Supplemental Material for

Essential role for the CRAC activation domain in store-dependent oligomerization of STIM1

Elizabeth D. Covington,*† Minnie M. Wu,† and Richard S. Lewis

Department of Molecular and Cellular Physiology, Stanford University School of Medicine, Stanford, CA 94305

SUPPLEMENTAL FIGURE LEGENDS

Supplemental Figure 1. Orthogonally tagged STIM1-ΔC proteins do not co-immunoprecipitate, even after store depletion. Cells co-expressing GFP-labeled and HA-labeled STIM1-ΔC were treated with either 2 Ca^{2+} Ringer ("-" TG) or 0 Ca^{2+} Ringer + 1 μM thapsigargin ("+" TG) for 10 min prior to lysis and immunoprecipitation with anti-HA antibodies; immunoprecipitates were washed in buffer containing low salt (10 mM NaCl). Whole cell lysates (top) and immunoprecipitates (bottom) were fractionated by SDS-PAGE and blotted with anti-HA (left) or anti-GFP (right) antibodies. Representative of 2 experiments.

Supplemental Figure 2. STIM1-ΔC associates with STIM1-WT upon store depletion. (A) Schematic of some of the possible *inter*- (red bar) vs. *intra*- (blue bar) oligomers that could form between the EF-SAM regions of CFP-STIM1-WT and YFP-STIM1-ΔC in store-depleted cells. (B) Time course of mean FRET-E (± sem) of HEK 293 cells co-expressing either CFP-STIM1-ΔC and YFP-STIM1-WT (blue, n=4) or CFP-STIM1-WT and YFP-STIM1-ΔC (red, n=4). Stores were depleted with 0 Ca^{2+} Ringer's solution + 1 μM ionomycin at 140 s. The FRET-E time course for CFP-STIM1-ΔC + YFP-STIM1-ΔC (black, n=14) from Figure 2C is shown for comparison. (C) STIM1-ΔC is weakly recruited by STIM1-WT into peripheral puncta after store depletion. HEK 293 cells co-expressing CFP-STIM1-WT and YFP-STIM1-ΔC were treated with 1 μM ionomycin in 0 Ca^{2+} Ringer + 1 mM EGTA for 5 min at room temperature before imaging. Similar results were seen with cells co-expressing CFP-STIM1-ΔC and YFP-STIM1-WT. Arrowheads mark locations of puncta. Bar, 5 μm.

Supplemental Figure 3. STIM1 point mutants and their predicted effect on the coiled-coil forming ability of the CAD region. The sequence of part of the hSTIM1 CAD region, including the predicted CC2 domain, is shown in at the top. The predicted heptad repeat (abcdefg) of the most probable coiled-coil forming region is below the sequence. Arrowheads denote the positions of the three lysine substitutions introduced into STIM1 and CAD. The coiled-coil forming probability from COILS is plotted versus residue in hSTIM1 for STIM1-WT (black), STIM1-A369K (green), STIM1-L373K (blue), STIM1-A376K (red), and the triple mutant STIM1-3K (gray). Only the triple mutant (3K) was predicted to have a severe defect in coiled-coil formation.

Supplemental Figure 4. Resting puncta of STIM1-A369K and STIM1-A376K are peripherally located. Confocal images focused near the equator of resting HEK 293 cells co-expressing mCherry-myc-Orai1 and (A) GFP-STIM1-A369K or (B) GFP-STIM1-A376K. Bars, 5 μm.

