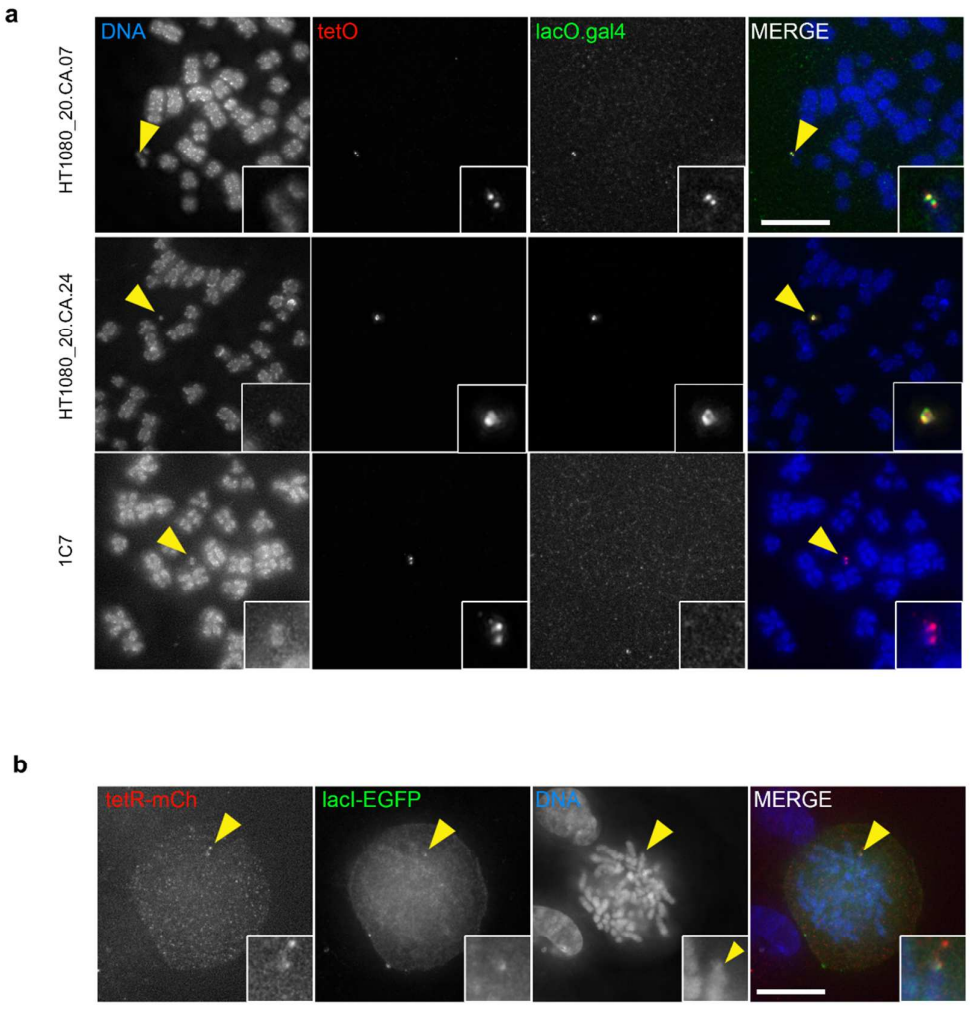
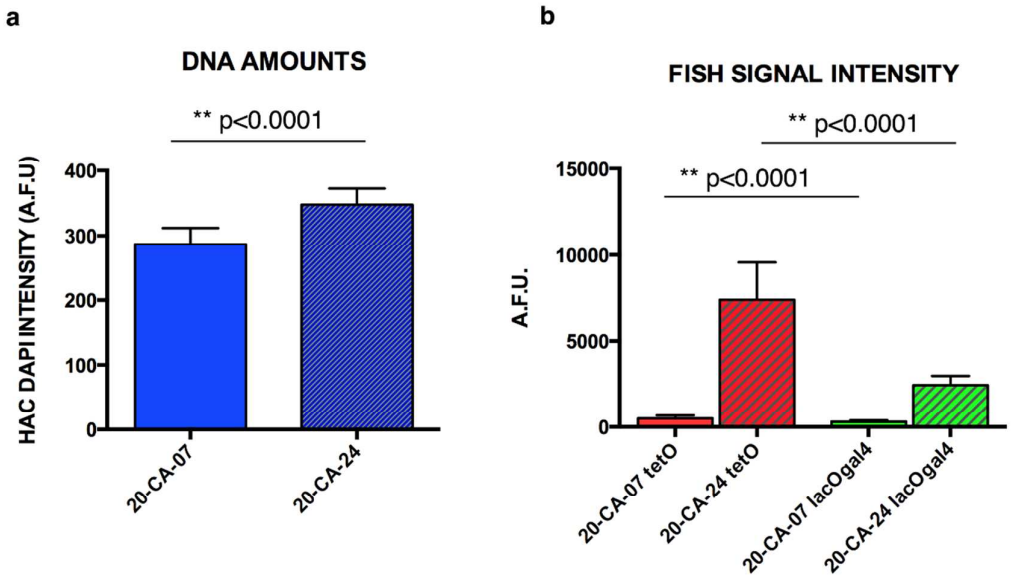
SUPPLEMENTARY FIGURES

