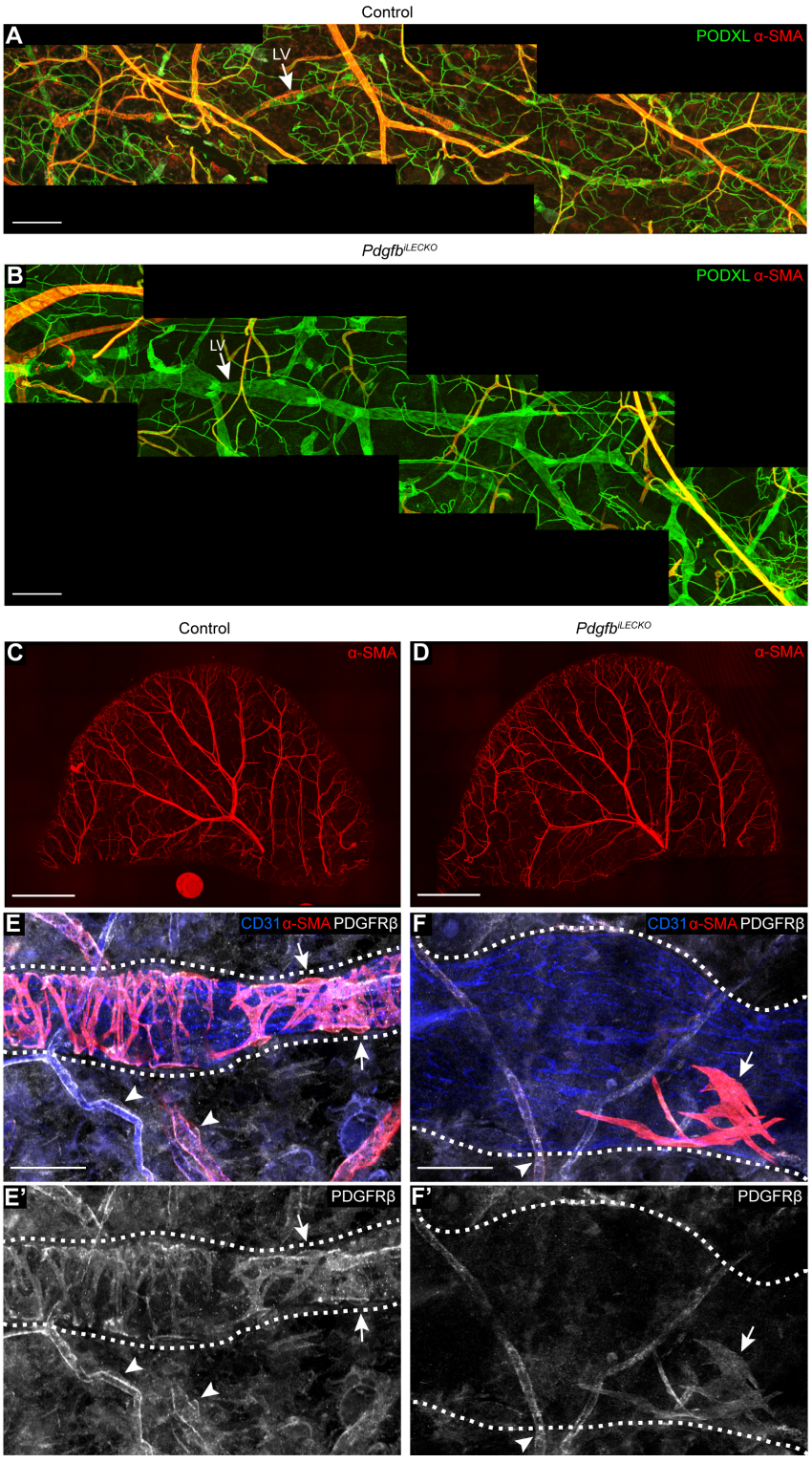
