Supplementary data for

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

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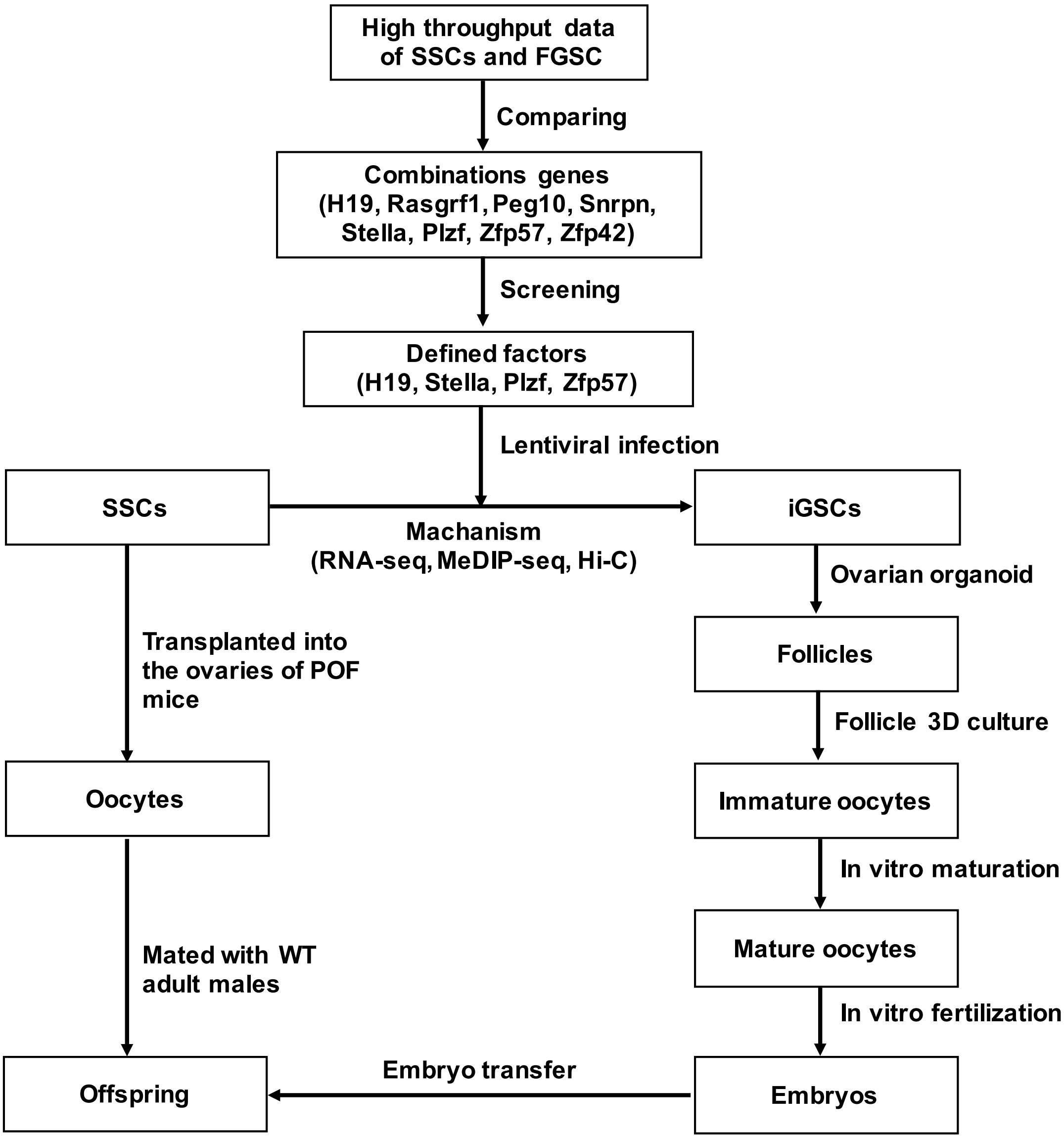

