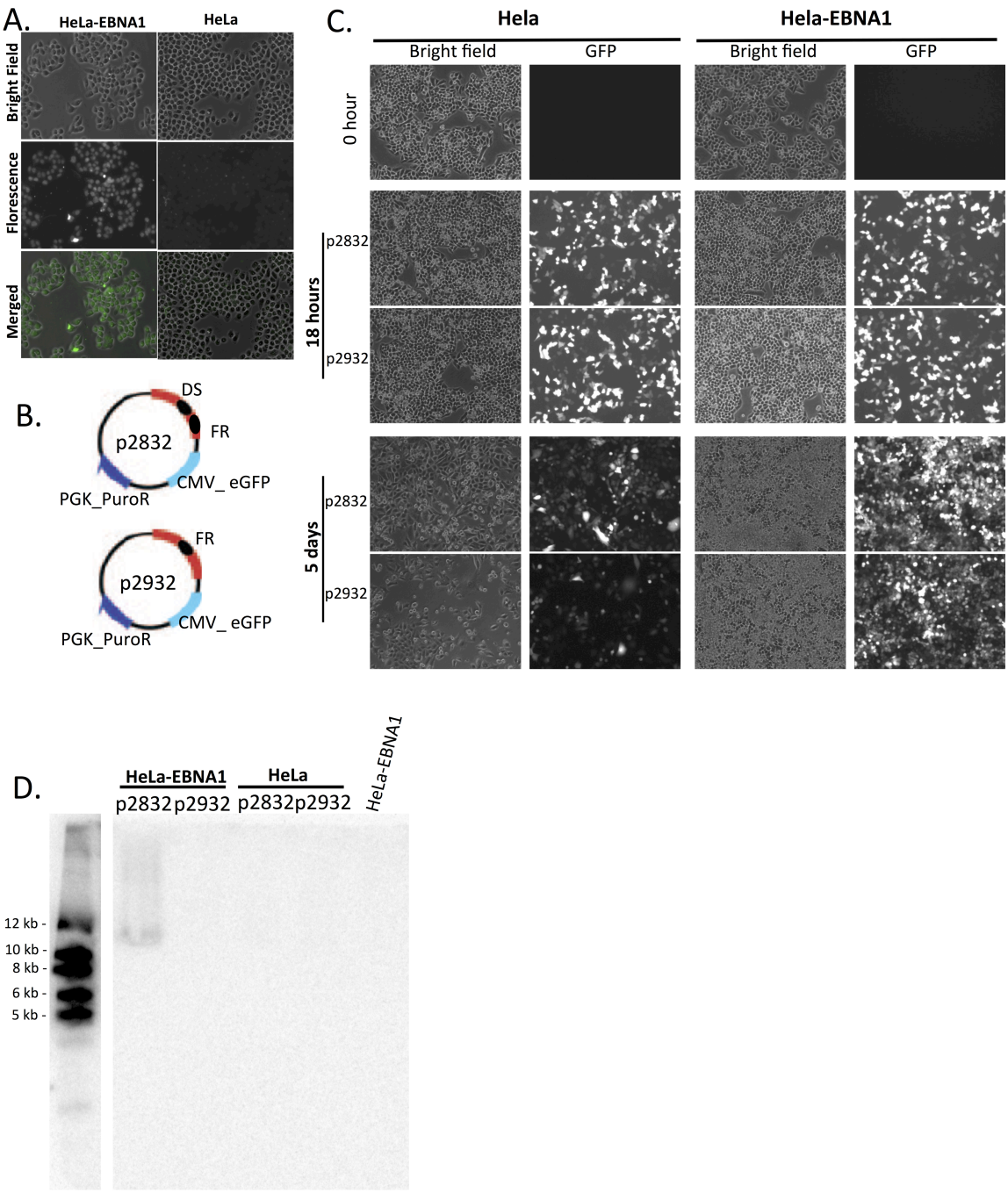
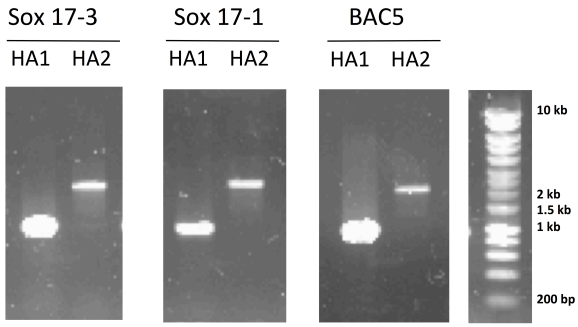
SUPPLEMENTARY DATA

