Supplemental Figure 1

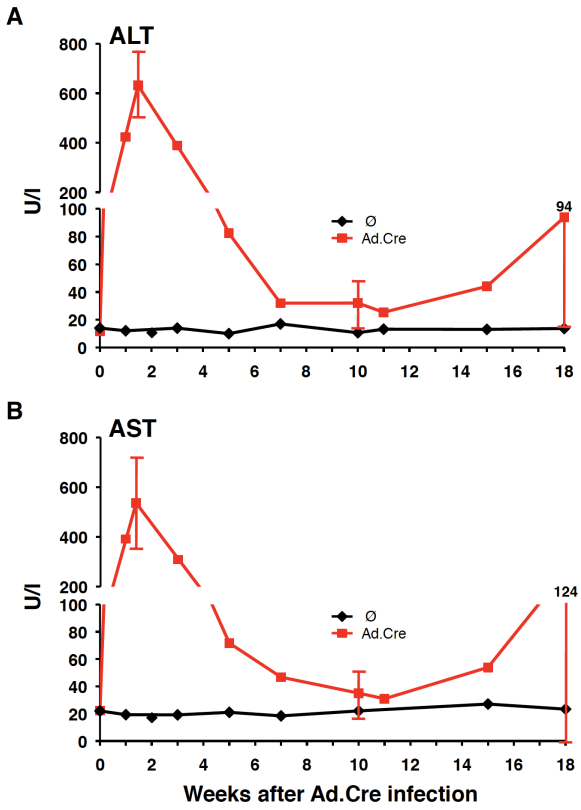

Figure S-1: Intravenous administration of Ad.Cre into LoxP-TAg mice induces acute liver damage and hepatitis. (**A**) Liver enzymes alanine aminotransferase (ALT) and (**B**) aspartate aminotransferase (AST) were determined before and after Ad.Cre infection and followed over a time period of 18 weeks until LoxP-TAg mice (red line, n=12) succumbed to HCC. As control non-treated wildtype mice (black line, n=3) were used. Mean values at three time points from two independent experiments are shown, error bars represent SEM. The last data point is given as value (U/l).

Supplemental Figure 2

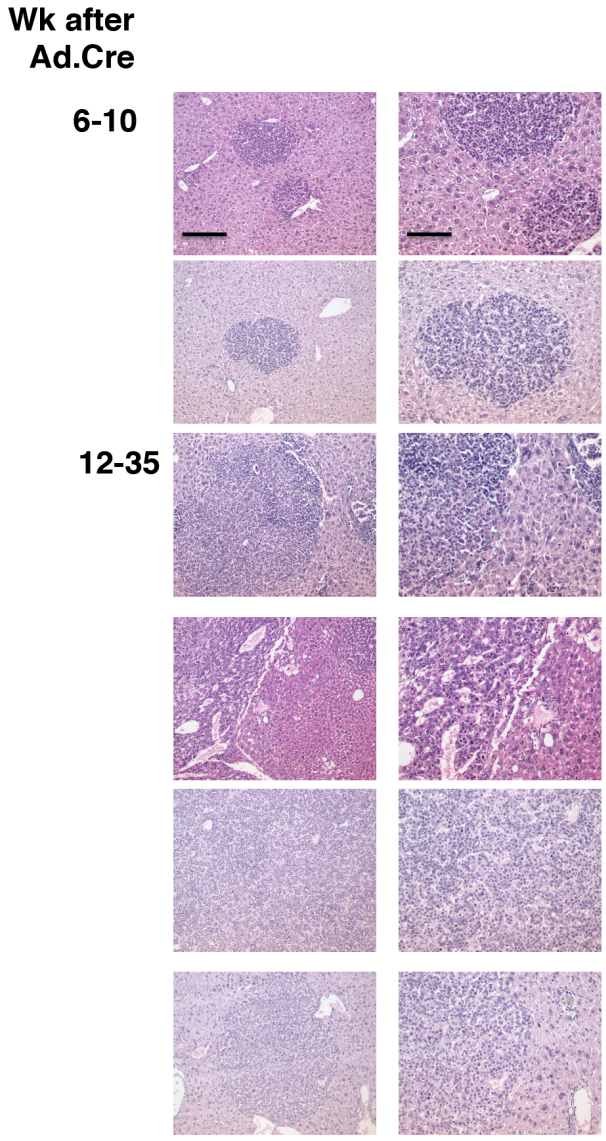

Figure S-2: Intravenous injection of Cre expressing adenoviruses induced the development of well to moderately differentiated HCC (G1-2). Histology of Ad.Cre induced liver tumors in LoxP-TAg mice reveals that HCC were of classical type resembling hepatocytes with a predominantly trabecular (plate like) architectural pattern according to the recent WHO classification of tumours of the digestive system. In some tumors, a clear cell change, a pseudoglandular or a more compact growth pattern were observed. Scale bars: left, 200 μm, right, 100 μm.