Fig. S1. Schematic representation of experimental protocol

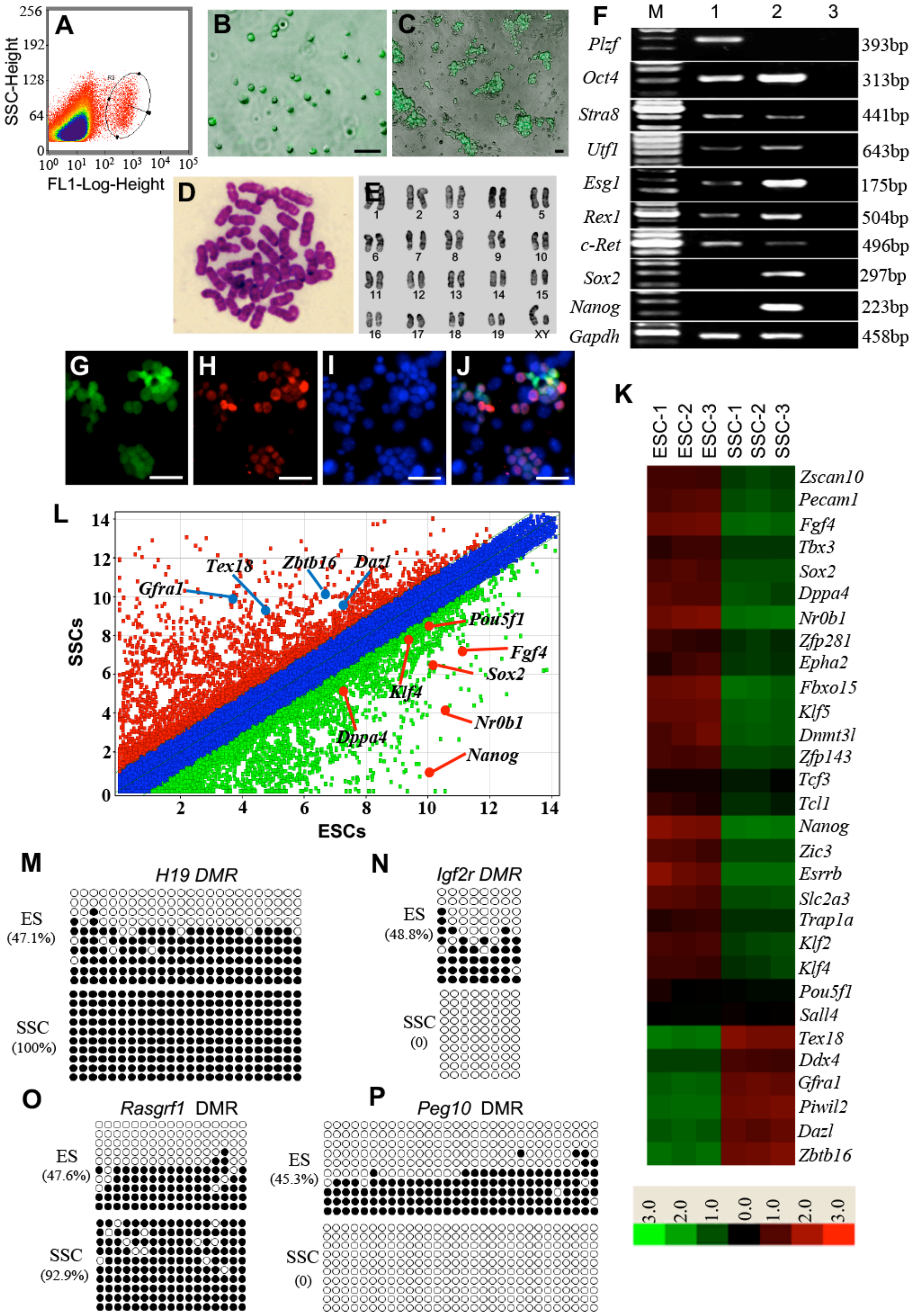

Fig. S2. Characterization of spermatogonial stem cells from neonatal mice. (**A**) Representative example of spermatogonial stem cell (SSC) purification by fluorescence-activated cell sorting (FACS). (**B**) Merge of bright field and fluorescence images of SSCs after purification by FACS. (**C**) Merge of bright field and fluorescence microscopies of cultured SSCs. (**D, E**) Cytogenetic analysis by G-band staining showing that SSCs possessed a normal karyotype (40, XY). (**F**) Gene expression profiles of SSCs. M, 100 bp DNA marker; lane 1, SSCs; lane 2, Embryonic stem cells (ESCs); lane 3, mock-transcribed SSC RNA samples. (**G-J**) Cultured SSCs were positive for EGFP (**G**) and MVH (mouse vasa homologue, expressed exclusively in germ cells) (**H**) staining. Cells were counterstained with 4',6-diamidino-2-phenylindole (DAPI) (**I**). (**K, L**) Global gene expression profiles of SSCs and ESCs. (**K**) Scatter plots of gene expression values in SSCs versus ESCs. Average expression levels of each gene (a dot on scatter plots) were calculated from three independent experiments. Genes whose expression level was at least two times greater in SSCs are shown in red, and genes whose expression level was at least two times greater in ESCs are shown in green. (**L**) Clustering analysis of some pluripotency-related genes and germ cell markers between SSCs and ESCs. (**M-P**) DMR methylation pattern of *H19* (**M**)*, Igf2r* (**N**)*, Rasgrf1* (**O**)*,* and *Peg10* (**P**) regions. DNA methylation levels were analyzed by bisulfite genomic sequencing. Black circles represent methylated cytosine-guanine sites (CpGs), and white circles represent unmethylated CpGs. The percentage of methylated CpG sites is shown beside the map. Scale bars: 50 μm (**B, C**), 30 μm (**G-J**).

