

Supplementary Information (SI)

SI Fig. 1. Single point in contrast to double point coiled-coil mutants of Orai2 and Orai3 exhibit clear stimulation by full-length STIM1. Time-course of whole-cell inward currents at -74mV activated by passive store-depletion (10mM EGTA pipette solution) of HEK293 cells expressing STIM1 with **a)** Orai2 L237S and **b)** Orai2 L237S L244S in comparison to Orai2 and with **c)** Orai3 L285S and **d)** Orai3 L285S L292S in comparison to Orai3.

SI Fig. 2. STIM1 C-terminus coiled-coil mutants fail to couple to and activate any of the Orai2 and Orai3 single point coiled-coil mutants. Time-course of constitutive whole-cell inward currents at -74mV of HEK293 cells expressing STIM1 C-terminus L373S with **a)** Orai2 L237S, **c)** Orai2 L237S L244S in comparison to Orai2 and with **e)** Orai3 L285S and **g)** Orai3 L285S L292S in comparison to Orai3. Values at $t = 0$ s were significantly different within each Orai subgroup with $p < 0.01$ **b)** Localization, overlay and calculated FRET of STIM1-C-terminus L373S-YFP with CFP-Orai2 and CFP-Orai2 L237S and **d)** corresponding average FRET values including CFP-Orai2 L237S L244S. Results within each Orai subgroup were significantly different with $p < 0.01$ except for comparison of STIM1 C-terminus L373S-YFP/CFP-Orai2 L237S with STIM1 C-terminus L373SYFP/ Orai2 L237S L244S ($p > 0.05$). **f)** Localization, overlay and calculated FRET of STIM1-C-terminus L373S-YFP with Orai3 and CFP-Orai3 L285S and **h)** corresponding average FRET values including CFP-Orai3 L285S L292S. Results within each Orai subgroup were significantly different with $p < 0.01$. Scale bars correspond to 5 μ m.

SI Fig. 3. STIM1 L373S single point coiled-coil mutant is still able to activate Orai3 but not Orai1. Time-course of constitutive whole-cell inward currents at -74mV of HEK293 cells expressing STIM1 L373S with **a)** Orai1 and **d)** Orai3 in comparison to currents mediated by STIM1. Localization and overlay of YFP-STIM1 L373S with **c)** CFP-Orai1 and **f)** CFP-Orai3 before (upper panel) and after (lower panel) application of 30 μ M BHQ for 5 min in comparison to YFP-STIM1 with **b)** CFP-Orai1 and **e)** CFP-Orai3. Scale bars correspond to 5 μ m.

