The mammalian un-coordinated 93 homologue B1 maintains STIM1 in a dimeric state primed for translocation to cortical ER domains.

Wen-An Wang & Nicolas Demaurex*

Supporting Information

Figures and figure legends S1-S8

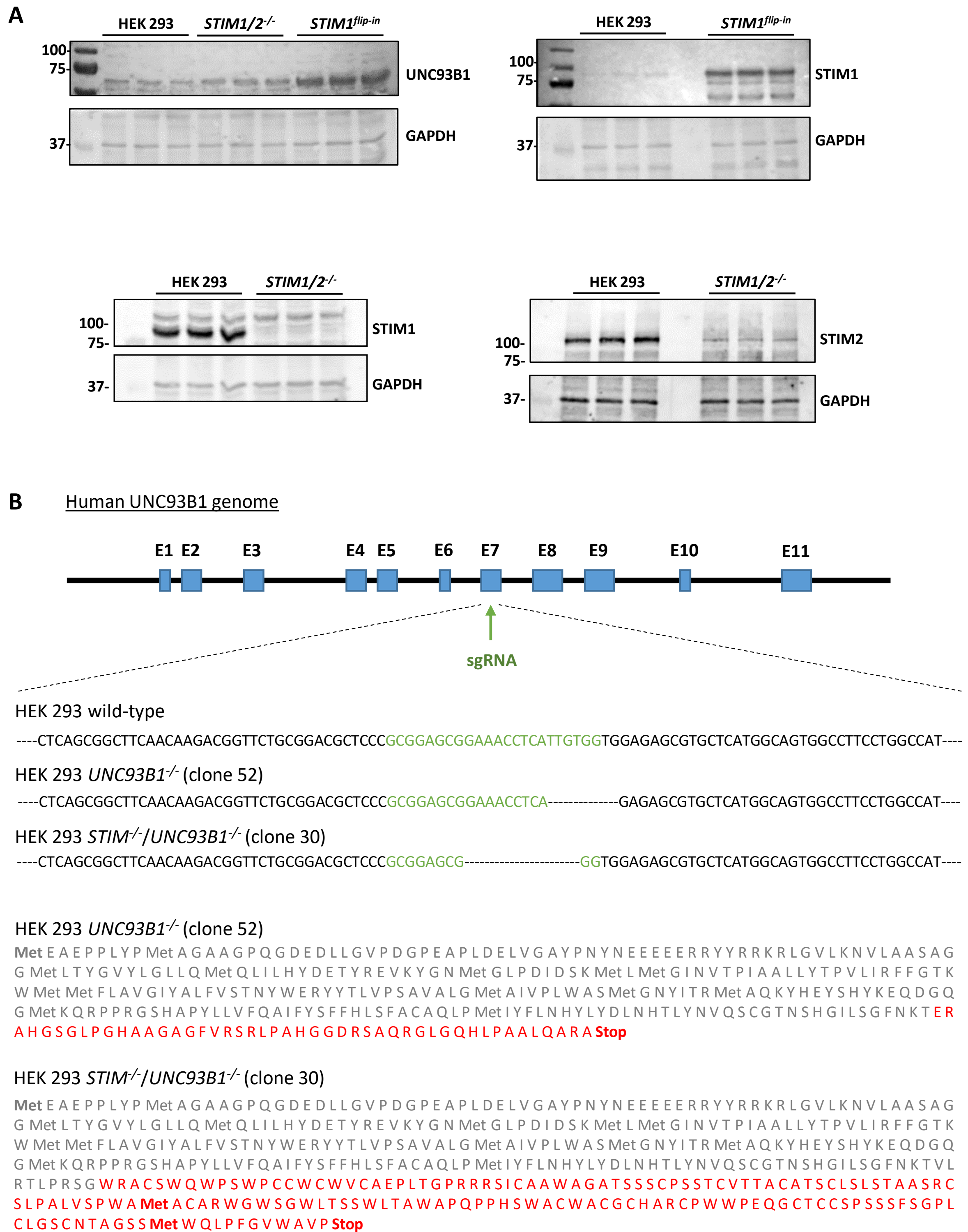


Figure S1

Figure S1

A. Western blot of protein lysates from WT, SKO and *STIM1*$^{flip\text{-}in}$ cells probed with αSTIM1, αSTIM2, αUNC93B1 and αGAPDH antibodies as indicated. B. Structure of the human *UNC93B1* gene with our sgRNA designed to target exon 7 (upper panel), sequencing results (middle panel) reveal base pair deletions within the sgRNA region translating in early stop codons within the amino acid sequence (in red, lower panel).

