AUF1/hnRNP D is a novel protein partner of the EBER1 non-coding RNA of Epstein-Barr Virus

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Supplementary Information

Figure Legends

Supplementary Figure S1. Determining accessible region(s) of EBER1 by RNase H digestion. (*A*) The sequence and secondary structure of EBER1 are shown with the four stem-loops indicated. (*B*) DNA oligonucleotides complementary to the indicated EBER1 sequences were added to whole cell lysate from BJAB-B1 cells and incubated for 10 min at 30°C to allow cleavage of EBER1 by endogenous RNase H. RNA was isolated by TRIZOL and Northern blots were probed for EBER1 and U6 as a loading control. Only the stem-loop III region of EBER1 is partially accessible for oligonucleotide-targeted RNase H cleavage. The arrow indicates the cleavage product of EBER1.

Supplementary Figure S2. Proteins enriched by MS2-aptamer selection are not visualized by silver staining. Proteins eluted using maltose competition after MS2-selection from EBER1 and EBER1-MS2 containing lysates were resolved by SDS-PAGE and silver stained. The MS2-MBP fusion protein is indicated. Although proteins selectively bound to EBER1-MS2, such as La and L22, can be detected by Western blot, their presence is not apparent by silver staining.

Supplementary Figure S3. Peptide mass spectrum and SILAC pair of identified AUF1 protein. (*A*) Fragmentation pattern (MS/MS) of the identified AUF1 peptide is shown. (*B*) Precursor ion SILAC pair of the AUF1 peptide is shown.

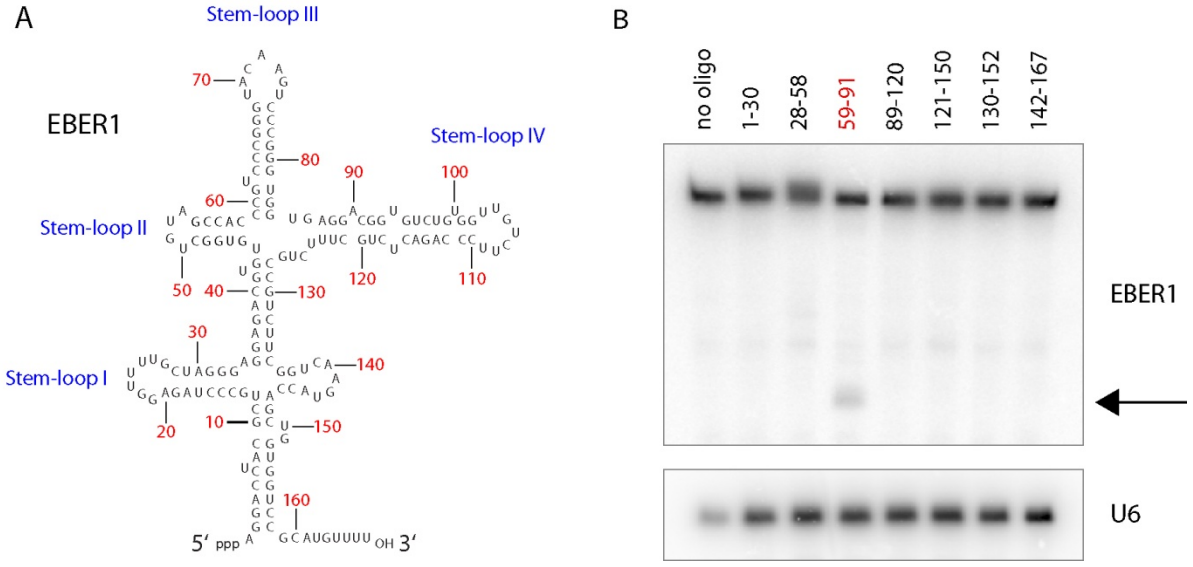
Supplementary Figure S4. Knockdown of AUF1 does not affect the level of EBER1. (*A*) BJAB-B1 cells that express a doxycycline (Dox)-inducible short-hairpin RNA construct against AUF1 were generated. Five days after Dox addition, the AUF1 level decreased to approximately 10% of the endogenous level as estimated by Western blot using anti-AUF1 antibodies. The lanes marked 30%, 20%, and 10% indicate fractions of the –Dox sample (outermost left lane) for comparison. Nucleolin served as a loading control. (*B*) RNA was isolated from BJAB-B1 cells carrying the AUF1 short-hairpin RNA construct before and after five days of doxycycline treatment. The level of EBER1 was examined by Northern blot. The level of U6 served as a loading control.

