**Supplemental informations for:**

***Genetic and epigenetic evolution as a contributor to WT1-mutant leukemogenesis***

Elodie Pronier,[1-2] Robert L. Bowman,[1-2] Jihae Ahn,[1-2] Jacob Glass,[1-2] Cyriac Kandoth,[1-3] Tiffany R. Merlinsky,[1-2] Justin T. Whitfield,[1-2] Benjamin H. Durham,[1] Antoine Gruet,[2] Amritha Varshini Hanasoge Somasundara,[1-2] Raajit Rampal,[1-2] Ari Melnick,[4] Richard P. Koche,[2] Barry S. Taylor,[1-3] and Ross L. Levine,[1-2-4-5-6].

[1]Human Oncology and Pathogenesis Program, Memorial Sloan Kettering Cancer Center, New York, NY 10065, USA.

[2]Center for Epigenetics Research, Memorial Sloan Kettering Cancer Center, New York, NY 10065, USA.

[3]Department of Epidemiology and Biostatistics, Memorial Sloan Kettering Cancer Center, New York, NY 10065, USA.

[4]Division of Hematology/Oncology, Weill Cornell Medical College, Cornell University, New York, NY 10065, USA.

[5]Leukemia Service, Department of Medicine, Memorial Sloan Kettering Cancer Center, New York, NY 10065, USA.

[6]Center for Hematologic Malignancies Memorial Sloan Kettering Cancer Center, New York, NY 10065, USA.

**Supplemental Methods:**

***qPCR and Western Blot analysis***

Total RNA was isolated from splenocytes or bone marrow cells using RNeasy Mini Kit (Qiagen) and cDNA was synthesized by SuperScript II Reverse Transcriptase (Invitrogen, Carlsbad, CA, USA). PCRs were carried out in the ABI Prism GeneAmp 7500 Sequence Detection System (Applied Biosystem, Invitrogen), using the Power SYBR Green PCR Master Mix (Invitrogen) and specific primers to determine *Wt1* expression. *Gapdh* and *Actin*, used as housekeeping genes. List of Primers used: *mActin*-F (5'-GGCTGTATTCCCCTCCATCG-3'); *mActin*-R (5'-CCAGTTGGTAACAATGCCATGT-3'); *mGapdh*-F (5'-AGGTCGGTGTGAACGGATTTG-3'); *mGapdh*-R (5'-TGTAGACCATGTAGTTGAGGTCA-3'); *mWt1* (exon 2)-F (5'-AAGGAGACACACAGGTGTGAAA-3'); *mWt1* (exon 2)-R (5'-GTGGGTCTTCAGATGGTCGG-3'). For protein expression analysis, total proteins were isolated using RIPA buffer and Western Blots were performed using anti-Wt1 (F6 from Abcam, Cambridge, UK). Normalizations were done using Gapdh of Histone H3 antibodies (Cell Stem Technology, Vancouver, Canada).

***Histopathology***

For histological analysis spleens, livers and sterna were fixed in 10% neutral-buffered formalin, embedded in paraffin, sectioned and stained with hematoxylin and eosin (H/E) or c-Kit by the Laboratory of Comparative Pathology of MSKCC and Weill Cornell Medical College using standard methods.

***Bone marrow transplantation***

Freshly dissected femurs and tibias were isolated. Bone marrow was spun at 8000 rpm by centrifugation at 4°C into PBS, and red blood cells were lysed in ammonium chloride-potassium bicarbonate lysis buffer for 10 min on ice. After centrifugation, cells were resuspended in PBS, passed through a cell strainer, and counted. For non-competitive transplantations $2\times10^6$ mononuclear cells from freshly harvested bone marrow were injected through tail veins into lethally irradiated (9.5 Gy) recipient mice (Figure 1D, Figure 2, supplemental Figure 4E-H and supplemental Figure 5B-F). For all competitive transplant, $1\times10^6$ total bone marrow cells from donor mice ($CD45.2^+$) were mixed with $1\times10^6$ wild-type ($CD45.1^+$) bone marrow cells from 6-8 weeks old females and transplanted into lethally irradiated recipient mice (Figure 3A, D and E and Figure 7E-F). Chimerism was measured by flow cytometry in peripheral blood starting 4 weeks post-transplant and subsequently every 4 weeks (week 4, 12 and 16 post transplantation). Chimerism in the bone marrow and spleens, was evaluated at 16-18 weeks post-transplantation via animal sacrifice and subsequent flow analysis.

***Flow cytometry***

Analysis of mature blood lineages was performed as described[1]. Analysis of the hematopoietic stem and myeloid progenitor populations was performed on single-cell suspensions prepared from bone marrow, spleens and livers by flow cytometry after red blood cell lysis. Populations were defined as follows: long-term (LT)-HSC, $Lineage^-Sca1^+c\text{-}Kit^+$ (LSK) $CD150^+CD48^-$; short-term (ST)-HSC, LSK $CD150^+CD48^+$; multipotent progenitors (MPP) LSK $CD150^-CD48^+$, myeloid progenitors (MP), $Lineage^-Sca1^-c\text{-}Kit^+$ (LK); common myeloid progenitors (CMP), LK $CD16/32^-CD34^+$; granulocyte/macrophage progenitors (GMP), LK $CD16/32^+CD34^+$; megakaryocyte/erythroid progenitors (MEP), LK $CD16/32^-CD34^-$. All antibodies were purchased from eBioscience (San Diego, CA, USA). Flow cytometry data was collected

using BD LSRII Fortessa cytometer operated by BD FACSDiva software version 6.2. Flow cytometry data was analyzed using FlowJo version 9.7.6.

