eLIFE
elifesciences.org

RESEARCH ARTICLE

Figures and figure supplements

BubR1 alterations that reinforce mitotic surveillance act against aneuploidy and cancer

Robbyn L Weaver *et al*

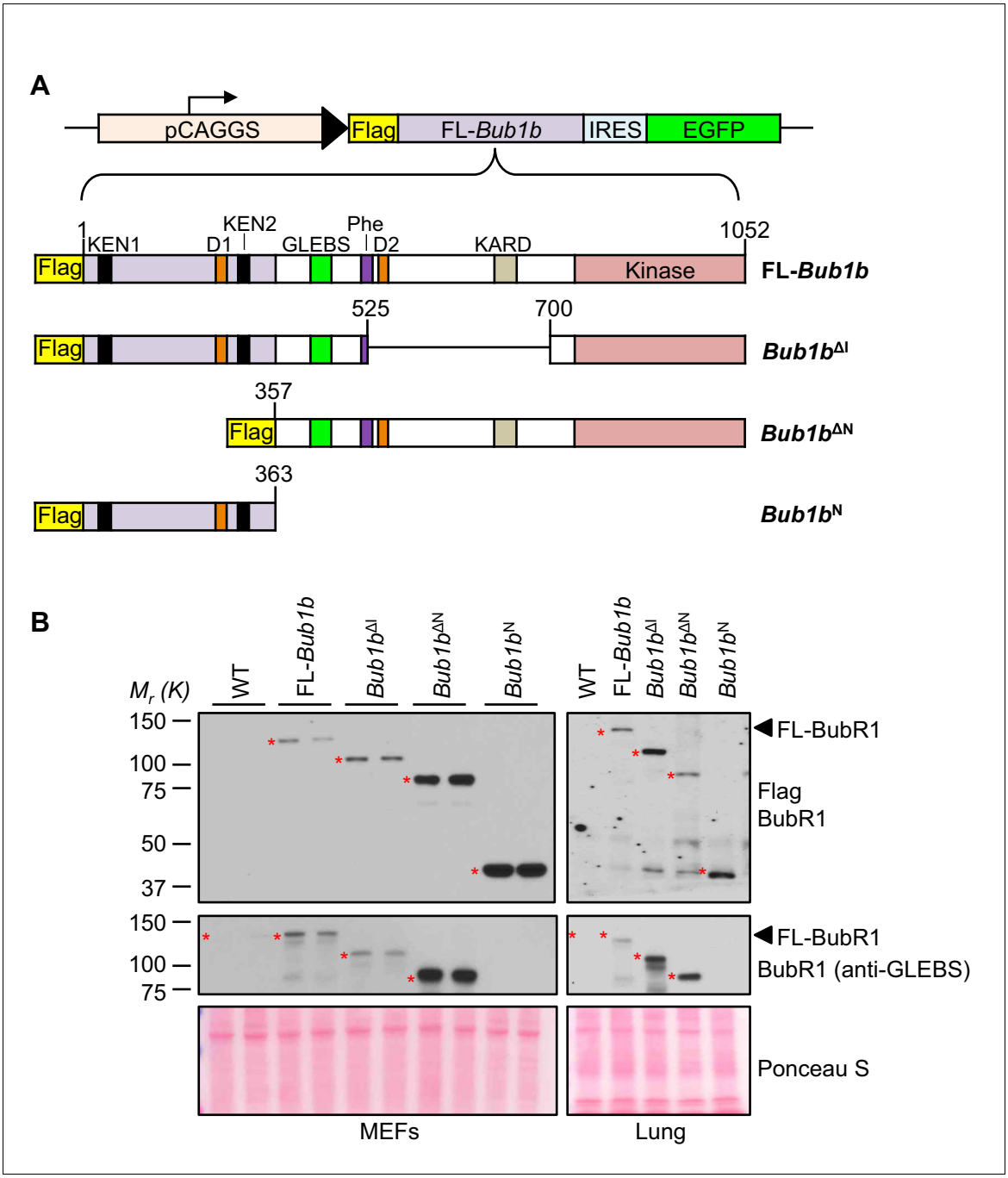


Figure 1. BubR1 transgenic mutant proteins are highly overexpressed in vitro and in vivo. (**A**) (top) *Bub1b* transgenic vector design. pCAGGS, promoter consisting of the CMV immediate enhancer and the chicken-actin promoter. FL, full-length. IRES, internal ribosome entry site. (bottom) Schematics of the Flag-*Bub1b* transgenic mouse constructs. KEN, KEN-box. D, destruction-(D-)box. GLEBs, GLEBs-binding motif. Phe, Phe box. KARD, kinetochore attachment regulatory domain. (**B**) Western blots of MEF (left) and lung tissue extracts (right) from wild-type (WT) and Flag-*Bub1b* transgenic mice. Blots were probed with the indicated antibodies. Ponceau S was used to normalize loading.

DOI: 10.7554/eLife.16620.003

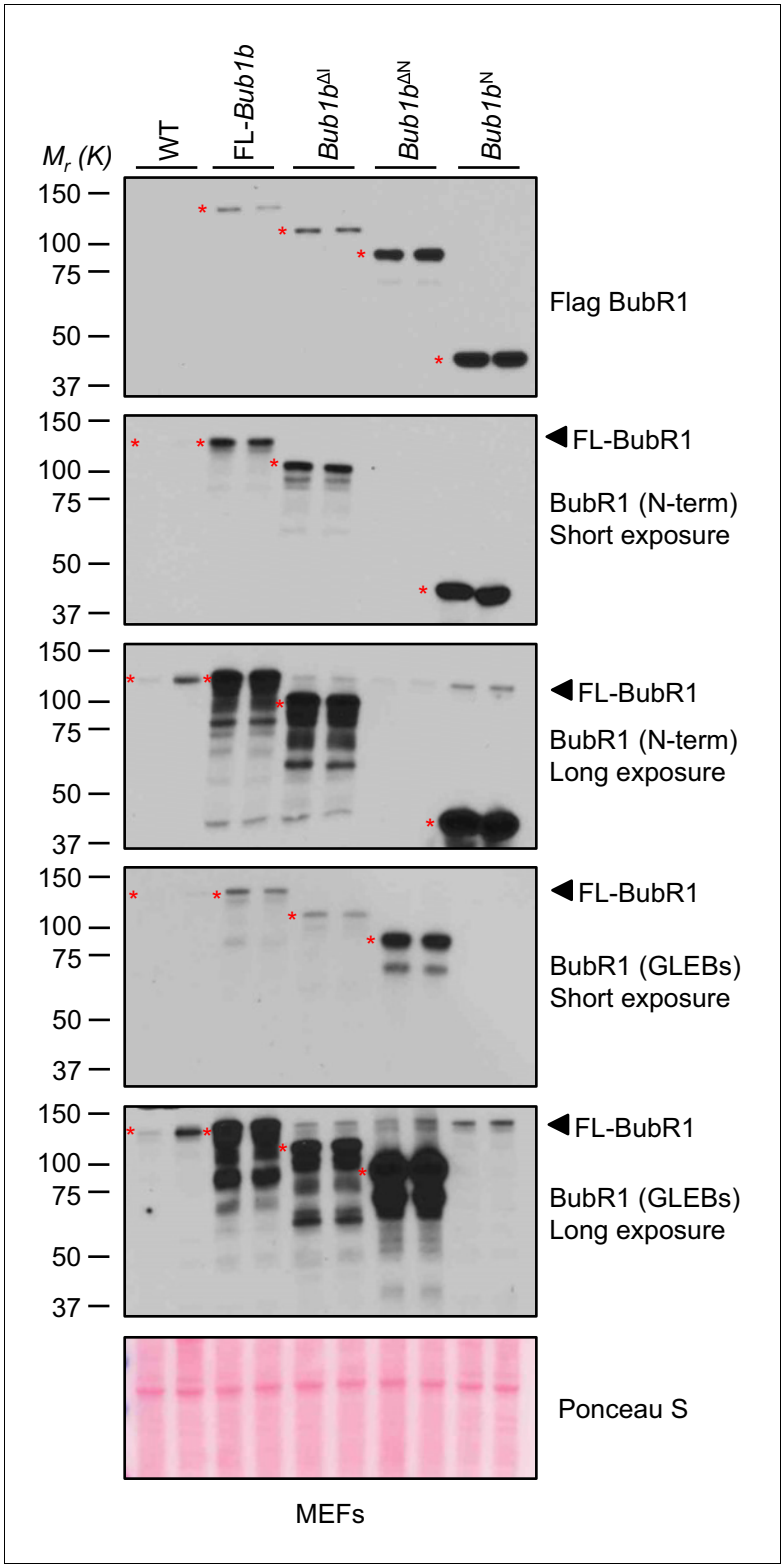


Figure 1—figure supplement 1. Analysis of BubR1 overexpression in transgenic MEFs. Full scan and multiple exposures of Western blots of MEF lysates from ***Figure 1B***. WT, wild-type. FL, full-length.
DOI: 10.7554/eLife.16620.004

