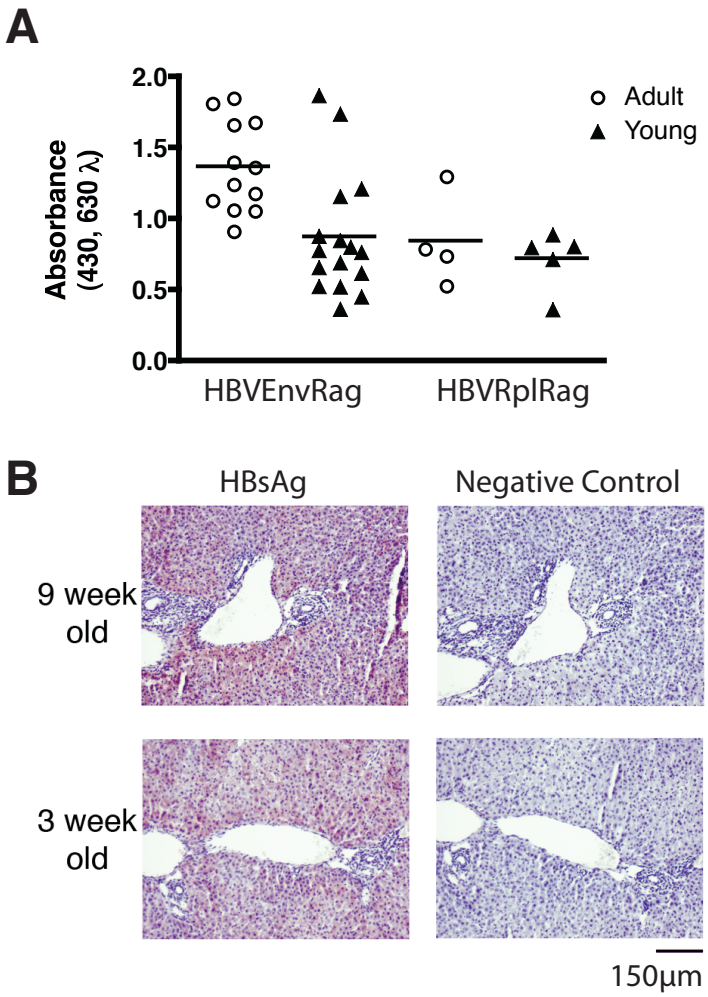
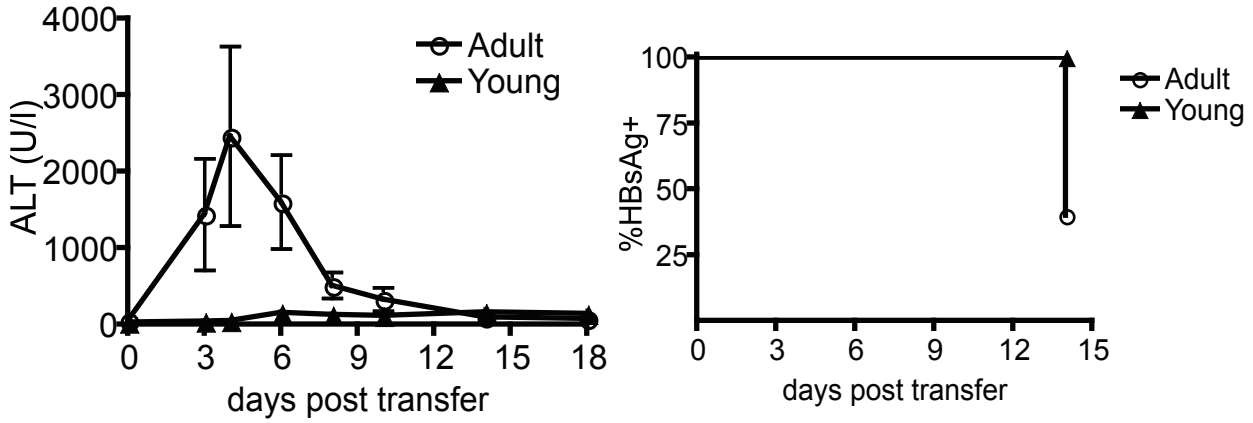
Publicover et al. Supplemental Figure 1



