Supplemental Materials for "*U2AF1* is a haplo-essential gene required for hematopoietic cancer cell survival in mice"

Authors and affiliations

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Supplemental methods

Animal models

All mice used in this experiment were of the C57BL/6 background. The *U2af1* KO mouse was generated by Ingenious Targeting Laboratory (2200 Smithtown Avenue, Ronkonkoma, NY 11779) using standard gene targeting techniques. To create the conditional *U2af1* KO mouse, exon 2 was flanked with loxP sites in targeting vector. The 5' single loxP site was engineered 443 bp upstream of exon 2 outside a potential regulatory region. The loxP/FRT-flanked Neo cassette, which served as the 3' loxP site after FLP-mediated Neo deletion, was placed 217 bp downstream of exon 2. The long homology arm extended about 6 kb 5' to the loxP cassette, and the short homology arm was about 2.1 kb 3' to the Neo cassette. The targeting vector was linearized and then transfected by electroporation of FLP C57BL/6 embryonic stem (ES) cells. After selection with G418 antibiotic, surviving clones were expanded for PCR analysis to identify recombinant ES cell clones. The Neo cassette in the targeting vector was removed during ES cell clone expansion. Secondary confirmation of positive clones identified by PCR was performed by Southern Blotting analysis. DNA was digested with restriction enzymes AvrII and MfeI to confirm the integration of targeting vector. The digested DNA was separated by gel electrophoresis on a 0.8% agarose gel. After transfer to a nylon membrane, the digested DNA was hybridized with a probe targeted against the target region. DNA from the C57Bl/6 mouse strain was used as wild type control. Targeted ES cells were confirmed to have a normal karyotype. The ES cell clones were injected into C57BL/6 blastocysts and implanted to create chimeras. Chimeras were bred to wild-type C57BL/6 mice, and germline transmission of the *U2af1* KO allele was verified in two mice obtained from one targeted ES cell clone by PCR and whole-genome sequencing. The mice were genotyped using the 5'

GAGGGTGATGGACCTACCCA 3' forward, and 5' GAGAAGAAAGGGGCGGCAAA 3' reverse primers (Targeted=295 bp, wild-type=112 bp). Mice carrying the *U2af1* floxed allele were crossed to the existing C57BL/6 Cre-expressing lines, including Cre-*ERT2*, *Mx1*-Cre, *Vav1*-Cre, *Lyz2*-Cre, *CD19*-Cre, or *CD4*-Cre as well as existing C57BL/6 doxycycline-inducible transgenic *U2AF1*(WT), transgenic *U2AF1*(S34F), or *U2af1*(S34F) KI mice. Before use in this study, *U2af1*(S34F) KI mice of mixed background (1) were serially backcrossed to C57BL/6 mice to achieve 100% C57BL/6 background using the Jackson Laboratory custom panel of 150 polymorphic SNPs. C57BL/6-CD45.1 mice were bred to C57BL/6-CD45.2 mice to generate C57BL/6-CD45.1/45.2 mice as a source of competitor bone marrow cells for competitive repopulation transplantation assays. Donor bone marrow from 7-12 weeks-old male and female mice were used for the experiments, and 8-week-old male and female C57BL/6-CD45.1 (Charles River) mice were used as recipients for the bone marrow transplantation assay. No gender bias was noted. Deletion of the *U2af1* KO allele and expression of *U2af1*(S34F) KI was achieved by six doses (300μg/mouse/dose) of polyinosinic-polycytidylic acid (Sigma) given every other day by intraperitoneal injection, or by six doses of tamoxifen (Sigma) dissolved in corn oil given by oral gavage, Monday, Wednesday, Friday, for two consecutive weeks (4mg/mouse/dose). The expression of transgenic U2AF1(WT) or U2AF1(S34F) was induced by Modified LabDiet® 5053 rodent chow with doxycycline (TestDiet).

Apoptosis assay

Apoptosis was assessed by flow cytometry using Annexin V. After staining with other antibodies as described, cells were washed and incubated in binding buffer containing Annexin V-APC and

SYTOX™ Green (Thermo Fisher) followed by flow cytometry analysis within an hour of staining.

Western Blot

Bulk bone marrow was lysed and sonicated in RIPA buffer (Cell Signaling) and protease inhibitor cocktail (Roche). Then, 10 μg of the protein sample was resolved in 10% SDS-PAGE gel (Bio-Rad) and transferred to a nitrocellulose membrane (Millipore). Protein was probed by anti-U2AF1 (Abcam) and anti-β-Actin (Sigma) antibodies. Detection was done with horseradish-peroxidase conjugated secondary mouse or rabbit antibodies (Cell Signaling), using SuperSignal West Pico and Femto chemiluminescence substrate (Thermo Scientific).

***U2af1* and *Mdm2* mRNA RT-PCR**

We flow sorted donor neutrophils (from *Lyz2*-Cre or *Lyz2*-Cre/ *U2af1*$^{flox/flox}$ bone marrow), B cells (from *CD19*-Cre or *CD19*-Cre/ *U2af1*$^{flox/flox}$ bone marrow), and T cells (from *CD4*-Cre or *CD4*-Cre/ *U2af1*$^{flox/flox}$ spleen) four weeks post-transplant to detect the *U2af1* mRNA levels. mRNA was isolated from the sorted cells using the NucleoSpin RNA Plus XS kit (Macherey-Nagel). Genomic DNA was removed using Turbo DNA-free kit (Ambion). Reverse transcription PCR was then performed using the Superscript III RT- PCR kit (Invitrogen). Quantitative-RT-PCR was performed for *U2af1* (Forward primer 5'- TGAGTCGTCCGCTCGAAAAT -3', Reverse primer 5'- CTCCATGACGACATGCTCCG -3') and mouse *Gapdh* (Forward primer 5'-GGATGCAGGGATGATGTTC -3', Reverse primer 5'- TGCACCACCAACTGCTTAG -3') using Sybergreen (Applied Biosystems). PCR data was analyzed using the delta-delta CT method to determine fold changes. RT-PCR and Gel electrophoresis was performed for

validation of *Mdm2* altered junctions in fetal liver KL cells (Forward primer 5'-GGGGACCCTCTCGGATCAC -3', Reverse primer 5'- TAAGTGTCGTTTTGCGCTCC -3') as previously described (2).

Detection of recombined *U2af1* KO and *U2af1* (S34F) alleles using cDNA and DNA-based Sanger sequencing

We flow sorted tg*U2AF1*(WT)/*U2af1*$^{flox/S34F}$ MLL-AF9 leukemia cells to detect recombination of the *U2af1* KO and *U2af1*(S34F) alleles. Genomic DNA was isolated using the DNeasy Blood & Tissue Kit (Qiagen), and cDNA was prepared from isolated mRNA as described above. Recombination of the *U2af1* KO allele was detected by PCR using *U2af1* KO allele-specific primers (Forward primer 5'- TGATGTGGGAAGCAGGCTAGCC -3', Reverse primer 5'-TACGGTGTTGACGAGGCGT -3') and the PCR product was visualized after agarose gel electrophoresis. To detect the RNA expression of *U2af1*(S34F), we performed PCR on the cDNA using mouse *U2af1* cDNA-specific primers to avoid detection of the human tg*U2AF1* (Forward primer 5'- CGGAATACTTGGCCTCCATC -3', Reverse primer 5'-CAAGTCGATCACGGCTTTCT -3'). The PCR product was gel purified following denaturing agarose gel electrophoresis followed by Sanger sequencing to detect the presence of the *U2af1*(S34F) mutation in the cDNA.

RNA-Sequencing analysis

Sequence read quality of the Illumina NovaSeq 6000-sequenced samples were assessed using FastQC [Andrews S. (2010). FastQC: a quality control tool for high throughput sequence data. Available online at http://www.bioinformatics.babraham.ac.uk/projects/fastqc/]. Analysis of

differentially expressed genes and splicing was done using samples within the same sequencing batch. Gene counts were determined by excluding secondary read alignments from the BAM files (using samtools view –F 0x0100) and processing the resulting reads using HTseq5 version 0.11.1 (with parameters: mode intersection-strict; minaqual 1; stranded reverse; type exon; idattr gene_id). Consensus sequence logos or skipped exons were created using seqLogo (version 1.48.0 [Bembom O (2019). *seqLogo: Sequence logos for DNA sequence alignments*. R package version 1.48.0.]). Volcano plots were generated using enhanced volcano plot R package (version 1.4.0 [Blighe K, Rana S, Lewis M (2020). *EnhancedVolcano: Publication-ready volcano plots with enhanced colouring and labeling*. R package version 1.6.0.]) (3). We used the enrichment map function from clusterProfiler to organize the top 100 statistically significant mutually overlapping gene sets into clusters that share a common function. Gene-set enrichment analysis was performed using clusterProfiler using differentially expressed genes (FDR 10% and $|\log_2 FC| > 0$) against GO-BP and GO-MF categories. Alternative splicing events were detected and computed via rMATS (version4.1.0). rMATS by default would compare two isoforms, an inclusion isoform and an exclusion or skipping isoform. For an event to be consider for any downstream analysis we required that each isoform was supported by at least 5 reads in half of the samples. Dysregulated events at |ΔPSI| 10% and FDR 10% were further used to plot sequence context of splice sites in exon skipping event. To elucidate the effect of dox dosage on positive and negative skipped exon events, we queried for junctions in the lowest dose (1250 ppm) that were significant (FDR 10% and |ΔPSI| 10%). We then queried the corresponding |ΔPSI| values for those junctions in the higher doses (2500 ppm and 10,000 ppm).

Supplemental figure legends

Supplemental Figure 1. Generation of a conditional *U2af1* knock-out allele. (A) Targeting strategy to insert loxP sites flanking *U2af1* exon 2. (B-C) Successful targeting of five embryonic stem cell clones was verified by PCR and Southern blotting after digestion with the restriction enzyme MfeI. Wild-type C57BL/6 (B6) DNA was used as a control, and clone 243 was used to generate the *U2af1* knock-out mouse. (D) Whole-genome sequencing was performed on a heterozygous knock-out (*U2af1*$^{wt/flox}$) mouse. The heterozygous *U2af1*$^{wt/flox}$ mouse has sequence coverage for both *U2af1* wild-type allele and the targeted allele containing the LoxP sequences flanking exon 2, validating correct targeting of *U2af1* in the *U2af1*$^{wt/flox}$ mouse. Neo (neomycin resistance gene), LA (long arm), MA (middle arm), SA (short arm). Sequence coverage (number of reads covering the *U2af1* exon 2 targeted locus).

