Supplementary Information

This Supplementary Information contains detailed experimental procedures, ten supplementary figures, a supplementary table, and references cited in the supplement.

Experimental Procedures

Isolation of Spindle Matrix

The spindle matrix was isolated as described previously (Tsai et al., 2006) with modifications. Briefly, 20 µl protein A Dynal beads were incubated with 20 µl AurA antibody at 4°C for one hour. After washing the beads with XB buffer (10 mM Hepes, pH 7.7, 50 mM sucrose, 100 mM KCl, 1mM MgCl₂, 0.1 mM CaCl₂, and 5mM EGTA), the beads were incubated with 100-200 µl CSF egg extracts for one hour to allow AurA present in the egg extracts to bind to the beads. After this incubation, the AurA-coated beads (AurA-beads) were washed using XB buffer and then used to stimulate spindle assembly in 2 ml CSF egg extracts in the presence of RanGTP or DMSO (as a control). After incubation at room temperature for 10-15 min, the egg extracts were diluted in 100 ml BRB80 plus 30% glycerol (to preserve microtubules), 100 ml XB plus 10 µM nocodazole or just ice-cold XB (to depolymerize microtubules but preserve the spindle matrix), or 100 ml XB plus 10 µM nocodazole and 0.5% Triton X100 (to depolymerize microtubules and disrupt the membranous spindle matrix). AurA-beads with associated materials were then retrieved using a magnet. After washing the beads using additional 100 ml of the respective buffers as above, the AurA-bead associated proteins were eluted using XB buffer containing 0.5% Triton X100. For mass-spectrometry analyses, proteins present in the spindle matrix preparation were TCA precipitated and washed using cold acetone. Typically, matrix preparation from 2 ml of CSF egg extracts yielded 100-400 µg of proteins.

Mass Spectrometry

Urea was used to denature the protein mixture and the proteins were then reduced with TCEP, alkylated using iodoacetamide (IAM), and subsequently digested with trypsin. The digestion process was stopped by adding formic acid to a final concentration of 1%. The protein digest (peptide mixture) was stored at -80°C prior to use.
The protein digest was pressure-loaded onto a fused silica capillary desalting column containing 5 cm of 5 µm Polaris C18-A material (Metachem, Ventura, CA) packed into a 250-µm i.d capillary with a 2 µm filtered union (UpChurch Scientific, Oak Harbor, WA). The desalting column was washed with buffer containing 95% water, 5% acetonitrile, and 0.1% formic acid. After desalting, a 100-µm i.d capillary with a 5-µm pulled tip packed with 10 cm 3-µm Aqua C18 material (Phenomenex, Ventura, CA) followed by 3 cm 5-µm Partisphere strong cation exchanger (Whatman, Clifton, NJ) was attached to the filter union and the entire split-column (desalting column–filter union–analytical column) was placed inline with an Agilent 1100 quaternary HPLC (Palo Alto, CA) and analyzed using a modified 12-step separation described previously (Washburn et al., 2001). The buffer solutions used were 5% acetonitrile/0.1% formic acid (buffer A), 80% acetonitrile/0.1% formic acid (buffer B), and 500 mM ammonium acetate/5% acetonitrile/0.1% formic acid (buffer C). Step 1 consisted of a 100 min gradient from 0-100% buffer B. Steps 2-11 had the following profile: 3 min of 100% buffer A, 2 min of X% buffer C, a 10 min gradient from 0-15% buffer B, and a 97 min gradient from 15-45% buffer B. The 2 min buffer C percentages (X) were 10, 15, 20, 25, 30, 35, 40, 45, 50, 60% respectively for the 12-step analysis. The final step, the gradient contained: 3 min of 100% buffer A, 20 min of 100% buffer C, a 10 min gradient from 0-15% buffer B, and a 107 min gradient from 15-70% buffer B.

As peptides eluted from the microcapillary column, they were electrosprayed directly into an LCQ-Deca or LTQ-Orbitrap mass spectrometer (ThermoFinnigan, Palo Alto, CA) with the application of a distal 2.5 kV spray voltage. A cycle of one full-scan mass spectrum followed by 3 data-dependent MS/MS spectra at 35% normalized collision energy was repeated continuously throughout each step of the multidimensional separation. Application of mass spectrometer scan functions and HPLC solvent gradients were controlled by the Xcaliber datasystem.

