

Expanded View Figures

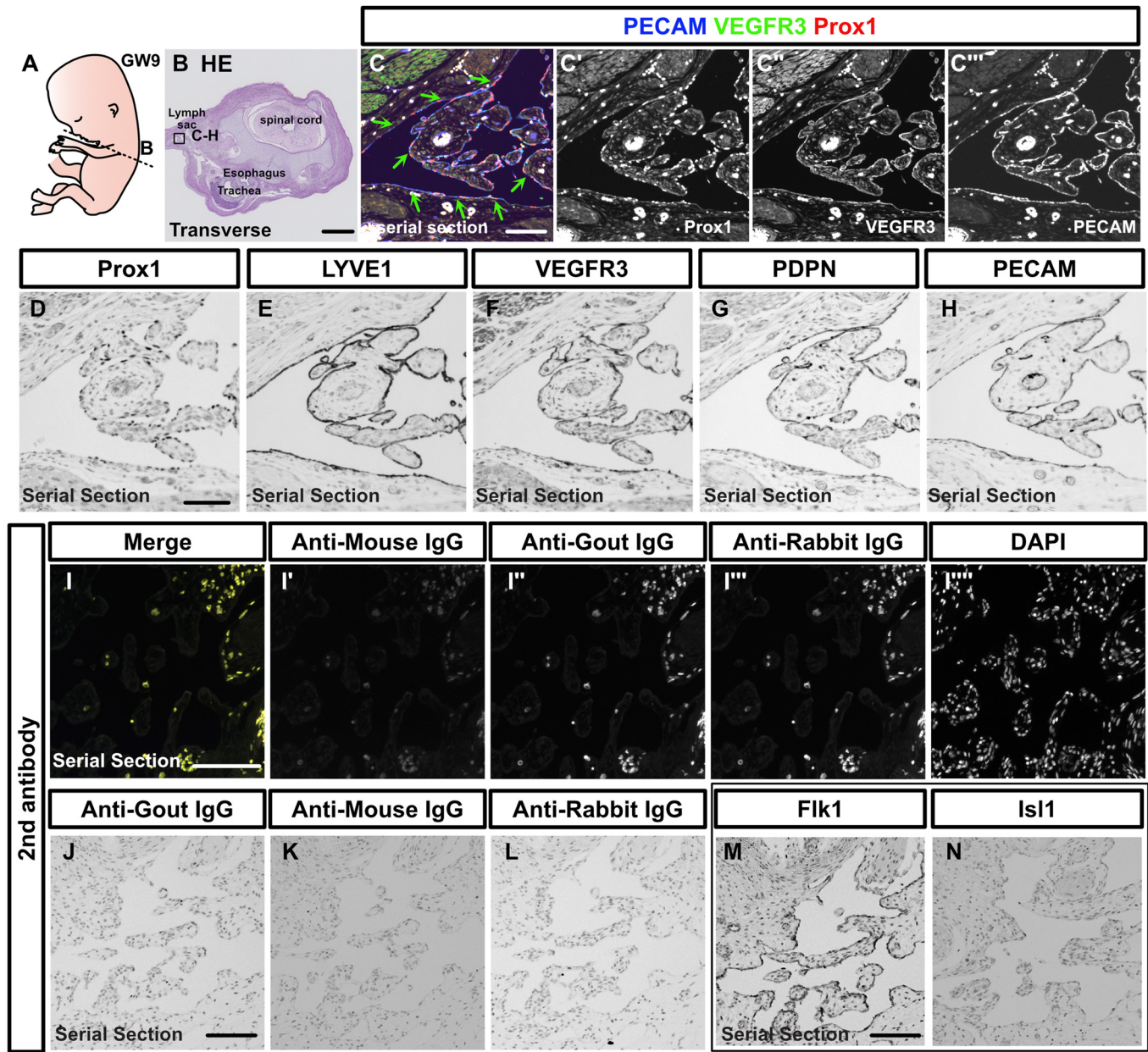


Figure EV1. Multiple lymphatic markers are expressed in fetal lymph sacs.

