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Online Supplemental Material

Experimental Procedures

Cell culture, expression constructs, transfection of plasmids and siRNAs

HeLa, MRC5-SV, B16F1 and HEK293 cells were cultured as described previously [1]. The GFP-STIM1 fusion [2] was a gift of Dr. R. Lewis (Stanford University, Stanford, USA); mCherry-α-tubulin [3] was a gift of Dr. R.Tsien (UCSD, La Jolla, USA); pDsRed2-ER and pYFP-ER were purchased from Clontech. EB3-mRFP was generated by substituting the GFP-encoding part of EB3-GFP [4] for mRFP (a gift of Dr. R.Tsien). FuGENE 6 (Roche) reagent was used for plasmid transfection of HeLa and MRC5-SV cells; HEK293 cells were transfected with Lipofectamine 2000 (Invitrogen). A HeLa cell line stably expressing mCherry-α-tubulin was generated as described previously [5]. siRNAs were synthesized by Ambion; they were directed against the following target sequences: EB1#1 AUUCCAAGCUAAGCUAGAA and EB1#2 UUCGUUCAGUGGUUCAAGA [6]; STIM1#1 GGCUCUGGAUACAGUGCUC [7] ; STIM1#2 GGGAAGACCUCAAUUACCA [8]. We used two control siRNAs, with the sequences GCACUCAUUAUGACUCCAU [5] and CGUACGCGGAAUACUUCGA (luciferase GL2, Qiagen); both gave the same phenotypes in the assays described in this study. Synthetic siRNAs were transfected using HiPerFect (Qiagen) at a concentration 5 nM. Cells were analyzed by different methods 3 days after siRNA transfection and 1-3 days after plasmid transfection.

GST pull down assays and mass spectrometry

GST fusions of EB1, EB2, EB3, EB1-N and EB1-C, expression and purification of the GST tagged proteins from *E. coli* and Western blotting were performed as described by [9]. Purified untagged EB1 was prepared as described previously [10]. Lysates of untransfected B16F1 cells or transfected HEK293 cells were prepared in a buffer containing 20 mM Tris-HCl pH 7.5, 100 mM NaCl, 1% Triton X-100, 1mM DTT and protease inhibitors (Complete, Roche). Cell lysates were centrifuged at 16 000 g for 15 min at 4 °C and the supernatant was incubated with individual GST fusion proteins for 2 hrs at 4°C. Beads were washed four times with a buffer containing 20 mM Tris-HCL pH 8, 150 mM NaCl, 0.05% Triton X-100 and 1mM DTT. The proteins retained on the beads were analyzed by Western blotting or mass spectrometry .

Antibodies, immunofluorescent staining and immunoprecipitation

We used rabbit polyclonal antibodies against GFP (Abcam) and EB1 [5], mouse monoclonal antibodies against EB1, p150^{Glued} and GM130 (BD Biosciences) and STIM1 (Abnova) and rat monoclonal antibodies against EB1 (clone KT51, Absea) and α-tubulin (Abcam). Secondary Alexa 594-conjugated goat antibodies against rat and mouse IgG were purchased from Molecular Probes. Cells were fixed with –20°C methanol fixation for 15 min, post-fixed in 4% paraformaldehyde in PBS for 15 min at room temperature and rinsed with 1% Triton X-100 in PBS; subsequent washing and staining steps were carried out in PBS supplemented with 1% bovine serum albumin and 0.15% Tween-20. Immunoprecipitation of the endogenous EB1 was performed using rabbit polyclonal antibodies as described previously [5].

Image acquisition and processing

Images of fixed cells were collected with a Leica DMRBE microscope equipped with a PL Fluotar 100x 1.3 N.A. oil objective, FITC/EGFP filter 41012 (Chroma) and Texas Red filter 41004 (Chroma) and an ORCA-ER-1394 CCD camera (Hamamatsu). 12-bit images were projected onto the CCD chip at a magnification of 0.1 μm/pixel. Images of fixed samples were prepared using Adobe Photoshop by converting them to 8 bit and linear adjustment of "Levels"; no image filtering was performed.

Simultaneous dual color (green and red) time-lapse live cell imaging was performed on the inverted research microscope Nikon Eclipse TE2000E (Nikon) with a CFI Apo TIRF 100x 1.49 N.A. oil objective (Nikon), equipped with QuantEM EMCCD camera (Roper Scientific) controlled by MetaMorph 7.1 software (Molecular Devices). For excitation we used HBO 103 W/2 Mercury Short Arc Lamp (Osram) and Chroma ET-GFP/mCherry filter cube. For separation of emissions we used DualView (Optical Insight) with emitters HQ530/30M and HQ630/50M (Chroma) and the beam splitter 565DCXR (Chroma). The same setup was used for dual color Total Internal Reflection Fluorescence microscopy (TIRFM); for excitation we used simultaneously 113 mW 488nm laser line of argon laser (Spectra-Physics Lasers) and 11 mW 561nm diode-pumped solid-state laser (Melles Griot) and Chroma ET-GFP/mCherry filter cube. The 16-bit images were projected onto the CCD chip at a magnification of 0.067 μm/pixel with intermediate magnification 2.5X. FRAP assay was performed on the same microscope using the FRAP scanning head

FRAP L5 D – CURIE (Curie Institute) and 113 mW 488nm laser line of argon laser (Spectra-Physics Lasers). During imaging cells were maintained at 37°C in the standard culture medium in a closed chamber. All live images with the exception of those used for the analysis of ER extension events in HeLa cells (Fig.4D,E, Fig.S5; Video 9) were acquired with wide field fluorescence microscopy. To improve contrast of dual color images of HeLa cells which are rather thick and have a dense microtubule system, we used TIRFM setup in a semi-TIRF mode which allowed optimal visualization of the ~0.5-1μm-thick part of the cell proximal to the coveslip. Images of live cells were adjusted using MetaMorph and Adobe Photoshop software as described in Online Supplemental Material. Image analysis was performed using MetaMorph software.

Intracellular Ca^{2+} Measurements

Intracellular Ca^{2+} concentration was measured in individual cells using a microscope-based imaging system as previously described [11, 12]. Briefly, cells were loaded with the Ca^{2+} indicator dye Fura-5F/AM, and fluorescence emission intensity at 510 nm was measured when cells were sequentially excited at 340 nm and 380 nm. Data representing relative intracellular Ca^{2+} concentrations are reported as 340/380 ratios.

