

Supplementary Material.

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**Compensatory increase of VE-cadherin expression through ETS1
regulates endothelial barrier function in response to TNF α**

Number of supplementary figures: 5

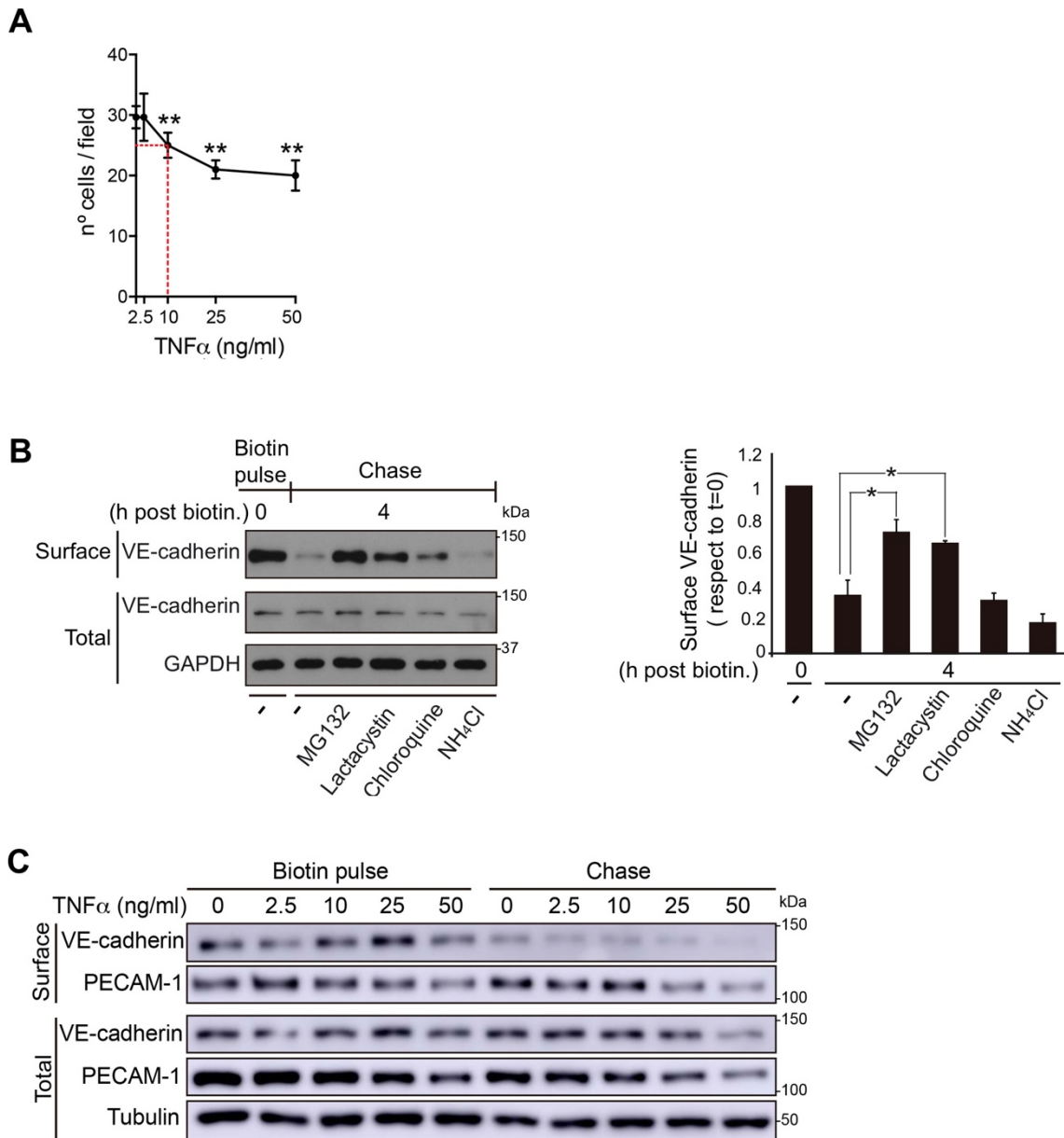


Figure S1. Effect of TNF α on cell density and surface VE-cadherin and PECAM-1 degradation. **(A)** HUVECs were stimulated with TNF α for 24 h at the indicated concentrations as in Fig. 1. Cells were fixed and stained for VE-cadherin and nuclei and the number of cells per field were quantified by confocal microscopy. Graphs show the mean \pm SEM from three independent experiments. **, $p < 0.005$. Discontinuous red line point at cell number decrease (15%) caused by TNF α at the concentration used through the manuscript. **(B)** Surface VE-cadherin degradation is

