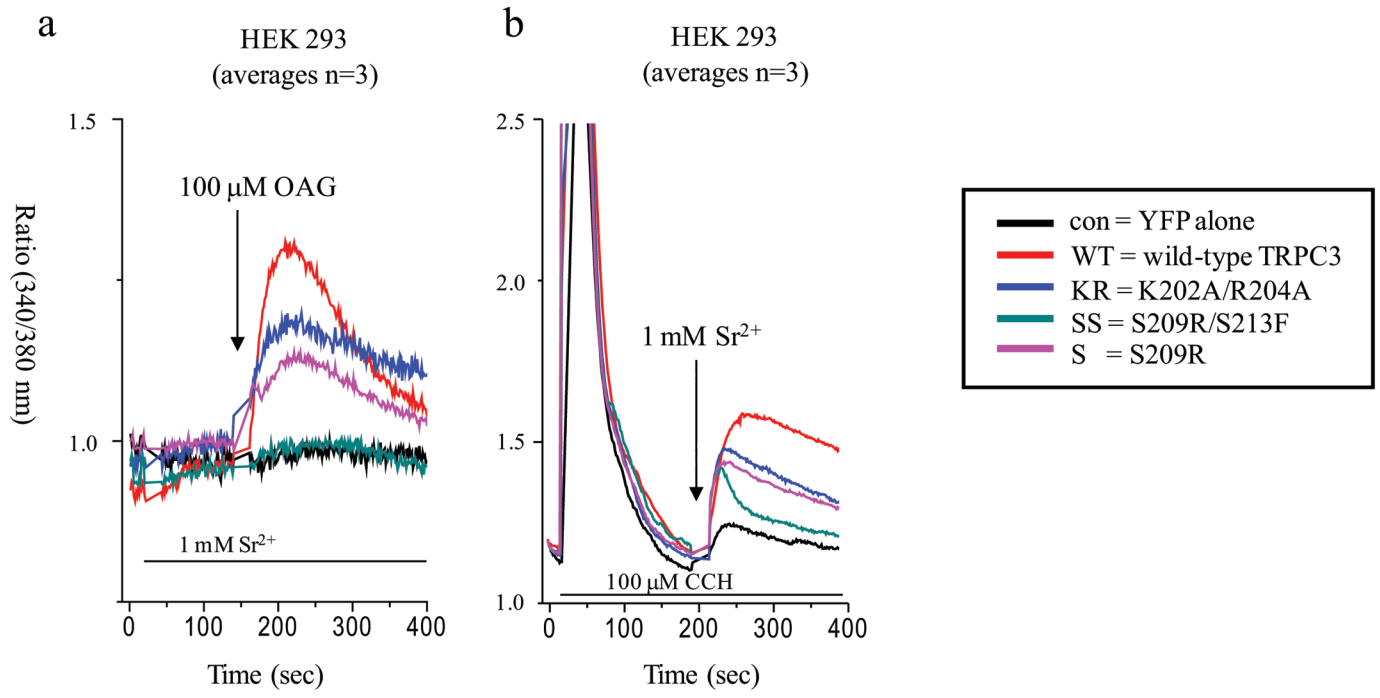
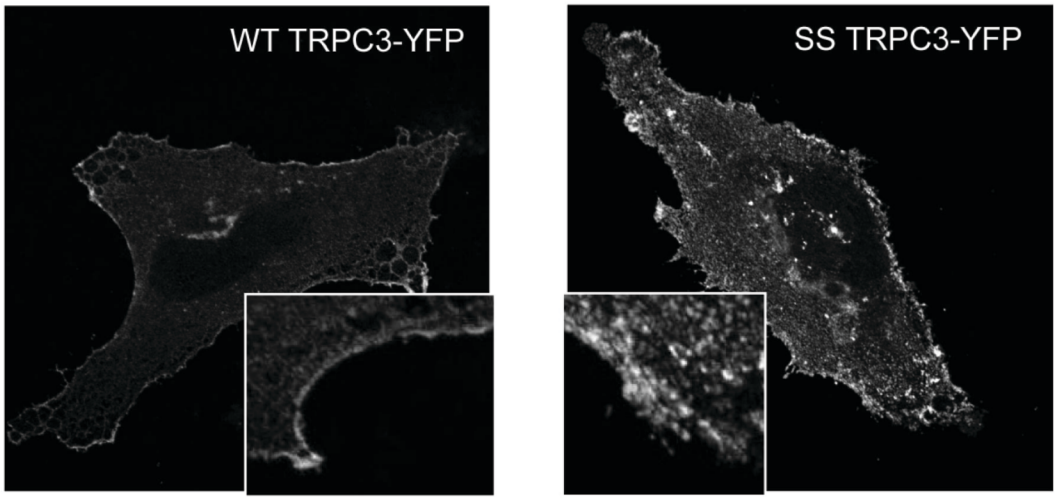
Supplemental Figure 1: Cell imaging and surface expression of WT and SS TRPC3. (a) Fura2AM measurements were made in HEK293 cells transfected for 24 h with either YFP alone (con) or YFP + full-length WT, K202A/R204A (KR), S209R/S213F (SS), or S209R (S) TRPC3 mutants. Cells were acclimated first in nominally Ca^{2+}-free medium, then in 1 mM Sr^{2+} containing media (bar), and then challenged with 100 μM OAG (arrow). **(b)** Fura2AM measurements were made in HEK293 cells transfected as in (a). Ca^{2+} pools were released in cells by carbachol (CCH, 100 μM) (first bar), in nominally Ca^{2+}-free medium followed by replacement with CCH and 1mM Sr^{2+} containing media (arrow). For a-b, these traces are quantified in the bar graphs in main text Figure 2b. **(c)** Confocal microscopy of HeLa cells transfected with either YFP-tagged full-length WT or SS TRPC3. Inset: magnification. **(d)** Total Internal Reflection Fluorescence of HEK293 cells stably transfected with either YFP-tagged full-length WT or SS TRPC3. These cells were stimulated with 100 μM carbachol (CCH). Arrows depict TRPC3 regions which increase over time. See Supplemental Movies for real-time TIRF experiments. **(e)** Graphical schematic of data collection for Fluorescence Recovery After Photobleaching (FRAP) and Fluorescence Loss In Photobleaching (FLIP) experiments (see Methods).


