Expanded View Figures

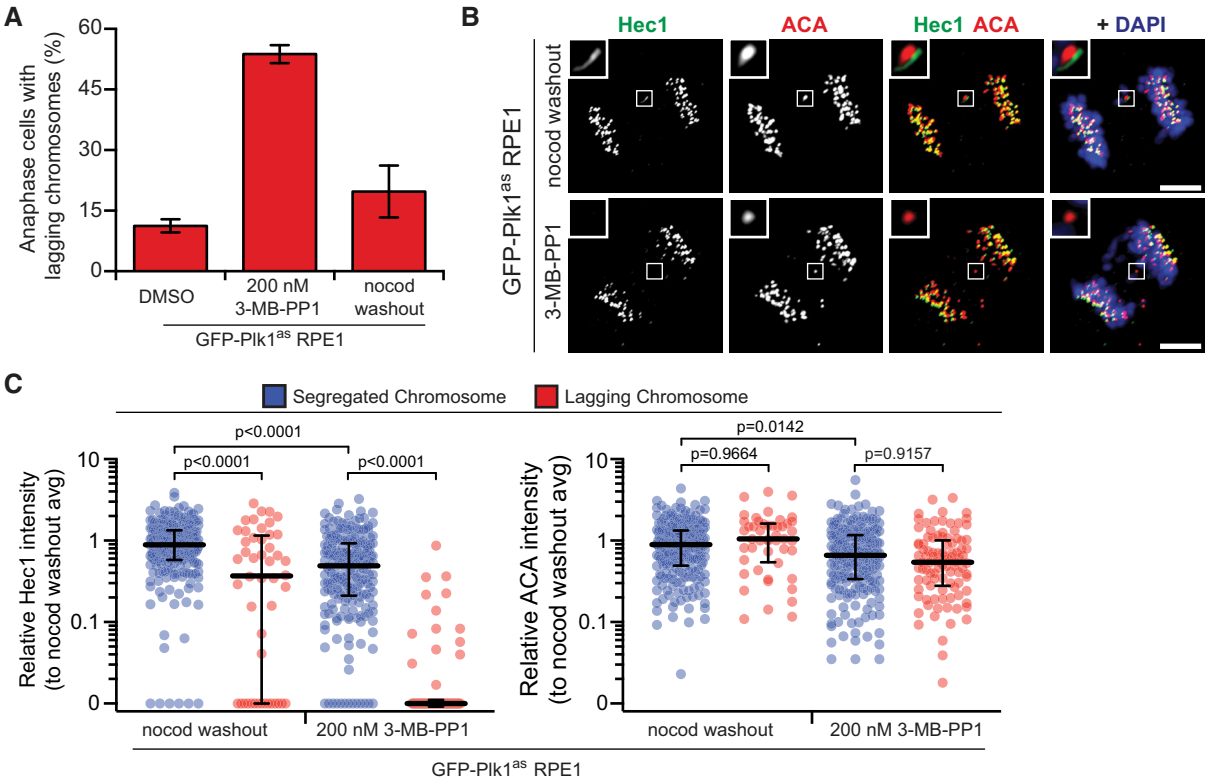

Figure EV1. Defects in outer kinetochore integrity are specific to loss of Plk1 activity (related to Fig 1).

A Graph shows average percentage (± SEM) of EGFP-Plk1^{as}-expressing RPE1 cells exhibiting lagging chromosomes in anaphase after Plk1^{as} inhibition (200 nM 3-MB-PP1) or nocodazole washout (n = 30 cells/experiment; four independent experiments).

B Representative maximum-intensity micrographs of anaphase cells from (A). Insets highlight presence/absence of Hec1 at lagging kinetochores, marked by ACA. Scale bars, 5 μm.

C Graph shows relative volume intensities of Hec1 (left) and ACA (right) at segregated (blue) and lagging (red) kinetochores from (A, B). Each circle represents a single kinetochore (n = 10 segregated kinetochores/cell, 1–8 lagging kinetochores/cell; eight cells/experiment; three independent experiments). Bars indicate median kinetochore intensity and interquartile range. Significance determined by Kruskal–Wallis test with Dunn's correction for multiple comparisons.

