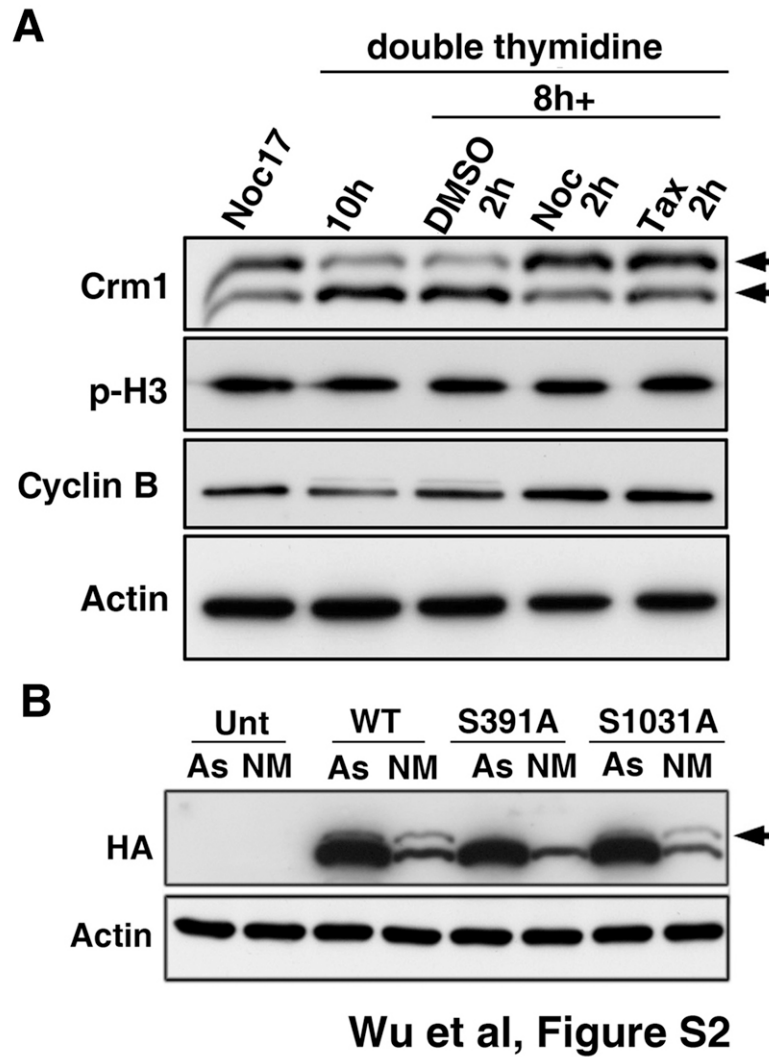
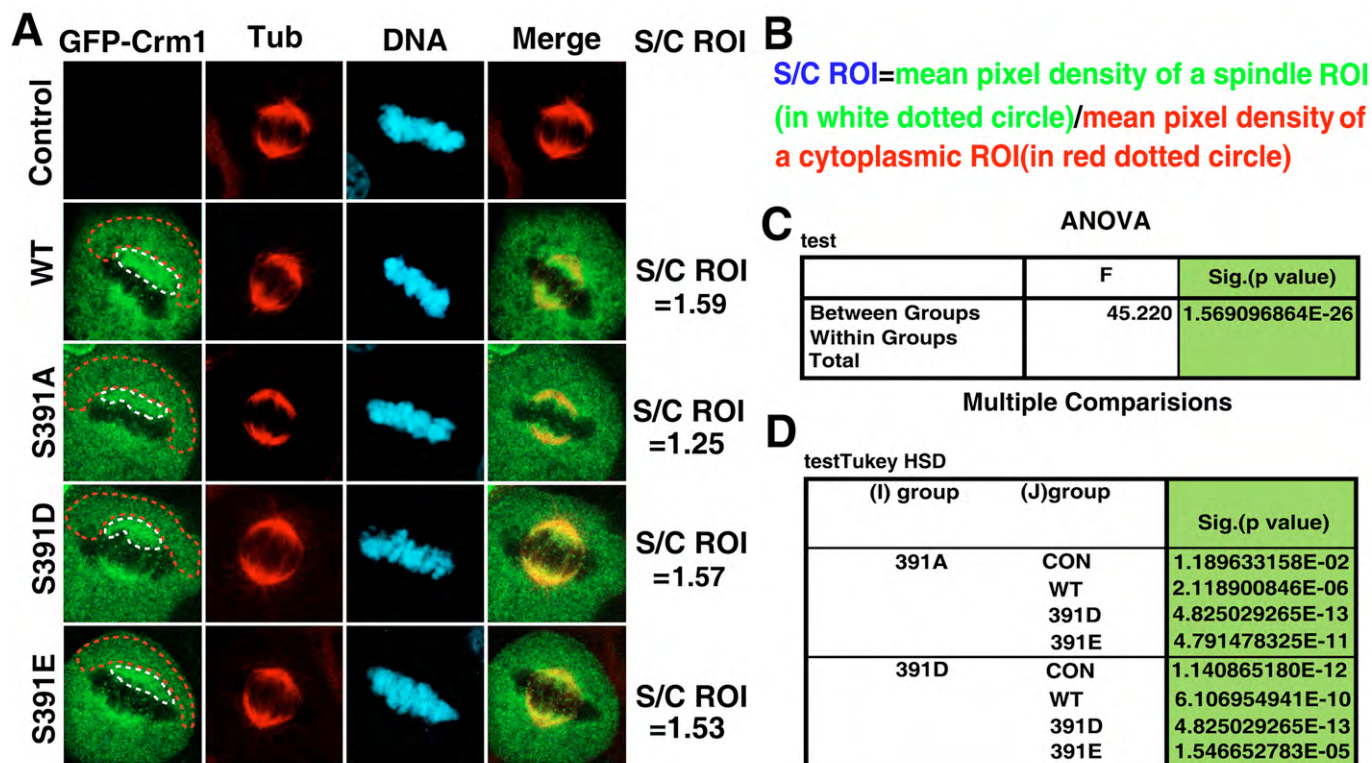


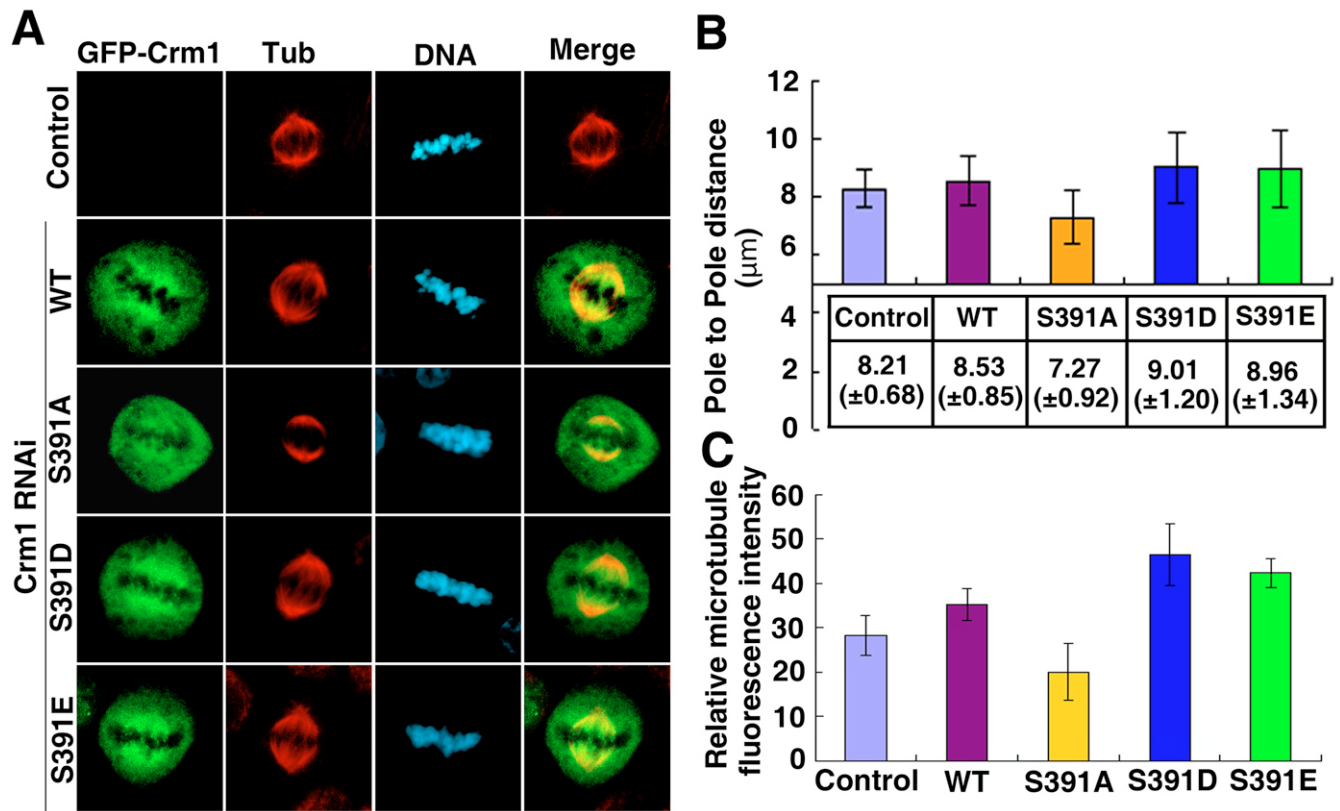
**Fig. S1. Crm1 localizes to mitotic spindle and kinetochore in mitosis.** (A) Asynchronous HeLa cells were fixed with 3.7% PFA and immuno-stained using rabbit anti-Crm1 antibody (Ra-Crm1). DNA was counter-stained using DAPI. The typical kinetochore staining was indicated by the white arrow head. (B) Asynchronous HeLa cells were fixed with 3.7% PFA and immuno-stained using mouse anti-Crm1 antibody (Mo-Crm1). DNA was counter-stained using DAPI. The typical kinetochore staining was indicated by the white arrow head. (C) HeLa cells