Supplemental Figure 2. Embryonic deletion of *U2af1* in hematopoietic cells is lethal and reduces the number of myeloid, lymphoid, and hematopoietic progenitor cells. (A-B) *U2af1*$^{wt/flox}$ mice were intercrossed with *Vav1*-Cre/*U2af1*$^{wt/flox}$ mice, and the frequency of observed versus expected alive genotypes was analyzed at E16.5 (A, n=74) or E18.5 (B, n=87). (C) Absolute numbers of hematopoietic myeloid progenitor cells (n=3-10). (D) Apoptotic cells as a percent of Annexin V^{+}/ SYTOX Green^{-} myeloid progenitor cells (n=3-10). All data are presented as mean +/- SD. ***p<0.001, by one-way ANOVA with Tukey's multiple comparison test.

Supplemental Figure 3. *U2af1* deletion induces multi-lineage bone marrow failure. (A) Experimental design of a non-competitive transplant of whole bone marrow cells (BM) from

CD45.2 *Mx1*-Cre*, U2af1*$^{flox/flox}$, *Mx1*-Cre/*U2af1*$^{wt/flox}$*,* or *Mx1*-Cre/*U2af1*$^{flox/flox}$ mice transplanted into lethally irradiated congenic wild-type CD45.1 recipient mice, followed by pIpC-induced Cre-activation and *U2af1* deletion. Analysis of the spleen was done 8-11 days post-pIpC. (B) Survival curve analyzed by Mantel-Cox log-rank test, n=5 per group. (C) Spleen cellularity of *Mx1*-Cre, *U2af1*$^{flox/flox}$, and *Mx1*-Cre/*U2af1*$^{wt/flox}$ mice compared to *Mx1*-Cre/*U2af1*$^{flox/flox}$ mice 8-11 days post pIpC. (D) Total number of neutrophils, monocytes, B cells, and T cells per spleen at 8-11 days post pIpC for *Mx1*-Cre, *U2af1*$^{flox/flox}$, and *Mx1*-Cre/*U2af1*$^{wt/flox}$ mice compared to *Mx1*-Cre/*U2af1*$^{flox/flox}$ mice. (E) Number of progenitor (KL and KLS) cells per spleen of pIpC-treated mice transplanted with *Mx1*-Cre, *U2af1*$^{flox/flox}$, and *Mx1*-Cre/*U2af1*$^{wt/flox}$ bone marrow cells compared to *Mx1*-Cre/*U2af1*$^{flox/flox}$ bone marrow cells 8-11 days post pIpC. (F-H) Absolute numbers of peripheral blood neutrophils, B cells and T cells of adult *Lineage*-Cre, *U2af1*$^{flox/flox}$, *Lineage*-Cre/*U2af1*$^{wt/flox}$ and *Lineage*-Cre/*U2af1*$^{flox/flox}$ mice (i.e., *Lyz2*-Cre*, CD19*-Cre*,* and *CD4*-Cre, respectively). All data are presented as mean +/- SD. ns (not significant), *p<0.05, **p<0.01, ***p<0.001, by one-way ANOVA with Tukey's multiple comparison test, n=5 per genotype (A-E), n=12-49 per genotype (F-H).

Supplemental Figure 4. Hematopoietic stem cells are dependent on *U2af1* expression for survival. (A) Experimental design of a competitive transplant of whole bone marrow cells (BM) from CD45.2 *Mx1*-Cre*, U2af1*$^{flox/flox}$, *Mx1*-Cre/*U2af1*$^{wt/flox}$*,* or *Mx1*-Cre/*U2af1*$^{flox/flox}$ mice mixed at a 1:1 ratio with congenic wild-type CD45.1/45.2 competitor cells followed by transplantation into lethally irradiated congenic wild-type CD45.1 recipient mice followed by pIpC-induced *U2af1* deletion. Spleen and bone marrow chimerism of *Mx1*-Cre*, U2af1*$^{flox/flox}$, and *Mx1*-Cre/*U2af1*$^{wt/flox}$ mice was compared to *Mx1*-Cre/*U2af1*$^{flox/flox}$ mice. (B) Spleen chimerism of

mature hematopoietic cells (neutrophils, monocytes, B cells, and T cells) at ten months post-pIpC (n=8). (C) Bone marrow chimerism of myeloid progenitor cells ten months post-pIpC (n=8 for KL and KLS; n=3-4 for all other). (D) Experimental design of a competitive transplant of whole bone marrow cells from CD45.2 *Lineage*-Cre, or *Lineage*-Cre/*U2af1*$^{flox/flox}$ mice mixed at a 1:1 ratio with congenic wild-type CD45.1/45.2 competitor cells followed by transplantation into lethally irradiated congenic wild-type CD45.1 recipient mice. (E) Peripheral blood neutrophil chimerism of *Lyz2*-Cre/*U2af1*$^{flox/flox}$ compared to *Lyz2*-Cre mice. (F) Peripheral blood B cell chimerism of *CD19*-Cre/*U2af1*$^{flox/flox}$ compared to *CD19*-Cre mice. (G) Peripheral blood T cell chimerism of *CD4*-Cre/*U2af1*$^{flox/flox}$ compared to *CD4*-Cre mice. (H) Relative *U2af1* mRNA expression in *Lyz2*-Cre/*U2af1*$^{flox/flox}$ compared to *Lyz2*-Cre bone marrow cells. (I) Relative *U2af1* mRNA expression in *CD19*-Cre/*U2af1*$^{flox/flox}$ compared to *CD19*-Cre bone marrow cells. (J) Relative *U2af1* mRNA expression in *CD4*-Cre/*U2af1*$^{flox/flox}$ compared to *CD4*-Cre splenic cells. All data are presented as mean +/- SD. ns (not significant), *p<0.05, **p<0.001, ***p<0.001, by one-way ANOVA with Tukey's (B, E-G) or Dunnett's (C, n=10 per genotype) multiple comparison test, or t-test (H-I, n=2-3 per genotype).

Supplemental Figure 5. *U2af1* deletion alters gene expression and pre-mRNA splicing. Differential gene expression from RNA-seq of E14.5 hematopoietic progenitor cells (Lineage$^-$, cKit$^+$, Sca1$^-$). (A) *U2af1* exon 2 expression levels, counts per million (CPM) was calculated using deepTools (4). (B) cKit expression levels, transcripts per million (TPM). (C-D) Volcano plot comparing gene expression of (C) *Vav1*-Cre/*U2af1*$^{flox/flox}$ compared to *U2af1*$^{flox/flox}$ control, and (D) *Vav1*-Cre/*U2af1*$^{wt/flox}$ compared to *U2af1*$^{flox/flox}$ control. Orange indicates genes that are significantly upregulated or downregulated at FDR<0.1 and fold-change>2. Blue are genes that

are significantly regulated at FDR<0.1 and fold change<2. (E) Venn diagram comparing differentially expressed genes in *Vav1*-Cre/*U2af1*$^{flox/flox}$ compared with *Vav1*-Cre/*U2af1*$^{wt/flox}$. (F) Absolute value of delta PSI (|ΔΨ|) of *Vav1*-Cre/*U2af1*$^{wt/flox}$ KL cells and *Vav1*-Cre/*U2af1*$^{flox/flox}$ KL cells compared to *Vav1*-Cre control cells (n=1-3, FDR<0.1, |ΔΨ|>0.1). SE (skipped exon). (G) Alternative splicing of *Mdm2* detected by gel electrophoresis (top) and quantification of the proportion of the *Mdm2* shorter transcript (bottom) (n=1-3). All data are presented as mean +/- SD. Box plots represent mean ± minimum and maximum value. n=3-4 per genotype, ns (not significant), *p<0.05, ***p<0.001, by one-way ANOVA with Tukey's multiple comparison test.

Supplemental Figure 6. Survival of mutant U2AF1(S34F) hematopoietic cells is dependent on the expression of the residual wild-type allele and the ratio of U2AF1(WT:S34F) expression. (A) Experimental design of a competitive transplant of whole bone marrow cells (BM) from CD45.2 tg*U2AF1*(WT)/*U2af1*$^{wt/wt}$, tg*U2AF1*(WT)/*U2af1*$^{flox/flox}$, tgU2AF1(S34F)/*U2af1*$^{wt/wt}$, or tgU*2AF1*(S34F)/*U2af1*$^{flox/flox}$ mice. All the donor test mice have both *Mx1*-Cre and rtTA. Transgenic U2AF1(WT) and U2AF1(S34F) were induced by 625 ppm or 10,000 ppm doxycycline (dox) chow followed by pIpC-induced *U2af1* deletion after four weeks, and analysis of the peripheral blood chimerism was performed. (B, C) Peripheral blood B cell and T cells chimerism in tg*U2AF1*(WT)/*U2af1*$^{wt/wt}$, tg*U2AF1*(WT)/*U2af1*$^{flox/flox}$, tg*U2AF1*(S34F)/*U2af1*$^{wt/wt}$, and tg*U2AF1*(S34F)/*U2af1*$^{flox/flox}$ mice after induction of transgenic U2AF1(WT) or U2AF1(S34F) by 10,000 ppm dox chow and *U2af1* deletion induced by pIpC Cre-activation (n=7-8). (D) Peripheral blood chimerism in tg*U2AF1*(WT)/*U2af1*$^{wt/wt}$ and tg*U2AF1*(S34F)/*U2af1*$^{wt/wt}$ mice after induction of transgenic U2AF1(WT) or U2AF1(S34F) by 625 ppm or 10,000 ppm dox chow and *U2af1* deletion induced by pIpC Cre-activation (n=7-8).

(E) Experimental design of a competitive transplant of whole bone marrow cells from CD45.2 *Mx1*-Cre/rtTA/tg*U2AF1*(WT)/*U2af1*$^{wt/S34F}$ (tg*U2AF1*[WT]/*U2af1*$^{wt/S34F}$) mice. Transgenic U2AF1(WT) was induced by 10,000 ppm dox chow starting two weeks post-transplant followed by pIpC-induced *U2af1* S34F mutant allele activation after two weeks of dox chow and analysis of peripheral blood chimerism was performed. (F, G) Peripheral blood B cell and T cell chimerism of tgU2AF1(WT)/*U2af1*$^{wt/S34F}$ with or without dox chow treatment (n=9-10). All data are presented as mean +/- SD. ***p<0.001, by two-way ANOVA with Sidak's (F, G) or Tukey's (B-D) multiple comparison test.

