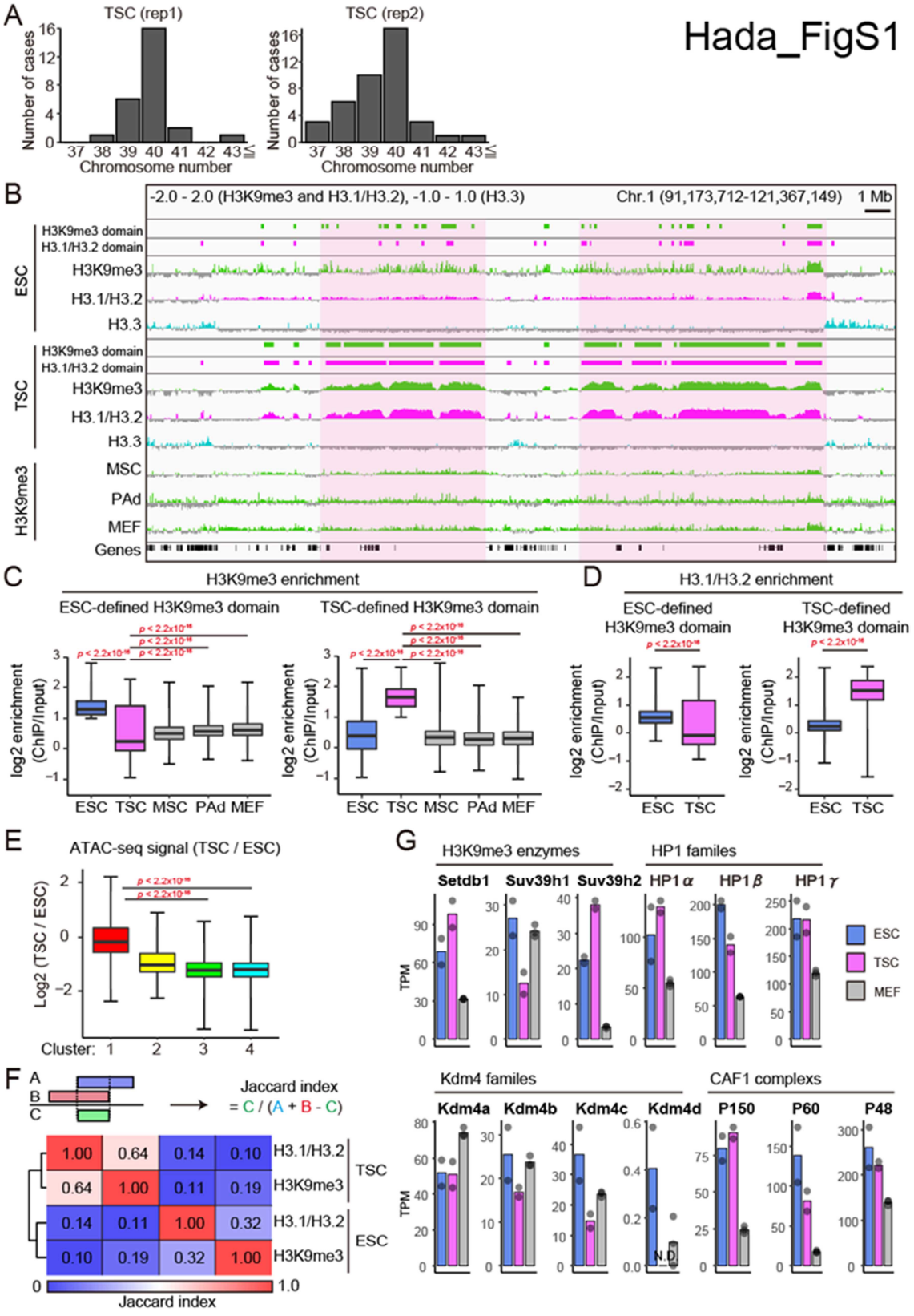

Hada_FigS1
A
TSC (rep1)
TSC (rep2)
Number of cases
Chromosome number
B
-2.0 - 2.0 (H3K9me3 and H3.1/H3.2), -1.0 - 1.0 (H3.3)
Chr.1 (91,173,712-121,367,149)
1 Mb
ESC
TSC
H3K9me3
H3K9me3 domain
H3.1/H3.2 domain
H3.1/H3.2
H3.3
MSC
PAd
MEF
Genes
C
H3K9me3 enrichment
ESC-defined H3K9me3 domain
TSC-defined H3K9me3 domain
log2 enrichment (ChIP/Input)
ESC TSC MSC PAd MEF
D
H3.1/H3.2 enrichment
E
ATAC-seq signal (TSC / ESC)
Log2 (TSC / ESC)
Cluster: 1 2 3 4
F
Jaccard index
= C / (A + B - C)
1.00 0.64 0.14 0.10
0.64 1.00 0.11 0.19
0.14 0.11 1.00 0.32
0.10 0.19 0.32 1.00
Jaccard index
G
H3K9me3 enzymes
HP1 familes
Setdb1 Suv39h1 Suv39h2 HP1α HP1β HP1γ
Kdm4 familes
CAF1 complexes
Kdm4a Kdm4b Kdm4c Kdm4d P150 P60 P48
TPM
N.D
ESC
TSC
MEF

Figure S1. Large H3K9me3 domains in TSCs. Related to Figure 1

