Expanded View Figures

Figure EV1. Expression of SOX7 in lymphatic endothelial cells.

A Whole-mount immune staining on the skin at E14.5 using SOX7 (magenta), PROX1 (yellow), and PECAM (CD31, blue). During mid-organogenesis SOX7 expression is not observed in the developing lymphatic endothelial cells (LECs) (PROX1/PECAM double-positive cells), but often, SOX7-positive cells are in close vicinity of PROX1-positive lymphatic endothelial cells (LECs).

B, C Transverse sections of a E10.5 wild-type embryo stained for SOX7 (intensity levels shown as a heat map), PROX1 (red), and Endomucin (white). High levels of SOX7 are indicated by red to white signals and weak expression (limit of detection) by a blue to yellow signal according to a 16-color scaling system. (B) Expression of SOX7 and PROX1 at the early steps of lymphangiogenesis, in migrating LECs at the mesenchyme (inset 1) and the LEC progenitors at the cardinal vein (CV) (inset 2). (C) White arrowheads indicate cells that have a high expression level for PROX1 and low for SOX7 (blue to yellow). Asterisks indicate cells with high level of SOX7 (red to white) but low level of PROX1.

D Quantitation of SOX7/PROX1 double-positive cells either in the LEC progenitor of the CV wall ($n = 6$) or in the migrating LECs at the mesenchyme ($n = 5$). Mean ± SEM, scale bars = 100 μm. Dors., dorsal; Med., medial. Scale bars = 100 μm.

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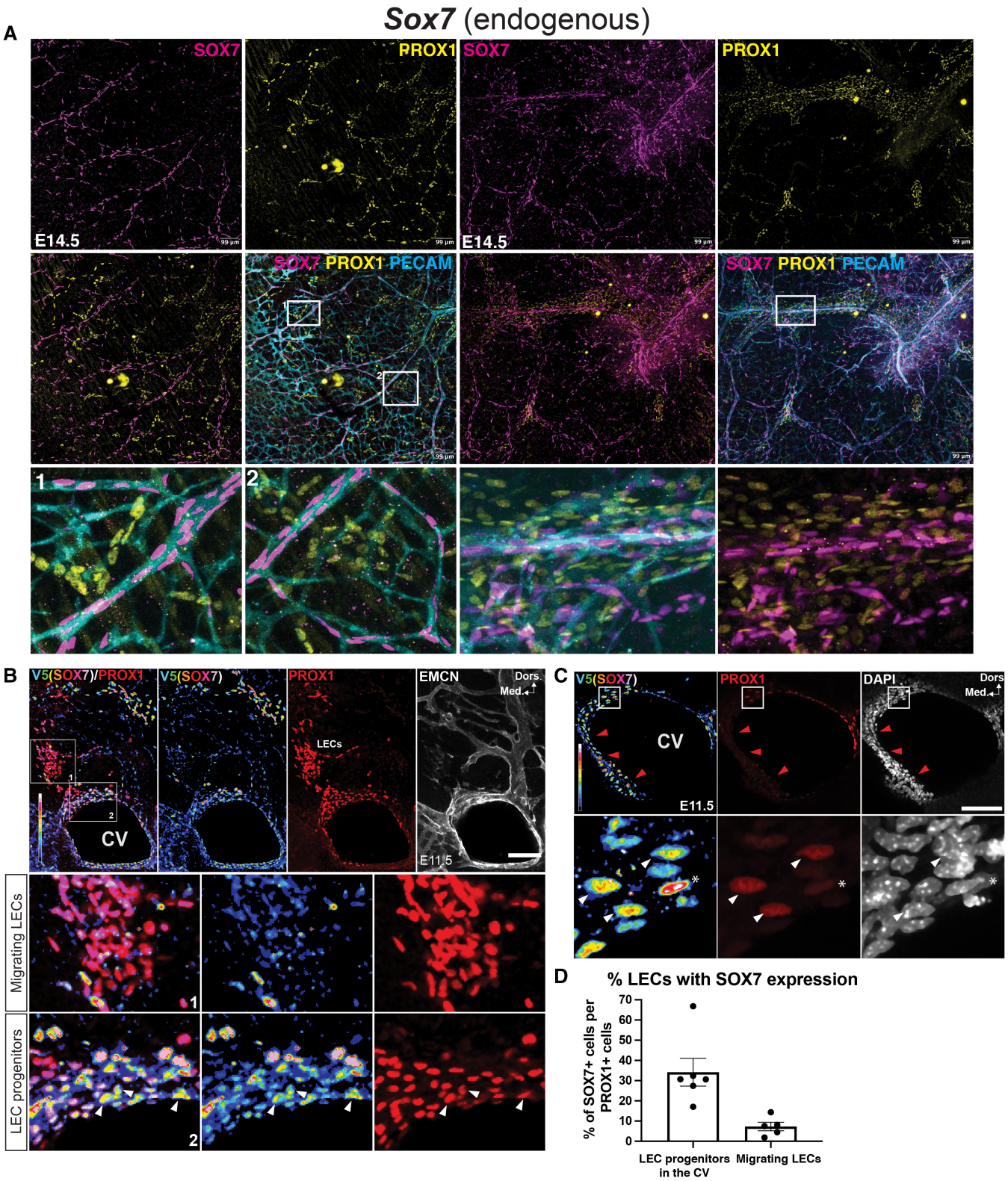
A
Sox7 (endogenous)
SOX7
PROX1
SOX7
PROX1
E14.5
E14.5
SOX7 PROX1 PECAM
SOX7 PROX1 PECAM
1
2
B
V5(SOX7)/PROX1
V5(SOX7)
PROX1
EMCN
Dors
Med.
CV
LECs
E11.5
Migrating LECs
LEC progenitors
C
V5(SOX7)
PROX1
DAPI
CV
E11.5
D
% LECs with SOX7 expression
% of SOX7+ cells per PROX1+ cells
70
60
50
40
30
20
10
0
LEC progenitors in the CV
Migrating LECs

