

SUPPLEMENTARY FIGURE LEGENDS.

Supp. Fig. 1. The tTS destabilizes the alphoid^{tetO} HAC kinetochore in 1C7 cells.

(A-B) 1C7 cells transfected with tetR:EYFP (A) or tTS:EYFP (B; green in A'' and B'', respectively) and stained with a human autoantibody ACA (A', B'; red in A'' and B'') 48 hours post transfection. Arrows indicate the targeted HAC. Scale bar: 5 μm .

Supp. Fig. 2. KAP1 subdomain targeting and CENP-C dissociation

(A-D) Cells were transfected with the following KAP1 deletion constructs: KAP1[20-559] (A), KAP1[RBCC] (B), KAP1[HP1D] (C) and KAP1[Ph/BrD] (D). Cells were fixed 96 hours post-transfection for co-staining with antibody against CENP-A (A'-D') and CENP-C (A''-D''). Colors in merge: blue=EYFP, green=CENP-C, red=CENP-A. Arrows indicate the position of the targeted HAC. Scale bar: 5 μm . (E) Schematic of the targeting constructs.

Supp. Fig 3. Dissociation of CENP-H following targeting with KAP1 subdomains.

(A-D) Cells were transfected and treated the same as for Supp. Fig. 2, but staining with anti-CENP-A (A'-D') was combined with anti-CENP-H (A''-D''). Colours in merge: blue=EYFP, green=CENP-H, red=CENP-A. Arrows indicate the targeted HAC. Scale bar: 5 μm .

Supp. Fig. 4. HP1 α recruitment to targeted alphoid^{tetO} HACs.

Analysis of HP1 α recruitment to the alphoid^{tetO} HAC following targeting with TetR:EYFP-tagged constructs. (A-D) Cells transiently expressing TetR:EYFP:EZH2

(A), TetR:EYFP:KAP1 Δ 20 (B), tetR:EYFP:KAP1[RBCC] (C) and TetR:EYFP:KAP1[Ph/BrD] (D) were fixed and immunostained with an antibody against HP1 α (A'-D'). Merge: green= various TetR:EYFP fusions, red=HP1 α . (E) Frequency of the HACs targeted with TetR:EYFP, TetR:EYFP:KAP1[HP1D] and TetR:EYFP:KAP1 Δ 20 positive or not for HP1 α and CENP-C. (F) Quantification of the amount of CENP-C associated to HACs targeted with TetR:EYFP:KAP1 Δ 20 and positive for both HP1 α and CENP-C. Scale bar: 5 μ m.

Supp. Fig. 5. HAC sister kinetochores display unequal CENP-C levels after KAP1 targeting.

48 hours after washing out Doxycycline, mitotic 1C7-KAP1 cells were imaged. (A) Maximum intensity projections of 1C7-KAP1 cells in metaphase (top panel) and late anaphase (bottom panel). Arrows point to the CENP-C spots associated with the two targeted HAC^{tetO} sister kinetochores. Unequal levels of CENP-C at the sister kinetochores are evident. Note that in the anaphase cell, the HAC^{tetO} sister chromatid with the lower level of CENP-C also appears to be lagging behind the endogenous chromosomes. Scale bar 5 μ m. (B) Fluorescence quantification of the fusion construct bound to the HAC^{tetO} sister chromatids (EYFP) and HAC^{tetO} associated CENP-C antibody staining in metaphase and anaphase 1C7-KAP1 cells. The ratio of fluorescent signal of both sister kinetochores of each quantified HAC^{tetO} kinetochore pair is plotted along the x-axis. While levels of the fusion construct on both sister chromatids were mostly similar, CENP-C staining appears to be generally unevenly distributed on the HAC^{tetO} sister kinetochores.

