Figure S1. Related to Figure 1. Transcriptome analysis of SUV420H2 KO and SUV420H1/H2 DKO ES cells.

(**A**) Boxplot of RNA-Seq data: upregulated and downregulated genes in WT and SUV420H2 KO, and WT and SUV420H1/H2 DKO ES cells (log2 RPKM). P < 2.2e-16 for all. (**B**) GSEA of downregulated (left) and upregulated (right) DE genes in SUV420H1/H2 DKO ES cells relative to undifferentiated ES cells and differentiated embryoid bodies (EB). (**C**) DAVID gene ontology (GO) functional annotation of downregulated (left) and upregulated (right) genes between WT and SUV420H2 KO ES cells (top) and WT and SUV420H1/H2 DKO ES cells (bottom). (**D**) Word frequency map of GO terms enriched in DE genes between WT and SUV420H2 KO ES cells (top), and WT and SUV420H1/H2 DKO ES cells (bottom).

A

SUV420H2 KO DE

| Upregulated | Downregulated |

SUV420H1/H2 DKO DE

| Upregulated | Downregulated |
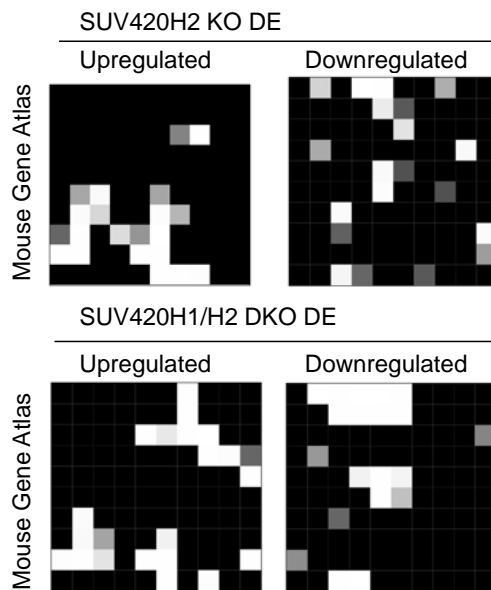
B

SUV420H2 KO DE

Upregulated		Downregulated	
Node Name	p-value	Node Name	p-value
PLACENTA	6.25E-09	EMBRYONIC_STEM_LINE_BRUCE4_P13	7.85E-08
OSTEOBLAST_DAY5	3.71E-07	EMBRYONIC_STEM_LINE_V26_2_P16	1.83E-04
MACROPHAGE_PERI_LPS_THIO_0HRS	5.91E-06	ADIPOSE_BROWN	6.53E-04
SPINAL_CORD	2.95E-05	RETINA	8.77E-04
DORSAL_ROOT_GANGLIA	4.75E-05	NUCLEUS_ACCUMBENS	1.24E-03
HYPOTHALAMUS	4.36E-05	MAST_CELLS	1.89E-03
MEF	6.75E-05	HEART	1.84E-03
OSTEOBLAST_DAY14	9.86E-05	NEURO2A	2.60E-03
OSTEOBLAST_DAY21	2.11E-04	BAF3	5.54E-03
LUNG	3.05E-04	MACROPHAGE_BONE_MARROW_24H_LPS	7.92E-03
CEREBRAL_CORTEX	4.92E-04	MEGA_ERYTHROCYTE_PROGENITOR	1.24E-02
CEREBRAL_CORTEX_PREFRONTAL	7.10E-04	B-CELLS_GL7NEGATIVE_ALUM	2.44E-02
PITUITARY	4.40E-03	OVARY	2.31E-02
NUCLEUS_ACCUMBENS	4.94E-03	RETINAL_PIGMENT_EPITHELIUM	2.83E-02
INTESTINE_SMALL	9.10E-03	PROSTATE	4.99E-02
KIDNEY	1.13E-02	LIVER	4.99E-02
HIPPOCAMPUS	1.10E-02	RAW_264_7	4.80E-02
UMBILICAL_CORD	1.33E-02	OSTEOBLAST_DAY14	4.76E-02
MACROPHAGE_PERI_LPS_THIO_7HRS	1.56E-02	OLFACTORY_BULB	4.53E-02
CEREBELLUM	1.93E-02	MIMCD-3	4.41E-02

