Supplemental Materials

Molecular Biology of the Cell

Perpelescu et al.

Supplementary Figure legend

Figure S1, related to Figure 1. Localization profile of chicken HJURP and FACS analysis of conditional HJURP knockout cells at the indicated time after tetracycline addition.

(A) Localization of GFP-HJURP (full length) throughout the cell-cycle. CENP-T was used as a centromere marker.

(B) DNA was stained with propidium iodide (x axis) and the replication stage was recognized by BrdU incorporation followed by staining with FITC-anti BrdU (y axis). cell stages and the percentage represent the gated events were written.

Figure S2, related to Figure 3. Rescue assay with different HJURP mutant proteins

(A) Schematic of chicken (Gallus gallus) HJURP in comparison with the human homologue (upper).

(B) Sequence alignment of chicken (ggHJURP) human HJURP (hsHJURP). Alignment was generated with ClustalW2 program.

(C) Cell proliferation assay for HJURP-conditional knockout cell line alone or stably expressing GFP-HJURP full length or functional mutant proteins.

(D) Same as in (C) for HJURP mutant proteins that cannot rescue HJURP-deficiency.

Figure S3, related to Figure 4. HJURP centromere recruitment is mediated by Mis18 complex components.

(A) Mass-spectrometry results for IP with anti-FLAG in cells expressing Mis18α and IP with anti-GFP in cells expressing CENP-C$^{644\text{-}864}$. For IP with cells expressing GFP-CENP-C$^{644\text{-}864}$, we used nuclear soluble fraction.

(B) Immunoprecipitation (IP) experiments were performed with anti-Mis18α and anti-GFP in cells expressing GFP fused HJURP mutant proteins. Mis18α IP detected M18BP1/KNL2 but not HJURP. Full-length HJURP was co-precipitated with M18BP1/KNL2. Whole cell lysate was used for IP.

(C) Immunoblot showing the different cellular localization of M18BP/KNL2, HJURP, CENP-C, Mis18α and CENP-A. Loading control are actin and

post-blotting CBB (Coomassie Brilliant Blue) staining of the same gel.

(D) Western blot analysis showing the levels of GFP-HJURP^{FL} stable expressed in Mis18α, CENP-C and M18BP1/KNL2 conditional knockout cell lines before and after 48hrs tetracycline supplementation of the culture media. Also, the efficient knockout of HJURP, Mis18α and M18BP1/KNL2 is shown.

(E) Western blot analysis showing the levels of GFP-HJURP$^{401\text{-}end}$ stable expressed in Mis18α, CENP-C and M18BP1/KNL2 conditional knockout cell lines before and after 48hrs tetracycline supplementation. The efficiency of depletion for HJURP, Mis18α and M18BP1/KNL2 is shown.

(F) Immunofluorescent staining showing the retention of GFP-HJURP$^{401\text{-}end}$ (green) at centromere marked by CENP-T (red) in the CENP-C-deficient cells before and at 48hours after tetracycline addition. GFP-HJURP$^{401\text{-}end}$ is stably expressed in CENP-C knockout cell line.

(G) Similar to D but for Mis18α conditional cell line stably expressing GFP-HJURP$^{401\text{-}end}$ showing loss of HJURP$^{401\text{-}end}$ from centromere after Mis18α deficiency following 48hrs tetracycline treatment.

(H) Immunofluorescence image showing that GFP-HJURP$^{401\text{-}end}$ cannot localize to centromere in the M18BP1/KNL2-deficient cells at 48hrs upon tetracycline addition.

