Supporting information

Table S1. Candidate list

Candidate	EdU [&]	Description
PRMT5	57.10 ± 11.99 %	Histone-Arginine N-Methyltransferase PRMT5
SIRT5	55.69 ± 3.88 %	NAD-Dependent Deacetylase Sirtuin-5
UBE2B	55.50 ± 0.90 %	Ubiquitin-Conjugating Enzyme E2B
L3MBTL1	52.55 ± 9.18 %	L(3)Mbt-Like 1
MBD1	46.84 ± 16.41 %	Methyl-CpG Binding Domain Protein 1
DNMT1	45.74 ± 11.58 %	DNA (Cytosine-5-)-Methyltransferase 1
SMARCD3	44.54 ± 0.76%	SWI/SNF Related, Matrix Associated, Actin Dependent Regulator Of Chromatin, Subfamily D, Member 3
HDAC7	38.90 ± 5.22 %	Histone Deacetylase 7
KDM4D	31.16 ± 4.33 %	Jumonji Domain-Containing Protein 2D
HLTF	24.81 ± 10.56 %	Helicase-Like Transcription Factor

[&] EdU positive cell number represented by mean ± SD

Table S2. Primer list

Name	Sequence
KDM4A FWD	AGCTTGCTTAAAGGCTGACG
KDM4A REV	GAAGTTTCAGTGAGCGGGAG
KDM4B FWD	ATCTTGACCATGTCCTTCCG
KDM4B REV	TCAACTGCGCAGAATCTACC
KDM4D FWD	GGGCAGGGGTGTTTACTCAAT
KDM4D REV	TGTTTGCCAAATGGCGATACT
Myc -7kb FWD	GAGTTGGCAACCCTTGATGT
Myc -7kb REV	GTTAGGATTTCCCGCCTTTC
Myc origin FWD	TACAGACTGGCAGAGAGCAG
Myc origin REV	ATGTATGCACAGCTATCTGG
Myc +7kb FWD	GGTTCTAAGATGCTTCCTGG
Myc +7kb REV	TGGTTGTGAAGGCAGCAGAA
MCM4 -5kb FWD	TACCTGTGGGTAAGAGATGAGTTG
MCM4 -5kb REV	TGCCTGTTCCCAAATGCTATATGC
MCM4 Origin FWD	AAACCAGAAGTAGGCCTCGCTCGG
MCM4 Origin REV	GTCTGACCTGCGGAGGTAGTTTGG
MCM4 +5kb FWD	ATCTCGCCTAATCCCACCAGTACC
MCM4 +5kb REV	ATATTCACTACTAGACCCTCCGG
LNMB origin FWD	GCGTCACAGCACAACCTGC
LMNB2 origin REV	GAGGCAGAACCTAAAATCAAA
LMNB2 -6kb FWD	GCTGCGCTCAGGTTAAGAAG
LMNB2 -6kb REV	GTGCTCACGGCAGATAAGGT
LMNB2 +6kb FWD	CTCCTCGATGCTGACGCTAC
LMNB2 +6kb REV	TACCAGTCCCACCTTCCTTG

Fig. S1. (Relate to Fig. 1)

(*A*) Heatmap of DNA replication defects screening. The color scheme represents the degree of defect. (*B*) Expression of *KDM4A*, *KDM4B,* and *KDM4D* in HeLa cells with shRNAs targeting *KDM4D* was examined by quantitative polymerase chain reaction (qPCR). (*C*) Analysis of Kdm4d depletion in U2OS and HCT116 cells by Western blot analysis. Two shRNAs targeting human (sh-h1 and sh-h2) or non-target shRNA (NT) was used for depletion of human Kdm4d, and proteins in whole cell extracts were analyzed. (*D-F*) Depletion of Kdm4d in U2OS and HCT116 cells results in reduced 5-ethynyl-2′-deoxyuridine (EdU)-positive cells and EdU intensity. Representative images of EdU staining in U2OS and HCT116 cells with and without Kdm4d are shown in *D*. Scale bar: 50 μm. Quantification of EdU-positive cell numbers (mean ± SD) from 3 independent experiments is shown in *E*. Five hundred cells were counted. Quantification of the EdU fluorescence intensity (mean ± SD) from 3 independent experiments is shown in *F*. Five hundred cells were counted. (*G*) Depletion of Kdm4d results in reduced S phase cells. U2OS and HCT116 cells with and without Kdm4d depletion were stained with 5-bromo-2′-deoxyuridine (BrdU) and propidium iodide (PI) and analyzed by flow cytometry. *** $P<0.001$.

