Table S1. Table of calculated non-linear regression constants

Condition	Yo	A1	K1	A2	K2	B	R2
WT Ctl	2.64±.026	1.64 ±.021	0.060±.003	0.42±.021	0.011±.001	0.43±.004	0.85
WT PHA	2.87±.061	1.61±.051	0.046±.002	0.85±.012	0.005±.001	0.40±.030	0.91
WT OKT3	4.12±.063	2.46±.051	0.024±.008	1.04±.015	0.005±.0011	0.62±.030	0.9
STIM1	2.79±.045	1.58±.004	0.075±.008	0.60±.001	0.005±.008	0.47±.014	0.81
STIM1 PHA	2.6±.031	1.42±.001	0.06±.002	0.63±.001	0.006±.001	0.54±.012	0.9
STIM2	2.71±.026	1.95±.007	0.076±.002	0.30±.007	0.007±.001	0.44±.007	0.94
Delta K	6.12±.071	4.6±.001	0.081±.004	0.55±.001	0.004±.001	1.05±.062	0.92
Delta 597	3.27±.036	2.6±.010	0.076±.005	0.33±.010	0.009±.014	0.60±.001	0.91
Delta P	4.84 ±.159	3.4 ±.001	0.071±.009	0.69±.007	0.011±.009	0.73±.112	0.65
NN	4.57±.157	2.6±.0103	0.066±.001	1.11±.007	0.007±.004	0.79±.263	0.65
Ctl-Ru360	3.45±.04	1.73±.14	0.050±.008	1.15±.14	0.012±.003	0.56±.006	0.91
PHA-Ru360	4.02±.02	1.82±.013	0.051±.003	1.48±.013	0.008±.001	0.71±.006	0.98
Scr Ctl	2.9±.030	2.2±.011	0.046±.026	0.12±.011	0.010±.007	0.50±.002	0.78
Scr PHA	3.1±.012	2.28±.003	0.050±.027	0.35±.003	0.004±.003	0.46±.003	0.78
S1KD Ctl	2.0±.011	1.5±.004	0.030±.001			0.50±.001	0.86
S1KD PHA	1.70±.023	1.15±.005	0.03±.002			0.55±.004	0.83
Ctl-BTP2	2.29±.012	1.86±.016	.020 ± .001			0.43±.001	0.94
PHA-BTP2	2.16±.023	1.69±.023	.013 ± .001			0.47±.001	0.78

Constants were obtained from the equations: $\mathbf{Y = B + A_1^{(-K_1 * t)} + A_2^{(-K_2 * t)}}$ or $\mathbf{Y = B + A_1^{(-K_1 * t)}}$ **,**where B is the plateau of decay, K_1 and K_2 are rate constants for the fast and slow phase, respectively. A_1 and A_2 are the amplitudes for the fast and slow phase of decay, respectively. t is time.