reduced in the presence of proteasome inhibitors. HUVECs stimulated with TNF α for 24 h were pulsed for 30 min with sulfo-NHS-biotin to label surface proteins and then lysed (chase) after 4 h of incubation at 37°C in the presence of 20 μ M MG132 and 10 μ M lactacystin as proteasome inhibitors, and of 300 mM chloroquine and 10 mM NH $_4$ Cl as lysosomal inhibitors, as indicated. Surface-biotinylated proteins were isolated by pull-down assay with neutravidin-agarose. Surface VE-cadherin levels were detected with a specific antibody from the pull-down fraction and compared with total VE-cadherin levels from the lysate. Immunoblot of GAPDH is shown as loading control. Graphs show the mean + SEM from three independent experiments. *, p<0.05. **(C)** HUVECs were stimulated with TNF α for 24 h at the indicated concentrations. Then cells were pulsed for 30 min with sulfo-NHS-biotin to label surface proteins and lysed (chase) after 4 h of incubation at 37°C. Surface-biotinylated proteins were isolated by pull-down assay with neutravidin-agarose. Surface VE-cadherin and PECAM-1 levels were detected with a specific antibody from the pull-down fraction and compared with total VE-cadherin and PECAM-1 levels from the lysate. Immunoblot of tubulin is shown as loading control. See also Fig. 1.

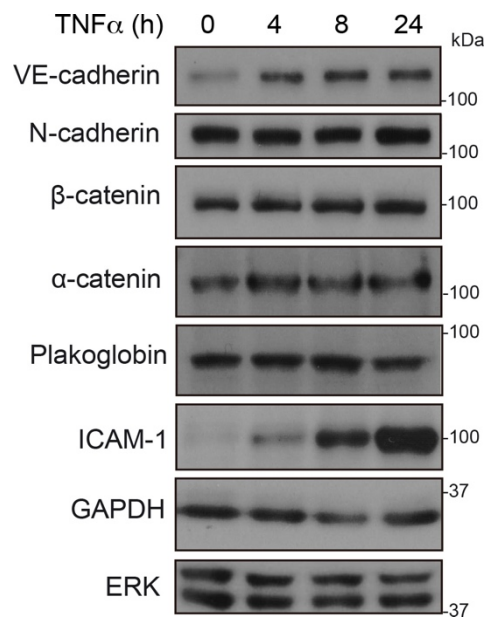


Figure S2. Effect of TNF α stimulation on endothelial junctional protein expression. HUVECs were cultured at confluence for 48 h on dishes pre-coated with fibronectin, starved for 12 h and then stimulated or not with 10 ng/ml TNF α for the indicated times. Cell lysates were immunoblotted with the indicated antibodies against junctional proteins. Note that VE-cadherin is the only junctional protein whose expression in the lysates is induced by TNF α . Immunoblot of ERK is shown as a loading control. Immunoblot of ICAM-1 is shown as a control of TNF α stimulation. See also Fig. 2.

