Nunes Bastos et al., http://www.jcb.org/cgi/content/full/jcb.201409129/DC1

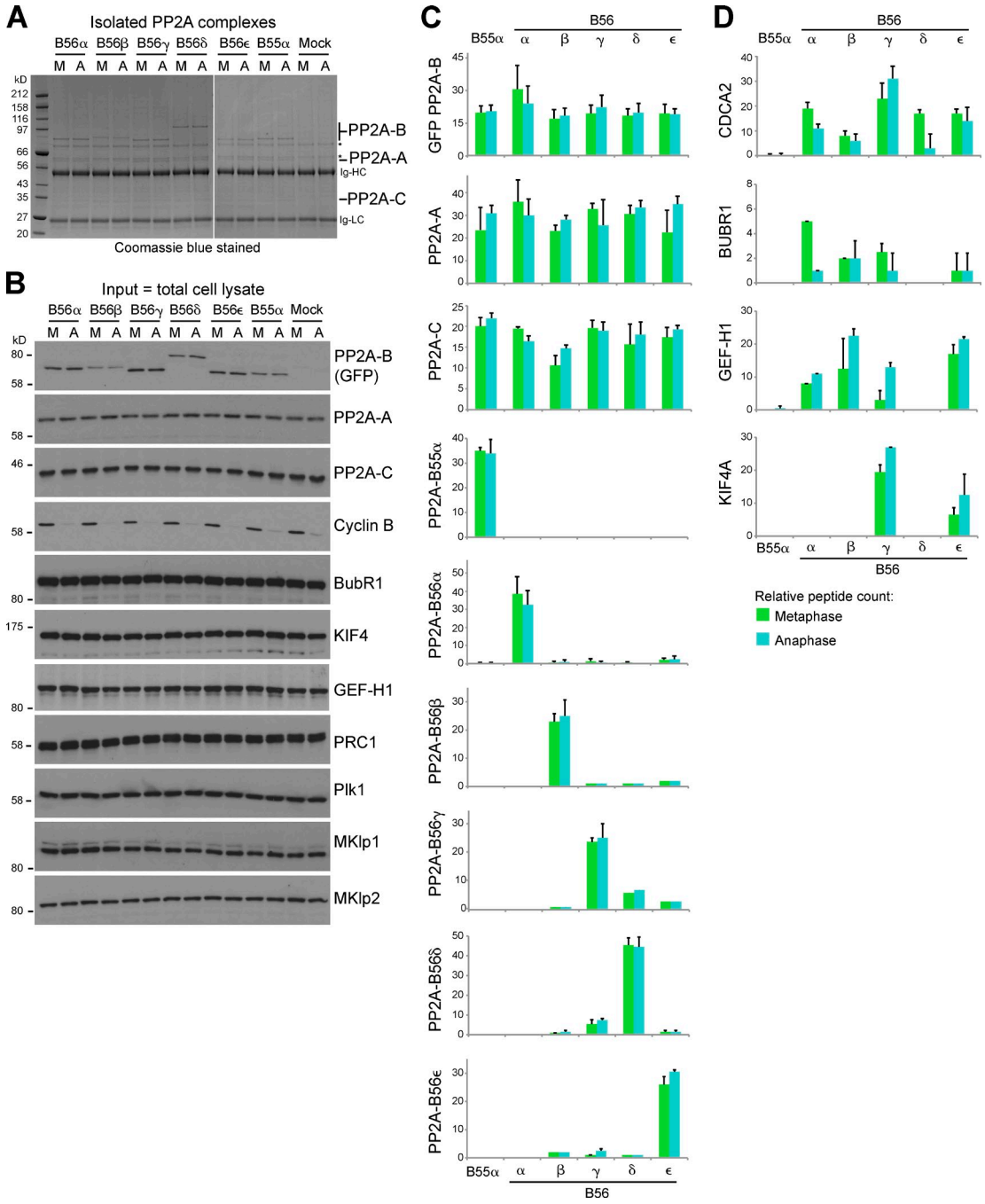


Figure S1. **Analysis of the subunit composition of PP2A–B56 complexes from metaphase and anaphase cells.** (A) PP2A–B56 and –B55α complexes were immune precipitated using GFP antibodies from synchronized HeLa cells in metaphase (M) and anaphase (A). Untransfected cells were used as a negative control (Mock). A Coomassie blue–stained gel of the complexes representative of three independent experiments is shown. White lines indicate that intervening lanes have been spliced out. Asterisks mark nonspecifically bound proteins. HC, immunoglobulin heavy chain; LC, immunoglobulin light chain. (B) The input fractions corresponding to the samples in Fig. 1 B were analyzed by blotting with the antibodies shown in the figure. Cyclin B was used to confirm the metaphase and anaphase states of the extracts. (C and D) Mass spectrometry analysis of PP2A–B56 complexes showing the relative abundance of core PP2A subunits (C), or associated proteins were calculated using MaxQuant (D). The number of peptides identified in metaphase and anaphase samples is plotted as a measure of relative protein abundance (means ± SEM, $n = 3$). Similar data were collected for PP2A complexes isolated in four independent experiments.

