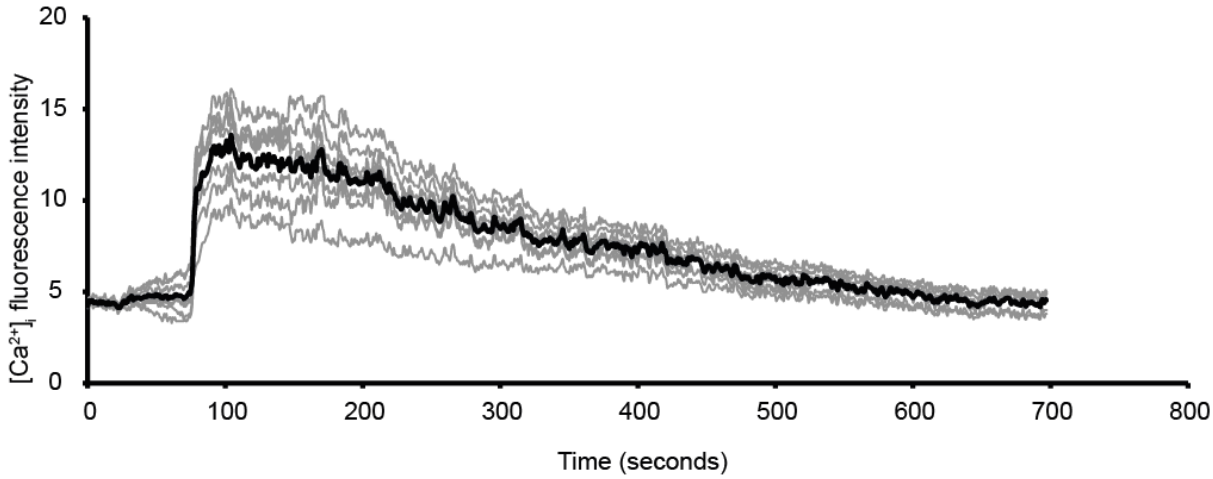
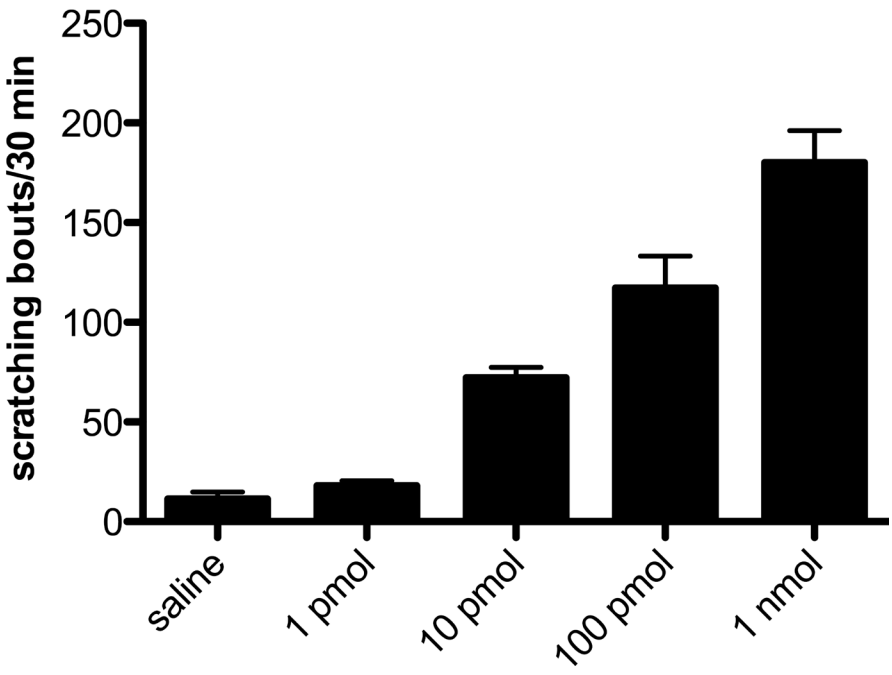
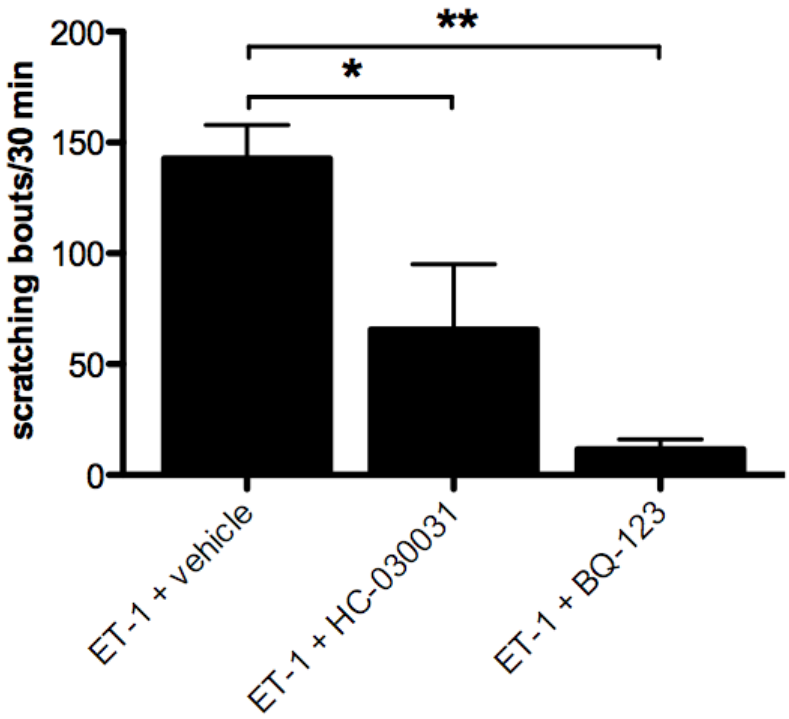
Supplemental Figures with legends



