HBV infection-induced liver cirrhosis development in dual-humanized mice with human bone mesenchymal stem cell transplantation

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● Supplemental Materials and Methods

Isolation, culture, phenotypic identification and multi-lineage differentiation of hBMSCs

The isolation, culture and identification of hBMSCs were performed using the standard procedures described in previous studies [1, 2]. Signed informed consent was obtained, and the protocol was approved by the Clinical Research Ethics Committee of the First Affiliated Hospital, Zhejiang University. BM mononuclear cells were isolated by BM aspiration from the iliac crest of healthy male volunteers and purified by Ficoll-Paque density-gradient centrifugation as previously described [3]. The purified mononuclear cells were allowed to attach in Dulbecco's modified Eagle's medium (DMEM, #11995-073, Gibco) supplemented with 10% foetal bovine serum (FBS, #10270-106, Gibco) overnight at 37ºC in 5% CO_2. After two days of incubation, the floating cells were washed out, and all the attached cells were maintained in the same culture medium. To collect a sufficient number of cells for transplantation, the freshly isolated hBMSCs were passaged and cultured in DMEM with 5% FBS *in vitro*. The cultured hBMSCs from passages 3-7 that showed typical stem cell characteristics were used for transplantation. Cryopreserved hBMSCs from the same passages were also used in this study after thawing and culturing for one or two passages. hBMSCs were subjected to phenotypic analyses based on hCD90, hCD29, hCD45 and hCD34 using standard flow cytometry methods (FC500, Beckman Coulter, Fullerton, CA, USA) prior to transplantation (Figure S1A).

To induce osteogenic differentiation, the hBMSCs were cultured in a commercially available osteogenic differentiation medium (Cambrex, Walkersville, MD, USA). On day 21, the alkaline phosphatase activity of the cultured cells was assessed as

previously described [4]. To induce adipogenic differentiation, the hBMSCs were cultured in a commercially available adipogenic differentiation medium purchased from Cambrex, and on day 21, the cells were stained with Oil red O. Hepatogenic differentiation was performed as previously described [5].

qRT-PCR

Total RNA from tissues or purified cells was extracted using TRIzol reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions and was then used for cDNA synthesis with a SuperScript First-Strand Synthesis System (Invitrogen, Carlsbad, CA, USA) as previously described [6]. Detailed primer information is presented in ***Table S3***.

Two-step collagenase perfusion for the isolation of total liver cells

Liver cells from hBMSC-FRGS mice were harvested using a standard collagenase perfusion protocol [7]. Briefly, the liver was perfused with calcium- and magnesium-free Earle’s balanced salt solution (EBSS) supplemented with 0.5 mM EGTA and 10 mM HEPES for five min. The solution was changed to EBSS supplemented with 0.1 mg/mL collagenase IV (Sigma-Aldrich, St Louis, MO, USA) and 0.05 mg/mL DNase I (Sigma-Aldrich, St Louis, MO, USA) for 10 min. The liver was gently minced in the second solution and filtered sequentially through 70- and 40-mm nylon mesh (BD Biosciences, San Jose, CA, USA). After centrifugation at 150 g for three min, the pellet contained the total population of liver cells, including parenchymal and non-parenchymal cells. To harvest the parenchymal cells (mainly hepatocytes), the pellet was further centrifuged at 50 g for three min. The number and viability of the resulting cells were assessed using the trypan blue exclusion test.

Identifying the chimerism of hBMSC-Heps in hBMSC-FRGS mice

To identify the chimerism of hBMSC-Heps, 3×10^7 liver cells, including parenchymal and non-parenchymal cells, were isolated from the livers of FRGS mice after hBMSC transplantation through collagenase perfusion and centrifugation at 150 g for 3 min [7]. To determine the ratio of HLA^+ cells in the total population of liver cells and the proportions of $hALB^+$, $hNTCP^+$ and $hCD45^+$ cells in HLA^+ cells, at least $1x10^5$ total liver cells (per mice) were analysed using standard flow cytometry methods using a FACSAria III (BD Biosciences, San Jose, CA, USA) according to the instructions provided by the manufacturer. Different fluorescently labelled antibodies were used to characterize the various hBMSC-derived human immune cell lineages (Table S2). The fluorescent dye and protein antibody labelling kits were purchased from Expedeon, and the assays were performed according to the instructions provided by the manufacturer (Table S1). For the detection of intracellular markers, such as hALB, fixation/permeabilization solution (#554714, BD Bioscience) was used. Dead cells were excluded using fixable viability dye (#L23101, eBioscience). To exclude non-specific reactions, background signals and other interferences, a less than 0.5% positive rate in the FACS analysis was recognized as a negative result. The isolated hBMSC-Heps and mouse livers were assessed by IHC, IF and qRT-PCR for human hepatocyte-specific markers and genes. The serum hALB levels were measured by ELISA. The chimeric rates of hBMSC-Heps were evaluated by the linear relationship between the percentages of $hALB^+/hNTCP^+$ cells in perfused liver cells and the serum hALB levels.

Identification of the chimerism of hBMSC-derived immune cell lineages in

hBMSC-FRGS mice

To identify the chimerism of hBMSC-derived immune cell lineages, BM, lymph node, peripheral blood, spleen and liver cells were collected and analysed by FACS with human leukocyte markers as previously described [8, 9, 10]. For further FACS analysis of multiple hBMSC-derived immune cell lineages, non-parenchymal cells (including immune cell lineages and other cells) were separated from the parenchymal cells by centrifugation at 50 g for 3 min to pellet the hepatocytes and leave most non-parenchymal cells in suspension. FACS analysis of multiple immune cell lineages was then performed using standard flow cytometry methods according to the instructions provided by the instrument's manufacturer. The gating scheme used in this study, which was adapted from previous studies, was defined by a FACS specialist with 10 years of experience according to the cell linages and antibodies [8, 11]. Different fluorescent labelled antibodies were used to characterize various hBMSC-derived human immune cell lineages (Tables S1-S2): $hCD45^+$ for total human immune cells, $hCD45^+hCD3^+$ for T cells, $hCD45^+hCD3^+hCD4^+$ for helper T (T_H) cells, $hCD45^+hCD3^+hCD8^+$ for cytotoxic T (T_C) cells, $hCD45^+hCD19^+$ for B cells, $hCD45^+hCD3^-hNKp46^+$ for NK cells, $hCD45^+hCD3^-hCD14^+$ for monocytes, $hCD45^+hCD3^-hCD14^+hCD68^+CD86^+$ for M1 macrophages, $hCD45^+hCD3^-hCD14^+hCD68^+CD163^+$ for M2 macrophages, $hCD45^+hCD3^-hCD14^-hCD11c^+HLA\text{-}DR^+$ for myeloid DCs and $hCD45^+hCD3^-hCD14^-hCD123^+HLA\text{-}DR^+$ for plasmacytoid DCs.

Isolation and measurement of hBMSC-derived $hCD45^+$ cells from peripheral blood, spleen, liver, bone marrow, thymus and mesentery lymph nodes

Hepatic $hCD45^+$ cells were directly measured by FACS analysis of the total

population of perfused liver cells. As previously described [6], heparinized blood was treated twice with ammonium-chloride-potassium (ACK) lysis buffer to eliminate RBCs. Bone marrow cells were harvested by flushing the diaphysis of femurs and tibias from mice with PBS containing 1% BSA, 100 U/mL penicillin and 100 μg/mL streptomycin. The spleen, thymus and mesentery lymph nodes were gently ground and sequentially filtered through 70- and 40-mm nylon mesh (BD Biosciences, San Jose, CA, USA). Single-cell suspensions of the spleen and bone marrow were treated with ACK lysis buffer, and the amount of $hCD45^+$ cells in these single-cell suspensions was assessed by FACS.

Harvest of infection source and establishment of HBV infection

For each genotype inoculation, 1×10^7 DNA copies of HBV dissolved in 200 μL of saline were inoculated into one hBMSC-FRGS mouse with 40~60% chimerism of hBMSC-Heps via intraperitoneal injection (1.5~2.5 mg/mL of the serum hALB levels). These genotypes and amounts of virus have been commonly used [12]. Uninfected hBMSC-FRGS mice with similar serum hALB levels were used as controls. The HBV inocula used in this study were harvested from cell culture supernatants of stable HBV-replicating cell lines generated from HepG2 cells. These stable HBV-replicating cell lines, named HepG2-pTSMP-1.3HBV-A, -B, -C and -D, were generated using the 1.3-copy genome of the indicated HBV genotype donated by the Sleeping Beauty transposon-based system [13]. In brief, the infectious inoculums were prepared from freshly collected culture supernatants of the HepG2-pTSMP-1.3HBV cell lines by precipitating the viral particles in the presence of 6% PEG. The pellet was resuspended in PBS, dialyzed three times and finally resuspended in PBS containing 25% FBS and stored at -80°C.