Supplemental Figure S1. Hela-EBNA1 supports efficient retention of FR containing plasmids.



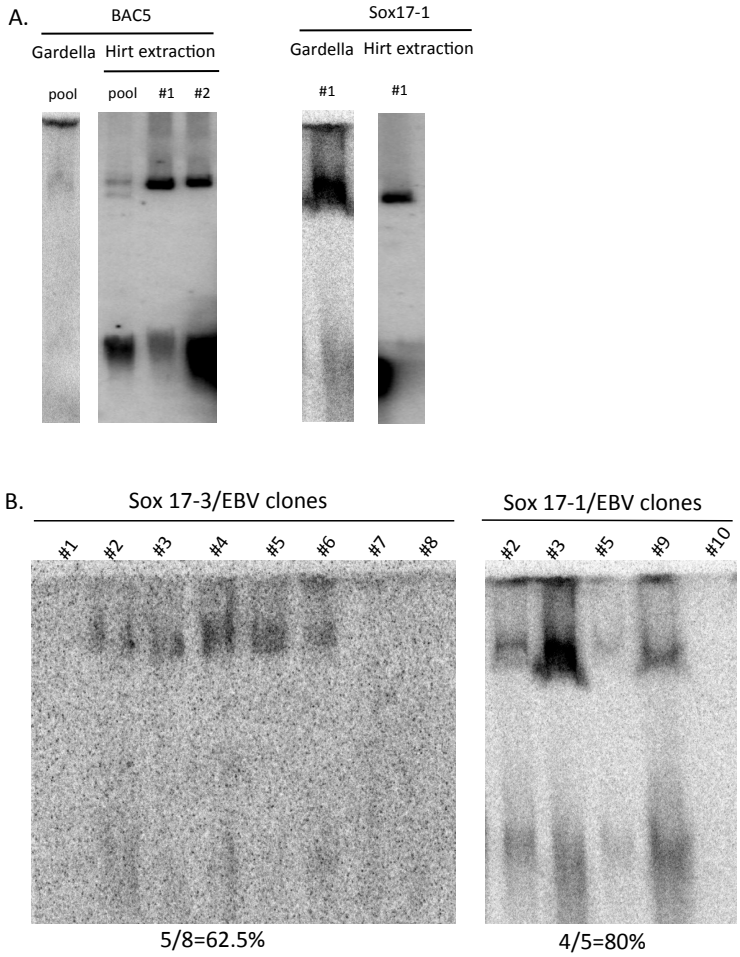
A. Immunofluorescence staining of EBNA1 in HeLa-EBNA1. B. Maps of plasmids used for HeLa-EBNA1 functional essay (kind gifts from M. Calos). FR, family of repeats, DS, Dyad symmetry; Both are components of the EBV latent origin (OriP), FR is responsible for even segregation of plasmid into daughter cells while DS is required for pre-RC (pre-replicative complex) assembly on small plasmids. DS is not necessary for the replication of native EBV or plasmids carrying >10kb segments of mammalian genomic DNA. C. Live cell imaging of HeLa or HeLa-EBNA1 cells transfected with p2832 and p2932. GFP images were obtained 18 hours after transfection and 5 days after puromycin selection. D. Hirt extraction results of p2832 (12 kb) or p2932 (9.5kb) transfected HeLa-EBNA1 cells.

