Supplementary Materials

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Supplemental Figure Legends

Supplemental Figure 1. Stimulation of G9a activity by nucleosomes that are pre-methylated at H3K9. (*A, B*) HMT activity assay at nucleosomes flanked by nucleosomes pre-modified at H3K9 with wild-type G9a or its catalytic mutant. (*C*) G9a HMT activity assay at H3K27A-containing nucleosomes flanked by nucleosomes pre-modified at H3K9. (*D*) G9a HMT activity assay at nucleosomes flanked by nucleosomes pre-modified at H3K27. (*E*) G9a 3A mutant HMT activity assay at nucleosomes flanked by nucleosomes pre-modified at H3K9. (1 μg of recombinant enzymes and 1 μg of recombinant oligonucleosomes were used in the assay.)

Supplemental Figure 2. ITC experiments showing titration of H3K9 mono-, di- or tri- methylated peptides with the ankyrin repeat domains of wild-type GLP, GLP 3A, wild-type G9a or G9a 3A.

Supplemental Figure 3. Stimulation of G9a activities by nucleosomes pre-methylated at H3K9 is a *cis* event. (*A*) HMT assay of G9a at nucleosomes exist *in cis* and in *trans* with pre-modified nucleosomes. The untagged unmodified histones exist *in cis* with pre-modified nucleosomes. (*B, C*) HMT assay of G9a (*B*) and GLP (*C*) at nucleosomes exist *in cis* and in *trans* with pre-modified nucleosomes. The Flag-tagged unmodified histones exist *in cis* with pre-modified nucleosomes. (*D*) Methylated H3K9 peptides did not stimulate the HMT activity of G9a. (1 μg of recombinant enzymes, 0.5 μg of peptides and 1 μg of recombinant oligonucleosomes were used in the assay.)

Supplemental Figure 4. Targeting vector design for G9a 3A and GLP 3A mutants, and cDNA sequencing results. cDNA fragments covering the targeted vector were amplified and cloned. 24 clones were randomly picked and sent for sequencing. The number of clones corresponding to wild-type or 3A mutant alleles were counted and summarized in the inset.

Supplemental Figure 5. Phenotypes of GLP$^{M/M}$ mice. (*A*) Statistics of G9a$^{+/M}$ intercross showed Medelian ratio of the three genotypes. (*B*) The rare, survived GLP$^{M/M}$ mice displayed severe growth retardation at indicated time. (*C*) Gross weight of littermate embryos at given developmental stages. Error bars represent the Standard Error. (*D*) Alzarin red staining of skulls of GLP$^{+/+}$ and GLP$^{M/M}$ at indicated stages. (*E*) Alzarin red staining of adult GLP$^{+/+}$ and GLP$^{M/M}$ mice skulls. Note the bone loss at metopic suture (arrow) and bent nose defects (arrowhead). (*F*) Photos showing malocclusion of adult GLP$^{M/M}$ survived mice.

Supplemental Figure 6. Analysis of H3K9me2 levels in ES cells and tissues. (*A*) Karyotype of WT and GLP 3A ES cells. (*B*) RT-qPCR for *GLP* expression in WT and GLP 3A ES cells. (*C*) Western blots for H3K9me2 in ESCs and ESCs treated with 0.5 μM UNC0638, an inhibitor of G9a and GLP. (*D*) Western blots for H3K9me2 in livers (*left*) or brains (*right*) from multiple pairs of neonatal mice. (*E*) MA-plot of H3K9me2 fold changes for all 10-kb genomic windows between GLP 3A and wild-type ESCs (*left*) or livers (*right*).

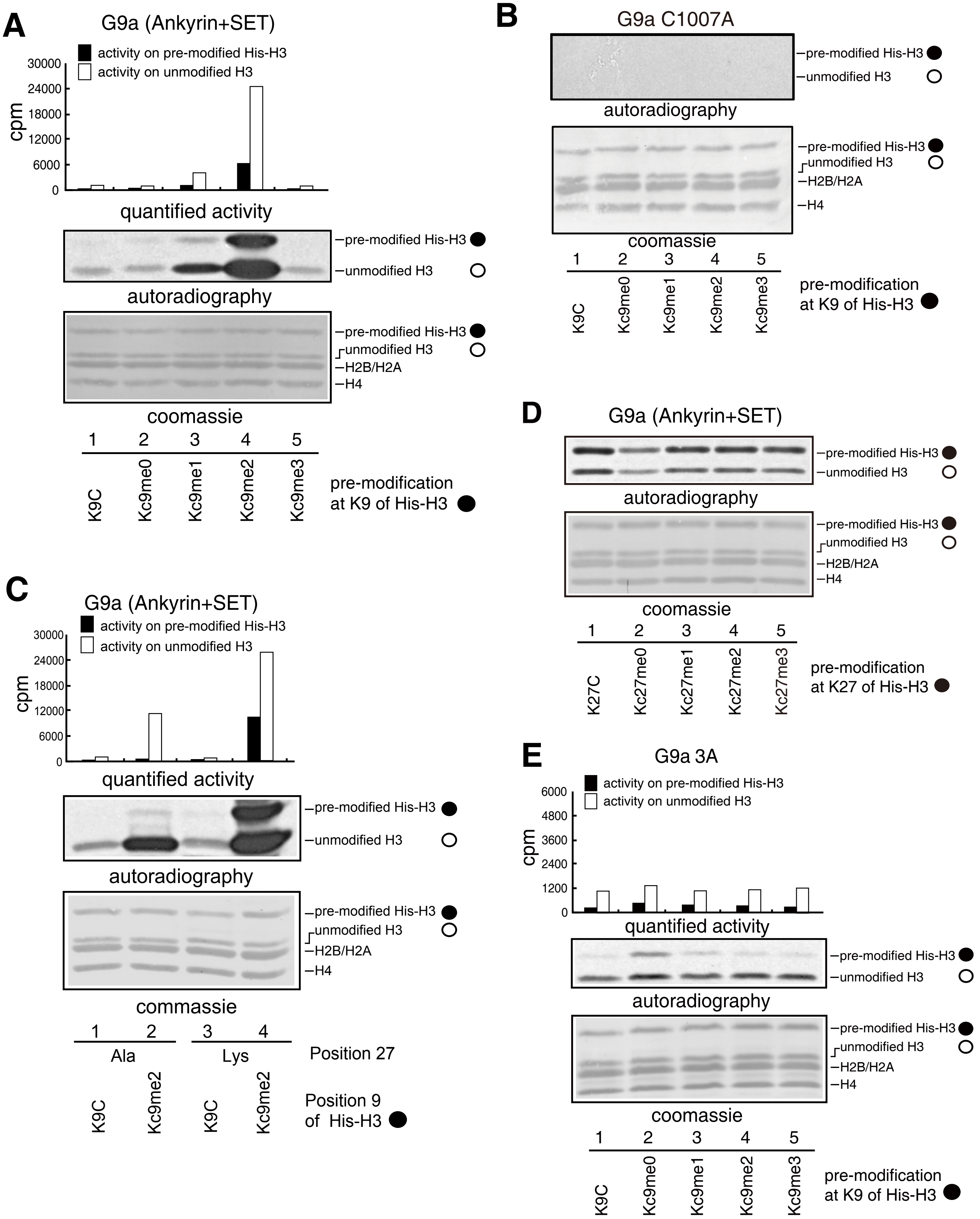
Supplemental Figure 7. Defective H3K9me2 establishment and inefficient gene repression during ES cell differentiation. (*A*) RT-qPCR showing inefficient repression of *Oct4* in a second line of GLP 3A ES cells (line 1-7 and 1-6, wild-type and GLP 3A, respectively). (*B*) ChIP-qPCR results showing H3K9me2 occupancy at indicated loci in a second line of GLP 3A ES cells after RA treatment. (*C*) ChIP-qPCR results showing H3K9me2 occupancy at indicated loci on day 5 of EB differentiation. (*D*)

RT-qPCR showing inefficient repression of *Oct4* during EB differentiation. (*E*) Bisulfite sequencing of *Oct4* promoter region during EB differentiation.

