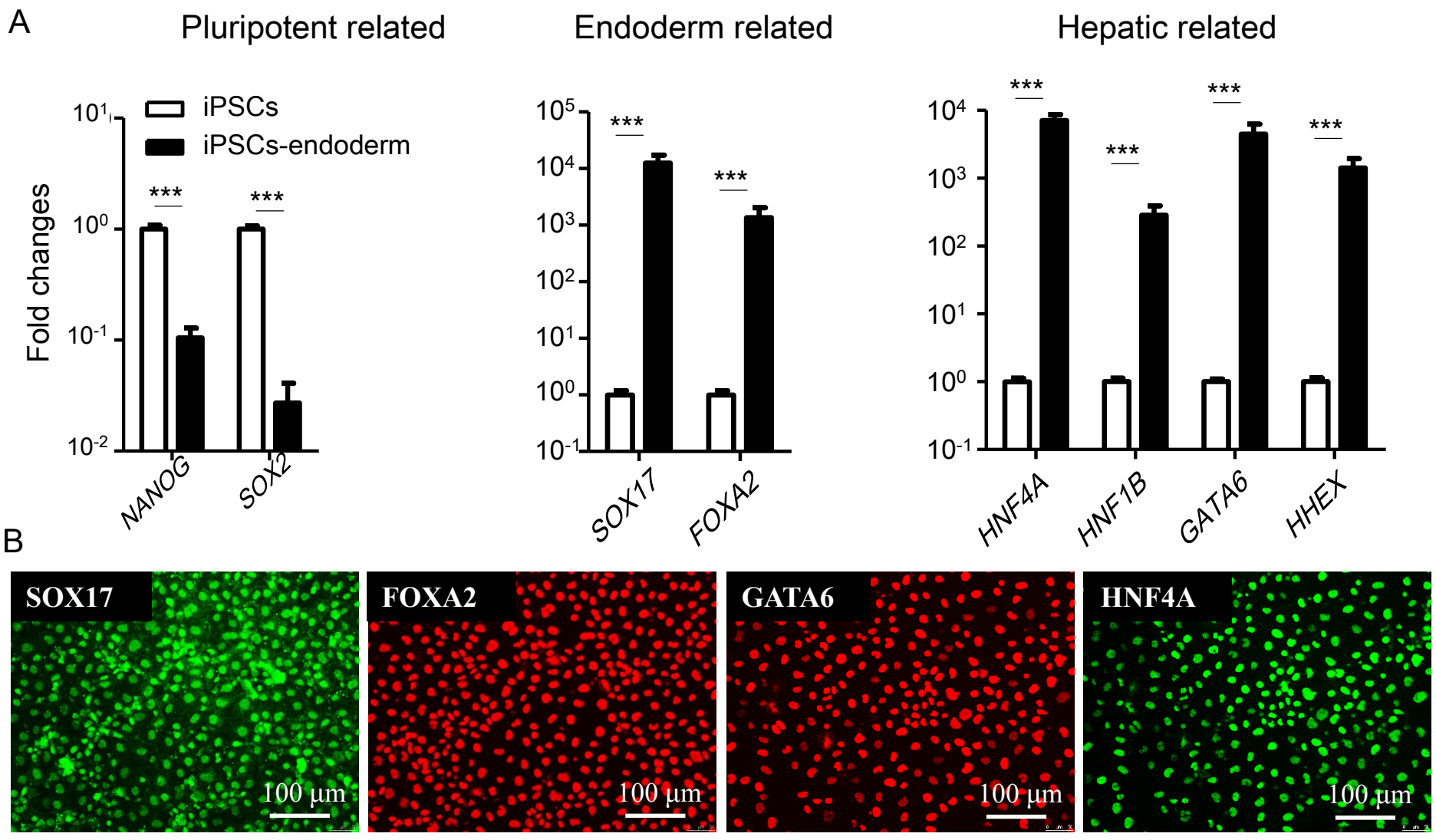
