Supplementary Material

Loss of FBW7 Promotes Chromosomal Instability and Tumorigenesis via Cyclin E1/ CDK2-mediated CENP-A Phosphorylation

Mamoru Takada[*], Weiguo Zhang[*], Aussie Suzuki, Taruho S. Kuroda, Zhouliang Yu, Hiroyuki Inuzuka, Daming Gao, Lixin Wan, Ming Zhuang, Lianxin Hu, Bo Zhai, Christopher J. Fry, Kerry Bloom, Guohong Li, Gary H. Karpen[+], Wenyi Wei[+], Qing Zhang[+]

[*]Contributed equally to this work

+ Corresponding authors

E-mail:

ghkarpen@lbl.gov (G.K)

Wwei2@bidmc.harvard.edu (W.W)

Qing_Zhang@med.unc.edu (Q.Z)

Figure S1

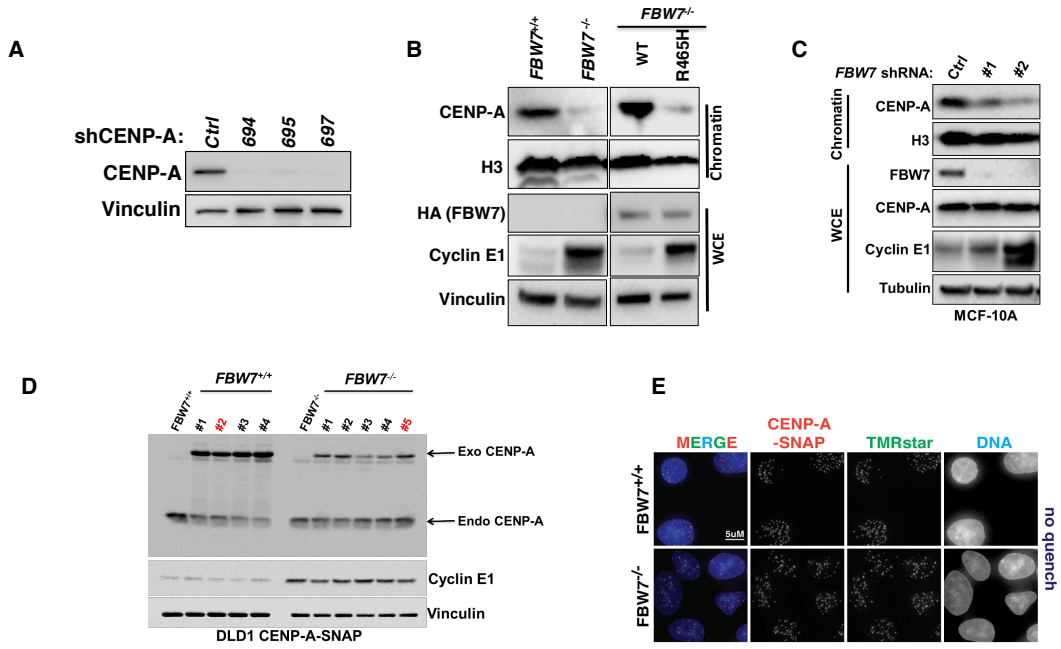

Figure S1. FBW7 Loss Leads to Defects in CENP-A Centromeric Incorporation in Cancer Cells, Related to Figure 1.