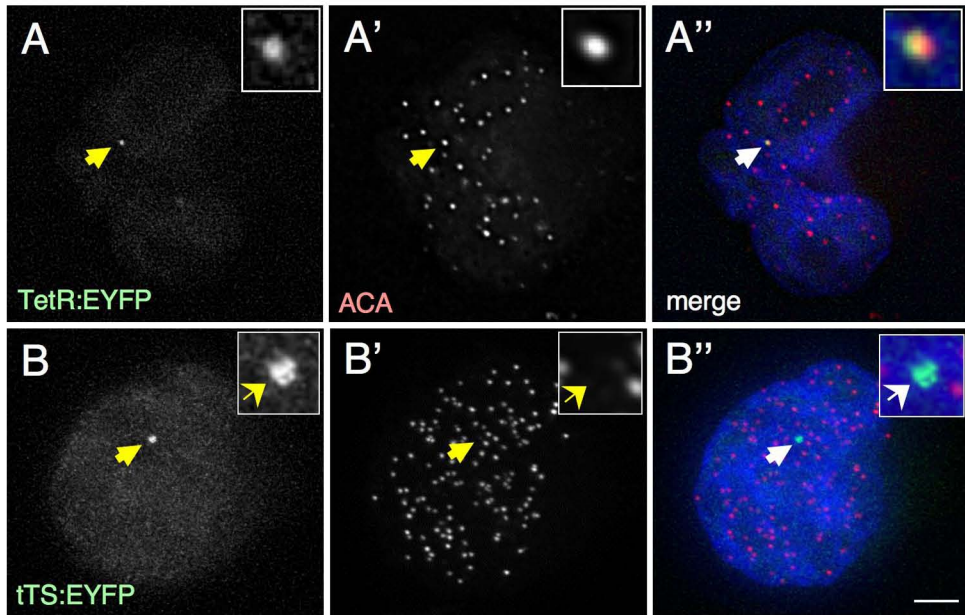
Supp. Fig. 6. HAC metaphase defects after KAP1 targeting.