PHA and PMA/ionomycin stimulation in vitro

PHA and PMA/ionomycin stimulation *in vitro* was performed as previously described [10, 14]. The $hCD45^+$ leukocytes cells collected from hBMSC-FRGS mice were briefly cultured with PHA (10 μg/mL) or PMA (10 μg/mL) and ionomycin (10 μg/mL) for 24 h and used for measurements of intracellular human cytokines using various ELISA kits. Detailed information for these human cytokine kits is presented in ***Table S1***.

Detect isotype of HBV antigen specific antibodies

A captured-ELISA method was used to distinguish and measure the Ig isotype of HBV antigen specific antibodies. Firstly, HBsAg or HBcAg (purchased from AOKEBOTAI, Wuhan, China) protein was anchored on the bottom of 96-well plates. Then, mice serum samples were diluted by ten times and incubated in the wells. After that, HRP labelled anti-human IgG (#I5260, Sigma-Aldrich) or anti-human IgM (#ab99737, Abcam) was used to measure the levels of hIgG and hIgM in HBsAb and HBcAb.

Cell fusion detection

To detect the existence or absence of cell fusion between human-derived cells and the recipient mouse liver cells, liver tissues collected from hBMSC-FRGS mice were co-stained for human major histocompatibility complex (MHC) and mouse MHC antibodies through IF staining. FRGS mouse liver tissues were used as controls. The total liver cells collected from hBMSC-FRGS mice by collagenase perfusion were further analysed by FACS using human and murine MHC antibodies. hBMSCs, pig

hepatocytes (#M00615, BioreclamationIVT) and FRGS mice liver cells were used as controls.

IF staining

The indicated cells cultured on slides were fixed with paraformaldehyde for 20 min, treated with 0.1 Triton-X100 for 10 min, incubated with goat serum for 30 min and incubated with antibodies. The nuclei were stained with DAPI. The slides were washed three times with PBS between each of these steps. Photomicrographs were obtained with an Axio Imager microscope (Zeiss, Jena, Germany) and a BX51 microscope (Olympus, Shinjuku, Japan). Detailed information regarding the antibodies used for IF staining is presented in ***Table S2***.

H&E, M&T, SR&FG, V.G. and IHC staining

To observe the rescue of mice from FHF by hBMSC transplantation, liver tissues were collected on days 0, 3 and 7 after transplantation for H&E staining. For observations of hBMSC transdifferentiation, IHC was performed to detect the expression of HLA and human hepatic-specific markers (hALB, hFAH, hNTCP, hCK18, hAAT) in liver tissue from hBMSC-FRGS mice. In a preliminary tumourigenicity assay, H&E staining was performed to observe the liver, heart, spleen, lung, kidney, colon, muscle/bone and brain tissues collected from hBMSC-FRGS mice 60 weeks after hBMSC transplantation. For observations of the HBsAg and HBcAg distribution, IHC assays were performed using liver tissue collected from hBMSC-FRGS mice after HBV infection. To observe the recruitment of Kupffer cells near the $HBsAg^+$ hepatocytes, uninfected controls and HBV-infected liver tissues from hBMSC-FRGS mice were collected for frozen sectioning and double immune

staining for HBsAg and $hCD68^{+}$. To observe the phenotype of chronic hepatitis, uninfected controls and HBV-infected hBMSC-FRGS mouse liver tissues were collected for H&E staining. To observe the progression of liver cirrhosis, uninfected controls and HBV-infected hBMSC-FRGS mouse liver tissues were collected from weeks 0 to 54 post-infection for M&T staining. To observe the typical phenotype of cirrhosis, serial sections were also analysed by SR&FG, V.G. staining and IHC staining for HLA.

Mouse tissues were fixed in 4% formaldehyde (pH 7.4) for at least 48 h to obtain paraffin sections, and the sections (4 μm) were applied to poly-L-lysine-coated slides. After the sections were dewaxed, rehydrated and washed, endogenous peroxidases were inactivated with 3% H_2O_2 for 10 min at room temperature. The sections were then incubated overnight with primary antibodies. The sections were subsequently washed three times with PBS and treated with reagents from an UltraSensitive™ SP Kit (Maixin Biotech, Fuzhou, China). After reaction with the DAB chromogen, the sections were rinsed with distilled water and counterstained with haematoxylin. Brown staining indicated positive expression. H&E staining was performed as previously described [1, 2]. M&T, SR&FG and V.G. staining was performed according to recommended protocols (Maixin Biotech, Fuzhou, China and Solarbio LIFE SCIENCE, Beijing, China). Livers were frozen in OCT (Sakura Finetek Europe B.V., Flemingweg, Netherlands) to obtain frozen sections, and 10-μm cryostat sections were fixed in 4% paraformaldehyde for 15 min at 20°C (used throughout). The sections were permeabilized in 0.1% Triton X-100 for 10 min and then were incubated in 0.3% H_2O_2 for 5 min, 10% goat serum for 20 min and antibodies. The nuclei were stained with DAPI. The slides were washed three times with PBS

between each of the steps. Photomicrographs were obtained with an Axio Imager microscope (Zeiss, Jena, Germany) and a BX51 microscope (Olympus, Shinjuku, Japan). Fields of the whole liver lobes were visualized using a scanning system (NanoZoomer 2.0-RS, Hamamatsu Photonics, Hamamatsu, Japan) and NDP.scan 2.5 software as previously described [1]. Detailed information regarding the antibodies used for the IF staining of frozen sections and the IHC staining of paraffin sections is presented in ***Table S2.***

Criteria of liver fibrosis and cirrhosis

Fibrosis is defined as the presence of excess collagen due to new fibre formation that causes only minor clinical symptoms or disturbance of liver cell function. Cirrhosis is a diffuse process characterized by fibrosis and the conversion of the normal liver architecture into structurally abnormal nodules that affect the whole organ. Liver cirrhosis is defined by its pathological features on microscopy: (a) presence of parenchymal nodules, (b) differences in liver cell size and appearance, (c) fragmentation of the biopsy specimen, (d) fibre formation, accumulation and septa; and (e) altered architecture and vascular relationships.

Serological analysis

Biochemical markers of liver function in mouse serum were detected using various reagents (Wantai, Beijing, China). Serum hALB, human hepatocellular carcinoma (HCC) markers, HBV antigens and antibodies, human cytokines, hIgM, hIgG and liver cirrhosis markers were measured using respective ELISA kits. The total HBV DNA levels in the mouse serum samples were measured using a qRT-PCR assay with Premix Ex Taq™ as described previously [15]. The primer sequences were 5’-GTT

CAA GCC TCC AAG CTG TG-3' and 5'-TCA GAA GGC AAA AAA GAG AGT AAC TC-3', and the probe sequence was 5'-Hex- CCT TGG GTG GCT TTG GGG CAT GGA-BHQ-1-3'. The materials and reagents are presented in ***Table S1.***

FISH analysis for intracellular HBV DNA

For the analysis of intracellular HBV DNA, liver cells from uninfected controls and HBV-infected hBMSC-FRGS mice were isolated, cultured *in vitro* for 24 h and then co-stained for hALB by IF staining and for HBV DNA using molecular probes. HBV DNA was coupled with a digoxin (Dig)-labelled HBV X-specific probe. The signal was then amplified by a cascade reaction of a biotin-anti-Dig secondary antibody and TRITC-labelled anti-biotin third antibody. Photomicrographs were obtained with an Axio Imager microscope (Zeiss, Jena, Germany).

Detection of the percentage HBV-infected human cells

To detect the percentage of HBV-infected human cells, serial sections of HBV-infected hBMSC-FRGS mice at 0, 4, 8, 16, 32 and 56 w.p.i. were stained with hALB and HBsAg antibodies. At each time point, 10 pairs of images were randomly selected from the full set of scanned images and used to calculate the percentage of $HBsAg^+$ or $hALB^+$ cells. The results are representative of eight independent samples per time point.

Qualitative and quantitative analysis of intrahepatic HBV cccDNA levels

Liver cells from HBV-infected hBMSC-FRGS mice at 0 to 56 w.p.i. were collected by collagenase perfusion to analyse the intrahepatic HBV cccDNA levels. For qualitative analysis, HBV cccDNA was isolated using the "Hirt" method [16] and measured with

the southern blot technique. Southern blot detection was performed according to previously described methods using DIG-labelled DNA fragments from the HBX gene as a probe [12, 15]. For quantitative analysis, the HBV cccDNA levels were measured by qRT-PCR as reported previously [17, 18, 19].

- **Supplemental References**

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- **Supplemental Figures and Legends**

Figure S1. Phenotypes and multi-potential stem cell characteristics of hBMSCs. **(A)** Flow cytometry analysis of hBMSCs and controls for stem cell-related markers, including hCD90, hCD29, hCD45 and hCD34. Most hBMSCs were positive for hCD90 and hCD29 but negative for hCD45 and hCD34. **(B)** Morphology of undifferentiated hBMSCs and hBMSCs that differentiated into hepatocyte-like cells, adipocytes and osteocytes. Undifferentiated hBMSCs exhibited a fibroblast-like morphology (bar=50 μm). Differentiated hepatocytes exhibited a polygonal morphology with a low cytoplasm/nucleus ratio under phase-contrast microscopy (bar=100 μm). Differentiated osteocytes exhibited mineralization (bar=20 μm). Differentiated adipocytes contained lipid droplets (bar=20 μm). **(C)** IF staining of cluttered hBMSC-derived hepatocyte-like cells for the human-specific hepatic markers hALB and hHNF-4A (bar=20 μm).