Supplemental Figure 7. Hematopoietic stem cells expressing mutant U2AF1(S34F) are more sensitive to decreased levels of the wild-type U2AF1 expression than wild-type cells. (A) Experimental design of a competitive transplant of test whole bone marrow cells (BM) from CD45.2 tg*U2AF1*(WT)/*U2af1*$^{flox/flox}$ or tg*U2AF1*(WT)/*U2af1*$^{flox/S34F}$ mice mixed at a 1:1 ratio with congenic wild-type CD45.1/45.2 competitor cells followed by transplantation into lethally irradiated congenic wild-type CD45.1 recipient mice. All the donor test mice have both *Mx1*-Cre and rtTA. Transgenic U2AF1(WT) expression was induced by 10,000 ppm doxycycline (dox) chow starting two weeks post-transplant followed by pIpC induction of *U2af1* deletion and S34F mutant allele activation two weeks later. Two weeks post-pIpC, the dox dose was reduced every two weeks (2500 ppm, 1250 ppm, 625 ppm, 350 ppm, 0 ppm), and analysis of the peripheral blood chimerism was performed. (B) A doxycycline dose-dependent curve showing peripheral blood neutrophil chimerism of tg*U2AF1*(WT)/*U2af1*$^{flox/flox}$ and tg*U2AF1*(WT)/*U2af1*$^{flox/S34F}$ (n=8-9), by two-way ANOVA with Tukey's multiple comparison test. (C) Western blot showing U2AF1 protein expression levels in bulk bone marrow cells of tg*U2AF1*(WT)/*U2af1*$^{wt/wt}$ mice

after one week on doxycycline chow (0 ppm, 1250 ppm, 2500 ppm, or 10,000 ppm). Densitometry ratio was obtained by first normalizing U2AF1 expression for each sample to its corresponding actin expression level, then dividing each sample's normalized value by that the normalized value for 0 ppm dox. (D-E) Gene ontology (GO) enrichment analysis of the differentially regulated biological processes in tg*U2AF1*(WT)/*U2af1*$^{flox/S34F}$ bone marrow KL cells at 1250 ppm, 2500 ppm and 10,000 ppm dox; biological processes significantly upregulated (D) or downregulated (E) in *Vav1*-Cre/*U2af1*$^{flox/flox}$ cells, n=3-6 per genotype. All data are presented as mean +/- SD. *p<0.05, ***p<0.001, by one-way ANOVA with Tukey's multiple comparison test.

Supplemental Figure 8. Hematopoietic cancer cells expressing mutant U2AF1(S34F) are sensitive to decreased levels of the wild-type U2AF1 expression. (A) Experimental design of transplantation of tg*U2AF1*(WT)/*U2af1*$^{flox/S34F}$ MLL-AF9 AML tumor cells (GFP^{+}/ CD45.2^{+}) isolated from the spleen of primary mice into sub-lethally irradiated CD45.2 secondary recipients. Secondary recipients were treated with or without 10,000 ppm doxycycline chow followed by pIpC induction and analysis of the peripheral blood analysis and tumor watch. (B) CD45.2^{+} MLL-AF9 AML cells chimerism up to 21 days post-second pIpC dose (n=10). (C) White blood cell count (WBC) up to 21 days post-second pIpC dose (n=10). (D) Spleen weight at the time of death (n=10). (E) Verification of recombination of the *U2af1*-KO allele in AML cells harvested from the mouse that received tg*U2AF1*(WT)/*U2af1*$^{flox/S34F}$ MLL-AF9 AML tumor cells that was treated with pIpC and died unexpectedly (pIpC*). Genomic DNA PCR was performed using *U2af1*-KO allele-specific primers that do not amplify the MG-S34F targeted allele and showed recombination (i.e., deletion) of the *U2af1* allele. (F) Verification of RNA

expression of mutant *U2af1*(S34F) from the mini-gene S34F (MG-S34F) allele in AML cells harvested from the mouse that that received tg*U2AF1*(WT)/*U2af1*$^{flox/S34F}$ MLL-AF9 AML tumor cells that was treated with pIpC and died unexpectedly (pIpC*). Sanger sequencing of the cDNA PCR product amplified using mouse *U2af1* cDNA-specific primers that do not amplify human tg*U2AF1*. All data are presented as mean +/- SD. ns (not significant), *p<0.05, **p<0.01, ***p<0.001, by one-way ANOVA with Tukey's (B) or Dunnett's (C, D) multiple comparison test.

Supplemental Tables

Supplemental Table 1: GO enrichment analysis of overlapping pathways of differentially spliced junctions between homozygous *U2af1* KO and heterozygous *U2af1*$^{wt/S34F}$ KI.

ID	Description	*U2af1* KO p-adjust	*U2af1*$^{wt/S34F}$ KI p-adjust
GO:0043484	Regulation of RNA splicing	0.00758156	0.00846466
GO:1903311	Regulation of mRNA metabolic process	0.00758156	0.08006101
GO:0006397	mRNA processing	0.01413557	0.08006101
GO:0048024	Regulation of mRNA splicing via spliceosome	0.09055668	0.06208035
GO:0016569	Covalent chromatin modification	0.00758156	0.08006101
GO:0016570	Histone modification	0.01201304	0.08151051
GO:0033044	Regulation of chromosome organization	0.09505715	0.08151051

Supplemental Table 2: Differential gene expression data (homozygous *U2af1* KO and heterozygous *U2af1* KO). Submitted as a separate Excel file.

Supplemental Table 3: GO enrichment analysis of differentially expressed genes (homozygous *U2af1* KO and heterozygous *U2af1* KO). Submitted as a separate Excel file.

Supplemental Table 4: Differentially spliced junctions (homozygous *U2af1* KO, heterozygous *U2af1* KO, and heterozygous *U2af1*$^{wt/S34F}$ KI). Submitted as a separate Excel file.

Supplemental Table 5: GO enrichment analysis of differentially spliced genes (homozygous *U2af1* KO, heterozygous *U2af1* KO, and heterozygous *U2af1*$^{wt/S34F}$ KI). Submitted as a separate Excel file.

Supplemental Table 6: Differential gene expression data (hemizygous *U2af1*$^{-/S34F}$). Submitted as a separate Excel file.

Supplemental Table 7: GO enrichment analysis of differentially expressed genes (hemizygous *U2af1*$^{-/S34F}$). Submitted as a separate Excel file.

Supplemental Table 8: Differentially spliced junctions (hemizygous *U2af1*$^{-/S34F}$). Submitted as a separate Excel file.

Supplemental Table 9: Antibodies and other resources

Reagent or Resource	Source	Catalog number
Antibodies (clone)		
Anti-CD45.1 BUV737 (A20)	BD Biosciences	Cat# 564574
Anti-CD45.2 BUV395 (104)	BD Biosciences	Cat# 564616
Anti-cKit BV421 (2B8)	Biolegend	Cat# 105828
Anti-Sca-1 PE (D7)	Biolegend	Cat# 108108
Anti-CD34 FITC (RAM34)	eBioscience	Cat# 11-0341-85
Anti-CD16/32 BV711 (93)	Biolegend	Cat# 101337

Reagent or Resource	Source	Catalog number
Anti-FLT3 APC (A2F10)	eBioscience	Cat# 17-1351-82
Anti-CD48 PE-Cy7(HM48-1)	eBioscience	Cat# 25-0481-80
Anti-CD150 APC/Fire 750 (TC15-12F12.2)	Biolegend	Cat# 115939
Anti-CD45.1 BV421 (A20)	Biolegend	Cat# 110732
Anti-CD45.2 FITC (104)	eBioscience	Cat# 11-0454-85
Anti-CD115 PE (AFS98)	eBioscience	Cat# 12-1152-83
Anti-B220 BV605 (RA3-6B2)	Biolegend	Cat# 103244
Anti-CD3e APC (145-2C11)	eBioscience	Cat# 17-0031-83
Anti-GR-1 APC/Fire 750	Biolegend	Cat# 108456
Anti-TER-119 BV605 (TER119)	Biolegend	Cat# 116239
Anti-GR-1 BV605 (RB6-8C5)	Biolegend	Cat# 108440
Anti-CD3e BV605 (145-2C11)	Biolegend	Cat# 100351
Anti-CD41 BV605 (MWReg30)	Biolegend	Cat# 133921
Anti-CD45.1 FITC (A20)	eBioscience	Cat# 11-0453-82
Anti-CD45.2 APC (104)	eBioscience	Cat# 17-0454-82
Anti-U2AF35/U2AF1	Abcam	Cat# ab86305
Anti-U2AF35/U2AF1	Abcam	Cat# ab86305
Anti-β-Actin (AC-15)	Sigma	Cat# A5441
Bacterial and Virus Strains		
MSCV MLL-AF9-IRES-GFP	Krivtsov et al. 2006 (5)	N/A
Chemicals, Peptides, and Recombinant Proteins		
Polybrene	American Bioanalytical	Cat# AB01643-00001
Polyinosinic-polycytidylic acid (pIpC)	Sigma	Cat# P1530
Tamoxifen	Sigma	Cat# T5648
Recombinant murine IL-3	PeproTech	Cat# 213-13
Recombinant murine SCF	PeproTech	Cat# 250-03
Recombinant murine TPO	PeproTech	Cat# 315-14
Recombinant murine FLT3	PeproTech	Cat# 250-31L
Doxycycline chow	Test Diet	N/A
Critical Commercial Assays		
MethoCult GF M3434	StemCell Technologies	Cat# M3434
Mouse CD117 MicroBeads	Milteny Biotec	Cat# 130091224
KAPA RNA Hyper Prep Kit with RiboErase	KapaBiosystems	Cat# KR1351
Experimental Models: Organisms/Strains		
Mice: *Mx1*-Cre: B6.Cg-Tg(Mx1-cre)1Cgn/J	Jackson Laboratory	Cat# 003556
Mice: *Vav1*-Cre: B6.Cg-*Commd10*$^{Tg(Vav1\text{-}icre)A2Kio}$/J	Jackson Laboratory	Cat# 008610
Mice: Cre-*ERT2*: B6.Cg-Tg(CAG-cre/Esr1*)5Amc/J	Jackson Laboratory	Cat# 004682
Mice: *Lyz2*-Cre: B6.129P2-*Lyz2*$^{tm1(cre)Ifo}$/J	Jackson Laboratory	Cat# 004781
Mice: *CD19*-Cre: B6.129P2(C)-*Cd19*$^{tm1(cre)Cgn}$/J	Jackson Laboratory	Cat# 006785
Mice: *CD4*-Cre: B6.Cg-Tg(Cd4-cre)1Cwi/BfluJ	Jackson Laboratory	Cat# 022071
Mice: Transgenic *U2AF1*(S34F): B6.Cg-*Gt(ROSA)26Sor*$^{tm1(rtTA*M2)Jae}$ *Col1a1*$^{tm1(tetO\text{-}U2AF1*S34F)Mjwa}$/J	Shirai et al. 2015 (2), Jackson Laboratory	Cat# 029784

