

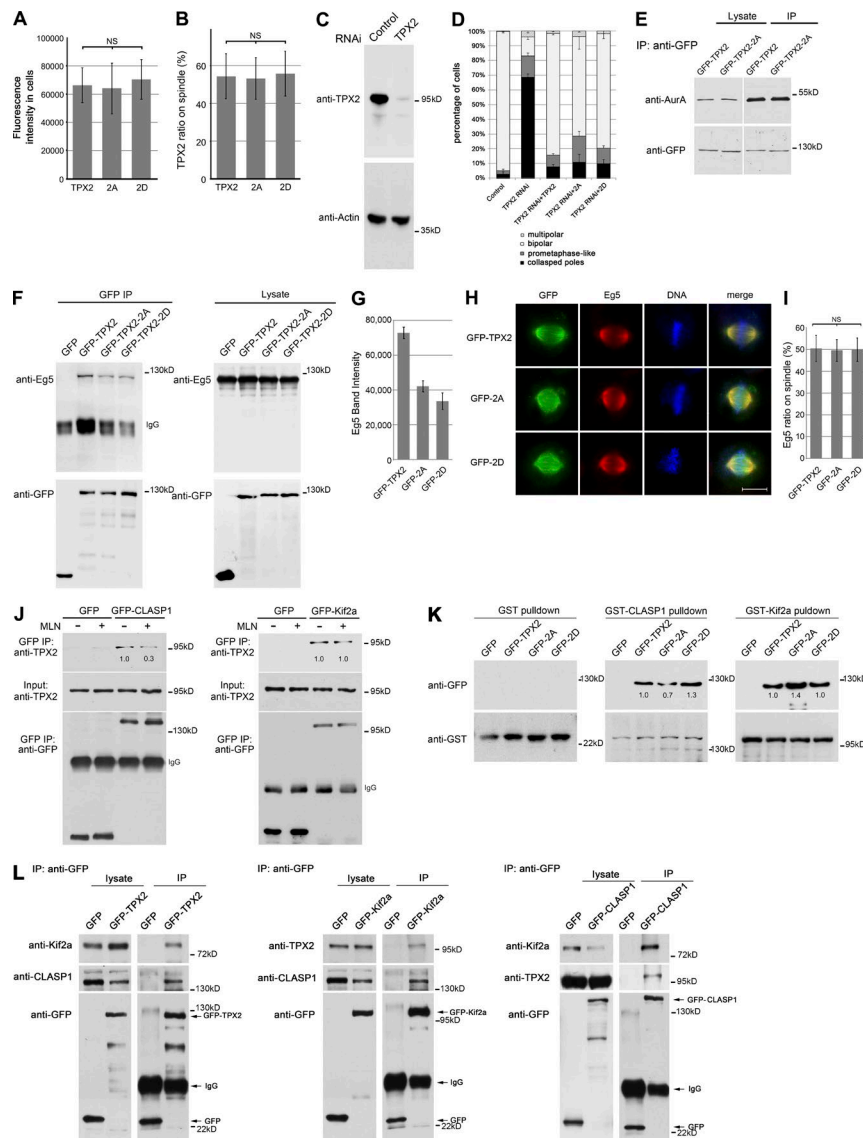
Fu et al., <http://www.jcb.org/cgi/content/full/jcb.201412109/DC1>

