Supplemental Materials

Molecular Biology of the Cell

Garcia-Alvarez et al.

Supplementary Figure Legends

Figure S1.Gisela Garcia-Alvarez et al.,

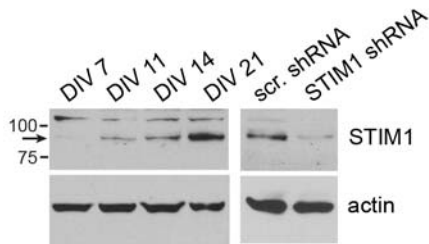


Figure S1. Expression and RNAi-mediated silencing of STIM1 in hippocampal neurons (DIV, *days in vitro*). The arrow points to the expected molecular weight of STIM1. The STIM1 Ab occasionally lights up a band of higher molecular weight.

Figure S2. Gisela Garcia-Alvarez et al.,

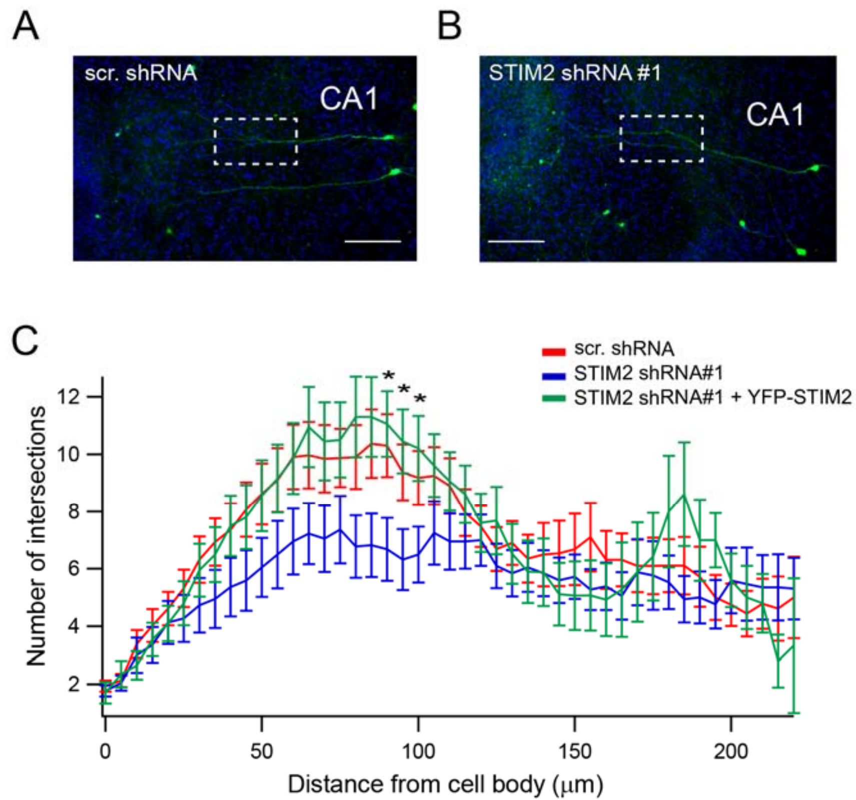


Figure S2. (A, B) Low power confocal images of CA1 pyramidal neurons in organotypic slices (DIV 14-16) biolistically transfected with scr. (A) or STIM2 (B) shRNAs vectors co-expressing GFP. Primary and secondary apical dendrites (white box) were selected for high-resolution dendritic spine analysis. Nuclei are stained with Hoechst (blue). Scale bar: 50 μm. (C) Sholl analysis of the apical dendrites of CA1 pyramidal neurons expressingscr. shRNA ($n = 30$), STIM2 shRNA#1 ($n = 22$) or STIM2 shRNA#1 with YFP-STIM2 ($n = 20$). The y axis corresponds to the number of apical dendrite intersections with concentric circles centered at the centroid of the cell body. STIM2-silenced cells show a significant decrease in the number of intersections between 50 and 100 μmfrom the soma. *, $p < 0.05$, unpaired t-test.

