

**Figure 1**

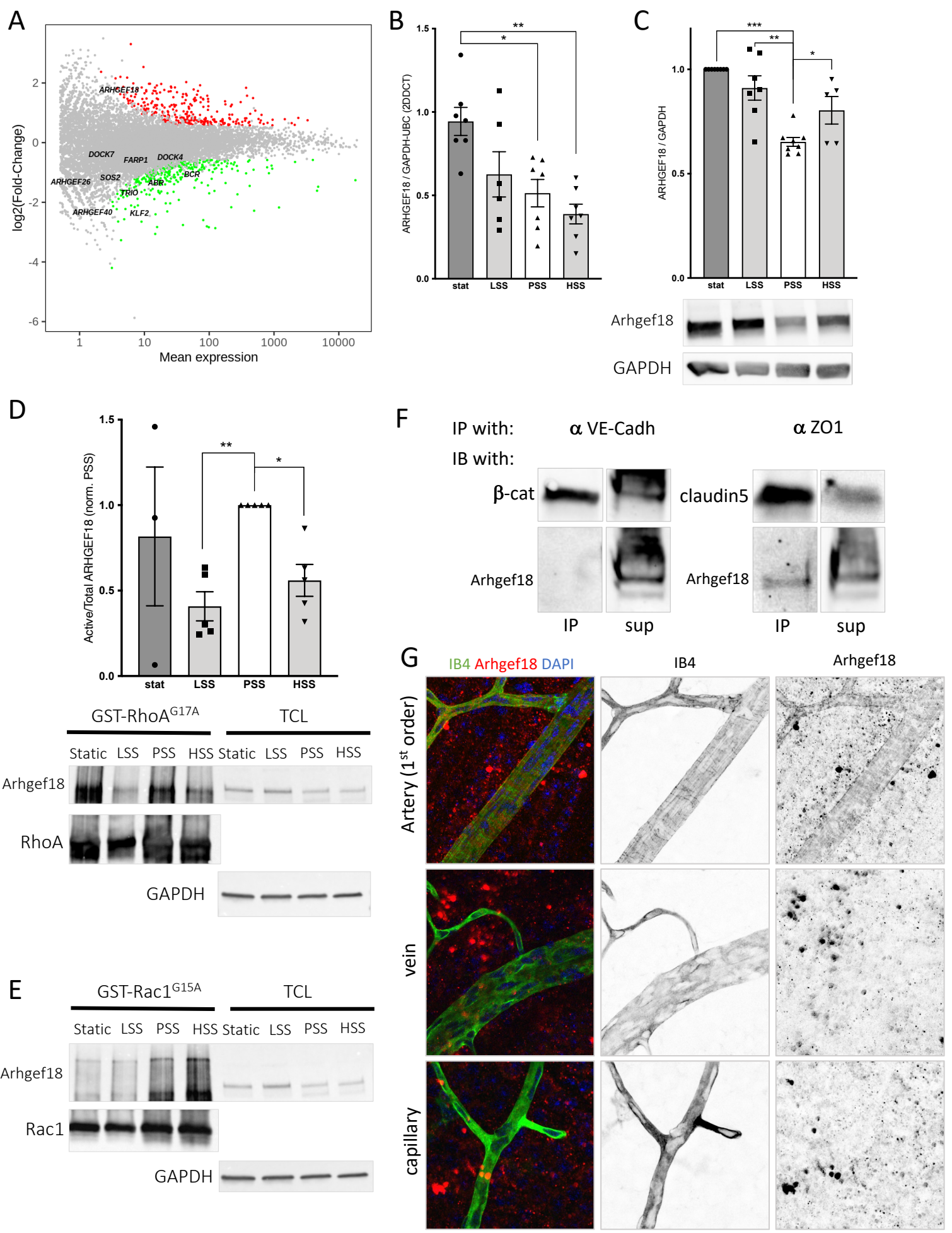
