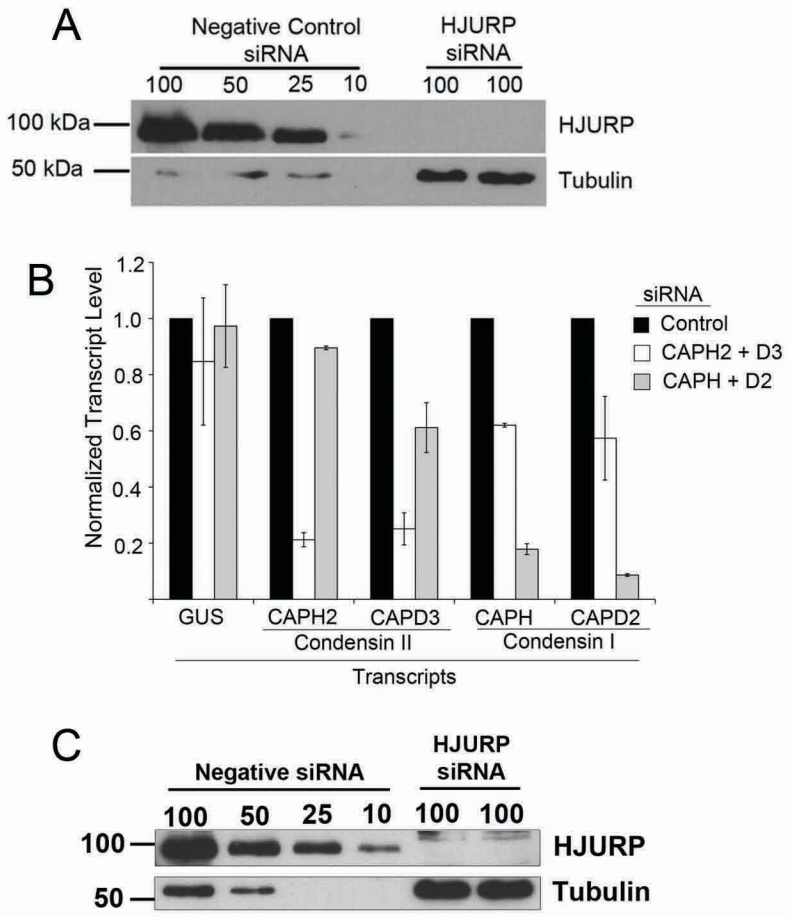
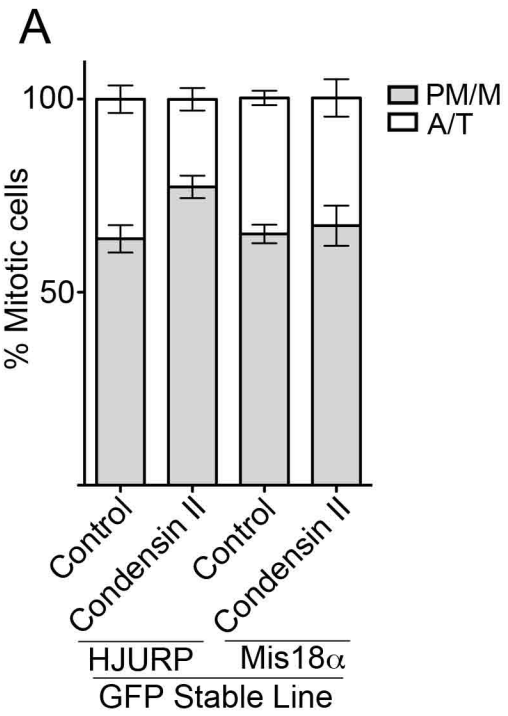
Supplemental Materials