SUV420H1/H2 DKO DE

Upregulated		Downregulated	
Node Name	p-value	Node Name	p-value
MACROPHAGE_PERI_LPS_THIO_0HRS	6.32E-27	EMBRYONIC_STEM_LINE_V26_2_P16	1.93E-87
MAST_CELLS_IGE	3.00E-15	EMBRYONIC_STEM_LINE_BRUCE4_P13	2.74E-81
MACROPHAGE_PERI_LPS_THIO_1HRS	4.87E-13	MEGA_ERYTHROCYTE_PROGENITOR	1.56E-32
MEF	8.06E-13	MIMCD-3	2.18E-19
OSTEOBLAST_DAY5	4.37E-11	NIH_3T3	2.28E-13
RETINAL_PIGMENT_EPITHELIUM	8.36E-09	NEURO2A	4.61E-10
3T3-L1	1.48E-07	BAF3	2.39E-08
MACROPHAGE_PERI_LPS_THIO_7HRS	1.30E-07	ADIPOSE_BROWN	6.95E-08
OSTEOCLASTS	9.75E-07	GRANULO_MONO_PROGENITOR	3.02E-07
SPINAL_CORD	3.47E-06	RAW_264_7	3.73E-07
OSTEOBLAST_DAY21	5.16E-06	COMMON_MYELOID_PROGENITOR	5.57E-07
DORSAL_ROOT_GANGLIA	4.89E-06	HEART	3.20E-06
MAST_CELLS	1.29E-05	STEM_CELLS__HSC	8.43E-04
CEREBRAL_CORTEX	1.58E-05	LIVER	1.07E-02
HYPOTHALAMUS	2.37E-05	SKELETAL_MUSCLE	1.26E-02
PLACENTA	5.88E-05	KIDNEY	6.40E-02
MACROPHAGE_BONE_MARROW_24H_LPS	1.02E-04	B-CELLS_GL7NEGATIVE_ALUM	1.05E-01
MIN6	1.16E-04	DORSAL_ROOT_GANGLIA	1.16E-01
OLFACTORY_BULB	4.03E-04	SPLEEN	1.24E-01
NK_CELLS	6.82E-04	NUCLEUS_ACCUMBENS	1.56E-01

Figure S2. Related to Figure 1. Gene ontology functional annotation of RNA-Seq data from SUV420H2 KO and SUV420H1/H2 DKO ES cells.

