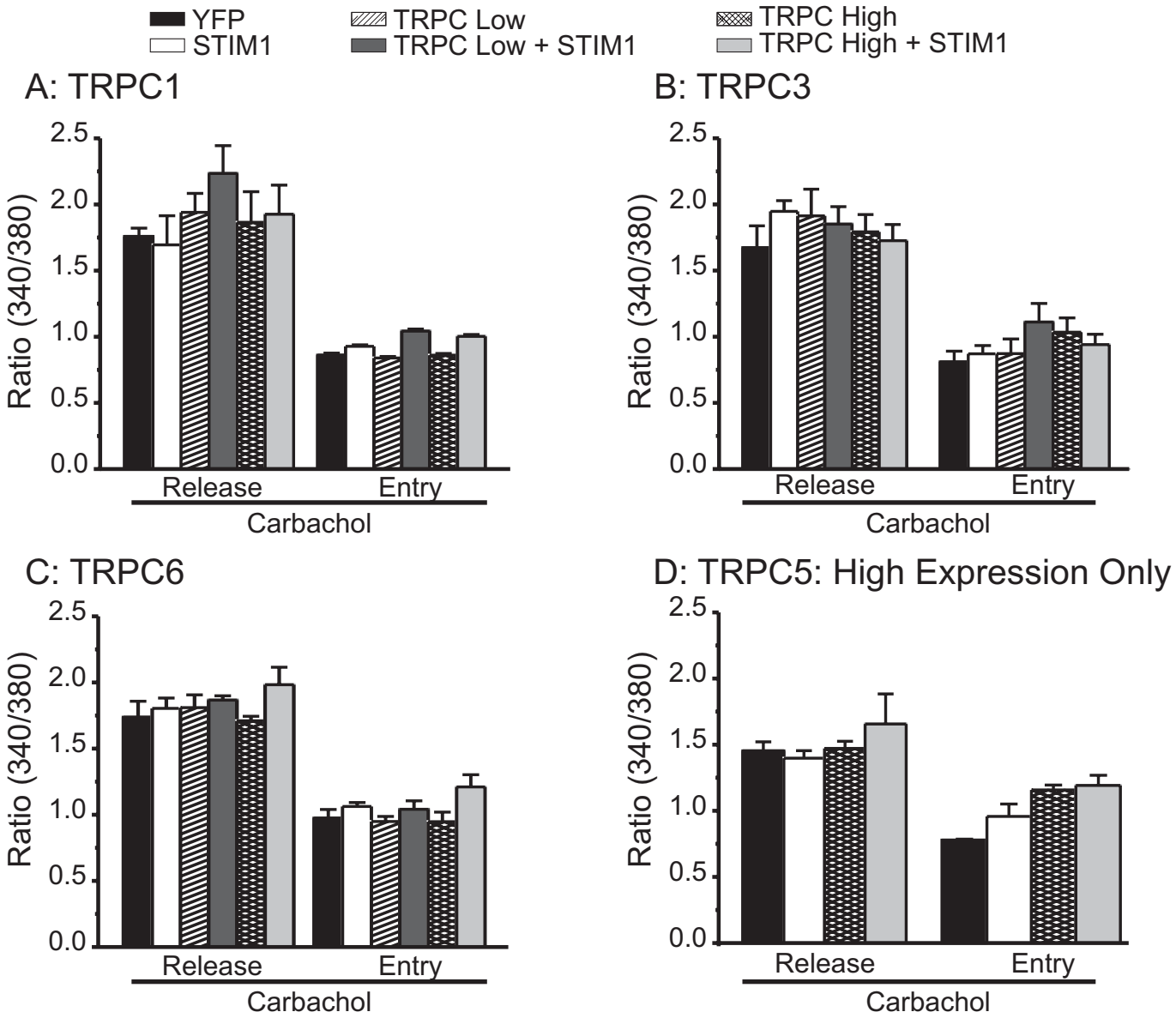
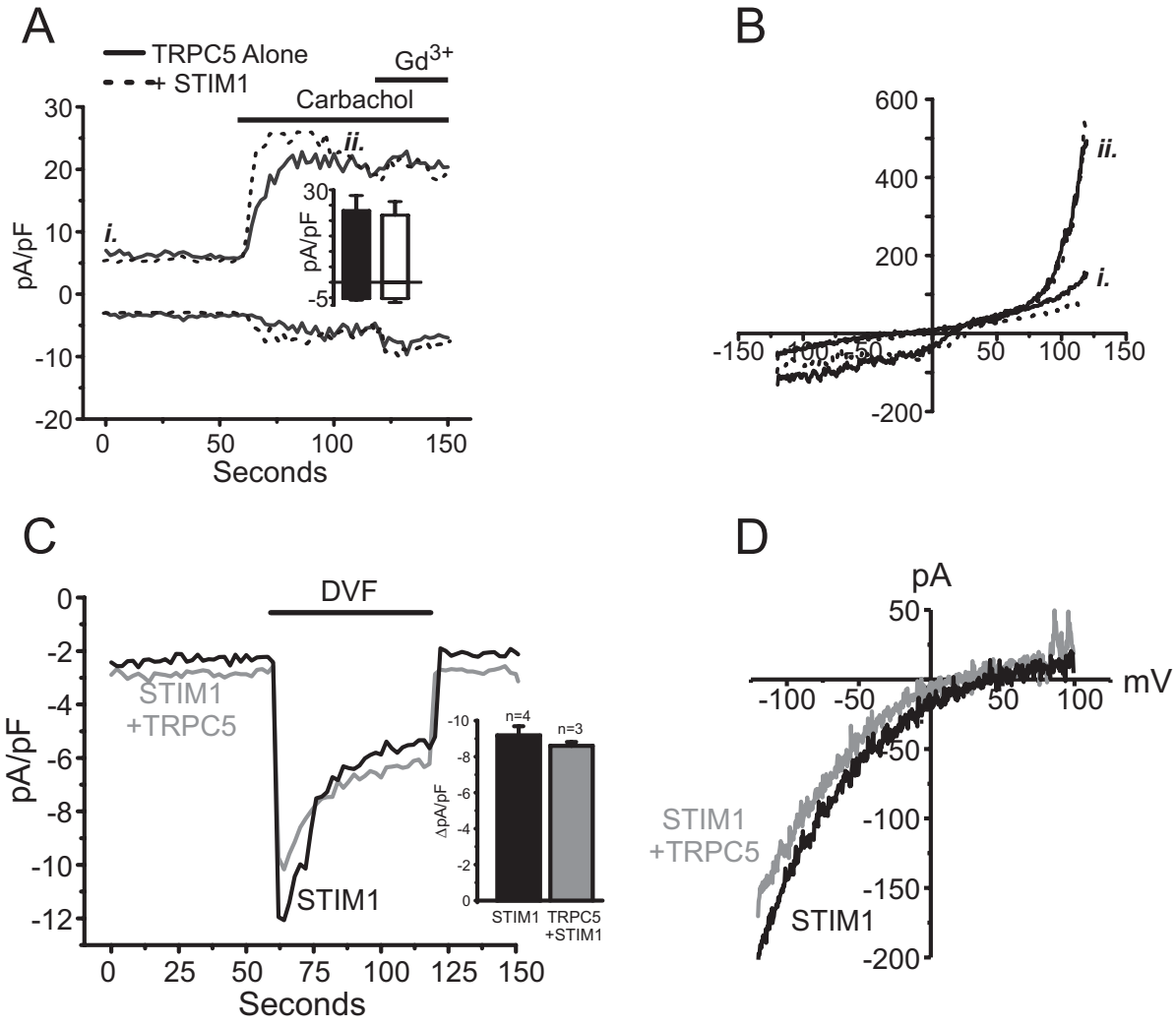
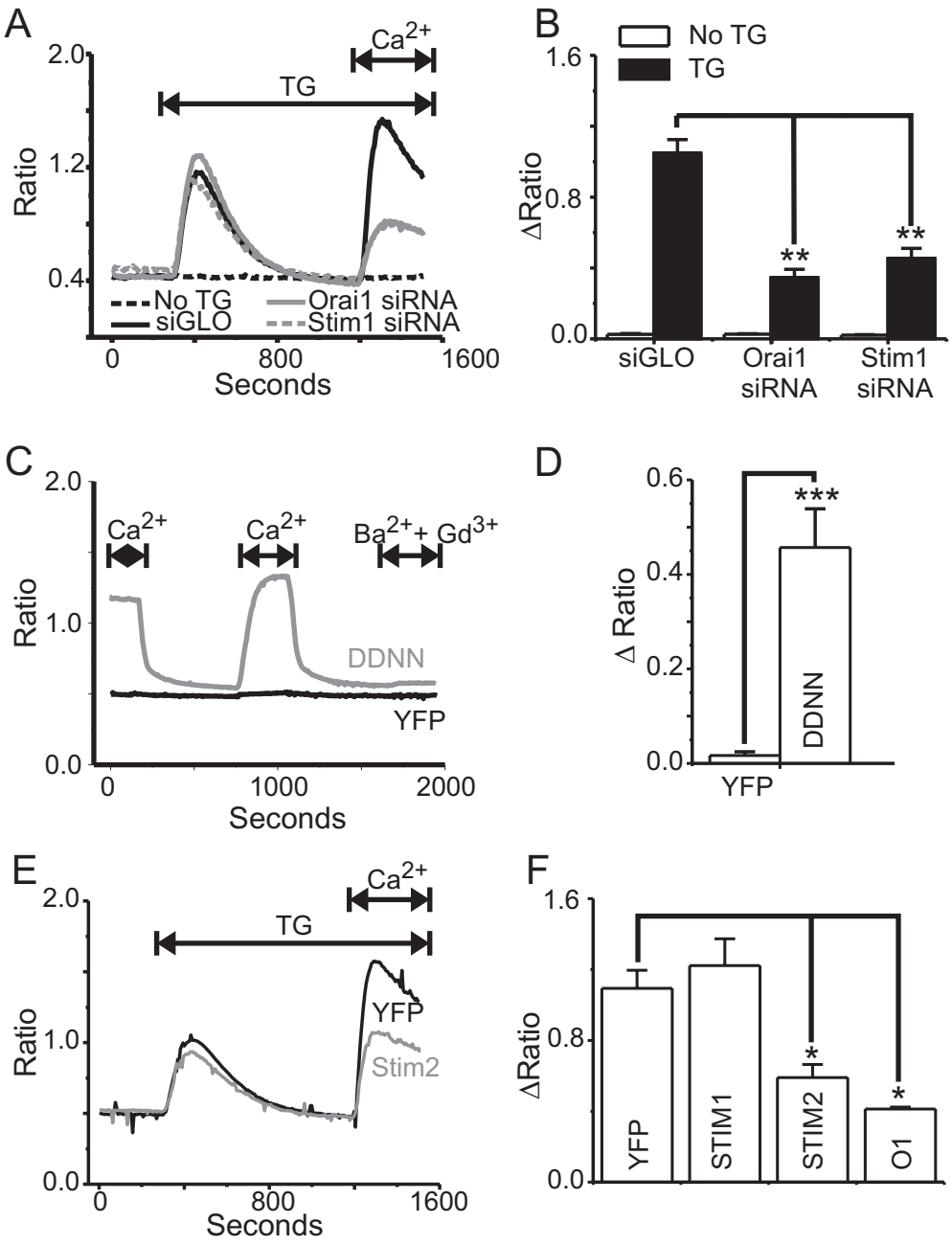


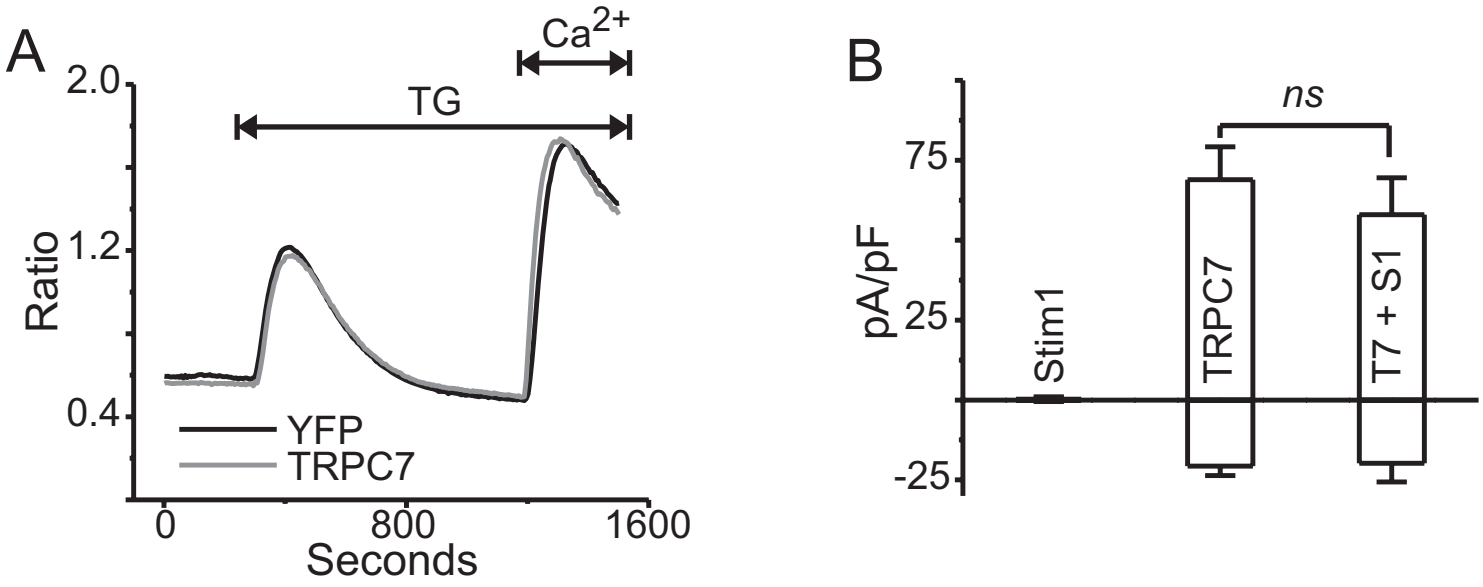
Supplemental Figure 1: Carbachol-evoked Ca^{2+} entry in cells co-expressing TRPC channels with STIM1 in HEK293 cells. Experiments were carried out as described in Figure 1, but without extracellular Gd^{3+}. Bar graphs depict the ratios (fura-5f; 340/380) for the carbachol release and Ca^{2+} entry phases (means $\pm$ SEM) in HEK293 cells co-expressing TRPC1 **(A)**, TRPC3 **(B)**, TRPC6 **(C)** and TRPC5 **(D)** with STIM1. Two concentrations of TRPC plasmid (see figure 1 and methods) were used for TRPC1, TRPC3, TRPC6, but not TRPC5, and are indicated as high and low TRPC. Each condition is representative of at least three coverslips with at least 25 cells per coverslip.



