

Supplementary Information

Supplementary methods

1.1 Human albumin measurement

Mouse serum was collected at Day 14 after hepatectomy and analyzed for human albumin using a commercial human albumin sandwich ELISA kit (USCN business, USCN-CEB028Hu) according to the manufacturer's instructions. Samples and standards were added to the antibody-coated plate and processed according to the supplied protocol. Absorbance was measured at 450 nm, and human albumin concentrations were calculated from the corresponding standard curve. Serum from model and scaffold-only mice served as negative controls, whereas conditioned medium from cultured dBDOs was included as an assay-positive reference.

1.2 Human-specific Alu DNA and transcript detection

For detection of donor-derived human DNA, genomic DNA was extracted from cultured dBDOs and recipient tissues using a genomic DNA extraction kit (Qiagen DNeasy Blood & Tissue Kit, Qiagen, Germany). Equal amounts of genomic DNA were subjected to quantitative PCR using human AluYb8-specific primers. At Day 14, liver tissue from the predefined patch-placement region was analyzed; for the 2-month biodistribution assessment, heart, liver, spleen, lung, and kidney tissues were examined. Cultured dBDOs served as the human positive control, whereas mouse tissues from the indicated non-OTP groups and no-template reactions served as negative controls.

For human-specific transcript detection, total RNA was extracted from cultured dBDOs and recipient liver tissue, reverse-transcribed to cDNA, and analyzed by qRT-PCR using primers specific for human SOX9, KRT19, KI67, HNF4A, CYP3A4, ALB, and CDH1. Primer specificity was assessed using mouse liver cDNA and no-template controls. Primer sequences are provided in Supplementary Table S3. Specific amplification with $Ct \leq 35$ was considered detectable, whereas $Ct > 35$ or

absence of specific amplification by 40 cycles was classified as not detected (ND).

1.3 Multiplex immunohistochemical

Paraffin-embedded liver sections were subjected to multiplex immunohistochemical staining using a TSA Fluorescence Triple Staining Plus Kit (RK05903P). Sections were sequentially stained for F4/80 and CD206 with TSA-based fluorescence amplification, followed by DAPI nuclear counterstaining. Fluorescence images were acquired for colocalization analysis.

37 **Supplementary tables**

38 **Table S1. Clinical background of bile donors used for BDO establishment**

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Donor ID	Age	Sex	ERCP indication	Hepatobiliary condition	Obstruction	Inflammation/cholangitis	Malignancy
BDO-01	56	M	bile duct stone	Choledocholithiasis	Yes	Yes	No
BDO-02	59	F	biliary obstruction	Choledocholithiasis	Yes	No	No
BDO-03	48	M	bile duct stone	Choledocholithiasis	Yes	Yes	No
BDO-04	67	M	bile duct stone	Choledocholithiasis	Yes	Yes	No
BDO-05	46	M	bile duct stone	Choledocholithiasis	Yes	Yes	No

40 **Table S2 Antibody list**

Primary antibody			
Antibody	Source	Product code	Species
CD206	Proteintech	83485-1-RR	Rabbit IgG
p-p65	Proteintech	82335-1-RR	Rabbit IgG
p65	Cell Signaling Technology	8242	Rabbit IgG
β-actin	Proteintech	66009-1-Ig	Mouse IgG
SOX9	Abcam	ab185966	Rabbit IgG
F-actin	Abcam	ab205	Mouse IgG1
EPCAM	Proteintech	66316-1-Ig	Mouse IgG1
ALB	Proteintech	66051-1-Ig	Mouse IgG1
HNF4A	Abcam	ab201460	Rabbit IgG
CYP3A4	Santa Cruz	sc-53850	Mouse IgG1
Cytokeratin 19	Abcam	ab52625	Rabbit IgG
HMGB1	Proteintech	10829-1-AP	Rabbit IgG
PCNA	Abcam	ab92552	Rabbit IgG
PCNA	Proteintech	60097-1-Ig	Mouse IgG
Ki67	Abcam	ab16667	Rabbit IgG
PPARα	Affinity	AF5301	Rabbit IgG
YAP	Cell Signaling Technology	14074	Rabbit IgG
F4/80	Cell Signaling Technology	#70076	Rabbit IgG
Secondary antibody			
Secondary antibody		Company	Code number
Goat Anti-Rabbit IgG H&L (HRP)		Abcam	ab6721
Goat anti-Mouse IgG (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor 488		ThermoFisher	A-11001
Goat Anti-Rabbit IgG H&L (Alexa Fluor® 647)		Abcam	ab150083
Goat Anti-Mouse IgG H&L (Alexa Fluor® 555)		Abcam	ab150114