(A) Histograms showing the distribution of TSC chromosome numbers in two TSC lines used in this study.

(B) IGV snapshot of ChIP-seq data shown by $\log_2$-transformed enrichment (ChIP/Input). Extension of Fig. 1B with the inclusion of data from other cell types. Light magenta areas correspond to TSC-defined large domains for H3K9me3 and H3.1/H3.2. MSC, 10T1/2 mesenchymal cells; PAd, 3T3-L1 preadipocytes; MEF, mouse embryonic fibroblasts.

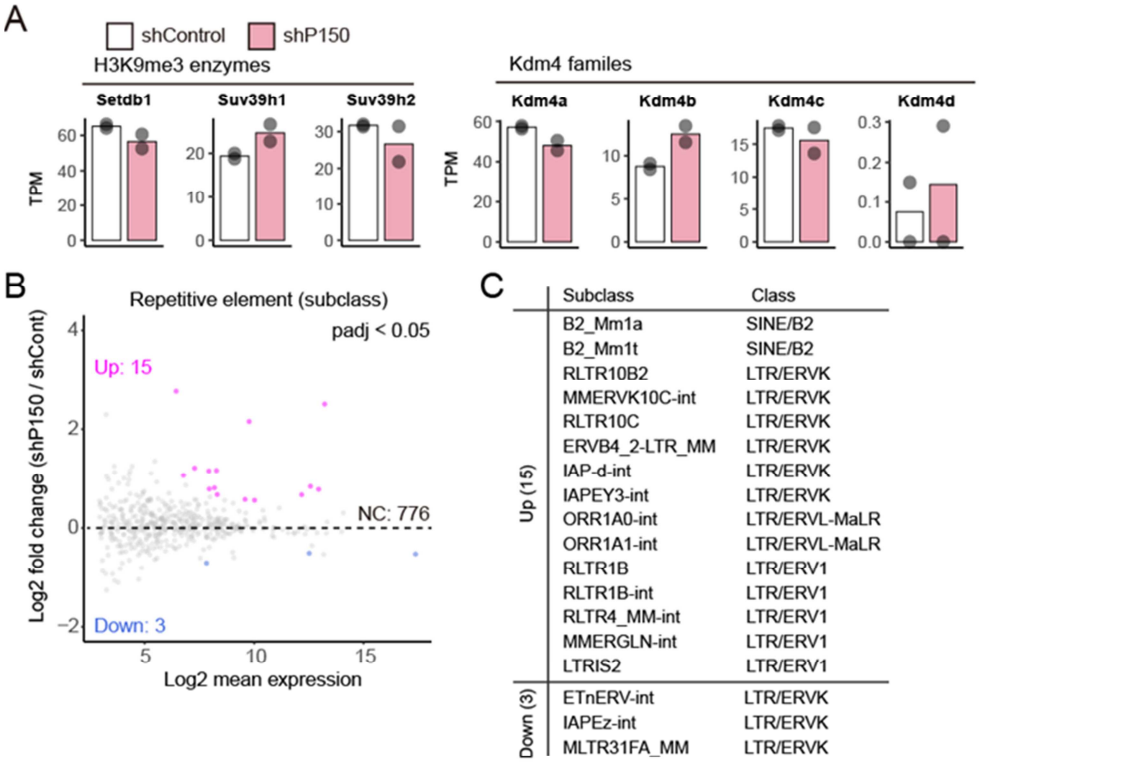
(C, D) Boxplots showing the H3K9me3 (B) or H3.1/H3.2 (C) enrichment within ESC-defined (left panel) or TSCs-defined (right panel) H3K9me3 domains.

(E) Boxplot showing the $\log_2$-transformed ratio of ATAC-seq intensity (ESC/TSC) within each cluster. *P* values from Wilcoxon rank-sum tests.

