Supporting Information

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SI Materials and Methods

Plasmid Constructs. pitEBLN was constructed inserting the coding sequence of an EBLN element (itEBLN) in the genome of the thirteen-lined ground squirrel (*Ictidomys tridecemlineatus*) (TLS) (corresponding to the nucleotide numbers 135–1131 of the GenBank accession number AB516287) into the KpnI and NotI sites of pcDNA3 vector (Invitrogen). The expression plasmids pNL-itEBLN and pNL-DsRed, in which the nuclear localization signal (NLS) of Borna disease virus nucleoprotein (BDV N) was fused to the coding sequences of the N terminus of itEBLN and Ds-Red, respectively, were constructed by PCR amplification. The BDV N sequence containing the NLS (amino acids 1–38) was amplified from prBDVP/M-GFP plasmid and fused to the N terminus of the coding sequences of itEBLN and Ds-Red. pNLsv-itEBLN was generated by PCR amplification of the itEBLN sequence with a forward primer containing the SV40 NLS sequence (5′-CCG AAG AAG AAG CGA AAG GTC-3′). The amplified fragment then was inserted into the KpnI and NotI sites of the pcDNA3 vector. To generate phsEBLN-1, the coding sequence of hsEBLN1 was amplified and cloned into the KpnI and NotI sites of pEF4A (Invitrogen). Nucleotide sequences of all plasmids were confirmed by DNA sequencing. The HA tag was fused at the N terminus of the all constructs.

Indirect Immunofluorescence Assay. Human oligodendroglioma (OL) and OL/BDV cells were seeded into eight-well chamber slides. One day after seeding, the cells were transfected with the EBLN expression constructs using Lipofectamine 2000. At 24 h posttransfection, the cells were fixed for 15 min in 4% (wt/vol) paraformaldehyde, permeabilized by incubation for 5 min in PBS containing 0.4% Triton X-100, and treated with 1% BSA. The cells were incubated with anti-BDV phosphoprotein (P) or anti-HA monoclonal antibody (Sigma-Aldrich) for 60 min in a humidified chamber at 37 °C and then were incubated with appropriate Alexa Fluor-conjugated secondary antibodies (Invitrogen). The cells were counterstained with DAPI in PBS for 15 min at room temperature. For immunofluorescence imaging, the cells were examined with an inverted Ti-E microscope with a C1confocal laser-scanning system (Nikon).

Immunoblotting. For Western blotting, total lysate proteins from OL or OL/BDV cells were subjected to SDS/PAGE and transferred onto polyvinylidene difluoride membranes. Mouse anti-HA (Sigma-Aldrich) and anti-BDV P antibodies were diluted 1:2,000, and 1:1,000, respectively, in PBS with 5% (wt/vol) low-fat milk powder and incubated with the membranes for 1 h at room temperature. After three 10-min washings with PBS-0.1% Tween-20, the membranes were reacted with HRP-coupled goat anti-rabbit or anti-mouse antibodies (Jackson ImmunoResearch) and visualized with ECL prime Western Blot Detection Reagents (GE Healthcare), according to the manufacturer's instructions.

Quantitative RT-PCR. Total RNA was extracted using TRIzol (Invitrogen) and reverse transcribed using a Verso cDNA Kit (ABgene) with a BDV-specific primer or an anchored oligo(dT) primer. Quantitative real-time PCR reactions were carried out using a gene-specific, double fluorescent-labeled probe in a LightCycler (Roche). The Taqman probe was labeled with 6-carboxy fluorescein as the 5′ fluorescent reporter and tetramethylrhodamine as the 3′ quencher. The sequence information for primers used in quantitative real-time PCR is available upon request.

Immunoprecipitation. BDV-infected OL cells were seeded in 10-cm plates. One day after seeding, the cells were transfected with HA-tagged itEBLN constructs using Lipofectamine 2000. At 24 h posttransfection, the medium was removed from the plates by aspiration, and the OL/BDV cells were washed with PBS. The cells then were scraped into 1.0 mL PBS. After centrifugation ($520 \times g$ for 3 min), the PBS was aspirated, and the cells were lysed using lysis buffer [20 mM Tris·HCl (pH 7.4), 150 mM NaCl, 1% Triton-X100, protease inhibitor]. The cell lysates were homogenized by sonication. After centrifugation ($18{,}800 \times g$ for 20 min), the supernatants were incubated with 40 μL of Dynabeads Protein G (Life Technologies) for 1 h with rotation. After incubation, the Dynabeads were removed, and anti-HA antibodies (Sigma-Aldrich) were added to the cell lysates, with rotation for 3 h. After incubation, Dynabeads were added to the cell lysates. Thirteen minutes after the beads were added, the cell lysates were mixed with RNase A (25 μg/mL) and incubated with rotation for 30 min. Then the beads were washed three times and collected. All steps of the harvesting procedure were carried out at 4 °C.

