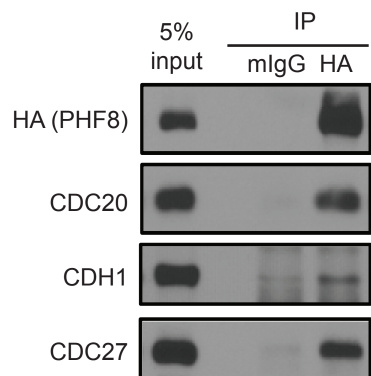
Lim et al. Supplementary Figure 1



SUP. FIG. 1. PHF8 interacts with the CDC20-containing APC (APC^{cdc20}). HEK293T cells were transiently transfected with Flag-HA-PHF8 and lysates were used for semi-endogenous IP using antibodies against HA or mouse IgG control. Immunocomplexes were immunoblotted for HA (PHF8), CDC20, CDH1 and CDC27.

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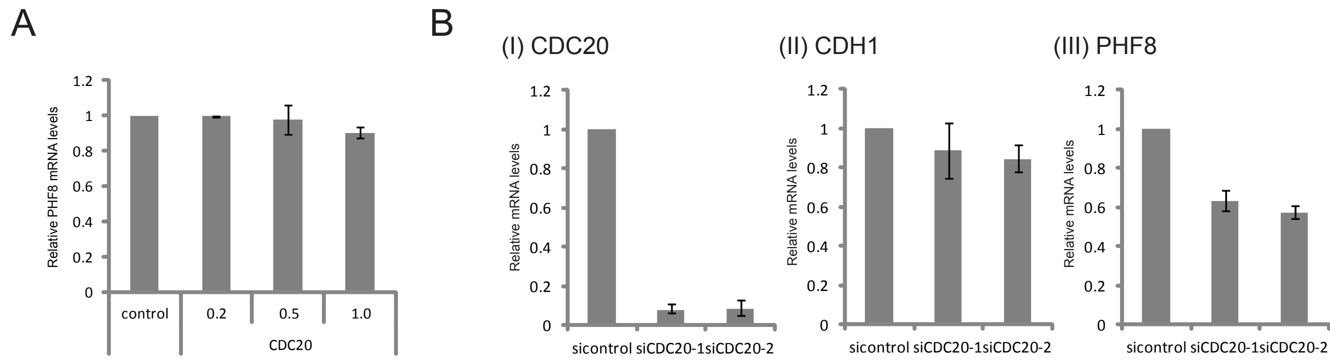
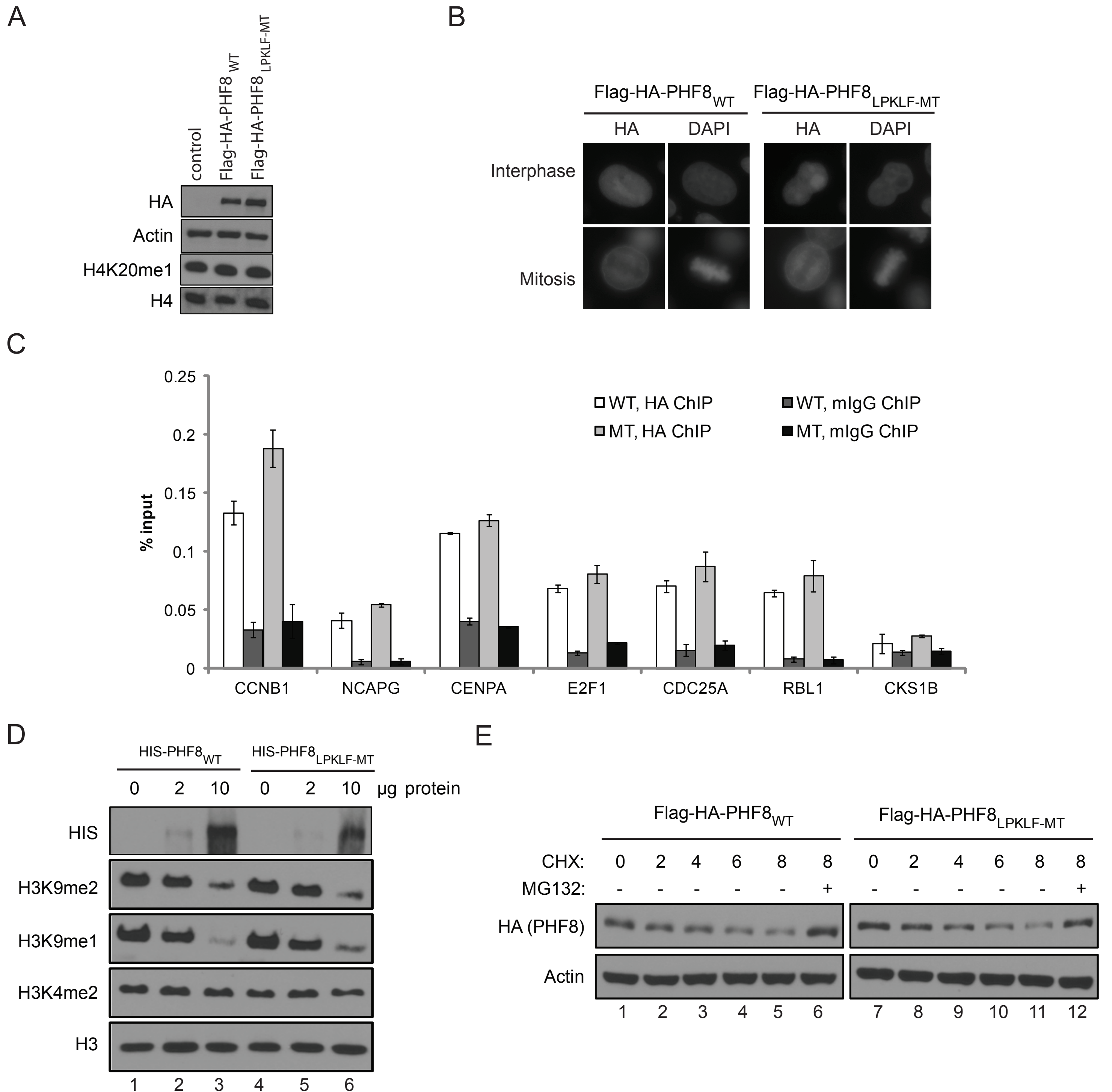
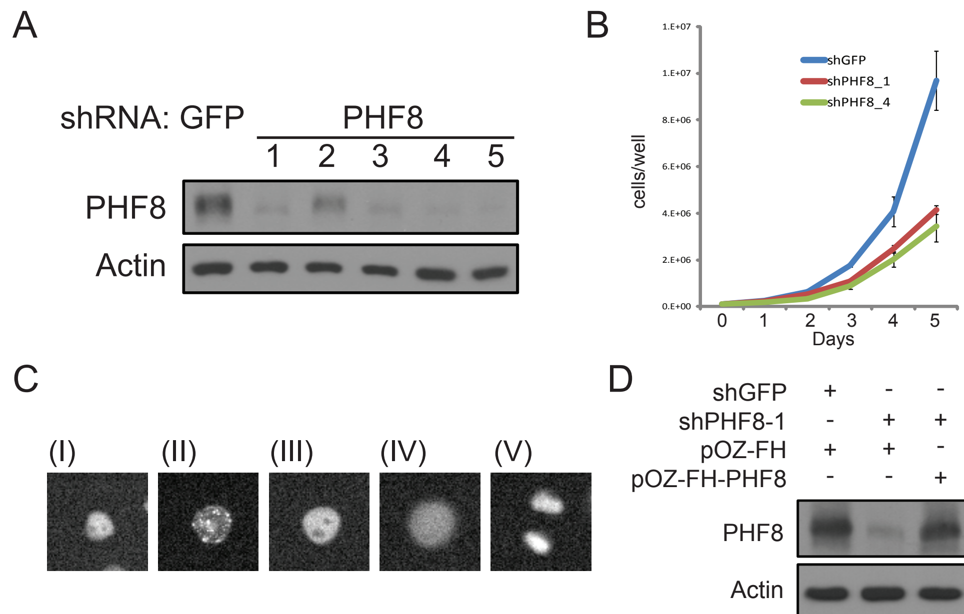


