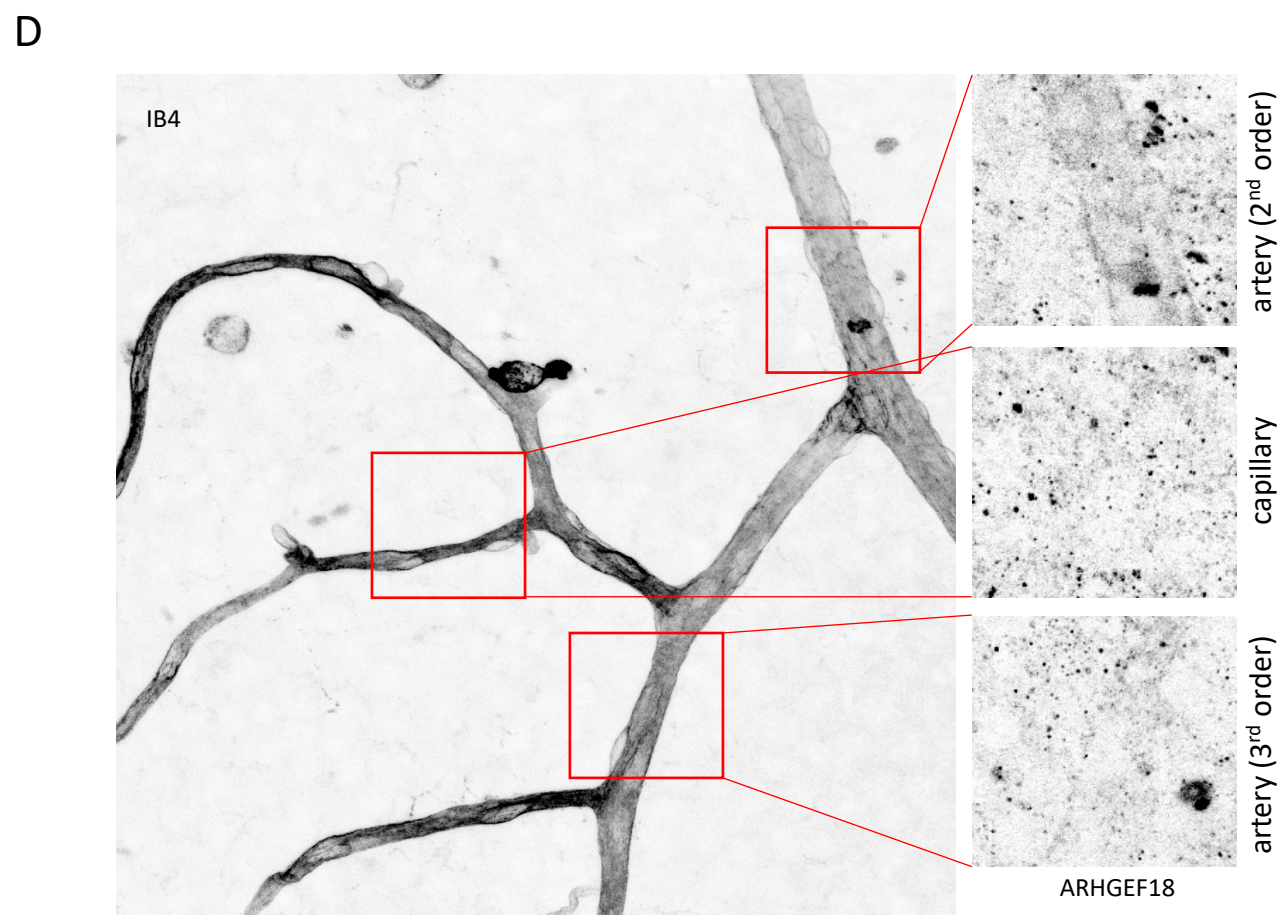
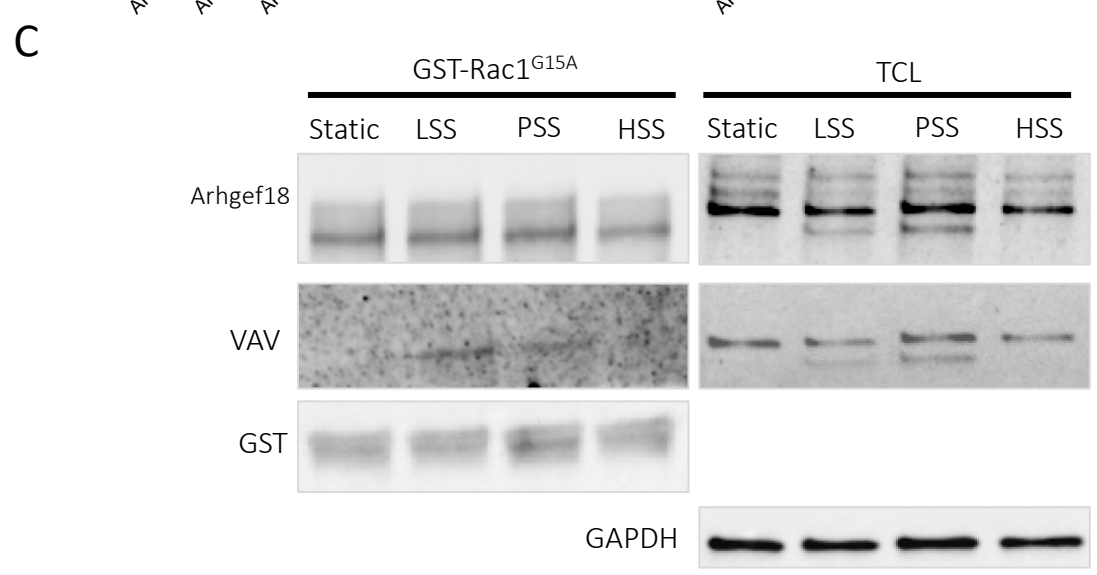
