Developmental Cell, Volume *56*

Supplemental information

TAOK2 is an ER-localized kinase that catalyzes the dynamic tethering of ER to microtubules

Kimya Nourbakhsh, Amy A. Ferreccio, Matthew J. Bernard, and Smita Yadav

TAOK2 is an ER-localized Kinase that Catalyzes the Dynamic Tethering of ER to Microtubules

Kimya Nourbakhsh[1,3], Amy A. Ferreccio[1,3], Matthew J. Bernard[1] and Smita Yadav[1,2]*

[1.] Department of Pharmacology, University of Washington, Seattle, WA 98195, United States

[2.] Institute of Stem Cell and Regenerative Medicine, Seattle, WA 98109, United States

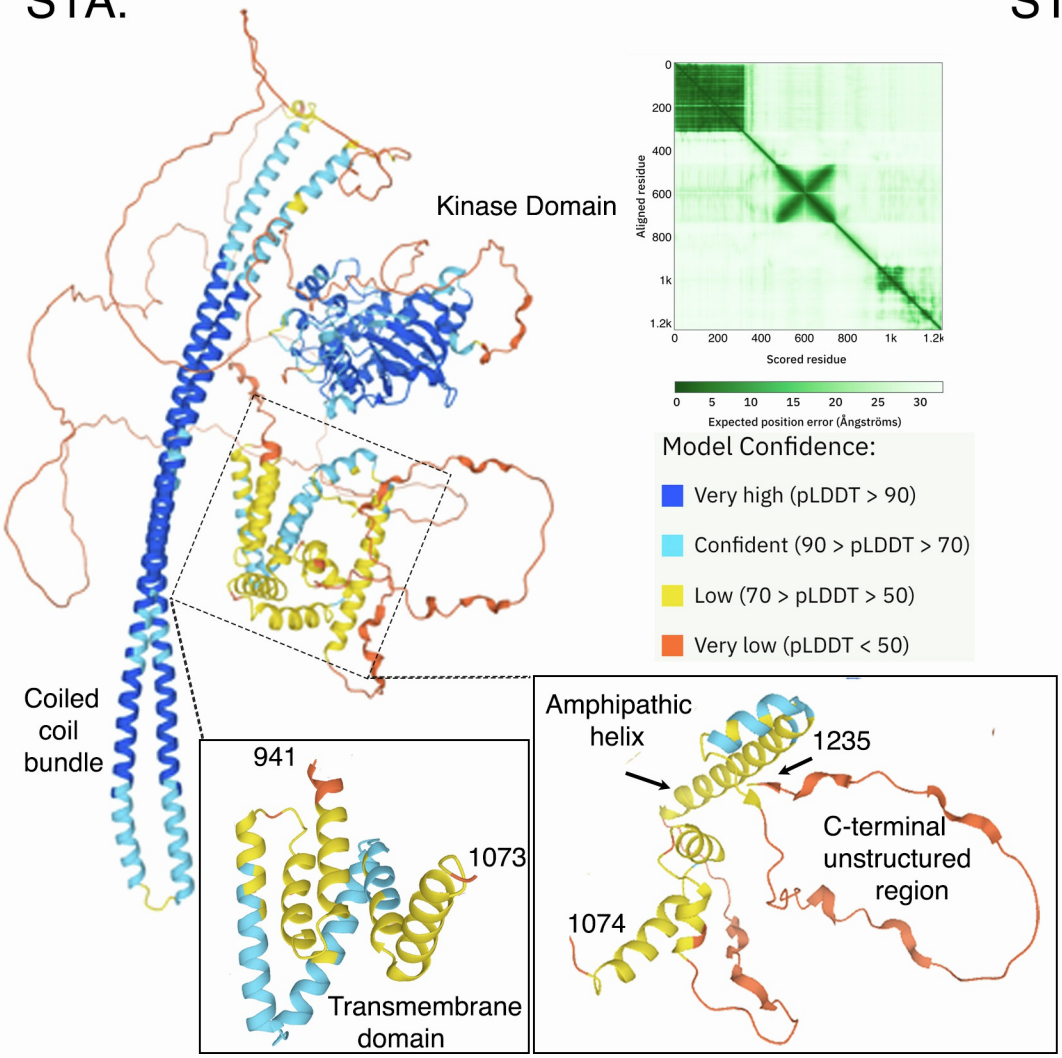
[3.] These authors contributed equally

** Corresponding author, smitay@uw.edu*

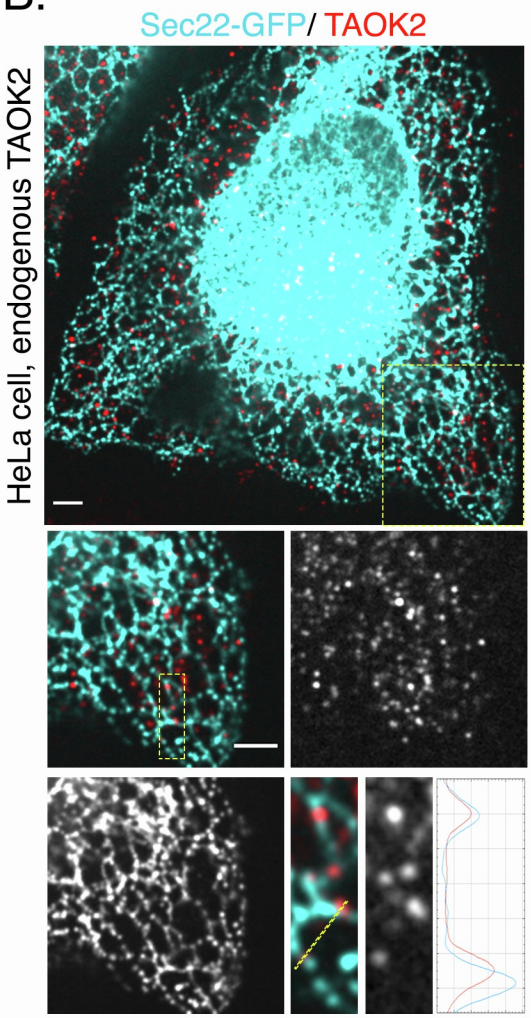
Supplementary Information

1. Supplementary Figures S1-S5 and Figure Legends
2. Movie Legends movie 1-3

S1A.



S1B.



S1C.