(**A**) Network2Canvas (Tan et al., 2013)analyses of upregulated (left) and downregulated (right) DE genes between WT and SUV420H2 KO ES cells (top), and WT and SUV420H1/H2 DKO ES cells (bottom). In each canvas, each node (square) represents a gene list (e.g. WT and H2 KO, WT and H1/H2 DKO DE genes in ES cells) associated with a functional term in a gene-set library (mouse gene atlas). The terms are organized on the canvas based on the similarity of their gene-set content. The brightness (white) of each node is determined by its p value. (**B**) List of significantly enriched GO terms enriched in upregulated and downregulated genes between WT and SUV420H2 KO ES cells, and WT and SUV420H1/H2 DKO ES cells.
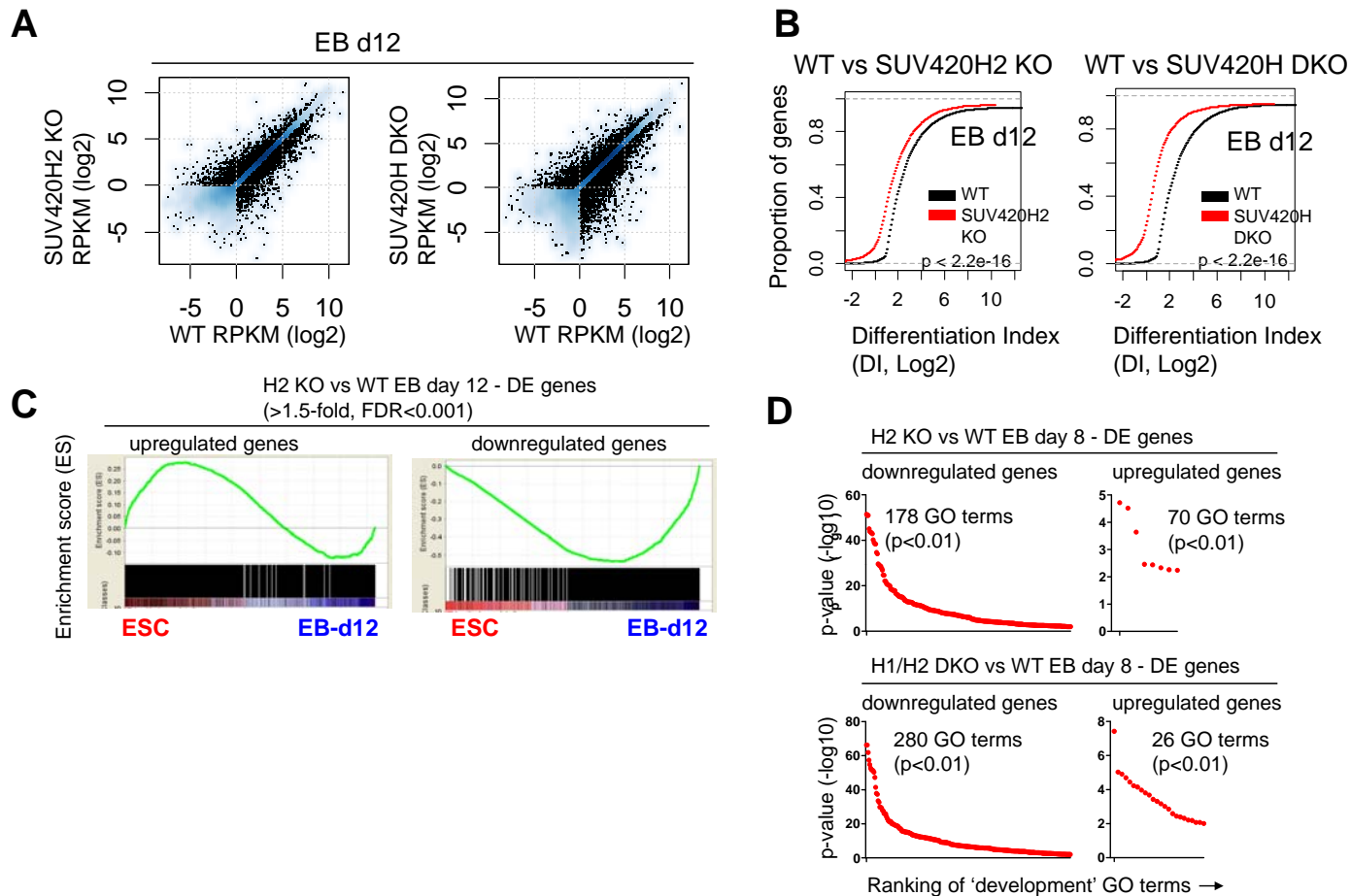
Figure S3. Related to Figure 1. RNA-Seq analysis of differentiated SUV420H2 KO and SUV420H1/H2 DKO EBs.

