BMP2/SMAD pathway activation in JAK2/p53-mutant Megakaryocyte/Erythroid Progenitors Promotes Leukemic Transformation

Supplemental Methods:

Murine model

We generated *Rosa-Cre*ERT2, *Jak2* $^{\text{Lox-STOP-Lox (LSL) V617F/+}}$ (J^{VF}), *Rosa-Cre*ERT2 *Jak2* $^{\text{LSL V617F/+}}$ *Trp53*$^{\text{flox (fl)/+}}$ ($J^{VF}P^{+/-}$), *Rosa-Cre*ERT2 *Jak2* $^{\text{LSL V617F/+}}$ *Trp53*$^{\text{fl/fl}}$ ($J^{VF}P^{-/-}$), *Rosa-Cre*ERT2 *Jak2* $^{\text{LSL V617F/+}}$ *Trp53* $^{\text{LSL R172H /+}}$ ($J^{VF}P^{R172H/+}$), *Rosa-Cre*ERT2, *Jak2* $^{\text{LSL V617F/+}}$ *Trp53* $^{\text{LSL R172H /fl}}$ ($J^{VF}P^{R172H/-}$). *Trp53* $^{\text{R172H}}$ corresponds to human *TP53*$^{\text{R175H}}$. At 8 to 10 weeks old, these mice were given tamoxifen to induce mutant *Trp53* and *Jak2* expression from the endogenous locus and/or simultaneously delete *Trp53* expression. *Rosa-Cre*ERT2 mice treated with tamoxifen were utilized as wild type (WT) control mice (**Supplemental Figure 1A**).

Murine transplantation

Primary transplant, noncompetitive: Freshly dissected femurs and tibias were isolated from 8-10 weeks old *Rosa-Cre*ERT2, *Jak2* $^{\text{LSL V617F/+}}$ *Rosa-Cre*ERT2, *Jak2* $^{\text{LSL V617F/+}}$ *Trp53*$^{\text{fl/+}}$ *Rosa-Cre*ERT2, *Jak2* $^{\text{LSL V617F/+}}$ *Trp53*$^{\text{fl/fl}}$ *Rosa-Cre*ERT2, *Jak2* $^{\text{LSL V617F/+}}$ *Trp53* $^{\text{LSL R172H /+}}$ *Rosa-Cre*ERT2 and *Jak2* $^{\text{LSL V617F/+}}$ *Trp53* $^{\text{LSL R172H /fl}}$ *Rosa-Cre*ERT2 mice after 2 weeks tamoxifen oral gavage. Bones were transected at the epiphyses and were centrifuged at 4℃ to extract whole BM cells into PBS plus 2% FBS and RBCs were lysed in ammonium chloride-potassium bicarbonate (ACK) lysis buffer for 10 min. After centrifugation, cells were re-suspended in PBS plus 2% FBS, passed through a cell strainer, and counted. A total of 2×10^6 cells were transplanted via tail-vein injection into lethally irradiated (1×900 cGy) CD45.1 host mice (The Jackson Laboratory).

Primary transplant, competitive: *Jak2* $^{\text{LSL V617F/+}}$ tdTomato *Rosa-Cre*ERT2, *Jak2* $^{\text{LSL V617F/+}}$ *Trp53*$^{\text{fl/fl}}$ *Rosa-Cre*ERT2 and *Jak2* $^{\text{LSL V617F/+}}$ *Trp53* $^{\text{LSL R172H /fl}}$ *Rosa-Cre*ERT2 mice were used for competitive transplant. BM single cells suspension was prepared as

described above. 1×10^6 cells from *Jak2* $^{\text{LSL V617F/+}}$ tdTomato *Rosa-Cre*ERT2 and 1×10^6 cells from *Jak2* $^{\text{LSL V617F/+}}$ *Trp53*$^{\text{fl/fl}}$ *Rosa-Cre*ERT2 or *Jak2* $^{\text{LSL V617F/+}}$ *Trp53* $^{\text{LSL R172H /fl}}$ *Rosa-Cre*ERT2 in 1:1 ratio were transplanted via tail-vein injection into lethally irradiated CD45.1 host mice. Oral gavage of tamoxifen was run at 2 weeks after transplant. Chimerism was followed via FACS in the peripheral blood every 4 weeks (week 1, 5, 9 and 13 after tamoxifen induction).Chimerism in the BM was evaluated at 13 weeks via animal sacrifice and subsequent flow cytometry analysis.

Secondary transplant and tertiary transplant (whole spleen cells): Spleens from $J^{VF}P^{-/-}$ and $J^{VF}P^{R172H/-}$ PEL mice were isolated and single-cell suspensions were made by mechanical disruption using glass slides. RBCs were lysed in ACK lysis buffer for 10 min. After centrifugation, cells were re-suspended in PBS plus 2% FBS, passed through a cell strainer, and counted. A total of 1×10^6 cells were transplanted via tail-vein injection into lethally irradiated CD45.1 host mice.

Secondary transplant (fractionated HSPCs): $J^{VF}P^{-/-}$ and $J^{VF}P^{R172H/-}$ mice at PEL or MPN stage were used for this transplant. BM single cells suspension was prepared as described above. Lineage negative BM cells were separated via lineage cell depletion kit (Miltenyi 130-110-470). MEPs (Lin-c-Kit+Sca-1-CD34-FcgRII/III-), GMPs (Lin-c-Kit+Sca-1-CD34+FcgRII/III+) and LSKs (Lin-c-Kit+Sca-1+) were isolated by FACS. For PEL MEPs transplant, 5000 CD45.2+MEPs, 5000 CD45-MEPs or CD150+MEPs with 1×10^6 supporter CD45.1+ BM cells were transplanted via tail-vein injection into lethally irradiated CD45.1 host mice. For MPN-MEPs, GMPs and LSKs transplant, 20,000 MEPs, 20,000 GMPs or 4,000 LSKs with 1×10^6 supporter CD45.1+ BM cells were transplanted via tail-vein injection into lethally irradiated CD45.1 host mice. For transduced erythroleukemic blasts (Lin-CD45-c-Kit+ cells from spleen) transplant, 5000 GFP+ erythroleukemic blasts with 1×10^6 supporter CD45.1+ BM cells were transplanted via tail-vein injection into lethally irradiated CD45.1 host mice.

Secondary transplant (in vivo treatment test): $J^{VF}P^{-/-}$ mice at PEL-stage were used for this transplant.10,000 whole BM cells from leukemic $J^{VF}P^{-/-}$ with 1×10^6 CD45.1 support BM cells were transplanted into lethally irradiated CD45.1 recipients. Mice were allowed to engraft for 2 weeks, and then were randomized to treatment.

All donor mice are CD45.2+.

Flow Cytometry and FACS

Antibodies for surface markers: c-Kit (2B8), Sca-1 (D7), Mac-1/CD11b (M1/70), Gr-1 (RB6-8C5), NK1.1 (PK136), Ter-119, CD34 (RAM34), FcgRII/III (2.4G2), CD4 (RM4-5), CD8 (53-6.7), CD19 (HIB19), CD45.1 (A20), CD45.2 (104), CD45R/B220 (RA3-6B2), CD71 (R17217), CD41(MWReg30), CD55(RIKO-3), CD105 (MJ7/18), CD150 (9D1), and CD48 (HM48-1). All were purchased from Biolegend, BD Biosciences or Invitrogen.

Antibodies for intracellular markers: Alexa 488-conjugated anti-gH2AX (S139) antibody (Biolegend 613405) and isotype IgG1κ (Biolegend 400134), Alexa 488-conjugated anti-pHH3 (S10) antibody (Biolegend 650803) and isotype IgG2bκ (Biolegend 400329), Propidium iodide (PI)/RNase solution (BD Biosciences 550825), phospho-Smad1 (Ser463/465)/Smad5 (Ser463/465)/Smad9 (Ser465/467) (D5B10) Rabbit mAb (CST13820) and Alexa 488 conjugated anti-rabbit IgG (CST 4412).

Instruments: Cell populations were analyzed using a Fortessa Flow Cytometer (BD) and sorted with a FACS-SH800 (Sony).

Histology

Hematoxylin and eosin (H&E) staining and immunohistochemistry (IHC) were performed on Formalin-fixed paraffin-embedded tissue sections in the Laboratory of Comparative Pathology (LCP) at MSKCC. Peripheral blood was smeared on a slide and stained using the Wright-Giemsa staining method. The following antibodies were used for IHC: CD45 (30-F11, BD Bioscience 550539), CD117 (D13A2, Cell Signaling 3074), GATA1 (N6, Santa Cruz sc-265), CD71 (EPR20584, Abcam ab214039), Ter119 (TER-119, BD Bioscience 550565), CD41 (EPR17876, Abcam ab181582) and BMP2 (Polyclonal, Novus NBP1-19751).

Sparse whole genome sequencing and copy number inference:

Copy number alterations were inferred from sparse whole genome sequencing data as described previously (1, 2). In brief, 1μg of bulk genomic DNA (gDNA) was extracted from isolated cells using a Qiagen DNA isolation kit, with DNA sonicated using the Covaris instrument. Sonicated DNA was subsequently end-repaired/A-tailed, followed by ligation of TruSeq dual indexed adaptors. Indexed libraries were enriched via PCR

and sequenced in multiplex fashion using the Illumina HiSeq2500 instrument to achieve roughly 1 million uniquely mappable reads per sample – a read count sufficient to allow copy number inference to a resolution of approximately 400kb. For data analysis, uniquely mapped reads where counted in genomic bins corrected for mappability. Read counts where subsequently corrected for GC content, normalized and segmented using Circular Binary Segmentation (CBS). Segmented copy number calls are illustrated as relative gains and losses to the median copy number of the entire genome.