Figure EV1.

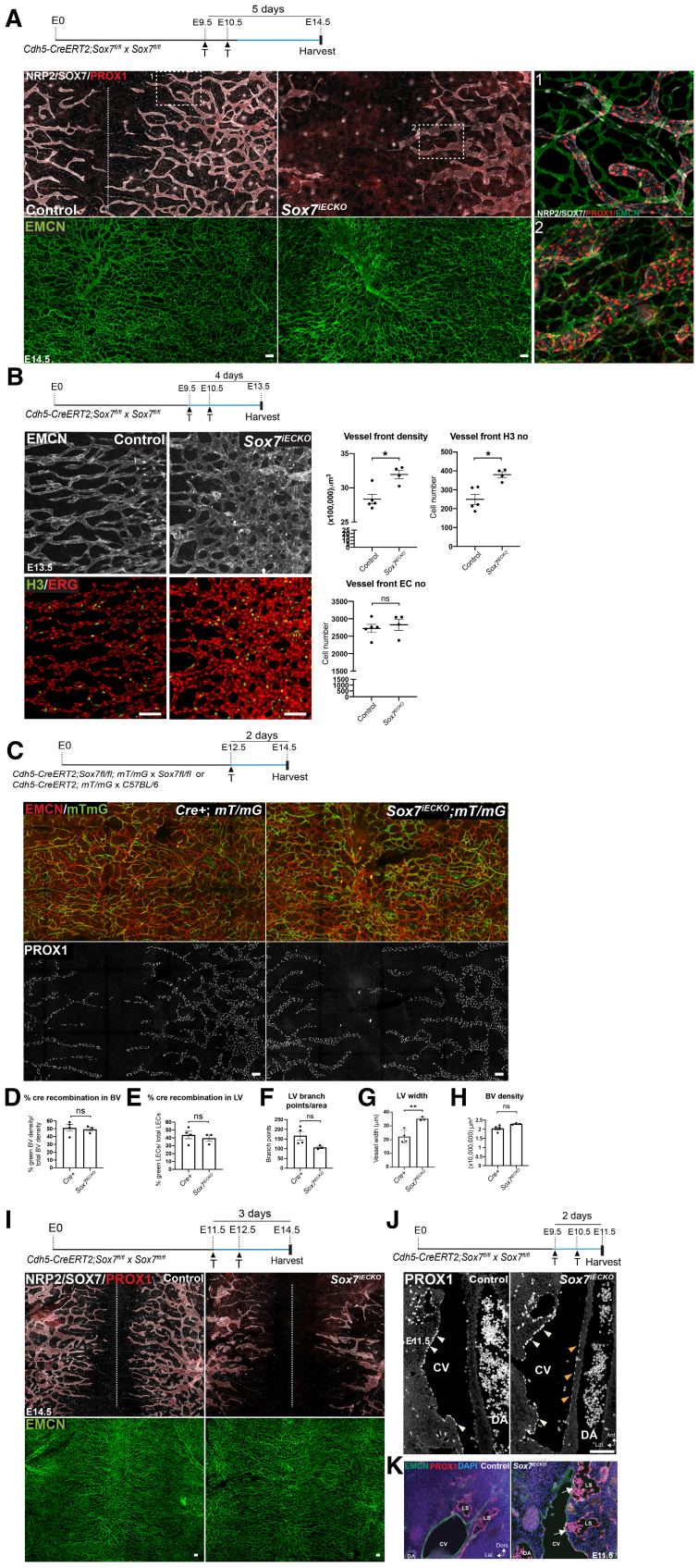

Figure EV2. Vascular defects associated with loss of SOX7 function.

A Whole-mount immunostaining of *Sox7*^{iECKO} mutant and sibling control embryonic skin at E14.5, following injection with tamoxifen at E9.5 and E10.5. Dermal lymphatic structures are marked by NRP2 (membranous white), lymphatic endothelial cells by PROX1 (red), blood vessels by EMCN (green), and arterioles by SOX7 (white nuclei). Dashed line represents the midline of the embryo. A higher magnification of some areas showing the morphogenic defects is shown in insets 1 and 2, right panel.

B Whole-mount immunostaining of *Sox7*^{iECKO} mutant and sibling control for EMCN (gray), showing dermal blood vessels at E13.5, after injection with tamoxifen at E9.5 and E10.5. Endothelial cells are marked by ERG (red), and proliferative cells are stained by phosphohistone 3, H3 (green). (Right panel) Quantitation of EMCN^{+} (blood) vessel front density (μm^3), number of H3^{+} proliferative and ERG^{+} endothelial cells in this region. Scored sibling control, $n = 5$; *Sox7*^{iECKO} mutants, $n = 4$; Mean ± SEM; Mann–Whitney *U*-test. $P < 0.05$ (*); ns = not significant.

C Whole-mount immunostaining of *Sox7*^{iECKO}; *mT/mG* mutant and *Cre*+; *mT/mG* control embryonic skin at E14.5, after injection with tamoxifen at E12.5. Blood vessels are stained with EMCN (red), cells after *Cre* excision are shown in green, and lymphatic endothelial cells are marked by PROX1 (white).

D–H Graphs showing quantitation of the Cre activity in (D) blood vessels, (E) lymphatic vessels (F) lymphatic vessel branching points, (G) lymphatic vessel width (μm), and (H) EMCN^{+} (blood) vessel density (μm^3), from $n = 4$ *Cre*+; *mT/mG* controls and $n = 3$ *Sox7*^{iECKO}; *mT/mG* mutants. Mean ± SEM; Mann–Whitney *U*-test; ns = not significant.

