	Poly(A) Selected		Ribodepleted	
	Uninduced	Induced (24h)	Uninduced	Induced (24h)
Number of reads/fragments	53,936,975	45,537,672	70,911,665	83,393,856
Number of mapped reads	47,852,873	38,802,797	62,571,694	69,131,398
Number of reads mapped to EBV	3,900	1,701,413	38,104	3,460,879
Strand specificity	99.86%	99.87%	99.94%	99.94%
Strand specificity standard deviation	0.28	0.24	0.11	0.14

	Time Course (Ribodepleted)								
	0	5m	30m	1h	2h	4h	8h	24h	48h
Number of reads/fragments	45,549,671	74,971,661	70,376,626	56,918,756	67,248,128	67,827,695	59,551,327	67,001,626	67,472,115
Number of mapped reads	38,169,413	64,251,923	59,470,292	48,923,989	57,399,365	57,846,467	50,557,152	55,525,060	55,234,594
Number of reads mapped to EBV	25,679	26,144	32,612	17,452	33,100	154,174	375,296	852,760	394,352
Strand specificity	99.94%	99.94%	99.93%	99.96%	99.94%	99.96%	99.94%	99.94%	99.93%
Strand specificity standard deviation	0.13	0.12	0.11	0.08	0.10	0.07	0.13	0.13	0.13

Table S1: Information about RNA-seq reactions

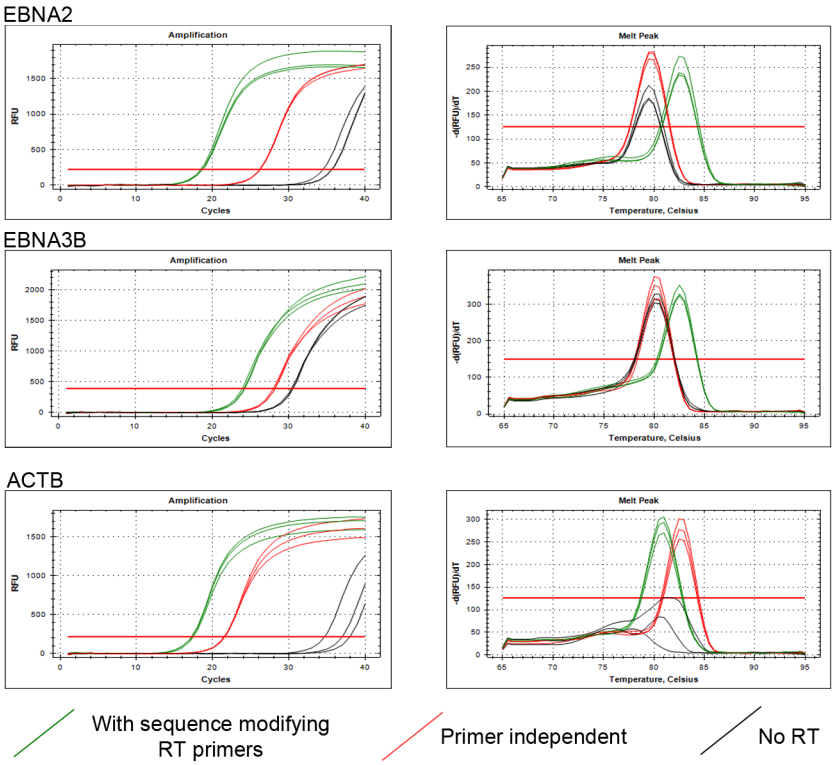

Figure S1: Strand specific qRT-PCR. Representative Ct curves and melting curves are shown for each set of primers. Sequence modifying RT primers increase (EBNA2 and EBNA3B) or decrease (ACTB) the melting temperature of the PCR amplicons relative to the unmodified amplicon.

