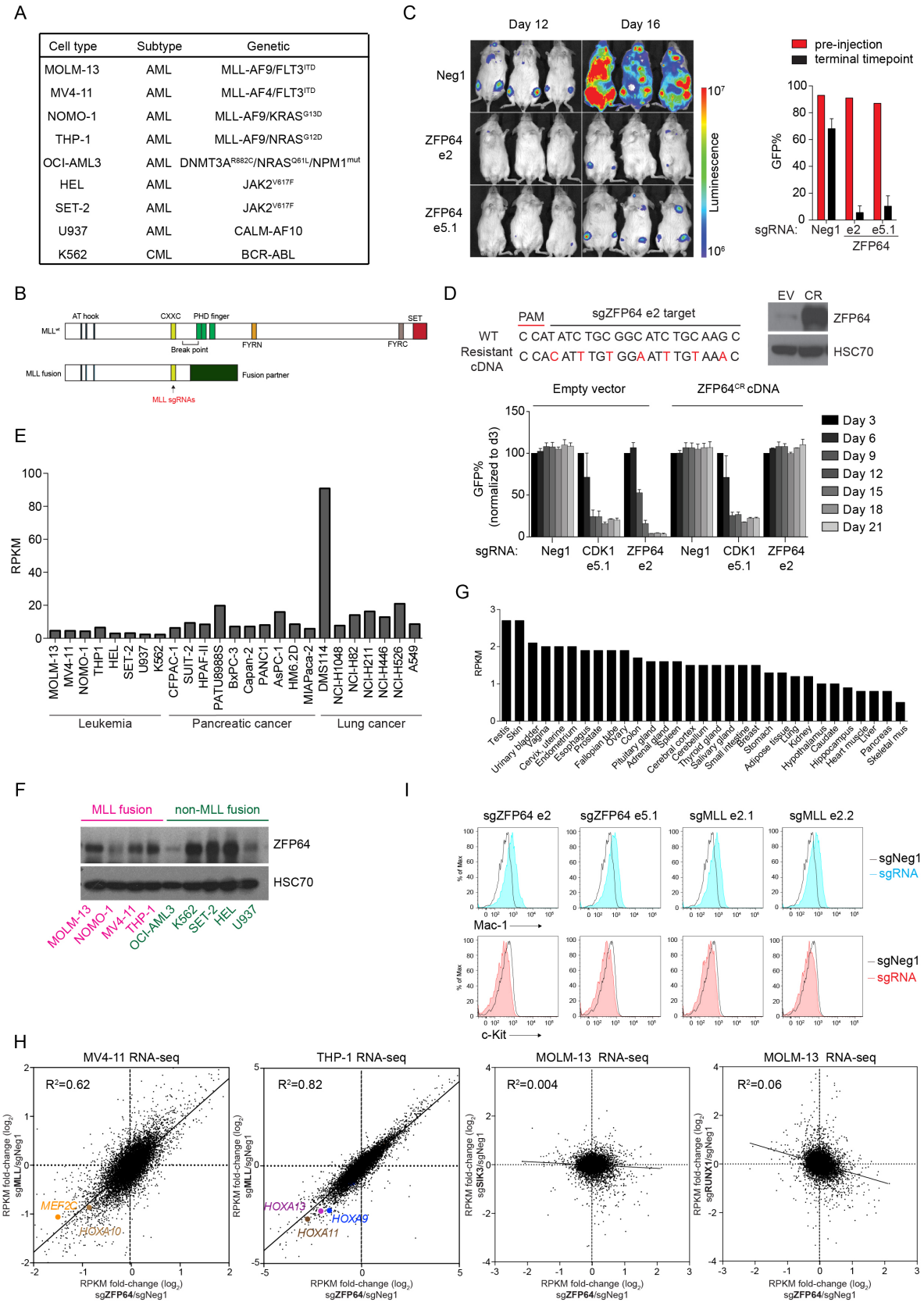
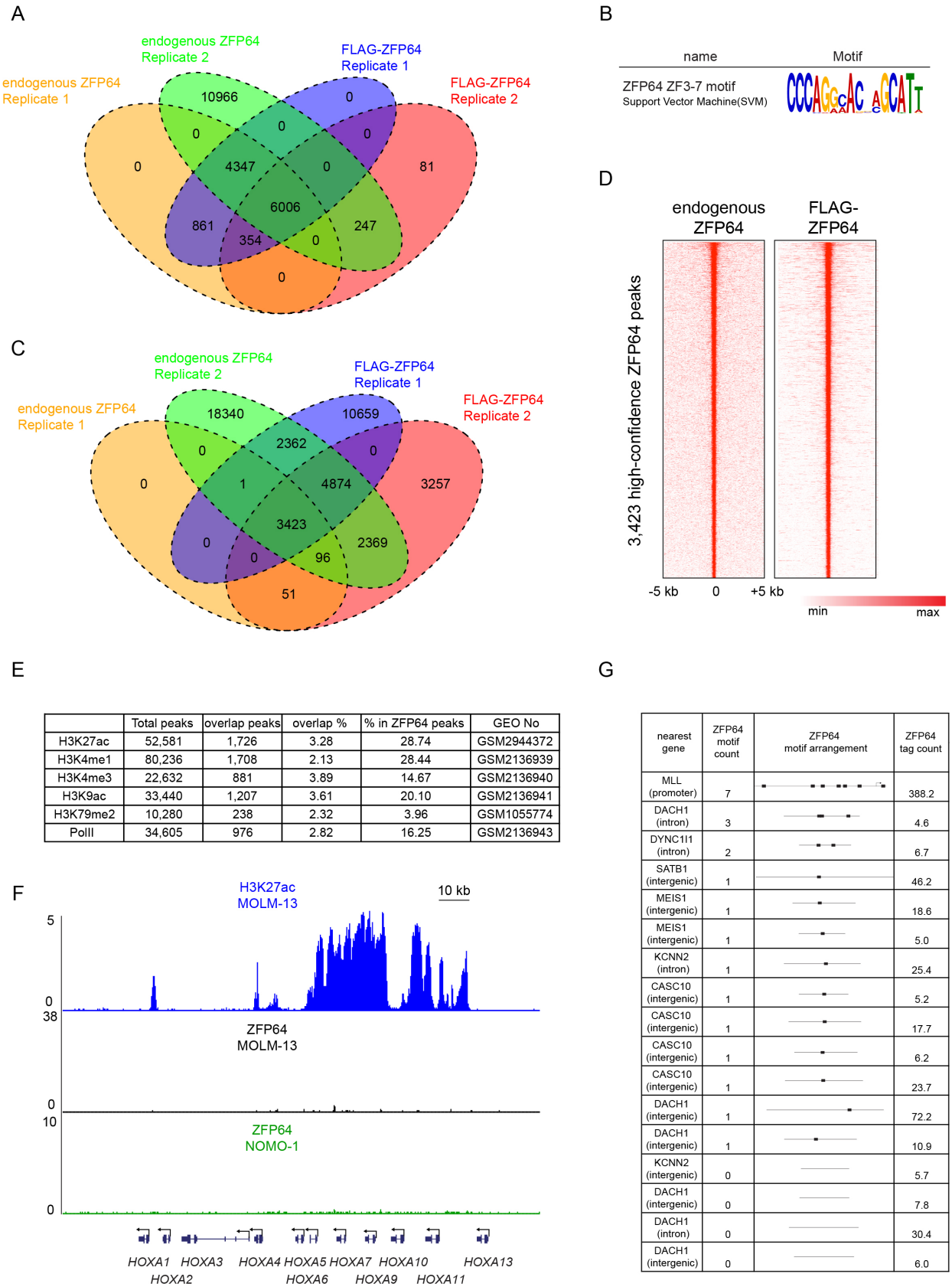