***M-IMPACT sequencing and analysis***

DNA from diseased mice and their littermates underwent targeted capture and deep sequencing using the mouse Integrated Mutation Profiling of Actionable Cancer Targets (M-IMPACT) v1 assay targeting the exons and select introns of 578 known cancer genes. M-IMPACT is a solution-phase hybridization-based exon capture and massively parallel DNA sequencing assay, a process described previously[2]. The genes in this capture design included all those genes part of the solid tumor and hematological malignancy clinical sequencing tests performed at the point of care at MSKCC[3] as well as those key cancer-associated genes of interest whose clinical significance is still investigational (supplemental Table 1). Each of the 578 human genes were mapped to their mouse orthologs using the Mouse/Human Orthology with Phenotype Annotations from the Jackson Laboratory Mouse Genome Informatics (ftp://ftp.informatics.jax.org/pub/reports/HOM_Mouse_HumanSequence.rpt) after which bait design was performed as previously described[2]. A uniform distribution of 1,598 heterozygous SNPs was then added to the design. We first filtered mouse dbSNP v142 (mm10 build) to identify SNPs where the average heterozygosity from all observations (avHet) was between 47.5 and 52.5%, location type was 'exact', mapping weight corresponded to those that align to exactly one location in the genome, and the allele frequency was between 45 and 55%. From 32,412 candidate SNPs, we selected approximately one SNP per ~1.28Mb chromosome bin across the mouse genome to standardize their genomic representation. In total, 250ng of mouse genomic DNA were used for library construction and samples were molecularly barcoded prior to capture and sequencing. Pools of 12 samples were equimolarly mixed and sequenced in one

lane of a Hiseq 4000 using SBS chemistry for paired end 125/125 reads. Average coverage was greater than 400-fold, with a minimum of 99% of the targeted sequences covered 30-fold. The resulting paired end reads were aligned to the mouse reference genome mm10 (GRCm38) using BWA-MEM (Burrows-Wheeler Aligner v0.7.12, http://arxiv.org/abs/1303.3997), and PCR duplicates were identified by MarkDuplicates in Picard Tools v1.124 (https://github.com/broadinstitute/picard). Variant calling was performed in tumor-vs-normal paired mode using MuTect v1.1.7[4] for single nucleotide variants and HaplotypeCaller from GATK 3.4[5] for small insertions and deletions. Resulting variants were annotated using vcf2maf v1.6.14 (https://github.com/mskcc/vcf2maf), which uses Ensembl's Variant Effect Predictor v88. Known and potential oncogenic mutations in human tumors[6] were re-mapped to mouse genome mm10 using UCSC's liftOver, in order to annotate likely oncogenic mutations in the mice. Copy-number variants including chromosomal instability (CIS) and whole-genome doubling (WGD) were called using FACETS[7].

***In vitro colony-forming assays***

Sorted LSK or total BM cells were isolated from *Wt1*$^{+/+}$, *Wt1*$^{fl/+}$ and *Wt1*$^{fl/fl}$ mice and seeded in duplicates at a density of 1.500 or 10.000 cells/replicate into cytokine-supplemented methylcellulose medium (Methocult, STEMCELL Technologies, Vancouver, Canada). Colonies propagated in culture were scored between days 10-14.

***Vector construction and viral transduction***

The human *WT1* cDNA (Isoform D) was clone into Migr1-IRES-GFP plasmid (Addgene, Cambridge, MA, USA), the murine *Npm1*, *Gata1* cDNA were cloned into MSCV-IRES-GFP plasmid (Origene, Rockville, MD, USA) and two shRNA-*Cebpa* were cloned in a pRFP-C-RS plasmid (Origene). Viruses were produced by transfecting 293T cells with

the corresponding vectors and EcoPack plasmids. c-Kit$^{+}$ cells were isolated from bone marrow from *Tet2*$^{fl/fl}$ or *Wt1*$^{fl/+}$ mice using immunomagnetic beads (Miltenyi Biotec, Bergisch Gladbach, Germany) and transduced with corresponding viral supernatants. GFP and RFP-positive cells were FACS sorted using ARIA III (Biosciences).

***5-hmC quantification***

Genomic DNA was purified using Puregene DNA purification kit (Qiagen). DNA was denatured, and 2-fold serial dilutions were spotted on a nitrocellulose membrane in an assembled Bio-Dot apparatus (Bio-Rad, Hercules, CA, USA). The blotted membranes were washed, blocked with 5% TBS-T, and incubated with rabbit polyclonal anti-5hmC antibody (1:10000) and HRP-conjugated anti-rabbit IgG secondary antibodies (1:5000).

***Statistics***

For the *in vitro* experiments, normal distribution was assumed, whereas for the *in vivo* experiments, normal distribution was tested before the use of the appropriate statistical test. Two-tailed unpaired t-test was used for comparison of two groups, whereas the one-, two-way analysis of variance (ANOVA) and non-parametric one-way ANOVA (Kruskal–Wallis) was used for comparison of multiple groups. Mantel-Cox and log-rank p value were used for survival curve (Kaplan–Meier) analyses. Analyses were performed using Prism 6.0 software (GraphPad).

