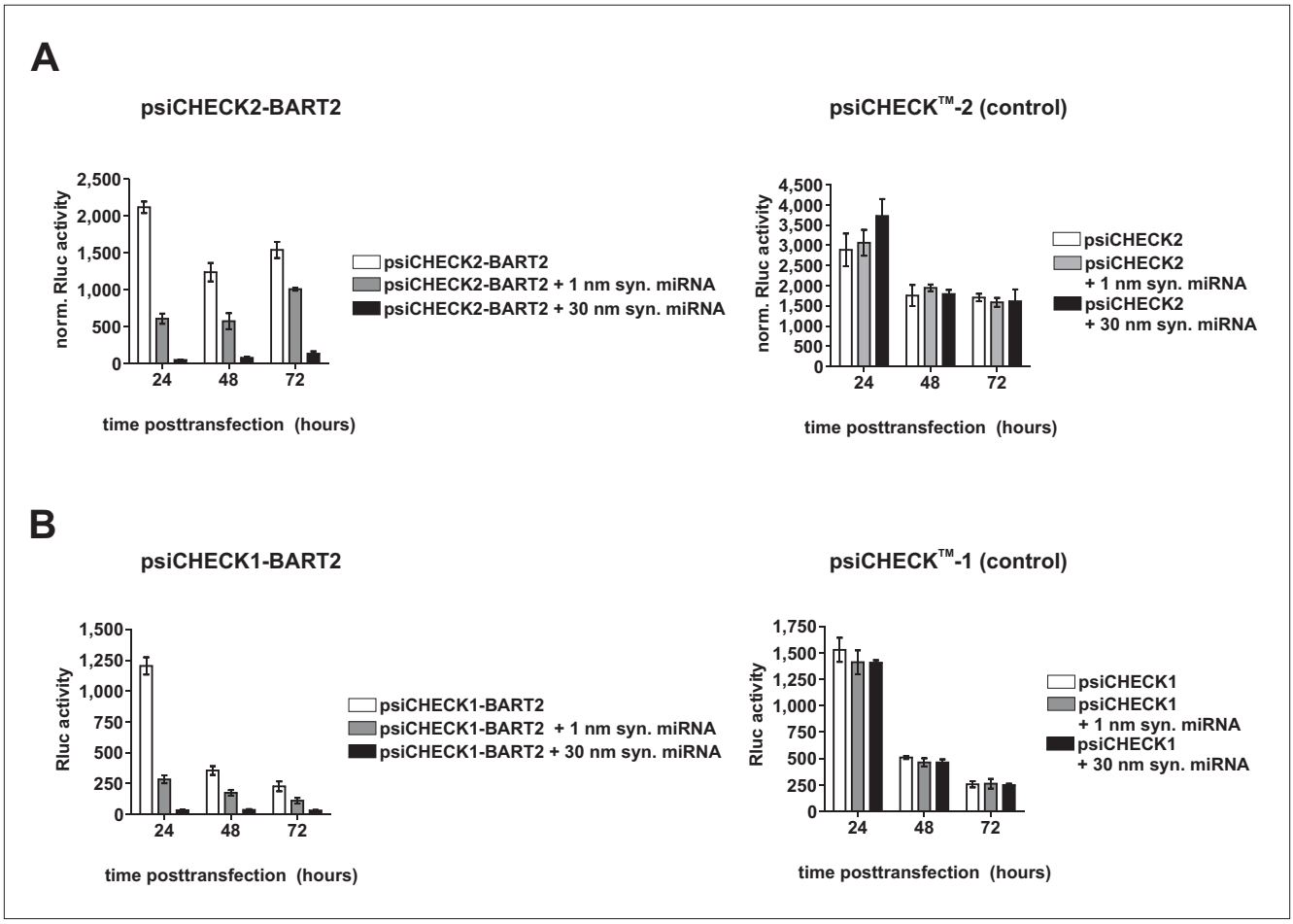
TABLE S1
Mutations in protein E resulting from BHK-21 cell adaptation.