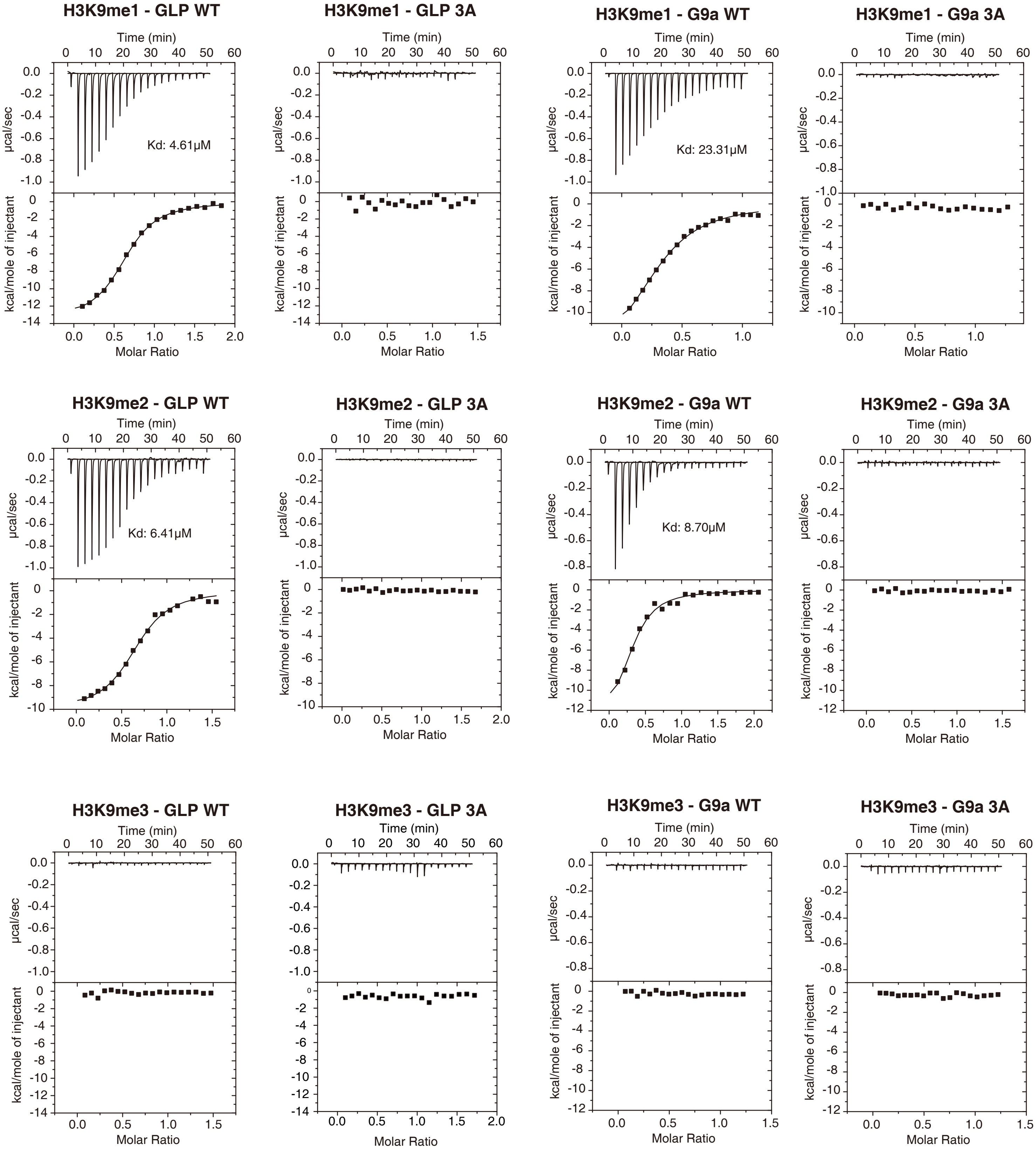
Supplemental Figure 8. The H3K9 methylation binding activity of GLP was required for efficient H3K9me2 establishment during ES cell differentiation at genome-wide level. (*A*) Western blot showing GLP protein levels were comparable in RA induced WT and GLP 3A cells. (*B*) Pairwise cross correlation between every two samples. Pearson correlation coefficients were calculated based on the 10-kb windows which were used in Fig. 5A-C. Color depth and box height indicates the degree of correlation. (*C*) For genes displaying a more than 2-fold increase of H3K9me2 during RA-induced WT differentiation, H3K9me2 ratio between GLP 3A and WT cells before and after RA treatment were plotted. The categories were divided according to different levels of H3K9me1 ratio between GLP 3A and WT ESCs. (*D*) Clustering of the 10-kb genomic windows according to their H3K9me1 levels for wild-type and GLP 3A cells with or without RA treatment. Windows were selected and clustered as described in Fig. 5A, except that the H3K9me1 data were used. (*E*) Accumulative distribution of the H3K9me2 enrichment level in WT and GLP 3A ES cells. (*F*) Accumulative distribution of the H3K9me2 enrichment level in RA-treated WT and GLP 3A cells. P values in (*E*) and (*F*) were calculated by *t*-test.

Supplemental Figure 9. Analysis of H3K9me2 establishment and gene expression at the control gene set. (*A*) MA-plot of H3K9me2 fold changes against the average H3K9me2 fold enrichment in GLP 3A cells. Red dots represent genes with significantly increased H3K9me2 level after RA treatment in GLP 3A cells. (*B*) Box plot of H3K9me2 fold changes between ES cells and RA-treated cells at genes acquired or lost H3K9me2 (Supplemental Fig. 8A) in wild-type and GLP 3A cells. (*C*) Histogram showing the expression change of genes acquired H3K9me2 (Fig. S9A) in GLP 3A cells during differentiation. Black bars: unchanged, red bars: repressed, green bars: activated. (*D*) Expression comparison of the genes acquired H3K9me2 in GLP 3A cells (Supplemental Fig. 9A) between wild-type and GLP 3A ES cells (*left*) or RA-treated cells (*right*). Note: red bars and red dots in (*C, D*) represent the same set of genes, and all the dots shown in Supplemental Fig. 9C-D represent the red dots in Supplemental Fig. 9A. (*E*) GO term analysis of the 329 genes that failed to be activated in GLP 3A cells during differentiation. F.E. represents fold enrichment.