Supplemental Figure 3

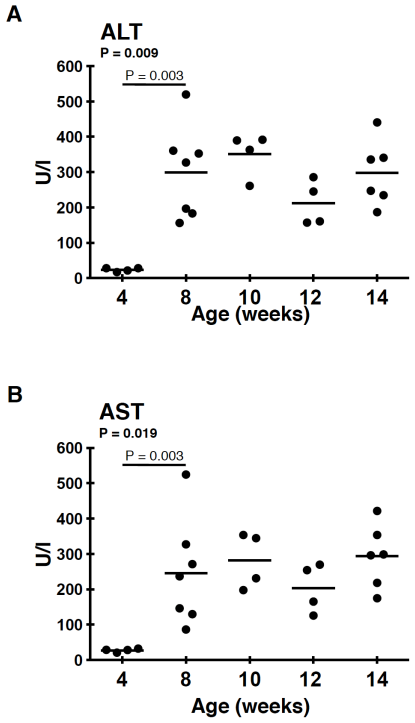

Figure S-3: Liver tumor development in LoxP-TAg x Alb-Cre mice induces ALT and AST. (**A**) Liver enzymes alanine aminotransferase (ALT) and (**B**) aspartate aminotransferase (AST) were determined over a time period of 14 weeks of age (n=3 to 7 per time point) until LoxP-TAg x Alb-Cre mice succumbed to HCC and cholangiocellular carcinoma (CCC). Mean values are shown.

Supplemental Figure 4

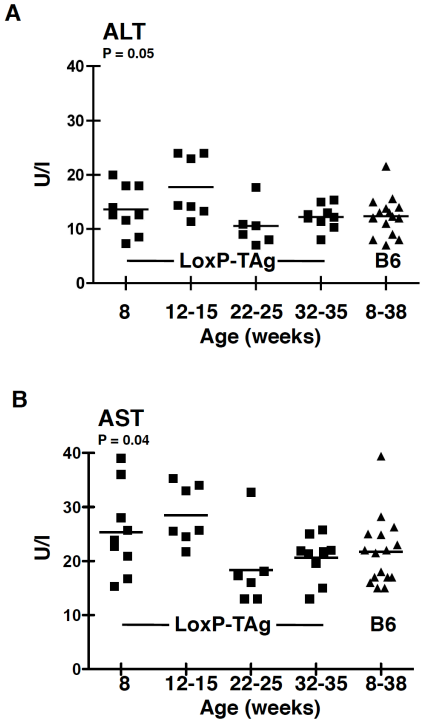

Figure S-4: Normal ALT and AST values in non-treated LoxP-TAg mice. (**A**) Liver enzymes alanine aminotransferase (ALT) and (**B**) aspartate aminotransferase (AST) were determined over a time period of 35 weeks of age (n= 6 to 9 per time point). As control non-treated wildtype mice (n=16) were observed up to 38 weeks of age. Mean values are shown.

Supplemental Figure 5

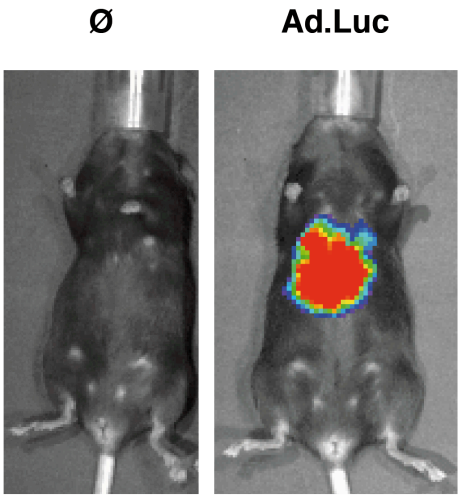

Figure S-5: Tropism of adenovirus results in selective infection of the liver. LoxP-TAg mice were injected i.v. with 1 x 10^9 pfu Ad.Luc and distribution of adenovirus was monitored by BL imaging seven days later. Non-infected LoxP-TAg mice injected with luciferin were used as control; one representative picture (n=4) is shown.

