Supplemental Information

Figure S1

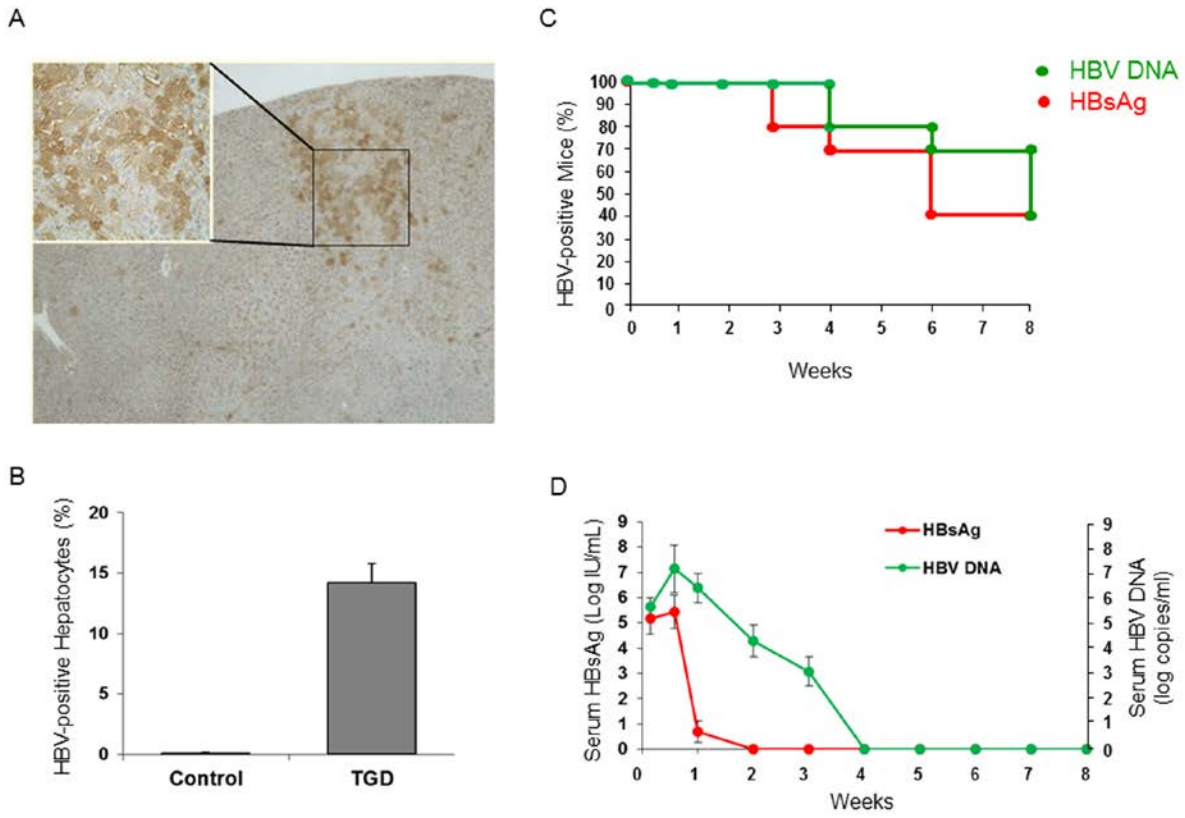


Figure S2

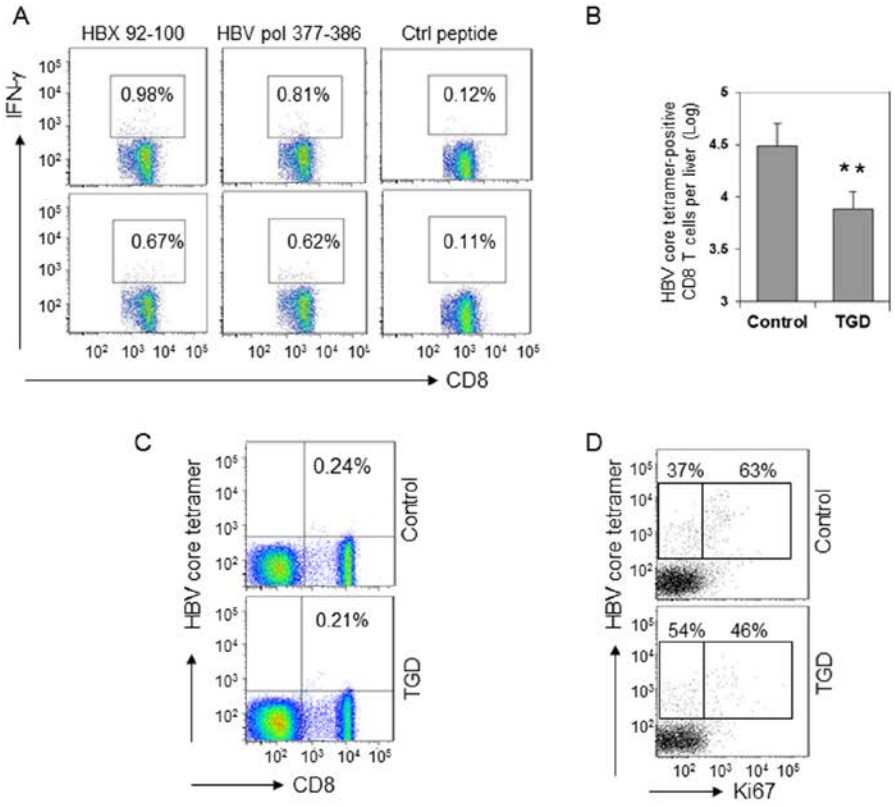

A
HBX 92-100
HBV pol 377-386
Ctrl peptide
IFN-γ
0.98%
0.81%
0.12%
0.67%
0.62%
0.11%
CD8
B
HBV core tetramer-positive CD8 T cells per liver (Log)
**
Control
TGD
C
HBV core tetramer
0.24%
0.21%
Control
TGD
CD8
D
37%
63%
54%
46%
Control
TGD
Ki67