(A) Immunoblot analysis of CENP-A using a specific anti- CENP-A antibody with DLD1 whole cell lysates after infection with the lentivirus encoding CENP-A shRNA (694, 695 and 697) or control (Ctrl). Vinculin serves as the loading control.
(B) Immunoblot analysis of CENP-A using whole cell and chromatin bound extracts prepared from DLD1 *FBW7*$^{+/+}$, or *FBW7*$^{-/-}$ cells infected with the lentivirus encoding either wild type FBW7 or FBW7 disease mutant R465H. Vinculin serves as the loading control.
(C) Immunoblot analysis of whole-cell and chromatin-bound extracts prepared from MCF-10A cells infected with the lentivirus encoding FBW7 shRNA (#1 and #2) or control shRNA (Ctrl). Tubulin serves as the loading control.
(**D**) Multiple stable DLD1 cell line clones expressing CENP-A-SNAP were established followed by immunoblot analysis. SNAP-tagged (top) and endogenous (bottom) CENP-A are shown. #2 clone from *FBW7*$^{+/+}$ and #5 clone *FBW7*$^{-/-}$ cells were picked for further analysis. Vinculin serves as the loading control.
(**E**) DLD1 *FBW7*$^{+/+}$ and *FBW7*$^{-/-}$ cells expressing CENP-A-SNAP were labeled with TMR only (for total CENP-A-SNAP; no quench).