Figure S1. CRISPR screening identifies ZFP64 as essential in *MLL*-rearranged leukemia. Related to Figure 1. (A) Known genetic mutations in nine leukemia cell lines. **(B)** Illustration of MLL^{WT} and MLL^{fusion} protein domain architecture and location of MLL sgRNAs. **(C)** Left:

Bioluminescence imaging of NSG mice transplanted with luciferase^{+}/Cas9^{+} MOLM-13 cells transduced with either Neg1 or ZFP64 targeting sgRNAs. Irradiated mice were tail vein injected with 500,000 MOLM-13 cells and imaged on day 12 and 16 post-transplantation. Percentage of sgRNA^{+}/GFP^{+} MOLM-13 cells from pre-transplantation and terminally-diseased mice collected from bone marrow. Three mice were used for each cohort. All mice were sacrificed for flow cytometry analysis on day 16. **(D)** top: Design of CRISPR resistant ZFP64 cDNA and Western blot of ZFP64 expression in cells transduced with empty vector (EV) or the CRISPR-resistant ZFP64 cDNA (CR). Competition-based proliferation assay for rescue experiment in MOLM-13 after infection of CRISPR-resistant cDNA. **(E)** ZFP64 mRNA expression level (RPKM values from RNA-seq) across different cancer cell lines. **(F)** Western blotting of ZFP64 and HSC70 in MLL^{WT} and MLL^{fusion} leukemia cell lines. **(G)** ZFP64 mRNA expression level from the GTEx database across different human tissues. **(H)** Comparison of global mRNA changes after knock out of ZFP64 and MLL in MV4-11 and THP-1 (left two panels). or knock out ZFP64 and SIK3 or RUNX1 in MOLM-13 (right two panels). Plotted is the fold change based on two independent sgRNA targeting ZFP64, MLL, SIK3 and RUNX1 compared to a negative control sgRNA Neg1. **(I)** Flow cytometry analysis of Mac-1 (top) and c-Kit (bottom) cell surface expression on day 8 post-infection with the indicated sgRNAs expressed in MOLM-13 cells. All bar graphs represent the mean ± SEM.



	Total peaks	overlap peaks	overlap %	% in ZFP64 peaks	GEO No
H3K27ac	52,581	1,726	3.28	28.74	GSM2944372
H3K4me1	80,236	1,708	2.13	28.44	GSM2136939
H3K4me3	22,632	881	3.89	14.67	GSM2136940
H3K9ac	33,440	1,207	3.61	20.10	GSM2136941
H3K79me2	10,280	238	2.32	3.96	GSM1055774
PolII	34,605	976	2.82	16.25	GSM2136943

nearest gene	ZFP64 motif count	ZFP64 motif arrangement	ZFP64 tag count
MLL (promoter)	7		388.2
DACH1 (intron)	3		4.6
DYNC1I1 (intron)	2		6.7
SATB1 (intergenic)	1		46.2
MEIS1 (intergenic)	1		18.6
MEIS1 (intergenic)	1		5.0
KCNN2 (intron)	1		25.4
CASC10 (intergenic)	1		5.2
CASC10 (intergenic)	1		17.7
CASC10 (intergenic)	1		6.2
CASC10 (intergenic)	1		23.7
DACH1 (intergenic)	1		72.2
DACH1 (intergenic)	1		10.9
KCNN2 (intergenic)	0		5.7
DACH1 (intergenic)	0		7.8
DACH1 (intron)	0		30.4
DACH1 (intergenic)	0		6.0

Figure S2. Genomic binding features of ZFP64 in leukemia cell lines. Related to Figure 2. (A) Overlap of ZFP64 binding peaks obtained by ChIP-seq using endogenous or Flag-tagged protein in MOLM-13. **(B)** Predicted binding motif for ZFP64 ZF3-7 region using Support Vector Machine

Prediction software: http://zf.princeton.edu. **(C)** Overlap of ZFP64 binding peaks obtained by ChIP-seq using endogenous or Flag-tagged protein in NOMO-1. **(D)** Density plot showing endogenous ZFP64 and FLAG-ZFP64 binding intensities around the summit of 3,423 high confidence overlapped ZFP64 peaks in NOMO-1 cells. **(E)** Overlap of ZFP64 peaks with histone marks or Pol II in MOLM-13 cells, which were datasets obtained from the indicated GEO accessions. **(F)** Sequencing depth normalized ChIP-seq pileup tracks showing ZFP64 and H3K27ac enrichment at the *HOXA* cluster in MOLM-13 and NOMO-1 cells. **(G)** ZFP64 motif counts and distribution at ZFP64 peaks identified in the vicinity of the seven ZFP64 target genes identified in Figure 2F.

