Redli et al., http://dx.doi.org/10.1083/jcb.201603019

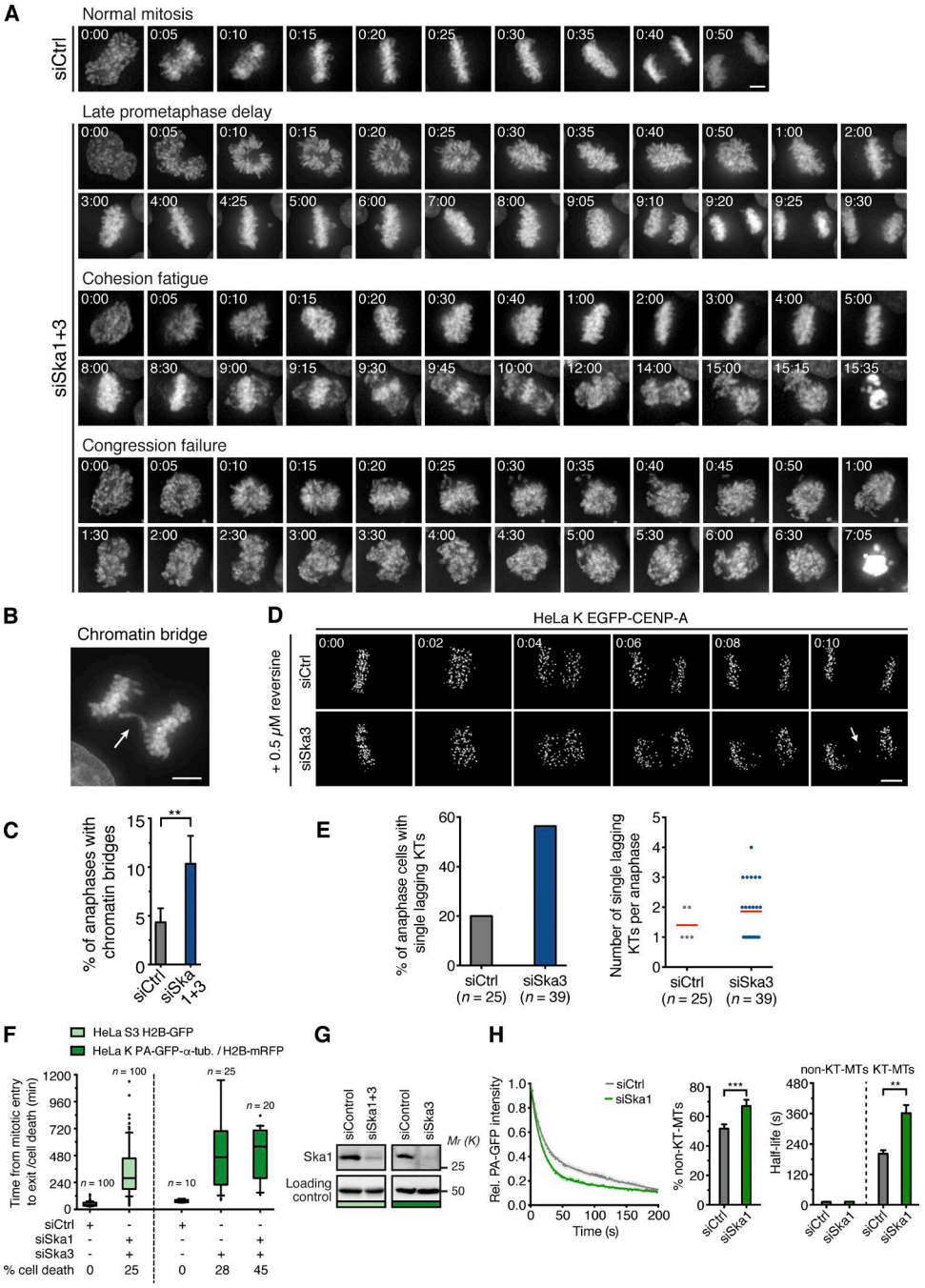

Figure S1. **Consequences of Ska complex depletion on mitotic fidelity.** (A) Selected images from time-lapse imaging sequences of HeLa S3 H2B–GFP cells treated with control or Ska1 and Ska3 siRNAs for 36–40 h that illustrate previously described Ska RNAi phenotypes. Representative image (B) and quantification (C) of anaphase cells with chromatin bridges. Bars represent mean ± 95% CI ($n \geq 275$ anaphase cells per condition from three independent experiments). (D) Selected images from time-lapse imaging sequences of HeLa K-CENP-A cells treated with control or Ska3 siRNAs for 36–40 h that were subjected to 0.5 µM reversine upon chromosome alignment to induce premature anaphase onset. (E) Quantification of anaphase cells with single lagging KTs and number of single lagging KTs per anaphase from D. The number of cells (*n*) analyzed is indicated below the graphs. (F) Box-and-whisker plot showing the elapsed time between mitotic entry and exit/cell death after the indicated siRNA treatments. The number of cells (*n*) analyzed is given above each box. Horizontal lines indicate mean. Percentage of mitotic cell death is displayed below the plot. (G) Western blotting of mitotic cell extracts of HeLa S3 H2B–GFP cells (lime green) or HeLa K PA-GFP–α-tubulin/histone H2B–mRFP cells (dark green) treated with the indicated siRNAs for 48 h. (H) Quantification of fluorescence intensity decay (left), relative abundance of non-KT-MTs (middle), and half-lives of non-KT-MTs and KT-MTs (right) after photoactivation of spindles in HeLa K PA-GFP–α-tubulin/H2B–mRFP cells treated with siRNAs against Ska1 for 48 h, as described in Fig. 1 (F–I) (see Table S1). Horizontal lines/bars represent mean. Asterisks show statistical significance (Student's *t* test, unpaired). ***, $P \leq 0.001$; **, $P \leq 0.01$. Bars, 5 µm.

