

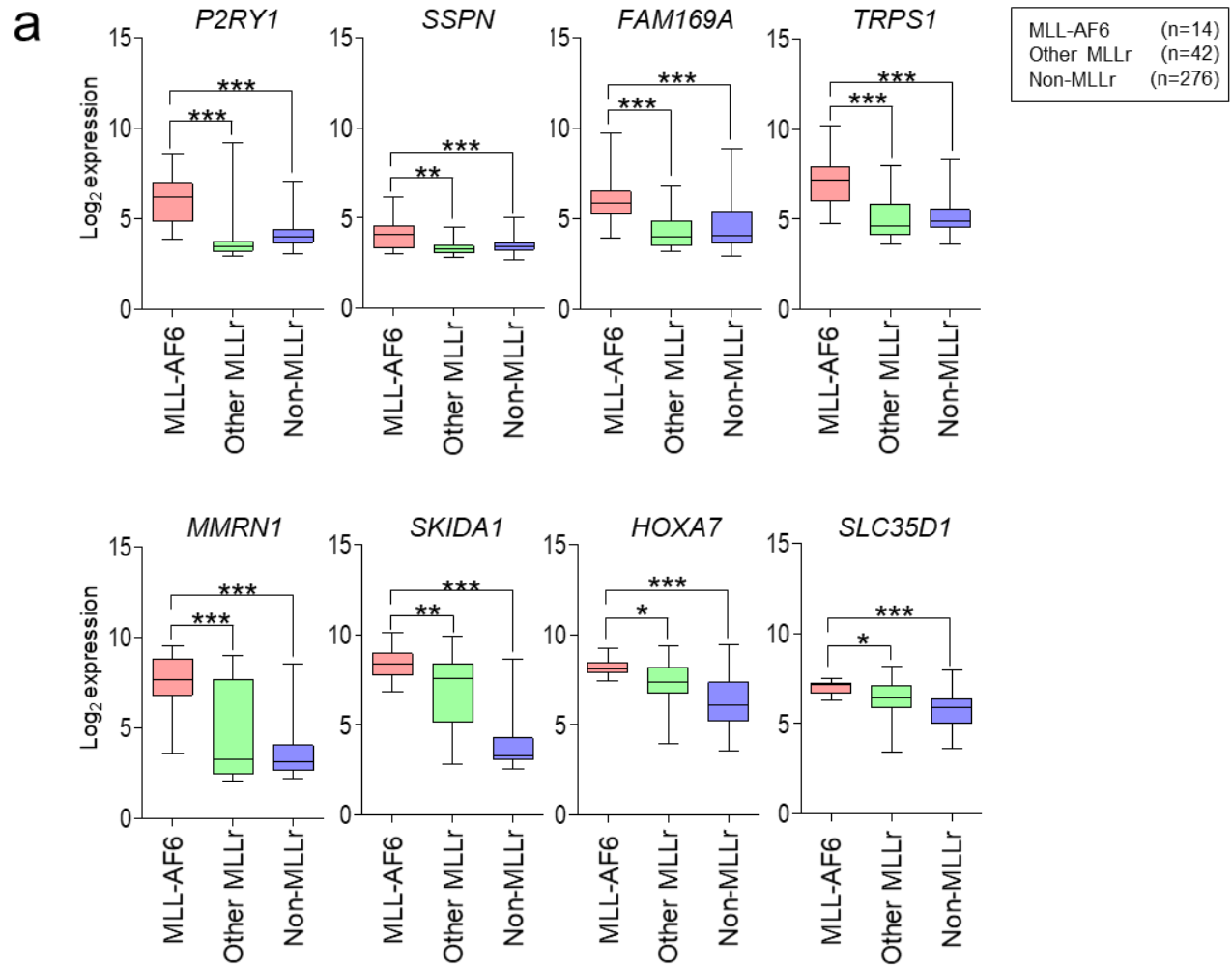
The basic helix-loop-helix transcription factor SHARP1 is an oncogenic driver