Supplemental Figure 6

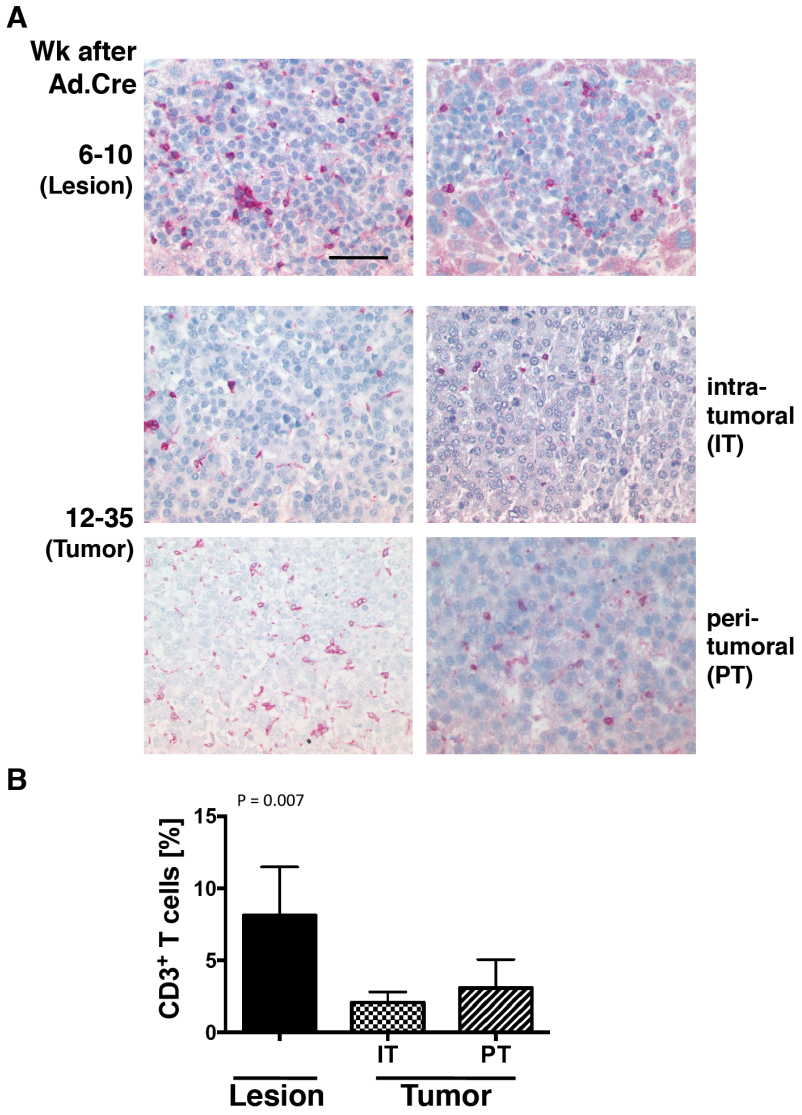

Figure S-6: Virus-induced HCC are only moderately infiltrated by CD3$^+$ T cells. Immunohistology of liver tissue sections of LoxP-TAg mice at 6-10 weeks or 12-35 weeks after Ad.Cre injection as indicated. (**A**) Tissues were stained with antibodies specific for CD3 and counterstained with hematoxylin. Scale bar, 50 µm. At least five mice were analyzed for each time point and representative stainings are shown. (**B**) CD3$^+$ T cells were quantified in at least five lesions and five HCCs. Whereas all cells in lesions were counted, the percentage of intra- and peritumoral CD3$^+$ T cells was determined by counting cells in five high power fields (HPFs, x400 magnification) per tumor. Bars represent means, error bars indicate SEM.

