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Supplemental information

Macrophages activated by hepatitis B virus have distinct metabolic profiles and suppress the virus via IL-1β to downregulate PPARα and FOXO3

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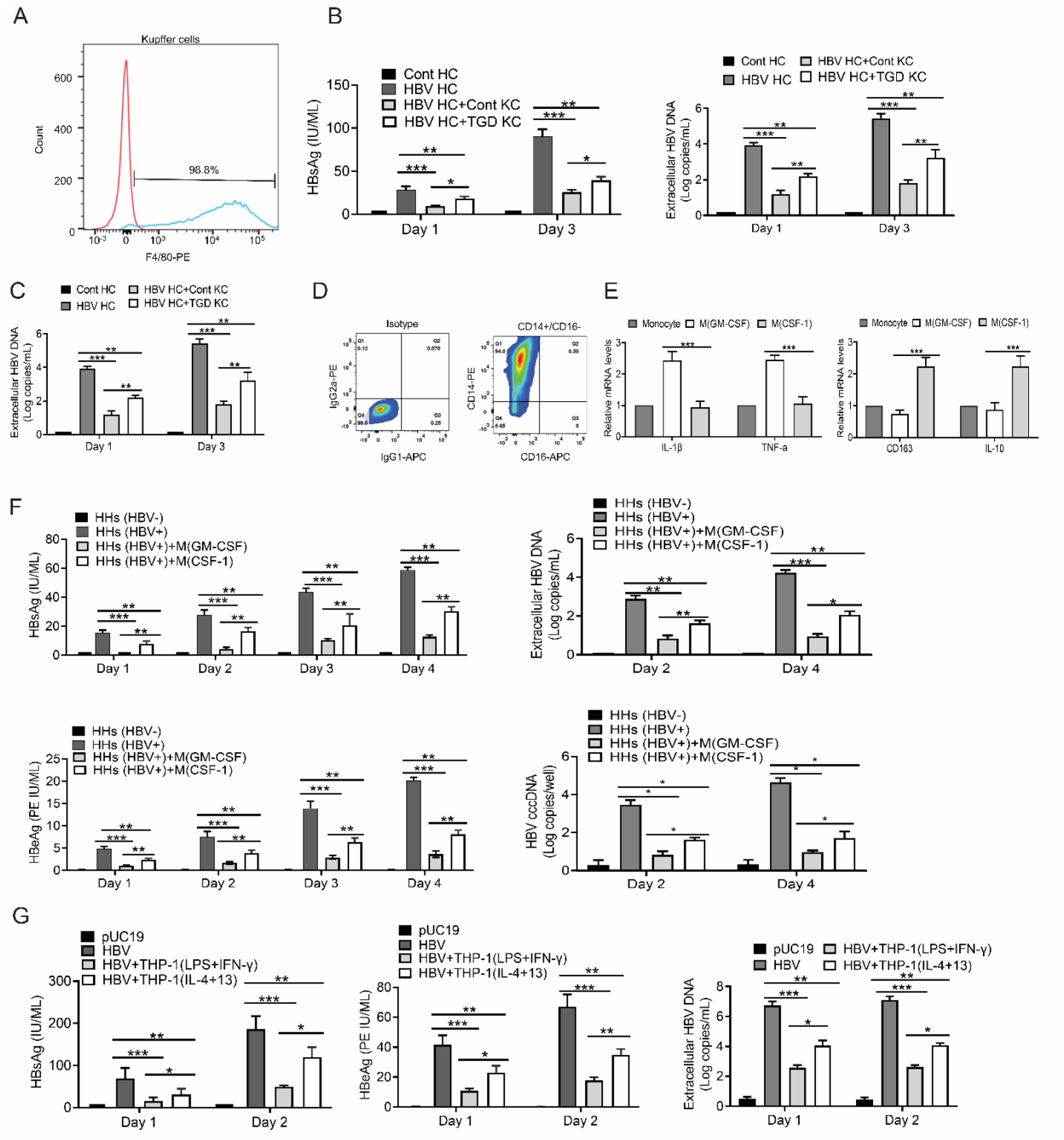


Figure S1. Effects of macrophages on HBV replication. Related to Figure 1.