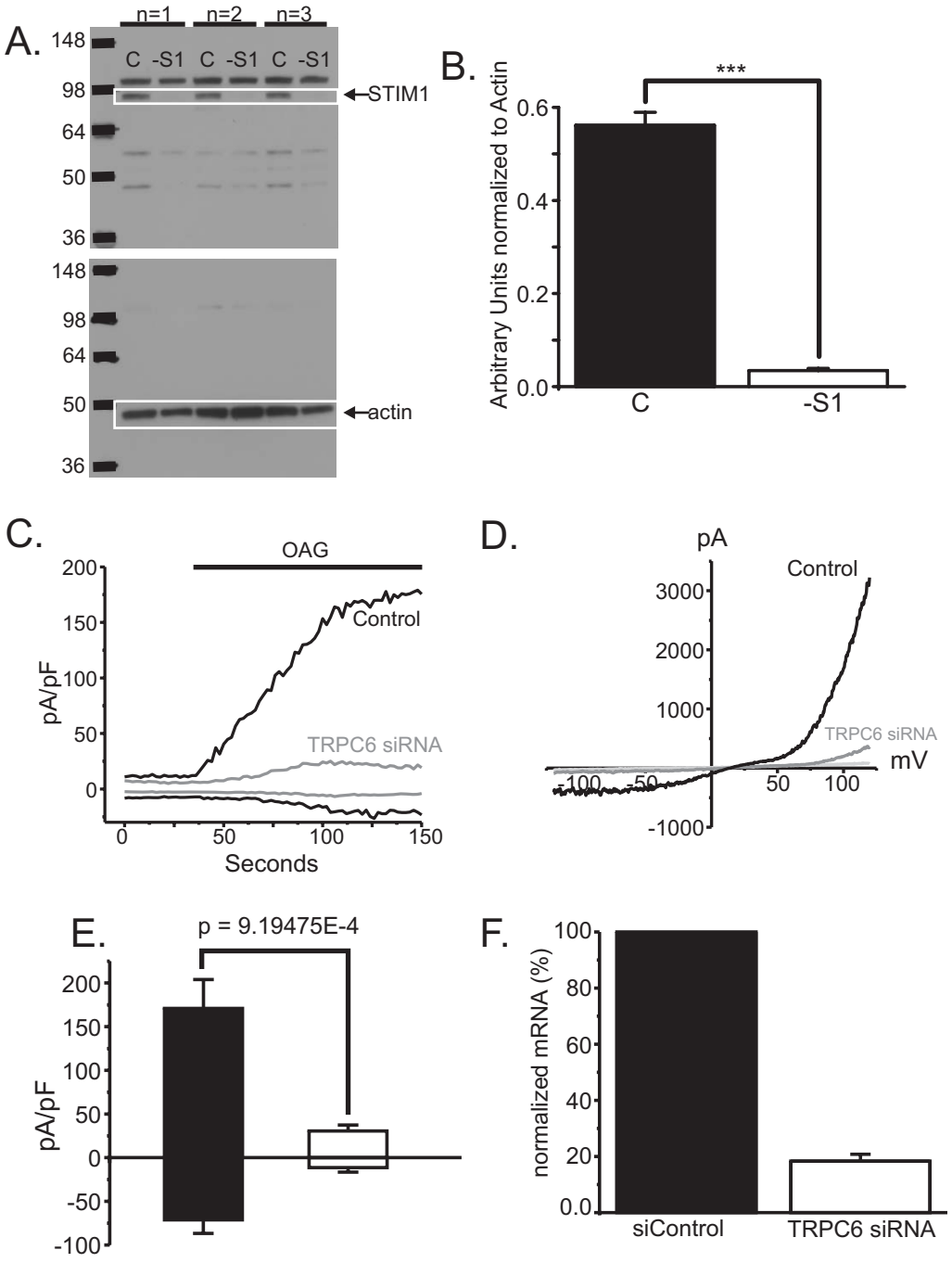
Supplemental Figure 2: STIM1 has no effect on carbachol-activated TRPC5 currents, and conversely, TRPC5 does not alter STIM1-mediated CRAC currents. (A) Whole-cell patch clamp experiments on HEK293 cells expressing TRPC5 alone (black trace), or with eYFP- STIM1 (broken black trace). Internal Ca^{2+} was clamped to 100 nM, and the external Ca^{2+} concentration was 1 mM throughout. Carbachol (200 μM) and 5 μM Gd^{3+} were added as indicated. Inset is a summary of the data in which there was no significant difference (unpaired t test) in the TRPC5 whole-cell currents activated by the focal application of carbachol in the absence (n=5; black bar) or presence of co-expressed STIM1 (n=5; open bar). **(B)** Current-voltage (I-V) relationships for the traces shown in (A). *i.* and *ii.* indicate where in the traces shown in panel (E) the I-V traces were taken. **(C)** Na^+-I_{CRAC} measurements taken from HEK293 cells expressing eYFP-STIM1 (+100 mV to -120 mV ramps every 2 seconds from a 0 mV holding potential) with (n=4; gray trace) or without (n=3; black trace) the co-expression of TRPC5. Stores were depleted using 25 μM IP_3 in the patch pipet and all external divalent cations were removed (DVF) in order to amplify the CRAC currents. Little to no Ca^{2+} I_{CRAC} currents can be detected in HEK293 cells expressing STIM1 alone; however, Na^+ -I_{CRAC} is easily detected in these cells and is 2-3 times bigger than endogenous Na^+- I_{CRAC} . External solution exchanges were focally applied as indicated by the lines above the graph. The internal pipet solution contained 100 nM clamped Ca^{2+}. Inset bar graph shows there was no significant difference (unpaired t test) in the peak Na^+ CRAC currents seen in cells expressing STIM1 alone, or in cells expressing STIM1 with TRPC5. (D) Leak subtracted I-V plots taken from the peak Na^+ CRAC currents shown in (C).