Figure S3. Gisela Garcia-Alvarez et al.,

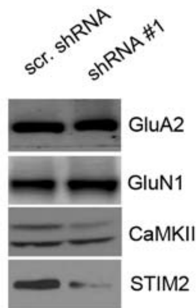


Figure S3. Immunoblot analysis of GluA2, GluN1, CamKII and STIM2 from lysates of hippocampal neurons (DIV 21) transfected with the indicated shRNAs.

Figure S4. Gisela Garcia-Alvarez et al.,

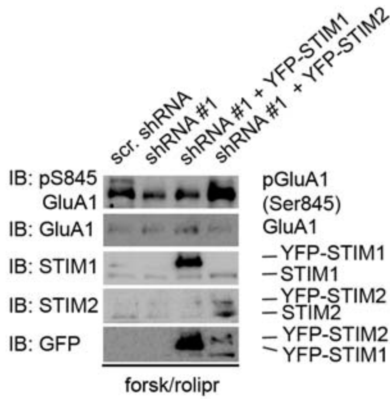


Figure S4. Immunoblot analysis of GluA1 pSer845 in hippocampal neurons (DIV 21) transduced with scr. or STIM2 shRNAs or co-transduced with STIM2 shRNA and YFP-STIM1 or YFP-STIM2. Cells were treated with 50 μM forskolin/ 0.1 μM rolipram for 30 min before they were harvested.

Figure S5. Gisela Garcia-Alvarez et al.,

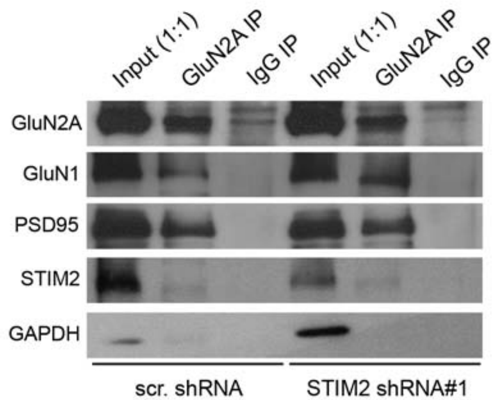


Figure S5. Co-IPs of cortical neurons (DIV 21) transduced with the indicated shRNAs. IPs were performed with a NR2A Ab or control IgG and fractions were immunoblotted with the indicated Abs. The ratio indicated in the input lane reflects the fraction of input loaded relative to the IP fraction. Note that co-IP of PSD95 is not affected by STIM2 silencing.