Fig. S2. (Relate to Fig. S3)

(*A*) HeLa cells stably expressing wild-type H3.1, H3.1 K9M, H3.1 K27M, wild-type H3.3, H3.3 K9M, and H3.3 K27M mutants were stained with H3K9me3 and H3K27me3 antibody. Representative images are shown. Scale bar: 200 μm. (*B-D*) Expression of H3K9M mutant rescued reduction of 5-ethynyl-2′-deoxyuridine (EdU)-positive cells in Kdm4d-depleted cells. HeLa cells stably expressing wild-type H3.3 and K9M, K27M mutants were infected with virus expressing shRNA targeting *KDM4D*. (*B*) The whole cell lysates were subjected to Western blotting using indicated antibodies. (*C*) Representative EdU staining images are shown. Scale bar: 50 μm. (*D*) Quantification of EdU-positive cell numbers (mean ± SD) from 3 independent experiments is shown. Five hundred cells were counted. (*E*) Expression of H3K9M mutant rescued reduction of S phase cells in Kdm4d-depleted cells. HeLa cells stably expressing wild-type H3.3 and K9M, K27M mutants with and without Kdm4d depletion were stained with 5-bromo-2′-deoxyuridine (BrdU) and propidium iodide (PI) and analyzed by flow cytometry. *** $P<0.001$.

Fig S3. (Relate to Fig. 4)

(*A*) Kdm4d was stained in HeLa and U2OS cells transduced with control shRNA (NT) and shRNAs targeting *KDM4D* (sh-h1 and sh-h2). Representative images are shown. Scale bar: 50 μm. (*B*) Kdm4d and EdU or PCNA were co-stained in HeLa and NIH-3T3, respectively. Representative images are shown. Scale bar: 5 μm. (*C*) Kdm4d dissociated from chromatin at different stages in M phase. Representative images from different mitotic stages are shown. Inter, interphase; Prometa, prometaphase; Meta, metaphase; Ana, anaphase; Tel, telophase. Scale bar: 5 μm. (*D*) FACS of synchronized HeLa cells. Asy, asynchronized cells; G1, mimosine arrested; S1, thymidine arrested and release for 2 hours; S2, thymidine arrested and release for 4 hours; G2/M, nocodazole arrested. (*E*) Western blot analysis of the whole cell lysates (WCE) and the chromatin fractions (Chr.) of cells synchronized as in *C*.