a
HEK 293
(averages n=3)
Ratio (340/380 nm)
1.5
1.0
100 µM OAG
1 mM Sr2+
0
100
200
300
400
Time (sec)
b
HEK 293
(averages n=3)
2.5
2.0
1.5
1.0
1 mM Sr2+
100 µM CCH
0
100
200
300
400
Time (sec)
con = YFP alone
WT = wild-type TRPC3
KR = K202A/R204A
SS = S209R/S213F
S = S209R

SF 1-1

c

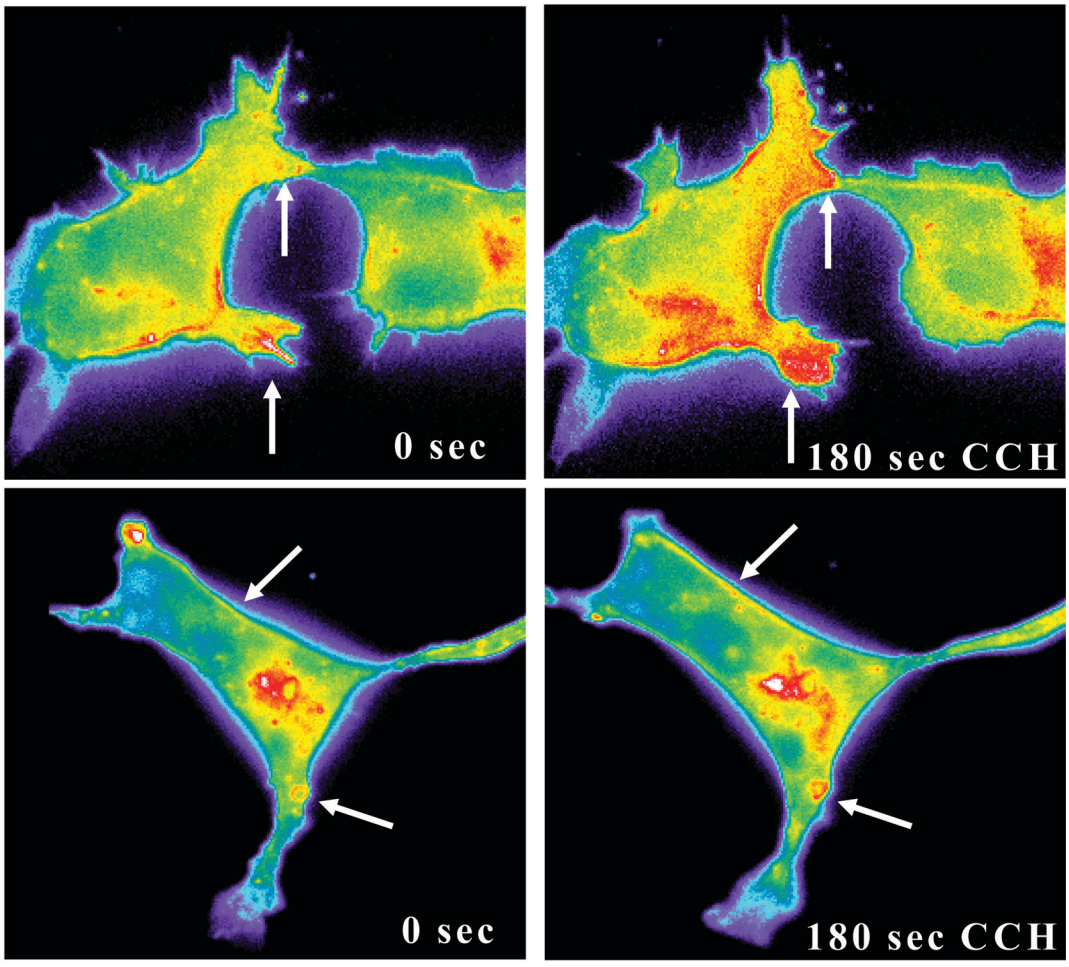