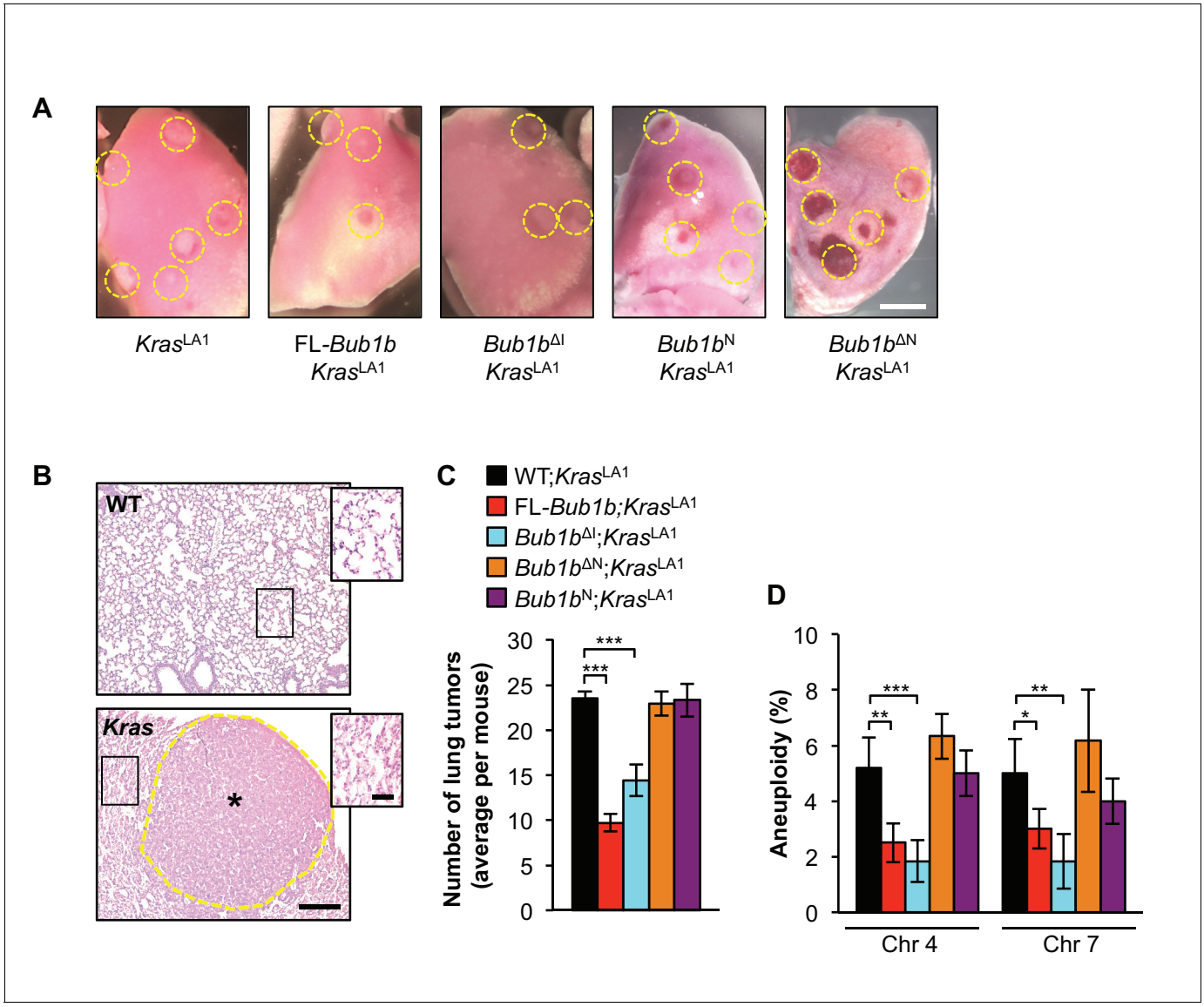


Figure 2. Select BubR1 domain overexpression protects against aneuploidy and cancer. (**A**) Lung lobes of *Kras*^{LA1} mice and *Kras*^{LA1} mice expressing various BubR1 transgenic proteins sacrificed at 6 weeks of age. Entire lungs were inspected using a dissection microscope to quantitate the number of lung tumors (adenomas) per mouse. (**B**) Hematoxylin-eosin stained lung sections of representative normal (WT) lung and a *Kras*^{LA1} (*Kras*) hyperplastic tumor-bearing lung (the dashed line marks the adenoma boundary). Insets highlight normal and hyperplastic lung architecture. (**C**) Quantification of the number of lung tumors from mice shown in **A**. n = 20, except for full-length (FL)-*Bub1b* where n = 7. Data are mean ± s.e.m. ***p<0.001. (**D**) Interphase FISH on the lungs of wild-type and *Kras*^{LA1} with and without FL-BubR1 and mutant overexpression. n = 5, ≥ 100 cells per animal. Data are mean ± s.d. *p<0.05, **p<0.01. ***p<0.001. Scale bars: **A**, 2 mm; **B**, 200 μm (main image) and 50 μm (insets). (See associated ***Figure 2—source data 1***).

DOI: 10.7554/eLife.16620.011

The following source data is available for figure 2:

Source data 1. Source file for tumor incidence and tissue aneuploidy rate data.

