Appendix

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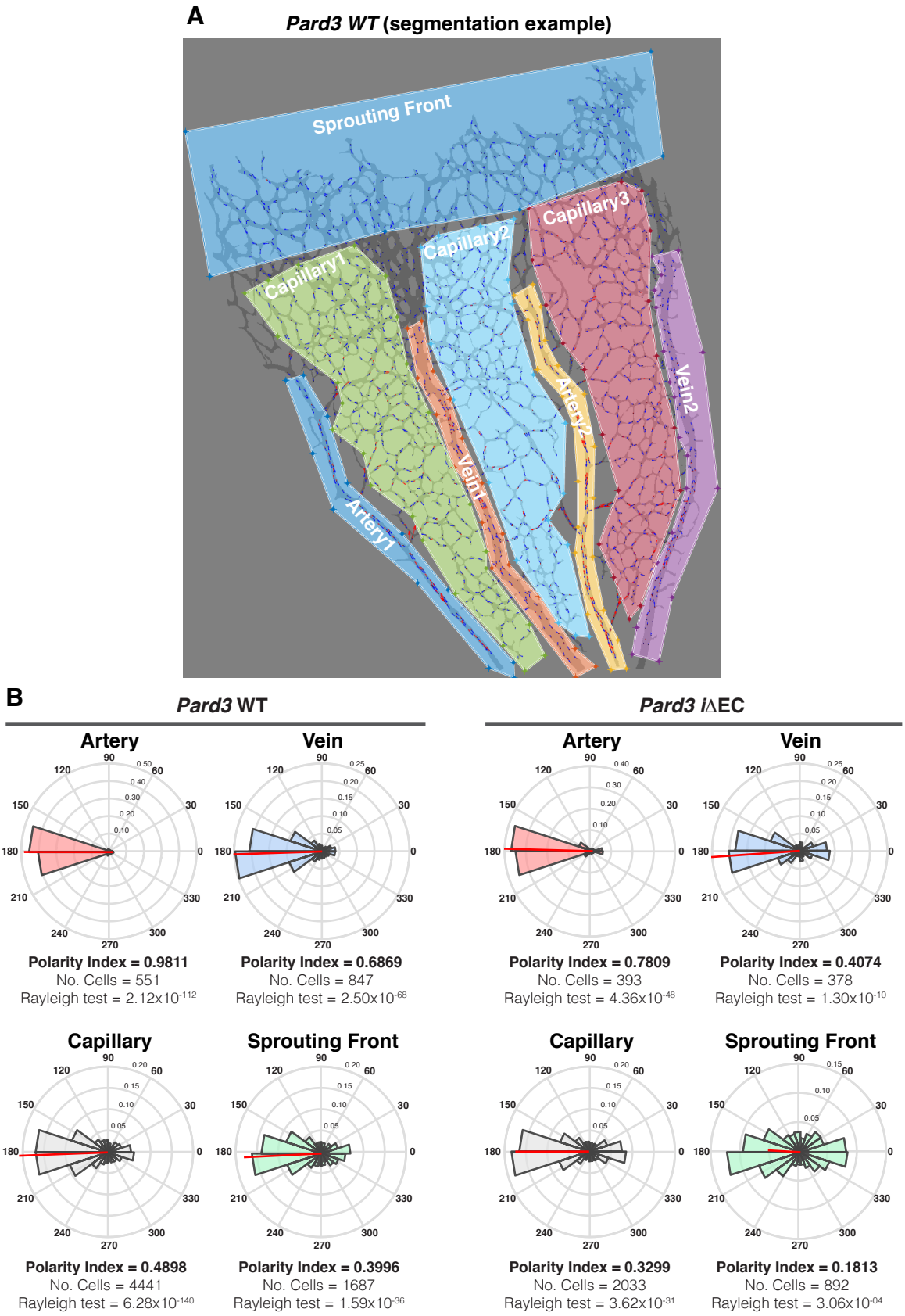
