Supporting Information

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SI Methods

In Vitro T-Cell Proliferation Assays with Hepatocytes. Mice were treated with 10^{11} vector genome copies (vgc) of recombinant adenoassociated virus (rAAV).GFP–membrane-anchored form of ovalbumin (rAAV.GFP-mOVA) or rAAV.GFP-mOVA-T4. Four days later, hepatocytes from treated and untreated mice were harvested by retrograde perfusion as described previously (1). Different numbers of hepatocytes were then cocultured with 10^5 OT-I lymph node (LN) cells labeled with 5 μM carboxyfluorescein succinimidyl ester (CFSE) in RPMI medium supplemented with 10% FCS and 50 μM β-mercaptoethanol. Medium was also supplemented with 20 ng/mL IL-2 (BD Biosciences) in some cultures. After 3 d of incubation at 37 °C with 5% CO_2, cells were restimulated with synthetic SIINFEKL peptide and the percentages of $CD8^+$ OT-I cells producing IFN-γ were determined by intracellular cytokine staining as described earlier.

Quantification of Cytokine mRNA Expression by Quantitative PCR. $CD8^+$ $CD45.1^+$ OT-I were sorted after purification from the livers of AAV.mOVA-treated B6 mice and restimulated ex vivo with SIINFEKL (0.1 μg/mL) for 8 h. Total RNA was isolated using the RNeasy Micro Kit (Qiagen), and cDNA was synthesized by using MMLV SuperScript II and random hexamers (Promega). Total cDNA was diluted 1:5 and amplified by using TaqMan gene-specific primer and probe sets (Applied Biosystems) in TaqMan quantitative PCR master mix (Applied Biosystems). Real-time acquisition and analysis was performed on the MX3005P real-time PCR system (Stratagene).

1. Bertolino P, Trescol-Biémont MC, Rabourdin-Combe C (1998) Hepatocytes induce functional activation of naive CD8+ T lymphocytes but fail to promote survival. *Eur J Immunol* 28(1):221–236.

