Subepithelial telocytes are an important source of Wnts that supports intestinal crypts

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Tissues that undergo rapid cellular turnover, such as the mammalian haematopoietic system or the intestinal epithelium, are dependent on stem and progenitor cells that proliferate to provide differentiated cells to maintain organisational health. Stem and progenitor cells, in turn, are thought to rely on signals and growth factors provided by local niche cells to support their function and self-renewal. Several cell types have been hypothesized to provide the signals required for the proliferation and differentiation of the intestinal stem cells in intestinal crypts1,2. Here we identify subepithelial telocytes as an important source of Wnt proteins, without which intestinal stem cells cannot proliferate and support epithelial renewal. Telocytes are large but rare mesenchymal cells that are marked by expression of FOXL1 and form a subepithelial plexus that extends from the stomach to the colon. While supporting the entire epithelium, FOXL1+ telocytes compartmentalize the production of Wnt ligands and inhibitors to enable localized pathway activation. Conditional genetic ablation of porcupine (Porcn), which is required for functional maturation of all Wnt proteins, in mouse FOXL1+ telocytes causes rapid cessation of Wnt signalling to intestinal crypts, followed by loss of proliferation of stem and transit amplifying cells and impaired epithelial renewal. Thus, FOXL1+ telocytes are an important source of niche signals to intestinal stem cells.

After the cloning of Foxl1, which encodes the gene for the winged-helix transcription factor FoxL1 (previously known as Fkh61), initial expression profiling focused on measuring its steady-state mRNA levels, both by RNAseq protection assays and by in situ hybridization2. Foxl1 transcripts were found at low levels in the adult stomach and intestine, and in about one to two cell layers of the mesodermal tissue surrounding the primitive fetal gut tube. In order to visualize subepithelial FoxL1-expressing cells in their entirety, we used the Fox1cre transgenic line3 in conjunction with genetic lineage labelling using the Rosa26-mTmG allele11. In this model, Cre-labelled cells produce a membrane-targeted version of green fluorescent protein (GFP), which allows us to map the size and location of Foxl1-expressing cells (Fig. 1a). Figure 1b shows an example of duodenal crypts, where Foxl1-expressing cells, identified both by nuclear FOXL1 staining (white) and membrane GFP expression (green), are in close contact with the entire crypt base, identified by EPCAM staining (red). FOXL1+ cells are large, flat cells with cytoplasmic processes in excess of 100 μm. Cells with these properties, that is, very large mesenchymal cells with extended cell bodies, were identified previously by electron microscopy and termed ‘telocytes’, with the long processes termed ‘telopodes’12,13.

Intestinal telocytes express platelet-derived growth factor receptor alpha (PDGFRα)14. PDGFRα staining overlapped with Foxl1cre-induced GFP expression, further supporting the notion that these cells are intestinal telocytes (Fig. 1c, d), although PDGFRα is also present in numerous cells deeper in the submucosa that do not express Foxl1. Immunoelectron microscopy confirmed that FOXL1+ cells form long telopodes that encompass all crypt cells, and are separated from the epithelium by sub-micrometre distances (Fig. 1e–g).

In order to gain a better understanding of the three-dimensional nature of the FOXL1+ telocyte network, we performed confocal imaging of immunofluorescence-stained jejunal following tissue clearing. FOXL1+ PDGFRα+ telocytes form a plexus that supports the entire epithelium, visualized by EPCAM staining (Fig. 1h and Supplementary Video). Thus, FOXL1+ PDGFRα+ positive telocytes maintain a subepithelial sheath that is juxtaposed to the epithelium, and therefore in an ideal position to act as a niche to provide signals to the stem and progenitor cells in the intestinal crypt.

