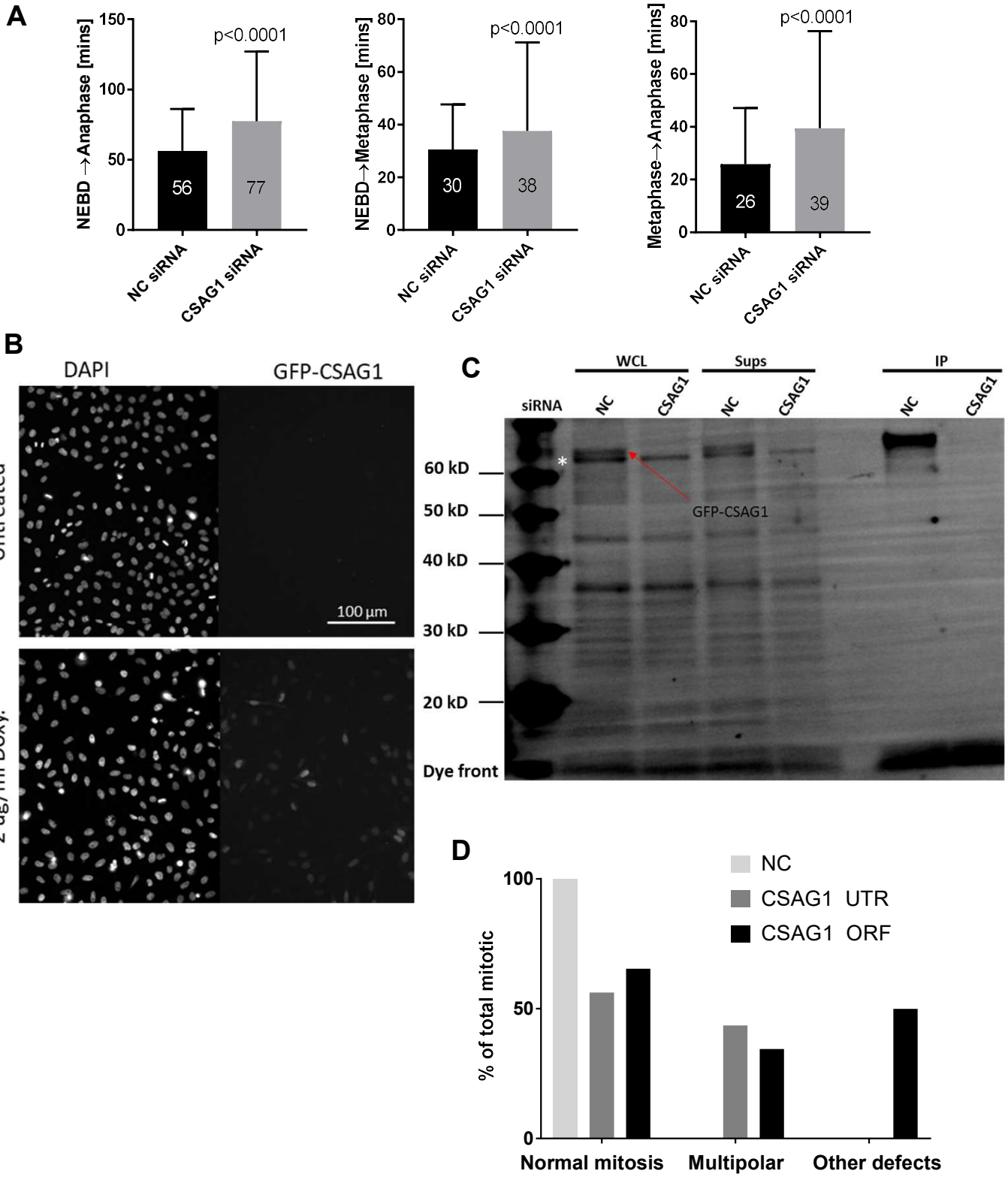


**Figure S1: CSAG1 depletion causes mitotic delay in cells that do not show multipolar phenotype.**
**(A)** Elapsed times from NEBD-anaphase (left), NEBD-metaphase (middle), and metaphase-anaphase (right) were determined in CSAG1-depleted HeLa-H2B-GFP cells. The graphs were plotted excluding the cells that showed multipolar mitosis. Totals of >180 cells from three independent experiments were analyzed. The Mann-Whitney test was used for statistical analysis. **(B)** HeLa cells expressing inducible GFP-CSAG1 were analyzed by live cell imaging for 24 h with or without pretreatment with 2 ug/ml doxycycline. The upper panels show untreated cells and lower panels show doxycycline-treated cells. About 90% of cells were GFP positive after doxycycline treatment whereas 0% were positive in the absence of doxycycline. **(C)** Western blot of whole cells lysate (WCL), soluble fraction (sups) and immuno-precipitated samples (IP) of HeLa cells expressing GFP-CSAG1 that were transfected with either control siRNA or CSAG1 siRNA targeting the ORF. GFP-CSAG1 in significantly