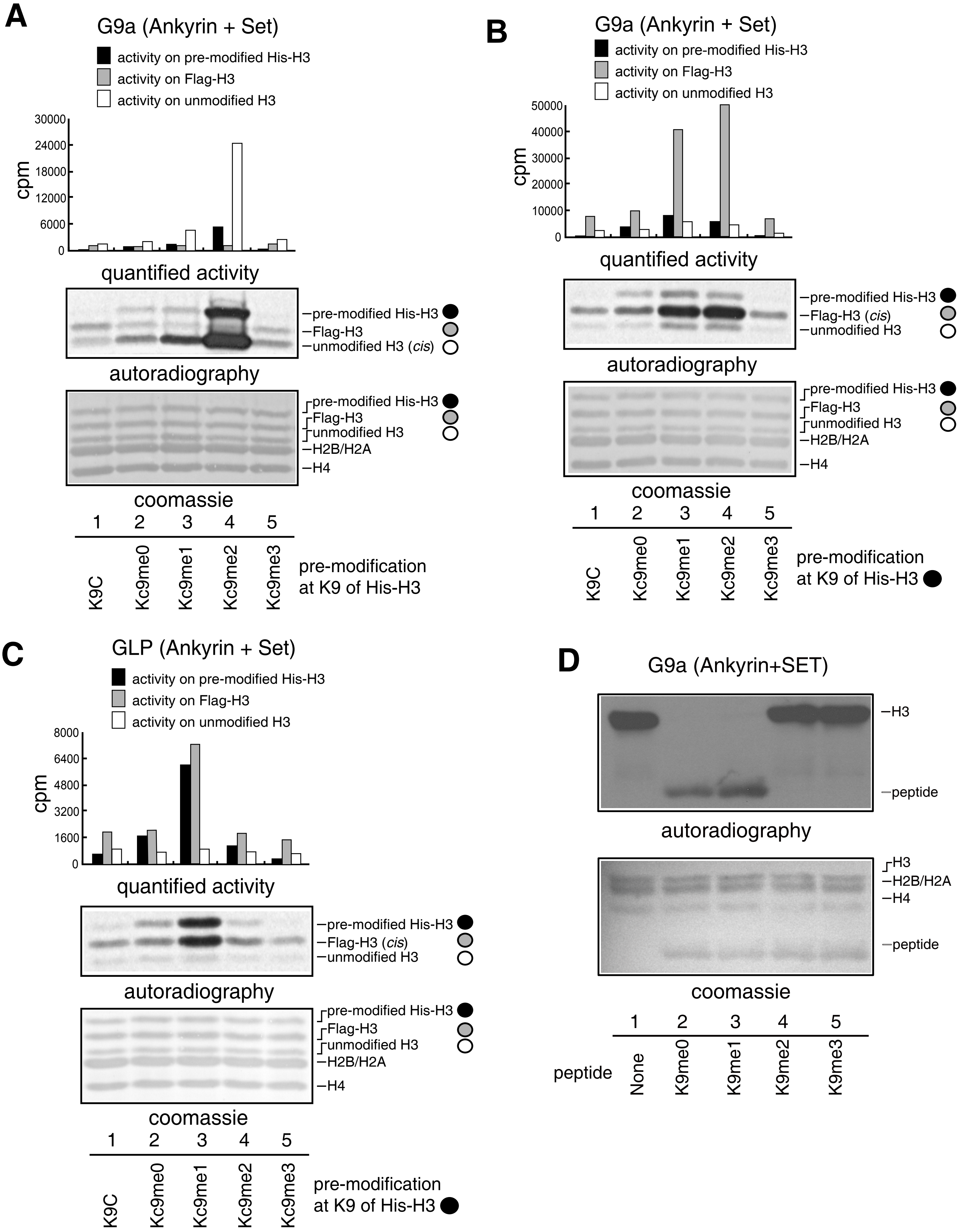
Liu_FigS1


A
G9a (Ankyrin+SET)
■ activity on pre-modified His-H3
□ activity on unmodified H3
cpm
30000
24000
18000
12000
6000
0
quantified activity
—pre-modified His-H3 ●
—unmodified H3 ○
autoradiography
—pre-modified His-H3 ●
unmodified H3 ○
—H2B/H2A
—H4
coomassie
1 2 3 4 5
K9C
Kc9me0
Kc9me1
Kc9me2
Kc9me3
pre-modification at K9 of His-H3 ●
B
G9a C1007A
—pre-modified His-H3 ●
—unmodified H3 ○
autoradiography
—pre-modified His-H3 ●
unmodified H3 ○
—H2B/H2A
—H4
coomassie
1 2 3 4 5
K9C
Kc9me0
Kc9me1
Kc9me2
Kc9me3
pre-modification at K9 of His-H3 ●
C
G9a (Ankyrin+SET)
■ activity on pre-modified His-H3
□ activity on unmodified H3
cpm
30000
24000
18000
12000
6000
0
quantified activity
—pre-modified His-H3 ●
—unmodified H3 ○
autoradiography
—pre-modified His-H3 ●
unmodified H3 ○
—H2B/H2A
—H4
commassie
1 2 3 4
Ala
Lys
Position 27
K9C
Kc9me2
K9C
Kc9me2
Position 9 of His-H3 ●
D
G9a (Ankyrin+SET)
—pre-modified His-H3 ●
—unmodified H3 ○
autoradiography
—pre-modified His-H3 ●
unmodified H3 ○
—H2B/H2A
—H4
coomassie
1 2 3 4 5
K27C
Kc27me0
Kc27me1
Kc27me2
Kc27me3
pre-modification at K27 of His-H3 ●
E
G9a 3A
■ activity on pre-modified His-H3
□ activity on unmodified H3
cpm
6000
4800
3600
2400
1200
0
quantified activity
—pre-modified His-H3 ●
—unmodified H3 ○
autoradiography
—pre-modified His-H3 ●
unmodified H3 ○
—H2B/H2A
—H4
coomassie
1 2 3 4 5
K9C
Kc9me0
Kc9me1
Kc9me2
Kc9me3
pre-modification at K9 of His-H3 ●

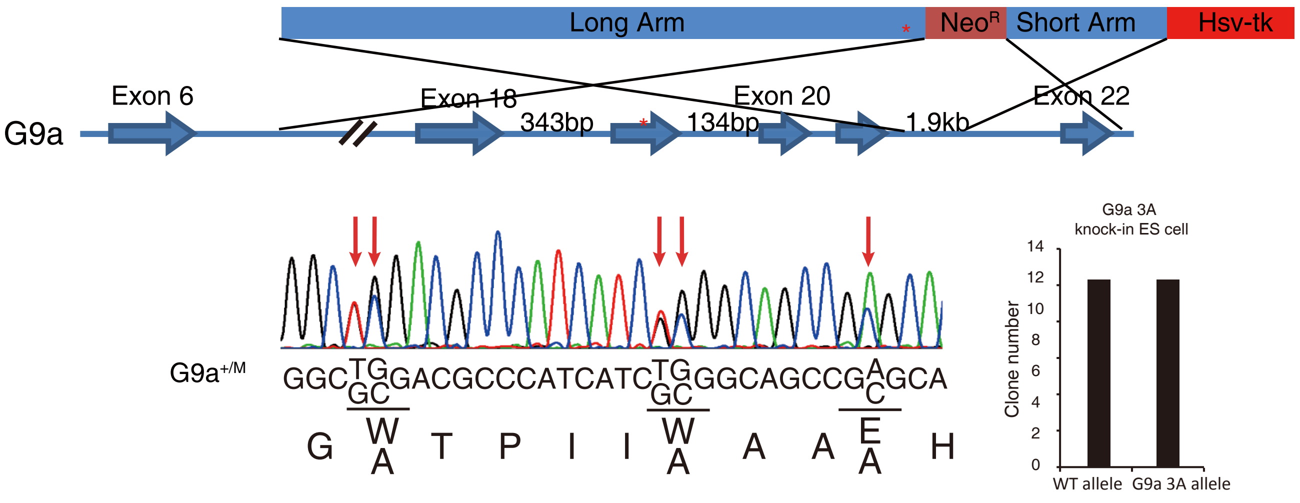
Liu_FigS2

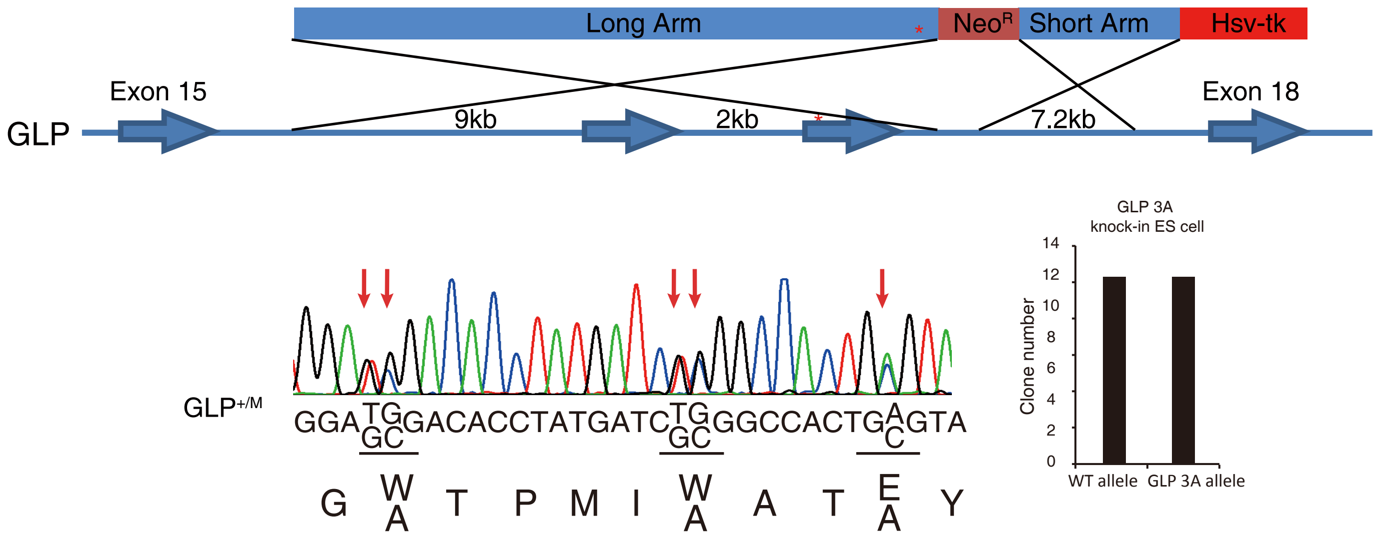

H3K9me1 - GLP WT
Kd: 4.61μM
H3K9me1 - GLP 3A
H3K9me1 - G9a WT
Kd: 23.31μM
H3K9me1 - G9a 3A
H3K9me2 - GLP WT
Kd: 6.41μM
H3K9me2 - GLP 3A
H3K9me2 - G9a WT
Kd: 8.70μM
H3K9me2 - G9a 3A
H3K9me3 - GLP WT
H3K9me3 - GLP 3A
H3K9me3 - G9a WT
H3K9me3 - G9a 3A
Time (min)
μcal/sec
kcal/mole of injectant
Molar Ratio

Liu_FigS3


A
G9a (Ankyrin + Set)
■ activity on pre-modified His-H3
▨ activity on Flag-H3
☐ activity on unmodified H3
cpm
30000
24000
18000
12000
6000
0
quantified activity
—pre-modified His-H3 ●
—Flag-H3 ◐
—unmodified H3 (cis) ○
autoradiography
pre-modified His-H3 ●
Flag-H3 ◐
unmodified H3 ○
—H2B/H2A
—H4
coomassie
1 2 3 4 5
K9C
Kc9me0
Kc9me1
Kc9me2
Kc9me3
pre-modification at K9 of His-H3
B
G9a (Ankyrin + Set)
■ activity on pre-modified His-H3
▨ activity on Flag-H3
☐ activity on unmodified H3
cpm
50000
40000
30000
20000
10000
0
quantified activity
—pre-modified His-H3 ●
—Flag-H3 (cis) ◐
—unmodified H3 ○
autoradiography
pre-modified His-H3 ●
Flag-H3 ◐
unmodified H3 ○
—H2B/H2A
—H4
coomassie
1 2 3 4 5
K9C
Kc9me0
Kc9me1
Kc9me2
Kc9me3
pre-modification at K9 of His-H3 ●
C
GLP (Ankyrin + Set)
■ activity on pre-modified His-H3
▨ activity on Flag-H3
☐ activity on unmodified H3
cpm
8000
6400
4800
3200
1600
0
quantified activity
—pre-modified His-H3 ●
—Flag-H3 (cis) ◐
—unmodified H3 ○
autoradiography
pre-modified His-H3 ●
Flag-H3 ◐
unmodified H3 ○
—H2B/H2A
—H4
coomassie
1 2 3 4 5
K9C
Kc9me0
Kc9me1
Kc9me2
Kc9me3
pre-modification at K9 of His-H3 ●
D
G9a (Ankyrin+SET)
—H3
—peptide
autoradiography
H3
—H2B/H2A
—H4
—peptide
coomassie
1 2 3 4 5
peptide
None
K9me0
K9me1
K9me2
K9me3

Liu_FigS4


Long Arm
*
Neo^R
Short Arm
Hsv-tk
G9a
Exon 6
Exon 18
343bp
*
134bp
Exon 20
1.9kb
Exon 22
G9a+/M
GGC TG/GC GACGCCCATCATC TG/GC GGCAGCCG A/C GCA
G W/A T P I I W/A A A E/A H
G9a 3A
knock-in ES cell
Clone number
14
12
10
8
6
4
2
0
WT allele
G9a 3A allele


