Supplemental Information for:

The microtubule-associated protein EML3 regulates mitotic spindle assembly by recruiting the Augmin complex to spindle microtubules

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Including 4 supplemental figures and 13 movies with legends

Supplemental Figure Legends

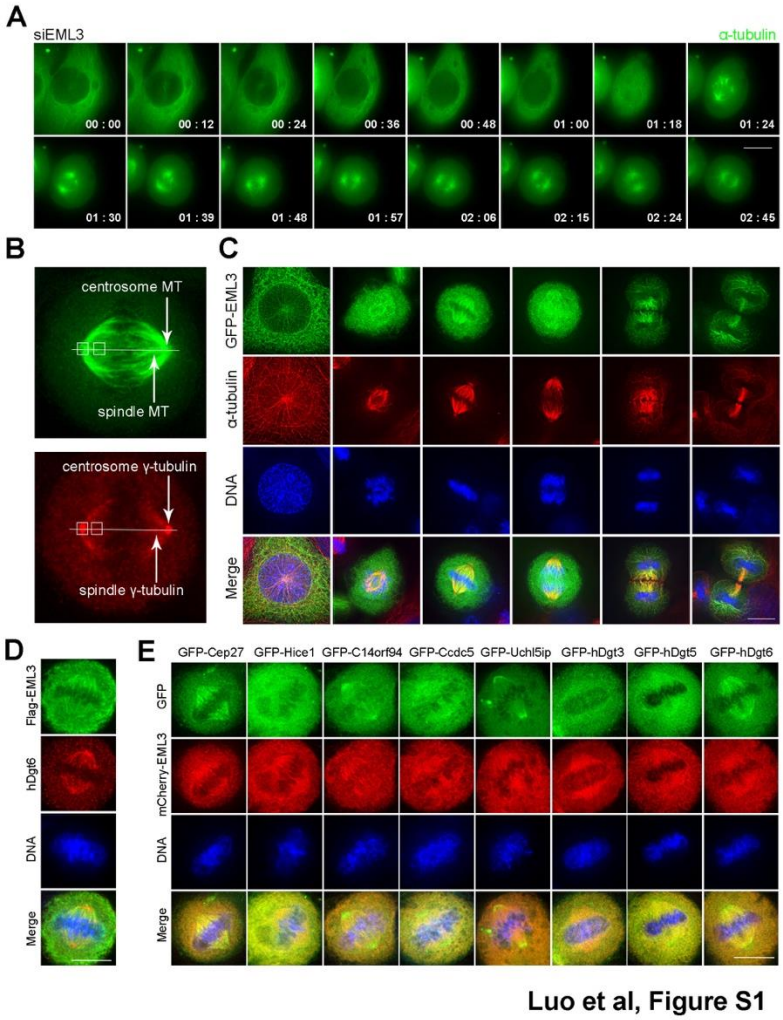


Figure S1. EML3 colocalizes with Augmin on spindle microtubules. Relate to Figure 1.

(A) GFP-α-tubulin expressing HeLa cells were transfected with EML3 siRNA and subjected to live cell imaging. (B) The fluorescence intensity of α-tubulin and γ-tubulin on spindles and centrosomes was measured in the indicated square area along the spindle axis. (C) HeLa cells were transfected with GFP-EML3 and immunostained with anti-α-tubulin antibodies. The DNA was stained with DAPI. Scale bar, 10 μm. Note that GFP-EML3 is associated with microtubules both in interphase and mitosis. (D) HeLa cells were transfected with Flag-EML3 and immunostained with anti-Flag and hDgt6 antibodies. Note that Flag-EML3 co-localizes with hDgt6. (E) HeLa cells were co-transfected with mCherry-EML3 and GFP-tagged Augmin subunits, followed by fixation and staining of DNA with DAPI. Scale bar, 10 μm. Note that mCherry-EML3 colocalized with GFP-tagged Augmin subunits on the microtubules in mitosis.