Reagent or Resource	Source	Catalog number
Mice: Transgenic *U2AF1*(WT): B6.Cg-*Gt(ROSA)26Sor*$^{tm1(rtTA*M2)Jae}$ *Col1a1*$^{tm1(tetO-U2AF1*WT)Mjwa}$	Shirai et al. 2015 (2)	N/A
Mice: *U2af1*(S34F) KI: *U2af1*$^{wt/S34F}$	Fei et al. 2018 (1)	N/A
Mice: *U2af1* KO: U2af1$^{flox/flox}$	This paper	N/A
Oligonucleotides		
U2af1 KO genotyping primer, forward: GAGGGTGATGGACCTACCCA	This paper	N/A
U2af1 KO genotyping primer, reverse: GAGAAGAAAGGGGCGGCAAA	This paper	N/A
U2af1 KO allele-specific primer, forward: TGATGTGGGAAGCAGGCTAGCC	This paper	N/A
U2af1 KO allele-specific primer, reverse: TACGGTGTTGACGAGGCGT	This paper	N/A
U2af1 exon2-detecting RT-PCR primer, forward: TGAGTCGTCCGCTCGAAAAT	This paper	N/A
U2af1 exon2-detecting RT-PCR primer, reverse: CTCCATGACGACATGCTCCG	This paper	N/A
Mouse *Mdm2* RT-PCR primer, forward: GGGGACCCTCTCGGATCAC	This paper	N/A
Mouse *Mdm2* RT-PCR primer, reverse: TAAGTGTCGTTTTGCGCTCC	This paper	N/A
Mouse *Gapdh*-specific RT-PCR primer, forward: GGATGCAGGGATGATGTTC	Shirai et al. 2015 (2)	N/A
Mouse *Gapdh*-specific RT-PCR primer, reverse: TGCACCACCAACTGCTTAG	Shirai et al. 2015 (2)	N/A
Mouse *U2af1*cDNA-specific Sanger primer, forward: CGGAATACTTGGCCTCCATC	This paper	N/A
Mouse *U2af1*cDNA-specific Sanger primer, reverse: CAAGTCGATCACGGCTTTCT	This paper	N/A
U2af1 KO P1 primer: TCGTTCGAACATAACTTCGTATAG	This paper	N/A
U2af1 KO P2 primer: TCTCCAACTGTGGGACTTACAGTG	This paper	N/A

Reagent or Resource	Source	Catalog number
U2af1 KO Southern blot probe: ATCCTGTGGGAGCTAGAGTGGTGACCTCTAA CACTGCCTGCAAGAGCTGCCTGACTTCTGTA GCATGGAAGAAAGTAGCTGAGAAGTAGCTTC AAGTTAATGGCGTTTCTGCCACATCTTGCCAG AAATAATCAGTTCTTGTTTTCCCTTTACAGAG TCAACTGTTCATTTTATTTCAAAATCGGAGCA TGTCGTCATGGAGACAGATGTTCTCGGTTGC ACAATAAACCAACCTTTAGCCAGGTTTGTGT GTTGCTGGGTTTTTTGCTTTTTTTTTTTTTATT TTTCACAAATTATCAAAAGTTCTTGTGCTTTC AGGAGCGATTAACATTCTCATGCACTCAGCA AGTTAAATCTTCCCTCTGGTTTTATTTTGCAT TTGTTACAGAGGGTGATGGACCTACCCAGTG GGAAGTCTTGA	This paper	N/A

References

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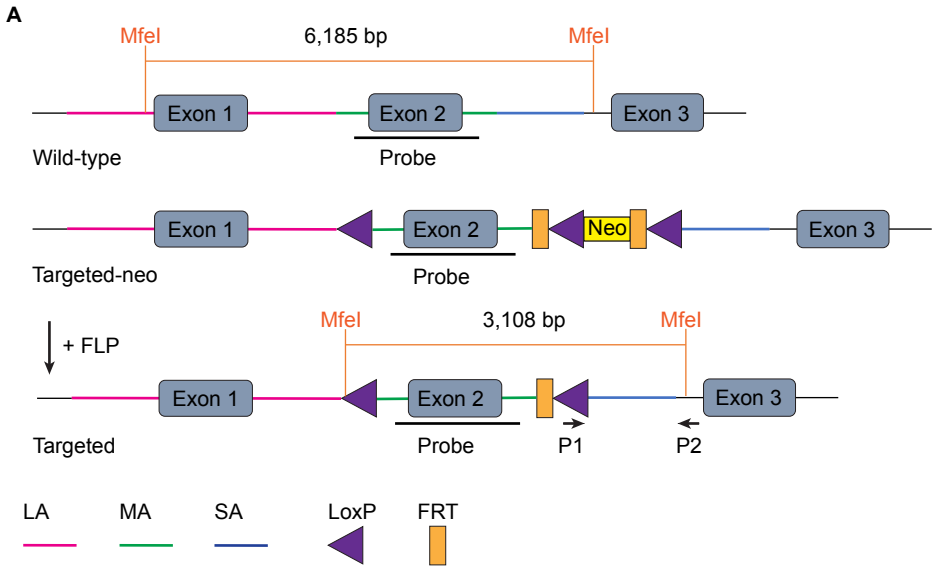
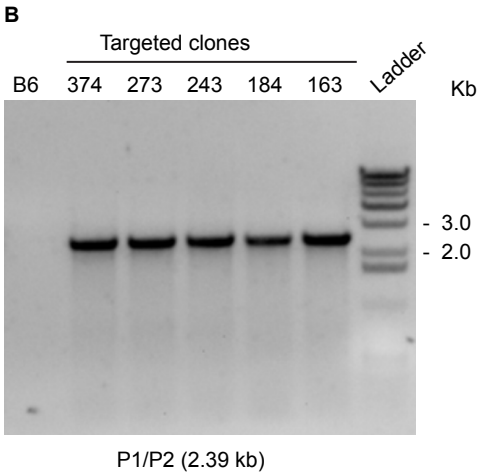
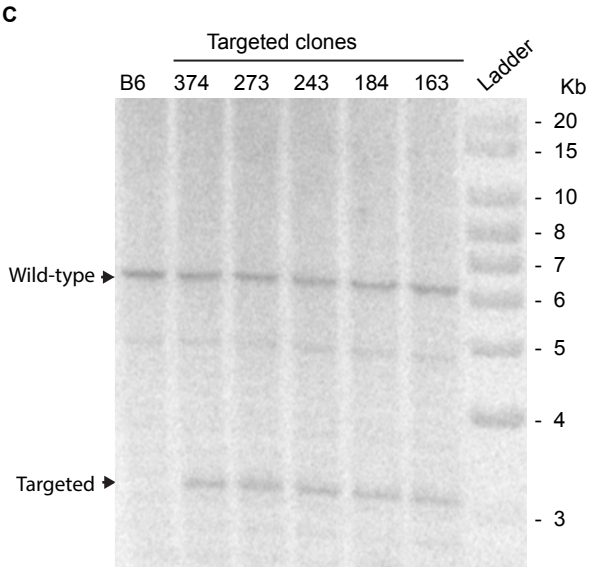
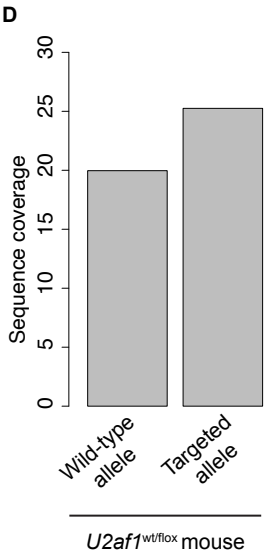
5. Krivtsov AV, Twomey D, Feng Z, Stubbs MC, Wang Y, Faber J, et al. Transformation from committed progenitor to leukaemia stem cell initiated by MLL-AF9. *Nature.* 2006;442(7104):818-22.





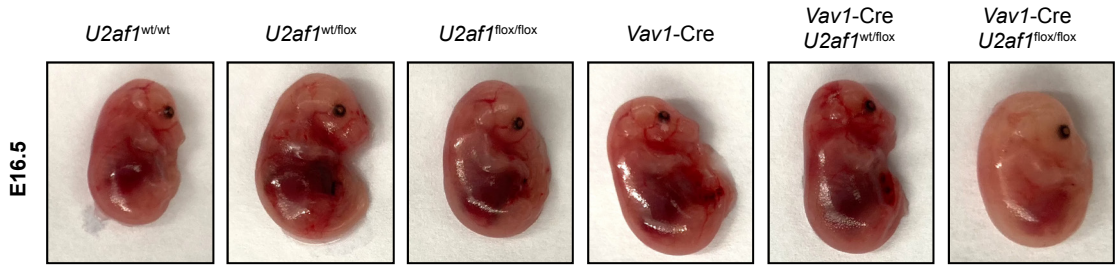




Supplemental Figure 1. Generation of a conditional U2af1 knock-out allele. (**A**) Targeting strategy to insert loxP sites flanking *U2af1* exon 2. (**B-C**) Successful targeting of five embryonic stem cell clones was verified by PCR and Southern blotting after digestion with the restriction enzyme MfeI. Wild-type C57BL/6 (B6) DNA was used as a control, and clone 243 was used to generate the *U2af1* knock-out mouse. (**D**) Whole-genome sequencing was performed on a heterozygous knock-out (*U2af1*$^{wt/flox}$) mouse. The heterozygous *U2af1*$^{wt/flox}$ mouse has sequence coverage for both U2af1 wild-type allele and the targeted allele containing the LoxP sequences flanking exon 2, validating correct targeting of U2af1 in the *U2af1*$^{wt/flox}$ mouse. Neo (neomycin resistance gene), LA (long arm), MA (middle arm), SA (short arm). Sequence coverage (number of reads covering the *U2af1* exon 2 targeted locus).

