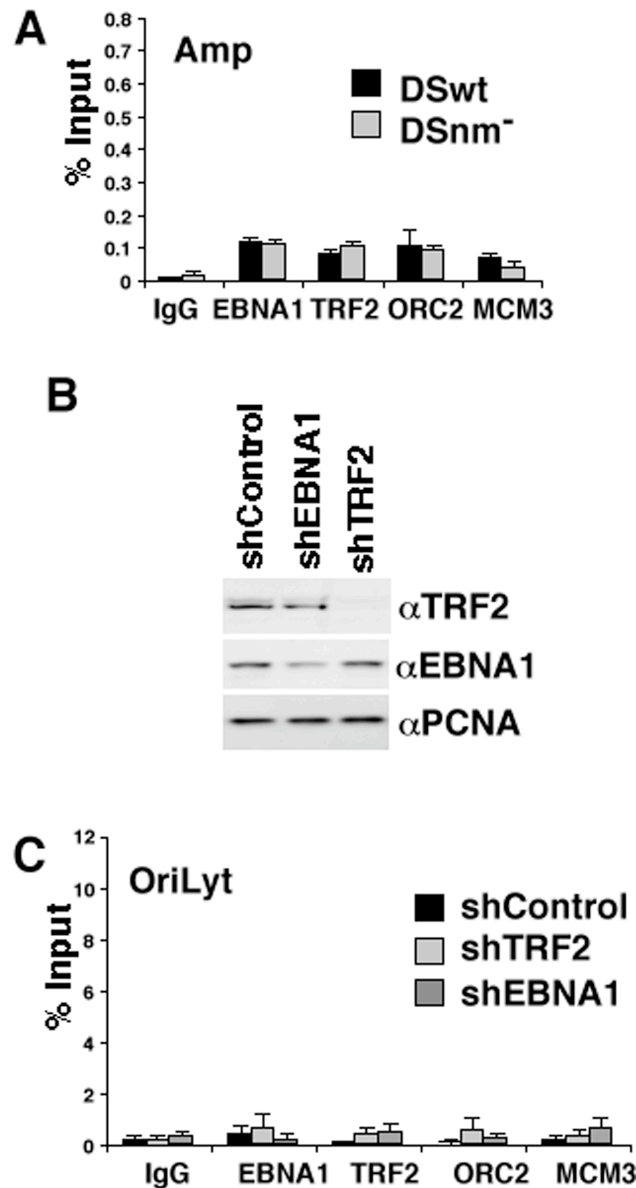
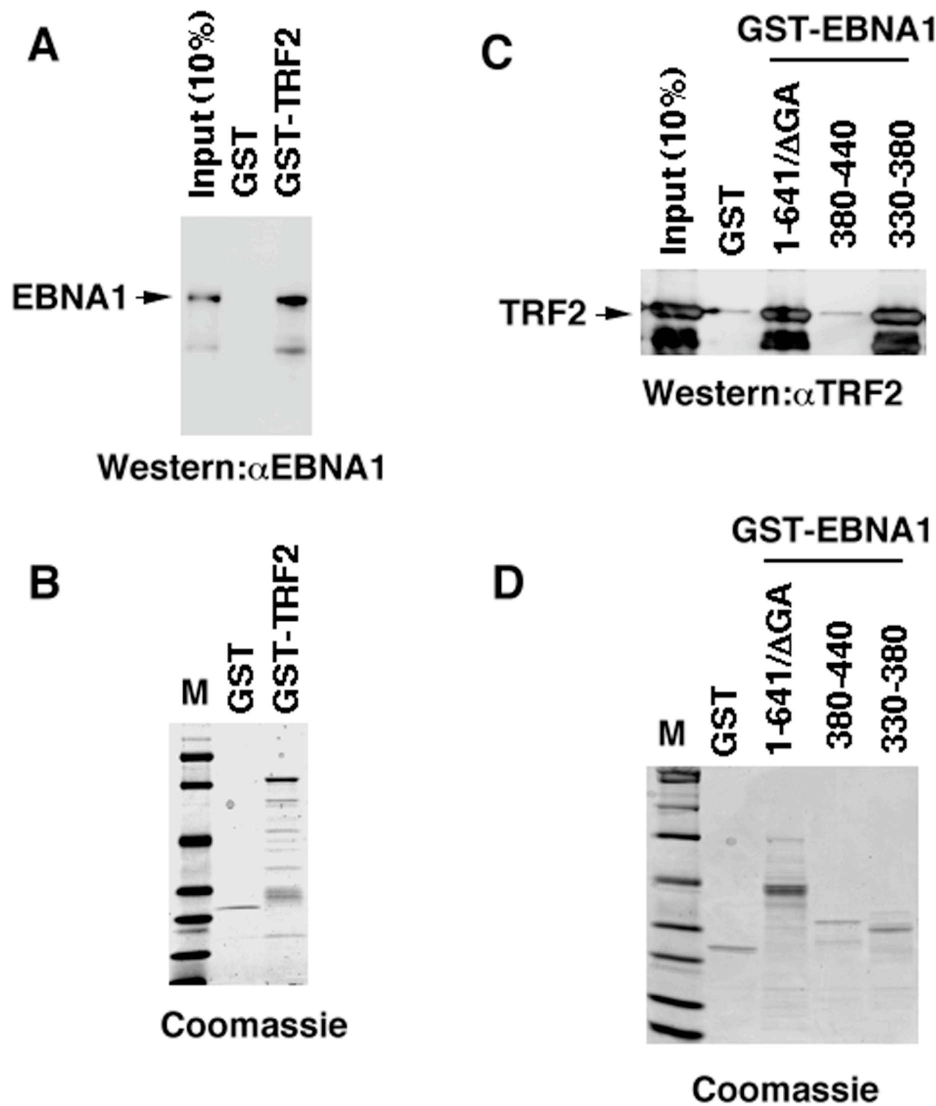
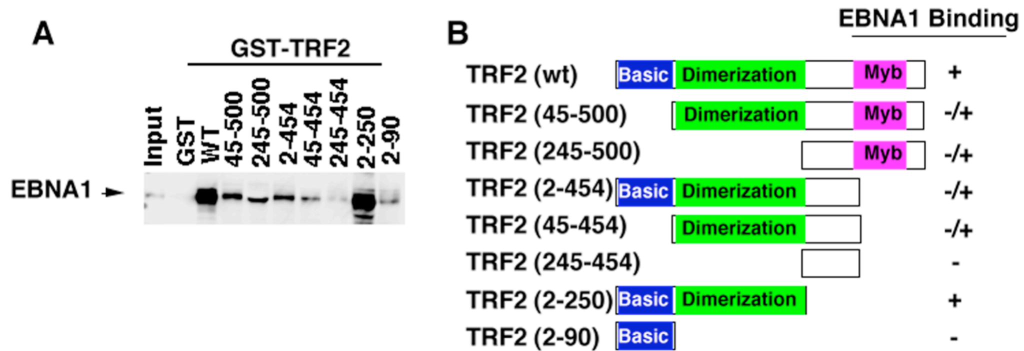


