Supplemental Information

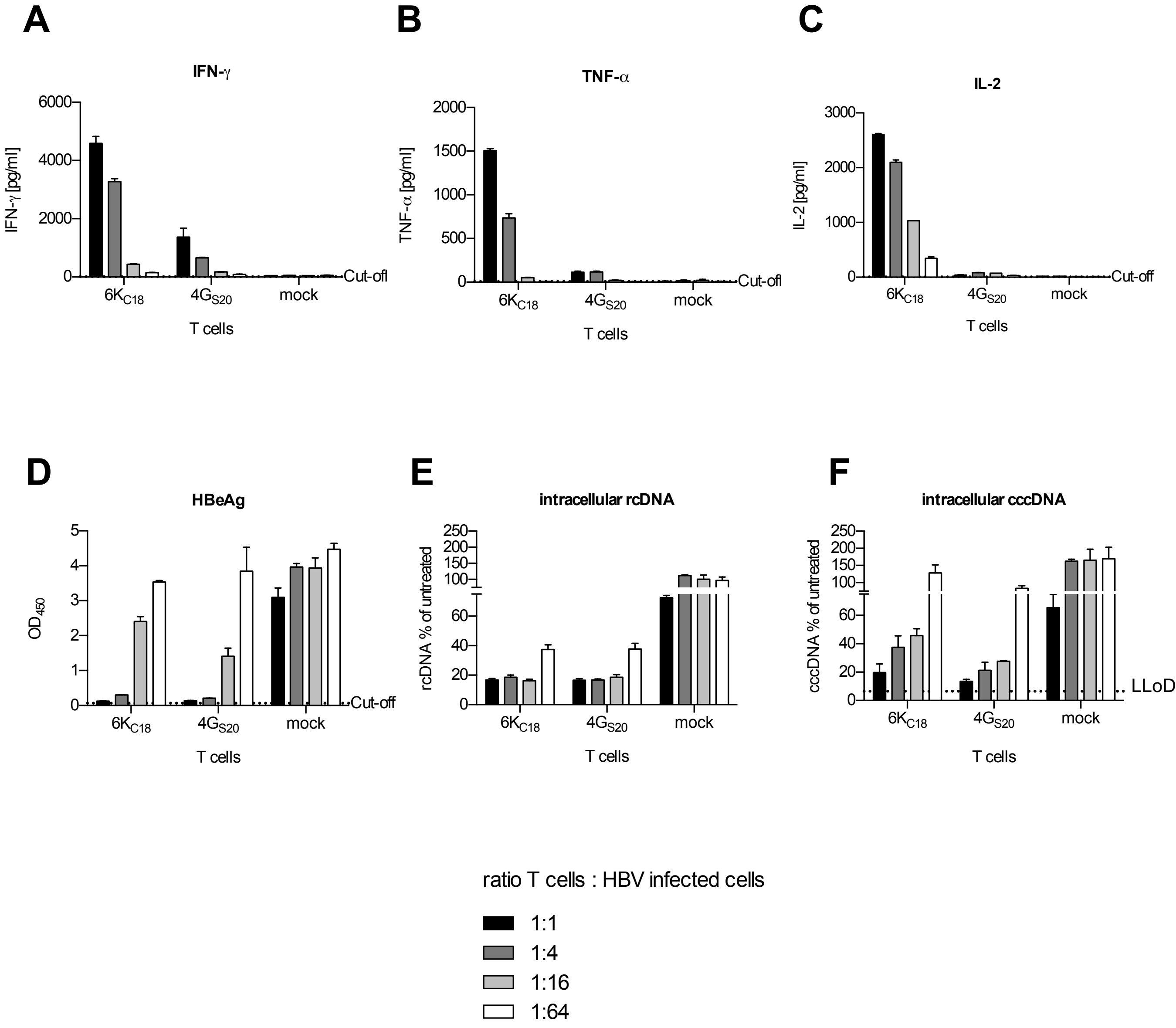
T Cell Receptor Grafting allows Virological Control of Hepatitis B Virus Infection