Bmp2 knockdown and overexpression

Lentiviral constructs expressing pGFP-C-*Bmp2* shRNA and pGFP only were purchased from OriGene (Catalog# TL513403, Lot#12156.). Whole spleen cells were positively selected for c-Kit (CD117) using antibodies conjugated to magnetic beads (Miltenyi 130-091-224) and separated using MACS columns and separator (Miltenyi). c-Kit+ spleen cells were transduced at a cell density of 1×10^6 using virus concentrated through Amicon Ultra-15 Centrifugal Filter (Millipore). After 48 hours, Lin-CD45-c-Kit+ GFP+ cells were sorted by FACS and either used for in vitro colony-forming assays or transplanting in to lethally irradiated recipient mice. The mouse *Bmp2* cDNA was cloned in MIGR1 (Addgene plasmid # 27490). Whole BM was positively selected for c-Kit (CD117) using antibodies conjugated to magnetic beads and separated using MACS columns and separator. c-Kit+ BM cells were transduced at a cell density of 1 $\times10^6$ using viral supernatant. After 48 hours, GFP expressed Lin-c-Kit+ cells were sorted by FACS and used for in vitro colony-forming assays. Knockdown/overexpression was confirmed using qRT-PCR and western blot.

RT-qPCR

Total RNA was isolated using the Trizol and cDNA was synthesized using the high-capacity cDNA reverse transcription kit (Thermo Fisher, 4374966). Primers were listed below.

Gene	**Forward (**5’-3’**)**	**Reverse (**5’-3’**)**
Bmp2	AACACCGTGCGCAGCTTCCATC	CGGAAGATCTGGAGTTCTGCAG

Bmp4	GCCGAGCCAACACTGTGAGGA	GATGCTGCTGAGGTTGAAGAGG
Gapdh	CATCACTGCCACCCAGAAGACTG	ATGCCAGTGAGCTTCCCGTTCAG

Western blot

The following antibodies were used for western blot analysis: β-Actin (13E5, CST 4970), p53 (1C12, CST 2524), BMP2 (Polyclonal, Novus NBP1-19751), Phospho-Rb (Ser780) (D59B7, CST 8180), Rb (D20, CST 9313), Phospho-CHK1 (Ser317) (D12H3, CST 12302), CHK1 (2G1D5, CST 2360), Phospho-RPA32 (Ser4, Ser8) (Polyclonal, Benthyl A300-245), RPA32 (Polyclonal, Benthyl A300-244), RAD51 (D4B10, CST 8875), Phospho-CDC2 (Tyr15) (Polyclonal, Cell Signaling 9111), CDC2 (BD Transduction Laboratories 610037), Poly/Mono-ADP Ribose (E6F6A, CST 83732) and Caspase-3 (Polyclonal, CST 9662).

RNA-Seq data analysis

Fastq files were mapped to the mouse genome (mm10) and reads counts per gene were quantified using STAR (3) with default parameters. Differentially expressed genes (DEGs) were identified with DESeq2 (4), with a fold change cutoff of ± 2 and a FDR adjusted P-value of 0.05. The heatmaps were plotted using the R package, heatmap. GO and KEGG enrichment analyses were conducted with the cluster Profiler package and Gene Set Enrichment Analysis (GSEA) desktop software. For GSEA, gene lists were sorted by correlation and ranked GSEA was performed.

Single cell RNA-sequencing data analysis

Raw sequencing data from the Illumina NovaSeq were aligned to mouse mm10 by CellRanger (3.1.0). Seurat version 3 was used to perform quality control, count normalization, and clustering on the single cell transcriptomic data using standard methods as follows: unique molecular identifiers (UMIs) which barcode each individual mRNA molecule within a cell during reverse transcription were used to remove PCR duplicates. Cells expressing fewer than 200, or greater than 5,000 genes were removed to exclude non-cells or cell aggregates. Cells expressing greater than 50 percent mitochondrial related genes were also removed, resulting in a final collection

of 24,153 cells. After quality control, the objects of wild type, *Jak2* $^{V617F/+}$, MPN *Jak2* $^{V617F/+}$ *Trp53* $^{-/-}$ and PEL *Jak2* $^{V617F/+}$ *Trp53* $^{-/-}$ samples were merged. Normalization of the integrated object was run by Seurat. Single cell data then were processed for dimension reduction and unsupervised clustering by following the workflow in Seurat. In brief, a total 2,000 highly-variable genes were selected for downstream analysis. RunPCA function was implemented with default parameters. The FinderNeighbours function was used and took first 20 principal components as input for construct a K-nearest neighbor (KNN) graph. The Finder Clusters function with default parameters and a resolution of 0.45 implements modularity optimization technique to iteratively group cells together in order to cluster the cells. Non-linear dimensional reduction technique t-SNE was used to visualize the results. The Find All Markers function were used to identify differentially expressed genes (markers) in each cell cluster or groups of clusters using the following parameters: only.pos = TRUE, min.pct = 0.25, logfc.threshold = 0.25. For pathway (gene set) scoring, the gene lists were applied as inputs in the AddModuleScore function with default parameters, which calculates module scores for feature expression programs on single cell level. For copy number variation (CNV) analysis, initial CNVs for each region were estimated by inferCNV R package (https://github.com/broadinstitute/inferCNV). Raw count data were extracted from the Seurat object by "GetAssayData()" function. For the inferCNV analysis the following parameters were used: "denoise," default hidden Markov model (HMM) settings, and a value of 0.1 for "cutoff." The chromosomal expression patterns were estimated from the moving averages of 101 genes as the window size and adjusted as centered values across genes.

Drugs

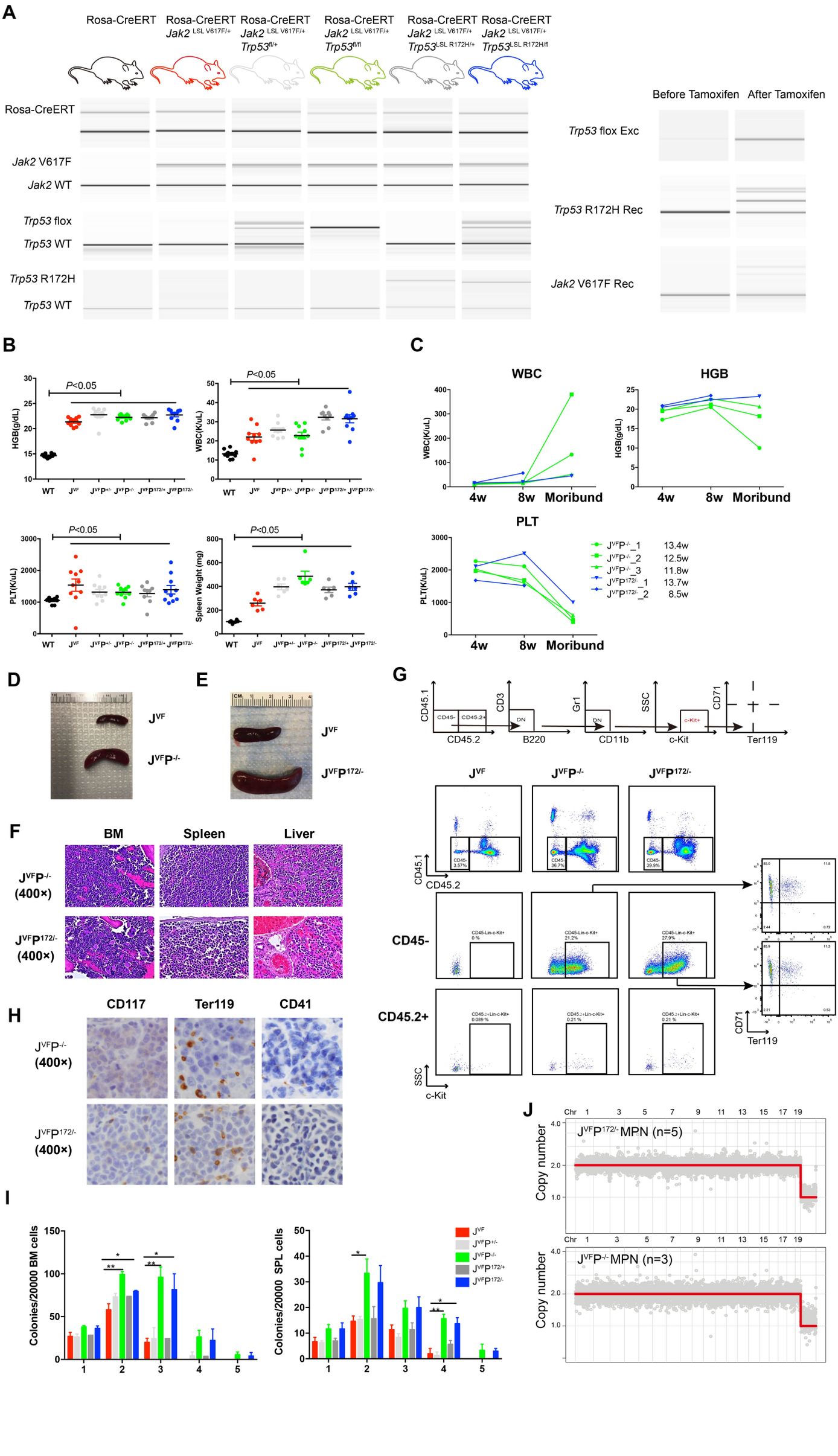
WEE1 inhibitor Adavosertib and the PARP inhibitor Olaparib were provided by AstraZeneca.