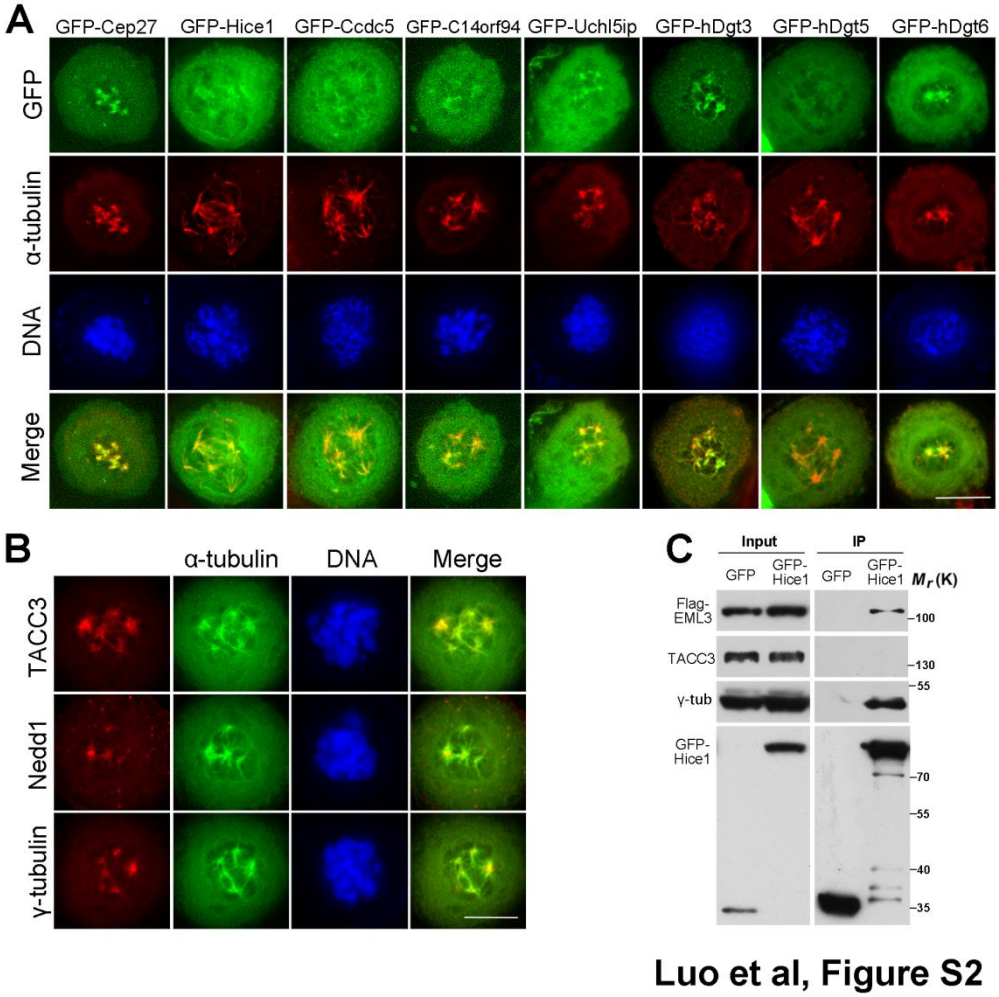


Figure S2. Augmin subunits localize to the acentrosomal microtubule asters. Relate to Figure 3.

(A) Augmin subunits load onto acentrosomal microtubule asters. HeLa cells transiently expressing GFP-tagged Augmin subunits were treated as the same as in A. Scale bar, 10 μm. Note that many small acentrosomal microtubule asters were assembled and EML3 co-localized with the Augmin subunits on the small acentrosomal microtubule asters. (B) HeLa cells were treated as the same as in A, and stained with the indicated antibodies. Note that γ-tubulin and Nedd1 were localized to the centers of the asters, TACC3 situated on the aster microtubules. Scale bar, 10 μm. (C) HEK 293 cells co-transfected with GFP-Hice1 and Flag-EML3 were arrested in mitosis with nocodazole, processed for IP assay with GFP-Trap beads, and analyzed by western blotting with indicated antibodies.