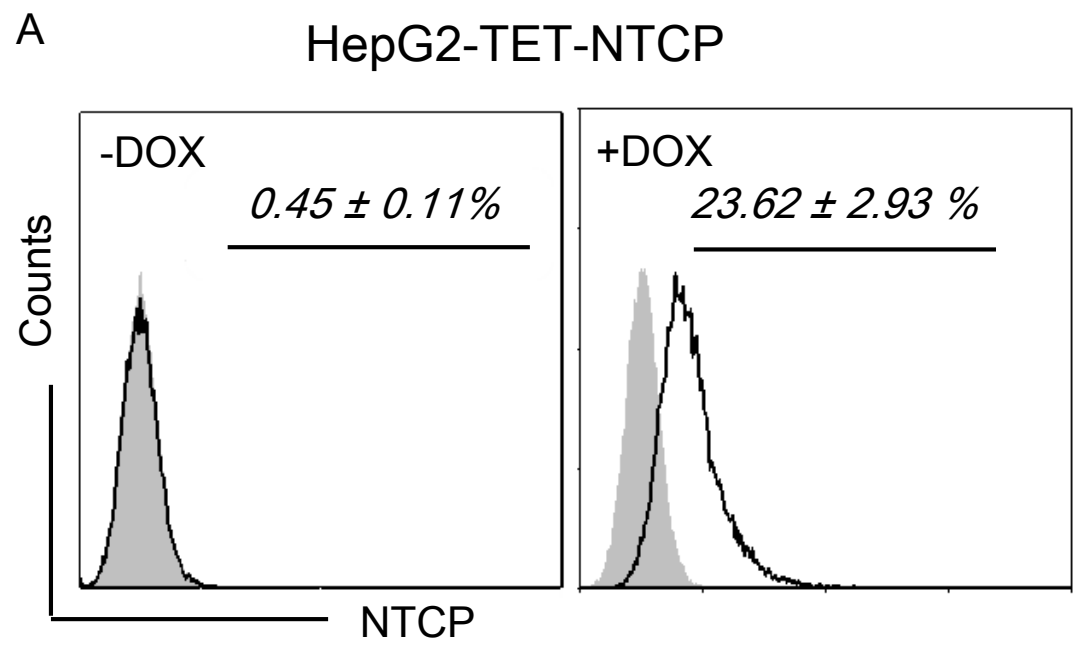
Supplementary information, Figure S1

