A
Breast Cancer

Melanoma

Pancreatic

B
All cancers

C
3T3 cells

D
RNA level relative to asynchronous population

E
siRNA loading

DMSO

MG132

Luc

Cenpa #1

Cenpa #2

HJURP

β-catenin

actin

CENP-A
Supplemental Figure S1 related to Figure 1

(A) Box plot comparisons of relative expression (mRNA) of genes coding for CENP-A (CENPA), HJURP (HJURP), CAF-1 p150 (CHAF1A), H3.1 (HIST1H3E), and H4 (HIST2H4A), from breast (top), melanoma (middle), and pancreatic (bottom) cancers classified according to p53 status (TCGA data). Tumors are either WT for TP53, (diploid with no mutations) or TP53 loss-of-function (LOF) (homozygous deletion or heterozygous deletion + TP53 mutation featuring nonsense or in frame truncations resulting in p53 loss). All other TP53 mutants were excluded. mRNA levels are expressed in RSEM units. We used Wilcoxon rank sum tests to compute significance.

(B) Box plot comparison of relative expression (mRNA) of genes coding for CENP-A and HJURP as in (A), for all cancers. We compare WT and p53 LOF tumors to p53 gain-of-function (GOF) single missense mutations (R175H, R248Q, R248W, R249S, R273H).

(C) Cell cycle profiles of NIH-3T3 cells sorted according to DNA content, measured by Hoechst 33342 incorporation. Profiles of the starting (Asy = asynchronous) population, and 5 isolated fractions for each cell cycle phase are shown.

(D) RT-qPCR analysis of Hjurp and Cenpa mRNA levels in NIH-3T3 cell sorted populations shown in (B). Expression normalized to Gapdh and Idh mRNA and set to 1 for the asynchronous population. Error bars represent the standard error of at least 3 experiments.

(E) CENP-A depletion leads to proteosome-dependent degradation of HJURP. Western blot of RIPA- soluble extracts of NIH-3T3 cells transfected for 48 hours with Control siRNA or two siRNAs against Cenpa (Cenpa #1 and Cenpa #2). Prior to harvest, cells were treated for 4 hours with DMSO alone or the protease inhibitor MG132. * marks a non-specific band detected with the HJURP antibody. We measured β-catenin levels as a control for efficient inhibition of the proteasome. γ tubulin is used as a loading control. A two-fold dilution series of each extract is represented by 4X, 2X, 1X. Molecular weight protein markers are indicated on the right.