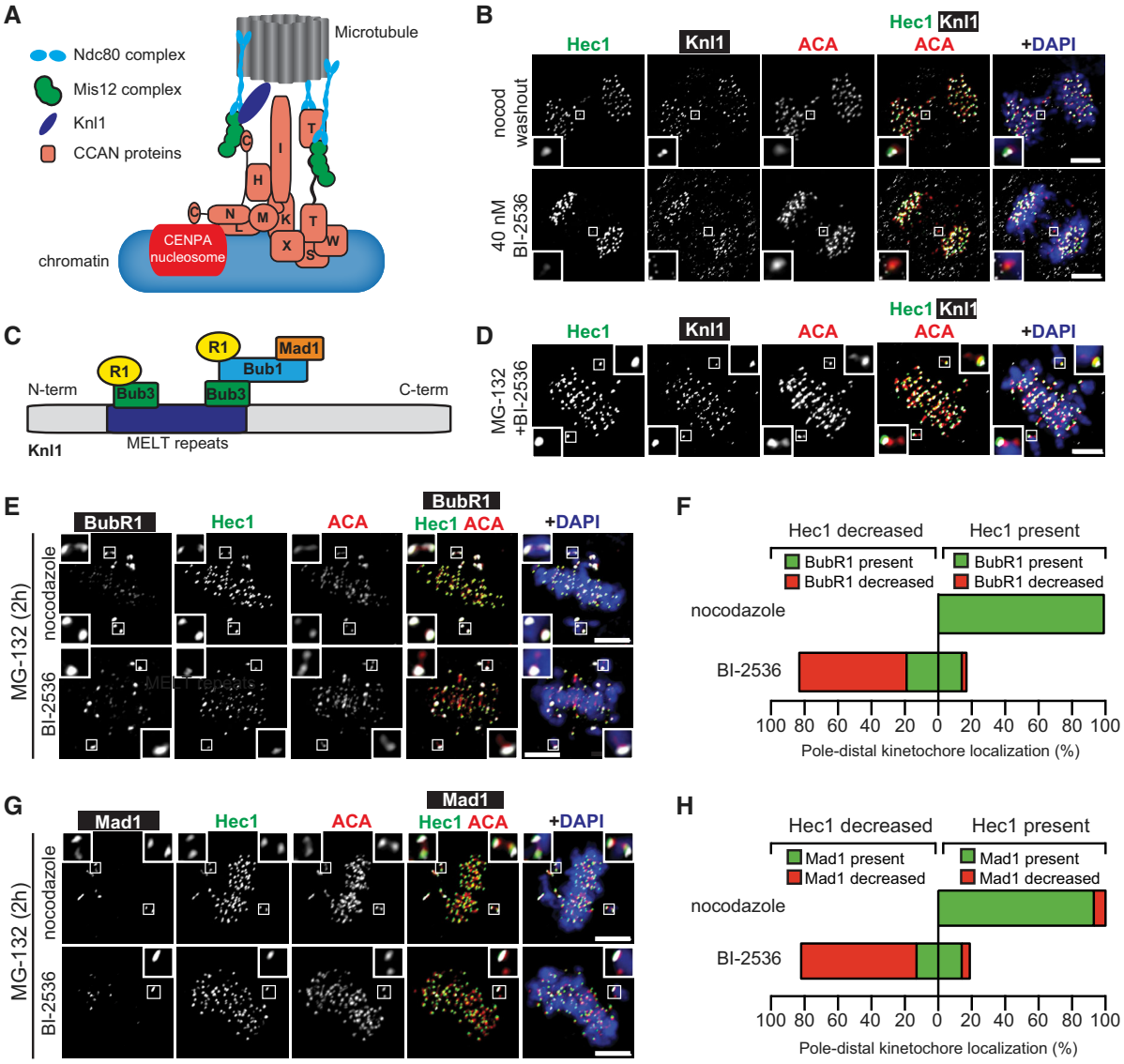

Figure EV2. Kinetochore disruption after Plk1 inhibition extends to Knl1, impairing checkpoint protein recruitment.

