Table S1. List of endothelial cell donors. HPMEC – human pulmonary microvascular endothelial cells, HDMEC – human dermal microvascular endothelial cells, HPAEC – human pulmonary artery endothelial cells

Donor #	*type*	*Company*
525116	HPMEC	Lonza
612039	HPMEC	Lonza
560121	HPMEC	Lonza
560758	HPMEC	Lonza
429Z007.1	HPMEC	PromoCell
431Z036.1	HPMEC	PromoCell
435Z034.2	HDMEC	PromoCell
423Z018.1	HDMEC	PromoCell
28074	HPAEC	Lonza
21304	HPAEC	Lonza
27930	HPAEC	Lonza

Table S2. List of all agonists, antagonists and inhibitors used in this study.

Agonists	*Target*	*Company*	*Cat #*	*stock [c]*	*diluent*
PGD$_2$	DP1, DP2	CaymanChem	12010	30 mM	EtOH
PGE$_2$	EP1-4	CaymanChem	14010	30 mM	EtOH
DK-PGD$_2$	DP2	CaymanChem	12610	30 mM	EtOH
BW245c	DP1	CaymanChem	12050	30 mM	EtOH
S1P	S1PRs	Sigma-Aldrich	73914	125 μM	0.1% BSA in a.d.
Antagonists					
ONO-AE3-208	EP4	CaymanChem	14522	10 mM	EtOH
BWA868c	DP1	CaymanChem	12060	30 mM	EtOH
Cay10471	DP2	CaymanChem	10006735	10 mM	EtOH
MK 0524	DP1	CaymanChem	10009835	30 mM	EtOH
T0070907	PPARγ	CaymanChem	10026	100 mM	DMSO
GW627368X	EP4	CaymanChem	10009162	10 mM	DMSO
Inhibitors					
Diclofenac	COX-1/2	Sigma-Aldrich	D6899	3 mM	a.d.

