Supplementary Information

Antibodies

The antibodies used in this study were from the following sources:

Santa Cruz Biotechnology, Pol-II N-term. (sc-9001), Brg-1 (sc-10768), CBP (sc-369).

Covance, Pol-II CTD-Ser5-P (H-14), Pol-II CTD-Ser-2P (H5), Pol-II CTD (8WG16).

Abcam, Histone 3 (ab8580), monomethyl H3-K4 (ab8895), dimethyl H3-K4 (ab7766), trimethyl H3-K4 (ab 8580), dimethyl H3-K9 (ab7312).

Upstate Biotechnology, Acetyl-H3 (06-599), Acetyl-H4 (06-866), dimethyl H3-K79 (07-366), dimethyl H3-K36 (07-274), dimethyl H3-K4 (07-030), FACT/cdc68 (07-255).

Anti-PCAF was obtained from Y. Nakatani, anti-TBP was from L. Tora, anti-Elp1 and anti-Elp3 was from J. Svejstrup.

Nucleotide positions of oligonucleotide primers used for real-time PCR amplifications.

HNF-4α gene (TSS: +1; polyA site: 28309)

Prom/TSS:	**nt -18**	**to**	**+92**
CR 0.5:	**nt +492**	**to**	**+578**
CR 1.0:	**nt +985**	**to**	**+1070**
CR 2.5	**nt +2509**	**to**	**+2599**
CR 5.0	**nt +5172**	**to**	**+5260**
CR 8.0	**nt +7948**	**to**	**+8034**
CR 10.0	**nt +10235**	**to**	**+10329**
CR 12.5	**nt +12563**	**to**	**+12649**
CR 16.2	**nt +16193**	**to**	**+16283**
CR 20.0	**nt +19963**	**to**	**+20045**
CR 28.0	**nt + 28061**	**to**	**+28149**
Control	**nt +32177**	**to**	**+32297**

HNF-1α gene (TSS: +1; polyA site: 23764)

Prom/TSS:	**nt -89**	**to**	**-19**
CR 0.5:	**nt +450**	**to**	**+535**
CR 1.0:	**nt +953**	**to**	**+1033**
CR 2.5	**nt +2513**	**to**	**+2591**
CR 5.0	**nt +6056**	**to**	**+6138**
CR 8.0	**nt +8137**	**to**	**+8212**
CR 10.0	**nt +9820**	**to**	**+9930**
CR 15.0	**nt +15649**	**to**	**+15720**
CR 22.0	**nt +21978**	**to**	**+22061**
Control	**nt +31801**	**to**	**+31888**

Albumin gene (TSS: +1; polyA site: 17262)

Prom/TSS:	**nt -20**	**to**	**+73**
CR 0.5:	**nt +439**	**to**	**+547**
CR 1.0:	**nt +1057**	**to**	**+1149**
CR 2.5	**nt +2561**	**to**	**+2632**
CR 5.0	**nt +4984**	**to**	**+5060**
CR 8.0	**nt +7940**	**to**	**+8020**
CR 10.0	**nt +10022**	**to**	**+10112**
CR 12.5	**nt +12986**	**to**	**+13068**
CR 15.0	**nt +15040**	**to**	**+15121**
Control	**nt +26283**	**to**	**+26358**

RT-PCR primer sets

HNF-4α mRNA : **5' ggagatgacttgaggccttact**
3' ggggaatcgtttccaaggcctc

HNF-1α mRNA : **5' gcctccctgggtcctacgttcacc**
3' gggcttgtggctgtagagggcgtg

Albumin mRNA : **5' caccttccatgcagatatatg**
3' tgcagcacttctctacaaaag

Supplementary Table 1. Analysis of simultaneous modification of individual nucleosomes by sequential chromatin immunoprecipitation (ReChIP) assays.

