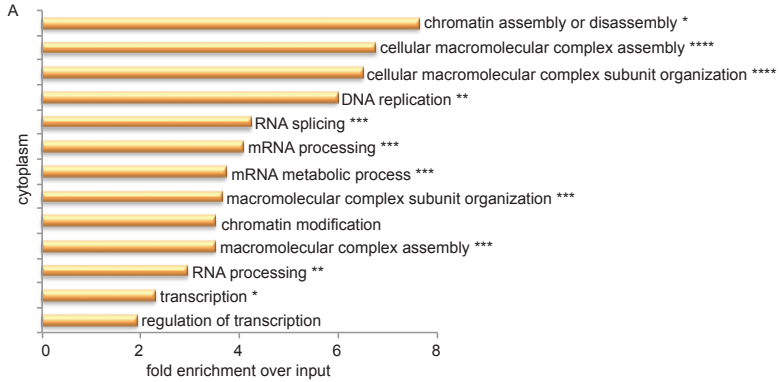
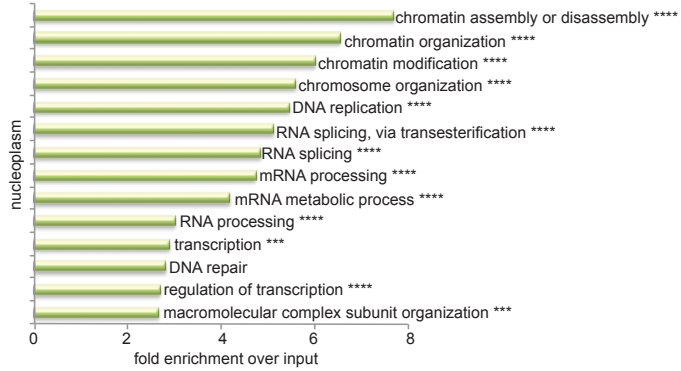
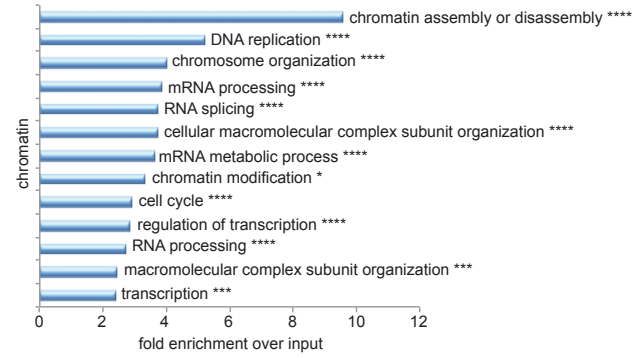
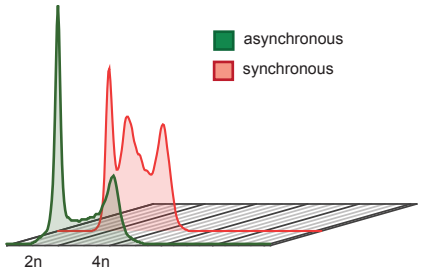

A
cytoplasm
chromatin assembly or disassembly *
cellular macromolecular complex assembly ****
cellular macromolecular complex subunit organization ****
DNA replication **
RNA splicing ***
mRNA processing ***
mRNA metabolic process ***
macromolecular complex subunit organization ***
chromatin modification
macromolecular complex assembly ***
RNA processing **
transcription *
regulation of transcription
0 2 4 6 8
fold enrichment over input

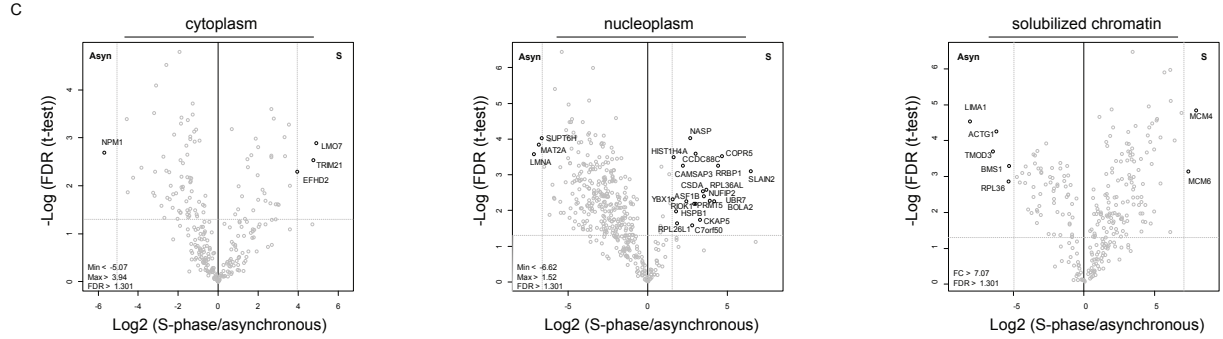

nucleoplasm
chromatin assembly or disassembly ****
chromatin organization ****
chromatin modification ****
chromosome organization ****
DNA replication ****
RNA splicing, via transesterification ****
RNA splicing ****
mRNA processing ****
mRNA metabolic process ****
RNA processing ****
transcription ***
DNA repair
regulation of transcription ****
macromolecular complex subunit organization ***
0 2 4 6 8
fold enrichment over input

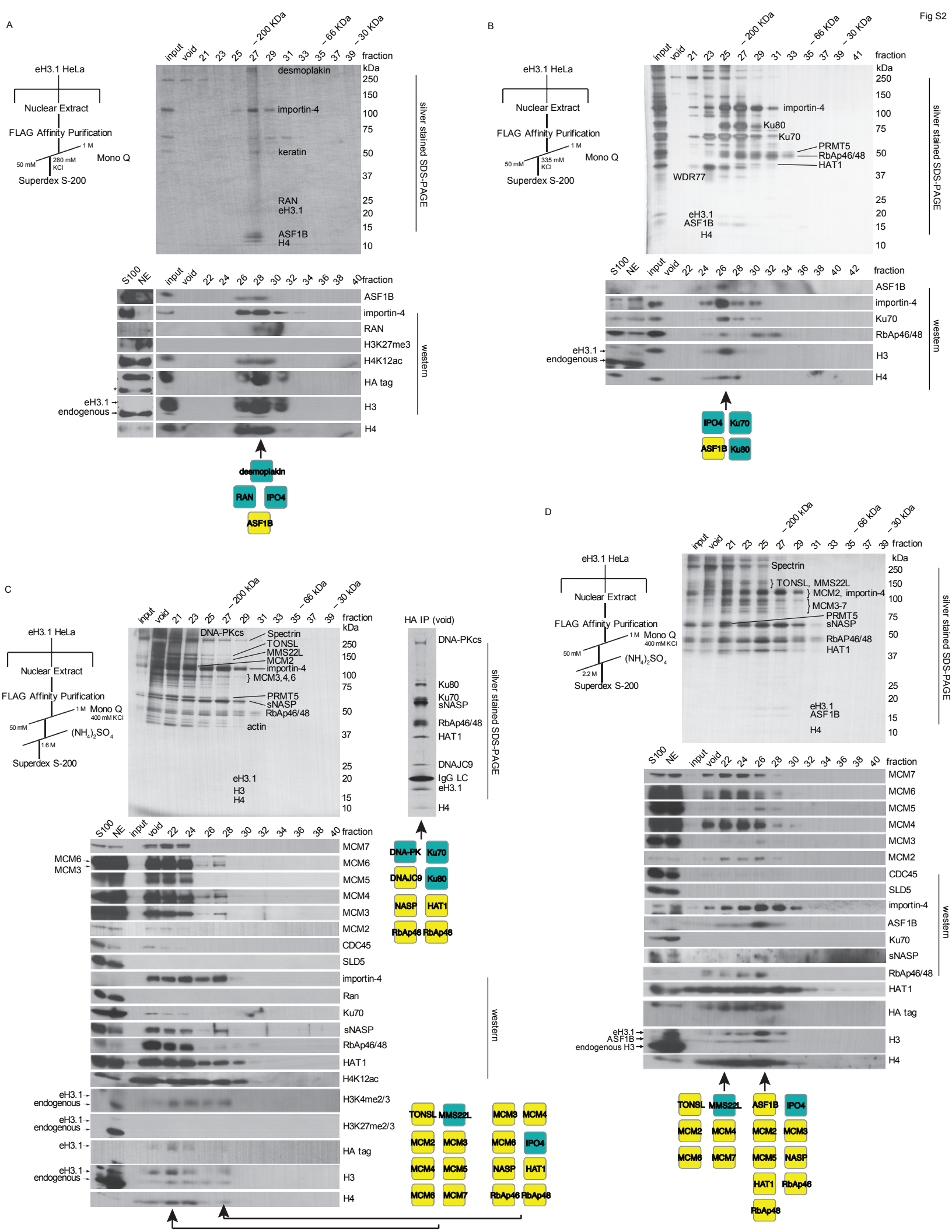

chromatin
chromatin assembly or disassembly ****
DNA replication ****
chromosome organization ****
mRNA processing ****
RNA splicing ****
cellular macromolecular complex subunit organization ****
mRNA metabolic process ****
chromatin modification *
cell cycle ****
regulation of transcription ****
RNA processing ****
macromolecular complex subunit organization ***
transcription ***
0 2 4 6 8 10 12
fold enrichment over input