Figure S1. Assessing the binding capacity of TPX2 phospho mutants to its interacting partners. (A and B) Isogenic stable HeLa cell lines were induced to express RNAi-resistant GFP-TPX2, -2A, and -2D using tetracycline and fixed. Quantification of total fluorescence of TPX2 in single mitotic cells shows that the expression levels of exogenous GFP-TPX2, -2A, and 2D are comparable (A), and the proportion on spindle MTs reveals that all three proteins localize to the spindle with similar affinity (B). Error bars indicate SD. (C) HeLa cells were transfected with scramble or TPX2 siRNA, and the efficacy of RNAi was assessed 48 h later by Western blotting. TPX2 protein level was reduced by 90%. (D) Isogenic stable HeLa cell lines were depleted of endogenous TPX2 and induced to express RNAi-resistant GFP-TPX2-WT, -2A, and -2D. These cells were synchronized in prometaphase by thymidine followed by Eg5 inhibitor, STLC, and then released into medium that contained MG132 to allow full establishment of bipolar spindles. Spindle morphology was quantified according to α -tubulin staining. Note that expression of exogenous TPX2-WT, -2A, or -2D greatly rescues the defect of collapsed poles. Three independent experiments were performed, each measuring no less than 100 cells. Error bars indicate SD. (E) Mitotic HeLa cell lysate transfected with GFP-TPX2 or GFP-TPX2-2A was immunoprecipitated with GFP antibody. The immunoprecipitated complexes were subjected to Western blotting for Aurora A (top) or GFP (bottom) antibody. TPX2 and 2A bind to Aurora A with similar affinity. (F and G) Mitotic HeLa cell lysate transfected with GFP-TPX2, -2A, or -2D was immunoprecipitated with GFP antibody. The immunoprecipitated complexes were subjected to Western blotting for Eg5 (top) or GFP (bottom) antibody. TPX2-2A and -2D bind to Eg5 with similar affinity, albeit less strong than WT. (H and I) Isogenic stable cell lines were induced to express RNAi-resistant GFP-TPX2, -2A, and -2D, fixed, and immunostained for Eg5 and DNA. Quantification of the proportion of Eg5 on spindle MTs shows that Eg5 localizes to the spindle similarly when different TPX2 constructs are expressed. Error bars indicate SD. Bar, 10 μ m. (J) Mitotic HeLa cells transfected with GFP-CLASP1 (left) or GFP-Kif2a (right) were treated with DMSO or MLN8237 (MLN) for 30 min before performing immunoprecipitation with GFP antibody. The immunoprecipitated complexes were subjected to Western blotting for TPX2 (top) or GFP (bottom). Note that CLASP1 binds 70% less TPX2 in the presence of MLN, whereas Kif2a binds a similar amount of TPX2 with or without MLN pretreatment. (K) Recombinant GST, GST-CLASP1, GST-Kif2a and His-GFP, His-GFP-TPX2-WT, His-GFP-TPX2-2A, and His-GFP-TPX2-2D were purified from *E. coli* and subjected to an in vitro GST pull-down assay. The pull-down complexes were analyzed by Western blotting for GFP and GST. Note that both CLASP1 and Kif2a directly interact with TPX2, and phospho-null TPX2 binds less CLASP1 and more Kif2a in vitro. (L) Mitotic HeLa cell lysate transfected with GFP-TPX2, GFP-Kif2a, or GFP-CLASP1 was immunoprecipitated with GFP antibody. Note that GFP-TPX2 pulls down endogenous CLASP1 and Kif2a (left), GFP-Kif2a pulls down endogenous TPX2 and CLASP1 (middle), and GFP-CLASP1 pulls down endogenous TPX2 and Kif2a (right).

