Hori et al., https://doi.org/10.1083/jcb.201605001

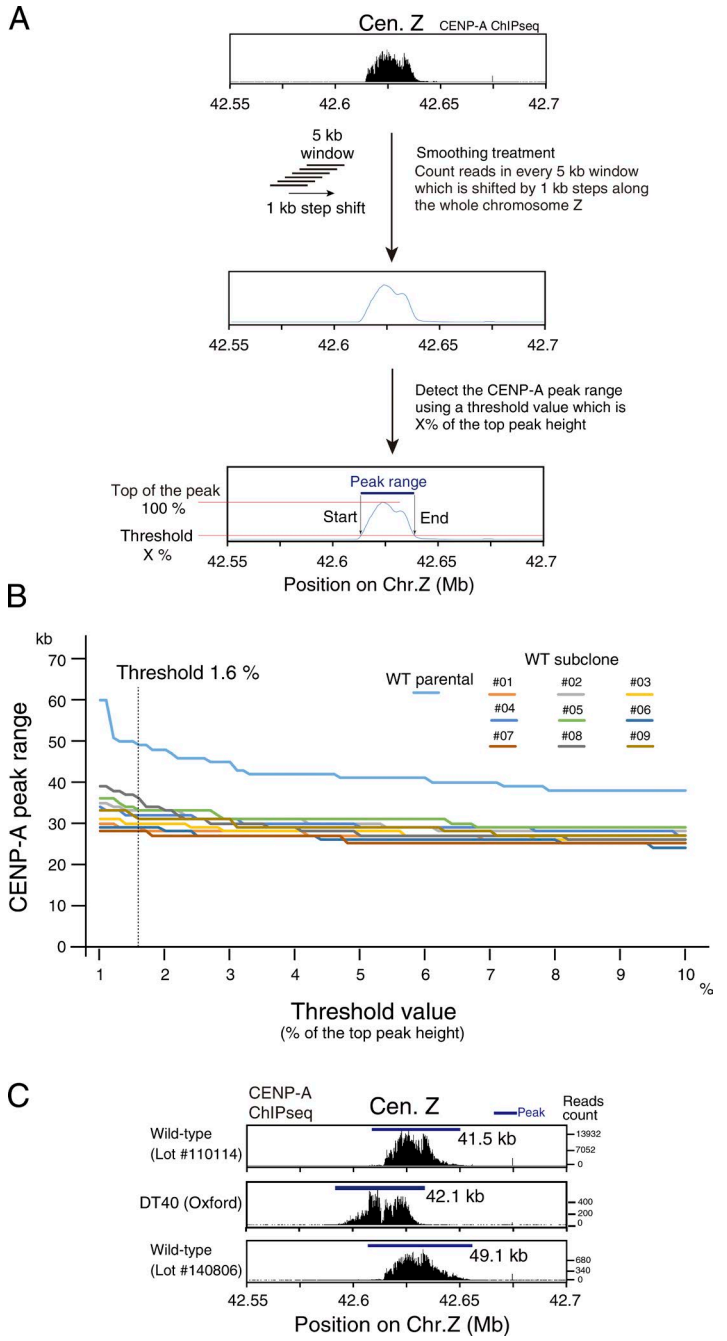

Figure S1. **A method for identification of centromere region.** (A) The sequences obtained with ChIP using anti–CENP-A antibody were mapped to the chicken reference genome (Galgal4). To define the edges of a CENP-A–associated region, we plotted the read counts in 5-kb regions, and these values were shifted in 1-kb steps (smoothing method). Using this method, the height of peak was defined. Afterward, we determined the threshold value and the start and end position of the centromere. We used 1.6% of the peak height as a threshold, after testing different threshold values. (B) CENP-A–associated regions were determined using different threshold values for each dataset. If the applied threshold value was too small, data were shown to vary considerably. Values between 1 and 2% generated the best results, and therefore, we used 1.6% of the peak height as a threshold for all further analyses. (C) ChIP-seq profiles of CENP-A at chicken chromosome Z in DT40 cells obtained from a different laboratory (DT40 Oxford). The profiles of our DT40 cell batches are presented as well. Centromere sizes in the wild-type lot 110114, DT40 (Oxford), and wild-type lot 140806 cells were 41.5, 42.1, and 49.1 kb, respectively. Defined CENP-A peak ranges are indicated by blue bars. WT, wild-type.