(A-H) Schema showing the positions of the sections in a GW9 fetus (A), and immunostaining of transverse sections (B-H); (C-C''') Fluorescent immunostaining of PECAM, Prox1, and VEGFR3; These markers were expressed in lymph sacs (green arrows). (D-H) Immunostaining of Prox1, LYVE1, VEGFR3, PDPN, and PECAM using the enzyme-antibody method, with color development by DAB. (I-L) Imaging of the secondary antibody-only staining. (M, N) Immunostaining of Flk1, and Isl1 using the enzyme-antibody method, with color development by DAB. Scale bars, 1 mm (B) or 100 μm (C, D, I, J, M).

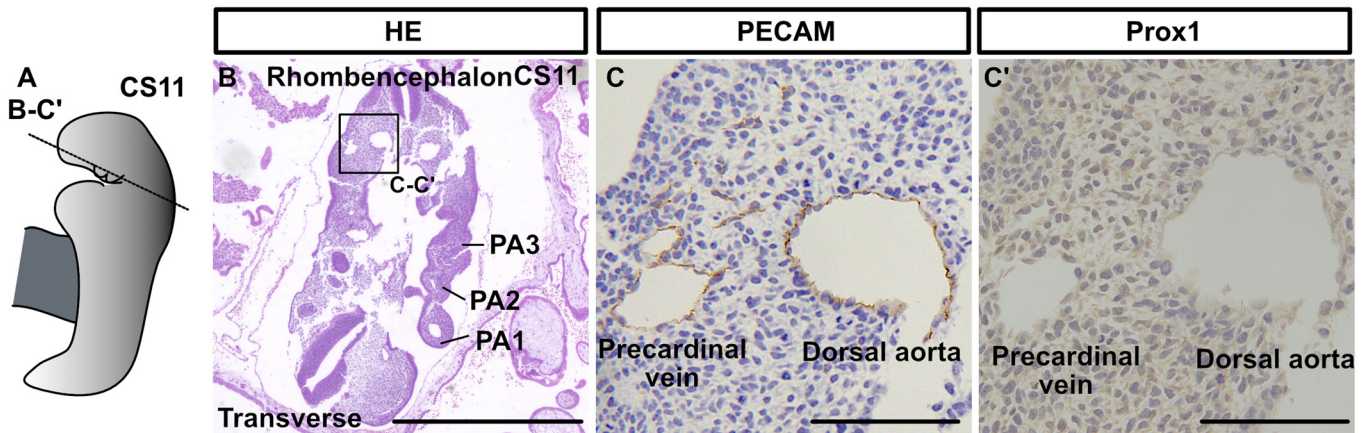


Figure EV2. Prox1 expression is not observed in the precardial vein of the CS11 embryo.

(A-C') Immunostaining of transverse sections of a CS11 embryo with the indicated antibodies and schema showing a CS11 embryo ($n = 1$). Scale bars, 1 mm (B) or 100 μm (C, C').

