

Supplementary Figure Legends

Supplementary Figure S1: Neoantigen load tends to increase with increasing chronic liver disease severity and fibrosis stage. Predicted neoantigen load was calculated in 20 human liver cirrhosis samples. There is a trend towards greater neoantigen load with more severe fibrosis stage, though this is not statistically significant, due to low sample number. One-way ANOVA was performed and found no significance.

Supplementary Figure S2: Animals injected with one dose of 25 mg/kg DEN at 2 weeks and weekly 4% CCl₄ starting at 6 weeks do not have any microscopic tumors prior to initiation of anti-PD-1 or IgG control antibody therapy. Representative 4X image of H&E stained liver at 10 weeks of age. Livers show centrilobular necrosis and immune infiltrate due to weekly CCl₄ injections, but no nodules of tumor cells. This shows that our intervention began in a preventive time frame.

Supplementary Figure S3: Whole section imaging of liver sections for quantification of immune cells reveals CD4+ T cell and CD8+ T cell tissue infiltration is associated with tumor prevention. (A) Whole liver section from an untreated animal sacrificed at 10 weeks immunostained for CD3 (red), CD4 (white), and CD8 (green) imaged using an AxioScan slide scanner microscope system. (B) Inset of (A). (C) Whole liver section from an IgG control treated animal sacrificed at 23 weeks immunostained for CD3 (red), CD4 (white), and CD8 (green) imaged using an AxioScan slide scanner microscope system. (D) Inset of (C).

Supplementary Figure S4: Image analysis pipeline for quantifying CD3+ cells, CD3+ CD4+ T cells, and CD3+ CD8+ T cells in whole liver sections. In brief, regions of interest are detected in the CD3 channel of each whole liver section image. Then intensities are measured both within the ROI (C_i), and in a small 2 μm band around each ROI (b_g). The ratio of C_i / b_g is calculated, and cells with a ratio > 1.7 are counted as positives. Then, intensities in the CD4 channel and CD8 channel are measured in each of the CD3+ ROIs. CD3+ CD4+ T cells and CD3+ CD8+ T cells are quantified using the same C_i / b_g calculation in those channels. Finally, the surface area of each section is measured, and CD3+ cells / mm², CD3+ CD4+ T cells / mm², and CD3+ CD8+ T cells / mm² are calculated.

Supplementary Figure S5: Tumor burden after preventive immunotherapy correlates with adaptive immune infiltration into liver parenchyma. Images of livers from (A) two IgG control-treated animals and (B) two anti-PD-1 treated animals that were randomly selected and sacrificed at 21 weeks of age. (C) There was no difference in surface tumor number in these animals. There were also no differences in (D) CD3+ cells, (E) CD3+ CD4+ T cells, or (F) CD3+ CD8+ T cells in the liver parenchyma of these animals. Two tailed student's t tests were performed for the data shown in S3C-F. All data are presented as mean ± SEM.