Chen et al., https://doi.org/10.1083/jcb.201606047

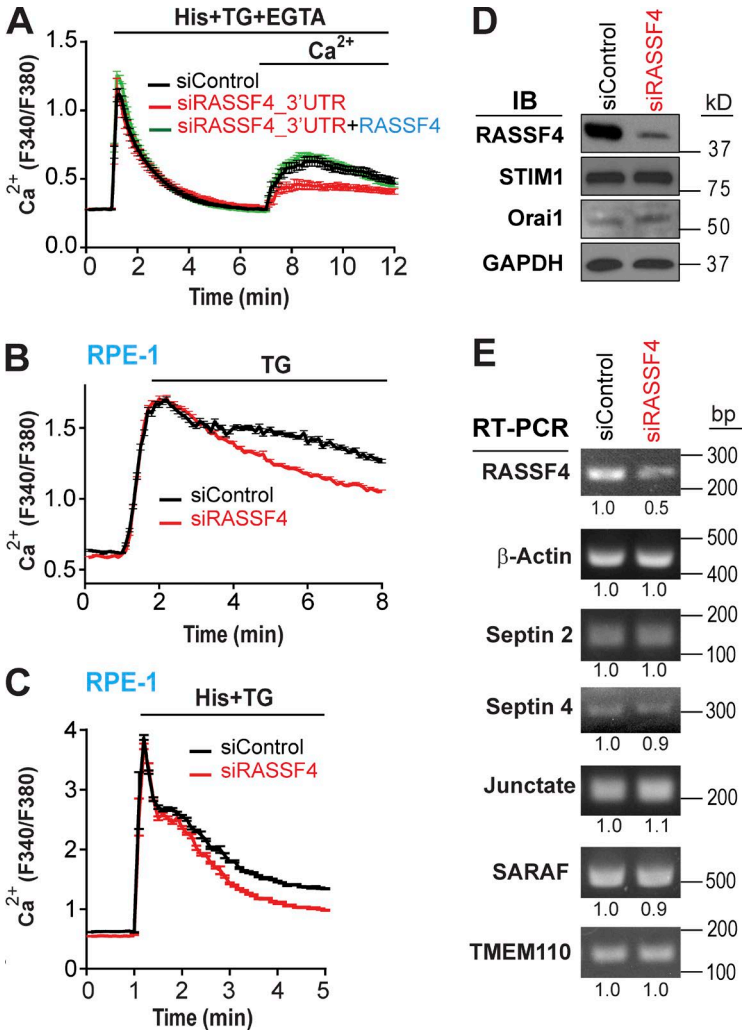


Figure S1. **RASSF4 regulates SOCE.** (A) Fura-2 ratios of HeLa cells treated with siControl and a control vector, siRASSF4_3′UTR (3′ untranslated region) and a control vector, or siRASSF4_3′UTR and RASSF4-YFP. Cells were stimulated as described in Fig. 1 C. Shown are mean Fura-2 ratios ± SEM derived from >1,000 cells for each condition across three independent experiments. (B) Fura-2 ratios of RPE-1 cells treated with siControl and with siRASSF4 and stimulated by 1 µM TG. Shown are mean Fura-2 ratios ± SEM derived from >1,000 cells for each condition across two independent experiments. (C) Fura-2 ratios of RPE-1 cells treated with siControl and with siRASSF4 and stimulated by 1 µM TG and 100 µM histamine (His). Shown are mean Fura-2 ratios ± SEM derived from >1,000 cells for each condition across two independent experiments. (D) Immunoblot (IB) analyses of the levels of the indicated proteins in HeLa cells treated with siControl or siRASSF4. (E) RT-PCR analyses of the expression levels of the indicated genes in HeLa cells treated with siControl or siRASSF4.

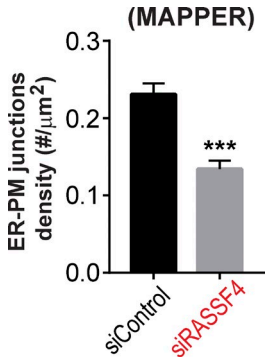


Figure S2. **RASSF4 regulates the formation of ER–PM junctions.** Densities of ER–PM junctions determined using TIRFM imaging of HeLa cells transfected with MAPPER. At least 30 cells treated with siRASSF4 or siControl for each condition from three independent experiments were analyzed. Means ± SEM are plotted. ***, P < 0.001.

