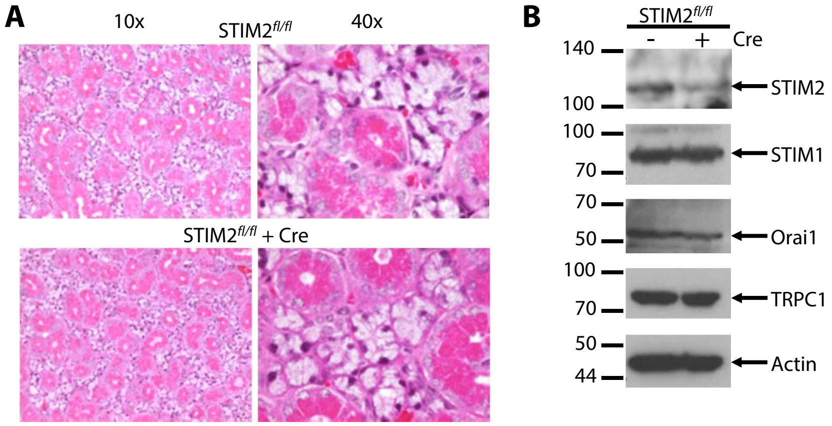

Figure S1. Effects and specificity of targeted knockout of STIM2 in mouse salivary glands. (A) H&E staining of salivary gland sections from STIM2$^{fl/fl}$ and STIM2$^{fl/fl}$+Cre mice, using 10x and 40x objectives. (B) Western blots showing expression of STIM2, STIM1, Orai1, TRPC1 and actin in salivary glands from STIM2$^{fl/fl}$ and STIM2$^{fl/fl}$+Cre mice. These data is representative of results obtained from 3 mice.

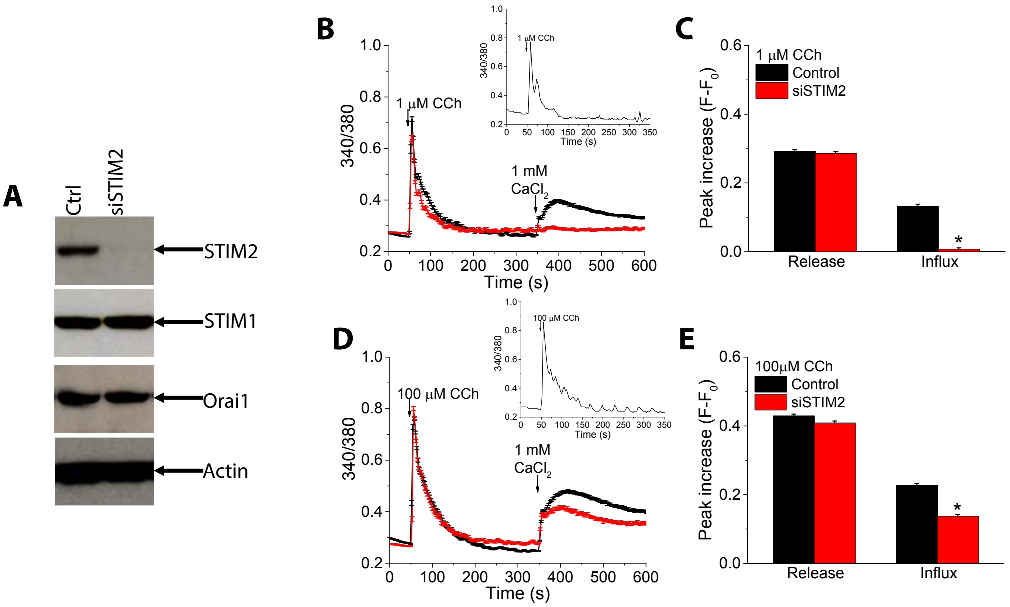

Figure S2. Specificity of siRNA-mediated STIM2 knockdown and its effects of CCh-stimulated Ca^{2+} signaling. (A) Knockdown of endogenous STIM2, but not Orai1 or STIM1 proteins, by treatment with siSTIM2. Protein loading is indicated by actin expression. Effect of siSTIM2 on Ca^{2+} release and influx in control (black trace) and siSTIM2 (red trace) cells stimulated with 1 μM (B, C) and 100 μM (D, E) CCh. Insets show the ER-Ca^{2+} store release induced by 1 and 100 μM CCh in a Ca^{2+}-free media in a single cell. These traces represent average fluorescence changes obtained from at least 159 cells from 3 or more separate experiments. *$P < 0.05$, Student's t-test.