Figure S2. Flow cytometry configuration, cell markers, fluorescent dye and protein antibody. **(A)** Violet (407 nm), yellow-green (561 nm), blue (488 nm) and red laser (533 nm) settings were used for flow cytometry. Antibodies and indicated fluorescent dyes for the indicated cell markers used in total liver cell and immune cell lineage analyses of hBMSC-FRGS mice are listed.

Figure S3. Cell fusion detection of human-derived hepatocytes and mouse hepatocytes in the livers of hBMSC-FRGS mice. **(A)** IF staining of frozen sections of FRGS and hBMSC-FRGS mouse liver tissues for the expression of human and mouse MHC. The nuclei were stained with DAPI. No cell fusion was observed between the human MHC-positive cells (red) and the mouse MHC-positive cells (green) (bar=100

μm). Representative FACS contour plots of **(B)** pig hepatocytes, **(C)** hBMSCs, **(D)** FRGS mouse liver cells and **(E)** hBMSC-FRGS mouse liver cells for the frequencies of human MHC- or mouse MHC-positive cells. **(B)** The pig hepatocytes were negative for both human and mouse MHC. **(C)** More than 99.5% of hBMSCs were positive for human MHC and negative for mouse MHC. **(D)** More than 99.5% of FRGS mouse liver cells were positive for mouse MHC and negative for human MHC. **(E)** Some of the hBMSC-FRGS mouse liver cells were positive for only human MHC, and the rest were positive for only mouse MHC. No hBMSC-FRGS mouse liver cells were positive for both human and mouse MHC. These results indicated that no cell fusion occurred between hBMSC-derived liver cells and the recipient mouse hepatocytes.

Figure S4. Safety and long-term tumourigenicity assay of hBMSC-FRGS mice. **(A)** Detection of human HCC markers, including hAFP, hGPC-3, hCA19-9 and hDCP, in serum of hBMSC-FRGS mice from weeks 0 to 60 after hBMSC transplantation (n=8). **(B)** qRT-PCR results for the expression of human HCC-related genes, including hAFP, hGOLM1, hCEA and hEGFR, in liver tissues collected from hBMSC-FRGS mice from weeks 0 to 60 after hBMSC transplantation (n=8). **(C)** H&E staining of main organs, including the heart, liver, spleen, lung, kidney, colon, muscle & bone (hind leg) and brain, collected from hBMSC-FRGS mice 60 weeks after hBMSC transplantation (bar=200 μm).

Figure S5. Supplementary information of hBMSC-derived human immune cell chimerism in hBMSC-FRGS mice. Reconstitution and maintenance of **(A)** $hCD3^{-}hCD14^{+}hCD68^{+}$ macrophages and **(B)** $hCD3^{-}hCD14^{-}HLA\text{-}DR^{+}$ dendritic cells in the peripheral blood, spleen and livers of hBMSC-FRGS mice from week 3 to 60

after transplantation, and proportions of these cells in the total population of $hCD45^+$ cells (10 different donors, n=3).

Figure S6. Supplementary information for HBV infection in hBMSC-FRGS mice. **(A)** Serum hALB, HBV DNA, **(B)** HBsAg and HBeAg levels in hBMSC-FRGS mice without HBV infection (control group) from 0 to 56 w.p.i. (n=8/group).

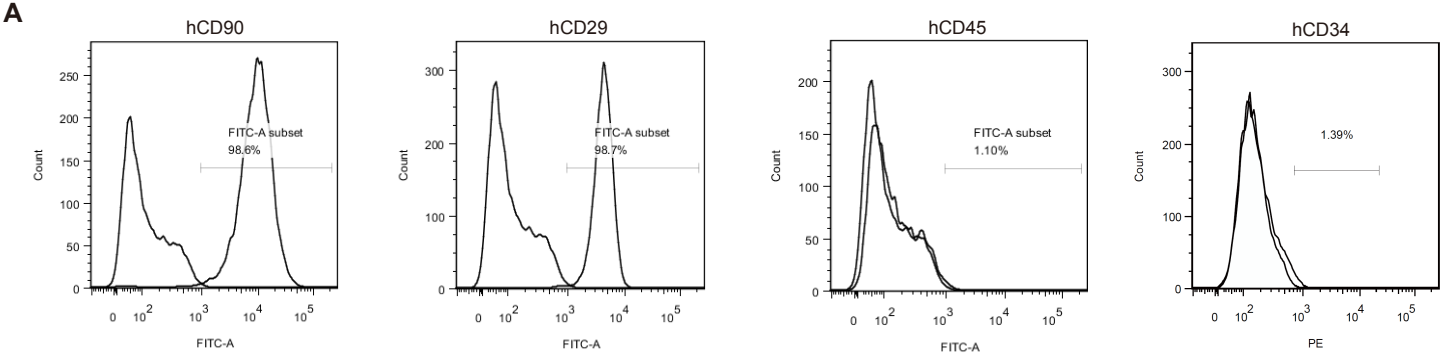
Figure S7. Serological analysis of the hALB, HBV DNA, HBsAg and HBeAg levels in individual animals (genotypes A to D). The serum hALB levels (first line), HBV DNA levels (second line), HBsAg levels (third line) and HBeAg levels (fourth line) in individual animals infected with genotypes A to D from weeks 0 to 56 w.p.i. are shown (n=8/group).

Figure S8. Follow-up of HBV-induced immune and inflammatory responses and chronic hepatitis. **(A)** Plasma samples from uninfected controls and HBV-infected hBMSC-FRGS mice were analysed by ELISA for various human cytokines from week 0 to 48 post-infection (n=8/group). **(B)** qRT-PCR analysis for the expression of human cytokine genes in HLA^+ liver cells collected from uninfected controls and HBV-infected hBMSC-FRGS mice from weeks 0 to 48 post-infection (n=4/group). **(C)** The serum hIgM and hIgG concentrations in individual animals from 0 to 48 w.p.i. were measured by ELISA, respectively (n=8/group). **(D)** The serum HBsAb, HBeAb and HBcAb concentrations in individual animals from 0 to 48 w.p.i. were measured by ELISA (n=8/group). **(E)** Serum of HBV-infected hBMSC-FRGS mice and uninfected controls were collected at 24 w.p.i. and measured by captured-ELISA method for Ig isotype of HBsAb and HBsAb (n=6/group). **(F)** Temporal changes in eight typical

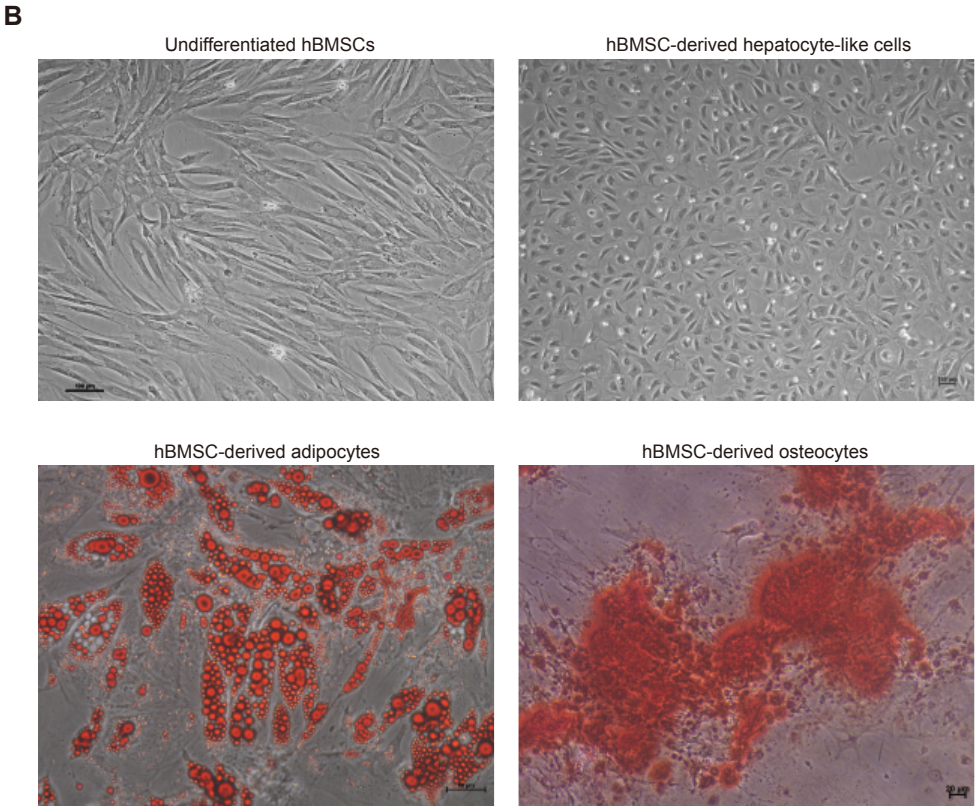
biochemical markers of liver function from weeks 0 to 48 post-infection (n=8/group) (***NS***, no significant difference; ***U.D.***, undetectable; ***a***, $p<0.05$; ***b***, $p<0.01$; ***c***, $p<0.001$).