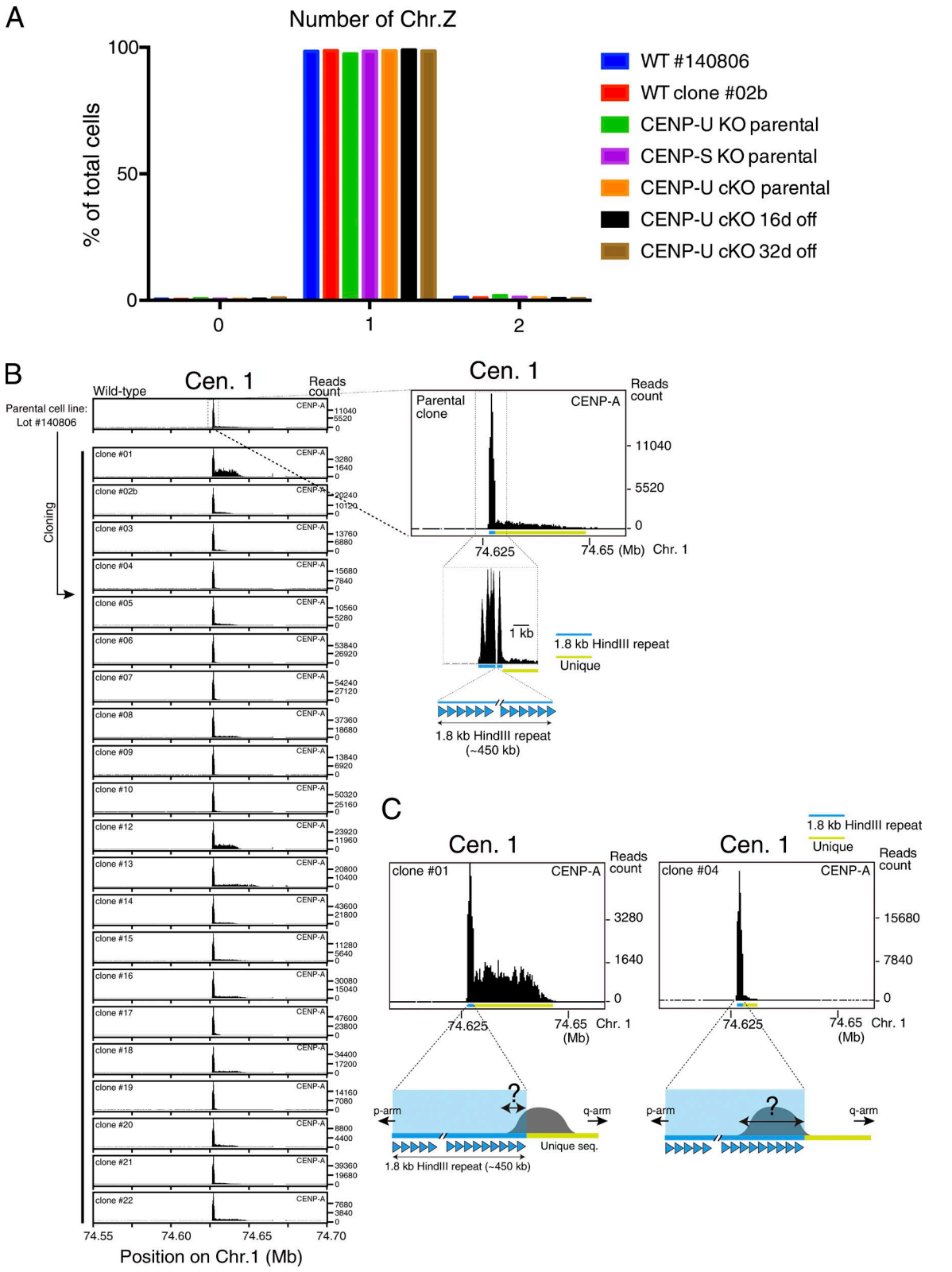

Figure S2. **Characterization of cells showing the centromere drift by various methods.** (A) FISH analysis, using a chromosome Z–specific probe, for the determination of chromosome Z copy numbers. (B) ChIP-seq profiles of CENP-A at chicken chromosome 1 in 21 independent clones isolated from a laboratory stock of DT40 cells (lot 140806). This centromere contains 1.8-kb–long repetitive sequences. (C) Schematic representation of CENP-A–associated region in clones 01 and 04 shown in Fig. 1. In clone 01, the centromere drift and a unique sequence near the repetitive region were detected. In clone 04, most of the CENP-A–associated region is within the repetitive sequence. The predicted peak images of the CENP-A–associated region around the boundary between repetitive and unique sequence are also shown. As shown in these illustrations, we could not define the exact length