Silencing Retinoid X Receptor Alpha Expression Enhances Early-stage Hepatitis B Virus Infection In Cell Cultures

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Supplementary Figure Legends

Figure S1 RXRα negatively regulates HDV infection.

A-B: Activation of RXRα inhibited early HDV infection in HepG2-NTCP cells. Cells were inoculated with various concentration of Bexarotene, Myr-59 (250 nM) or DMSO for 24 h in the presence of HDV (A) or 24 h post HDV inoculation (B). At 7dpi, the intracellular HDV delta antigen was stained with mouse monoclonal antibody 4G5 (left), and the levels of HDV total RNAs in infected cells were quantified by qRT-PCR (right). C-D: Decreased RXRα expression enhanced HDV infection in HepG2-NTCP and PTHs. siRNAs against hRXRα, tsRXRα and control siRNA were transfected into HepG2-NTCP (C) or PTHs (D), respectively. Three days after transfection, cells were inoculated with

HDV and the intracellular HDV delta antigen was detected at 7 dpi. HDV total RNAs were quantified by qRT-PCR in viral infected HepG2-NTCP cells. E-H: AA biosynthesis/metabolism pathways had little effects on HDV infection. Cells were transfected with siRNAs against hPLA2G2A or control siRNA. 3 d post transfection, cells were incubated with HDV in the presence of 5 μM of Bexarotene or Indomethacin for 24 h (E). Cells were inoculated with indicated concentrations of AA (F), Indomethacin (Indo), Zileuton, Baicalein, PD146176 (G) or vehicles for 24 h in the presence of HDV. Cells were incubated with HDV in the presence of 5 μM of Bexarotene or DMSO plus 25 μM of Indomethacin (Indo) or vehicle for 24 h (H). At 7dpi, the intracellular HDV delta antigen was stained with mouse monoclonal antibody 4G5. (Student's t-test ***P <0.001)

Figure S2 RXRα silencing didn't alter the surface expression of NTCP in HepG2-NTCP cells.

A: Activation of RXRα didn't affect the mRNA expression of NTCP in HepG2-NTCP cells. Cells were incubated with Bexarotene for 24 h, and the mRNA expression of NTCP was evaluated by qRT-PCR. B-D: Silencing of RXRα didn't alter the mRNA and cell surface expression of NTCP in HepG2-NTCP cells. Cells were transfected with siRNA against hRXRα or control siRNA. 3 d post transfection, the mRNA expression of NTCP was evaluated by qRT-PCR (B). For the cell surface expression of NTCP, total surface proteins were biotinylated and pulled down with streptavidin T1 Dynabeads. NTCP surface expression was then detected by western blotting. The expression of tubulin was used as internal control (C). (D) Cells were transfected with siRNA against hRXRα or control siRNA. The cell surface expression of NTCP was examined by FACS with a monoclonal antibody (36#) against human NTCP.

Figure S3 Arachidonic acid biosynthesis was down regulated in RXRα knockdown cells

A: Gene expression in RXRα-stable-knockdown mono-clone cell line (RXRα-stable-knockdown) and wild-type HepG2-NTCP (WT) cells analyzed by RNAseq. Expression profiles of target genes regulated by RXRα and its heterodimers, including PPAR, FXR, LXR, VDR, CAR, PXR and RAR are presented in heatmap. The expression levels of genes (Reads Per Kilobase per Million (RPKM) >0.5, 21,172 genes in total) in RXRα-stable-knockdown and wild-type HepG2-NTCP cells are compared to the average among them. The color-coding in the heat maps represents log2 fold changes in genes expression levels. B: Cellular level of AA was decreased in RXRα-stable-knockdown cells. RXRα-stable-knockdown cells and wild-type HepG2-NTCP (WT) cells were subjected to LC-MS/MS-based analysis. The relative abundance of AA in both samples was normalized according to the protein concentration that reflects cells numbers.