Database Search and Interpretation of MS/MS Data

Tandem mass spectra were extracted from raw files. The tandem mass spectra were searched against a target protein database comprised of the following sets of proteins: *Xenopus laevis* and *Xenopus tropicalis* proteins from NCBI, *Xenopus tropicalis*
predicted proteins (v4.1) from JGI (Joint Genome Institute), human, mouse and rat IPI
proteins from EBI (European Bioinformatics Institute), for a total of 196,321 proteins. To
calculate confidence levels and false-positive rates, we used a decoy database containing
the reverse sequences of the 196,321 proteins appended to the target database (Peng et al,
2003), and the SEQUEST algorithm (Eng et al, 1994) to find the best matching sequences
from the combined database.

SEQUEST searches were done on an Intel Xeon 80-processor cluster running
under the Linux operating system. The peptide mass search tolerance was set to 50 ppm
for LTQ-Orbitrap data or 3 amu for LCQ-Deca data. No differential modifications were
considered. The mass of the amino acid cysteine was statically modified by + 57.02146 Da,
due to carboxyamidomethylation of the sample. No enzymatic cleavage conditions
were imposed on the database search.

The validity of peptide/spectrum matches was assessed in DTASelect (Tabb et al.,
2002) using SEQUEST-defined parameters, the cross-correlation score (XCorr), and
normalized difference in cross-correlation scores (DeltaCN). The distribution of XCorr
and DeltaCN values for (a) direct and (b) decoy database hits was obtained, and the two
subsets were separated by quadratic discriminative analysis. The discriminative score was
set such that a false-positive rate of 5% was determined based on the number of accepted
decoy database peptides.

**De novo and BLAST Analysis of MS/MS Data**

The procedure of de novo peptide sequence and BLAST analysis is as following.
Tandem mass spectra were used to first search against the target database containing
"Xenopus, human, rat, and mouse" protein sequences using SEQUEST. SEQUEST search
hits were filtered by using DTASelect to get a list of identified proteins. Tandem mass
spectra matched to the identified proteins were removed. The remaining tandem mass
spectra were used for de novo sequencing using PepNovo software (Frank et al., 2005).
The outputs from PepNovo analysis were filtered with the criteria that the number of
consecutive amino acids should be at least 6 and the average probability score is at least
0.9. PepNovo outputs were used to BLAST against the "Xenopus, human, rat, and
mouse" protein sequence database (Altschul et al., 1997). BLAST search hits to Xenopus
as top-hit (lowest E-value) and with E-value < 1000 were collected for further analysis. Proteins identified by two independent matrix preparations were considered as candidate components of the matrix.

**Plasmids, Protein Purification, Antibodies, and Protein Pull-down**

GST- and double 6His-tagged LB3 fusion constructs were made by cloning the full length LB3 (aa, 1-583), Rod region (aa, 29-385), coil1 (aa, 29-216), coil2 (aa, 242-385), or LB3T (aa, 384-583) into either pGEX-4T-1 or pET-28a at the BamHI and NotI sites. GST- and double 6His-tagged *Xenopus* XNudel fusion constructs were made by cloning the full length XNudel (aa, 1-345), N-XNudel (aa, 1-201), C-XNudel (aa, 167-345) into either pGEX-4T-1 or pET-28a at EcoRI and SalI sites. The N-terminal XNudE (aa, 1-198) was similarly cloned to make 6His-N-XNudE and GST-N-XNudE. The N-terminal human hNudel (aa, 1-197) were cloned into the BamHI and SalI sites of pET28a or pGEX-4T-1 to make the 6His-N-hNudel or GST-N-hNudel, respectively. All proteins were expressed in either BL21plysS, BL21-CondonPlus (DE3)-RIPL (Stratagene, #230280), or ArcticExpress (DE3)RP (Stratagene, #230194) competent cells with IPTG induction at either 13 °C overnight (full length LB3), 25°C for 5 h (GST-XNudel, 6His-XNudel, GST-C-XNudel, and 6His-C-XNudel), or 37°C for 3 h (GST, GST-LB3-Rod, GST-LB3-Coil1, GST-LB3-Coil2, GST-N-XNudel and 6His-N-XNudel).

