Supplemental Methods

Construction of different expression vectors

The cDNA encoding mBuGZ was cloned into pET-30a to express 6His-GFP-tagged fusion protein in bacteria. The constructs that express His-mBuGZ, His-xBuGZ and GST-mBuGZ in bacteria have been described previously (Jiang et al., 2014). The 9 Y and 5 F residues from the 93 amino acid (aa) to 464 aa of the wild type mBuGZ (isoform 3 as listed in the protein database) were changed to S by synthesizing the mutated mBuGZ14S cDNA (Biosune Biotechnology Co). The mBuGZ14S cDNA contained in the pUC57-Simple plasmid was shipped to us from the company and confirmed by further sequencing. To create Flag-mBuGZ14S for mammalian expression, we first created Flag tag sequence using primers synthesized by Invitrogen and subcloned it into the pCDNA3 vector at the HindIII and EcoRI sites. Then the cDNA encoding mBuGZ14S was amplified by PCR from pUC57-simple plasmid and subcloned into the pCDNA3-Flag vector at the BamHI and XhoI sites. The Flag-mBuGZ14S-res1 that is insensitive to the siRNA oligo 1 (Jiang et al., 2014) was created using the Quick Change Site-directed Mutagenesis Kit (Agilent Technologies, #200518). The last Y in Flag-mBuGZ14S-res1 was mutated into S using the same site directed mutagenesis to create Flag-mBuGZ15S-res1. To create His-GFP-xBuGZ-A, -B, and -C and His-GFP-xBuGZ-A3S, -B3S, and -C5S for bacterial expression, the cDNA encoding GFP was amplified by PCR with primers that harbored a 5' BglII site and a 3' BamHI site and was subcloned into the BamHI site of the pET30a plasmid. The cDNA encoding each xBuGZ fragment was amplified by PCR using the corresponding wild type and mutant xBuGZ cDNA as templates and subcloned into GFP-pET30a vector at the EcoRI and XhoI sites. All plasmid constructs were transformed into DH5α for plasmid purification and sequencing. The primer sequences and restriction sites used for each of the constructs are listed in Table S1.

The construct for YFP-xBuGZ expression in baculovirus was made in three steps. First, the cDNA encoding YFP was amplified by PCR with primers that harbor a 5' BglII site and a 3' BamHI site and subcloned into BamHI site of pFASTBacHTb vector (Invitrogen) behind the 6His tag already present in the vector. Second, xBuGZ was amplified by PCR and subcloned into YFP-pFASTBacHTb vector at BamHI and NotI

sites. The internal BamHI site of xBuGZ was eliminated by PCR mutagenesis prior to subcloning without changing amino acid sequence. Third, a short double-stranded DNA encoding a flexible amino acid linker containing 3×GGGGS was made from two complimentary DNA oligos with cohesive ends compatible to BglII and BamHI at 5' and 3' end, respectively. After annealing at 65°C, this short double-strand DNA was inserted into the BamHI site of the above-mentioned YFP-xBuGZ-pFASTBacHTb vector by ligation. The final construct expresses His-tagged (at the N-terminus) YFP-xBuGZ fusion protein in which the two fusion moieties of YFP and xBuGZ were separated by a 15 amino acid flexible linker.

The above YFP-xBuGZ construct was transformed into DH10Bac *E. coli* to generate recombinant bacmid according to the protocol in the user's manual of Bac-to-Bac Baculovirus Expression system (Invitrogen). The bacmid DNA was purified using Pure-link DNA isolation kit (K2100-02, Invitrogen). YFP-xBuGZΔN construct was created based on the above-mentioned YFP-xBuGZ-pFASTBacHTb construct in which the full-length xBuGZ was replaced by a truncated xBuGZ (a.a. 93-452) using 5' BamHI and 3' NotI as cloning sites. Constructs expressing YFP-xBuGZ5S and YFP-xBuGZ13S were created by multiple rounds of PCR-based mutagenesis to convert either the last 5 or the last 13 phenylalanine (F) and tyrosine (Y) within the disordered region of xBuGZ (a.a. 123-452) to serines (S). Mutagenesis were made using PCR by including a desired change (F/Y>S/S) in one of the primers. As the primers were extended by PCR, the resulting amplification products incorporated the mutation, replacing the original sequence. The mutated PCR product was purified with Zymoclean gel DNA recovery kit (D4002, Zymo Research) and was subjected to second round of mutagenesis using primers containing another "F/Y>S/S" substitution. The PCR product was subcloned into YFP-xBuGZ-pFASTBacHTb construct after two to three rounds of PCR amplifications and mutagenesis were confirmed by DNA sequencing. The plasmid DNA containing "F/Y>S/S" mutations was used as template for a new round of mutagenesis until all of desired mutations had been obtained. All constructs were confirmed by DNA sequencing and all primers used in the PCR are listed in Table S1.