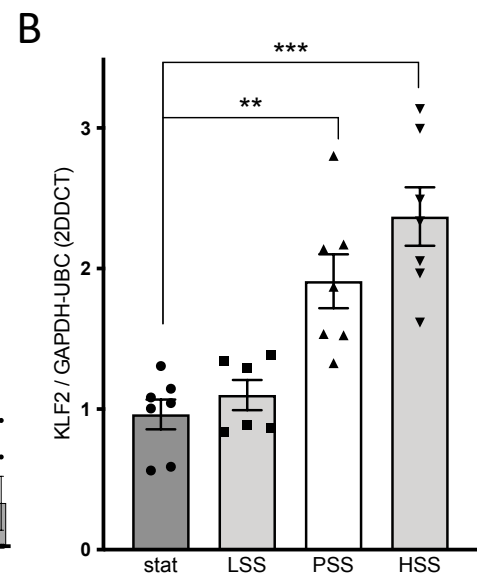
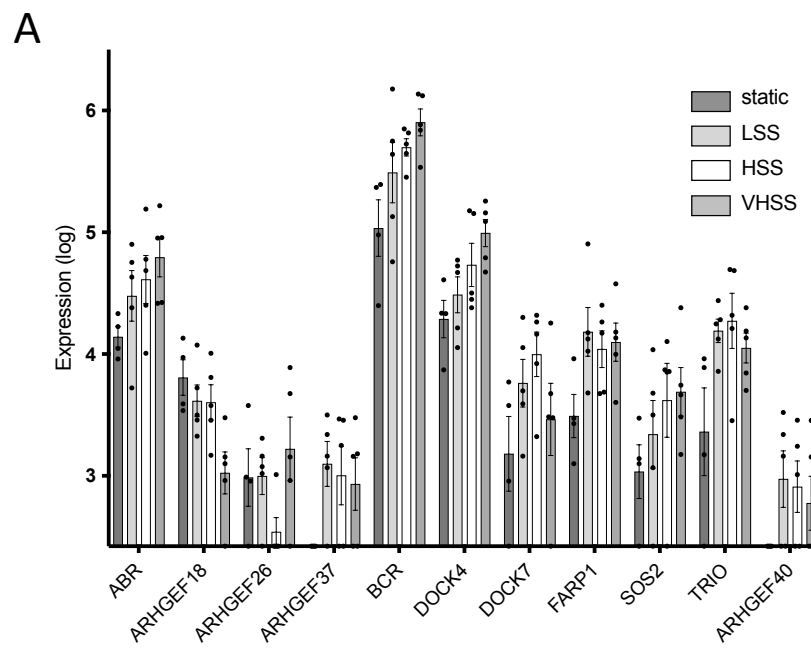


