ScienceAdvances

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Supplementary Materials for

The matrikine N-α-PGP couples extracellular matrix fragmentation to endothelial permeability

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Published 24 April 2015, *Sci. Adv.* **1**, e1500175 (2015)
DOI: 10.1126/sciadv.1500175

This PDF file includes:

SUPPLEMENTAL MATERIALS

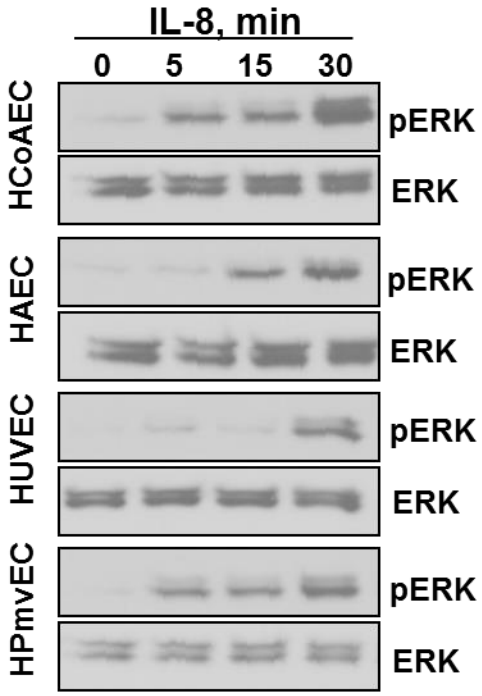

Fig. S1. Multiple endothelial cells demonstrate ERK phosphorylation with IL-8 stimulation. HUVEC, pulmonary microvascular (HPmvEC), coronary artery (HCoAEC), and aortic (HAEC) endothelial cells were grown to confluence, serum starved for two hours, and then stimulated with100 ng/ml IL-8 for 0-30 min. pERK was assessed by western blot analysis. Representative results from three independent experiments are shown.

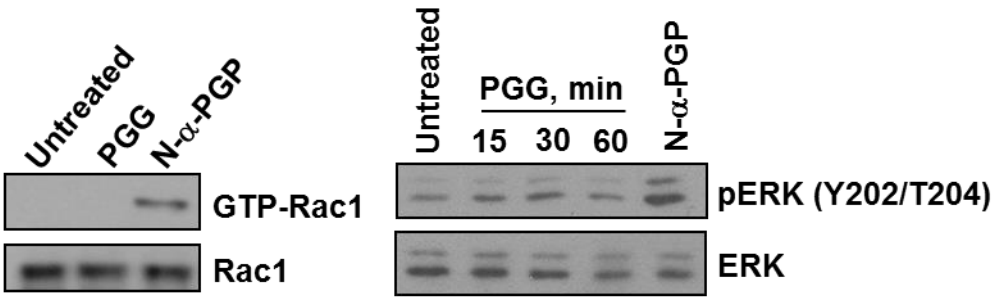

Fig. S2. PGG stimulation does not activate HUVECs. HUVECs were serum starved for two hours before stimulation with 0.5 mg/ml PGG for 0-60 min or with 0.5 mg/ml N-α-PGP for 60 min and activation of Rac1(GTP-Rac1) **(A)** and phosphorylation of ERK **(B)** were determined by Western blot.

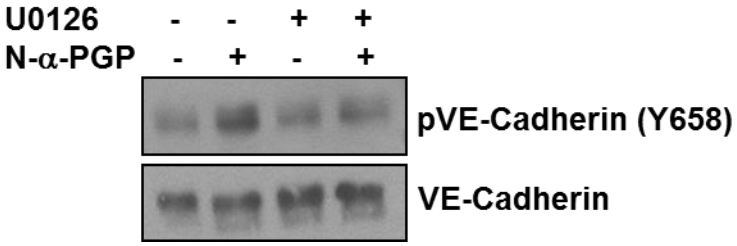

Fig. S3. Blockade of ERK mitigates N-α-PGP-mediated VE-cadherin activation in endothelial cells. HUVECs were untreated or treated with 0.5 mg/ml N-α-PGP alone or after pretreatment with 10 μM U0126 and phosphorylation of VE-Cadherin were determined by Western blot.

