Zhu et al., http://www.jcb.org/cgi/content/full/jcb.201207050/DC1



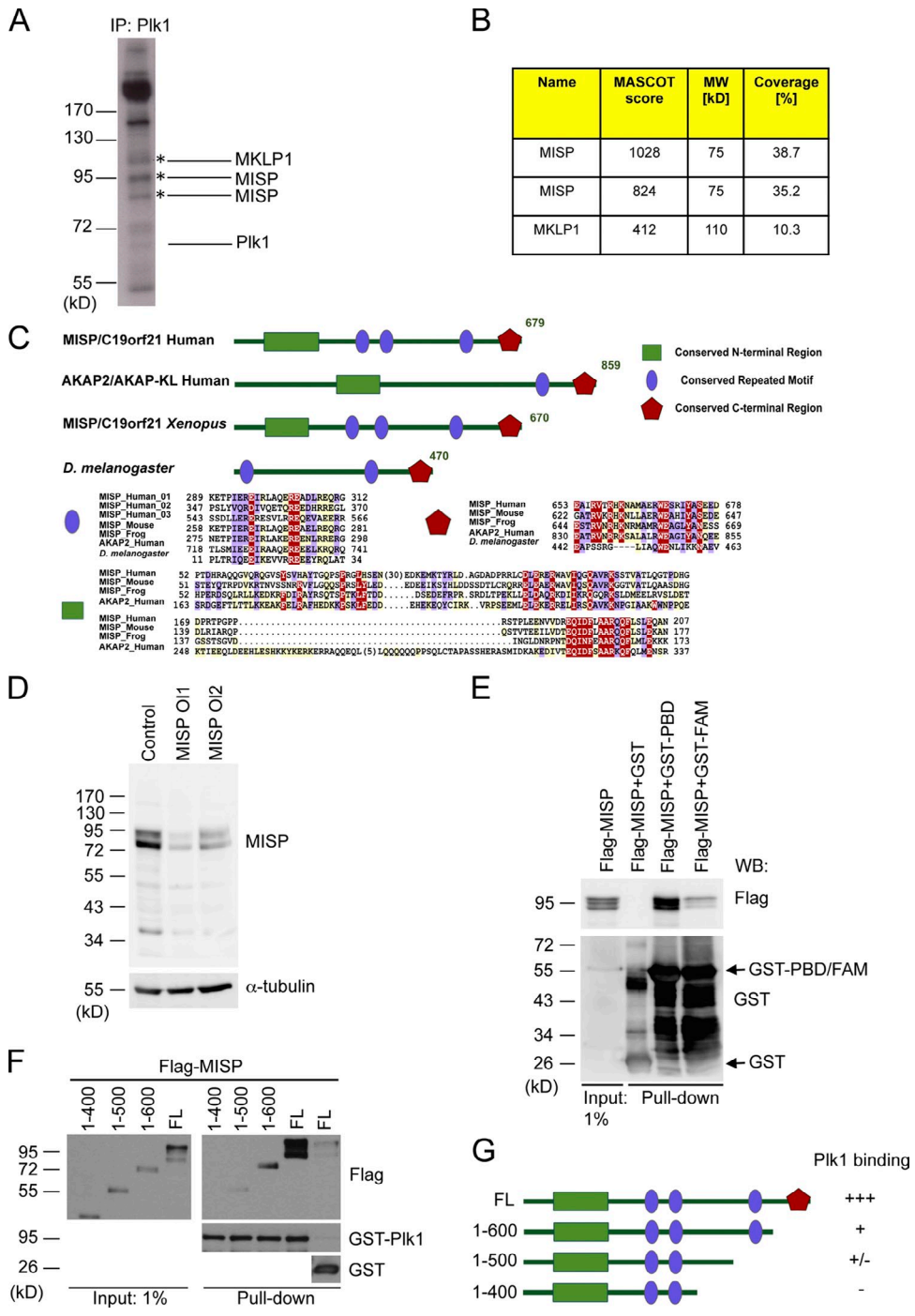
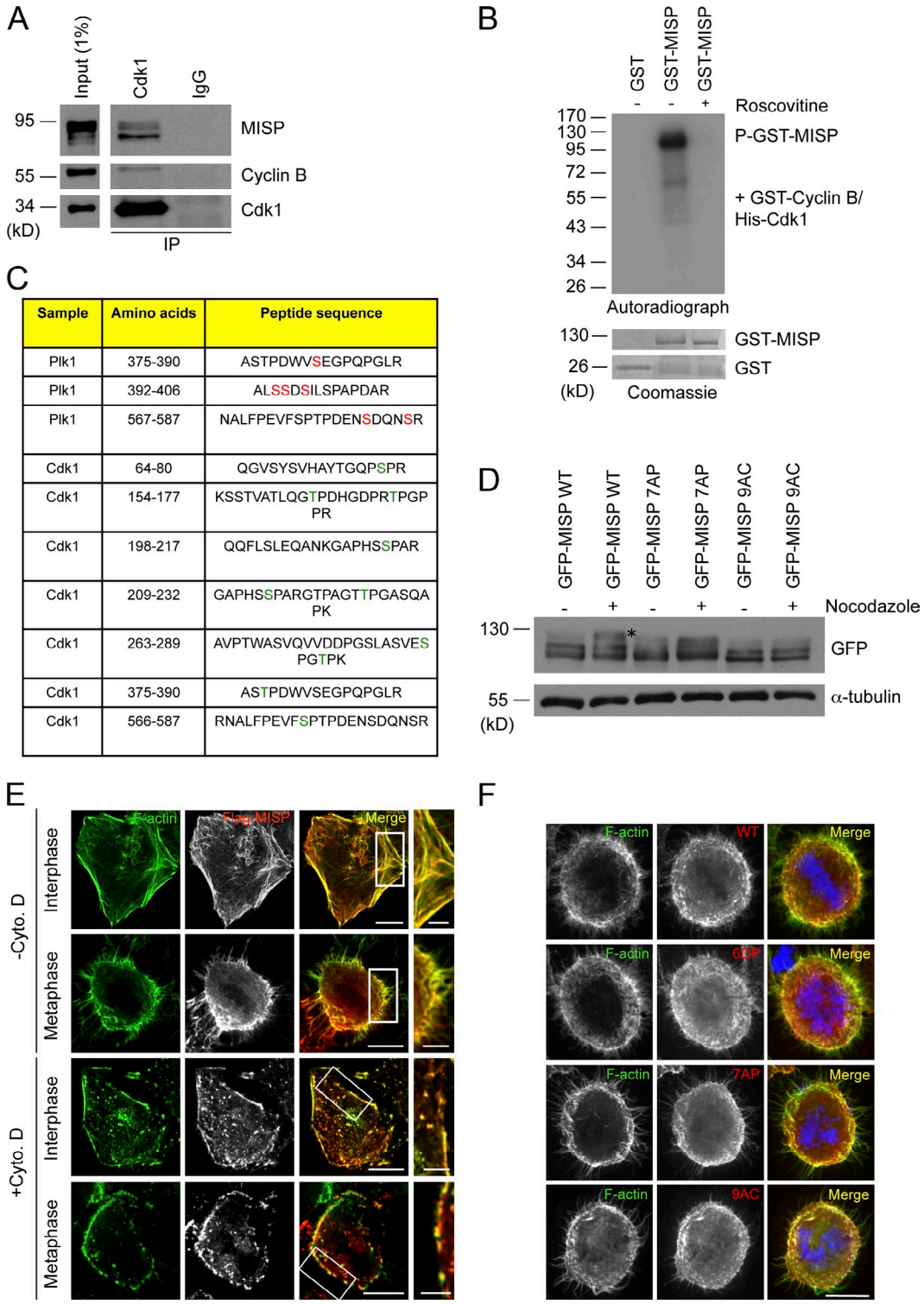