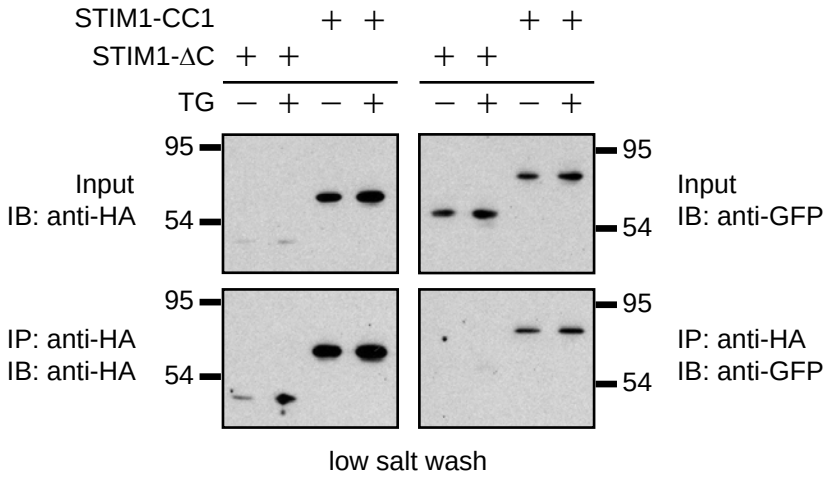
Supplemental Figure 5. STIM1-3K, similar to STIM1-A376K, forms puncta at rest, but cannot cluster Orai1 or activate SOCE. (A) Confocal images at the footprint of HEK 293 cells co-transfected with GFP-STIM1-3K and mCherry-myc-Orai1, before and after treatment with 1 μM thapsigargin to deplete stores. Bar, 5 μm. (B) Mean fura-2 ratios (± sem) for wild-type STIM1 (red, n=32) and STIM1-3K (black, n=33) in HEK 293 cells

co-transfected with mCherry-myc-Orai1. Extracellular solutions are indicated above the graph.

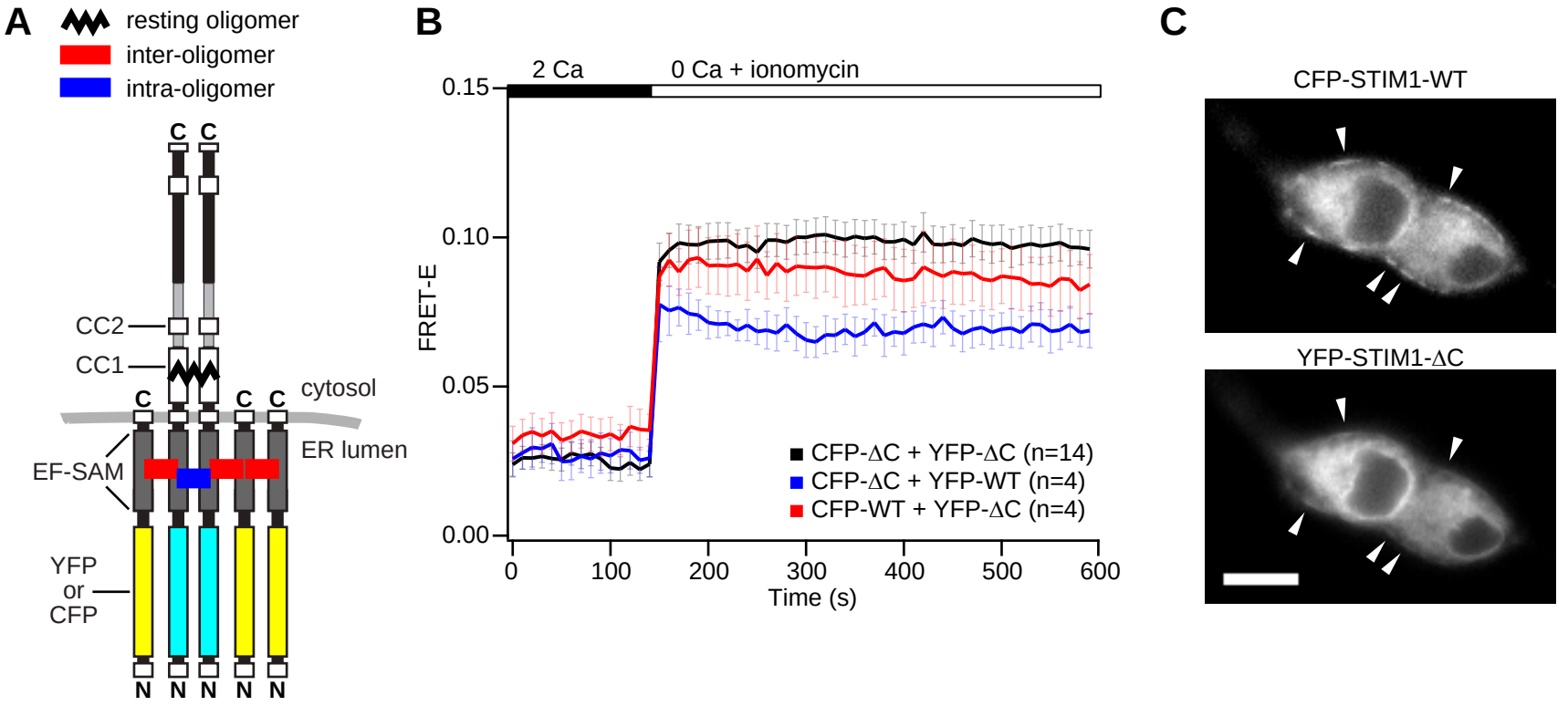
Supplemental Figure 6. Mutations in CC2 of the CAD prevent co-localization with or activation of Orai1. (A) Mutant CADs fail to co-localize with Orai1 at the plasma membrane. Confocal images of HEK 293 cells co-expressing GFP-myc-Orai1 (left) with mCherry-CAD constructs (middle). Bars, 5 μm. CAD mutation is indicated to the left of each row of images. Fluorescence intensity along a 5-pixel wide line for each channel was normalized to the peak intensity and overlaid (far right; mCherry intensity, red; GFP intensity, green) to show the mCherry-CAD mutants are not recruited to the plasma membrane. (B) Mutations in mCherry-CAD inhibit constitutive Ca^{2+} entry. Mean fura-2 ratios (± sem) for HEK 293 cells expressing GFP-myc-Orai1 plus mCherry-CAD (black; n=29), mCherry-CAD-A376K (blue; n=25), or mCherry-CAD-3K (gray; n=9). Extracellular solutions are displayed above the graph.

