Supplemental Material

Ca^{2+}/Calmodulin Binding to STIM1 Hydrophobic Residues Facilitates Slow Ca^{2+}-Dependent Inactivation of the Orai1 Channel

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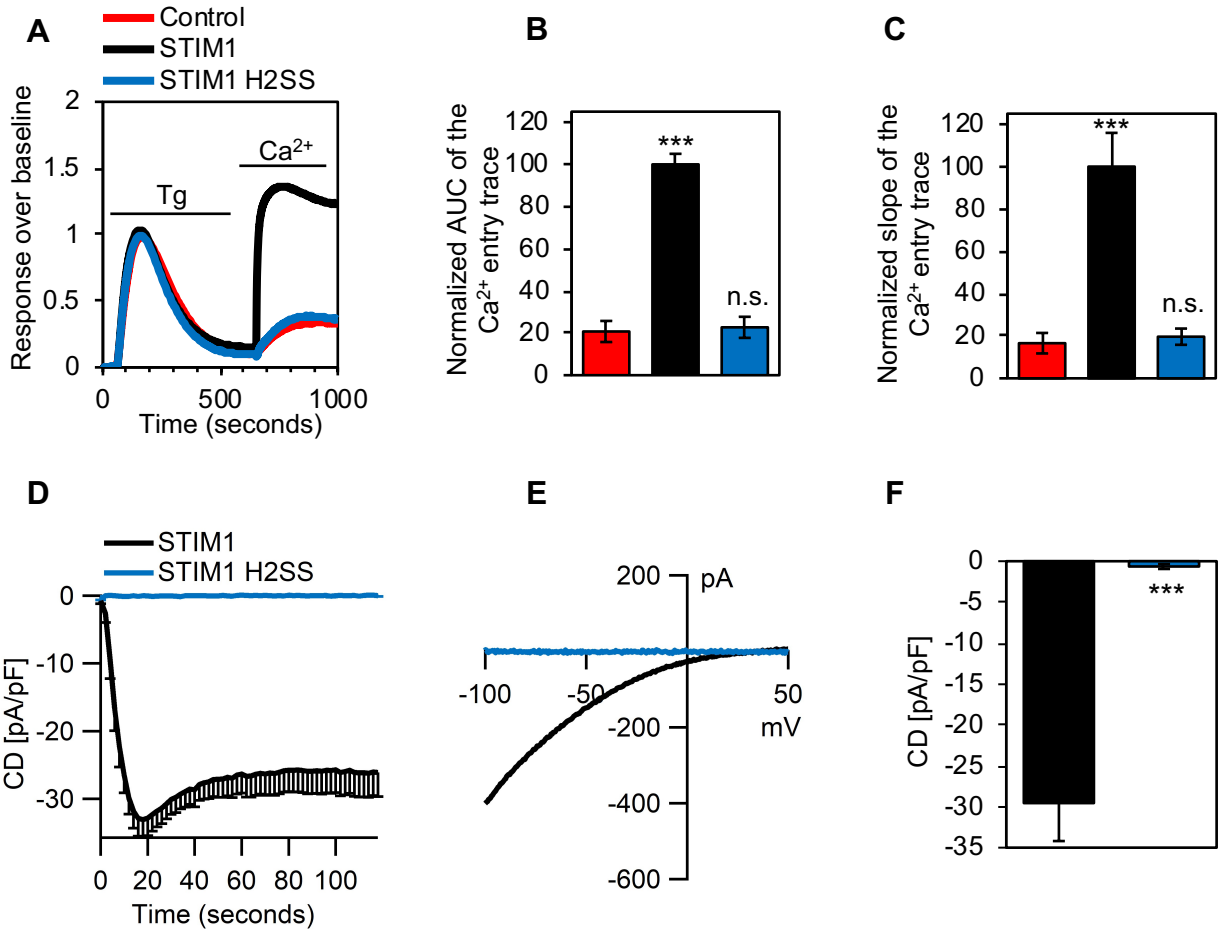
A
Control
STIM1
STIM1 H2SS
Response over baseline
Tg
Ca2+
Time (seconds)
B
Normalized AUC of the Ca2+ entry trace
***
n.s.
C
Normalized slope of the Ca2+ entry trace
***
n.s.
D
STIM1
STIM1 H2SS
CD [pA/pF]
Time (seconds)
E
pA
mV
F
CD [pA/pF]
***

Fig. S1. H2 site mutants of STIM1 CaM-binding domain show impaired Orai1-activating function in HEKO1 STIM1$^{-/-}$ cells.

(A), Representative FLIPR traces of SOCE in HEKO1 STIM1$^{-/-}$ cells transiently transfected with mCherry (Control, red), mCherry-STIM1 (STIM1, black) or mCherry-STIM1-L390S-F391S (STIM1 H2SS, blue). 1 µM thapsigargin (Tg) and 2 mM $CaCl_2$ (Ca^{2+}) administrations are indicated. (B), Quantified area under the curve (AUC) and (C), slope of the SOCE traces after extracellular Ca^{2+} application in HEKO1 STIM1$^{-/-}$ cells transiently transfected with control (red), STIM1 (black) or STIM1 H2SS (blue). The data was normalized by setting the AUC and slope of the Ca^{2+} entry traces to 100 (n = 15; mean ± standard deviation). (D), average current density (CD) of the I_{CRAC} recordings (STIM1, n = 9; STIM1 H2SS, n = 5; mean - SEM) from HEKO1 STIM1$^{-/-}$ cells. (E), average current-voltage (I-V) curves of the I_{CRAC} recordings at t = 2 min (STIM1, n = 9; STIM1 H2SS, n = 5). (F), quantified average CD values at t = 2 min (STIM1, n = 9; STIM1 H2SS, n = 5; mean ± SEM) from HEKO1 STIM1$^{-/-}$ cells transiently overexpressing STIM1 (black) or STIM1 H2SS (blue). p value (p) of the WT or mutant STIM compared to the mcherry control group is indicated above the respective bar as non-significant (n.s.) for p > 0.05 or as "***" for p <= 0.001.