Expanded View Figures

Figure EV1. Transcriptome analysis of dermal endothelial cells.

A Proportion of LEC subpopulations (of the total LEC population) sorted from mouse ear skin based on expression of LYVE1, PDPN, and *Prox1-GFP* as shown in Fig 1A ($n = 3$ samples). Horizontal line indicates mean. Col, collecting vessel; pre-col, pre-collecting vessel; cap, lymphatic capillary.

B Heat map showing differential expression of 1,191 genes between dermal LEC and BEC. Color coding shows $\log_2$ fold change. Gene ID list is provided in Dataset EV1.

C Intensity bar plots of cell lineage/phenotype genes showing expression in dermal EC populations (each bar represents one sample, $n = 3$). Y-axis represents expression level (signal intensity value given in arbitrary units (AU)).

D Intensity bar plot of *Foxp2* showing expression in dermal EC populations ($n = 3$). Y-axis represents expression level (signal intensity value given in arbitrary units (AU)).

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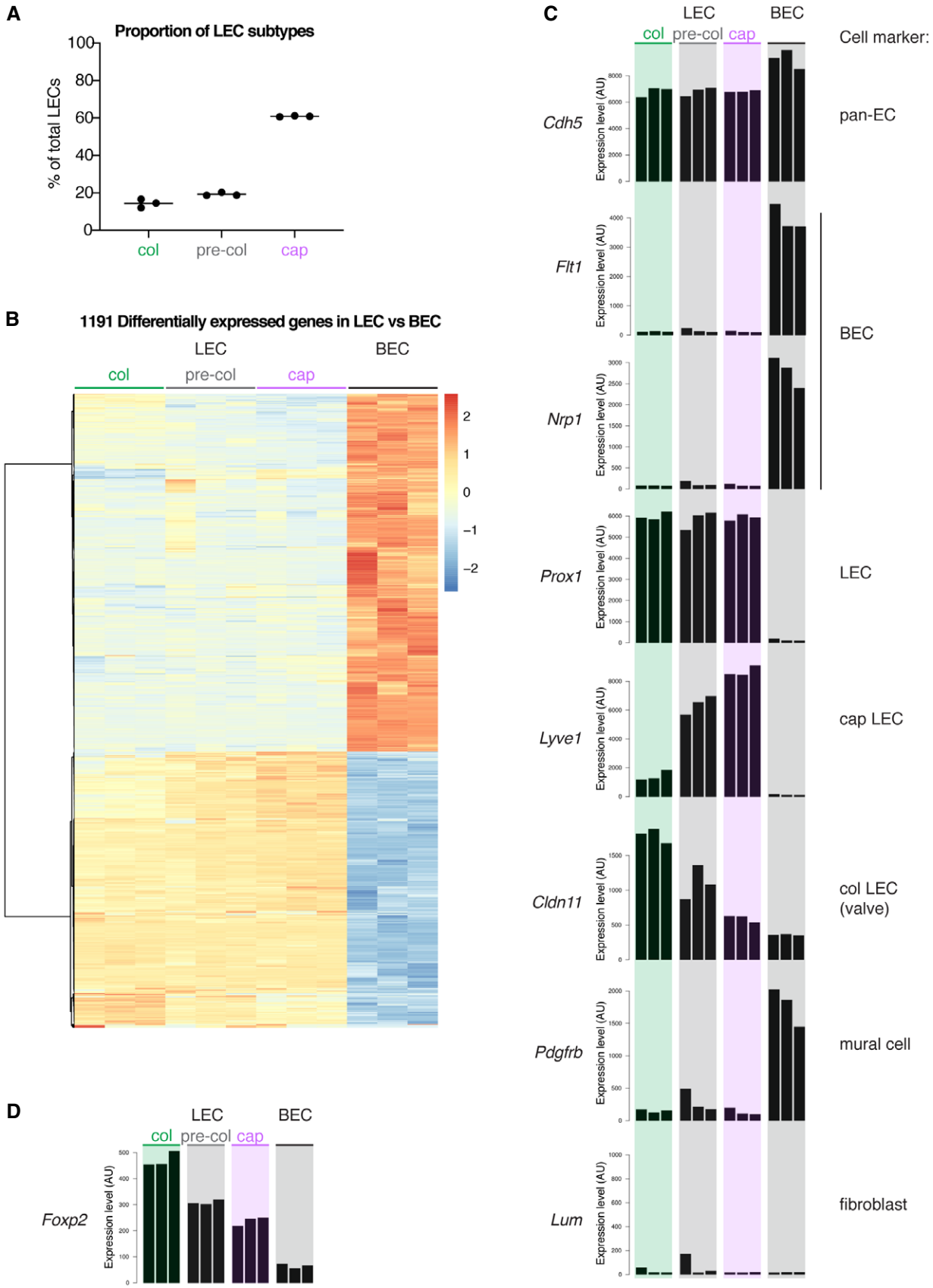

Figure EV1.

