**Figure S1**

Fig. S1 Generation of *Prdm14* reporter and deficient rats

- A.** Primers to confirm correct gene targeting into *Prdm14* locus in rat ES cells.
- B.** Representative genotyping result of *Prdm14-H2BVenus* knock-in ES cells using genomic DNA.
- C.** Representative images of *Prdm14-H2BVenus* knock-in ES cells.
- D.** FACS pattern of *Prdm14-H2BVenus* in knock-in ES cells (green) and the control (red)
- E.** Result of embryo manipulation and efficiency for germline transmission for generation of *Prdm14^{+/HV}* rats.
- F.** Deletion strategy for making *Prdm14*-mutant rats.
- G.** Result of embryo manipulation for generation of *Prdm14^{+/mut}* rats.

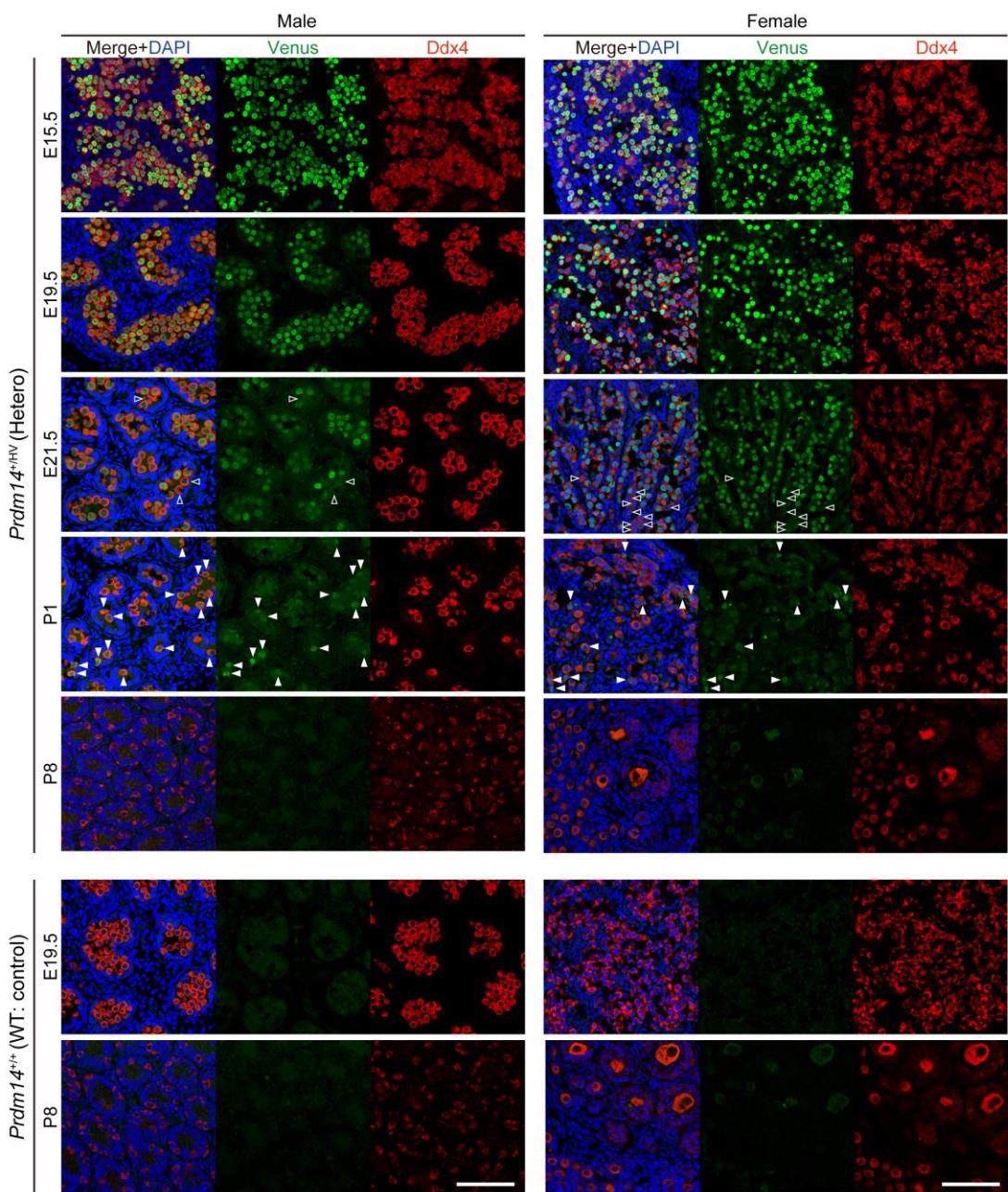


Figure S2

Fig. S2 Expression pattern of Prdm14-H2BVenus at late stage germ cells

Immunostaining of gonad cryosection at indicated stages. Arrowheads in E21.5 show PRDM14 negative germ cells and filled arrowheads in P1 show PRDM14 +ve germ cells. Scale bar: 100 μ m.

