

Supplemental Materials

Table1. Characteristics of PBC patients and Healthy Controls.

| | PBC | HC |
|---|-----------------------|-----------------------|
| Age (yrs) Mean±SD | 58.71±11.68 | 56.87±4.58 |
| ALT(U/L) Median(P ₂₅ ,P ₇₅) | 28.00(16.00,56.00) | NA |
| AST(U/L) Median(P ₂₅ ,P ₇₅) | 39.00(29.00,68.50) | NA |
| GGT (U/L) ^a Median(P ₂₅ ,P ₇₅) | 131.00(64.00,200.00) | NA |
| AKP (U/L) ^a Mean±SD | 195.29±72.34 | NA |
| TBiL (umol/L) Median(P ₂₅ ,P ₇₅) | 13.20(10.15,28.65) | NA |
| IL-6(pg/mL) Median(P ₂₅ ,P ₇₅) | 7.15(6.15,7.67) | 5.31(4.81,5.71) |
| IL-8(pg/mL) Median(P ₂₅ ,P ₇₅) | 13.81(12.98,15.06) | 10.05(9.37,11.06) |
| TNF-α(pg/mL) Median(P ₂₅ ,P ₇₅) | 6.62(8.54,10.62) | 9.84(5.74,7.43) |
| IL-22(pg/mL) Median(P ₂₅ ,P ₇₅) | 35.09(31.93,38.81) | 27.28(24.96,29.51) |
| IFN-γ(pg/mL) Median(P ₂₅ ,P ₇₅) | 95.86(87.93,102.31) | 77.52(70.82,85.20) |
| TGF-β(pg/mL) Median(P ₂₅ ,P ₇₅) | 536.19(499.67,581.41) | 423.16(397.07,472.72) |
| VEGF(pg/mL) Median(P ₂₅ ,P ₇₅) | 38.01(34.84,40.63) | 26.98(25.53,28.43) |

a, the normal range of alkaline phosphatase is 45-125U/L,g-glutamyl transpeptidase is 10-60U/L in our hospital. NA,not applicable. ALT: Alanine aminotransferase; AST: Aspartate transaminase; γ-GT: G-glutamyl transpeptidase; TBiL: Total bilirubin; IL-6: Interleukin-6; IL-8: Interleukin-8; TNF-α: Tumor necrosis factor-alpha;IL-22:Interleukin-22;IFN-γ:Interferon gamma; TGF-β: Transforming growth factor-beta; VEGF: Vascular endothelial growth factor.

Table 2. List of Primers Used in Real-Time PCR

| Gene Name | | Sequence | Real-time PCR product size |
|-----------------|---------|--------------------------|----------------------------|
| hIL-6 | Forward | AAAGCAGCAAAGAGGCACTG | 137bp |
| | Reverse | TACCTCAAACCTCCAAAAGACCAG | |
| hIL-8 | Forward | GACATACTCCAAACCTTTCCACC | 162bp |
| | Reverse | AACTTCTCCCACAACCCTCTGC | |
| hTNF- α | Forward | CACGCTCTTTCTCTGCCTGCTG | 129bp |
| | Reverse | GGCTTGTCGGGGTTC | |
| hER α | Forward | CCAGGCTTTGTGGATTTGAC | 147bp |
| | Reverse | G TTCCTGTCCAAGAGCAAGTTAG | |
| hER β | Forward | TCTCCTTTAGTGGTCCATCGC | 181bp |
| | Reverse | GAGCATCCCTCTTTGAACCTG | |
| β -actine | Forward | ACCACCATGTACCCTGGACT | 103bp |
| | Reverse | TTGTTTTCTGCGCAAGTTAGGT | |