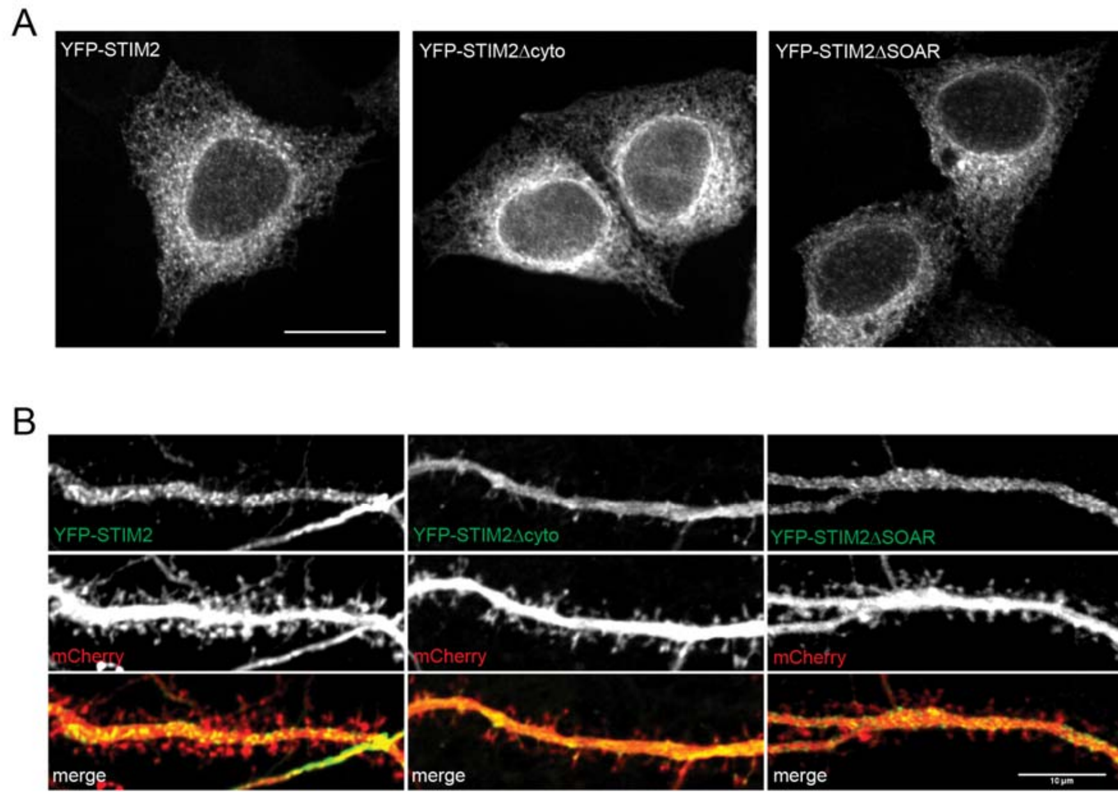


Figure S6. (A)Expression of YFP-STIM2, YFP-STIM2Δcyto and YFP-STIM2ΔSOAR in Hela cells. All three STIM2 variants are localized in the ER. (B) Hippocampal neurons (DIV 21) co-expressing mCherry and the indicated YFP-STIM2 variants. Scale bar: 10 μm. Both YFP-STIM2 and YFP-STIM2ΔSOAR are localized to puncta, while YFP-STIM2Δcyto is more evenly distributed. Spine size and density is reduced in neurons expressing YFP-STIM2Δcyto and YFP-STIM2ΔSOAR.