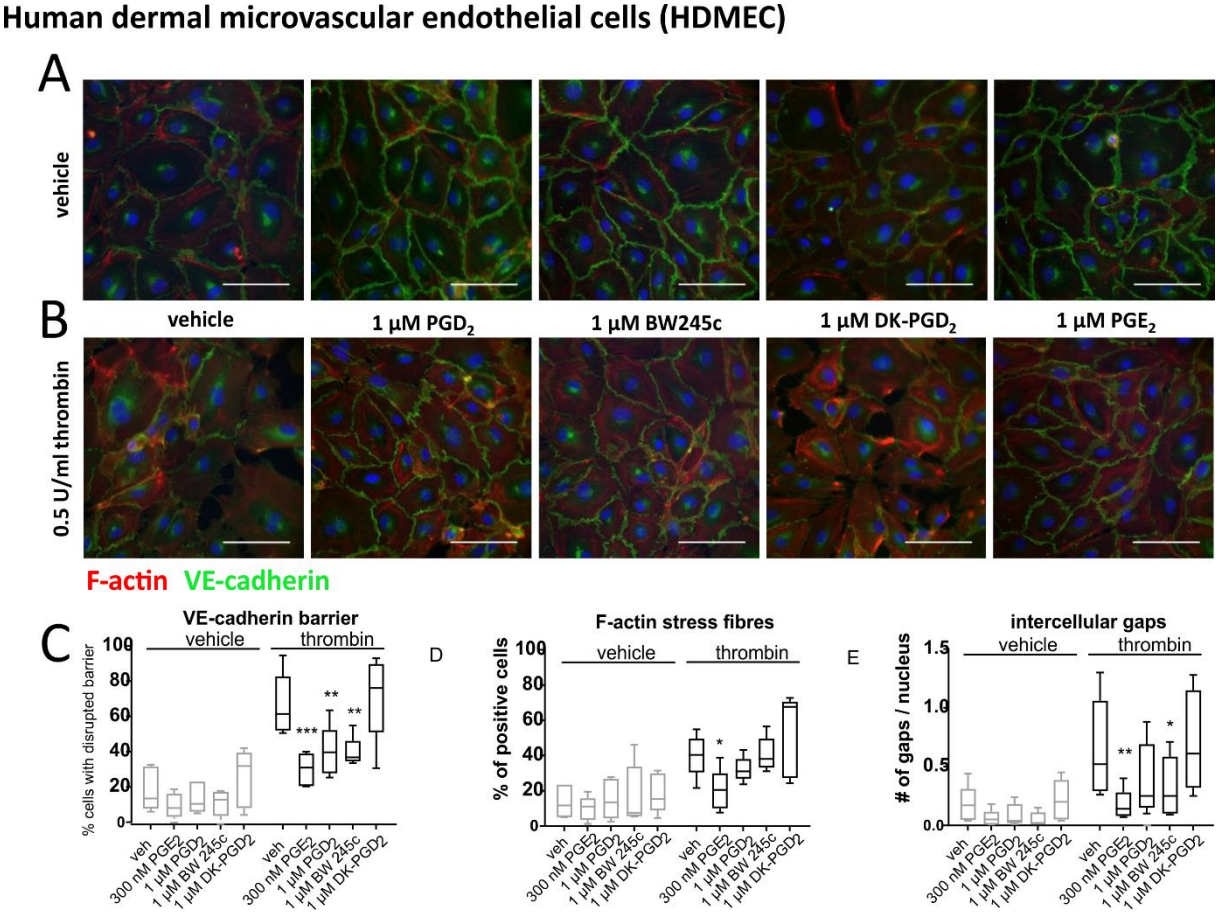

Figure S1. PGD_2 and DP_1 agonist BW245c but not DP2 agonist DK-PGD_2 protect HDMEC against thrombin-induced barrier disruption. Representative images of 5 independent experiments of VE-cadherin and F-actin stained, confluent HDMEC were stimulated with vehicle (EtOH), 1 µM PGD_2, BW245c, DK-PGD_2 or 300 nM PGE_2 for 15 minutes followed by incubation with (A) vehicle (a.d.) or (B) 0.5 U/ml thrombin for 15 minutes (Scale bar 100 µm). Extent of barrier disruption was evaluated by quantifying (C) the percentage of cells with disrupted barrier, (D) the percentage of cells with actin stress fibres and (E) the ratio of intercellular gaps per nuclei. Data are displayed as box and whisker plot, n = 5, two-way ANOVA with Fisher's LSD post hoc test, * $p<0.05$, ** $p<0.01$, *** $p<0.001$ difference between veh/thrombin vs. agonist.

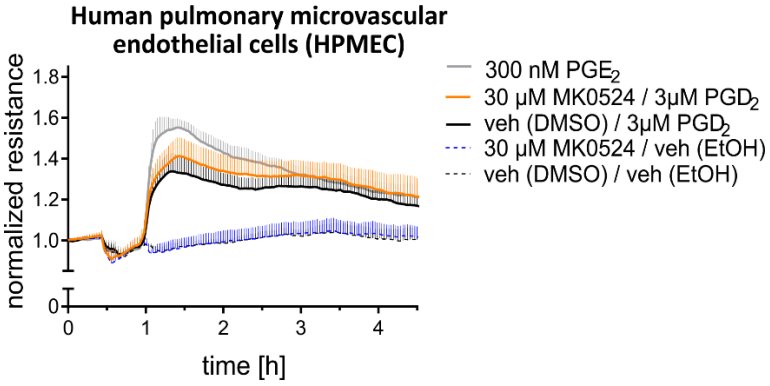

Figure S2. DP1 antagonist MK0524 does not block PGD_2-induced barrier enhancement in HPMEC. Pre-treatment with 30 µM of MK0524 did not affect PGD_2-induced increase in resistance. Data are displayed as mean + SEM, n = 5, two-way ANOVA for repeated measurements with Tukey's post hoc test, # $p<0.05$, ## $p<0.01$, ### $p<0.001$ difference between veh (DMSO) / 3 µM PGD_2 vs. 30 µM MK0524 / 3 µM PGD_2.