Mass spectrometry analysis

For mass spectrometry analysis, proteins bound to the beads were separated on a 3-8% NuPAGE tris-acetate gel and stained with the Colloidal Blue staining kit (Invitrogen). Gel lanes were cut into 2-mm slices using an automatic gel slicer and subjected to in-gel reduction with dithiothreitol, alkylation with iodoacetamide and digestion with trypsin (Promega, sequencing grade), essentially as described previously [13]. Nanoflow LC-MS/MS was performed on an 1100 series capillary LC system (Agilent Technologies) coupled to an LTQ linear ion trap mass spectrometer (Thermo) operating in positive mode and equipped with a nanospray source. Peptide mixtures were trapped on a ReproSil C18 reversed phase column (Dr Maisch GmbH; column dimensions 1.5 cm × 100 μm, packed in-house) at a flow rate of 8 μl/min. Peptide separation was performed on ReproSil C18 reversed phase column (Dr Maisch GmbH; column dimensions 15 cm × 50 μm, packed in-house) using a linear gradient from 0 to 80% B (A = 0.1 M acetic acid; B = 80% (v/v) acetonitrile, 0.1 M acetic acid) in 70 min and at a constant flow rate of 200 nl/min using a splitter. The column eluent was directly sprayed into the ESI source of the mass spectrometer. Mass spectra were acquired in continuum mode; fragmentation of the

peptides was performed in data-dependent mode. Peak lists were automatically created from raw data files using the Mascot Distiller software (version 2.1; MatrixScience). The Mascot search algorithm (version 2.1, MatrixScience) was used for searching against the NCBInr database (release NCBInr_20070217; taxonomy: *Mus musculus*). The peptide tolerance was typically set to 2 Da and the fragment ion tolerance to 0.8 Da. A maximum number of 2 missed cleavages by trypsin were allowed and carbamidomethylated cysteine and oxidized methionine were set as fixed and variable modifications, respectively. The Mascot score cut-off value for a positive protein hit was set to 60. Individual peptide MS/MS spectra with Mowse scores below 40 were checked manually and either interpreted as valid identifications or discarded.

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Suppl. Table S1

Identification of STIM1 and STIM2 as potential EB partners by mass spectrometry in B16F1mouse melanoma cell extract.

Identified proteins	NCBI identification	Mascot score			% Coverage			Unique peptides		
		EB1	EB2	EB3	EB1	EB2	EB3	EB1	EB2	EB3
CLIP-115	gi|85662406	3143	1652	3140	45	28.3	48.9	39	24	42
CLASP 1	gi|82881262	1560	160	1027	23.8	11.7	15.7	27	5	19
CLASP 2	gi|58037445	2349	363	2133	36	12.9	32.5	34	8	31
CLIP-170	gi|23821025	1698	252	2087	23.9	8	26.6	29	5	33
Dynactin 1 (p150^{Glued})	gi|74186248	2321		1126	35.6		20.8	31		18
melanophilin	gi|87080831	488		551	19.3		23.4	7		8
STIM 1	gi|17368305	1234		614	31.7		17.7	17		9
STIM 2	gi|94374457	232		368	14.1		12.5	5		6

Only proteins that were absent from control pull downs with GST alone are included in the table. Several known EBs-binding +TIPs are included in this table for comparison. A complete list of all proteins identified in this experiment will be published elsewhere (Montenegro Gouveia et al., in preparation).

Suppl. Table S2. Parameters of ER dynamics in MRC5-SV cells.

	control		GFP-STIM1 expression	
Type of elongation	Sliding ER tubule	TACs	Sliding ER tubule	TACs
Elongation rate, μm/s	1.39 ± 0.69	0.22 ± 0.17	1.38 ± 0.76	0.22 ± 0.09
Total n in total cells	71 in 5	4 in 5	45 in 5	50 in 5
Frequency of new tubule formation events, per 100 μm^2 per 1 min	5.31 ± 1.94	0.31 ± 0.20	3.65 ± 1.41*	3.88 ± 2.60**
Ratio Sliding/TACs	17.13		0.94	

The events of *de novo* ER tubule formation were identified by observing a luminal ER marker (YFP-ER in control cells or DsREd2-ER in GFP-STIM1-expressing cells). In control cells, ER tubule sliding along pre-existing MTs was distinguished from TAC-mediated tubule formation by simultaneous imaging with MTs, which were visualized with mCherry-α-tubulin. In GFP-STIM1 expressing cells TAC-dependent tubules were distinguished from the sliding ones by the presence of a GFP-STIM1 comet at their tip (this was possible because all motile GFP-STIM1 comets correspond to the growing MT ends, and the interaction of a growing MT tip with the ER membrane induces a GFP-STIM1 comet).

Values indicate mean ± SD; values significantly different in GFP-STIM1 expressing cells compared to control cells are marked with asterisks.
* $p < 0.05$; ** $p < 0.001$; Mann-Whitney U test.

Suppl. Figure S1.

GFP-STIM1 localizes to the growing MT tips in EB1-dependent manner.

A, B. Simultaneous imaging of GFP-STIM1 (green in overlay) and EB3-mRFP (red in overlay) in a transiently transfected HeLa cell. A single frame of Video 2 is shown in **A** and projection analysis is shown in **B**. Bars, 3 μm.

C. HeLa cells were transfected with the siRNA EB1#1; two days later the cells were transfected with GFP-STIM1, cultured for one more day and used for live imaging. A single frame from Video 8 is shown on the left, projection analysis is shown on the right. Bar, 10 μm.

D. MRC5-SV cells were transfected with GFP-STIM1 and imaged before and 18s after nocodazole addition. Single frames are shown on the left, projection analysis is shown on the right. Bar, 5 μm.

E. MRC5-SV cells were transfected with GFP-STIM1 and imaged after the addition of low dosages of nocodazole or taxol. Images show superimposition of 10 successive frames. Bar, 5 μm.

Suppl. Figure S2.

GFP-STIM1 accumulates at the tips of extending ER tubules in a MT polymerization–dependent manner.