suppl figure 1



# Suppl Figure 2

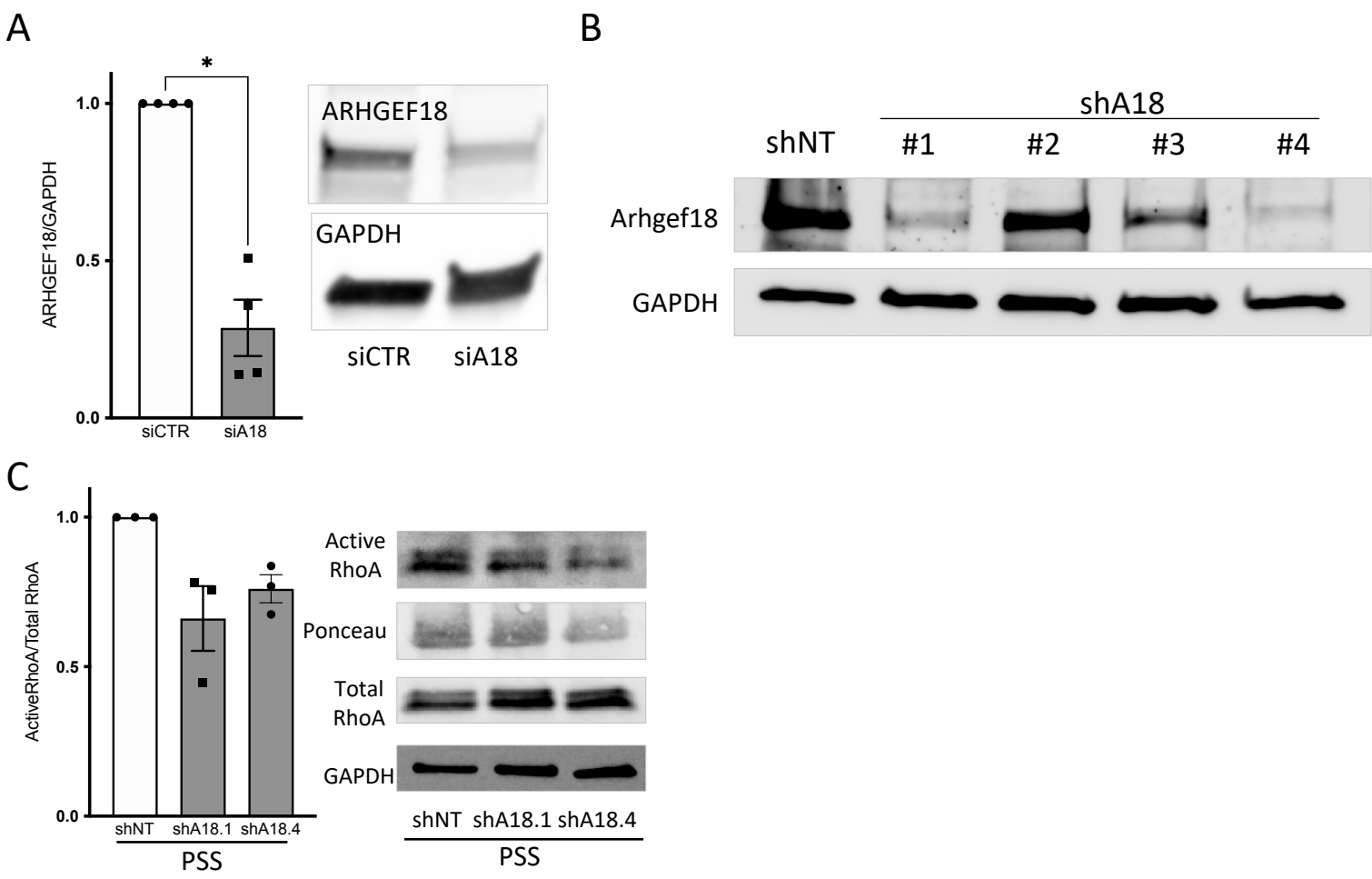


Figure suppl 3