Supplemental Figure 7. The resting FRET level of CFP/YFP-labeled, peripherally-localized STIM1-A369K is higher than for STIM1-WT. Comparison of mean FRET efficiencies (± sem) of STIM1-WT (black, n=7 cells) and STIM1-A369K (green, n=9 cells) in the cytosol (central, solid lines) versus the cell periphery (peripheral, circles). FRET efficiency was measured for HEK 293 cells expressing CFP- and YFP-labeled STIM1-WT or STIM1-A369K. Data analysis was restricted to an ROI encompassing the center of the cell or a peripheral ROI band, 10 pixels-wide (approx 1.5 μm), drawn from the edge of the cell (see Methods). The higher resting FRET-E of peripheral STIM1-A369K vs. STIM1-WT is consistent with the constitutive Ca^{2+} influx observed in Figure 4E.

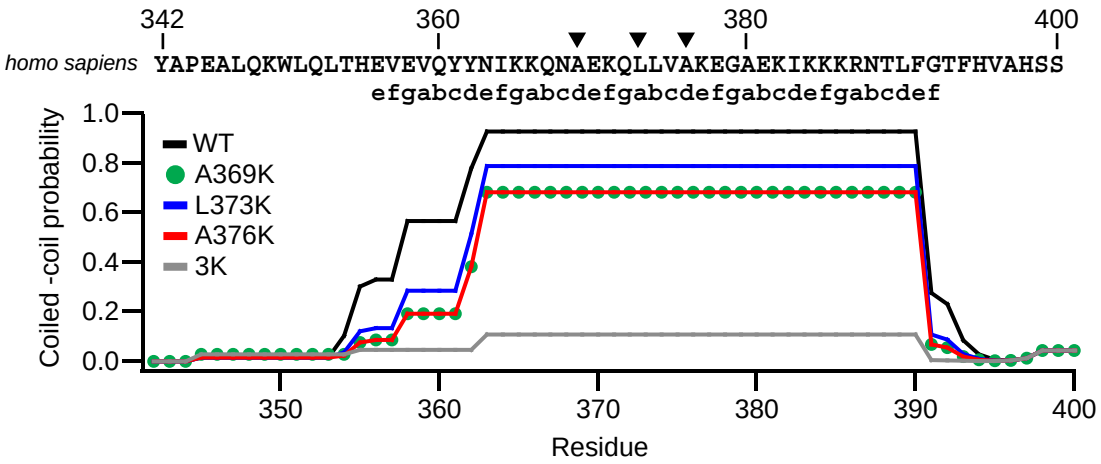
Supplemental Figure 1



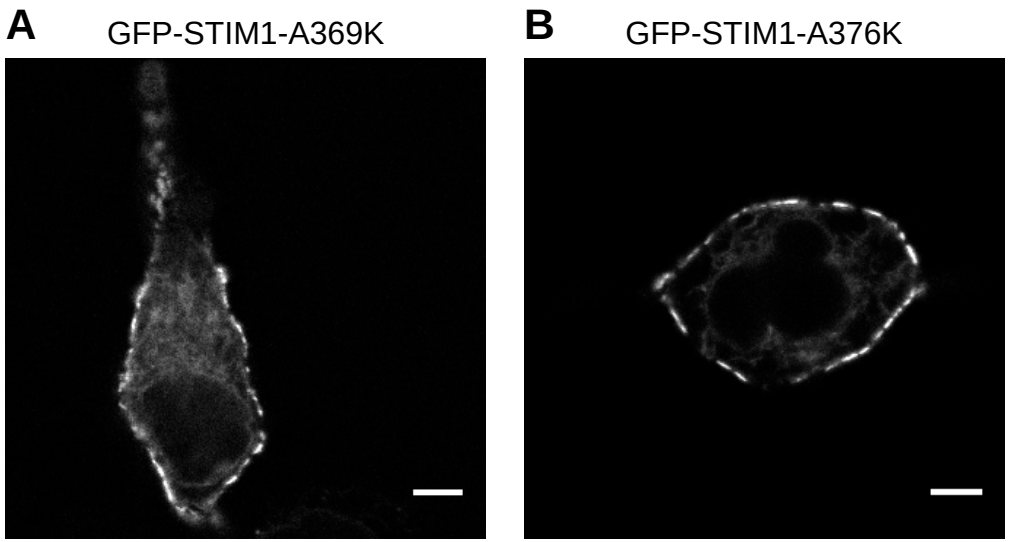
Supplemental Figure 2



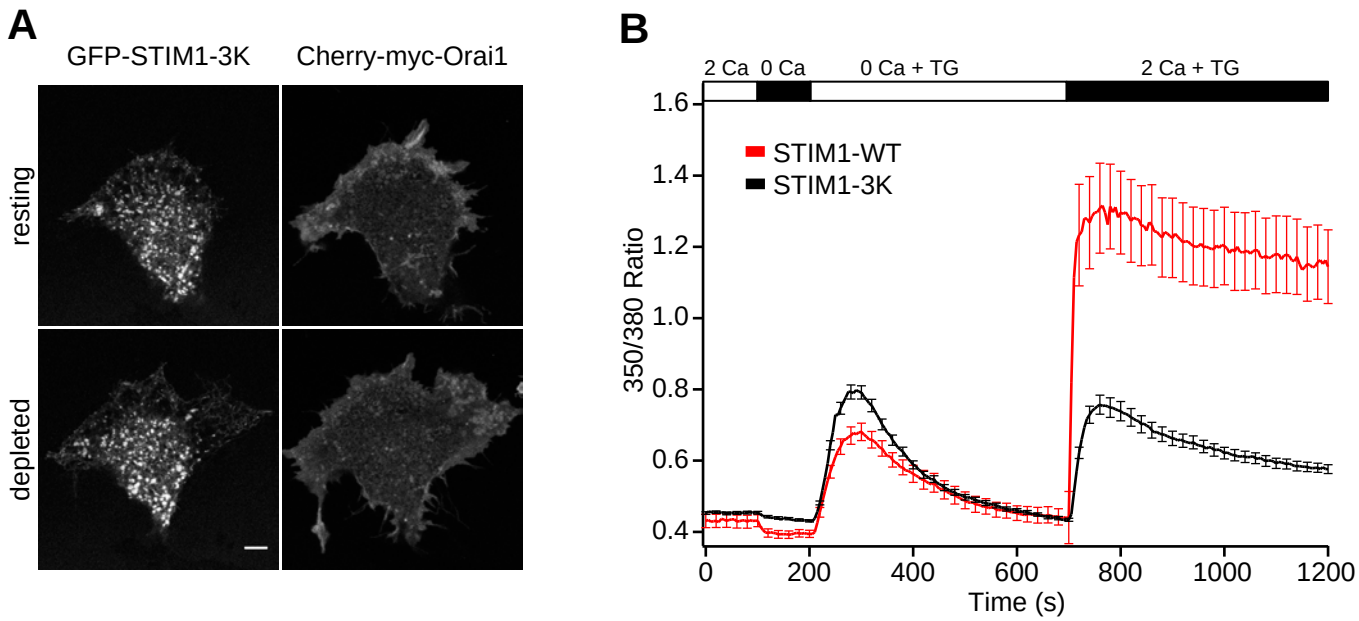
Supplemental Figure 3



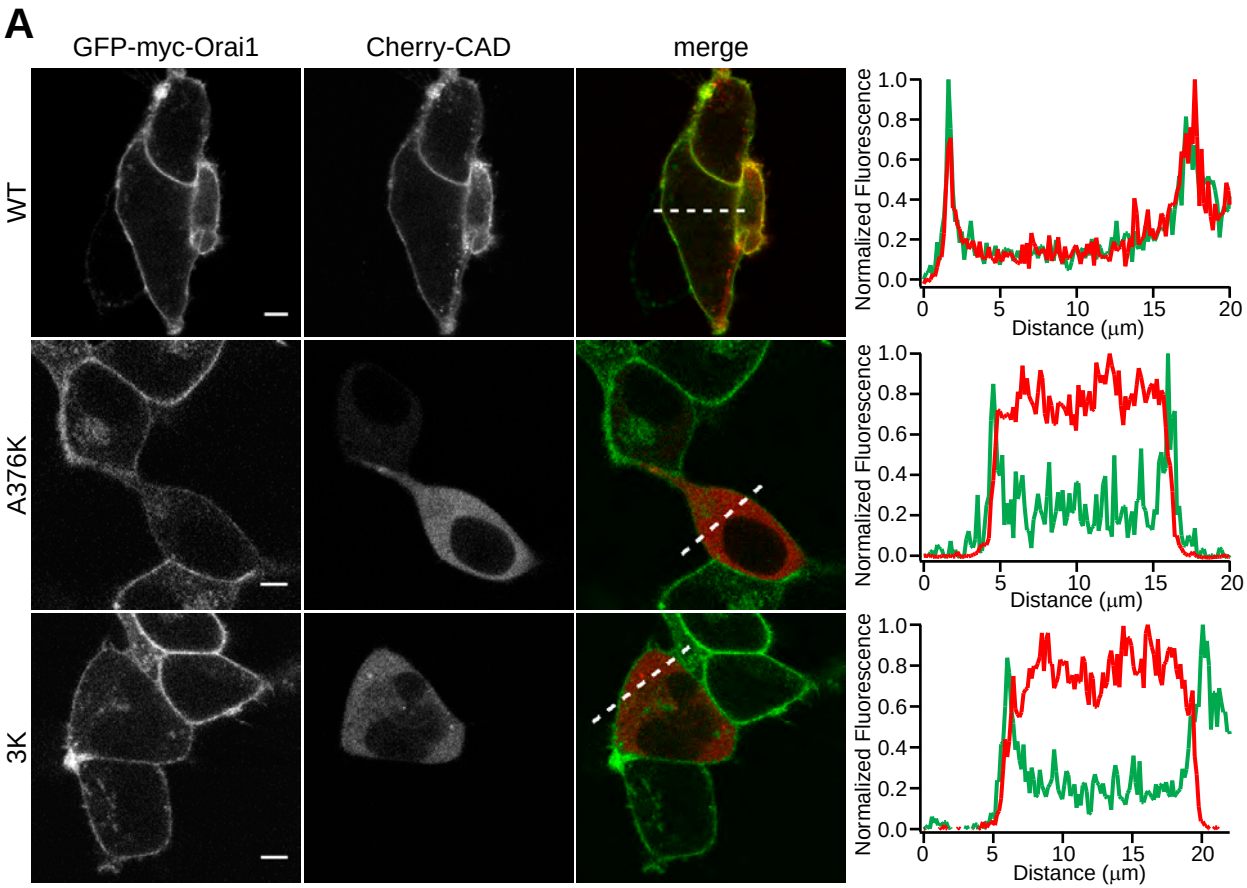
Supplemental Figure 4



Supplemental Figure 5



Supplemental Figure 6



Supplemental Figure 7