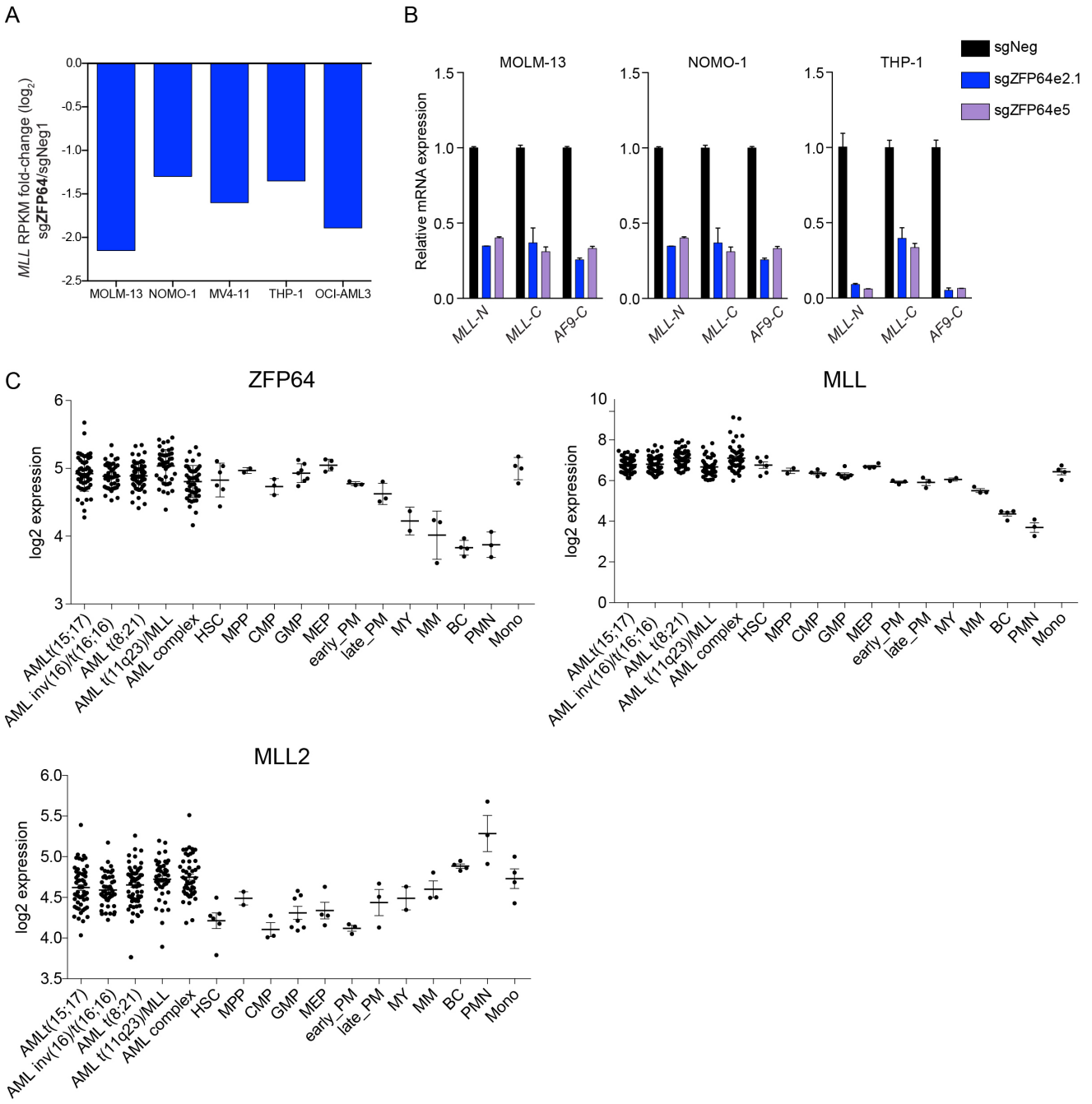