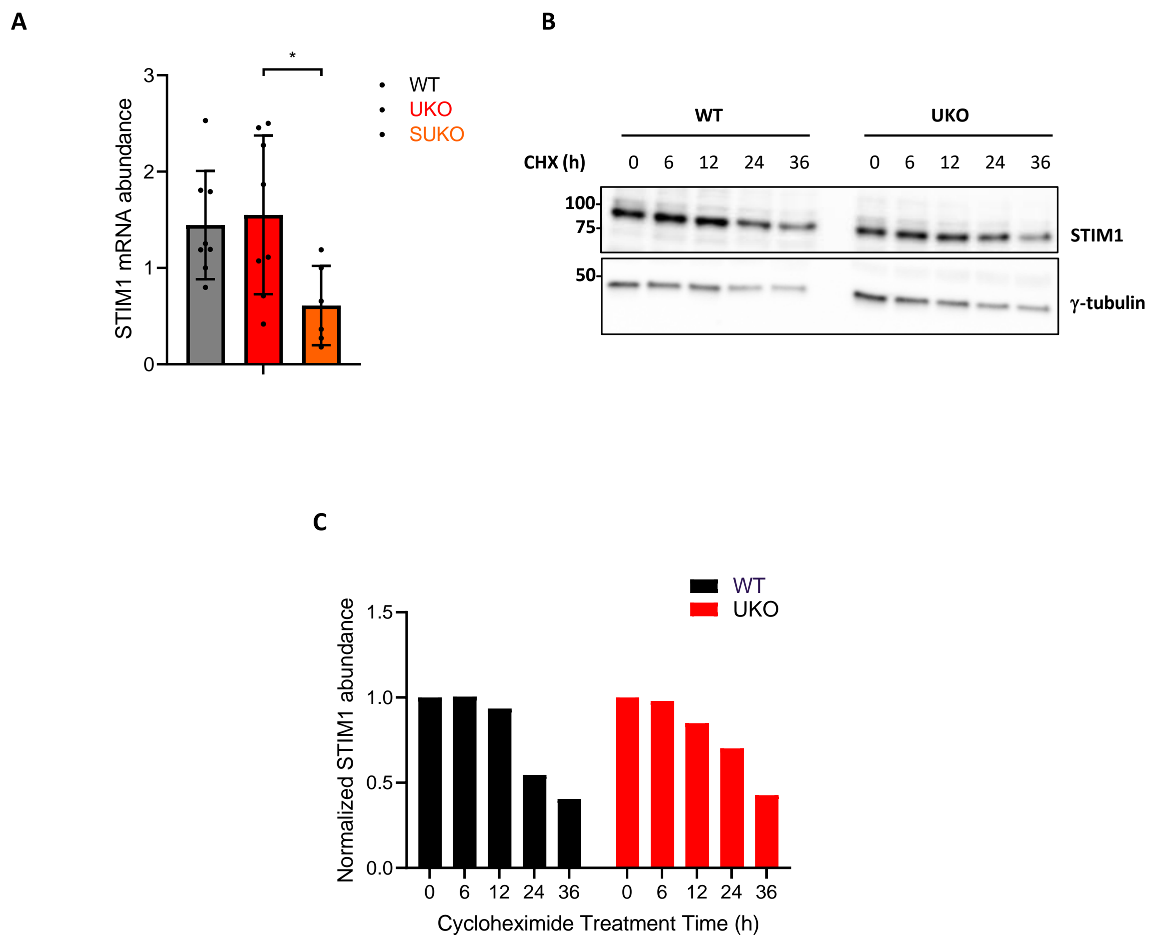


Figure S2

Figure S2

A. STIM1 mRNA levels assessed by qPCR in WT, UKO and SUKO cells. N=4, *$p<0.05$, ordinary one-way ANOVA ($F=4.241$, $P=0.0300$). B. Western blot of protein lysates of WT and UKO cells treated with 30 μg/ml of cycloheximide (CHX) for 0, 6, 12, 24, 36 hours and probed with αSTIM1 and αγ-tubulin antibodies as indicated. C. Quantification of protein degradation in the western blots. Representative of 2 experiments.

