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Supplemental Information

Evaluation of a Fully Human, Hepatitis B Virus-Specific Chimeric Antigen Receptor in an Immunocompetent Mouse Model

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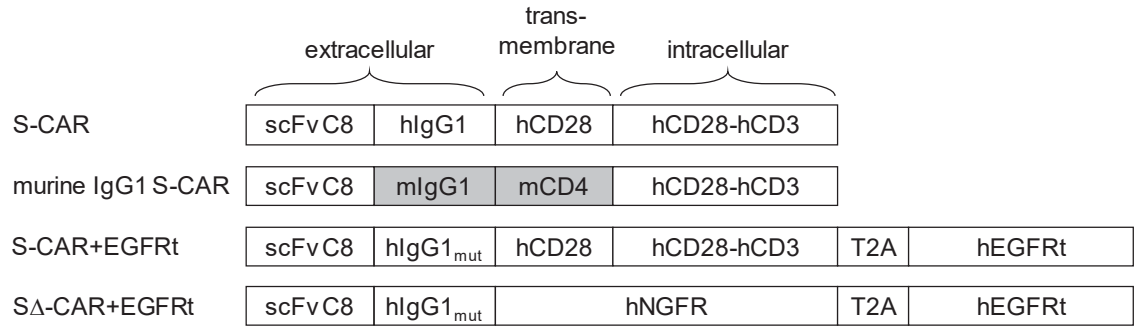

Figure S1: Scheme of CAR constructs. S-CAR and murine IgG1 S-CAR harbor the identical scFv C8 and intracellular signaling domains of human CD28 and CD3ζ. The S-CAR has a human IgG1 spacer and a human CD28 transmembrane domain, the murine construct harbors a murine IgG1 spacer and the murine CD4 transmembrane domain (grey boxes). The human IgG1 SΔ-CAR harbors the domain of human nerve growth factor receptor (NGFR) instead of CD3ζ and CD28 and serves as negative control. If indicated, human EGFRt is co-expressed utilizing a T2A element. If EGFRt is co-expressed, the S-CAR and SΔ-CAR contain a human IgG1 spacer with decreased Fc-receptor binding capacity ($hIgG1_{mut}$).[19]

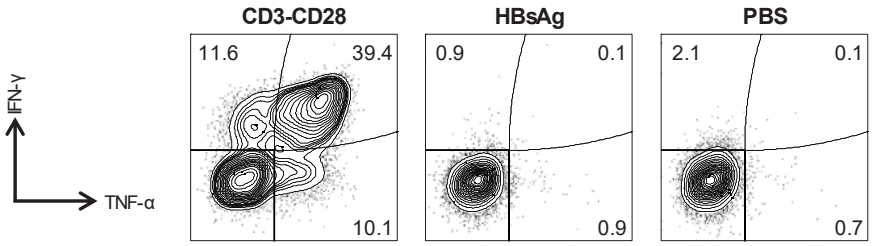

Figure S2: Intracellular cytokine staining of stimulated T cells. Exemplary flow cytometry plots of spleen-derived T cells cultured on HBsAg-, anti-CD3/anti-CD28- or PBS-coated control plates overnight (see also Figure 1E). Expression of IFN-γ and TNF-α was analyzed on transferred $CD45.1^+$ $CD8^+$ T cells.

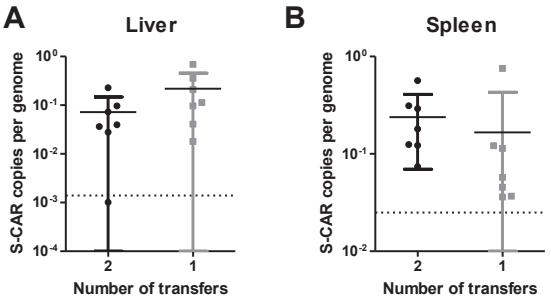

Figure S3: Quantitative PCR for S-CAR integrates. HBV-transgenic mice were injected once or twice with S-CAR T cells (n=7 per group, see also Figure 1). The number of S-CAR integrates normalized to the single copy gene *PRNP* in (A) liver and (B) spleen tissue was analyzed by quantitative PCR. A plasmid containing both the S-CAR and *PRNP* coding sequenced served as standard. Dotted line represents the background determined in untreated mice. Data points represent individual animals, mean values ± SD are indicated.

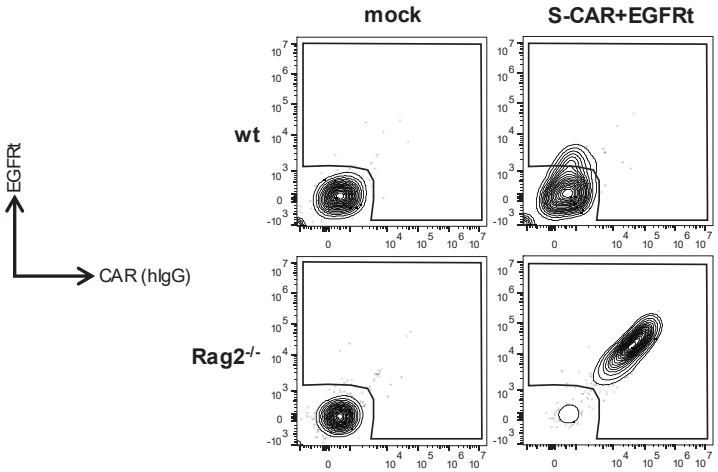

Figure S4: S-CAR and EGFRt-expression on transferred cells. Exemplary flow cytometry plots of S-CAR and EGFRt expression on CD45.1^{+} T cells isolated from blood of HBV-naïve wildtype or Rag2$^{-/-}$ mice on day 10 after transfer (see also Figure 3A).