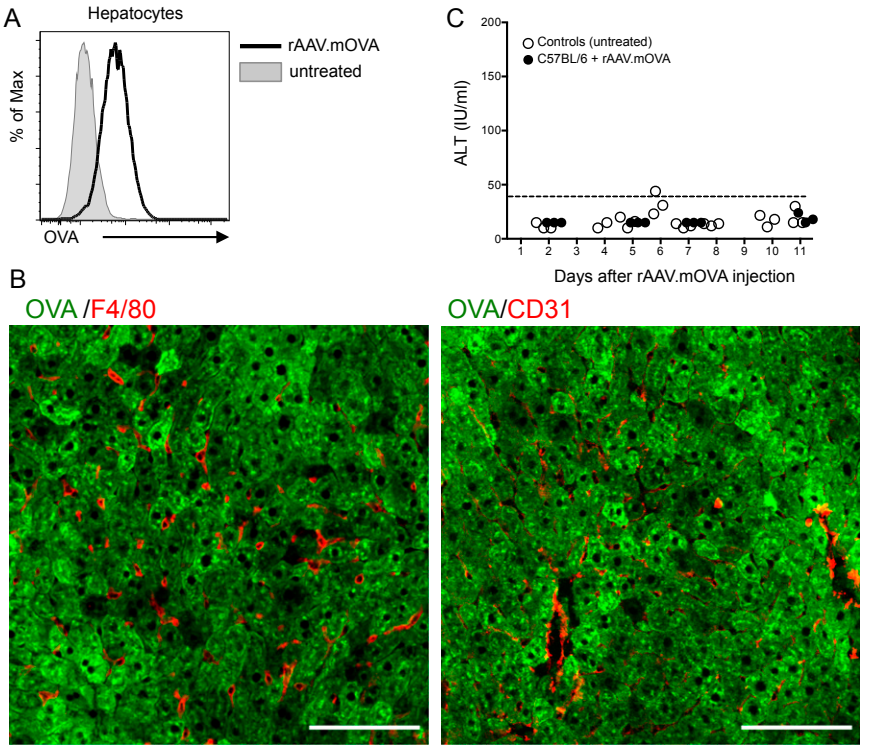

Fig. S1. Expression of OVA by most hepatocytes following administration of rAAV.mOVA does not lead to hepatotoxicity. (*A*) OVA expression on purified hepatocytes from B6 mice injected i.v. with 5×10^{10} vgc rAAV.mOVA 7 d earlier (black line) compared with untreated controls (shaded) assessed by flow cytometry. Overlays are representative of two independent experiments. (*B*) Merged confocal microscopic images of liver sections showing OVA expression in livers from B6 mice treated 3 wk earlier with 5×10^{10} vgc rAAV.mOVA compared with untreated mice. OVA expression (green) did not colocalize with $F4/80^+$ Kupffer cells (red, *Left*) or $CD31^+$ liver endothelial cells (red, *Right*). Images are representative of sections from four mice. (Scale bars: 100 μm.) (*C*) Serum alanine aminotransferase levels in B6 mice after treatment with 10^{11} vgc rAAV.mOVA, compared with age-matched controls that did not receive rAAV.mOVA. Dashed line indicates average alanine aminotransferase value from 15 control mice.