(F) Heatmap showing Jaccard indices of the H3K9me3 and H3.1/H3.2 domains defined in ESCs or TSCs. This shows the hierarchical clustering results. There is a stronger correlation between the H3K9me3 and H3.1/H3.2 domains in TSCs than in ESCs (0.64 vs. 0.32, Jaccard index).

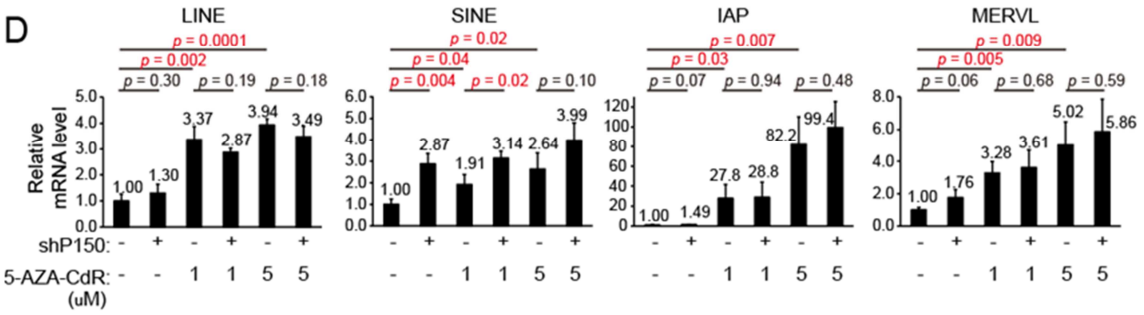
(G) Gene expression levels of H3K9me3-methytransferase enzymes, HP1 families, Kdm4 families, and the components of the CAF1 complex in ESCs, TSCs, and MEFs. Each dot represents a replicate RNA-seq result.

Hada_FigS2



C

	Subclass	Class
Up (15)	B2_Mm1a	SINE/B2
	B2_Mm1t	SINE/B2
	RLTR10B2	LTR/ERVK
	MMERVK10C-int	LTR/ERVK
	RLTR10C	LTR/ERVK
	ERVB4_2-LTR_MM	LTR/ERVK
	IAP-d-int	LTR/ERVK
	IAPEY3-int	LTR/ERVK
	ORR1A0-int	LTR/ERVL-MaLR
	ORR1A1-int	LTR/ERVL-MaLR
	RLTR1B	LTR/ERV1
	RLTR1B-int	LTR/ERV1
	RLTR4_MM-int	LTR/ERV1
	MMERGLN-int	LTR/ERV1
	LTRIS2	LTR/ERV1
Down (3)	ETnERV-int	LTR/ERVK
	IAPEz-int	LTR/ERVK
	MLTR31FA_MM	LTR/ERVK



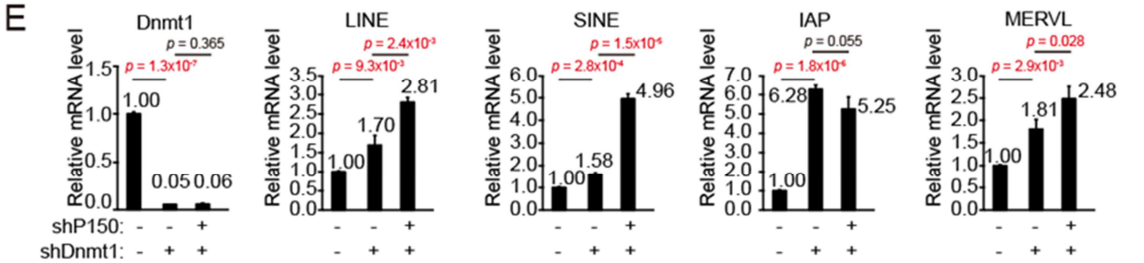


Figure S2. Knockdown of P150 does not derepress the expressions of repetitive elements. Related to Figure 4.

(A) Gene expression level of H3K9me3 enzyme and components of the CAF1 complex in control and shP150-expressing TSCs. Each dot represents a replicate RNA-seq result.

(B) Scatterplot showing the differentially expressed repetitive elements. Each dot corresponds to a subclass in the classes of LINE/L1, ERV1, ERVK, ERVL, and SINE/Alu. Genes with padj values (the threshold P) < 0.05 were extracted as DEGs.

(C) List of upregulated and downregulated repetitive elements.

(D) RT-qPCR analysis of expressions of LINE, SINE, IAP, and MERVL in 5-AZA-CdR-treated TSCs. Targets of shRNA and the concentration of 5-AZA-CdR are shown. The control shRNA result without 5-AZA-CdR treatment is set as 1.0.