Figure S3

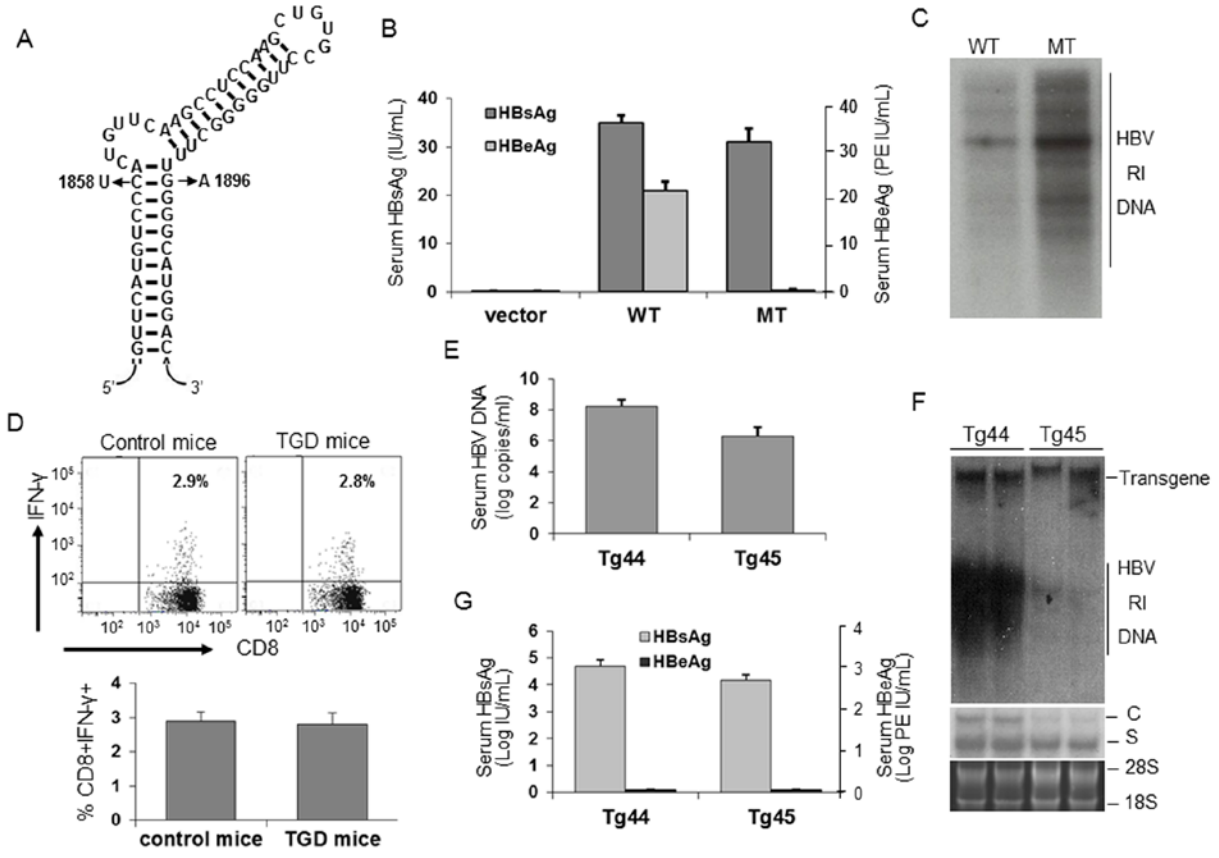