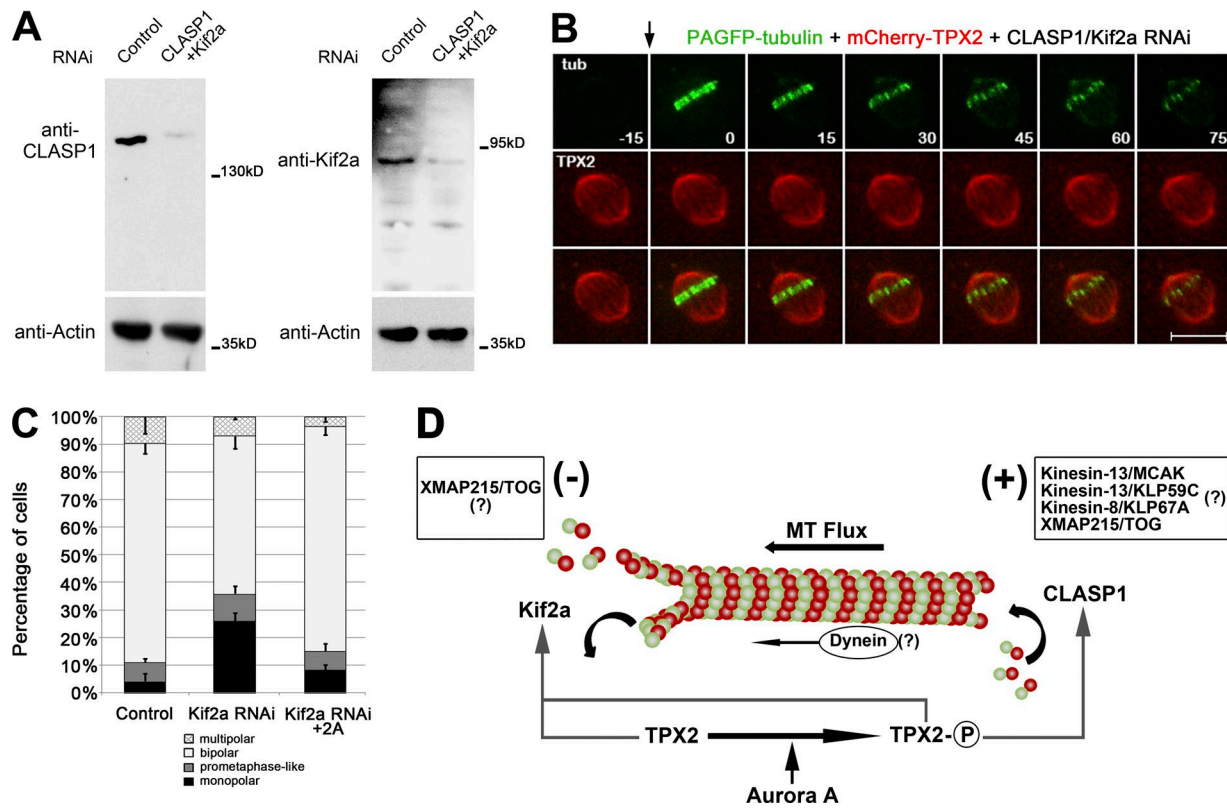


Figure S2. **TPX2 regulates metaphase spindle length by affecting the MT flux rate.** (A) HeLa cells were transfected with scramble or CLASP1/Kif2a siRNA mixture, and the efficacy of RNAi was assessed 48 h later by Western blotting. In the cases of CLASP1, the protein level was reduced by 90%, and Kif2a by 80%. (B) HeLa cells were cotransfected with photoactivatable GFP-tagged α -tubulin (PAGFP-tubulin), mCherry-TPX2, and CLASP1/Kif2a siRNA mixture. 405 nm laser was applied to activate GFP signal in one rectangular region near the MT plus ends (time point = 0, arrows). The movement of the tubulin subunits was tracked every 15 s. Note that no obvious MT flux can be observed. Bar, 10 μm . (C) GFP-TPX2-2A cell line with or without induction for the exogenous protein was depleted of endogenous Kif2a. Spindle morphology was quantified according to α -tubulin staining. Depletion of Kif2a causes an increase in cells forming monopolar spindles, and expressing TPX2-2A greatly restores the bipolarity. Three independent experiments were performed each measuring no less than 100 cells. Error bars indicate SD. (D) A model depicting the role of TPX2 in regulating metaphase spindle length. The metaphase spindle maintains a constant length by delicately balancing the MT polymerization at the kinetochore (+) and depolymerization at the centrosome (-). TPX2, when phosphorylated by Aurora A, binds to both CLASP1 and Kif2a; when dephosphorylated, it binds to Kif2a alone. Phosphorylation of TPX2 might regulate the activity of CLASP1 via a not-yet-known mechanism and somehow affect its ability in incorporating tubulin dimers at the plus end of the spindle MTs. TPX2 might also have an effect on Kif2a because they interact directly, but this is likely not to be affected by its phosphorylation state. Therefore, loss of phosphorylation in the mutant TPX2 likely destroys the balance between MT polymerization and depolymerization, resulting in slow MT flux and short spindles. Other possible candidates that might be involved in the TPX2 pathway include other Kinesin-13 family members, Kinesin-8, and MT polymerization factor XMAP215/TOG, as well as dynein, which can promote the poleward streaming of specific cargos.