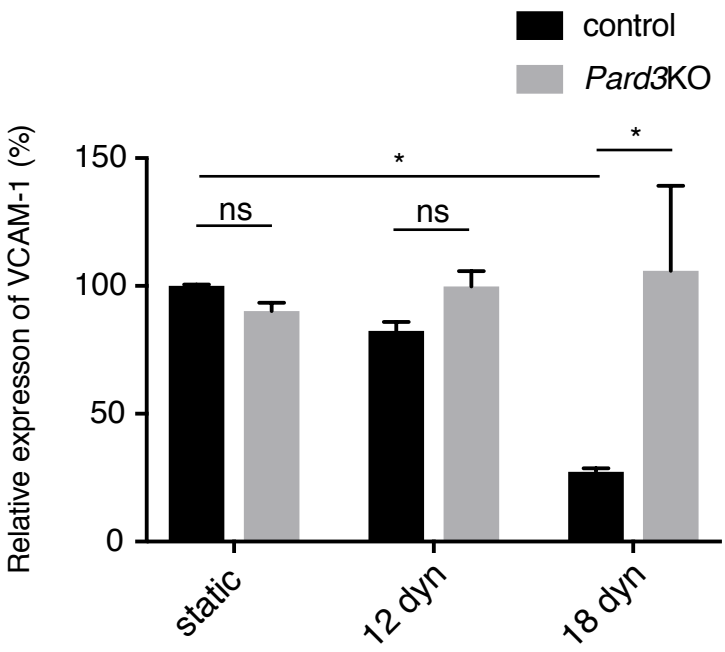
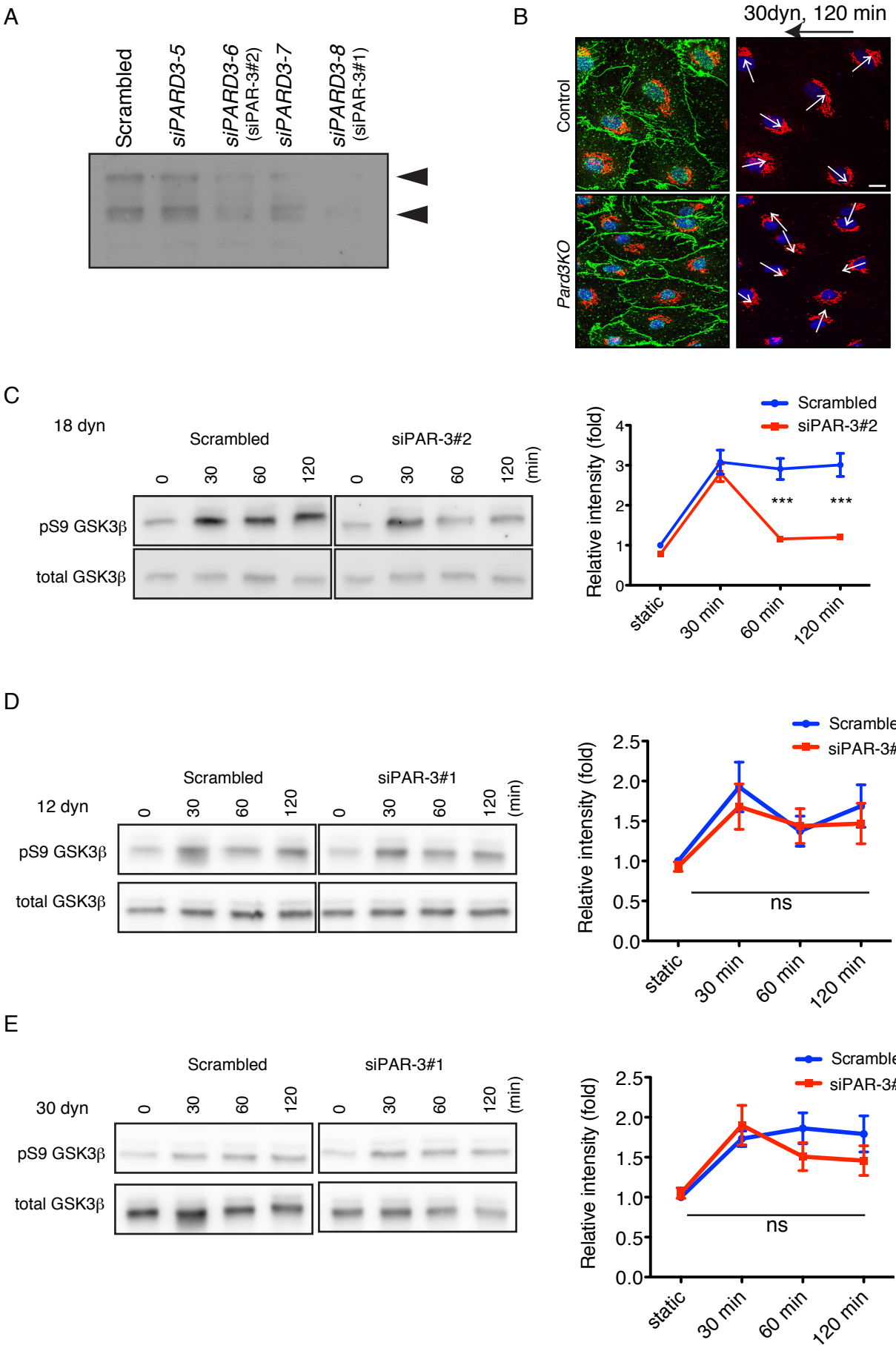


