Wang et al., http://www.jcb.org/cgi/content/full/jcb.201205106/DC1

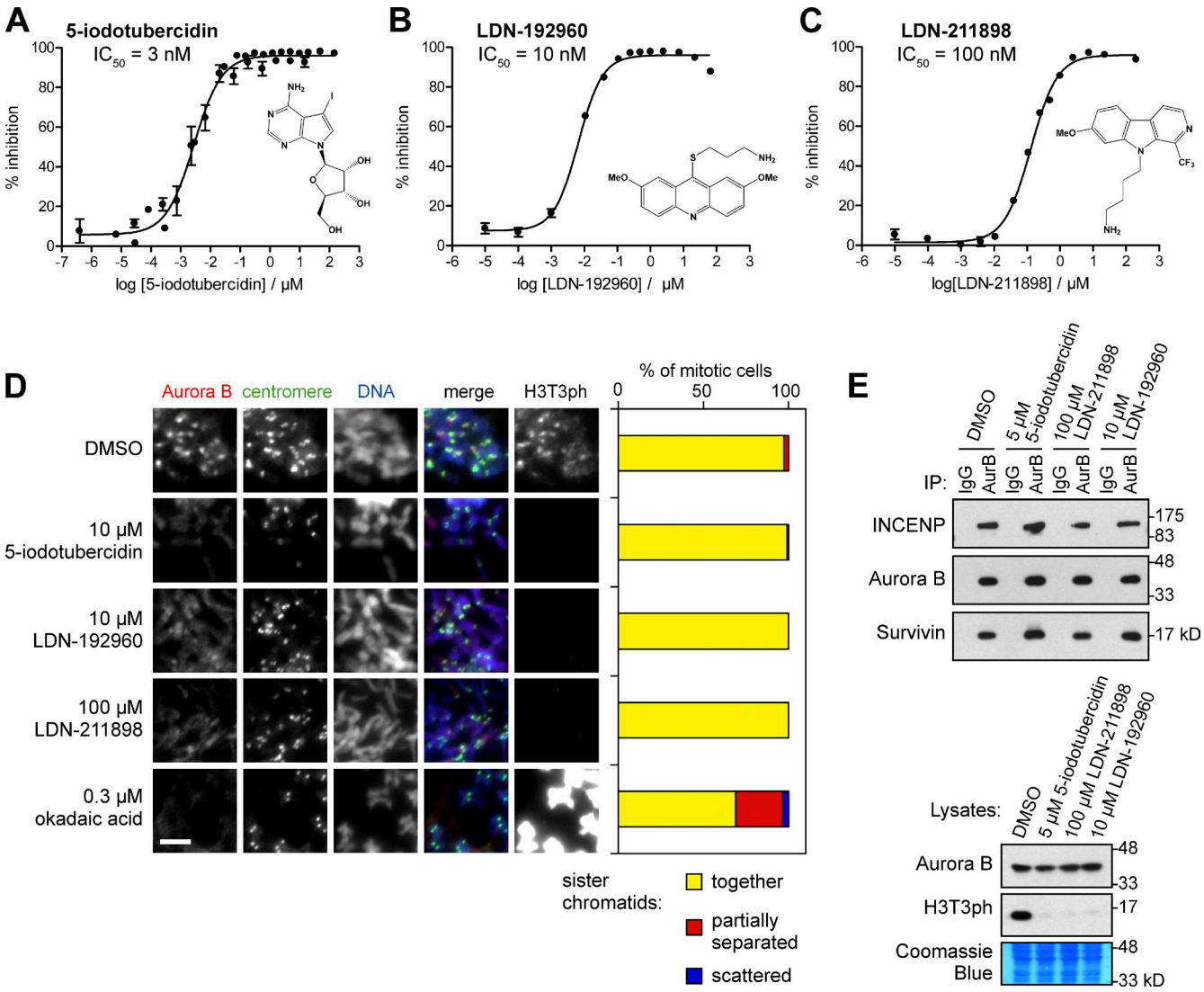


Figure S1. **Basic parameters of in vitro and cellular Haspin inhibitor activity.** (A) Determination of IC_{50} of 5-iodotubercidin using MBP-Haspin in a TR-FRET assay with H3(1–21)-biotin peptide as a substrate. Means ± SD are shown (error bars), *n* = 3. (B and C) As in A, but for LDN-192960 (B) or LDN-211898 (C). (D) Haspin inhibitors do not affect sister chromatid cohesion. HeLa cells were released from a thymidine block, then, after 6.5 h, 60 nM nocodazole was added for 2 h. Mitotic cells were collected by "shake-off" and replated with the addition of Haspin inhibitors or okadaic acid (a phosphatase inhibitor that induces premature chromatid separation) and 20 µM MG132 for 2 h before chromosomes spreads were prepared. Sister chromatid cohesion status was determined for at least 100 cells per condition by immunofluorescence microscopy. The result is representative of two experiments. Exposure times for the DNA channel only were adjusted separately to better reveal chromosomes. Bar, 5 µm. (E) Haspin inhibitors do not cause disassembly of the CPC. HeLa cells were released from a thymidine block, then, after 3 h, 3 µM nocodazole was added for 11 h and mitotic cells were collected by shake-off. Haspin inhibitors and 20 µM MG132 were added for 2 h before cells were lysed and subjected to immunoprecipitation followed by immunoblotting with the antibodies indicated.