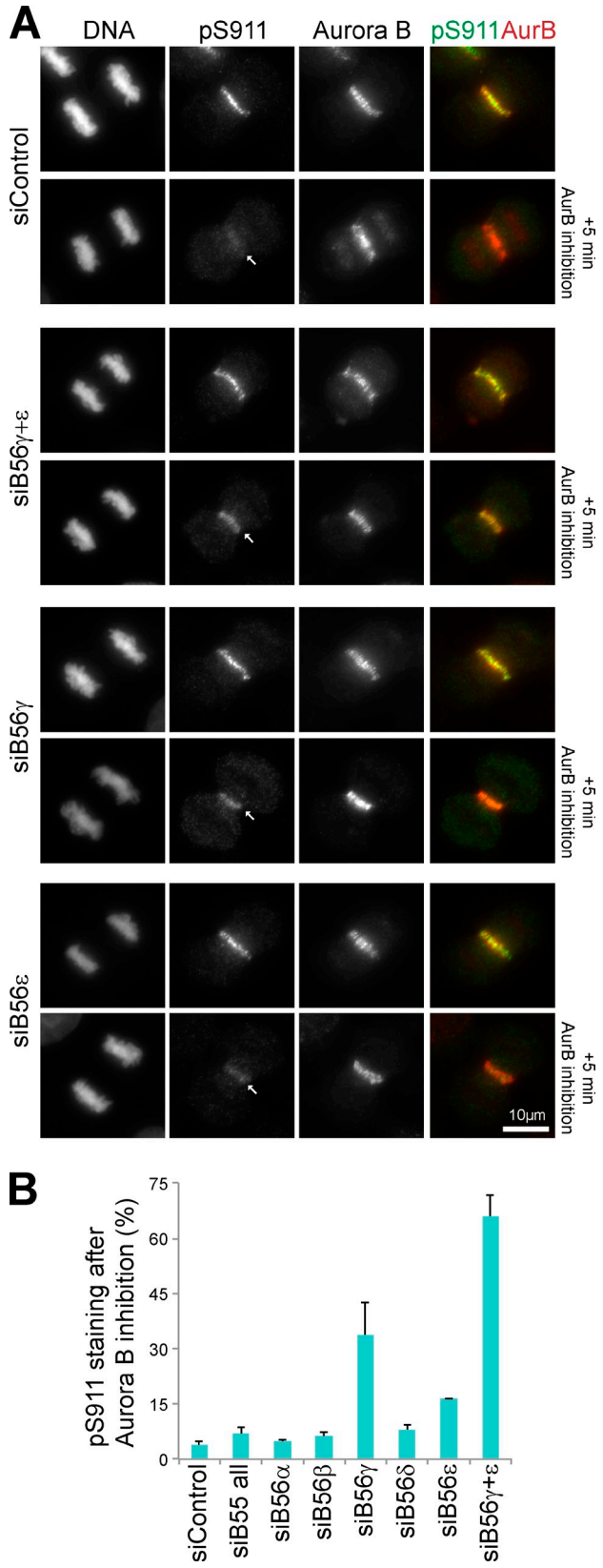


Figure S2. **PP2A–B56γ is a KIF4A pT799 and MKlp1 pS911 phosphatase.** (A) HeLa cells were treated with control, combined PP2A–B56γ, and -ε, or PP2A–B56γ or -ε siRNA for 36 h, arrested in interphase with 2 mM thymidine for 18 h, and then released to allow cells to enter mitosis. After 10 h, when the peak of anaphase cells was observed, Aurora B was inhibited for 5 min with 1 μM ZM447439. The cells were fixed and