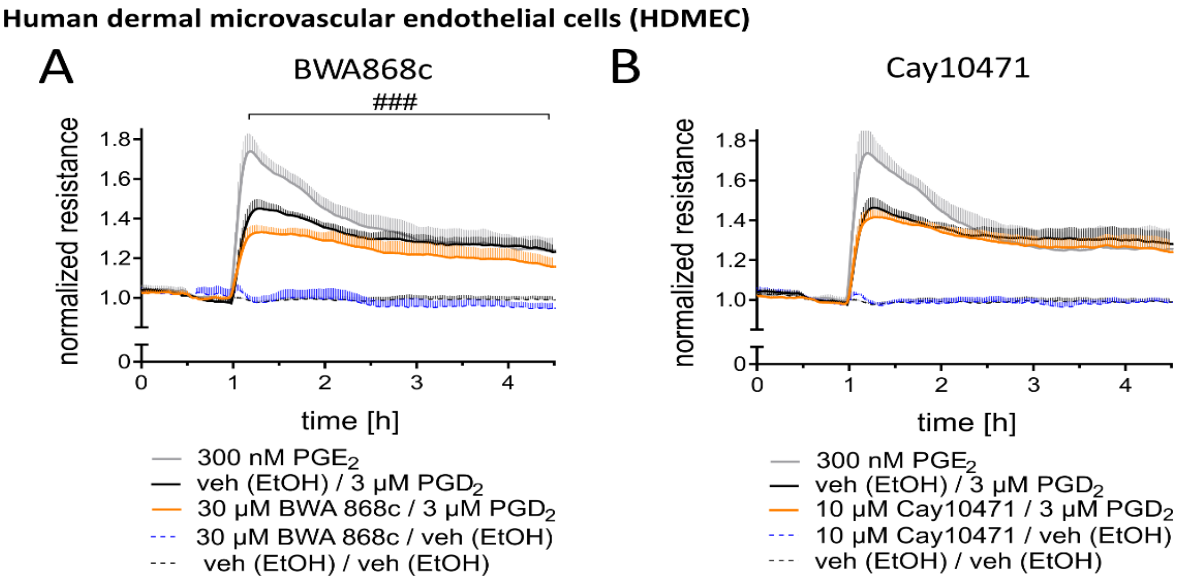

Figure S3. DP1 and DP2 receptor antagonists do not prevent the PGD_2-induced barrier increase in HDMEC. (A) Pre-treatment of HDMEC with 30 µM of the DP1 antagonist BWA868c for 30 minutes significantly diminished the PGD_2-induced barrier increase. (B) Pre-treatment with 10 µM of the DP2 antagonist Cay10471 did not affect PGD_2-induced increase in resistance. Data are displayed as mean + SEM, n = 5. Two-way ANOVA for repeated measurements with Tukey's post hoc test, # $p<0.05$, ## $p<0.01$, ### $p<0.001$ difference between veh (DMSO) / 3 µM PGD_2 vs. 30 µM BWA868c / 3 µM PGD_2.

