Maciejowski et al., http://www.jcb.org/cgi/content/full/jcb.201001050/DC1

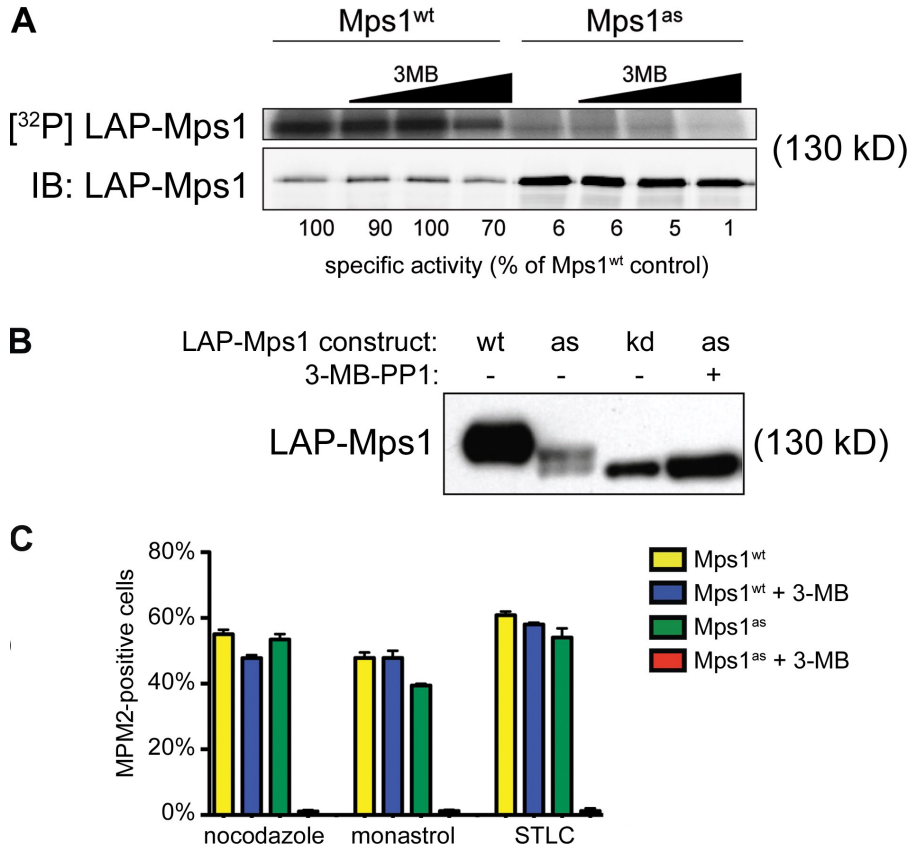

Figure S1. **Mps1^{as} kinase activity is analogue sensitive and required for mitotic arrest in response to several spindle poisons.** (A) Mps1 was immunoprecipitated from mitotic Mps1^{wt} or Mps1^{as} extracts and either immunoblotted (IB; bottom) or incubated with γ-[^{32}P]ATP. 3MB-PP1 (3MB) was added to the reaction at 0.1, 1.0, or 10 µM where indicated. (B) Inhibition of Mps1^{as} prevents its autophosphorylation and electrophoretic upshift during mitosis. HEK293 cells were transfected with the indicated localization and affinity purification–tagged forms of Mps1, synchronized in mitosis with nocodazole, and treated for 2 h with 3MB-PP1. Lysates were resolved by SDS-PAGE and immunoblotted with GFP-specific antibodies (reactive with the LAP tag). (C) Mps1^{as} inhibition overrides SAC arrest caused by MT-destabilizing and nondestabilizing spindle poisons. Cells were treated for 16 h with nocodazole, monastrol, or STLC in the presence or absence of 3MB-PP1. Mitotic indices were determined by MPM-2 staining and flow cytometry. LAP, localization and affinity purification. Error bars indicate SEM.