Figure S2

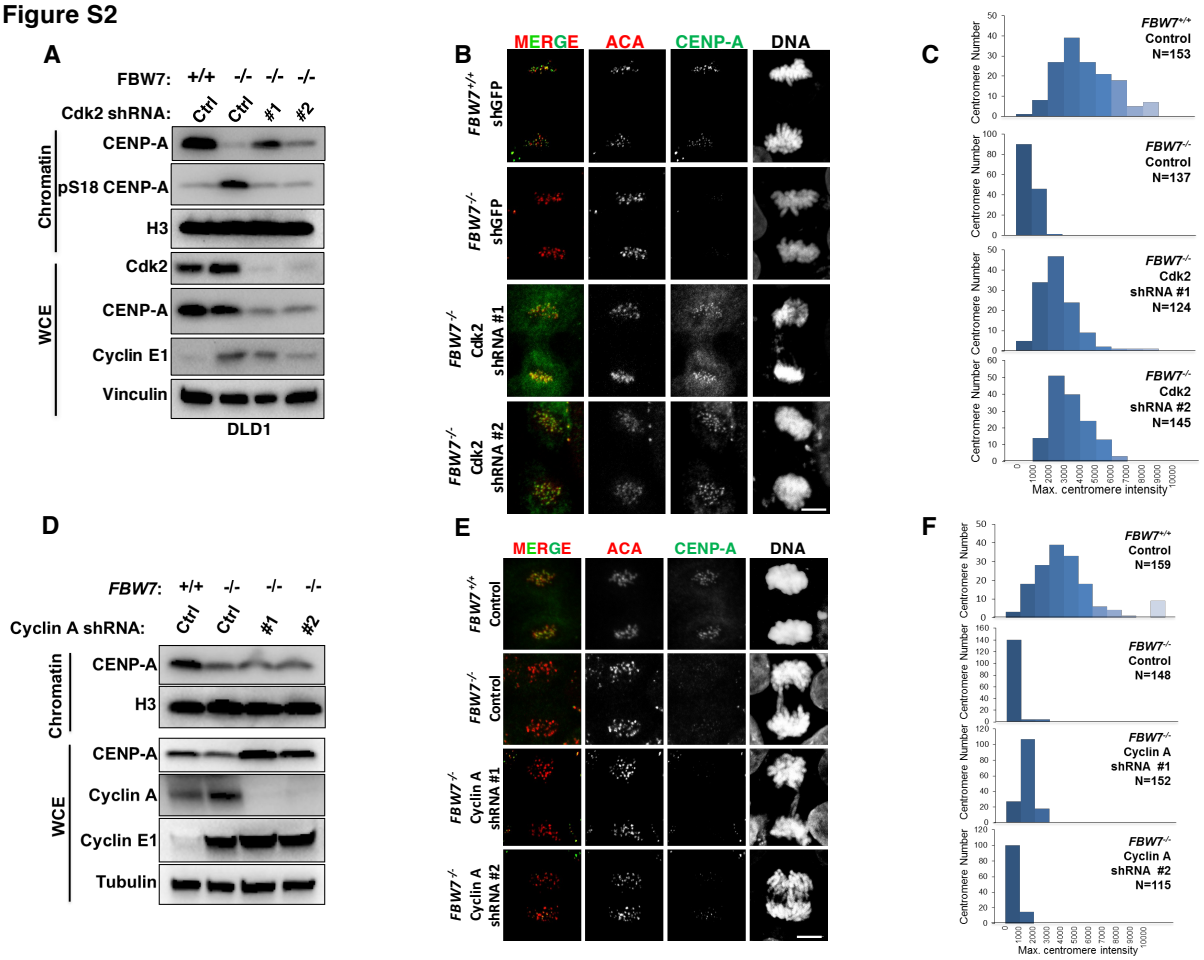

Figure S2. Cdk2/Cyclin E1 Is Required for De-localization of CENP-A in *FBW7*$^{-/-}$ Cancer Cells, Related to Figure 2.

(A, D) Immunoblot analysis of whole cell and chromatin extracts prepared from DLD1 *FBW7*$^{+/+}$, or *FBW7*$^{-/-}$ cells infected with the lentivirus encoding either Cdk2 shRNA (#1 and #2, panel **A**), Cyclin A shRNA (#1 and #2, panel **D**) or control (Ctrl).

(B, C, E, F) Representative images (**B, E**) or quantification (**C, F**) from cells described above stained with DAPI, CENP-A or ACA. Images are maximum-intensity projections of z stacks collected at 0.2-μM steps. Bars=5 μm. For each cell type, >3 cells and >100 kinetochores were quantified.

Figure S3

