EphA2 Stimulates VCAM-1 Expression Through Calcium-Dependent NFAT1 Activity

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Supplemental Material

Supplemental Figure I: NF-κB in ephrin-A1 and TNFα-induced VCAM-1 expression.

Supplemental Figure II: Ephrin-A1 stimulates VCAM-1 expression through NFAT1.

Supplemental Figure III: siRNA-mediated knockdown of EphA2 but not EphA4 blunts Fc-ephrin-A1-induced NFAT nuclear translocation.

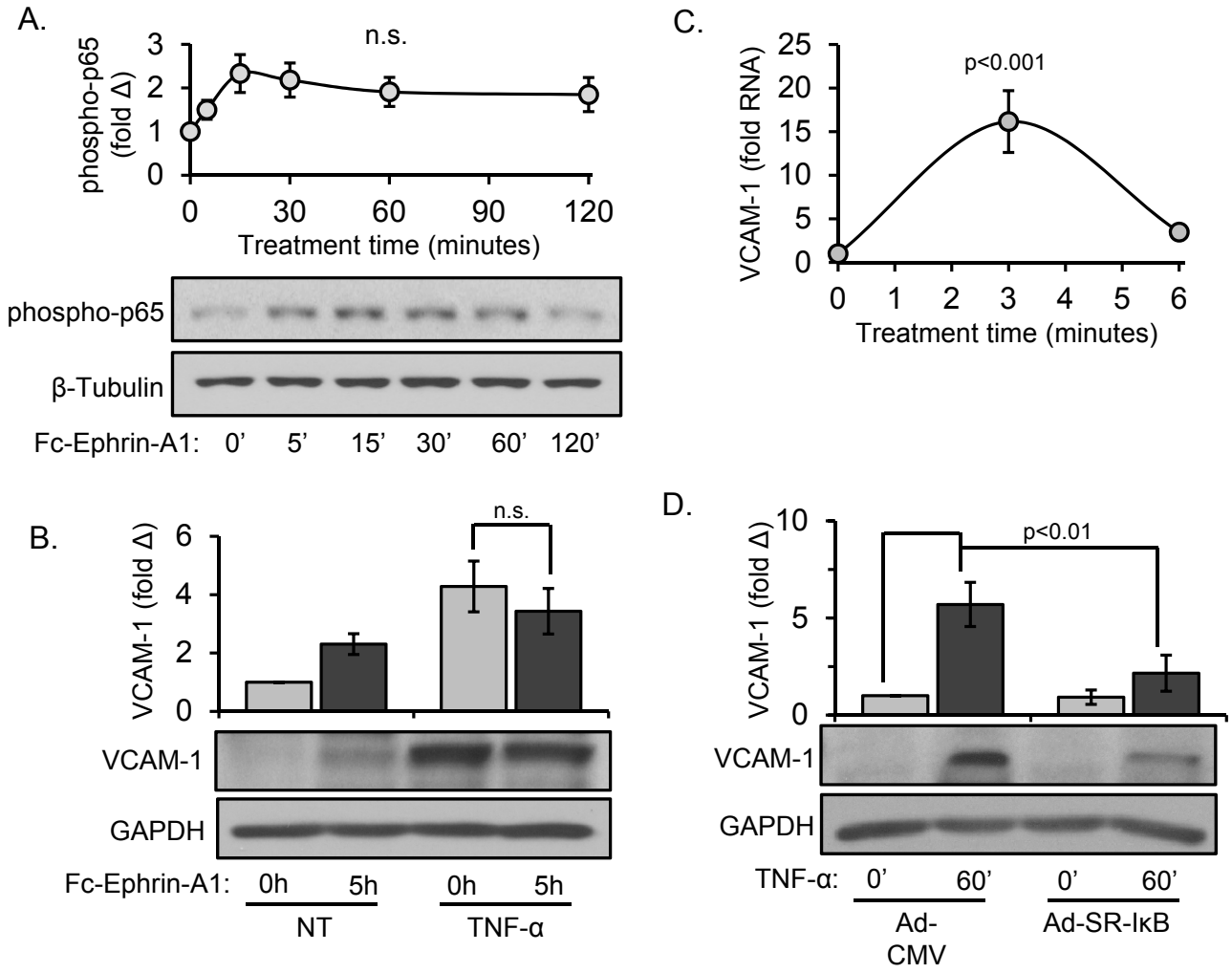
Movie 1. Fluo4-based calcium imaging in HAECs treated with Fc alone.

Movie 2. Fluo4-based calcium imaging in HAECs treated with ephrin-A1-Fc.

Movie 3. Fluo4-based calcium imaging in HAECs treated with EphA2 siRNA and Fc alone.