To purify 6His-tagged Nudel or NudE proteins, 25 ml of cold NaP buffer (50 mM NaH₂PO₄, pH 8.0, 300 mM NaCl) containing 1mM imidazole, 1mM PMSF, 1mg/ml lysozyme, and 1% Triton X-100 was used to resuspend the bacteria pellet from each liter of the culture. Following 30’ incubation on ice, the bacteria were broken by sonication. After binding the proteins to the Ni-column (5 ml for 2-liter culture). The column was washed with 50 ml of NaP buffer containing 1 mM imidazole and 0.1 mM PMSF, followed by 200 ml of NaP buffer containing 10-20 mM imidazole. After another 50 ml wash with NaP buffer containing 1 mM imidazole, the protein was eluted using NaP buffer containing 250-500 mM imidazole and concentrated using Millipore’s Amicon Ultra centrifugal filter devices to 1-20 mg/ml depending on the protein. To purify GST-tagged Nudel proteins, we used HEPES buffer (50 mM HEPES, pH 8.0, 1 mM EGTA, 1 mM MgCl₂) containing either 500 mM NaCl (for lysis) or 100 mM NaCl (for elution).
For protein addition experiments, the proteins were exchanged into XB buffer using desalting columns (PD-10 columns, GE Healthcare).

Rabbit antibodies to *Xenopus* Nudel and *Xenopus* LB3 were generated using full length GST-XNudel and 6His-LB3T, respectively. 6His-LB3T was also used to generate chicken antibody. 6His-N-XNudE and 6His-N-hNudel were also used to generate rabbit antibodies. Antibodies to GST-XNudel were affinity purified against 6His-N-XNudel or 6His-C-XNudel. Affinity purification against 6His-C-XNudel did not yield usable antibodies, so all experiments were carried out using the Nudel antibody recognizing the 6His-N-XNudel. Antibodies to 6His-N-XNudE or 6His-N-hNudel were purified using N-XNudE or N-hNudel that were cleaved from the GST-N-XNudE or GST-N-hNudel, respectively, with thrombin. Antibodies to LB3 were affinity purified against 6His-LB3T. Monoclonal antibody to LB3 (L6-5D5) was a kind gift from Dr. Reimer Stick via Dr. Robert Goldman. Monoclonal antibodies to lamin B1 (Santa Cruz, sc-56144), 6His (Sigma, H1029), γ-tubulin (Sigma, T6557), 70.1 (Sigma, D5167), and 74.1 (Abcam, ab23905) were purchased. Control mouse ascites fluid clone NS-1 (Sigma, M8273) was purchased. The following secondary antibodies (all from Invitrogen) were used for immunofluorescent staining: goat anti-mouse 647 (A-21235), 594 (A11005), 488 (A11029), goat anti-rabbit 488 (A-11008), goat-anti-chicken 488 (A11039).

To determine whether Nudel directly interacts with LB3, GST-LB3 (aa, 1-583) was expressed by freshly transforming the plasmid into ArcticExpress(DE3)RP competent cells. A single colony was used to inoculate 5 ml LB containing ampicillin (50 µg/ml) and gentamycin (20 µg/ml) and grown overnight at 37°C. The overnight culture was used to inoculate 100 ml LB medium and grown at 30°C to OD₆₀₀= 0.8~1.0. Protein expression was induced with IPTG (final 0.4 mM) for 20 h at 13 °C. To express GST, GST-LB3-Rod, GST-LB3T, GST-LB3-coil1, and GST-LB3-coil2, the plasmids were freshly transformed into BL21-CondonPlus (DE3)-RIPL competent cells and grown in 5 ml LB containing ampicillin (50 µg/ml) and chloramphenicol (34 µg/ml) at 37 °C overnight. The overnight cultures were used to inoculate 100 ml medium and grown to OD₆₀₀=0.6~0.8. Protein expression was induced by IPTG (final 0.1mM) for 3 h at 37 °C. Bacterial lysates were made from these cultures using 7 ml cold lysis buffer (50 mM HEPES, pH 8.0, 1 mM EGTA, 1 mM MgCl₂, 500 mM NaCl, 1mM DTT, 1% Triton X-
100, 1mM PMSF) per culture and sonication on ice. After clarifying the lysates in a SS34 rotor at 15K rpm at 4°C for 30’, the supernatants were snap frozen as small aliquots in liquid nitrogen and stored at −80°C.

