Supplemental Materials

Molecular Biology of the Cell

Martins et al.

Suppl. Figure 1

Centromere core domain bears MARKS of active transcription

(A) Human centromere core regions show evidence of H2A.Z accumulation. Mitotic stretched chromatin fibers from human HT1080 cells were immunostained with antibodies against H2A.Z (red) and CENP-A (green). (B) Human centromere core regions show punctual accumulations of the promoter-associated H3K4me3 MARK. Mitotic chromatin fibers as in A, but stained with antibodies against H3K4me3 (red) and CENP-A (green). (C, D, E, F) The α-satellite centromere array of both the HAC and chr. 21 shows enrichment of actively elongating RNA polymerase II. Chromatin analysis of HeLa 1C7^{HAC} cells by ChIP. Antibodies against the unphosphorylated (non-initiated) and Ser2 phosphorylated (elongating) forms of RNA polymerase II were used, as shown in E) and F) respectively. Mouse unspecific IgG was used as negative pulldown control for ChIP, as shown in D). Primer sets used are shown at the top: Sat2 was used as a *bona fide* heterochromatic transcriptionally silenced locus, the 5' UTR of PABPC1 as a *bona fide* actively transcribing promoter-proximal locus and 10kb downstream on the PABPC1 gene as a surrogate downstream region on an active gene. Mean of three independent experiments, error bars denote SEM

Suppl. Figure 2

EZH2 tethering affects the CCAN but has little effect on HAC CENP-A assembly

(A) EZH2 tethering slightly reduces HAC CENP-A and recruits RING1A, representative pictures for Figure 2 G, scale bar 5µm. (B) EZH2 tethering reduces HAC CENP-C, representative pictures for Figure 2 H, scale bar 5µm. (C) Experimental outline for detection of CENP-A assembly. A plasmid encoding CENP-A:SNAP was co-transfected into HeLa 1C7^{HAC} cells together with either TetR-EYFP, TetR-EYFP-EZH2 or TetR-EYFP-LSD1. 5h after transfection, cells were changed to medium containing 2 mM thymidine to induce an S-phase arrest. This allows the constructs time to express and tether to the HAC before the CENP-A assembly stage, in the next G1 phase (Silva et al., 2011). 16h later, cells were released from the arrest and a SNAP quench-chase-pulse assay was performed, as depicted (see material and methods). After a total of ~21h after transfection, samples were fixed and processed for microscopy analysis. (D) The first event of CENP-A assembly at the HAC after tethering of EZH2 showed little perturbation in recruitment of newly-synthesized CENP-A. On the other hand, LSD1 significantly affects HAC CENP-A assembly at the first event of assembly after tethering. Total of two independent experiments, No. of cells analyzed in each condition per experiment: EYFP only n≥30, EZH2 n≥18 and LSD1 n≥15. Red dotted line indicates level of the analyzed protein in the 'EYFP only' negative control. Mann-Whitney U statistical test was used to evaluate significance of differences observed. (E) EZH2 tethering to the HAC has little effect on subsequent CENP-A assembly, unlike LSD1. Samples were fixed and processed for fluorescence microscopy: CENP-A:SNAP~TMR-*Star* is depicted in red. Scale bar = 5 µm.

Suppl. Figure 3

Complementary chromatin analysis for Figure 4.

Chromatin analysis of 1C7-EZH2 cells by ChIP, after 5 days of TetR-EYFP-EZH2 tethering. Mouse antibodies against the epitopes described below were used, mouse unspecific IgG was used as negative pulldown control. Experimental details as in Figure A. Mean of two independent experiments, error bars denote SEM. (A) Tethering of EZH2 to the HAC does not affect the levels of heterochromatin-related H3K9me3. Antibody against H3K9me3 was used. (B) Tethering of EZH2 to the HAC induces PRC1-dependent H2AK119 monoubiquitination, Antibody against H2AK119ub was used.
(C and C') Tethering of EZH2 to the HAC does not reduce the levels of total RNA polymerase II on the alphoid^{tetO} array. Mouse antibodies against total RNA Polymerase II were used. C' shows a smaller scale to visualize low-level signals of RNA Polymerase II.

