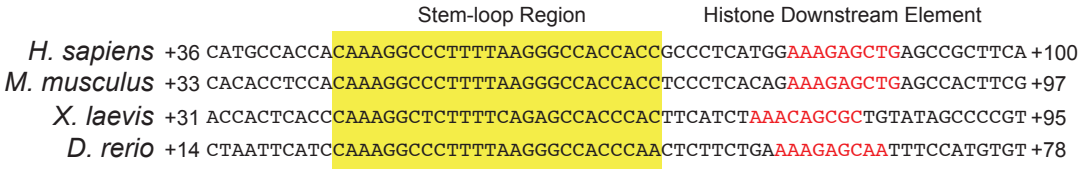
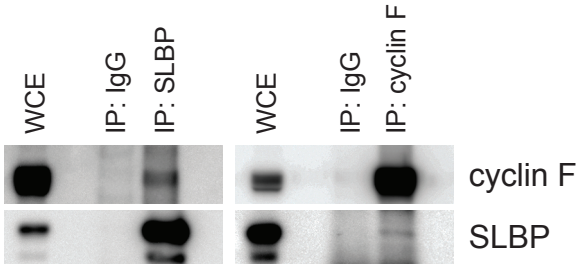
Figure S1

A

H2AFX Gene



B



C

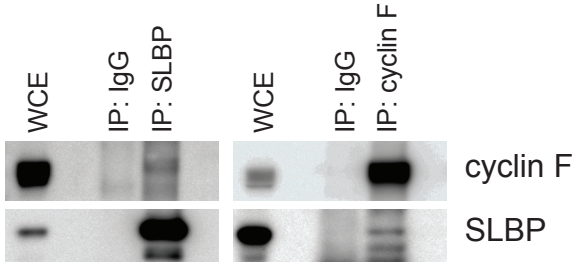


Figure S1. SLBP specifically interacts with cyclin F, related to Figure 1.

(a) Alignment of the 3' UTR region of *H2AFX* in vertebrates with the stem-loop region highlighted in yellow and the histone downstream element in red. Numbers refer to the position downstream from the stop codon.

(b) Co-immunoprecipitations of endogenous cyclin F and SLBP. Endogenous SLBP or endogenous cyclin F was immunoprecipitated from U2OS cell extracts using SLBP or cyclin F antibody, respectively (IgG was used as negative control). Whole cell extracts (WCE) and immunoprecipitations were immunoblotted as indicated.

(c) Co-immunoprecipitations of endogenous cyclin F and SLBP. Endogenous SLBP or endogenous cyclin F was immunoprecipitated from RPE-1 cell extracts using SLBP or cyclin F antibody, respectively (IgG was used as negative control). Whole cell extracts and immunoprecipitations were immunoblotted as indicated.

Figure S2

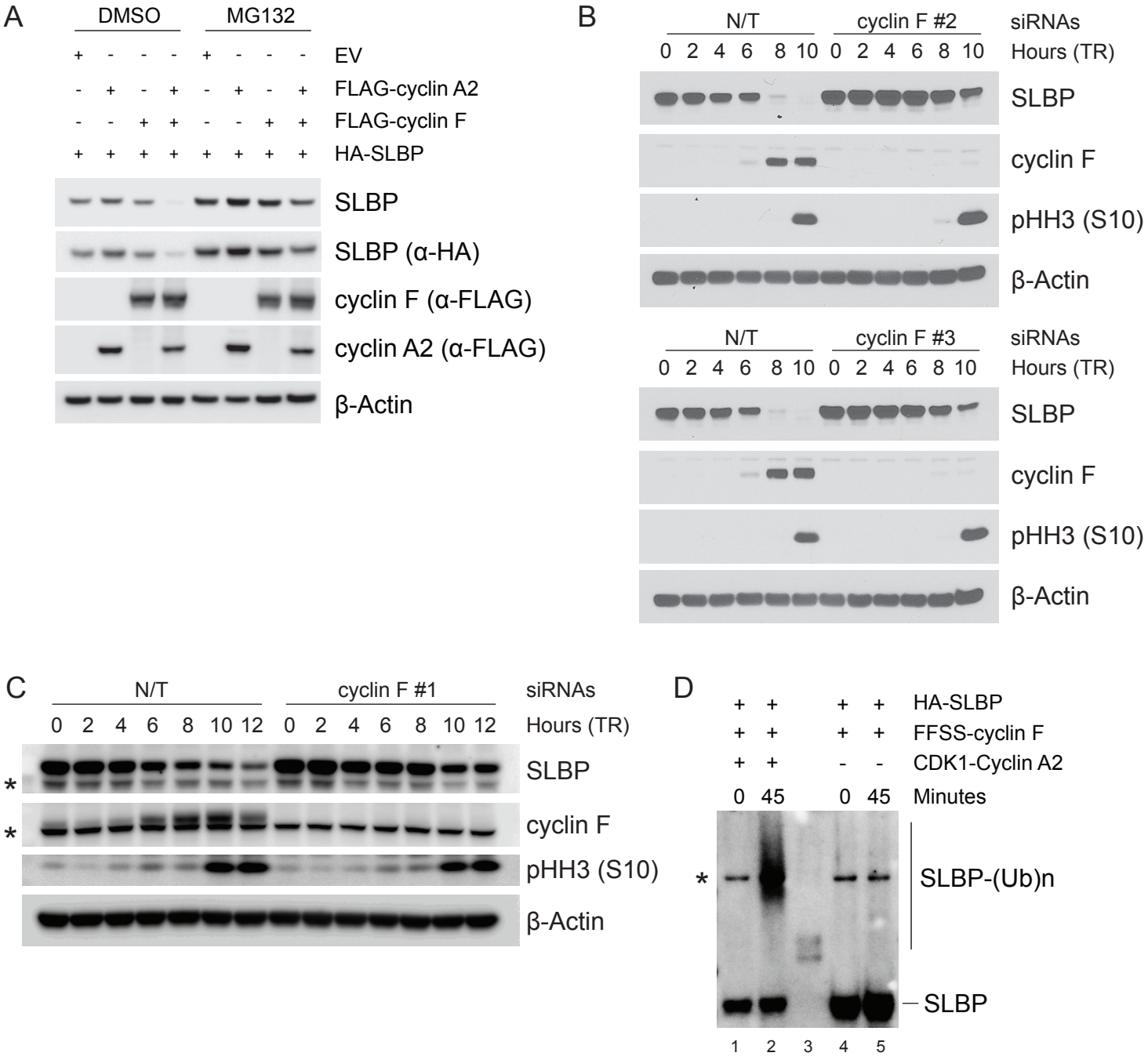

A
DMSO
MG132
EV
FLAG-cyclin A2
FLAG-cyclin F
HA-SLBP
SLBP
SLBP (α-HA)
cyclin F (α-FLAG)
cyclin A2 (α-FLAG)
β-Actin
B
N/T
cyclin F #2
siRNAs
Hours (TR)
SLBP
cyclin F
pHH3 (S10)
β-Actin
N/T
cyclin F #3
siRNAs
Hours (TR)
SLBP
cyclin F
pHH3 (S10)
β-Actin
C
N/T
cyclin F #1
siRNAs
Hours (TR)
SLBP
cyclin F
pHH3 (S10)
β-Actin
D
HA-SLBP
FFSS-cyclin F
CDK1-Cyclin A2
Minutes
SLBP-(Ub)n
SLBP

Figure S2. Cyclin F targets SLBP for ubiquitylation and degradation, related to Figure 2.