Figure S9. Supplementary information for liver cirrhosis progression in hBMSC-FRGS mice. Different fields of view obtained from M&T-stained liver tissues collected from uninfected controls and HBV-infected hBMSC-FRGS mice during the progression of HBV-induced liver cirrhosis (bar=500 μm).

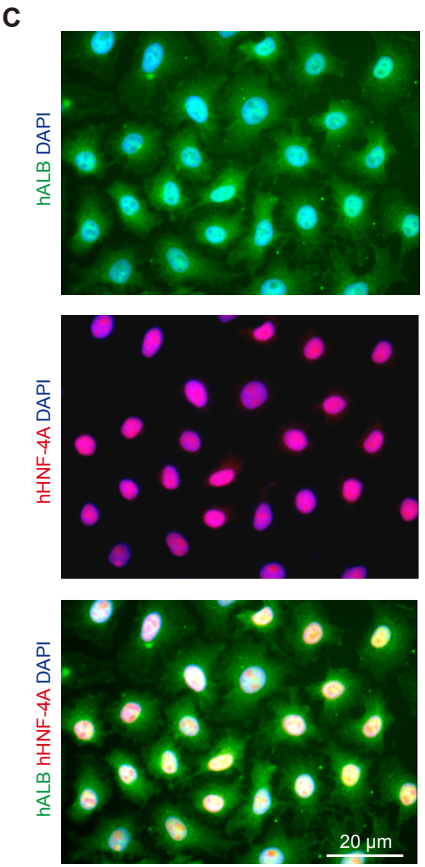
Supplementary Figure 1



FACS analysis for stem cell surface markers of hBMSCs and control



Morphology and *in vitro* multi-potential stem cell characteristics of hBMSCs



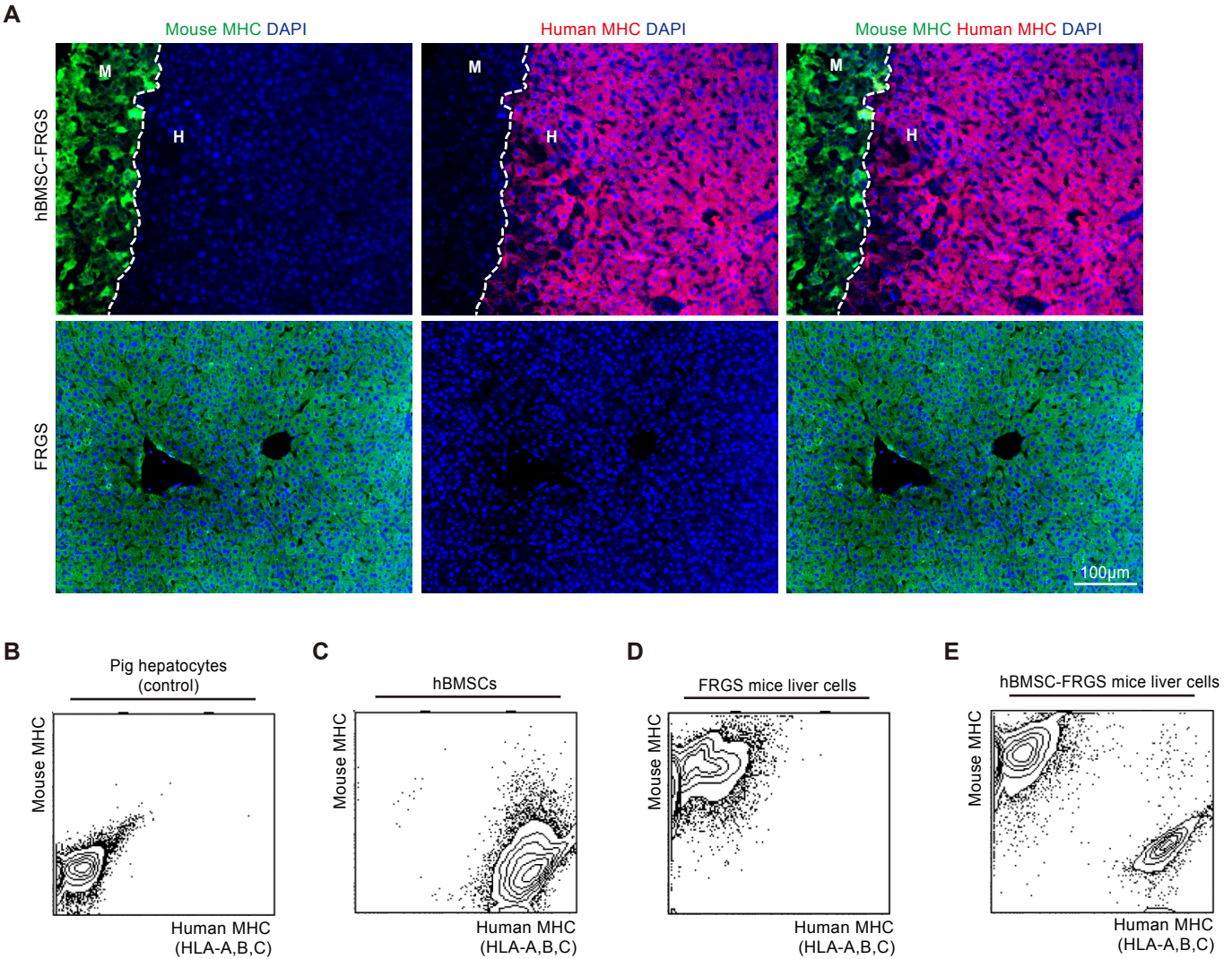
IF staining for hepatic markers of hBMSC-derived hepatocyte-like cells

Supplementary Figure 2

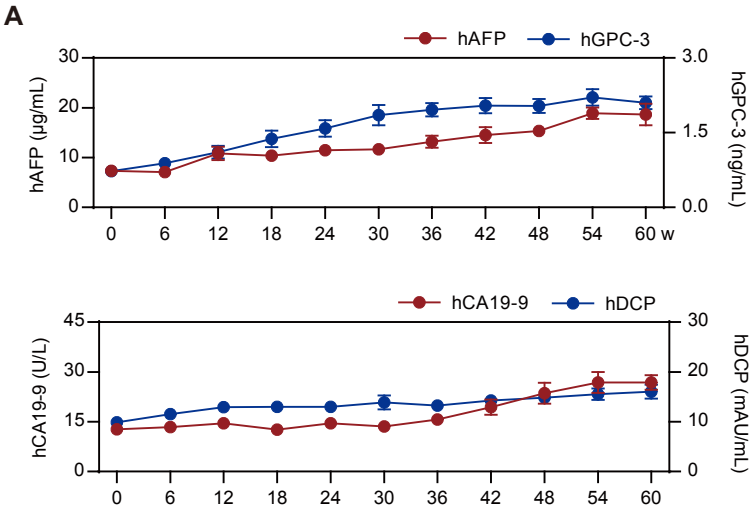
A

Laser	Filters	Fluorescent dye	Total liver cell analysis	Immune cell lineages analysis
407nm	780/60 735LP	BV786	hCD45	hCD45
	660/20 630LP	BV650	-	hCD123
	610/20 595LP	BV605	-	hNKp46
	510/50 502LP	BV510	-	hCD163
	450/40	BV421	-	hCD68
488nm	695/40 655LP	PerCP	hALB	hCD3
	530/30 450LP	BB515	-	hCD11c
	488/10	-	-	-
561nm	780/60 735LP	PE-Cy7	-	hCD19
	710/50 685LP	PE-Cy5.5	-	
	655/20 630LP	PE-Cy5	hNTCP	hCD86
	610/20 600LP	PE-CF594	-	mCD45
	560/20 545LP	PE	-	HLA-DR
633nm	780/60 755LP	APC-Cy7	HLA	hCD14
	730/45 685LP	AF700	-	hCD4
	660/20	APC	-	hCD8

