Samejima et al., http://www.jcb.org/cgi/content/full/jcb.201508072/DC1

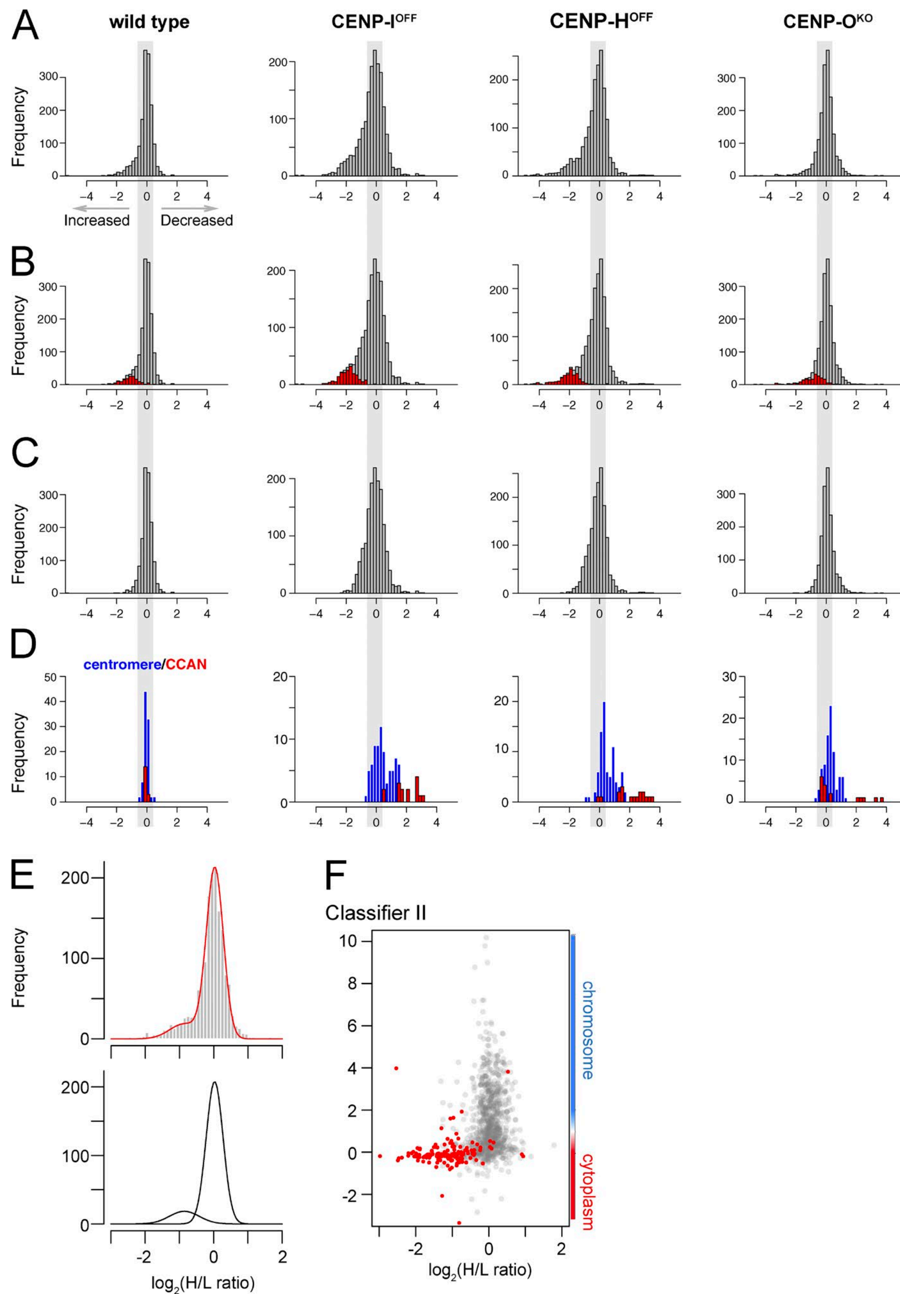

Figure S1. **Identifying contaminating proteins that were removed from statistical analyses.** (A) Histograms show the distribution of $\log_2$(H/L ratio) for all proteins in individual SILAC experiments, with proteins whose levels decreased on chromosomes yielding positive values on the abscissa. Wild-type chromosomes were labeled with heavy amino acids, and KO chromosomes were labeled with light amino acids. (For an as-yet-unknown reason, many of the KO cell lines will not grow in medium with dialyzed serum.) All quantified proteins (gray) are shown for representative wild-type, *CENP-I*^{OFF}, *CENP-H*^{OFF}, and *CENP-O*^{KO} experiments. Bin size is 0.2. The shaded areas span −0.6 to 0.4. (B) As in A, but with "noise" proteins highlighted in red. (C) Histograms after removal of the noise proteins. (D) Behavior of all centromere proteins (blue), with CENP-A and CCAN proteins (red). (E, top) Histogram of $\log_2$(H/L ratio) comparing proteins of chromosomes isolated from wild-type cells grown with dialyzed FBS (heavy amino acid label) versus cells grown with complete FBS (light amino acid label). Red line traces the contour of the histogram. Bin size is $2^{0.2}$. (E, bottom) Multipeak analysis was applied to the distribution of all quantified proteins. The distribution of $\log_2$H/L was fitted with two Gaussian curves. (F) Classifier II (the ratio of amounts of each protein in cytoplasm versus that in isolated chromosomes; Ohta et al., 2010) was plotted against the $\log_2$(H/L ratio) from the same experiment as E. Virtually all proteins in the small subsidiary peak are known to be cytoplasmic.