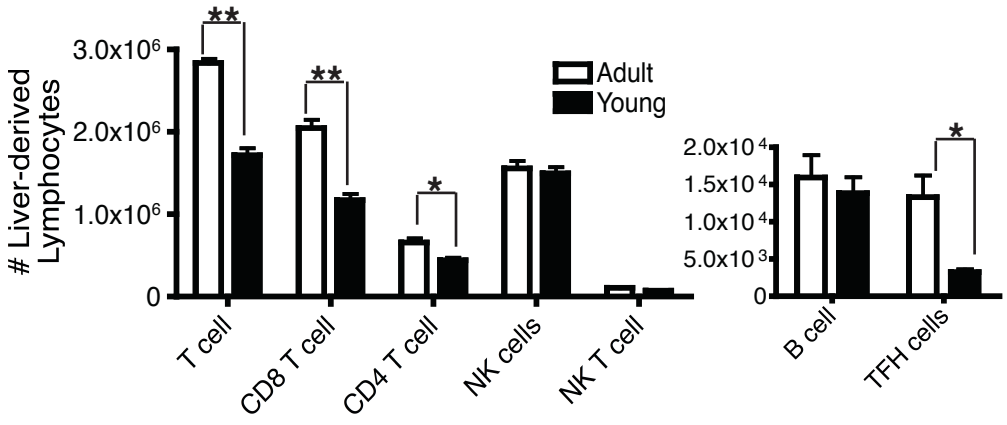
Supplemental Figure 1
Comparison of baseline protein expression of HBV transgenes in young and adult HBVtgRag mice**.** (**A**) Absorbance (430, 630λ) generated by presence of HBsAg using ETI-MAK-2 PLUS (Diasorin) on plasma from young (3-4 week-old) or adult (8-12 week-old) HBVEnvRag or HBVRplRag mice. (**B**) Representative liver sections from tissue from young and adult HBVEnvRag mice stained with goat polyclonal anti-HBsAg (Abcam ab17183).

Publicover et. al, Supplemental Figure 2



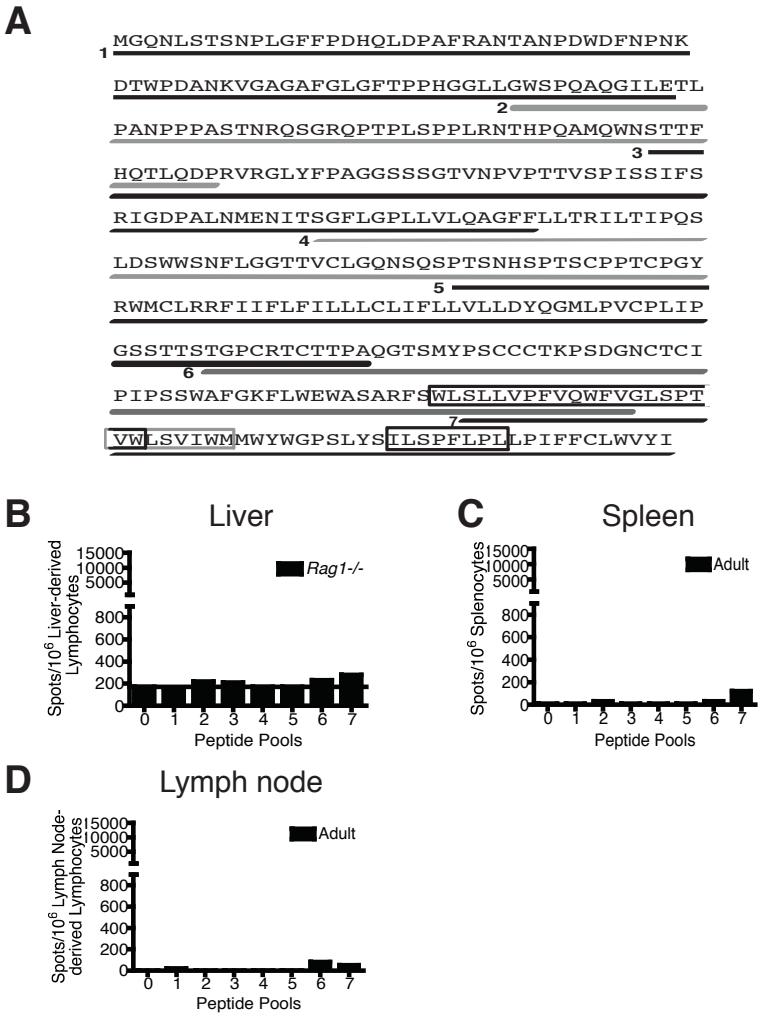
Supplemental Figure 2
Transfer of adult splenocytes into young and adult HBVEnvRag mice on B10.D2 (H-2^d) background results in a difference in disease and HBsAg clearance. (**A**) Depiction of plasma alanine transaminase (ALT) in young and adult HBVEnvRag after adoptive transfer of adult wild-type, syngeneic B10.D2 mouse splenocytes. Error bars show mean±SEM. (**B**) Depiction of percentage of mice with detectable circulating HBsAg**,** N≥5 mice.

