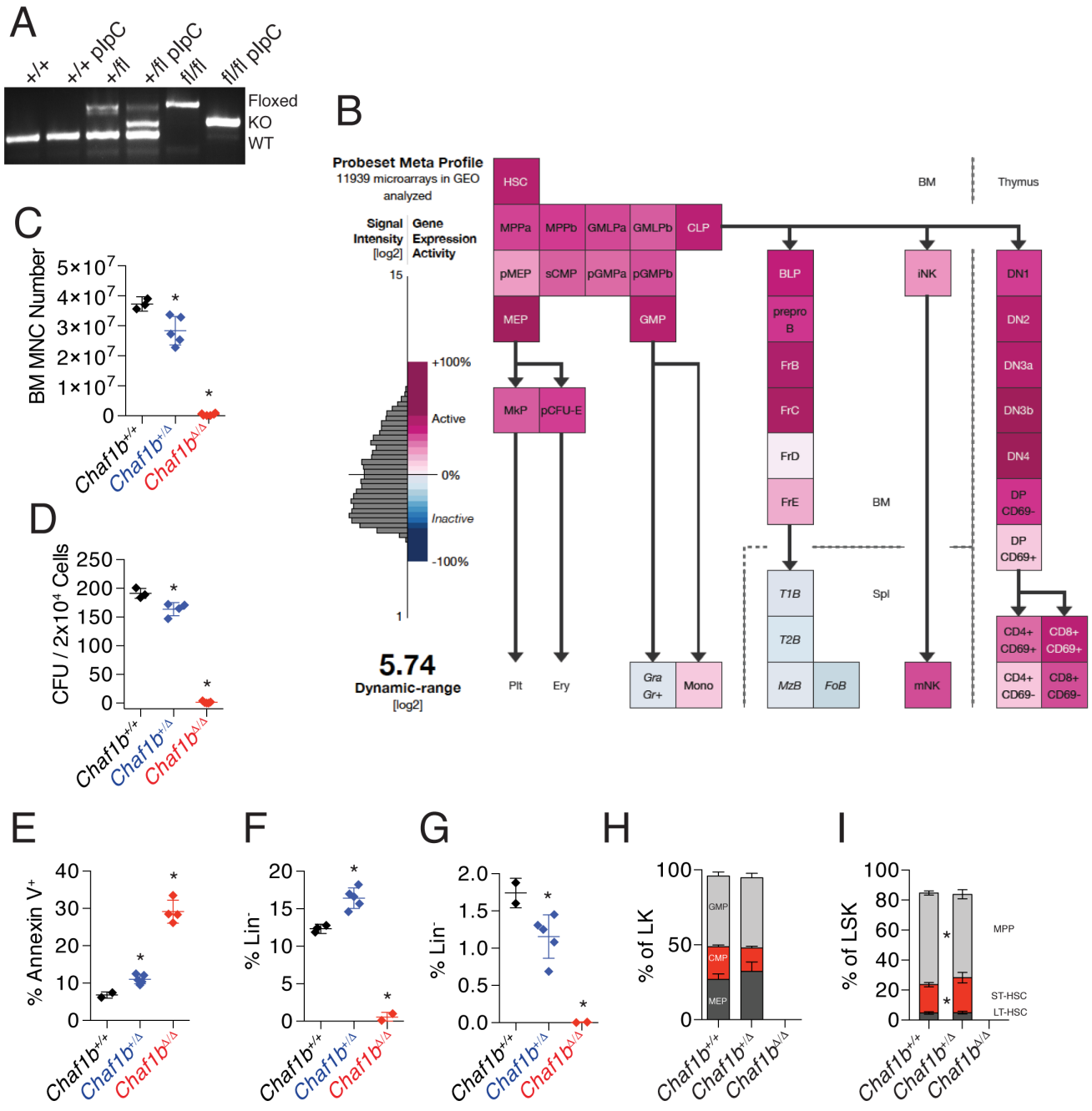

Figure S1. CHAF1B is necessary for hematopoiesis (Related to Figure 1). (A) Genomic PCR of bone marrow mononuclear cells from Mx1-Cre+ mice of indicated genotypes after three injections of pIpC. **(B)** Gene expression commons data of CHAF1B expression in mouse hematopoietic cells. **(C)** Total cell count of hind limb BM MNCs 4 weeks after pIpC (*Chaf1b*$^{+/+}$ and *Chaf1b*$^{+/fl}$) or 1 week after pIpC (*Chaf1b*$^{fl/fl}$). **(D)** Colony forming units from BM MNCs at same time point. **(E)** Percent Annexin V$^+$/PI$^+$ BM MNCs at same time point. **(F-I)** FACS analysis of LK (F), LSK (G), myeloid progenitors (H), and SLAM$^+$ LSK population (I). Horizontal line indicates mean and bars represent standard deviation, with each point represents a sample from an individual mouse (C-G). Horizontal line indicates mean and error bars indicate standard deviation from five individual mice (H, I). * indicates $p<0.05$ as determined by one-way ANOVA with Bonferroni correction (C-I).