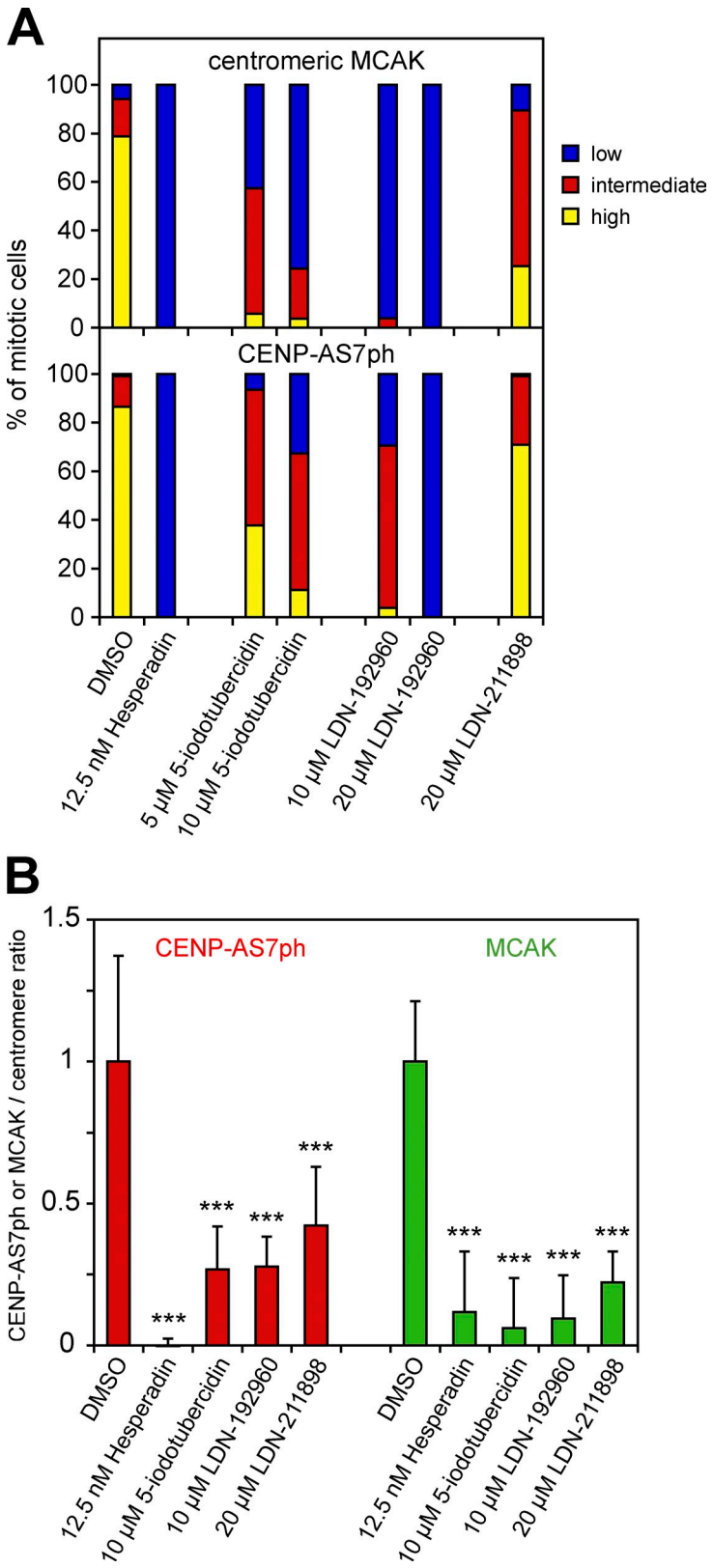


Figure S2. **Effect of Haspin inhibitors on centromeric MCAK and CENP-AS7ph in HeLa cells.** (A) Nocodazole-arrested HeLa cells were exposed to Haspin inhibitors for 1 h as described in Fig. 1 B. The mitotic centromeric staining intensity of MCAK and CENP-AS7ph in one experiment was classified by immunofluorescence microscopy for at least 100 cells per condition. Similar results were obtained in independent experiments (Fig. 3) and in U2OS cells (Fig. 2). (B) The intensities of CENP-AS7ph and MCAK at mitotic centromeres in each condition in A were quantified (18 centromeres/cell; n = 5–9 cells). Results are expressed as a ratio to centromeric autoantigen staining intensity at the same centromeres. Means + SD are shown (error bars); ***, $P < 0.001$ vs. DMSO.

