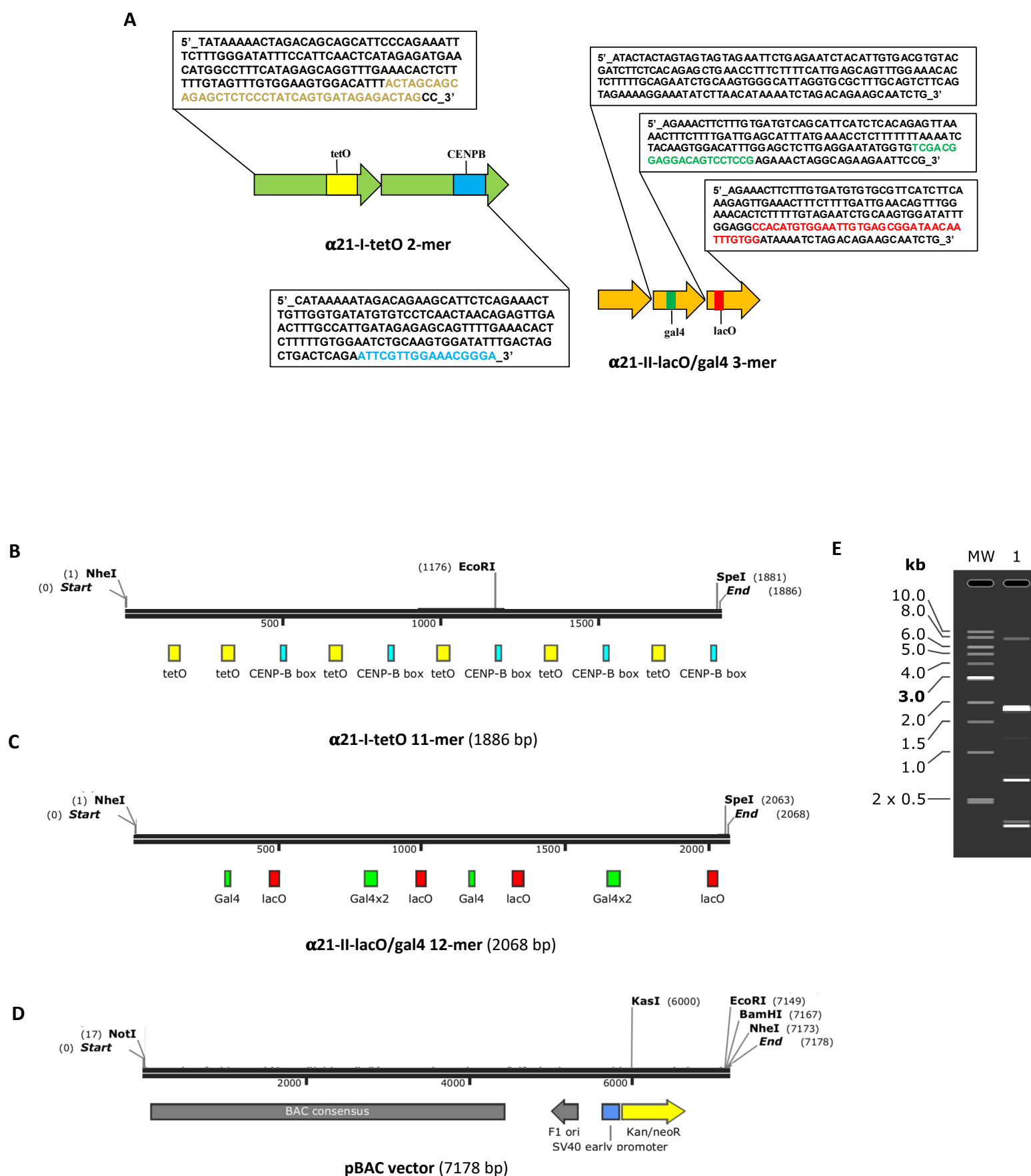


Supporting Information Available: Supplementary Figures.

