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Supplemental Information

Tracing and Characterizing the Development of Transplanted Female Germline Stem Cells In Vivo

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Supplemental material

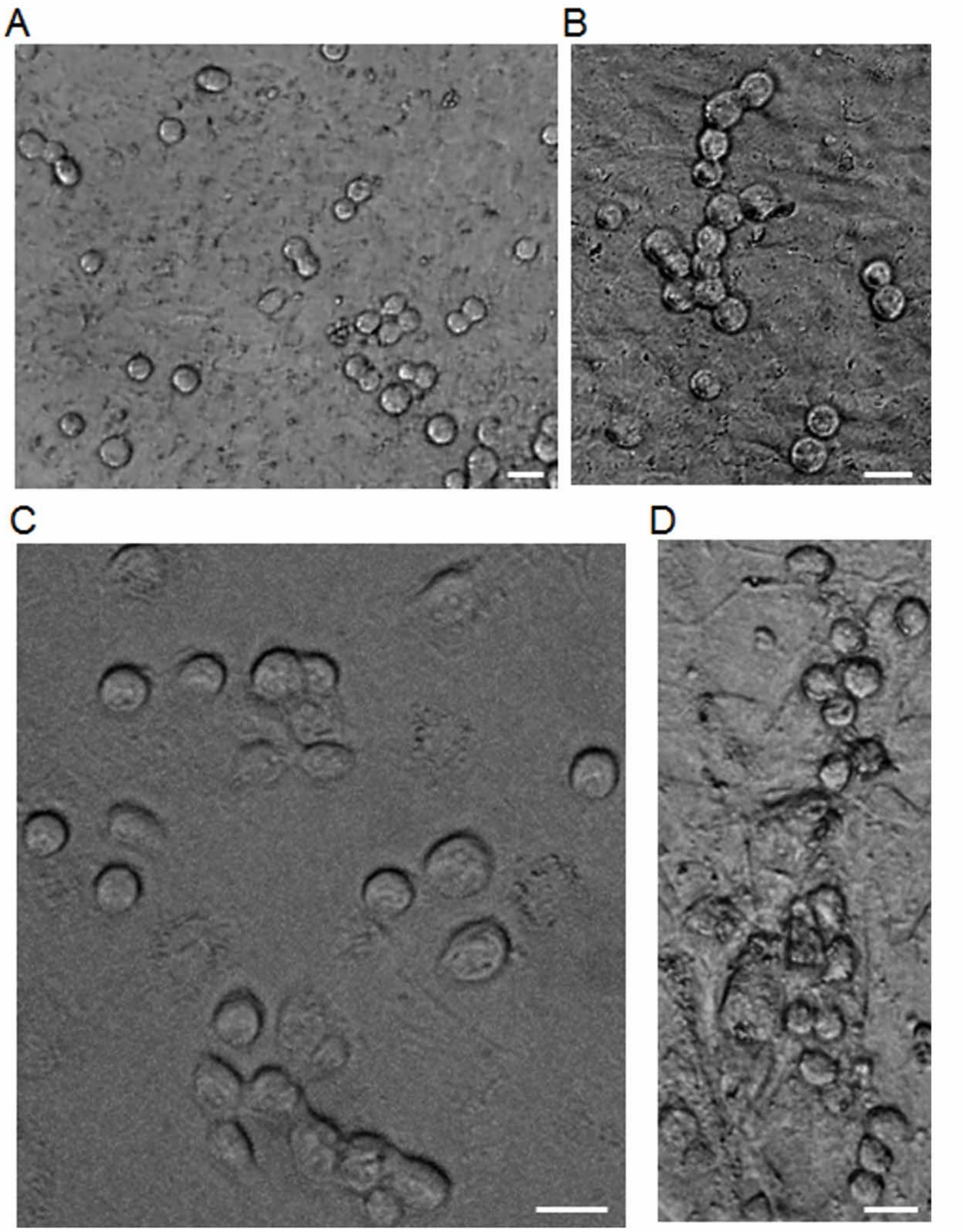


Figure S1. Representative morphology of FGSCs from 8-day-old or 10-day-old mouse. (**A,B**) FGSCs cultured for 3 days isolated from 8-day-old (**A**) and 10-day-old (**B**) mouse.(**C,D**) FGSCs cultured for 8 weeks isolated from 8-day-old (**C**) or 10-day-old (**D**) mouse. Scale bar, 20 μm.

