

## Appendix Figures

Transcription factor FOXP2 is a flow-induced regulator of collecting lymphatic vessels

Magda N. Hernández Vásquez<sup>1</sup>, Maria H. Ulvmar<sup>1</sup>, Alejandra González-Loyola<sup>2</sup>, Ioannis Kritikos<sup>3</sup>, Ying Sun<sup>1</sup>, Liqun He<sup>1</sup>, Cornelia Halin<sup>3</sup>, Tatiana V. Petrova<sup>2</sup> and Taija Mäkinen<sup>1</sup>

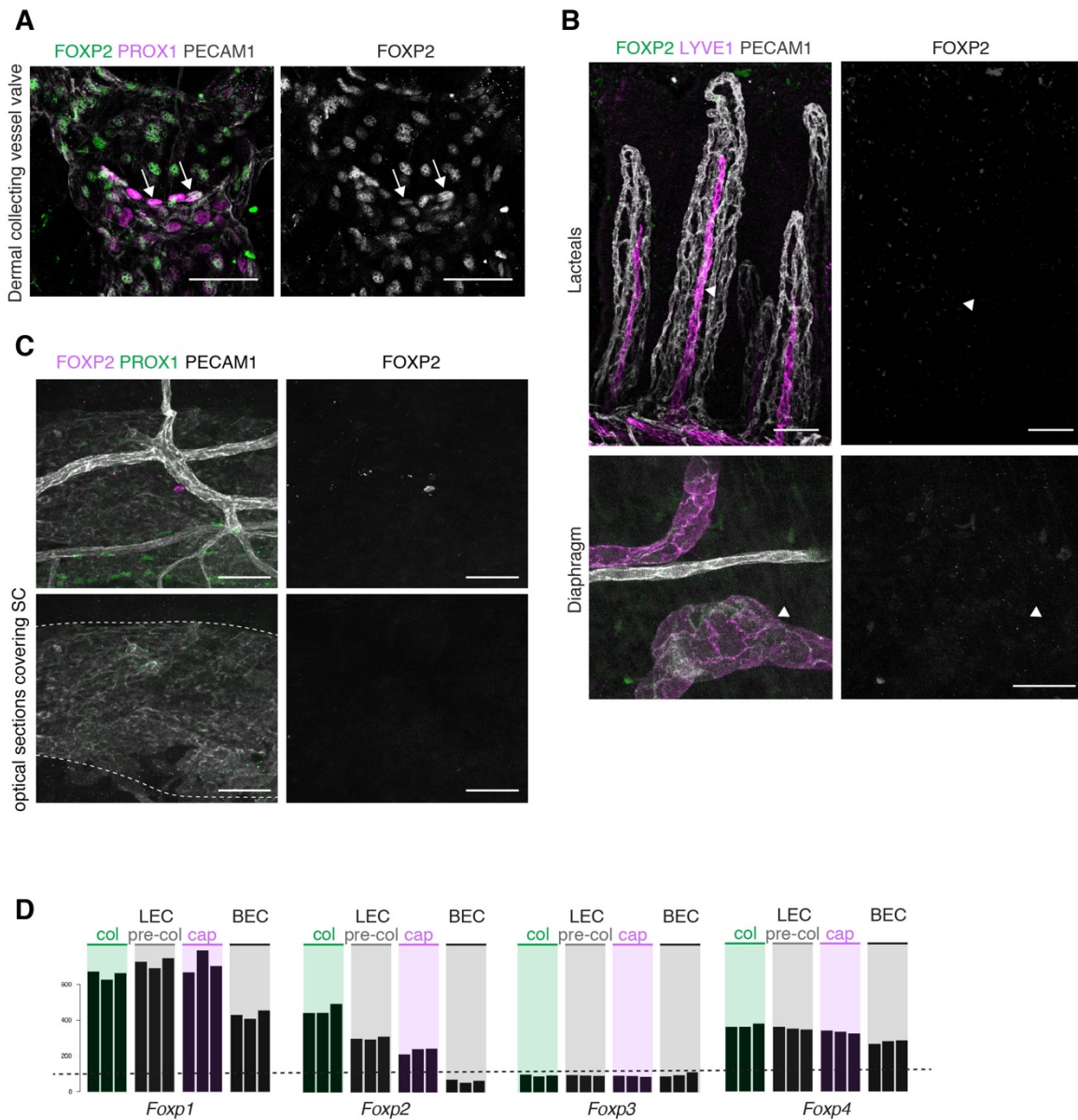
<sup>1</sup>Uppsala University, Department of Immunology, Genetics and Pathology, Dag Hammarskjölds väg 20, 751 85 Uppsala, Sweden.

<sup>2</sup>Vascular and Tumor Biology Laboratory, Department of Oncology UNIL CHUV, Ludwig Institute for Cancer Research Lausanne, Bâtiment CLE Chemin des Boveresses 155, CH-1066 Lausanne, Switzerland.

<sup>3</sup>Institute of Pharmaceutical Sciences, ETH Zürich, HCI G490.2, Vladimir-Prelog-Weg 4, CH-8093 Zürich, Switzerland.