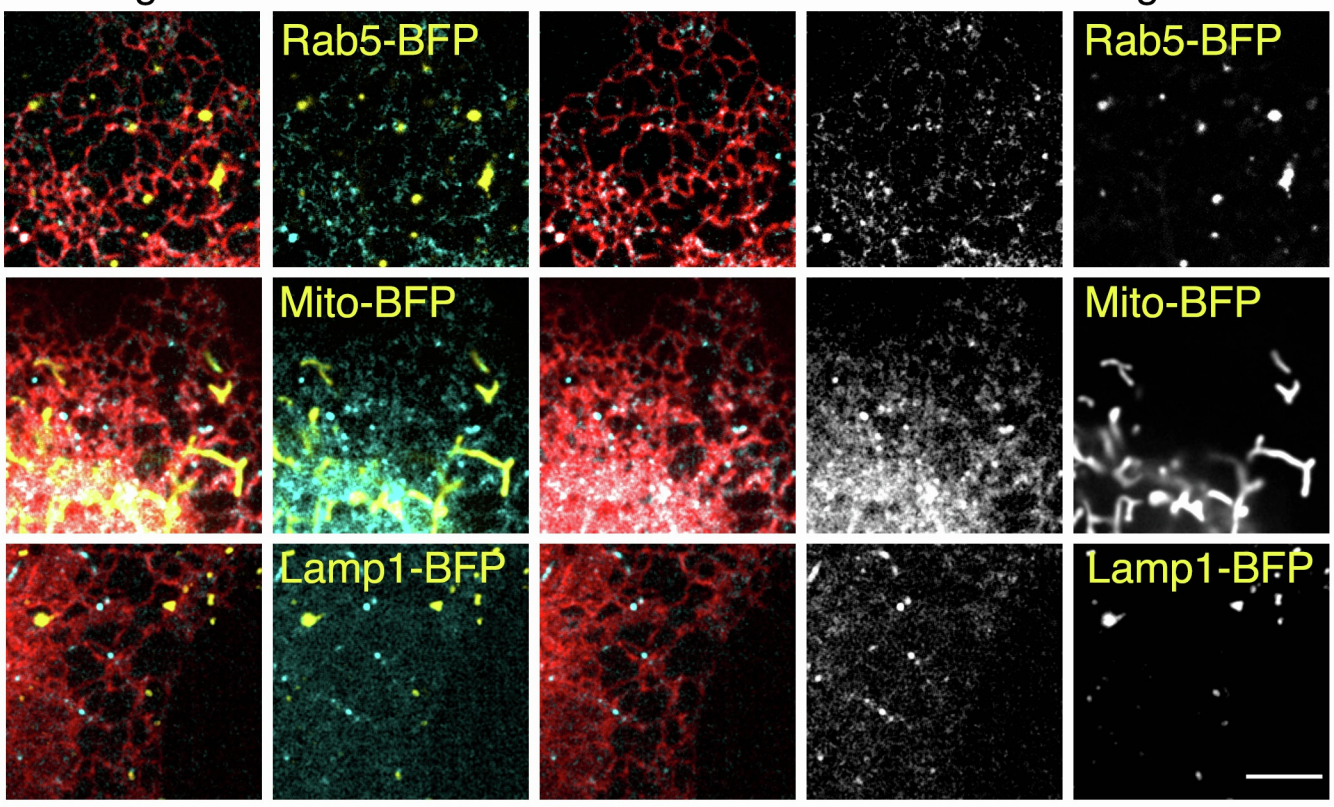


Figure S1

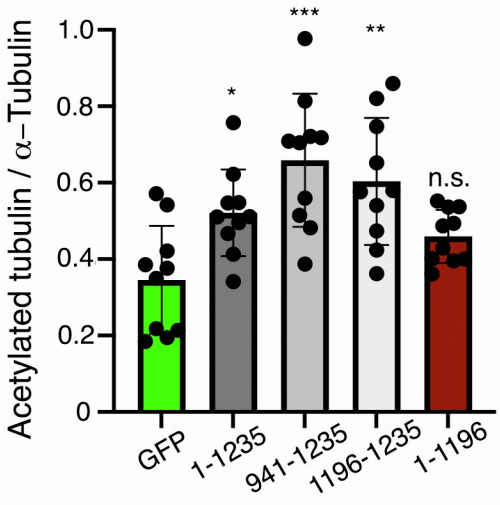
Figure S1. TAOK2 localizes to the ER membrane (related to main figure 1)

(A) Model of TAOK2 structure as determined by AlphaFold2.0 protein structure and folding prediction algorithm. Structure is for the TAOK2α isoform (1-1235 amino acids). Kinase domain (top center), coiled coil bundle (left), transmembrane domains (941-1073) and C terminal tail (1074-1235) spanning the amphipathic domain and microtubule