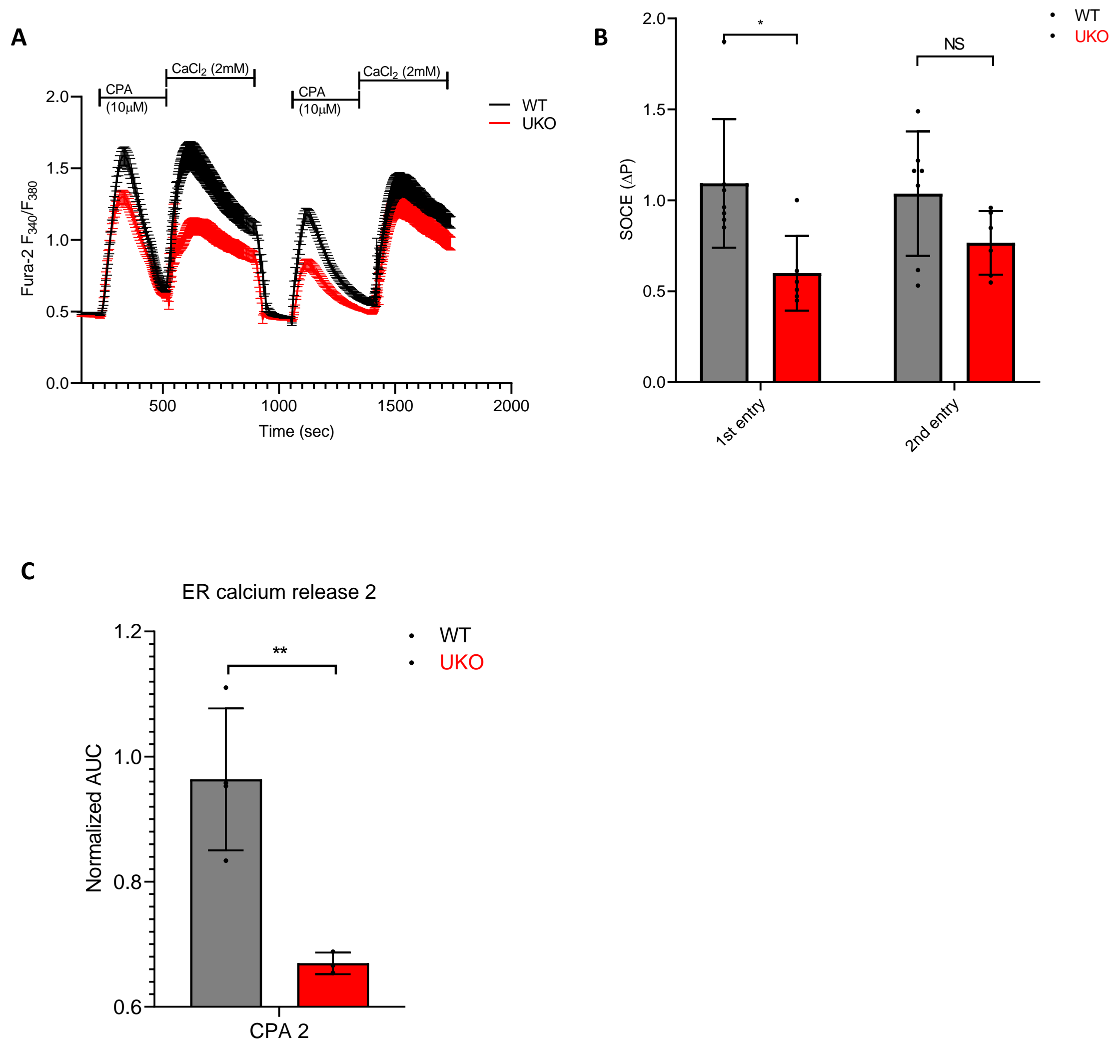
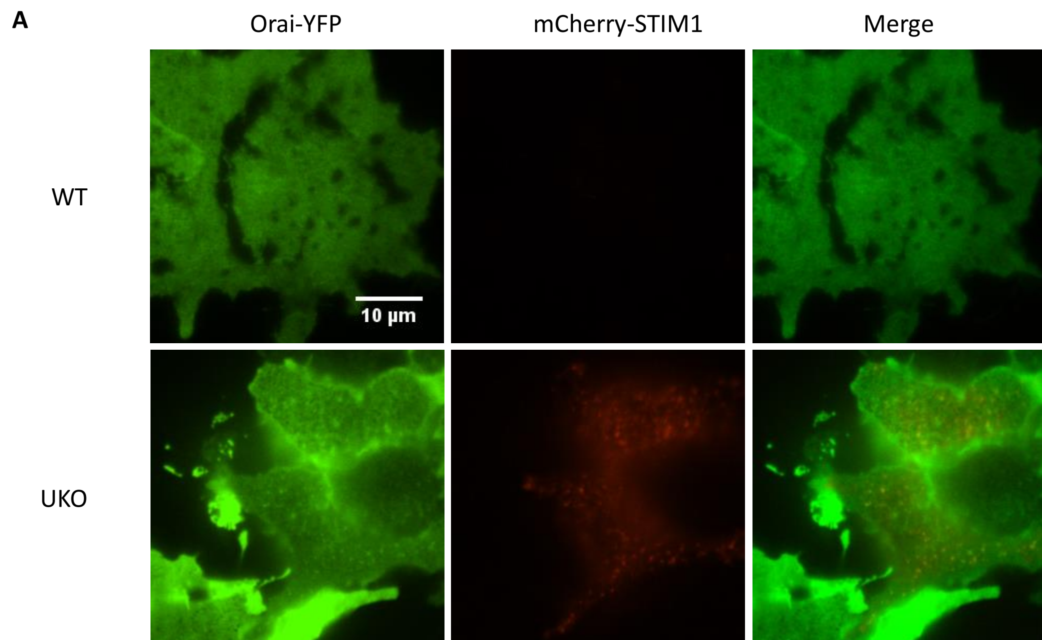
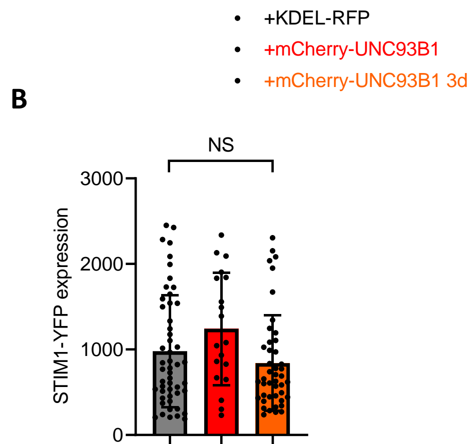


Figure S3

Figure S3

A. Representative Fura-2AM recordings from WT and UKO cells during successive additions of 10 μM cyclopiazonic acid (CPA) in Ca^{2+}-free medium followed by readmission of 2 mM Ca^{2+}. B. Peak amplitude of the two successive SOCE responses. Data are mean±SD of 7 independent experiments. NS=non-significant; *p>0.05, two-way ANOVA (Interaction: SS=0.08050, DF=1, MS=0.08050, F(1,11)=2.132, P=0.1722). C. The ER Ca^{2+} mobilized after the second addition of CPA in Ca^{2+}-free medium as quantified with area under the curve (AUC). Data are mean±SD of 7 independent experiments. NS=non-significant; **p>0.01, unpaired t-test.