in MLL-AF6 Acute Myelogenous Leukemia

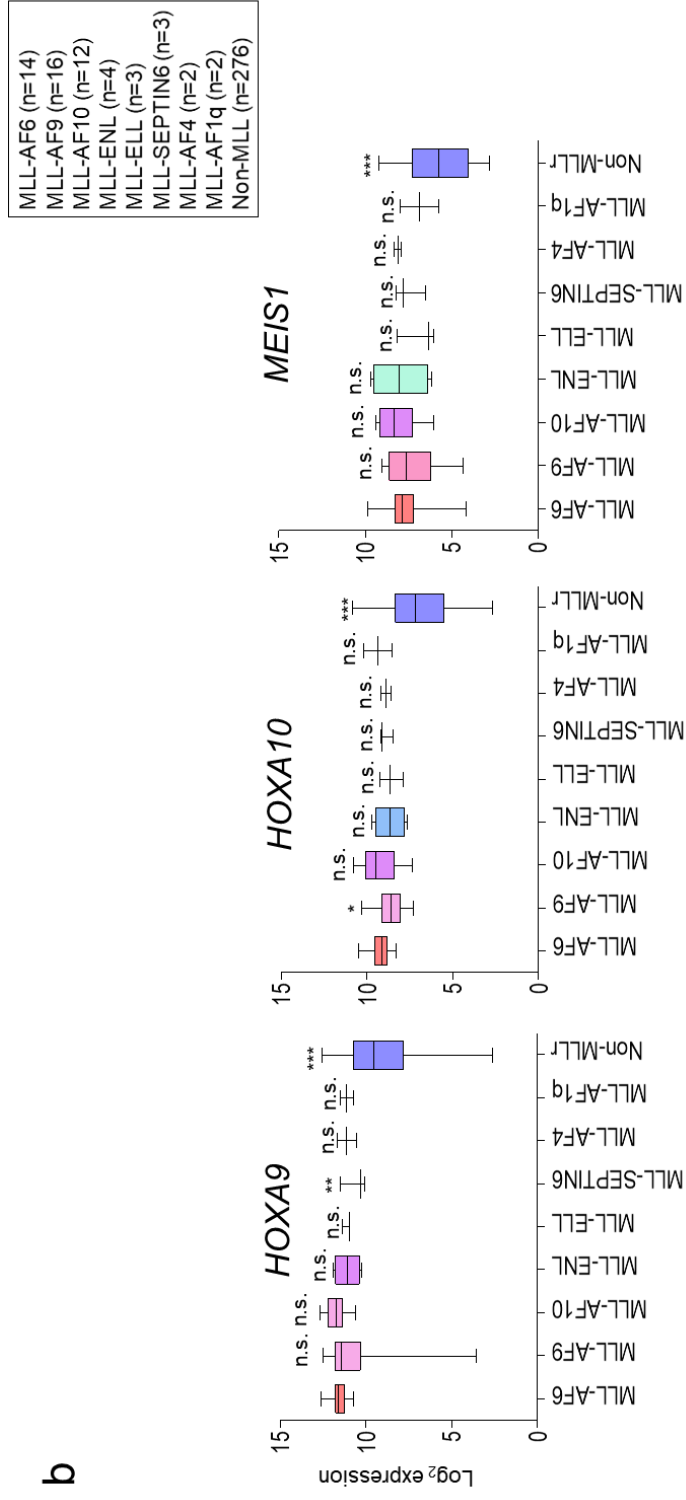
Numata *et al.*

Supplementary figures 1 – 8

Supplementary tables 1 – 5



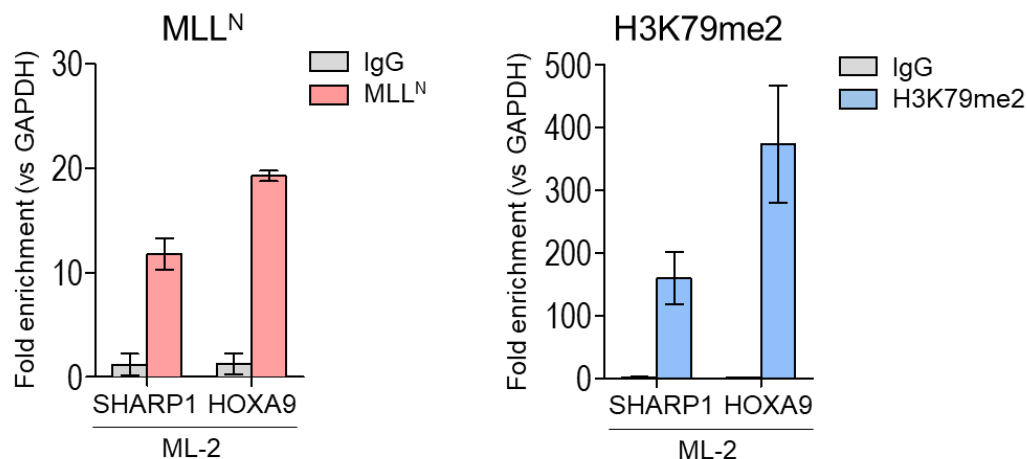
Numata et al., Supplementary Figure 1



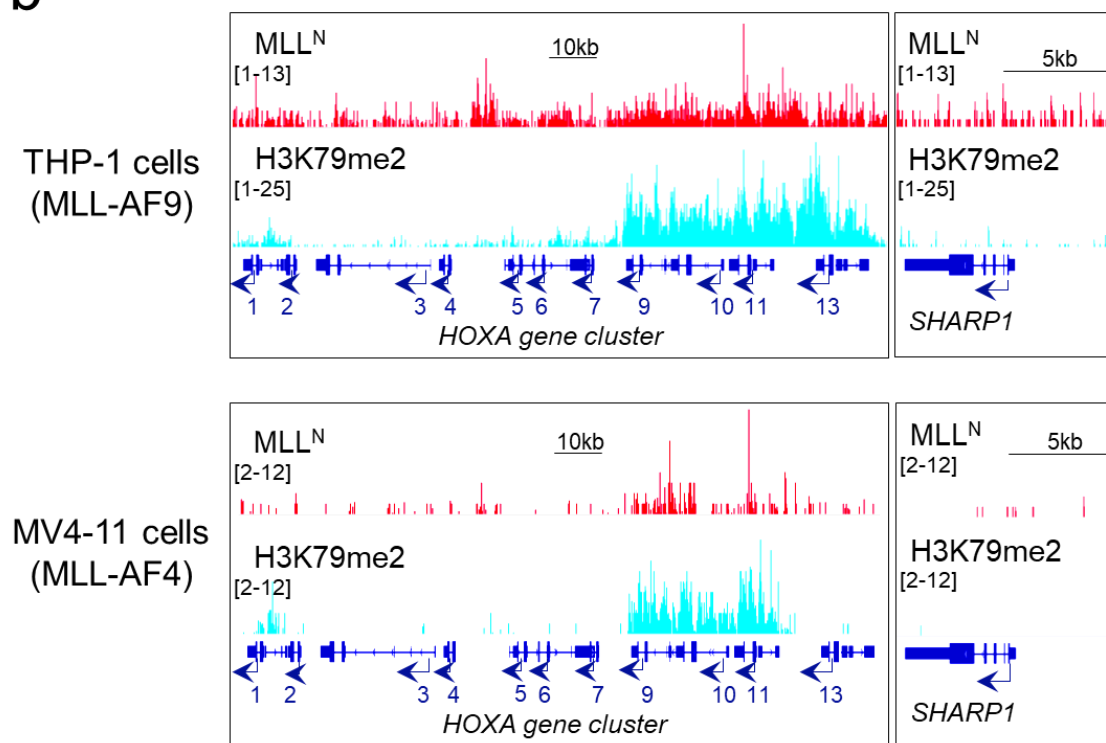
Supplementary Figure 1. Related to Figure 1.

(a) Box plots showing expression of MLL-AF6 specific target genes in AML patients. (b) Box plots showing expression of MLL-AF6 specific targets (SHARP1, P2RY1, and TRPS1) and the canonical target genes of MLL-FPs (HOXA9, HOXA10, and MEIS1) in AML patients according to the subtypes. Gene expression data of patients were obtained from GSE19577, GSE14468 and GSE61804. All box plots extend from the 25th to 75th percentiles and the whisker extends from the minimum level to the maximum. Median value is plotted in the box. *P* values are calculated based on the comparison between each subtype and MLL-AF6. **p* < 0.05, ** *p* < 0.01, ****p* < 0.001

a



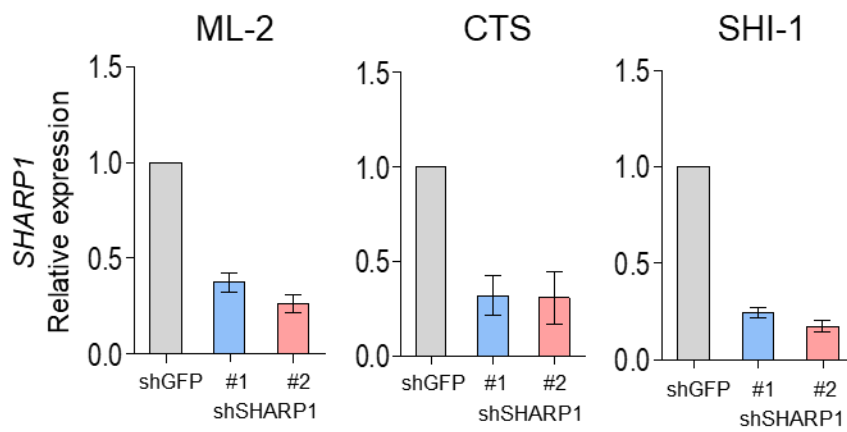
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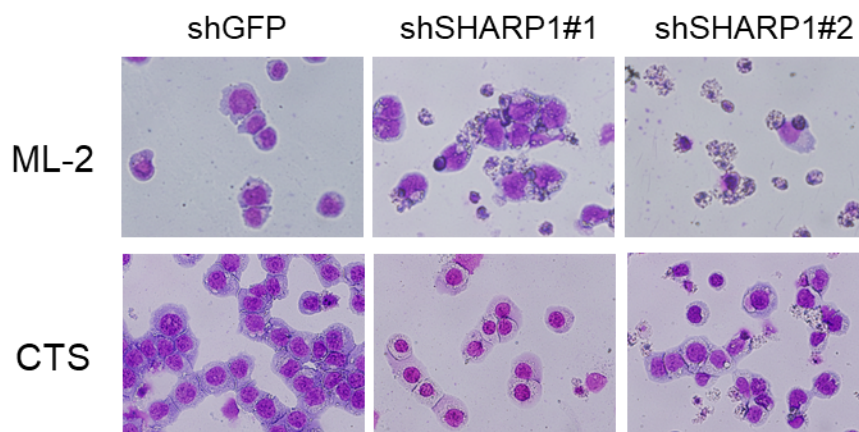
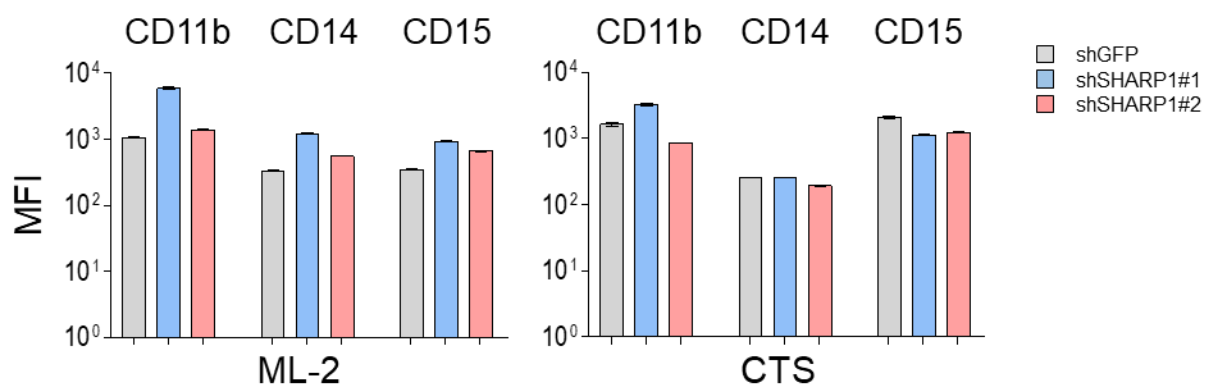
Supplementary Figure 2, Related to Figure 2

(a) MLL^N and H3K79me2 ChIP-qPCR in the *SHARP1* and *HOXA9* promoters, normalized to GAPDH promoter in the ML-2 cells (MLL-AF6). Data are from three independent experiments and presented as mean \pm s.e.m. (b) MLL^N ChIP-seq profiles of THP-1 (MLL-AF9) (top panel) and MV4-11 cells (MLL-AF4) (bottom panel) at the loci of the *HOXA* gene cluster and *SHARP1* gene. ChIP-seq data were obtained from GSE79899.