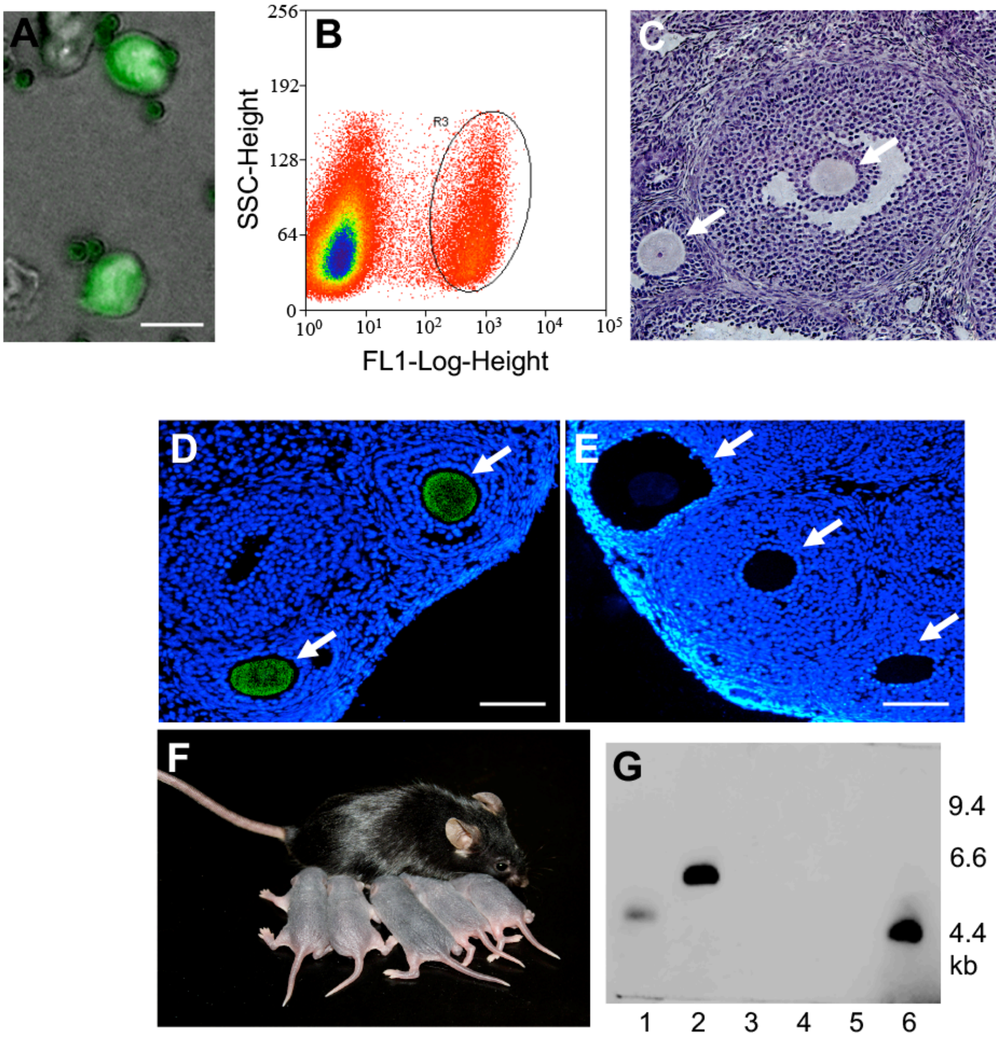

Fig. S3. Offspring derived from female germline stem cells. (**A**) Example of female germ cells isolated by magnetic activated cell sorting (MACS) with an anti-fragilis antibody. (**B**) Representative examples of female germline stem cell (FGSC) purification by FACS. (**C**) Representative morphology of ovaries from recipients of FGSC transplantation. (**D**) GFP-positive (green, arrows) oocytes in recipient ovaries at 6–8 weeks after transplantation of Pou5f1/GFP transgenic FGSCs. (**E**) Oocytes (arrows) in a wild-type ovary did not show a GFP signal. (**F**) Example of offspring from premature ovarian failure (POF) recipient mice transplanted with Pou5f1/GFP transgenic FGSCs. (**G**) Example of Southern blotting (see above). Lanes 1, 2, and 6, transgenic mice; lanes 3–5, wild-type mice. Scale bars, 10μm (**A**) and 100 μm (**C**-**E**).

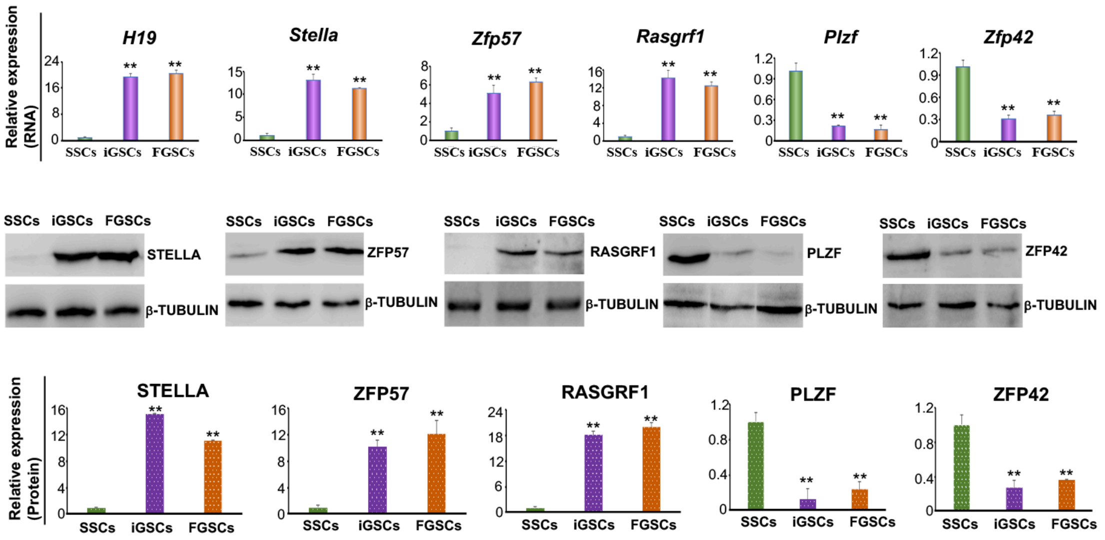

Fig. S4. Effects of specific gene expression levels examined by qRT-PCR and western blot analyses.