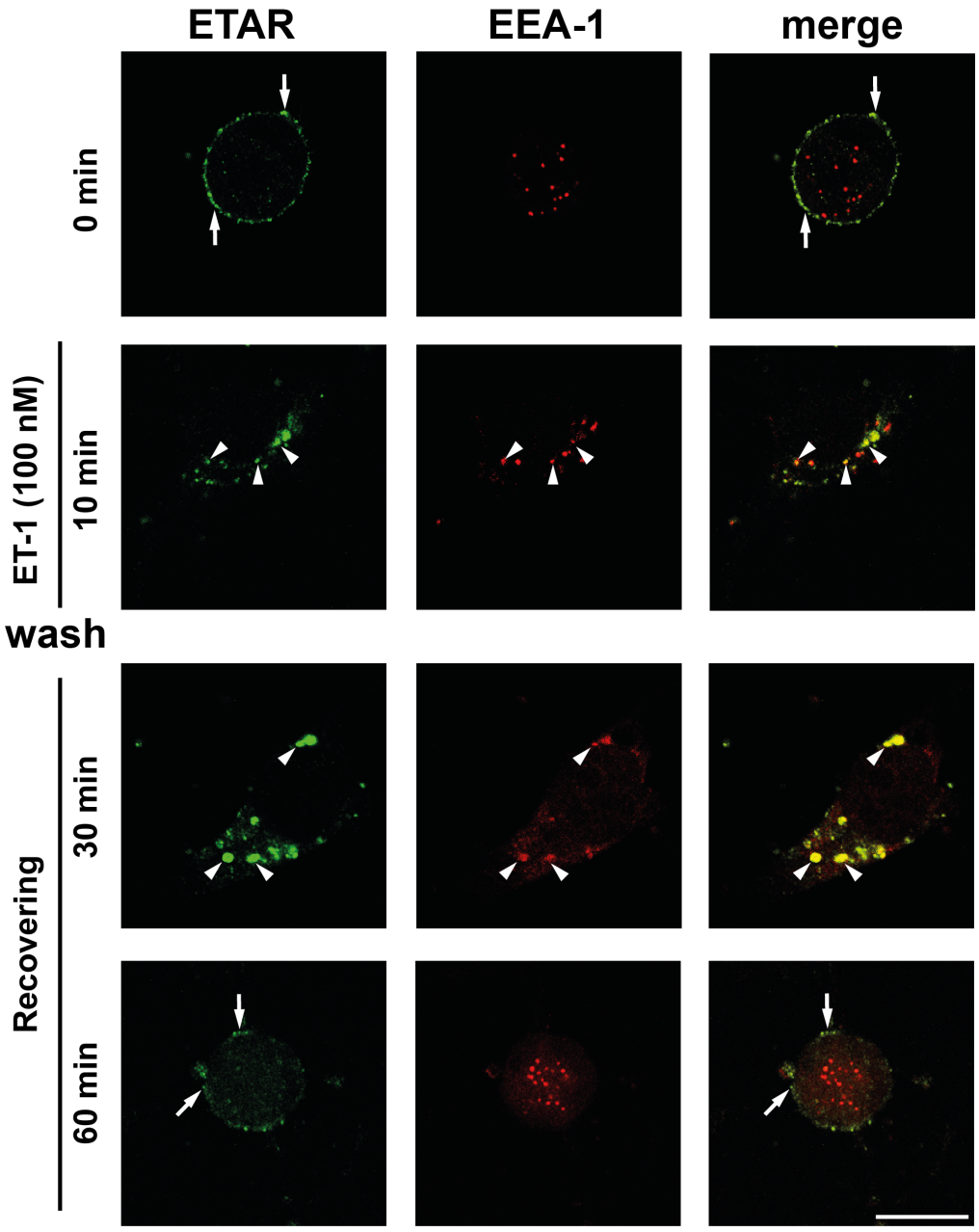
Supplemental Figure 1: ET-1 induces marked Ca2+ response in murine DRG neurons. DRG neurons treated *in vitro* with 100 nM ET-1 at indicated time point (arrow) release intracellular Ca2+ suggesting intact ETAR function in neuronal cells (n=7).



