

SUPPLEMENTARY MATERIAL

“Dynamic and functional changes in vascular endothelial cell monolayers under combined hypoxic and inflammatory stimuli” by Sone et al.

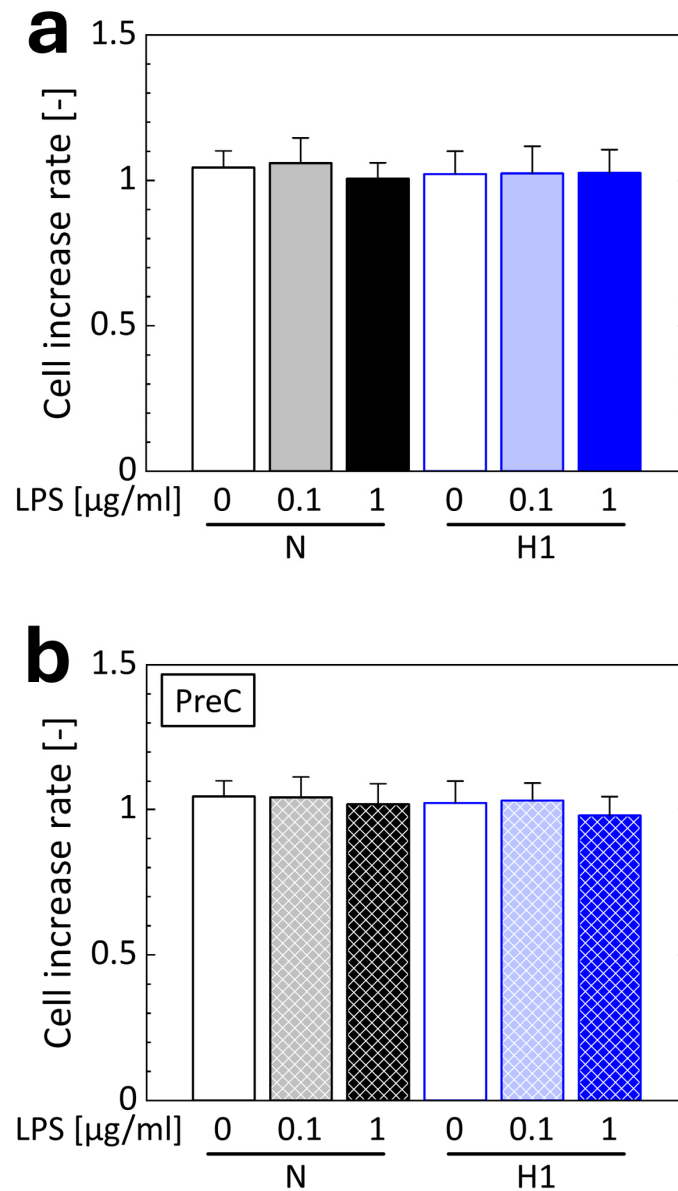


Fig. S1 Cell increase rate during the experimental period after 6 h of exposure to hypoxia and LPS with/without preconditioning (PreC) by LPS. Cell increase rates were calculated by measuring HUVEC density from phase-contrast microscope images at both the beginning and end of 6-h time-lapse observations under (a) acute or (b) long inflammatory stimulus with LPS and different oxygen conditions. Error bars show the standard deviation. No significant differences were detected among conditions by two-way ANOVA followed by post hoc Tukey's test for multiple comparisons.