Long Arm
*
Neo^R
Short Arm
Hsv-tk
GLP
Exon 15
9kb
2kb
*
7.2kb
Exon 18
GLP+/M
GGA TG/GC GACACCTATGATC TG/GC GGCCACTG A/C GTA
G W/A T P M I W/A A T E/A Y
GLP 3A
knock-in ES cell
Clone number
14
12
10
8
6
4
2
0
WT allele
GLP 3A allele

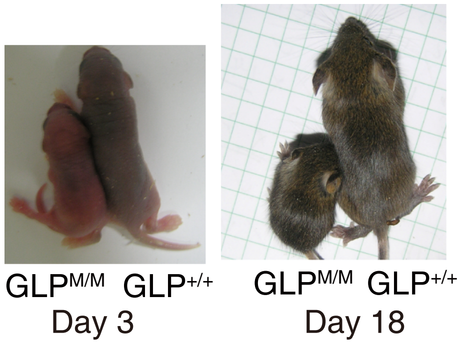
Liu_FigS5

A

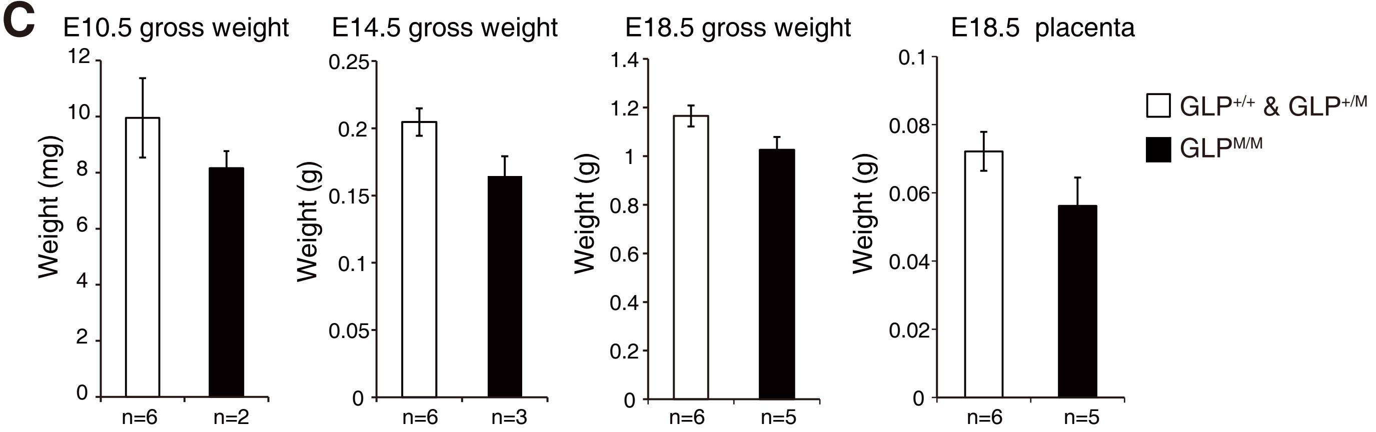
Statistics of G9a$^{+/M}$ intercross

	G9a$^{+/+}$	G9a$^{+/M}$	G9a$^{M/M}$
Day2	33	70	36
Day4	33	70	36
Day20	32	68	33

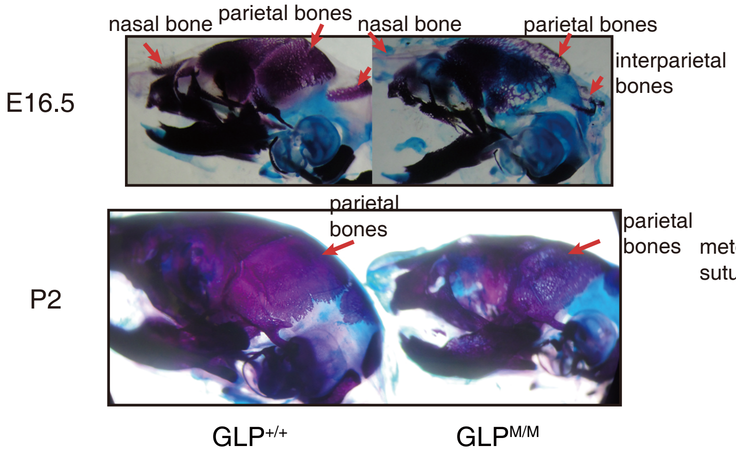
B



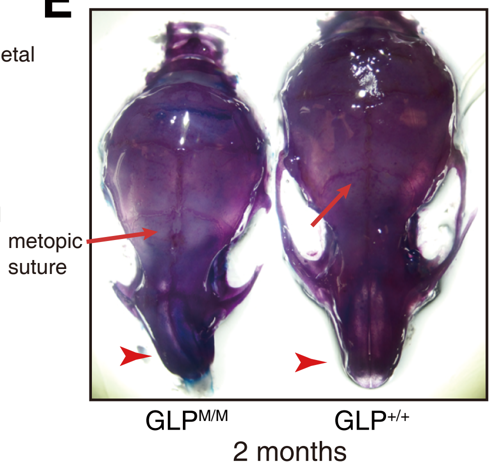
C



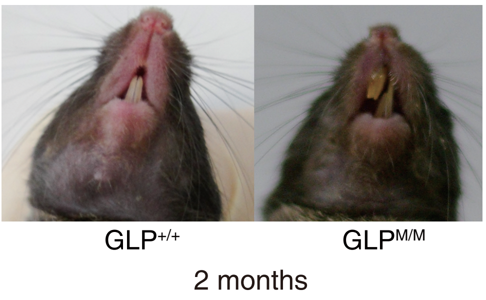
D



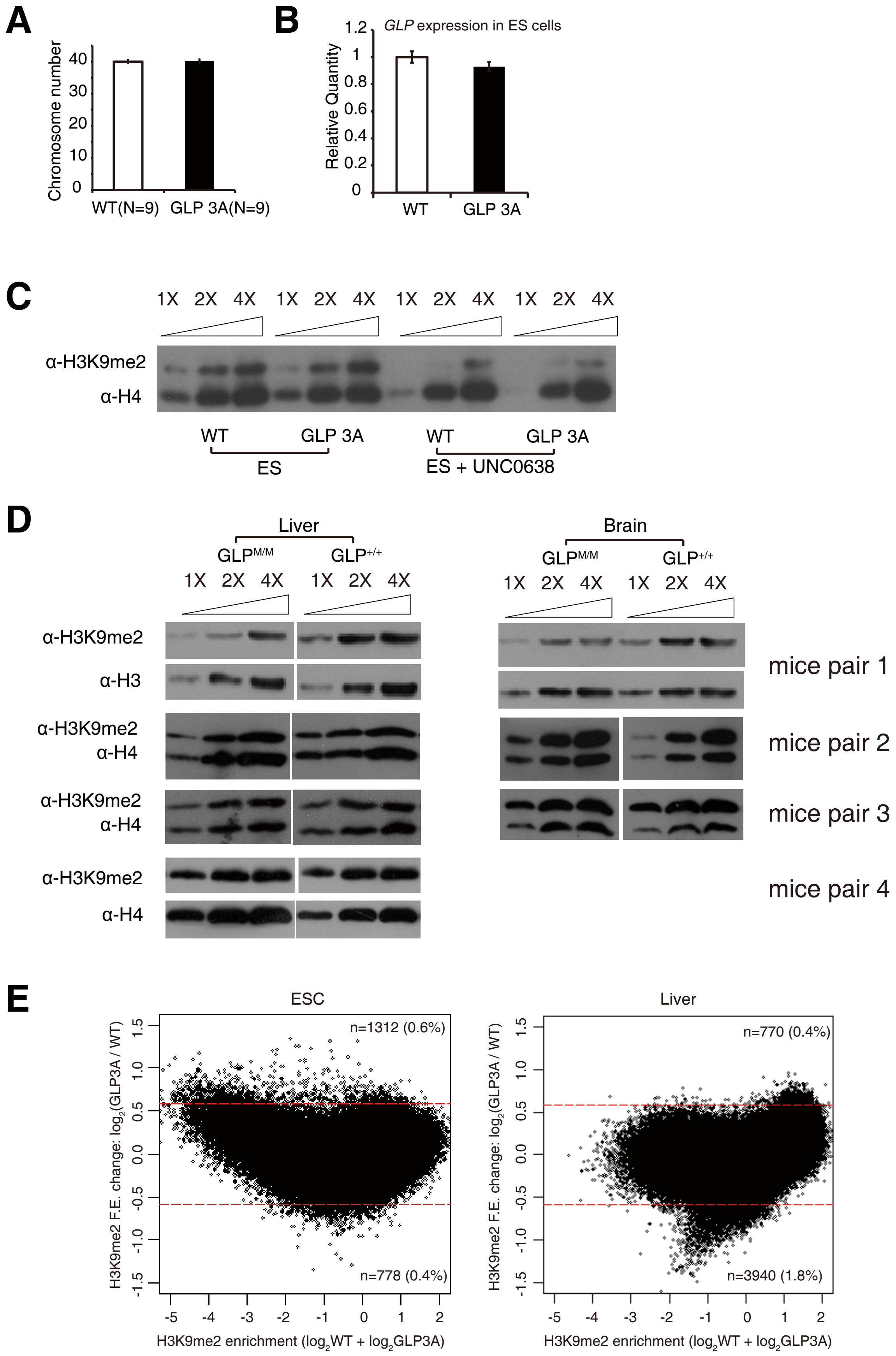
E



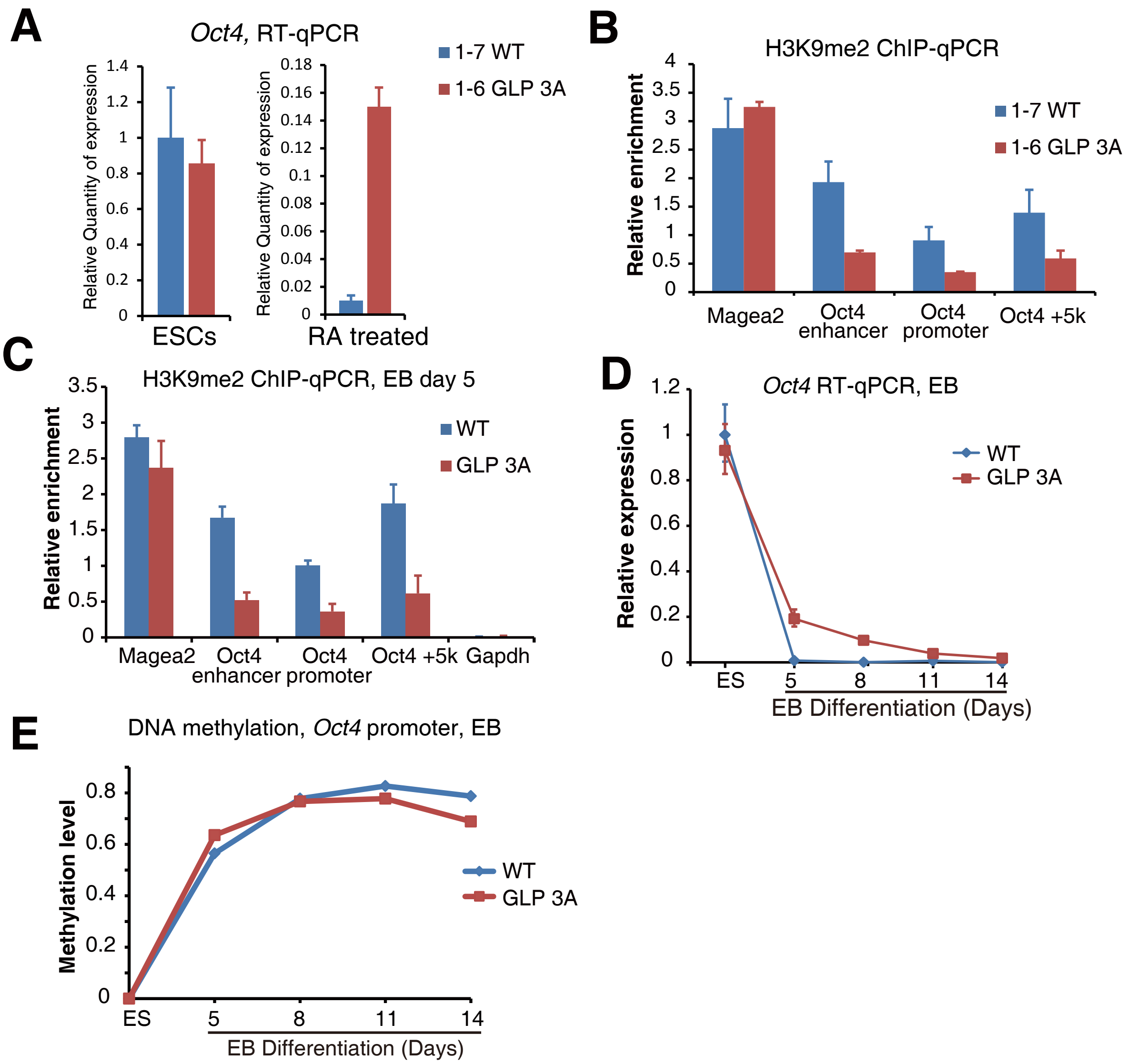