Supplemental Table 1

specificity	TCR	score
$Core_{18-27}$	FLP14	20
$Core_{18-27}$	FLP122	13
$Core_{18-27}$	5E	32
$Core_{18-27}$	6K	33
$Core_{18-27}$	7D	26
S_{20-28}	G6	12
S_{20-28}	FL6	18
S_{20-28}	4G	31
S_{20-28}	D1	20
$S_{172-180}$	WL12	12
$S_{172-180}$	WL31	18

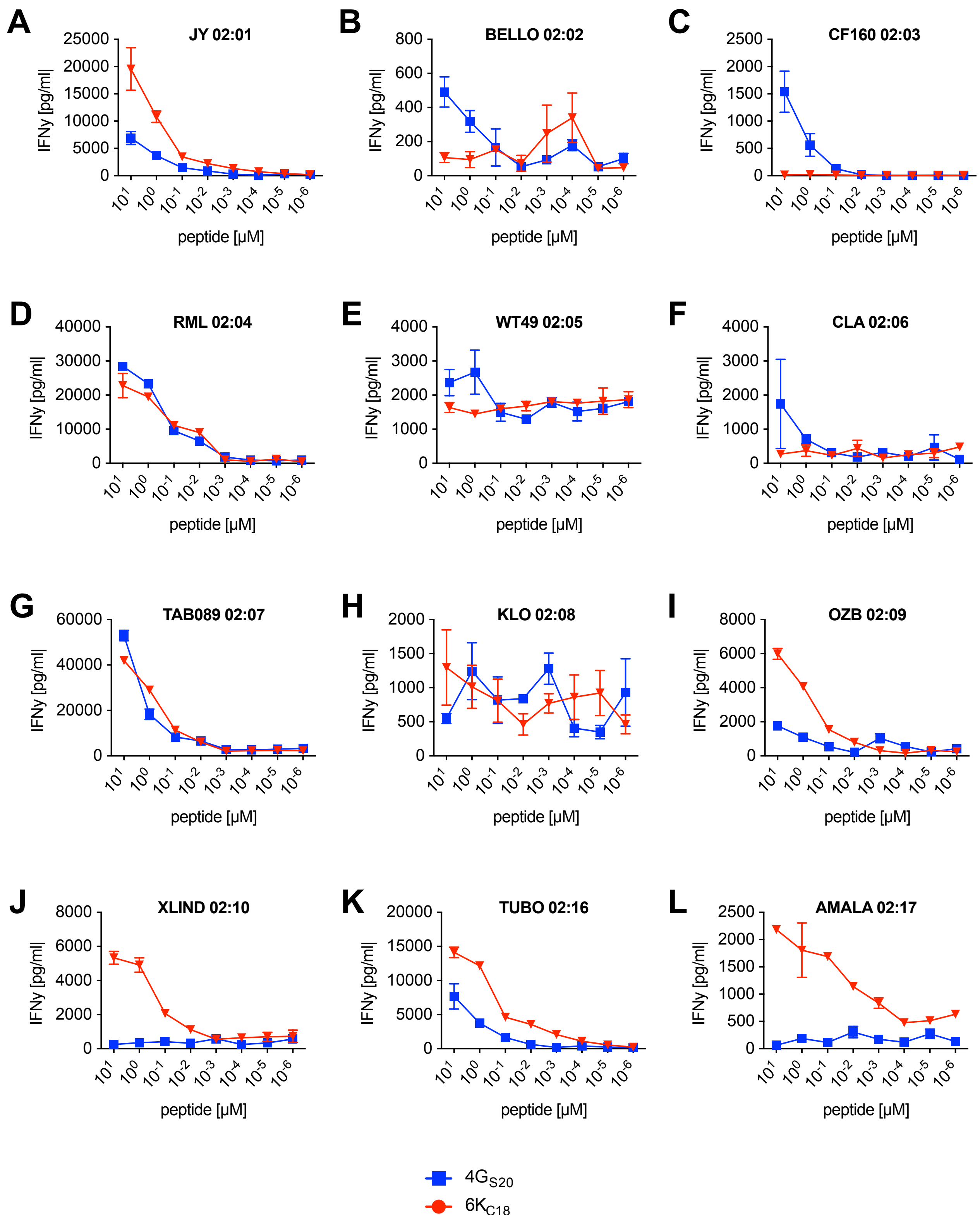
Supplemental Table 1. Scoring of TCR functionality. T cells expressing 11 different TCRs were scored according to frequency of streptamer-binding cells, peptide concentration of effector cell number necessary to induce a certain level of specific target cell lysis or cytokine secretion, and number of HLA-A2 subtypes recognized (Wisskirchen et al., Plos One 2017). TCRs 6K and 4G were identified to be the most potent TCRs recognizing a core- or S-derived peptide, respectively, and were chosen for further assessment of their antiviral functionality.