Cell culture and manipulations

For BuGZ depletion and rescue we used our previously published BuGZ siRNA oligo-1 (GCC UGC UAC ACU UAC AAC AAC UAG U), control oligo (Life Technologies cat# 12935-300) and a Bub3 oligo (CCG GUU CUA ACG AGU UCA AGC UGA A). Briefly, 50 pM of RNAi oligos were transfected into cells using Lipofectamine RNAiMAX (Invitrogen). To rescue BuGZ expression, 12 h after transfection of siRNA, Lipofectamine 3000 (Life Technologies) was used for transfection of RNAi insensitive FLAG-mBuGZ, called Res1 (Jiang et al., 2014), Flag-mBuGZΔN, Flag-mBuGZAA, or Flag-mBuGZ15S into cells. The cells were analyzed 72 h after siRNA transfection.

To assay for extractability of BuGZ in mitotic HeLa cells at room temperature or on ice, HeLa cells were treated by taxol (20 μM final) for 5 min at 37°C. They were then incubated either at room temperature or on ice for 5 min followed by extraction with PEM buffer (100 mM PIPES pH6.8, 5 mM EGTA, 2 mM MgCl2, 5 nM taxol, 0.05% Triton X100) for 20 sec. The mitotic cells were collected by shake-off and then processed for Western blotting.

To assay for temperature sensitivity of BuGZ and MT interaction, HeLa cells were first treated with MG132 (10 μM final) to enrich for metaphase cells. Cells were then incubated at room temperature or on ice for another 5 min in the presence of taxol (20 μM final) to stabilize MTs. For detergent extraction, cells were further incubated with PEM buffer (100 mM PIPES pH6.8, 5 mM EGTA, 2 mM MgCl2, 5 nM taxol, 0.05% Triton X100) for 20 sec at room temperature followed by fixation and then processed for immunofluorescence microscopy or by Western blotting.

To test MT re-growth after cold-mediated depolymerization, 330 nM nocodazole in DMEM complete medium was added 56 h after siRNA and/or plasmid transfection, and incubation was continued for another 16 h at 37°C. The cells were washed twice with cold complete DMEM medium, and incubated further in DMEM medium for 10 min on ice. The cells were then incubated with pre-warmed (37°C) complete DMEM medium at 37°C for 3 or 9 min followed by immunostaining.

Protein expression, purification, and interaction studies

For expression and purification of His-mBuGZ, His-xBuGZ, His-GFP-mBuGZ, His-GFP-xBuGZ-B, His-GFP-xBuGZ-B3S proteins, plasmids were transformed into BL21 (DE3). For each protein expression, a single colony of *E. coli* was used to inoculate a 20 ml of LB culture. After 12-15 h of incubation at 37°C with vigorous shaking, the culture was added into a 2-L fresh and pre-warmed LB medium for further incubation at 37°C with shaking. When OD_{600} reached 0.6-1, IPTG was added to the culture to a final concentration of 1 mM to induce protein expression by incubating the culture with shaking at 16°C for 16 h.

Bacteria were collected by centrifugation at 6000 rpm for 15 min at 4°C. The bacteria pellet was resuspended in 100 ml of ice-cold lysis buffer (50 mM NaH2PO4, 300 mM NaCl, 5 mM imidazole pH 7.8) containing 1 mM PMSF and 1 to 100 dilution of protease cocktail (AEBSF, Bestatin, E-64, Pepstain A, Phosphoramidon, cat# P8849, Sigma) by pipetting on ice. After sonication (Misonix, Sonicator 3000) to break bacteria on ice until the lysis buffer became clear (~15 min), the lysate was centrifuged at 12000 rpm in a Beckman JA20 rotor for 30 min at 4°C. The supernatant was transferred to a fresh tube containing 4 ml of 50% slurry of Ni-NTA beads (Qiagen). After rotating the mixture at 4°C for 4 h, the mixture was transferred to a column (Glass Econo-Column®, Bio-RAD) to allow the settlement of the beads. The column was gently tapped to dislodge any trapped air bubbles. The column was washed with 100 ml wash buffer (50 mM NaH2PO4, 300 mM NaCl, pH7.8) with 10 mM imidazole and then 100 ml wash buffer with 20 mM imidazole. His-tagged proteins were eluted with 5 ml Elution buffer (50 mM NaH2PO4, 300 mM NaCl, 300 mM imidazole, pH7.8) in 0.5 ml fractions. The peak fractions were exchanged into 2 ml of XB or PBS buffer using a PD10 column (GE Healthcare), followed by further concentrating to 100~350 μM with Amicon Ultra 30K device (Millipore) at 4°C. The protein stocks were divided in 5~10 μl aliquots, snap frozen in liquid nitrogen, and stored at -80°C.

