Supplemental Figure Legends

Figure S1. Schematic diagram of the construction of Rpb1 conditional knock-out cell line.

Figure S2. Amino-acid sequence comparison between human (denoted as query) and chicken (subject) Rpb1 using blastp.

Figure S3. Ser 2, Ser 5 and Ser 7 phosphorylation patterns in mutant cells. Cells were treated with tet for 24 hours. Cell lysates were analyzed by western blotting using indicated antibodies. (A) Ser 7 levels. (B) CTD phosphorylation status in various mutant cells. Thr4P was not detected in S5A and S7A samples. This negative result is difficult to interpret because the antibody could simply not recognize Thr4P on the CTD with S5A or S7A mutations. Samples were prepared as in Figure 4B.

Figure S4. Coomassie blue staining of purified GST-CTD, Fcp1, Fcp1 (M271E), and CDK9/cyclin T complex. Arrow indicates the purified protein resolved by SDS-PAGE.

Figure S5. *In vitro* phosphorylation of Thr 4. GST-CTD was incubated with HeLa nuclear extract (left lane) or the indicated amount of CDK9/cyclinT plus or minus indicated inhibitors. Samples were analyzed by western blotting with antibodies that detect P-Thr 4, P-Ser 2, P-Ser 5, and P-Ser 7.

Figure S6. Fcp1 mRNA levels in shRNA-treated HEK293 cells. Cells were transfected with plasmids expressing shRNAs targeting Fcp1 or GFP (control). RNA was extracted 22 h or 41 h after transfection, and RT-qPCR was performed to determine Fcp1 mRNA levels. Levels of Fcp1 mRNA in Fcp1 shRNA expressing cells relative to control were plotted.

Figure S1

Construction of targeting vectors

↓

Disrupt one allele of *Rpb1* with the targeting vector carrying hygromycin-resistance gene

↓

Introduce tetracycline repressive expression vector of HA-tagged human Rpb1 and the tTA expression vector (tet-off)

↓

Disrupt second allele of *Rpb1* with the targeting vector carrying zeocin-resistance gene