of CENP-A–associated region in the repetitive sequences because of technical difficulty of mapping. cKO, conditional knockout; KO, knockout; WT, wild-type.

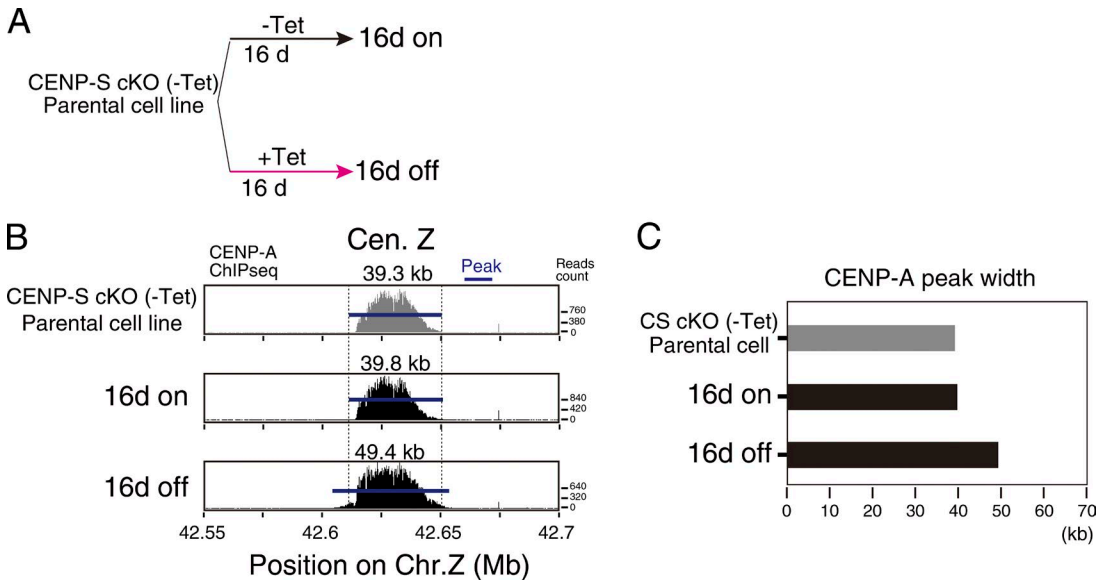

Figure S3. **CENP-S deficiency–dependent increase in CENP-A–associated region size.** (A) ChIP-seq samples were prepared after culturing CENP-S conditional knockout (cKO) cells for 16 d in the presence (16d off) or absence (16d on) of tetracycline. CENP-S expression is suppressed in the presence of tetracycline (Tet). (B) CENP-A–associated region size in the samples indicated in A, based on ChIP-seq analysis with anti–CENP-A antibody. CENP-A–associated region sizes in the parental CENP-S conditional knockout cells, 16 d on cells, 16 d off cells were 39.3, 39.8, and 49.4 kb, respectively. CENP-A peak ranges are indicated by blue bars. (C) Graphical representation of centromere size in the samples indicated in B.

