Supplementary Figures

Supp. Fig. 1. Sequence alignment of the sequence of the conserved N-terminal region of Orai proteins. Putative CaM binding domain (blue), conserved region (green) and transmembrane region 1 (red).

Supp. Fig. 2. Localization of Orai3 deletion mutants. Confocal fluorescence images from a representative cell expressing a) wild-type YFP-Orai3, b) YFP-Orai3 ΔN_{1-47}, c) YFP-Orai3 ΔN_{1-51}, d) YFP Orai3 ΔN_{1-53}, e) YFP-Orai3 ΔN_{1-55}, f) YFP-Orai3 ΔN_{1-57}, g) YFP-Orai3 ΔN_{1-62} and h) YFP-Orai3 ΔN-term. i) Block diagram comparing plasma membrane intensities of Orai3, Orai3 ΔN_{1-47} and Orai3 ΔN_{1-57}.

Supp. Fig. 3. Inactivation profile, store-operated and 2-APB-mediated currents of Orai3 deletion mutants. a) d) Store-operated currents of Orai3 ΔN_{1-47} and ΔN_{1-55}. b) e) Corresponding current/voltage relationships to a) d). c) f) Corresponding fast inactivation to a) d). g) h) 2-APB mediated currents of all Orai3 deletion mutants. i) Store-operated activation of Orai3 ΔN_{1-60} and ΔN_{1-62}. j-l) Fast inactivation upon voltage-steps to -90mV for 1600ms.

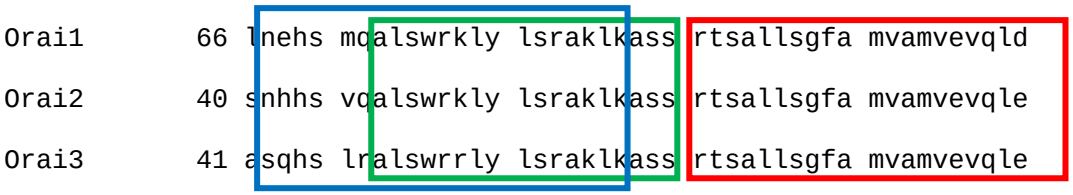
Supp. Fig. 4. Comparison of peptide aa 47-65 and peptide aa 52-65 with respect to CaM binding. The sensor chip was prepared and cleaned as described (Hahn et al., 2007, Chemical Monthly 138, 245–252) and immediately immersed in an ethanolic solution of octadecanethiol (20 µM) for 72 h to form a hydrophobic self-assembled monolayer. After rinsing in ethanol and water, the chip was mounted in the BIAcore setup and superfused with PBS. The flow was 20 µl/min (except for lipid vesicle injection which was at 4 µl/min) and all injection volumes were 100 µl. The surface was washed by injection of octylglucoside (40 mM, 100 µl), whereupon lipid vesicles (DOPC/biotin-cap-DOPE/PS at a molar ratio of 7/2/1, 1 mg/ml lipid concentration in PBS) were injected at a flow rate of 4 µl/min, yielding a phospholipid monolayer with biotin residues on 20% of the lipid head groups (Sagmeister et al., 2009, Biosens. Bioelectron. 24, 2643-2648). Adhering vesicles were removed by injection of 10 mM NaOH. Then the running buffer was changed to Hepes buffer (150 mM NaCl, 5 mM Hepes, pH 7.3). Streptavidin (2 µM, in Hepes buffer) was injected in flow cell 2 yielding a dense layer of streptavidin. The free binding sites were blocked with *D*-biotin (200 µM in Hepes buffer). After thorough rinsing of the complete microfluidics system to remove free *D*-biotin, streptavidin was applied to flow cell 1, followed by injection biotin-EG_8-Orai peptide aa 47-65 or aa 52-65 (2 µM in Hepes buffer), also in flow cell 1 only. Then the running buffer was changed to Hepes buffer (150 mM NaCl, 5 mM Hepes, pH 7.3) either containing 2 mM EGTA (Ca-free buffer, data not shown) or to 2 mM $CaCl_2$ (Ca-buffer) and CaM (2 µM) was injected in the corresponding buffer also. The resonance angle change is shown in kilo-resonance units (1000 RU, equivalent to a 0.1° change in the resonance angle). Rough estimates of the K_D values for the binding of CaM to the two peptides were calculated with the BIAevaluation software 3.1 (1:1 Langmuir binding). The sensorgrams show CaM binding of CaM to peptide aa 47-65 (flow cell 1, solid black line) and to the corresponding peptide-free control cell (flow cell 2, dotted black line), as well as of CaM binding to peptide aa 52-65 (flow cell 1, solid red line) and to the corresponding peptide-free control cell (flow cell 2, dotted red line). The curves were vertically displaced by small increments to avoid overlap of the data.