Molecular Biology of the Cell

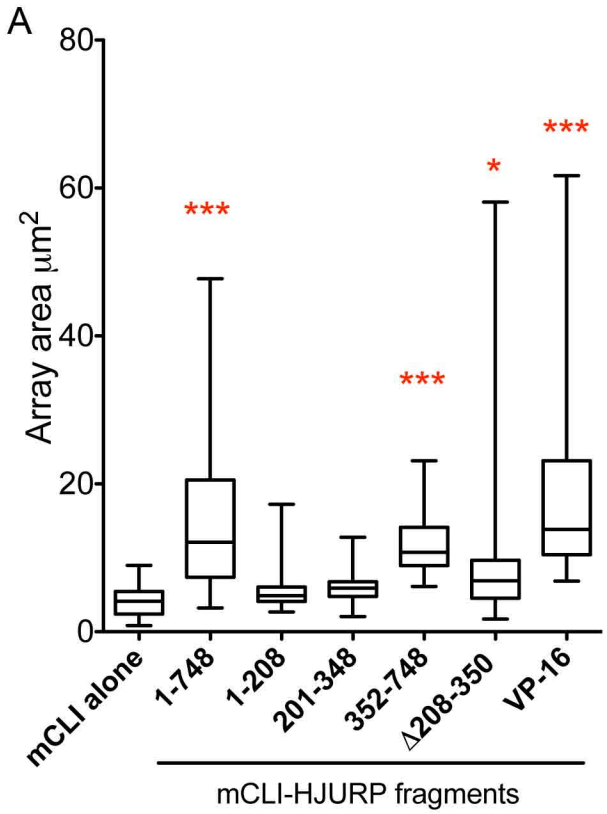
Barnhart-Dailey et al.



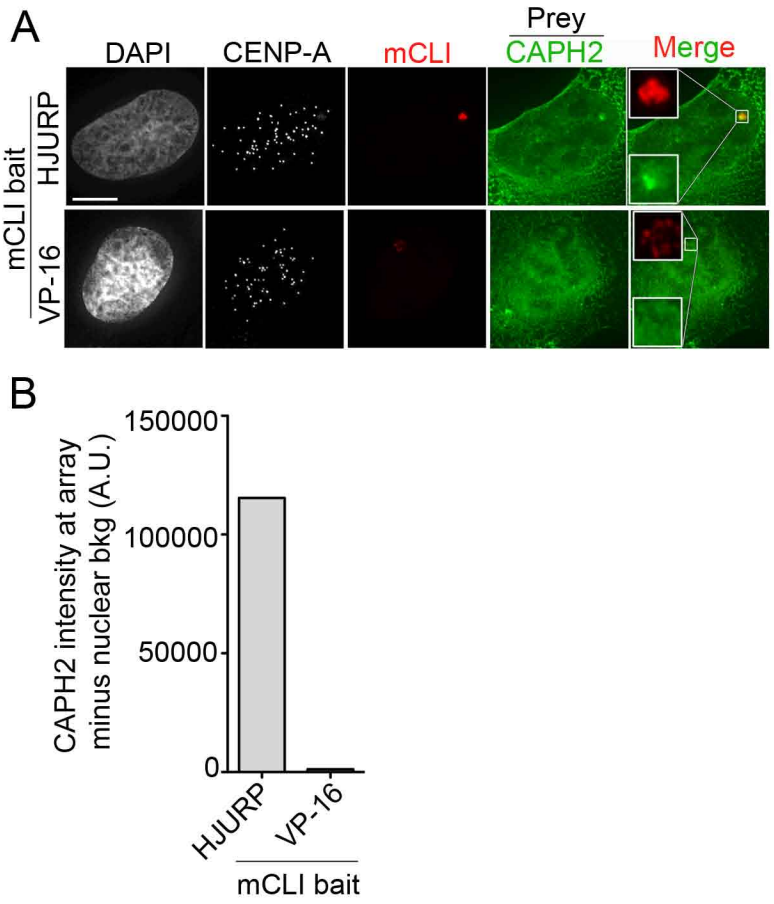
Supplemental Figure 1: (A) Immunoblot of cell lysates from experiment represented in Figure 1 demonstrating HJURP depletion efficiency in inducible mCherry-CENP-A HeLa TRex cells. Cells were treated with siRNA to negative control sequence or HJURP. Tubulin was used as a loading control. (B) qPCR analysis of CAPH, CAPD2, CAPH2, or CAPD3 transcript levels from experiment represented in Figure 1. Values normalized to negative control siRNA. GUS was used as an endogenous control transcript to control for cDNA input. Error bars represent standard deviation. N=3 biological replicates. (C) Immunoblot of cell lysates from experiment represented in Figure 2 demonstrating HJURP depletion efficiency in inducible CAPH2-GFP HeLa TRex cells. Cells were treated with siRNA to negative control sequence or HJURP. Tubulin was used as a loading control.



