Offspring production of ovarian organoids derived from spermatogonial stem cells by chromatin reorganization

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Abstract

Fate determination of germline stem cells remains poorly understood at the chromatin structure level^{1,2}. Here, we demonstrate successful production of offspring from oocytes transdifferentiated from mouse spermatogonial stem cells (SSCs) with tracking of transplanted SSCs in vivo, single cell whole exome sequencing, and in 3D cell culture reconstitution of the process of oogenesis derived from SSCs. Furthermore, we demonstrate direct induction of germline stem cells (iGSCs) differentiated into functional oocytes by transduction of *H19*, *Stella*, and *Zfp57* and inactivation of *Plzf* in SSCs after screening with ovarian organoids. Using high throughput chromosome conformation, we uncovered extensive chromatin reorganization during SSC conversion into iGSCs, which was highly similar to female germline stem cells. We observed that although topologically associating domains were stable during SSC conversion, chromatin interactions changed in a striking manner, altering 35% of inactive and active chromosomal compartments throughout the genome. These findings have important implications in various areas including mammalian gametogenesis, genetic and epigenetic reprogramming, biotechnology, and medicine.

Main

Cell fate decisions, which require key gene regulation, remain poorly understood at the chromatin structure level. Although three-dimensional chromatin architectures of mouse gametes were recently reported, how they affect fate decisions of germline stem cells remains to be explored¹⁻³.

To characterize spermatogonial stem cells (SSCs), we firstly isolated by magnetic activated cell sorting (MACS) using an anti-Thy-1 antibody after two step enzymatic digestion of the testes from 6-day-old *pou5f1* (also known as Oct4, a germ cell-specific transcriptional factor)/GFP transgenic×C57BL/6 F1 hybrid mice⁴. Then, the isolated SSCs were purified for GFP-positive SSCs by fluorescence-activated cell sorting (FACS) (Extended Data Fig. 1a). The purified SSCs were maintained on SIM mouse embryo derived thioguanine- and ouabain- resistant (STO) feeder layers (see METHODS) (Extended Data Fig. 1b). After 3–5 days of culture, SSCs expanded into clusters (Extended Data Fig. 1c). Next, we determined the expression patterns of *Oct4*,

1 *Mvh* (mouse vasa homologue, expressed exclusively in germ cells), *c-Ret*⁵, *Plzf*⁶,
2 *Rex-1*⁷, *Utf1*⁸, *Esg-1* (also known as *DPPA5*)⁹, *Stra8*¹⁰, *Sox2*¹¹, and *Nanog*¹². Reverse
3 transcription-polymerase chain reaction (RT-PCR) and immunocytochemical analyses
4 showed that SSCs expressed *Oct4*, *Mvh*, *c-Ret*, *Plzf*, *Rex-1*, *Utf1*, *Esg-1*, and *Stra8*.
5 Cytogenetic analysis by treatment with colchicines followed by G-band staining
6 demonstrated a normal karyotype (40, XY) in the metaphase spreads of examined
7 SSCs (Extended Data Fig. 1d–j). To verify the characterization of SSCs, we compared
8 the global expression profiles of SSCs and embryonic stem cells (ESCs) using
9 microarrays. Gene expression profiles by scatter plots showed a significant difference
10 between SSCs and ESCs (Extended Data Fig. 1k, n=3). More than two thousand
11 genes (2251) were differentially expressed between SSCs and ESCs, including
12 pluripotency-related genes *Dppa4*, *Fgf4*, *Nanog*, *Sox2*, and *Klf4* (Extended Data Fig.
13 1k, l, fold change>2, P<0.05, t-test) and SSC-related genes *Zbtb16*(or *Plzf*), *Gfra1*,
14 *Tex18*, *Piwi12*, and *Dazl* (Extended Data Fig. 1k, l, fold change>2, P<0.05, t-test).
15 Therefore, these results demonstrated that SSCs had their apparent original property
16 rather than a pluripotent identity. To determine the imprinting pattern of SSCs,
17 differentially methylated regions (DMRs) of two paternal (*H19* and *Rasgrf1*) and two
18 maternal (*Igf2r* and *Peg 10*) imprinted regions were examined in SSCs and ESCs by
19 bisulfite genomic sequencing. In SSCs, paternally imprinted regions (Extended Data
20 Fig. 1m, o) were methylated, while maternally imprinted regions were not methylated
21 (Extended Data Fig. 1n, p); this indicated an androgenetic imprinting pattern that was
22 different from that of ESCs.

23 For investigating SSC fate determination in the mouse ovary, *pou5f1*/GFP
24 transgenic mouse SSCs cultured for 3–5 days were directly transplanted into the
25 ovaries of premature ovarian failure (POF) mice (see METHODS). Phosphate
26 buffered saline (PBS) was injected into the ovaries of POF recipients as a control. For
27 the positive control, female germline stem cells (FGSCs) from *pou5f1*/GFP transgenic
28 mice were also transplanted into the ovaries of POF mice (see METHODS). At 8
29 weeks post-transplantation, recipient ovaries including positive control ovaries were
30 collected and evaluated for morphology and GFP expression. Histological analysis
31 showed that recipient ovaries injected with cells contained numerous oocytes at all
32 stages of development, including GFP-positive oocytes (Fig. 1a I, III, IV and

Extended Data Fig. 2a-e). Furthermore, DNA fluorescence in situ hybridization (FISH) analysis showed the presence of the *Sry* gene in oocytes from recipient ovaries (Fig. 1b). For confirmation, single cell whole exon sequencing was used. The results demonstrated that the germinal vesicle (GV) oocytes from recipient ovaries were derived from transplanted SSCs (Fig. 1c). Mature oocytes from recipient ovaries were then collected for karyotype analysis. The results showed that some mature oocytes had the karyotype of 20, Y (Fig. 1d I–V). PCR analysis of DNA fragment *Sry* confirmed that some mature oocytes contained a candidate of the Y chromosome (Fig. 1d VI). However, control ovaries consisted of stromal and interstitial cells as well as atretic follicles (Fig. 1a II). These results indicate that XY oocytes were regenerated in POF females by transplantation of SSCs.

To examine whether XY oocytes derived from SSCs could produce offspring, POF recipients were mated with wild-type C57BL/6 adult males at 35 days after cell transplantation or PBS injection (control)^{13,14}. Control recipients were not fertile ($n = 9$). All POF recipients produced offspring ($n=8$, Fig. 1e I) with more males than females per litter (male:female, 1.95:1.00). One hundred and sixteen of the 130 offspring were alive with a normal phenotype as well as fertile. Fourteen of the 130 offspring died at 1–6 weeks after birth. The offspring were examined for the presence of GFP transgenes by Southern blot analyses (Fig. 1e II). Sixty-four of the 130 F1 progeny were heterozygous for the GFP transgene. Furthermore, simple sequence length polymorphism (SSLP) analysis was performed with SSLP markers to confirm that the offspring were derived from transplanted SSCs. The offspring from eight recipients (see above) were distinct from POF (see METHODS) or C57BL/6 mice-their parents (POF mice and mated male); however, they had exactly the same profiles as the SSCs from which they were derived (Fig. 1f). After analysis of the methylation status, five of the 20 offspring demonstrated abnormal methylation patterns. Two offspring that did not survive showed high methylation in *Peg 10* with an increase of $21.46\% \pm 2.66\%$ and $28.16\% \pm 2.87\%$ compared with the control. The remaining three demonstrated that *H19* was highly methylated and increased by $16.66\% \pm 1.48\%$ and $14.23\% \pm 1.38\%$ or had low methylation with a decrease of

14.94%±1.26% compared with the control. Moreover, five out of six positive controls (see METHODS) with FGSC transplantation were fertile with approximately equal numbers of males and females per litter (male:female, 1.02:1.00), and their offspring showed no abnormal phenotype. Fifty of the 101 offspring were heterozygous for the GFP transgene (Extended Data Fig. 2f, g). Although approximately 11% of the offspring were abnormal, these results suggest that the XY oocytes derived from SSCs can produce offspring in previously sterile recipients and generate transgenic progeny. Control adult mice that received PBS injections into their ovaries did not produce transgenic offspring.