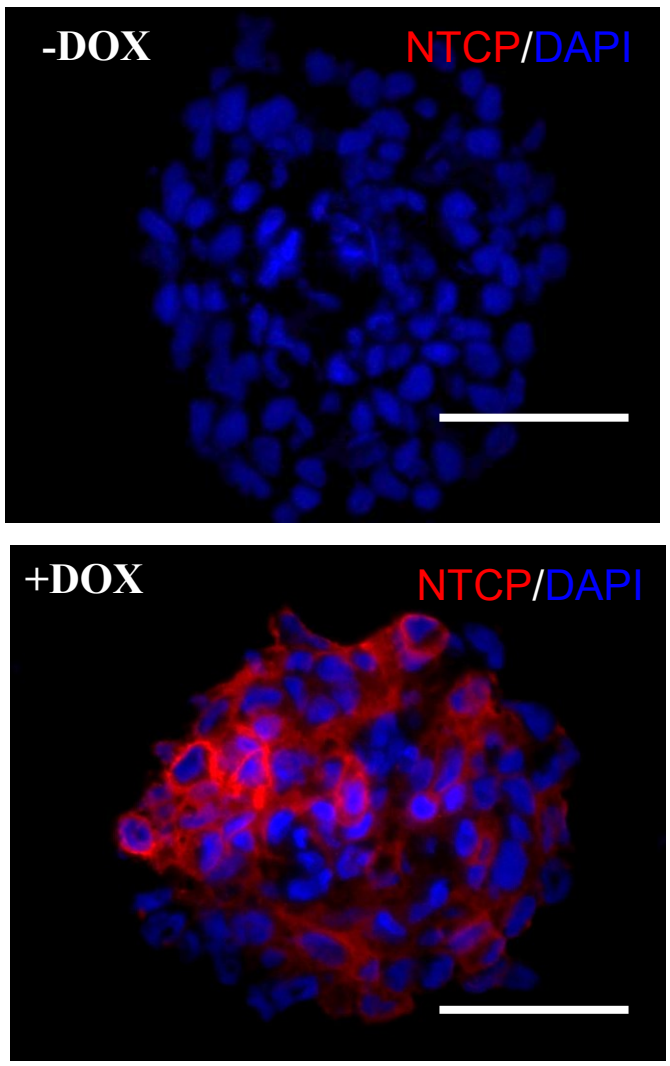


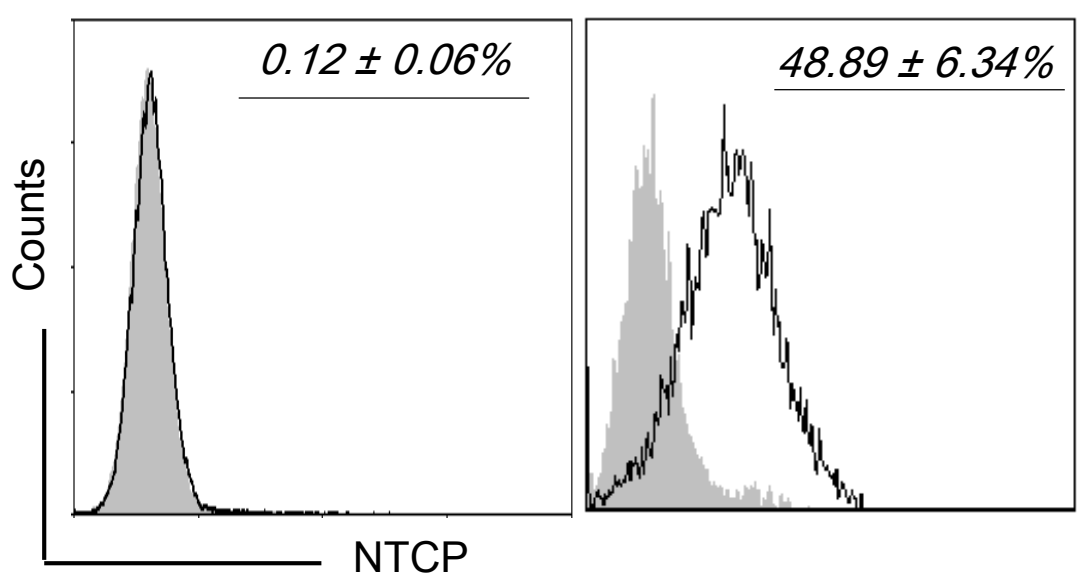
Supplementary information, Figure S1. In vitro generation and characteristic of functional liver organoids from hiPSCs