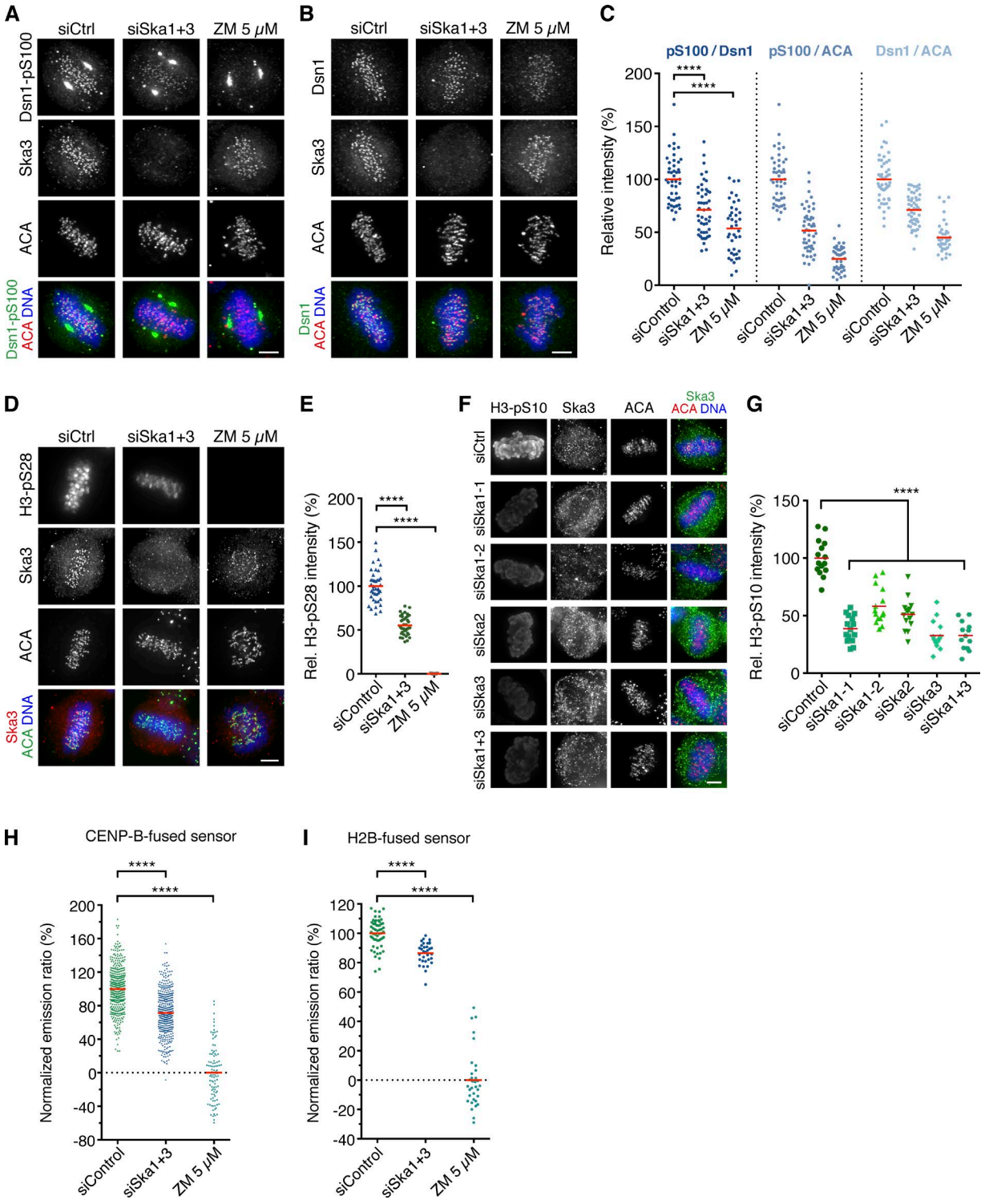

Figure S2. **Additional effect of Ska depletion on Aurora B substrate phosphorylation.** Immunofluorescence images (A and B) and quantification (C) of relative Dsn1-pS100 and Dsn1 intensities in HeLa S3 cells treated for 48 h with control or Ska1 and Ska3 siRNAs. ZM was added to siCtrl cells 1 h before fixation (n = 40–50 cells per condition from three experiments). Immunofluorescence images (D) and quantification (E) of relative histone H3-pS28 intensities in HeLa S3 cells treated as in A (n = 35–40 cells per condition from two experiments). Immunofluorescence images (F) and quantification (G) of relative histone H3-pS10 intensities in HeLa S3 cells treated for 48 h with control or the indicated panel of siRNAs targeting the Ska complex (n = 13–15 cells per condition from one experiment). (H and I) Data from Fig. 2 (L and N) normalized to control and ZM cells. Horizontal lines depict mean. Asterisks show statistical significance (Student's t test, unpaired). ****, $P \leq 0.0001$. Bars, 5 µm.