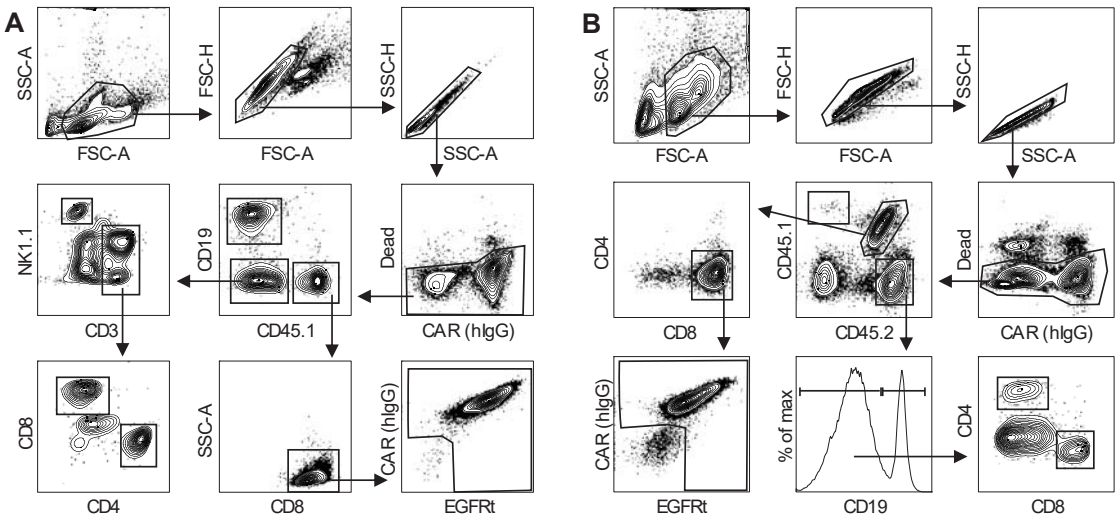

Figure S5: Gating strategy to differentiate between endogenous and transferred immune cells. A) Gating strategy applied to calculate results of figures 4C-E and S6. Lymphocytes were gated according to their size (FSC) and granularity (SSC). Subsequently duplets were excluded (FSC-A vs. FSC-H and SSC-A vs. SSC-H). After removal of dead cells, B cells ($CD19^+$) were identified. Moreover $CD45.1^+$ cells (transferred cells) were distinguished from $CD45.1^-$ cells (endogenous cells). All transferred cells were identified to be $CD8^+$ as well as CAR^+ ($hIgG^+$) and $EGFRt^+$. Within the population of $CD19^-$ CD45. 1^- cells, NK cells ($CD3^-$ $NK1.1^+$) as well as $CD3^+$ T cells were detected, whereby $CD3^+$ T cells were further analyzed according to the expression of CD4 and CD8. **B)** Gating strategy applied to calculate results of Figures 6D and S8. Lymphocytes were gated according to their size (FSC) and granularity (SSC). Subsequently duplets were excluded (FSC-A vs. FSC-H and SSC-A vs. SSC-H). After removal of dead cells, $CD45.1^+$ cells (cells of first transfer), $CD45.1^+/CD45.2^+$ (cells of second transfer) and $CD45.2^+$ cells (endogenous cells) were identified. All $CD45.1^+/CD45.2^+$ cells were identified to be $CD8^+$ as well as CAR^+ ($hIgG^+$) and $EGFRt^+$. Within the endogenous cells, $CD19^+$ B cells were distinguished from $CD19^-$ cells, which were used to further gate on $CD4^+$ and $CD8^+$ T cells.

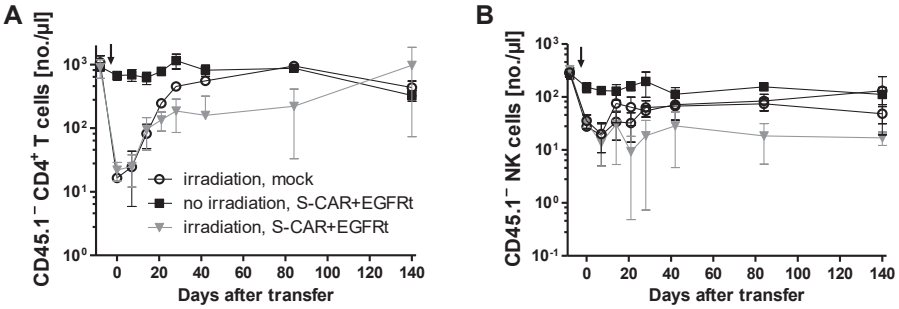

Figure S6: Concentration of $CD4^+$ T cells and NK cells in peripheral blood after irradiation. AAV-HBV infected $CD45.2^+$ wildtype mice were irradiated one day before transfer of $S\text{-}CAR^+/EGFRt^+$ T cells (see also Figure 4). **A)** Amount of (endogenous) $CD45.1^-$ $CD4^+$ T cells and **B)** $NK1.1^+$ NK cells per µl peripheral blood at indicated time points. Arrows mark time point of irradiation. All data are presented as mean values ± SD. (n=4 per group)