Tissue Samples. Tissues from a female captive breeding TLS and a wild-captured male TLS were provided by the Ground Squirrel Captive Breeding Colony at the University of Wisconsin-Oshkosh. A male TLS was captured in the wild in May 2014. Immediately after decapitation, tissue samples of brain (occipital cortex), liver, testis or ovary, heart, and kidney were rapidly dissected, cut into 5-mm cubes, and placed into excess cold RNAlater (Ambion). The fixed tissue samples were shipped to Japan and then were used for RNA and protein extractions. All animal procedures were preapproved by the University of Wisconsin Oshkosh's Institutional Animal Care and Use Committee, and conformed to guidelines set forth by the US Department of Agriculture Animal Welfare Act, the National Institutes of Health Office of Laboratory Animal Welfare, and the Association for Assessment and Accreditation of Laboratory Animal Care.

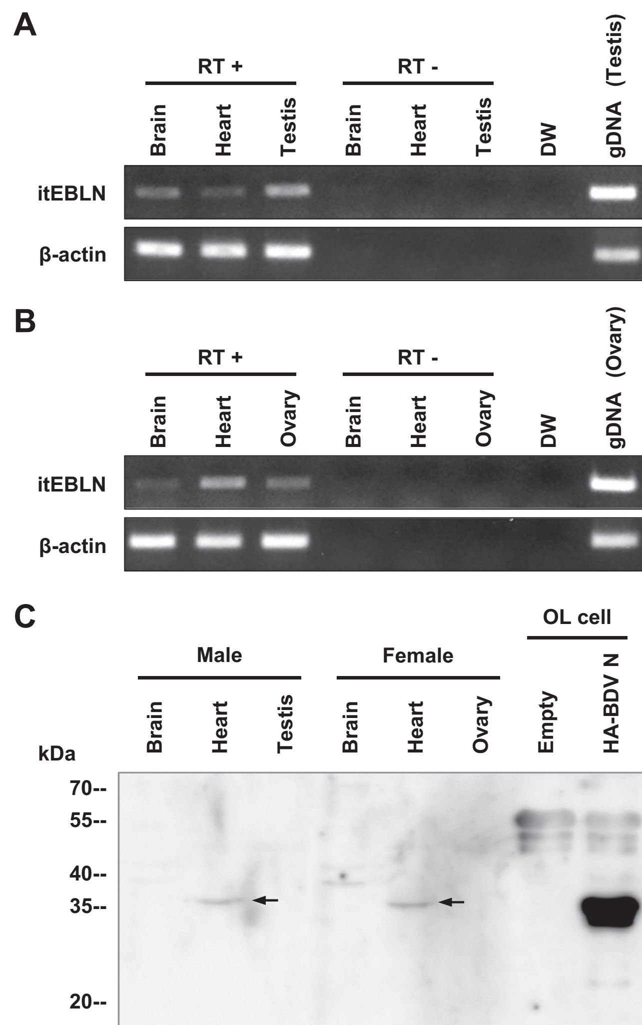

Fig. S1. Detection of itEBLN mRNA and protein in TLS tissue samples. (*A* and *B*) Total RNA and protein were extracted using TRIzol (Invitrogen) from tissue samples (brain, heart, testis, and ovary) from a wild-captured male (*A*) and a captive breeding female (*B*) TLS. Total RNAs were reverse-transcribed using M-MLV Reverse Transcriptase (Invitrogen) with an anchored oligo(dT) primer. PCR was performed using primers specific for predicted itEBLN mRNA (XM_005342477.1) (forward primer 5′-tgcactcttgtcctttccaa-3′, reverse primer 5′-ggtggtgtctctggcactct-3′; 37 cycles) and TLS β-actin mRNA (XM_005340038.1) (forward primer 5′-gcaagagaggcattctgacc-3′, reverse primer 5′-ggggtgttgaaggtctcaaac-3′; 35 cycles) by PrimeSTAR HS (TaKaRa). RT samples were reverse-transcribed without RT enzyme as a negative control, and gDNAs were used as positive control. DW, distilled water. (*C*) Detection of itEBLN protein in the TLS tissue samples. Immunoblot analysis was carried out using 12% SDS/PAGE and a rabbit anti-BDV N polyclonal antibody. As a positive control, OL cells transfected with HA-tagged BDV N expression plasmid were used. Arrows indicate the bands detected at the expected size for itEBLN in the heart samples of TLS.