References

1. Baslan T, Kendall J, Ward B, Cox H, Leotta A, Rodgers L, et al. Genome Res. 2015; 25(5): 714-24.
2. Baslan T, Kendall J, Rodgers L, Cox H, Riggs M, Stepansky A, et al. Nat Protoc. 2012; 7(6): 1024-41.
3. Dobin A, Davis CA, Schlesinger F, Drenkno J,Zalesski C, Jha S, Batut, et al.

Bioinformatics, et al. Bioinformatics. 2013. 29(1):15-21.
4. Love MI, Huber W, Anders S. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. Genome Biol. 2014; 15(12): 550.

Figure S1.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Rosa-CreERT Jak2 LSL V617F/+ Trp53fl/+
Rosa-CreERT Jak2 LSL V617F/+ Trp53fl/fl
Rosa-CreERT Jak2 LSL V617F/+ Trp53LSL R172H/+
Rosa-CreERT Jak2 LSL V617F/+ Trp53LSL R172H/fl
Before Tamoxifen
After Tamoxifen
Rosa-CreERT
Jak2 V617F
Jak2 WT
Trp53 flox
Trp53 WT
Trp53 R172H
Trp53 WT
Trp53 flox Exc
Trp53 R172H Rec
Jak2 V617F Rec
B
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HGB(g/dL)
WBC(K/uL)
PLT(K/uL)
Spleen Weight (mg)
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JVF
JVFP+/-
JVFP-/-
JVFP172/+
JVFP172/-
C
WBC
HGB
PLT
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HGB(g/dL)
PLT(K/uL)
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8w
Moribund
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JVFP172/-_2 8.5w
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JVFP172/- (400×)
G
CD45.1
CD45.2
CD3
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CD71
Ter119
JVF
JVFP-/-
JVFP172/-
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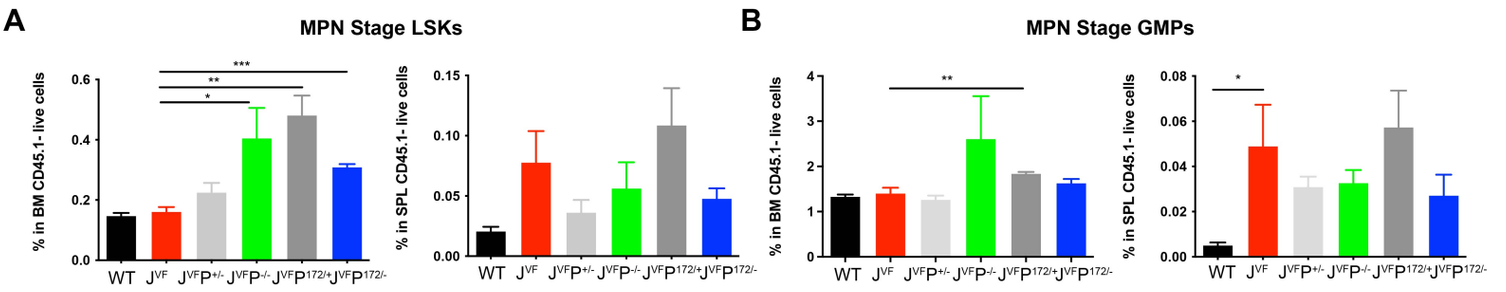
CD45.2+
H
CD117
Ter119
CD41
JVFP-/- (400×)
JVFP172/- (400×)
I
Colonies/20000 BM cells
Colonies/20000 SPL cells
JVF
JVFP+/-
JVFP-/-
JVFP172/+
JVFP172/-
J
Chr 1 3 5 7 9 11 13 15 17 19
Copy number
JVFP172/- MPN (n=5)
JVFP-/- MPN (n=3)

Figure S1. Related to Figure 1.

A. Genotyping analysis of different alleles using genomic DNA isolated from different groups of animals (left) and genotyping analysis to evaluate *Trp53* deletion or mutation and *Jak2* mutation in peripheral blood cells before and after tamoxifen treatment (right).
B. Peripheral blood (PB) count analysis of samples collected from recipient mice 8 weeks after transplant (MPN stage). Wild type (WT; n=10); *Jak2* $^{V617F/+}$ (J^{VF}; n=10); *Jak2* $^{V617F/+}$ *Trp53* $^{+/-}$ ($J^{VF}P^{+/-}$; n=10); *Jak2* $^{V617F/+}$ *Trp53* $^{-/-}$ ($J^{VF}P^{-/-}$; n=10); *Jak2* $^{V617F/+}$ *Trp53* $^{R172H/+}$ ($J^{VF}P^{172/+}$; n=9); *Jak2* $^{V617F/+}$ *Trp53* $^{R172H\ /-}$ ($J^{VF}P^{172/-}$; n=10). Spleen weight of recipient mice at 8 weeks after transplant (MPN stage) (n=6 for each group).
C. PB count analysis of samples collected from primary *Jak2* $^{V617F/+}$ *Trp53* $^{-/-}$ ($J^{VF}P^{-/-}$; n=3) and *Jak2* $^{V617F/+}$ *Trp53* $^{R172H\ /-}$ ($J^{VF}P^{172/-}$; n=2) mice at 4 weeks and 8 weeks after Tamoxifen exposure, and at moribund point. Legend indicates the time points when each mouse was moribund after Tamoxifen.
D. Whole spleen specimen from a representative moribund primary $J^{VF}P^{-/-}$ mouse.
E. Whole spleen specimen from a representative moribund primary $J^{VF}P^{172/-}$ mouse.
F. Histopathologic H&E sections of bone marrow (BM), spleen and liver from representative moribund primary $J^{VF}P^{-/-}$ and $J^{VF}P^{172/-}$ mice.
G. Gating strategy to analyze leukemia cell immunophenotye in PB by flow cytometry and representative flow cytometry analysis of PB cells from J^{VF}, PEL $J^{VF}P^{-/-}$ and PEL $J^{VF}P^{172/-}$.
H. Immunohistochemistry (IHC) of liver (CD117, Ter119 and CD41) from representative PEL $J^{VF}P^{-/-}$ and PEL $J^{VF}P^{172/-}$ mice. Magnification 400×.
I. Methylcellulose replating assay demonstrating enhanced replating capability of $J^{VF}P^{-/-}$ and $J^{VF}P^{172/-}$ hematopoietic stem and progenitor cells (HSPCs) at MPN stage (primary recipient mice, 8 weeks after transplant). N=3 for each genotype. Data are presented as comparison to J^{VF}.
J. Genome-wide copy number profile arising from primary transplant of BM cells derived from $J^{VF}P^{-/-}$ and $J^{VF}P^{172/-}$ donors at the MPN stage ($J^{VF}P^{-/-}$; n=3 and $J^{VF}P^{172/-}$; n=5).

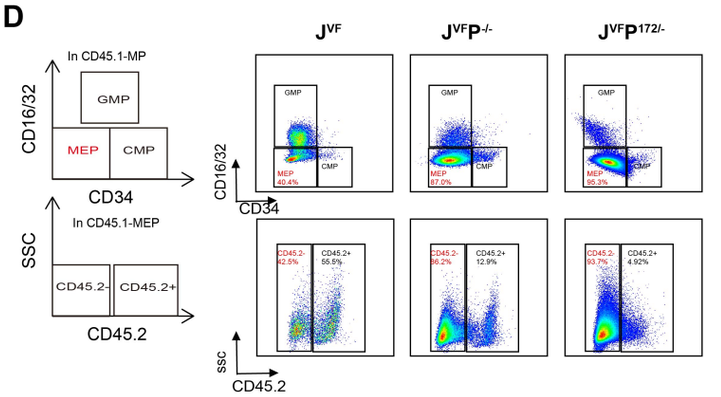
Data are represented as mean ± SEM. The unpaired Student's t test was used to compare the mean of two groups in B and I.* $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$

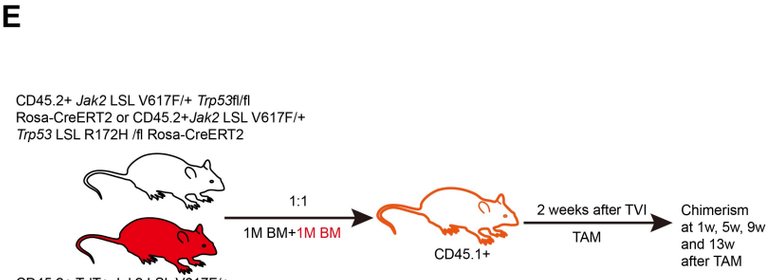
Figure S2.

