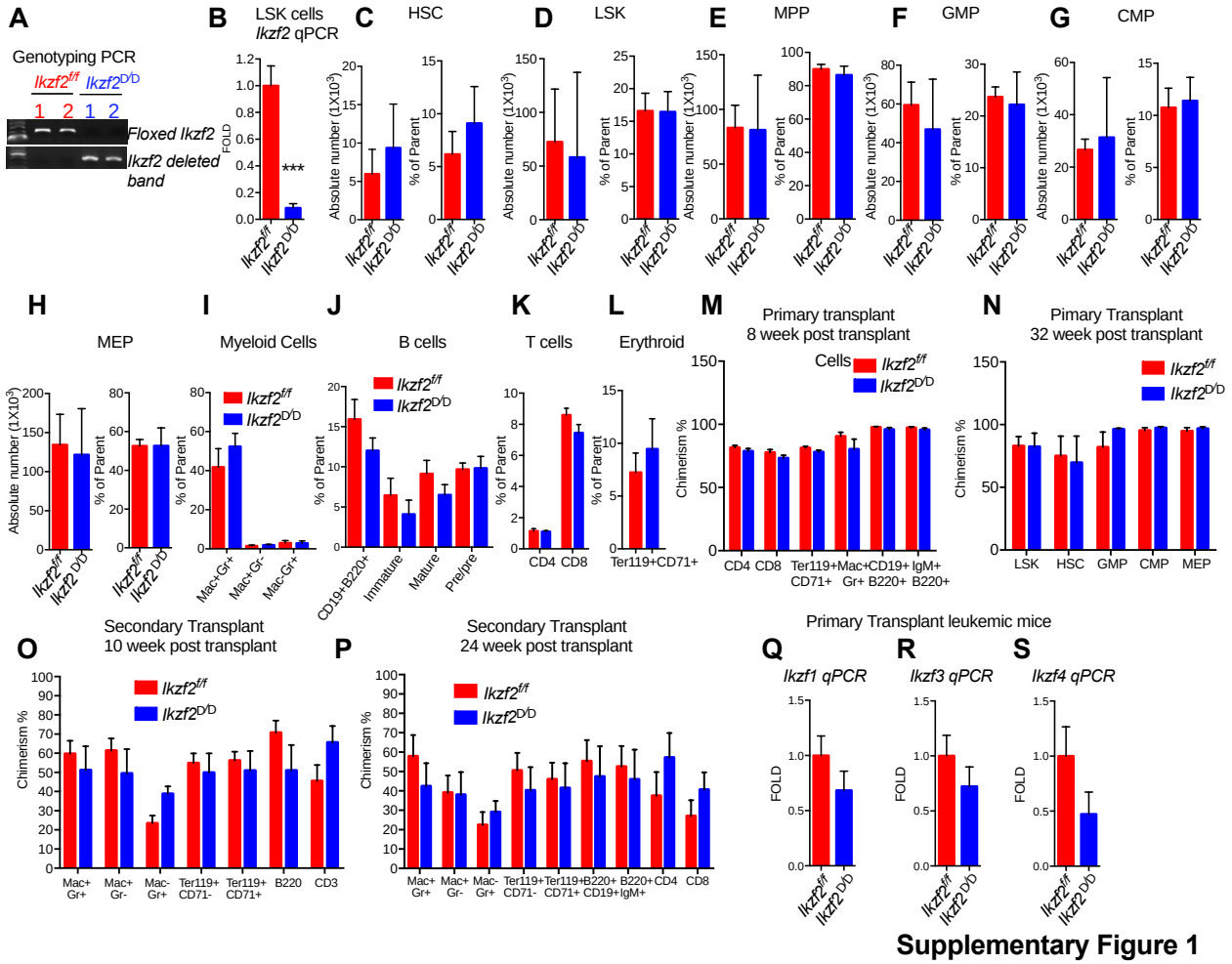
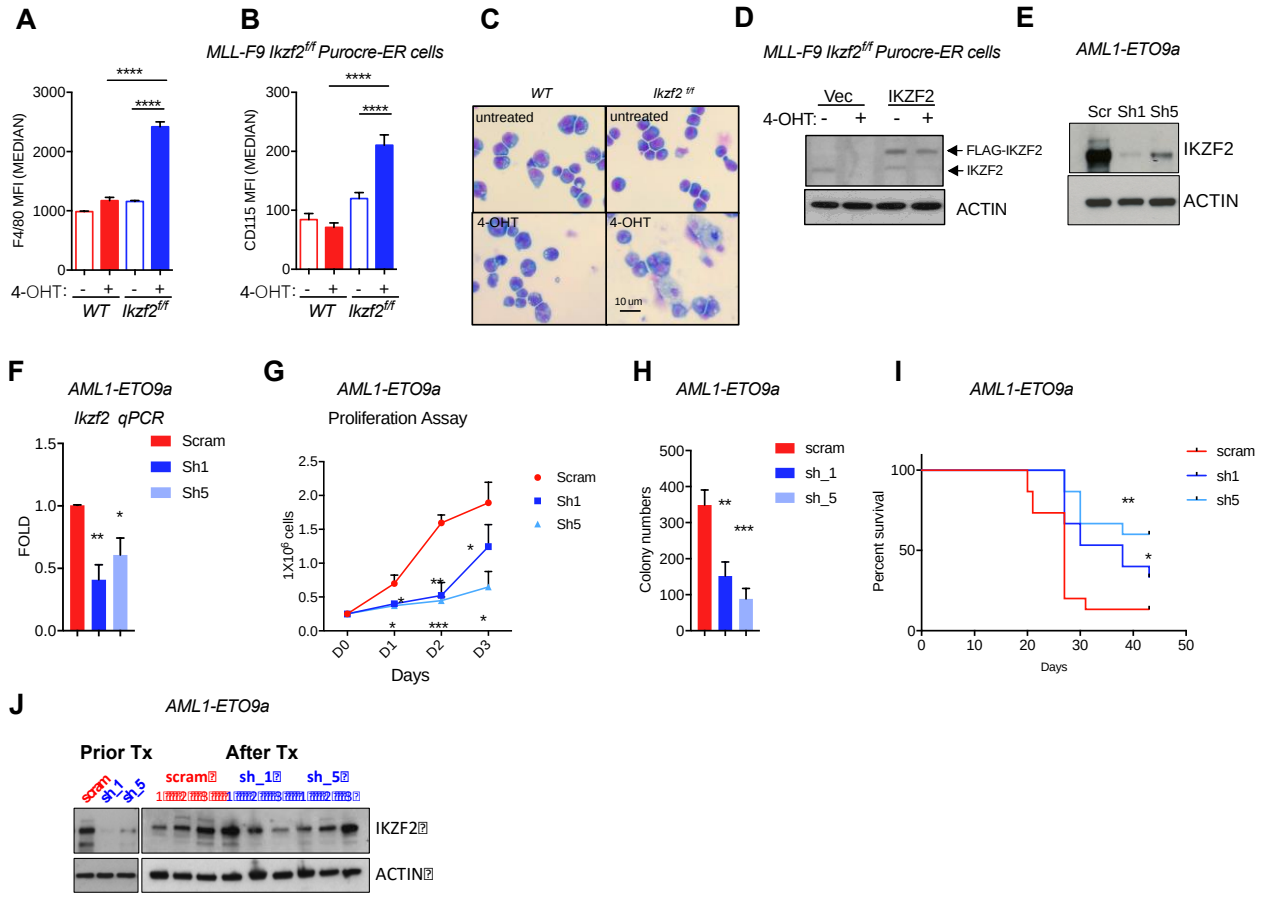