F



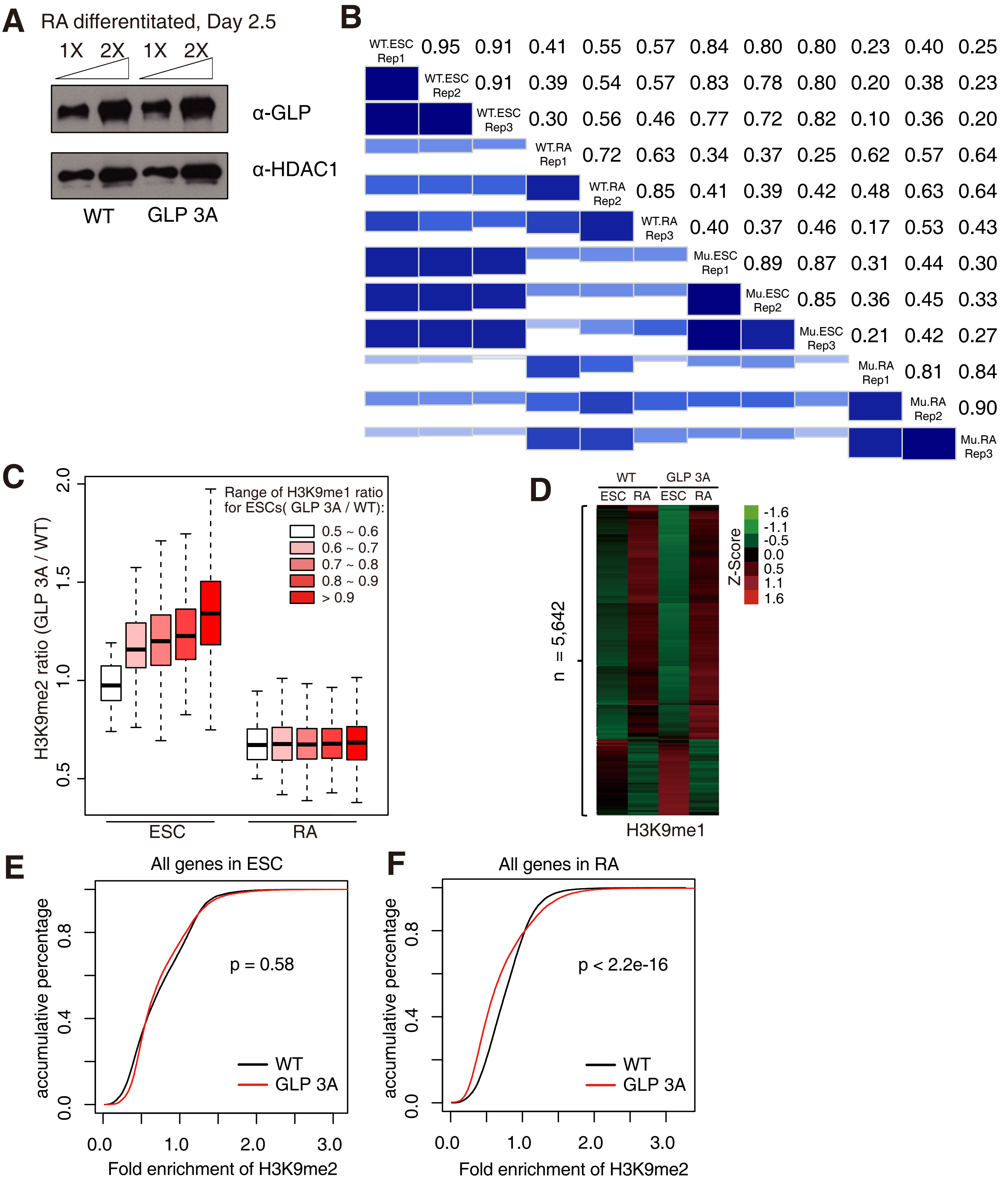
Liu_FigS6


A
Chromosome number
0
10
20
30
40
WT(N=9)
GLP 3A(N=9)
B
GLP expression in ES cells
Relative Quantity
0
0.2
0.4
0.6
0.8
1
1.2
WT
GLP 3A
C
1X 2X 4X 1X 2X 4X 1X 2X 4X 1X 2X 4X
α-H3K9me2
α-H4
WT
GLP 3A
ES
WT
GLP 3A
ES + UNC0638
D
Liver
GLP^M/M
GLP^+/+
1X 2X 4X 1X 2X 4X
Brain
GLP^M/M
GLP^+/+
1X 2X 4X 1X 2X 4X
α-H3K9me2
α-H3
α-H3K9me2
α-H4
α-H3K9me2
α-H4
α-H3K9me2
α-H4
mice pair 1
mice pair 2
mice pair 3
mice pair 4
E
ESC
n=1312 (0.6%)
n=778 (0.4%)
Liver
n=770 (0.4%)
n=3940 (1.8%)
H3K9me2 F.E. change: log2(GLP3A / WT)
-1.5
-1.0
-0.5
0.0
0.5
1.0
1.5
-5
-4
-3
-2
-1
0
1
2
H3K9me2 enrichment (log2WT + log2GLP3A)