For the molecular characterization of FOXL1+ telocytes, we sorted cells based on Fox1-lcre;Rosa26-mTmG activity (Extended Data Fig. 1), performed RNA sequencing analysis (RNA-seq), and compared the expression profiles to those of FOXL1+ mesenchymal cells, LGR5+ stem cells (sorted by Lgr5-eGFPhigh expression) and epithelial enterocytes (Fig. 2a). Note that while FOXL1+ cells express high levels of Pdgfra and Cldn4, these two genes are also active in FOXL1+ cells, confirming our observation from immunostaining and genetic lineage tracing that FOXL1+ cells constitute a small subset of PDGFRα+ cells. FOXL1+ telocytes express high levels of multiple regulators—both activators and repressors—of key signalling pathways, such as Wnt, Shh, Bmp and Tgf-β (Fig. 2b–g). While the Wnt ligands Wnt2b and Wnt5a are specifically expressed in FOXL1+ telocytes, the Wnt signalling enhancer and LGR5-ligand R-spondin3 is produced by both FOXL1+ telocytes and FOXL1+ mesenchymal cells (Fig. 2c; Extended Data Table 1). Notably, FOXL1+ telocytes also express the Wnt inhibitors Dkk3 and Sfrp1. Similarly, FOXL1+ telocytes express Bmp4, Bmp5, BMP6 and BMP7 as well as the Bmp inhibitors chordin-like1 and gremlin1 (Fig. 2d).

The results of the expression profiling of FOXL1+ telocytes summarized above present an apparent paradox. How can FOXL1+ telocytes provide Wnt ligands to activate Wnt signalling in intestinal crypts if they also produce Wnt pathway inhibitors? We speculated that there might be a regional differentiation of the transcriptome of FOXL1+ telocytes according to their position along the crypt–villus axis. In order to test this hypothesis, we performed single-molecule fluorescence in situ hybridization (smFISH) to detect mRNA encoding activators (Wnt2b, Wnt5a and R-spondin 3) and inhibitors (Sfrp1 and Dkk3) of the Wnt signalling pathway, and BMP5, which signals via activation of the Smad signalling cascade. We labelled telocytes by immunostaining for PDGFRα, as FOXL1 is a nuclear protein and thus does not show the full extent of the cells. As shown in Fig. 3a, telocytes express both Wnt2b, an activator of canonical Wnt signalling, and Wnt5a, which acts via the planar cell polarity pathway. However, the localization of the two mRNAs is clearly distinct, with Wnt2b enriched at the bottom of the crypts where LGR5+ stem cells are situated, and Wnt5a mRNA present near the crypt–villus junction. Likewise, there is a striking pattern of co-expression of Wnt2b with Sfrp1, which encodes a secreted frizzled protein that acts as a decoy receptor to limit Wnt signalling at the bottom of the crypt; whereas towards the crypt–villus junction, Sfrp1 mRNA predominates, as the density of Wnt2b mRNA

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**Fig. 1 | FOXL1+ cells are telocytes and co-express PDGFRα.** a. Schema for labelling FOXL1+ cells with GFP. b, Foxl1-cre-driven GFP expression is restricted to pericryptal mesenchymal telocytes. FOXL1 (white, *), GFP (green) and EPCAM (red) immunofluorescence in cleared whole mouse duodenum. c, d, GFP (green) and PDGFRα (red) immunofluorescence in the duodenum (c) and colon (d). e–g, Immunoelectron microscopy showing GFP in duodenal crypt (transverse section) of a Foxl1-cre;Rosa26-mTmG mouse. f, Inset showing the nucleus and the telopode of a telocyte. g, Inset showing contact between two telopodes. h, Confocal imaging of cleared whole small intestine showing expression of PDGFRα (green) and EPCAM (red). Experiments were repeated at least three times with similar results. Scale bars, 10 µm.

