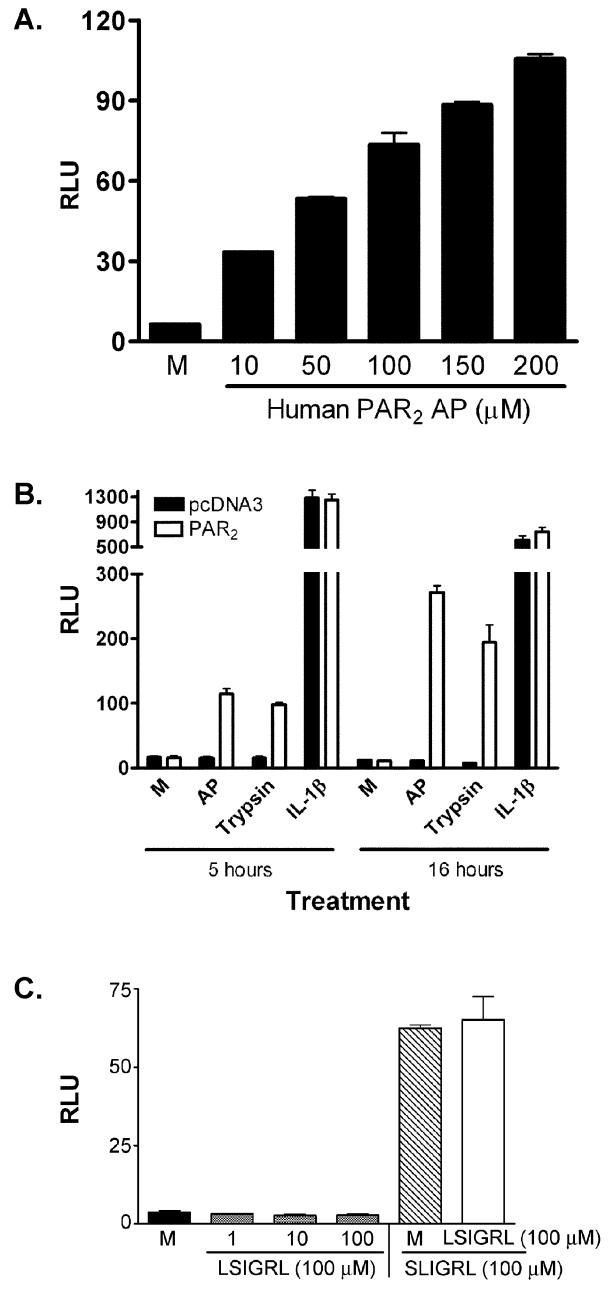
Supplementary Figure Legends

Supplemental Figure 1. HEK293T/huPAR$_2$ transfectants show dose-dependent ELAM (NF-κB) reporter activation by PAR$_2$ AP, respond to trypsin, and an inactive peptide does not antagonize AP activity. (*A*). HEK293T cells, co-transfected with huPAR$_2$ construct together with the NF-κB luciferase (ELAM-luc) and pCMV1-β-galactosidase reporter constructs, were treated with huPAR$_2$ AP at the indicated concentrations for 16 hr. Cell lysates were assayed for luciferase and β-galactosidase activities as described in the Experimental Procedures. A representative experiment is shown (n=6). *(B)* pcDNA3- or huPAR$_2$-transfected HEK293T cells were treated with huPAR$_2$ AP (100 μM), trypsin (10 nM), or rhIL-1β (30 ng/ml) in duplicate for 5 or 16 hr under serum-free conditions. RLU represents NF-κB-luciferase activity normalized to β-galactosidase activity in each sample. Data are representative of 2 separate experiments. (*C*). HEK293T cells co-transfected as described in (*A*) were treated with the indicated concentrations of scrambled peptide, LSIGRL-NH$_2$ (left), or 100 μM of rodent PAR$_2$ AP (SLIGRL-NH$_2$) after a 1 hr pre-treatment of transfectants with medium (M) or LSIGRL-NH$_2$ (100 μM) (and continuing its presence), for 16 hr. Reporter activities in cell lysates were analyzed. Results are representative of 2 individual experiments.

Supplemental Fig. 2. PAR$_2$ activation by AP does not require *de novo* protein synthesis. (*A*). HEK293T cells plated in 6-well plates (0.5 x 10^6 cells/dish) were transfected with the huPAR$_2$ (0.5 μg), ELAM-luc reporter (1.25 μg) and β-gal (0.25 μg) expression vectors, and 1 μg of blank pcDNA3 vector. Transfected cells were pre-treated with cycloheximide (+CHX, 5 μg/ml) or media (-CHX) for 1 hr. Cells were then stimulated for the indicated times with huPAR$_2$ AP (SLIGKV-NH$_2$) (100 μM), without or with CHX. Cells were processed at the indicated time points and total RNA isolated. Relative quantities of steady-state luciferase and human GAPDH (internal control) mRNA were determined by semi-quantitative RT-PCR. A representative experiment is shown (n=3). (*B*). Two 12-well culture plates with 2 x 10^5 cells/well of HEK293T cells were co-transfected with untagged huPAR$_2$ construct together with the NF-κB luciferase and pCMV1-βgal reporter constructs. Transfectants were treated with either media (M) or 100 μM of huPAR$_2$ AP (SLIGKV-NH$_2$) for 11 hr (left side of graph). Supernatants from the first set of cells were transferred to the second set of PAR$_2$ transfectants and incubated for an additional 5 hr (right side of graph). Luciferase and β-galactosidase activities were measured in cell lysates. A representative experiment is shown (n=2).

Supplementary Figure 1



Supplementary Figure 2