Supplemental Figure S2. Colony PCR confirmation of FR cassette recombineering.



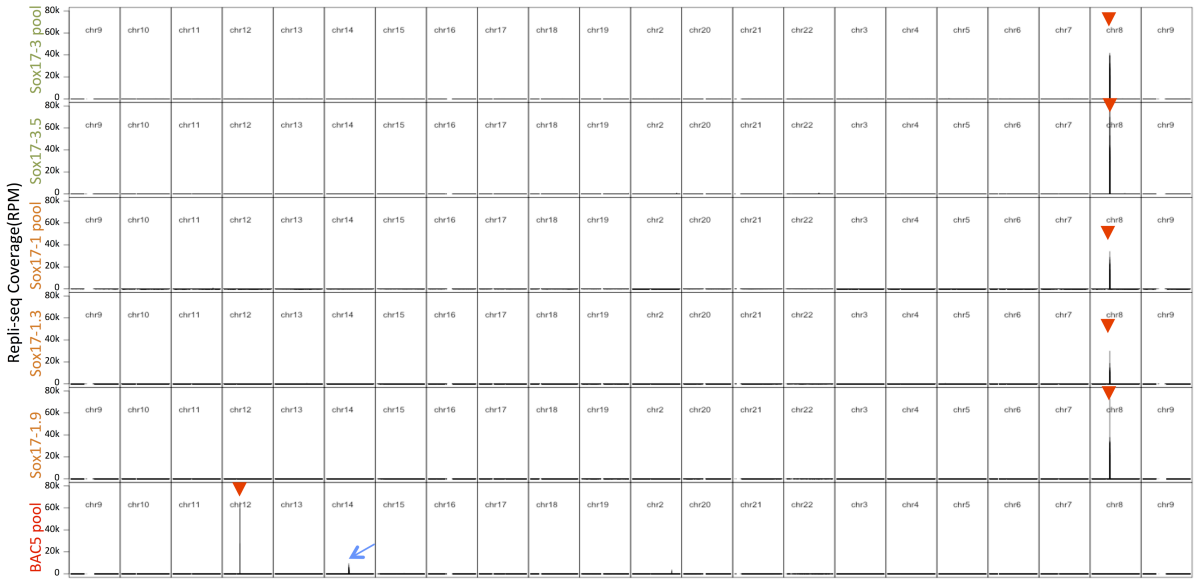
Colony PCR screening using 2 primer sets (HA1 and HA2) spanning across recombineering junctions to ensure complete insertion of FR cassette.

Supplemental Figure S3. Gardella Gel results of HeLa-EBNA1 E-BAC subclones



A. Comparison of Gardella Gel and Hirt extraction method. B. Gardella Gel results of HeLa-EBNA1 E-BAC subclones.Results indicate that 60-80% subclones retain extrachromosomal E-BACs. Subclone #5 and #6 of sox17-3/EBV, subclone #3 and #9 of sox17-1/EBV were used in the main text.