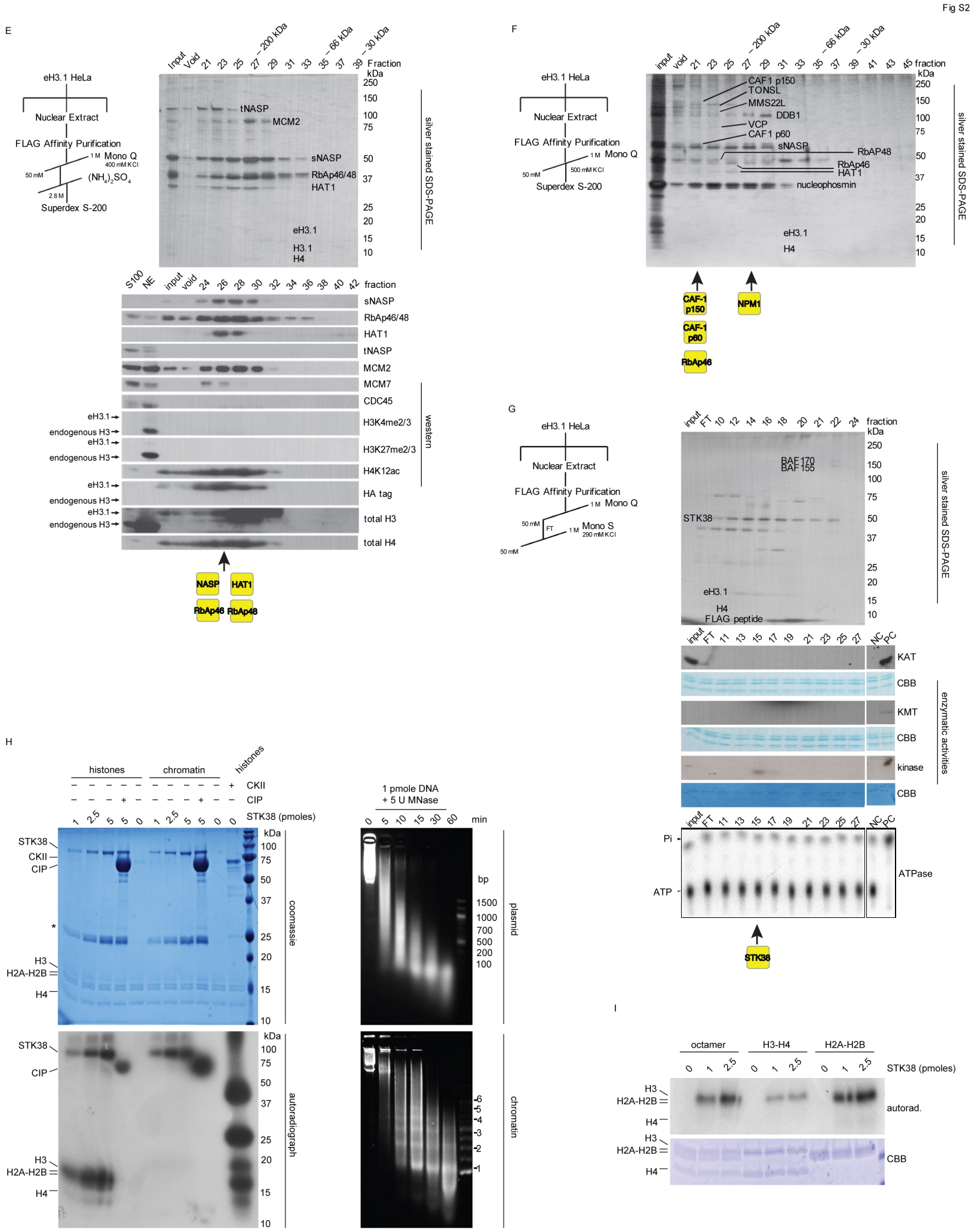
B

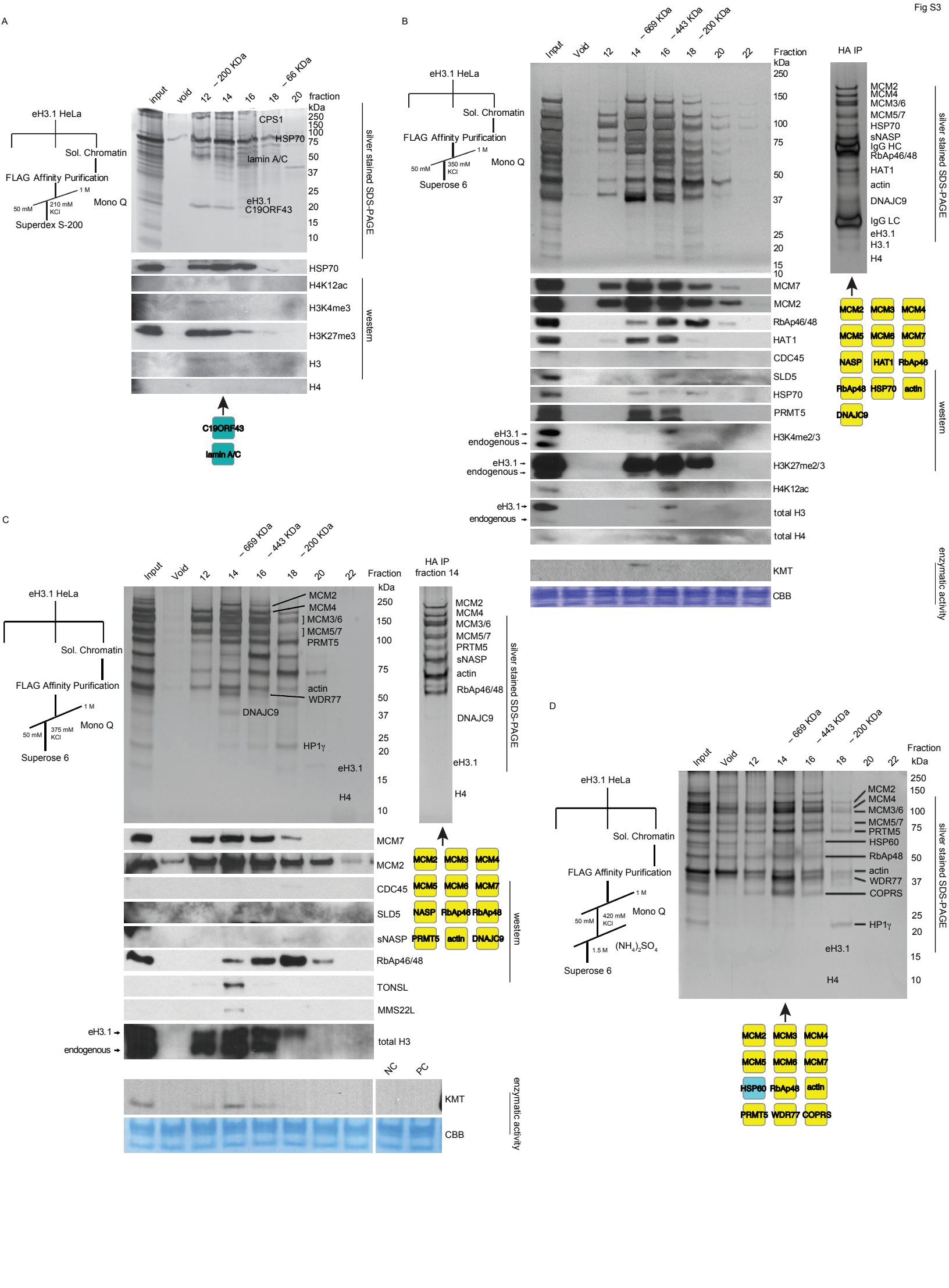

asynchronous
synchronous
2n
4n

C

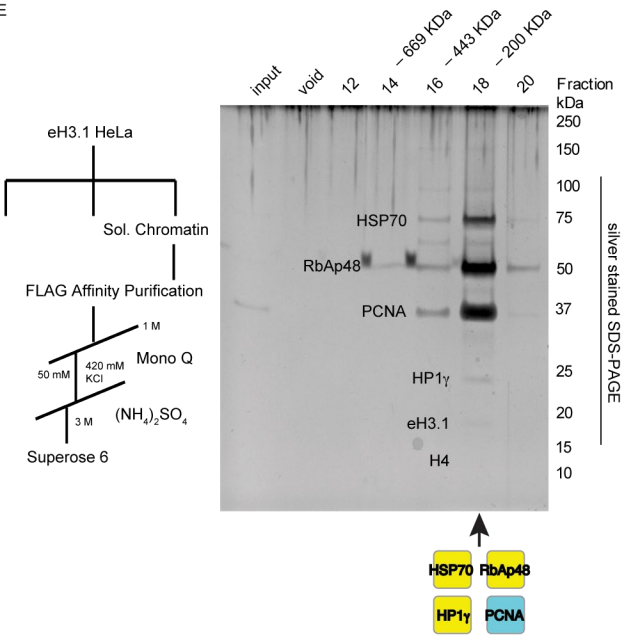

cytoplasm
nucleoplasm
solubilized chromatin
Asyn
S
-Log (FDR (t-test))
Log2 (S-phase/asynchronous)
NPM1
LMO7
TRIM21
EFHD2
Min < -5.07
Max > 3.94
FDR > 1.301
SUPT6H
MAT2A
LMNA
NASP
HIST1H4A
CCDC88C
COPR5
CAMSAP3
RRBP1
SLAIN2
CSDA
RPL36AL
NUFIP2
YBX1
ASF1B
UBR7
RDX
PHB1
BOLA2
HSPB1
CKAP5
RPL26L1
C7orf50
Min < -6.62
Max > 1.52
FDR > 1.301
LIMA1
ACTG1
TMOD3
BMS1
RPL36
MCM4
MCM6
FC > 7.07
FDR > 1.301


A
eH3.1 HeLa
Nuclear Extract
FLAG Affinity Purification
Mono Q
Superdex S-200
1 M
280 mM KCl
50 mM
desmoplakin
importin-4
keratin
RAN
eH3.1
ASF1B
H4
silver stained SDS-PAGE
S100
NE
input
void
fraction
ASF1B
importin-4
RAN
H3K27me3
H4K12ac
HA tag
H3
H4
western
eH3.1 endogenous
desmoplakin
RAN
IPO4
ASF1B
B
335 mM KCl
WDR77
Ku80
Ku70
PRMT5
RbAp46/48
HAT1
IPO4
Ku70
ASF1B
Ku80
C
DNA-PKcs
Spectrin
TONSL
MMS22L
MCM2
importin-4
} MCM3,4,6
PRMT5
sNASP
RbAp46/48
actin
eH3.1
H3
H4
HA IP (void)
Ku80
Ku70
sNASP
HAT1
DNAJC9
IgG LC
MCM7
MCM6
MCM5
MCM4
MCM3
MCM2
CDC45
SLD5
Ran
H4K12ac
H3K4me2/3
H3K27me2/3
DNA-PK
NASP
RbAp46
RbAp48
D
2.2 M
(NH4)2SO4
} TONSL, MMS22L
} MCM2, importin-4
} MCM3-7
endogenous H3