Figure S4 Prostanoid biosynthesis, but not leukotriene biosynthesis modulated HBV infection in HepG2-NTCP cells

A: Indomethacin treatment during HBV incubation enhanced viral infection in HepG2-NTCP cells. Cells were inoculated with HBV in the presence of drugs or DMSO for 24 h. Culture medium samples were collected at indicated time and HBV viral antigens were measured by ELISA. B-C: PGD_2, PGE_2 or PGD_2 plus PGE_2 treatment during HBV incubation reduce viral infection in HepG2-NTCP cells. Cells were inoculated with HBV in the presence of PGD_2 or PGE_2 or vehicle for 24 h. At 7 dpi, the copy numbers of HBV total RNA and HBV 3.5 kb were measured by qRT-PCR (B). Intracellular HBcAg (green) were stained with 1C10, and nuclei were stained with DAPI (blue) (C). (Student's t-test *P <0.01, * * P <0.01)

Figure S1

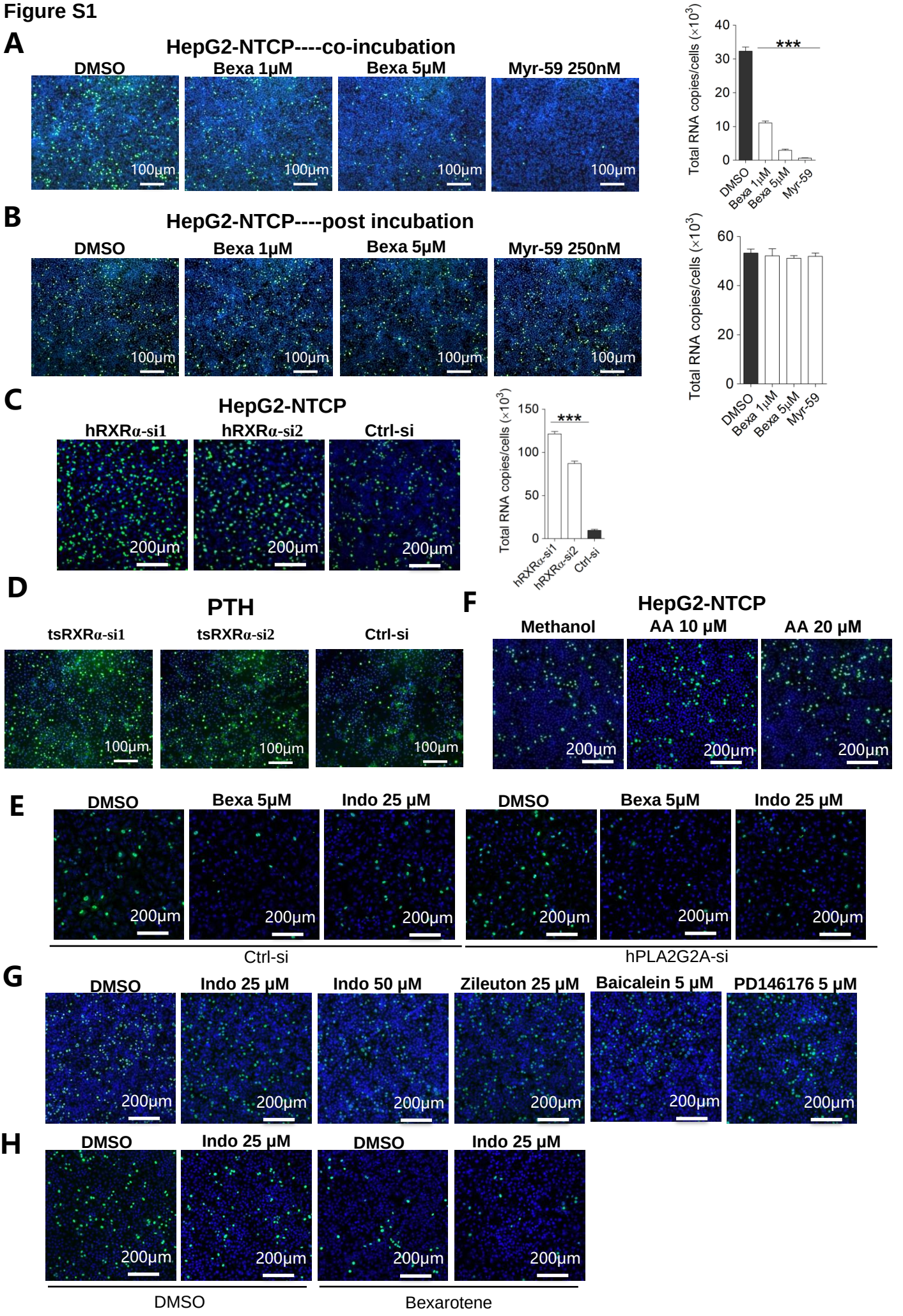

A
HepG2-NTCP----co-incubation
DMSO
Bexa 1μM
Bexa 5μM
Myr-59 250nM
100μm
Total RNA copies/cells (×10^3)
***
DMSO
Bexa 1μM
Bexa 5μM
Myr-59
B
HepG2-NTCP----post incubation
DMSO
Bexa 1μM
Bexa 5μM
Myr-59 250nM
100μm
Total RNA copies/cells (×10^3)
DMSO
Bexa 1μM
Bexa 5μM
Myr-59
C
HepG2-NTCP
hRXRα-si1
hRXRα-si2
Ctrl-si
200μm
Total RNA copies/cells (×10^3)
***
hRXRα-si1
hRXRα-si2
Ctrl-si
D
PTH
tsRXRα-si1
tsRXRα-si2
Ctrl-si
100μm
F
HepG2-NTCP
Methanol
AA 10 μM
AA 20 μM
200μm
E
DMSO
Bexa 5μM
Indo 25 μM
200μm
Ctrl-si
hPLA2G2A-si
G
DMSO
Indo 25 μM
Indo 50 μM
Zileuton 25 μM
Baicalein 5 μM
PD146176 5 μM
200μm
H
DMSO
Indo 25 μM
200μm
DMSO
Bexarotene

