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Supplemental Information

PRMT5 Protects Genomic Integrity during Global DNA Demethylation in Primordial Germ Cells and Preimplantation Embryos

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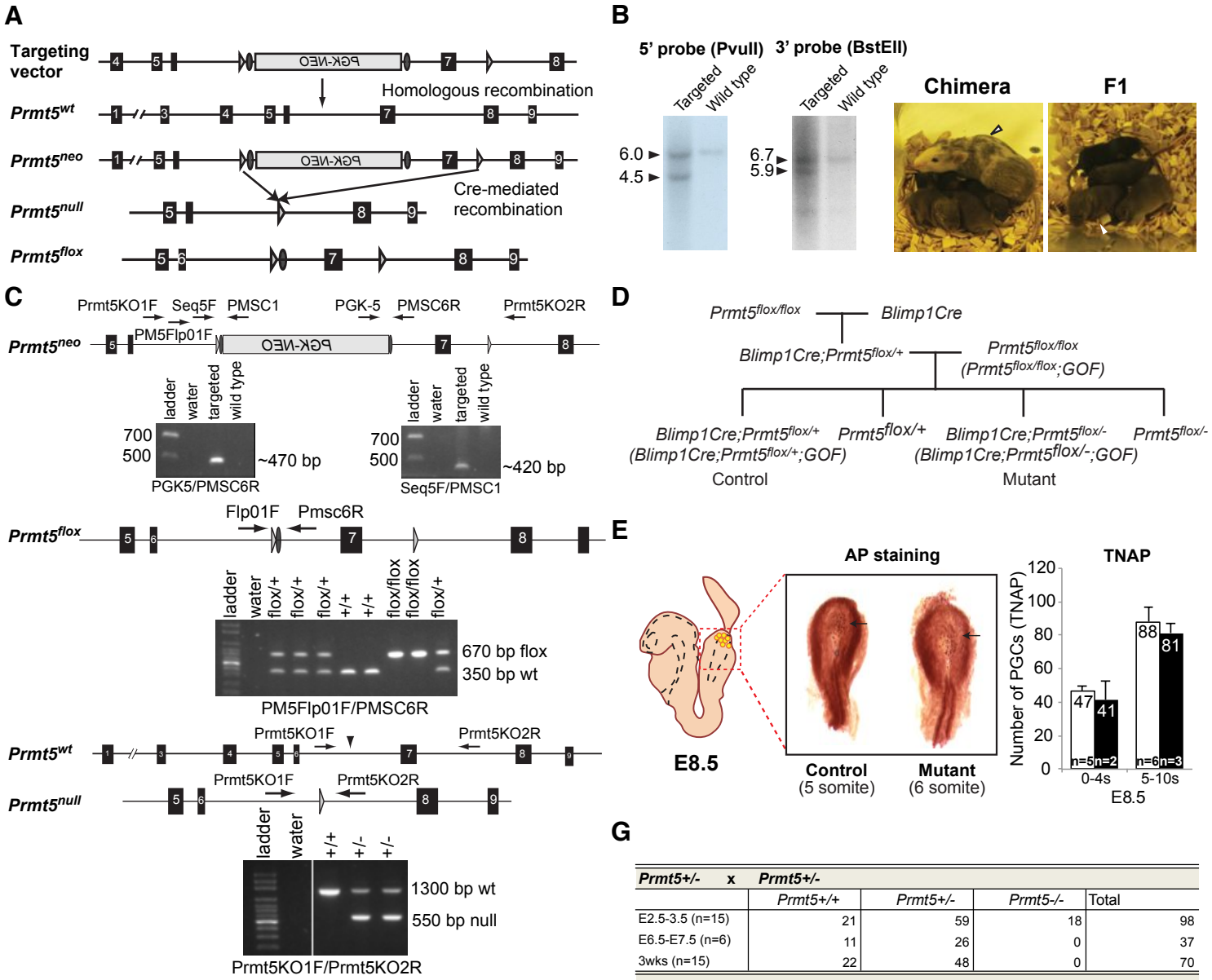
Figure S1



G

Prmt5+/- x ***Prmt5+/-***				
	Prmt5+/+	*Prmt5+/-*	*Prmt5-/-*	Total
E2.5-3.5 (n=15)	21	59	18	98
E6.5-E7.5 (n=6)	11	26	0	37
3wks (n=15)	22	48	0	70

Blimp1Cre;Prmt5+/- x ***Prmt5 flox/flox***					
	Blimp1Cre;Prmt5 flox/+	*Blimp1Cre;Prmt5 flox/-*	*Prmt5 flox/+*	*Prmt5 flox/-*	Total
E8.5-E16.5 (n=78)	88	68	64	69	289
3wks (n=17)	18	13	12	14	57

Zp3Cre;Prmt5+/- x ***Prmt5 flox/flox***					
	Zp3Cre;Prmt5 flox/+	*Zp3Cre;Prmt5 flox/-*	*Prmt5 flox/+*	*Prmt5 flox/-*	Total
3wks (n=12)	26	25	29	22	102

Zp3Cre;Prmt5 flox/+ x ***B6***					
	Zp3Cre;Prmt5 +/+	*Zp3Cre;Prmt5 +/-*	*Prmt5 +/+*	*Prmt5 +/-*	Total
3wks (n=4)	13	14	7	7	41

Zp3Cre;Prmt5 flox/- x ***B6***			
	Zp3Cre;Prmt5 +/-	*Prmt5 +/-*	Total
3wks (n=5)	9	12	21

Zp3Cre;Prmt5 flox/- x ***Prmt5+/-***			
	Prmt5 +/-	*Prmt5 -/-*	Total
E2.5-3.5 (n=8)	25	23	48

Sox2Cre;Prmt5+/- x ***Prmt5 flox/flox***					
	Sox2Cre;Prmt5 flox/+	*Sox2Cre;Prmt5 flox/-*	*Prmt5 flox/+*	*Prmt5 flox/-*	Total
E6.5-E8.5 (n=7)	17	16	19	14	66

n= number of litter that is genotyped.

Figure S1 related to Figure 1.

(A) A schematic diagram showing the targeting strategy for the generation of a conditional allele of *Prmt5*. FRT sites are shown as ovals and LoxP sites as triangles. Cre-mediated deletion removed exon7 and generated an early stop codon at exon8 to cut off the methyltransferase domain of *Prmt5*.

(B) Left panel: Southern blotting of the targeted allele and an example of a positive ES clone. The 5' probe hybridized on the left arm of the allele (4.5 kb targeted, 6.0 kb wild type) and the 3' probe detected the right arm (5.9 kb targeted, 6.7 kb wild type). Right panel: correctly targeted ES clone (E14 cells; 129P1 background) was injected into C57BL6/J blastocysts and showed high contribution to chimeras (white and agouti coat color; arrowhead). F1 agouti mice (white arrowhead) show successful germline transmission of the targeted allele.

(C) Schematic diagram of the targeted allele and location of primers for genotyping. An example for each genotyping PCR is shown.

(D) Breeding scheme to delete *Prmt5* in PGCs. *Blimp1Cre;Prmt5*$^{flox/+}$ (PGC: *Prmt5*$^{+/-}$) embryos were used as a control when littermates were available and *Blimp1Cre;Prmt5*$^{flox/-}$ (PGC: *Prmt5*$^{-/-}$) were used as the mutant. The GOF transgene refers to *Oct4ΔPE-GFP*, which is expressed in PGCs.

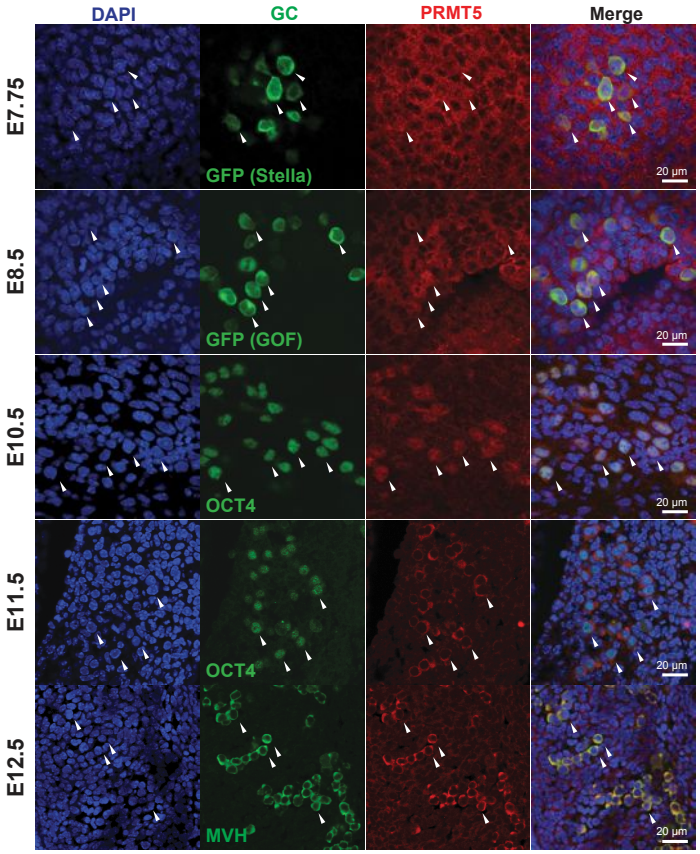
(E) Alkaline phosphatase (AP) staining with control (*Blimp1Cre;Prmt5*$^{flox/+}$) and mutant (*Blimp1Cre;Prmt5*$^{flox/-}$) embryos at E8.5. Shown is the basal allantoic region. Brown spot indicates AP positive PGCs (arrow). Right graph shows counted AP positive PGCs in control (white bar) and mutant embryos (black bar). n=number of embryos.