Figure S7.Gisela Garcia-Alvarez et al.,

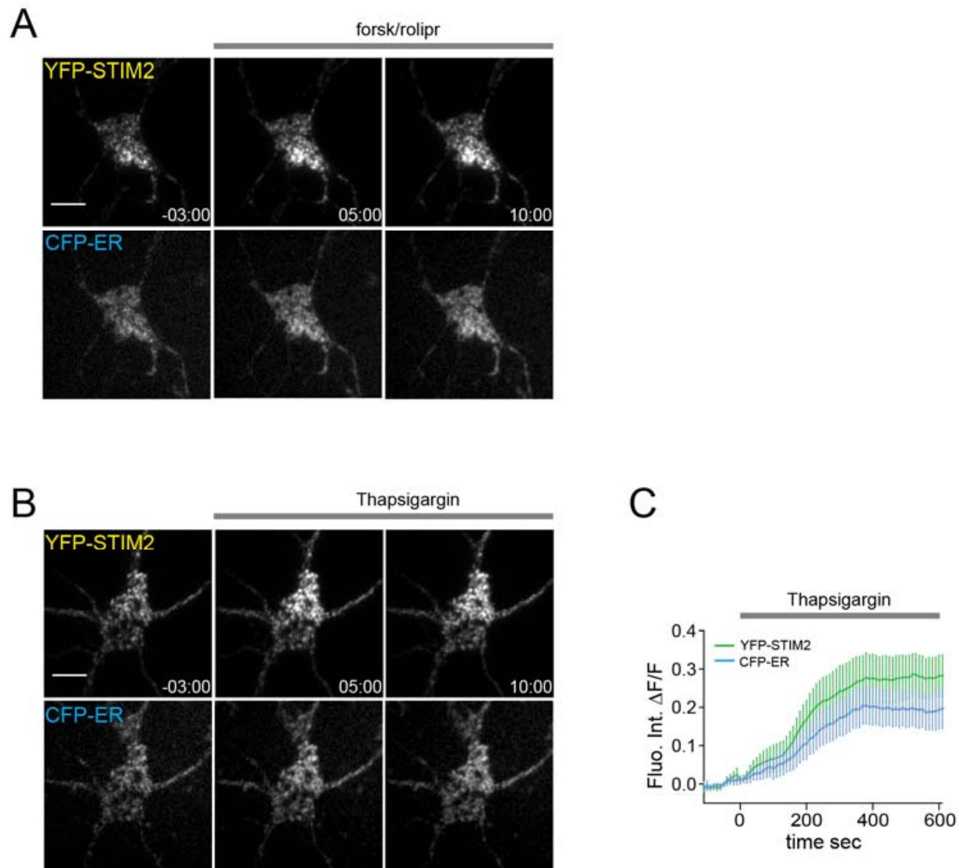


Figure S7. cAMP and thapsigargin trigger translocation of STIM2 to puncta near the PM and bulk movement of the ER towards the PM.(A, B) TIRF imaging of hippocampal neurons (DIV 6-8) electroporated with YFP-STIM2 and CFP-ER. Both forsk/rolipr treatment (A) and store depletion by thapsigargin (B) trigger translocation of STIM2 into puncta near the PM. Both stimulations also induce an increase in CFP-ER fluorescence but to a smaller degree. (C) Quantification of thapsigargin-induced increase in fluorescence intensity in the cell soma for YFP-STIM2 (green, n = 8) and CFP-ER (blue, n = 8).

Figure S8.Gisela Garcia-Alvarez et al.,

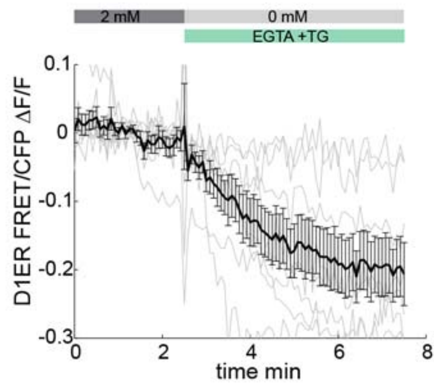
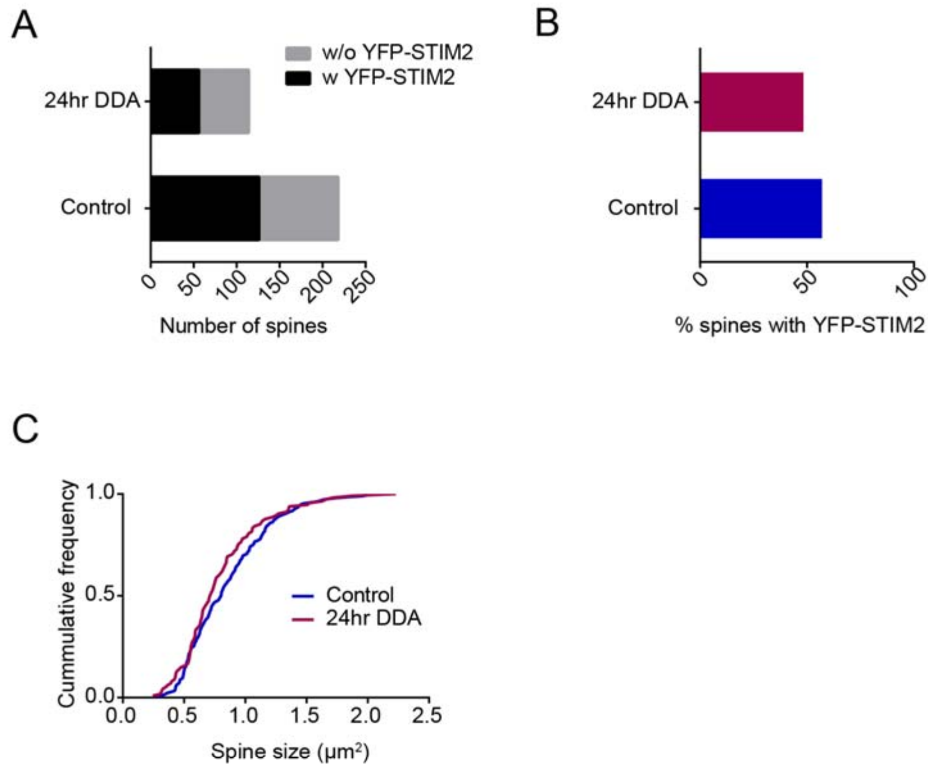