(A) The purity of Kupffer cells (KCs) isolated from mice was analyzed by flow cytometry using F4/80 as the marker. The isotype antibody was used as the negative control. (B) The ex vivo effects of KCs isolated from control mice and TGD mice on HBV replication in hepatocytes isolated from HBV transgenic mice were analyzed on day 1 and day 3 after co-culturing by measuring the levels of HBsAg and HBeAg in the incubation media using ELISA. Hepatocytes isolated from control mice (Cont HC) were used as the negative control. (C) The study was conducted the same way as in (B), with the exception that the levels of HBV DNA in the incubation media were quantified by qPCR. (D) The purity of human classical monocyte

(CD14+CD16-) isolated from the peripheral blood of healthy donors was analyzed by flow cytometry. (E) The levels of IL-1β, TNF-α, CD163 and IL-10 RNAs in monocytes, M(GM-CSF) and M(CSF-1) were quantified by RT-qPCR. (F) The ex vivo effects of M(GM-CSF) or M(CSF-1) on HBV replication in human hepatocytes (HHs) at different timepoints after HBV infection were determined by measuring the levels of HBsAg, HBeAg and HBV DNA in the incubation media and the levels of HBV cccDNA in infected HHs. (G) Effect of M1-like THP-1(LPS+IFN-γ) and M2-like THP-1(IL-4+3) macrophages on HBV replication in Huh7 cells transfected with the 1.3mer HBV genomic DNA. Huh7 cells transfected with the vector pUC19 were used as the negative control. HBsAg (left panel), HBeAg (middle panel) and HBV virion-associated DNA (right panel) in the incubation media were quantified. *, $p<0.05$; **, $p<0.01$; ***, $p<0.001$.

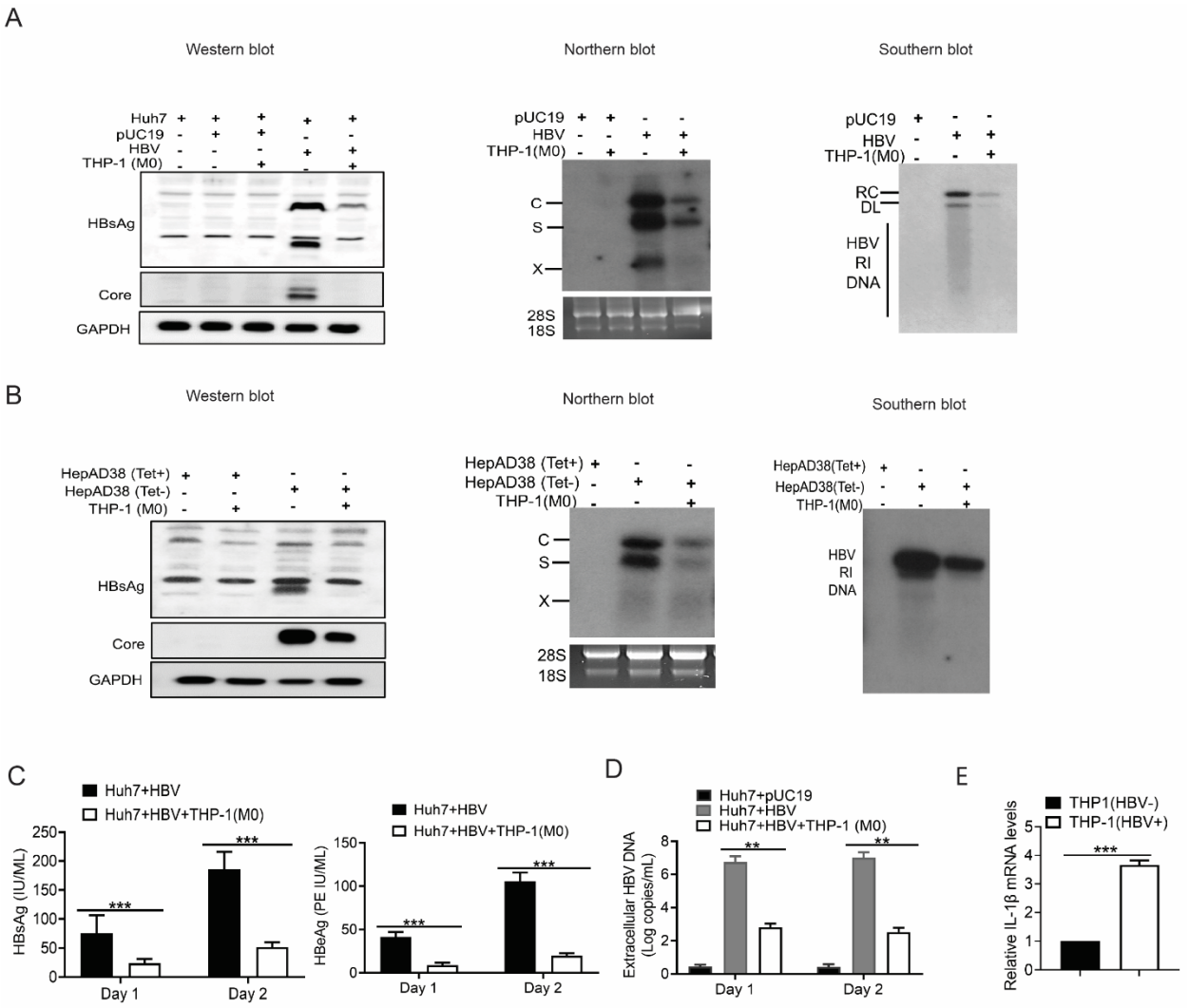
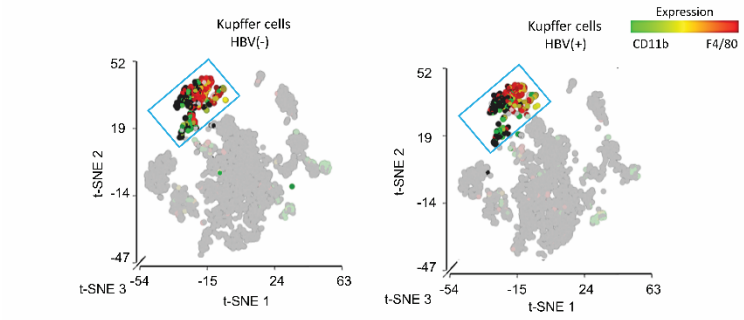