ReChIP assays were performed by the indicated 1st and 2nd IP antibodies. The nucleosomal DNAs obtained after the second immunoprecipitation were amplified by Real Time PCR, using primer sets corresponding to nucleosomes located at 0.5 and 1.0 kb of the coding regions of HNF4, HNF1 and Albumin genes. The results were

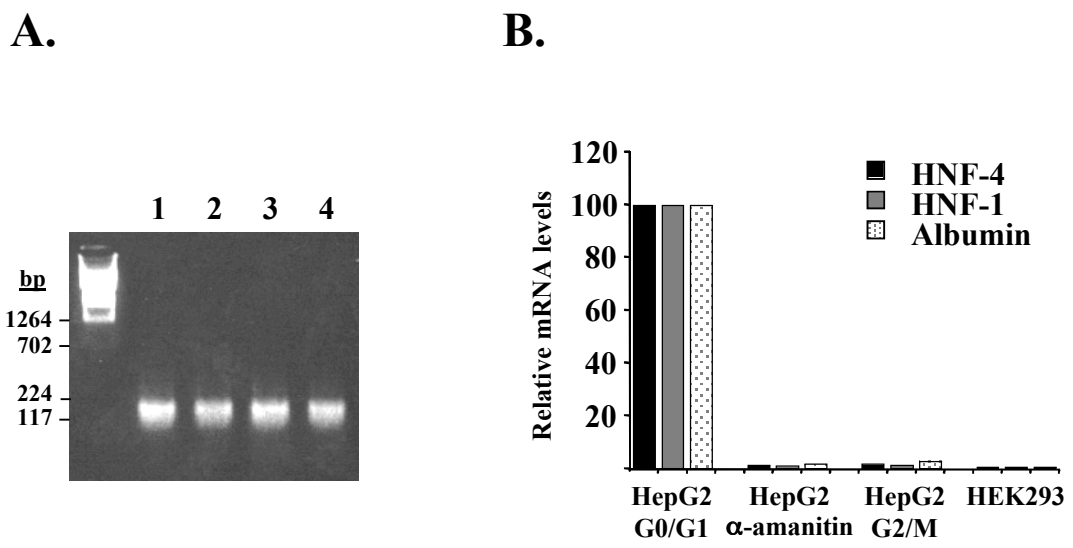
first normalized to those obtained with non-immune antibody and divided by the input values (which correspond to results obtained with the eluted material after the 1st IPs). The Re-ChIP efficiencies of the different antibodies were calculated from the data (% of input) obtained in experiments using the same antibody in the first and second IP. The data from the 6 regions (0.5 and 1kb segments of three genes) were averaged. The average percentages were as follows: **56% for the H3K4 Tri-Methyl antibody, 32% for the H3K4 Di-Methyl antibody, 41% for the H3K79 Di-Methyl antibody, 38% for the Acetyl-H3 antibody and 33% for H3K4 Mono-Methyl antibody (the latter obtained on coding region 10 kb)**. Using these values all the data were normalized to 100% Re-ChIP efficiency. The numbers indicate normalized average percentages and deviations of the recovered material corresponding to the 0.5 kb segments (Numbers at the top) and the 1.0 kb segments (Numbers at the bottom), from at least 3 experiments.

1st IP:	H3K4 Tri-Methyl			H3K4 Di-Methyl			H3K79 Di-Methyl			H3-acetyl		
Gene	HNF4	HNF1	Alb.	HNF4	HNF1	Alb.	HNF4	HNF1	Alb.	HNF4	HNF1	Alb.
2nd IP												
H3K4 Tri-Me	92±14 99±13	108±8 87±7	103±7 106±8	90±10 78±12	91±8 83±10	92±10 85±8	83±8 90±12	89±12 96±14	85±12 87±14	72±10 71±6	80±12 83±10	81±8 80±10
H3K4 Di-Me	96±8 87±7	87±6 78±4	93±8 100±9	109±9 93±8	96±10 100±9	93±9 106±12	93±10 109±11	84±8 81±8	78±6 90±12	87±6 81±10	90±10 93±12	78±10 90±12
H3K79 Di-Me	85±8 94±10	90±10 97±10	77±10 85±10	97±6 87±8	85±10 77±6	92±10 80±8	111±18 97±12	102±11 106±15	87±11 92±12	92±10 85±12	85±8 89±12	87±8 75±9
H3-acetyl	81±6 78±7	79±8 92±7	92±10 84±9	86±10 73±14	79±14 76±12	81±12 71±14	73±10 79±12	84±8 89±10	86±14 73±12	102±14 92±12	94±11 105±9	105±11 99±9
H3K4 Mono-Me	1.2±0.4 1.5±.05	1.1±0.6 0.9±0.4	1.2±0.5 0.7±0.3	0.6±0.2 0.6±0.3	0.7±0.2 0.6±0.3	0.9±0.1 0.7±0.2	1±0.4 1.2±0.5	1.1±0.6 0.9±0.5	0.8±0.3 0.7±0.2	0.3±0.1 0.5±0.3	0.2±0.1 0.2±0.1	0.4±0.2 0.3±0.1

Supplementary Figure 1. Analysis of the DNA after Mnase digestion and the expression of HNF-4, HNF-1 and albumin genes.

