Cell Reports, Volume *42*

Supplemental information

Cyclin E/CDK2 and feedback from soluble histone protein regulate the S phase burst of histone biosynthesis

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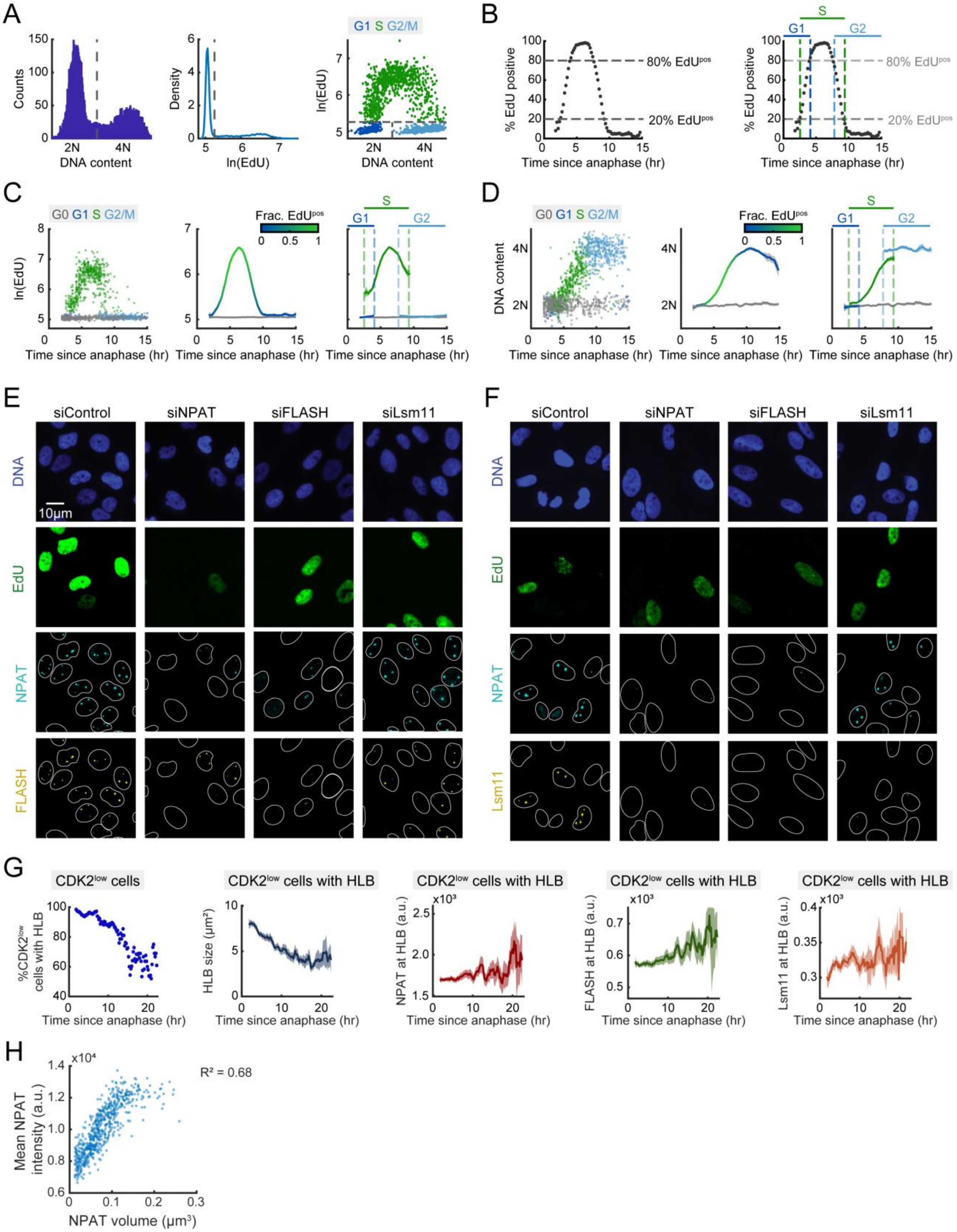

Figure S1. Determination of cell-cycle phase, representative images of cells treated with siControl, siNPAT, siFLASH, and siLsm11, and HLB quantification in CDK2^{low} cells, related to Figure 1-2. (A)

Column 1: Histogram of DNA content based on Hoestch signal of all cells in the population, with cutoff for 2N vs 4N DNA content being determined by local minima. Column 2: ksdensity plot of EdU signal for all cells in the population, with cutoffs for EdU$^{\text{neg}}$ vs. EdU$^{\text{pos}}$ being determined by local minima. Column 3: Representative scatter of EdU vs. DNA content in CDK2$^{\text{inc}}$ cells following timelapse imaging segmented by cell-cycle phase. (B) Column 1: Percent EdU$^{\text{pos}}$ cells at each timepoint for CDK2$^{\text{inc}}$ population aligned to anaphase. Column 2: Cutoff of timing for each cell-cycle phase, where G1 cells are plotted from anaphase to the timepoint when 80% of cells are EdU$^{\text{pos}}$; S cells from the timepoint when 20% of cells are EdU$^{\text{pos}}$ until the timepoint when 80% of cells are EdU$^{\text{neg}}$; and G2 cells between the timepoint when 20% of cells are EdU$^{\text{neg}}$ until the end of the cell cycle. (C-D) Column 1: Raw single-cell data. Column 2: Average EdU (C) or DNA content (D) signal and 95% confidence intervals as a function of time since anaphase for CDK2$^{\text{inc}}$ and CDK2$^{\text{low}}$ populations. Column 3: CDK2$^{\text{inc}}$ population from column 2 segmented into G1 cells defined as 2N DNA content and EdU$^{\text{neg}}$, S cells defined as EdU$^{\text{pos}}$, and G2/M populations defined as 4N DNA content and EdU$^{\text{neg}}$. (E-F) Representative images of cells treated with 25nM of siControl, siNPAT, siFLASH, and siLsm11 for 48 hr prior to fixation with live-cell imaging in the final 18 hr, stained for DNA, EdU, NPAT, and either FLASH (E) or Lsm11 (F) (see Figure 1E). (G) Column 1: Percent of CDK2$^{\text{low}}$ cells with HLB puncta as a function of time spent out of the cell cycle. Column 2-5: Average HLB size (2) and average HLB signal for NPAT (3), FLASH (4), or Lsm11 (5) with 95% confidence interval in CDK2$^{\text{low}}$ cells with HLBs. (H) Scatter of mean NPAT intensity vs NPAT volume.

