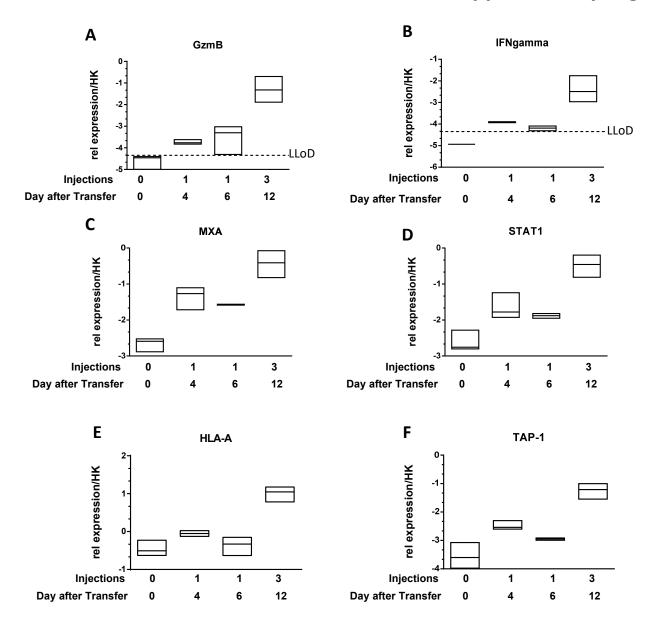
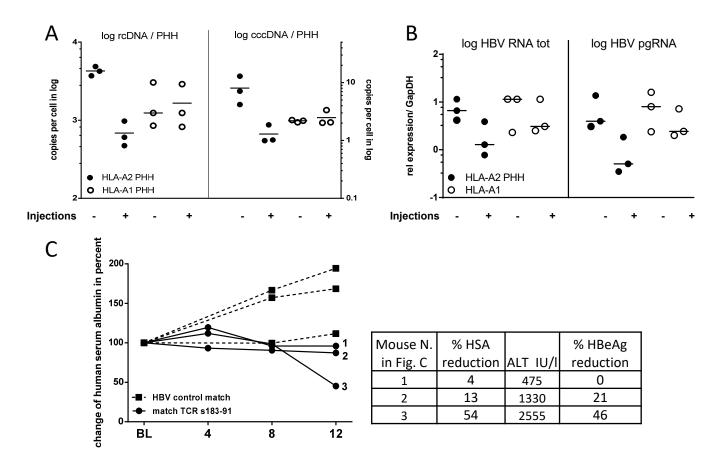


# T cell transfer results in a exclusive release of cytokines in humanized HBV+HLA-A2+ mice Median changes of human cytokines (e.g. IL-5 (A), GM-CSF (B) and MDC (C)) in the serum were determined at protein level by Multiplex measurement and in all groups of humanized mice (uninfected and HBV infected; harbouring matched PHHs; and receiving 0, 1 or 3 injections of redirected T cells). Median values for HBV infected HLA-A2 repopulated mice receiving 3 applications of effector T-cells were IL-5 = 50 ng/ml; GM-CSF = 4354 ng/ml; MDC = 254 ng/ml.

