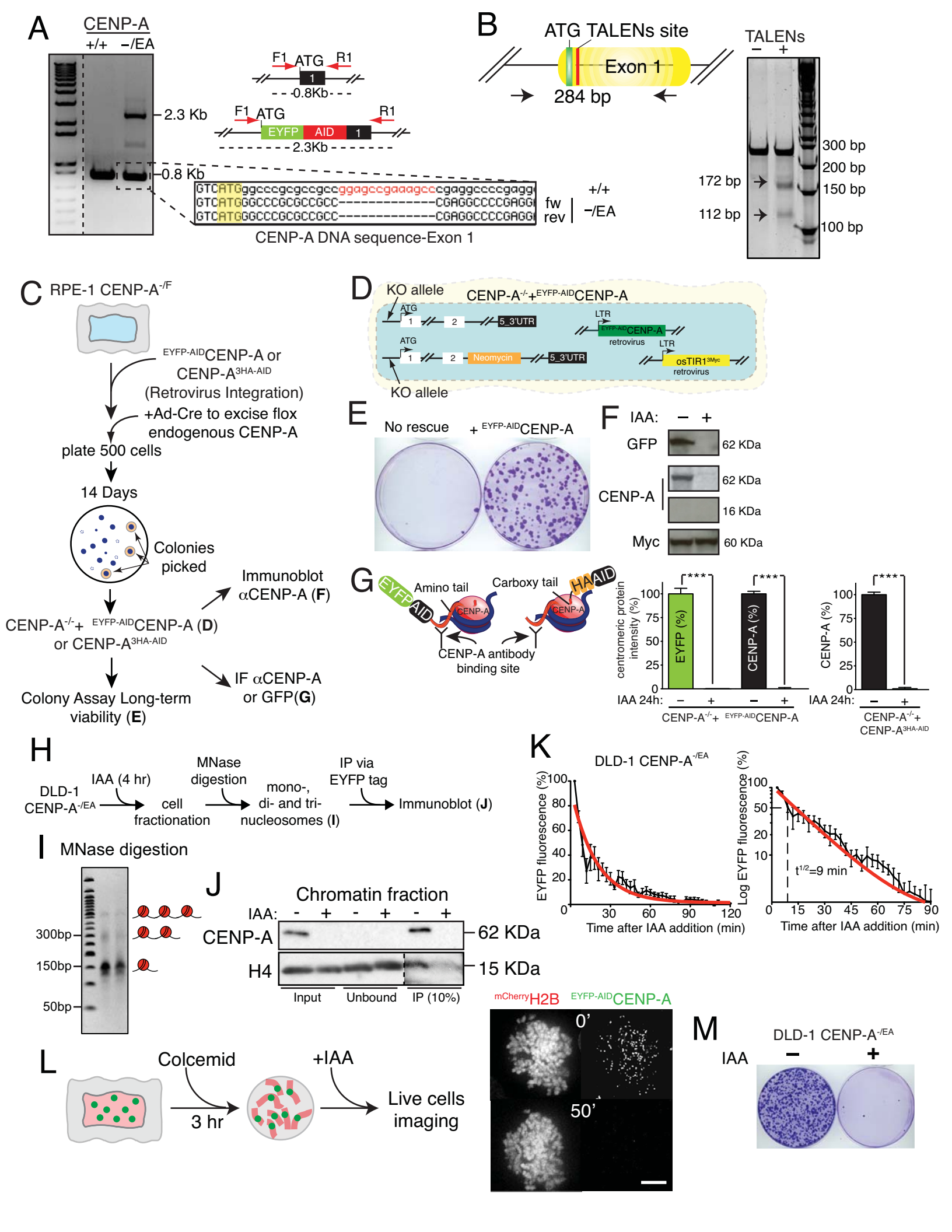
