Nunes Bastos et al., http://www.jcb.org/cgi/content/full/jcb.201301094/DC1

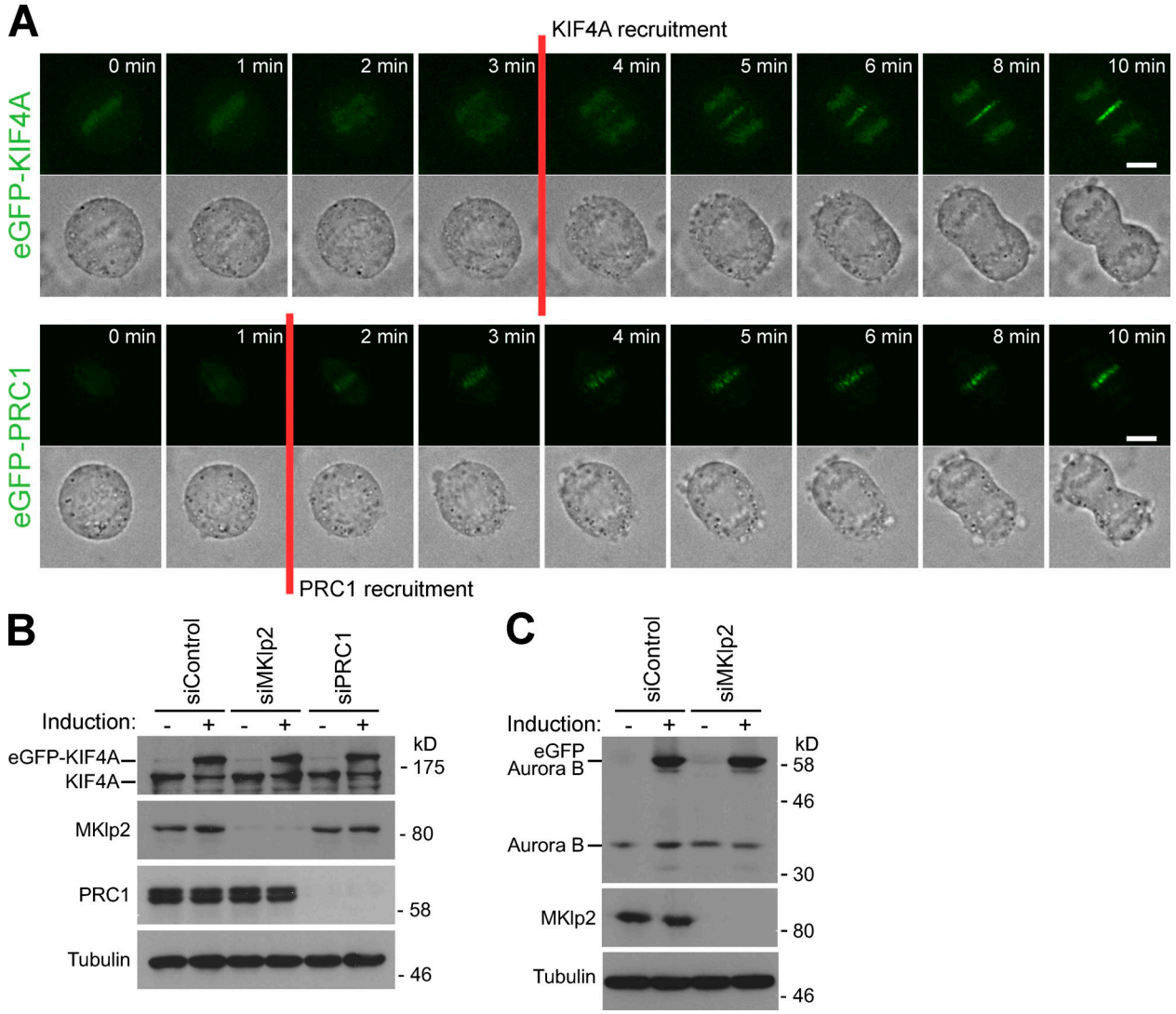

Figure S1. **Characterization of stable cell lines expressing eGFP-tagged KIF4A and Aurora B.** (A) HeLa cells stably expressing eGFP-KIF4A or eGFP-PRC1 were imaged every minute with a spinning disk confocal microscope. Maximum intensity projected images of eGFP fluorescence, and a single brightfield image collected at the center plane of the image stack, are shown at the time indicated. All times are relative to the onset of anaphase (t = 0). (B) HeLa cells stably expressing eGFP-KIF4A were treated with control, MKlp2, or PRC1 siRNA duplexes for 48 h in the presence or absence of 1 µg/ml doxycycline and analyzed by Western blotting as shown to confirm MKlp2 and PRC1 depletion as well as induction of eGFP-KIF4A. (C) HeLa