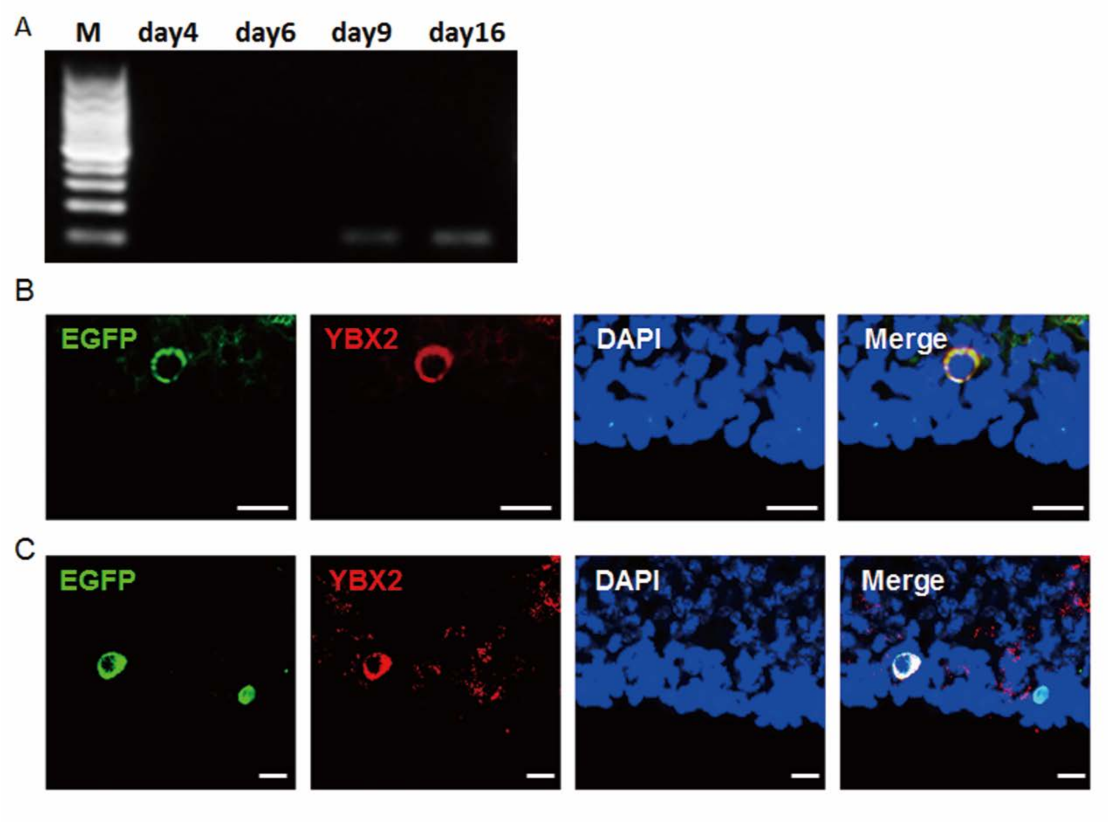


Figure S2. The expression of YBX2. (**A**) Ybx2 expression in single EGFP-positive FGSC on day 4, day 6, day 9 and day 16 post-transplantation. M: 100 bp DNA marker. (**B,C**) Immunofluoresence analysis of YBX2 and EGFP on day 9 (**B**) and day 16 (**C**) post-transplantation. Scale bar, 20 μm.