Fig. S1

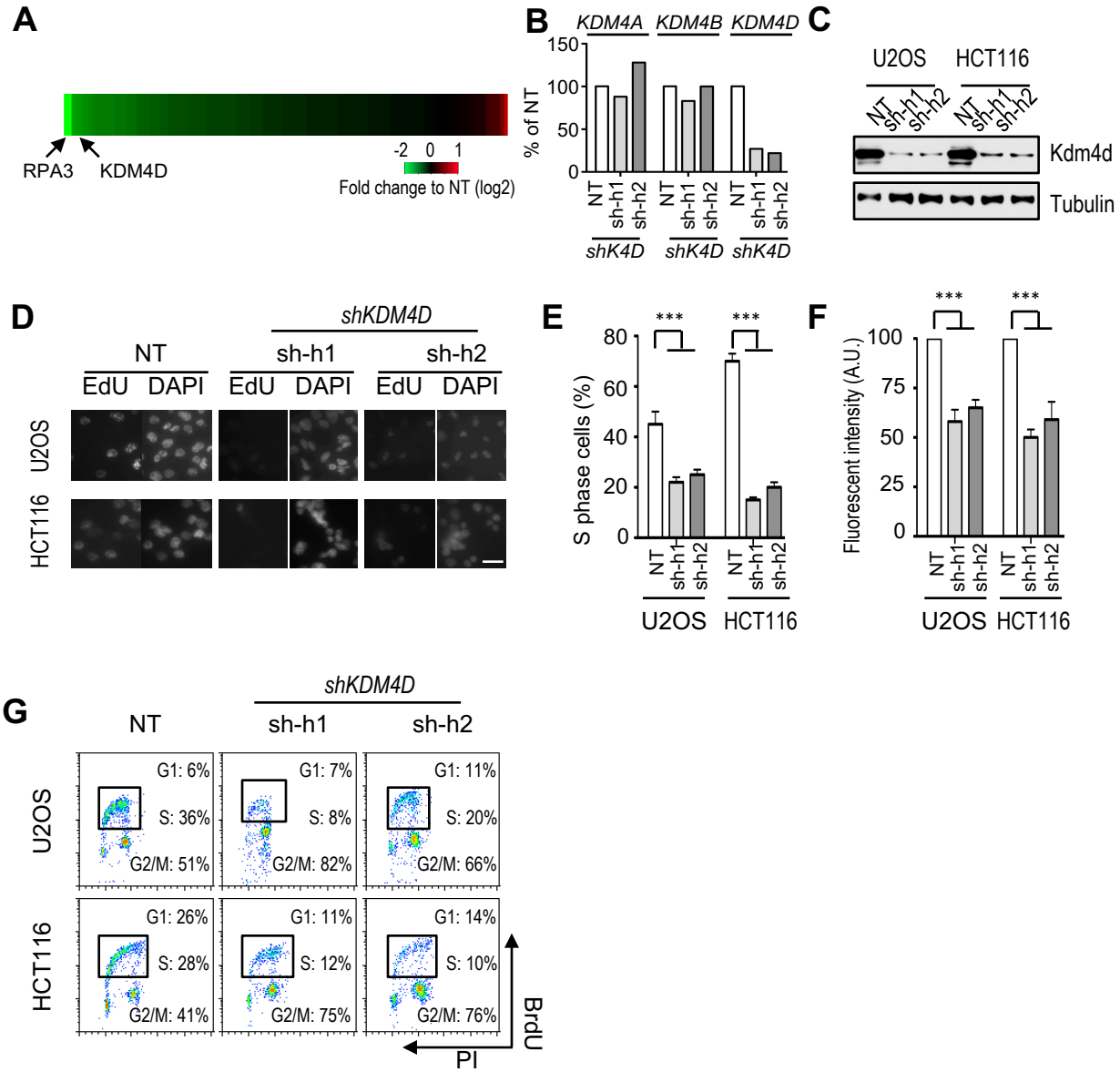

A
RPA3
KDM4D
-2 0 1
Fold change to NT (log2)
B
KDM4A
KDM4B
KDM4D
% of NT
150
100
50
0
NT
sh-h1
sh-h2
shK4D
C
U2OS
HCT116
NT
sh-h1
sh-h2
Kdm4d
Tubulin
D
shKDM4D
NT
sh-h1
sh-h2
EdU
DAPI
U2OS
HCT116
E
S phase cells (%)
80
60
40
20
0
***
F
Fluorescent intensity (A.U.)
100
75
50
25
0
G
shKDM4D
NT
sh-h1
sh-h2
U2OS
HCT116
G1: 6%
S: 36%
G2/M: 51%
G1: 7%
S: 8%
G2/M: 82%
G1: 11%
S: 20%
G2/M: 66%
G1: 26%
S: 28%
G2/M: 41%
G1: 11%
S: 12%
G2/M: 75%
G1: 14%
S: 10%
G2/M: 76%
BrdU
PI