cells stably expressing eGFP-Aurora B were treated with control or MKlp2 siRNA duplexes for 48 h in the presence or absence of 1 µg/ml doxycycline and analyzed by Western blotting as shown to confirm MKlp2 depletion as well as induction of eGFP-Aurora B.

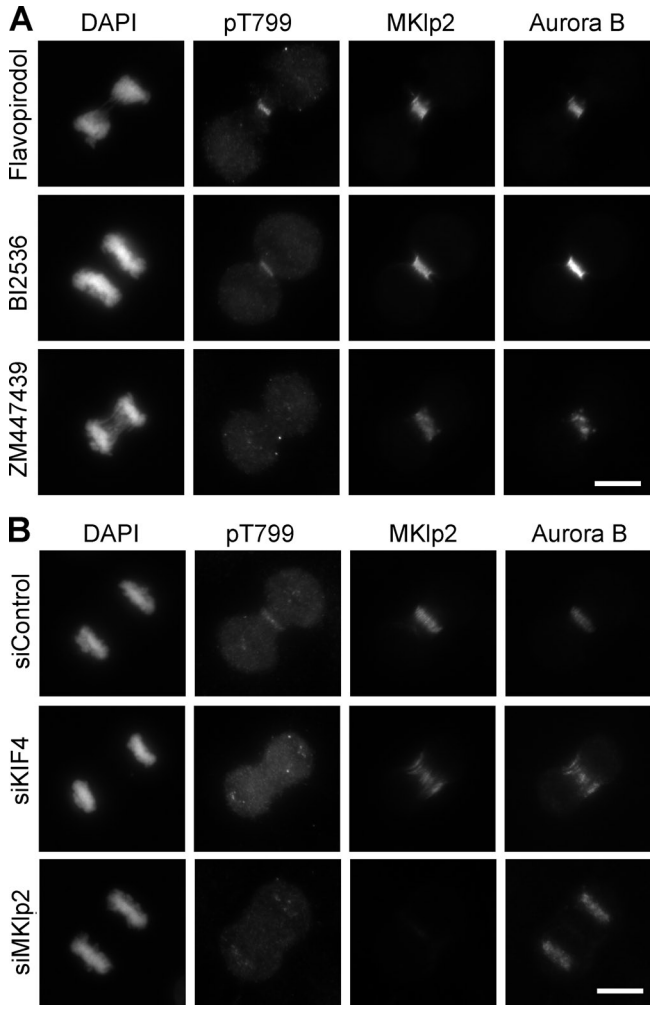

Figure S2. **KIF4A phosphorylation by Aurora B at T799.** (A) Thymidine synchronized HeLa cells were treated 8 h after the thymidine washout step for 5 min with DMSO, 1 µM BI2536, 5 µM flavopiridol, or 1 µM ZM447439. These cells were fixed, and then stained for DNA with DAPI, rabbit anti-pT799, sheep anti-MKlp2, and mouse anti–Aurora B. (B) HeLa cells were transfected with control, KIF4A, or MKlp2 siRNA duplexes for 30 h, fixed and then stained for DNA with DAPI, rabbit anti-pT799, sheep anti-MKlp2, and mouse anti–Aurora B. Bar, 10 µm.