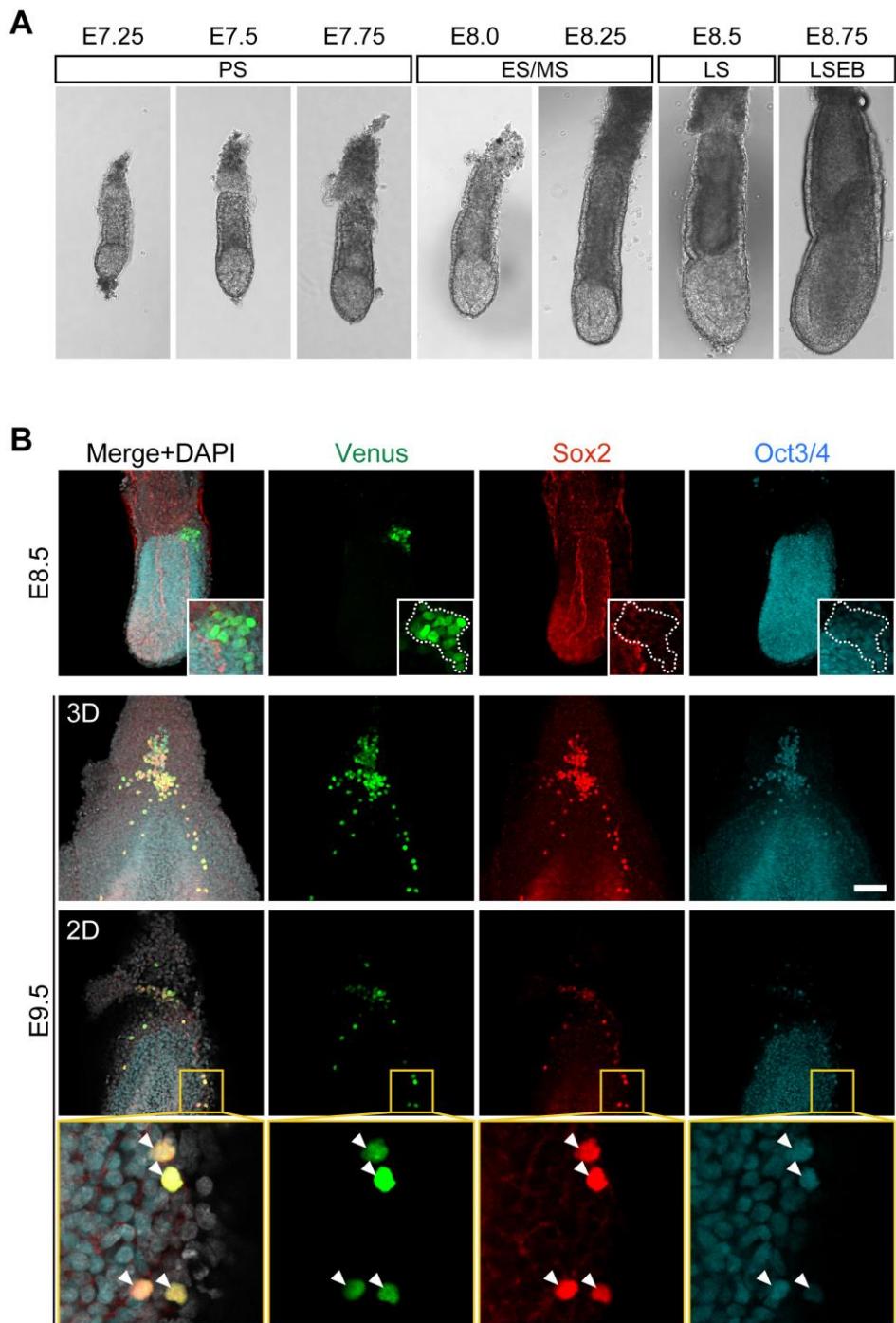


Figure S3

Fig. S3 Analysis of gastrulating rat embryos

- A.** Staging of peri-gastrulating rat embryos based on Theiler stages.
 - B.** Expression of pluripotency genes, *Oct3/4* and *Sox2* at E8.5 and E9.5 embryos.
- Dashed line at E8.5 shows cluster of PGCs. Scale bar: 100 µm.