Supp. Fig. 5. Comparison of peptide aa 47-65 and peptide aa 52-65 R52A R53A with respect to CaM binding. The sensor chip was prepared and cleaned as described (Hahn et al., 2007, Chemical Monthly 138, 245–252) and immediately immersed in an acetonitile

solution of N-(14-hydroxy-3,6,9,12-tetraoxatetradecyl)-16-sulfanylhexadecanoylamide (16 μM, Svedhem et al. 2001, J. Org. Chem. 66, 4494-4503) and N-(23-biotinamido-3,6,9,12,15,18,21-heptaoxatricosanyl)-16-sulfanylhexadecanoylamide (4 μM, Pollheimer et al., manuscript in preparation) for 72 h to form a protein-resistant self-assembled monolayer with biotin on 20% of the oligo(ethylene glycol) chains, in close analogy to Jung et al. (1999, Sensors and Actuators B 54, 137-144). After rinsing in acetonitrile, ethanol, and water, the chip was mounted in the BIAcore setup and superfused with Hepes buffer (150 mM NaCl, 5 mM Hepes, pH 7.3). The selective functionalization of the control cell (flow cell 2) with biotin-blocked streptavidin and of the measuring cell (flow cell 1) with functional streptavidin was performed as described in Supp. 5. The next step was injection biotin-EG_8-Orai peptide aa 47-65 or aa 52-65 R52A R53A (2 μM in Hepes buffer), in flow cell 1. Finally, the binding of CaM in Ca-free buffer or Ca-buffer was tested as described in Supp. 5. The sensorgrams show binding of CaM to peptide aa 47-65 (flow cell 1, solid black line) and to the corresponding peptide-free control cell (flow cell 2, dotted black line), as well as of CaM binding to peptide aa 52-65 R52A R53A (flow cell 1, solid red line) and to the corresponding peptide-free control cell (flow cell 2, dotted red line). The curves were vertically displaced by small increments to avoid overlap of the data.