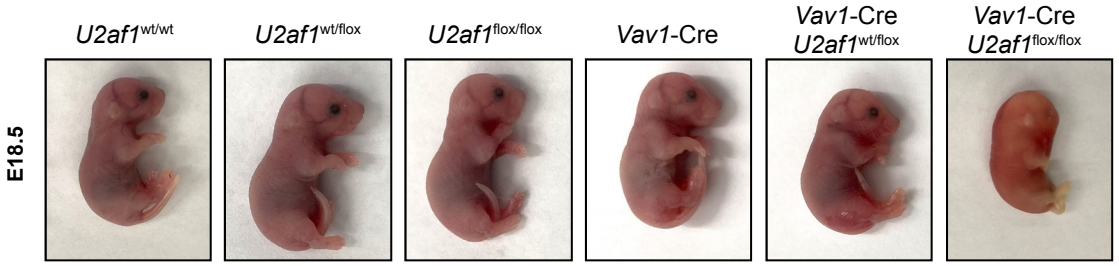
A

Vav1-Cre	-	-	-	+	+	+
U2af1	wt/wt	wt/flox	flox/flox	wt/wt	wt/flox	flox/flox
Live/Total E16.5, n=74	8/8	16/16	11/11	11/11	19/19	9/9
Expected genotype frequency	12.5%	25%	12.5%	12.5%	25%	12.5%
Observed genotype frequency alive	10.8%	21.6%	14.9%	14.9%	25.7%	12.2%

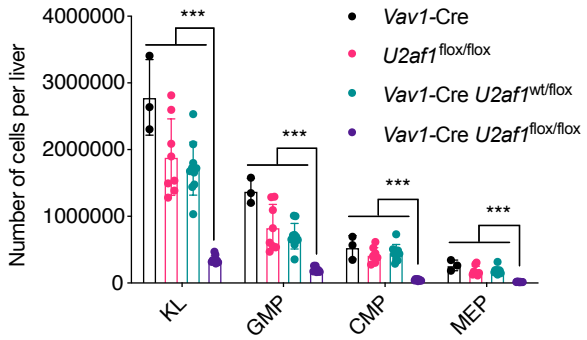


B

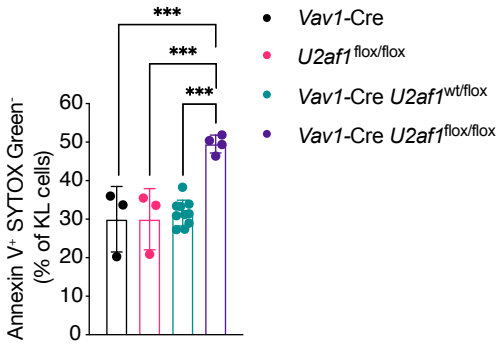
Vav1-Cre	-	-	-	+	+	+
U2af1	wt/wt	wt/flox	flox/flox	wt/wt	wt/flox	flox/flox
Live/Total E18.5, n=87	14/14	23/23	10/10	11/11	21/21	2/8
Expected genotype frequency	12.5%	25%	12.5%	12.5%	25%	12.5%
Observed genotype frequency alive	16.1%	26.4%	11.5%	12.6%	24.1%	2.3%