Figure S8. Thapsigargin treatment is effective in reducing neuronal ER Ca^{2+}. Quantification of ER Ca^{2+}level in hippocampal neurons (DIV 14) electroporated with D1ER, a FRET-based ER Ca^{2+}sensor, upon thapsigargin treatment. The 2 mM extracellular Ca^{2+} was simultaneously removed as done in Ca^{2+} addback assay.

Figure for Reviewer 3 (point #3).



Hippocampal neurons (DIV22) electroporated with YFP-STIM2 and mCherry were treated with 2',5'-dideoxyadenosine (DDA) for 24 hrs before fixation and spine analysis. Results shown were from one experiment. (A) Number of spines with and without YFP-STIM2 in control and DDA-treated neurons. Total number of spines detectable was markedly reduced in DDA-treated neurons (n = 114 spines) than in control neurons (n = 218 spines). (B) Percentage of spines containing YFP-STIM2 decreased from 57.8 % in control neurons to 49.1 % in DDA-treated neurons. (C) Cummulative distribution revealed a decrease in spine size in DDA-treated neurons compared to in control neurons.

Movie legends

Figure 4. mov 1 and 2. FRET imaging of PKA activity in control and STIM2-silenced cells.Time-lapse AKAR3EV FRET imaging of hippocampal neurons (DIV21) co-expressing AKAR3EV, mCherry and scramble (movie 1) or STIM2 (movie 2) shRNAs. A dendritic segment is shown. AKAR3EV FRET is displayed as the FRET/CFP ratio, and is pseudocolored. Forsk/rolipr was added at ~0.5 sec into the movie. Movie played at 20 f/sec and sped up 600x.

Figure 6. mov 3-6. cAMP-induced redistribution of STIM2 and GluA1 in hippocampal neurons.Movie 3: TIRF imaging of YFP-STIM2 in the soma and proximal dendrites of a hippocampal neuron (DIV 7) following forsk/rolipr treatment. Movies 4 and 5: Dual-color TIRF imaging of mCherry-STIM2 (movie 4) and SEP-GluA1 (movie 5) in a hippocampal neuron (DIV7) following forsk/rolipr treatment. Movie 6: Dual color confocal imaging of YFP-STIM2 (green) and mCherry (magenta) upon forsk/rolipr addition, in a dendritic segment of a DIV 19 neuron. Frame at which forsk/rolipr is added is marked in all movies. Time is in min and sec. Scale bar: 5 μm.

Figure 8. mov 7-10. STIM2 is required for PKA-dependent surface delivery of SEP-GluA1 and spine enlargement.Dual color imaging of SEP-GluA1 (movies 7 and 9) and mCherry (movies 8 and 10) in hippocampal neurons (DIV 18) co-expressing scrambled (movies 7 and 8) or STIM2 (movies 9 and 10) shRNAs. Time at which forsk/rolipr is added is marked. Time is in min and sec. SEP-GluA1 intensity is color coded, according to the scale shown in Figure 7A. Scale bar, 5 μm.