

**Title: Supplementary Movie 1: *In vivo* imaging of trunk perfusion in WT embryo.**

**Description:** Imaging of red blood cell perfusion of trunk aISV and vISV in WT embryo at 2dpf. Anterior to the left, dorsal is up. 25 frames/sec.

**Title: Supplementary Movie 2: *In vivo* imaging of trunk perfusion in *plgf<sup>musc</sup>* embryo.**

**Description:** Imaging of red blood cell perfusion of trunk aISV and vISV in *plgf<sup>musc</sup>* embryo at 2dpf. Anterior to the right, dorsal is up. 25 frames/sec.

**Title: Supplementary Movie 3: *In vivo* imaging of aISV perfusion in *plgf<sup>musc</sup>* embryo.**

**Description:** Imaging of red blood cell perfusion in aISV of *plgf<sup>musc</sup>; Tg(kdrl:hsa.HRAS-mcherry)<sup>s916</sup>* transgenic embryo at 2dpf. RBCs in transmitted light, endothelial cell membranes in red. Scale bar represents 10  $\mu\text{m}$ . aISV, arterial intersegmental vessel. Note that the aISV lumen is enlarged thereby allowing multiple erythrocytes to pass at the same time. Dorsal is up.

**Title: Supplementary Movie 4: *In vivo* imaging of aISV growth in WT embryo.**

**Description:** Time-lapse confocal imaging of a growing aISV in *Tg(fli1a:lifeactEGFP)<sup>mu240</sup>* transgenic line. Developmental stage and scale bar are indicated (bottom left and right corner, respectively). Scale bar represents 20  $\mu\text{m}$ . aISV, arterial intersegmental vessel.

**Title: Supplementary Movie 5: *In vivo* imaging of aISV growth in *plgf<sup>musc</sup>* embryo.**

**Description:** Time-lapse confocal imaging of a growing aISV in the *plgf<sup>musc</sup>; Tg(fli1a:lifeactEGFP)<sup>mu240</sup>* double transgenic line. Developmental stage and scale bar are indicated (bottom left and right corner, respectively). Scale bar represents 20  $\mu\text{m}$ . aISV, arterial intersegmental vessel.

**Title: Supplementary Movie 6: Endothelial shape change upon transfection with *Trion*.**

**Description:** Time-lapse confocal imaging of endothelial cell shape changes in cells transfected with *Trion-GFP* (green) and Lifeact-mScarlet for visualizing F-actin remodeling (in red). Cells were imaged for 17 hours (30 frames/hour). VE-Cadherin (in white) was visualized using a non-blocking alexa-647-labelled VE-Cadherin antibody. Movie framerate, 20 frames/second. Scale bar represents 20  $\mu\text{m}$ . EC, endothelial cell.

**Title: Supplementary Movie 7: Endothelial shape change upon control transfection.**

**Description:** Time-lapse confocal imaging of endothelial cell shape changes in cells transfected with control GFP construct (green), and Lifeact-mScarlet for visualizing F-actin remodeling (in red). Cells were imaged for 17 hours (30 frames/hour). VE-Cadherin (in white) was visualized using a non-blocking alexa-647-labelled VE-Cadherin antibody. Movie framerate, 20 frames/second. Scale bar represents 20  $\mu\text{m}$ . EC, endothelial cell.

**Title: Supplementary Movie 8: *In vivo* imaging of trunk perfusion in *plgf<sup>musc</sup>; flt1<sup>enh</sup>:Trion* embryo.**

**Description:** Imaging of red blood cell perfusion of trunk aISV and vISV in *plgf<sup>musc</sup>; flt1<sup>enh</sup>:Trion* embryo at 2dpf. Anterior to the right, dorsal is up. 25 frames/sec.