(A) Part of the input samples after crosslinking and MNase digestion, were de-crosslinked, extracted with phenol-chlorophorm, precipitated with ethanol. The DNAs were separated by electrophoresis on 1.5% agarose gel and stained with ethidium bromide. Order of samples: lambda BstEII marker; 1, G0/G1 enriched HepG2; 2, a-amanitin treated HepG2; 3, Nocodazol-arrested HepG2; 4, HEK 293.

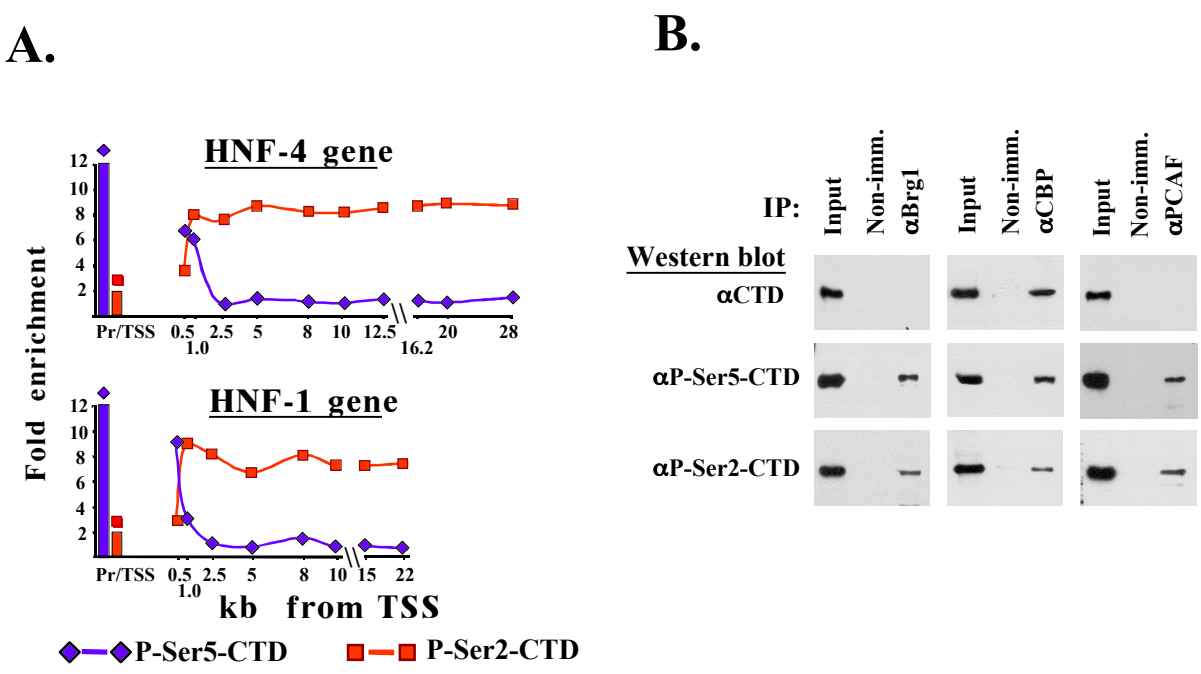
(B) Total RNAs, extracted from $2x10^6$ cells, were analyzed by RT-PCR. Real-time PCR values are expressed as percentage of those detected with RNAs from G0/G1-enriched HepG2 cells.



Supplementary Figure 2. Association of Brg-1, CBP and PCAF with initiating and elongating forms of RNA pol-II.

(A) Chromatin immunoprecipitation assays were performed with the antibodies H14 (P-Ser5-CTD) and H5 (P-Ser-2-CTD), raised against phosphorylated CTD peptides.

