Expanded View Figures

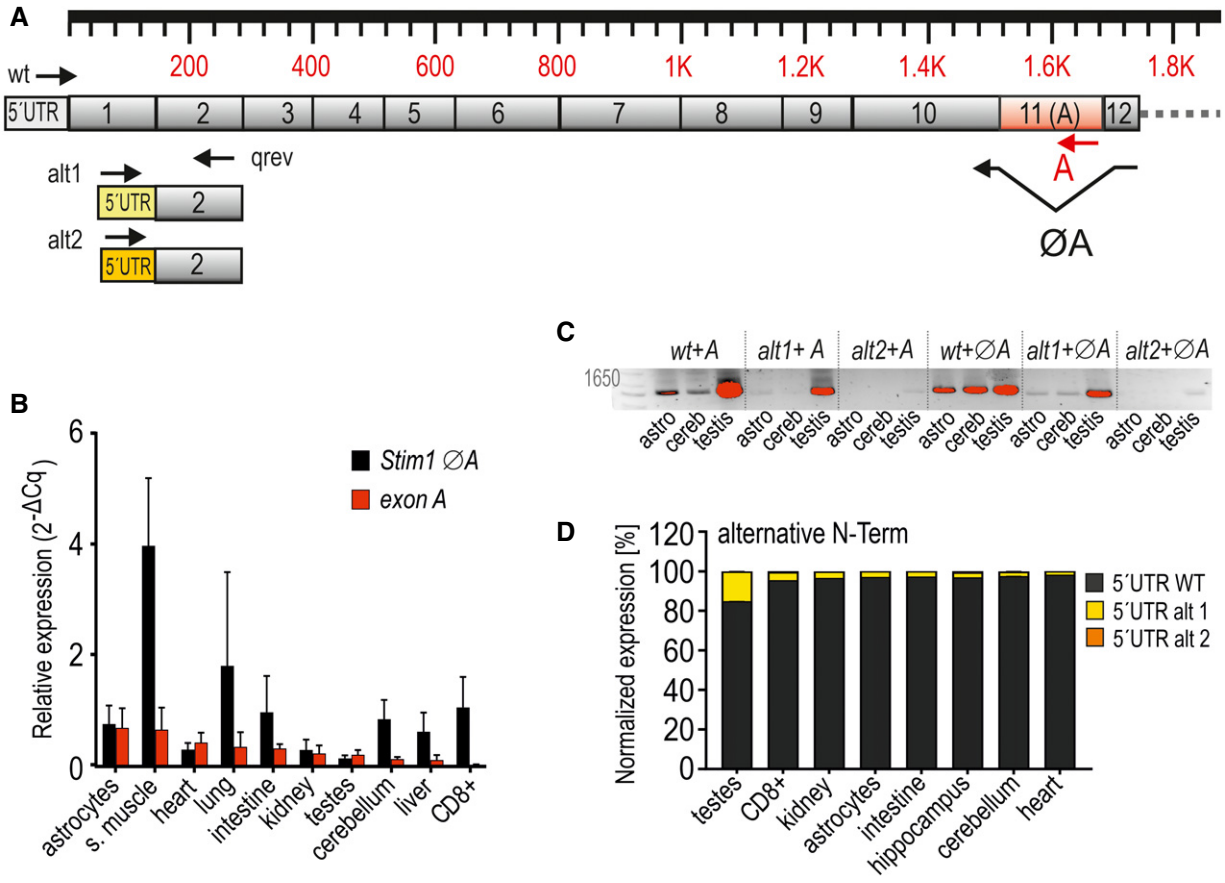

Figure EV1. Alternative splicing and PCR analysis of Stim1 and exon A.

A Schematic exon structure of Stim1 with conventional exons depicted in gray. Highlighted in red is the alternative exon 11 (A). Alternative usage of 5′UTR regions is indicated with alt1 and alt2. Annealing sites of primers are depicted as arrows.

B Expression of *exon A* and *ØA (wt)* normalized to *TATA box-binding protein Tbp* (mean ± SD) as determined by qRT–PCR from cDNA templates isolated from indicated tissues and cells derived from 3 to 4 mice.

