Supplemental Data

CDK2-dependent phosphorylation of Suv39H1 is involved in control of heterochromatin replication during cell cycle progression

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Inventory of Supplemental Information

The Supplemental Information contains 14 additional figures, and 1 table

Figure S1. Related to Figure 2

Figure S2. Related to Figure 2

Figure S3: Related to Figure 2B

Figure S4. Related to Figure 2D

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Figure S12. Related to Figure 5

Figure S13. Related to Figure 5C

Figure S14: Related to Discussion section **(**A summary model figure)

Table S1: primer sequences used in this study

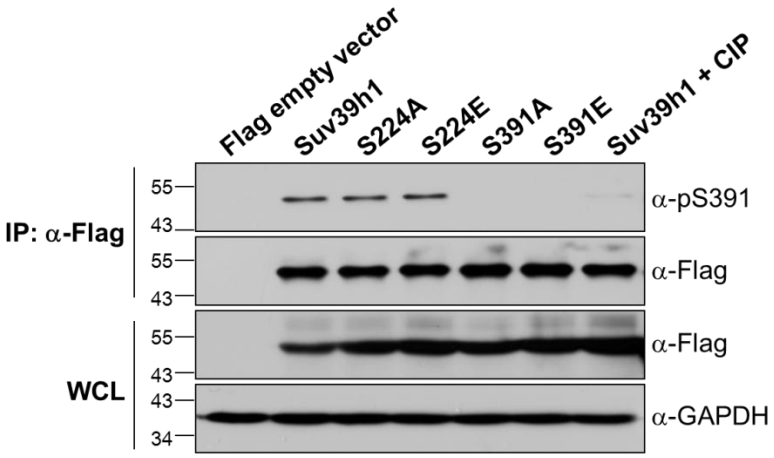

Figure S1. (Related to Figure 2) *In vivo* phosphorylation of Suv39H1 at Ser-391. 293T cells were transfected with Flag-tagged wild-type (WT) Suv39H1, Flag-Suv39H1 S224A, Flag- Suv39H1 S224E, Flag-Suv39H1 S391A, or Flag-Suv39H1 S391E. Total lysates including CIP-treated WT were immunoprecipitated using anti-Flag M2 agarose beads and analyzed by Western blotting by anti-phosphoSuv39H1 (pS391; top). Expression of Flag-Suv39H1-WT or four Suv39H1 point mutants was confirmed by Western blot analysis using anti-Flag antibody (middle panels).

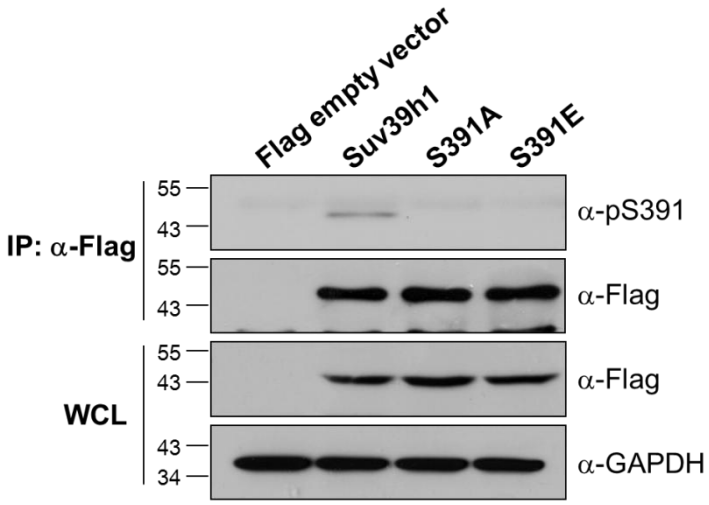

Figure S2. (Related to Figure 2) Suv39H1 is *in vivo* phosphorylated at Ser-391 site in the mouse fibroblast NIH3T3 cells. Immunoprecipitation (IP) and immunoblot of NIH3T3 cell lines stably expressing Flag-tagged wild type Suv39H1, Flag-Suv39H1 S391A, or Flag-Suv39H1-S391E. IP with anti-Flag antibody and immunoblot with anti-pS391 antibody were performed as mentioned in Figure 1D.