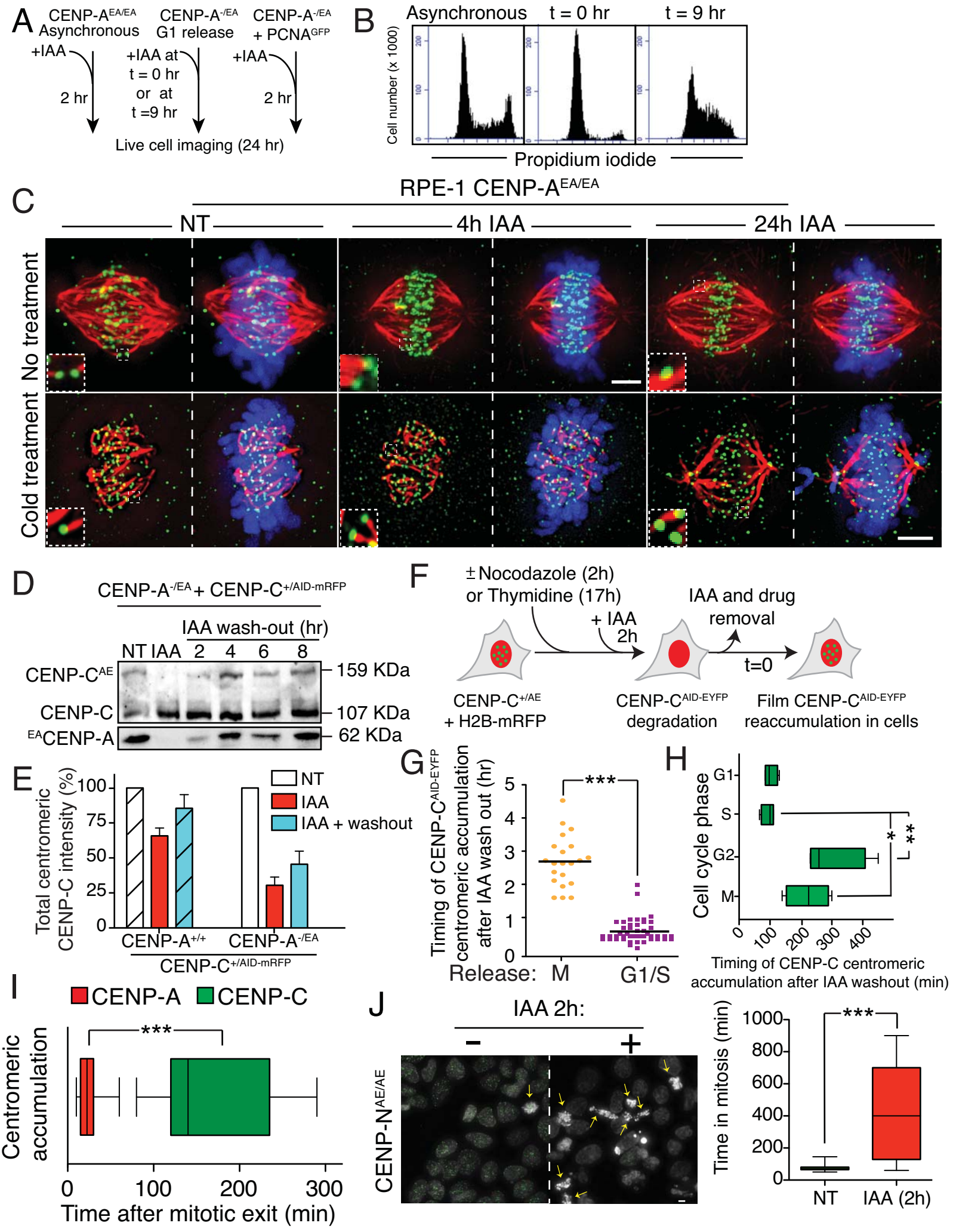
Supplementary Information