A Illustrative map of the kinetochore highlighting positions of KMN (Knl1, Mis12, Ndc80) and CCAN complexes relative to microtubules and centromeric chromatin.

B Representative maximum-intensity projection micrographs of kinetochore protein localization in anaphase cells after Plk1 inhibition (BI-2536) or nocodazole washout. Insets highlight lagging kinetochores, marked by ACA. Scale bars, 5 μm.

C Illustrative diagram of Knl1 indicating method of BubR1 and Mad1 recruitment.

D Representative maximum-intensity projection micrographs of metaphase cell with misaligned chromosome pairs after 2-h Plk1 inhibition (BI-2536). Inset highlights decreased localization of Knl1 and Hec1 to the pole-distal kinetochores, indicated by ACA. MG-132 was used to prevent mitotic cells from entering anaphase. Scale bar, 5 μm.

E Representative maximum-intensity projection micrographs of metaphase cells with misaligned chromosome pair after 2-h Plk1 inhibition (BI-2536) or nocodazole challenge. Insets highlight distribution of BubR1 and Hec1 at misaligned kinetochore pairs, indicated by ACA. MG-132 was used to prevent mitotic cells from entering anaphase. Scale bars, 5 μm.

F Graphs show average percentage of misaligned pole-distal kinetochores with localized (green) or decreased (red) BubR1 and Hec1 after 2-h Plk1 inhibition (BI-2536) or nocodazole challenge (n = 1–10 kinetochore pairs/cell; 10 cells/experiment; two independent experiments). "Decreased" intensity indicates ≤ 50% intensity of sister kinetochore.

G Representative maximum-intensity projection micrographs of metaphase cells with misaligned chromosome pair after 2-h Plk1 inhibition (BI-2536) or nocodazole challenge. Insets highlight distribution of Mad1 and Hec1 at misaligned kinetochore pairs, indicated by ACA. MG-132 was used to prevent mitotic cells from entering anaphase. Scale bars, 5 μm.

H Graphs show average percentage of misaligned pole-distal kinetochores with localized (green) or decreased (red) Mad1 and Hec1 after 2-h Plk1 inhibition (BI-2536) or nocodazole challenge (n = 1–10 kinetochore pairs/cell; 10 cells/experiment; two independent experiments). "Decreased" intensity indicates ≤ 50% intensity of sister kinetochore.