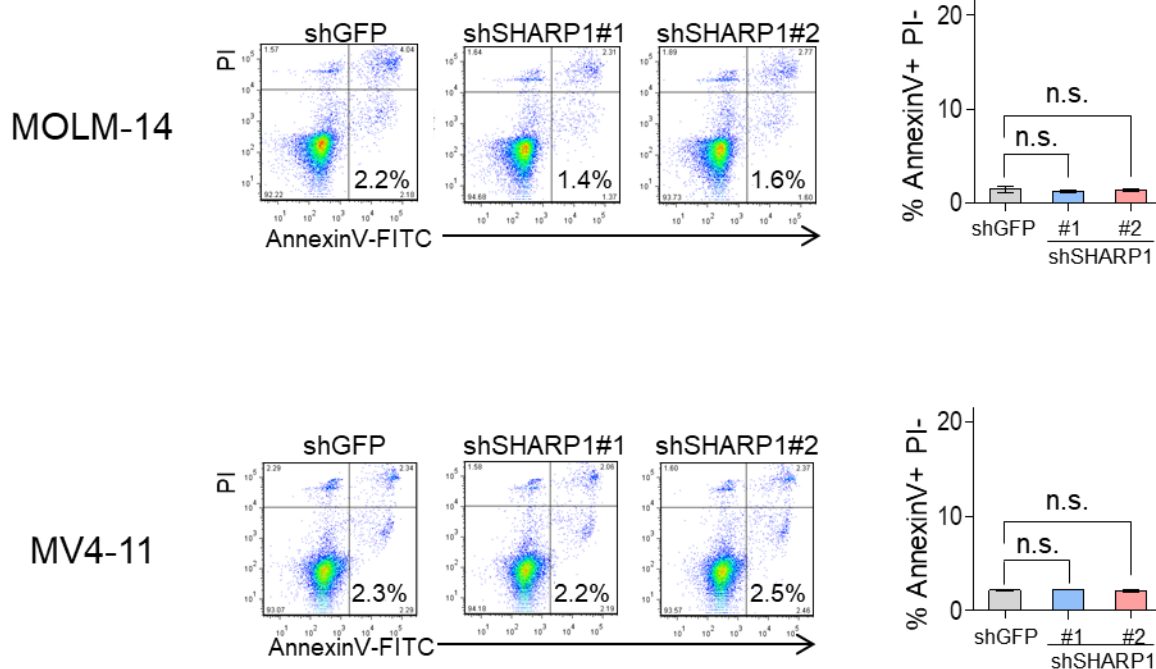
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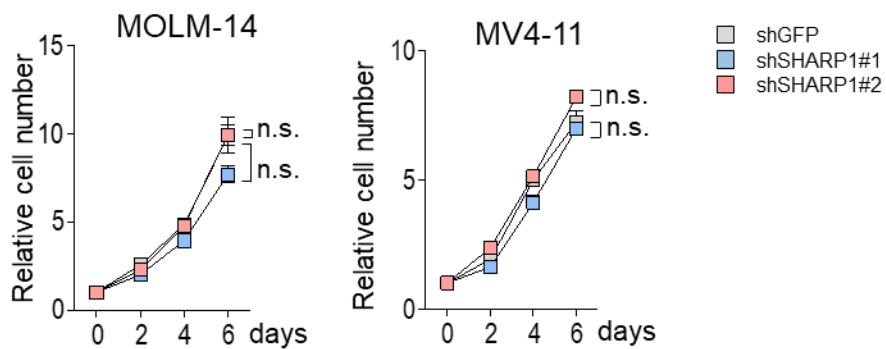
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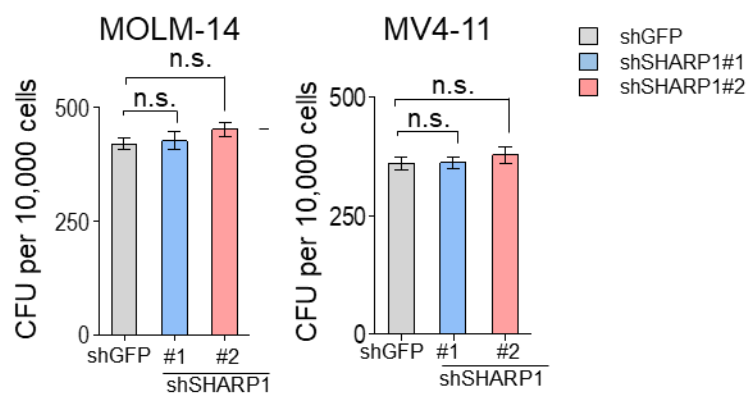
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d



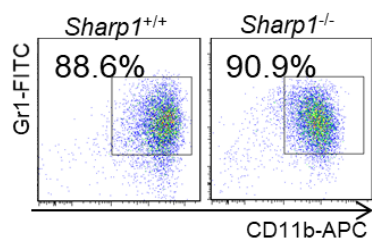
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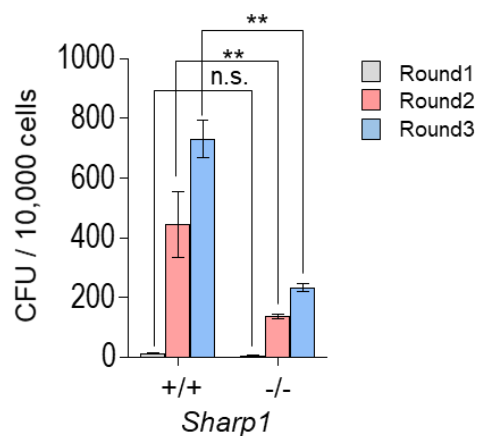
Supplementary Figure 3. Related to Figure 3

(a) qPCR for SHARP1 mRNA in ML-2, CTS and SHI-1 cells transduced with the indicated shRNAs. Expression is the value relative to shGFP. (b) Expression levels of mature granulocytic and monocytic markers, CD11b, CD14 and CD15, measured by FACS in ML-2 and CTS cells transduced with the indicated shRNAs (top panel). Giemsa staining of ML-2 and CTS cells transduced with the indicated shRNAs (bottom panel). (c) Representative AnnexinV and PI FACS plot and percentage of AnnexinV+ and PI- cell of MOLM-14 cells (top panel) and MV4-11 cells (bottom panel) transduced with the indicated shRNAs. (d) Cell count of MOLM-14 cells (left panel) and MV4-11 cells (right panel) transduced with the indicated shRNAs in culture. The value is determined as fold increase in cell number relative to the number of cells initially plated. (e) Colony-forming units (CFU) per 10,000 cells of MOLM-14 cells (left panel) and MV4-11 cells (right panel) transduced with the indicated shRNAs, with the number of colonies observed 7 days after the plating. The graphs are representative examples of three independent experiments and presented as mean \pm s.e.m.

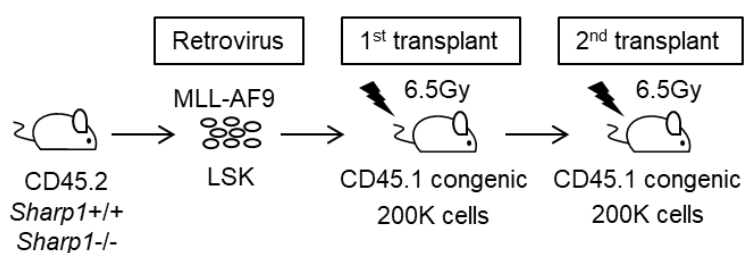
a



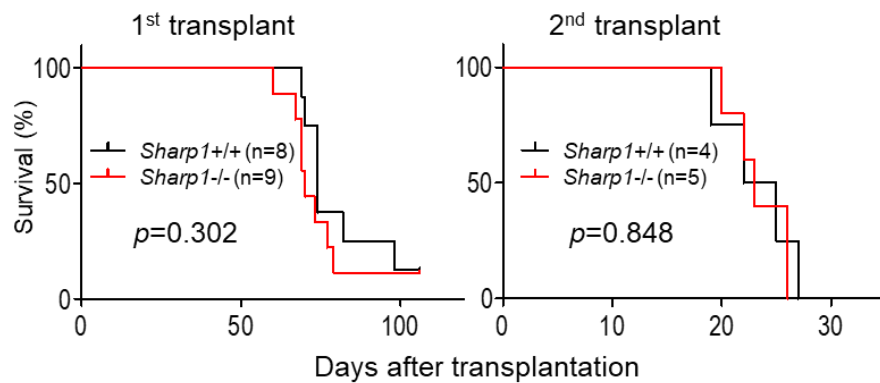
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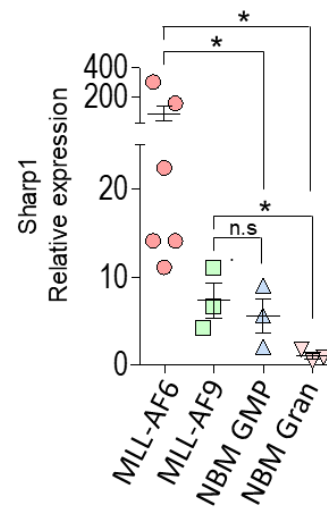
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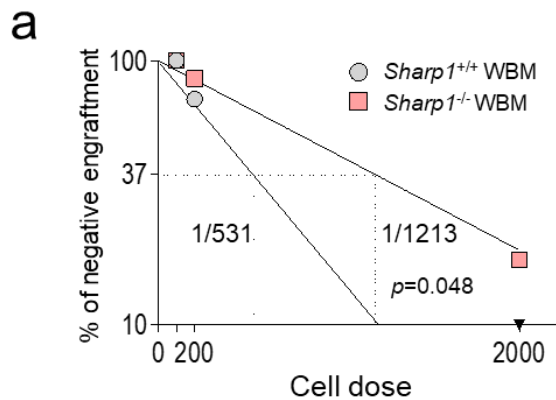