C Analytic PCR with reverse primers flanking the A splice site or within exon A in combination with forward primers specific for 5′URT wt, alt1, or alt2 as also depicted in (A) using cDNA of murine astrocytes, cerebellum, and testis. Abbreviations: astro = astrocytes, cereb = cerebellum. Red indicates saturated pixels (see text).

D Quantification of alternative N-term splicing via qRT–PCR. Expression levels were normalized to *Tbp*.

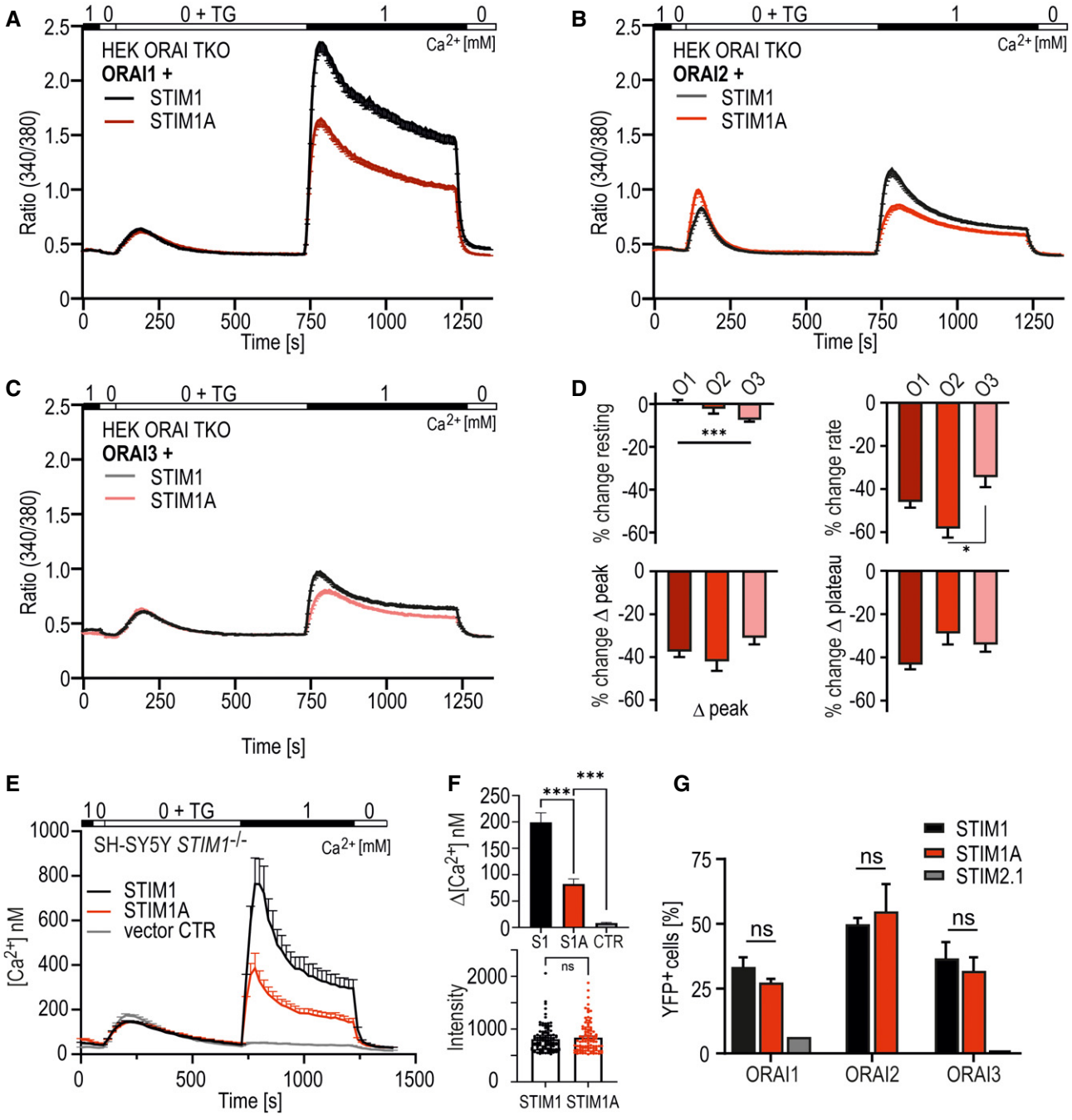

Figure EV2.