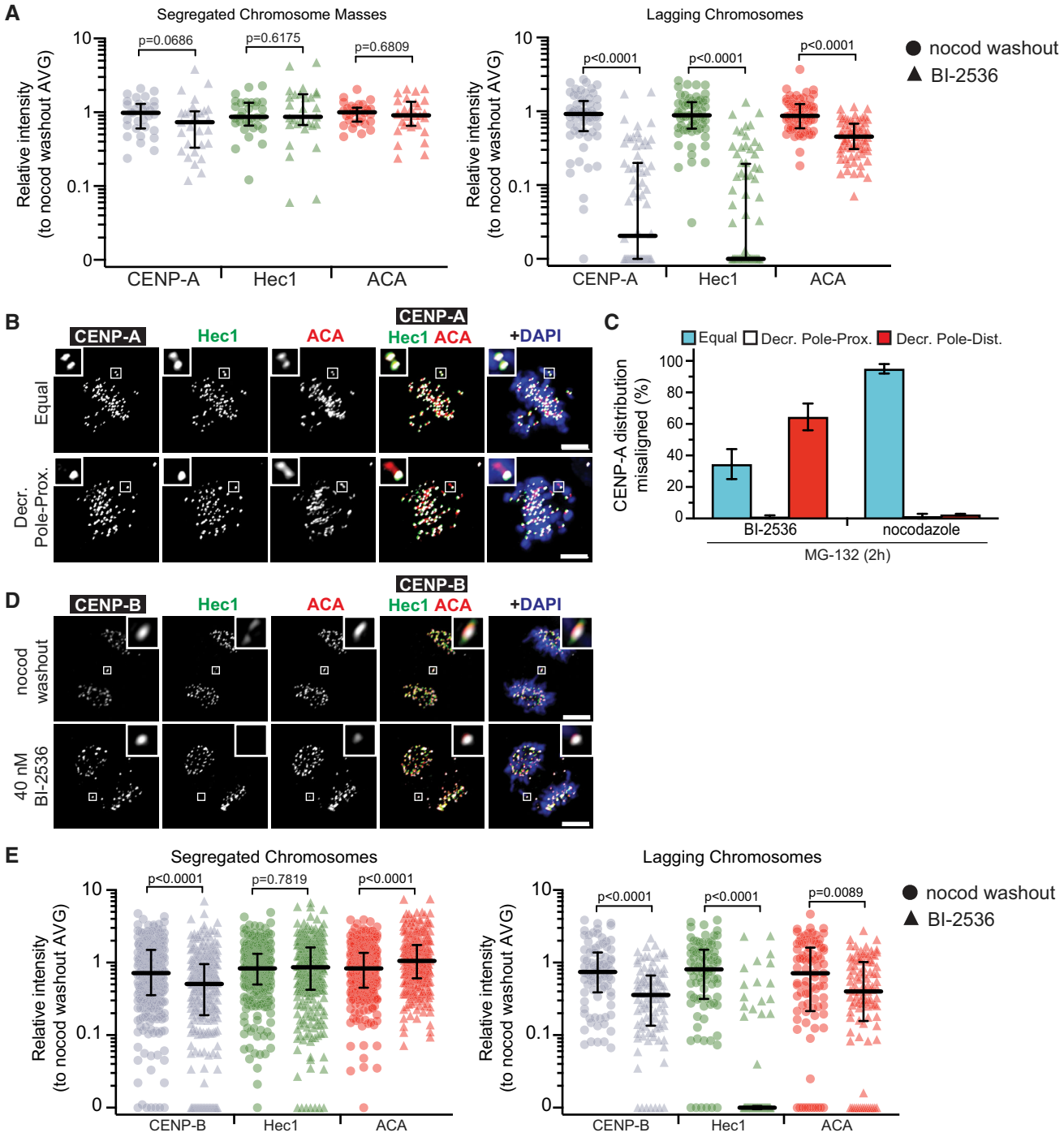
A
Segregated Chromosome Masses
Lagging Chromosomes
Relative intensity (to nocod washout AVG)
p=0.0686
p=0.6175
p=0.6809
p<0.0001
p<0.0001
p<0.0001
CENP-A
Hec1
ACA
nocod washout
BI-2536
B
CENP-A
Hec1
ACA
Hec1 ACA
+DAPI
Equal
Decr. Pole-Prox.
C
Equal
Decr. Pole-Prox.
Decr. Pole-Dist.
CENP-A distribution misaligned (%)
BI-2536
nocodazole
MG-132 (2h)
D
CENP-B
Hec1
ACA
Hec1 ACA
+DAPI
nocod washout
40 nM BI-2536
E
Segregated Chromosomes
Lagging Chromosomes
p<0.0001
p=0.7819
p<0.0001
p<0.0001
p<0.0001
p=0.0089
CENP-B
Hec1
ACA
nocod washout
BI-2536

Figure EV3.

Figure EV3. Kinetochore disruption after Plk1 inhibition includes CENP-A, but not CENP-B (related to Fig 3).