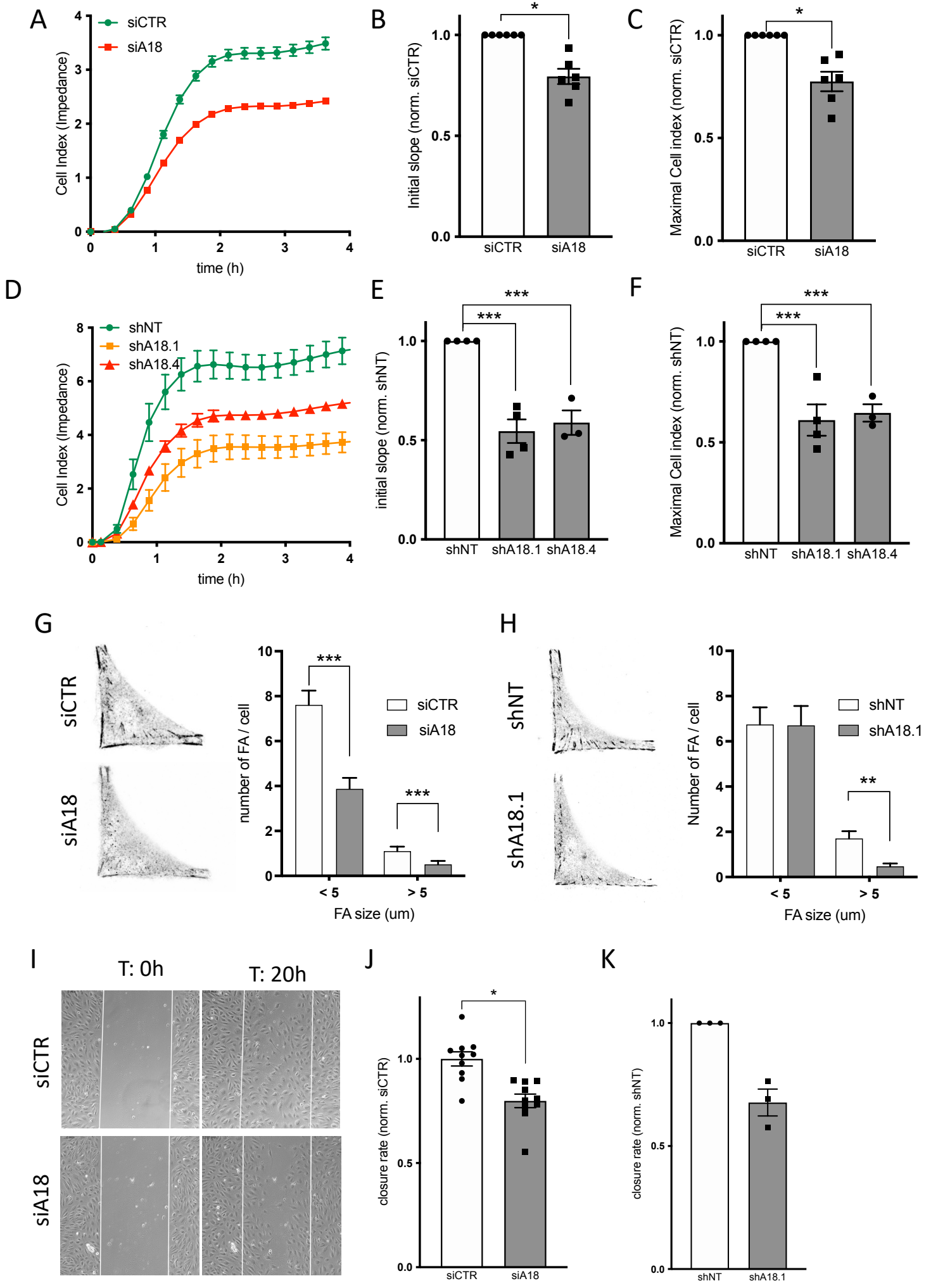


