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Supplementary Materials for

Cholesterol modulates Orai1 channel function

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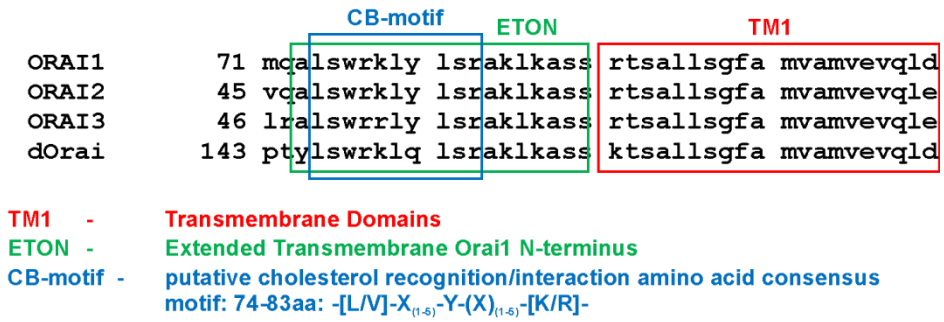
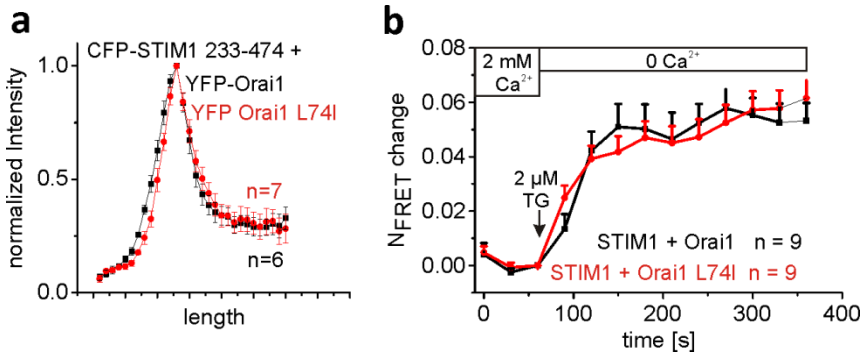
The PDF file includes:

Supplementary Figure 1



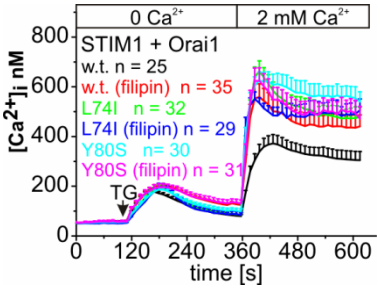
Supplementary Figure 1 Sequence alignment of the ETON region and TM1 in Orai1, Orai2, Orai3, and dOrai. The CB-motif is indicated in blue, the ETON region in green, and the first transmembrane domain in red. The CB-motif is not fully conserved in dOrai.

Supplementary Figure 2



Supplementary Figure 2 The colocalization of STIM1 233–474 and FRET of STIM1 with Orai1 L74I are similar to wild-type Orai1. **a)** Co-localization intensity plot of STIM1 233-474 in cells containing Orai1 L74I compared to Orai1. **b)** Time-course of FRET upon store-depletion through 2 µM TG of STIM1 and Orai1 compared to STIM1 and Orai1 L74I co-expressing cells. For a) - b) n represents the number of tested cells, which have been taken from 3 different transfections.

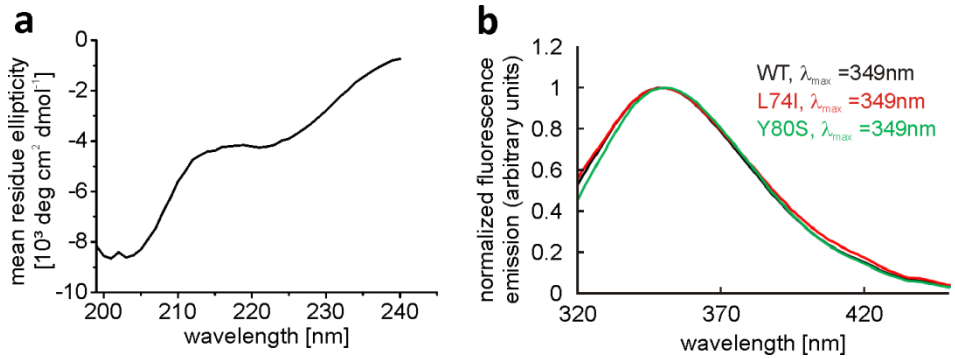
Supplementary Figure 3



Supplementary Figure 3

Orai1 L74I and Y80S are not affected by filipin. Changes in intracellular Ca^{2+} concentrations in HEK293 cells containing STIM1 with Orai1 and CB motif mutants after incubation with 1 µg/ml filipin for 60 minutes, each in comparison with untreated Orai1 wild-type (w.t.) and CB motif mutants ($t = 150s$, $p < 0.05$ as evaluated by Student's t-test). n represents the number of tested cells, which have been taken from 3-5 transfections.

Supplementary Figure 4



Supplementary Figure 4 CD spectra display reduced α-helicity of the Orai1 N-terminal peptide. **a**) Far-UV CD spectrum of Orai1 N-terminal domain (residues 72 to 90). The spectrum shows two minima at ~204 and ~222 nm, suggesting an α-helix and random coil mixture. The spectrum is an average of three consecutive acquisitions and is representative of two independent samples. **b)** Intrinsic fluorescence emission spectra of the WT, L74I and Y80S Orai1 N-terminal peptides. The data were collected using an excitation wavelength of 280nm as described in the Materials and Methods and are shown after buffer subtraction. The emission spectra are normalized to the maximum intensity to eliminate variations due to protein concentration error and cuvette positioning. The data show that WT (black line) and L74I (red line) have identical maximum emission wavelengths, suggesting only minor or no structural differences caused by the L74I mutation. The Y80S mutant shows a slight (2 nm) shift to higher wavelength that may be due to a small structural change or the elimination of the Y80 fluorophore compared to WT. The data are a representative spectrum of three independently measured samples for each of the wild-type, L74I and Y80S peptides.