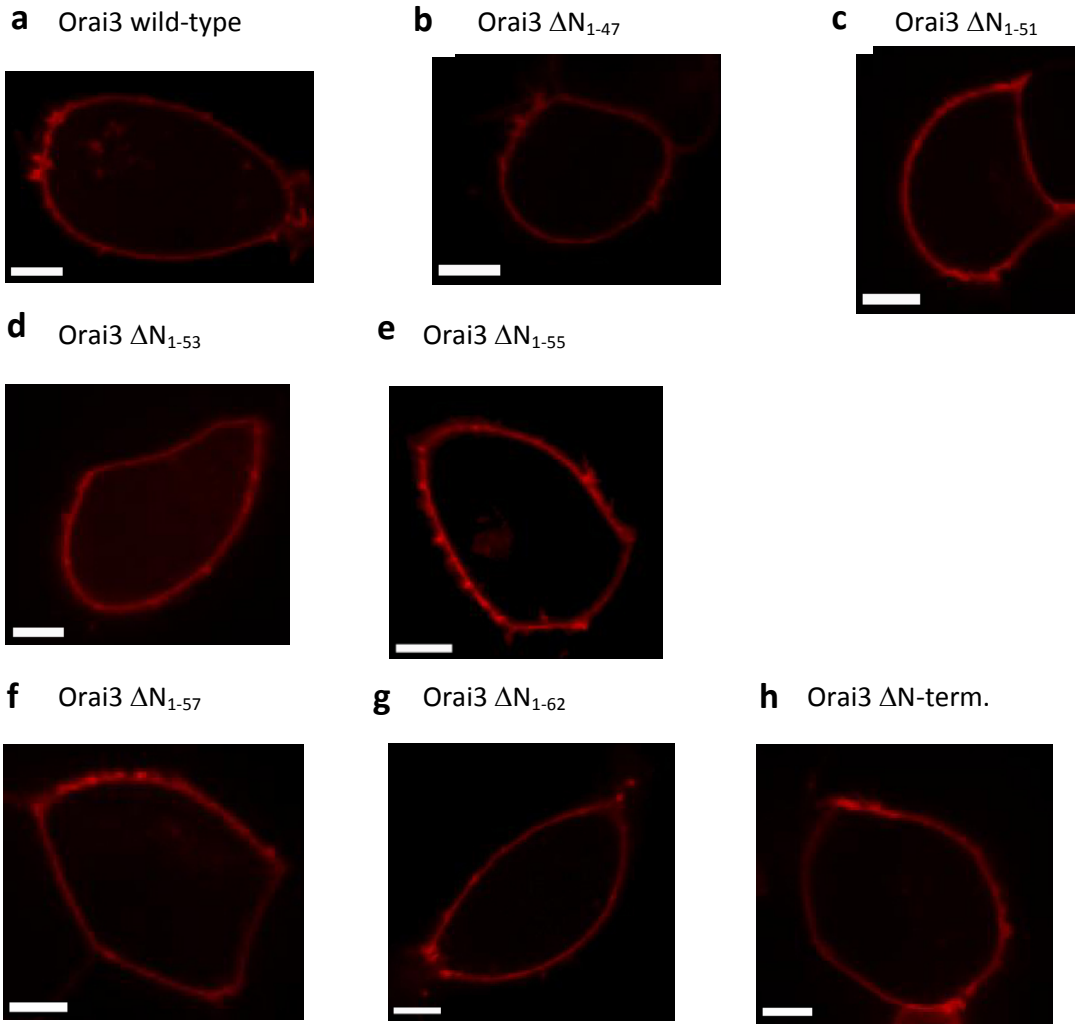
Supp. Fig. 6. Neither CRACR2A knock-down nor CRACR2A over-expression affects Orai3 gating. a) Time-course of whole cell inward currents at -74 mV activated by passive store-depletion of HEK 293 cells coexpressing STIM1 and Orai1 together with either siRNA CRACR2A or CRACR2B in comparison to control siRNA. b) d) Time-course of whole cell inward currents at -74 mV activated by passive store-depletion of HEK 293 cells coexpressing STIM1 and b) Orai3 or d) Orai3 ΔN_{1-53} together with either siRNA CRACR2A or CRACR2B in comparison to control siRNA. c) e) Corresponding, mean fast inactivation for 100 ms upon voltage steps from a holding potential of 0 mV to -90 mV of normalized current traces to b) d). f) h) Time-course of whole cell inward currents at -74 mV activated by passive store-depletion of HEK 293 cells coexpressing STIM1 and f) Orai3 or h) Orai3 ΔN_{1-53} together with either CRACR2A or CRACR2B in comparison to control cells. g) i) Corresponding, mean fast inactivation for 100 ms upon voltage steps from a holding potential of 0 mV to -90 mV of normalized current traces to f) h).