(F) Representative image of embryos from E11.5 and E12.5. Left panel: control (*Blimp1Cre;Prmt5*$^{flox/+}$) and mutant (*Blimp1Cre;Prmt5*$^{flox/-}$) embryo at E11.5. Right panel: female (left) and male (right) embryos at E12.5. No apparent morphological differences are observed between control and mutant at both stages.

(G) Genotyping results for embryos and/or litters from indicated matings.

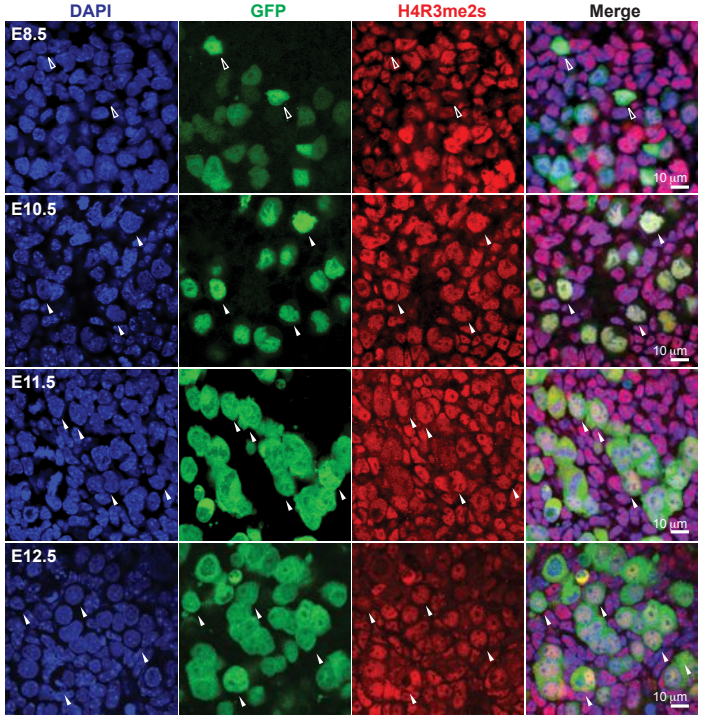
Figure S2

A



	PGCs	Nuclear PRMT5	%
E7.75	26	16	61.5
E8.5	136	135	99.3
E10.5	253	249	98.4
E11.5	176	11	6.3
E12.5	255	1	0.4

B



	PGCs	H2A/H4R3me2s (med/high)	%
E8.5	49	25	51.0
E10.5	217	181	83.4
E11.5	176	140	79.5
E12.5	263	164	62.4

C

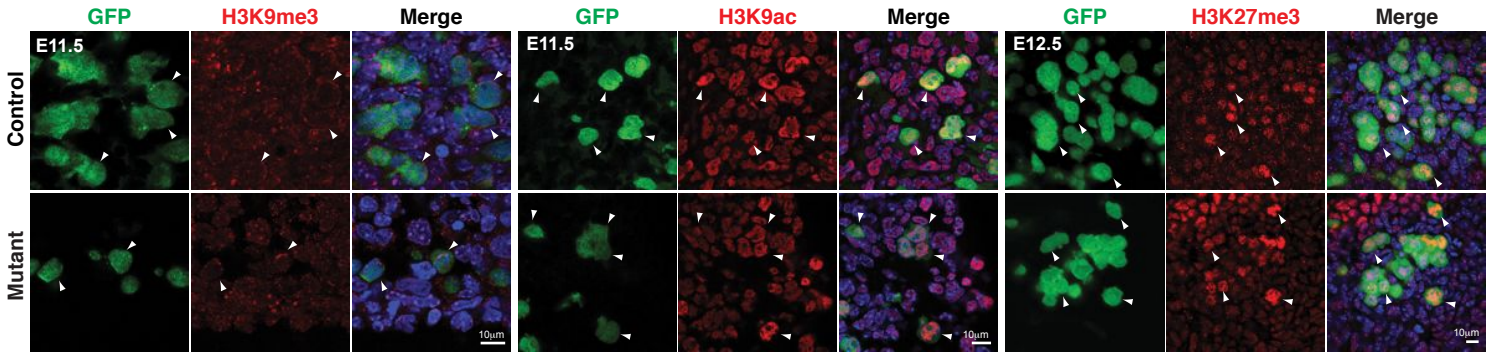


Figure S2 related to Figure 1 and 2

(A) Immunofluorescence stainings for PRMT5 (red) and germ cell markers (as indicated; green) in PGCs (arrowheads) during development. Scale bar represents 20 μm. Bottom table shows the number of PGCs (left), number of PGCs that has nuclear PRMT5 (middle) from each stage and the percentage of PGCs that shows nuclear PRMT5 (right).

(B) Genital ridges from mice with the GOF transgene at E8.5 to E12.5 were stained for H2A/H4R3me2s (red) and GFP (green). The intensity of H2A/H4R3me2s in PGCs were categorized either as medium/high (filled arrowhead) or low (empty arrowhead) compared to the intensity of surrounding somatic cells. The table shows the number of counted GFP positive PGCs (left), the number of medium/high H2A/H4R3me2s PGCs (middle) and the percentage of PGCs that shows medium/high H2A/H4R3me2s (right).

(C) Genital ridges from mice with the GOF transgene were stained for histone modifications (red) and GFP (green) in genital ridges. Stages are indicated and arrowheads show PGCs. Scale bar: 10 μm.