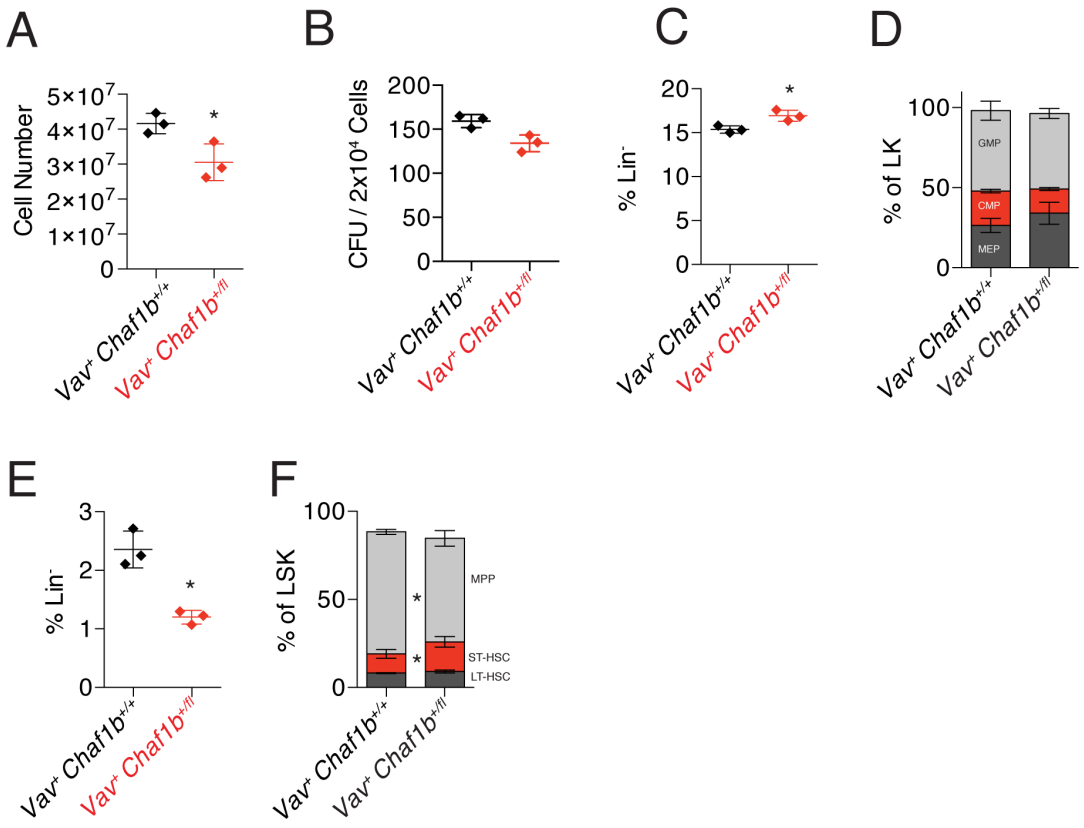

Figure S2: Confirmation of *Chaf1b* deletion phenotype with Vav-Cre (Related to Figure 1). Analysis of Vav-Cre^{+} *Chaf1b*$^{fl/+}$ mice for bone marrow cellularity **(A)**, CFU activity **(B)**, LK percentage **(C)**, percentages of myeloid progenitors **(D)**, LSK percentage **(E)**, and the SLAM^{+} LSK population **(F)**. Horizontal line indicates mean and bars represent standard deviation, with each point represents a sample from an individual mouse (A-C, E). Horizontal line indicates mean and error bars indicate standard deviation from three individual mice (F, D). * indicates $p<0.05$ as determined by Student's t-test with Welch's correction (A-C, E) or one-way ANOVA with Bonferroni correction (D, F).