enriched after IP in control but not detected in CSAG1 siRNA samples. The same band is completely removed with CSAG1 siRNA in WCL and sups samples. * shows a nonspecific band that was not altered with CSAG1 siRNA. **(D)** Mitotic multipolarity or other mitotic defects were assessed in HeLa cells transfected with negative control siRNA (NC), siRNA targeting the UTR of CSAG1 or siRNA targeting an ORF region of CSAG1. Totals of >100 cells were analyzed per siRNA transfection.

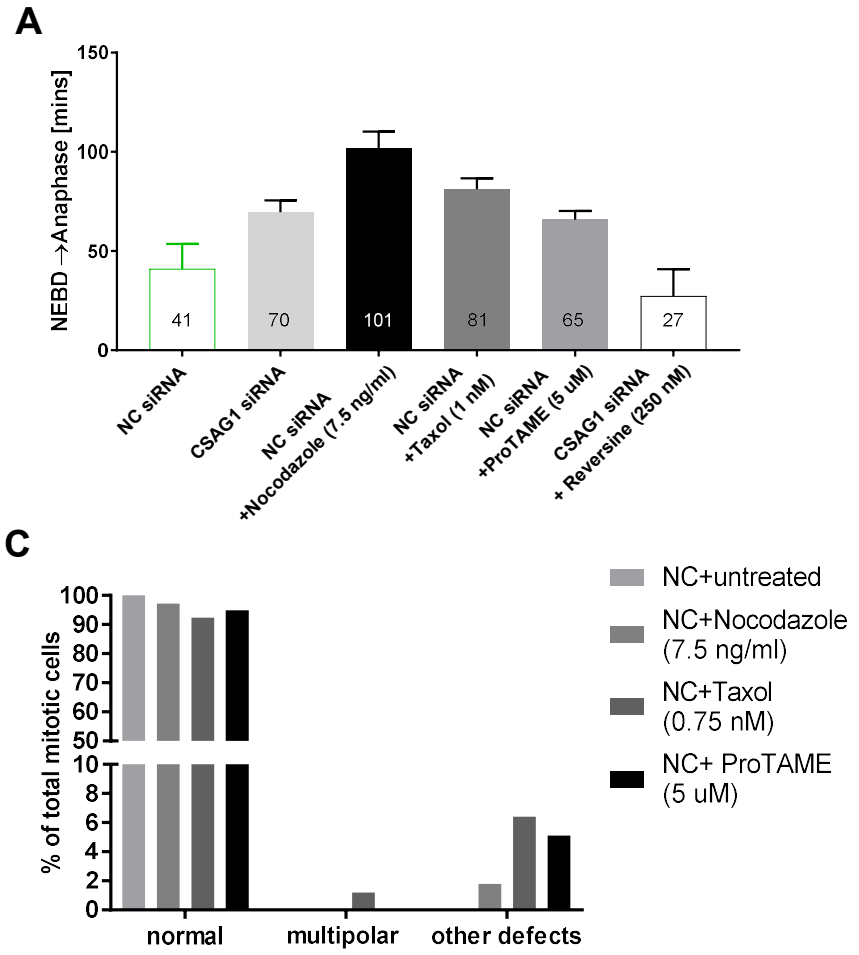



**Figure S2: Subtle modulations of mitotic duration with low concentrations of drugs do not induce significant levels of multipolar spindles or other mitotic defects in control cells.**

**(A)**.Elapsed times from NEBD to anaphase **(B)** multipolar mitosis and other defects were determined in HeLa cells that were transfected with NC siRNA and treated with either nocodazole (25 nM), Taxol (1 nM), ProTAME (5 uM) or reversine (250 nM). Totals of >200 cells for each treatment were analyzed from three independent experiments. Numbers within each bar show the average times from NEBD to anaphase.