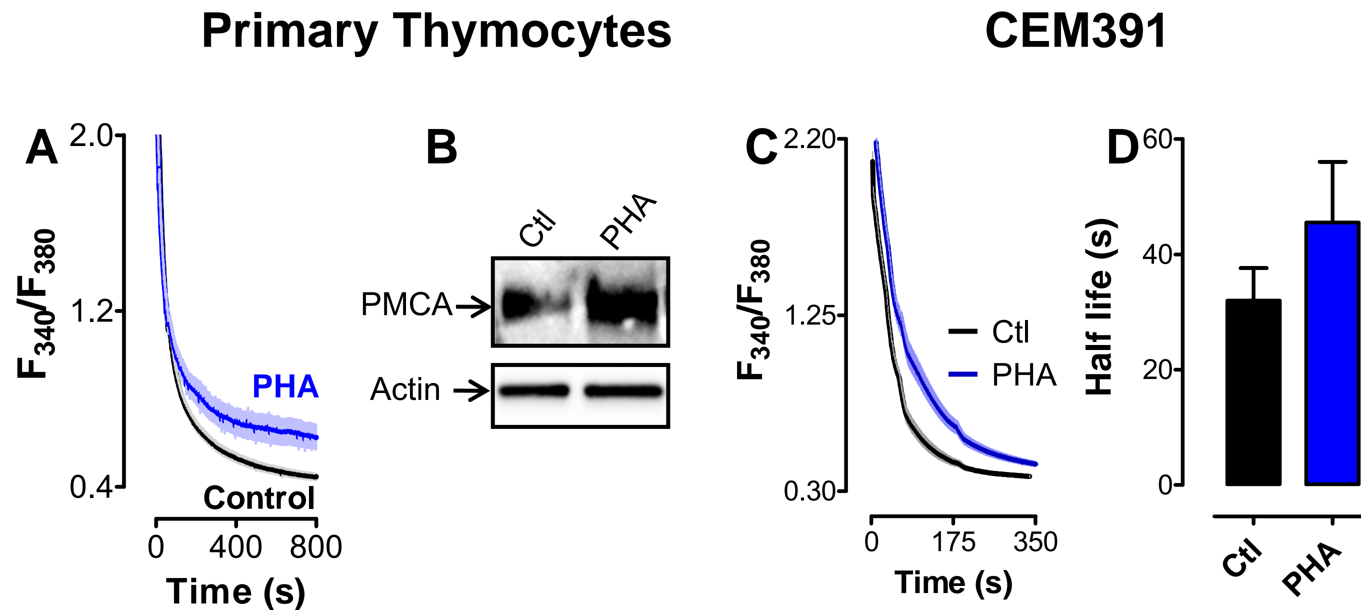

Figure S1: PHA-mediated changes in PMCA expression and function in T cells. Freshly isolated primary murine thymocytes were treated with PHA or vehicle for 2 hours before assessing $[Ca^{2+}]_c$ clearance **(A,** representative trace of multiple experiments**)** or analyzing PMCA and actin protein expression by Western blot **(B)** $[Ca^{2+}]_c$ clearance was analyzed in CEM391 T cells after treatment with PHA or vehicle for two hours. Representative traces of multiple experiments **(C)** are shown and analyzed by one-phase, nonlinear regression **(D)** (ctl, $\overline{R^2}$ =.74, n=5; PHA, $\overline{R^2}$ =.72, n=5.

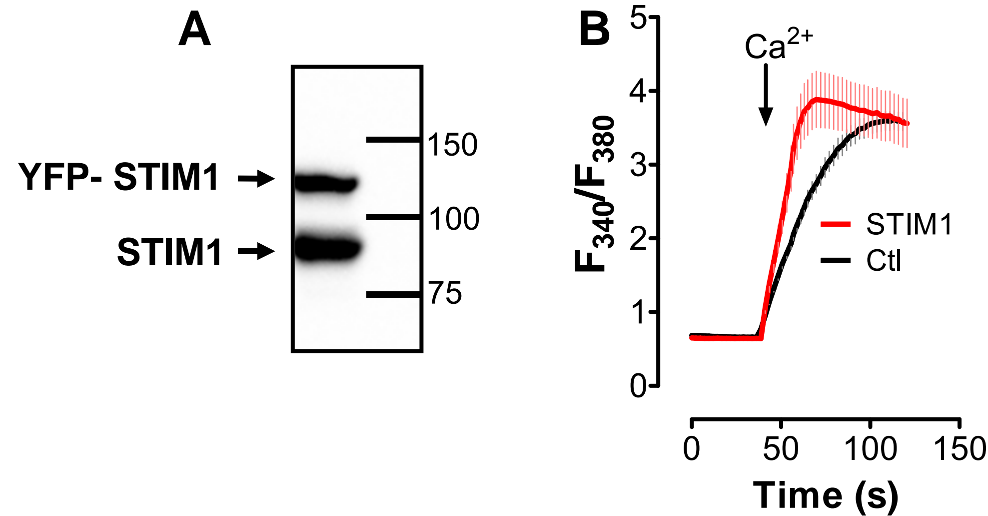

Figure S2: YFP-STIM1 transfections control STIM1 expression and function. (A) WT Jurkat cells were transfected with YFP or YFP-STIM1. 48 hours after transfection, cells were harvested for cell lysis and analyzed for expression of STIM1 by Western blot. **(B)** Representative traces of multiple experiments assessing the magnitude of SOCe in Jurkat cells 48 hours after transfection with YFP or STIM1.

