

Supporting Information

Schindl et al. 10.1073/pnas.0907714106

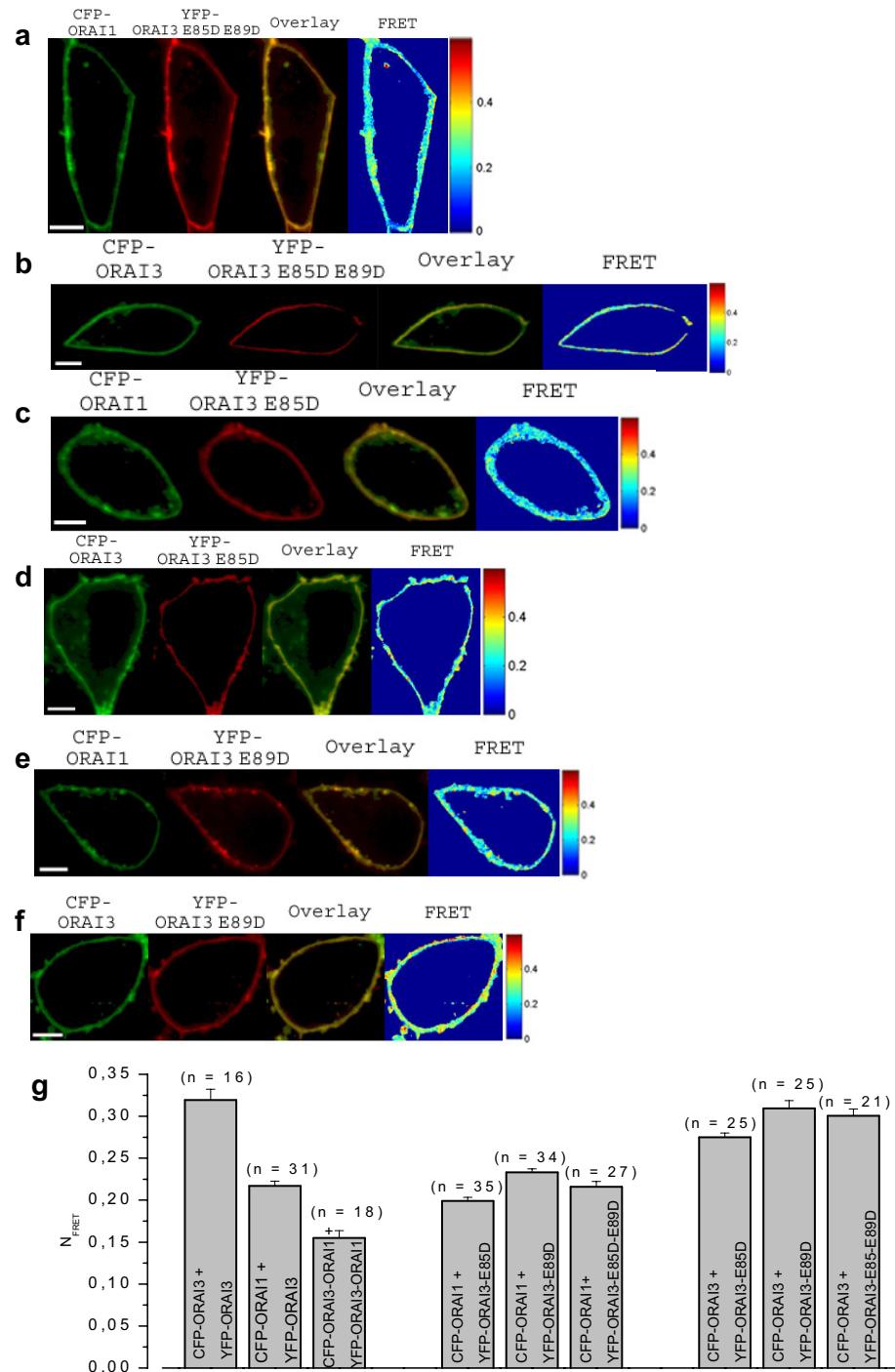


Fig. S1. FRET series of Orai3 pore mutants. (a) Co-expression of CFP-Orai1 and YFP-Orai3-E85D-E89D, (b) CFP-Orai3 and YFP-Orai3-E85-E89D, (c) CFP-Orai1 and YFP-Orai3-E85D, (d) CFP-Orai3 and YFP-Orai3-E85D, (e) CFP-Orai1 and YFP-Orai3-E89D, and (f) CFP-Orai3 and YFP-Orai3-E89D, overlay and calculated FRET. (g) Average FRET values determined for the constructs shown in (a–f).