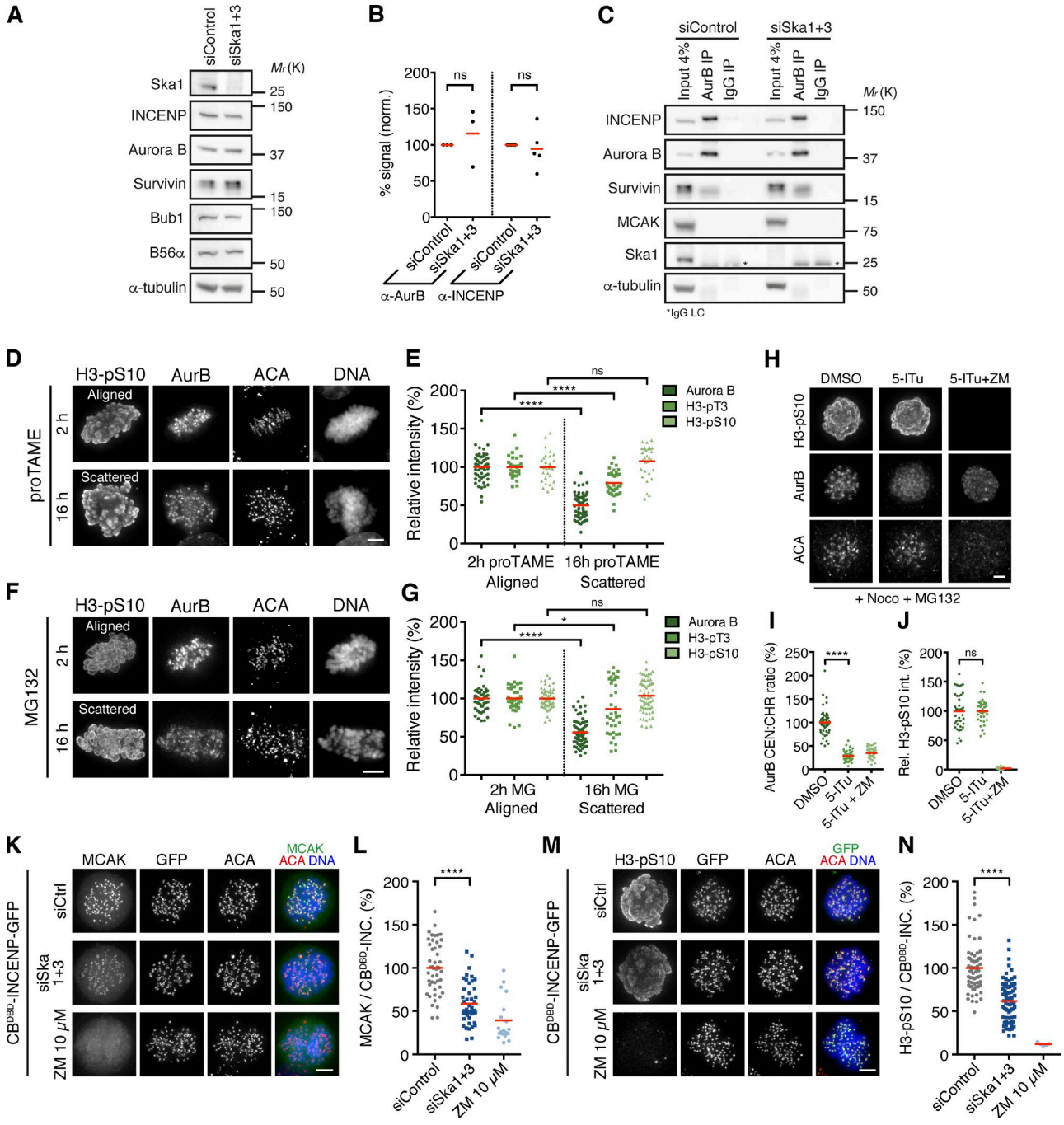

Figure S3. **Analysis of CPC complex formation in Ska-depleted cells, and effect of cohesion fatigue and Haspin inhibition on Aurora B activity.** (A) Western blotting (WB) analysis of CPC complex subunits and CPC-related proteins (Bub1, PP2A-B56α) in mitotic HeLa S3 cell extracts 48 h after transfection with control or Ska1 and Ska3 siRNAs. α-Tubulin is shown as loading control. (B) Scatter plot showing the corresponding quantification of the relative Aurora B and INCENP signal intensities from C normalized against α-tubulin (three to five experiments). (C) Mitotic HeLa S3 cell extracts prepared from cells treated for 48 h with control or Ska1 and Ska3 siRNAs were subjected to immunoprecipitation (IP) with Aurora B or control antibodies (IgG), before WB with the indicated antibodies. (D) Immunofluorescence analysis of histone H3-pS10 and Aurora levels in HeLa S3 cells treated for 2 or 16 h with the anaphase-promoting complex/cyclosome (APC/C) inhibitor proTAME. (E) Corresponding scatter plot of the relative Aurora B, histone H3-pS10, and histone H3-pT3 intensities in cells treated as in D (n = 30–65 cells per condition from two experiments). (F) Histone H3-pS10 and Aurora B levels in HeLa S3 cells treated for 2 or 16 h with the proteasome inhibitor MG132. (G) Scatter plot of the relative Aurora B, histone