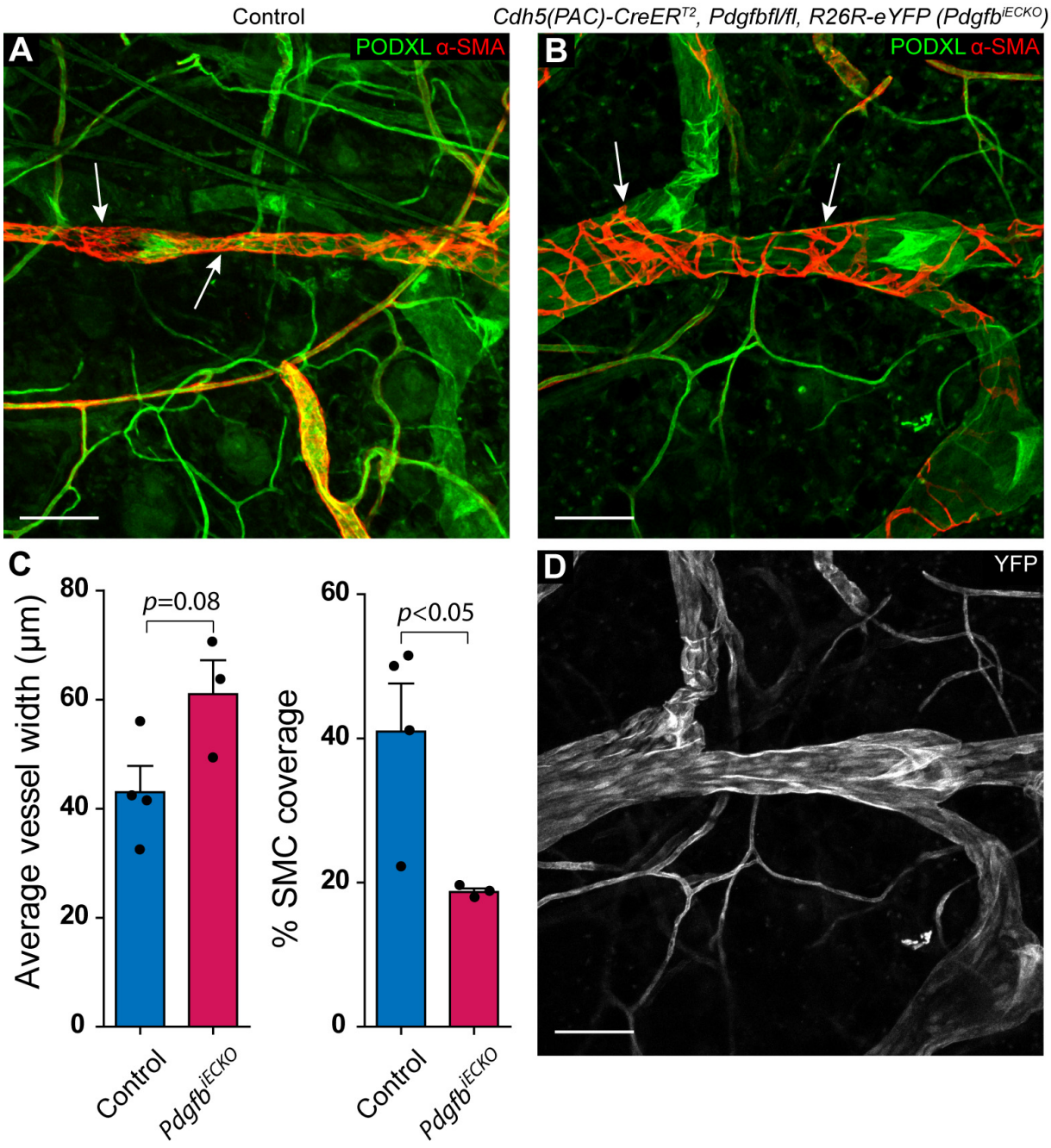
Supplementary Figures