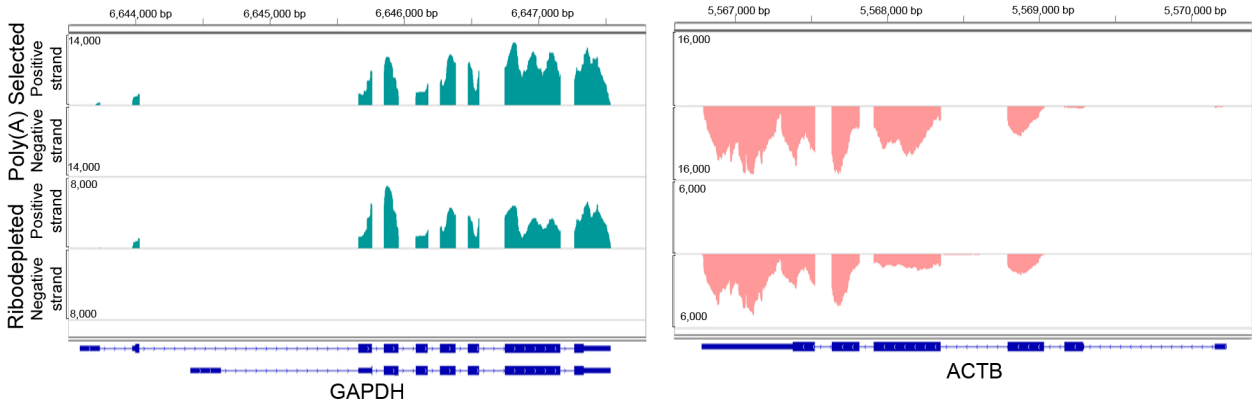

Figure S2: RNA-seq sense and antisense read coverage of cellular genes GAPDH and ACTB.

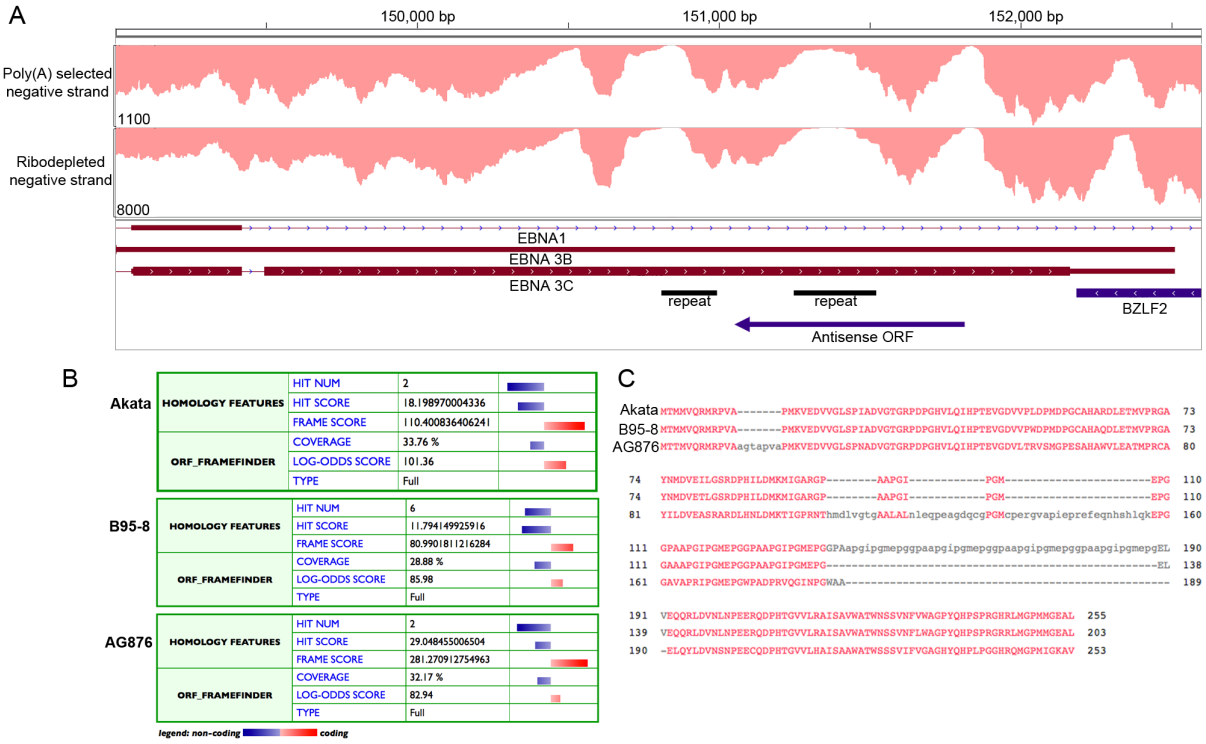

Figure S3: A novel antisense ORF in the EBNA3C locus. (A) Genomic location and RNA-seq read coverage of a novel antisense ORF. (B) Coding Potential Calculator output for corresponding regions in the EBV strains Akata, B95-8 and AG876. (C) BLAST multiple sequence alignment of translated ORFs from Akata, B95-8 and AG876 sequences.