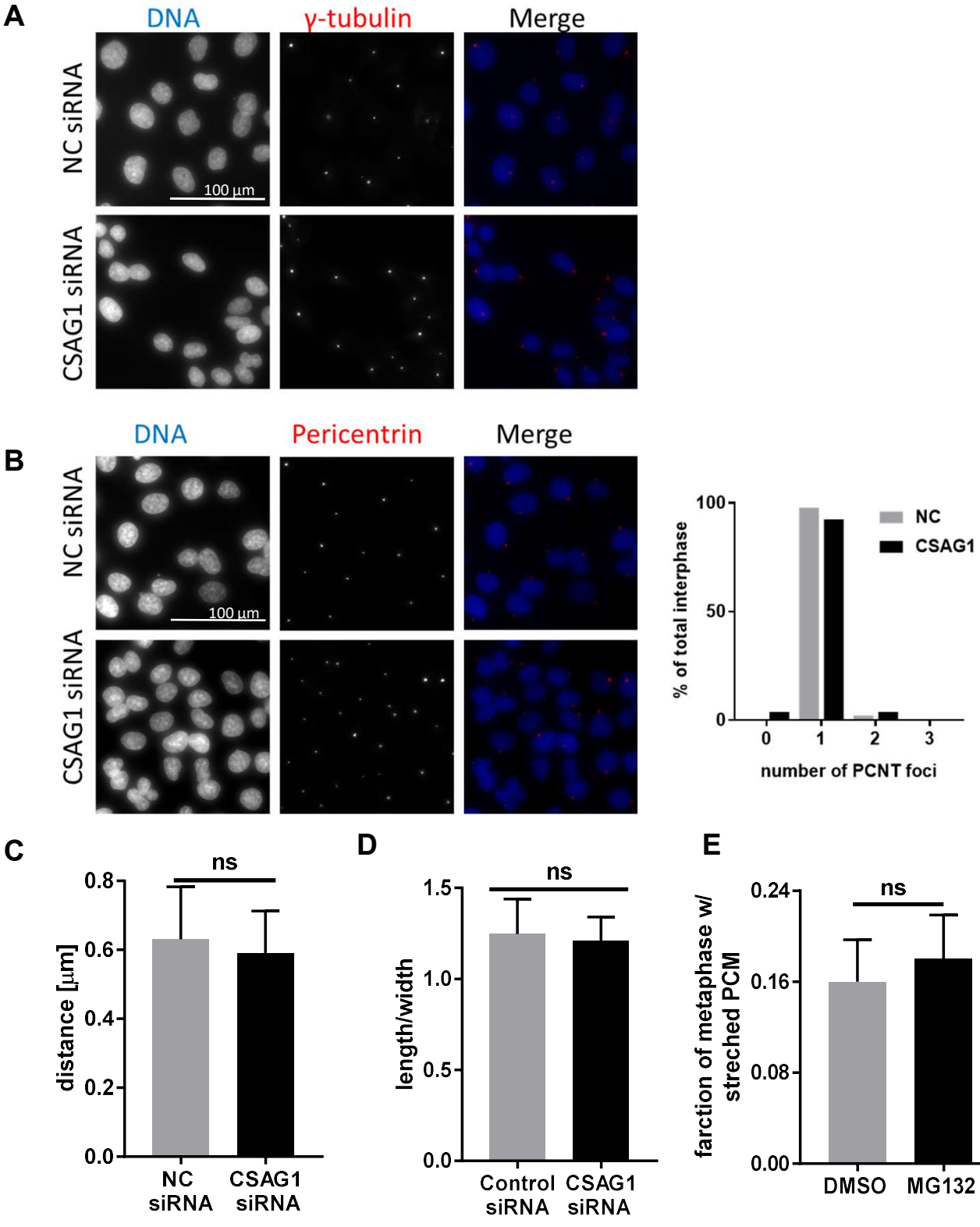


**Figure S3: Interphase centrosome numbers and centriole separation are not altered in CSAG1-depleted cells, and short delay at metaphase does not change pericentrin distribution at spindle poles of control cells at metaphase.**