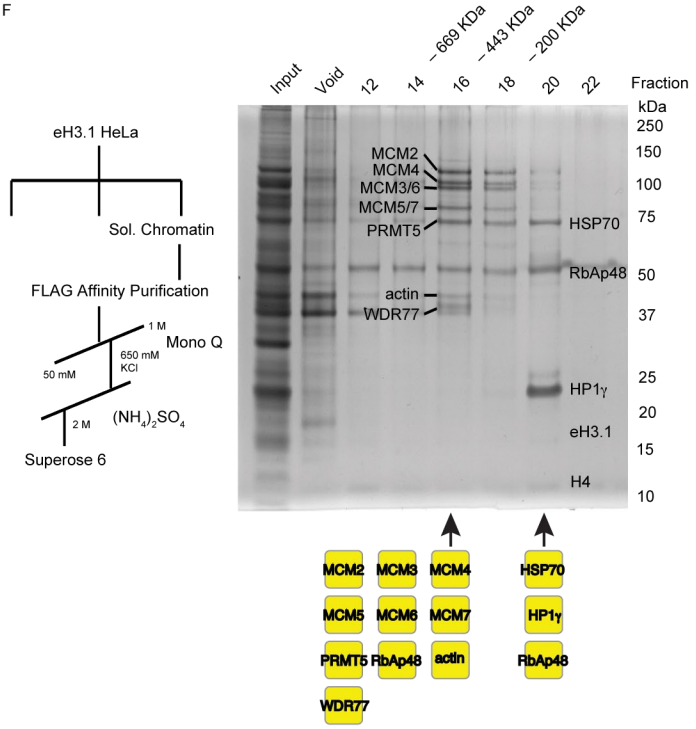

E
eH3.1 HeLa
Nuclear Extract
FLAG Affinity Purification
Mono Q
400 mM KCl
50 mM
(NH4)2SO4
2.8 M
Superdex S-200
Input
Void
Fraction
kDa
tNASP
MCM2
sNASP
RbAp46/48
HAT1
eH3.1
H3.1
H4
silver stained SDS-PAGE
S100
NE
input
void
fraction
sNASP
RbAp46/48
HAT1
tNASP
MCM2
MCM7
CDC45
H3K4me2/3
H3K27me2/3
H4K12ac
HA tag
total H3
total H4
endogenous H3
western
NASP
HAT1
RbAp46
RbAp48
F
eH3.1 HeLa
Nuclear Extract
FLAG Affinity Purification
Mono Q
Superdex S-200
CAF1 p150
TONSL
MMS22L
DDB1
VCP
CAF1 p60
sNASP
RbAP48
RbAp46
HAT1
nucleophosmin
eH3.1
H4
CAF-1 p150
CAF-1 p60
RbAp46
NPM1
G
eH3.1 HeLa
Nuclear Extract
FLAG Affinity Purification
Mono Q
Mono S
290 mM KCl
FT
BAF170
BAF155
STK38
eH3.1
H4
FLAG peptide
NC
PC
KAT
CBB
KMT
kinase
enzymatic activities
Pi
ATP
ATPase
H
histones
chromatin
CKII
CIP
STK38 (pmoles)
coomassie
1 pmole DNA
+ 5 U MNase
min
bp
plasmid
autoradiograph
chromatin
I
octamer
H3-H4
H2A-H2B
autorad.
CBB


A
eH3.1 HeLa
Sol. Chromatin
FLAG Affinity Purification
50 mM
210 mM KCl
1 M
Mono Q
Superdex S-200
input
void
– 200 KDa
– 66 KDa
fraction
kDa
CPS1
HSP70
lamin A/C
eH3.1
C19ORF43
silver stained SDS-PAGE
HSP70
H4K12ac
H3K4me3
H3K27me3
H3
H4
western
C19ORF43
lamin A/C
B
eH3.1 HeLa
Sol. Chromatin
FLAG Affinity Purification
50 mM
350 mM KCl
1 M
Mono Q
Superose 6
– 669 KDa
– 443 KDa
– 200 KDa
Input
Void
Fraction
HA IP
MCM7
MCM2
RbAp46/48
HAT1
CDC45
SLD5
HSP70
PRMT5
eH3.1 endogenous
H3K4me2/3
H3K27me2/3
H4K12ac
total H3
total H4
KMT
CBB
enzymatic activity
MCM2
MCM4
MCM3/6
MCM5/7
HSP70
sNASP
IgG HC
RbAp46/48
HAT1
actin
DNAJC9
IgG LC
eH3.1
H3.1
H4
C
eH3.1 HeLa
Sol. Chromatin
FLAG Affinity Purification
50 mM
375 mM KCl
1 M
Mono Q
Superose 6
– 669 KDa
– 443 KDa
– 200 KDa
Input
Void
Fraction
HA IP
fraction 14
MCM2
MCM4
] MCM3/6
] MCM5/7
PRMT5
actin
WDR77
DNAJC9
HP1γ
eH3.1
H4
sNASP
RbAp46/48
MCM7
CDC45
SLD5
TONSL
MMS22L
total H3
KMT
CBB
NC
PC
silver stained SDS-PAGE
western
enzymatic activity
D
eH3.1 HeLa
Sol. Chromatin
FLAG Affinity Purification
50 mM
420 mM KCl
1 M
Mono Q
1.5 M
(NH4)2SO4
Superose 6
– 669 KDa
– 443 KDa
– 200 KDa
Input
Void
Fraction
MCM2
MCM4
MCM3/6
MCM5/7
PRTM5
HSP60
RbAp48
actin
WDR77
COPRS
HP1γ
eH3.1
H4
silver stained SDS-PAGE

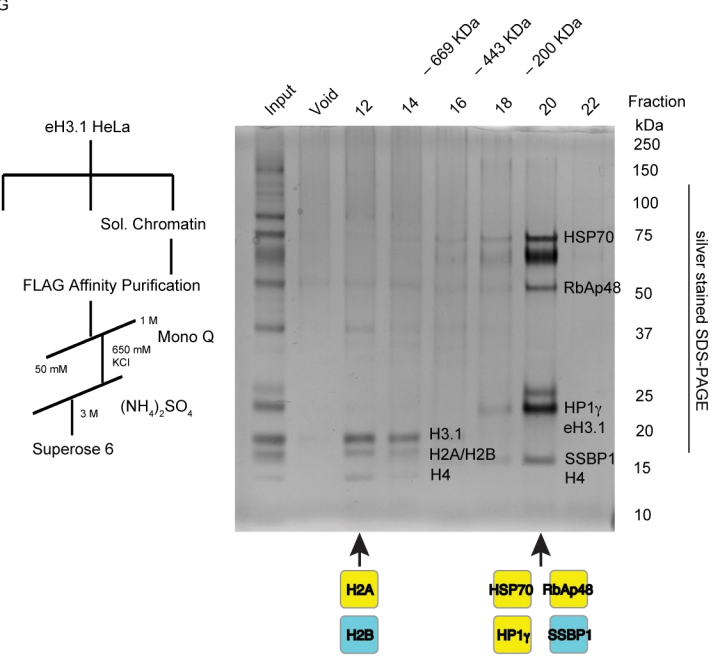
E


eH3.1 HeLa
Sol. Chromatin
FLAG Affinity Purification
1 M
420 mM KCl
Mono Q
50 mM
3 M
(NH4)2SO4
Superose 6
– 669 KDa
– 443 KDa
– 200 KDa
input
void
12
14
16
18
20
Fraction
kDa
250
150
100
75
50
37
25
20
15
10
HSP70
RbAp48
PCNA
HP1γ
eH3.1
H4
silver stained SDS-PAGE
HSP70
RbAp48
HP1γ
PCNA

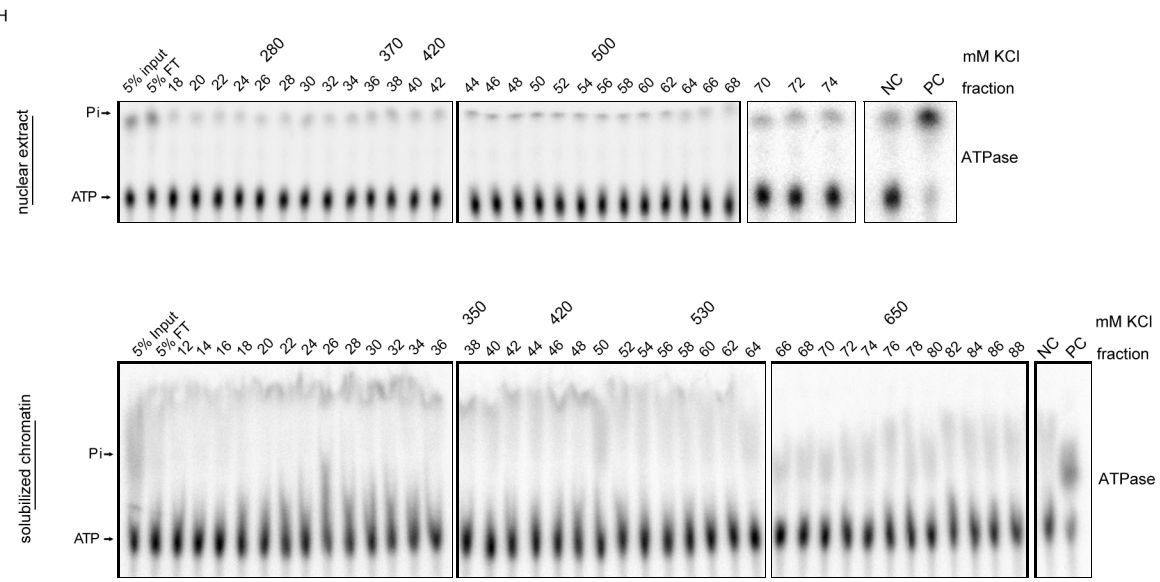
F


eH3.1 HeLa
Sol. Chromatin
FLAG Affinity Purification
1 M
650 mM KCl
Mono Q
50 mM
2 M
(NH4)2SO4
Superose 6
– 669 KDa
– 443 KDa
– 200 KDa
Input
Void
12
14
16
18
20
22
Fraction
kDa
250
150
100
75
50
37
25
20
15
10
MCM2
MCM4
MCM3/6
MCM5/7
PRMT5
actin
WDR77
HSP70
RbAp48
HP1γ
eH3.1
H4
MCM2
MCM3
MCM4
HSP70
MCM5
MCM6
MCM7
HP1γ
PRMT5
RbAp48
actin
RbAp48
WDR77

G


eH3.1 HeLa
Sol. Chromatin
FLAG Affinity Purification
1 M
650 mM KCl
Mono Q
50 mM
3 M
(NH4)2SO4
Superose 6
– 669 KDa
– 443 KDa
– 200 KDa
Input
Void
12
14
16
18
20
22
Fraction
kDa
250
150
100
75
50
37
25
20
15
10
HSP70
RbAp48
HP1γ
eH3.1
SSBP1
H4
H3.1
H2A/H2B
H4
silver stained SDS-PAGE
H2A
H2B
HSP70
RbAp48
HP1γ
SSBP1

H


nuclear extract
5% input
5% FT
18
20
22
24
26
28
30
32
34
36
38
40
42
280
370
420
44
46
48
50
52
54
56
58
60
62
64
66
68
500
70
72
74
NC
PC
mM KCl
fraction
Pi
ATP
ATPase
solubilized chromatin
5% Input
5% FT
12
14
16
18
20
22
24
26
28
30
32
34
36
350
420
530
38
40
42
44
46
48
50
52
54
56
58
60
62
64
650
66
68
70
72
74
76
78
80
82
84
86
88
NC
PC
mM KCl
fraction
Pi
ATP
ATPase