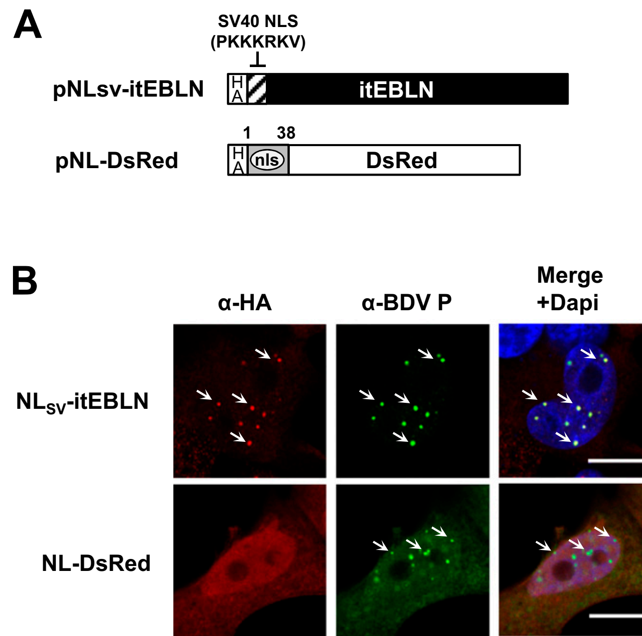

Fig. S2. Contraction and expression of control plasmids in human cells. (*A*) Schematic representations of the recombinant proteins of itEBLN and DsRed. The expression plasmids pNLsv-itEBLN and pNL-DsRed were generated by fusing the NLS of SV40 and BDV N to the N terminus of the itEBLN and DsRed ORFs, respectively. (*B*) Subcellular localization of NLsv-itEBLN and NL-DsRed in the transiently transfected BDV/OL cells. Arrows indicate the viral factories of BDV, vSPOT. (Scale bar, 10 μm.)

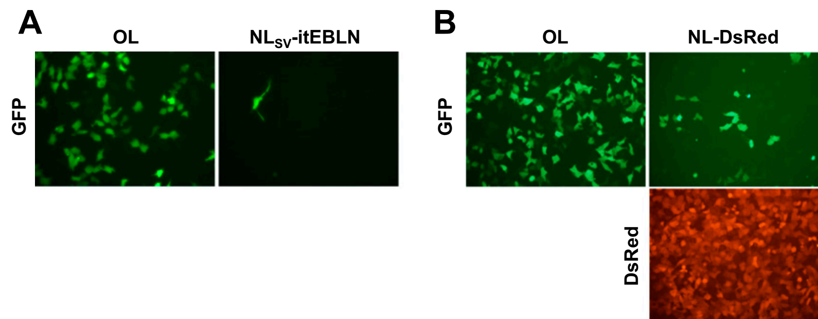

Fig. S3. Expression of NLsv-itEBLN in human cells inhibits BDV infection. OL cells stably expressing pNLsv-itEBLN (*A*) and pNL-DsRed (*B*) were inoculated with cell-free rBDV expressing GFP at a multiplicity of infection of 0.1. GFP expression was monitored by fluorescence microscopy at 12 (*A*) and 10 (*B*) d after infection.

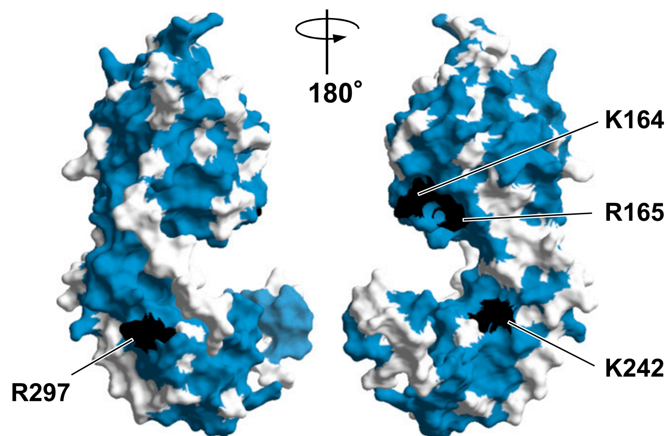

Fig. S4. itEBLN may conserve RNA-binding capacity. Amino acid identity between BDV and itEBLN in the 3D structure of BDV N. Amino acid residues identical to the itEBLN sequences are indicated by blue, and the predicted residues essential for the interaction with BDV RNA (K164, K165, K242, and R297) are indicated by black. The molecular model was created with CueMol ver. 2.0 (www.cuemol.org/).