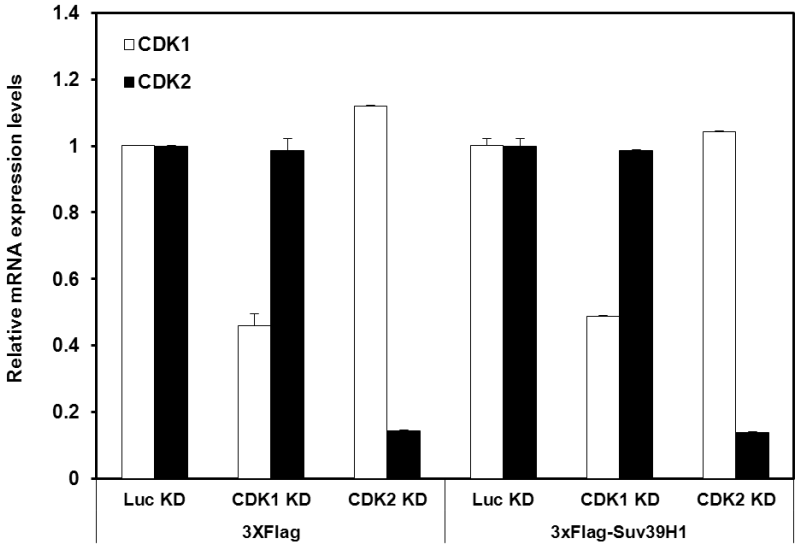

Figure S3. (Related to Figure 2B) RT-PCR of CDK1 or CDK2 mRNA levels after infection of 293T cells stably expressing Flag-empty vector or Flag-Suv39H1 with lentivirus containing shRNA constructs (CDK1-KD or CDK2-KD) compared with shLuc control. The efficiency in CDK1 KD or CDK2 KD was confirmed by RT-PCR.

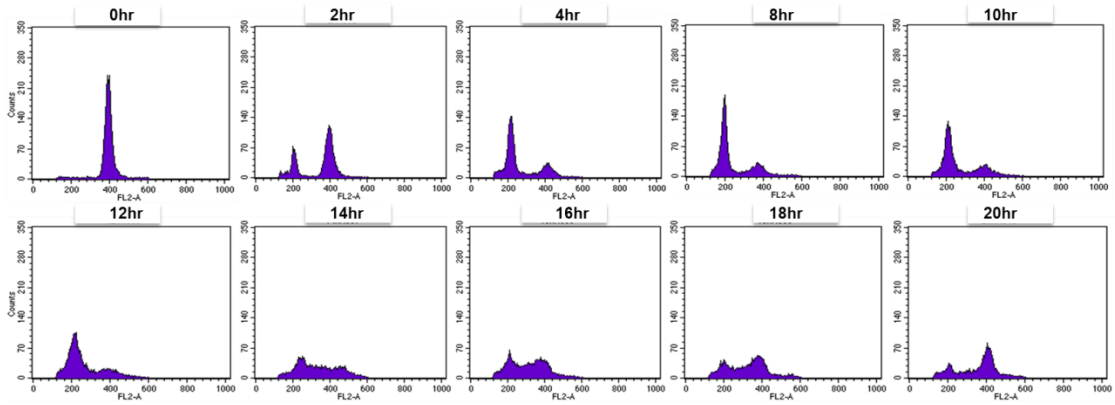

Figure S4. (Related to Figure 2D) Stably expressing Flag-Suv39H1 HeLa cells were synchronized to M phase by nocodazole for 24 h and then released at time intervals. Cells were stained with PI, and DNA contents were evaluated by flow cytometry.