DOI: 10.7554/eLife.16620.012

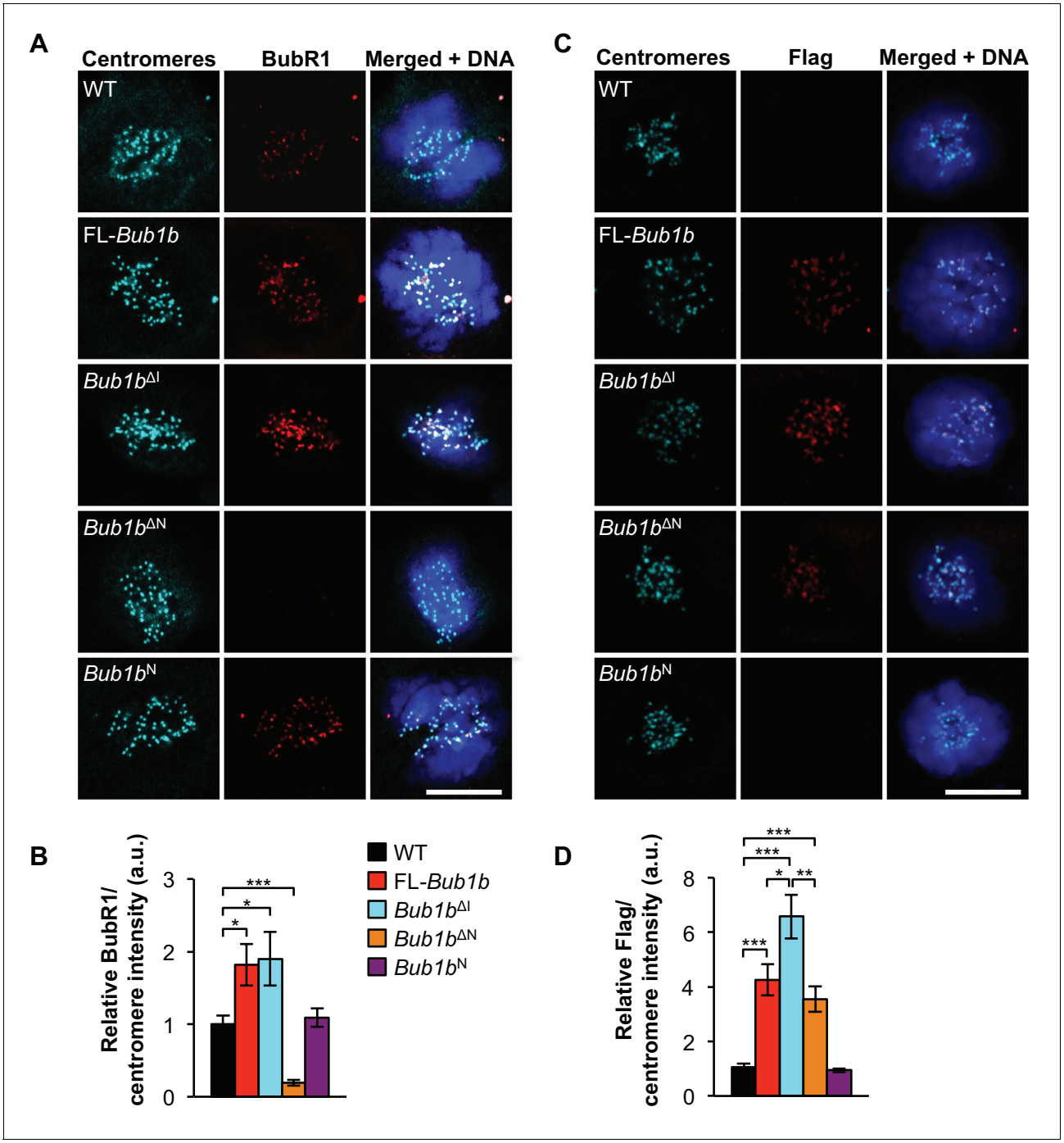


Figure 3. Increased BubR1 localization to kinetochore corresponds to phenotypic benefits. (**A**) MEFs of indicated genotypes were stained for BubR1 (red), centromeres (cyan), and DNA (blue). WT, wild-type. FL, full-length. (**B**) Quantification of immunostaining of BubR1 shown in **A**. Values were normalized to centromere stain and are relative to wild-type. n = 3 lines, ≥ 10 cells per line. Data are mean ± s.d. *p<0.05, ***p<0.001. (**C**) Same as in **A** except with anti-Flag antibody to detect transgenic BubR1. (**D**) Quantification of immunostaining of Flag shown in **C**. Values were normalized to centromere stain and are relative to wild-type. Wild-type and *Bub1b*^{N} represent background. n = 3 lines, ≥ 10 cells per line. Data are mean ± s.d. *p<0.05, **p<0.01, ***p<0.001. Scale bar 10 µm. (See associated ***Figure 3—source data 1***).

DOI: 10.7554/eLife.16620.013

The following source data is available for figure 3:

Source data 1. Source file for intensity of kinetochore-localized BubR1 and FLAG protein data.

