Jha et al., http://www.jcb.org/cgi/content/full/jcb.201301148/DC1

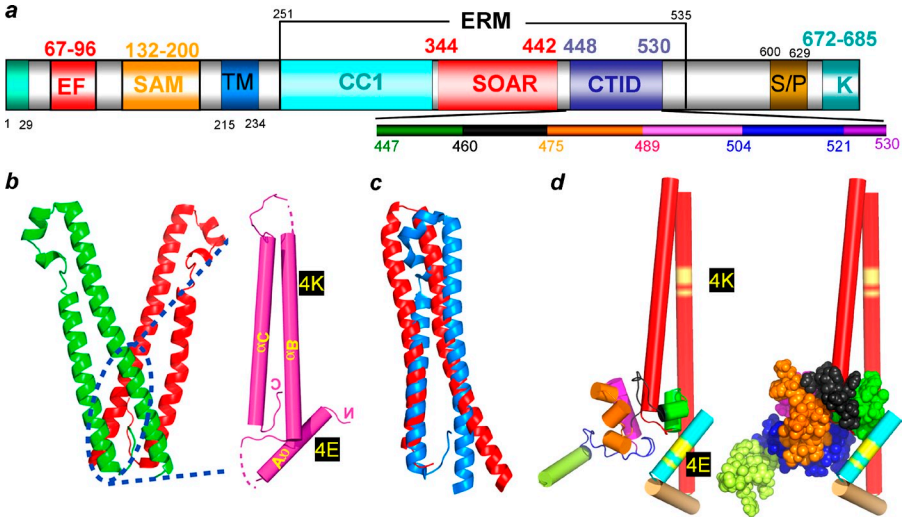

Figure S1. **Structural model of STIM1 ERM domain.** (a) Linear representation of known STIM1 domains. The boundaries of the different domains in CTID (STIM1(448–530)) are shown with different colors. (b) Crystal structure of human and *C. elegans* SOAR and part of coiled-coil region of STIM1. Reproduced from Yang et al. (2012). (c) Alignment of the crystal structure (blue) and predicted (red) SOAR domains. (d) Model of SOAR–CTID structure predicted by Robetta software. The position of the four lysines in SOAR(4K) and four glutamates (4E) in the second helix of CC1 are shown in yellow.

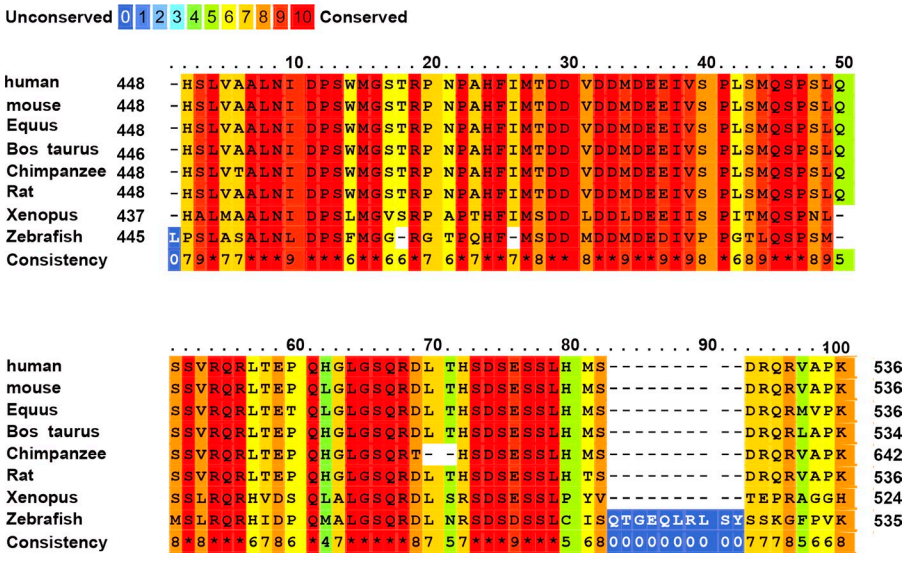

Figure S2. **Multiple sequence alignment of the C-terminal fragment of CTID.** CTID in all the phyla of vertebrates ranging from mammalia to pisces is highly conserved.