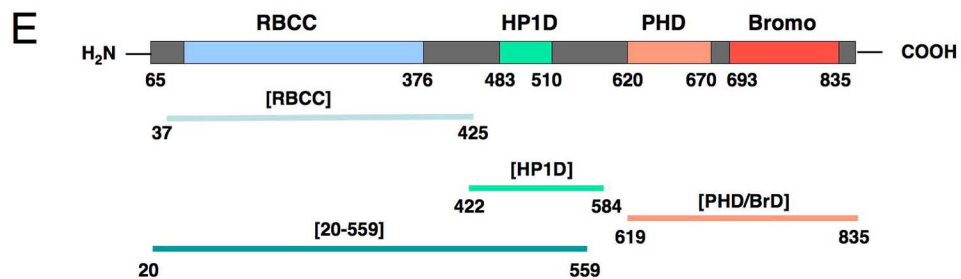
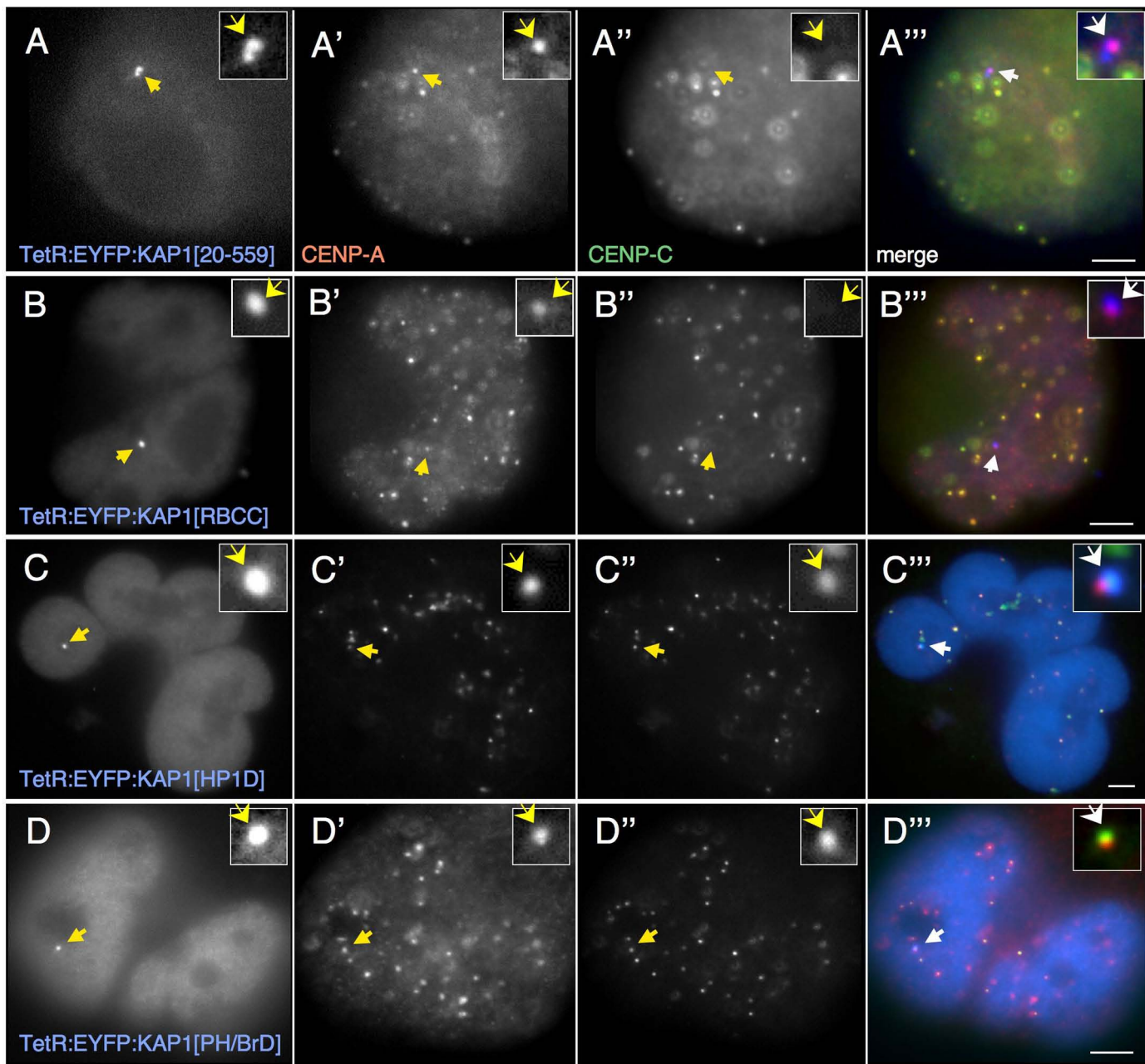
(A-B) Representative images of metaphase 1C7-KAP1 cells fixed and stained for CENP-C at 48 (A) and 72 (B) hours after washing out of Doxycycline. At the earlier time point, most HACs showed metaphase alignment and kinetochore tension (top panel), with a fraction of cells showing the HAC positioned more towards the edge of the plate and an apparently lower inter-kinetochore distance (bottom panel). Low but readily detectable levels of CENP-C were usually seen at the HAC sister kinetochores. By 72h, a large proportion of HACs failed to align at the metaphase plate. While in some cases the HAC sister chromatids could be resolved as distinct spots (top panel), other cells frequently showed an unresolvable EYFP spot (bottom panel) indicative of a lack of tension and / or higher chromatin compaction. CENP-C staining was generally extremely weak (bottom) or virtually undetectable (top). For the 72h time point (B), the DAPI signal associated with the magnified region around the HAC is shown in the greyscale inset. Scale bar: 5 μ m. **(C)** Quantification of the percentage of HACs that showed alignment or were unaligned at the indicated time points in late prometaphase and metaphase 1C7-KAP1 cells.