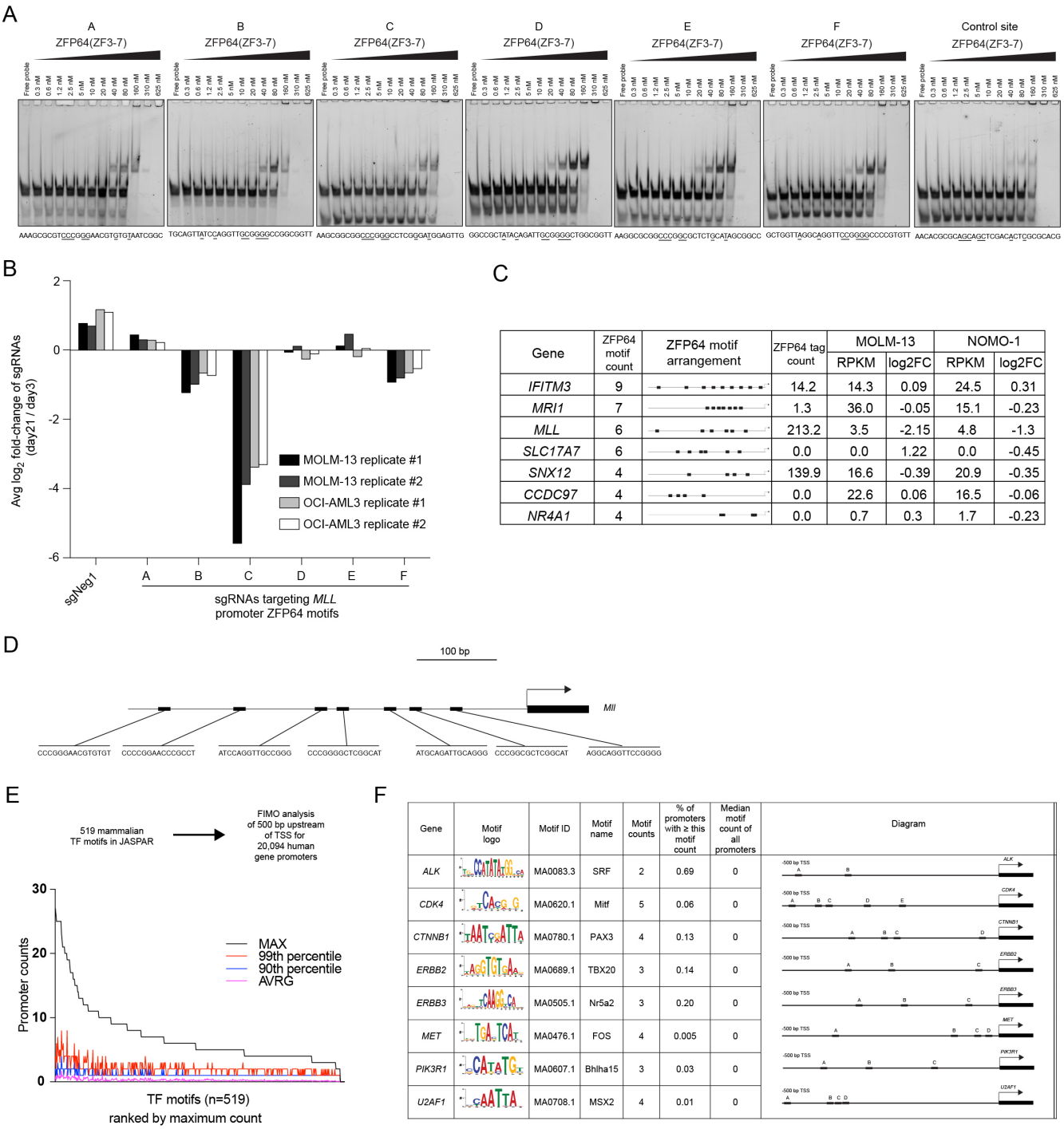