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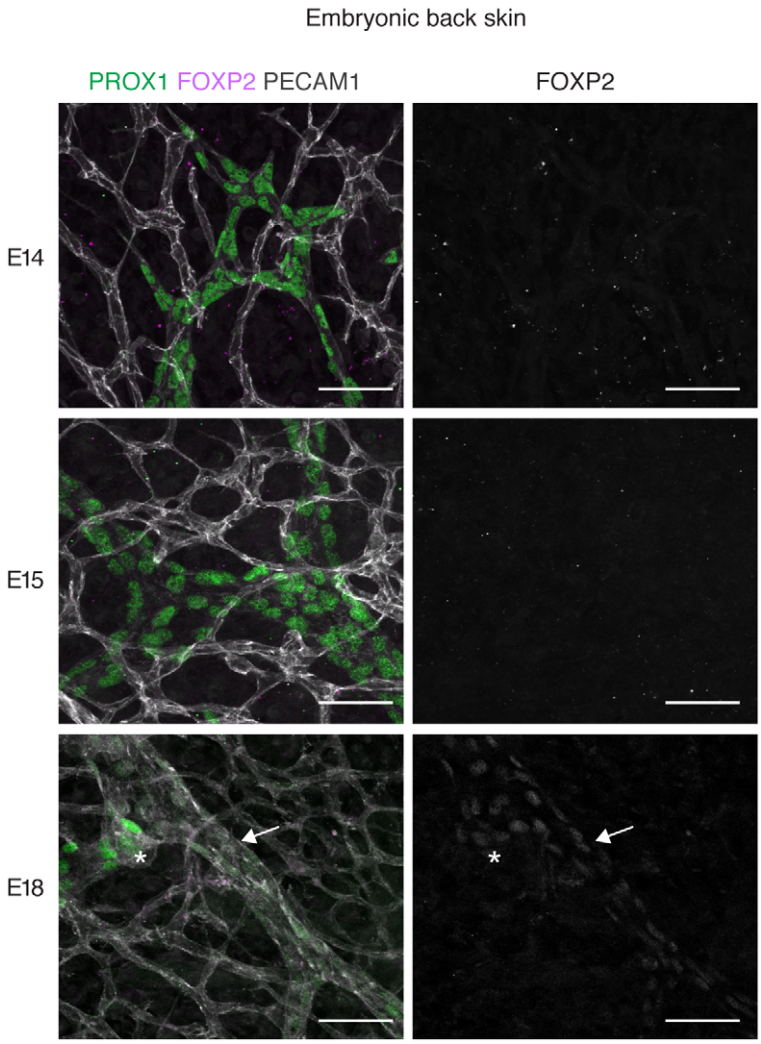

Figure EV2. FOXP2 expression in the developing dermal vasculature.

Whole-mount immunofluorescence of embryonic back skin at the indicated developmental stages. Note expression of FOXP2 (single channel images on the right) in the developing collecting vessel (arrow) including luminal valve (asterisk) at E18. Scale bar: 50 μm.