Figure 2



**Figure 3**

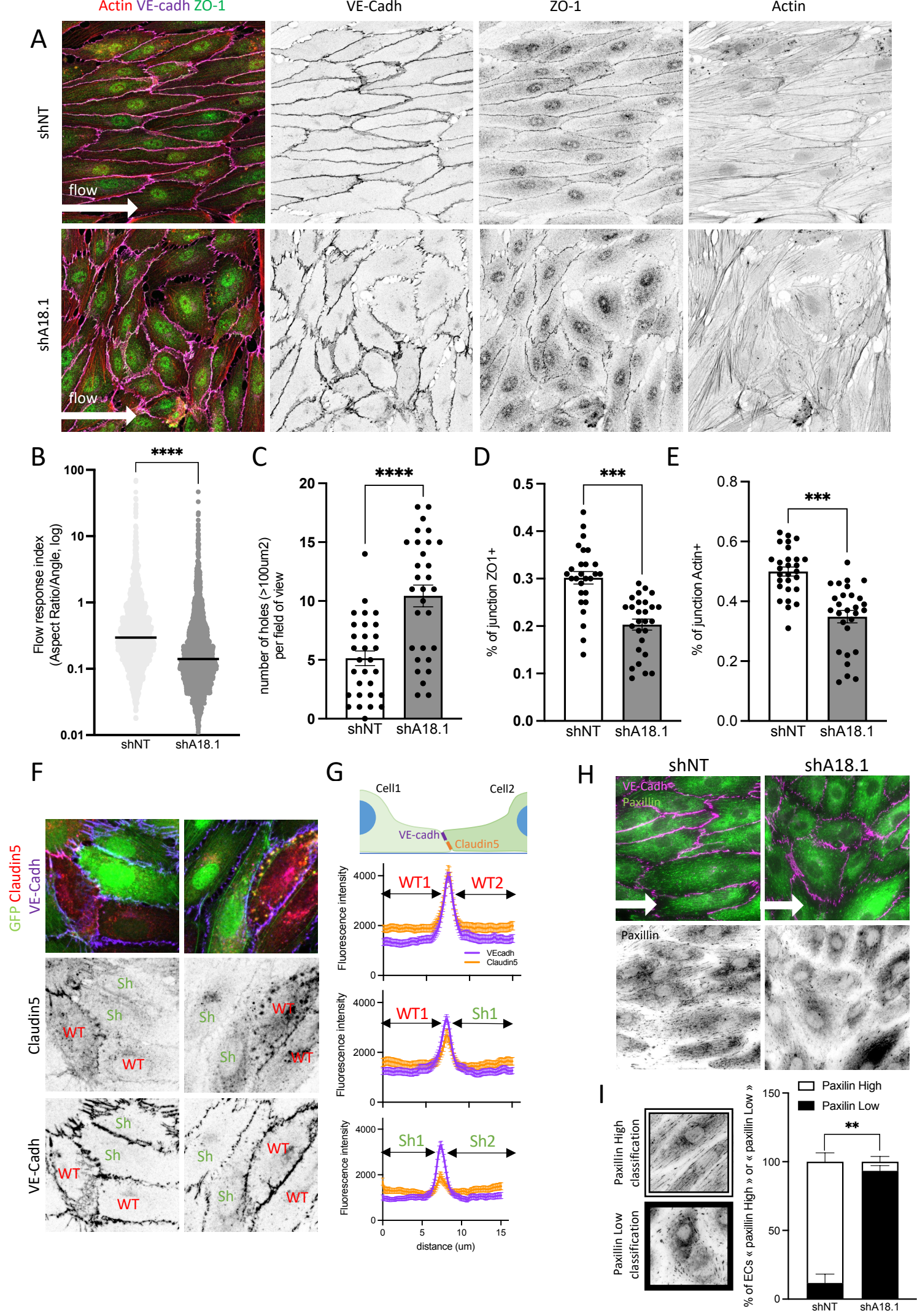


Figure 4