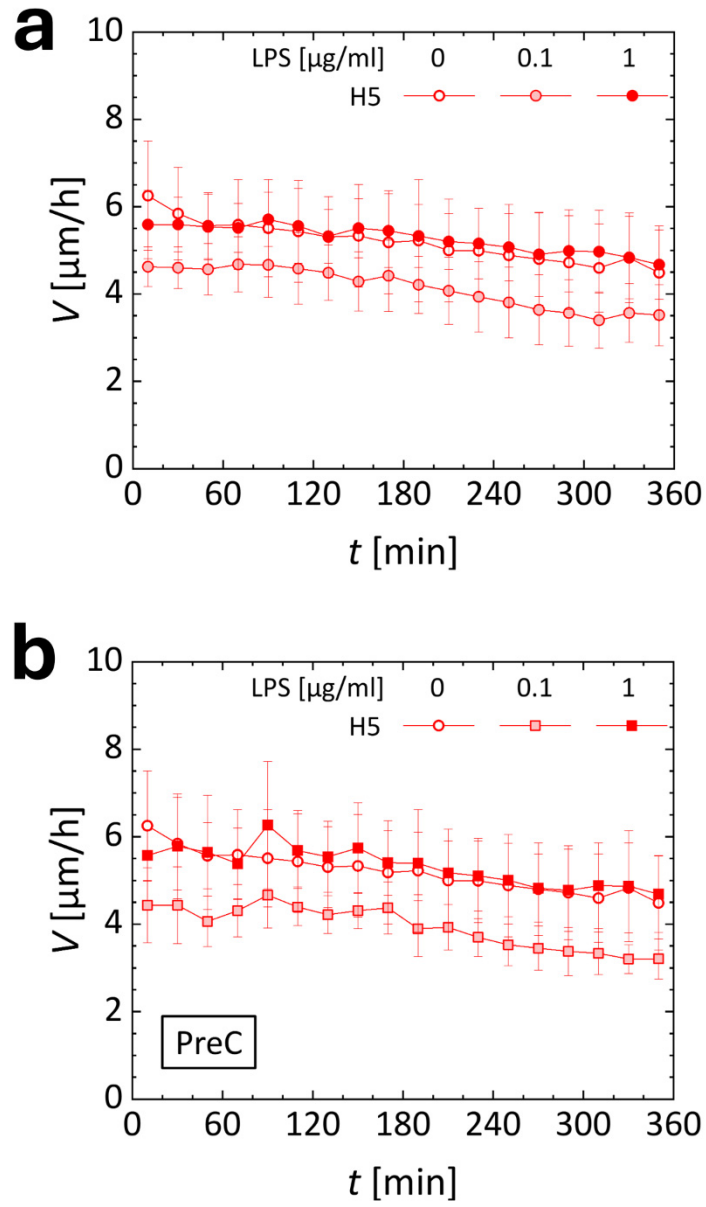


Fig. S2 Collective migration of HUVECs under exposure to hypoxia (H5: 5.3% O₂) and LPS at different concentrations of 0, 0.1, or 1 $\mu\text{g/ml}$. Average migration speed, V , over time for 6 h **(a)** without and **(b)** with preconditioning (PreC) by LPS.

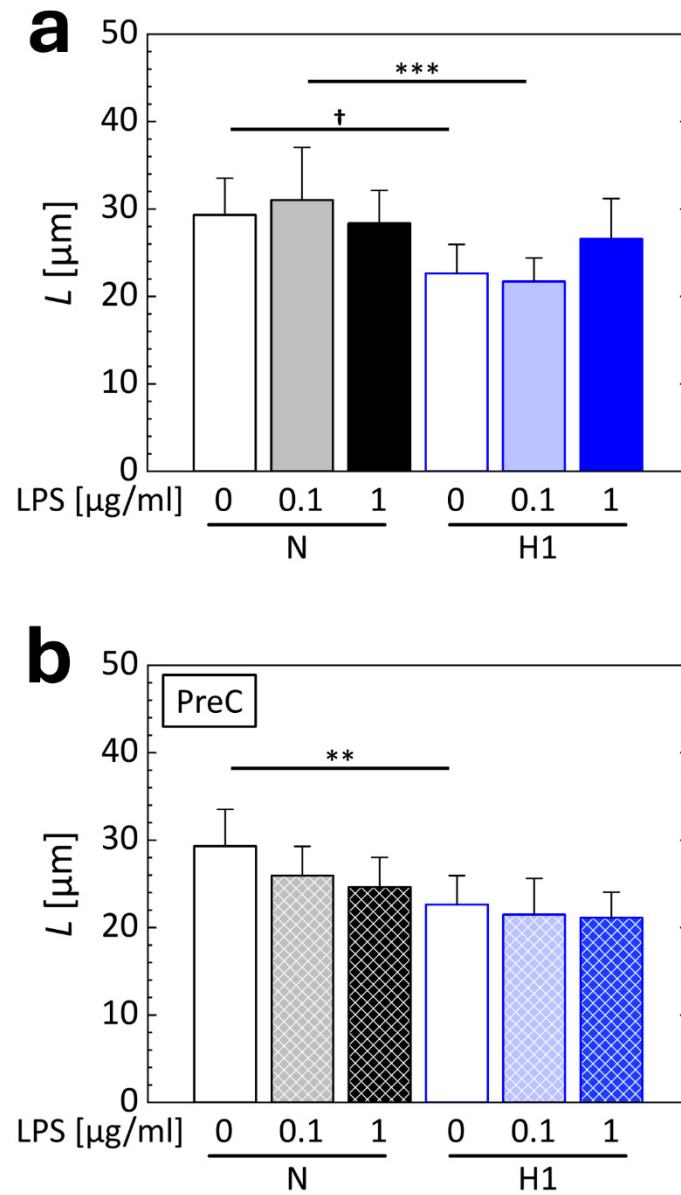


Fig. S3 Total migration distance during 6-h of exposure to hypoxia and LPS with/without preconditioning (PreC) by LPS. The total migration distance L was calculated by integrating the migration speed over time (multiplying the migration speed by the time interval and summing these values) under **(a)** acute or **(b)** long inflammatory stimulus with LPS and different oxygen conditions. Error bars show the standard deviation. Significant differences in migration distance between the oxygen and LPS conditions were assessed by two-way ANOVA followed by post hoc Tukey's test for multiple comparisons. $\dagger P < 0.1$; $** P < 0.01$; $*** P < 0.001$.