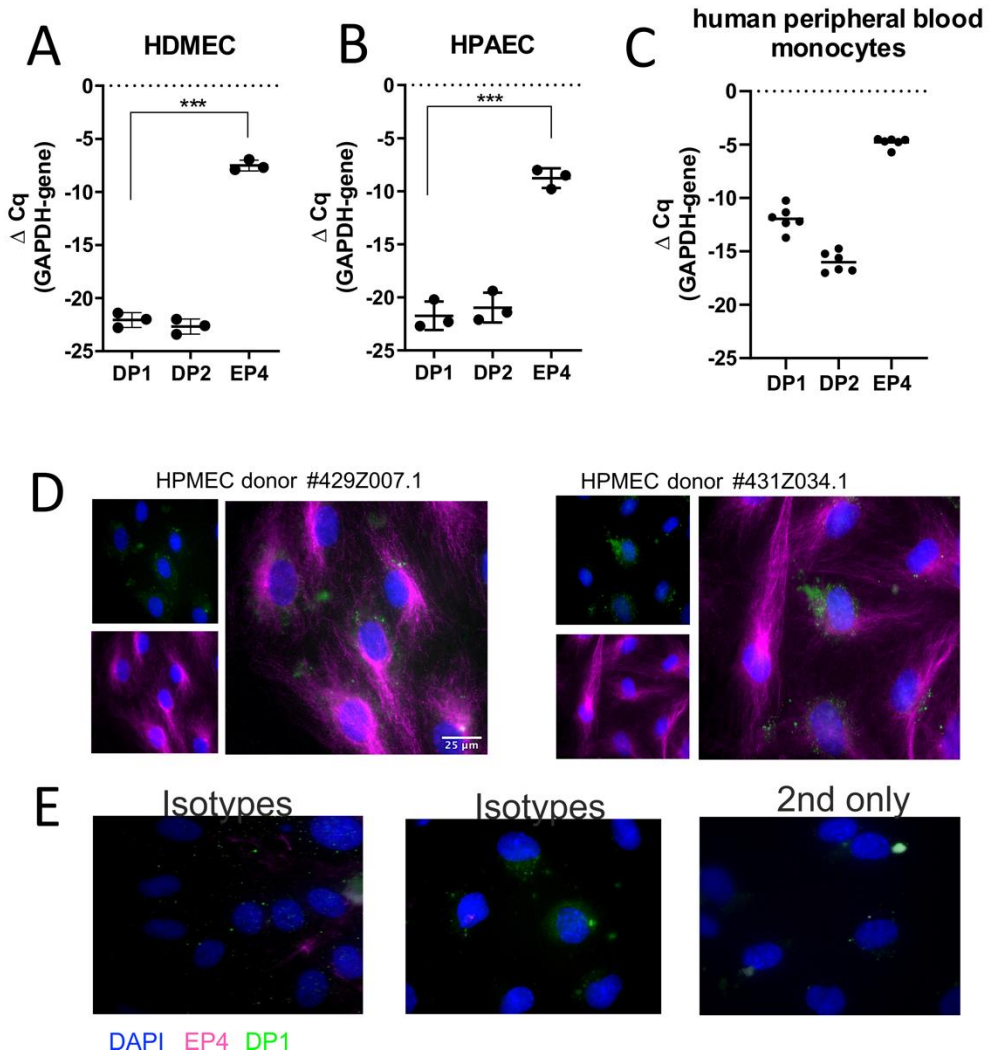

Figure S4. EP4, DP1, DP2 receptor mRNA expression levels in HDMEC, HPAEC and human peripheral blood monocytes, and EP4 / DP1 immunofluorescence staining in HPMEC. Quantitative RT-PCR analysis showed low DP1 and DP2 receptor mRNA expression in (A) HDMEC and (B) HPAEC during steady state, while EP4 receptor mRNA levels were significantly higher. (C) In human peripheral blood monocytes, DP1 receptor was expressed at higher levels than in endothelial cells and DP2 mRNA could robustly be detected. Likewise, EP4 receptor was more highly expressed than DP receptors in monocytes. (D) Immunofluorescence images from 2 HPMEC donors showing EP4 and DP1 staining during steady state. (E) Staining controls (EP4/mouse and DP1/rabbit IgG and 2nd antibody-only) on HPMEC (representative of 3 independent experiments). Data are shown as mean ± SD, n = 3-6, one-way ANOVA with Tukey's post hoc test, n.d. – non detectable, ***$p<0.001$.