I Whole-mount immunostaining of *Sox7*^{iECKO} mutant and sibling control embryonic skin at E14.5, after injection with tamoxifen at E11.5 and E12.5. Dermal lymphatic structures are marked by the NRP2 (membranous white), lymphatic endothelial cells by PROX1 (red), and blood vessels are stained by EMCN (green). Dashed line represents the midline of the embryo.

J Immunostaining of *Sox7*^{iECKO} mutant and sibling control coronal sections, at E11.5, after injection with tamoxifen at E9.5, E10.5. Lymphatic progenitor cells in the cardinal veins (CVs) are PROX1^{+} (white). Yellow arrowheads highlight the presence of PROX1-positive LEC progenitors in the lateral aspects of the cardinal vein.

K Transverse section of E11.5 control and *Sox7*^{iECKO} mutant embryos after Cre induction with tamoxifen at E9.5 and E10.5. Staining for PROX1 (red), Endomucin (green), and DAPI (blue) shows the presence of red blood cells in the developing lymph sacs of the *Sox7*^{iECKO} embryos (white arrows). Scored sibling controls, $n = 4$; *Sox7*^{iECKO} mutants, $n = 5$. CV, cardinal vein; DA, dorsal aorta; LS, lymph sac. Scale bars = 100 μm.

Source data are available online for this figure.