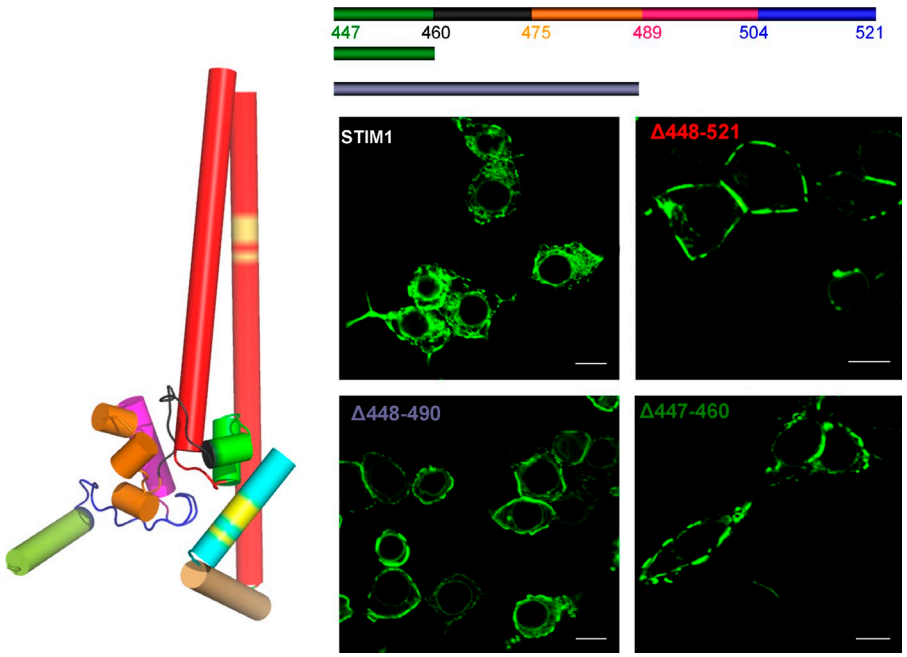

Figure S3. **Localization of STIM1 deletion constructs.** Shown are confocal images of STIM1 and of selective STIM1 deletion mutants expressed in HEK293 cells. All the deletion mutants make it to the ER–plasma membrane junctions in the absence of store depletion.