Figure S2. Effect of THP-1 macrophages on HBV replication. Related to Figure 2.

(A) THP-1 cells treated with PMA (THP-1(M0)) were co-cultured with Huh7 cells that had been transfected with the control vector pUC19 or the 1.3mer HBV genomic DNA as described in the legend to Figure 1. Huh7 cells were then lysed for western-blot analysis of HBV proteins (left panel), northern-blot analysis of HBV RNAs (middle panel), and Southern-blot analysis of HBV DNA. (B) THP-1(M0) macrophages were co-cultured with HepAD38 cells with or without the treatment of tetracycline. HepAD38 cells were lysed two days after co-culturing for western-blot analysis of HBV proteins (left panel), northern-blot analysis of HBV RNAs (middle panel) and Southern-blot analysis of HBV DNA (right panel). (C) Huh7 cells transfected with the 1.3mer HBV genomic DNA were co-cultured with THP-1 macrophages. HBsAg (left panel) and HBeAg (right panel) in the incubation media were then collected at the timepoints indicated and analyzed by ELISA. (D) Huh7 cells transfected with either pUC19 or the 1.3mer HBV genomic DNA were co-cultured with THP-1 macrophages and the virion-associated HBV DNA in the incubation media were quantified by qPCR at the timepoints indicated. (E) Analysis of IL-1β expression in THP-1(HBV-) and THP-1(HBV+) cells. The IL-1β RNA level was quantified by RT-qPCR. **, p<0.01; ***, p<0.001.

A



KC (HBV+) VS KC (HBV-)		
Genes	p value	Fold change (Log2)
IL-1β	6.33E-04	2.21
TNF-α	5.72E-08	3.78
CD206 (MRC1)	0.03	-1.53
CD163	3.87E-21	-1.73

B

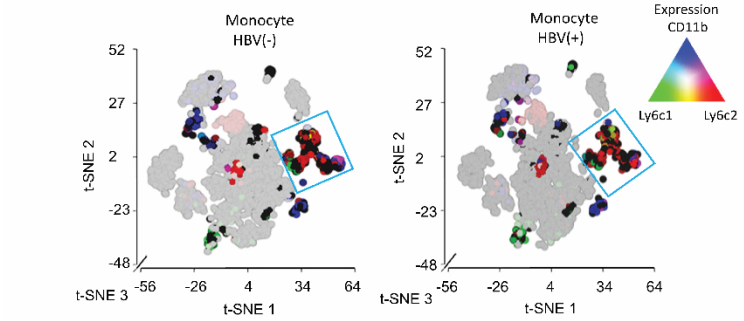


Figure S3. Single-cell RNA-sequencing for the analysis of Kupffer cells and monocyte-derived macrophages in the liver. Related to Figure 3.