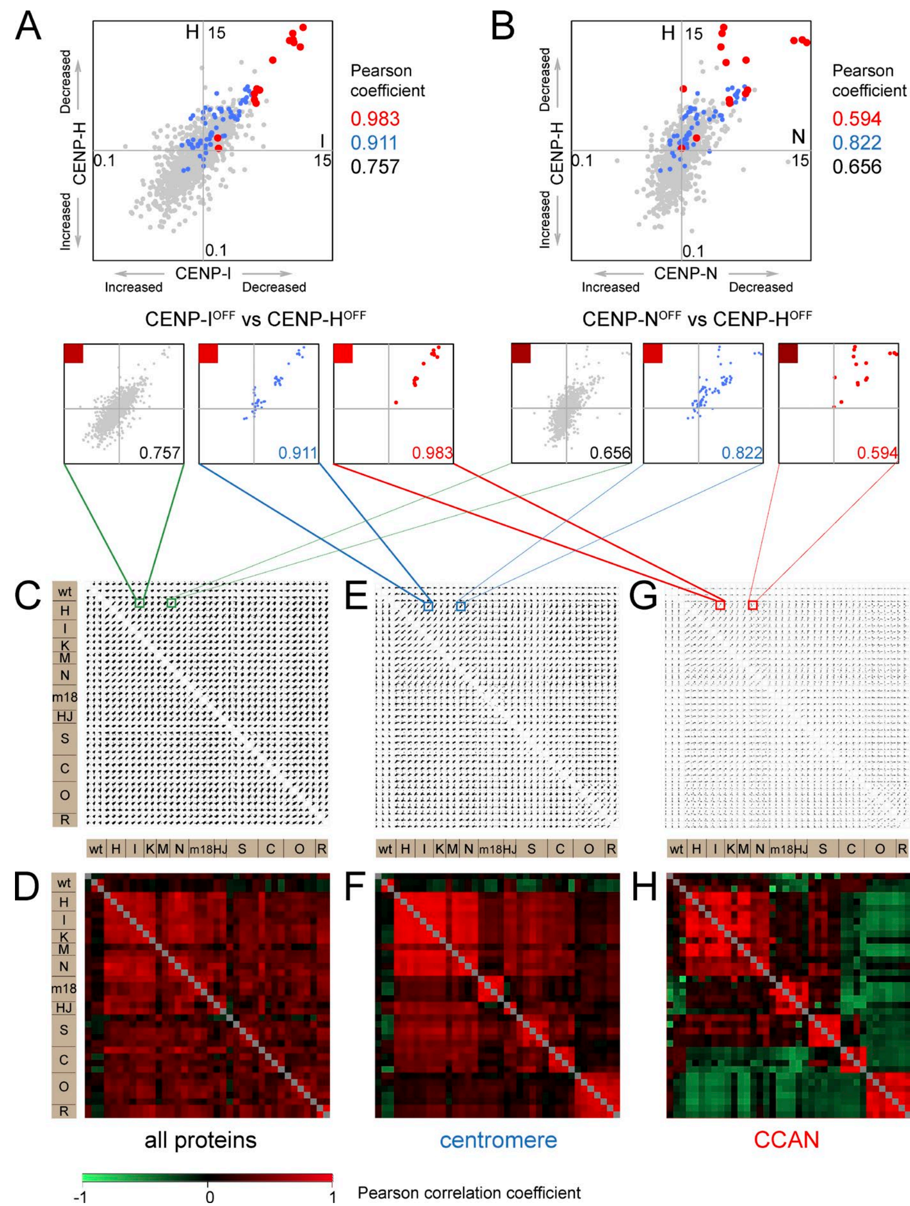

Figure S2. **Use of correlation analysis to compare results from all experiments.** (A) SILAC ratios are plotted for *CENP-I*^OFF chromosomes (abscissa) versus *CENP-H*^OFF chromosomes (ordinate). The behavior of the CCAN proteins (red) shows a high correlation between the two experiments (Pearson coefficient 0.983). (B) SILAC ratios are plotted for *CENP-N*^OFF chromosomes (abscissa) versus *CENP-H*^OFF chromosomes (ordinate). The divergent behavior of some CCAN proteins (red) leads to a poor correlation between the two experiments (Pearson coefficient 0.594). (C) First step in calculating the Pearson correlation coefficient. Each table entry is a diagrammatic plot of the distribution of CCAN proteins exactly as seen in A. It is necessary to blow this up online to see the pattern. The calculated correlation coefficients make up a table of numbers that maps directly onto the diagrammatic table shown in C and are not shown here. Instead, D