Figure S3

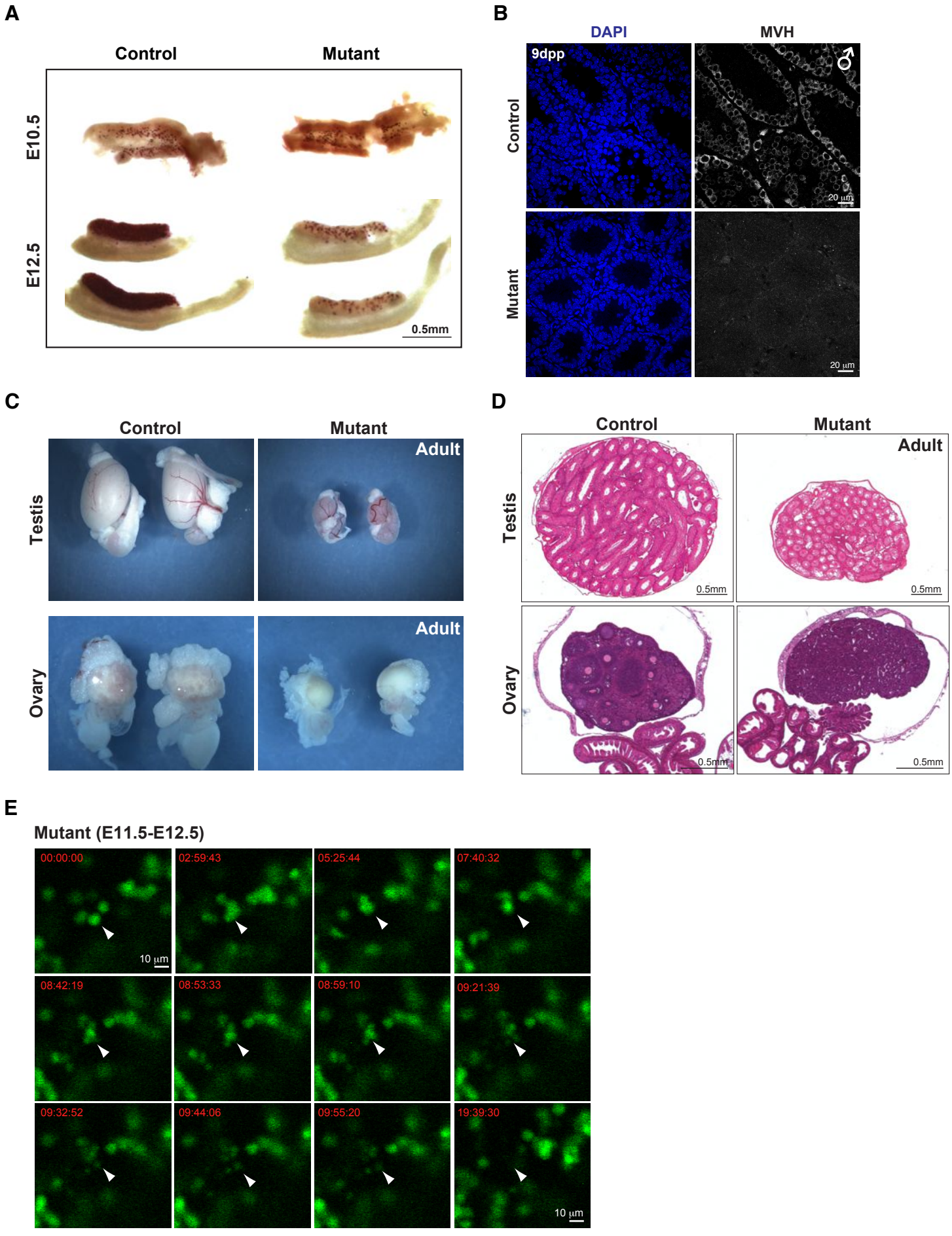

A
Control
Mutant
E10.5
E12.5
0.5mm
B
DAPI
MVH
9dpp
Control
Mutant
♂
20 μm
20 μm
C
Control
Mutant
Testis
Ovary
Adult
Adult
D
Control
Mutant
Testis
Ovary
Adult
0.5mm
0.5mm
0.5mm
0.5mm
E
Mutant (E11.5-E12.5)
00:00:00
02:59:43
05:25:44
07:40:32
08:42:19
08:53:33
08:59:10
09:21:39
09:32:52
09:44:06
09:55:20
19:39:30
10 μm
10 μm

Figure S3 related with Figure 3.

(A) Alkaline phosphatase (AP) staining of genital ridges at E10.5 and E12.5 female. Brown spots indicate AP positive PGCs.

(B) Postnatal day 9 testes were collected and stained for MVH (white) and DAPI (blue).

(C) Adult testis and ovary from control (left) and mutant (right) mice.

(D) Lack of germ cells in mutant adults is shown in hematoxylin and eosin (HnE) stainings of sections from testis and ovary.

(E) Captured images of mutant germ cells during live-imaging. E11.5 genital ridges with a GOF transgenes were imaged *ex-vivo* for 21 hours. Arrowhead indicates mutant PGCs that underwent fragmentation from ~9 hour after imaging. Scale bar, 10 μm. See also Movie S1 (Mutant) and S2 (Control).

Figure S4

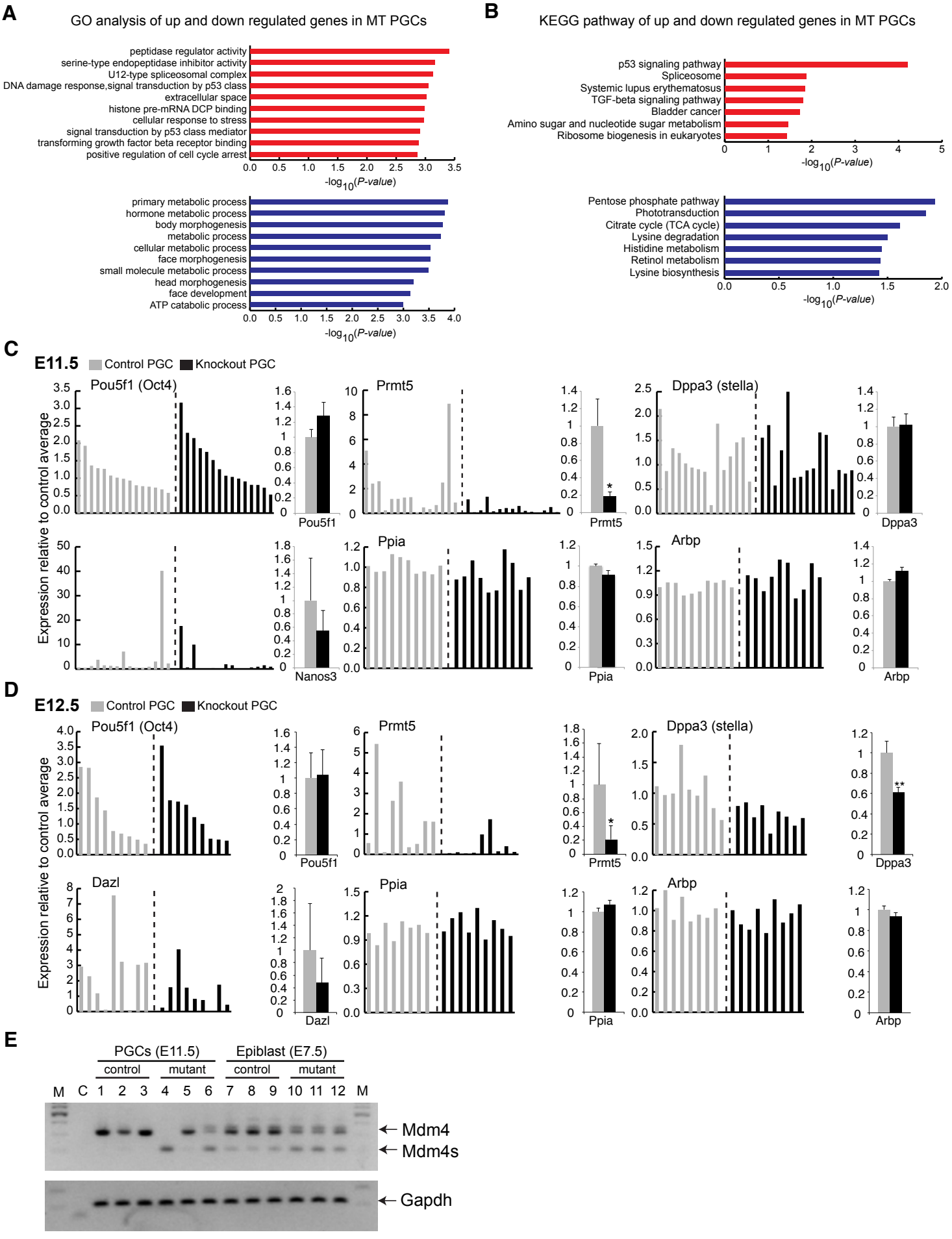

A
GO analysis of up and down regulated genes in MT PGCs
peptidase regulator activity
serine-type endopeptidase inhibitor activity
U12-type spliceosomal complex
DNA damage response,signal transduction by p53 class
extracellular space
histone pre-mRNA DCP binding
cellular response to stress
signal transduction by p53 class mediator
transforming growth factor beta receptor binding
positive regulation of cell cycle arrest
0.0 0.5 1.0 1.5 2.0 2.5 3.0 3.5
-log10(P-value)
primary metabolic process
hormone metabolic process
body morphogenesis
metabolic process
cellular metabolic process
face morphogenesis
small molecule metabolic process
head morphogenesis
face development
ATP catabolic process
0.0 0.5 1.0 1.5 2.0 2.5 3.0 3.5 4.0
-log10(P-value)
B
KEGG pathway of up and down regulated genes in MT PGCs
p53 signaling pathway
Spliceosome
Systemic lupus erythematosus
TGF-beta signaling pathway
Bladder cancer
Amino sugar and nucleotide sugar metabolism
Ribosome biogenesis in eukaryotes
0 1 2 3 4 5
-log10(P-value)
Pentose phosphate pathway
Phototransduction
Citrate cycle (TCA cycle)
Lysine degradation
Histidine metabolism
Retinol metabolism
Lysine biosynthesis
0.0 0.5 1.0 1.5 2.0
-log10(P-value)
C
E11.5
Control PGC
Knockout PGC
Expression relative to control average
Pou5f1 (Oct4)
Pou5f1
Prmt5
Prmt5
Dppa3 (stella)
Dppa3
Nanos3
Nanos3
Ppia
Ppia
Arbp
Arbp
D
E12.5
Control PGC
Knockout PGC
Expression relative to control average
Pou5f1 (Oct4)
Pou5f1
Prmt5
Prmt5
Dppa3 (stella)
Dppa3
Dazl
Dazl
Ppia
Ppia
Arbp
Arbp
E
PGCs (E11.5)
Epiblast (E7.5)
control
mutant
control
mutant
M C 1 2 3 4 5 6 7 8 9 10 11 12 M
Mdm4
Mdm4s
Gapdh

Figure S4 related to Figure 4.

(A) Gene Ontology (GO) analysis based on the RNA-seq data shows up (red) and down (blue) regulated genes in mutant (MT) PGCs.

(B) Analysis using the Kyoto Encyclopedia of Genes and Genomes (KEGG) shows up (red) and down (blue) regulated pathways in mutant (MT) PGCs.