For each protein pull-down, 20 µl of settled GST beads were blocked with 500 µl of binding buffer (50 mM HEPES, pH 8.0, 1 mM EGTA, 1 mM MgCl₂, 100 mM NaCl) containing 4% BSA at 4°C with rotation for 30’. The beads were then incubated with bacterial lysates diluted to a final volume of 500 µl with the binding buffer containing 1% BSA, 1mM DTT, 0.1% NP-40, 1mM PMSF for 20’ at 4°C. In each pull-down, 10-12 µg of GST-fusion proteins were captured by the GST beads. 12 µg each of the purified 6His-XNudel, 6His-N-XNudel, or 6His-C-XNudel proteins were added to the GST bead mixture and incubated with rotation for another 2 h at 4°C. The beads were washed four times with 1 ml of binding buffer containing 0.1% NP-40 and analyzed by Western blotting probing with anti-6His antibody (1:1000 dilution) to detect Nudel. All steps including washes were carried out at 4°C.

For reciprocal immunoprecipitation in HEK293T cells, the cells were transfected with 10 µg each of pFLAG-hLB1 and pEGFP-hNudel plasmids (control cells were transfected with pFLAG vector and pEGFP-hNudel) or pUHD-hNudel (expresses FLAG-hNudel) and pEGFP-hLB1 plasmids (control cells were transfected with pUHD vector and pEGFP-hLB1). 30–36 h after transfection, nocodazole was added to the medium to a final concentration of 400 ng/ml. Cell lysates were made in lysis buffer (50 mM Tris-HCl, pH7.4, 250 mM NaCl, 1 mM EDTA, 50 mM NaF, 1 mM DTT, 0.1% TritonX-100, and protease inhibitors) 10~12 h after nocodazole addition. 20 µl of settled anti-FLAG M2 affinity resin (Sigma, A2220) was used for immunoprecipitation. The immunoprecipitates were washed 4 times with 1 ml each of wash buffer (50 mM Tris-HCl, pH7.4, 150 mM NaCl, 1 mM EDTA, 1 mM DTT, 0.1% NP-40) and analyzed by Western blotting, probing with antibodies against GFP (rabbit, Santa Cruz, #8334), FLAG (mouse, Sigma, F1804), β-actin (mouse, Sigma, A1978), or α-tubulin (mouse, Sigma, T6199).

Biosensor Binding Studies
The Nudel and LB3-coil2 binding studies were carried out at 25°C using an optical binding sensor (Biacore 3000, Core Facility of Molecular Biology, Shanghai Institute of Biochemistry and Cell Biology) equipped with CM5 sensor chips. Purified 6His-Nudel was coupled to the chip through amine coupling (3000-6000 response unit). GST-LB3-coil2 or GST was exchanged into the injecting buffer (20 mM HEPES, 0.005% P20, 150 mM NaCl, and 3 mM EDTA, pH7.5) by dialyzing against 2L of the buffer with four changes. The proteins were injected at 50 µl/min for 3 min with increasing concentrations. No significant binding was detected for GST. The equilibrium response data for GST-LB3-coil2 were fitted to 1:1 binding site model to obtain the $K_D$ of ~50 µM. The affinity between Nudel and LB3 should be higher than this measured $K_D$ because Nudel binds to both coil2 domain and the LB3T region. Moreover, since lamin forms tetrameric and high-order associations, the interaction between Nudel and lamin B could be further strengthened by multi-valent interactions.

