SUPPLEMENTAL FIGURE LEGENDS

Figure S1. Knockdown of Cdc20 slows cyclin B1 proteolysis in stable HeLa and A549 cyclin B1-EYFP lines.

Changes of cyclin B1 level over time from 5 representative HeLa or A549 cells treated with Kinesin-5 inhibitor or Cdc20 siRNA. HeLa and A549 cells stably expressing full-length cyclin B1-EYFP were treated as indicated and monitored by time-lapse microscopy. Fluorescent intensity (with subtraction of background) was normalized by the highest intensity in the time course for each cell. T=0 marks mitotic entry.

(A) HeLa cells died in mitosis under Kinesin-5 inhibitor.

(B) A549 cells slipped out of mitotic arrest induced by Kinesin-5 inhibitor

(C) HeLa cells died in mitosis when treated with Cdc20 siRNA.

(D) A549 cells died in mitosis when treated with Cdc20 siRNA.

Figure S2. SAC-dependent mitotic arrest and death induced by paclitaxel.

(A) Knockdown of indicated SAC proteins abrogates paclitaxel-induced arrest, but has no effect on arrest induced by Cdc20 knockdown in HeLa. Numbers in parentheses indicate n. Error bars represent SD.

(B) Knockdown of Mad2 abrogates paclitaxel-induced arrest, but has no effect on arrest extended by Cdc20 co-knockdown in MDA-MB-435S, MCF7 and A549. Numbers in parentheses indicate n. Error bars represent SD.

(C) Knockdown of indicated SAC proteins attenuates paclitaxel-induced death in HeLa, but has no effect on death induced by co-Cdc20 knockdown.

(D) Knockdown of Mad2 attenuates paclitaxel-induced death in MDA-MB-435S, MCF7

and A549, but has no effect on death induced by co-Cdc20 knockdown after extended arrests.

Figure S3. Phase-contrast and IMS-RP time-lapse sequences of representative HeLa, HeLa stably expressing Bcl2, or A549 cells for indicated treatments. Scale bar: 5 μm.

(A) HeLa cells treated with Kinesin-5 inhibitor.

(B) HeLa cells stably expressing Bcl2 treated with Kinesin-5 inhibitor.

(C) A549 cells treated with Kinesin-5 inhibitor.

(D) A549 cells treated with Kinesin-5 inhibitor and Cdc20 siRNA.

Figure S4. Non-degradable cyclin B1 as an alternative approach to block mitotic exit.

(A) Expression of endogenous and non-degradable cyclin B1. HeLa cells were infected with adenovirus expressing EGFP or CT-cyclin-B1-EGFP for indicated times. Cell lysates were immunoblotted for cyclin B1 or actin.

(B) Knockdown of SAC proteins abrogates Kinesin-5 inhibitor-induced arrest, but has no effect on arrest induced by non-degradable cyclin B1. HeLa cells were transfected with indicated SAC protein (or Lamin A/C as control) siRNA for 6 hr, followed by infection of adenovirus expressing CT-cyclin-B1-EGFP or addition of Kinesin-5 inhibitor (with adenovirus expressing EGFP). Duration of mitosis was measured by time-lapse microscopy. Numbers in parentheses indicate n. Error bars represent SD.

(C) Cumulative survival curves for indicated treatments in various cancer cell lines: HeLa, MDA-MB-435S, MCF7, A549, and HeLa cells stably expressing Bcl2. Individual cells

were monitored by time-lapse microscopy, and time from mitotic entry to death was measured and plotted as survival curves as a function of time since mitotic entry.