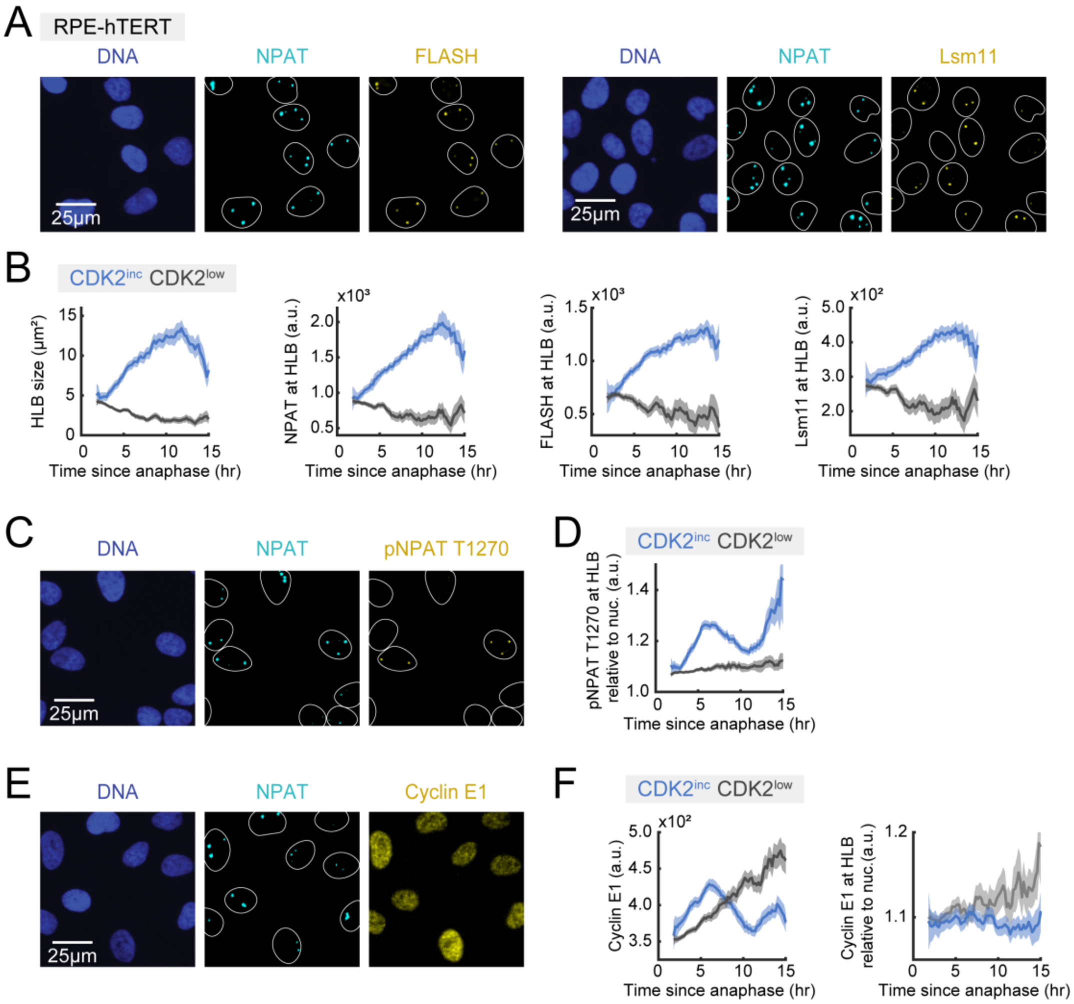

Figure S2. HLB formation and NPAT phosphorylation in RPE-hTERT cells related to Figure 1-3. (A) Representative images of cells following timelapse imaging, stained for DNA content, NPAT and either FLASH or Lsm11. (B) Average of the total HLB size per cell, or protein signal at the HLB, and 95% confidence intervals as a function of time since anaphase for CDK2^{inc} and CDK2^{low} populations. (C) Representative images of cells following timelapse imaging, stained for DNA content, NPAT and pNPAT T1270. (D) Average HLB signal for pNPAT T1270 normalized to nuclear signal for CDK2^{inc} and CDK2^{low} populations. (E) Representative images of cells following timelapse imaging, stained for DNA content, NPAT and Cyclin E1. (F) Average nuclear signal for Cyclin E1 (left) and average HLB signal for Cyclin E1 normalized to nuclear signal (right) and 95% confidence intervals as a function of time since anaphase for CDK2^{inc} and CDK2^{low} populations.