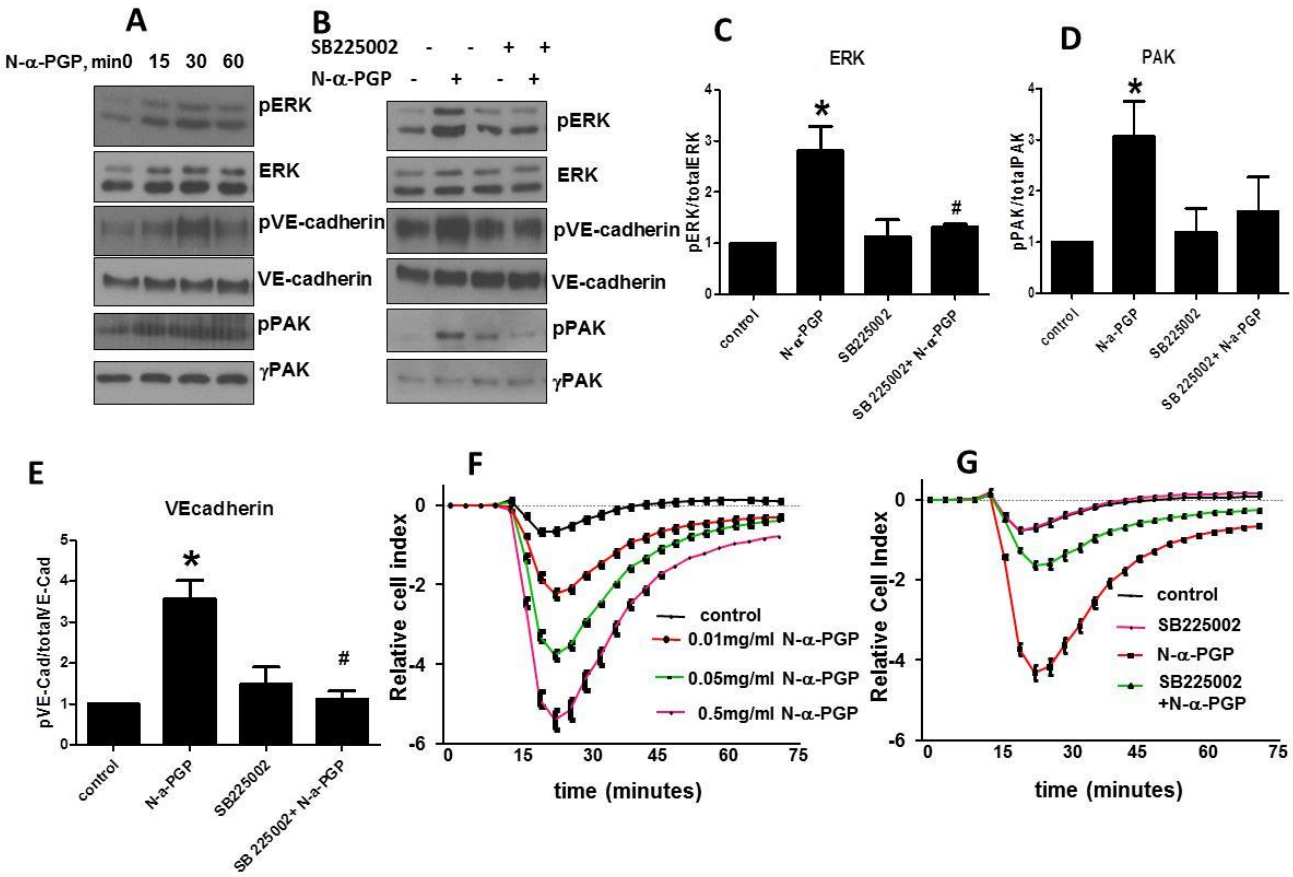

Fig S4: Effects of N-α-PGP on PMVEC permeability. (A) PMVECs were serum starved for two hours before stimulation with 0.05mg/ml N-α-PGP and time-dependent activation of ERK, VE-cadherin and PAK determined. **(B-E)** N-α-PGP-dependent activation of ERK, VE-cadherin and PAK was determined at 15min in the presence of absence of SB225002. Shown are representative Western blots together with quantification. Bar graphs show mean ± SEM, n = 3 *P<0.05 relative to time 0 by 1-way ANOVA with Tukey post-test. Shown are representative Western blots together with quantification. Bar graphs show mean ± SEM, n = 3 *P<0.05 relative to control, $^{\#}$P<0.05 relative to N-α-PGP by 1-way ANOVA with Tukey post-test. Changes in permeability in response to different doses of N-α-PGP peptide **(F)** and in the presence or absence of SB225002 **(G)**. Graphs present mean ± SEM from representative experiments.