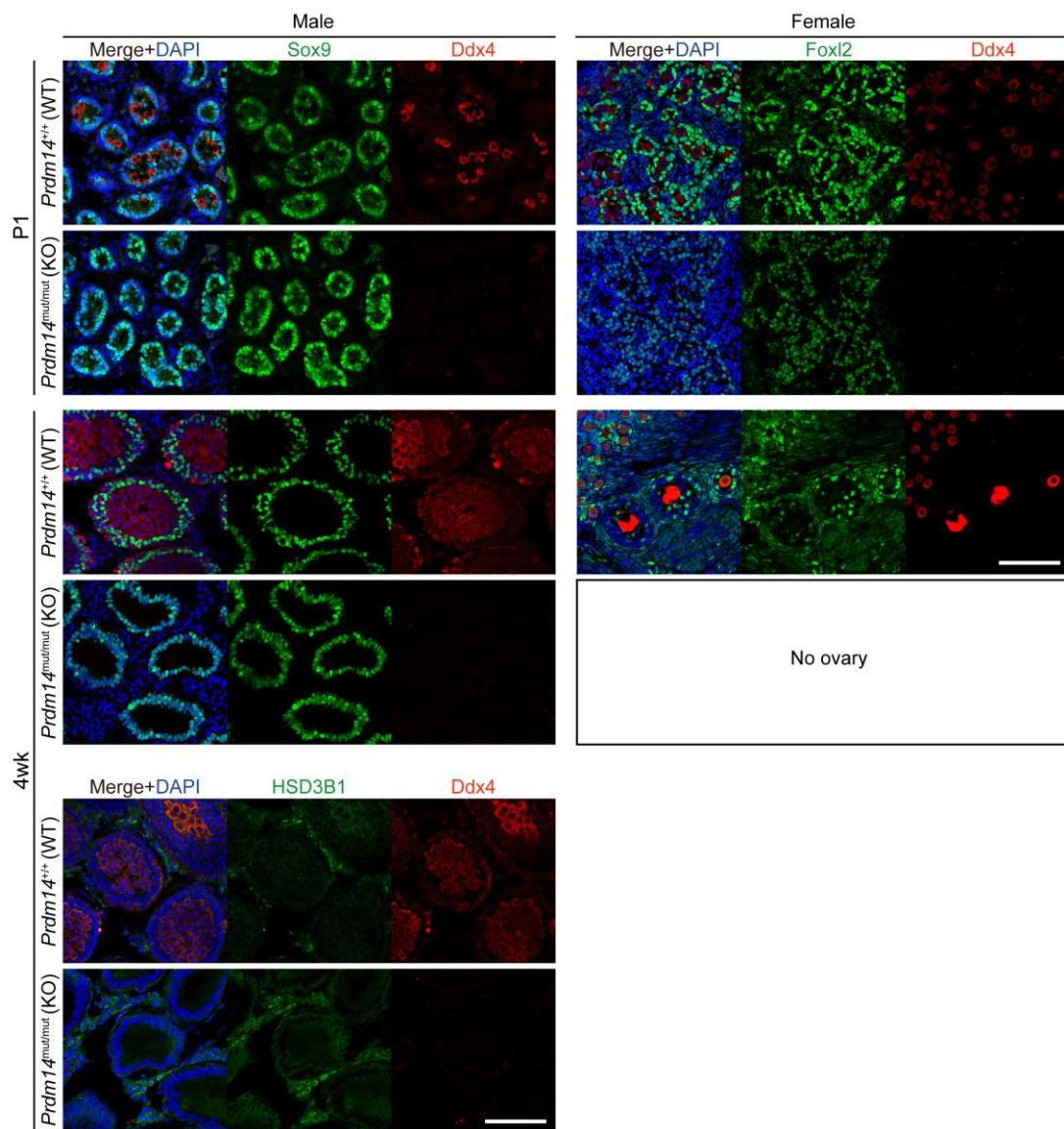


Figure S4

Fig. S4 Analysis of testes and ovaries of *Prdm14*-deficient rats

Immunostaining of gonad cryosections for indicated genotypes and stages.

Scale bar: 100 µm.