**Fig. 2 | Telocytes express key signalling molecules.** RNA-seq analysis of FOXL1+ cells (n = 3 mice) compared to FOXL1+ mesenchymal cells (Mes., n = 3 mice), LGR5+ stem cells (n = 2 mice) and differentiated enterocytes (Diff., n = 2 mice). a, Heat map showing hierarchical clustering. Only genes with more than tenfold difference in expression level in pairwise comparisons and a false discovery rate (FDR) of less than 2% were used for the analysis. Values are shown as normalized Z score ± s.e.m. b–g, Selected markers showing differential expression: Foxl1, Pdgfra and Cd34 (telocyte markers), Lgr5 and Slc5a1 (b), WNT pathway (c), BMP pathway (d), SHH pathway (e), growth factors (f) and TGF-β pathway (g). Data are shown as fragments per kilobase million (FPKM). Error bars indicate s.e.m.© 2018 Macmillan Publishers Limited, part of Springer Nature. All rights reserved.
The intestinal epithelium depends on Wnts secreted from FOXL1+ telocytes. a–c, Control and PorcnΔ mutant duodenum, one and three days after tamoxifen injection. Experiments were repeated at least three times with similar results. d, e, Duodenal crypt depth (control, n = 3 mice; PorcnΔ 24 h, n = 3 mice; PorcnΔ 72 h, n = 7 mice; **P < 0.001, unpaired two-tailed t-test). f–h, Control and PorcnΔ mutant colon, one and three days after tamoxifen injection. Experiments were repeated at least three times with similar results. i, Colonic crypt depth (control, n = 7 mice; PorcnΔ 24 h, n = 3 mice; PorcnΔ 72 h, n = 7 mice; **P < 0.001, unpaired two-tailed t-test). j–l, n–p, EdU incorporation (red) in the epithelium (EPCAM, green) of duodenum and colon. Experiments were repeated at least three times with similar results. m, Number of proliferating cells per duodenal crypt. n = 3 mice per group; **P < 0.001, unpaired two-tailed t-test. q, Number of proliferating cells per colonic crypt. n = 3 mice per group; **P < 0.001, unpaired two tailed t-test. Centre lines, mean; error bars, s.d. Scale bars, 100µm.
To address the question of whether Wnt production by FOXL1+ telocytes is important for intestinal stem cell activity, we derived a Foxl1-creERT2 mouse line to enable temporally controlled gene ablation in this cell type (Extended Data Fig. 3a). To confirm that the Foxl1-creERT2 transgene, which is based on the same 170-kb bacterial artificial chromosome (BAC) transgene as we employed previously to obtain faithful reproduction of the Foxl1 gene expression pattern, is active in subepithelial telocytes, we crossed these mice to Rosa26-mIRES2 reporter mice (Extended Data Fig. 3b, c) and treated them with tamoxifen to induce Cre activity. In both small and large bowel, Foxl1-creERT2 was active in subepithelial telocytes as intended.

Next, we crossed Foxl1-creERT2 mice with mice carrying a floxed allele of the X-linked Porcn gene, to obtain PorcnloxP/Y;Foxl1-creERT2 mice (hereafter referred to as PorcnΔ, Extended Data Fig. 3d). This model enables conditional ablation of all Wnt secretion from FOXL1-expressing telocytes. We analysed PorcnΔ mice after 24 and 72 h of tamoxifen treatment. Notably, while epithelial morphology was normal at the 24-h time point (Fig. 4a, b, f, g), stem and progenitor cell proliferation in duodenal and colonic crypts was already markedly reduced, as determined by labelling cells in S-phase with a short pulse of the thymidine analogue ethynyl deoxyuridine (EdU) (Fig. 4j, k, m, n, o, r). Consequently, duodenal crypt depth was significantly reduced even after one day of porcupine ablation (Fig. 4d). After three days of tamoxifen treatment, PorcnΔ mice exhibited marked changes in the epithelial architecture in both the small and large intestine (Fig. 4c, h), which we attribute to lack of resupply of the epithelium with newly differentiated cells from the crypts. Epithelial proliferation rates were reduced even further at this time point (Fig. 4l, m, p, r). As a result, the length of the villi was reduced in the duodenum (Fig. 4c, e), and epithelial cells appeared abnormal in colonic crypts (Fig. 4h), which also exhibited a significant reduction in depth (Fig. 4i). Thus, eliminating Wnt secretion from FOXL1+ telocytes results in a large and highly significant reduction in epithelial cell proliferation in the small and large intestine.