bp, base pair; h, human

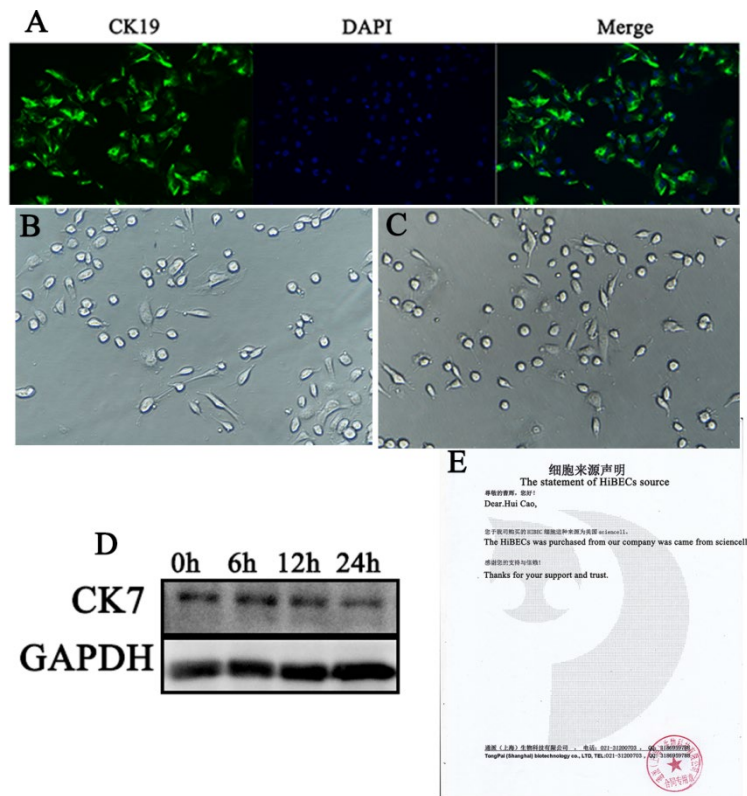


Figure1: (A-E): HiBECs identification. **A.** Immunofluorescence was used to detect the ck19 expression; **B.** Immunoblotting was used to detect the ck7 expression; **B-C.** The morphology of HiBECs was observed by optical microscope; **E.** Certificate was provided by tongpai biotechnology co., LTD.