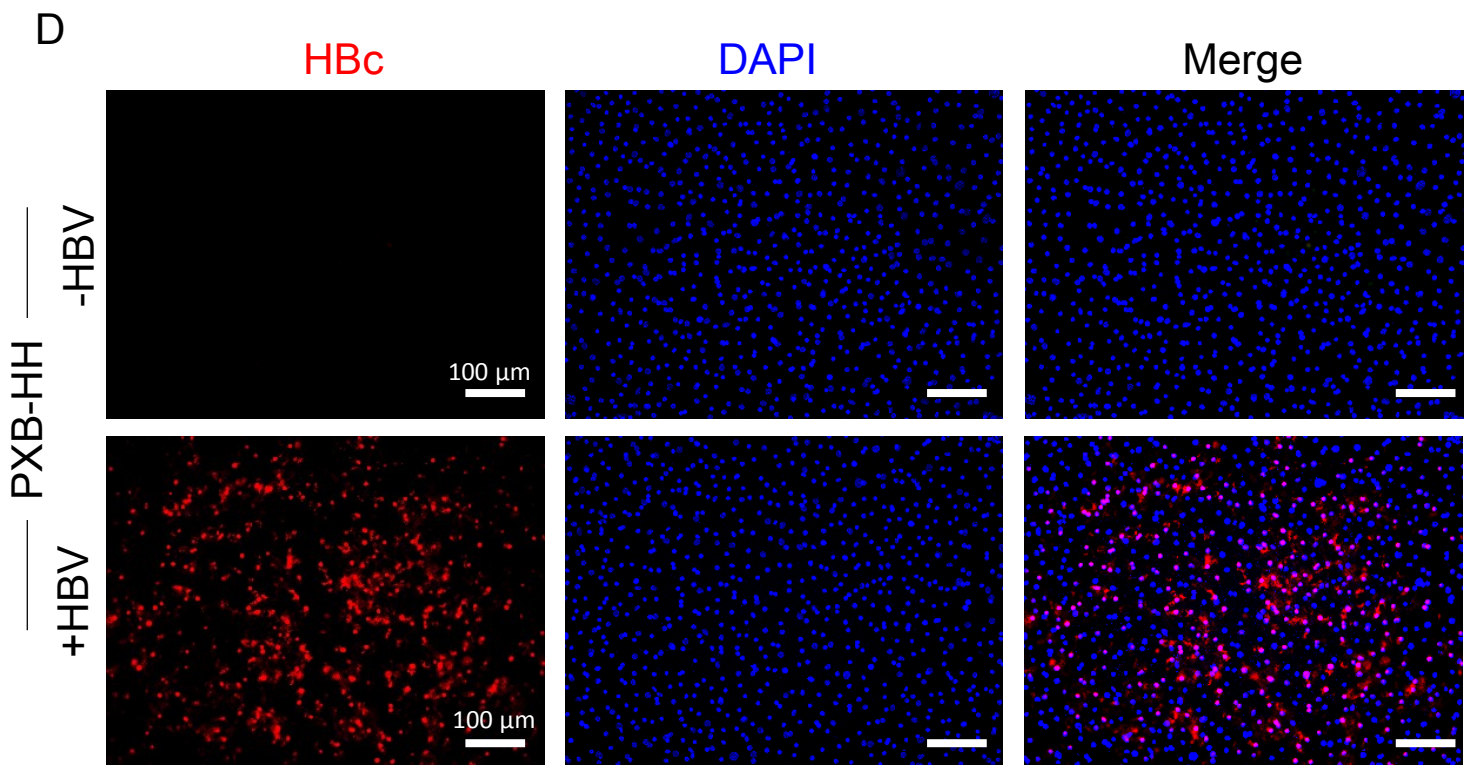
(A) Q-PCR Quantification of pluripotency genes (NANOG and SOX2), endoderm genes (*SOX17* and *FOXA2*), and hepatic genes (*HNF4*, *HNF1B*, *GATA6* and *HHEX*) in hiPSCs (n = 6) and hiPSC endoderm (n = 4). (B) Immunostaining of hiPSC-endoderm with *SOX17*, *FOXA2*, *GATA6* and *HNF4A*; Scale bar, 100 μm. (C) Morphology and diameters of hiPSC-LOs after 24 h of culture in a 3D microwell plate; Scale bar, 500 μm. (D) Q-PCR analysis expression of hepatic genes: *ALB*, *G6PC*, *CYP2C9*, *CYP2C19*, *CYP3A4* and *CYP3A7* in 1383D2-HLCs (n = 4) and 1383D2-LOs (n = 4). (E) ELISA for ALB secretion of 1383D2-HLCs (n = 4) and differentiated 1383D2-LOs (n = 4). (F) Indocyanine green uptake (left) and release (right) in hiPSC-LOs (Day 15), hiPSC-HLCs (positive control) and hiPSC-LOs (Day 1, negative control). Scale bar, 50 μm. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$.