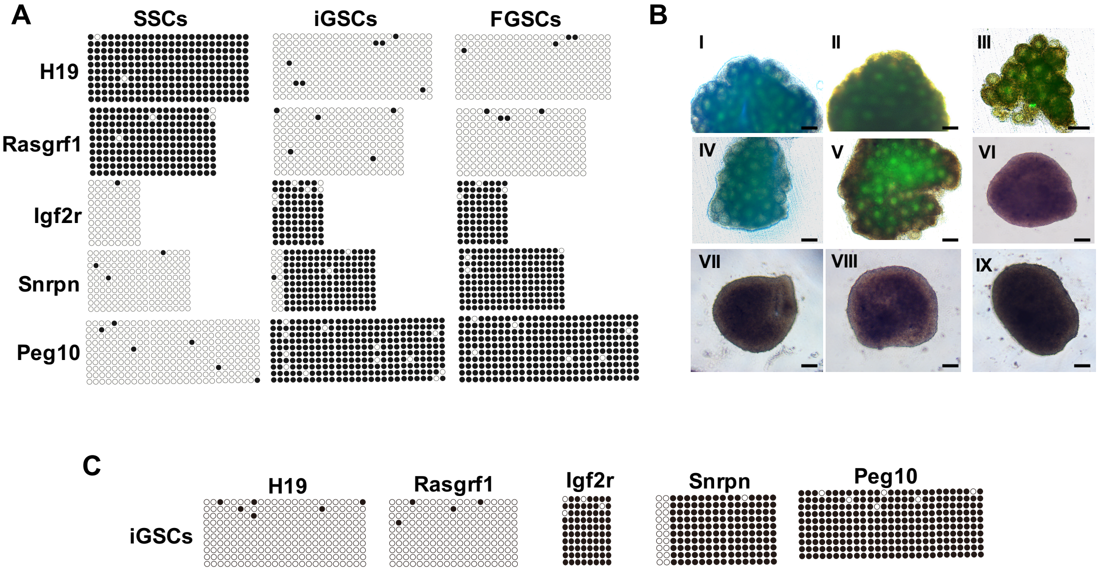

Fig. S5. Screening the critical imprinted genes and transcription factor genes required for SSC conversion. (**A**) Methylation analysis at paternally imprinted loci (*H19* and *Rasgrf1*) and maternally imprinted loci (*Igf2r*, *Snrpn*, and *Peg10*) in SSCs, iGSCs (induced by 6Gs, *Stella, H19, Zfp57, Rasgrf1, Plzf,* and *Zfp42*), and FGSCs. (**B**) Representative merged bright field and fluorescence images of ovarian organoids. I, Ovarian organoids formed by FGSCs co-cultured with somatic cells of gonads. II, Ovarian organoids formed by iGSCs induced by 6Gs co-cultured with somatic cells of gonads. III, Withdrawal of *Rasgrf1* from 6Gs, and ovarian organoids formed by iGSCs induced by remaining in 5Gs (*Stella, H19, Zfp57, Plzf,* and *Zfp42*) co-cultured with somatic cells of gonads. IV, Withdrawal of *Zfp42* from 5Gs, and ovarian organoids formed by iGSCs induced by remaining in 4Gs (*Stella, H19, Zfp57*, and *Plzf*) co-cultured with somatic cells of gonads. V–VIII, Removal of *Stella* (V), *H19* (VI), *Zfp57* (VII), or *Plzf* (VIII) from 4Gs failed to form ovarian organoids. (**C**) Methylation analysis at paternally imprinted loci (*H19* and *Rasgrf1*) and maternally imprinted loci (*Igf2r*, *Snrpn* and *Peg10*) in iGSCs induced by 4Gs. Scale bars: 100 µm.

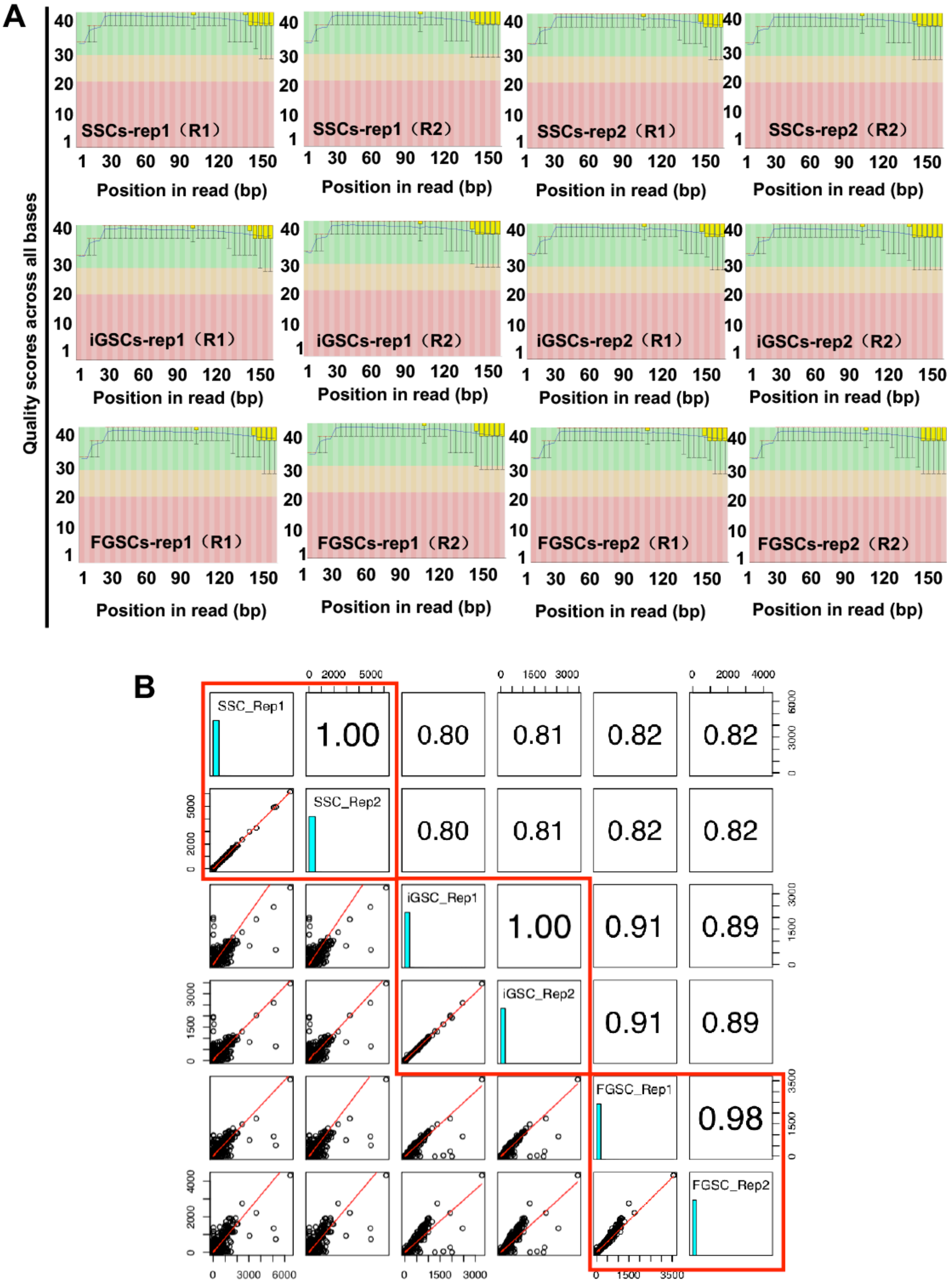

Fig. S6. Validate RNA-Seq data. (**A**) Fast-QC data showing the position-specific sequencing quality in each replicated sample of SSCs, iGSCs, and FGSCs. (**B**) Correlation plots of each replicated sample of SSCs, iGSCs, and FGSCs.