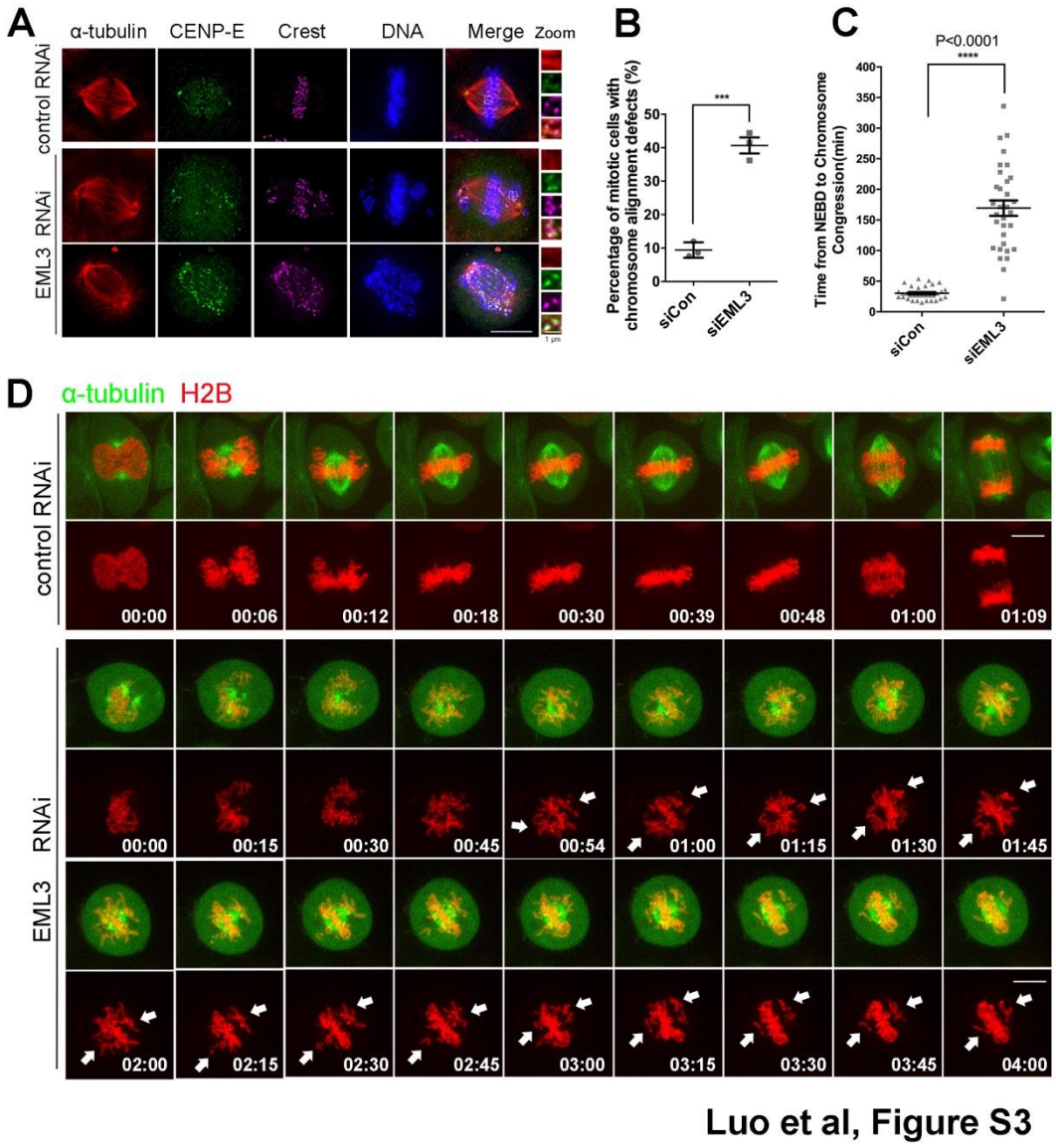


Figure S3. EML3 is required for chromosome congression. Relate to Figure 4.