Supplemental Figure S4. Global 4C-seq profiles

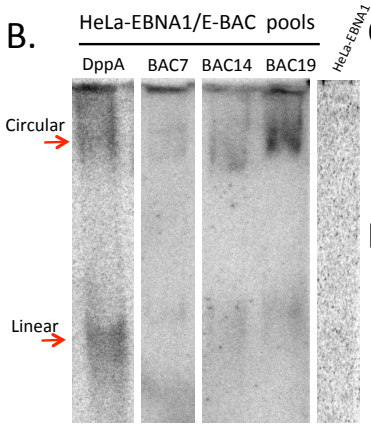


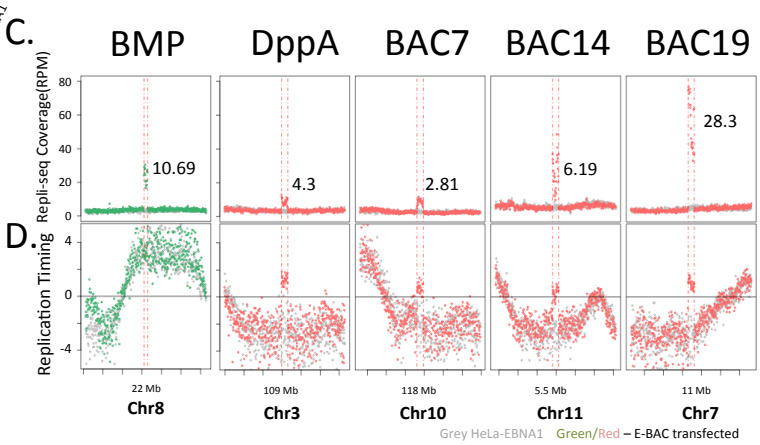
Results demonstrate little to no integration of E-BACs as the majority of the reads are restricted to the E-BAC corresponding regions, as indicated by red triangles. A potential integration site in HeLa-EBNA1/BAC5 pool is indicated by the blue arrow.

Supplemental Figure S5. Additional E-BAC replication timing.

A.

Name	BAC ID	CHR	Hg19_Start	Hg19_End	Length	Chromosome RT
BMP	RP11-747M1	chr8	22051650	22145837	94187	Early CTR
DppA2-2	RP11-708A5	chr3	108985191	109148512	163322	Late CTR
BAC7	RP11-96N16	chr10	117806914	117972936	166023	Late CTR
BAC14	RP11-57F14	chr11	5406818	5566563	159746	Late CTR
BAC19	RP11-170F19	chr7	10649849	10809019	159171	Late TTR



