E07-06-0556 Fukagawa

Supplemetary Figure legends

Supplementary Figure S1. Generation of knockout (KO) cell lines of CENP-O-class proteins.

(A) (a) Restriction maps of the chicken *CENP-Q* locus, gene disruption constructs, and targeted loci. Black boxes indicate the positions of exons. XbaI restriction sites are shown. The position of the probe used for Southern hybridization is indicated. Novel 9.8- and 17.5-kb XbaI fragments hybridize to the probe if targeted integration of the construct occurs. (b) Restriction analysis of genomic DNAs from cells with targeted integration of a *CENP-Q* disruption construct. Genomic DNAs from wild-type (WT) DT40 cells, a clone after first-round targeting (+/-, 1st), and a clone (#102) after second-round targeting (-/-, 2nd KO) were analyzed by Southern hybridization with the probe indicated in (a). M is λ-DNA digested with HindIII. (c) Western blot analysis of KO cell (#102) extracts with anti-CENP-Q antibody. Anti-α-tubulin antibody was used as a loading control.

(B) (a) Restriction maps of the chicken *CENP-R* locus, gene disruption constructs, and targeted loci. There are three alleles of the *CENP-R* locus. Black boxes indicate the positions of exons. EcoRV restriction sites are shown. The position of the probe used for Southern hybridization is indicated. Novel 14.0-kb EcoRV fragments hybridize to the probe if targeted integration of the construct occurs. (b) Restriction analysis of genomic DNAs from cells with targeted integration of a *CENP-R* disruption construct. Genomic DNAs from WT DT40 cells, a clone after first-round targeting (+/+/-, 1st), a clone after second-round targeting (+/-/-, 2nd), and a clone (#133) after third-round targeting (-/-/-, 3rd knockout) were

analyzed by Southern hybridization with the probe indicated in (a). M is λ-DNA digested with HindIII. (c) Western blot analysis of KO cell (#133) extracts with anti-CENP-R antibody. Anti-α-tubulin antibody was used as a loading control.

(C) Creation of CENP-P- and CENP-50-double KO cells. We previously created a tetracycline-repressible conditional KO cell line for CENP-50 (#17-15 cells). We performed two-round targeting with CENP-P disruption constructs into #17-15 cells. Both CENP-P and CENP-50 were not expressed in double KO cells (#4-46, with tetracycline) by Western blot analysis. +tet indicates addition of tetracycline to the culture medium. A Coomassie brilliant blue (CBB) staining gel is also shown.

Supplementary Figure S2. Potential phosphorylation sites for chicken CENP-50

Five potential CDK phosphprylation sites (T99, S112, S145, S148, S151) are selected. Additional phosphorylation sites around the CDK sites are choosen by using the NetPhos 2.0 software (http://www.cbs.dtu.dk/services/NetPhos/). Total16 potential serine/threonine phosphorylation sites, which are shown as red characters were chosen and mutated to alanine (16×A mutant). 62S and 63T for PLK1 phosphorylation are indicated by underline.

Supplementary Movie legends

Supplementary Movie1

Chromosome dynamics in control cells observed by time-lapse microscopy of living cells.

Supplementary Movie2

Chromosome dynamics in CENP-Q-deficient cells observed by time-lapse microscopy of living cells.