SUP. FIG. 2. PHF8 mRNA levels of CDC20-overexpressing and CDC20-knockdown cells (A) A portion of cells from Fig. 4A transiently transfected with 0.2, 0.5 and 1.0 µg of HA-CDC20 were lysed to obtain total RNA. RNA was reversed transcribed to obtain cDNA. qPCR was carried out on cDNA samples using gene specific primers to detect PHF8 and GAPDH mRNA levels. Relative PHF8 mRNA levels were obtained by normalizing against GAPDH and control sample. The mean of biological duplicate sets of data and their standard deviation is plotted here in graphical form. (B) A portion of cells from Fig. 4B transiently transfected with sicontrol, siCDC20-1 or siCDC20-2 were lysed to obtain total RNA. RNA was reversed transcribed to obtain cDNA. qPCR was carried out on cDNA using gene-specific primers against (I) CDC20, (II) CDH1 and (III) PHF8, and the data was normalized against GAPDH mRNA levels and control samples. The mean and standard deviation of duplicate sets of data was plotted graphically.



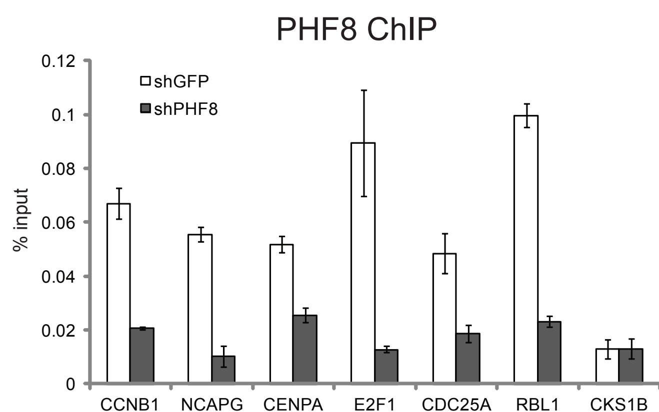
SUP. FIG. 3. PHF8LPKLF-MT retains the localization and catalytic ability of wild-type PHF8. (A) HeLa stable cell lines were generated by retrovirus transduction to incorporate either empty vector, Flag-HA-PHF8 WT or the LPKLF-MT, and selected by α-IL2R bead selection. The expression of the construct is shown by α-HA immunoblotting. (B) Cells from (A) were fixed and subjected to immunofluorescence staining for HA and DAPI. Representative images of single cells are presented here. (C) HeLa cell line stably overexpressing Flag-HA-tagged PHF8WT or PHF8LPKLF-MT was generated. HA and mIgG ChIP-qPCR were carried out using the WT or MT line to see if WT and MT PHF8 bind to a similar extent and fashion over TSS of its target genes. ChIP-qPCR data was normalized over the input DNA and is presented as the %input. (D) Sf9-purified full-length WT or LPKLF-MT PHF8 was incubated with calf histones in the presence of co-factors α-ketoglutarate and Fe(II). Reaction was carried out at 37°C for 4-6h before SDS loading dye was added to stop the reaction. Reaction was immunoblotted for PHF8 target methylation marks, H3K9me2 and H3K9me1, and H3K4me2 and H3 as control. (E) The two HeLa cell lines stably overexpressing either Flag-HA-tagged PHF8WT or PHF8LPKLF-MT were subjected to cycloheximide chase with DMSO or MG132 addition, harvested at time points 0, 2, 4, 6 and 8h post cycloheximide addition, and probed for exogenous HA-tagged PHF8.

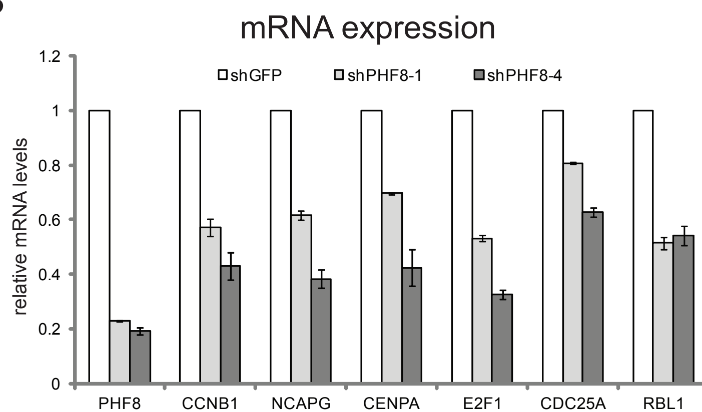
Lim et al. Supplementary Figure 4



SUP. FIG. 4. Determining the cell cycle effect of PHF8. (A) Five different shRNAs targeting different regions on PHF8 or an shRNA targeting GFP as control was transduced by lentivirus into HeLa cells. 48h post infection, cells were harvested and equal amounts of lysates were separated by SDS-PAGE and immunoblotted for PHF8 and actin as loading control. (B) Equal number of HeLa cells subjected to GFP or PHF8 RNAi (shRNA #1 and #4) were plated in triplicate and counted over five days. The mean and standard deviation from triplicate counts were plotting here in graphical form. (C) HeLa-RFP-ligase cells were used for live cell imaging and looks as follows in various stages of the cell cycle: (I) G1, (II) S, (III) G2, (IV) prophase of M, (V) telophase of M. (D) PHF8 rescue was carried out by transducing cells with shPHF8-1 together with exogenous pOZ-Flag-HA-PHF8. Immunoblotting shows that PHF8 rescue cells express PHF8 to similar levels as control transduced cells.

Lim et al. Supplementary Figure 5





SUP. FIG. 5. Validation of PHF8 target genes. (A) PHF8 ChIP was carried out using control GFP or PHF8 shRNA transduced HeLa cells, and qPCR was done using gene-specific primers for the genomic region corresponding to the TSS of CCNB1, NCAPG, CENPA, E2F1, CDC25A, RBL1 and CKS1B. Data was normalized against input DNA and the mean and standard deviation of duplicate sets of data is presented here in graphical form. (B) Total mRNA was harvested from control GFP or two different PHF8 shRNA (#1 and #4) transduced HeLa cells. mRNA was reverse transcribed and the qPCR was carried out on the cDNA obtained, using gene-specific primers against the various genes. Data was normalized against GAPDH and control sample, and the mean and standard deviation of duplicate sets of data is presented here.