The total number of centrosomes in interphase cells was determined using **(A)** γ-tubulin or **(B)** pericentrin labeling of HeLa cells. The graph at right shows the frequency distribution of pericentrin foci in interphase cells. Totals of >100 interphase cells were examined for each sample. CSAG1 depletion does not cause increased numbers of centrosomes in interphase. **(C**) Distances between centrioles were measured in spindle poles of metaphase cells transfected with negative control (NC) or CSAG1 siRNA. **(D)** PCM axis ratios were measured in early prophase cells depleted of CSAG1. **(E)** Pericentrin labeling was evaluated as normal or abnormal/dispersed in metaphase HeLa cells treated with MG132 for 1.5 h. Totals of 150 cells were examined for each sample. Metaphase delay induced by MG132 does not alter pericentrin distribution.

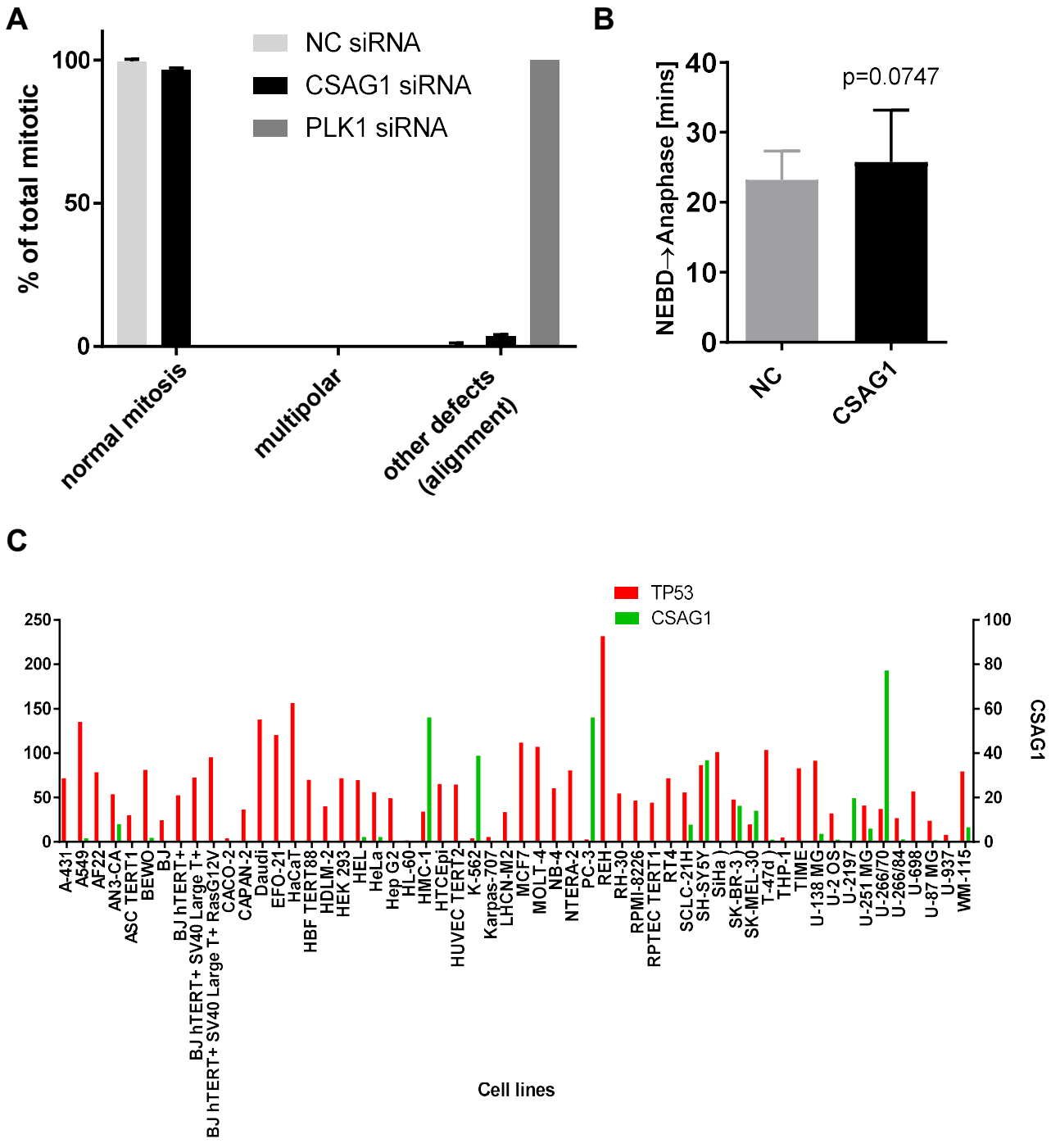
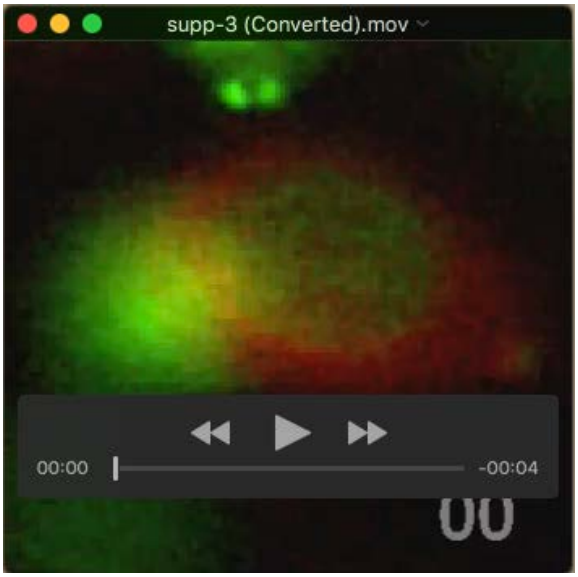
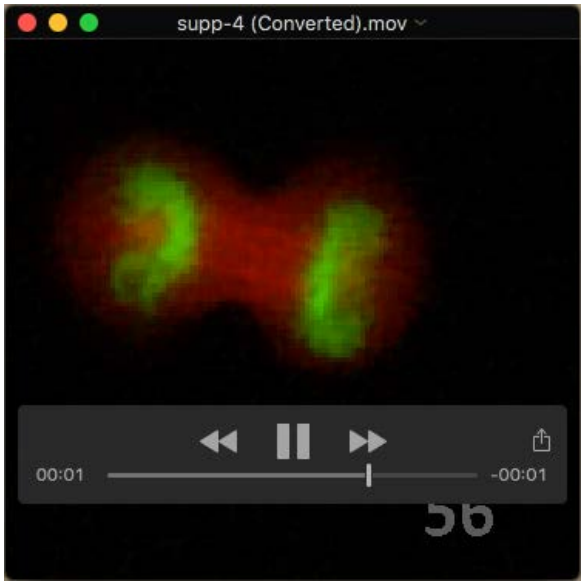


