Supplementary Data

Supplementary Methods

Real-time reverse transcrptase polymerase chain reaction analysis of microRNA

The procedure adapted from (Varkonyi-Gasic *et al*., 2007) was used for the amplification and detection of mature microRNAs by a stem-loop gene-specific reverse transcription primer. Briefly, stem-loop primers binding to the 3′ portion of microRNA molecule were designed to specifically reverse transcribe the mature microRNA of interest. For calibration, random hexamer was used for reverse transcription of 5S rRNA. One microgram of total liver RNA was transcribed using Expand Reverse Transcriptase (Roche Diagnostics GmbH). Quantification was used in a quantitative real-time polymerase chain reaction (FastStart SYBR Green Master; Roche Diagnostics GmbH) with the specific primer sets containing a microRNA-specific forward primer and stem-loop-specific oligonucleotide as a reverse primer. All samples were run along with negative reverse transcriptase controls to exclude DNA contamination and water blanks. microRNA levels were assessed from the cycle number at which the microRNA amplification exceeded the threshold crossing point (ct), and these values were standardized against the 5S rRNA value obtained from the same sample. microRNA levels were expressed as the fold-change compared with levels in the saline control mice, where fold-change $= 2^{(\text{saline control ct} - \text{experimental ct})}$. Stem-loop reverse transcription primer and amplification primer sets are listed in Supplementary Table S2.

Supplementary Reference

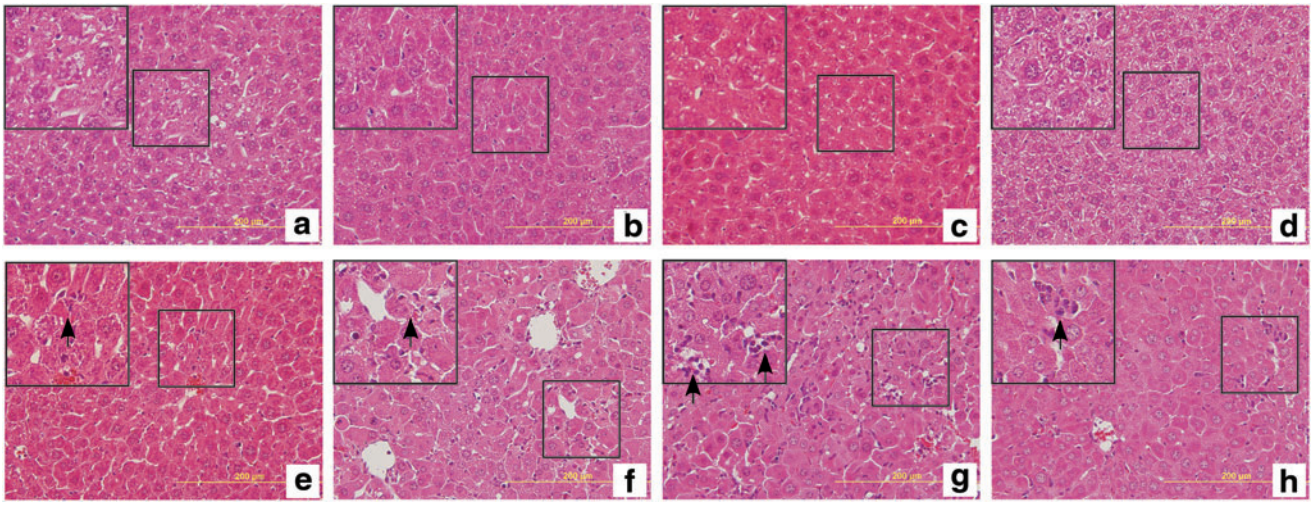
Varkonyi-Gasic, E., Wu, R., Wood, M., *et al.* (2007). Protocol: a highly sensitive RT-PCR method for detection and quantification of microRNAs. Plant Methods 3, 12.