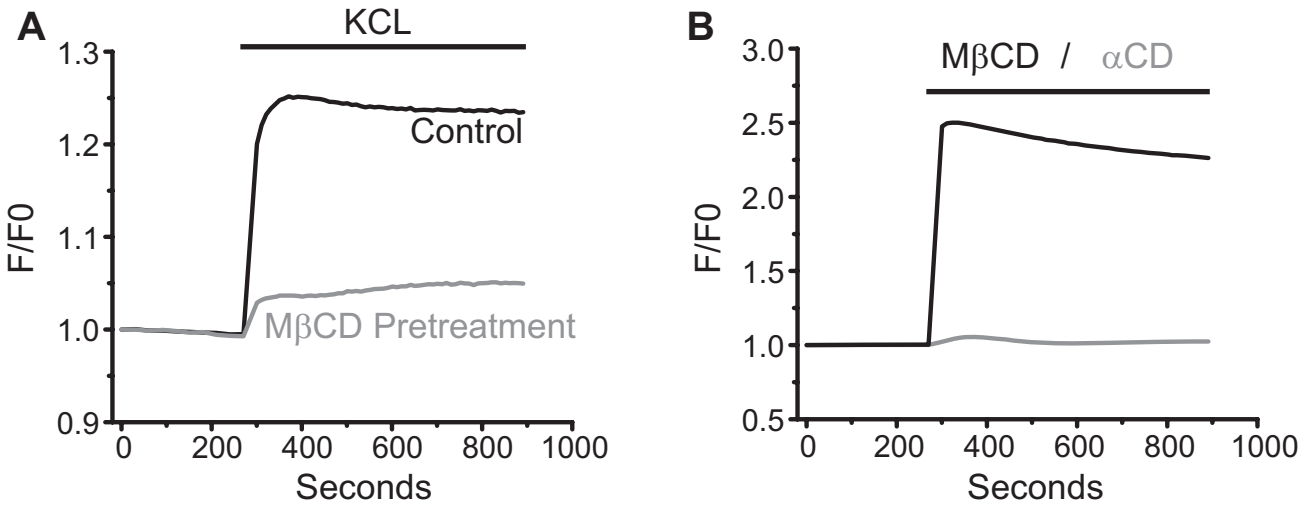
Supplemental Figure 3: Knockdown and over-expression controls on endogenous SOCE in HEK293 cells. (A) Representative traces (fura-2 AM) showing the inhibition of store-operated Ca^{2+} entry in WT HEK293 cells transfected with STIM1 (broken gray trace) or Orai1 siRNA (solid gray trace), when compared to control transfections (siGLO; solid black trace). Stores were depleted with thapsigargin under nominally Ca^{2+} free conditions, followed by the addition of 2 mM Ca^{2+}. **(B)** Bar graph showing significant reduction of SOCE (ANOVA) in HEK293 cells transfected with STIM1 (n=6 coverslips) or Orai1 siRNA (n=6 coverslips) compared to controls (n=6 coverslips). **(C)** Mean imaging experiments demonstrating that HEK293 cells transfected with the constitutively active mutant of STIM1 (D76N/D78N) show a significant increase in basal Ca^{2+} levels when compared to eYFP (control), but do not show any influx of Ba^{2+} in the presence of 5μM Gd^{3+}. **(D)** Summary of data in panel (C) showing significantly elevated basal Ca^{2+} levels (unpaired t test, p = 0.00062) in WT HEK293 cells that were transfected with the constitutively active mutant of STIM1 (n=8 coverslips) compared to eYFP controls (n=6 coverslips). **(E)** Mean traces showing that HEK293 cells transfected with STIM2 show a reduction in Ca^{2+} entry after store depletion when compared to eYFP (control). **(F)** Summary of data (Δ Ratios) depicting significant decreases in Ca^{2+} influx (ANOVA followed by Tukey's test) after store depletion with thapsigargin in HEK293 cells transiently transfected with STIM2 (n=4 coverslips) or Orai1 (n=3 coverslips) alone, but no significant difference in STIM1 (n=4 coverslips) expressing cells. All data presented as mean ± SEM and each n represents the mean of a single coverslip in which at least 25 cells were selected.



