Supplemental Material and Methods

Generation of recombinant adenoviruses

We designed an expression cassette for cloning into recombinant adenovirus that consists of the genes for GFP, ovalbumin and CBG99-luciferase separated by P2A linker sites from the Porcine Teschovirus 1 followed by a bGH poly(A) signal. The expression cassette was either cloned after a minimal CMV-promoter or the hepatocyte-restricted murine Transthyretin (TTR)-promoter.

Recombinant second generation serotype 5 adenoviruses were generated using the Gateway® technology from ThermoFisher. Expression cassettes (eGFP, ovalbumin, luciferase genes and the bGH poly(A) signal) with either CMV or TTR-promotor, were synthesized (Eurofins Genomics, Germany) and cloned into Gateway® pENTR™11 Dual Selection Vector (ThermoFisher Scientific, Germany). Recombination of pENTR™ with expression cassette into pAD/PL-DEST™ Gateway® vector (ThermoFisher Scientific, Germany) was performed *in vitro* via the LR Clonase® Enzyme Mix (ThermoFisher Scientific, Germany). The obtained pAD/PL-DEST™ vector with expression cassette was linearized using the PacI restriction enzyme. The resulting adenoviral DNA was transfected into HEK293 cells (CRL-1573™; ATCC, USA) by Lipofectamine 2000 (ThermoFisher Scientific, Germany). Cells were harvested after complete detachment from cell culture flasks. Supernatant was freeze/thawed, centrifuged and used for further infection of HEK293 cells. Cells from several cell culture dishes were harvested, resuspended in Tris-buffer and freeze/thawed three times to establish high-titer viral stocks. Cell debris was removed by centrifugation and supernatant was purified by a two-step CsCl gradient ultracentrifugation. The band containing adenovirus was harvested and dialyzed. By this, we generated recombinant Ad-CMV-GOL or Ad-TTR-GOL, which express identical expression cassettes driven by either minimal CMV or hepatocyte-specific TTR-promoter.

Virus titers in stocks were determined via adenovirus hexon titration. HEK293 cells were infected with serial dilutions of purified adenovirus. After 35 to 40 hours, cells were fixed with methanol, virus infected cells were stained with anti-Hexon antibody (anti-Hexon 2297HRP, Acris, Germany) and detected via DAB (Dako). The infected cells were counted and the titer was calculated.

Isolation of leukocytes from spleen and liver

Dendritic cells (DCs) from the spleen were obtained by immunomagnetic separation using $CD11c^+$ microbeads (Milteny Biotec, Germany). Splenic $CD11c^+$ DCs were used immediately for the *in vitro* CD8 T cell proliferation assay.

For isolation of lymphocytes from livers, mice were sacrificed and the liver was perfused via the portal vein with PBS, mechanically dissected and passed through a sieve. After washing in PBS, liver cells were incubated in Gey`s balanced-salt solution (PAN Biotech, Germany) and 0,125 u/mg collagenase type 2 (Worthington) for 10 minutes at 37 °C. Lymphocytes were isolated by percoll (GE Healthcare) gradient centrifugation (40%/80%). After gradient centrifugation, lymphocytes were washed and used directly for flow-cytometric analysis.

Isolation of murine hepatocytes

For hepatocyte isolation, murine livers were perfused via the portal vein for 8 minutes with buffer containing 0.12 U/mL collagenase (SERVA Electrophoresis GmbH, Germany), were mechanically dissected, filtered through a mesh (300 µm) and centrifuged at 50xg for two minutes to remove cellular debris. Hepatocytes were washed twice, filtered through a mesh (100 µm) and isolated by 80%/50% percoll gradient centrifugation.

Real-time PCR

Real-time PCR for adenoviral genomes from liver tissue was performed with 100 ng genomic DNA isolated from liver tissue by NucleoSpin® Tissue kit (Macherey-Nagel, Germany). We used 100 nM forward (TAAGCGACGGATGTGG) and reverse (CCACGTAAACGGTCAAAG) primer and SYBR Green Master I Mix (Roche Molecular Systems, Inc). PCR reactions were performed with the LightCycler® 480 II System (Roche Molecular Systems) and data were analyzed by LightCycler® 480 SW 1.5.1 software.

Liver immunohistochemistry

Mouse livers were fixed in 10% neutral-buffered formalin solution. 48 hours dehydrated livers (Leica ASP300S, Wetzlar, Germany) were embedded in paraffin. Serial 2µm-thin sections were prepared with a rotary microtome (HM355S, ThermoFisher Scientific, USA) and subjected to histological and immune-histochemical analysis. Hematoxylin-Eosin (HE) staining was performed on deparaffinized sections with Eosin and Mayer's Haemalaun according to standard protocol.

Immunohistochemistry was performed using a BondMax RXm system (Leica, Wetzlar, Germany, all reagents from Leica) with primary antibody against eGFP (A-11122, diluted 1:500 in antibody diluent, Invitrogen, ThermoFisher Scientific, Waltham, USA). Slides were deparaffinized, pretreated with Epitope Retrieval solution 1 for 30 minutes. Bound antibody was detected with a Polymer Refine detection kit without post primary reagent and visualized with DAB as a dark brown precipitate. Counterstaining was done with hematoxyline.