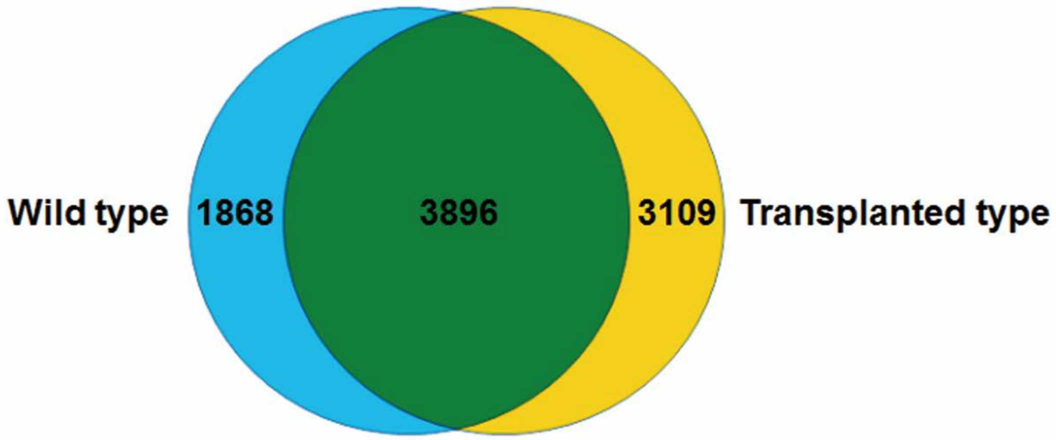


Figure S3. Venn diagram of differentially expressed genes (DEGs) during folliculogenesis between wild type and transplanted ovaries. In total, 3896 genes are the same DEGs in both ovaries.

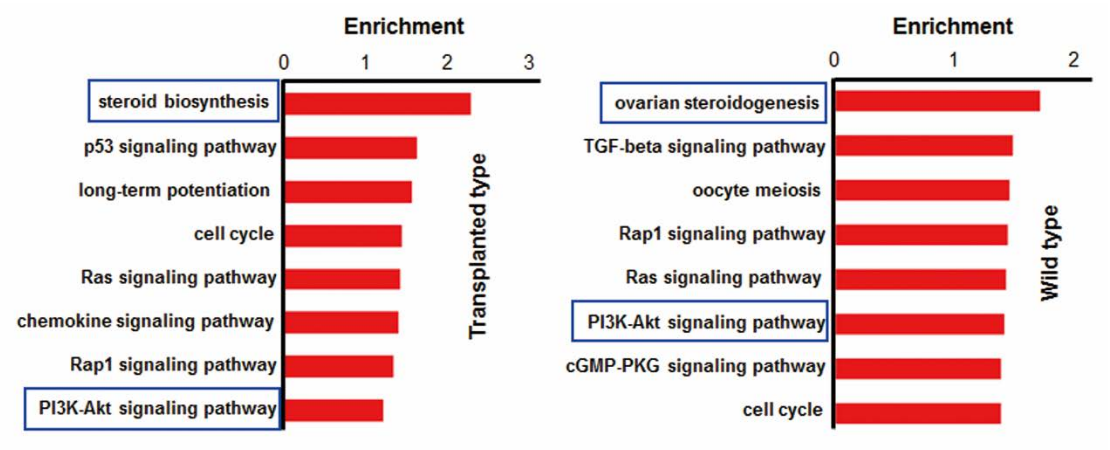
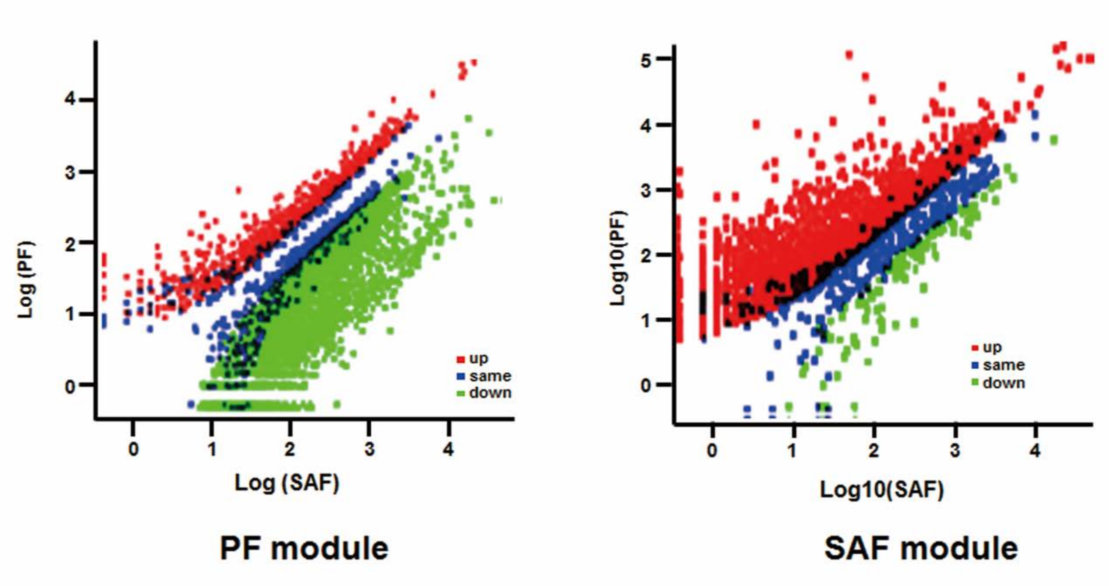