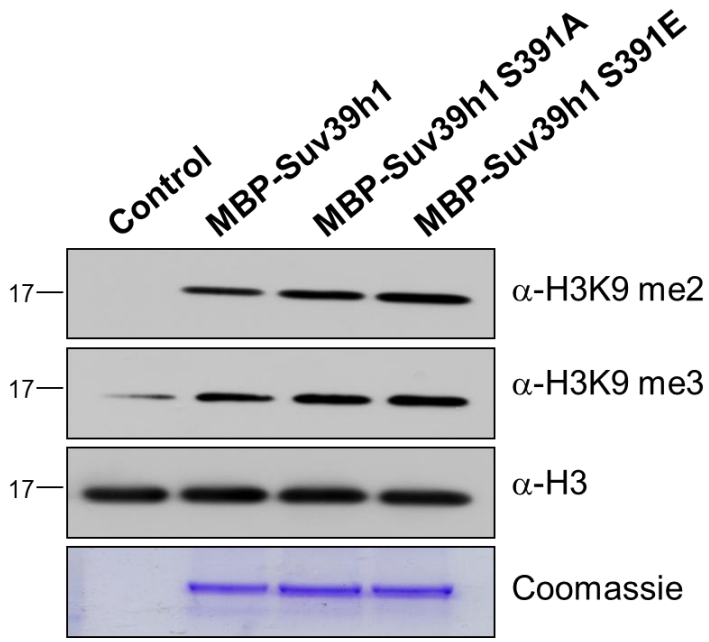

Figure S5. (Related to Figure 3) ***In vitro*** **histone methyltransferase assay with purified Suv39H1-WT, phospho-defective Suv39H1-S391A, or phospho-mimic-Suv39H1- S391E proteins.** Proteins were expressed in CodonPlus *E.coli* cells and purified using amylose resins (New England Biolabs., Beverly, MA). Purified proteins were incubated with 1 μg recombinant histone H3 at 30°C for 3 hr in reaction mixture containing S-adenosyl-methionine as a methyl donor and ATP. Reaction mixtures were separated by SDS-PAGE and subjected to Western blot analysis using anti-H3K9me2 or anti-H3K9me3. Equal loading of histone H3 was confirmed by Western blot analysis with anti-H3 antibody. The Suv39hH1 protein was stained with Coomassie blue to confirm equal loading (bottom).

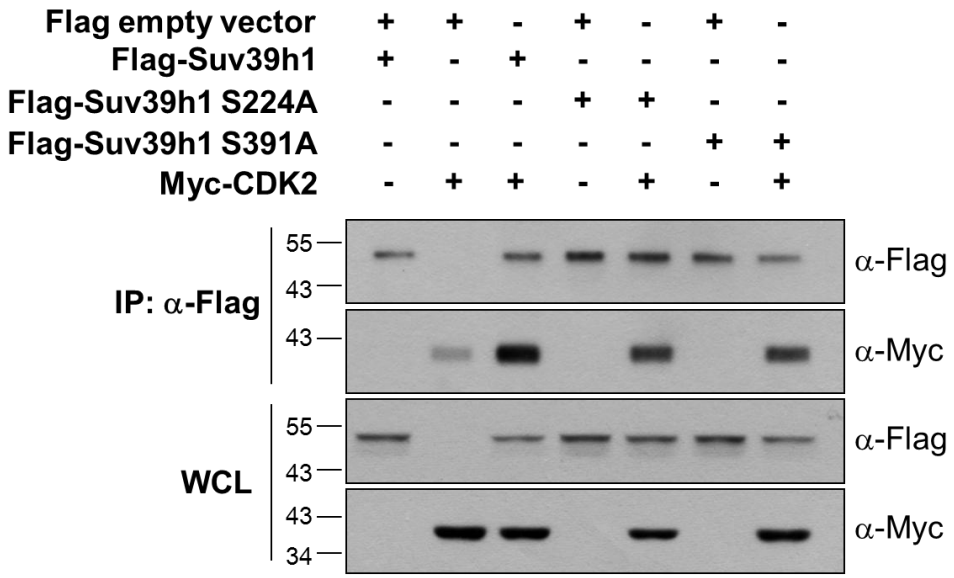

Figure S6. (Related to Figure 3) Phosphorylation does not affect the physical interaction between Suv39H1 and CDK2. Cell extracts from Flag-Suv39H1-WT, Suv39H1-S391A, or Suv39H1-S391E and Myc-CDK2 co-transfected 293T cells were immunoprecipitated (IP) and analyzed by immunoblotting as indicated.

