Wang et al., http://www.jcb.org/cgi/content/full/jcb.201308001/DC1

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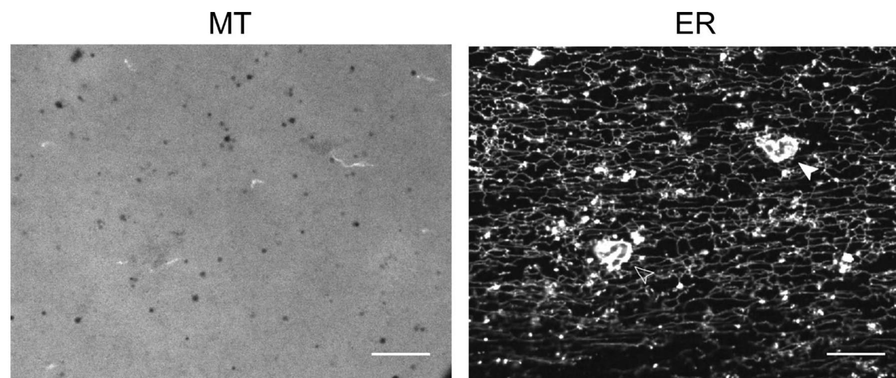



Figure S1. **The ER network is spatially homogeneous in the absence of MTs.** A crude interphase *X. laevis* egg extract containing Alexa fluor 488–labeled tubulin and the hydrophobic dye $DiIC_{18}$ was treated with 30 µM nocodazole to disassemble MTs. The left and right panels show tubulin and membrane staining, respectively. Arrowheads indicate NEs. Bars, 20 µm.

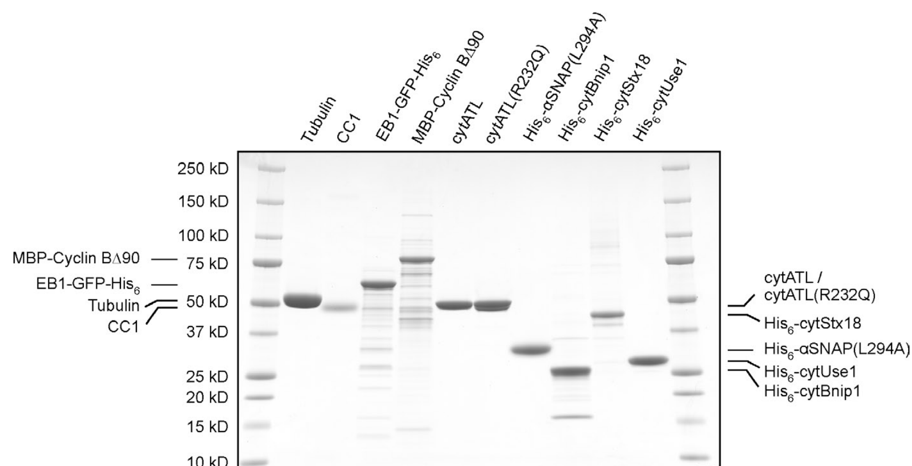



Figure S2. **SDS gel of purified proteins used in the study.** The indicated proteins were purified and separated in a 4–20% linear acrylamide SDS gel. The gel was stained with Coomassie blue.