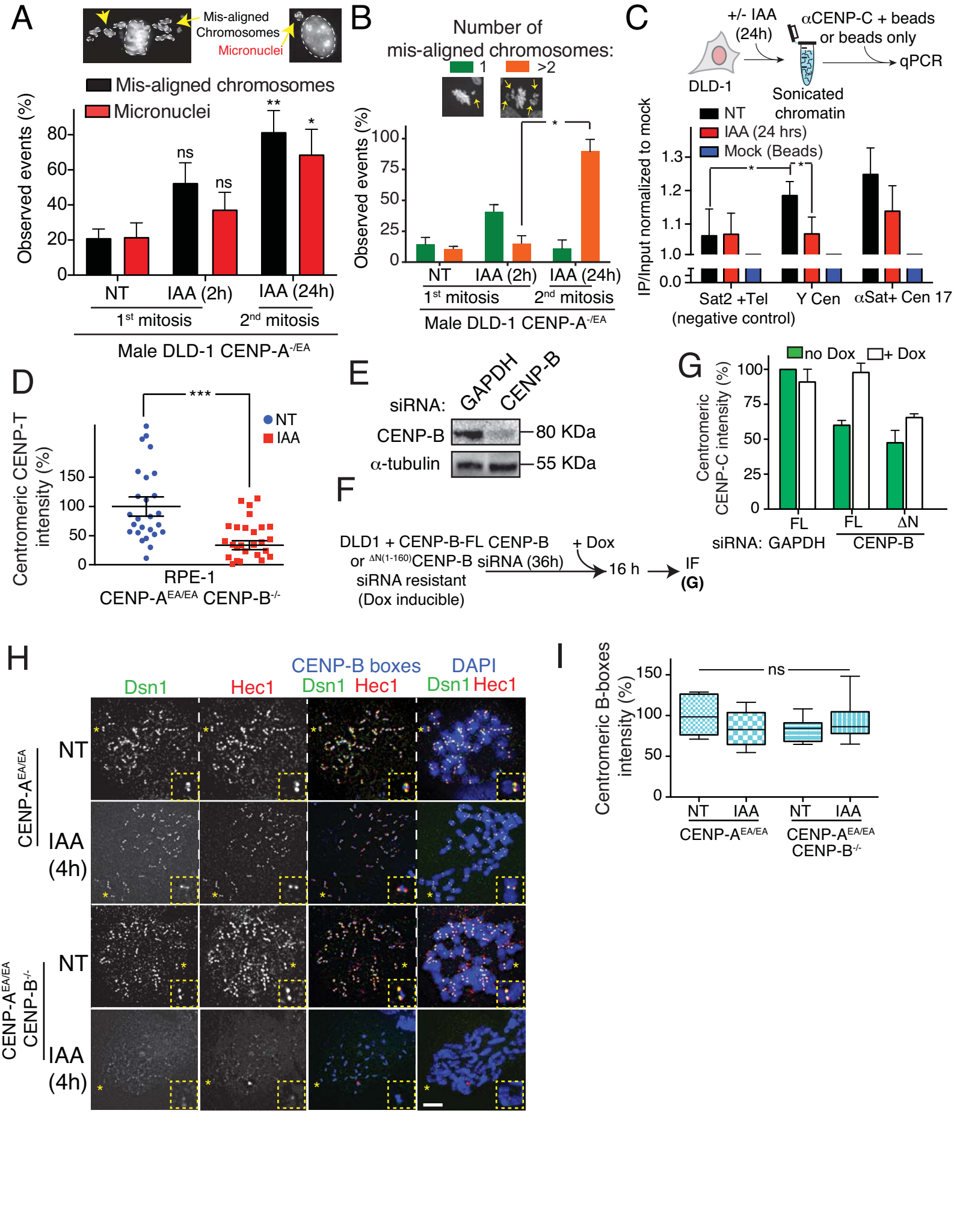

A
CENP-A
+/+ −/EA
2.3 Kb
0.8 Kb
F1 ATG R1
1
0.8Kb
F1 ATG R1
EYFP AID 1
2.3Kb
GTCATGggcccgcgccgccggagccgaaagcccgaggccccgaggg
GTCATGGGCCCGCGCCGCC----------CGAGGCCCCGAGGG
GTCATGGGCCCGCGCCGCC----------CGAGGCCCCGAGGG
fw
rev
+/+
−/EA
CENP-A DNA sequence-Exon 1
B
ATG TALENs site
Exon 1
284 bp
TALENs
− +
300 bp
200 bp
172 bp
150 bp
112 bp
100 bp
C
RPE-1 CENP-A-/F
EYFP-AIDCENP-A or
CENP-A3HA-AID
(Retrovirus Integration)
+Ad-Cre to excise flox
endogenous CENP-A
plate 500 cells
14 Days
Colonies
picked
Immunoblot
αCENP-A (F)
CENP-A-/-+ EYFP-AIDCENP-A (D)
or CENP-A3HA-AID
IF αCENP-A
or GFP(G)
Colony Assay Long-term
viability (E)
D
KO allele
CENP-A-/-+EYFP-AIDCENP-A
ATG
1
2
5_3'UTR
LTR
EYFP-AIDCENP-A
retrovirus
LTR
Neomycin
osTIR13Myc
retrovirus
KO allele
E
No rescue
+ EYFP-AIDCENP-A
F
IAA: − +
GFP
62 KDa
CENP-A
62 KDa
16 KDa
Myc
60 KDa
G
EYFP
AID
Amino tail
CENP-A
Carboxy tail
HA
AID
CENP-A
CENP-A antibody
binding site
centromeric protein
intensity (%)
EYFP (%)
CENP-A (%)
CENP-A (%)
***
IAA 24h: − + − +
CENP-A-/-+ EYFP-AIDCENP-A
IAA 24h: − +
CENP-A-/-+
CENP-A3HA-AID
H
DLD-1
CENP-A-/EA
IAA (4 hr)
cell
fractionation
MNase
digestion
mono-,
di- and tri-
nucleosomes (I)
IP via
EYFP tag
Immunoblot (J)
I
MNase digestion
300bp
150bp
50bp
J
Chromatin fraction
IAA: - + - + - +
CENP-A
62 KDa
H4
15 KDa
Input
Unbound
IP (10%)
K
DLD-1 CENP-A-/EA
EYFP fluorescence (%)
Time after IAA addition (min)
Log EYFP fluorescence (%)
t1/2=9 min
Time after IAA addition (min)
L
Colcemid
3 hr
+IAA
Live cells
imaging
mCherryH2B
EYFP-AIDCENP-A
0'
50'
M
DLD-1 CENP-A-/EA
IAA
− +

Supplementary Figure S1, related to Figure 1. CENP-A is rapidly and completely removed from chromatin following AID-tagging and IAA treatment. (A) DNA gel and relative DNA sequence showing CENP-A tagging or inactivation. (B) Schematic representation of the CENP-A exon 1 (yellow). Green denotes the ATG codon; red the TALENs recognition site. Arrows denote positions of the DNA primers used for PCR of the expected TALENs cleavage product. A