Suppl. Figure 4

Comparative analysis between the HAC and a non-centromeric integrated alphoid^{tetO} array reveals the latter is significantly more euchromatic and transcriptionally active

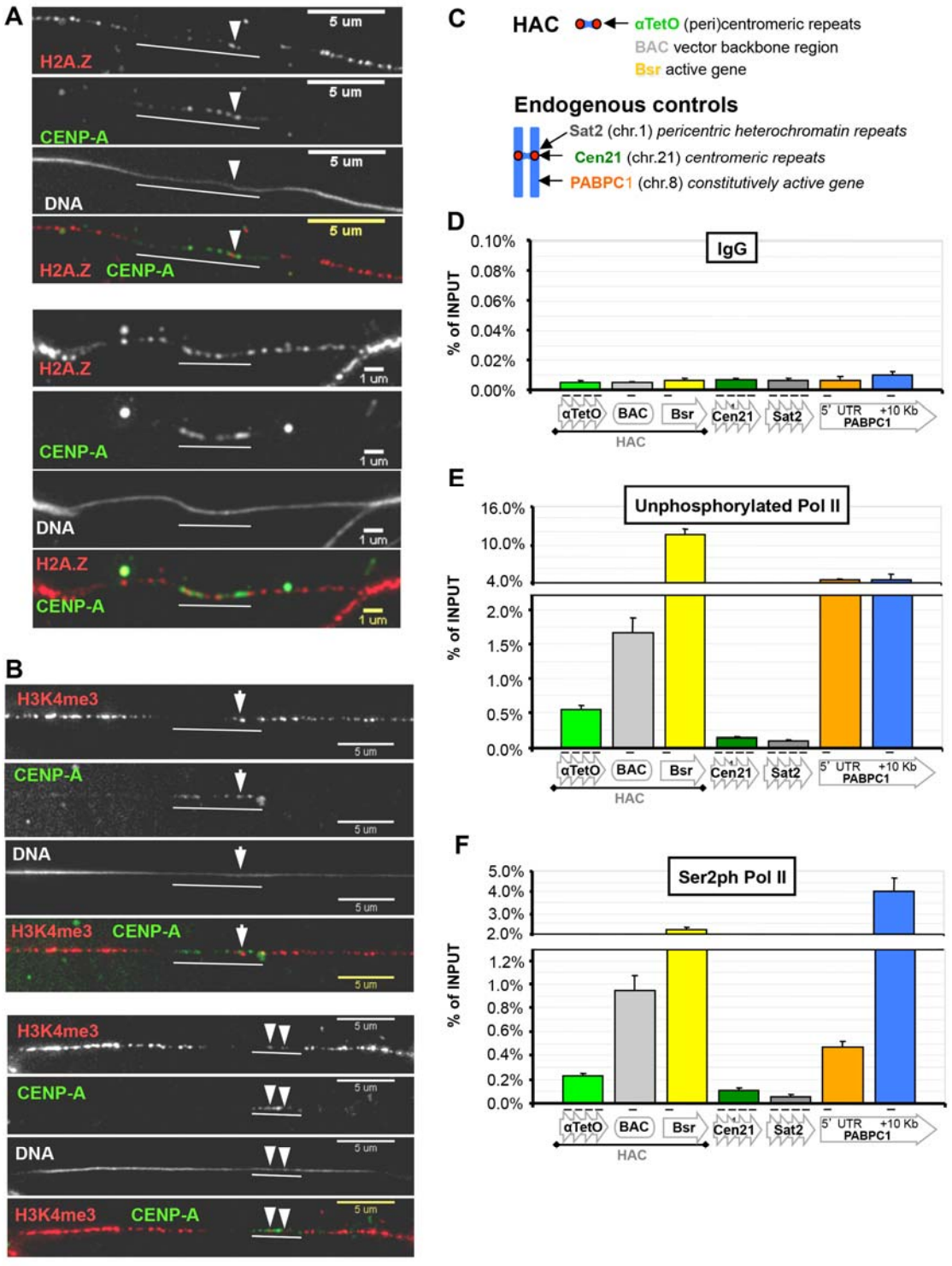
(A) Integrated alphoid^{TetO} array (red arrows) is significantly more enriched in euchromatic MARKS H3K4me2, H3K4me3 and H3K27ac, and shows reduced levels of heterochromatic H3K9me3, when compared to the HAC array. Chromatin analysis of HeLa HT1080Ab2.2.18.21^{HAC} and HT1080Ab2.5.30^{INT} cells by ChIP. Antibodies against H3K9me3, H3K4me2, H3K4me3 and H3K27ac were used; mouse

