Expanded View Figures

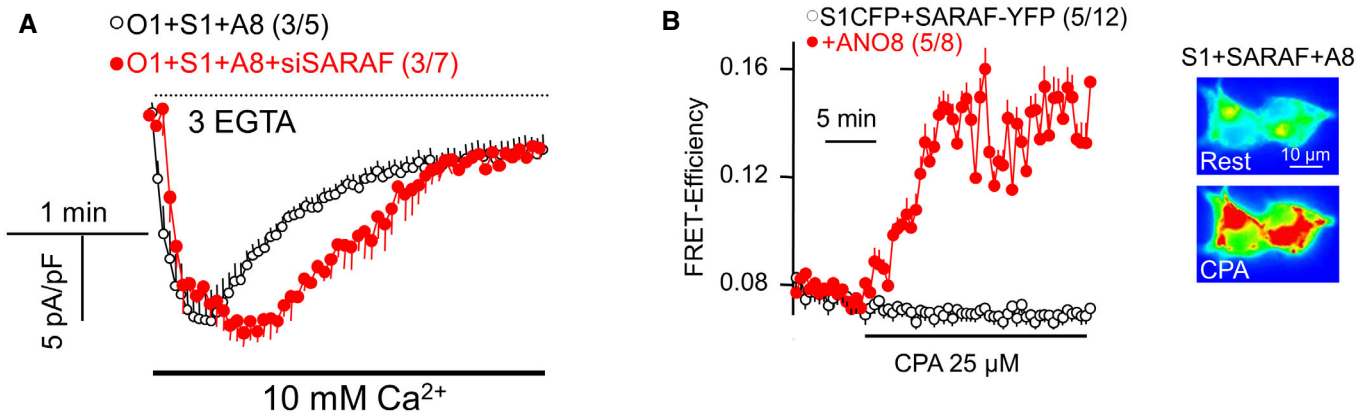
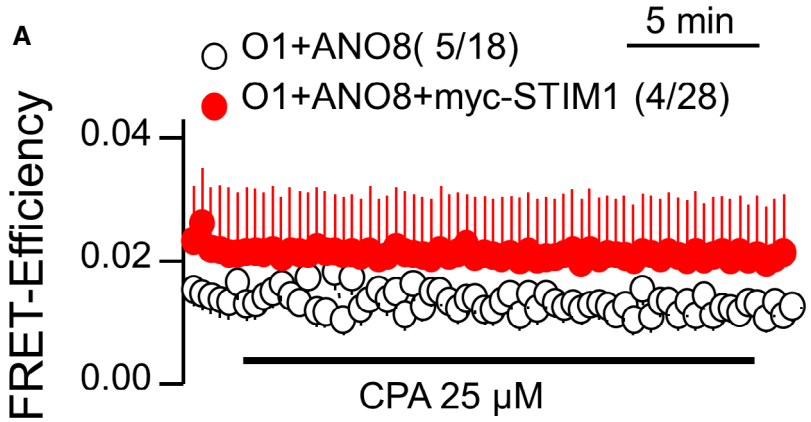


Figure EV1. SARAF modulates Orai1 SCDI and ANO8 increases STIM1-SARAF interaction.

A HEK cells transfected with STIM1-CFP, Orai1-mCherry, and ANO8 and treated with scrambled (black) or SARAF siRNA (red) were used to measure CRAC current with pipette solution containing 3 mM EGTA.

B FRET efficiency was measured with HEK cells transfected with STIM1-CFP, SARAF-YFP, and with (red) and without (black) untagged ANO8 before and after store depletion.



