Supplemental Figures

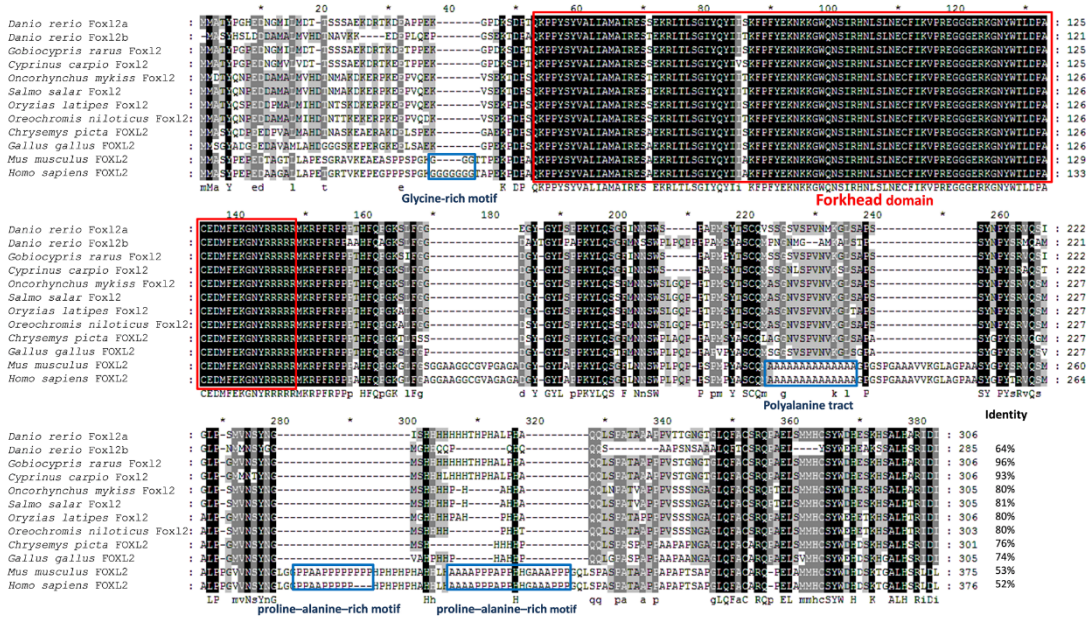

Figure S1. Amino acid sequence alignment of zebrafish Foxl2a, Foxl2b and other vertebrate homologs. The sequence sources were described in the Materials and Methods section. The forkhead domains are indicated by red box. Several motifs specific to mammals, such as glycine-rich motif, polyalanine tract and proline-alanine-rich motif, are indicated by blue boxes.

