

## Supplemental Figure Legends

**Supplemental Figure 1. Prox1-expressing LEC progenitors are located on ISVs. (A-B)** EM images of the budding LECs shown in Figure 1A and 1C to better highlight the Prox1-expressing LECs labeled by numerous small black aggregates. **(C)** EM image of WT embryos to show LacZ negative cells without black aggregates. Transverse sections **(D and E)** and frontal section **(F)** of E10.5 *Tie2Cre;COUP-TFII<sup>+/-</sup>* embryos (n=3 of each type of section) showing that Prox1-expressing LEC progenitors (arrowheads) are located in the ISVs, which are labeled by COUP-TFII ( $\beta$ -gal reporter). **(F)** Prox1-expressing LEC progenitors bud off from ISV (arrow). Panel **E** is the high-power magnification of the white-boxed area in **A**. **(G-I)** Higher magnification EM images of the ISV shown in Figure 2D to better highlight the Prox1-expressing LECs labeled by numerous small black aggregates. CV, cardinal vein; ISV, intersomitic vessel. Scale bars: 2  $\mu$ m **(A and B)**, 6  $\mu$ m **(C)**, 100  $\mu$ m **(D-F)**, 6  $\mu$ m **(G and H)**, 2  $\mu$ m **(I)**.

**Supplemental Figure 2. *Plexin<sup>-/-</sup>* embryos have more LECs. At E10.5, the number of LECs is higher in *Plexin<sup>-/-</sup>* embryos (B) when compared with WT littermates (A).** Prox1<sup>+</sup> cells are detected inside the mispatterned and excessively branched intersomitic vessels in *Plexin<sup>-/-</sup>* embryos (arrowheads). **(C)** Compared with wild-type littermates, the total number of Prox1<sup>+</sup> PECAM1<sup>+</sup> ECs was increased in E11.0 *Plexin<sup>-/-</sup>* embryos (n = 3 for each genotype, \*P < 0.05). Scale bars: 100  $\mu$ m **(A and B)**.

**Supplemental Figure 3. Podoplanin provides the first molecular distinction between LEC progenitors and differentiated LECs.** Podoplanin expression is turned on once Prox1-expressing LEC progenitors fully exit the CV in both the posterior (**A and B**) and anterior regions (**C and D**) of the embryo at E11.5. Prox1-expressing LECs organize into a lymph sac-like structure at approximately E11.5 (**C and D**). Panels **B** and **D** are the high-power magnifications of the white boxed areas in **A** and **C**, respectively. Scale bars: 100  $\mu\text{m}$  (**A and C**), 20  $\mu\text{m}$  (**B and D**).

**Supplemental Figure 4. The number of LECs is decreased in Prox1 heterozygotes animals.** WT embryos (**A**) have more Prox1<sup>+</sup> cells inside and outside the CV than *Prox1*<sup>+/*GFP*Cre</sup> heterozygous embryos (**B**). Podoplanin expression marks Prox1-expressing cells outside the CV. The level of podoplanin expression in Prox1-expressing LECs outside the CV is lower in Prox1-heterozygous embryos (**B**). In addition, the level of Prox1 expression is lower in Prox1-heterozygous embryos in and outside the CV. Scale bars: 100  $\mu\text{m}$  (**A and B**).

**Video 1. Prox1-expressing LEC progenitors are located inside the ISVs.** Frontal section of an E11.0 embryo showed that Prox1-expressing LEC progenitors (red) are located inside the ISVs and are negative for podoplanin (green), which is expressed as soon as the cells bud off.