DNA gel of a surveyor nuclease assay to identify TALENs cutting in the designed location is also shown. Arrows mark the expected band after TALENs cutting. (C) Schematic representation of the experiment shown in D-G (D) Schematic of the genotype of the indicated cell line. (E) Representative images of crystal violet–stained colonies from the colony formation assay in RPE-1 CENPA$^{-/F}$ cells following Ad-Cre expression to remove the endogenous CENP-A. (F) Immuno-blot to detect CENP-A targeting and degradation following IAA treatment. (G) (Left) Schematic of the CENP-A nucleosome tagged at the amino- or the carboxy- terminal tail. The binding site for the CENP-A antibody used in this study is also shown. (Right) Bar graphs represent CENP-A quantification using EYFP or CENP-A antibody in the indicated cell lines. Error bars represent the SEM of three independent experiments. Unpaired t test: *** $p < 0.0001$. (H) Schematic of the experiments shown in I, J. (I) Titration of MNase digestion time shows the ability to generate a pool of bulk soluble tri-, di- or mono-nucleosomes. (J) Immuno-blot on chromatin following treatment with IAA for 4 hours using CENP-A or H4 antibodies. A different exposure of the anti-H4 blot is shown for the IP lanes (bottom right). (K) Degradation kinetics of CENP-A in DLD-1 CENP-A$^{-/EA}$ cells following IAA treatment measured by EYFP intensity during live cell imaging on a linear (left) or log2 (right) graph. Red lines show the dose –response equation (variable slope). IAA was added at the

microscope stage. (L) (Left) Schematic representation of the experimental design. (Right) Representative images of CENP-A degradation in DLD-1 CENP-A$^{-/EA}$ cells following IAA treatment measured by EYFP intensity during live cell imaging after treatment with colcemid to block cells in mitosis. IAA was added at the microscope stage. (M) Representative images of crystal violet–stained colonies from the colony formation assay +/- IAA treatment in DLD-1 CENP-A$^{-/EA}$ cells. Scale bar = 5 μm.