(E) RT-qPCR analysis of expressions of Dnmt1, LINE, SINE, IAP, and MERVL in Dnmt1-knockdown TSCs. Knockdown of P150 or Dnmt1 was introduced by adding DOX to the culture medium for 3 days. Data are presented as the mean ± S.D. the control shRNA result without shDnmt1 is set as 1.0. P values are from two-tailed, unpaired Student's t tests.

Hada_FigS3

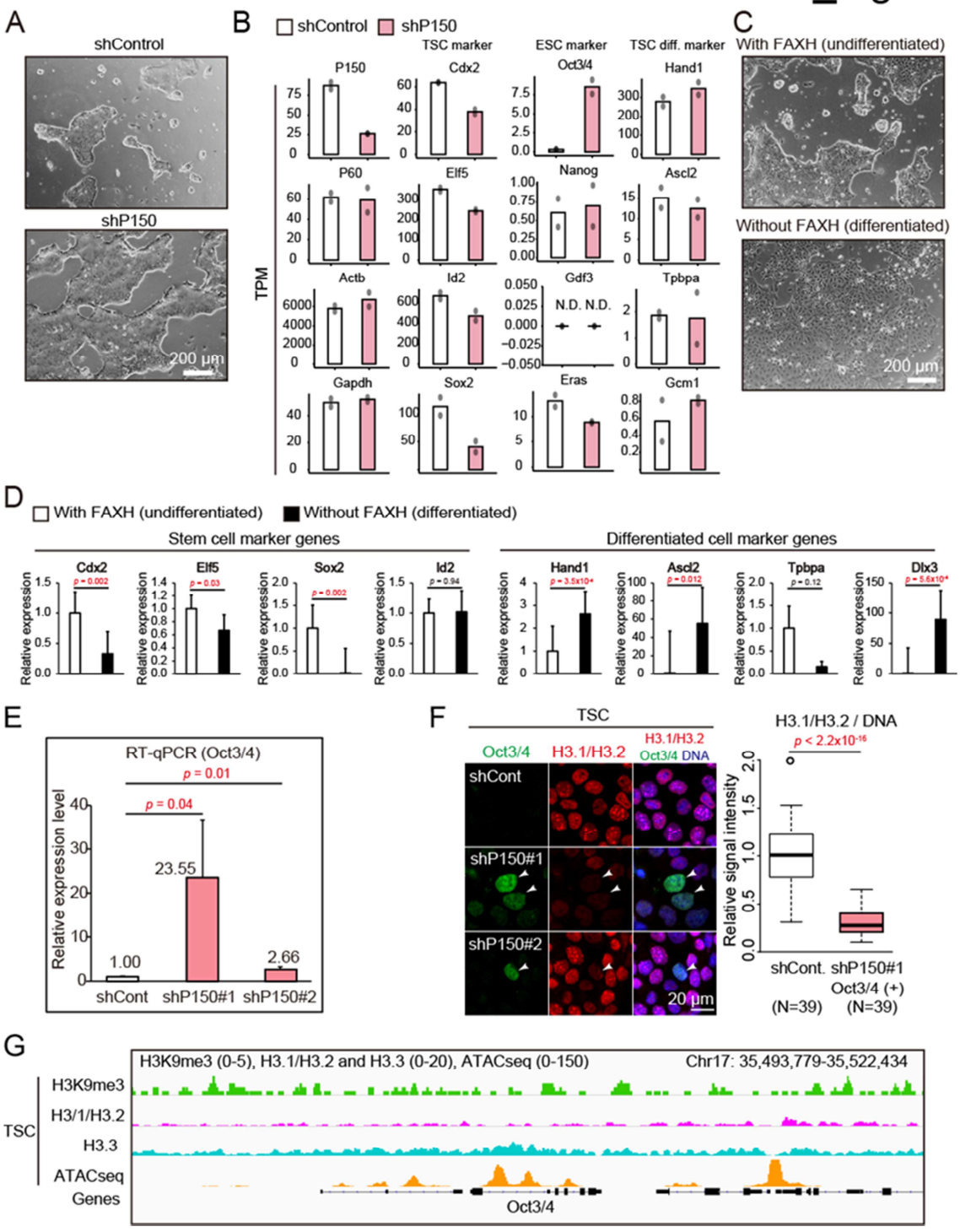

A
shControl
shP150
200 μm
B
shControl
shP150
TSC marker
ESC marker
TSC diff. marker
P150
Cdx2
Oct3/4
Hand1
P60
Elf5
Nanog
Ascl2
Actb
Id2
Gdf3
Tpbpa
N.D. N.D.
Gapdh
Sox2
Eras
Gcm1
TPM
C
With FAXH (undifferentiated)
Without FAXH (differentiated)
200 μm
D
With FAXH (undifferentiated)
Without FAXH (differentiated)
Stem cell marker genes
Differentiated cell marker genes
Cdx2
Elf5
Sox2
Id2
Hand1
Ascl2
Tpbpa
Dlx3
Relative expression
E
RT-qPCR (Oct3/4)
p = 0.01
p = 0.04
Relative expression level
23.55
1.00
2.66
shCont
shP150#1
shP150#2
F
TSC
Oct3/4
H3.1/H3.2
H3.1/H3.2
Oct3/4 DNA
shCont
shP150#1
shP150#2
20 μm
H3.1/H3.2 / DNA
p < 2.2x10-16
Relative signal intensity
shCont.
shP150#1
Oct3/4 (+)
(N=39)
(N=39)
G
H3K9me3 (0-5), H3.1/H3.2 and H3.3 (0-20), ATACseq (0-150)
Chr17: 35,493,779-35,522,434
TSC
H3K9me3
H3/1/H3.2
H3.3
ATACseq
Genes
Oct3/4

Figure S3. P150 knockdown causes repression of TSC marker genes and overexpression of Oct3/4 in TSCs. Related to Figure 4.