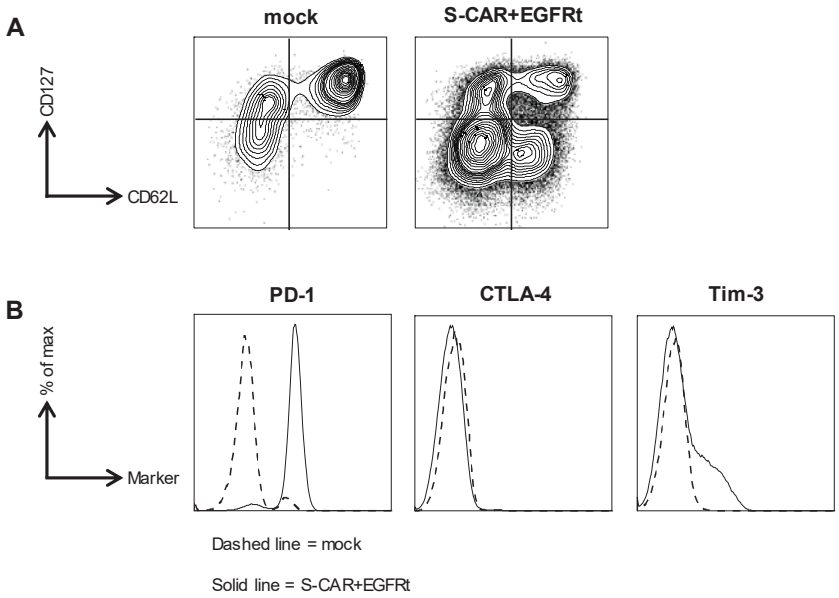

Figure S7: Memory and exhaustion marker expression of transferred T cells. Exemplary flow cytometry plots pregated on transferred CD45.1$^+$ T cells in splenocyte population. **A)** Memory marker expression (see also Figure 4F). **B)** Exhaustion marker expression (see also Figure 4G).

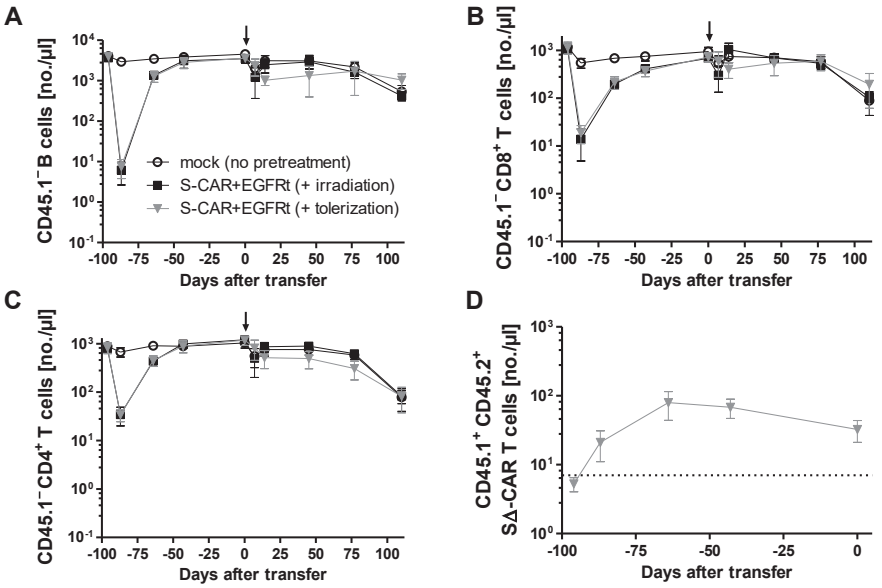

Figure S8: Concentration of endogenous immune cells and SΔ-CAR T cells after tolerization.
AAV-HBV infected CD45.2$^+$ wildtype mice were irradiated one day before SΔ-CAR$^+$/EGFRt$^+$ T-cell transfer (see also Figure 6). Cell concentrations in peripheral blood monitored over time. Arrows mark time of S-CAR$^+$/EGFRt$^+$ T-cell transfer on day 0. **A)** Concentration of CD45.1$^-$ CD19$^+$ B cells. **B)** Concentration of CD45.1$^-$ CD8$^+$ T cells. **C)** Concentration of CD45.1$^-$ CD4$^+$ T cells. **D)** Concentration of CD45.1$^+$ CD45.2$^+$ SΔ-CAR$^+$/EGFRt$^+$ T cells. The dotted line represents the background determined in untreated mice. All data are presented as mean values ± SD. (n=5 per group)