then stained for DNA, mouse anti–Aurora B, and rabbit anti-MKlp1 pS911. Arrows indicate the central spindle region. (B) Central spindle pS911 staining intensity after 5 min Aurora B inhibition was scored in anaphase cells after PP2A–B55 and –B56 subunit depletion in the combinations indicated (means ± SEM, $n = 3$ measuring >100 cells per condition). siControl, control siRNA.

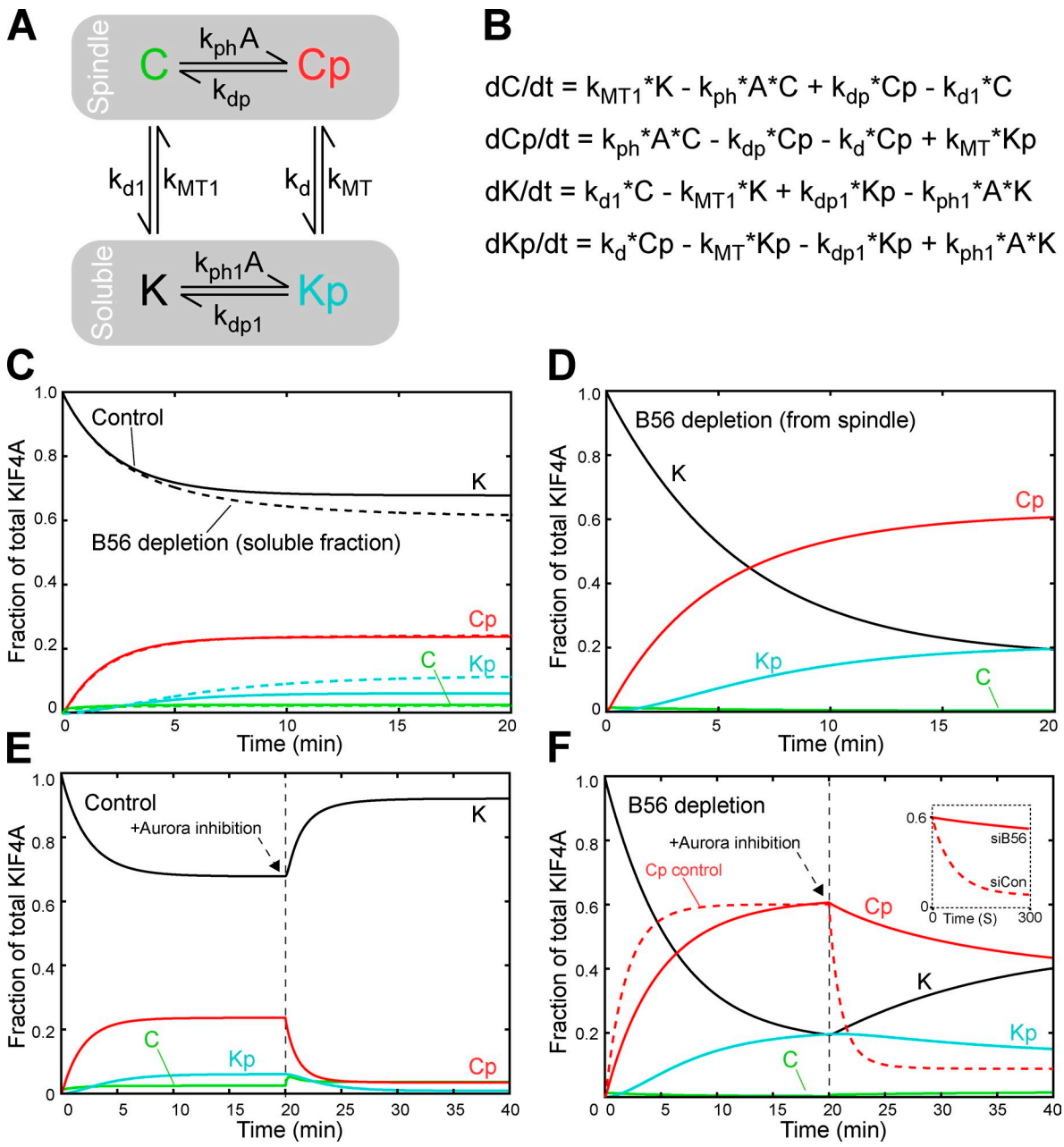
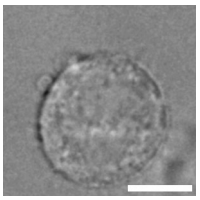