Manuscript title: Analysis of complex DNA rearrangements during early stages of HAC formation

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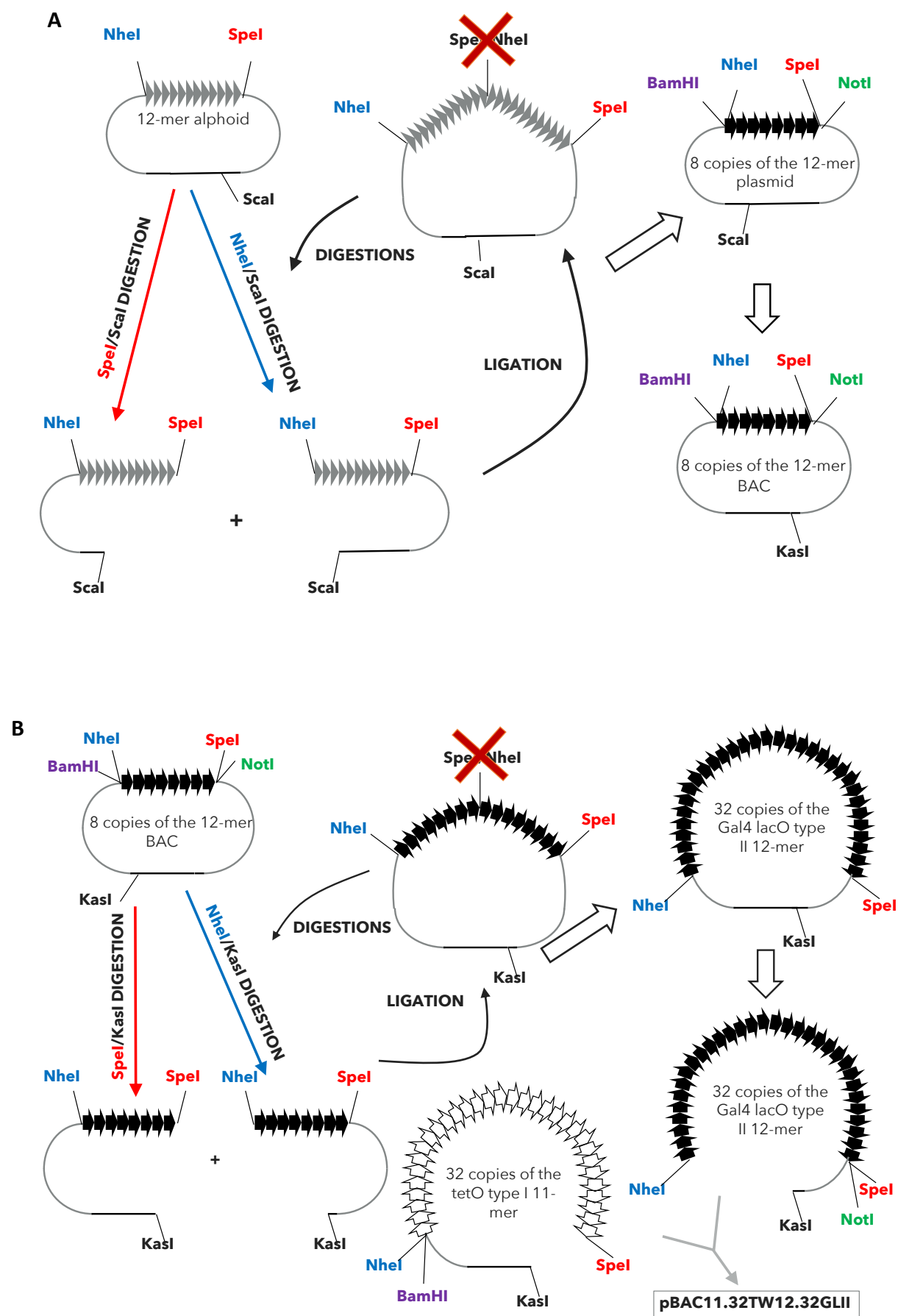
Fig. Supplemental 1



Supplementary Figure 1: Sequences of $\alpha 21$ -I^{TetO} array, $\alpha 21$ -II^{LacO/Gal4} array and BAC vector.