Figure 1 Supplementary information

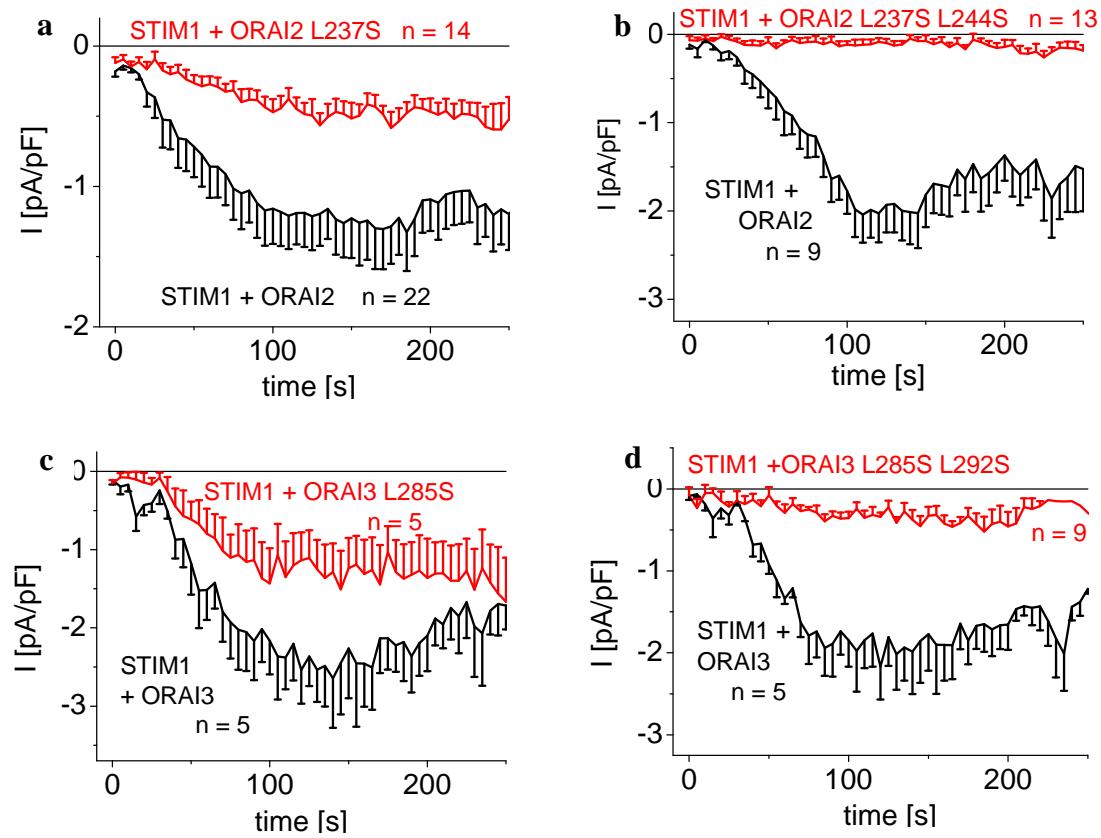


Figure 2 Supplementary information

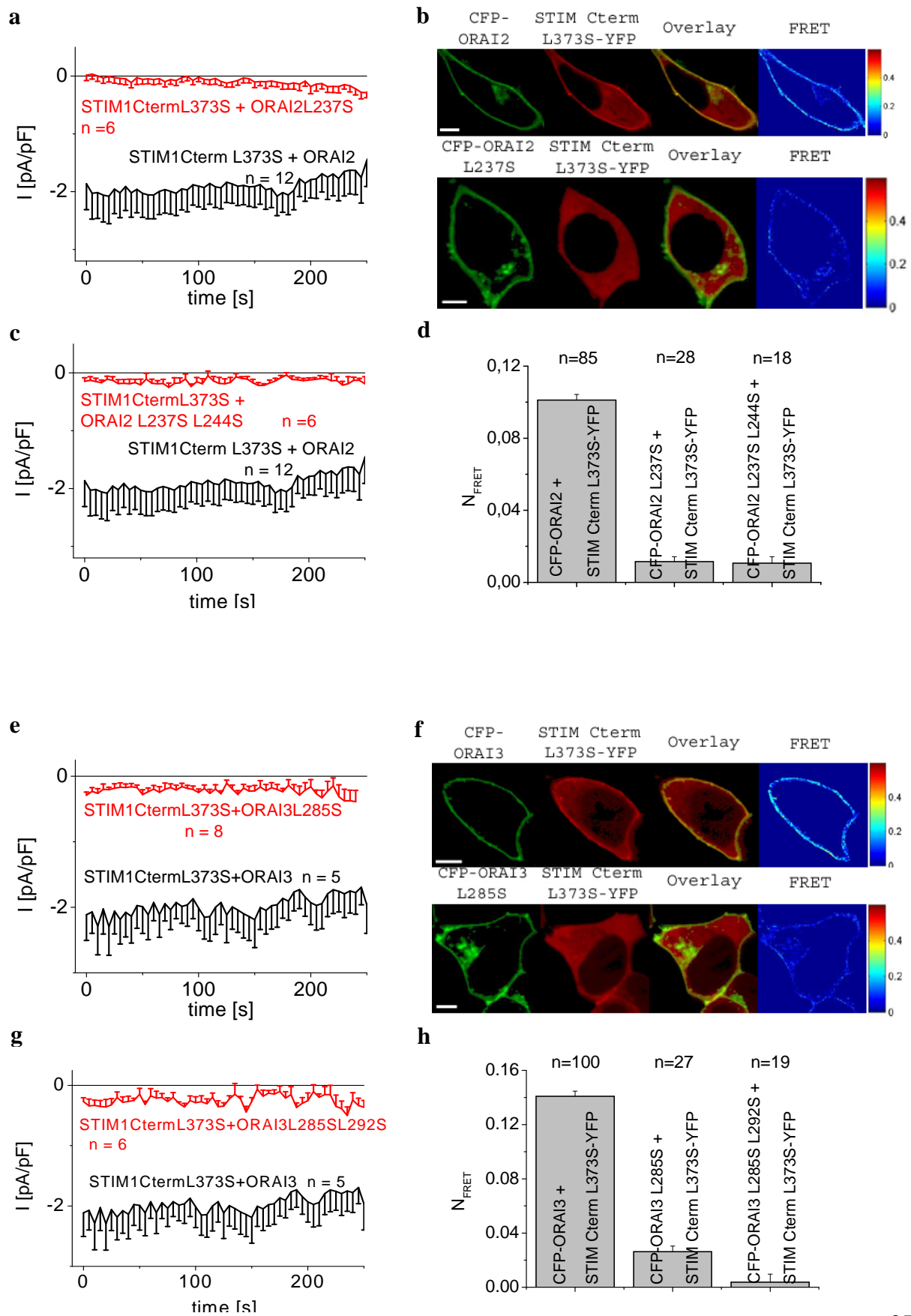


Figure 3 Supplementary information