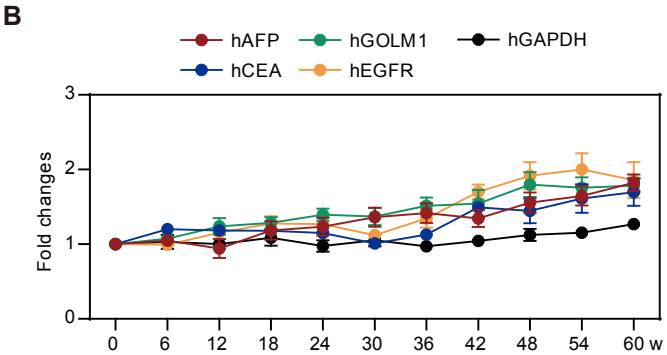
Supplementary Figure 3



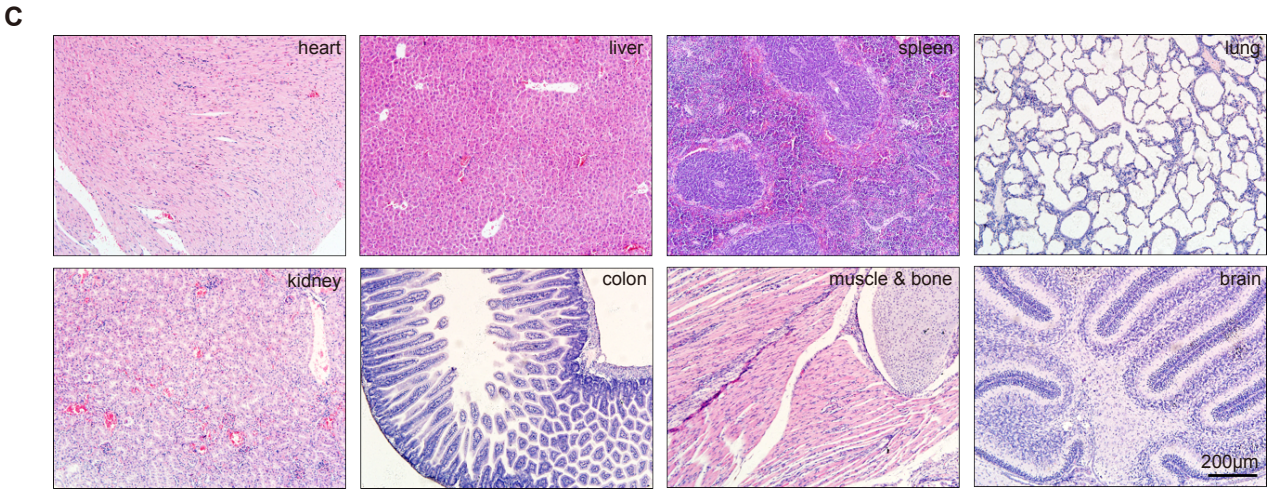
Supplementary Figure 4



Concentration of HCC markers in serum of hBMSC-FRGS mice (n=8/group)

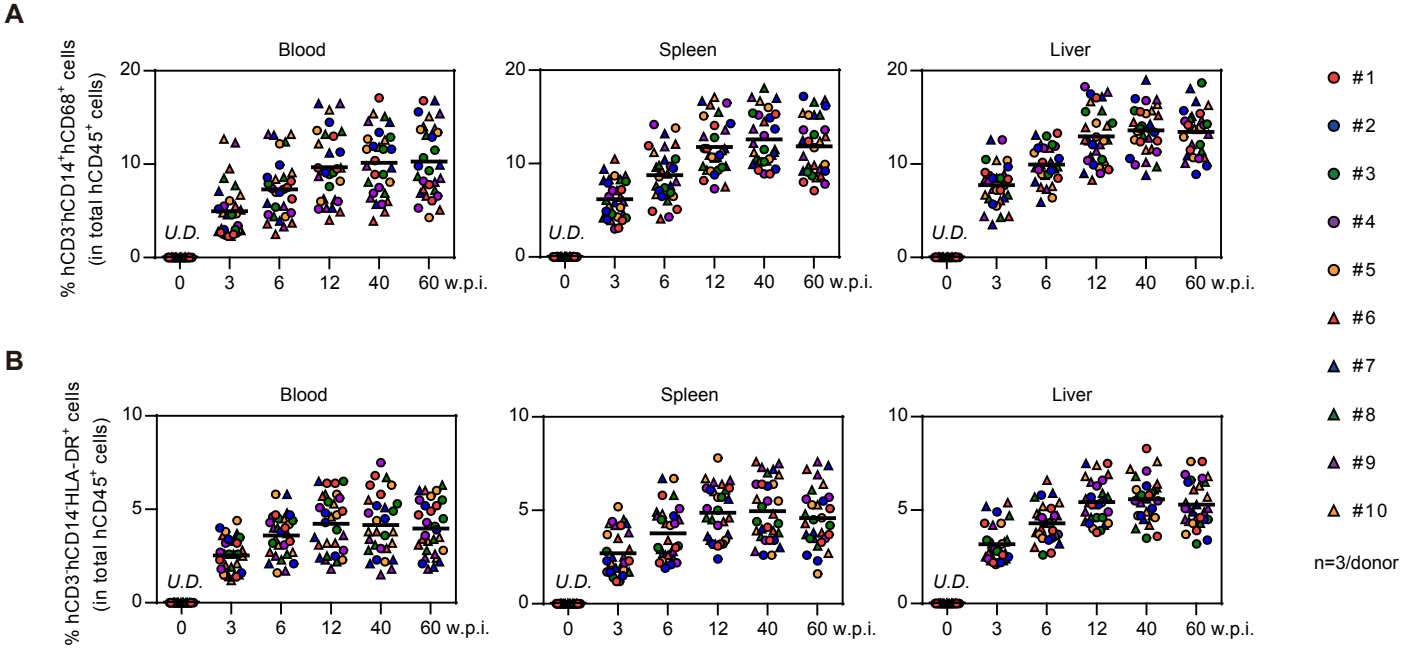


Fold changes of HCC related genes expression in liver tissues collected from hBMSC-FRGS mice (n=8/group)



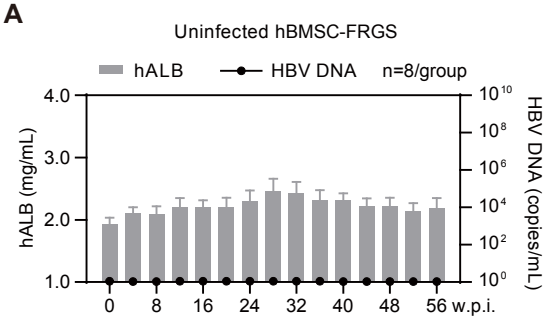
H&E staining of main organs collected from hBMSC-FRGS mice at 60 weeks after hBMSCs transplantation

