1 **Three-dimensional Genome Structure Reveals Distinct Chromatin Signatures of** 2 **Mouse Female Germline Stem Cells During Development**

3 Geng G. Tian ^{1,9}, Xinyan Zhao ^{2,9}, Wenhai Xie ³, Xiaoyong Li ¹, Changliang Hou ¹,
4 Yinjuan Wang ¹, Lijuan Wang ³, Xiaodong Zhao ⁴, Hua Li ⁵, Jing Li ^{6,*},
5 and Ji Wu ^{1, 2, 7, 8,*}

6
7 ¹Renji Hospital, Key Laboratory for the Genetics of Developmental &
8 Neuropsychiatric Disorders (Ministry of Education), Bio-X Institutes, School of
9 Medicine, Shanghai Jiao Tong University, Shanghai 200240, China

10 ²Key Laboratory of Fertility Preservation and Maintenance of Ministry of Education,
11 Ningxia Medical University, Yinchuan 750004, China

12 ³School of Life Sciences, Shandong University of Technology, No.266 Xincun Road,
13 Zibo, Shandong 255000, China.

14 ⁴Shanghai Center for Systems Biomedicine, Shanghai Jiao Tong University, Shanghai
15 200240, China

16 ⁵State Key laboratory for Oncogenes and Bio-ID Center, School of Biomedical
17 Engineering, Shanghai Jiao Tong University, 800 Dongchuan Road, Shanghai 200240,
18 China

19 ⁶Department of Bioinformatics and Biostatistics, School of Life Sciences and
20 Biotechnology, Shanghai Jiao Tong University, 800 Dongchuan Road, Shanghai
21 200240, China

22 ⁷Shanghai Key Laboratory of Reproductive Medicine, Shanghai 200025, China

23 ⁸Lead Contact

24 ⁹These authors contributed equally

25 * Correspondence: jiwu@sjtu.edu.cn (W. J), jing.li@sjtu.edu.cn

26

27 **SUMMARY**

28 The three-dimensional configuration of the genome ensures cell-type-specific gene
 29 expression profiles by placing genes and regulatory elements in close spatial
 30 proximity. Here, we revealed the distinct features of the chromatin architecture in
 31 female germline stem cells (FGSCs) by *in situ* high-throughput chromosome
 32 conformation analysis. We also showed that the X chromosome structures were
 33 similar in spermatogonial stem cells and FGSCs. Using integrative analysis of the
 34 three-dimensional chromatin structure, we observed that the TADs were attenuated in
 35 germinal vesicle oocytes and disappeared in metaphase II oocytes during FGSC
 36 development. Finally, we identified conserved compartments belonging to the
 37 paternal/maternal genomes during early embryonic development, which were related
 38 to imprinted genes. These results will provide a valuable resource for studying and
 39 further our understanding of the fundamental characteristics of oogenesis and early
 40 embryo development.

41

42 **INTRODUCTION**

43 The chromatin architecture of germline stem cells (GSCs) carries the information
 44 necessary for the cells to exert their unique functions, and is thus an essential factor in

the transmission of the genome from generation to generation. GSCs can renew themselves and differentiate into gametes, including sperm and metaphase II (MII) oocytes¹⁻³. During this process, spermatogonial stem cells (SSCs) differentiate into sperm by packaging the chromatin into a highly condensed configuration. Recent identified female GSCs (FGSCs) in postnatal ovaries were shown to differentiate into MII oocytes after transplantation into the ovaries of infertile mice⁴⁻¹⁰, thus reshaping the idea that female mammals lose the ability to produce oocytes at birth^{11,12}. Unlike other stem cells, GSCs can undergo meiosis to produce haploid gametes with chromatin remodeling. It is therefore necessary to characterize the chromatin structure of GSCs during their development to further our understanding of GSC biology.

High-throughput chromosome conformation (Hi-C) is a powerful technology for studying the genome-wide architecture, allowing the high-order chromatin structure to be displayed and revealing the chromatin organization in the nucleus¹³. The spatial organization of chromatin, as the structural and functional basis of the genome, can affect DNA localization, with important roles in gene transcription, the prevention of DNA damage, and ensuring DNA duplication and other biological processes^{14,15}. Previous studies reported that the chromatin architecture changed dynamically during spermatogenesis, with dissolved and reappeared topologically associated domains (TADs) and A/B compartments^{16,17}. However, the signature of the chromatin architecture during the development of FGSCs is unknown. Furthermore, the paternal and maternal chromatin architectures have been shown differences during early embryonic development^{18,19}, but the respective changes in high-order allelic genome

67 structure in early embryonic development remain to be explored.