Fig. S2

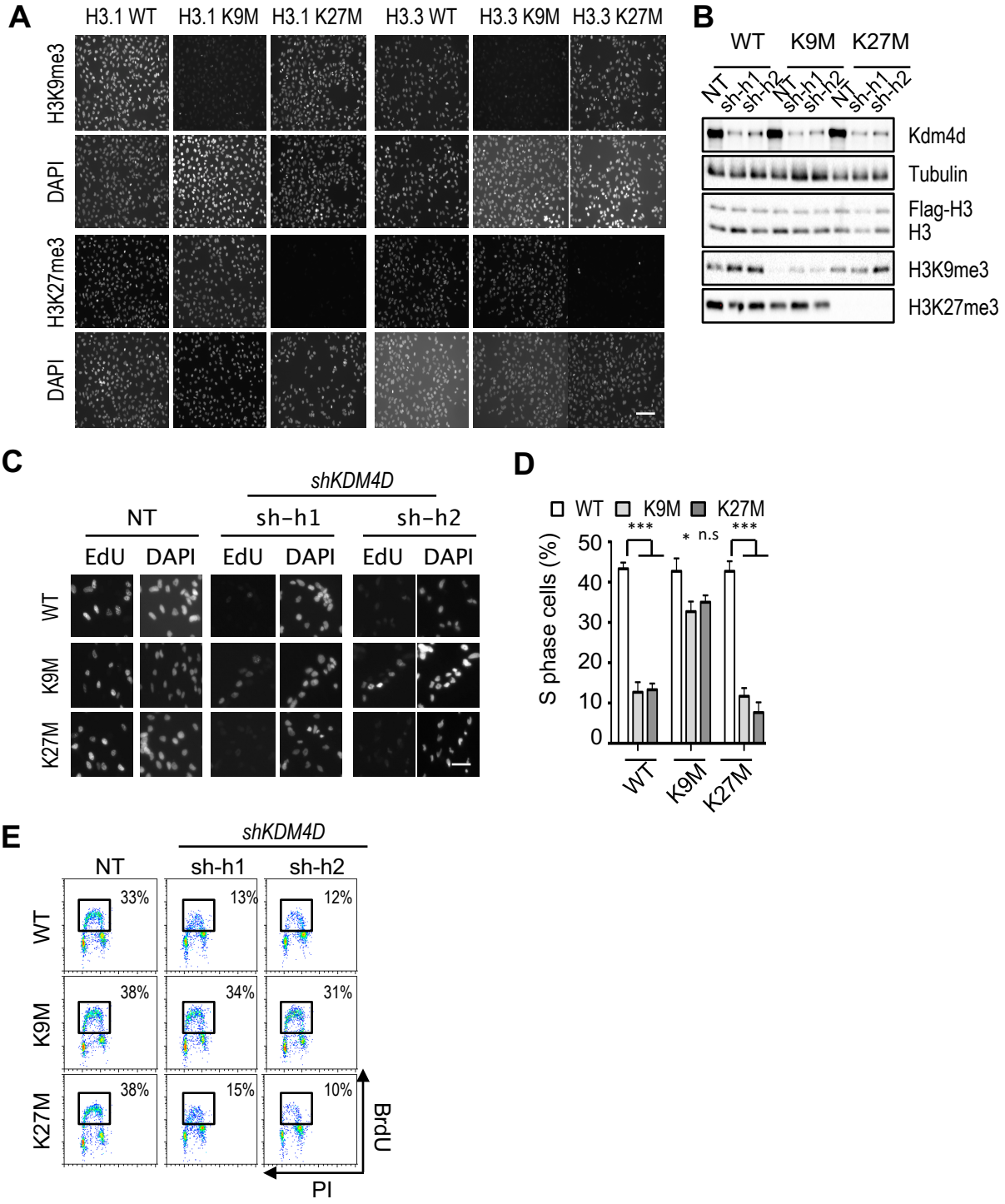
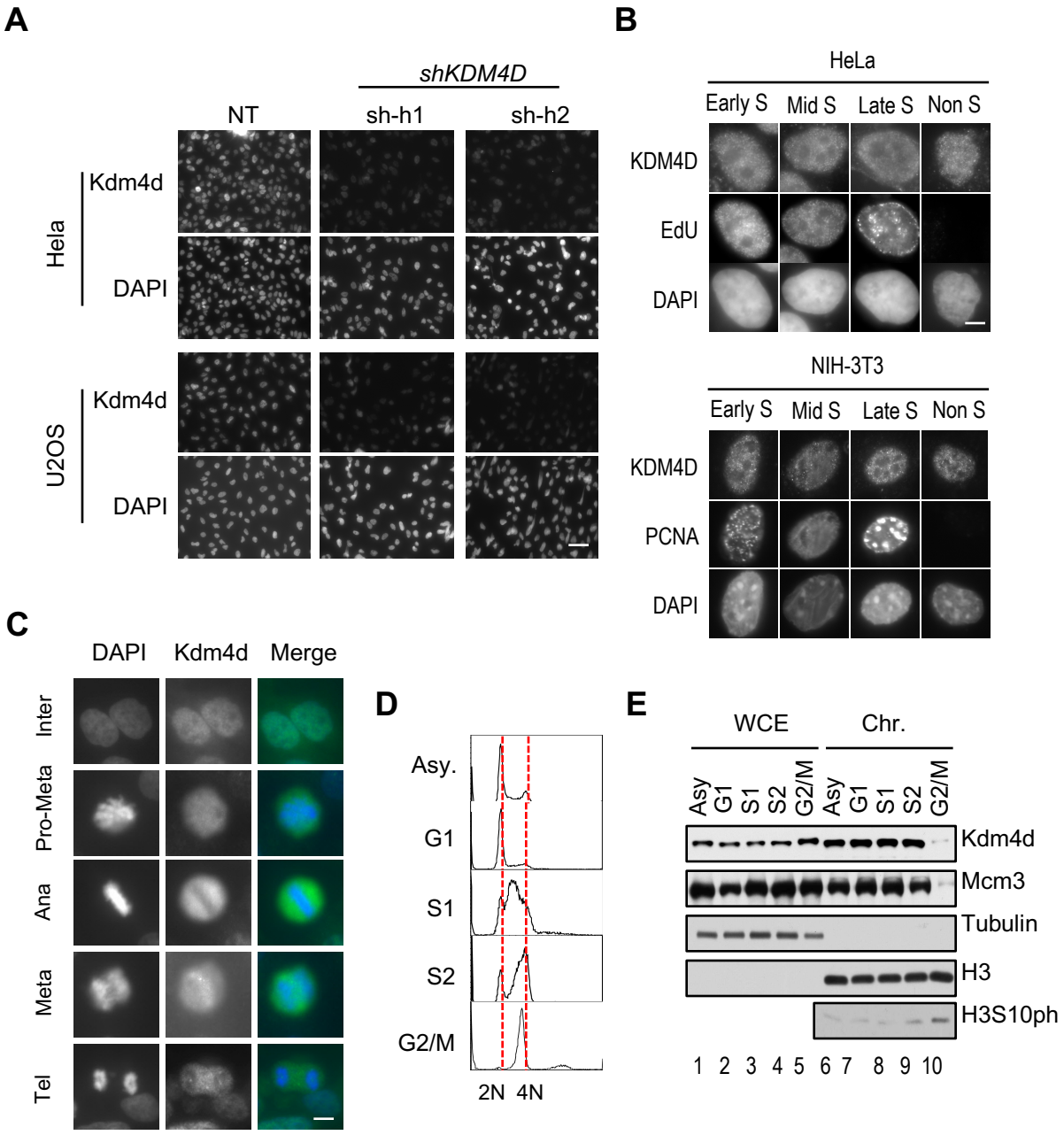