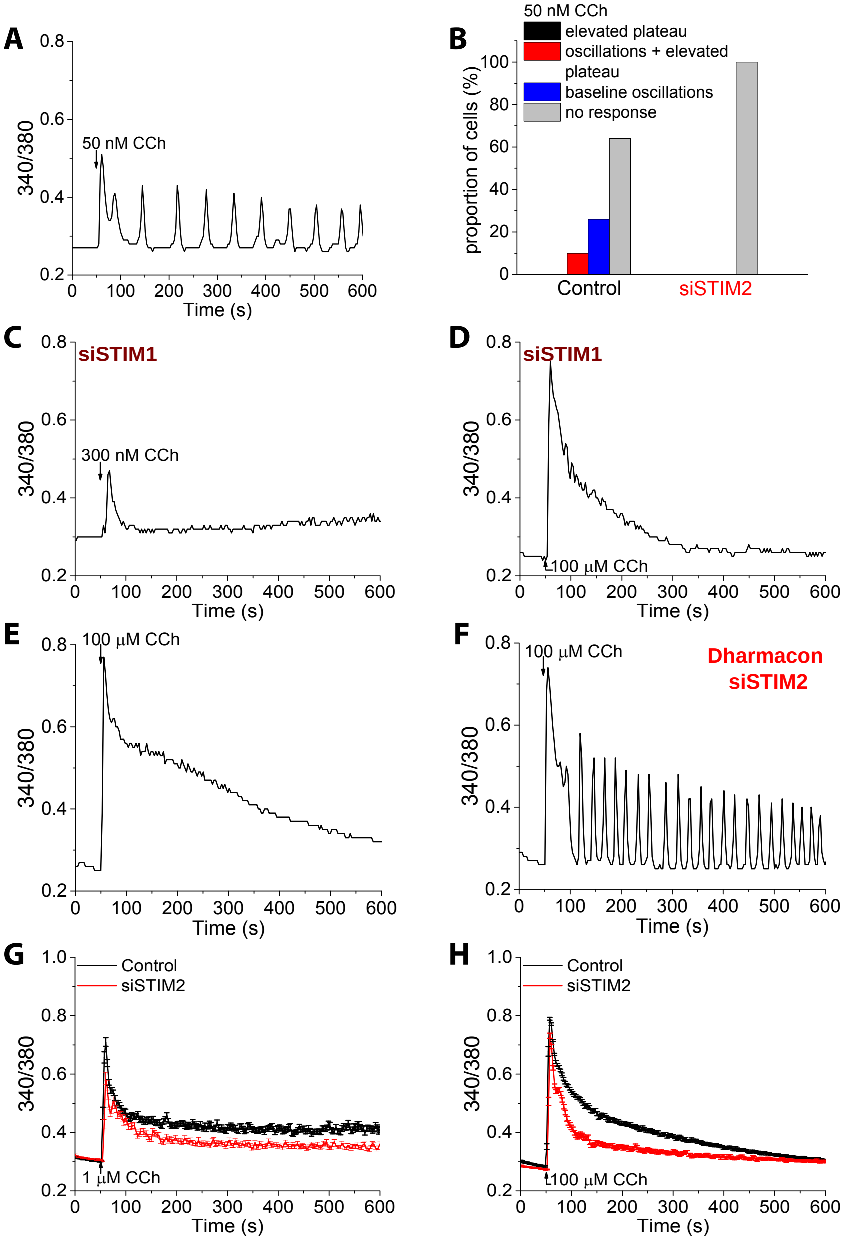

Figure S3. Effect of STIM1 or STIM2 knockdown on CCh-stimulated [Ca^{2+}]$_i$ responses. (A and B) 50 nM CCh-induced responses in control and siSTIM2 cells. (C and D) Effect of siRNA-mediated knockdown of STIM1 in cells stimulated with 300 nM and 100 μM CCh respectively. (E and F) Effect of siRNA-mediated knockdown of STIM2 in cells stimulated with 100 μM CCh using the Dharmacon SMARTpool siRNA. (G and H) Averaged traces from a cell population stimulated with 1 and 100 μM CCh respectively, with and without treatment with siSTIM2. These data represent [Ca^{2+}]$_i$ responses obtained from 3 or more separate experiments for each condition, with greater than 50 cells analyzed per experiment.