For understand how the SSCs transdifferentiated into oocytes in recipient ovaries, pou5f1/GFP transgenic mouse SSCs cultured for 3 days were directly transplanted into the ovaries of POF mice, and then monitored by confocal laser scanning microscopy. At 2 hours after SSC transplantation, the SSCs were observed in ovaries of recipient mice, indicating that the SSCs had been successfully transplanted into the mouse ovary (Fig. 2a). The transplanted cells were found to migrate toward the edge of the ovarian cortex at 2 days post-transplantation (Fig. 2a). At 3 days after transplantation, the cells continuously migrated toward the edge of the ovarian cortex and some of them reached the edge (Fig. 2a). When the transplanted cells had been in the ovary for 4 days, all of them had migrated into the edge of the ovarian cortex (Fig. 2a). Five days post-transplantation, transplanted cells settled in the edge of the ovarian cortex and began to transdifferentiate into early primary oocytes (Fig. 2a, b). At 6–15 days after transplantation, the transplanted cells continued to transdifferentiate into oocytes at various stages of development (Fig. 2a). This was confirmed by dual immunofluorescence analysis of the expression of MVH and GFP in transplanted cells (Fig. 2c).

To explore the mechanism of SSC fate determination when the SSCs were transplanted into the recipient ovary, we performed bisulfite sequencing to analyze the methylation status of these transplanted cells, mainly the differentially methylated regions (DMRs) of paternally imprinted gene *H19* and maternally imprinted gene *Peg10*. It is noteworthy that no obvious change of methylation levels, including the

1 maternally or paternally imprinting gene, was observed at 2 hours after SSC
2 transplantation (Extended Data Fig. 3a–d). At 3–4 days after transplantation,
3 methylation levels of *H19* were reduced gradually to 68.3% and 60.8%, with a further
4 reduction to 37.9% and 23.8% at 5–6 days, suggesting that the bulk of methylation
5 erasure occurred at 5–6 days. The low levels of methylation were present at 9 days
6 and persisted to 15 days (Extended Data Fig. 3a, c). In contrast, the maternally
7 imprinted gene *Peg10* showed evidence of robust de novo methylation with an
8 increase to 45.7% methylation at 3 days and further increase to 78% methylation at 6
9 day, indicating that the bulk of methylation establishment occurred at 6 days. The high
10 methylation levels were maintained from 9 to 15 days (Extended Data Fig. 3b, d).
11 These results suggested that the maternal DNA methylation pattern was directly
12 constructed during SSC development in recipient ovaries, and that the SSCs did not
13 dedifferentiate into PGCs.

14 Furthermore, we determined expression patterns of imprinted genes (*H19*, *Grb10*,
15 *Gtl2*, *Rasgrf1*, *Peg10*, *Igf2r*, and *Snrpn*) and important transcription factor genes (*Plzf*,
16 *Stella* or *Dppa3*, *Zfp42*, *Zfp57*, and *Nanos2*) during transdifferentiation of the
17 transplanted cells into oocytes in recipient ovaries based on data from our previous
18 studies^{15,16}. After comparing the expression of imprinted genes in the cultured SSCs (0
19 hour) and transplanted SSCs at 2 hours to 15 days post-transplantation, we observed
20 that paternally imprinted genes (*H19*, *Grb10*, *Rasgrf1*, and *Gtl2*) and transcription
21 factor genes (*Stella* and *ZFP57*) were gradually upregulated, especially at 3 and 6
22 days with a further increase from 9 to 15 days (Extended Data Fig. 3e). In contrast,
23 along with transdifferentiation into oocytes of the SSCs in recipient ovaries, the
24 expression levels of these maternally imprinted genes (*Peg 10*, *Igf2r*, and *Snrpn*) and
25 transcription factor genes (*Plzf*, *Zfp42*, and *Nanos2*) underwent obvious reductions at
26 5–6 days with a continuous decrease from 9 to 15 days after transplantation (Extended
27 Data Fig. 3e), suggesting establishment of the maternal imprinting pattern.

28 Based on the above results, we further screened for the critical imprinted genes
29 and transcription factor genes required for SSC conversion. The spatial organization
30 of the mammal genome is known to play an important role in the regulation of gene

1 expression¹⁷. Therefore, we used in situ high throughput chromosome conformation
 2 capture (Hi-C) to further screen for the critical genes and found 6.28 billion unique
 3 read pairs in SSCs and FGSCs. The compartment status was divided into two groups,
 4 compartment A and B. By comparing A/B compartment statuses and chromatin loops
 5 between SSCs and FGSCs (Extended Data Fig. 4, Supplementary Table 1-3), we
 6 established combinations of six genes (6Gs), including imprinted genes, *H19* and
 7 *Rasgrf1*, and transcription factor genes *Stella*, *Zfp57*, *Zfp42*, and *Plzf*. After
 8 overexpressing *Stella*, *H19*, *Zfp57*, and *Rasgrf1* and knockdown of *Plzf* and *Zfp42* in
 9 SSCs, the cells converted to induced germline stem cells (or induction of germline
 10 stem cells, iGSCs) with a maternal imprinted pattern (Extended Data Fig. 5, Extended
 11 Data Fig. 6a) and formed ovarian organoids when three-dimensional (3D) co-cultured
 12 with somatic cells from the fetal ovary for 2 weeks, modifying the method previously
 13 described in ref¹⁸ (Extended Data Fig. 6b I-II). Upon withdrawal of *Rasgrf1* from the
 14 6Gs, we found that the 3D co-cultured cells still formed ovarian organoids (Extended
 15 Data Fig. 6b III). For the remaining five genes (5Gs), removal of *Zfp42* further
 16 promoted the formation of the organoids (Extended Data Fig. 6b IV). However,
 17 removal of any factor from the four genes (4Gs, *Stella*, *H19*, *Zfp57*, and *Plzf*) led to
 18 the failure to form the organoids (Extended Data Fig. 6b V–VIII).

19 The morphology of iGSCs induced by the overexpression of *Stella*, *H19*, and
 20 *Zfp57* and the inactivation of *Plzf* was similar to that of FGSCs (Extended Data Fig.
 21 7a). Furthermore, the iGSCs expressed *Stella*, *Mvh*, *Fragilis*, *Dazl*, and *Oct4* with a
 22 maternal imprinted pattern (Extended Data Fig. 7a, b, Extended Data Fig. 6c). For
 23 confirmation, we performed genome-wide DNA methylation analysis in SSCs, iGSCs,
 24 and FGSCs by MeDIP-seq. A total of 38.7 million reads, yielding 467,163 DNA
 25 methylationsites (peaks) in three kinds of cell populations were generated. We
 26 observed widespread variation in terms of DNA methylation during SSC transition
 27 into iGSCs (Extended Data Fig. 7c). Subsequently, we performed pair-wise
 28 correlation analysis of the MeDIP data sets from SSCs, iGSCs, and FGSCs. We found
 29 that the overall DNA methylation pattern of iGSCs was similar to that of FGSCs ($r =$
 30 0.79), but it was less similar to that of SSCs ($r = 0.59$). Such a trend was evidenced

1 more clearly by individual regions of interest. For example, at the maternally
 2 imprinted region *Igf2r*, the DNA methylation signal was relatively low in the *Igf2r*
 3 promoter of SSCs. It increased remarkably and appeared to be almost at the same
 4 level in iGSCs and FGSCs (Extended Data Fig. 7c, d). A similar phenomenon was
 5 also observed at the promoter region of *Nr0b1*, the gene encoding the orphan nuclear
 6 receptor and required for development of male characteristics in mice¹⁹. These
 7 observations suggest that DNA methylation contributed to the SSC transition. Next,
 8 we compared global gene expression profiles among SSCs, iGSCs and FGSCs by
 9 RNA sequencing. A total of 380,702,626 raw reads were generated. We detected
 10 expression of 18229, 19755, and 18978 out of 24550 genes in SSCs, iGSCs, and
 11 FGSCs, respectively. On average, 77% of the known mouse genes were expressed in
 12 the sampled SSCs, iGSCs, and FGSCs. Hierarchical clustering was performed, and the
 13 results indicated that iGSCs were clustered with FGSCs, but separated from SSCs,
 14 suggesting that the global gene expression profile of iGSCs was similar to that of
 15 FGSCs (Extended Data Fig. 7e, f). Among these genes, *Igf2r*, a maternal imprinted
 16 gene, showed high expression in SSCs and low level expression in iGSCs and FGSCs
 17 (Extended Data Fig. 7f), which was consistent with the results from the analysis of
 18 genome-wide DNA methylation in SSCs, iGSCs, and FGSCs.