were transfected with GFP-Crm1. The cells were fixed 24 hours after transfection. DNA was counter-stained with DAPI. The typical kinetochore staining was indicated by the white arrow head. (D) HeLa cells were transfected with GFP-Crm1 (green) and RFP-H2B (red). The living cells were observed under the microscope 24 hours after transfection. The typical kinetochore staining was indicated by the white arrow head. (E) HeLa cells were treated with nocodazole, taxol and monastrol for 3 h, followed by pre-extracted with 0.2% triton X-100 before fixation and subjected for immunofluorescence using anti-C terminal Crm1 (green) and CREST antibodies (red). DNA was simultaneously stained using DAPI.



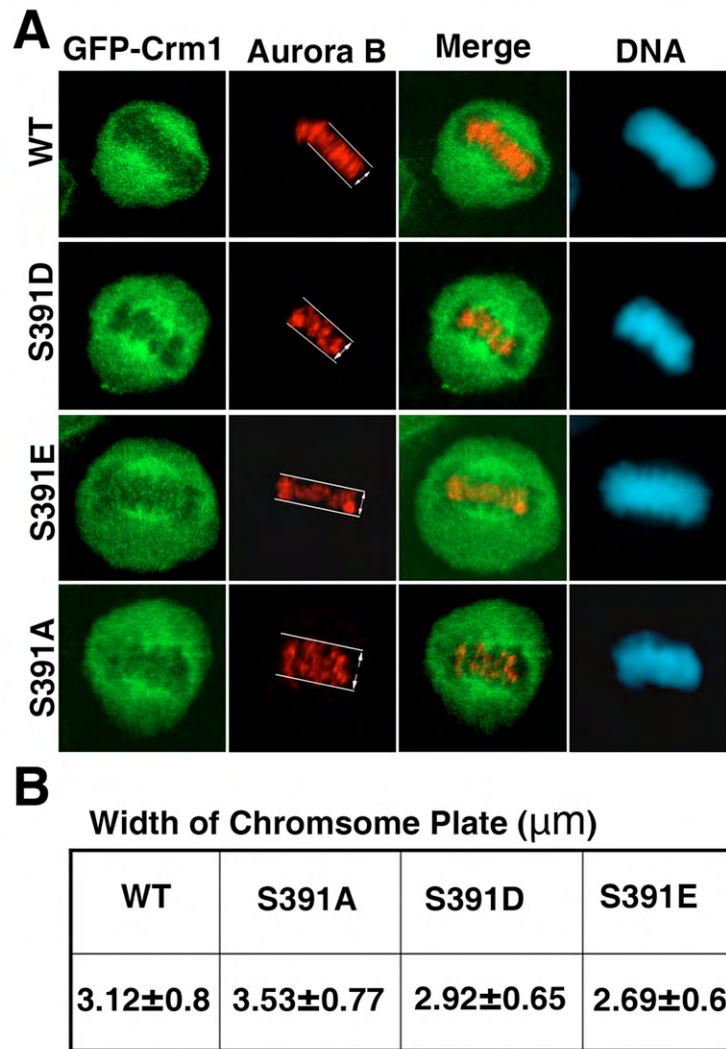
**Fig. S2. Crm1 is phosphorylated at Ser391 in mitosis.** (A) HeLa cells were synchronized at the G1/S boundary by double thymidine block. Then the cells were released into cell cycle either for 8h, followed by treatment of DMSO, Nocodazole, and Taxol for 2h, or for 10h. Samples were analyzed by SDS-PAGE and immunoblotting with Crm1 antibody. (B) HeLa cells were transiently transfected with either HA-Crm1 WT, HA-Crm1 S391A or HA-Crm1 S1031A. After treatment with 100 ng/ml nocodazole for 17h, samples were analyzed by Phos-tag acrylamide gel and immunoblotting with HA antibody.