Figure EV2. STIM1A reduces SOCE independent of the ORAI homolog.

A–C Traces showing average changes (mean ± s.e.m.) in intracellular Ca^{2+} (Fura-2 ratio) in HEK293 triple ORAI-deficient cells (HEK ORAI TKO) co-transfected with either STIM1-mCherry (black traces, n = 60–98) or Stim1A-IRES-mCherry (red traces, n = 51–78), and ORAI1 IRES-GFP (A) co-transfected with ORAI2 IRES-GFP (B) or ORAI3 IRES-GFP (C).

D Relative change compared with the average of each respective control. Statistical analysis: Kruskal–Wallis ANOVA with Dunn's multiple comparisons test with significance indicated (*P < 0.05 and ***P < 0.001, error bars indicate s.e.m., data were obtained from three technical replicates each derived from three biological replicates with multiple cells, total n indicated above).

E Calibrated traces showing mean $[Ca^{2+}]_i$ (+s.e.m.) after expression of mCherry-tagged STIM1 (black trace, n = 68), STIM1A (red trace, n = 54) or vector control (gray trace, n = 47 cells) in SH-SY5Y STIM1$^{-/-}$ cells. Solution exchanges similar as indicated in C, and data were obtained from three technical replicates each (with multiple cells) derived from three biological replicates.

F Analysis of $[Ca^{2+}]_i$ plateau (upper panel) and mCherry expression levels (lower panel) (mean ± s.e.m.). ***P < 0.001, Kruskal–Wallis ANOVA with Dunn's multiple comparisons test (for replicates, see under C).

G Bimolecular fluorescence complementation (BiFC) assay: Analysis for interaction via flow cytometry. HEKS1$^{-/-}$/S2$^{-/-}$ were transfected with STIM1-EYFP$_N$ (black), STIM1A-EYFP$_N$ (red), or STIM2.1-EYFP$_N$ (gray) or with ORAI1-EYFP$_C$, ORAI2-EYFP$_C$ or ORAI3-EYFP$_C$ and screened for YFP$^+$ cells via FACS, and results (mean ± SD) were obtained from 2–3 transfections (1 transfection for STIM2.1) each with 10,000 sorted cells. n.s.: non-significant, 2-way ANOVA.

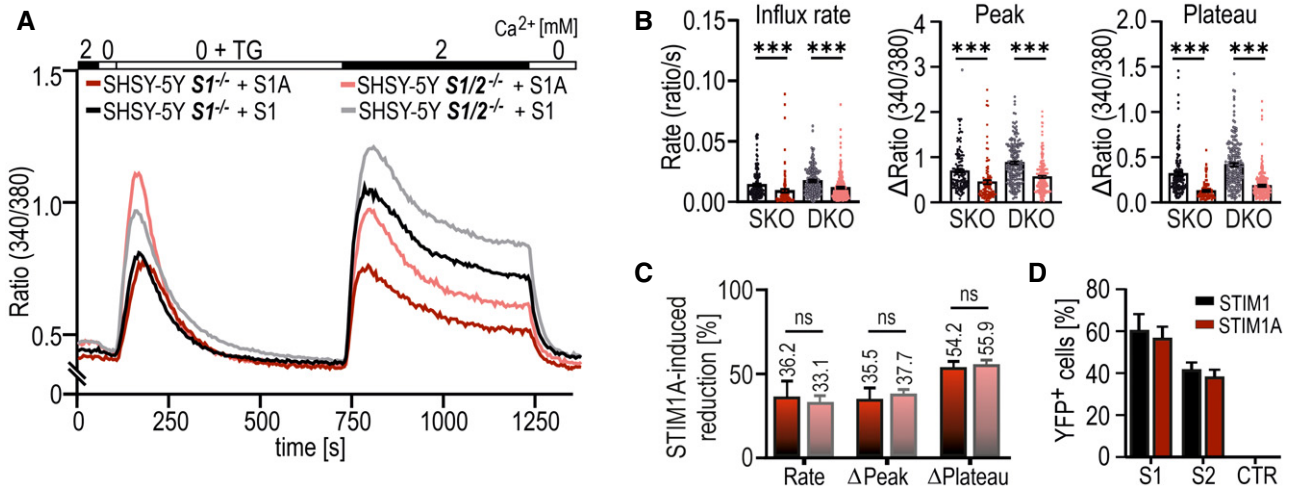

Figure EV3. STIM2 has no impact on STIM1A-mediated SOCE.