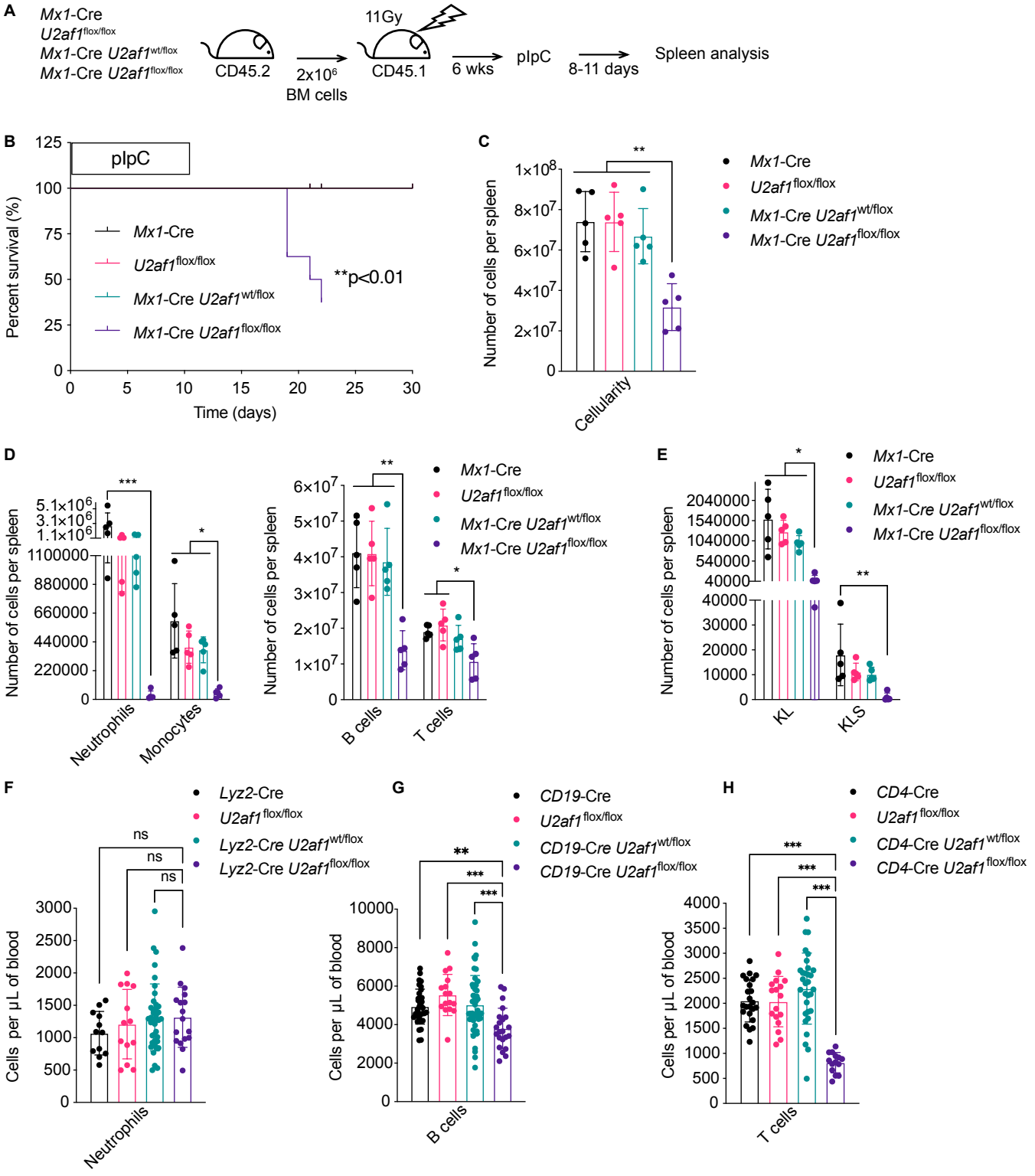
C



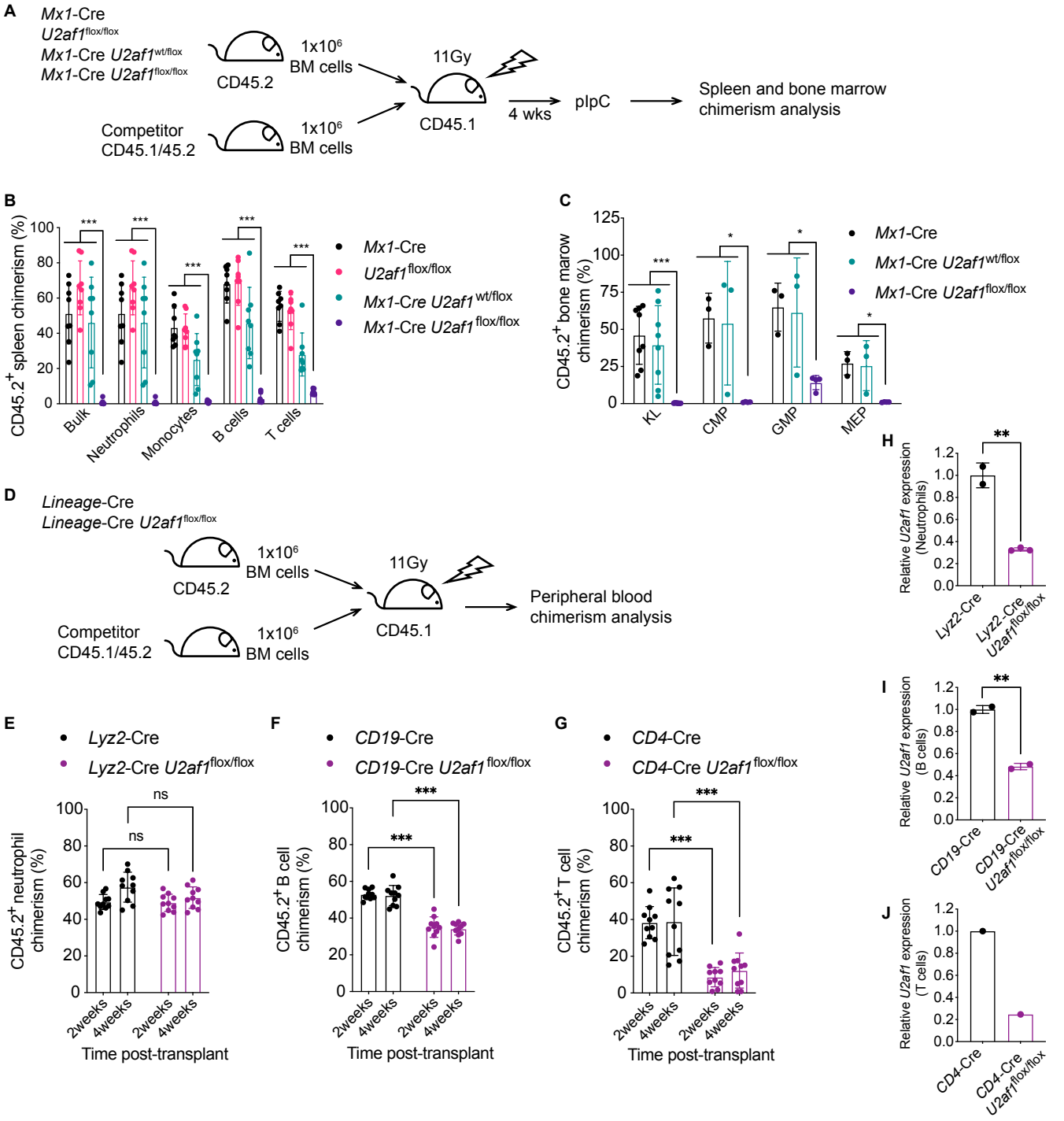
D



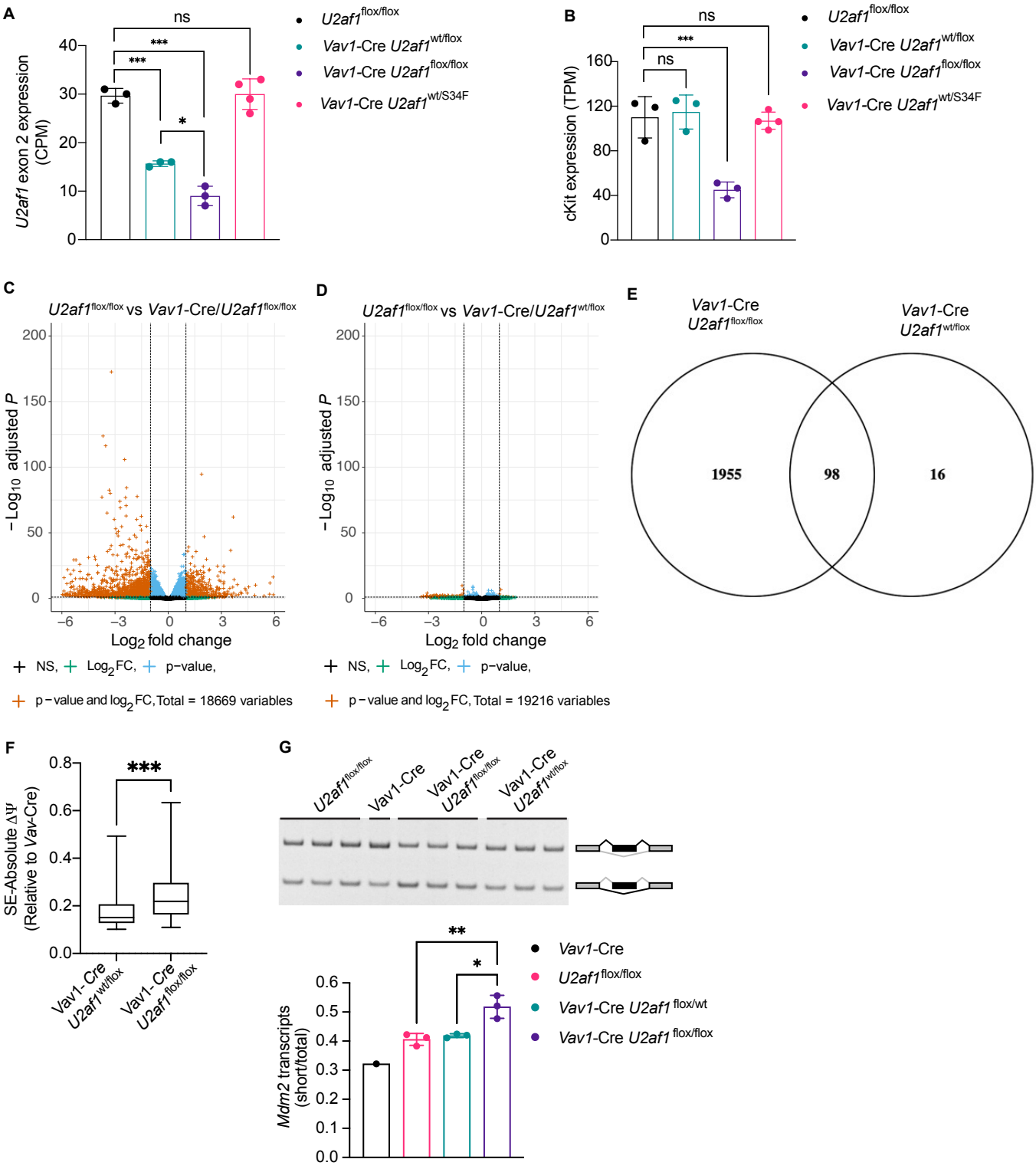
Supplemental Figure 2. Embryonic deletion of *U2af1* in hematopoietic cells is lethal and reduces the number of myeloid, lymphoid, and hematopoietic progenitor cells. (A-B) *U2af1*$^{wt/flox}$ mice were intercrossed with *Vav1*-Cre/*U2af1*$^{wt/flox}$ mice, and the frequency of observed versus expected alive genotypes was analyzed at E16.5 (A, n=74) or E18.5 (B, n=87). (C) Absolute numbers of hematopoietic myeloid progenitor cells (n=3-10). (D) Apoptotic cells as a percent of Annexin V+/ SYTOX Green- myeloid progenitor cells (n=3-10). All data are presented as mean +/- SD. ***p<0.001, by one-way ANOVA with Tukey's multiple comparison test.



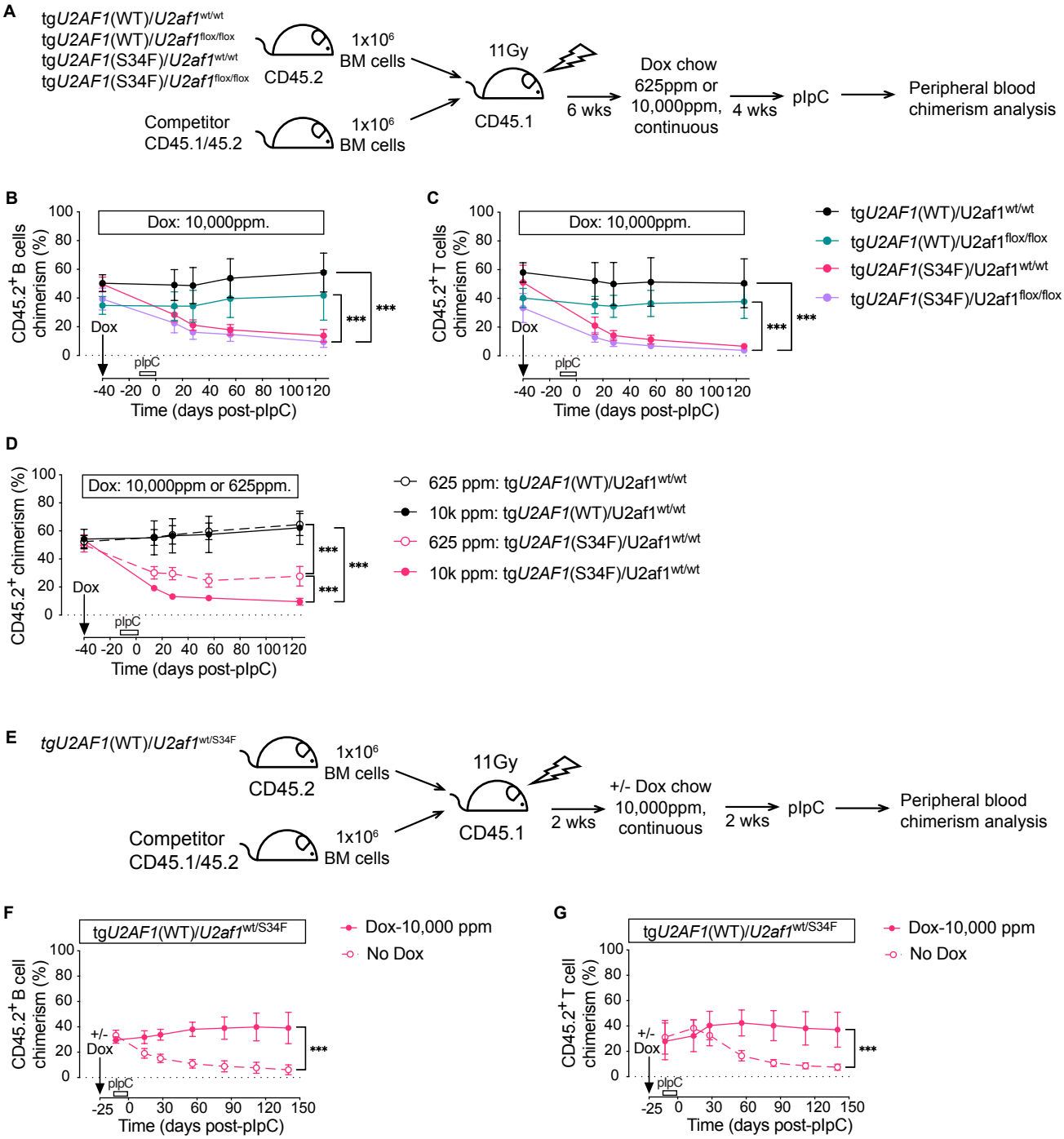
Supplemental Figure 3. *U2af1* deletion induces multi-lineage bone marrow failure. (**A**) Experimental design of a non-competitive transplant of whole bone marrow cells (BM) from CD45.2 *Mx1*-Cre, *U2af1*$^{flox/flox}$, *Mx1*-Cre/*U2af1*$^{wt/flox}$, or *Mx1*-Cre/*U2af1*$^{flox/flox}$ mice transplanted into lethally irradiated congenic wild-type CD45.1 recipient mice, followed by pIpC-induced Cre-activation and *U2af1* deletion. Analysis of the spleen was done 8-11 days post-pIpC. (**B**) Survival curve analyzed by Mantel-Cox log-rank test, n=5 per group. (**C**) Spleen cellularity of *Mx1*-Cre, *U2af1*$^{flox/flox}$, and *Mx1*-Cre/*U2af1*$^{wt/flox}$ mice compared to *Mx1*-Cre/*U2af1*$^{flox/flox}$ mice 8-11 days post pIpC. (**D**) Total number of neutrophils, monocytes, B cells, and T cells per spleen at 8-11 days post pIpC for *Mx1*-Cre, *U2af1*$^{flox/flox}$, and *Mx1*-Cre/*U2af1*$^{wt/flox}$ mice compared to *Mx1*-Cre/*U2af1*$^{flox/flox}$ mice. (**E**) Number of progenitor (KL and KLS) cells per spleen of pIpC-treated mice transplanted with *Mx1*-Cre, *U2af1*$^{flox/flox}$, and *Mx1*-Cre/*U2af1*$^{wt/flox}$ bone marrow cells compared to *Mx1*-Cre/*U2af1*$^{flox/flox}$ bone marrow cells 8-11 days post pIpC. (**F-H**) Absolute numbers of peripheral blood neutrophils, B cells and T cells of adult *Lineage*-Cre, *U2af1*$^{flox/flox}$, *Lineage*-Cre/*U2af1*$^{wt/flox}$ and *Lineage*-Cre/*U2af1*$^{flox/flox}$ mice (i.e., *Lyz2*-Cre, *CD19*-Cre, and *CD4*-Cre, respectively). All data are presented as mean +/- SD. ns (not significant), *p<0.05, **p<0.01, ***p<0.001, by one-way ANOVA with Tukey's multiple comparison test, n=5 per genotype (**A-E**), n=12-49 per genotype (**F-H**).