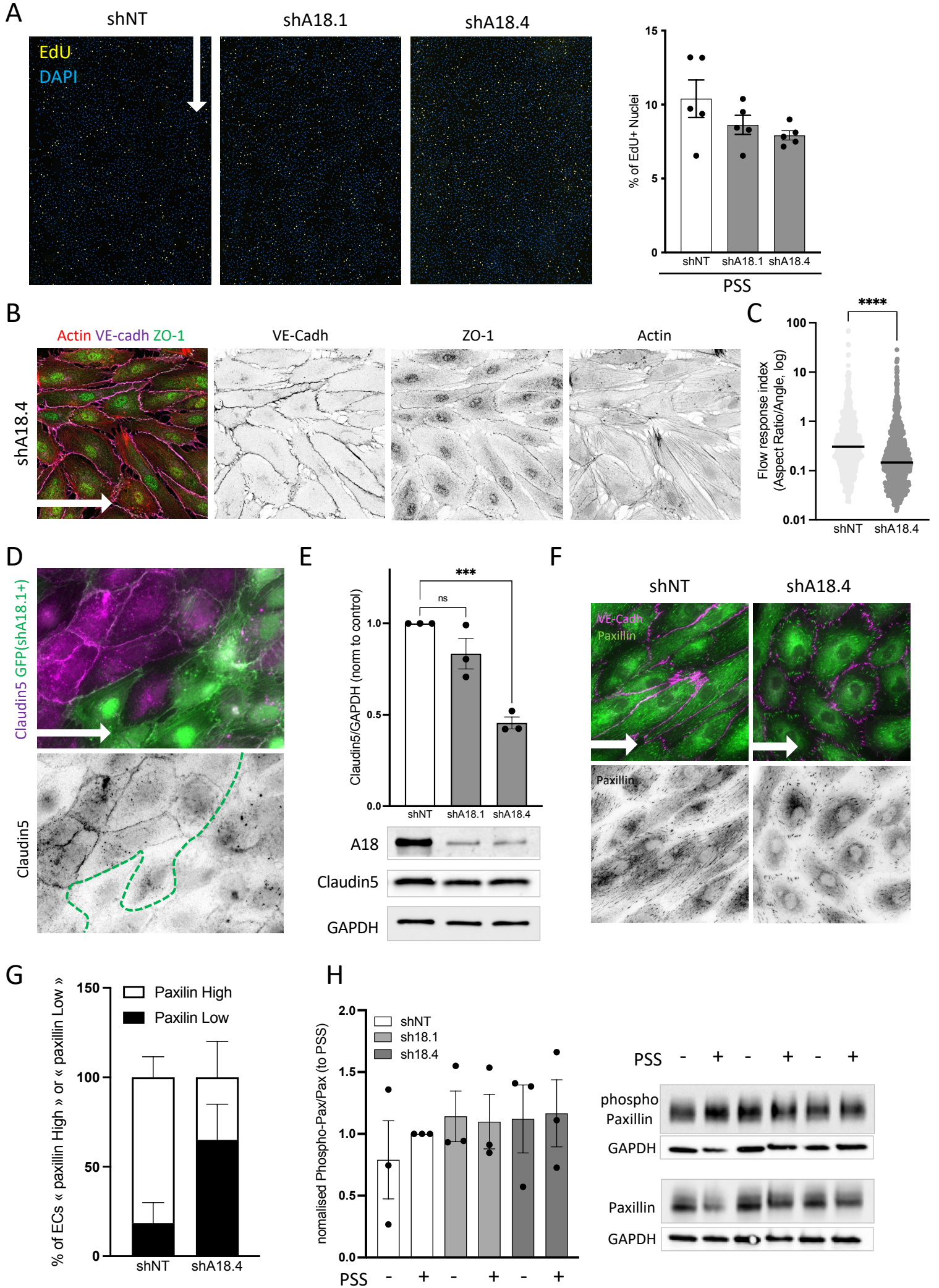
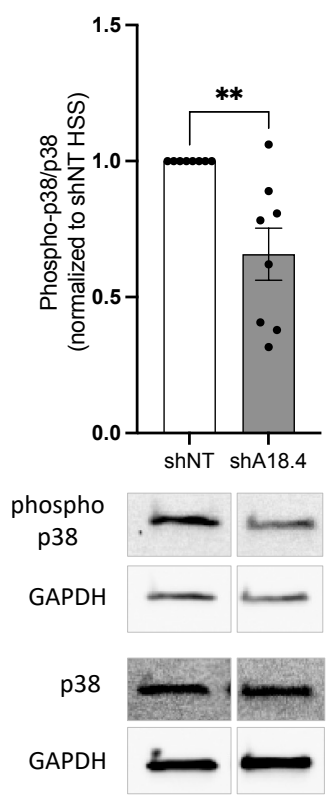
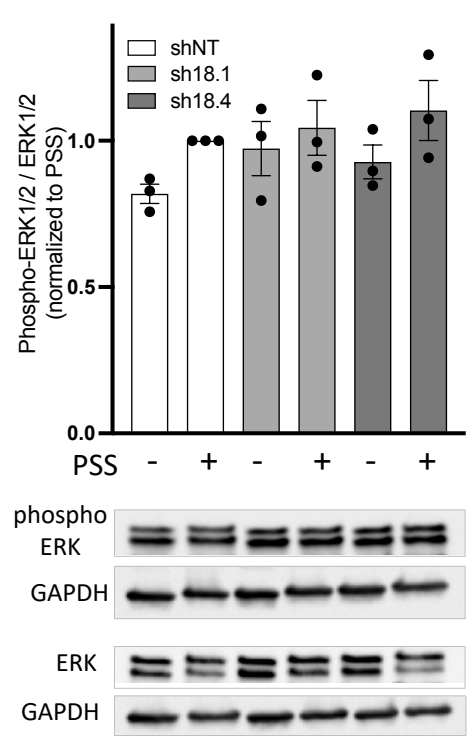