In order to test directly whether FOXL1+ telocytes are a relevant source of Wnt signals to the intestinal crypt, we stained control and PorcnΔ intestines for the presence of nuclear β-catenin, a marker of active Wnt signalling, as well as cyclin D1 and SOX9, proteins encoded by direct target genes of β-catenin. As shown in Fig. 5a, b, while the control duodenum showed the typical distribution of β-catenin protein at the adherens junctions of all epithelial cells and in the nuclei of cells with active Wnt signalling in the crypts, the PorcnΔ intestine had lost...
nuclear β-catenin protein expression. Likewise, expression of cyclin D1 and SOX9 was reduced markedly in the duodenum and colon of PorcnΔ mice (Fig. 5c–j). Indeed, we found that expression of Olfm4, Lgr5 and CD44, all markers of stem cells in the crypt base, was lost as early as 24 h following porcupine ablation (Fig. 5k–p). Taken together, these findings demonstrate that FOXL1+ telocytes are necessary for Wnt pathway activation and proliferation in intestinal stem and progenitor cells. Recently, systemic administration of ligand-specific pharmacological perturbations showed that Wnt and R-spondin ligands represent non-equivalent ligand families that cooperate to maintain intestinal stem cells, with the former having a ‘priming’ effect and the latter driving stem-cell expansion. Therefore, we evaluated whether Wnt production by FOXL1+ telocytes might indirectly influence R-spondin gene expression. Remarkably, we found reduced Rspo3 mRNA levels in telocytes in the PorcnΔ intestine (Fig. 5o, p). Telocytes were still present even after 72 h of Porcn gene ablation (Fig. 5q, r), suggesting the possibility that reciprocal signalling from the epithelial compartment might be responsible for maintaining Rspo3 gene expression. In sum, sub-epithelial FOXL1+ telocytes contribute important Wnt signals to intestinal crypt cells to maintain their proliferation and allow for homeostatic renewal of the intestinal epithelium.

Online content
Any Methods, including any statements of data availability and Nature Research reporting summaries, along with any additional references and Source Data files, are available in the online version of the paper at https://doi.org/10.1038/s41586-018-0084-4.

Received: 11 January 2017; Accepted: 27 February 2018; Published online 2 May 2018.

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Acknowledgements We thank A. Rustgi, C. Lengner and Y. Dor for critical reading of the manuscript; M. Sundaram for suggesting the porcupine gene ablation experiment; and all members of the Kaestner laboratory. We acknowledge funding from NIDDK through R37-DK053839. We thank the University of Pennsylvania’s Diabetes Research Center (DRC) for the use of the Functional Genomics Core (P30-DK019525) and the Center for Molecular Studies in Digestive and Liver Diseases for the use of the Molecular Pathology and Imaging and Transgenic and Chimeric Mouse Cores (P30-DK053056).

Reviewer information Nature thanks L. Samuelson and the other anonymous reviewer(s) for their contribution to the peer review of this work.

Author contributions M.S.-C. and K.H.K. came up with the study concept and design, analysed and interpreted the data and wrote and revised the manuscript. B.T., E.E.M. and S.I. acquired and interpreted smFISH data and reviewed the manuscript. Y.J.W., K.J.W. and A.K. acquired and interpreted immunostaining and RNA-seq data.

Competing interests The authors declare no competing interests.