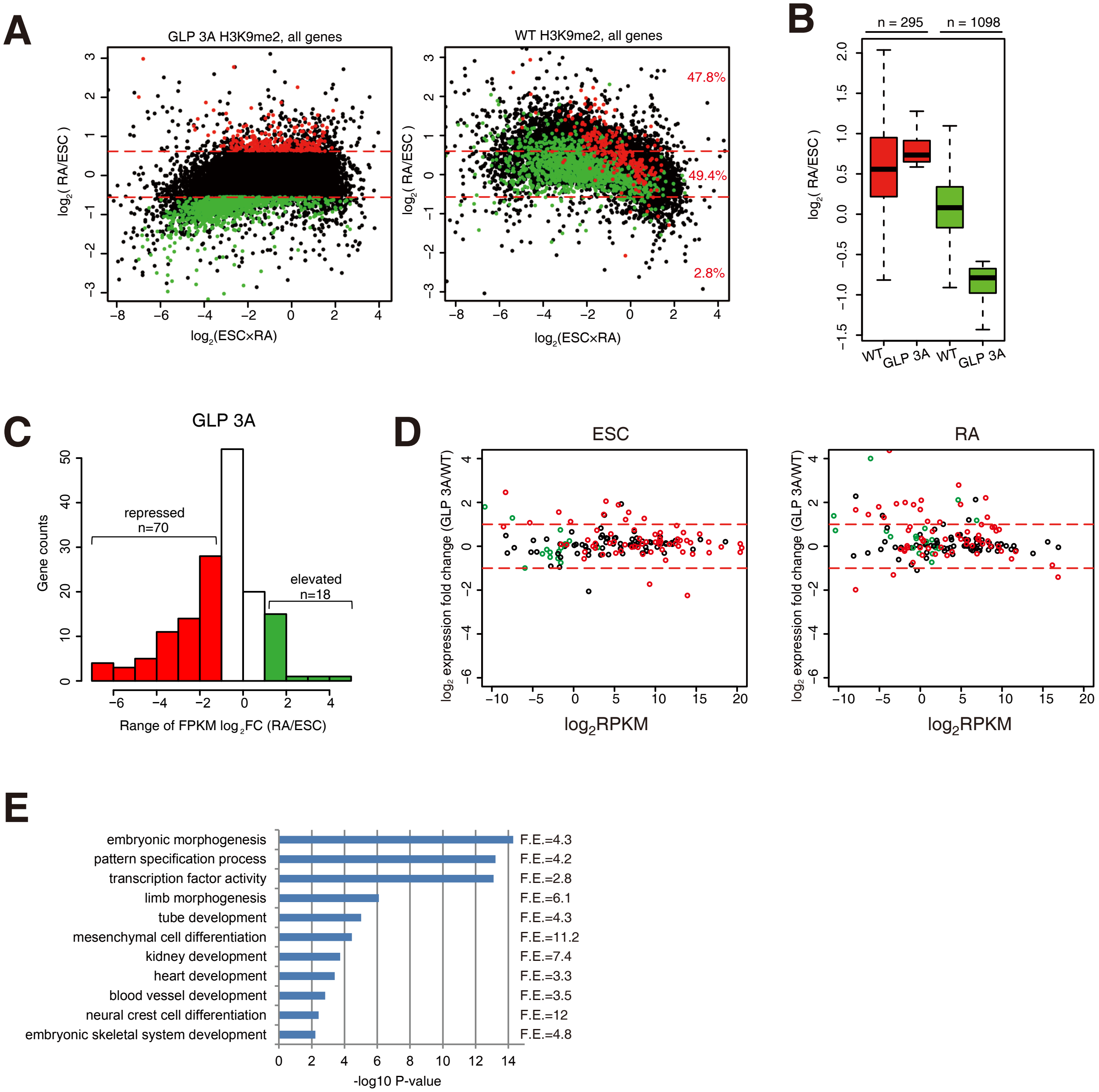
Liu_FigS7


A
Oct4, RT-qPCR
Relative Quantity of expression
0
0.2
0.4
0.6
0.8
1
1.2
1.4
ESCs
Relative Quantity of expression
0
0.02
0.04
0.06
0.08
0.1
0.12
0.14
0.16
0.18
RA treated
1-7 WT
1-6 GLP 3A
B
H3K9me2 ChIP-qPCR
Relative enrichment
0
0.5
1
1.5
2
2.5
3
3.5
4
1-7 WT
1-6 GLP 3A
Magea2
Oct4 enhancer
Oct4 promoter
Oct4 +5k
C
H3K9me2 ChIP-qPCR, EB day 5
Relative enrichment
0
0.5
1
1.5
2
2.5
3
3.5
WT
GLP 3A
Magea2
Oct4 enhancer
Oct4 promoter
Oct4 +5k
Gapdh
D
Oct4 RT-qPCR, EB
Relative expression
0
0.2
0.4
0.6
0.8
1
1.2
WT
GLP 3A
ES
5
8
11
14
EB Differentiation (Days)
E
DNA methylation, *Oct4* promoter, EB
Methylation level
0
0.2
0.4
0.6
0.8
WT
GLP 3A
ES
5
8
11
14
EB Differentiation (Days)

Liu_FigS8


A
RA differentitated, Day 2.5
1X 2X 1X 2X
α-GLP
α-HDAC1
WT
GLP 3A
B
WT.ESC Rep1 0.95 0.91 0.41 0.55 0.57 0.84 0.80 0.80 0.23 0.40 0.25
WT.ESC Rep2 0.91 0.39 0.54 0.57 0.83 0.78 0.80 0.20 0.38 0.23
WT.ESC Rep3 0.30 0.56 0.46 0.77 0.72 0.82 0.10 0.36 0.20
WT.RA Rep1 0.72 0.63 0.34 0.37 0.25 0.62 0.57 0.64
WT.RA Rep2 0.85 0.41 0.39 0.42 0.48 0.63 0.64
WT.RA Rep3 0.40 0.37 0.46 0.17 0.53 0.43
Mu.ESC Rep1 0.89 0.87 0.31 0.44 0.30
Mu.ESC Rep2 0.85 0.36 0.45 0.33
Mu.ESC Rep3 0.21 0.42 0.27
Mu.RA Rep1 0.81 0.84
Mu.RA Rep2 0.90
Mu.RA Rep3
C
H3K9me2 ratio (GLP 3A / WT)
2.0
1.5
1.0
0.5
Range of H3K9me1 ratio for ESCs(GLP 3A / WT):
0.5 ~ 0.6
0.6 ~ 0.7
0.7 ~ 0.8
0.8 ~ 0.9
> 0.9
ESC
RA
D
WT
GLP 3A
ESC RA ESC RA
n = 5,642
Z-Score
-1.6
-1.1
-0.5
0.0
0.5
1.1
1.6
H3K9me1
E
All genes in ESC
accumulative percentage
0.0
0.4
0.8
p = 0.58
WT
GLP 3A
0.0
1.0
2.0
3.0
Fold enrichment of H3K9me2
F
All genes in RA
accumulative percentage
0.0
0.4
0.8
p < 2.2e-16
WT
GLP 3A
0.0
1.0
2.0
3.0
Fold enrichment of H3K9me2

Liu_FigS9


A
GLP 3A H3K9me2, all genes
log2(RA/ESC)
log2(ESC×RA)
WT H3K9me2, all genes
47.8%
49.4%
2.8%
B
n = 295
n = 1098
log2(RA/ESC)
WT
GLP 3A
WT
GLP 3A
C
GLP 3A
Gene counts
repressed
n=70
elevated
n=18
Range of FPKM log2FC (RA/ESC)
D
ESC
RA
log2 expression fold change (GLP 3A/WT)
log2RPKM
E
embryonic morphogenesis
pattern specification process
transcription factor activity
limb morphogenesis
tube development
mesenchymal cell differentiation
kidney development
heart development
blood vessel development
neural crest cell differentiation
embryonic skeletal system development
F.E.=4.3
F.E.=4.2
F.E.=2.8
F.E.=6.1
F.E.=4.3
F.E.=11.2
F.E.=7.4
F.E.=3.3
F.E.=3.5
F.E.=12
F.E.=4.8
-log10 P-value

Supplemental Table 1. List of genes that are inefficiently repressed during differentiation of GLP 3A cells.