The same procedure as above was used to induce the expression of GST-mBuGZ. 100 ml of ice-cold PBST (PBS plus 1% Triton X-100) containing 1 mM PMSF and the protease cocktail (as above) were used to resuspend and sonicate bacterial pellet as above. After centrifugation, bacterial lysates were added to 4 ml of the 50% slurry of Glutathione Sepharose 4B and incubated with rotation at 4°C for 3 h. After packing the

Glutathione Sepharase beads into a column, the beads were washed by 200 ml PBS. To elute proteins, 5 ml of Glutathione Elution Buffer (50 mM Tris-HCl, 10 mM reduced glutathione, pH8.0) was added and incubated at 4°C for 2 h. The eluted fractions were exchanged into 2 ml of XB buffer with a PD10 column (GE Healthcare), and further concentrated to 100~200 μM using Amicon Ultra 30K device (Millipore) at 4°C and divided in 5~10 μl aliquots, snap frozen in liquid nitrogen, and stored in -80°C.

Production of recombinant baculovirus and expression of wild type (YFP-xBuGZ) and mutant fusion proteins (YFP-xBuGZΔN, YFP-xBuGZ5S, YFP-xBuGZ13S) were carried out based on the user manual of Bac-to-Bac Baculovirus Expression system (Invitrogen). The Sf9 cells were grown in Sf-900 II medium (10902-104, Invitrogen) to reach log phase (1.5-2.5×10^6 cells/ml). Cells were seeded in a 6-well plate with a density of 8×10^5 cells per well. 1 μg/well of bacmid DNA was transfected into Sf9 cells using CellFectin II (10362-100, Invitrogen) according to manufacturer's instructions. Cells were incubated at 28°C for 72-96 h until the expression of YFP was clearly visible under an inverted fluorescent microscope. The culture medium that contains baculovirus (P1) was collected and mixed with 10 ml of fresh Sf-900 II medium. This medium mixture was distributed into a 6-well plate (2 ml per well) that was plated with freshly prepared log-phase cells at a density of 2×10^6 cells per well. After incubated at 28°C for 48-72 h, the medium (also known as P2 viral stock) was combined, clarified by centrifuging at 1,000 ×g for 5 min at room temperature, and then stored in 1 ml aliquots at -80°C. 1 ml of the P2 viral stock was added into 50 ml suspension culture at 2×10^6 cells/ml and incubated at 28°C for 48-72 h to produce the P3 viral stocks. The P3 viral stock was titrated according to Hopkins and Esposito (Hopkins and Esposito, 2009). The titers of P3 viral stocks (stored at -80°C as 5-10 ml aliquots) were usually in the range of 10^8-10^9/ml. For protein production, a 400 ml of Sf9 cell suspension was cultured to a density of 1.5~2.5×10^6 cells/ml, and was infected with P3 virus at MOI >10, and continued to culture for additional 48 h. Sf9 cells were harvested by centrifugation at 1,000 ×g for 10 min and resuspended in 40 ml of His-binding buffer (20 mM phosphate buffer, pH 7.4, 1 mM MgCl2, 1 mM β-mercaptoethanol, 0.01% Triton X-100, 0.5 M NaCl, 25 mM imidazole, 10% glycerol) supplemented with 1 mM phenylmethylsulfonyl fluoride (PMSF), 10 μg/ml each of leupeptin, pepstatin, and chymostatin. Cells were lysed by

passing through a high-pressure homogenizor (Avestin EmulsiFlex C3) twice at 500 psi. Cell lysate was centrifuged at 14,000 rpm for 45 min at 4°C. The supernatant was loaded onto a column packed with 5 ml (slurry volume) nickel-nitrilotriacetic acid (NTA) resin (Qiagen) and followed by washing with 20 volume of His-binding buffer. The protein was then eluted with 5×2 ml of His-elution buffer (same as His-binding buffer except with 500 mM imidazole). The eluted fractions were combined and concentrated by Amicon Ultra 30K device (Millipore) to a final of 2-3 ml.