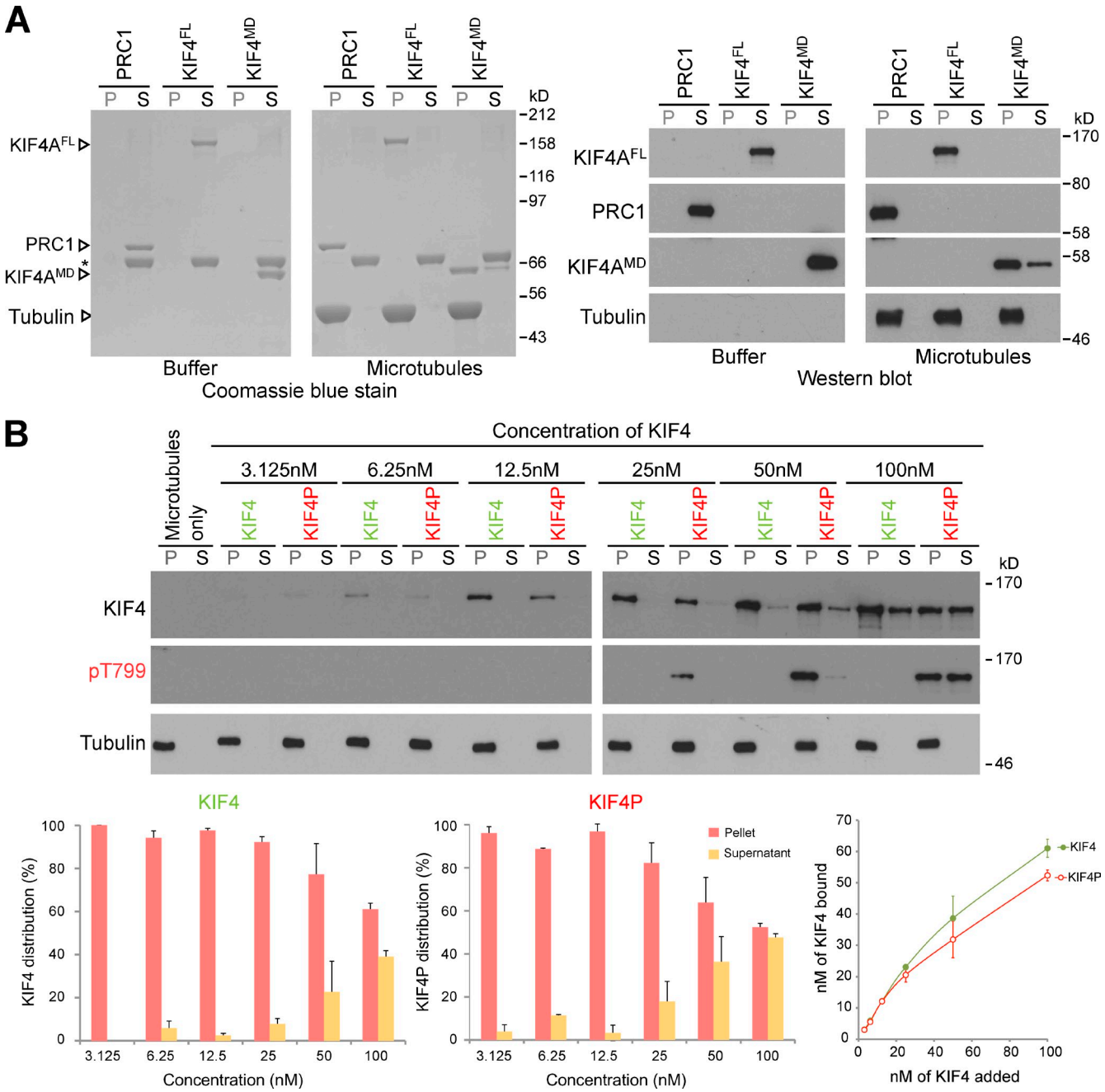

Figure S3. **Phosphorylation does not regulate binding of KIF4A to microtubules.** (A) Pelleting assays were performed in the presence or absence of polymerized microtubules as described in Materials and methods using 10 nM of the full-length KIF4A ($KIF4^{FL}$), 33 nM of the KIF4A motor domain ($KIF4^{MD}$), or 10 nM PRC1 as indicated. Equivalent aliquots of the pellet fraction (P) and supernatant fractions (S) were analyzed by Coomassie blue staining and Western blotting. An asterisk marks BSA used as a carrier protein in the reactions. (B) Microtubule binding assays were performed as described in Materials and methods using 175 nM microtubules and a range of concentrations of KIF4A (green) and phosphorylated KIF4A (red) as indicated in the figure. Equivalent aliquots of the microtubule pellet fraction (P) and supernatant fractions (S) were analyzed by Western