68 In this study, we used *in situ* Hi-C technology to compared the chromatin
69 organization of FGSCs with pluripotent stem cells (induced pluripotent stem cells,
70 iPSCs), adult stem cells (ASCs) including SSCs and neural stem cells (NSCs), and
71 somatic cells (mouse SIM embryonic fibroblast cells, STOs) to explore the
72 chromosome structure character of FGSCs. Together with RNA sequencing
73 (RNA-Seq) and chromatin immunoprecipitation sequencing (ChIP-Seq), we identified
74 the distinct features of chromatin organization in FGSCs at three major levels: A/B
75 compartments, TADs, and chromatin loops, and demonstrated that FGSCs were most
76 similar to other ASCs, and largely different from iPSCs and STOs. We also identified
77 similarities in X chromosomes between SSCs and FGSCs by principal component
78 analysis of the X chromosome. Further analysis of Hi-C data during female germline
79 cell development showed that TADs were attenuated but still present in germinal
80 vesicle (GV) oocytes, disappeared in MII oocytes. Finally, we found conserved
81 compartment regions in the paternal/maternal genomes in early embryonic
82 development, related to imprinted genes. Together , these findings revealed the unique
83 chromatin signature of FGSCs, and presented a whole landscape of high-order
84 genome structure during development of female germline cells and early embryos.

85

86 **RESULTS**

87 **Biological Characterization of FGSCs and Other ASCs**

88 FGSCs were isolated and cultured from the ovaries of Ddx4-Cre;mT/mG neonatal

89 mice, as described previously ²⁰. After culture for at least 18 passages, the cells
 90 exhibited a characteristic morphology similar to that previously described for FGSCs
 91 ^{2,20}. The expression of female germline marker genes was determined by
 92 reverse-transcription polymerase chain reaction (RT-PCR). FGSCs after long-term
 93 culture expressed *Oct4*, *Fragilis*, *Mvh* (mouse vasa homologue, expressed exclusively
 94 in germ cells), *Stella*, *Gfra1*, and *Dazl* genes. Furthermore, immunofluorescence
 95 analysis revealed that these cells also expressed MVH, confirming their identity as
 96 FGSCs (Figure 1A).

97 We isolated and cultured SSCs from the testes of 6-day-old DBA/2×CAG-EGFP
 98 F₁ mice, as described previously ²¹. Long-term cultured SSCs (>20 passages) were
 99 assessed by RT-PCR and were shown to express male germline marker genes (*Etv5*,
 100 *Oct4*, *Plzf* (promyelocytic leukaemia zinc finger), *Gfra1*, and *Mvh*). These results
 101 were confirmed by immunofluorescence and most of the cultured cells were also
 102 positive for PLZF expression, confirming their identity as SSCs (Figure 1B).

103 Isolated primary NSCs could self-proliferate and be cultured for 5–8 passages in
 104 NSC proliferation medium. Morphologically, cultured NSCs were spindle-shaped
 105 with a high nucleus-to-cytoplasm ratio, as reported previously ²². The cultured NSCs
 106 were positive for several NSC-specific markers including *Nestin*, *Sox2*, *Pax6*, *Olig2*,
 107 and *Ascl1*, as determined by RT-PCR. Immunocytochemical staining confirmed that
 108 most of the cultured NSCs were positive for NESTIN and SOX2, as typical markers
 109 specific for NSCs. Following the removal of epidermal growth factor and basic
 110 fibroblast growth factor from the medium, NSCs spontaneously differentiated into

neurons and astrocytes, characterized by prominent dendrites with long axons and by extensive cytoplasm with thick processes, respectively. The differentiation potential of cultured NSCs was confirmed by immunochemical staining of the neural- and astrocyte-specific markers TUJ-1 (β 3 Tubulin) and GFAP (glial fibrillary acidic protein). These results confirmed the identity of the cultured cells as NSCs (Figure 1C). The morphology of STOs is shown in Figure 1D.