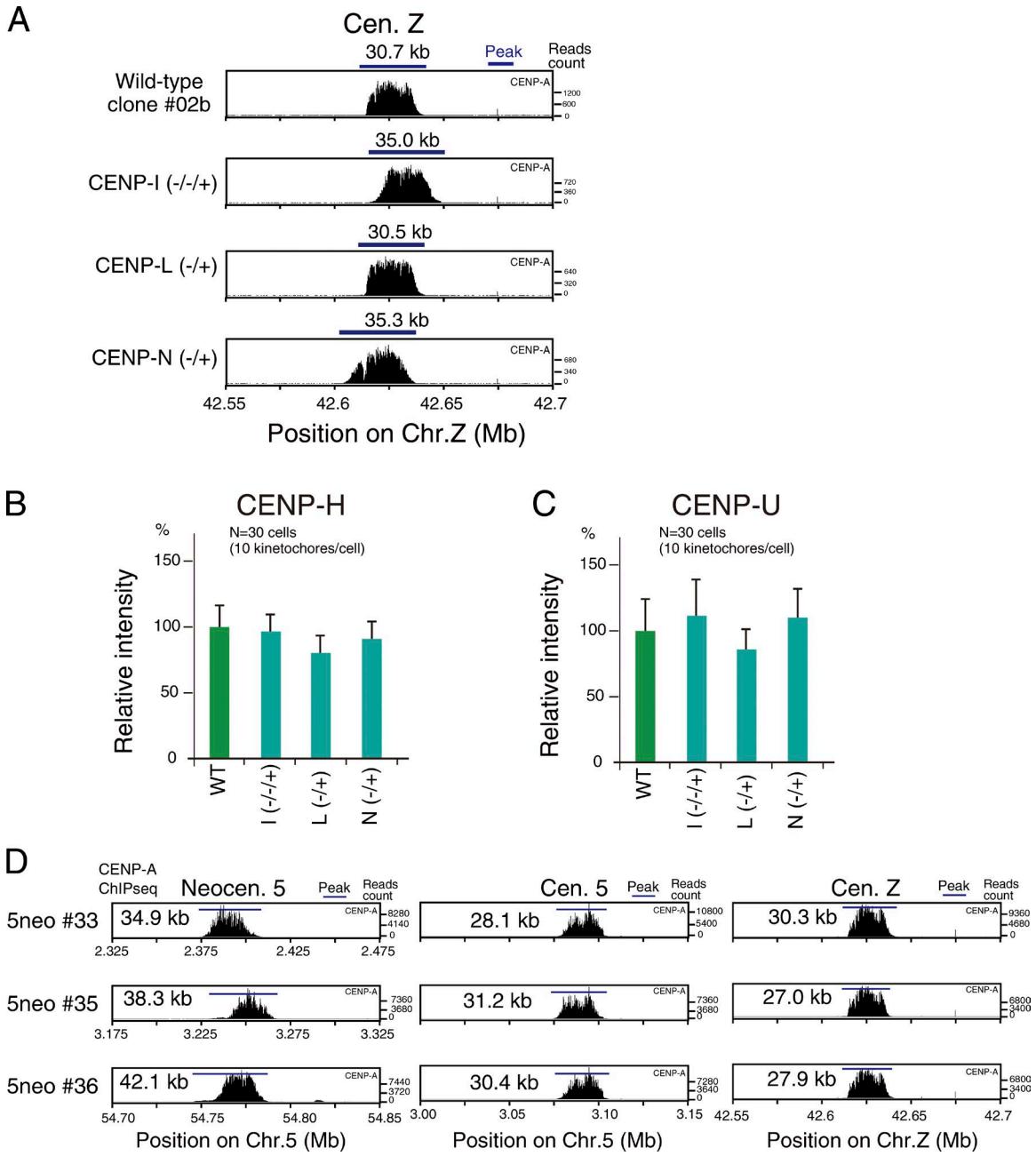

Figure S4. **ChIP-seq analysis of CENP-I (−/−/+), CENP-L (−/+), and CENP-N (−/+) cells and cells with neocentromere on chromosome 5, using anti–CENP-A antibody.** (A) ChIP-seq profiles of CENP-A at chicken chromosome Z in CENP-I (−/−/+), CENP-L (−/+), and CENP-N (−/+) cells. The results of the wild-type clone 02 analyses are presented as well. CENP-A–associated region sizes in the wild-type clone 02b, CENP-I (−/−/+), CENP-L (−/+) and CENP-N (−/+) cells were 30.7, 35.0, 30.5, and 35.3 kb, respectively. Defined CENP-A peak ranges are indicated by blue bars. (B) CENP-H signal intensity at kinetochore in wild-type (WT) DT40 cells, CENP-I (−/−/+), CENP-L (−/+), and CENP-N (−/+) cells. Signal intensities of 10 kinetochores in each cell were measured. Error bars indicate SD (n = 30 cells in each measurement). (C) CENP-U signal intensity at kinetochore in wild-type DT40 cells, CENP-I (−/−/+), CENP-L (−/+), and CENP-N (−/+) cells. Signal intensities of 10 kinetochores in each cell were measured. Error bars indicate SD (n = 30 cells in each measurement). (D) ChIP-seq profiles of CENP-A on chicken chromosomes 5 and Z in three independent cell lines with neocentromere on chromosome 5 (Shang et al., 2013). CENP-A–associated region sizes of neocentromeres on chicken chromosome 5 in the 5neo #33, 5neo #35, and 5neo #36 were 34.9, 38.3, and 42.1 kb, respectively. CENP-A–associated region sizes of native centromere on chicken chromosome 5 in the 5neo #33, 5neo #35, and 5neo #36 were 28.1, 31.2, and 30.4 kb, respectively. CENP-A–associated region sizes of chicken chromosome Z in the 5neo #33, 5neo #35, and 5neo #36 were 30.3, 27.0, and 27.9 kb, respectively. Defined CENP-A–associated region sizes are indicated by blue bars.

