

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.

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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.

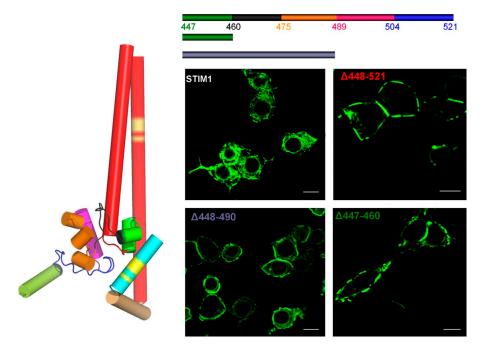


Figure S3. Localization of STIM1 deletion constructs. Shown are confocal images of STIM1 and of selective STIM1 deletion mutants expressed in HEK293 cells. All the deletion mutants make it to the ER-plasma membrane junctions in the absence of store depletion.

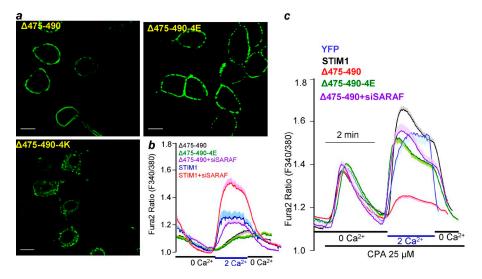


Figure S4. **Properties of STIM1**(Δ 475–490). (a) STIM1(Δ 475–490) and STIM1(Δ 475–490)^{4E/4A} cluster at the ER–plasma membrane junction in the absence of store depletion, which required intact SOAR as evident from trapping of STIM1(Δ 475–490)^{4E/4A} in an intracellular vesicular compartment. Bars, 5 µm. (b) In spite of clustering of STIM1(Δ 475–490) at the ER–plasma membrane domain it did not activate Ca²⁺ influx, even when the 4E/4A mutations were inserted into STIM1(Δ 475–490)^{4E/4A} or when SARAF was knocked down with siSARAF. (c) SOC influx in HEK293 cells is strongly inhibited by STIM1(Δ 475–490), but the inhibition is relieved by the STIM1(Δ 475–490)^{4E/4A} mutation and by siSARAF, suggesting that STIM1(Δ 475–490) inhibits SOC probably by recruiting SARAF to the native Ca²⁺ influx channels. The results in b and c are given as mean ± SEM of 30–50 cells.

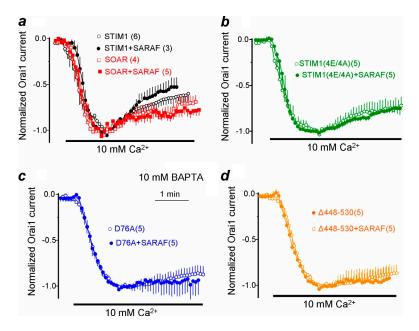


Figure S5. **BAPTA inhibits residual SCDI.** Orail current was measured in pipette solution containing 10 mM BAPTA in HEK cells expressing Orail with and without SARAF and STIM1 or SOAR (a), STIM1^{4E/4A} (b), STIM1^{D76A} (c), or STIM1(Δ 448–530) (d). The results are plotted as mean ± SEM of the number of experiments listed in parentheses.

	Table S1.	Parameters	of FCDI and SCDI
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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	STIM 1	76	4.0	STIM 1	76	4.0	94*	6.0
∆490–521	17.5	2.5	73	8	Δ447–460	96	10	Δ447–460	96	10	100	
∆447–460	25	7.0	NA	NA	∆490–521	68	7.0	∆490–521	68	7.0	70	7.7
∆448–521	10.3	0.07	NA	NA	∆448–521	49	6.0	∆448–521	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	∆448–530	32	9.0	∆448–530	32	9.0	28	5.0
					∆448–490	53	6.9	∆448–490	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