Supplementary Table S1. Primer Sets for Quantitative Real-Time Polymerase Chain Reaction of Mouse Cytokine And Chemokine

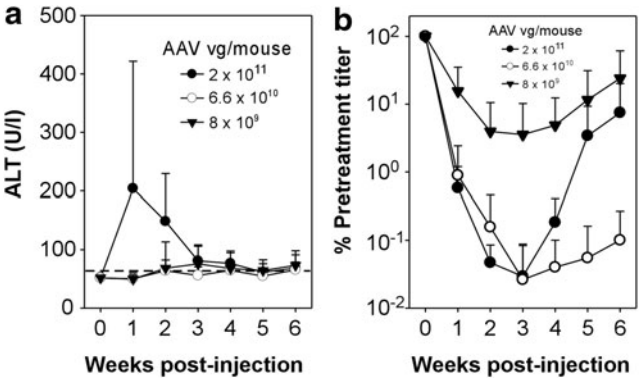
	Forward	*Reverse*
OAS-1	5′-AGGTGGTAAAGGGTGGCT CC-3′	5′-ACAACCAGGTCAGCGTCAGAT-3′
IFN-β	5′-AGCTCTTCAACTGGAGAGCAGTTGAGG-3′	5′-CCACAGCCCTCTCCATCAACTATAAGC-3′
TNF-α	5′-ACACCCATTCCCTTCACAGA-3′	5′-CCTCCCTCTCATCAGTTCTATG-3′
IL-6	5′-CTCCTTCTGTGACTCCAGCT-3′	5′-ACGGCCTTCCTACTTCACA-3′
IL-7	5′-CTTGTGCAGTTCACCAGTGT-3′	5′-GGAATTCCTCCACTGATCCTTG-3′
IFN-γ	5′-TGGACCTGTGGGTTGTTGACCTCAAACTTGGC-3′	5′-TGCATCTTGGCTTTGCAGCTCTTCCTCATGGC-3′
MIG	5′-GAAGTCCGCTGTTCTTTTCCT-3′	5′-GCATCGTGCATTCCTTATCA-3′
IP-10	5′-TGCTGTCCATCACAGCACCG-3′	5′-CGCTGAGAGACATCCCGAGC-3′
MIP-2	5′-GAACAAAGGCAAGGCTAACTGA-3′	5′-AACATAACAACATCTGGGCAA T-3′
MCP-1	5′-CTGTCACACTGGTCACTCCT-3′	5′-TCCCAATGAGTAGGCTGGAG-3′
MIP-1α	5′-AGGCATTCAGTTCCAGGTCA-3′	5′-TCCACCACTGCCCTTGCTGT-3′
MIP-1β	5′-TCAGTTCAACTCCAAGTCAC-3′	5′-GCTCTGCGTGTCTGCCCTCT-3′
RANTES	5′-CTCTATCCTAGCTCATCTC-3′	5′-CATGAAGATCTCTGCAGCTG-3′
GAPDH	5′-TCAACGGCACAGTCAAGG-3′	5′-GTAGCCCAAGATGCCCTTC-3′

Supplementary Table S2. Primer Sets for Quantitative Real-Time Polymerase Chain Reaction of Mouse microRNA

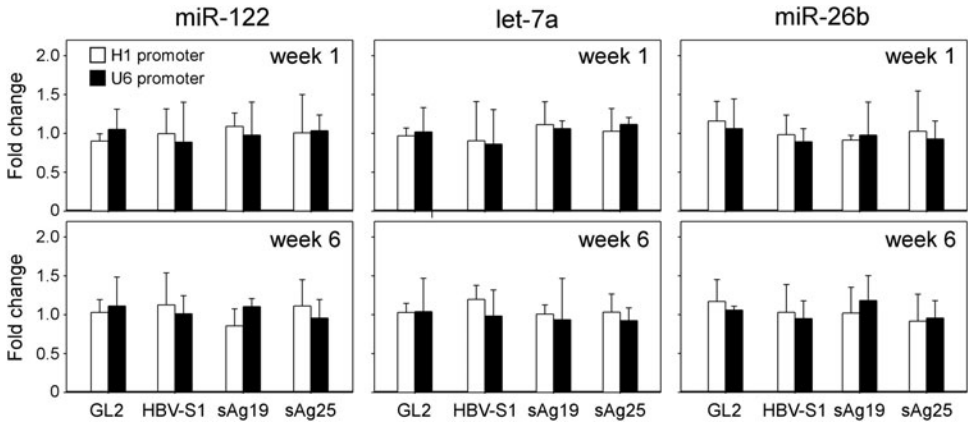
	Reverse transcription primer	*Forward*
miR-122	5′-GTTGGCTCTGGTGCAGGGTCCGAGGTATTCGCACCAGAG CCAACCAAACA-3′	5′-TTCCGTGGAGTGTGACAATGG-3′
let-7a	5′-GTTGGCTCTGGTGCAGGGTCCGAGGTATTCGCACCAG AGCCAACAACTAT-3′	5′-GCCGGTGAGGTAGTAGGTTGT-3′
miR-26b	5′-GTTGGCTCTGGTGCAGGGTCCGAGGTATTCGCACCAG AGCCAACAACCTA-3′	5′-GGTCGGTTCAAGTAATTCAGGA-3′
Universal reverse primer for microRNA		5′-GTGCAGGGTCCGAGGT-3′
	Forward	*Reverse*
5S rRNA	5′-CGGCCATACCACCCTGAAC-3′	5′-CGGTCTCCCATCCAAGTACTA-3′