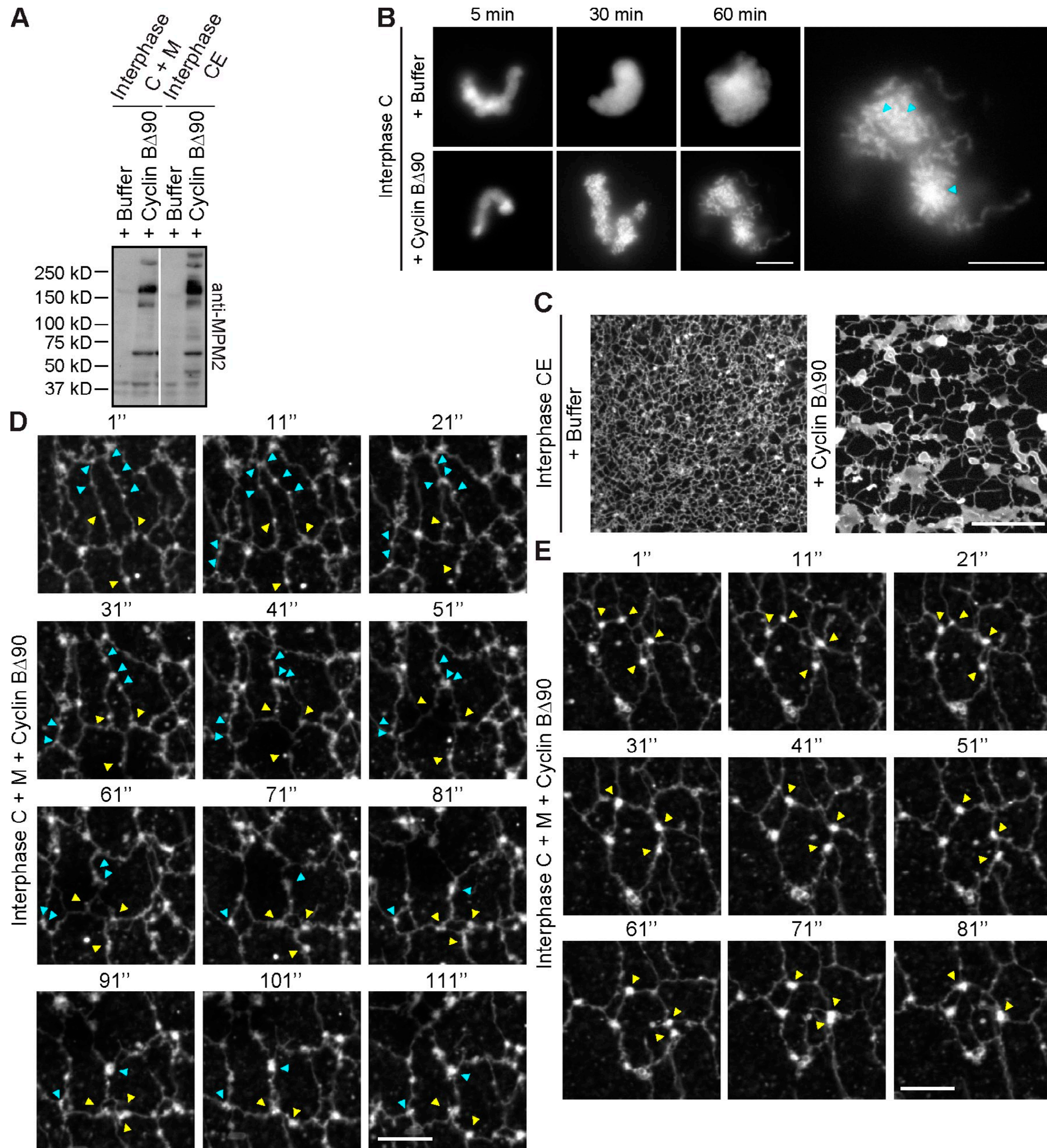



Figure S3. **The ER network undergoes tubule-to-sheet conversion during the transition from interphase to mitosis.** (A) A crude interphase extract (CE) or a mixture of interphase cytosol and light membranes (C + M) was incubated for 40 min with either buffer or cyclin BΔ90. Aliquots were subjected to immunoblotting with anti-MPM2 antibodies to detect mitotically phosphorylated proteins. (B) Interphase cytosol was incubated for 40 min with either buffer or cyclin BΔ90. Demembranated sperm was added and aliquots were fixed with the Hoechst-containing solution. The DNA was visualized by confocal microscopy. The right panel shows a magnified view of the 60-min time point in the presence of cyclin BΔ90. Arrowheads point to condensed chromatids. Bars, 20 µm. (C) The crude extracts (CE) used in A were incubated with $DiOC_{18}$ to stain the membranes. The samples were analyzed by confocal microscopy. Bar, 20 µm. (D) Time course of the conversion of interphase ER into mitotic ER. An ER network was formed by incubating interphase cytosol, light membranes, and an energy regenerating system at room temperature for 20 min. Cyclin BΔ90 and octadecyl rhodamine were added, and the changes of ER morphology followed over time. Arrowheads point to sheet-like structures that appear in tubules. Arrowheads of the same color point to sheets that eventually merge. (E) As in D, but the start is at a later time point. Note the merging of sheets. Bars, 10 µm.

