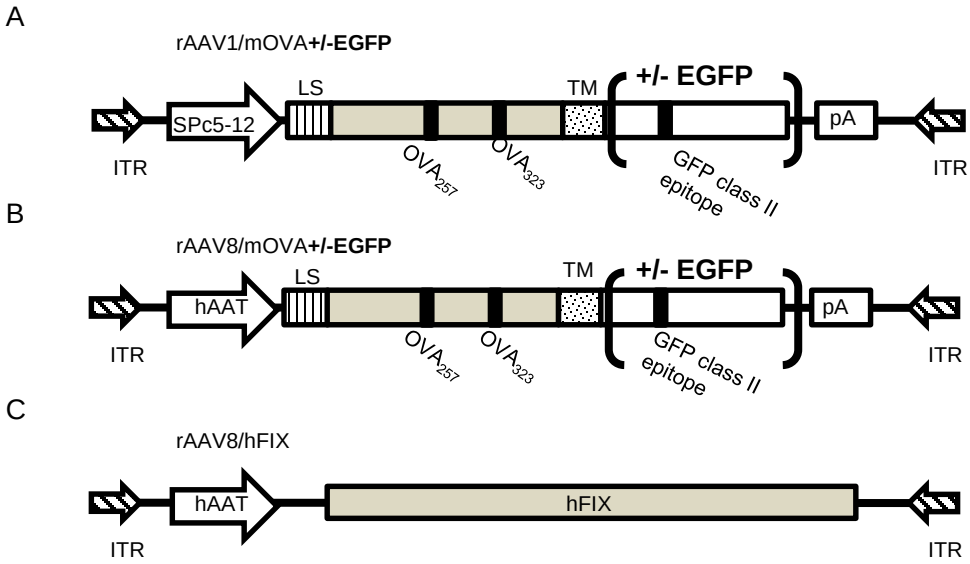

Figure S1. **rAAV constructs.**
The mOVA construct comprises the leader peptide from the H-2K^b gene (LS), the full-length OVA cDNA including the MHC I and MHC II epitope, OVA_{257} and OVA_{323} respectively, the H-2D^b transmembrane sequence (TM) followed by a STOP codon and a poly A chain (pA). The mOVA-GFP construct comprises the leader peptide from the H-2K^b gene (LS), the full-length OVA cDNA, the H-2D^b transmembrane sequence (TM)and the full-length EGFP cDNA, including the EGFP MHC II epitope (HDFFKSAMPEGYVQE). The muscle targeting construct (A) contains the muscle-specific promotor SPc5-12 and two ITR sequences for encapsulation in rAAV1, which have a strong tropism for muscle. The liver targeting construct (B) contains the liver-specific promotor hAAT and two ITR sequences for encapsulation in rAAV8, which have a strong tropism for liver. The liver targeting construct (C) contains the liver-specific promotor hAAT and two ITR sequences for encapsulation in rAAV8 and the full hFIX transgene cassette.

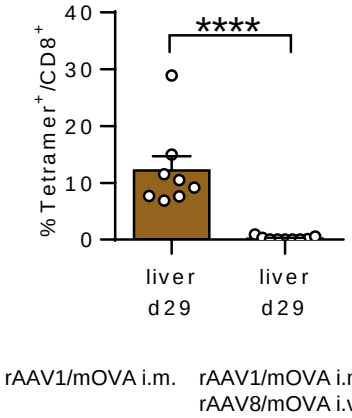

Figure S2. Presence of OVA-specific CD8⁺ T cells in liver

Adult male C57/Bl6 mice were injected in the left tibialis anterior muscle with 10^9 viral genomes (vg) of rAAV1 encoding mOVA under the muscle-specific SPc5-12 promotor and injected i.v. with 10^{10} vg rAAV8 encoding mOVA under the liver-specific promotor hAAT. Experimental conditions listed correspond to rAAV1/mOVA i.m. injection and to simultaneous injections of rAAV1/mOVA i.m. and rAAV8/mOVA i.v. Lymphocytes were extracted liver at day 29 in order to analyze OVA-specific CD8⁺ T cells by Kb/OVA_{257} tetramer staining and cytometry. Each dot represents an individual animal, mean ± SEM (n=9 mice per group, pooled from three independent experiments). ****p < 0.0001 (Mann-Whitney test).