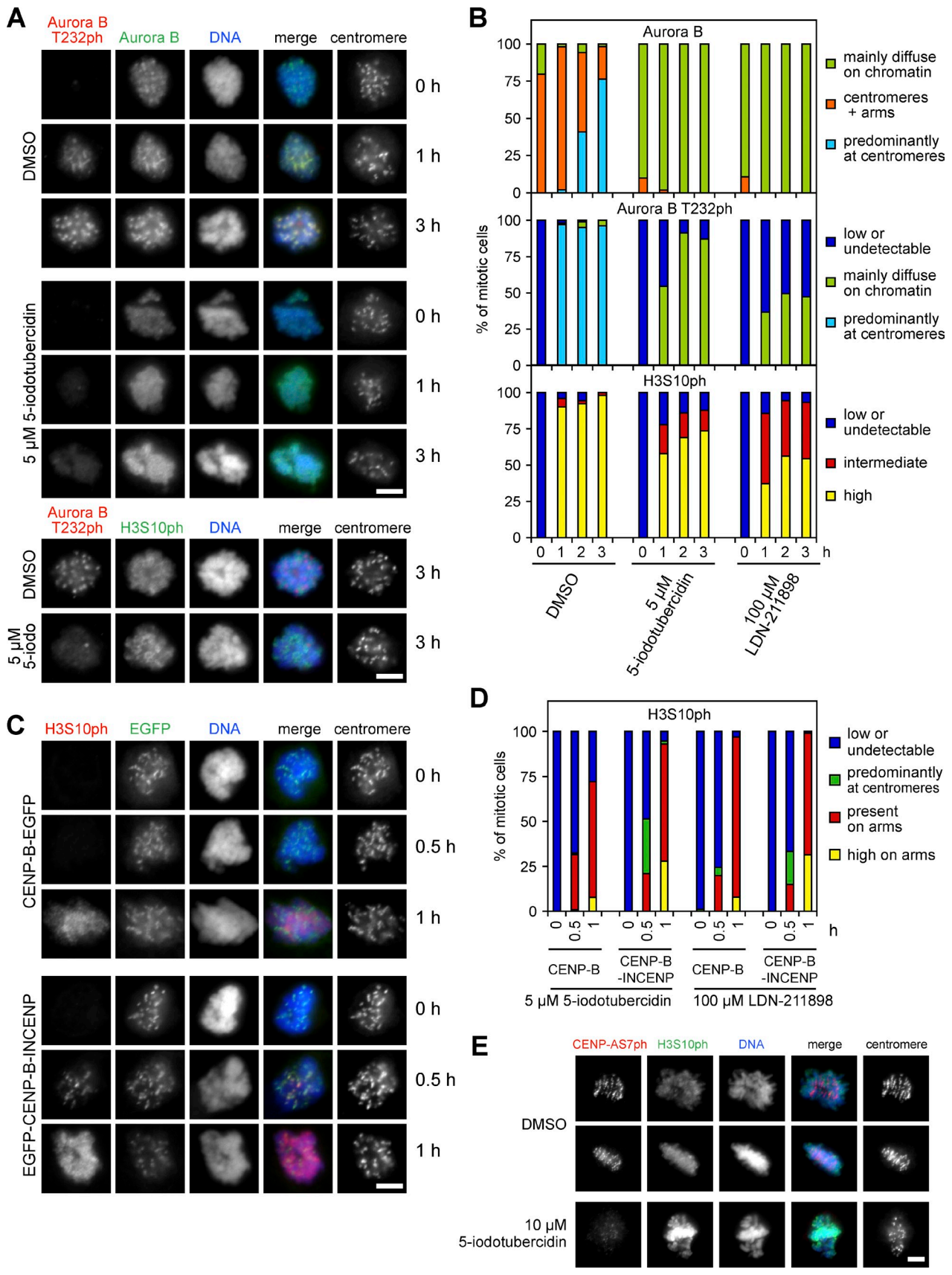


Figure S3. **Effect of Haspin inhibitors on Aurora B localization and autophosphorylation, and on H3S10ph recovery in Aurora B reactivation assays.** (A) Immunofluorescence microscopy of Aurora B, Aurora B-T232ph, and H3S10ph during Aurora B reactivation in the presence or absence of Haspin inhibitors in HeLa cells treated as in Fig. 4 A. (B) Approximately 100 mitotic cells in each condition from one experiment as in A were classified according to the localization of Aurora B (top), the localization and intensity of Aurora B T232ph (middle), and the intensity of H3S10ph (bottom). Similar results were obtained in a second experiment. (C) HeLa cells were transfected twice with plasmids encoding CENP-B–EGFP or EGFP–CENP-B–INCENP between and after double thymidine treatments, and then treated as in A. (D) The intensity and localization of H3S10ph staining in EGFP-positive cells from C was classified for at least 100 mitotic cells per condition in one experiment. Similar results were obtained in a duplicate experiment. (E) HeLa cells were released from a thymidine block and, after 7 h, DMSO or 10 µM 5-iodotubercidin were added for 3 h before analysis by immunofluorescence microscopy. Bars, 5 µm.