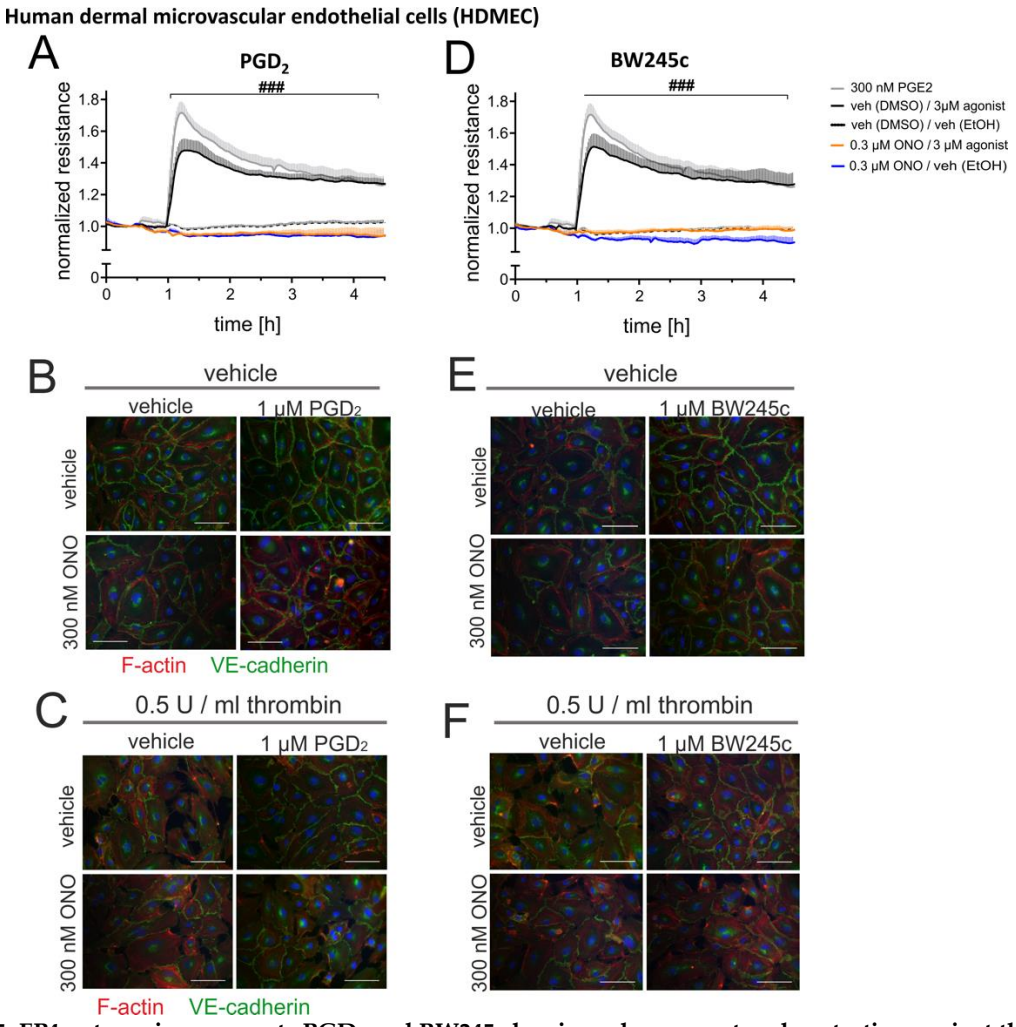

Figure S5. EP4 antagonism prevents PGD_2 and BW245c barrier enhancement and protection against thrombin-induced barrier disruption in HDMEC. Pre-treatment of HDMEC with 300 nM ONO-AE3-208 (ONO; EP4 antagonist) for 30 minutes completely abolished (A) PGD_2 as well as (B) BW245c-induced barrier increase. VE-Cadherin and F-actin staining revealed that pre-treatment with the EP4 antagonist before (C) PGD_2 or (D) BW245c stimulation inhibits enhancement of VE-Cadherin staining. In the thrombin barrier disruption assay, EP4 antagonism abolished the barrier protective effect by (E) PGD_2 or (F) BW245c in HDMEC (representative images of 5 independent experiments are shown, scale bar 100 µm). Data are displayed as mean + SEM, n = 5, two-way ANOVA for repeated measurements with Tukey's post hoc test, # $p<0.05$, ## $p<0.01$, ### $p<0.001$ difference between veh (DMSO) / 3 µM PGD_2 or BW245c vs. 300 nM ONO-AE3-208 / 3 µM PGD_2 or BW245c.