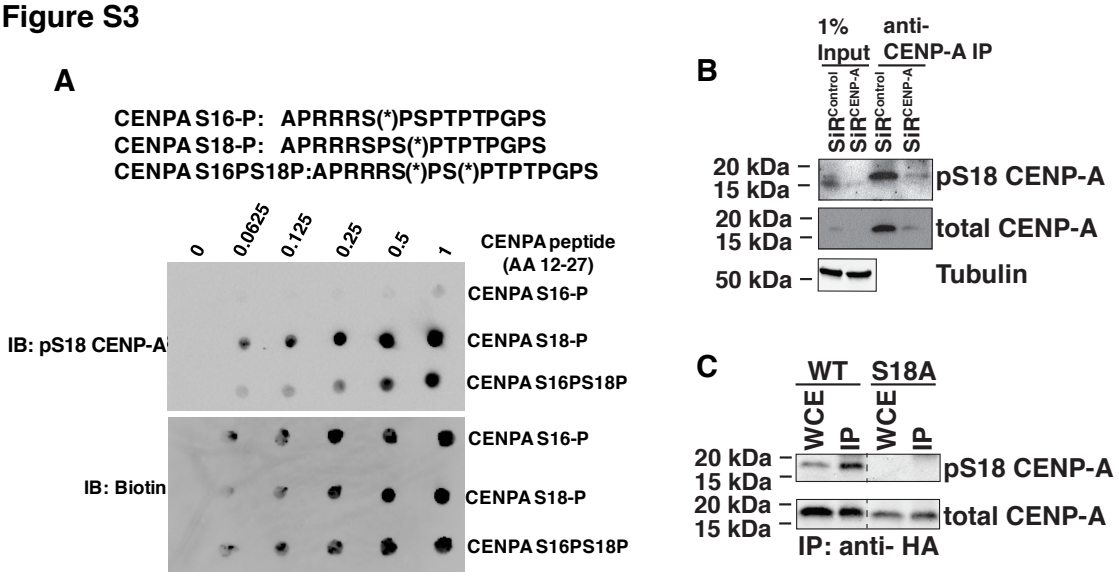

Figure S3. Characterization of CENP-A S18-P Antibody, Related to Figure 3.
(A) Dot blot analysis of pS18 CENP-A and biotin antibodies for biotinylated CENP-A peptides (AA12-27) that are either WT (Non-phospho) or phosphorylated on Ser16, Ser18 or Ser16Ser18 residues. **(B)** Immunoblots of pS18 CENP-A and CENP-A on total CENP-A immunoprecipitated using an anti- CENP-A antibody from chromatin extracts using HeLa cells transfected with either CENP-A siRNA or control siRNA. Anti- tubulin was used to control the number of cells used for chromatin extraction. **(C)** Immunoblots of whole cell extracts (WCE) and anti- HA antibody immunoprecipitates (IP) from HeLa cells transfected with HA- tagged CENP-A WT or S18A mutant.