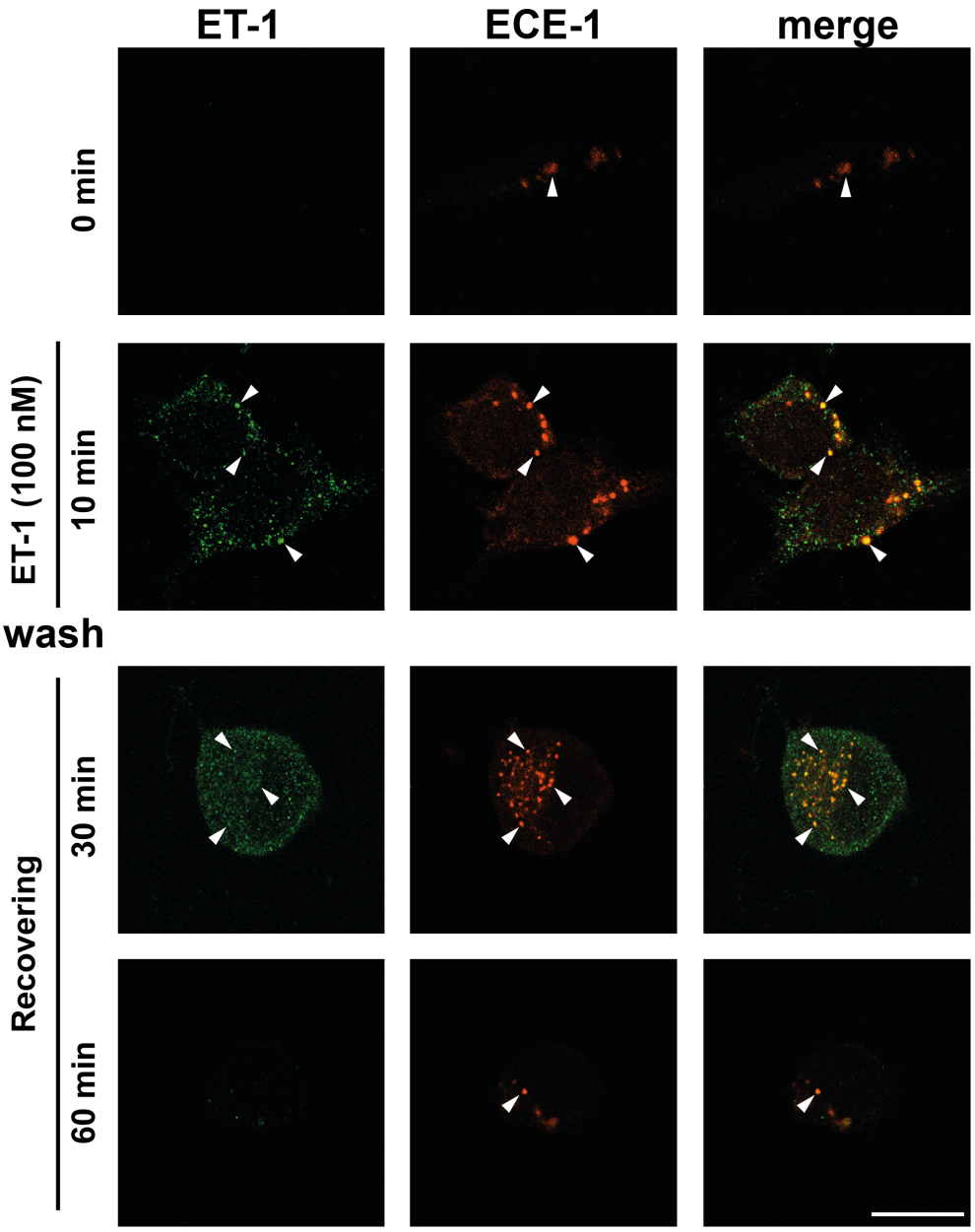
Supplemental Figure 2: Dose dependency of ET-1 activated itch in the mouse cheek model.