Supplementary Figure 1

Supplemental Figure 1. *Ikzf2* knockout mice have normal hematopoiesis and HSC function, Related to Figure 1

(A) Genotyping PCR showing deletion of floxed *Ikzf2*$^{f/f}$ allele in *Ikzf2*$^{f/f}$ *and Ikzf2*$^{\Delta/\Delta}$ bone marrow cells. PCR bands represents floxed bands and deleted bands from 6-8 week old mice with genotypes *Ikzf2*$^{f/f}$ *vavcre- and Ikzf2*$^{f/f}$ *vavcre*+. (B) QPCR of *Ikzf2* in sorted LSK cells from *Ikzf2*$^{f/f}$ and *Ikzf2*$^{\Delta/\Delta}$ mice. Result is from *Ikzf2*$^{f/f}$ n=3 *and Ikzf2*$^{\Delta/\Delta}$ n=3 mice. (C-H) Absolute number and frequency of (C) HSCs, (D) $Lin^{-}Sca^{+}Kit^{+}$ cells, (E) Multi Potent Progenitors, (F) Granulocyte Monocyte Progenitors, (G) Common Myeloid Progenitors and (H) Megakaryocyte Erythrocyte Progenitors in *Ikzf2*$^{f/f}$ *and Ikzf2*$^{\Delta/\Delta}$ primary mice. All data are from 6-8 week old *Ikzf2*$^{f/f}$ n=4 and *Ikzf2*$^{\Delta/\Delta}$ n=4 mice. (I-L) Frequency of (I) myeloid, (J) B cells, (K) T cells, (L) Erythroid cells in *Ikzf2*$^{f/f}$ *and Ikzf2*$^{\Delta/\Delta}$ primary mice. All data are from 6-8 week old *Ikzf2*$^{f/f}$ n=4 and *Ikzf2*$^{\Delta/\Delta}$ n=4 mice. (M-N) Chimerism of stem cells, progenitors and mature cells in CD45.1 mice at (M) 8 and (N) 32 weeks after non-competitive primary transplantation with *Ikzf2*$^{f/f}$ and *Ikzf2*$^{\Delta/\Delta}$ bone marrow cells. All data are from *Ikzf2*$^{f/f}$ n=5 and *Ikzf2*$^{\Delta/\Delta}$ n=5 mice. (O-P) Chimerism of mature cells in CD45.1 mice at (O) 10 and (P) 24 weeks after non-competitive secondary transplantation with *Ikzf2*$^{f/f}$ and *Ikzf2*$^{\Delta/\Delta}$ bone marrow cells. All data are from *Ikzf2*$^{f/f}$ n=5 and *Ikzf2*$^{\Delta/\Delta}$ n=5 mice. (Q-S) QPCR of other members of the *Ikaros* family, (Q) *Ikzf1* (R) *Ikzf3* and (S) *Ikzf4* in *Ikzf2*$^{f/f}$ and *Ikzf2*$^{\Delta/\Delta}$ leukemic cells from primary initiation transplant. *Ikzf2*$^{f/f}$ n=16 *and Ikzf2*$^{\Delta/\Delta}$ n=4.