A Graph shows relative volume intensities of CENP-A (gray), Hec1 (green), and ACA (red) at segregated (left) and lagging (right) kinetochores after Plk1 inhibition or nocodazole washout (Fig 3E and F). Note CENP-A data are identical to data presented in Fig 3F. Each symbol in the left graph represents all segregated kinetochores/cell (n = 10 cells/experiment; three independent experiments), whereas each symbol in the right graph represents a single kinetochore (n = 1–6 kinetochores/cell; 10 cells/experiment; three independent experiments). Bars indicate median kinetochore intensity and interquartile range. Significance determined by two-tailed Mann–Whitney test.

B Representative maximum-intensity projection micrographs of metaphase cells with misaligned chromosomes. Insets highlight distribution of CENP-A and Hec1 at misaligned kinetochore pair, indicated by ACA. Scale bars, 5 μm.

C Graph shows average percentage (± SEM) of misaligned chromosome pairs exhibiting each of the three distribution types after 2-h Plk1 inhibition (BI-2536) or nocodazole challenge (n = 1–8 chromosomes/cell; 10 cells/experiment; two independent experiments). "Decreased" intensity indicates ≤ 50% intensity of sister kinetochore.

D Representative maximum-intensity micrographs of anaphase cells after Plk1 inhibition (BI-2536) or nocodazole washout. Insets highlight the presence of CENP-B and Hec1 at lagging kinetochores, marked by ACA. Scale bars, 5 μm.

E Graph shows relative volume intensities of CENP-B (gray), Hec1 (green), and ACA (red) at segregated (left) and lagging (right) kinetochores from (B). Each symbol represents a single kinetochore (n = 10 segregated kinetochores/cell, 1–8 lagging kinetochores/cell; 10 cells/experiment; three independent experiments). Bars indicate median kinetochore intensity and interquartile range. Significance determined by two-tailed Mann–Whitney test.