Supplemental Figure 3: ET-1 induced scratching behavior in mice is dependent on TRPA1 and ETAR. Scratching elicited by ET-1 (100 pmol/10 µl into the cheek) is eliminated in WT mice pre-treated (30 min before agonist) intraperitoneally with TRPA1 antagonist (HC-030031, 30 mg/kg) or with ETAR inhibitor BQ-123 (1 nmol) locally into the cheek of ET-1 injection.



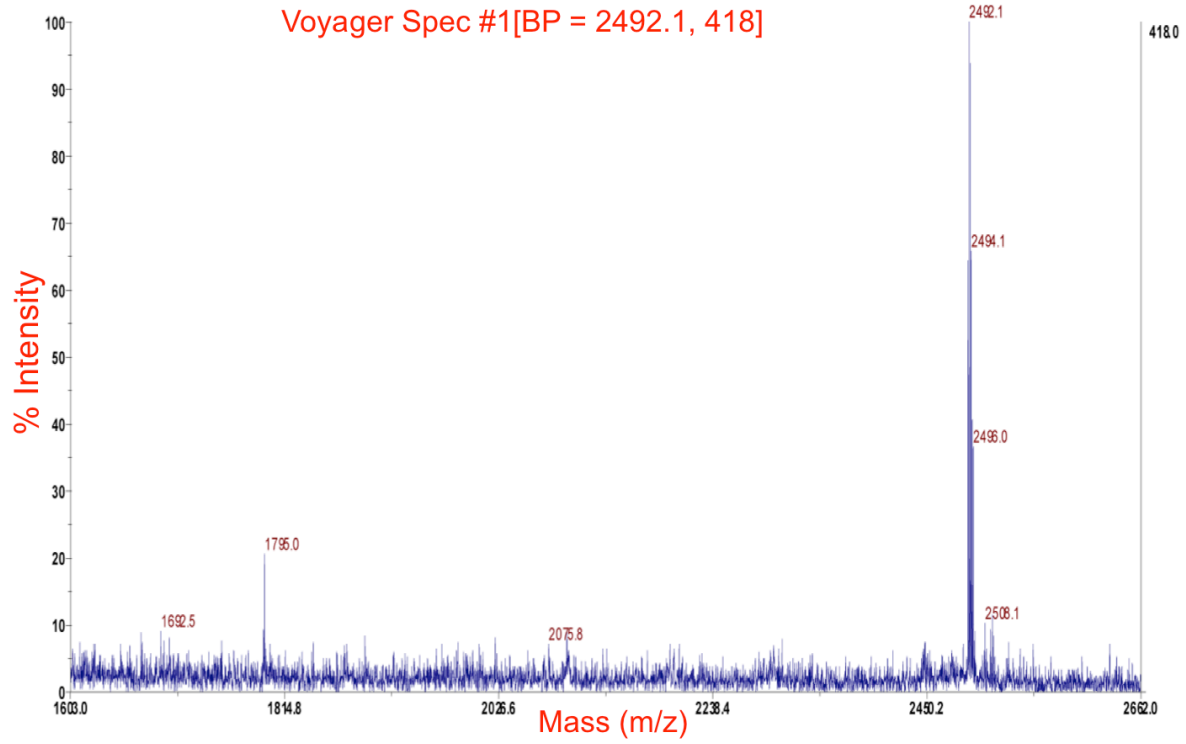
Supplemental Figure 4: ET-1 activated ETAR localizes in EEA-1+ acidic endosomes of murine DRG neurons. After ET-1 stimulation, ETAR trafficked from plasma membrane (arrows) to endosomes containing the endosomal marker EEA-1 (arrowheads). Recovery of ETAR to plasma membrane was observed 60 min after stimulation (arrows). Scale bar 30μM.