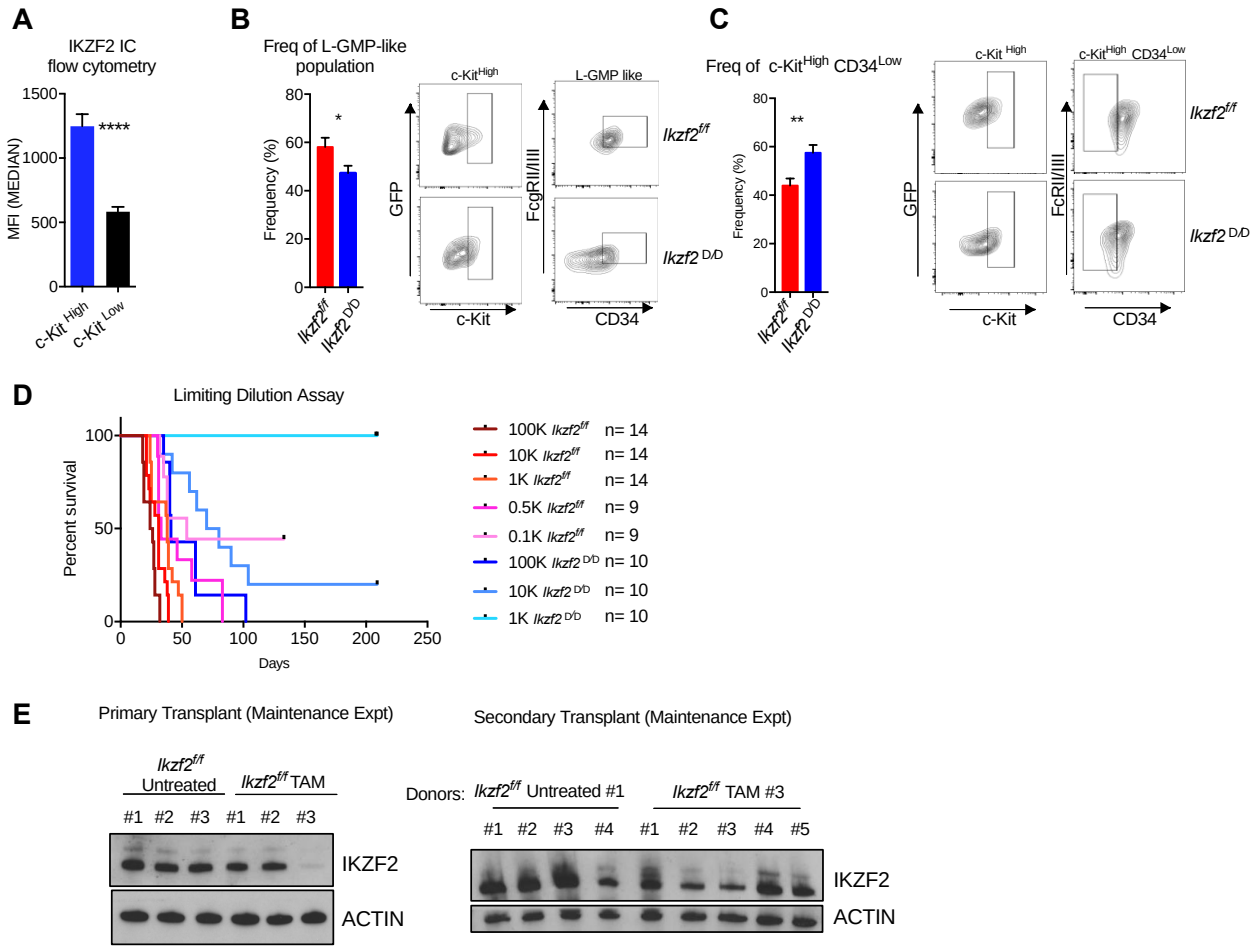


Supplementary Figure 2

Supplemental Figure 2. Acute deletion *of Ikzf2* increases protein expression of myeloid genes, Related to Figure 2.