binding domain are shown (middle center, zoom in bottom). Expected position error at each residue is plotted for the entire length of the protein. Structure is color coded according to confidence of the algorithm in the prediction.

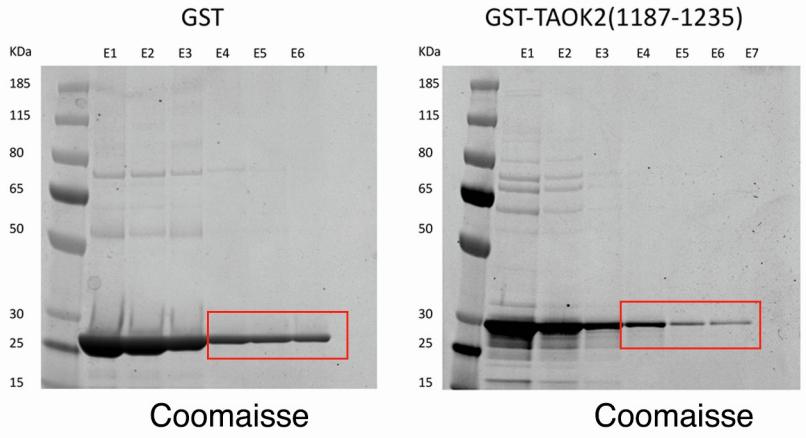
(B) Confocal image of HeLa cell expressing EGFP-Sec22b (cyan) and immunostained for TAOK2 (red). Scale bar is 5μm (top) and 1μm (bottom). RGB profile of fluorescence intensity peaks of TAOK2 (red) and EGFP-Sec22b (cyan).