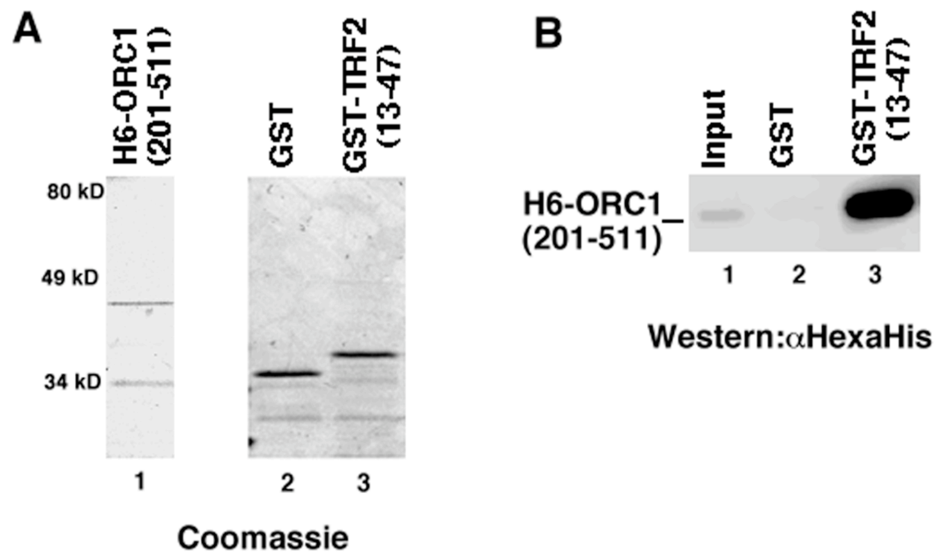
Supplementary Figure 1. A) Control ChIP assay for the experiment described in Fig 2B, analyzed with primers specific for the ampicillin gene on the transfected OriP-containing plasmid. B) Western blot of EBV ZKO-293 cells transfected with shTRF2, shEBNA1, or control vectors and analyzed for TRF2, EBNA1, or PCNA as described for Fig. 2C. C) Same as in Fig 2C, except that ChIP DNA was analyzed with primers specific for the control OriLyt region of the EBV genome.



