Supplemental Figure Legends

Figure S1. **HOXA and HOXB gene cluster expression in primary AML samples.** Heatmap showing RNA-seq expression of the HOXA and HOXB cluster genes from primary AML samples with t(8;21) creating the RUNX1-RUNX1T1 fusion, t(9;11) or t(11;19) with MLL rearrangements, and the NPM1c mutation in NPM1 in log2 transcripts per million (TPM) from ref. 1. These recurrent mutations are associated with canonical HOX expression patterns, including little to no HOX expression in t(8;21) samples, HOXA genes only in MLL-rearranged samples, and combined HOXA/HOXB expression in NPM1-mutant samples.

Figure S2. **HOX gene expression and histone modifications in primary AML samples and normal hematopoietic cells.** A. HOXA and HOXB expression in purified bone marrow cells from normal donors, including CD34+ hematopoietic stem/progenitor cells (HSPCs), promyelocytes, and neutrophils (1). HOXA and HOXB genes are expressed in HSPCs and then downregulated in promyelocytes and neutrophils. B. ChIP-seq signal for H3K27ac at the HOXA locus from a subset of the primary AML samples shown in Figure S1, including samples with t(8;21), t(9;11) or t(11;19), and the NPM1c mutation. High H3K27ac signal is present at the active HOXA chromatin domain shown in Figure 2.

Figure S3. A. CTCF ChIP-seq in the NPM1-mutant OCI-AML3 cells compared to primary AML samples with NPM1 mutations. Top track (black) shows normalized CTCF ChIP-seq signal from OCI-AML3 cells. Bottom tracks (purple) show data from three distinct primary AML samples. Red dashed box highlights three CTCF binding sites that are conserved between the OCI-AML3 cell line and primary AML samples. B. Representative flow cytometry for CD11b and CD14 from wild type OCI-AML3 cells and a triple mutant with deletions at CTCF binding sites CBSA6/7, CBSA7/9, and CBSA10. C. Mean percentage of CD11b, CD14 double positive cells in wild type
OCI-AML3 cells (N=4) and mutant clones with single deletions (CBSA6/7, N=4; CBSA7/9, N=5; CBSA10, N=5), double deletions (CBSA6/7+CBSA7/9, N=9; CBSA7/9+CBSA10, N=13), and triple deletion mutants (N=9), which demonstrates little difference in these flow markers in the mutant OCI-AML3 clones. Bars show mean percent double positive cells +/- one standard deviation. D. Deletion allele fraction over time in bulk edited cultures where CBSA6/7, CBSA7/9, or CBSA10 were targeted with CRISPR/Cas9. DNA was prepared from edited cultures on days 2, 7, and 14 for PCR amplification and direct sequencing of amplicons. Reads were mapped to the genome and counted if they were unmodified or contained deletions >5 bp. Bars represent the fraction of reads with deletions and error bars show the 95% confidence interval of the point estimate. E. Proliferation curves of wild type OCI-AML3 cells and selected deletion mutants using the Incucyte platform from triplicate cultures. Note that biological replicates (independent mutant clones) were included for CTCF binding site and triple mutants. 500 cells from wild type cells and the indicated deletion mutants were seeded in 96-well plates on day 0 and then monitored via automated phase contrast microscopy. X-axis indicates the time after seeding and Y-axis shows the percent confluence. All deletion mutants showed similar growth properties to wild type, with no consistent growth deficits observed in single mutants at each site or triple mutant lines.