Supplementary Figure S5. TNFα mRNA levels are not affected by EBER1. (*A*) A firefly luciferase reporter containing TNFα-ARE in the 3′-UTR was expressed in HEK293T cells with empty vector (-EBER1) or EBER1-expression vector (+EBER1). A plasmid expressing Renilla luciferase was co-transfected to control for transfection efficiency. The luciferase reporters were described by Vasudevan and Steitz (2007). (*B*) TNFα mRNA levels were measured by quantitative PCR and normalized to GAPDH levels in BJAB cells and in BJAB cells expressing EBER1 (BJAB-EBER1). Real-time primers for TNFα were 5′-CCCAGGGACCTCTCTCTAATCA-3′ and 5′-AGCTGCCCCTCAGCTTGAG-3′. All data represent mean +/- s.d. (n=3).

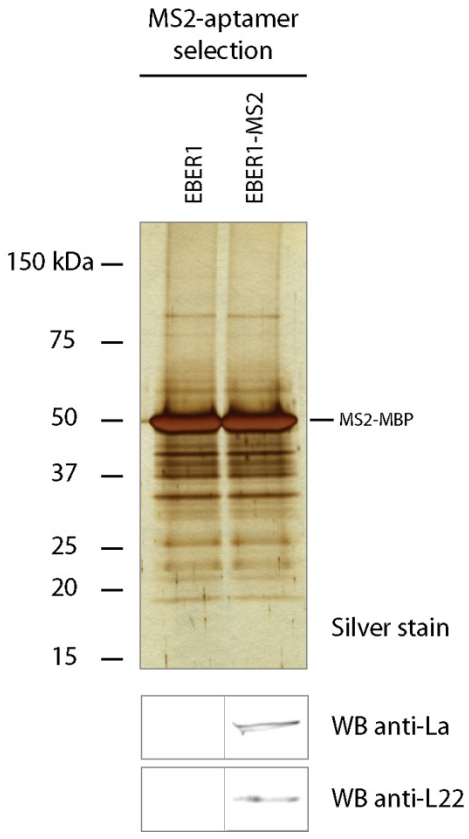
Supplementary Figure S6. hTERT mRNA levels are reduced over twofold in BJAB-B1 cells compared to BJAB cells. hTERT mRNA levels of BJAB and BJAB-B1 cells were measured by quantitative PCR. RNA was isolated with TRIZOL, and cDNA was prepared using High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems). For real-time PCR analysis, FastStart Universal SYBR Green Master Mix (Roche) and the following primers were used as described (Li et al. 2011): 5′-CACGCGAAAACCTTCCTCAG-3′ and 5′-CAAGTTCACCACGCAGCCAT-3′. All data represent mean +/- s.d. (n=3).

Supplementary Table S1. Proteins identified by mass spectrometry analysis after SILAC and MS2-aptamer selection are shown in Sheets I and II. Sheet I shows the results for the MS2-selection where EBER1-MS2 and EBER1-expressing cells were grown in heavy and light amino acid-containing medium, respectively. For the results shown in Sheet II, the isotope labels were reversed. The SILAC ratio reveals the relative abundance of selected peptides from the EBER1-MS2-containing sample compared to the unmodified EBER1-containing lysate. Among the proteins that are enriched in the selection with EBER1-MS2-containing cell lysate are the known EBER1-interacting proteins La and L22. PEP: posterior error probability (also known as local false discovery rate (Kall et al. 2008)).