Supplemental Figure 4: Unlike Orai1, the transient expression of TRPC7 does not suppress SOCE in HEK293 cells. (A) Ca^{2+} imaging experiment (fura-2 AM) showing the effects of transiently expressing TRPC7 (gray trace) on endogenous SOCE in HEK293 cells. Stores were depleted with thapsigargin under nominally Ca^{2+} free conditions, and SOCE was assessed by the re-addition of extracellular Ca^{2+} (2 mM), as indicated by the lines above the graph. Results were compared to an eYFP control cell (black trace). Shown are the means of coverslips in which at least 25 cells were selected. **(B)** Summary of whole-cell currents recorded at -100 and +100 mV from a 0 mV holding potential showing transient co-expression of TRPC7 with Stim1 does not alter the OAG-activated non-selective currents (Stim1: n=3; TRPC7: n=5; Stim1 + TRPC7: n=5).



Supplemental Figure 5: Further verification of effective RNAi directed against STIM1 and TRPC6. (A) Western blot analysis of STIM1 protein expression in A10 vascular smooth muscle cells treated 72 hours earlier with either siControl (C) or STIM1 siRNA (-S1). Note the non-specific band that runs just above STIM1; the identity of this band is not known, but it was only detected in cells from rat (not human) and was not detected by two other commercial STIM1 antobodies. The membrane was stripped and re-probed with antibodies directed against actin. (B) Bar graph showing in three independent experiments the significant knockdown (unpaired t test; $p = 0.00005$) of STIM1 compared to siControl treated control cells. (C) Whole-cell currents recorded every 2 seconds (250 msec ramps going from -120 mV to +120 mV) from human TRPC6 stably expressing HEK293 cells, activated by 30 μM OAG, and treated 72 hours earlier with either siControl (black trace) or TRPC6 siRNA (gray trace). The internal pipet solution contained 100 nM clamped Ca^{2+}, while the external solution was nominally Ca^{2+} free (NF) HBSS. (D) The current-voltage relationships taken from the experiments shown in panel C. (E) Bar graph showing the significant reduction (unpaired t test; $p = 0.00092$) in peak OAG-activated TRPC6 currents in cells treated with siRNA directed against TRPC6 (n=6, siControl; n=7, TRPC6 siRNA). (F) Results from qRT-PCR anaylsis of TRPC6 message in cells treated with siControl or TRPC6 siRNA (n=2).



Supplemental Figure 6: Experiments with a membrane potential-sensitive dye suggest MβCD, but not αCD, depolarizes HEK293 cells. (A) Experiments examining effects of MβCD on the membrane potential of HEK293 cells. Cells were pretreated with MβCD for 1 hr and washed 3 times in HBSS, followed by loading of a membrane potential-sensitive dye for 1 hour. The data were normalized by dividing all readings (F) by the first fluorescent reading (F0). Application of a depolarizing concentration of KCl (60 mM) is indicated by the line above the graph. The depolarizing effects of KCl treatment in the wells pretreated with MβCD (gray trace) were significantly smaller than the effects of KCl in untreated control wells (black trace), suggesting the cells in those wells were already significantly depolarized. **(B)** The acute effects of 10 mM MβCD and 10 mM αCD on membrane potential-sensitive dye fluorescence in HEK293 cells. Experiments were carried out as in panel A; however, rather than pretreatment with MβCD and αCD, the drugs were applied directly in HBSS. Shown are the means of multiple wells from a single 96-well plate taken from a FLIPR experiment.