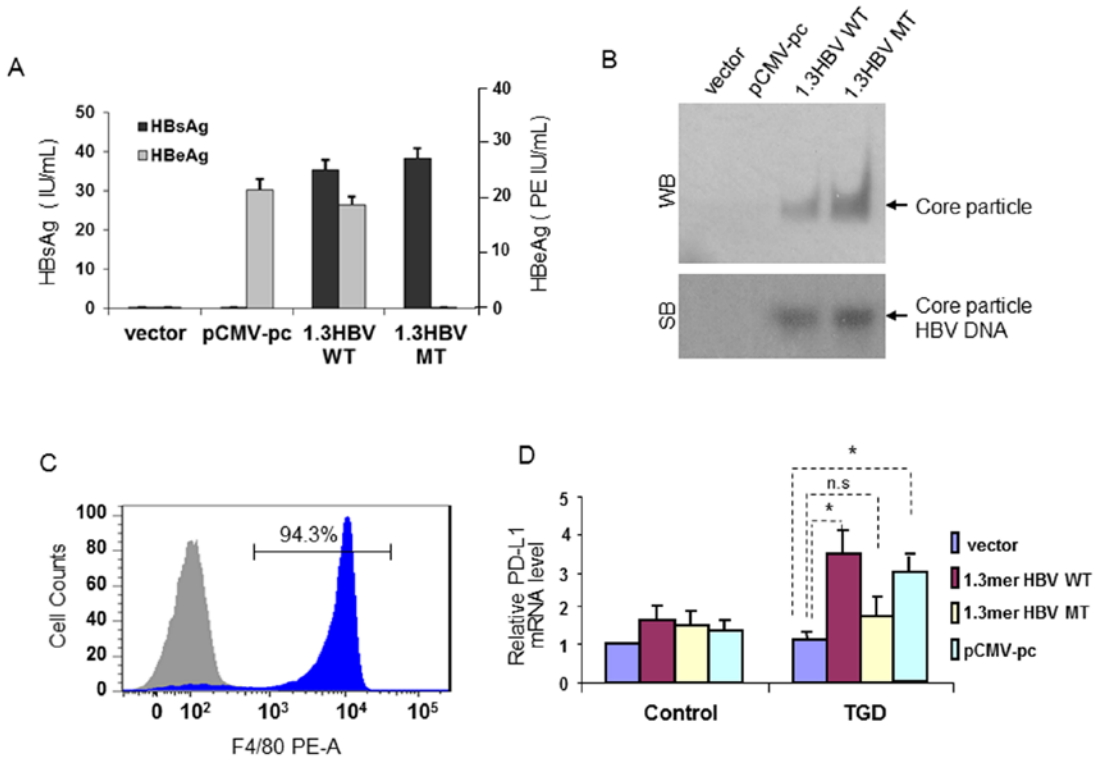