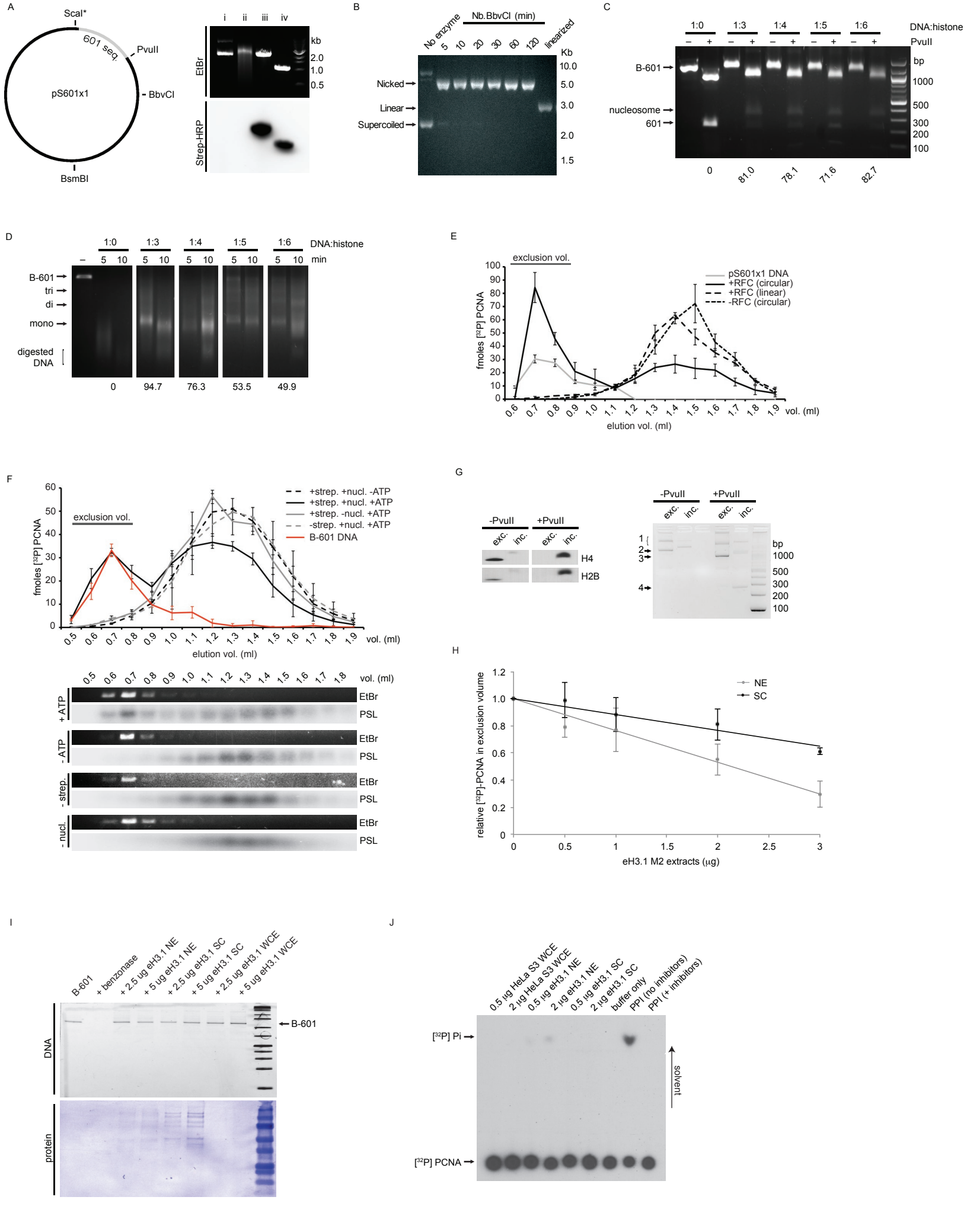
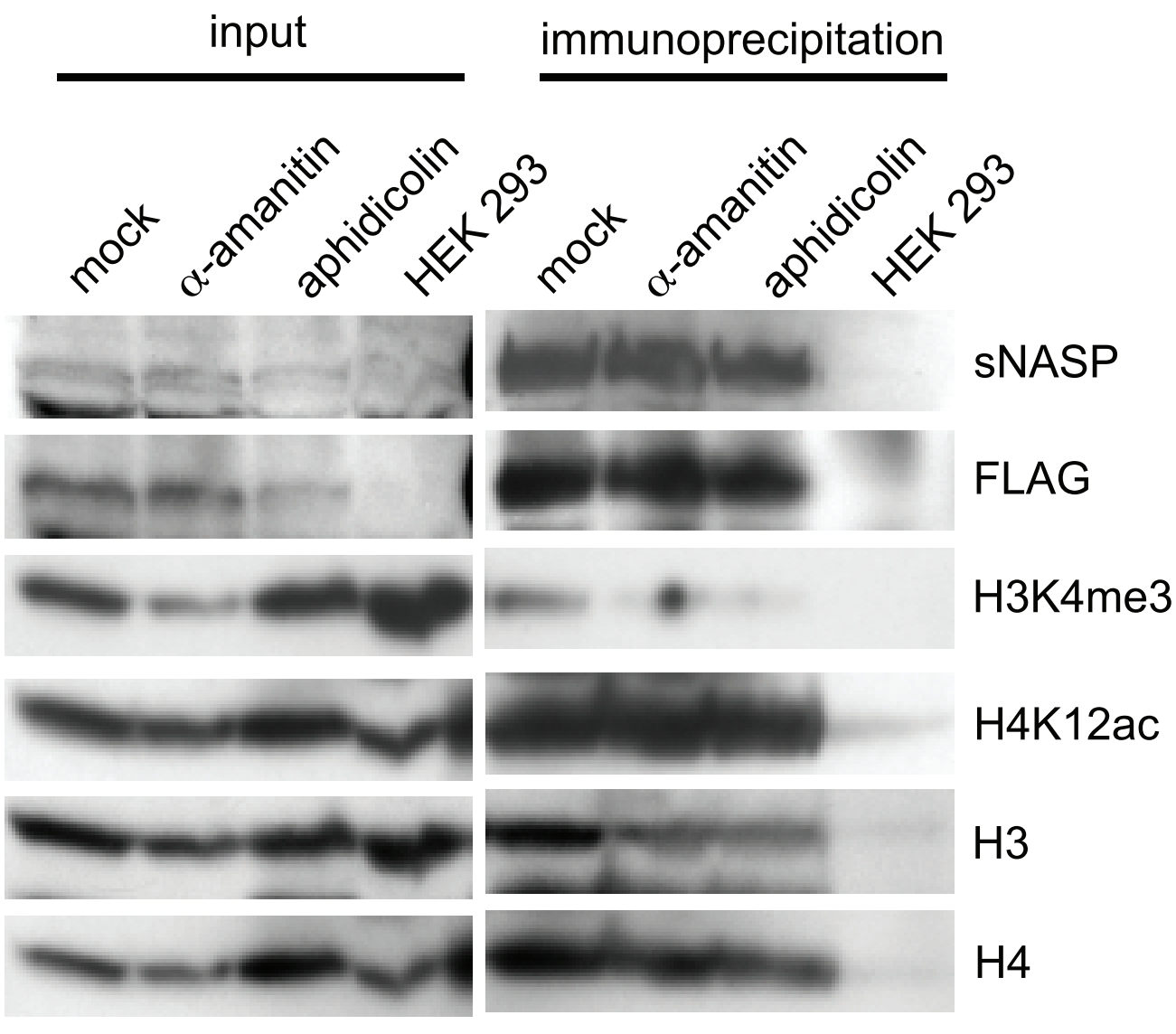
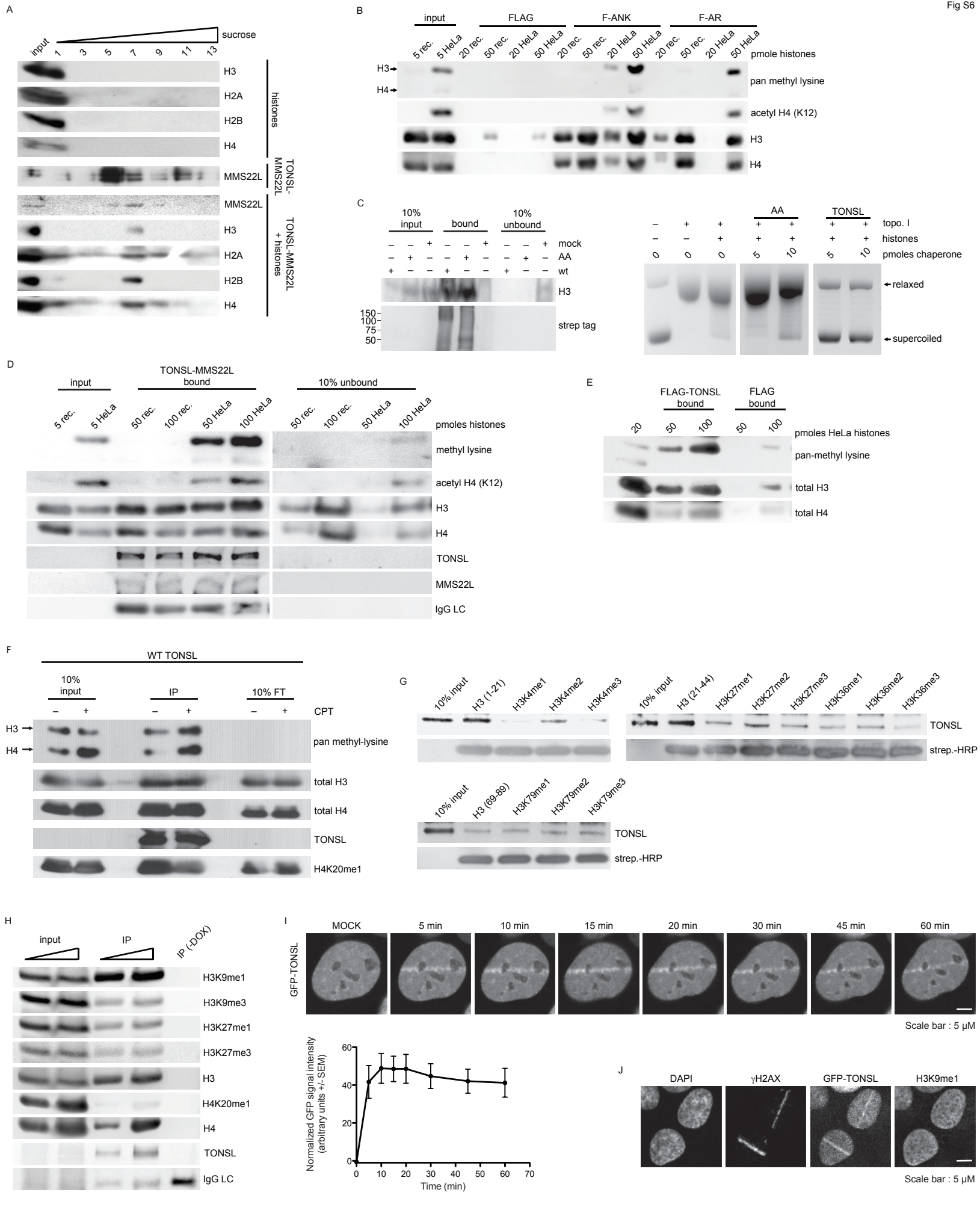
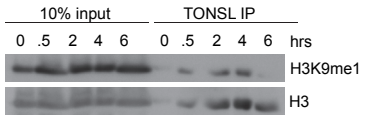