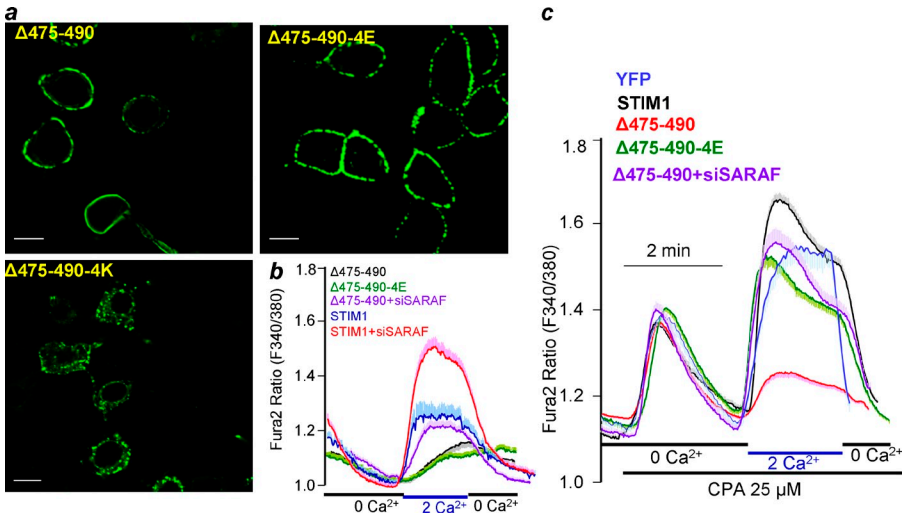

Figure S4. **Properties of STIM1(Δ475–490).** (a) STIM1(Δ475–490) and STIM1(Δ475–490)$^{4E/4A}$ cluster at the ER–plasma membrane junction in the absence of store depletion, which required intact SOAR as evident from trapping of STIM1(Δ475–490)$^{4K/4A}$ in an intracellular vesicular compartment. Bars, 5 µm. (b) In spite of clustering of STIM1(Δ475–490) at the ER–plasma membrane domain it did not activate Ca^{2+} influx, even when the 4E/4A mutations were inserted into STIM1(Δ475–490)$^{4E/4A}$ or when SARAF was knocked down with siSARAF. (c) SOC influx in HEK293 cells is strongly inhibited by STIM1(Δ475–490), but the inhibition is relieved by the STIM1(Δ475–490)$^{4E/4A}$ mutation and by siSARAF, suggesting that STIM1(Δ475–490) inhibits SOC probably by recruiting SARAF to the native Ca^{2+} influx channels. The results in b and c are given as mean ± SEM of 30–50 cells.

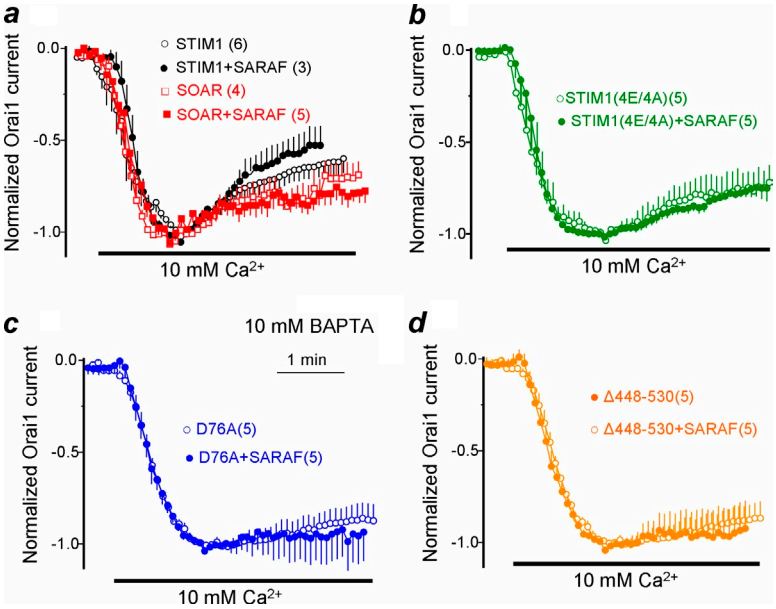

Figure S5. **BAPTA inhibits residual SCDI.** Orai1 current was measured in pipette solution containing 10 mM BAPTA in HEK cells expressing Orai1 with and without SARAF and STIM1 or SOAR (a), STIM1$^{4E/4A}$ (b), STIM1^{D76A} (c), or STIM1(Δ448–530) (d). The results are plotted as mean ± SEM of the number of experiments listed in parentheses.

Table S1. **Parameters of FCDI and SCDI**

Construct	τ_1	± SEM	τ_2	± SEM	Construct	Inhibition	± SEM	Construct	Control inhibition	± SEM	SARAF inhibition	± SEM
	ms			*ms*		%			%		%	
STIM1	12.3	1.2	69.3	6.4	STIM1	76	4.0	STIM1	76	4.0	94*	6.0
Δ490–521	17.5	2.5	73	8	Δ447–460	96	10	Δ447–460	96	10	100	
Δ447–460	25	7.0	NA	NA	Δ490–521	68	7.0	Δ490–521	68	7.0	70	7.7
Δ448–521	10.3	0.07	NA	NA	Δ448–521	49	6.0	Δ448–521	49	6.0	54	5.0
SOAR	NA	NA	NA	NA	SOAR	39	7.0	SOAR	39	7.0	55*	5.0
Δ490-504	22.0	4.0	NA	NA	Δ448–530	32	9.0	Δ448–530	32	9.0	28	5.0
					Δ448–490	53	6.9	Δ448–490	53	6.9	89*	5.8

The table lists the mean fitted parameters for FCDI, the time courses, and the mean extent of SCDI. *, $P < 0.05$.

Reference

Yang, X., H. Jin, X. Cai, S. Li, and Y. Shen. 2012. Structural and mechanistic insights into the activation of Stromal interaction molecule 1 (STIM1). *Proc. Natl. Acad. Sci. USA*. 109:5657–5662. http://dx.doi.org/10.1073/pnas.1118947109