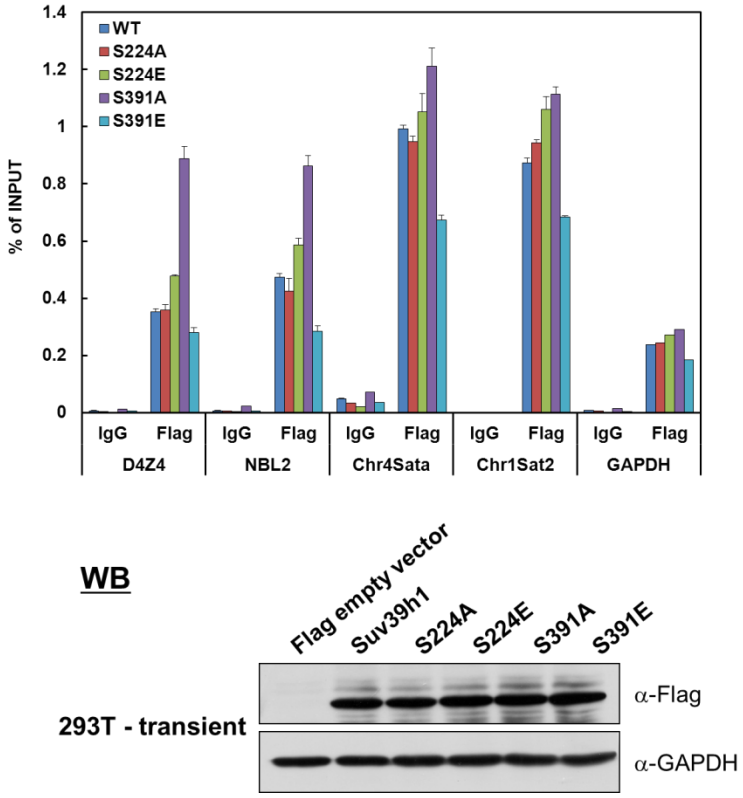

Figure S7. (Related to Figure 3) Phospho-mimic form of Suv39H1-S391 shows lower occupancy on heterochromatic regions compared with its phospho-defective form using ChIP assay. ChIP assay of asynchronously growing *293T* cell lines transiently transfected with Flag-Suv39H1-WT, Flag-Suv39H1-S224A, Flag-Suv39H1-S224E, Flag-Suv39H1-S310A, or Flag-Suv39H1-S391E. ChIP assay using anti-Flag antibody was performed as mentioned in Figure 2A & 2B. Error bars, SEM of triplicates from representative experiment. The protein expression of each gene transiently expressing 293T cells were confirmed by Western blot analysis. qPCR data are presented as means±s.d., and are representative of 3 independent experiments.

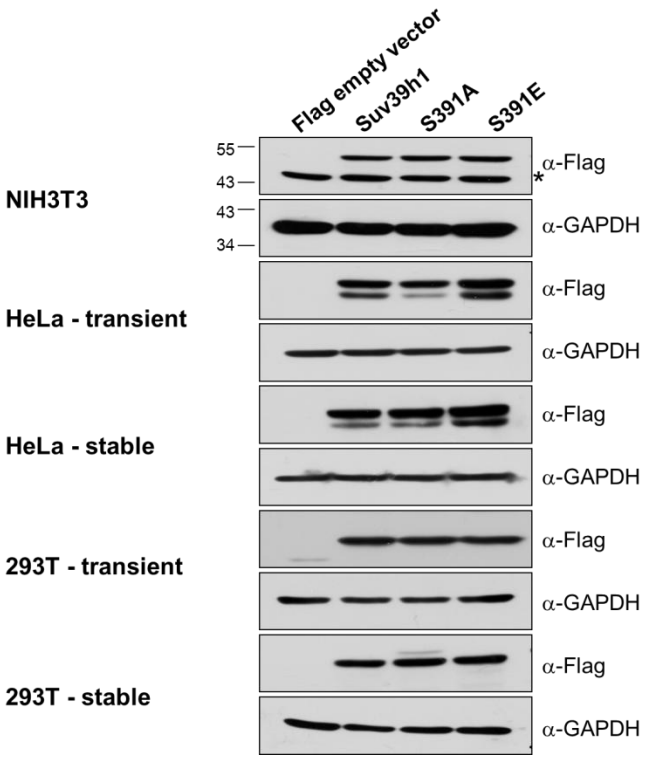

Figure S8. (Related to Figure 3, 4, and 5) Immunoblot of NIH3T3, *HeLa*, or 293T cell lines stably or transiently expressing Flag-tagged Suv39H1-WT, Flag-Suv39H1-S319A, or Flag-Suv39H1-S319E.

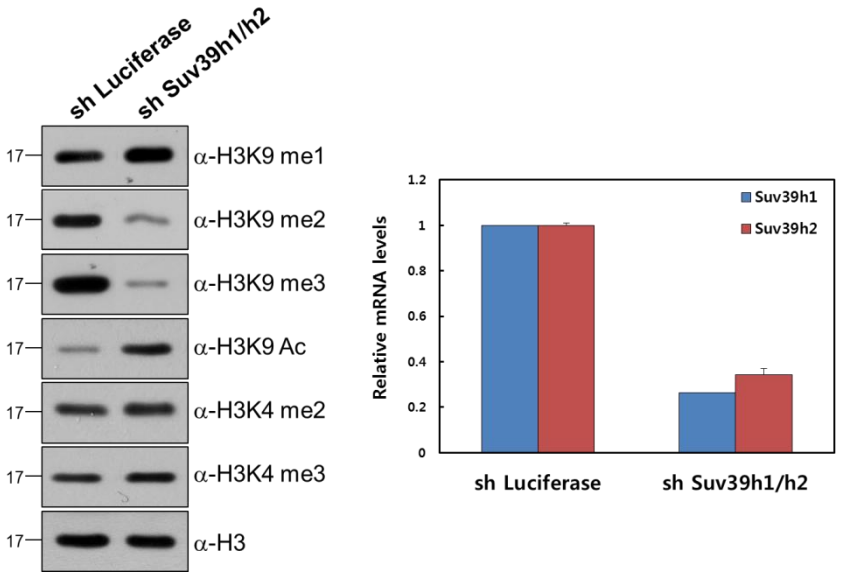

Figure S9. (Related to Figure 3) Establishment of HeLa cell lines with double knock-down of Suv39H1 and Suv39H2 genes. The global histone modifications and mRNA levels of Suv39H1/Suv39H2 using immunoblot and qRT-PCR were characterized for the double knock-down mutant. The data suggest that the double knock-down mutant cells have peculiar characters which are expected in cells lacking both Suv39H1 and Suv39H2 genes. qPCR data are presented as means±s.d. for representative experiment.

