

Supplemental Fig. 1, PKC inhibition enhances SOCE in HEK293 cells.

HEK293 cells were incubated with different concentrations of the PKC inhibitors Calphostin C (A) and Go6983 (B) after depletion of Ca²⁺ stores with TG. Fura2 emission ratios were monitored for 60 s after addition of 2 mM Ca²⁺.

Supplemental Fig. 2, Comparable protein levels of ectopically expressed wild-type and mutant Orai1 following knockdown of endogenous Orai1.

HEK293 cells were transfected with the following vectors: shLu (Lu, luciferase) as negative control for knockdown or shOrai1 targeting the 3'UTR of Orai1. shRNA vectors also contained the coding sequences of mRFP, wild-type Orai1, Orai1-R91W or Orai1-S27/30A. Orai1 protein expression was analyzed by Western blotting using anti-Orai1, anti-STIM1 and anti-Actin antibodies.

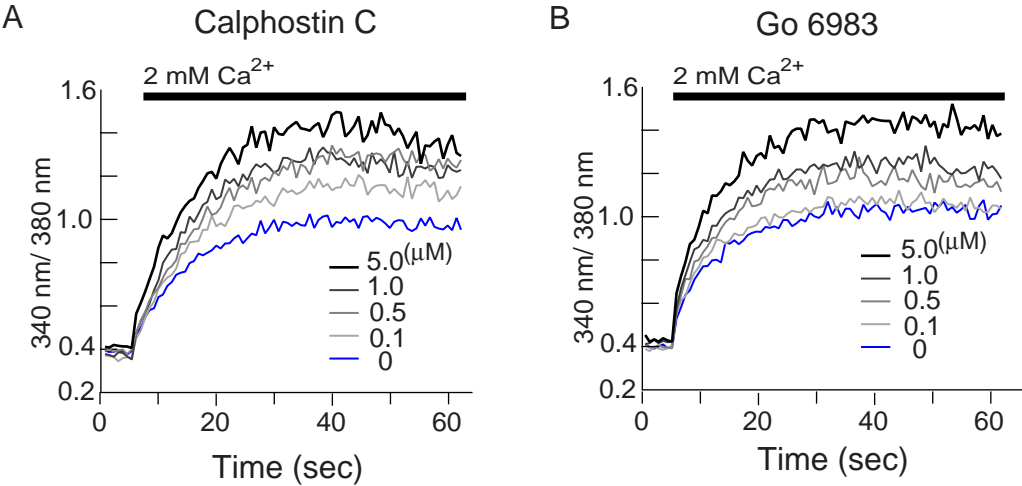
Supplemental Fig. 3, Alignment of N- and C-terminal protein regions of human Orai1, 2, and 3.

Conserved residues are boxed. * represent critical PKC phosphorylation sites in Orai1 identified in this study.

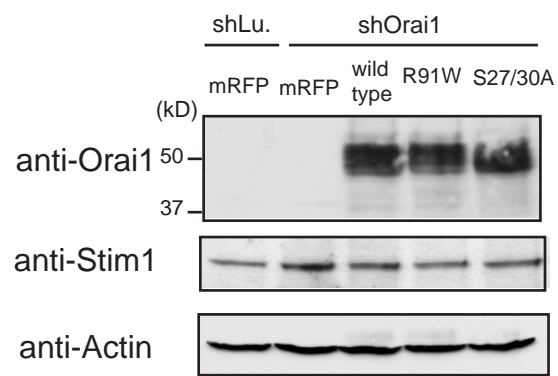
Supplemental Fig. 4, Mutant Orai1-S27/30A protein is able to bind to STIM1.

Flag-tagged wild-type and mutant (S27/30A) Orai1 were expressed in HEK293 cells and cell lysates were incubated with the purified GST-tagged CCb9 domain, a minimal CRAC channel activation domain in STIM1, or GST alone. Protein binding was detected by Western blot analysis using anti-FLAG antibody; GST proteins were detected by Ponceau red staining.

Supplemental Fig.1



Supplemental Fig. 2



Supplemental Fig.3

Intracellular N-terminal region of Orai

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N-Orai1 1: MHPEPAPPPSRSSPELPPSGGSTTSGSRR-S 30
N-Orai2 1: -----MSA 3
N-Orai3 1: -----MKGGE 5

31: RRRSGDGE--PPGAPPEPPSAVTYPDWIGQS 59
4: ELNVPI-DPSAPACPEPGHKGMDYRDWVRRS 33
6: GDAGEQA-PLNPEGESEFAGSAT-YREFVHRG 34

60: YSEVMSLNEHSMQALSWRKLYLSRAKLKA 88
34: YLELVTSNHHSVQALSWRKLYLSRAKLKA 62
35: YLDLMGASQHSLRALSWRRLYLSRAKLKA 63
    
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Intracellular C-terminal region of Orai

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C-Orai1 257: YRSLVSHKTD RQFQELNELAEFARLQDQLDH--RGDHPLTPGSHYA 301
C-Orai2 219: YRSLVRHKTERHNREIEELH---KLVQLDGHESLQ-VL----- 254
C-Orai3 267: YRSLVAHKTD RYKQEELEELNR---LQGETQAV----- 295
    
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Supplemental Fig.4