19 Hi-C interaction maps provide information on multiple hierarchical levels of
 20 genome organization²⁰. To understand how genome organization is involved in SSCs
 21 transition to iGSCs, we also performed Hi-C experiments using two biological
 22 replicates of iGSCs, generating a total of 2.96 billion unique read pairs. The Hi-C data
 23 analysis showed the high order chromatin organization of the whole genome in SSCs,
 24 iGSCs, and FGSCs (Fig. 3a–c, Extended Data Fig. 9). To examine the characteristics
 25 of their chromatin organization, we analyzed the pattern of compartment A/B in SSCs,
 26 iGSCs, and FGSCs. We found a large degree of spatial plasticity in the arrangement of
 27 the A/B compartments or redistribution of the spatial organization of their genomes
 28 during SSC transition into iGSCs with 35% of the genome switching compartments.
 29 Furthermore, we found that the regions that changed their A/B compartment status
 30 corresponded to a single or series of topologically associated domains (TADs),

1 suggesting that TADs are the units of dynamic alterations in chromosome
2 compartments (Fig. 3b). Interestingly, we observed that iGSCs and FGSCs were
3 highly similar in their status of A/B compartments compared with SSCs (Fig. 3c). For
4 SSC transition into iGSCs, genes that changed from compartment B to A tended to
5 show higher expression, while genes that changed from A to B tended to show
6 reduced expression (Fig. 3d). Moreover, we identified 4353 genes with co-variation
7 between compartment switching and gene expression. For example, at the
8 compartment B region, *Dppa3* expression was relatively low in SSCs. It increased
9 remarkably and appeared to be almost at the same level in iGSCs and FGSCs when
10 changing from compartment B to A (Fig. 3e). DNA methylation that changed from
11 compartment B to A also tended to show a reduced signal, whereas DNA methylation
12 that changed from A to B tended to show higher signal (Fig. 3f). Take together, these
13 results demonstrate that, when SSCs convert to iGSCs, there is a high degree of
14 plasticity in A and B compartments, corresponding changes in gene expression,
15 indicating that the A and B compartments have a contributory to cell type-specific
16 patterns of gene expression.

17 Developmental feature of ovarian organoids from FGSCs and iGSCs were
18 explored. At 2 weeks of 3D co-culture with the germline stem cells and somatic cells
19 from the fetal ovary, ovarian organoids were generated in FGSC and iGSC groups.
20 The organoids were completely filled with follicles which possessed oocytes. In
21 contrast, ovarian organoids were not formed in the SSC group. When the ovarian
22 organoids were 3D co-cultured for 3 weeks, the follicles grew obviously (Fig. 4a).
23 After these follicles were 3D cultured individually for 2 weeks (see METHODS), a
24 large number of immature oocytes were obtained from the cultured follicles.
25 Moreover, Oct4-EGFP cells were detectable in iGSC and FGSC groups at 3 days of
26 3D co-culture. A number of EGFP-positive oocytes were observed in ovarian
27 organoids after 3 weeks of 3D co-culture (Fig. 4a). After 2-3 weeks of 3D co-culture,
28 however, the EGFP expression became weak (Fig. 4a).

29 For comparison between iGSC and FGSC groups, 905 immature oocytes from
30 the iGSC group were obtained from 27 ovarian organoids in 8 cultures with

1 33.52±4.27 immature oocytes per organoid. In the FGSC group, 1140 immature
2 oocytes were obtained from 36 ovarian organoids in 12 experiments with 31.67±3.86
3 immature oocytes per organoid. No difference in immature oocytes per organoid was
4 observed in both groups (Fig. 4b).

5 To evaluate oogenesis during the ovarian organoid development,
6 immunofluorescence analysis of SCP3 and H2AX was performed. Furthermore, the
7 gene expression profiles during iGSC or FGSC differentiation were analyzed by
8 qRT-PCR. The results showed progression of meiotic prophase I from 7 to 21 days of
9 the germ cell differentiation in both groups (Fig. 4c). The expression dynamics of
10 genes involved in oogenesis were similar between both groups (Extended Data Fig.
11 10).

12 After in vitro maturation for 17-20 hours, 48.9% and 51.3% of these immature
13 oocytes reached mature oocytes in iGSC and FGSC groups, respectively. In addition,
14 no difference in the number of mature oocytes was observed between the two groups
15 (Fig. 4d, Extended Data Fig. 11a).

16 To determine whether these mature oocytes could develop into offspring
17 following in vitro fertilization, embryo culture and transfer into pseudopregnant ICR
18 females were performed. The fertilization rate of the iGSC group (47.2%) was similar
19 to that of the FGSC group (49.9%). Subsequently, these zygotes developed to 2-cell
20 embryos (Fig. 4e, Extended Data Fig. 11b). After the embryo transfer, 53
21 (male:female, 1.65:1.00) out of 342 (for iGSC group) or 51 (male:female, 1.13:1.00)
22 out of 355 (for FGSC group) were delivered as viable offspring with colored eyes (Fig.
23 4f, Extended Data Fig. 11c), indicating that the offspring were derived from C57BL/6
24 iGSC- or FGSC-derived oocytes, but not ICR oocytes among gonadal somatic cells.
25 The offspring were confirmed for the presence of GFP transgenes by Southern blot
26 analysis, and live imaging by a Lumazine imaging system (Fig. 4g, h). All of the
27 obtained offspring grew up normally and were fertile with no difference between the
28 two groups. After analysis of the methylation status, 10 offspring per group
29 demonstrated no observably abnormal methylation patterns (Extended Data Table 1).
30 Our findings provide a new strategy to investigate stem cell biology, biotechnology,

- 1 and medicine.
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METHODS

Mice. C57BL/6, pou5f1-GFP transgenic mice [CBA-Tg (pou5f1-EGFP) 2Mnn] (The Jackson Laboratory) or pou5f1/GFP transgenic mice²¹×C57BL/6 F1 hybrid mice were used in this study. Premature ovarian failure (POF) *Pten* (phosphatase and tensin homolog deleted on chromosome 10)^{loxp/loxp}; *Gdf9-Cre* (*Gdf9* promoter-mediated Cre recombinase⁺) mice were produced and genotyped as described by Reddy *et al*^{22,23}. POF (*Pten*^{loxp/loxp}; *Gdf9-Cre*⁺) mice were used as recipients. *Pten*^{loxp/loxp} mice (B6.129S4-*Pten*^{tm1Hwu}) and *Gdf9-Cre* [Tg (*Gdf9-iCre*)] mice were purchased from The Jackson Laboratory. Animal experimentation was approved by the Institutional Animal Care and Use Committee of Shanghai and performed in accordance with the National Research Council Guide for Care and Use of Laboratory Animals.