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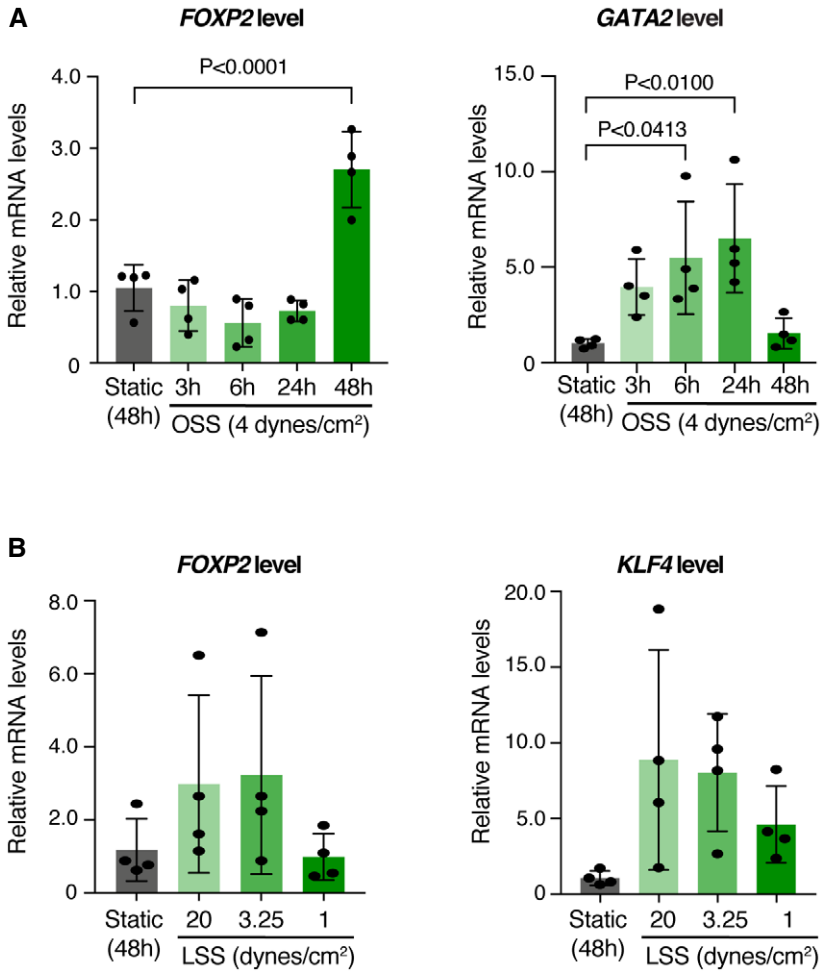

Figure EV3. Regulation of *FOXP2* expression by oscillatory and laminar flow.

A, B qRT–PCR analysis of HDLECs grown under static conditions or exposed to oscillatory shear stress (OSS) for the indicated times (A), or to laminar shear stress (LSS) at the indicated dyn/cm^2 for 48 h (n = 4 independent experiments). *GATA2* and *KLF4* are shown as controls. Data are presented as mean ± SD. *P*, Student's *t*-test.

Source data are available online for this figure.

Figure EV4. Analysis of FOXP2 expression and function in venous and lymphovenous valves.

A, B Whole-mount immunofluorescence of venous valves of the proximal femoral vein in P7 *Foxp2*$^{flox/flox}$;*Tie2-Cre* (A) and *Foxp2*$^{flox/-}$;*Tie2-Cre* (B) mice with respective littermate controls. Note expression of FOXP2 in ECs of the valve leaflet but apparently normal PROX1^{high} integrinα9$^+$ valve leaflets in *Foxp2*-deficient mice.

C Left: Immunofluorescence of a coronal vibratome section of an E14 *Vegfr3-CreER*^{T2};*R26-mTmG* (Martinez-Corral *et al*, 2016) embryo showing no expression of FOXP2 in LVV (arrows). FOXP2$^+$ cells are likely (PROX1$^-$) neuronal cells. Maximum intensity projection is shown above, and a single optical section at the level of LVV leaflets below. GFP expression in LECs was induced by intraperitoneal injection of 1 mg of 4-OHT into pregnant female. Right: Visualization of FOXP2$^+$ neuronal cells confirmed successful staining (arrowheads). LS, lymph sac; IJV, internal jugular vein; SVC, superior vena cava; A, artery.

Data information: Scale bar: 50 μm (A–C, lymphovenous connection), 200 μm (C, brain).