unspecific IgG was used as negative pulldown control. Pulldown DNA was quantified by qPCR. Primer sets used are shown on the left. (B) The integrated alphoid^{TetO} array is transcribed ~10-fold more than the HAC alphoid^{TetO} array. Quantification of transcripts from HT1080Ab2.2.18.21^{HAC} and HT1080Ab2.5.30^{INT} cells. Transcripts were using specific primers against alphoid^{tetO} repeats, the Bsr gene, endogenous Cen21 and 5S rDNA. Transcript level was normalized to its genomic copy number (for repeats) and further normalized to β-actin. Mean of three independent experiments, error bars denote SEM. (C) HeLa 1F10^{INT} cells, derived from HT1080Ab2.5.30^{INT}, have low or undetectable levels of CENP-A and detectable levels of "open chromatin" mark H3K9ac. Microscopy images of cells transfected for 24h with TetR-EYFP, immunostained with antibodies against CENP-A (red) and H3K9ac (blue). (D) Quantification of CENP-A signals co-localizing with TetR-EYFP, in HeLa 1C7^{HAC} and 1F10^{INT} cells, transfected with TetR-EYFP for 3 days. Total of two independent experiments, n≥5 cells for each condition. Blue line indicates median.

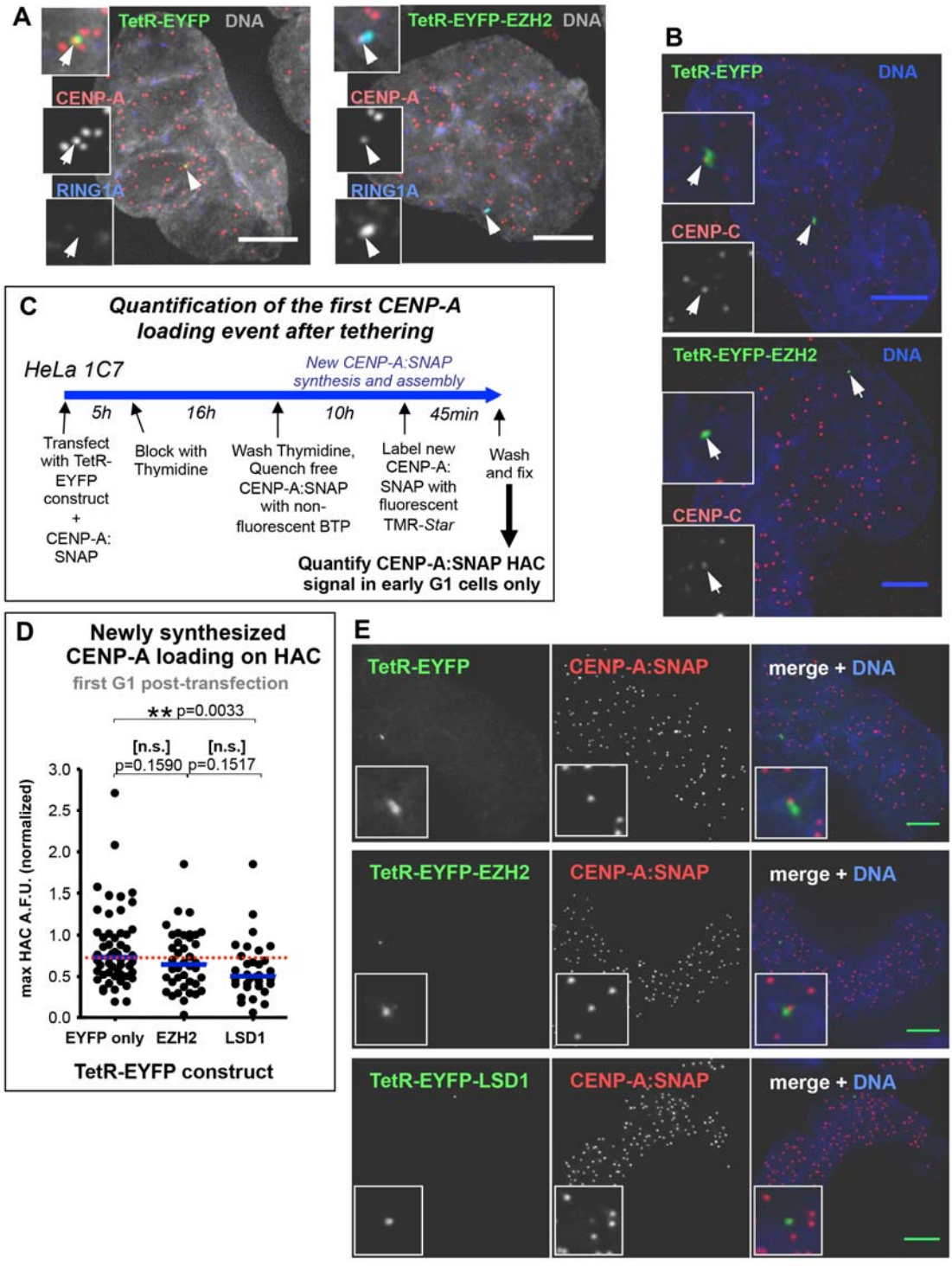
Suppl. Figure 5

BMI1 tethering recruits PRC1 to the HAC in higher amounts than EZH2

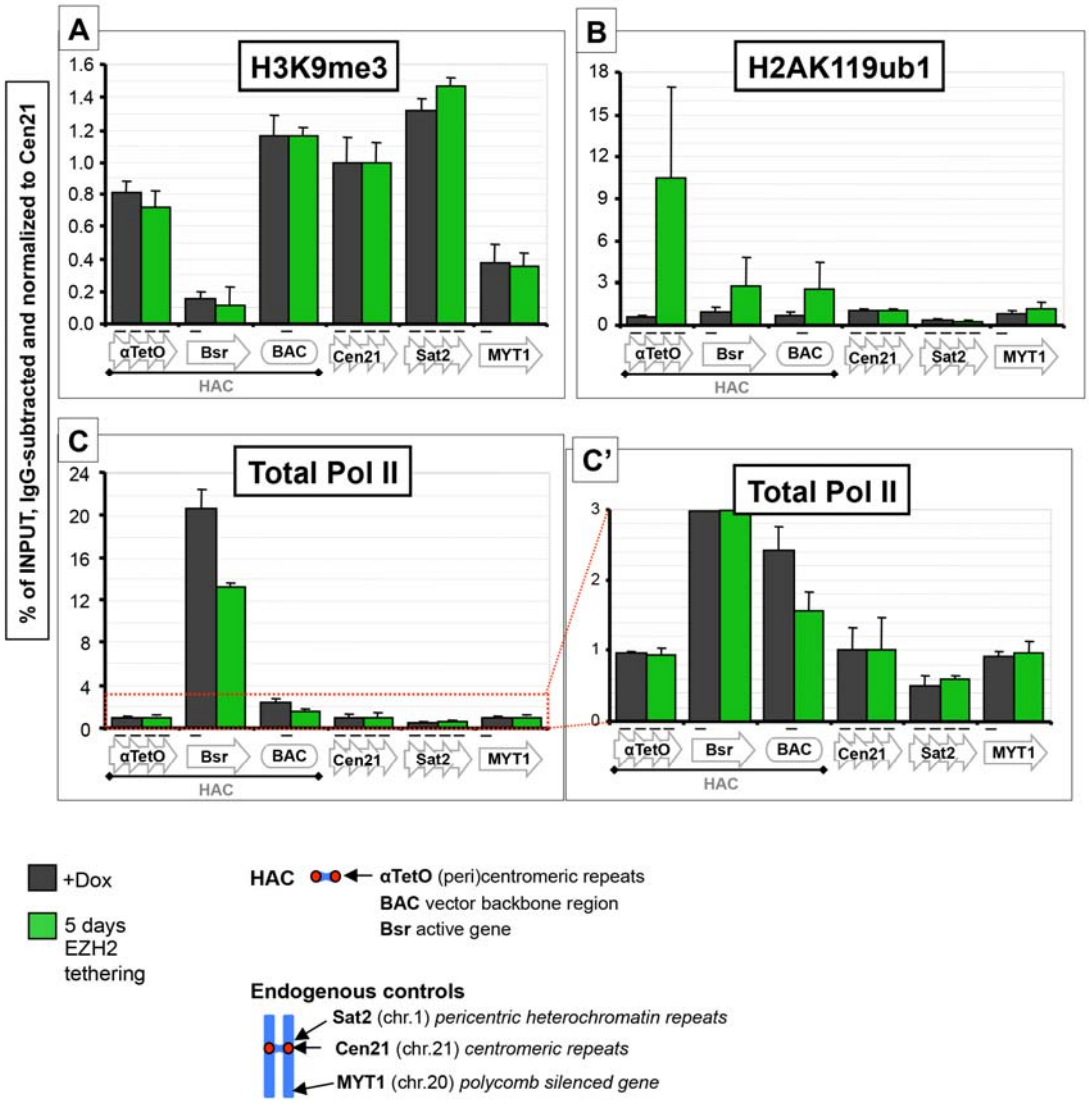
(A) Tethering of TetR-EYFP-BMI1 to the HAC recruits ~3x the amount of RING1A than TetR-EYFP-EZH2. HeLa 1C7^{HAC} cells were transfected with TetR-EYFP, TetR-EYFP-EZH2 or TetR-EYFP-BMI1. Cells were fixed 3 days later and processed for immunofluorescence. Antibody against RING1A (as surrogate for the presence of PRC1) was used. Total of three independent experiments, No. of cells analyzed in each condition per experiment: EYFP only n≥17, EZH2 n≥18 and BMI1 n≥16. Red dotted line indicates mean level of local RING1A background signal. Mann-Whitney U statistical test was used to evaluate significance of differences observed. (B) Microscopy images of quantification in A. Samples were fixed and processed for immunofluorescence: antibodies against H3K27me3 (red) and RING1A (blue) were used. Scale bar = 5 μm.