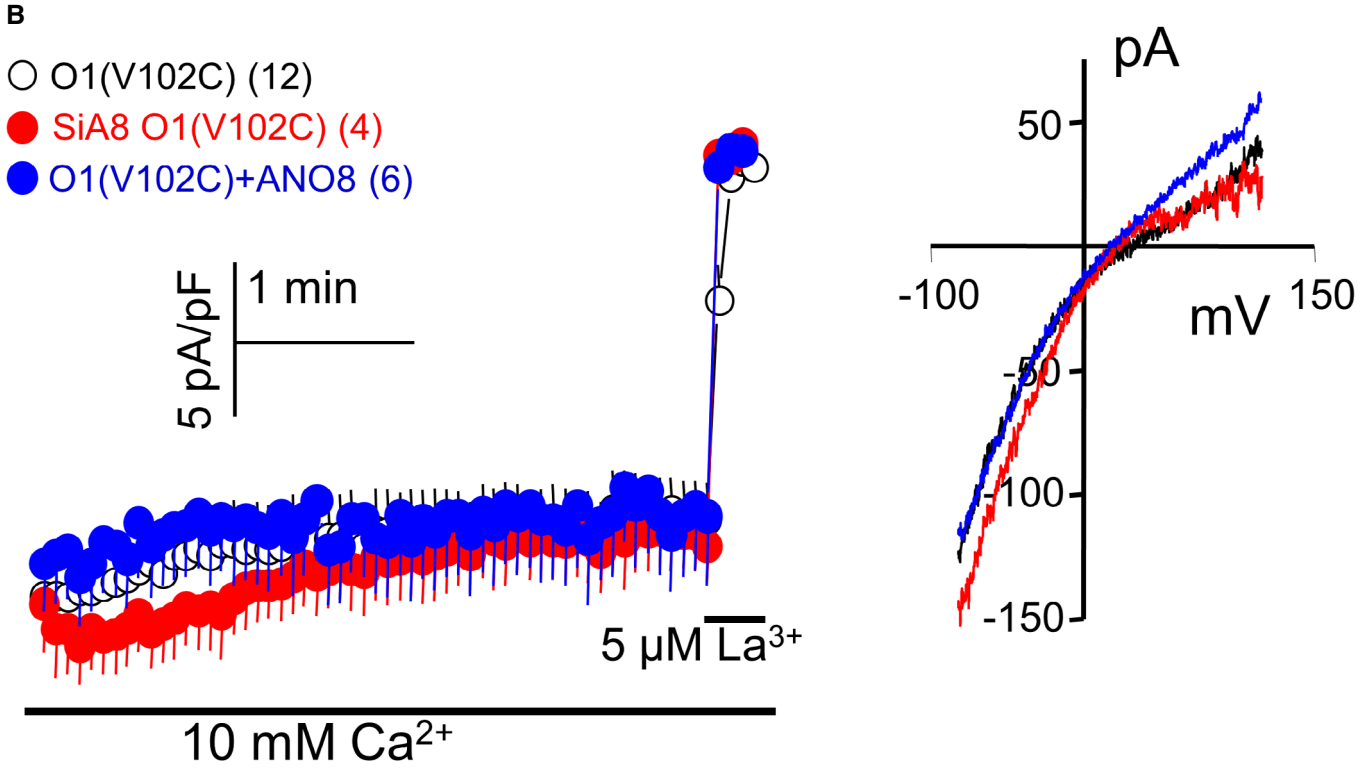


Figure EV2. ANO8 does not interact with or activate Orai1.

A FRET efficiency was measured with HEK cells transfected with Orai1-mCherry, ANO8-YFP, and with (red) and without (black) myc-STIM1 before and after store depletion.

B Effect of ANO8 on Orai1 current was measured using the STIM1-independent constitutively active Orai1 (V102C) mutant transfected alone in control cells (black), together with ANO8 (blue) or in cells treated with siANO8 (red).