**Supplemental Table 1: Gene content of mouse IMPACT**

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| *Abl1* | *Cd274* | *Erbb3* | *Hist1h1c* | *Lmo1* | *Pak7* | *Rb1* | *Spry4* |
| *Actg1* | *Cd276* | *Erbb4* | *Hist1h1d* | *Ltb* | *Palb2* | *Rbm10* | *Src* |
| *Acvr1* | *Cd28* | *Ercc2* | *Hist1h1e* | *Lyn* | *Park2* | *Recql* | *Srsf2* |
| *Ago2* | *Cd48* | *Ercc3* | *Hist1h2ad* | *Malt1* | *Parp1* | *Recql4* | *Stag1* |
| *Akt1* | *Cd79a* | *Ercc4* | *Hist1h2ae* | *Map2k1* | *Pax5* | *Rel* | *Stag2* |
| *Akt2* | *Cd79b* | *Ercc5* | *Hist1h2an* | *Map2k2* | *Pbrm1* | *Ret* | *Stat3* |
| *Akt3* | *Cdc42* | *Erf* | *Hist1h2ao* | *Map2k4* | *Pcbp1* | *Rfwd2* | *Stat5a* |
| *Alk* | *Cdc73* | *Erg* | *Hist1h2bf* | *Map3k1* | *Pdcd1* | *Rheb* | *Stat5b* |
| *Alox12b* | *Cdh1* | *Errfi1* | *Hist1h2bg* | *Map3k13* | *Pdcd1lg2* | *Rhoa* | *Stat6* |
| *Amer1* | *Cdk12* | *Esco1* | *Hist1h2bk* | *Map3k14* | *Pdgfra* | *Rictor* | *Stk11* |
| *Ankrd11* | *Cdk4* | *Esco2* | *Hist1h2bp* | *Mapk1* | *Pdgfrb* | *Rit1* | *Stk19* |
| *Apc* | *Cdk6* | *Esr1* | *Hist1h3a* | *Mapk3* | *Pdpk1* | *Rnf43* | *Stk40* |
| *Ar* | *Cdk8* | *Etnk1* | *Hist1h3b* | *Mapkap1* | *Pds5a* | *Robo1* | *Sufu* |
| *Araf* | *Cdkn1a* | *Etv1* | *Hist1h3c* | *Max* | *Pds5b* | *Ros1* | *Suz12* |
| *Arhgef28* | *Cdkn1b* | *Etv6* | *Hist1h3d* | *Mcl1* | *Pgr* | *Rps6ka4* | *Syk* |
| *Arid1a* | *Cdkn2a* | *Ezh1* | *Hist1h3e* | *Mdc1* | *Phf6* | *Rps6kb2* | *Tap1* |
| *Arid1b* | *Cdkn2b* | *Ezh2* | *Hist1h3g* | *Mdm2* | *Phox2b* | *Rptor* | *Tap2* |
| *Arid2* | *Cdkn2c* | *Fam175a* | *Hist1h3h* | *Mdm4* | *Piga* | *Rragc* | *Tbl1xr1* |
| *Arid3a* | *Cebpa* | *Fam46c* | *Hist1h3i* | *Med12* | *Pik3c2g* | *Rras* | *Tbx3* |
| *Arid3b* | *Cenpa* | *Fam58b* | *Hist2h2be* | *Mef2b* | *Pik3c3* | *Rras2* | *Tceb1* |
| *Arid3c* | *Chek1* | *Fanca* | *Hist2h3b* | *Men1* | *Pik3ca* | *Rtel1* | *Tcf3* |
| *Arid4a* | *Chek2* | *Fancc* | *Hist2h3c1* | *Met* | *Pik3cb* | *Runx1* | *Tcf7l2* |
| *Arid4b* | *Cic* | *Fancd2* | *Hist2h3c2* | *Mga* | *Pik3cd* | *Runx1t1* | *Tek* |
| *Arid5a* | *Ciita* | *Fas* | *Hist3h2ba* | *Mgam* | *Pik3cg* | *Rxra* | *Tert* |
| *Arid5b* | *Crbn* | *Fat1* | *Hnf1a* | *Mitf* | *Pik3r1* | *Rybp* | *Tet1* |
| *Asxl1* | *Crebbp* | *Fbxo11* | *Hoxb13* | *Mlh1* | *Pik3r2* | *Samhd1* | *Tet2* |
| *Asxl2* | *Crkl* | *Fbxw7* | *Hras* | *Mob3b* | *Pik3r3* | *Sdha* | *Tet3* |
| *Atm* | *Crlf2* | *Fgf15* | *Icosl* | *Mpeg1* | *Pim1* | *Sdhaf2* | *Tgfbr1* |
| *Atp6ap1* | *Csde1* | *Fgf3* | *Id3* | *Mpl* | *Plcg1* | *Sdhb* | *Tgfbr2* |
| *Atp6v1b2* | *Csf1r* | *Fgf4* | *Idh1* | *Mre11a* | *Plcg2* | *Sdhc* | *Tmem127* |
| *Atr* | *Csf3r* | *Fgfr1* | *Idh2* | *Msh2* | *Plk1* | *Sdhd* | *Tmprss2* |
| *Atrx* | *Ctcf* | *Fgfr2* | *Ifngr1* | *Msh3* | *Plk2* | *Sesn1* | *Tnfaip3* |
| *Atxn2* | *Ctla4* | *Fgfr3* | *Igf1* | *Msh6* | *Pmaip1* | *Sesn2* | *Tnfrsf14* |
| *Aurka* | *Ctnnb1* | *Fgfr4* | *Igf1r* | *Msi1* | *Pms1* | *Sesn3* | *Top1* |
| *Aurkb* | *Cul3* | *Fh1* | *Igf2* | *Msi2* | *Pms2* | *Setbp1* | *Traf2* |