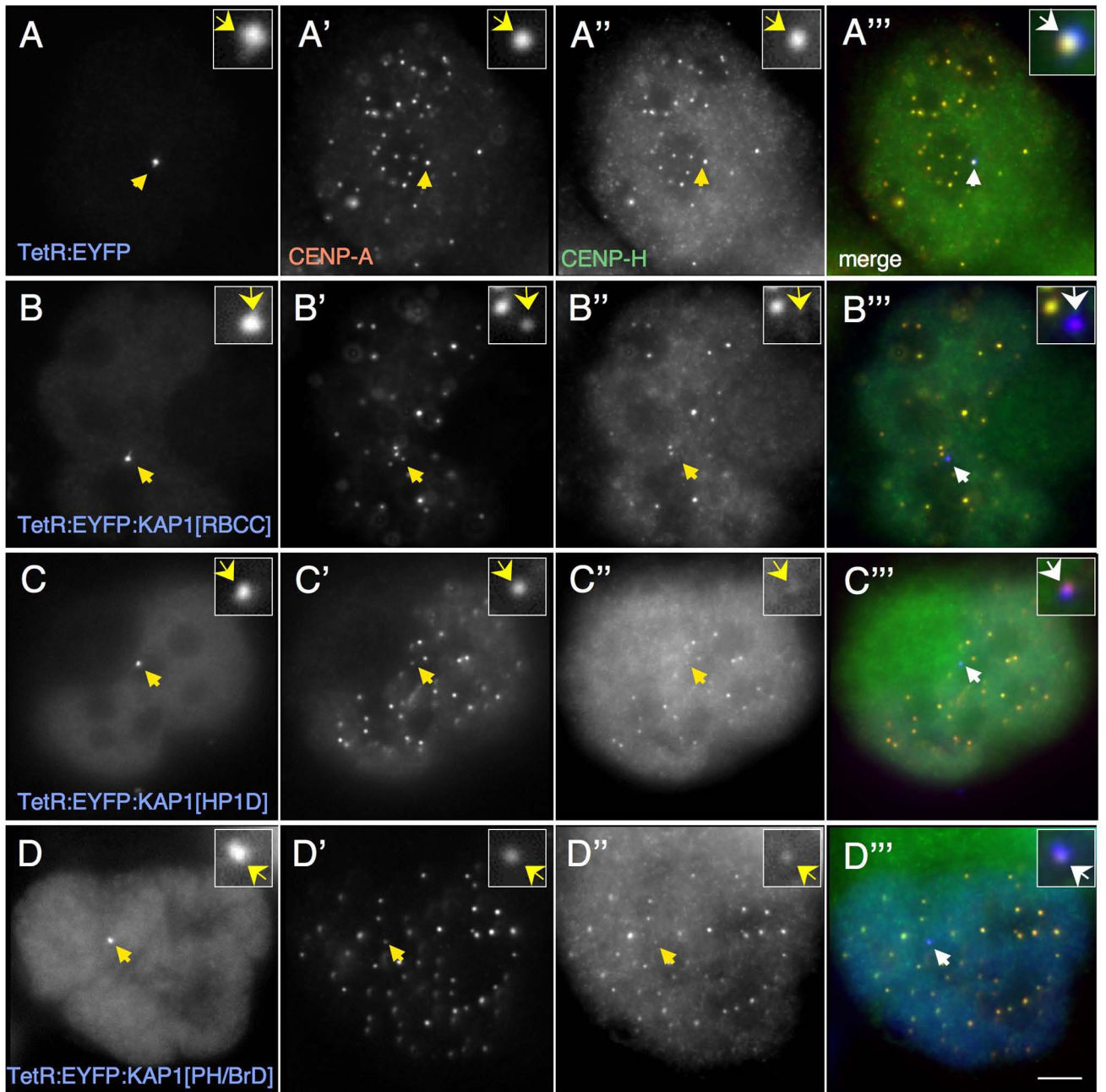
Supp. Fig. 7. HAC sister kinetochores display unequal CENP-C levels after KAP1 targeting.