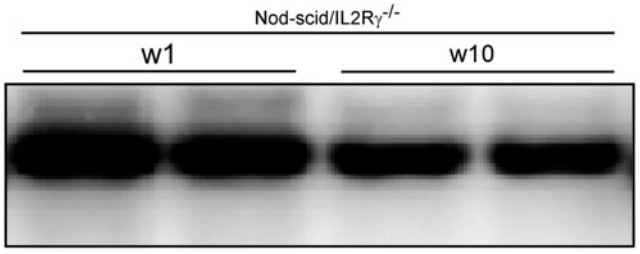
SUPPLEMENTARY FIG. S1. Pathological analysis of ICR/HBV mice injected with shRNA-encoding AAV-H1 or AAV-U6 vectors. ICR/HBV mice were injected intravenously with 10^{12} vg per mouse of different AAV8-H1 **(a–d)** or AAV8-U6 **(e–h)** vectors expressing GL2 **(a, e)**, HBV-S1 **(b, f)**, sAg19 **(c, g)**, or sAg25 **(d, h)**, and euthanized at day 7 **(b, c, f, g)** or day 14 **(a, d, e, h)**, the time when serum ALT activity peaked. Histologic analysis of hematoxylin and eosin–stained liver sections revealing focal necrosis (arrow, inset) and mononuclear cell aggregation. AAV, adeno-associated virus; ALT, alanine aminotransferase; HBV, hepatitis B virus.



