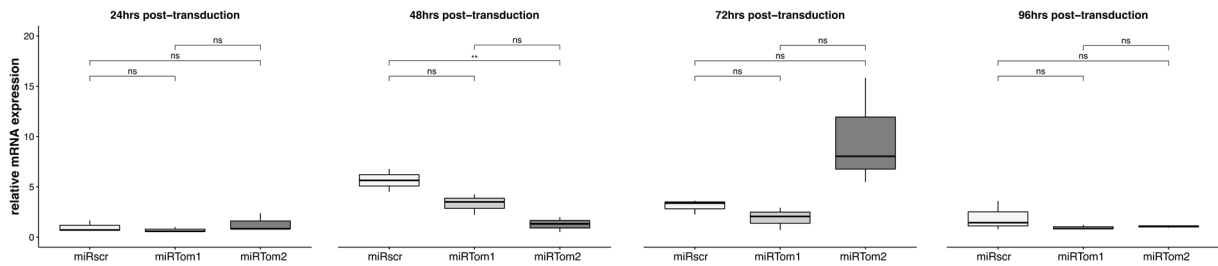
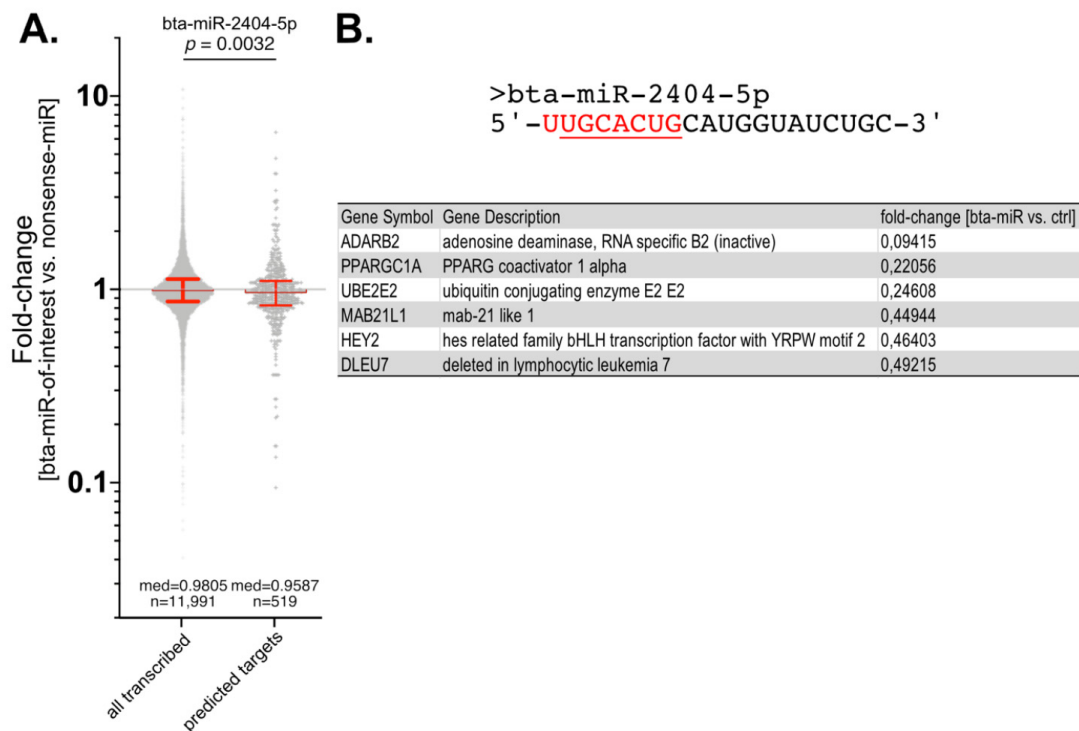


## Supplementary Material

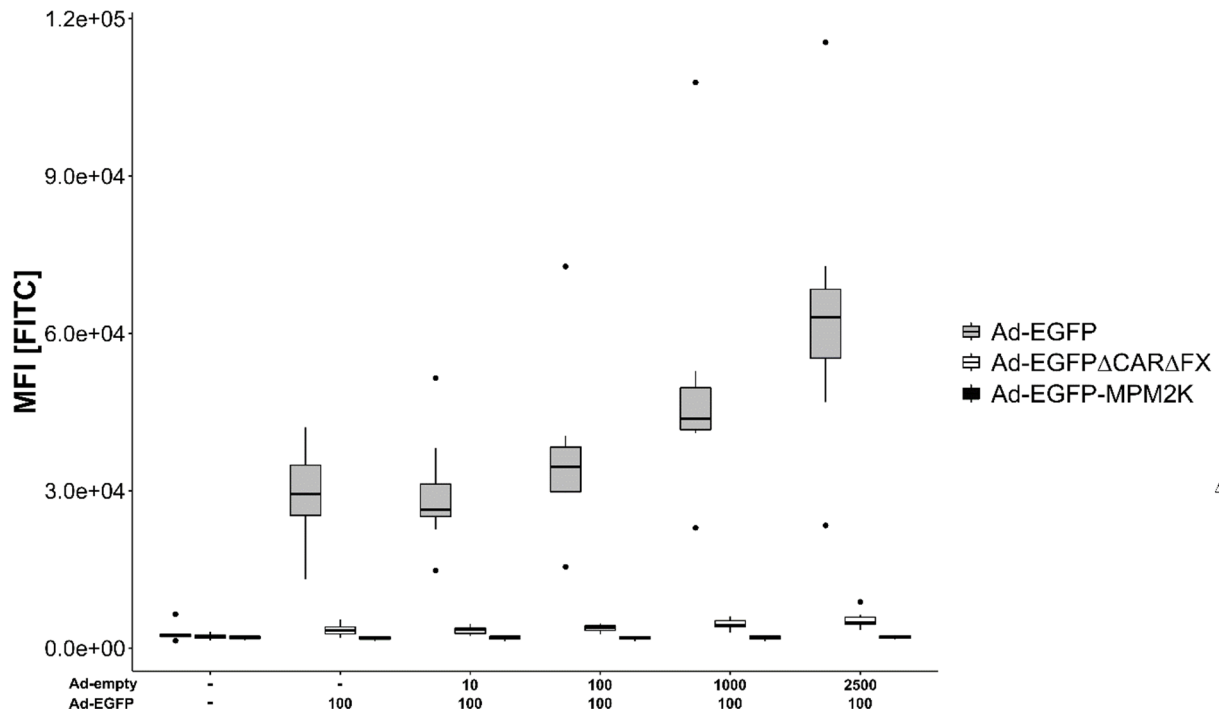
### An adenoviral vector as a versatile tool for delivery and expression of miRNAs