Supplementary figure 1.



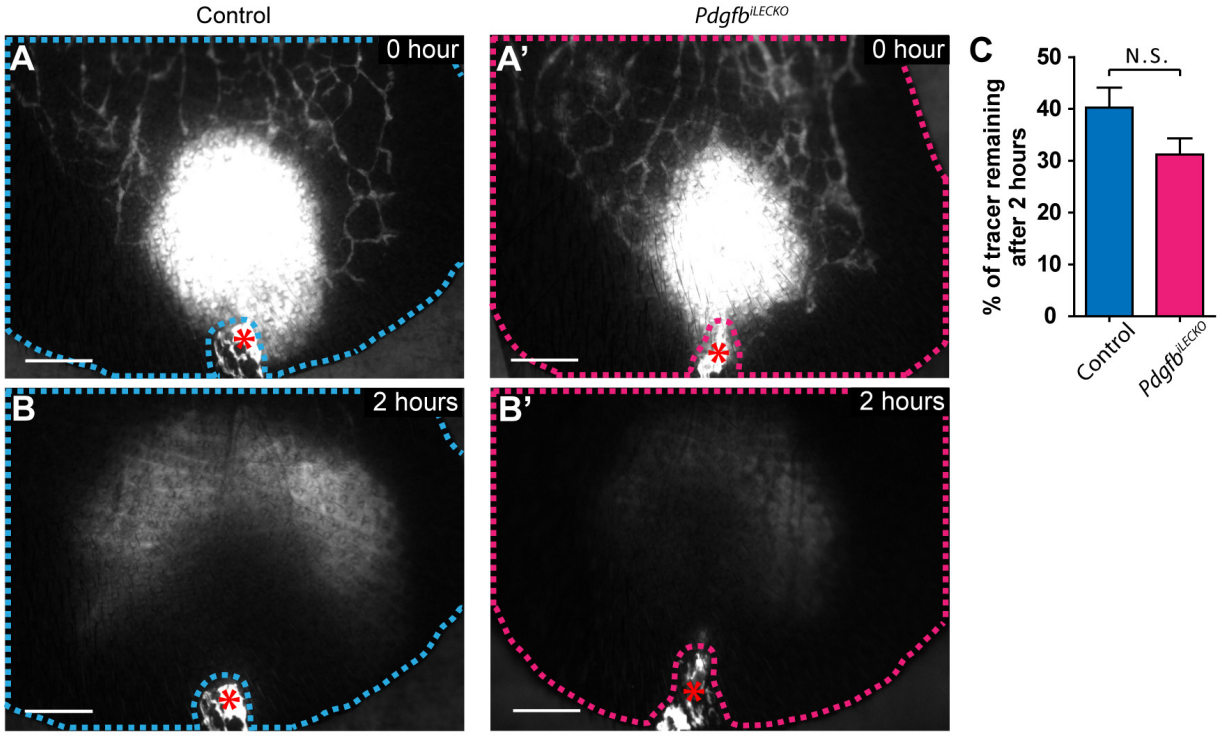
Supplementary Figure 1. ***LEC-specific deletion of Pdgfb alters collecting vessel morphology but not blood vessel patterning.*** (A,B,C,D) Whole-mount immunofluorescence staining of dorsal mouse ear skin from *Pdgfb*^{iLECKO} and Ctrl mice at four months age with antibodies against Podocalyxin (A,B, green), α-SMA (A,B,C,D, red). Collecting vessels (LV, arrow) from the control (A) and the *Pdgfb*^{iLECKO} mice (B) are traced respectively. (C,D) Tiled confocal images indicate normal blood vessel morphology of the control mice (C) and the *Pdgfb*^{iLECKO} mice (D). (E,E',F,F') Immuno-stained dorsal ear skin with antibodies against CD31 (blue), α-SMA (red), PDGFRβ (grey) showing collecting vessels (dashed lines) of control mice (E,E') and *Pdgfb*^{iLECKO} mice (F,F'). Perivascular SMCs of collecting vessels are identified by expression of both α-SMA (red, arrow) and PDGFRβ (grey). PDGFRβ+/α-SMA- cells (arrowheads) are only found in blood vessels but not lymphatic vessels. Scale bars, A,B, 200 µm; C,D, 2 mm; E,F, 50 µm.

Supplementary figure 2.



