Supplemental material

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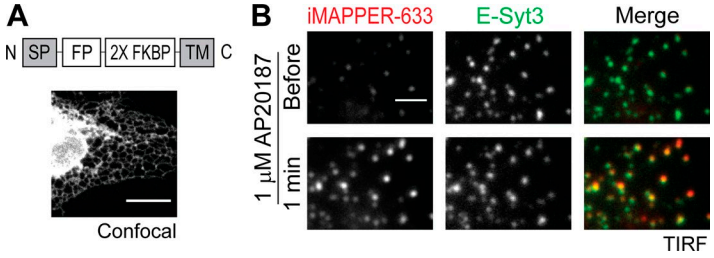


Figure S1. **Localization of 2× FKBP-TM and translocation iMAPPER-633 to ER–PM junctions. (A)** Diagram of 2× FKBP-TM construct and its ER localization as shown by confocal microscopy in HeLa cells. **(B)** Translocation of mCherry–iMAPPER-633 to ER–PM junctions after 1 µM AP20187 treatment, monitored by TIRF microscopy in HeLa cells cotransfected with GFP-E-Syt3. Bars: (A) 10 µm; (B) 2 µm.

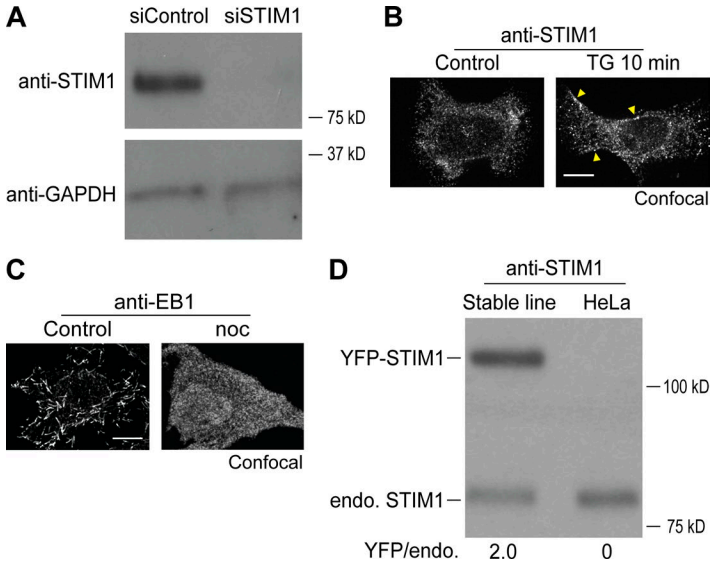


Figure S2. **Localization of endogenous STIM1 in HeLa cells and protein levels of YFP-STIM1 in HeLa cells stably expressing STIM1. (A)** Endogenous STIM1 protein detected by Western blotting using anti-STIM1 antibody in HeLa cells transfected with siControl or siSTIM1. **(B)** Localization of endogenous STIM1 in control and TG-treated HeLa cells, visualized by anti-STIM1 immunostaining using confocal microscopy. Yellow arrowheads indicate STIM1 puncta at the cell periphery, indicating STIM1 translocation to ER–PM junctions after ER Ca^{2+} store depletion. **(C)** Localization of endogenous EB1 after 10 µM nocodazole (noc) treatment for 20 min, visualized by anti-EB1 immunostaining using confocal microscopy. Bars, 10 µm. **(D)** Endogenous (endo.) STIM1 and exogenous YFP-STIM1 protein levels detected by Western blotting using anti-STIM1 antibody in HeLa cells stably expressing YFP-STIM1. The intensity of bands was measured by ImageJ. Ratio of YFP-STIM1 to endogenous STIM1 is indicated.

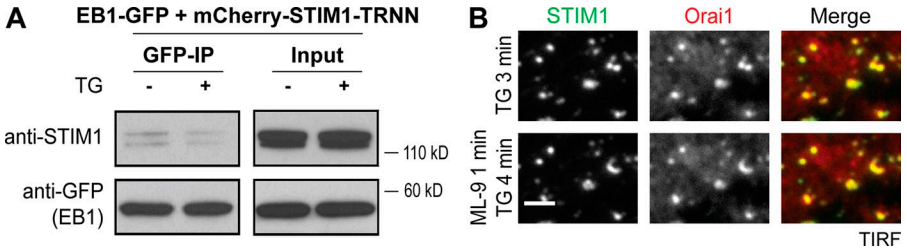


Figure S3. **Lack of interaction between STIM1-TRNN with EB1 and minimal disruption of STIM1–Orai1 complexes at ER–PM junctions after ML-9 treatment. (A)** IP of EB1-GFP with mCherry-STIM1-TRNN after 1 µM TG treatment in HeLa cells. Protein levels of EB1-GFP and mCherry-STIM1-TRNN in total cell lysates (Input) and IP were assessed by Western blotting using antibodies against GFP and STIM1. **(B)** YFP-STIM1 and Orai1-mCherry puncta remain unchanged after 100 µM ML-9 treatment, monitored by TIRF microscopy. Bar, 2 µm.