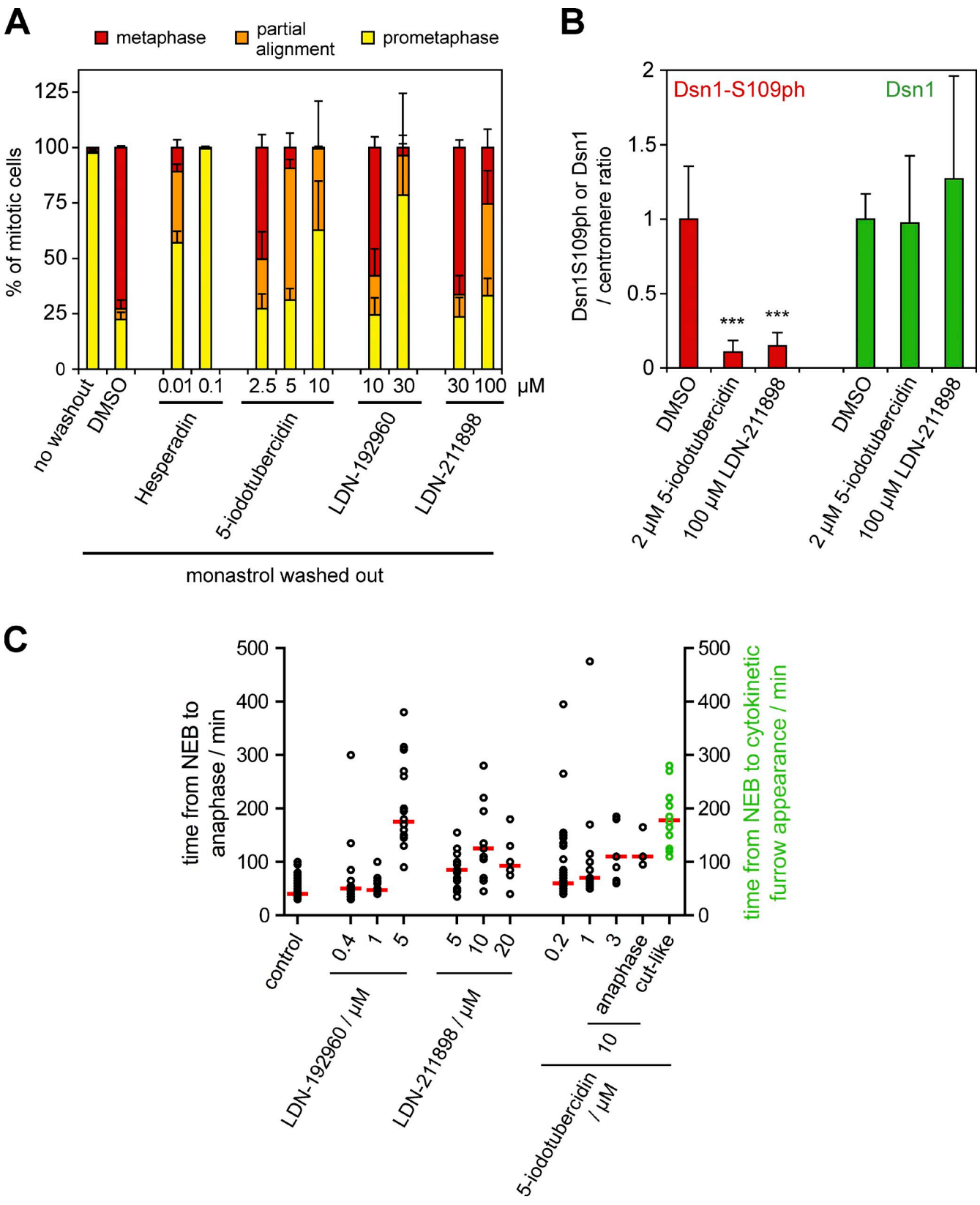


Figure S4. **Effect of Haspin inhibitors on error correction and progression to anaphase.** (A) HeLa cells were synchronized by single thymidine treatment and, 7 h after release, 100 µM monastrol was added for 3 h. Monastrol was then removed by washing into fresh medium containing Haspin inhibitors or control compounds in the continued presence of MG132. Chromosome alignment was determined by fluorescence microscopy of Hoechst-stained cells. Means ± SD are shown, *n* = 3. (B) Immunofluorescence microscopy of Dsn1-S109ph or total Dsn1 during Aurora B reactivation for 2 h in the presence or absence of Haspin inhibitors in HeLa cells treated as in Fig. 5 (B and C). The intensities of Dsn1-S109ph (10 centromeres/cell; *n* = 15 cells) and total Dsn1 (10 centromeres/cell; *n* = 6–8 cells) at kinetochores were quantified. Results are expressed as a ratio to centromeric autoantigen staining intensity at the same centromeres. Means + SD are shown (error bars); ***, P < 0.001 vs. DMSO. (C) The length of mitosis (NEB to anaphase onset; or, for cut-like cells in green, from NEB to onset of cytokinetic furrowing) for individual U2OS cells treated as described in Fig. 6 was determined by live imaging. Note that cells in 10 µM 5-iodotubercidin that underwent anaphase and those that displayed a cut-like phenotype are shown separately. Red lines indicate median mitotic duration. The results are derived from 1–5 independent movies of each condition.