Figure S1. **Identification and characterization of MISP and its binding activity to Plk1.** (A) Plk1 immunoprecipitated from HeLa cells arrested in late mitotic stages by nocodazole block and release for 1.5 h was subjected to an in vitro kinase assay. Indicated bands were sent for mass spectrometry analysis. (B) Depicted proteins from mass spectrometry analysis are shown with MASCOT score, molecular weight, and coverage. (C) Computational analysis of MISP in different species and homologies with human MISP. Schematic representation of conserved domains in the MISP family and multiple sequence alignment of the conserved regions of representative proteins of different species. The coloring scheme indicates average BLOSUM62 score (correlated with amino acid conservation) in each alignment column: red (greater than 3), violet (between 3 and 1.5), and light yellow (between 1.5 and 0.5). (D) HeLa cells were transfected with control or MISP siRNA (OI1/OI2) for 48 h. Cell lysates were analyzed by Western blot. (E) Western blot analysis of a GST pull-down assay using lysates from mitotic HeLa cells expressing Flag-MISP and bacterially purified GST-Plk1 PBD, FAM, or GST as bait. (F) Western blot analysis of a GST pull-down assay using lysates from mitotic HeLa cells expressing Flag-MISP full length (FL) or indicated truncations, and bacterially purified GST-Plk1 or GST as bait. (G) Schematic overview of MISP FL and truncations and their binding activities to Plk1.