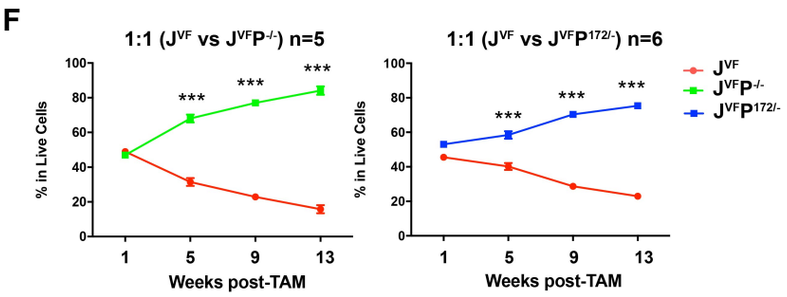


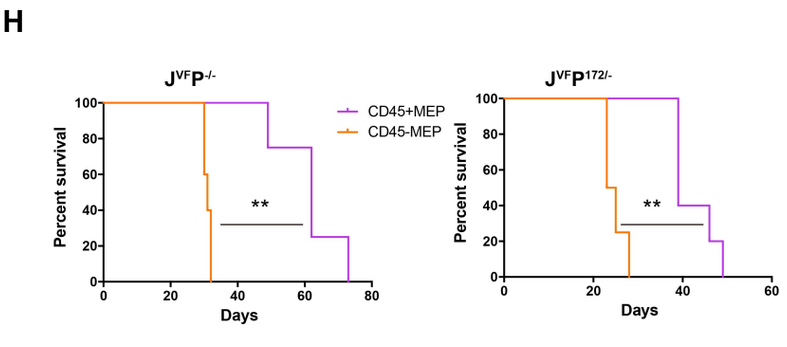
C

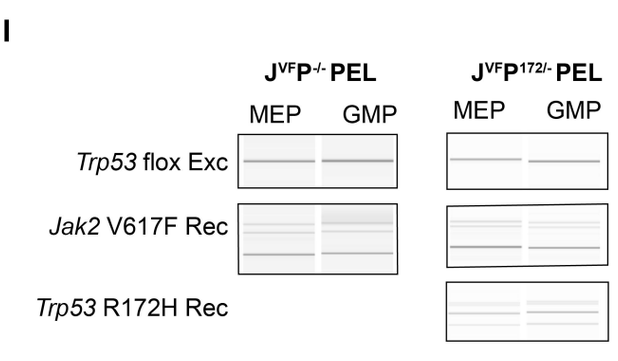
	MEP(BM/SPL)	GMP(BM/SPL)	LSK(BM/SPL)
$J^{VF}P^{-/-}$ vs WT	**/***	NS/**	*/NS
J^{VF}	*/**	NS/NS	*/NS
$J^{VF}P^{+/-}$	**/NS	NS/NS	NS/NS
$J^{VF}P^{172/-}$ vs WT	***/***	*/*	***/*
J^{VF}	**/**	NS/NS	***/NS
$J^{VF}P^{172/+}$	**/NS	NS/NS	*/NS











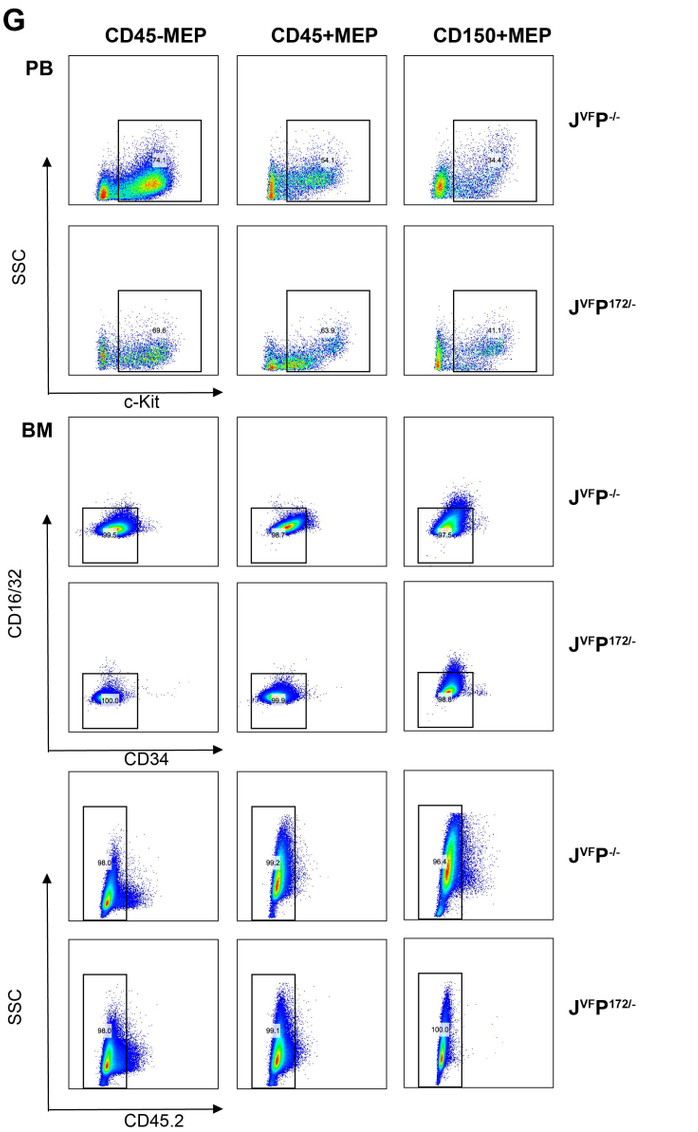
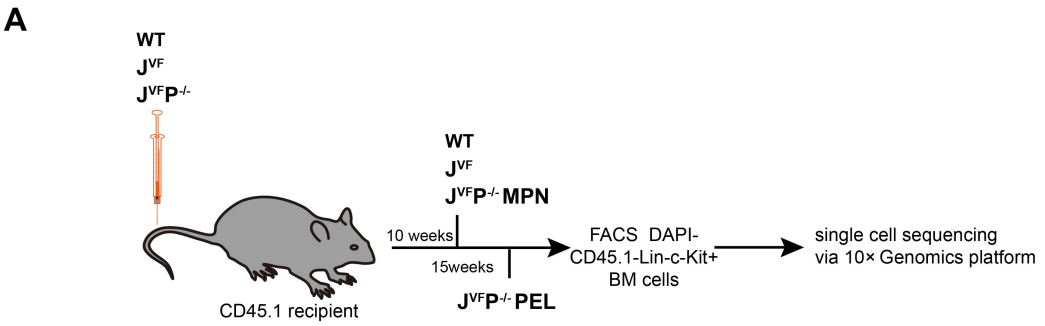


Figure S2. Related to Figure 2

A. Percentage of LSKs in BM (left) and SPL (right) at MPN-stage (8 weeks after transplantation) (n=5 mice for each group). Data are presented as comparison to J^{VF}. BM: $J^{VF}P^{-/-}$ *vs.* J^{VF} P=0.042; $J^{VF}P^{172/+}$ *vs.* J^{VF} P=0.0013; $J^{VF}P^{172/-}$ *vs.* J^{VF} P<0.0001.
B. Percentage of GMPs in BM (left) and SPL (right) at MPN-stage (n=5 mice for each group). Data are presented as comparison to J^{VF}. BM: $J^{VF}P^{172/+}$ *vs.* J^{VF} P=0.0078. SPL: WT *vs.* J^{VF} P=0.0439.
C. Statistic analysis for percentage of MEPs, GMPs and LSKs in BM and SPL at MPN-stage. NS indicate not significant.
D. Gating strategy to analyze MEPs in BM and SPL by flow cytometry and representative flow cytometry analysis of BM and SPL cells from J^{VF} , PEL $J^{VF}P^{-/-}$ and PEL $J^{VF}P^{172/-}$.
E. Experimental design to evaluate the competitive advantage of $J^{VF}P^{-/-}$ and $J^{VF}P^{172/-}$ HSPCs relative to *Jak2*^{VF} HSPCs at the MPN stage of disease in vivo. TAM indicate Tamoxifen.
F. Percentage of CD45.2+ chimerism in PB of $J^{VF}P^{-/-}$ or $J^{VF}P^{172/-}$ vs. J^{VF} recipient mice at 1 week, 5weeks, 9 weeks and 13 weeks post-Tamoxifen. Proportion of $J^{VF}P^{-/-}$ or $J^{VF}P^{172/-}$ cells at 5th, 9th and 13th week are compared to 1st week. $J^{VF}P^{-/-}$: P=0.0003, P<0.0001 and P<0.0001 for 5th, 9th and 13th, respectively. $J^{VF}P^{172/-}$: P<0.0001, P<0.0001 and P<0.0001 for 5th, 9th and 13th, respectively.
G. Representative flow cytometry analysis of leukemia cells in PB and MEPs in BM from recipients that were transplanted with CD45+MEPs, CD45-MEPs and CD150+MEPs from PEL $J^{VF}P^{-/-}$ and PEL $J^{VF}P^{172/-}$ mice.
H. Kaplan-Meier comparative survival analysis of recipients that were transplanted with CD45+MEPs and CD45-MEPs from PEL $J^{VF}P^{-/-}$ (CD45+MEPS, n=4; CD45-MEPs, n=5) and PEL $J^{VF}P^{172/-}$ (CD45+MEPS, n=5; CD45-MEPS, n=4). P value was determined by the log-rank test. $J^{VF}P^{-/-}$ P=0.006 and $J^{VF}P^{172/-}$ P=0.0025.
I. Genotyping analysis to evaluate *Trp53* deletion or mutation and *Jak2* mutation in MEPs and GMPs sorted from PEL $J^{VF}P^{-/-}$ and PEL $J^{VF}P^{172/-}$ BM.