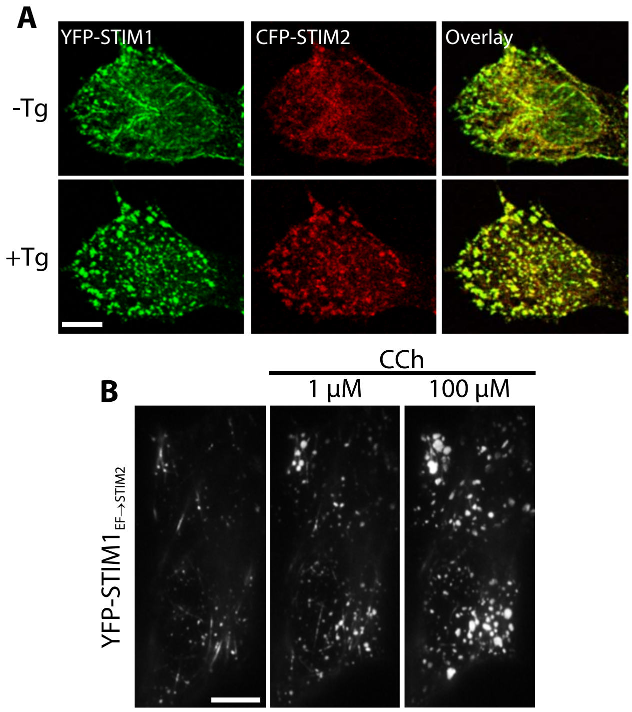

Figure S4. Store depletion-induced clustering of STIM proteins in cells co-expressing YFP-STIM1 and CFP-STIM2, or expressing only YFP-STIM1$_{EF\rightarrow STIM2}$. (A) Confocal images of co-expressed STIM1+STIM2 before and after Tg stimulation. YFP-STIM1 and CFP-STIM2 were pseudocolored green and red respectively for the overlay image. (B) Clustering of YFP-STIM1$_{EF\rightarrow STIM2}$ in response to stimulation with 1 and 100 µM CCh. These images are representative of at least 9 cells from 3 or more separate experiments. The bar indicates a scale of 10 µm.

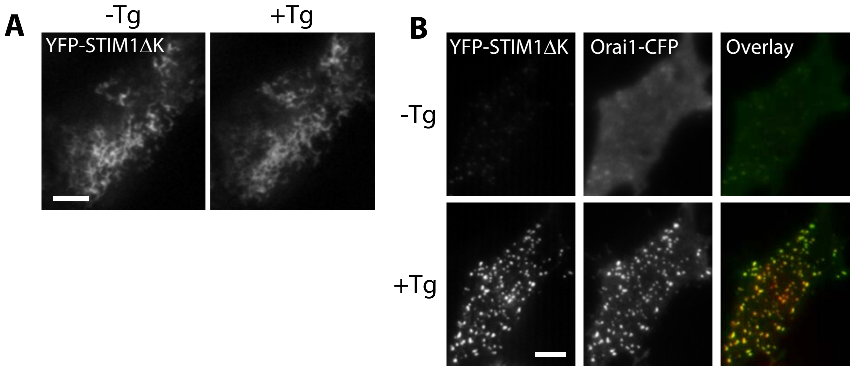

Figure S5. Effect of deleting the polybasic tail domain of STIM1 on store depletion-induced clustering. Different clustering patterns of (A) YFP-STIM1ΔK alone; and (B) co-expressed Orai1-CFP and YFP-STIM1ΔK following stimulation with 1 μM Tg. These images are representative of at least 15 cells from 3 or more separate experiments. The bar indicates a scale of 10 μm