DOI: 10.7554/eLife.16620.014

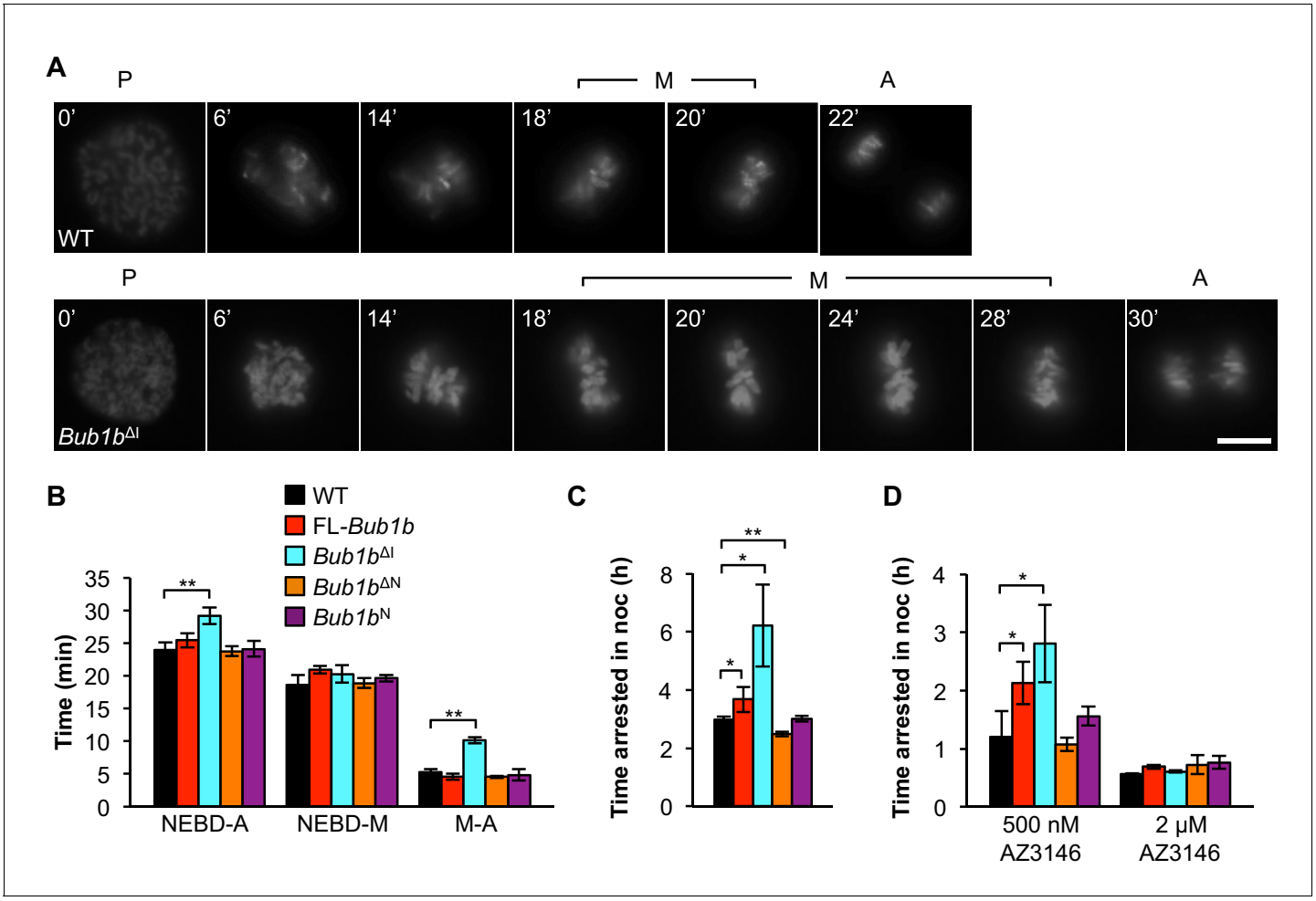


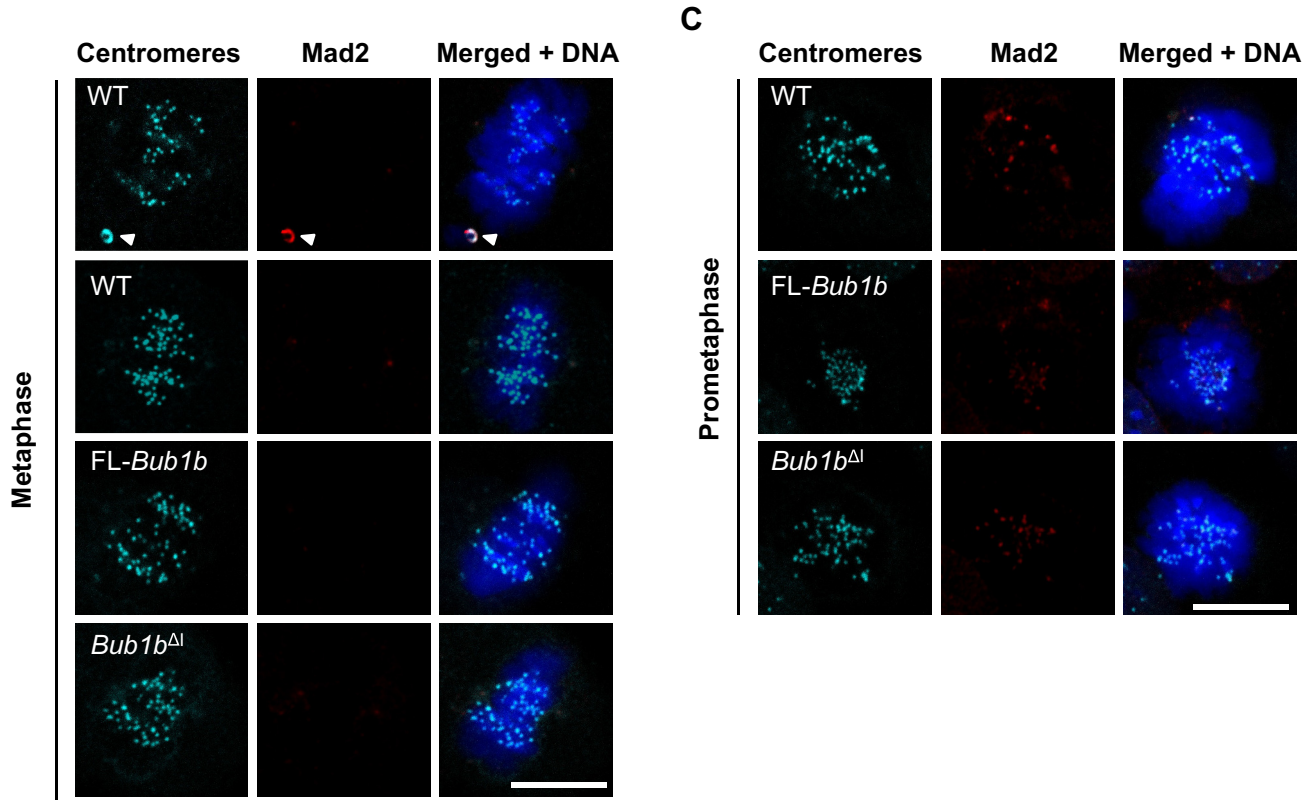
Figure 4. *Bub1b*$^{\Delta I}$ MEFs have an increased time in mitosis and duration of mitotic arrest. (**A**) Representative time-lapse images of live MEF cells of indicated genotypes progressing from prophase (t = 0) to anaphase (A). Time is indicated in min. WT, wild-type. P, prophase. M, metaphase. (**B**) Analysis of the time from nuclear envelope breakdown (NEBD) to anaphase onset in H2B-RFP MEFs of the indicated genotypes by live cell time-lapse imaging. n = 3 lines, ≥ 20 cells per line. Data are mean ± s.d. **p<0.01. FL, full-length. (**C**) In a nocodazole challenge, H2B-RFP MEFs of indicated genotypes were treated with 100 ng/ml of nocodazole (noc) and monitored by live cell time-lapse imaging. The point of time in which 50% of cells are arrested in mitosis is plotted. n ≥ 3 lines, ≥ 20 cells per line. Data are mean ± s.d. *p<0.05, **p<0.01. (**D**) H2B-RFP wild-type and mutant transgenic MEFs were treated concurrently with 100 ng/ml nocodazole and indicated concentrations of the Mps1 kinase inhibitor, AZ3146. The point of time in which 50% of cells are arrested in mitosis is plotted. n = 3 lines, ≥ 20 cells per line. Data are mean ± s.d. *p<0.05. Scale bar, 10 μm (See associated ***Figure 4—source data 1***).

DOI: 10.7554/eLife.16620.015

The following source data is available for figure 4:

Source data 1. Source file for mitotic timing and nocodazole arrest data.

DOI: 10.7554/eLife.16620.016



B

Mitotic MEF genotype (n)	Normal metaphases inspected	Metaphases with Mad2-positive kinetochore (%)
WT (3)	45	2
FL-*Bub1b* (3)	45	0
Bub1b$^{\Delta I}$ (3)	45	0

Figure 4—figure supplement 1. *Bub1b*$^{\Delta I}$ MEFs do not have persistent Mad2 signaling. (**A**) MEFs of indicated genotypes and cell stage were stained for Mad2 (red), centromeres (cyan), and DNA (blue). White arrowhead depicts misaligned chromosome. WT, wild-type. FL, full-length (**B**) Table depicting the number of metaphases with at least one Mad2-positive kinetochore. *n* = 3 lines, 15 cells per line. (**C**) MEFs of indicated genotypes and cell stage were stained for Mad2 (red), centromeres (cyan), and DNA (blue). Scale bar 10 μm.

DOI: 10.7554/eLife.16620.017

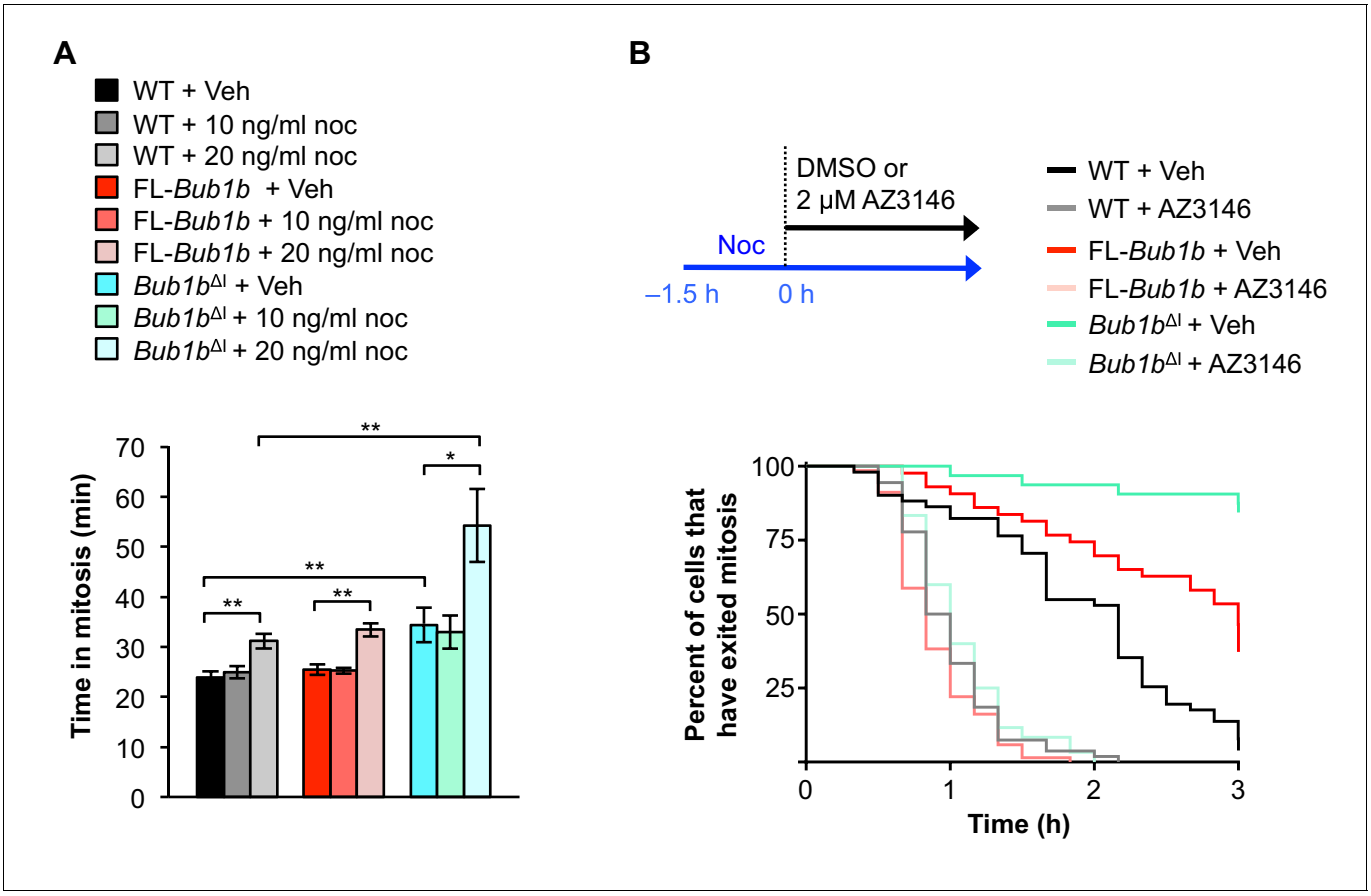


Figure 5. *Bub1b*$^{\Delta I}$ MEFs have a lower threshold to checkpoint activation. (**A**) Analysis of the time from NEBD to anaphase onset in H2B-RFP MEFs of the indicated genotypes treated with either DMSO vehicle (Veh) or indicated concentration of nocodoazole (Noc). n = 3 lines, ≥ 20 cells per line. Data are mean ± s.d. *p<0.05, **p<0.01. WT, wild-type. FL, full-length. (**B**) (top) Strategy for analyzing the checkpoint silencing efficiency. MEFs of indicated genotypes were treated with 100 ng/ml nocodazole for 1.5 hr before addition of either DMSO vehicle (Veh) or 2 μM AZ3146, at which point cells were marked and monitored for time of escape (time point zero). (bottom) Analysis of duration of mitotic arrest from time point zero as outlined in (top). n = 3 lines, ≥ 20 cells per line. (See associated ***Figure 5—source data 1***).