Supplemental Figure 1



Supplemental Figure 1. Recognition of HBV-infected cells by TCR-grafted T cells. HepG2-NTCP cells HBV-infected at an MOI of 100 four days before were co-cultured with TCR-grafted T cells for three days at indicated E:T ratios. 6K: C18-specific TCR, 4G: S20-specific TCR, mock: non-transduced T cells. (**A-C**) Cytokines were measured in the supernatant of co-cultures after 24 hours. (**D**) HBeAg, (**E**) intracellular rcDNA, and (**F**) nuclear cccDNA were determined after three days of co-culture. Data are presented as mean values +/- SEM from triplicate co-culture experiments. For the next experiments, in which T cell titration was not required, an E:T of 1:2 was chosen to guarantee the strongest specific antiviral effect and at the same time reduce background antiviral activity as mock T cells from our vaccinated donors in the lab had background antiviral activity at an E:T of 1:1.

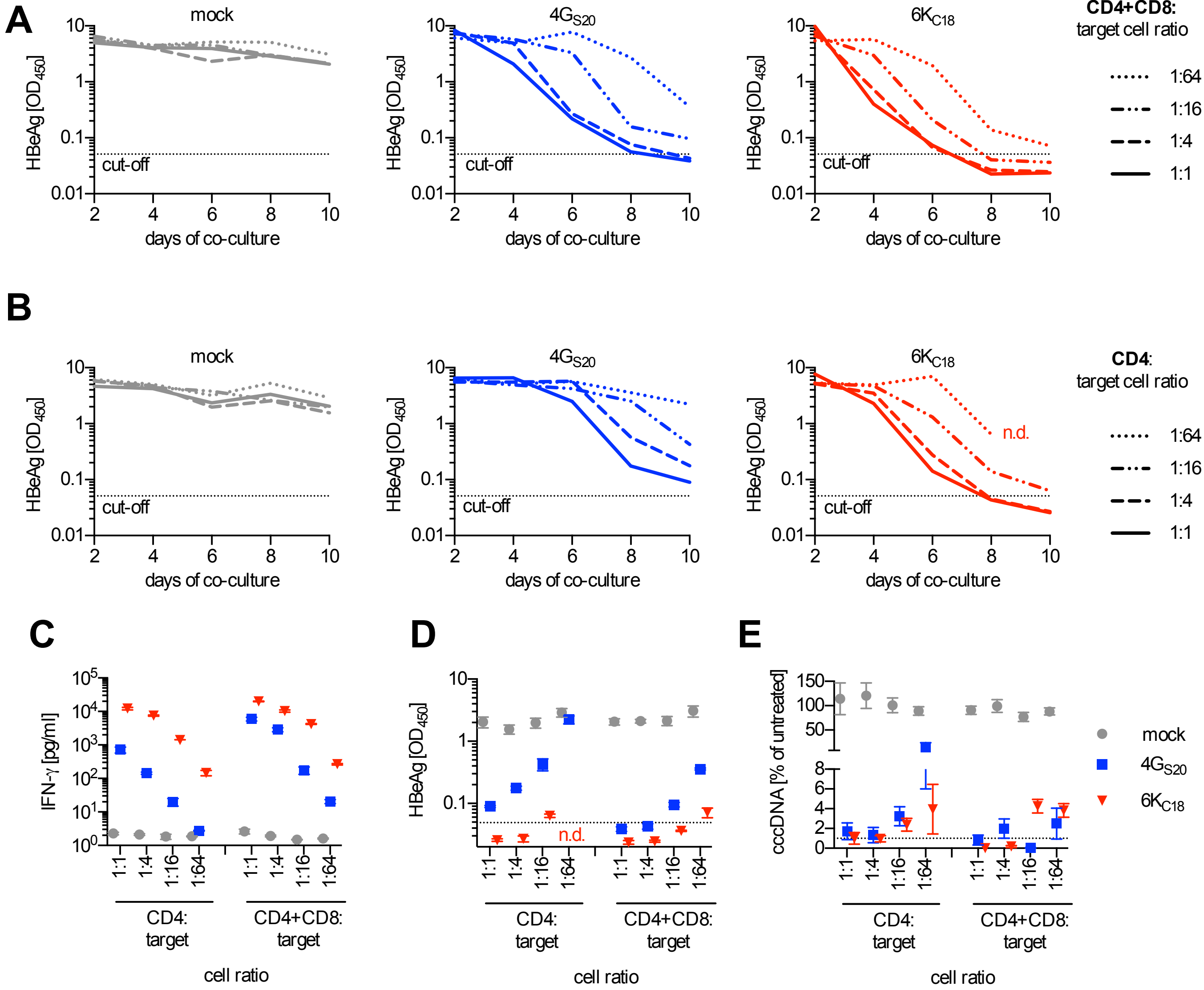
Supplemental Figure 2



Supplemental Figure 2. Recognition of peptides presented by different HLA-A*02 subtypes. Peptides C18 or S20 were titrated on LCL with varying HLA-A*02 subtypes and co-cultured with 6K- or 4G-transduced T cells (E:T 1:1). When using lymphoblastoid cell lines (21), however, the loaded peptide competes with the endogenous peptides for binding to the MHC-molecule, which prevents a reliable quantitative analysis and only allows a qualitative conclusion.