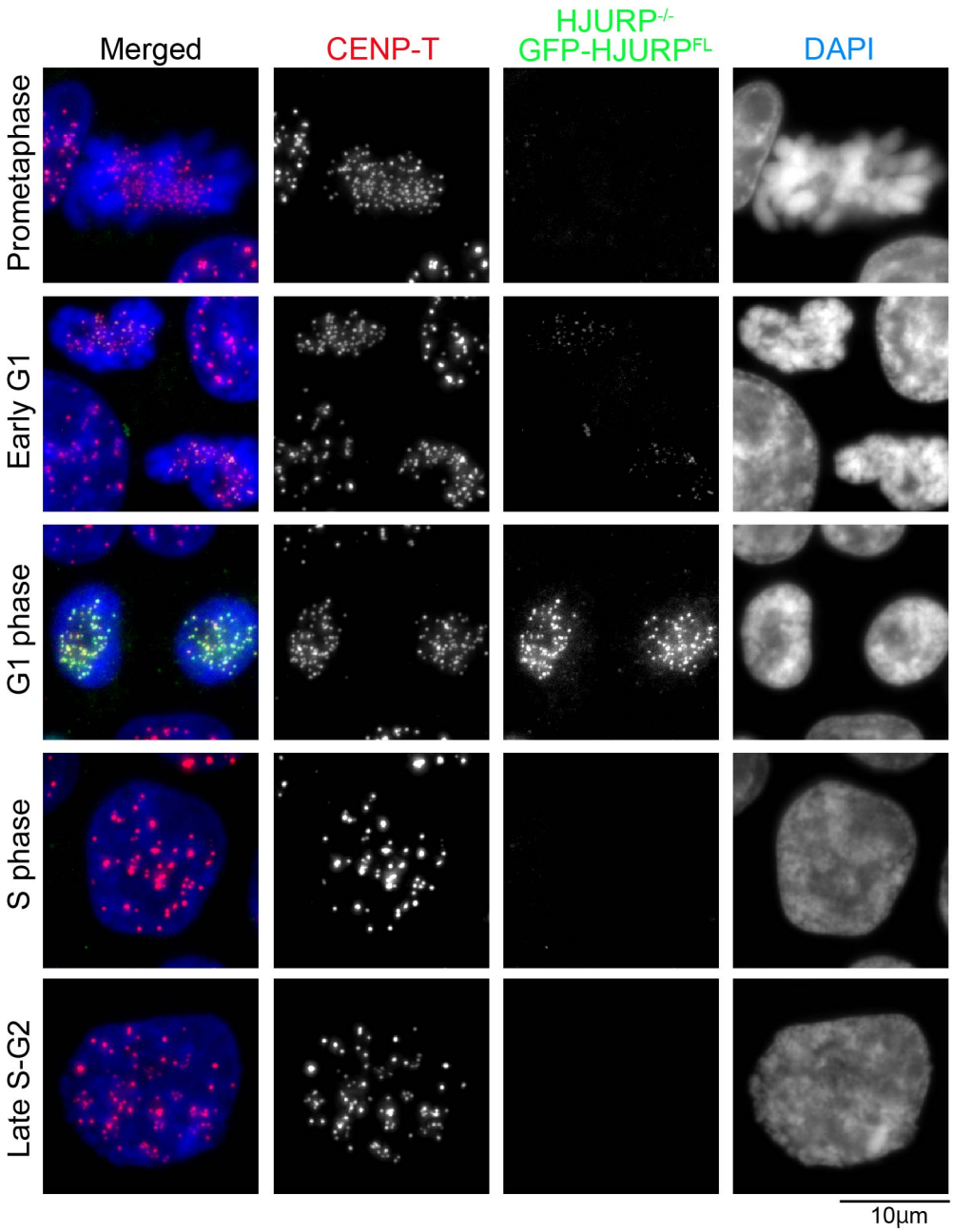
Figure S4, related to Figure 5. LacI-HJURPs at the LacO site were not visible after IPTG addition.

(A) An experimental scheme for Figure 5.

(B) Although LacI-HJURPs localize at the LacO site was detected in the absence of IPTG (Figure 5B), these signals were not detected in the presence of IPTG. After cells were cultured for 1 week in the presence of IPTG, ChIP-seq analysis with anti-CENP-A was performed. Arrows shows artificial kinetochores.

FIGURE S1

A


Merged
CENP-T
HJURP-/-
GFP-HJURPFL
DAPI
Prometaphase
Early G1
G1 phase
S phase
Late S-G2
10μm