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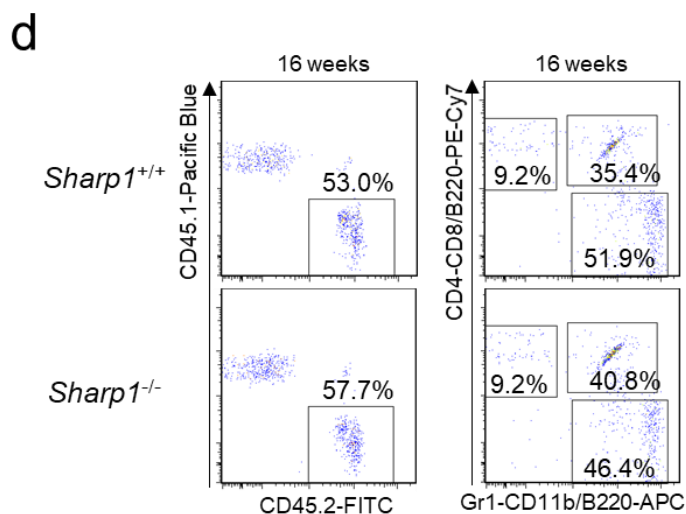
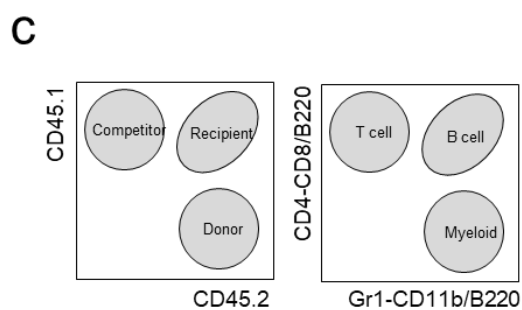
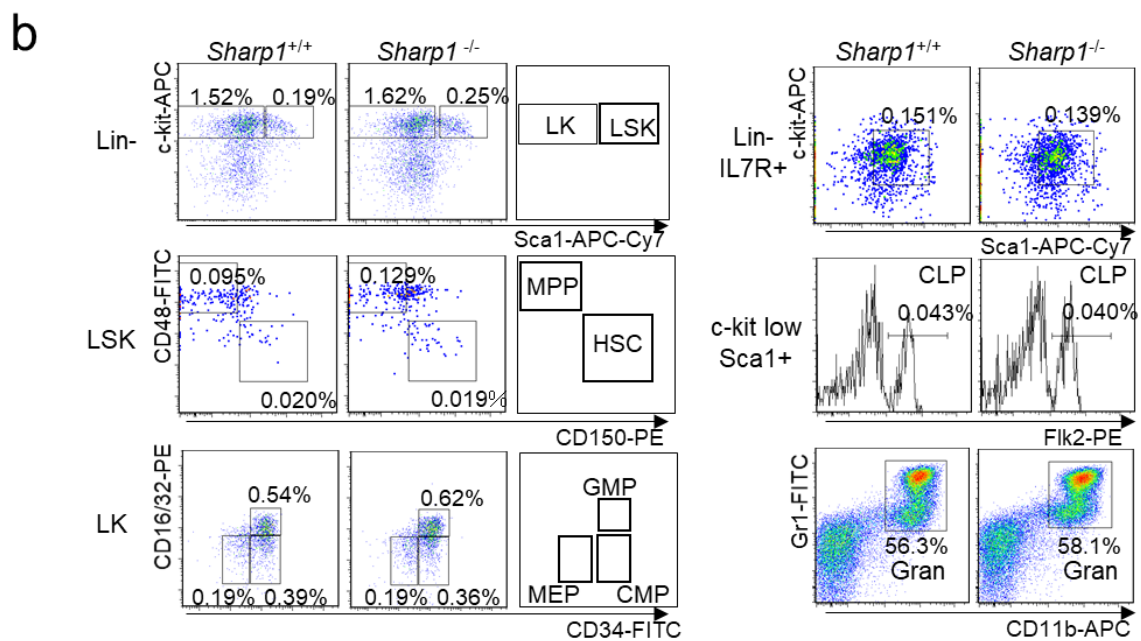


Supplementary Figure 4. Related to Figure 4

(a) Representative flow cytometry plot for Gr1 and CD11b of leukemic BM cells. Percentage of Gr1⁺CD11b⁺ cells is shown. (b) Number of colonies per 10,000 cells observed 7 days after each replating of MLL-AF6 transduced *Sharp1*^{+/+} and *Sharp1*^{-/-} LSK cells. The graph is a representative example of three independent experiments and presented as mean ± s.e.m. (c) Experimental strategy for development of MLL-AF9 AML in *Sharp1*^{+/+} and *Sharp1*^{-/-} mice. (d) Kaplan Meyer survival curve of sublethally irradiated congenic mice transplanted with 200,000 cells from (left panel) the first plate and (right panel) whole bone marrow cells isolated from leukemic recipients following the first transplant. *P* values were determined by Log-rank (Mantle-Cox) Test. (e) qPCR for Sharp1 mRNA expression in murine MLL-AF6 and MLL-AF9 AML cells, normal bone marrow GMP (NBM GMP) and normal bone marrow granulocytes (NBM Gran, Gr1⁺CD11b⁺ cells from C57BL/6 mice). Values relative to the average expression of NBM Gr are shown. *P* values were determined by Mann–Whitney U test.



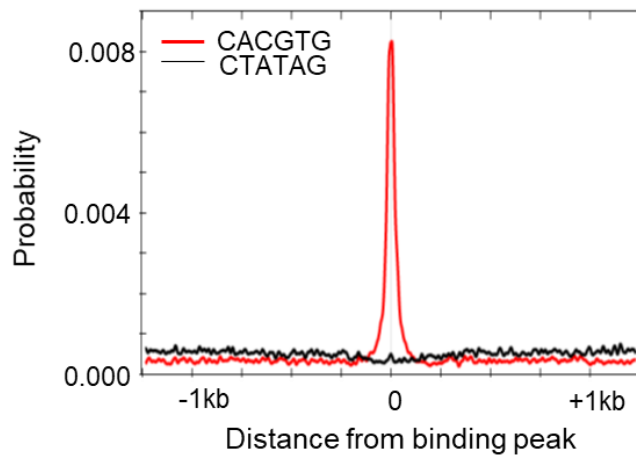
| Cell dose | Response / tested | |
|-----------|------------------------------|------------------------------|
| | <i>Sharp1</i> ^{+/+} | <i>Sharp1</i> ^{-/-} |
| 2,000 | 16 / 16 | 14 / 17 |
| 200 | 4 / 14 | 2 / 14 |
| 100 | 0 / 5 | 0 / 5 |
| Frequency | 1 in 531 | 1 in 1213 |
| C.I 95% | 291 - 970 | 712 - 2067 |



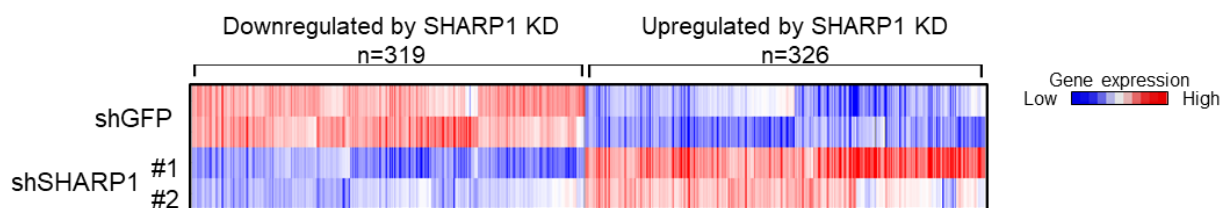
Supplementary Figure 5. Related to Figure 5

(a) Limiting Dilution Assay (LDA). The indicated numbers of leukemic whole bone marrow (WBM) cells (ML-AF6 AML *SharpI*^{+/+} and *SharpI*^{-/-}) were transplanted into sublethally irradiated congenic mice. Inverted triangle represents 0% of negative engraftment of *SharpI*^{+/+} WBM cells. (b) Representative flow cytometry plots for HSPC and mature myeloid cells from age and gender-matched *SharpI*^{+/+} and *SharpI*^{-/-} mice. Percentages of the gated populations are shown. (c) Cartoon showing the chimerism analysis of peripheral blood by flow cytometry. When staining for CD45.1 and CD45.2, CD45.1 and CD45.2 double positive cells are recipient-derived cells. CD45.2 only positive cells are donor-derived cells, while CD45.1 only positive cells are competitor-derived cells. When staining for B220/CD4-CD8 and CD11b-Gr1/B220, the double positive population is B220⁺ B lineage cells. CD4-CD8 only positive are T lineage cells, while CD11b-Gr1 only positive cells are from the myeloid lineage. (d) Representative flow cytometry plots for identification of donor cell, myeloid, B and T cell in peripheral blood of recipients of *SharpI*^{+/+} (upper) and *SharpI*^{-/-} mice (lower) 16 weeks after transplantation. Percentages of the gated populations are shown.