DOI: 10.7554/eLife.16620.018

The following source data is available for figure 5:

Source data 1. Source file for low-dose nocodazole challenge and SAC silencing data.

DOI: 10.7554/eLife.16620.019

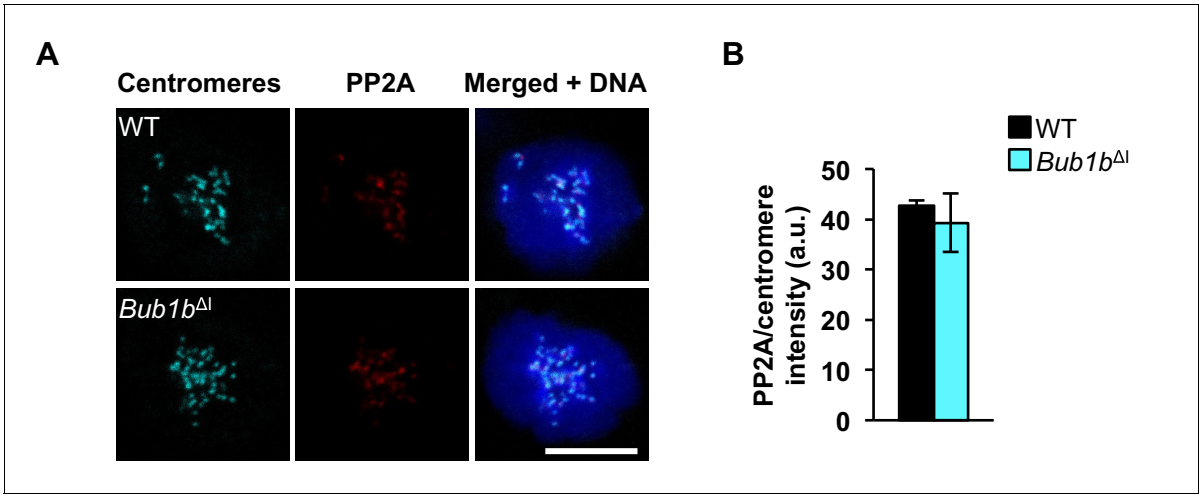


Figure 5—figure supplement 1. PP2A localization is normal in *Bub1b*$^{\Delta I}$ MEFs. (**A**) Wild-type (WT) and *Bub1b*$^{\Delta I}$ MEFs were arrested in 100 ng/ml nocodazole and stained for PP2A (red), centromeres (cyan), and DNA (blue). (**B**) Quantification of immunostaining of PP2A in **A**. Values were normalized to centromere stain. n = 3 lines, $\geq$ 10 cells per line. Data are mean ± s.d. Scale bar 10 µm. (See associated ***Figure 5—figure supplement 1—source data 1***).

DOI: 10.7554/eLife.16620.020

The following source data is available for figure 5:

Figure supplement 1—Source data 1. Source file for intensity of kinetochore-localized PP2A protein data.

DOI: 10.7554/eLife.16620.021

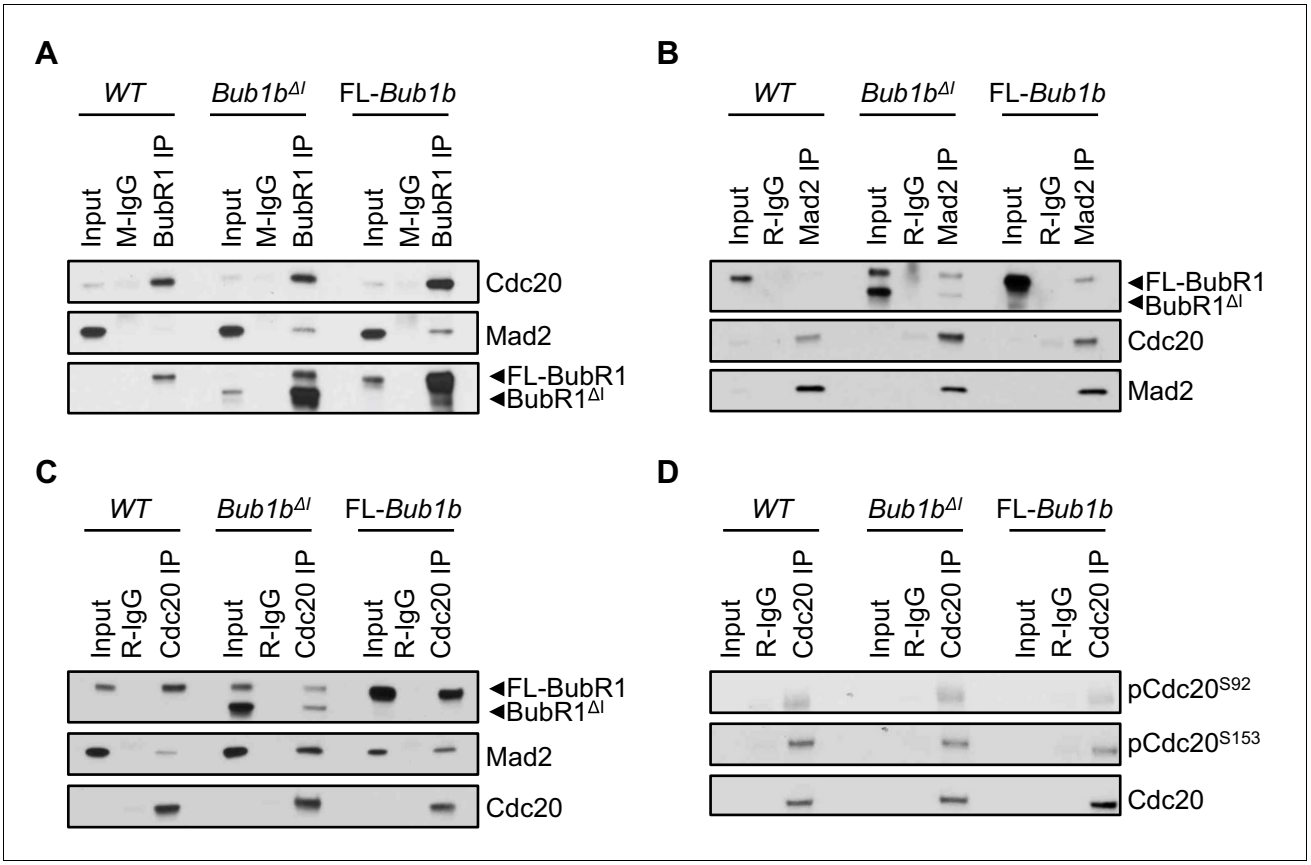


Figure 6. Composition of mitotic checkpoint complexes is unique in *Bub1b*$^{\Delta I}$ MEFs. (**A**–**D**) Immunoblots of mitotic wild-type (WT) and indicated mutant MEF extracts subjected to co-immunoprecipitation with the indicated antibodies. Each blot is a representative of at least 3 experiments. FL, full-length.
DOI: 10.7554/eLife.16620.022

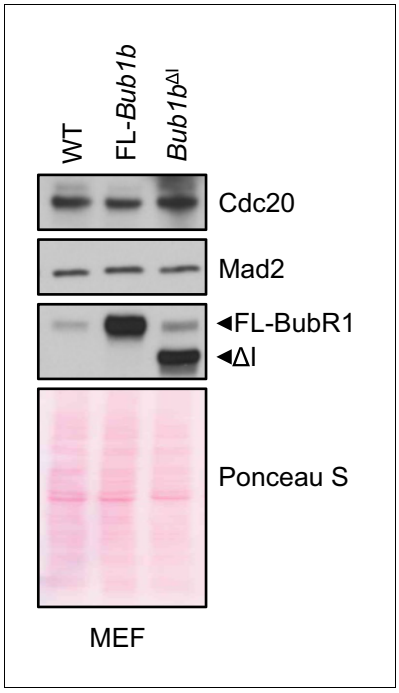


Figure 6—figure supplement 1. Mitotic checkpoint components have a normal expression in *Bub1b* transgenic MEFs. Western blot analysis of mitotic MEF lysates of indicated genotypes. Blots were probed with indicated antibodies. Ponceau S was used to normalize loading. Blot is a representative of at least three experiments. WT, wild-type. FL, full-length.
DOI: 10.7554/eLife.16620.023