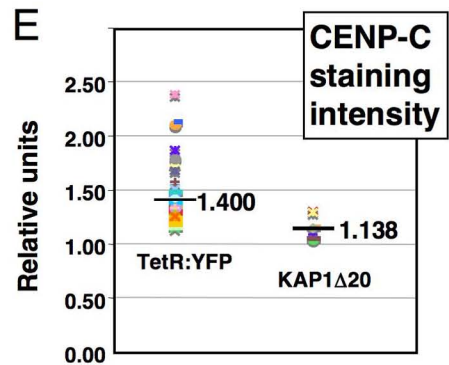
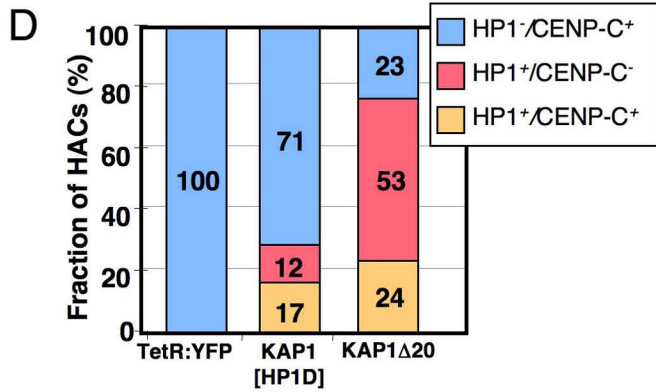
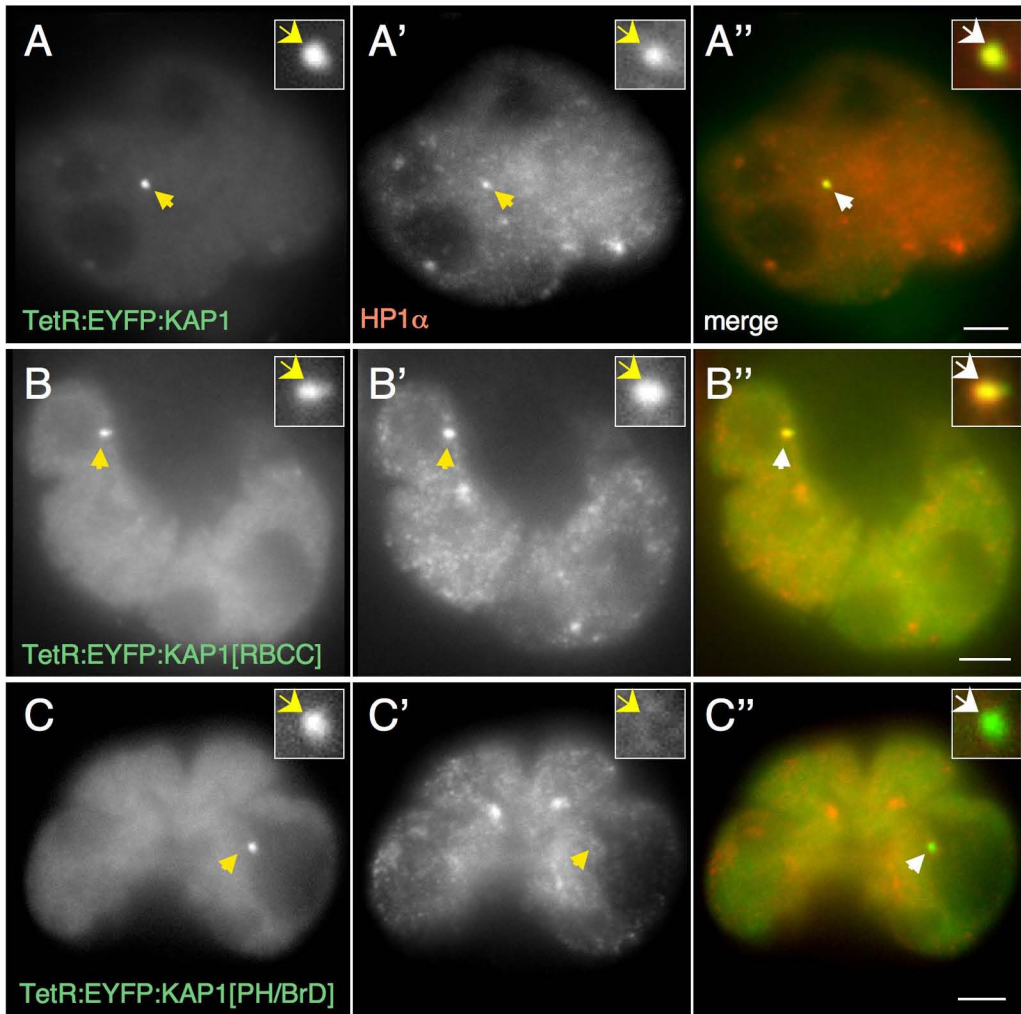
48 hours after washing out Doxycycline, mitotic 1C7-KAP1 cells were imaged. **(A)** Maximum intensity projections of 1C7-KAP1 cells in metaphase (top panel) and late anaphase (bottom panel). Arrows point to the CENP-C spots associated with the two targeted HAC^{tetO} sister kinetochores. Unequal levels of CENP-C at the sister kinetochores are evident. Note that in the anaphase cell, the HAC^{tetO} sister chromatid with the lower level of CENP-C also appears to be lagging behind the endogenous chromosomes. Scale bar 5 μ m. **(B)** Fluorescence quantification of the fusion construct