Supplemental Figure 7

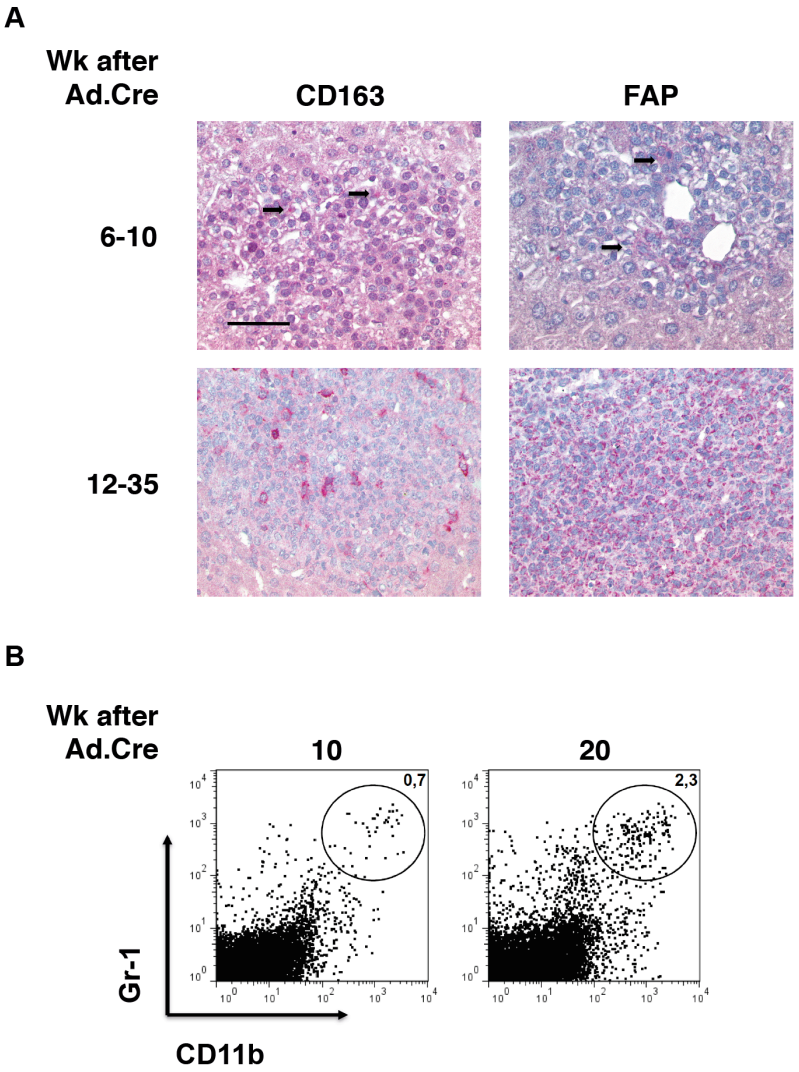

Figure S-7: Detection of M2 macrophages, FAP$^+$ stromal cells and CD11b$^+$ Gr-1$^+$ immature myeloid cells in virus-induced HCC. (**A**) Immunohistology of liver tissue sections of LoxP-TAg mice at 6-10 weeks or 12-35 weeks after Ad.Cre injection as indicated. (**A**) Tissues were stained with antibodies specific for CD163 (M2 macrophage marker) or fibroblast activation protein (FAP) and counterstained with hematoxylin. Scale bar, 50 µm. At least five mice were analyzed for each time point and representative stainings are shown. (**B**) For analysis of immature myeloid cells, single cell preparations of liver tumors were double-stained with antibodies against Gr-1 and CD11b. Shown is a representative plot (n = 3) of flow cytometry analysis of HCC-bearing LoxP-TAg mice 10 and 20 weeks after Ad.Cre infection. Numbers show percentage of Gr-1/CD11b double-positive cells of total liver cells.