(A) Representative images of control and P150-knockdown TSCs.

(B) Expression levels of marker genes for TSCs, ESCs, and differentiated TSCs in TSCs with or without P150 knockdown. Dots correspond to the levels in replicate RNA-seq experiments. N.D. not detected.

(C) Representative images of undifferentiated and differentiated TSCs. F, FGF2; A, activin A; X, XAV939; H, heparin.

(D) The expression levels of marker genes for TSCs, ESCs, and differentiated TSCs analyzed by RT-qPCR.

(E) Effect of P150 knockdown on the Oct3/4 expression in TSCs analyzed by RT‐qPCR. Data are presented as the mean ± S.D. (three replicates). *P* values are from two-tailed, unpaired Student's *t* tests

(F) Immunostaining of P150 knockdown TSCs for Oct3/4 and H3.1/H3.2. The left panel shows representative images. Arrowheads highlight Oct3/4-expressing cells. The right panel shows the relative fluorescent intensity of H3.1/H3.2 in Oct3/4-positive P150 knockdown TSCs. *P* values are from two-tailed, unpaired Student's *t* tests.

(G) IGV snapshots of the *Oct3/4* locus in TSCs. ChIP-seq data and ATAC-seq data are visualized through the IGV genome browser. Note that there is no H3.1/H3.2 enrichment on the *Oct3/4* promoter in TSCs, suggesting that overexpression of Oct3/4 in TSCs was an indirect effect of P150 knockdown.