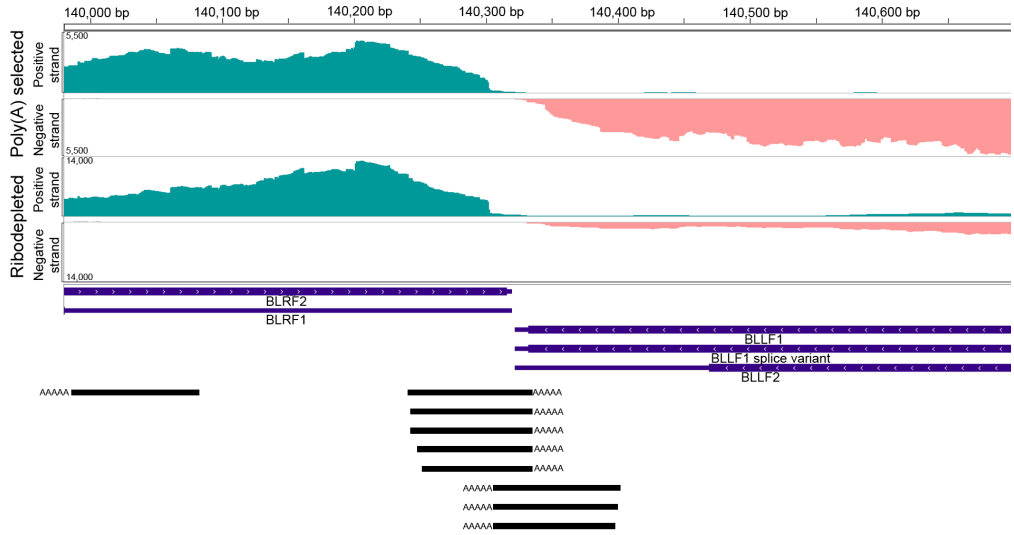

Figure S4: Poly(A) tail-containing RNA-seq reads (i.e.RNA-seq reads with 5 or more Ts at their 5' ends with at least one 5'-most mismatch to the genome (see methods)) align at the 3' ends of known polyadenylated transcripts. Representative results shown.

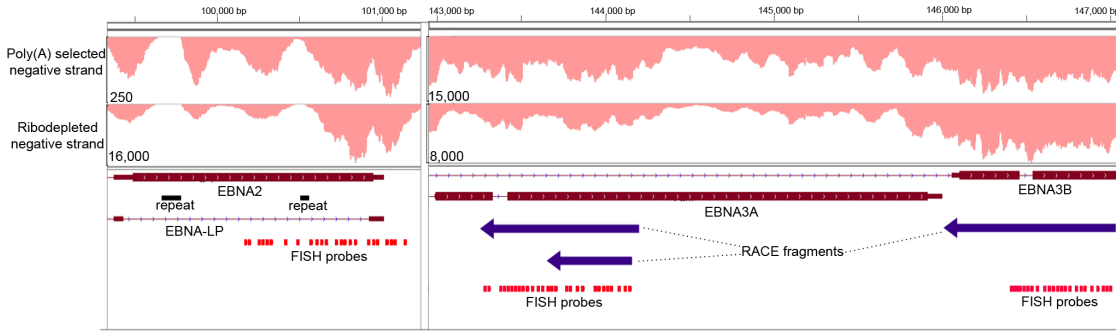

Figure S5: Genomic location of Stellaris FISH probes relative to known transcripts, RACE fragments and RNA-seq read coverage.

