Figure S3

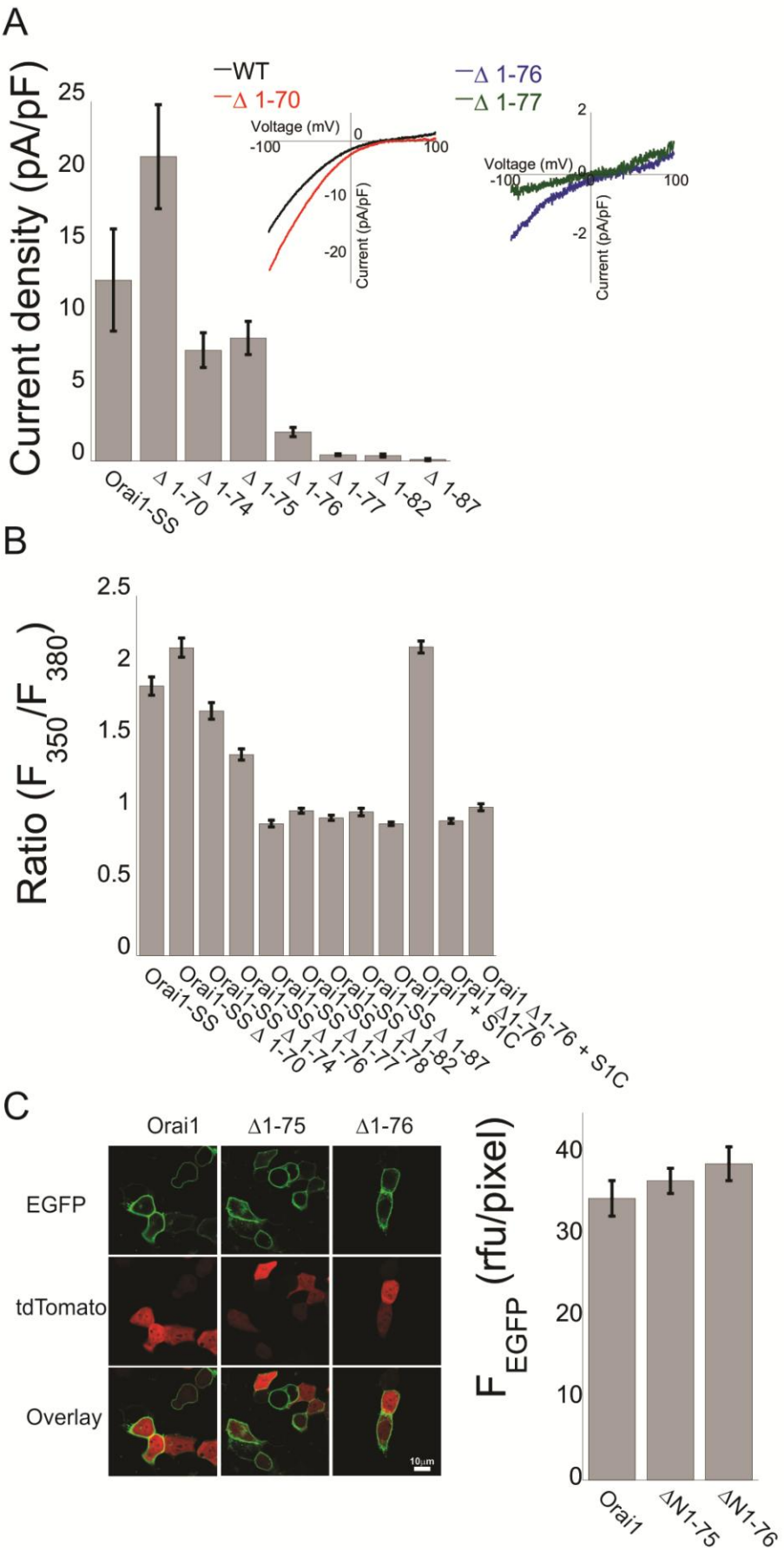
A
Current density (pA/pF)
25
20
15
10
5
0
WT
Δ 1-70
Δ 1-76
Δ 1-77
Voltage (mV)
-100
0
100
-10
-20
Current (pA/pF)
2
-2
Orai1-SS
Δ 1-70
Δ 1-74
Δ 1-75
Δ 1-76
Δ 1-77
Δ 1-82
Δ 1-87
B
Ratio (F350/F380)
2.5
2
1.5
1
0.5
0
Orai1-SS
Orai1-SS Δ 1-70
Orai1-SS Δ 1-74
Orai1-SS Δ 1-76
Orai1-SS Δ 1-77
Orai1-SS Δ 1-78
Orai1-SS Δ 1-82
Orai1-SS Δ 1-87
Orai1
Orai1 + S1C
Orai1 Δ1-76
Orai1 Δ1-76 + S1C
C
Orai1
Δ1-75
Δ1-76
EGFP
tdTomato
Overlay
10μm
F EGFP (rfu/pixel)
40
30
20
10
0
Orai1
ΔN1-75
ΔN1-76

Figure S3. N-terminal truncations beyond arginine 77 of the N' terminal region of Orai1 abolish channel activation by tethered STIM1 fragments. (A) Left, summary of current densities recorded from cells expressing Orai1-SS-EGFP or the indicated N terminal deletion mutants Orai1-SS-EGFP ΔN_{1-70}, ΔN_{1-74}, ΔN_{1-75}, ΔN_{1-76}, ΔN_{1-77}, ΔN_{1-78}, ΔN_{1-82} and ΔN_{1-87} (n=3-7 cells). Right, representative plots of the current-voltage relationship of currents recorded from cells expressing Orai1-SS-EGFP, Orai1-SS-EGFP ΔN_{1-70}, Orai1-SS-EGFP ΔN_{1-76} and Orai1-SS-EGFP ΔN_{1-77}. (B) Basal Ca^{2+} levels measured from HEK293 cells expressing Orai1-SS-EGFP or the indicated N terminal deletion mutants Orai1-SS-EGFP ΔN_{1-70}, ΔN_{1-74}, ΔN_{1-75}, ΔN_{1-76}, ΔN_{1-77}, ΔN_{1-78}, ΔN_{1-82} and ΔN_{1-87} or from cells expressing Orai1-EGFP or Orai1-EGFP ΔN_{1-76} with or without mCherry-S1C (n=23-76 cells, *** $p<0.001$). (C) left - Orai1 N terminal deletion mutants localize to cell surface. Fluorescent images of HEK293 cells expressing Orai1-EGFP, Orai1-EGFP ΔN_{1-75} or Orai1-EGFP ΔN_{1-75} together with td-Tomato (as a marker for cytosol and nucleus regions). Right- Quantitation of expression levels for ΔN Orai1 constructs (n = 6 regions).