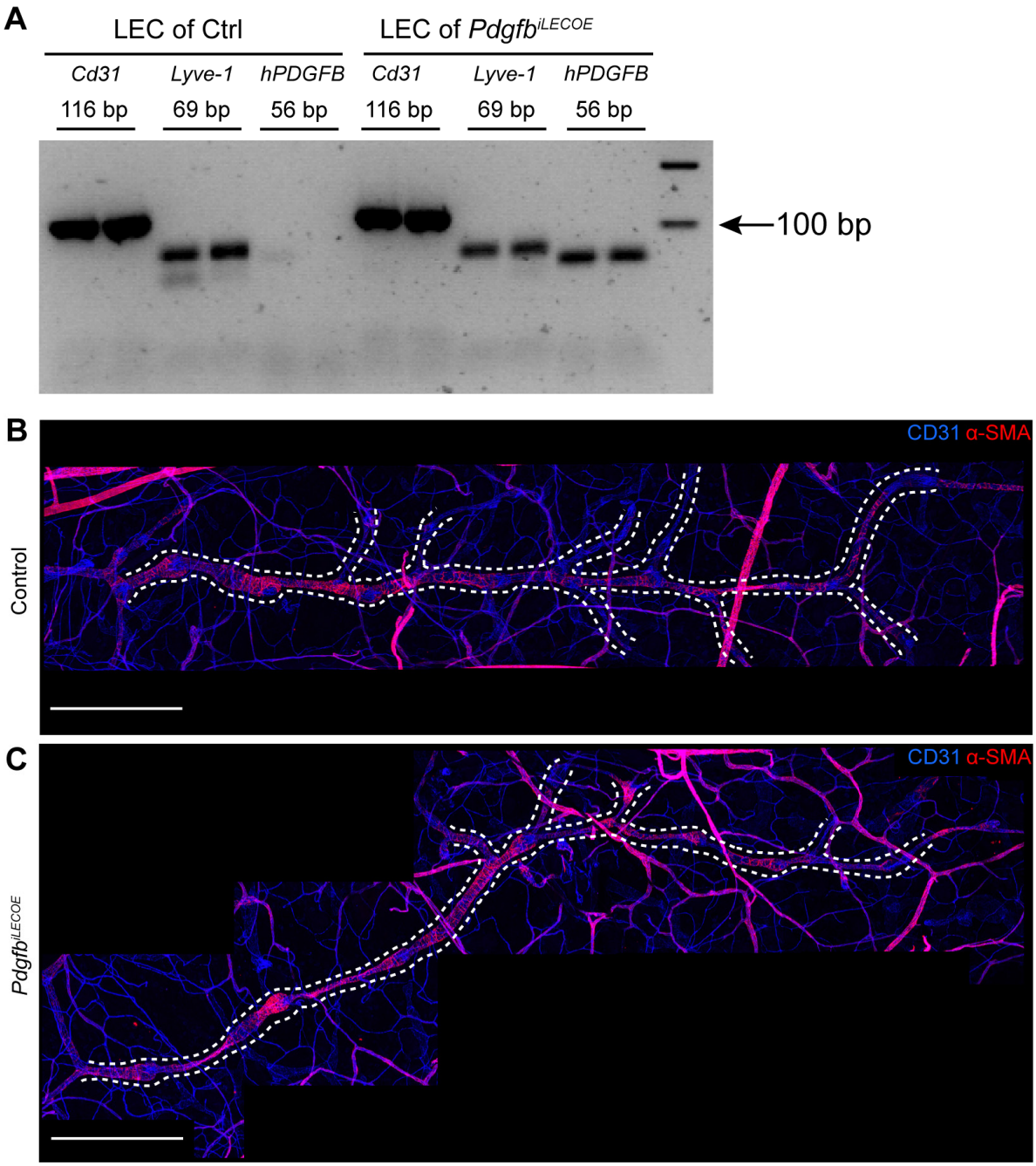
Supplementary Figure 2. ***Endothelial cell-specific deletion of Pdgfb alters collecting vessel morphology.*** (A,B) Whole-mount immunofluorescence staining of dorsal ear skin of mice at 4 months age with antibodies against podocalyxin (green), and α-SMA (red). Collecting vessels in control ears are covered by SMCs (A, arrows) forming concentric rings on the endothelium. In *Pdgfb*^{iECKO} mouse ears, SMCs sparsely cover collecting vessels (B, arrows) and the vessels are dilated compared to the controls (A). (C) Quantification of vessel width and SMCs coverage of collecting vessels comparing the controls (n=4) and mutants (n=4). Error bars indicate mean ± SEM. *p* value is calculated by student's t-test.(D) Vascular specific deletion of *Pdgfb* indicated by YFP expression is shown in both blood and lymphatic vasculature. Scale bars, A,B,D, 100 µm.