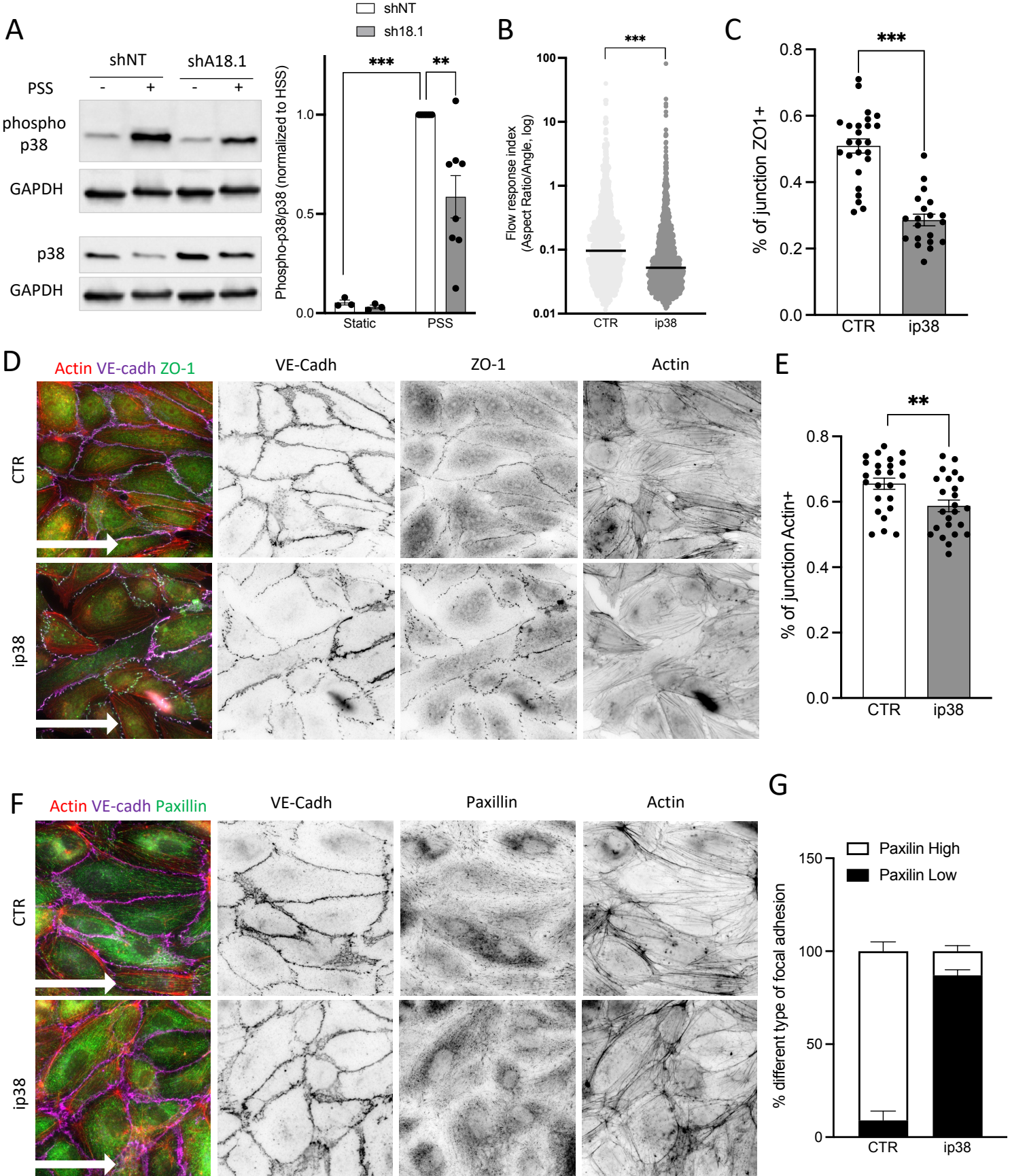
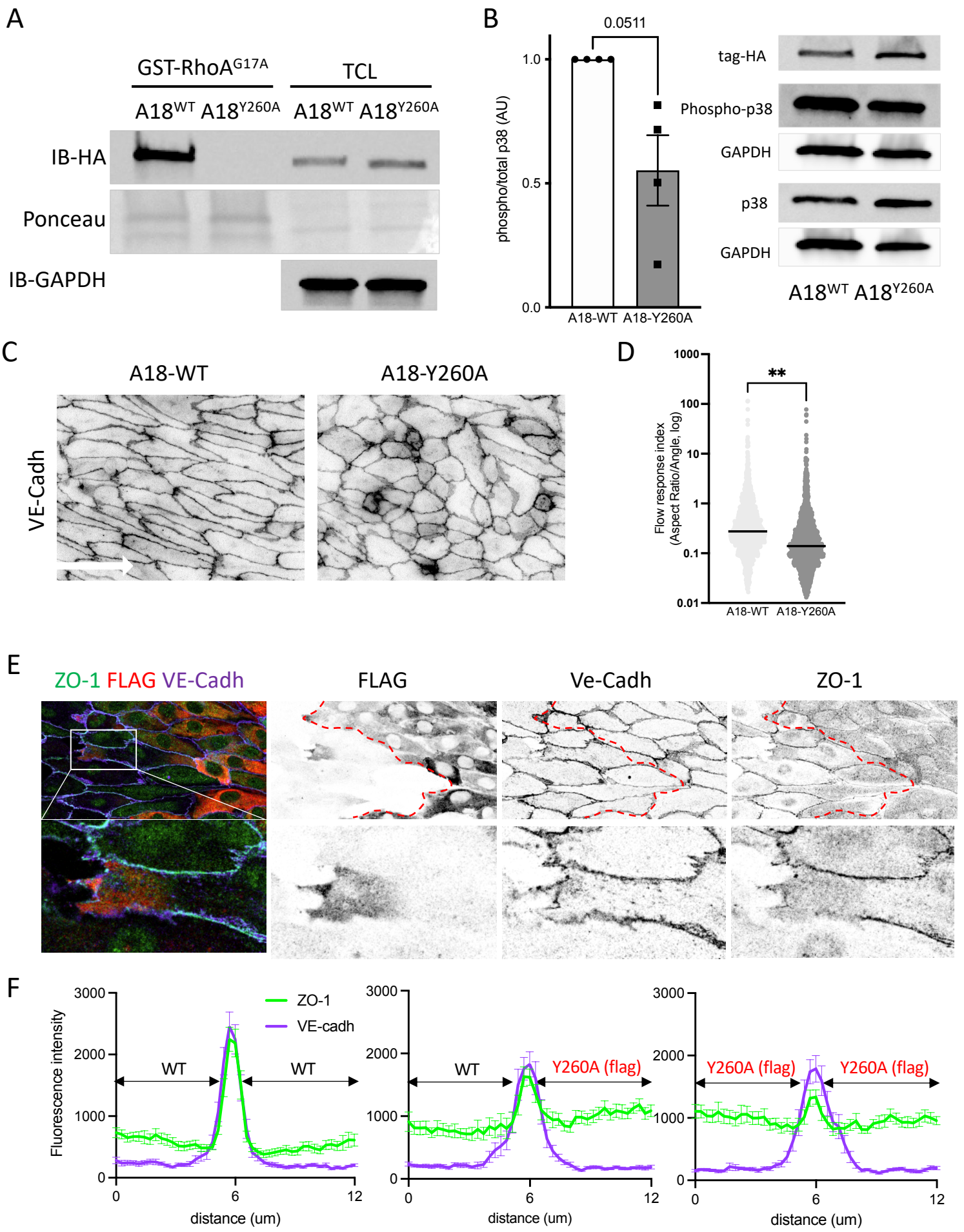