(A) Control or EML3 knockdown HeLa cells were stained with the indicated antibodies. Selected areas in merged panels were magnified to the right. The DNA was stained with DAPI. Scale bar, 10 μm. (B) Quantification of the percentage of mitotic cells with chromosome alignment defects in A. Error bars, s.e.m. 203 control knockdown and 208 EML3 knockdown cells from three independent experiments were counted. (C) Quantification of the time from NEBD to chromosome congression in cells in D. Error bars, s.e.m. 32 control and 32 EML3 knockdown cells from three independent experiments were analyzed. (D) RFP-H2B-expressing HeLa cells were co-transfected with EML3 or control siRNA and GFP-α-tubulin and subjected to live cell imaging. Images were captured every 3 minutes with a 6 μm Z-section by 4 slices. Arrows indicate misaligned chromosomes. Scale bar, 10 μm.

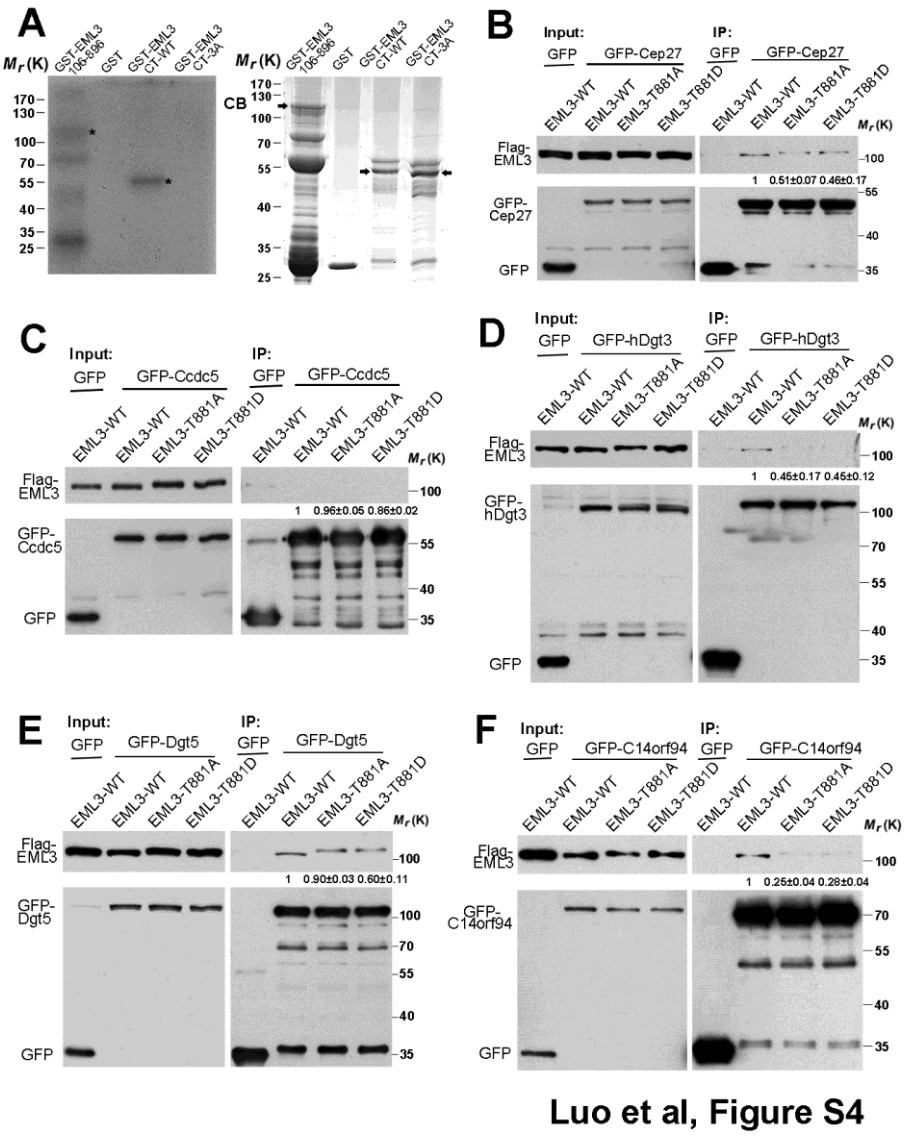
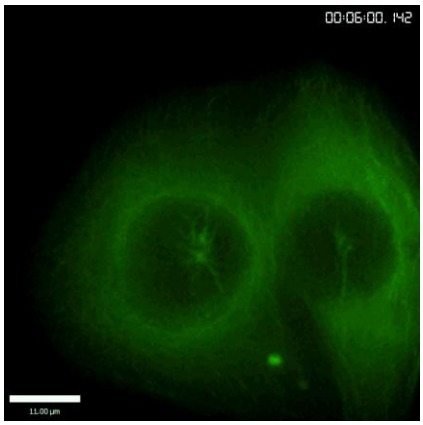