Figure S4. A. Heatmap representation HOXA cluster genes from RNA-sequencing of wild type OCI-AML3 cells (N=4) and mutant clones with homozygous deletions of CBSA6/7, CBSA7/9, or CBSA10, and double mutants (CBSA6/7+CBSA7/9, N=1; CBSA7/9+CBSA10, N=2), and triple mutants (N=3). Expression values are indicated in absolute normalized read counts obtained from the ‘vst’ function in DESeq2. B. Allelic ratio of a heterozygous common SNP in wild type OCI-AML3 cells and mutant clones from RNA-seq data, demonstrating that expression of both alleles is balanced in nearly all clones with deletions, including a mutant with a heterozygous deletion, and multiple compound heterozygotes. Note that double mutants A91-10 (*) and A95-5
(**) show skewed expression because these clones contain a 29 kbp deletion and a 29 kbp inversion, respectively, that disrupt CTCF binding sites but also involve the HOXA9 gene. C. Statistical analysis of the H3K4me3 ChIP-seq signal in triple mutant OCI-AML3 clones at peaks overlapping the HOXA cluster, which shows this histone modification does not appear altered by loss of CTCF binding sites. Left panel shows log2 normalized H3K4me3 signal at all peaks identified in wild type OCI-AML3 cells from two independent triple mutant OCI-AML3 clones (Y-axis) vs. signal in wild type OCI-AML3 cells (N=2 replicates). Dashed lines indicate a 2-fold change in signal. The numbered points in red correspond to the numbered peaks in the panel on the right, which shows the mean ChIP-seq signal from wild type OCI-AML3 cell in gray and triple mutant clones in red, with peaks indicated by the blue rectangles; CTCF ChIP-seq from wild type OCI-AML3 cells is included for reference in the bottom track. D. Statistical analysis of the H3K27me3 ChIP-seq signal in the same triple mutant clones. Figure components are identical to panel (C). E. ChIP-seq for H3K4me3 (yellow panel) and H3K27me3 (blue panel) from a mutant OCI-AML3 clone with deletion of CBSA6/7. Mean ChIP-seq signals from 2 replicates of wild type OCI-AML3 cells are shown in gray. F. Histone ChIP-seq for a mutant clone with deletion of CBSA10, displayed as in panel (E). G. Histone ChIP-seq from double mutant clones with deletion of CBSA7/9 and either CBSA6/7 (N=1) or CBSA10 (N=2). H. ChIP-seq for histone H3K79 dimethylation (H3K79me2; top panel in orange) and H3K27 acetylation (bottom panel in white). Data are from three mutant clones with deletions of either CBSA7/9 (N=2) or CBSA10 (N=1) (shown in blue). Data from wild type OCI-AML3 cells are shown in gray.

Figure S5. A. Chromatin contact matrix for chromosome 7p for the MLL-rearranged MOLM13 cell line from in situ Hi-C data. Data were generated using the approach described in the Methods section for comparison with Hi-C data from OCI-AML3 cells. Chromatin interactions involving the HOXA gene cluster are similar between MOLM13 and OCI-AML3 cells. Data shown were normalized using KR normalization and were visualized using juicebox (see ref.
25). B. Chromatin contact matrix for chromosome 7p from normal hematopoietic stem/progenitors (HSPCs) from reference 31. Data are presented as in A. C. Chromatin contact matrix and comparative loop analysis for a double mutant OCI-AML3 clone lacking CTCF binding sites CBSA7/9 and CBSA10 (see Table S3). The loop shown in red was found to be significantly different from wild type OCI-AML3 cells in a pairwise comparison of normalized interaction frequencies (see Methods). D. Normalized read depth of interacting reads between the HOXA cluster and the SNX10 gene and the distal intergenic locus from wild type (black) and mutant (red) OCI-AML3 cells. Note that these interactions involve the posterior HOXA cluster in wild type cells, but in mutant clones lacking CTCF binding sites these interactions are reduced and occur across the HOXA cluster.