| *Axin1* | *Cux1* | *Flcn* | *Ikbke* | *Mst1* | *Pnrc1* | *Setd1a* | *Traf3* |
| *Axin2* | *Cxcr4* | *Flt1* | *Ikzf1* | *Mst1r* | *Pold1* | *Setd1b* | *Traf5* |
| *Axl* | *Cyld* | *Flt3* | *Ikzf3* | *Mtor* | *Pole* | *Setd2* | *Traf7* |
| *B2m* | *Cysltr2* | *Flt4* | *Il10* | *Mutyh* | *Pot1a* | *Setd3* | *Trp53* |
| *Babam1* | *Daxx* | *Foxa1* | *Il7r* | *Myc* | *Pparg* | *Setd4* | *Trp53bp1* |
| *Bach2* | *Dcun1d1* | *Foxl2* | *Inha* | *Mycl* | *Ppm1d* | *Setd5* | *Trp63* |
| *Bap1* | *Ddr2* | *Foxo1* | *Inhba* | *Mycn* | *Ppp2r1a* | *Setd6* | *Tsc1* |
| *Bard1* | *Ddx3x* | *Foxp1* | *Inpp4a* | *Myd88* | *Ppp4r2* | *Setd7* | *Tsc2* |
| *Bbc3* | *Dicer1* | *Fubp1* | *Inpp4b* | *Myod1* | *Ppp6c* | *Setd8* | *Tshr* |
| *Bcl10* | *Dis3* | *Furin* | *Inppl1* | *Nbn* | *Prdm1* | *Setdb1* | *Tyk2* |
| *Bcl11b* | *Dnajb1* | *Fyn* | *Insr* | *Ncoa3* | *Prdm14* | *Setdb2* | *U2af1* |
| *Bcl2* | *Dnmt1* | *Gata1* | *Irf1* | *Ncor1* | *Prex2* | *Sf3b1* | *U2af2* |
| *Bcl2l1* | *Dnmt3a* | *Gata2* | *Irf4* | *Ncor2* | *Prkar1a* | *Sgk1* | *Ubr5* |
| *Bcl2l11* | *Dnmt3b* | *Gata3* | *Irf8* | *Ncstn* | *Prkci* | *Sh2b3* | *Upf1* |
| *Bcl6* | *Dot1l* | *Gli1* | *Irs1* | *Negr1* | *Prkd1* | *Sh2d1a* | *Vav1* |
| *Bcor* | *Drosha* | *Gm10499* | *Irs2* | *Nf1* | *Ptch1* | *Shoc2* | *Vav2* |
| *Bcorl1* | *Dtx1* | *Gm12657* | *Jak1* | *Nf2* | *Pten* | *Shq1* | *Vegfa* |
| *Bcr* | *Dusp1* | *Gna11* | *Jak2* | *Nfe2* | *Ptp4a1* | *Slx4* | *Vhl* |
| *Birc3* | *Dusp22* | *Gna12* | *Jak3* | *Nfe2l2* | *Ptpn1* | *Smad2* | *Vtcn1* |
| *Blm* | *Dusp4* | *Gna13* | *Jarid2* | *Nfkbia* | *Ptpn11* | *Smad3* | *Wapl* |
| *Bmpr1a* | *E2f3* | *Gnaq* | *Jun* | *Nipbl* | *Ptpn2* | *Smad4* | *Whsc1* |
| *Braf* | *Eed* | *Gnas* | *Kdm5a* | *Nkx2-1* | *Ptprd* | *Smarca4* | *Whsc1l1* |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| *Brca1* | *Egfl7* | *Gnb1* | *Kdm5c* | *Nkx3-1* | *Ptprs* | *Smarcb1* | *Wt1* |
| *Brca2* | *Egfr* | *Gps2* | *Kdm6a* | *Notch1* | *Ptprt* | *Smarcd1* | *Wwtr1* |
| *Brd4* | *Egr1* | *Grem1* | *Kdr* | *Notch2* | *Rab35* | *Smc1a* | *Xbp1* |
| *Brip1* | *Eif1a* | *Grin2a* | *Keap1* | *Notch3* | *Rac1* | *Smc3* | *Xiap* |
| *Btg1* | *Eif4a2* | *Gsk3b* | *Kit* | *Notch4* | *Rac2* | *Smg1* | *Xpo1* |
| *Btk* | *Eif4e* | *Gtf2i* | *Klf4* | *Npm1* | *Rad21* | *Smo* | *Xrcc2* |
| *Calr* | *Elf3* | *H2-Q2* | *Kmt2a* | *Nras* | *Rad50* | *Smyd3* | *Yap1* |
| *Card11* | *Ep300* | *H3f3a* | *Kmt2b* | *Nsd1* | *Rad51* | *Socs1* | *Yes1* |
| *Carm1* | *Ep400* | *H3f3c* | *Kmt2c* | *Nt5c2* | *Rad51b* | *Sos1* | *Zfhx3* |
| *Casp8* | *Epas1* | *Hdac1* | *Kmt2d* | *Nthl1* | *Rad51c* | *Sox17* | *Zrsr2* |
| *Cbfb* | *Epcam* | *Hdac4* | *Knstrn* | *Ntrk1* | *Rad51d* | *Sox2* | |
| *Cbl* | *Epha3* | *Hdac7* | *Kras* | *Ntrk2* | *Rad52* | *Sox9* | |
| *Ccnd1* | *Epha5* | *Hdac8* | *Ksr2* | *Ntrk3* | *Rad54l* | *Sp140* | |
| *Ccnd2* | *Epha7* | *Hgf* | *Lats1* | *Nuf2* | *Raf1* | *Spen* | |
| *Ccnd3* | *Ephb1* | *Hif1a* | *Lats2* | *Nup93* | *Rara* | *Spop* | |
| *Ccne1* | *Erbb2* | *Hist1h1b* | *Lck* | *Pak1* | *Rasa1* | *Spred1* | |