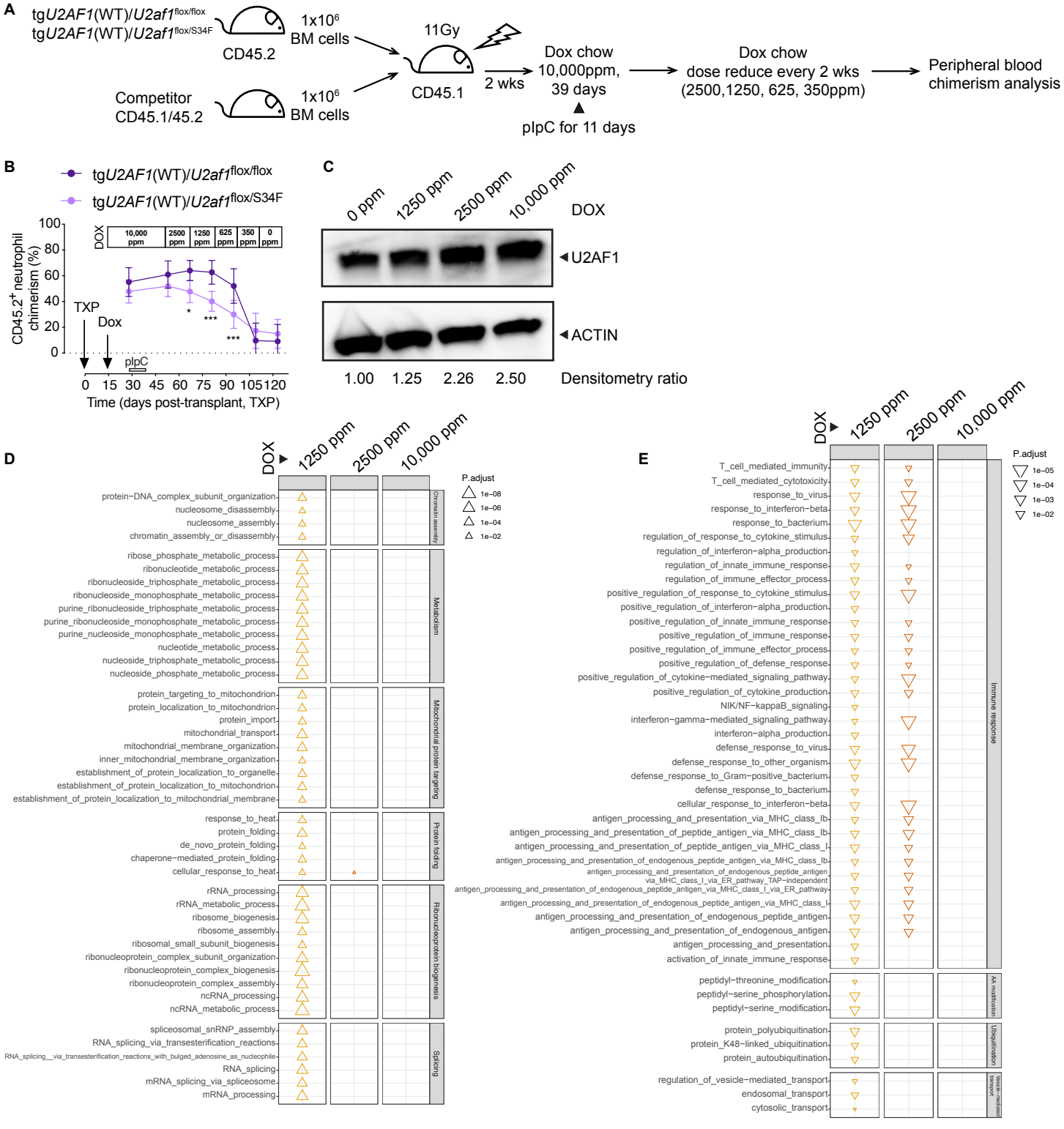
Supplemental Figure 4. Hematopoietic stem cells are dependent on U2af1 expression for survival. (**A**) Experimental design of a competitive transplant of whole bone marrow cells (BM) from CD45.2 *Mx1*-Cre, *U2af1*$^{flox/flox}$, *Mx1*-Cre/*U2af1*$^{wt/flox}$, or *Mx1*-Cre/*U2af1*$^{flox/flox}$ mice mixed at a 1:1 ratio with congenic wild-type CD45.1/45.2 competitor cells followed by transplantation into lethally irradiated congenic wild-type CD45.1 recipient mice followed by pIpC-induced *U2af1* deletion. Spleen and bone marrow chimerism of *Mx1*-Cre, *U2af1*$^{flox/flox}$, and *Mx1*-Cre/*U2af1*$^{wt/flox}$ mice was compared to *Mx1*-Cre/*U2af1*$^{flox/flox}$ mice. (**B**) Spleen chimerism of mature hematopoietic cells (neutrophils, monocytes, B cells, and T cells) at ten months post-pIpC (n=8). (**C**) Bone marrow chimerism of myeloid progenitor cells ten months post-pIpC (n=8 for KL and KLS; n=3-4 for all other). (**D**) Experimental design of a competitive transplant of whole bone marrow cells from CD45.2 *Lineage*-Cre, or *Lineage*-Cre/*U2af1*$^{flox/flox}$ mice mixed at a 1:1 ratio with congenic wild-type CD45.1/45.2 competitor cells followed by transplantation into lethally irradiated congenic wild-type CD45.1 recipient mice. (**E**) Peripheral blood neutrophil chimerism of *Lyz2*-Cre/*U2af1*$^{flox/flox}$ compared to *Lyz2*-Cre mice. (**F**) Peripheral blood B cell chimerism of *CD19*-Cre/*U2af1*$^{flox/flox}$ compared to *CD19*-Cre mice. (**G**) Peripheral blood T cell chimerism of *CD4*-Cre/*U2af1*$^{flox/flox}$ compared to *CD4*-Cre mice. (**H**) Relative *U2af1* mRNA expression in *Lyz2*-Cre/U2af1$^{flox/flox}$ compared to *Lyz2*-Cre bone marrow cells. (I) Relative *U2af1* mRNA expression in *CD19*-Cre/*U2af1*$^{flox/flox}$ compared to *CD19*-Cre bone marrow cells. (**J**) Relative *U2af1* mRNA expression in *CD4*-Cre/U2af1flox/flox compared to *CD4*-Cre splenic cells. All data are presented as mean +/- SD. ns (not significant), *p<0.05, **p<0.001, ***p<0.001, by one-way ANOVA with Tukey's (**B, E-G**) or Dunnett's (**C**, n=10 per genotype) multiple comparison test, or t-test (**H-I**, n=2-3 per genotype).



