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SUPPLEMENTARY DATA

Oligomerization and Ca^{2+}/calmodulin control binding of the ER Ca^{2+}-sensors STIM1 and STIM2 to plasma membrane lipids

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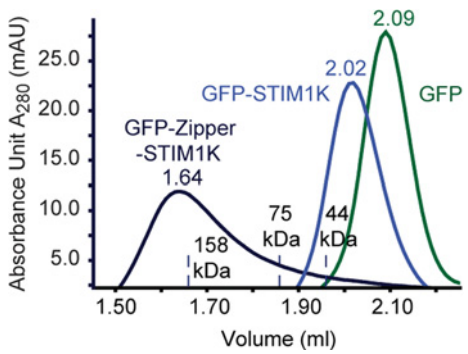

Figure S1 Addition of a leucine zipper to STIM1 K-rich domain leads to tetramerization

Elution profiles of 5 μM GFP (green), GFP–STIM1K (blue) and a GFP-tagged construct with yeast GCN4 leucine zipper fused to STIM1 K-rich domain (GFP–Zipper–STIM1K, black) S200 gel-filtration column run are shown. Positions where markers eluted are indicated.

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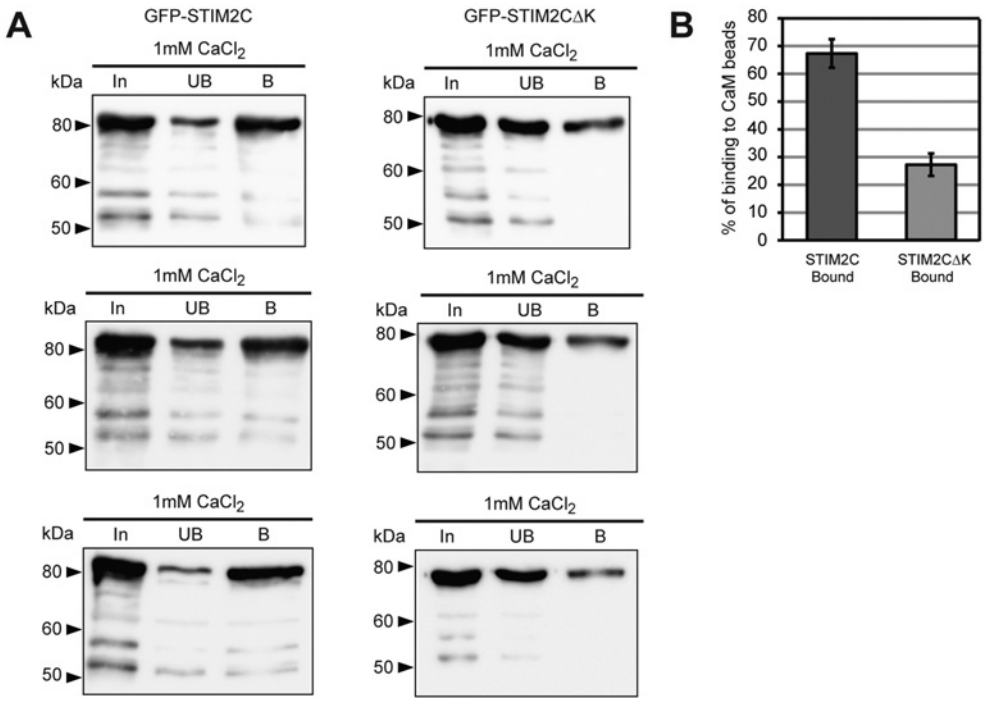


Figure S2 **Western blot quantification of GFP–STIM2C and GFP–STIM2CΔK binding to Ca^{2+}/calmodulin beads**

(**A**) 1 μM reduced GFP–STIM2C and GFP–STIM2CΔK were incubated with Ca^{2+}/CaM beads in presence of 1 mM $CaCl_2$. Input (In), unbound (UB) and bound (B) material of three separate experiments were detected by a rabbit polyclonal GFP antibody from Santa Cruz Biotechnology, Inc. (1:5000). (**B**) Quantification of bound GFP–STIM2C and GFP–STIM2CΔK from three experiments. Bars indicate means $\pm$ S.D.