d

Total Internal Reflection Fluorescence

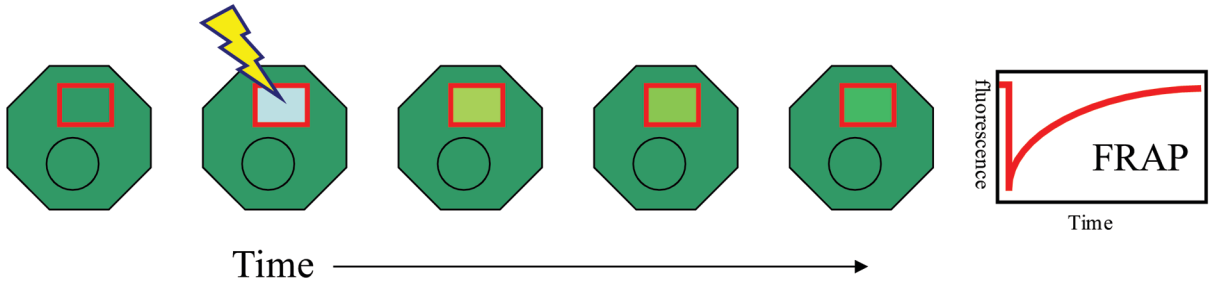
WT TRPC3-YFP

SS TRPC3-YFP

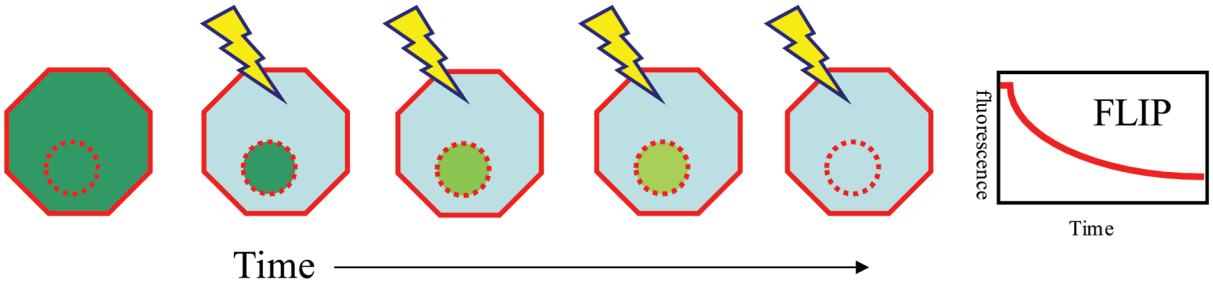


SF 1-3

e Fluorescence recovery after photobleaching (**FRAP)**



Fluorescence loss in photobleaching (**FLIP)**



Bleached Region of Interest (ROI)
Unbleached ROI