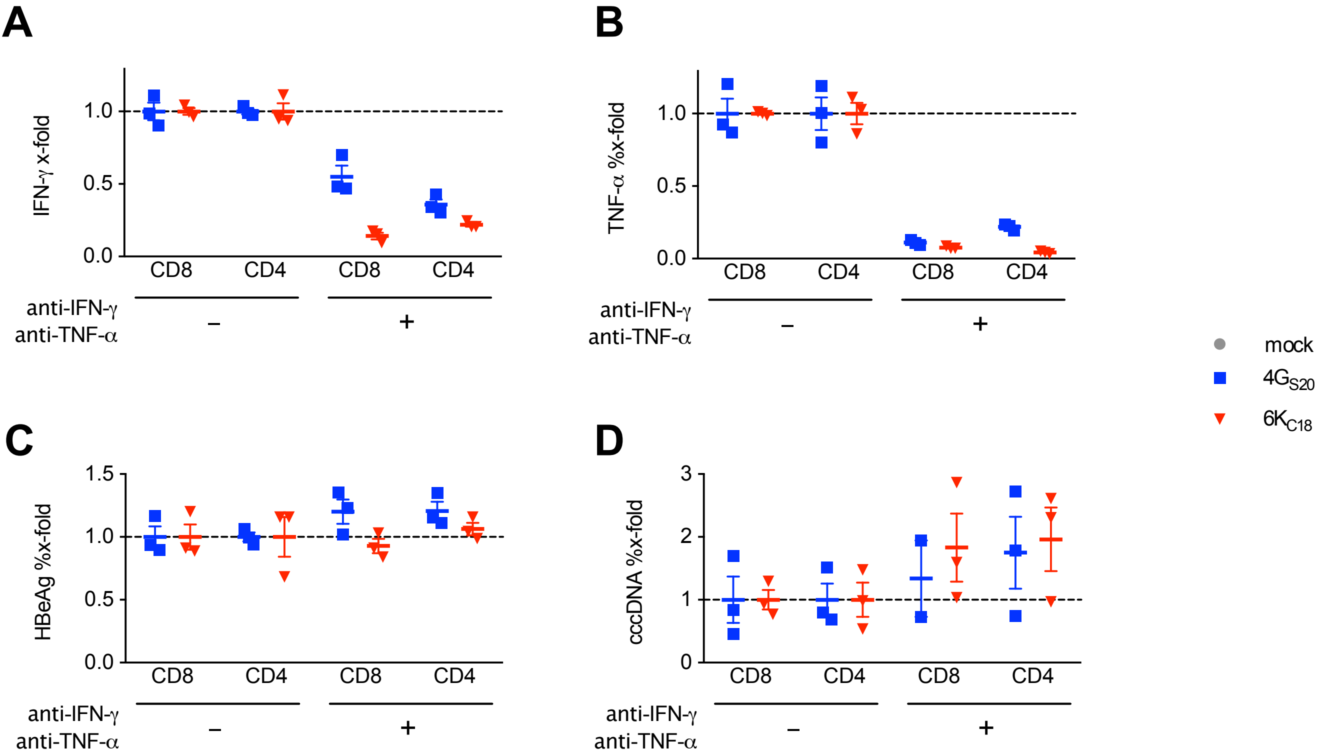
T-cell activation was measured by IFN-γ ELISA. Both donors, from which TCRs originated, carried the HLA-A subtype 02:01. The HLA-A subtype used is indicated in the title of each individual graph. Data are presented as mean values +/- SEM from duplicate co-cultures.