A Traces showing mean changes in Fura-2 ratio in SH-SY5Y STIM1$^{-/-}$ or in SH-SY5Y STIM1$^{-/-}$;STIM2$^{-/-}$ cells transfected with either STIM1-mCherry (black traces, n = 136 and gray traces, n = 187) or STIM1A-IRES-mCherry (red traces, n = 107 and light red traces, n = 217).

B Quantification of changes in ratio of resting, influx rate (Δratio/time), Δpeak, and Δplateau measured in A. Bars underlying scatter plots depict mean ± s.e.m., ***P < 0.001, Kruskal–Wallis ANOVA with Dunn's multiple comparisons test, and data were obtained from three technical replicates each derived from three biological replicates.

C STIM1A induced reduction in SOCE parameter (B) after expression in single KO (dark red) or in double KO (dark red) in %. ns: not significant, Kruskal–Wallis ANOVA with Dunn's multiple comparisons test.

D Percentage of YFP$^+$ cells from BiFC assay after transfection of HEKS1$^{-/-}$;S2$^{-/-}$cells with STIM1(A)-YFP$_C$ with STIM1-YFP$_N$, STIM2-YFP$_N$, or negative control. Results (mean ± SD) were obtained from 2-3 transfections each with 10,000 sorted cells.

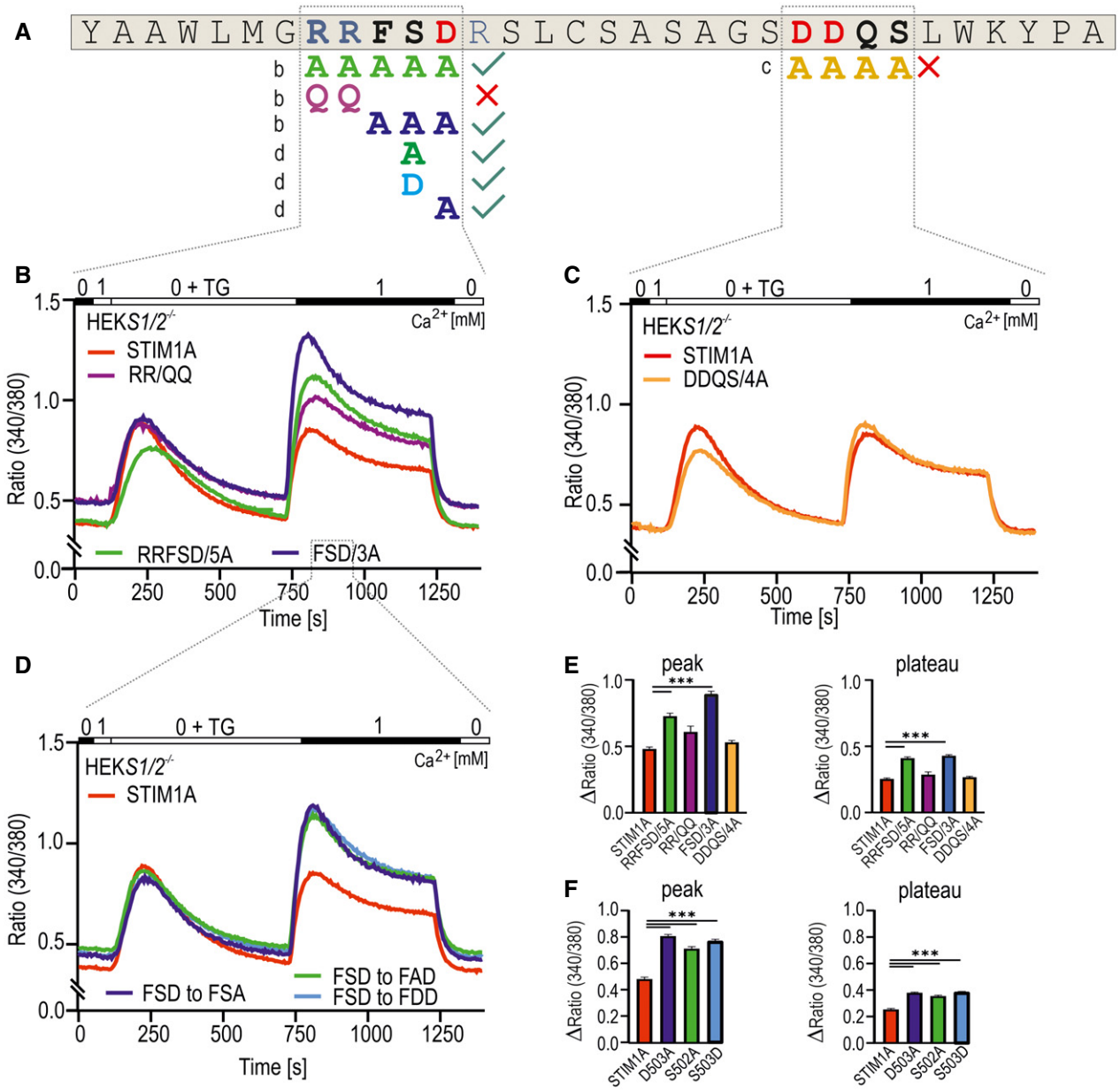

Figure EV4. Site-directed mutagenesis of exon A.

A Amino acids of exon A. Positive charged amino acids are marked blue, and negative charged ones are shown in red. Mutated amino acids depicted below are marked in the color code used for B–F.

B–D Traces showing average changes (mean ± s.e.m.) in intracellular Ca^{2+} (Fura-2 ratio) over time in response to perfusion of different $[Ca^{2+}]o$ as indicated in the upper bar with mutant constructs as indicated expressed in HEKS1$^{-/-}$;S2$^{-/-}$ cells.

E Quantification of changes in ratio measured in B and C.

F Quantification of changes in ratio measured in D.

Data information: All constructs were directly tagged with mCherry. ***$P < 0.001$, Kruskal–Wallis ANOVA with Dunn's multiple comparisons test. Data were obtained from three technical replicates (each with multiple cells) from three biological replicates (129 < total n < 708) and are shown as mean ± s.e.m.

