Endothelial FOXC1 and FOXC2 promote intestinal regeneration after ischemia-reperfusion injury

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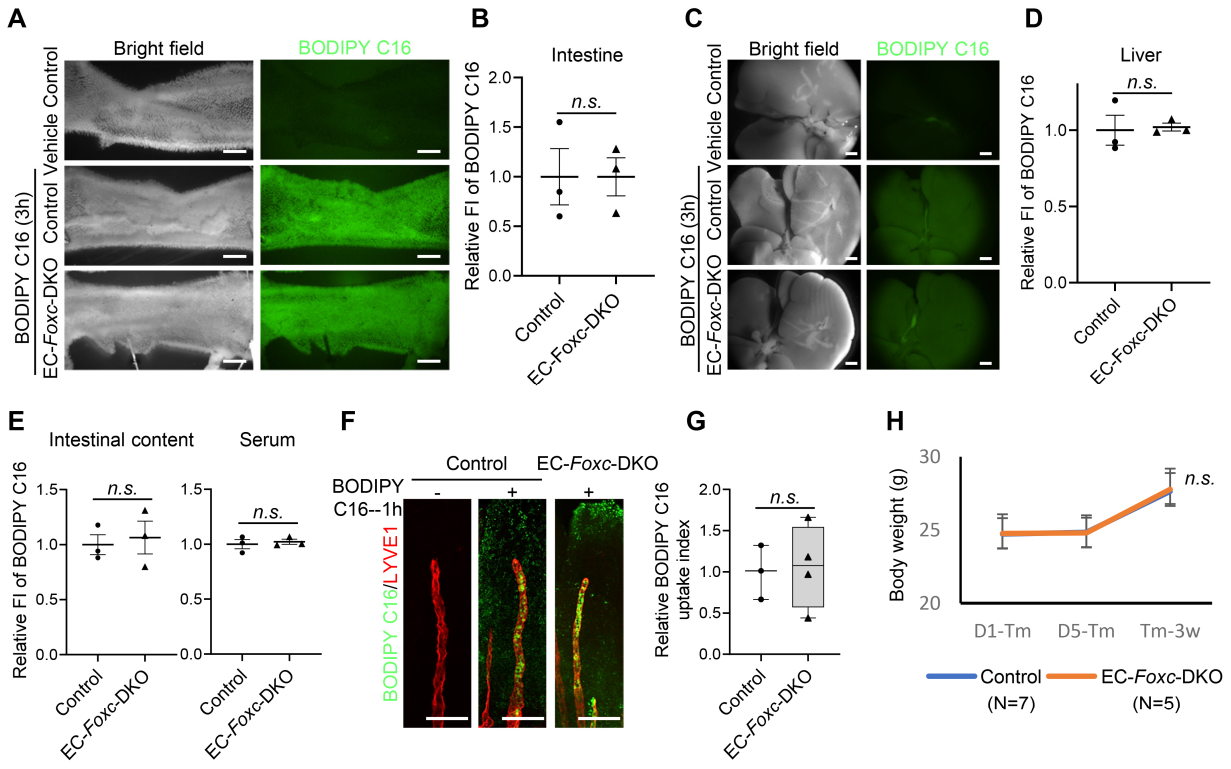
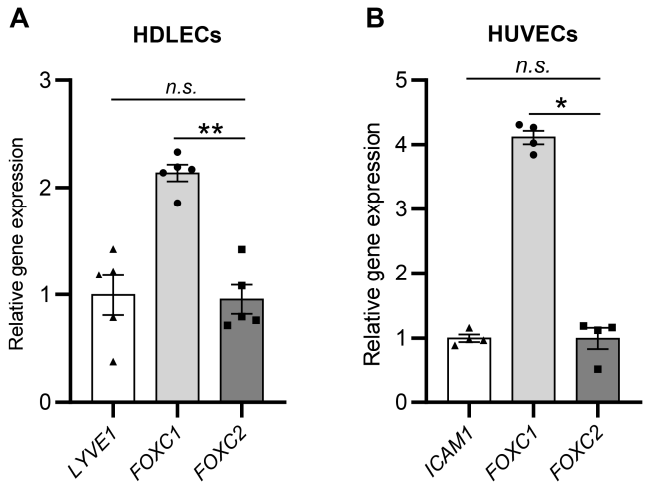
Contents