B

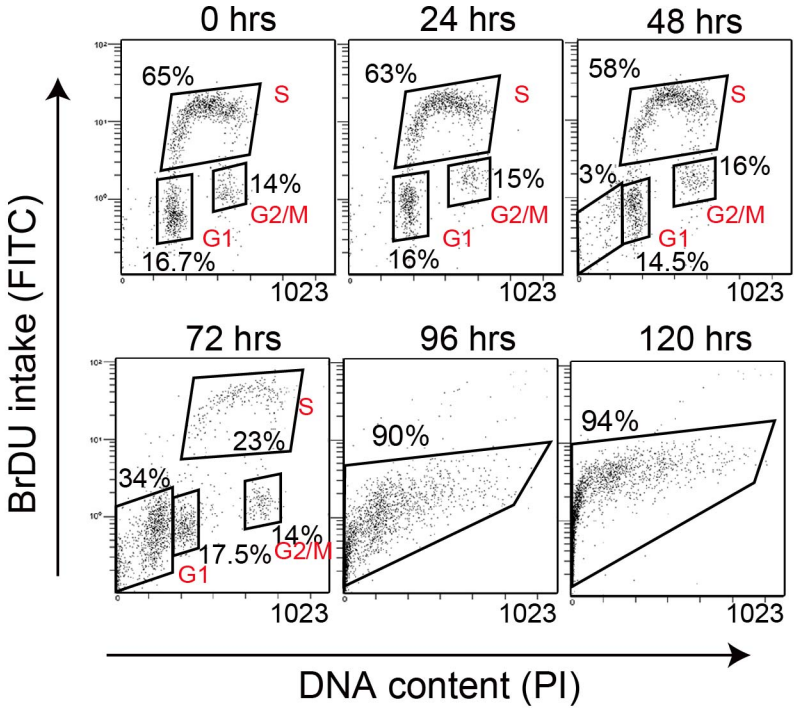
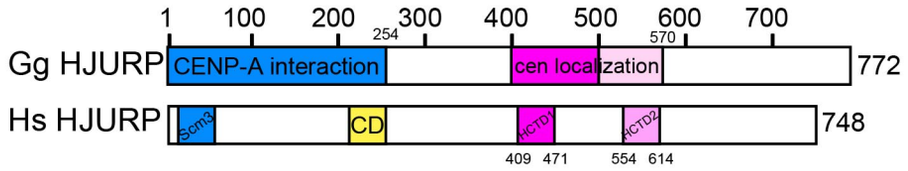

0 hrs
24 hrs
48 hrs
72 hrs
96 hrs
120 hrs
65%
S
14%
G2/M
G1
16.7%
63%
15%
16%
58%
3%
14.5%
23%
34%
17.5%
90%
94%
1023
BrDU intake (FITC)
DNA content (PI)