**Figure S1: Stable co-cistronic expression of EGFP reporter gene over 96 hpt.** We monitored Ad5dE1-driven, co-cistronic expression of EGFP over 96 h using quantitative PCR. The expression level of EGFP oscillated only slightly over time and was independent of inserted miRNA (96 hpt: miRScr 1.94-fold over 24 hpt; miRTom1 0.97-fold over 24 hpt; miRTom2 1.06-fold over 24 hpt). hpt, hours post-transduction; ns,  $p > 0.05$ ; \*\*,  $p < 0.01$ .



**Figure S2: Effects of Ad5-mediated expression of bta-miR-2404-5p.** The cattle-specific miRNA bta-miR-2404-5p that does not occur in human was used to target endogenous mRNAs. Putative human targets for bta-miR-2404-5p were predicted using the 'custom prediction' option of miRDB\* (<http://mirdb.org/mirdb/index.html>). **A.** Effects of Ad5-mediated expression of bta-miR-2404-5p were studied in HIEC-6 in comparison to HIEC-6 expressing an Ad5-mediated nonsense-miR. Relative fold-changes were compared for the whole HIEC-6 transcriptome and the lists of expressed predicted targets. Predicted targets for each bta-miR comprised of hundreds of mRNAs. Statistical power calculations were done using the nonparametric Wilcoxon–Mann–Whitney method and GraphPad Prism 8.4.3 software. **B.** Sequence of bta-miR-2404-5p. miRDB considers nucleotides 1-8 (red) for putative target interactions within the 3'-UTR of mRNAs, whereas mainly the seed region comprises of nucleotides 2-8 (underlined). Below is a list of experimentally confirmed targets exhibiting fold changes below 0.5x that were previously predicted using miRDB.



**Figure S3: Direct comparison of transgene expression driven from different Ad-EGFP vectors.**

A549 cells were transduced with escalating doses of Ad-empty followed by transduction with an EGFP-expressing vector. Compared to Ad-EGFP, transgene expression from Ad-EGFPΔCARΔFX was significantly reduced due to its ablated binding to CAR [50]. The enhancing effect of Ad-empty co-transduction was similar for Ad-EGFP and Ad-EGFPΔCARΔFX. EGFP expression driven from Ad-EGFP/MPM2K was not detectable. MFI, mean fluorescence intensity.

**Table S1 Primers used for PCR amplification and homologous recombination.** Homology arms are displayed *italic*. Tan, annealing temperature.

target		homology arms ( <i>italic</i> )	5' - 3'	Tan
miR expression cassette	forward	Ad5 bp 391-440 (AY339865)	<i>AGGTGTTTTCTCAGGTGTTTTCCGCGTTCCGGGTCAAAGTTGGCGTTTT</i> tctgcttagggtaggcgtt	63.5 °C
miR expression cassette	reverse	Ad5 bp 3522-3571 (AY339865)	<i>ATAAGACCCACCTTATATATTCTTTCCACCCCTTAAGCCACGCCACA</i> gctgccaggaacagctatg	63.5 °C
rps1neo counter-selection marker	forward	miRNA cassette	<i>G TAGTGAGTCGACCAGTGGATCCTGGAGGCTTGCTGAAGGCTGTATGCTG</i> ggcctgggtgatgagcggggatcg	65°C
rps1neo counter-selection marker	reverse	miRNA cassette	<i>ATCTGGGCCATTGTTCATGTGAGTGTAGTAACAGGCCTTGTCCTG</i> tcagaagaactcgtcaagaaggcg	65°C
inserted miRNA oligo	forward	none	ggatcactctcggcatggac	58°C
inserted miRNA oligo	reverse	none	attgccgtcatagcgcgggt	58°C
miRNA-Tom1 ( <i>target</i> , <i>loop</i> , <i>revTarget</i> )	oligo	miRNA cassette	<i>G TAGTGAGTCGACCAGTGGATCCTGGAGGCTTGCTGAAGGCTGTATGCTG</i> <u><i>TACTGTTCCACGATGGTGTAG</i></u> <i>GTTTTGGCCACTGACTGACCTACACCAGTGGAAACAGTACAGGACACAAGGCCTGTACTAGCACTCACATGG</i> <i>AACAAATGGCCAGAT</i>	-
miRNA-Tom2 ( <i>target</i> , <i>loop</i> , <i>revTarget</i> )	oligo	miRNA cassette	<i>G TAGTGAGTCGACCAGTGGATCCTGGAGGCTTGCTGAAGGCTGTATGCTG</i> <u><i>TTGGTGTCACGTAAGTAG</i></u> <i>GTTTTGGCCACTGACTGACCTACTACTGTGGACACCAACAGGACACAAGGCCTGTACTAGCACTCACATGG</i> <i>AACAAATGGCCAGAT</i>	-