Name	H3K9me2 Fold Enrichment				P_value(WT.RA vs WT.ESC)	H3K9me2 FE ratio (Log2)		Expression FC (Log2)		
	WT.ESC	WT.RA	Mut.ESC	Mut.RA		wRA/wES	muRA/muES	WT.RA/WT.ES	Mu.RA/Mu.ESC	Mu.RA/WT.RA
Esrrb	**0.445**	**0.948**	**0.469**	**0.662**	**0.008**	**1.092**	**0.498**	**-8.112**	**-5.351**	**2.336**
Pou5f1	**0.221**	**0.618**	**0.248**	**0.405**	**0.017**	**1.481**	**0.707**	**-7.331**	**-5.589**	**1.441**
Fgf4	**0.100**	**0.539**	**0.220**	**0.248**	**0.007**	**2.433**	**0.174**	**-9.220**	**-7.414**	**1.790**
Nanog	**0.293**	**0.950**	**0.331**	**0.513**	**0.002**	**1.695**	**0.631**	**-6.872**	**-4.359**	**2.909**
Nr5a2	**0.607**	**0.949**	**0.581**	**0.787**	**0.001**	**0.645**	**0.438**	**-7.825**	**-5.193**	**2.765**
4930548H24Rik	0.690	1.097	0.601	1.093	0.044	0.670	0.863	-6.124	-5.337	1.094
4930500J02Rik	0.647	1.060	0.415	0.748	0.037	0.713	0.852	-2.501	-2.380	1.131
AU018091	0.521	1.055	0.478	0.749	0.000	1.017	0.649	-2.964	-2.156	1.613
Cela1	0.418	0.768	0.472	0.468	0.033	0.875	-0.011	-1.719	0.741	1.230
Hormad1	0.435	0.824	0.532	0.715	0.041	0.923	0.426	-3.856	-2.696	2.542
Gm9926	0.531	0.798	0.516	0.688	0.010	0.588	0.415	-1.289	1.078	2.716
Mylpf	0.292	1.101	0.288	0.517	0.010	1.914	0.846	-6.482	-3.241	2.816
Sox15	0.087	0.330	0.178	0.177	0.013	1.925	-0.002	-4.258	-3.302	1.417
Arhgef15	0.486	0.830	0.505	0.544	0.028	0.771	0.106	-2.473	-1.773	1.035
Lgals7	0.506	1.220	0.448	0.595	0.015	1.271	0.408	-4.226	-2.577	4.041
Prdm14	0.526	0.859	0.504	0.565	0.008	0.708	0.165	-7.007	-4.467	2.660
Snord1c	0.066	0.240	0.270	0.136	0.007	1.852	-0.987	-5.199	-1.797	2.933
4921517D22Rik	0.639	1.071	0.617	0.875	0.002	0.744	0.503	-2.179	inf	2.391
Mospd4	0.259	0.667	0.425	0.529	0.022	1.367	0.315	-2.866	-1.452	1.530
Ttc9b	0.315	0.589	0.593	0.566	0.025	0.904	-0.066	-4.175	-1.520	3.672
BC055111	0.517	0.823	0.589	0.761	0.009	0.671	0.369	-3.941	-2.036	2.680
4930539E08Rik	0.474	0.770	0.497	0.514	0.044	0.699	0.051	-3.351	-2.132	1.553
F13b	0.667	1.107	0.696	1.092	0.044	0.730	0.650	-1.148	-0.092	1.350
Klf2	0.133	0.239	0.175	0.118	0.010	0.845	-0.573	-6.703	-4.281	2.429
Spp1	0.384	0.823	0.426	0.668	0.038	1.099	0.650	-5.401	-3.754	2.482
BC064078	0.670	1.043	1.219	1.356	0.010	0.640	0.154	-3.611	-1.360	1.080
Rhd	0.423	0.807	0.436	0.482	0.046	0.930	0.143	-2.234	-0.538	1.743
Tmc8	0.266	0.589	0.344	0.296	0.041	1.147	-0.213	-1.386	-0.325	1.065
Npb	0.178	0.313	0.280	0.114	0.026	0.814	-1.304	-1.344	0.864	2.324
Gjb3	0.290	0.661	0.261	0.269	0.007	1.188	0.045	-3.591	-3.036	1.509
Kcnip2	0.424	0.783	0.446	0.490	0.054	0.885	0.135	-3.381	-1.399	1.374
Cidea	0.423	0.649	0.505	0.481	0.048	0.617	-0.070	-2.630	-0.916	2.824

Chrna9	0.483	1.034	0.433	0.695	0.009	1.097	0.685	-9.684	-6.266	3.067
Trh	0.484	1.002	0.445	0.524	0.018	1.050	0.235	-9.053	-8.166	1.261
Gng3	0.263	0.799	0.333	0.423	0.027	1.605	0.346	-1.274	-0.144	1.447
1700019D03Rik	0.592	0.917	0.761	1.000	0.009	0.630	0.393	-6.732	-4.555	1.320
Prr15l	0.305	0.926	0.318	0.450	0.017	1.604	0.499	-2.975	-1.109	2.113
Zp3	0.345	0.554	0.348	0.337	0.016	0.681	-0.046	-4.546	-4.835	1.481
Gsdma	0.659	1.015	0.594	0.600	0.012	0.622	0.014	-4.228	-1.497	2.083
Aspdh	0.389	0.883	0.425	0.497	0.025	1.181	0.225	-1.792	0.719	2.308
Tsks	0.411	0.680	0.428	0.385	0.041	0.727	-0.155	-3.974	-3.121	1.053
Ndufa4l2	0.392	0.703	0.348	0.424	0.026	0.842	0.283	-4.286	-2.231	1.100
Pycard	0.192	0.587	0.255	0.237	0.000	1.615	-0.105	-3.573	-2.386	1.104
Upp1	0.528	0.847	0.482	0.661	0.005	0.682	0.455	-6.117	-4.302	2.040
Pla2g10	0.596	0.979	0.512	0.727	0.031	0.715	0.505	-4.737	-3.534	1.528
Ngfr	0.353	0.574	0.387	0.325	0.017	0.700	-0.254	-3.074	-1.235	1.601
Zfp296	0.194	0.592	0.405	0.275	0.046	1.610	-0.559	-4.965	-3.594	1.095
D930028M14Rik	0.284	0.556	0.459	0.340	0.045	0.968	-0.431	-4.480	-1.351	2.918
Gm6792	0.582	0.985	0.517	0.719	0.025	0.758	0.475	-8.255	-5.733	2.731
1700023L04Rik	0.404	0.704	0.329	0.436	0.004	0.800	0.406	-3.186	-1.434	1.501
Cpne9	0.540	0.819	0.513	0.590	0.038	0.602	0.201	-4.675	-4.412	1.300
Gpa33	0.590	0.893	0.630	0.628	0.015	0.599	-0.006	-5.353	-2.752	2.663
E230025N22Rik	0.449	0.969	0.437	0.504	0.014	1.111	0.207	-1.296	1.070	2.038
Gtsf1l	0.281	0.925	0.289	0.505	0.035	1.720	0.807	-6.100	-4.449	1.521
Morn3	0.435	0.822	0.356	0.481	0.028	0.919	0.434	-3.909	-1.984	1.912
Pfkp	0.620	1.024	0.598	0.761	0.010	0.723	0.348	-5.072	-3.779	1.019
Lpar5	0.346	0.666	0.500	0.531	0.017	0.943	0.086	-2.763	-1.379	1.156
Otx1	0.118	0.264	0.228	0.174	0.024	1.165	-0.390	-1.282	0.309	1.110
Cd68	0.351	1.190	0.231	0.573	0.015	1.763	1.311	-1.221	-0.779	1.186
Gm13939	0.402	0.659	0.437	0.532	0.003	0.715	0.282	-6.325	-4.666	1.281
Lefty2	0.308	0.561	0.406	0.323	0.004	0.864	-0.331	-6.109	-4.609	2.308
Wnt8a	0.418	0.750	0.409	0.392	0.015	0.844	-0.063	-1.872	-1.686	2.062
Ttll13	0.504	0.827	0.448	0.551	0.013	0.715	0.297	-1.335	-1.013	1.052
Gm6537	0.514	0.964	0.510	0.470	0.047	0.907	-0.120	inf	1.371	2.286
Tspan1	0.666	1.053	0.432	0.743	0.037	0.660	0.783	-1.507	-2.164	1.066
Gm15698	0.714	1.089	0.497	0.893	0.012	0.610	0.846	-1.042	-0.356	2.668
Rasgrp2	0.347	0.753	0.379	0.381	0.007	1.116	0.006	-5.407	-4.486	1.719
2410007B07Rik	0.516	1.061	0.459	1.149	0.010	1.041	1.324	-8.316	-6.778	1.807