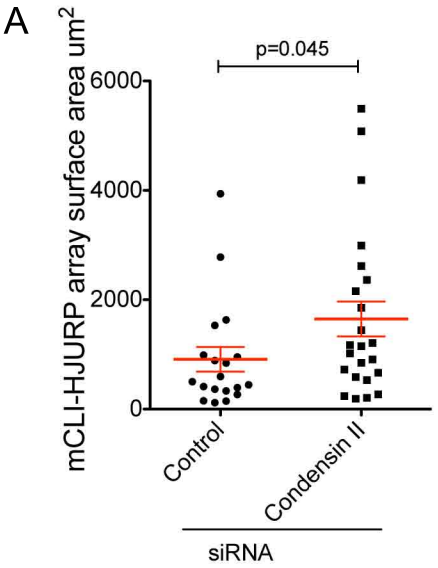
Supplemental Figure 2: (A) Graph displaying the mitotic index classification for HeLa cell lines stably expressing HJURP-GFP or Mis18α-GFP. Cells were treated with siRNA to negative control sequences or CAPH2 + CAPD3 (condensin II) for 48 hours prior to fixation and classification. Gray bars represent prometaphase and metaphase cells. White bars represent anaphase and telophase cells. Error bars are standard deviation. n≥100 cells, N=3 biological replicates.