## Supplemental figures:

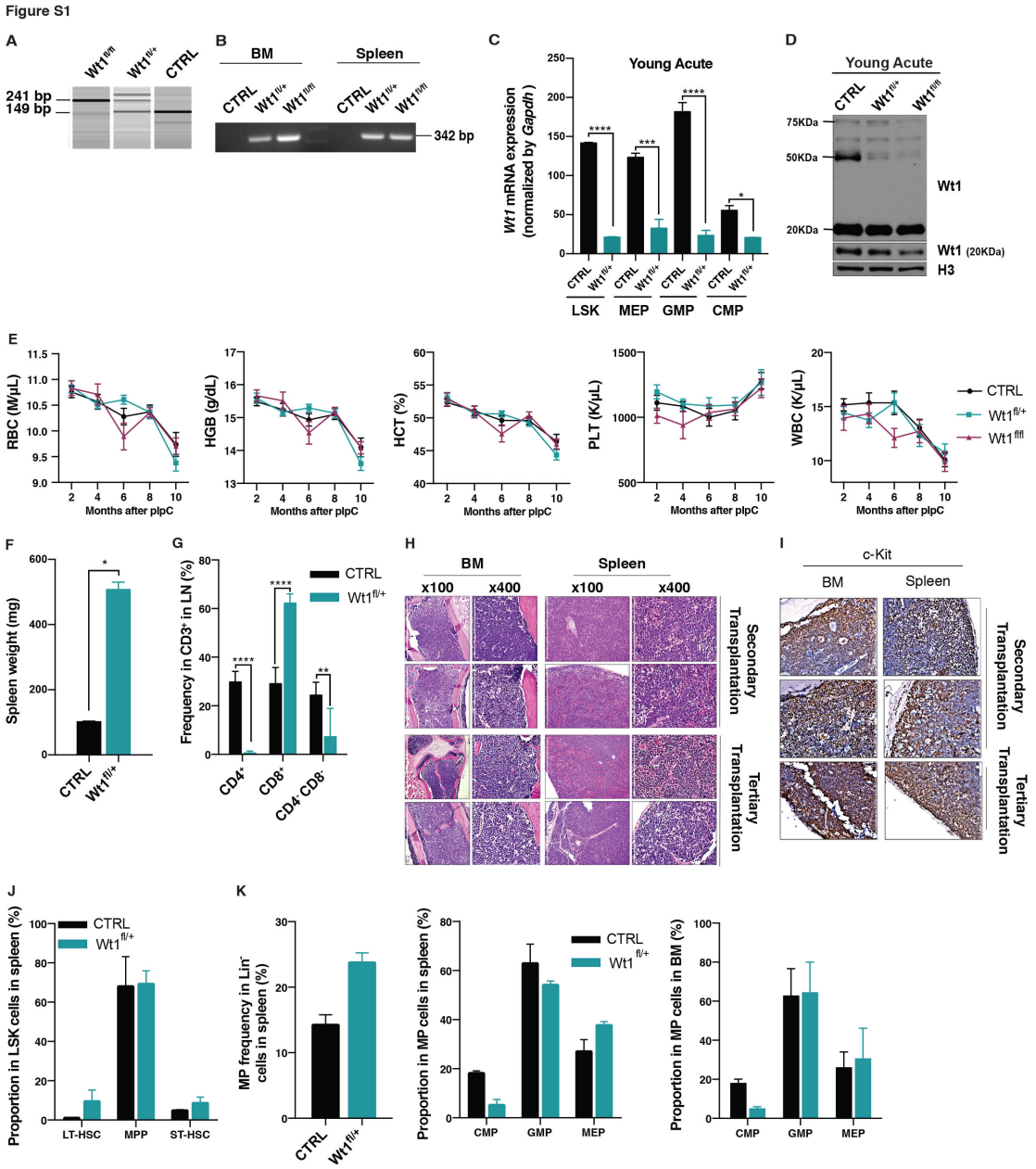



**Figure S1. *Wt1*-haploinsufficient but not *Wt1*-deficient hematopoietic cells progress to leukemic transformation over time.** (A) Representative results of PCR analysis of floxed *Wt1* genotyping in peripheral blood of $Wt1^{fl/fl}$, $Wt1^{fl/+}$ and control animals (CTRL) (floxed allele: 241 bp; wild-type allele: 149 bp), (n=18 to 20). (B) Representative results of PCR analysis of *Wt1* excision in bone marrow (BM) and spleen

compartments of control, *Wt1*$^{fl/+}$ and *Wt1*$^{fl/fl}$ animals (excised allele: 342 bp), (n=18 to 20). Confirmation of *Wt1* mRNA silencing by qPCR in different hematopoietic lineages (LSK, MEP, GMP and CMP) isolated from *Wt1*$^{fl/+}$ and *Wt1*$^{fl/fl}$ mice compared to control littermates (n=6 to 7). *Gapdh* was used as a housekeeping gene. (D) Western Blot analysis of Wt1 protein levels in splenocytes isolated from *Wt1*$^{fl/+}$ and *Wt1*$^{fl/fl}$ mice compared to control littermates. Histone H3 was used loading control. Blot is representative of 4 independent experiments. (E) Complete blood counts from control, *Wt1*$^{fl/+}$ and *Wt1*$^{fl/fl}$ animals were measured overtime (0-10 weeks post *Wt1*-depletion) (n=10 to 15). RBC: Red blood cell, HGB: Hemoglobin, HCT: Hematocrit, PLT: Platelet, WBC: White blood cells. (F) Spleen weights of *Wt1*$^{fl/+}$ (T-ALL) mice compared to controls (n=3 to 5). (G) Proportions of $CD4^+$, $CD8^+$ and $CD4^+CD8^+$ cells in $CD3^+$ cells of the lymph node (LN) of control and T-ALL mice at disease onset (n=6). Representative H&E-stained (H) and c-Kit immunohistochemical stains (I) of bone marrow and spleen sections of T-ALL mice at time of sacrifice. (J) Stem cell percentages in spleen of diseased *Wt1*-heterozygous aged mice compared to controls (n=4). (K) Quantification of progenitors in the spleen and bone marrow compartments of diseased *Wt1*-heterozygous aged mice compared to controls (n=4). In C, F-G, J-K data are shown as mean ± s.e.m., $^*p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, $^{****}p < 0.0001$ by one way ANOVA.