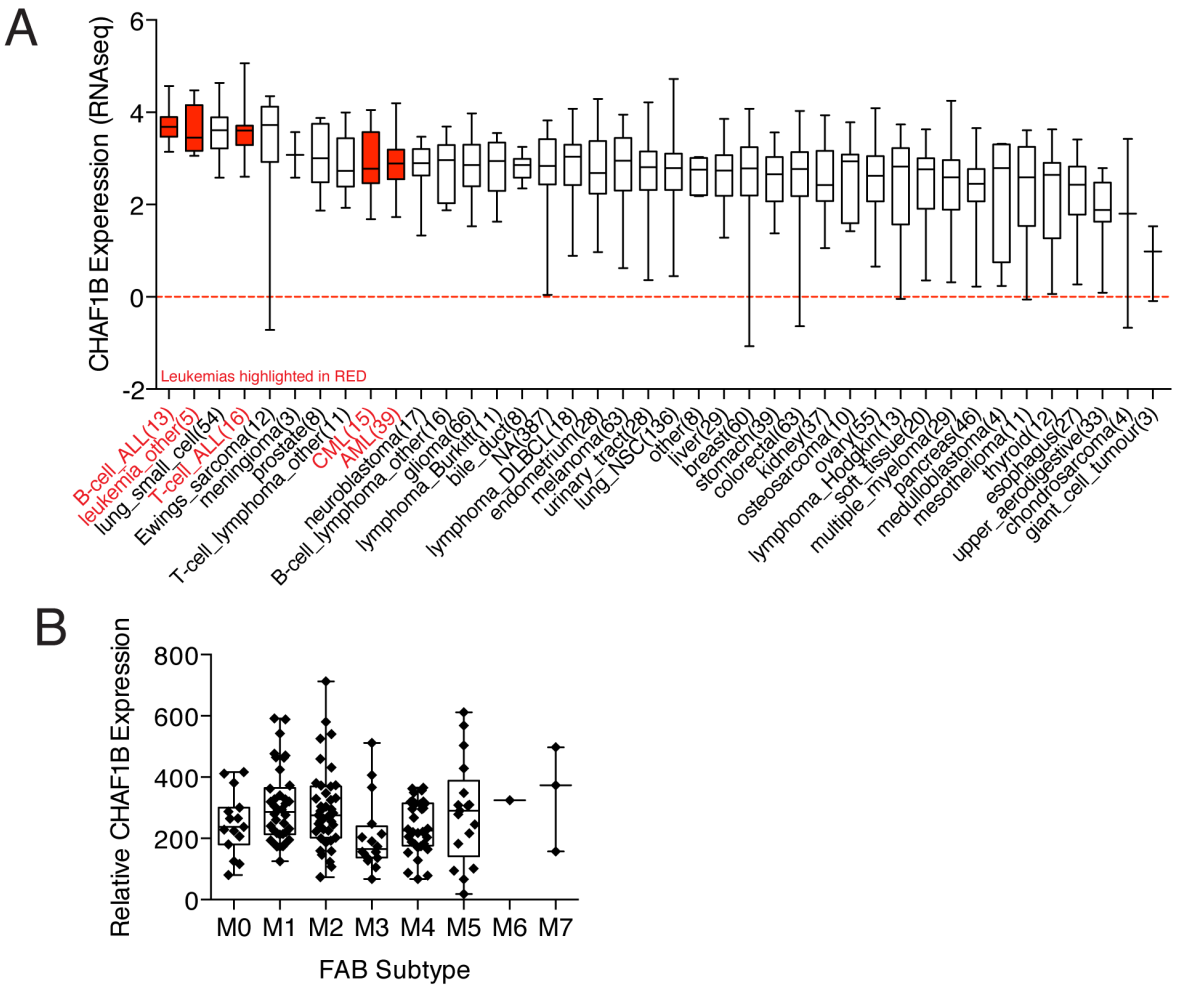

Figure S3: CHAF1B expression in human AML (Related to Figure 2). (A) CHAF1B expression relative to healthy tissue control analyzed from Broad Institute's Cancer Cell Line Encyclopedia. Leukemia samples are highlighted in red. Box plot horizontal line indicates mean, box indicates standard deviation, and whiskers indicate range. (**B)** CHAF1B expression levels in different FAB subtypes of AML analyzed from TCGA. Each individual point is an individual patient. Box plot horizontal line indicates mean, box indicates standard deviation, and whiskers indicate range.

Table S1. Characteristics of AML Patient Samples used in study, Related to Figure 2.

Patient	Gender	Age (year)	Disease status	FAB subtype	Cytogenetics	Blast purity (%)	Gene mutation
AML#1	Male	66	Newly diagnosed	M2	46, XY	NA	CEBPA double mutation
AML#2	Female	40	Newly diagnosed	M5	46,XX t(8;21)	79.5	FLT-3-ITD RUNX1-RUNX1T1
AML#3	Male	27	Newly diagnosed	M1	46,XY	96	CEBPA double mutation, NPM-1
AML#4	Female	61	Relapsed	M4	46,XX	93	CEBPA double mutation
AML#5	Female	40	Newly diagnosed	M4	46, XY	53.5	NPM-1,IDH-1