Figure S3. **A model describing the regulation of KIF4A by Aurora B and PP2A–B56.** (A) The biochemical reactions providing the basis for the mathematical modeling are described. KIF4A is present in soluble cytoplasmic (K) and central spindle–associated fractions (C), which can also be phosphorylated (Kp and Cp). Aurora B kinase is described by the term A, k_{ph} and k_{dp} are rate constants for phosphorylation and dephosphorylation of KIF4A, and k_{MT} and k_d are rate constants for microtubule binding and release of KIF4A at the spindle. (B) The time-dependent change of each component is described by a nonlinear ordinary differential equation. Each term on the right hand side of the equation corresponds to a biochemical reaction. Parameters were defined based on the assumption that $k_{ph} >> k_{ph1}$ because Aurora B is predominantly spindle associated and $k_{dp} > k_{dp1}$ because B56 is enriched at spindle but also present in cytoplasm. Phosphorylation does not alter microtubule binding, so $k_{MT} = k_{MT1}$; however, phosphorylation increases microtubule residence time, so $k_d < k_{d1}$. (C) Simulation of KIF4A behavior in control cells entering anaphase (A = 1, $k_{MT1} = 0.2$, $k_{d1} = 5$, $k_{ph} = 10$, $k_{dp} = 1.0$, $k_{MT} = 0.2$, $k_d = 0.1$, $k_{ph1} = 0.001$, and $k_{dp1} = 0.2$). Because all available KIF4A is cytoplasmic at anaphase onset, K = 1 provides the starting condition for the simulation. A 95% reduction in soluble B56, $k_{dp1} = 0.01$ is plotted in the dotted lines. (D) To mimic 95% depletion of B56γ and -ε, k_{dp} is reduced to 0.05, and because it is assumed to contribute 50% of soluble PP2A–B56, k_{dp1} is reduced to 0.1. (E and F) To simulate the requirement for Aurora B, A was reduced to 0.1 equal to 90% inhibition. In F, to facilitate comparison with the experiment in Fig. 4 C, the control curve has been normalized to the same value as the B56 depletion condition and is plotted as a dotted red line. An enlarged boxed region shows a time window comparable to the experimental data in Fig. 4 C. siCon, control siRNA.