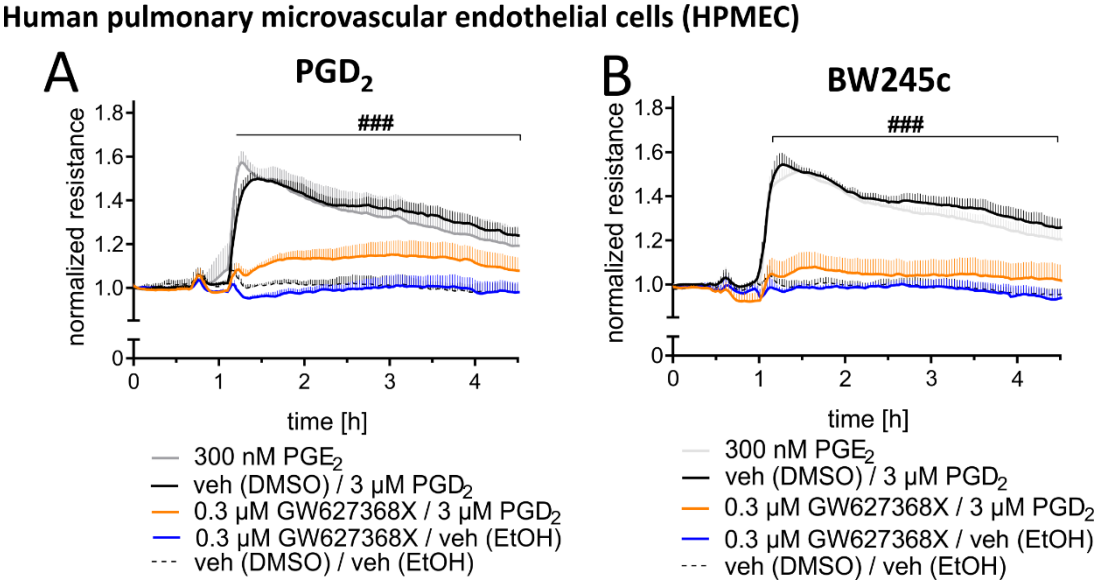

Figure S6. EP4 antagonist GW627368X strongly attenuates the barrier-protective effect of PGD_2 and BW245c. Pre-treatment of HPMEC with 300 nM GW6227368X for 30 minutes significantly reduced (A) PGD_2- as well as (B) BW245c-induced barrier increase. Data are displayed as mean + SEM, n = 5, two-way ANOVA for repeated measurements with Tukey's post hoc test, #p<0.05, #p0.01, ###p<0.001 difference between veh (DMSO)/ 3 µM PGD_2 or BW245c vs. 300 nM GW627368X/3 µM PGD_2 or BW245c.

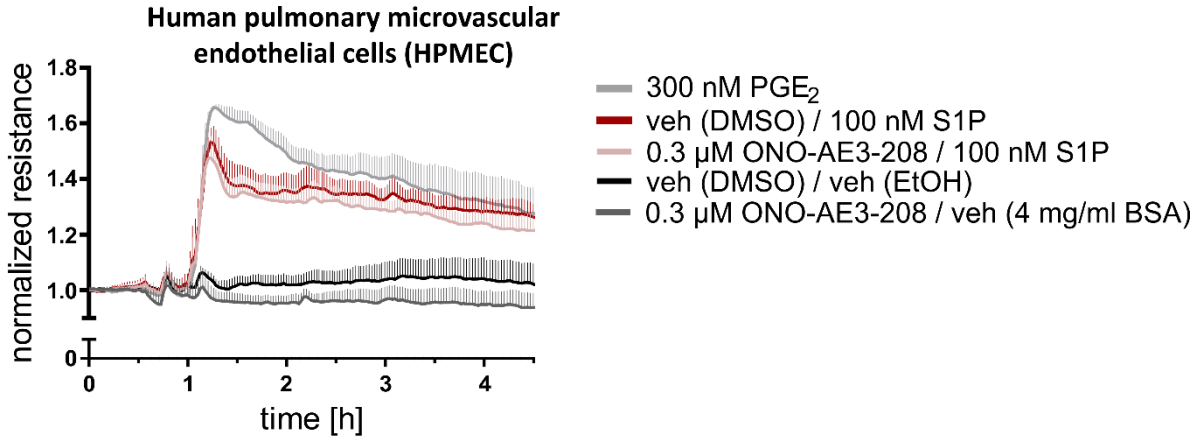

Figure S7. ONO-AE3-208 does not inhibit sphingosine-1-phosphate-induced barrier enhancement in HPMEC. Pre-treatment of HPMEC with 0.3 µM ONO-AE3-208 for 30 minutes did not affect subsequent sphingosine-1-phosphate- (S1P) induced barrier enhancement. Data are displayed as mean + SEM, n = 4, two-way ANOVA for repeated measurements with Tukey's post hoc test.