(a) Cyclin F and cyclin A2 cooperate in promoting the proteasomal degradation of SLBP. HEK293T cells were transfected with the indicated constructs, lysed, and immunoblotted as indicated. Where indicated, cells were treated with MG132 for four hours prior to collection.

(b) Knockdown of cyclin F stabilizes SLBP in G2. HeLa cells were synchronized at G1/S by double-thymidine block before trypsinization and release into fresh media. Cells were transfected with either siRNAs targeting cyclin F (#2 for top panels and #3 for bottom panels) or a non-targeting (N/T) siRNA between the first and second thymidine treatment, collected at the indicated time points, and immunoblotted as indicated.

(c) Knockdown of cyclin F stabilizes SLBP in G2. RPE-1 cells were synchronized at G1/S by double-thymidine block before trypsinization and release into fresh media. Cells were transfected with either an siRNA targeting cyclin F (oligo #1) or a non-targeting (N/T) siRNA between the first and second thymidine treatment, collected at the indicated time points, and immunoblotted as indicated (asterisks denote non-specific bands).

(d) Cyclin A2 promotes cyclin F-mediated ubiquitylation of SLBP. *In vitro* translated SLBP was incubated at 30°C with or without CDK1-cyclin A2 (as indicated) and then with an ubiquitylation mix containing *in vitro* translated cyclin F. The samples were then immunoblotted with an anti-SLBP antibody. The bracket indicates a ladder of bands corresponding to poly-ubiquitylated SLBP. Lane #3 contained molecular weight markers that crossreacted with the anti-SLBP antibody.

Figure S3

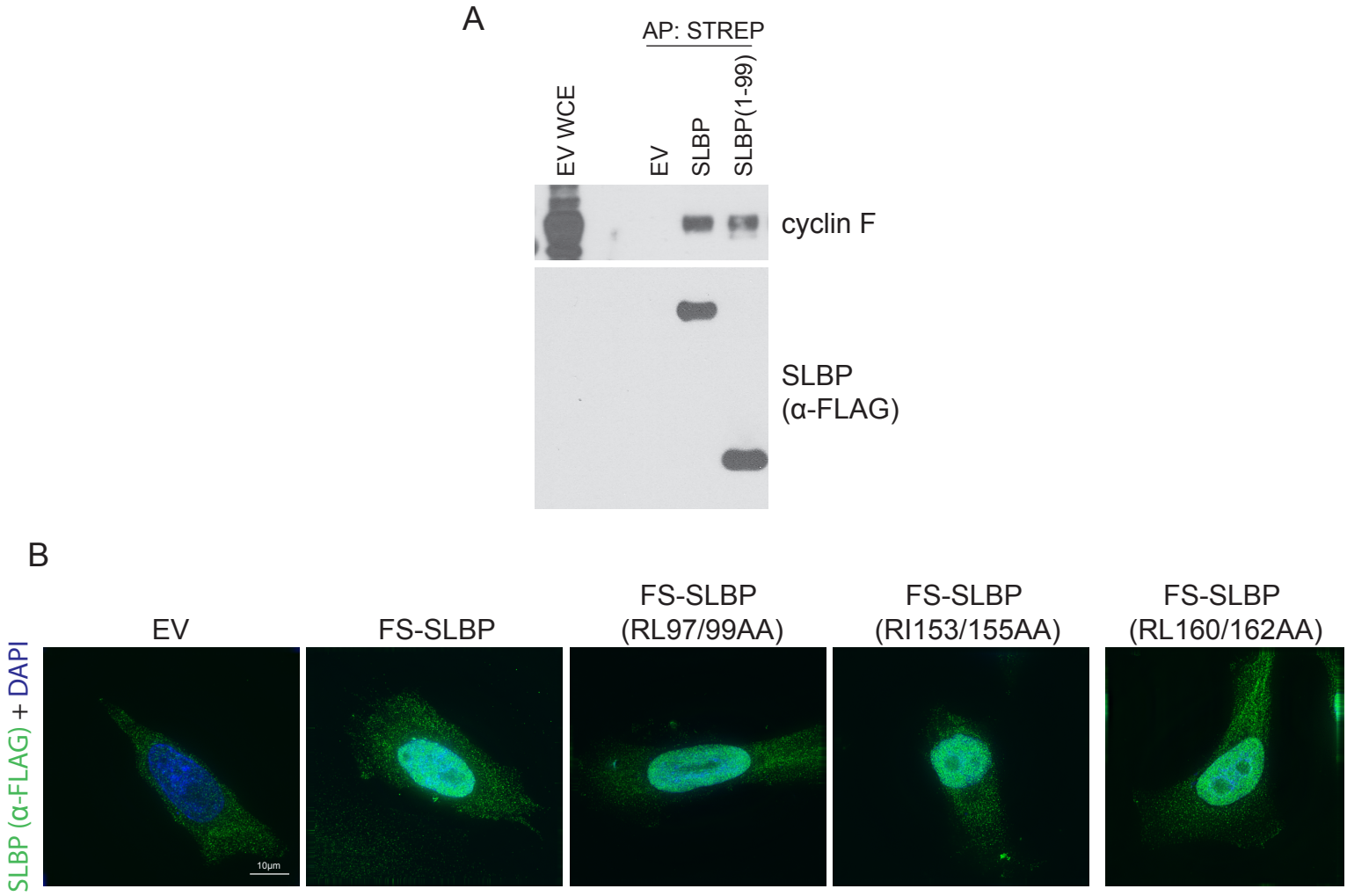

A
AP: STREP
EV WCE
EV
SLBP
SLBP(1-99)
cyclin F
SLBP
(α-FLAG)
B
EV
FS-SLBP
FS-SLBP
(RL97/99AA)
FS-SLBP
(RI153/155AA)
FS-SLBP
(RL160/162AA)
SLBP (α-FLAG) + DAPI
10μm

Figure S3. Cyclin F co-immunoprecipitates with SLBP independent of the RNA-binding domain and the SLBP CY mutants are not mislocalized *in vivo*, related to Figure 3.

(a) Cyclin F co-immunoprecipitates with SLBP independent of the RNA-binding domain. HEK293T cells were transfected with either empty vector (EV) or FLAG-STREP-tagged SLBP constructs. Whole cell extracts (WCE) were affinity precipitated with anti-STREP resin, and affinity precipitations were probed with the indicated antibodies.

(b) SLBP CY mutants are not mislocalized *in vivo*. HeLa cells were transfected with either empty vector (EV) or FLAG-STREP-tagged SLBP constructs and fixed with 4% paraformaldehyde. Cells were stained with an anti-FLAG antibody (green) and DAPI (blue).

