SUPPLEMENTARY INFORMATION

SUPPLEMENTARY METHODS

Generation of mice by CRISPR/Cas9 gene editing

Single guide RNA sequences (sgRNA) were designed using the Breaking Cas online software. For *U2af1* mutant mice, single guide RNA (sgRNA) (CTGCCGCCAGTATGAAATGG) was designed against the exon 6 of the *U2af1* gene and was produced by *in vitro* transcription (MEGAshortscript T7 kit, Life Technologies) from g-Blocks containing T7 promoter. Capped polyadenylated Cas9 mRNA was produced by *in vitro* transcription (mMESSAGE mMACHINE T7 ULTRA kit, Life Technologies) from the plasmid pMJ920 (Addgene 42234) linearized with BstBI and treated with Antarctic phosphatase (NEB). Both the Cas9 mRNA and the sgRNAs were purified using MEGAclear kit (Life Technologies) and eluted in TE buffer. Cas9 mRNA (50 ng/µl) and sgRNA (20 ng/ µl) were injected into one-cell embryos [30] obtained from the cross of female C57BL6J/CBAF1 with male C57BL6J. Microinjected embryos were allowed to develop to blastocysts stage and transferred into the uterus of CD1 pseudopregnant females to produce founder mice (F0) [31]. For genotyping, DNA was extracted from the tail of 3-week-old mice using a conventional DNA isopropanol-ethanol precipitation protocol. PCR products were visualized by 3% agarose gel electrophoresis. PCR products were sequenced by Sanger using DNA extracted from a tail biopsy using specific primers (sequences in **Supplementary Table 1**). Wild-type (*U2af1*$^{+/+}$) and heterozygous mutant mice (*U2af1*$^{mut/+}$) were analyzed after backcrossing to C57BL/6J background for six generations. *Tet2* knockout mice were also generated by CRISPR/Cas9 system. The sgRNA (CTGCCGCCAGTATGAAATGG) was designed against

the exon 3 of the *Tet2* gene [32]. Primers for genotyping are indicated in **Supplementary Table 1**. Pure C57BL/6 background was generated by backcrossing for seven generations, then heterozygotes animals were cross to obtain knockout mice.

Table 1. Primers used in this study.

Gene	Forward	Reverse	Application
U2af1	TCAGCCCTTAGCAACACAGCAGT	TGCCCACCACACCAGAGTCC	Genotyping/ Sequencing
Tet2	CTCTCAAGTCACAGAAACACGTG	GTTGCTTGAGGCTCTGCAGCTC	
U2af1	AAGTTTCGACGTGAGGAGGA	CCTCTTGTGCACTCTCCCAT	RT-qPCR
β-actin	GTGGGCCGCTCTAGGCACCAA	CTCTTTGATGTCACGCACGTATTC	
Tet2	CTCTCCAAGCCGTGACAATG	GCAGCCTGGATATCTGTTGG	
Hprt	GCCCTTGACTATAATGAG	GATAAGCGACAATCTACC	