(A,B) Median Flourescence Intensity of myeloid markers (A) F4/80 and (B) CD115, is increased in *Ikzf2* deleted leukemic cells. CD115 and F4/80 was measured by flow cytometry, 24 hr after MLL-AF9 *WT* or *Ikzf2*$^{f/f}$ leukemic cells were treated with 10 nM 4-OHT. Results were combined from at least three independent experiments, using one MLL-AF9 *WT* cre-ER line and three MLL-AF9 *Ikzf2*$^{f/f}$ cre-ER lines. Mean +/- S.E.M Student's *t* test **** p<0.0001 (C) Morphological analysis revealed more differentiation in *Ikzf2* deleted cells, showing morphological characteristics of high cytoplasmic /nuclear

ratio and lobulated nuclei. Representative images of Wright-Giemsa staining was taken of cytospins from MLL-AF9 *WT* or *Ikzf2*$^{f/f}$ leukemic cells treated with 10 nM 4-OHT for 24hr. (D) Representative western blot analysis showing the expression of Flag-CBP-IKZF2 and deletion of endogenous IKZF2. ACTIN is used to show equal protein loading. (E) Western blot analysis and (F) qPCR showing depletion of IKZF2 in mouse AML1-ETO9a cells. Experiments were performed 5 days after AML1-ETO9a cells were transduced with lentivirus expressing scramble or *Ikzf2* shRNAs and selected by puromycin. (G) Proliferation assay and (H) Colony Assay show reduction in both experiments when IKZF2 is depleted.n=3 independent experiments * $p < 0.05$, ***$p < 0.001$, ****$p<0.0001$ Student's *t* test. (I) Depletion of IKZF2 leads to delay in leukemia progression in AML1-ETO9a mouse model. Cells transduced with lentivirus expressing scramble or *Ikzf2* shRNAs as in (F) were injected into sublethally irradiated BL6 mice. Survival analysis is from the result of two combined transplants with n=15 mice for each conditions. * $p < 0.05$, ** $p<0.01$ log-rank test. (J) Western blot analysis of IKZF2 in prior and after transplant of AML1-ETO9a cells shows the reexpression of IKZF2. ACTIN was used as control for equal loading.