Supplementary Figure 2. The EBNA1 RGG-motif (aa 330-380) can bind TRF2 in vitro. Partially purified EBNA1 protein from baculovirus was assayed for binding to purified GST or GST-TRF2. EBNA1 protein was detected by Western blot with anti-EBNA1 antibody. B) Coomassie stain of purified GST or GST-TRF2 proteins, as indicated above each lane. Marker lanes (M) are indicated. C) GST, GST-EBNA1 (1-641/DGA), GST-EBNA1 (380-440), or GST-EBNA1 (330-380) were assayed for binding to purified TRF2. D) Purified GST fusion proteins used in panel C are shown by Coomassie staining of SDS-PAGE.



Supplemenal Figure 3. The TRF2 dimerization domain is required for EBNA1 interaction in vitro. GST-TRF2 fragments described in Figure 4 were assayed for binding to purified EBNA1 in vitro. A) Western blot of EBNA1 input (10%) or fractions bound to GST fusion proteins as indicated above each lane. B) Summary of GST-TRF2 fusions binding to EBNA1. We found evidence that the TRF2 dimerization domain between aa 45-250 contribute to EBNA1 binding, but this is further stabilized by additional TRF2 regions.



Supplementary Figure 4. Direct interaction between ORC1 (aa201-511) and TRF2 (aa 13-47). A) Proteins encoding H6-ORC1 (aa 201-511) and GST or GST-TRF2 (aa 13-47) were purified and analyzed by Coomassie Blue staining of SDS-PAGE. B) H6-ORC1 (aa 201-511) was assayed for binding to GST or GST-TRF2 (aa 13-47) and detected by Western blotting with antibody specific for the hexahistidine tag.

Supplemental Methods

Cells and Plasmids

HeLa N2, 293, and D98/HR1 cells were cultured in Dulbecco's modified Eagle's medium supplemented with 10 % fetal bovine serum and antibiotics in a 5 % CO_2 incubator at 37 °C. EBV positive Raji, and ZKO-293 cells (kindly provided by H. Delecluse) were cultured in RPMI 1640 medium supplemented with 10 % fetal bovine serum and antibiotics in a 5 % CO_2 incubator at 37 °C. Stable cell line expressing Flag-ORC2 was generated from 293 cells and maintained under puromycin selection (2.5 μg/ml). DSwt and DSnm$^-$ were described previously (Deng et al., 2003). DS(1+2) and DS(1+2)nm$^-$ were generated by cloning a 72 bp oligonucleotide DS(1+2) or DS(1+2)nm$^-$ fragment into *Bam HI*/*HinD III* site of pBluescript-KS vector. Mammalian cell expression plasmid pCMV-FLAG-TRF2ΔBΔM (aa 45 to 454) was described previously. pCMV-FLAG-TRF2 (aa 1 to 500) and pCMV-FLAG-TRF2ΔB (aa 45 to 500) were cloned into *Hind*III-*Bam*HI sites of p3XFLAG-CMV (Sigma). PCMV-FLAG-ORC2 was generated by PCR amplification of ORC2 cDNA and cloning into the *BglII-Asp*718 site of p3xFLAG-CMV. Small hairpin RNA targeting TRF2 or EBNA1 was cloned into the pENTR/D-TOPO vector (Invitrogen) containing the U6 promoter, as was described previously (Deng et al., 2003). GST-hRAP1, -TRF1, -TRF2, and ORC1 truncation mutants were generated by PCR-based site directed mutagenesis in the pGEX-2T vector (Amersham). All transfections were performed by the use of Lipofectamine 2000 reagent using 2-5 μg of plasmid DNA for 1.5 X 10^6 cells, which were seeded in 6-cm plates 12 to 16 h prior to transfection. ORC1 subdomains containing amino acid residues 1-200,

201-511, 512-862 were subcloned as *BamHI-HinDIII* fragments into pRSETA in frame with hexahistidine amino-terminal tag (Invitrogen).