Additional information Extended data is available for this paper at https://doi.org/10.1038/s41586-018-0084-4. Supplementary information is available for this paper at https://doi.org/10.1038/s41586-018-0084-4. Reprints and permissions information is available at http://www.nature.com/reprints. Correspondence and requests for materials should be addressed to K.H.K. Publisher’s note: Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.
METHODS
Foxl1-creERT2 mice. Foxl1-creERT2 mice for tamoxifen-inducible gene ablation of loxP-flanked targets were derived by introducing a creERT2 cassette, cloned from a K18ires-CreERT2 T2 plasmid19 (Addgene, #44580), into the coding region of the mouse Forkhead box I1 (Fox1) gene in the BAC RP23-4461414 by BAC recombining as described previously19,20. The targeting primers were: forward, ccwccgg gggcacatggatacgcgtccagcacttgatccatggagcaagattgc; reverse, aagccagtggctccgccaattggtacaagagccagtacagtgctccgaacagtgcatt.

The resulting Foxl1-creERT2 BAC DNA was linearized and microinjected into fertilized oocytes. The positive transgenic founders were identified by genomic PCR, and the fidelity of transgene expression was tested by crossing Foxl1-creERT2 transgenic mice to the Rosa26-mTmG reporter line1,20 and visualizing fluorescent protein expression in tissue sections (Extended Data Fig. 3b, c).

Foxl1-creERT2;Porcn Δ Foxl1-creERT2;Rosa26-m1mG and Foxl1-cre, Rosa26-m1mG mice. Foxl1-creERT2 mice were crossed with Porcn-ex3-7Neo;Fox15, Rosa26-membrane-targeted dimer tomato protein or membrane-targeted green fluorescent protein (mG11), or Rosa26-YFP mice to obtain Foxl1-creERT2;PorcnΔ, Foxl1-creERT2;Rosa26-m1mG, or Foxl1-creERT2;Rosa26-YFP mice. Non-inducible Foxl1-cre mice10 were also crossed to Rosa26-YFP and Rosa26-m1mG reporter lines (Jackson Laboratories). The animal experiments were performed according to a protocol approved by the University of Pennsylvania Institutional Animal Care and Use Committee.

Tamoxifen treatment. For Foxl1-creERT2;PorcnΔ and control (Foxl1-creERT2;Rosa26-YFP or Foxl1-creERT2;Rosa26-m1mG) mice, tamoxifen (Sigma-Aldrich, Cat.10540-29-1) was dissolved in corn oil (10 mg/ml) by shaking overnight at 37 °C and administered intraperitoneally at 75 mg tamoxifen per kg body weight. Mice were injected on three consecutive days and killed on day 4, corresponding to 72 h tamoxifen treatment, or day 2, corresponding to 24 h tamoxifen treatment.

EdU treatment. For EdU treatment, 10 µM EdU solution was injected intra peritoneally two hours before mice were killed, and EdU was detected according to the manufacturer's protocol (Click-IT Plus EdU Alexa Fluor 555 imaging kit C10638, Molecular Probes).

FACS, RNA isolation and sequencing library preparation. Isolation of FOXL1+ cells using FACS was performed using Foxl1-cre Rosa26-YFP and Foxl1-cre Rosa26-m1mG mice. Small intestines were dissected and washed thoroughly with Hank's balanced salt solution (HBSS) and were incubated in 5 mM EDTA in HBSS for 10 min at 4 °C. Intestinal villi were scraped off using a coverslip and the remaining tissue was cut into small pieces and incubated in 5 mM EDTA in HBSS on ice for 10 min while pipetting to completely remove the remaining epithelium. After vigorous washes, the remaining mesenchymal fraction was incubated with 6 mg/ml Dispase II/0.05% trypsin solution (Sigma-Aldrich, 04942078001) supplementing 1 U/ml DNaseI (Invitrogen) at 37 °C, until the solution became homogeneous. After vigorous washes, the remaining mesenchymal fraction was incubated with ice for 10 min while pipetting to completely remove the remaining epithelium. Mice were injected with tamoxifen 2 h before mice were killed, and EdU was detected according to the manufacturer's protocol (Click-IT Plus EdU Alexa Fluor 555 imaging kit C10638, Molecular Probes).