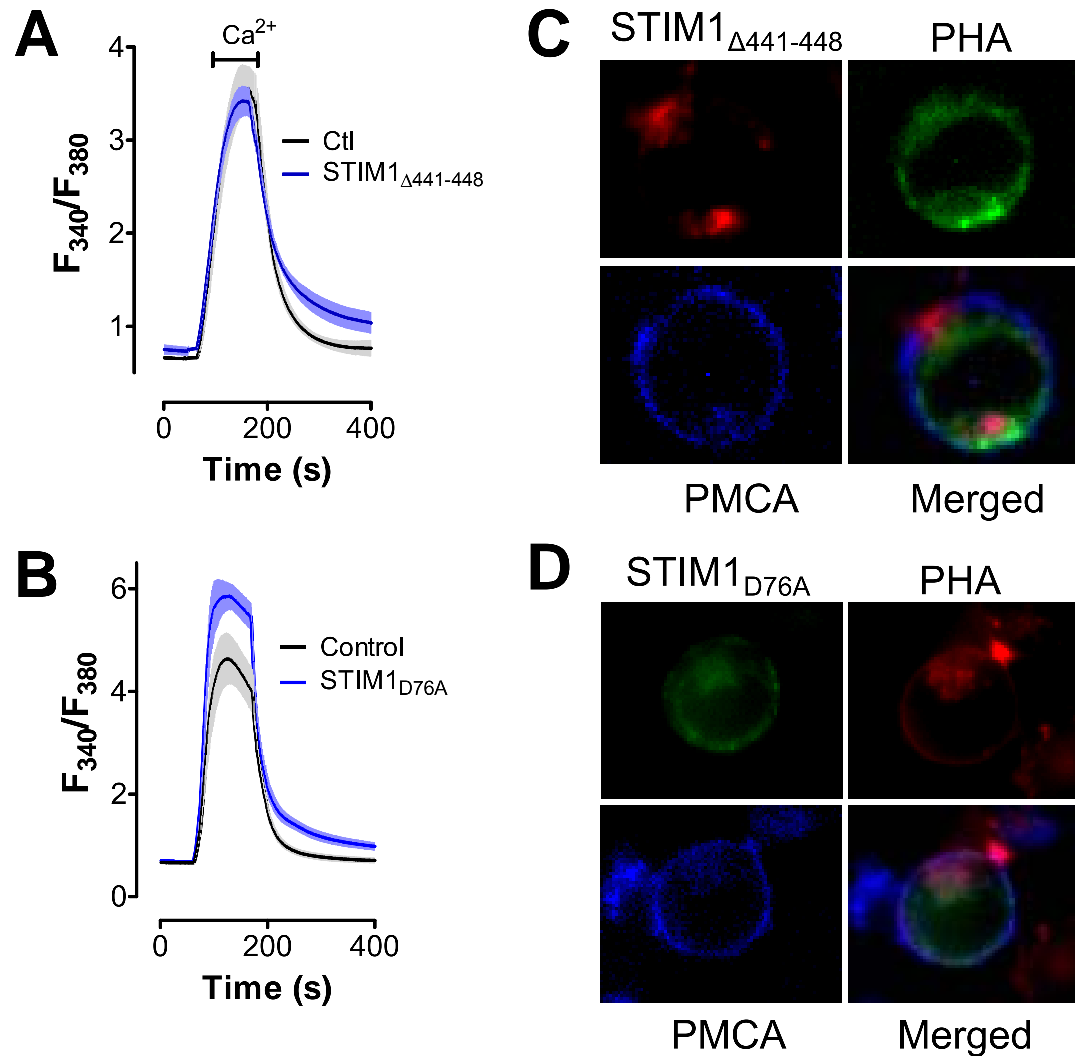

Figure S3: Control of Orai1 function and PMCA function by STIM1 are independent. (A,B) $[Ca^{2+}]_c$ clearance was analyzed in Jurkat T cells after transfection with YFP **(A,B)**, YFP-$STIM1_{Δ441\text{-}448}$ **(A)** or YFP-$STIM1_{D76A}$ **(B)**. **(C,D)** Co-localization of YFP-$STIM1_{Δ441\text{-}448}$ **(C)** or YFP-$STIM1_{D76A}$ **(D)** with CFP-PMCA and Alexafluor594-tagged PHA was determined by fluorescence microscopy.