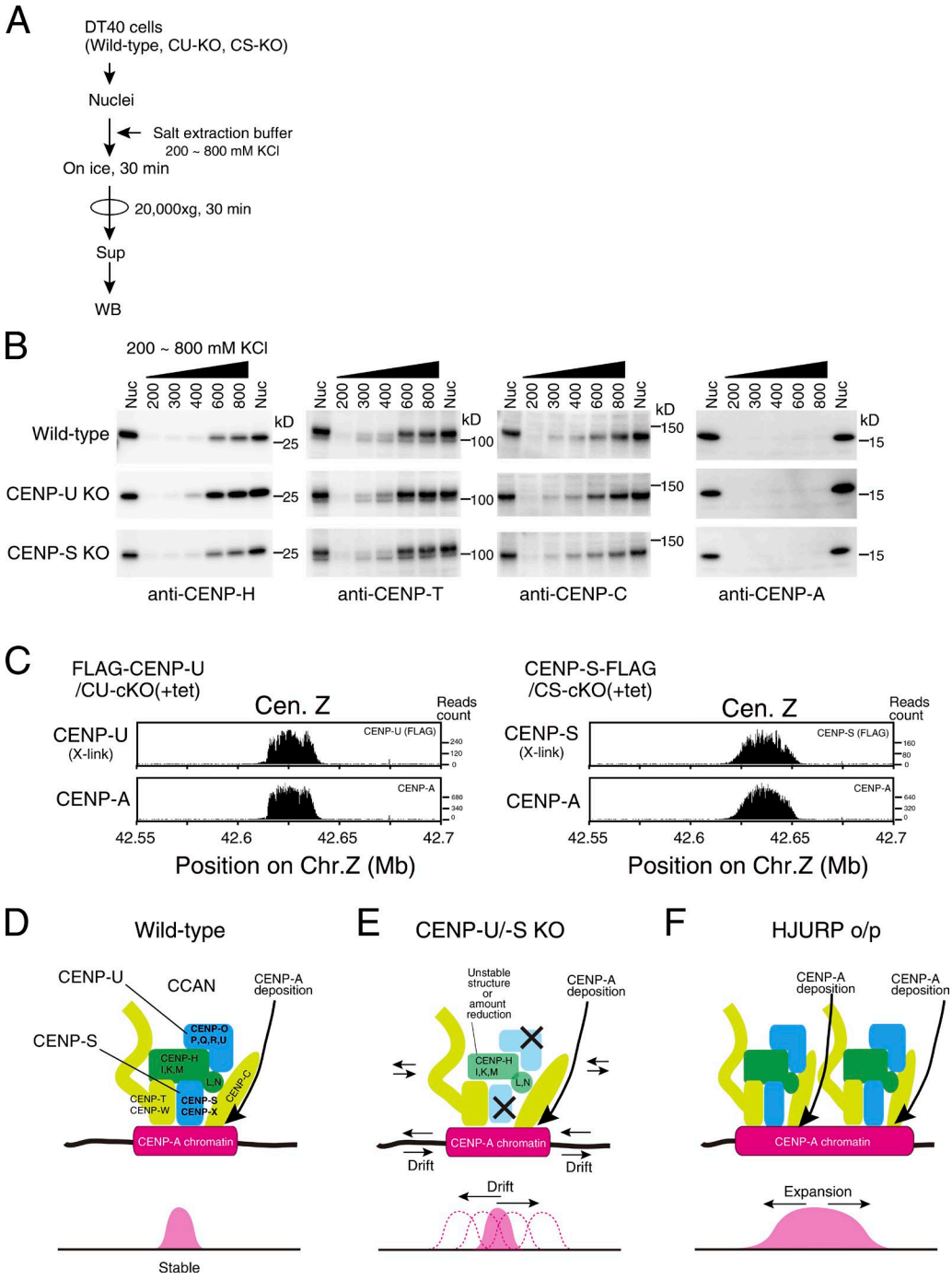

Figure S5. **Salt extraction of CCAN proteins, ChIP-seq with CENP-U and CENP-S, and summary of this study.** (A) Experimental design of CCAN protein salt extraction. Sup, Supernatant; WB, Western blot analysis. (B) Western blot analysis of the cell extracts, obtained using the indicated salt concentrations and anti–CENP-H, anti–CENP-T, anti–CENP-C, and anti–CENP-A antibodies. Independent salt-extraction experiments were performed three times. (C) ChIP-seq profiles of CENP-U and CENP-S at chicken chromosome Z. CENP-U (CU) or CENP-S (CS) knockout cells expressing CENP-U–FLAG or CENP-S–FLAG, respectively, were prepared. Anti-FLAG antibodies were used for ChIP-seq analysis because endogenous antibodies cannot be used for ChIP analyses. CENP-A ChIP-seq profiles are presented for both cell lines as well. D–F summarize centromere drift and expansion model. (D) In the wild-type cells, centromere can drift after a large number of cell divisions, but this drift is usually suppressed, and CENP-A must be incorporated at an existing centromere. (E) CCAN structure is partially disrupted in CENP-U– or CENP-S–deficient cells, and therefore, centromere drift occurs more frequently. CENP-A–associated region appears wider in ChIP-seq analyses, because of the frequent centromere drifts. (F) In contrast to E, if CENP-A levels are increased in HJURP-overexpressing (o/p) cells, centromeres are expanded together with the recruitment of other kinetochore components. cKO, conditional knockout; KO, knockout; Nuc, isolated whole nuclei before salt extraction; Tet, tetracycline.

Reference

Shang, W.H., T. Hori, N.M. Martins, A. Toyoda, S. Misu, N. Monma, I. Hiratani, K. Maeshima, K. Ikeo, A. Fujiyama, et al. 2013. Chromosome engineering allows the efficient isolation of vertebrate neocentromeres. *Dev. Cell.* 24:635–648. http://dx.doi.org/10.1016/j.devcel.2013.02.009