Figure S6. A. Chromatin contact matrix from in situ Hi-C data for chromosome 7p from the NPM1-mutant IMS-M2 cell line at 5 kbp resolution. B. Chromatin contact matrix from in situ Hi-C data for chromosome 7p from the RUNX1-RUNX1T1 containing Kasum-1 cell line at 5 kbp resolution. C. Focused view of chromatin loop anchors at the HOXA locus in Kasumi-1, IMS-M2, and OCI-AML3 cells. Note the loop anchors involve the posterior HOXA cluster in IMS-M2 and OCI-AML3 cell lines, which have high HOXA expression, whereas interactions occur at the anterior and central portions of the HOXA cluster in Kasumi-1 cells that have low HOXA expression. D. ChIP-seq for H3K27ac from primary AML samples with NPM1c (purple), MLL rearrangements (N=1 each of t(9;11) and t(11;19); green), and t(8;21) and the RUNX1-RUNX1T1 gene fusion for a 480 kbp region that includes HOXA interacting regions in the SNX10 gene and the distal intergenic locus, which is demarcated by the dashed box. Note that the intergenic locus does not possess the enhancer associated H3K27ac modification, and therefore does not appear to be functionally active in primary AML samples that express the HOXA genes.
Figure S1

A

RUNX1-RUNX1T1
MLL rearranged
Normal karyotype w/ NPM1c

RNA-seq expression

log2(TPM)

HOXA1
HOXA2
HOXA3
HOXA4
HOXA5
HOXA6
HOXA7
HOXA9
HOXA10
HOXA11
HOXA13
HOXB1
HOXB2
HOXB3
HOXB4
HOXB5
HOXB6
HOXB7
HOXB8
HOXB9
HOXB13
Figure S2

A

- CD34
- Pro
- PMN

RNA-seq expression

B

H3K27ac ChIP-seq

RUNX1-RUNX1T1

Normal karyotype w/ NPM1c

MLL rearranged
Figure S3

A

OCI-AML3

NPM1c mutant primary AML sample 1

NPM1c mutant primary AML sample 2

NPM1c mutant primary AML sample 3

Conserved CTCF binding sites

HOXA11

HOXA2

HOXA5

HOXA6

HOXA7

HOXA10

HOXA13

B

OCI-AML3 WT

OCI-AML3 triple mutant

C

CD11b+CD14+

WT

Deletion mutants

D

Deletion stability over time

E

Experiment 1

Experiment 2
Figure S4

A) RNA-seq expression

B) Heterozygous common SNPs: rs10259620, rs7810502

C) H3K4me3 differential analysis

D) H3K27me3 differential analysis

E) CBSA6/7 deletion mutant histone ChIP-seq

F) CBSA10 deletion mutant histone ChIP-seq

G) Double deletion mutant histone ChIP-seq (A7/9 + A6/7 or A10)

H) H3K79me2 and H3K27ac ChIP-seq (A7/9 or A10 mutants)
Figure S5

A

C

D

HOXA cluster

MOLM13 monocytic AML

Human HSPCs

ΔCBSA7/9
ΔCBSA10

MUT vs. WT P<0.05

ΔCBSA6/7 CBSA7/9

26 mb
26.5 mb
27 mb
27.5 mb
28 mb

CTCF ChIP-seq

HOXA-SNX10+intergenic locus interacting reads

0
20
40
60
80
100

Relative read depth (rpm)

Normalized Signal

WT OCI-AML3
Deletion mutants

HOXA
HOXA
HOXA2 HOXA5
HOXA6
HOXA11
HOXA7
HOXA3
HOXA1 HOXA9 HOXA4
HOXA10

0
1
2
3
4
Figure S6

A. IMS-M2 (NPM1c)

B. Kasumi-1 (RUNX1-RUNX1T1)

C. Kasumi-1

D. HOXA loops

HOXA11
HOXA2 HOXA5
HOXA6
HOXA13
HOXA7
HOXA3
HOXA1 HOXA9
HOXA4
HOXA10

IMS-M2

OCI-AML3

SKAP2, SNX10, distal enhancer

NPM1c
MLX
RUNX1-RUNX1T1
HOXA loops

intergenic loop

AC010677.1
AC010677.2
SNX10
AC010677.1
AC004540.2
AC010677.2
SNX10
AC004540.2
SNX10
AC004540.2
SNX10
AC004540.2
SNX10
AC004540.2
SNX10
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