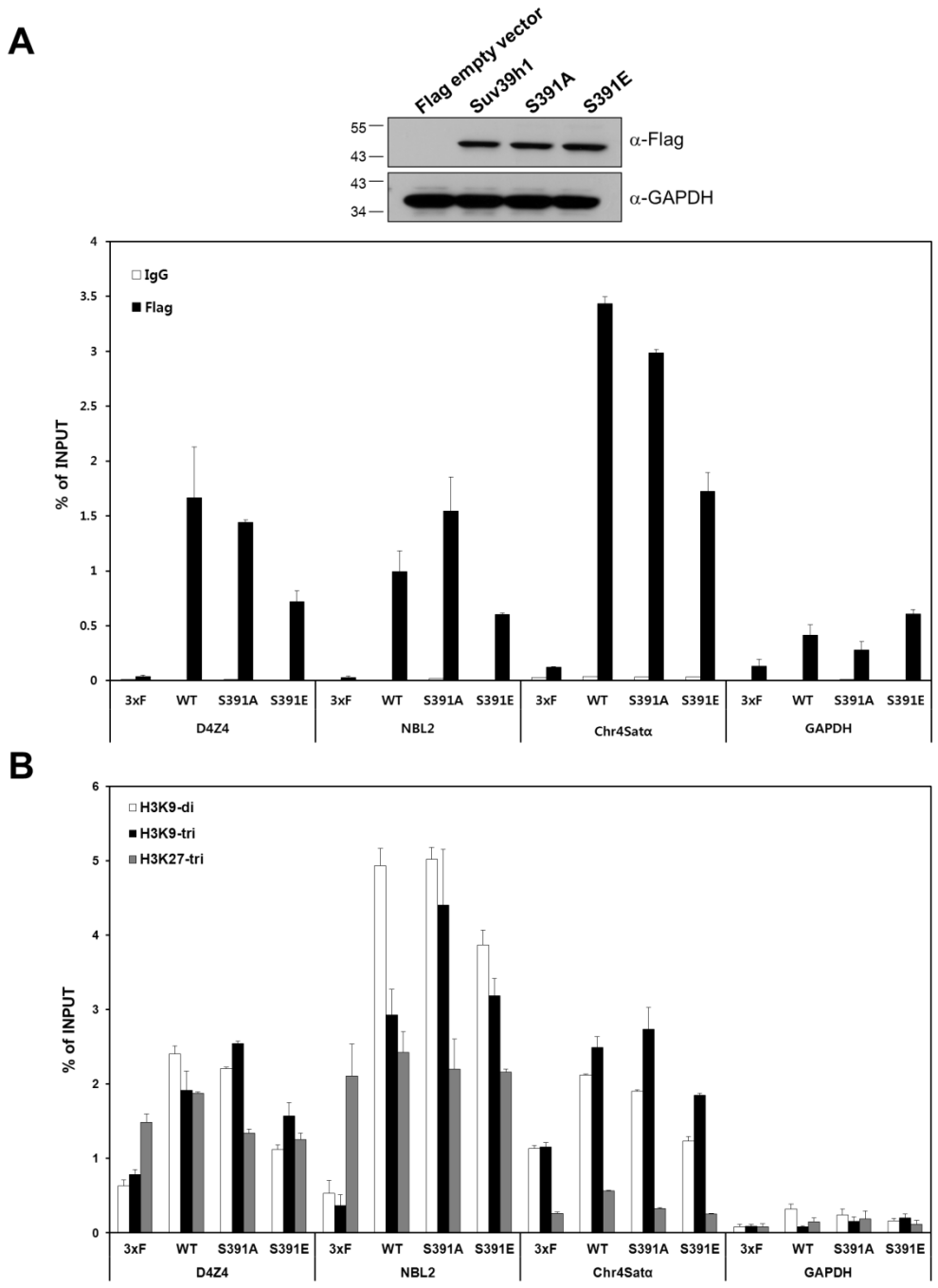

Figure S10. (Related to Figure 3) ChIP assay of asynchronously growing Suv39H double knock-down *HeLa* cell lines transfected with Flag-empty vector, Flag-Suv39H1-WT, Flag-Suv39H1-S391A, or Flag-Suv39H1-S391E. ChIP assay was carried out to reveal the heterochromatin occupancy of Flag-tagged target proteins (A),

and alteration in histone H3K9 methylations at heterochromatic loci (B). The expression of each gene was confirmed by Western blot (A). qPCR data are presented as means±s.d., and are representative of 2 independent experiments.