Figure S4. Pathway enrichment analysis of DEGs in transplanted and wild type ovaries (*P*-value < 0.05). Blue boxes indicate steroid biosynthesis or ovarian steroidogenesis and PI3K-Akt signaling pathway, respectively.



PF module

SAF module

Figure S5. Scatter plot of FPKM in PF and SAF modules. Red, blue, and green dots represented up-, same-, and down-regulated genes, respectively.

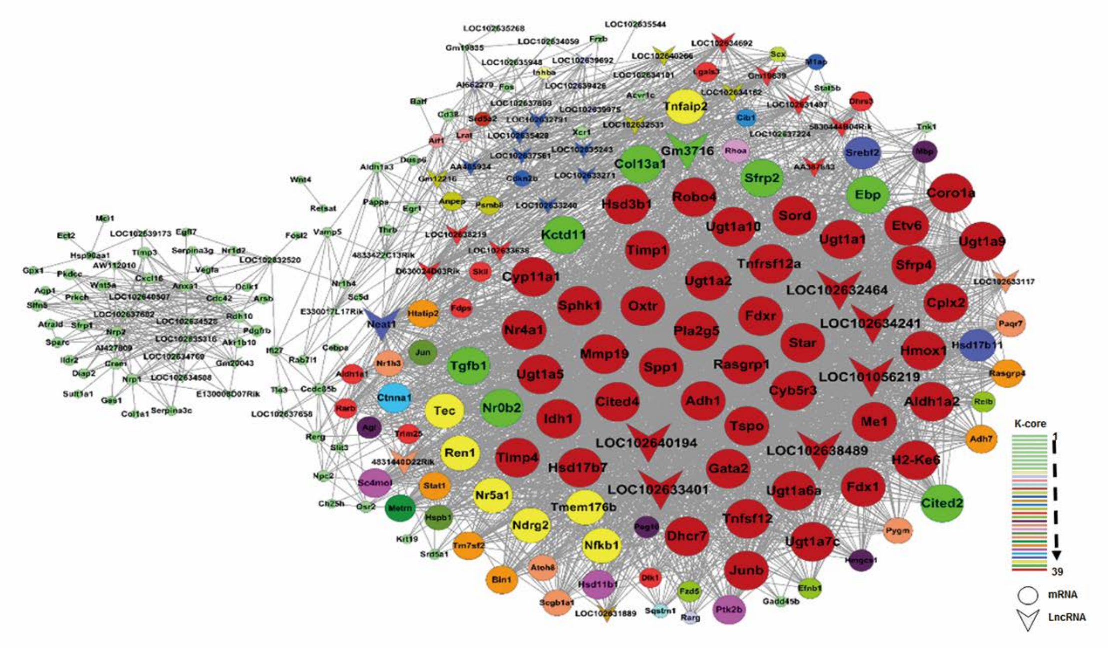


Figure S6. Gene co-expression network of SAF module. Color legend in bottom right corner indicates k-core values from 1 to 39. Cycle with color represents mRNA, and quadrangle with color represents lncRNA.