Figure S4

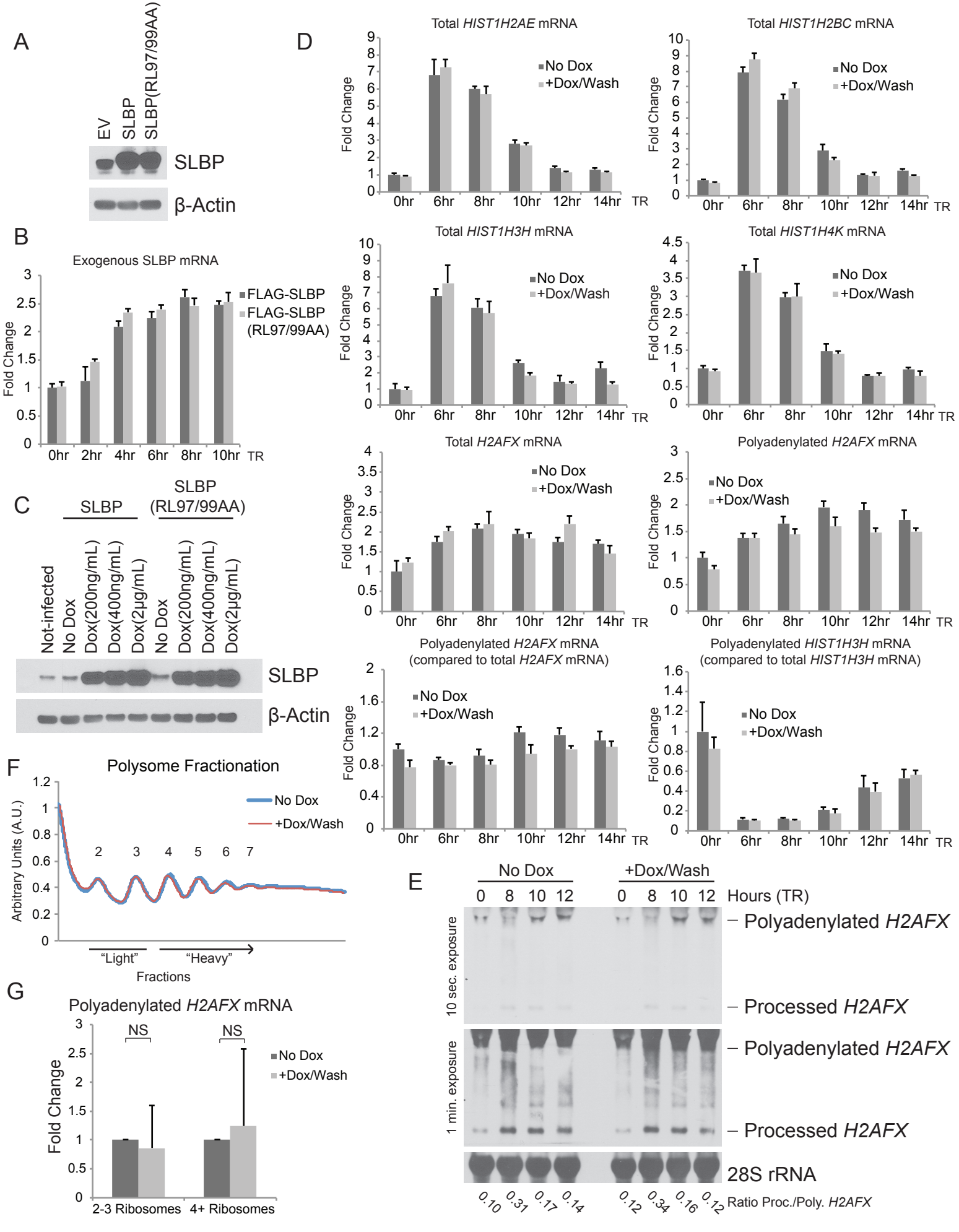

A
EV
SLBP
SLBP(RL97/99AA)
SLBP
β-Actin
B
Exogenous SLBP mRNA
Fold Change
FLAG-SLBP
FLAG-SLBP (RL97/99AA)
0hr 2hr 4hr 6hr 8hr 10hr TR
C
SLBP
SLBP (RL97/99AA)
Not-infected
No Dox
Dox(200ng/mL)
Dox(400ng/mL)
Dox(2μg/mL)
No Dox
Dox(200ng/mL)
Dox(400ng/mL)
Dox(2μg/mL)
SLBP
β-Actin
D
Total HIST1H2AE mRNA
Total HIST1H2BC mRNA
Total HIST1H3H mRNA
Total HIST1H4K mRNA
Total H2AFX mRNA
Polyadenylated H2AFX mRNA
Polyadenylated H2AFX mRNA (compared to total H2AFX mRNA)
Polyadenylated HIST1H3H mRNA (compared to total HIST1H3H mRNA)
Fold Change
No Dox
+Dox/Wash
0hr 6hr 8hr 10hr 12hr 14hr TR
E
No Dox
+Dox/Wash
0 8 10 12
0 8 10 12
Hours (TR)
10 sec. exposure
1 min. exposure
Polyadenylated H2AFX
Processed H2AFX
Polyadenylated H2AFX
Processed H2AFX
28S rRNA
0.10 0.31 0.17 0.14
0.12 0.34 0.16 0.12
Ratio Proc./Poly. H2AFX
F
Polysome Fractionation
Arbitrary Units (A.U.)
No Dox
+Dox/Wash
2 3 4 5 6 7
"Light"
"Heavy"
Fractions
G
Polyadenylated H2AFX mRNA
Fold Change
NS
NS
No Dox
+Dox/Wash
2-3 Ribosomes
4+ Ribosomes

Figure S4. Expression of SLBP(RL97/99AA) does not affect the processing or degradation of canonical histone and *H2AFX* mRNAs in cells progressing through the cell cycle, related to Figure 4.

(a) Expression of exogenous SLBP and SLBP(RL97/99AA) in HeLa cells. HeLa cells infected with retroviruses expressing an empty vector (EV), FLAG-tagged SLBP, or FLAG-tagged SLBP(RL97/99AA) were collected, lysed, and immunoblotted as indicated.

(b) Exogenous SLBP and SLBP(RL97/99AA) mRNA levels increase after release from thymidine block. OligodT primed cDNAs corresponding to the samples in Figure 4A were analyzed by qPCR for exogenous SLBP constructs. Exogenous FLAG-tagged SLBP mRNA is presented as a ratio to *GAPDH* mRNA. Each sample is presented relative to the "FLAG-SLBP 0hr" sample. The data are presented as the mean ± SD of one representative experiment performed in triplicate.

(c) Expression of SLBP and SLBP(RL97/99AA) from a doxycycline-inducible promoter depends on the presence of doxycycline. U2OS cells infected with lentiviruses expressing either wild type SLBP or untagged SLBP(RL97/99AA) under the control of a doxycycline-inducible promoter were treated with thymidine and the indicated concentrations of doxycycline. Cells were collected after 16 hours of doxycycline and thymidine, lysed, and immunoblotted.