Global Chromosome Organization Map in FGSCs

To study the signature of the chromatin architecture in FGSCs, we performed *in situ* Hi-C²³ with two biological replicates of FGSCs and other cells (SSCs, NSCs, iPSCs and STOs) generating approximately 400 million reads for each replicate. After filtering artificial reads and normalization, we obtained a total of over 2 billion valid Hi-C reads, including an average of 1 billion long-range (>20 kb) intra-chromosomal *cis* contacts and 400 million inter-chromosomal *trans* contacts (Table S1). We confirmed the high reproducibility of the Hi-C data (Figure S1) and combined the two biological replicates into a single set of merged Hi-C data per cell type, to reach a maximum resolution of 20 kb.

Analysis of the Hi-C data showed that the high-order chromatin organization of the whole genome in FGSCs differed from that in the other cells (Figure S2). An overview of the intra-chromosomal contact heat maps revealed that FGSCs displayed a distinct chromatin organization (Figure 2A). We further examined the characteristics of the chromatin organization by analyzing the patterns of compartment status and

133 TADs in the autosomes across cells, avoiding sex chromosome effects. The
 134 compartment status was classified as active (A) or inactive (B) (Table S2). FGSCs
 135 were more similar to SSCs and NSCs in terms of A/B compartments, compared with
 136 iPSCs and STOs (Figure 2B), suggesting that FGSCs were ASCs. The patterns of
 137 TADs and directional indexes (DI) were almost the same for these cells (Figure 2B).
 138 We counted the numbers of compartments and TADs in the cells and showed that
 139 FGSCs had the lowest number of TADs, and the number of compartments was similar
 140 to that in SSCs (Figure 2C and Table S3). We also calculated the average
 141 intra-chromosomal contact probability of cells and found that the chromatin
 142 interaction frequency decreased monotonically from 10^5 to 10^8 bp for FGSCs and the
 143 other cells (Figure 2D). The contact probability curves were similar in five cell types
 144 from 10^5 to $10^{6.5}$ bp, but changes occurred at long-distance genome, as reported
 145 previously¹⁸.

146

147 **FGSCs Exhibited Distinct Compartment Status**

148 Systematic analysis of the compartment status in FGSCs showed that genes had
 149 higher expression levels in compartment A than in compartment B (Figure S3A),
 150 indicating that compartment status was correlated with gene expression. Combining
 151 with the ChIP-Seq data analysis, we observed that H3K27ac and H3K4me3 were
 152 highly correlated with compartment status (Figure S3B). The genome browser
 153 showed that H3K27ac was highly enriched in compartment A but not in compartment
 154 B (Figure S3C). By k-means clustering of the compartment status in FGSCs

155 compared with other cells, we obtained activate and inactive compartments of FGSCs
156 (Figure 3A). Furthermore, switching compartments of FGSCs accounted for a high
157 proportion (about 50%) of the total number in the genome compared with iPSCs and
158 STOs, but a smaller proportion (about 30%–40%) compared with SSCs and NSCs
159 (Figure S4A). These results suggested that FGSCs had a unique A/B compartment
160 status that was more similar to other ASCs than to iPSCs or STOs. Additional
161 RNA-Seq data revealed that the genes located in the switching compartment tended to
162 be significantly differentially expressed compared with the stable compartments
163 (Figure 3B). This was consistent with ChIP-Seq signal results, which showed
164 dramatic differences of H3K27ac and H3K4me3 enrichment in the switching
165 compartment compared with the stable compartment (Figure S4B). By calculating the
166 covariation between gene expression and compartment status, we identified a subset
167 of 1206 genes that were highly correlated with compartment status (Table S4). Gene
168 Ontology (GO) analysis showed that these genes were particularly associated with
169 stem cell population maintenance and cell proliferation (Figure S4C). Among these,
170 *Coprs*, as an ASC marker located in the A compartment of FGSCs, SSCs, and NSCs,
171 showed higher expression than in iPSCs in which it was located in the B compartment,
172 consistent with a previous report²⁴ (Figure 3C). In addition, *Nanog* as a pluripotent
173 stem cell marker, showed higher expression in iPSCs, being located in the A
174 compartment in iPSCs while switching to the B compartment in other types of cells
175 (Figure 3C). These findings suggested that FGSCs had a unique compartment status
176 characteristic of ASCs, which could work together with histone modification to

177 regulate gene expression to determine their features.