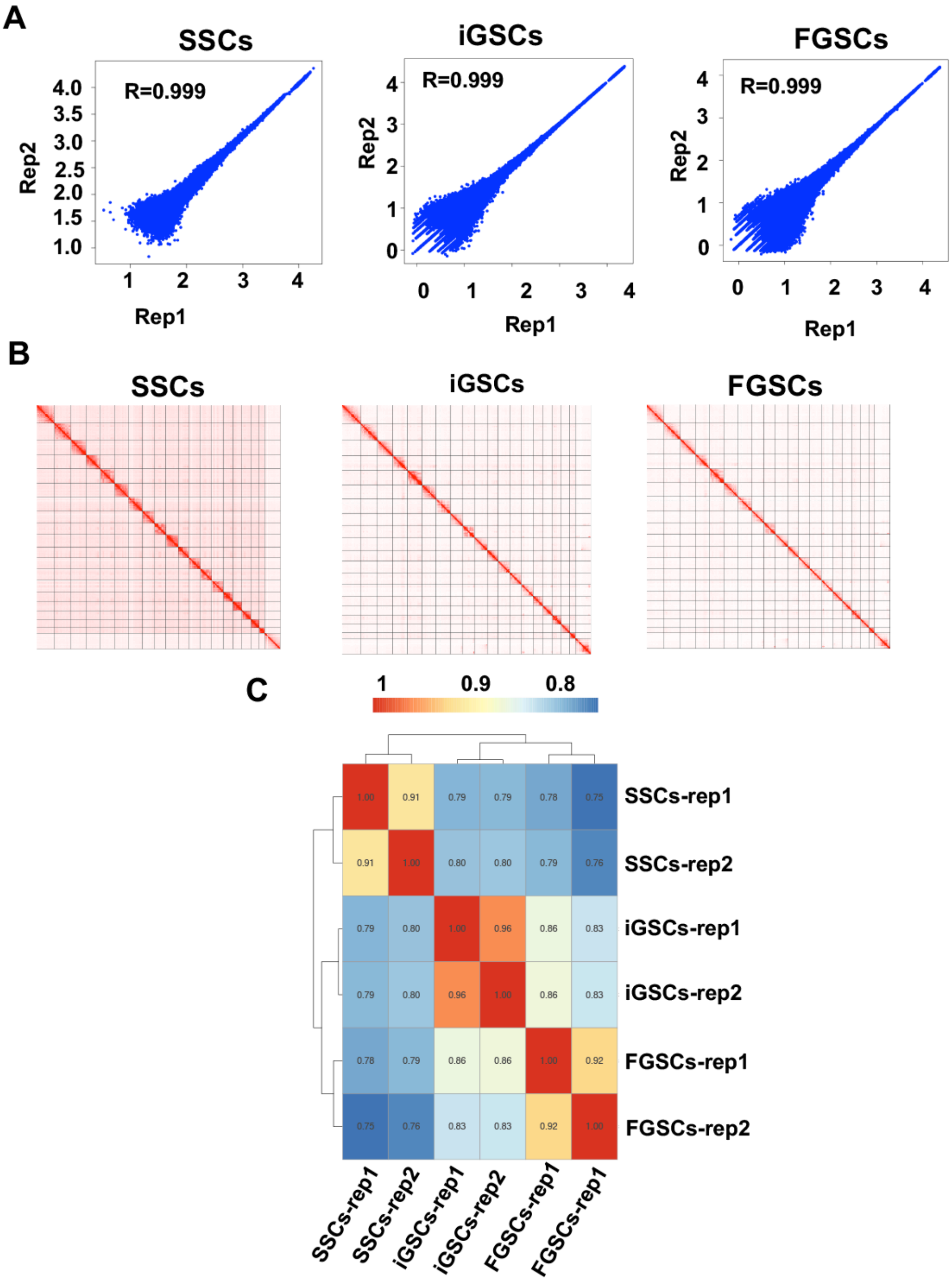

Fig. S7. Validate Hi-C data and overview of whole genome interaction frequency heat maps in SSCs, iGSCs, and FGSCs. (**A**) Correlation between Hi-C replicates of SSCs, iGSCs, and FGSCs according to the normalized interaction frequency at a 400kb resolution. R indicates Pearson's correlation coefficient. (**B**) Hieratical clustering of PC1 values for the A/B compartment status in SSCs, iGSCs, and FGSCs. (**C**) verview of whole genome interaction frequency heat maps in SSCs, iGSCs, and FGSCs.