Hada_FigS4

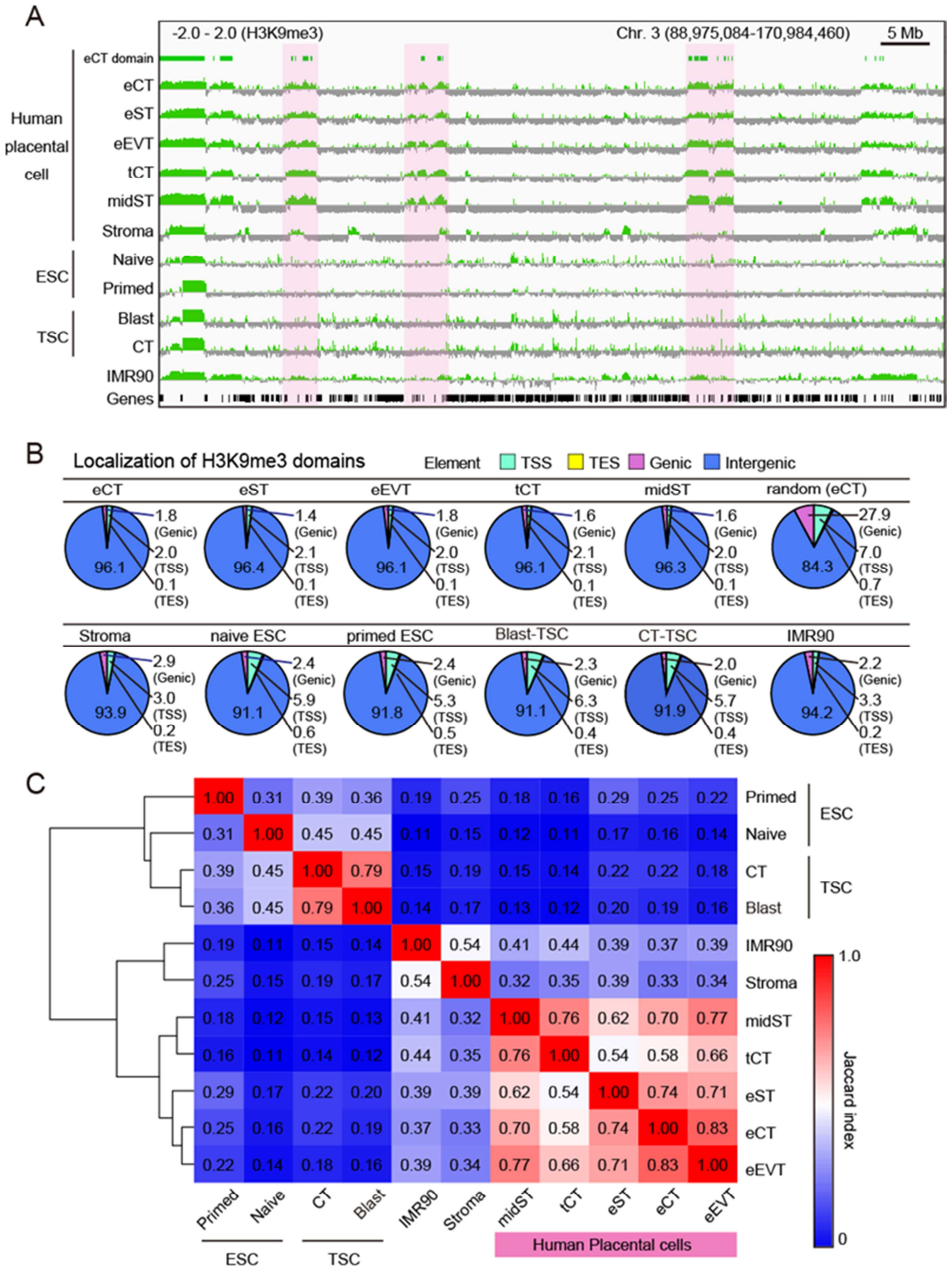

A
-2.0 - 2.0 (H3K9me3)
Chr. 3 (88,975,084-170,984,460)
5 Mb
eCT domain
eCT
eST
eEVT
tCT
midST
Stroma
Human placental cell
Naive
Primed
ESC
Blast
CT
TSC
IMR90
Genes
B
Localization of H3K9me3 domains
Element
TSS
TES
Genic
Intergenic
eCT
eST
eEVT
tCT
midST
random (eCT)
96.1
1.8 (Genic)
2.0 (TSS)
0.1 (TES)
96.4
1.4 (Genic)
2.1 (TSS)
0.1 (TES)
96.1
1.8 (Genic)
2.0 (TSS)
0.1 (TES)
96.1
1.6 (Genic)
2.1 (TSS)
0.1 (TES)
96.3
1.6 (Genic)
2.0 (TSS)
0.1 (TES)
84.3
27.9 (Genic)
7.0 (TSS)
0.7 (TES)
Stroma
naive ESC
primed ESC
Blast-TSC
CT-TSC
IMR90
93.9
2.9 (Genic)
3.0 (TSS)
0.2 (TES)
91.1
2.4 (Genic)
5.9 (TSS)
0.6 (TES)
91.8
2.4 (Genic)
5.3 (TSS)
0.5 (TES)
91.1
2.3 (Genic)
6.3 (TSS)
0.4 (TES)
91.9
2.0 (Genic)
5.7 (TSS)
0.4 (TES)
94.2
2.2 (Genic)
3.3 (TSS)
0.2 (TES)
C
1.00 0.31 0.39 0.36 0.19 0.25 0.18 0.16 0.29 0.25 0.22 Primed
0.31 1.00 0.45 0.45 0.11 0.15 0.12 0.11 0.17 0.16 0.14 Naive
0.39 0.45 1.00 0.79 0.15 0.19 0.15 0.14 0.22 0.22 0.18 CT
0.36 0.45 0.79 1.00 0.14 0.17 0.13 0.12 0.20 0.19 0.16 Blast
0.19 0.11 0.15 0.14 1.00 0.54 0.41 0.44 0.39 0.37 0.39 IMR90
0.25 0.15 0.19 0.17 0.54 1.00 0.32 0.35 0.39 0.33 0.34 Stroma
0.18 0.12 0.15 0.13 0.41 0.32 1.00 0.76 0.62 0.70 0.77 midST
0.16 0.11 0.14 0.12 0.44 0.35 0.76 1.00 0.54 0.58 0.66 tCT
0.29 0.17 0.22 0.20 0.39 0.39 0.62 0.54 1.00 0.74 0.71 eST
0.25 0.16 0.22 0.19 0.37 0.33 0.70 0.58 0.74 1.00 0.83 eCT
0.22 0.14 0.18 0.16 0.39 0.34 0.77 0.66 0.71 0.83 1.00 eEVT
ESC
TSC
TSC
ESC
Primed
Naive
CT
Blast
IMR90
Stroma
midST
tCT
eST
eCT
eEVT
Human Placental cells
1.0
Jaccard index
0