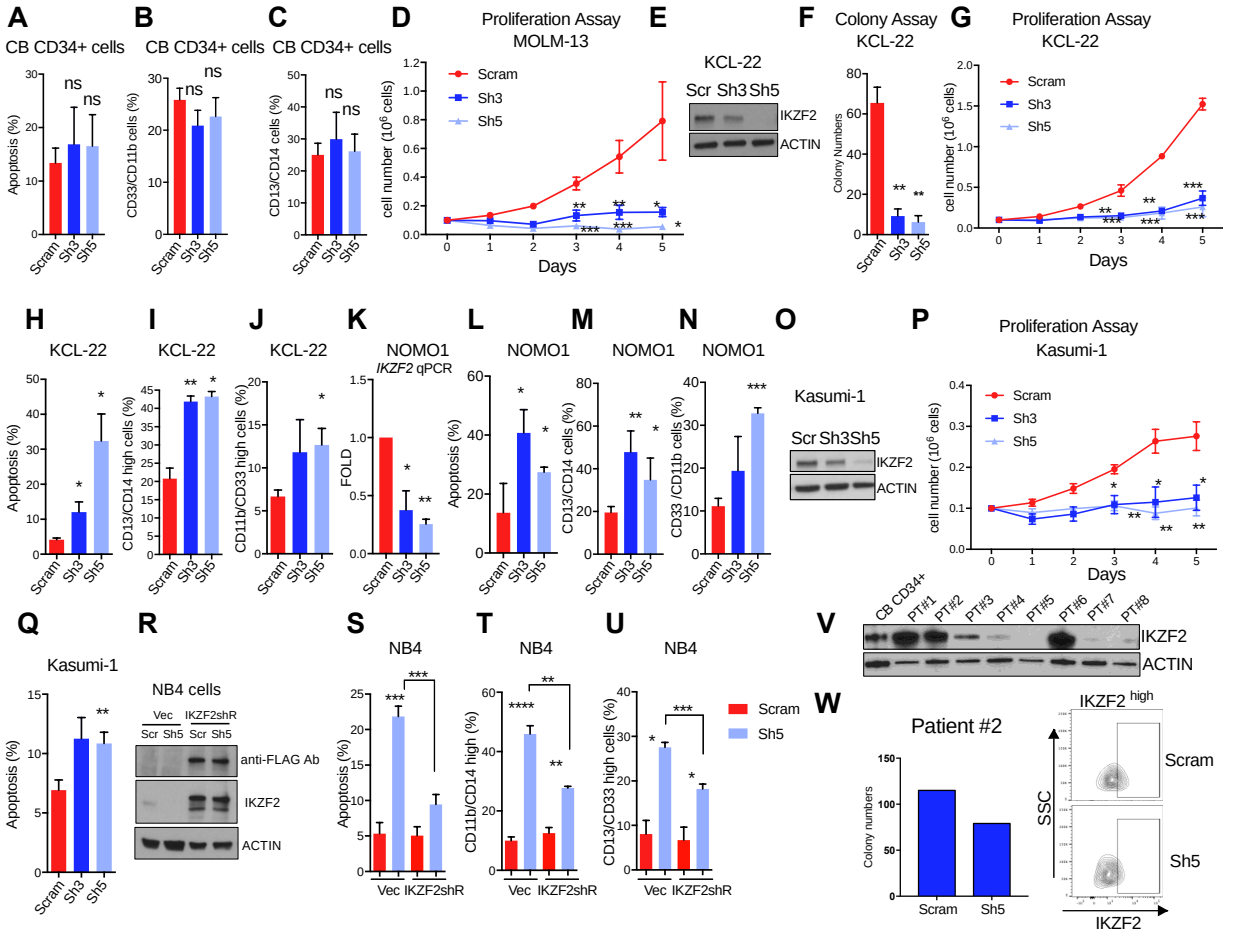


Supplementary Figure 3

Supplemental Figure 3. IKZF2 is required for maintaining LSCs, Related to Figure 3.

(A) IKZF2 is highly expressed in LSCs. Median Flourescence Intensity of IKZF2 in c-Kit^{High} and c-Kit^{Low} cells of primary leukemic bone marrow cells. Result represent leukemic cells isolated from *Ikzf2*$^{f/f}$ n=15 mice ****, p<0.0001. (B) *Ikzf2* deletion reduces L-GMP frequency in primary MLL-AF9 leukemic mice. Left, frequency of L-GMP like population (c-Kit ^{High}/ CD34^{High}/ FcgRII/III +) in bone marrow leukemic cells from primary *Ikzf2*$^{f/f}$ and *Ikzf2*$^{\Delta/\Delta}$ transplanted mice. Right, representative cytometric flow plot showing the gating for L-GMP like population shown in left panel. (C) *Ikzf2* deletion leads to increase in cells

with reduced CD34 expression. Left, frequency of c-Kit ^{High} CD34^{Low} population in bone marrow leukemic cells from primary transplanted mice. Right, representative cytometric flow plot showing the gating for c-Kit ^{High} CD34^{Low} population shown in left panel. (B-C) Result represent leukemic cells isolated from *Ikzf2*$^{f/f}$ n=15 and *Ikzf2*$^{\Delta/\Delta}$ n=10 mice.*, $p<0.05$ **, $p<0.01$. (D) Survival curve for individual condition of limiting dilution assay experiment. (E) Western blot analysis of IKZF2 in primary and secondary transplant of maintenance experiment shows the reexpression of IKZF2 in secondary transplant recipients from deleted donor. *Ikzf2*$^{f/f\ TAM}$ #3 mouse from primary transplant was used as donor for secondary transplant.