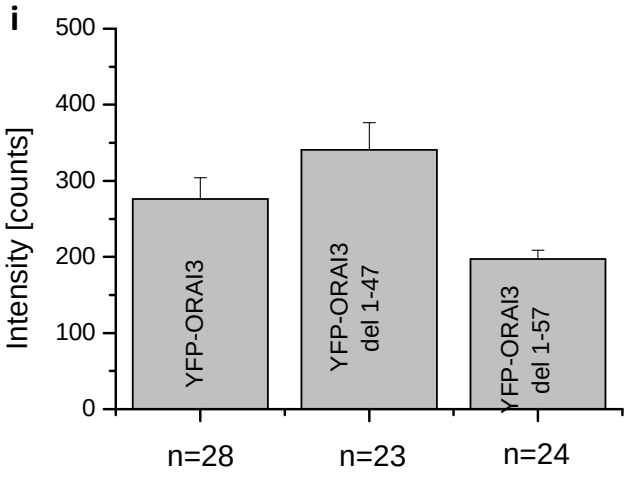
Supplementary Figure 1



CaM binding domain; conserved region; 1st transmembrane domain

Supplementary Figure 2





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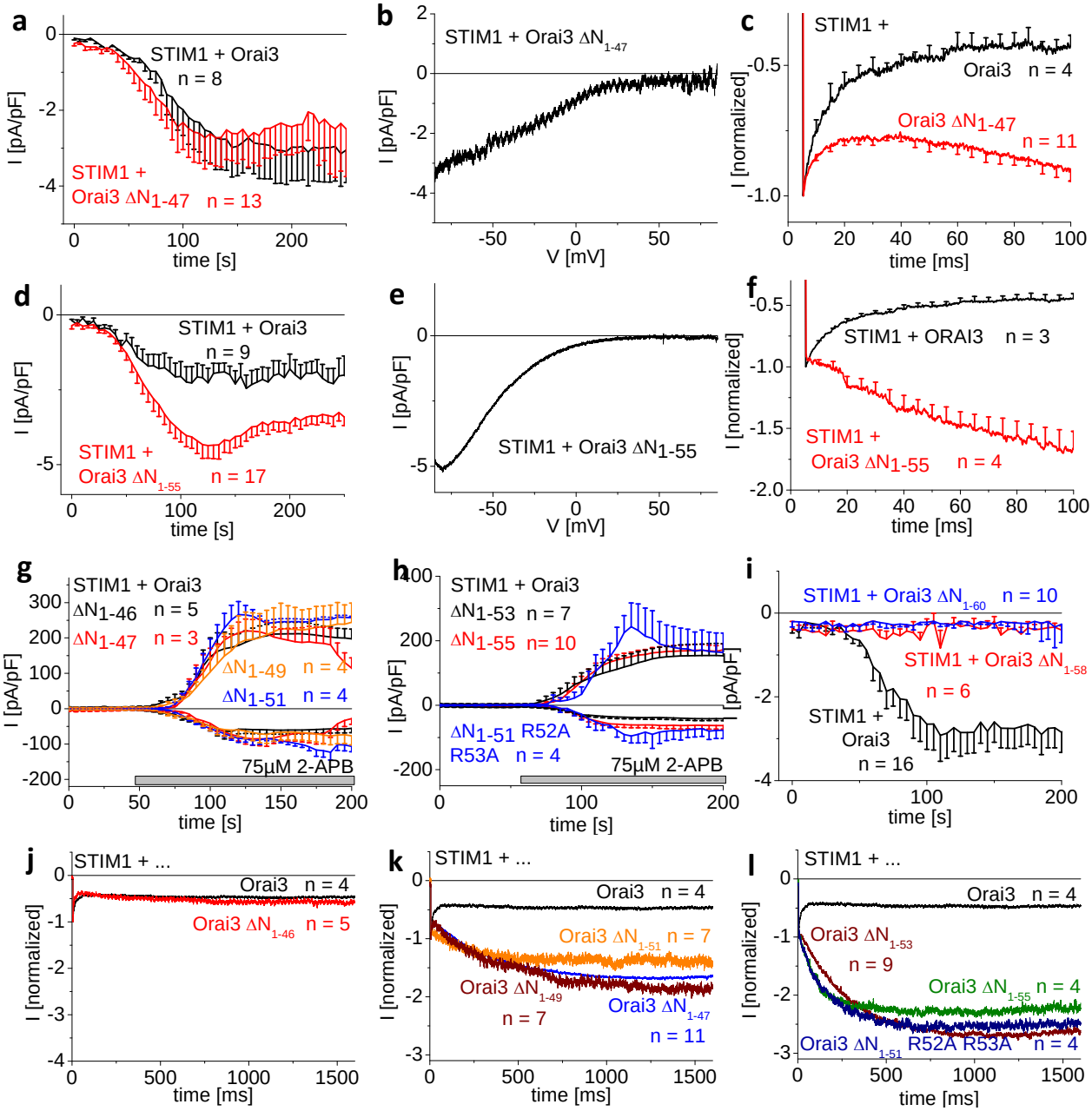