| Sample | Amino acids | Peptide sequence |
|---|---|---|
| Plk1 | 375-390 | ASTPDWVSEGPQPGLR |
| Plk1 | 392-406 | ALSSDSILSPAPDAR |
| Plk1 | 567-587 | NALFPEVFSPTPDENSDQNSR |
| Cdk1 | 64-80 | QGVSYSVHAYTGQPSPR |
| Cdk1 | 154-177 | KSSTVATLQGTPDHGDPRTPGP PR |
| Cdk1 | 198-217 | QQFLSLEQANKGAPHSSPAR |
| Cdk1 | 209-232 | GAPHSSPARGTPAGTTPGASQA PK |
| Cdk1 | 263-289 | AVPTWASVQVVDDPGSLASVES PGTPK |
| Cdk1 | 375-390 | ASTPDWVSEGPQPGLR |
| Cdk1 | 566-587 | RNALFPEVFSPTPDENSDQNSR |

Figure S2. **Identification of the mitotic phosphorylation sites on MISP and localization of Flag-MISP WT and phospho-mutants.** (A) Cdk1 was immunoprecipitated from mitotically arrested HeLa cell lysates and Western blot analysis was performed. (B) GST-MISP or GST alone was incubated with GST-cyclin B/His-Cdk1 (in the presence or absence of 5 µg/ml of the Cdk inhibitor roscovitine) and analyzed by autoradiography. (C) Phospho-peptides in MISP were identified by mass spectrometry analysis from in vitro kinase assays using His-Plk1 or GST-cyclin B/His-Cdk1. The possible phosphorylation sites for Plk1 (red) and for Cdk1 (green) were mutated to alanine or aspartic acid. For the MISP mutants of Plk1 phosphorylation sites, S471 has additionally been included into the analysis based on the mass spectrometry analyses of the Plk1-dependent phosphoproteome (Santamaria et al., 2011). S382 has been excluded from the phospho-mimicking mutant (6DP) due to technical constraints. (D) HeLa cells transfected with GFP-MISP WT/7AP/9AC were analyzed by Western blot with indicated antibodies with and without nocodazole treatment. Asterisk indicates highest phosphorylated band with slowest migrating form of GFP-MISP. (E) HeLa cells were transfected with Flag-MISP in the presence or absence of Cyto. D (2 µM, 10 min). Cells were fixed and stained with Flag antibody (red) and Atto 488 Phalloidin (green). Bars, 10 µm. Insets show higher magnification of the framed regions. Bars, 5 µm. (F) Representative images of mitotic HeLa cells transfected with Flag-MISP WT, 6DP, 7AP, or 9AC. Flag-MISP (red), Atto 488 Phalloidin (green), DNA (blue). Bar, 10 µm.