Supplemental Figure 8

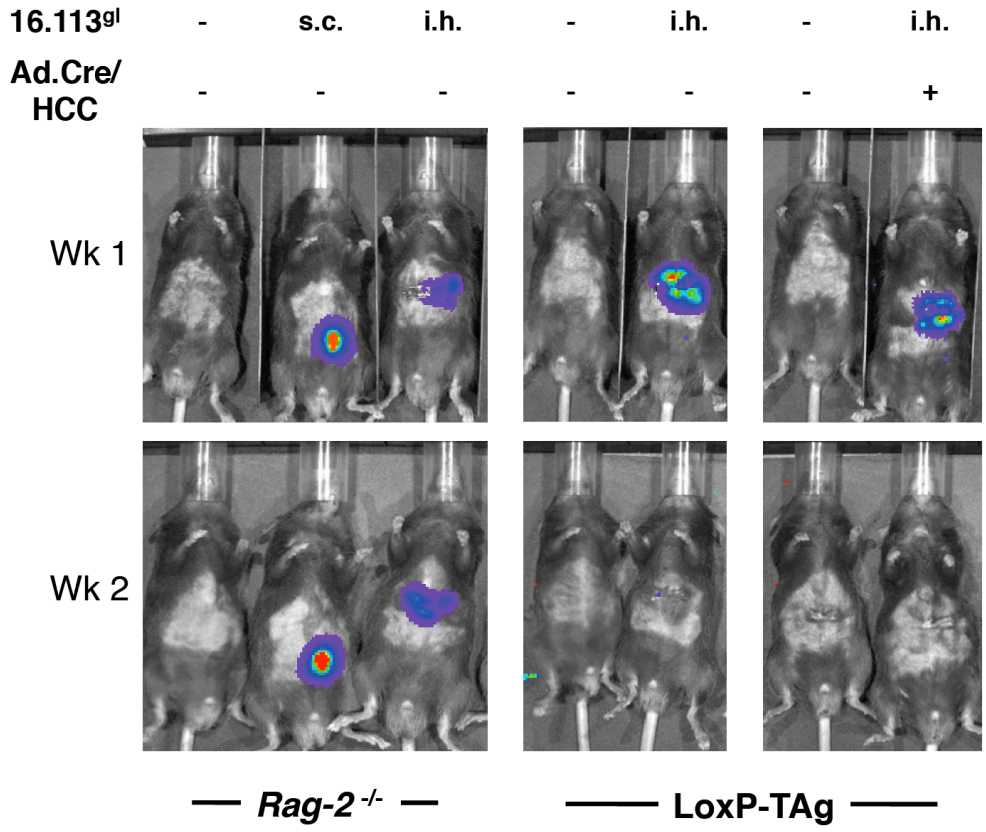

Figure S-8: TAg^{+} 16.113^{gl} cells grow in the liver of HCC-bearing LoxP-TAg mice but lose Fluc expression. One x 10^{6} 16.113^{gl} cells were injected intra-hepatically (i.h.) into the indicated mice and followed by BL imaging until mice had to be sacrificed due to 16113^{gl} or primary HCC tumor growth (in Rag-2 deficient and HCC-bearing mice only). Overlays of luminescent and light photographs from representative mice of two experiments (see also Figure 5B) are shown: Rag-2 deficient mice (n=4) and HCC-bearing mice 2-6 months after Ad.Cre injection (n=14). Age-matched untreated LoxP-TAg mice injected into the liver with 1 x 10^{6} 16113^{gl} cells served as control (n=3). BL images taken directly (data not shown) and one week after 16113^{gl} cell inoculation served an injection control. Rag-2 deficient mice (n=2) injected subcutaneously (s.c.) with 1 x 10^{6} 16113^{gl} cells served as additional control for stable luciferase expression in the absence of T cells. Untreated mice injected with luciferin were used as background controls (n=2).

Supplemental Figure 9

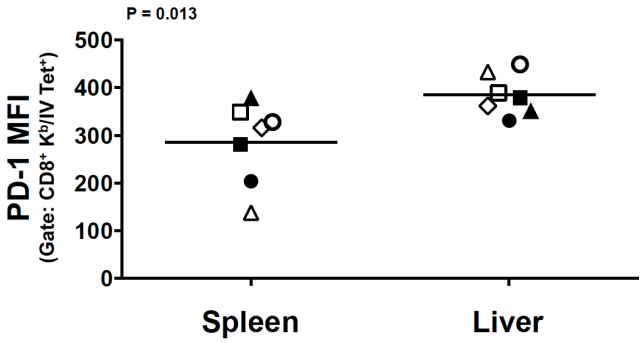

Figure S-9: pIV-specific CD8$^+$ T cells in the liver of HCC-bearing LoxP-TAg mice have higher PD-1 expression than in the spleen. Relative mean fluorescence intensity (MFI) of PD-1 on K^b/IV tetramer positive CD8$^+$ T cells in spleen and liver as measured in Figure 6B was calculated using FlowJo software. Individual mice were analysed, mean values from combined data of two independent experiments are shown.