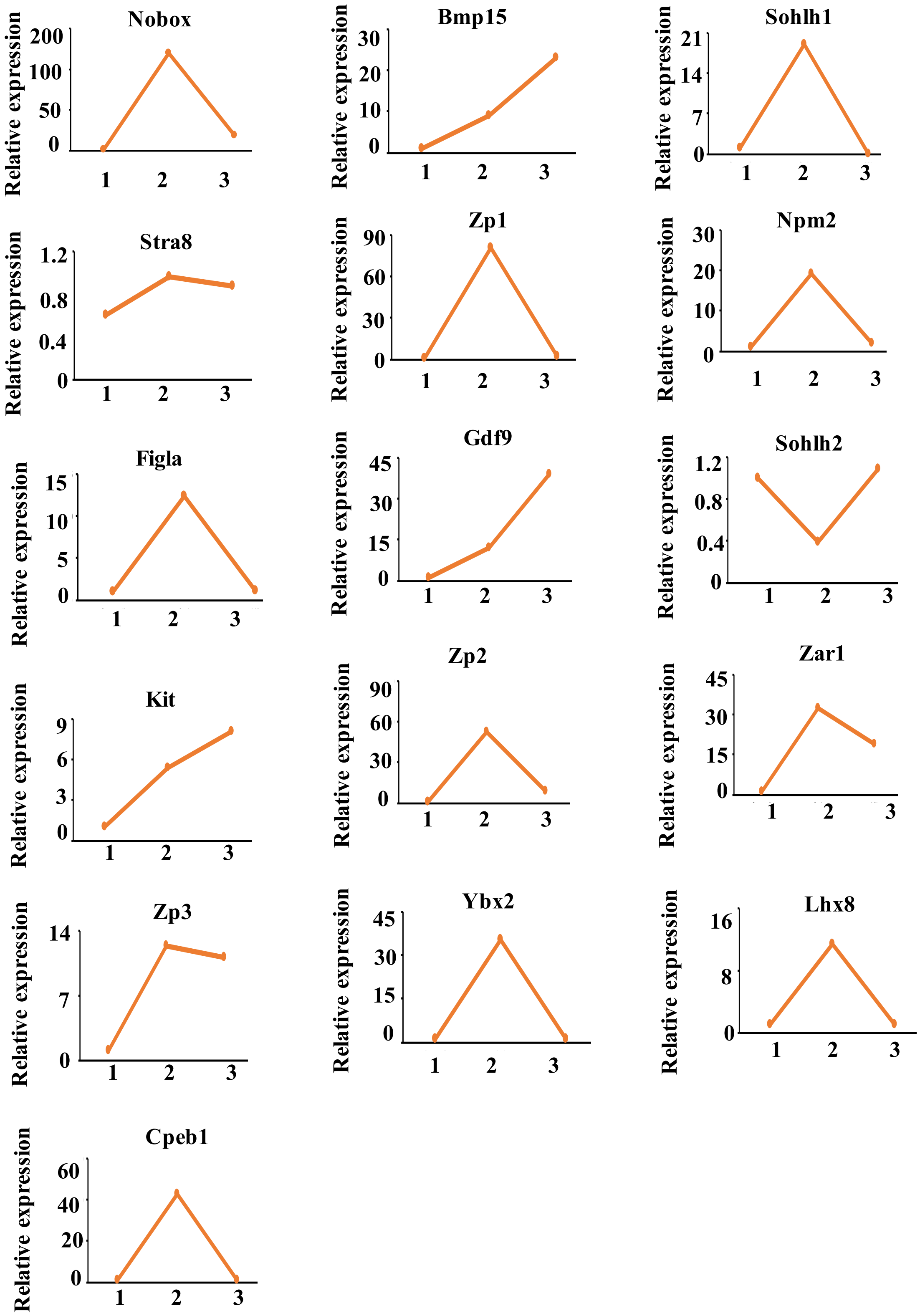

Fig. S8. Gene expression dynamics during oogenesis in vitro determined by qRT-PCR. Relative mRNA expression of genes in oogenesis of iGSCs (red) and control (blue) are shown. 1, GSCs, 2, immature oocytes, and 3, Mature oocytes.

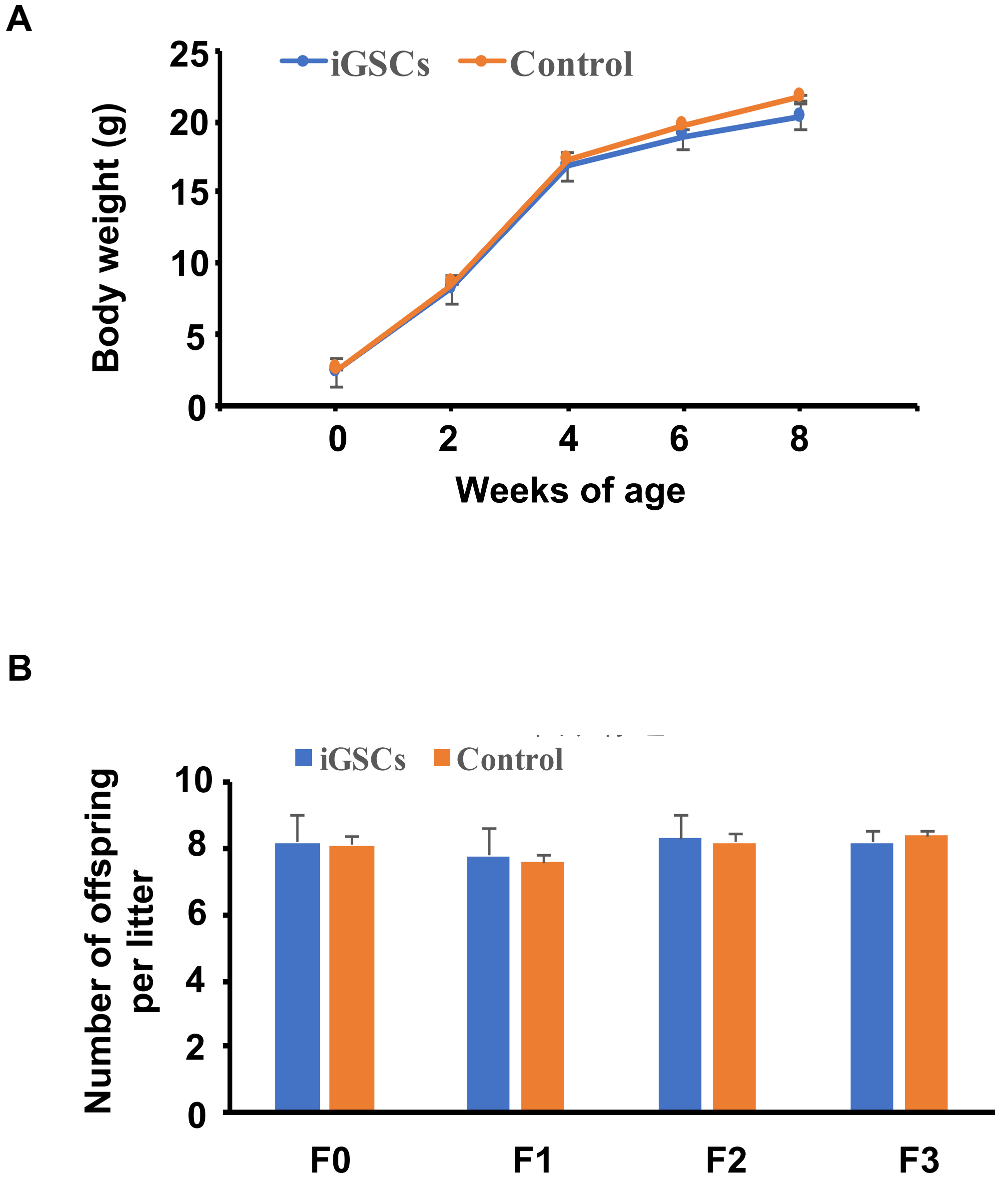

Fig. S9. Fertile offspring following in vitro production of functional oocytes from iGSCs. (**A**) The mean body weight of offspring from in vitro production of functional oocytes from iGSCs at postnatal week 0, 2, 4, 6, 8. (**B**) The mean number of F0, F1, F2, F3 generation offspring from in vitro production of functional oocytes from iGSCs per litter. F0 (offspring from in vitro offspring production of functional oocytes derived from iGSCs), F1(offspring produced by F0 generation), F2(offspring produced by F1 generation), F3 (offspring produced by F2 generation). Control, wild-type mice.