Simultaneous imaging of GFP-STIM1 (green in overlay) and mCherry-α-tubulin (red in overlay) in transiently transfected MRC5-SV cells. Successive frames are shown; time is indicated above the panels. Tips of extending/retracting ER tubules and MTs are indicated by green and red arrows, respectively. Bars, 3 μm.

Suppl. Figure S3.

Analysis of GFP-STIM1 dynamics in after Ca^{2+} store depletion.

Representative frames of simultaneous two-color videos of a HeLa cell expressing GFP-STIM1 and EB3-RFP before and 120s after the addition of 2μM thapsigargin in normal culture medium. Kymographs illustrating the changes of fluorescent intensity over time in the indicated boxed areas are shown on the right. In kymographs motile comets appear as slopes and immobile structures as vertical lines. Bars, 5 μm.

Suppl. Figure S4.

EB1 depletion or inhibition of MT dynamics with taxol have no effect on thapsigargin-induced SOCE.

Single-cell Ca^{2+} concentrations were measured in live cells plated on glass coverslips and mounted in Teflon chambers. Prior to experiments, cells were incubated in 1 μM Fura-5F/AM (Invitrogen) for 25 minutes at 37°C. Cells were then bathed in room temperature Hepes-buffered saline solution (HBSS; in mM: 120 NaCl, 5.4 KCl, 0.8 $MgCl_2$, 11 glucose, and 20 Hepes, pH 7.4) throughout the course of the experiments. Fura-5F/AM fluorescence emission at 510 nm was measured when cells were excited consecutively at 340 nm and 380 nm using a microscope-based digital fluorescence imaging system (InCyt Im2; Intracellular Imaging Inc.), and relative Ca^{2+} concentrations are reported as the ratio of fluorescence emission at the two excitation wavelengths. At the end of each experiment, Fura-5F/AM fluorescence was quenched by treating cells with 10 μM ionomycin and 20 mM $MnCl_2$ to obtain background fluorescence values; these background values were subtracted from each experimental measurement.

To monitor SOCE, HeLa cells were treated with thapsigargin (Tg; 2 μM) in nominally Ca^{2+}-free extracellular medium to deplete intracellular Ca^{2+} stores. Fifteen minutes later, extracellular Ca^{2+} was restored to 1.8 mM to reveal SOCE. Gadolinium (5 μM), which inhibits SOCE, was added was added in the continued presence of 1.8 mM extracellular Ca^{2+} at the end of each experiment to demonstrate the specificity of the SOCE response.

A. SOCE was monitored in cells pretreated for 20 min with 500 nM taxol (red trace) or in cells left untreated (control; black trace). For taxol treated cells, the drug was present throughout the course of the experiment. Each trace represents the average response of all the cells measured in a single experiment.

B. The peak SOCE responses above baseline from experiments performed as described in panel A were averaged for untreated control cells (n = 104 cells, 3 experiments) and for taxol treated cells (n = 81 cells, 3 experiments). Data are reported as mean ± SEM.

C. SOCE responses of cells treated with siRNA targeted to EB1 (red trace), STIM1 (blue trace), or control siRNA (black trace).

D. The peak SOCE responses above baseline from experiments performed as described in panel **C** were averaged for cells treated with control siRNA (n = 107 cells, 3 experiments), EB1 siRNA (n = 115 cells, 3 experiments), and STIM1 siRNA (n = 106 cells, 3

experiments). Data are reported as mean ± SEM; * indicates significant difference ($p<0.01$) compared to control siRNA based on one-way ANOVA.

Suppl. Figure S5.

Quantification of ER protrusion events and MT density after STIM1 and EB1 depletion.

HeLa cells were transfected with the indicated siRNAs; one day later cells were transfected with plasmid DNA, cultured for two more days and used for dual color imaging. The following combinations of fluorescent markers were used: mCherry-α-tubulin (stably expressed in HeLa cells) together with transiently expressed YFP-ER; transiently expressed EB3-mRFP and YFP-ER, transiently expressed EB3-mRFP and GFP-STIM1; mCherry-α-tubulin (stably expressed in HeLa cells) together with transiently expressed GFP-STIM1.

A. Total number of ER protrusion events (a sum of TAC and sliding events).

B. MT density, determined by projecting all frames of 50s-long movies, using either mCherry-α-tubulin or EB3-mRFP as markers. Since most of the MTs underwent elongation during the 50s period, the numbers of MTs visualized by the two markers are not significantly different.

Number of analysed cells: ER-MT: control, n = 20; EB1 #1, n = 20, EB1 #2, n = 15. ER-EB3: control, n = 20; STIM1 #1, n = 20, STIM1 #2, n = 20. STIM1-EB3: n = 20. STIM1-MT: control, n=10; EB1#1, n = 15, EB1 #2, n = 15. Values obtained in EB1 or STIM1 siRNA-treated cells that were significantly different from the corresponding values in cells treated with the control siRNAs are indicated by asterisks ($p<0.001$, ***; $p<0.01$, **; $p<0.05$, *; $p>0.05$, n.s., Kolmogorov-Smirnov test).

Supplemental Videos

Video 1
Dynamic behavior of GFP-STIM1 in a HeLa cell
Exposure: 1 s
Delay between frames: none
Filter set: ET-GFP Filter Set 49002 (Chroma Technology Corporation)
Image Processing:
none

Video 2
Simultaneous imaging of GFP-STIM1 and EB3-mRFP comets in a HeLa cell
Exposure: 0.5 s
Delay between frames: 2 s
Filter set:
1) ET-GFP/mCherry Filter Set 59022 (Chroma Technology Corporation)
2) DualView, beam splitter 565DCXR, emitters HQ530/30M and HQ630/50M (Roper Scientific, Inc.)
Image Processing:
MetaMorph 7.1
Blur filter (Sensitivity 1)

Video 3
Simultaneous imaging of GFP-STIM1 and DsRed2-ER in an MRC5-SV cell
Exposure: 1 s
Delay between frames: none
Filter set:
1) ET-GFP/mCherry Filter Set 59022 (Chroma Technology Corporation)
2) DualView, beam splitter 565DCXR, emitters HQ530/30M and HQ630/50M (Roper Scientific, Inc.)
Image Processing:
MetaMorph 7.1
Low Pass filter (Horizontal Size 24; Vertical Size 24)
Subtraction low pass filtered image from original one (plus Value 10000)
Blur filter (Sensitivity 1)
Equalize Light (average, by multiplication)

