Supplementary Information for

Pseudouridylation of Epstein-Barr Virus Noncoding RNA EBER2 Facilitates Lytic Replication

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Supplementary Figure Legends

Supplementary Figure 1. (**A**) Experimental outline of the 2CIMPL approach. This technique utilizes crosslinking via UV light irradiation and the crosslinking agent psoralen (AMT), followed by partial RNase digest and proximity ligation; RNA-RNA interactions can subsequently be identified computationally by mapping hybrid reads generated by next-generation sequencing to their individual loci. Here, 2CIMPL was performed with anti-DKC1 antibody to uncover EBER2 interactions with H/ACA snoRNAs. (**B**) Model of how H/ACA snoRNAs engage in RNA-RNA interactions with substrate RNAs, which are targeted for pseudouridylation by H/ACA snRNP enzyme complexes. Box H and Box ACA regions are indicated; the position of the nucleotide within the substrate RNA to be pseudouridylated is indicated by Ψ. (**C**) Nucleotide sequences of SNORA22 and EBER2. Box H and Box ACA of SNORA22 are indicated in yellow; bulge regions predicted to engage in putative RNA-RNA interactions are highlighted in red. The complementary base-pairing regions in EBER2 are highlighted in red as well. (**D**) Sequencing track of 2CIMPL result for DKC1 showing the RNA hybrids between EBER2 and SNORA22. The 3′ end region of EBER2 surrounding the pseudouridylation site and the 5′ end region of SNORA22 upstream of the Box H form RNA hybrids, indicating RNA duplex formation between these regions. (**E**) Identifying ASOs that knock down SNORA22 *in vivo*. DNA ASOs complementary to the indicated regions within SNORA22 were incubated with BJAB-B1 cell lysate to mediate RNase H digestion, if a particular ASO is accessible to SNORA22. RNase H digest was performed as described previously (Lee et al. 2015). Arrows indicate two ASOs that efficiently knock down SNORA22 as determined by Northern blot analysis. (**F**) The EBV genomic region containing the EBER1 and EBER2 loci were cloned into the pCR-Blunt II-TOPO vector. The EBER2 U160C mutant was generated by site-directed mutagenesis. Both vectors were transfected into HEK293T cells, and RNA was harvested 48 hours after transfection to examine EBER2 levels by Northern blot analysis. EBER1 levels, expressed from the same plasmid, were used for normalization and account for transfection efficiency bias.

Supplementary Figure 1