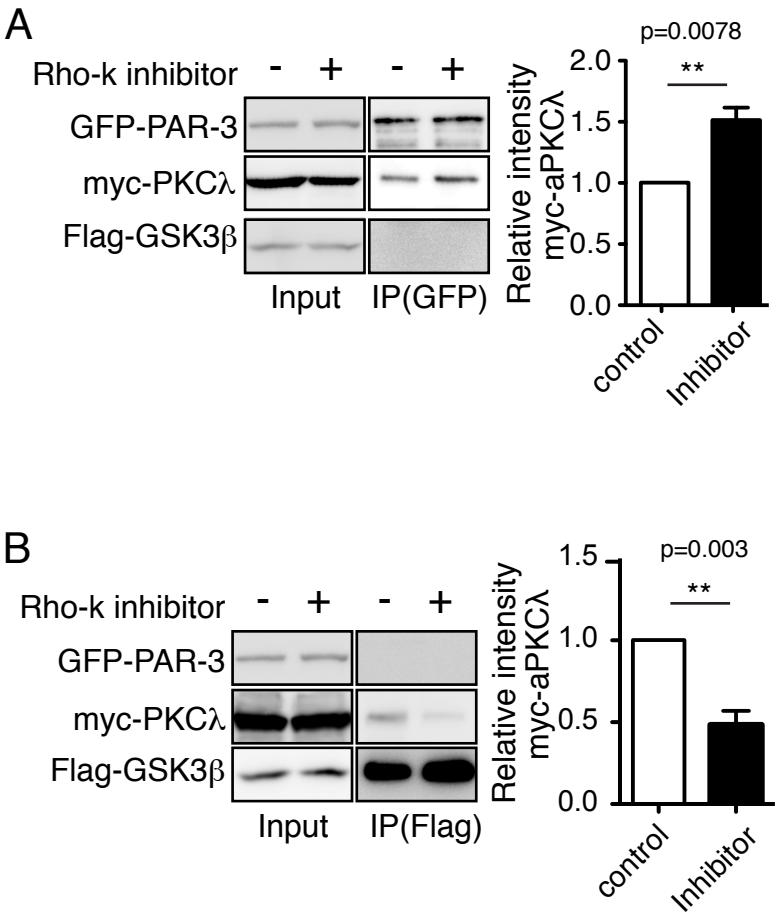
Appendix Figure S1



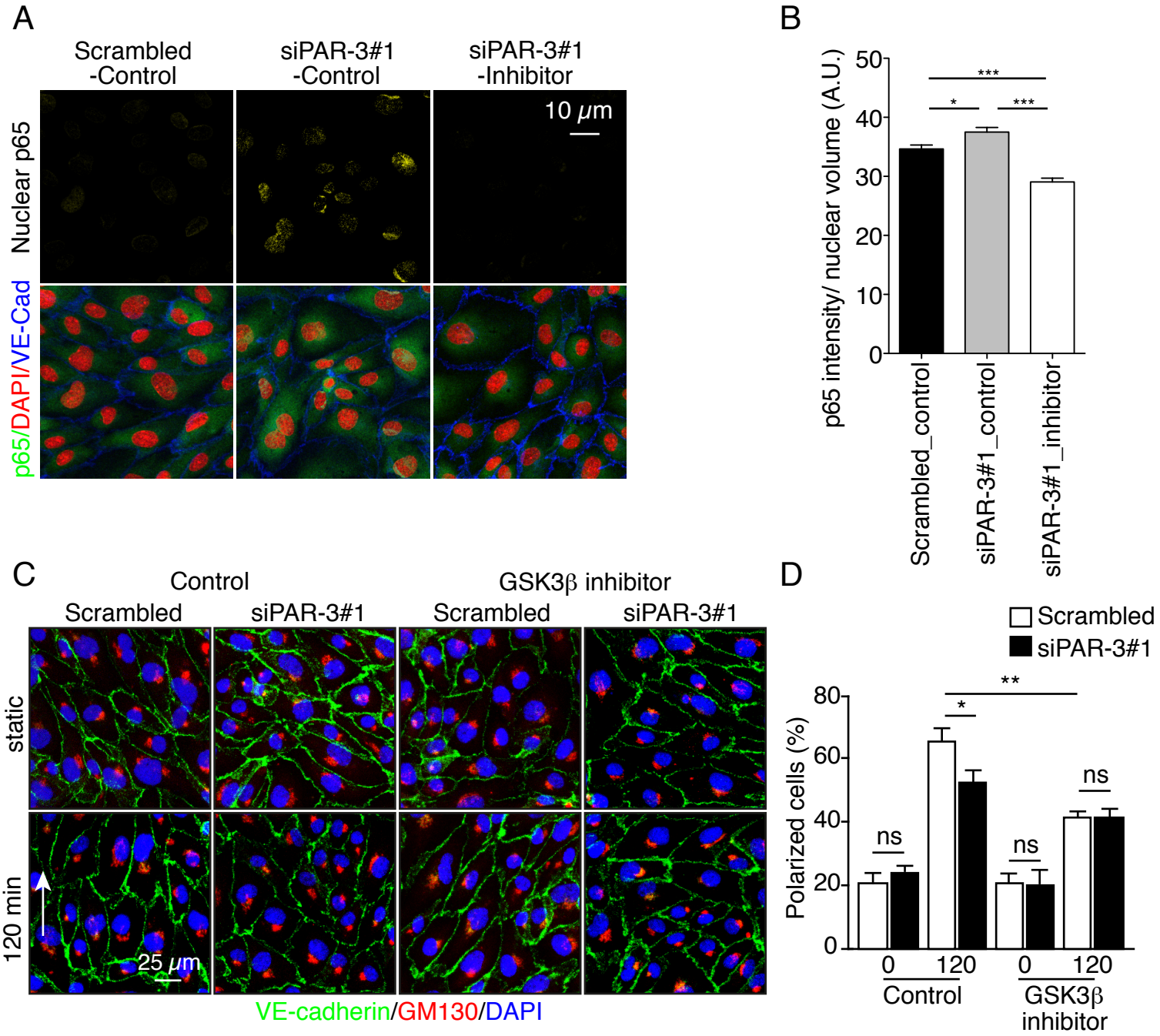
Appendix Figure S2



Appendix Figure S3



Appendix Figure S4



Appendix Figure S5

Appendix legends

Appendix Figure S1. Flow-dependent polarity patterns of endothelial cells in control and *Pard3*$^{i\Delta EC}$.

A. Overview of segmentation strategy for each specific vascular bed in a *Pard3* WT P6 mouse retina. The network is segmented in 4 types of vascular beds: arteries, veins, capillary network and vascular sprouting front. The specific image contains 2 arteries, 2 veins, 3 capillary networks and 1 sprouting front. All endothelial cells in each specific vascular bed is then pooled together to obtain a global polarity pattern for each specific mouse retina.