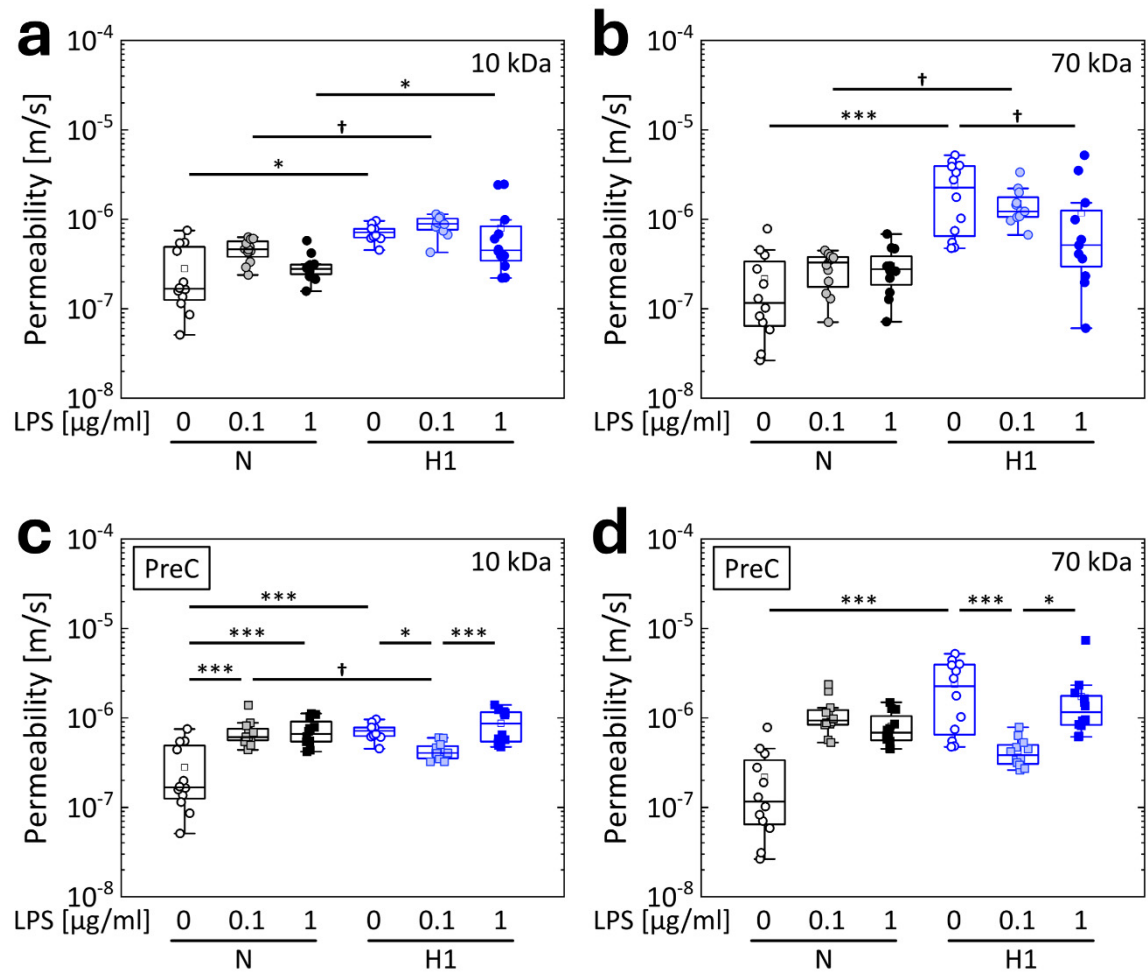


Fig. S4 Box-whisker plots of permeability values P_m of HUVEC monolayers under different oxygen and LPS conditions measured by observing diffusion of fluorescently labeled dextran of different molecular sizes at four arbitrary locations in three microfluidic devices ($N = 3$, 12 interfaces in total): **(a)** 10 kDa or **(b)** 70 kDa under acute inflammatory stimulus, and **(c)** 10 kDa or **(d)** 70 kDa under long inflammatory stimulus (preconditioning (PreC)). Significant differences in permeability between the oxygen and LPS conditions were assessed by two-way ANOVA followed by post hoc Tukey's test for multiple comparisons. $\dagger P < 0.1$; $* P < 0.05$; $*** P < 0.001$.