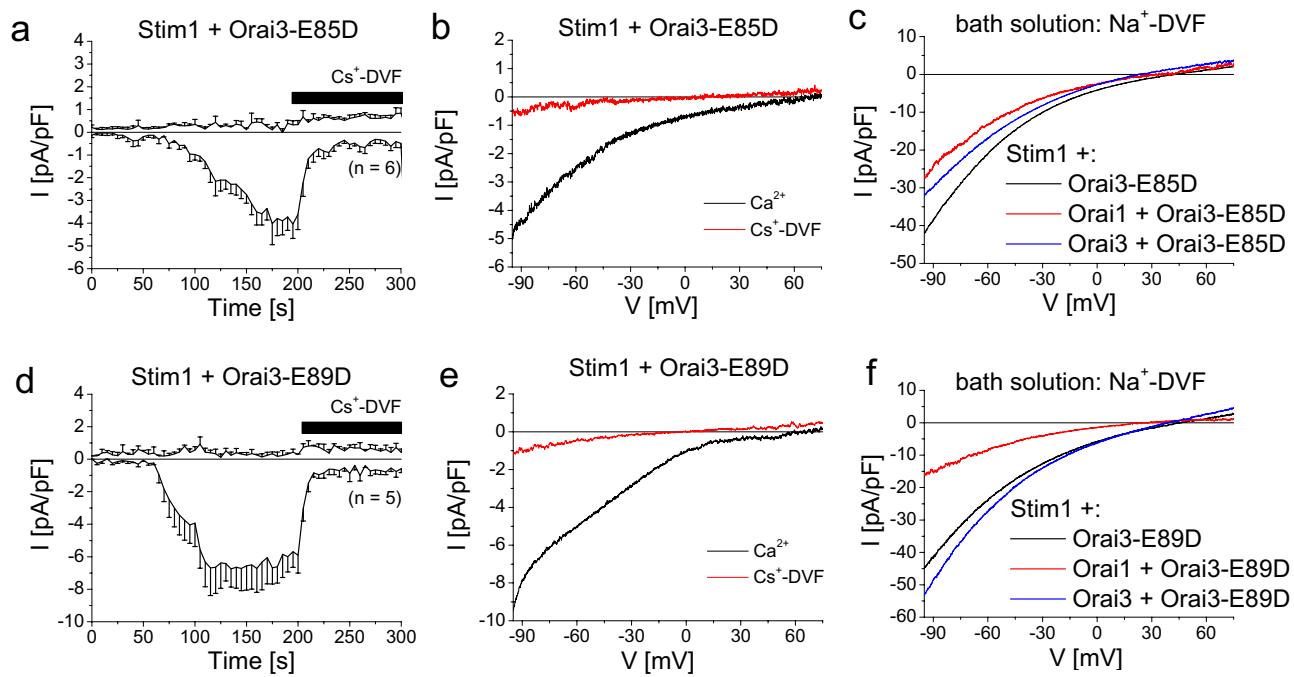


Fig. S2. Store-operated activation of Orai3 single point mutants. (a) Time course of co-expression of Stim1 and Orai3-E85D; (d) Stim1 and Orai3-E89D showing activation of Ca²⁺ currents upon passive store-depletion, followed by perfusion of a Cs⁺-DVF solution after 200 s. (b and e) Corresponding representative current voltage-relationships were taken from voltage ramps for a 10 mM Ca²⁺ containing bath solutions as well as a Cs⁺-DVF solution for (a and d). (c) Representative current voltage-relationships for Stim1, Orai3-E85D co-expression and in combination with overexpressed Orai1 or Orai3 in a Na⁺-DVF solution. (f) Representative current voltage-relationships for Stim1, Orai3-E89D co-expression and in combination with overexpressed Orai1, or Orai3 in a Na⁺-DVF solution.