Figure S2

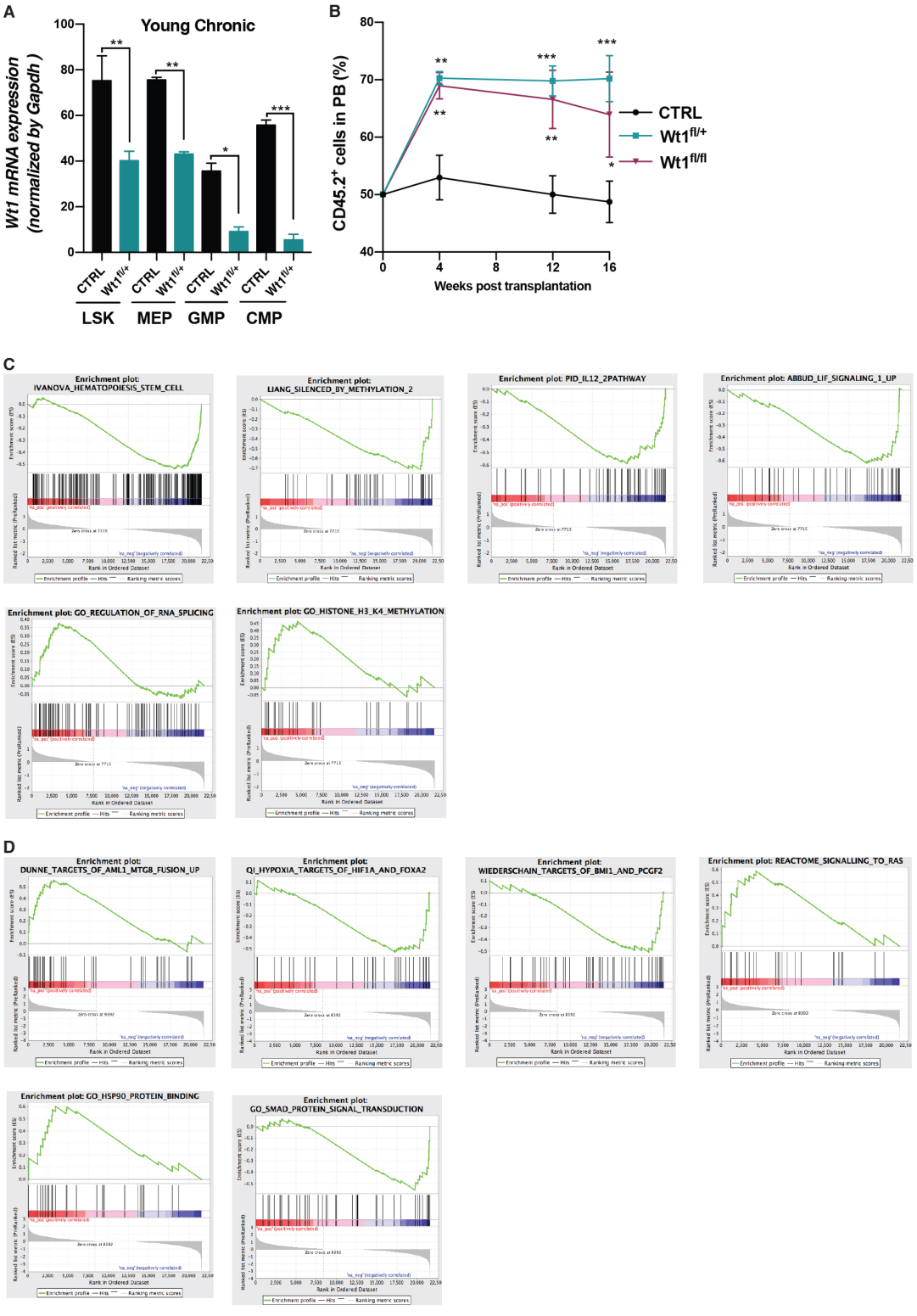
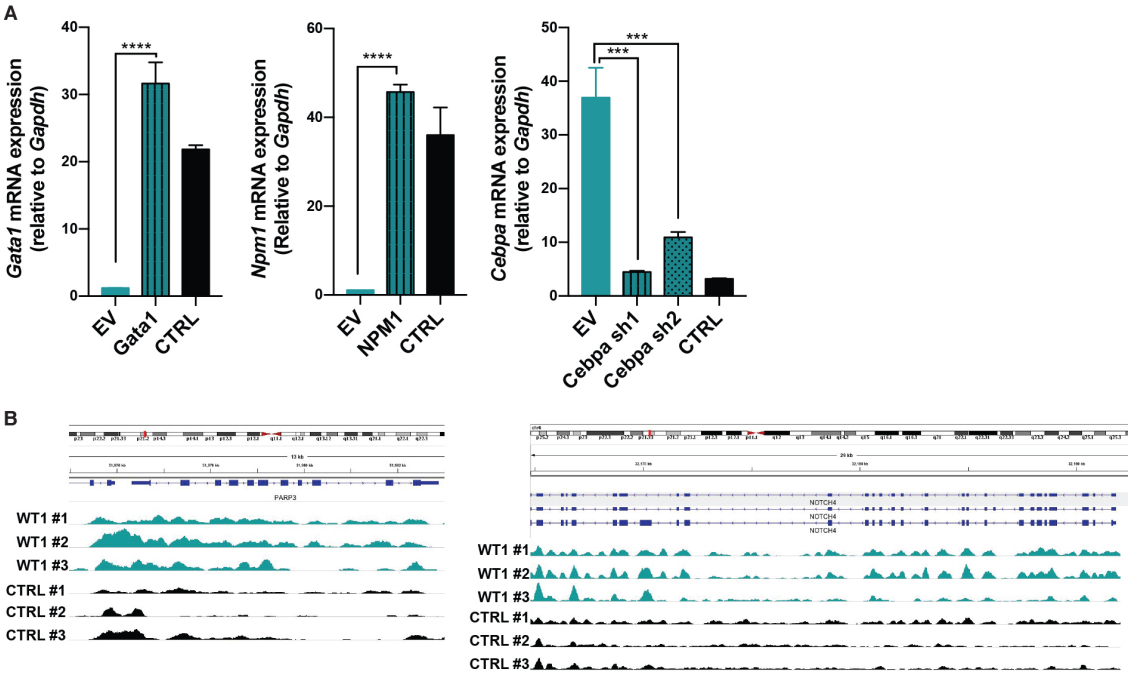



**Figure S2. Gene signatures deregulated by Wt1 depletion in elderly mice.** (A) Confirmation of *Wt1* mRNA silencing by qPCR in different hematopoietic lineages (LSK,

MEP, GMP and CMP) isolated from *Wt1*$^{fl/+}$ and *Wt1*$^{fl/fl}$ mice compared to control littermates of the Young Chronic cohort (n=6 to 7). *Gapdh* was used as a housekeeping gene. (B) Quantification of donor-derived (CD45.2) cells in the peripheral blood (PB) of recipient animals at indicated time points after transplantation (n=5). GSEA plots from comparison of RNAseq data performed on MP-sorted cells from Young Chronic and Young Acute Wt1 heterozygous mice compared to age-matched controls (C) or younger controls (D). In A, B data are shown as mean ± s.e.m., $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ by one way ANOVA.

Figure S3




**S3. Wt1 directly targets a subset of genes involved in hematopoietic differentiation.** (A) Relative mRNA expression of *Gata1*, *Npm1* and *Cebpa* in WT BM cells (CTRL) and EV or *Gata1*, *Npm1* or sh*Cebpa* transduced $Wt1^{fl/+}$ c-$Kit^{+}$ cells from the Young Chronic cohort (n=3 for each group in triplicate). (B) CHIP-Seq signals for WT1 at *Parp3* and *Notch4* loci. In A data are shown as mean ± s.e.m., ***p < 0.001, ****p <0.0001 by one way ANOVA.