Supplementary Information, Figure S2





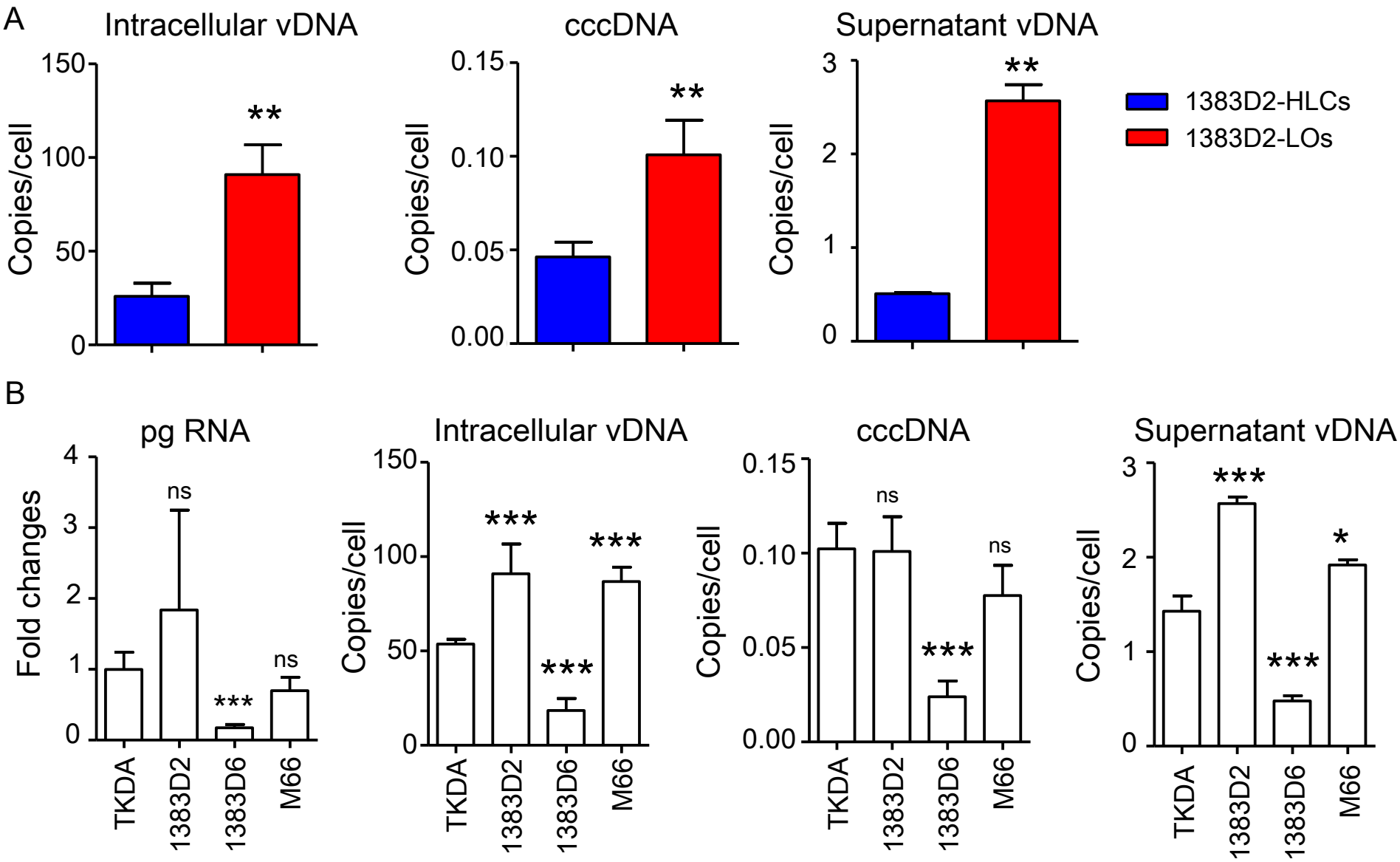




Supplementary Information, Figure S2, Quality of anti-NTCP antibody and anti-HBc antibody with positive controls.