Figure S2

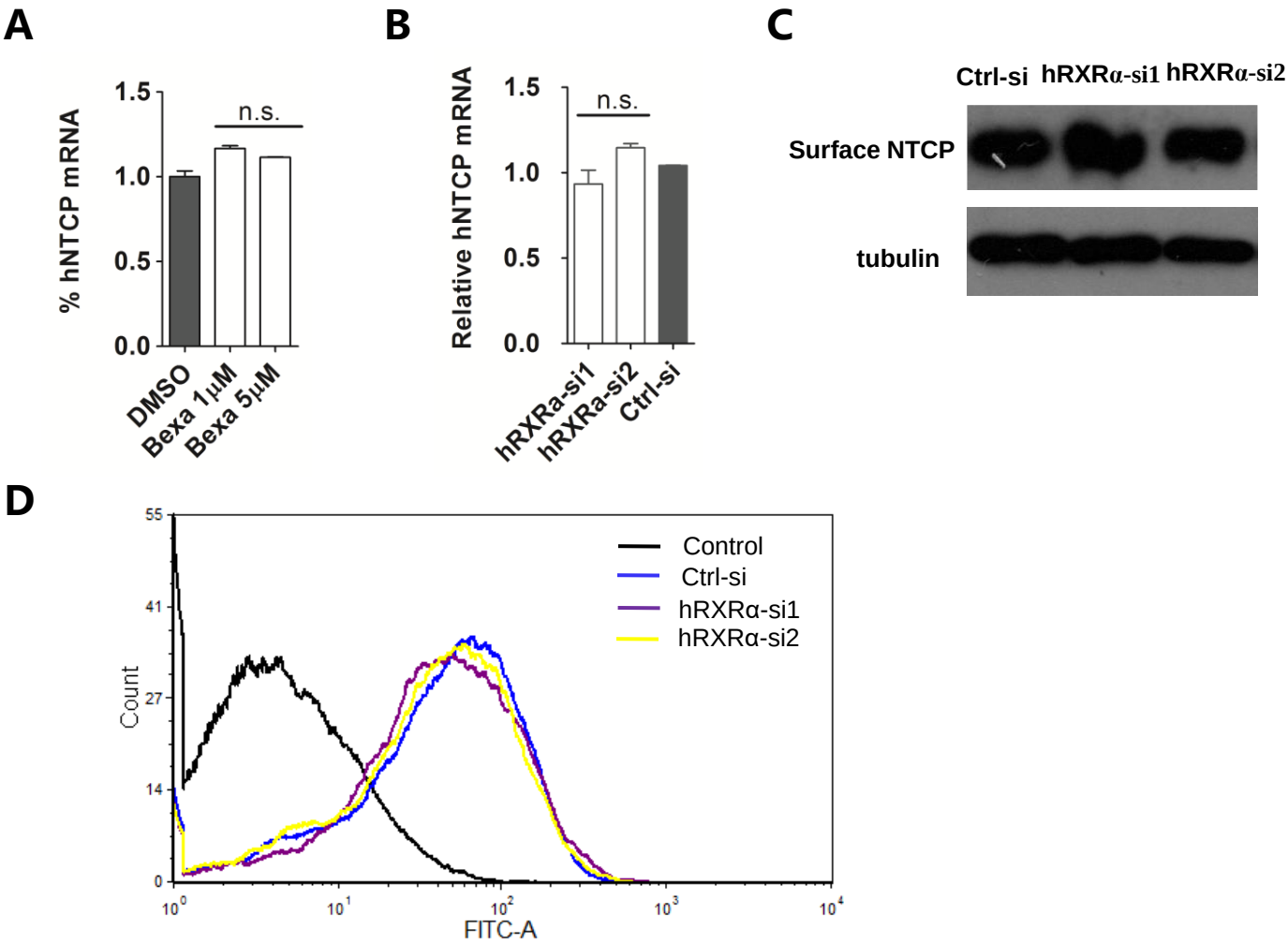