(A) Sequences of the $\alpha 21$ -I^{TetO} and $\alpha 21$ -II^{LacO/Gal4} high ordered repeats (HOR) synthetic DNA, adapted from⁴: both constructs are derived from a chromosome 21 alphoid type I and type II DNA; base pairs in color represent the corresponding feature on the synthetic DNA (colored boxes). (B) Map of $\alpha 21$ -I^{TetO} 11-mer (1886 bp) with CENP-B boxes (blue) and TetO (yellow), showing single cut restriction site relevant for the cloning. (C) Map of $\alpha 21$ -II^{LacO/Gal4} 12-mer (2068 bp) with LacO (red) and Gal4 (green) sequences, showing single cut restriction site relevant for the cloning. (D) Map of the BAC vector (7178 bp) showing single cut restriction site relevant for the cloning. (E) Pattern predicted in silico using Snapgene of pBAC11.32TW12.32GLII digested with EcoRI (lane 1) run on 1.2% agarose gel. Predicted fragments are 7499, 1880, 677, 370, 342, 340 and 339 bp. (MW 1 kb marker)

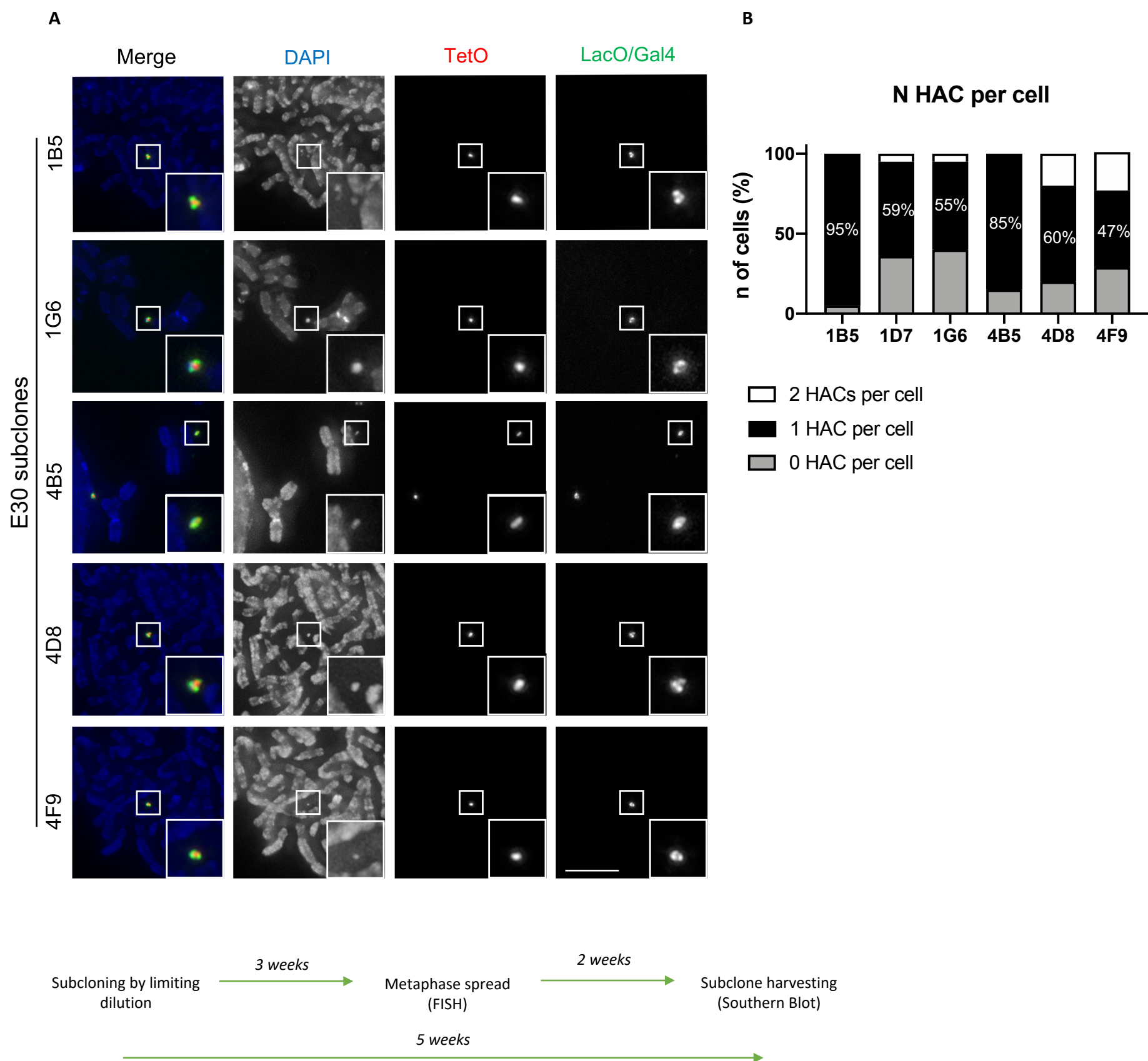
Fig. Supplemental 2



Supplementary Figure 2: Schematic of tandem ligation amplification.

(A) Multiple cycles of SpeI/ScaI and NheI/ScaI digestions and ligation are performed in plasmid to elongate the synthetic arrays (schematic only showed for $\alpha 21$ -II^{LacO/Gal4}12-mer, in grey). 8 copies of $\alpha 21$ -II^{LacO/Gal4}12-mer (in black) are then transferred in BAC. Relevant restriction sites are shown. (B) Multiple cycles of SpeI/KasI and NheI/KasI digestions and ligation are performed to elongate 8 copies of the $\alpha 21$ -II^{LacO/Gal4}12-mer (in black). 32 copies of the $\alpha 21$ -II^{LacO/Gal4}12-mer are ligated with 32 copies of the $\alpha 21$ -I^{TetO} 11-mer (in white) to form the final pBAC11.32TW12.32GLII. Relevant restriction sites are shown.