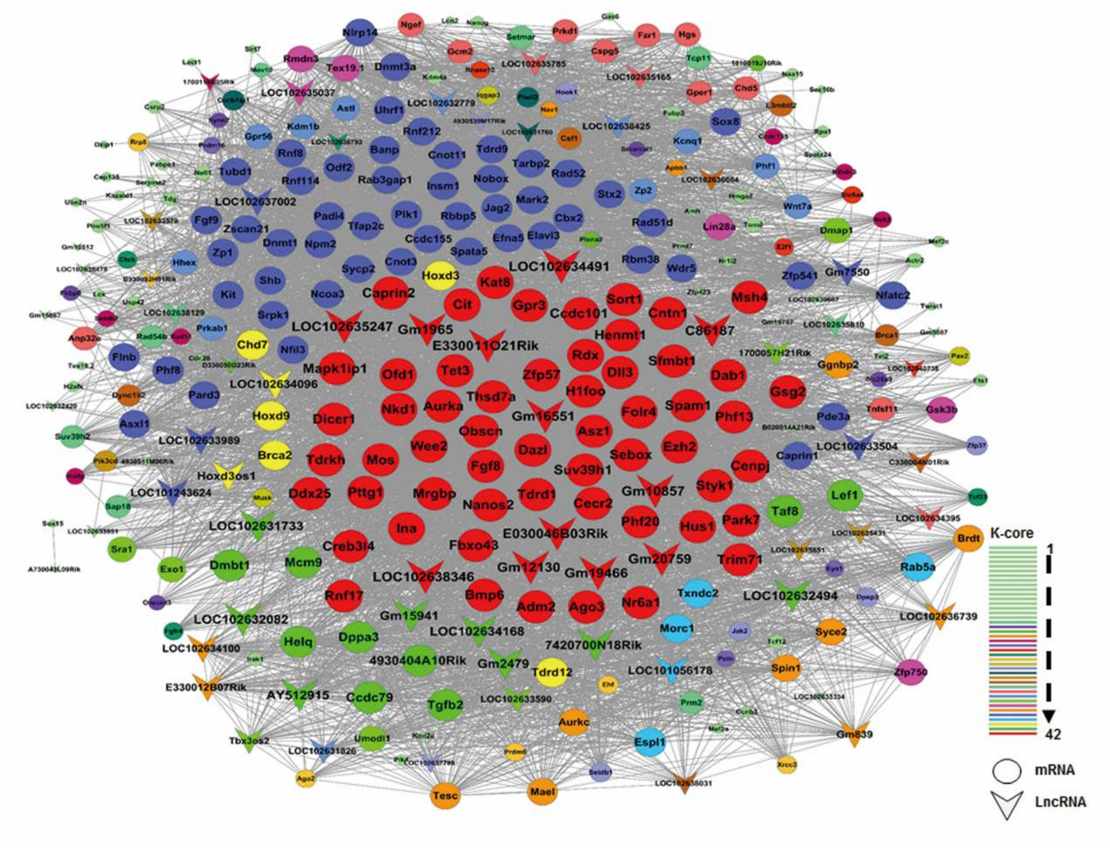


Figure S7. Gene co-expression network of PF module. Color legend in bottom right corner indicates k-core values from 1 to 42. Cycle with color represents mRNA, and quadrangle with color represents lncRNA.