Egg Extract Manipulations and Image Acquisition

For immunoprecipitations, fresh egg extracts that were able to make spindles in the presence of RanGTP or RanGTP plus AurA-beads were used. 30 µg of non-immunized rabbit IgG (Sigma), anti-Nudel antibody, or anti-LB3 antibody was bound to 15 µl of settled Affi-Prep protein A beads (Bio-Rad, #156-0005). After washing with XB buffer, the beads were added to 100 µl of egg extracts in the presence or absence of nocodazole (to depolymerize microtubules) and incubated for 1 h with rotation. The beads were then washed four times with 1 ml each of XB buffer plus 0.1% NP-40. The bound proteins were analyzed using either conventional Western or the One-Step™ Complete IP-Western Kit (GenScript Corporation).

To immunodeplete Nudel and NudE from 100 µl of egg extracts, 50-100 µl of protein A Dynabeads slurry (Invitrogen, #100.02) was bound to 60-100 µg of either rabbit control IgG or anti-Nudel antibody. Over 95% of Nudel/NudE was removed from the egg extracts. Endogenous Nudel/NudE concentration in egg extracts was estimated to be 50-100 nM by analyzing the proteins on the control IgG beads and Nudel antibody beads by SDS-PAGE followed by Coomassie Blue staining. BSA was used as standards. We found that our Nudel and NudE antibodies recognized the purified 6His-Nudel/NudE
much better than the proteins in the egg extract, which could be due to post-translational modifications that mask certain epitopes. For add-back experiments, purified 6His-Nudel was added to the egg extracts to the estimated endogenous Nudel concentration of 50-100 nM. Monoclonal antibody to LB3 was used to detect LB3 containing matrices in the Nudel depletion and add-back experiments.

To immunodeplete LB3 from 100 µl of egg extracts, 100 µl of protein A Dynabeads slurry was bound to 100 µg of either rabbit control IgG or anti-LB3 antibody raised against LB3T. 95-99% of LB3 (estimated at ~200 nM) was removed from the egg extracts.

To inhibit dynein, 70.1 ascites (Sigma, D5167) or control ascites NS-1 (Sigma, M8273) were dialyzed against XB buffer and concentrated using Millipore’s Amicon Ultra centrifugal filter devices to a final concentration of 1-10 mg/ml of antibody. 0.05~0.2 mg/ml (final) of each antibody was added to the egg extracts to inhibit dynein without completely disrupting MT aster assembly. We found an increasing disruption of MT asters as the concentration of 70.1 antibody used was increased. When 70.1 antibody was added at a final concentration of 0.5-2 mg/ml, no or few MTs were found to associate with AurA beads. Since the secondary antibody can recognize mouse IgM, for immunostaining, we directly labeled anti-LB3 and anti-γ tubulin antibodies using Zenon Alexa Fluor 488 (Invitrogen, Z25302) and 647 (Invitrogen, Z25308) rabbit IgG labeling kits. To inhibit Eg5 in egg extracts, 100 µM (final) of Monastrol was used.

Spindle assembly assay and assay for matrix formation were carried out as described previously (Tsai et al, 2006) with some modifications. Briefly, to make AurA-beads, 20-µl of Dynabeads protein A slurry (Invitrogen) was coupled with 20-30 µg of antibody against Xenopus AurA (also called Eg2). After washes, the beads were resuspended in 50 µl of XB buffer. To induce spindle assembly, 1 µl of such resuspended AurA-beads were added into 100 µl of egg extracts and incubated at 4°C with rotation for 30’. This corresponds to 1:250 dilution of the beads from the original slurry. Then, GST-RanL43E (1 mg/ml final), energy mix, and rhodamine tubulin were added to the egg extracts and incubated at room temperature for 3-15’. In general, the spindle matrices are fragile and easily fragmented especially when the quality of egg extracts are not good. We found that the amount of AurA-beads used affected the size of
MT balls and spindles as well as matrix assembly. As the concentration of AurA-beads in the egg extracts increased, there was a corresponding decrease of the size of MT balls and quality of spindles and matrices. We routinely used 1:250 to 400 dilution of the beads (from the slurry). We found that at 1:100 dilution, only small asters and weak spindles (with a few microtubules) were assembled, and after MT depolymerization, very few matrices could be detected.