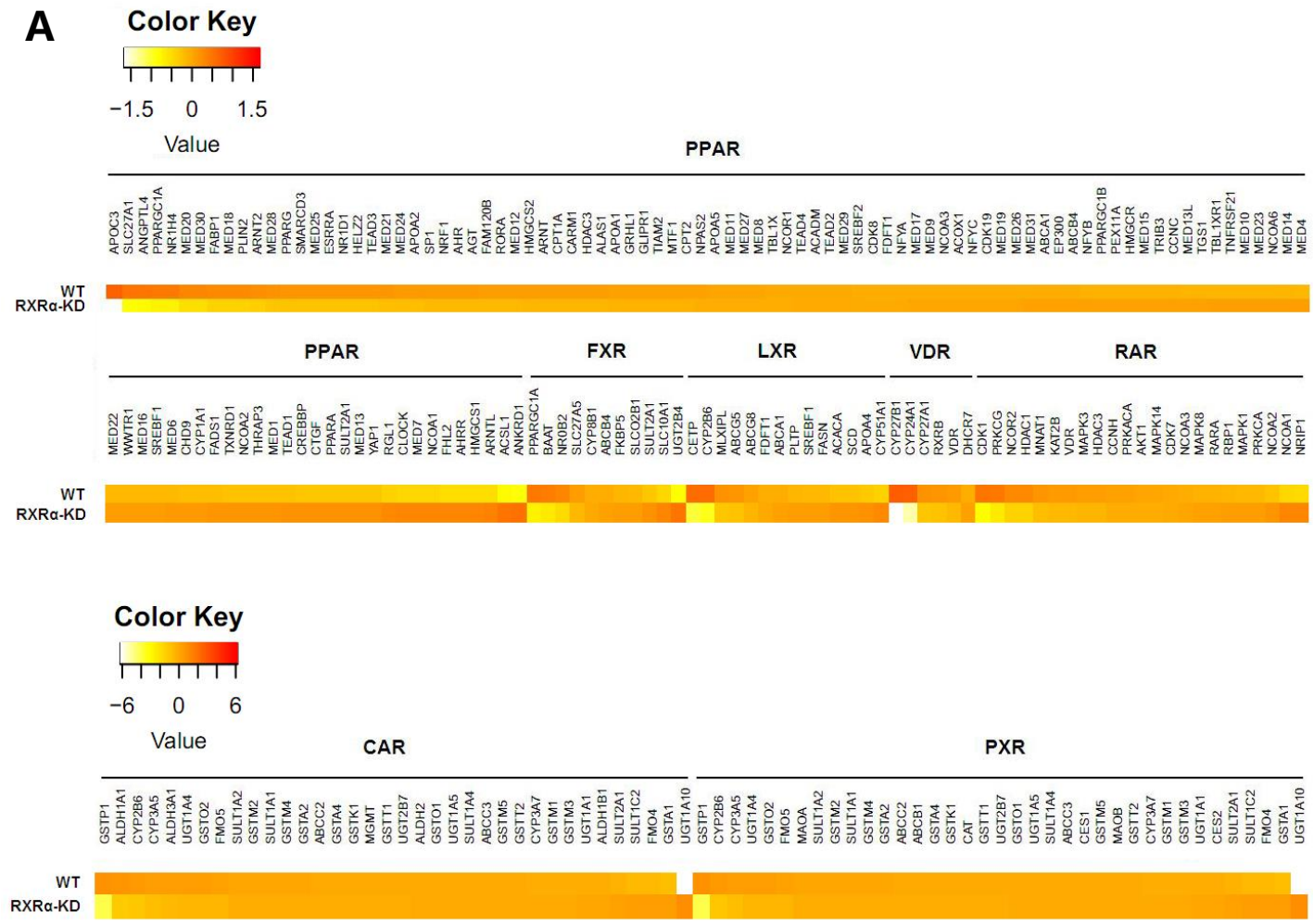
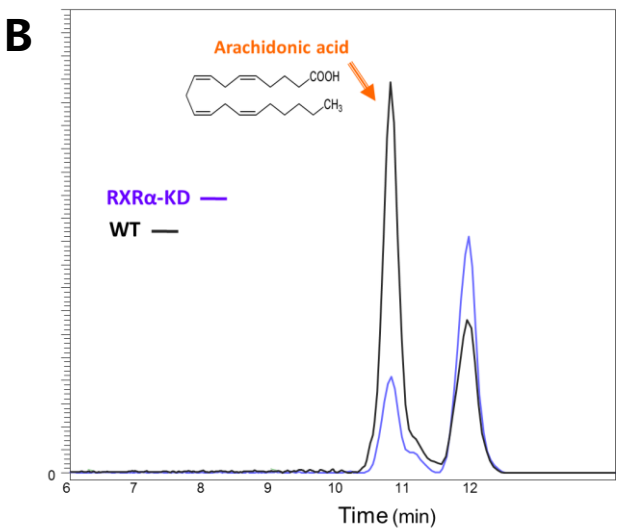