(**A**) RNA-Seq analysis of WT, SUV420H2 KO, and SUV420H1/H2 DKO ES cells differentiated for 12 days in the absence of LIF. Scatter plots show log2 adjusted differentially expressed (DE) genes between WT and SUV420H2 KO day 12 EBs (left) and between WT and SUV420H1/H2 DKO day 12 EBs (right) (fold-change > 1.5, RPKM>1, and FDR<0.001). (**B**) ECDF function for the differentiation index (DI) of genes in WT (black) and SUV420H2 KO (red) day 12 EBs (left), and WT (black) and SUV420H1/H2 DKO (red) day 12 EBs (right). Y-axis shows the percentage of regions that exhibit a DI less than the value specified by the x-axis. A line shifted to the left means a systematic decrease in the differentiation index. P-value for all <2.2E-16 (Kolmogorov-Smirnov test). Note the decreased DI for genes in SUV420H2 KO and SUV420H1/H2 DKO day 12 EBs. (**C**) GSEA of upregulated (left) and downregulated (right) DE genes in SUV420H2 KO day 12 EBs relative to undifferentiated ES cells and differentiated EBs. (**D**) DAVID GO functional annotation of downregulated (left) and upregulated (right) genes between WT and SUV420H2 KO day 12 EBs (top) and WT and SUV420H1/H2 DKO day 12 EBs (bottom).

A Upregulated genes
(SUV420H2 KO vs WT ESC,
1.5 FC, FDR<0.01)

C

B SUV420H2 KO vs WT ESC
(1.5 FC, FDR<0.05)

D H4K20me3 density
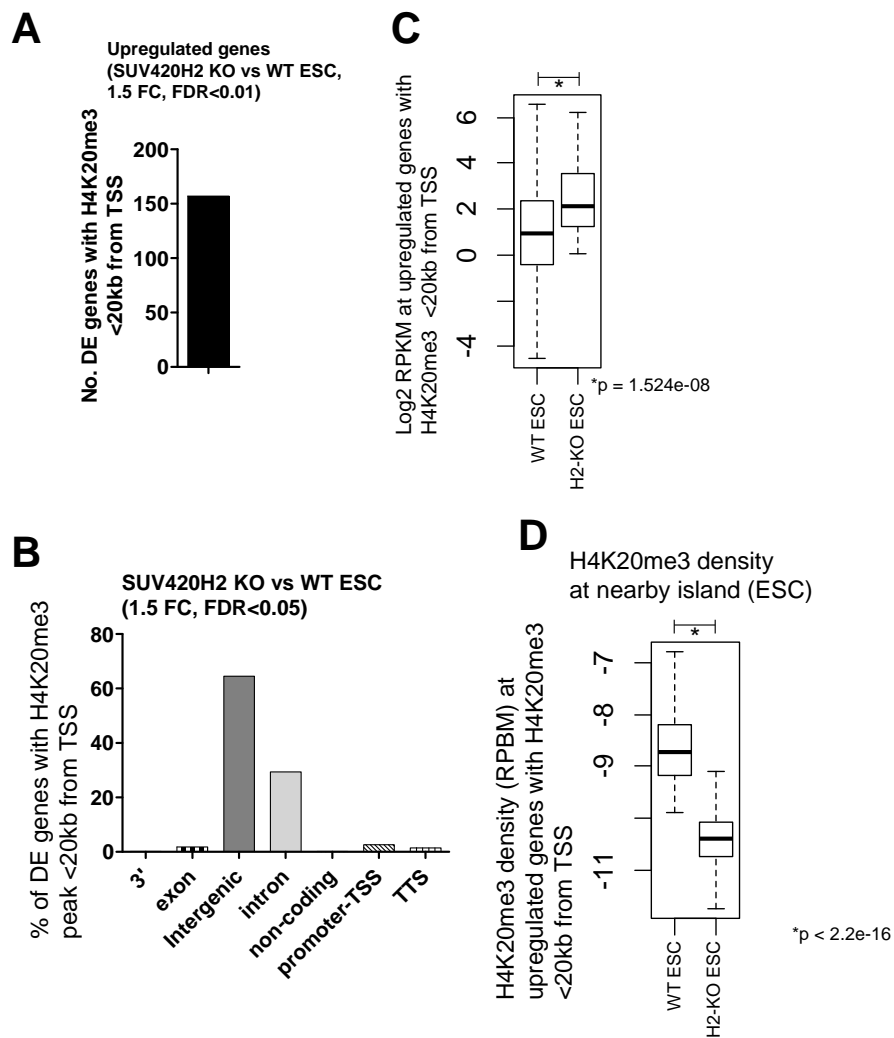at nearby island (ESC)

*p = 1.524e-08

*p < 2.2e-16

Figure S4. Related to Figure 2. Upregulated genes in SUV420H2 KO and SUV420H1/H2 DKO ES cells is associated with LTR/ERV elements and decreased H4K20me3.