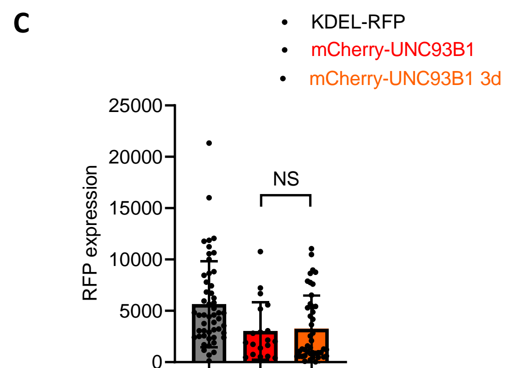


Figure S4

Figure S4

A. Corresponding representative TIRF images to Fig. 2C of WT and UKO cells expressing Orai1-YFP (green) and mCherry-STIM1 (red) at rest before TG treatment. B, C. Wide-field YFP (B) and RFP/mCherry (C) fluorescence levels of WT cells co-expressing YFP-STIM and RFP/mCherry constructs used for the TIRF experiments of Fig. 3. Data are mean±SD of 26-69 cells from 4 independent experiments. NS=non-significant, ordinary one-way ANOVA (B. F=2.779, P=0.664) (C. F=6.458, P=0.0022).

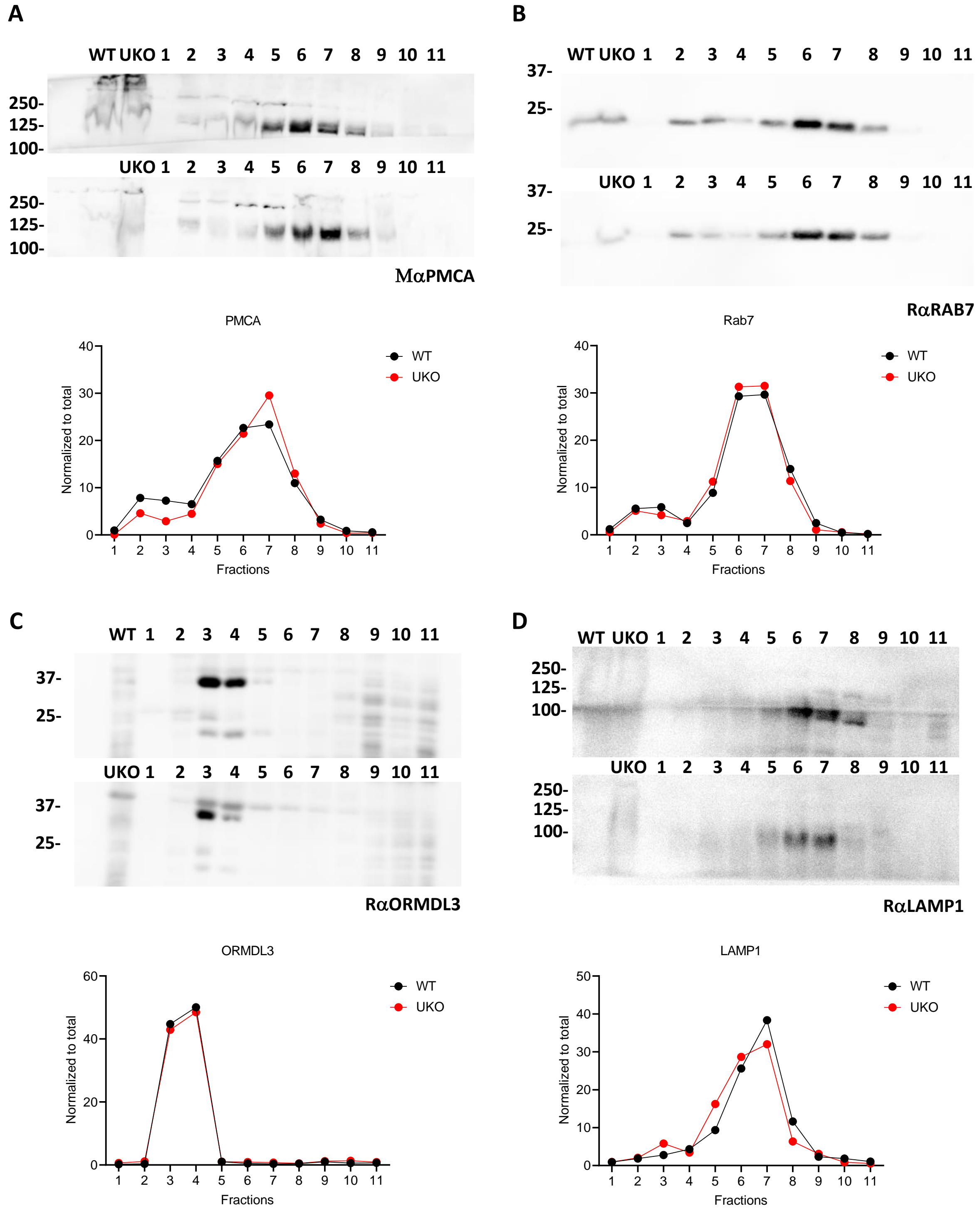
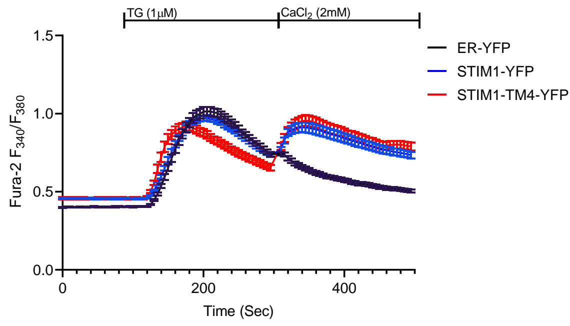