Appendix Figure S1



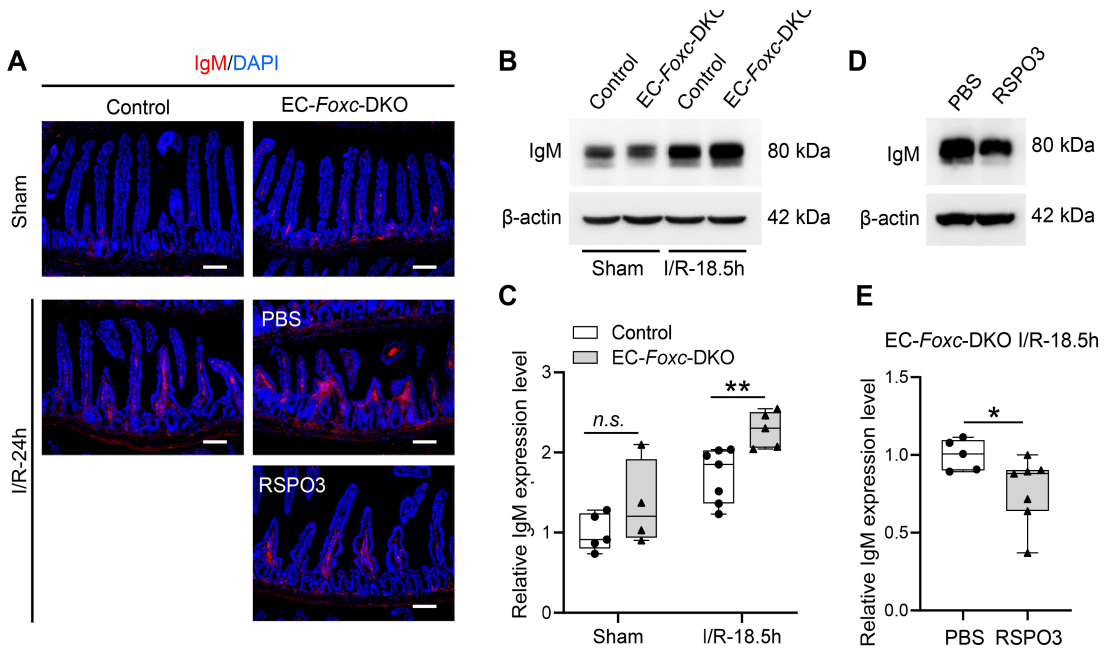
Appendix Figure S1. EC-iKO of *Foxc1/c2* does not affect the lacteal permeability. Mice were treated with 50 µg BODIPY C16 in 200 µL Intralipid by oral gavage after being fasted for 9~12h. Mice treated with Intralipid only were used as vehicle control. 3h after the treatment, the distal jejunum **(A)** and liver **(C)** were dissected and imaged under Nikon AZ100 fluorescent microscope. Scale bars = 1 mm. The quantification of fluorescent intensity (FI) of BODIPY C16 (green) in intestine **(B)** and liver **(D)** was then performed based on the images as shown in A and C. **(E)** Intestinal content and blood serum were also collected 3h post BODIPY dose for the detection of FI of BODIPY. Data are box-and-whisker plots, Mann-Whitney *U* test, each symbol represents one mouse, N = 3, *n.s.* = not significant. **(F)** Representative confocal images of whole-mount intestines immunostained with LYVE1 show the uptake of BODIPY C16 (green) by the lacteals (LYVE1+, red). Scale bars = 50 µm. **(G)** Quantification of the BODIPY C16 uptake index (= ratio of BODIPY C16 intensity in lacteal vs. lacteal area) was performed based on the images as shown in F. Data are box-and-whisker plots, Mann-Whitney *U* test, each symbol represents one mouse, N = 3~4, *n.s.* = not significant. **(H)** Mouse body weight was monitored at Tm-day 1 (D1-Tm), day 5 (D5-Tm) and 3 weeks post Tm treatment (Tm-3w). Data are Mean ± SEM, unpaired *t* test, N=7 in control, N=5 in EC-*Foxc*-DKO, *n.s.* = not significant.

