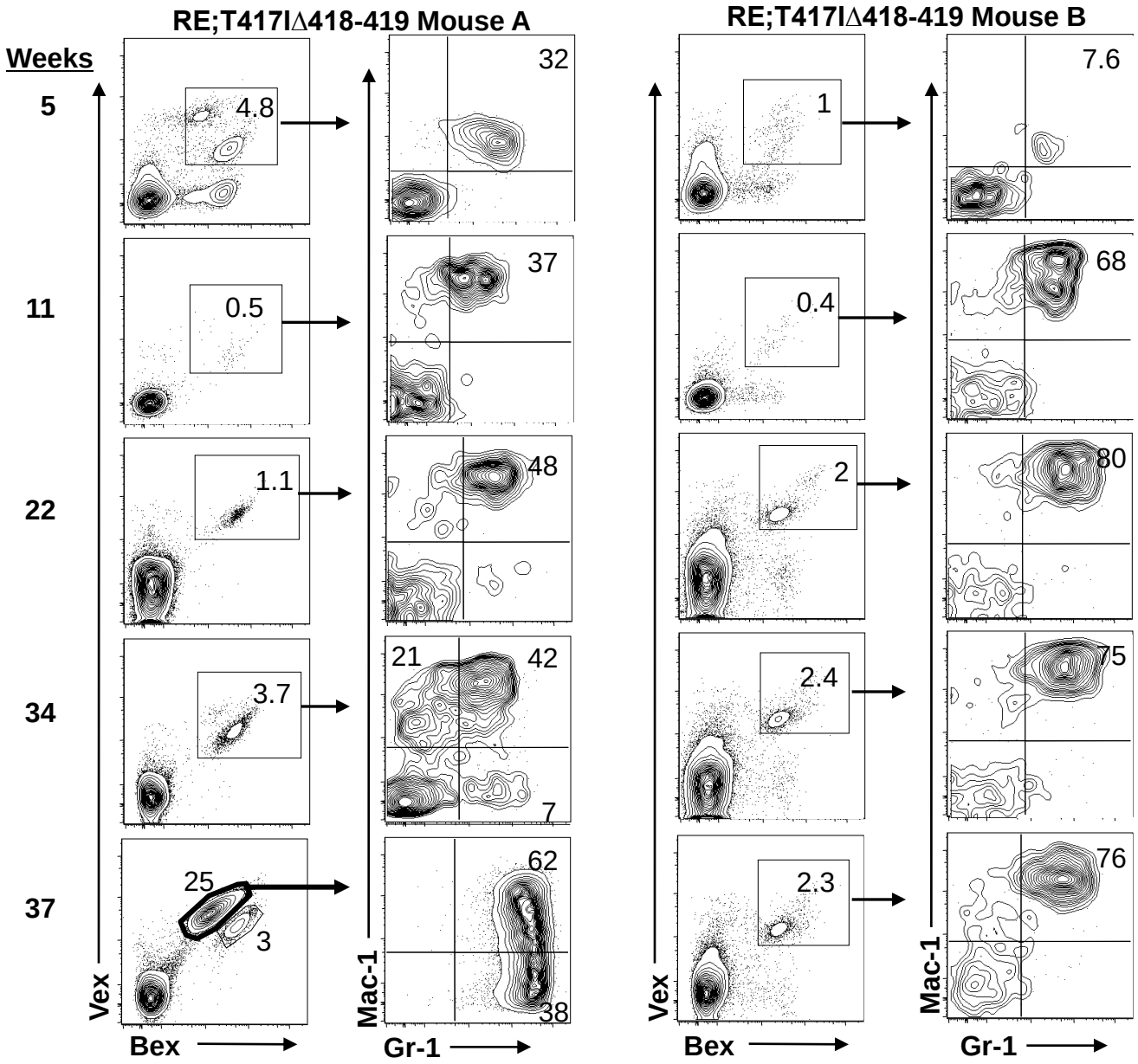

RE;T417IΔ418-419 Mouse A
RE;T417IΔ418-419 Mouse B
Weeks
5
11
22
34
37
4.8
32
0.5
37
1.1
48
3.7
21
42
7
25
3
62
38
1
7.6
0.4
68
2
80
2.4
75
2.3
76
Vex
Bex
Mac-1
Gr-1