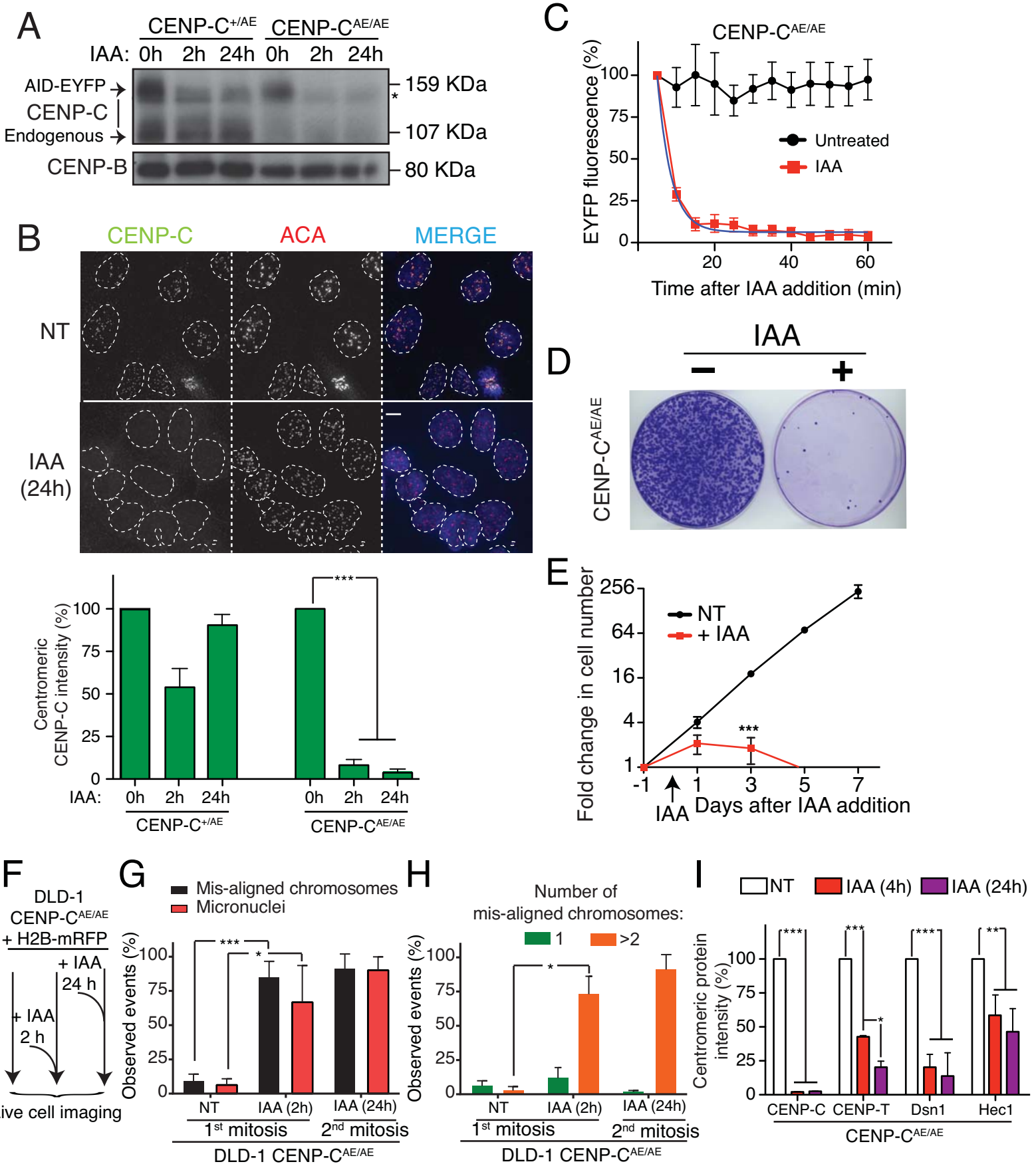

A
CENP-A EA/EA Asynchronous
+IAA
2 hr
CENP-A -/EA G1 release
+IAA at t = 0 hr or at t =9 hr
CENP-A -/EA + PCNA GFP
+IAA
2 hr
Live cell imaging (24 hr)
B
Asynchronous
t = 0 hr
t = 9 hr
Cell number (x 1000)
Propidium iodide
C
RPE-1 CENP-A EA/EA
NT
4h IAA
24h IAA
No treatment
Cold treatment
D
CENP-A -/EA + CENP-C +/AID-mRFP
IAA wash-out (hr)
NT IAA 2 4 6 8
CENP-C AE
CENP-C
EA CENP-A
159 KDa
107 KDa
62 KDa
E
Total centromeric CENP-C intensity (%)
NT
IAA
IAA + washout
CENP-A +/+
CENP-A -/EA
CENP-C +/AID-mRFP
F
± Nocodazole (2h) or Thymidine (17h)
+ IAA 2h
IAA and drug removal
t=0
CENP-C +/AE + H2B-mRFP
CENP-C AID-EYFP degradation
Film CENP-C AID-EYFP reaccumulation in cells
G
Timing of CENP-C AID-EYFP centromeric accumulation after IAA wash out (hr)
***
Release: M
G1/S
H
Cell cycle phase
G1
S
G2
M
*
**
Timing of CENP-C centromeric accumulation after IAA washout (min)
I
CENP-A
CENP-C
Centromeric accumulation
***
Time after mitotic exit (min)
J
IAA 2h:
-
+
CENP-N AE/AE
Time in mitosis (min)
***
NT
IAA (2h)

Supplementary Figure S2, related to Figure 2 and 3. CENP-C deposition at centromeres occurs following CENP-A loading as visualized by rapid removal and re-activation of endogenous CENP-C in human cells. (A) A schematic of the experiments shows in Figure 2D. (B) FACS analysis of the cell synchronization experiment described in 2D. (C) Representative images