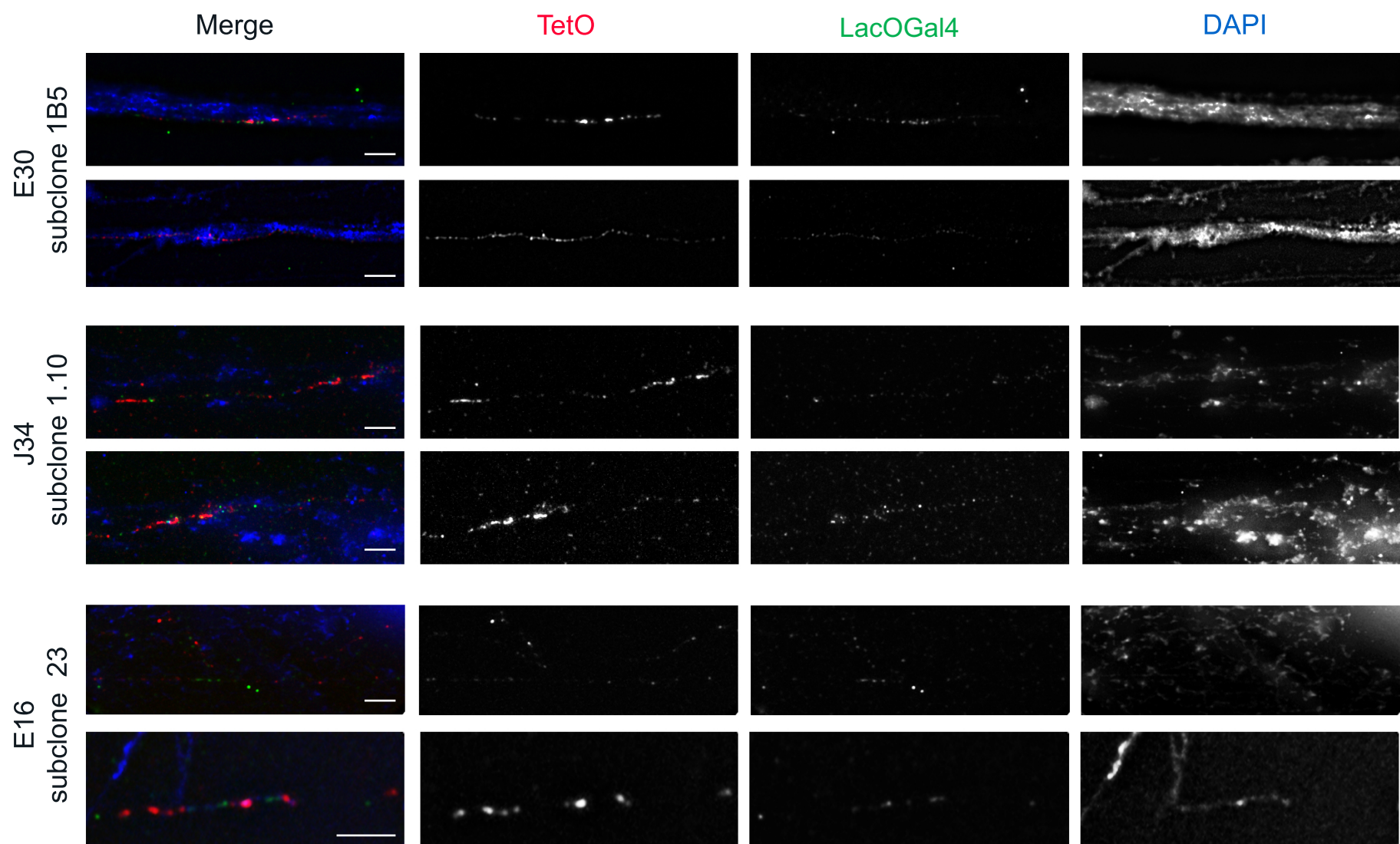
Fig. Supplemental 3



Supplementary Figure 3: Screening of HT1080 subclones by FISH.

(A) Representative pictures of FISH staining of 6 HT1080 subclones originated from clone E30: slides have been hybridized with DNA probes (TetO-dig/rhodamine α -dig antibody, Gal4-biotin and LacO-biotin/Fitc-streptavidin). DAPI stains DNA. Scalebar = 10 μ m. Timeline of the experiments performed on HT1080 subclones. (B) Number of metaphases (%) containing 0, 1 or 2 HACs for each subclone; 50 metaphases for each condition were analyzed. Percentages indicate the cells containing 1 HAC.