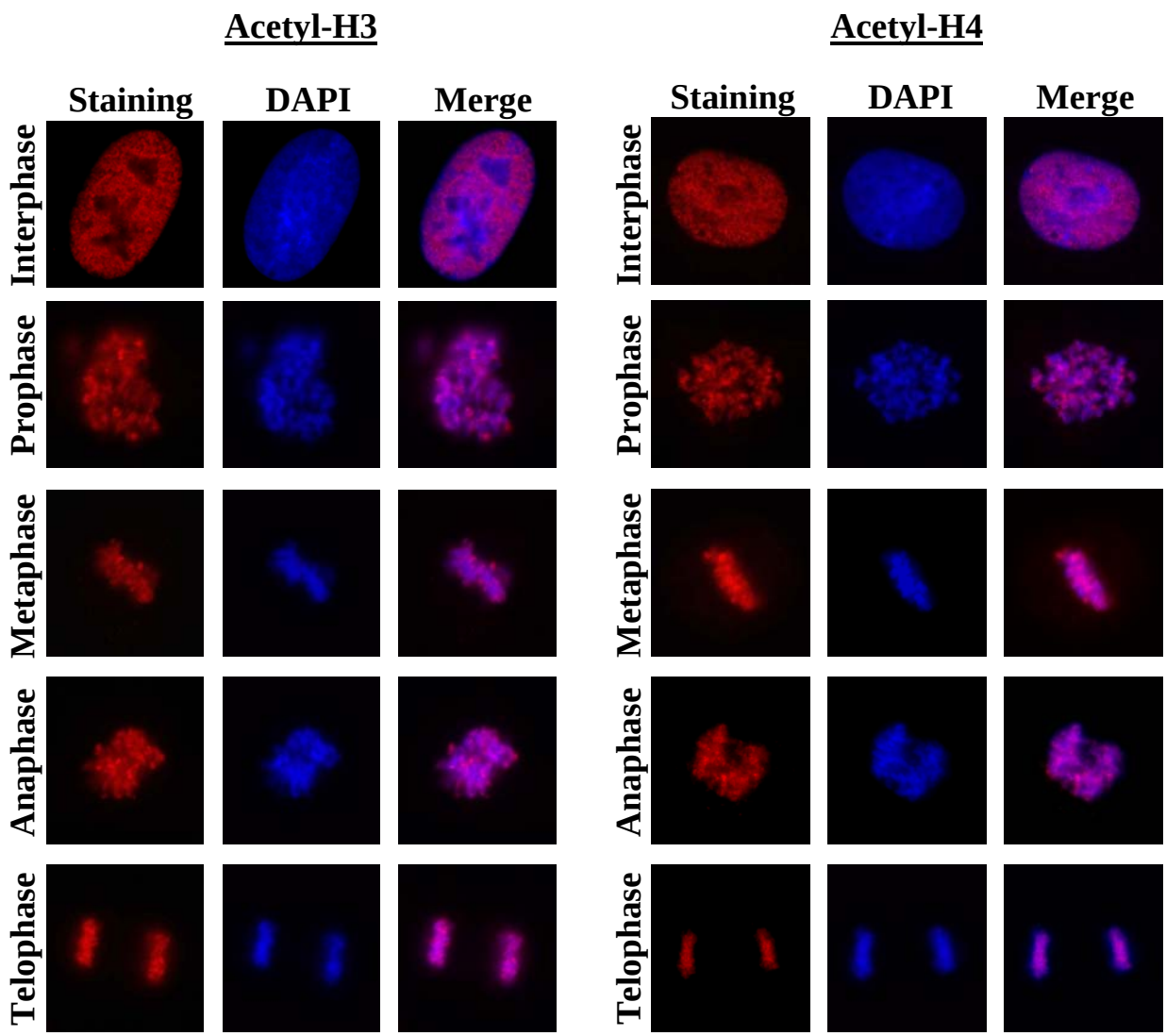
(B) HepG2 nuclear extracts were immunoprecipitated with the antibodies αBrg1, αCBP and αPCAF and the presence of the different CTD-phosphorylated forms of RNA pol-II in the precipitates was detected by western blot analysis using antibodies raised against non-phosphorylated CTD, 8WG16 (αCTD), and H14 (αP-Ser5-CTD), or H5 (αP-Ser-2-CTD) as indicated.