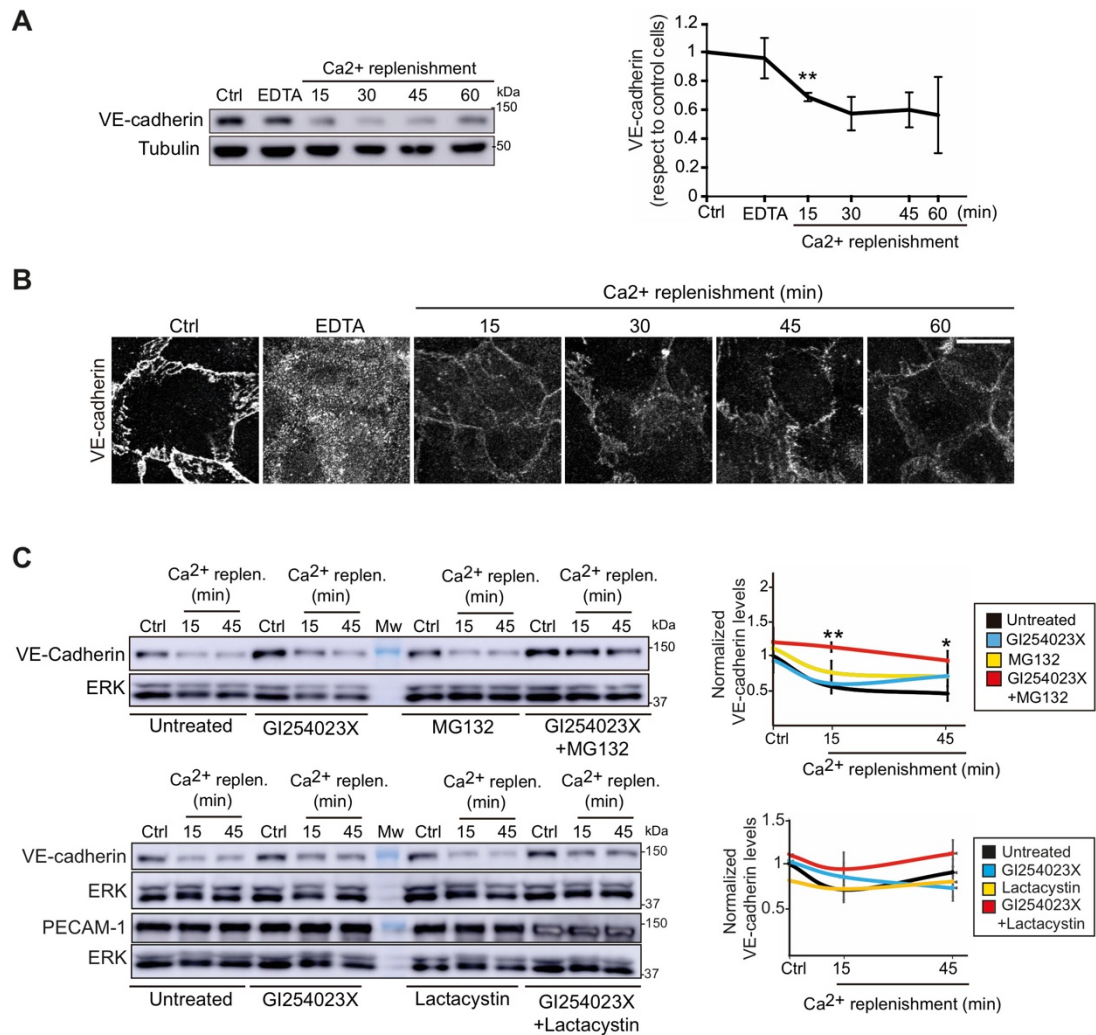


Figure S3. (A,B) Ca²⁺ switch (CS) assays induced with EDTA. HUVECs were cultured at confluence for 48 h, starved for 12 h and then stimulated with 10 ng/ml TNF α for 24 h before performing a Ca²⁺ switch (CS) assay by adding 3 mM EDTA in the culture medium. Ca²⁺ was restored by adding fresh medium and cells were lysed (A) or fixed for immunofluorescence (B) at the indicated times. (A) Graph show VE-cadherin expression levels detected by western blot from at least three independent experiments. *, $p < 0.05$, t-test shows comparison between control cell lysates and lysates from cells 15 min post-Ca²⁺ restoration. Tubulin was immunoblotted as a loading control (C) Simultaneous inhibition of proteasome and ADAM-10 prevents

reduction of VE-cadherin protein levels during Ca²⁺ switch assays. HUVECs were cultured at confluence for 48 h, starved for 12 h and then stimulated with 10 ng/ml TNF α for 24 h before performing a Ca²⁺ switch (CS) assay with PBS. Cells were lysed before Ca²⁺ depletion (Ctrl) or 15 and 45 min after Ca²⁺ replenishment in the presence or absence of the indicated inhibitors. VE-cadherin expression levels were detected by western blot. Graphs on the right-hand side show the quantification of VE-cadherin expression levels with respect to untreated control cells from three independent experiments. *, p=0.016. **, p=0.0015, t-tests comparing with control cells. See also Fig. 3.

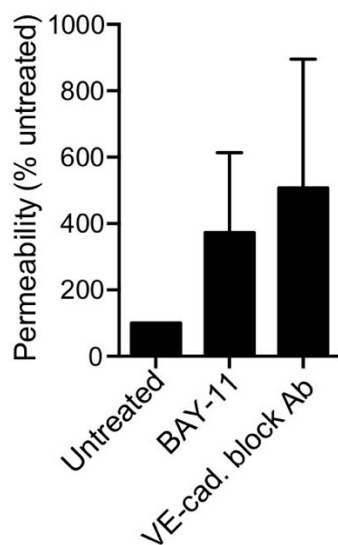


Figure S4. The NF- κ B inhibitor BAY11 and the anti-VE-cadherin blocking antibody increase endothelial permeability. HUVECs were cultured at confluence for 48 h on transwells of 0.4 μ m pore diameter precoated with fibronectin, starved for 12 h and incubated with 0.1 mg/ml of FITC-dextran for 90 min in the presence or absence of 10 μ M BAY11 and 20 μ g/ml VE-cadherin blocking antibody. Graph shows the mean fluorescence values + SEM of two independent experiments performed in triplicates.