blotting with the antibodies shown in the figure. The amount of KIF4A present in the pellet and supernatant was measured using densitometry in ImageJ and is plotted in the graphs ($n = 2$) as a ratio of the total. In addition, the amount of KIF4A or phosphorylated KIF4A bound to the microtubule pellet is plotted as a function of the amount of KIF4A added. Error bars show the standard deviation from the mean ($n = 2$).

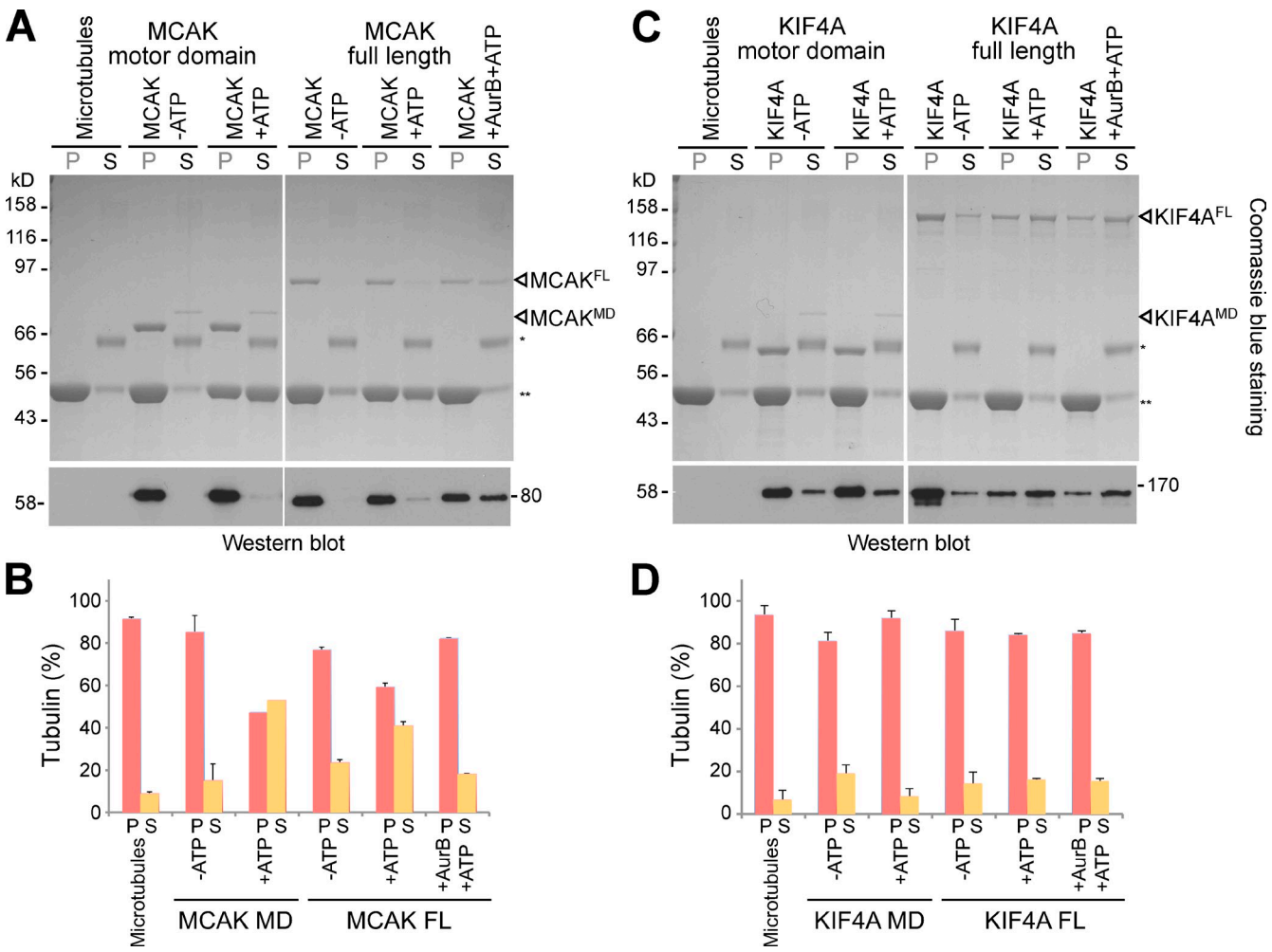

Figure S4. **KIF4A does not show ATP-dependent microtubule-destabilizing activity.** (A and B) Microtubule-destabilizing assays were performed using either 250 nM MCAK or (C and D) 200 nM KIF4A as described in Materials and methods. Motor domain–only and full-length proteins were incubated with microtubules in the presence or absence of ATP. In the case of the full-length protein the role of Aurora B phosphorylation was also addressed in the presence