

Supporting Information for

Gonadal sex and temperature independently influence germ cell differentiation and meiotic progression in *Trachemys scripta*

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Figures S1 to S6



Fig. S1. Identification of germ cell clusters. (A) UMAP coordinates of SCVI integrated datasets of *Trachemys scripta* gonads. On the left, cells are colored based on the sample of origin (temperature and developmental stage). On the right, the result of the leiden clustering with 1.5 resolution. (B) Same UMAPs than in A showing the SCVI normalized expression of two germ cell markers (*Dazl* and *Ddx4*).



Fig. S2. GO Analysis of germ cell clusters. Summary of the results of the GO analysis of differentially expressed genes in each of the 7 subclusters of the germ cells. Rows are enriched GO terms and columns are differentially expressed genes belonging to those terms. The heatmap in the center allows to see which genes are shared among different GO terms. The heatmap at the bottom show the log2 Fold Change of the genes in the different clusters. The heatmap on the right shows the Odds Ratio of the different terms in the different clusters.



Fig. S3. Per cluster expression of meiotic genes. Violin plots showing SCVI normalized expression of meiotic genes per cluster. The genes correspond to those of the dotplot of Figure 1E.





Fig. S4. Top 50 GO terms for differentially expressed genes in FPT and MPT St. 15 germ cells. Summary of the results of the GO analysis of differentially expressed genes between FPT and MPT St. 15 germ cells in cluster 0. Rows are enriched GO terms and columns are differentially expressed genes belonging to those terms. The heatmap in the center allows to see which genes are shared among different GO terms. The heatmap at the bottom show the log2 Fold Change of the genes in the different groups of cells. The heatmap on the right shows the Odds Ratio of the different terms in the different clusters.



Fig. S5. Expression of differentially expressed genes between FPT and MPT St. 15 cells belonging to selected GO terms. (A) Violin plots with SCVI normalized expression of differentially expressed genes of the term *meiotic cell cycle process.* (B) Same as in (A) but for the term *WNT signaling pathway.*



Fig. S6. Addition of E2 at St. 15 sex reversed the experimental MPT gonad. (A) Schematic of the experimental question. Yellow crescents represent bipotential, undifferentiated gonadal somatic cells. Gray circles represent undifferentiated germ cells. Blue and red crescents represent male and female gonadal soma cells, respectively. (B) Top panel: Representative image of a wildtype MPT St. 26 control testis. HuC/D marks the germ cells, which are arranged in testes cords. Bottom panel: HuC/D and DAPI. (C) Top panel: Representative image of a wildtype FPT St. 26 control ovary. HuC/D marks the germ cells, which are arranged with the outer cortex. Bottom panel: HuC/D marks the germ cells, which are arranged with the outer cortex. Bottom panel: HuC/D marks the germ cells, which are arranged with the outer cortex. Bottom panel: HuC/D marks the germ cells, which are arranged with the outer cortex. Bottom panel: HuC/D and DAPI. (D) Top panel: Representative image of a MPT + E2 St. 26 experimental ovary. HuC/D marks the germ cells, which are arranged with the outer cortex. Bottom panel: HuC/D and DAPI. Dashed lines mark border of the gonads. Scale bars = 100μ m. All images are representative of 1 Z stack in the approximate middle depth of the gonad.