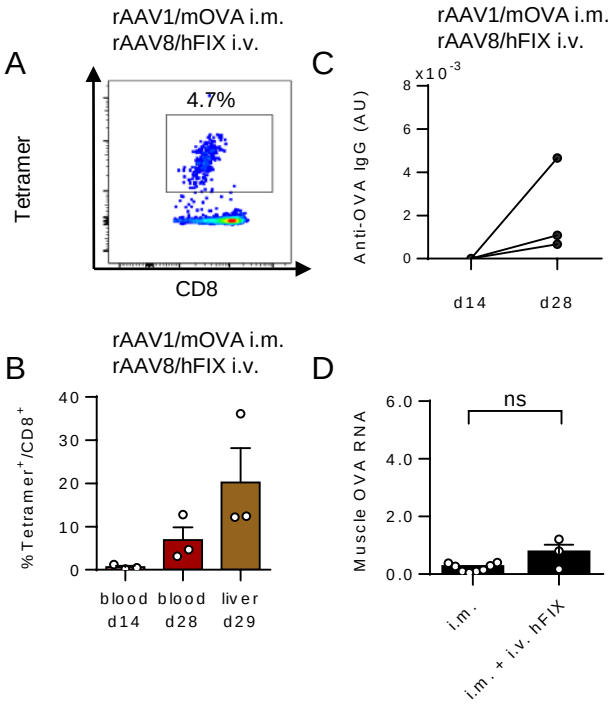

Figure S3. Lack of tolerance after i.m. and i.v. disparate transgene injections

Male C57/Bl6 mice were injected i.m. in the left tibialis anterior muscle with 10^9 viral genomes (vg) of rAAV1 encoding mOVA under the muscle-specific Spc512 promotor and injected i.v. with 10^{10}vg of rAAV8 encoding hFIX under the liver-specific promotor hAAT. Lymphocytes were extracted from blood at day 14 and 28, and from liver at day 29 in order to analyze OVA-specific tetramer staining of $CD8^+$ T cells by cytometry. (A) Representative dot plots at d28 in blood. (B) Frequencies of $CD8^+$ Kb/OVA_{257} $tetramer^+$ ($Tetramer^+$) in blood gated on $CD8^+$T cells (C) Quantities of anti-OVA IgG relative to a control serum in arbitrary unit (AU). (D) RT-qPCR performed in muscle at day 29 in the experimental conditions listed. RT-qPCR results are expressed relatively to OVA RNA expression in the "rAAV1/mOVA i.m. & rAAV8/mOVA i.v." group from Fig. 1. Each dot represents an individual animal, mean ± SEM (n=9 mice per group, pooled from three independent experiments). ns $p > 0.05$ (Mann-Whitney test)