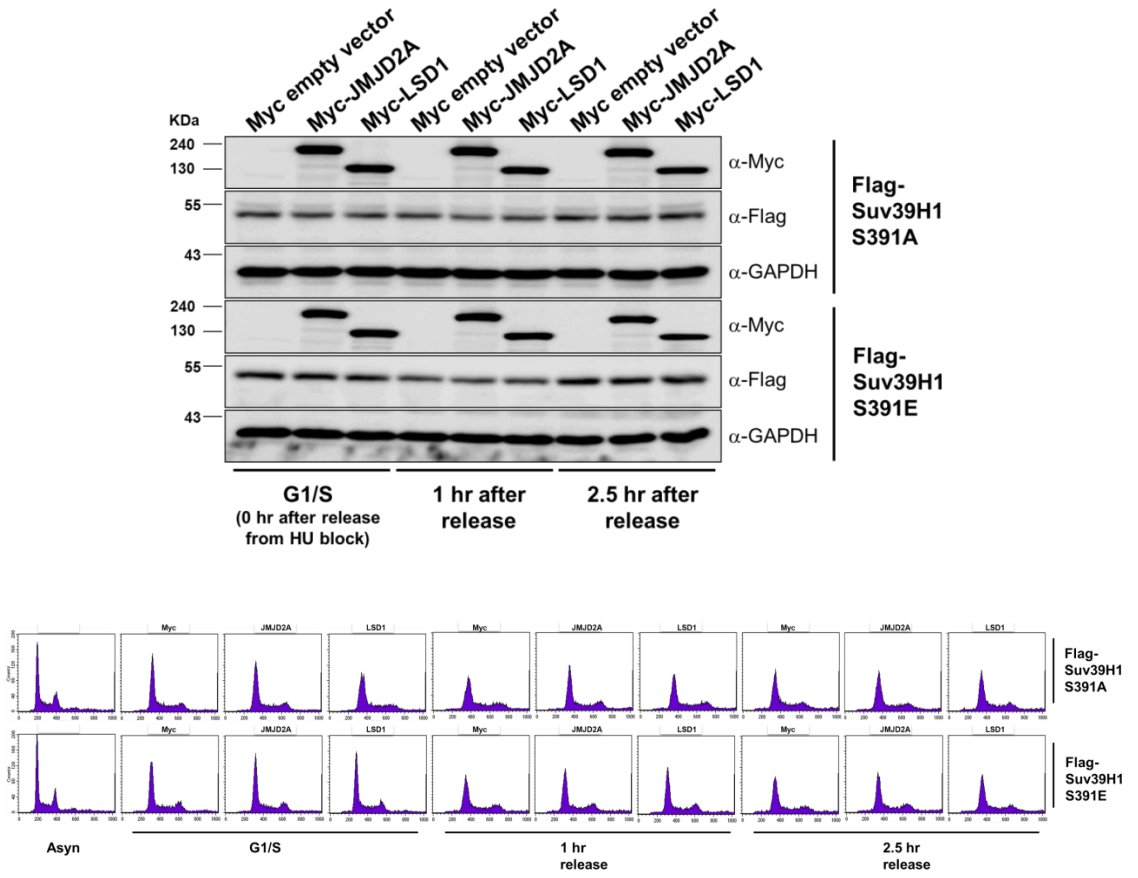

Figure S11. (Related to Figure 4) Immunoblot and cell cycle profile of HeLa cells overexpressing Flag-Suv39H1 S391A or Flag-Suv39H1 S391E transfected with Myc-empty vector, Myc-JMJD2A, Myc-LSD1 at time intervals after release from HU blockage. Cells stably expressing Flag-tagged Suv39H1-S391A or Suv39H1-S391E were transfected with empty Myc vector (control), Myc-tagged Jmjd2A or LSD1 and synchronized in G1/S phase with HU treatment and released from the block and collected at different time points indicated. Protein expression of each construct was confirmed by Western blot analysis using anti-Myc, anti-Flag antibodies or anti-GAPDH antibody. Cells were stained with PI, and DNA contents were evaluated by flow cytometry as shown in bottom panel.