Video 4
Simultaneous imaging of GFP-STIM1 and EB3-mRFP comets in an MRC5-SV cell
Exposure: 1 s
Delay between frames: none
Filter set:
1) ET-GFP/mCherry Filter Set 59022 (Chroma Technology Corporation)
2) DualView, beam splitter 565DCXR, emitters HQ530/30M and HQ630/50M (Roper Scientific, Inc.)
Image Processing:
MetaMorph 7.1
Low Pass filter (Horizontal Size 24; Vertical Size 24)

Subtraction low pass filtered image from original one (plus Value 10000)
Blur filter (Sensitivity 1)
Equalize Light (average, by multiplication)

Video 5

Simultaneous imaging of GFP-STIM1 and MTs labeled with mCherry-α-tubulin in an MRC5-SV cell

Exposure: 1 s
Delay between frames: none
Filter set:
1) ET-GFP/mCherry Filter Set 59022 (Chroma Technology Corporation)
2) DualView, beam splitter 565DCXR, emitters HQ530/30M and HQ630/50M (Roper Scientific, Inc.)
Image Processing:
MetaMorph 7.1
Low Pass filter (Horizontal Size 24; Vertical Size 24)
Subtraction low pass filtered image from original one (plus Value 10000)
Blur filter (Sensitivity 1)
Equalize Light (average, by multiplication)

Video 6

Dynamics of GFP-STIM1 in a HeLa cell before and after thapsigargin addition

Note that at the moment of thapsigargin addition the image becomes temporarily unfocused.
Exposure: 1 s
Delay between frames: 2 s
Filter set: ET-GFP Filter Set 49002 (Chroma Technology Corporation)
Image Processing:
none

Video 7

Dynamics of GFP-STIM1 and EB3-mRFP in an MRC5-SV cell before and after thapsigargin addition

Note that at the moment of thapsigargin addition the image becomes temporarily unfocused.
Exposure: 1 s
Delay between frames: 2 s
Filter set:
1) ET-GFP/mCherry Filter Set 59022 (Chroma Technology Corporation)
2) DualView, beam splitter 565DCXR, emitters HQ530/30M and HQ630/50M (Roper Scientific, Inc.)
Image Processing:
MetaMorph 7.1
Low Pass filter (Horizontal Size 24; Vertical Size 24)
Subtraction low pass filtered image from original one (plus Value 10000)
Blur filter (Sensitivity 1)
Equalize Light (average, by multiplication)

Blur filter (Sensitivity 1) (only for red channel)

Video 8
Dynamics of GFP-STIM1 in a HeLa cell after EB1 knockdown
Exposure: 1 s
Delay between frames: none
Filter set: ET-GFP Filter Set 49002 (Chroma Technology Corporation)
Image Processing:
none

Video 9
Dynamics of YFP-ER and EB3-mRFP in HeLa cells, visualized by using semi-TIRFM imaging
Colocalization of a protruding ER tubule and an EB3-mRFP positive MT tip (a TAC-mediated event) can be observed at the beginning of the movie in the area indicated with a black box. Several ER sliding events, which do not colocalize with growing MT tips are visible in the region indicated with a grey box.
Exposure: 500 ms
Delay between frames: 500 ms
Filter set:
1) ET-GFP/mCherry Filter Set 59022 (Chroma Technology Corporation)
2) DualView, beam splitter 565DCXR, emitters HQ530/30M and HQ630/50M (Roper Scientific, Inc.)
Image Processing:
MetaMorph 7.1
Blur filter (Sensitivity 1)
Equalize Light (average, by multiplication)

Details of acquisition and processing for live cell images shown in Figures 2,3, S1-S3.

Figure 2A. Representative frame of a simultaneous two-color video (Video 3) of an MRC5-SV cell expressing GFP-STIM1 and DsRed2-ER.
Exposure: 1 s
Delay between frames: none
Filter set:
1) ET-GFP/mCherry Filter Set 59022 (Chroma Technology Corporation)
2) DualView, beam splitter 565DCXR, emitters HQ530/30M and HQ630/50M (Roper Scientific, Inc.)
Image Processing:
Adobe Photoshop
    Levels
    Blur filter (Sensitivity 0.3)

Figure 2B. Representative frame of a simultaneous two-color video of an MRC5-SV cell expressing GFP-STIM1 and EB3-mRFP.
Exposure: 1 s
Delay between frames: no
Filter set:
1) ET-GFP/mCherry Filter Set 59022 (Chroma Technology Corporation)
2) DualView, beam splitter 565DCXR, emitters HQ530/30M and HQ630/50M (Roper Scientific, Inc.)
Image Processing:
Adobe Photoshop
Green channel:
    Levels
    Blur filter (Sensitivity 0.3)
Red channel:
    Unsharp Mask filter (Amount 100%; Radius 4.0 pixels; Threshold 0 levels)
    Levels
    Blur filter (Sensitivity 0.3)

Figure 2C. Successive frames of a simultaneous two-color video (Video 4) of an MRC5-SV cell expressing GFP-STIM1 and EB3-RFP.
Exposure: 1 s
Delay between frames: none
Filter set:
1) ET-GFP/mCherry Filter Set 59022 (Chroma Technology Corporation)
2) DualView, beam splitter 565DCXR, emitters HQ530/30M and HQ630/50M (Roper Scientific, Inc.)
Image Processing:
MetaMorph 7.1
    Low Pass filter (Horizontal Size 24; Vertical Size 24)
    Subtraction low pass filtered image from original one (plus Value 10000)
    Blur filter (Sensitivity 1)
    Equalize Light (average, by multiplication)
Adobe Photoshop

Green channel:

Unsharp Mask filter (Amount 100%; Radius 4.0 pixels; Threshold 0 levels)
Levels
Blur filter (Sensitivity 0.3)

Red channel:

Levels
Blur filter (Sensitivity 0.3)

