Supplementary Information

Figure Legends

Fig. S1. Characterization of methylation-specific monoclonal antibodies directed against the RG-repeat of EBNA2 by dot-blot analysis. The OVA-coupled peptides containing non-methylated (NMA)-, symmetrically dimethylated (SDMA)-, -asymmetrically dimethylated (ADMA) Argines or citrulline instead of Arginine residues were spotted onto nitrocellulose strips. OVA-HA conjugate served as internal control and was detected by the HA-specific antibody 3F10 (Roche, Penzberg, Germany). Each strip was incubated with the indicated antibody, and bound antibody was visualized by ECL using peroxidise-coupled secondary anti-rat or anti-mouse antibody.

Fig. S2. (A) The methylation of EBNA2-methylation does not change during lytic viral replication. B95.8 cells were treated with the phorbol ester TPA. Treated and untreated cells were analyzed for the amount of EBNA2 using R3, the level of methylation using aDMA-6F12, and the amount of the lytic cycle marker BZLF1 using BZ.1. (B) Inhibition of methylation does not induce lytic cycle replication. B95.8 cells treated with either TPA or the methylation inhibitor AdOx were tested for the amount of EBNA2 using R3, for EBNA2-methylation using 6F12, and for lytic cycle induction using BZ.1 against BZLF1. Staining for β–actin served as loading control in both (A) and (B).

Fig. S3. Characterization of *in vitro* generated EBNA2 using reticulocyte lysate. EBNA2 was synthesized *in vitro* in the TNT® Coupled Reticulocyte Lysate System (Promega, Mannheim, Germany) in the presence of ^{35}S-labelled Methionine (ICN). The radioactive EBNA2 was

then precipitated using a control antibody, R3, and the sDMA- or aDMA-specific antibodies as indicated. The precipitate was analysed by SDS-PAGE and fluorography.

Fig. S4. (A) Precipitation of sDMA-EBNA2 under EMSA condition. Nuclear extract from Raji cells prepared for electrophoretic mobility shift analysis (EMSA) was precipitated with R3 or the sDMA-7D9 antibody in the buffer used for gel shift analysis. Precipitated EBNA2 was visualized in western blot using R3. (B) The sDMA-7D9 and the aDMA-6F12 precipitate EBNA2 under ChIP condition. Extract from Mutu III cells

Fig S5. Remethylation generates DNA-binding EBNA2 but is not shifted with the antibody against non-methylated EBNA2 ("NMA"). The DNA probe (lane 1) was either incubated in vitro generated RBPJκ alone ("IVT RBPJκ", lane 2), or in the indicated combinations of IVT-RBPJκ plus unmethylated *E.coli*-EBNA2 alone ("E.coli E2 + jκ"), remethylated *E.coli*-EBNA2 ("Rem. E2 + jκ") or Reticulocyte-derived EBNA2 ("Retic E2 + jκ"). The antibodies used are indicated. The supershifted EBNA2-containing complexes are indicated by an arrow.

Fig. S6. Mutu III cells contain sDMA- and aDMA-modified EBNA2. Extract of Mutu III cells was subjected to immunoprecipitation using R3, aDMA-6F12 or sDMA-7D9 antibody in combination with the proper rat or mouse control antibody. Precipitated EBNA2 was visualized in a Western blot using R3.

