Supplementary methods

Cells.

K562 cells (a human chronic myelogenous leukemia cell line) were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal calf serum (FCS) and antibiotics.

In vitro Ad gene expression analysis in K562 cells.

K562 cells were seeded onto 12-well plates at 1×10^5 cells/well. On the following day, cells were transduced with Ad vectors at an MOI of 100 and harvested 12 h after transduction. After total RNA isolation, mRNA levels of Ad genes were determined by real-time RT-PCR.

Supplementary Figure S1

Suppression of the leaky expression of Ad genes in cultured cells. K562 cells were transduced with Ad vectors at an MOI of 100 and harvested 12 h after transduction. The Ad gene expression levels were determined by real-time RT-PCR. The data are expressed as the mean values ± S.D. (n=4). *$p<0.05$ in comparison with Ad-L2.

Supplementary Figure S2

The leaky expression of Ad genes in the mouse liver and spleen following intravenous administration of the Ad vectors. (A) The Ad gene expression levels in the liver were determined 2 days following intravenous administration of Ad vectors. (B) The Ad gene expression levels in the liver and spleen were determined 2 days following intravenous administration of Ad-L2. The data are expressed as the mean values ± S.D. (n=5-6).

Supplementary Figure S3

Chemokine mRNA levels in the liver after administration of Ad-L2 and Ad-E4-122aT-L2. C57BL/6 mice were treated with Ad vectors at 1×10^{10} IFU/mouse. Ten days after administration, chemokine mRNA levels in the liver were determined by real-time RT-PCR. The data are expressed as the mean values ± S.D. (n=3-6).

Supplementary Table S1 Oligonucleotide sequences.

Ad-E2A-122aT	
E2A-3'-UTR-F	5'-accagcacactggtt-3'
E2A-3'-UTR-R	5'-aaccagtgtgctggt-3'
E2A-miR-122aT-BstXI-S3	5'-acaaacaccattgtcacactccacagcacaaacaccattgtcacactccattaattaacagactagttgtg-3'
E2A-miR-122aT-BstXI-AS3	5'-actagtctgttaattaatggagtgtgacaatggtgtttgtgctgtggagtgtgacaatggtgtttgtcaca-3'
E2A-miR-122aT-BstXI-S4	5'-acaaacaccattgtcacactccaggacacaaacaccattgtcacactcca-3'
E2A-miR-122aT-BstXI-AS4	5'-ctagtggagtgtgacaatggtgtttgtgtcctggagtgtgacaatggtgtttgtat-3'
Ad-E4-122aT	
E4-3'-UTR-F1	5'-aatttcaagtcatttttcattcagtagtatagccccaccaccacatagcttatacagatcaccgtaccttaatca aactaggtaccacctgccacc-3'
E4-3'-UTR-R1	5'-gggaggtggcaggtggtacctagtttgattaaggtacggtgatctgtataagctatgtggtggtggggctata ctactgaatgaaaaatgacttga-3'
E4-3'-UTR-F2	5'-catgcggccgctatcacagaaccctagtattcaacctgccacc-3'
E4-3'-UTR-R2	5'-gggaggtggcaggttgaatactagggttctgtgatagcggccgcatggtac-3'
E4-miR-122aT-S1	5'-ggccacaaacaccattgtcacactccacagcacaaacaccattgtcacactccattaattaagcggtac-3'
E4-miR-122aT-AS1	5'-cgcttaattaatggagtgtgacaatggtgtttgtgctgtggagtgtgacaatggtgtttgt-3'
E4-miR-122aT-S2	5'-acaaacaccattgtcacactccaggacacaaacaccattgtcacactccagtac-3'
E4-miR-122aT-AS2	5'-tggagtgtgacaatggtgtttgtgtcctggagtgtgacaatggtgtttgtat-3'
Ad-pIX-122aT	
pIX-miR-122aT-S1	5'-ctagctaaacaaacaccattgtcacactccacagcacaaacaccattgtcacactccagaattccagggtacc-3'
pIX-miR-122aT-AS1	5'-ctagggtaccctggaattctggagtgtgacaatggtgtttgtgctgtggagtgtgacaatggtgtttgtttag-3'
pIX-miR-122aT-S2	5'-aattgacaaacaccattgtcacactccaggacacaaacaccattgtcacactccaggtac-3'
pIX-miR-122aT-AS2	5'-ctggagtgtgacaatggtgtttgtgtcctggagtgtgacaatggtgtttgtc-3'
Ad-E2A-142-3pT	
E2A-miR-142-3pT-BstXI-S3	5'-tccataaagtaggaaacactacacagctccataaagtaggaaacactacattaattaacagactagttgtg-3'
E2A-miR-142-3pT-BstXI-AS3	5'-actagtctgttaattaatgtagtgtttcctactttatggagctgtgtagtgtttcctactttatggacaca-3'
E2A-miR-142-3pT-BstXI-S4	5'-tccataaagtaggaaacactacaggactccataaagtaggaaacactaca-3'
E2A-miR-142-3pT-BstXI-AS4	5'-ctagtgtagtgtttcctactttatggagtcctgtagtgtttcctactttatggaat-3'
Ad-E4-142-3pT	
E4-miR-142-3pT-S1	5'-ggcctccataaagtaggaaacactacacagctccataaagtaggaaacactacattaattaagcggtac-3'
E4-miR-142-3pT-AS1	5'-cgcttaattaatgtagtgtttcctactttatggagctgtgtagtgtttcctactttatgga-3'
E4-miR-142-3pT-S2	5'-tccataaagtaggaaacactacaggactccataaagtaggaaacactacagtac-3'
E4-miR-142-3pT-AS2	5'-tgtagtgtttcctactttatggagtcctgtagtgtttcctactttatggaat-3'