A
ScaI*
601 seq.
PvuII
pS601x1
BbvCI
BsmBI
i ii iii iv
EtBr
Strep-HRP
kb
2.0
1.0
0.5
B
No enzyme
Nb.BbvCI (min)
5 10 20 30 60 120
linearized
Kb
Nicked
Linear
Supercoiled
10.0
5.0
3.0
2.0
1.5
C
1:0 1:3 1:4 1:5 1:6
DNA:histone
PvuII
B-601
nucleosome
601
bp
1000
500
300
200
100
0 81.0 78.1 71.6 82.7
D
1:0 1:3 1:4 1:5 1:6
DNA:histone
min
B-601
tri
di
mono
digested DNA
0 94.7 76.3 53.5 49.9
E
exclusion vol.
pS601x1 DNA
+RFC (circular)
+RFC (linear)
-RFC (circular)
fmoles [32P] PCNA
elution vol. (ml)
vol. (ml)
F
exclusion vol.
+strep. +nucl. -ATP
+strep. +nucl. +ATP
+strep. -nucl. +ATP
-strep. +nucl. +ATP
B-601 DNA
fmoles [32P] PCNA
elution vol. (ml)
vol. (ml)
+ ATP
- ATP
- strep.
- nucl.
EtBr
PSL
G
-PvuII
+PvuII
exc.
inc.
H4
H2B
bp
1000
500
300
200
100
H
NE
SC
relative [32P]-PCNA in exclusion volume
eH3.1 M2 extracts (μg)
I
B-601
+ benzonase
+ 2.5 ug eH3.1 NE
+ 5 ug eH3.1 NE
+ 2.5 ug eH3.1 SC
+ 5 ug eH3.1 SC
+ 2.5 ug eH3.1 WCE
+ 5 ug eH3.1 WCE
DNA
protein
B-601
J
0.5 μg HeLa S3 WCE
2 μg HeLa S3 WCE
0.5 μg eH3.1 NE
2 μg eH3.1 NE
0.5 μg eH3.1 SC
2 μg eH3.1 SC
buffer only
PPI (no inhibitors)
PPI (+ inhibitors)
[32P] Pi
[32P] PCNA
solvent

Fig S5

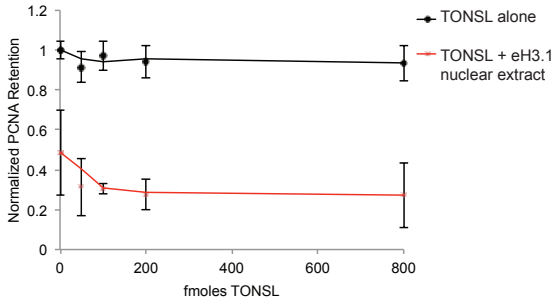

input
immunoprecipitation
mock
α-amanitin
aphidicolin
HEK 293
mock
α-amanitin
aphidicolin
HEK 293
sNASP
FLAG
H3K4me3
H4K12ac
H3
H4

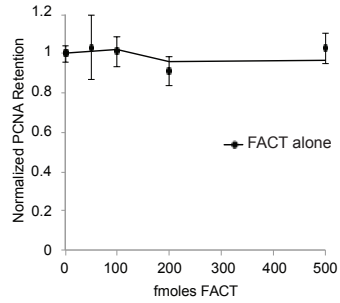

A
input
sucrose
1 3 5 7 9 11 13
H3
H2A
H2B
H4
histones
MMS22L
TONSL-MMS22L
MMS22L
H3
H2A
H2B
H4
TONSL-MMS22L + histones
B
input
FLAG
F-ANK
F-AR
5 rec. 5 HeLa 20 rec. 50 rec. 20 HeLa 50 HeLa 20 rec. 50 rec. 20 HeLa 50 HeLa 20 rec. 50 rec. 20 HeLa 50 HeLa
pmole histones
H3
H4
pan methyl lysine
acetyl H4 (K12)
H3
H4
C
10% input
bound
10% unbound
mock
AA
wt
H3
150
100
75
50
strep tag
AA
TONSL
topo. I
histones
pmoles chaperone
0 0 0 5 10 5 10
relaxed
supercoiled
D
input
TONSL-MMS22L bound
10% unbound
5 rec. 5 HeLa 50 rec. 100 rec. 50 HeLa 100 HeLa 50 rec. 100 rec. 50 HeLa 100 HeLa
pmoles histones
methyl lysine
acetyl H4 (K12)
H3
H4
TONSL
MMS22L
IgG LC
E
FLAG-TONSL bound
FLAG bound
20 50 100 50 100
pmoles HeLa histones
pan-methyl lysine
total H3
total H4
F
WT TONSL
10% input
IP
10% FT
CPT
H3
H4
pan methyl-lysine
total H3
total H4
TONSL
H4K20me1
G
10% input
H3 (1-21)
H3K4me1
H3K4me2
H3K4me3
10% input
H3 (21-44)
H3K27me1
H3K27me2
H3K27me3
H3K36me1
H3K36me2
H3K36me3
TONSL
strep.-HRP
10% input
H3 (69-89)
H3K79me1
H3K79me2
H3K79me3
TONSL
strep.-HRP
H
input
IP
IP (-DOX)
H3K9me1
H3K9me3
H3K27me1
H3K27me3
H3
H4K20me1
H4
TONSL
IgG LC
I
MOCK
5 min
10 min
15 min
20 min
30 min
45 min
60 min
GFP-TONSL
Scale bar : 5 µM
Normalized GFP signal intensity (arbitrary units +/- SEM)
Time (min)
J
DAPI
γH2AX
GFP-TONSL
H3K9me1
Scale bar : 5 µM

A

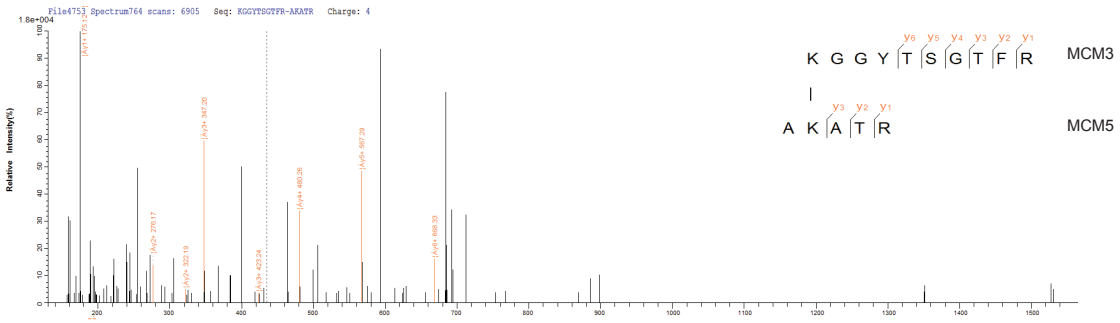

10% input
TONSL IP
0 .5 2 4 6 0 .5 2 4 6 hrs
H3K9me1
H3

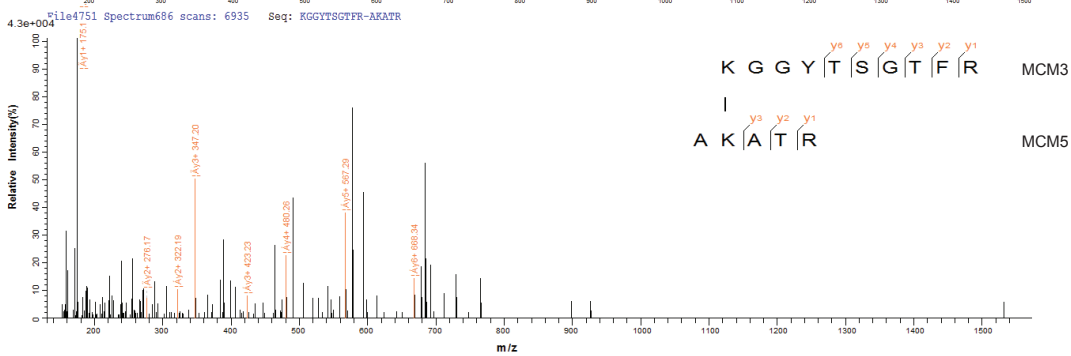
B

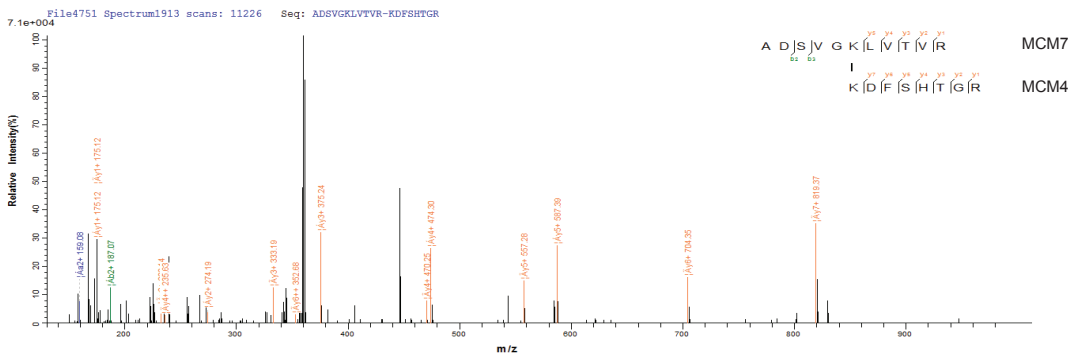

TONSL alone
TONSL + eH3.1 nuclear extract
Normalized PCNA Retention
fmoles TONSL


FACT alone
Normalized PCNA Retention
fmoles FACT

C


File4753 Spectrum764 scans: 6905 Seq: KGGYTSGTFR-AKATR Charge: 4
K G G Y T S G T F R MCM3
A K A T R MCM5
Relative Intensity(%)