Figure S5

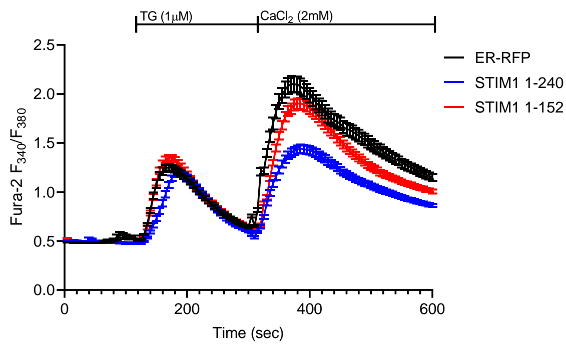
Figure S5

Immunoblot of protein lysates of WT (top) and UKO (bottom) cells, subjected to subcellular fractionation (1-11; light to heavy) through an iodixanol gradient (6%, 9%, 12%, 15%, 18%, 21%, 25%, and 27%). Blots were probed with αPMCA (A), αRab7 (B), αORMDL3 (C) and αLAMP1 (D) antibodies. Graphs show the quantification of the respective immunoreactivity through fractions 1 to 11. Representative of 2 biological experiments.

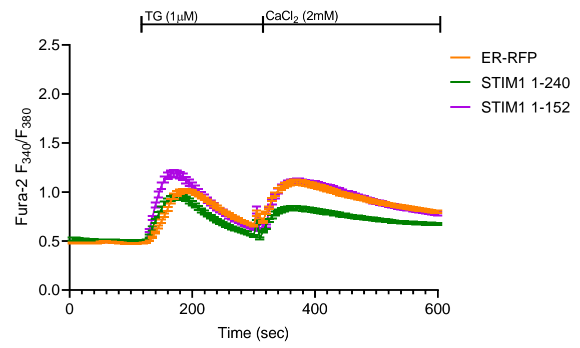
A



B



C



D

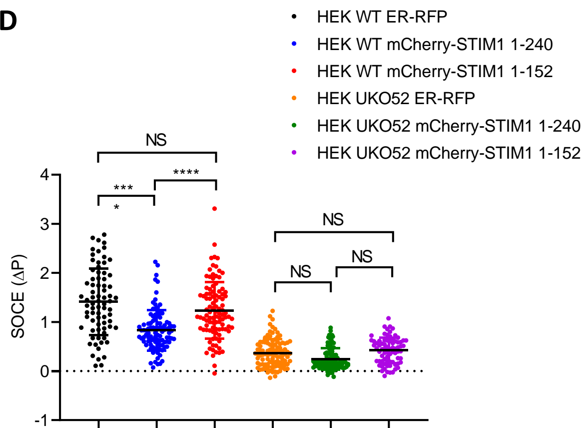


Figure S6

Figure S6

