# nature research

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# Life Sciences Reporting Summary

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For final submission: please carefully check your responses for accuracy; you will not be able to make changes later.

## ▶ Experimental design

1. **Sample size**

   Describe how sample size was determined.

   > Before carrying out our experiments, there was no information available regarding variance and difference between the means. Hence, no power calculation was possible in advanced. Based on prior experience with similar mouse models, we employed n=4 for the quantitative experiments, and determined statistical significance once the results were obtained using the statistical tools as described in the paper.

2. **Data exclusions**

   Describe any data exclusions.

   > For the Single Cell Sorting and qPCR assay, outliers were removed using the identifyOutliers function from SINGuLAR software with a predetermined detection limit set to 24 cycles

3. **Replication**

   Describe the measures taken to verify the reproducibility of the experimental findings.

   > Key experiments were repeated by a second investigator on a second set of experimental mice. Only congruent results were reported.

4. **Randomization**

   Describe how samples/organisms/participants were allocated into experimental groups.

   > Mice were assigned to experimental groups based on genotype, i.e. control versus Porcupine deficient, as described in the text.

5. **Blinding**

   Describe whether the investigators were blinded to group allocation during data collection and/or analysis.

   > For key experiments and quantification, such as single molecule RNA-FISH and quantification of the number of proliferating cells per crypt, the investigator was blinded to the genotype of the sample.

   Note: all in vivo studies must report how sample size was determined and whether blinding and randomization were used.

6. Statistical parameters

For all figures and tables that use statistical methods, confirm that the following items are present in relevant figure legends (or in the Methods section if additional space is needed).

| n/a | Confirmed | |
|---|---|---|
| ☐ | ☒ | The <u>exact sample size</u> (n) for each experimental group/condition, given as a discrete number and unit of measurement (animals, litters, cultures, etc.) |
| ☐ | ☒ | A description of how samples were collected, noting whether measurements were taken from distinct samples or whether the same sample was measured repeatedly |
| ☐ | ☒ | A statement indicating how many times each experiment was replicated |
| ☐ | ☒ | The statistical test(s) used and whether they are one- or two-sided<br>*Only common tests should be described solely by name; describe more complex techniques in the Methods section.* |
| ☒ | ☐ | A description of any assumptions or corrections, such as an adjustment for multiple comparisons |
| ☐ | ☒ | Test values indicating whether an effect is present<br>*Provide confidence intervals or give results of significance tests (e.g. P values) as exact values whenever appropriate and with effect sizes noted.* |
| ☐ | ☒ | A clear description of statistics including <u>central tendency</u> (e.g. median, mean) and <u>variation</u> (e.g. standard deviation, interquartile range) |
| ☐ | ☒ | Clearly defined error bars in <u>all</u> relevant figure captions (with explicit mention of central tendency and variation) |

*See the web collection on statistics for biologists for further resources and guidance.*

## ▶ Software

Policy information about availability of computer code

### 7. Software

Describe the software used to analyze the data in this study.

> Standard statistical software available in Excel and GraphPad.
> For single-cell qPCR analysis, Singular V3.6.2 was used with default parameters. For bulk RNA-seq analysis, R version 3.4.1 was used. Hierarchical clustering was performed with the aheatmap function in R version 3.4.1.
> Also used were:
> SINGuLAR Fluidigm PN100-5066F1. MATLAB release2013b Mathworks. TransQuant.

For manuscripts utilizing custom algorithms or software that are central to the paper but not yet described in the published literature, software must be made available to editors and reviewers upon request. We strongly encourage code deposition in a community repository (e.g. GitHub). *Nature Methods* guidance for providing algorithms and software for publication provides further information on this topic.

## ▶ Materials and reagents

Policy information about availability of materials

### 8. Materials availability

Indicate whether there are restrictions on availability of unique materials or if these materials are only available for distribution by a third party.

> No restrictions

### 9. Antibodies

Describe the antibodies used and how they were validated for use in the system under study (i.e. assay and species).

> Novel antibodies, i.e. those against Foxl1, were validated in a previous publication (Aoki et al., CMGH 2016) by Western blot on tissue from Foxl1 null animals. The following commercially available antibodies were used: Sox9 (rabbit, Millipore, AB5535), GFP (goat, Abcam, ab6673), Ecad (mouse, BD Transduction Lab, 610181), PDGFRA (goat, R&D, AF1062). These antibodies were validated by the supplier through Western blot analysis.

### 10. Eukaryotic cell lines

a. State the source of each eukaryotic cell line used.

> No cell lines were used

b. Describe the method of cell line authentication used.

> No cell lines were used

c. Report whether the cell lines were tested for mycoplasma contamination.

> No cell lines were used

d. If any of the cell lines used are listed in the database of commonly misidentified cell lines maintained by ICLAC, provide a scientific rationale for their use.

> No cell lines were used

## ▶ Animals and human research participants

### 11. Description of research animals

Provide all relevant details on animals and/or animal-derived materials used in the study.

> The mice used were: Foxl1-CreER transgenic mice, developed for this study, details described in the method section, and previously published mice such as the Porcupine loxP mice and the Rosa26 mTmG mice. The first description of these mice are referenced in the text. Male mice between 2 and 6 months of age were used

### 12. Description of human research participants

Describe the covariate-relevant population characteristics of the human research participants.

> The study did not involve human research participants.