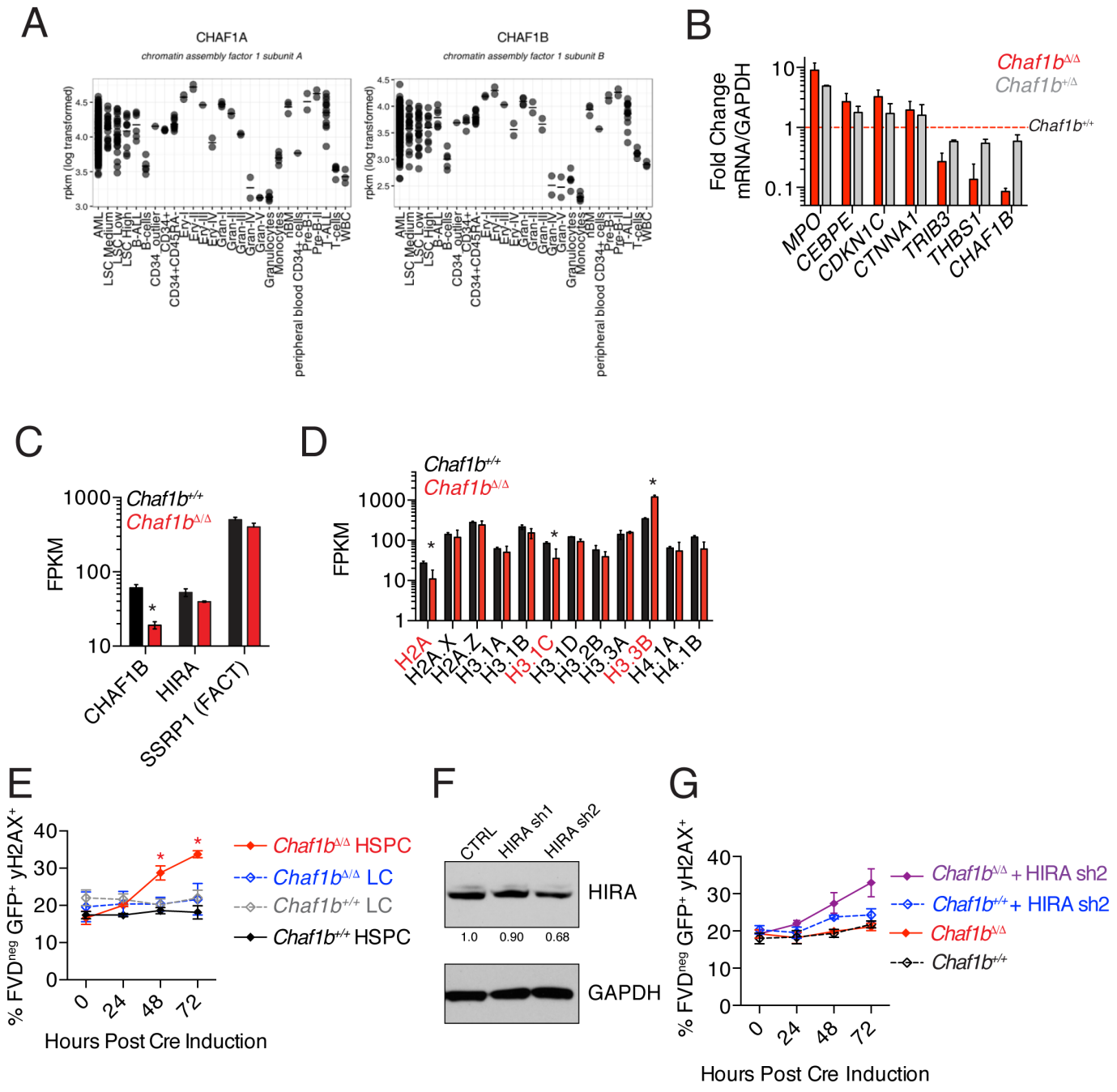

Figure S4: Loss of *Chaf1b* induces differentiation of MLL-AF9 leukemic cells (Related to Figure 4). (A) Analysis of CHAF1B and CHAF1A expression in hematopoietic cells at different stages of differentiation or disease. Data analyzed from Leucegene AML RNA-seq dataset. Horizontal bars represent mean value. **(B)** qRT-PCR confirmation of genes identified by RNA-seq in *Chaf1b*$^{\Delta/\Delta}$ leukemic cells. Results shown are mean +/- SD from three biological replicates analyzed with ΔΔCT method. **(C)** Fragments of exon per million mapped reads (FPKM) of CHAF1B, HIRA, and SSRP1 in MLL-AF9 leukemic cells after *Chaf1b* deletion. **(D)** FPKM for various histone variants in MLL-AF9 leukemic cells 48 hours after *Chaf1b* deletion. **(E)** γH2A.X incorporation in HSPCs and MLL-AF9 LCs following *Chaf1b* deletion. **(F)** Western blot for HIRA protein levels in MLL-AF9 LCs expressing *Hira* shRNA. Numbers indicate the levels relative to control hairpin as determined by Image J. **(G)** γH2A.X incorporation in MLL-AF9 LCs following *Chaf1b* deletion and *Hira* knockdown. Bar chart height indicates mean, error bar indicates standard deviation ((B-D). Line indicates mean value from three independent replicates, whiskers indicate standard deviation (E, G). * indicates $p<0.05$ as determined by Two-way ANOVA with Bonferroni post-hoc test (E, G). D is representative of three biological replicates.