Supplemental Figure 3



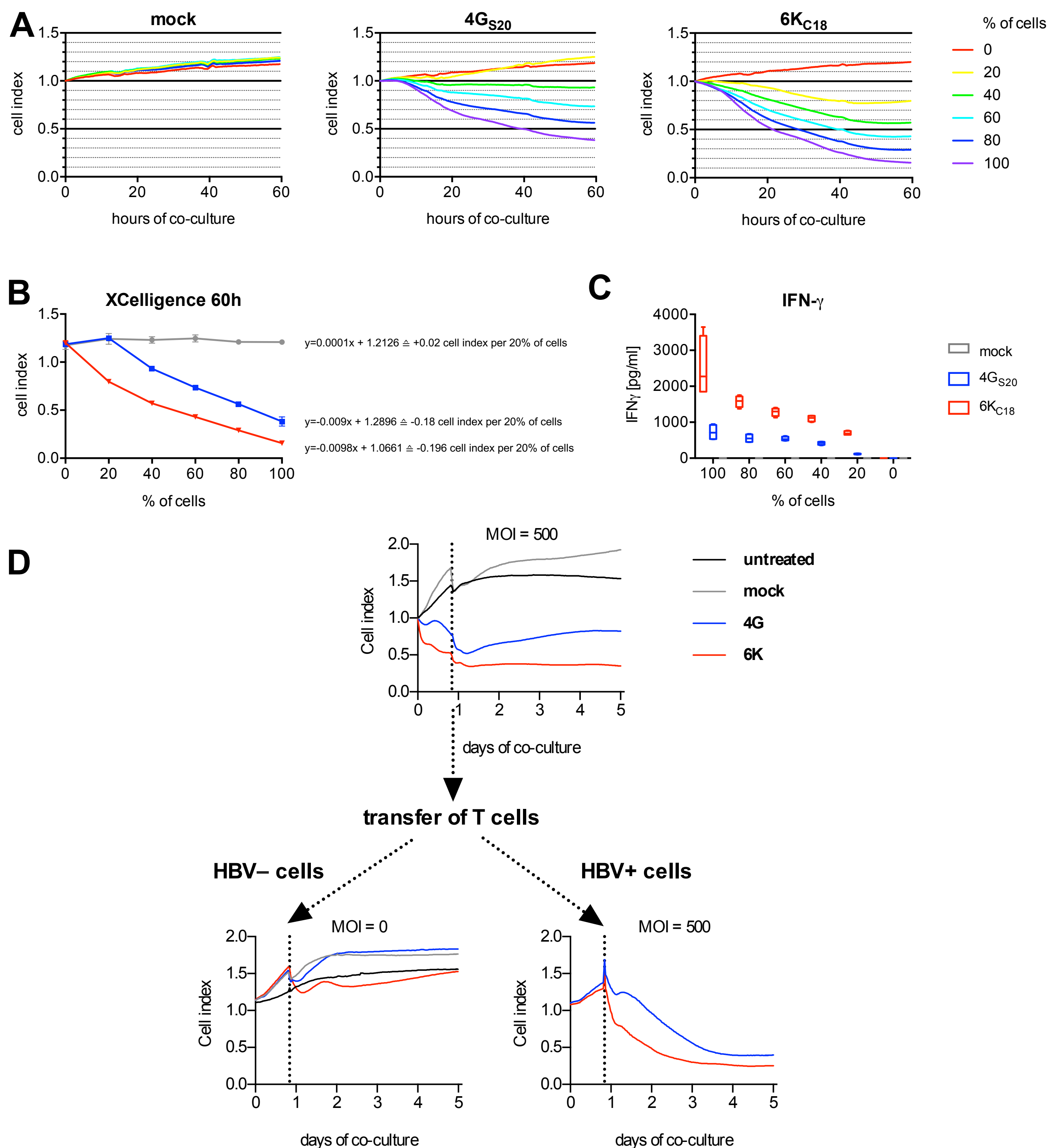
Supplemental Figure 3. Viral decline during co-culture with CD4$^+$, TCR-grafted T cells. HepG2-NTCP cells were infected with HBV at an MOI of 500. After three weeks, T cells grafted with TCR 4G$_{S20}$ (blue lines) or 6K$_{C18}$ (red lines) or non-transduced T cells (mock, grey lines) were added at decreasing E:T ratios. (**A,B**) HBeAg secretion into the cell culture supernatant determined every other day after starting the co-culture with CD4$^+$ and CD8$^+$ T cells (**A**) or CD4$^+$ T cells only (**B**). IFN-γ was measured in the cell culture medium after 2 days (**C**), secreted HBeAg (**D**) and intracellular HBV cccDNA (**E**) were measured after 10 days of co-culture. n.d.= not determined. Data are presented as mean (A,B) values or mean ± SEM (C-E) of triplicate co-cultures (n=3).