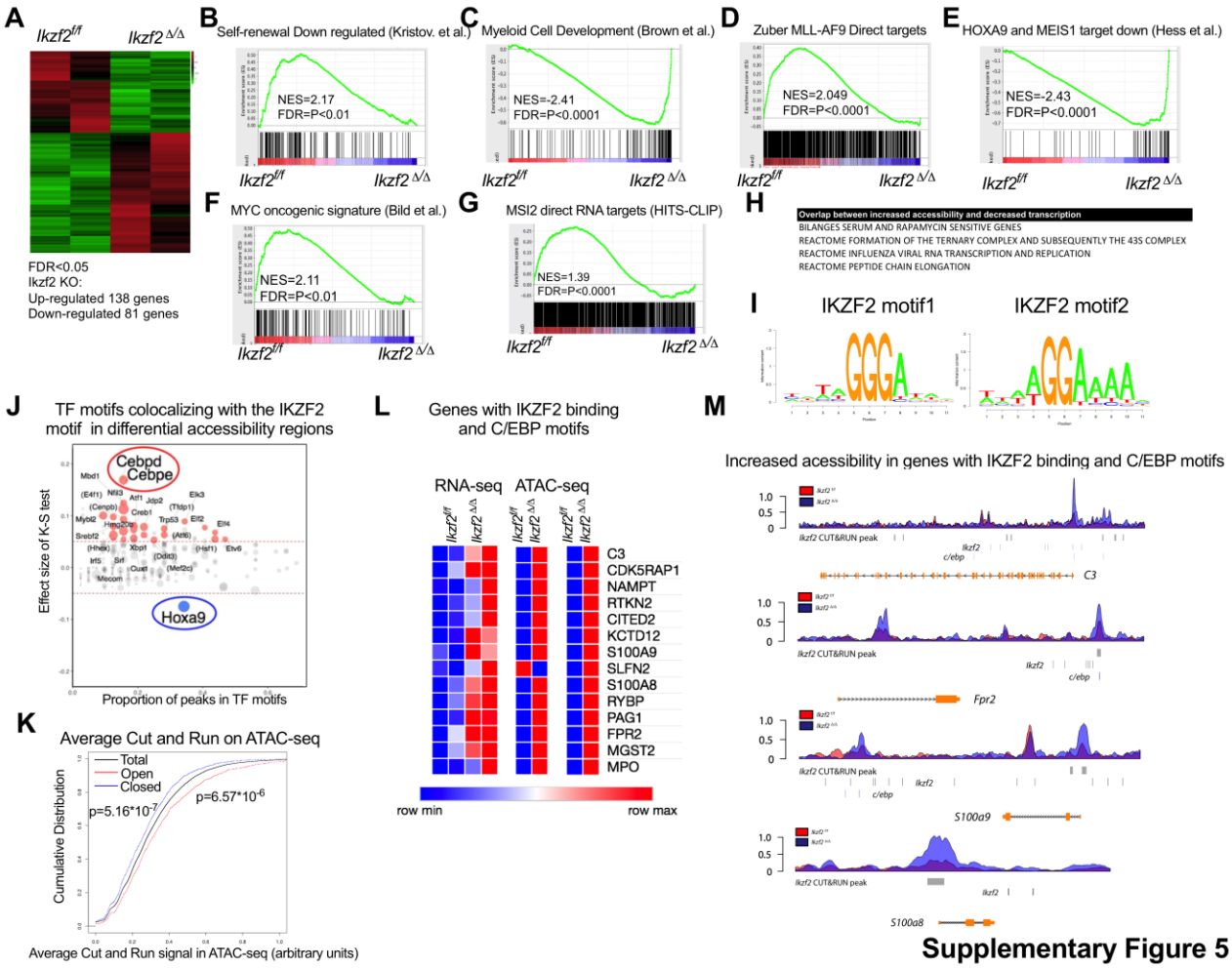
Supplementary Figure 4

Supplemental Figure 4. *IKZF2* Knockdown in different human cell lines leads to reduced cell proliferation, increased apoptosis and differentiation, Related to Figure 4.

(A) Apoptosis and myeloid markers, (B) CD33/CD11b and (C) CD13/CD14 were measured in human cord blood CD34$^+$ HSPCs transduced with lentivirus expressing either Scramble control or two independent *IKZF2* shRNAs. Apoptosis was measured at day 6 and differentiation was measured at day 9 post transduction. n=3 independent experiments were performed. ns, nonsignificant. (D) *IKZF2* knockdown inhibits cell

proliferation in MOLM-13 cells. Cells post-transduced with *IKZF2* or scramble shRNA virus for 4 days were plated and viable cells were counted daily for five days. (E,O) Western blot analysis shows IKZF2 is reduced at day 4 post transduction and puromycin selection in (E) KCL-22 cells and (O) Kasumi-1 cells. ACTIN was used as loading control. (K) QPCR for *IKZF2* was performed to examine knock down in NOMO1 cells which were transduced with virus expressing *IKZF2* shRNA or scramble shRNA. Cells were prepared four days post infection and after puromycin selection. (F) *IKZF2* knockdown reduces colony formation in KCL-22 cells. Colonies were measured a week after plating shRNA virus-transduced and selected KCL-22 cells. (G,P) *IKZF2* knockdown inhibits cell proliferation in (G) KCL-22 and (P) Kasumi-1 cells. Cells post-transduced with *IKZF2* or scramble shRNA virus for 4 days were plated and viable cells were counted daily for five days. (H, L, Q) *IKZF2* knockdown leads to increased apoptosis in (H) KCL-22, (L) NOMO1 and (Q) Kasumi-1 cells. At day 7 post-transduction, cells were stained for Annexin V-PE and 7-AAD, and measured with flow cytometry. (I,J, M,N) *IKZF2* knockdown leads to increased differentiation in human leukemic cells. Flow cytometry was used to measure different myeloid markers in cells at day 7 post-transduction. Frequency of high CD13/CD14 cells was increased in both (I) KCL-22 and (M) NOMO1 cells. High CD11b/CD33 cells were increased in both (J) KCL-22 and (N) NOMO1 cells at 7 days post-transduction. Results shown in (A-U) are from more than three independent experiments using triplicates are shown. Mean +/- SEM Student's *t* test p value. *$p<0.05$, ** $p<0.01$, *** $p<0.001$ (R-U) shRNA resistant IKZF2 is able to rescue the apoptosis and differentiation caused by IKZF2 depletion. (R) Western blot showing efficient overexpression of Flag-human IKZF2 and knockdown of endogenous IKZF2 in NB4 cells.

(S) Apoptosis and differentiation markers (T) CD11b/CD14 and (U) CD13/CD33 were measured at day 4 post transduction of scramble or shRNA virus. Results are from more than three independent experiments are shown. Mean +/- SEM Student's *t* test p value. *p<0.05, ** p<0.01, *** p<0.001 (V) Western blot analysis of CB-CD34+ cells and eight AML patient samples. ACTIN was used as control for equal loading. (W) Patient #2 AML cells were transduced with lentivirus expressing Scramble or IKZF2 shRNA and sorted for GFP at day 4 post transduction. Left, graph showing number of colonies scored after 5000 GFP+ sorted cells were plated on methylcellulose and incubated for 2 weeks. Right, flow plot of intracellular IKZF2 staining in patient cells.