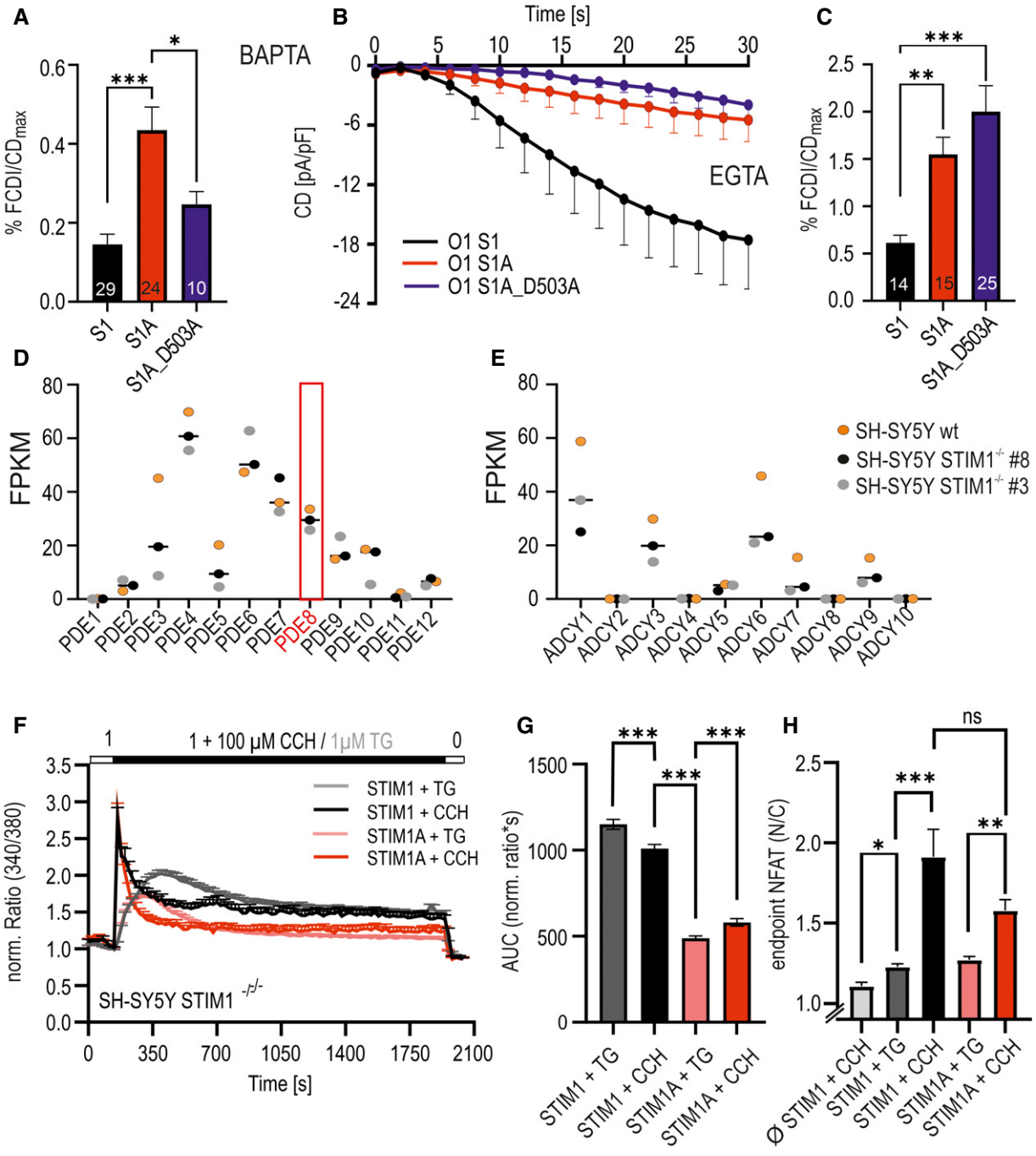

Figure EV5.

Figure EV5. Ca^{2+} dependence of FCDI, expression of PDE and ADCY genes, receptor-mediated Ca^{2+} entry, and NFAT translocation.

A Normalized FCDI of STIM1, STIMA, and STIM1A_D503A with intracellular BAPTA buffering. Number of patch-clamped cells from > 3 transfections each indicated in bars with mean + s.e.m. *P < 0.05 and ***P < 0.001, ordinary one-way ANOVA.

B Current densities over time (data points: mean ± s.e.m.) measured with intracellular EGTA buffering after expression of indicated constructs in HEKS1$^{-/-}$;S2$^{-/-}$ cells. Number of patch-clamped cells from > 3 transfections (biological replicates).

C Normalized FCDI of STIM1, STIMA, and STIM1A_D503A with intracellular EGTA buffering. Number of patch-clamped cells from > 3 transfections indicated in bars with mean + s.e.m. **P < 0.01 and ***P < 0.001, Kruskal–Wallis ANOVA.

D, E FPKM counts of PDE genes (E) and ADCY genes (F) from RNA-seq analysis of mock-transfected SH-SY5Y cell line and two independent CRISPR/Cas9-generated STIM1-deficient cell lines, horizontal lines indicate median, and red box highlights expression of PDE8. Variants of one gene are summed.

F Traces showing average changes (mean + s.e.m.) of normalized ratio over time in response to perfusion of different stimuli as indicated in the upper bar with mCherry-tagged constructs expressed in SH-SY5Y STIM1$^{-/-}$ cells (clone#8). Darker colors indicate responses to carbachol (CCH) (26 < total n < 105 from 3 transfections with technical replicates).

G Quantification of the area under the curve (AUC) of ratios (mean ± s.e.m.) shown in (F). ***P < 0.001, ordinary one-way ANOVA indicated only for selections (26 < total n < 105 from 3 transfections with technical replicates).

H Normalized NFAT ratios (nuclear/cytoplasm), mean + s.e.m, after 30 min with stimulation as shown in (F). *P < 0.05, **P < 0.01, and ***P < 0.001, Kruskal–Wallis ANOVA (13 < total n < 103, 3 transfections).