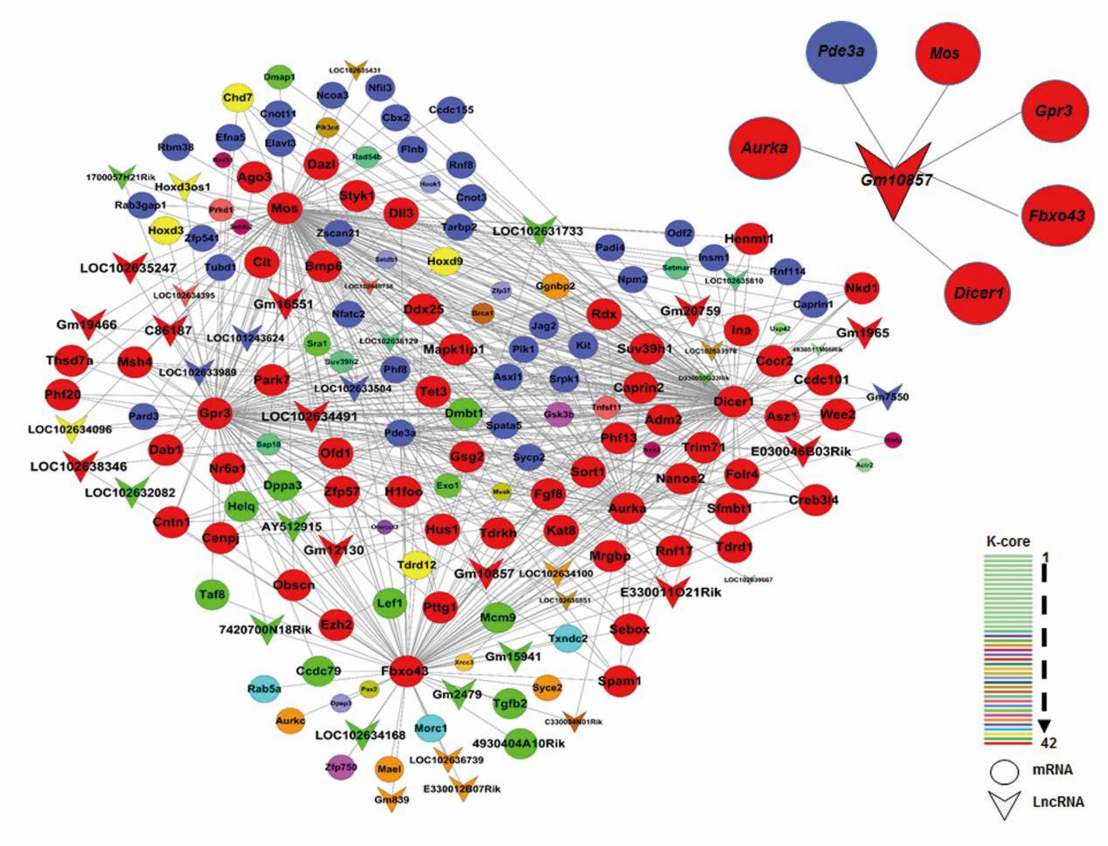


Figure S8. Sub-network consists of *Mos*, *Pde3a*, *Fbxo43*, *Gpr3*, *Aurka*, and *Dicer1* in the PF module. Top right image is potential network extracted from sub-network. Color legend in bottom right corner indicates k-core values from 1 to 42. Cycle with color represents mRNA, and quadrangle with color represents lncRNA.

Table S1. The fertility results using FGSCs isolated from different donor mouse.

Group	POF recipient number	Recipient number that generated offspring
Group A [a.]	8	6
Group B [a.]	8	7
Group C [a.]	8	5
Group D [a.]	8	6
Group E [b.]	8	0

[a.] FGSCs isolated from different donor mouse were used for transplantation.

[b.] The PBS was injected into each ovary for the control.

Table S3. Validation of gene expression specificity.

Gene	RNA-Seq data		qPCR data	
	SAF-TF/PF-TF[a.]	*FDR* value	SAF-TF/PF-TF[a.]	*P* value
BMP15	0.0058	0.0000^{***}	0.0065 ± 0.0003	0.0016^{***}
WEE2	0.0061	0.0000^{***}	0.0064 ± 0.0004	0.0022^{***}
AMH	0.0601	0.0001^{***}	0.0593 ± 0.0021	0.0011^{***}
NR5A1	1.3737	0.0002^{***}	1.1900 ± 0.0761	0.0084^{*}
ASZ1	0.0093	0.0000^{***}	0.0096 ± 0.0013	0.0047^{***}
PDK4	1.4366	0.0003^{***}	1.3984 ± 0.0846	0.0025^{***}
ACTG2	8.0656	0.0000^{***}	7.4050 ± 0.1907	0.0006^{***}

[a.] the expression level is normalized to *Gapdh*. Data are the mean ± SD.

* $P < 0.05$; *** $P < 0.005$ or $FDR < 0.005$, one-tail and two-sample T-test.

Table S5. Primers list.