Figure 2D. Successive frames of a simultaneous two-color video of an MRC5-SV cell expressing GFP-STIM1 and mCherry-α-tubulin.
Exposure: 1 s
Delay between frames: none
Filter set:
1) ET-GFP/mCherry Filter Set 59022 (Chroma Technology Corporation)
2) DualView, beam splitter 565DCXR, emitters HQ530/30M and HQ630/50M (Roper Scientific, Inc.)
Image Processing:
Adobe Photoshop
Green channel:

Unsharp Mask filter (Amount 100%; Radius 4.0 pixels; Threshold 0 levels)
Blur filter (Sensitivity 0.3)
Levels

Red channel:

Unsharp Mask filter (Amount 100%; Radius 4.0 pixels; Threshold 0 levels)
Blur filter (Sensitivity 0.3)
Unsharp Mask filter (Amount 100%; Radius 4.0 pixels; Threshold 0 levels)
Blur filter (Sensitivity 0.3)
Levels

Figure 3A. Projections of 5 adjacent frames of a video of a HeLa cell expressing GFP-STIM1 before and after FRAP.
Exposure: 1 s
Delay between frames: none
Filter set: ET-GFP Filter Set 49002 (Chroma Technology Corporation)
Image Processing:
Adobe Photoshop

Unsharp Mask filter (Amount 100%; Radius 4.0 pixels; Threshold 0 levels)
Blur filter (Sensitivity 0.3)
Levels

Figure 3C. Representative frames of a simultaneous two-color video (Video 7) of an MRC5-SV cell expressing GFP-STIM1 and EB3-RFP before and 120s after addition of 2μM thapsigargin.
Exposure: 1 s
Delay between frames: 2 s
Filter set:

1) ET-GFP/mCherry Filter Set 59022 (Chroma Technology Corporation)
2) DualView, beam splitter 565DCXR, emitters HQ530/30M and HQ630/50M (Roper Scientific, Inc.)
Image Processing:
Adobe Photoshop
    Unsharp Mask filter (Amount 100%; Radius 4.0 pixels; Threshold 0 levels)
    Blur filter (Sensitivity 0.3)
    Levels

Suppl. Figure S1A. Representative frame of a simultaneous two-color video (Video 2) of a HeLa cell expressing GFP-STIM1 and EB3-mRFP.
Exposure: 0.5 s
Delay between frames: 2 s
Filter set:
1) ET-GFP/mCherry Filter Set 59022 (Chroma Technology Corporation)
2) DualView, beam splitter 565DCXR, emitters HQ530/30M and HQ630/50M (Roper Scientific, Inc.)
Image Processing:
MetaMorph 7.1
    Blur filter (Sensitivity 1)
Adobe Photoshop
    Levels

Suppl. Figure S1B. Projection of 10 adjacent frames of a simultaneous two-color video (Video 2) of a HeLa cell expressing GFP-STIM1 and EB3-RFP.
Image Processing:
MetaMorph 7.1
    Blur filter (Sensitivity 1)
Adobe Photoshop
    Levels
    Projection of 10 adjacent frames. Blending mode for each layer "Lighten", opacity 100%, fill 100%.

Suppl. Figure S1C. Left: Representative frame of a video (Video 8) of a HeLa cell expressing GFP-STIM1 transfected with siRNA for EB1.
Exposure: 1 s
Delay between frames: no
Filter set: ET-GFP Filter Set 49002 (Chroma Technology Corporation)
Image Processing:
Adobe Photoshop
    Unsharp Mask filter (Amount 100%; Radius 4.0 pixels; Threshold 0 levels)
    Blur filter (Sensitivity 0.3)
    Levels

Right: Projection of 20 frames (every 5th frame) of Video 6.
Image Processing:
Adobe Photoshop
    Unsharp Mask filter (Amount 100%; Radius 4.0 pixels; Threshold 0 levels)
    Blur filter (Sensitivity 0.3)

Levels
Projection of 20 frames (every 5th frame). Blending mode for each layer "Lighten", opacity 100%, fill 100%.

Suppl. Figure S1D. Left: Representative frames of a video of a HeLa cell expressing GFP-STIM1 before and 18 s after addition of 10 μM nocodazole.
Exposure: 1 s
Delay between frames: 2 s
Filter set: ET-GFP Filter Set 49002 (Chroma Technology Corporation)
Image Processing:
Adobe Photoshop
Unsharp Mask filter (Amount 100%; Radius 4.0 pixels; Threshold 0 levels)
Blur filter (Sensitivity 0.3)
Levels

Right: Projections of 15 frames (every 2nd frame) of a video of a HeLa cell expressing GFP-STIM1 before and 18 s after addition of 10 μM nocodazole.
Image Processing:
Adobe Photoshop
Unsharp Mask filter (Amount 100%; Radius 4.0 pixels; Threshold 0 levels)
Blur filter (Sensitivity 0.3)
Levels
Projection of 15 frames (every 2nd frame). Blending mode for frames layer "Lighten", opacity 100%, fill 100%.

Suppl. Figure S1E. Left: Projections of 10 frames (every 5th frame) of a video of a HeLa cell expressing GFP-STIM1 15 minutes after addition of 100 nM nocodazole.
Exposure: 1 s
Delay between frames: none
Filter set: ET-GFP Filter Set 49002 (Chroma Technology Corporation)
Image Processing:
Adobe Photoshop
Unsharp Mask filter (Amount 100%; Radius 4.0 pixels; Threshold 0 levels)
Blur filter (Sensitivity 0.3)
Levels
Projection of 10 frames (every 5th frame). Blending mode for each layer "Lighten", opacity 100%, fill 100%.

Right: Projections of 10 frames (every 5th frame) of a video of a HeLa cell expressing GFP-STIM1 30 minutes after addition of 100 nM taxol.
Exposure: 1 s
Delay between frames: none
Filter set: ET-GFP Filter Set 49002 (Chroma Technology Corporation)
Image Processing:
Adobe Photoshop
Unsharp Mask filter (Amount 100%; Radius 4.0 pixels; Threshold 0 levels)
Blur filter (Sensitivity 0.3)
Levels

Projection of 20 frames (every 5th frame).
Projection of 10 frames (every 5th frame). Blending mode for each layer "Lighten", opacity 100%, fill 100%.