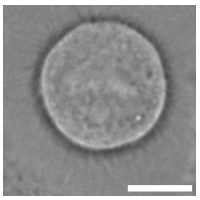
Table S1. **siRNA duplexes used to target PP2A and KIF4A**

Gene	Accession	Company	Catalog no.	Sequence 5′→3′
PPP2R2A	NM_002717	Thermo Fisher Scientific	J-004824-06	CAUACCAGGUGCAUGAAUA
PPP2R2A	NM_002717	Thermo Fisher Scientific	J-004824-07	GUAUAGAGAUCCUACUACA
PPP2R2A	NM_002717	Thermo Fisher Scientific	J-004824-08	GCAAGUGGCAAGCGAAAGA
PPP2R2A	NM_002717	Thermo Fisher Scientific	J-004824-09	AGACAUAACCCUAGAAGCA
PPP2R5A	NM_006243	Thermo Fisher Scientific	J-009352-07	GCUCAAAGAUGCCACUUCA
PPP2R5A	NM_006243	Thermo Fisher Scientific	J-009352-08	CAAUACAAGUGCCGAAUAA
PPP2R5A	NM_006243	Thermo Fisher Scientific	J-009352-09	UGAAUGAACUGGUUGAGUA
PPP2R5A	NM_006243	Thermo Fisher Scientific	J-009352-10	GGAAAUGAAUGGCAAGCUU
PPP2R5B	NM_006244	Thermo Fisher Scientific	J-009366-05	CGCAUGAUCUCAGUGAAUA
PPP2R5B	NM_006244	Thermo Fisher Scientific	J-009366-06	UCAAGUCGCUGUCUGUCUU
PPP2R5B	NM_006244	Thermo Fisher Scientific	J-009366-07	CAAACCAUCGUAUCACUGA
PPP2R5B	NM_006244	Thermo Fisher Scientific	J-009366-08	GAACAAUGAGUAUAUCCUA
PPP2R5C	NM_178587	Thermo Fisher Scientific	J-009433-05	GGAUUUGCCUUACCACUAA
PPP2R5C	NM_178587	Thermo Fisher Scientific	J-009433-06	GGAAGAUGAACCAACGUUA
PPP2R5C	NM_178587	Thermo Fisher Scientific	J-009433-07	CAUCAGAAUUUGUGAAGAU
PPP2R5C	NM_178587	Thermo Fisher Scientific	J-009433-08	CAGAAGUAGUCCAUAUGUU
PPP2R5D	NM_180977	Thermo Fisher Scientific	J-009799-06	GUACAUCGACCAGAAGUUU
PPP2R5D	NM_180977	Thermo Fisher Scientific	J-009799-07	UCCAUGGACUGAUCUAUAA
PPP2R5D	NM_180977	Thermo Fisher Scientific	J-009799-08	UGACUGAGCCGGUAAUUGU
PPP2R5D	NM_180977	Thermo Fisher Scientific	J-009799-09	GUAGGCAGAUCAACCACAU
PPP2R5E	NM_006246	Thermo Fisher Scientific	J-008531-06	UUAAUGAACUGGUGGACUA
PPP2R5E	NM_006246	Thermo Fisher Scientific	J-008531-07	GCACAGCUGGCAUAUUGUA
PPP2R5E	NM_006246	Thermo Fisher Scientific	J-008531-08	GACACGCUAUCUGAUCUUA
PPP2R5E	NM_006246	Thermo Fisher Scientific	J-008531-09	GGAUAAAGUAGACGGAUUU
KIF4A	NM_012310	QIAGEN	Custom	CAGGUCCAGACUACUACUC
Control	None	Thermo Fisher Scientific	Custom	CGUACGCGGAAUACUUCGA

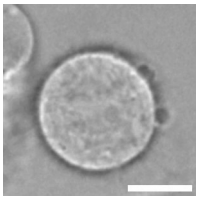
Accession numbers were obtained from GenBank.



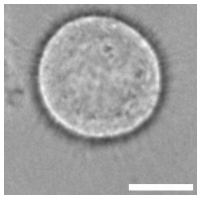
Video 1. **Central spindle localization of PP2A–B56β.** HeLa cells expressing an EGFP-tagged PP2A–B56β subunit were imaged at 1-min intervals for 20 min from 1 min before the onset of anaphase using a spinning-disk confocal microscope. A maximum intensity projection of the GFP signal, 17 planes spaced by 0.6 µm through the cell volume, is shown with grayscale and a heatmap scale together with a single brightfield image taken at the cell equator. PP2A–B56β accumulated at the central spindle 3–4 min after anaphase onset, spread out toward the cell cortex during furrowing, and then concentrated at the midbody. Time is in minutes relative to metaphase–anaphase transition. Supplemental to Fig. 1 E. Bar, 10 µm.