(C) Confocal images of HeLa cells expressing GFP-tagged TAOK2 (cyan), ER-mRFP (red) along with the indicated organellar constructs (yellow). Endosomes visualized through expression of BFP-Rab5, mitochondria marked by BFP-mito, and lysosomes are marked by BFP-Lamp1. Greyscale images for TAOK2 and organelles is displayed on right. Scale bar is 3μm.

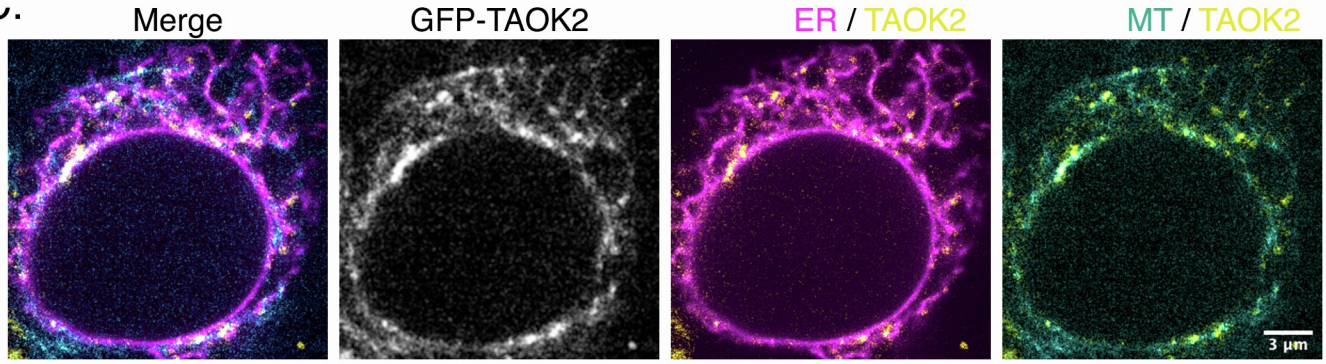
S2A.



S2B.



S2C.



S2D.

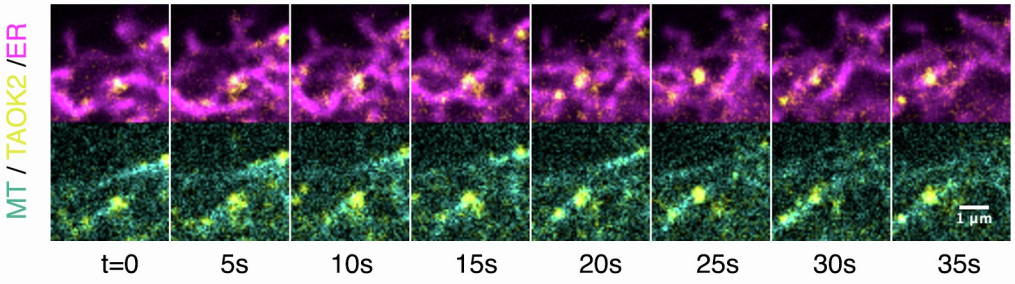


Figure S2

Figure S2. TAOK2 is an ER-microtubule tether (related to main figure 2 and 3)