File4751 Spectrum686 scans: 6935 Seq: KGGYTSGTFR-AKATR
K G G Y T S G T F R MCM3
A K A T R MCM5
Relative Intensity(%)
m/z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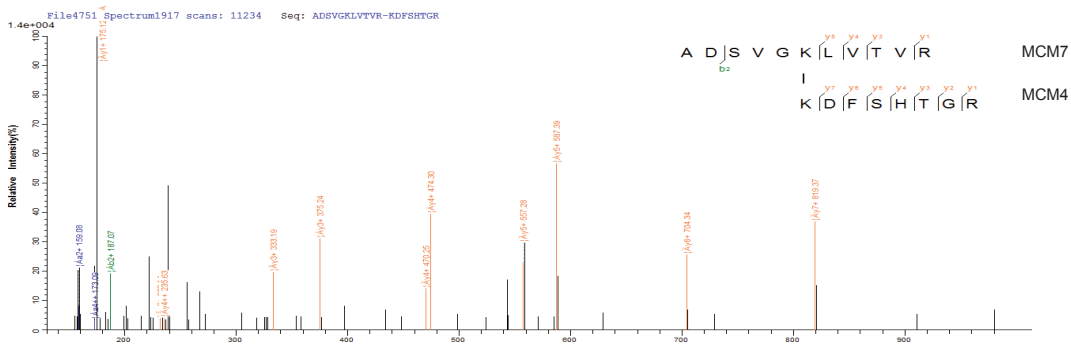
Relative Intensity(%)
m/z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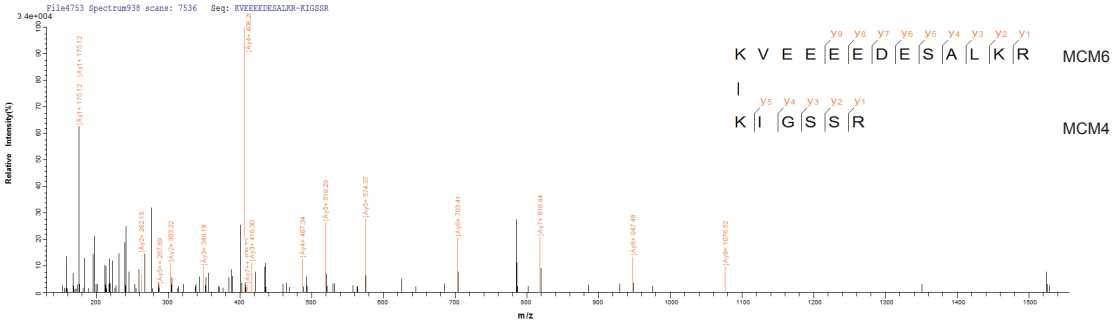
Relative Intensity(%)
m/z

C (cont'd)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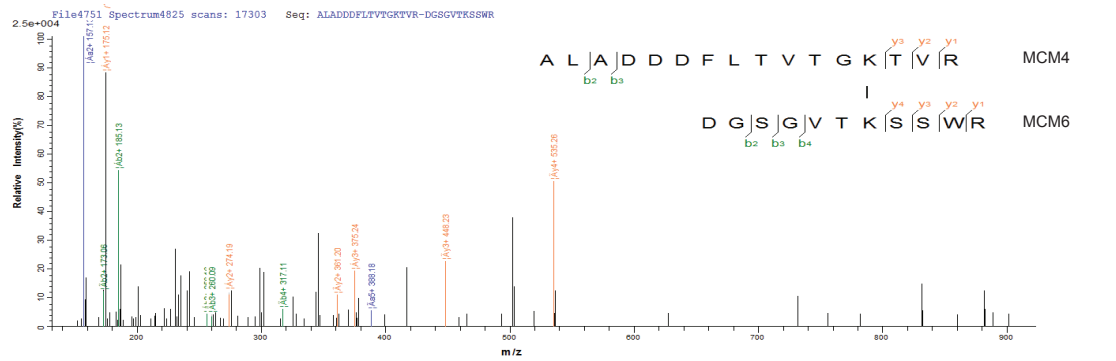
Relative Intensity(%)
m/z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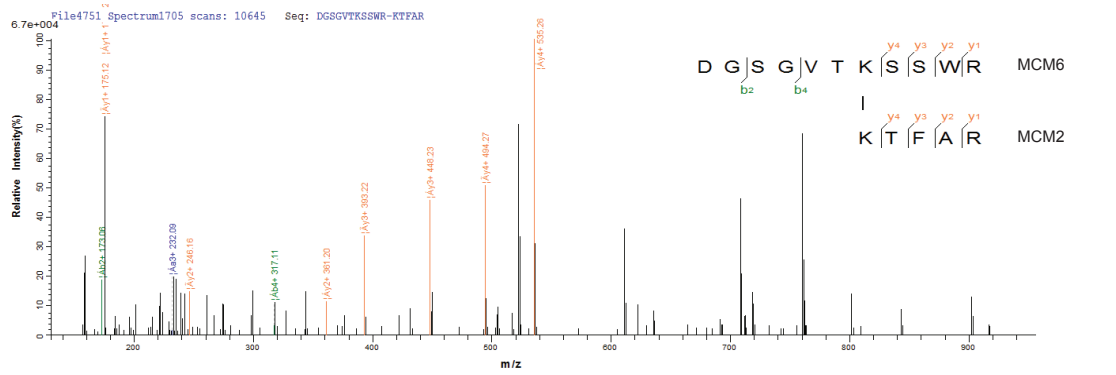
Relative Intensity(%)
m/z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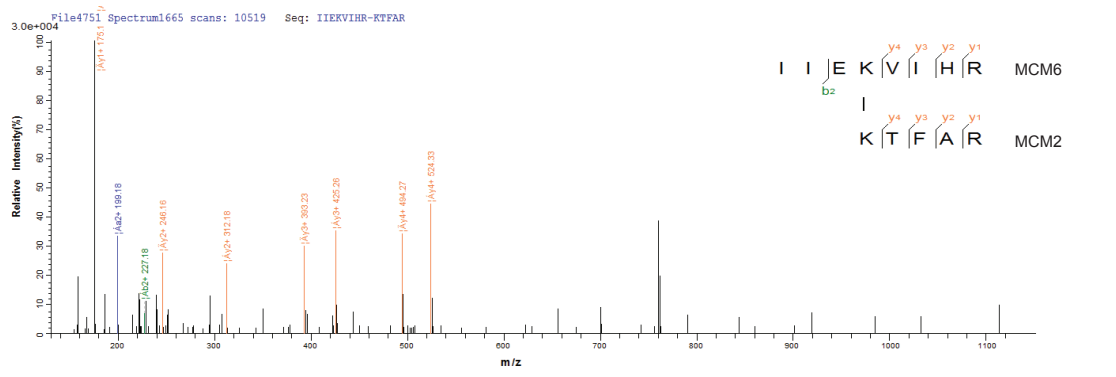
Relative Intensity(%)
m/z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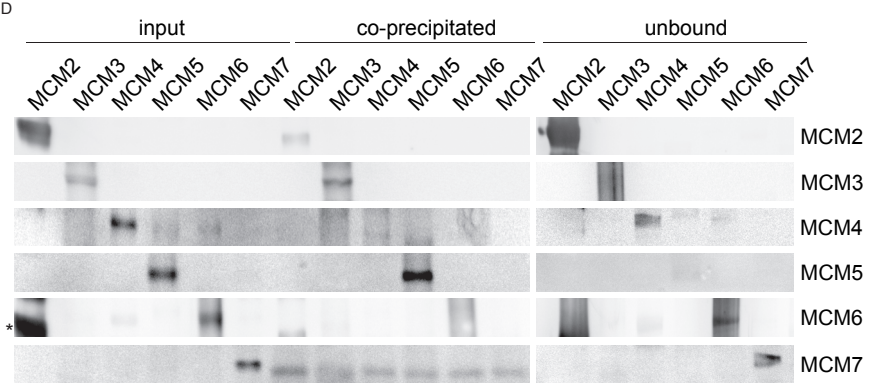
Relative Intensity(%)
m/z

D


input
co-precipitated
unbound
MCM2
MCM3
MCM4
MCM5
MCM6
MCM7
*

SUPPLEMENTAL DATA

Figure S1, Related to Figure 1. (A) Ontology groups associated with H3.1-associated proteins. (B) Cell cycle distribution of cells used to compare H3.1 interactions between asynchronous and synchronized, replicating cells. (C) Quantitative MS analysis of affinity purified H3.1 material. Each volcano plot represents three independent H3.1 pull-downs comparing asynchronous cell extracts to extracts from replicating cells. The *x* axis denotes the intensity of the MS signal whereas a false discovery rate (FDR) adapted t-test is on the *y* axis.

Figure S2, Related to Figure 2. (A-G) Purification schemes (left panels) and silver and western analyses of soluble H3.1 protein complexes from nuclear extracts (right panels). (G) Figure is composed of separate panels, as extraneous samples are not shown. (H) *In vitro* kinase assay using recombinant STK38 and free or nucleosomal HeLa histones (left panels). Micrococcal nuclease digest of the chromatin template used for the kinase assay compared (right panels). (I) *In vitro* kinase assay comparing the phosphorylation of H3-H4 and H2A-H2B by STK38. NC: negative control, PC: positive control, CBB: Coomassie Brilliant Blue, KAT: lysine acetyltransferase, KMT: lysine methyltransferase.

Figure S3, Related to Figure 2. (A-G) Purification schemes (left panels) and silver and western analyses of soluble H3.1 protein complexes from solubilized chromatin (right panels). (H) ATPase activity within eH3.1 fractions. Figures are composed of separate panels as extraneous samples are not shown. Controls were loaded on the last TLC plate.

NC: negative control, PC: positive control, CBB: Coomassie Brilliant Blue, KMT: lysine methyltransferase.

Figure S4, Related to Figure 3. Nucleosome disassembly assay. (A-D) Substrate preparation. (A) The pS601x1 plasmid (i) encompassing the DNA substrate is first digested with with BsmBI (ii), the overhang is filled in with biotinylated dNTPs (iii), further digested with ScaI and gel purified (iv). (B) The substrate is nicked with Nb.BbvCI alongside a circular plasmid control, to allow for PCNA loading by RFC. (C-D) Careful histone titration allows a single nucleosome to be formed by salt dialysis over the DNA substrate. (C) PvuII digest releasing the nucleosome and relative quantification of assembled histones (percentage). (D) Micrococcal nuclease digest and relative quantification of mononucleosomes (percentage). (E) Radiolabeled PCNA loading by RFC over circular and linear nicked DNA in the absence of histones. (F) Radiolabeled PCNA loading by RFC over linear nicked DNA in the presence or absence of ATP, streptavidin (strep.), or nucleosomal histones (nucl.). (G) Histone and DNA distribution within the inclusion (inc.) and exclusion (exc.) volumes of the gel filtration resin. (1) DNA substrates co-bound by the same streptavidin complex; (2) Full length DNA substrate; (3-4) cleaved DNA substrates. (H) Nucleosome disassembly assay comparing affinity purified eH3.1 material from nuclear extracts or solubilized chromatin. Data represents mean normalized [32P]-PCNA retention +/- SD. (I) B-601 substrate after incubation with either benzonase or the indicated eH3.1 purified extracts at 37 °C for 1 hour. (J) Phosphatase activity with the eH3.1 affinity purified extracts as observed by thin layer chromatography (TLC) resolving free inorganic phosphate (Pi) from radiolabeled

PCNA. EtBr = ethidium bromide; PSL = photostimulated luminescence; WCE = whole cell extract; NE = nuclear extract; SC = solubilized chromatin; PP1 = Protein Phosphatase 1

Figure S5, related to Figure 4. FLAG-sNASP immunoprecipitation from mock-treated 293 cells, or cells exposed to either 50 nM α-amanitin or 1 mM aphidicolin for 24 hrs.