shows a color-coding of that table. E and F correspond to C and D, but in this case, the plots in E correspond to all centromere proteins shown in blue and red in A. G and H correspond to C and D, but in this case, the plots in G correspond to all proteins shown in A. In the colored matrices shown in D, F, and H red shows a high positive correlation, and green shows a high negative correlation. The matrix (H) is shown in Fig. 2. wt, wild type.

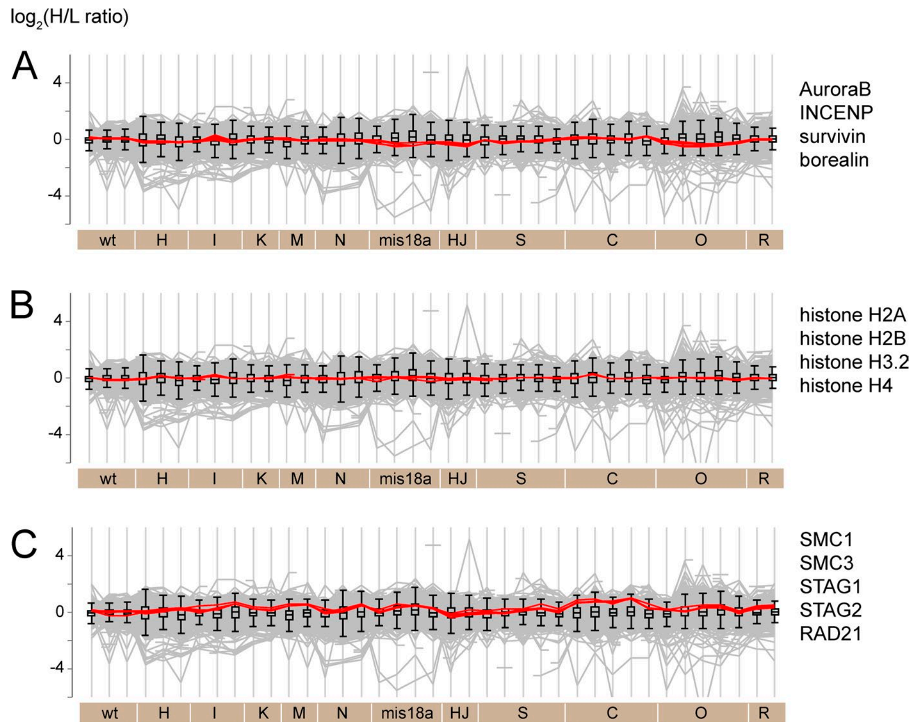

Figure S3. **Coordinated behavior among members of protein complexes.** (A) Profile plot showing the behavior of core histones (H2A, H2B, H3.2, and H4 [red]) in all experiments. (B) Profile plot showing the behavior of chromosomal passenger complex components (Aurora B, INCENP, survivin, and borealin [red]) in all experiments. (C) Profile plot showing the behavior of cohesin subunits (SMC1, SMC3, RAD21, STAG1, and STAG2 [red]) in all experiments. Gray lines show behavior of other proteins.

