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Sample-size estimation

- You should state whether an appropriate sample size was computed when the study was being designed
- You should state the statistical method of sample size computation and any required assumptions
- If no explicit power analysis was used, you should describe how you decided what sample (replicate) size (number) to use

Please outline where this information can be found within the submission (e.g., sections or figure legends), or explain why this information doesn't apply to your submission:

For the mutant phenotype analysis (foxl2l and wnt9b) we choose to raise three separate populations produced by crossing parents that were heterozygous for the mutation. Three biological replicates would allow us to calculate p. We cannot control the numbers of progeny produced in each cross and choose to raise all fish for analysis. The statistical analysis for foxl2l and wnt9b can be found in the materials and methods.

Replicates

- You should report how often each experiment was performed
- You should include a definition of biological versus technical replication
- The data obtained should be provided and sufficient information should be provided to indicate the number of independent biological and/or technical replicates
- If you encountered any outliers, you should describe how these were handled
- Criteria for exclusion/inclusion of data should be clearly stated
- High-throughput sequence data should be uploaded before submission, with a private link for reviewers provided (these are available from both GEO and ArrayExpress)

Please outline where this information can be found within the submission (e.g., sections or figure legends), or explain why this information doesn't apply to your submission:



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Information on data replicates are listed in the text or Materials and Methods. The raw and processed scRNA-seq data reported in this paper are archived at NCBI GEO https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE191137) and in an interactively browsable forms at the Broad Institute Single-Cell Portal (https://singlecell.broadinstitute.org/single_cell/study/SCP928/40dpf-ovary-all-cells). Analysis code and objects are archived at github (https://github.com/yulongliu68/zeb_ov_ssRNAseq). Gene expression tables for the cell clusters identified are archived at Dryad: (https://datadryad.org/stash/share/CEd0Zs4oZKdinTWeJPKbWYjBq6hYq4QhVacQcFjf37E). This information is also detailed in the Data Availability statement.



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Statistical reporting

- Statistical analysis methods should be described and justified
- Raw data should be presented in figures whenever informative to do so (typically when N per group is less than 10)
- For each experiment, you should identify the statistical tests used, exact values of N, definitions of center, methods of multiple test correction, and dispersion and precision measures (e.g., mean, median, SD, SEM, confidence intervals; and, for the major substantive results, a measure of effect size (e.g., Pearson's r, Cohen's d)
- Report exact p-values wherever possible alongside the summary statistics and 95% confidence intervals. These should be reported for all key questions and not only when the p-value is less than 0.05.

Please outline where this information can be found within the submission (e.g., sections or figure legends), or explain why this information doesn't apply to your submission:

For the statistical analysis pertaining to the phenotypes of foxl2l and wnt9b mutants vs. wildtype, we used one-way ANOVA. This information is detailed in Materials and Methods and the *n*, N and p values are presented in the figure legends (Figs 3 and 5, respectively).

(For large datasets, or papers with a very large number of statistical tests, you may upload a single table file with tests, Ns, etc., with reference to sections in the manuscript.)

Group allocation

- Indicate how samples were allocated into experimental groups (in the case of clinical studies, please specify allocation to treatment method); if randomization was used, please also state if restricted randomization was applied
- Indicate if masking was used during group allocation, data collection and/or data analysis

Please outline where this information can be found within the submission (e.g., sections or figure legends), or explain why this information doesn't apply to your submission:

The information for foxl2l and wnt9b phenotypic analysis can be found in the Materials and Methods.

Additional data files ("source data")

- We encourage you to upload relevant additional data files, such as numerical data that are represented as a graph in a figure, or as a summary table
- Where provided, these should be in the most useful format, and they can be uploaded as "Source data" files linked to a main figure or table
- Include model definition files including the full list of parameters used
- Include code used for data analysis (e.g., R, MatLab)
- Avoid stating that data files are "available upon request"

Please indicate the figures or tables for which source data files have been provided:

scRNA-seq analysis code and objects have been archived at github (https://github.com/yulongliu68/zeb_ov_ssRNAseq).