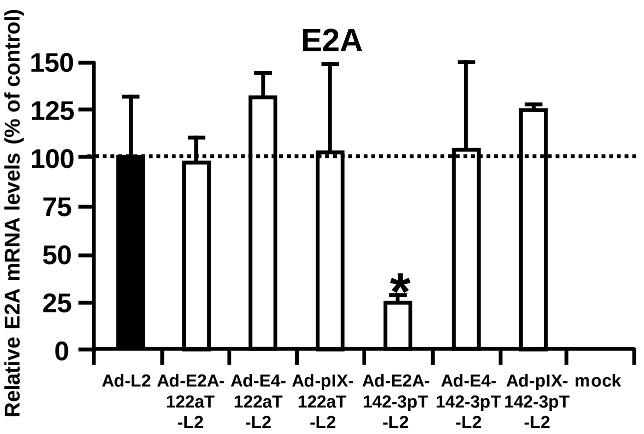
Ad-pIX-142-3pT	
pIX-miR-142-3pT-S1	5’-ctagctaatccataaagtaggaaacactacacagctccataaagtaggaaacactacagaattccagggtacc-3’
pIX-miR-142-3pT-AS1	5’-ctagggtaccctggaattctgtagtgtttcctactttatggagctgtgtagtgtttcctactttatggattag-3’
pIX-miR-142-3pT-S2	5’-aattgtccataaagtaggaaacactacaggactccataaagtaggaaacactacaggtac-3’
pIX-miR-142-3pT--AS2	5’-ctgtagtgtttcctactttatggagtcctgtagtgtttcctactttatggac-3’

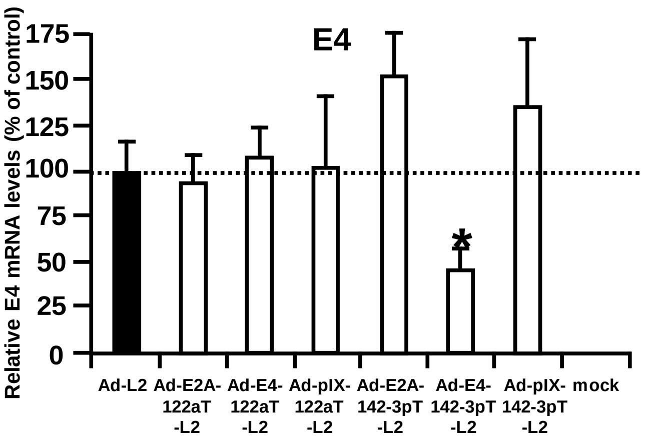
Underlines indicate miRNA-targeted sequences.

Supplementary Table S2 Sequences of primers for IFN-γ and chemokines.

Gene	Forward primer	Reverse primer	Amplicon size
IFN-γ	5’-ATG AAC GCT ACA CAC TGC ATC-3’	5’-TCT AGG CTT TCA ATG ACT GTG C-3’	92 bp
CCL2	5’-TTA AAA ACC TGG ATC GGA ACC AA-3’	5’-GCA TTA GCT TCA GAT TTA CGG GT-3’	121 bp
CCL3	5’-TTC TCT GTA CCA TGA CAC TCT GC-3’	5’-CGT GGA ATC TTC CGG CTG TAG-3’	100 bp
CCL4	5’-TTC CTG CTG TTT CTC TTA CAC CT-3’	5’-CTG TCT GCC TCT TTT GGT CAG-3’	121 bp
CCL5	5’-GCT GCT TTG CCT ACC TCT CC-3’	5’-TCG AGT GAC AAA CAC GAC TGC-3’	104 bp
CXCL2	5’-CGC TGT CAA TGC CTG AAG AC-3’	5’-ACA CTC AAG CTC TGG ATG TTC TTG-3’	62 bp
CX_3CL	5’-ACG AAA TGC GAA ATC ATG TGC-3’	5’-CTG TGT CGT CTC CAG GAC AA-3’	120 bp
CXCL10	5’-CCA AGT GCT GCC GTC ATT TTC-3’	5’-TCC CTA TGG CCC TCA TTC TCA-3’	133 bp