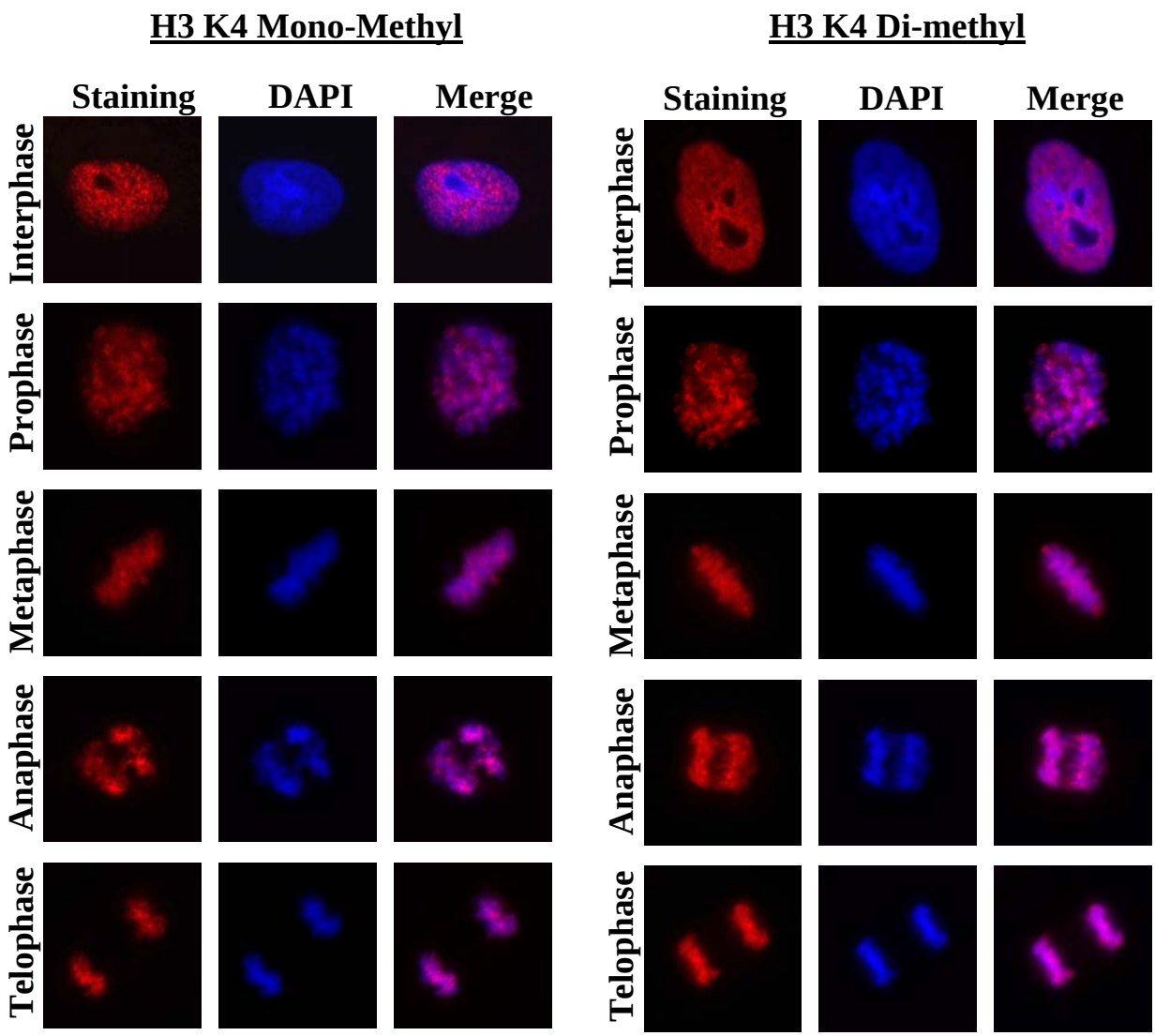
Supplementary Figure 3. Immunocytological examination of histone modifications and chromatin modifying activities at the different stages of mitosis.