Supplemental Figure 4



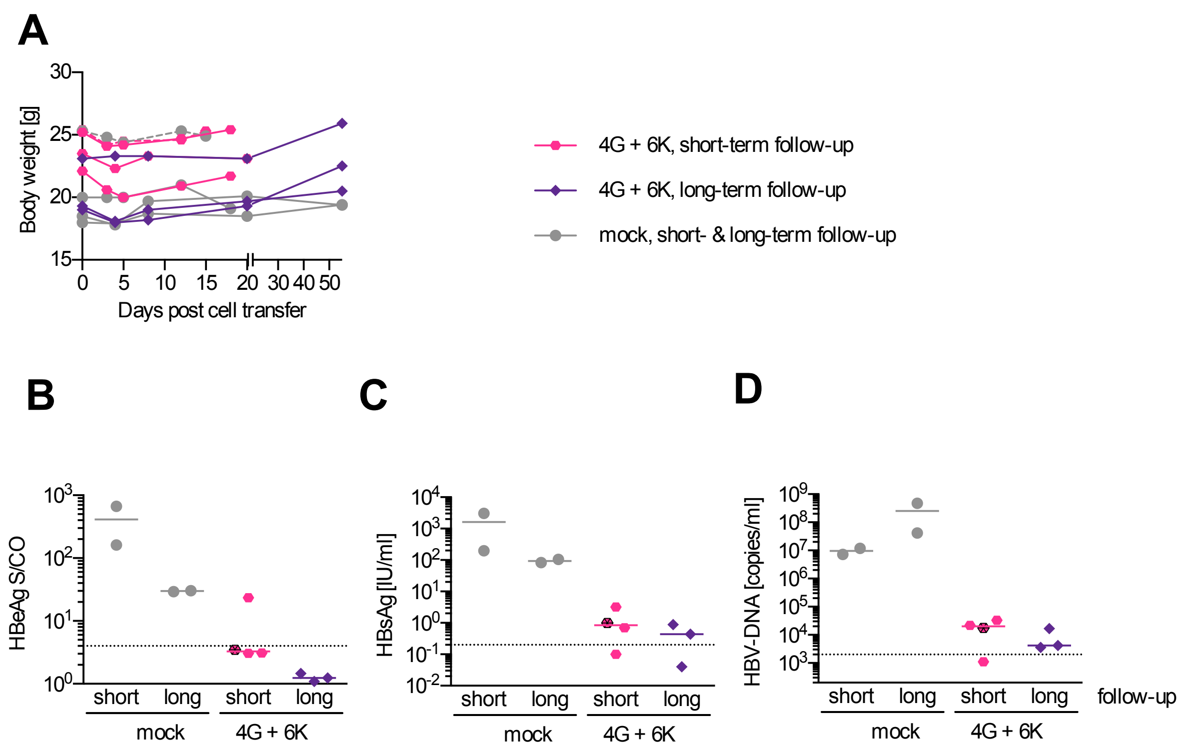
Supplemental Figure 4. Contribution of cytokines to antiviral effect of TCR-grafted T cells . HepG2-NTCP cells were infected with HBV at an MOI of 500. After 12 days T cells grafted with TCR $4G_{S20}$ (blue squares) or $6K_{C18}$ (red triangles) were added at an E:T ratio of 1:2. Cytokine-blocking antibodies against IFN-γ (10ng/ml) or TNF-α (5ng/ml) were given every other day when medium was exchanged. (**A, B**) IFN-γ and TNF-α were measured in the cell culture medium after 2 days. (**C**) Secreted HBeAg and (**D**) intracellular HBV cccDNA were measured after 10 days of co-culture. (**A-D**) The increase or decrease of each parameter is given relative to the amounts measured in co-cultures in which anti-cytokine antibodies were not added (=1). Data are presented as mean values ± SEM of triplicate co-cultures (n=3).