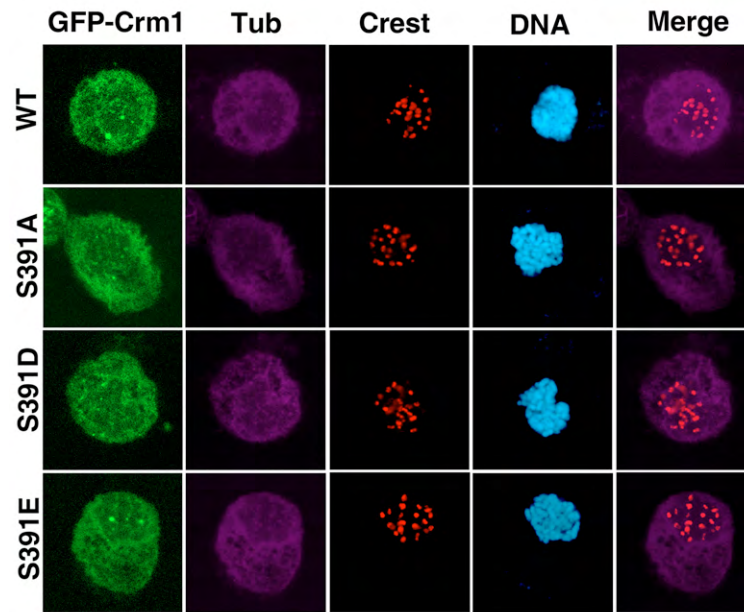
**Fig. S3. Phosphorylation of Crm1 regulates its spindle localization and the spindle size.** (A) Immunofluorescence of HeLa cells transiently transfected with GFP-Crm1 WT, GFP-Crm1 S391A, GFP-Crm1 S391D and GFP-Crm1 S391E plasmids. The cells were fixed 24h after transfection followed by immunofluorescence staining for tubulin (red). DNA was stained using DAPI. The mean pixel densities of a spindle ROI (in white dotted circle) and a cytoplasmic ROI (in red dotted circle) were measured by software Image J. The S/C ROI value was calculated by the formula given in B. (B) The formula for calculating the value of S/C ROI. (C) The results of ANOVA test of the spindle length data in Fig 5B. (D) The results of Tukey's post-hoc test (Multiple comparisons) of the spindle length data in Fig 5B, showing that the spindle length in S391A-expressing cells were significantly different from the other groups.



