

Supporting Information

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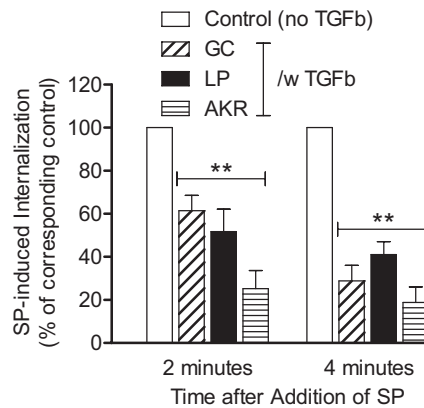


Fig. S1. Overnight treatment with TGF- β (TGF β) leads to a significant reduction in substance P (SP)-induced NK-1R internalization. Receptor internalization after either 2 or 4 min of SP exposure was measured in Granuloma (GC) or lamina propria T cells (LP), or in the AKR T cell line. The experimental design was as described for Figs. 1 and 3. Internalization at each time-point observed with TGF- β pretreated cells is expressed as a percentage of the corresponding control value, observed in the same cell type without TGF- β exposure (open bar, 100%). Data represent the mean \pm SEM of $n = 6$ (GC), $n = 7$ (LP), or $n = 5$ (AKR) experiments. **, $P < 0.01$ vs. control.

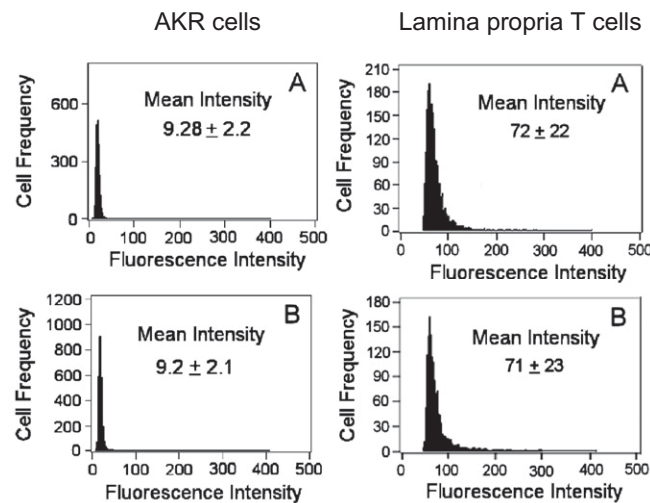


Fig. S2. Overnight treatment with TGF- β does not alter NK-1R expression in AKR or lamina propria T cells. (Left) ImageStream analysis of AKR cells expressing recombinant NK-1R-eGFP. Culture of cells in the absence (A) or presence (B) of TGF- β for 18 h results in similar intensity of eGFP fluorescence, suggesting that NK-1R expression is not affected by TGF- β treatment. (Right) ImageStream analysis of Alexa smSP (10^{-9} M) binding to IL-10 $^{-/-}$ mouse intestinal lamina propria T cells expressing endogenous NK-1R. The fluorescence intensity of labeled ligand is similar after prior overnight incubation of cells in the absence (A) or presence of TGF- β (B), suggesting that NK-1R expression is not affected by TGF- β treatment.

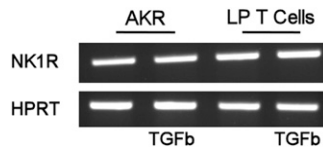


Fig. S3. Overnight treatment with TGF- β does not alter the abundance of NK-1R mRNA transcripts in either AKR or lamina propria T cells. Following culture of cells either in the absence or presence TGF- β (as indicated in the bottom labels), total RNA was extracted by homogenization in guanidinium/acid-phenol 1. Cellular RNA (5 μ g) was reverse-transcribed with Moloney-monkey leukemia virus (400 U) using an 18-mer of oligo-dT (0.5 μ g) as the primer. The first strand cDNA was diluted to 250 μ L, and 15 μ L (0.3 μ g RNA) was added to PCR buffer containing 2 U Taq DNA polymerase, 1.4 mM Mg Cl₂, 50 mM KCl, and 100 mM Tris (pH 8.3), in a total volume of 50 μ L. The sense primer to amplify NK-1R was 5'-CCA ACA CCT CCA CCA AGA CTT CTG-3' and the antisense primer was 5'-GCC ACA GCT GTC ATG GAG TAG AT-3'. Forty cycles of PCR were performed at 93 °C for 1.1 min, 63 °C for 1.36 min, and 72 °C for 1.14 min. Products of RT PCR amplification were analyzed by agarose gel electrophoresis using 1.7% Nusieve GTG agarose (FMC Bioproducts) in 0.5 \times TBE buffer. In each sample, transcripts of the housekeeping gene hypoxanthine phosphoribosyltransferase (HPRT) were also amplified as a control.

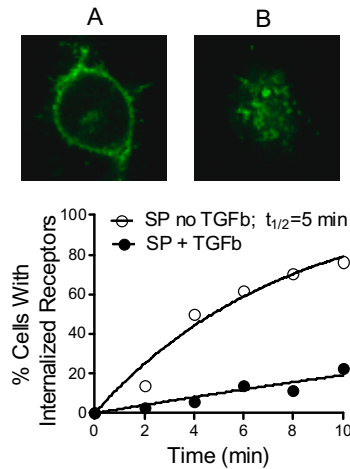


Fig. S4. SP induces internalization of NK-1Rs in HEK 293 cells, and this process is inhibited by exposure to TGF- β (TGF β). (Upper) Higher magnification view of SP-induced NK1R internalization in HEK 293 cells, demonstrating movement of the receptors from the cell surface (A) to intracellular clusters (B). Both panels show the same cell pre- and postexposure to SP, as highlighted by arrows "A" and "B" in Fig. 4. (Lower) Semiquantitative assessment of agonist-induced NK-1R internalization in the absence vs. presence of TGF- β . The percentage of cells that show receptor internalization over time (as shown in the Upper panels) was visually quantified from representative fields of > 40 cells.

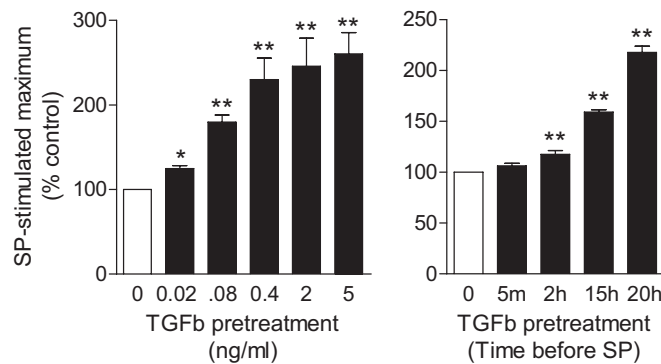


Fig. S5. Amplification of SP-induced signaling by TGF- β (TGF β) is evident at low concentrations and requires overnight treatment. HEK293 cells were transiently cotransfected with cDNAs encoding NK-1R-eGFP, nuclear factor of activated T cell-luciferase, and beta-galactosidase, as described in *Materials and Methods*. Four hours after transfection, the cells were either pretreated with increasing concentrations of TGF- β for 18 h (Left), or with 5 ng/mL of TGF- β for indicated periods of time (Right). Following further incubation for 4 h with SP (10⁻⁶ M), luciferase activity was determined and normalized to the value observed in control cells without prior TGF- β exposure (open bars, 100%). Data represent the mean \pm SEM of four experiments. *, $P < 0.05$; **, $P < 0.01$ vs. control.