of the microtubules/kinetochore interactions following IAA (4h or 24h) and cold treatment (15 min). Microtubules are stained with α-tubulin and centromere with ACA (top images) or CENP-C (bottom images). (D) Immuno-blot of the experiment shown in Fig. 3A-C. (E) Bar graphs show centromere intensities of CENP-C in the indicated cell lines from the experiment shown in Fig. 3A-C. Error bars represent the SEM of three independent experiments. Individual Σn = ~30 cells, Σn = 25 centromeres for cell. (F) Schematic representation of the experiment shown in G and H. (G) Scatter plot graph showing the time required for CENP-C to be loaded at centromere measured after nocodazole (M) or thymidine (G1/S) release. Each individual point represents a single cell. *** $p < 0.0001$. (H) Box and whiskers graph shows distribution of CENP-C loading at different cell cycle phases of four independent experiments. * $p = 0.01$, **$p = 0.008$. (I) Box and whiskers graph shows differences in timing of $^{EYFP\text{-}AID}$CENP-A or CENP-C$^{AID\text{-}EYFP}$ loading at centromere following mitotic exit. *** $p < 0.0001$. n = 30 cells. (J) (left) Representative images of DLD-1 CENP-N$^{AE/AE}$ cells with or without treatment with IAA for 24 hours. Yellow arrows marked mitotic cells. Box and whiskers graph shows differences in mitotic timing following IAA addition. *** $p < 0.0001$. Σn = ~33 cells. Scale bars = 5 μm.