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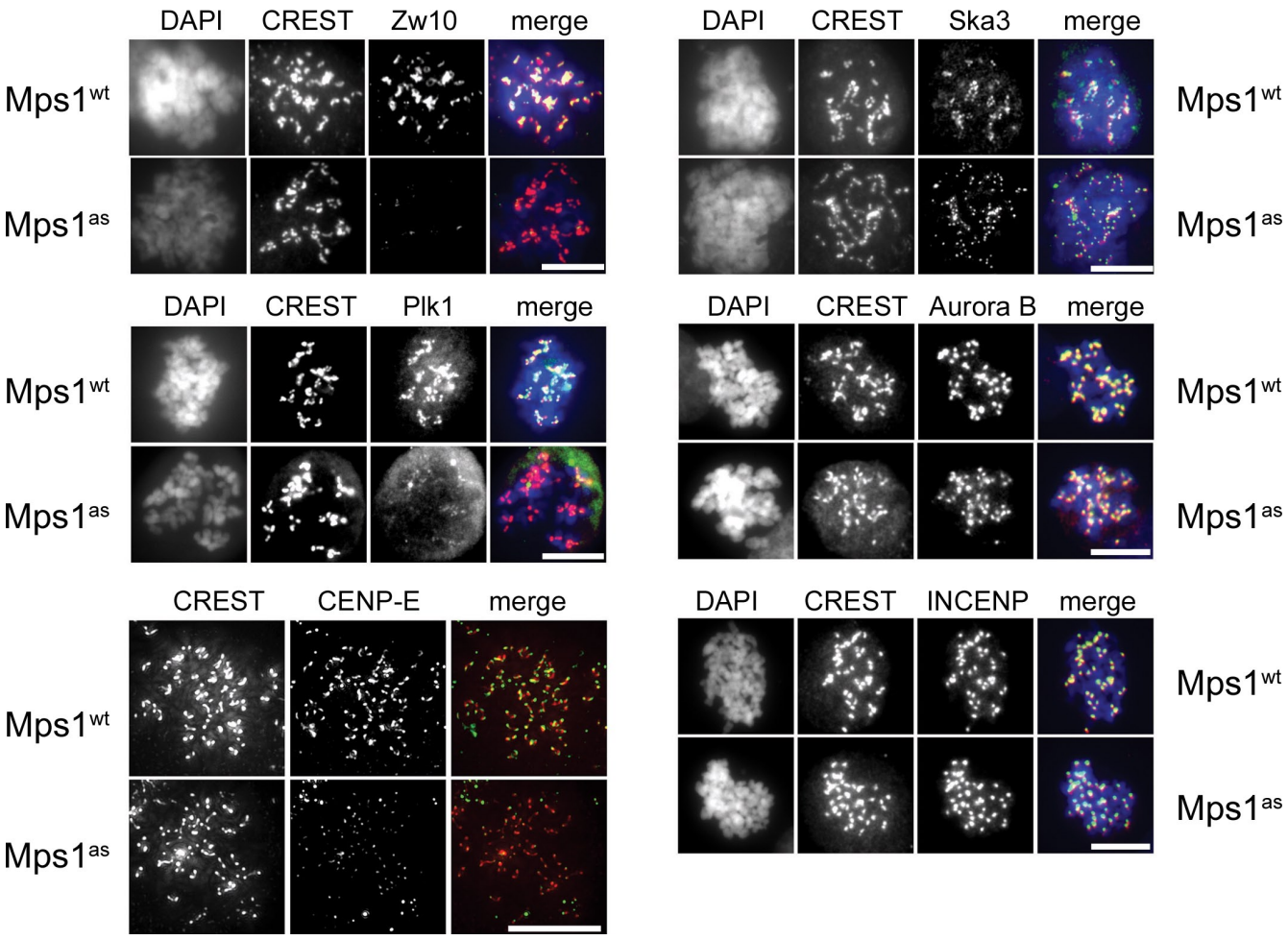


Figure S2. **Mps1 kinase activity is continuously required to maintain SAC effectors at kinetochores.** Cells treated as in Fig. 7 were stained with antibodies to the indicated SAC/kinetochore proteins (green), CREST antiserum (red), and DAPI (blue). All images were acquired via widefield microscopy except for CENP-E, which was acquired and deconvolved on an image restoration microscope (Deltavision). Bars, 10 µm.