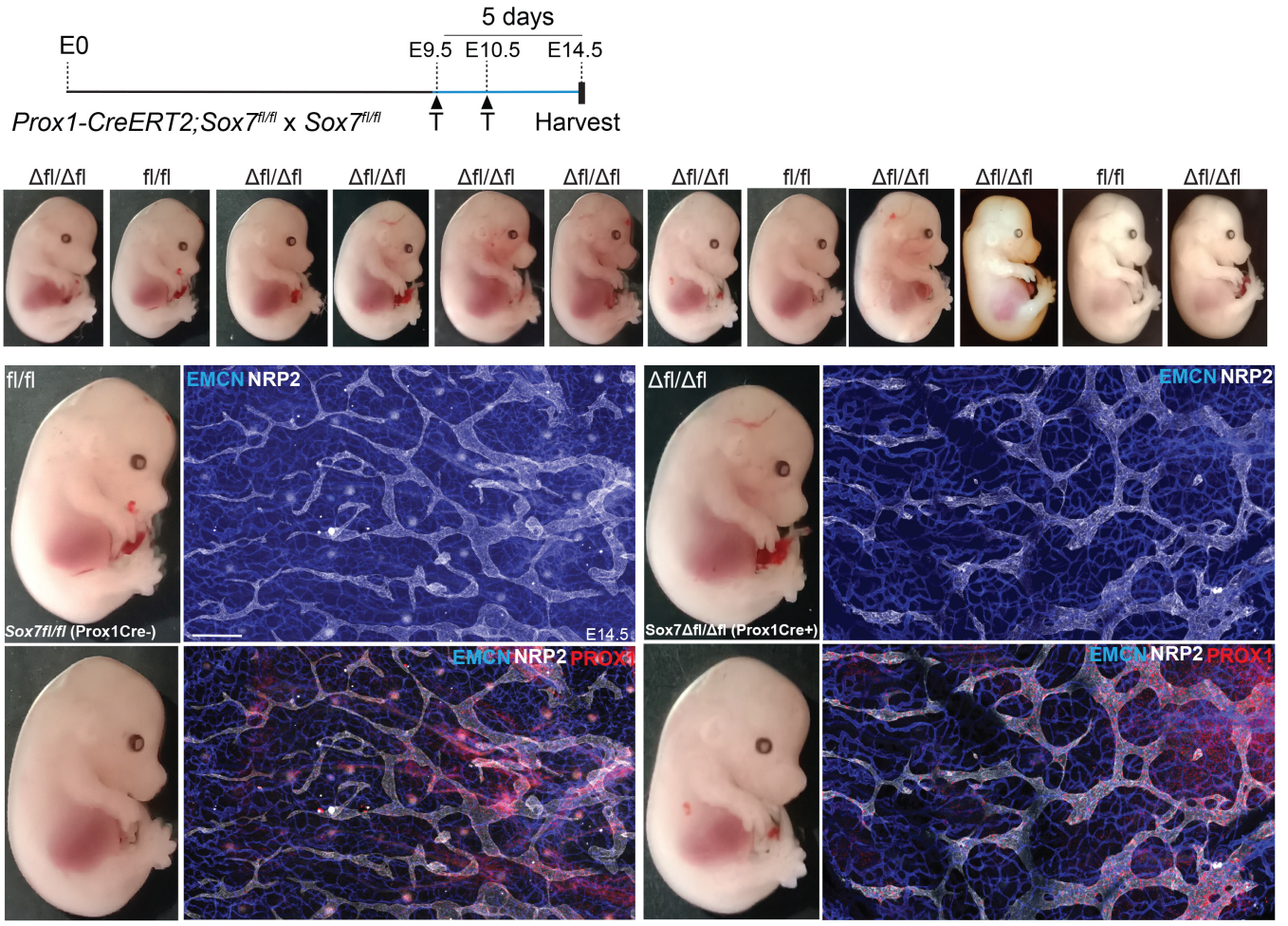

Figure EV3. Conditional deletion of *Sox7* gene in lymphatic endothelial cells using *Prox1-CreERT2: Sox7 fl/fl* genetic background.

Genetic disruption of *Sox7* exon 2 in lymphatic endothelial cells was performed upon tamoxifen treatment at E9.5 and E10.5. Embryos are collected at E14.5. (Top panel) Brightfield images of *Sox7Δfl/Δfl* (*Prox1Cre*$^{+}$) mutants and sibling controls *Sox7fl/fl* (*Prox1Cre*$^{-}$). (Bottom panel) Whole-mount immunostaining of *Sox7Δfl/Δfl* mutant and *Sox7fl/fl* sibling control embryonic skin at E14.5. At that time point, *Sox7Δfl/Δfl* mutant and *Sox7fl/fl* sibling control embryos display no significant morphological difference (e.g., lack of edema). The lymphatic vasculature in the Sox7-null embryos appears patterned properly and does not reproduce the phenotypic outcome obtained when using the *Cdh5-CreERT2* driver to delete *Sox7* function.

Source data are available online for this figure.