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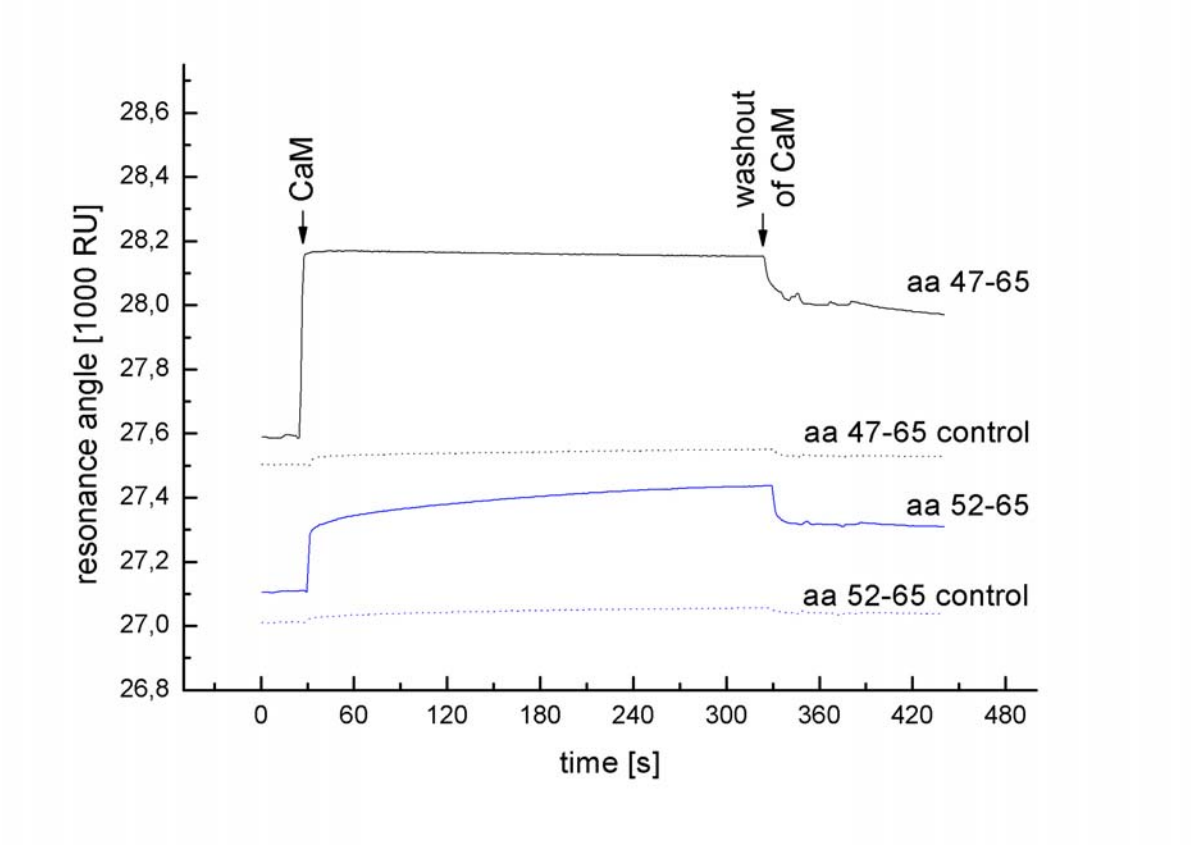
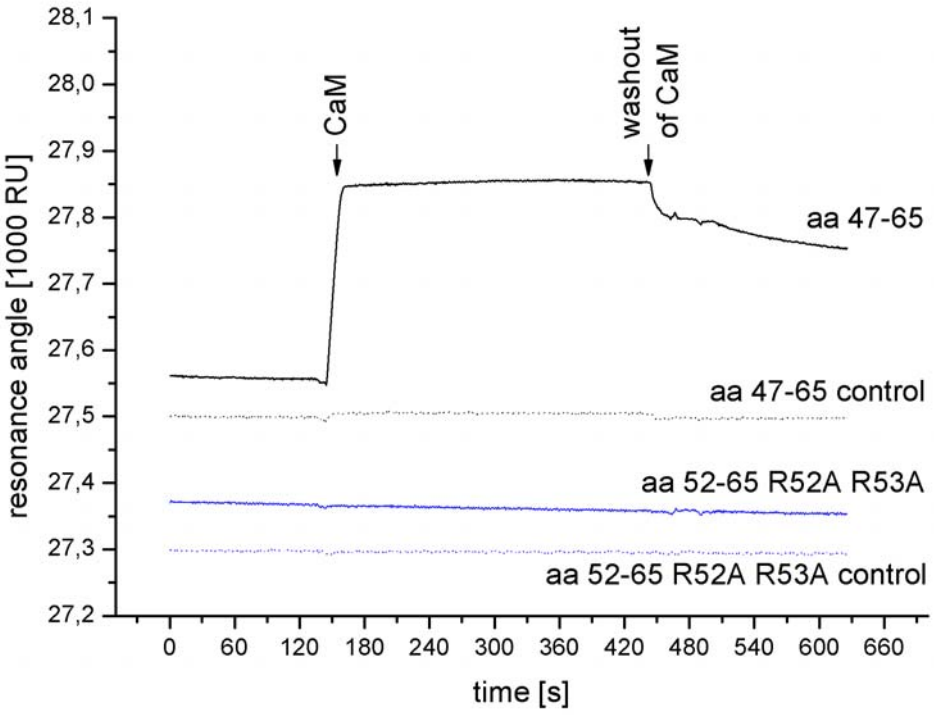


Supplementary Figure 5



Supplementary Figure 6