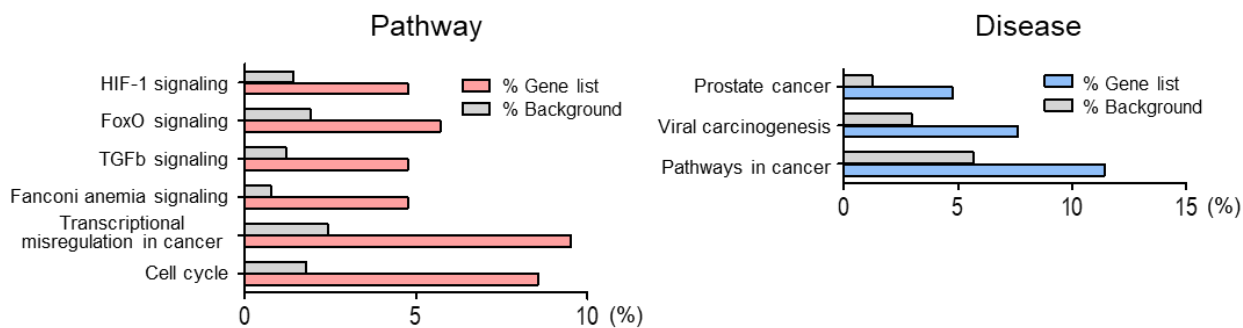
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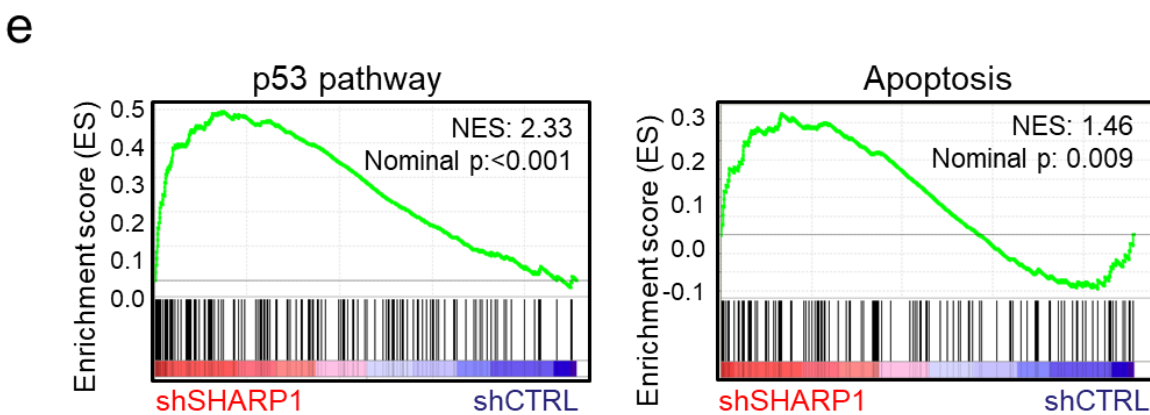
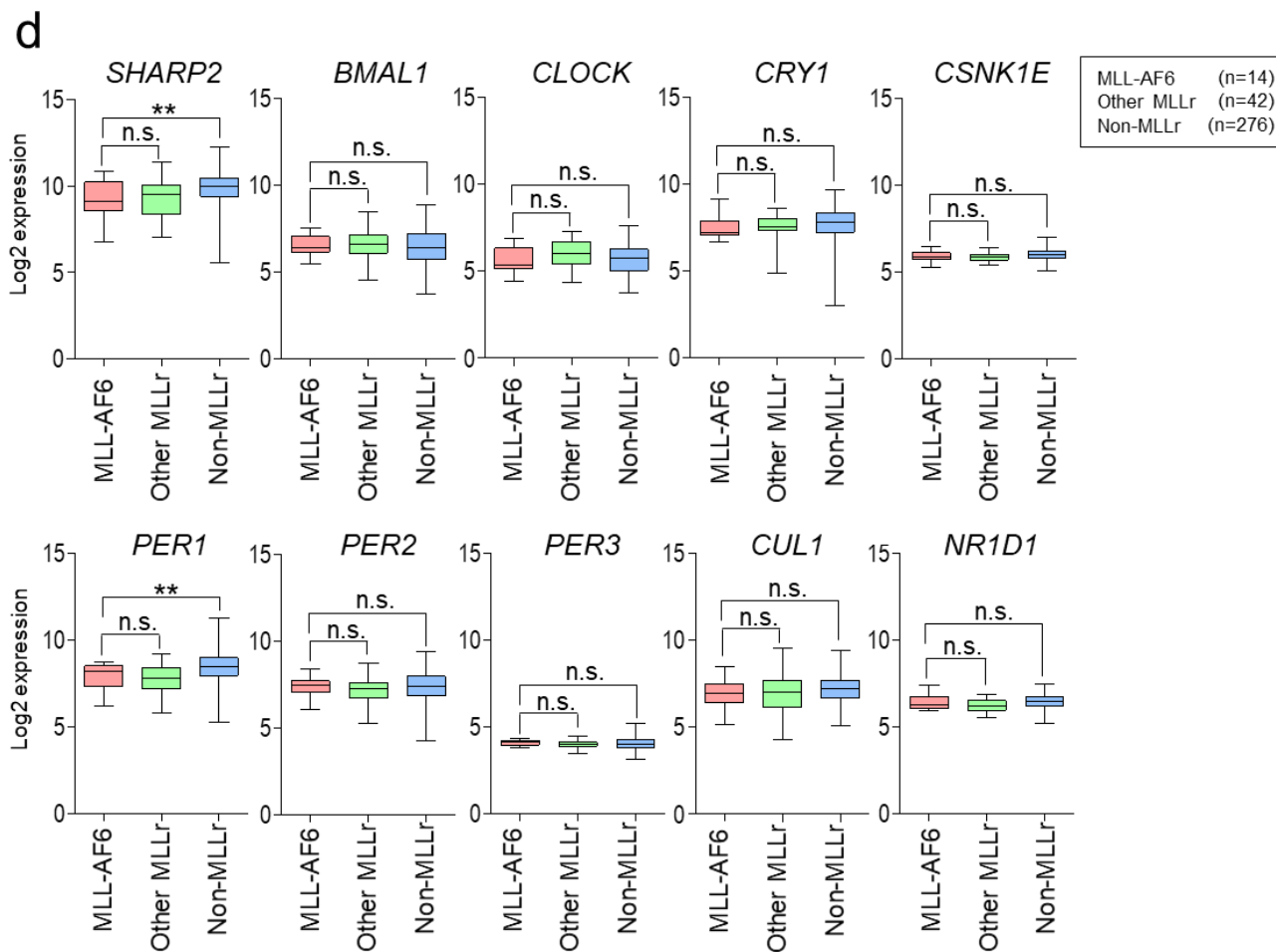