(C and D) qRT-PCR analysis of selected genes in PGCs at E11.5 (C) and E12.5 (D). E12.5 PGCs are from male. Gene expression levels relative to the average of controls are shown. Each sample was ordered according to the level of *Pou5f1* (Oct4), which values were normalized to *Gapdh*. Transcript levels are shown for PGC genes and two housekeeping genes (*Arbp*, *Ppia*). Right graph shows the average gene expression values +/-SE of all cells.

(E) Semi-quantitative PCR of the skipped exon on the *Mdm4* in PGCs and post-implantation epiblast (E7.5) using previously reported primer set (Bezzi et al., 2013). Mdm4s indicates the short form of *Mdm4* with skipped exon7. Mdm4s band become comparable to wild type full length Mdm4 band upon loss of PRMT5 in PGCs and the E7.5 epiblasts. Housekeeping gene *Gapdh* was used as a loading control.

See also TableS2.

Figure S5

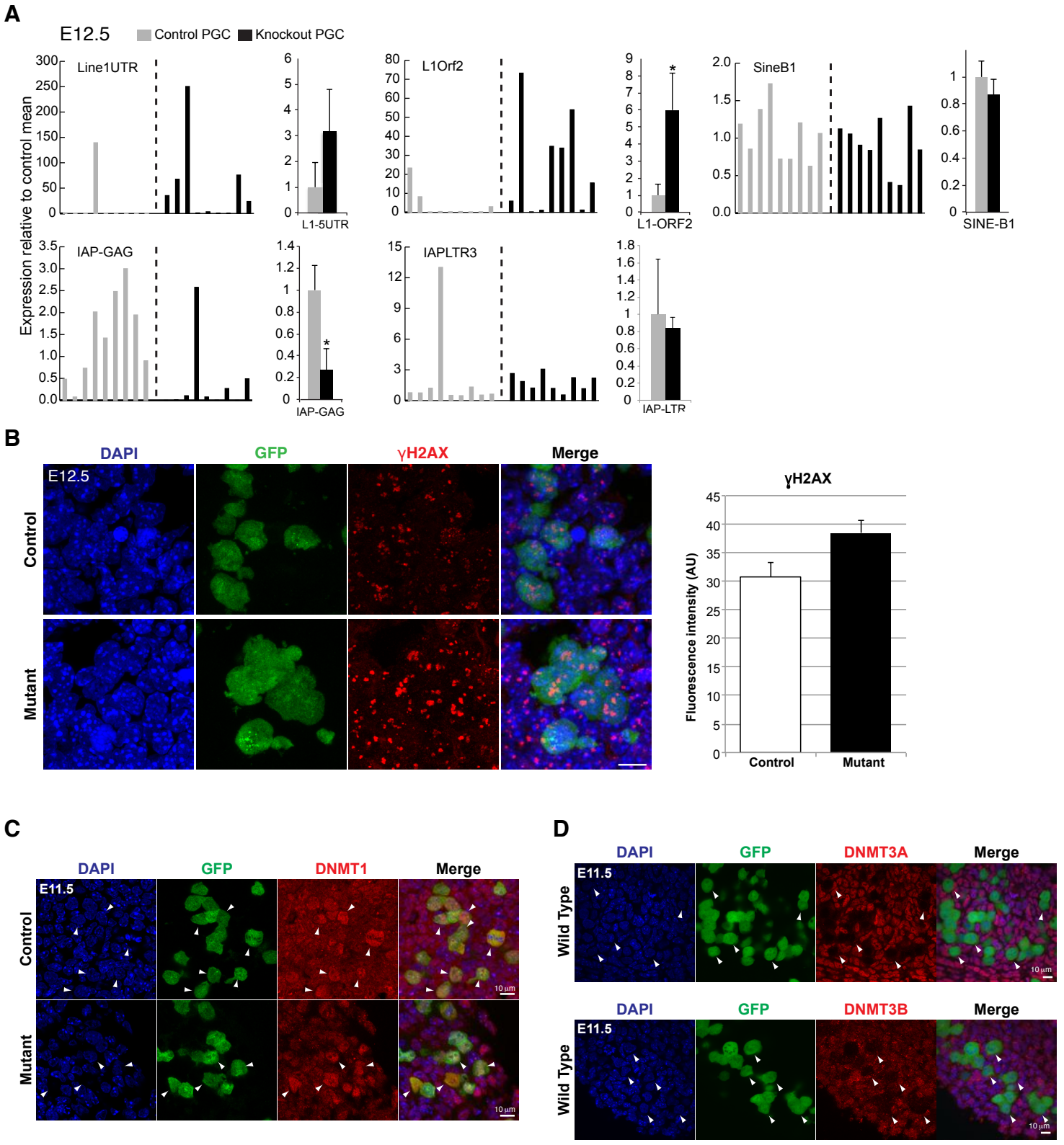

A
E12.5
Control PGC
Knockout PGC
Expression relative to control mean
Line1UTR
L1-5UTR
L1Orf2
L1-ORF2
SineB1
SINE-B1
IAP-GAG
IAP-GAG
IAPLTR3
IAP-LTR
B
DAPI
GFP
γH2AX
Merge
E12.5
Control
Mutant
γH2AX
Fluorescence intensity (AU)
Control
Mutant
C
DAPI
GFP
DNMT1
Merge
E11.5
Control
Mutant
10 μm
D
DAPI
GFP
DNMT3A
Merge
E11.5
Wild Type
DAPI
GFP
DNMT3B
Merge
E11.5
Wild Type
10 μm

Figure S5 related to Figure 5.

(A) Single cell qPCR analysis of indicated TEs in male PGCs at E12.5. Each bar represents one cell. Chart on the right shows the average mean values +/- SE of all cells. Significance was tested by Student's t-test: * $p<0.05$

(B) Immunofluorescence staining of γH2AX (red) in female genital ridges at E12.5. The quantification shows the mean fluorescence intensity +/- SE in GFP positive area (PGCs). Scale bars, 10 μm.

(C) Immunofluorescence staining of DNMT1 (red) in genital ridges at E11.5. Arrowheads indicate PGCs. Scale bars, 10 μm.

(D) Immunofluorescence staining of DNMT3A or DNMT3B (red) in wildtype genital ridges at E11.5. Arrowheads indicate PGCs. Scale bars, 10 μm.