(d) Expression of SLBP(RL97/99AA) does not affect the processing or disappearance rate of canonical histone and *H2AFX* mRNAs. Random primed cDNAs corresponding to the samples in Figure 4B were analyzed by qPCR for polyadenylated *H2AFX* mRNA, total *H2AFX* mRNA, polyadenylated *HIST1H3H* mRNA, and selected total canonical histone mRNAs. All total histone mRNAs are presented as a ratio to U6 snRNA (which is not polyadenylated), polyadenylated *H2AFX* mRNA is presented as a ratio to *GAPDH* mRNA (which is polyadenylated) unless otherwise indicated, and polyadenylated *HIST1H3H* mRNA is presented as a ratio to total *HIST1H3H* mRNA. Polyadenylated *HIST1H3H* mRNA (as a ratio to total *HIST1H3H* mRNA) levels show an apparent increase 10-14 hours after thymidine release due to the rapid degradation of processed *HIST1H3H* mRNA as the cells enter G2 (*i.e.,* the denominator of the ratio decreases). Each sample is presented relative to the "No Dox 0hr" sample. The data are presented as mean ± SD of one representative experiment performed in triplicate.

(e) Expression of SLBP(RL97/99AA) does not affect the processing or disappearance rate of *H2AFX* mRNA. U2OS cells were prepared as in Figure 4B, samples were collected at the indicated time points, lysed, and total RNA was extracted. Total RNA was subjected to Northern blot in which *H2AFX* mRNA was probed with a biotin-incorporated RNA complementary to the 5' UTR of *H2AFX*. 28S rRNA is presented as a loading control. Densitometric analysis of the bands (using multiple Northern blot exposures in the linear range) was conducted, and the ratio of processed *H2AFX* mRNA to polyadenylated *H2AFX* mRNA is presented (bottom).

(f) Expression of SLBP(RL97/99AA) does not affect the loading of total cellular mRNA at the polyribosomes. Density sedimentation peak analysis comparing ribosome loading on the total cellular mRNA for the polysome fractionation showed in Figure 4D. The number of ribosomes bound to the total mRNA is indicated above each peak in the curve.

(g) Expression of SLBP(RL97/99AA) does not affect the loading of polyadenylated *H2AFX* mRNA at the light and heavy polyribosome fractions. Random primed cDNAs were prepared from the mRNA corresponding to Figure 4D, and analyzed by qPCR for polyadenylated *H2AFX* mRNA. Polyadenylated *H2AFX* mRNA is presented as a ratio to *GAPDH* mRNA. mRNA from the corresponding unfractionated sample was used to normalize each data point. The data are presented as mean ± SD (NS: not significant, n=3, each in triplicate).

Figure S5

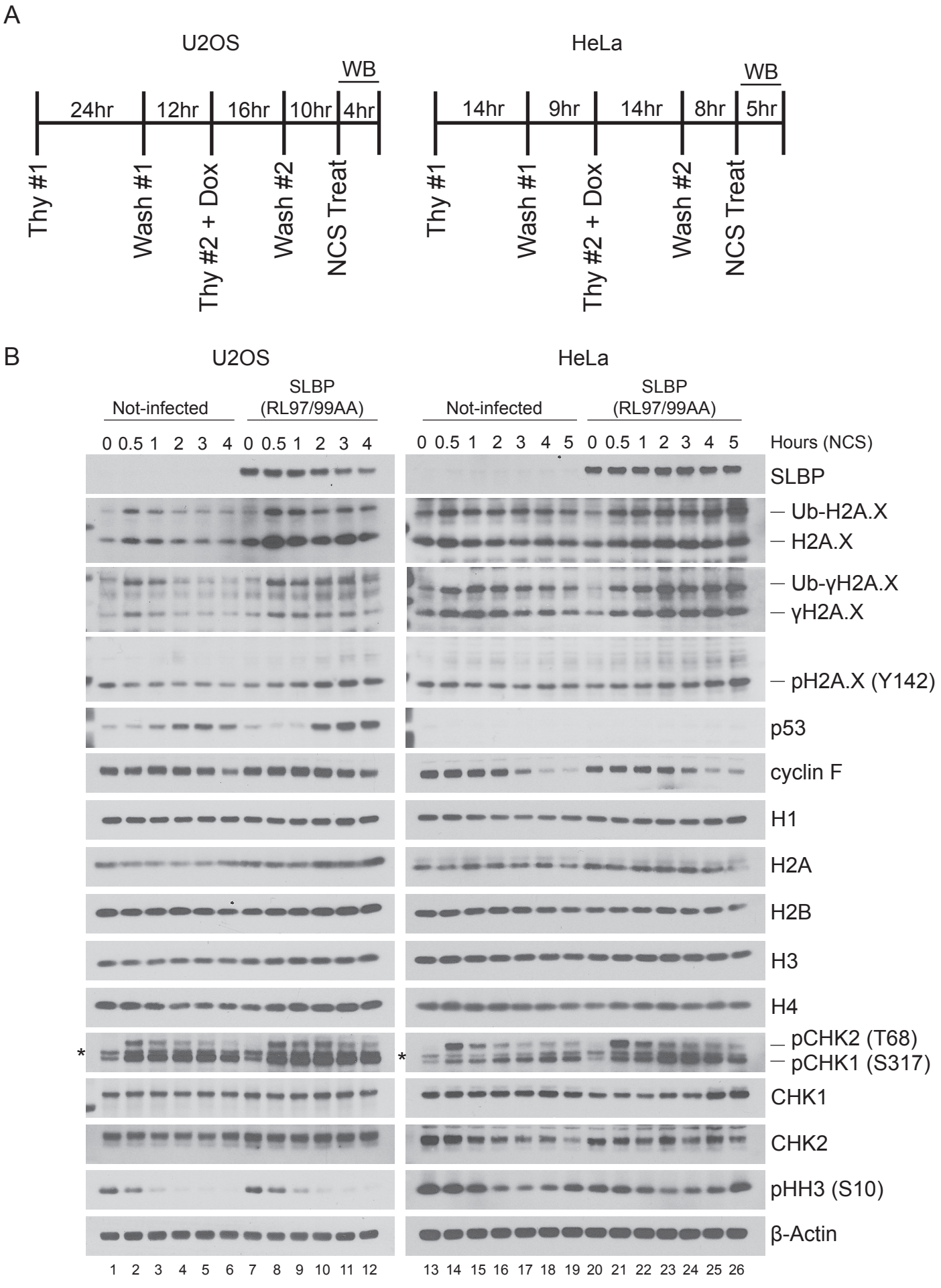

A
U2OS
HeLa
WB
24hr
12hr
16hr
10hr
4hr
14hr
9hr
14hr
8hr
5hr
Thy #1
Wash #1
Thy #2 + Dox
Wash #2
NCS Treat
B
U2OS
HeLa
Not-infected
SLBP (RL97/99AA)
0 0.5 1 2 3 4
0 0.5 1 2 3 4 5
Hours (NCS)
SLBP
Ub-H2A.X
H2A.X
Ub-γH2A.X
γH2A.X
pH2A.X (Y142)
p53
cyclin F
H1
H2A
H2B
H3
H4
pCHK2 (T68)
pCHK1 (S317)
CHK1
CHK2
pHH3 (S10)
β-Actin
1 2 3 4 5 6 7 8 9 10 11 12
13 14 15 16 17 18 19 20 21 22 23 24 25 26

Figure S5. SLBP(RL97/99AA) expression in G2 leads to persistent DNA damage response signaling, related to Figure 5.