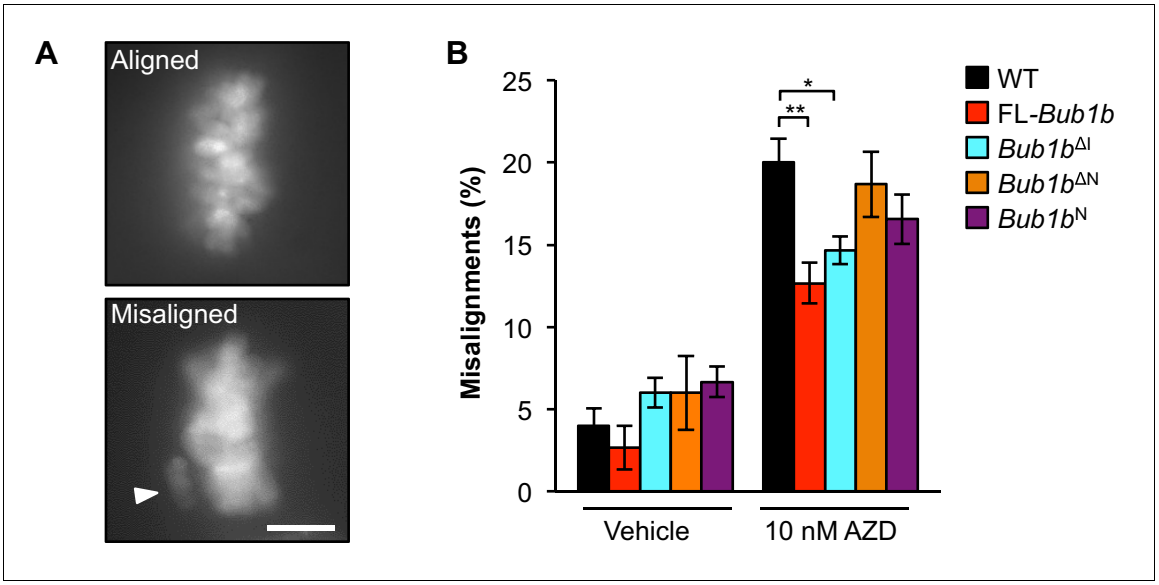


Figure 7. Overexpression of FL-BubR1 and BubR1$^{\Delta I}$improves error correction rates. (**A**) Representative images of MEFs with aligned or misaligned chromosomes after monastrol washout. White arrowhead depicts misaligned chromosome. (**B**) Analysis of chromosome misalignment in MEFs expressing the indicated *Bub1b* transgenes. MEFs were treated with 100 μM monastrol for 1 hr and then with monastrol and 10 μM MG132 for 1 hr and released for 90 min into MG132. Cells were treated with DMSO (Vehicle) or 10 nM AZD1152-HQPA (AZD) throughout the duration of the experiment. *n* = 6 lines (≥25 cells per line were analyzed). Data are mean ± s.d. *p<0.05, **p<0.01. Scale bar, 10 μm. WT, wild-type. (See associated ***Figure 7—source data 1***).

DOI: 10.7554/eLife.16620.024

The following source data is available for figure 7:

Source data 1. Source file for monastrol washout data.

DOI: 10.7554/eLife.16620.025

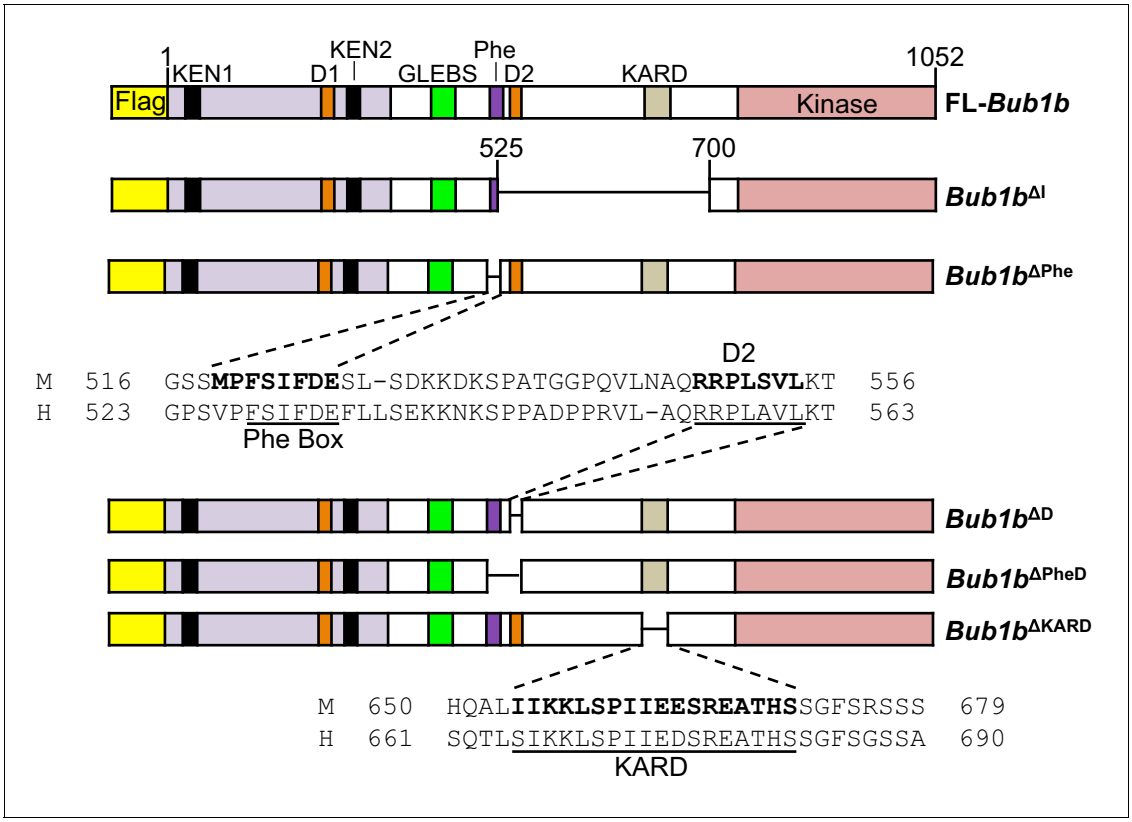


Figure 8. Schematics of pTripZ-Flag-*Bub1b* mutants. Schematics of the pTripZ-Flag-*Bub1b* constructs. D, destruction-(D-)box. GLEBs, GLEBs-binding motif. Phe, Phe box. KARD, kinetochore attachment regulatory domain. FL, full-length. Sequence alignment of the Phe box, D-box2, and KARD region of human and mouse BubR1. Residues characterized in human BubR1 are underlined, and homologous residues deleted in mouse *Bub1b* constructs are bold.