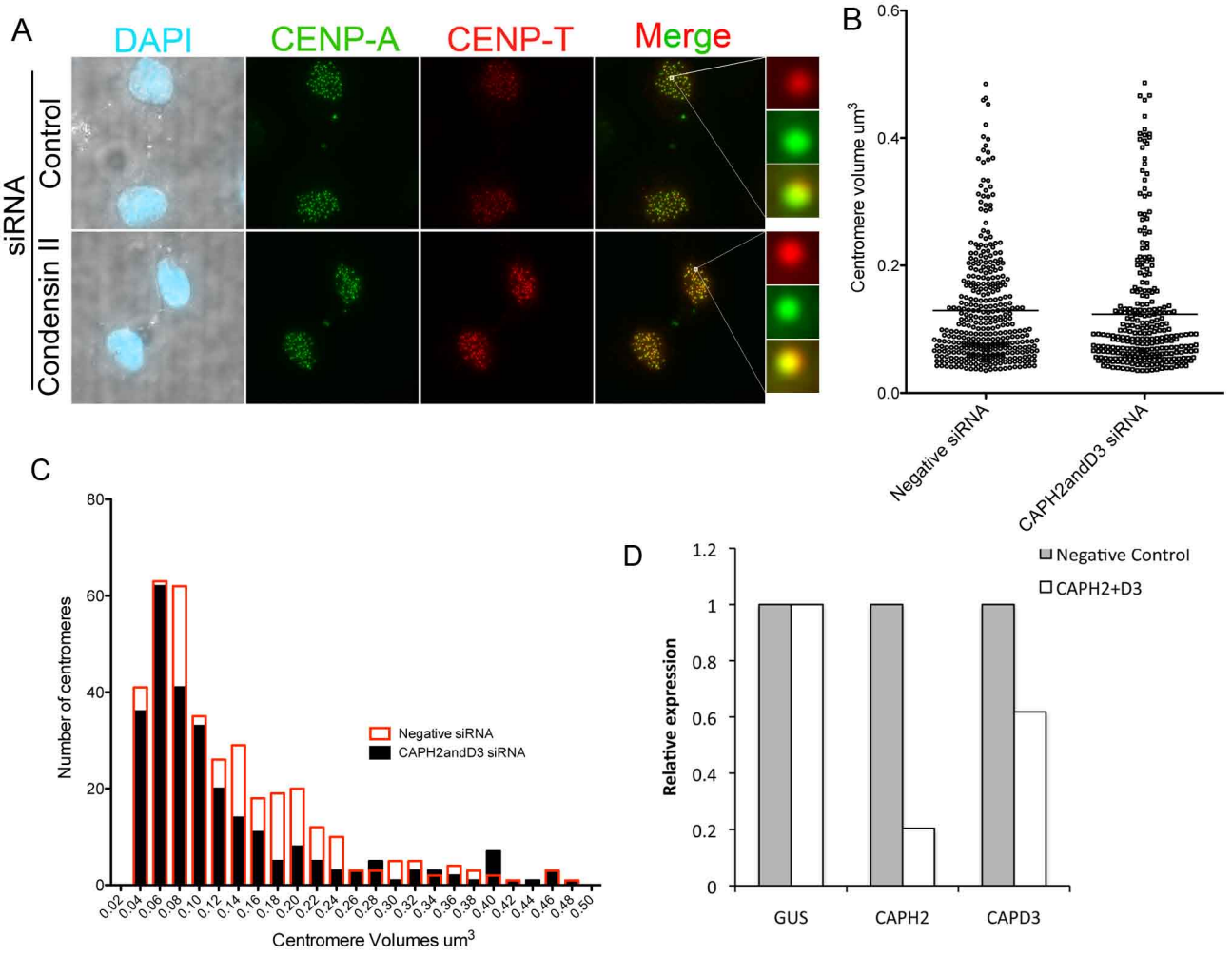
Supplemental Figure 3. Measuring mCLI-HJURP array areas gives same statistically significant fragments as measuring the asymmetry index. Indicated mCLI-HJURP fragements or mCLI alone were targeted to array in U2OS-LacO cells for 48 hours. Array area measurements were made using ImageJ. Box plots display the mean and upper and lower quartile; the whiskers mark the min and max. Statistically significant groups are marked with red asterisks, determined by Kruskal-Wallis test followed by Dunn's multiple comparison test to compare to mCLI alone. $p<0.0001$