Loss of SUV420H2-dependent silencing of LTR/ERV elements influences the expression of nearby genes. **(A)** Number of differentially expressed (DE) genes between WT and SUV420H2 KO ES cells (fold-change>1.5, FDR < 0.01). **(B)** Annotation of LTR/ERV elements nearby DE genes in WT and SUV420H2 KO ES using HOMER software[69]. **(C)** Expression of upregulated genes between WT and SUV420H2 KO ES cells (p = 1.524e-8) (log2 RPKM). **(D)** Density of H4K20me3 marks nearby LTR/ERV element and within 20 kb of TSS of DE genes.
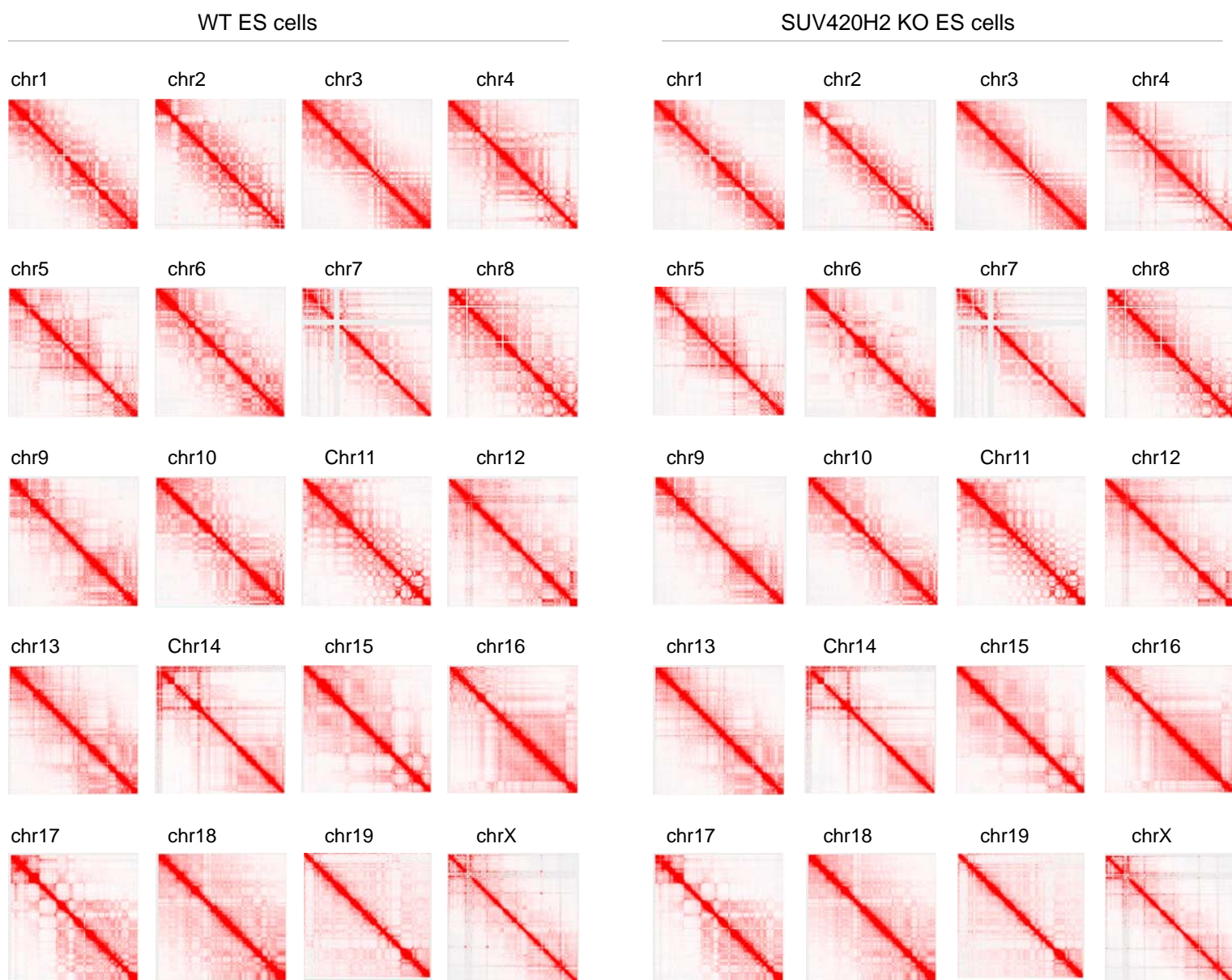
WT ES cells