All images shown were acquired on Leica SP5 confocal microscope at 12-bit resolution with 4-line averaging. To quantify the amount of LB3 on MT structures stimulated by AurA-beads, 25-50 randomly selected structures for each experimental condition were captured using a Hamamatsu camera (C4742-98) driven by the Metamorph software in rhodamine (for MT) and fluorescein (for LB3) channels (chicken anti-LB3 antibody was used for these experiments to avoid the staining of the rabbit Aurora A antibody on the beads by the secondary anti-rabbit antibody). The fluorescence intensity of the structure and the background was measured in each channel. After background subtraction, the LB3 intensity was divided by the MT intensity and used to compare the amount of LB3 on different structures in different experimental conditions.

**Supplementary Figure Legends:**

**Supplementary Figure S1.** Interaction between human lamin B1 and Nudel in HEK293T cells. Nudel and lamin B1 were tagged with either FLAG or GFP and expressed in HEK293T cells. Cells were arrested using nocodazole to enrich for mitotic cells and the cell lysates were used for immunoprecipitation using FLAG antibody-coupled beads. Anti-GFP antibody was used to probe whether FLAG-beads could pull down the GFP-tagged protein. Either actin or tubulin was used as loading controls.

**Supplementary Figure S2.** Sequence comparisons of Nudel from *Xenopus* (xNudel, accession, Q6DK98), human (hNudel, accession, NP_110435), and mouse (mNudel, accession, NP_076157). Identical amino acids are shaded.

**Supplementary Figure S3.** Schematic representation of different *Xenopus* Nudel and
LB3 fusion proteins used in this study. (a) Nudel fusion proteins. (b) LB3 fusion proteins. Each of the fusion proteins was made with either GST-tag at the N-terminus or 6His-tags at both the N- and the C-terminus of the protein. The colored regions represent different domains. Numbers indicate positions of amino acid residues.

**Supplementary Figure S4.** Proteome analysis of the spindle matrix. (a) Proteins identified using SEQUEST and de novo/BLAST were grouped by their known or predicted functions (see Supplementary Table S1 for details). More than a quarter of identified proteins are either uncharacterized or poorly characterized. (b) A list of some spindle assembly factors (SAF), signaling molecules (Signaling), transcription factors (Transcription), and proteins involved in membrane trafficking (Membrane) found in the matrix proteome. Red asterisks indicate proteins that have been confirmed as matrix components by immunostaining and/or Western blotting analyses (data not shown). Consistent with the membranous nature of the matrix, mass-spectrometry identified LB3 as well as a number of proteins involved in membrane trafficking. Among the known spindle assembly factors (SAF) identified, Eg5 and XMAP215 were previously shown as components of the mitotic lamin B-containing matrix. In addition to a large number of proteins that have not been characterized previously, the matrix proteome contains proteins with known or predicted functions in regulating cell polarity, cell signaling, and transcription during animal development.

**Supplementary Figure S5.** Time course analyses of spindle assembly using AurA-beads in the presence of GST or GST-N-Nudel. The definition of early and late asters as well as MT ball and spindles can be found in the main text. Addition of GST-N-Nudel severely blocked the formation of MT ball and spindles. Error bar, standard deviation from >3 independent experiments.

**Supplementary Figure S6.** The xNudel may compensate for xNudE (accession, Q66J96)’s function in mitosis. (a) Sequence comparisons between the full-length xNudE and xNudel. Identical amino acids are shaded. The two proteins are more similar to each other within the N-terminal ~200 amino acids, sharing ~65% identity. (b) Rabbit
antibodies against the N-terminal xNudE or xNudel could recognize both proteins by either immunoprecipitate or immunoblotting, presumably due to cross-reactivity. The asterisk indicates a non-specific band in the egg extracts recognized by the xNudE antibody, which could not be depleted by either xNudE or xNudel antibody. (e) Purified xNudel could rescue the spindle assembly defects caused by immunodepletion using anti-xNudE antibodies. AurA-bead-based spindle assembly assay was shown (see also Fig. 3e), but similar results were observed when using the sperm-based spindle assembly assay (not shown).