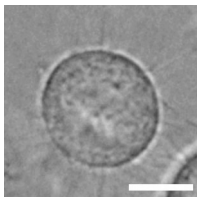
Video 2. **Central spindle localization of PP2A–B56γ.** HeLa cells expressing an EGFP-tagged PP2A–B56γ subunit were imaged at 1-min intervals for 20 min from 1 min before the onset of anaphase using a spinning-disk confocal microscope. A maximum intensity projection of the GFP signal, 17 planes spaced by 0.6 µm through the cell volume, is shown with grayscale and a heatmap scale together with a single brightfield image taken at the cell equator. PP2A–B56γ accumulated at the central spindle 3–4 min after anaphase onset but was absent from the cell cortex and then became increasingly concentrated at the midbody. Time is in minutes relative to metaphase–anaphase transition. Supplemental to Fig. 1 E. Bar, 10 µm.



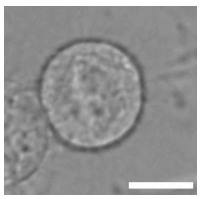
Video 3. **Central spindle localization of PP2A–B56ε.** HeLa cells expressing an EGFP-tagged PP2A–B56ε subunit were imaged at 1-min intervals for 20 min from 1 min before the onset of anaphase using a spinning-disk confocal microscope. A maximum intensity projection of the GFP signal, 17 planes spaced by 0.6 µm through the cell volume, is shown with grayscale and a heatmap scale together with a single brightfield image taken at the cell equator. PP2A–B56ε accumulated at the central spindle 3–4 min after anaphase onset but was absent from the cell cortex and became increasingly concentrated at the midbody. The cytoplasmic signal is greater than seen for B56γ, which otherwise shows a similar pattern of localization. Time is in minutes relative to metaphase–anaphase transition. Supplemental to Fig. 1 E. Bar, 10 µm.



Video 4. **Cytoplasmic localization of PP2A–B56δ in anaphase.** HeLa cells expressing an EGFP-tagged PP2A–B56δ subunit were imaged at 1-min intervals for 20 min from 1 min before the onset of anaphase using a spinning-disk confocal microscope. A maximum intensity projection of the GFP signal, 17 planes spaced by 0.6 µm through the cell volume, is shown with gray-scale and a heatmap scale together with a single brightfield image taken at the cell equator. PP2A–B56δ was present in the cytoplasm during exit from mitosis and cytokinesis. Time is in minutes relative to metaphase–anaphase transition. Supplemental to Fig. 1 E. Bar, 10 µm.



Video 5. **Central spindle formation in control cells.** HeLa cells stably expressing EGFP-tubulin (green) and mCherry-histone H2B (red) were treated with control siRNA. Images corresponding to 17 planes spaced by 0.6 µm through the cell volume were collected at 1-min intervals for 20 min from 1 min before the onset of anaphase using a spinning-disk confocal microscope. A maximum intensity projection is shown together with a single brightfield image taken at the cell equator. Time is in minutes relative to metaphase–anaphase transition. Supplemental to Fig. 5 A. Bar, 10 µm.



Video 6. **Central spindle formation in PP2A–B56γ and -ε–depleted cells.** HeLa cells stably expressing EGFP-tubulin (green) and mCherry-histone H2B (red) were depleted of PP2A–B56γ and -ε using siRNA. Images corresponding to 17 planes spaced by 0.6 µm through the cell volume were collected at 1-min intervals for 20 min from 1 min before the onset of anaphase using a spinning-disk confocal microscope. A maximum intensity projection is shown together with a single brightfield image taken at the cell equator. Time is in minutes relative to metaphase–anaphase transition. Supplemental to Fig. 5 A. Bar, 10 µm.