A
1858 U
A 1896
5'
3'
B
HBsAg
HBeAg
Serum HBsAg (IU/mL)
Serum HBeAg (PE IU/mL)
vector
WT
MT
C
WT
MT
HBV
RI
DNA
D
Control mice
TGD mice
2.9%
2.8%
IFN-γ
CD8
% CD8+IFN-γ+
control mice
TGD mice
E
Serum HBV DNA (log copies/ml)
Tg44
Tg45
F
Tg44
Tg45
Transgene
HBV
RI
DNA
C
S
28S
18S
G
HBsAg
HBeAg
Serum HBsAg (Log IU/mL)
Serum HBeAg (Log PE IU/mL)
Tg44
Tg45

Figure S4


A
HBsAg
HBeAg
HBsAg (IU/mL)
HBeAg (PE IU/mL)
vector
pCMV-pc
1.3HBV WT
1.3HBV MT
B
vector
pCMV-pc
1.3HBV WT
1.3HBV MT
WB
SB
Core particle
Core particle
HBV DNA
C
94.3%
Cell Counts
F4/80 PE-A
D
Relative PD-L1 mRNA level
n.s
*
*
vector
1.3mer HBV WT
1.3mer HBV MT
pCMV-pc
Control
TGD

Supplemental Figure Legends

Figure S1. HBV persists in TGD mice but not in control mice. **Related to Figure 1.** (A) Immunostaining of the HBV core protein in the liver tissue section of TGD mice at three months after the injection of the HBV DNA. (B) Quantitative analysis of HBV-positive hepatocytes. Results were obtained from multiple liver tissue sections of at least three mice. (C) Persistence analysis of HBV in 6-8-month old TGD mice. Ten 6-8-month old mice were injected with the HBV genomic DNA. Mouse sera were collected at different time points for the analysis of HBsAg and HBV DNA using ELISA and PCR, respectively. (D) Persistence analysis of HBV in the offspring of TGD mice. Five nine-week old mice born to female TGD mice were injected with the HBV genomic DNA and analyzed for serum HBsAg and HBV DNA at different time points after DNA injection.

Figure S2. CTL responses are impaired in TGD mice. Related to Figure 2. (A) Analysis of $CD8^+$ T cell responses to HBV X (HBX) and polymerase (pol) peptides. Control mice and TGD mice injected with the HBV DNA were sacrificed at 14 days after injection. Intrahepatic mononuclear cells were then isolated and stimulated with HBX, pol or the control (Ctrl) peptide and analyzed by flow cytometry for $CD8^+IFN\text{-}\gamma^+$ cells. (B) Total number of HBV-specific $CD8^+$ T cells per mouse liver. $CD8^+$ T cells stained by the HBV core tetramer as shown in Figure 2B were quantified. The results represent the mean of five different mice. **, $p<0.01$. (C) $CD8^+$ T cells in the liver of control and TGD mice at 6 days after HBV DNA injection were stained by the HBV core tetramer followed by flow cytometry analysis. (D) HBV tetramer-stained CD8+ T cells were analyzed for the expression of Ki67.