Isolation and culture of spermatogonial stem cells. Testes from 6-day-old pou5f1-GFP transgenic mice or pou5f1/GFP transgenic mice×C57BL/6 F1 hybrid mice were collected and decapsulated. Spermatogonial stem cells (SSCs) were isolated using methods described by Wu *et al*^{24,25} and Yuan *et al*²⁶. The SSCs were purified by both magnetic activated cell sorting (MACS) with an anti-Thy-1 antibody and fluorescence activated cell sorting (FACS), according to the manufacturers' instructions. SSCs were cultured on mitotically inactivated SIM mouse embryo derived thioguanine- and ouabain- resistant (STO) feeder cells (5×10^4 cells/cm²; ATCC) in culture medium. For mitotic inactivation, STO cells were treated with 10 µg/ml mitomycin C (Sigma) for 2–3 hours. Mitomycin C-treated STO cells were washed with phosphate buffered saline (PBS) and transferred to 0.2% (w/v) gelatin-coated tissue culture plates. The SSC culture medium consisted of high glucose Dulbecco's modified Eagle's medium (DMEM; Life Technologies) supplemented with 10% fetal bovine serum (FBS; GIBCO), 2 mM L-glutamine (Sigma), 0.1 mM β-mercaptoethanol (Sigma), 1 mM nonessential amino acids (Life Technologies), 10 ng/ml glial cell line-derived neurotrophic factor (GDNF; R&D Systems), 10 ng/ml leukemia inhibitory factor (LIF; Chemicon), and 15 mg/l

1 penicillin (Sigma). SSCs were cultured on STO feeders in 24-well plates with 500 μ l
2 culture medium per well. The medium was replaced every 1–2 days, and cells were
3 subcultured at a split ratio of 1:1–3 by trypsinization every 3 days. All cultures were
4 maintained at 37°C with 5% CO₂.

5 **Isolation and purification of female germline stem cells.** Ovaries were collected
6 from 5-day-old pou5f1/GFP transgenic mice $\times$ C57BL/6 F1 hybrid mice. Female
7 germline stem cells (FGSCs) were isolated and purified using a method described
8 elsewhere²⁷. Briefly, dissected ovarian tissues were incubated in 1 mg/ml collagenase
9 (type IV; Sigma) at 37°C with gentle agitation for 15–20 min. After washing, ovarian
10 tissues were incubated in 0.05% trypsin and 1 mM EDTA at 37°C for 5–7 min. Sheep
11 anti-mouse IgG magnetic beads (DynaL Biotech) were incubated with an anti-fragilis
12 antibody (Abcam) for 30 min at room temperature. The magnetic bead/antibody
13 mixture was incubated with the isolated cell suspension for another 30 min at room
14 temperature. Then, the mixture of cells and magnetic beads was placed on a magnetic
15 bead separator for 2–3 min, and the supernatant was removed. The fraction on the
16 inner side of the eppendorf tube was collected and rinsed twice with PBS,
17 resuspended in PBS, and further purified by FACS, in accordance with the
18 manufacturers' instructions. The purified FGSCs were placed in FGSC culture
19 medium²⁷ and cultured on mitotically inactivated STO feeder cells in 24-well plates at
20 37°C with 5% CO₂.

21 **Preparation of ovarian tissue for analysis.** Ovaries from recipient and control mice
22 were fixed with 4% (w/v) paraformaldehyde (4°C, overnight) and dehydrated via a
23 graded ethanol series. The tissues were vitrified in xylene, embedded in paraffin,
24 sectioned (6 μ m thickness), and then mounted on slides. Prior to immunofluorescence
25 staining, the sections were dewaxed in xylene and rehydrated via a graded ethanol
26 series. Sections were counterstained with hematoxylin.

27 **Immunofluorescence.** After equilibration in PBS, tissue sections were digested with
28 0.125% trypsin for 10 min at 37°C and then washed in PBS twice. The sections were
29 blocked in 10% goat serum at room temperature for 10 min and then incubated
30 overnight at 4°C with appropriate primary antibodies. The primary antibodies used
31 were mouse monoclonal anti-GFP (1:200 dilution; Abcam) and rabbit polyclonal

1 anti-MVH (1:200; Abcam). After washing in PBS, the sections were incubated at
2 37°C for 30 min with TRITC-conjugated goat anti-rabbit IgG (1:200; Sino-American
3 Biotechnology Co.) or fluorescein isothiocyanate (FITC)-conjugated goat anti-mouse
4 IgG (1:200; Sino-American Biotechnology Co.) as appropriate. Sections were stained
5 with 4',6-diamidino-2-phenylindole (DAPI, 1:1000) at 37°C for 20 min, covered with
6 mounting medium (glycerol:PBS, 3:1), and viewed under a Nikon Eclipse E600
7 microscope equipped with a Nikon Dxm 1200 digital camera using fluorescein optics
8 for TRITC and FITC, and ultraviolet optics for DAPI or under a confocal microscope
9 (FluoView™ FV1000).

10 Cultured germline stem cells were fixed with 4% paraformaldehyde in PBS at room
11 temperature for 20 min. After fixation, the cells were permeabilized with 0.5% Triton
12 X-100 for 30 min at room temperature for PLZF staining. The cells were incubated in
13 blocking solution (10% normal goat or bovine serum in PBS, 10 min, 37°C), followed
14 by rinsing and overnight incubation at 4°C with appropriate primary antibodies: rabbit
15 polyclonal anti-MVH (1:200; Abcam), mouse monoclonal anti-GFP (1:200; Abcam),
16 and anti-PLZF (1:150, Santa Cruz Biotechnology). After washing in PBS, the cells
17 were incubated with TRITC-conjugated goat anti-rabbit IgG (1:200) or fluorescein
18 isothiocyanate (FITC)-conjugated goat anti-mouse IgG (1:150) at 37°C for 30 min,
19 rinsed, and then incubated with DAPI (1:1000) at 37°C for 20 min. Petri dishes were
20 then covered with mounting medium (glycerol:PBS, 3:1) and viewed as described
21 above.

22 **Karyotypic analysis.** Karyotypic analysis was performed using standard protocols for
23 mouse chromosome analysis. After culture for 3 days, SSCs were treated with culture
24 medium containing colchicine (100 ng/ml; Sigma) for 3 hours, hypotonically treated
25 with 75 mM KCl for 15 min at 37°C, immersed twice in methanol:acetic acid (3:1) for
26 30 min at -30°C, dried in air for 3–4 days, digested with 0.025% trypsin, and then
27 stained with Giemsa. To verify the chromosomal type of recipient mouse oocytes,
28 karyotypic analysis of mature oocytes from recipients was performed as
29 described previously²⁸. To collect mature oocytes, recipient mice were superovulated
30 with 10 IU pregnant mare serum gonadotropin (PMSG; ProSpec-Tany) for 48 hours,
31 followed by 10 IU human chorionic gonadotropin (hCG; ProSpec-Tany). These

1 oocytes were hypotonically treated with 75 mM KCl at 37°C for 15 min and then
2 fixed with two solutions consisting of methanol/acetic acid/water (5:1:2) for 5–10 min
3 and methanol/acetic acid (3:1) for 15 min at room temperature. Fixed cells were
4 mounted on slides and immediately exposed to steam from boiling water (90–100°C)
5 for 30 sec to cause expansion of the cells, followed by drying at 37°C and Giemsa
6 staining (Amresco)²⁸.

7 **Embryonic stem cell culture.** Embryonic stem cells (ESCs) were cultured with
8 mouse embryonic fibroblasts in the presence of leukemia inhibitory factor (LIF; 1000
9 U/ml) in Glasgow modification of Eagle's medium (GMEM; Invitrogen) containing
10 10% fetal calf serum. The medium was replaced every 1–2 days, and cells subcultured
11 at a split ratio of 1:1–3 by trypsinization every 3 days. All cultures were maintained at
12 37°C with 5% CO₂.