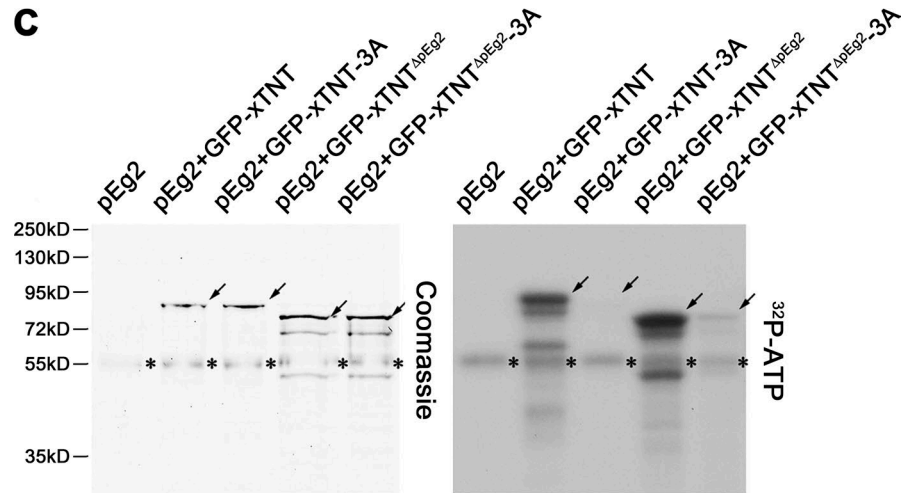
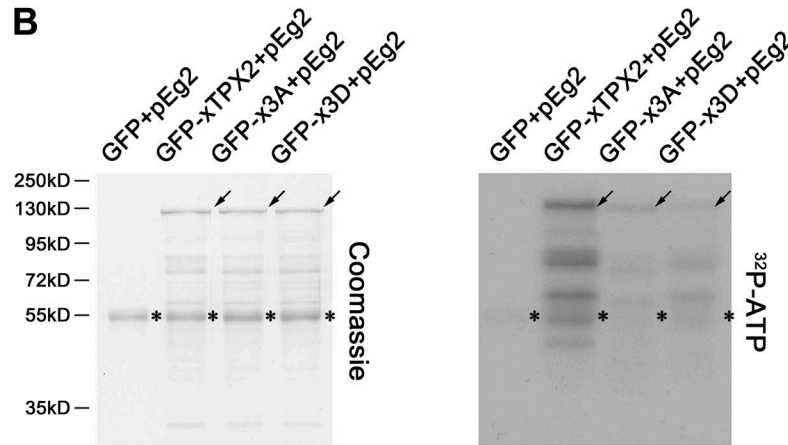
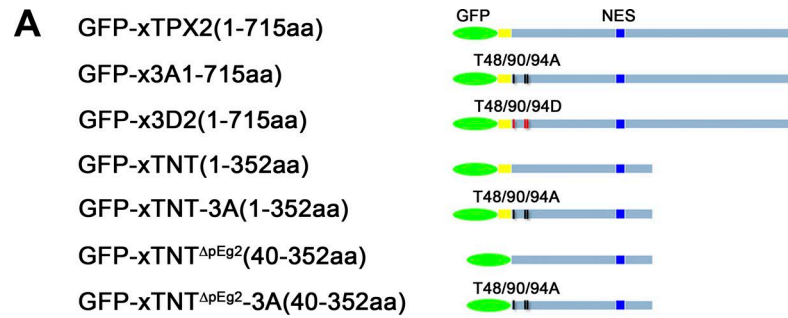
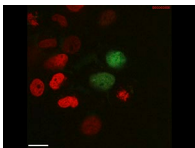
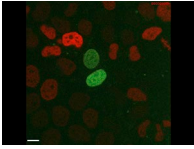


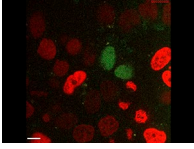
Figure S3. **Xenopus TPX2 is phosphorylated by Aurora A.** (A) Scheme of different xTPX2 constructs. (B) GFP, GFP-xTPX2, GFP-xTPX2-3A, or GFP-xTPX2-3D were incubated with pEg2 in the presence of γ -[³²P]ATP and analyzed by autoradiography (right). Total inputs were seen by Coomassie blue staining (left). xTPX2 is indicated by arrows and pEg2 by asterisks. Note that phosphorylation of xTPX2 by pEg2 is abolished after triple serine-to-alanine or serine-to-aspartic acid mutation. (C) GFP fusion protein of xTNT, xTNT-3A, xTNT^{ΔpEg2}, or xTNT^{ΔpEg2}-3A was incubated with pEg2 in the presence of γ -[³²P]ATP and analyzed by autoradiography (right). Total inputs were seen using Coomassie blue staining (left). Different xTPX2 mutants were indicated by arrows and pEg2 by asterisks. The phosphorylation of the N terminus of xTPX2 by pEg2 is abolished after triple serine-to-alanine mutation; destroying the interaction between xTPX2 and pEg2 did not harm the phosphorylation in vitro.