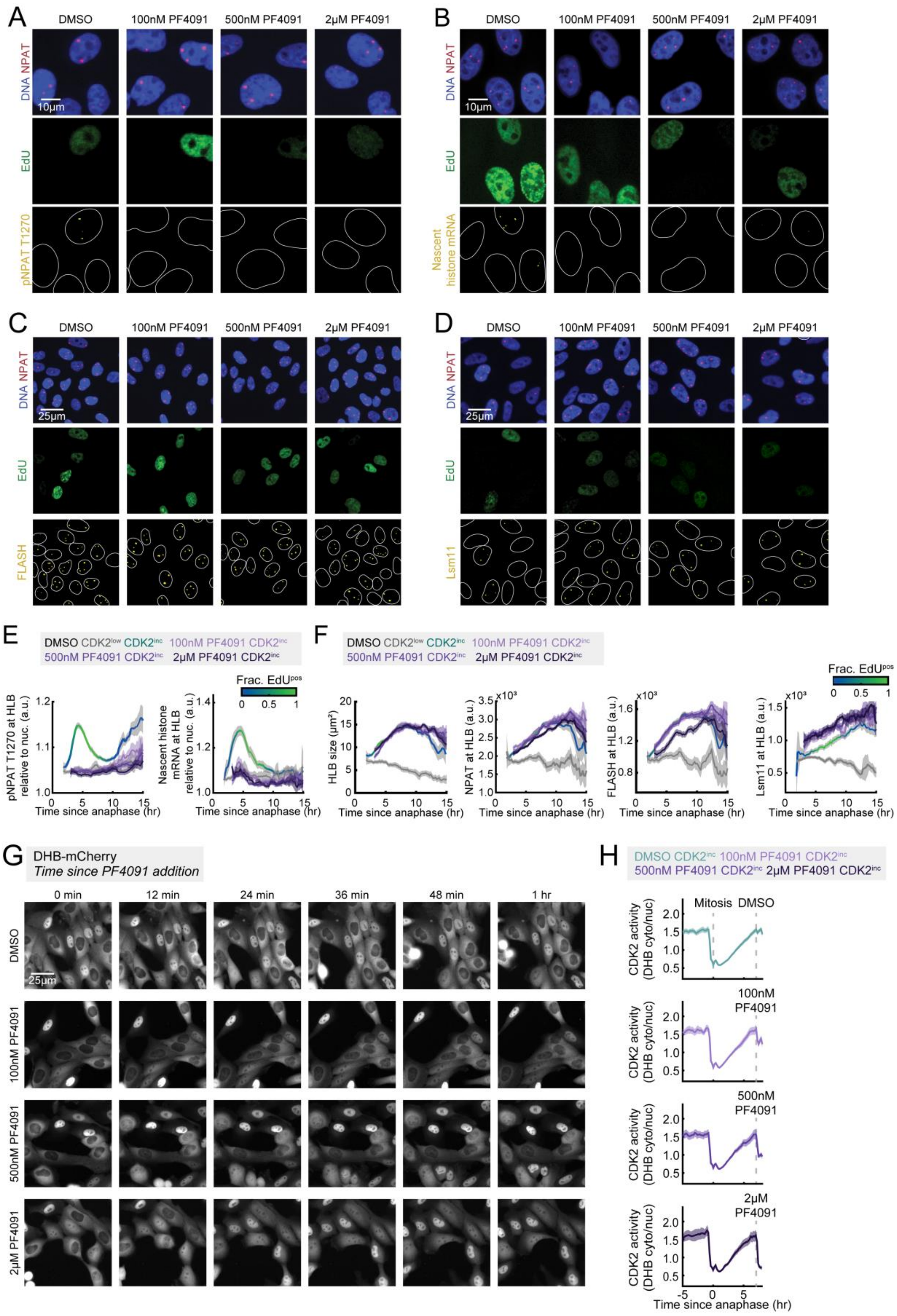
A
DMSO
100nM PF4091
500nM PF4091
2μM PF4091
DNA NPAT
10μm
EdU
pNPAT T1270
B
DMSO
100nM PF4091
500nM PF4091
2μM PF4091
DNA NPAT
10μm
EdU
Nascent histone mRNA
C
DMSO
100nM PF4091
500nM PF4091
2μM PF4091
DNA NPAT
25μm
EdU
FLASH
D
DMSO
100nM PF4091
500nM PF4091
2μM PF4091
DNA NPAT
25μm
EdU
Lsm11
E
DMSO CDK2^low CDK2^inc 100nM PF4091 CDK2^inc
500nM PF4091 CDK2^inc 2μM PF4091 CDK2^inc
Frac. EdU^pos
0 0.5 1
pNPAT T1270 at HLB relative to nuc. (a.u.)
Nascent histone mRNA at HLB relative to nuc. (a.u.)
Time since anaphase (hr)
F
DMSO CDK2^low CDK2^inc 100nM PF4091 CDK2^inc
500nM PF4091 CDK2^inc 2μM PF4091 CDK2^inc
Frac. EdU^pos
0 0.5 1
HLB size (μm²)
NPAT at HLB (a.u.)
FLASH at HLB (a.u.)
Lsm11 at HLB (a.u.)
x10³
Time since anaphase (hr)
G
DHB-mCherry
Time since PF4091 addition
0 min
12 min
24 min
36 min
48 min
1 hr
DMSO
100nM PF4091
500nM PF4091
2μM PF4091
25μm
H
DMSO CDK2^inc 100nM PF4091 CDK2^inc
500nM PF4091 CDK2^inc 2μM PF4091 CDK2^inc
Mitosis
DMSO
100nM PF4091
500nM PF4091
2μM PF4091
CDK2 activity (DHB cyto/nuc)
Time since anaphase (hr)

Figure S3. Representative images and additional data on PF4091 treatment, related to Figure 3. (A-D) Representative images of cells treated with 100nM, 500nM, and 2µM of PF-07104091 (PF4091) for the last 1 hr of live-cell imaging, before staining for DNA, EdU, NPAT, and either pNPAT T1270 (A), nascent histone mRNA (B), FLASH (C) or Lsm11 (D). (E) Average HLB signal for pNPAT T1270 and nascent histone mRNA FISH normalized to nuclear signal for $\mathrm{CDK2}^{\mathrm{inc}}$ and $\mathrm{CDK2}^{\mathrm{low}}$ populations for cells treated with DMSO, and $\mathrm{CDK2}^{\mathrm{inc}}$ population for cells treated with 100nM, 500nM, or 2µM PF-07104091 (PF4091) for the last 1 hr of live-cell imaging. (F) Average HLB size and average HLB signal with 95% confidence interval for NPAT, FLASH, and Lsm11 for $\mathrm{CDK2}^{\mathrm{inc}}$ and $\mathrm{CDK2}^{\mathrm{low}}$ populations for cells treated with DMSO, and $\mathrm{CDK2}^{\mathrm{inc}}$ population for cells treated with 100nM, 500nM, or 2µM PF-07104091 (PF4091) in the for the last 1 hr of live-cell imaging. (G) Representative images of the final 1 hr of live-cell imaging of the CDK2 activity sensor in cells treated with 100nM, 500nM, or 2µM PF-07104091 (PF4091). (H) Average CDK2 activity and 95% confidence interval of $\mathrm{CDK2}^{\mathrm{inc}}$ cells aligned to anaphase that completed their last mitosis 10 hr before the final frame of the movie, with drug added in the final 1 hr of imaging (see Figure 3D, 3F).