Figure S6

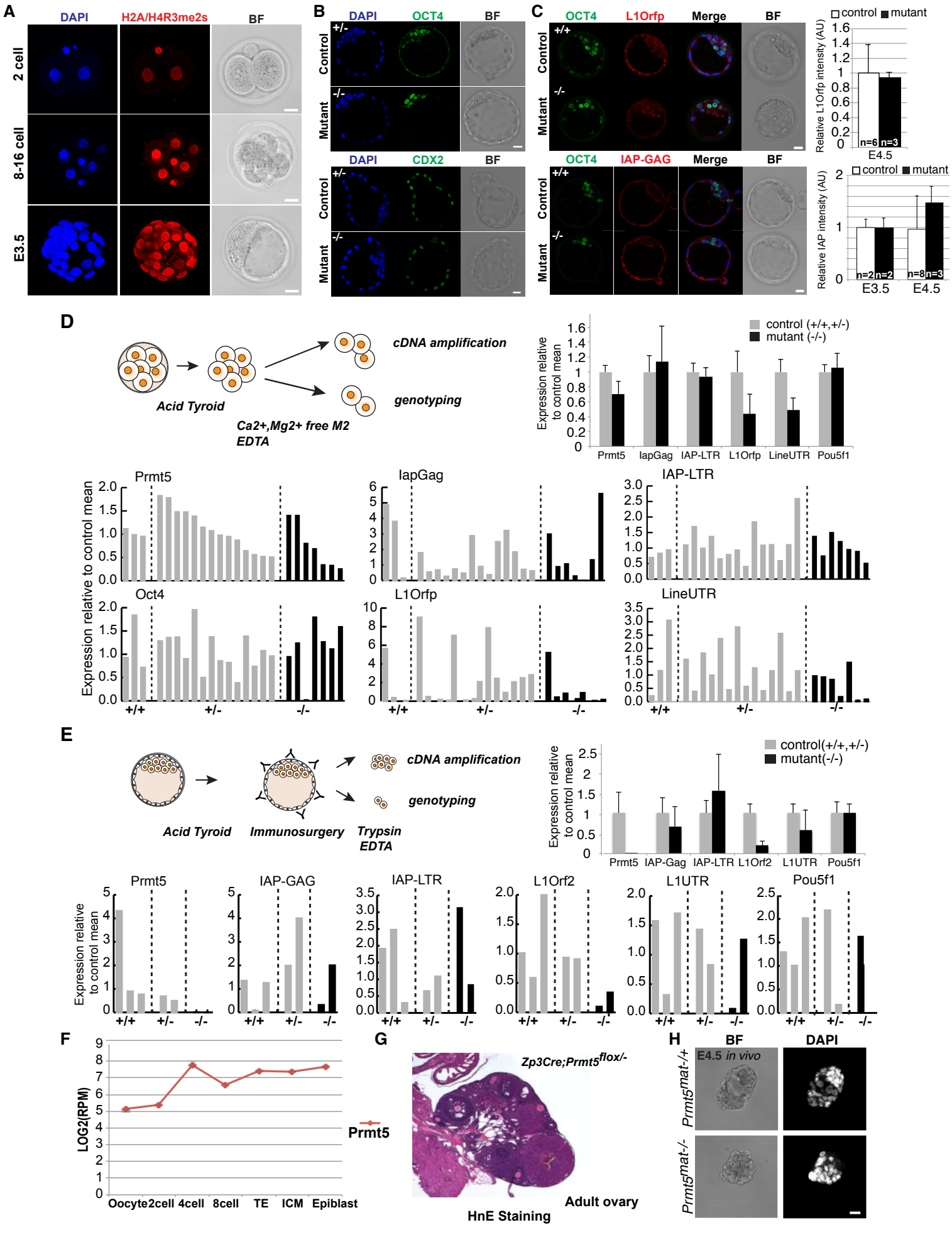

A
DAPI
H2A/H4R3me2s
BF
2 cell
8-16 cell
E3.5
B
DAPI
OCT4
BF
Control
Mutant
+/-
-/-
DAPI
CDX2
BF
C
OCT4
L1Orfp
Merge
BF
+/+
control
mutant
Relative L1Orfp intensity (AU)
n=6
n=3
E4.5
OCT4
IAP-GAG
Merge
BF
Relative IAP intensity (AU)
n=2
n=2
n=8
n=3
E3.5
E4.5
D
cDNA amplification
genotyping
Acid Tyroid
Ca2+,Mg2+ free M2
EDTA
control (+/+,+/-)
mutant (-/-)
Expression relative to control mean
Prmt5
IapGag
IAP-LTR
L1Orfp
LineUTR
Pou5f1
Oct4
+/+
+/-
-/-
E
cDNA amplification
genotyping
Acid Tyroid
Immunosurgery
Trypsin
EDTA
control(+/+,+/-)
mutant(-/-)
Prmt5
IAP-Gag
IAP-LTR
L1Orf2
L1UTR
Pou5f1
IAP-GAG
F
LOG2(RPM)
Oocyte
2cell
4cell
8cell
TE
ICM
Epiblast
Prmt5
G
Zp3Cre;Prmt5flox/-
Adult ovary
HnE Staining
H
BF
DAPI
E4.5 in vivo
Prmt5mat-/+
Prmt5mat-/-

Figure S6 related to Figure 6.

(A) Immunofluorescence staining for H2A/H4R3me2s (red) in wild type 2-cell to blastocyst stage preimplantation embryos (stages are indicated). Scale bars, 20 μm.

(B) Immunofluorescent staining for OCT4 (top panel; green) or CDX2 (bottom panel; green) in *Prmt5* heterozygous (+/-) and null (-/-) preimplantation embryos (E4.5).

(C) Immunofluorescent staining of E4.5 control (+/+) and *Prmt5* mutant (-/-; zygotic *Prmt5* null) embryos for L1Orfp (top panel; red), IAP-GAG (bottom panel; red) and OCT4 (green) antibody. The quantification shows the relative fluorescence intensity +/- SD (n=number of embryos), which was measured from 10 z-sections (increment: 4 μm). Bottom panel: Immunofluorescence staining with IAP-GAG (red) at E4.5 embryos.

(D and E) Schematic diagram showing the experimental scheme for cDNA amplification from preimplantation embryos. From each embryo, two blastomeres were used for genotyping and the rest was used for cDNA amplification. qPCR analysis for indicated genes and TEs using cDNAs from control (+/+), *Prmt5* heterozygous (+/-) and *Prmt5* mutant (-/-; zygotic *Prmt5* null) embryos at E2.5 (D) and E4.5 (E) is shown. Each bar corresponds to one single embryo. Each sample was ranked in order of the level of *Prmt5* expression. Average expression +/- SE is shown in the top right graph.

(F) Single cell RNA-seq analysis of *Prmt5* in oocyte and preimplantation embryos. Values are shown in log 2 reads per million (RPM).

(G) Hematoxylin and Eosin staining of ovary sections from adult maternal *Prmt* knockout mice (*Zp3Cre;Prmt5*$^{flox/-}$).

(H) A representative bright field (BF) and DAPI staining image of control (*Prmt5*$^{mat-/+}$) and mutant (*Prmt5*$^{mat-/-}$) embryo at E4.5 *in vivo.* Note that the mutant embryo does not form a blastocoel and looks morphologically abnormal.