42 **Table S3 Primer sequences used for Real-time PCR studies**

Primer	Sequence
Pcna (F)	TTTGAGGCACGCCTGATCC
Pcna (R)	GGAGACGTGAGACGAGTCCAT
Ki67 (F)	GAGGAGAAACGCCAACCAAGAG
Ki67 (R)	TTTGTCTCTCGGTGGCGTTATCC
Ccl5 (F)	CCTGCTGCTTTGCCTACCTCTC
Ccl5 (R)	ACACACTTGGCGGTTCTTCGA
Ccl2 (F)	GTCTGGACCCATTCTTCTTGG
Ccl2 (R)	GCTACAAGAGGATCACCAGCAG
Cxcl1(F)	TCCAGAGCTTGAAGGTGTTGCC
Cxcl1 (R)	AACCAAGGGAGCTTCAGGGTCA
Cxcl2 (F)	CATCCAGAGCTTGAGTGTGACG
Cxcl2 (R)	GGCTTCAGGGTCAAGGCAAAC
IL-6 (F)	TACCACTTCACAAGTCGGAGGC
IL-6 (R)	CTGCAAGTGCATCATCGTTGTTC
TNF- α (F)	GGTGCCTATGTCTCAGCCTCTT
TNF- α (R)	GCCATAGAAGTATGAGAGGGAG
IL-1 β (F)	TGGACCTTCCAGGATGAGGACA
IL-1 β (R)	GTTTCATCTCGGAGCCTGTAGTG
Acox1 (F)	GCCATTCGATACAGTGCTGTGAG
Acox1 (R)	CCGAGAAAGTGGAAGGCATAGG
Cpt1a (F)	GGCATAAACGCAGAGCATTCTG
Cpt1a (R)	CAGTGTCCATCCTCTGAGTAGC
β -actin (F)	CATTGCTGACAGGATGCAGAAGG
β -actin (R)	TGCTGGAAGGTGGACAGTGAGG
hALB (F)	GATGAGATGCCTGCTGACTTGC
hALB (R)	CACGACAGAGTAATCAGGATGCC
hKRT19 (F)	AGCTAGAGGTGAAGATCCGCGA

hKRT19 (R)	GCAGGACAATCCTGGAGTTCTC
hKI67 (F)	GAAAGAGTGGCAACCTGCCTTC
hKI67 (R)	GCACCAAGTTTTACTACATCTGCC
hCDH1 (F)	GCCTCCTGAAAAGAGAGTGGAAG
hCDH1 (R)	TGGCAGTGTCTCTCCAAATCCG
hGAPDH (F)	GTCTCCTCTGACTTCAACAGCG
hGAPDH (R)	ACCACCCTGTTGCTGTAGCCAA
hHNF4A (F)	GGTGTCCATACGCATCCTTGAC
hHNF4A (R)	AGCCGCTTGATCTTCCCTGGAT
hSOX9 (F)	AGGAAGCTCGCGGACCAGTAC
hSOX9 (R)	GGTGGTCCTTCTTGTGCTGCAC
hCYP3A4 (F)	CCGAGTGGATTCCTTCAGCTG
hCYP3A4 (R)	TGCTCGTGGTTTCATAGCCAGC
hAluYb8 (F)	CGAGGCGGGTGGATCATGAGGT
hAluYb8 (R)	TCTGTGCGCCAGGCCGGACT

44 **Table S4. Survival outcome and timing of death after 70% partial hepatectomy**

Group	Total mice	Deaths	Timing of death	Cause of death
Control	10	0	None	Not applicable
Model	18	7	POD1: 1; POD2: 2; POD3: 2; POD5: 1; POD11: 1	Not pathologically confirmed;
Scaffold	14	5	POD1: 2; POD2: 1; POD5: 1; POD8: 1	Not pathologically confirmed;
OTP	14	1	POD3: 1	Not pathologically confirmed;

45 Abbreviation: POD, postoperative day. Causes of death were not pathologically adjudicated
 46 because spontaneous deaths were not followed by complete necropsy-based confirmation.