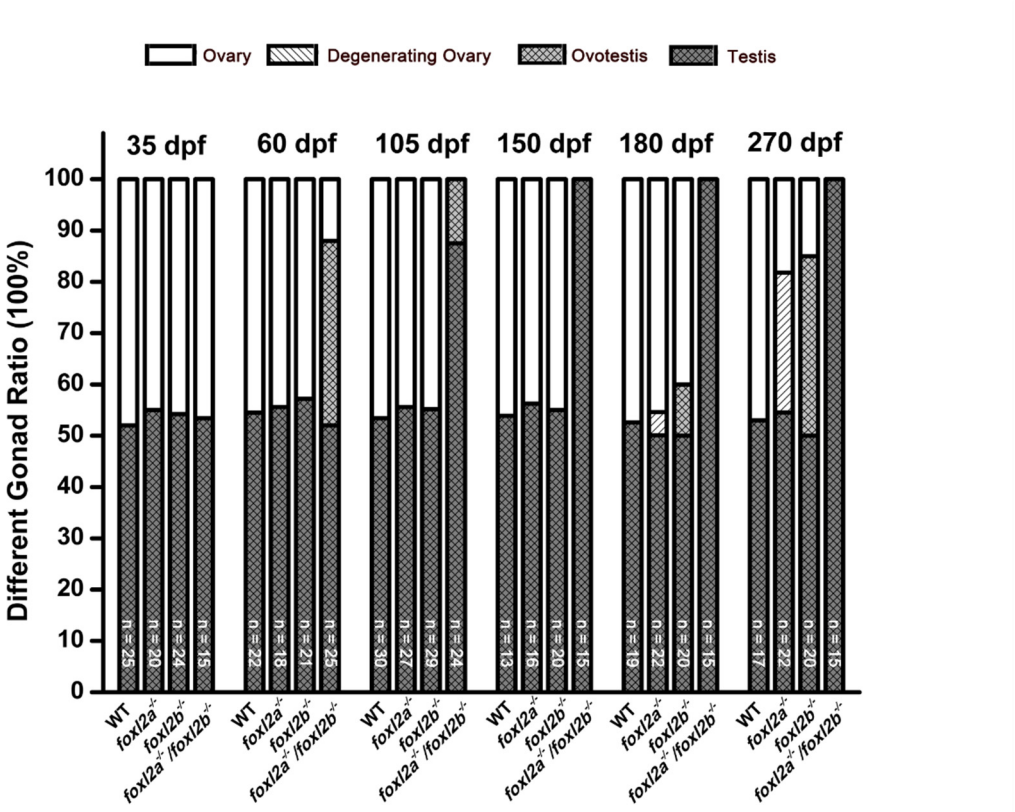

Figure S2. Different gonad ratio of ovary, degenerating ovary, ovotestis, and testis in wide-type as well as *foxl2a*$^{-/-}$, *foxl2b*$^{-/-}$ and *foxl2a*$^{-/-}$/*foxl2b*$^{-/-}$ mutants at different development stages as indicated.

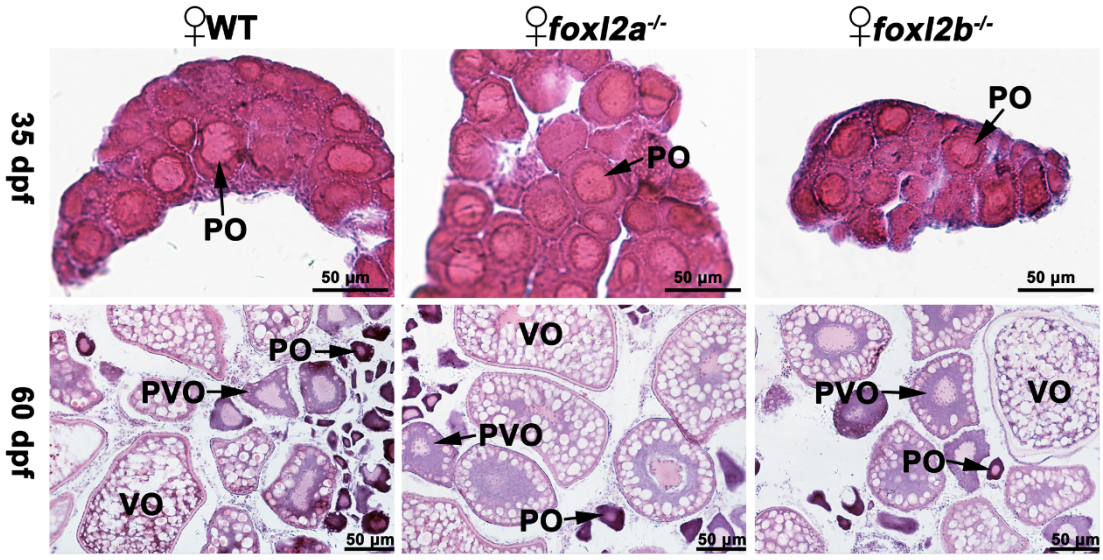

Figure S3. Normal ovary differentiation and oogenesis in female *foxl2a*$^{-/-}$ or *foxl2b*$^{-/-}$ mutants. Both control (WT) and mutant fish (*foxl2a*$^{-/-}$ *or foxl2b*$^{-/-}$) contain primary-growth oocytes (PO) at 35 dpf and previtellogenic oocytes (PVO) and vitellogenic oocytes (PO) at 60 dpf in their ovaries (n = 8). Bars are shown at bottom-right of the images.