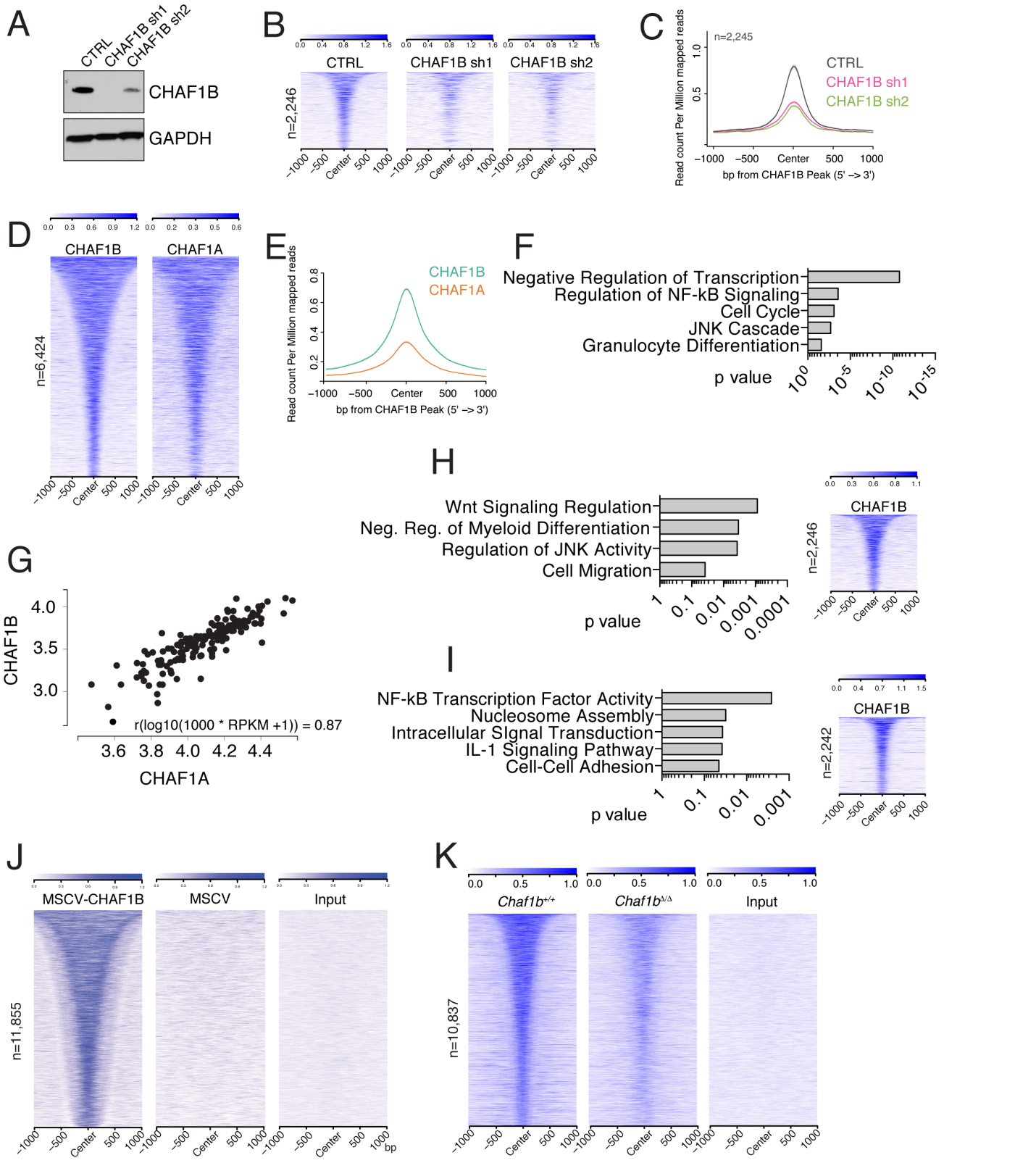

Figure S5: CHAF1B chromatin occupancy in AML cells and confirmation of the CHAF1B antibody for ChIP-seq (Related to Figure 5). (A) Western blot analysis for CHAF1B expression after introduction of shRNA targeting *CHAF1B* in U937 cells. **(B)** Heatmap of the CHAF1B ChIP-seq signal associated with genomic regions centered on CHAF1B peaks in U937 cells. **(C)** Metaplot of CHAF1B ChIP-seq signal from (B). **(D)** Heatmap of CHAF1B and CHAF1A ChIP-seq at regions centered on CHAF1B peaks in MOLM13 cells. **(E)** Metaplot of CHAF1B and CHAF1A ChIP-seq signal from (D). **(F)** GO analysis of genes associated with CHAF1B peaks in MOLM13 cells. **(G)** Co-expression analysis of CHAF1A and CHAF1B in human AML patient samples from Leucegene AML RNA-seq database. **(H-I)** GO and heatmaps of CHAF1B peaks in U937 (H and JURKAT (I). **(J)** CHAF1B