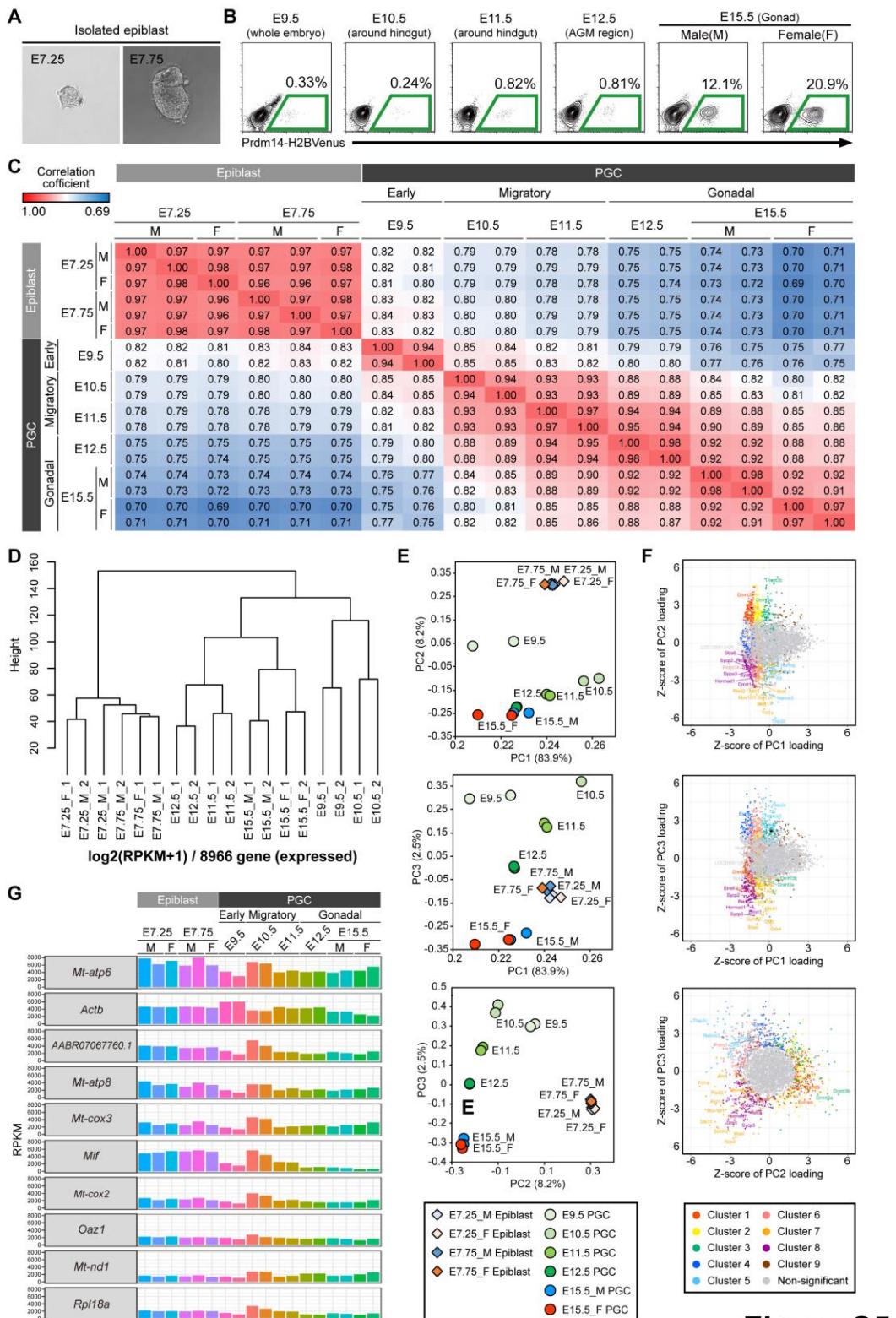


Figure S5

Fig. S5 Collection of rat epiblast and developing PGCs and transcriptome analysis

- A.** Images of isolated epiblast from indicated stages for RNA-seq.
- B.** FACS pattern of Prdm14-H2BVenus +ve rat PGCs.
- C.** Heat map of the correlation coefficients of rat epiblast and PGC replicates with all expressed genes (8966 genes).
- D.** Hierarchical clustering of rat epiblast and PGC replicates.
- E.** Principal component analysis (PCA) of all expressed genes. PC2/PC3 scatter plot is used in **Fig.4A**.
- F.** Scatter plot of normalized loading score of PC1-3 in PCA.
- G.** Expression patterns of top 10 genes contributing to PC1.