b



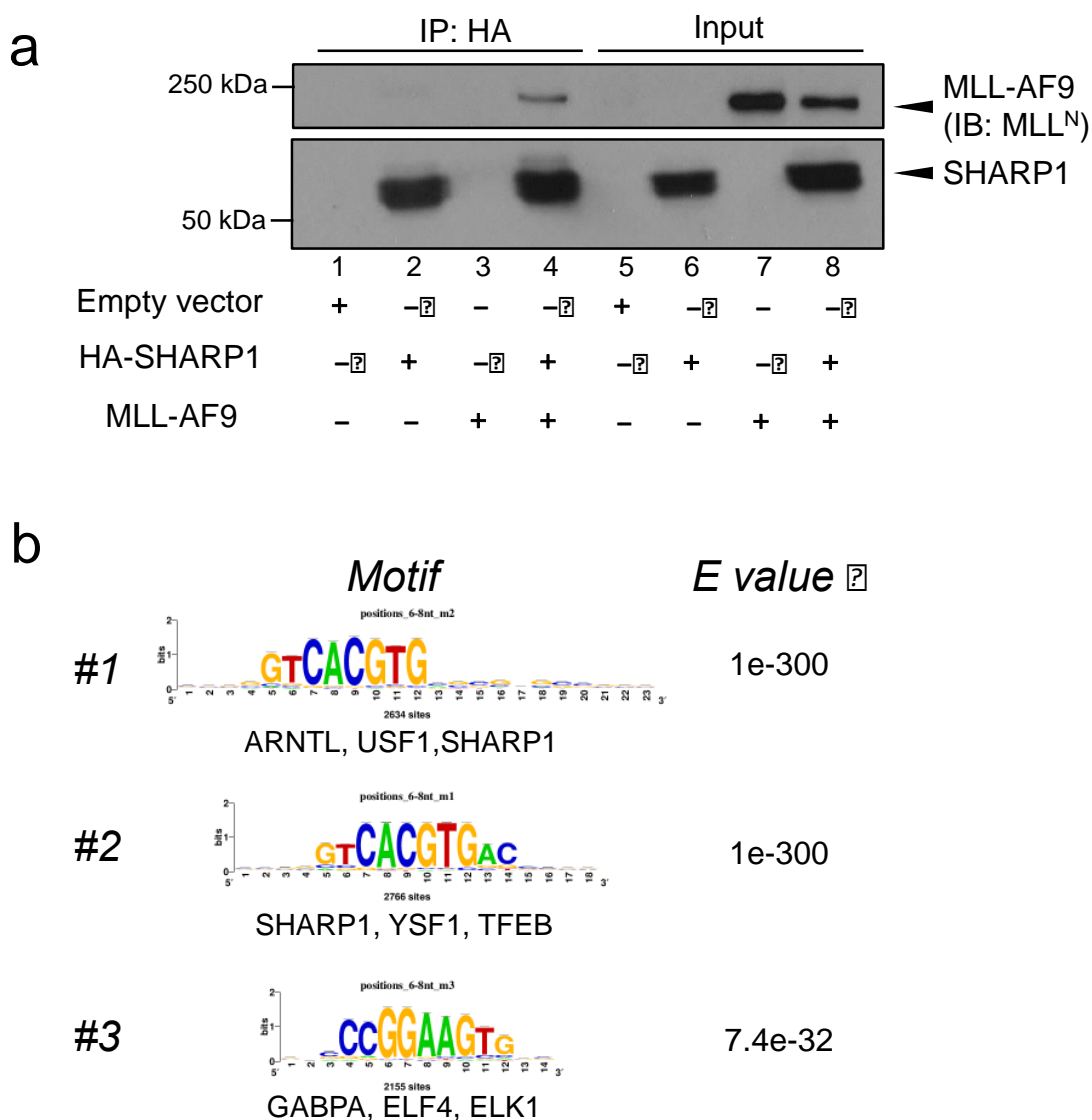
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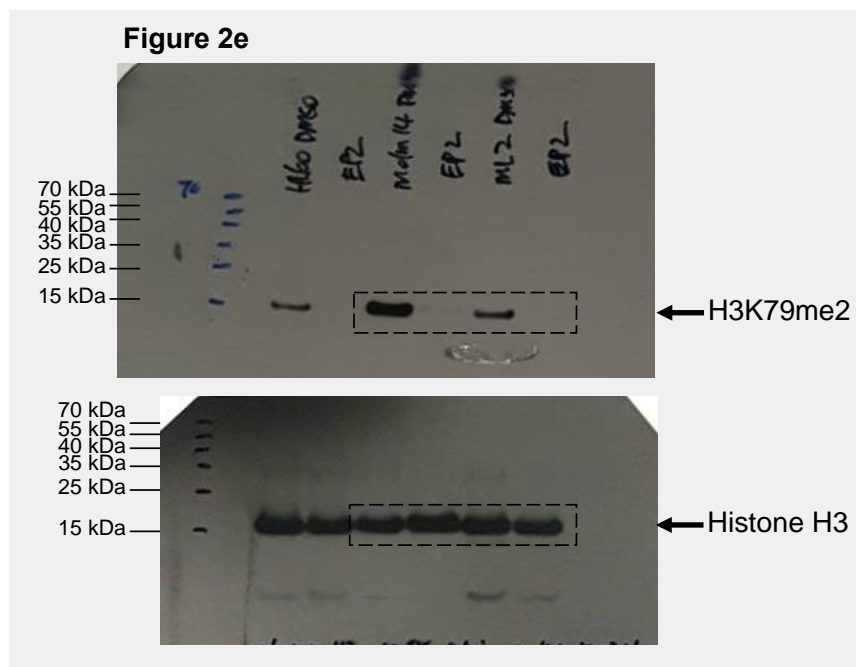
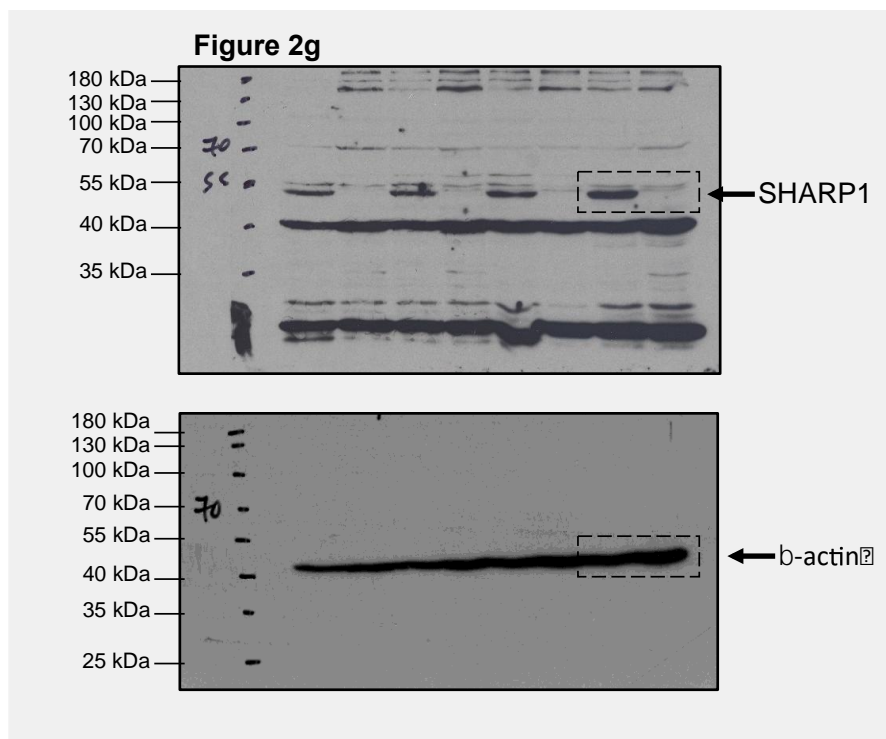
Supplementary Figure 6. Related to Figure 6

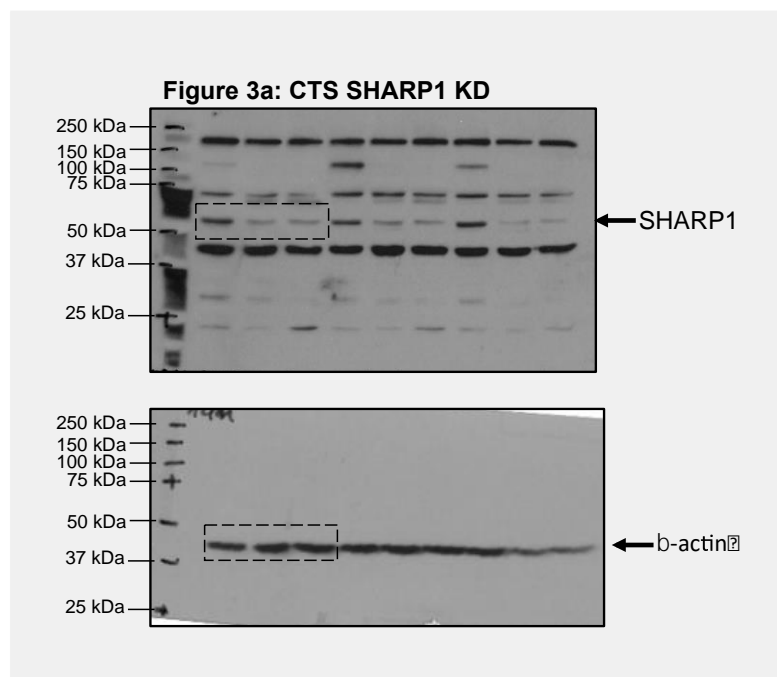
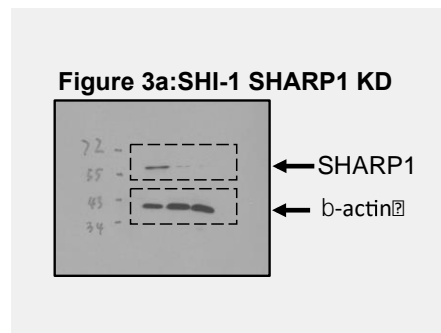
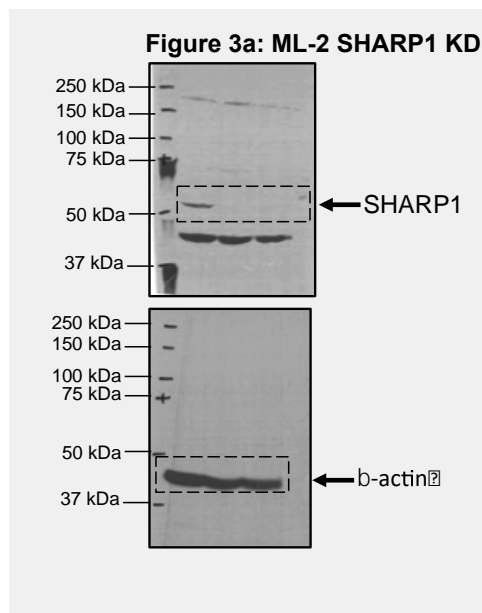
(a) Distributions of *cis* elements around the SHARP1 binding sites with CACGTG motif compared to control CTATAG motif. (b) Heatmap images representing the relative expression levels of 319 SHARP1-bound genes downregulated and 326 SHARP1-bound genes upregulated by SHARP1 knockdown (KD) in ML-2 cells. Each row corresponds to a gene and is normalized across the row. (c) Pathway analysis of SHARP1-bound genes downregulated by SHARP1 KD. (d) Box plot showing the average expression of circadian clock genes in AML patients. Other MLLr: MLL-rearranged AML other than MLL-AF6 AML, Non-MLLr: non MLL-rearranged AML. Gene expression data of patients were obtained from GSE19577, GSE14468 and GSE61804. Box plot extends from the 25th to 75th percentiles and the whisker extends from the minimum level to the maximum. Median value is plotted in the box. (e) Enriched gene sets in ML-2 shSHARP1 cells over shGFP: p53 pathway (HALLMARK P53 PATHWAY) and Apoptosis (HALLMARK APOPTOSIS).