Functional SOCE assessment of the STIM1-TM4, $STIM1^{1-240}$, and $STIM1^{1-152}$ truncation constructs. A. Averaged Fura-2 responses evoked by Tg and subsequent Ca^{2+} readmission to SKO cells expressing ER-YFP, STIM1-YFP or STIM1-TM4-YFP. B, C. Representative averaged Fura-2 responses evoked by Tg and subsequent Ca^{2+} readmission to WT (B) and UKO cells (C) expressing ER-RFP, $STIM1^{1-240}$ or $STIM1^{1-152}$. D. Peak amplitude of the SOCE responses in B-C. Data are mean±SD of 75-100 cells from 3 independent experiments. NS=non-significant, ****$p>0.001$, ordinary one-way ANOVA ($F=112.6$, $P<0.0001$).

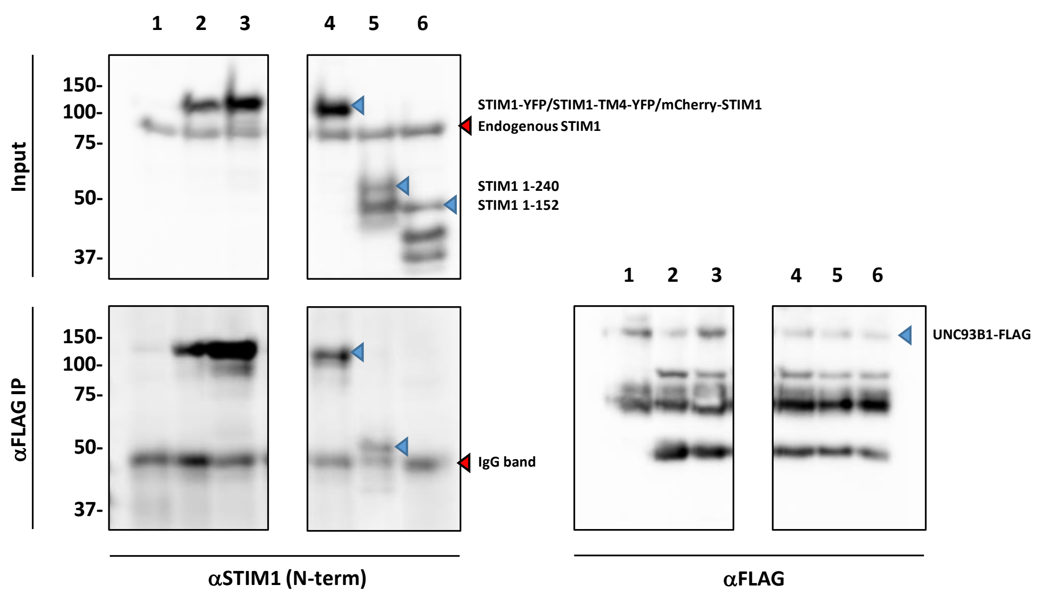
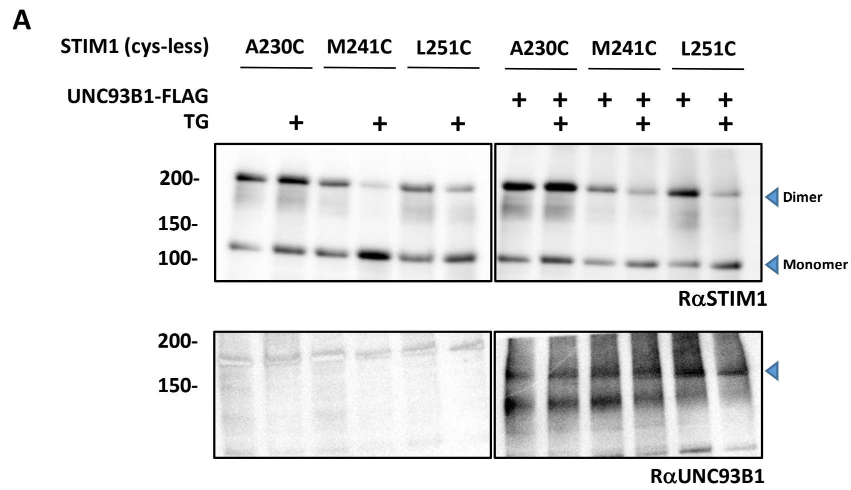