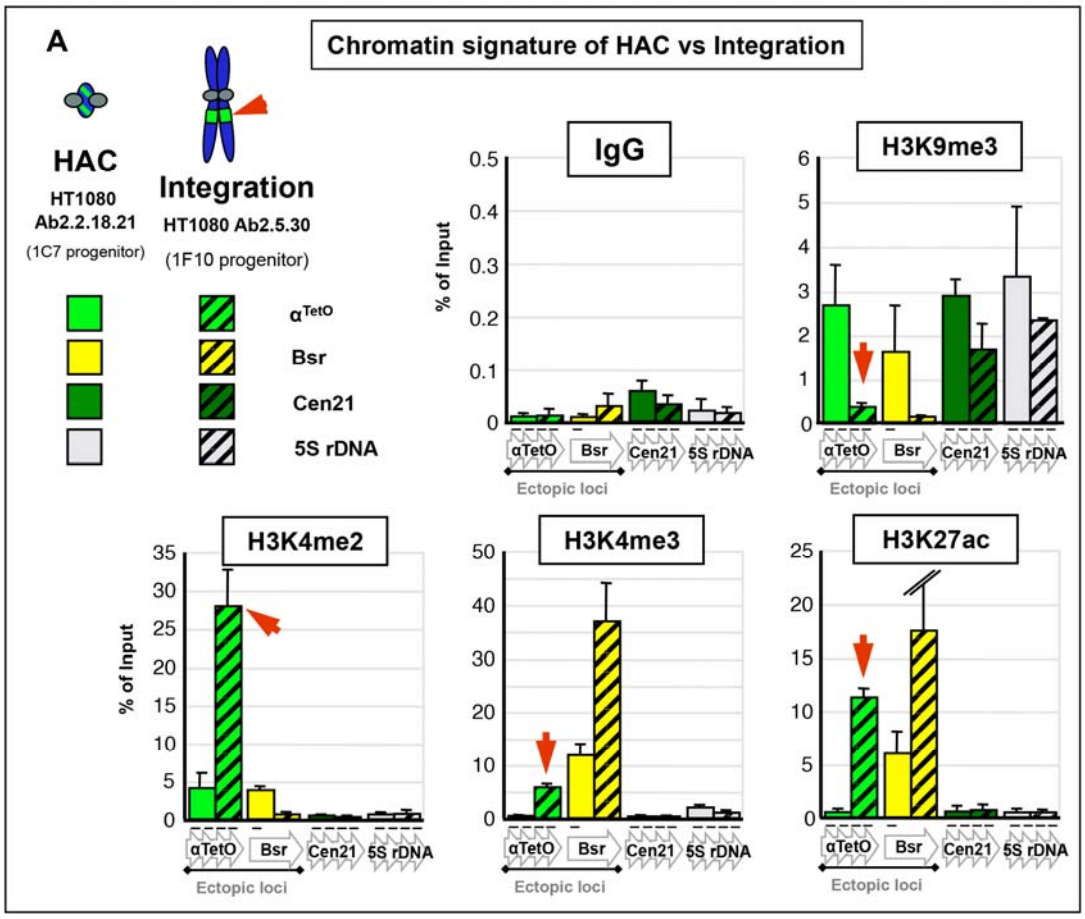


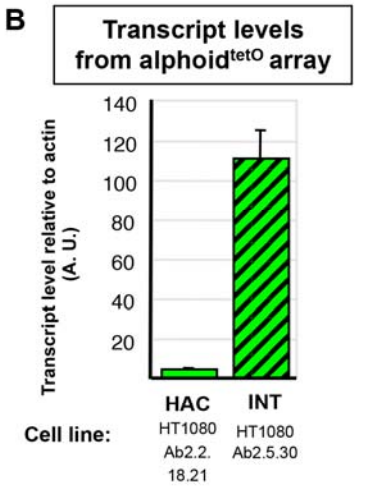
Martins et al. Supplemental Figure 1

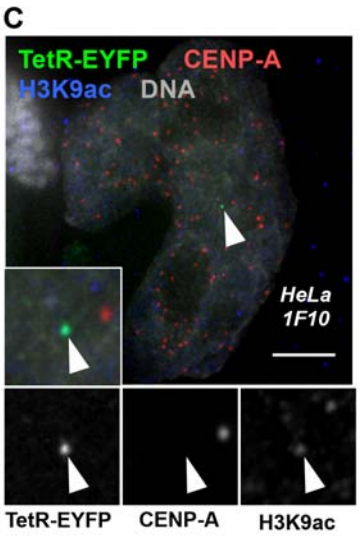


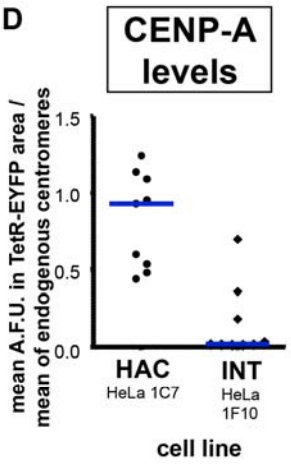
Martins et al. Supplemental Figure 2


A
H3K9me3
B
H2AK119ub1
C
Total Pol II
C'
Total Pol II
% of INPUT, IgG-subtracted and normalized to Cen21
αTetO
Bsr
BAC
Cen21
Sat2
MYT1
HAC
+Dox
5 days EZH2 tethering
HAC
αTetO (peri)centromeric repeats
BAC vector backbone region
Bsr active gene
Endogenous controls
Sat2 (chr.1) pericentric heterochromatin repeats
Cen21 (chr.21) centromeric repeats
MYT1 (chr.20) polycomb silenced gene