Figure S1: The alphoid^{hybrid} HACs contain both alphoid DNA arrays (Related to Figure 5). (**a**) Representative images of FISH experiments using specific oligonucleotide probes that recognize tetO sequences (red; second panel) and lacO+gal4 sequences (green; third panel) in the indicated cell lines. (**b**) Representative image of a cell containing the alphoid^{hybrid} HAC tethered with tetR-mCherry (first panel) and lacI-GFP (second panel).



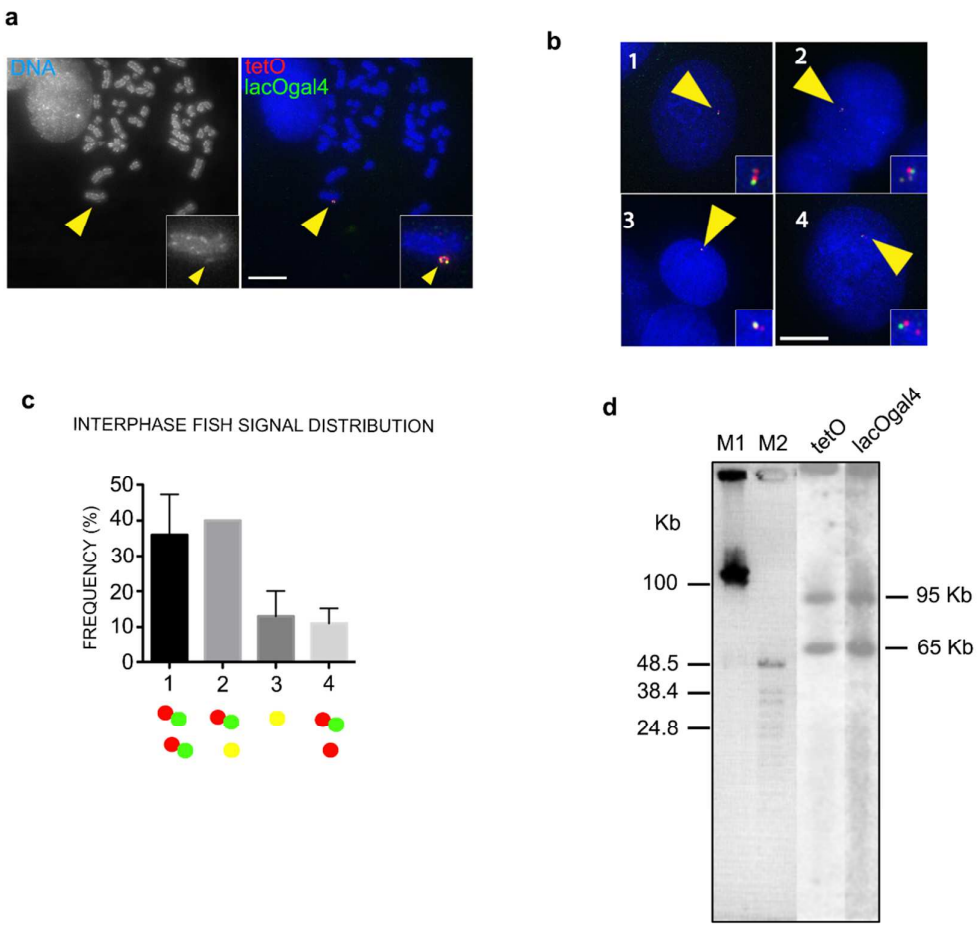
Supplementary Figure 1

Figure S2: The alphoid^{hybrid} HAC in clone 20.CA.24 is larger than in clone 20.CA.07. (Related to Figure 6). (**a**) Quantification of the alphoid^{hybrid} HAC-associated DAPI intensity in metaphase spreads. (**b**) Quantification of the alphoid^{hybrid} HAC-associated tetO (green bars) and lacO+gal4 (red bars) fluorescence signals in the indicated cell lines. n=16 metaphases per cell line; Mann-Whitney test <0.0001.



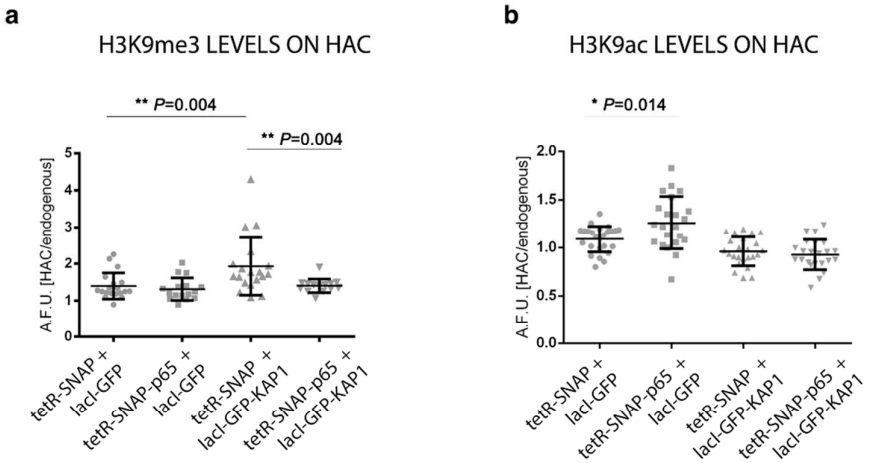
Supplementary Figure 2

Figure S3: Structural analysis of the hybrid HAC propagated in clone 20.CA.07. (Related to Figure 6). (**a**-**b**) Representative FISH images recognizing the tetO and lacO+gal4 domains of the alphoid^{hybrid} HAC in a metaphase chromosome spreads (**a**) and in interphase nuclei (**b**). (**c**) Quantification of the frequency of the indicated distribution of FISH signals in interphase nuclei of 20.CA.07 cells (2 independent FISH experiments). (**d**) Southern blot for the HAC clone 20.CA.07. Genomic DNA was digested with SpeI endonuclease and separated by CHEF gel electrophoresis (range 10-100kb). The transferred membrane was hybridized with radioactively labeled either tetO-specific or lacO+gal4-specific probes. Arrows indicate to the fragments of 95kb and 65kb in size that are specific to both probes.



Supplementary Figure 3

Figure S4: The alphoid^{hybrid} HAC shows epigenetically distinct centromeric domains (Related to Figure 8). Quantification of fluorescence signals of HAC-associated H3K9me3 (**a**) and H3K9ac (**b**) in clone 20.CA.20-5B10 cells expressing the indicated fusion proteins. Solid bars indicate the medians and error bars represent the standard error of the mean (s.e.m). N= two independent experiments per time point and staining. Asterisks indicate a significant difference (** p<0.001; Mann-Whitney test).



Supplementary figure 4