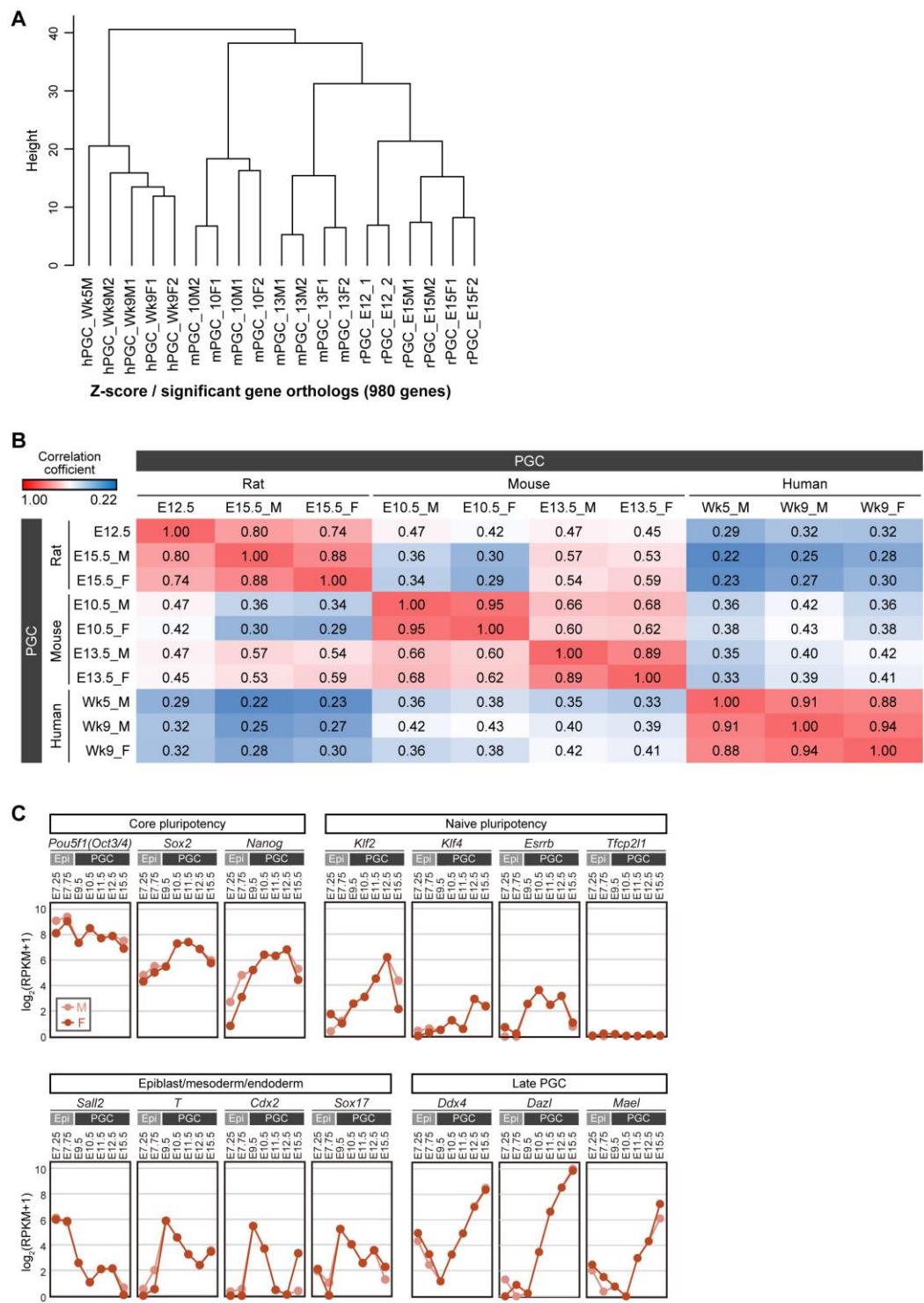


Figure S6

Fig. S6 Comparative analysis of rat, mouse and human PGCs

- A.** Hierarchical clustering of rat, mouse and human early to late gonadal PGCs (duplicates are combined) with significant orthologous genes (980 genes). For human PGCs, published data (Tang et al., 2015) was used.
- B.** Heat map of the correlation coefficients between rat, mouse and human early to late gonadal PGCs.
- C.** Expression changes of representative genes.

Table S1 List of primers to check correct gene targeting

[Click here to Download Table S1](#)

Table S2 List of antibodies

[Click here to Download Table S2](#)

Table S3 List of primers for single cell RT-qPCR analysis

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Table S4 List of samples used for transcriptome analysis

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