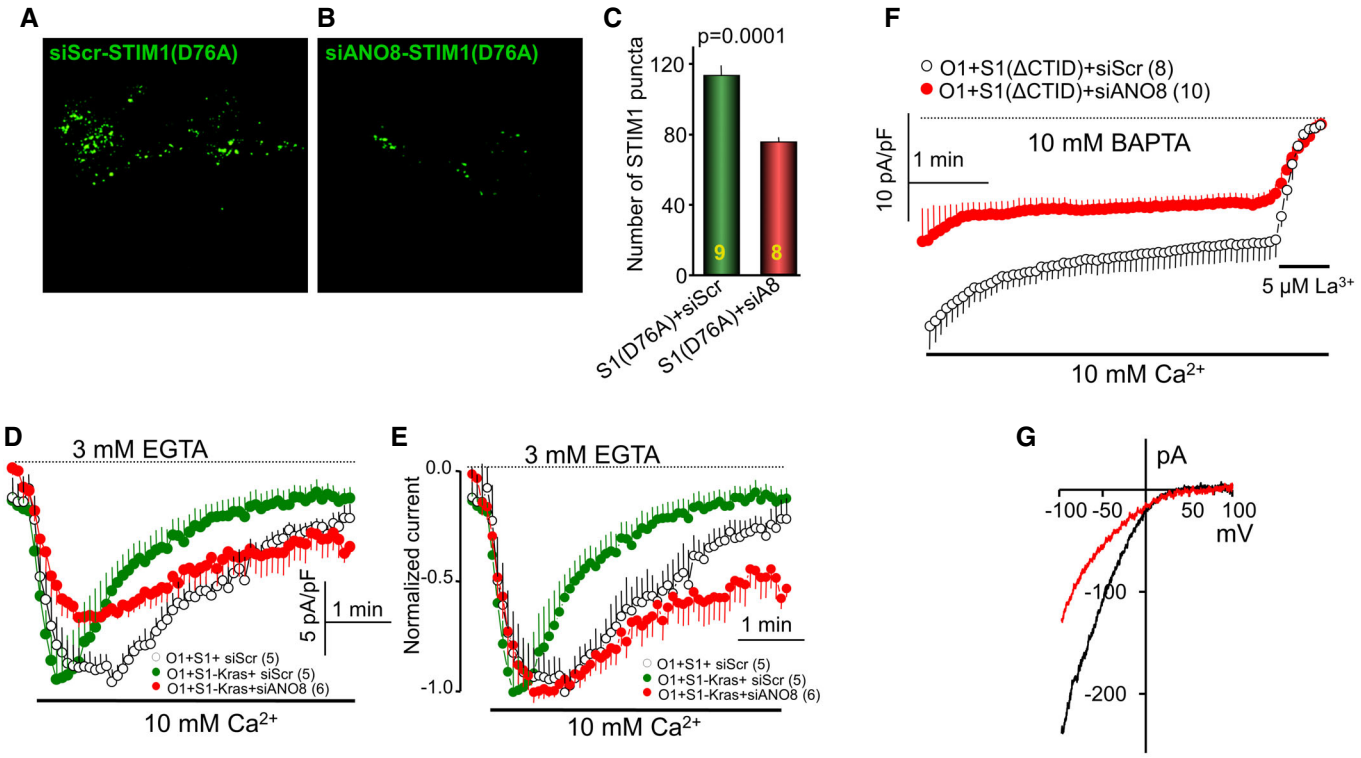


Figure EV3. Knockdown of ANO8 reduces clustering of constitutively active STIM1 mutants at the ER/PM junctions and activation of Orai1.

A–C HEK cells treated with scrambled (A) or ANO8 (B) siRNA were transfected with the constitutively active STIM1(D76A). The average number of puncta at the TIRF plane was analyzed in three separate experiments with the total number of cells indicated (C).

D, E Orai1 current was measured in cells transfected with Orai1 and the constitutively active STIM1-Kras with pipette solution containing 3 mM EGTA and treated with scrambled (black, green) or ANO8 (red) siRNA; (D) shows current density and (E) is the normalized current, which better demonstrates the effect of ANO8 knockdown on the rate of SCDI. Current started by addition of 10 mM Ca^{2+} to the media.