A
% hNTCP mRNA
1.5
1.0
0.5
0.0
n.s.
DMSO
Bexa 1μM
Bexa 5μM
B
Relative hNTCP mRNA
1.5
1.0
0.5
0.0
n.s.
hRXRa-si1
hRXRa-si2
Ctrl-si
C
Ctrl-si
hRXRα-si1
hRXRα-si2
Surface NTCP
tubulin
D
Count
55
41
27
14
0
10^0
10^1
10^2
10^3
10^4
FITC-A
Control
Ctrl-si
hRXRα-si1
hRXRα-si2

Figure S3


A
Color Key
−1.5 0 1.5
Value
PPAR
APOC3 SLC27A1 ANGPTL4 PPARGC1A NR1H4 MED20 MED30 FABP1 MED18 PLIN2 ARNT2 MED28 PPARG SMARCD3 MED25 ESRRA NR1D1 HELZ2 TEAD3 MED21 MED24 APOA2 SP1 NRF1 AHR AGT FAM120B RORA MED12 HMGCS2 ARNT CPT1A CARM1 HDAC3 ALAS1 APOA1 GRHL1 GLIPR1 TIAM2 MTF1 CPT2 NPAS2 APOA5 MED11 MED27 MED8 TBL1X NCOR1 TEAD4 ACADM TEAD2 MED29 SREBF2 CDK8 FDFT1 NFYA MED17 MED9 NCOA3 ACOX1 NFYC CDK19 MED19 MED26 MED31 ABCA1 EP300 ABCB4 NFYB PPARGC1B PEX11A HMGCR MED15 TRIB3 CCNC MED13L TGS1 TBL1XR1 TNFRSF21 MED10 MED23 NCOA6 MED14 MED4
WT
RXRα-KD
PPAR
FXR
LXR
VDR
RAR
MED22 WWTR1 MED16 SREBF1 MED6 CHD9 CYP1A1 FADS1 TXNRD1 NCOA2 THRAP3 MED1 TEAD1 CREBBP CTGF PPARA SULT2A1 MED13 YAP1 RGL1 CLOCK MED7 NCOA1 FHL2 AHRR HMGCS1 ARNTL ACSL1 ANKRD1 PPARGC1A BAAT NR0B2 SLC27A5 CYP8B1 ABCB4 FKBP5 SLCO2B1 SULT2A1 SLC10A1 UGT2B4 CETP CYP2B6 MLXIPL ABCG5 ABCG8 FDFT1 ABCA1 PLTP SREBF1 FASN ACACA SCD APOA4 CYP51A1 CYP27B1 CYP24A1 CYP27A1 RXRB VDR DHCR7 CDK1 PRKCG NCOR2 HDAC1 MNAT1 KAT2B VDR MAPK3 HDAC3 CCNH PRKACA AKT1 MAPK14 CDK7 NCOA3 MAPK8 RARA RBP1 MAPK1 PRKCA NCOA2 NCOA1 NRIP1
WT
RXRα-KD
Color Key
−6 0 6
Value
CAR
PXR
GSTP1 ALDH1A1 CYP2B6 CYP3A5 ALDH3A1 UGT1A4 GSTO2 FMO5 SULT1A2 GSTM2 SULT1A1 GSTM4 GSTA2 ABCC2 GSTA4 GSTK1 MGMT GSTT1 UGT2B7 ALDH2 GSTO1 UGT1A5 SULT1A4 ABCC3 GSTM5 GSTT2 CYP3A7 GSTM1 GSTM3 UGT1A1 ALDH1B1 SULT2A1 SULT1C2 FMO4 GSTA1 UGT1A10 GSTP1 CYP2B6 CYP3A5 UGT1A4 GSTO2 FMO5 MAOA SULT1A2 GSTM2 SULT1A1 GSTM4 GSTA2 ABCC2 ABCB1 GSTA4 GSTK1 CAT GSTT1 UGT2B7 GSTO1 UGT1A5 SULT1A4 ABCC3 CES1 GSTM5 MAOB GSTT2 CYP3A7 GSTM1 GSTM3 UGT1A1 CES2 SULT2A1 SULT1C2 FMO4 GSTA1 UGT1A10
WT
RXRα-KD


B
Arachidonic acid
COOH
CH3
RXRα-KD
WT
0
6 7 8 9 10 11 12
Time (min)