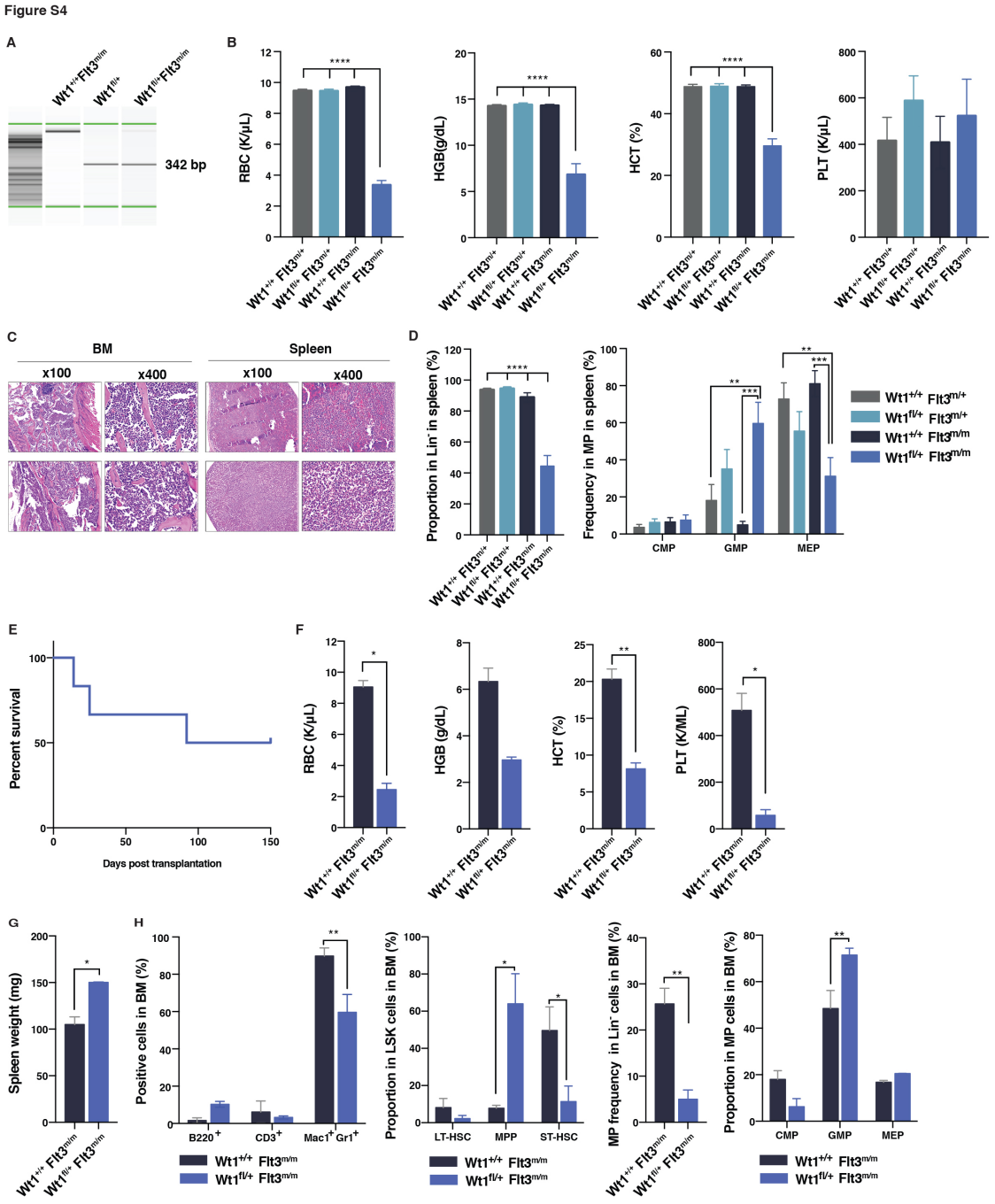



**Figure S4. Wt1 loss cooperates with *Flt3-ITD* mutations to induce leukemogenesis *in vivo*.** (A) Representative results of PCR analysis of *Wt1* excision in the peripheral blood of control, $Wt1^{fl/+}$ (one-month post Poly(I:C) treatment) and $Wt1^{fl/+}$ $Flt3^{m/m}$ mice, showing *Wt1* excision in these animals before Poly(I:C) treatment. (B) RBC, HGB, HCT

and PLT counts of primary *Wt1*$^{fl/+}$ *Flt3*$^{m/m}$ mice compared to littermates (n=7). (C) Representative H&E-stained bone marrow and spleen sections of diseased mice at time of sacrifice. (D) Proportion of splenic Lin$^-$ and myeloid progenitors (MP) cells of *Wt1*$^{+/+}$ *Flt3*$^{m/+}$, *Wt1*$^{fl/+}$ *Flt3*$^{m/+}$, *Wt1*$^{+/+}$ *Flt3*$^{m/m}$ and *Wt1*$^{fl/+}$ *Flt3*$^{m/m}$ mice (n=7). (E) Kaplan-Meyer survival curve of primary transplanted mice with total bone marrow cells from diseased *Wt1*$^{fl/+}$ *Flt3*$^{m/m}$ mice (n=6). (F) RBC, HCT, WBC, HGB and PLT counts of secondary recipients transplanted with *Wt1*$^{fl/+}$ *Flt3*$^{m/m}$ or *Wt*$^{+/+}$ *Flt3*$^{m/m}$ total bone marrow cells (n=3 to 6). (G) Spleen weights of transplanted recipients with *Wt1*$^{fl/+}$ *Flt3*$^{m/m}$ cells compared to controls (n=3 to 6). (H) Quantification of mature, stem/progenitor cells in the bone marrow of primary recipient transplanted with total bone marrow cells from each group (n=3 to 6). In (B, D-H) data are shown as mean ± s.e.m., $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, $^{****}p < 0.0001$ by two-way ANOVA (B, D, F-H) or Mantel-Cox (E).