Appendix Figure S2



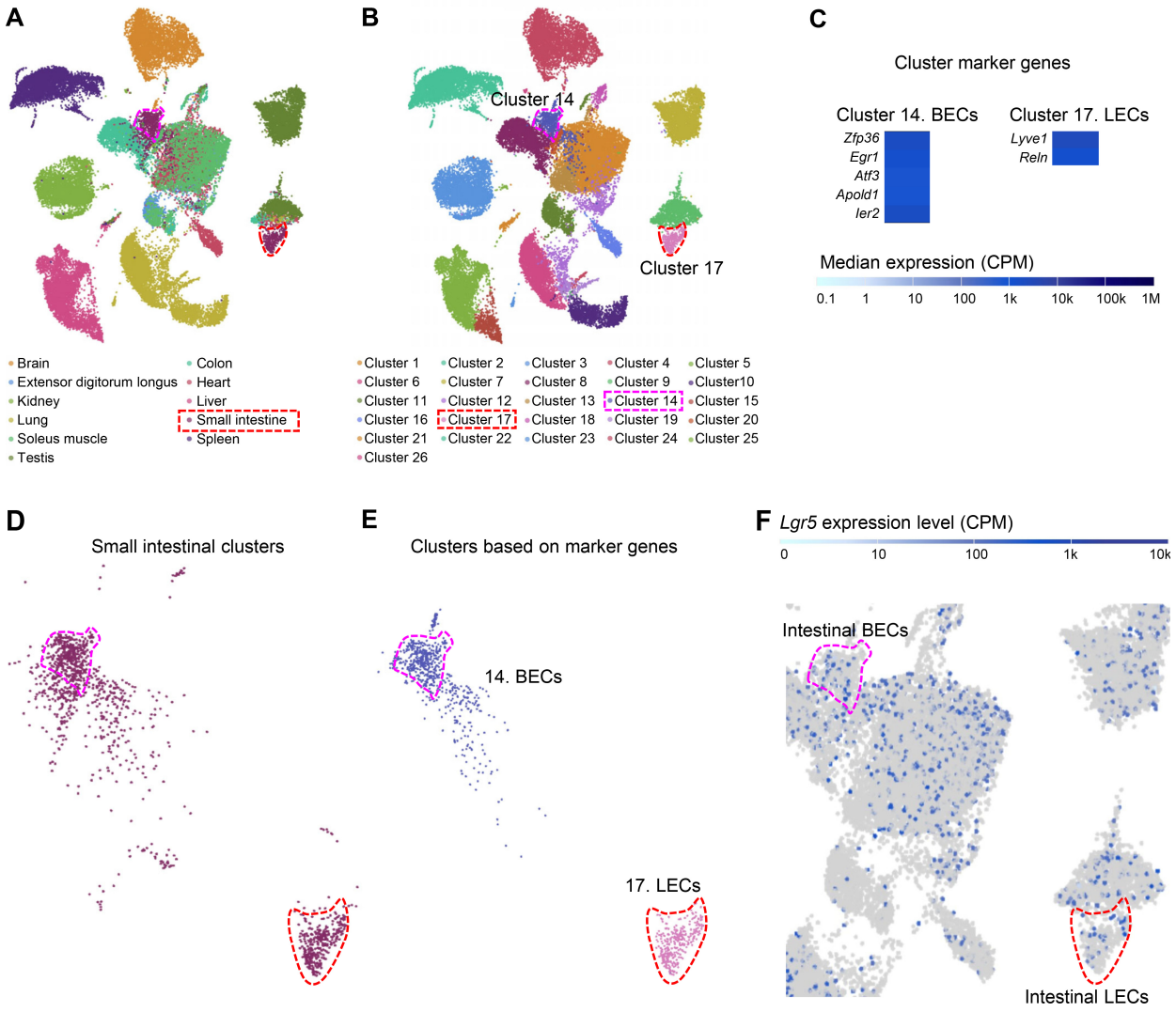
Appendix Figure S2. FOXC1 and FOXC2 are expressed in HDLECs and HUVECs. Before the ChIP assay experiment, the expression of *FOXC1* and *FOXC2* in **(A)** HDLECs and **(B)** HUVECs were confirmed by qPCR. Relative mRNA expression of *FOXC1* and *FOXC2* as well as the EC markers (*LYVE1* for HDLECs and *ICAM1* for HUVECs) show *FOXC2* has similar expression level as the EC markers, while the expression level of *FOXC1* is higher than that of *FOXC2* in both cell types. Data are box-and-whisker plots, Mann-Whitney *U* test, each symbol represents one experiment, N = 5 in HDLECs, N = 4 in HUVECs, **P*<0.05, ***P*<0.01, *n.s.* = not significant.