Supplemental Figure 5: ET-1 trafficks to ECE-1 containing vesicles in cultured DRG neurons. Murine DRG neurons were treated with 100nM ET-1. Upon ETAR activation by ET-1, ET-1 trafficked to intracellular vesicles where it co-localized with ECE-1 (arrowheads). Scale bar 30μM.

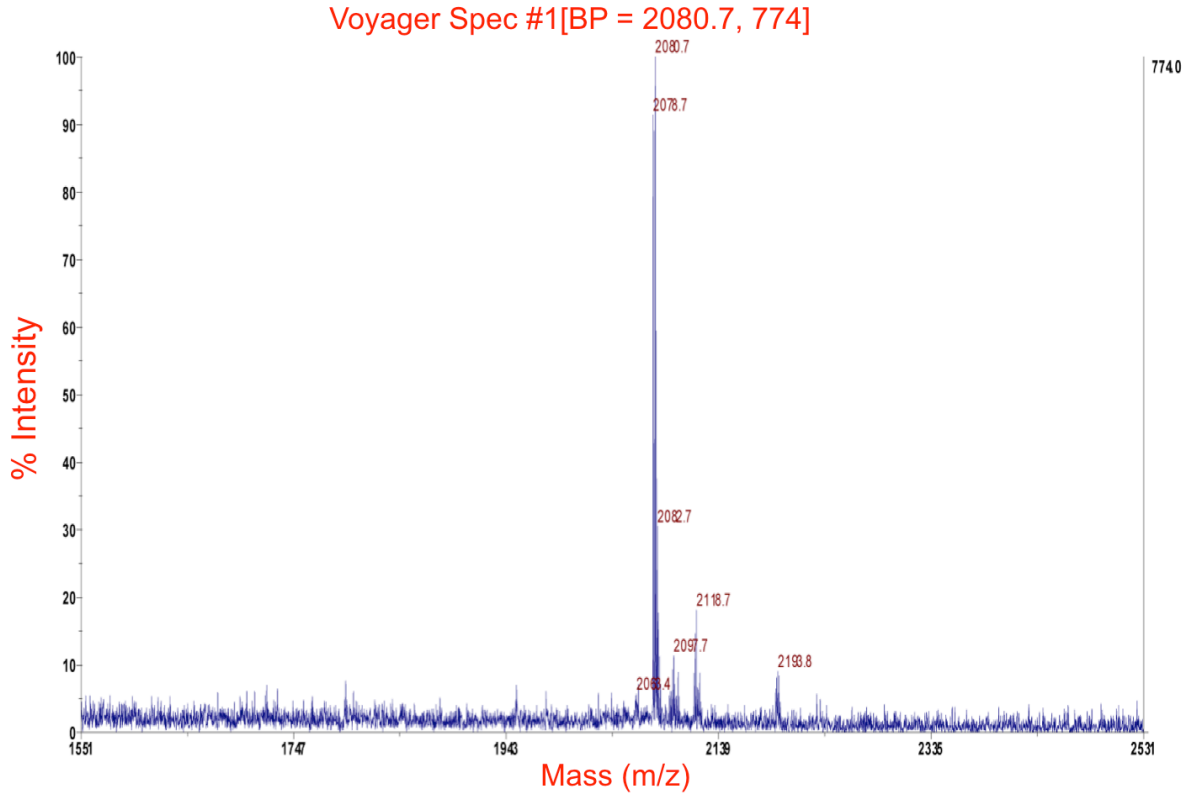
A

Sample A: ET-1 (in the absence of ECE)

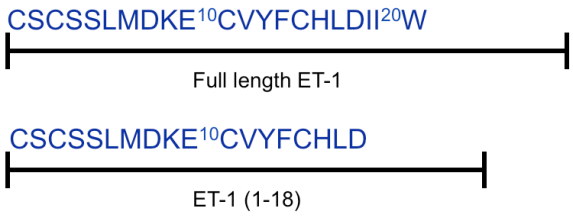


B

Sample B: ET-1 (in the presence of ECE at pH 5.5)