Supplemental Figure 5



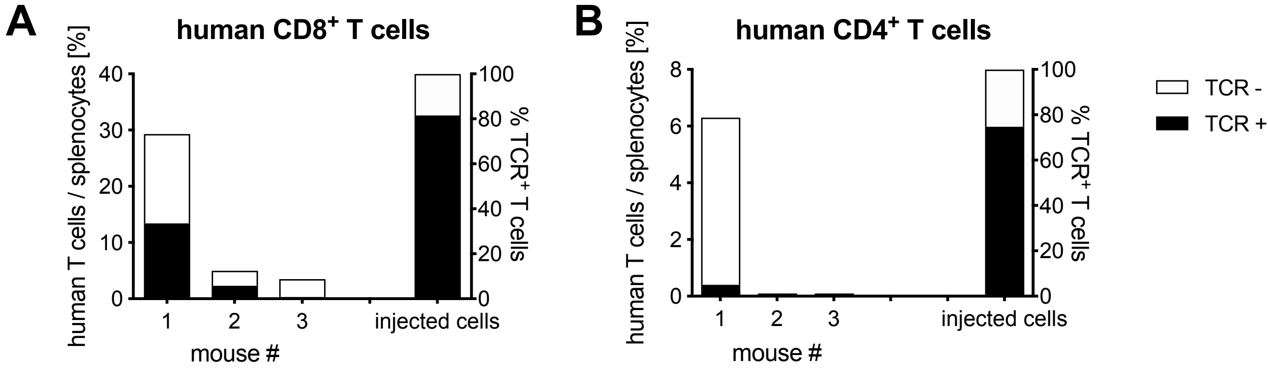
Supplemental Figure 5. Specificity of TCR-grafted T cells. HepG2-NTCP cells were infected with HBV at an MOI of 100, which results in approximately 45% of cells showing strong viral infection. After 1 week, these cells from infection (given as % of cells) were mixed with uninfected HepG2-NTCP cells and seeded for one day in a 96 well plate. Non-transduced T cells (mock), or T cells grafted with TCR 4G$_{S20}$ or 6K$_{C18}$ were added at an E:T of 1:1. (**A**) Killing of target cells determined by detachment from the bottom of the 96-well plate was measured in real-time (XCelligence™) and is given as normalized cell index relative to the settlement of T cells. (**B**) Target cell index after 60 hours of co-culture (endpoint of Figure A). Formulas for trendlines of each curve are calculated. (**C**) IFN-γ was measured in the cell culture medium after 3 days. (**D**) T cells were incubated with target cells infected with HBV at an MOI of 500 two weeks before (E:T 1:1). After 22 hours, when cytotoxic activity of T cells was detectable, T cells were resuspended and, including ¾ of medium per well, transferred to either uninfected (left) or infected (right) HepG2-NTCP cells. Data are presented as mean values (A,D) or mean values ± SEM (B,C) of quadruplicate co-cultures (n=4).

Supplemental Figure 6



Supplemental Figure 6. Safety and endpoint serology in HBV-infected humanized mice treated with TCR-grafted T cells. USG mice were repopulated with HLA-A*02-matched PHH, infected with 1x10^7 HBV virions, followed until a stable viremia had established (week 12-14) and injected with 2x10^6 TCR-grafted T cells (1x10^6 with 6K$_{C18}$ plus 1x10^6 with 4G$_{S20}$; colored symbols, n=7) or equal numbers of mock-treated human T cells (grey circles, n=4). 4 mice where sacrificed within 3 weeks (pink hexagons) and 3 mice 8 weeks (purple diamonds) after T cell transfer, respectively. 2/11 mice received a second dosage of either effector cells or mock cells and where sacrificed on day 15 (presented by broken lines in A and crossed dots in B-D). (**A**) Progression of body weight after T cell transfer. (**B-D**) The amount of serum obtained by withdrawal before and during treatment is limited and had to be diluted strongly for measurement of antigens. Serum obtained at the endpoint of treatment (day 15 and 18 ("short"), or 55 ("long") was available in higher amounts and could be used in 1:10 or 1:4 dilution, respectively, for measurement of HBeAg (**B**), HBsAg (**C**) and for viremia (**D**). Each data point or line presents one mouse. For antigen measurements (B, C) dotted line represents the technical cut-off of the respective test. For viremia (D) dotted line (LLoD) represents 10 copies per PCR.

Supplemental Figure 7



Supplemental Figure 7. FACS Analysis of splenocytes isolated from mice 55 days after T cell transfer. HBV-infected USG mice were used for transfer of 2x10^6 TCR-grafted (1x10^6 6K + 1x10^6 4G) or equal numbers of mock-treated human T cells. Splenocytes and injected cells were stained for human CD8 **(A)** and CD4 **(B)** markers, as well as for the mTRBC to determine TCR expression. Data represent the frequency of total human T cells (full bar) and TCR$^+$ T cells (black bar) assessed by flow cytometry in splenocytes of individual mice 55 days post T-cell transfer or before performing the adoptive cell transfer (injected cells).