Figure S1


E2A
Relative E2A mRNA levels (% of control)
0
25
50
75
100
125
150
*
Ad-L2
Ad-E2A-122aT-L2
Ad-E4-122aT-L2
Ad-pIX-122aT-L2
Ad-E2A-142-3pT-L2
Ad-E4-142-3pT-L2
Ad-pIX-142-3pT-L2
mock


E4
Relative E4 mRNA levels (% of control)
0
25
50
75
100
125
150
175
*
Ad-L2
Ad-E2A-122aT-L2
Ad-E4-122aT-L2
Ad-pIX-122aT-L2
Ad-E2A-142-3pT-L2
Ad-E4-142-3pT-L2
Ad-pIX-142-3pT-L2
mock

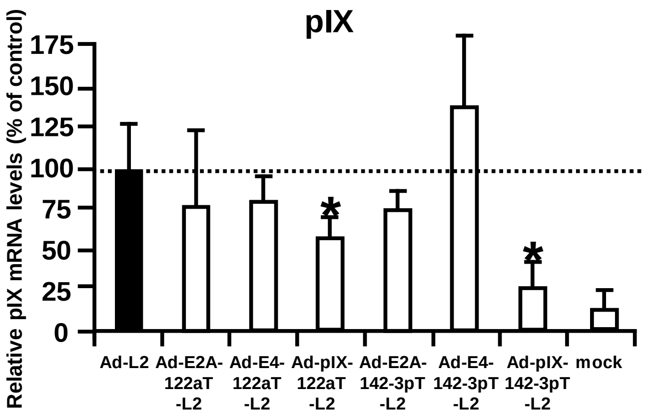

pIX
Relative pIX mRNA levels (% of control)
0
25
50
75
100
125
150
175
*
*
Ad-L2
Ad-E2A-122aT-L2
Ad-E4-122aT-L2
Ad-pIX-122aT-L2
Ad-E2A-142-3pT-L2
Ad-E4-142-3pT-L2
Ad-pIX-142-3pT-L2
mock