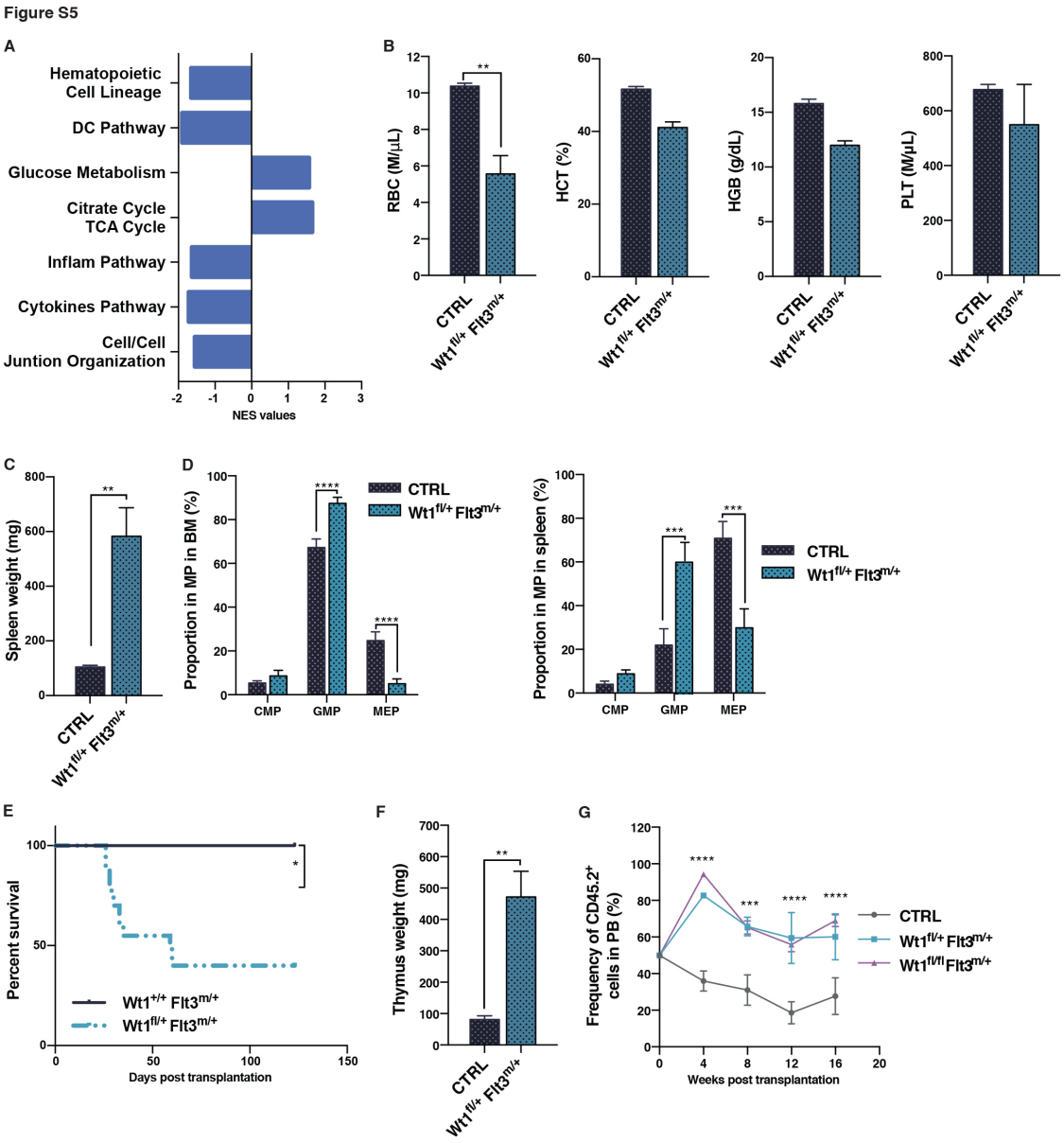



**Figure S5. Role of genetic background on malignant phenotypes in *Wt1*-deficient/haploinsufficent mice.** (A) Normalized Enrichment Scores (NES) of gene signatures defined by RNAseq performed in myeloid progenitors from diseased *Wt1*$^{fl/+}$ *Flt3*$^{m/m}$ mice compared to age-matched *Wt1*$^{+/+}$ *Flt3*$^{m/m}$ controls. (B) RBC, HCT, HGB and PLT counts of *Wt1*$^{fl/+}$ *Flt3*$^{m/+}$ (129S1/SvImJ) mice compared to control (n=10). (C) Spleen weights of transplanted recipients with *Wt1*$^{fl/+}$ *Flt3*$^{m/m}$ cells compared to controls (n=3 to 6). (D) Quantification of myeloid progenitor cells in the bone marrow and the spleen of

secondary recipient transplanted with cells from *Wt1*$^{fl/+}$ *Flt3*$^{m/+}$ compared to *Wt1*$^{+/+}$ *Flt3*$^{m/+}$ controls (n=3 to 6). (E) Kaplan-Meyer survival curves of secondary recipient transplanted with total bone marrow from *Wt1*$^{fl/+}$ *Flt3*$^{m/+}$ compared to *Wt1*$^{+/+}$ *Flt3*$^{m/+}$ controls (n=3 to 6). (F) Thymus weights of transplanted mice with *Wt1*$^{fl/+}$ *Flt3*$^{m/+}$ (129S1/SvImJ) or control cells (n=10). (G) Quantification of donor-derived (CD45.2) cells in the peripheral blood of recipient animals at indicated time points after transplantation (n=5). Graphs of mean ± s.e.m., $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, $^{****}p < 0.0001$ by two-way ANOVA (B-D, F) or Mantel-Cox (E).

Supplemental References:


1. Mayle A, Luo M, Jeong M, Goodell MA. Flow cytometry analysis of murine hematopoietic stem cells. *Cytometry A.* 2013;83(1):27-37.
2. MacGregor GA, Markandu ND, Smith SJ, Sagnella GA. Captopril: contrasting effects of adding hydrochlorothiazide, propranolol, or nifedipine. *J Cardiovasc Pharmacol.* 1985;7 Suppl 1:S82-87.
3. Zehir A, Benayed R, Shah RH, et al. Mutational landscape of metastatic cancer revealed from prospective clinical sequencing of 10,000 patients. *Nat Med.* 2017;23(6):703-713.
4. Cibulskis K, Lawrence MS, Carter SL, et al. Sensitive detection of somatic point mutations in impure and heterogeneous cancer samples. *Nat Biotechnol.* 2013;31(3):213-219.
5. DePristo MA, Banks E, Poplin R, et al. A framework for variation discovery and genotyping using next-generation DNA sequencing data. *Nat Genet.* 2011;43(5):491-498.
6. Chang MT, Asthana S, Gao SP, et al. Identifying recurrent mutations in cancer reveals widespread lineage diversity and mutational specificity. *Nat Biotechnol.* 2016;34(2):155-163.
7. Shen R, Seshan VE. FACETS: allele-specific copy number and clonal heterogeneity analysis tool for high-throughput DNA sequencing. *Nucleic Acids Res.* 2016;44(16):e131.