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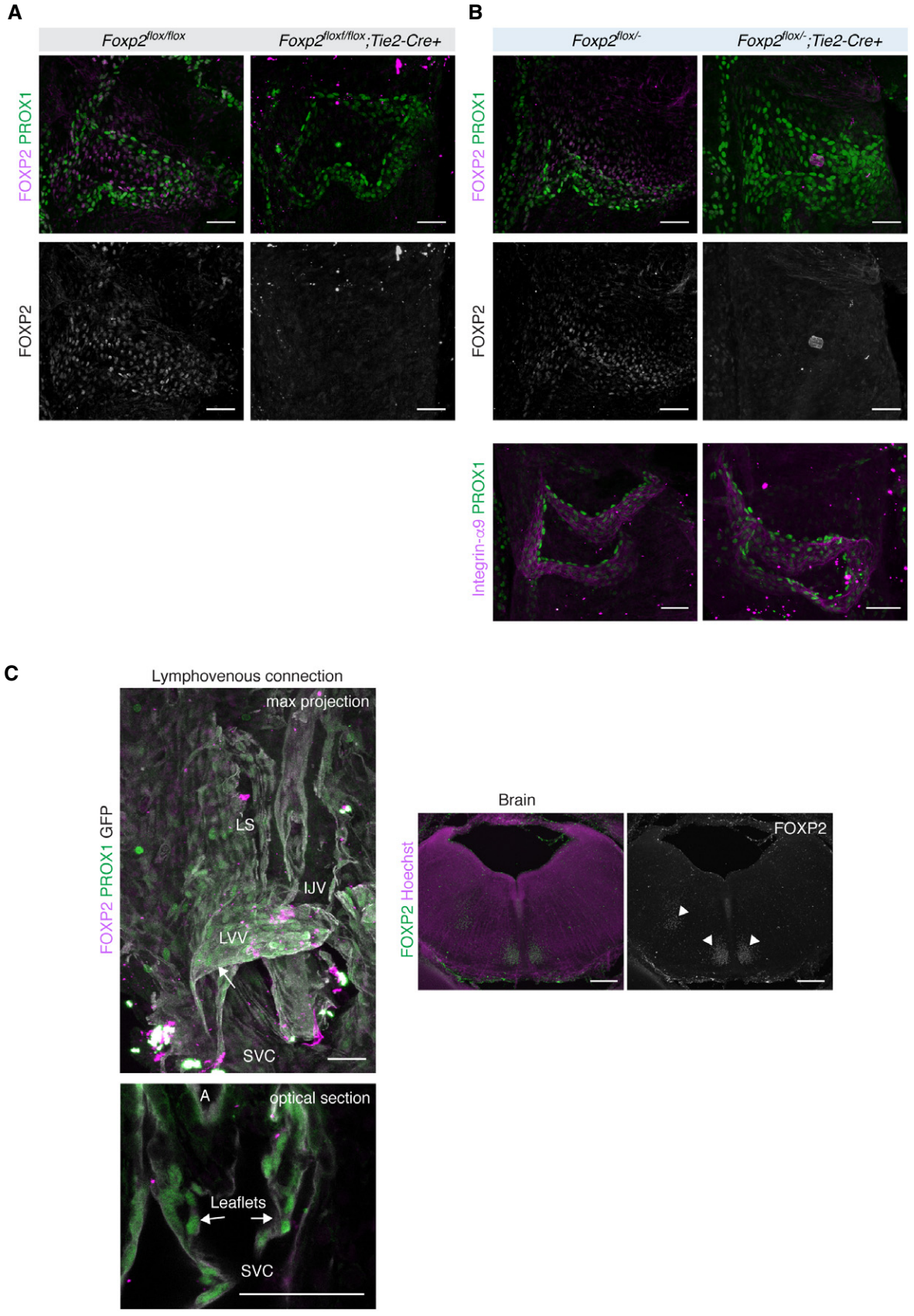
A
Foxp2flox/flox
Foxp2floxf/flox;Tie2-Cre+
FOXP2 PROX1
FOXP2
B
Foxp2flox/-
Foxp2flox/-;Tie2-Cre+
FOXP2 PROX1
FOXP2
Integrin-α9 PROX1
C
Lymphovenous connection
max projection
FOXP2 PROX1 GFP
LS
IJV
LVV
SVC
A
optical section
Leaflets
SVC
Brain
FOXP2 Hoechst
FOXP2

Figure EV4.