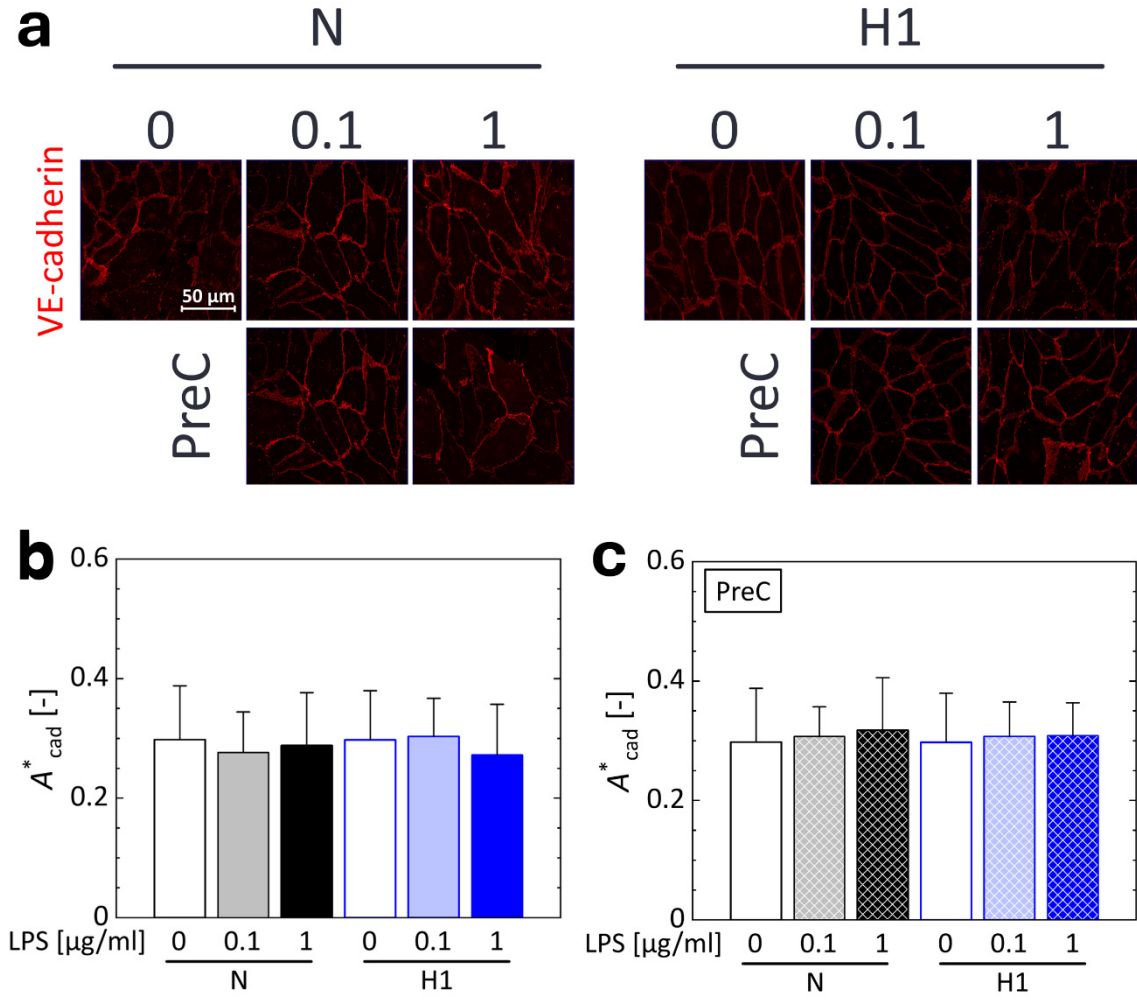


Fig. S5 Expression and localization of VE-cadherin in HUVECs after 6 h of exposure to hypoxia and LPS with/without preconditioning (PreC) by LPS. **(a)** Representative images of maximum intensity projections of confocal microscopy images of HUVECs to the xy -plane. VE-cadherin was stained with red. Variation of the ratio A_{cad}^* of the VE-cadherin area to the total cell area under **(b)** acute or **(c)** long inflammatory stimulus with LPS and oxygen conditions. Error bars show the standard deviation. No significant difference in the values of A_{cad}^* between the oxygen and LPS conditions was confirmed by two-way ANOVA followed by post hoc Tukey's test for multiple comparisons.