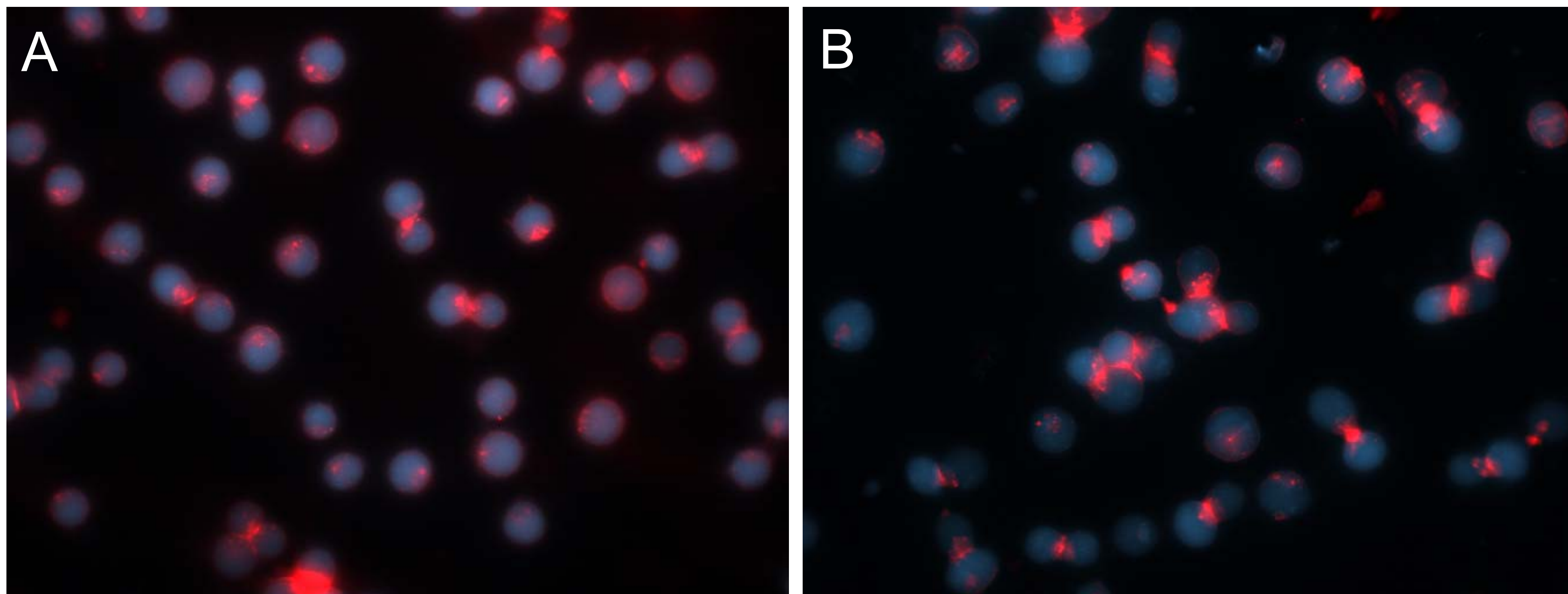

Figure S4: Localization of tagged PHA in full fields of Jurkat T cells. Jurkat cells were transfected with Scr RNA **(A)** or STIM1 siRNA **(B)** and treated with 1 μg/ml Alexa Fluor 594 conjugated PHA for two hours, mounted on a cover slip and PHA cytolocalization was assessed. See **supplemental movie 1 or 2** for Fura-2 based Ca^{2+} images of these fields.