178 We then identified the TADs in FGSCs using DI (Table S3). Well-defined TADs
179 were conserved in ~90% of the genome across cell types (Figure 3D). We classified
180 the TADs into five types: stable, merge, split, rearrangement, and unique. Most of the
181 TADs belonged to the stable type (Figure 3E), suggesting that TAD structure was
182 highly stable across all five types of cells, in accordance with a previous report²⁵.
183 However, the absolute DI showed that the strength of the TADs differed between
184 FGSCs and the other cells (Figure S5A), suggesting that, although TAD domains were
185 stable, FGSCs had a distinct frequency of intra-TAD interactions. We further explored
186 the relationship between TADs and gene expression, and observed that gene
187 expression was higher in TAD boundaries than in TADs in FGSCs and other cells
188 (Figure 3F), illustrating that genes were more activated in these TAD boundaries
189 (Figure S5B). We classified the boundaries into cell-type-specific and common
190 boundaries, and identified 417, 369, 286, 263, and 48 cell-type-specific boundaries
191 and 833 common boundaries, suggesting that most boundaries were stable across cell
192 types. Further study of the relationship between cell-type-specific boundaries and
193 gene expression indicated that gene expression changed significantly between specific
194 and common boundaries (Figure S5C). These results revealed that FGSCs had stable
195 TADs, but that gene expression was activated in the TAD boundary.

196

197 **Cell-type-specific Chromatin Loops Revealed FGSC Signature**

198 We systematically analyzed the chromatin loops and identified 4832, 1906, 7004,

199 3060, and 6951 chromatin loops in FGSCs, SSCs, NSCs, iPSCs and STOs,
200 respectively. Using a Venn diagram, we observed that only a few (n=177) chromatin
201 loops were shared across all cell types (cell-type shared loops) (Figure 4A),
202 suggesting that most chromatin loops were cell-type-specific and that FGSCs had
203 distinct chromatin loops. Furthermore, genes located in these cell-type-specific loops
204 were highly enriched in cell-type-related GO categories (Figure 4B). For instance,
205 *Sox17*, as a transcription factor involved in embryonic development ²⁶, formed
206 chromatin loops in iPSCs but gradually disappeared in FGSCs, SSCs, NSCs, and
207 STOs, which was also most highly expressed in iPSCs (Figure S6A), suggesting that
208 its expression could be regulated by *cis* regulation of the chromatin loops. Based on
209 previous results, both A/B compartment status and TADs could affect gene expression
210 ^{27,28}. We therefore investigated if the formation of cell-type-specific chromatin loops
211 was related to compartment A/B status or to TADs, and found that 60% of
212 cell-type-specific loops were commonly localized in the switching compartments
213 (Figure S6B), while about 30% were localized in the stable TAD type (Figure S6C).
214 This suggested that the chromatin loops relied more on compartment switching to
215 regulate gene expression, and were not dependent on TAD type to exert their function.

216 We further examined the specific chromatin loops in FGSCs as GSCs by
217 comparing them with the loops in SSCs using ChIP-Seq data. K-means clustering of
218 ChIP-Seq signals identified four major classes on active promoter (H3K4me3) and
219 enhancer (H3K27ac) sites (Figure 4C). Cluster 4 of active promoters and most of the
220 active enhancers were obviously different between FGSCs and SSCs (Figure 4C).

221 Meanwhile, GO enrichment analysis further revealed that specific loops with different
222 active promoters and enhancers were enriched in reproductive processes such as sex
223 differentiation, sexual reproduction, and reproductive development (Fisher's exact test,
224 $P < 0.05$; Figure S7). We divided these loops into two subsets: FGSCs lost and FGSCs
225 gained (Figure 4D). *Dmrt1*, as a conserved transcriptional regulator in male germ cells
226 required for the maintenance and replenishment of SSCs ²⁹, was looped in SSCs but
227 not in FGSCs. *Hif1an*, as a heterodimeric transcription factor related to interface with
228 stem cell signaling pathways ³⁰, gained loops in FGSCs and could be related to female
229 germline cell development (Figure 4D). Overall, these findings indicated that FGSCs
230 possessed cell-type-specific chromatin loops that provided the spatial space for
231 histone modification to regulate gene expression.