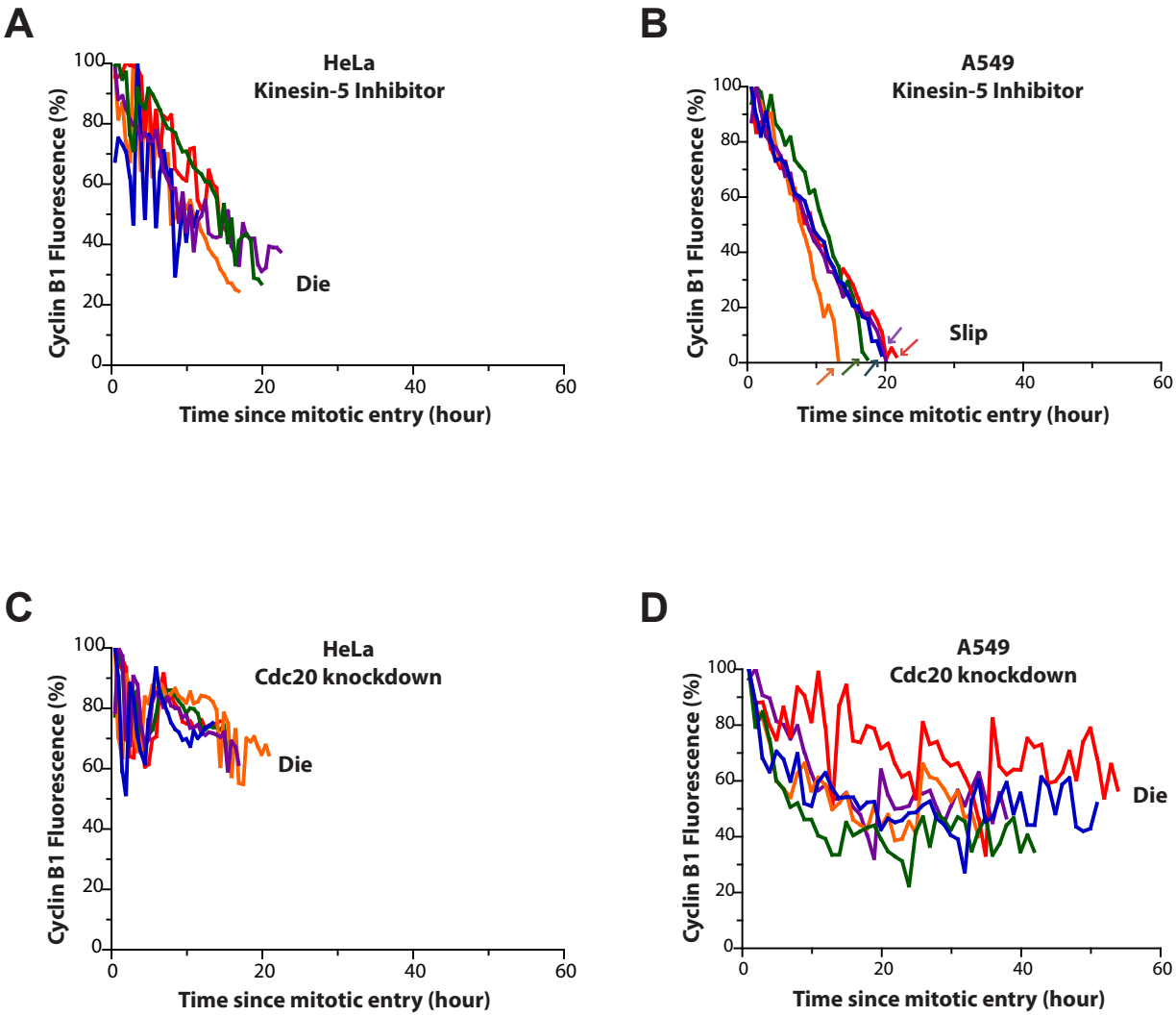

Figure S1.
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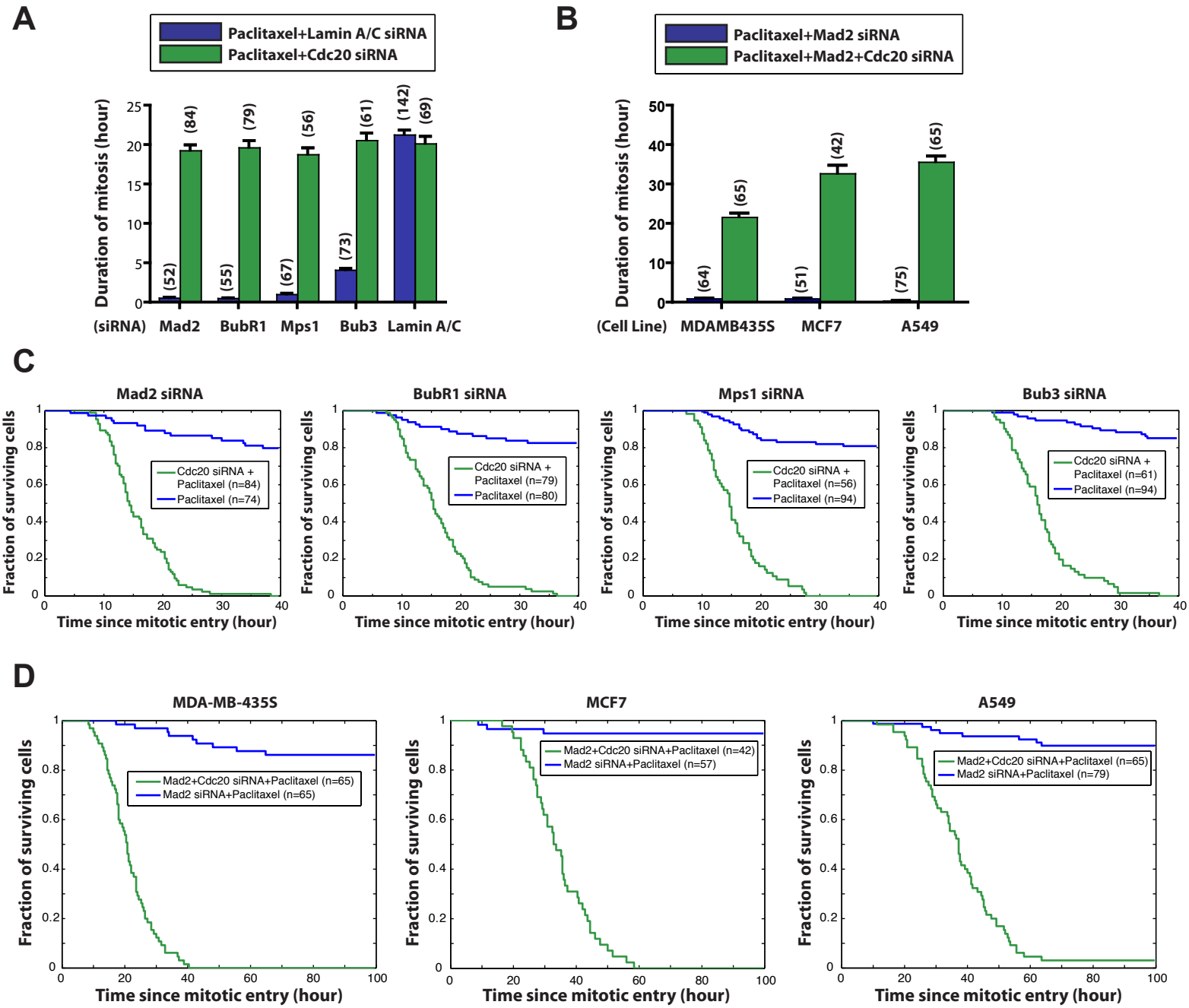