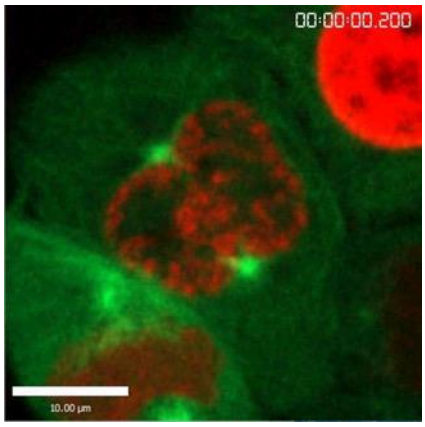
Figure S4. Thr881 phosphorylation of EML3 is required for its binding with Augmin subunits. Relate to Figure 5&6.

(A) GST-EML3-aa106-end, GST-EML3-CT, and GST-EML3-CT-3A (T881A, T885A and S889A) were subjected to CDK1/Cyclin B kinase assays in vitro followed by autoradiography (left panel). Coomassie Blue (CB) staining in the right panel showed the loading of the GST-tagged proteins. (B, C, D, E, F) HEK 293 cells co-transfected with one of the GFP-tagged Augmin subunits as indicated and Flag-EML3-WT, Flag-EML3-T881A or Flag-EML3-T881D were arrested in mitosis with nocodazole, followed by IPs and Western blotting analysis with GFP-Trap beads and mouse anti-Flag antibodies. The control HEK 293 cells were co-transfected with GFP and Flag-EML3 as indicated. The experiment was independently performed twice.

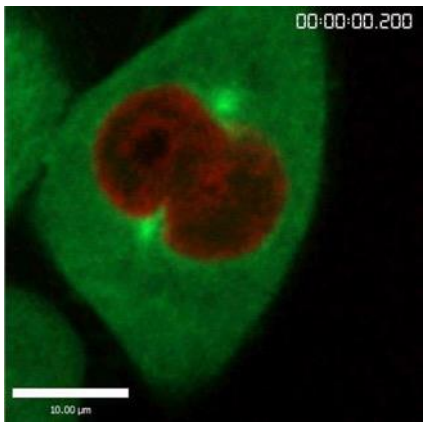
Supplemental Movie Legends



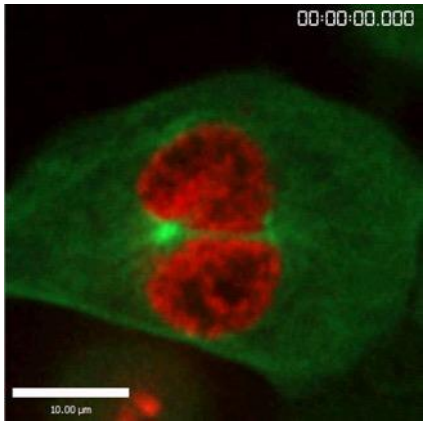
Movie S1. GFP-α-tubulin expressing HeLa cells were transfected with EML3 siRNAs. Images were captured every 3 minutes.