Figure S2

```
Query  13    CPLRTIKRVQFGVLSPDELKRMSVTEGGIKYPETTEGGRPKLGGLMDPRQGVIERTGRCQTCAGNMTECPGHFGHIELAKPVFHVGFLVK  102
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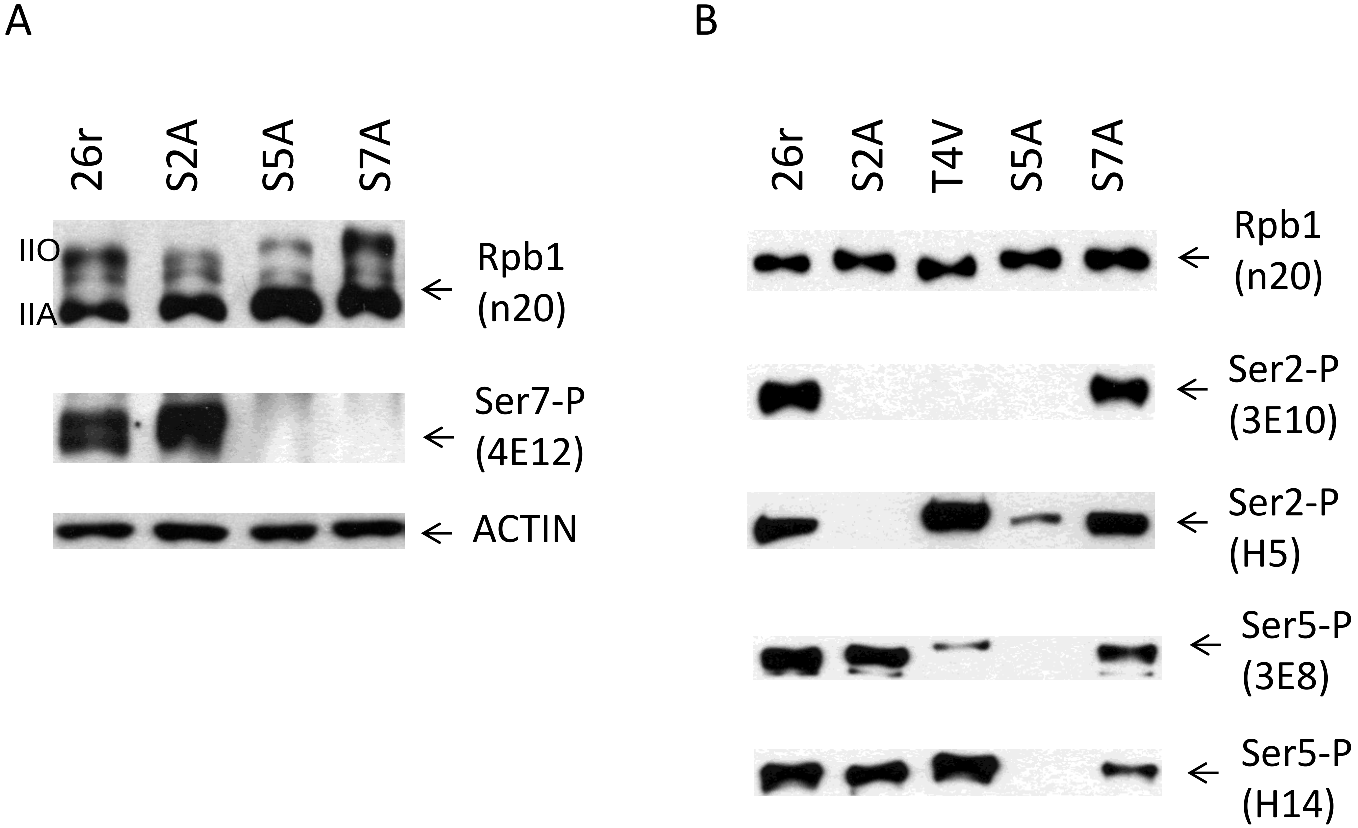
Figure S3


A
26r
S2A
S5A
S7A
IIO
IIA
Rpb1 (n20)
Ser7-P (4E12)
ACTIN
B
26r
S2A
T4V
S5A
S7A
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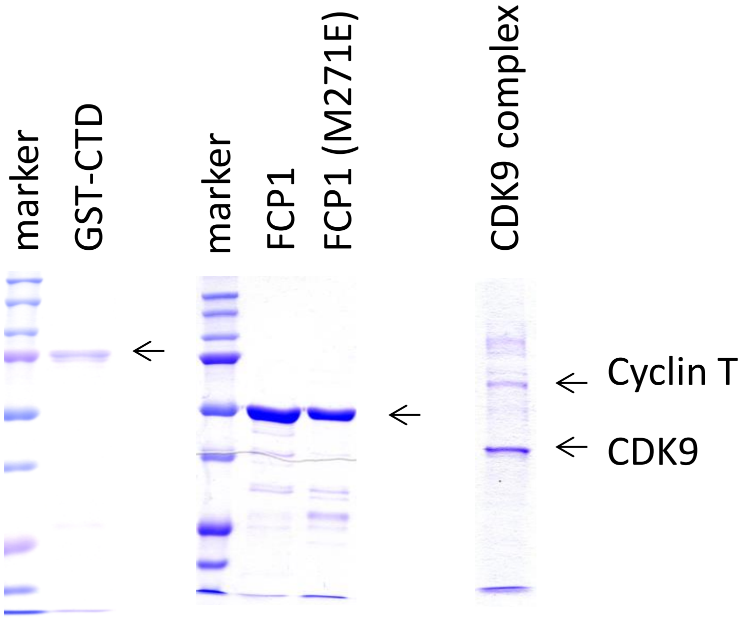
Ser2-P (3E10)
Ser2-P (H5)
Ser5-P (3E8)
Ser5-P (H14)

Figure S4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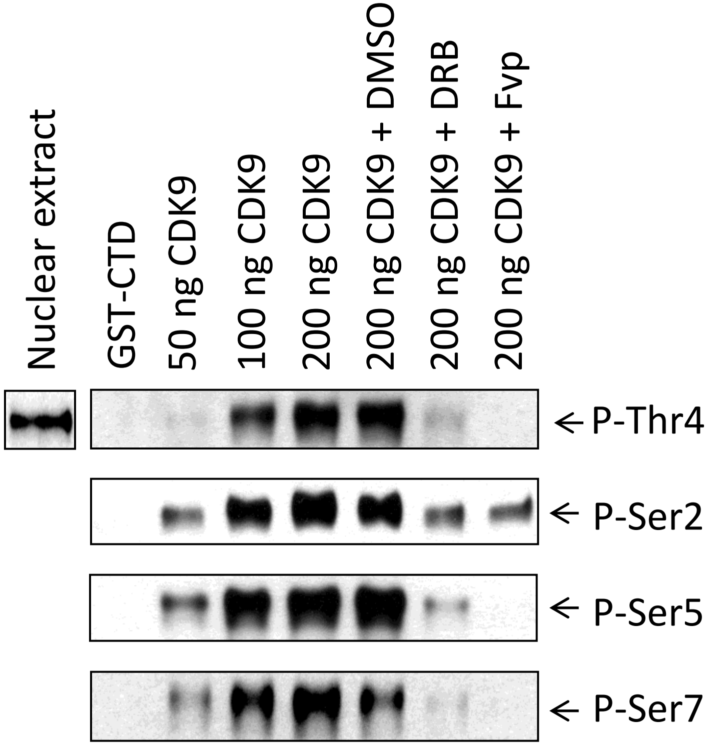
GST-CTD
marker
FCP1
FCP1 (M271E)
CDK9 complex
Cyclin T
CDK9

Figure S5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50 ng CDK9
100 ng CDK9
200 ng CDK9
200 ng CDK9 + DMSO
200 ng CDK9 + DRB
200 ng CDK9 + Fvp
P-Thr4
P-Ser2
P-Ser5
P-Ser7

Figure S6

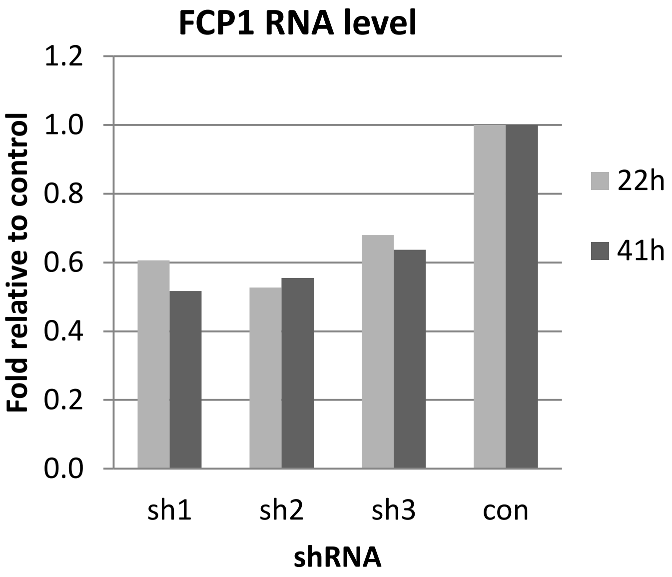
FCP1 RNA level
Fold relative to control
1.2
1.0
0.8
0.6
0.4
0.2
0.0
sh1
sh2
sh3
con
shRNA
22h
41h

Table S1. Primer sequence list.

Amplicon	Primer	Sequence
Unspliced egr1	egr1_F2	CGTTGCTGCTGGAGACCT
	egr1_2R1	GGCATGGTTTTCTCGTTGTTC
Spliced egr1	egr1_1F1	GCCACTATCCCAAACTGGAG