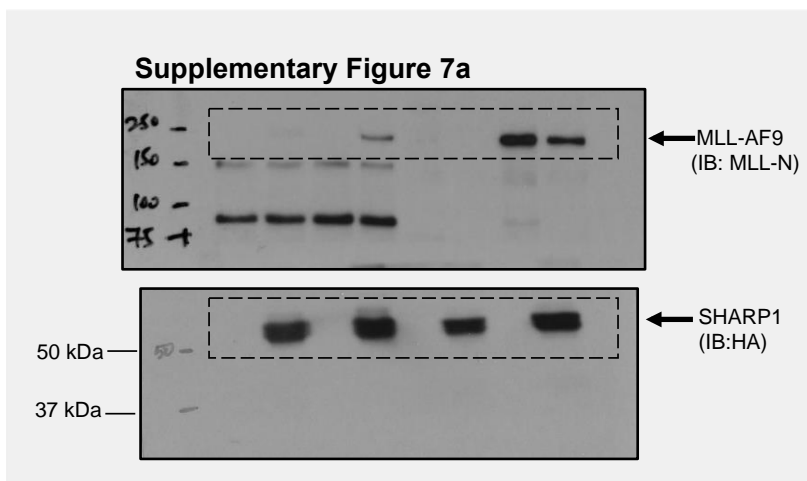
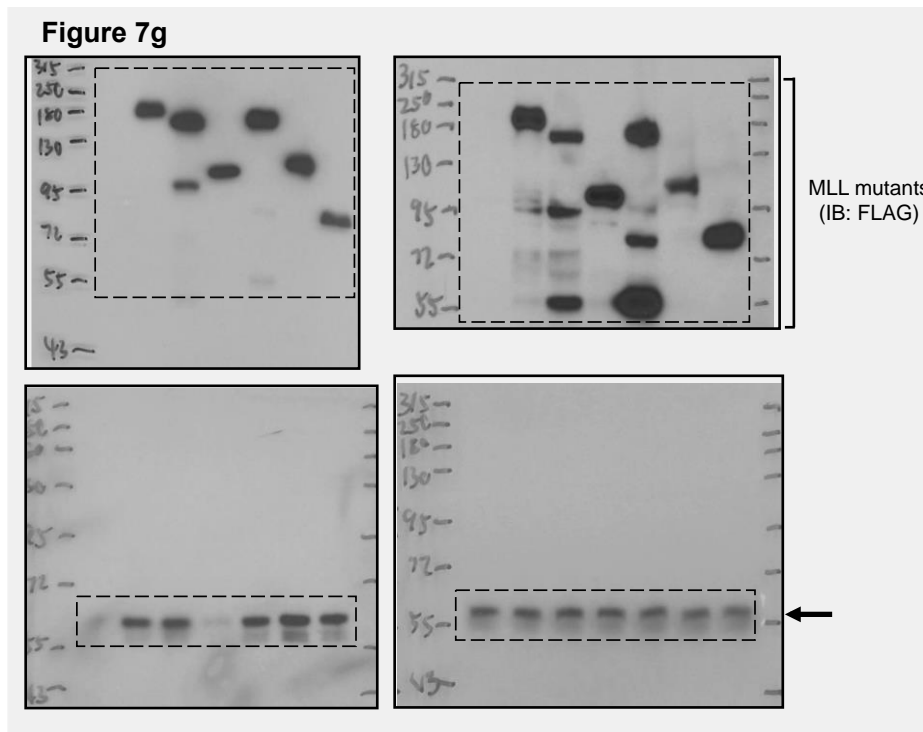
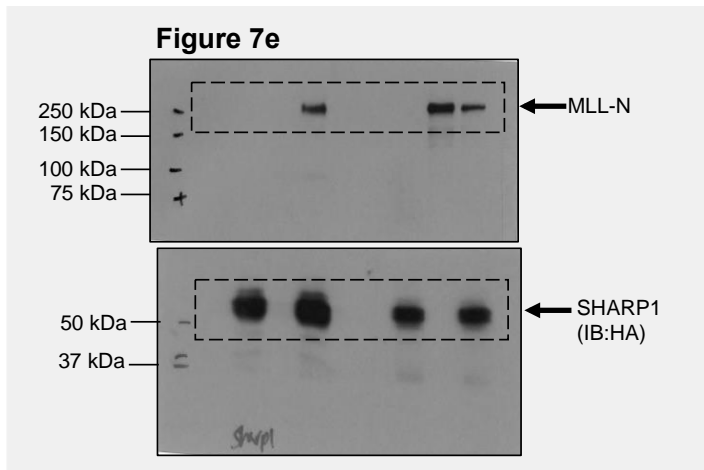


Supplementary Figure 7. Related to Figure 7.

(a) Co-immunoprecipitation studies of SHARP1 and MLL-AF9 with an anti-HA antibody in 293T cells transfected with plasmids encoding MLL-AF9 and/or HA-tagged SHARP1. Proteins present in immunoprecipitates (IP, lane 1-4) or whole cell lysates of transfected cells (input, lane 5-8) were separated by SDS-PAGE and immunoblotted with antibodies specific for MLL^N and SHARP1. Interaction of SHARP1 and MLL-AF9 was detected (lane 4) and not observed in negative control lanes with either empty vector, SHARP1 or MLL-AF9 only (lane 1-3). Western blots are representative of three independent experiments (b) Top 3 enriched motifs within SHARP1 ChIP-seq peaks on gene promoters revealed by peak-motifs module from the RSAT suite, using oligomer length ranging from 6 to 8 nucleotides and the “merge lengths for assembly” option.







Supplementary Figure 8. Uncropped Western blot images from Figures 2, 3, 7 and Supplementary Figure 7.

Shown are the uncropped Western blot images presented in the main and supplementary figures. Images are labeled corresponding to their figures from the manuscript, with the respective data denoted by dotted boxes. All data are representative of at least three independent experiments.

Supplementary Table 1. MLL-AF6 bound genes in human MLL-AF6 AML cells

| | | | | |
|---------------------|---------------------|---------------------|-------------------|------------------|
| <i>ADAMTS19-AS1</i> | <i>DLX5</i> | <i>JMJD1C</i> | <i>MMRN1</i> | <i>SKIDA1</i> |
| <i>ANO6</i> | <i>DLX6</i> | <i>JMJD1C-AS1</i> | <i>MYB</i> | <i>SLC35D1</i> |
| <i>ANXA2R</i> | <i>DLX6-AS1</i> | <i>KLRF2</i> | <i>NPAS3</i> | <i>SSPN</i> |
| <i>APOLD1</i> | <i>DOCK8</i> | <i>LINC00938</i> | <i>NSMAF</i> | <i>SUPT3H</i> |
| <i>ARID2</i> | <i>EIF4E3</i> | <i>LOC100130992</i> | <i>P2RY1</i> | <i>SYDE2</i> |
| <i>BAZ2B</i> | <i>EMB</i> | <i>LOC100132356</i> | <i>PARP8</i> | <i>TAPT1</i> |
| <i>BHLHE41</i> | <i>FAM169A</i> | <i>LOC100499489</i> | <i>PDCD6IPP2</i> | <i>TAPT1-AS1</i> |
| <i>BMI1</i> | <i>FLJ32255</i> | <i>LOC100506159</i> | <i>PGM5P3-AS1</i> | <i>TCF4</i> |
| <i>C9orf66</i> | <i>FOXD4L1</i> | <i>LOC100996255</i> | <i>PGM5P4-AS1</i> | <i>TCTEX1D1</i> |
| <i>CDK13</i> | <i>FOXP1</i> | <i>LOC153684</i> | <i>PLEKHA8P1</i> | <i>TMEM117</i> |
| <i>CDK6</i> | <i>FRY</i> | <i>LOC643072</i> | <i>PRDM8</i> | <i>TRPS1</i> |
| <i>CDKN1B</i> | <i>FUT4</i> | <i>LOC646762</i> | <i>PROK2</i> | <i>WHAMMP2</i> |
| <i>CDKN2C</i> | <i>HOTTIP</i> | <i>LOC648987</i> | <i>PTPRK</i> | <i>WHAMMP3</i> |
| <i>CHSY3</i> | <i>HOXA10</i> | <i>MBNL1</i> | <i>RBMS1</i> | <i>ZNF521</i> |
| <i>CLEC2B</i> | <i>HOXA10-AS</i> | <i>MBNL1-AS1</i> | <i>REEP3</i> | <i>ZNRF2P1</i> |
| <i>COMMD3</i> | <i>HOXA10-HOXA9</i> | <i>MCOLN3</i> | <i>RNF220</i> | <i>ZNRF2P2</i> |
| <i>COMMD3-BMI1</i> | <i>HOXA11</i> | <i>MEF2C</i> | <i>RUNX2</i> | |
| <i>CPEB2</i> | <i>HOXA11-AS</i> | <i>MEF2C-AS1</i> | <i>SATB1</i> | |
| <i>DACH1</i> | <i>HOXA13</i> | <i>MIR196B</i> | <i>SATB1-AS1</i> | |
| <i>DHRS7</i> | <i>HOXA7</i> | <i>MIR4785</i> | <i>SCAF11</i> | |
| <i>DLEU1</i> | <i>HOXA9</i> | <i>MIR591</i> | <i>SENP6</i> | |

List of 101 MLL^N-bound genes in ML-2 cells from ChIP-seq analysis.

