

Supplementary Movie Legends

Supplementary Movie 1. Upstream migration of arterial ECs in a venous ISV.

Time-lapse imaging of *Tg(flt4:mCitrine; flt1^{enh}:tdTomato; gata1a:dsRed)* in which venous ECs are labelled with mCitrine (*green*), arterial ECs are labelled with mCitrine and tdTomato, and erythrocytes are labelled with dsRed (*red*). *Orange arrows* depict first erythrocyte passing through venous ISVs. *Green arrow* depicts arterial ECs migrating upstream in a venous ISV. *Red arrow* points to arterial ISVs.

Supplementary Movie 2. Displacement of arterial ECs by venous ECs in a venous ISV. Time-lapse imaging of *Tg(dll4:GAL4; UAS:lifeactGFP; fli1a:lifeactCherry)* in which venous ECs express lifeactCherry (*magenta*) and arterial ECs express lifeactCherry and lifeactGFP (*green*). *Red arrow* points to an arterial EC migrating dorsally in a venous ISV.

Supplementary Movie 3. Migration and proliferation of venous ECs in a venous ISV. Time-lapse imaging and cell tracing in *Tg(kdl:H2B-Cherry)*, in which all ECs express nuclear-localized mCherry (*grey*).

Supplementary Movie 4. Arterial ECs do not migrate in an arterial ISV. Time-lapse imaging of *Tg(dll4:GAL4; UAS:lifeactGFP; fli1a:lifeactCherry)* in which venous ECs express lifeactCherry (*magenta*) and arterial ECs express lifeactCherry and lifeactGFP (*green*). *Red arrow* points to an arterial EC in an arterial ISV.

Supplementary Movie 5. Flow-induced changes in the planar polarization and migration of HUVECs in the microfluidic model. Positions of MTOC and nuclei in individual HUVECs were monitored for 300 min after their exposure to flow with a shear stress $\tau = 7.2 \text{ dyn/cm}^2$. The direction of flow is from left to right. *Left panel* shows fluorescence images (with an inverted grayscale) of a fragment of a microchannel with ECs expressing GFP- α -tubulin. The arrows show the direction of cell polarization and their color indicates the polarization angle, β , with red corresponding to 90° (polarization against the flow) and blue corresponding to $\beta = -90^\circ$ (polarization along the flow). *Right panel* shows phase contrast images of the same area. The arrows show the direction of cell migration and their color represents the angle between the directions of migration and flow, β^* , with red corresponding to 90° (migration against the flow) and blue corresponding to $\beta^* = -90^\circ$ (migration along the flow). The dials in the top left corners show color-coded polarization angle, β (*left panel*), and migration angle, β^* (*right panel*) averaged over all cells in the field of view. Images were acquired every 10 min. Frame rate is 3 per sec (1 sec = 30 min). Scale bar is 30 μm .

Supplementary Movie 6. Migration of HUVECs exposed to low and high shear stress in the microfluidic model. Phase-contrast images of HUVECs exposed to a shear stress of 0.23 dyn/cm^2 (*upper panel*) and 14.5 (*lower panel*) acquired every 10 min. Direction of the flow was from left to right. Frame rate is 6 per sec. Scale bar is 50 μm .

Supplementary Movie 7. Blood flow in arterial and venous ISVs. Time-lapse imaging of *Tg(gata1:dsRed)*, in which all erythrocytes express dsRed (grey).

Supplementary Movie 8. Blood flow in the dorsal aorta and posterior cardinal vein. Time-lapse imaging of *Tg(gata1:dsRed)*, in which all erythrocytes express dsRed (grey).

Supplementary Movie 9. Expression of *Tp1:d2GFP* during remodeling of the ISVs. Time-lapse imaging of *Tg(Tp1:d2GFP; fli1a:lifectCherry)* which expresses a destabilized GFP (green) under the control of 12xCSL Notch responsive elements. All ECs are marked by lifectCherry (magenta). Red arrows point to arterial ISVs. Green arrow depicts a venous sprout that did not anastomose with an arterial ISV with high Notch activity.

Supplementary Movie 10. Expression of *Tp1:d2GFP* during remodeling of the ISVs. Time-lapse imaging of *Tg(Tp1:d2GFP; fli1a:lifectCherry)* which expresses a destabilized GFP (green) under the control of 12xCSL Notch responsive elements. All ECs are marked by lifectCherry (magenta). Red arrows point to arterial ISVs. Green arrows depict venous sprouts. The sprout on the left anastomoses with an ISV. Orange arrow highlights the formation of a lumen in the sprout on the right. This sprout detaches from an ISV after d2GFP becomes expressed. Blue arrow points to a venous EC migrating from the sprout into this ISV.

Supplementary Movie 11. Expression of *Tp1:d2GFP* during remodeling of the ISVs. Time-lapse imaging of *Tg(Tp1:d2GFP; fli1a:lifectCherry)* which expresses a destabilized GFP (green) under the control of 12xCSL Notch responsive elements. All ECs express lifectCherry (magenta). Red arrows point to arterial ISVs. Green arrows point to ECs expressing d2GFP in venous ISV.