Flow cytometry

Flow-cytometric analysis was performed with an SP6800 Spectral Analyzer (Sony Biotechnology, Weybridge, UK). The data were analyzed by FlowJo software V10.1 (TreeStar Inc., Ashland, USA). Transferred CD45.1$^+$ OT-1 x CD45.1 T cells were stained by H-2K$^{b/SIINFEKL}$ MHC Dextramers (Immudex). For phenotypic analysis, the following antibodies were used: anti-CD8alpha (clone 53-6.7), anti-CD45.1 (clone A20), anti-CD25 (clone PC61), anti-CD44 (clone IM7), anti-CD69 (clone H1.2F3), anti-CD62L (clone MEL-14), anti-PD-I (clone J43), anti-LAG-3 (clone C9B7W), anti-Tim-3 (clone RMT3-23), anti-CD160 (clone 7H1), anti-CTLA-4 (clone UC10-4B9), anti-KLRG-1 (clone 2F1/KLRG1), anti-TNF (MP6-XT22), anti-IFNγ (clone XMG1.2), fixable viability dye eFluor^{TM} 520. Antibodies were purchased from eBioscience or BioLegend.

For *ex vivo* restimulation and intracellular cytokine staining, transferred CD45.1$^+$ OT-1 CD8 T cells were isolated from liver or spleen and incubated over night with 10 nM SIINFEKL peptide and 3 µg/ml brefeldin A (eBioscience) at 37°C. After staining with anti-CD8a, anti-CD45.1 and viability dye aFluor780, CD8 T cells were fixed in IC fixation buffer (eBioscience) for 20 minutes. Intracellular staining for TNF and IFNγ was performed in Permeabilisation Buffer (eBioscience).

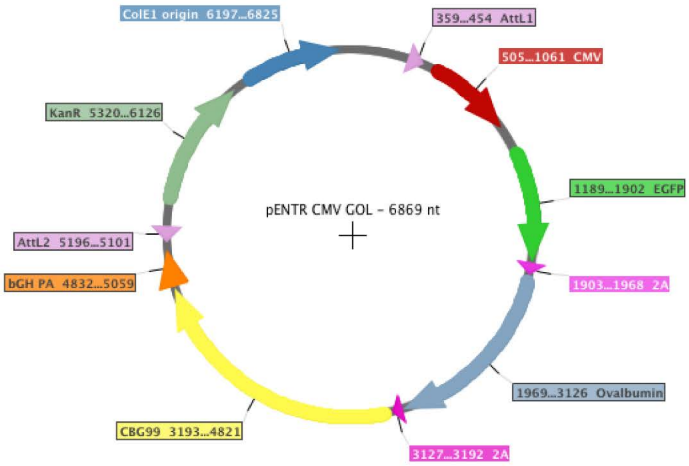
Figure Legends

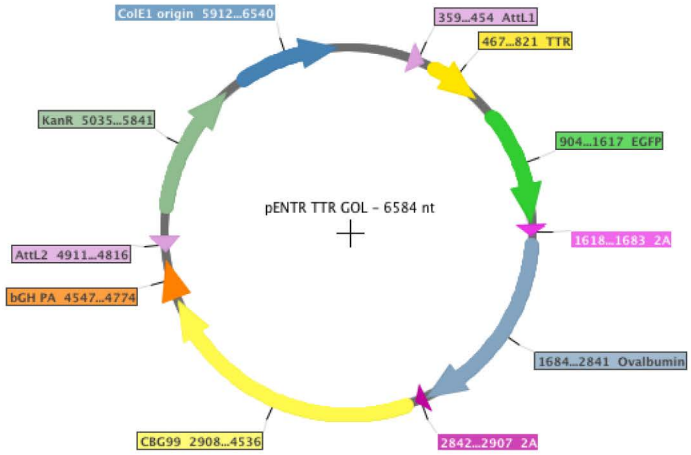
Supplemental Figure 1: Vector maps of expression cassettes.

pENTR vector with expression cassette consisting of CMV promoter, eGFP, ovalbumin and luciferase genes linked by P2A sequences and followed by an bGH polyadenylation signal.