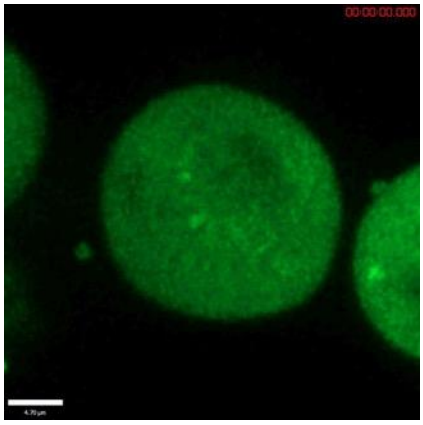
Movie S2. RFP-H2B-expressing HeLa cells were co-transfected with GFP-α-tubulin and negative control siRNAs. Acentrosomal microtubule asters were indicated by arrows. Images were captured every 5 seconds.



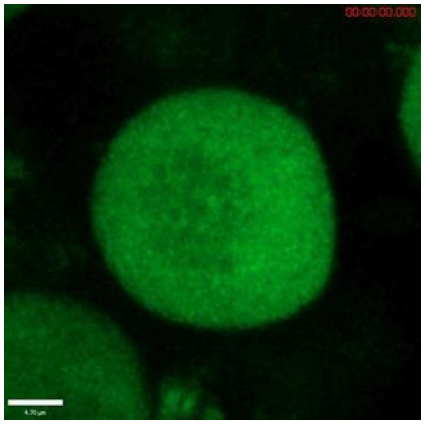
Movie S3. RFP-H2B-expressing HeLa cells were co-transfected with GFP-α-tubulin and EML3 siRNAs. Acentrosomal microtubule aster was indicated by arrow. Images were captured every 5 seconds.



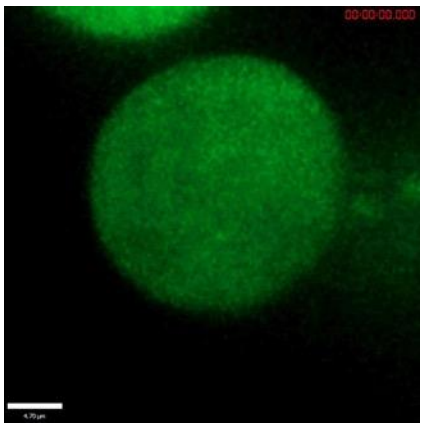
Movie S4. RFP-H2B-expressing HeLa cells were co-transfected with GFP-α-tubulin and hDgt6 siRNAs. Acentrosomal microtubule asters were indicated by arrows. Images were captured every 5 seconds.



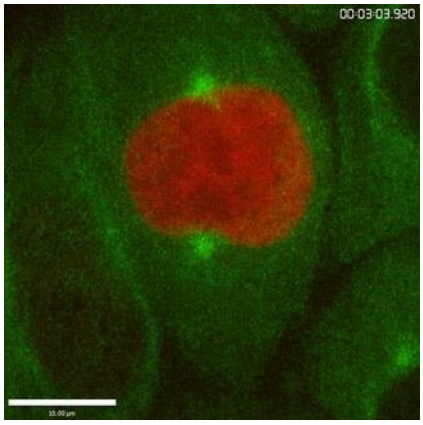
Movie S5. GFP-α-tubulin expressing HeLa cells were transfected with negative control siRNAs, and released from 500 ng/ml nocodazole into medium with 30 ng/ml nocodazole. Images were captured every 1 minute.



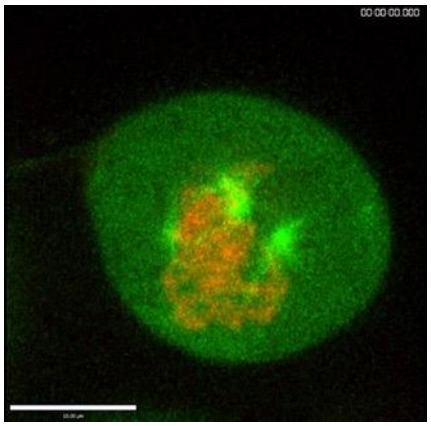
Movie S6. GFP-α-tubulin expressing HeLa cells were transfected with EML3 siRNAs, and released from 500 ng/ml nocodazole into medium with 30 ng/ml nocodazole. Images were captured every 1 minute.