Slc35f2	0.515	0.847	0.500	0.679	0.000	0.718	0.441	-1.595	-1.548	1.075
Napsa	0.606	1.098	0.604	0.829	0.025	0.857	0.456	-4.406	-1.142	1.332
Gm7325	0.146	0.425	0.203	0.291	0.022	1.542	0.516	-8.293	-5.287	2.492
Mmp21	0.613	1.140	0.465	0.824	0.008	0.895	0.825	-1.326	0.724	1.994
Xist	0.246	0.634	0.487	0.535	0.000	1.366	0.136	-3.393	0.065	1.410
D7Ertd143e	0.074	0.553	0.183	0.364	0.002	2.907	0.987	-6.287	-4.789	1.860
4930578M01Rik	0.235	0.513	0.248	0.269	0.034	1.128	0.117	-2.994	0.974	2.948
Nlrp4f	0.618	0.974	0.587	0.777	0.011	0.656	0.405	-5.147	-4.281	1.437
Ctf2	0.538	0.839	0.535	0.591	0.013	0.640	0.143	-1.120	0.882	2.147
Trim12a	0.733	1.101	0.685	0.969	0.025	0.588	0.501	-1.321	-0.715	2.768
Nr0b1	0.215	0.568	0.315	0.378	0.009	1.402	0.266	-8.383	-6.297	1.719
Phlda2	0.129	0.304	0.288	0.228	0.021	1.245	-0.338	-1.660	0.230	1.112
Rnf135	0.525	0.862	0.516	0.667	0.027	0.715	0.372	-1.174	-0.988	1.287
Neat1	0.150	0.823	0.268	0.279	0.036	2.457	0.061	-1.257	0.335	1.108
Trim34a	0.472	0.735	0.565	0.697	0.002	0.637	0.303	-1.375	-0.947	1.403
Rnf39	0.338	0.659	0.327	0.246	0.032	0.962	-0.412	-3.026	-0.944	1.088
Hsd17b14	0.299	0.686	0.319	0.334	0.009	1.199	0.063	-6.302	-5.164	1.246
Zyg11a	0.463	0.870	0.493	0.622	0.001	0.910	0.335	-4.820	-2.518	2.209
Gdf3	0.367	1.002	0.284	0.605	0.030	1.449	1.092	-5.052	-4.629	1.245
Syngr1	0.450	0.840	0.466	0.511	0.104	0.900	0.133	-2.474	-1.171	1.086
Ooep	0.333	1.301	0.447	0.638	0.022	1.967	0.514	-4.984	-3.467	2.123
Gm15441	0.278	0.444	0.329	0.356	0.026	0.673	0.117	-2.213	0.281	1.225
Tdrd12	0.595	0.924	0.503	0.680	0.004	0.637	0.434	-4.031	-4.089	1.077
Fgr	0.502	0.781	0.412	0.451	0.038	0.637	0.130	-5.926	-3.195	3.059
Saa3	0.308	0.548	0.385	0.323	0.047	0.831	-0.256	-3.361	-3.352	2.749
Tdgf1	0.242	0.658	0.273	0.397	0.010	1.442	0.538	-9.641	-7.110	2.985
Tuba4a	0.351	0.853	0.343	0.451	0.041	1.280	0.396	-3.644	-2.196	1.055
Ttll6	0.470	0.754	0.507	0.506	0.042	0.681	-0.002	-1.729	-1.569	1.347
Lctl	0.457	0.768	0.533	0.551	0.009	0.749	0.048	-2.586	-3.405	1.145
Parp9	0.469	0.816	0.458	0.560	0.000	0.799	0.289	-1.164	-0.659	1.266
Dnajc22	0.424	0.780	0.421	0.535	0.030	0.879	0.345	-1.530	-0.006	1.459
Gstm3	0.709	1.342	0.572	0.900	0.044	0.920	0.653	-6.112	-4.242	4.138
2410017I17Rik	0.489	0.803	0.459	0.586	0.002	0.716	0.351	-3.380	-2.755	2.430
Calcoco2	0.400	0.739	0.381	0.458	0.007	0.887	0.267	-2.146	-1.448	2.798
Tm4sf5	0.404	0.740	0.383	0.356	0.032	0.872	-0.109	-2.770	-1.149	2.068
Tchh	0.150	0.324	0.290	0.390	0.020	1.110	0.431	-1.611	0.826	1.472

Gngt2	0.799	1.284	0.563	0.728	0.131	0.684	0.372	-3.843	-2.640	1.270
Slc10a1	0.594	0.904	0.495	0.578	0.007	0.606	0.222	-2.922	-0.897	1.701
Cbln1	0.107	0.278	0.311	0.141	0.015	1.377	-1.140	-2.234	-1.136	1.181
Mt1	0.054	0.241	0.209	0.169	0.017	2.158	-0.302	-2.878	-2.184	1.958
Phkg1	0.348	0.790	0.428	0.535	0.002	1.184	0.325	-2.274	-1.130	1.466
Dlgap3	0.628	0.986	0.546	0.693	0.024	0.651	0.344	-4.643	-3.475	1.102

Supplemental Table 2. Antibodies for western blot and ChIP experiments.

Antibody	Brand	Purpose
H3K9me1	Ab8896 (abcam)	Western, ChIP-seq
H3K9me2	Ab1220 (abcam)	Western, ChIP-seq
H3K27me3	Ab8898 (abcam)	Western
H3	07-690 (millipore)	Western
H4	04-858 (millipore)	Western
HDAC1	Ab46985 (abcam)	Western
GLP	Ab41969 (abcam)	Western

Supplemental Table 3. Sequencing depth of the ChIP-seq and RNA-seq experiments.

Replicate	Experiment	Raw Reads#	Uniquely mapped#	%
Rep1	WT-ESC-H3K9me2-ChIP	133,115,195	89,755,597	67.43%
	WT-ESC-input	142,175,701	97,958,070	68.90%
	Mut-ESC-H3K9me2-ChIP	125,814,498	78,711,670	62.56%
	Mut-ESC-input	145,872,824	101,144,050	69.34%
	WT-RA-H3K9me2-ChIP	164,656,848	113,632,886	69.01%
	WT-RA-input	143,064,229	92,922,316	64.95%
	Mut-RA-H3K9me2-ChIP	141,392,832	91,562,507	64.76%
	Mut-RA-input	158,087,474	102,324,012	64.73%
Rep2	WT-ESC-H3K9me2-ChIP	87,970,077	59,388,157	67.51%
	WT-ESC-input	54,065,164	37,514,449	69.39%
	Mut-ESC-H3K9me2-ChIP	87,325,816	56,437,180	64.63%
	Mut-ESC-input	56,927,763	39,612,709	69.58%
	WT-RA-H3K9me2-ChIP	160,111,523	107,076,147	66.88%
	WT-RA-input	87,417,918	60,307,324	68.99%
	Mut-RA-H3K9me2-ChIP	135,473,619	89,927,104	66.38%
	Mut-RA-input	78,979,683	55,164,902	69.85%
Rep3	WT-ESC-H3K9me2-ChIP	43,039,558	30,038,960	69.79%
	WT-ESC-input	64,985,331	46,385,557	71.38%
	Mut-ESC-H3K9me2-ChIP	51,521,142	34,389,342	66.75%
	Mut-ESC-input	73,513,062	52,689,438	71.67%
	WT-RA-H3K9me2-ChIP	63,770,383	47,600,048	74.64%
	WT-RA-input	99,818,550	70,430,875	70.56%
	Mut-RA-H3K9me2-ChIP	57,593,598	41,274,037	71.66%
	Mut-RA-input	79,698,645	55,021,699	69.04%
Rep1	WT-RA-RNASeq	61,395,164	58,039,354	94.53%
	Mut-RA-RNASeq	58,600,606	55,525,098	94.75%
	WT-ESC-RNASeq	60,040,755	57,755,123	96.19%
	Mut-ESC-RNASeq	56,570,531	55,627,909	98.33%
Rep2	WT-RA-RNASeq	28,985,182	27,982,795	96.54%
	Mut-RA-RNASeq	24,102,364	22,745,515	94.37%
	WT-ESC-RNASeq	29,192,128	28,801,683	98.66%
	Mut-ESC-RNASeq	25,702,281	25,294,095	98.41%
H3K9me1	WT-ESC-H3K9me1-ChIP	80,565,505	63,530,116	78.86%
	Mut-ESC-H3K9me1-ChIP	87,248,558	68,447,868	78.45%
	WT-RA-H3K9me1-ChIP	169,158,915	140,252,959	82.91%
	Mut-RA-H3K9me1-ChIP	179,858,424	149,948,834	83.37%

Supplemental Table 4. Primers sequences for ChIP-qPCR and RT-qPCR.

Olfr18_ChIP_F	TGTCACCCCTATGCTCAACCC
Olfr18_ChIP_R	GGAAACAGAATAAATGGCACCG
Gapdh_ChIP_F	CTCTCTTTGGACCCGCCTCATTT
Gapdh_ChIP_R	GAGTCCTATCCTGGGAACCATCAC
Magea2_ChIP_F	TTGGTGGACAGGGAAGCTAGGGGA
Magea2_ChIP_R	CGCTCCAGAACAAAATGGCGCAGA
Oct4_ChIP_enh_F	TCACCCTGGGGCCTTCGTTCA
Oct4_ChIP_enh_R	CCCATTCCTGTGCTGGCGGA
Oct4_ChIP_prom_F	GTGGGTAAGCAAGAACTGAGGA
Oct4_ChIP_prom_R	TGGAGAGCCTAAAACATCCATT
Oct4_ChIP_5k_F	TGTCTACAATGCCAGAATAGGTCAA
Oct4_ChIP_5k_R	GCCTACAGAGTTCCAGATAGCC
Nanog_ChIP_F	GAATAGGGGGTGGGTAGGGT
Nanog_ChIP_R	AGCCTTCCCACAGAAAGAGC
Nanog_ChIP_F	ACAATGTCCATGGTGGACCC
Nanog_ChIP_R	ACCCTACCCACCCCCTATTC
Fgf4_ChIP_F	TCTCCAGGTGACAGTAGCCA
Fgf4_ChIP_R	GAGCAAATAGGGACGCAACG
Fgf4_ChIP_F	CTGCCCCAAGGTGAAGGAAT
Fgf4_ChIP_R	TGTGGAAGAGAAGCAGGCAG
Esrrb_ChIP_F	ATGCTTAACGCCATCCCCAA
Esrrb_ChIP_R	AATCCAGGTCTTGAGCTGCC
Esrrb_RT_F	GAACACTCTCGCCTGGTAGG
Esrrb_RT_R	CGCCTCCAGGTTCTCAATGT
Fgf4_RT_F	TGGGCCTCAAAAGGCTTCG
Fgf4_RT_R	ACTCCGAAGATGCTCACCAC
Oct4_RT_F	TGAGAACCTTCAGGAGATATGCAA
Oct4_RT_R	CTCAATGCTAGTTCGCTTTCTCTTC
Nanog_RT_F	TCGAATTCTGGGAACGCCTC
Nanog_RT_R	GTCTTCAGAGGAAGGGCGAG
Gapdh_RT_F	CTTTGGCATTGTGGAAGGGC
Gapdh_RT_R	CAGGGATGATGTTCTGGGCA