Immunofluorescence and immunohistochemistry. Mouse intestines were rinsed in PBS and fixed with 4% paraformaldehyde overnight, rinsed in PBS, and either dehydrated and embedded in paraffin or immersed in OCT media for cryosectioning at 4 °C. After overnight fixation, the OCT blocks were frozen for cryosectioning. Antigen retrieval was performed using citrate buffer pH 6.0 with a pressure cooker (PickCell Laboratories, Agoura Hills, CA). FOXL1 staining required the use of cryosections, antigen retrieval, and amplification of signal using tyramide (TSA systems, PerkinElmer) as previously described16. CD44 immunohistochemistry was performed as described16. Antibodies used were as follows: rabbit SOX9 (1:300; Millipore AB5533), guinea pig FOX1 (1:1,500), goat GFP (1:200; Abcam AB6673), mouse E-cadherin (1:250; BD Transduction 610182), mouse β-catenin (1:200; BD Transduction 610153), rabbit EPCAM (1:100; Abcam AB19196), goat PDGFRα (1:100; R&D AF1062), rat CD44v (v6) (1:60; Biosciences BMS145). Cy2-, Cy3- and Cy5-conjugated donkey secondary antibodies were purchased from Jackson ImmunoResearch Laboratories. For immunohistochemistry, horseradish peroxidase-conjugated antibodies were incubated for 2 h at room temperature and 3,3-diaminobenzidine tetrahydrochloride (DAB) was used as a substrate to develop the signal. For the studies requiring quantification, we used n = 3 per genotype or condition based on the severity of the phenotype observed in pilot experiments. Mice were assigned to condition by genotype and littermate to ensure the same colony. The investigator counting the number of EdU-positive cells or quantifying villus length or crypt depth was blinded to the genotype of the mice.

Clearing of adult mouse intestine. Foxl1-cre; Rosa26-m1mG and C57BL/6 adult mice were anaesthetized, intestine was dissected and fixed in 4%PFA for 24 h at 4 °C. Following washes with PBS for another 24 h at 4 °C, the intestine was incubated in hydrogel solution for 24 h at 4 °C. After 3 h at 37 °C, the hydrogel-embedded intestine was placed in an X-CLARITY ETC chamber (LOGOS Biosystems) for electrophoretic tissue clearing for 7 h. The cleared intestine was immunostained with goat anti-GFP (1:100; Abcam) or PDGFRα (1:100; R&D) and APC-conjugated anti-mouse EPCAM (1:100; Biolegend) or rabbit anti-EPCAM (1:100; Abcam). For Foxl1 antibody staining, following clearing, antigen retrieval was performed (as described above) in citrate buffer. Primary and secondary antibodies were incubated for 48 h at 4 °C each. The stained intestine was placed en bloc on an image slide using 1 mm deep adhesive silicone isolate, mounted in X-CLARITY mounting solution and imaged using confocal scanning. Z-stacks projections were compiled using Velocity software.

Single-molecule FISH and immunofluorescence. Libraries of 48 probes each were designed against the mouse Wnt2b, Wnt5a, Sfrp1, Rspo3, Bmp5 and Dkk3 mRNAs (Stellaris RNA FISH probes, Biosearch Technologies). Hybridization conditions and imaging were as described previously24,25, except for the addition of immunofluorescence detection of PDGFRα. For immunofluorescence, a goat anti-PDGFRα antibody was diluted in hybridization buffer (1:100), added to the FISH probes and incubated overnight at 30 °C. Secondary antibody Cy2-conjugated donkey anti-goat (1:200) was added to glucose oxido (GLOX) buffer for 20 min in room temperature. smFISH imaging was performed on a Nikon-Ti-E inverted fluorescence microscope with a 100 x 0.9 oil-immersion objective and a Photometrics Coolsnap-HQ camera using Chameleon software24,25. Single-cell segmentation and automatic transcript quantification were performed in a custom Matlab program (MATLAB release 2013b, Mathworks) using

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TransQuant software\textsuperscript{31}. Images were filtered with a Laplacian of Gaussian filter of size 15 pixels and standard deviation of 1.5 pixels\textsuperscript{32}.