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Supplementary figures

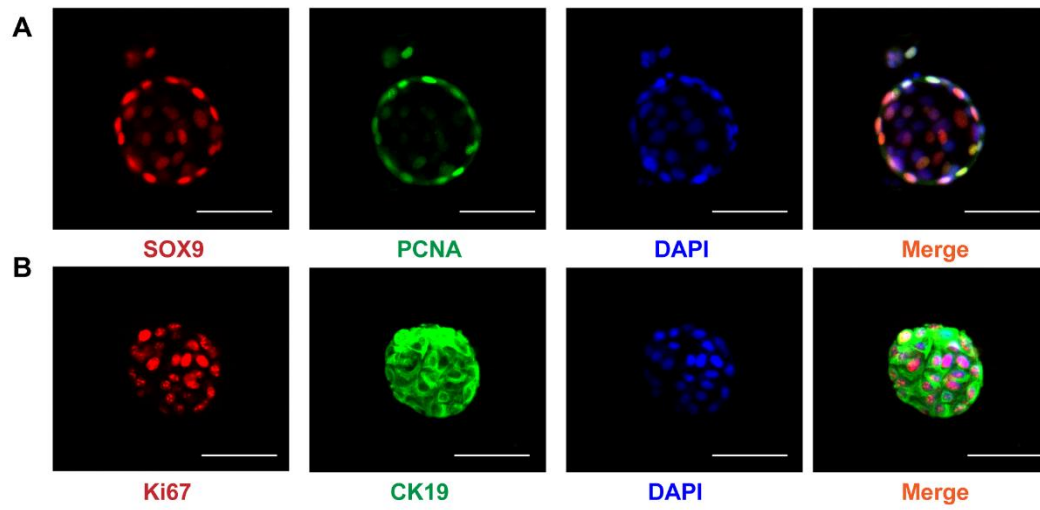
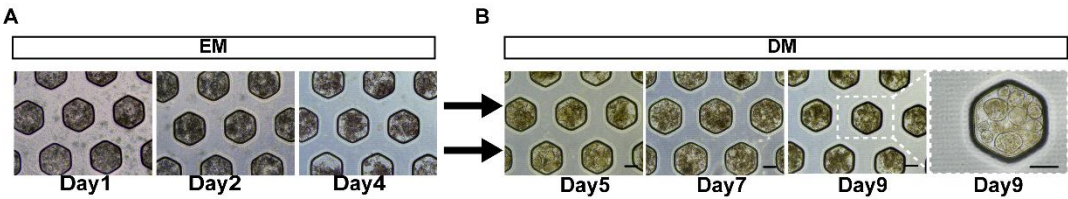


Figure S1. Morphological and phenotypic features of bile-derived organoids cultured in expansion medium (EM).

(A) Representative immunofluorescence images of BDOs maintained in EM showing SOX9 and PCNA expression. (B) Representative immunofluorescence images showing Ki67 and CK19 expression in expansion-stage BDOs. Scale bars, 50 μm .



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61 **Figure S2. Time-course morphology of bile-derived organoids cultured in the matrix-free**
62 **microwell system during expansion and maturation.**

63 (A) Bright-field images of bile-derived liver organoids cultured in expansion medium (EM) within
64 hexagonal microwells at Days 1, 2, and 4, showing progressive epithelial compaction and growth.

65 (B) Bright-field images of organoids after transfer to differentiation medium (DM) at Days 5, 7,
66 and 9, showing continued enlargement while maintaining a stable spheroid morphology. The
67 rightmost panel shows a magnified view of a representative Day 9 organoid. Scale bars, 100 μ m.