H3-pS10, and histone H3-pT3 intensities in cells treated as in F (n = 35–67 cells per condition from two experiments). (H–J) HeLa S3 cells were treated with 3.3 μM nocodazole for 50 min before MG132 was added for 10 min. Cells were then treated 1.5 h with DMSO, the Haspin kinase inhibitor 5-iodotubercidin (5-ITu), or 5-ITu and ZM as control. Shown are the corresponding immunofluorescence images (H) and scatter plots of the relative Aurora B (I) and H3-pS10 (J) intensities. Immunofluorescence images (K) and quantification (L) of normalized KT MCAK fluorescence intensities in HeLa S3 cells cotransfected with CB^{DBD}-INCENP–GFP and control or Ska1 and Ska3 siRNAs for 48 h. ZM was added to siControl cells 1 h before fixation. The MCAK signal intensities were divided by the respective GFP signals to normalize for differences in the CB-INCENP–GFP expression levels (n = 45–47 cells for siControl and siSka1+3 from two experiments, and n = 19 cells for ZM from one experiment). Immunofluorescence images (M) and quantification (N) of normalized histone H3-pS10 intensities in cells treated as in K (n = 64–72 cells for siControl and siSka1+3 from three experiments, and n = 5 cells for ZM from one experiment). Asterisks show statistical significance (Student's t test, unpaired). ****, $P \leq 0.0001$; *, $P \leq 0.05$; ns, nonsignificant. Bars, 5 μm.