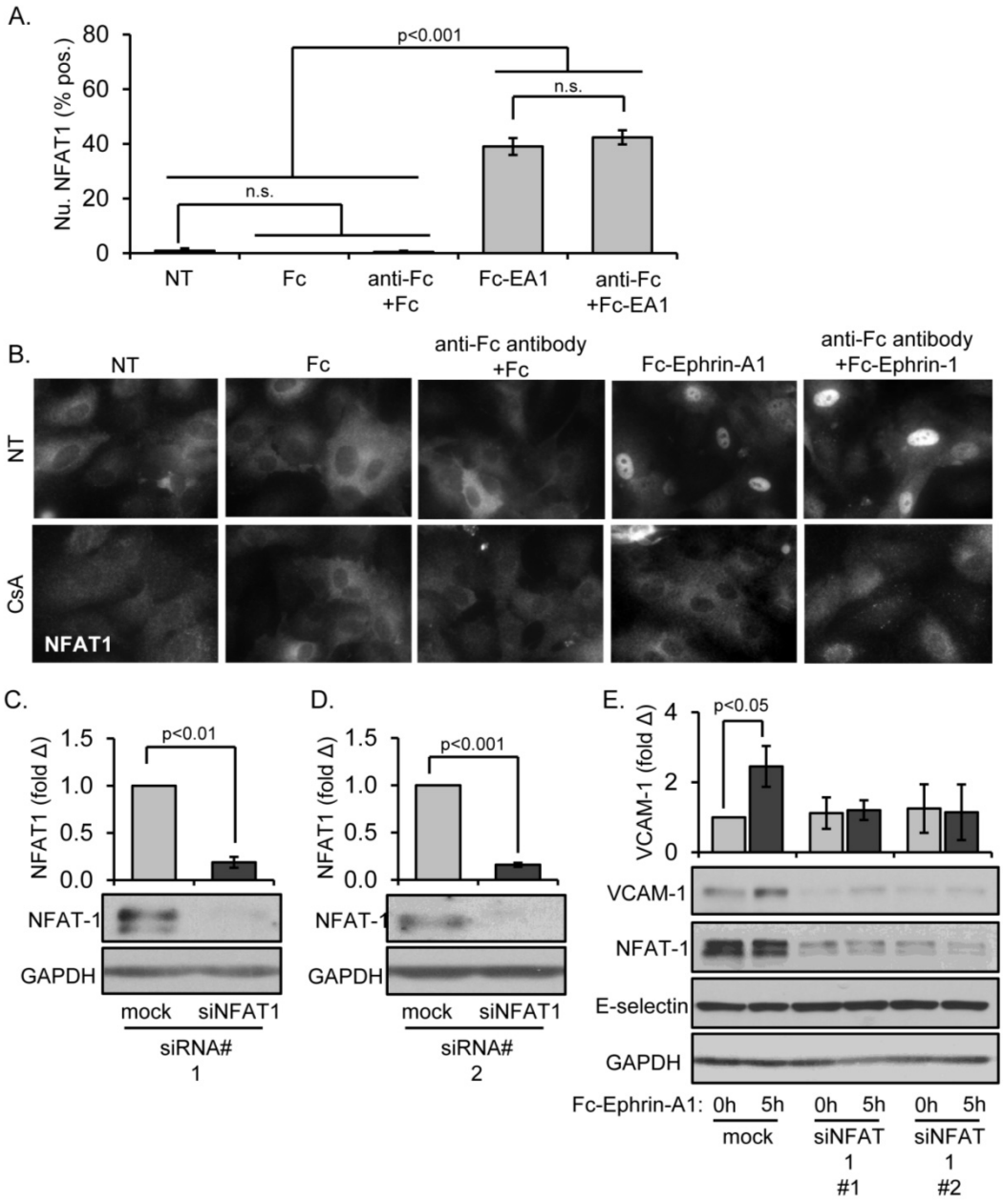
Movie 4. Fluo4-based calcium imaging in HAECs treated with EphA2 siRNA and ephrin-A1-Fc.