Figure S7

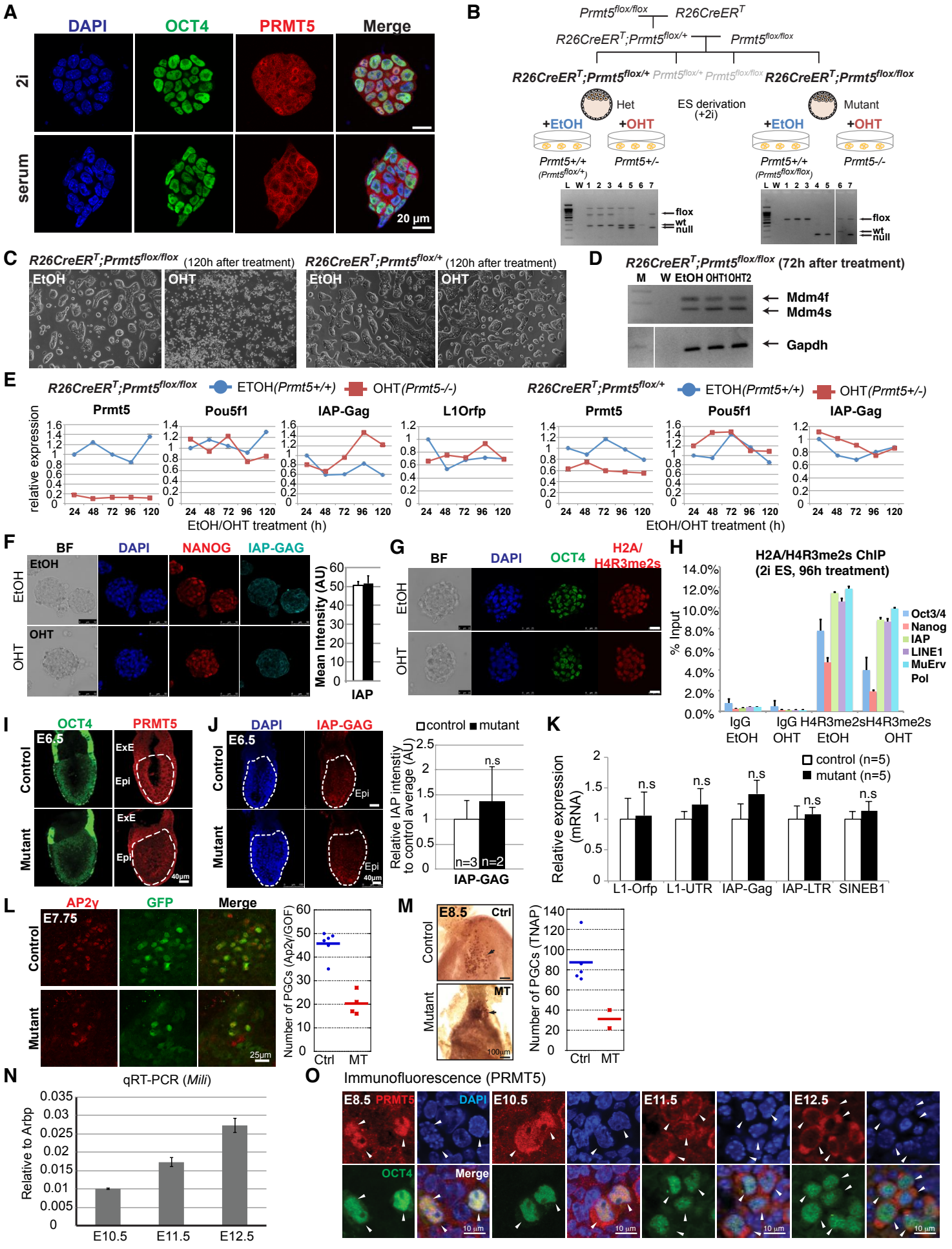

A
DAPI
OCT4
PRMT5
Merge
2i
serum
20 µm
B
Prmt5flox/flox
R26CreERT
R26CreERT;Prmt5flox/+
Prmt5flox/flox
R26CreERT;Prmt5flox/+
Prmt5flox/+
Prmt5flox/flox
R26CreERT;Prmt5flox/flox
Het
ES derivation (+2i)
Mutant
+EtOH
+OHT
+EtOH
+OHT
Prmt5+/+ (Prmt5flox/+)
Prmt5+/-
Prmt5+/+ (Prmt5flox/flox)
Prmt5-/-
L W 1 2 3 4 5 6 7
L W 1 2 3 4 5 6 7
flox
wt
null
flox
wt
null
C
R26CreERT;Prmt5flox/flox (120h after treatment)
R26CreERT;Prmt5flox/+ (120h after treatment)
EtOH
OHT
EtOH
OHT
D
R26CreERT;Prmt5flox/flox (72h after treatment)
M W EtOH OHT1OHT2
Mdm4f
Mdm4s
Gapdh
E
R26CreERT;Prmt5flox/flox
ETOH(Prmt5+/+)
OHT(Prmt5-/-)
R26CreERT;Prmt5flox/+
ETOH(Prmt5+/+)
OHT(Prmt5+/-)
Prmt5
Pou5f1
IAP-Gag
L1Orfp
Prmt5
Pou5f1
IAP-Gag
relative expression
EtOH/OHT treatment (h)
EtOH/OHT treatment (h)
F
BF
DAPI
NANOG
IAP-GAG
EtOH
OHT
Mean Intensity (AU)
IAP
G
BF
DAPI
OCT4
H2A/ H4R3me2s
EtOH
OHT
H
H2A/H4R3me2s ChIP (2i ES, 96h treatment)
% Input
Oct3/4
Nanog
IAP
LINE1
MuErv
Pol
IgG EtOH
IgG OHT
H4R3me2s EtOH
H4R3me2s OHT
I
OCT4
PRMT5
E6.5
Control
Mutant
ExE
Epi
40µm
J
DAPI
IAP-GAG
E6.5
Control
Mutant
Epi
control
mutant
Relative IAP intensity to control average (AU)
n.s
n=3
n=2
IAP-GAG
K
Relative expression (mRNA)
control (n=5)
mutant (n=5)
n.s
L1-Orfp
L1-UTR
IAP-Gag
IAP-LTR
SINEB1
L
AP2γ
GFP
Merge
E7.75
Control
Mutant
25µm
Number of PGCs (Ap2γ/GOF)
Ctrl
MT
M
E8.5
Ctrl
MT
Control
Mutant
100µm
Number of PGCs (TNAP)
Ctrl
MT
N
qRT-PCR (Mili)
Relative to Arbp
E10.5
E11.5
E12.5
O
Immunofluorescence (PRMT5)
E8.5
PRMT5
DAPI
OCT4
Merge
E10.5
E11.5
E12.5
10 µm

Figure S7 related to Figure 6 and 7

(A) A representative image of wild type ES cells grown in 2i (upper panel) and serum (bottom panel). PRMT5 (red), OCT4 (green) immunofluoresence staining is shown. Note that there is heterogeneous nuclear localization of PRMT5 in '2i' cultured ES cells.

(B) Top panel: Schematic diagram of the derivation protocol of inducible *Prmt5* knockout ES cells. *R26Cre;Prmt5*$^{flox/+}$ (het) and *R26Cre;Prmt5*$^{flox/flox}$ (conditional null) are shown. Each ES cell line was derived in 2i and cultured with 2i and EtOH or OHT.
Bottom panel: genotyping result of induced *Prmt5* excision. Lane L:DNA ladder, lane W:water, lane 1: no treatment (0h), 2: no treatment (24h), 3: EtOH (24h), 4: 50 nM OHT (24h), 5: 100 nM OHT (24h), 6: *Prmt5*$^{flox/+}$ control, 7: *Prmt5*$^{flox/-}$ control.

(C) Bright field image of *R26CreER*^{T}*;Prmt5*$^{flox/flox}$ cells 120 hour after EtOH or OHT treatment (left panel), and *R26CreER*^{T}*;Prmt5*$^{flox/+}$ cells (right panel).

(D) Semi-quantitative PCR of the skipped exon of the Mdm4 in *R26CreER*^{T}*;Prmt5*$^{flox/flox}$ cells 72h after EtOH or OHT treatment using previously reported primer set (Bezzi et al., 2013). M; DNA ladder, W; water, EtOH (72h), OHT-1;50 nM OHT, OHT-2; 100 nM OHT. Mdm4s indicates the short form of Mdm4 with skipped exon7. Mdm4s band become more prominent upon OHT treatment compared to EtOH treated control.

(E) Time-course qRT-PCR for ES cells. After treatment with EtOH (control, blue) and OHT (experimental, red) for 24h, 48h, 72h, 96h, 120h are shown. Homozygous *R26CreER*^{T}*;Prmt5*$^{flox/flox}$ line showed significant reduction in *Prmt5* whereas the heterozygous *R26CreER*^{T}*;Prmt5*$^{flox/+}$ line showed about 40% reduction after OHT treatment. At 96h OHT treatment, IAP-Gag is slightly upregulated (~1.8 fold) compared to the IAP-Gag expression of 96h EtOH treated ES cells. Graphs are shown for the relative expression of 24h EtOH treated ES cells.

(F) Immunofluorescence staining with IAP-GAG antibody in *R26CreER*^{T}*;Prmt5*$^{flox/flox}$ ES cells after 96h EtOH (control, top panel) or OHT (mutant, bottom panel) treatment. Right graph shows mean intensity of IAP-GAG staining (n=3, for EtOH and OHT).

(G) Immunofluoresence staining with an H2A/H4R3me2s antibody (red) in *R26CreER*^{T}*;Prmt5*$^{flox/flox}$ ES cells after 96h EtOH (control, top panel) or OHT (mutant, bottom panel) treatment. Only some cells showed slightly lower staining but overall the levels were not significantly different between EtOH and OHT treated cells.

(H) ChIP with *R26CreER^{T};Prmt5$^{flox/flox}$* ES cells with 96h EtOH or OHT treatment using a H2A/H4R3me2s antibody and IgG, respectively. ChIP-qPCR results +/- SE are shown. The Oct4 and Nanog locus served as negative contols.

(I) Immunofluorescence staining for epiblast marker OCT4 (green) and PRMT5 (red) in *Sox2Cre;Prmt5$^{flox/+}$* and *Sox2Cre;Prmt5$^{flox/-}$* embryos at E6.5. PRMT5 is deleted specifically in the epiblast (Epi) but not in the extraembryonic ectoderm (ExE). Dashed line indicates epiblast.

(J) Immunofluorescence staining for IAP-GAG (red) in control (*Sox2Cre;Prmt5$^{flox/+}$*) and mutant (*Sox2Cre;Prmt5$^{flox/-}$*) embryos at E6.5. The epiblast (Epi) is indicated by the dashed line. The measured intensity of IAP-GAG in control and mutant embryos is not significantly different.

(K) qRT-PCR analysis of TEs with epiblasts of E7.5 from control (*Sox2Cre;Prmt5$^{flox/+}$*,white) and mutant (*Sox2Cre;Prmt5$^{flox/-}$*, black). Shown are the mean values +/- SE. Control and mutant epiblasts do not show significant difference. Significance is shown by Student's t test, n.s : not significant $p>0.05$.

(L) Immunofluorescence staining AP2γ (red) and GFP (green, Stella-GFP) of E7.75 embryos. Right graph shows the quantification of counted germ cells with AP2γ and GFP positive PGCs at E7.5 from control (*Sox2Cre;Prmt5$^{flox/+}$;Stella-GFP,* n=6) and mutant (*Sox2cre;Prmt5$^{flox/-}$;Stella-GFP*, n=4) embryos.

(M) AP staining of E8.5 control (*Sox2Cre;Prmt5$^{flox/+}$*) and mutant (*Sox2Cre;Prmt5$^{flox/-}$*) embryos. Arrow indicates AP positive PGCs. Right graph shows the quantification of the counted germ cells of control (n=5) and mutant (n=2) at E8.5.

(N) qRT-PCR analysis of *Mili* by qRT-PCR. *Mili* expression was calculated relative to the expression of the housekeeping gene *Arbp*.

(O) Immunofluorescence staining of PRMT5 (red) and OCT4 (green) in genital ridges from E8.5 to E12.5. White arrowheads indicate OCT4 positive PGCs. Scale bar, 10 μm.

Table S1 related to Figure 4

Microarray data with E11.5 female and male PGCs. List of up- and downregulated genes in female mutant PGCs (genotype: *Blimp1Cre;Prmt5$^{flox/-}$;GOF*) compared to control (*Blimp1Cre;Prmt5$^{flox/+}$;GOF*) are shown.

Table S2 related to Figure 4

List of differentially expressed genes of E11.5 female mutant PGCs (*Blimp1Cre;Prmt5$^{flox/-}$;GOF*) compared with control (*Blimp1Cre;Prmt5$^{flox/+}$;GOF*) from RNA-Seq analysis.

Table S3 related to Figure 5

List of repeats and their expressions of E11.5 female PGCs of mutants (*Blimp1Cre;Prmt5$^{flox/-}$;GOF*) and control (*Blimp1Cre;Prmt5$^{flox/+}$;GOF*) identified in the RNA-seq analysis.

Tables S1-S3 are provided separately as Excel files.