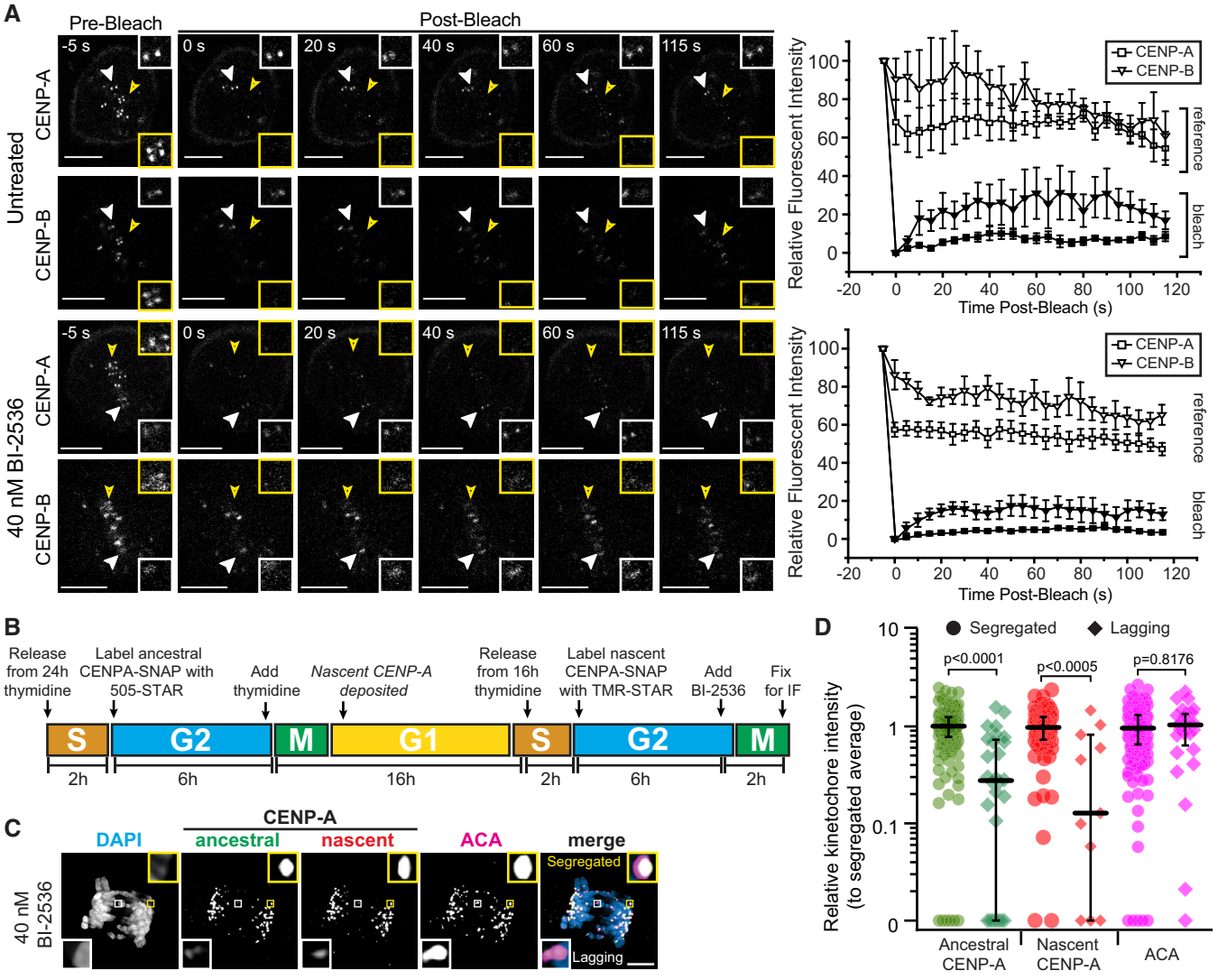

Figure EV4.

Figure EV4. CENP-A loss is not the result of dysregulated turnover; both ancestral and nascent CENP-A pools are equally affected.

A Representative single-plane images of untreated and 40 nM BI-2536-treated RPE1 cell in mitosis before and after photobleaching CENP-A and CENPB. The recovery of the CENP-A and CENPB loci was recorded after photobleaching. White arrowheads mark the kinetochore(s) which was not photobleached (reference), and the yellow arrowhead marks the photobleached kinetochore. The inset on the top right on each image highlights the reference kinetochore(s), and the inset on the bottom right highlights the photobleached kinetochore. Average intensities (± SD) of the CENP-A and CENPB loci are plotted as a function of time on the right for both untreated (n = 3 kinetochores, two independent experiments) and 40 nM BI-2536-treated (n = 10 kinetochores, two independent experiments) conditions. Scale bars, 5 μm.

B Illustrative strategy to differentially label ancestral and nascent CENP-A pools in CENPA-SNAP-expressing RPE1 cells and then determine localization at anaphase kinetochores after Plk1 inhibition (BI-2536).

C Representative maximum-intensity projection micrographs of anaphase cell as described in (A). Insets highlight intensities of ancestral (green) and nascent (red) CENP-A at segregated (yellow box) and lagging (white box) kinetochores, indicated by ACA. Scale bar, 5 μm.

D Graph shows relative volume intensities of ancestral and nascent CENP-A at segregated (circles) and lagging (diamonds) kinetochores from (A, B). Each symbol represents a single kinetochore (n = 20 segregated kinetochores/cell, 1–8 lagging kinetochores/cell; eight cells from two independent experiments). Bars indicate median kinetochore intensity and interquartile range. Significance determined by two-tailed Mann–Whitney test.