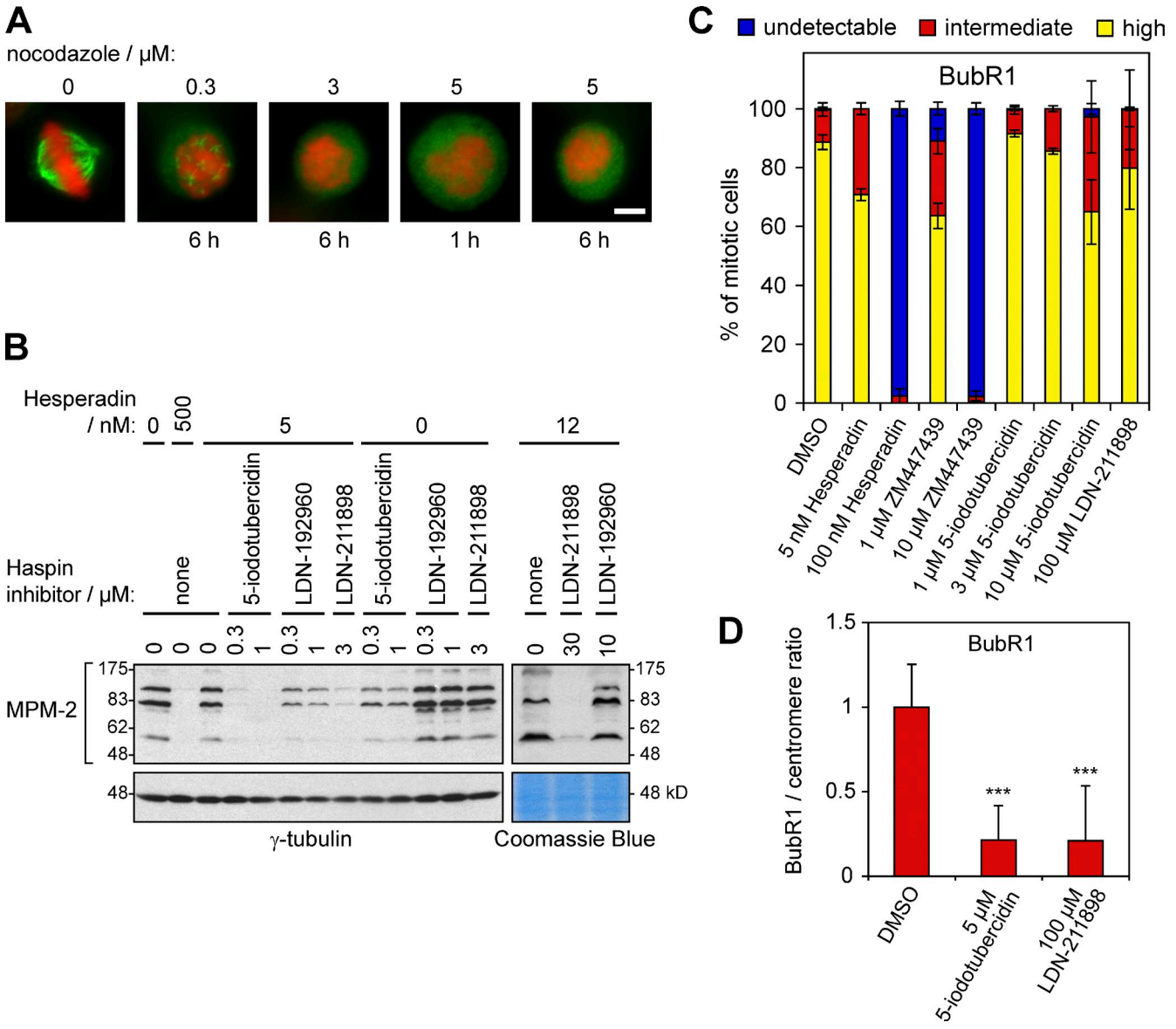
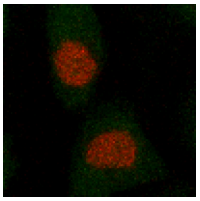
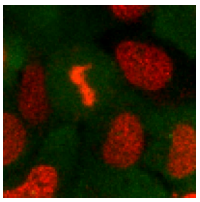