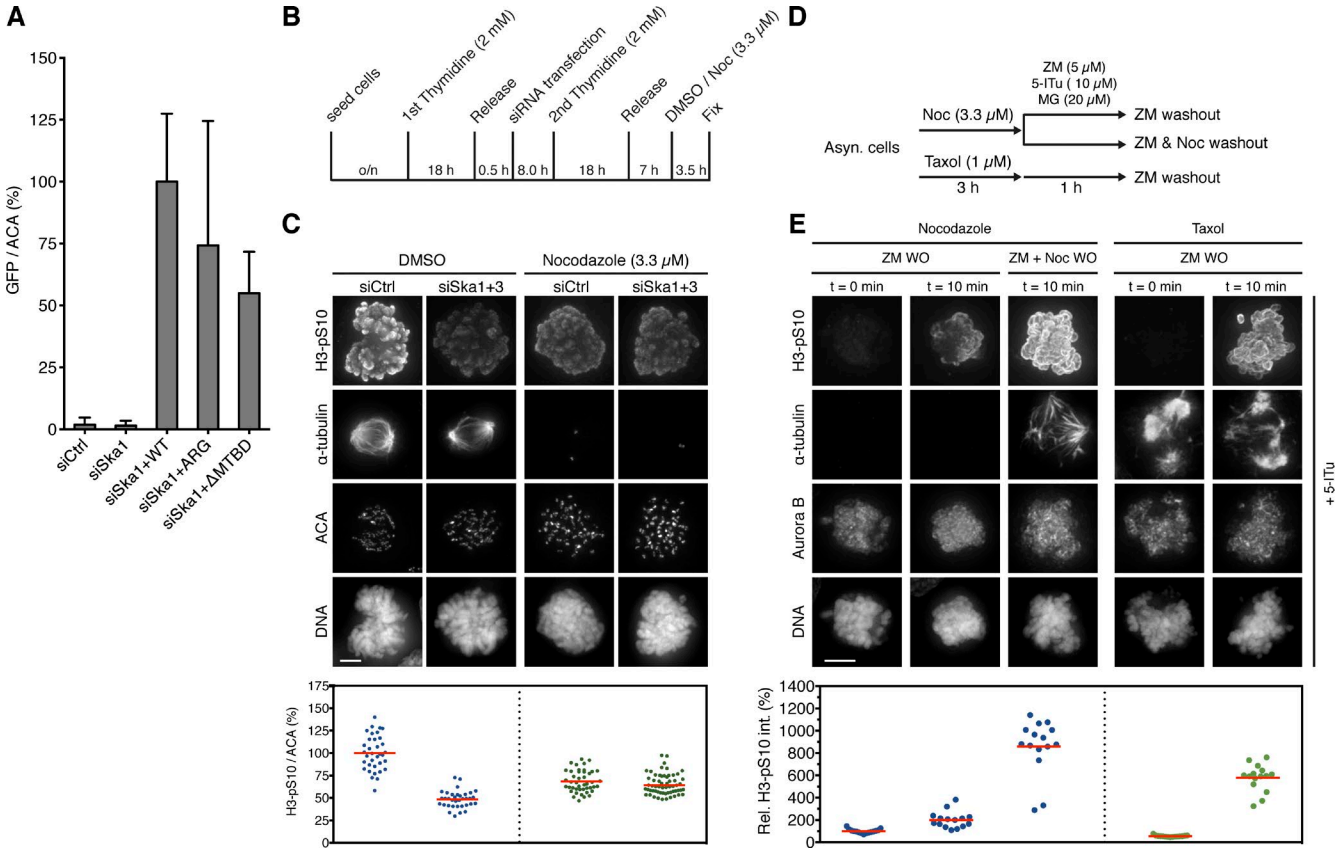

Figure S4. **Microtubule dependence of Aurora B activity.** (A) Construct expression levels at KTs of HeLa cells stably expressing RNAi-resistant GFP-Ska1 wild-type, GFP-Ska1$^{R155A,\ R236A,\ R245A}$, and GFP-Ska1$^{\Delta MTBD}$, respectively, depleted of endogenous Ska1. Data represent mean ± SD (n = 100 cells per conditions from five experiments). (B) Scheme representing the experimental setup followed in C. (C) Immunofluorescence images and quantification of relative H3-pS10 levels in HeLa S3 cells depleted of Ska1 and Ska3 or treated with control siRNAs, synchronized by a double thymidine arrest/release as depicted in B and released into 3.3 µM nocodazole or DMSO (as control; n = 35–60 cells per condition from two experiments). (D) Scheme representing the experimental setup followed in E. (E) Asynchronously (Asyn.) growing cells were treated with 3.3 µM nocodazole (Noc) or 1 µM taxol, and after 3 h, the Aurora B inhibitor ZM (5 µM), the Haspin inhibitor 5-ITu (10 µM), and MG132 (20 µM) were added to the cells. ZM was washed out (WO) from cells to allow Aurora B reactivation, and cells were then fixed at the indicated times. Shown are immunofluorescence images and the quantification of relative H3-pS10 intensities (n = 15 cells per condition from one experiment). Bars, 5 µm.