FIGURE S2

A



B

```
>ggHJURP        ----------MALDLDERLRRSNARFQTSILSILERYNYPFEDDFLISMETLTYDTP 47
>hsHJURP        MLGTLRAMEGEDVEDDQLLQKLRASRRRFQRRMQRLIEKYNQPFEDTPVVQMATLTYETP 60
                          :* ::** *. ***  :  ::*:** ****  ::.* ****:**

>ggHJURP        EGPKEWGDLSTKEVRKRFKHHARSQRTADQTAGEESDAEIVSVRRRFEDIHFQNLDLDDV 107
>hsHJURP        QGLRIWGGRLIKERNEGEIQDS-SMKPADRTDGSVQAAAWGPELPSHRTVLGADSKSGEV 119
                :* : **.   ** .:   :.: * :.**:* *. . *   .    .. :   : . .:*

>ggHJURP        KAREKVQVQVDVIVQDNERRIPKWITVEPSSSHFTSPVREMIGHQSSVCRKKVELANECS 167
>hsHJURP        DATS----------DQEESVAWALAPAVPQSPLKNELRRKYLTQVDILLQGAEYFECAG 168
                .* .           *:*. :.  ::   ..* :.. :*.    * .:  : .*  : ..

>ggHJURP        SSGPDQLQSSGVPMLPDTITVPRHRSFQEMNETGYNSTLEEYQSADEGCSWSNVTLADLY 227
>hsHJURP        NRAGRDVRVTPLPSLASPAVPAPGYCSRISRKSPGDPAKPASSPREWDPLHPSSTDMALV 228