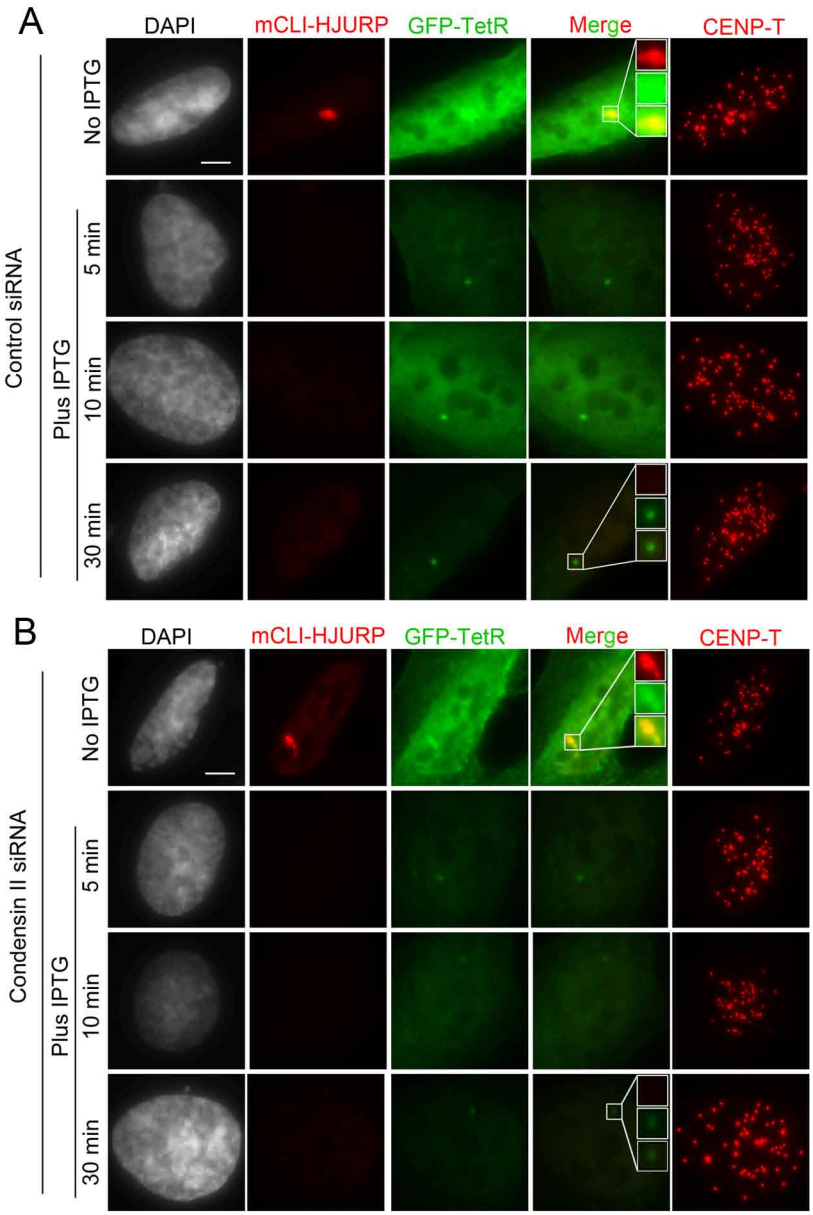
Supplemental Figure 4: (A) Representative images of U2OS-LacO cells co-transfected for 48 hours with mCLI-HJURP or VP-16 and CAPH2-GFP. Cells were pre-extracted then fixed and stained with an antibody to CENP-A. Scale bar represents 5μm. (B) Quantification of experiment in A. Graph displays the CAPH2-GFP intensity at mCLI-HJURP or VP-16 arrays minus the average nuclear background signal. n≥30 cells measured.



Supplemental Figure 5. Condensin II depletion significantly increases the surface area of mCLI-HJURP arrays. (A) Surface area measurements of U2OS-LacO cells transfected for 72 hours with mCLI-HJURP and treated with siRNA to control or condensin II (CAPH2 and CAPD3) for the final 48 hours of transfection. Red bars mark mean and whiskers mark SEM. N=3 biological reps. p=0.045 by Mann-Whitney test.



Supplemental Figure 6: Effect of condensin II depletion on endogenous centromere volume (A) Representative images of HeLa TRex cells treated with control siRNA or CAPH2 and CAPD3 siRNA for 48 hours then fixed and stained with antibodies to endogenous CENP-A and CENP-T. Midbody positive G1 pairs were imaged then assessed for centromere volume based on size of CENP-A foci. Scale bar represents 5υm. (B) Centromere volume measurements for the experiment represented in (A). n>100 centromeres per condition. (C) Histogram of data graphed in (B). (D) rtPCR results of CAPH2 and CAPD3 gene targets following siRNA depletion.



Supplemental Figure 7: mCLI-HJURP arrays re-condense following mCLI-HJURP removal. (A) Representative images of U2OS-LacO cells expressing mCLI-HJURP and GFP-TetR following 48 hours of either control (A) or condensin II siRNA (B). Cells were stained with an antibody to CENP-T to mark centromeres. Cells were treated with 5mM IPTG to remove mCLI-HJURP from the LacO array for the indicated timepoints. Scale bar represents 5υm.