Figure S2

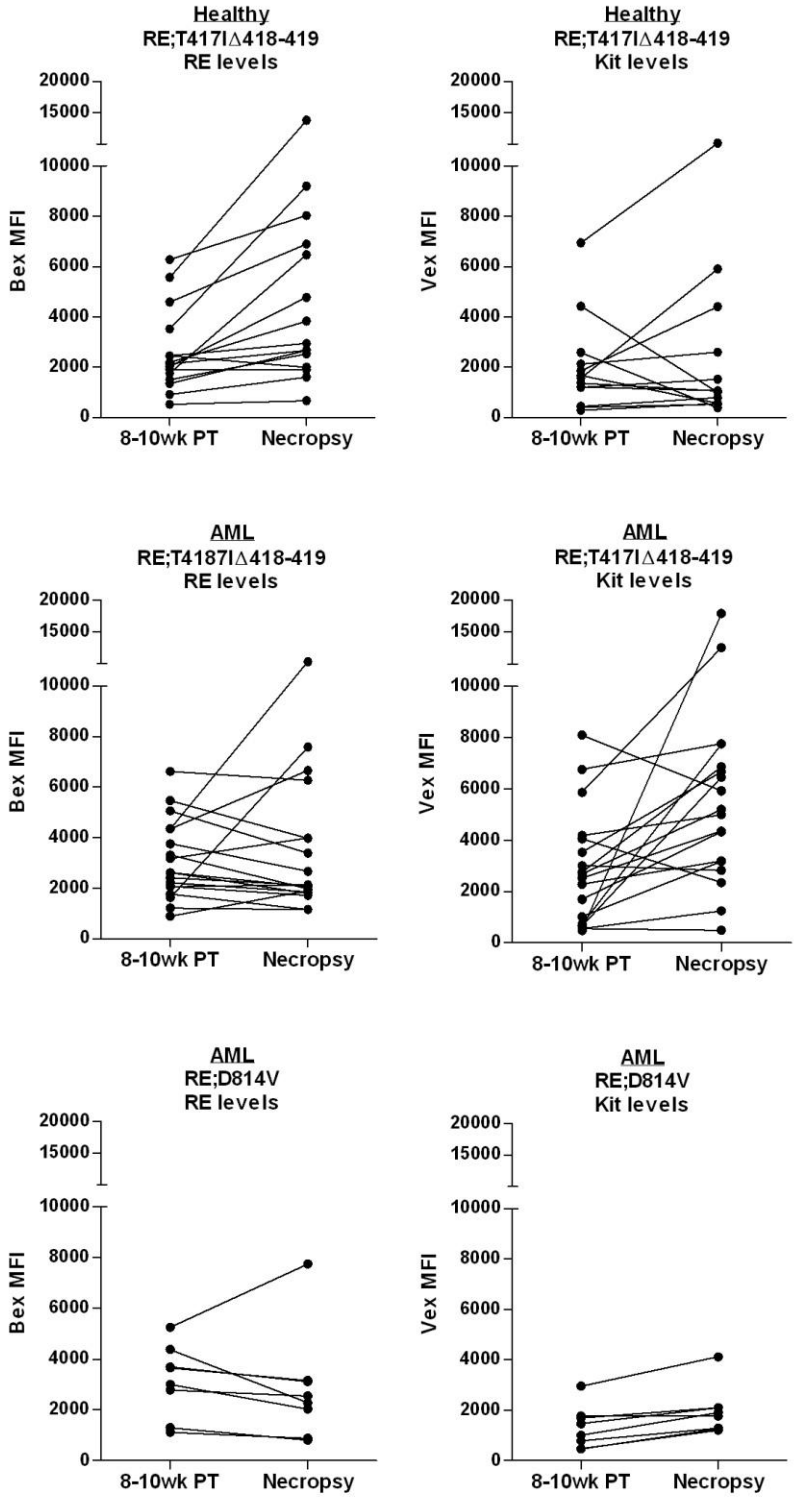

Healthy
RE;T417IΔ418-419
RE levels
Bex MFI
20000
15000
10000
8000
6000
4000
2000
0
8-10wk PT
Necropsy
Healthy
RE;T417IΔ418-419
Kit levels
Vex MFI
AML
RE;T4187IΔ418-419
RE levels
AML
RE;T417IΔ418-419
Kit levels
AML
RE;D814V
RE levels
AML
RE;D814V
Kit levels