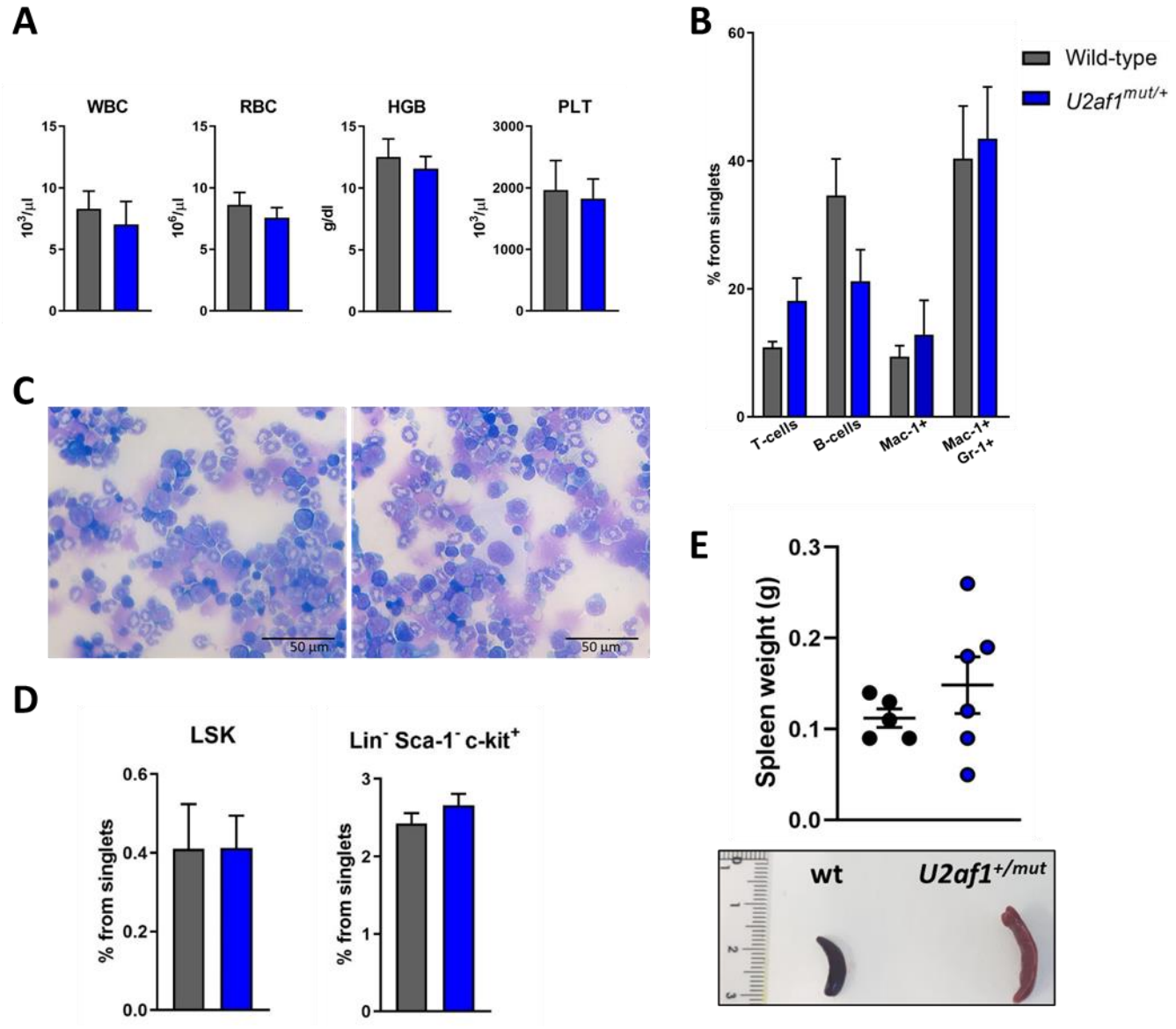
Table 2. FACS-antibodies used in flow cytometry analysis.

Stain	Antibody	Conjugate	Clone	Dilution	Company
Mature cells	CD4	PE	-	1/100	Miltenyi Biotec
	Gr-1	PE-Cy5	RB6-8C5	1/400	BioLegend
	CD8	FITC	53-67	1/200	Miltenyi Biotec
	B220	PE-Cy7	RAS-6B2	1/100	BD Bioscience
	Mac-1	V450	M1/70	1/200	BD Bioscience