Table S1. **Details regarding the RT-PCR or qRT-PCR analysis of cells and tissues**

Gene	Product Size(bp)	Primer Sequence (5’-3’)
Primers for gene expression dynamic during oogenesis *in vivo*		
Plzf	393	F：CACCAACCTTTCTTCTCCGGG
		R：CCGTGTAGGCGTACTCCAGG
Mvh	193	F: GCTTCATCAGATATTGGCGAGT
		R: GCTTGGAAAACCCTCTGCTT
Stra8	173	F:ACAACCTAAGGAAGGCAGTTTAC
		R:GACCTCCTCTAAGCTGTTGGG
Sycp3	206	F: AGCCAGTAACCAGAAAATTGAGC
		R: CCACTGCTGCAACACATTCATA
Zp1	164	F: CCCTGAGATTGGGTCAGCG
		R: AGAGCAGTTATTCACCTCAAACC
Zp3	186	F: ATGGCGTCAAGCTATTTCCTC
		R: CGTGCCAAAAAGGTCTCTACT
Gapdh	123	F: AGGTCGGTGTGAACGGATTTG
		R: TGTAGACCATGTAGTTGAGGTCA
Primers for gene expression dynamic in transplanted SSCs		
H19	106	F: GAACAGAAGCATTCTAGGCTGG
		R: TTCTAAGTGAATTACGGTGGGTG
Grb10	181	F: CCTGCCAAGCATGATGTCAAA
		R: CCAGGCACCTCTCTAATCCCA

Rasgrf1	71	F: GCCAGAAGACTTGACAACGCT
		R: TCAATCTACAGGGATGGTGGAAG
Zfp57	122	F: ATGGCAGCTAGGAAACAGTCT
		R: TGGTAAAGGGTCTTCTGTGTAGA
Igf2r	194	F: GGGAAGCTGTTGACTCCAAAA
		R: GCAGCCCATAGTGGTGTTGAA
Snrpn	156	F: TGCTACGTGGGGAGAACTTG
		R: CCTGGGGAATAGGTACACCTG
Gtl2	71	F: TCCTCACCTCCAATTTCCCCT
		R: GAGCGAGAGCCGTTCGATG
Peg10	162	F: TGCTTGCACAGAGCTACAGTC
		R: AGTTTGGGATAGGGGCTGCT
Stella	130	F: GACCCAATGAAGGACCCTGAA
		R: GCTTGACACCGGGGTTTAG
Zfp42	161	F: GGAGGAAATAGGTAGAGCGCA
		R: AGTGAGGCGATCCTGCTTTC
Plzf	150	F: CTGCGGAAAACGGTTCCTG
		R: GTGCCAGTATGGGTCTGTCT
Nanos2	183	F: CTGCAAGCACAATGGGGAGT
		R: CGTCGGTAGAGAGACTGCTG
Gapdh	123	F: AGGTCGGTGTGAACGGATTTG
		R: TGTAGACCATGTAGTTGAGGTCA

Primers for RT-PCR analysis of germ cell or germline stem cell markers

Dazl	170	F: ATGTCTGCCACAACTTCTGAG

		R: CTGATTTCGGTTTCATCCATCCT
Mvh	193	F: GCTTCATCAGATATTGGCGAGT
		R: GCTTGGAAAACCCTCTGCTT
Stella	386	F: ATCGCCATGGAGGAACCATC
		R: AATGGCTCACTGTCCCGTTC
Fragilis	183	F: GCCTATGCCTACTCCGTGAA
		R: AGTGTGAAGGTTTTGAGCGTT
Oct4	313	F: GGCGTTCTCTTTGGAAAGGTGTTC
		R: CTCGAACCACATCCTTCTCT
Plzf	150	F: CTGCGGAAAACGGTTCCTG
		R: GTGCCAGTATGGGTCTGTCT
Gapdh	222	F: CAGGAGAGTGTTTCCTCGTCC
		R: TTCCCATTCTCGGCCTTGAC

Primers for gene expression dynamic during oogenesis *in vitro*

Stella	130	F: GACCCAATGAAGGACCCTGAA
		R: GCTTGACACCGGGGTTTAG
Bmp15	100	F: TCCTTGCTGACGACCCTACAT
		R: TACCTCAGGGGATAGCCTTGG
Sohlh1	181	F: CGGGCCAATGAGGATTACAGA
		R: TCCTGCGTTCTCTCTCGCT
Nobox	189	F: ATGGAACCTACGGAGAAGCTC
		R: CTCAGAGGTCTTCGACAGTGG
Zp1	164	F: CCCTGAGATTGGGTCAGCG
		R: AGAGCAGTTATTCACCTCAAACC

Npm2	162	F: GTGACCGAAACCACAGCAAAA
		R: CACACGGTTCACCTCCTCTT
Stra8	173	F:ACAACCTAAGGAAGGCAGTTTAC
		R:GACCTCCTCTAAGCTGTTGGG
Gdf9	116	F: TCTTAGTAGCCTTAGCTCTCAGG
		R: TGTCAGTCCCATCTACAGGCA
Sohlh2	154	F: GGGCAGGGCAGAGTAAATCTT
		R: CAAACGAGTTAGCAGCCAAAAG
Figla	238	F: CCGCCATCTGTAGGCTCAAG
		R: ACACAGCCGAGTATCTGTATGTA
Zp2	111	F: GTGGCAGAGGAAAGCATCTGT
		R: GACTGAGGAAGGCTTACTGAGT
Zar1	136	F: TCGGTGCAGTGTTCACTCG
		R: CTACGGTCTGCCAGGATCG
Kit	90	F: GCCACGTCTCAGCCATCTG
		R: GTCGCCAGCTTCAACTATTAACT
Ybx2	102	F: GGAGTTTGATGTCGTGGAAGG
		R: CGTCGATTAGGGGCATAGCG
Lhx8	159	F: TCAGAGAGTGGTTACGGTCAC
		R: CTGCTCGTCACATACCAGCTC
Zp3	186	F: ATGGCGTCAAGCTATTTCCTC
		R: CGTGCCAAAAAGGTCTCTACT
Cpeb1	229	F: AAGGATTGCTGGGACAACCAA
		R: GGCCACGGGGAGATTCTTG