13 **Microarrays.** Total RNA was extracted from cultured SSCs and ESCs using Trizol
14 reagent (Invitrogen), in accordance with the manufacturer's instructions. RNA was
15 labeled using an Illumina labeling kit. An Illumina sentrix mouse WG-6 Beadchip
16 (45281 transcripts) was used in this study. Microarray experiments, including RNA
17 labeling, hybridization, washing, scanning, image analysis, normalization, and data
18 processing, were performed by Shanghai Biotechnology Corporation using the
19 Illumina manual. Three biological repeats were included in microarray experiments.
20 Differentially expressed genes were identified by the Illumina system. The data were
21 analyzed using GeneSpring GX 11 software. Hierarchical clustering of samples was
22 performed by cluster 3.0 and TreeView software²⁹.

23 **Transplantation.** For injection into the ovary, SSCs were collected and transplanted
24 into the ovaries of POF mice. For the positive control, FGSCs from pou5f1/GFP
25 transgenic mice were also transplanted into ovaries of POF mice. Recipient mice were
26 anesthetized by injection of pentobarbital sodium (45 mg/kg). Approximately 6 µl of a
27 singlecell suspension containing 1×10^4 cells or 6 µl PBS for the control was
28 microinjected into the ovaries of recipients as described elsewhere. In detail, after
29 anesthetization of recipient mice for 20–30 min and disinfection of the abdominal
30 surface using 75% ethanol, the recipient abdominal cavity was carefully opened. To
31 expose and find the ovaries, the intestines were carefully moved away from the inside
32 of the abdominal cavity. The Y-shaped uterus was located, and then following the

uterus and oviduct until posterior to the kidneys, the ovaries were located caudal to the kidneys in the lower abdominal cavity. By gently holding an ovary with forceps without causing damage, the ovary was injected at 1–2 sites using a glass pipette with a 45 μ m tip and mouth pipetting to carefully transplant the 6 μ l single cell suspension of $\sim 1 \times 10^4$ SSCs or FGSCs into each ovary. At 35 days after transplantation, recipients were mated with 8-week-old male mice.

Reverse transcription-polymerase chain reaction and Southern blotting. Reverse transcription-polymerase chain reaction (RT-PCR), PCR, and Southern blotting were performed as described elsewhere. Twenty-five cycles of PCR were performed using Taq polymerase (Takara) with primer sets specific for each gene. The glyceraldehyde-3-phosphate dehydrogenase gene (*Gapdh*) was amplified in each sample as a loading control. PCR products were isolated, subcloned, and sequenced to confirm the gene sequence.

Bisulfite genomic sequencing. Genomic DNA was extracted from SSCs, transplanted SSCs, ESCs, induced germline stem cells (iGSCs), and FGSCs. For bisulfite sequencing analysis of methylation, 500 ng genomic DNA was processed using an EZ DNA Methylation-Gold Kit™ (ZYMO Research), in accordance with the manufacturer's instructions. The methylation status of imprinted genes was analyzed using specific primers (outside, 5'-GTTTTTTTGGTTATTGAAT-TTTAAAATTAGT-3' and 5'-AAAAACCATTCCGTAAATACACAAATACCTA-3', inside, 5'-TTAGTGTGGTTTATTATAGGAAGGTATAGAAGT-3' and 5'-TAAACCTAAAATACTCAAACTTTATCACAA-3' for *H19*; 5'-GTG TAG AAT ATG GGG TTG TTT TAT ATT G-3' and 5'-ATA ATA CAA CAA CAA CAA TAA CAA TC-3' for *Rasgrfl*; 5'-GTA AAG TGA TTG GTT TTG TAT TTT TAA GTG-3' and 5'-TTA ATT ACT CTC CTA CAA CTT TCC AAA TT-3' for *Peg10*; 5'-TTA GTG GGG TAT TTT TAT TTG TAT GG-3' and 5'-AAA TAT CCT AAA AAT ACA AAC TAC ACA A-3' for *Igf2r*; outside, 5'-TATGTAATATGATATAGTTTAGAAATTAG-3' and 5'-AATAAACCCAAATCTAAAATATTTTAATC-3', inside, 5'-AATTTGTGTGATGTTTGTAAATTATTTGG-3' and 5'-ATAAAATACACTTTCACTACTAAAATCC-3' for *Snrpn*). PCR products were sequenced and CpG islands were analyzed.

PCR amplification of lineage-specific microsatellite loci. Genomic DNA was

1 extracted from mouse tail tips or donor SSCs. DNA samples from donor SSCs, female
2 recipients, mated males, and their corresponding offspring were analyzed by simple
3 sequence length polymorphism (SSLP). Sequences for the primer pairs were designed
4 according to the Mouse Genome Informatics website
5 (<http://www.informatics.jax.org/>). Amplification of lineage-specific microsatellite
6 DNA was performed in accordance with a previously described procedure³⁰. PCR
7 products were separated and analyzed by 3% agarose gel electrophoresis (Bio-Rad)
8 and visualized by ethidium bromide staining.

9 **Flow cytometry and cell sorting.** After MACS, the cells were suspended in PBS and
10 subjected to flow cytometry to analyze and sort GFP-positive cells using a FACS Aria
11 II cell sorter equipped with BD software (Becton Dickinson).

12 **Quantitative reverse transcription-PCR analysis.** Total RNA from cells was
13 isolated using Trizol reagent. Complementary DNA was synthesized from 2 µg total
14 RNA using a High Capacity cDNA Reverse Transcription Kit (Invitrogen). Primers
15 were designed using Primer Premier Software (Primer Premier 5.0). Primer details are
16 listed in Supplementary Table 4. *Gapdh* was amplified in each sample as an internal
17 control. The mRNA level of each gene was normalized to *Gapdh* expression. The
18 specificity of all quantitative real-time PCRs (qPCRs) was verified by a single peak in
19 the melting curve. qPCRs were performed with a 7500 real-time PCR amplification
20 system using SYBR Green PCR master mix (Applied Biosystems, UK). The relative
21 levels of transcripts were calculated using the $\Delta\Delta CT$ method within the ABI 7500
22 System Software (V2.0.4). All gene expression levels were normalized to the internal
23 standard gene, *Gapdh*. The means and standard error were calculated from triplicate
24 measurements. Significance was determined using the Student's *t*-test. A *P*-value of
25 less than 0.05 was considered as significant, and a *P*-value of less than 0.01 was
26 extremely significant.

27 **Single cell whole genome amplification and exome sequencing.** Single cell whole
28 genome amplification was performed on lysed single cells using a recently developed
29 method named multiple annealing and looping based amplification cycles
30 (MALBAC)³¹. In brief, amplification was initiated by primers, each with a 27 fixed

1 and eight degenerate base hybridizing uniformly throughout the genome. Fragments
2 with variable length at random starting positions were generated by polymerase
3 extension for multiple cycles. All fragments were flanked by the 27 base-fixed
4 sequence and their complementary sequences, and further amplified by PCR to about
5 1 µg for barcoded massively parallel sequencing on an Illumina HiSeq 2500
6 sequencing platform.

7 **Sry DNA in situ hybridization.** We used a commercially available SRY DNA FISH
8 kit (Mice SRY DNA biotin labelled POD and fluorescent FISH in situ hybridization
9 double staining system, TBD Science), according to the manufacturer's instructions.
10 Briefly, sections were dewaxed with a graded series of ethanol, quenched in 3% H₂O₂
11 for 10 min at room temperature, and then washed twice with PBS. The sections were
12 covered with SRY reagent B for 10 min at 37°C. After washing with PBS, the sections
13 were incubated in Tris buffered saline (TBS) for 20 min at 95–100°C (pH 8.9) and
14 then rinsed three times with cold TBS (5 min per rinse) and once with 0.2× saline
15 sodium citrate (5 min per rinse) at 0 °C. The sections were incubated for 8 hours at
16 37°C with SRY reagent A and then washed three times with 2×saline sodium citrate at
17 37°C (3 min per rinse), three times with 0.2×saline sodium citrate (3 min per rinse) at
18 37 °C, and three times with TBS (2 min per rinse) at 37°C. The sections were covered
19 with SRY reagent C for 45 min at 37°C. After washing in PBS, the sections were
20 incubated at 37°C for 120 min with a fluorescein isothiocyanate (FITC)-conjugated
21 mouse anti-digoxin monoclonal antibody, then incubated at 37°C for 120 min with
22 DAPI. Finally, the sections were mounted in anti-fade mounting medium. Images
23 were obtained using a Leica DMI3000 B microscope and Leica DFC550 digital
24 camera.