Figure S2.
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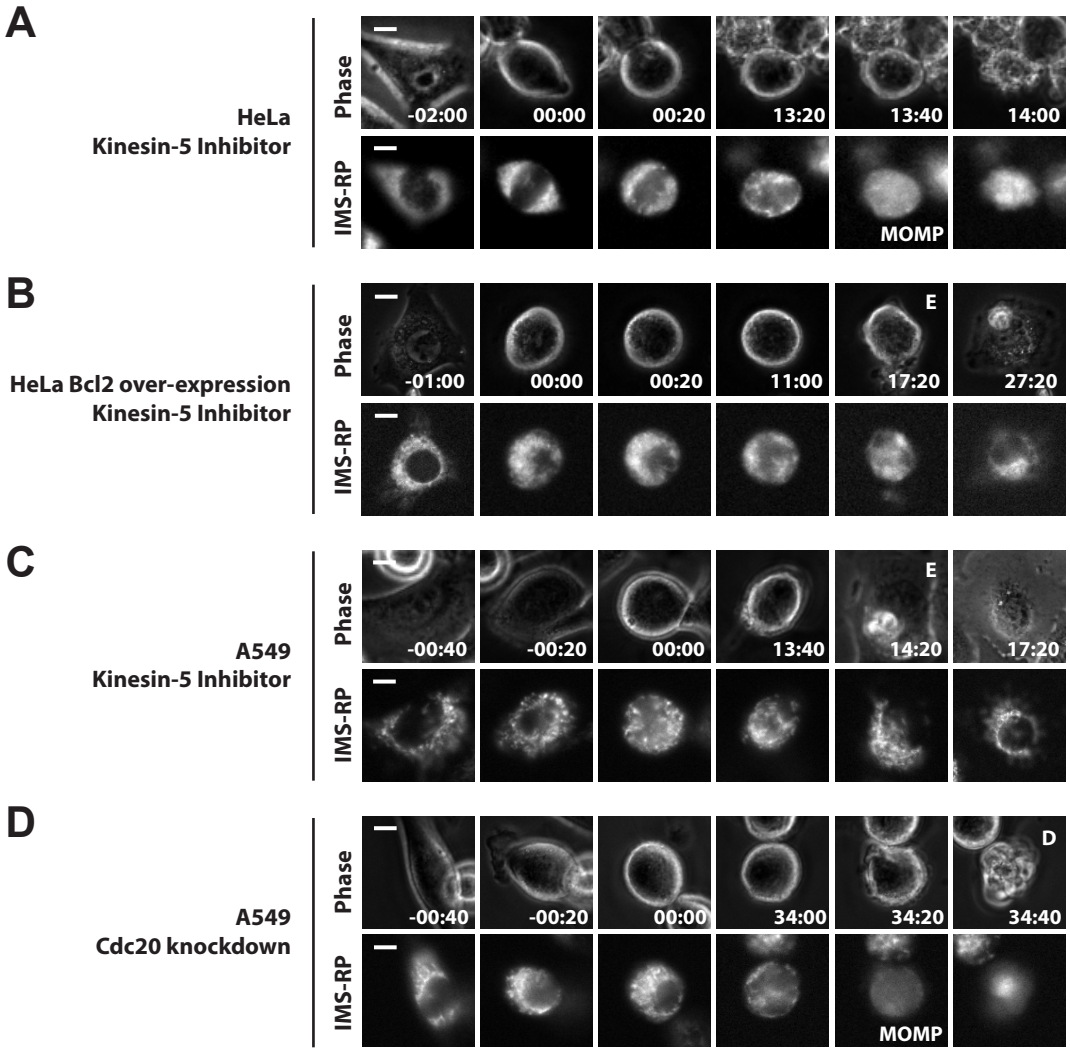