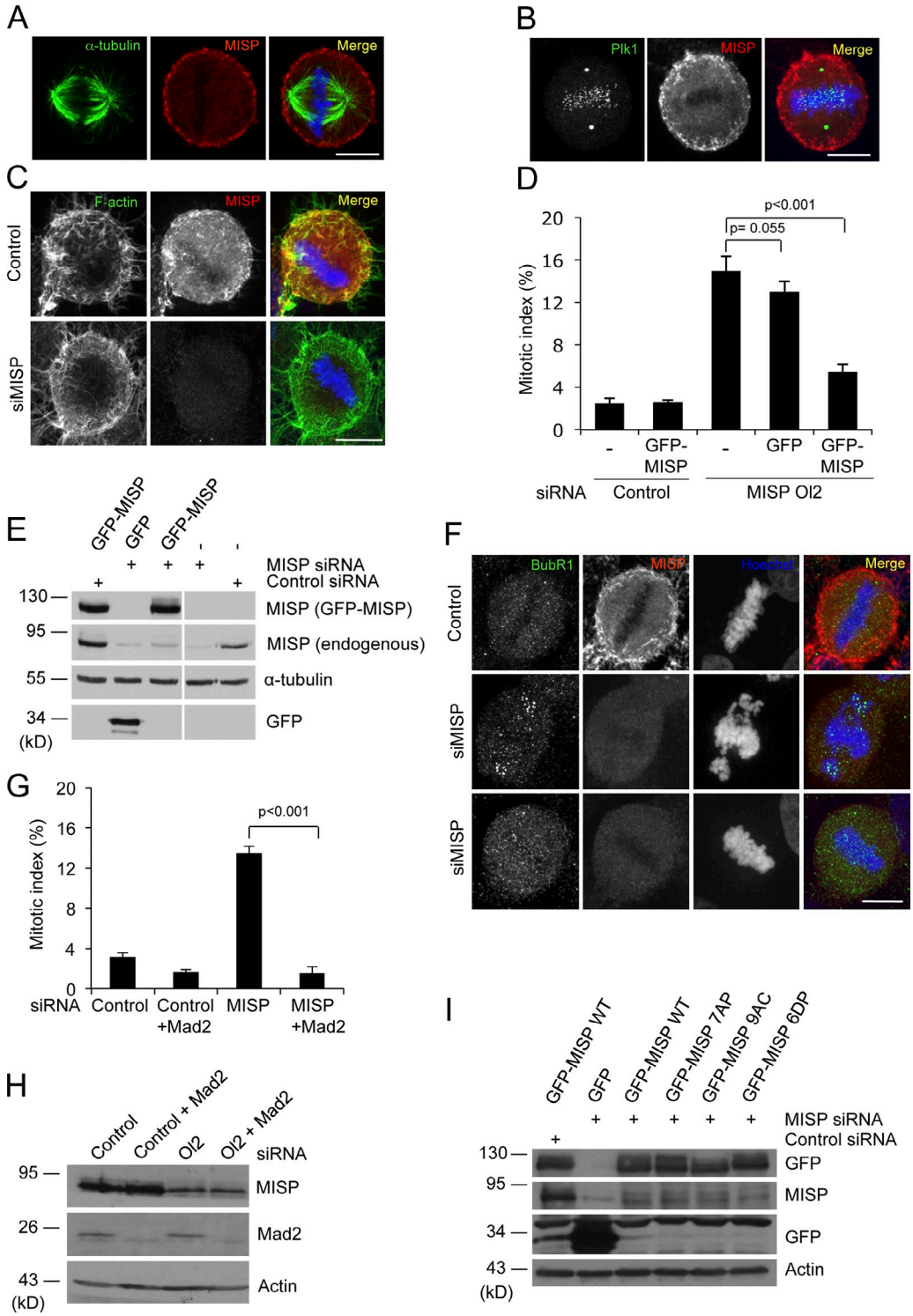



Figure S3. **MISP is required for mitotic progression.** (A and B) A representative image of a mitotic HeLa cell with co-stained MISP (red), α-tubulin (A, green) or Plk1 (B, green), and Hoechst 33342. (C) HeLa cells transfected with control or MISP siRNA were stained with MISP antibody (red), Atto 488 Phalloidin (green), and Hoechst 33342. (D) HeLa cells transfected with GFP alone or an siRNA (OI2)-resistant version of GFP-MISP were treated with MISP or control siRNA for 48 h. The mitotic index was evaluated by α-tubulin and p-Ser10 H3 staining. *n* = 200 cells from three independent experiments. (E) Lysates prepared from cells from D were analyzed by Western blot with indicated antibodies. The dividing white lane marks the grouping of images from different parts of the same gel, as an intervening lane was removed for presentation purposes. (F) HeLa cells were transfected with control or MISP siRNA for 48 h, and stained with BubR1 (green), CREST (red), and Hoechst 33342. (G) Quantification of the mitotic index of HeLa cells transfected with MISP or control siRNA and in combination with Mad2 siRNA for 48 h. *n* = 200 cells from three independent experiments. (H) Lysates prepared from cells from G were analyzed by Western blot with indicated antibodies. (I) HeLa cells transfected with GFP or siRNA-resistant versions of GFP-MISP WT/7AP/9AC/6DP were treated with MISP or control siRNA for 48 h. Lysates were analyzed by Western blot with indicated antibodies. Bars: (A, B, C, and F) 10 μm. Error bars in D and G represent SD. Student's *t* test was used to calculate p-value for comparison of control and experimental samples.