	egr1_2R1	GGCATGGTTTTCTCGTTGTTC
Uncut egr1	egr1_2F2	CATGCGGTTCATTATTTTGTG
	egr1_3'R3	GCACCCCTTACTTCCTAGCAG
Total egr1	egr1_2F5	AAGACACTCAAATGTGGCAGAA
	egr1_2R7	TTAAGGCAAGATGCAAAGCA
Rplp1_A	rplp1_p_F1	TATGACAGCCATGAGGCAAG
	rplp1_p_R1	ACGACCTAACATTTGAAGAAGC
Rplp1_B	rplp1_p_F2	TGGCTGATGGAGCCCTTAT
	rplp1_p_R2	GGAACGGCCTTAGCTTCAG
Rplp1_C	rplp1_F3	AGCTGAGAACAGAGGGACGA
	rplp1_R1	GCACACAGAAAGAGGATCTGC
Rplp1_D	rplp1_3'_F1	GATGACATGGGCTTTGGTCT
	rplp1_3'_R1	CAGCCATCCTTGAGAAACACT
Rplp1_E	rplp1_3'_F2	TGATAACAGTGCCTGCCTTG
	rplp1_3'_R2	GAACAGCAGTGAGCATCCAA
Actb_A	actb_p_F1	CTGACTGACCGCGTTACTCC
	actb_p_R1	CAGAAAAGAAACGAGCCGTCA
Actb_B	actb_2F	ATGATATTGCTGCGCTCGTT
	actb_2R	CCCACGATAGATGGGAACAC
Actb_C	actb_3'_F	GGCTTGACTGGTGTTGGTTT
	actb_3'_R	GCAGATAGCTGACCCACACA
H2A_TSS	H2A_F3	TCTTCGGTTGTACAGCAAGC
	H2A_R2	CGACTTGGCCTTAGCTCTGA
H2A_3'	H2A_F2	TTCAGAGCCACCCACTTTCT
	H2A_R1	GCGTTAAAAACAAAGGCACA
U1	U1_F1	CATGATCAGGCAGGTGGTTT
	U1_R1	AGTCGAGTTTCCCGCATTT
U2	U2_F1	CTCGGCCTTTTGGCTAAGAT
	U2_R1	CGCCCAAAAATCCGTTTAAT
U1_uncleaved	U1_F4	AGACACCATGATCAGGCAGG
	U1_3'_R4	TTGAACCGCGCAAATCAGG
U2_uncleaved	U2_F1	CTCGGCCTTTTGGCTAAGAT
	U2_3'_R	CGCCTTCCTTCTCTCCTTTC