of ATP. Equivalent aliquots of the microtubule pellet fraction (P) and supernatant fractions (S) were analyzed by SDS-PAGE analysis followed by Coomassie blue staining (top panels) and Western blotting using a His-tag antibody (bottom panels) to confirm the distribution of the motor proteins. The amount of tubulin present in the pellet and supernatant was measured using densitometry in ImageJ and is plotted in the graphs ($n = 2$) as a ratio of the total. Error bars show the standard deviation from the mean. Single and double asterisks mark BSA used as a carrier protein in the assays and tubulin, respectively.

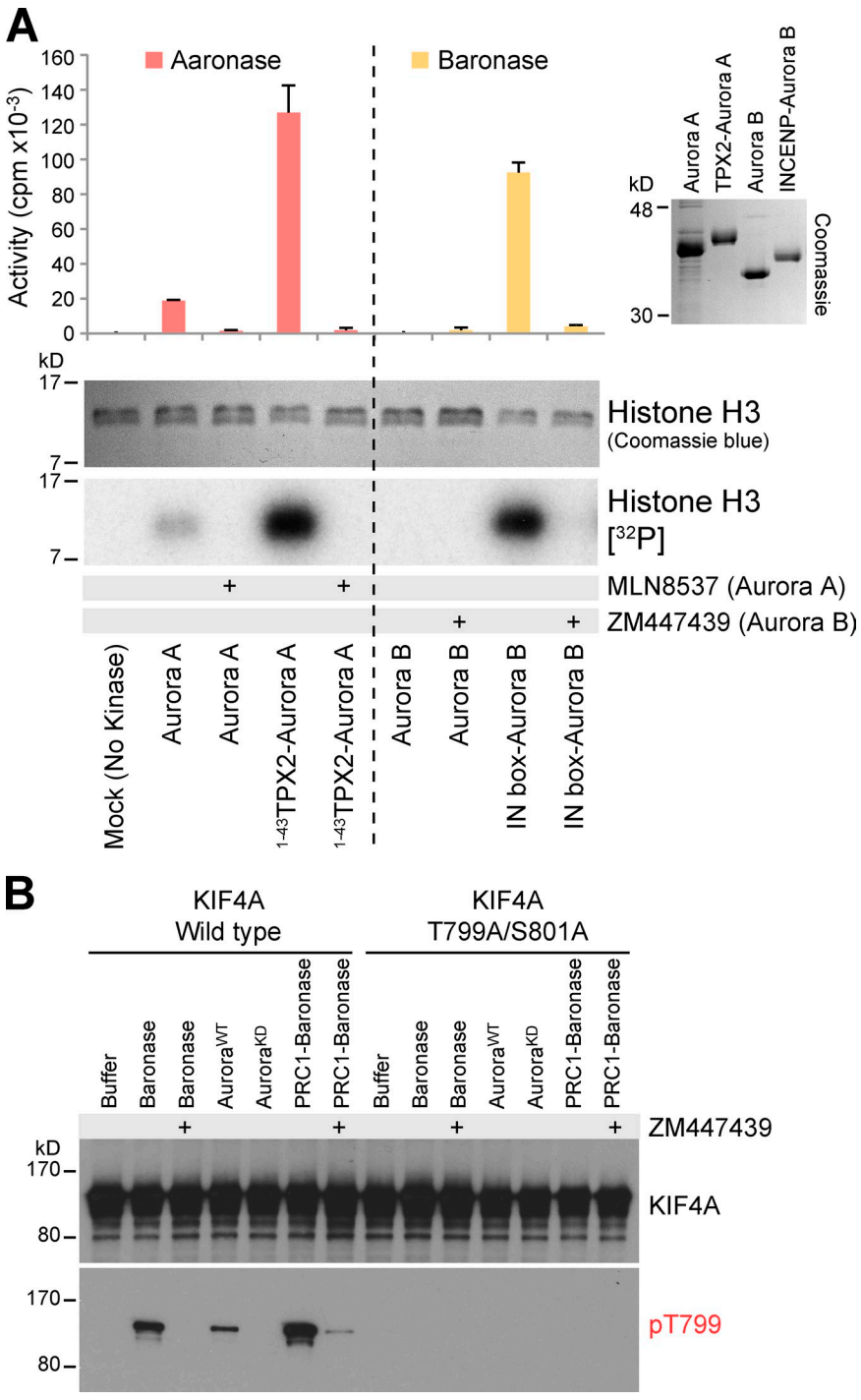

Figure S5. **Characterization of the A- and B-type Aurora kinase modules: Aaronase and Baronase.** (A) Recombinant Aurora A, Aurora A minikinase (Aaronase), Aurora B, and Aurora B mini-kinase (Baronase) were purified from bacteria as described in Materials and methods. The inset gel shows 2 µg of each protein. Kinase assays were performed using 10 ng of each kinase, 2 µg of the model substrate histone H3, in the presence and absence of 1 µM MLN8537 (Aurora A inhibitor) or ZM447439 (Aurora B inhibitor) for 30 min at 30°C. Samples were analyzed by autoradiography and the level of kinase activity measured by scintillation counting of the excised gel bands. These values are plotted on the bar graph; errors indicate the standard error (n = 3). (B) In vitro kinase assays were performed using 500 ng of full-length wild-type KIF4A or KIF4A$^{T799A/S801A}$ mutant as a substrate in the absence or presence 50 ng of Baronase, wild-type Aurora kinase (Aur^{WT}), inactive "kinase-dead" mutant Aurora (Aur^{KD}), as well as with the PRC1-Baronase protein with and without 1 µM ZM447439 for 30 min at 30°C. Samples were analyzed by Western blotting for KIF4A and KIF4A pT799.