232

233 **X-Chromosome Structure Was Similar Between SSCs and FGSCs**

234 One female X chromosomes is randomly inactivated during mammalian development
235 to ensure matched dosages in males and females ³¹. To dissect the high-order
236 organization of the X chromosome in SSCs and FGSCs, we performed Pearson's
237 correlation analysis of the Hi-C matrix in the X chromosome. The results showed that
238 SSCs and FGSCs had a strong correlation (Figure 5A). Furthermore, upon extracting
239 the eigenvectors of the whole chromosome interactions and using the first principal
240 component (PC1) score to compare the structure of the X chromosome in SSCs and
241 FGSCs, we found that the X chromosome was more similar (correlation = 0.87) than
242 the autosomes (mean correlation = 0.21) between SSCs and FGSCs (Figure 5B and

Figure S8). By analyzing the PC1 score of the X chromosome in FGSCs, SSCs, and female embryonic stem cells (fESCs), considering that fESCs have two activated X chromosomes (Xa), we observed that FGSCs were more highly correlated with SSCs than with fESCs in the X chromosome (Figure 5C). This suggested that the X chromosome is similar between SSCs and FGSCs, probably due to one of the X chromosomes being inactivated (Xi) in FGSCs^{32,33}.

To identify the difference between the active X chromosome and the inactive one, we deconvoluted the respective Hi-C data of these chromosomes from FGSCs (Figure 5D). As expected, we visualized that the X chromosome was separated by a region containing the DXZ4 macrosatellite (which reportedly plays a crucial role in shaping Xi-chromosome structure) into two parts in FGSCs³⁴, as well as in deconvoluted Xi, whereas this was not the case in SSCs (Figure 5D). This demonstrated that one of the X chromosomes in FGSCs was inactivated. Deconvoluted Xi displayed that the long-range contacts were attenuated for intra-TADs and inter-TADs (Figure 5D), consistent with a previous report^{32,33}. We next investigated the structure of the region containing Xist, which is a key factor for inactivation of the X chromosome. Notably, FGSCs were similar to SSCs in the Xist region, while in deconvoluted Xi it was shown that the Xist region lost most long-range contacts and retained a sub-TAD-like structure (Figure 5E), in support of a previous report³². Taken together, our data suggested that one of the X chromosomes was inactivated in FGSCs, to maintain relative consistency with SSCs to balance the gene expression between males and females.

265

266 **TADs Were Attenuated and then Disappeared During Female Germline Cell** 267 **Development**

268 FGSCs are derived from primordial germ cells and undergo meiosis into GV oocytes,
269 and then to MII oocytes. To explore the dynamic changes of TADs during female
270 germline cell development, we applied Hi-C to obtain the data of whole genome of
271 chromatin architecture in mouse GV oocytes (Figure S9). After analysis of Hi-C data
272 for MII oocytes, zygotes, two-cell embryos, and eight-cell embryos, we observed that
273 TADs were attenuated in GV oocytes, disappeared in MII oocytes, and recovered in
274 two-cell embryos (Figure 6A). A snapshot of TAD signals also showed that the TAD
275 strength was weakened during female germline cell development and reprogramed at
276 early embryonic development (Figure 6B), in contrast to changes in TADs during
277 spermatogenesis³⁵.

278 We further examined the changes in TADs during female germline cell
279 development by calculating the DI value, which reflected the degree of interactions of
280 a given bin in upstream or downstream regions and was associated with calling TADs.
281 Expectedly, the DI value was significantly reduced during female germline cell
282 development and reestablished in early embryonic development (Figure 6C).
283 Meanwhile, the insulation score showed that FGSCs had the strongest TAD
284 boundaries, while these were decreased in GV oocytes and weakest in MII oocytes,
285 and gradually increased in early embryonic development (Figure 6D). We further
286 calculated the proportion of *cis*-short interactions (<2 Mb) and *cis*-long interactions

(>2 Mb) versus total *cis*-interactions. The relative proportions of *cis*-short interactions in FGSCs was similar to GV oocytes, lowest in MII oocytes, highest in zygotes, and then reduced during early embryonic development, with similar interactions in eight-cell embryos and FGSCs (Figure 6E). Overall, these results demonstrated that TADs were attenuated and then disappeared during female germ cell development, and reestablished during early embryonic development (Figure 6F), thus revealing the pattern of TADs throughout female germ cell and early embryonic development.