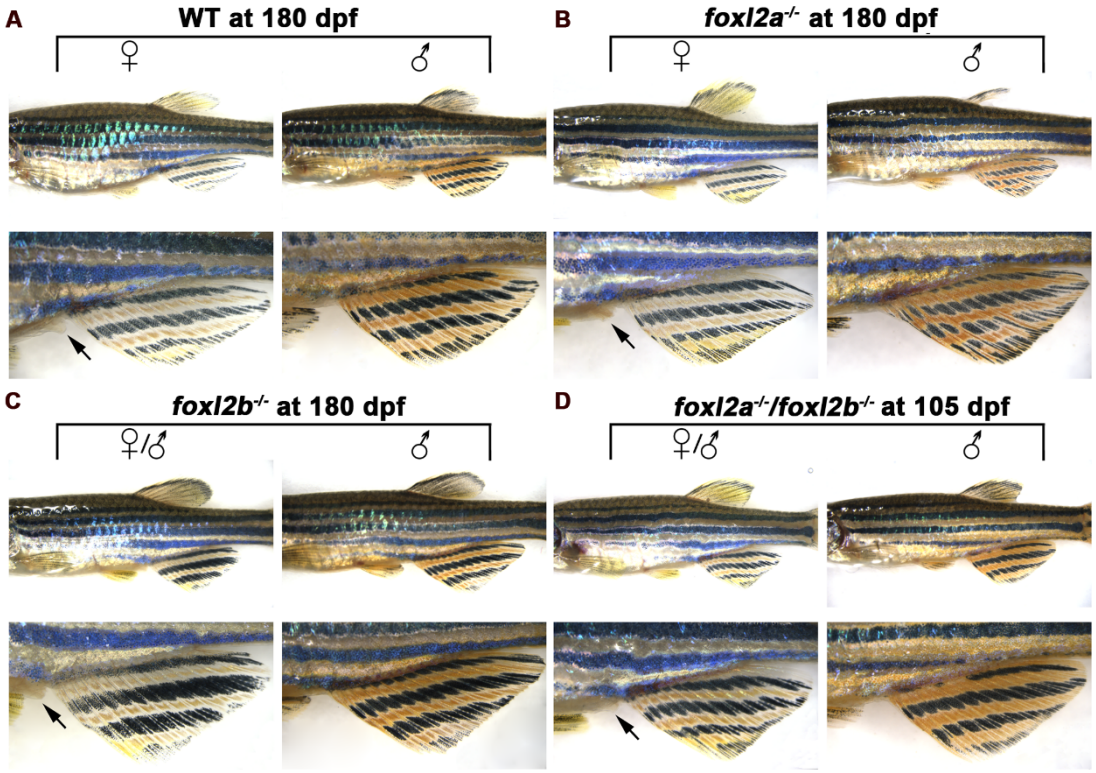

Figure S4. Representative secondary sexual characteristics of wide-type (A), *foxl2a*$^{-/-}$ mutants (B), and *foxl2b*$^{-/-}$ mutants (C) at 180 dpf as well as homozygous double mutants (*foxl2a*$^{-/-}$/*foxl2b*$^{-/-}$) (D) at 105 dpf. The arrows indicate the feminine urogenital papilla. A mixed secondary sexual characteristics, including a slim and reddish body, a anal fin with distinct markings and a feminine urogenital papilla, are clearly visible in *foxl2b*$^{-/-}$ female individuals (n = 5) at 180 dpf and *foxl2a*$^{-/-}$/*foxl2b*$^{-/-}$ female individuals (n = 3) at 105 dpf.