F, G Orai1 current was measured in cells transfected with Orai1 and the constitutively active STIM1(ΔCTID) with pipette solution containing 10 mM BAPTA. The cells were treated with scrambled (black) or ANO8 (red) siRNA. Current measurement started on establishing the whole-cell configuration in media already containing 10 mM Ca^{2+}.

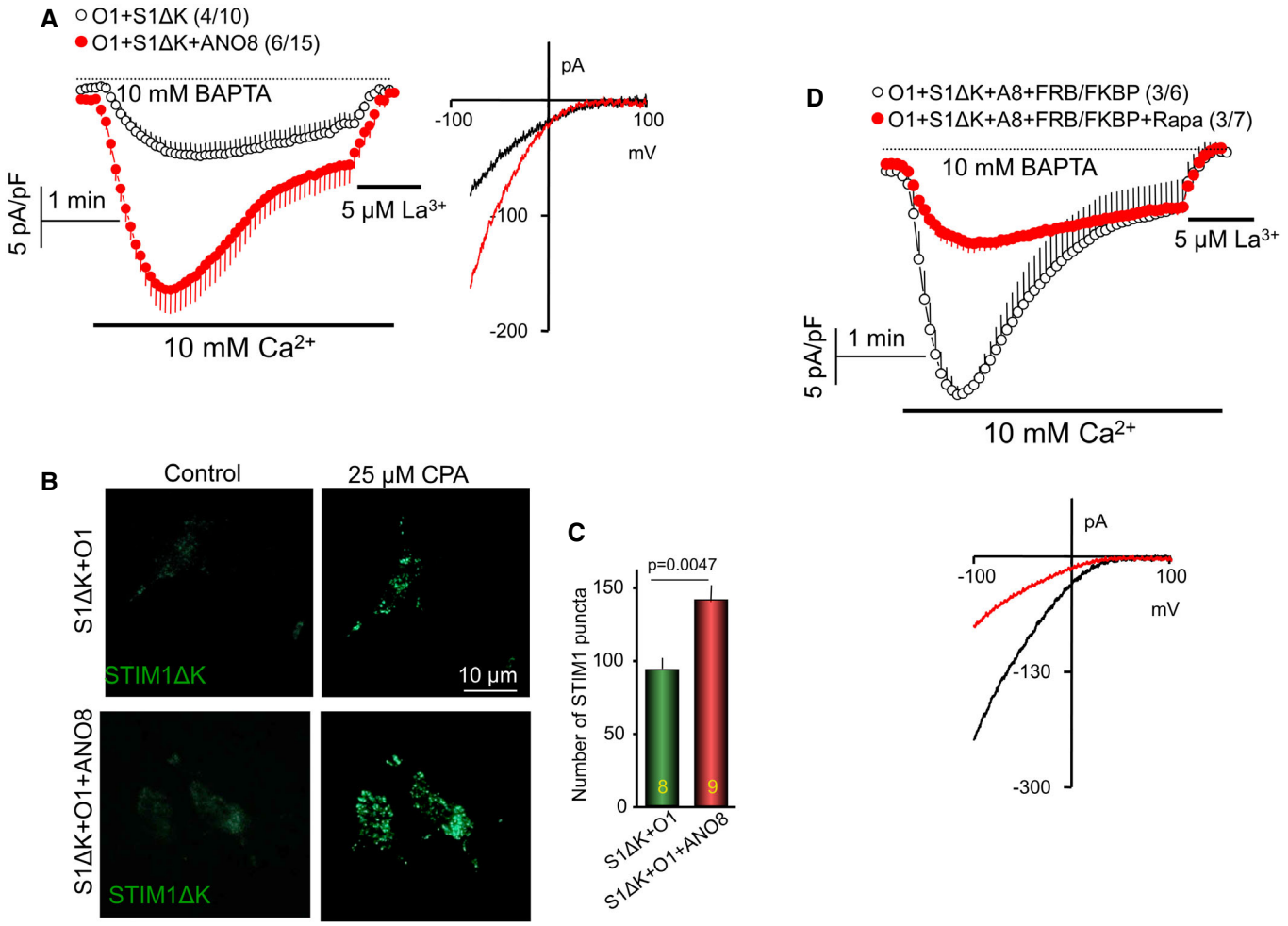


Figure EV4. ANO8 markedly increases current activated by STIM1(ΔK) that is PI(4,5)P_2-dependent.