Supplementary Figure 5

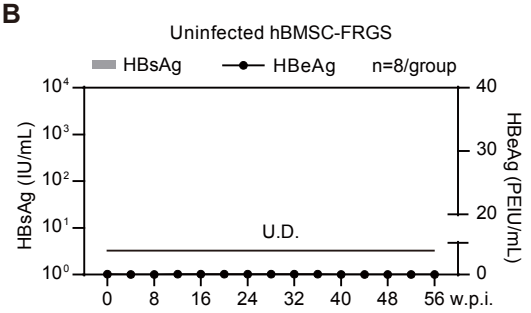


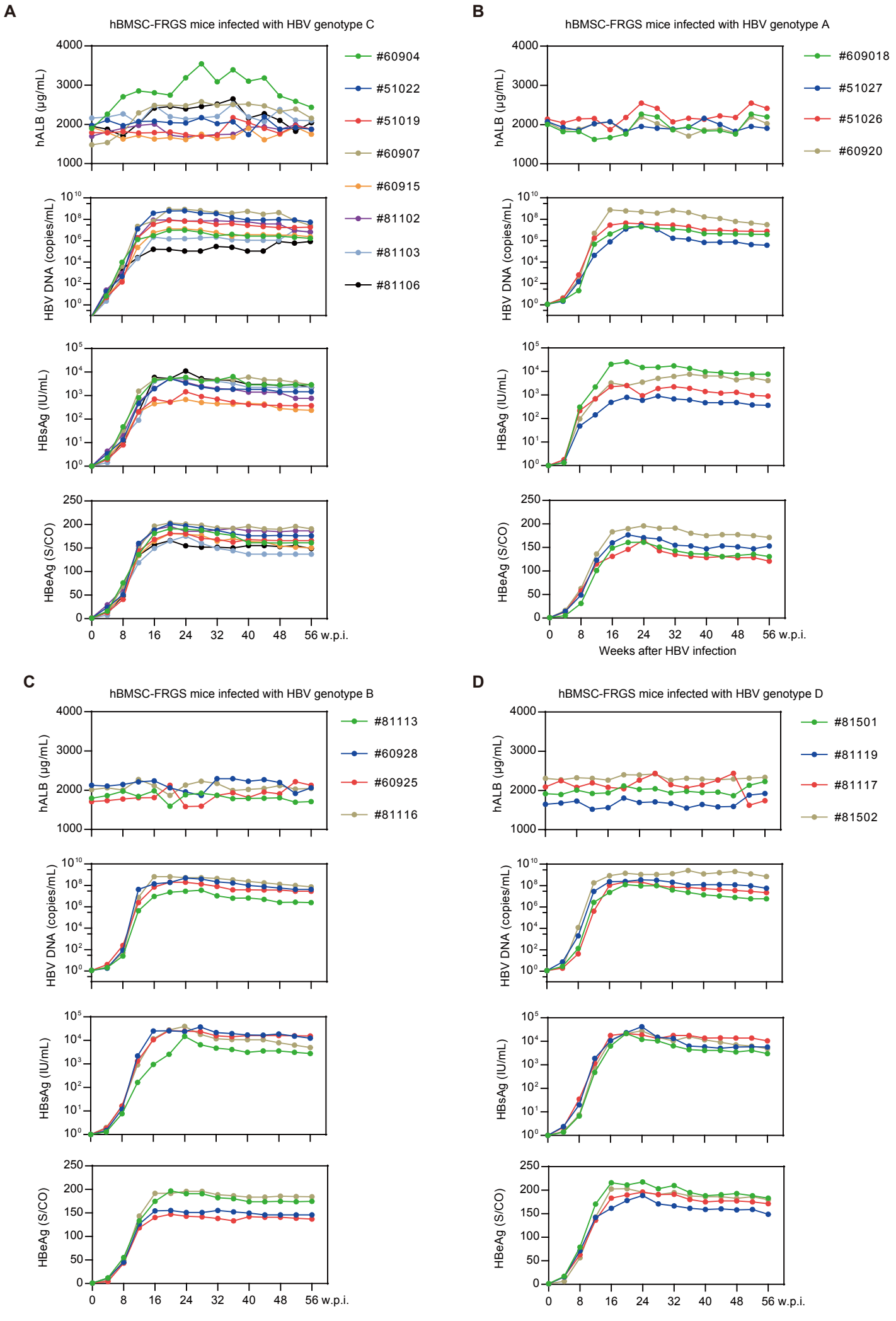
Supplementary Figure 6

A

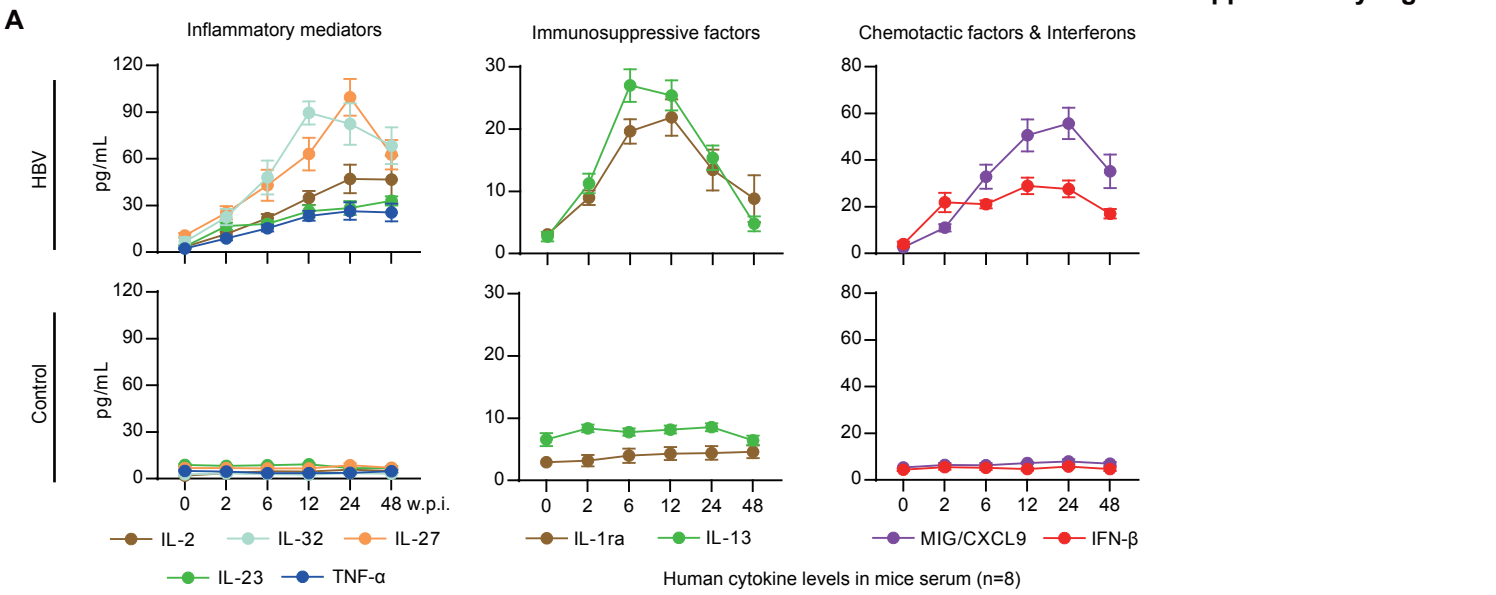


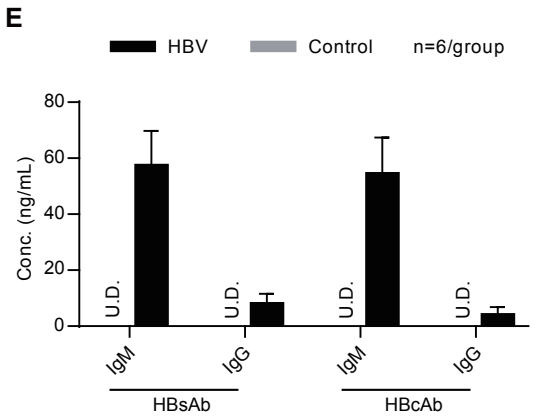
B

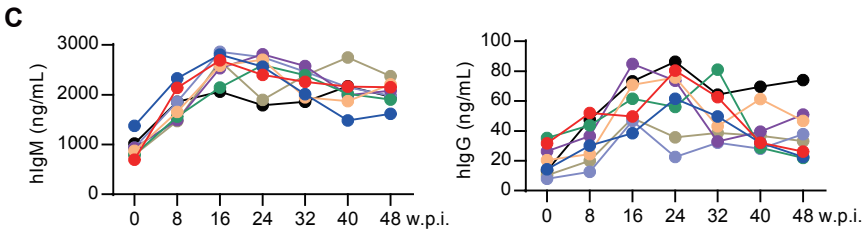


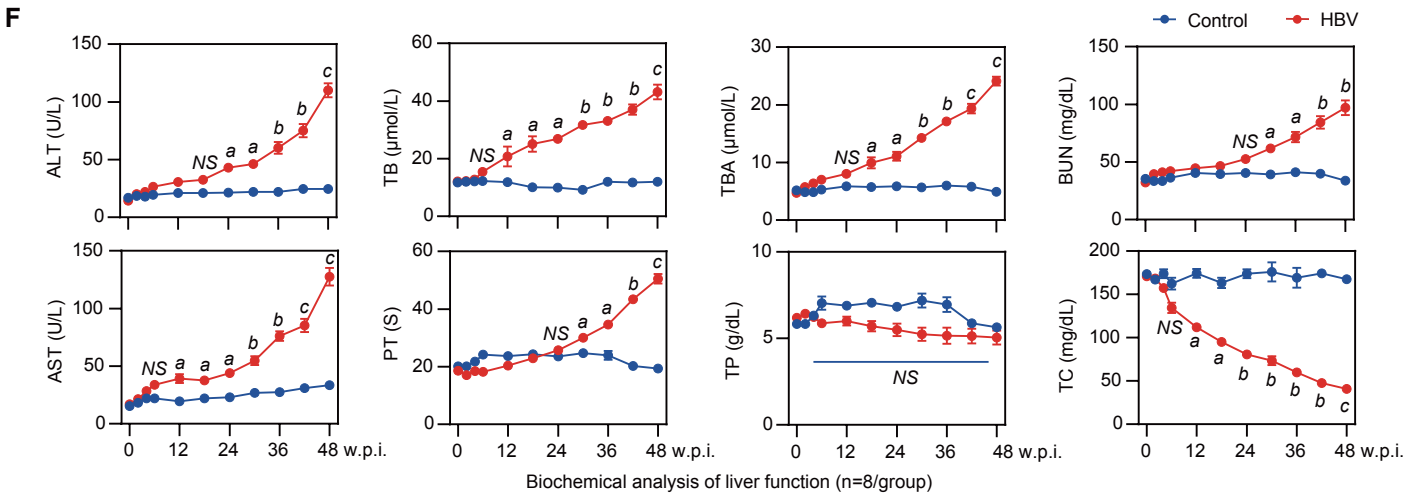

A
hBMSC-FRGS mice infected with HBV genotype C
hALB (μg/mL)
HBV DNA (copies/mL)
HBsAg (IU/mL)
HBeAg (S/CO)
#60904
#51022
#51019
#60907
#60915
#81102
#81103
#81106
B
hBMSC-FRGS mice infected with HBV genotype A
#609018
#51027
#51026
#60920
C
hBMSC-FRGS mice infected with HBV genotype B
#81113
#60928
#60925
#81116
D
hBMSC-FRGS mice infected with HBV genotype D
#81501
#81119
#81117
#81502
0 8 16 24 32 40 48 56 w.p.i.
Weeks after HBV infection