**Olfm4 mRNA in-situ hybridization.** In situ hybridization for Olfm4 mRNA was performed using the RNAscope kit (Advanced Cell Diagnostics) according to the manufacturer’s instructions.

**Reporting summary.** Further information on experimental design is available in the Nature Research Reporting Summary linked to this paper.

**Data availability.** RNA-seq data that support the findings of this study have been deposited in the NCBI GEO under accession code GSE94072. Source data for Figs. 2, 3 and 4 are provided in the online version of the paper.

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Extended Data Fig. 1 | Fluorescence-activated cell sorting plots.

a–d, Representative FACS plots of mesenchymal cells isolated from Foxl1-cre;Rosa26-mTmG (c, d) as compared to control Rosa26-mTmG (a, b) mice, showing sorting strategy. In a and c, Allophycocyanin+ (APC+), CD45+ and EPCAM+ -labelled cells were gated out to exclude immune and epithelial cell contamination. In d, FOXL1+, GFP+ and FOXL1−Tomato+ cells were gated and sorted based on fluorescence activity as compared to the negative control (b). Experiments were repeated at least three times with similar results.
Extended Data Fig. 2 | Single-cell qPCR of FOXL1⁺ telocytes showing heterogeneity within the FOXL1⁺ cell population. Hierarchical clustering of FOXL1⁺ single cells based on qPCR-based detection of 30 genes. Note that all FOXL1⁺ cells express Pdgfra and Wnt3a. The population is clustered into three main groups. Jaccard coefficients for the three clusters were: 0.83 (green), 0.69 (turquoise) and 0.79 (yellow), respectively, indicating underlying cluster stability. Values between 0.6 and 0.75 indicate that the cluster is measuring a pattern in the data. Clusters with stability values above about 0.85 are considered to be highly stable.
Extended Data Fig. 3 | Derivation of Foxl1-creERT2;PorcnΔ mice.

a, Schema for the generation of Foxl1-creERT2 mice using BAC recombinaseering. The coding sequence of exon 1 of Foxl1 was targeted by the sequence of creERT2. FRT, flipase recognition target; Flp, flipase; LA, left homology arm; RA, right homology arm. 

b, c, Tamoxifen induction of Foxl1-creERT2;Rosa26-mTmG-driven expression of membrane-bound GFP to mesenchymal telocytes in the duodenum (b) and colon (c). Immunofluorescence staining for GFP (green), EPCAM (red).

d, Schema for the generation of Foxl1-creERT2;PorcnΔ mice. Foxl1-creERT2 mice were crossed with mice carrying loxP sites flanking exons 3–7 of the X-linked porcupine homologue (Porcn) gene. 

e, Foxl1-creERT2;Rosa26-mTmG (control, n = 5 mice) and Foxl1-creERT2;PorcnΔ (PorcnΔ, n = 8 mice) male mice were treated with tamoxifen for three consecutive days to induce Cre expression and weighed every day. The slope of the weight loss was significantly different in PorcnΔ mice as compared to control mice (*P = 0.0107, two-tailed linear regression analysis).
Extended Data Table 1  |  Wnt pathway gene expression levels in different cell populations as assessed by RNA-seq (mean of 2–3 samples each in FPKM)