Movie S1 related to Figure 3

Time lapse imaging of E11.5 mutant (*Blimp1Cre;Prmt5$^{flox/-}$:GOF*) genital ridges for 24h. Green fluorescent cells are *GOF* positive PGCs.

Movie S2 related to Figure 3

Time lapse imaging of E11.5 control (*Blimp1Cre;Prmt5$^{flox/+}$:GOF*) genital ridges for 24h. Green fluorescent cells are *GOF* positive PGCs.

Movie S1 and S2 are provided separately as Mov files.

Supplemental Experimental Procedure

Targeted disruption of *Prmt5* locus and generation of cell type-specific *Prmt5* knockout mice

The *Prmt5* targeting vector was constructed by the following scheme: *Pgk-neo* flanked by frt site and the 5' *loxP* site, we inserted, the sixth intron of *Prmt5.* 3' *loxP* site to PGK-neo cassette was inserted into the seventh intron of *Prmt5* (Figure S1). The targeting vector was linearized and electroporated into E14Tg2a ES cells (129/Ola) and selection was performed with G418 (Invitrogen). The homologous recombinants were identified from G418-resistant colonies by PCR and a single integration site was confirmed by Southern hybridization by use of a 5' and 3' DNA probes. We obtained one ES cell clone with successful gene targeting, which, upon injection into C57BL/6 blastocysts, generated high contribution chimera with germline transmission. The germline transmitted *Prmt5* targeted mice were bred with *FlpE* mice to remove *Pgk-neo* cassette to get the floxed allele ($Prmt5^{flox}$). The GOF reporter mice were bred with this $Prmt5^{flox}$ mice to generate $Prmt5^{flox/flox}$;*GOF* to trace the germ cell lineage.

Cell type-specific *Prmt5* knockout mice were generated using Cre-mediated recombination of *loxP* sites in a functional *Prmt5* allele, $Prmt5^{floxed}$. $Prmt5^{+/-}$ animals (129/Ola and C57BL/6 mixed background) were crossed with *B Lymphocyte Induced Maturation Protein 1 Cre* (*Blimp1-Cre*) animals expressing Cre in primordial germ cells (PGCs) (Ohinata et al., 2005). $Prmt5^{flox/flox}$ animals (female) were crossed with *Blimp1-Cre*; $Prmt5^{+/-}$ animals to produce *Prmt5 germ cell knockout* mutants (*Blimp1Cre;*$Prmt5^{flox/-}$). As controls, we used $Prmt5^{flox/-}$, $Prmt5^{flox/+}$, or *Blimp1Cre*;$Prmt5^{flox/+}$ littermates. For *Prmt5* epiblast knockout, we used Sox2 promoter driven *Cre* mice (Hayashi et.al, 2002) expressing Cre in early epiblast cells.

All husbandry and experiments involving mice were carried out according to the local ethics committee and were performed in a facility designated by the Home Office.

Histological analysis

Dissected testes were fixed in Bouin's fixative or phosphate buffered formalin overnight at 4 C, progressively dehydrated in a graded ethanol series and embedded in paraffin wax. Sections (6-7 μm) were deparaffinized, rehydrated, and stained with hematoxylin and eosin.

Time lapse imaging

E11.5 gonads were cultured *ex vivo* as previously described (Martineau et al. 1997) with some deviations. 1.5% agar (in DMEM) was poured in 35mm glass bottom culture dishes (MatTek) with a thin glass capillary in the center to generate a small groove. One mutant and one control gonad were placed into the groove and covered with medium containing DMEM/10% FCS/Penicillin (10000U/ml)/ Streptomycin (10mg/ml). Mutant and control gonads were imaged simultaneously using a Zeiss LSM 510 Meta confocal microscope (512x512, 5 minutes interval, 37 Z-sections, 223 frames) for ~24h at 37C and 5% CO_2. Movies and cell count data were generated using the Volocity 3D image analysis software (PerkinElmer).

Single-cell cDNA library preparation

For isolation of PGCs, genital ridges of embryos from timed crosses of mice bearing a GOF reporter were dissected and single PGCs from individual genotyped embryos sorted by GFP for GOF transgene or SSEA1 positive cells using a MoFlo MLS high-speed flow sorter (Beckman Coulter). For the RNA-seq analysis 50 pg of total RNAs of purified PGCs from control and mutant embryos were used to generate cDNA library. Generation of cDNA library and subsequent amplification were performed as described by Tang et al (Tang et al., 2010).

Quantitative real-time PCR and gene expression analysis

RNA was extracted using the Picopure kit (Life Technologies) and cDNA was synthesized using Superscript III reverse transcriptase (Life Technologies). All quantitative PCR runs were performed and analysed as detailed previously (Grabole et

al., 2013). Relative expression levels of single cell cDNA library was normalized with housekeeping genes (*Gapdh*, *Arbp* or *Ppia*) and analysed as previously described (Livak and Schmittgen, 2001).

Generation of maternal PRMT5 deleted zygotes

The *Prmt5*$^{flox/flox}$ mice were mated with Zp3-Cre transgenic mice, which express Cre recombinase under the control of the Zona pellucida glycoprotein 3 promoter (de Vries et al., 2000). Then the *Zp3-Cre;Prmt5*$^{flox/+}$ female mice were mated with *Prmt5*$^{flox/flox}$ male mice. From this mating we obtained *Zp3Cre;Prmt5*$^{flox/-}$ mice and following the deletion of the floxed allele in the oocyte, we generate ooctyes that are the null mutants for maternal PRMT5.

Isolation of preimplantation embryos and immunostaining

The morulae were flushed out from the oviduct at E2.5 from *Zp3Cre;Prmt5*$^{flox/-}$females mated with *Prmt5*$^{+/-}$ males and cultured for 2 days in M16 medium. The developed blastocysts were fixed in 2% PFA followed by immunostaining.

After all images were acquired with confocal microscope, each embryo was retrieved and genotyped to identify control and mutant.

Whole Mount Alkaline Phosphatase Staining of Embryos

Embryos were dissected in PBS, fixed in 4% (w/v) paraformaldehyde (PFA) in PBS for 2 h at 4 °C, washed twice in PBS, and then incubated in freshly prepared alkaline phosphatase (AP)-staining solution, i.e., 250 mM Tris-maleic acid, pH 9, 0.1 mg/ml 1-Naphthyl phosphate, disodium salt (Sigma N7255), and 0.5 mg/ml Fast Red TR salt (Sigma F6760), for 15 min in the dark. Embryos were cleared in 40% glycerol for 1 h and 80% glycerol overnight at 4 °C.

Wholemount immunofluorescence staining

Embryos from timed mating were dissected and processed for immunostaining as described previously (Seki et al., 2007). Images were acquired using confocal

microscope (Olympus, Leica) and analyzed with ImageJ software. Primary antibodies used were as follows: anti-PRMT5 (Millipore, 07-405, 1:500), anti-H2A/H4R3me2s (Abcam, ab5823, 1:800) anti-GFP (Nacalia Tesque, GF090R, 1:500), anti-OCT-3/4 (BD transduction Laboratories, 1:200), anti-MVH (R&D systems, AF2030, 1:500), anti-CDX2 (Biogenex, 1:200), anti-DNMT3a (IMGENEX, IMG-268A, 1:200), anti DNMT3b (IMGENEX, IMG-184A, 1:200), anti-Ki67 (BD Biosceince, 550609, 1:200), anti-γH2AX (Millipore, 05-636, 1:500), anti-LINE1 Orf1p (gift from Alex Bortvin, 1:1000), anti-IAP-GAG (gift from Bryan R. Cullen, 1:2000).

Cryosections

Genital ridges were dissected and fixed in 4% PFA for 1h at room temperature and washed several times with PBS, and then transferred into 10% sucrose/PBS (2h), 20% sucrose/PBS (2h) and finally into OCT (overnight). Next day, the tissues were embedded in OCT in tissue molds and frozen at -80 °C. The OCT blocks containing the tissues were cut into 8µm thick sections using a Leica Cryostat CM3050S and collected on SuperFrost Plus slides (VWR). For immunofluorescence staining, cryosections were washed with PBS and permeabilized in PBS/1%BSA/0.1%Triton X-100 before incubation with primary antibodies in permeabilization buffer over night at 4 °C. Next day, cryosections were washed several times with PBS, incubated with secondary antibodies in permeabilization buffer for 2h at room temperature, washed several times with PBS, incubated with DAPI in PBS for 15 minutes and mounted using Vectashield Mounting Medium (VECTOR Labs). Imaging was performed using a Leica SP5 confocal microscope.

TUNEL assay

The TUNEL assay was performed on cryosections using the ‘In Situ Cell Death Detection Kit, TMR red’ from Roche according to manufacturer’s instructions. Briefly, cryosections on slides were first permeabilized with PBS/1%BSA/0.1% Triton X-100,

then incubated with the TUNEL Reaction Mixture for 30 minutes at 37C, washed several times with PBS, which was then followed by immunofluorescent staining as described in the 'cryosections' section.