Figure S3. ZFP64 maintains *MLL* expression via promoter activation. Related to Figure 3. (A) RNA-seq analysis evaluating MLL mRNA levels after ZFP64 sgRNA transduction in the indicated cell lines compared to a control sgRNA. **(B)** RT-PCR analysis of *MLL* or *AF9* exons regions in the indicated MLL-AF9 leukemia cell lines after knock out ZFP64. RT-PCR primers were designed to detect an N-terminal region of MLL (MLL-N, found on MLL^{fusion} and MLL^{WT}), a C-terminal region of MLL (MLL-C, found only on MLL^{WT}), and the C-terminus of AF9 (AF9-C, found on MLL^{fusion} and $AF9^{WT}$). GAPDH is used as normalization control. Primer sequences are provided in Table S2. (n=3) **(C)** ZFP64, MLL, or MLL2/KMT2B mRNA expression within human leukemia patient samples or from purified normal human hematopoietic cells. Data was retrieved from BloodSpot. (http://servers.binf.ku.dk/bloodspot/). Abbreviations: HSC, Hematopoietic stem cell; MPP, Multipotential progenitors; CMP, Common myeloid progenitor cell; GMP, Granulocyte monocyte progenitors; MEP, Megakaryocyte-erythroid progenitor cell; early_PM, Early Promyelocyte; late_PM,

Late Promyelocyte; MY, Myelocyte; MM, Metamyelocytes; BC, Band cell; PMN, Polymorphonuclear cells; Mono, Monocytes. All bar graphs represent the mean ± SEM.