B. Angular histograms of endothelial cell polarity patterns for the designated vascular beds, as described in (A) for control and *Pard3*$^{i\Delta EC}$ P6 mouse retinas. Polarity indexes are calculated as described in the material and methods and Figure 1B; total number of cells for each angular histogram; statistical Rayleigh test for non-uniform distribution of cell polarities. n=3 animals.

Appendix Figure S2. The effect of PAR-3 on VCAM-1 in isolated ECs from mouse aorta.

Effect of laminar flow on VCAM-1 expression level of the control and KO aortic ECs. In B, data are mean ± SEM (n=3 experiments); statistical significance (*$p < 0.05$) was evaluated with tow-way ANOVA followed by Tukey's post-test for multiple comparisons. ns: not significant, $P \geq 0.05$; differences: **$P<0.01$.

Appendix Figure S3. Further confirmation of the effect of loss of PAR-3 on endothelial cellular response.

A. WB of *siPARD3*s transfected ECs. Most efficient one (#8; siPAR-3#1) and second best (#6; siPAR-3#2) were used in this study. B Endothelial cells isolated from P56 *Pard3*$^{flox/flox}$; *Cdh5-CreERT2* mouse aorta were seeded in the fibronectin-coated flow chamber and gene knockout was induced by incubating cells with 1μM 4-Hydroxytamoxifen. PBS was used as control. After 48 hours, ECs were exposed to 30dyn/cm^2 laminar flow for 120 min, and axial polarity was analyzed. EC junction (VE-cadherin) is green, Golgi (GM130) is red, and nuclear stain (DAPI) is blue. White arrows show the direction of the vector from the center of EC nuclei to Golgi. C. Confirmation of PAR-3 KD phenotype using another siRNA. HUVECs transfected with siPAR-3#2 were treated with 18dyn/cm^2 of laminar flow and subjected to western blotting with indicated antibodies. Quantitation is shown on the right side. D, E, Effect of low flow (12dyn/cm^2) and high flow (30dyn/cm^2) on GSK3β S9 phosphorylation. HUVECs were transfected with siPAR-3#1 and subjected to 12dyn/cm^2 (upper panels) or 30dyn/cm^2 (lower panels) of laminar flow. Quantitation is shown on the right side of images. In C, D and E, data are means ± SEM (n=3 experiments); ns: not significant, P≥0.05; differences: ***P<0.001, analyzed with 2-way ANOVA followed with Bonferroni multiple comparisons. Scale bar, 10 μm.

Appendix Figure S4. Rho-Kinase regulates balance between the PAR-3/aPKCλ complex versus the aPKCλ/GSK3β complex

A, B. HEK293 cells were transfected with indicated cDNAs and were treated with or without Rho-kinase inhibitor (Y-27632, 20μM). After immunoprecipitation with indicated antibodies, samples were analyzed by Western blotting. In A and B data are presented as mean ± SEM (n=5 experiments). Differences **P<0.01 were analyzed with Mann–Whitney U test.

Appendix Figure S5. GSK3β indirectly controls EC polarity toward the flow axis and anti-inflammatory effects *in vitro*

A, Representative images of control and PAR-3KD ECs treated with control or GSK3β inhibitor (6-BIO, 1μM) under static condition or after subjected to 18dyn/cm^2 flow for 120 min. The direction of flow is indicated on the picture. EC junction (VE-cadherin) is green, Golgi (GM130) is red, and nuclear stain (DAPI) is blue. B, Quantitative analysis of the percentile of ECs polarized at 90° degrees toward the flow direction. C, Nuclear translocation of p65 on the control (scrambled) and PAR-3 KD ECs, subjected to 12 dyn/cm^2 flow for 60 min, with culture medium containing control (DMSO) or GSK3β inhibitor (1μM 6-BIO). D, Quantification and statistical analysis/ In B and D, data are means ± SEM of three independent experiments, n=100 cells from each experiment; ns: not significant, P≥0.05; differences: statistical significance (*p < 0.05; **p<0.01; ***p<0.001) was evaluated with one-way ANOVA with Tukey's multiple comparison post hoc analysis. Scale bars, 10 μm (A) and 25 μm (C).