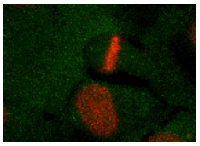
Figure S5. **Effect of Haspin inhibitors on the spindle checkpoint.** (A) Confirmation that 5 μM nocodazole severely disrupts microtubules in mitosis. HeLa cells were treated with increasing concentrations of nocodazole for 1 or 6 h, followed by immunofluorescence microscopy with antibodies to α-tubulin (green) and Hoechst-33342 to stain DNA (shown red). At 0.33 μM nocodazole, multiple small microtubule asters were visible, which is consistent with previous studies (Jordan et al., 1992), whereas at 3.3 and 5 μM nocodazole, residual microtubules were not observed. Bar, 5 μm. (B) HeLa cells were synchronized and treated essentially as in Fig. 7 A, except that Hesperadin was used instead of ZM447439. Note that 5 μM nocodazole was used throughout. Cell lysates were prepared and analyzed by immunoblotting. In the right panel, note that 30 μM LDN-211898 but not 10 μM LDN-192960 is able to drive mitotic exit in the presence of 12 nM Hesperadin. (C) HeLa cells treated with 5 μM nocodazole were then exposed to Haspin inhibitors as in Fig. 7 A. The kinetochore staining intensity of BubR1 was classified for at least 100 cells in each condition by immunofluorescence microscopy. Means ± SD are shown (error bars), $n = 3$. (D) Immunofluorescence microscopy of BubR1 after Aurora B reactivation for 2 h in the presence or absence of Haspin inhibitors in HeLa cells treated as in Fig. 7 A. The intensity of BubR1 at kinetochores was quantified (10 centromeres/cell; $n = 15$ cells). Results are expressed as a ratio to centromeric autoantigen staining intensity at the same centromeres. Means + SD are shown (error bars); ***, $P < 0.001$ vs. DMSO.



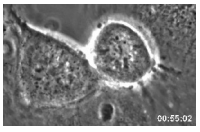
Video 1. **Mitosis in vehicle-treated U2OS cells.** U2OS cells stably expressing Histone-H2B-mRFP and γ-tubulin–GFP were exposed to 0.1% DMSO (vehicle), and mitotic progression was followed by live laser-scanning confocal fluorescence microscopy (TE-2000; Nikon). Maximum intensity projections of H2B-mRFP (red) and γ-tubulin–GFP (green) fluorescence are shown for 44 frames taken every 5 min. See Fig. 6 A.



Video 2. **Mitosis in LDN-211898-treated U2OS cells.** U2OS cells stably expressing Histone-H2B-mRFP and γ-tubulin–GFP were exposed to 10 μM LDN-211898, and mitotic progression was followed by live laser-scanning confocal fluorescence microscopy (TE-2000; Nikon). Maximum intensity projections of H2B-mRFP (red) and γ-tubulin–GFP (green) fluorescence are shown for 60 frames taken every 5 min. See Fig. 6 B.



Video 3. **Mitosis in 5-iodotubercidin-treated U2OS cells.** U2OS cells stably expressing Histone-H2B-mRFP and γ-tubulin–GFP were exposed to 10 μM 5-iodotubercidin, and mitotic progression was followed by live laser-scanning confocal fluorescence microscopy (TE-2000; Nikon). Maximum intensity projections of H2B-mRFP (red) and γ-tubulin–GFP (green) fluorescence are shown for 73 frames taken every 5 min. See Fig. 6 C.



Video 4. **Effects of anti-H3T3ph microinjection in an error correction assay.** LLC-PK cells arrested in mitosis with monopolar spindles with 10 μM 5-S-trityl-L-cysteine (a kinesin-5/Eg5 inhibitor) and 25 μM MG132 were injected or not injected with H3T3ph antibodies. Then, 5-S-trityl-L-cysteine was washed out, and the cells were followed for ∼3 h in the presence of 25 μM MG132 to counter mitotic exit. Time-lapse phase-contrast images were collected using a microscope (Axiovert 200M; Carl Zeiss). Times of frame acquisition are shown in hours:minutes:seconds. Of 10 control cells observed for ∼3 h after washout, 60% formed bipolar spindles and then aligned all chromosomes within 20 min, whereas 40% failed to form bipolar spindles and align chromosomes. Of 10 injected cells observed for 3 h, 30% formed bipolar spindles and then aligned all chromosomes within 20 min, whereas 70% failed to align all chromosomes (20% formed bipolar spindles but then did not align all chromosomes for at least 55 min [see video], and 50% failed to form bipolar spindles).

Reference

Jordan, M.A., D. Thrower, and L. Wilson. 1992. Effects of vinblastine, podophyllotoxin and nocodazole on mitotic spindles. Implications for the role of microtubule dynamics in mitosis. *J. Cell Sci.* 102:401–416.