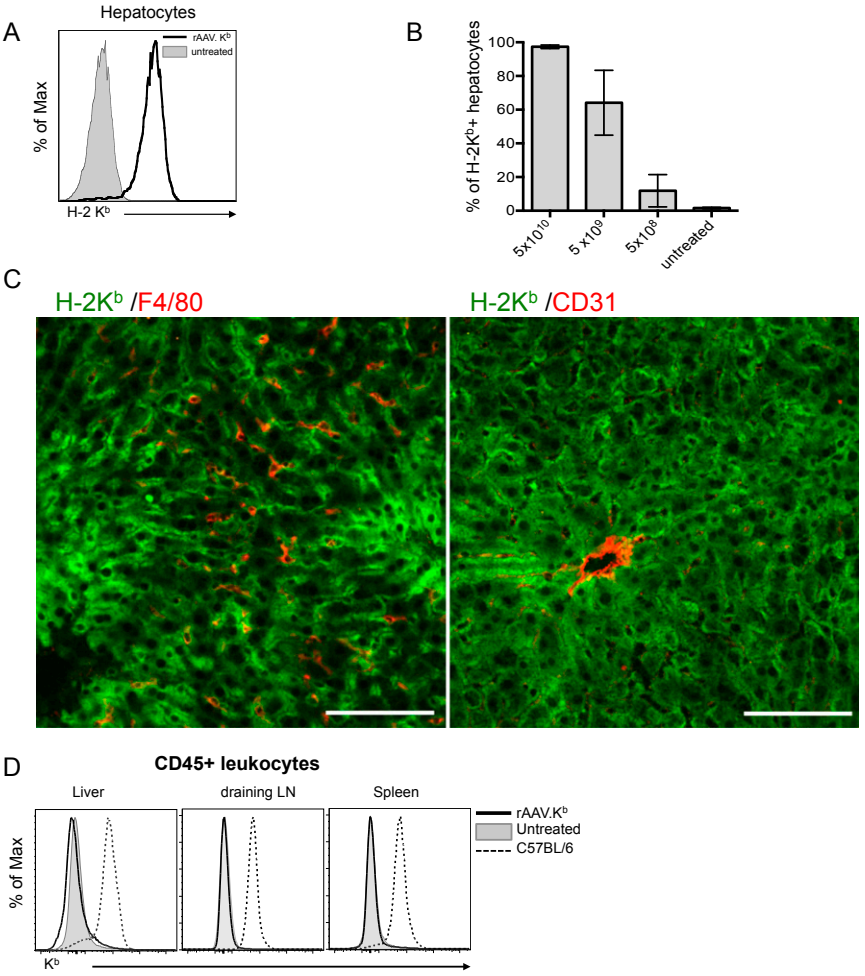

Fig. S2. Hepatocyte-specific H-2K^b expression induced by rAAV.K^b-treatment. (*A*) Surface H-2K^b expression on purified, live (PI$^-$) hepatocytes from B10.BR mice treated with rAAV.K^b (5×10^{10} vgc) 7 d earlier compared with untreated controls. (*B*) Percentages of H-2K^{b+} hepatocytes 7 d after B10.BR mice were treated with indicated doses of rAAV.K^b. Representative data from three mice per group are shown. (*C*) Merged confocal microscopic images of liver sections showing that H-2K^b expression (green) in liver does not colocalize with F4/80$^+$ Kupffer cells (red, *Left*) or CD31$^+$ liver endothelial cells (red, *Right*). (Scale bars: 100 μm.) (*D*) Lack of H-2K^b expression by CD45$^+$ leukocytes isolated from the liver, liver-draining LN, and spleen of rAAV.K^b-treated B10.BR mice (black line), compared with untreated B10.BR (shaded) or B6 mice (dashed). B6 leukocytes were used as positive controls for H-2K^b expression. Plots represent three mice from two independent experiments.