A Current density recorded with pipette solution containing 10 mM BAPTA in cells transfected with STIM1(ΔK), Orai1 and with (red) or without (black) ANO8.

B, C STIM1(ΔK) puncta were recorded with TIRF microscopy of cells expressing STIM1(ΔK), Orai1, and with or without ANO8. Example images are shown in (B) and the average of the indicated number of cells in (C).

D HEK cells expressing STIM1(ΔK), Orai1, ANO8, FRB, and FKB were used to measure CRAC current without (black) and with treatment with 0.2 μM rapamycin to deplete PI(4,5)P_2 (red).

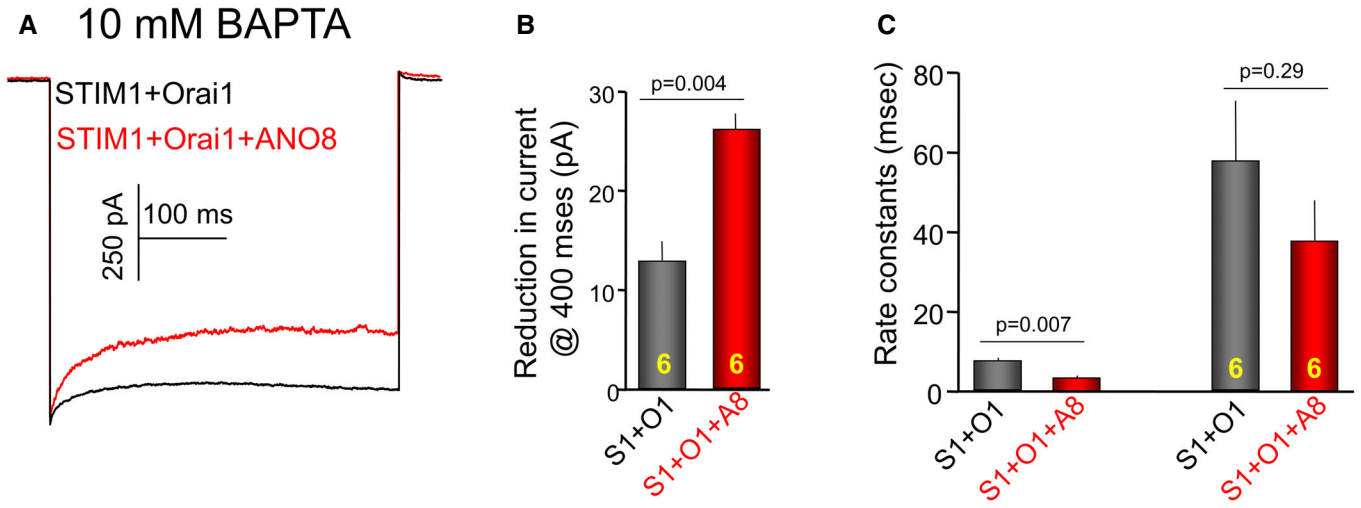


Figure EV5. Effect of ANO8 on fast Ca^{2+}-dependent inactivation.

A The current traces from HEK cells transfected with STIM1, Orai1, and with (red) and without (black) ANO8 with pipette solution containing 10 mM BAPTA.
B The extent of reduction in current at 400 ms.
C The current traces were fit to two exponentials, and the left and right columns show the effect of ANO8 on the first and second exponentials, respectively.