Supplementary Methods

Bone marrow transplantation: 8-10 week old transplant recipient Boy/J (CD45.1$^+$) or F1 (CD45.1$^+$CD45.2$^+$) mice of either gender were lethally irradiated and equal numbers of bone marrow cells from age matched Boy/J (CD45.1$^+$) and leukemic (CD45.2$^+$; from *Tet2$^{-/-}$Flt3^{ITD}* or *Dnmt3a$^{+/-}$Flt3^{ITD}*) mice of either gender were transplanted via tail vein. Development of leukemia was monitored by analysis of PB on Element HT5 hematology analyzer (Heska, USA) and mice were randomly assigned to vehicle or SHP099 treatment group. Vehicle or SHP099 (100mg/kg body weight) was orally administered once a day for 4 weeks.

Phenotypic analysis: PB differential analysis was done using Element HT5 hematology analyzer (Heska, USA) on samples collected from the tail vein. For flow cytometric analysis, single cell suspensions were prepared from PB, spleen and bone marrow followed by red blood cells (RBC) lysis and staining with fluorochrome tagged antibodies. Anti-mouse CD45.1 (A20) and CD45.2 (104) were used to discriminate between normal and leukemic cells. GR-1 (RB6-8C5), CD11b (M1/70) and c-KIT (2B8) were used to define mature and immature myeloid committed cells. Anti-mouse Ter119 (TER119), B220 (RA36B2) GR-1 (RB6-8C5) CD11b (M1/70) CD3 (17A2) were included in the lineage cocktail along with anti-mouse c-KIT (2B8), Sca-1 (D7), CD48 (HM48-1) and CD150 (TC15-12F12.2) for analysis of stem and progenitor cells in the bone marrow. All the antibodies were procured from Biolegend (USA) and clone names are mentioned in parenthesis. Cells were acquired on BD flow cytometers (Canto II or LSR Fortessa) and data was analyzed using FloJo™ software.

Histology: Femur and section of spleen were fixed in formalin, sectioned and stained with hematoxylin and eosin (H&E). Images were acquired at 20x magnification using Aperio whole slide digital imaging system, ScanScope CS (Leica). One representative image from each group is shown at same magnification.

Cell culture and RNA isolation: Cells from the hind limbs of C57BL/6 or *Tet2$^{-/-}$Flt3^{ITD}* mice were isolated and the lineage positive cells were removed using mouse hematopoietic progenitor cell enrichment kit (Stem Cell Technologies). The cells were expanded in a cytokine cocktail of IL-3, IL-6 and SCF for 5 days and treated with SHP099 for 24h and total RNA was isolated using a kit (Qiagen RNeasy Plus).

RNA sequencing and library preparation: The RNA integrity and concentration was assessed using Agilent 2100 Bioanalyzer. RNA (600 ng/ sample) was used to prepare single indexed strand specific cDNA library using TruSeq stranded mRNA library prep kit (Illumina). The library prep was assessed for quantity and size distribution using Qubit and Agilent 2100 Bioanalyzer. The pooled libraries were sequenced with 75bp single-end configuration on NextSeq500 (Illumina) using NextSeq 500/550 high output kit. The quality of sequencing was confirmed using a Phred quality score. The sequencing data was next assessed using FastQC (Babraham Bioinfomatics, Cambridge, UK) and then mapped to the mouse genome (UCSC mm10) using STAR RNA-seq aligner (Dobin et al, 2013) with the parameter: "—outSAMmapqUNIQUE 60". Uniquely mapped sequencing reads were assigned to mm10 refGene genes using featureCounts (from subread) (Liao et al, 2014). Genes with readcount per million (CPM) >0.5 in more than 3 of the samples were kept. The data were normalized using TMM (trimmed mean of M values) method.

References:

Dobin A, Davis CA, Schlesinger F, Drenkow J, Zaleski C, Jha S, Batut P, Chaisson M, Gingeras TR. STAR: ultrafast universal RNA-seq aligner. *Bioinformatics.* 2013;29(1):15-21.

Liao Y, Smyth GK, Shi W. FeatureCounts: an efficient general purpose program for assigning sequence reads to genomic features. *Bioinformatics.* 2014;30(7):923-930.