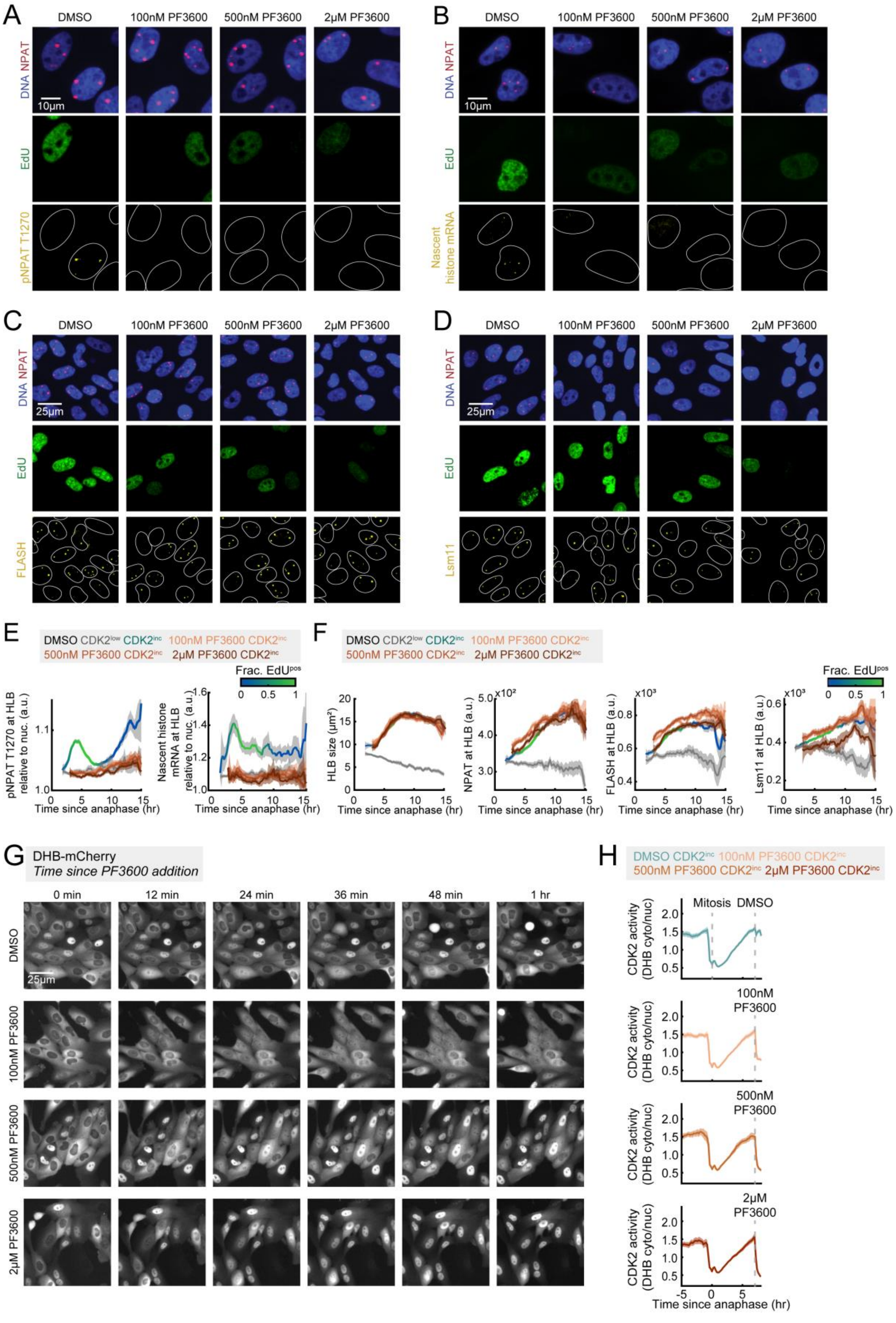
A
DMSO
100nM PF3600
500nM PF3600
2μM PF3600
DNA NPAT
10μm
EdU
pNPAT T1270
B
DMSO
100nM PF3600
500nM PF3600
2μM PF3600
DNA NPAT
10μm
EdU
Nascent histone mRNA
C
DMSO
100nM PF3600
500nM PF3600
2μM PF3600
DNA NPAT
25μm
EdU
FLASH
D
DMSO
100nM PF3600
500nM PF3600
2μM PF3600
DNA NPAT
25μm
EdU
Lsm11
E
DMSO CDK2low CDK2inc 100nM PF3600 CDK2inc
500nM PF3600 CDK2inc 2μM PF3600 CDK2inc
Frac. EdUpos
0 0.5 1
pNPAT T1270 at HLB relative to nuc. (a.u.)
1.0
1.1
Nascent histone mRNA at HLB relative to nuc. (a.u.)
1.0
1.2
1.4
1.6
0 5 10 15
Time since anaphase (hr)
F
DMSO CDK2low CDK2inc 100nM PF3600 CDK2inc
500nM PF3600 CDK2inc 2μM PF3600 CDK2inc
Frac. EdUpos
0 0.5 1
HLB size (μm²)
0 5 10 15 20
NPAT at HLB (a.u.)
x10²
3.0 4.0 5.0
FLASH at HLB (a.u.)
x10³
0.4 0.6 0.8
Lsm11 at HLB (a.u.)
x10³
0.2 0.4 0.6
0 5 10 15
Time since anaphase (hr)
G
DHB-mCherry
Time since PF3600 addition
0 min
12 min
24 min
36 min
48 min
1 hr
DMSO
25μm
100nM PF3600
500nM PF3600
2μM PF3600
H
DMSO CDK2inc 100nM PF3600 CDK2inc
500nM PF3600 CDK2inc 2μM PF3600 CDK2inc
Mitosis
DMSO
100nM PF3600
500nM PF3600
2μM PF3600
CDK2 activity (DHB cyto/nuc)
0.5 1.0 1.5 2.0
-5 0 5
Time since anaphase (hr)