25 **In situ high throughput chromosome conformation capture library generation**
26 **using a low amount of cells.** In situ high throughput chromosome conformation
27 capture (Hi-C) assays were carried out according to the protocol with minor
28 modifications³²⁻³⁴. Cells were fixed in a 1% final concentration of formaldehyde prior
29 to 10 min incubation at room temperature. The reaction was quenched for 5 min by
30 adding a 2.5 M glycine solution. Cells were pelleted twice (3000g, 4°C for 5 min),

1 resuspended in ice-cold Hi-C lysis buffer for at least 15 min, and then washed once
2 with 100 μ l of 1 $\times$ NEBuffer 2. The supernatant was discarded, and 1 μ l of 5% sodium
3 dodecyl sulfate (SDS) was added to the remaining 9 μ l solution. The pellet was gently
4 mixed and incubated at 62°C for 10 min. After incubation, 9.5 μ l water and 2.5 μ l of
5 10% Triton X-100 were added to quench the SDS, and then the solution was
6 incubated at 37°C for 30 min. Chromatin digestion was performed with Dpn II
7 restriction enzyme (NEB, R0543M) at 37°C overnight and then inactivated for 20 min
8 at 65 °C. To fill the overhangs generated by the Dpn II restriction enzyme, a master
9 mix of 3.75 μ l biotin-14-dATP (Life Technologies), 0.45 μ l of 10 mM
10 dCTP/dGTP/dTTP mix, and 1 μ l of 5 U/ μ l large DNA Polymerase I (NEB, M0210L)
11 were added, followed by incubation at 24°C for 4 hours. The above biotin-labelled
12 products were ligated by adding a master mix of 66.3 μ l water, 12 μ l of 10 $\times$ NEB T4
13 DNA ligase buffer, 10 μ l of 10% Triton X-100, 5 μ l of 10 mg/ml bovine serum albumin,
14 and 2 μ l of 400 U/ml T4 DNA ligase, followed by incubation at 16°C for 20 hours and
15 then inactivation at 75°C for 20 min. The samples were pelleted (3000g, 4°C for 5 min)
16 and washed once with 100 μ l of 10 mM Tris buffer. To remove biotin from unligated
17 DNA ends, a master mix of 40 μ l water, 5 μ l of 10 $\times$ NEBuffer 2.1, 0.125 μ l of 10 mM
18 dATP/dGTP, and 5 μ l of 3,000 U/ml T4 DNA polymerase (NEB, M0203L) were
19 added to the tube containing the DNA sample, followed by incubation at 20°C for 4
20 hours. The samples were pelleted (3000g, 4°C for 5 min) and resuspended in 50 μ l of
21 10 mM Tris buffer. To digest the proteins, 2 μ l of 20 mg/ml proteinase K (NEB,
22 P8107S) was added, followed by incubation at 62°C for 18 hours and inactivation at
23 75°C for 30 min. The DNA was sheared to an average size of 400 bp (Covaris, M220)
24 to perform the End Repair/dA-Tailing and Adaptor Ligation (NEB, E7337A) with a
25 KAPA Hyper Prep Kit (KAPA, kk8502) and then processed by 3 μ l of USER™
26 Enzyme (NEB, M5505L) at 37°C for 15 min to open up the loop. Biotin-labeled
27 ligation products were isolated using MyOneStreptavidin T1 Dynabeads (Life
28 Technologies, 65601) and then resuspended in 20 μ l of 10 mM Tris buffer at 98°C for
29 10 min, and the supernatant was transferred to a fresh PCR tube. Hi-C DNA was
30 amplified using Index Primers set 1 (NEB, E7335S). The Hi-C libraries were purified

1 with AMPure XP beads (Beckman Coulter, A63881) and sequenced using an Illumina
2 sequencing platform.

3 **Hi-C data processing, mapping, and ICE normalization.** For Hi-C pair-end raw
4 data, we first trimmed the adaptor sequences and low quality reads with BBmap
5 (version 38.16). Then, we used HiCPro (version 2.7)³⁵ to map, process, and perform
6 iterative correction for normalization. Briefly, reads were independently aligned to the
7 mouse reference genome (mm9) by the bowtie2 algorithm³⁶. We discarded the uncut
8 DNA reads, re-ligation reads, continuous reads, and PCR artifacts. We then used the
9 unique mapped reads (MAPQ>10) to build the contact matrix. Valid read pairs were
10 then binned at a specific resolution by dividing the genome into sequential bins of
11 equal size. We generated the raw contact matrices at binning resolutions of 10, 20, 40,
12 100, and 200 kb. ICE³⁷ normalization was applied to remove bias in the raw matrix,
13 such as GC content, mappability, and effective fragment length in the Hi-C data.

14 **Validation of Hi-C data.** The data reproducibility was confirmed by calculating
15 Pearson's correlation coefficient (PCC) between the two libraries. Briefly, the
16 interaction frequency was generated for each pair of 40kb bins. For each possible
17 interaction I_{ij} between two replicates, they were correlated by comparing each point
18 interaction in the normalized interaction matrix. Considering that the interaction
19 matrix was highly skewed toward proximal interactions, we restricted the correlation
20 to a maximum distance of 2 Mb between points i and j . We used R to calculate
21 Pearson's correlation between two duplicates.

22 **Contact probability $p(s)$ calculation.** $P(s)$ was calculated with normalized interaction
23 matrices at a 40kb resolution, as described previously³⁰. $P(s)$ calculations only
24 considered intra interactions. Briefly, we divided the genome into 40kb bins. For each
25 distance separated by 40, 80, 120, and 160 kb, we counted the number of interactions
26 at corresponding distances. Then, we divided the number of interactions in each bin
27 by the total number of possible region reads as $P(s)$. Furthermore, we normalized the
28 sum of $P(s)$ over the range of distances as 1. We used LOWESS fitting to construct
29 the curve (log-log axis).

30 **Identification of A and B compartments.** We used the R package (HiTC)³⁸ `pca.hic`

1 function to generate PC1 eigenvectors using 400kb normalized matrices with the
2 following options: normPerExpected=TRUE, npc=1, for which a positive value
3 indicated the A compartment, while a negative value indicated the B compartment. To
4 investigate compartment switching, we defined switched bins only if PC1
5 eigenvectors changed in the same direction for two replicates.

6 **Identification of concordant genes with an A/B compartment switch.** We used a
7 previously described method with minor modifications to define genes with
8 concordant changes in expression and compartment status³⁹. Briefly, we calculated the
9 covariance between the vector of the gene expression values (FPKM) and the vector
10 of PC1 values for each gene across five cell types. The calculated covariance as a
11 metric to quantitatively define “concordance” was used. We compared these observed
12 covariance values with a random background distribution to calculate a P-value for
13 the covariance for each gene. Then, we produced the background distribution by
14 randomly shuffling the vector of FPKM for each gene and calculating the covariance
15 between the PC1 values and random gene expression vector. A rank-based P-value
16 could be calculated for observed covariance values with 1000 repeats for each gene.
17 Concordant genes were defined as those with a P-value of <0.01.