Supplementary Figure 5

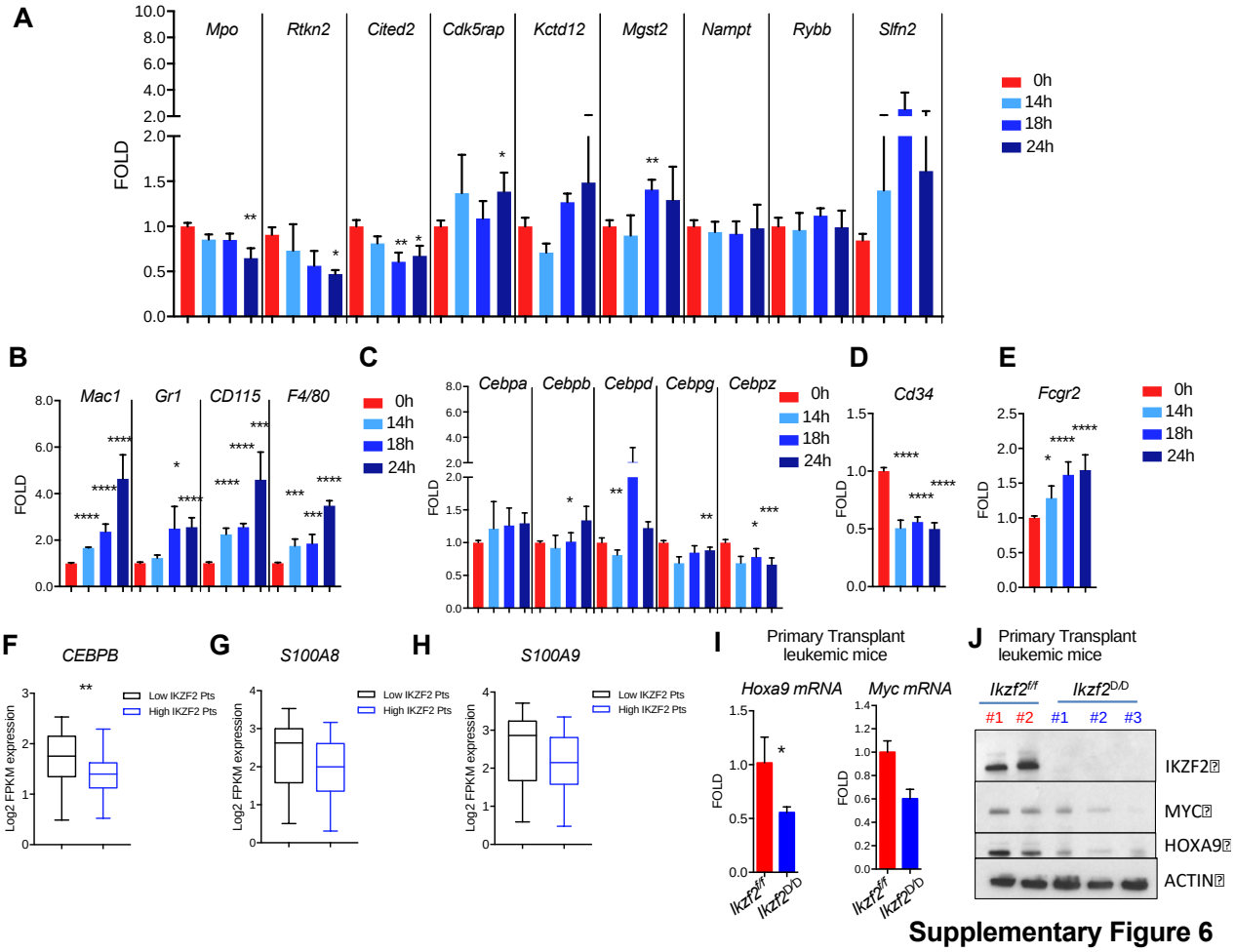
Supplemental Figure 5. *Ikzf2* deleted LSCs have increased differentiation and reduced self-renewal program, Related to Figure 5.

(A) Gene expression heat map of the top 138 up regulated and 81 down regulated genes from RNA sequencing analysis of *Ikzf2*$^{f/f}$ n=2 and *Ikzf2*$^{\Delta/\Delta}$ n=2 LSCs is shown.