                . .  ::: : :* *... . .   . :  .::  :.:    .. : .   .. *   *

>ggHJURP        PKMVEVLTKLITKEARKKTSKYVFGLYKYKRGHARRPKLSITVEKIRKFRPLKLKKALPS 287
>hsHJURP        PRNDSLSLQETSSSSFLSSQPFEDDDICNVTISDLYAGMLHSMSRLLSTKPSSIISTKTF 288
                *:  .:  :  :..:  .:. :  .           . :  ::.:: . :* .: .: .

>ggHJURP        ICSGSKDIQNQTFGSNINENSGLLDDPCFIDSSSGLVPFSYSDTNEMEMDCSDSSLDYHL 347
>hsHJURP        IMQNWNSRRRHRYKSRMNKTYCKGARRSQRSSKENFIPCSEPVKGTGALRDCKNVLD--V 346
                * .. :. :.: : *.:*:.       .  .*...::* * . ..   :  ... **  :

>ggHJURP        VFGKDQKLSEQIAIPNALARMGETFLAEDQLQTTVSLNSSKYNKSGYLTYKHSLEPPFRT 407
>hsHJURP        SCRKTGLKLEKAFLEVNRPQIHKLDPSWKERKVTPSKYSSLIYFDSSATYNLDEENRFRT 406
                   *     *:  :    .:: :   : .: :.* *  **    ..  **: . *  ***

>ggHJURP        SSASSGSVALHLVKEGKTRKNDFPSDDTSRFCSSTCSIISDGNTFTPIKDYYLASGTLLI 467