Appendix Figure S3



Appendix Figure S3. RSPO3 treatment alleviates IgM accumulation in intestinal mucosa of EC-*Foxc*-DKO mice after I/R. (A) Representative immunostaining images of small intestine labeled with IgM in control and EC-*Foxc*-DKO mice in sham or at 24h after I/R. Note that after I/R, more IgM accumulation in the intestinal mucosa was found in EC-*Foxc*-DKO mice compared with control mice. The accumulation of IgM was alleviated in RSPO3-treated compared with PBS-treated EC-*Foxc*-DKO mice. Paraffin sections (4 μm), scale bars = 100 μm. **(B-E)** Representative Western blots (**B** and **D**) and densitometry measurements (**C** and **E**) show IgM (heavy chain) in intestinal tissue lysates from control and EC-*Foxc*-DKO mice in sham and after I/R at 18.5h (**B** and **C**) as well as IgM (heavy chain) in PBS- and RSPO3- treated EC-*Foxc*-DKO mice after I/R at 18.5h (**D** and **E**). Data are box-and-whisker plots, Mann-Whitney *U* test, each symbol represents one mouse, N = 4~7, **P*<0.05, ***P*<0.01, *n.s.*=not significant.

Appendix Figure S4



Appendix Figure S4. *Lgr5* is expressed in both intestinal BECs and LECs by scRNAseq. The scRNAseq data for mouse small intestinal ECs from the publication entitled "Single-Cell Transcriptome Atlas of Murine Endothelial Cells" (PMID: 32059779) was examined. The data is available from the website of "Single Cell Expression Atlas" (https://www.ebi.ac.uk/gxa/sc/home) and was exported to check *Lgr5* expression in mouse small intestinal ECs. **(A)** Color plot by organism parts. Pink and red circled area indicate the major cell populations from small intestine. **(B)** Color plot by marker genes. Pink (Cluster 14) and red (Cluster 17) circled area indicate the major cell populations from small intestine. **(C)** Cluster 14 and cluster 17 were identified as BECs and LECs respectively according to their marker genes. CPM: counts per million. **(D)** Small intestinal clusters identified from A. **(E)** Small intestinal clusters based on marker genes identified from B. Pink and red circled area in D and E indicate the intestinal major cell populations. **(F)** *Lgr5* expression in small intestinal BECs (pink circled) and LECs (red circled).