Supplementary Figure legends

Supplementary Figure S1. Generating mice with CENP-U conditional alleles

(A) Targeting construct for creating CENP-U^{LF} alleles in mouse ES cells.

(B) Restriction analysis of genomic DNA from ES cells with CENP-U^{LF} alleles. DNA was digested with Hind III, and a novel 10-kb fragment was detected with a probe indicated in (A) if a CENP-U^{LF} allele was created.

(C) Strategy for generating conditional CENP-U-deficient mice. Mice with CENP-U$^{flox/+}$ alleles were generated from ES cells with these alleles. Then, the CENP-U$^{flox/flox}$ mice were obtained by intercrossing with the CENP-U$^{flox/+}$ mice. The ERT2-Cre gene was integrated into the CENP-U$^{+/-}$ mice (Figure 1). Finally, the CENP-U$^{flox/-}$ mice with ERT2-Cre genes were obtained by crosses with the CENP-U$^{flox/flox}$ mice and CENP-U$^{+/-}$ mice with ERT2-Cre genes.

(D) PCR genotyping for distinguishing between wild-type CENP-U (WT), CENP-U-disrupted (KO), and CENP-U flox alleles. (a) Primer design. (b) PCR results with DNA from mice with the indicated alleles.

(E) Immunofluorescence analysis using anti-CENP-U antibody for MEF cells from CENP-U$^{flox/-}$ mice. CENP-U signals were not detected in the presence of OHT. Bar, 10 μm.

Supplementary Movie legends

Supplementary Movie1

Chromosome dynamics in CENP-U-deficient ES cells observed by time-lapse microscopy. Selected images were used in Figure 4A-(a).

Supplementary Movie2

Chromosome dynamics in CENP-U-deficient ES cells observed by time-lapse

microscopy. Selected images were used in Figure 4A-(b).

Supplementary Movie3

Chromosome dynamics in CENP-U-deficient ES cells observed by time-lapse

microscopy. Selected images were used in Figure 4A-(c).

A

Mouse CENP-U locus

Probe

Wild-type (+) 12.6 kb / HindIII

2 kb

Stop

HindIII   ATG

Wild-type (+) allele

HindIII

loxP Frt   SA   IRES   βgeo   Frt   loxP

LF allele / KO2 construct

LF allele 10.0 kb / HindIII

B

+/+   LF/+ (ES #40)

WT (+) allele (12.6 kb)

LF allele (10 kb)

C

Mouse CENP-U cKO strategy

LF/+ —— Flippase/-

flox/+ —— flox/+

+/- —— ERT2-Cre /ERT2-Cre

flox/flox —— +/-; ERT2-Cre

flox/-; ERT2-Cre

D

(a)

550 bp / flox

flox allele

loxP Frt   loxP

WT (+) allele

350 bp / WT (+)

KO (-) allele

420 bp / KO (-)

SA   IRES   βgeo

(b)

flox/+ flox/- flox/+ flox/- flox/+ flox/- flox/+ flox/-
  1   2   3   4   5   6   7   8

flox (550 bp)
KO (-) (420 bp)
WT (+) (350 bp)

2   4   6   8

*

ERT2-Cre (310 bp)

flox/-; ERT2-Cre

E

DNA   CENP-U   CENP-U DNA

- OHT

+ OHT 6 day

CENP-U cKO

Sup. Figure 1