18 **Direct induction of germline stem cells from SSCs.** Knockdowns of specific genes
19 were accomplished by small interfering RNAs (siRNAs) targeting *Plzf* and *Eed*. The
20 interfering fragment was inserted downstream of the U6 promoter in a lentiviral
21 vector (pLKD-CMV-G&PR-U6-shRNA) by molecular biological methods. At least
22 four independent siRNAs were screened for knockdown efficiency against each target
23 and the best siRNA target was selected (target Seq: CCAGGCATCTGATGACAAT
24 for *Plzf*; GCAACAGAGTAACCTTATA for *Eed*). For *Stella*, *Zfp57*, *H19*, and *Rasgrf1*
25 overexpression, cDNAs of candidate genes were inserted into the EcoRI and BamHI
26 restriction sites of the overexpression plasmid (pHBLV-CMVIE-ZsGreen-T2A-puro).
27 Lentivirus particles were generated by cotransfection of knockdown or overexpression
28 plasmids and lentivirus packaging plasmids into HEK293T cells using transgene
29 reagent. Enhancing buffer was added to the medium after 12 hours of transfection.

1 Virus particles were harvested at 48 hours after transfection, and a standardized virus
 2 titer was obtained using HEK293T cells.
 3 For lentivirus infection, 1×10^4 SSCs, which were passaged for 2-3 times, were seeded
 4 in the well of a 48-well plate pre-coated with laminin and incubated with a 1:1
 5 mixture of culture medium and lentivirus-concentrated solution (lentivirus titer:
 6 1×10^9 TU/ml) containing 5 µg/ml polybrene. After overnight infection, cells were
 7 re-plated onto puromycin-resistant STO feeder layers and cultured in SSC medium. At
 8 12 hours after re-plating, the SSCs were incubated with a 1:1 mixture of culture
 9 medium and lentivirus-concentrated solution again. After overnight infection, the
 10 mixture was changed to fresh culture medium, and the cells were cultured for 12
 11 hours. SSCs were then infected for a third time. After overnight infection, the mixture
 12 was changed to fresh culture medium, and the cells were cultured at 37°C with 5%
 13 CO₂. At day 6, the cells were subcultured at a 1:1–2 split ratio, and 100 ng/ml
 14 puromycin was added to the FGSC culture medium to screen for puromycin-resistant
 15 iGSCs. After 72 hours, the surviving iGSCs were passaged and analyzed by qRT-PCR
 16 and western blotting.

17 **RNA-seq library generation and data analysis.** Total RNA was extracted from
 18 $1\text{--}2 \times 10^6$ cells using Trizol Reagent. The RNA quality was assessed using an Agilent
 19 Bioanalyzer 2100. RNA-Seq libraries were prepared using the KAPA Stranded
 20 mRNA-Seq kit, following the manufacturer's instructions. After preparation, libraries
 21 were quantified using a Qubitfluorometer and sequenced with the HiSeq Platform
 22 (2×100 bp). All RNA-Seq data were trimmed and aligned to the mm9 reference
 23 genome using Hisat2 (version 4.8.2)⁴⁰ with the default parameters. Gene expression as
 24 FPKM was calculated by Cufflinks (version 2.2.1)⁴¹ using the RefSeq database from
 25 the UCSC genome browser. Sequencing depth was normalized.

26 **GO term enrichment analysis.** GO term enrichment analysis was performed using
 27 the DAVID tool (version 6.8)⁴², focusing on enriched biological processes (BP). The
 28 GO results were displayed by Cytoscape (version 3.5.1)⁴³. For the Benjamin-corrected
 29 P-value, a threshold of <0.05 was used for significance.

1 **MeDIP-seq and bioinformatics.** The DNA methylome assay was performed as
2 described previously⁴⁴. Briefly, genomic DNA (gDNA) was extracted and fragmented
3 with Bioruptor (Connecticut, USA) into fragment sizes of 200–500 bp. Sonicated
4 gDNA was used for end-repair and adaptor ligation. The adaptor-ligated gDNA was
5 denatured and incubated with an antibody (Epigentek, A-1014) conjugated on Protein
6 A+G Magnetic beads (Millipore, 16-663). Immunoprecipitated DNA was amplified
7 by PCR and subjected to Illumina sequencing.

8 MedIP and input raw sequencing reads were mapped using Bowtie2 (version
9 2.2.6) to the UCSC mm10 genome reference³⁶. Duplicate reads were removed by
10 samtools (version: 1.6-1). The normalized coverage was calculated by binning the
11 unique tags in 1 kb bins, and the number of reads in each bin was normalized using
12 reads per kilobase per million reads (RPKM). We identified the enriched MeDIP
13 regions over the background with MACS (version 2.1.1) and default parameters⁴⁵.
14 Genome-wide pairwise correlation analysis of read depth in 1 kb bins was performed
15 to evaluate DNA methylation patterns of SSCs, iGSCs, and FGSCs.

16 **Ovarian organoid generation and culture.** Ovarian organoids were formed using a
17 modified method described elsewhere⁴⁶. Briefly, iGSCs, FGSCs (positive control),
18 and SSCs (negative control) were purified by a FACS Aria II (BD Bioscience) and
19 co-cultured with E12.5 female gonadal somatic cells in a 96-well U-bottom,
20 low-binding culture plate (Thermo Fisher Scientific) for 2 days in GMEM
21 supplemented with 15% Knockout serum replacement (Invitrogen), 1 μ M retinoic
22 acid, 2 mM L-glutamine (Sigma), 1 mM non-essential amino acids (Life
23 Technologies), 2 mM L-glutamine (Sigma), 30 mg/ml pyruvate (Amresco), 50
24 mM β -mercaptoethanol (Biotech), 30 mg/l penicillin (Amresco), and 75 mg/l
25 streptomycin (Amresco). One thousand iGSCs, FGSCs or SSCs were 3D co-cultured
26 with 3×10^4 gonadal somatic cells. The co-cultures from 96-well U-bottom,
27 low-binding culture plates were transferred onto transwell-COL membranes (Coaster)
28 soaked in α -MEM- based medium, α -MEM supplemented with 2% FBS, 2 mM
29 L-glutamine, 150 μ M ascorbic acid (Sigma), 50 mM β -mercaptoethanol, 30
30 mg/l penicillin, and 75 mg/l streptomycin. At 4 days of culture, the culture medium

was changed to StemPro-34-based medium, StemPro-34 SFM (Life Technologies) supplemented with 10% FBS, 2 mM L-glutamine, 150 μ M ascorbic acid, 50 mM β -mercaptoethanol, 30 mg/l penicillin, and 75 mg/l streptomycin. From 7 to 10 days of culture, 600 nM ICI182780 was added to the StemPro-34-based medium. At 11 days of culture, the culture medium was changed to StemPro-34-based medium without ICI182780. After 21 days of culture, individual follicles were manually dissociated using sharpened tungsten needles.

Follicle 3D culture. The single follicles were cultured on transwell-COL membranes with medium, α -MEM supplemented with 5% FBS, 2% polyvinylpyrrolidone (Sigma), 2 mM L-glutamine, 150 μ M ascorbic acid, 50 mM β -mercaptoethanol, 30 mg/l penicillin, 75 mg/l streptomycin, 30 mg/ml pyruvate (Amresco), 0.1 IU/ml follicle-stimulating hormone (FSH; MSD), 15 ng /ml BMP15, and 15 ng/ml GDF9 (R&D Systems). At 2 days of culture, the culture medium was changed to medium without BMP15 and GDF9, and then follicles were incubated in 0.1% Type IV Collagenase (Invitrogen) for 5 min. After washing with α -MEM supplemented with 5% FBS several times, the follicles were cultured in medium without BMP15 and GDF9. After 14 days of culture, cumulus-oocyte complexes grown on the membrane were picked up by a fine glass capillary.

In vitro maturation, in vitro fertilization, and embryo transfer. The cumulus-oocyte complexes were cultured with α -MEM containing 5% FBS, 30 mg/ml pyruvate (Amresco), 0.1 IU /ml FSH, 4 ng/ml EGF, 1.2 IU/ml hCG (gonadotropin, ASKA), 4 ng/ml bFGF, 30 mg/l penicillin, 75 mg/l streptomycin. After 17-20 hours of culture, mature oocytes with expanded cumulus cells were fertilized in HTF medium (SAGE) by sperm. Embryos developed to the 2-cell stage were transferred into the oviducts of pseudopregnant females at 0.5 day post-coitum.