Gene	ZFP64 motif count	ZFP64 motif arrangement	ZFP64 tag count	MOLM-13		NOMO-1	
				RPKM	log2FC	RPKM	log2FC
IFITM3	9		14.2	14.3	0.09	24.5	0.31
MRI1	7		1.3	36.0	-0.05	15.1	-0.23
MLL	6		213.2	3.5	-2.15	4.8	-1.3
SLC17A7	6		0.0	0.0	1.22	0.0	-0.45
SNX12	4		139.9	16.6	-0.39	20.9	-0.35
CCDC97	4		0.0	22.6	0.06	16.5	-0.06
NR4A1	4		0.0	0.7	0.3	1.7	-0.23

Gene	Motif logo	Motif ID	Motif name	Motif counts	% of promoters with ≥ this motif count	Median motif count of all promoters	Diagram
ALK		MA0083.3	SRF	2	0.69	0	
CDK4		MA0620.1	Mitf	5	0.06	0	
CTNNB1		MA0780.1	PAX3	4	0.13	0	
ERBB2		MA0689.1	TBX20	3	0.14	0	
ERBB3		MA0505.1	Nr5a2	3	0.20	0	
MET		MA0476.1	FOS	4	0.005	0	
PIK3R1		MA0607.1	Bhlha15	3	0.03	0	
U2AF1		MA0708.1	MSX2	4	0.01	0	

Figure S4. An exceptional density of ZFP64 motifs at the *MLL* promoter. Related to Figure 4. (A) EMSA assays showing the binding activity of recombinant ZFP64 (ZF3-7) to the six predicted ZFP64 motifs present in *MLL* promoter or to a control site. **(B)** CRISPR tiling experiment of the *MLL* promoter evaluating effect of indel mutagenesis in the vicinity of each ZFP64 motif on cell growth. ~10 sgRNAs were designed in the vicinity of each motif, which were pooled and subjected to a negative selection CRISPR screen in Cas9^{+} MOLM-13 cells or OCI-AML3 cells. Average fold-change of sgRNAs targeting each motifs over 18 days in culture was quantified by next-gen sequencing. **(C)** Evaluation of ZFP64 occupancy and changes in mRNA abundance following ZFP64 knockout among

the 7 promoters harboring exceptional density of ZFP64 motifs. **(D)** Distribution of ZFP64 binding motifs in the mouse *Mll* promoter, identified using FIMO ($p < 10^{-4}$). **(E)** FIMO analysis that interrogates all vertebrate TF motifs in the JASPAR database (n=519) at all human protein coding gene promoters. The motif counts at each promoter were calculated ($p < 10^{-4}$) and plotted is the average, 90^{th} percentile, 99^{th} percentile, and the maximum motif counts for each TF. **(F)** Using the analysis in (E), several human oncogene promoters were identified that possess motif counts that are in the top 1% among all human promoters in the genome.