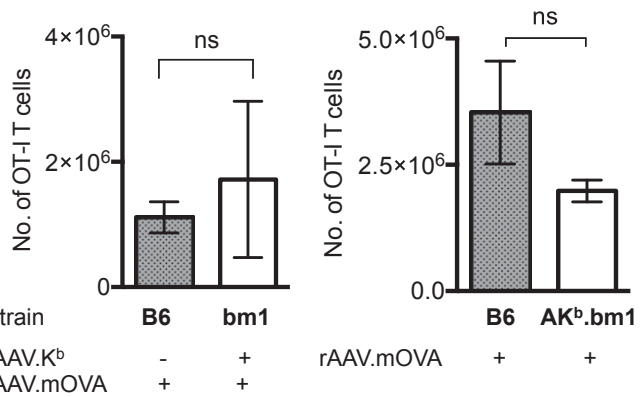

Fig. S3. Expansion of OT-I T cells in response to rAAV.mOVA treatment. Absolute OT-I T-cell counts performed 7 d after OT-I T cells were transferred into B6, Alb-K^b.bm1, or bm1 mice treated with rAAV.mOVA and/or rAAV.K^b (5×10^{10} vgc each) the following day.

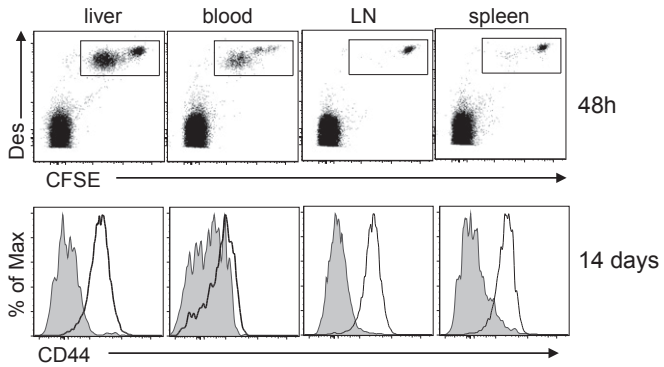

Fig. S4. Des T-cell proliferation in response to rAAV.K^b treatment. (*Upper*) Des T cell (rectangle gate, CD8$^+$Des$^+$) proliferation 48 h after transfer of 4×10^6 Des recombinase activating gene$^{-/-}$ LN cells into B10.BR mice pretreated with rAAV.K^b (5×10^{10} vgc) 7 d earlier. (*Lower*) CD44 expression by Des T cells 2 wk after transfer into B10.BR mice treated with rAAV.K^b (5×10^{10} vgc) 7 d before T-cell transfer, compared with untreated B10.BR controls (shaded). Plots representative of four or five mice in each group.