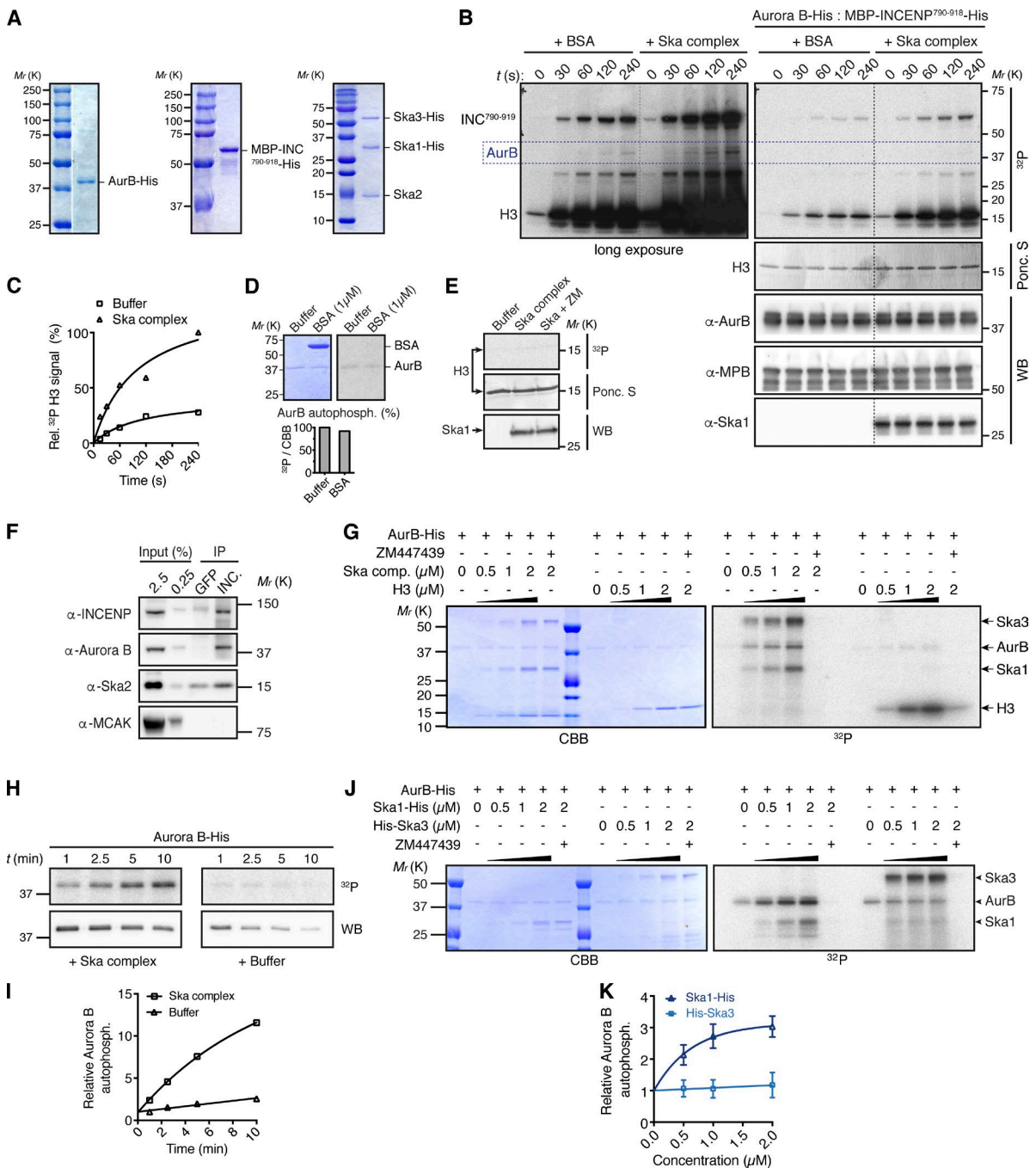

Figure S5. **Aurora B activity is enhanced by Ska in vitro.** (A) Coomassie-stained gels of recombinant Aurora B–His (left), MBP–INCENP$^{790–919}$–His (middle), and the Ska complex (right), separately expressed and purified from *E. coli*. (B) In vitro Aurora B kinase activation assay, performed as described in Fig. 5 A, showing Aurora B autophosphorylation after prolonged exposure (dashed blue frame). (C) Aurora B kinase activation assay as described in Fig. 5 A, where BSA was omitted in the control reaction. Shown is the quantification of the relative histone H3 ^{32}P signal intensities normalized to the H3 and Aurora B protein levels (one experiment). (D) Aurora B–His was preincubated for 30 min at 30°C in the presence or absence of BSA, before incubation with γ-[^{32}P]ATP for another 30 min. Autophosphorylation of Aurora B is visualized by autoradiography (right) and Aurora B levels by Coomassie staining (left). The bar graph shows the quantification of the ^{32}P autophosphorylation signals normalized to the Aurora B levels (one experiment). (E) Histone H3 was incubated with Ska complex dialysis buffer, Ska complex, or Ska complex and 10 µM ZM for 30 min at 30°C in the presence of γ-[^{32}P]ATP. Incorporation of ^{32}P into histone H3 was monitored by autoradiography (^{32}P), and levels of histone H3 and the Ska complex were monitored by Ponceau S staining (Ponc. S) and Western blotting (WB), respectively. (F) Immunoprecipitates (IP) from mitotic HeLa S3 cell extracts, obtained using beads coupled to anti-INCENP antibody