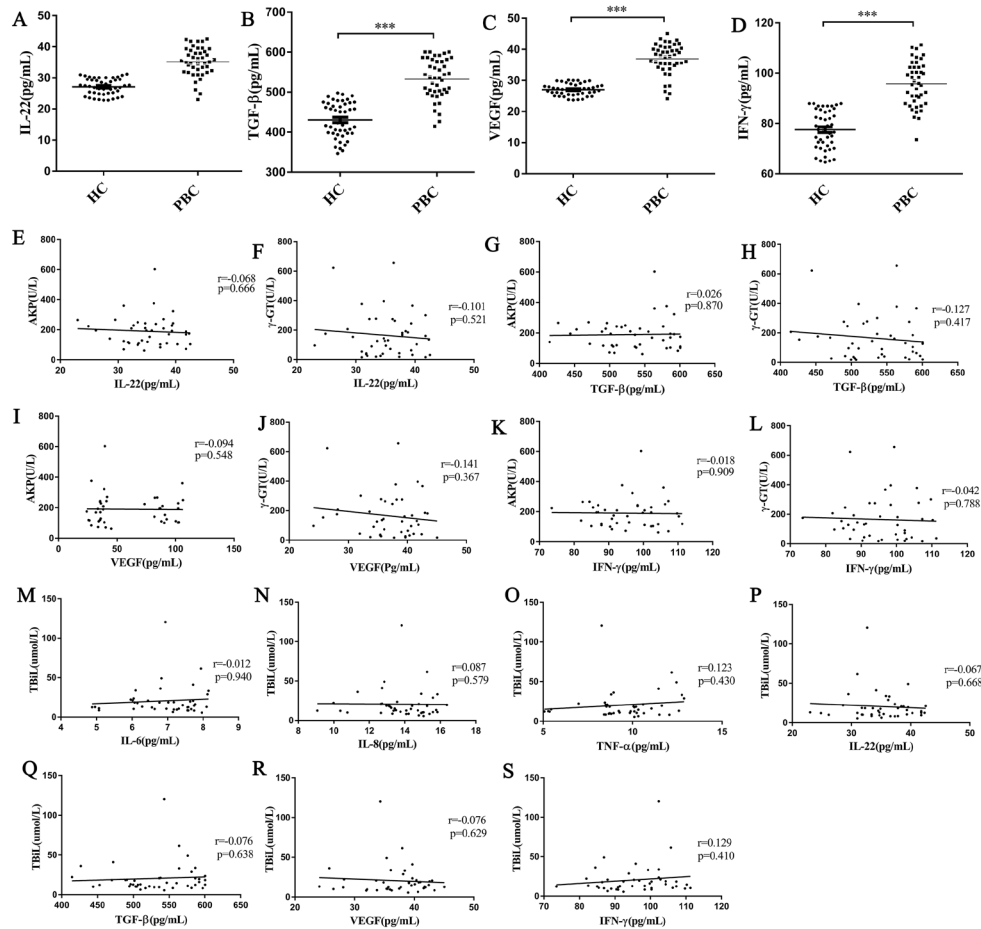


Figure 2: Serum levels of cytokines and correlation with AKP and γ -GT and TBIl. **(A-D):** Serum levels of IL-22(A), TGF- β (B), VEGF(C) and IFN- γ (D) in PBC patients (n=43) and HC(n=45), ***P <0.001, compared with HC by a Mann-Whitney test. Data are represented as median with interquartile range **(E-L):** Serum IL-22, TGF- β , VEGF and IFN- γ concentration with the levels of AKP and γ -GT in PBC patients(n=43). **(M-S):** Serum IL-6, IL-8, TNF- α , IL-22, TGF- β , VEGF and IFN- γ concentration with the levels of TBIl in PBC patients (n=43). The p values were determined by Spearman's rank correlation, P<0.05 was considered significant. PBC, primary biliary cirrhosis; HC: healthy control; IL-6, interleukin-6; IL-8, interleukin-8; TNF- α , tumor necrosis factor-alpha; AKP, Alkaline phosphatase; GGT, g-glutamyl transpeptidase; IL-22: Interleukin-22; IFN- γ : Interferon gamma; TGF- β : Transforming growth factor-beta; VEGF: Vascular endothelial growth factor.