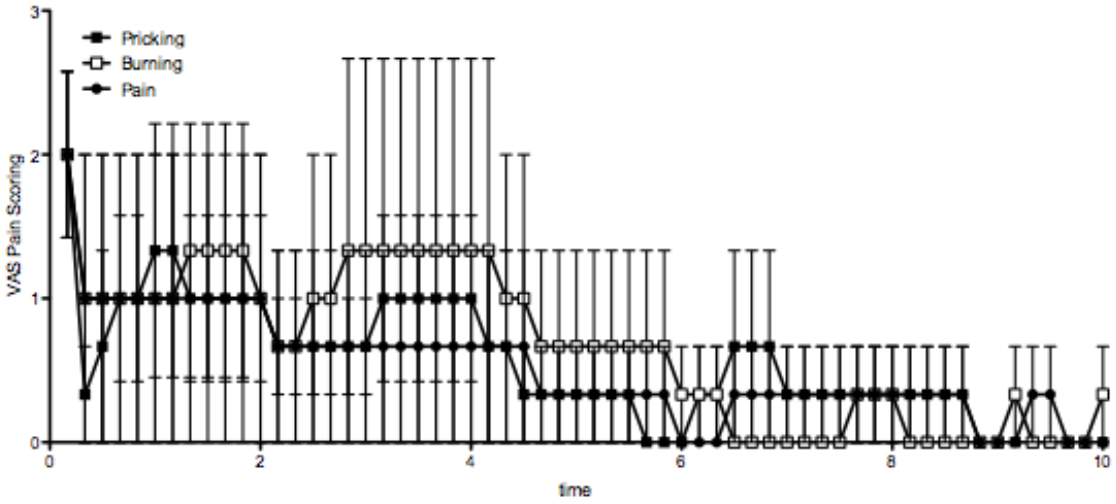


C

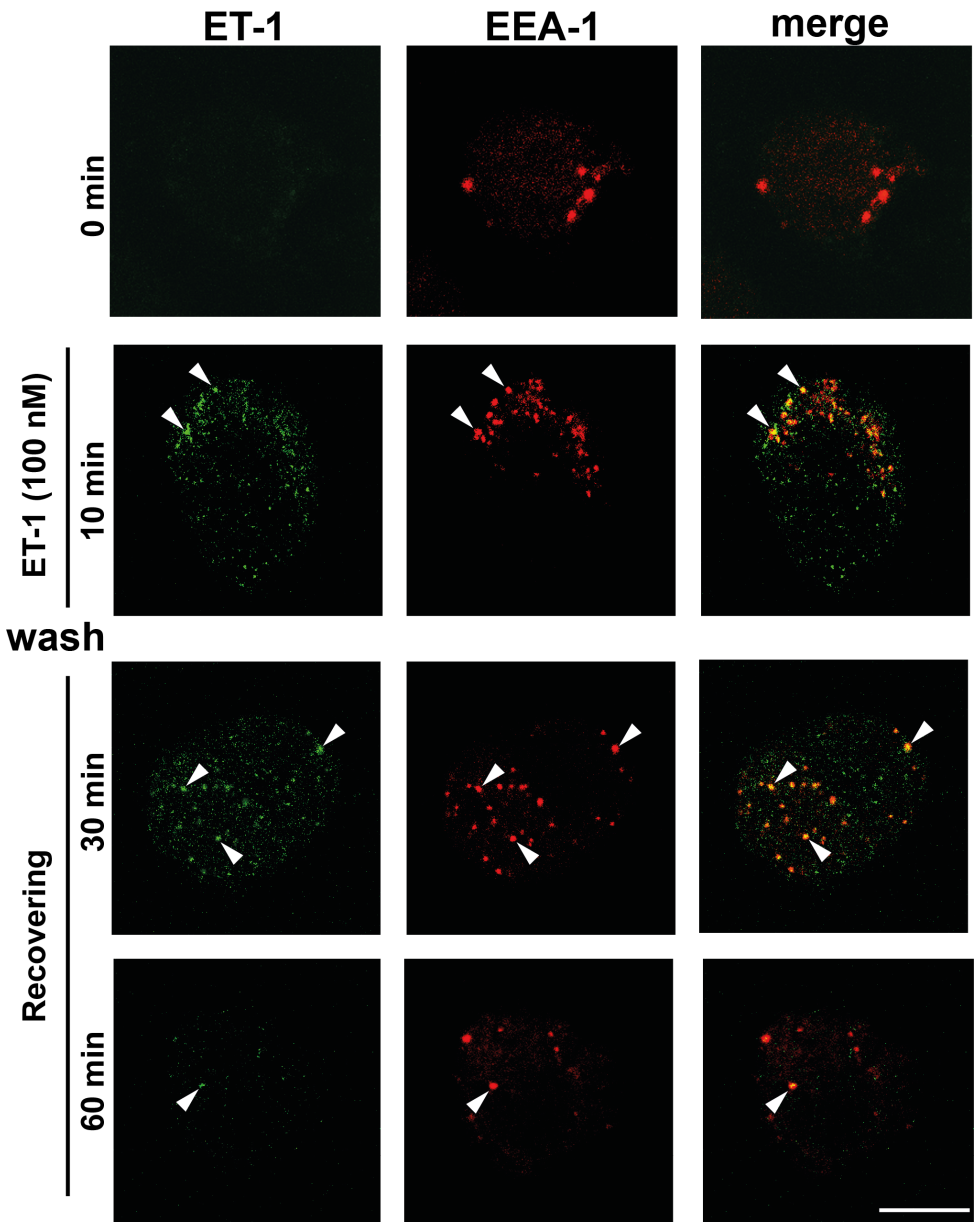


ET-1 sample	Peak intensity	Observed MH+	Predicted MH+	Corresponding peptide
Sample A (in the absence of ECE at pH 5.5)	strong	2491.1	2491.1	Full length ET-1
Sample B (in the presence of ECE at pH 5.5)	strong	2078.7	2078.7	ET-1 (1-18)
- "-	weak	2095.7	*Ammonium adduct of 2078.7*	ET-1 (1-18)
- "-	weak	2100.6	*Sodium adduct of 2078.7*	ET-1 (1-18)
- "-	weak	2116.7	*Potassium adduct of 2078.7*	ET-1 (1-18)
- "-	weak	2191.8	2191.9	ET-1 (1-19)