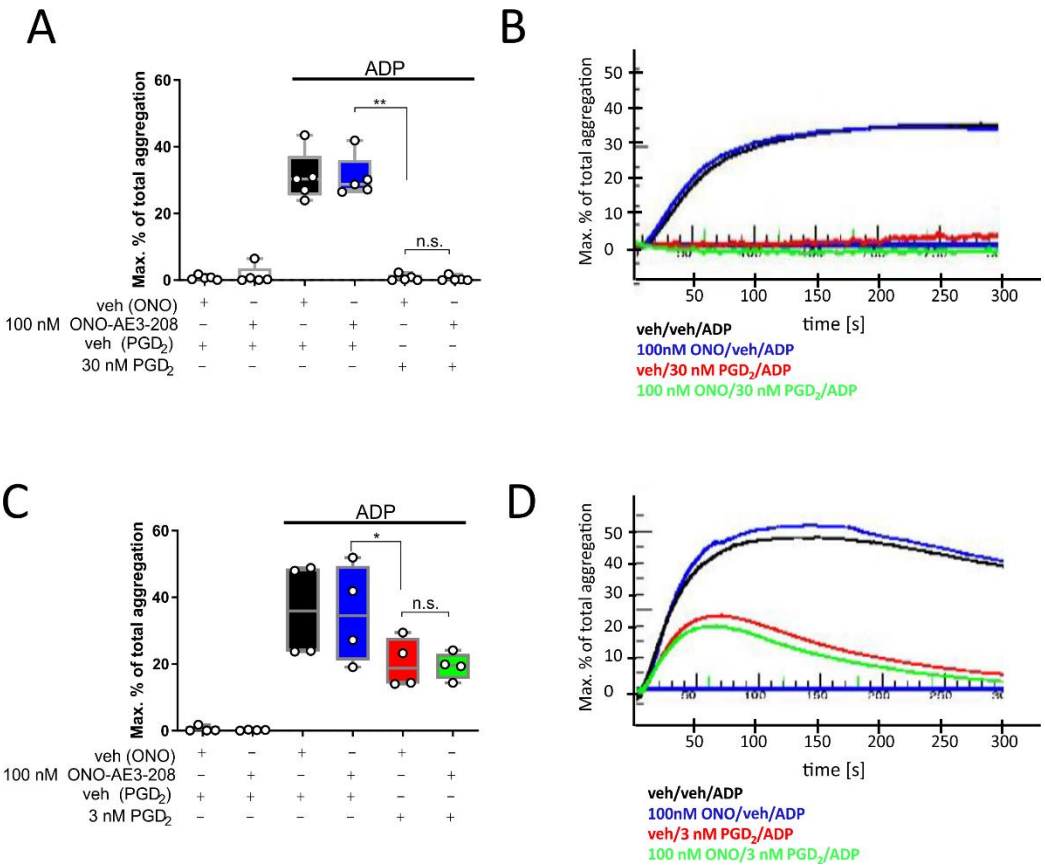

Figure S8. ONO-AE3-208 does not affect PGD_2-induced inhibition of platelet aggregation. (A) Pre-treatment of washed platelets with 100 nM of ONO-AE3-208 for 10 minutes did not affect the complete inhibition of aggregation caused by 30 nM of PGD_2. Pre-treatment with ONO-AE3-208 alone did not affect ADP-induced platelet aggregation. (B) Representative graph from one donor showing the aggregatory time course using 30 nM PGD_2 to inhibit aggregation. (C) Further, ONO-AE3-208 did not reverse the partial inhibition of platelet aggregation induced by a low concentration (3 nM) of PGD_2. (D) Representative graph from one donor showing the aggregatory time course using 3 nM PGD_2 to inhibit aggregation. Data are shown as box and whisker plot, n = 4-5, one-way ANOVA for repeated measurements with Tukey's post hoc test, * $p<0.05$.