Figure 5



## Figures Legends

### Figure 1: ARHGEF18 expression and activity is shear-stress dependent and bound to RhoA but not RAC1.

**A.** MA plot comparing gene expression between static condition and high shear stress. 24h of SS. Green dots: up-regulated genes in HSS condition; red dots: down-regulated genes in HSS condition. N=4. **B.** qPCR analysis of Arhgef18 RNA expression level. 24h of SS, LSS: low shear stress; PSS: physiological shear stress; HSS: high shear stress. N>5. \*p<0.05; \*\*p<0.01; One-way ANOVA. **C.** Western-Blot analysis of ARHGEF18 protein expression level (left, quantification; right, representative blot). 24h of SS, LSS: low shear stress; PSS: physiological shear stress; HSS: high shear stress. N>5. \*p<0.05; \*\*p<0.01; \*\*\*p<0.001; One-way ANOVA. **D.** Quantification and representative blot of ARHGEF18 binding on nucleotide-free RhoA (GST-RhoA<sup>G17A</sup>) by pull-down assay. 24h of SS, LSS: low shear stress; PSS: physiological shear stress; HSS: high shear stress. N=5. \*p<0.05; \*\*p<0.01; \*\*\*p<0.001; One-way ANOVA. **E.** Representative blot of ARHGEF18 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. **F.** Representative blot from Co-immunoprecipitation of ARHGEF18 with VE-cadherin (left) or ZO-1 (right). Co-immunoprecipitation efficiency was assessed by the presence of  $\beta$ -catenin and Claudin 5 in the immunoprecipitated fraction of VE-cadherin complex and ZO-1 complex respectively (upper lanes). Static condition. Representative of N=2. **G.** Immunofluorescent staining of ARHGEF18 in mouse retina. Retinas from 4 weeks-old animals. Green: Isolectin B4; Representative of N=5.