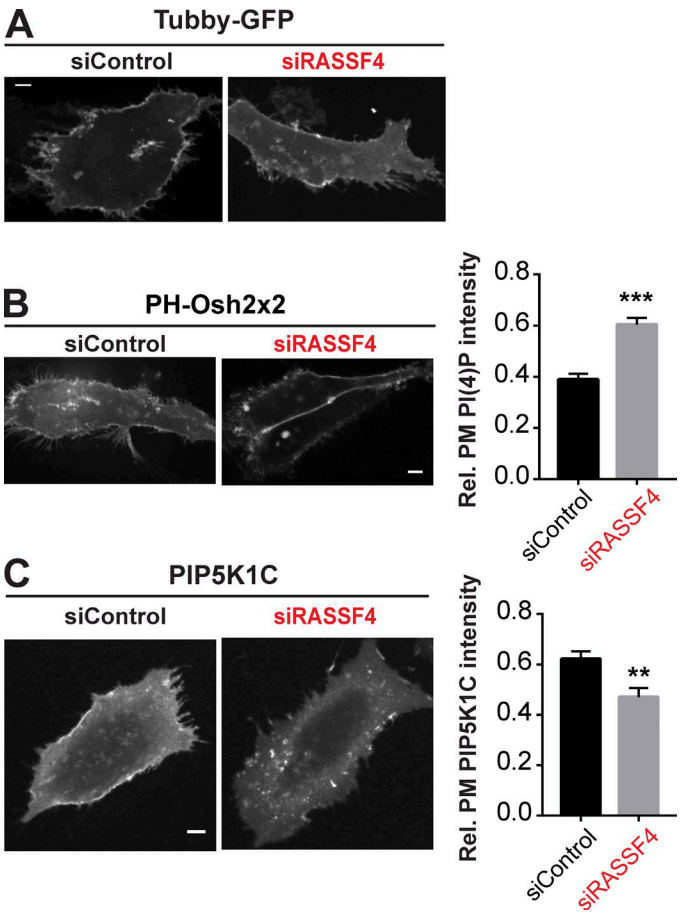


Figure S3. **RASSF4 regulates PM PI(4,5)P_2, PI(4)P, and PIP5K levels.** (A) Confocal images of HeLa cells cotransfected with Tubby-GFP and either siRASSF4 or siControl. (B) Confocal images of HeLa cells expressing GFP-PH-Osh2×2 and treated with either siRASSF4 or siControl. Relative (Rel.) GFP-PH-Osh2×2 fluorescence intensity in the PM from >60 cells across four independent experiments was evaluated. Means ± SEM are shown. (C) Confocal images of HeLa cells expressing YFP-PIP5K1C and treated with either siRASSF4 or siControl. Relative YFP-PIP5K1C fluorescence intensity in the PM from >30 cells across two independent experiments was evaluated. Means ± SEM are shown. **, P < 0.01; ***, P < 0.001. Bars, 5 µm.

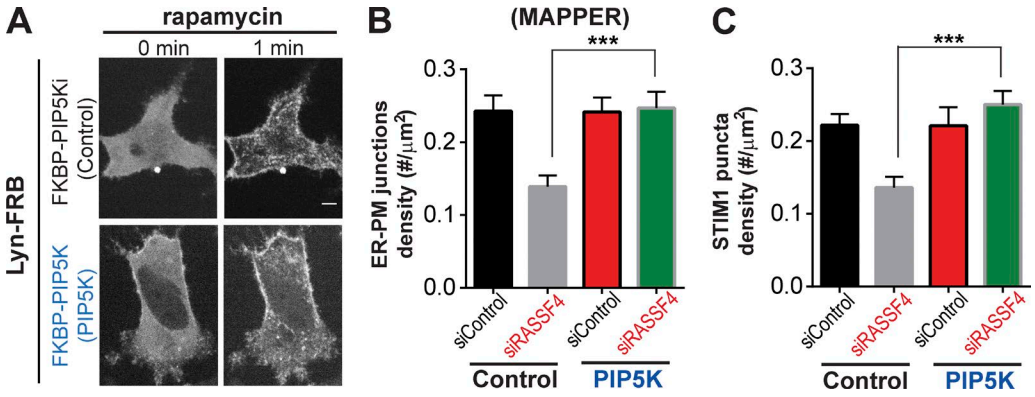


Figure S4. **Reconstitution of ER–PM junctions and STIM1 translocation in RASSF4-knockdown cells by targeting of PIP5K to the PM.** (A) GFP-FKBP-PIP5K or GFP-FKBP-PIP5Ki (the kinase-dead mutant) translocation to the PM induced by 5 µM rapamycin and monitored by confocal microscopy in HeLa cells coexpressing Lyn-FRB. Bar, 5 µm. (B) The density of MAPPER puncta in HeLa cells treated with the indicated siRNA and expressing MAPPER, mCherry-KRAS-tail, Lyn-FRB, and either CFP-FRBP-PIP5K or CFP-FRBP-PIP5Ki. More than 30 cells for each condition across three independent experiments were evaluated. Means ± SEM are plotted. (C) The density of STIM1 puncta in HeLa cells treated with the indicated siRNA and expressing mCherry-STIM1, Lyn-FRB, and either CFP-FRBP-PIP5K or CFP-FRBP-PIP5Ki. Cells were stimulated with 1 µM TG for 4 min. More than 20 cells for each condition across two independent experiments were evaluated. Means ± SEM are plotted. (B and C) Cells were treated with 5 µM rapamycin for 10 min after the transfection and before the experiments. ***, $P < 0.001$.

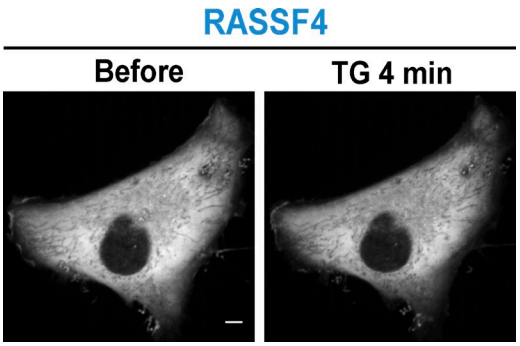
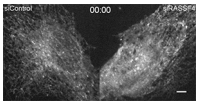


Figure S5. **RASSF4-YFP is diffusely distributed in the cytoplasm of HeLa cells.** Confocal images of a HeLa cell transfected with RASSF4-YFP. 1 µM TG was used for stimulation. Bar, 5 µm.



Video 1. **RASSF4 regulates STIM1 translocation to ER–PM junctions during SOCE.** HeLa cells cotransfected with mCherry-STIM1 and either siControl or siRASSF4 were imaged by confocal microscopy. 1 µM TG was used for stimulation. The images were taken every 10 s. The playback rate is five frames per second. Bar, 5 µm.

Table S1 is available as an Excel file and shows oligonucleotides used in this study.