(A) Non-parenchymal cells were isolated from control mice 4 days after the hydrodynamic injection of 20 μg pUC19 or pHBV1.3mer and subjected to scRNA-seq. The t-SNE plot was used to separate cell clusters. Itgam (i.e., CD11b) and Adgre1 (i.e., F4/80) were used as the markers for the identification of the Kupffer cell cluster (boxed). The fold-change of expression of IL-1β and TNF-α (i.e., M1 markers) as well as CD206 and CD163 (i.e., M2 markers) is shown in the Table to the right. (B) t-SNE plots were used to identify monocyte-derived macrophages (boxed) using three different makers Itgam (i.e., CD11b), Ly6c1 and Ly6c2.

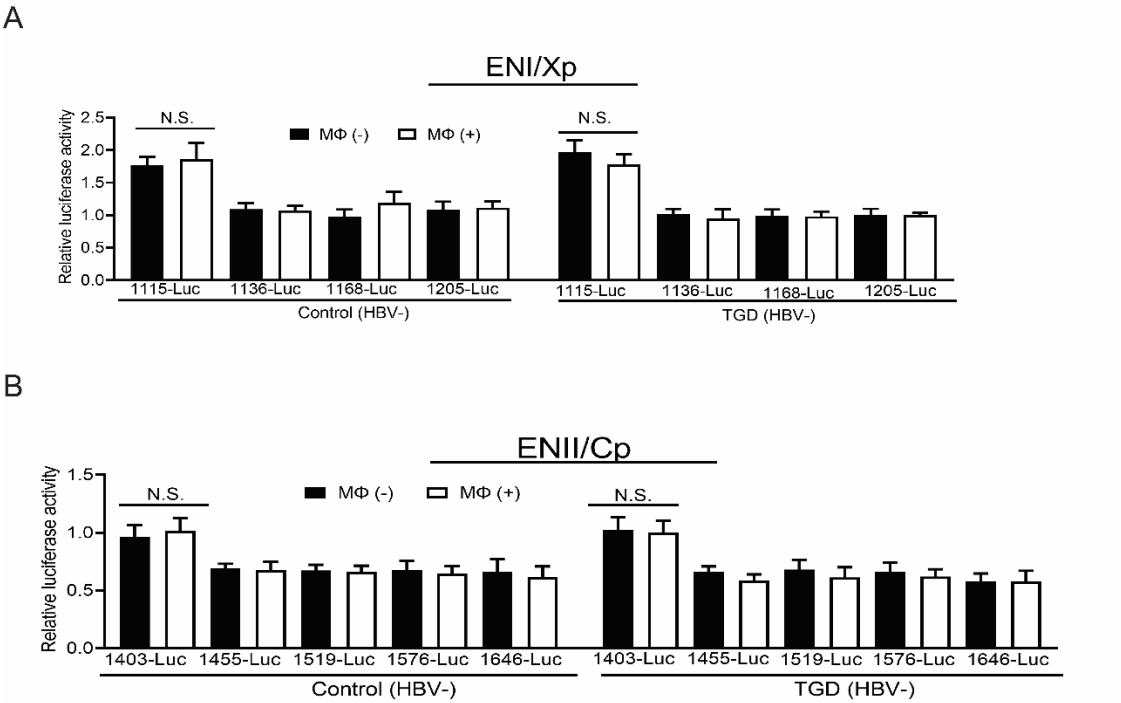


Figure S4. Lack of effect of hepatic macrophages on HBV ENI and ENII reporter constructs in the absence of replicating HBV. Related to Figure 4.

(A-B) Paired hepatocytes and Kupffer cells were isolated from control mice and TGD mice after the hydrodynamic injection of 20 μg pUC19. Hepatocytes were then transfected with either ENI (A) or ENII (B) luciferase reporter constructs ex vivo. These hepatocytes, with or without co-culturing with their paired Kupffer cells were then lysed for the analysis of the luciferase reporter activities. The results represent the mean ± SEM of at least three independent experiments. No statistical difference of the luciferase activities was observed whether or not hepatocytes were co-cultured with Kupffer cells.