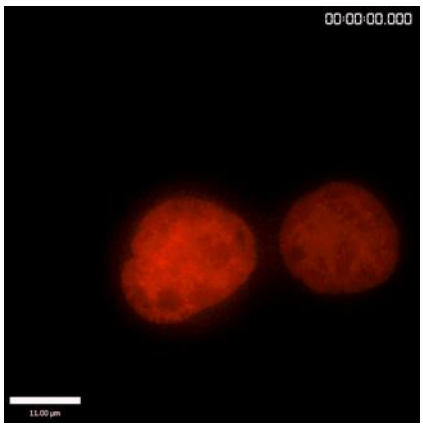
Movie S7. GFP-α-tubulin expressing HeLa cells were transfected with hDgt6 siRNAs, and released from 500 ng/ml nocodazole into medium with 30 ng/ml nocodazole. Images were captured every 1 minute.



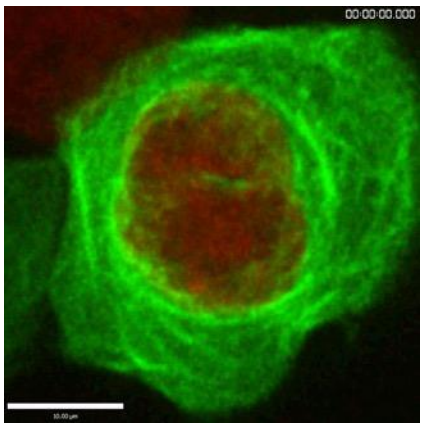
Movie S8. RFP-H2B-expressing HeLa cells were co-transfected with negative control siRNA and GFP-α-tubulin. Images were captured every 3 minutes.



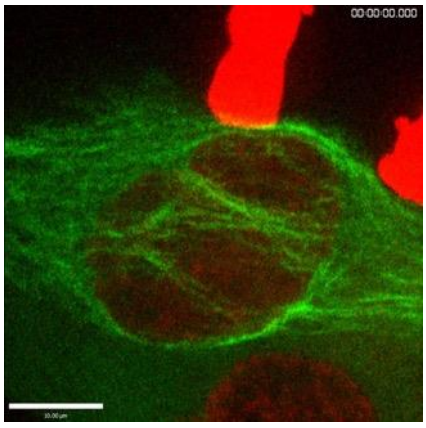
Movie S9. RFP-H2B-expressing HeLa cells were co-transfected with EML3 siRNA and GFP-α-tubulin. Images were captured every 3 minutes.



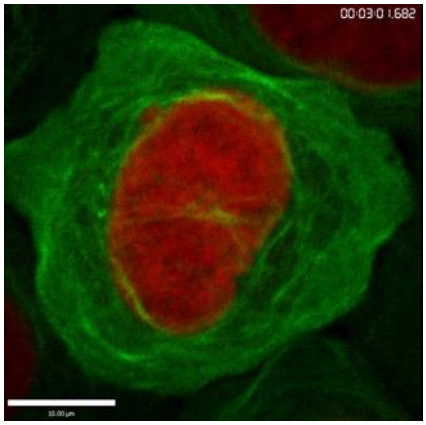
Movie S10. RFP-H2B-expressing HeLa cells were transfected with EML3 siRNA. Images were captured every 3 minutes.



Movie S11. RFP-H2B-expressing HeLa cells were co-transfected with EML3 siRNA and siRNA-resistant EML3-WT. Images were captured every 3 minutes.



Movie S12. RFP-H2B-expressing HeLa cells were co-transfected with EML3 siRNA and siRNA-resistant EML3-T881A. Images were captured every 3 minutes.



Movie S13. RFP-H2B-expressing HeLa cells were co-transfected with EML3 siRNA and siRNA-resistant EML3-T881D. Images were captured every 3 minutes.