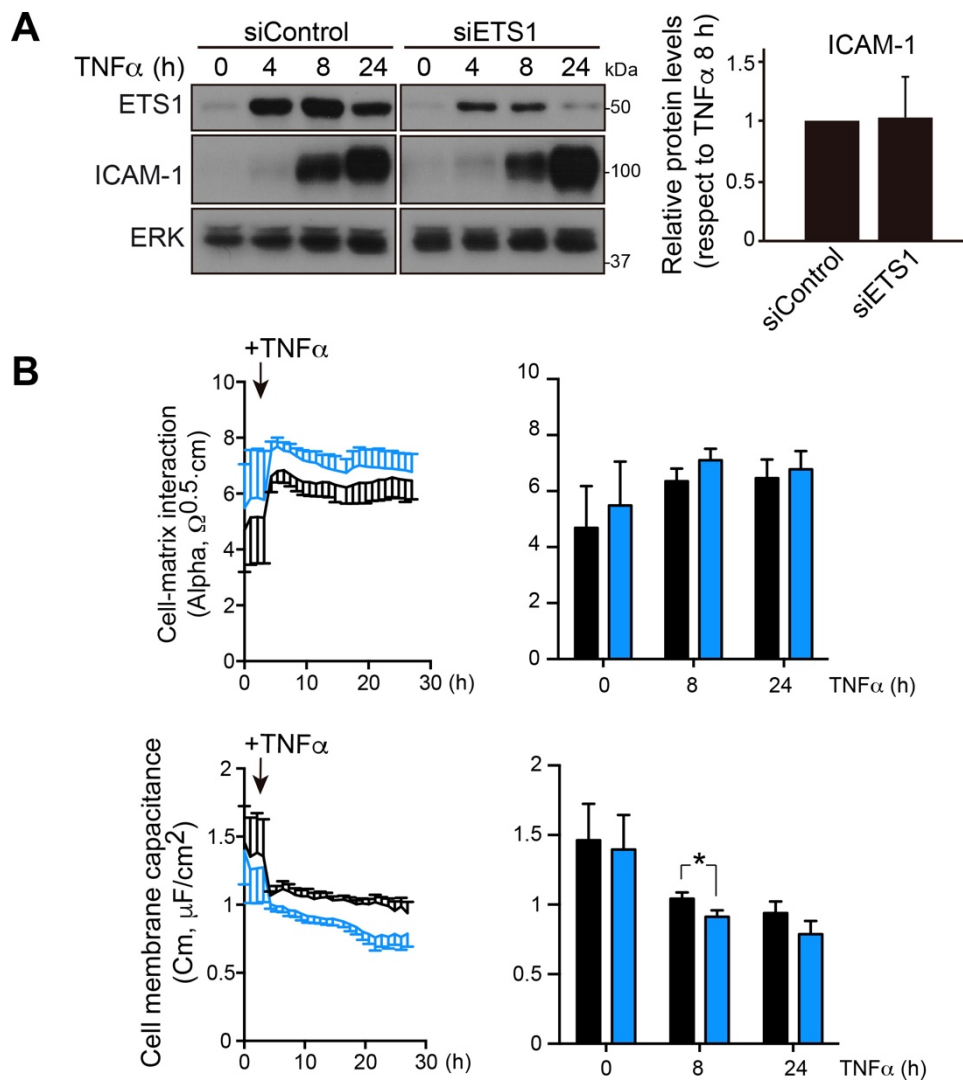


Figure S5. (A) siRNA-mediated knockdown of ETS1 (siETS1) does not reduce ICAM-1 protein expression. Graphs show quantifications of ICAM-1 levels with respect to siControl after 8 h of TNF α stimulation. Mean + SEM from three independent experiments. **(B)** Effect of ETS1 knockdown on Alpha and Cm. ECIS mathematical modelling reveals minor changes in cell-matrix interactions (Alpha), and endothelial Cell membrane capacitance (Cm). Graphs show the mean + SEM from three independent experiments measured at the indicated times of TNF α stimulation. *, $p < 0.05$. See Fig. 6F.