For further purification of the above protein, 1 ml of the protein solution was loaded onto a size exclusion column (Superdex-200, GE healthcare), and eluted with His-binding buffer in 1 ml fractions at 0.4 ml/min by FPLC (AKTA^{TM}, GE Healthcare). The peak of YFP-BuGZ was eluted at fractions 10 and 11 of total 32 fractions. The FPLC purification process was entirely operated in cold room. Fractions 10 and 11 were combined and were exchanged into the XB buffer using PD-10 column (GE Healthcare), and further concentrated to >200 μM concentrations with Amicon Ultra 30K device (Millipore) at 4°C. The protein was divided into 5-50 μl aliquots, snap-frozen in liquid nitrogen and stored at -80°C.

To study whether BuGZ, BuGZΔN, or BuGZ13S directly binds to tubulin, 20 μl of 2.5 μM purified YFP-xBuGZ or YFP-xBuGZ-ΔN were bound to 60 μl Ni-NTA bead slurry via their His tag by incubating 2.5 h at 4°C. After incubation, the protein-coated beads were washed with 1 ml of lysis buffer (50 mM NaH_2PO_4, 300 mM NaCl, 10% Glycerol, 5 mM imidazole, 0.5% Triton, 1 mM PMSF, 1 mM $MgCl_2$) for 4 times and 1 ml of wash buffer (50 mM NaH_2PO_4, 300 mM NaCl, 10% Glycerol, 10 mM imidazole, 1 mM PMSF, 1 mM $MgCl_2$) for another 4 times. 25 μl of 2 μM tubulin were added to the beads, followed by incubation for 1.5 h at 4°C in a final volume of 200 μl of the lysis buffer. The beads were then washed with 1 ml of lysis buffer followed by 1 ml of wash buffer for 3 times each. 30 μl of SDS buffer were added into the pelleted bead. 10% of the initial mixture was loaded as input and 33% of each pull-down samples were loaded for SDS-PAGE and Coomassie blue staining.

Xenopus egg extract assays

Spindle assembly assays were based on previous studies (Ma et al., 2009; Tsai and Zheng, 2005). To prepare Aurora A beads, 20 μl of Dynabeads protein A slurry (Invitrogen, mixed well before use) were incubated with 30 μg of *Xenopus* Aurora A (Eg2) antibody for at least 2 h at room temperature. After washing with XB buffer for 3 times, the beads were resuspended in 50 μl of XB buffer. To induce spindle assembly, 1 μl of the resuspended Aurora A beads or 1 μl sperm chromatin (~250 sperm/μl final) was added into 100 μl of egg extracts that were mock-depleted, depleted of xBuGZ and supplemented with wild type or mutant xBuGZ (0.1 μM final), or containing GFP-BuGZ-B or GFP-BuGZ-B3S (2 μM final). For Aurora A bead assays, egg extracts were incubated at 4°C with rotation for 30 min followed by addition of purified RanL43E (1 mg/ml final), energy mix (10 mM creatine phosphate, 0.5 mM Na-ATP, 0.5 mM MaCl2, 50 μM EGTA, pH7.7), and rhodamine tubulin (1 μM final) to the egg extracts with beads and then incubated at room temperature for 5 min for MT aster assembly or 12 min for spindle assembly. After the incubation, 10 μl of egg extracts were then diluted with 1 ml BRB80 (80mM PIPES, 1mM $MgCl_2$, 1mM EGTA, pH6.8) buffer containing 30% glycerol. The mixture was spun through 2 ml cushion of BRB80 with 60% glycerol onto coverslips. The MT structures on coverslips were fixed with ice-cold methanol for 5 min. For sperm spindle assembly, the egg extracts were incubated at room temperature for 60-90 min. The egg extracts were diluted and the structures spun onto coverslips and fixed as described for Aurora A spindles.