Figure S6, related to Figure 5. TONSL binds histone H3 monomethylated on lysine 9. (A) Sucrose density sedimentation of recombinant TONSL-MMS22L with or without HeLa histones. (B) Pull-down assay with immobilized FLAG, FLAG-ankyrin repeats (ANK), and FLAG-acidic region (AR) of TONSL, incubated with recombinant (rec.) or HeLa (modified) histones. (C) Left panel: Binding of HeLa histones to immobilized full-length TONSL or a protein fragment encompassing the acidic region and ankyrin repeats (AA); Right panel: Supercoiling assay comparing the full-length TONSL protein to the AA protein fragment. (D) Pull-down assay as in (B) using immobilized recombinant TONSL-MMS22L. (E) Histones bound to immobilized TONSL (F) Exogenous FLAG-TONSL immunoprecipitated from untreated or camptothecin-treated U2OS cells. (G) Pull-down assay with immobilized histone peptides incubated with recombinant TONSL. (H) Western analysis of histone marks co-immunoprecipitating with TONSL. (I) UV microirradiation of U2OS cells expressing GFP-TONSL. Data represents arbitrary units +/- SEM. (J) Histone marks over UV microirradiated U2OS cells.

Figure S7, related to Figure 5. (A) Immunoprecipitated TONSL from U2OS cells exposed to 1 μM camptothecin and collected at the indicated time points. (B) Nucleosome disassembly (as in Figure 3). Left panel: Recombinant TONSL was titrated over 100 fmoles of the nucleosomal substrate either by itself (black line) or in the presence of 1.5 μg of eH3.1-purified nuclear extracts (red line). Right panel: Recombinant FACT was titrated over 100 fmoles of the nucleosomal template by itself. Data represents mean normalized [32P]-PCNA retention +/- SD. (C) Representative MS/MS HCD spectrum of the (M + H)+4 ion of cross-linked peptides within the TONSL-MCM2-7 complex, acquired after reduction and alkylation on the QExactive with 70,000 resolution (@ 400 m/z). N-terminal fragment ions (*b*) are indicated in blue and C-terminal fragment ions (*y*) are indicated in green and red. Doubly charged ions are indicated with +2. The mass accuracy for precursor ion is better than 1 ppm and mass accuracy of all the fragment ions is better than 10 ppm. (D) Binding of purified recombinant MCM subunits to immobilized TONSL. Asterisk denotes degraded MCM2 protein.

Table S1, Related to Figure 1. Quantitative mass spectrometry of eH3.1-associated polypeptides. Raw data can be found through the following link: https://chorusproject.org/anonymous/download/experiment/a2600c95c5944e79aa6fe2d541a864fe

Table S2, Related to Figure 2. Polypeptides co-purifying with soluble H3.1. Histone species solely acetylated on H4K5 and K12 (with or without concomitant H3K9me1) are considered as newly-synthesized, whereas histones containing an assortment of any additional modifications, such as H3K27me2/3 or H3K4me2/3 are considered evicted, pre-existing, nuclear histones. Complexes containing both endogenous and exogenous H3 are considered to be in (H3-H4)2 tetrameric forms.

Table S3, Related to Figure 5. MS analysis of polypeptides co-purifying with immunoprecipitated TONSL from DSS-crosslinked cells.

figure	purification scheme	proteins co-eluting with eH3.1	theoretical MW (kDa)	approx. MW (kDa)	endo. H3	H4K12 ac	H3K4/27 me3
S2A	NE / M2 / Q(0.28) / GF	desmoplakin, importin-4, ASF1B, RAN, H4	541.1 • without desmoplakin: 209.3	200	no	yes	no
S2B	NE / M2 / Q(0.34) / GF	importin-4, Ku80, Ku70, ASF1B, H4	317.5	275	no	n.d.	n.d.
S2C	NE / M2 / Q(0.4) / AS(1.6) / GF / HA	DNA-PKcs, Ku80, Ku70, DNAJC9, sNASP, HAT1, RbAp46/48, H4	824.4	>700	no	n.d.	n.d.
S2C	NE / M2 / Q(0.4) / AS(1.6) / GF	MCM2-7/TONSL/MMS22L, H4	839.2	700	yes	yes	yes
S2C	NE / M2 / Q(0.4) / AS(1.6) / GF	MCM3, 4, 6, importin-4, sNASP, HAT1, RbAp46/48, H4	• MCM3, 4, 6: 280.4 • sNASP, HAT1, RbAp46, H3, H4: 172.9	200	yes	yes	yes
S2D	NE / M2 / Q(0.4) / AS(2.2) / GF	MCM2, 4, 6, 7, TONSL, MMS22L H4	692.7	700	no	n.d.	n.d.
S2D	NE / M2 / Q(0.4) / AS(2.2) / GF	MCM2, 3, 5, importin-4, ASF1B, sNASP, HAT1, RbAp46/48, H4	• MCM2, 3, 5, ASF1B, H3, H4: 324.4 • (sNASP, HAT1, RbAp46, H3, H4)$_2$: 345.8	330	no	n.d.	n.d
S2E	NE / M2 / Q(0.4) / AS(2.8) / GF	sNASP, HAT1, RbAp46/48, H4	172.9 • (sNASP, HAT1, RbAp46, H3, H4)$_2$: 345.8	275	yes	yes	no
4A	NE / M2 / Q(0.4) / AS(3.4) / GF	sNASP, HAT1, RbAp46/48, H4	172.9 • (sNASP, HAT1, RbAp46, H3, H4)$_2$: 345.8	275	no	yes	yes
S2F	NE / M2 / Q(0.5) / GF	CAF-1 p150, CAF-1 p60, RbAp48	216.2	700			
S2F	NE / M2 / Q(0.5) / GF	Nucleophosmin, H4	59.3	450 & 200	n.d	n.d,	n.d.
S2G	NE / M2 / S(0.29)	STK38, H4	81	n.d.	n.d.	n.d.	n.d.
S2H	SNP / M2 / Q(0.21) / GF	lamin A/C, C19ORF43, H4	100.2	225	no	no	yes
S2I	SNP / M2 / Q(350) / GF / HA	MCM2-7, HSP70 protein 9, sNASP, RbAp46/48, HAT1, DNAJC9, H4	822.4 • MCM2-7, H3, H4: 572.7	600	yes	yes	yes
S2J	SNP / M2 / Q(375) / GF / HA	MCM2-7, PRMT5, sNASP, RbAp46/48, DNAJC9, actin, H4	905.1 • MCM2-7, actin, DNAJC9, H3, H4: 688.1	670	yes	n.d.	n.d.
S2K	SNP / M2 / Q(420) / AS(1.5) / GF	MCM2-7, HSP60, RbAp48, actin, PRMT5, WDR77, COPRS, H4	846.8 • MCM2-7, actin, HSP60, RbAp48, H3, H4: 723.3	670	n.d.	n.d.	n.d.
S2L	SNP / M2 / Q(420) / AS(3) / GF	HSP70, RbAp48, PCNA, HP1γ, H4	196.5	200	n.d.	n.d.	n.d.
S2M	SNP / M2 / Q(650) / AS(2) / GF	MCM2-7, RbAp48, actin, PRMT5, WDR77, H4	765.6 • MCM2-7, actin, RbAp48, H3, H4: 662.2	450	n.d.	n.d.	n.d.
S2M	SNP / M2 / Q(650) / AS(2) / GF	HSP70, RbAp48, HP1γ, H4	167.7	150	n.d.	n.d.	n.d.
S2N	SNP / M2 / Q(650) / AS(3) / GF	HSP70, RbAp48, HP1γ, SSBP1, H4	184.9	150	n.d.	n.d.	n.d.
S2N	SNP / M2 / Q(650) / AS(3) / GF	H3, H2A, H2B, H4		>700	n.d.	n.d.	n.d.

NE: nuclear extract (nucleosol)
SNP: solubilized nuclear pellet (chromatin)
M2: FLAG affinity purification
Q(x): anion exchange [KCl] (M)
S(x): cation exchange [KCl] (M)
AS(x): salting out [$(NH_4)_2SO_4$] (M)
GF: gel filtration
HA: hemagglutinin affinity purification
n.d.: not determined

EXTENDED EXPERIMENTAL PROCEDURES

Constructs, Antibodies, Cell lines and Tissue Culture

The FLAG-HA H3.1 (eH3.1) Hela S3 lineage, FLAG-ASF1B and FLAG-sNASP 293 clones, as well as the inducible FLAG-TONSL U2OS cells were cultured as previously described (Campos et al., 2010; O'Donnell et al., 2010; Tagami et al., 2004). Thymidine-based cell synchronization, laser irradiation and immunofluorescence were performed as before (Campos et al., 2010; O'Donnell et al., 2010). TONSL (GenBank accession number BC008782.2) and MMS22L (NM_199467.2) cDNAs were obtained from OpenBiosystems and subcloned into pFASTBAC vectors (Invitrogen). Baculoviruses were generated using the Bac-to-Bac system (Invitrogen) as directed by the manufacturer. TONSL was further subcloned into pET28 (EMD Biosciences).

Thymidine, α-amanitin, and aphidicolin were purchased from Sigma-Aldrich. The following antibodies and reagents were used in addition to the ones that had been previously described (Campos et al., 2010; O'Donnell et al., 2010): CDC45 (Abcam), H2A (Cell Signaling), H2B (Millipore), H3K9me1 (Abcam), H3K27me1 (Millipore), H3K27me3 (Cell Signaling), H4me3 (Upstate), H4K20me1 (Abcam), Ku70 (Abcam), MCM2 (Bethyl), MCM3 (Bethyl), MCM4 (Bethyl), MCM5 (Pierce), MCM6 (Bethyl), MMS22L (SCBT), nucleophosmin (Abcam), pan-methyl lysine (Abcam), PRMT5 (Abcam), TONSL (Pierce), RAN (SCBT), SLD5 (Proteintech), streptavidin-HRP (Jackson ImmunoResearch). Restriction enzymes were obtained from NEB.