ChIP-seq in MLL-AF9 LCs expressing MSCV-CHAF1B, MSCV, or input. **(K)** CHAF1B ChIP-seq in MLL-AF9 LCs expressing cells replete or deleted for *Chaf1b*.

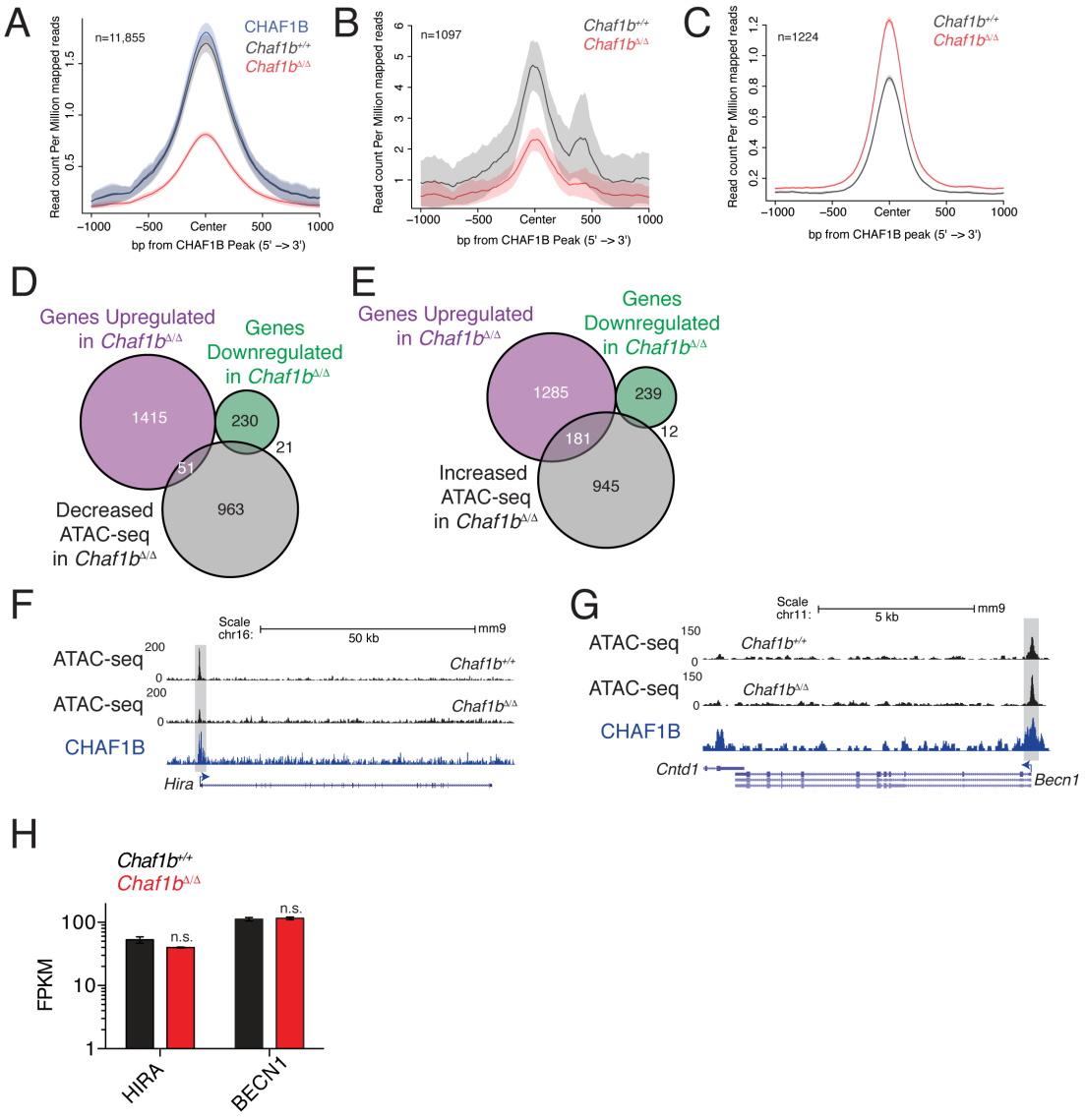

Figure S6: Loss of *Chaf1b* leads to global reduction of chromatin accessibility at CHAF1B sites with no discernable relationship with changes in transcription (Related to Figure 5). (A) Global change in ATAC-seq enrichment at CHAF1B sites. **(B, C)** Meta-analysis of ATAC-seq signal at the 1097 most reduced locations (B) or 1224 most increased locations (C). **(D-E)** Venn diagrams showing overlap in genes with most significantly changed ATAC-seq signal. **(F, G)** Track examples of *Hira* and *Becn1*. **(H)** Counts per million reads of HIRA and BECN1 in leukemic cells 48 hours following *Chaf1b* deletion. Box height represents mean, error bars represent standard deviation.