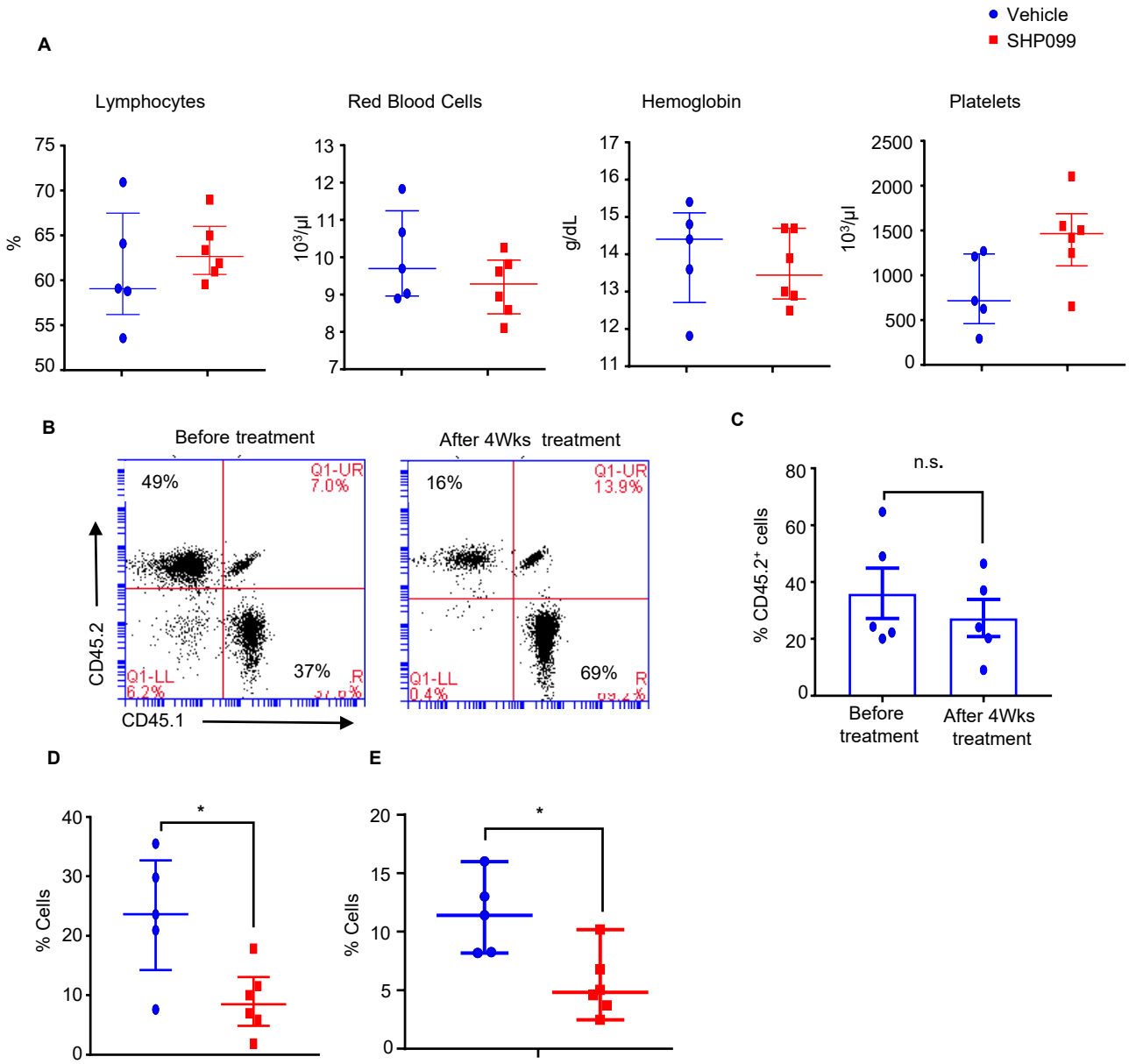

Figure S1: Mice transplanted with *Tet2*$^{-/-}$*Flt3*^{ITD} BM cells along with normal BM cells were treated with vehicle (n=5) or SHP099 (n=6) for 4 weeks and peripheral blood was analyzed for (A) percent lymphocytes, red blood cells, hemoglobin and platelet numbers. (B) Representative flow plots showing expression of CD45.1 and CD45.2 in PB of leukemic mice before and after treatment with SHP099. (C) Percent CD45.1^{-}CD45.2^{+} (leukemic cells) in peripheral blood in the vehicle treated mice. (D) Percent CD45.1^{-}CD45.2^{+} cells in the spleen of vehicle and SHP099 treated mice. (E) Percent CD45.1^{-} CD11b^{-}KIT^{+} cells in spleen. Data points shown are values from individual mice in each group. Median value for each group is indicated with the interquartile range. * p<0.05, ** p<0.001 Students't' test with Welch's correction for unequal variance.