294

295 **Identification of Conserved Allelic Chromatin Structures**

Previous findings have reported the chromatin structure of the maternal genome is different with the paternal genome during early embryonic development^{18,19}. We aimed to investigate how the conserved structures in these respective genomes affected their genome organization. Early embryonic Hi-C data were analyzed with single nucleotide polymorphisms between two mouse strains to track the maternal and paternal genomes. The correlation of compartment status according to the PC1 score, showed that the paternal genome was clustered in pachytene spermatocytes (PACs), sperm, paternal zygotes, and eight-cell embryos, while the maternal genome was clustered within MII oocytes, maternal zygotes, and eight-cell embryos (Figure 7A), indicating that the allelic chromatin structure was conserved in early embryonic development. FGSCs and SSCs, as early-stage germ cells, were clustered together (Figure 7A). Interestingly, the paternal genome clustered with the maternal genome at the two-cell embryo stage, but was completely separate at the eight-cell stage,

309 suggesting that the two-cell stage plays an important role in allelic chromatin structure
 310 development, possibly because of the reestablishment of TADs at this stage.
 311 Furthermore, we observed some regions of compartments in allelic genome were
 312 conserved during the early embryonic development (Figure 7B). We then
 313 systematically identified the conserved A/B compartment regions by calculating
 314 Pearson's correlation for the whole genome, with a sliding window of 2 Mb. The
 315 paternal genome had more conserved regions than the maternal genome (Figure 7C),
 316 indicating that the paternal genome was more conserved. Comparing the conserved
 317 regions, some allelic-specific conserved regions were identified by Venn diagrams
 318 (Figure 7D). By enriching for imprinted genes with Fisher's exact test ($P < 0.05$), we
 319 found that the allelic-specific regions were significantly related to imprinted genes
 320 (Table S5) such as *Igf2r*, *Dlk1*, and *Dio3*, which were reported to highly express in
 321 maternal or paternal ^{36,37}. The results suggested that those imprinted genes were
 322 affected by the allelic-specific conserved region, which could regulate paternal or
 323 maternal development.

324

325 **DISCUSSION**

326 Stem cells, including pluripotent stem cells and ASCs, have important implications in
 327 basic biology and regenerative medicine. However, regenerative medicine requires
 328 stem cells to be transplanted safely, with a particular focus on avoiding the
 329 development of cancer. Although gene mutations have been reported to be responsible
 330 for many diseases, including cancer ^{38,39}, recent studies have revealed that diseases

331 such as cancer can also be caused by disruption of chromatin organization. The
 332 chromatin architecture thus plays crucial roles in preventing DNA damage, in gene
 333 mutation, and in ensuring appropriate gene transcription, DNA duplication, and
 334 developmental processes⁴⁰⁻⁴². It is therefore essential that stem cells are characterized
 335 or identified in terms of their chromatin organization before their use in a clinical
 336 context. FGSCs not only have the abilities of self-renewal and differentiation, but are
 337 also responsible for passing on genetic information to the next generation. The
 338 stability of that genetic information is affected by the high-order genome organization
 339⁴³.

340 To identify the chromosome structure character of FGSCs, we compared FGSCs
 341 with pluripotent stem cells (iPSCs), ASCs (SSCs and NSCs) and somatic cells (STOs)
 342 by Hi-C technology. The results revealed that FGSCs had a distinct high-order
 343 genome structure in terms of the A/B compartment status, chromatin loops, and TADs.
 344 For further characterization, we identified FGSCs specific activated and repressed
 345 compartment regions, and obtained partially genes highly related with the switch of
 346 FGSCs compartment status. These genes were related to stem cell maintenance and
 347 differentiation pathways, strongly supporting the role of FGSCs as stem cells, with
 348 some shared characteristics with SSCs and NSCs, and belonging to ASCs. Moreover,
 349 FGSC-specific loops, which included active promoters and enhancers, could be
 350 significantly enriched in reproductive-related pathways. Among these, *hiflan*, which
 351 is related to Notch signaling, could be a potential marker for female germline cells.
 352 Our findings indicated that FGSCs belong to ASCs in the high-order organization, and

353 further confirmed the existence of FGSCs, consistent with the previous reports for
354 cellular and molecular characteristic^{4-7,44,45}.

355 Previous studies indicated that both FGSCs and SSCs have their own unique
356 epigenetic signatures^{44,46}. We analyzed and compared the 3D genomic architectures
357 of FGSCs and SSCs, and revealed that GSCs had their own unique high-order
358 chromatin organization, especially in terms of A/B compartments and chromatin loops.
359 Although FGSCs have one more X chromosome than SSCs, the architecture of the X
360 chromosome in FGSCs was more similar to SSCs than to autosomes, suggesting that
361 FGSCs maintain a balance of gene expression with SSCs by inactivating one X
362 chromosome. Furthermore, analysis of the X-chromosome matrix of FGSCs indicated
363 that it was separated into two domains by a region containing *Dxz4*, which has been
364 reported to be an essential regulator of X chromosome inactivation³². Meanwhile,
365 deconvoluted Xi data showed that *Xist* was also located in a region showing moderate
366 interactions with a TAD-like structure, consistent with previous findings³². These
367 results indicate that these differences could reflect Xi, supporting inactivation of one
368 X chromosome in FGSCs⁴⁷.