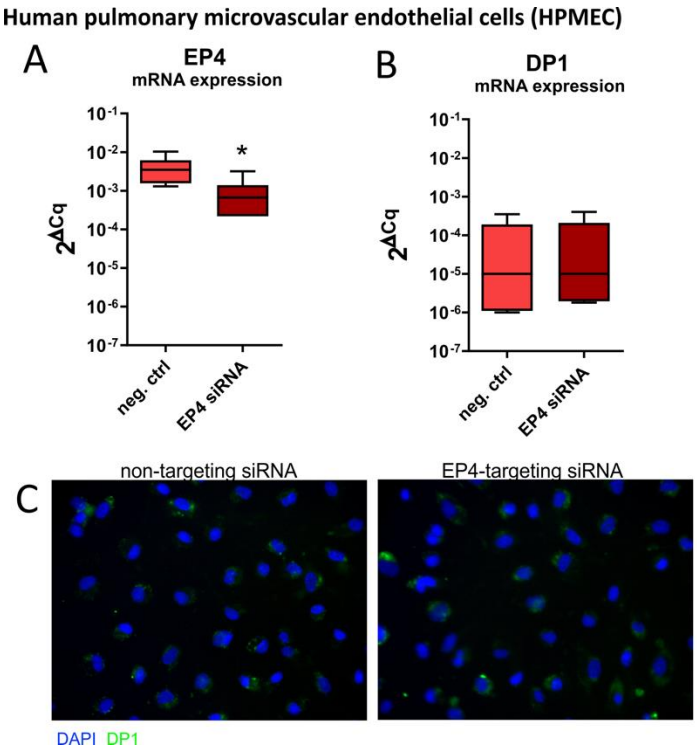

Figure S9. Transient knock-down of EP4 receptor does not affect DP1 receptor expression in HPMEC. PTGER4-targeting siRNA (A) reduced EP4 receptor mRNA levels but (B) did not affect DP1 receptor mRNA expression levels. Data are shown as box and whisker plot, n = 5, Student's t-test, difference between control siRNA- vs. EP4 siRNA-transfected cells *p<0.05. (C) Immunofluorescence staining of DP1 receptor in HPMEC transfected with non-targeting or EP4-targeting siRNA (representative of 3 experiments).

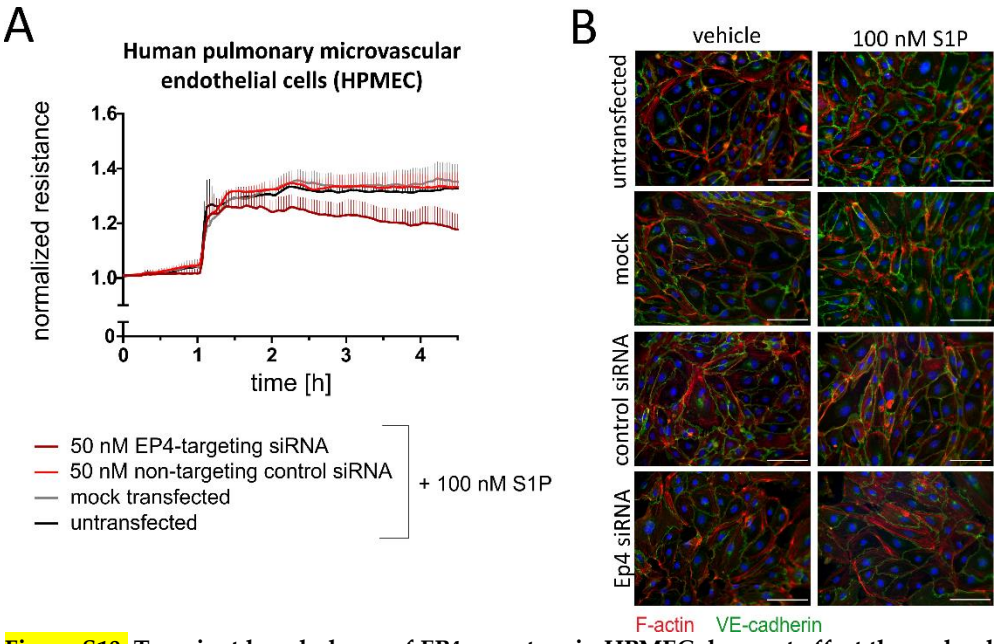

Figure S10. Transient knock-down of EP4 receptors in HPMEC does not affect the early phase of sphingosine-1-phosphate barrier enhancement. Primary HPMEC were transfected with PTGER4-specific or control siRNA for 48 h. To rule out a harmful or off-target effect of EP4 receptor knock down, cells were stimulated with sphingosine-1-phosphate. (A) EP4 receptor knock down did not reduce S1P-induced increase in endothelial resistance or (B) barrier protective effect against thrombin-induced barrier disruption (representative of 3 experiments). Data are shown as mean + SEM, n = 4.