A
H3.1 WT
H3.1 K9M
H3.1 K27M
H3.3 WT
H3.3 K9M
H3.3 K27M
H3K9me3
DAPI
H3K27me3
DAPI
B
WT
K9M
K27M
NT
sh-h1
sh-h2
Kdm4d
Tubulin
Flag-H3
H3
H3K9me3
H3K27me3
C
shKDM4D
NT
sh-h1
sh-h2
EdU
DAPI
WT
K9M
K27M
D
WT
K9M
K27M
S phase cells (%)
50
40
30
20
10
0
***
* n.s
***
E
shKDM4D
NT
sh-h1
sh-h2
33%
13%
12%
38%
34%
31%
38%
15%
10%
BrdU
PI

Fig. S3


A
shKDM4D
NT
sh-h1
sh-h2
Hela
Kdm4d
DAPI
U2OS
Kdm4d
DAPI
B
HeLa
Early S
Mid S
Late S
Non S
KDM4D
EdU
DAPI
NIH-3T3
Early S
Mid S
Late S
Non S
KDM4D
PCNA
DAPI
C
DAPI
Kdm4d
Merge
Inter
Pro-Meta
Ana
Meta
Tel
D
Asy.
G1
S1
S2
G2/M
2N
4N
E
WCE
Chr.
Asy
G1
S1
S2
G2/M
Asy
G1
S1
S2
G2/M
Kdm4d
Mcm3
Tubulin
H3
H3S10ph
1 2 3 4 5 6 7 8 9 10