Figure S1

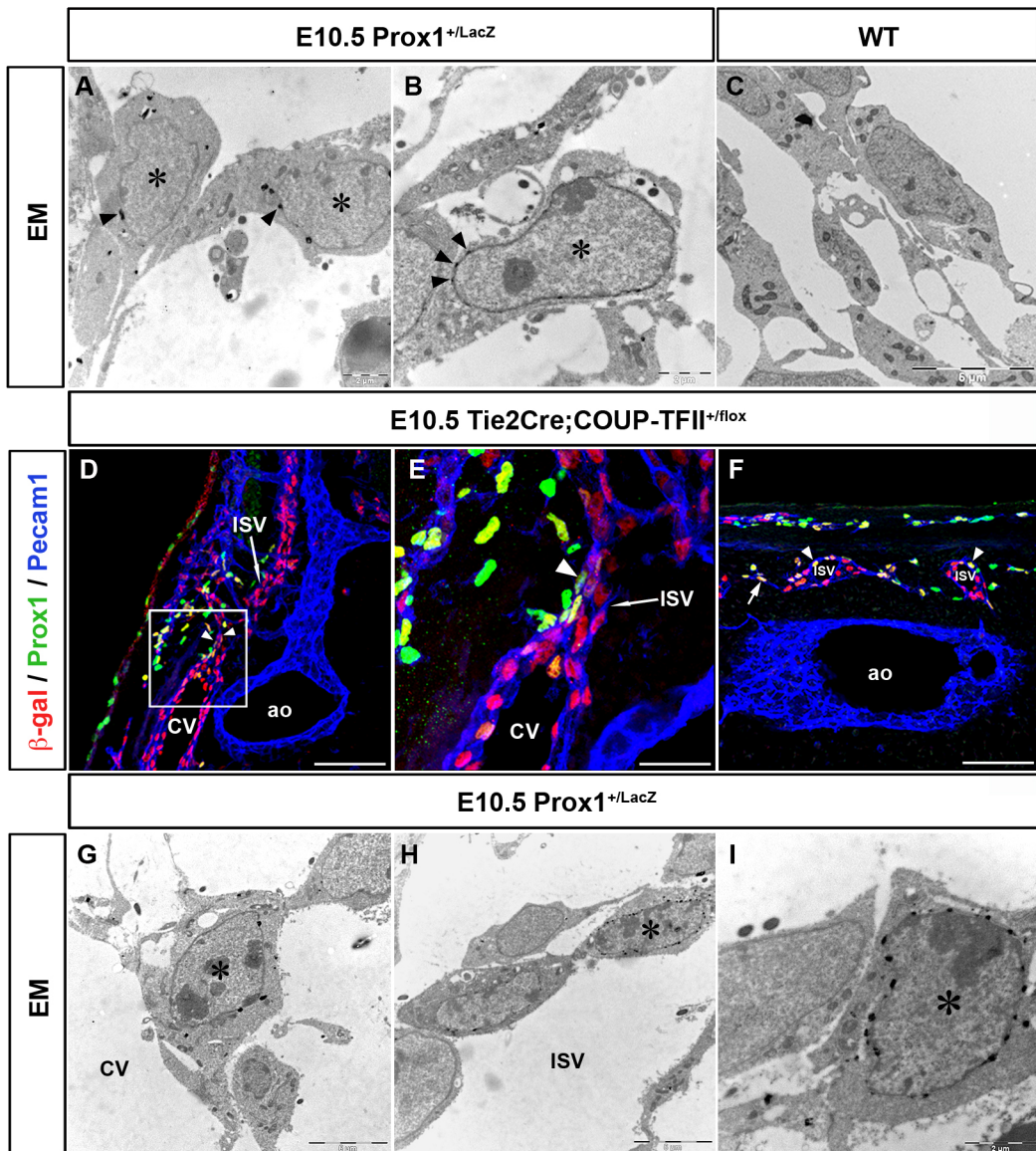


Figure S2

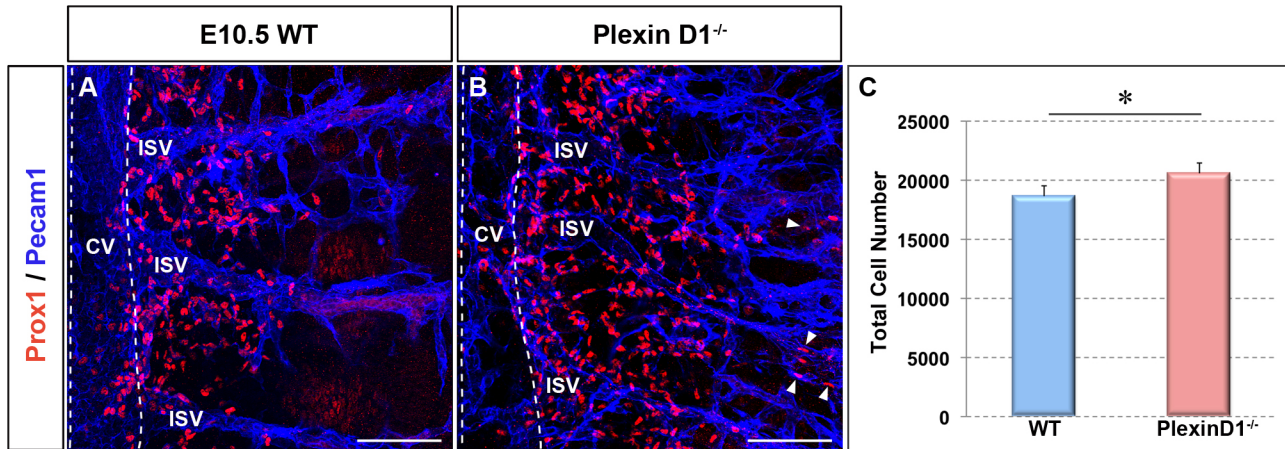


Figure S3

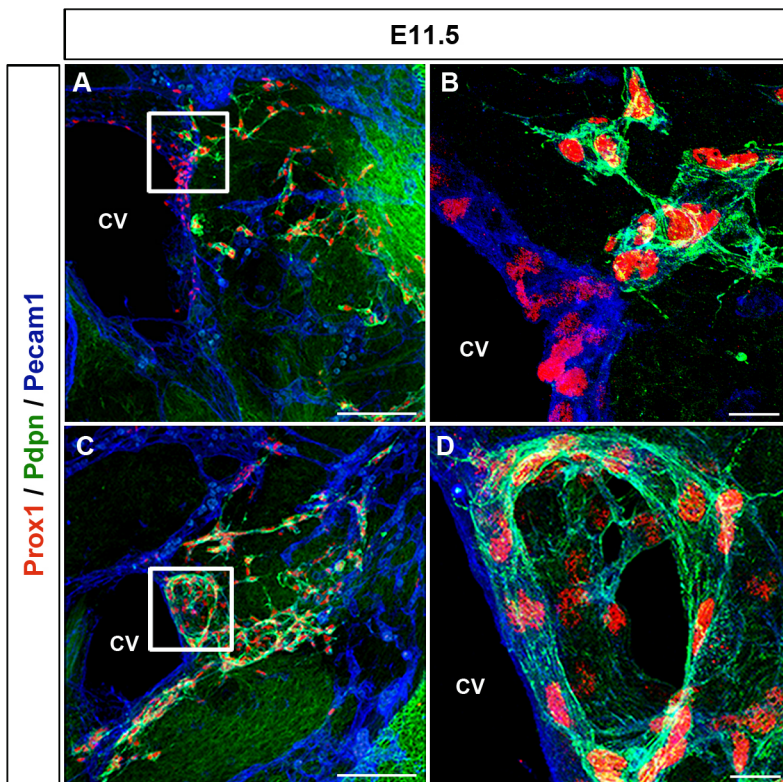


Figure S4