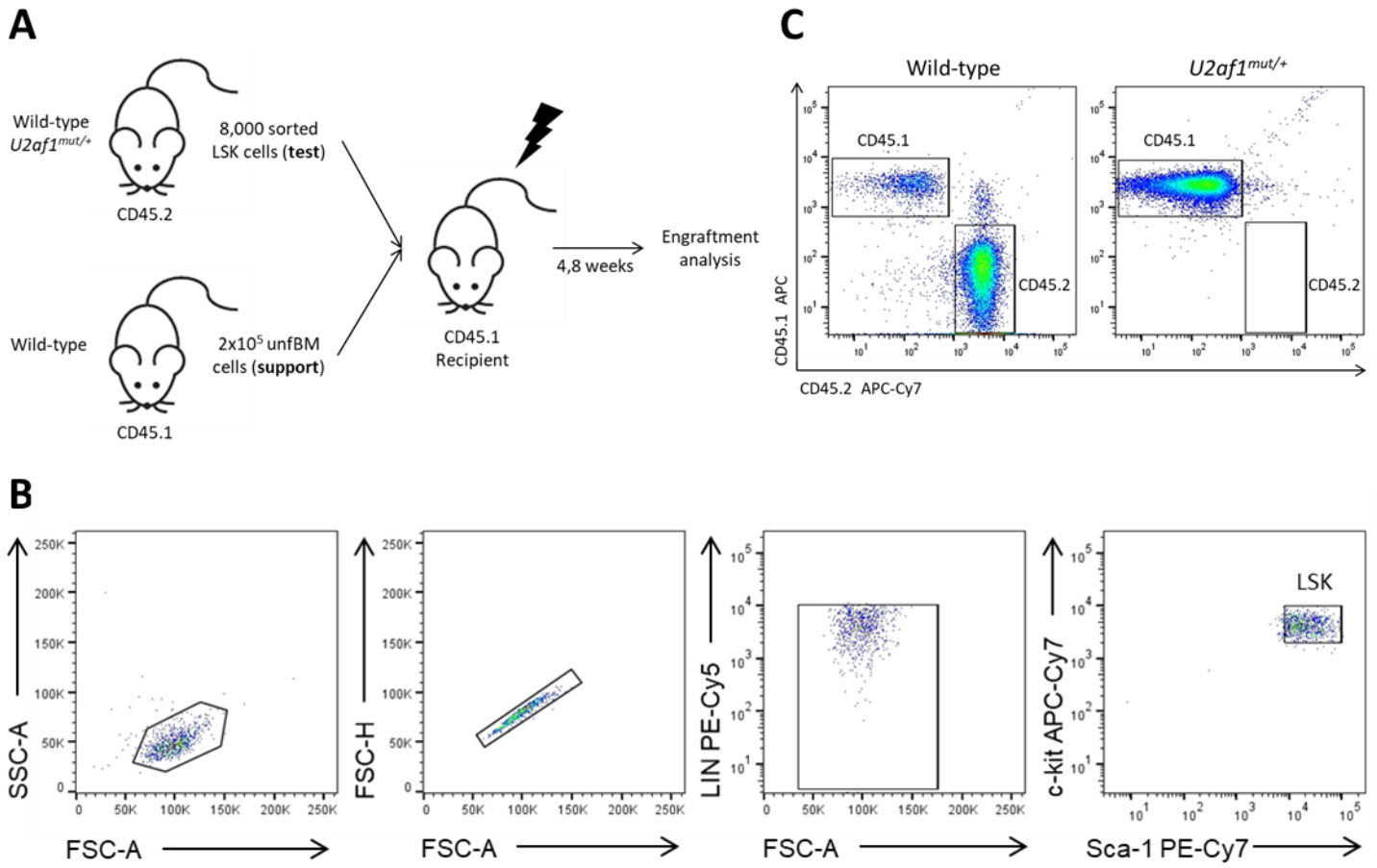
Stain	Antibody	Conjugate	Clone	Dilution	Company
Reconstitution analysis	CD4	PE	-	1/100	Miltenyi Biotec
	Gr-1	PE-Cy5	RB6-8C5	1/400	BioLegend
	CD8	FITC	53-67	1/200	Miltenyi Biotec
	B220	PE-Cy7	RAS-6B2	1/100	BD Bioscience
	Mac-1	V450	M1/70	1/200	BD Bioscience
	CD45.1	APC	A20	1/200	BioLegend
	CD45.2	APC-Cy7	104	1/200	BD Bioscience