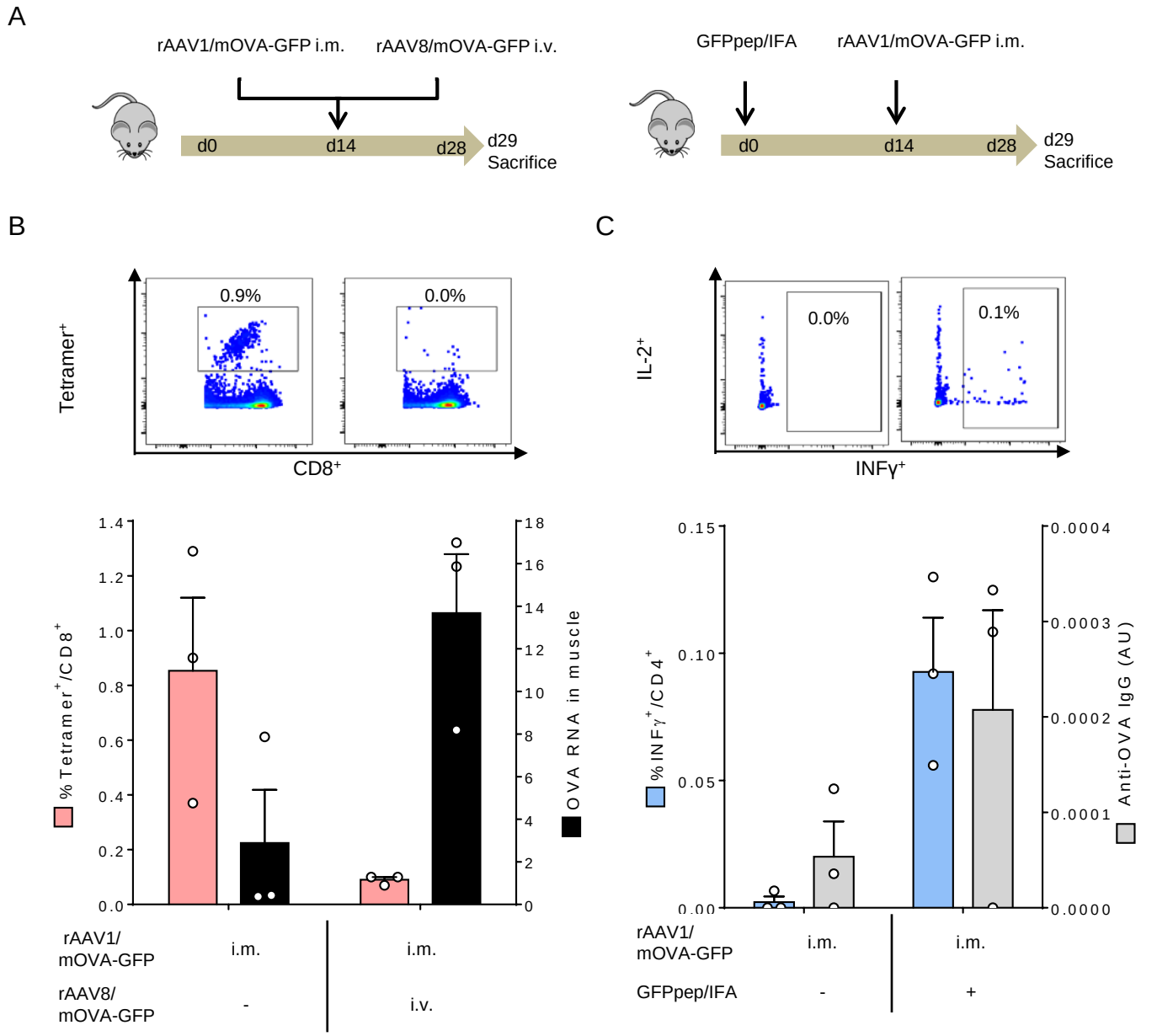

Figure S4. Tolerance induction with muscle and liver transduction of mOVA-GFP transgene bearing an additional CD4 epitope

Twelve male C57/Bl6 mice were immunized or not with the MHCII GFP epitope (HDFFKSAMPEGYVQE) at day 0 and injected at day 14 with $2{,}5x10^{10}$vg of rAAV1 i.m. encoding mOVA/GFP under the muscle-specific Spc512 promotor and injected with 10^{10}vg of rAAV8 encoding for mOVA/GFP i.v. under the liver-specific promotor hAAT. Mice were euthanized at day 29 to collect spleen and injected muscle. Lymphocytes were extracted from spleen to perform Kb/OVA_{257} tetramer staining in $CD8^+$T cells and intracellular INFγ staining gated on $CD4^+$T cells. (A) Time line of the experiment. (B) Representative dot plots (upper panel) and frequencies of $CD8^+$ Kb/OVA_{257} $Tetramer^+$ ($Tetramer^+$) in $CD8^+$T cells (left axis) and expression of OVA RNA in muscle (right axis, lower panel) at d28 in the two experimental conditions listed (lowed panel). (C) Representative dot plots of $CD4^+CD44^{hi}$ $INFγ^+$T cells (upper panel), frequencies of $INFγ^+$ gated on $CD4^+CD44^{hi}$T cells in spleen (left axis) and amounts of blood anti-OVA IgG relative to a control serum in arbitrary unit (AU, right axis, lower panel) at d28 in the two experimental conditions listed (lowed panel). Each dot represents an individual animal, mean ± SEM (n=3 mice per group).