Supplementary figure 3.



Supplementary Figure 3. ***Loss of collecting vessel SMC coverage does not affect lymphatic drainage in the ear skin.*** (A,A',B,B') Ears at the time of 0 hour (A,A') or 2 hours following (B,B') subcutaneous injection with Tritc-Dextran (white) and imaged by fluorescent microscopy through the intact skin of control ears (n=12) versus *Pdgfb*^{iLECKO} ears (n=9) over time. Total fluorescence intensity within the area surrounding the injection site are measured (dashed line) at 0 hour (A,A') and 2 hours (B,B') post-injection, and plotted (C) as percentage remaining tracer to indicate the draining capacity. Error bars indicate mean ± SEM. Scale bars, A,A',B,B', 1mm.

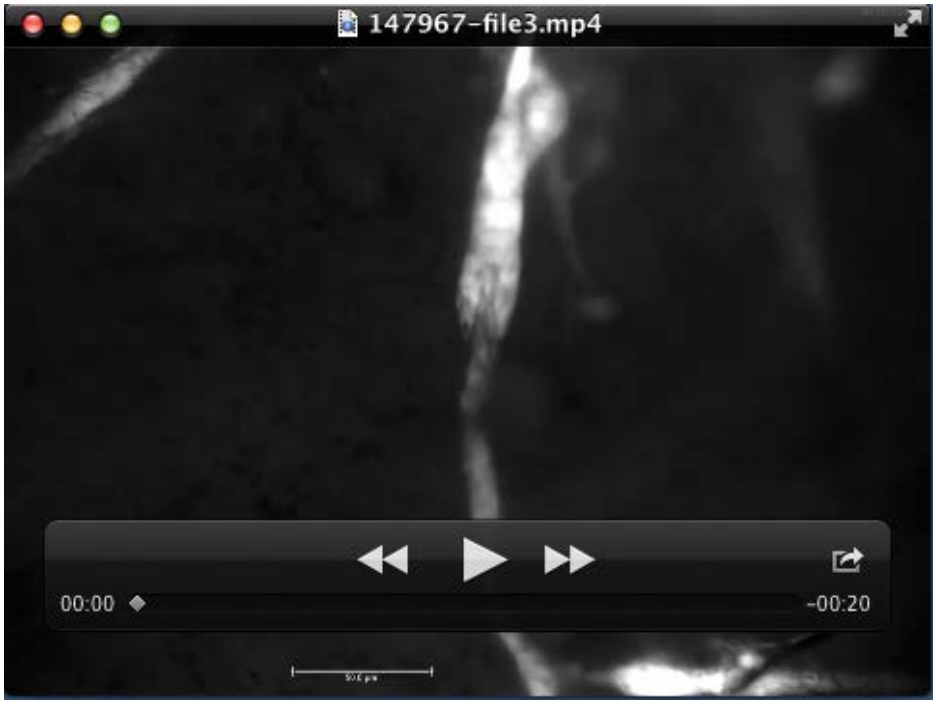
Supplementary figure 4.