DOI: 10.7554/eLife.16620.026

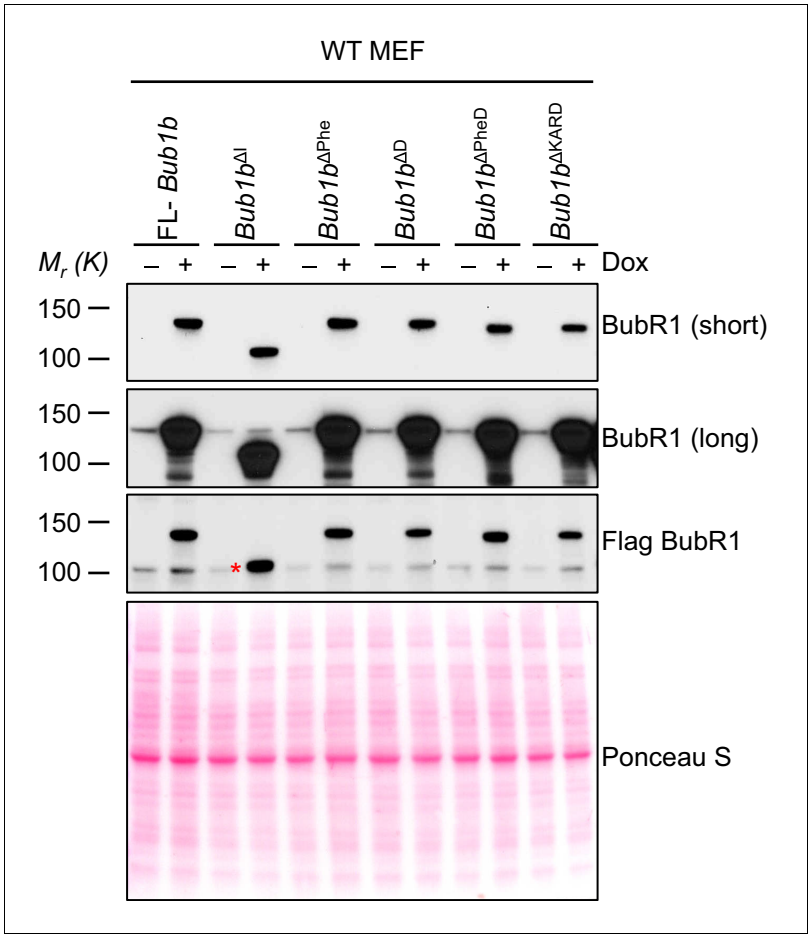


Figure 8—figure supplement 1. Protein levels of *Bub1b* deletion constructs in wild-type MEFs. Western blots of wild-type (WT) MEFs infected with the indicated constructs with or without doxycycline (Dox). Blots were probed with the indicated antibodies. Ponceau S was used to normalize loading. Asterisk indicates specific band for BubR1$^{\Delta I}$.

DOI: 10.7554/eLife.16620.027

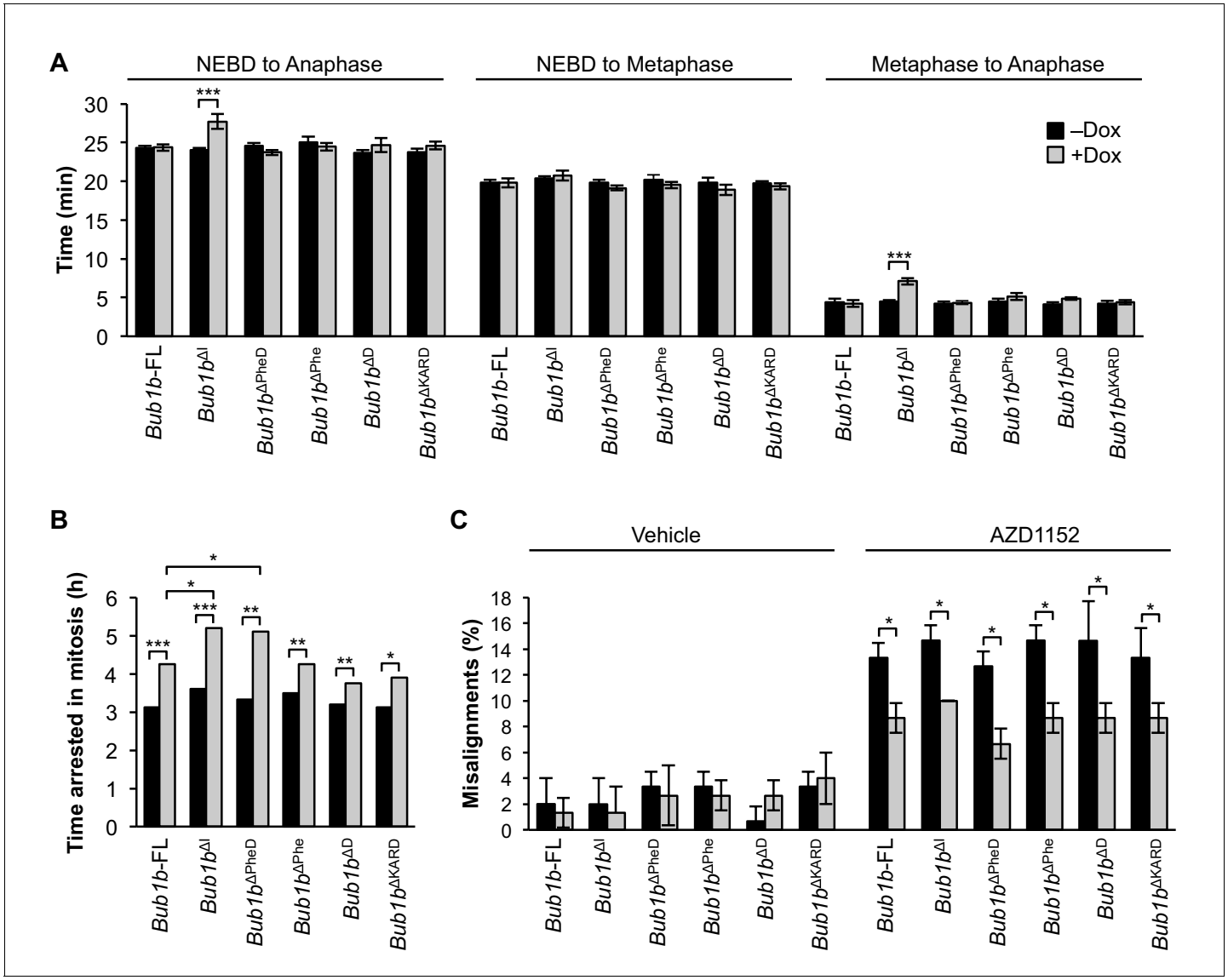


Figure 9. BubR1 deletion constructs extend nocodazole arrest and positively impact error attachment machinery. (**A**) Analysis of the time from nuclear envelope breakdown (NEBD) to anaphase onset in H2B-RFP wild-type MEFs infected with the indicated constructs with and without the addition of doxycycline (Dox) by live cell time-lapse imaging. n = 1 line, ≥ 20 cells per treatment. Data are mean ± s.e.m. ***p<0.001. FL, full-length. (**B**) In a nocodazole challenge, H2B-RFP wild-type MEFs infected with the indicated constructs with and without the addition of Dox were treated with 100 ng/ml of nocodazole and monitored by live cell time-lapse imaging. The point of time in which 50% of cells are arrested in mitosis is plotted. n = 1 line, ≥ 15 cells per treatment. *p<0.05, **p<0.01, ***p<0.001. (**C**) Analysis of chromosome misalignment in wild-type MEFs infected with the indicated constructs with and without addition of Dox. MEFs were treated with 100 µM monastrol for 1 hr and then with monastrol and 10 µM MG132 for 1 hr and released for 90 min into MG132. Cells were treated with DMSO (Vehicle) or 50 nM AZD1152-HQPA (AZD1152) throughout the duration of the experiment. n = 3 lines, 50 cells per line per treatment. Data are mean ± s.d. *p<0.05. (See associated ***Figure 9—source data 1***).

DOI: 10.7554/eLife.16620.028

The following source data is available for figure 9:

Source data 1. Source file for mitotic timing, nocodazole challenge and monastrol washout data.