Publicover et al. Supplemental Figure 3



Supplemental Figure 3
Absolute numbers of various liver lymphocyte populations in young and adult HBVEnvRag mice eight days post transfer. Absolute numbers of populations of liver-derived lymphocytes in young and adult HBVEnvRag eight days post transfer of adult, syngeneic splenocytes. Adult mice have more total liver-derived lymphocytes compared to the young (mean±SEM: $5.5X10^6 \pm 1.5X10^5$ vs $4.1X10^6 \pm 6.1X10^5$). T follicular helper cells (TFH) are defined as $CD4^+$, $CXCR5^+$, $ICOS^+$ cells. B cells are defined as $CD19^+$, $B220^+$ cells. Error bars indicate mean±SEM; N=4 mice. Statistical significance as determined by two-tailed unpaired t test was observed between young and adult T cell ($p<0.0001$), CD8 T cell ($p=0.0005$), CD4 T cell ($p=0.015$) and TFH cell ($p=0.0146$) numbers.

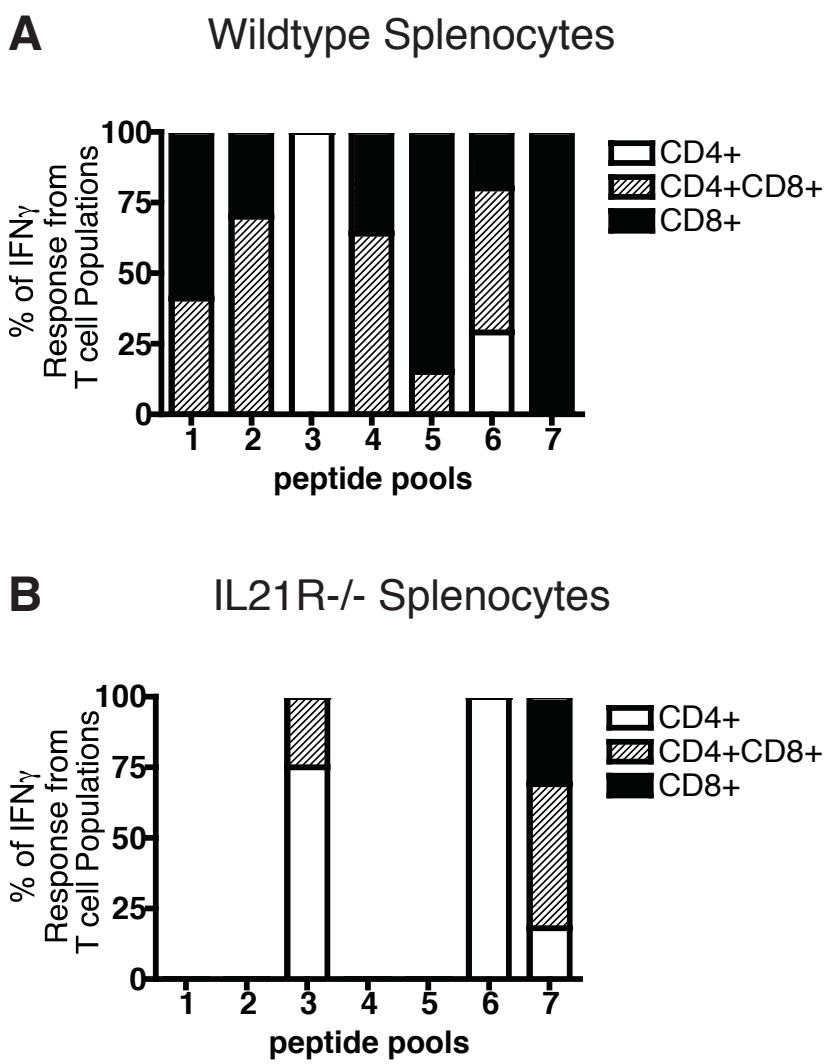
Publicover et al. Supplemental Figure 4



Supplemental Figure 4

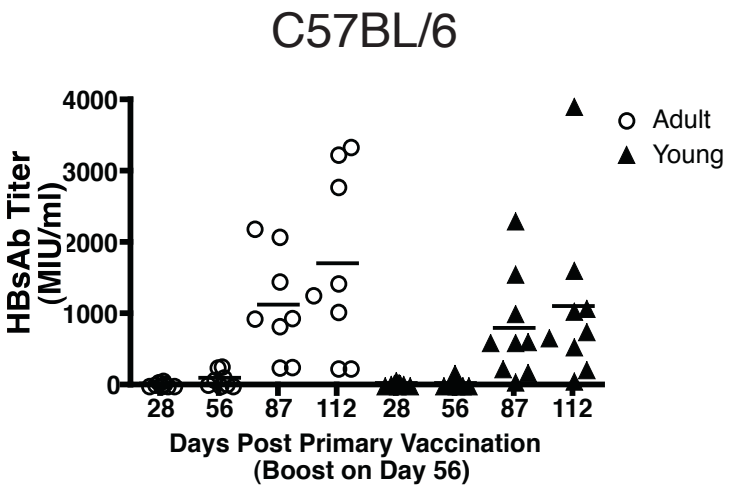
Amino acid sequences of peptide pools spanning all HBV envelope proteins; Post adoptive transfer, IFNγ responses were obtained from ELISpot assays on *Rag1-/-* liver and adult HBVEnvRag spleen and pancreaticoduodenal lymph node**.** (**A**) Eleven to fourteen 15mer overlapping peptides were grouped together sequentially throughout the envelope protein to establish peptide pools. Underlined regions indicate the amino acids that are included in the peptides in the pools. Boxed amino acids indicate peptides previously identified in alternate publications by alternate methods to be dominant or subdominant epitopes for H-2b: WLSLLVPFVQWFVGLSPTVW, ILSPFLPL (Schirmberk et al, J Immunol 2001); VWLSVIWM (Schirmberk et al, Eur J Immunol 1998). (**B**) Day 8 or any other day examined