Stain		Antibody	Conjugate	Clone	Dilution	Company
Myeloerythroid progenitors	Lineage antibodies	Mac-1	PE-Cy5	M1/70	1/800	BioLegend
		B220		RA3-6B2	1/400	
		CD5		53-7.3	1/400	
		Ter119		TER-119	1/200	
		Gr-1		RB6-8C5	1/800	
		CD4		GK15	1/800	
		CD8		53-6.7	1/800	
		CD150	APC	9D1	1/100	eBioscience
		c-kit	APC-eF780	2B8	1/200	eBioscience
		Sca-1	Pacific Blue	E13-161.7	1/100	BioLegend
		CD16/32	PE-Cy7	93	1/400	Invitrogen
		CD41	FITC	MWReg30	1/25	Invitrogen
		CD105		MJ7/18	1/25	eBioscience

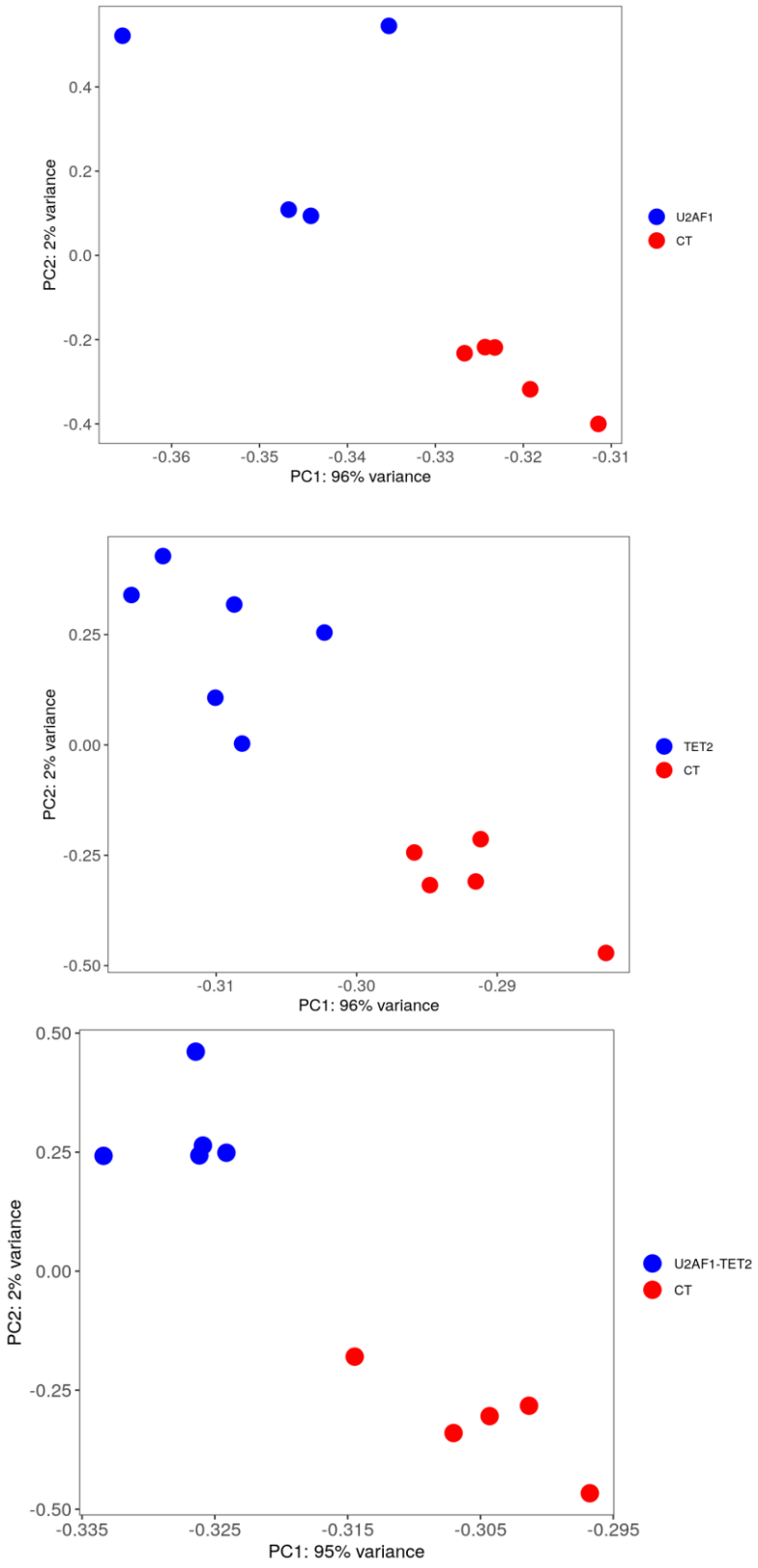
Stain		Antibody	Conjugate	Clone	Dilution	Company
HSPC	Lineage antibodies	Mac-1	PE-Cy5	M1/70	1/800	BioLegend
		B220		RA3-6B2	1/400	
		CD5		53-7.3	1/400	
		Ter119		TER-119	1/200	
		Gr-1		RB6-8C5	1/800	
		CD4		GK15	1/800	
		CD8		53-6.7	1/800	
		c-kit	APC-eF780	2B8	1/200	eBioscience
		Sca-1	Pacific Blue	E13-161.7	1/100	BioLegend