>hsHJURP        LKWLIS--PVKIVSRPTIRQG--HGENRQREIEIRFDQLHREYCLSPRNQ----PRRMCL 458
                .   .  .:::*.. . *:.   .:: .*  .   . :     ::* ::    .  : :

>ggHJURP        KPEKKISERQNSFQRRHSFSSLSMKQSPSKTPQKHEDAFEKLYYKLCSRGSQKPLTLTKP 527
>hsHJURP        PDSWAMNMYRGGPASPGGLQGLETRRLSLPSSKAKAKSLSEAFENLGKRSLEAGRCLPKS 518
                  .  :.  :..     .:..*. :: .  :.: : .::.: : :* .*. :    *.*.

>ggHJURP        LSNSQKLEEKGGIMRNTFSDSVGSSIQYNVAFEKIYQQLSSEVVPKIPGFQRASNLRKYE 587
>hsHJURP        DSSSS-LPKTNPTHSATRPQQTSDLHVQGNSSGIFRKSVSPSKTLSVP----DKEVPGHG 573
                 *.*. * :..     * .:....    . :   : :.:*.. . .:*     .::  :

>ggHJURP        GIQMSETVNALVNSPVRSLYAIPRVKRLGNFQNDLLSSPVKRLKNIPEHFFSTKCQQISH 647
>hsHJURP        RNRYDEIKEEFDKLHQKYCLKSPGQMTVPLCIGVSTDKASMEVRYQTEGFLGKLNPDPHF 633
                  : .*  : : :   :     *    :    .   ...  .::  .* *:..   :  .