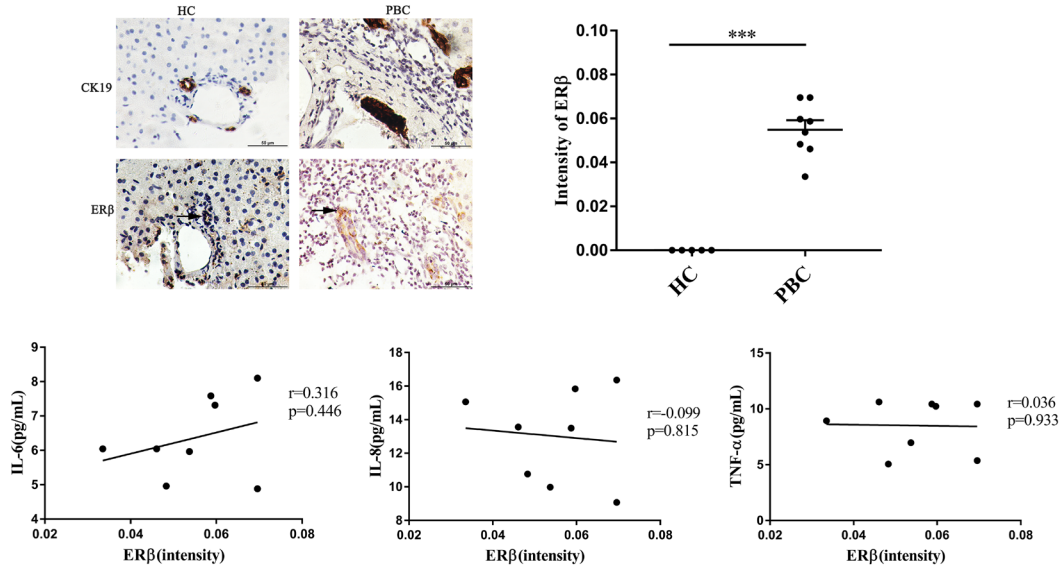


Figure3: ERβ expression in liver biopsies of PBC patients and HC. ERβ expression in small bile duct of PBC patients (upper right panel) were higher than HC (lower right panel)(original magnification, ×400), However,the expression levels of ERβ have not a linear relationship with the levels of cytokines.

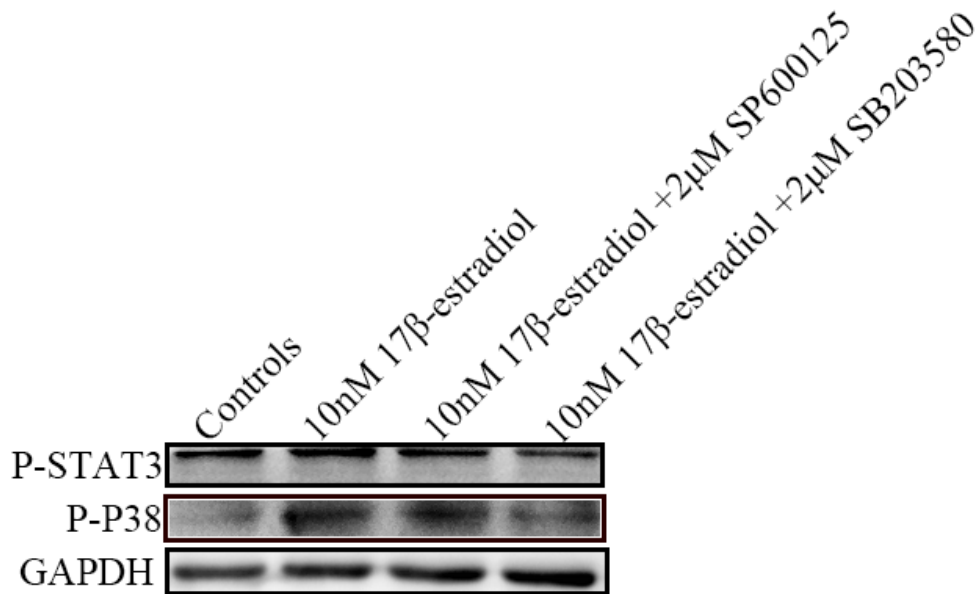


Figure4: *In vitro*, SB203580 can block STAT3 activation, which should be up-regulated in HiBECs by treated with 17β-estradiol.

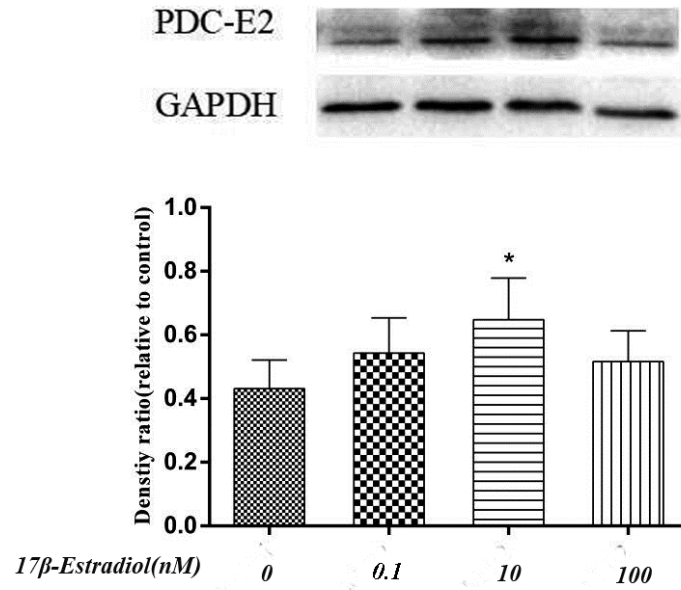


Figure5: *In vitro* 17β-estradiol promoted PDC-E2 expression in HiBECs. HiBECs were treated with 3 concentrations of 17β-estradiol(0.1nM,10nM and 100nM) for 48h. Immunoblotting analysis of PDC-E2 expression in HiBECs. 10nM 17β-estradiol had significantly induced PDC-E2 expression, *p< 0.05 compared with controls by Student t test.