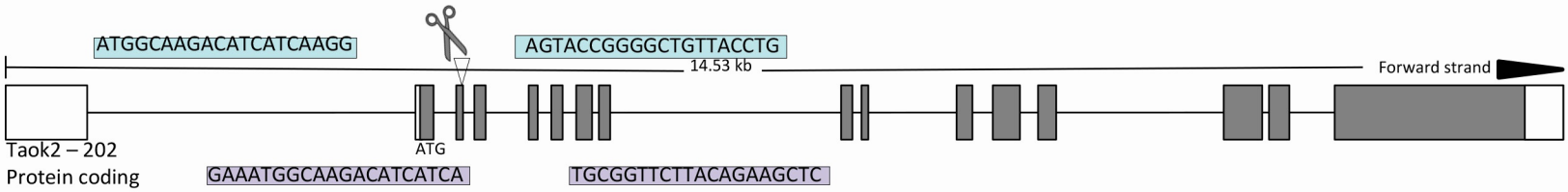
(A) Fraction of acetylated tubulin relative to α-tubulin for TAOK2 domain dissection constructs. Values indicate mean, error bars depict S.E.M., n=10 cells from 3 experiments for each construct. Ordinary one-way ANOVA with multiple comparisons, where p values are denoted by *<0.05, **<0.001, ***<0.0005 and ****<0.0001.

(B) Coomassie stained gels showing eluate fractions obtained after affinity purification of GST (left) and GST-TAOK2-C (right) proteins. Fraction E4 was concentrated and used for downstream microtubule binding assays. Molecular weight proteins ladder is shown on the left.

(C) Confocal images of HEK293T cells expressing GFP-TAOK2 (yellow), ER-mRFP (magenta) and live-stained with microtubule dye (cyan). Scale bar is 3μm.

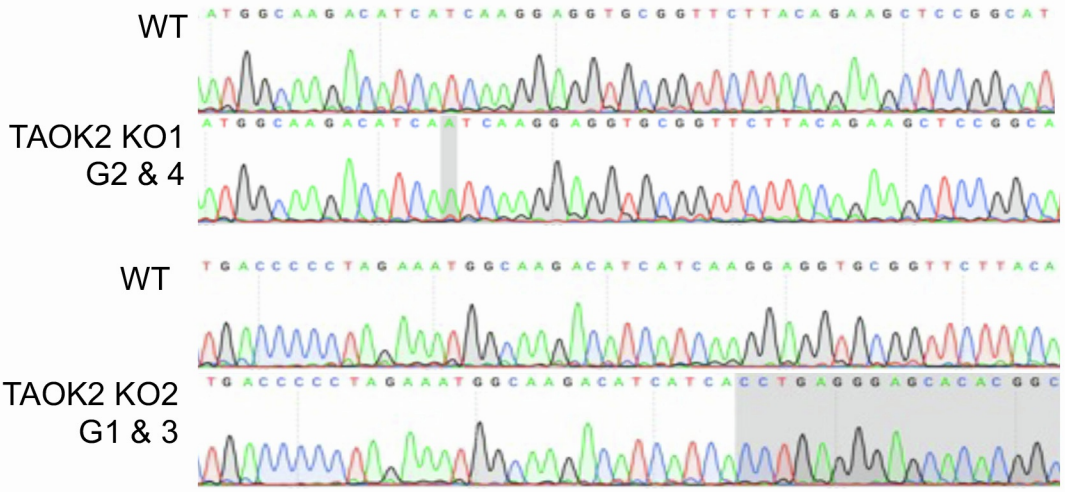
(D) Montage of confocal time lapse images shows TAOK2 (yellow) punctae colocalized with both ER membrane (magenta) and microtubules (cyan). Scale bar is 1μm.