(B-G) Gene Set Enrichment Analysis of *Ikzf2*$^{f/f}$ and *Ikzf2*$^{\Delta/\Delta}$ LSCs, showing (B) loss of self-renewal and (C) enrichment of myeloid developmental signature. GSEA plots shows that (D) MLL-AF9 direct target genes are downregulated, (E) genes repressed by HOXA9 and Meis1 are upregulated, (F) MYC targets are down-regulated in MLL-AF9 *Ikzf2*$^{\Delta/\Delta}$ LSCs. (G) GSEA plot shows that there is enrichment of MSI2 direct RNA targets in the gene

expression found in *Ikzf2*$^{f/f}$ compared to *Ikzf2*$^{\Delta/\Delta}$ LSCs, indicating that IKZF2 transcriptionally regulates MSI2 binding targets. The normalized enrichment scores (NES) and p values are shown in each plot. (H) Overlap of gene sets from genes with increased accessibility (ATAC-seq data) and decreased RNA expression (RNA-seq data) leads to 4 gene sets as listed. (I) The IKZF2 motif1 and motif2 obtained from TRANSFAC were used for motif enrichment analysis. (J) TF motifs enriched together with IKZF2 motifs in differentially accessible regions in *Ikzf2*$^{\Delta/\Delta}$ LSCs. C/EBPε and C/EBPδ motifs are top enriched TF motifs and HOXA9 is the top TF motif lost in the differential accessible regions that colocalize with IKZF2 motifs. TF symbol annotations are written where the effect size ≥ 0.05 and odds ratio (circle size) ≥ 1.3; the odds ratio was defined as the ratio of foreground occurrence over background occurrence. The foreground occurrence is the number of peaks containing a particular TF motif and IKZF2 motif within the group of differentially upregulated or downregulated peaks, respectively. The background occurrence is the number of peaks containing a particular TF motif and IKZF2 motif found among all the IKZF2-containing peaks in all samples. The list of significant motifs is in **Table S7**. (K) The cumulative distribution plot of the average CUT&RUN signal for ATAC-seq peaks. Black, all peaks in the atlas. Red, differentially opened peaks upon *Ikzf2*$^{\Delta/\Delta}$ under nominal p-value ≤ 0.05. Blue, differentially closed peaks upon *Ikzf2*$^{\Delta/\Delta}$ under nominal p-value ≤ 0.05. (L) Heatmap of 14 genes with increased RNA expression and accessibility in *Ikzf2*$^{\Delta/\Delta}$ LSCs, that contains IKZF2 and C/EBP motifs and are bound by IKZF2 is shown. (M) Representative signal tracks of IKZF2 targets that are related to myeloid differentiation. Representative genes with increased accessibility in *Ikzf2*$^{\Delta/\Delta}$ LSCs and having IKZF2 binding with IKZF2 and C/EBP motifs are shown *C3*, *Frp2*, *S100a9* and

*S100a*8. IKZF2 CUT&RUN peaks are marked with grey rectangles, and IKZF2 and C/EBP motifs are marked with black blocks. ATAC-seq peaks in *Ikzf2*$^{f/f}$ and *Ikzf2*$^{\Delta/\Delta}$ LSCs are indicated as red and blue respectively.



Supplementary Figure 6

Supplemental Figure 6. IKZF2 negatively and positively regulates the expression of TFs required for maintaining LSC program, Related to Figure 6.

(A) QPCR analysis of 10 genes out of the 14 target genes performed in sorted MLL-AF9 *Ikzf2*$^{f/f}$ cre-ER c-Kit ^{High} cells treated with 4-OHT for different time points. These genes do not exhibit any changes or increased expression after acute IKZF2 deletion as seen in the endpoint *Ikzf2*$^{\Delta/\Delta}$ LSCs, suggesting the upregulation of these genes in the *Ikzf2*$^{\Delta/\Delta}$ LSCs are not direct effects of *Ikzf2* deletion. (B) Acute deletion of *Ikzf2* increases the expression of myeloid genes reported to be regulated by C/EBP proteins in MLL-AF9 *Ikzf2*$^{f/f}$ cre-ER

c-Kit ^{High} cells. (C) QPCR of different members of the *C/ebp* family shows that members excluding *C/ebp*ε are not increased by *Ikzf2* deletion. (D-E) Acute deletion of *Ikzf2* leads to transcriptional changes in L-GMP markers, *Cd34* and *Fcgr2*. (A-E) Experiments performed with sorted MLL-AF9 *Ikzf2*$^{f/f}$ cre-ER c-Kit ^{High} cells were conducted in four different experiments. Student's *t* test *p<0.05, ** p<0.01, *** p<0.001, **** p<0.0001. (F-H) Box and whiskers plot showing correlation of the indicated genes in patients with high versus low IKZF2 mRNA expression in AML patients from TCGA dataset. Patients with top and lowest 25% of IKZF2 mRNA expression were defined as high (n=45) versus low IKZF2 (n=24) patients. (F) CEBPB (G) S100A8 (H) S100A9 **P < 0.01 two-tailed t test. (I) QPCR showing *Hoxa9* and *c-Myc* expression is reduced in primary *Ikzf2*$^{\Delta/\Delta}$ leukemic mice. Result is from *Ikzf2*$^{f/f}$ n=13 and *Ikzf2*$^{\Delta/\Delta}$ n=4 mice. Student's *t* test * p<0.05 (J) Western blot showing reduction of HOXA9, MYC and IKZF2 in *Ikzf2* deleted leukemic cells from primary transplant mice. ACTIN was used to show equal loading.