A
Mis-aligned Chromosomes
Micronuclei
Mis-aligned chromosomes
Micronuclei
Observed events (%)
ns
ns
**
*
NT
IAA (2h)
IAA (24h)
1st mitosis
2nd mitosis
Male DLD-1 CENP-A-/EA
B
Number of mis-aligned chromosomes:
1
>2
Observed events (%)
*
NT
IAA (2h)
IAA (24h)
1st mitosis
2nd mitosis
Male DLD-1 CENP-A-/EA
C
+/- IAA (24h)
αCENP-C + beads or beads only
qPCR
DLD-1
Sonicated chromatin
NT
IAA (24 hrs)
Mock (Beads)
IP/Input normalized to mock
Sat2 +Tel (negative control)
Y Cen
αSat+ Cen 17
D
***
NT
IAA
Centromeric CENP-T intensity (%)
RPE-1
CENP-AEA/EA CENP-B-/-
E
siRNA:
GAPDH
CENP-B
CENP-B
80 KDa
α-tubulin
55 KDa
F
DLD1 + CENP-B-FL CENP-B or ΔN(1-160)CENP-B siRNA resistant (Dox inducible)
siRNA (36h)
+ Dox
16 h
IF
(G)
G
no Dox
+ Dox
Centromeric CENP-C intensity (%)
FL
FL
ΔN
siRNA: GAPDH
CENP-B
H
Dsn1
Hec1
CENP-B boxes
Dsn1 Hec1
DAPI
Dsn1 Hec1
CENP-AEA/EA
NT
IAA (4h)
CENP-AEA/EA CENP-B-/-
NT
IAA (4h)
I
Centromeric B-boxes intensity (%)
ns
NT
IAA
NT
IAA
CENP-AEA/EA
CENP-AEA/EA CENP-B-/-

Supplementary Figure S3, related to Figure 4. CENP-B binding to DNA sequences is required to preserve CENP-C at centromeres. (A) Bar graph shows the percentage of chromosome mis-segregation events observed by live cell imaging of DLD-1 CENP-$A^{-/EA}$ cells in non-treated conditions or following IAA treatment for 2 or 24 hours, respectively. Error bars represent the SEM of three independent experiments. Individual Σn = ~45 cells. Representative images of the type of chromosome mis-segregation defects analyzed are also shown. Unpaired t test: * p = 0.03, ** p =0.004. (B) Bar graph shows the number (1 or >2) of mis-aligned chromosomes in percentage from analysis in A. Representative images of a single or several mis-aligned chromosomes are indicated by yellow arrows. Error bars represent the SEM of three independent experiments. Individual Σn = ~45 cells. Unpaired t test: * p = 0.03. (C) Bar graph shows the immuno-precipitation enrichment of CENP-C over the input (adjusted to 100%) at the indicated chromosomal regions (Sat2 +Tel = regions inside the satellite 2 and telomeric repeats, act as negative control; Y Cen = a region inside the Y centromere; Sat+Cen17 = a region inside the alpha satellite repeats combined with a region inside the centromere of chromosome 17). A schematic of the experimental design is also shown. Values are normalized relative to the mock (beads only). Unpaired t test: * p = 0.037, 0.012. (D) Quantification of CENP-T intensity in CENP-$A^{EA/EA}$ CENP-B KO cells. Each dot represents an average of 25 centromeres in a single cell. Unpaired t test: *** p = 0.0007 (E) Immuno-blot shows the level of CENP-B depletion achieved by siRNA for one experiment described in Figure 4 H-K. (F) Schematic of the experimental design shown in G. (G) Bar graphs show CENP-C intensity at centromere following CENP-B depletion by siRNA and rescue with siRNA-resistant CENP-B variants (FL is full length; N is lacking the CENP-B DNA binding site). Values represent the mean of two independent experiments.

Error bars represent the SEM (standard error of the mean). Individual Σn = ~30 cells, Σn = 25 centromeres for cell. (H) Representative images of IF-FISH on chromosome spreads stained with Dns1, Hec1 and with a CENP-B boxes FISH probe. (I) Box & whisker plots of CENP-B boxes intensities at the centromere measured on metaphase spreads used to show the homogeneity of the IF-FISH analysis. Scale bar = 5 μm.