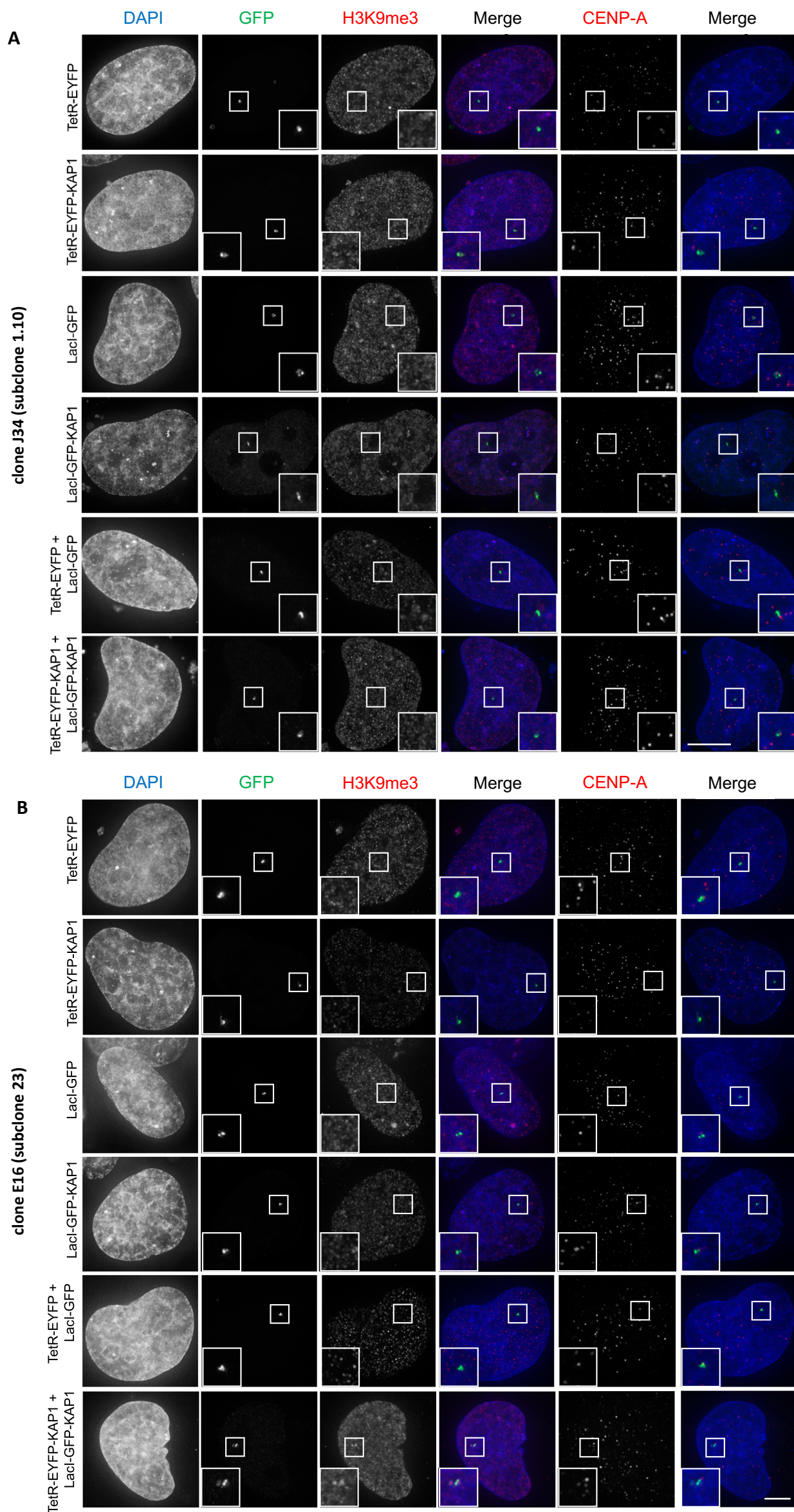
Fig. Supplemental 4



Supplementary Figure 4: **FISH on DNA fibers using TetO and LacOGal4 specific probes.**

Representative pictures of FISH staining of stretched DNA fibers from HT1080 clone E30 (subclone 1B5), clone J34 (subclone 1.10) and clone E16 (subclone 23). Fibers were hybridized with TetO (red) and LacOGal4 (green) specific probes; DAPI stains DNA. Scalebar = 5 μ m.

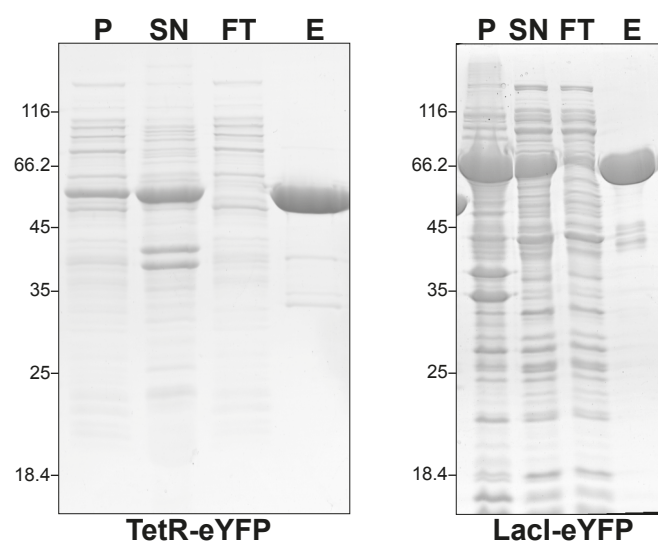
Fig. Supplemental 5



Supplementary Figure 5: CENP-A and H3K9me3 have been quantified after transfection with KAP1.

(A, B) Representative images of HT1080 subclones J34 1.10 (A) and E16 23 (B) transfected with the corresponding GFP-targeted protein (green) which localizes the HAC; cells are fixed, permeabilized and stained for α -H3K9me3 rabbit/TRITC α -rabbit and α -CENP-A mouse/CY5 α -mouse antibodies. DAPI stained the nuclei. First merge image from left represent the overlay between DAPI, GFP and H3K9me3; second merge image from left represent the overlay between DAPI, GFP and CENP-A. Scalebar = 10 μ m.