Western blotting

Primary antibodies to EBNA1 (Advanced Biotechnologies, Inc), FLAG (Sigma M2 and polyclonal), PCNA (Santa Cruz Biotechnology), TRF2 (IMGENEX), and ORC2 (BD Pharmingen) were used according to manufacturer's specifications. Rabbit polyclonal antibodies to EBNA1, TRF1, hRap1and TRF2 were generated against recombinant protein.

GST-pulldown assay. The GST-fused proteins were expressed in BL21 (DE3) Gold by IPTG induction for 1 hr at 37 ºC. Pellets from 100 ml culture were resuspended in 5 ml PBS 150 (0.1 M phosphate (pH 7.4), 150 mM NaCl supplemented with 0.1% NP-40, 1 mM PMSF, 1 mM EDTA, 1 mM DTT, bacterial protease inhibitors cocktail (Sigma)) then sonicated for 4x15 seconds in a Branson 250 sonicator, at 30% output. The extract was spun for 30 minutes at 15,000 rpm (SS34 rotor). GST-fused proteins were purified by incubating 25 μl Glutathione Sepharose (Amersham) per 250 μl soluble extract for 2 hr at 4 ºC. Unbound proteins were washed away with PBS 500 (supplemented with NaCl to 500 mM) with three washes, 5 minutes each. GST-Sepharose beads were washed once with PBS 150. The purity of each protein was checked by Coomassie Blue staining of SDS-PAGE. Purified GST proteins bound to beads were incubated with 100 μl HeLa or Raji nuclear extract for 1 hr at 4 ºC. Alternatively, purified GST protein was incubated with purified hexa-histidine tagged ORC1 (201-511) in PBS150 for 1 hr at 4°C. Beads

were washed 3 times with PBS 150 then boiled for 15 minutes at 100°C. Samples were loaded on 8-16% Tris-glycine gel (Invitrogen) and analyzed by Western blot with indicated antibodies.

CoImmunoprecipitation Assay

For coimmunoprecipitations, approximately 2 x 10^7 Raji cells were collected and then resuspended in 2 ml cold D75 buffer (20 MM HEPES (PH 7.9), 20 % glycerol, 0.2 mM EDTA, 75 mM NaCl) supplemented with 0.05 % Igepal, 1 mM phenylmethylsulfonyl fluoride (PMSF), 1 mM dithiothreitol (DTT), and protease inhibitor cocktail at 4 °C. After 10 min on ice, lysates were sonicated and centrifuged for 10 min at 14,000 rpm. Supernatants were collected and pre-cleared with protein G-sepharose beads preblocked with BSA for 1 h, and then incubated overnight with indicated antibodies with rotation at 4 °C. The immune complexes were collected with protein G-sepharose beads for 2 h at 4 °C; beads were washed 4 times with cold D75 buffer and resuspended in 50 µl 2 x Laemmli buffer. The sample (25 µl) was subject to 4-20% Tris-glycine PAGE gel followed by Western blotting analysis.

Chromatin Immunoprecipitation Assay

ChIP assays were performed as described previously (Chau and Lieberman, 2004). Real-time PCR analysis of ChIP DNA was performed with ABI Prism 1000 Sequence Detection System (Applied Biosystems) and primers designed with computer software (Primer Express version 2.0, Applied Biosystems). Primer sequences are available upon request.

ChIP assays were performed in triplicate and quantified by the standard curve method using the ABI software.

DNA replication assays and DNA affinity

DNA replication for OriP and DNA affinity purification assays were performed as described previously (Deng et al., 2002). All DNA replication assays were performed in duplicate with two independent transfections. Each experiment was repeated at least three times. The quantitation was an average value for all experiments, and standard deviations were less than 10% of the average value. DNA templates for DNA affinity were amplified with one 5'-biotin labeled primer and PCR. The FR fragments was 450 bp and consisted of 10 EBNA1 binding sites. The Qp fragment was 150 bp and contained two EBNA1 binding sites. The DS template was 270 bp (120 bp DS plus ~75 bp flanking sequence on each side), and the DS (1+2) template was 222 bp (72 bp DS (1+2) plus ~75 bp flanking sequence on each side. Primer sequences are available upon request.