(a) Schematic representation of the experiments. Uninfected U2OS and HeLa cells or U2OS and HeLa cells infected with lentiviruses expressing untagged SLBP(RL97/99AA) under the control of a doxycycline-inducible promoter were synchronized at G1/S using a double-thymidine block before trypsinization and release into fresh medium. Doxycycline was added to all cells with the second thymidine treatment and was not re-added after release from double-thymidine. Neocarzinostatin (NCS) was added at 10 hours (U2OS) or 8 hours (HeLa) after the release from double-thymidine block.

(b) Expression of SLBP(RL97/99AA) in G2 leads to persistent DNA damage response signaling after genotoxic stress. Cells were collected at the listed time points, lysed, and immunoblotted as indicated (asterisk denotes a non-specific band).

Figure S6

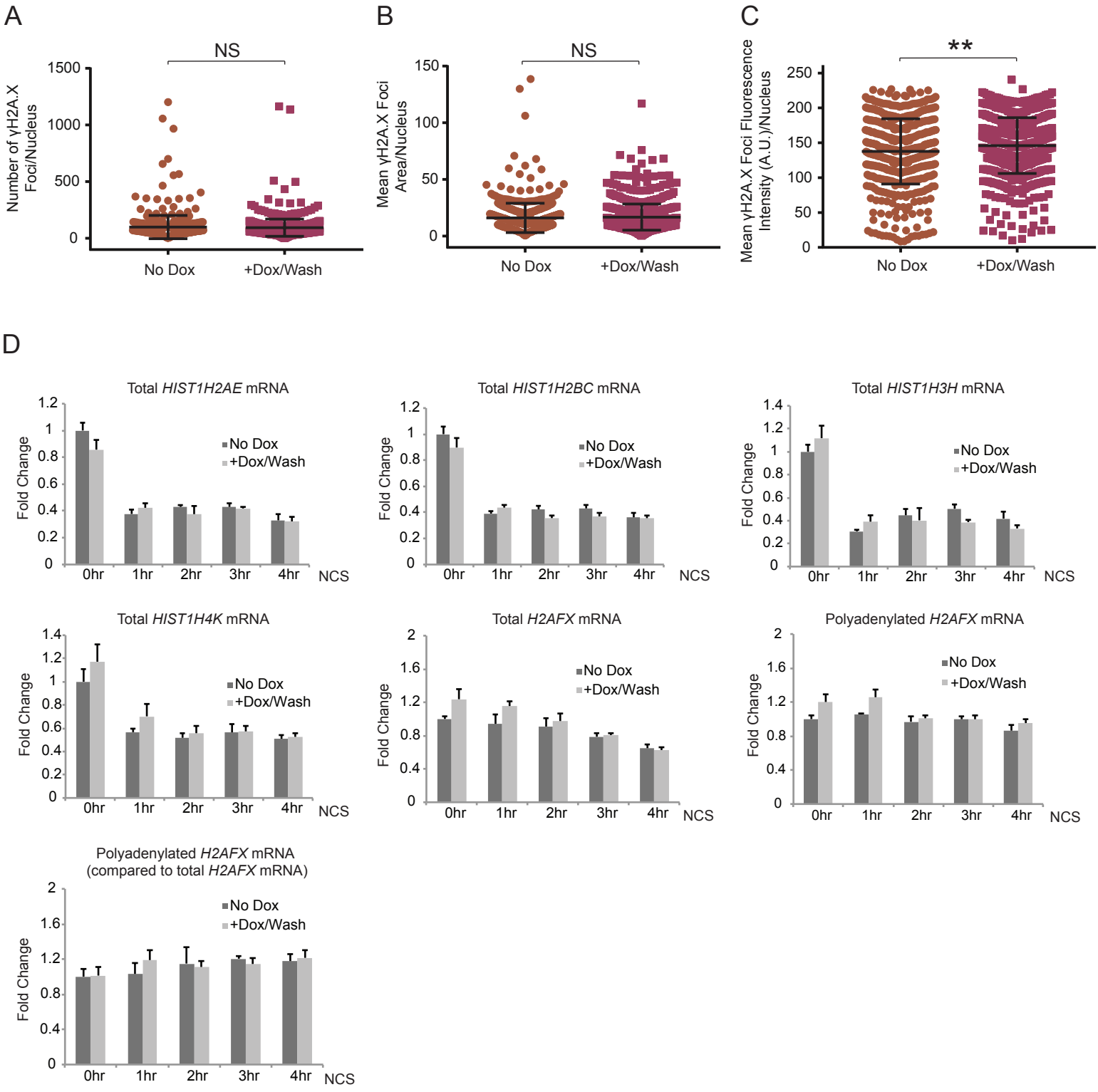

A
NS
Number of γH2A.X Foci/Nucleus
1500
1000
500
0
No Dox
+Dox/Wash
B
NS
Mean γH2A.X Foci Area/Nucleus
150
100
50
0
No Dox
+Dox/Wash
C
**
Mean γH2A.X Foci Fluorescence Intensity (A.U.)/Nucleus
250
200
150
100
50
0
No Dox
+Dox/Wash
D
Total HIST1H2AE mRNA
Total HIST1H2BC mRNA
Total HIST1H3H mRNA
Total HIST1H4K mRNA
Total H2AFX mRNA
Polyadenylated H2AFX mRNA
Polyadenylated H2AFX mRNA (compared to total H2AFX mRNA)
Fold Change
No Dox
+Dox/Wash
0hr
1hr
2hr
3hr
4hr
NCS

Figure S6. SLBP(RL97/99AA) expression in G2 leads to persistent DNA damage response signaling without affecting the degradation rate of canonical histone and *H2AFX* mRNAs, related to Figure 5.

(a-c) Samples corresponding to Figure 5D were analyzed for additional metrics (NS: not significant, **$p \leq 0.01$).

(d) Expression of SLBP(RL97/99AA) does not affect the disappearance rate of canonical histone and *H2AFX* mRNAs after genotoxic stress. Random primed cDNAs corresponding to the samples in Figure 5B were analyzed by qPCR for polyadenylated and total *H2AFX* mRNA and select total canonical histone mRNA. All total histone mRNA are presented as a ratio to U6 snRNA (which is not polyadenylated) and polyadenylated *H2AFX* mRNA is presented as a ratio to *GAPDH* mRNA (which is polyadenylated) unless otherwise indicated. Each sample is presented relative to the "No Dox 0hr" sample. The data are presented as mean ± SD of one representative experiment performed in triplicate.