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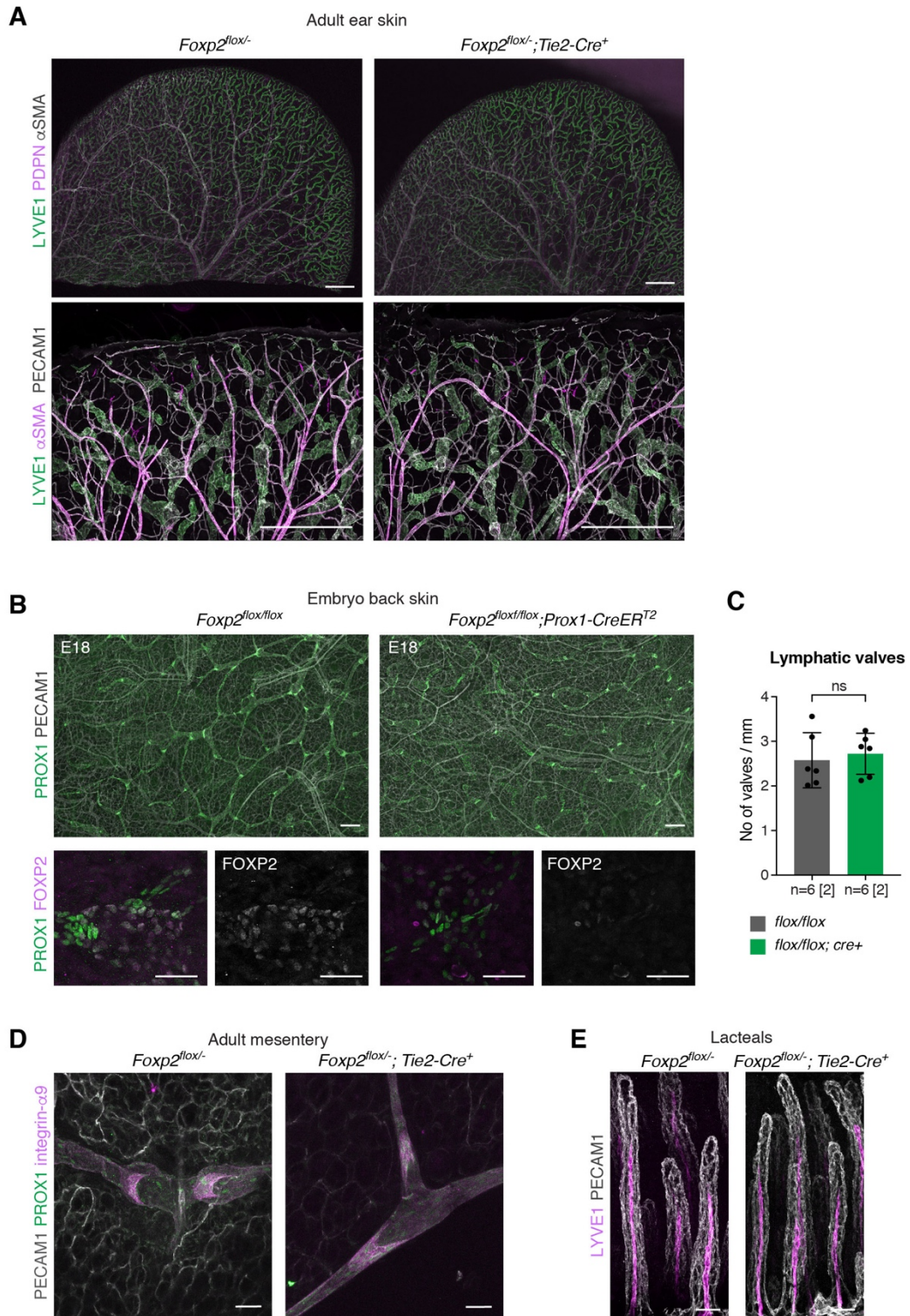
**Appendix Figure S1 - Endothelial expression of FOXP2.**

A, B. Whole-mount immunofluorescence of ear skin (A), intestine and diaphragm (B), showing nuclear FOXP2 staining in collecting vessel and PROX1<sup>high</sup> valve LECs (arrows), but not in LYVE1<sup>+</sup> lymphatic capillaries (arrowheads) or PECAM1<sup>+</sup>LYVE1<sup>-</sup> blood vessels.

C. Whole-mount immunofluorescence showing no expression of FOXP2 in the Schlemm’s canal (SC, dotted) in an adult mouse. Maximum intensity projection of the full z stack (upper panels) and partial z stack covering the SC (dotted outline, lower panels) are shown.

D. Intensity bar plots showing expression of *Foxp1-4* in dermal EC populations (n = 3 samples). Dotted line indicates the threshold of detection.

Scale bar: 50  $\mu$ m (A-C).



**Appendix Figure S2 - Normal gross morphology of dermal blood and lymphatic vessels in mice lacking endothelial *Foxp2*.**

A. Whole-mount immunofluorescence of 3 weeks old ear skin of control and *Foxp2<sup>fllox/-</sup>;Tie2-Cre* mice showing normal gross morphology and organization of the dermal blood and lymphatic vessels.

B. Whole-mount immunofluorescence of back skin of E18 control and *Foxp2<sup>flox/flox</sup>;Prox1-CreER<sup>T2</sup>* embryos showing normal gross morphology and organization of the dermal blood and lymphatic vessels. Pregnant females were administered with 1 mg of 4-OHT at E15 and E16.

C. Quantification of lymphatic valve numbers (clusters of PROX1<sup>high</sup> LECs) in *Foxp2<sup>flox/flox</sup>;Prox1-CreER<sup>T2</sup>* and littermate control embryos (n = images [mice], as indicated). Data are presented as mean ± SD. P, Student's t-test. ns = not significant.

D. Whole-mount immunofluorescence of mesenteries of 9-week-old control and *Foxp2<sup>flox/-</sup>;Tie2-Cre* mice showing valves of normal morphology.

E. Whole-mount immunofluorescence of intestinal villi of P7 control and *Foxp2<sup>flox/-</sup>;Tie2-Cre* mice showing normal morphology of lacteal lymphatic vessels.

Scale bar: 500 μm (A), 200 μm (B, upper panels), 50 μm (B, lower panels, D, E).