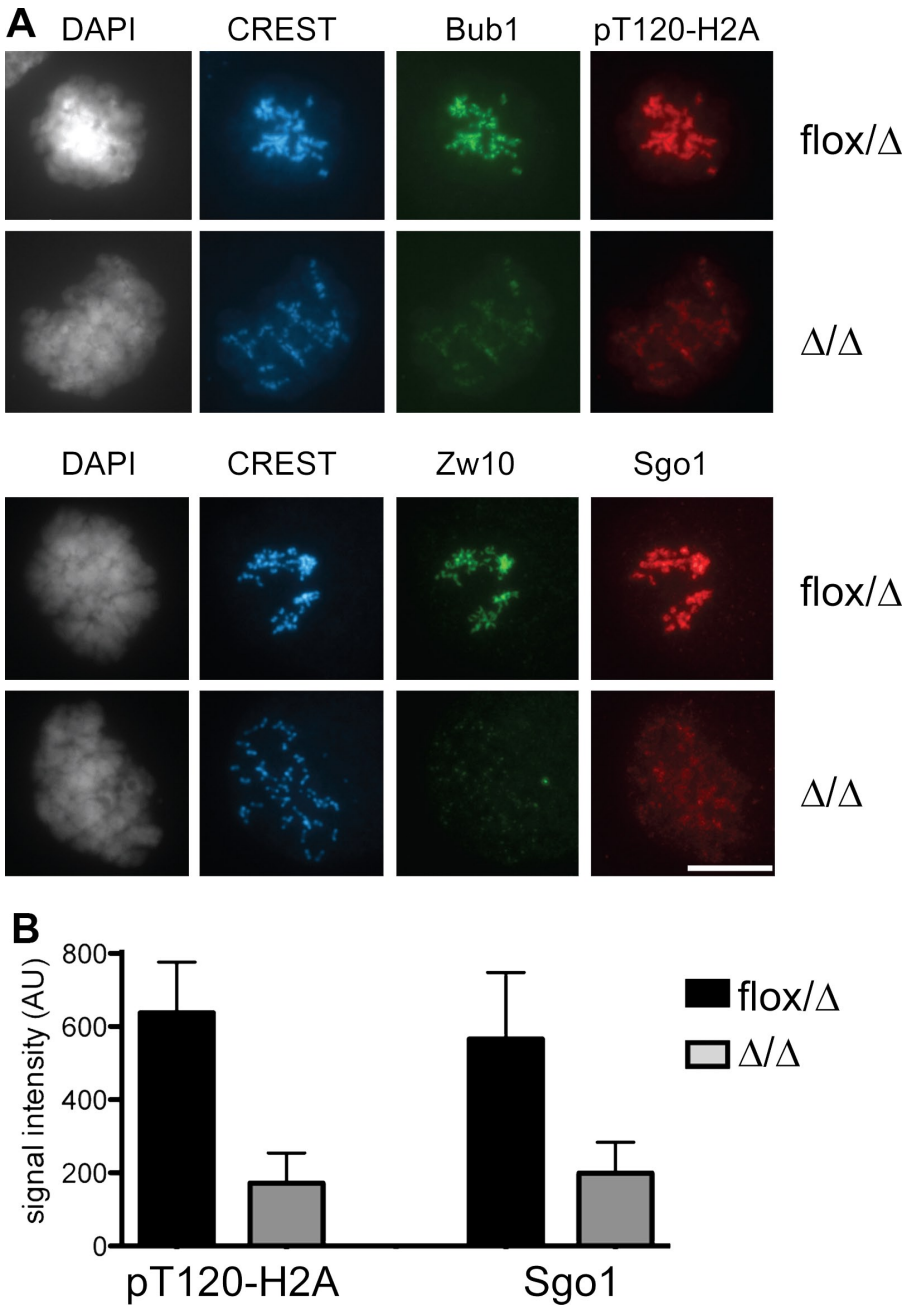
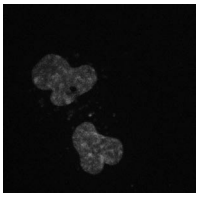
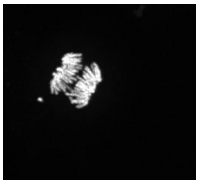


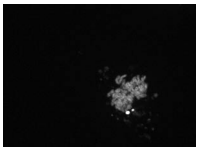
Figure S3. **Mps1 promotes Bub1-catalyzed histone H2A phosphorylation and targeting of Sgo1 to centromeres.** (A) *MPS1*$^{flox/\Delta}$ cells were infected with Adβgal (top) or AdCre (bottom). 3 d later, both populations were treated with nocodazole and MG132 for 60 min, fixed, and stained with the indicated antibodies. Bub1 and Zw10 were used to verify functional inactivation of Mps1 (Fig. 7 and Fig. S2). Bar, 10 µm. (B) Quantification of results in A. At least 100 centromeres in at least five cells were scored per sample. Error bars indicate SD.



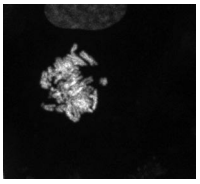
Video 1. **Mps1^{wt} cells treated with 10 µM 3MB-PP1 undergo normal mitosis, example 1.** Mps1^{wt} cells expressing an H2B::mCherry fusion protein were imaged by time-lapse spinning-disk confocal microscopy. Cells were treated with 3MB-PP1 immediately before imaging. Images were collected every minute and correspond to Fig. 2 A. This video is shown at 5 frame/s.



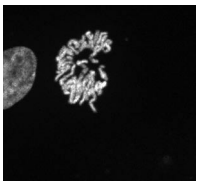
Video 2. **Mps1^{wt} cells treated with 10 µM 3MB-PP1 undergo normal mitosis, example 2.** Mps1^{wt} cells expressing an H2B::mCherry fusion protein were imaged by time-lapse spinning-disk confocal microscopy. Cells were treated with 3MB-PP1 immediately before imaging. Images were collected every minute and correspond to Fig. 2 A. This video is shown at 5 frame/s.



