Supplemental Materials
Molecular Biology of the Cell

Nagpal et al.

Legends for Supplemental Figures

Supplemental Figure S1 Generation of a CENP-N conditional knockout cell line

(A) Restriction map of the chicken CENP-N locus, gene disruption constructs, and targeted loci. Black boxes indicate the position of exons. ApaLI restriction sites are shown. Novel 10.7 kb and 9.8 kb ApaLI fragments hybridize to the probe if targeted integration of the construct occurs.

(B) Restriction enzyme analysis of genomic DNA from cells with targeted integration of CENP-N disruption constructs. Genomic DNA from wild-type (CI18), first-round targeting, and second-round targeting and integration were analyzed by Southern blot.

(C) Growth curve for the cell line in the presence or absence of tetracycline.

(D) Immunostaining for CENP-T (red) after CENP-N depletion (36 h after tetracycline addition).

(E) Quantification of CENP-T signal intensity in CENP-N- and CENP-H-deficient cells. Results were plotted as an average of the signal intensities at 20 kinetochores per cell with antibody (n > 7 cells). Error bars indicate the S.D.

Supplemental Figure S2 CENP-C$^{\Delta 166\text{-}224}$ localizes to centromeres

(A) Immunostaining for CENP-T (red) in CENP-C-deficient cells expressing GFP-CENP-C$^{\Delta 166\text{-}224}$ showing its dependency on the middle region of CENP-C for localization to the kinetochore (36 h after tetracycline addition). Bars, 10 μm.

(B) Quantification of CENP-T signal intensity in CENP-C-deficient cells expressing GFP-CENP-C$^{\Delta 166\text{-}224}$. Results are plotted as an average of the signal intensities in 20 kinetochores per cell with antibody (n > 7 cells). Error bars indicate the S.D.

Supplemental Figure S3 Characterization of CENP-C and CENP-N/L interactions

(A) His pull-down of CENP-C$^{1\text{-}165}$ with CENP-N^{ct}/L.

(B) His pull-down of CENP-C$^{601\text{-}864}$ with CENP-N^{ct}/L.

(C) Details of changed residues seen between free and bound CENP-C$^{166\text{-}224}$ by

NMR experiments.

(D) Diagram of MBP-CENP-N^{ct} and MBP-CENP-L proteins.

Fig S1

A

Chicken CENP-N knockout (conditional)

ATG

ApaLI

hisD

puro^r

Wild-type allele   6.8 kb   / ApaLI

1st allele   10.7 kb

2nd allele   9.8 kb

5 kb

B

| Marker | Wild Type (cl18) | 1st K.O | 2nd K.O |

kb

23

9.4

6.6

4.4

2.5
2.0

10.7kb
9.8kb

6.8kb

C

Growth Curve (CENP-N)

8.5

8

7.5

7

6.5

6

5.5

5

Cell Number

0   12   24   36   48   60   72   96

Time (hr)

tet−
tet+

D

CENP-T   DAPI   Merge

CENP-N K.O

On

Off

E

120

100

80

60

40

20

0

Normalised CENP-T Signal (%)