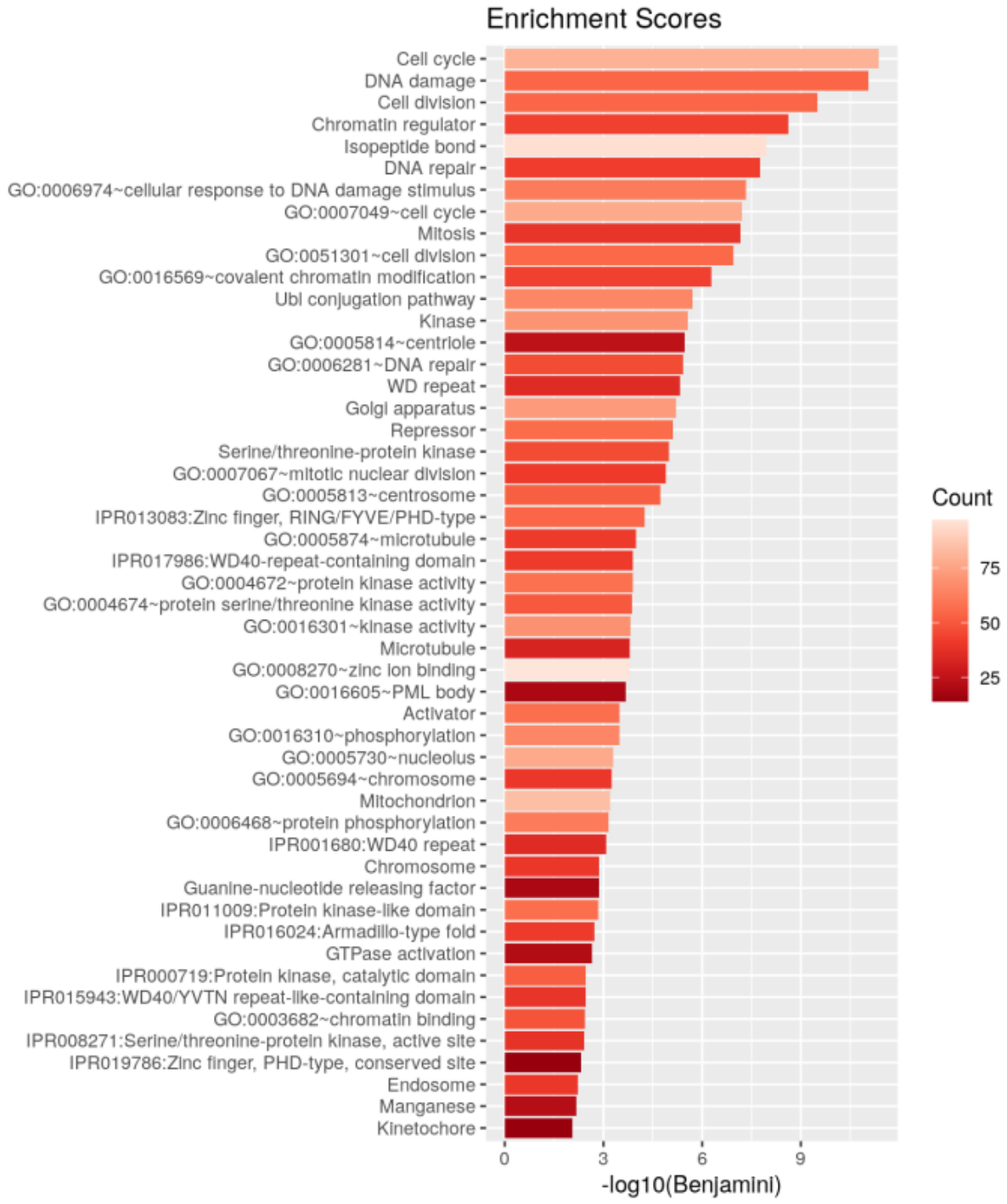
Supplementary Figure 1. Hematological characterization of aged *U2af1*$^{mut/+}$ mice (23 month-old). a) Blood counts from WT and *U2af1*$^{mut/+}$ mutant mice (WT n=5, *U2af1*$^{mut/+}$ n=6). b) Frequencies of peripheral blood mature cells (WT n=5, *U2af1*$^{mut/+}$ n=6). c) Representative May Grünwald-Giemsa (MGG) stain of unfractionated bone marrow cells (WT n=4, *U2af1*$^{mut/+}$ n=6). d) Left panel: Frequencies of LSK (Lin$^-$ c-kit$^+$ Sca-1$^+$) cells. Right panel: Frequencies of progenitors (Lin$^-$ c-kit$^+$ Sca-1$^-$). e) Upper panel: Quantification of the spleen weight. Lower panel: Images of spleens from WT and mutant aged *U2af1*$^{mut/+}$ mice. a-e) Mean values ± SEM are represented.