post adoptive transfer of syngeneic, wild-type splenocytes into *Rag1-/-* mice did not induced non-specific stimulation of liver-derived lymphocytes by any of the pools. (**C**) HBV-specific T cell responses in the splenocytes eight days post transfer of adult splenocytes into an adult HBVEnvRag mice. (**D**) HBV-specific T cell responses in pancreaticoduodenal lymph node-derived lymphocytes eight days post transfer of adult splenocytes into an adult HBVEnvRag mice. **B-D** Samples are pooled where N≥4 mice.

Publicover et al. Supplemental figure 5



Supplemental Figure 5
Contributions of CD4 and CD8 populations to IFNγ responses in the liver following peptide pool stimulations. Two months post transfer of (**A**) wild-type or (**B)** IL-21R-/- C57BL/6 splenocytes into adult HBVEnvRag mice, intracellular cytokine staining was used to identify the contributions of liver-derived $CD4^+$ ($CD8^-$; open bars), $CD4^+CD8^+$ (striped bars) and $CD8^+$ ($CD4^-$; closed bars) to the IFNγ response following peptide stimulation. Percent of IFNγ response over unstimulated background of specified populations over total IFNγ response over background was used following individual peptide pool stimulations. (**B**) At two months post transfer of IL-21R-/- splenocytes, no IFNγ response over background was observed in pools 1, 2, 4 and 5. Samples were pooled from N≥4 mice.

Publicover et al. Supplemental figure 6



Supplemental Figure 6
HBsAb response in young and adult C57BL/6 wild-type mice after i.m. vaccination with the HBV vaccine. HBsAb response in plasma after i.m. vaccination of wild-type C57/BL6 young-(3 week-old; during weaning; closed triangle) or adult-aged mice (10 week-old; open circles) with 50µl (0.5µg) of RecombivaxHB® (Merck) at day 0 and day 56, N≥8 mice.