CENP-H K.O   CENP-N K.O

Tet−
Tet+

Fig S2

A
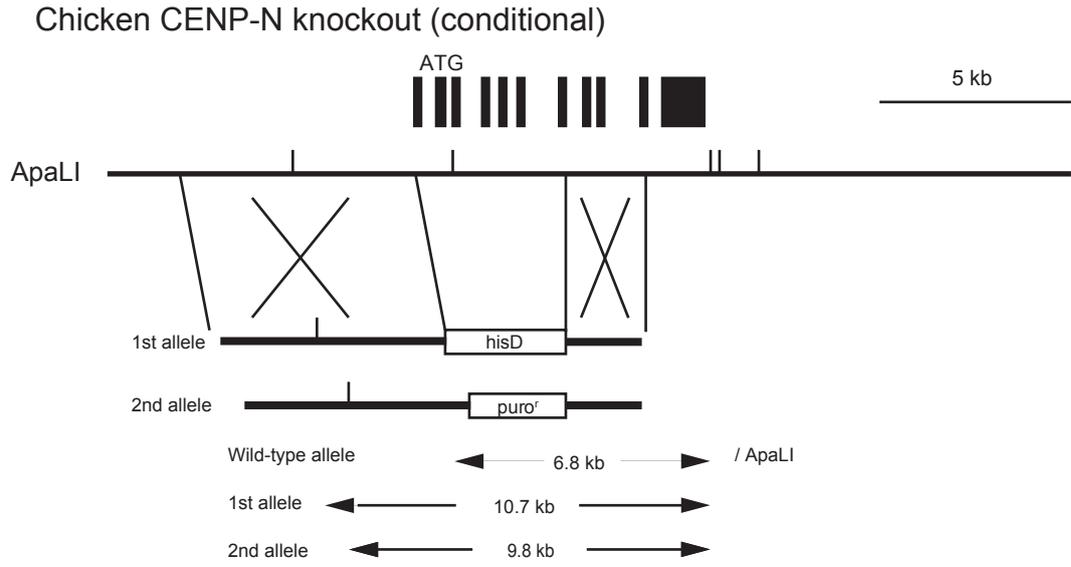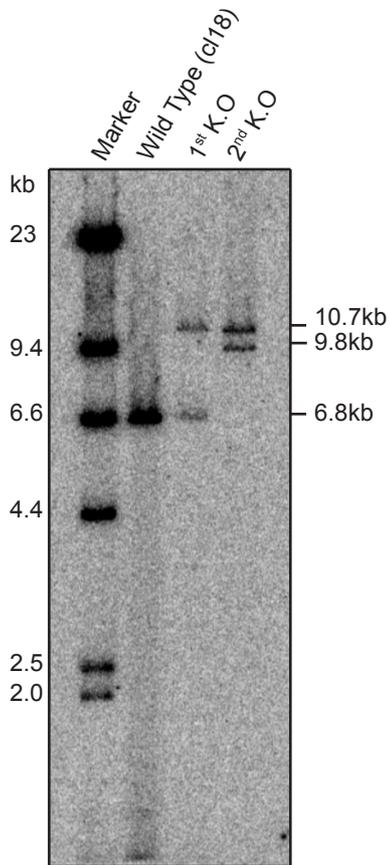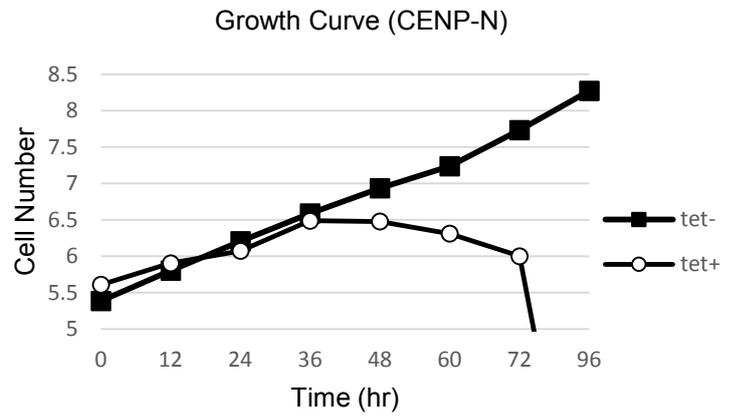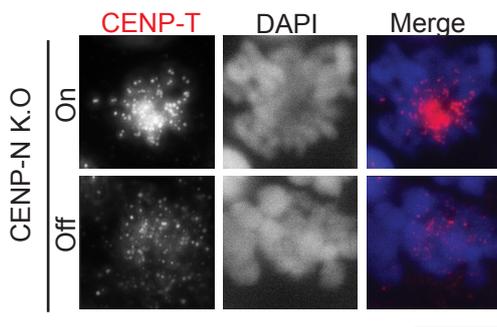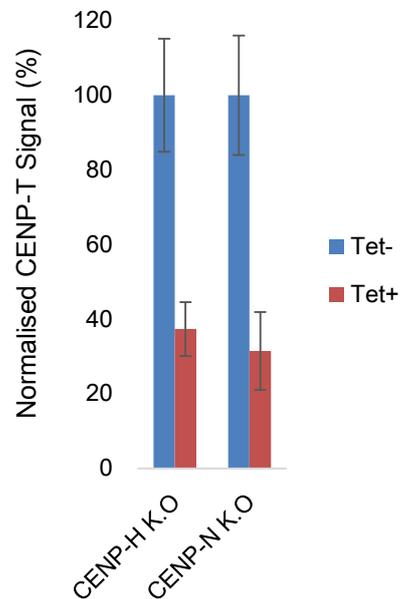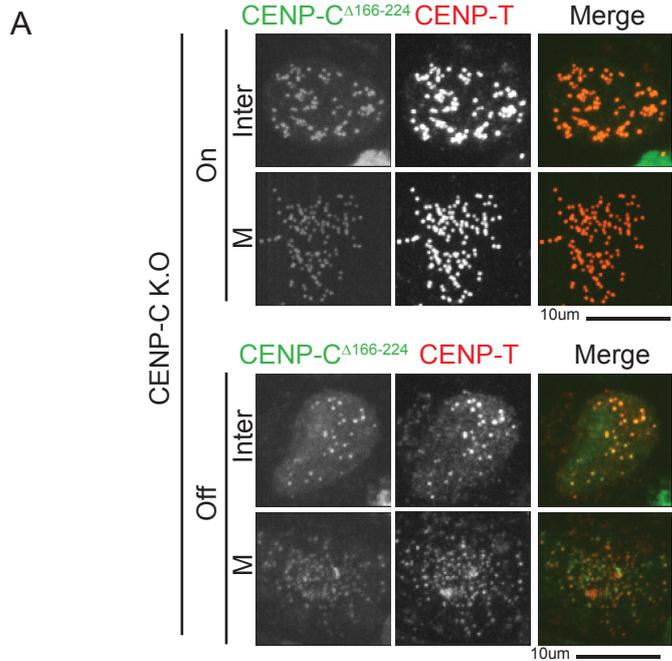
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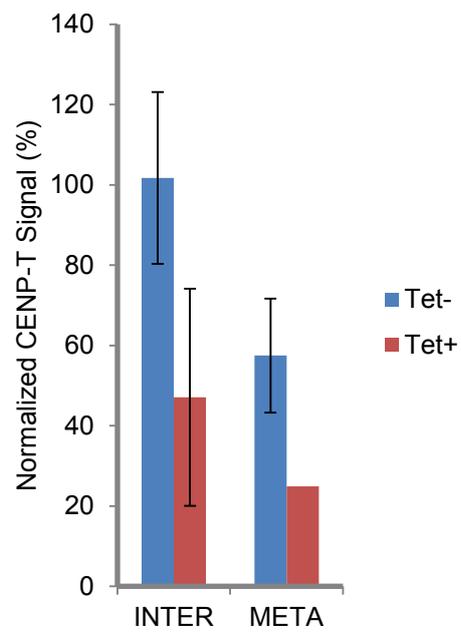
Fig S3