Figure S3. The precore mutation abolishes HBeAg expression without affecting HBV replication. Related to Figure 5. (A) The epsilon structure in the HBV pregenomic RNA and the locations of nucleotide muations are illustrated. A G→A mutation at nucleotide 1896 was created and a compensatory mutation of C→U at nucleotide 1858 was also created to maintain the epsilon structure. (B-C) Huh7 cells were transfected with the pUC19 control, the 1.3mer wild-type HBV DNA (WT) or the HBeAg-negative HBV DNA (MT) for 48 hours. The incubation media were then harvested for the analysis of HBsAg and HBeAg by ELISA (B), and the cells were lysed for the analysis of the HBV RI DNA by Southern-blot (C) using our previous procedures (Tian et al., 2011). (D) The HBV-specific CD8+ T cells in the liver of control and TGD mice injected with the HBeAg-negative HBV genomic DNA were analyzed by flow cytometry as described in the Figure 2 legend. The results shown in the histogram represent the average of three independent experiments. There is no statistical significance between the results of control mice and TGD mice. (E) HBV DNA levels in the sera of nine-week old male Tg44 and Tg45 mice were analyzed by real-time PCR. (F) HBV DNA (top panel) and RNA (middle panel) in the liver of TG44 and Tg45 mice were analyzed by Southern-blot and Northern-blot, respectively. Two mice each were analyzed. In Southern-blot, the HBV transgene served as the loading control. In Northern-blot, the 28S and 18S ribosomal RNAs were stained by ethidium bromide to serve as the loading control (bottom panel). C and S mark HBV C gene and S gene transcripts, respectively. (G) HBsAg and HBeAg in the sera of Tg44 and Tg45 mice were analyzed by ELISA.

Figure S4. HBeAg expressed by HBV plasmids induces the expression of PD-L1 in hepatic macrophages of TGD mice. Related to Figure 6. (A) Huh7 cells were transfected with the pUC19 vector, the precore protein expression plasmid pCMV-pc, the 1.3mer wild-type HBV DNA or the HBeAg-negative 1.3mer HBV mutant DNA. The incubation media were harvested at 48 hours after DNA transfection for analysis of HBsAg and HBeAg by ELISA. (B) HBV particles in the incubation media harvested in (A) were precipitated in 26% polyethylene glycol 8000, 0.5 M NaCl and 60 mM EDTA at 4°C overnight. After the centrifugation, the pellet was resuspended in a buffer containing 50 mM Tris-HCl, pH 8.0, 40 mM $MgCl_2$, 50 mM NaCl and 1% Nonidet P-40, subjected to electrophoresis in a 1% agarose gel, and transferred to a filter paper. The filter paper was then used for Western-blot (WB) analysis using the anti-core antibody (upper panel) or Southern-blot (SB) analysis using a ^{32}P-labeled HBV DNA probe to reveal the encapsidated HBV DNA (lower panel). (C) Hepatic macrophages purified as described in Experimental Procedures were analyzed by flow cytometry for its purity. Blue curve, anti-F4/80 antibody; grey curve, isotype antibody control. (D) Hepatic macrophages isolated from control mice and TGD mice were treated with the incubation media of Huh7 cells that had been transfected with various DNA plasmids. Cells were then lysed and the level of PD-L1 mRNA was then quantified by real-time RT-PCR. The PD-L1 mRNA level of hepatic

macrophages isolated from control mice and treated with the control media was arbitrarily defined as one. The results represent the mean of three independent experiments. *, $p<0.05$; n.s., not significant.

Supplemental Experimental Procedures

Primers used in real-time PCR analysis

The following primers were used in the real-time PCR analysis for quantification of gene expression in hepatic macrophages:

TNF-α: 510-ACGGCATGGATCTCAAAGAC-529 (forward), 634-AGATAGCAAATCGGCTGACG-615 (reverse) (Genebank reference number NM_013693);

IL-1β: 937-TTCGTGAATGAGCAGACAG-955 (forward), 998-TGGTTTCTTGTGACCCTGAGC-978 (reverse) (Genebank reference number NM_008361);

Arg-1: 108-CTCCAAGCCAAAGTCCTTAGAG-129 (forward), 271-AGGAGCTGTCATTAGGGACATC-250 (reverse) (Genebank reference number NM_007482)

IL-10: 97-GCTCTTACTGACTGGCATGAG-117 (forward), 182-CGCAGCTCTAGGAGCATGTG-163 (reverse) (Genebank reference number NM_010548)

Mrc-1: 1203-CTATGCAGGCCACTGCTACA-1222 (forward), 1583-GTCTGCACCCTCCGGTACTA-1564 (reverse) (Genebank reference number NM_008625)

iNOS: 3465-CACCAAGCTGAACTTGAGCG-3484 (forward), 3548-CCATAGGAAAAGACTGCACCGA-3527 (reverse) (Genebank reference number AF065919)