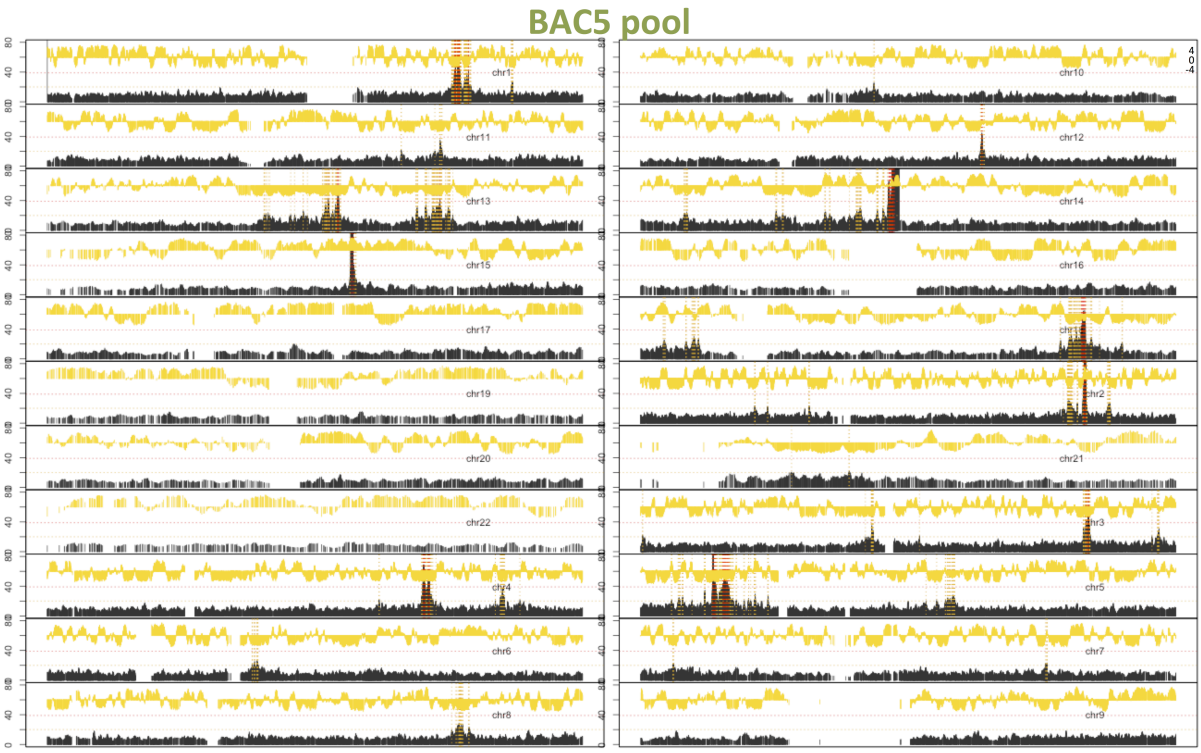


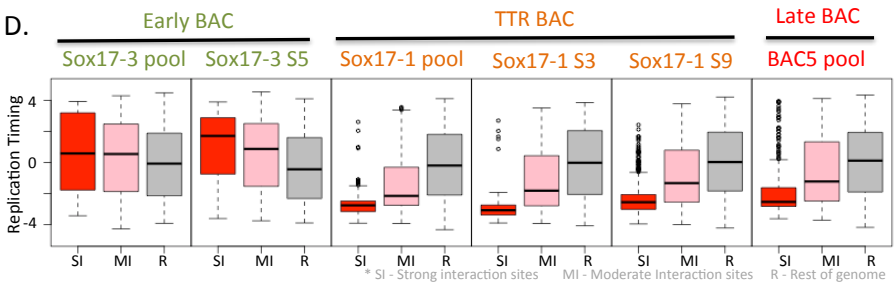
A. Summary of additional E-BACs transfected into HeLa-EBNA1 cells. B. Gardellla gel results of E-BAC transfected cells. C. Repli-seq read coverage for E-BAC regions. The numbers on each plot represent copy number estimation of E-BACs. C. E-BAC cell lines repli-seq profiles, as in Figure 4B. Each data point is the log2 ration for reads within a 6kb window. HeLa-EBNA1 cells without E-BAC transfection were used as control (grey data points). Vertical pink dashed lines indicate the human chromosomal map positions of the segments contained in each BAC. RT of the 18kb bacterial

sequences of each E-BAC (pBAC3.6 E-BAC backbone) was plotted separately and indicated as horizontal lines next to the chromosomal regions.

Supplemental Figure S6. Exemplary 4C-seq profiles.