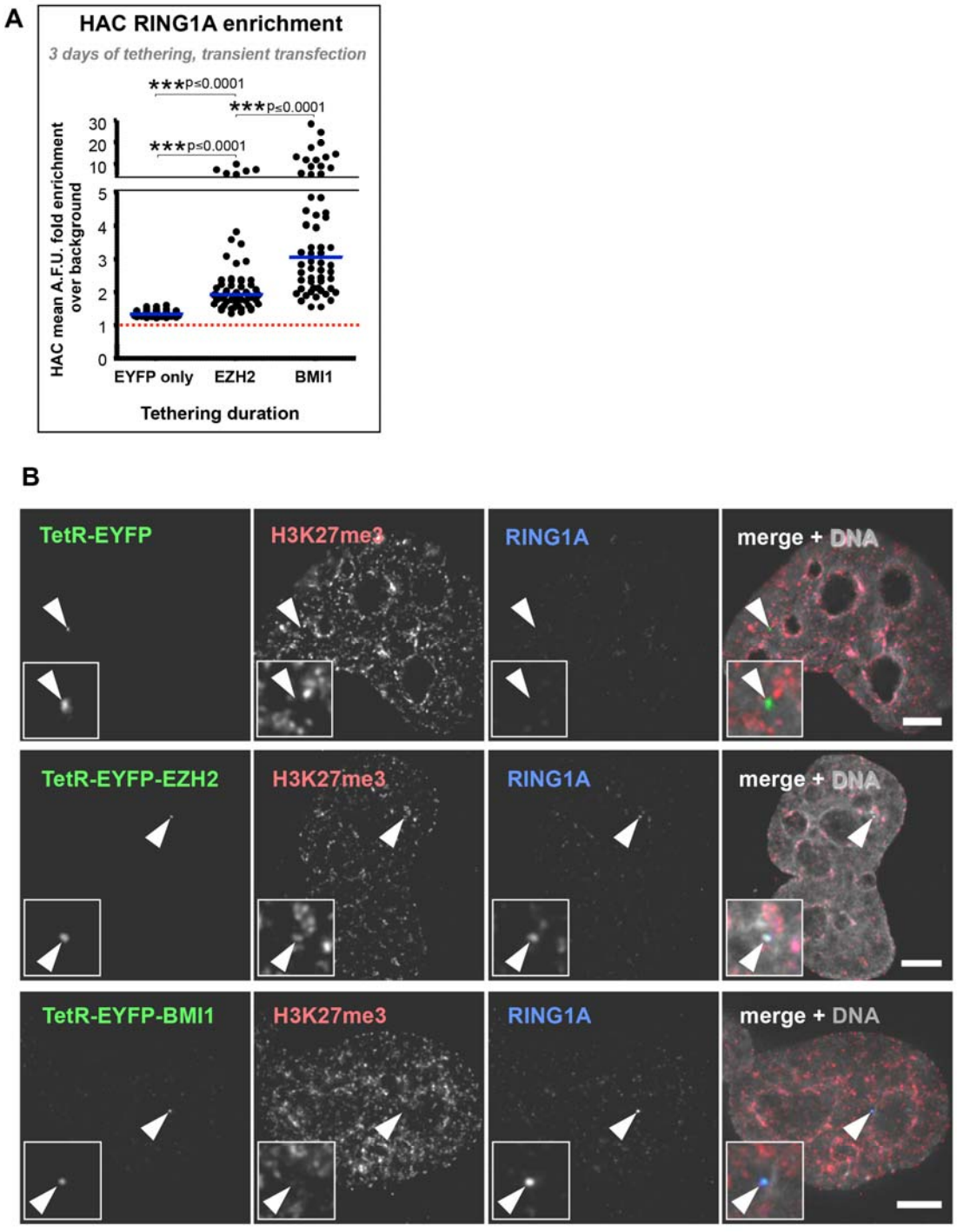








Martins et al. Supplemental Figure 4



Martins et al. Supplemental Figure 5