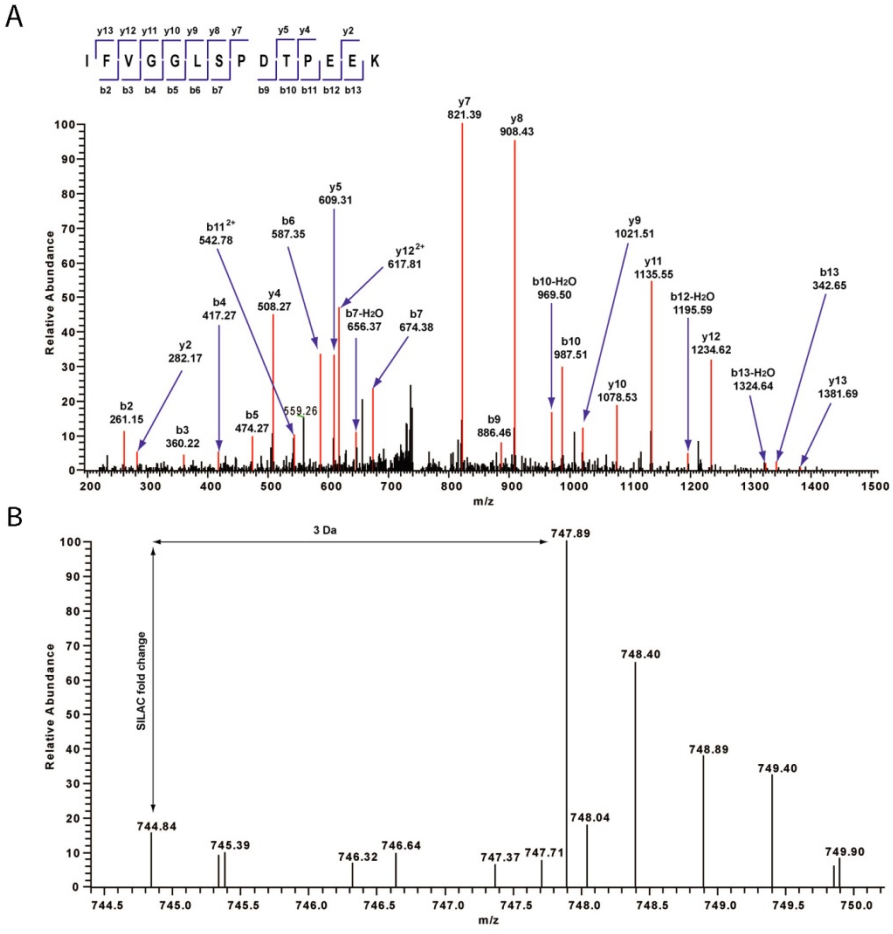
Supplementary Figure S1



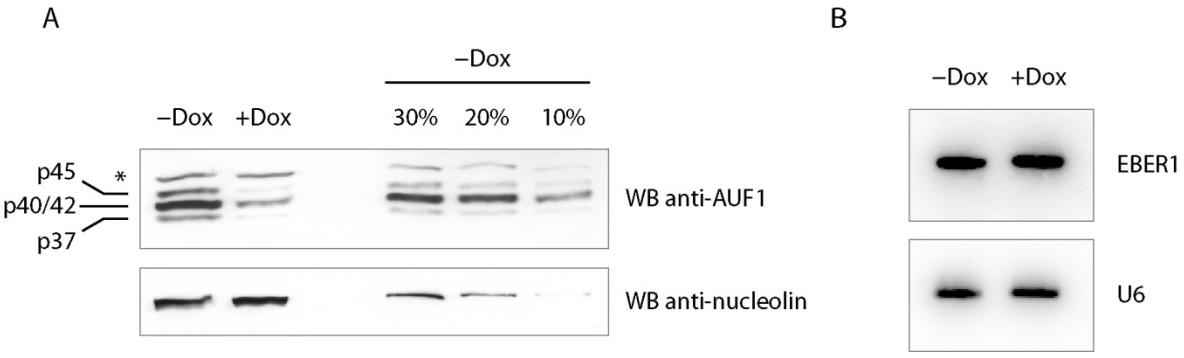
Supplementary Figure S2



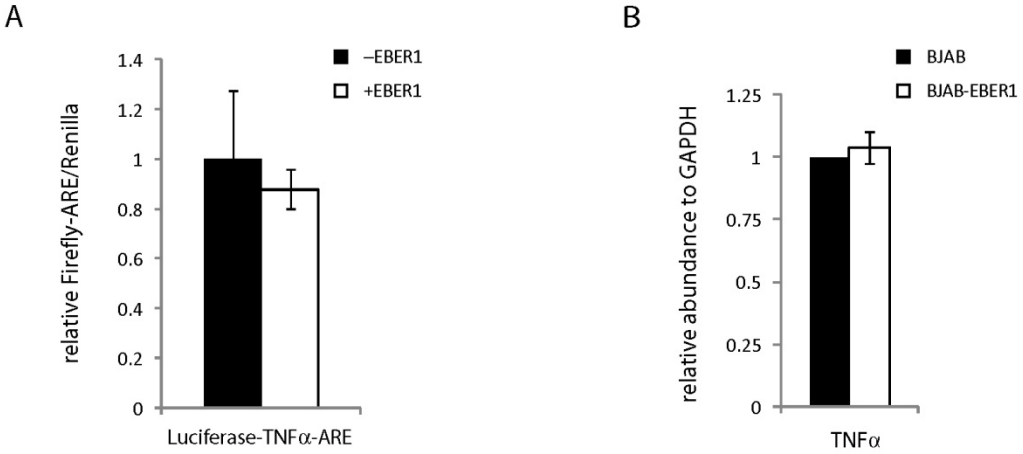
Supplementary Figure S3



Supplementary Figure S4



Supplementary Figure S5



Supplementary Figure S6