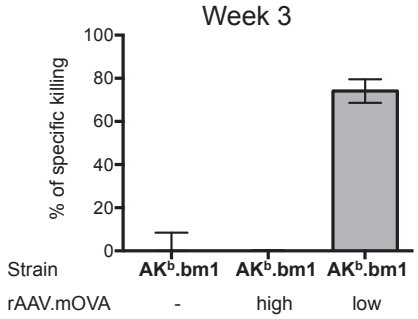

Fig. S5. Cytotoxicity of transferred OT-I.bm1 cells transferred into Alb-K^b.bm1 mice treated with rAAV.mOVA. In vivo cytotoxicity to SIINFEKL-coated splenocytes measured 3 wk after 2×10^6 OT-I.bm1 LN cells were transferred into Alb-K^b.bm1 mice treated with low-dose (5×10^8 vgc) or high-dose (5×10^{10} vgc) rAAV.mOVA 1 d after OT-Ibm1 transfer.

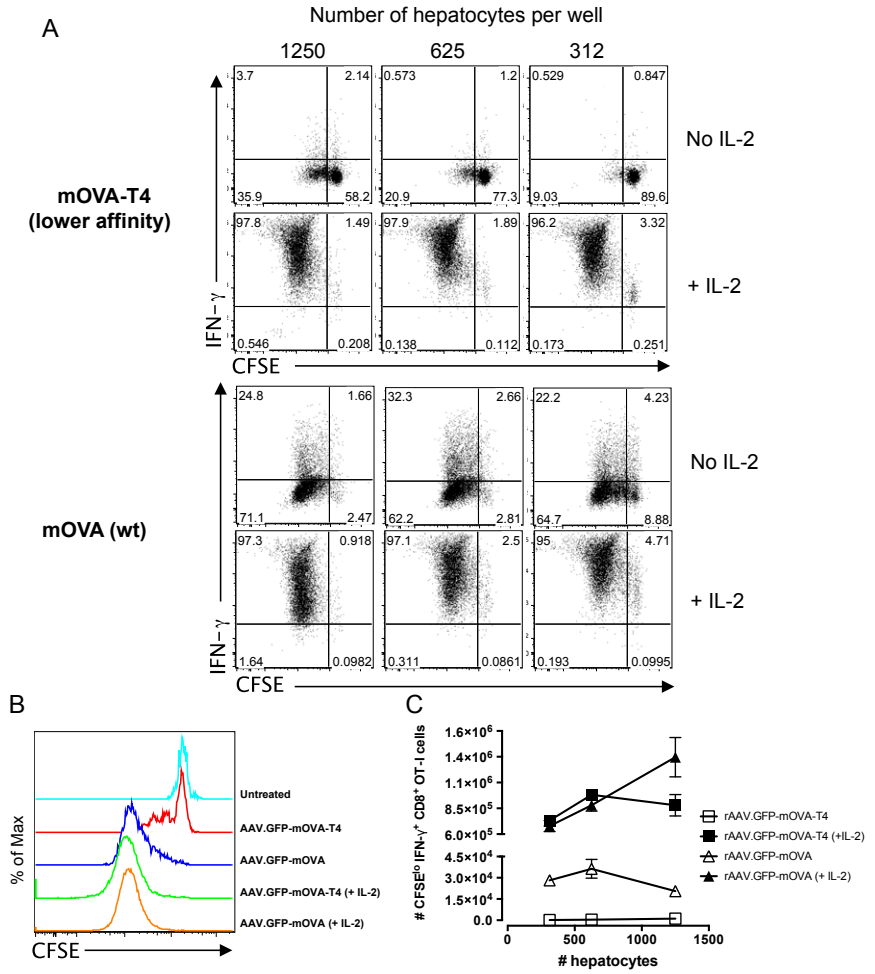

Fig. S6. Effects of in vitro stimulation of OT-I T cells by hepatocytes transduced with high- vs. low-affinity ligand. C57BL/6 mice were administered 1×10^{11} vgc of rAAV.GFP-mOVA or rAAV.GFP-mOVA-T4. Hepatocytes were harvested 4 d later by retrograde perfusion and collagenase digestion. Varying numbers of hepatocytes were then cocultured in RPMI medium supplemented with 10% FCS and 50 μM β-mercaptoethanol with 1×10^5 OT-I LN cells previously labeled with 5 μM CFSE. Medium was also supplemented with 20 ng/mL IL-2 (BD Biosciences) in some cultures. After 3 d of incubation at 37 °C with 5% CO_2, cells were restimulated with synthetic SIINFEKL peptide and the percentages of $CD8^+$ OT-I cells producing IFN-γ determined by intracellular cytokine staining as described for Fig. 7. (*A*) Representative FACS plots demonstrating division (CFSE dilution) and IFN-γ production by OT-I T cells cocultured with varying numbers of hepatocytes as indicated, transduced with high- or low-affinity ligand, in the presence or absence of IL-2. (*B*) CFSE division profiles of OT-I T cells under conditions as per *A*; (*C*) Numbers of dividing ($CFSE^{low}$) cells producing IFN-γ following restimulation under the aforementioned conditions. Plots represent mean ± SEM of two replicates per group. This experiment was repeated once with similar results.

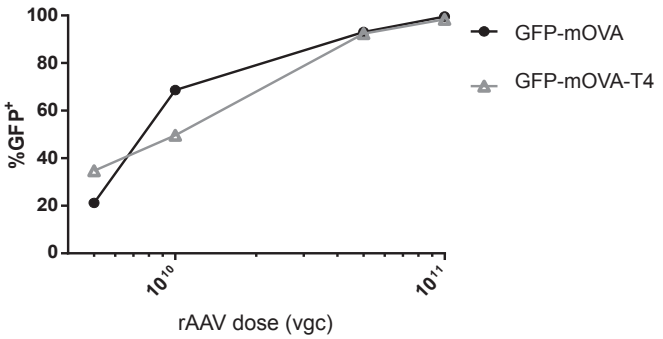

Fig. S7. Treatment with rAAV.GFP-mOVA and rAAV.GFP-mOVA-T4 lead to the same level of hepatocyte transduction. Percentage of GFP-expressing hepatocytes isolated from B6 mice treated with various doses of rAAV.GFP-mOVA or rAAV.GFP-mOVA-T4 7 d earlier (n = 1 mouse per data point).