Figure S4. Human placental cells also possess lineage-specific H3K9me3 domains.

(A) IGV snapshots showing H3K9me3 enrichment specific for human placental cells (light magenta areas). CT, cytotrophoblast; ST, syncytiotrophoblast; EVT, extravillous cytotrophoblast; eCT, early CT; eST, early ST; eEVT, early EVT; tCT, term CT; midST, middle ST; blast-TSC, TSC line established from blastocyst cells; CT-TSC, TSC line established from CT cells.

(B) Distributions of the H3K9me3 domains in human placental cells. H3K9me3 domains are preferentially distributed in intergenic regions, while randomly mapped eCT domains are not.

(C) Heatmap showing Jaccard indices and hierarchal clustering of the H3K9me3 domains in human placental cells. The H3K9me3 domains were defined for each cell and subjected to the calculation for the Jaccard index. Note that the different placental cell types share similar H3K9me3 domain patterns with each other, but not with stroma cells, IMR90 cells, TSCs, or ESCs.

Hada_FigS5

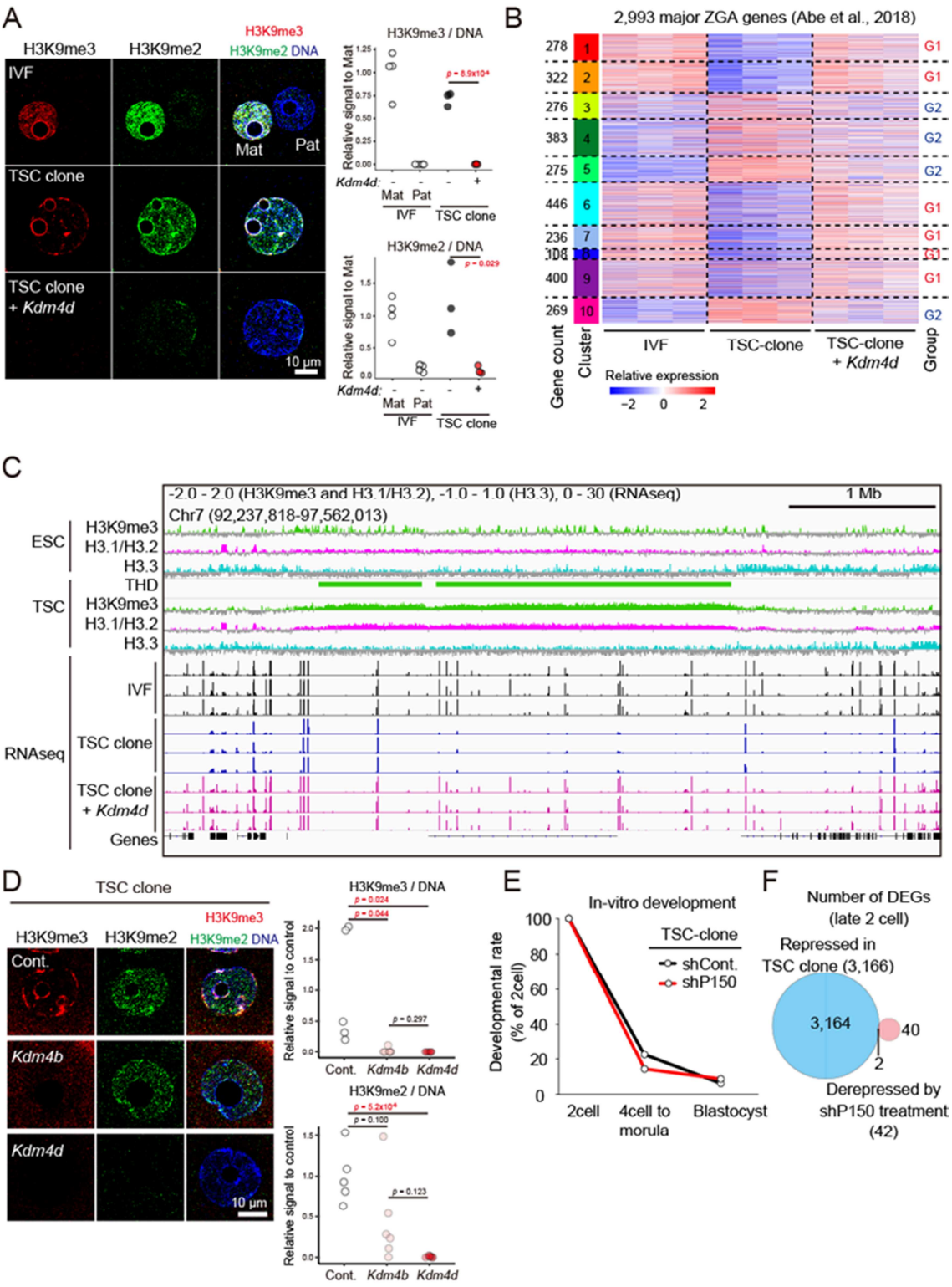

A
H3K9me3
H3K9me2
H3K9me2 DNA
IVF
TSC clone
TSC clone + Kdm4d
Mat
Pat
10 μm
H3K9me3 / DNA
Relative signal to Mat
p = 8.9x10-4
Kdm4d:
Mat Pat
IVF
TSC clone
H3K9me2 / DNA
p = 0.029
B
2,993 major ZGA genes (Abe et al., 2018)
278
322
276
383
275
446
236
400
269
G1
G2
Gene count
Cluster
Group
IVF
TSC-clone
TSC-clone + Kdm4d
Relative expression
−2 0 2
C
-2.0 - 2.0 (H3K9me3 and H3.1/H3.2), -1.0 - 1.0 (H3.3), 0 - 30 (RNAseq)
1 Mb
Chr7 (92,237,818-97,562,013)
ESC
TSC
RNAseq
H3K9me3
H3.1/H3.2
H3.3
THD
IVF
TSC clone
TSC clone + Kdm4d
Genes
D
TSC clone
H3K9me3
H3K9me2
H3K9me2 DNA
Cont.
Kdm4b
Kdm4d
10 μm
H3K9me3 / DNA
p = 0.024
p = 0.044
p = 0.297
Relative signal to control
Cont. Kdm4b Kdm4d
H3K9me2 / DNA
p = 5.2x10-4
p = 0.100
p = 0.123
E
In-vitro development
TSC-clone
shCont.
shP150