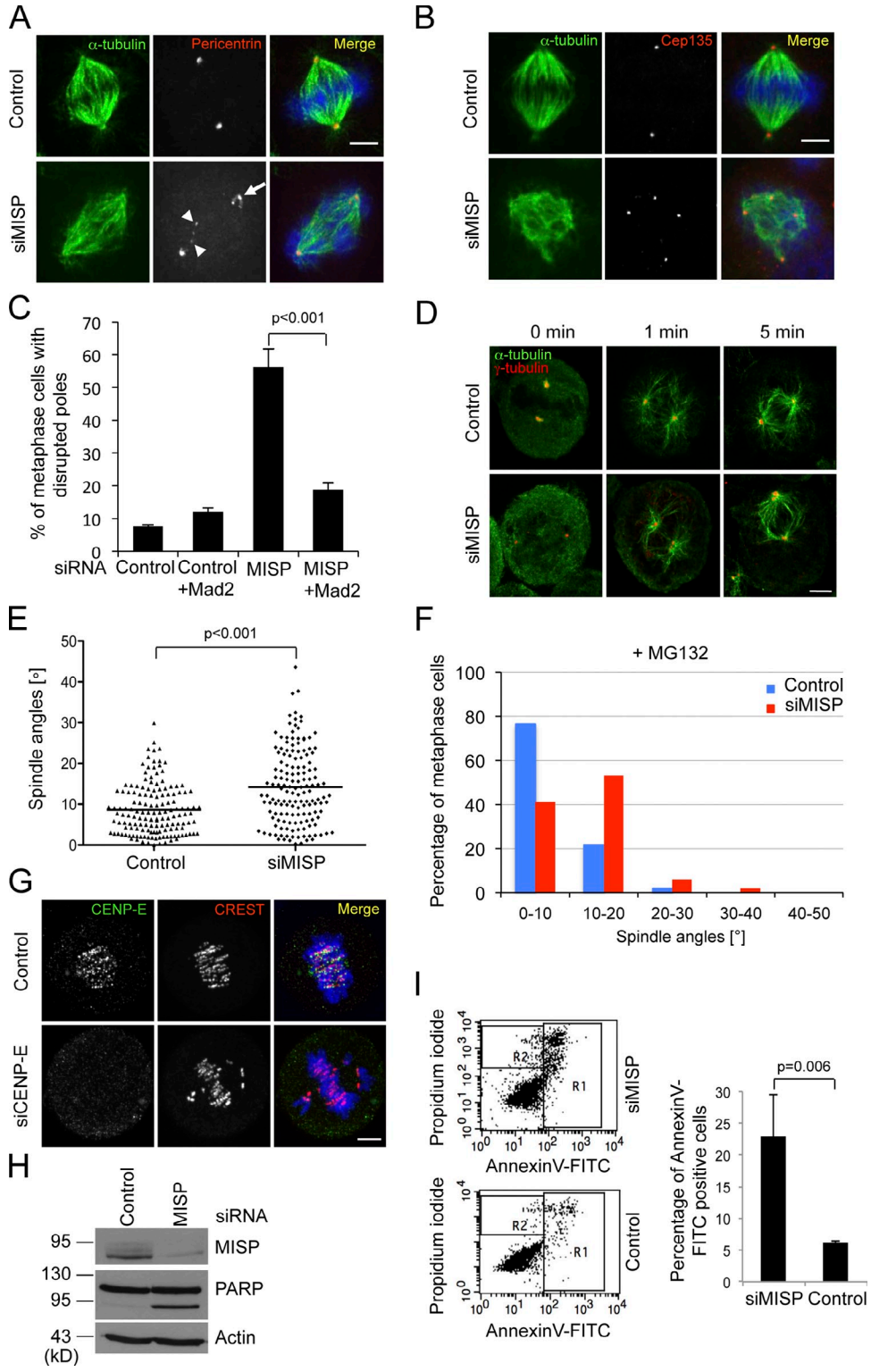



Figure S4. **MISP knockdown leads to mitotic defects and apoptosis.** (A and B) MISP or control siRNA-treated HeLa cells were stained with α-tubulin (green) and pericentrin (A, red); or α-tubulin (green) and Cep135 (B, red). (C) Quantification of the percentage of HeLa cells in metaphase with disrupted poles in MISP or control siRNA and in combination with Mad2 siRNA-treated cells (48 h after transfection). *n* = 200 cells from three independent experiments. (D) MISP or control siRNA-transfected HeLa cells were exposed at 4°C for 30 min and shifted back to 37°C for indicated time points. Cells were stained with α-tubulin (green) and γ-tubulin (red). (E) Scatter plots showing spindle angles from the spindle orientation assay described in Fig. 5, C and D, with calculated mean value. *n* = 150 cells from three independent experiments. (F) Quantification of the percentage of metaphase cells with indicated spindle angles with MG132 treatment for 2 h before fixation. *n* = 50 cells were calculated from a single experiment. (G) HeLa cells transfected with control or CENP-E siRNA were stained with CENP-E antibody (green), CREST (red), and Hoechst 33342. (H) Control or MISP siRNA-treated HeLa cell lysates were analyzed by Western blot with indicated antibodies. (I) HeLa cells treated with MISP or control siRNAs for 48 h were stained with propidium iodide and AnnexinV-FITC to analyze the DNA content and the apoptotic cells by FACS, respectively (left panel). Quantification of gate R1 of the AnnexinV-FITC–positive cells from three independent experiments was shown in the right panel. Bars: (A, B, D, and G) 5 µm. Error bars in C, E, and I represent SD. Student's *t* test was used to calculate p-value for comparison of control and experimental samples.