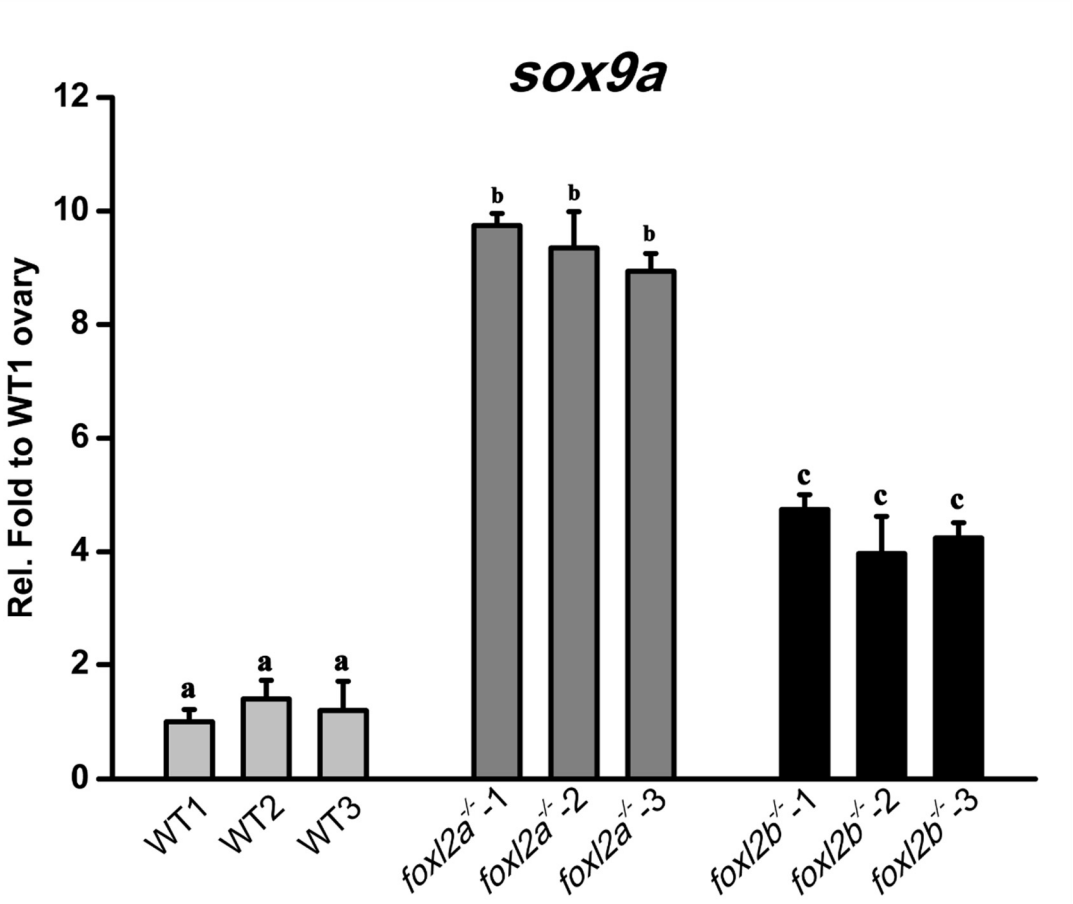

Figure S5. Relative expression of *sox9a* in ovaries of WT, *foxl2a*$^{-/-}$ and *foxl2b*$^{-/-}$mutants at 105 dpf**.** All the samples were analyzed in triplicates. The qPCR quantification is normalized to *actb1*, and the data are presented as mean ± SEM. A significant difference among the groups of $p < 0.05$ (ANOVA) is indicated by differences in the letters.