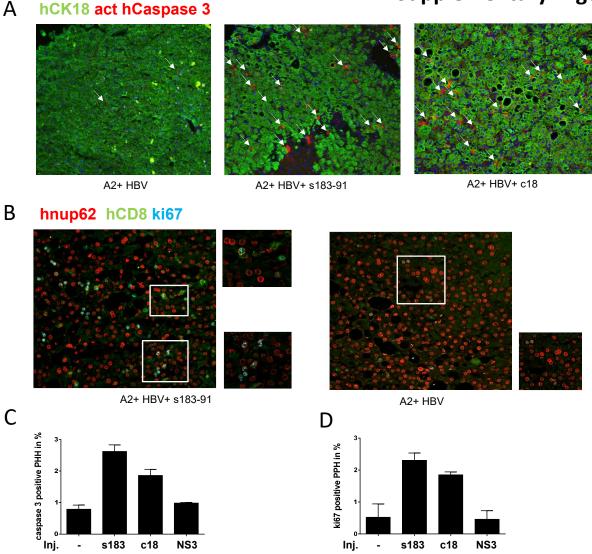


#### Enhancement of human T-cell response related genes after adoptive T-Cell transfer.

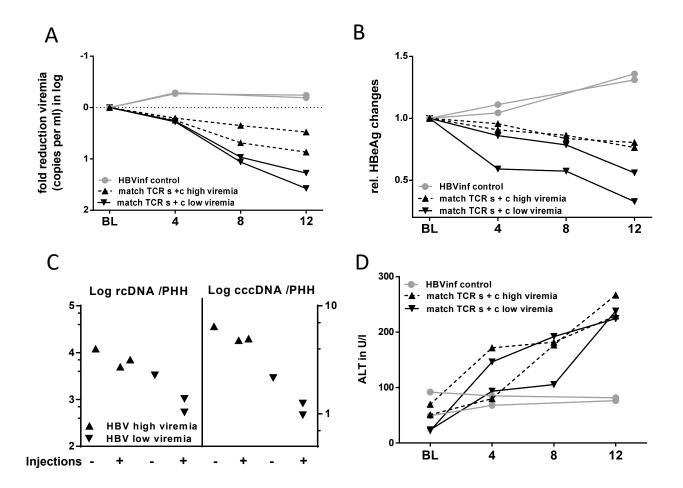
Gene expression was measured in *humanized HBV+HLA-A2+ mice* that received HBV-specific T-cells only once and were sacrificed 4 or 6 days after injection or three times (day 12; 4 days after the last T cell injection) and in untreated HBV-infected control mice (BL; no T-cells recipient). Transcript levels of human Granzyme-B (GzmB) (A), IFN gamma (B) and human interferon-stimulated genes (MXA (C), STAT1 (D), HLA-A (E) and TAP-1 (F) were determined by qRT-PCR and normalized against human housekeeper transcripts (HK).



Adoptive transfer of effector T cells results in reduction of intrahepatic HBV markers in haplotype matched but not in mismatched treated mice harbouring comparable levels of human repopulation and infection at baseline. A) Intrahepatic levels of rcDNA and cccDNA copies expressed per human hepatocyte (beta-Globin) show median reduction 0.83log and 0.85log, respectively, in mice receiving TCR matched T cells (black dots), but not in mice harbouring mismatched hepatocytes (white dots). (B) HBV RNA amounts relative to human GAPDH show a similar pattern of reduction of HBV transcript levels only in haplotype matched treated mice. C) Levels of human serum albumin (HSA) in mouse serum monitored from BL to days 12 in treated and control mice. The same mouse numbers are shown in figure and in the adjacent table. Note that mouse n.3 showed the stronger HSA decrease (54%) and also the highest ALT levels (2555 IU/L) and the most pronounced HBeAg decrease (46%, see also figure 2).



Haplotype-matched effector T cell transfer increases apoptosis and proliferation events in HBV infected mice. Liver tissues of humanized HBV-infected mice that underwent different treatment regimens were used for immunofluorescence. A) Human HLA-A2-presenting hepatocytes (A2+) are visualized using human specific CK18-Ab (green) and caspase 3 positive cells are identified in red (pointed out with white arrows). B) Human cell proliferation was determined using a nup62-Ab (red) to visualize human hepatocytes, a human specific CD8-Ab (green) to identify immune cells, while the human specific ki67-Ab (light blue) was used to identify all human proliferating cells. A tissue from a representative infected mouse that received HBV-specific T cells is shown on the left side and from an untreated control on the right side. To quantify the level of apoptotic human cells (C) and of proliferating human hepatocytes (D) three tissue slides per mouse and treatment group were used for counting (5 fields of vision per slide). Median values are expressed in percent.



Transfer of c18 and s183 mRNA redirected effector T cells results in a stronger reduction of serological and intrahepatic HBV markers in mice displaying lower baseline viremia. Experiment was performed in mice displaying very high (>10E9; n=2) and lower viral load (10E7; n=2). Both groups received three injections of effector T-cells (at day 0, after determining BL parameters, day 4 and 8) and were sacrificed on day 12. A) Median viremia changes relative to baseline levels were -0.7 log in high viremic and -1.42 log in lower viremic treated mice. A light viremia increase was determined in untreated controls (+0.2log; BL viral load= 10E9). B) Median changes in levels of circulating HBeAg were determined by ELISA. (C) Intrahepatic levels of rcDNA and cccDNA are normalized per human cell amounts (beta-Globin). Median rcDNA reduction of 0.5log was determined in mice displaying high titers and 0.76log in mice with lower titer compared to matched controls. Median cccDNA reduction differed between 0.25log in mice with higher BL viral load and 0.5log in mice with lower viral load. (D) Changes in ALT levels (IU/ml) were determined longitudinally both in HBV-infected control and treated mice.