Supplemental Experimental Procedures

Tandem affinity purification and mass spectrometry

HEK293T cells were transiently transfected with FLAG-STREP-tagged SLBP and control plasmids using polyethylenimine. 48 hours after transfection cells were treated with MLN4924 for 4 hours before collection. MLN4924 was utilized to inhibit Cullin-RING E3 ubiquitin ligases (CRLs) and increase the probability of capturing SLBP in complex with a CRL (including CRL1, aka SCF). Lysis of cell pellets was carried out with lysis buffer (50 mM Tris-Cl pH 8.0, 150 mM NaCl, 10% glycerol, 1 mM EDTA, 50 mM NaF, and 0.5% NP-40) supplemented with protease and phosphatase inhibitors. The first purification of the soluble fraction was carried out with Strep-Tactin (IBA) Superflow resin (1.5 hours at 4°C). STREP-purified fractions were incubated with D-Desthiobiotin (IBA) for 30 minutes at room temperature to elute the affinity precipitated proteins, which were then subjected to a second purification with an anti-FLAG antibodys (1.5 hours at 4°C) conjugated to agarose resin. FLAG-immunoprecipitates were eluted with 3x-FLAG-peptide (50 $\mu g\text{-}ml^{-1}$) for 1 hour at 4°C. Following sample filtration and concentration (Amicon), gel digestion was performed as follows: samples were reduced with DTT at 57°C for 1 hour (2 µl of 0.2 M in 100 mM ammonium bicarbonate). Samples were alkylated with iodoacetamide at room temperature in the dark for 45 minutes (2 µl of 0.5 M in 100 mM ammonium bicarbonate), immediately loaded onto a NuPAGE® 4-12% Bis-Tris Gel 1.0 mm (Life Technologies Corporation), and run for approximately 20 minutes at 200 V. The gel was stained using GelCode Blue Stain Reagent (Thermo Fisher Scientific). The SLBP region was excised, prepped and analyzed by LC-MS (Young et al., 2015) separately from the remainder of the gel. Protein digestion and peptide extraction was performed as described (Young et al., 2015). Briefly, gel plugs were destained in 1:1 v/v solution of methanol and 100 mM ammonium bicarbonate solution under agitation at 4°C. After 15 minutes, the solution was discarded and a fresh aliquot added. This process was repeated at least five times. Gel pieces were partially dehydrated with an acetonitrile rinse and further dried in a SpeedVac concentrator for 20 minutes. 100 ng of sequencing grade modified trypsin (Promega) was added to each gel sample until absorbed, then, 100 µl of 100 mM ammonium bicarbonate was added to cover the gel pieces and digestion proceeded overnight on a shaker at RT. Peptide extraction was performed using a slurry of R2 20 µm Poros beads (Life Technologies Corporation) in 5% formic acid; 0.2% trifluoroacetic acid (TFA) was added to each sample at a volume equal to that of the ammonium bicarbonate. Samples were incubated with agitation at 4°C for 3 hours. The beads were loaded onto equilibrated C18 ziptips (Millipore) using microcentrifugation. Gel pieces were rinsed three times with 0.1% TFA and each rinse was added to its corresponding ziptip followed by microcentrifugation. Extracted porous beads were further washed with 0.5% acetic acid. Peptides were eluted by the addition of 40% acetonitrile in 0.5% acetic acid followed by the addition of 80% acetonitrile in 0.5% acetic acid. The organic solvent was removed using a SpeedVac concentrator and the sample reconstituted in 0.5% acetic acid. Liquid chromatographic separation was performed inline with MS using the autosampler of an EASY-nLC 1000 (Thermo Fisher Scientific). Peptides were gradient eluted from the column directly to Q Exactive mass spectrometer (Thermo Fisher Scientific) using a linear gradient of 0-40% B in 120 min, 40-50%B in 10 min and 50-100% in 10 min (Solvent A: 2% acetonitrile in 0.5% acetic acid, Solvent B: 95% acetonitrile, 0.5% acetic acid). High resolution full MS spectra were acquired with a resolution of 70,000, an AGC target of 1e6, maximum injection time of 120 ms, and a scan range of 400 to 1500 m/z. Following each full MS scan twenty data-dependent high resolution HCD MS/MS spectra were acquired. All MS/MS spectra were collected using the following instrument parameters: resolution of 17,000, AGC target of 5e4, maximum injection time of 250 ms, one microscan, 2 m/z isolation window, and Normalized collision energy of 27. The MS/MS spectra were searched using the Uniprot human database using Sequest within Proteome Discoverer (Thermo Fisher Scientific). The data was filtered using a 1% false discovery rate searched against a decoy database and only proteins with at least 2 unique peptides were retained for further analysis.

Antibodies

Antibodies used were:
cyclin F (1:1500, Santa Cruz Biotechnology cat. No. sc-952),
SLBP (1:10,000, Bethyl cat. No. A303-968A),
FLAG (1:1,000 (IF), 1:7,000 (WB), Sigma-Aldrich cat. No. F7425),
HA.11 (16B12) (1:5000, Covance cat. No. MMS-101P)
c-MYC (9E10) (1:4000, Sigma-Aldrich cat. No. M5546)
pHH3 (S10) (1:1000, Cell Signaling cat. No. 9701S),
PCNA (1:5000, Zymed cat. No. 13-3900),
cyclin A2 (1:5000, produced in our laboratory),
CUL1 (1:1500, Life Technologies cat. No. 322400)
SKP1 (1:5000, produced in our laboratory)
FBXL1 (1:500, Invitrogen cat. No. 32-3300),

FBXO7 (1:1000, Santa Cruz Biotechnology cat. No. sc-86450)
FBXW1 (1:2000, Cell Signaling cat. No. D13F10)
FBXO3 (1:1000, Santa Cruz Biotechnology cat. No. sc-134722)
FBXO18 (1:2000, previously described in Jeong, Y. T. et al., 2013)
FBXO28 (1:5000, Bethyl cat. No. A302-377A)
CDC20 (1:1000, Santa Cruz Biotechnology cat. No. sc-8358)
CDH1 (1:1000, Thermo Fisher Scientific cat. No. MA5-11496)
CTIF (1:500, Sigma-Aldrich cat. No. HPA016865)
CBP80 (1:1000, Bethyl cat. No. A301-793A-T)
eiF4GI (1:10000, Bethyl cat. No. A301-776A)
SLIP1 (1:1000, gift from William Marzluff)
UPF1 (1:10000, Bethyl cat. No. A301-902A)
H2A.X (1:10000, Bethyl cat. No. A300-082A)
H1 (1:1000, Santa Cruz Biotechnology cat. No. sc-8030)
H2A (1:1500, Active Motif cat. No. 39111)
H2B (1:10000, Abcam cat. No. ab1790)
H3 (1:30000, Abcam cat. No. ab1791)
H4 (1:10000, gift from CD Allis)
FLASH (1:1000, Bethyl cat. No. A301-622A-T)
ARS2 (1:1000, Bethyl cat. No. A304-550A-T)
CSTF64 (1:1000, Bethyl cat. No. A301-092A-T)
ZFP100 (1:1000, Thermo Fisher Scientific cat. No. PA5-26756)
pCHK1 (S317) (1:1000, Cell Signaling cat. No. 2344S)
pCHK2 (T68) (1:1000, Cell Signaling cat. No. 2661S)
CHK1 (1:1000, Santa Cruz Biotechnology cat. No. sc-8408)
CHK2 (1:1000, Santa Cruz Biotechnology cat. No. sc-9064)
H2A.Z (1:1000, Active Motif cat. No. 39113)
β-Actin (1:7000, Sigma-Aldrich cat. No. A5441)
γH2A.X (1:1500(WB), EMD Millipore cat. No. 05-636)
pH2A.X (Y142) (1:1000, EMD Millipore cat. No. 07-1590)
R2 (1:1000, Santa Cruz Biotechnology cat. No. sc-10844)
p53 (1:1000, Santa Cruz Biotechnology cat. No. sc-6243)