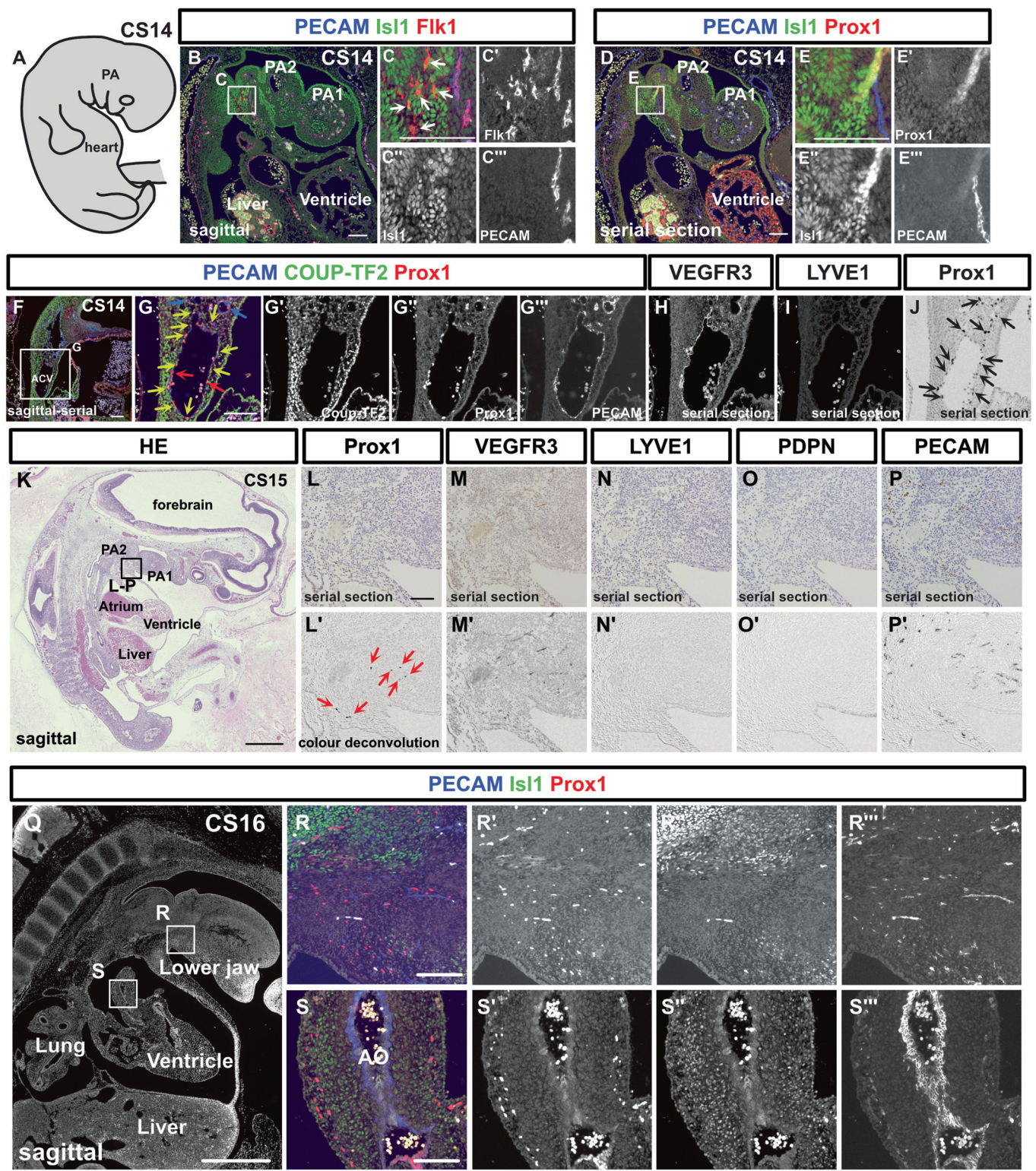
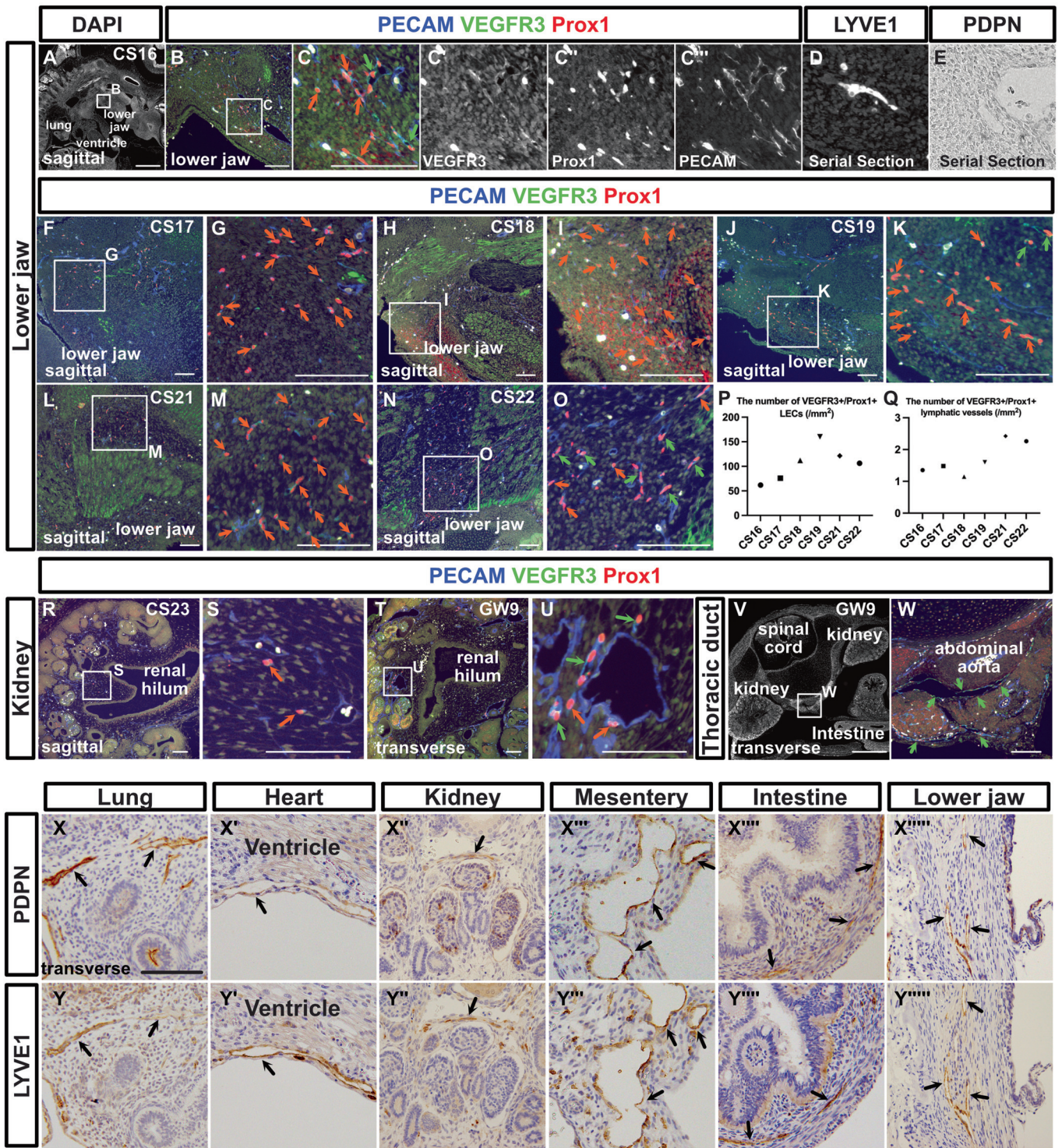



