Supporting information for:

Pro-inflammatory cytokine-induced and constitutive cleavage of Endomucin from the endothelial surface is mediated by ADAM10 and ADAM17

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Running title: EMCN cleavage by ADAM10 and ADAM17 under inflammation

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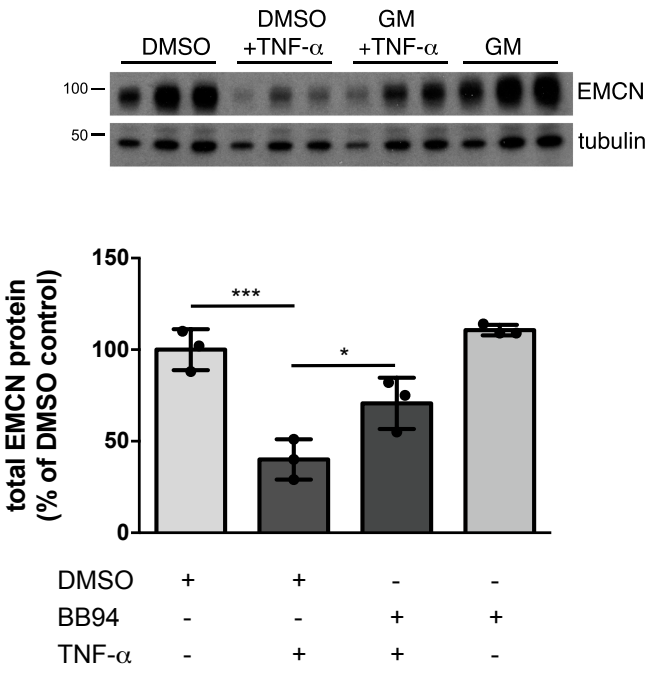

Figure S1. The pan-MMP inhibitor GM6001 blocks TNF-α-induced reduction of total EMCN protein. After overnight serum starvation, confluent HUVECs were pretreated with a pan-MMP inhibitor GM6001 (GM; 10 μM) or DMSO as vehicle control for 30 minutes before TNF-α treatment (1 ng/ml, 24 hours). Total cell lysates were harvested for protein analysis. GM6001 blocked TNF-α-induced reduction of total EMCN protein. n = 3, One-way ANOVA. * $P < 0.05$, *** $P < 0.001$

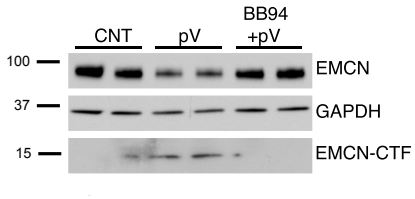

Figure S2. The pan-MMP inhibitor BB94 blocks pervanadate-induced reduction of total EMCN protein and the release of EMCN-CTF. After overnight serum starvation, confluent HUVECs were pretreated with a pan-MMP inhibitor BB94 (5 μM) or DMSO as vehicle control for 30 minutes before the treatment of pervanadate (50 μM) for 30 minutes. Total cell lysates were harvested for protein analysis. BB94 blocked pervanadate-induced reduction of total EMCN protein as well as the release of EMCN-CTF detected by an antibody against C-terminal region of EMCN (NBP1-71558, Novus).

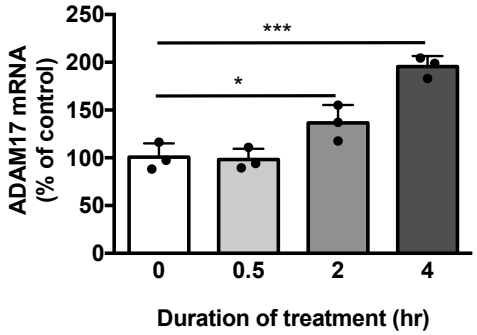

Figure S3. TNF-α treatment increases ADAM17 gene expression. Following overnight serum starvation, confluent HUVECs were treated with TNF-α (1 ng/ml) for varying times (0.5~4 hours). Total mRNA was harvested for gene expression analysis. ADAM17 mRNA levels were increased as early as 2 hours after TNF-α treatment, and was further elevated at 4-hour time point. n = 3, One-way ANOVA. * $P < 0.05$, *** $P < 0.001$

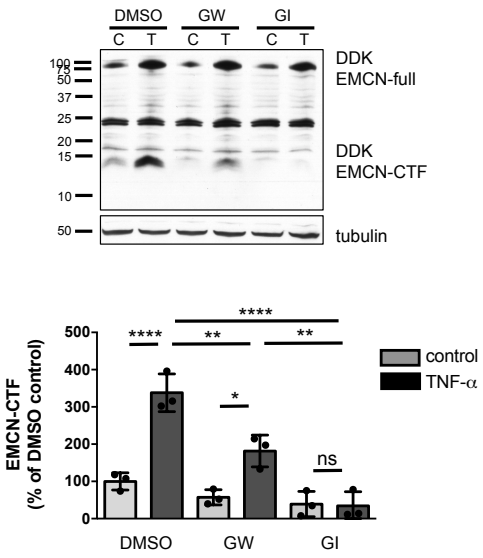

Figure S4. Inhibition of ADAM10 and ADAM17 blocks TNF-α-induced EMCN-CTF release. HUVECs were transduced with AdEMCN or AdGFP for 24 hours followed by overnight serum starvation. The cells were pretreated with GW280264 (GW; 10 μM), GI254023X (GI; 5 μM) or DMSO as vehicle control for 30 minutes before TNF-α stimulation (1 ng/ml) for 24 hours. Cell lysates were harvested for protein analysis. Pretreatment with either GW or GI reduced TNF-α-induced EMCN-CTF release. n = 3, One-way ANOVA. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