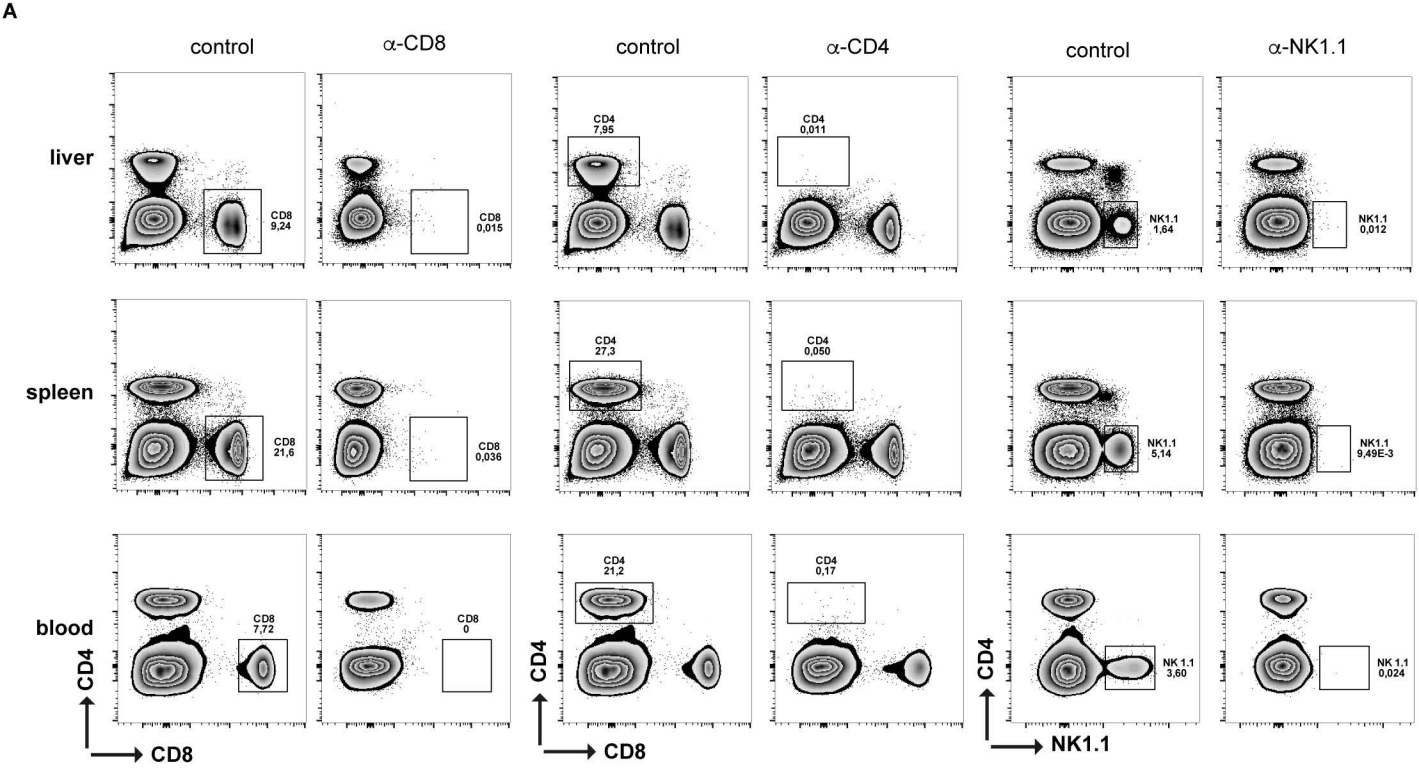
Supplemental Figure 2: Effect of CD8 T cell depletion on anti-viral immunity.

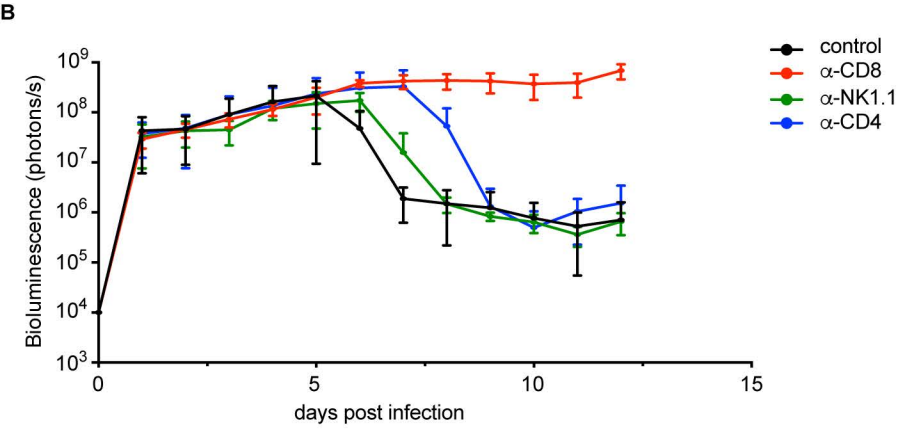
(A) Flow cytometric analysis of CD8 T cells, CD4 T cells and NK1.1 cells in liver, spleen and blood after infection with 10^7 pfu Ad-CMV-GOL and treatment by α-CD8, α-CD4 or α-NK1.1 depleting antibody. (**B**) Quantification of bioluminescence in mice infected with 10^7 pfu Ad-CMV-GOL and treated with α-CD8, α-CD4 or α-NK1.1 depleting antibody as in (A). (A-B) All experiments have been performed at least three times and were reproducible.





Supplemental Figure 1





Supplemental Figure 2