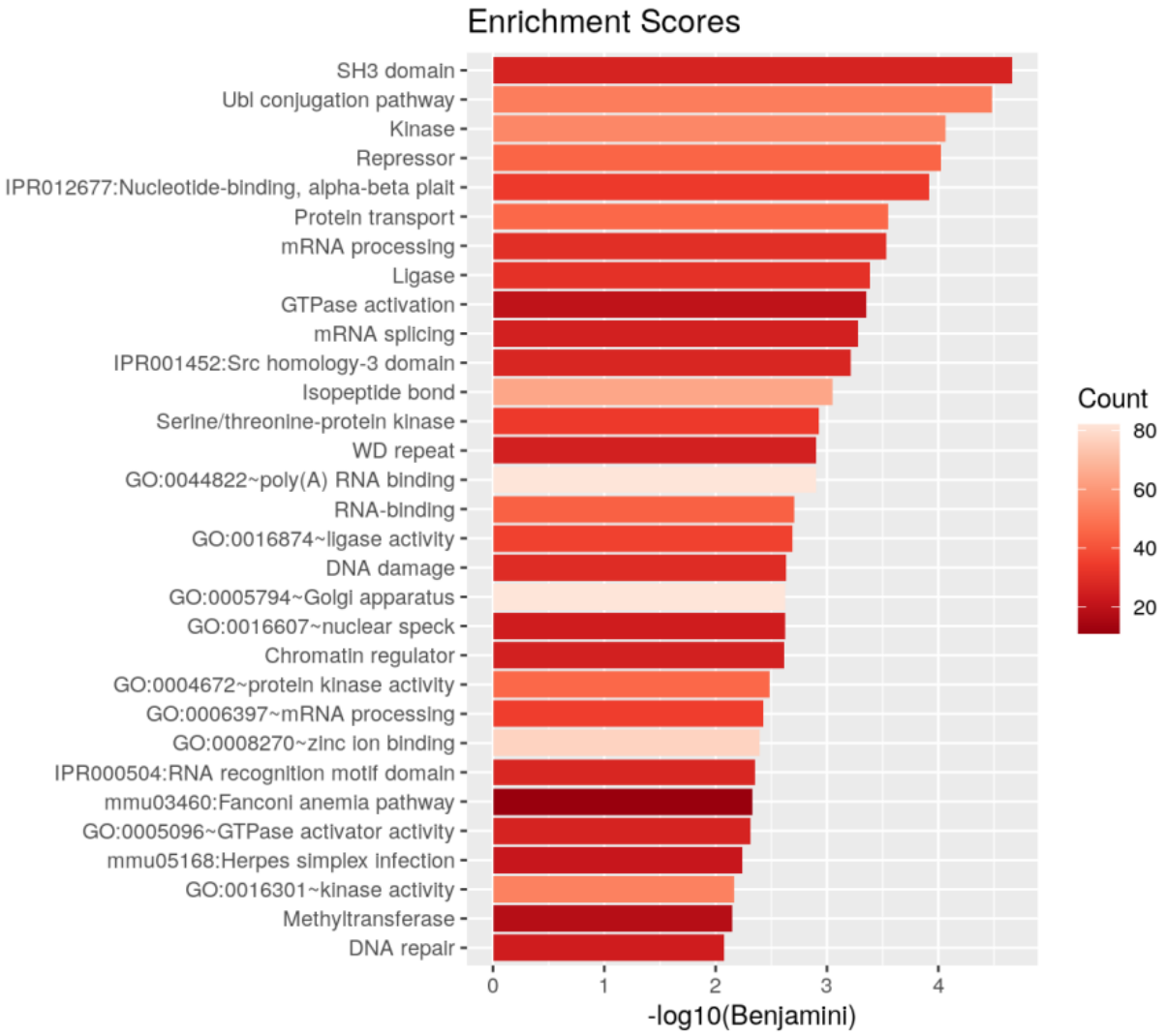
Supplementary Figure 2. Competitive transplantation of *U2af1*$^{mut/+}$ and WT LSK cells. a) Scheme of competitive transplantation assay using FACS-sorted LSK. b) Purity check of sorted LSK fraction (85%). c) Peripheral blood CD45.2+ donor chimerism at 8 weeks post-transplantation (n=3, WT n=13, *U2af1*$^{mut/+}$ n=14).