Data Availability

Original data of Hi-C have been deposited in the Gene Expression Omnibus database (accession number: 135104). Original data of RNA-Seq have been deposited in the

- 1 Gene Expression Omnibus database (accession number: 134727). Original data of
- 2 MeDIP-Seq have been deposited in the Gene Expression Omnibus database
- 3 (accession number: 134640). Original data of Microarrays have been deposited in the
- 4 Gene Expression Omnibus database (accession number: GSE38776). All other
- 5 relevant data are available from the corresponding author upon request.
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23 **Author contributions** H.L., X.L. and G.G.T. conducted all the major experiments,
24 data analysis and wrote the manuscript; D.L. performed embryo transfer; C. H. carried
25 out in situ Hi-C library generation using a low amount of cells; X.D. and W.X. were
26 responsible for karyotype analysis; L.H, Y.Y., and H.W. were responsible for
27 immunofluorescence and histological analysis of ovarian tissue; Q.L., A.J.C. and J.X.
28 conducted Gdf9-Cre⁺ and GFP transgenic mice study; X.Z. carried out MeDIP-seq and
29 bioinformatics; J.W. initiated and supervised the entire project, conducted SSC and
30 FGSC transplantation, analyzed data and wrote the manuscript.

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Figure legends

Fig. 1: SSCs transdifferentiate into oocytes in the ovaries of POF recipients and GFP-expressing offspring are generated from the transplanted SSCs from pou5f1/GFP transgenic mice. **a**, SSCs were transplanted into the ovaries of POF recipient mice. I, II, Representative morphologies of the ovaries from recipients with (I) or without (II) SSC transplantations. III, Follicles containing GFP-positive (green) oocytes in recipient ovaries at 8 weeks after transplantation of pou5f1/GFP transgenic SSCs. IV, Oocytes in a wild-type ovary without a GFP signal. **b**, DNA fluorescence *in situ* hybridization for SRY. SRY was only localized in oocytes (green) derived from SSCs in ovary (I). Nuclei were counterstained with DAPI (blue) (II). **c**, Circos plot showing the coverage from the single cell exon sequencing as a histogram. Grey represents FGSCs; Red represents GV oocytes derived from SSCs in the ovary; Blue represents SSCs. **d**, Karyotype analysis of mature oocytes from POF recipient ovaries at 2 months after pou5f1/GFP transgenic SSC transplantation. I, II, Representative morphologies of mature oocytes derived from pou5f1/GFP transgenic SSCs (I) emitting GFP fluorescence (II) under UV light. III–V, Cytogenetic analysis by G-band staining showing that some mature oocytes from SSCs had a karyotype of 20, Y. III: An example of 20, Y in mature oocytes derived from pou5f1/GFP transgenic SSCs. Arrow indicates the Y chromosome. IV: Example of 20, X in mature oocytes derived from pou5f1/GFP transgenic SSCs. V: Representative karyotype (20, X) of wild-type mature oocytes. VI, PCR analysis of *Sry*. M, 100 bp DNA marker; lane 1, SSCs; lane 2, mature oocytes derived from pou5f1/GFP transgenic SSCs; lane 3, wild-type mature oocytes; lane 4, mock. **e**, Example of offspring from POF recipient mice transplanted with pou5f1/GFP transgenic SSCs (I) and an example of a Southern blot of tail DNA (II). Genomic DNA was digested with *EcoRI*. Marker sizes are indicated to the right of the blot. Lanes 1, 3, 5, and 7: transgenic mice; lanes 2, 4, 6, and 8: wild-type mice. **f**, SSLP analysis of parents and their offspring mice through SSLP markers. M: DNA marker; lane 1: donor SSCs; lane 2: female recipients (POF); lane 3:

mated males (C57BL/6); lanes 4–11: offspring from eight corresponding recipients females. Scale bars, 50 μ m (**a** I, III, IV), 100 μ m (**a** II), 25 μ m (**b** I, II), 10 μ m (**d** I, II).

Fig. 2: Tracking of transplanted SSCs in recipient ovaries. **a**, Transplanted SSCs from pou5f1/GFP transgenic mice were monitored by confocal laser scanning microscopy at 2 hours, and 2, 3, 4, 5, 6, 9, 12, and 15 days after transplantation into recipient ovaries. **b**, Gene expression dynamics during oogenesis in transplanted cells at 4, 6, 9, and 15 days after transplantation. **c**, Dual immunofluorescence analysis of MVH and GFP expression in transplanted cells at 2, 3, 4, 5, 6, 9, 12, and 15 days after transplantation. Scale bars, 50 μ m.

Fig. 3: Reorganization of the chromosome structure during SSC conversion to iGSCs. **a**, Contact matrices from chromosome 16 in SSCs, iGSCs, and FGSCs. **b**, First principal component (PC1) value and normalized Hi-C interaction heat maps at a 40kb resolution in SSCs, iGSCs, and FGSCs. The PC1 value was used to indicate the A/B compartment status, where a positive PC1 value represents the A compartment (blue) and a negative value represents the B compartment (yellow). Dashed lines indicate TAD boundaries in SSCs. **c**, Hierarchical clustering of PC1 values for the A/B compartment status in SSCs, iGSCs, and FGSCs. **d**, Expression of genes that changed compartment status (“A to B” or “B to A”) or remained the same (“stable”) compared with SSCs (P-value by Wilcoxon’s test). **e**, IGV snapshot of Dppa3 (Stella) showing concordance between its expression and PC1 values. **f**, Relative MeDIP-seq signal that changed compartment status (“A to B” or “B to A”) or remained the same (“stable”) compared with SSCs (P-value by Wilcoxon’s test). ***p<0.0001.

Fig. 4: Offspring production of ovarian organoids derived from iGSCs and FGSCs. **a**, Ovarian organoid formation and development. Representative ovarian organoids with a merge of bright field and fluorescence. I–III, Ovarian organoids or co-cultures with somatic cells of gonad and iGSCs at 3 days (I), 2 weeks (II), and 3

1 weeks (III). IV–VI, Ovarian organoids or co-cultures with somatic cells of gonads and
2 FGSCs at 3 days (IV), 2 weeks (V), and 3 weeks (VI). VII–IX, Images of aggregates
3 formed by somatic cells of gonads and SSCs at 3 days (VII), 2 weeks (VIII), and 3
4 weeks (IX). **b**, Follicle growth in vitro. I–III, VII, Representative follicles isolated
5 from ovarian organoids formed by somatic cells of gonads and iGSCs at 0 days (I), 2
6 days (II), 7 days (III), and 11 days (VII). IV–VI, VIII, Representative follicles isolated
7 from ovarian organoids formed by somatic cells of gonads and FGSCs at 0 days (IV),
8 2 days (V), 7 days (VI), and 11 days (VIII). IX, X, Cumulus-oocytes complexes
9 derived from iGSCs (IX) and FGSCs (X) before in vitro maturation. **c**, Representative
10 views of each stage of meiotic prophase I during ovarian organoid development after
11 stained with anti-Sycp3 and -H2AX antibodies. **d**, Mature oocytes derived from
12 iGSCs or FGSCs after in vitro maturation. **e**, Two-cell embryos derived from iGSCs
13 or FGSCs after in vitro fertilization. **f**, Representative offspring derived from iGSCs
14 or FGSCs. **g**, Offspring were identified by Southern blotting. Lanes 1–4, offspring
15 derived from iGSCs, lane W, wild-type mice, lanes 5–8, offspring derived from
16 FGSCs. **f**, Offspring were identified by fluorescence. Lanes 1–4, offspring derived
17 from iGSCs, lane W, wild-type mice, lanes 5–8, offspring derived from FGSCs. Scale
18 bars, 100 μm (**a**), 20 μm (**b** I–VI), 40 μm (**b** VII–VIII), 50 μm (**b** IX–X, **d**, **e**), 5 μm (**c**).
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