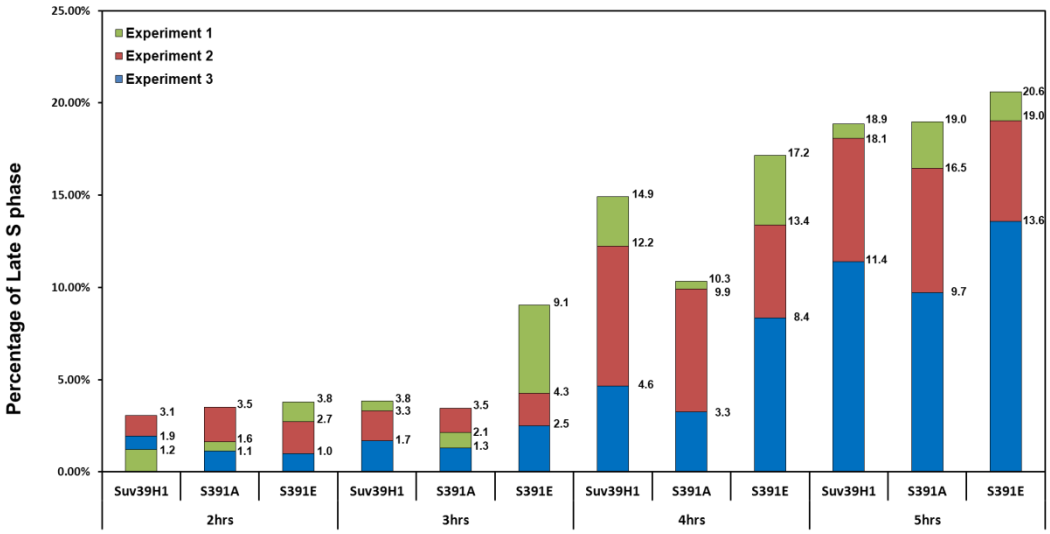

Figure S12. **(Related to Figure 5) Overexpression of phospho-mimic Suv39H1-S391E results in early appearance of late S phase cells whereas delay of S phase is caused by overexpression of Suv39H1-S391A.** Mouse fibroblast NIH3T3 cells stably expressing Flag empty vector, wild type Suv39H1, Suv39H1 S391A, or Suv39H1 S391E were synchronized at the G1-S border with aphidicolin, released into S phase by wash-out, pulse-labeled with BrdU at the times indicated. The cells co-stained with propidium iodide (PI) and anti-BrdU antibody were subjected to FACS for analysis of DNA contents. We conducted three independent experiments and each FACS data are separately presented here. The proportion of BrdU-stained late S phase cells over total BrdU-stained S phase cells at the time intervals in each FACS data was displayed as a graph. The graph depicts indicates the percent proportion of late S phase cells among total BrdU-stained S phase cells at each interval after aphidicolin-blockage and release. The data showed that overexpression of phospho-mimic form of Suv39H1 causes early appearance of late S phase compared with that of overexpression of phospho-defective form Suv39H1-S391A.

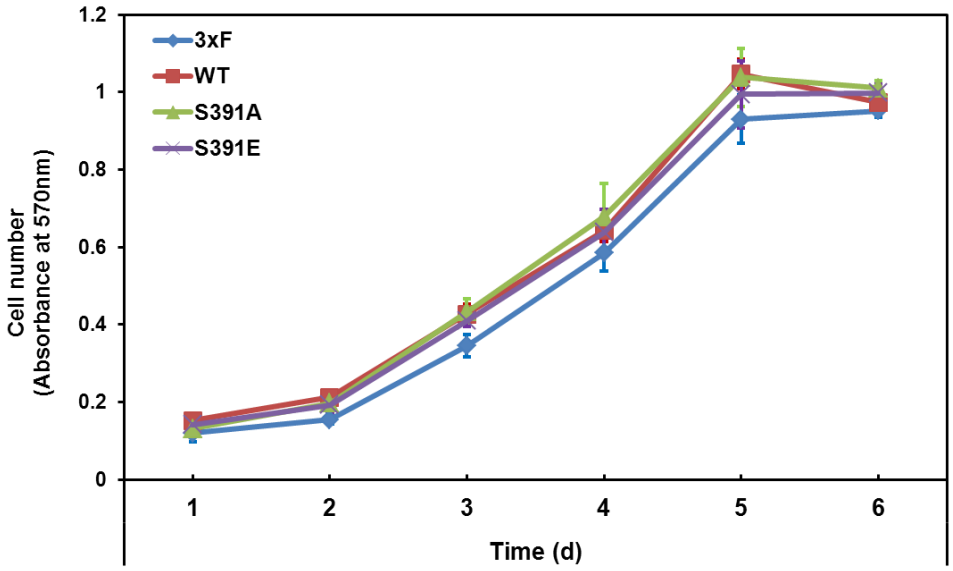

Figure S13. (Related to Figure 5C) Cells overexpressing Suv39H1 wild-type or phospho mutants show little differences in growth rates compared to control cells. *Hela* cells stably expressing Flag empty vector, Suv39H1-WT, Suv39H1 S391A, or Suv39H1 S391E were subjected to MTT assay for investigation of cell growth rates at indicated time points (d, day).