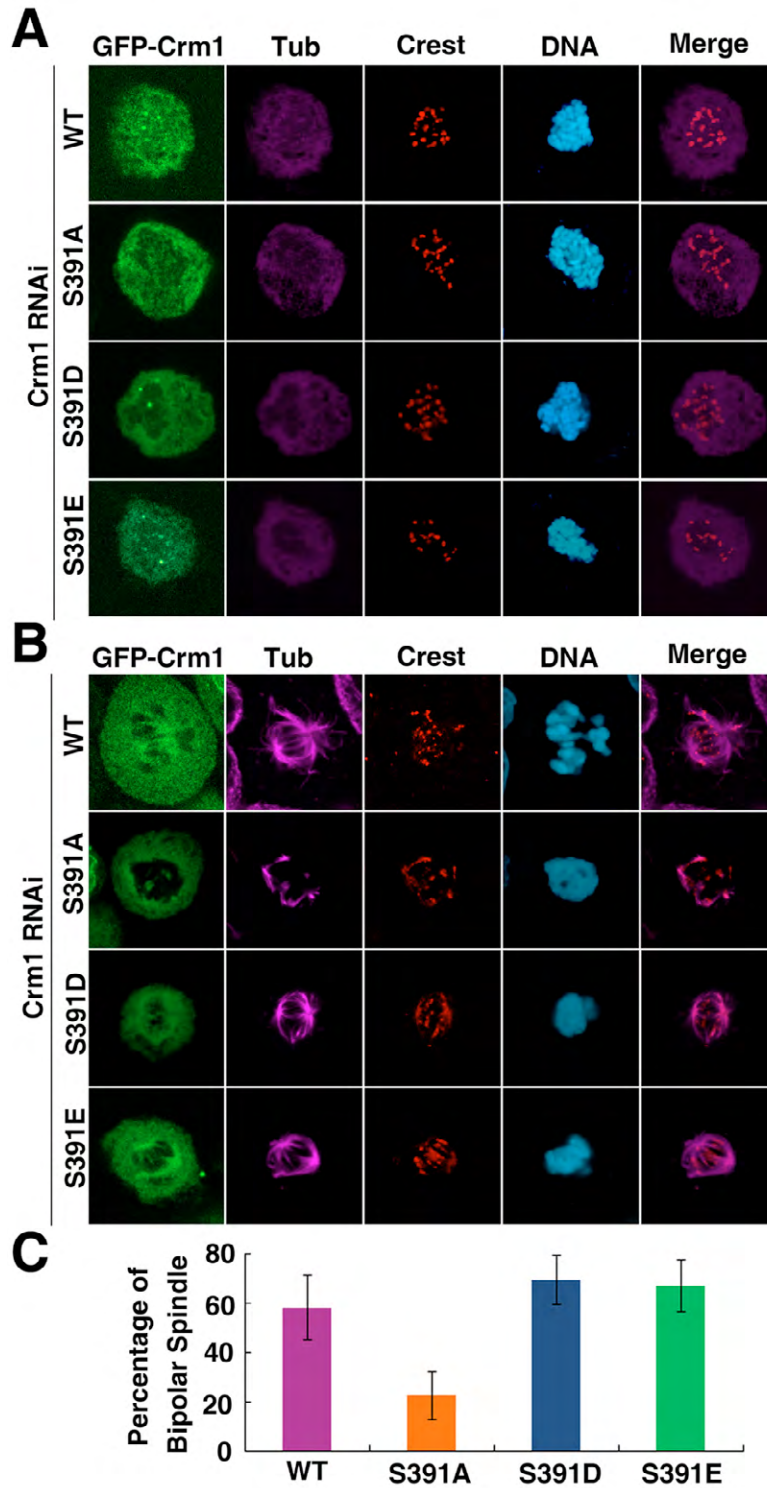
**Fig. S4. Phosphorylation of Crm1 at Ser391 regulates spindle assembly.** (A) HeLa cells were exposed to Crm1 siRNA for 48h, followed by transiently transfected with siRNA-resistant GFP-Crm1 WT, GFP-Crm1 S391A, GFP-Crm1 S391D, GFP-Crm1 S391E plasmid. Then the cells were fixed 24h after transfection. Overlay shows GFP-Crm1 (green) and Tubulin (red). DNA was simultaneously stained using DAPI. The morphology of the spindles in cells overexpressing phosphorylation-mimetic mutants GFP-Crm1 S391D and GFP-Crm1 S391E were much better than those in the S391A cells. (B) Pole-to-pole distance is decreased in cells transfected with GFP-Crm1 S391A compared to GFP-Crm1 S391D, GFP-Crm1 S391E and GFP-Crm1 WT. The pole-to-pole distance was measured by ZEN 2009. (C) The relative microtubule intensity of half spindle is measured by Image J software. The microtubule intensity is significantly decreased in cells transfected with GFP-Crm1 S391A compared to GFP-Crm1 S391D, GFP-Crm1 S391E and GFP-Crm1 WT.