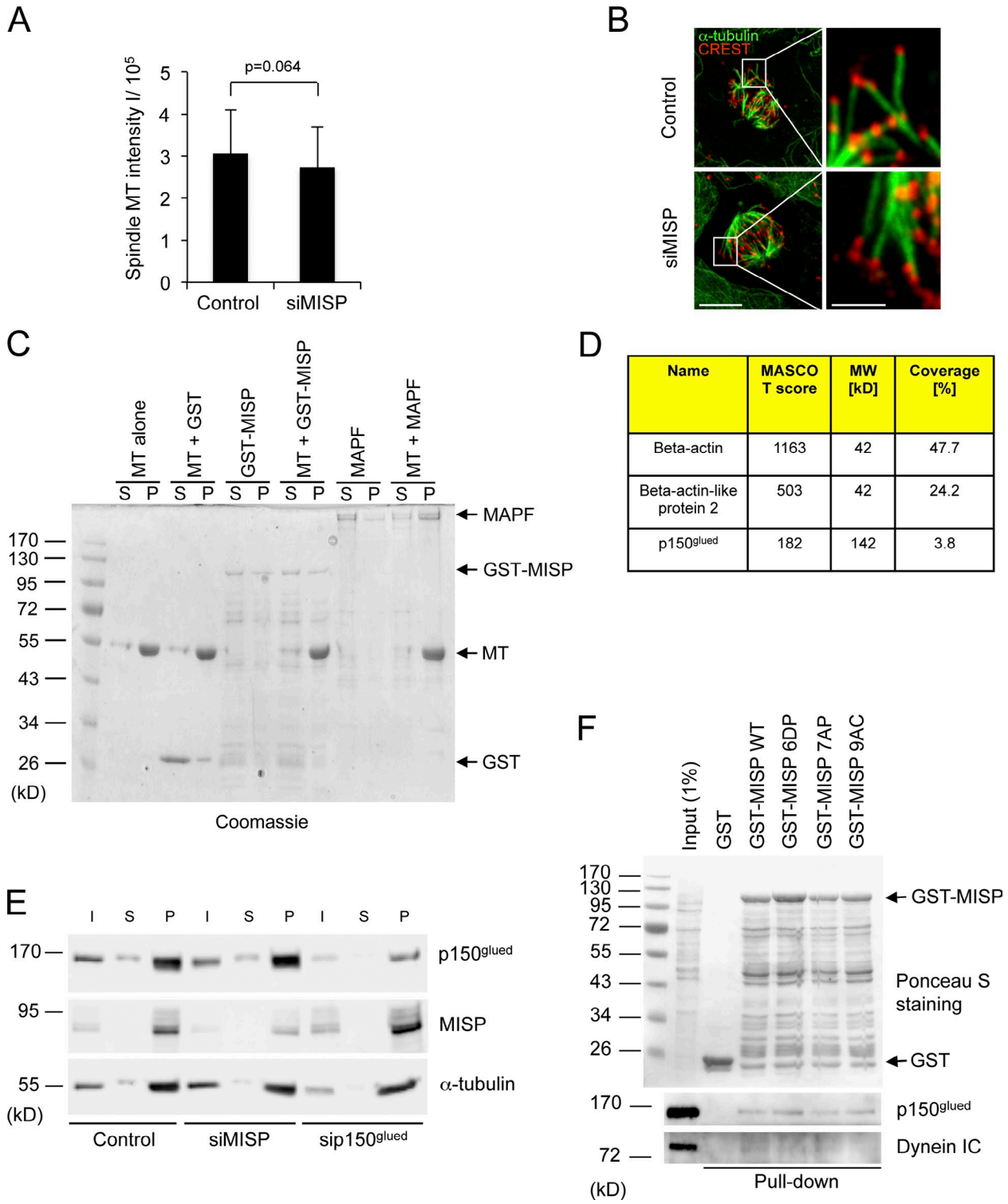
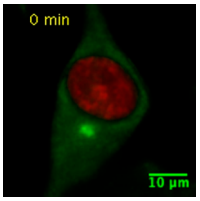
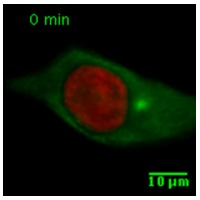


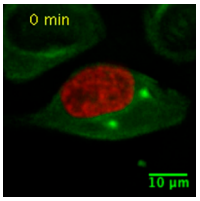

Figure S5. **MISP does not directly bind to MTs and associates with p150$^{glued}$ in a phosphorylation-independent manner.** (A) Quantification of the measured spindle intensity ($I_{spindle}$) as described in Fig. 6 B; $n$ = 60 cells from three independent experiments. Error bars represent SD. Student's $t$ test was used to calculate p-value for comparison of control and MISP siRNA-treated cells. (B) HeLa cells treated with MISP or control siRNA were stained with α-tubulin (green) and CREST (red) after cold treatment for 10 min at 4°C before fixation. Bar, 10 µm. Enlargements of the framed insets are shown on the right. Bar, 2.5 µm. (C) In vitro MT-binding assay using polymerized MT and GST (negative control), GST-MISP, or MAPF (MT-associated protein fraction containing 60% MAP2 and 40% tau proteins, positive control). P, pellet; S, supernatant. (D) GST pull-down was performed using GST-MISP and mitotic HeLa cell lysate and analyzed by mass spectrometry. Candidate binding proteins are shown with MASCOT score, molecular weight, and coverage. (E) Taxol-stabilized MTs were pelleted by high-speed centrifugation in control, MISP, or p150$^{glued}$ siRNA-treated mitotic HeLa cell lysates. Input (I), supernatant (S), and pellet (P) were analyzed by Western blot with indicated antibodies. (F) GST pull-down assays were performed using bacterially purified GST, GST-MISP WT, 6DP, 7AP, or 9AC and mitotic HeLa cell lysates. Ponceau S staining image and Western blot analyses with indicated antibodies were shown.