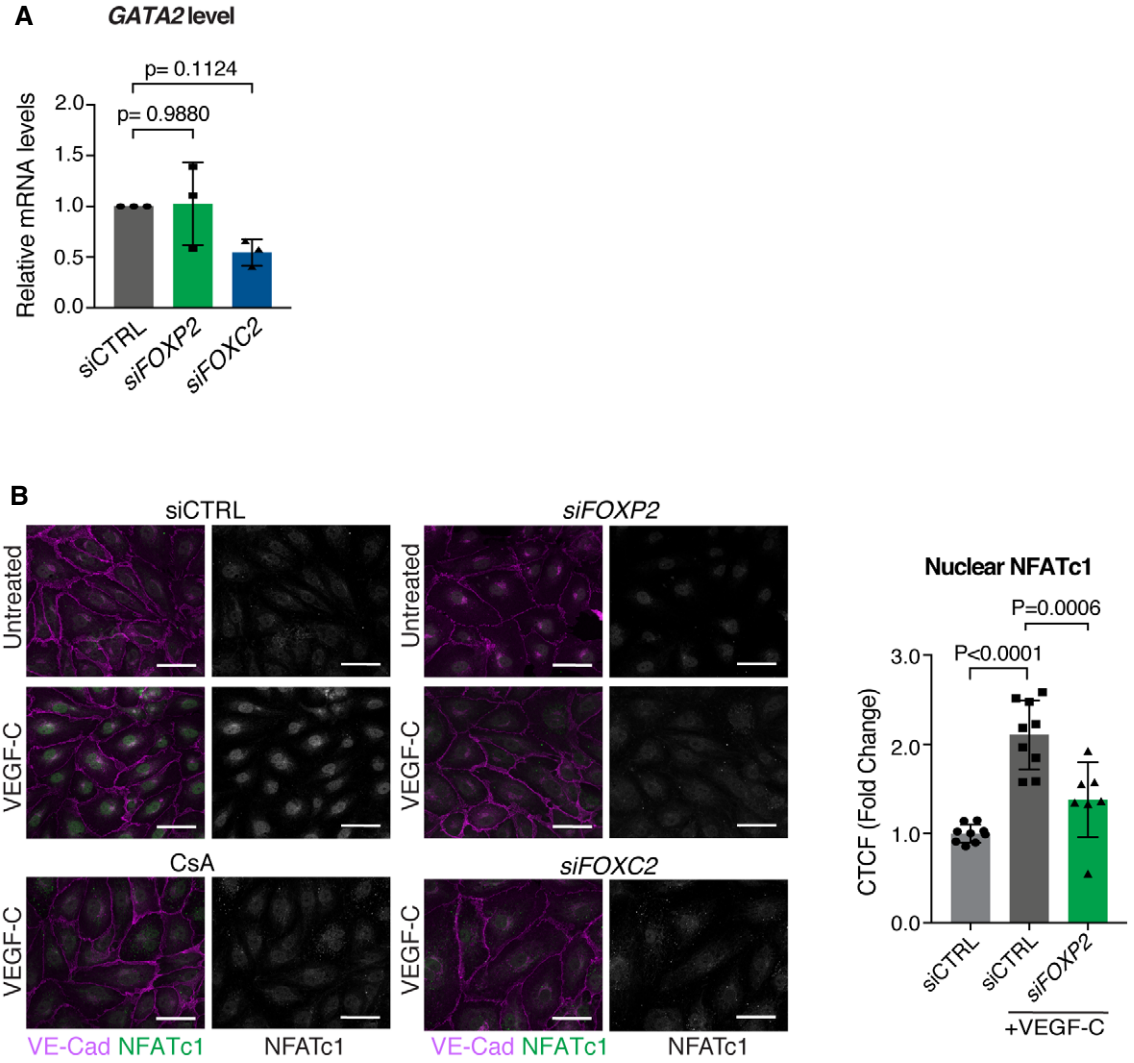

Figure EV5. Analysis of FOXC2 pathway components in *FOXP2* silenced LECs.

A qRT–PCR analysis of *GATA2* expression in control (siCTRL) and *FOXP2* or *FOXC2* siRNA-treated HDLECs (n = 3 independent experiments).

B Left: NFATc1 immunofluorescence in control (siCTRL) and *FOXP2* or *FOXC2* siRNA-transfected HDLECs under basal conditions (untreated) or after 30-min stimulation with VEGF-C (50 ng/ml) in the absence or presence of the calcineurin inhibitor cyclosporin A (CsA). Note nuclear translocation of NFATc1 upon VEGF-C stimulation, which is abrogated in cells treated with *FOXP2* or *FOXC2* siRNA, or CsA. Right: Quantification of nuclear NFATc1 in CTRL and *FOXP2* siRNA-treated LECs after VEGF-C stimulation (n = 7–9 images from two independent experiments).

Data information: In (A, B), data are presented as mean ± SD. *P*, one-way ANOVA (A), or Student's *t*-test (B). Scale bar: 50 μm (B).

Source data are available online for this figure.

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