A
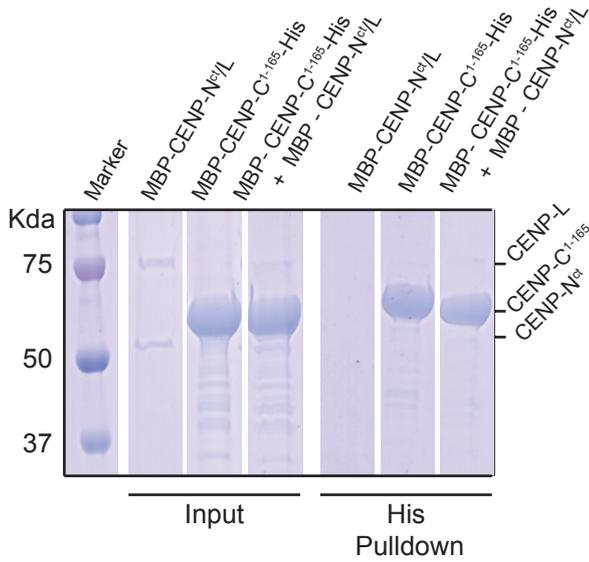
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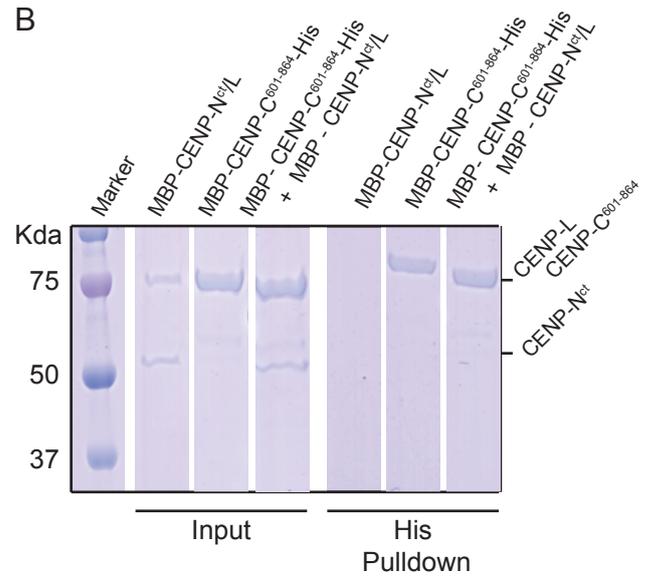
C

Residues where signal disappeared on addition of CENP-N^{ct}/L

GRSPLLVEEA KTPVQTRTVS IAPSSPSPVK DHNVEIESDC EFLIDESGCL
SFKSWFSIPR PHHHHHH

Residues where signal shifted on addition of CENP-N^{ct}/L

GRSPLLVEEA KTPVQTRTVS IAPSSPSPVK DHNVEIESDC EFLIDESGCL
SFKSWFSIPR PHHHHHH

Residues where signal strength increased on addition of CENP-N^{ct}/L

GRSPLLVEEA KTPVQTRTVS IAPSSPSPVK DHNVEIESDC EFLIDESGCL
SFKSWFSIPR PHHHHHH

D

MBP-CENP-N^{ct}

1   388   397   490   MW: 54 kDa   Linker region: gagaacctgtactttcagggagat
MBP   CENP-N (aa 252-344)

MBP-CENP-L

1   388   397   727   MW: 80 kDa   Linker region: gagaacctgtactttcagggagat
MBP   CENP-L (aa 13-344)