369 Interestingly, recent studies reported that the chromatin architecture changed
370 dynamically during spermatogenesis, with dissolved and then reappeared TADs and
371 compartments^{16,17}. On the contrary, TADs were attenuated and then disappeared in
372 oogenesis. During early embryonic development, TADs recovered and started to
373 appear in two-cell embryos, suggesting that this stage could play an essential role in
374 genome organization, including paternal/maternal chromatin reprogramming. We

375 therefore further analyzed paternal and maternal chromatin structures during early
376 embryonic development, and found that the paternal genome clustered with the
377 maternal genome at the two-cell stage, but was completely separate at the eight-cell
378 stage, consistent with the above results.

379 In conclusion, we present a comprehensive overview of the chromatin
380 organization of FGSCs to create a rich resource of high-resolution genome-wide maps.
381 Our findings revealed that the chromatin architecture of FGSCs included unique
382 features, especially in terms of compartment status and chromatin loops, which may
383 contribute to their cell-type-specific gene regulation. These data will provide a
384 valuable resource for future studies of the features of chromatin organization in
385 mammalian stem cells, with important implications for their role in medical research
386 and their potential and actual clinical applications.

387

388 **EXPERIMENTAL PROCEDURES**

389 Additional information and details regarding this work may be found in the
390 Supplemental Experimental Procedures.

391

392 **ACCESSION NUMBERS**

393 The accession number for the expression and sequencing data reported in this paper is
394 GEO: GSE126014 and GEO: GSE137771.

395

396 **SUPPLEMENTAL INFORMATION**

397 Supplemental Information includes Supplemental Experimental Procedures, eight
398 figures and four tables and can be found with this article online.

399

400 **AUTHOR CONTRIBUTIONS**

401 T.G.G performed the Hi-C experiments, analyzed the data and wrote the manuscript.
402 Z.X carried out ChIP-Seq experiments. X.W and W.L performed the NSC culture and
403 identification. L.X undertook the FGSC culture and identification. W.Y performed the
404 SSC culture and identification. L.H finished RNA-Seq experiment. H.C did the Hi-C
405 experiment of GV stage oocyte. W.J and L.J supervised the experiment work and
406 devised this study.

407

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550

551 **Figure Legends**

Figure 1. Morphology and biological characteristics of FGSCs and other ASCs

(A) Morphology and biological characteristics of FGSCs. (I) Representative morphology of cultured FGSCs (>18 passages) from Ddx4-Cre;mT/mG mice. (II) Representative field under UV light for FGSCs with GFP expression. (III) Merged images from I and II. Bar=25 μ m. (IV) RT-PCR analysis of female germline markers. Marker, 100-bp DNA markers. (V–VIII) Immunofluorescence of FGSCs for GFP (V), MVH (VI), DAPI (VII), and merged (VIII). Bar=10 μ m.

(B) Morphology and biological characteristics of SSCs. (I) Representative view of cultured SSCs (>20 passages) from CAG-EGFP mice. (II) Representative image under UV light for SSCs with GFP expression. (III) Merged images from I and II. Bar=25 μ m. (IV) RT-PCR analysis of male germline markers. Marker, 100-bp DNA markers. (V–VIII) Immunofluorescence of SSCs for GFP (V), PLZF (VI), DAPI (VII), and merged (VIII). Bar=50 μ m.

(C) Morphology and biological characteristics of NSCs. (I) Representative morphology of cultured NSCs. (II) Representative view of differentiated NSCs *in vitro*. Bar=10 μ m. (III) RT-PCR analysis of NSC markers. Marker, 100-bp DNA markers. (V–VIII) Immunofluorescence of NSCs for Nestin (V), SOX2 (VI), DAPI (VII), and merged (VIII). (IX–XII) Immunofluorescence of differentiated NCs for TUJ-1 (IX), GFAP (X), DAPI (XI), and merged (XII). Bar=10 μ m.

(D) Representative morphology of cultured STOs. Bar=10 μ m.