A-C

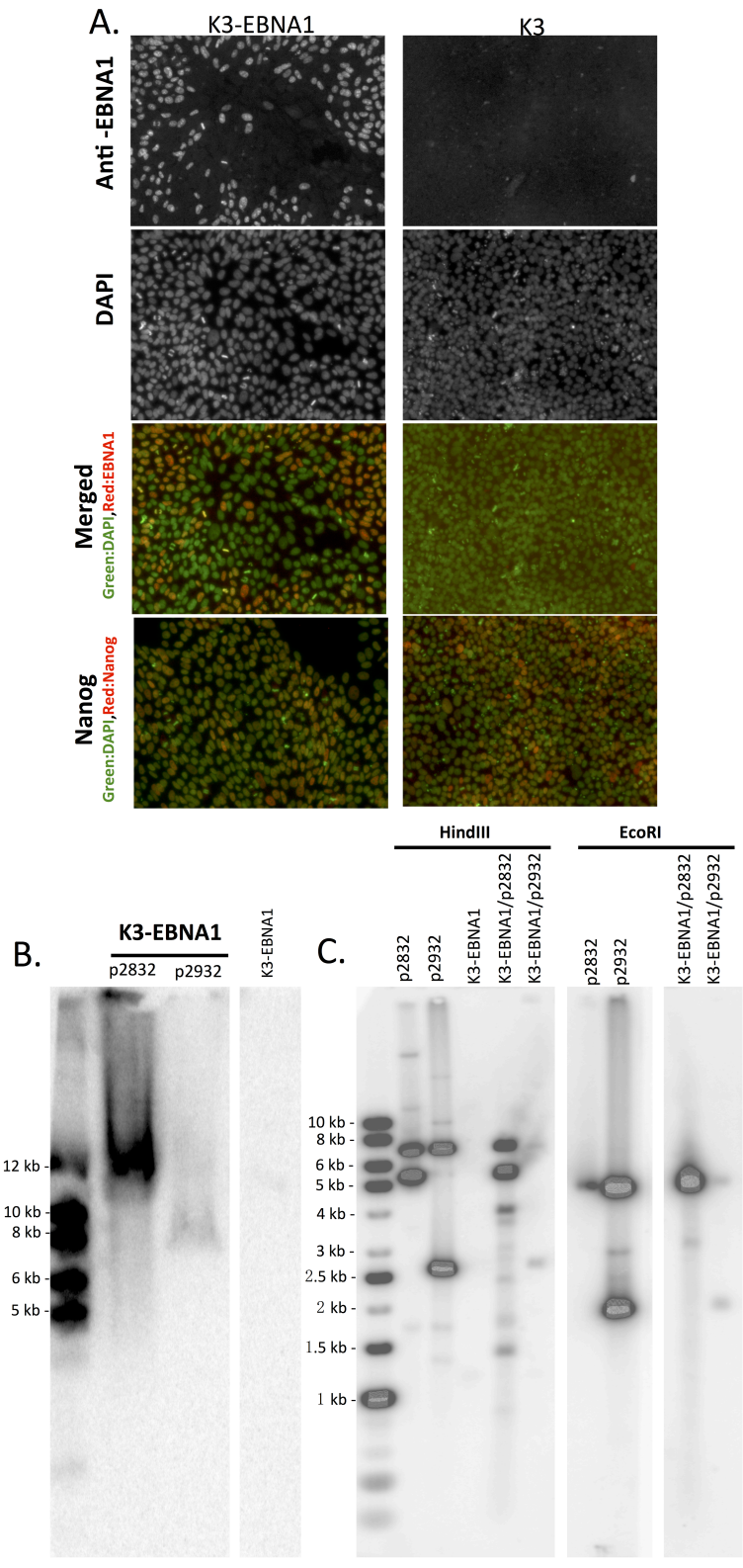




Cutoff: SIs: 34-400RPM ; MIs: 13-34RPM

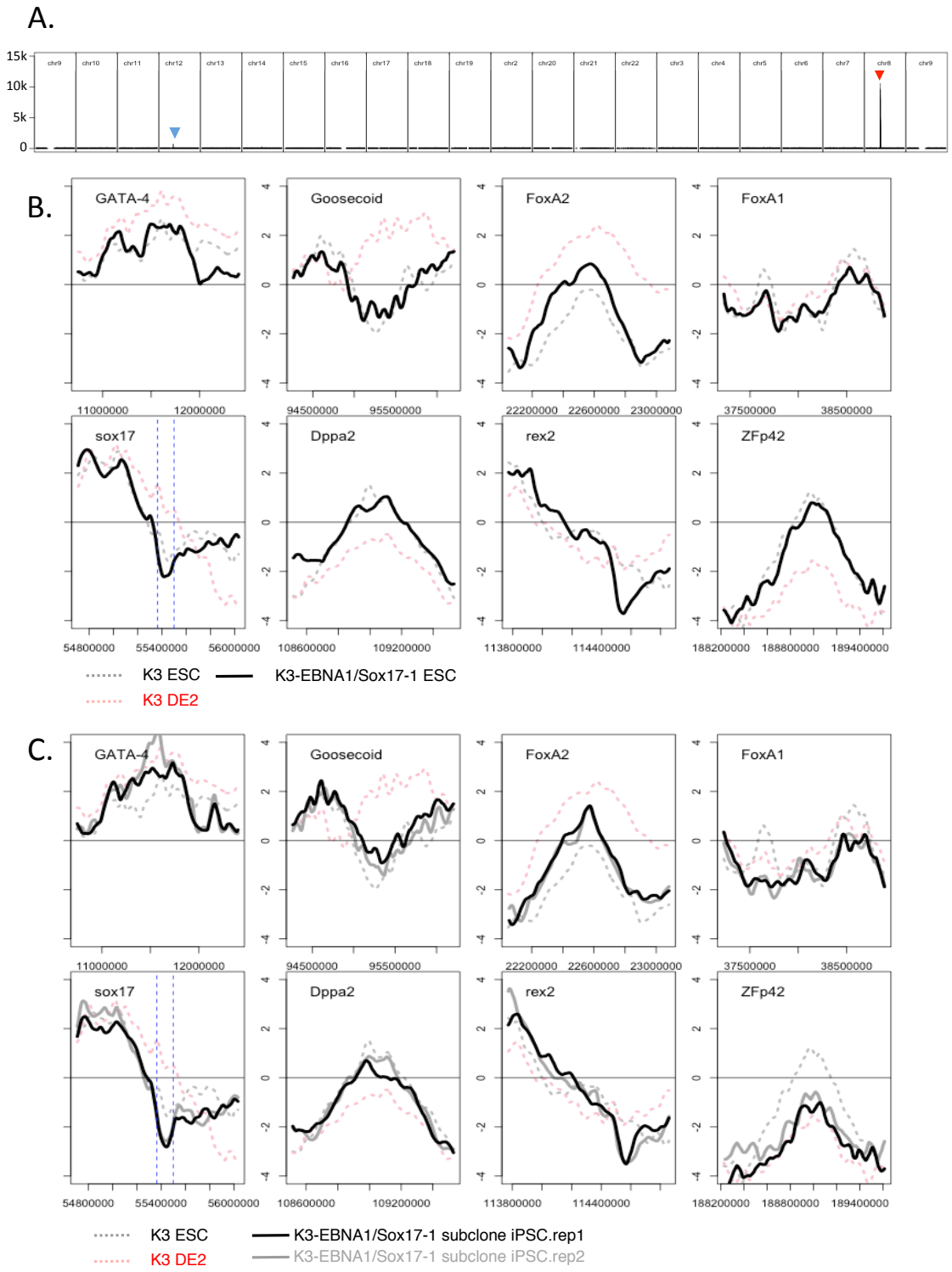
A-C: These figures illustrate the definition of Strong Interaction Sites (SIs) and Moderate Interaction sites (MIs) in three E-BAC transfected pools (sox17-3, sox17-1, BAC5). Genome-wide 4C-seq and corresponding repli-seq profiles for chr12 are plotted as black or yellow histogram, respectively (BAC region removed). SIs and MIs are marked with red and with deep yellow dashed lines, respectively. D. Compartment analysis using different cutoffs (SIs: 34-400RPM; MIs: 13-34RPM), shows that interaction preferences of SIs and MIs are conserved.

Supplemental Figure S7. K3-EBNA1 supports efficient retention of FR containing plasmids.



A. Immunofluorescence staining of EBNA1 in K3-EBNA1. B. Hirt extraction results of p2832 or p2932 transfected K3-EBNA1 cells. C. HindIII or EcoRI restriction mapping of p2832 or p2932 purified from transfected K3-EBNA1 cells. Plasmids p2832 or p2932 were prepared from E.coli was used as control.