Figure S2

Figure S2A

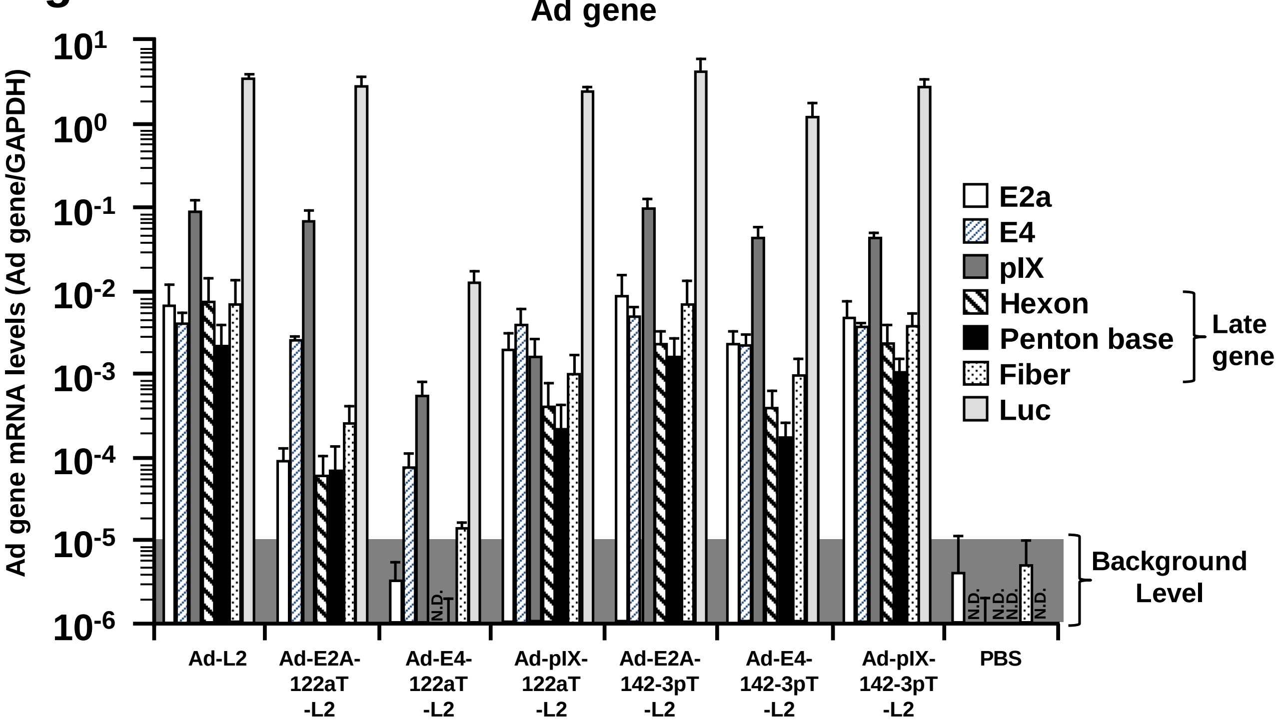


Figure S2B

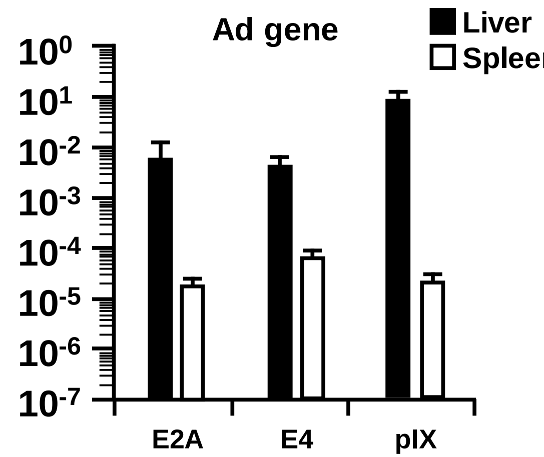
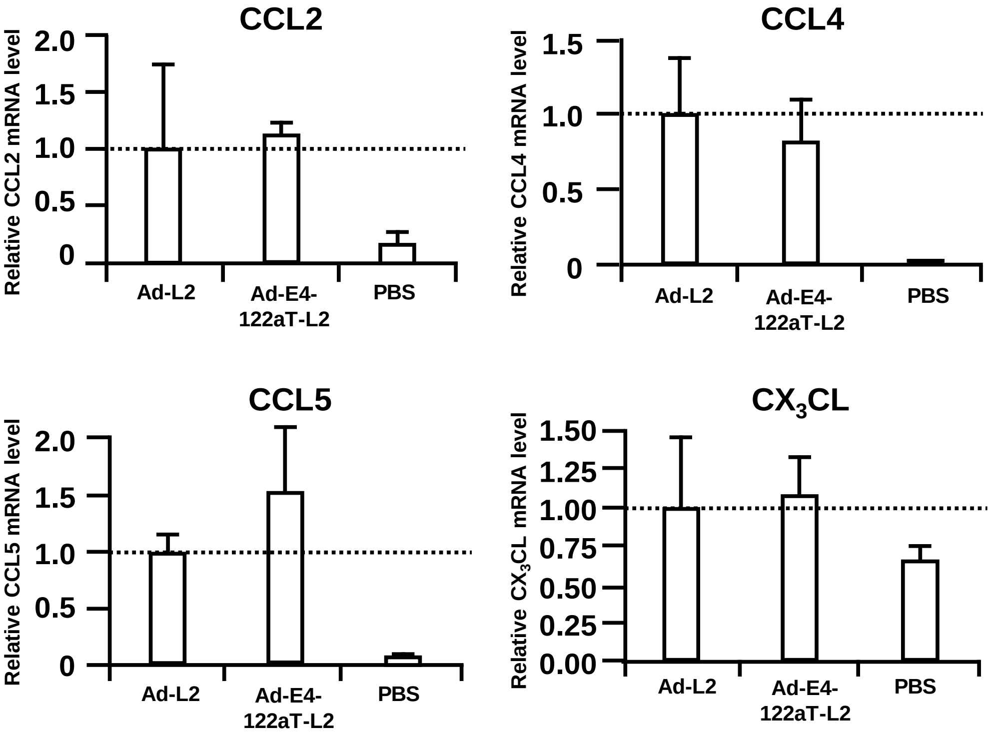


Figure S3


CCL2
Relative CCL2 mRNA level
2.0
1.5
1.0
0.5
0
Ad-L2
Ad-E4-122aT-L2
PBS
CCL4
Relative CCL4 mRNA level
1.5
1.0
0.5
0
Ad-L2
Ad-E4-122aT-L2
PBS
CCL5
Relative CCL5 mRNA level
2.0
1.5
1.0
0.5
0
Ad-L2
Ad-E4-122aT-L2
PBS
CX_3CL
Relative CX_3CL mRNA level
1.50
1.25
1.00
0.75
0.50
0.25
0.00
Ad-L2
Ad-E4-122aT-L2
PBS