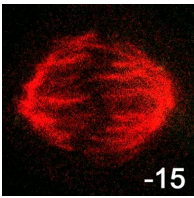
Video 1. **Spindle formation in the background of GFP-TPX2.** HeLa cells stably expressing RFP-H2B (red) were depleted of endogenous TPX2 and transiently expressing RNAi-resistant GFP-TPX2 (green) for 48 h. Spindle morphology visualized by GFP and DNA movement by RFP signals were then recorded every 3 min using a microscope (Eclipse Ti; Nikon; 60x/1.4 NA oil objective lens). Bar, 17 μ m.



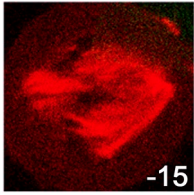
Video 2. **Spindle formation in the background of GFP-TPX2-2A.** HeLa cells stably expressing RFP-H2B (red) were depleted of endogenous TPX2 and transiently expressing RNAi-resistant GFP-TPX2-2A (green) for 48 h. Spindle morphology visualized by GFP and DNA movement by RFP signals were then recorded every 3 min using a microscope (Eclipse Ti; Nikon; 60 \times /1.4 NA oil objective lens). Note that expression of TPX2-2A results in a shorter bipolar spindle compared with -WT (Video 1) and -2D (Video 3). Bar, 11 μ m.



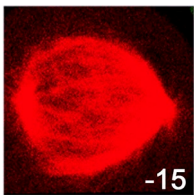
Video 3. **Spindle formation in the background of GFP-TPX2-2D.** HeLa cells stably expressing RFP-H2B (red) were depleted of endogenous TPX2 and transiently expressing RNAi-resistant GFP-TPX2-2D (green) for 48 h. Spindle morphology visualized by GFP and DNA movement by RFP signals were then recorded every 3 min using a microscope (Eclipse Ti; Nikon; 60 \times /1.4 NA oil objective lens). Bar, 11 μ m.



Video 4. **MT flux on metaphase spindles in the background of GFP-TPX2.** U2OS cells were cotransfected with photoactivatable GFP-tagged α -tubulin (PAGFP- α -tubulin) and mCherry-tagged TPX2-WT for 24 h. 405 nm laser was applied to activate GFP signal in a rectangular region near the MT plus ends, and the movement of the tubulin subunits was tracked every 15 s using a confocal microscope (LSM 510 Meta; Carl Zeiss; 100 \times /1.4 NA oil objective lens). Green indicates photoactivatable GFP-tagged α -tubulin, and red indicates mCherry-TPX2.



Video 5. **MT flux on metaphase spindles in the background of GFP-TPX2-2A.** U2OS cells were cotransfected with photoactivatable GFP-tagged α -tubulin (PAGFP- α -tubulin) and mCherry-tagged TPX2-2A for 24 h. 405 nm laser was applied to activate GFP signal in a rectangular region near the MT plus ends, and the movement of the tubulin subunits was tracked every 15 s using a confocal microscope (LSM 510 Meta; Carl Zeiss; 100 \times /1.4 NA oil objective lens). Green indicates photoactivatable GFP-tagged α -tubulin, and red indicates mCherry-TPX2-2A.



Video 6. **MT flux on metaphase spindles in the background of GFP-TPX2-2D.** U2OS cells were cotransfected with photoactivatable GFP-tagged α -tubulin (PAGFP- α -tubulin) and mCherry-tagged TPX2-2D for 24 h. 405 nm laser was applied to activate GFP signal in a rectangular region near the MT plus ends, and the movement of the tubulin subunits was tracked every 15 s using a confocal microscope (Carl Zeiss LSM 510 Meta; 100 \times /1.4 NA oil objective lens). Green indicates photoactivatable GFP tagged α -tubulin, and red indicates mCherry-TPX2-2D.