Figure EV3. LECs bud from the cardinal veins and form luminal structures.

(A–J) Immunostaining of sagittal sections of a CS14 embryo with the indicated antibodies and schema showing the CS14 embryo. (B–C'') Cardiovascular progenitor cells, which were composed of $FLK1^{+}/Isl1^{+}/PECAM^{-}$ cells, were observed in the second pharyngeal arch (white arrows). (F–G'') $PECAM^{+}/Prox1^{+}/Coup-TF2^{+}$ cells (yellow arrows: Frequency of $Coup-TF2^{+}$ cells among $PECAM^{+}/Prox1^{+}$ cells=44.1%, $n=1$ [the ACVs could not be identified in another embryo]), and $PECAM^{+}/Prox1^{+}/Coup-TF2^{-}$ cells (red arrows) were observed in and around the ACVs. (K–P') HE staining of a sagittal section of a CS15 embryo (K) and immunostaining of sagittal sections of the same CS15 embryo with the indicated antibodies (L–P'); At this stage, scattered $Prox1^{+}$ cells were observed in the pharyngeal arch (red arrows). (Q–S'') Immunostaining of sagittal sections of a CS16 embryo with the indicated antibodies. PA1 first pharyngeal arch, PA2 second pharyngeal arch, ACV anterior cardinal vein. scale bars, 1 mm (K) or 100 μ m (B–G, L).