Figure S3

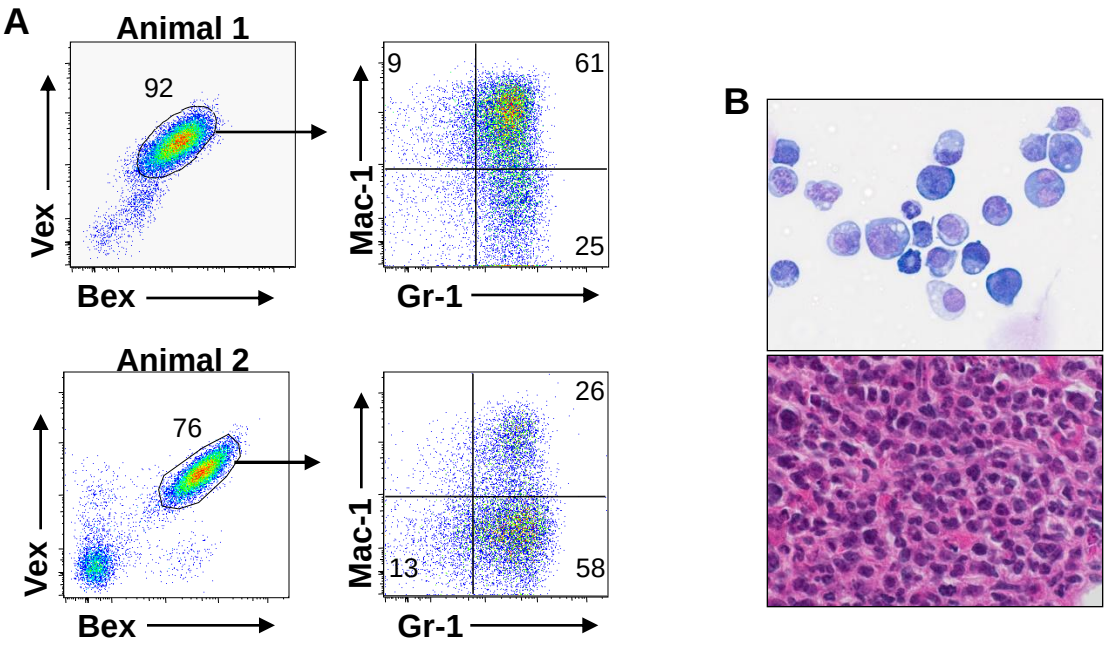
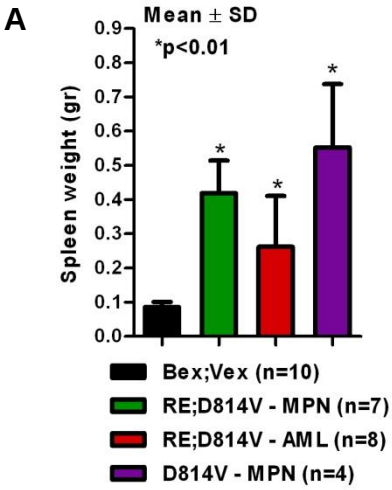
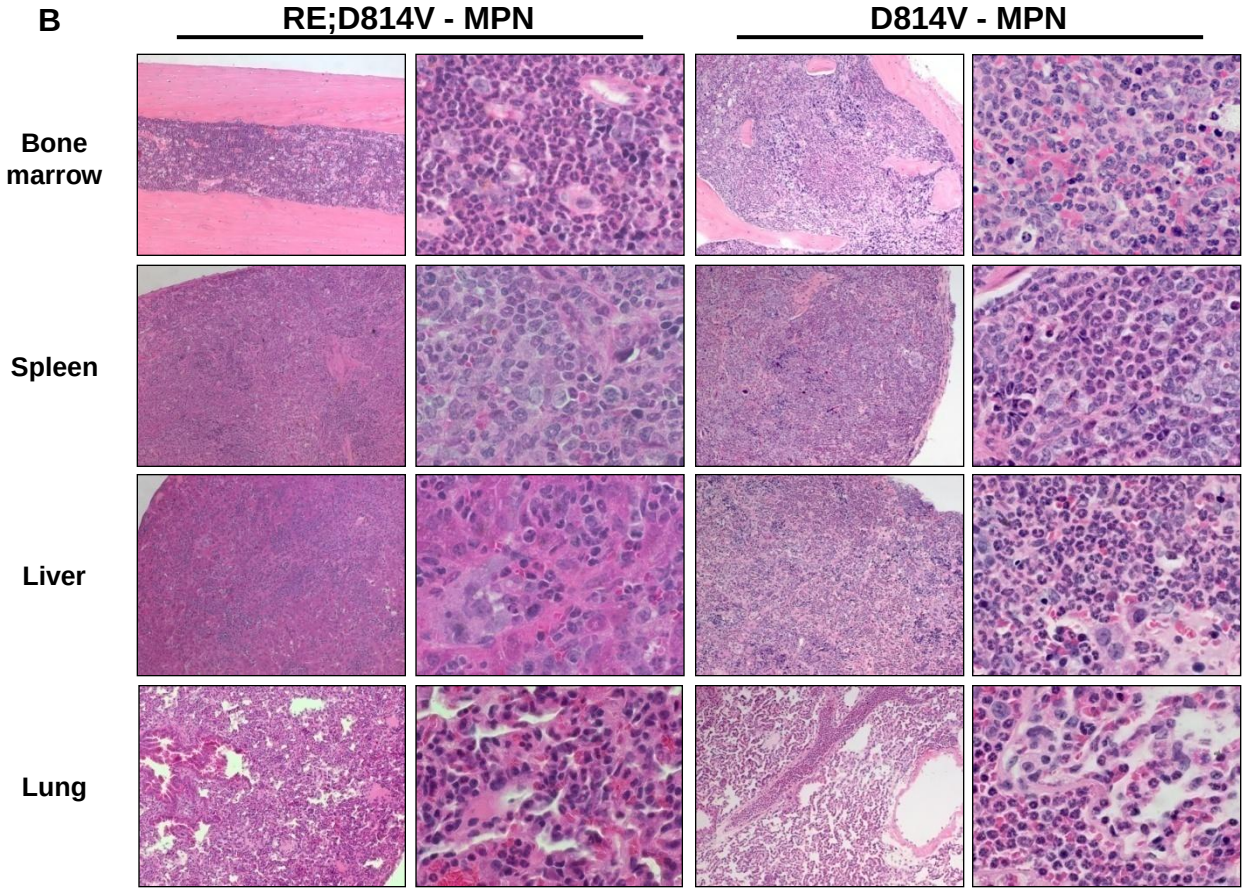

A
Animal 1
92
Vex
Bex
9
61
25
Mac-1
Gr-1
Animal 2
76
Vex
Bex
26
13
58
Mac-1
Gr-1
B

Figure S4


A
Mean ± SD
*p<0.01
Spleen weight (gr)
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
*
*
*
Bex;Vex (n=10)
RE;D814V - MPN (n=7)
RE;D814V - AML (n=8)
D814V - MPN (n=4)


B
RE;D814V - MPN
D814V - MPN
Bone marrow
Spleen
Liver
Lung