Figure S4

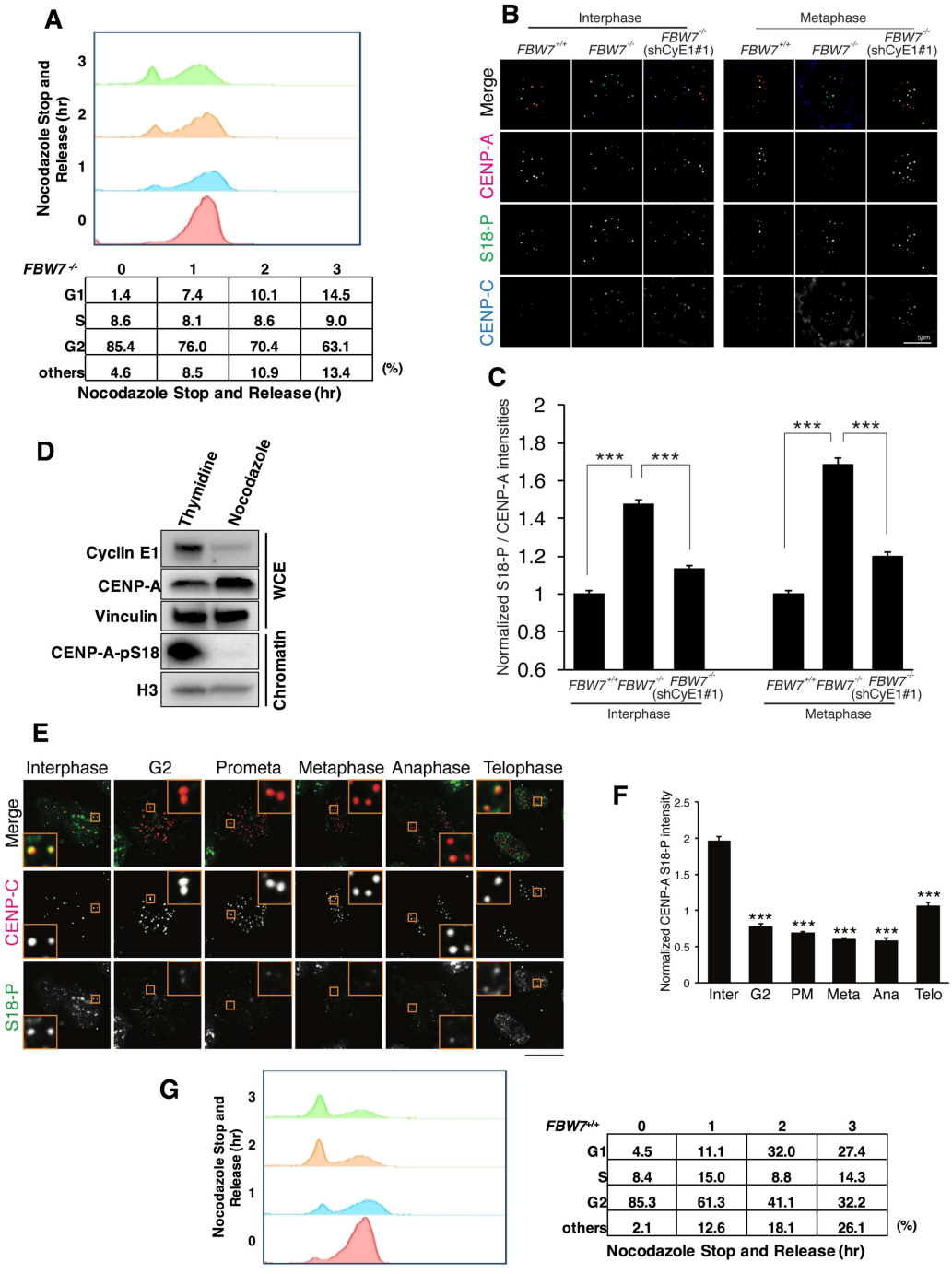

Figure S4. Cyclin E1 Overexpression Enhances CENP-A Ser18 Phosphorylation, Related to Figure 4.