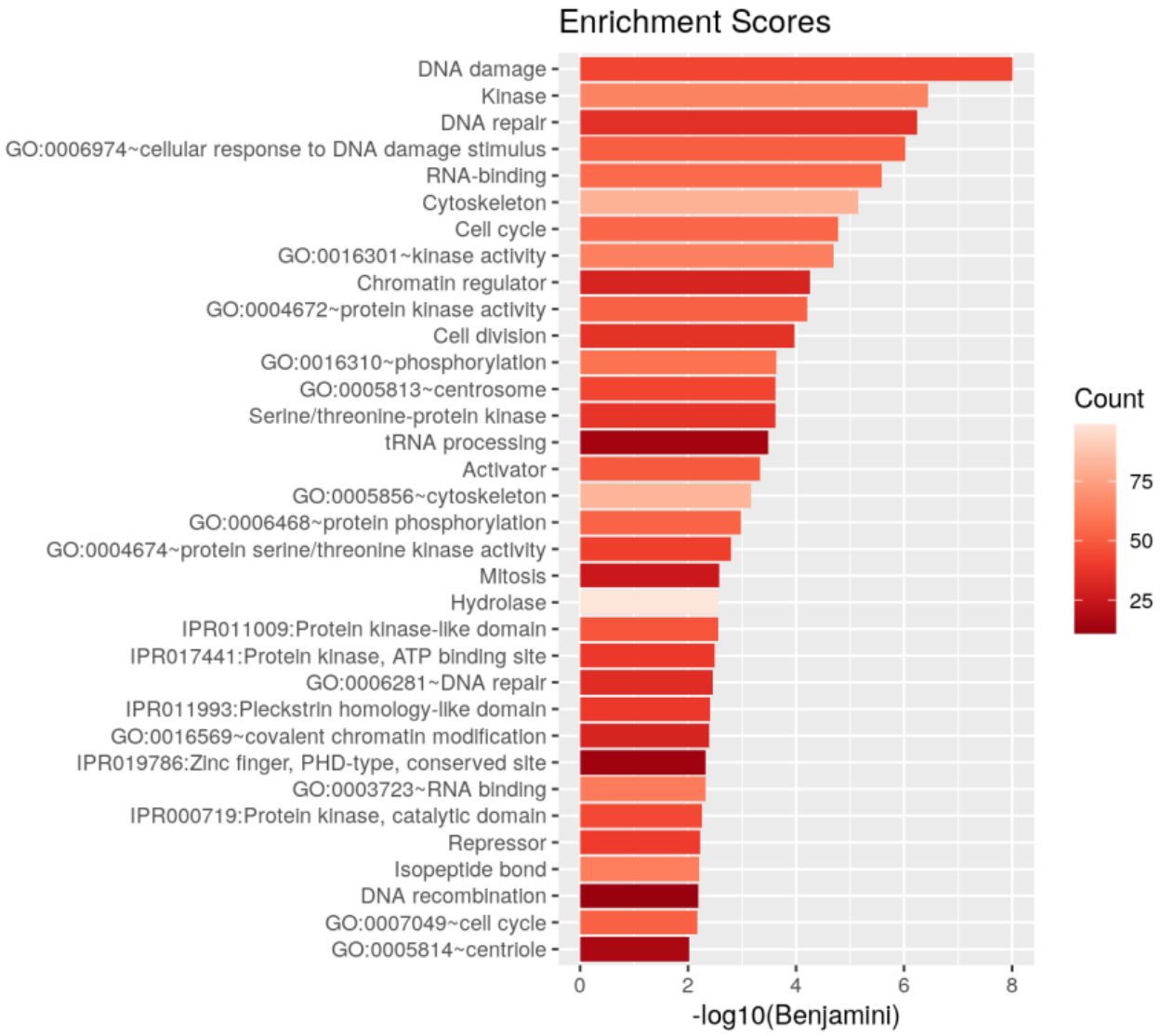
Supplementary Figure 3. Principal components analysis of aberrantly spliced genes in mutated samples compared to WT controls.



Supplementary Figure 4. Top specific functional enrichment terms in *U2af1* aberrant splicing events versus control samples. DAVID annotation database was mined for functional information.



Supplementary Figure 5. Top specific functional enrichment terms in *Tet2* aberrant splicing events versus control samples. DAVID annotation database was mined for functional information.



Supplementary Figure 6. Top specific functional enrichment terms in *U2af1*$^{mut/+}$ *Tet2*$^{mut/mut}$ aberrant splicing events versus control samples. DAVID annotation database was mined for functional information.