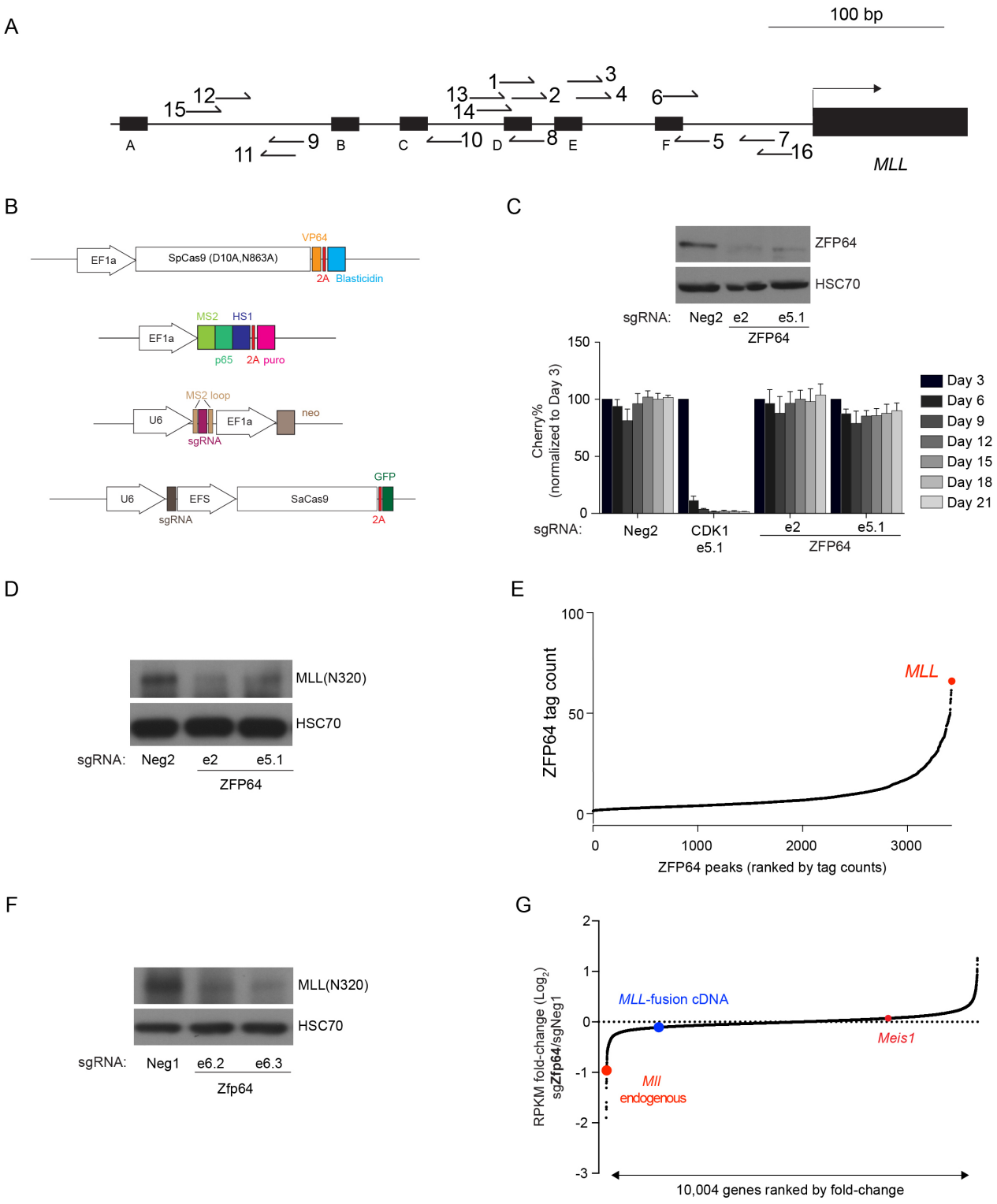


Figure S5. MLL promoter activation is a critical function of ZFP64 underlying its essential function in MLL-fusion leukemia. Related to Figure 6. (A) Schematic showing the position of sgRNAs used to activate endogenous *MLL* expression using CRISPR-activation relative to the position of six ZFP64 motifs. **(B)** Vectors used for dual CRISPR-activation/editing experiment. **(C)** Western blot and competition-based proliferation assay in MLL-AF9/FLT3^{ITD} cells after transduction of sgRNAs targeting ZFP64. The presence of GFP in this engineered cell line led us to use sgRNA mCherry vector for this experiment. (n=3) **(D)** Western blotting of MLL in the human retroviral MLL-

AF9/Nras^{G12D} AML cell line after ZFP64 sgRNA transduction. **(E)** Ranking of ZFP64 occupied sites in human retroviral MLL-AF9/Nras^{G12D} AML cells using ChIP-seq. The *MLL* promoter peak of ZFP64 is indicated. **(F)** Western blot of endogenous MLL^{WT} in mouse retroviral MLL-AF9/Nras^{G12D} AML cells after transduction of sgRNA targeting mouse Zfp64. **(G)** RNA-seq analysis of mouse retroviral MLL-AF9/Nras^{G12D} AML cells after transduction with sgRNAs targeting Zfp64. To evaluate endogenous *Mll*, a custom transcript was inserted into the analysis representing the C-terminal portion of the mouse gene. To evaluate the MLL fusion cDNA, a custom transcript of the human *MLL* N-terminus was inserted into the analysis. All bar graphs represent the mean ± SEM.