(A, G) FACS analysis of **(A)** DLD1 *FBW7*$^{-/-}$ or **(G)** *FBW7*$^{+/+}$ cells synchronized by nocodazole followed by mitotic shake-off and release for the indicated periods of time.
(**B**) Representative IF images and (**C**) quantification from the staining of CENP-A (red) and CENP-A-S18P (green) in DLD1 *FBW7*$^{-/-}$ cells infected with the lentivirus encoding either Cyclin E1 shRNA or control. Centromeres were recognized by CENP-C staining (blue). The values were normalized for the value of *FBW7*$^{+/+}$ .The statistical significance was calculated using unpaired two-tailed student's t-test. *** denotes p value of < 0.005.
(D) Immunoblot of total CENP-A and pS18-CENP-A in whole cell extract and chromatin extract using DLD1 *FBW7*$^{+/+}$ cells that were synchronized by either thymidine or nocodazole.
(E) Representative IF images and **(F)** quantification of DLD1 cells stained with CENP-C (red) and pS18-CENP-A (S18-P, green) antibodies. Images are maximum-intensity projections of z stacks collected at 0.2-µm steps. Bars=5 µm. The values were normalized for the value of Telophase (Telo). The statistical significance from the value of interphase was determined using unpaired two-tailed student's t-test (*** denotes p value of < 0.005).

Figure S5

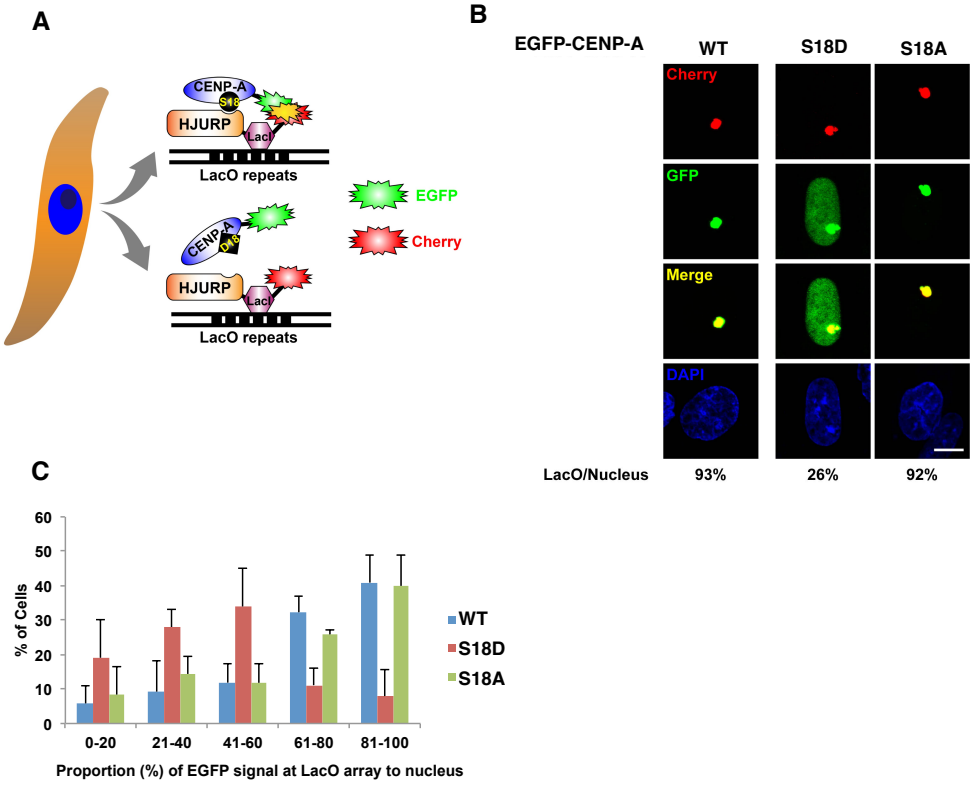

Figure S5. CENP-A S18 Phosphorylation May Affect Its Binding to HJURP. Related to Figure 5.