Biochemical fractionation

The eH3.1 chromatin pellet from a ~200 L culture was resuspended and dounce homogenized with a loose pestle in 50 ml of buffer E (50 mM Tris, pH7.6, 25% glycerol (v/v), 5 mM $MgCl_2$, 0.5 mM EDTA, 5 mM DTT, 0.2 mM PMSF). The soluble material was then separated from insoluble chromatin and lamina by adding ammonium sulfate to 300 mM (final concentration). Chromatin was then sonicated on ice and centrifuged at 185,000 x *g* for 1 hour to separate the solubilized protein complexes from the insoluble chromatin and lamina. The supernatant was collected, diluted two folds in buffer E, and two volumes of saturated ammonium sulfate solution slowly added to precipitate the protein complexes. Precipitates were pelleted by centrifugation (35,000 x *g* for 30 min) and re-suspended in BC50 (20 mM Tris, pH 7.6, 50 mM NaCl, 10% glycerol (v/v), 0.2 mM EDTA, 10 mM β-mercaptoethanol) and dialyzed against the same buffer. Salting-out was performed by adding ammonium sulfate to the indicated concentrations and centrifuging at 30,000 x *g* for 30 min. Ion exchange, gel filtration chromatography, immunoprecipitations, and silver stains were performed as described (Campos et al., 2010). Protein-protein interaction plots were generated by comparing the purified proteins to experimental results in STRING (Franceschini et al., 2013), and organized using Cytoscape (Shannon et al., 2003).

Density gradient sedimentation of recombinant proteins were performed as described (Campos et al., 2010) at 4°C using an SW60-Ti rotor (Beckman Coulter). Histone–TONSL-MMS22L interactions were resolved over 15-30% linear sucrose gradients in BC300 + 0.05% NP-40 (v/v), centrifuged at 42,000 RPM in for 16 hrs. *In vitro* translation was performed as described by the manufacturer (TNT system—Promega).

Enzymatic Assays

Histone acetyltrasferase and methyltransferase reactions were performed as described (Campos et al., 2010) using purified HeLa histones. Recombinant p300 (Abcam) and G9A (Abcam) were respectively used as positive controls. Kinase reactions were performed using [γ -^{32}P] ATP (Perkin-Elmer) and 1 µl of 1:10 dilutions of the eH3.1 fractions in 20 mM HEPES, pH 7.2, 25 mM KCl, 5 mM $Mg(OAc)_2$, 10 mM $(NH_4)_2SO_2$, 0.1 mM EDTA and 20 pmoles of HeLa histones at 30°C for 30 min. CKII (Abcam) was used as a positive control. Samples were resolved by SDS-PAGE, coomassie-stained, and visualized by autoradiography. ATPase assays were performed on chromatinized plasmids generated by salt dialysis (Margueron et al., 2008), and performed as described using [γ -^{32}P] ATP (Lee and Hurwitz, 2000) and recombinant RSF (Loyola et al., 2003) as a positive control. Recombinant PRC2 was purified as described (Margueron et al., 2008). Histone tetramers and sNASP-bound histones were selected by gel filtration and methylated using recombinant PRC2 as described (Margueron et al., 2008).

Label-free quantitative mass spectrometry

S100, NE and SNP extracts were obtained as described above. 2-5 mg extract was incubated with 15 µl M2 resin (Sigma-Aldrich) (Flag IP) or 15 µl agarose beads (Chromotek) (control) for 90 min at 4°C in 430 µl final volume containing 300 mM KCl, 0.15% NP-40 and 50 mg/ml ethidium bromide. All IPs were performed in triplicate. Beads were washed twice with 1 ml BC300 + 1% NP-40, twice with 1 ml PBS + 1% NP-40 and finally twice with 1 ml PBS. Bound proteins were eluted from the beads using two

30 µl 200 mM glycine pH 2.5 elutions, which were incubated at 25°C for 5 min and subsequently neutralized in 10 µl 1 M tris, pH 8. The eluted proteins were partially denaturated and reduced in 2 M urea, 25 mM tris, pH 8.5 and 10 mM DTT for 20 min, after which they were alkylated with 50 mM IAA for 10 minutes and digested with 0.5 µg trypsin (Promega) overnight at 25°C. Digested peptides were desalted and concentrated using C18 stagetips prior to online LC-MS/MS analysis. 120 minute gradients (6-75% acetonitrile) were applied (nanoLC1000, Thermo Scientific) and spectra were recorded on an Orbitrap Velos (Thermo Scientific), selecting the 15 most intense precursor ions for fragmentation for each full scan. Data analysis was done as described before (Smits et al., 2013). Top ontology terms, along with the terms 'replication', 'transcription', 'DNA repair', 'chromatin assembly or disassembly' and 'chromatin modification', were queried and compared to the input material using the DAVID functional annotation tool (Dennis et al., 2003).

Crosslinking and Mass Spectrometry Analysis

In vivo crosslinking of cells was performed after a 3 hr release from a double-thymidine block, after which cells were trypsinized, washed, and resuspended at 2.5 x 10^7 in PBS, pH 8 at room temperature (RT). DSS (Pierce) was added to 2 mM (final concentration) and the sample allowed to incubate for 30 min at RT with gentle rotation. The reaction was quenched by adding tris, pH 6.8 to 20 mM. The ensuing partial crosslinking allowed the immunoprecipitation of lysine-containing epitopes from cells in S-phase. Cells were gently pelleted at 800 x *g* and lysed in 5 pellet volumes of 20 mM tris, pH 6.8, 150 mM NaCl, 0.2 mM EDTA, 30% glycerol (v/v), 0.5 mM $MgCL_2$, 0.5% NP-40 (v/v), 1 mM

DTT in the presence of protease inhibitors, and the DNA digested with 0.2 U/ml benzonase (Sigma-Aldrich) at 4°C with gentle rotation overnight. The samples were then centrifuged at 30,000 x *g* for 30 min to remove insoluble material. The resulting partial crosslinking allowed for subsequent immunoprecipitation using the lysine-containing FLAG epitope from the aforementioned stable clones. Immunoprecipitates were resolved by SDS-PAGE, coomassie-stained, and high molecular weight species excised for MS analysis.

For the analysis of protein-protein interactions, recombinant proteins were pre-incubated in 10 mM HEPES, pH 8, 150 mM NaCl for 30 min at RT. Crosslinking reactions were performed with DSS as described above. Crosslinked proteins were reduced with 20mM DTT, alkylated with 50 mM iodoacetamide, and digested using trypsin as described (Muellenbeck et al., 2013). Tryptic peptides were separated on an EASY spray 50cm C18 analytical HPLC column with <2 μm bead size using the auto sampler of an EASY-nLC 1000 HPLC and gradient eluted using a two hour gradient into a Q Exactive Orbitrap Mass spectrometer. High resolution full MS spectra were acquired with a resolution of 70,000, AGC target of 1e6, maximum ion time of 120ms, and scan range of 400 to 1500 m/z. Following each full MS twenty data-dependent high resolution HCD MS/MS spectra were acquired. All MS/MS spectra were collected using the following instrument parameters: resolution of 17,500, AGC target of 5e4, maximum ion time of 250ms, one microscan, 2m/z isolation window, fixed first mass of 150 m/z, and Normalized Collision Energy (NCE) of 27. For discovery of linear peptides, ions with charge state +2, +3, +4, +5 were selected for MS/MS, for the crosslink analysis only ions with charge state of +4, +5 and +6 were selected for MS/MS. The spectra for the linear

peptide analysis were searched against the Uniprot human database using Sequest within Proteome Discoverer. Cysteine carbamidomethylation was selected as static modification and oxidation of methionine, deamidation of glutamine and asparagine and monolink mass addition of 156.07864 Da on lysine residues and N-termini was selected as variable modification. Proteins with an FDR of 1% and at least two unique peptides were extracted to create a focused database for subsequent search of the crosslinked data. For identification of crosslinked peptides the data was searched against the focused database using the pLink (Yang et al., 2012) search algorithm including the static and variable modifications as above and the crosslink mass shift of 138.06809 Da. Hits with pLink scores below 1E-04 was excluded from the analysis and higher scoring spectra were manually verified.

DNA Supercoiling Assay

Chromatin assembly and DNA supercoiling assay was done as described (Fyodorov and Kadonaga, 2003), with the following modifications. The drosophila topoisomerase fragment ND423 was expressed from BL21 cultures and purified over NiNTA resin (Qiagen) followed by anion exchange chromatography. One ng ND423 was used per 20 fmoles of circular plasmid, which was relaxed at 30°C for 30 min in 50 mM tris, pH 7.6, 10 mM $MgCl_2$, 0.1 mM EDTA, 0.5 mM DTT, 50 μg/ml BSA. HeLa histone:histone chaperones were simultaneously incubated in Assembly Buffer (10 mM tris, pH 7.6, 100 mM KCl, 2 mM $MgCl_2$, 1 mM DTT, 0.1 mg/ml BSA) at the desired molar ratios. Chaperone-mediated chromatin assembly was then performed by mixing 100 fmoles of the relaxed plasmid (directly from ND423 mixture) and 10 pmoles HeLa histone (from

the histone:chaperone mixture), with an equal reaction volume of 2X Assembly Buffer. Reactions proceeded at 37°C for 2.5 hrs and were stopped by adding an equal volume of 2X Stop Buffer (20 mM EDTA, pH 8, 0.5 mg/ml glycogen, 0.1% SDS (w/v)) and 2.5 μg proteinase K, and incubating at 50°C for 30 min. DNA was then extracted with phenol:chloroform, ethanol precipitated and resolved over an 0.8% agarose gel in the absence of intercalating agents. Gels were then stained with ethidium bromide and visualized under UV light. Yeast NAP-1 served as a positive control. The protein was expressed from BL21 cultures, purified over glutathione sepharose (GE Life Science), and the elutions further resolved by anion exchange chromatography.

Peptide pulldowns

Biotinylated peptides spanning histone H3 1-21, 21-44, and 69-89 were purchased from Anaspec. 250 pmoles of peptides were immobilized onto 10 μl Strep-Tactin macroprep slurry (IBA) per pulldown. 10 pmoles of recombinant TONSL, TONSL-MMS22L, or protein fragments were incubated with the beads in 500 ul BC150 for 3 hrs at 4°C with gentle rotation. The resin was collected by centrigugation (1000 x *g*, 2 min, 4°C) and washed 3 x BC300 + 0.05% NP-40 (v/v) followed by a BC150 wash. Beads were resuspended in Laemmli buffer, boiled and resolved by SDS-PAGE for western analysis.

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