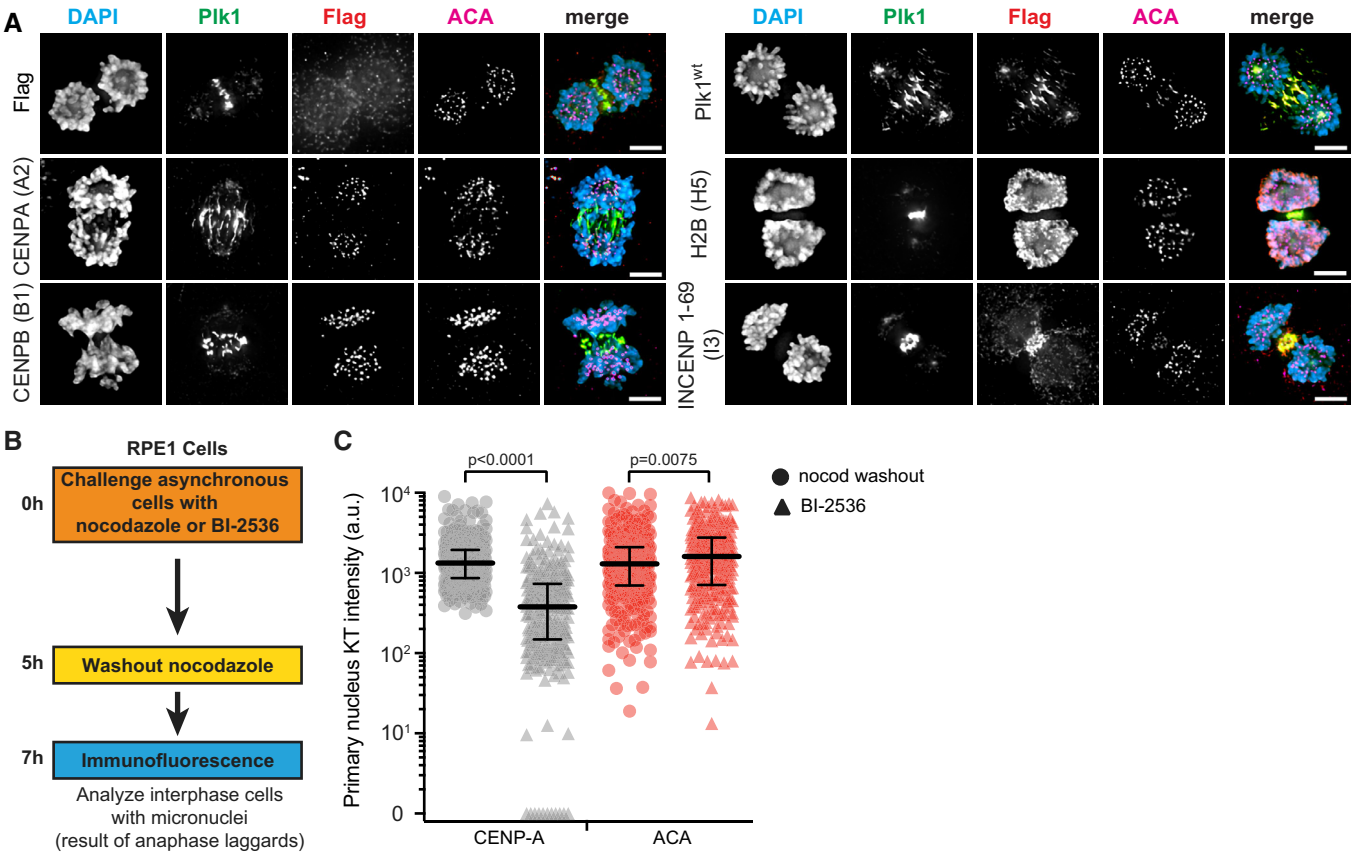

Figure EV5. Centromere-localized Plk1 activity promotes kinetochore integrity, which, when lost, is not restored in subsequent G1 (related to Fig 6).

A Representative maximum-intensity micrographs of anaphase cells indicating localization of the Flag tag, Flag-Plk1^{wt}, or Flag-tagged chromatin-tethered Plk1 constructs. Parentheses indicate clone number. Scale bars, 5 μm.

B Illustrative strategy to analyze the presence of CENP-A in micronuclei after mitosis compromised by Plk1 inhibition (BI-2536) or nocodazole washout.

C Graph shows raw kinetochore protein intensities in the primary nuclei of G1 cells following anaphase exposure to nocodazole washout (circle) or 40 nM BI-2536 (triangle). Bars indicate median protein intensity and interquartile range. Significance determined by two-tailed Mann–Whitney test.