Gene	PCR primers[a.]	Product size
Cre[b.]	F:TCTGATGAAGTCAGGAAGAACC R:GAGATGTCCTTCACTCTGATTC	500bp
Pten[b.]	F: AATTGAAAGCTCAGGGTAGC R: ATCTGAACACTTCATCGGGA	551bp (wild type) 619bp (loxp type)
DDx4[b.]	F: GGAAACCAGCAGCAAGTGAT R: TGGAGTCCTCATCCTCTGG	213bp
Dppa3 [b.]	F: CCCAATGAAGGACCCTGAAAC R: AATGGCTCACTGTCCCGTTCA	354bp
Ifitm3 [b.]	F:GTTATCACCATTGTTAGTGTCATC R: AATGAGTGTTACACCTGCGTG	151bp
Pou5f1 [b.]	F: AGCTGCTGAAGCAGAAGAGG R: GGTTCTCATTGTTGTCGGCT	198bp
Dazl[b.]	F: GTGTGTCGAAGGGCTATGGAT R: ACAGGCAGCTGATATCCAGTG	328bp
Nanog[b.]	F: CAGGAGTTTGAGGGTAGCTC R: CGGTTCATCATGGTACAGTC	223bp
Prdm1 [b.]	F: CGGAAAGCAACCCAAAGCAATAC R: CCTCGGAACCATAGGAAACATTC	483bp
Figla[b.]	F: CCAAAGAGCGTGAACGGATAA R: TCTTCCAGAACACAGCCGAGT	154bp

Kit[b.]	F:CTGGTGGTTCAGAGTTCCATAGAC R:TCAACGACCTTCCCGAAGGCACCA	401bp
Sycp3[b.]	F: GAGCCGCTGAGCAAACATCTA R: ATATCCAGTTCCCACTGCTGC	437bp
Zp3[b.]	F: CCGAGCTGTGCAATTCCCAGA R: AACCCTCTGAGCCAAGGGTGA	183bp
Gapdh[b.]	F: GTCCCGTAGACAAAATGGTGA R: TGCATTGCTGACAATCTTGAG	458bp
Pou5f1 [c.]	F: GGCTTCAGACTTCGCCTCC R: AACCTGAGGTCCACAGTATGC	211bp
Zp1[c.]	F: CCCTGAGATTGGGTCAGCG R:AGAGCAGTTATTCACCTCAAACC	164bp
Zp2[c.]	F: GTGGCAGAGGAAAGCATCTGT R: GACTGAGGAAGGCTTACTGAGT	111bp
eGfp[c.]	F:CGACGGCAACTACAAGACCCG R: ACGAACTCCAGCAGGACCATG	366bp
Stra8[c.]	F:ACAACCTAAGGAAGGCAGTTTAC R: GACCTCCTCTAAGCTGTTGGG	173bp
Sycp3[c.]	F:AGCCAGTAACCAGAAAATTGAGC R: CCACTGCTGCAACACATTCATA	106bp
Dppa3 [c.]	F: GACCCAATGAAGGACCCTGAA R: GCTTGACACCGGGGTTTAG	130bp

Gapdh[c.]	F: AGGTCGGTGTGAACGGATTTG R:TGTAGACCATGTAGTTGAGGTCA	123bp
Ybx2[c.]	F: GGAGTTTGATGTCGTGGAAGG R: CGTCGATTAGGGGCATAGCG	102bp
Bmp15[d.]	F: TCCTTGCTGACGACCCTACAT R: TACCTCAGGGGATAGCCTTGG	100bp
Wee2[d.]	F: ATGGCCGACACAGAGACTGA R:GGCCTGAAACTGATTTCCAAAGA	247bp
Nobox[d.]	F: ATGGAACCTACGGAGAAGCTC R: CTCAGAGGTCTTCGACAGTGG	189bp
Amh[d.]	F: CCACACCTCTCTCCACTGGTA R: GGCACAAAGGTTCAGGGGG	151bp
Nr5a1[d.]	F: CCCAAGAGTTAGTGCTCCAGT R: CTGGGCGTCCTTTACGAGG	131bp
Asz1[d.]	F: CACGGCAGGGTCATAAAAATGT R:CTCTTCTTTGGTTAGCTGCTGAA	188bp
Pdk4[d.]	F: AGGGAGGTCGAGCTGTTCTC R: GGAGTGTTCACTAAGCGGTCA	185bp
Actg2[d.]	F: CCGCCCTAGACATCAGGGT R: TCTTCTGGTGCTACTCGAAGC	192bp
Gapdh[d.]	F: AGGTCGGTGTGAACGGATTTG R:TGTAGACCATGTAGTTGAGGTCA	123bp

[a.] F, forward; R, reverse.

[b.] Primers used for identification POF mouse and characterization of FGSCs.

[c.] Primers used for single cell PCR.

[d.] Primers used for validation the RNA-seq analysis.