Developmental rate (% of 2cell)
2cell
4cell to morula
Blastocyst
F
Number of DEGs (late 2 cell)
Repressed in TSC clone (3,166)
3,164
40
2
Derepressed by shP150 treatment (42)

Figure S5. Analyses of TSC-cloned embryos by immunostaining, RNA-seq, and *in vitro* culture. Related to Figure 6.

(A) Immunostaining of TSC-derived or IVF-derived 1-cell embryos treated with *Kdm4d* mRNA. The left panel shows representative images of the nuclei of embryos double-immunostained for H3K9me3 and H3K9me2. The right panel shows the corresponding relative fluorescent intensities normalized against the DNA signal.

(B) Heatmap showing the normalized gene expression levels of major ZGA genes. The list of 2,993 major ZGA genes was obtained from Abe et al., 2018. Group 1 (G1): genes repressed in TSC-derived embryos. Group 2 (G2): genes hyperactivated in TSC-derived embryos.

(C) IGV snapshot of ChIP-seq data shown by $\log_2$-transformed enrichment (upper) and RNA-seq data (lower).

(D) Immunostaining of TSC-cloned 1-cell embryos treated with *Kdm4b* mRNA. The left panel shows representative images of the nuclei of embryos double-immunostained for H3K9me3 and H3K9me2. The right panel shows the corresponding relative fluorescent intensities normalized against the DNA signal. In the *Kdm4b*-treated cloned embryos, H3K9me2 was demethylated to various extents, but its average level was not significantly different from that of the control non-treated TSC-cloned embryos.

(E) *In vitro* development of cloned embryos derived from TSCs with or without P150 knockdown.

(F) Venn diagram showing the overlap between the genes that failed to be activated in TSC-cloned embryos and derepressed genes in P150-knockdown TSC-cloned embryos at the late 2-cell stage. Genes with padj values < 0.01 and fold change > 1.5 were classed as DEGs.