Suppl. Figure S2. Successive frames of a simultaneous two-color video (Video 5) of a MRC5-SV cell expressing GFP-STIM1 and mCherry-α-tubulin.
Exposure: 1 s
Delay between frames: none
Filter set:
1) ET-GFP/mCherry Filter Set 59022 (Chroma Technology Corporation)
2) DualView, beam splitter 565DCXR, emitters HQ530/30M and HQ630/50M (Roper Scientific, Inc.)
Image Processing:
Adobe Photoshop
Green channel:
Unsharp Mask filter (Amount 100%; Radius 4.0 pixels; Threshold 0 levels)
Blur filter (Sensitivity 0.3)
Levels
Red channel:
Unsharp Mask filter (Amount 100%; Radius 4.0 pixels; Threshold 0 levels)
Unsharp Mask filter (Amount 100%; Radius 4.0 pixels; Threshold 0 levels)
Blur filter (Sensitivity 0.3)
Levels

Suppl. Figure S3. Representative frames of a simultaneous two-color video of a HeLa cell expressing GFP-STIM1 and EB3-RFP before and 120s after addition of 2μM thapsigargin.
Exposure: 1 s
Delay between frames: 2 s
Filter set:
1) ET-GFP/mCherry Filter Set 59022 (Chroma Technology Corporation)
2) DualView, beam splitter 565DCXR, emitters HQ530/30M and HQ630/50M (Roper Scientific, Inc.)
Image Processing:
Adobe Photoshop
Unsharp Mask filter (Amount 100%; Radius 4.0 pixels; Threshold 0 levels)
Blur filter (Sensitivity 0.3)
Levels

Supplemental Figure 1
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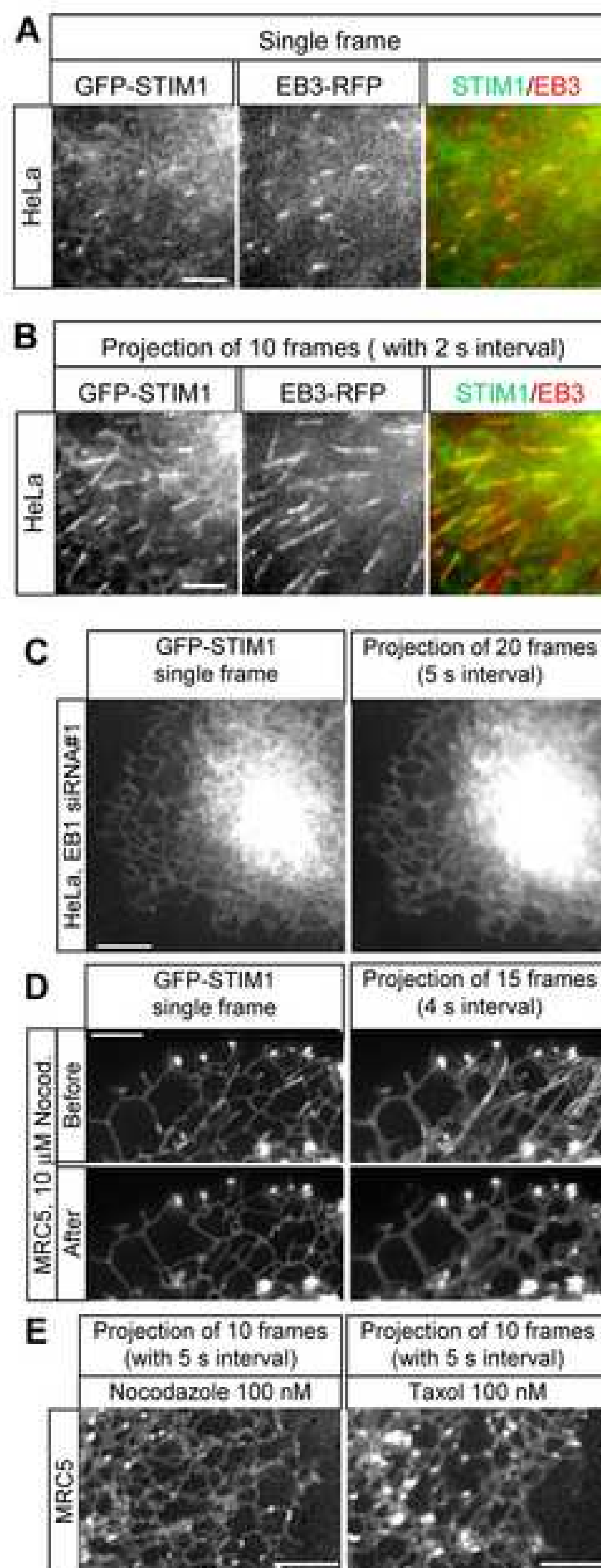