### Figure 2: ARHGEF18 participates to endothelial cell adhesion and migration.

**A.** Representative curves of impedance measurement during adhesion of HUVECs. Green: non-targeting siRNA (siCTR); red: siRNA targeting arhgef18 RNA (siA18). **B.** Quantification of adhesion speed (slope of the linear part of the curve) of HUVECs transfected with a control siRNA (siCTR) or with an siRNA targeting Arhgef18 (siA18). N=6. \*p<0.05; Wilcoxon T-test. **C.** Quantification of maximal adhesion (plateau of the curve) of HUVECs transfected with a control siRNA (siCTR) or with an siRNA targeting Arhgef18 (siA18). N=6. \*p<0.05; Wilcoxon T-test. **D.** Representative curves of impedance measurement during adhesion of HUVECs. Green: non-targeting shRNA (shNT); red and orange: shRNAs targeting arhgef18 RNA (shA18.4 and shA18.1 respectively). **E.** Quantification of adhesion speed (slope of the linear part of the curve) of HUVECs expressing a non-targeting shRNA (shNT) or a shRNA targeting Arhgef18 (shA18.1 or A18.4). N<3. \*\*\*p<0.001; One-way ANOVA. **F.** Quantification of maximal adhesion (plateau of the curve) of HUVECs expressing a non-targeting shRNA (shNT) or a shRNA targeting Arhgef18 (shA18.1 or A18.4). N<3. \*\*\*p<0.001; One-way ANOVA. **G.** Representative images of HUVECs adhesion on L-shaped micropattern immune-stained for paxillin (siRNA transfected HUVECs) and Quantification of focal adhesion number and size. N=39 (cells) from 4 different experiments. \*\*\*p<0.001; 2-way ANOVA. **H.** Representative images of HUVECs adhesion on L-shaped micropattern immune-stained for paxillin. (shRNA expressing HUVECs) and Quantification of focal adhesion number and size. N=51 (cells) from 4 different experiments. \*\*\*p<0.001; 2-way ANOVA. **I.** Representative images of wound assay experiment (phase contrast images) at starting point (T:0h) and 20h post wound. siRNA transfected HUVECs. **J.** Quantification of closure speed of HUVECs transfected with a control siRNA (siCTR) or with an siRNA targeting Arhgef18 (siA18). N=10. \*p<0.05; paired T-test. **K.** Quantification of closure speed of HUVECs expressing a control shRNA (shNT) or a shRNA targeting Arhgef18 (shA18.1). N=3.

### Figure 3: ARHGEF18 participates in ECs response to flow, tight junction formation and focal adhesion formation.

**A.** Representative images of HUVECs expressing a non-targeting shRNA (shNT) or a shRNA targeting Arhgef18 (shA18.1), exposed to physiological SS and stained for ZO-1 (green), VE-cadherin (magenta) and actin (red). Flow direction is indicated by the arrow. **B.** Quantification of cell orientation and elongation with the flow in HUVECs expressing a non-targeting shRNA (shNT) or a shRNA targeting Arhgef18 (shA18.1), exposed to physiological SS. 24h of SS. Each dot represents a cell. N=5 with in-between 200 and 600 cells analyzed by experiment. \*\*\*p<0.001; Mann-Whitney T-test. **C.** Quantification of number of holes in-between cells in HUVECs expressing a non-targeting shRNA (shNT) or a shRNA targeting Arhgef18 (shA18.1), exposed to physiological SS. 24h of SS. N=3, 10 areas analyzed by experiment. \*\*\*p<0.001; Unpaired T-test. **D.** Quantification of ZO-1 localized at junction in HUVECs expressing a non-targeting shRNA (shNT) or a shRNA targeting Arhgef18 (shA18.1), exposed to physiological SS. 24h of SS. N=3, 9 areas analyzed by experiment. \*\*\*p<0.001; Unpaired T-test. **E.** Quantification of Actin localized at junction in HUVECs expressing a non-targeting shRNA (shNT) or a shRNA targeting Arhgef18 (shA18.1), exposed to physiological SS. 24h of SS. N=3, 9 areas analyzed by experiment. \*\*\*p<0.001; Unpaired T-test. **F.** Representative images of Claudin5 staining in a mix population of WT cells (GFP negative, non-green, indicated as "WT" on images) and cells expressing a shRNA targeting Arhgef18 (shA18.1, GFP positive, green, indicated as "KO" on images), exposed to physiological SS. Green: GFP; red: Claudin5; Magenta: VE-cadherin. **G.** Quantification of the Claudin5 staining at junction using plot profile analysis. N=3, 12 couple of cells analyzed per experiment. **H.** Representative images of Paxillin staining in HUVECs expressing a non-targeting shRNA (shNT) or a shRNA targeting Arhgef18 (shA18.1), exposed to physiological SS. Flow direction is indicated by the arrow. Green: paxillin; magenta: VE-cadherin. **I.** 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=4. Quantified blindly.