Supplementary Table 2. MLL-AF6 target genes in human MLL-AF6 AML cells

| | | | | |
|---------------------|-------------------|---------------------|-------------------|------------------|
| <i>ADAMTS19-AS1</i> | <i>DLX5</i> | <i>KLRF2</i> | <i>MYB</i> | <i>SKIDA1</i> |
| <i>ANO6</i> | <i>DLX6</i> | <i>LINC00938</i> | <i>NPAS3</i> | <i>SLC35D1</i> |
| <i>ANXA2R</i> | <i>DLX6-AS1</i> | <i>LOC100130992</i> | <i>NSMAF</i> | <i>SSPN</i> |
| <i>APOLD1</i> | <i>DOCK8</i> | <i>LOC100132356</i> | <i>P2RY1</i> | <i>SUPT3H</i> |
| <i>ARID2</i> | <i>EIF4E3</i> | <i>LOC100499489</i> | <i>PARP8</i> | <i>SYDE2</i> |
| <i>BAZ2B</i> | <i>EMB</i> | <i>LOC100506159</i> | <i>PDCD6IPP2</i> | <i>TAPT1</i> |
| <i>BHLHE41</i> | <i>FAM169A</i> | <i>LOC100996255</i> | <i>PGM5P3-AS1</i> | <i>TAPT1-AS1</i> |
| <i>BMI1</i> | <i>FLJ32255</i> | <i>LOC153684</i> | <i>PLEKHA8P1</i> | <i>TCF4</i> |
| <i>C9orf66</i> | <i>FOXD4L1</i> | <i>LOC643072</i> | <i>PRDM8</i> | <i>TCTEX1D1</i> |
| <i>CDK13</i> | <i>FOXP1</i> | <i>LOC646762</i> | <i>PROK2</i> | <i>TMEM117</i> |
| <i>CDK6</i> | <i>FRY</i> | <i>LOC648987</i> | <i>PTPRK</i> | <i>TRPS1</i> |
| <i>CDKN2C</i> | <i>FUT4</i> | <i>MBNL1</i> | <i>RBMS1</i> | <i>WHAMMP2</i> |
| <i>CHSY3</i> | <i>HOTTIP</i> | <i>MBNL1-AS1</i> | <i>REEP3</i> | <i>WHAMMP3</i> |
| <i>CLEC2B</i> | <i>HOXA10</i> | <i>MCOLN3</i> | <i>RNF220</i> | <i>ZNF521</i> |
| <i>COMMD3</i> | <i>HOXA11</i> | <i>MEF2C</i> | <i>RUNX2</i> | <i>ZNRF2P1</i> |
| <i>CPEB2</i> | <i>HOXA7</i> | <i>MEF2C-AS1</i> | <i>SATB1</i> | <i>ZNRF2P2</i> |
| <i>DACH1</i> | <i>HOXA9</i> | <i>MIR4785</i> | <i>SATB1-AS1</i> | |
| <i>DHRS7</i> | <i>JMJD1C</i> | <i>MIR591</i> | <i>SCAF11</i> | |
| <i>DLEU1</i> | <i>JMJD1C-AS1</i> | <i>MMRN1</i> | <i>SENP6</i> | |

List of 92 MLL + H3K79me2 overlapped genes in ML-2 cells from ChIP-seq analysis.

Supplementary Table 3. MLL-AF6 and SHARP1 co-target genes in human MLL-AF6 AML cells

| | | | | |
|--------------|----------|--------------|------------|----------|
| ADAMTS19-AS1 | DLEU1 | LINC00938 | NPAS3 | SKIDA1 |
| ANO6 | DLX6 | LOC100130992 | NSMAF | SLC35D1 |
| ANXA2R | DOCK8 | LOC100132356 | P2RY1 | SSPN |
| APOLD1 | EMB | LOC100499489 | PARP8 | SUPT3H |
| ARID2 | FAM169A | LOC100506159 | PDCD6IPP2 | SYDE2 |
| BAZ2B | FLJ32255 | LOC100996255 | PGM5P3-AS1 | TAPT1 |
| BHLHE41 | FOXD4L1 | LOC153684 | PRDM8 | TCF4 |
| BMI1 | FOXP1 | LOC646762 | PROK2 | TCTEX1D1 |
| CDK13 | FRY | LOC648987 | PTPRK | TMEM117 |
| CDK6 | FUT4 | MBNL1 | REEP3 | TRPS1 |
| CDKN2C | HOXA10 | MBNL1-AS1 | RNF220 | WHAMMP2 |
| CHSY3 | HOXA11 | MEF2C | RUNX2 | WHAMMP3 |
| CLEC2B | HOXA7 | MEF2C-AS1 | SATB1 | ZNF521 |
| CPEB2 | HOXA9 | MIR4785 | SATB1-AS1 | ZNRF2P1 |
| DACH1 | JMJD1C | MIR591 | SCAF11 | |
| DHRS7 | KLRF2 | MYB | SENP6 | |

List of 78 MLL + H3K79me2 + SHARP1 overlapped genes in ML-2 cells from ChIP-seq analysis.

Supplementary Table 4. Primary antibodies for FACS

| Antigen | Species | Clone | Cat # | Company | Dilution | Fluorochrome |
|----------------|---------|--------------|------------|---------------|----------|--------------|
| CD11b | mouse | M1/70 | 17-0112-82 | eBioscience | 1:200 | APC |
| CD11b | mouse | M1/70 | 25-0112-82 | eBioscience | 1:200 | PE-CY7 |
| Gr1/Ly6G | mouse | RB6-8C5 | 11-5931-82 | eBioscience | 1:200 | FITC |
| Gr1/Ly6G | mouse | RB6-8C5 | 25-5931-82 | eBioscience | 1:200 | PE-CY7 |
| CD3e | mouse | 145-2C11 | 25-0031-82 | eBioscience | 1:200 | PE-CY7 |
| CD4 | mouse | GK1.5 | 25-0041-82 | eBioscience | 1:200 | PE-CY7 |
| CD8a | mouse | 53-6.7 | 25-0081-82 | eBioscience | 1:200 | PE-CY7 |
| B220/CD45R | mouse | RA3-6B2 | 553092 | BD Pharmingen | 1:100 | APC |
| B220/CD45R | mouse | RA3-6B2 | 25-0452-82 | eBioscience | 1:100 | PE-CY7 |
| CD19 | mouse | 1D3 | 25-0193-82 | eBioscience | 1:100 | PE-CY7 |
| Ter119 | mouse | TER-119 | 25-5921-82 | eBioscience | 1:100 | PE-CY7 |
| NK1.1 | mouse | 145-2C11 | 25-5941-82 | eBioscience | 1:200 | PE-CY7 |
| CD117/c-kit | mouse | 2B8 | 553356 | BD Pharmingen | 1:100 | APC |
| Sca-1/Ly-6A/E | mouse | D7 | 560654 | BD Pharmingen | 1:100 | APC-CY7 |
| CD150 (SLAMF7) | mouse | TC15-12F12.2 | 115904 | Biolegend | 1:100 | PE |
| CD48 | mouse | HM48-1 | 11-0481-82 | eBioscience | 1:100 | FITC |
| CD34 | mouse | RAM34 | 553733 | BD Pharmingen | 1:50 | FITC |
| CD16/32 | mouse | 2.4G2 | 553145 | BD Pharmingen | 1:100 | PE |
| CD127/IL7Ra | mouse | A7R34 | 11-1271-85 | eBioscience | 1:100 | FITC |
| CD135/Flt3 | mouse | A2F10 | 25-0452-82 | eBioscience | 1:100 | PE |
| CD45.1 | mouse | A20 | 110721 | Biolegend | 1:200 | Pacific Blue |
| CD45.2 | mouse | 104 | 109805 | Biolegend | 1:200 | FITC |
| CD11b | human | ICRF44 | 550019 | BD Pharmingen | 1:200 | APC |
| CD14 | human | M5E2 | 555398 | BD Pharmingen | 1:200 | PE |
| CD15 | human | HI98 | 551376 | BD Pharmingen | 1:200 | APC |

Supplementary Table 5. Primary and secondary antibodies for Western blotting

| Antigen | Company | Catalog no. | Dilution |
|-------------------------------|------------------------------|--------------------|-----------------|
| SHARP-1 | Santa Cruz Biotechnology | H-72 | 1:1000 |
| H3K79me2 | Abcam | ab3594 | 1:1000 |
| Histone H3 | Cell Signaling Technology | #9715 | 1:5000 |
| MLL | Abcam | ab25735 | 1:100 |
| β -actin | Santa Cruz Biotechnology | sc-47778 | 1:5000 |
| FLAG | Sigma | F1804 | 1:10000 |
| HA | Santa Cruz Biotechnology | sc-7392 | 1:2000 |
| secondary anti- rabbit IgG | Cell Signaling Technology | #7074 | 1:2000 |
| secondary anti- mouse IgG | Santa Cruz Biotechnology | sc-358914 | 1:2000 |