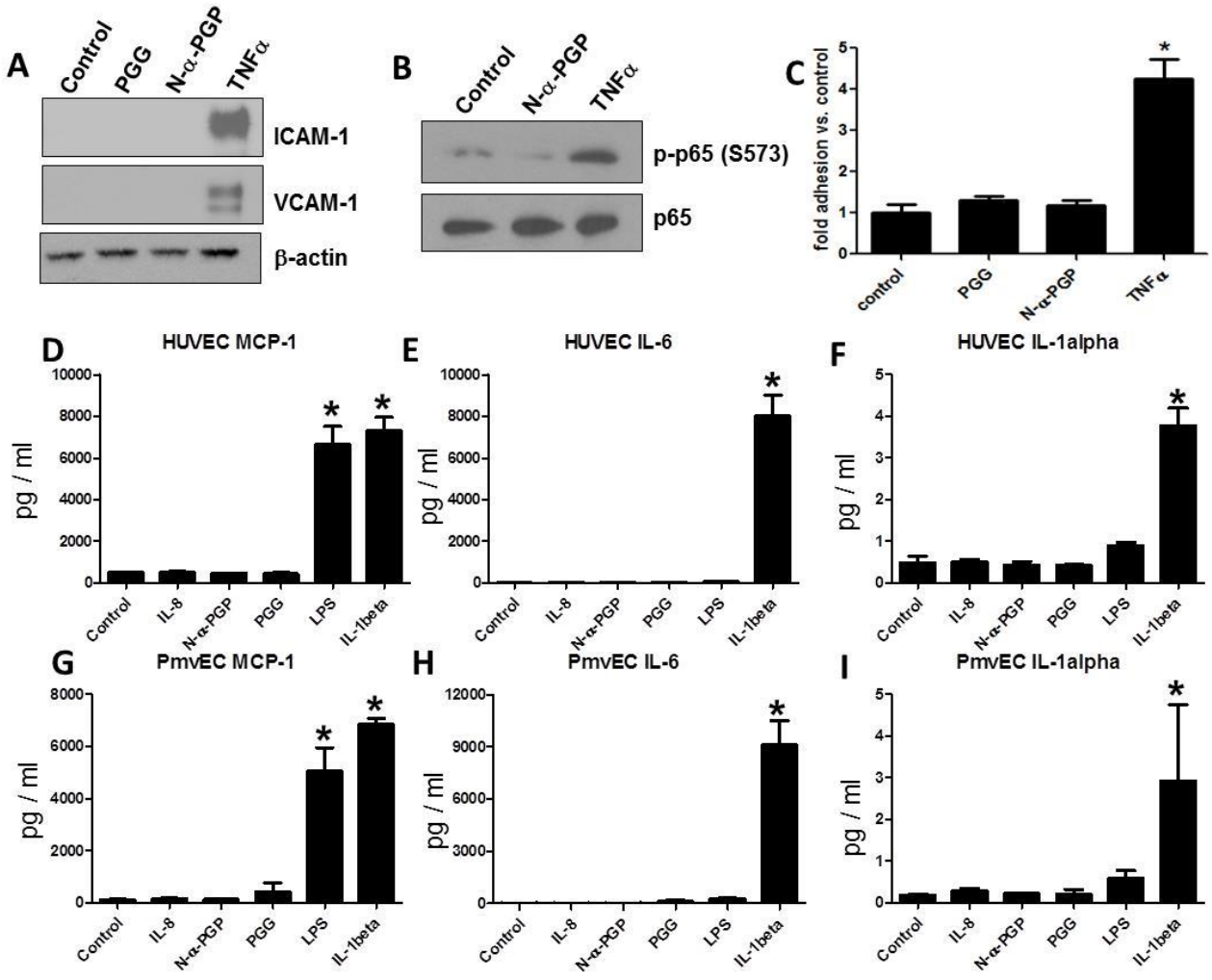

Fig. S5. N-α-PGP does not induce proinflammatory signaling in endothelial cells. HUVEC were untreated or treated with 0.5 mg/ml N-α-PGP, 0.5 mg/ml PGG control peptide, or 10 ng/ml TNF**α** for six hours. Lysates were collected and Western blot analysis was conducted for ICAM-1, VCAM-1 and b-actin **(A)** and for phosphorylation of p65 **(B)**. Cells were treated as described above and their ability to support THP-1 monocyte adhesion was determined **(C)**. Blots are representative of three separate experiments and THP-1 adhesion is representative of two separate experiments with 4-6 replicates per condition in each experiment. HUVEC **(D-F)** or PMVEC **(G-I)** were treated with IL8 (100ng/ml), N-α-PGP (0.05mg/ml), PGG (0.1mg/ml), LPS (100ng/ml) or IL1β (1ng/ml) for 12h and indicated cytokines measured. Data shown mean ± SEM (n=3), *P<0.05 relative to control by 1-way ANOVA with Tukey post-test.