Supplemental Figure 10

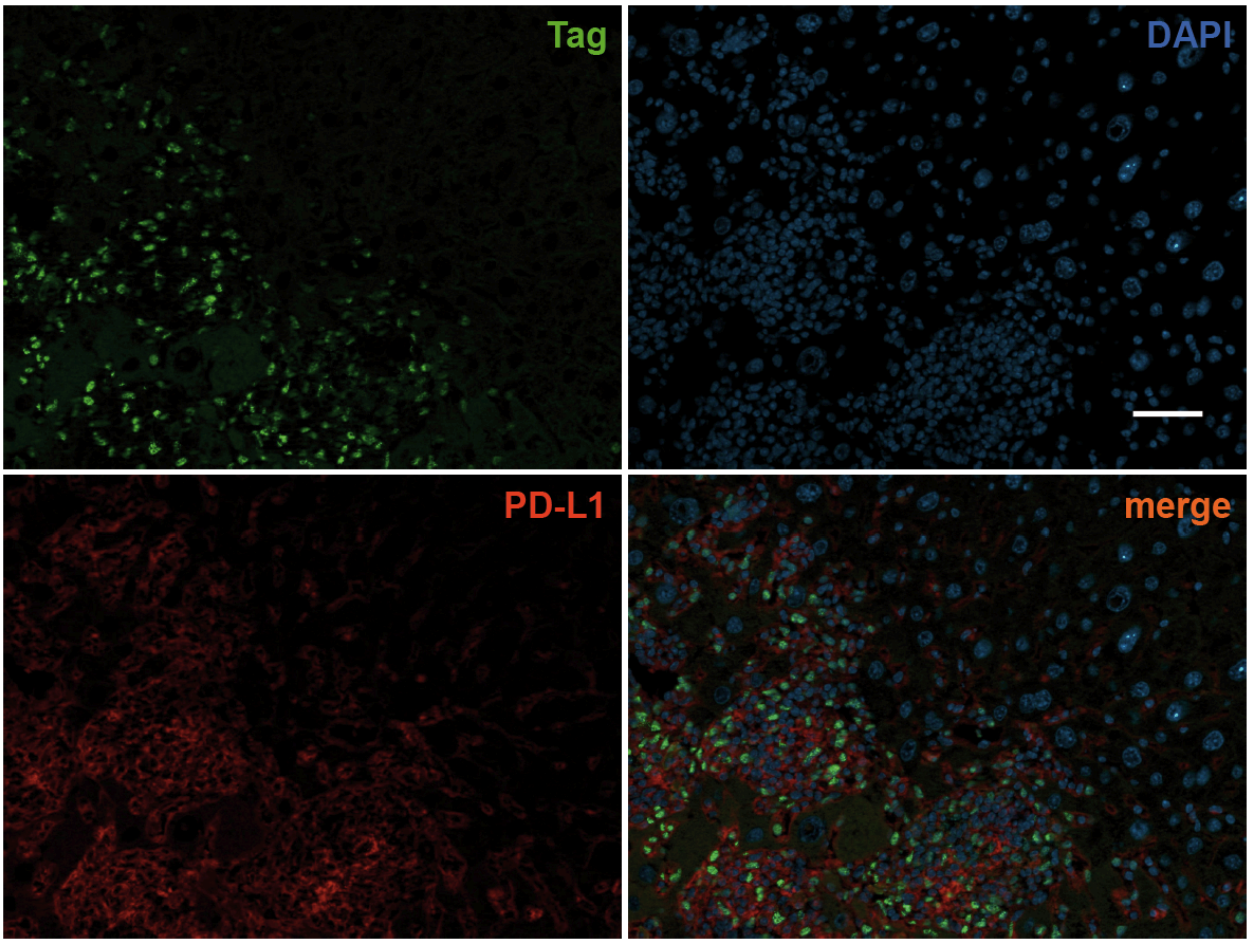

Figure S-10: PD-L1 is expressed by hepatocellular carcinoma cells and stromal cells. Liver obtained from LoxP-TAg mice 2-5 months after Ad.Cre infection were double-stained for PD-L1 (red) and TAg (green), nuclei were counterstained with DAPI. Cells that are either double-positive for TAg and PD-L1 or single-positive for PD-L1 are shown in the overlay (merge). Scale bar, 50 μm. Immunohistochemical analysis is representative for 5 mice.