Supplemental Figure I



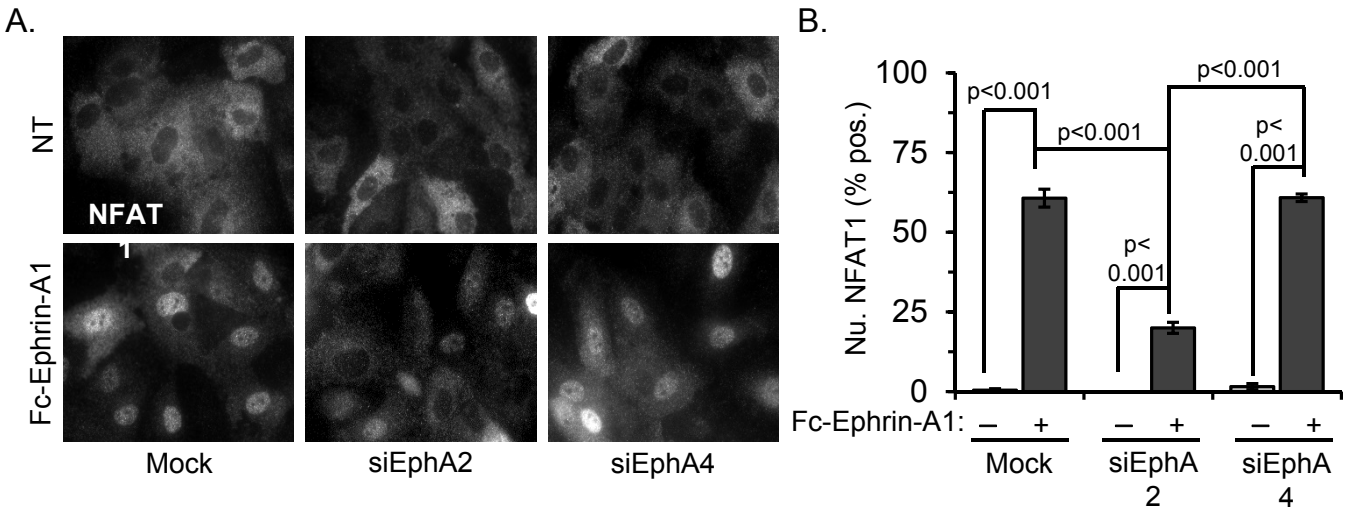
Supplemental Figure I: A) HAECs were treated with fc-ephrin-A1 for the indicated times, and phosphorylation of the NF-κB subunit p65 was assessed by Western blotting and normalized to β-tubulin, n=4. B) HAECs were treated with either vehicle, Fc-ephrin-A1 (2μg/mL) for 5 hours or TNF-α (10ng/mL) for 1 hour or both, and VCAM-1 expression was assessed with Western blot. n=4. C) HAECs were treated with fc-ephrin-A1 (2μg/mL) for 0, 3, or 6 hours, and VCAM-1 gene expression was measured with qRT-PCR. D) HAECs were transduced with either control CMV or SR-IκB adenovirus (20MOI) for 24 hours, then treated with either vehicle or TNF-α (10ng/mL), n=4. Statistical comparisons were made with either 1-way ANOVA with Bonferroni posttest (A/C) or 2-way ANOVA with Bonferroni posttest (B/D).

Supplemental Figure II



Supplemental Figure II: A-B) HAECs were treated with either vehicle or Cyclosporine A (3μM) for 30 minutes, then treated with either vehicle, recombinant Fc (2μg/mL), Fc conjugated with anti-Fc antibody (2μg/mL), Fc-ephrin-A1 (2ugmL), or Fc-ephrinA1 conjugated with anti-Fc antibody (2μg/mL) for 15 minutes and NFAT1 nuclear translocation was assessed with immunocytochemistry. A) Nuclear translocation was quantified by scoring cells for positive nuclear NFAT1 staining. At least 100 cells were assessed for each condition per experiment. Cyclosporine A-treated cells were not quantified. B) Representative images shown. n=4. C-E) HAECs were treated with either mock or two different siRNA oligonucleotides directed against NFAT1 for 48 hours. C/D) Knockdown efficiency of each siRNA directed against NFAT1 assessed with Western blot and normalized to GAPDH. E) Following transfection, cells were treated with either vehicle or Fc-Ephrin-A1 (2μg/mL) for 5 hours, and VCAM-1, NFAT1, and E-selectin expression were assessed with Western blot and normalized to GAPDH. n=4. Statistical comparisons were made with either 1-way ANOVA with Bonferroni posttest (A/B), Student's T-test (C/D), or 2-way ANOVA with Bonferroni posttest (E).

Supplemental Figure III



Supplemental Figure III: A/B) HAECs were treated with either mock or siRNA (Sigma) directed against EphA2 or EphA4 for 48 hours. 48 hours following transfection, cells were treated with either vehicle or Fc-ephrin-A1 (2µg/mL) for 15 minutes, and NFAT nuclear translocation was assessed with immunocytochemistry. A) Representative images shown. B) Nuclear translocation was quantified by scoring cells for positive nuclear NFAT1 staining. At least 100 cells were assessed for each condition per experiment, n=4. Statistical comparisons were made with 1-way ANOVA with Bonferroni posttest.