Supporting Information

Kar et al. 10.1073/pnas.1201204109

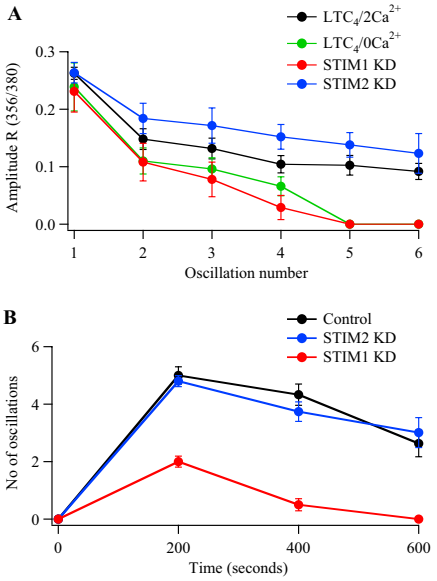

Fig. S1. Knockdown of STIM1 but not STIM2 alters the pattern of Ca^{2+} oscillations to LTC_4. (*A*) The amplitude of each Ca^{2+} oscillation is plotted against oscillation number for the conditions shown. Each point represents >16 cells. (*B*) The number of oscillations (measured in 200-s bins) is plotted against time for control cells (stimulated with 120 nM LTC_4) and after knockdown of STIM1 and STIM2. Each point represents >14 cells.

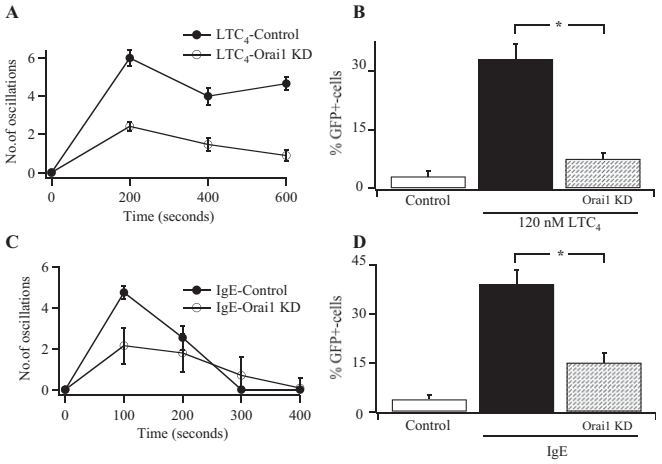

Fig. S2. Ca^{2+} responses and gene expression are reduced by Orai1 knockdown. (*A*) Ca^{2+} oscillations to CysLT1 receptor activation run down more quickly when Orai1 expression is reduced. Each point represents >23 cells. (*B*) NFAT-driven gene expression in response to LTC_4 is reduced after knockdown of Orai1. Each histogram is the aggregate of several experiments. (*C*) The number of Ca^{2+} oscillations to IgE is reduced by Orai1 knockdown. Each point represents >21 cells. (*D*) Orai1 knockdown reduces the fraction of cells expressing NFAT-driven GFP in response to IgE. Each histogram is the aggregate of several experiments. *$P < 0.05$.

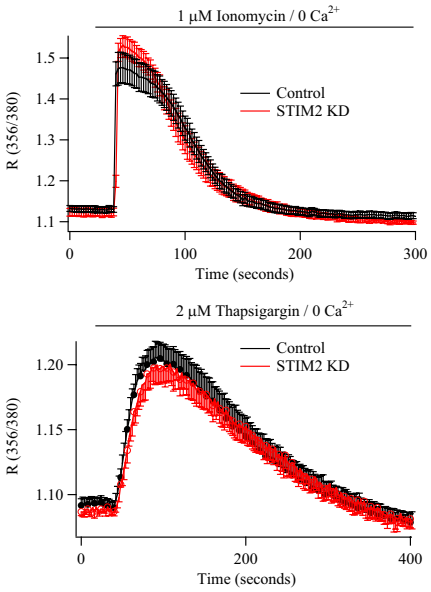

Fig. S3. STIM2 knockdown does not affect the Ca^{2+} content of the stores. (*Upper*) Stimulation with ionomycin in Ca^{2+}-free external solution releases Ca^{2+} rapidly from internal stores. The extent of Ca^{2+} release and subsequent Ca^{2+} removal were unaffected by knockdown of STIM2. (*Lower*) Thapsigargin (2 μM) was used to release Ca^{2+}. Graphs are averages of >15 cells each.