Supplemental Figure 11

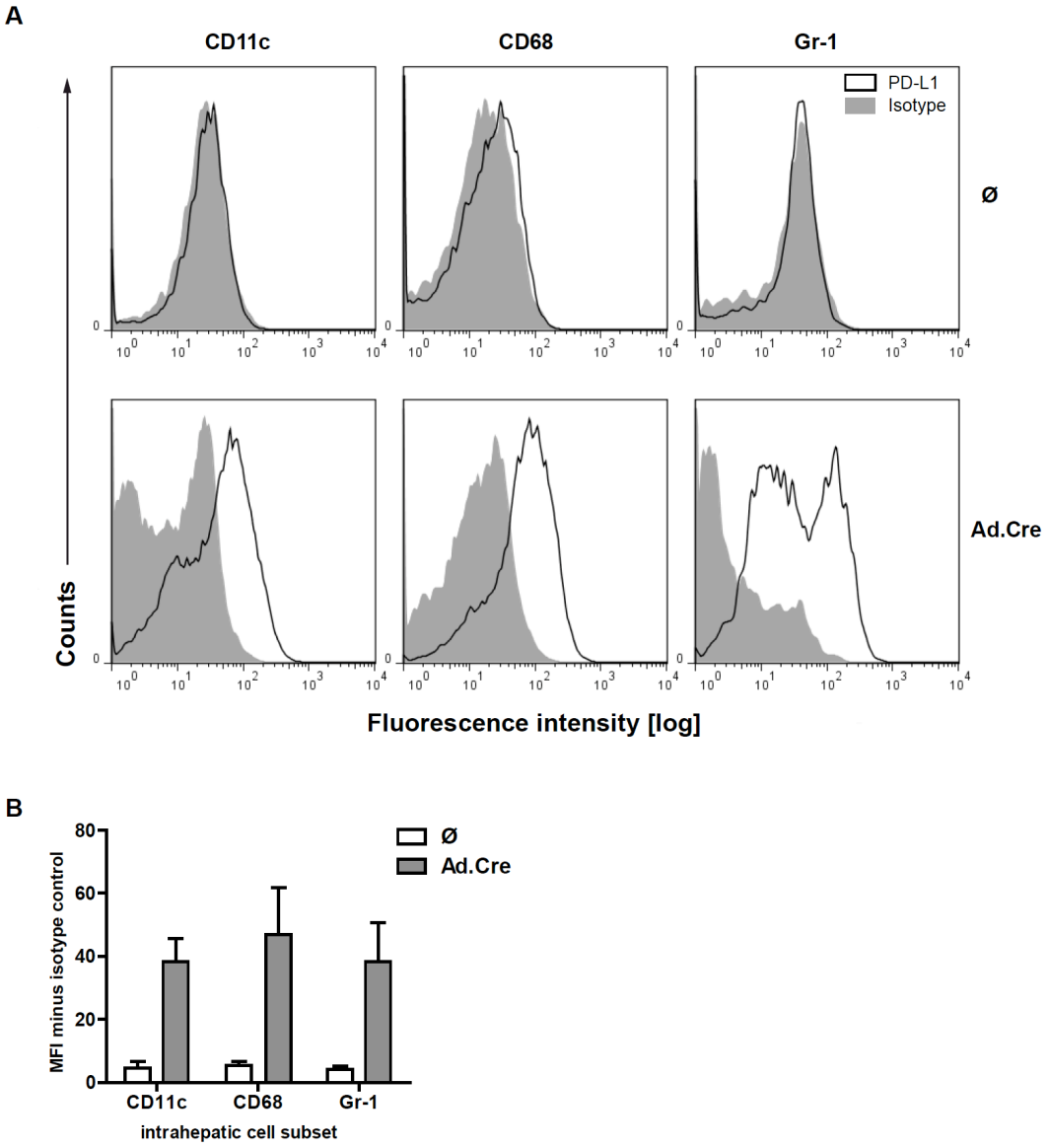

Figure S-11: PD-L1 is expressed on stromal cells upon virus-induced HCC development. Single cell suspensions of livers obtained from HCC-bearing LoxP-TAg mice 10-20 weeks after Ad.Cre infection (Ad.Cre) or age-matched untreated LoxP-TAg mice (Ø) were double-stained with CD11c, CD68 or Gr-1, respectively, and PD-L1 (open histogram) or isotype antibody (filled histogram). (**A**) The flow cytometry profiles for each marker are representative of three mice and data are summarized in (**B**) as MFI minus the MFI of isotype control stained cells (n = 3), open bars: untreated LoxP-TAg mice; filled bars: Ad.Cre infected LoxP-TAg mice.

Supplemental Figure 12

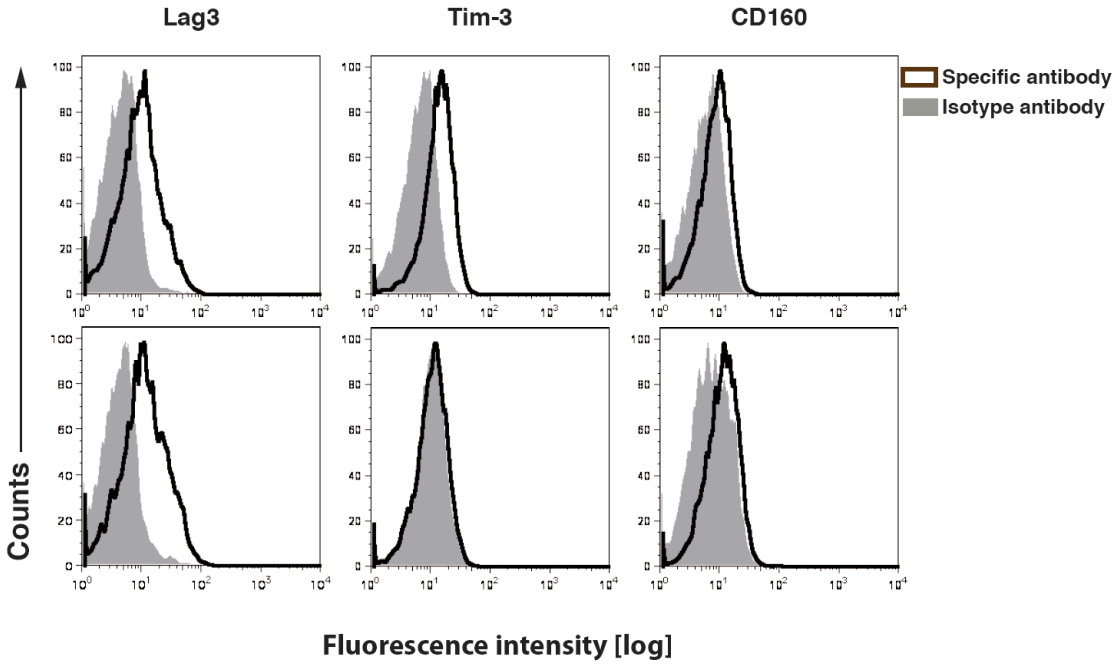

Figure S-12: Analysis of inhibitory receptors on pIV-specific CD8$^+$ T cells in the liver of virus-infected LoxP-TAg mice. Mice that showed PD-1 expression on TAg-specific CD8$^+$ T cells in the liver (see Figure 6B) were analyzed for the expression level of additional markers associated with an exhausted phenotype, notably Lag3, Tim-3, and CD160. Shown is expression of the respective marker on K^b/IV tetramer positive CD8$^+$ T cells (open histogram). Isotype antibody staining is also shown (filled histogram). The flow cytometry profiles for each marker are representative for 5 to 9 mice.