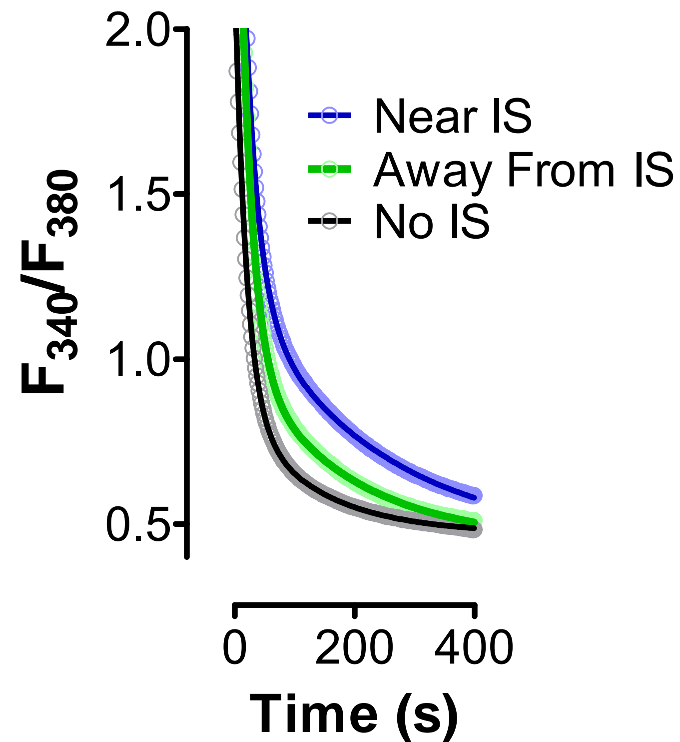

Figure S5: Comparison of $[Ca^{2+}]_c$ clearance rates at the IS vs. other regions of the cell. Jurkat T cells were treated with PHA or vehicle and loaded with fura-2 for 1 hr and depleting ER Ca^{2+} stores with Tg (2 μM) in the absence of $[Ca^{2+}]_e$ (not depicted). Once $[Ca^{2+}]$ had returned to baseline, $[Ca^{2+}]_e$ was elevated to 1 mM; upon reaching peak $[Ca^{2+}]$, $[Ca^{2+}]_e$ was again removed. $[Ca^{2+}]_c$ was assessed as global (away from IS) or local Ca^{2+} signals (near IS) in PHA treated cells and compared to global Ca^{2+} signals in vehicle treated (Control) cells.

Supplemental Movie 1: Comparison of local changes in $[Ca^{2+}]_c$ during Jurkat T cell activation. Jurkat cells were transfected with Scr RNA and treated with 1 µg/ml Alexa Fluor 594 for two hours, mounted on a cover slip before loading with fura-2 and assessing changes $[Ca^{2+}]_c$. High resolution images were taken every two seconds. The images were recorded for 30 seconds before the addition of 1 mM $[Ca^{2+}]_e$. Ca^{2+} was then removed and the recording continued. Movie is shown in 10x speed. Note that localization of PHA for these images is depicted in figure S4A.

Supplemental Movie 2: Comparison of local changes in $[Ca^{2+}]_c$ during T cell activation after STIM1 knockdown. Jurkat cells were transfected with STIM1 siRNA and treated with 1 µg/ml Alexa Fluor 594 for two hours, mounted on a cover slip before loading with fura-2 and assessing changes $[Ca^{2+}]_c$. High resolution images were taken every two seconds. The images were recorded for 30 seconds before the addition of 10 mM $[Ca^{2+}]_e$. Ca^{2+} was then removed and the recording continued. Movie is shown in 10x speed. Note that localization of PHA for these images is depicted in figure S4B.