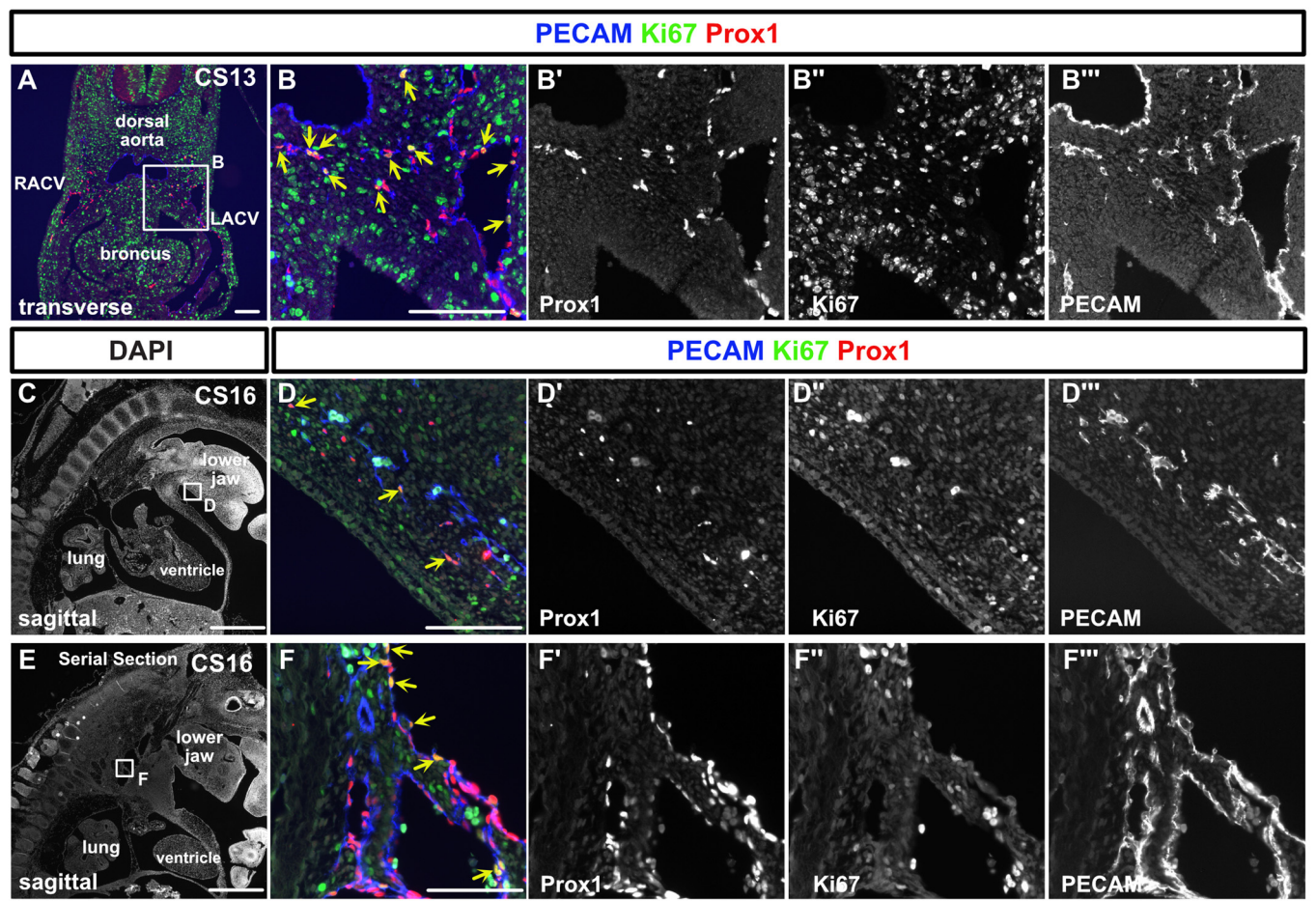


Figure EV5. Proliferation activity of lymphatic endothelial cells at CS13 and CS16.

(A–F'') Immunostaining of transverse and sagittal sections using the indicated antibodies in a CS13 and 16 embryos. We detected Prox1⁺/PECAM⁺/Ki67⁺ LECs (indicated by yellow arrows) as well as Prox1⁺/PECAM⁺/Ki67[−] LECs. At CS13, within CV, 19.6% of LECs were Ki67 positive, while outside the CV, the percentage was 43.6%. At CS16, 22.7% of LECs in the lymph sacs were Ki67 positive, and in the lower jaw, the positivity rate was 31% (based on an average of two sections at *n* = 1). CV cardinal vein, RACV right anterior cardinal vein, LACV left anterior cardinal vein. scale bar, 1 mm (C, E), 100 μm (A, B, D, F).