Data are represented as mean ± SEM. The unpaired Student's t test was used to compare the mean of two groups in A, B and F.* P≤ 0.05, ** P ≤ 0.01, *** P ≤ 0.001

Figure S3.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WT
JVF
JVFP-/-
CD45.1 recipient
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JVF
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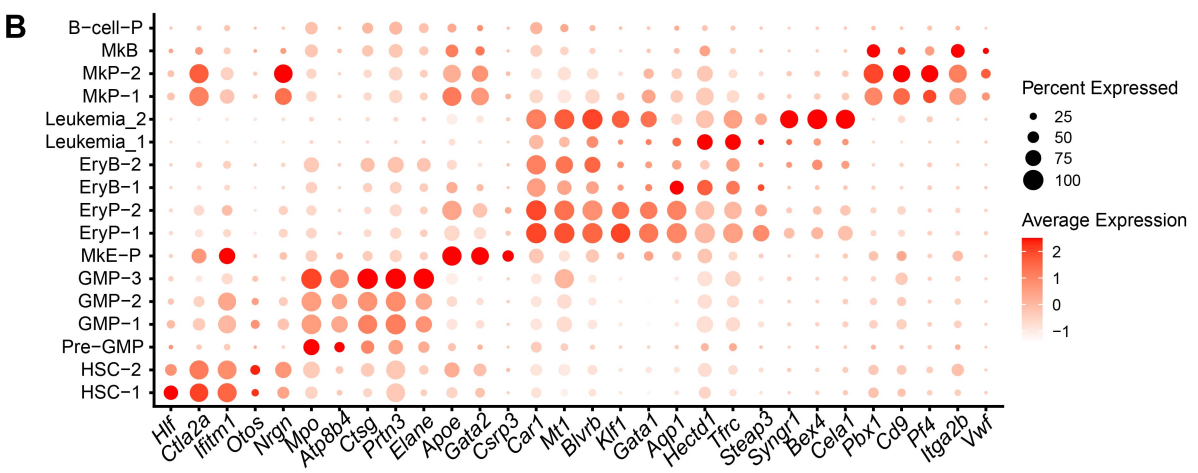
BM cells
single cell sequencing
via 10× Genomics platform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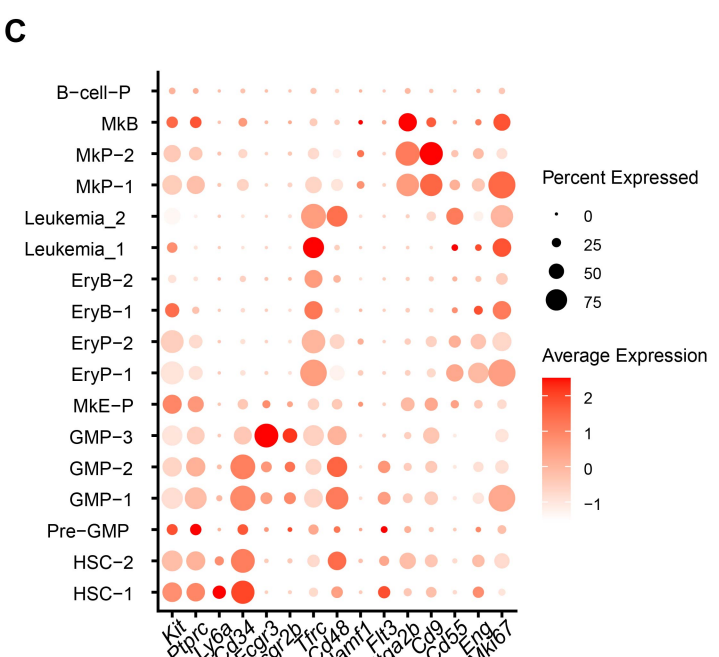
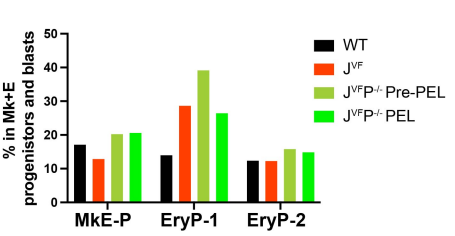
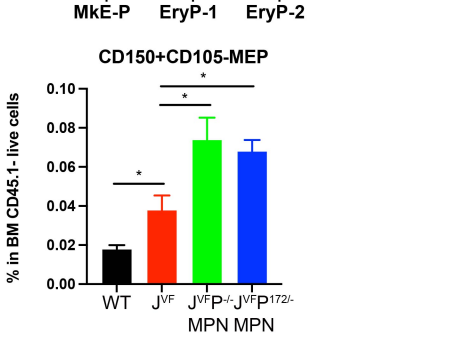
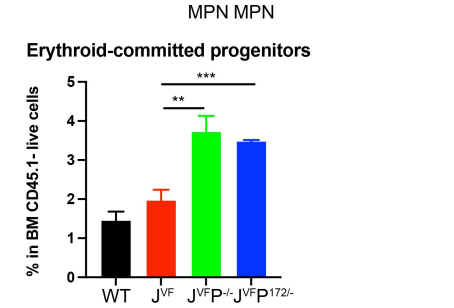
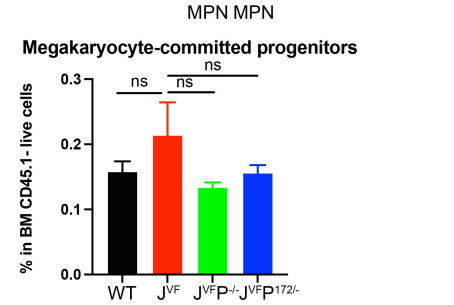
HSC−2
HSC−1
Hlf Ctla2a Ifitm1 Otos Nrgn Mpo Atp8b4 Ctsg Prtn3 Elane Apoe Gata2 Csrp3 Car1 Mt1 Blvrb Klf1 Gata1 Aqp1 Hectd1 Tfrc Steap3 Syngr1 Bex4 Cela1 Pbx1 Cd9 Pf4 Itga2b Vwf
Percent Expressed
25
50
75
100
Average Expression
2
1
0
−1

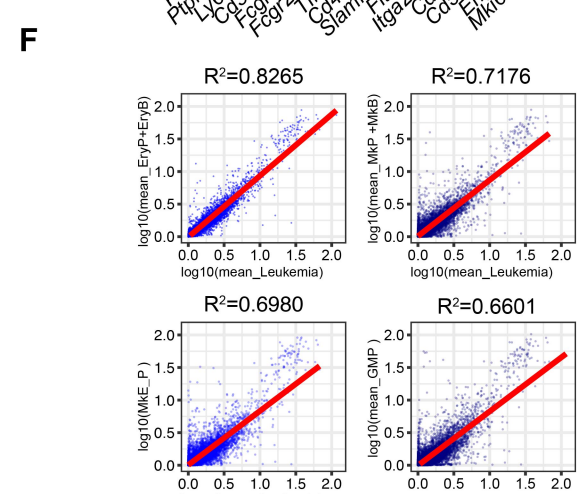

C
B−cell−P
MkB
MkP−2
MkP−1
Leukemia_2
Leukemia_1
EryB−2
EryB−1
EryP−2
EryP−1
MkE−P
GMP−3
GMP−2
GMP−1
Pre−GMP
HSC−2
HSC−1
Kit Ptprc Ly6a Cd34 Fcgr3 Fcgr2b Tfrc Cd48 Slamf1 Flt3 Itga2b Cd9 Cd55 Eng Mki67
Percent Expressed
0
25
50
75
Average Expression
2
1
0
−1


D
% in Mk+E progenistors and blasts
WT
JVF
JVFP-/- Pre-PEL
JVFP-/- PEL
MkE-P
EryP-1
EryP-2


E
CD150+CD105-MEP
% in BM CD45.1- live cells
WT
JVF
JVFP-/-
JVFP172/-
MPN MPN


Erythroid-committed progenitors
% in BM CD45.1- live cells
WT
JVF
JVFP-/-
JVFP172/-
MPN MPN


Megakaryocyte-committed progenitors
% in BM CD45.1- live cells
ns
WT
JVF
JVFP-/-
JVFP172/-
MPN MPN


F
R2=0.8265
R2=0.7176
R2=0.6980
R2=0.6601
log10(mean_EryP+EryB)
log10(mean_MkP +MkB)
log10(MkE_P)
log10(mean_GMP)
log10(mean_Leukemia)

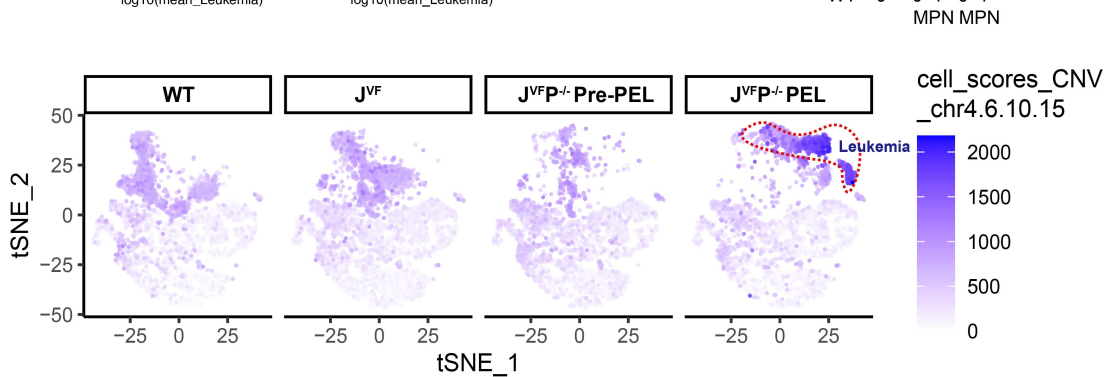
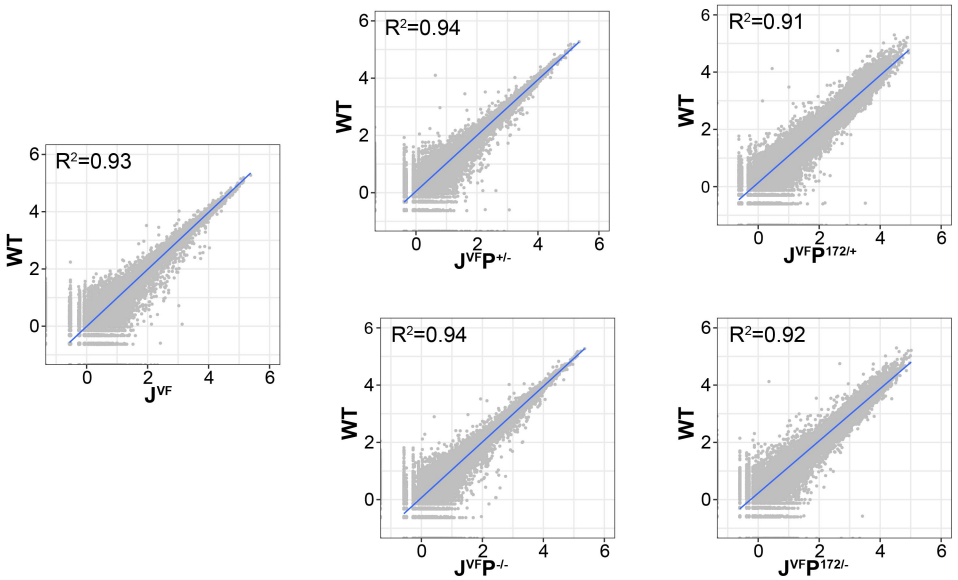

G
WT
JVF
JVFP-/- Pre-PEL
JVFP-/- PEL
Leukemia
cell_scores_CNV
_chr4.6.10.15
2000
1500
1000
500
0
tSNE_2
tSNE_1

Figure S3. Related to Figure 3.