Virus	Mutation (position no.)	
	Nucleotide[a]	Amino acid[b]
TNd/c BART(+)	1337 (A→G)	Glu→Gly (122)
	1573 (G→A)	Glu→Lys (201)
TNd/c BART(-)	1337 (A→G)	Glu→Gly (122)
TNd/c matBART(+)	1337 (A→G)	Glu→Gly (122)
	1573 (G→A)	Glu→Lys (201)
TNd/c	1337 (A→G)	Glu→Gly (122)
	1573 (G→A)	Glu→Lys (201)

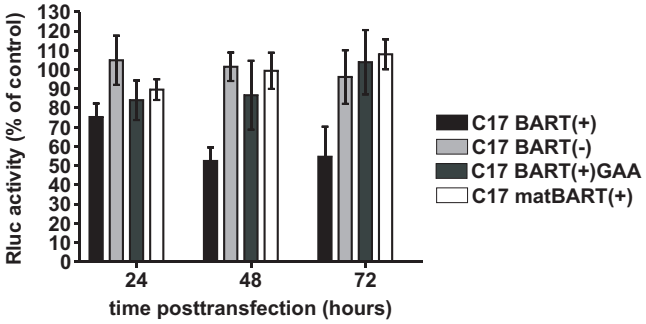
Viral RNA was extracted from BHK-21 cell culture supernatant, infected with viral passage 5, and subjected to RT-PCR and direct sequencing of both strands, including the entire protein E coding region.

[a]Numbers are according to the TBE virus genomic sequence (GenBank accession no. U27495)

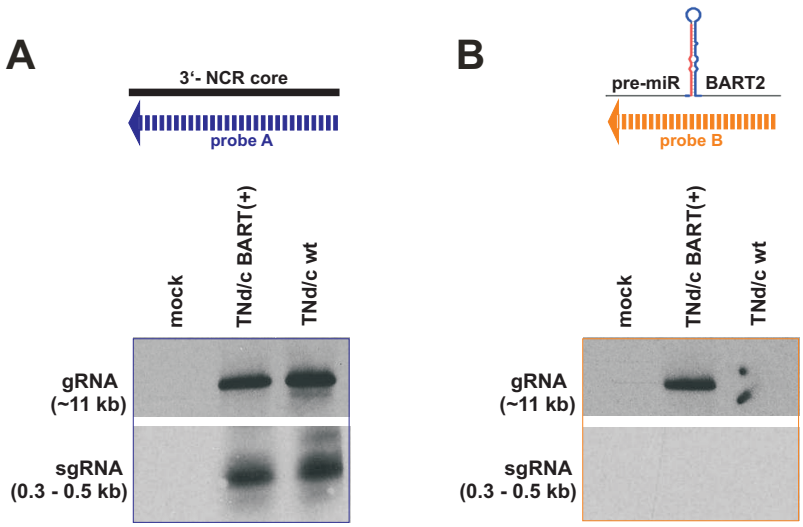
[b]Numbers start from the amino terminus of protein E.



Supplementary Figure S1



Supplementary Figure S2



Supplementary Figure S3

FIGURE LEGENDS – SUPPLEMENTARY MATERIAL

Fig. S1

miR-BART2 specifically downregulates the expression of reporter mRNAs fused to the corresponding miR-BART2 target site. Time course of *Renilla* luciferase (Rluc) activity in cells transfected with the reporter constructs psiCHECK2-BART2 **(A)** and psiCHECK1-BART2 **(B)**, either individually or together with synthetic pre-miR-BART2 precursor molecules at a concentration of 1mM or 30 nM. In the case of psiCHECK2-BART2, the Rluc light units were normalized to those of firefly luciferase (fluc) expressed from a different promoter on the same plasmid as an internal standard (Fig. 1D). psiCHECK-1-BART2 does not contain the fluc cistron (Fig. 3D). Data from control experiments using the parental vectors without miR-BART2 are shown at the right. Error bars represent the standard deviation of one experiment, measured in triplicate.

Fig. S2

Downregulation of Renilla luciferase in cells co-transfected with psiCHECK1-BART2 and the C17 BART mutants (complete data set). Rluc levels are shown as a percentage of the level obtained with the parental replicon C17. Error bars represent the standard deviation from a minimum of three independent experiments, each measured in triplicate.

Fig. S3

A subgenomic RNA is produced from the 3'-terminal region of TNd/c and TNd/c BART(+) (A), but the miR-BART2 sequence insertion is not contained on this fragment (B). BHK-21 cells were infected with the indicated viruses at an MOI of 10, and total RNA was subjected to northern blot analysis using two biotinylated probes, one specific for the conserved "core" region of the 3'-NCR (probe A) and one specific for the miR-BART2 sequence insertion including the flanking EBV elements (probe B). gRNA, genomic RNA; sgRNA, subgenomic RNA.