Figure 2. Overall chromosome structure in FGSCs

574 (A) Contact matrices from chromosome 19 in FGSCs, SSCs, NSCs, iPSCs and STOs.

575 (B) First principal component (PC1) value, normalized Hi-C interaction heat maps,

576 and directional indexes (DIs) in FGSCs and other cells at 20-kb resolution. PC1 value

577 was used to indicate A/B compartment status, where a positive PC1 value represented

578 the A compartment (blue) and a negative one represented the B compartment

579 (yellow).

580 (C) Numbers of identified A/B compartments and TADs in FGSCs and other cells.

581 (D) The average contact probability across the genome decreased as a function of

582 genomic distance.

583

584 **Figure 3. FGSCs exhibited specific compartment status**

585 (A) K-means clustering (k=2) of PC1 values of the genome that change A/B

586 compartment status in FGSCs.

587 (B) Genes that changed compartment status (A to B or B to A) or that remained the

588 same (stable) compared with FGSCs (P value by Wilcoxon's test).

589 (C) IGV snapshots for *Coprs* and *Nanog* showed concordance between their

590 expression and PC1 values.

591 (D) Percentages of TADs and TAD boundaries in the genome.

592 (E) Numbers of TAD types, including stable, merged, split, unique, and rearrangement,

593 in stem cells.

594 (F) Comparison of gene expression between TADs and TAD boundaries.

595 Genome-wide average distribution of RNA-Seq, H3K27ac, H3K4me3, and

596 H3K27me3 reads around the domain boundaries in FGSCs.

597

598 **Figure 4. Cell-type-specific chromatin loops revealed FGSCs signature**

599 (A) Venn diagram showing that most chromatin loops were cell-type-specific in five
600 types of cells.

601 (B) GO enrichment of cell-type-specific chromatin loops.

602 (C) K-means clustering of H3K4me3 and H3K27ac ChIP-Seq data at promoter and
603 enhancer regions between FGSCs and SSCs.

604 (D) Chromatin loops gained or lost in FGSCs compared with SSCs.

605

606 **Figure 5. X-Chromosome structure conformation between SSCs and FGSCs**

607 (A) Pearson's correlation heat map showing similarity between FGSCs and SSCs.

608 (B) PC1 values in the X chromosome in FGSCs and SSCs.

609 (C) Spearman's correlation of PC1 values in the X chromosome in FGSCs, SSCs, and
610 fESCs.

611 (D) Deconvolution of Xi signal from Hi-C data obtained in FGSCs ($X_a + X_i$) by
612 subtracting the X_a contribution estimated from SSCs. A small region containing *Dxz4*
613 showed two domains in FGSCs and deconvoluted X_i , but not in SSCs (blue pixels
614 represent negative values, red pixels represent positive values).

615 (E) Normalized chromatin interaction maps around *Xist* at 20-kb resolution. Plots
616 show TAD signal (insulation score).

617

618 **Figure 6. TADs were attenuated and then disappeared during female germline**

619 **cell development**

620 (A) Normalized Hi-C interaction frequencies during female germline cell
621 development displayed as a heatmap.

622 (B) TAD signals at chromosome 17:33–37 Mb during female germline cell
623 development.

624 (C) Boxplot of absolute DI values during female germline cell development. P value
625 calculated by Kruskal-Wallis test.

626 (D) Average insulation scores (IS) of different stages in female germline cell
627 development at TADs (defined in FGSCs) and nearby regions.

628 (E) Relative proportions of *cis* interactions at different genome distances versus total
629 paired loci.

630 (F) Graphical model for dynamic changes in TADs during female germline cell
631 development.

632

633 **Figure 7. Identification of conserved allelic chromatin structures**

634 (A) Hierarchical clustering of PC1 values based on maternal (black) and paternal (red)
635 genome architectures. Reference shown in green.

636 (B) Example of conserved compartment region at chromosome 19:34–60 Mb during
637 early embryonic development.

638 (C) Numbers of conserved compartment regions in paternal and maternal genomes.

639 (D) Venn diagram showing overlap of conserved compartment regions between

640 paternal and maternal genomes.

D

A **B** **C** **D** **E** **F**

A

B

C

D

A

Chr6:105,000,000-125,000,000

B

TAD Signal

Chr17:33Mb-37Mb

C **D**

Insulation Score around FGSC TADs

E

Relative Proportion of cis interactions

F

A **B** **C** **D**