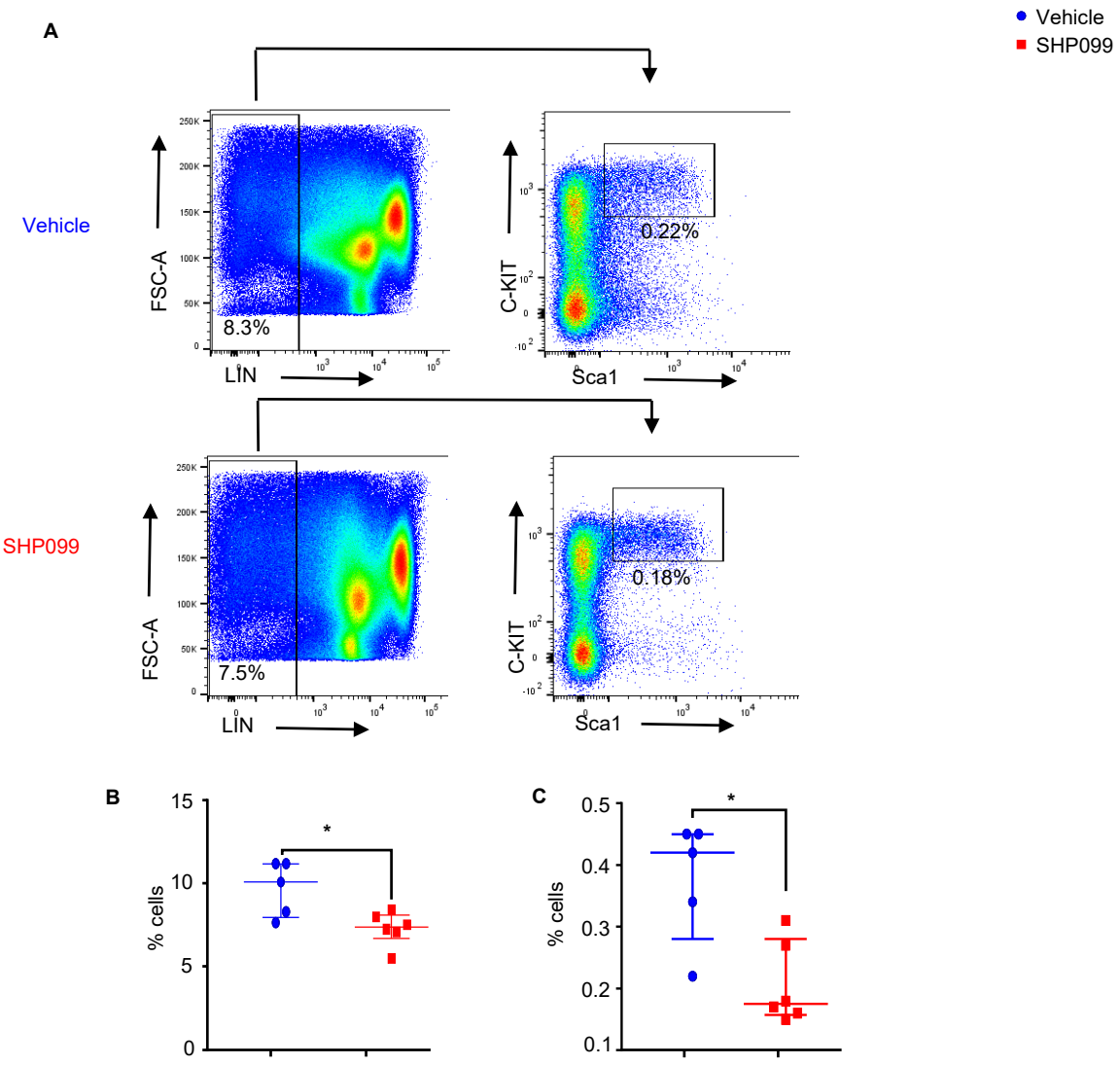

Figure S2: Mice transplanted with *Tet2*$^{-/-}$*Flt3*^{ITD} BM cells along with normal BM cells were treated with vehicle (n=5) or SHP099 (n=6) and BM progenitor cells were analyzed by flow cytometry. (A) representative flow plots of bone marrow cells gated on CD45.1^{-} cells from vehicle (top panel) and SHP099 (bottom panel) treated mice showing the gating and analysis of stem and progenitor cell compartment. Quantification of (B) percent lin^{-} cells and (C) percent lin^{-}KIT^{+}Sca1^{+} cells within the CD45.1^{-} gate. Data points shown are values from individual mice in each group. Median value for each group is indicated with the interquartile range. * p<0.05, ** p<0.001 Student's 't' test with Welch's correction for unequal variance.