Figure S4. DHB sensor translocation during PF3600 treatment, related to Figure 3. (A-D) Representative images of cells treated with 100nM, 500nM, and 2µM of PF-06873600 (PF3600) for the last 1 hr of live-cell imaging, before staining for DNA, EdU, NPAT, and either pNPAT T1270 (A), nascent histone mRNA (B), FLASH (C) or Lsm11 (D). (E) Average HLB signal for pNPAT T1270 and nascent histone mRNA FISH normalized to nuclear signal for $CDK2^{inc}$ and $CDK2^{low}$ populations for cells treated with DMSO, and $CDK2^{inc}$ population for cells treated with 100nM, 500nM, or 2µM PF-06873600 (PF3600) for the last 1 hr of live-cell imaging. (F) Average HLB size and average HLB signal with 95% confidence interval for NPAT, FLASH, and Lsm11 for $CDK2^{inc}$ and $CDK2^{low}$ populations for cells treated with DMSO, and $CDK2^{inc}$ population for cells treated with 100nM, 500nM, or 2µM PF-06873600 (PF3600) in the for the last 1 hr of live-cell imaging. (G) Representative images of the final 1 hr of live-cell imaging of the CDK2 activity sensor in cells treated with 100nM, 500nM, or 2µM PF-06873600 (PF3600). (H) Average CDK2 activity and 95% confidence interval of $CDK2^{inc}$ cells aligned to anaphase that completed their last mitosis 10 hr before the final frame of the movie, with drug added in the final 1 hr of imaging (see Figure 3E, 3G).

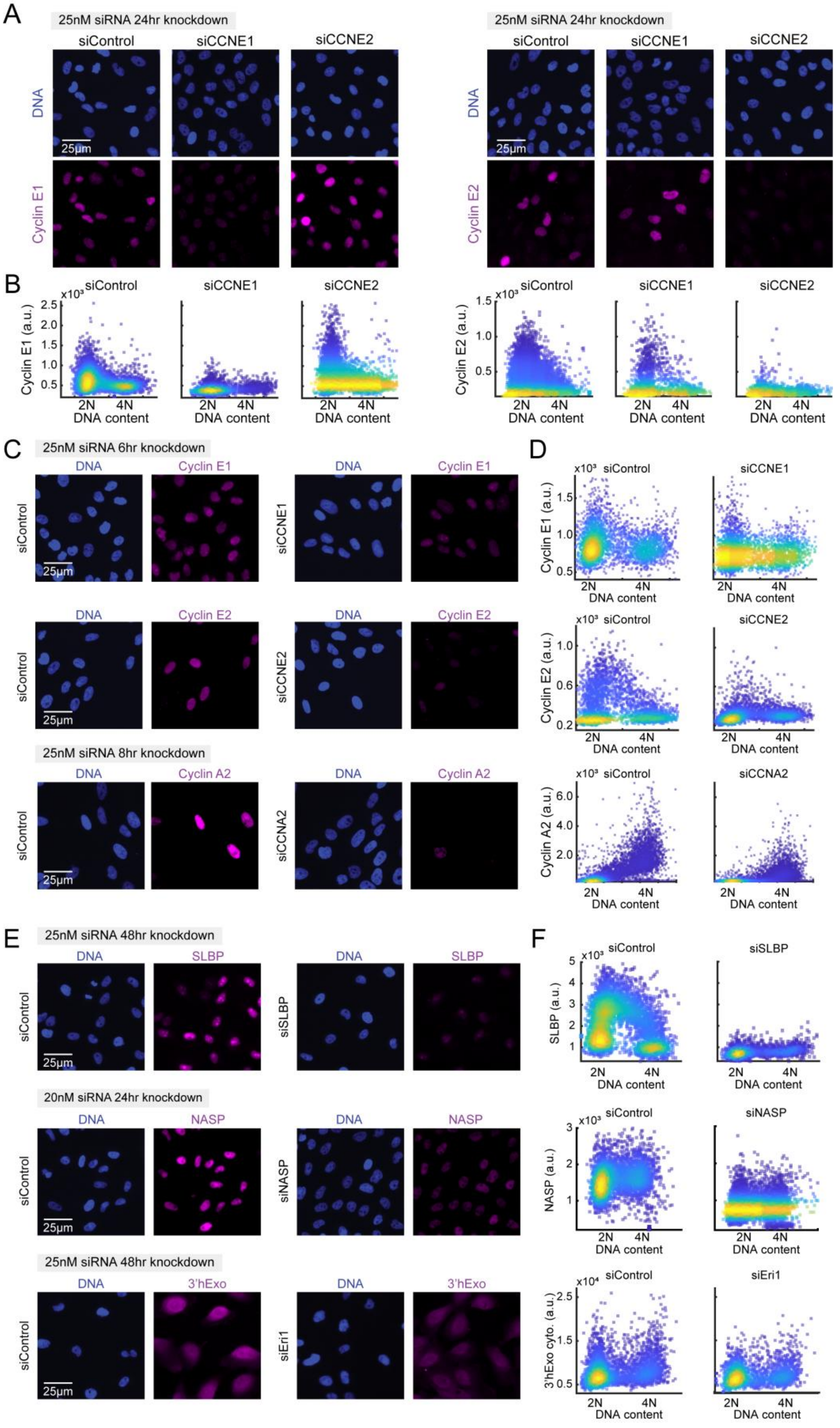
A
25nM siRNA 24hr knockdown
siControl
siCCNE1
siCCNE2
DNA
25µm
Cyclin E1
25nM siRNA 24hr knockdown
siControl
siCCNE1
siCCNE2
DNA
25µm
Cyclin E2
B
x10³
siControl
siCCNE1
siCCNE2
Cyclin E1 (a.u.)
2.5
2.0
1.5
1.0
0.5
2N
4N
DNA content
x10³
siControl
siCCNE1
siCCNE2
Cyclin E2 (a.u.)
1.5
1.0
0.5
2N
4N
DNA content
C
25nM siRNA 6hr knockdown
DNA
Cyclin E1
siControl
25µm
siCCNE1
DNA
Cyclin E2
siControl
25µm
siCCNE2
25nM siRNA 8hr knockdown
DNA
Cyclin A2
siControl
25µm
siCCNA2
D
x10³
siControl
siCCNE1
Cyclin E1 (a.u.)
1.5
1.0
0.5
2N
4N
DNA content
x10³
siControl
siCCNE2
Cyclin E2 (a.u.)
1.0
0.6
0.2
2N
4N
DNA content
x10³
siControl
siCCNA2
Cyclin A2 (a.u.)
6.0
4.0
2.0
2N
4N
DNA content
E
25nM siRNA 48hr knockdown
DNA
SLBP
siControl
25µm
siSLBP
20nM siRNA 24hr knockdown
DNA
NASP
siControl
25µm
siNASP
25nM siRNA 48hr knockdown
DNA
3'hExo
siControl
25µm
siEri1
F
x10³
siControl
siSLBP
SLBP (a.u.)
5
4
3
2
1
2N
4N
DNA content
x10³
siControl
siNASP
NASP (a.u.)
3
2
1
2N
4N
DNA content
x10⁴
siControl
siEri1
3'hExo cyto. (a.u.)
2.5
1.5
0.5
2N
4N
DNA content