DOI: 10.7554/eLife.16620.029

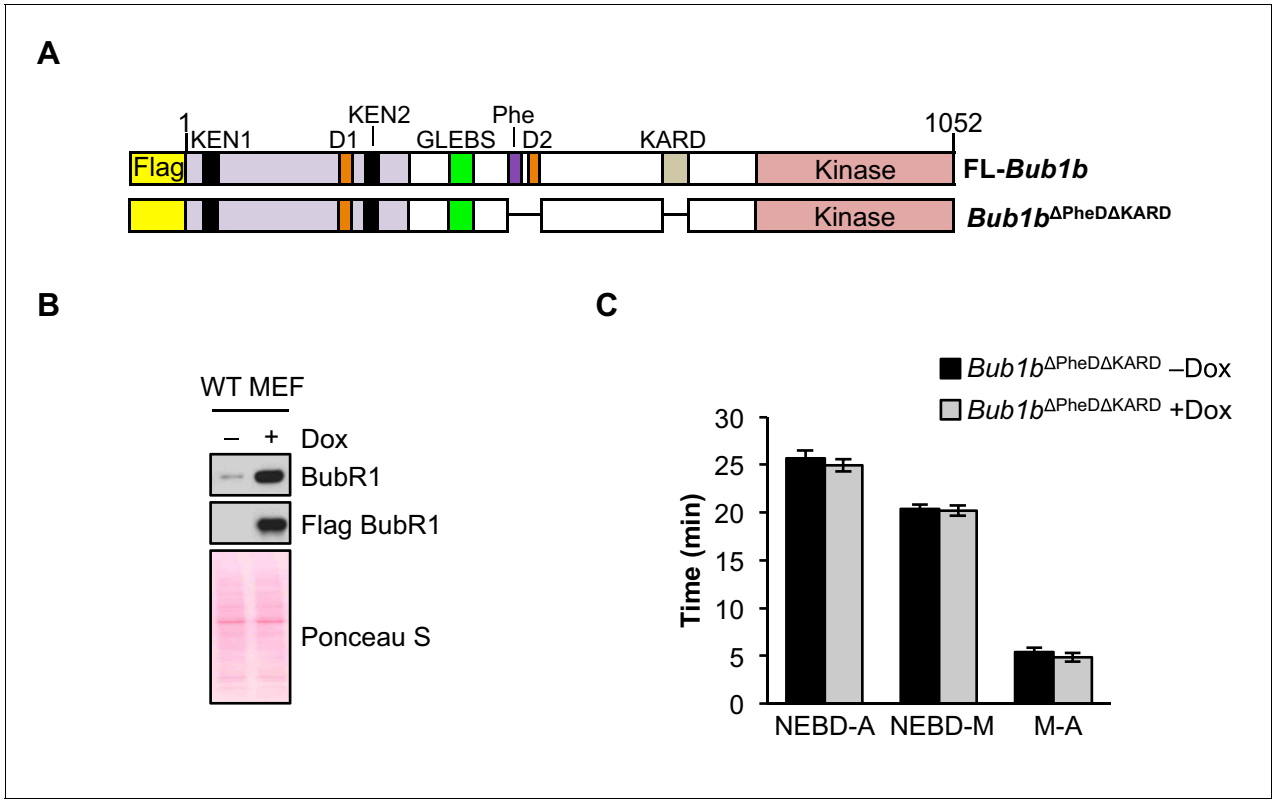


Figure 9—figure supplement 1. Combined loss of Phe, D-box2 and KARD does not impact mitotic timing. (**A**) Schematic of the pTripZ-Flag-*Bub1b*$^{\Delta PheD\Delta KARD}$ construct. D, destruction-(D-)box. GLEBs, GLEBs-binding motif. Phe, Phe box. KARD, kinetochore attachment regulatory domain. FL, full length. (**B**) Western blot of wild-type (WT) MEFs infected with *Bub1b*$^{\Delta PheD\Delta KARD}$ with or without doxycycline (Dox). Blot was probed with the indicated antibodies. Ponceau S was used to normalize loading. (**C**) Analysis of the time from nuclear envelope breakdown (NEBD) to anaphase onset in H2B-RFP WT MEFs infected with *Bub1b*$^{\Delta PheD\Delta KARD}$ with and without the addition of Dox by live cell time-lapse imaging. n = 1 line, $\geq$ 20 cells. Data are mean ± s.e.m. M, metaphase. (See associated ***Figure 9—figure supplement 1—source data 1***).

DOI: 10.7554/eLife.16620.030

The following source data is available for figure 9:

Figure supplement 1—Source data 1. Source file for mitotic timing data.

DOI: 10.7554/eLife.16620.031

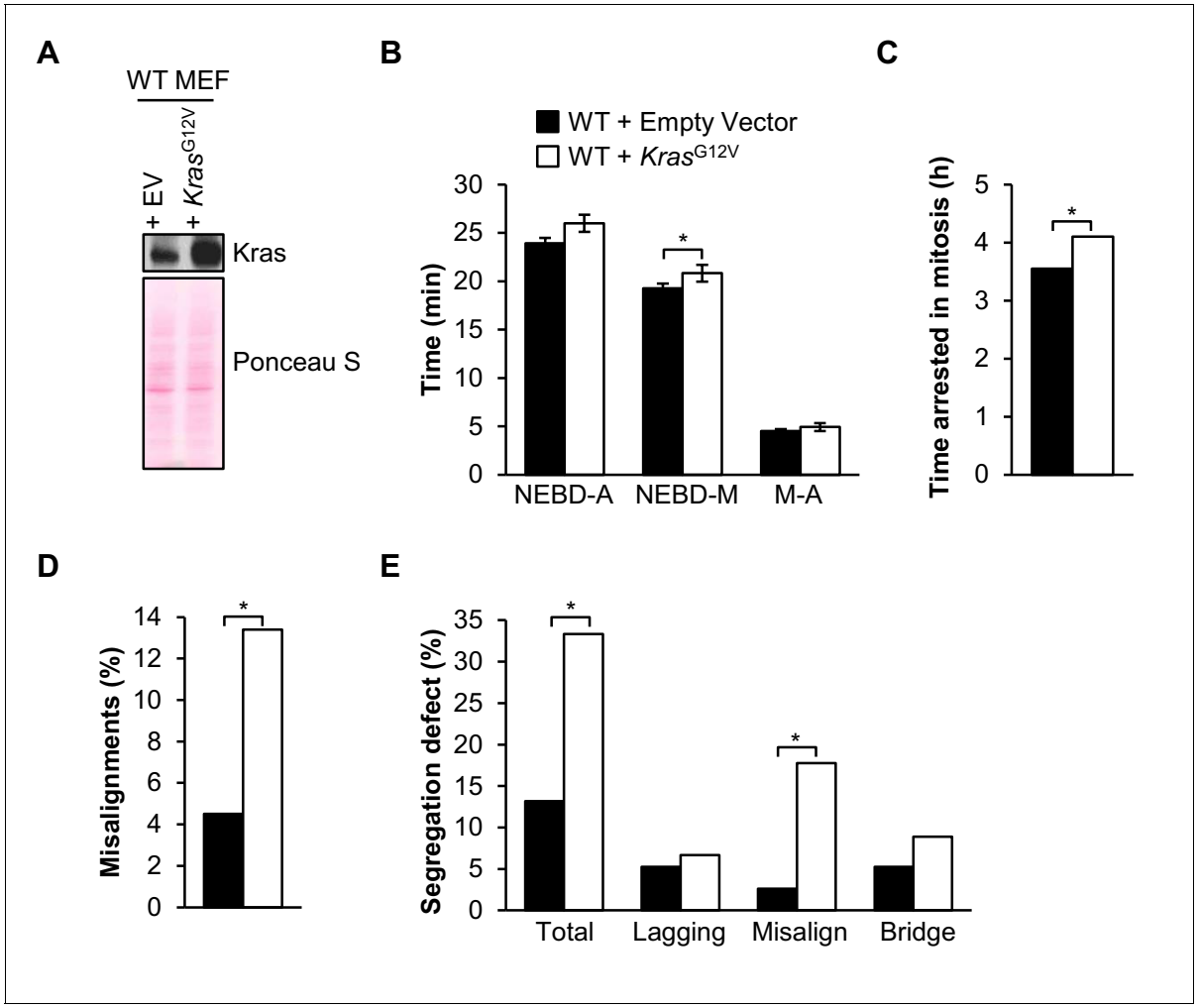


Figure 10. Oncogenic Kras increases microtubule-kinetochore malattachment. (**A**) Western blot of wild-type (WT) MEFs infected with pBABE-Puro-KRas (G12V) or empty vector (EV). Blot was probed with the indicated antibody. Ponceau S was used to normalize loading. (**B**) Analysis of the time from nuclear envelope breakdown (NEBD) to anaphase (A) onset in H2B-RFP wild-type MEFs infected with $Kras^{G12V}$ or EV by live cell time-lapse imaging. n = 1 line, $\geq$ 19 cells. Data are mean ± s.d. *p<0.05. M, metaphase (**C**) In a nocodazole challenge, H2B-RFP wild-type MEFs infected with $Kras^{G12V}$ or EV were treated with 100 ng/ml of nocodazole and monitored by live cell time-lapse imaging. The point of time in which 50% of cells are arrested in mitosis is plotted. n = 1 line, $\geq$ 20 cells. *p<0.05. (**D**) Analysis of chromosome misalignments in wild-type MEFs infected with $Kras^{G12V}$ or EV. MEFs were treated with 100 μM monastrol for 1 hr and then with monastrol and 10 μM MG132 for 1 hr and released for 90 min into MG132. n = 1 line, ≈200 cells. *p<0.05. (**E**) Live-cell imaging of chromosome segregation defects in primary H2B-RFP wild-type MEFs infected with Kras or EV. n = 1 line, ≈40 cells. *p<0.05. (See associated ***Figure 10—source data 1***).

DOI: 10.7554/eLife.16620.032

The following source data is available for figure 10:

Source data 1. Source file for mitotic timing, nocodazole challenge, missegregation assay and monastrol washout data.

DOI: 10.7554/eLife.16620.033