S3A



Guide	Sequence
# 1	GAAATGGCAAGACATCATCA
# 2	ATGGCAAGACATCATCAAGG
# 3	TGCGGTTCTTACAGAAGCTC
# 4	AGTACCGGGGCTGTTACCTG

S3B



S3C

Mutation Pattern

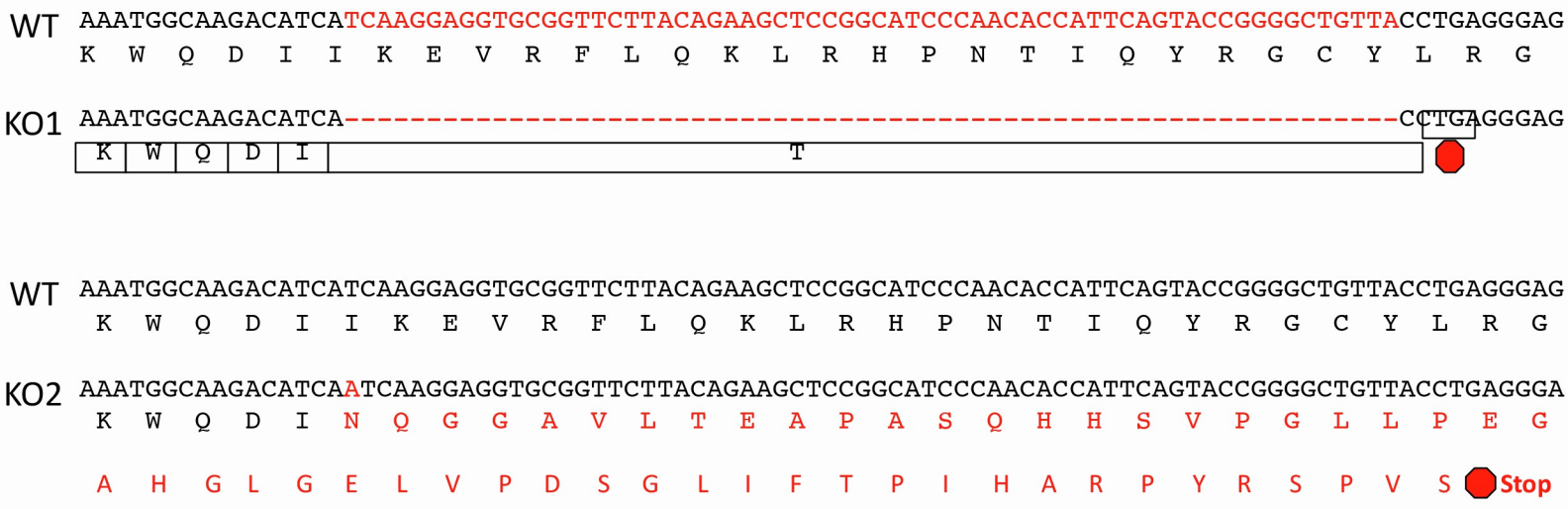


Figure S3

Figure S3. Generation of TAOK2 knockout HEK293 cell lines using genome editing

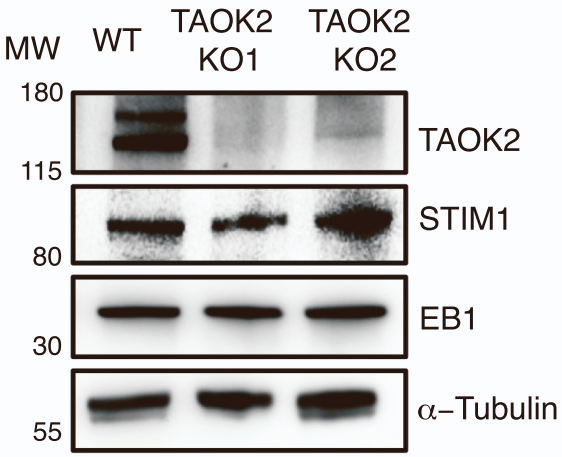
(related to main figure 3)

(A) Genomic structure of TAOK2 as visualized through the UCSC genomic browser, shows the exons in gray, and region targeted by RNA guides (purple and blue) is marked by scissors. RNA guide sequences used for generation of TAOK2 knockout are shown.

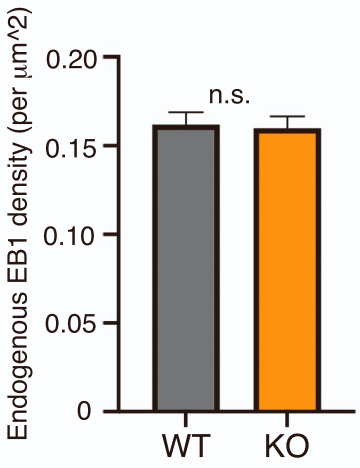
(B) Sequence peaks show the result of DNA sequencing in wildtype and two separate KO cell lines performed after PCR of the surrounding genomic region. The change in nucleotide sequence is shown in gray.