**Figure S4: RPE1 cells are resistant to CSAG1 depletion phenotypes.** **(A)** Mitotic phenotypes were examined in RPE1 cells treated with NC siRNA, siRNA to CSAG1, or siRNA to Plk1, n=2 independent experiments **(B)** Elapsed times from NEBD-metaphase, metaphase to anaphase were determined in cells from A. Totals of >60 cells per siRNA treatment were examined. The Mann-Whitney test was used for statistical analysis. RPE1 cells do not show any discernable mitotic phenotype after CSAG1 depletion beyond a marginal increase in mitotic duration. (**C**) Numbers of p53 and CSAG1 transcripts in different cell lines were plotted from publicly available data (www.sciencemag.org/content/356/6340/eaal3321/suppl/DC1). No correlation between p53 and CSAG1 transcript levels was evident**.**

**Supplemental Movies:** HeLa cells stably expressing GFP-Tubulin were transfected with siRNA targeting the UTR of CSAG1 or non-targeting control siRNA. Cells were labeled with sirDNA dye to visualize DNA and imaged every 7 minutes. Videos show HeLa cells from NEBD to mitotic exit.




**Movie 1:** pole fragments causing metaphase plate to bend in a CSAG1-depleted cell.




**Movie 2:** mitosis proceeds unperturbed in a control siRNA-depleted cell.