The spindle matrix isolation and assays were based on previous studies (Ma et al., 2009; Tsai and Zheng, 2005) with some modifications. Briefly, spindle assembly was induced by Aurora A beads and RanL43E in 100 μl egg extracts for 12 min. 10 μl of the egg extract was diluted into 1 ml XB buffer containing nocodazole (20 μg/ml final) and incubated at room temperature or placed on the ice for 15 min to depolymerize MTs. The mixture was then layered onto the cushion and the bead-associated spindle matrix was spun onto coverslips as described above. The spindle matrix was fixed by ice-cold methanol as above and processed for immunostaining with antibodies to known spindle matrix components (see Table S2 for the source of antibodies and its dilutions). For Western blotting the whole 100-μl reaction was used.

To test whether temperature affects the binding of BuGZ to MTs, 12 min after spindle assembly induced by Aurora A beads, 10 μl of egg extracts were then diluted with 1 ml BRB80 buffer containing 30% glycerol that was either pre-warmed at RT or pre-cooled on ice. The mixtures were incubated at RT or 0°C for another 5 min, followed by spinning through a 2-ml cushion of BRB80 and 60% glycerol onto coverslips, and then processed for immunostaining.

To isolate spindle matrix for Western blotting analyses, 200 μl of mock depleted, BuGZ depleted and rescued (by various proteins as above) extracts or egg extracts containing 2 μM GFP-BuGZ-A, -B, -C or their mutants were used to induce spindle assembly in the presence of Aurora A beads and RanL43E. After 12 min incubation, the egg extract was then diluted into 2 ml of BRB80 buffer with 30% glycerol at RT. Spindles associated with Aurora A beads were collected with a magnet (for 5 min at room temperature). The collected Aurora A-associated MT structures were then washed with 2 ml of BRB80 buffer with 30% glycerol for three times. 2 ml of XB buffer containing 20 μg/ml nocodazole were added to depolymerize MTs at RT or on ice for 15 min. The bead associated spindle matrix was washed with 2 ml of XB buffer containing 20 μg/ml nocodazole two times, then 2 ml of XB for two more times. The beads were then re-suspended in 30 μl of XB buffer and pipetted repeatedly to release the spindle matrix from the Aurora A beads. After removing the beads by magnet, the supernatant was mixed with 30 μl of 2× SDS sample buffer. 10 μl of this sample were analyzed by Western blotting using the antibodies to the spindle matrix markers (see Table S2 for the antibodies sources and dilutions).

To extract xBuGZ on spindles assembled from Aurora A beads, 10 μl of spindle assembly reaction mix were diluted with 1 ml of ice-cold or pre-warmed (RT) BRB80 buffer containing 30% glycerol and Taxol (20 μM final). The mixture was incubated either on ice or at RT for another 5 min and then spun through a 2-ml cushion of BRB80 with 60% glycerol onto coverslips at room temperature. The coverslips were processed by fixation with ice-cold methanol for 5 min followed by immunostaining for xBuGZ.

Assays for BuGZ phase transitions *in vitro*

5 μl of wild type or mutant mBuGZ or xBuGZ at the indicated concentrations were prepared by diluting the stock solutions into ice-cold XB or PBS buffer. To test whether GFP-xBuGZ-B could dominant negatively inhibit droplet formation, 25 μM of YFP-xBuGZ were mixed with either 25 μM or 50 μM of xBuGZ-B or xBuGZ-B3S in a final volume of 10 μl on ice in the ice-cold XB. After incubation of the mixtures at 37°C for 5 min, 3 μl of the mixture were loaded into a flow cell made with two pieces of double sticky scotch taps and imaged immediately using a SP5 confocal (Leica) under DIC and fluorescence with resolution set to 12 bit. Fluorescence images were taken using the Leica/EYFP or Leica/ALEXA 568 with laser power set at 15%, while the DIC images were taken using Scan-DIC (laser power 18%) with the sequential scan and format 8192×8192. For DIC imaging, the light angle was set to be the same for all samples imaged based on the shadows around the edge of the droplets.

To assay whether YFP-xBuGZ and its mutant proteins exhibit different abilities to incorporate into preformed His-xBuGZ droplets, we first used 25 μM His-xBuGZ to form droplets. We then determined the liquid phase concentration of His-xBuGZ to be ~2.5 μM. To assay for protein exchange into the droplets, we first formed His-xBuGZ droplets and then added 2.5 μM YFP-xBuGZ or its mutants, and incubated at RT for 5 min. Droplets were imaged using SP5 confocal and quantified.