or control antibody (anti-GFP), analyzed by WB. (G) Uncropped Coomassie-stained gel (CBB) and autoradiograph (^{32}P) of the Aurora B autophosphorylation assay shown in Fig. 5 E. comp., complex. (H) Aurora B–His was preincubated with Ska complex or Ska complex dialysis buffer, as control, before addition of γ-[^{32}P]ATP. Reactions were stopped at the indicated time-points. Autophosphorylation of Aurora B was detected by autoradiography (^{32}P), and levels of Aurora B by WB. (I) Quantification of the relative Aurora B autophosphorylation signals normalized to the Aurora B protein levels from H (one experiment). (J) Aurora B–His was preincubated with purified Ska1-His or His-Ska3 at the indicated concentrations before addition of γ-[^{32}P]ATP. Autophosphorylation of Aurora B and phosphorylation of Ska1 and Ska3 were detected by autoradiography (^{32}P) and the Aurora B levels were visualized by Coomassie brilliant blue (CBB) staining. (K) Quantification of the relative Aurora B autophosphorylation signals normalized to the Aurora B protein levels from J. Data represent mean ± SD (three experiments).

Table S1. **Double exponential decay curve-fitting parameters and sample sizes analyzed related to Fig. 1 (F–I)**

Parameter	siCtrl	siSka1	siSka3
Fibers, *n*	111	83	111
Cells, *n*	32	23	31
Experiments, *n*	3	2	3
P_{fast}	0.52	0.67	0.66
k_{fast}	0.060	0.058	0.049
$t_{1/2}$ (fast) = ln(2)/k_{fast}	11.6	11.9	14
k_{slow}	0.00344	0.00192	0.00188
$t_{1/2}$ (slow) = ln(2)/k_{slow}	202	362	368

Double exponential fit: $I_t = P_{fast} \exp(-k_{fast}\, t) + P_{slow} \exp(-k_{slow}\, t)$.