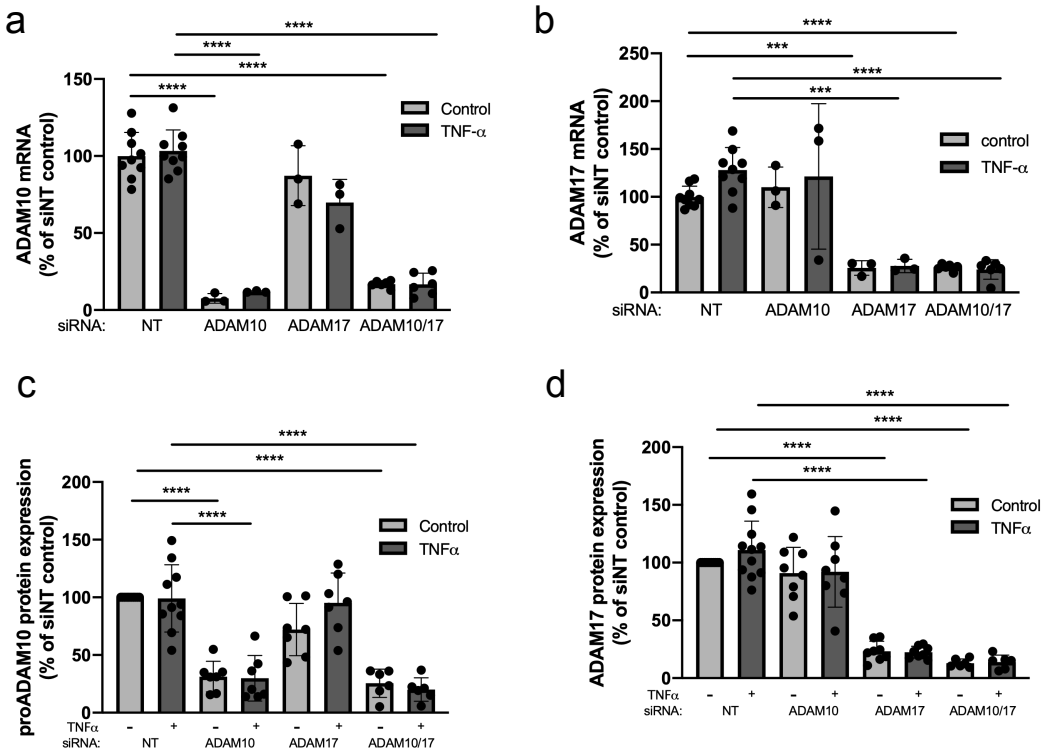

Figure S5. Knockdown of ADAM10 and ADAM17 by siRNA. Following overnight serum starvation, HUVECs at 70% confluency were treated with siRNA targeting ADAM10 (50 nM), ADAM17 (50 nM) or a combination of ADAM10/17 (50 nM) overnight before TNF-α treatment (1 ng/ml, 24 hours). **(a)** Total RNA was collected in RNA-Bee for q-PCR analysis using primers for **(a)** ADAM10 and **(b)** ADAM17. siRNA against ADAM10 and ADAM17 alone and in combination reduced message expression of ADAM10 and ADAM17. **(c-d)** Total protein lysate was harvested for analysis using western blot. siRNA against ADAM10 and ADAM17 alone and in combination reduced the protein expression of ADAM10 and ADAM17.

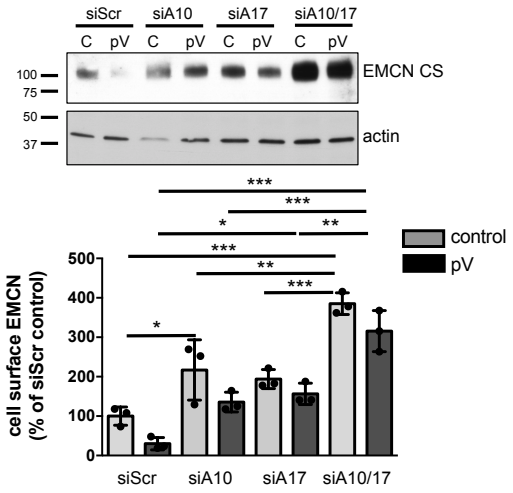

Figure S6. Inhibition of ADAM10 and/or ADAM17 blocks pervanadate-induced reduction of cell surface. Inhibition of ADAM10 and/or ADAM17 was achieved by siRNA knockdown (25 nmol on 1.20 x 10^5 cells, 72 hours). The cells were then treated with pervanadate (50 µM, 30 minutes). Cell surface protein were harvested for protein analysis. siRNA knockdown of ADAM10 alone or ADAM10 and ADAM17 together led to an increase in the basal level of cell surface EMCN protein. siRNA knockdown of ADAM17 alone or knockdown of ADAM10 and ADAM17 together blocked pervanadate-induced reduction of cell surface EMCN. siRNA knockdown of ADAM10 and ADAM17 had a synergistic effect on pervanadate-induced reduction of cell surface EMCN protein compared to knockdown of ADAM10 or ADAM17. n = 3, One-way ANOVA. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$