**Wnt pathway**

| Gene name | FoxI1* Expression level mean (FPKM) | Mes Expression level mean (FPKM) | Lgr5 Expression level mean (FPKM) | Diff Expression level mean (FPKM) |
|-----------|-------------------------------------|----------------------------------|-----------------------------------|----------------------------------|
| Wnt2      | 0.9742                              | 0                                | 0.1736                            | 0                                |
| Wnt2b     | 9.5831                              | 0.1205                           | 0.1559                            | 0.1191                           |
| Wnt3      | 0.3438                              | 0                                | 1.0915                            | 0.0909                           |
| Wnt4      | 1.7951                              | 0                                | 0.0102                            | 0.9198                           |
| Wnt5a     | 4.9932                              | 0                                | 0.1219                            | 0.0786                           |
| Wnt5b     | 0.8935                              | 0                                | 0.5514                            | 0.3542                           |
| Wnt6      | 0.0866                              | 0                                | 0.0824                            | 0.3045                           |
| Wnt9a     | 0.5072                              | 0                                | 0.0505                            | 0.0553                           |
| Wisp1     | 2.4082                              | 0                                | 0.0286                            | 0                                |
| Rsps2     | 0.4112                              | 0                                | 0                                 | 0                                |
| Rsps3     | 2.1902                              | 13.3906                          | 0.0141                            | 0                                |
| Lgr4      | 7.5384                              | 0                                | 48.6203                           | 9.8020                           |
| Lgr5      | 1.9220                              | 0                                | 19.3406                           | 0.0408                           |
| Lgr6      | 0.2264                              | 0                                | 0                                 | 0                                |
| Znrf1     | 4.2325                              | 0                                | 3.6232                            | 0.6523                           |
| Znrf2     | 2.2594                              | 5.0671                           | 12.7731                           | 7.7214                           |
| Znrf3     | 0.9130                              | 0.1328                           | 1.9908                            | 0.1333                           |
| Dkk2      | 1.8779                              | 0                                | 0                                 | 0                                |
| Dkk3      | 36.5672                             | 22.1995                          | 0.0017                            | 0.0077                           |
| Wi1       | 1.2579                              | 0                                | 0.0125                            | 0.0844                           |
| Sfrp1     | 47.2422                             | 0.6198                           | 0.0722                            | 0.0034                           |
| Sfrp4     | 0.7134                              | 0                                | 0.0096                            | 0                                |
| Fzd2      | 2.50329                             | 0                                | 1.9856                            | 0.0100                           |
| Fzd4      | 4.5451                              | 0                                | 0.2025                            | 0.9071                           |
| Fzd6      | 0.5785                              | 0                                | 2.7795                            | 0.2181                           |
| Fzd7      | 1.1146                              | 0                                | 16.7951                           | 2.5999                           |
| Fzd8      | 0.6245                              | 0                                | 0.7510                            | 0.3280                           |
| Lrp1      | 11.4018                             | 0.0144                           | 6.4567                            | 12.5752                          |
| Lrp6      | 6.0929                              | 0.0013                           | 9.5234                            | 4.2674                           |
| Tcf4      | 15.0228                             | 8.338                            | 6.0325                            | 4.9127                           |
| Tcf15     | 1.71113                             | 0                                | 0.0090                            | 0                                |
| Tcf19     | 1.456                               | 8.768                            | 13.6231                           | 0.7681                           |
| Tcf21     | 19.6602                             | 0.0065                           | 0                                 | 0                                |
| Tcf7f1    | 1.8914                              | 0                                | 0.1070                            | 0.0623                           |
Life Sciences Reporting Summary

Nature Research wishes to improve the reproducibility of the work that we publish. This form is intended for publication with all accepted life science papers and provides structure for consistency and transparency in reporting. Every life science submission will use this form; some list items might not apply to an individual manuscript, but all fields must be completed for clarity.

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

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 advance. 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 exact sample size (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
  - 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
  - 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 central tendency (e.g. median, mean) and variation (e.g. standard deviation, interquartile range)
- Clearly defined error bars in all relevant figure captions (with explicit mention of central tendency and variation)

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

7. Software

Policy information about availability of computer code

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.

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.

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.

b. Describe the method of cell line authentication used.

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

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
11. Description of research animals

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

The mice used were: FoxI1-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.