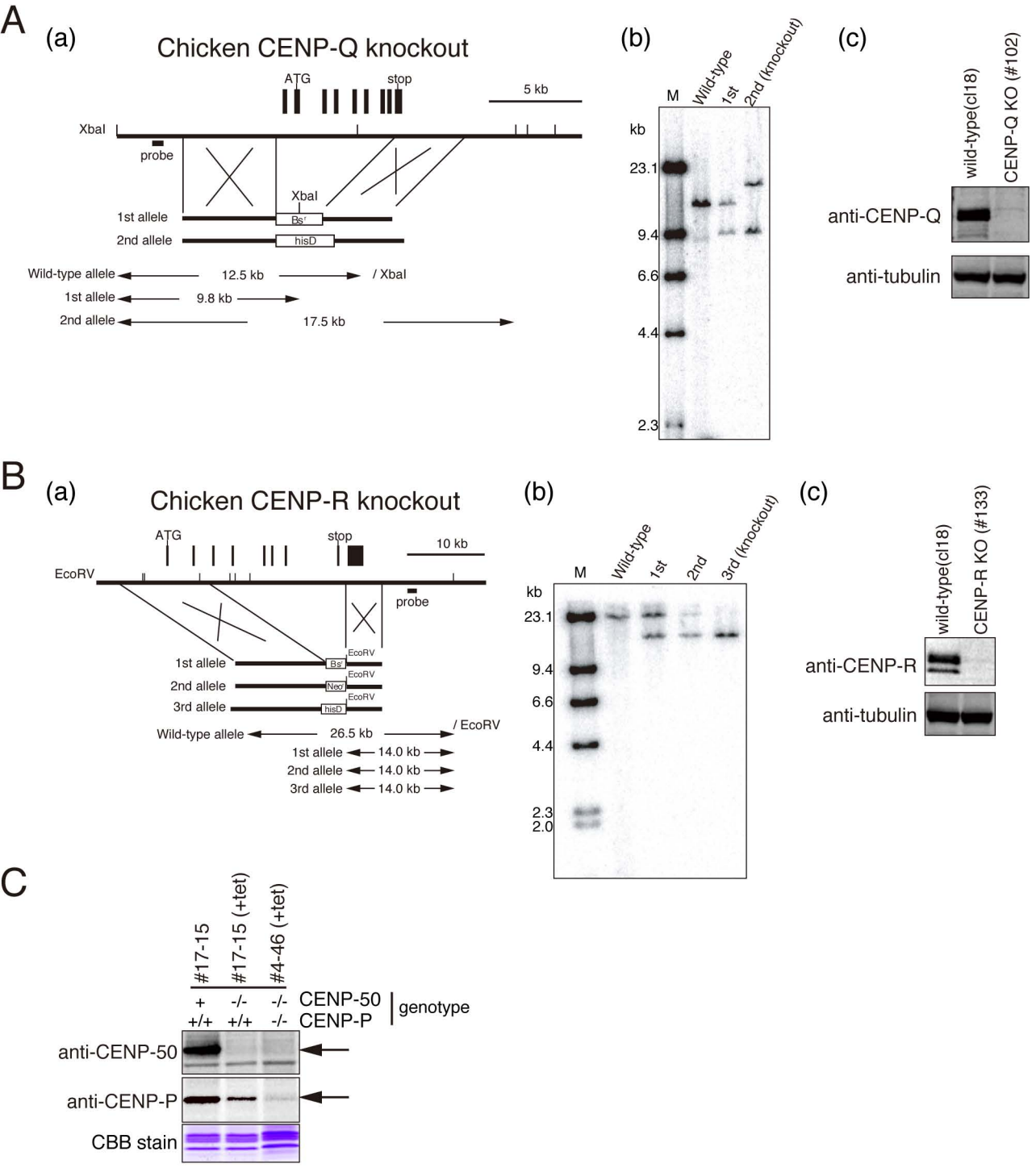

Fig. S1 Hori et al.

mutated sites of CENP-50 mutants

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  1 MSSKKRTKRNRAGDEYKEHKGRSHPRRKFLPPEEPDVSRI
 41 SKVAGVNQLEELCDSFDQPLHSTAVDACGEEHSENESSGY
 81 VPAPQRTNAERSEKMLLETPEGDVHEFSQSGSVREPLMEN
121 LNAPNTTRSEVKKKRPSKKSSSDSSVNSPSSVQLWCPNKL
161 KRSSRDITELDVVLAEFEKIAANYRQSIESKACRKAVSAF
201 CSAFEDQVTDLITEVQELKNTKKKNAKVVADIKKKRQRLM
241 QVREKLSRTEPQLIKLQKEYAEVEERRSSLRQVVQFLTDL
281 KELQQDYLDYREENPRKKVVYGASSLPALLVESRRILQAE
321 RHFQNINRKLEYALEVQRGKLAKEH
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Fig. S2 Hori et al.