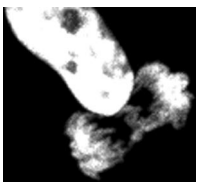
Video 3. **Mps1^{wt} cells treated with 10 µM 3MB-PP1 undergo normal mitosis, example 3.** Mps1^{wt} cells expressing an H2B::mCherry fusion protein were imaged by time-lapse spinning-disk confocal microscopy. Cells were treated with 3MB-PP1 immediately before imaging. Images were collected every minute and correspond to Fig. 2 A. This video is shown at 5 frame/s.



Video 4. **Mps1^{as} cells treated with 10 µM 3MB-PP1 undergo accelerated mitosis, example 1.** Mps1^{as} cells expressing an H2B::mCherry fusion protein were imaged by time-lapse spinning-disk confocal microscopy. Cells were treated with 3MB-PP1 immediately before imaging. Images were collected every minute and correspond to Fig. 2 A. This video is shown at 5 frame/s.



Video 5. **Mps1^{as} cells treated with 10 µM 3MB-PP1 undergo accelerated mitosis, example 2.** Mps1^{as} cells expressing an H2B::mCherry fusion protein were imaged by time-lapse spinning-disk confocal microscopy. Cells were treated with 3MB-PP1 immediately before imaging. Images were collected every minute and correspond to Fig. 2 A. This video is shown at 5 frame/s.



Video 6. **Mps1^{as} cells treated with 10 µM 3MB-PP1 undergo accelerated mitosis, example 3.** Mps1^{as} cells expressing an H2B::mCherry fusion protein were imaged by time-lapse spinning-disk confocal microscopy. Cells were treated with 3MB-PP1 immediately before imaging. Images were collected every minute and correspond to Fig. 2 A. This video is shown at 5 frame/s.

Table S1. **List of antibodies used in this study**

Antibody	Source	Dilution
ACA (CREST)	Immunovision	1:10,000
Anti-actin	Abcam	1:5,000
Anti-APC8	Bethyl Laboratories, Inc.	1:500
Anti–aurora B	Transduction Laboratories	1:250
Anti-Bub1	Santa Cruz Biotechnology, Inc.	1:300
Anti-BubR1	Bethyl Laboratories, Inc.	1:500 (IB)
Anti-BubR1	Millipore	1:250 (IF)
Anti-Cdc20	Santa Cruz Biotechnology, Inc.	1:500
Anti-Cdc20	Santa Cruz Biotechnology, Inc.	1 µg antibody/mg extract
Anti–CENP-E	Santa Cruz Biotechnology, Inc.	1:200
Anti–cyclin B1	BD	1:5,000
Anti-GFP	Santa Cruz Biotechnology, Inc.	1:1,000
Anti-GFP	Invitrogen	1:1,000
Anti–human KNL1	Bethyl Laboratories, Inc.	1:1,000
Anti–human KNL1	I. Cheeseman[a]	1:250
Anti-INCENP	Millipore	1:250
Anti-Mad1	A. Musacchio[b]	1:1,000 IF and IB
Anti-Mad2	Bethyl Laboratories, Inc.	1 µg/1 mg extract
Anti-Mad2	D. Cleveland[c]	1:1,000 (IF)
Anti-Mad2	BD	1:500 (IB)
Anti-Mps1	Santa Cruz Biotechnology, Inc.	1:500 (IB)
Anti-Mps1	Santa Cruz Biotechnology, Inc.	1:1,000 (IB)
Anti-pCENP-A	Millipore	1:500
Anti-pH2A (T120)	Active Motif	1:1,000
Anti-pH3 (S10)	US Biologicals	1:1,000
Anti–MPM-2	Millipore	1:500
Anti-Plk1	Santa Cruz Biotechnology, Inc.	1:500
Anti-securin	Thermo Fisher Scientific	1:250
Anti-Sgo1	Novus Biologicals	1:500
Anti-Ska3	G. Gorbsky[d]	1:250
Anti-tubulin	Santa Cruz Biotechnology, Inc.	1:2,000
Anti-Zw10	Abcam	1:250
Anti-Zwint	Abcam	1:250

IB, immunoblot; IF, immunofluorescence.

[a]Whitehead Institute, Cambridge, MA.

[b]European Institute of Oncology, Milan, Italy.

[c]Unversity of California, San Diego, La Jolla, CA.

[d]Oklahoma Medical Research Foundation, Oklahoma City, OK.