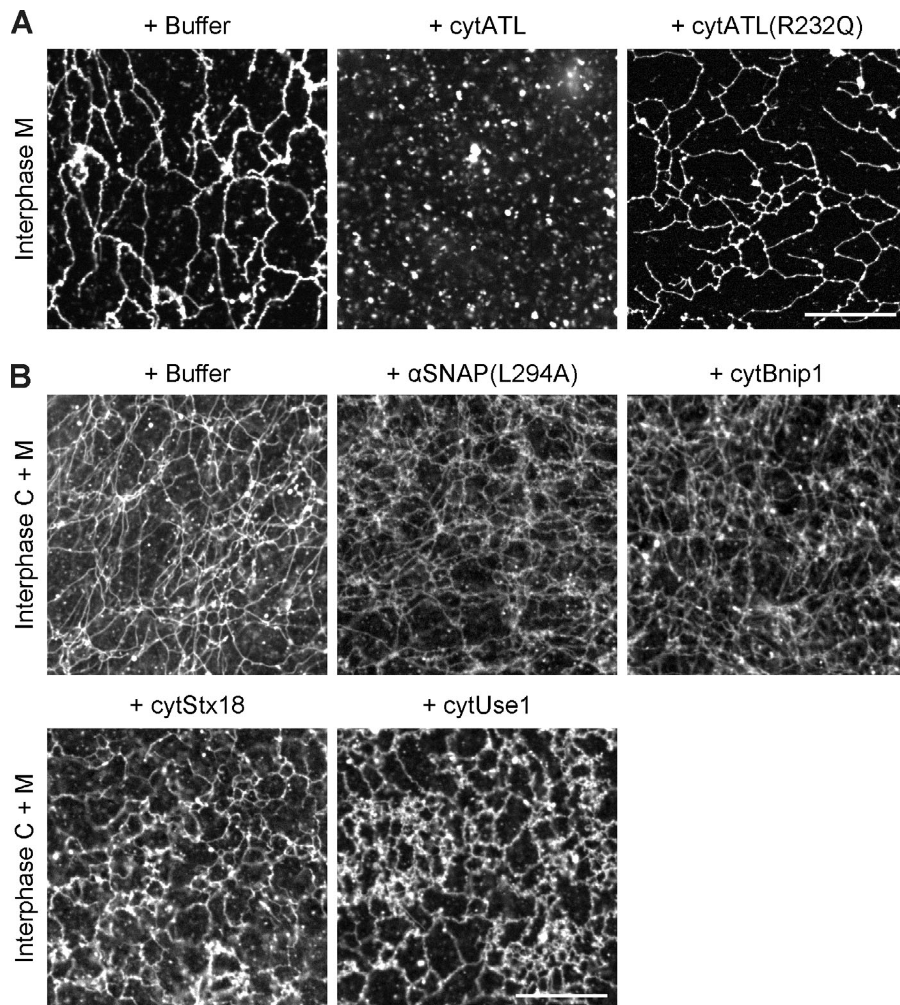
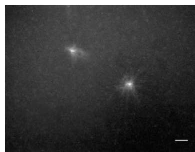
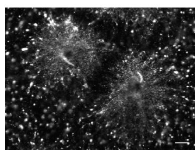



Figure S4. **ER network formation requires ATL, but not ER SNARE, function.** (A) $DiOC_{18}$ prelabeled interphase light membranes were incubated for 5 min on ice with buffer, 2 μM of the cytoplasmic fragment of *X. laevis* ATL2 (cytATL), or 2 μM of mutant fragment (cytATL(R232Q)). An energy regenerating system was added and the samples were incubated for 15 min at room temperature. The membranes were visualized by confocal microscopy. (B) Interphase cytosol and light membranes were mixed and incubated on ice for 5 min with buffer, 50 μM of dominant-negative αSNAP mutant (αSNAP(L294A)), 20 μM of cytoplasmic fragment of Bnip1 (cytBnip1), 30 μM of cytoplasmic fragment of syntaxin 18 (cytStx18), or 70 μM of cytoplasmic fragment of Use1 (cytUse1). An energy regenerating system was added and the samples were incubated for 30 min at room temperature. The membranes were stained with octadecyl rhodamine and visualized by confocal microscopy. Bars, 20 μm.

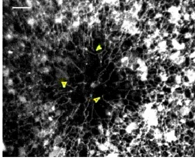
Figure S5. **ER SNARE–mediated fusion is required for NE assembly from a preformed ER network.** (A) Interphase cytosol, light membranes, and an energy regenerating system were mixed and incubated at room temperature for 20 min. The samples then received buffer, 50 µM of dominant-negative αSNAP mutant (αSNAP(L294A)), 20 µM of the cytoplasmic fragment of Bnip1 (cytBnip1), 30 µM of the cytoplasmic fragment of syntaxin 18 (cytStx18), or 70 µM of the cytoplasmic fragment of Use1 (cytUse1). Aliquots were stained with octadecyl rhodamine and visualized by confocal microscopy. Bar, 20 µm. (B) The samples in A received demembranated sperm and were incubated at room temperature for 1.5 h. Fluorescently labeled dextran was added to detect nuclei with closed NE. The samples were also stained for membranes and DNA and visualized by confocal microscopy. Bar, 20 µm. Quantification of the NE fusion at 1.5 h was determined as described in Fig. 8 A and the data plotted are the mean ± SD of three independent experiments. **, $P < 0.005$; ***, $P < 0.001$; Student's *t* test.