(A) Schematic of the LacI-LacO targeting system.
(B) Representative images and **(C)** quantification of EGFP-CENP-A constructs (green) at LacO array marked by mCherry-LacI-HJURP (red) from stably transfected cells described in (A). DNA was stained by DAPI (blue). Bar =5 μm.

Figure S6

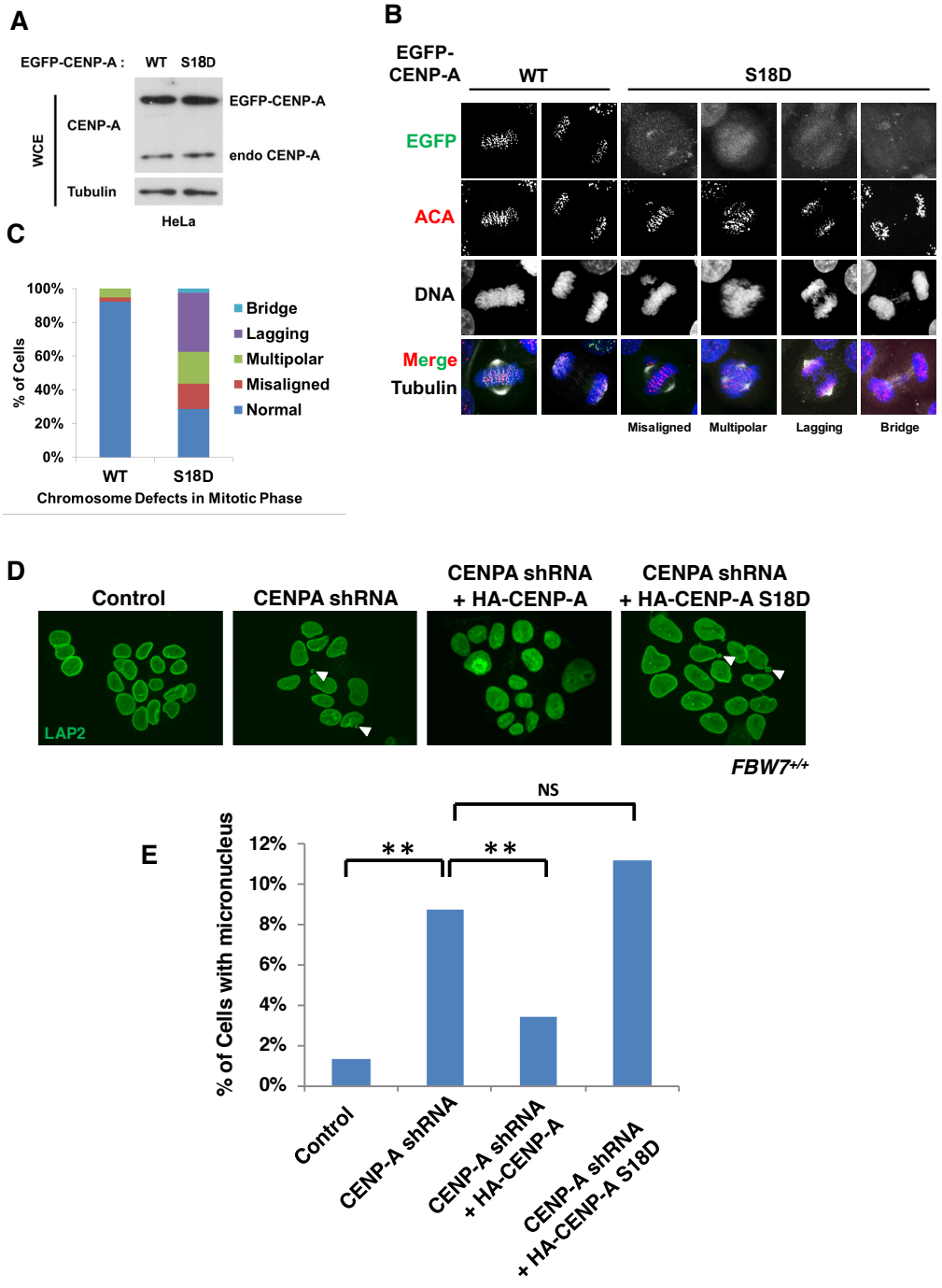

Figure S6. CENP-A S18D Is a Loss-of-function Mutation, Related to Figure 6.

(A) Immunoblot analysis of Hela cells transfected with EGFP-CENP-A WT or the S18D mutant.

(B) Representative images and (**C**) quantifications of chromosome segregation defects for cells in (**A**) stained with DAPI for DNA (blue), ACA for centromere (red) or EGFP

(green). In the merged images in (B), tubulin (grey) was used to identify mitotic spindles.

(D) Representative images of LAP2 staining (green) for micronuclei and (**E**) quantification of micronuclei in DLD1 *FBW7*$^{+/+}$ cells infected with lentivirus encoding CENP-A shRNA, control, CENP-A shRNA followed by another round of infection with HA-CENP-A or HA-CENP-A S19D that is resistant to CENP-A shRNA targeting. More than 200 cells were quantified for each cell type. Statistical significance was evaluated by Fisher's exact test (** denotes $P < 0.01$, and NS denotes insignificance).

Figure S7

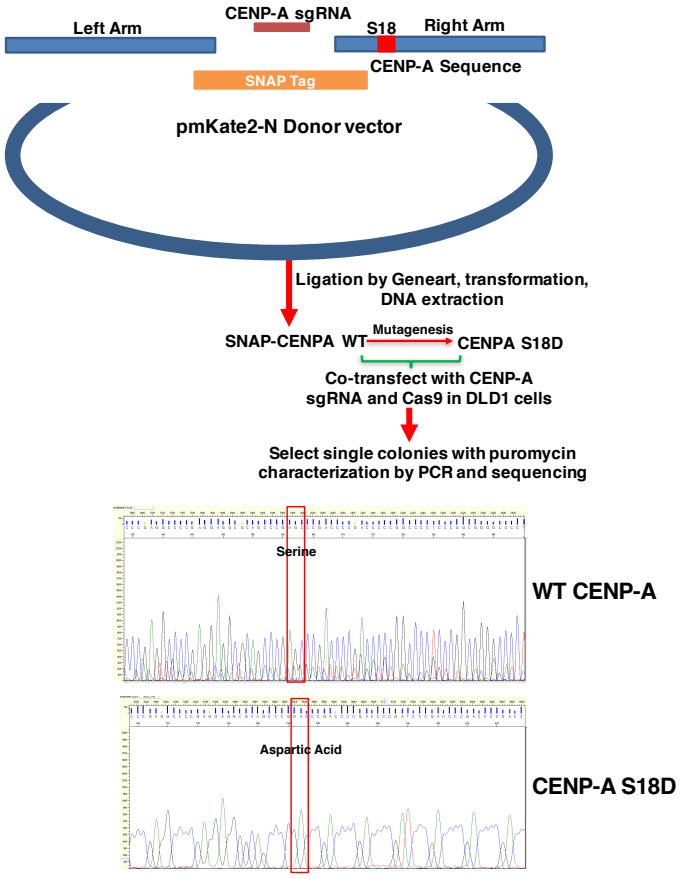

Figure S7. Ser18 CENP-A Phosphorylation Contributes to Elevated Tumor Progression, Related to Figure 7.

The schematic on CRISPR knockin of SNAP-tagged CENP-A WT or S18D mutant. The genomic DNA sequencing results were shown to confirm the correct gene editing.