(A) Flow cytometry analysis of surface NTCP expression in HepG2-TET-NTCP cells with or without DOX induction (n = 4). (B) Immunostaining of NTCP in HepG2-TET-NTCP organoids with or without DOX induction. (C) Flow cytometry analysis of surface NTCP expression in hiPSC-endoderm and hiPSC-HLCs (n = 4). (D) Immunostaining of HBc in PXB-HH infected with HBV at 500GEq/cells. Scale bar: 100μm.

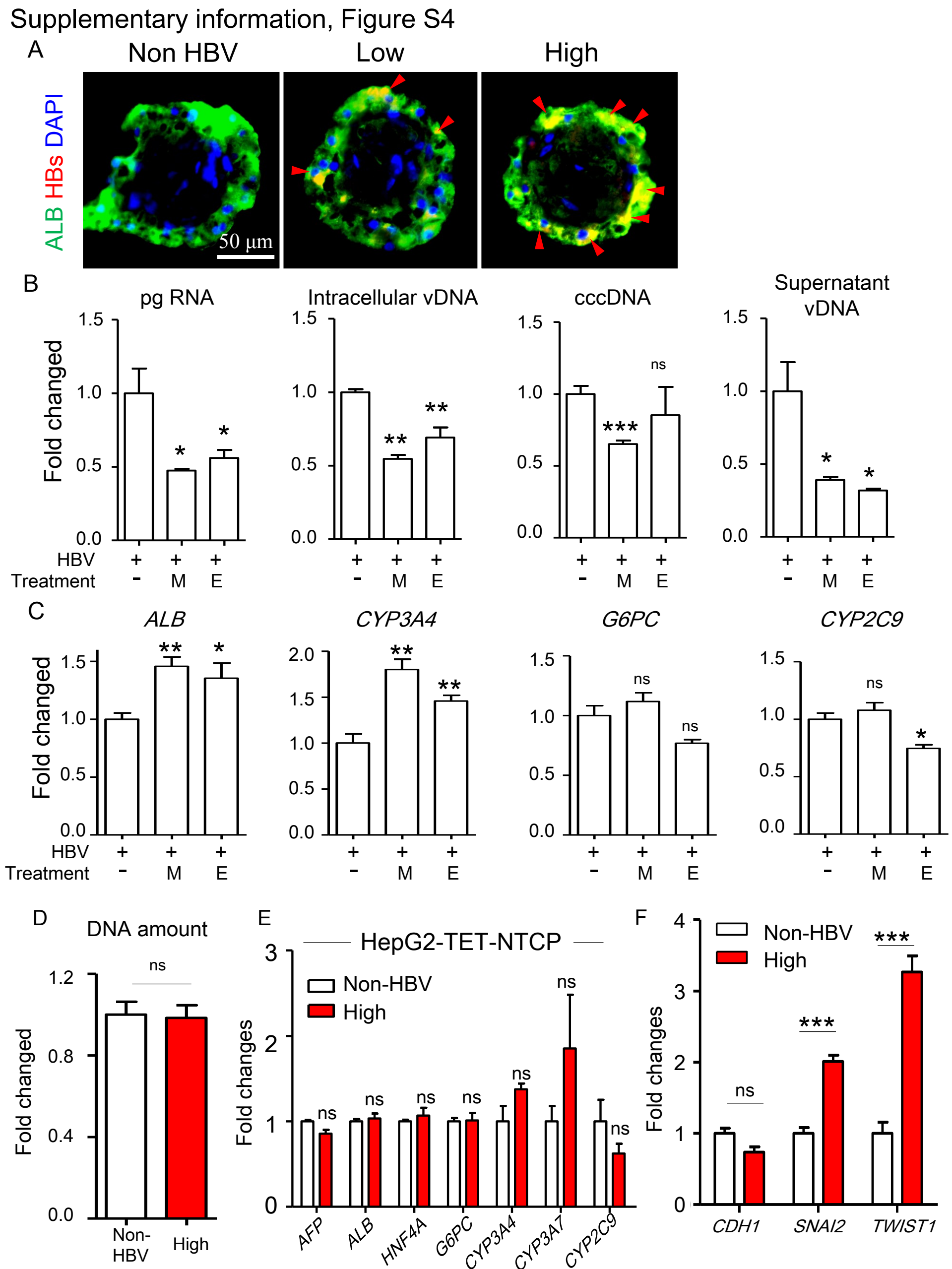
Supplementary Information, Figure S3



Supplementary Information, Figure S3. Susceptibility of different hiPSC-derived liver organoids to HBV infection

(A) Q-PCR quantification of intracellular vDNA, cccDNA, and supernatant vDNA in 1383D2-HLCs (n = 4), and 1383D2-LOs (n = 6) infected with HBV at 500GEq/cell. (B) Q-PCR quantification of HBV pgRNA, intracellular vDNA, cccDNA, and supernatant vDNA in TKDA-LOs (n = 8), and 1383D2-LOs (n = 6), 1383D6-LOs (n = 6) and M66-LOs (n = 6) infected with HBV at 500GEq/cell.