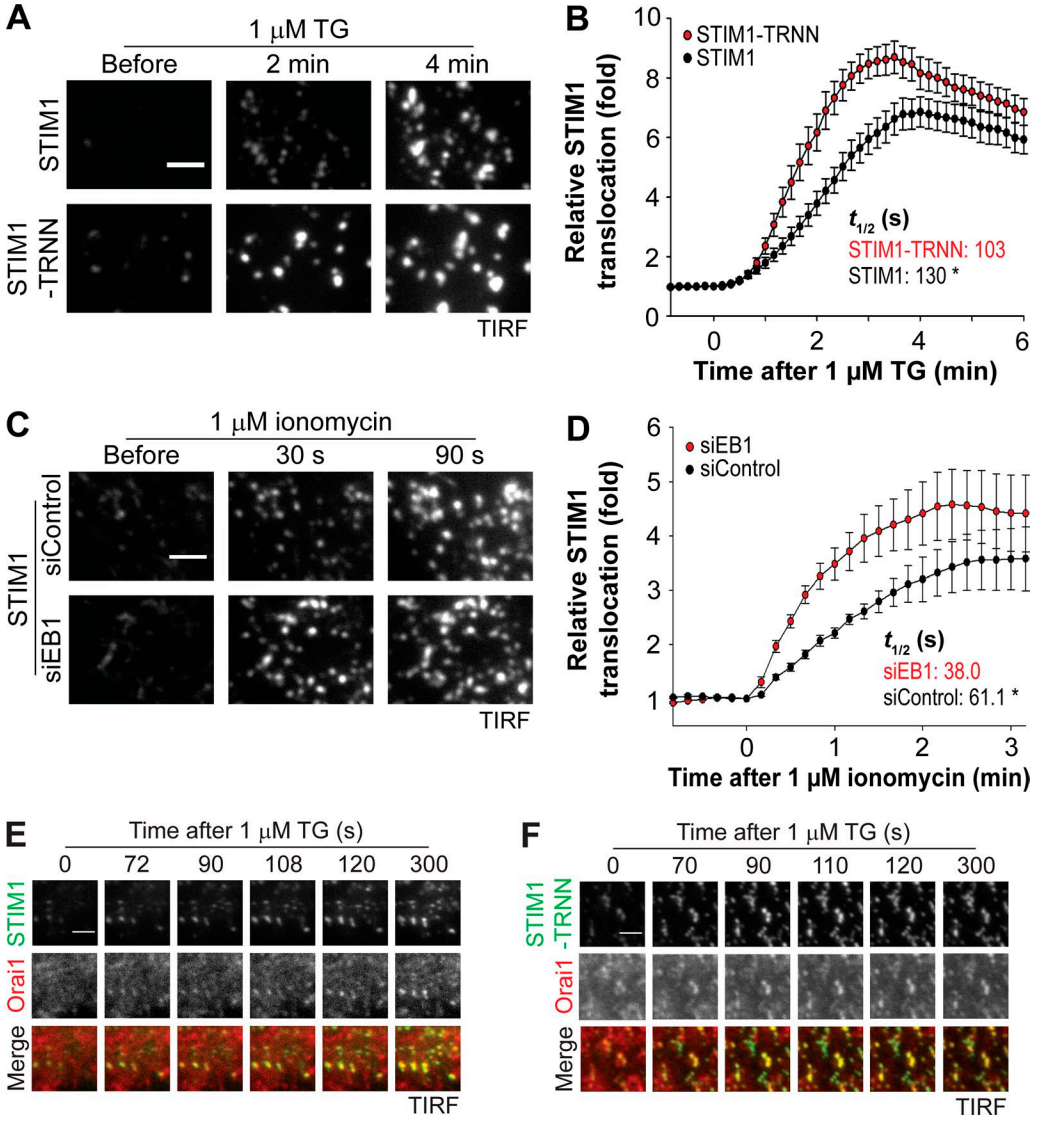
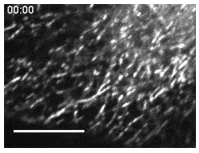
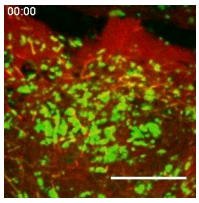