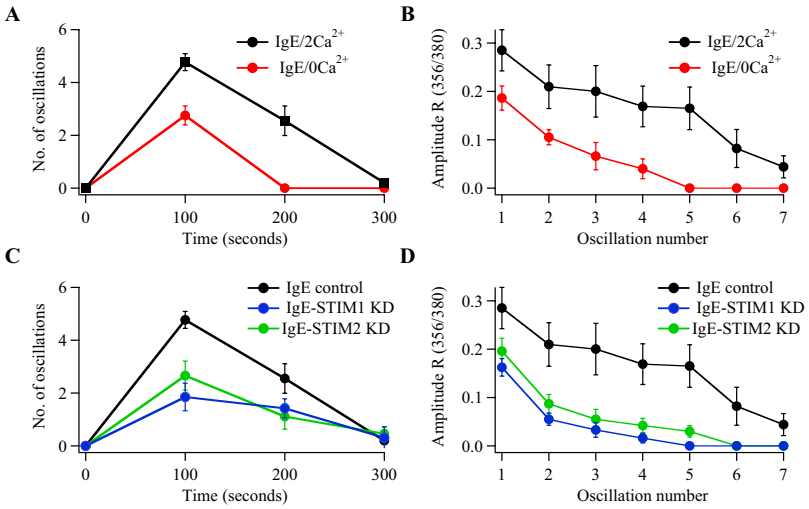

Fig. S4. Effect of STIM protein knockdown on the pattern of Ca^{2+} oscillations evoked by IgE. (*A*) The number of Ca^{2+} oscillations per 100-s bin is compared for cells stimulated with IgE in 2 mM external Ca^{2+} or Ca^{2+}-free solution. (*B*) The amplitude of each Ca^{2+} oscillation is compared for the two conditions shown. (*C*) The number of Ca^{2+} oscillations per 100-s bin is compared between control cells and cells in which either STIM1 or STIM2 had been knocked down. (*D*) The amplitude of each Ca^{2+} oscillation is compared for the different conditions. Each graph represents >18 cells.

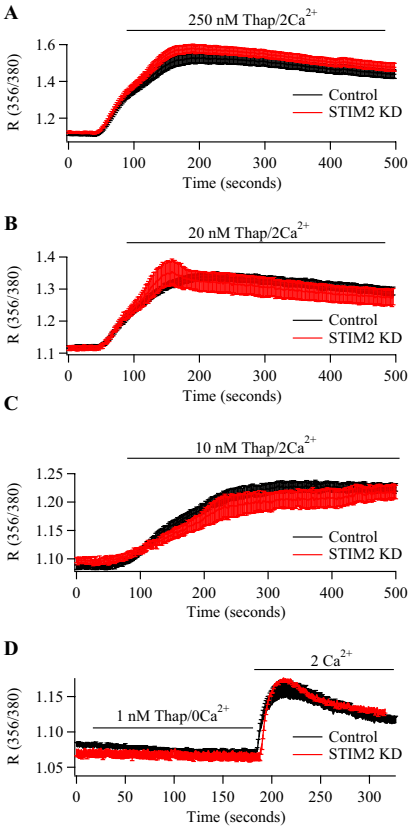

Fig. S5. STIM2 knockdown has no effect on store-operated Ca^{2+} entry evoked by a range of thapsigargin concentrations. (*A*) Responses are compared between control (mock-transfected) and STIM2 knockdown cells after stimulation with 250 nM thapsigargin. (*B*) As in *A*, but 20 nM thapsigargin was used. (*C*) As in *A*, but 10 nM thapsigargin was used. (*D*) Cells were stimulated with 1 nM thapsigargin in Ca^{2+}-free solution and then 2 mM external Ca^{2+} was readmitted, as shown. Each graph summarizes data from >30 cells.

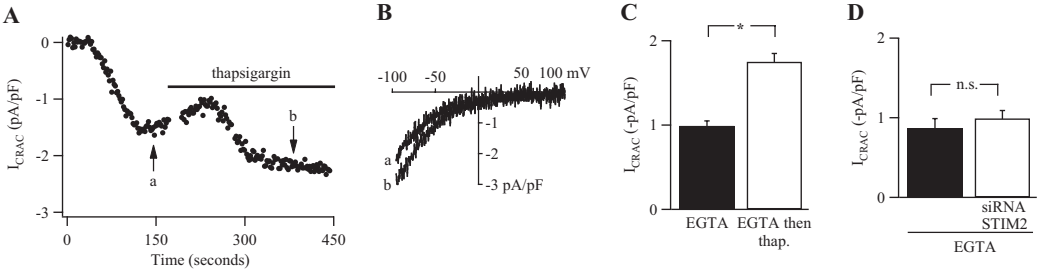

Fig. S6. Partial activation of I_{CRAC} is unaffected by STIM2 knockdown. (*A*) Whole cell dialysis with 2 mM EGTA led to activation of submaximal I_{CRAC}. Subsequent application of thapsigargin (2 μM) produced a larger current. (*B*) Current–voltage relationships, taken at the indicated points in *A*, are shown. (*C*) Histograms compare the size of I_{CRAC} for cells dialyzed with 2 mM EGTA versus 2 mM EGTA followed by thapsigargin (six cells for each bar). (*D*) The size of I_{CRAC} is compared for the conditions shown (five cells for EGTA and seven cells for STIM2). EGTA refers to 2 mM EGTA.