Supplementary Figure 8

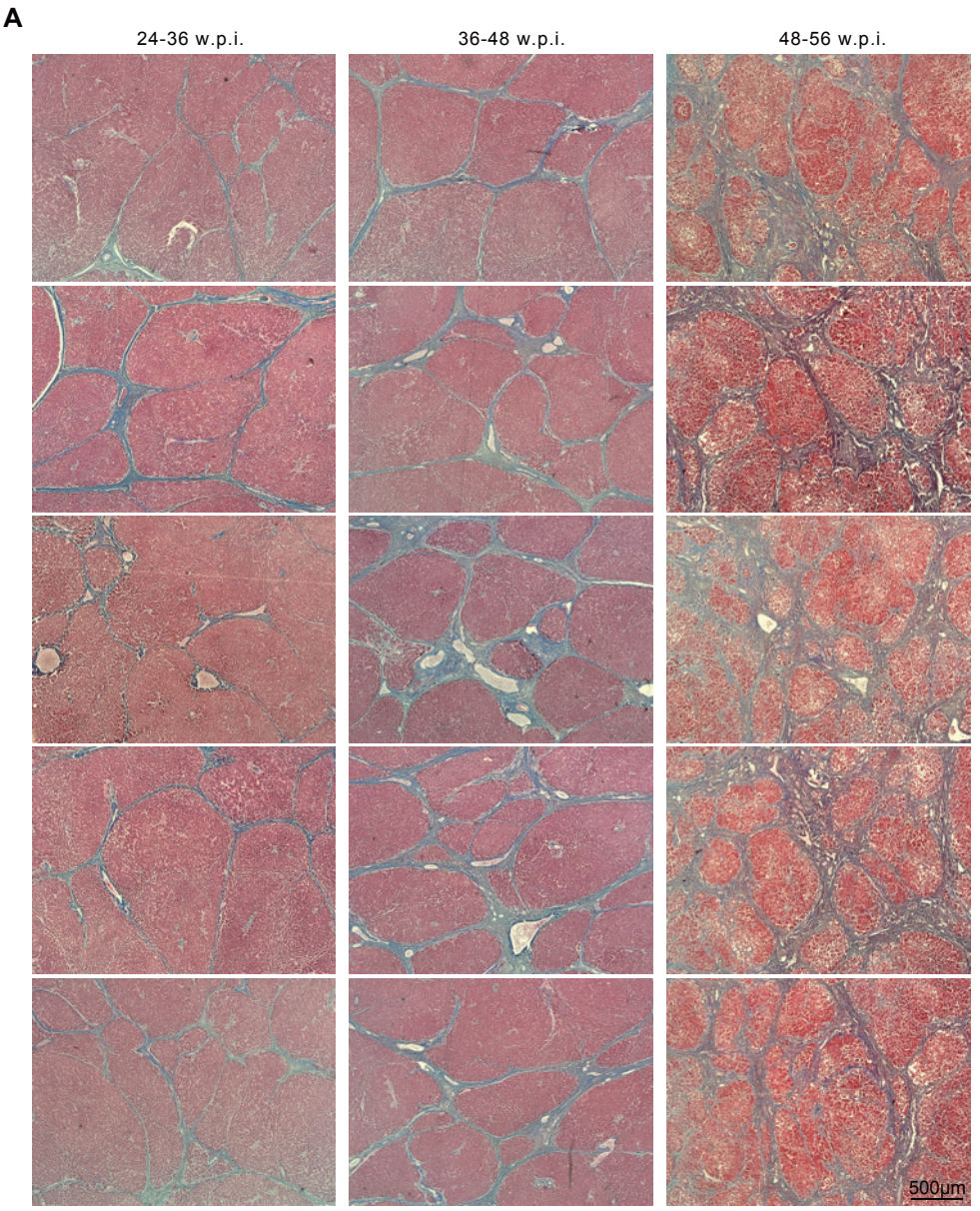








Supplementary Figure 9



Tab. S1 Reagents

Name	Company	Cat. No.	Speices specific test
Kits for HBV infection marker (antigens and antibodies) assays (HBsAg; HBeAg; HBsAb; HBeAb; HBcAb)	Wantai, Beijing,China	(http://www.bjwtdr.com/index.asp)	-
Kits for detection of liver biochemical markers and liver cirrhosis markers (ALT; AST; TB; TBA; TC; TP; PT; BUN; GGT; HA)			
hALB ELISA Kit	Bethyl, Montgomery, TX, USA	#E88-129	Passed
Human IgM/human IgG	Jackson ImmunoResearch, West Grove, PA, USA	#109–035–043/008	Passed
UltraSensitiveTM SP Kit for IHC assay	Maixin Biotech, Fuzhou, China	#KIT-9730	-
Dimethyl sulfoxide (DMSO)	Sigma-Aldrich, St Louis, MO, USA	#D4540	-
Collagenase, Type I/IV, powder		#C0130-5G/#C5138-5G	-
Biotin-11-dUTP	Fermentas (MBI), Hanover, MD, USA	#R0081	-
Ficoll-Paque^{TM} PLUS	GE Healthcare, Fairfield, CT, USA	#17-1440-03	-
Proteinase K	TaKaRa, Kusatsu, Japan	#D9034	-
DIG Easy Hyb™ Granules	Roche, AG, Basel, Switzerland	#11796895001	-
Hepes Buffer Solution	Invitrogen, Carlsbad, CA, USA USA	#15630	-
M&T stain Kit	Maixin Biotech, Fuzhou, China	#MST-8004	-
V.G. stain Kit	Maixin Biotech, Fuzhou, China	#MST-8002	-
SR/FG stain Kit	Solarbio LIFE SCIENCE, Beijing, China	#G1470	-
EndoFree Plasmid Giga Kit	Qiagen, Hilden, Germany	#12391	-
Busulfan	Sigma-Aldrich, St Louis, MO, USA	#B2635-25G	-
Nitisinone (NTBC)	SOBI, Stockholm, Sweden	#1052201/1042275	-
DNase I (Amplification Grade)	Sigma-Aldrich, St Louis, MO, USA	#AMPD1	-
Williams' Medium E (1X) without Phenol Red	GIBCO, Grand Island, NY, USA	#A12176-01	-
DMEM, High Glucose, Pyruvate		#11995-073	-
Foetal Bovine Serum, Qualified, Australia Origin		#10099-141	-

TRIzol	Invitrogen Corporation, Carlsbad, CA, USA	#15596-026	-
Trypsin ethylenediaminetetraacetic acid (Trypsin - EDTA)		#25200-072	-
Human IL-17 Quantikine ELISA Kit	R&D Systems, Minneapolis, MN, USA	#D1700	Passed
Human IL-1ra Quantikine ELISA Kit		#DRA00B	Passed
Human IL-6 Quantikine ELISA Kit		#D6050	Passed
Human IL-8 Quantikine ELISA Kit		#D8000C	Passed
Human CXCL10/IP-10 Quantikine ELISA Kit		#DIP100	Passed
Human IL-2 Quantikine ELISA Kit		#D2050	Passed
Human IL-4 Quantikine ELISA Kit		#D4050	Passed
Human IL-5 Quantikine ELISA Kit		#D5000B	Passed
Human IL-10 Quantikine ELISA Kit		#D1000B	Passed
Human TNF-alpha Quantikine ELISA Kit		#DTA00C	Passed
Human IFN-alpha ELISA Kit		#41100-1	Passed
Human IFN-beta ELISA Kit		#41410-1	Passed
Human IFN-gamma Quantikine ELISA Kit		#DIF50	Passed
Human alpha-Fetoprotein (AFP) Quantikine ELISA Kit		#DAFP00	Passed
Human IL-27 Quantikine ELISA Kit	Enzyme-linked Biotechnology, Shanghai, China	#ml028587	Passed
Human IL-32 Quantikine ELISA Kit		#ml027388	Passed
Human IL-16 Quantikine ELISA Kit		#ml028600	Passed
Human IL-23 Quantikine ELISA Kit		#ml027404	Passed
Human IL-13 Quantikine ELISA Kit		#ml027429	Passed
Human IL-1α Quantikine ELISA Kit		#ml027418	Passed
Human IL-1β Quantikine ELISA Kit		#ml027417	Passed
Human IL-17E (IL-25) Quantikine ELISA Kit	Boster Biotechnology, Wuhan, China	#EK0793	Passed
Human DCP ELISA Kit	Abnova, Taipei City, Taiwan, China	#KA0432	Passed
Human CA19-9 ELISA Kit		#KA0207	Passed
Human GPC3 ELISA Kit		#KA1175	Passed
Human IL-16 Quantikine ELISA Kit	Abcam, Cambridge, UK	#ab720	Passed