Plasmids
SLBP, cyclin A2, and cyclin F complementary DNAs were inserted into pcDNA3 either by sub-cloning or site-directed mutagenesis. FLAG-TR-TUBE constructs were a gift from K. Tanaka. Where specified, we also used retroviral (pBabe) and lentiviral (pTRIPZ) vectors. Specific details will be provided on request.

Immunoprecipitation and immunoblotting
HEK293T cells were transiently transfected using polyethylenimine. Where indicated, 48 hours after transfection, HEK293T cells were incubated with MLN4924 for 4 hours before collection. Cell lysis was carried out with lysis buffer (50 mM Tris pH 8.0, 150 mM NaCl, 10% glycerol, 1 mM EDTA, 50 mM NaF, and 0.5% NP-40) supplemented with protease and phosphatase inhibitors. Where indicated, SUPERase-In™ RNase Inhibitor (Thermo Fisher Scientific) was used at 1 U/μL. Lysates were then immunoprecipitated with either Strep-Tactin (IBA) Superflow resin or an anti-FLAG antibody conjugated to agarose resin. Elution of the immunoprecipitate was carried out with either D-Desthiobiotin (IBA) or FLAG peptide for the Strep-Tactin Superflow resin or anti-FLAG agarose, respectively. For immunoprecipitation of endogenous proteins, HEK293T, U2OS, or RPE-1 cells were collected and lysed with lysis buffer. SLBP or cyclin F was immunoprecipitated with the listed antibodies mixed with Dynabeads Protein G (Thermo Fisher Scientific). Rabbit IgG (Bethyl) was used as a negative control. Elution of the immunoprecipitate was carried out with NuPAGE® LDS sample buffer (Thermo Fisher Scientific) supplemented with β-mercaptoethanol (Sigma-Aldrich) and incubation at 95°C for 5 minutes. Immunoblotting was performed as described previously (Skaar et al., 2015).

Immunofluorescence microscopy
Cells were fixed with 4% paraformaldehyde (Electron Microscopy Sciences) for 1 hour, and then blocked with 3% BSA in PBS before incubation with primary antibodies. Alexa-488-conjugated secondary antibodies (Life Technology Corporation) were added for 1 hour at 1:1000. Slides were mounted in ProlongGold with DAPI (Invitrogen). Imaging was performed using a DeltaVision Elite inverted microscope system (Applied Precision), using a x100/1.4NA Oil PSF

Objective from Olympus. The system was equipped with a CoolSNAP Hq@ camera and SoftWorx imaging software version 5.0. Serial optical sections obtained 0.2 μm apart along the z-axis were processed using the SoftWorx deconvolution algorithm and projected into one picture using SoftWorx software (Applied Precision).

Clonogenic assay
150 untreated U2OS cells or 20,000 U2OS cells treated with NCS cells were plated. Colonies were observed after 12 days of growth. Cells were washed with PBS, fixed with 6% glutaraldehyde (Electron Microscopy Sciences) and stained with crystal violet (Sigma-Aldrich).

qRT-PCR
Total RNA was generated using RNeasy mini kits (Qiagen). cDNA was generated using Random Hexamers or OligodT EcoDry kits (Takara Clontech). qPCR was performed using Absolute SYBR green (Thermo Fisher Scientific) on a Roche Lightcycler 480. Analysis of the qPCR experiments was conducted via absolute relative quantification with in-experiment standard curves for each primer set to control for primer efficiency. The oligos used for qRT-PCR analysis are listed here including previously validated oligos for the canonical histones (Skaar et al., 2015).

Total *HIST1H2AE*
F: 5'-CCGGTCTTCAGTTTCCAGTT-3'
R: 5'-CCGACTCGTTCGGAGTAGTT-3'

Total *HIST1H2BC*
F: 5'-CCTGAGCCAGCCAAGTCTGC-3'
R: 5'-TGCGGCTGCGCTTGCGCTTC-3'

Total *HIST1H3H*
F: 5'-TATCGGCCTGGTACAGTGG-3'
R: 5'-CGCAAGTCGGTCTTGAAGT-3'

Polyadenylated *HIST1H3H*
F: GGGCCTGCTTAAACCACTTA
R: GGCTTCAAAGGGAATGTTCT

Total *HIST1H4K*
F: 5'-CCATCCGGCGCCTTGCTC-3'
R: 5'-TTCAGCACCCCGCGAGTC-3'

Polyadenylated *H2AFX*
F: 5'-TCCCTTCCAGCAAACTCAAC-3'
R: 5'-CCCCAATGCCTAAGGTTCTAG-3'

Total *H2AFX*
F: 5'-CTGCTGCCCAAGAAGACC-3'
R: 5'-CGGGCCCTCTTAGTACTCCT-3'

U6 snRNA
F: 5'-CTCGCTTCGGCAGCACA-3'
R: 5'-AACGCTTCACGAATTTGCGT-3'

GAPDH
F: 5'-ATGGGTGTGAACCATGAGAA-3'
R: 5'-GTGCTAAGCAGTTGGTGGTG-3'

FLAG-SLBP
F: 5'-GGATGACGATGACAAGGCAGCC-3'
R: 5'-CTGCCGTCGGCTCTGCGCTT-3'