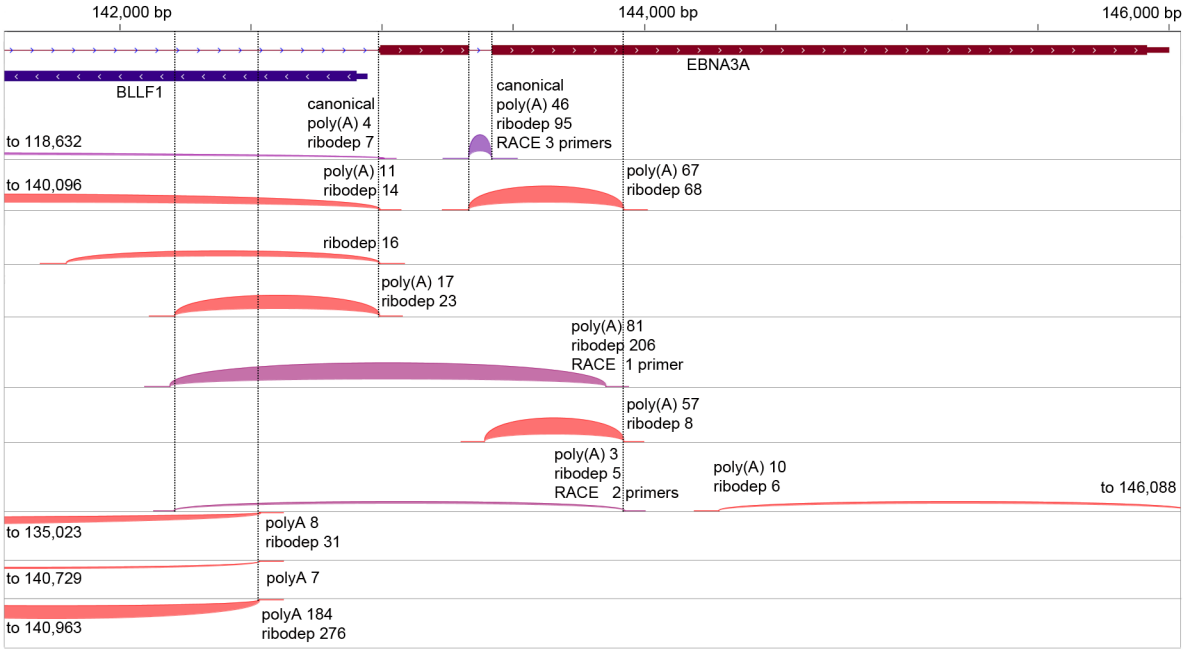

Figure S6: Splice junctions in the EBNA3A region that are annotated, detected in fragments from at least 2 RACE primers, or detected in RNA-seq reads by TopHat analysis (at least 5 reads from poly(A) selected RNA or 10 reads from ribodepleted RNA).

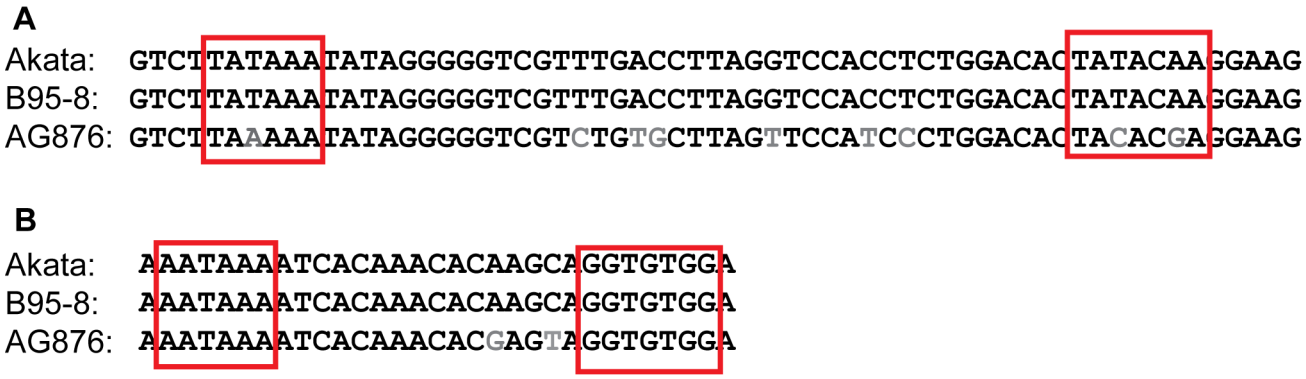

Figure S7: Sequence motif conservation. (A) TATA boxes for EBNA3A antisense transcripts. Red boxes indicate the locations of TATA motifs. (B) Polyadenylation signal for EBNA3B antisense transcript. Red boxes indicate AATAAA signal and downstream GT-rich element.

Video S1: Successive Z-plane images of FISH staining for EBNA2 antisense transcript. Blue = DAPI, red = EBNA2 antisense transcript.

Video S2: Successive Z-plane images of FISH staining for EBNA3A antisense transcript. Blue = DAPI, red = EBNA3A antisense transcript.

Video S3: Successive Z-plane images of FISH staining for EBNA3B antisense transcript. Blue = DAPI, red = EBNA3B antisense transcript.