Figure S5. Validation of antibodies and siRNA knockdowns, related to Figure 4-6. (A-B) Representative images (A) and single cells plotted as either Cyclin E1 or Cyclin E2 (B) in cells treated with siControl, siCCNE1, or siCCNE2 (see Figure 4A-C). (C-D) Representative images (C) and single cells plotted as Cyclin E1, Cyclin E2, or Cyclin A2 vs. DNA content (D) for cells treated with siControl, siCCNE1, or siCCNE2 for 6 hr, or siControl or siCCNA2 for 8 hr (see Figure 4E). (E-F) Representative images (E) and single cells plotted as SLBP, NASP, or 3'hExo vs. DNA content (F) in cells treated with siControl and either siSLBP, siNASP, or siEri1 (see Figure 5A, 6B-G).

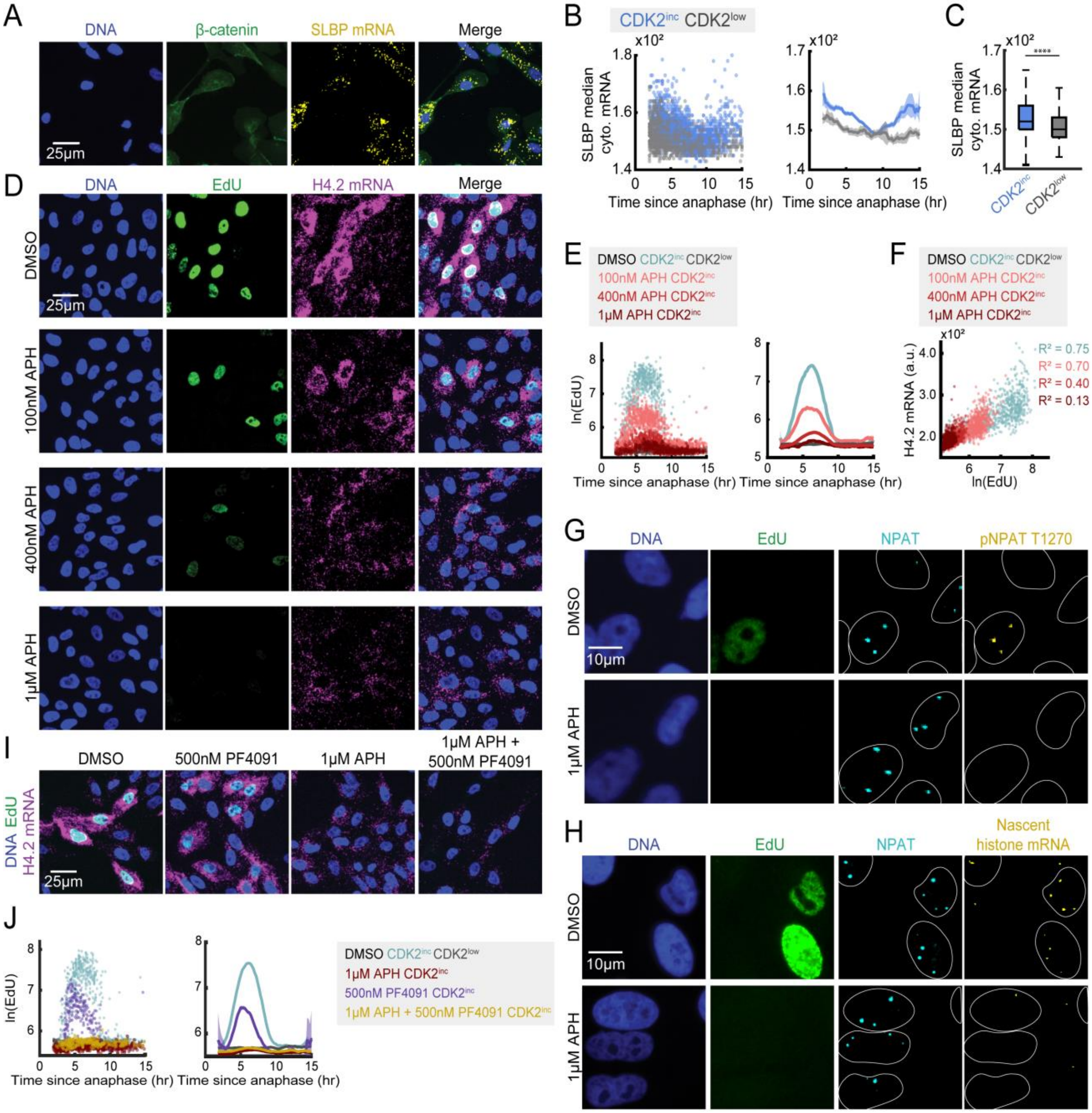

Figure S6. SLBP expression and EdU incorporation upon DNA synthesis and CDK2 inhibition, related to Figure 5. (A) Representative images of cells following live cell imaging, stained for DNA, β-catenin as a cytoplasmic marker, and SLBP mRNA. (B) Column 1: Raw single-cell data for CDK2^{inc} and CDK2^{low} populations. Column 2: Average cytoplasmic signal for SLBP mRNA and 95% confidence interval as a function of time since anaphase for data in column 1. (C) Median for CDK2^{inc} vs CDK2^{low} cells of SLBP mRNA. p-values are indicated as four stars $p \leq 0.0001$. (D) Representative images of cells treated with DMSO, 100nM, 400nM, or 1µM APH during the last 1 hr of live-cell imaging, before staining for DNA, EdU, and histone H4.2 mRNA. (E) Column 1: Raw single-cell data. Column 2: Average EdU signal and 95% confidence intervals as a function of time since anaphase for CDK2^{inc} and CDK2^{low} cells treated with DMSO, as well as CDK2^{inc} cells treated with 100nM, 400nM, or 1µM APH (see Figure 5E). (F) Representative scatter of histone H4.2 mRNA vs. EdU of CDK2^{inc} cells for cells treated with DMSO, 100nM APH, 400nM APH, or 1µM APH, with the R-squared value reported from linear regression. (G-H) Representative images of cells treated with DMSO or 1µM APH in the last 1 hr of imaging, followed by staining for DNA, EdU, NPAT and nascent histone RNA FISH (G) or pNPAT T1270 (H). (I) Representative images of cells treated with DMSO, 1µM APH, 500nM PF-07104091 (PF4091), or a combination of 1µM APH and 500nM PF-07104091 (PF4091). (J) Column 1: Raw single-cell data. Column 2: Average EdU signal and 95% confidence intervals as a function of time since anaphase for CDK2^{inc} and CDK2^{low} cells treated with DMSO, as well as CDK2^{inc} cells treated with 1µM APH, 500nM PF-07104091 (PF4091), or a combination of 1µM APH and 500nM PF-07104091 (PF4091) (see Figure 5G).