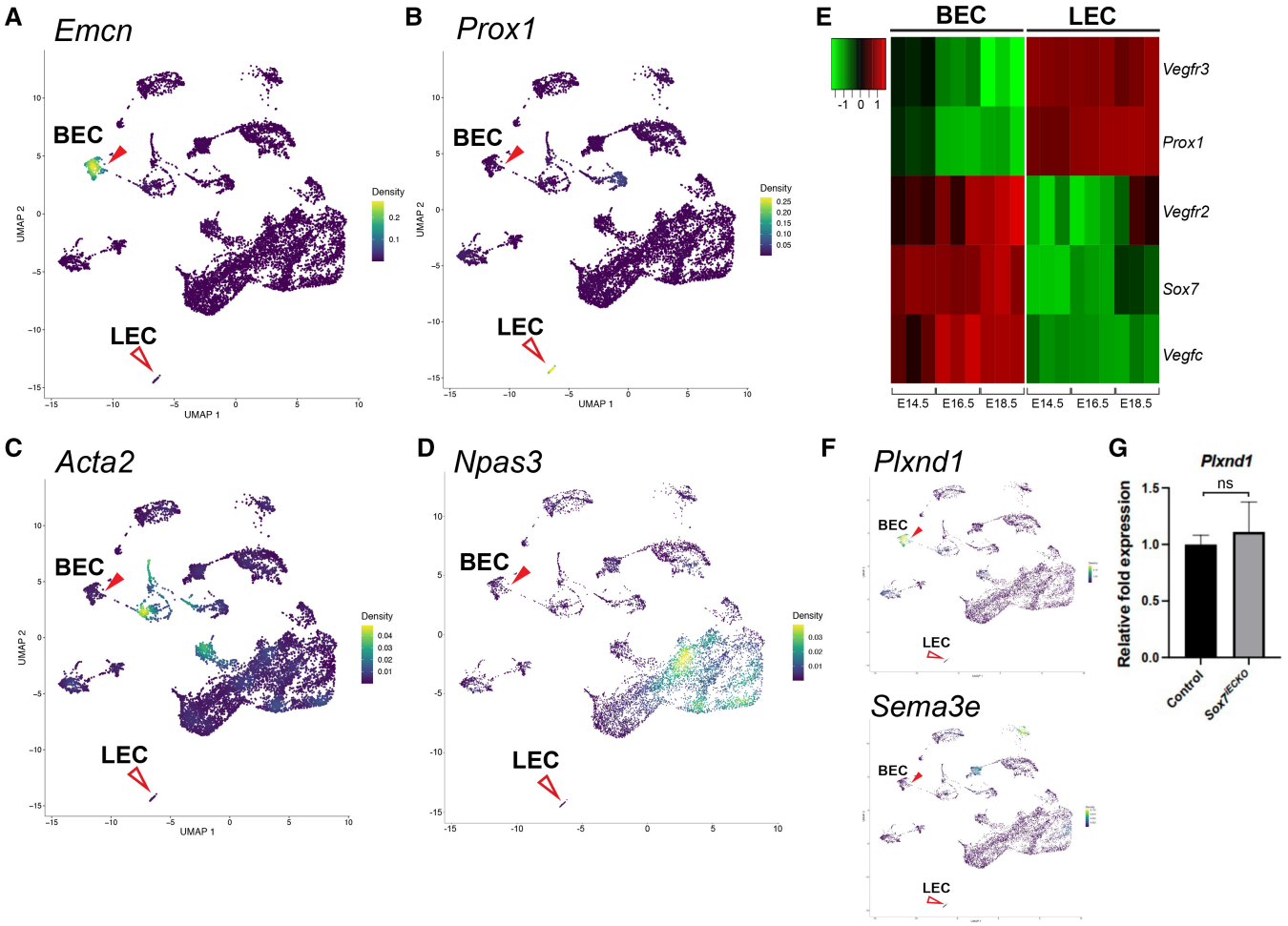

Figure EV4. Visualization of endothelial and non-endothelial cell specific markers shows that *Sox7* is highly expressed and limited to blood vascular endothelial cells.

A–D Nebulosa plots from single-nuclei RNA-Seq on E14.5 embryonic skins, showing markers for BECs (*Emcn*), LECs (*Prox1*), SMCs (*Acta2*), and neuronal cells (*Npas3*).

E Microarray analysis of dermal BEC and LEC populations sorted from different embryonic stages reveals that *Sox7* and *Vegfc* mRNA are preferentially expressed by BECs. BECs, blood endothelial cells; LECs, lymphatic endothelial cells; SMC, smooth muscle cells.

F Expression profiles of *Sema3e* and *Plxnd1* in BECs and LECs during development. Single Cell RNA sequencing reveals that *Plxnd1*, but not *Sema3e*, is expressed in BECs at E14.5. (A–D, F) BECs (red arrowheads), LECs (empty red arrowheads).

G qPCR analysis shows that the expression levels of *Plxnd1* are not affected in the $Sox7^{iECKO}$ endothelial cells. Expression is normalized to endothelial markers *Pecam* and *Cdh5*. Scored sibling controls, $n = 9$; $Sox7^{iECKO}$ mutants, $n = 7$. Mean ± SEM; *t*-test. ns = not significant.

Figure EV5. Downstream analyses of SOX7 ChIP-Seq.

A MSigDB pathway analysis on the top 2k peaks from human SOX7 HUVEC ChIP-Seq identified enrichment for genes within the VEGF/VEGFR gene set. These include *VEGFR1*, *VEGFR2*, *NRP1*, *NRP2*, *PDGFC*, *VEGFA*, and *VEGFC*.