Figure suppl 4

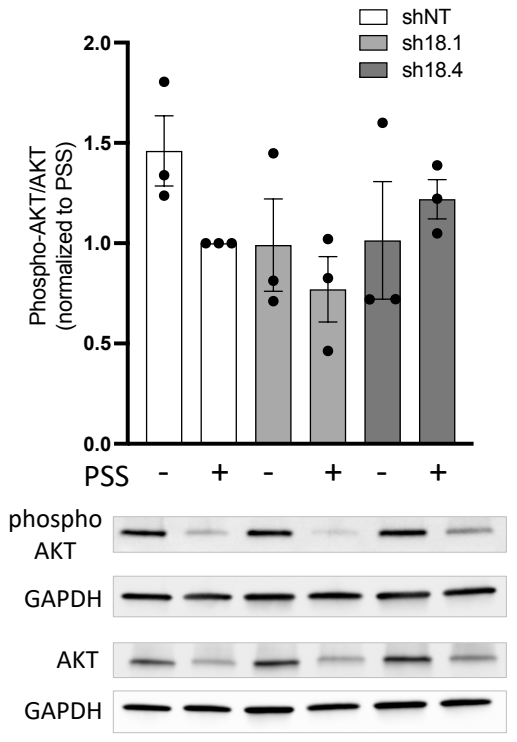
A



B



C



**Supplemental Figure 1: ARHGEF18 expression and activity is shear-stress dependent and bound to RhoA but not RAC1.** **A.** extracted data from the 3'RNA sequencing analysis for the RhoGEFs with significant expression level changes. Static, N=4; SS N=5. LSS: low shear stress; PSS: physiological shear stress; HSS: high shear stress. **B.** Quantification of Klf2 expression level by qPCR. LSS: low shear stress; PSS: physiological shear stress; HSS: high shear stress. N>6. \*\*p<0.01; \*\*\*p<0.001; One-way ANOVA. **C.** Representative blot of VAV binding on nucleotide-free Rac1 (GST-Rac1<sup>G15A</sup>) by pull-down assay. 24h of SS, LSS: low shear stress; PSS: physiological shear stress; HSS: high shear stress. Representative of N=3. **D.** Second representative image from ARHGEF18 staining in the retina showing ARHGEF18 staining in different area of the arterial tree.