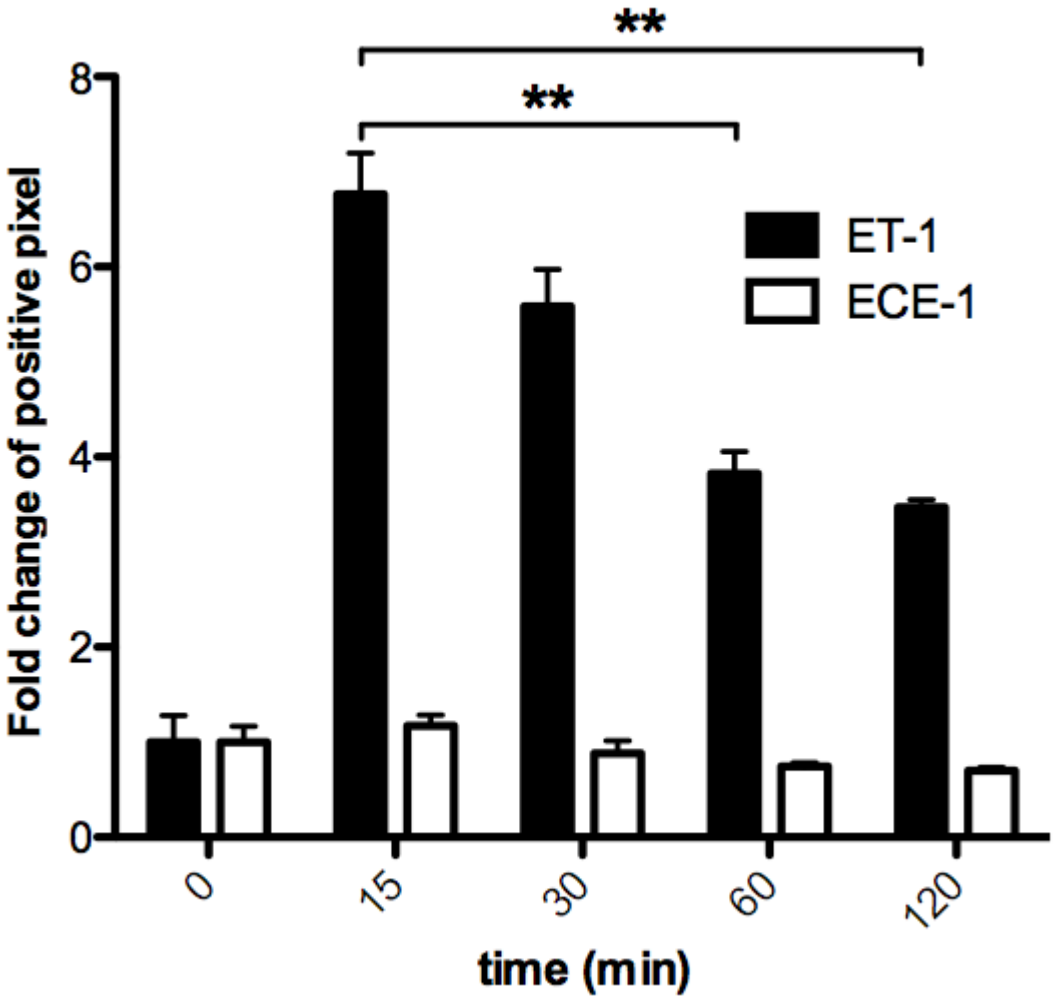
Supplemental Figure 6: Raw MALDI-TOFF data of (rh)ET-1 degradation by ECE-1 in (A) absence or (B) presence of the converting enzyme at pH 5.5. (C) Tabular summarization of two representative traces.



Supplemental Figure 7: The time course of pain, pricking and burning intensities (0: no sensation, 10: worst imaginable pain, pricking or burning) for ten minutes after the termination of a 60-second iontophoresis with endothelin-1 on the forearm skin of healthy human subjects (n = 4), ET-1 significantly induced instant pain, pricking or burning (pain-related sensations) that were only described for seconds after agonist application. Error bars as SEM.



Supplemental Figure 8: ET-1 co-localizes with EEA-1 in acidic endosomes of murine DRG neurons. After ET-1 treatment, the receptor bound agonist ET-1 trafficked to endosomes containing the endosomal marker EEA-1 (arrowheads). Scale bar 30μM.



Supplemental Figure 9: Stimulation with ET-1 (100 nM) increased immunofluorescence for ET-1 (derived from external application) in DRG neurons. While immunofluorescence for ET-1 decreased (degradation) over a time period of 120 min, ECE-1 immunofluorescence was constant (n = 10 cells/group, error bars are indicated as +/- SEM, Students t´test, ***; p< 0.001). Subcellular distribution was analysed from captured images using Image J (http://rsbweb.nih.gov/ij/).