Figure S4. **EB1 binding impedes STIM1 translocation to ER–PM junctions during ER Ca^{2+} depletion. (A)** Translocation of YFP-STIM1 and YFP-STIM1-TRNN to ER–PM junctions after 1 µM TG treatment in HeLa cells, monitored by TIRF microscopy. **(B)** Relative translocation to ER–PM junctions of YFP-STIM1 and YFP-STIM1-TRNN as described in A. 19–22 cells from four independent experiments. Mean times to the half-maximal translocation ($t_{1/2}$) are indicated. **(C)** Translocation of YFP-STIM1 to ER–PM junctions after 1 µM ionomycin treatment in HeLa cells transfected with siControl or siEB1, monitored by TIRF microscopy. **(D)** Relative translocation to ER–PM junctions of YFP-STIM1 as described in C. Means ± SEM are shown. 9–10 cells from three independent experiments. $t_{1/2}$ values are indicated. *, $P < 0.05$. **(E and F)** Translocation to ER–PM junctions of YFP-STIM1 (E) and YFP-STIM1-TRNN (F) and corresponding Orai1-mCherry after 1 µM TG treatment in HeLa cells, monitored by TIRF microscopy. Representative images are shown. Bars, 2 µm.



Video 1. **iMAPPER-633 tracks MT plus ends.** iMAPPER-633 displayed MT plus end–tracking movements toward the cell periphery, monitored by confocal microscopy in a HeLa cell transfected with YFP–iMAPPER-633. 6 s per frame; 36× playback speed. Bar, 10 µm.



Video 2. **Activated STIM1 retains EB1 binding ability.** The activated mutant of YFP-STIM1-D76A (green) trapped by EB1-mCherry (red) after 100 µM ML-9 treatment in a HeLa cell, monitored by confocal microscopy. 10 s per frame; 60× playback speed. Bar, 10 µm.

Table S1. **Oligonucleotides used in this study**

Name	Sequence (5′–3′)
2× FKBP F	ATCGACTAGTGGAGCAGGTGCTCTCGAG (SpeI)
2× FKBP R	ATCGAAGCTTTGCACTGCCTCCAGCTGA (HindIII)
MAPPER TM F	ATCGAAGCTTCTGGATACAGTGCTCTTTGG (HindIII)
MAPPER cytosol R	ATCGCAATTGCCATTAGAATTGCTCTAGCAGC (MfeI)
STIM1 633 F	CTAGGAATTCCCAGCCGAGCCCTGCAAGCCAG (EcoRI)
CR	CCTCTACAAATGTGGTATGG (BamHI)
CT-TRNN F	GCCGAAACACACGCAATAACCACCTGGCTGGCAAGAAGGC
CT-TRNN R	GCCAGCCAGGTGGTTATTGCGTGTGTTTCGGCTGGCTTG
STIM1-2K F	CGGAAGAAGTTTCCTCTCAAAATCTTTAAGAAGCCTCTTAAGAAGGGGGCGGGGCGGAAGAAGTTTCCTCTCAAAATCTTTAAGAAG CCTCTTAAGAAGTAG
STIM1-2K R	CTACTTCTTAAGAGGCTTCTTAAAGATTTTGAGAGGAAACTTCTTCCGCCCCGCCCCCTTCTTAAGAGGCTTCTTAAAGATTTTGAG AGGAAACTTCTTCCG
EB1 F	GGACTCAGATCTCGAGATGGCAGTGAACGTATACTCAA (XhoI)
EB1 R	GGCGACCGGTGGATCCGAATACTCTTCTTGCTCCTCCTG (BamHI)
siEB1 F	GCGTAATACGACTCACTATAGGCGAGTACATCCAGAACTTCAAAA
siEB1 R	GCGTAATACGACTCACTATAGGTCTTCTTGCTCCTCCTGTGG
siSTIM1 F	GCGTAATACGACTCACTATAGGGGTGTTCTGTCTCTCCTTC
siSTIM1 R	GCGTAATACGACTCACTATAGGGAGGCCTGAGTGAGATTAG

F, forward; R, reverse.