Gapdh	123	F: AGGTCGGTGTGAACGGATTTG R: TGTAGACCATGTAGTTGAGGTCA

Primers for gene expression profiles of SSCs

Gapdh	458	F: GTCCCGTAGACAAAATGGTGA R: TGCATTGCTGACAATCTTGAG
Oct4	313	F: GGCGTTCTCTTTGGAAAGGTGTTC R: CTCGAACCACATCCTTCTCT
Rex-1	504	F: CACCATCCGGGATGAAAGTGAGAT R: ACCAGAAAATGTCGCTTTAGTTTC
Esg-1	175	F: GCCGTGCGTGGTGGATAAGC R: GCCAAACAGATATTTCAGCACCAGC
Stra8	441	F: TCACAGCCTCAAAGTGGCAGG R: GCAACAGAGTGGAGGAGGAGT
Utf1	643	F: GATGTCCCGGTGACTACGTCT R: TCGGGGAGGATTCGAAGGTAT
c-Ret	496	F: TGGAAGCAGGAGCCAGACA R: TGCTCTAATCCGCTTCTCCTG
Sox-2	297	F: TAGAGCTAGACTCCGGGCGATGA R: TTGCCTTAAACAAGACCACGAAA
Nanog	223	F: CAGGAGTTTGAGGGTAGCTC R: CGGTTCATCATGGTACAGTC

Primers for effects of specific gene expression levels examined by qRT-PCR

H19	106	F: GAACAGAAGCATTCTAGGCTGG R: TTCTAAGTGAATTACGGTGGGTG

Stella	130	F: GACCCAATGAAGGACCCTGAA
		R: GCTTGACACCGGGGTTTAG
Zfp57	122	F: ATGGCAGCTAGGAAACAGTCT
		R: TGGTAAAGGGTCTTCTGTGTAGA
Rasgrf1	71	F: GCCAGAAGACTTGACAACGCT
		R: TCAATCTACAGGGATGGTGGAAG
Plzf	150	F: CTGCGGAAAACGGTTCCTG
		R: GTGCCAGTATGGGTCTGTCT
Zfp42	161	F: GGAGGAAATAGGTAGAGCGCA
		R: AGTGAGGCGATCCTGCTTTC
Gapdh	123	F: AGGTCGGTGTGAACGGATTTG
		R: TGTAGACCATGTAGTTGAGGTCA

F, forward primer; R, reverse primer

Table S2. Number of valid Hi-C reads

Cell Type	RE	Replicate	Total Reads	Unique Read pairs (%)	Valid HiC pairs (%)	Duplication Remaining Read pairs	Cis short-range contacts(<20kb)(%)	Cis long-range contacts(>20kb)(%)	Trans Contacts (%)
SSCs	Mbol	R1	358740361	192240210(53.6%)	67216789(18.7%)	60006537	46582509(7.8%)	21665016(36.1%)	33683271(56.1%)
	Mbol	R2	387705044	201493073(52.0%)	89231944(23.0%)	75753385	5161733(7.2%)	27178344(35.9%)	43413308(56.8%)
	Mbol	Comb	746445405	393733283(52.7%)	156448733(21.0%)	135759922	9819983(7.2%)	48843360(36.0%)	77096579(28.3%)
iGSCs	Mbol	R1	344949297	143578429(41.6%)	103008967(29.9%)	47297743	5533433(11.7%)	28381393(60.0%)	13382917(28.3%)
	Mbol	R2	359258923	152003521(42.3%)	108556313(30.2%)	48746196	5693933(11.7%)	29242847(60.0%)	13809416(28.3%)
	Mbol	Comb	704208220	295581950(42.0%)	211565280(30.0%)	96043939	11227366(11.7%)	57624240(60.0%)	27192333(28.3%)
FGSCs	Mbol	R1	352273659	155092100(44.0%)	94903762(27.0%)	46337591	6457272(13.9%)	27845481(60.0%)	12034838(26.0%)
	Mbol	R2	184015447	78861662(42.9%)	47072757(25.6%)	30656444	4232968(13.8%)	18433037(60.1%)	7990439(26.1%)
	Mbol	Comb	536289106	233953762(43.6%)	141976519(26.5%)	76994035	10690240(13.9%)	46278518(60.1%)	20025277(26.0%)

Table S2. The DNA methlytion analysis of offspring derived from iGSCs

Source of offspring mice	Number of selected offspring	The DNA methylation levels (%)	
		H19	Peg10
Wide type	WT-1	49.55	48.51
	WT-2	50.77	48.96
	WT-3	49.22	49.03
IGSCs	I-1	49.17	51.13
	I-2	51.32	48.54
	I-3	51.08	49.16
	I-4	49.16	51.83
	I-5	49.00	48.77
	I-6	49.68	50.98
	I-7	50.33	50.75
	I-8	51.71	49.66
	I-9	48.52	49.53
	I-10	49.31	49.19

Table S4. Number of offspring following *in vitro* offspring production of functional oocytes derived from iGSCs

Group	Number of male offspring	Number of female offspring	Male to female ratio
2 ♂ (F0) × 4 ♀ (WT)	73	78	0.94
2 ♂ (WT) × 4 ♀ (F0)	73	77	0.95
2 ♂ (F0) × 4 ♀ (F0)	78	71	1.10
2 ♂ (F1) × 4 ♀ (WT)	73	76	0.96
2 ♂ (WT) × 4 ♀ (F1)	81	78	1.04
2 ♂ (F1) × 4 ♀ (F1)	77	75	1.03
2 ♂ (F2) × 4 ♀ (WT)	76	74	1.03
2 ♂ (WT) × 4 ♀ (F2)	71	73	0.97
2 ♂ (F2) × 4 ♀ (F2)	75	81	0.93
2 ♂ (F3) × 4 ♀ (WT)	69	76	0.91
2 ♂ (WT) × 4 ♀ (F3)	72	80	0.90
2 ♂ (F3) × 4 ♀ (F3)	74	69	1.07
2 ♂ (WT) × 4 ♀ (WT)	73	75	0.97

Notes: F0 (offspring from *in vitro* offspring production of functional oocytes derived from iGSCs), F1(offspring produced by F0 generation), F2(offspring produced by F1 generation), F3 (offspring produced by F2 generation)