Figure S7

Figure S7

Immunoblot of input and immunoprecipitated (IP) protein lysates from WT cells expressing UNC93B1-FLAG with KDEL-GFP (1), STIM1-YFP (2), STIM1^{TM}-YFP (3), mCherry-STIM1 (4), mCherry-STIM1$^{1-240}$ (5), or mCherry-STIM1$^{1-152}$ (6). UNC93B1 was immunoprecipitated with αFLAG antibody and the blots were probed with αSTIM1 (N-term) and αFLAG antibodies. The blue arrows indicate the locations of the expected products and the red arrows additional reactivities. Representative of 2 biological experiments.



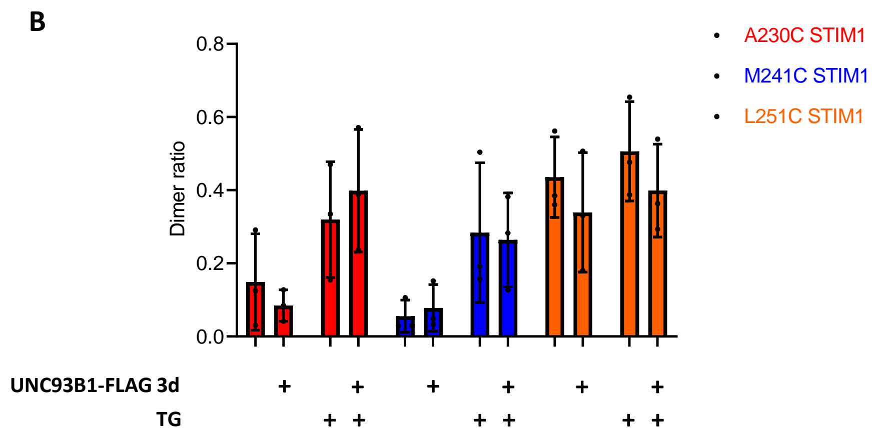


Figure S8

Figure S8

A. Immunoblot analysis of protein lysates from SUKO cells expressing the single cysteine STIM1 constructs with or without mCherry-UNC93B1-3d and treated or not with Tg (0.5 μM for 5 min). Cells were subjected to *in-vivo* crosslinking with aldrithiol-4 and to electrophoresis under non-reducing conditions. Blots were probed with αSTIM1 and αUNC93B1 antibodies. Representative of 3 independent experiments. B. Quantification of the STIM1 dimer ratio from the *in-vivo* crosslink immunoblots. Data are mean±SD of 3 experiments. Non-significant where there is no indication, unpaired t-test.