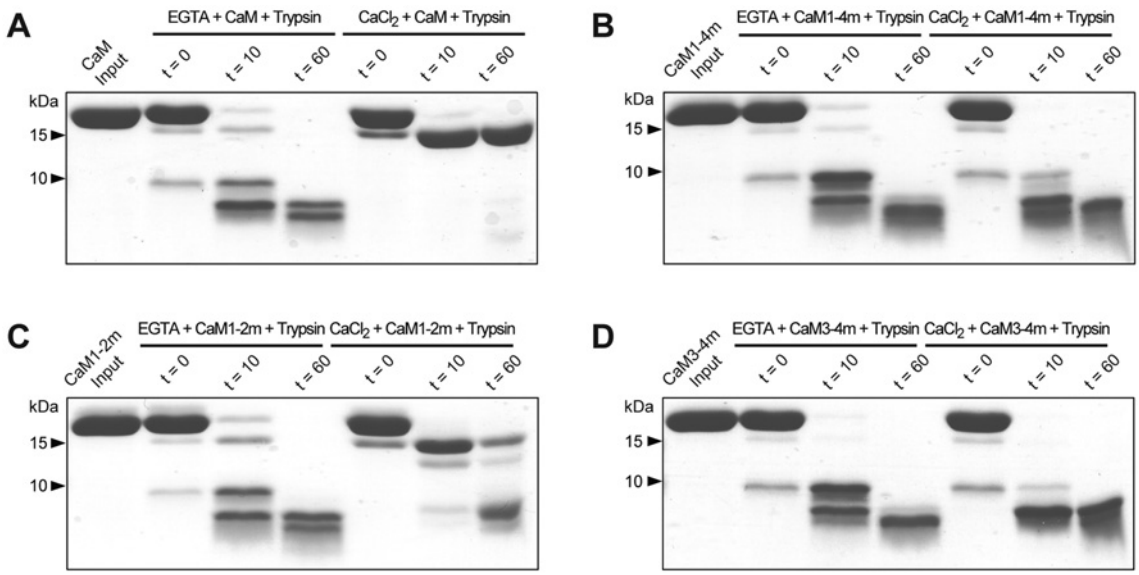
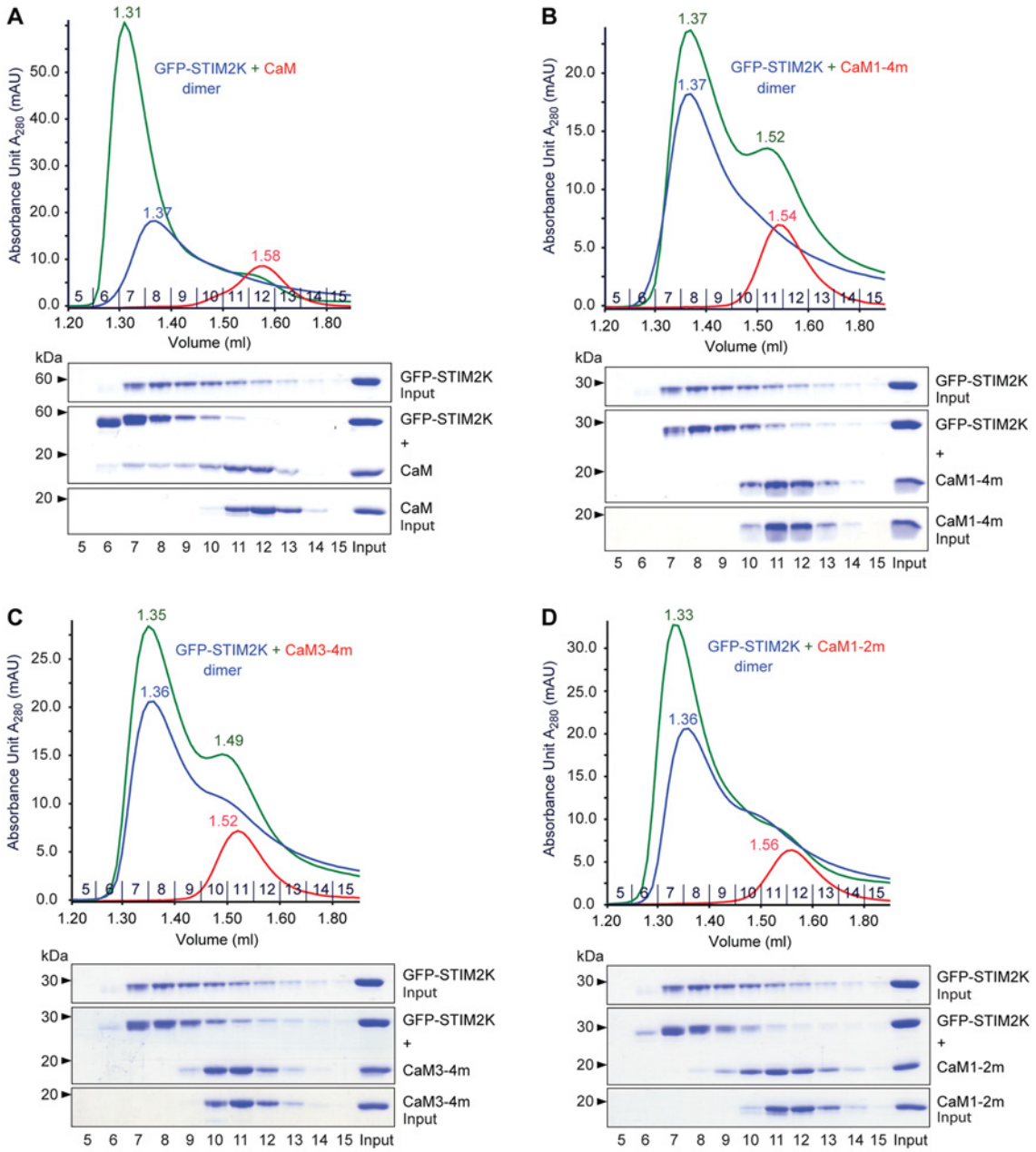


Figure S3 Protease protection assays with purified CaM and CaM mutants
14 μM CaM (**A**), CaM1–4m (**B**), CaM1–2m (**C**) and CaM3–4m (**D**) in 1 mM EGTA or 0.2 mM $CaCl_2$ were incubated with 0.02 μM trypsin in 20 mM ammonium bicarbonate buffer (pH 8.1) at 20 °C for the indicated times. The proteins were separated by SDS/PAGE and stained with Coomassie Brilliant Blue. The first lane of each gel shows the input of CaM or Ca^{2+}-binding mutants of CaM without protease.



Figuere S4 **In solution binding of GFP-STIM2K dimer and calmodulin**

10 μM oxidized GFP–STIM2K dimer and 10 μM purified CaM (**A**), CaM1–4m (**B**), CaM3–4m (**C**) or CaM1–2m (**D**) in 1 mM $CaCl_2$ were separated by gel filtration. The elution profiles of GFP–STIM2K dimer (blue), CaM (red) and complexes (green) with indicated volume for elution peaks are shown. Proteins of the indicated eluted fractions separated by SDS/PAGE and Coomassie Brilliant Blue stained are shown below the chromatograms.

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