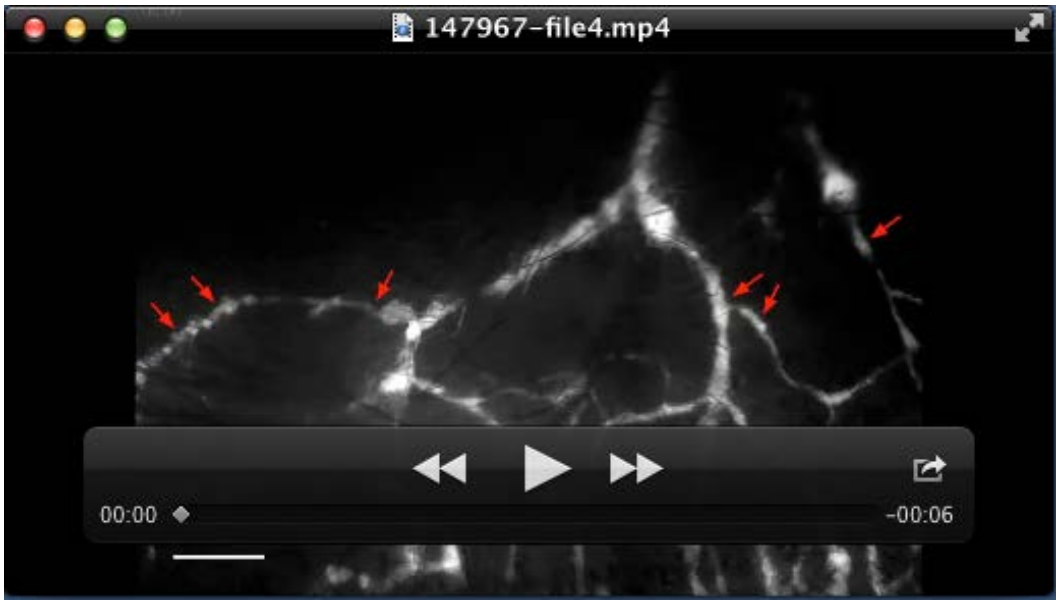
Supplementary Figure 4. ***Postnatal LEC-specific overexpression of PDGFB increases SMC coverage of collecting vessels but not capillaries***

(A) Electrophoresis of amplified cDNA of *cd31* (116 bp), *Lyve1* (69 bp) and human *PDGFB* (56 bp) by PCR reactions. Isolated mRNA from FACS sorted capillary LECs (CD31+;Podoplanin+;LYVE1+) from control mice and *Pdgfb*^{iLECOE} mice were reverse transcribed into cDNA, pre-amplified, followed by PCR and products run in an agarose gel. (B,C) Tiled images of complete collecting vessels (dashed line) indicate the morphology of collecting vessels of *Pdgfb*^{iLECOE} mice (C) compared to control mice (B). Scale bars, B,C, 500 µm.

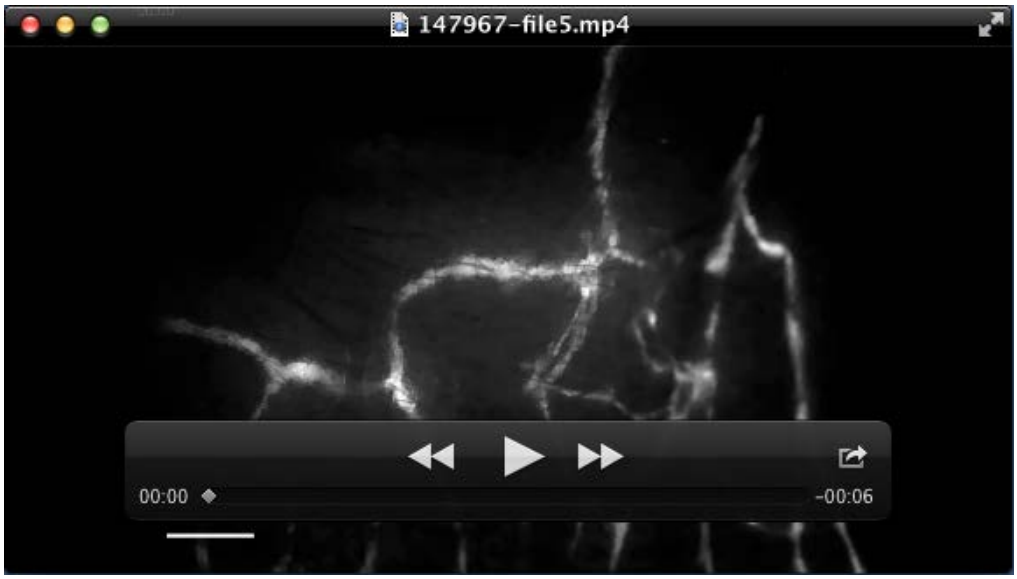
Supplementary Movies



Supplementary Movie 1. ***Pulsatile contraction of a dermal lymphatic vessel of the ear***. A wild-type mouse was anaesthetized and subcutaneously injected with Tritc Dextran, followed by time-lapse imaging utilizing a 25x/1.0 water immersion objective with images acquired at a rate of 5 frames/second.