Figure S3.
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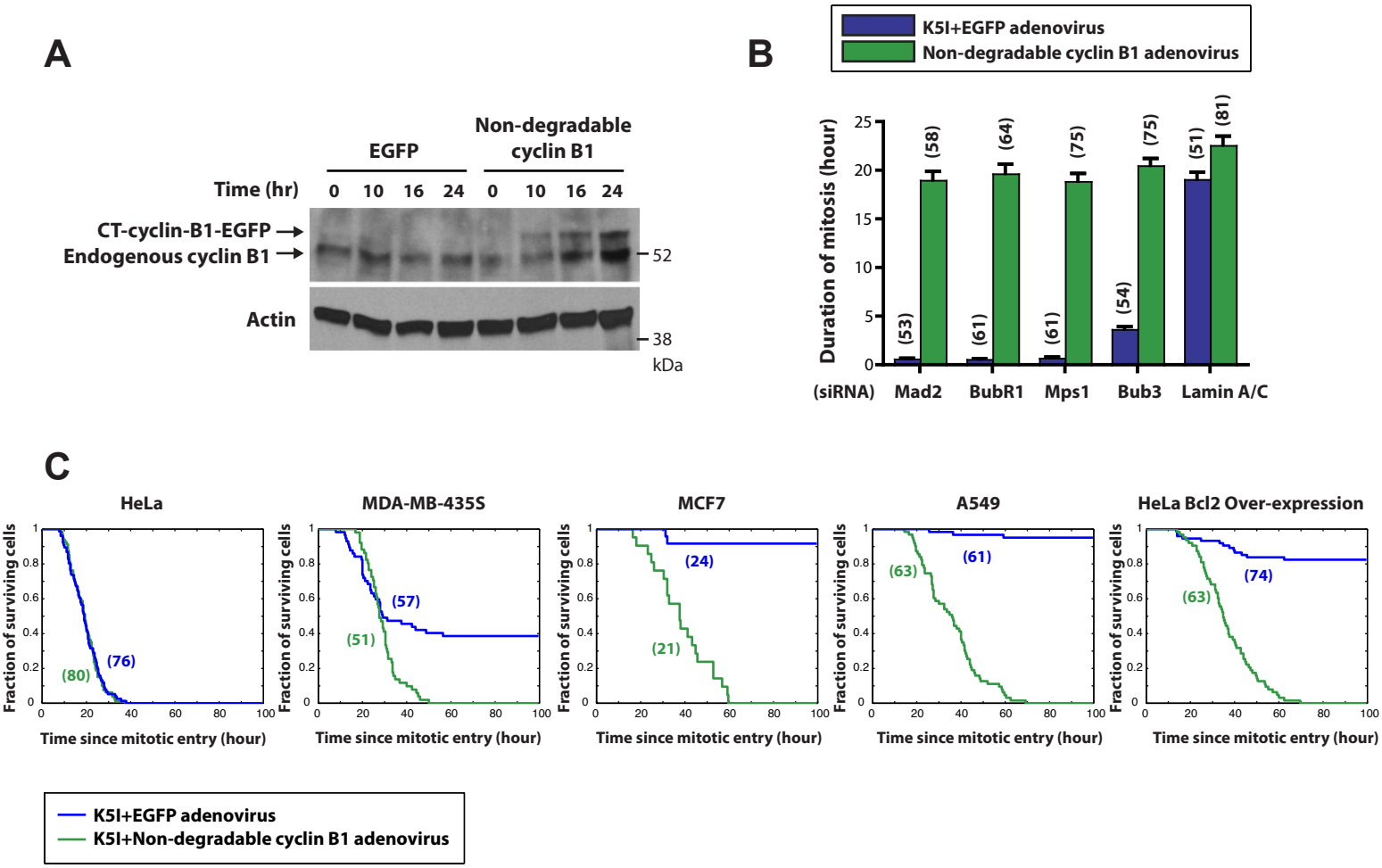

Figure S4.
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