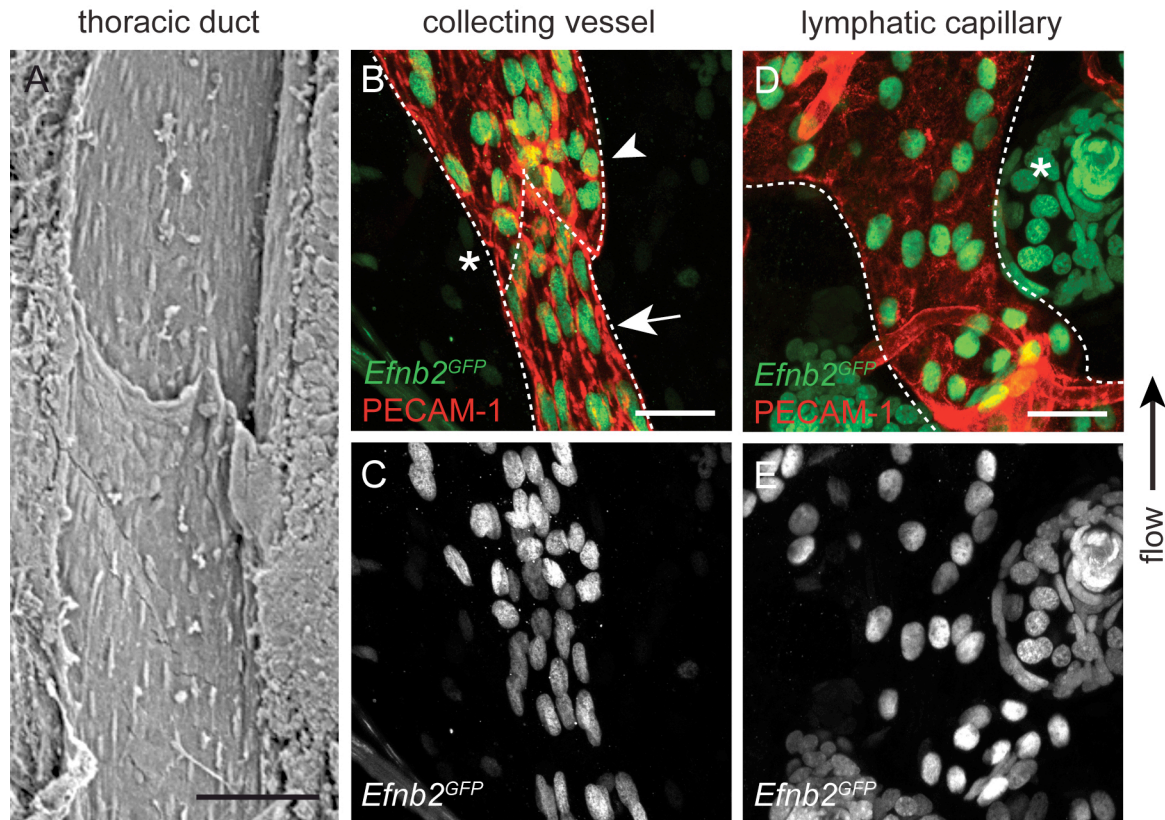


## Supplemental Data

Bazigou, Lyons et al. "Venous valve formation and maintenance in mice are controlled by genes regulating lymphangiogenesis"

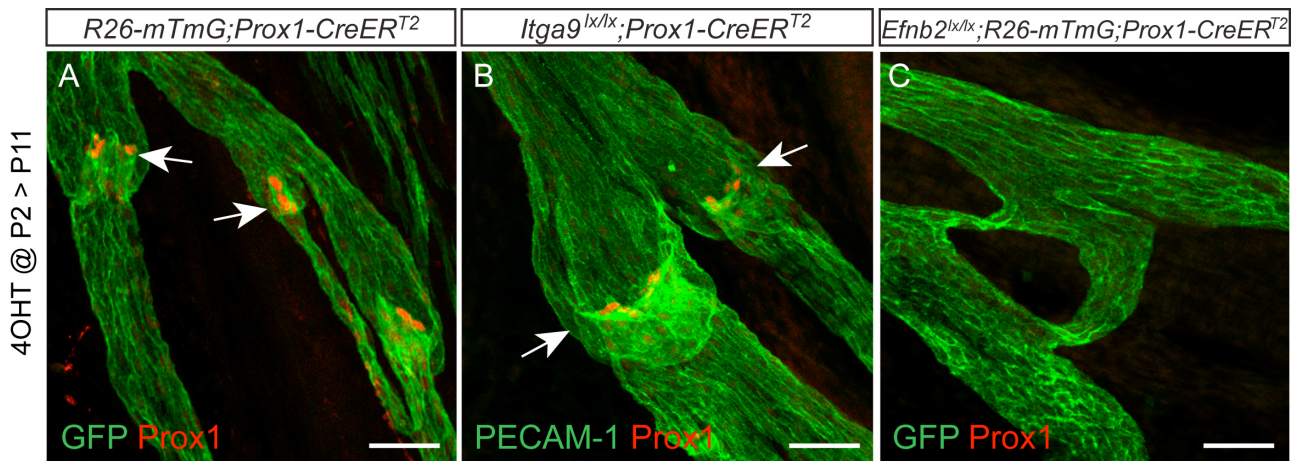


### Supplemental Figure 1. Flow-induced endothelial cell alignment in lymphatic vessels.

(A) SEM image of mouse thoracic duct at postnatal day P7 showing the exposed luminal side of the vessel and a valve.

(B-D) Immunofluorescence of dermal and collecting lymphatic vessels (B, C) and lymphatic capillaries (D, E) in adult *Efnb2<sup>GFP</sup>* reporter mouse ear skin (green nuclei), co-stained with antibodies against PECAM-1 (red). Dashed lines depict vessel (and valve, in B) borders. \* show the position of a luminal valve in (B) and *Efnb2* expressing hair follicle keratinocytes in (D). Direction of lymph flow is shown on the right. Note the alignment of endothelial cells in the direction of flow upstream of the valve (arrow in B) and rounded endothelial cells downstream of the valve (arrowhead in B).

Scale bars: A: 25 $\mu$ m, B, D: 20 $\mu$ m.



**Supplemental Figure 2. Deletion of *Itga9* and *Efnb2* in mesenteric lymphatic vessels after the formation of luminal valves.**

Immunofluorescence of mesenteric lymphatic vessels of control *R26-mTmG;Prox1-CreER<sup>T2</sup>* (A; n=3 mice), *Itga9<sup>lx/lx</sup>;Prox1-CreER<sup>T2</sup>* (B; n=3 mice) and *Efnb2<sup>lx/lx</sup>;R26-mTmG;Prox1-CreER<sup>T2</sup>* (C; n=4 mice) at P11, following 4-OHT administration at P2, using antibodies against Prox-1 (red) and PECAM-1 (green in B). GFP (A, C) shows efficient Cre-mediated recombination in lymphatic endothelial cells in wild type and *Efnb2* conditional mutants. Arrows point to valves (A, B), which are absent in *Efnb2* mutants (C).

Scale bars: 25µm.