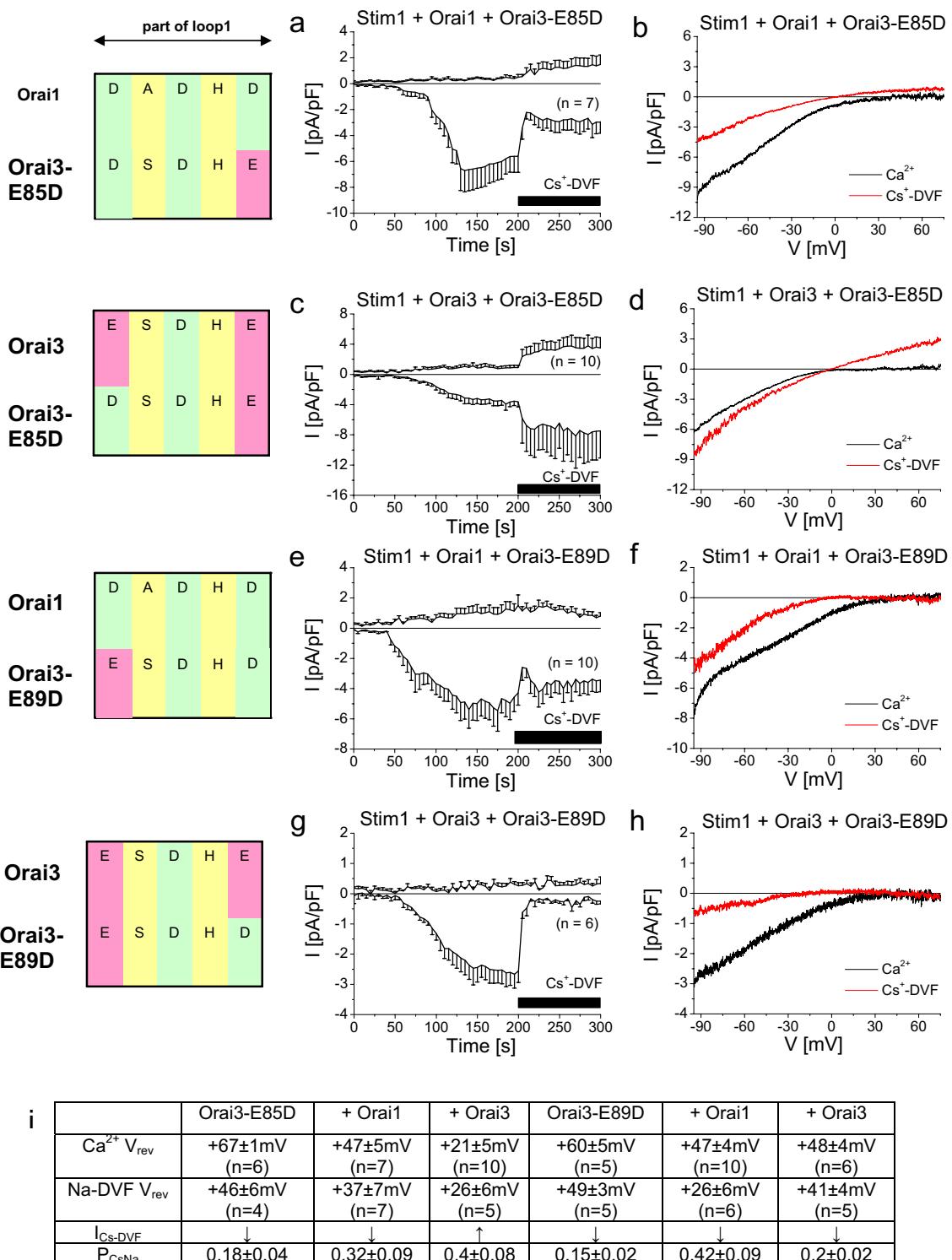


Fig. S3. Single point glutamate to aspartate mutations within the loop domain of Orai3 tune Ca^{2+} selectivity and Cs^+ permeation. Time course of co-expressing (a) Stim1, Orai1 and Orai3-E85D, (c) Stim1, Orai3 and Orai3-E85D, (e) Stim1, Orai1, and Orai3-E89D, or (g) Stim1, Orai3 and Orai3-E89D showing activation of Ca^{2+} currents upon passive store-depletion, followed by perfusion of a Cs^+ -DVF solution after 200 s. (b, d, f, and h) Corresponding representative current voltage relationships were taken from voltage ramps for a 10 mM Ca^{2+} containing bath solutions for (a, c, e, and g) as well as a Cs^+ -DVF solution. (i) Summary of reversal potentials in a Ca^{2+} and Na^+ -DVF solution, decrease (↓) or increase (↑) of inward currents when switching from Ca^{2+} to Cs^+ -DVF solution, and calculated permeability ratios P_{CsNa} for Stim1, Orai3-E85D, or Orai3-E89D as well as a co-expressed with Orai1 or Orai3.