Supplementary Figure S7. Nudel enhances the loading of LB3 onto microtubules in a time window between 6’-11’ when the late asters undergo transformation into microtubule balls and spindles. To determine whether Nudel could enhance the loading of LB3 on MTs, we used AurA-bead-based assay. Fluorescence intensity of LB3 was measured and normalized using the rhodamine tubulin intensity of each of the MT structures formed in egg extracts that were mock depleted, Nudel depleted, or Nudel depleted and Nudel rescued. 20-50 randomly selected MT structures at each time point (3’, 6’, 9’, 11’, 13’ and 15’) were analyzed in each condition. Quantifications for four independent experiments are shown. The arbitrary unit represents LB3 intensity after normalizing against rhodamine tubulin intensity on each structure analyzed. The variations in different experiments reflect the variations in the speed of formation and transformation of various structures in different egg extracts. Since depletion of Nudel only transiently affected the recruitment of LB3, other proteins, possibly dynein and dynactin, may also facilitate the loading of LB3 onto microtubules. Error bars, standard error of the mean (SEM). Student T tests (two tailed) were used to calculate the p values.

Supplementary Figure S8. Nudel localizes to the nuclear envelope in prophase in tissue culture cells. HeLa cells cultured either in the absence or presence of nocodazole were immunostained using antibodies to lamin B1 and human Nudel. 5-10 μg/ml of nocodazole were incubated with cells for 1-3 hr to depolymerize microtubules before fixation. White arrows point to nuclei in prophase with Nudel staining the nuclear envelope. Scale bar, 10 μm.
Supplementary Figure S9. Effects of depleting different amounts of LB3 on spindle assembly. (a) A gradual reduction of LB3 from egg extracts by immunodepletion using different amounts of antibodies. 25, 50, 75, or 100 µg of LB3 antibody was used to deplete LB3 in 100 µl of egg extracts, which resulted in the reduction of LB3 from ~200 nM (in the mock depletion using 100 µg of control IgG) to 38, 20, 14, and 10 nM, respectively, in the egg extracts. (b) Reducing LB3 to 14 nM caused a maximum spindle elongation in the AurA-bead-based assay. Error bars, standard deviation from >50 spindles measured. (c and d) Reducing LB3 to 38 nM caused a maximum drop of the percentages of normal spindle assembly (c) and a maximum increase in the percentages of aster and half spindles in the sperm-based assay (d). (e) Reducing LB3 to 20 nM caused a maximum increase in the percentages of multipolar spindle assembly in the sperm-based assay.

Supplementary Figure S10. A model for assembly of MT and lamin B spindle matrix during spindle morphogenesis in both ‘open’ and ‘closed’ mitosis. In ‘open’ mitosis, the nuclear envelope remnants are organized along MTs by Nudel and dynein to assemble into the mitotic spindle matrix, which helps to organize MTs. By tethering to the membrane system and nuclear proteins, the spindle matrix could also facilitate the partitioning of both membrane compartments and nuclear contents during division. In ‘closed’ mitosis (S. cerevisiae mitosis as an example), the intact nucleus might play an equivalent function to the spindle matrix. Here, dynein and Nudel are known to regulate the orientation of the mitotic spindle/nucleus for proper cell division.

Legend to Supplementary Table S1:
This table lists proteins identified by mass spectrometry. For proteins identified by de novo BLAST, the E-value was provided in the third column. For proteins identified by SEQUEST, protein sequence coverage was listed on the fourth column. The proteins are categorized according to their known or predicted functions. ‘Spindle Assembly Factors/cytoskeleton-Associated’ includes proteins with functions in regulating mitotic spindle assembly, microtubule, actin, or intermediate filaments. ‘DNA/RNA-
Associated’ includes proteins with functions in regulating DNA replication, transcription, chromatin structure, RNA processing, or translation. ‘Protein Modification/Stability’ includes proteins with functions in regulating post-translational protein modifications, protein folding, or protein degradation. ‘Proteins Involved in Signaling’ includes proteins with functions in regulating signal transduction pathways. ‘Proteins Involved in Transport’ includes proteins with functions in regulating membrane-related functions such as membrane trafficking and nucleocytoplasmic transport. ‘Proteins in other Categories’ includes transporter proteins and proteins that regulate metabolism. ‘Uncharacterized/Poorly Characterized Proteins’ includes proteins with no known or only predicted functions based on limited domain homology.

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