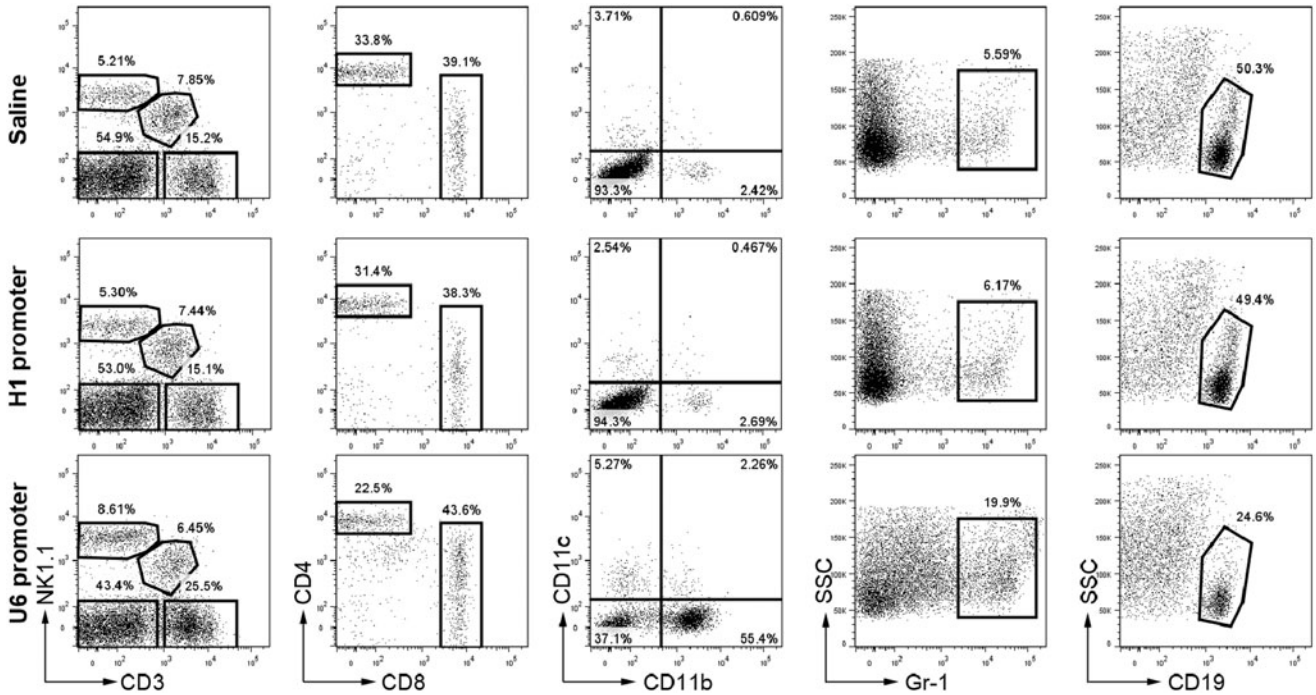
SUPPLEMENTARY FIG. S2. *In vivo* HBV DNA inhibition and liver injury with lower doses of AAV8-U6/HBV-S1. Groups of ICR/HBV mice ($n=7$) were injected intravenously with 2×10^{11}, 6.6×10^{10}, or 8×10^{9} vg per mouse of AAV8-U6/HBV-S1 as in Figure 2, and serum were samples collected at the indicated times for measurement of ALT activity **(a)** and HBV DNA levels **(b)** in which the results are displayed as a percentage of the pretreatment titer for each group (mean ± SD). The dashed lines represent the mean value for ALT activity in untreated ICR/HBV mice ($n=6$).



SUPPLEMENTARY FIG. S3. Measurement of endogenous microRNA in the liver by real-time reverse transcriptase polymerase chain reaction. ICR/HBV mice ($n=3$) were injected intravenously with 10^{12} vg per mouse of the different AAV8-H1 or AAV8-U6 vectors as in Figure 2, and then were euthanized at 1 or 6 weeks after AAV transduction. The amounts of miR-122, let-7a, and miR-26b transcripts were analyzed by real-time reverse transcriptase polymerase chain reaction, normalized to 5S rRNA expression. microRNA levels were expressed as the fold-change compared with levels in the saline control mice (mean ± SD). Differences were nonsignificant.



SUPPLEMENTARY FIG. S4. The copy number of AAV vectors in the liver of Nod-scid/IL2R$\gamma^{-/-}$ (NSG) mice. NSG mice were injected intravenously with 10^{12} vg per mouse of AAV8-U6/sAg19, and then were euthanized at 1 or 10 weeks after AAV transduction. Southern blot analysis of AAV vectors was performed as in Figure 3.



SUPPLEMENTARY FIG. S5. Representative flow cytometry data for liver leukocytes. Livers from groups of C57BL/6 mice ($n = 3$) injected with saline, AAV8-H1/sAg19, or AAV8-U6/sAg19 were harvested at day 4 for isolation of hepatic leukocytes. The cells were multiple-stained with different antibodies and analyzed for T cells ($CD3^+$ $NK1.1^-$), NK cells ($NK1.1^+CD3^-$), NKT cells ($NK1.1^+$ $CD3^+$), CD4 cells ($NK1.1^-CD3^+CD4^+$), CD8 cells ($NK1.1^-CD3^+CD8^+$), dendritic cells ($NK1.1^-CD3^-CD11c^+CD11b^-$), macrophages ($NK1.1^-CD3^-CD11c^-CD11b^+$), granulocytes ($Gr\text{-}1^+$), and B cells ($NK1.1^-CD3^-CD19^+$) by flow cytometry. The percentages of the individual cell populations are shown above the boxes.