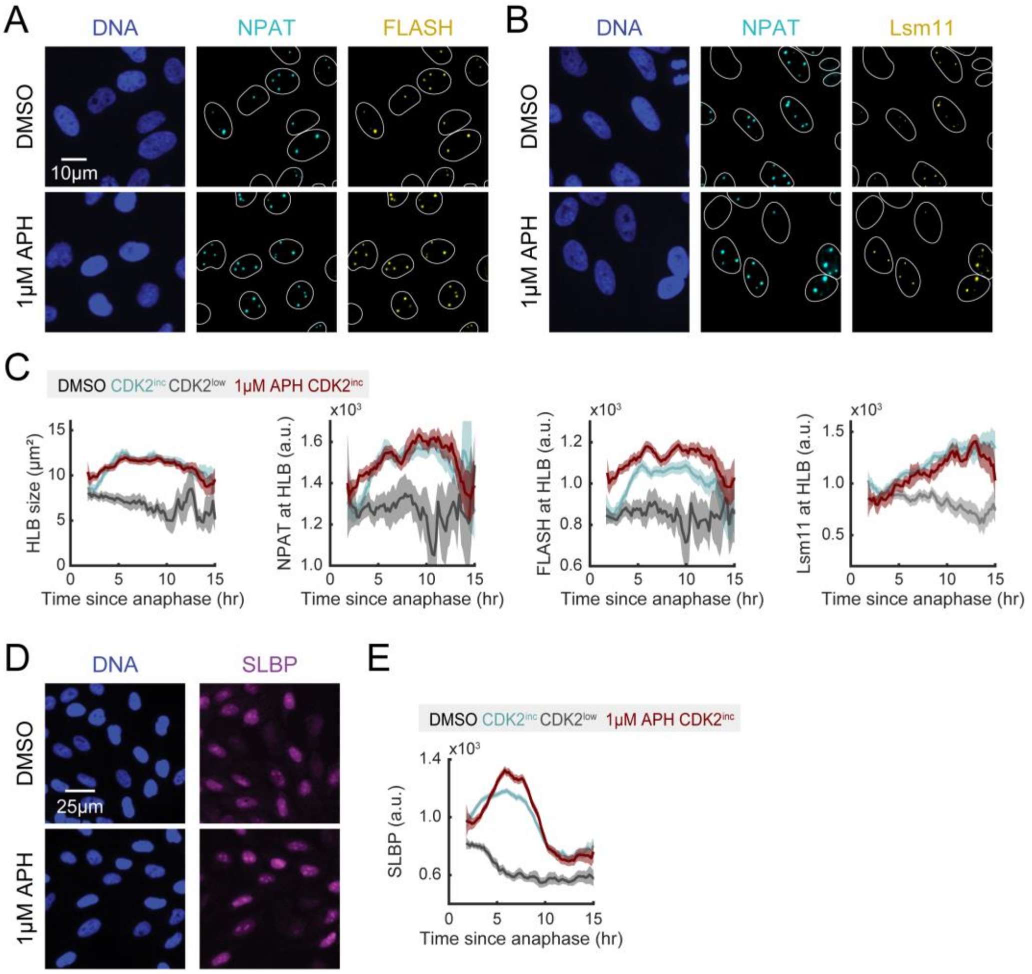

Figure S7. HLB formation and SLBP levels upon DNA synthesis inhibition, related to Figure 5. (A-B) Representative images of cells treated with DMSO or 1µM APH in the last 1 hr of imaging, followed by staining for DNA, NPAT, and FLASH (A) or Lsm11 (B). (C) Column 1-4: Average HLB size (1) and signal at the HLB for NPAT (2), FLASH (3), and Lsm11 (4) in CDK2$^{\text{inc}}$ and CDK2$^{\text{low}}$ cells treated with DMSO for 1 hr before fixation, with CDK2$^{\text{inc}}$ cells plotted for cells treated with 1µM APH for 1 hr before fixation. (D) Representative images of cells treated with DMSO or 1µM APH in the last 1 hr of imaging, followed by staining for SLBP. (E) Average nuclear signal for SLBP in CDK2$^{\text{inc}}$ and CDK2$^{\text{low}}$ cells treated with DMSO, and CDK2$^{\text{inc}}$ cells plotted for cells treated with 1µM APH for 1 hr before fixation.

Gene	Probe sequence (reverse complement)	Position relative to the cleavage site	Gene	Probe sequence (reverse complement)	Position relative to the cleavage site
HIST1			H3C11	Agcttccttttggtgacaat	position 1
H1-1	Agctcttttcctgaaatgcg	position 1	H3C12	Cagctatcctcagtgagaat	position 2
	Ggggatttcatctgtgtact	position 23		Aagacagtcgaccaatggtg	position 25
H1-5	Agccatttttagaggtctc	position 1	H4C1	Cagctcttcttttgtttagt	position 1
	Cctgatctaacatgttgtgc	position 23	H4C3	Caacgcatttcagctcttta	position 11
H1-2	Ccagctcttttattgagatc	position 3	H4C4	Tactctttctcctgagtgga	position 1
H1-3	Cttcagctcttttgactgag	position 6		Ctacacttttagcagtgact	position 23
H1-4	Acagctcttttactgagagc	position 3	H4C5	Aagtcatgagctcttttgcg	position 9
H2AC1	Acgttttgtaacgtacagcc	position 15	H4C6	Cttcgtgtgacgaaggaggt	position 1
H2AC4	Tccaaagcacaacagctcat	position 13	H4C7	Gagcccttcggaagaaaacg	position 1
H2AC6	Tacaagcagtgaggttagct	position 16	H4C11	Accactcttcagctgagaaa	position 2
H2AC7	Atgtgagcccttttaaggaa	position 5	H4C12	Cggctcctcagctgagaaag	position 1