**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 shRNA targeting Arhgef18 (shA18.1), under static condition or exposed to physiological SS (PSS). Static: N=3; PSS: N=8. \*\* $p < 0.01$ ; \*\*\* $p < 0.001$  2-way ANOVA. **B.** Quantification of cell orientation and elongation with the flow in HUVECs treated with an inhibitor for p38 (SB239063, 100nM, ip38) or vehicle (CTR), exposed to physiological SS. 24h of SS. Each dot represents a cell. N=4 with in-between 200 and 450 cells analyzed by experiment. \*\*\* $p < 0.001$ ; Mann-Whitney T-test. **C.** Quantification of Actin localized at junction in HUVECs treated with an inhibitor for p38 (SB239063, 100nM, ip38) or vehicle (CTR), exposed to physiological SS. 24h of SS. N=2, 10 to 14 areas analyzed by experiment. \*\* $p < 0.01$ ; Unpaired T-test. **D.** Representative images of HUVECs treated with an inhibitor for p38 (SB239063, 100nM, ip38) or vehicle (CTR) exposed to physiological SS. 24h and stained for ZO-1 (green), VE-cadherin (magenta) and actin (red). Flow direction is indicated by the arrow. **E.** Quantification of ZO-1 localized at junction in HUVECs treated with an inhibitor for p38 (SB239063, 100nM, ip38) or vehicle (CTR) exposed to physiological SS. 24h of SS. N=2, 10 to 15 areas analyzed by experiment. \*\*\* $p < 0.001$ ; Unpaired T-test. **F.** Representative images of Paxillin staining in HUVECs treated with an inhibitor for p38 (SB239063, 100nM, ip38) or vehicle (CTR), exposed to physiological SS. Flow direction is indicated by the arrow. Green: paxillin; magenta: VE-cadherin; red: Actin. **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.

**Figure 5: GEF activity of ARHGEF18 is important for p38 activation and flow response of ECs.** **A.** Representative blot of ARHGEF18 binding on nucleotide-free RhoA (GST-RhoA<sup>G17A</sup>) by pull-down assay with HUVECs exposed to physiological SS, over-expressing WT form (A18<sup>WT</sup>) or mutant form (A18<sup>Y260A</sup>) of ARHGEF18. **B.** Quantification of p38 phosphorylation by Western blot in HUVECs exposed to physiological SS, over-expressing WT form (A18<sup>WT</sup>) or mutant form (A18<sup>Y260A</sup>) of ARHGEF18. N=4. Paired T-test. **C.** Representative images of HUVECs exposed to physiological SS, over-expressing WT form (A18<sup>WT</sup>) or mutant form (A18<sup>Y260A</sup>) of ARHGEF18 and stained VE-cadherin. Flow direction is indicated by the arrow. **D.** Quantification of cell orientation and elongation with the flow in HUVECs exposed to physiological SS, over-expressing WT form (A18<sup>WT</sup>) or mutant form (A18<sup>Y260A</sup>) of ARHGEF18. Each dot represents a cell. N=1 with in-between 1000 and 1350 cells analyzed by experiment. \*\* $p < 0.01$ ; Unpaired T-test. **E.** Representative images of ZO-1 staining in a mix population of WT cells (FLAG negative, non-red) and cells over-expressing the mutant form (A18<sup>Y260A</sup>) of ARHGEF18 (FLAG positive, red), exposed to physiological SS. Green: ZO-1; red: FLAG; Magenta: VE-cadherin. **F.** Quantification of the ZO-1 staining at junction using plot profile analysis. N=1, 25 couples of cells analyzed per experiment.