Supplemental Figure 13

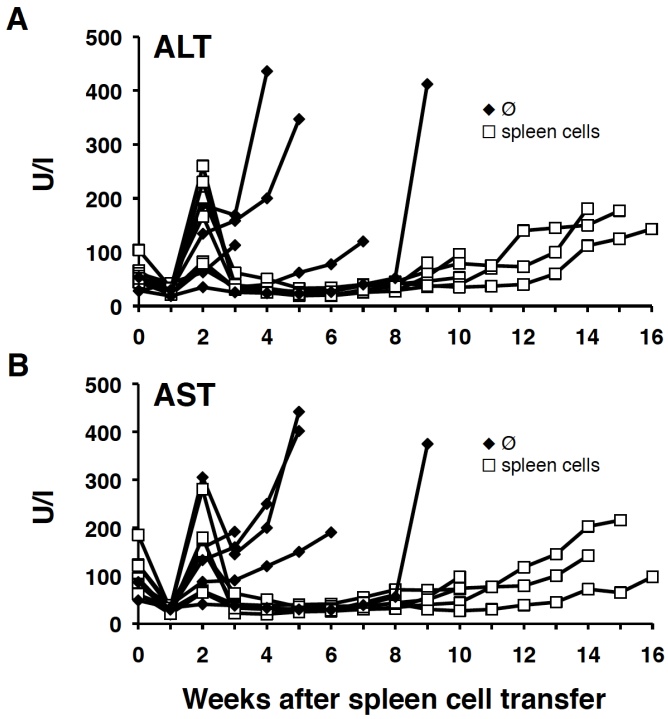

Figure S-13: Liver damage after adoptive spleen cell transfer. After irradiation (5 Gy) and adoptive cell transfer of 1 x 10^7 spleen cells or 1 x 10^6 $CD8^+$ T cells obtained from Ad.Cre-treated HCC-bearing LoxP-TAg mice the serum of recipient HCC-bearing LoxP-TAg mice was analyzed for liver enzymes (**A**) alanine aminotransferase (ALT) and (**B**) aspartate aminotransferase (AST), until mice had to be sacrificed due to tumor load. A representative analysis for spleen cell transfer into LoxP-TAg mice 14 weeks after Ad.Cre-injection is shown (G, n=6; see also Figure 6E). Irradiated Ad.Cre-treated HCC-bearing LoxP-TAg mice served as control (F, n=5).

Supplemental Figure 14

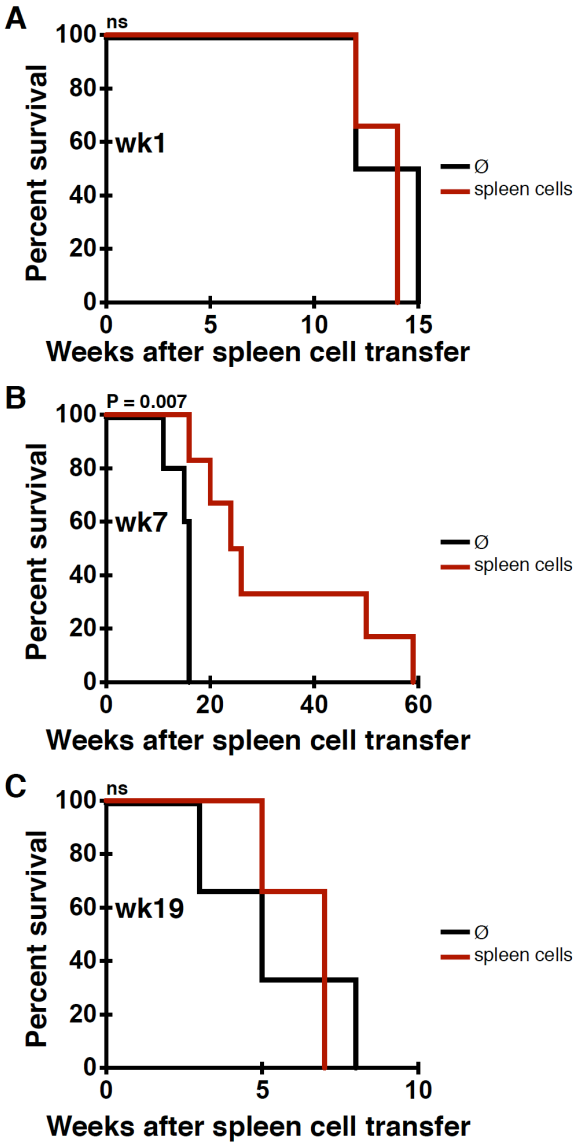

Figure S-14: Delay of HCC progression depends on the time point of adoptive spleen cell transfer. One day after whole body irradiation (5 Gy) HCC-bearing LoxP-TAg mice one (**A**; n=3), seven (**B**; n=6) and 19 weeks (**C**; n=3) after Ad.Cre-injection received an i.v. injection of 5 x 10^6 spleen cells obtained from Ad.Cre-treated HCC-bearing LoxP-TAg donor mice (red line, 16-24 weeks after Ad.Cre-injection). Survival of HCC-bearing LoxP-TAg mice was monitored, irradiated Ad.Cre-treated HCC-bearing LoxP-TAg mice served as control (black line, n=4, n=5 and n=3, respectively); ns, not significant.