H2AC8	Gatttcaggcatcgtatgtg	position 25	H4C13	Tagtctacagctctttcaca	position 8
H2AC12	Tttctctaagacagtgtaga	position 2	HIST2		
	Aacaaacatgactgtgtcca	position 24	H2AC21	Cagcccttcatttgacactt	position 1
H2AC13	Tgctcagttcttccacaaaa	position 4		Agctaatgcgctatttcgtg	position 23
H2AC14	Ggactcgtgtcatcttttta	position 8		Aaaggctctttttagagcca	position 62
H2AC15	Acacagctctacttggtgaa	position 5	H2AC20	Acgaaatagcgcattagctc	position 15
H2AC16	Cagctctcccgcagaaatag	position 1		Agtgtcaaatgaagggctga	position 37
	Gttccaaatgtttaaccagt	position 25		Aaggctcttttaagagccac	position 60
H2AC17	Cagttcttccgtagaaatgg	position 1	H2BC18	Acagctgctttctcgattac	position 1
	Taggtttggagaacagagtg	position 23		Tcaaacgctctgacaagtgt	position 23
H2BC1	Cacagctgtttcagtatgtt	position 1		Actccctacctgaccaaaag	position 46
H2BC3	Tatagctctccttgtgacaa	position 3		Cgaatgcatccgtgaccatg	position 71
H2BC6	Catgtcagtatatagctcca	position 13	H2BC21	Gctcttttctagtgattagg	position 1
H2BC7	Atgcaggtaattacaactct	position 14		Ccatgaccttcacactaaaa	position 63
H2BC10	Ccaatgccaagtctggaaaa	position 24		Aaaaagctacgtatgccatt	position 85
H2BC12	Gtttacagctctaatatcga	position 6	H3C15	Ggtacagcttcttccaagtg	position 7
H2BC13	Cccagtgataggaagagcga	position 24		Tctaatgccggaggagcacg	position 37
H2BC14	Agtgaactggctctttcttg	position 8		Cgttttgttgggagcctaag	position 75
H2BC17	Aaatgcacaggctctttctc	position 8	H3C13	Gtacagctgctcttgatgaa	position 7
H3C1	Ggtttcttacagctacttta	position 4		Gacgcacatcagatggagag	position 30
H3C2	Acagctactttcgttggaat	position 1		Tttgggttctgtaacgtcac	position 91
H3C3	Accgaatgggttacaactgt	position 16	H4C14	Caactcctcagatgagcaag	position 1
H3C6	Caaaggattacagcccttttt	position 12		Tactgttgcactttacgcag	position 30
H3C7	Acggcgctttcaactgaaat	position 5		Ccaaagttaccttccaacat	position 53
H3C8	Cacagctattttcgggtgaa	position 3		Tttccgttaacttcagatcc	position 89
H3C10	Aaacagatcttttcagcgcg	position 5		Cgacttaagtctttacggct	position 133

Supplementary Table S1. Nascent histone mRNA probe set. Related to Key Resources Table.