To assay whether GFP-xBuGZ-A, -B, -C fragments and their mutants could incorporate into and disrupt preformed His-xBuGZ droplets, 10 μl His-xBuGZ (25 μM final) was incubated at 37°C for 5 min to form droplets. 25 μM each GFP-xBuGZ fragments or the mutants were added to the reaction and incubated for another 5 min at RT. Droplets were imaged using SP5 confocal and quantified.

To visualize the temperature dependent phase transitions, 100 μM YFP-xBuGZ was placed in a flow chamber and sealed with Beewax. The sample was placed in a modified temperature-controlled stage on an inverted microscope (Nikon Eclipse TE200). The images were captured using 60× oil lens using Hoffman modulation contrast. The temperature was increased from 4-20°C at 1°C/min using a Physitemp TS-4 controller.

To estimate protein concentrations in the BuGZ droplets, different concentrations of YFP-xBuGZ alone or a mixture of YFP-xBuGZ and cycled tubulin were prepared in the XB buffer in the presence of 20 μg/ml nocodazole at 4°C and placed on ice (each

reaction is 100 μl total). The protein samples were then incubated at 37°C for 5 min. The droplets were pelleted by centrifugation at 2,000 rpm for 5 min on a bench top microfuge. For protein mixtures with high concentrations of BuGZ, the droplets formed a yellow pellet, which made it easy to remove the supernatants. In this case 95 μl of the supernatant were carefully removed. When it was not possible to see the pellet because not many droplets were formed, 97 μl of the supernatant were removed. Care was taken to avoid the disruption of the settled droplets. 500 μl of 2× SDS sample buffer were added to resuspend each pellet. 5 μl were analyzed by SDS-PAGE followed Coomassie blue staining. The estimated protein concentration in the pellet represents an underestimation because it was not possible to completely remove all the supernatant.

Assays for the phase transition property of BuGZ in spindle and spindle matrix

To assay for protein incorporation into spindles, Aurora A spindles were retrieved from 10 μl reaction using magnet for 5 min and re-suspended with 50 μl XB buffer briefly and then retrieved again by magnet for 5 min. After removing the buffer, the spindles were resuspended in 50 μl XB buffer containing different YFP-xBuGZ proteins (0.1 μM final) or different GFP-xBuGZ fragments (0.5 μM final) and incubated for 5-10 min followed by dilution in 500 μl of BRB80+30% glycerol and spin-down through a 2-ml cushion (as above) onto coverslips for microscopic analyses of incorporations of YFP or GFP-tagged proteins. For spindle matrix incorporation assays, the retrieved spindles were first washed and retrieved as above. The spindles were then resuspended in 50 μl XB buffer containing 20 μg/ml nocodazole and incubated at RT for 10 min to depolymerize MTs. Different YFP-xBuGZ proteins (0.1 μM final), GFP-xBuGZ fragments (0.5 μM final), and tubulin (20 μM final) were added into the spindle matrix and incubated for another 5-10 min at RT. The matrices were diluted in 1 ml BRB80+30% glycerol and spun through a 2-ml cushion (as above) onto coverslips for microscopy or retrieved by magnet for Western blotting analyses (100-μl spindle reaction). For MT assembly assays, the retrieved spindle matrices (from 10-μl spindle reaction) were re-suspended in BRB80 containing 25 μM tubulin (labeled/unlabeled: 1/5)

and incubated at 30°C for 15 min followed by dilution in 1 ml BRB80+30% glycerol and spun through a 2-ml cushion (as above) onto coverslips for microscopy.

Estimation of xBuGZ concentration in Aurora A asters and spindles

MT asters and spindles were induced by Aurora A beads. Half of the reaction was diluted and spun down onto coverslips to allow both 3D imaging by confocal microscopy and counting the number of structures, while the other half was retrieved by magnet and washed with 500 μl XB buffer two times at room temperature. The bead-associated MT asters and spindles were analyzed by Western blotting to determine the amount of xBuGZ using His-xBuGZ as standards. To estimate the number of MT asters and spindles, structures under the coverslip were counted under a microscope. MT asters appeared as ball-like structures by 9-15 min of reaction (Ma et al., 2009). Since spin-down flattened MT balls and spindles, we estimated the volumes of both structures by treating them as cylinders. The volumes were determined by multiplying the bottom surface area by the height of each structure. The average volumes of MT balls or spindles were determined from 5 structures each in each of three experiments.