Fluorescent dye: BV786, BV650, BV605, BV510, BV421, PerCP, BB515, PE-Cy7, PE-Cy5.5, PE-Cy5, PE-CF594, PE, APC-Cy7, AF700, APC	Expedeon, Cambridge, UK	https://www.expedeon.com/products/immunoreagents/lightning-link-antibody-labeling-kits/fluorescent-dyes-and-proteins/	-

Tab. S2 Antibodies

Antibodies	Company	Cat. No.	Speices specific test
Purified NA/LE Hamster Anti-Mouse CD95 Clone JO2	BD Biosciences, San Jose, CA	#554254	-
anti-human albumin	House keeping	#5D2[1]	Passed
anti-HBsAg		#83H12[2]	-
anti-HBcAg	DAKO, Chattanooga, TN, USA	#B058601	-
anti-HLA (human MHC)	eBioscience, San Diego, CA, USA	#12-9983-80/-41	Passed
anti-mouse MHC	Abcam, Cambridge, UK	#ab25333	Passed
anti-biotin antibody (Texas Red)		#ab6653	-
anti-biotin antibody (FITC)		#ab6650	-
anti-human CK18		#ab82254	Passed
anti-human AAT		#ab9399	Passed
anti-human FAH		#ab47381	Passed
anti-human AFP		#ab3969	Passed
anti-total CD45 (human+mouse)		#ab10558	-
anti-mouse CD45		#ab25386	Passed
anti-human CD45		#ab10559	Passed
anti-human CD68		#ab845	Passed
anti-human CD86		#ab53004	Passed
anti-human CD19		#ab134114	Passed
anti-human CD3		#ab828	Passed
anti-human CD4		#ab133616	Passed
anti-human CD8		#ab17147	Passed
anti-human CD123		#ab21562	Passed
anti-human CD163		#ab156769	Passed
anti-human Nkp46		#ab14823	Passed

anti-human CD11c		#ab52632	Passed
anti-HLA-DR		#ab92511	Passed
anti-human NTCP	Sigma-Aldrich, St Louis, MO, USA	#HPA042727	Passed
anti-rabbit IgG (whole molecule)–TRITC		#T6778	Passed
anti-mouse IgG (whole molecule)–TRITC		#T5393	Passed
anti-rabbit IgG (whole molecule)–FITC		#F9887	Passed
anti-mouse IgG (whole molecule)–FITC		#F9006	Passed
anti-human IgM (μ-chain specific)		#12386	Passed
anti-human IgG (Fab specific)		#15260	Passed
anti-human IgG (Fc specific)		#12136	Passed

1.Science Translational Medicine 2016; Vol. 8, 352

2.Journal of Hepatology 2012; Vol. 57, 720-729

Tab. S3 Primers

Gene		Primer		Speices specific test
Human hepatic-specific genes	hALB	F	TTTATGCCCCGGAACTCCTTTT	Passed
		R	ACAGGCAGGCAGCTTTATCAG	Passed
	hAAT	F	GCCTATGATGAAGCGTTTAGGC	Passed
		R	TTCCAGTAATGGACAGTTTGGGT	Passed
	hHNF-4α	F	AACGGACAGATGTGTGAGTGG	Passed
		R	CAGGAGCTTATAGGGCTCAGAC	Passed
	hHNF-1α	F	GCCACCTGCTGCCATCCAA	Passed
		R	TGCAGCCCGTAGTTTAAAC	Passed
	hFAH	F	CCTACGGCGTCTTCTCGAC	Passed
		R	CTGCAAGAACACTCTCGCCT	Passed
	hNTCP	F	AAGGACAAGGTGCCCTATAAAGG	Passed
		R	ACGATCCCTATGGTGCAAGGA	Passed
	hCK18	F	TCGCAAATACTGTGGACAATGC	Passed
		R	GCAGTCGTGTGATATTGGTGTC	Passed
	hTransferrin	F	TGTCTACATAGCGGGCAAGTG	Passed
		R	GTTCCAGCCAGCGGTTCTG	Passed
	hCYP3A4	F	AAGTCGCCTCGAAGATACACAA	Passed
		R	AAGGAGAGAACACTGCTCGTG	Passed
	hCYP2B6	F	GCACTCCTCACAGGACTCTTG	Passed
		R	CCCAGGTGTACCGTGAAGAC	Passed
	hCYP1A2	F	CTTCGCTACCTGCCTAACCC	Passed
		R	TGACTGTGTCAAATCCTGCTCC	Passed
	hASGPR1	F	ATGACCAAGGAGTATCAAGACCTT	Passed
		R	TGAAGTTGCTGAACGTCTCTCT	Passed
	hMRP2	F	CAGCCATAGAGCTGGCCCT	Passed
		R	GCAAAACCAGGAGCCATGTG	Passed
	hTAT	F	TAGCTTCTAGGGGTGCCTCA	Passed
		R	AGCCATTGTGGACAACATGA	Passed
	hTTR	F	GGCTCACAACAGATGAGAAA	Passed
		R	TGTGGTGGAGTAAGAGTAGG	Passed
	hCYP7A1	F	CAAGAACCTGTACATGAGGGA	Passed
		R	CACTTCTTCAGAGGCTGCTTT	Passed
	hHNF1β	F	CTACAACCAGCAGGGAAAC	Passed
		R	CCATCAGGTGAGAGGAGAT	Passed
	hCDH1	F	CAGGTCTCCTCTTGGCTCTG	Passed
		R	ACTTTGAATCGGGTGTCGAG	Passed
	hBSEP	F	TTGGCTGATGTTTGTGGGAAG	Passed
		R	CCAAAAATGAGTAGCACGCCT	Passed

Human cytokine genes	hIL-1ra	F	GCTCATTGCTGGGTACTTACAA	Passed
		R	CCAGACTTGGCACAAGACAGG	Passed
	hIL-6	F	ATGGATGCTTCCAATCTG	Passed
		R	CTGGCTTGTTCCTCACTAC	Passed
	hIL-8	F	TGCTAAAGAACTTAGATGTCAGTG	Passed
		R	TGGTCCACTCTCAATCACTCTCA	Passed
	hIL-10	F	AAAAGAAGGCATGCACAGCTCAG	Passed
		R	GTGGGTGCAGCTGTTCTCAGACT	Passed
	hIL-2	F	TGCAACTCCTGTCTTGCATT	Passed
		R	TCAGTTCTGTGGCCTTCTTG	Passed
	hIL-4	F	ACTGCACAGCAGTTCCACAG	Passed
		R	CTCTGGTTGGCTTCCTTCAC	Passed
	hIL-5	F	ACTCTGATGATAGCCAATGAGA	Passed
		R	TCCAGTGTGCCTATTCCCTGA	Passed
	hIL-17A	F	TCAACCCGATTGTCCACCAT	Passed
		R	GAGTTTAGTCCGAAATGAGGCTG	Passed
	hIL-23	F	AGAAGCTCTGCACACTGGC	Passed
		R	CCACACTGGATATGGGGAAC	Passed
	hIL-32	F	GAAGGTCCTCTCTGATGACA	Passed
		R	AAGTAGAGGAGTGAGCTCTG	Passed
	hTNF-α	F	CAGCCTCTTCTCCTTCCTGAT	Passed
		R	GCCAGAGGGCTGATTAGAGA	Passed
	hIFN-α	F	CCCATTTCAACCAGTCTAGCAG	Passed
		R	TGTGGGTTTGAGGCAGATC	Passed
	hIFN-β	F	GACGCCGCATTGACCATCTA	Passed
		R	CCTTAGGATTTCCACTCTGACT	Passed
	hIFN-γ	F	TCGGTAACTGACTTGAATGTCCA	Passed
		R	TCGCTTCCCTGTTTTAGCTGC	Passed
Human fibrosis genes	hCOL1A1	F	GATGGACTCAACGGTCTCC	Passed
		R	CCTTGGGGTTCTTGCTGATG	Passed
	hTIMP-1	F	CTGTGTCCCACCCCACC	Passed
		R	GAACTTGGCCCTGATGACGA	Passed
	hMMP-2	F	ACCCAGATGTGGCCAACTAC	Passed
		R	TCATGATGTCTGCCTCTCCA	Passed
	hCOL1A2	F	GGCCCTCAAGGTTTCCAAGG	Passed
		R	CACCCTGTGGTCCAACAACTC	Passed
Control	hGAPDH	F	GGAGTCAACGGATTTGGTCGT	Passed
		R	CACTTGATTTTGGAGGGATCTCG	Passed

Human HCC marker genes	hGLOM1	F	TGGCCTGCATCATCGTCTTG	Passed
		R	CCCTGGAACTCGTTCTTCTTCA	Passed
	hEGFR	F	AGGCACGAGTAACAAGCTCAC	Passed
		R	ATGAGGACATAACCAGCCACC	Passed
	hHGF	F	GCTATCGGGGTAAAGACCTACA	Passed
		R	CGTAGCGTACCTCTGGATTGC	Passed
	hAFP	F	CTTGCACACAAAAAGCCCACT	Passed
		R	GGGATGCCTTCTTGCTATCTCAT	Passed