Annexin-V-PI staining and flow cytometry

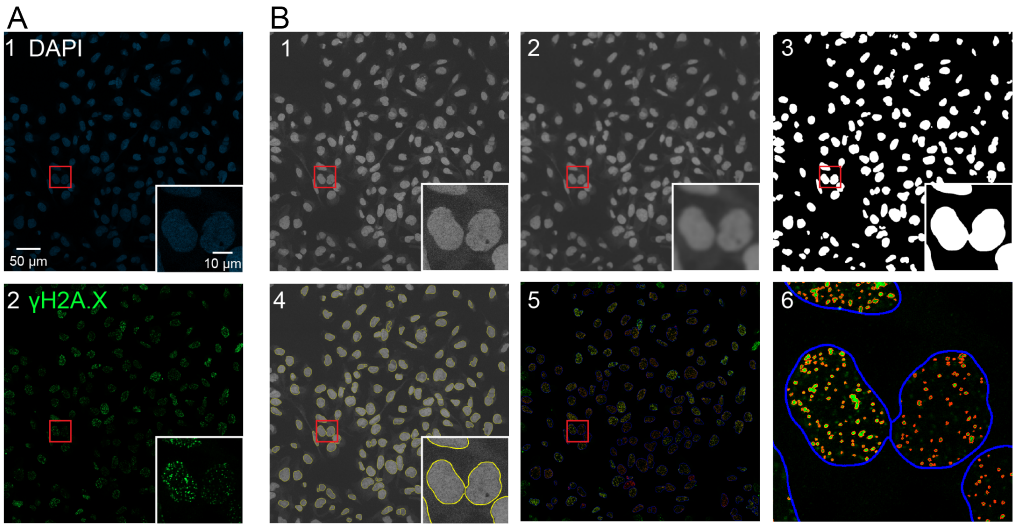
Annexin-V staining was performed as previously described by Rieger et al., 2011. Briefly cells were collected, washed with phosphate buffered saline (PBS pH 7.4) followed by washing with Annexin binding buffer (10 mM HEPES, 140 mM NaCl, and 2.5 mM $CaCl_2$, pH 7.4). $1x10^5$ cells were resuspended in 100 µl Annexin binding buffer and 5 µl Annexin-V Alexa-488 conjugate (Molecular Probes) was added along with 1 µg/ml of PI for 15 minutes at room temperature. Following washing, cells were fixed with 1% PFA (in Annexin binding buffer). Finally, cells were resuspended in Annexin binding buffer supplemented with 50 µg/ml RNAse A before flow cytometry analysis. One- and two-dimensional flow cytometry was performed to detect either Alexa-488 or both Alexa-488 and PI using a LSRII flow-cytometer (BD Biosciences) and FlowJo software (Tree Star Inc., Stanford). γH2A.X flow cytometry analyses were conducted as previously described by Huang and Darzynkiewicz, 2006.

Northern blot

U2OS cells were lysed and total RNA was purified via RNeasy mini kits (Qiagen). Preparation of the total RNA for gel electrophoresis and handling of the membrane was performed using a NorthernMax®-Gly Kit (Thermo Fisher Scientific), as recommended by the manufacturer. The membrane was developed using a Chemiluminescent Nucleic Acid Detection Module (Thermo Fisher Scientific) with stabilized streptavidin-horseradish peroxidase conjugate per the manufacturer's recommendations. For the generation of the 5' UTR H2AFX probe, the 5' UTR and a portion of the ORF of *H2AFX* (specifically 5'-acagcagttacac…cggcaagactggc-3'; 97 nucleotides) was cloned into pcDNA3 using EcoRI/XhoI sites. This construct was then digested with EcoRI, and the linearized plasmid was purified using a QIAquick Gel Extraction Kit (Qiagen). This linearized plasmid was then used as the template in an Sp6 MAXIscript *in vitro* Transcription Kit (Thermo Fisher Scientific) reaction with inclusion of Bio-16-UTP (Thermo Fisher Scientific) to generate biotin-incorporated RNA complementary to the cloned region of *H2AFX,* as recommended by the manufacturer. Free nucleotides were removed from the post-reaction solution via NucAway™ Spin Columns (Thermo Fisher Scientific). The concentration of the purified biotin-incorporated RNA probe was then quantified using a NorthernMax®-Gly Kit (Thermo Fisher Scientific), as recommended by the manufacturer.

Automated analysis of γH2A.X foci

Tile image scans were acquired on a Zeiss LSM 800 with a 63x NA=1.4 Plan Apo objective and the automated processing of the images of nuclei and γH2A.X foci was implemented in Python. Segmentation of the nuclei was achieved by using fluorescent images of cell cultures stained for DAPI (Panel A1, below). First, the contrast of the gray-scale DAPI image was enhanced by applying a contrast limited adaptive histogram equalization (CLAHE) with the default parameters of OpenCV package (Panel B1, below), followed by the implementation of a Gaussian filter (Panel B2, below). Then, the Otsu algorithm (Otsu, 1979) was used to determine a threshold value to generate a binary image (Panel B3, below). After segmentation, nuclei found at the edges of the image were eliminated from further analysis and the watershed algorithm was used to separate touching nuclei (nuclei are outlined in yellow; Panel B4, below). Then, all nuclei were labeled and the area of each nucleus was measured. All nuclei where the area was less than 1000px (9.8 μm^2) were eliminated from further analysis. The following steps were achieved to segment the foci. The green channel was obtained from the γH2A.X immunofluorescent image (Panel A2, below) and using only the intensities inside each individual nucleus, the Otsu algorithm (Otsu, 1979) was applied to determine the threshold value to segment the foci. Then, a binary image of the foci was generated, and the foci labeled and their sizes and intensities measured (foci outlined in red; nuclei outlined in blue; Panel B5, below). The red box in B5 is magnified in B6 to show how foci were segmented. Foci that covered more than 80% of the nuclei or less than or equal to 4px (0.039 μm^2) in area were eliminated from the analysis. For each individual focus, the area and mean fluorescence intensity were calculated. The following Python packages were used for the analysis: OpenCV (Itseez, 2015), mahotas (Cohelo, 2013), scikit-image (Walt et al., 2014).



***In vitro* ubiquitylation**

HA-tagged SLBP and FFSS-tagged cyclin F were *in vitro* translated (IVT) using the TNT T7 Quick Coupled Transcription/Translation System (Promega) according to the manufacturer's recommendations. HA-tagged SLBP was purified from the IVT lysate on Anti-HA Affinity Matrix beads (Roche). Phosphorylation of HA-tagged SLBP with recombinant CDK1-cyclin A2 (Thermo Fisher Scientific) was carried out on the HA beads in PK buffer (New England Biolabs) supplemented with 200 nM ATP at 30°C for 15 minutes. FFSS-tagged cyclin F was purified from the IVT lysate on anti-FLAG M2 Magnetic Beads (Sigma-Aldrich) and eluted with 3xFLAG peptides (Sigma-Aldrich) according to the manufacturer's recommendations. *In vitro* ubiquitylation reaction of purified HA-tagged SLBP by FFSS-tagged cyclin F was carried out in buffer containing 50 mM Tris HCL pH 7.5, 5 mM $MgCl_2$, 1 mM DTT, 1 mM ATP, 0.1 µM E1 (Boston Biochem), 0.25 µM UBC H5C (Boston Biochem), 0.25 µM UBC H3 (Bostom Biochem), 10 µM Ubiquitin, 3 µM Ubiquitin Aldehyde (Boston Biochem), 2.5 µM MG132, 0.20 nM Okadaic Acid, and 0.2 µM NaV. Reactions were incubated at 30°C, and terminated by adding NuPAGE® LDS sample buffer (Thermo Fisher Scientific) followed by incubation at 95°C. Samples were resolved by SDS PAGE and immunoblotted as indicated.

Supplemental References

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