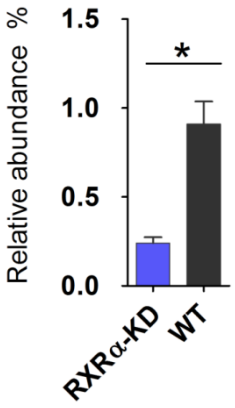
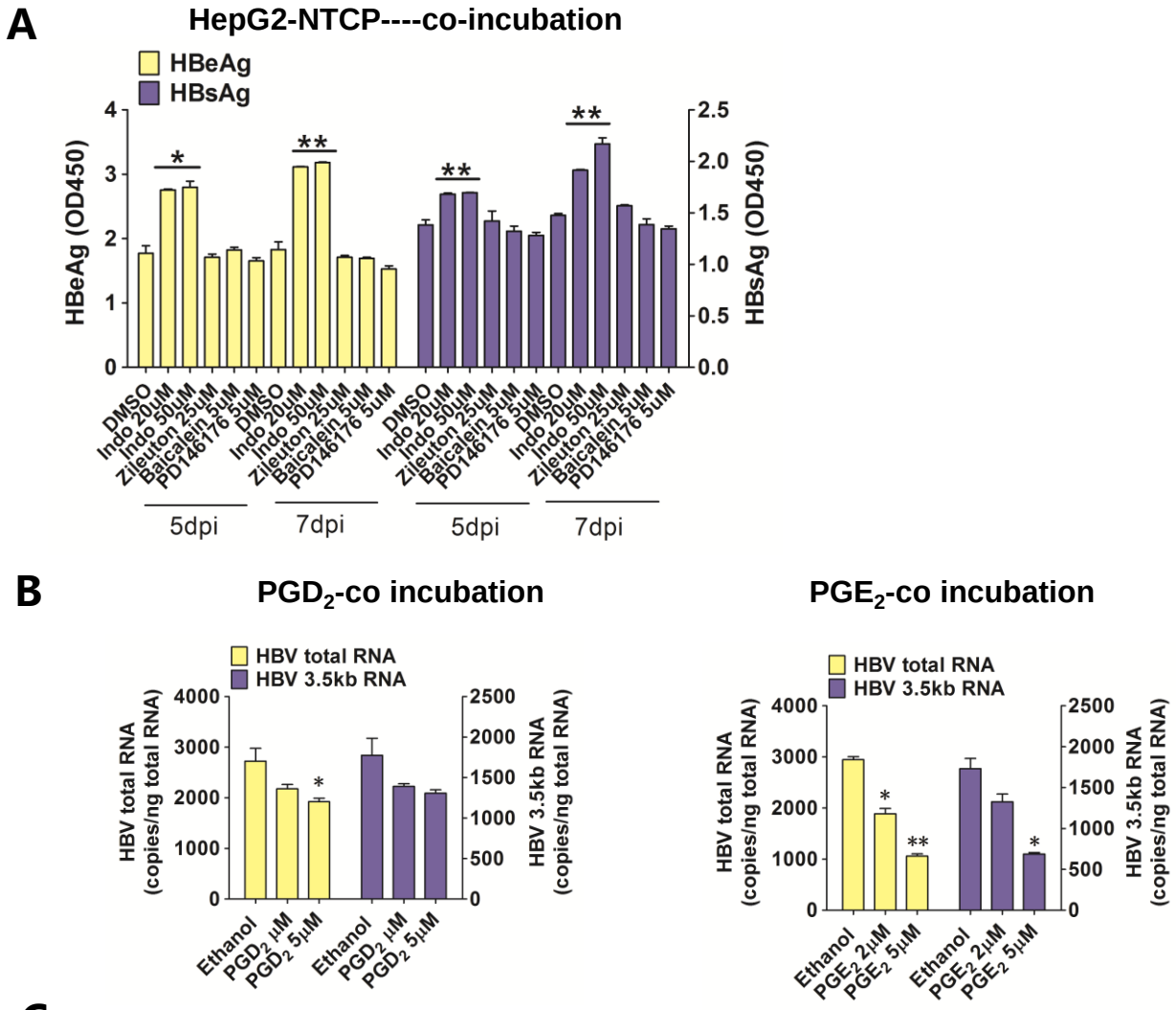

Relative abundance %
1.5
1.0
0.5
0.0
*
RXRα-KD
WT

Figure S4


A
HepG2-NTCP----co-incubation
HBeAg
HBsAg
HBeAg (OD450)
HBsAg (OD450)
DMSO
Indo 20uM
Indo 50uM
Zileuton 25uM
Baicalein 5uM
PD146176 5uM
5dpi
7dpi
B
PGD2-co incubation
PGE2-co incubation
HBV total RNA
HBV 3.5kb RNA
HBV total RNA (copies/ng total RNA)
HBV 3.5kb RNA (copies/ng total RNA)
Ethanol
PGD2 μM
PGD2 5μM
PGE2 2μM
PGE2 5μM
C
co-incubation---HBcAg
Ethanol
PGD2 2μM
PGE2 2μM
PGD2 2μM +PGD2 2μM
100μm