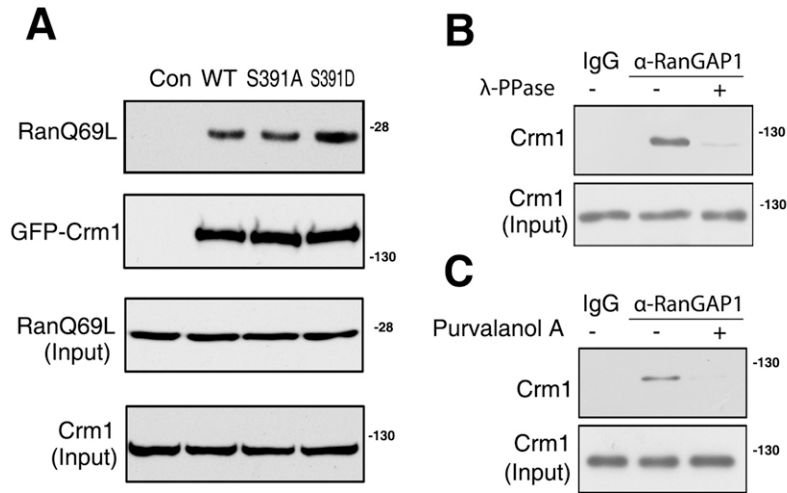
**Fig. S5. Phosphorylation of Crm1 regulates chromosome congression.** (A) HeLa cells were transiently transfected with GFP-Crm1 WT, GFP-Crm1 S391A, GFP-Crm1 S391D or GFP-Crm1 S391E plasmid. The cells were fixed and performed the immunofluorescence microscopy 24h after transfection. Overlay shows GFP-Crm1 (green) and Aurora B (red). DNA was simultaneously stained using DAPI. (B) The metaphase plate is bordered in cells transfected with GFP-Crm1 S391A. The distance of metaphase plate was measured by LSM Image Browser.



**Fig. S6. Microtubules depolymerize completely after cold treatment.** HeLa cells were transiently transfected with GFP-Crm1 WT, GFP-Crm1 S391A, GFP-Crm1 S391D and GFP-Crm1 S391E plasmids. The cells were arrested by nocodazole 24h after transfection, followed by incubation on ice for 1 h to depolymerize the microtubules.



**Fig. S7. Phosphorylation of Crm1 promotes bipolar spindle formation.** (A) HeLa cells were exposed to Crm1 siRNA for 48h followed by transiently transfected with siRNA-resistant GFP-Crm1 WT, GFP-Crm1 S391A, GFP-Crm1 S391D and GFP-Crm1 S391E plasmids. The cells were arrested by nocodazole 24h after transfection, followed by incubation in medium on ice for 1 h to depolymerize the microtubules. (B) The HeLa cells were exposed to Crm1 SiRNA for 48h followed by transiently transfected with GFP-Crm1 WT, GFP-Crm1 S391A, GFP-Crm1 S391D, GFP-Crm1 S391E plasmid. The cells were arrested by nocodazole 24h after transfection, followed by incubation in medium on ice for 1 h to depolymerize the microtubules. Then the cells were moved to pre-warmed medium at 37°C and incubated for 12 min to allow microtubule regrow from the kinetochore as well as the centrosome. The cells were finally double stained with anti tubulin (purple) and crest (red) antibodies after fixation. (C) Percentage of bipolar spindle formation as treated in (B). The bipolar spindle assembly is inhibited in GFP-Crm1 S391A transfected cells.



**Fig. S8. RanGTP dependent binding of RanGAP1 to Crm1 in mitosis.** (A) HeLa cells were transfected with GFP-Crm1 WT, GFP-Crm1 S391A and GFP-Crm1 S391D. GFP-Crm1 and its mutants were precipitated from asynchronous cells using GFP beads in the presence of 2  $\mu$ M RanGTP and immunoblotted for GFP and Ran. (B) RanGAP1 and Crm1 interaction in mitosis is dependent on phosphorylation. Mitotic cell extracts were pretreated with  $\lambda$ -protein phosphatase ( $\lambda$ -PPase) to induce de-phosphorylation. RanGAP1 was precipitated from the extracts using RanGAP1 antibody in the presence of 2.5  $\mu$ M RanQ69L. IgG was used as a control for the precipitation. (C) Inhibition of CDK1 activity significantly reduces the RanGAP1 and Crm1 interaction in mitosis. HeLa cells were arrested in mitosis by 100 ng/ml nocodazole for 17 h and treated with 10  $\mu$ M purvalanol A for 15min. RanGAP1 was precipitated from mitotic (NM) cells using RanGAP1 antibody in the presence of 2.5  $\mu$ M RanQ69L. IgG was used as a control for the precipitation.