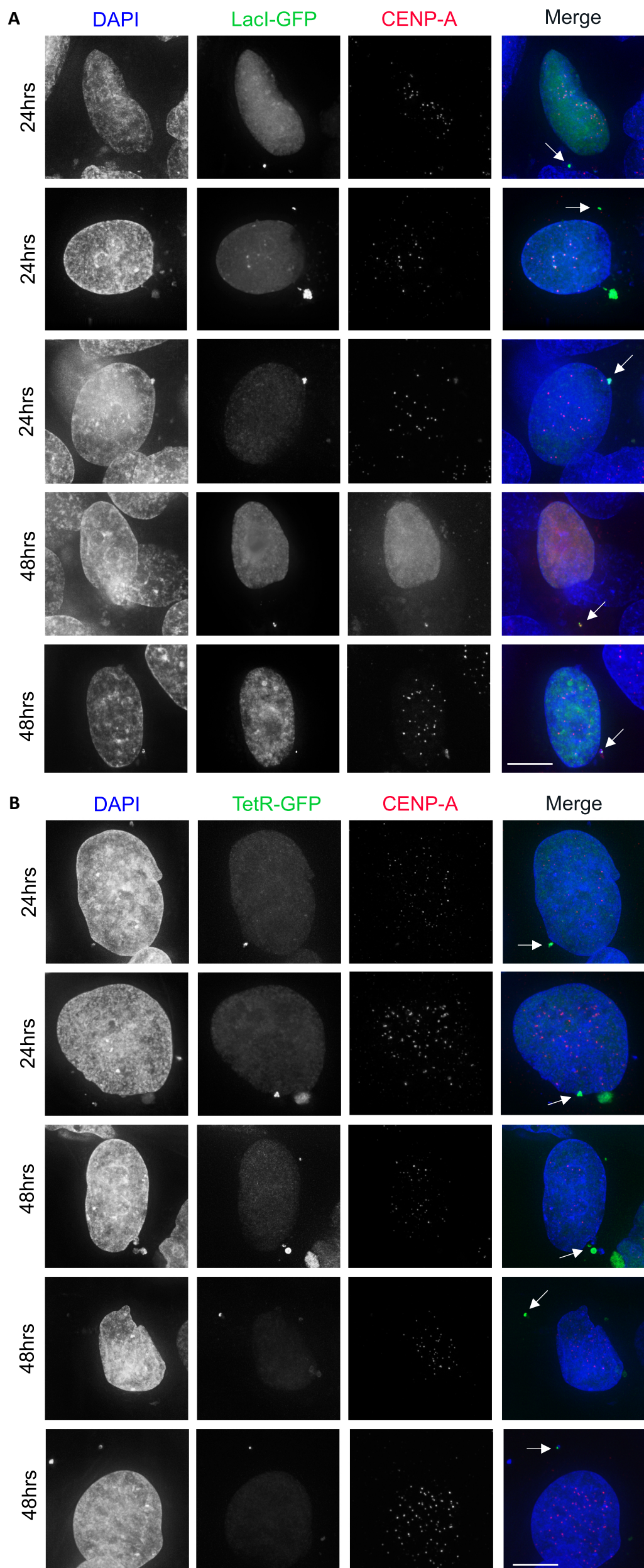
Fig. Supplemental 6



Supplementary Figure 6: SDS-PAGE analysis of TetR-eYFP and LacI-eYFP proteins after Ni-NTA affinity purification to assess protein purity.

P= Pellet (insoluble fraction), SN= Supernatant (soluble fraction, input of affinity purification), FT= Flow-Through (unbound fraction), E= Pool of elutions.

Fig. Supplemental 7



Supplementary Figure 7: HAC-seeding DNA is found in nanonuclei after transfection.

Representative images of indirect immunofluorescence on HT1080 (A) or HT1080 constitutively expressing TetR-EYFP (B) at the indicated timepoints after transfection with HAC-seeding DNA. The experiment was performed using HT1080 (where the synthetic DNA was localized by co-transfecting a plasmid encoding LacI-GFP) or HT1080 cells constitutively expressing TetR-EYFP. After fixation, cells were stained for CENP-A; DAPI stains the nuclei. White arrowheads indicate nanonuclei containing HAC DNA. Scalebar = 10 μ m.

HT1080

HT1080 TetR-EYFP