SUV420H2 KO ES cells

Figure S5. Related to Figure 3. Hi-C interaction matrices in WT and SUV420H2 KO ES cells.

Contact matrices showing observed normalized Hi-C signal from WT and SUV420H2 KO ES cells for all chromosomes at 250 kb resolution as evaluated using Juicer and Juicebox(Durand et al., 2016a; Durand et al., 2016b).
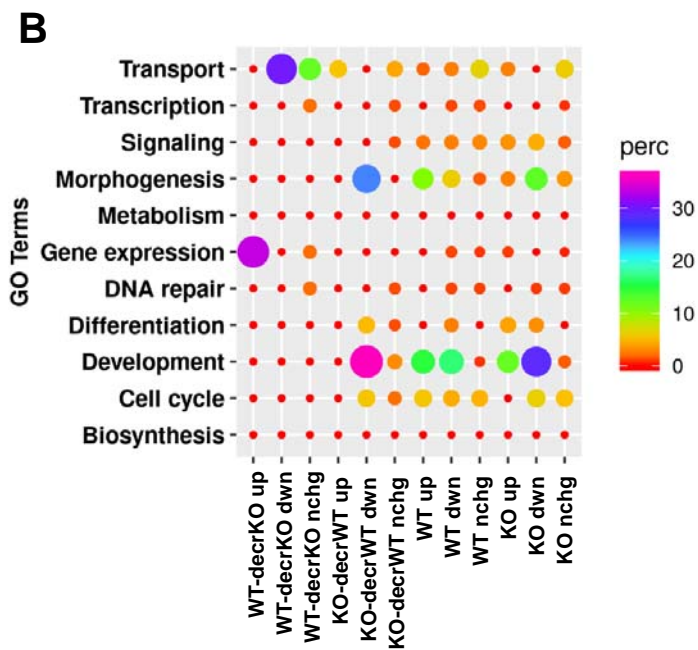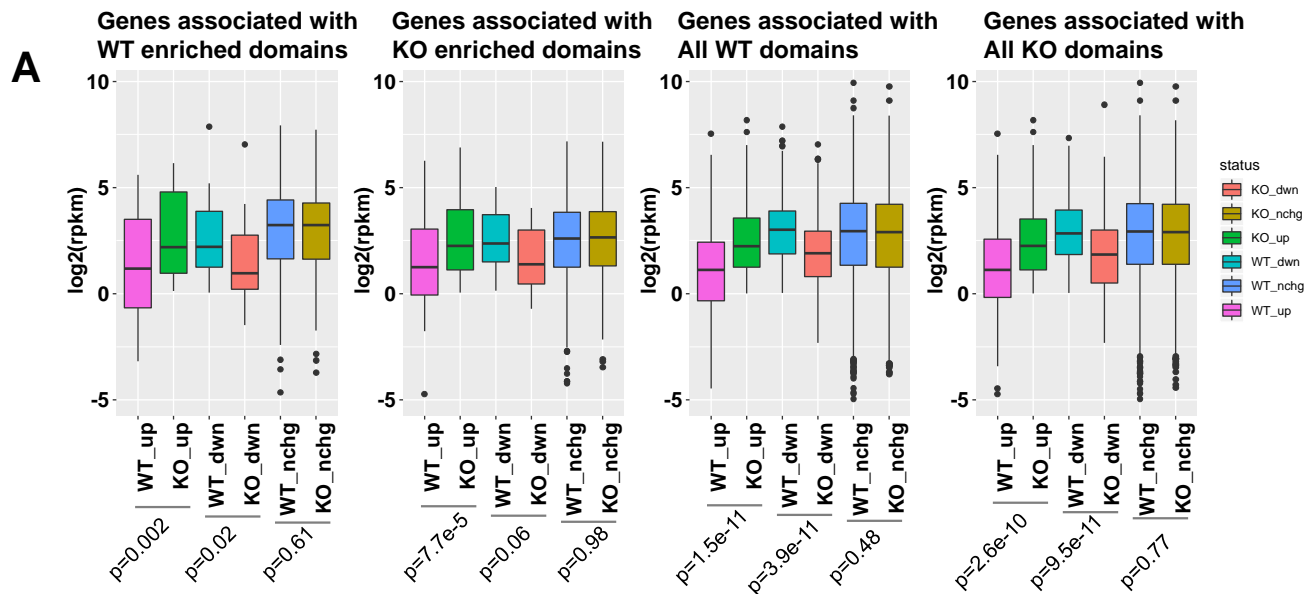
Figure S6. Related to Figure 3. Expression and GO enrichment analysis of genes nearby differential domain boundaries.