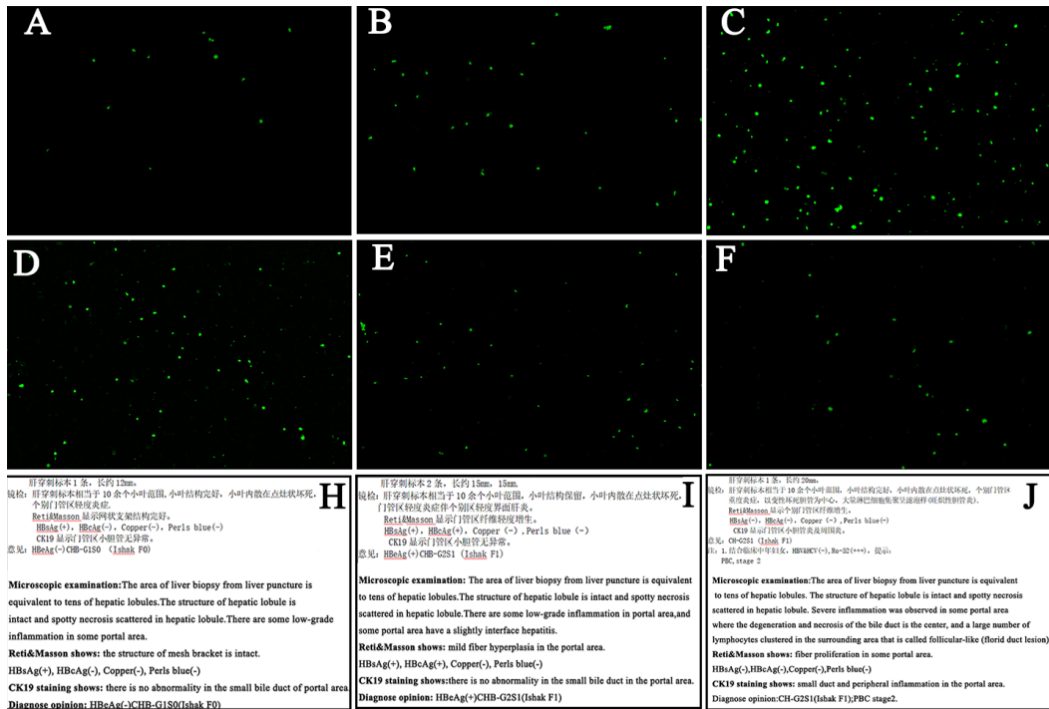


Figure6: PBMCs adhere to HiBECs and the results of liver pathological of three patients. **(A-C):** PBMCs were extracted from 10mL peripheral blood of PBC patients(n=1) and CHB patients (n=2).Meanwhile, HiBECs were also isolated from those patients. PBMCs were stimulated with 10 mg/mL phytohemagglutinin for 72h. Then, BCECF-AM was added to PBMCs for 1h and co-cultured with HiBECs. **A:** PBMCs and HiBECs from CHB patient;**B:** PBMCs and HiBECs from CHB patient 2; **C:**PBMCs and HiBECs from PBC patient. **(D-F):** Fulvestrant was added to HiBECs which was isolated from PBC patient for 12h **(D)**, 24h**(E)** and 48h**(F)**. **(H-J)**.The results of liver pathological of two CHB patients **(A:** patient 1; **B:** patient 2) and PBC patient**(J)**.