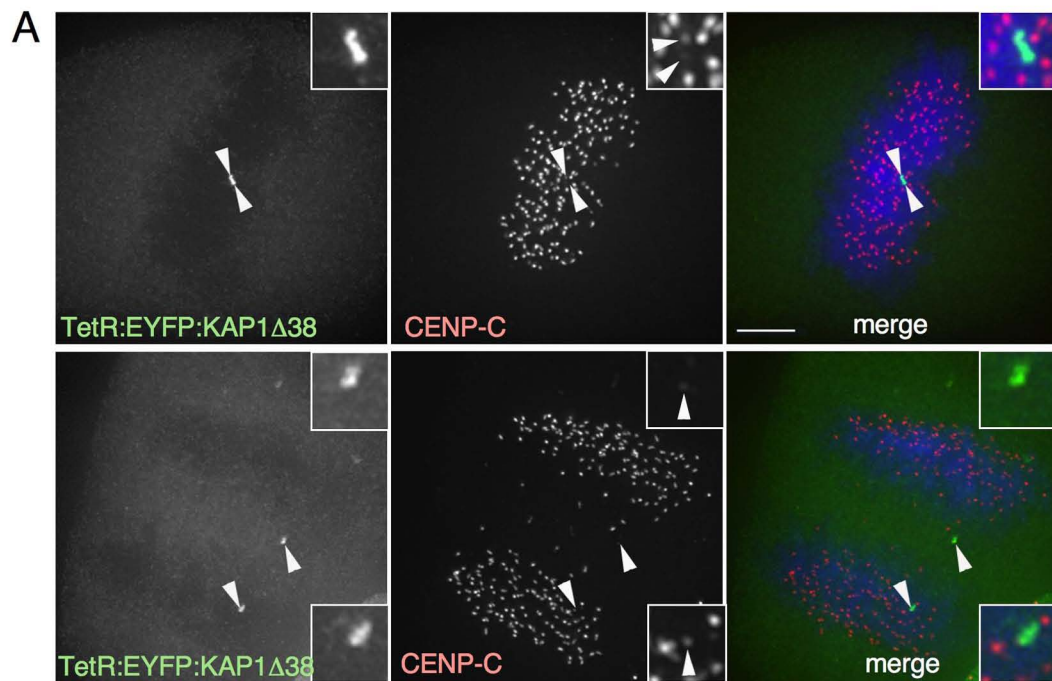
bound to the HAC^{tetO} sister chromatids (EYFP) and HAC^{tetO} associated CENP-C antibody staining in metaphase and anaphase 1C7-KAP1 cells. The ratio of fluorescent signal of both sister kinetochores of each quantified HAC^{tetO} kinetochore pair is plotted along the x-axis. While levels of the fusion construct on both sister chromatids were mostly similar, CENP-C staining appears to be generally unevenly distributed on the HAC^{tetO} sister kinetochores.











B

Sister Kinetochore Staining
(weaker kinetochore normalized to 1.0)