HepG2 cells grown on glass cover slips were fixed for 10 min at –20 ^{0}C and immunostained with the indicated antibodies. Fluorescence images of representative cells at the interphase and various stages of the mitotic phase are shown. Left panels correspond to immunofluorescence stainings using Alexa Red 568 secondary antibodies, middle panels show DNA stainings with DAPI, while panels at the right are merged images of the former two pictures. Note that, immunofluorescence signals obtained by antibodies against the factots (CBP, PCAF, Elp3 and Brg1) were excluded from mitotic chromatin, while those obtained with histone modification state antibodies overlapped with it. In the case of CBP and Brg1 staining two populations of telophase cells were observed. In some cells they were excluded, in others they were partially associated with mitotic chromatin. Representative images of both populations are shown.

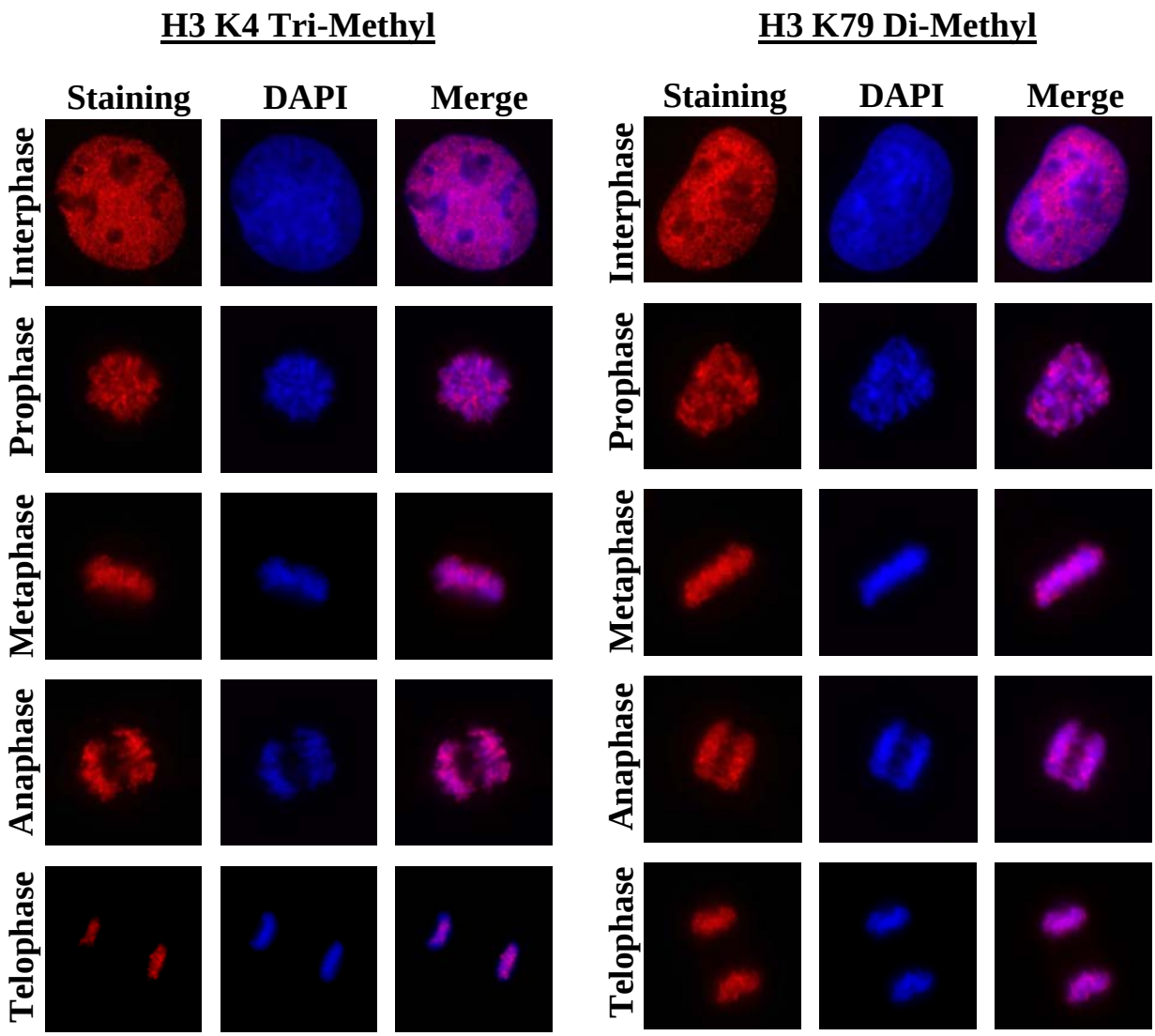
Supplementary Figure 3a



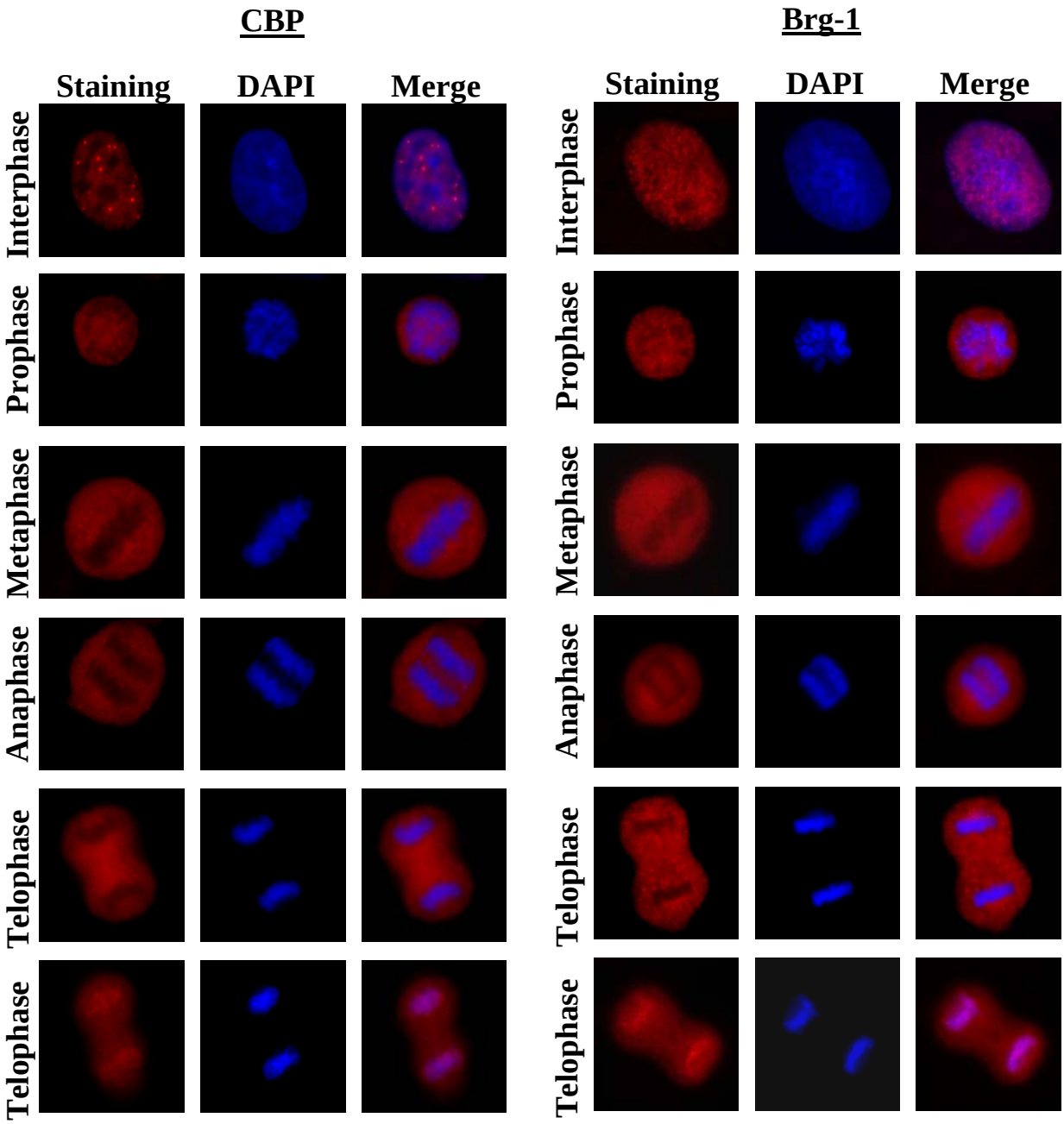
Supplementary Figure 3b



Supplementary Figure 3c



Supplementary Figure 3d



Supplementary Figure 3e