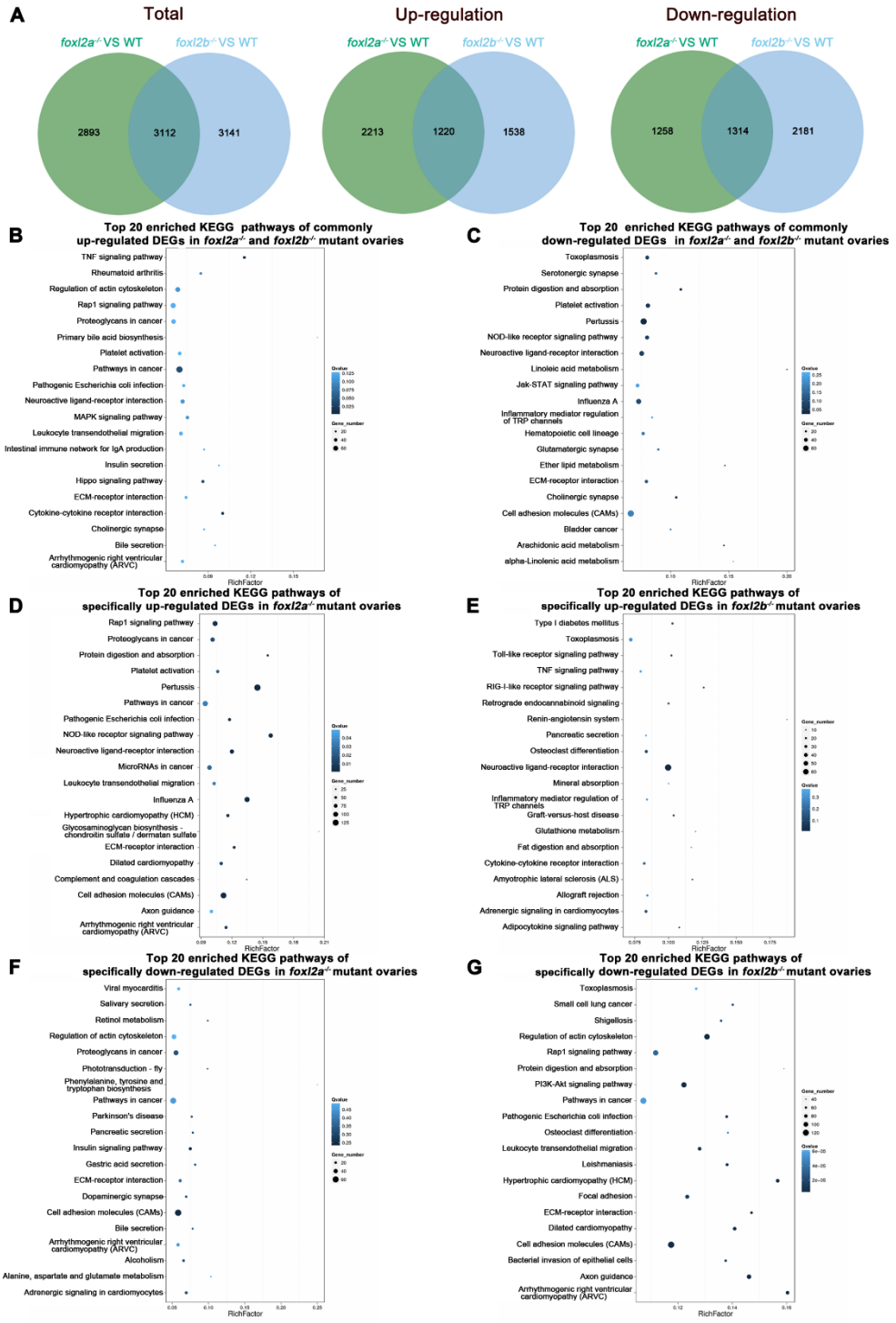

Figure S6. The analyses of DEGs in ovarian transcriptome profiles of *foxl2a*$^{-/-}$ or *foxl2b*$^{-/-}$ mutants VS controls. (A) Venn diagram of up- or down-regulated DEGs between *foxl2a*$^{-/-}$ ovaries vs WT ovaries (green circle) and *foxl2b*$^{-/-}$ ovaries vs WT ovaries (blue circle). (B-G) Top 20 enriched KEGG pathways of commonly up-regulated DEGs (B), commonly down-regulated DEGs (C), specifically up-regulated DEGs in *foxl2a*$^{-/-}$ (D) or *foxl2b*$^{-/-}$ (E) mutant ovaries, or specifically down-regulated DEGs in *foxl2a*$^{-/-}$ (F) or *foxl2b*$^{-/-}$ (G) mutant ovaries, respectively. The x-axis indicates the rich factor of each pathway showed at y-axis. The color and size of dot indicates Q value and number of DEGs assigned to the corresponding pathway respectively.

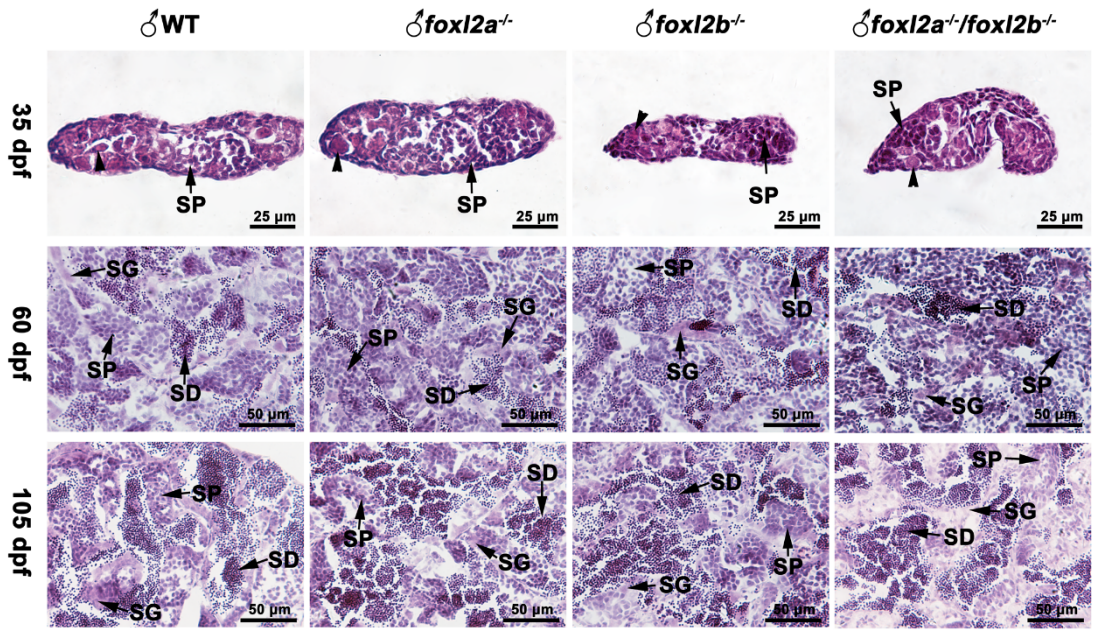

Figure S7. Normal testis differentiation and development in *foxl2a*$^{-/-}$, *foxl2b*$^{-/-}$ and *foxl2a*$^{-/-}$/*foxl2b*$^{-/-}$ mutants. Testis histology were examined in WT and mutants at 35, 60 and 105 dpf (n = 10). Arrowheads indicate the degenerating ocoyte-like cells. SG, spermatogonia; SP, spermatocyte; SD, spermatid. Bars are shown at bottom-right of the images.