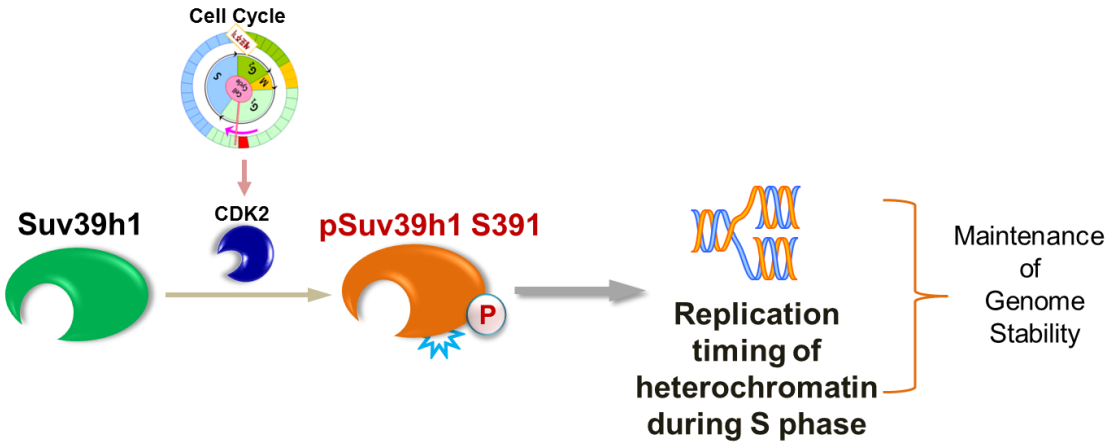

Figure S14. (Related to Discussion section) Model depicting the functional role of CDK2-mediated Suv39H1 phosphorylation during cell cycle progression.

CDK2-dependent phosphorylation of Suv39H1 at Ser-391 results in its preferential dissociation from heterochromatin during S phase. These phosphorylation-mediated dissociation of Suv39H1 from chromatin is accompanied by enhanced preoccupancy of JMJD2A at heterochromatic loci and decreased levels of inactive histone mark H3K9me3, thereby allowing the cell proper replication of heterochromatin. Collectively, the CDK2-mediated phosphorylation of Suv39H1 is important for maintenance of genome stability.

Supplementary Table S1. Primer sequence for shRNA and ChIP

Use	Name	Direction	Sequence
shRNA	*human Suv39h1*		CCGGCGTTGGGATTCATGGCCTATTCTCGAGAATAG GCCATGAATCCCAACGTTTTTTG
	human Suv39h2		CCGGGCACAGATTGCTTCTTTCAAACTCGAGTTTG AAAGAAGCAATCTGTGCTTTTTG
	mouse Suv39h1		CCGGGCCTTTGTACTCAGGAAAGAACTC GAGTTCTTTCCTGAGTACAAAGGCTTTTTG
	mouse Suv39h2		CCGGCCACCTTTGGATGTTCATGTACTCGAGTACAT GAACATCCAAAGGTGGTTTTTG-
	human CDK1		CCGGCCGGGCTGTACTTCGTCTTCTAATTCTCGAGA ATTAGAAGACGAAGTACAGCTTTTT-
	human CDK2		CCGGCCGGGCCCTCTGAACTTGCCTTAAACTCGAG TTTAAGGCAAGTTCAGAGGGCTTTTT
	Luciferase		CCGGAGAGCTGTTTCTGAGGAGCCTCTCGAGAGGC TCCTCAGAAACAGCTCTTTTTTG
ChIP	*D4Z4*	For	CTCAGCGAGGAAGAATACCG
		Rev	ACCGGGCCTAGACCTAGAAG
	NBL2	For	TCCCACAGCAGTTGGTGTTA
		Rev	TTGGCAGAAACCTCTTTGCT
	Chr4Satα	For	CTGCACTACCTGAAGAGGAC
		Rev	GATGGTTCAACACTCTTACA
	Chr1Sat2	For	CATCGATGGAAATGAAAGGAGTC
		Rev	ACCATTGGATGATTGCAGTCAA
	GAPDH	For	CCCAACTTTCCCGCCTCTC
		Rev	CAGCCGCCTGGTTCAACTG