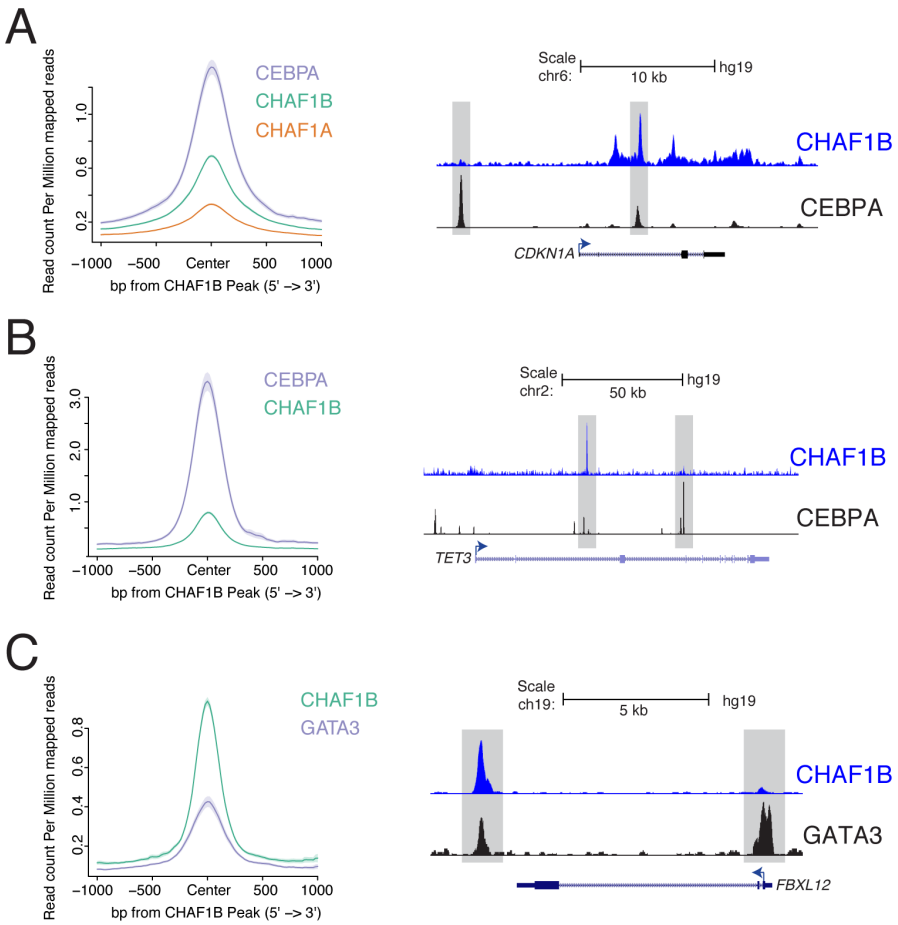

Figure S7: CHAF1B co-occupies chromatin with pro-differentiation transcription factors in human leukemia cell lines (Related to Figure 6): A-C) Meta plots and example tracks for ChIP-seq signal of CHAF1B, CHAF1A, and CEBPA/GATA3 in (A) MOLM13, (B) U937, and (C) JURKAT cells. Gray boxes indicate regions of interest where CHAF1B and CEBPA/GATA3 occupancies are inversely related.