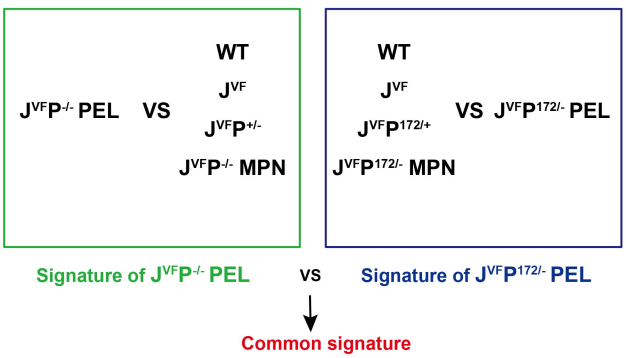
A. Experimental design for single-cell RNA-sequencing.
B. Representative differentially expressed genes (FDR < 0.05, logistic regression with Bonferroni correction) when comparing each cell cluster with the remaining clusters.
C. Expressed genes encoding conventional surface markers and *Mki*67 in each cell cluster. In B and C, the dot size encodes the fraction of cells within the cluster that show detectable expression of the gene (UMIs > 0), while the color encodes the average expression level across all cells within a cluster.
D. Percentage of MkE-P, EryP-1 and EryP-2 in progenitors and blasts of erythrocyte and megakaryocyte (MkE-P+ EryP-1+ EryP-2+ EryB-1+ EryB-2+ MkP-1+ MkP-2+MkB).
E. Percentage of CD150+CD105-MEPs (Lin-c-Kit+Sca-1-CD34-FcgRII/III-CD71-CD41-CD150+CD105-), Erythroid-committed progenitors (Lin-c-Kit+Sca-1-CD34-FcgRII/III-CD71+CD41-) and Megakaryocyte-committed progenitors (Lin-c-Kit+Sca-1-CD34-FcgRII/III-CD71-CD41+) in BM from MPN $J^{VF}P^{-/-}$, MPN $J^{VF}P^{172/-}$, J^{VF} and WT mice (n=5 for each group).
F. Pearson's correlation coefficient scatter plots of average normalized single cell RNA-seq data between Leukemia (Leukemia-1+Leukemia-2) vs. erythroid progenitors and erythroid blasts (EryP-1+EryP-2+EryB-1+EryB-2), MkE-P, GMPs (GMP-1+GMP-2+GMP-3) or progenitors and blasts of megakaryoctye (MkP-1+MkP-2+MkB).
G. tSNE plots showing the accumulation scores of CNV based on chromosome 4, 6, 10, 15 from WT, J^{VF}, Pre-PEL $J^{VF}P^{-/-}$ and PEL $J^{VF}P^{-/-}$. The color key from white to blue indicates low to high CNV level, respectively.

Figure S4.

A



B



C

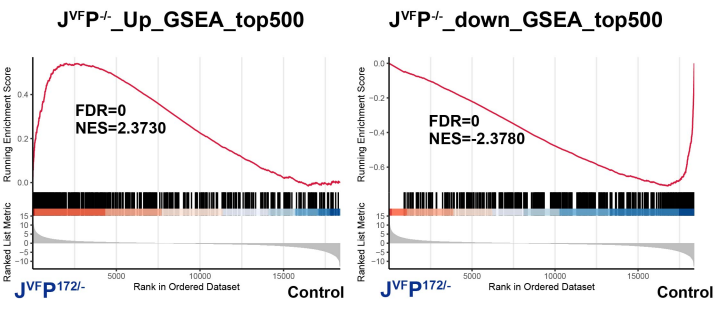
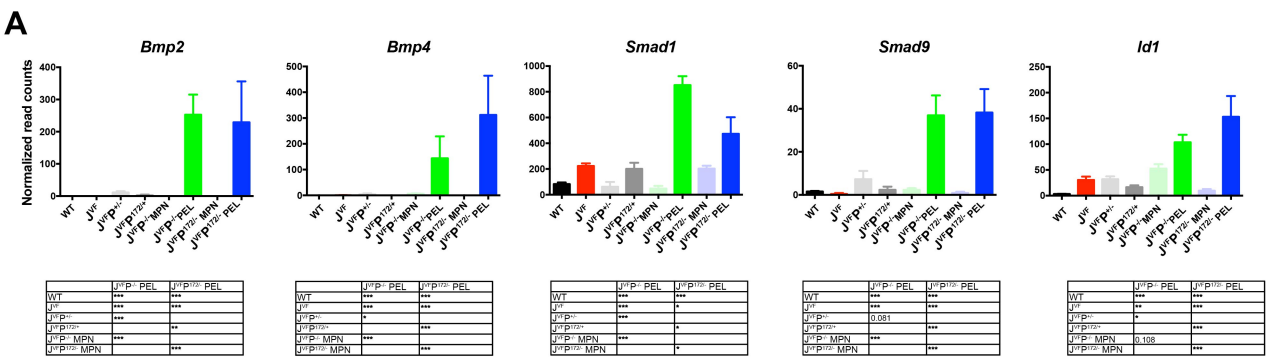
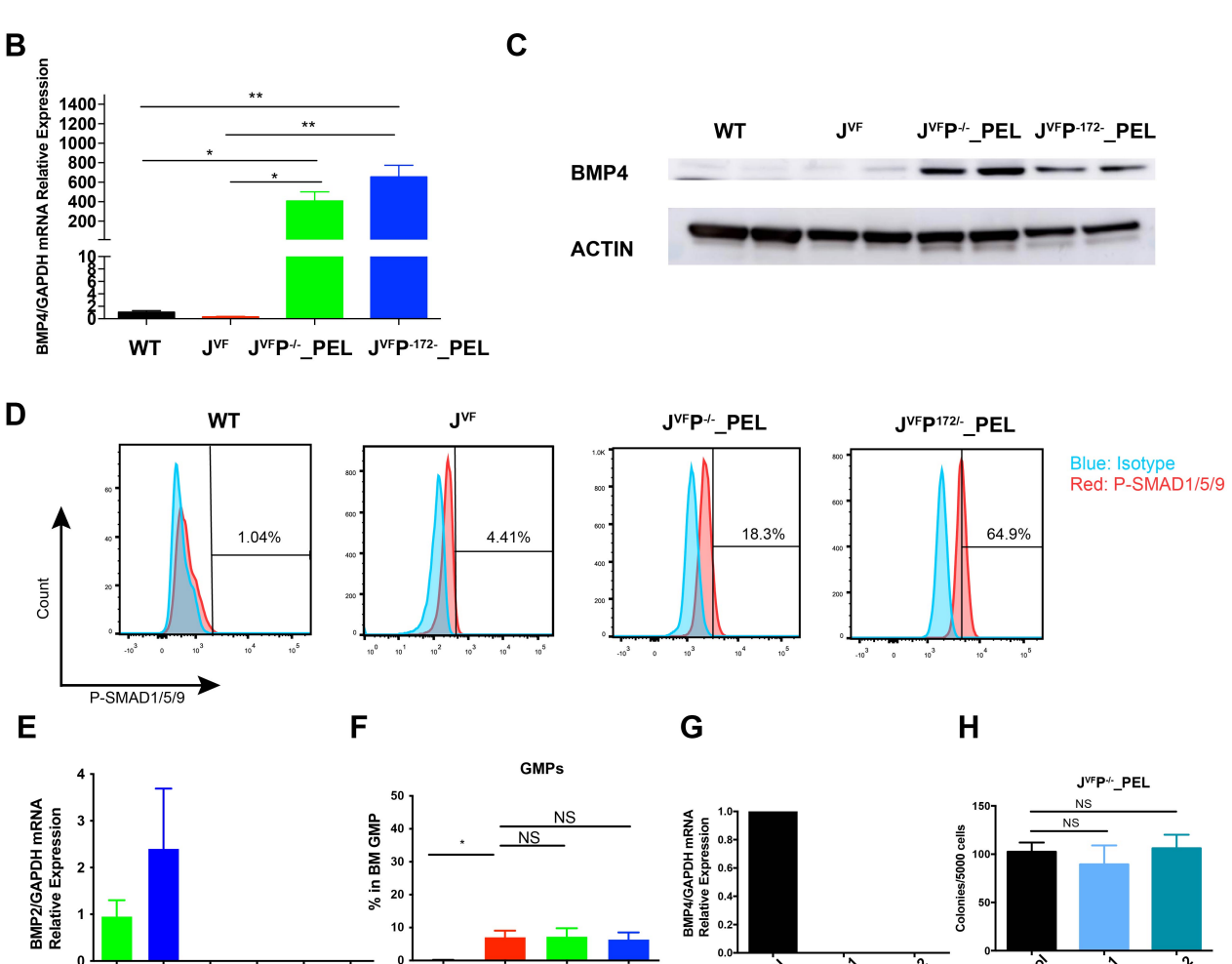


Figure S4. Related to Figure 4

A. Pearson's correlation coefficient scatter plots of bulk RNA-seq data between WT vs. J^{VF}, $J^{VF}P^{+/-}$, $J^{VF}P^{-/-}$, $J^{VF}P^{172/+}$ and $J^{VF}P^{172/-}$ LSKs. The normalized counts per million (CPM) value of each gene was set to a baseline of 1 and log-transformed for the correlation analysis.
B. Strategy for analyzing RNA-sequencing data from MEPs.
C. Gene set enrichment analysis (GSEA) comparing gene expression of $J^{VF}P^{172/-}$ PEL MEPs to signatures of $J^{VF}P^{-/-}$ PEL MEPs.