(C) Genome editing leads to frameshift mediated early termination. Resulting changes in protein sequence for both TAOK2 knockout cell lines is depicted.

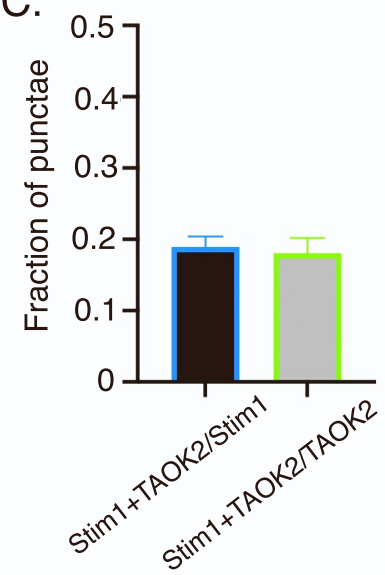
S4A.



S4B.



S4C.



S4D.

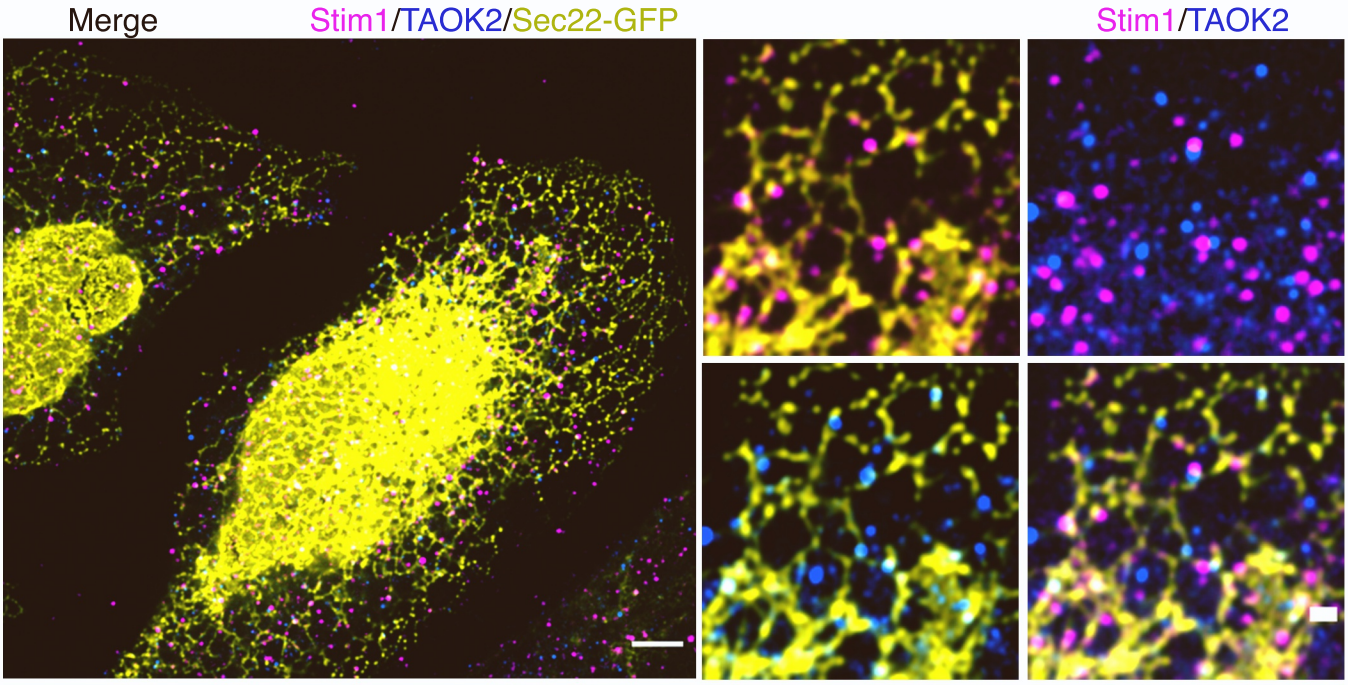


Figure S4

Figure S4. Role of TAOK2 in ER motility on microtubule plus tips (related to main figures 4 and 5)

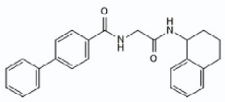
(A) Western blot of HEK293T WT and TAOK2 KO (1 and 2) cell lysate. Probed for TAOK2, EB1, STIM1 and α-tubulin.