Assays for tubulin-MT binding, MT polymerization, bundling, and binding to BuGZ

Protein purification from HEK293T cells and the MT spin-down assay to determine mBuGZAA and MT binding were described (Jiang et al., 2014). To assay for tubulin and xBuGZ interaction, 50 μM purified His-tagged YFP-xBuGZ, YFP-BuGZΔN, or YFP was incubated with beads coated with anti-6His antibody at 4°C for 2 h. The beads were washed with XB buffer three times. The beads were then incubated with 20 μM cycled tubulin plus 20 μg/ml nocodazole and incubated at 4°C for 2 h. After washes in XB buffer three times, proteins remained on the beads were analyzed by Coomassie blue staining.

To assay MT polymerization stimulated by BuGZ, different concentrations of purified wild type or mutant YFP-xBuGZ were mixed with 5-30 μM (final concentration) cycled tubulin (5 unlabeled tubulin:1 rhodamine tubulin) in BRB80 with a final concentration of 1 mM GTP in a total of 5 μl of reaction mix. After incubating the mixture at 37°C for 5 min, 45 μl of 1% glutaraldehyde (in BRB80) were added to fix the

tubulin by gentle mixing to prevent the breakage of the MTs. 3 μl of the MT containing solution were gently squashed into a thin and even layer under 18×18 mm coverslips for microscopic analyses.

To make taxol stabilized MT seeds for MT bundling assay, we followed the protocol of the Kinesin Motility Assay Biochem Kit (Cytoskeleton) with modifications. Briefly, 2 μl of the Cushion Buffer (80 mM PIPES, 2 mM $MgCl_2$, 0.5 mM EGTA, 60% glycerol, pH 6.9) containing 1 mM GTP were added to 2 μl of the tubulin stock to yield a final concentration of 18 μM tubulin. The mixture was incubated at 35°C for 15 min and then diluted by 100 μl of the pre-warmed (RT) General Tubulin Buffer (80 mM PIPES, 2 mM MgCl2, 0.5 mM EGTA, pH 7.0). Taxol was added to this mixture to a final concentration of 20 μM. This mixture was then layered onto the 400 μl of the Cushion Buffer containing 20 μM Taxol and centrifuged at 100,000 ×g at 25°C for 30 min to separate the MT seeds from the tubulin. After removing the supernatant, the pelleted MT seeds were resuspended into 100 μl of General Tubulin Buffer containing 20 μM taxol.

To assay for MT bundling, 5 μl of taxol stabilized MTs were mixed with wild type or mutant YFP-xBuGZ at different final concentrations. After incubating the mixture at 37°C for 5 min, 3 μl of the mixture were gently squashed under the coverslip for microscopy analyses.

To visualize the binding of wild type and mutant YFP-xBuGZ to MTs by microscopy, 5 μl of the taxol stabilized rhodomain MTs were mixed with 4 μM (final) YFP-xBuGZ, YFP-xBuGZ13S, or YFP-xBuGZΔN. The BuGZ proteins were diluted with the General Tubulin Buffer containing 20 μM taxol and the final MT protein mixture was 10 μl. After incubating the mixture at 37°C for 5 min, 2-3 μl of the mixture were either directly placed onto coverslip for visualization or the whole 10-μl reaction was diluted by 90 μl of the General Tubulin Buffer containing 20 μM taxol and 1% glutaraldehyde, and layered onto 400 μl of the Cushion Buffer containing 20 μM Taxol and centrifuged at 100,000 ×g at 25°C for 30 min at 25°C in a bench top ultracentrifuge (Beckman). After removing the supernatant, the pelleted MTs were gently resuspended in 10 μl of the General Tubulin Buffer containing 20 μM taxol. 3 μl of the resuspended MT solution were gently spread into a thin and even layer under an 18×18-mm coverslip for

microscopic analyses immediately. Fixation and spin down tend to deform the droplets formed on MT bundles.

Sequence analysis for protein disorder and low complexity

The PONDR program (http://www.disprot.org/index.php) was used to analyze disordered regions of BuGZ and other spindle matrix proteins at default settings (Xue et al., 2010). All four predictors (PONDR-FIT (P-FIT), VLXT, VL3, and VSL2B) indicated that these proteins have a high disorder disposition. The low complexity region of BuGZ and other spindle matrix proteins were analyzed using the SEG program (http://mendel.imp.ac.at/METHODS/seg.server.html) (Wootton, 1994). Three outputs (LC1, LC2, and LC3) were determined at three levels of stringency.

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