**Supplemental Figure 2: ARHGEF18 participates to endothelial cell adhesion and migration.** **A.** Silencing efficiency of the siRNA transfection assessed by Western Blot. N=4. Wilcoxon T-test. **B.** Representative Blot of the silencing efficiency by shRNA expression following infection, selection and induction. **C.** Quantification of RhoA activity by pull-down assay using GST-RBD coated beads in HUVECs expressing a non-targeting shRNA (shNT) or a shRNA targeting Arhgef18 (shA18.1 or A18.4) and exposed to physiological SS (PSS). 24h of SS. N=3.

**Supplemental Figure 3: ARHGEF18 participates in ECs response to flow, tight junction formation and focal adhesion formation.** **A.** Representative images and Quantification of proliferation by Edu incorporation and staining in HUVECs expressing a non-targeting shRNA (shNT) or a shRNA targeting Arhgef18 (shA18.1 and shA18.4), exposed to physiological SS. 8h of SS. N=5. \*\*p<0.01; 2-way ANOVA. Flow direction is represented by the arrow. **B.** Representative images of HUVECs expressing a second shRNA targeting Arhgef18 (shA18.4), exposed to physiological SS and stained for ZO-1 (green), VE-cadherin (magenta) and actin (red). Flow direction is indicated by the arrow. **C.** Quantification of cell orientation and elongation with the flow in HUVECs expressing a non-targeting shRNA (shNT) or a second shRNA targeting Arhgef18 (shA18.4), exposed to physiological SS. 24h of SS. Each dot represents a cell. N=3 with in-between 200 and 350 cells analyzed by experiment. \*\*\*p<0.001; Mann-Whitney T-test. **D.** Representative images of Claudin5 staining in a mix population of WT (GFP negative) and HUVECs expressing the shRNA targeting Arhgef18 (shA18.1, GFP+), the green dotted line indicate the separation between WT and shA18+ cells. **E.** Quantification of Claudin5 protein level by Western blot in HUVECs expressing a non-targeting shRNA (shNT) or a shRNA targeting Arhgef18 (shA18.1 and shA18.4) exposed to physiological SS. N=3. \*\*\*p<0.001, One-way ANOVA. **F.** Representative images of Paxillin staining in HUVECs expressing a non-targeting shRNA (shNT) or a second shRNA targeting Arhgef18 (shA18.4), exposed to physiological SS. Flow direction is indicated by the arrow. Green: paxillin; magenta: VE-cadherin. **G.** Quantification of focal adhesion type by hand classification of paxillin staining within each cell based on the representative images for each category on the left (white box: paxillin high = numerous, elongated and aligned focal adhesions; black box: paxillin low = few, short and misaligned focal adhesions). N=2. Quantified blindly. **H.** Quantification of FAK phosphorylation level by Western blot in HUVECs expressing a non-targeting shRNA (shNT) or a shRNA targeting Arhgef18 (shA18.1 and shA18.4), under static condition or exposed to physiological SS (PSS).

**Supplemental Figure 4: p38 mediates ARHGEF18 effects in flow response of ECs.** **A.** Quantification of p38 phosphorylation by Western blot in HUVECs expressing a non-targeting shRNA (shNT) or a second shRNA targeting Arhgef18 (shA18.4), exposed to physiological SS. N=8. \*\*p<0.01; paired T-test. Images originate from the same blot. **B.** Quantification of ERK1/2 phosphorylation level by Western blot in HUVECs expressing a non-targeting shRNA (shNT) or a shRNA targeting Arhgef18 (shA18.1 and shA18.4), under static condition or exposed to physiological SS (PSS). N=3. **C.** Quantification of AKT phosphorylation level by Western blot in HUVECs expressing a non-targeting shRNA (shNT) or a shRNA targeting Arhgef18 (shA18.1 and shA18.4), under static condition or exposed to physiological SS (PSS). N=3.