B–F Binding profiles and motif analysis of mouse SOX7-V5 ChIP-Seq. (B) Image from Integrative Genome Viewer (IGV) illustrating ChIP-Seq tracks at E9.5 (pink) and E10.5 (light blue) mapped to the mouse reference genome GRCm38/mm10 (top track, dark blue). The height of a graph peak indicates the number of "positive bindings" on a given chromosomal location. Total number of binding events is indicated on the left under each track name. Top DNA-binding motifs in SOX7-V5 ChIP-Seq by MEME-ChIP in (C) E9.5 and (D) E10.5 embryos. The different height of the letters in the position weight matrix reveals the information content of each position (in bits), correlated by the degree of certainty of the nucleotide at a given position. (E) Spaced motif analysis (SPAMO) found a SOX7 primary motif among the topmost enriched motif next to a MEF2A/MEF2C-binding motif found in E9.5 SOX7-V5 ChIP-Seq, with 2,093 total occurrences. SOX7 is best gapped at 134 bp from the MEF2A/MEF2C-binding motif (24/2093). (F) ChIP–PCR on both bound fraction (left) and corresponding assumed unbound controls (right), as predicted by the peak location from SOX7-V5 ChIP-Seq.

G A total of 712 genes were found overlapping between E9.5 and E10.5 SOX7-V5 mouse ChIP-Seq.

H–J KEGG pathway analysis (H) and gene ontological analysis by DAVID bioinformatics database, showing the top biological processes (I) and molecular functions (J) associated with the 712 overlapping genes from the SOX7-V5 mouse ChIP-Seq.