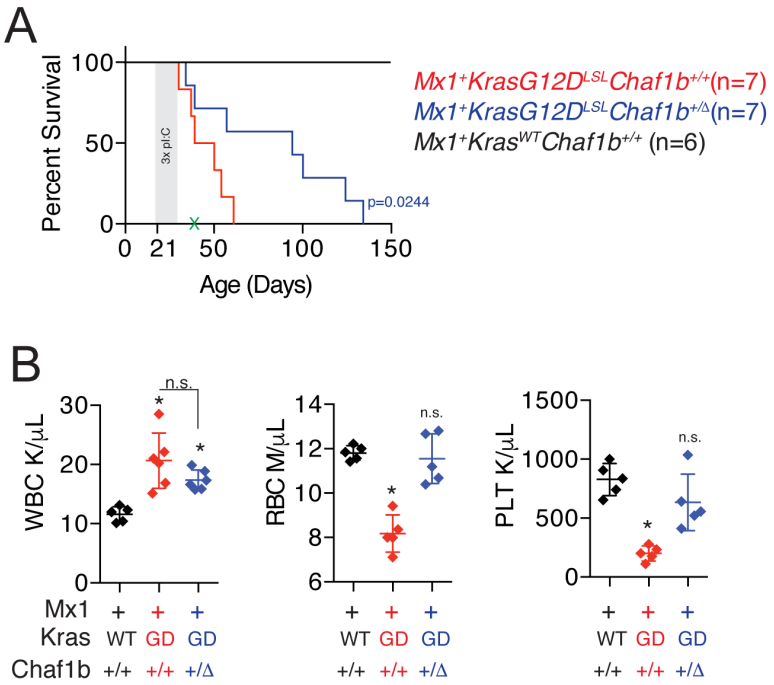

Figure S8: KrasG12D-mediated MPD is partially alleviated by heterozygous *Chaf1b* deletion (Related to Figure 8). (A) Survival of mice treated with pIpC at the days indicated by shading. **(B)** Peripheral white blood cells, red blood cells, and platelet counts at 40 days of age. Horizontal bars indicate mean, error bars indicate standard deviation, each point represents an individual mouse (B). "GD" refers to activation of the *Kras*^{G12D} allele in (B). * indicates $p<0.05$ as measured by log-rank test (A), or One-way ANOVA with Bonferroni correction (B).

Table S2. Oligonucleotides used in study, Related to STAR Methods

Primer	Sequence
Chaf1b Genotype F	GGAAAGCAAGGAATGCTGAG
Chaf1b Flox R	TGGGTATTGGGGATACATGC
Chaf1b Knockout R	CTGAGTTGAACTGATGGCG
CreTV F (Mx1)	GCCTGCATTACCGGTCGATGCAACGA
CreTV R (Mx1)	GTGGCAGATGGCGCGGCAACACCAT
Vav-Cre F	AGATGCCAGATCAGGAACCTG
Vav-Cre R	ATCAGCCACACCAGACACAGAGATC
Kras WT F	TGTCTTTCCCCAGCACAGT
Kras G12D F	GCAGGTCGAGGGACCTAATA
Kras R	CTGCATAGTACGCTATACCCTGT
mChaf1b F	CCGCCGTCAGGATCTGGAAGTTG
mChaf1b R	GCTCCTTGCTGTCATTCATCTTCCAC
mChaf1a F	GTGTCTTCCTCAACTTTCTCCTTGG
mChaf1a R	CCGCGGCCGTGGATTGC
mGAPDH f	TGCCCCCATGTTTGTGATG
mGAPDH r	TGTGGTCATGAGCCCTTCC
hCHAF1B F	TTCAGTCAGAGACGCCTGGA
hCHAF1B R	GCTTTAGCTCTGGGGGACTG
mCebpa F	ATAGACATCAGCGCCTACATCGA
mCebpa R	GTCGGCTGTGCTGGAAGAG
mFli1 F	ACTTGGCCAAATGGACGGGACTAT
mFli1 R	CCCGTAGTCAGGACTCCCG
mMpo F	TCCCACTCAGCAAGGTCTT
mMpo R	TAAGAGCAGGCAAATCCAG
mCdkn1c F	AGAGAACTGCGCAGGAGAAC
mCdkn1c R	TCTGGCCGTTAGCCTCTAAA
mCtnna1 F	AAGTCTGGAGATTAGGACTCTGG
mCtnna1 R	ACGGCCTCTCTTTTTATTAGACG
mTrib3 F	GGCCTTATATCCTTTTGGAACGA
mTrib3 R	CGCTGGCAGGGTACACCTT
mThbs1 F	GGGGAGATAACGGTGTGTTTG
mThbs1 R	CGGGGATCAGGTTGGCATT
CHAF1B sh1	GCTGTTGTATTCAGTATCCAT
CHAF1B sh2	CGTCATACCAAAGCCGTCAAT
CHAF1B sg1	AGATGTGTATGATATTTGCT
CHAF1B sg2	ATTGGACAAAAATTCCACGA
Cebpa sh1	AGCCGAGATAAAGCCAAACAA
Cebpa sh2	GCACGAGACGTCTATAGACAT
Cebpa sh3	CAACGCAACGTGGAGACGCAA
Fli1 sh1	CGGGAGTATGACCACATGAAT

Fli1 sh2	GCCTATAACACAACCTCCCAT
Fli1 sh3	GCCAGTGAGAGTCAATGTCAA
Hira sh2	ACTTGGGATCCCGTTGGTAAA
Hira sh3	AGGTCATTCTGGCTTAGTAAA