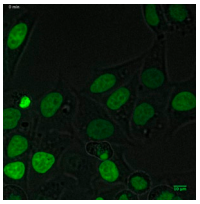

Video 1. **Mitotic progression in MISP siRNA-treated HeLa GFP-α-tubulin/RFP-H2B cells.** HeLa GFP-α-tubulin/RFP-H2B cells were transfected with MISP siRNA and 24 h later analyzed by time-lapse confocal microscopy using a laser scanning microscope (Ti TIRF; Nikon). Frames were taken every 6 min for 246 min.



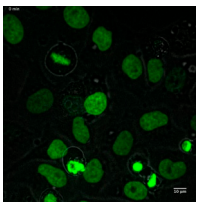

Video 2. **Mitotic progression in MISP siRNA-treated HeLa GFP-α-tubulin/RFP-H2B cells.** HeLa GFP-α-tubulin/RFP-H2B cells were transfected with MISP siRNA and 24 h later analyzed by time-lapse confocal microscopy using a laser scanning microscope (Ti TIRF; Nikon). Frames were taken every 6 min for 282 min.



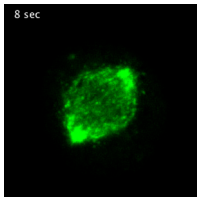

Video 3. **Mitotic progression in control siRNA-treated HeLa GFP-α-tubulin/RFP-H2B cells.** HeLa GFP-α-tubulin/RFP-H2B cells transfected with control siRNA and 24 h later analyzed by time-lapse confocal microscopy using a laser scanning microscope (Ti TIRF; Nikon). Frames were taken every 6 min for 54 min.



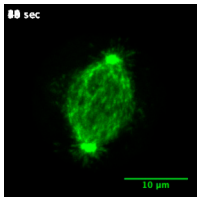
Video 4. **Mitotic progression in MISP siRNA-treated HeLa GFP-H2B cells.** HeLa GFP-H2B cells were transfected with MISP siRNA and 28 h later monitored by a spinning disk microscope (UltraView ERS-VoX; PerkinElmer). Frames were taken every 3 min for 474 min.



Video 5. **Mitotic progression in control siRNA-treated HeLa GFP-H2B cells.** HeLa GFP-H2B cells were transfected with control siRNA and 28 h later monitored by a spinning disk microscope (UltraView ERS-VoX; PerkinElmer). Frames were taken every 3 min for 474 min.




Video 6. **EB3 dynamics in MISP siRNA-treated HeLa cells.** HeLa cells were transfected with GFP-EB3 and MISP siRNA and 48 h later monitored by a spinning disk microscope (UltraView ERS-VoX; PerkinElmer). Frames were taken every 2 s for 1 min.




Video 7. **EB3 dynamics in control siRNA-treated HeLa cells.** HeLa cells were transfected with GFP-EB3 and control siRNA and 48 h later monitored by a spinning disk microscope (UltraView ERS-VoX; PerkinElmer). Frames were taken every 2 s for 1 min.

## Reference

Santamaria, A., B. Wang, S. Elowe, R. Malik, F. Zhang, M. Bauer, A. Schmidt, H.H. Sillje, R. Korner, and E.A. Nigg. 2011. The Plk1-dependent phosphoproteome of the early mitotic spindle. *Mol. Cell. Proteomics.* 10:M110 004457.