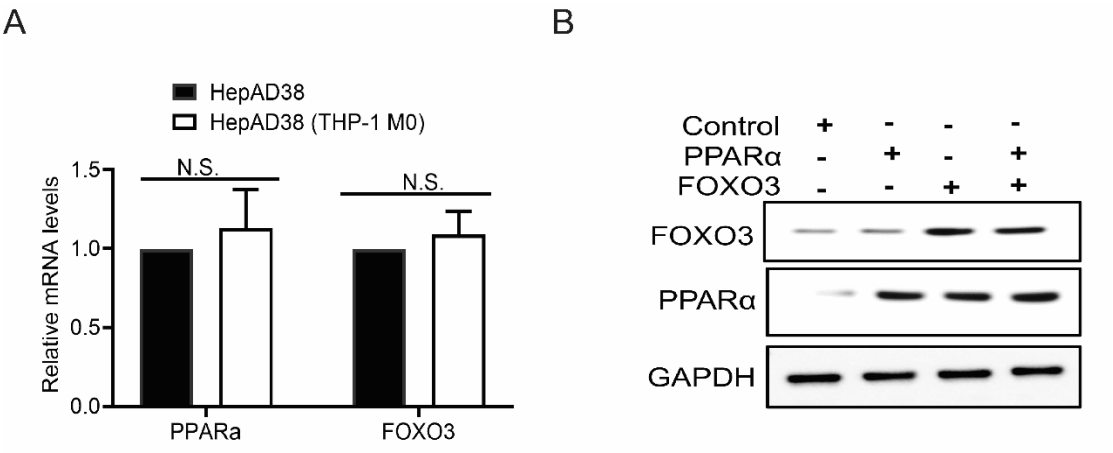


Figure S5. Effects of THP-1 macrophages on PPARα and FOXO3 RNAs in HepAD38 cells and the over-expression of PPARα and FOXO3 in Huh7 cells. Related to Figure 6.

(A) HepAD38 cells with replicating HBV were co-cultured with THP-1(M0) for 2 days and lysed for the analysis of PPARα and FOXO3 RNAs by RT-qPCR. The results represent the means ± SEM of at least three independent experiments. N.S., no statistical significance. (B) Huh7 cells were transduced with the lentiviral vector that expressed PPARα, FOXO3 or both and lysed for western-blot analysis of PPARα and FOXO3. Cells transduced with the control lentiviral vector was used as the control. Note that the over-expression of FOXO3 also increased the PPARα protein level, suggesting a role of FOXO3 in the regulation of PPARα expression.

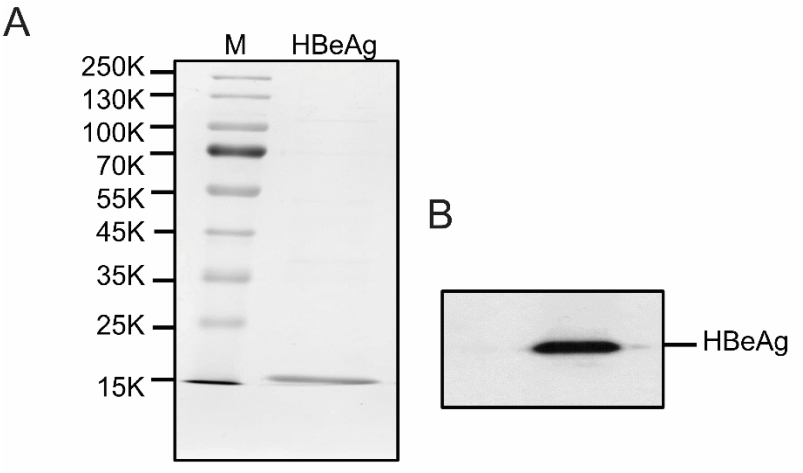


Figure S6. Analysis of the purity of HBeAg. Related to Figure 7.

(A) Coomassie blue staining of 6xHis-tagged HBeAg purified from E. coli. (B) Western-blot analysis of the purified HBeAg using the anti-HBeAg antibody.