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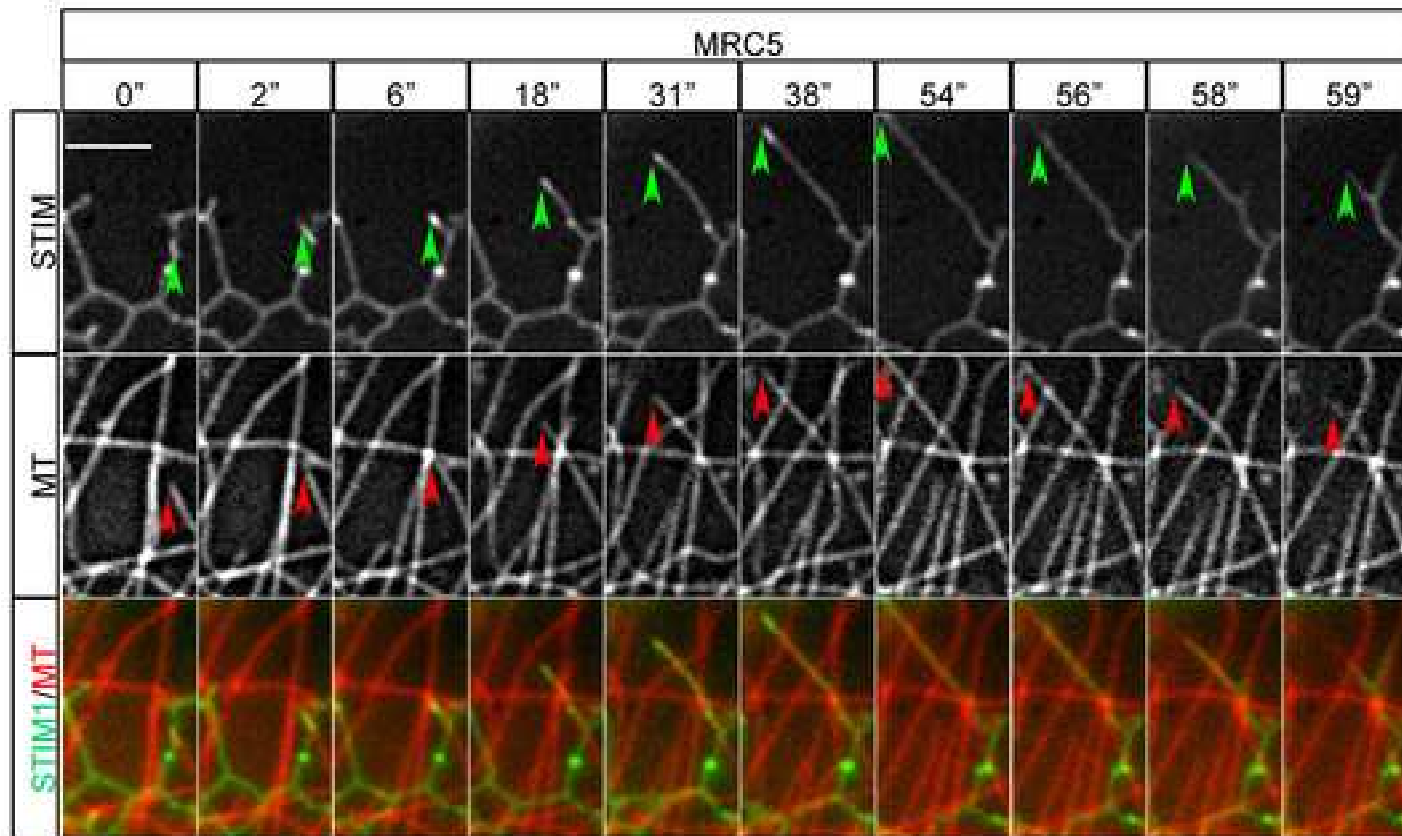

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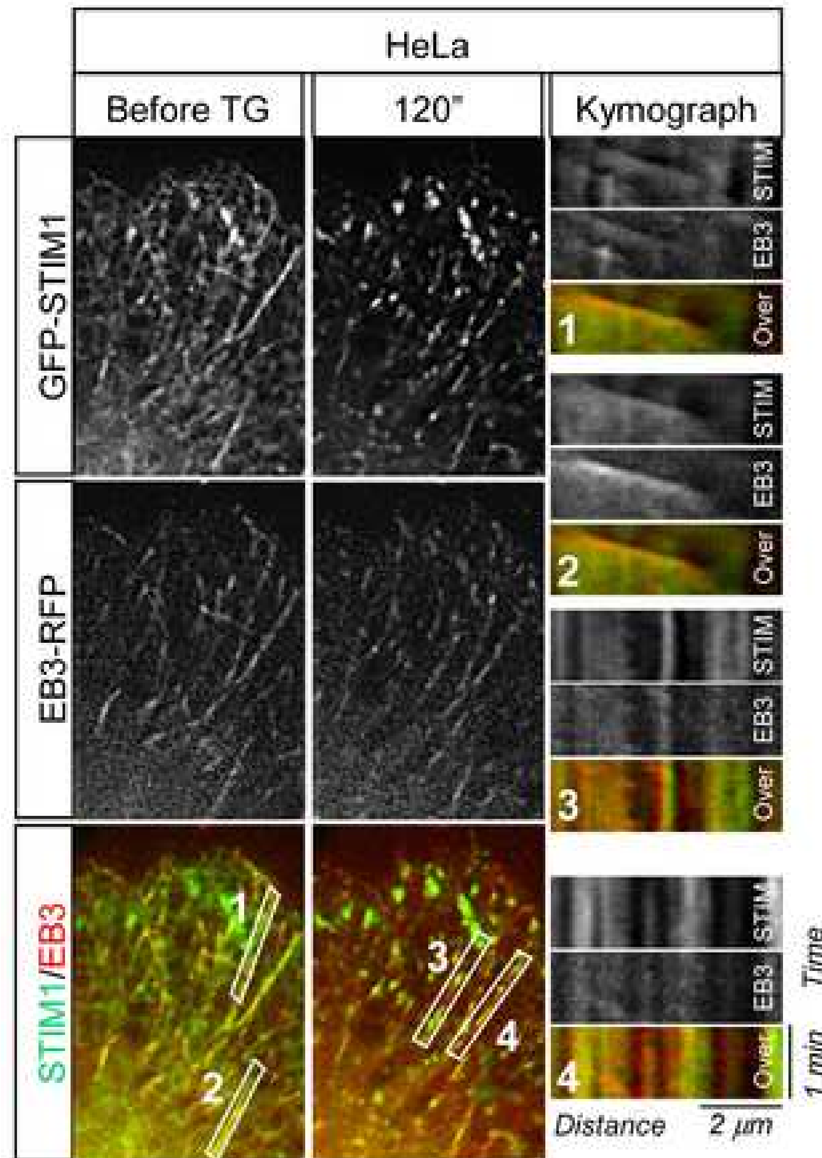