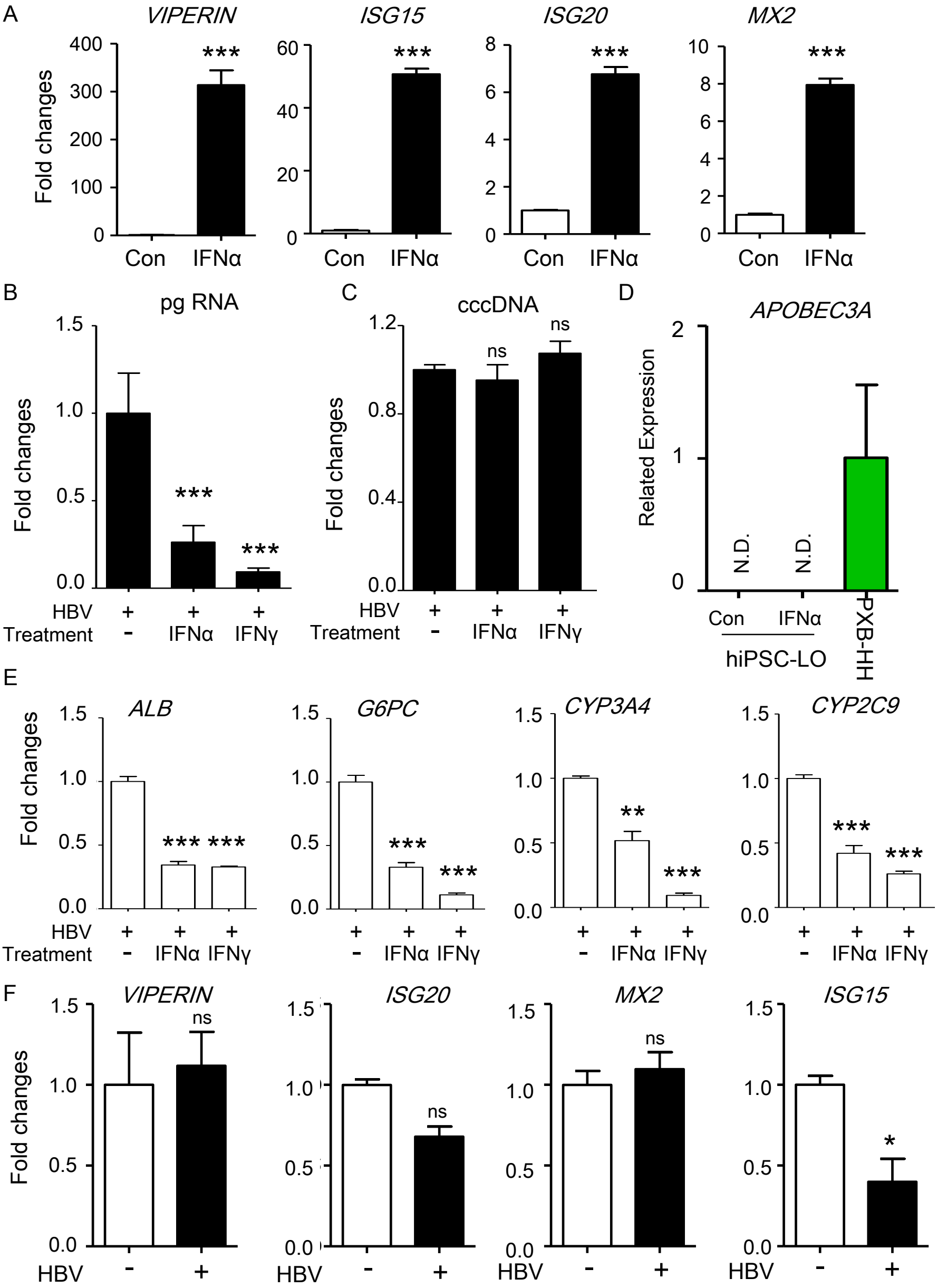
Supplementary information, Figure S4



Supplementary information, Figure S4. HBV infection in hiPSC-LOs and HepG2-Tet-NTCP organoids.

(A) Immunofluorescence analysis of HBs and ALB in hiPSC-LOs at doses of 0 (Non-HBV), 500 (low dose), and 5,000 (high dose) GEq/cell infection. (B) Q-PCR quantification of HBV pg RNA, intracellular vDNA, cccDNA, supernatant vDNA in high-dose HBV infected hiPSC-LOs with Myrcludex (M, $n = 4$) and Entecavir (E, $n = 4$) treatment; no treated infected hiPSC-LOs as a control. (C) Q-PCR quantification of *ALB*, *CYP3A4*, *G6PC*, and *CYP2C9* in high-does HBV infected hiPSC-LOs with Myrcludex (M, $n = 4$) and Entecavir (E, $n = 4$) treatment; no treated infected hiPSC-LOs as a control. (D) The total genome DNA amount in non- and high-dose HBV-infected hiPSC-LOs ($n = 4$). (E) Q-PCR quantification of *AFP*, *ALB*, *HNF4A*, *G6PC*, *CYP3A4*, *CYP3A7*, *CYP2C9* and *CYP2C19* in non-, and high-dose HBV-infected HepG2-TET-NTCP organoids with DOX, $n = 4$. (F) Q-PCR analysis of the expression of EMT genes *CDH1, SNAI2*, and *TWIST1* in non- and high-dose HBV-infected, hiPSC-LOs, $n = 4$. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$; ns, not significant.

Supplementary Information, Figure S5



Supplementary Information, Figure S5. Interferons suppressed virus replication and aggravated hepatic injury in hiPSC-LOs.

(A) Q-PCR analysis of *VIPERIN*, *ISG15*, *ISG20*, and *MX2* in hiPSC-LOs with IFNα treatment (48h, $n = 4$). (B) Q-PCR quantification of HBV pg RNA in high-does HBV infected hiPSC-LOs with IFNα and IFNγ treatment, $n = 4$. (C) Q-PCR quantification of HBV cccDNA in high-does HBV infected hiPSC-LOs with IFNα and IFNγ treatment, $n = 4$. (D) Q-PCR analysis of APOBEC3A expression in hiPSC-LOs with or without IFNα treatment, PXB-HHs as a control ($n = 4$). (E) Q-PCR quantification of functional hepatic genes, *ALB*, *G6PC*, *CYP3A4* and *CYP2C19* in high-does HBV infected hiPSC-LOs with IFNα and IFNγ treatment ($n = 4$). (F) Q-PCR analysis of the IFN-stimulated genes *VIPERIN*, *ISG15*, *ISG20*, and *MX2* in non-HBV and high-dose HBV infected hiPSC-LOs. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$; ns, not significant.