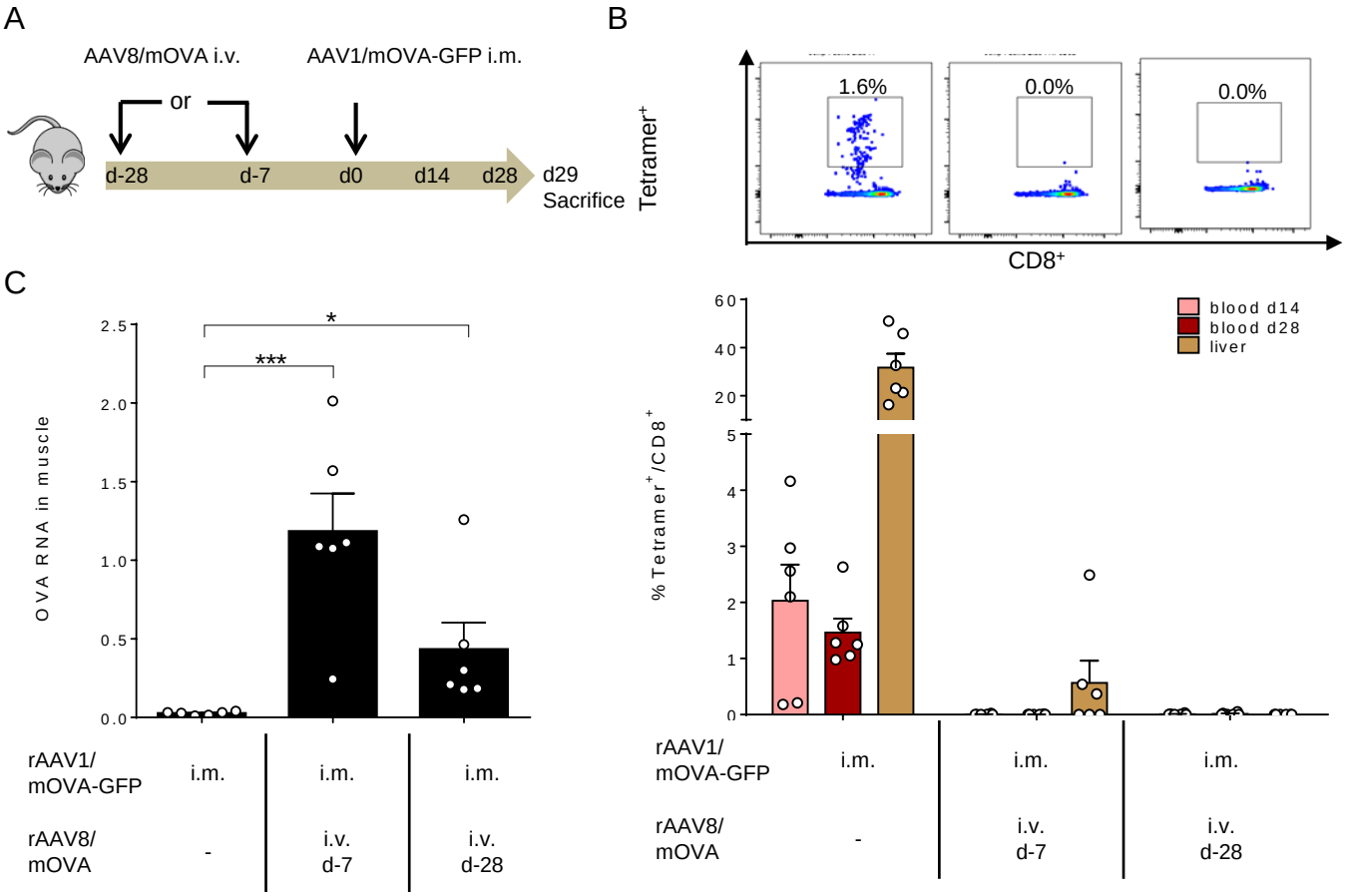

Figure S5. Induction of sustained immune tolerance with mOVA liver delivery and disparate mOVA-GFP muscle delivery

Male C57/Bl6 mice were injected i.v. with 10^{10}vg of rAAV8/mOVA at day -28 or -7 or none (control group). At day 0, mice were injected in the left tibialis anterior muscle with $2{,}5x10^{10}$vg of rAAV1/mOVA-GFP i.m. Blood was collected at d14 and 28 and mice euthanized at day 29 to collect injected muscle and liver. (A) Time line of the experiment.(B) Representative dot plots in blood (upper panel) and frequencies of $CD8^+$ Kb/OVA_{257} $Tetramer^+$ ($Tetramer^+$) gated on $CD8^+$ T cells (lower panel) at d28 in the three experimental conditions listed (lowed panel). (C) RT-qPCR to quantify OVA RNA expression in muscle at day 29 relative to the "rAAV1/mOVA-GFP i.m. & rAAV8/mOVA i.v. d-7" group. Each dot represents an individual animal, mean ± SEM (n=6 mice per group, pooled from two independent experiments). *$p < 0.05$,**$p < 0.01$ (Kruskal-Wallis test followed by Dunn's multiple comparisons test).