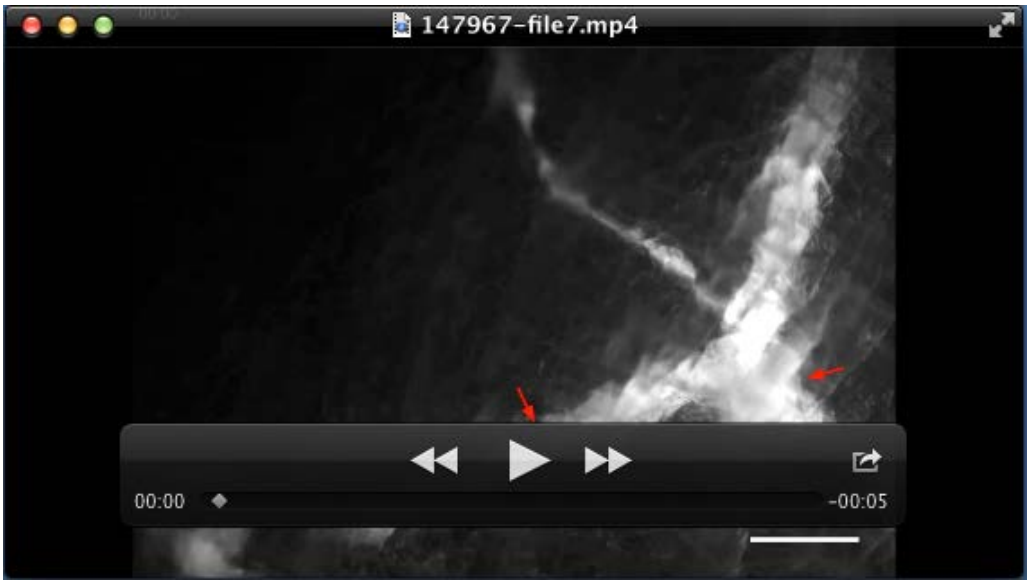


Supplementary Movie 2. ***Dermal lymphatic vessels show pulsatile contraction- overview of control*****.** A wild-type mouse was anaesthetized and the ear was subcutaneously injected with Tritc Dextran, followed by time-lapse imaging utilizing a Leica M205FA microscope with a PLANAPO 1.0x objective. Arrows indicate contracting sites within the lymphatic vasculature. Scale bar, 500 µm.



Supplementary Movie 3. ***Dermal lymphatic vessel contraction is lacking in the absence of SMCs coverage of collecting vessels - overview of Pdgfb^{iLECKO}.*** A *Pdgfb^{iLECKO}* mouse was anaesthetized and the ear was subcutaneously injected with Tritc Dextran, followed by time-lapse imaging utilizing a Leica M205FA microscope with a PLANAPO 1.0x objective. Contraction was not observed on the lymphatic vessels. Scale bar, 500 µm.

Supplementary Movie 4. ***Dermal lymphatic vessel contraction is associated with SMCs coverage (Control).*** A control mouse was anaesthetized and the ear was subcutaneously injected with Tritc Dextran, followed by time-lapse imaging for 5 minutes utilizing a Leica M205FA microscope with a PLANAPO 1.0x objective. A contraction site on the lymphatic vessel is indicated by the arrow. Scale bar, 200 µm.



Supplementary Movie 5. ***Dermal lymphatic vessel contraction is associated with SMCs coverage in the Pdgfb^{iLECKO} mouse (mutant).*** An anaesthetized *Pdgfb^{iLECKO}* mouse following subcutaneous ear injection with Tritc Dextran, and time-lapse imaged utilizing a Leica M205FA microscope with a PLANAPO 1.0x objective. Arrows indicate contracting sites within the lymphatic vasculature. Scale bar, 200 µm.