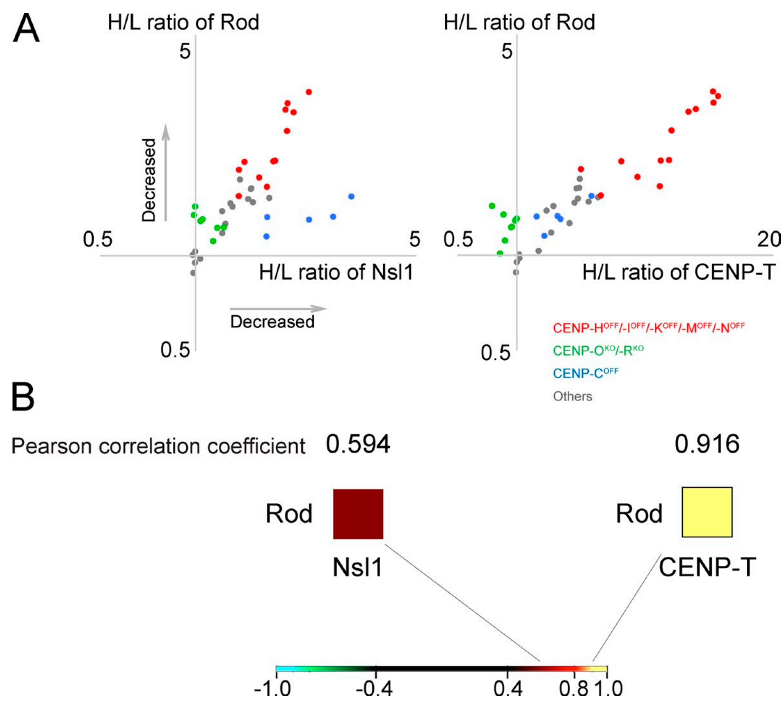

Figure S4. **Correlation revealed by comparisons in the abundance of two proteins on chromosomes as a result of depletion of CCAN proteins.** (A) Scatterplots showing coordinated behavior of two proteins across all the experiments. Each dot represents respective H/L ratios of a pair of proteins in one experiment. Axes are plotted in a log scale. (B) Pearson correlation coefficients were calculated from the distribution of paired H/L ratios and translated into a coded color. Yellow tiles in Fig. 9 B indicate coordinated behavior of a protein cohort throughout this study.

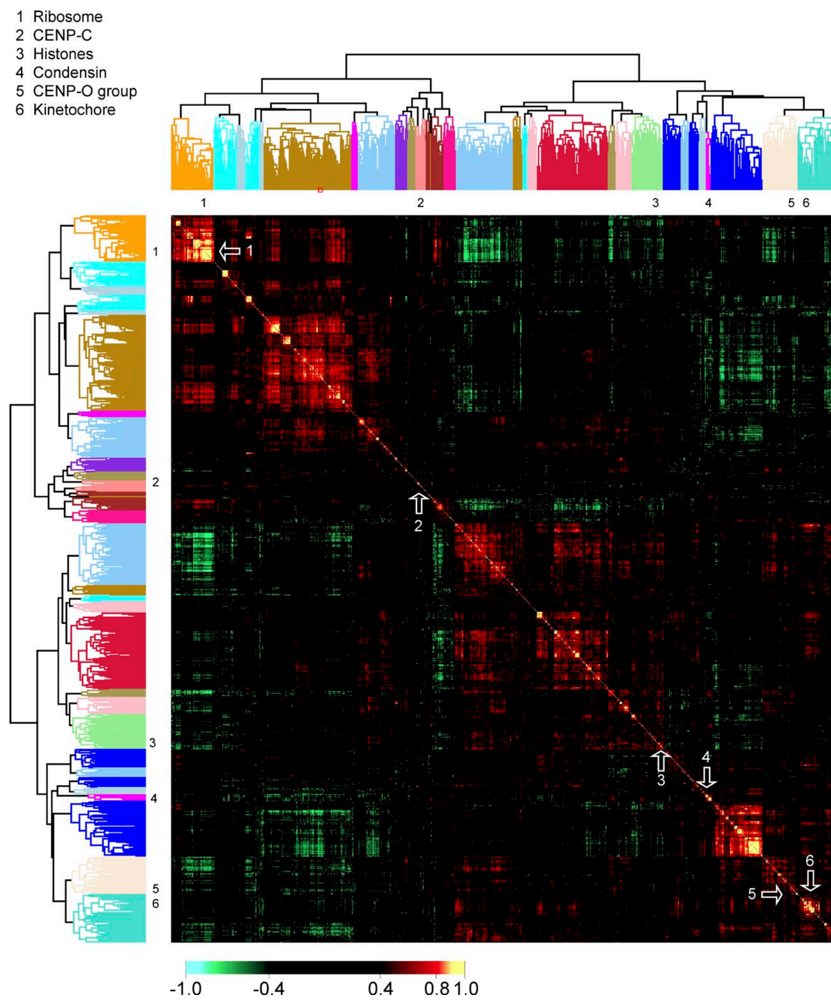

Figure S5. **Blowup of the correlation matrix shown in Fig. 9 B.**

Provided online as an Excel table is Table S1, which shows the H/L ratio for a protein in each experiment. NaN is a missing value where the protein was not quantified in the given experiment. Proteins marked with a + in the exclusion list column were removed from the analysis.

Reference

Ohta, S., J.C. Bukowski-Wills, L. Sanchez-Pulido, Fde.L. Alves, L. Wood, Z.A. Chen, M. Platani, L. Fischer, D.F. Hudson, C.P. Ponting, et al. 2010. The protein composition of mitotic chromosomes determined using multiclassifier combinatorial proteomics. *Cell.* 142:810–821.