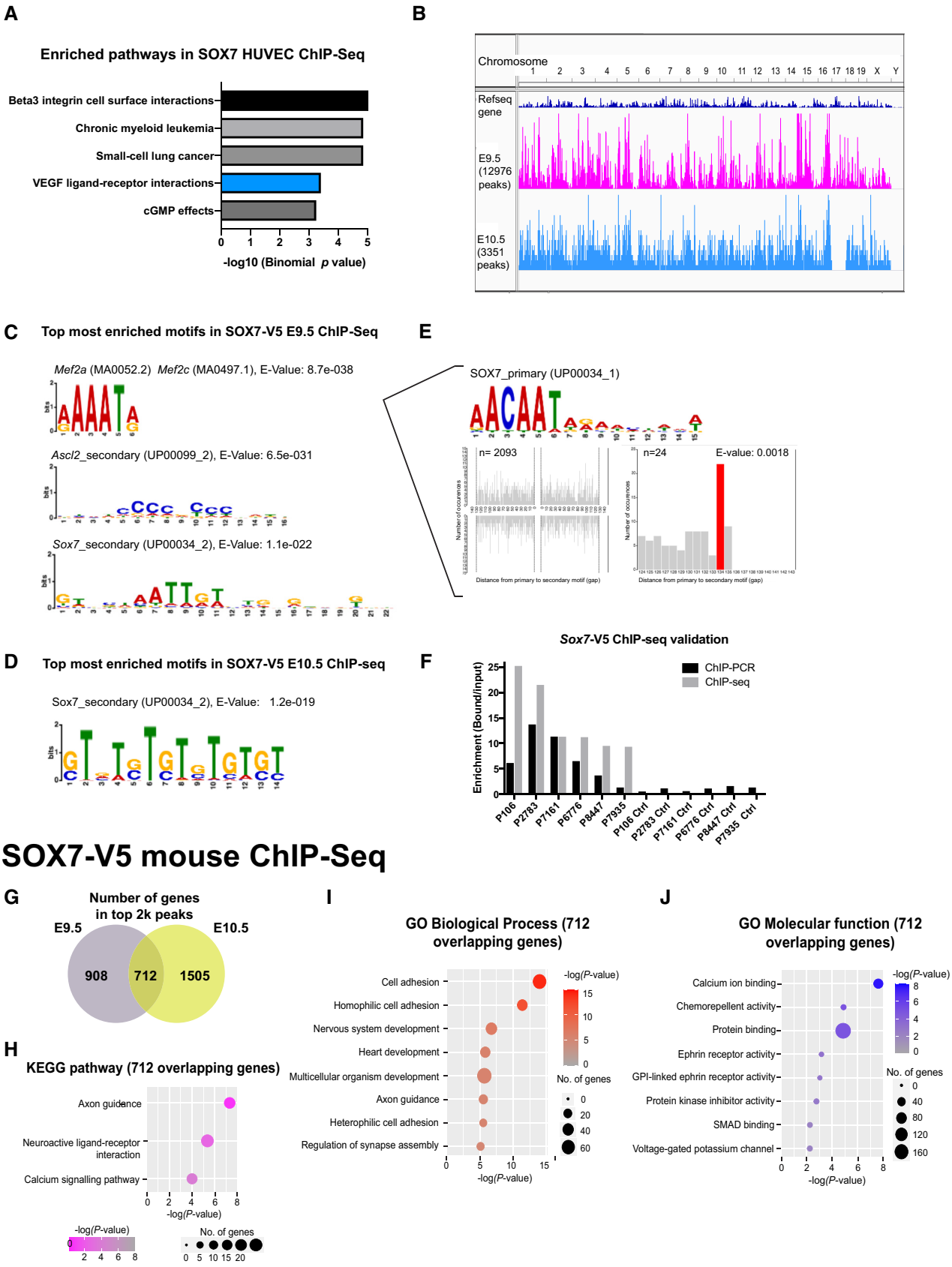
A
Enriched pathways in SOX7 HUVEC ChIP-Seq
Beta3 integrin cell surface interactions
Chronic myeloid leukemia
Small-cell lung cancer
VEGF ligand-receptor interactions
cGMP effects
0 1 2 3 4 5
-log10 (Binomial p value)
B
Chromosome
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 X Y
Refseq gene
E9.5 (12976 peaks)
E10.5 (3351 peaks)
C
Top most enriched motifs in SOX7-V5 E9.5 ChIP-Seq
Mef2a (MA0052.2) Mef2c (MA0497.1), E-Value: 8.7e-038
Ascl2_secondary (UP00099_2), E-Value: 6.5e-031
Sox7_secondary (UP00034_2), E-Value: 1.1e-022
E
SOX7_primary (UP00034_1)
n= 2093
n=24
E-value: 0.0018
Number of occurrences
Distance from primary to secondary motif (gap)
D
Top most enriched motifs in SOX7-V5 E10.5 ChIP-Seq
Sox7_secondary (UP00034_2), E-Value: 1.2e-019
F
Sox7-V5 ChIP-seq validation
ChIP-PCR
ChIP-seq
Enrichment (Bound/input)
P106
P2783
P7161
P6776
P8447
P7935
P106 Ctrl
P2783 Ctrl
P7161 Ctrl
P6776 Ctrl
P8447 Ctrl
P7935 Ctrl
SOX7-V5 mouse ChIP-Seq
G
Number of genes in top 2k peaks
E9.5
E10.5
908
712
1505
H
KEGG pathway (712 overlapping genes)
Axon guidance
Neuroactive ligand-receptor interaction
Calcium signalling pathway
-log(P-value)
No. of genes
I
GO Biological Process (712 overlapping genes)
Cell adhesion
Homophilic cell adhesion
Nervous system development
Heart development
Multicellular organism development
Axon guidance
Heterophilic cell adhesion
Regulation of synapse assembly
-log(P-value)
No. of genes
J
GO Molecular function (712 overlapping genes)
Calcium ion binding
Chemorepellent activity
Protein binding
Ephrin receptor activity
GPI-linked ephrin receptor activity
Protein kinase inhibitor activity
SMAD binding
Voltage-gated potassium channel
-log(P-value)
No. of genes

Figure EV5.