Figure S5.


A
Bmp2
Bmp4
Smad1
Smad9
Id1
Normalized read counts
WT
JVF
JVFP+/-
JVFP172+/-
JVFP+/-MPN
JVFP+/-PEL
JVFP172-MPN
JVFP172-PEL


B
BMP4/GAPDH mRNA Relative Expression
WT
JVF
JVFP-/-_PEL
JVFP-172-_PEL
C
WT
JVF
JVFP-/-_PEL
JVFP-172-_PEL
BMP4
ACTIN
D
WT
JVF
JVFP-/-_PEL
JVFP172/-_PEL
1.04%
4.41%
18.3%
64.9%
Blue: Isotype
Red: P-SMAD1/5/9
Count
P-SMAD1/5/9
E
BMP2/GAPDH mRNA Relative Expression
JVFP-/- MEP
JVFP172/- MEP
WT GMP
JVF GMP
JVFP-/- GMP
JVFP172/- GMP
F
GMPs
% in BM GMP
NS
G
BMP4/GAPDH mRNA Relative Expression
sh. Control
sh. Bmp4_1
sh. Bmp4_2
H
JVFP-/-_PEL
Colonies/5000 cells
NS

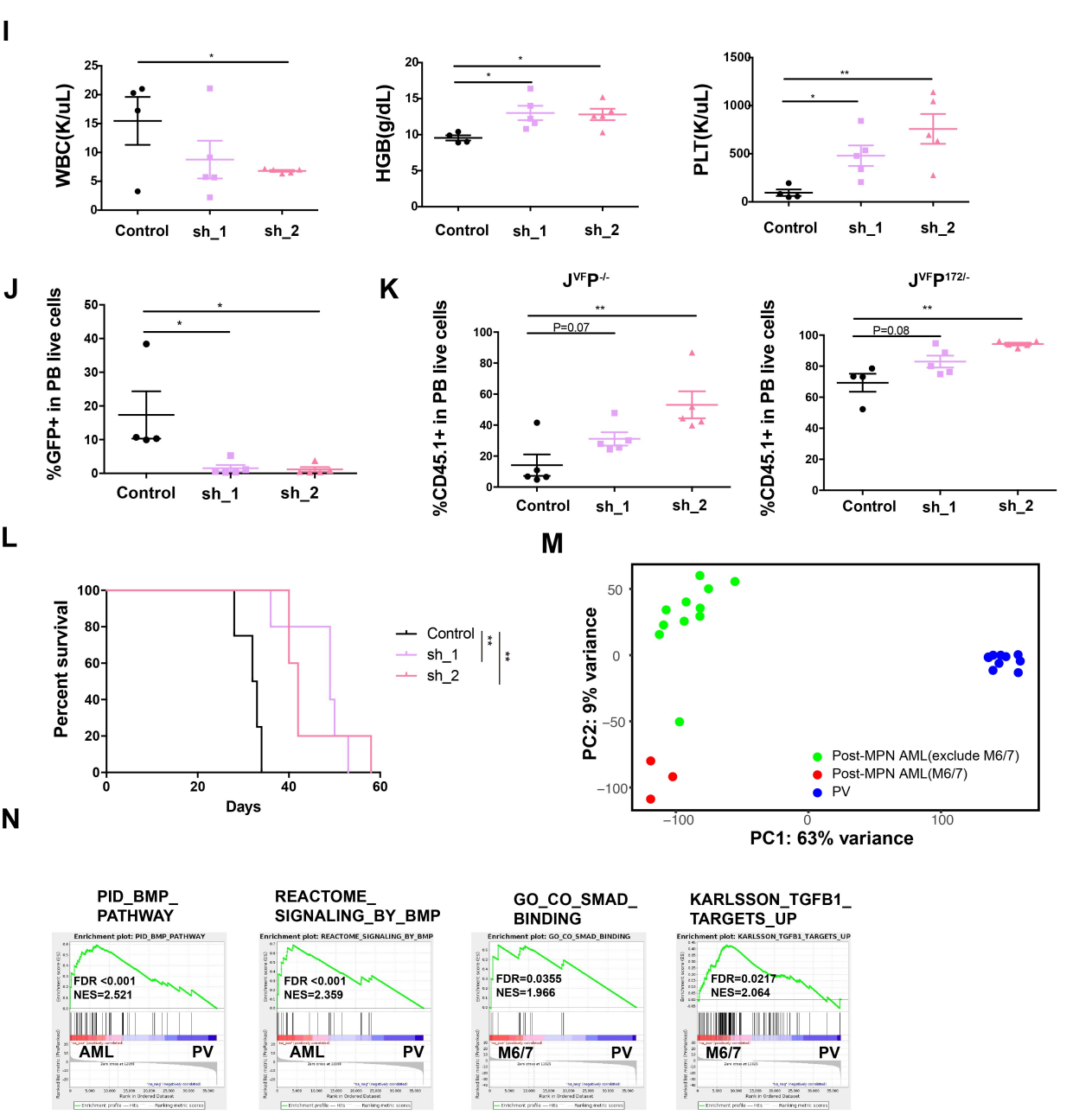

I
WBC(K/uL)
HGB(g/dL)
PLT(K/uL)
Control
sh_1
sh_2
J
%GFP+ in PB live cells
K
JVFP-/-
JVFP172/-
%CD45.1+ in PB live cells
P=0.07
P=0.08
L
Percent survival
Days
Control
sh_1
sh_2
M
PC2: 9% variance
PC1: 63% variance
Post-MPN AML(exclude M6/7)
Post-MPN AML(M6/7)
PV
N
PID_BMP_ PATHWAY
REACTOME_ SIGNALING_BY_BMP
GO_CO_SMAD_ BINDING
KARLSSON_TGFB1_ TARGETS_UP
FDR <0.001
NES=2.521
FDR <0.001
NES=2.359
FDR=0.0355
NES=1.966
FDR=0.0217
NES=2.064
AML
PV
M6/7
PV

Figure S5. Related to Figure 5.

A. Histogram represents the normalized read counts of *Bmp2*, *Bmp4*, *Smad1*, *Smad9* and *Id1* gene expression in MEPs from WT, J^{VF}, $J^{VF}P^{+/-}$, $J^{VF}P^{172/+}$, PEL $J^{VF}P^{-/-}$ (MPN and PEL) and PEL $J^{VF}P^{172/-}$ (MPN and PEL) mice (n=2-3 mice for each group). The FDR adjusted P values were calculated by DESeq2.

B. RT-qPCR for *Bmp4* in MEPs from independent WT, J^{VF}, PEL $J^{VF}P^{-/-}$ and PEL $J^{VF}P^{172/-}$ mice (n=4-6 mice for each group). $J^{VF}P^{-/-}$ *vs.* WT P=0.0132*;* $J^{VF}P^{-/-}$ *vs.* J^{VF} P=0.0131*;* $J^{VF}P^{172/-}$ *vs.* WT P=0.0053*;* $J^{VF}P^{172/-}$ *vs.* J^{VF} P=0.0053.

C. Western blot for BMP4 in c-kit+ spleen cells. Side-by side duplicates for each murine genotype are presented.

D. Representative examples of P-SMAD1/5/9 measured by flow cytometry. The percentages within the histogram plots indicate the fraction of P-SMAD1/5/9 positive cells within CD45-MEPs.

E. RT-qPCR for *Bmp2* in GMPs from WT, J^{VF}, PEL $J^{VF}P^{-/-}$ and PEL $J^{VF}P^{172/-}$ mice (n=3 mice per genotype).

F. The fraction of P-SMAD1/5/9 positive cells within GMPs measured by flow cytometry in WT (n=4), J^{VF}(n=10), PEL $J^{VF}P^{-/-}$ (n=6) and PEL $J^{VF}P^{172/-}$ mice (n=10).

G. RT-qPCR for *Bmp4* in $J^{VF}P^{-/-}$ erythroleukemic blasts with and without *Bmp4* knock down.

H. Total number of CFUs generated by $J^{VF}P^{-/-}$ erythroleukemic blasts containing sh. Control, sh.*Bmp4*_1 and sh.*Bmp4*_2 (n=2 for each). NS indicates not significant.