(B) Endogenous EB1 density over 100 μm^2 region in cell periphery of WT and TAOK2 KO HEK293T cells. Values indicate mean, n=10 cells from 3 experiments, error bars indicate S.E.M., t-test.

(C) Fraction of TAOK2 punctae positive for STIM1 and fraction of STIM1 puncta positive for TAOK2. Values indicate mean, n=10 cells from 3 experiments, error bars indicate S.E.M., t-test.

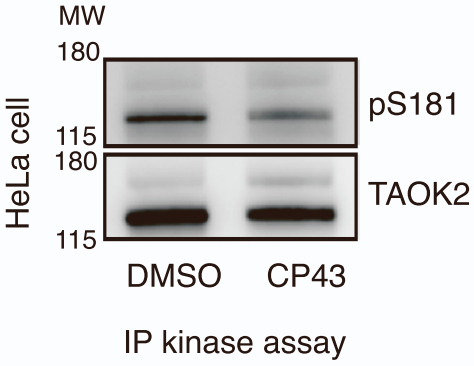
(D) Confocal image of HeLa cell expressing EGFP-Sec22b (yellow) and immunostained for TAOK2 (blue) and STIM1 (magenta). Scale bar is 3μm (top) and 1μm (right).

A.

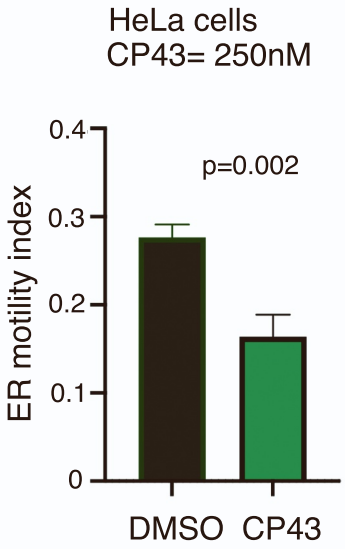


CP43 (TAOK inhibitor)

N-(2-Oxo-2-((1,2,3,4-tetrahydronaphthalen-1-yl)amino)ethyl)-[1,1'-biphenyl]-4-carboxamide



B.



C.

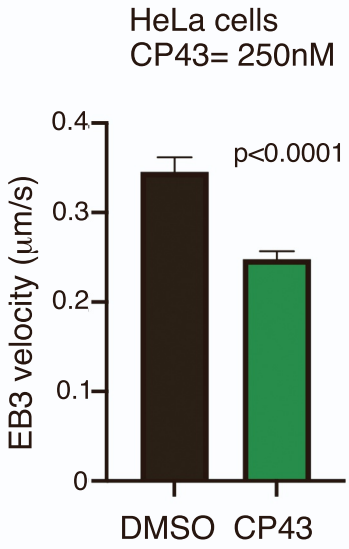


Figure S5

Figure S5. Effect of TAOK2 kinase inhibition on ER and microtubule dynamics (related to main figure 7)

(A) Kinase activity of endogenous TAOK2 was measured in the presence of DMSO and 250nM CP43 using an *in vitro* kinase reaction with autophosphorylation at S181 the readout. Western blot probed with: phospho-S181 antibody to measure kinase autophosphorylation activity and TAOK2 to measure immunoprecipitated TAOK2.

(B) ER motility index calculated by averaging the normalized pixel difference between successive time frame over 30s is plotted for DMSO and CP43 treated HeLa cells. Error bars indicate S.E.M, n=10 cells from 3 experiments, two tailed t-test.

(C) EB3 comet tracks generated using the Manual tracking function in Fiji were used to measure EB3 comet velocity by dividing the total distance traveled over time. Mean values for DMSO and CP43 treated HeLa cells. Error bars indicate S.E.M, n=10 cells with 5 comets per cell, two tailed t-test.