(**A**) Boxplots showing expression of genes nearby WT ES cell enriched domains (within 10 kb), SUV420H2 KO ES cell enriched domains, all WT ES cells domains, and all SUV420H2 KO ES cell domains. Up: genes upregulated in SUV420H2 KO ES cells; dwn: genes downregulated in SUV420H2 KO ES cells; nchg: genes with no change in expression between WT and SUV420H2 KO ES cells. (**B**) Bubble plot shows enrichment of top biological process GO terms identified from DAVID annotation of genes nearby chromatin domains and juicer arrowhead TADCompare differential domain boundaries. WT-decrKO: domains enriched in WT ES cells but decreased in SUV420H2 KO ES cells; KO-decrWT: domains enriched in SUV420H2 KO ES cells but decreased in WT ES cells; WT: all domains in WT ES cells; KO: all domains in SUV420H2 KO ES cells; up: genes upregulated in SUV420H2 KO ES cells; dwn: genes downregulated in SUV420H2 KO ES cells; nchg: genes with no change in expression between WT and SUV420H2 KO ES cells.
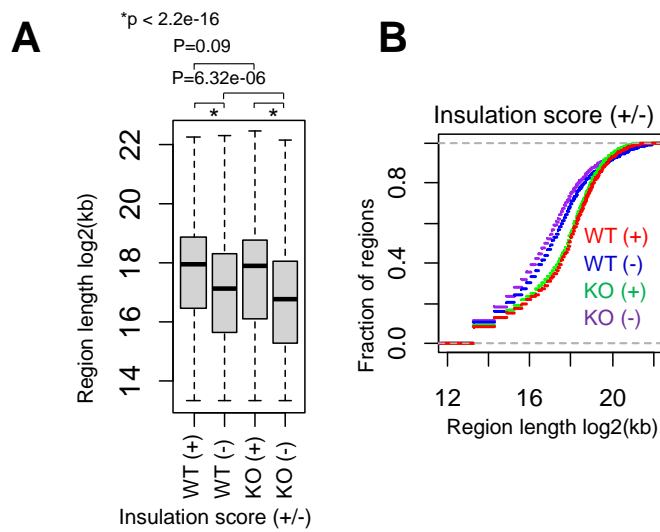
Figure S7. Related to Figure 5. Length of genomic regions with positive or negative insulation scores.

(**A**) Boxplot showing length of regions with positive or negative insulation scores in WT and SUV420H2 KO ES cells. K-S test p-values are shown. (**B**) Empirical cumulative distribution function (ECDF) for the length of regions with positive or negative insulation scores in WT and SUV420H2 KO ES cells.

Table S1. Statistics of Hi-C libraries.

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