RNA-seq analysis and repeat analysis

For the RNA-seq analysis 50 pg of total RNAs of purified PGCs from control and mutant embryos were used to generate cDNA libraries. RNA-seq data was mapped with LifeScope version 2.5 on mm9 and read counts were generated for each transcript using NCBI RefSeq annotation. Differentially expressed genes were identified using the Bioconductor package DeSeq (Anders & Huber, 2010). From an initial pool of 21358 genes, 11343 passed a minimum expression filter requiring a non-zero read count at least 1 sample. Of these, 422 genes were found to be differentially expressed. GO and KEGG terms overrepresented among the differentially expressed genes were identified using the Bioconductor package goseq (Young et al., 2010).

For repeat analysis, reads were aligned by using the BWA software (http://bio-bwa.sourceforge.net/bwa.shtml) and RepeatMasker annotations were downloaded from the UCSC browser (GRCm38/mm10). Unique read counts on repeat classes were normalized using the Bioconductor/DESeq package.

Bisulfite sequencing

We isolated PGCs from dissected genital ridges of individual embryos with a GOF transgene by FACS sorting using a MoFlo 3 laser Cytometer (Beckman Coulter). The genomic DNA is prepared from each sample followed by genotyping and bisulfite sequencing. The bisulfite sequencing was performed as previously described (Hackett et al., 2012).

Chromatin immunoprecipitation q-PCR

Low cell number Chip-qPCR was performed as previously described (Ng et al., 2013). Briefly FACS-purified PGCs were fixed in 1% formaldehyde (room temperature, 10 min),

quenched with 1 vol. 250 mM glycine (room temperature, 5 min), and rinsed with chilled TBSE buffer (20 mM Tris-HCl, 150 mM NaCl, 1 mM EDTA) twice before storage at −80°C. After thawing on ice, fixed cells were pooled (22,000 cells per ChIP) and lysed with 100 µl 1% SDS Lysis Buffer (50mMTris-HCl pH8, 10mM EDTA, 1% SDS, Roche protease inhibitor cocktail; on ice, 5 min) and then centrifuged (2,000 rpm, 10 min). Since pellet was not visible, supernatant was carefully removed such that ~10 µl remains. Samples were resuspended in 100 µl of Dilution buffer (16.7mM Tris-HCl, pH8, 167mM NaCl, 1.2mM EDTA, 1.1% Triton X-100, 0.01% SDS, Roche protease inhibitor cocktail). Samples were sonicated 9 times (30 s pulses with 30 s break interval) using the Bioruptor water bath sonicator (Diagenode). Chromatin extracts were then precleared with Dynal Magnetic Beads (Invitrogen) (4°C, 1 hr) followed by centrifugation (2,000 rpm, 30 min). Supernatant (precleared chromatin) was immunoprecipitated overnight with Dynal Magnetic Beads coupled with anti-H4R3me2s (1 µg per ChIP, ab5823, Abcam) or normal rabbit serum. On the next day, beads were washed (nutate in dilution buffer for 5 min at 4°C thrice then once in TE buffer) and elution was performed in a PCR machine (68°C, 10 min). After reverse crosslinking (with protease K at 42°C for 2 hr and 68°C for 6 hr) DNA was purified (phenol-chloroform extraction) and used for qPCR analysis.

Analysis of staining intensity

For each analysis, images were taken using the same microsocope settings and between control and mutant and analysed using ImageJ software. For analysis of blastocyst L1Orfp1 and IAP-GAG stainings, an intensity threshold (minimum:50, maximum:255) and a value of integrated density were used. Mean intensity of 10-Z sections (4 µm increment) of each embryo was calculated.

For γH2AX staining, Images (z stacks; increment: 1µm) were acquired using a Leica SP5 confocal microscope. Maximum projections of image stacks were used for

quantification of mean fluorescence intensity in GFP positive cells as defined by the Otsu thresholding algorithm using ImageJ.

Blastocyst genotyping

Nested PCR was used to determine the genotype of blastocysts obtained from a *Prmt5*$^{+/-}$ cross. Blastocysts were washed three times in PBS-BSA, transferred to PCR tubes containing 4.5 µl PBS-BSA. These were centrifuged briefly to collect embryos, heated for 10 min at 99°C, then centrifuged at 11,000 rpm for 1 min. Proteinase K (0.4 mg/ml) was added to the tubes and incubated 10 min at 55 °C, then at for 3 min at 99 °C to inactivate proteinase. Diagnositc regions were amplified from DNA in lysate (5 µl) by PCR using Taq polymerase (1U, Qiagen) and PM5OutF, Pm5OutR and Pm5Exon7R primers (0.2 µM, each). PCR product (1 µl) was used for the 2nd PCR, with PM5Flp03F, PMSC6R and PM5DelinR1 primers. Final PCR product was resolved by agarose gel electrophoresis (2% agarose), to determine the presence of 210 bp (*Prmt5*$^{-/-}$) and/or 260 bp (*Prmt5*$^{+/+}$) bands.

Semiquantitative RT-PCR for splicing defects

PCR for skipped exon of Mdm4 were performed using previously reported method (Bezzi et al., 2013). cDNA of PGCs and epiblasts from individual embryos were used as a template.

Primer sequence

Probe (for Southern anlalysis)

PR1AF: AAAGGGACGCACAGTCATTC

PR1AR: GCACCAATTTCTTCCCAAAA

PR2F: TTATCCCAGCACTCCAGAGG

PR2R: AAACAGGCAGGCAGAAAGAA

Genotyping

PGK5: AAAGCGCATGCTCCAGACTGCCTTG

PMSC6R: TACCCAGCTCACACATGGAA

Seq5F: AGGCAGGAGGATCAGGAGTT

PMSC1: ATTAAGGGCCAGCTCATTCC

PM5Flp01F: CTGGGGGTGTAGCTCAGTTG

PM5KO1F: CTGCACACACATGGCACATA

PM5KO2R: GTCCTGGTTGTGGGAGCTTA

PM5OutF: TGAAGCCCCTAAGTAGATCC

PM5OutR: TTCTCAGCTTTCTGGATGAA

PM5Exon7R: AGCCTCTGCTGCACCTTAGA

PM5Flp03F: GCCTGCAGTTCCACCTCTTA

PM5DelinR1: GGGCACAAGGACCCTCAACT

Splicing (skipped exon) for Mdm4

Mdm4sF: TGTGGTGGAGATCTTTTGGG

Mdm4sR: TCAGTTCTTTTTCTGGGATTGG

SUPPLEMENTAL REFERENCES

Anders, S., and Huber, W. (2010). Differential expression analysis for sequence count data. Genome Biol, *11*, R106.

Bezzi, M., Teo, S.X., Muller, J., Mok, W.C., Sahu, S.K., Vardy, L.A., Bonday, Z.Q., and Guccione, E. (2013). Regulation of constitutive and alternative splicing by PRMT5 reveals a role for Mdm4 pre-mRNA in sensing defects in the spliceosomal machinery. Genes Dev. *27*, 1903-1916.

Grabole, N., Tischler, J., Hackett, J.A., Kim, S., Tang, F., Leitch, H.G., Magnúsdóttir, E., and Surani, M.A. (2013). Prdm14 promotes germline fate and naive pluripotency by repressing FGF signalling and DNA methylation. EMBO Rep.*14*, 629-637.

Hackett, J. A., Sengupta, R., Zylicz, J. J., Murakami, K., Lee, C., Down, T. A., and Surani, M. A. (2013). Germline DNA Demethylation Dynamics and Imprint Erasure Through 5-Hydroxymethylcytosine. Science *25*, 448-452.

Livak, K.J., and Schmittgen, T.D. (2001). Analysis of Relative Gene Expression Data

Using Real-Time Quantitative PCR and the 2−ΔΔCT Method. Methods *25*, 402–408.

Martineau, J., Nordqvist, K., Tilmann, C., Lovell-Badge, R., and Capel B. (1997). Male-specific cell migration into the developing gonad. Curr Biol. *7*, 958-968.

Ng, J. H., Kumar, V., Muratani, M., Kraus, P., Yeo, J. C., Yaw, L. P., Xue, K., Lufkin, T., Prabhakar, S., and Ng, H.H. (2013). In Vivo epigenomic profiling of germ cells reveals germ cell molecular signatures. Dev. Cell *24*, 324–333.

Seki, Y., Yamaji, M., Yabuta, Y., Sano, M., Shigeta, M., Matsui, Y., Saga, Y., Tachibana, M., Shinkai, Y., Saitou, M. (2007) Cellular dynamics associated with the genome-wide epigenetic reprogramming in migrating primordial germ cells in mice. Development *134* 2627-2638.

Young, M. D., Wakefield, M. J., Smyth, G. K., and Oshlack, A. (2010). Gene ontology analysis for RNA-seq: accounting for selection bias. Genome Biol., *11*, R14.