>ggHJURP        KKYDDLQTVGMDFVSLHKGSNTRFFNNHNCHHQDSGFHASSDKTFLVIPDASLKESENTD 707
>hsHJURP        QGFQKLPSSPLGCRKSLLGSTAIEAPSSTCVARAITRDGTRDHQFPAK-RPRLSEPQGSG 692
                : ::.* :  :.  .   **.:    . .*  :    ..: *: * .   . *.*.:.:.

>ggHJURP        VCSSWPRTMPHYSSPGSGLKRPQKVSRKLSYTDGKDRNPSNPLDNVLIKTHQGAFMAYRE 767
>hsHJURP        RQG---NSLGASDGVDNTVRPGDQGSSSQPNSEERGENTSYRME------EKSDFMLEKL 743
                  .   .::   .. .. ::  :: * . . :: :..*.*  ::      .:. **  :

>ggHJURP        NGIVL 772
>hsHJURP        ETKSV 748
                :   :
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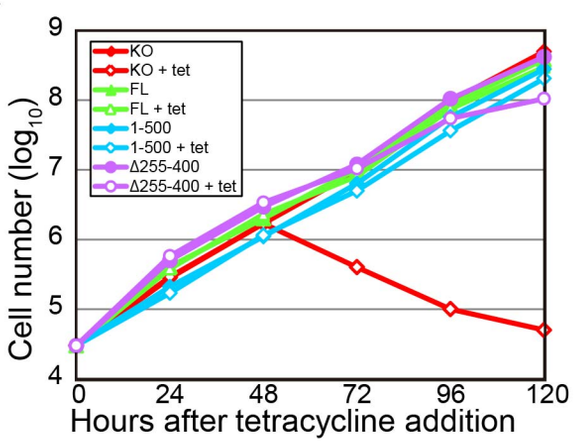
C



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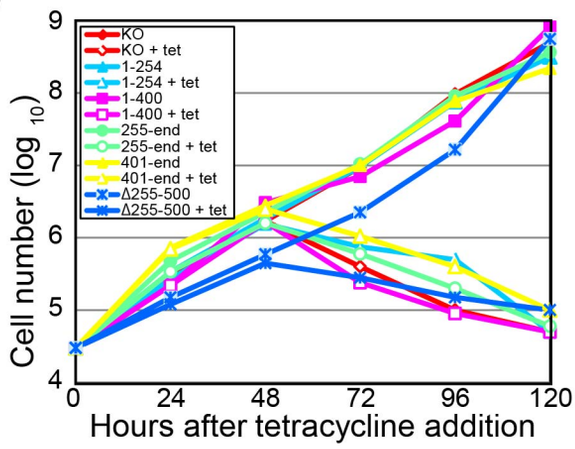
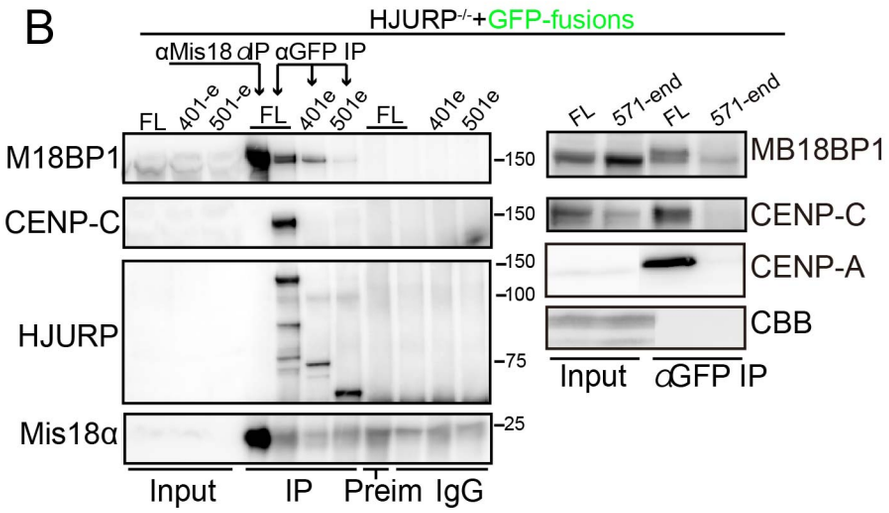
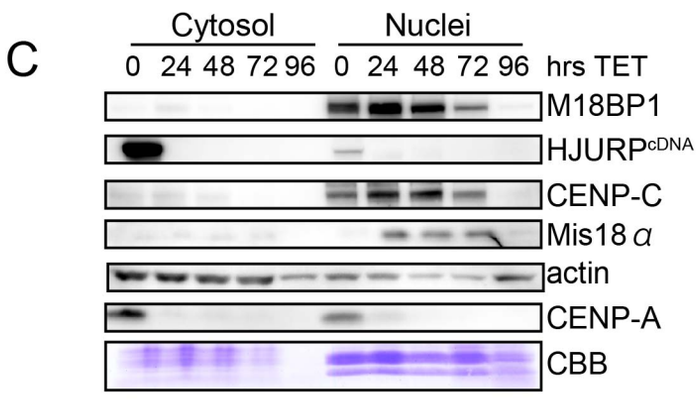


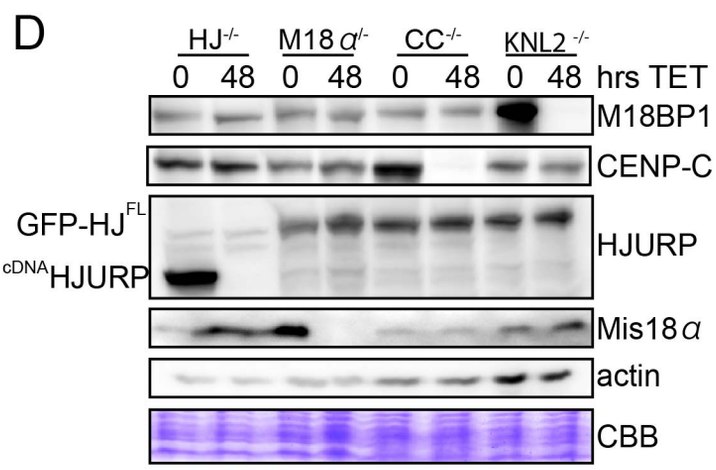
FIGURE S3