Supplementary Information

Figure S1

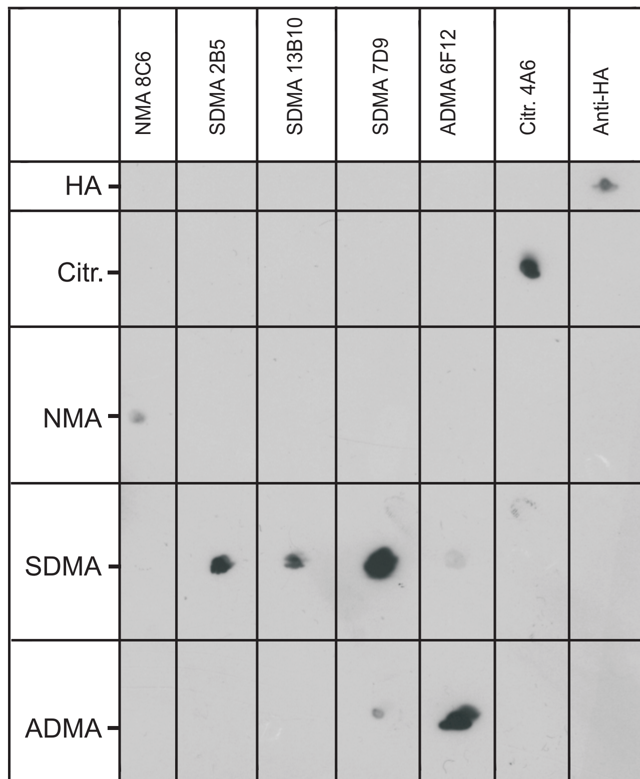

Figure S2A

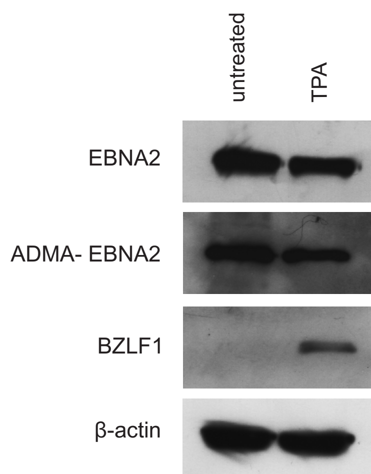

Figure S2A

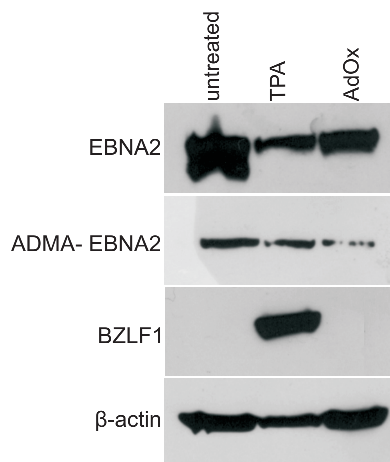

Figure S3

Figure S4A

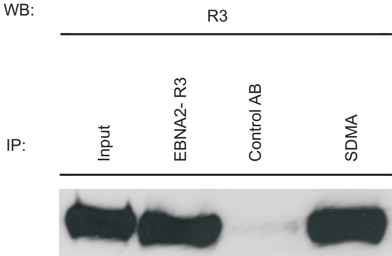

Figure S4B

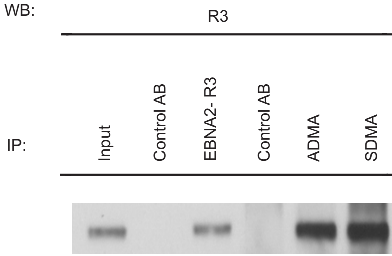

Figure S5

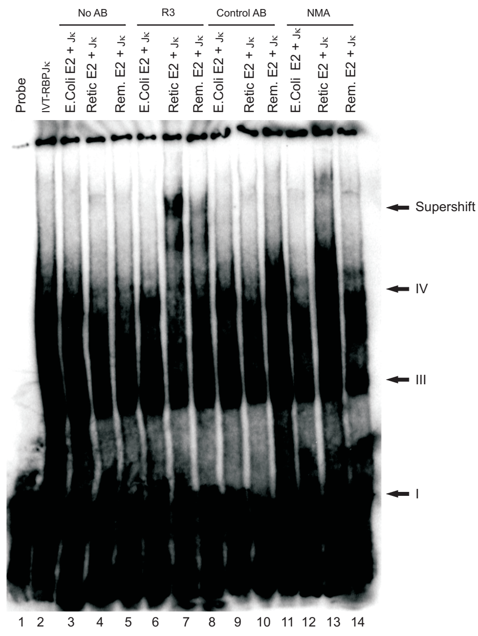

Figure S6

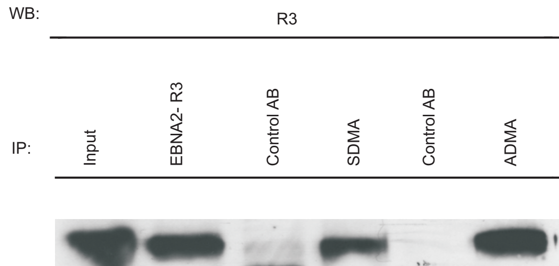

Table S1

Antigen / Antibody	OVA	NMA	SDMA	ADMA	CITR
control	0.076 0,055	0,051 0,054	0,055 0,057	0,055 0,051	0,056 0,052
SDMA	0,066 0,058	0,061 0,059	0,568 0,528	0,06 0,069	0,06 0,06
ADMA	0,07 0,065	0,076 0,074	0,093 0,094	0,569 0,487	0,055 0,063
NMA	0,096 0,057	0,134 0,139	0,063 0,074	0,07 0,076	0,067 0,053