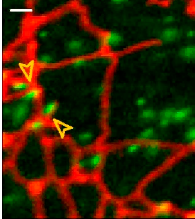
Video 1. **MT aster formation in crude interphase *X. laevis* egg extracts.** Demembranated sperm was added to a crude interphase *X. laevis* egg extract containing Alexa fluor 488–labeled tubulin and the hydrophobic dye $DiIC_{18}$. The formation of MT asters (Alexa fluor 488 fluorescence; shown in white) was followed over time by spinning-disk confocal microscopy. The images were acquired at 20-s intervals for 15 min. The video is shown at 10 frames per second. Still images of this video were used for Fig. 1 A. Bar, 30 µm.



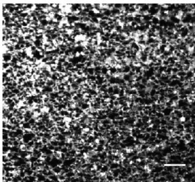
Video 2. **ER network formation in crude interphase *X. laevis* egg extracts.** Demembranated sperm was added to a crude interphase *X. laevis* egg extract containing Alexa fluor 488–labeled tubulin and the hydrophobic dye $DiIC_{18}$. The formation of the ER network ($DiIC_{18}$ fluorescence; shown in white) was followed over time by spinning-disk confocal microscopy. The images were acquired at 20-s intervals for 15 min. The video is shown at 10 frames per second. Still images of this video were used for Fig. 1 B. Bar, 30 µm.



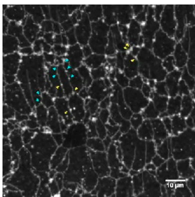
Video 3. **ER tubule extension toward the minus end of MTs.** Demembranated sperm was added to a crude interphase *X. laevis* egg extract containing Alexa fluor 488–labeled tubulin and the hydrophobic dye $DiIC_{18}$. The samples were immediately imaged. Shown is the $DiIC_{18}$ fluorescence (in white). The arrowheads point to tubules extending toward the minus end of MTs. Time-lapsed images were acquired by spinning-disk confocal microscopy at 2-s intervals for 2 min. The video is shown at four frames per second. Bar, 5 µm.



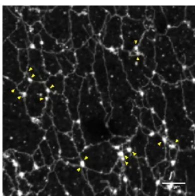
Video 4. **The leading ends of ER tubules track with the plus ends of MTs.** Demembranated sperm was added to a crude interphase *X. laevis* egg extract containing the hydrophobic dye $DiIC_{18}$ (in red) and a GFP fusion of the plus end tracking protein EB-1 (EB-1–GFP; in green). The samples were imaged after 30-min incubation. Shown is the merged image of the two fluorescence channels. The yellow arrowheads point at EB-1 comets, marking the growing plus ends of MTs and the tip of extending ER tubules. The blue arrowhead indicates the fusion site of an ER tubule with another. Time-lapsed images were acquired by spinning-disk confocal microscopy at 2-s intervals for 1 min. The video is shown at four frames per second. Still images of this video were used for Fig. 3 A. Bar, 2 µm.



Video 5. **The ER network undergoes tubule-to-sheet conversion during the transition from interphase to mitosis.** A crude interphase extract containing the hydrophobic dye $DiIC_{18}$ was incubated for 10 min with 0.05 mg/ml cyclin BΔ90. The ER network (in white) was imaged at 5-s intervals for 12 min using a spinning-disk confocal microscope. The video is shown at four frames per second. Bar, 5 µm.



Video 6. **Tubule-to-sheet conversion of the ER in a fractionated system.** An ER network was formed by incubating interphase cytosol, light membranes, and an energy regenerating system at room temperature for 20 min. Cyclin BΔ90 and the fluorescent hydrophobic dye octadecyl rhodamine were added, and the changes of ER morphology (in white) were followed over time. Time-lapsed images were acquired with a spinning-disk confocal microscope at 2-s intervals for 2 min. The video is shown at five frames per second. Arrowheads point to sheet-like structures that appear in tubules. Arrowheads of the same color point to sheets that eventually merge. Still images of this video were used for Fig. S3 D. Bar, 10 µm.



Video 7. **Merging of ER sheets during the transition from interphase to mitosis.** An ER network was formed by incubating interphase cytosol, light membranes, and an energy regenerating system at room temperature for 20 min. Cyclin BΔ90 and the hydrophobic fluorescent dye octadecyl rhodamine was added, and changes in ER morphology were recorded over time (shown in white). Time-lapsed images were acquired in a spinning-disk confocal microscope at 2-s intervals for 2 min. The video is shown at five frames per second. Arrowheads show the gradual change from smaller to larger sheets. This video was taken with the same sample used for Video 6, but at a later time point. Still images of this video were used for Fig. S3 E. Bar, 10 µm.