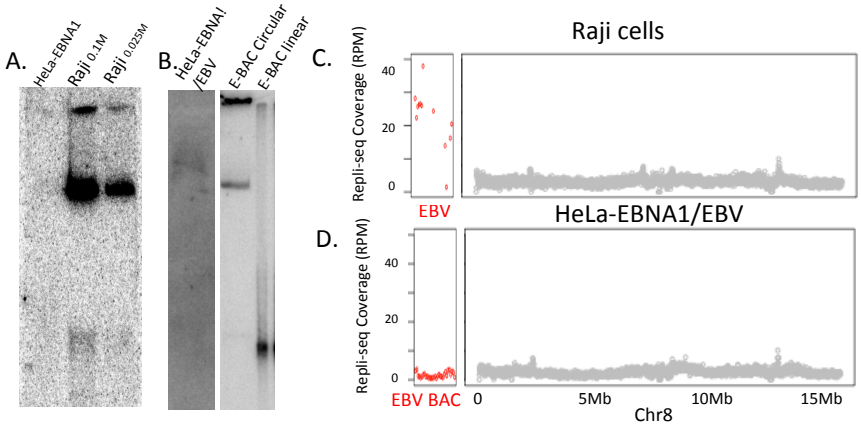
Supplemental Figure S8. Global 4C-seq profiles of Sox17-1 E-BAC transfected K3-EBNA1 pool and their unaltered pluripotency RT signatures.



A. 4C-seq result of Sox17-1 E-BAC transfected K3-EBNA1 pool shows the extra-chromosomal status of E-BACs as the majority of the reads are restricted to the E-BAC corresponding regions (indicated

by red triangle). The blue triangle points to the signal at the BAC5 corresponding position caused by contamination of 1% of HeLa-EBNA1/BAC5 population, which is likely result from the residues left in the dounce homogenizer during the fixation step. B&C. RT signatures of pluripotency markers in Sox17-1 E-BAC transfected K3-EBNA1 pool (B) and the subclone (C). RT of K3 iPSC and definitive endoderm 2 (unpublished) are plotted as dashed lines for comparison.

Supplemental Figure S9. Episome detection and copy number of EBV in Raji cells and EBV BAC in HeLa-EBNA1 cells.



A,B. Gardellla gel detecting episomal EBV genome in Raji cells and EBV BAC in HeLa-EBNA1. C,D. Read coverage of EBV genome in Raji cells and EBV BAC in HeLa-EBNA1/EBV (red). Coverage from 15Mb of chr8 is plotted for comparison.

SUPPLEMENTARY methods

Immuno-fluorescence

Cells were fixed in 4% formaldehyde for 10min at RT, permeabilized with 0.1% Triton in PBST, blocked with 5% goat serum, and stained with mouse anti-EBNA1 (Millipore MAB8173 1:50); washed 3 times using PBS, and then stained with Rabbit anti Mouse IgG (049K4841, Sigma,1:400), and finally FITC-conjugated Mouse anti Rabbit IgG, 1:400. Images were obtained with Nikon Eclipse Ti-U microscope. For EBNA1 staining: Primary antibody: sc-57719, Santa Cruz, 1:50; Secondary antibody: Alexa 594- rabbit anti mouse, 1:200. For Nanog staining: Primary antibody: RCAB004P-F, Reprocell, 1:200; Secondary antibody: Alexa 488- goat anti rabbit, 1:500.

HeLa-EBNA1 functional assay

HeLa or HeLa-EBNA1 cells were transfected with 1ug of p2832 or p2932 using Fugene HD reagent. Two days post transfection, 2ug/ml puromycin was added to media. Images were obtained with Nikon Eclipse Ti-U microscope.

Colony PCR

Colony PCR was performed using primer sets: HA1: 5'-ATCCTTCTATAGTGTCACCTA & 5'-GTAAACTCCTCTTCAGACC; HA2: 5'-GAGACGCTGTCGAACTTT & 5'-CTACGATTCCATCAATGCC, using standard PCR conditions.

Cell culture and transfection

Raji cells were maintained in RPMI media supplemented with 10% fetal bovine serum. EBV BAC (3991.1, gift from Wolfgang Hammerschmidt lab) was purified using Qiagen midi kit, and electroporated into HeLa-EBNA1 cells, which are expanded under 0.2mg/ml hygromycin B selection.