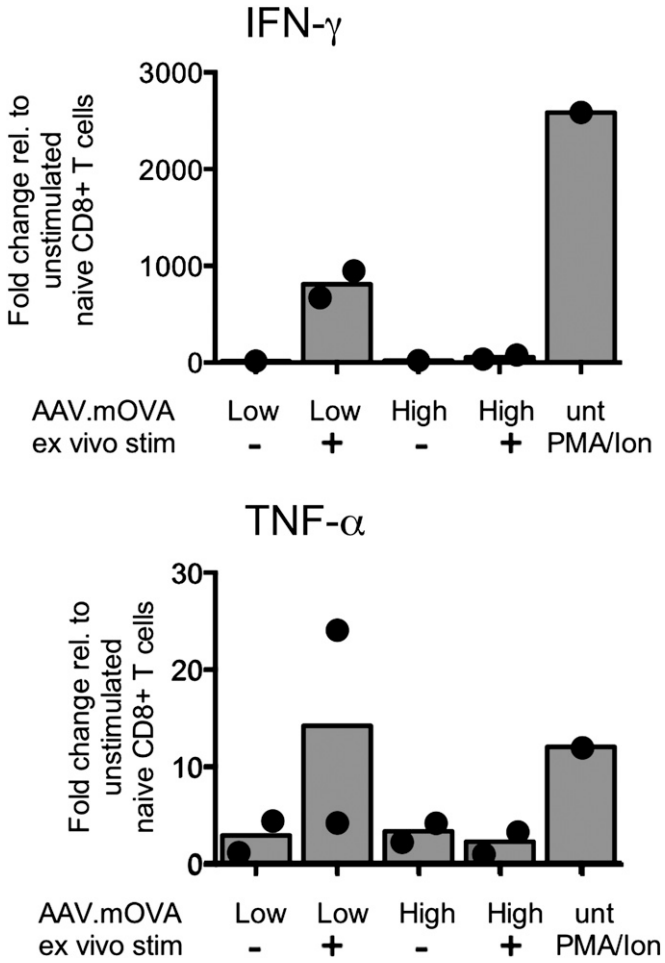

Fig. S8. Intrahepatic OT-I T cells mice treated with a high dose of rAAV.mOVA did not express IFN-γ and TNF-α upon peptide restimulation. IFN-γ and TNF-α mRNA expression was analyzed in sorted OT-I T cells transferred into B6 mice treated with high- and low-dose AAV.mOVA at week 3 after transfer. T cells were restimulated ex vivo with SIINFEKL (0.1 μg/mL) for 8 h. In the first experiment, 5,000–10,000 cells per group were sorted; in the second experiment, 88,000 cells per group were sorted. The relative gene expression to unstimulated OT-I sorted from untreated B6 mice was calculated by using the 2^-[δ][δ]Ct method after Ct values of the gene of interest were normalized to the average Ct values of the three housekeeping genes (HRPT, GAPDH, and β-actin). Ct values obtained for IL-2, IL-17A, and IL-10 in the positive control sample (OT-I sorted from untreated B6 mice stimulated ex vivo with PMA and ionomycin for 8 h) were 32, 34, and 40, respectively, whereas the same cytokines were not detected in OT-I sorted from all AAV-treated mice (all Ct values >45).

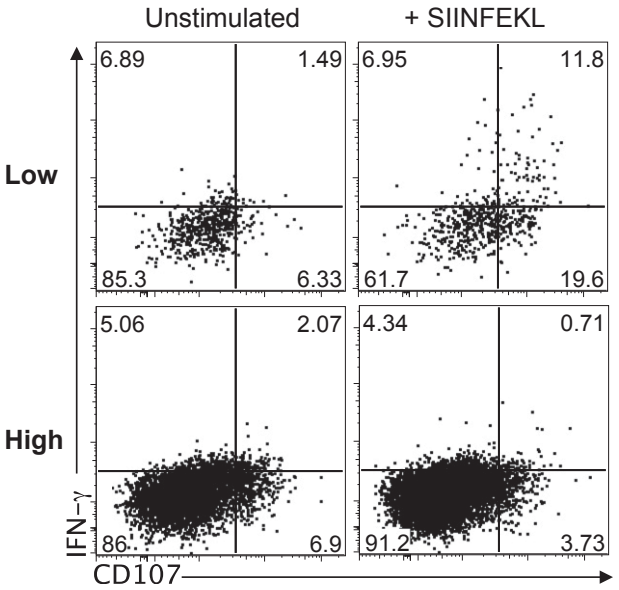

Fig. S9. Function of OT-I T cells following adoptive transfer of a small inoculum into mice treated with rAAV.mOVA. 1000 OT-I LN cells (500 CD8 OT-I T cells) were adoptively transferred into C57BL/6 mice. One day later, mice were administered a low dose (5×10^8 vgc) or a high dose (5×10^{10} vgc) of rAAV.mOVA. Liver leukocytes were harvested 3 wk later, and CD107 degranulation assay and intracellular staining for IFN-γ was performed as described for Fig. 7.