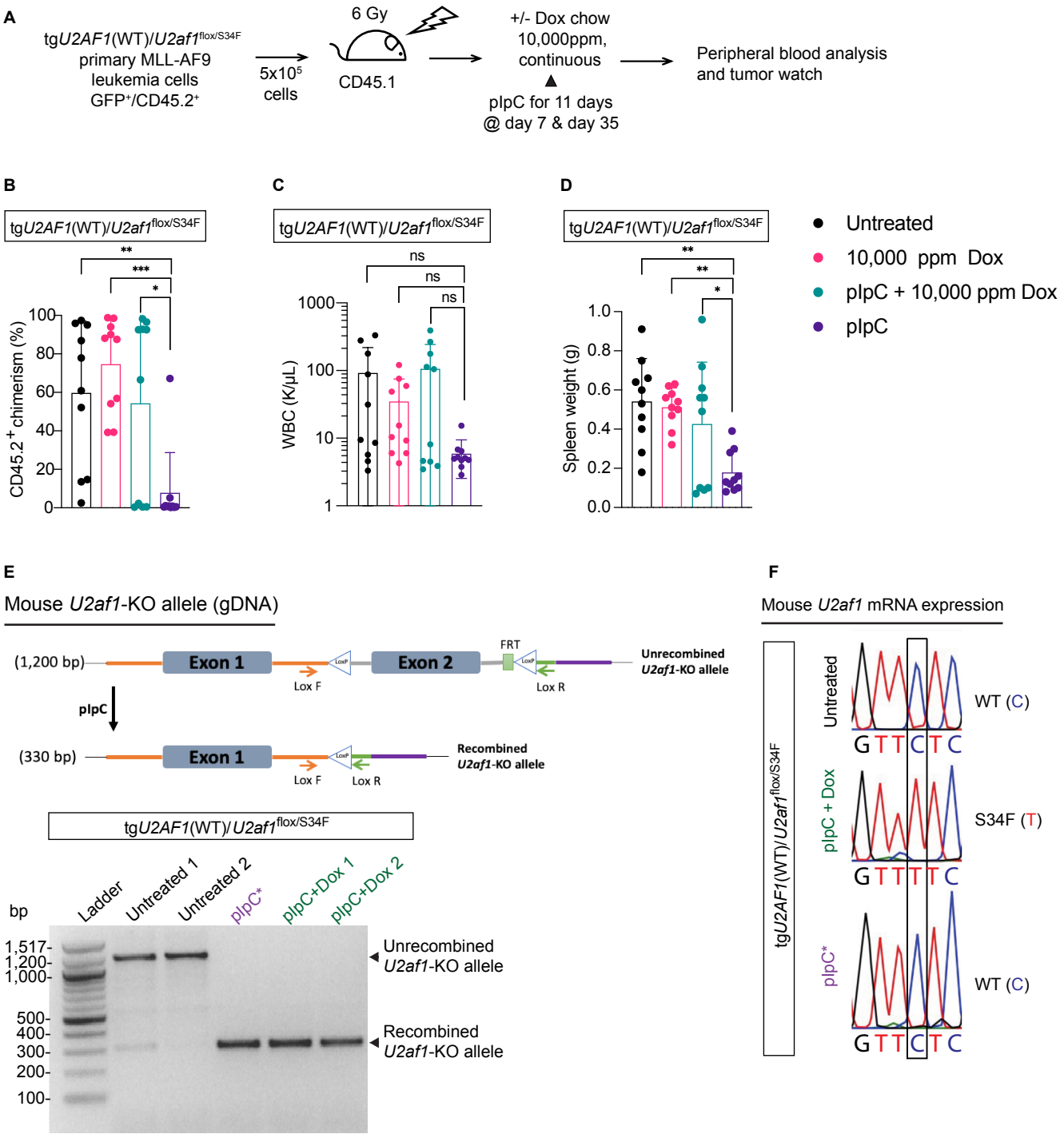
Supplemental Figure 5. *U2af1* deletion alters gene expression and pre-mRNA splicing. Differential gene expression from RNA-seq of E14.5 hematopoietic progenitor cells (Lineage-, cKit+, Sca1-). (**A**) *U2af1* exon 2 expression levels, counts per million (CPM) was calculated using deepTools (4). (**B**) cKit expression levels, transcripts per million (TPM). (**C-D**) Volcano plot comparing gene expression of (**C**) *Vav1*-Cre/*U2af1*$^{flox/flox}$ compared to *U2af1*$^{flox/flox}$ control, and (**D**) *Vav1*-Cre/*U2af1*$^{wt/flox}$ compared to *U2af1*$^{flox/flox}$ control. Orange indicates genes that are significantly upregulated or downregulated at FDR<0.1 and fold-change>2. Blue are genes that are significantly regulated at FDR<0.1 and fold change<2. (**E**) Venn diagram comparing differentially expressed genes in *Vav1*-Cre/*U2af1*$^{flox/flox}$ compared with *Vav1*-Cre/*U2af1*$^{wt/flox}$. (**F**) Absolute value of delta PSI (|Δψ|) of *Vav1*-Cre/*U2af1*$^{wt/flox}$ KL cells and *Vav1*-Cre/*U2af1*$^{flox/flox}$ KL cells compared to *Vav1*-Cre control cells (n=1-3, FDR<0.1, |Δψ|>0.1). SE (skipped exon). (**G**) Alternative splicing of Mdm2 detected by gel electrophoresis (top) and quantification of the proportion of the *Mdm2* shorter transcript (bottom) (n=1-3). All data are presented as mean +/- SD. n=3-4 per genotype, ns (not significant), *p<0.05, ***p<0.001, by one-way ANOVA with Tukey's multiple comparison test.



Supplemental Figure 6. Survival of mutant U2AF1(S34F) hematopoietic cells is dependent on the expression of the residual wild-type allele and the ratio of U2AF1(WT:S34F) expression. (**A**) Experimental design of a competitive transplant of whole bone marrow cells (BM) from CD45.2 tg*U2AF1*(WT)/*U2af1*$^{wt/wt}$, tg*U2AF1*(WT)/*U2af1*$^{flox/flox}$, tg*U2AF1*(S34F)/*U2af1*$^{wt/wt}$, or tg*U2AF1*(S34F)/*U2af1*$^{flox/flox}$ mice. All the donor test mice have both *Mx1*-Cre and rtTA. Transgenic U2AF1(WT) and U2AF1(S34F) were induced by 625 ppm or 10,000 ppm doxycycline (dox) chow followed by pIpC-induced *U2af1* deletion after four weeks, and analysis of the peripheral blood chimerism was performed. (**B, C**) Peripheral blood B cell and T cells chimerism in tg*U2AF1*(WT)/*U2af1*$^{wt/wt}$, tg*U2AF1*(WT)/*U2af1*$^{flox/flox}$, tg*U2AF1*(S34F)/*U2af1*$^{wt/wt}$, and tgU2AF1(S34F)/*U2af1*$^{flox/flox}$ mice after induction of transgenic U2AF1(WT) or U2AF1(S34F) by 10,000 ppm dox chow and U2af1 deletion induced by pIpC Cre-activation (n=7-8). (**D**) Peripheral blood chimerism in tgU2AF1(WT)/*U2af1*$^{wt/wt}$ and tgU2AF1(S34F)/*U2af1*$^{wt/wt}$ mice after induction of transgenic U2AF1(WT) or U2AF1(S34F) by 625 ppm or 10,000 ppm dox chow and *U2af1* deletion induced by pIpC Cre-activation (n=7-8). (**E**) Experimental design of a competitive transplant of whole bone marrow cells from CD45.2 *Mx1*-Cre/rtTA/tg*U2AF1*(WT)/*U2af1*$^{wt/S34F}$ (tg*U2AF1*[WT]/*U2af1*$^{wt/S34F}$) mice. Transgenic U2AF1(WT) was induced by 10,000 ppm dox chow starting two weeks post-transplant followed by pIpC-induced *U2af1* S34F mutant allele activation after two weeks of dox chow and analysis of peripheral blood chimerism was performed. (**F, G**) Peripheral blood B cell and T cell chimerism of tg*U2AF1*(WT)/*U2af1*$^{wt/S34F}$ with or without dox chow treatment (n=9-10). All data are presented as mean +/- SD. ***p<0.001, by two-way ANOVA with Sidak's (**F, G**) or Tukey's (**B-D**) multiple



Supplemental Figure 7. Hematopoietic stem cells expressing mutant U2AF1(S34F) are more sensitive to decreased levels of the wild-type U2AF1 expression than wild-type cells. (**A**) Experimental design of a competitive transplant of test whole bone marrow cells (BM) from CD45.2 tg*U2AF1*(WT)/*U2af1*$^{flox/flox}$ or tg*U2AF1*(WT)/*U2af1*$^{flox/S34F}$ mice mixed at a 1:1 ratio with congenic wild-type CD45.1/45.2 competitor cells followed by transplantation into lethally irradiated congenic wild-type CD45.1 recipient mice. All the donor test mice have both *Mx1*-Cre and rtTA. Transgenic U2AF1(WT) expression was induced by 10,000 ppm doxycycline (dox) chow starting two weeks post-transplant followed by pIpC induction of *U2af1* deletion and S34F mutant allele activation two weeks later. Two weeks post-pIpC, the dox dose was reduced every two weeks (2500 ppm, 1250 ppm, 625 ppm, 350 ppm, 0 ppm), and analysis of the peripheral blood chimerism was performed. (**B**) A doxycycline dose-dependent curve showing peripheral blood neutrophil chimerism of tg*U2AF1*(WT)/*U2af1*$^{flox/flox}$ and tg*U2AF1*(WT)/*U2af1*$^{flox/S34F}$ (n=8-9), by two-way ANOVA with Tukey's multiple comparison test. (**C**) Western blot showing U2AF1 protein expression levels in bulk bone marrow cells of tg*U2AF1*(WT)/*U2af1*$^{wt/wt}$ mice after one week on doxycycline chow (0 ppm, 1250 ppm, 2500 ppm, or 10,000 ppm). Densitometry ratio was obtained by first normalizing U2AF1 expression for each sample to its corresponding actin expression level, then dividing each sample's normalized value by that the normalized value for 0 ppm dox. (**D-E**) Gene ontology (GO) enrichment analysis of the differentially regulated biological processes in tg*U2AF1*(WT)/*U2af1*$^{flox/S34F}$ bone marrow KL cells at 1250 ppm, 2500 ppm and 10,000 ppm dox; biological processes significantly upregulated (D) or downregulated (E) in *Vav1*-Cre/*U2af1*$^{flox/flox}$ cells, n=3-6 per genotype. All data are presented as mean +/- SD. *p<0.05, ***p<0.001, by one-way ANOVA with Tukey's multiple comparison test.



Supplemental Figure 8. Hematopoietic cancer cells expressing mutant U2AF1(S34F) are sensitive to decreased levels of the wild-type U2AF1 expression. (**A**) Experimental design of transplantation of tg*U2AF1*(WT)/*U2af1*$^{flox/S34F}$ MLL-AF9 AML tumor cells (GFP$^+$/CD45.2$^+$) isolated from the spleen of primary mice into sub-lethally irradiated CD45.2 secondary recipients. Secondary recipients were treated with or without 10,000 ppm doxycycline chow followed by pIpC induction and analysis of the peripheral blood analysis and tumor watch. (**B**) CD45.2$^+$ MLL-AF9 AML cells chimerism up to 21 days post-second pIpC dose (n=10). (**C**) White blood cell count (WBC) up to 21 days post-second pIpC dose (n=10). (**D**) Spleen weight at the time of death (n=10). (**E**) Verification of recombination of the U2af1-KO allele in AML cells harvested from the mouse that received tg*U2AF1*(WT)/*U2af1*$^{flox/S34F}$ MLL-AF9 AML tumor cells that was treated with pIpC and died unexpectedly (pIpC*). Genomic DNA PCR was performed using *U2af1*-KO allele-specific primers that do not amplify the MG-S34F targeted allele and showed recombination (i.e., deletion) of the *U2af1* allele. (**F**) Verification of RNA expression of mutant U2af1(S34F) from the mini-gene S34F (MG-S34F) allele in AML cells harvested from the mouse that that received tg*U2AF1*(WT)/*U2af1*$^{flox/S34F}$ MLL-AF9 AML tumor cells that was treated with pIpC and died unexpectedly (pIpC*). Sanger sequencing of the cDNA PCR product amplified using mouse *U2af1* cDNA-specific primers that do not amplify human tgU2AF1. All data are presented as mean +/- SD. ns (not significant), *p<0.05, **p<0.01, ***p<0.001, by one-way ANOVA with Tukey's (**B**) or Dunnett's (**C, D**) multiple comparison test.