I. PB count of recipients transplanted with $J^{VF}P^{172/-}$ erythroleukemic blasts (Lin-CD45-c-Kit+ cells from spleen) containing sh. Control (Control), sh. *Bmp2*_1 (sh_1) and sh. *Bmp2*_2 (sh_2) at 3 weeks after transplantation. N=4-5 mice for each arm**.** WBC: sh_1 *vs*. Control P=0.2387; sh_2 *vs*. Control P=0.0492. HGB: sh_1 *vs*. Control P=0.022; sh_2 *vs*. Control P=0.0109. PLT: sh_1 *vs*. Control P=0.018; sh_2 *vs*. Control P=0.0075.

J. Percentage of GFP+ cells in PB samples in recipients transplanted with $J^{VF}P^{172/-}$ erythroleukemic blasts containing sh. Control (Control), sh. *Bmp2*_1 (sh_1) and sh. *Bmp2*_2 (sh_2) at 3 weeks after transplantation. N=4-5 mice for each arm. sh_1 *vs*. Control P=0.0395; sh_2 *vs*. Control P=0.0355.

K. Percentage of CD45.1+ cells in PB samples in recipients transplanted with $J^{VF}P^{-/-}$ or $J^{VF}P^{172/-}$ erythroleukemic blasts containing sh. Control (Control), sh. *Bmp2*_1 (sh_1) and sh. *Bmp2*_2 (sh_2) at 3 weeks after transplantation. N=4-5 mice for each arm. $J^{VF}P^{-/-}$: sh_1 *vs*. Control P=0.07; sh_2 *vs*. Control P=0.008. $J^{VF}P^{172/-}$: sh_1 *vs*. Control P=0.0806; sh_2 *vs*. Control P=0.002.

L. Kaplan-Meier comparative survival analysis of recipients that were transplanted with $J^{VF}P^{172/-}$ erythroleukemic blasts containing sh. Control (Control), sh. *Bmp2*_1 (sh_1) and sh. *Bmp2*_2 (sh_2). P value was determined by the log-rank test. N=4-5 mice for each arm. sh_1 *vs*. Control P=0.0027; sh_2 *vs*. Control P=0.0027.

M. Principal component analysis (PCA) based on bulk RNA-sequencing data from post-MPN AML (excluding M6 or M7 cases) (n=11), post-MPN AML M6 or M7 (n=3) and PV (n=9). The top two components (PC1 and PC2) of PCA results were used for plot in the plane.

N. Gene set enrichment analysis (GSEA) comparing gene expression of post-MPN AML (include M6 and M7, n=14) vs. PV (n=9) and post-MPN M6 and M7 (n=3) vs. PV (n=9) to previously described signatures related to BMP.

Data are represented as mean ± SEM. The unpaired Student's t test was used to compare the mean of two groups in B, F, H, I, J and K.* $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$

Figure S6.

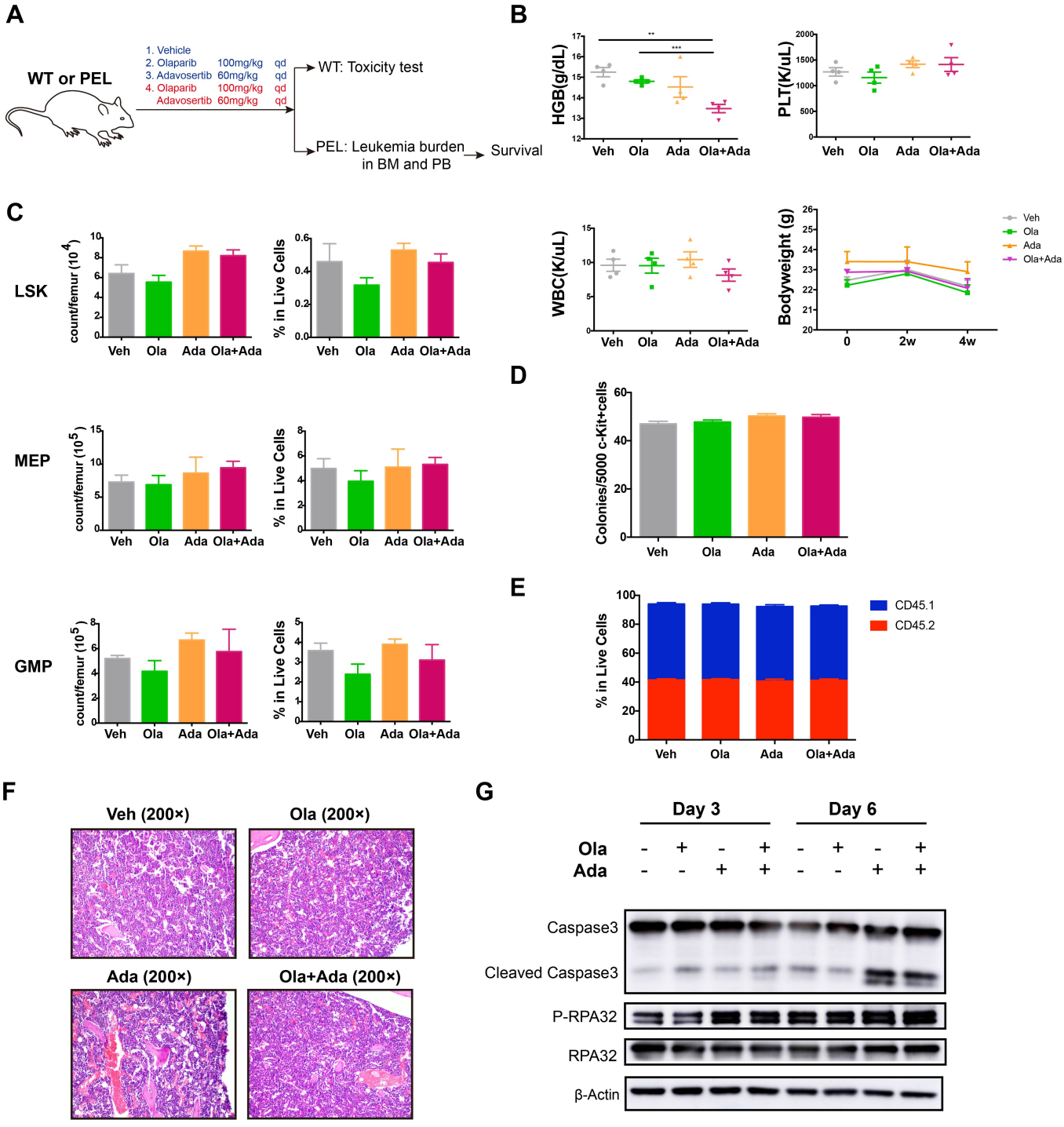

A
WT or PEL
1. Vehicle
2. Olaparib 100mg/kg qd
3. Adavosertib 60mg/kg qd
4. Olaparib 100mg/kg qd
Adavosertib 60mg/kg qd
WT: Toxicity test
PEL: Leukemia burden in BM and PB
Survival
B
HGB(g/dL)
PLT(K/uL)
Veh
Ola
Ada
Ola+Ada
C
LSK
MEP
GMP
count/femur (10^4)
count/femur (10^5)
% in Live Cells
WBC(K/uL)
Bodyweight (g)
0
2w
4w
D
Colonies/5000 c-Kit+cells
E
CD45.1
CD45.2
F
Veh (200×)
Ola (200×)
Ada (200×)
Ola+Ada (200×)
G
Day 3
Day 6
Ola
Ada
Caspase3
Cleaved Caspase3
P-RPA32
RPA32
β-Actin

Figure S6. Related to Figure 6

A. Experimental design to evaluate the toxicity and efficacy of the WEE1 inhibitor (Adavosertib, Ada) and PARP inhibitor (Olaparib, Ola) in vivo.
B. Complete blood count of PB and body weight of WT mice collected from vehicle (Veh), Olaparib (Ola), Adavosertib (Ada) and combination of Olaparib and Adavosertib (Ola+Ada) cohorts after 4 weeks exposure. N=4 mice for each arm. HGB: Ola+Ada *vs.* Veh P=0.0011; Ola+Ada *vs.* Ola P=0.0009.
C. The absolute number (left) and percentage (right) of LSKs (top), MEPs (middle) and GMPs (bottom) per femur in vehicle (Veh), Olaparib (Ola), Adavosertib (Ada) and combination of Olaparib and Adavosertib (Ola+Ada) cohorts after 4 weeks treatment. N=4. B6-CD45.2 WT mice for each arm.
D. Total number of CFUs generated by c-Kit+ BM cells from WT mice after 4 weeks treatment with vehicle (Veh), Olaparib (Ola), Adavosertib (Ada) and combination of Olaparib and Adavosertib (Ola+Ada). N=4 mice for each arm.
E. Percentage of CD45.2+ cells in PB of CD45.1+ recipient mice that were transplanted with 1 million CD45.1+ whole BM cells and 1 million CD45.2+ whole BM cells from WT mice treated with vehicle (Veh), Olaparib (Ola), Adavosertib (Ada) and combination of Olaparib or Adavosertib (Ola+Ada). N=5 mice for each arm.
F. Histopathologic H&E sections of BM from representative WT mice after 4 weeks treatment with vehicle (Veh), Olaparib (Ola), Adavosertib (Ada) and combination of Olaparib or Adavosertib (Ola+Ada). Magnification, 200×
G. Western blot analysis of Caspase3 and RPA32 in c-Kit+ spleen cells from $J^{VF}P^{-/-}$ PEL mice treated with vehicle (Veh), Olaparib (Ola), Adavosertib (Ada) or combination of Olaparib and Adavosertib (Ola+Ada) after 3 and 6 days.

Data are represented as mean ± SEM. The unpaired Student's t test was used to compare the mean of two groups in B, C, D and E. ** $P \leq 0.01$, *** $P \leq 0.001$