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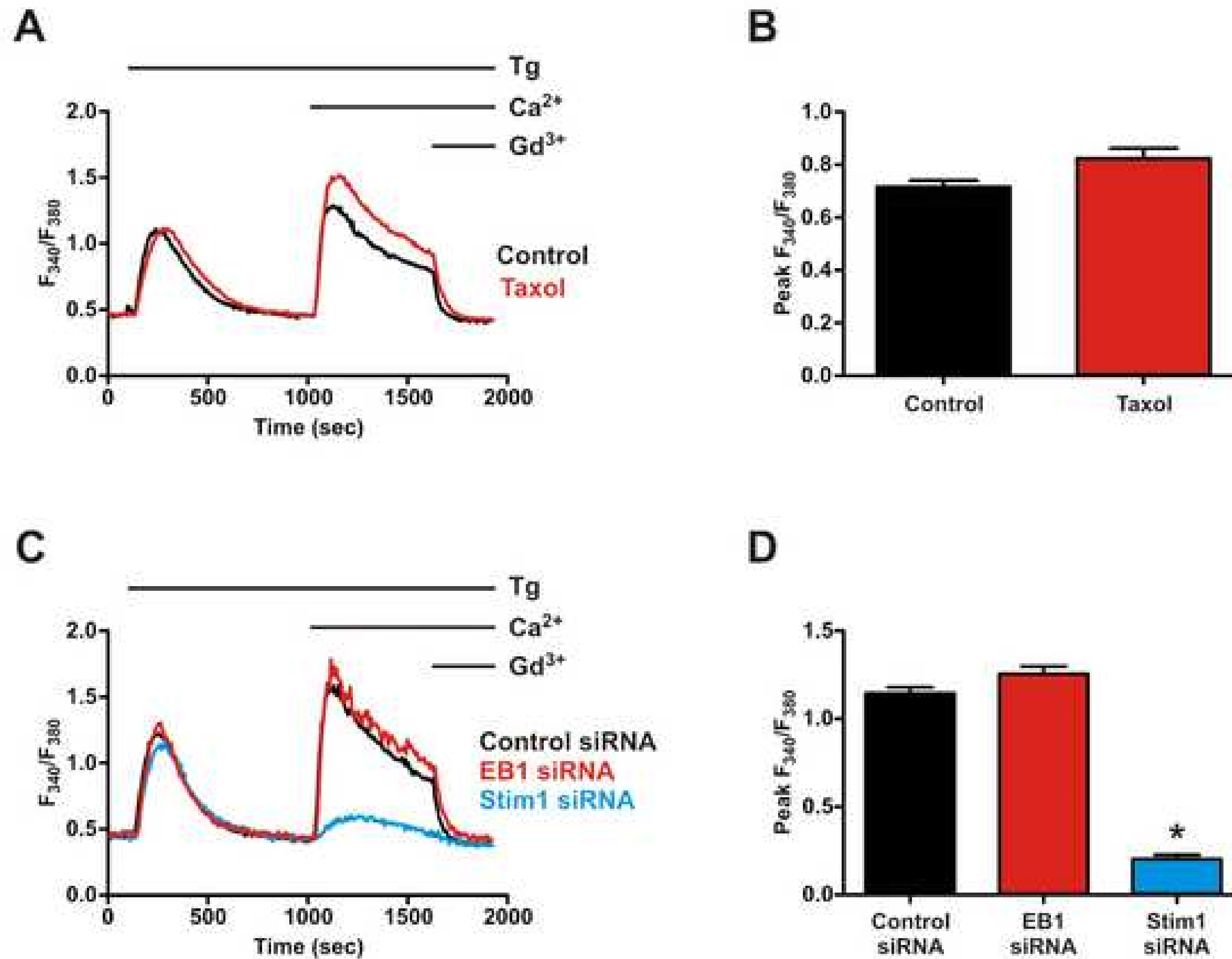

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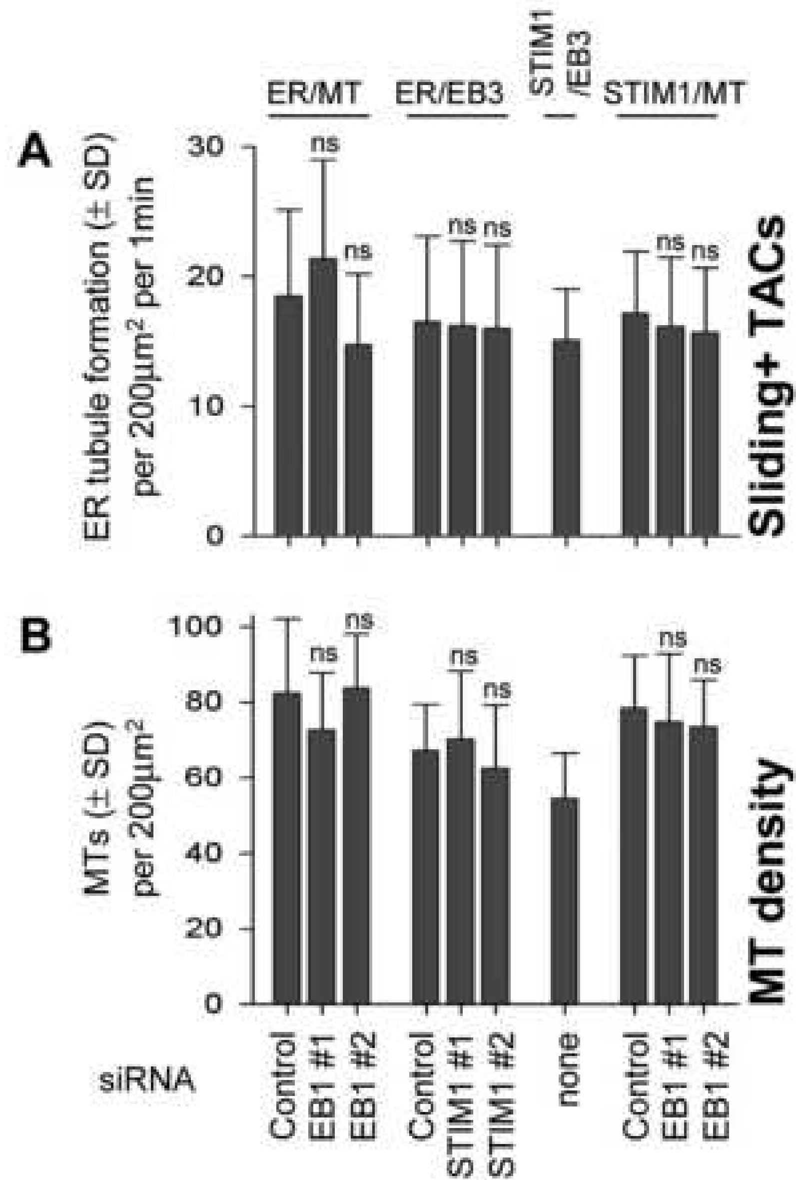

Supplemental Video 1

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