A
CENP-C+/AE
CENP-CAE/AE
IAA: 0h 2h 24h 0h 2h 24h
AID-EYFP
CENP-C
Endogenous
159 KDa
107 KDa
CENP-B
80 KDa
B
CENP-C
ACA
MERGE
NT
IAA (24h)
Centromeric CENP-C intensity (%)
IAA:
CENP-C+/AE
CENP-CAE/AE
C
CENP-CAE/AE
EYFP fluorescence (%)
Untreated
IAA
Time after IAA addition (min)
D
IAA
CENP-CAE/AE
E
Fold change in cell number
NT
+ IAA
Days after IAA addition
F
DLD-1
CENP-CAE/AE
+ H2B-mRFP
+ IAA 24 h
+ IAA 2 h
Live cell imaging
G
Mis-aligned chromosomes
Micronuclei
Observed events (%)
NT
IAA (2h)
IAA (24h)
1st mitosis
2nd mitosis
DLD-1 CENP-CAE/AE
H
Number of mis-aligned chromosomes:
1
>2
Observed events (%)
NT
IAA (2h)
IAA (24h)
1st mitosis
2nd mitosis
DLD-1 CENP-CAE/AE
I
NT
IAA (4h)
IAA (24h)
Centromeric protein intensity (%)
CENP-C
CENP-T
Dsn1
Hec1
CENP-CAE/AE

Supplementary Figure S4, related to Figure 4. Rapid CENP-C depletion induces chromosome mis-segregation in the first mitosis. (A) Immuno-blot shows CENP-C targeting and degradation following IAA treatment for 2 or 24 hours. CENP-B was used as a loading control. The asterisk marks a non-specific band. (B) (Top) Representative immunofluorescence images of DLD-1 cells to monitor CENP-C depletion following treatment with IAA for 24 hours. ACA was used to mark centromere position. Scale bar = 5 μm. (Bottom) Bar graphs represent CENP-C quantification using a CENP-C antibody in the indicated cell lines. Error bars represent the SEM of three independent experiments. Unpaired t test: *** $p < 0.0001$. (C) Degradation kinetics of CENP-C in DLD-1 cells +/- IAA treatment measured by EYFP intensity during live cell imaging. IAA was added at the microscope stage. $\Sigma n = 10$ cells. (D) Representative images of crystal violet–stained colonies from the colony formation assay +/- IAA treatment in DLD-1 cells. (E) Cell counting experiment on DLD-1 cells +/- IAA treatment. IAA was added at day 0 and kept for a maximum of 7 days. Error bars represent the SEM of three independent experiments. Unpaired t test: *** $p = 0.0002$. (F) Schematic of the experiments shown in G-H. (G) Bar graph shows the percentage of chromosome mis-segregation events observed by live cell imaging in non-treated conditions or following IAA treatment for 2 or 24 hours, respectively. Error bars represent the SEM of three independent experiments. Individual $\Sigma n = \sim 37$ cells. Unpaired t test: *** $p = 0.0005$, * $p = 0.01$. (H) Bar graph shows the number (1 or >2) of mis-aligned chromosomes in percentage from analysis in F. Error bars represent the SEM of three independent experiments. * $p = 0.01$. (I) Bar graphs showing centromere intensities of CENP-C, CENP-T, Dsn1 and Hec1 for the indicated cell line following IAA treatment. Values represent the mean of three independent experiments.

Error bars represent the SEM (standard error of the mean). Individual Σn = ~30 cells, Σn = 25 centromeres for cell. Unpaired t test: * p = 0.01, ** p = 0.08, *** p < 0.0001.

Supplementary Movie S1, related to Figure 1. Rapid CENP-A depletion following IAA treatment. DLD-1 cells were treated with IAA and immediately filmed every 3 minutes. Green is CENP-A, red is H2B. Time (in minutes) is indicated in yellow.

Supplementary Movies S2, related to Figure 4. Rapid CENP-A depletion following IAA treatment causes mitotic defects only in siRNA-depleted CENP-B cells. DLD-1 cells were treated with IAA for 2 hours and then filmed every 5 minutes following siRNA against GAPDH for 48 hours. Time (in minutes) is indicated in white.

Supplementary Movies S3, related to Figure 4. Rapid CENP-A depletion following IAA treatment causes mitotic defects only in siRNA-depleted CENP-B cells. DLD-1 cells were treated with IAA for 2 hours and then filmed every 5 minutes following siRNA against CENP-B for 48 hours. Time (in minutes) is indicated in white.