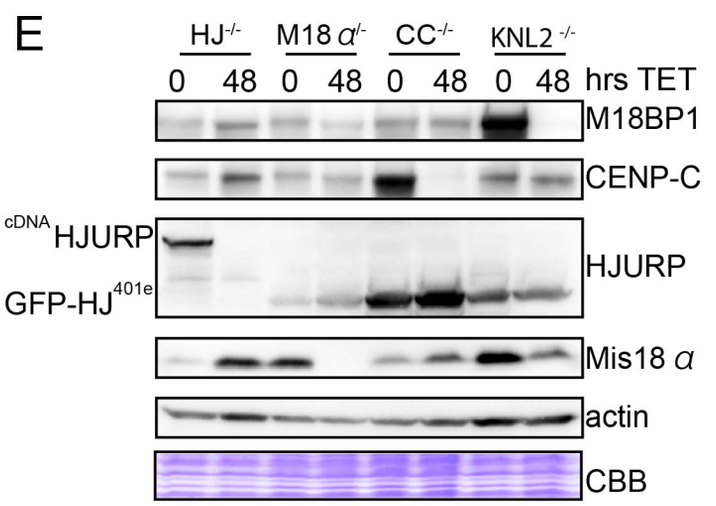
A

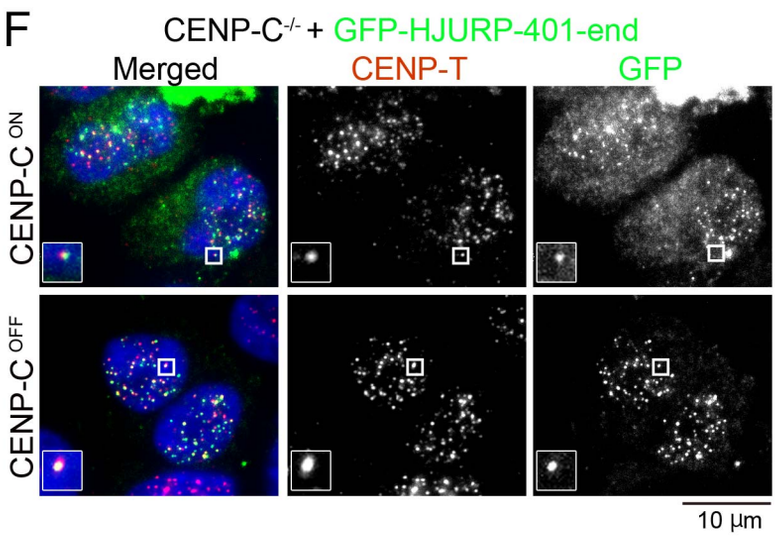
Protein name	Predicted size (kDa)	Peptide count: FLAG-IP Mis18α-flag	Peptide count: GFP-IP gfp-CENP-C$^{644-864}$
CENP-C	96.2 (864 aa)	0	35
HJURP	87.6 (772 aa)	6	6
Mis18α	22.2 (204 aa)	32	0
Mis18β	24.1 (213 aa)	46	0
Mis18BP1	122.2 (1088 aa)	133	0

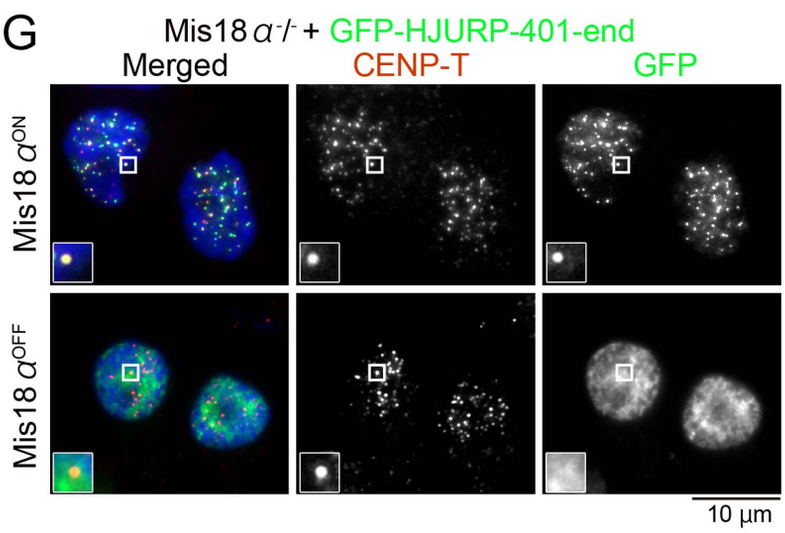












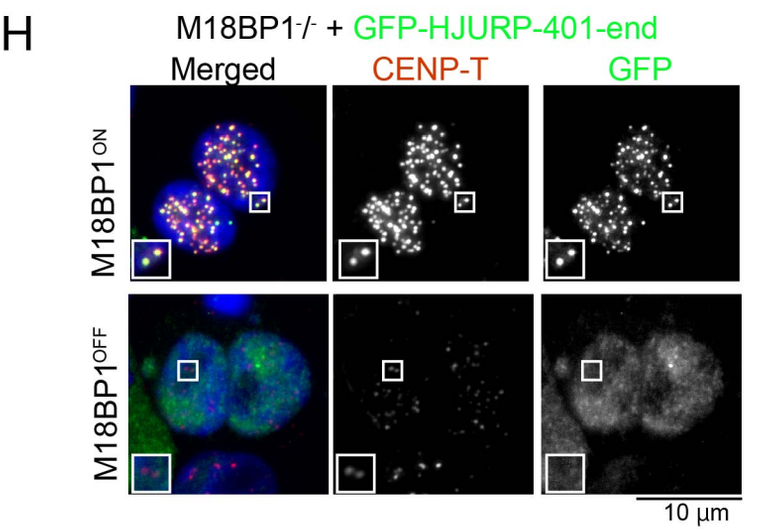
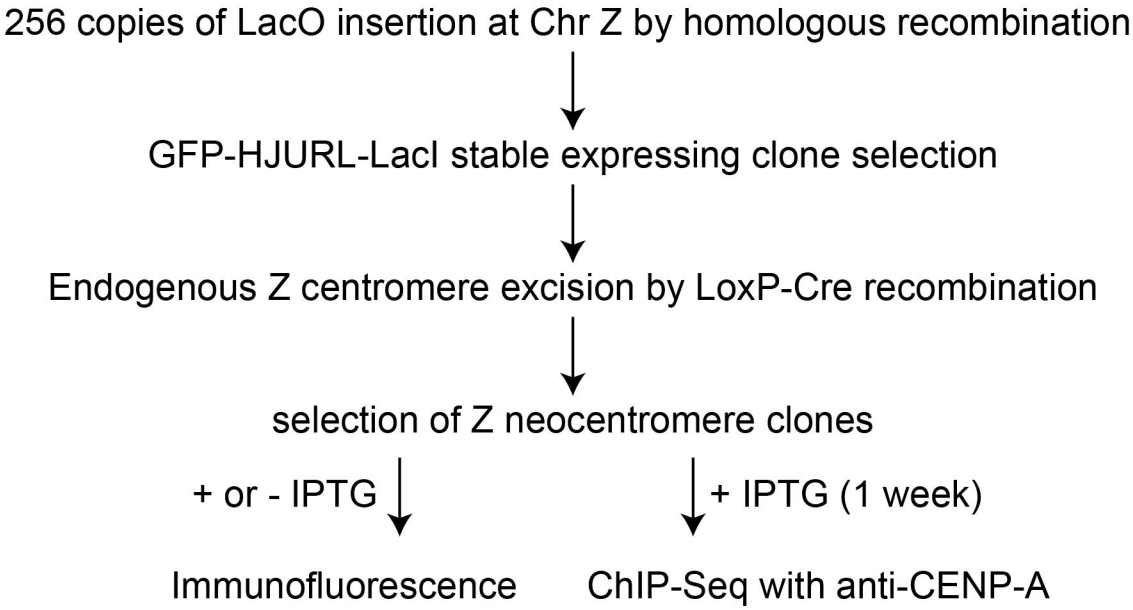
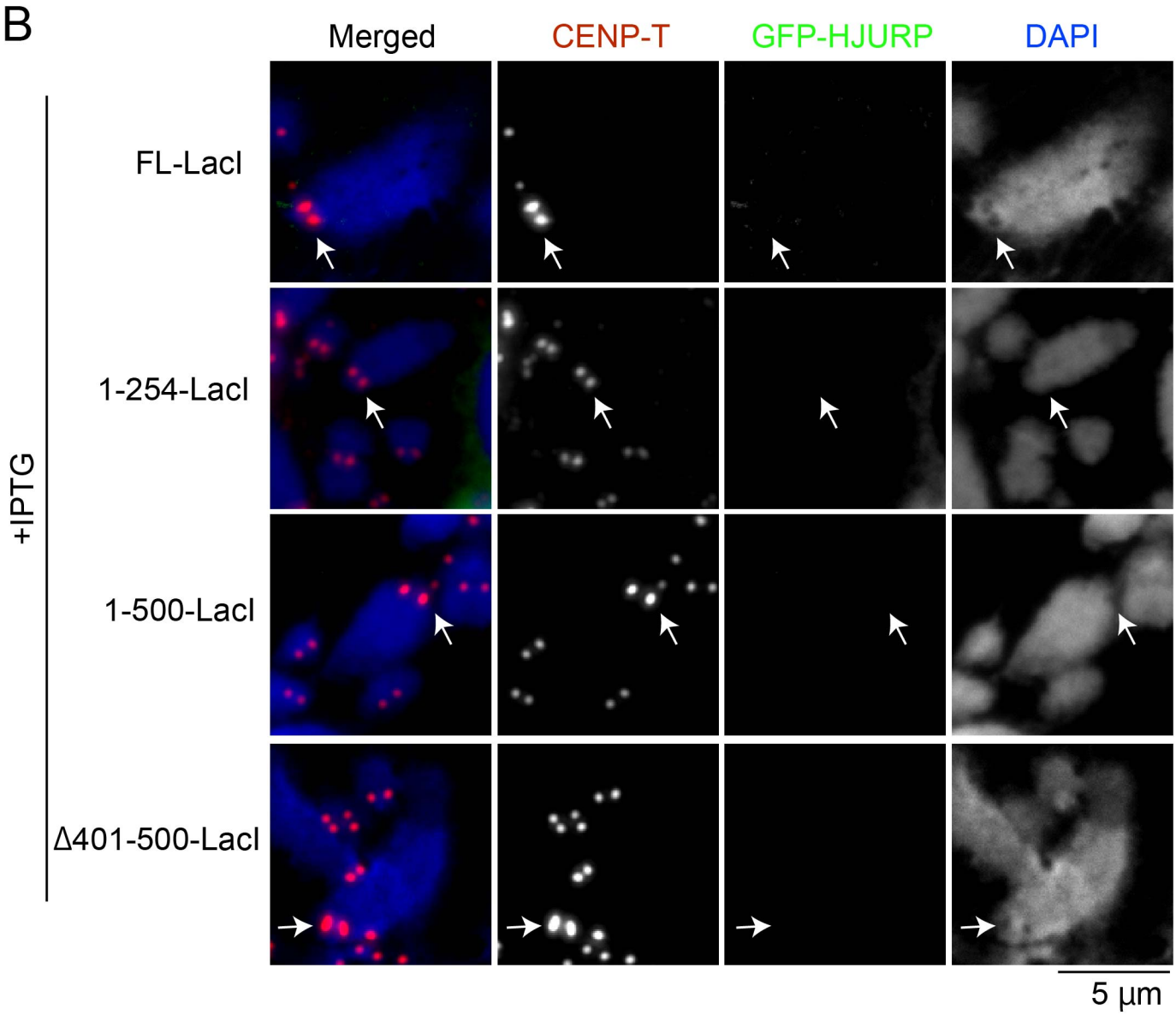


Figure S4

A


256 copies of LacO insertion at Chr Z by homologous recombination
GFP-HJURL-LacI stable expressing clone selection
Endogenous Z centromere excision by LoxP-Cre recombination
selection of Z neocentromere clones
+ or - IPTG
+ IPTG (1 week)
Immunofluorescence
ChIP-Seq with anti-CENP-A

B


Merged
CENP-T
GFP-HJURP
DAPI
+IPTG
FL-LacI
1-254-LacI
1-500-LacI
Δ401-500-LacI
5 μm