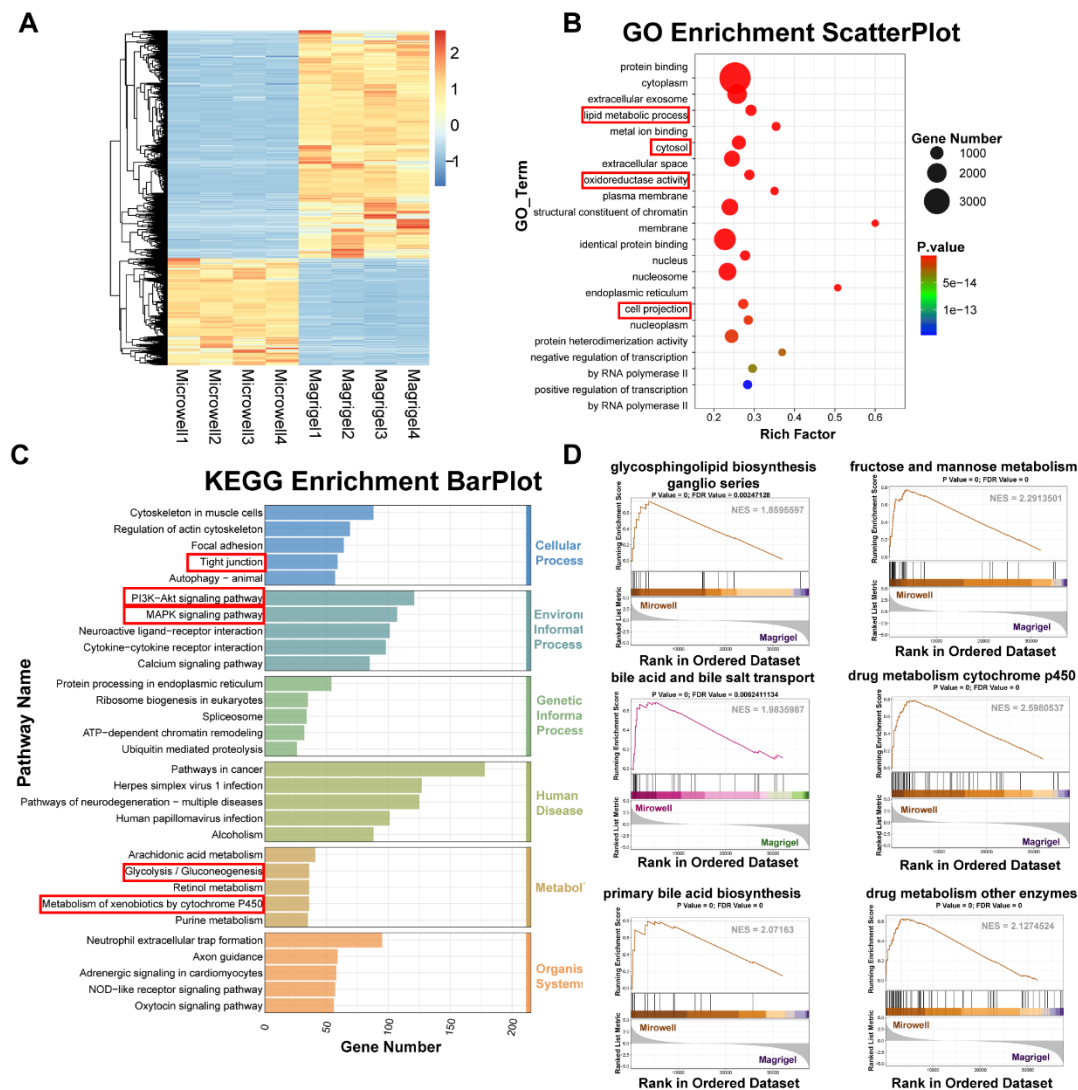


Figure S3. Supplementary transcriptomic analyses of microwell-cultured differentiated bile-derived organoids.

(A) Heatmap of differentially expressed genes between microwell-cultured dBDOs (Microwell1–Microwell4) and Matrigel-cultured dBDOs (Matrigel1–Matrigel4). (B) Gene Ontology enrichment analysis of differentially expressed genes in microwell-cultured dBDOs, showing enrichment of terms related to lipid metabolic processes, oxidoreductase activity, cytosolic/extracellular organization, and cell projection. (C) KEGG enrichment analysis showing pathway-level remodeling in microwell-cultured dBDOs, including junctional/cytoskeletal organization, intracellular signaling, and metabolism-related programs. (D) Additional gene set enrichment analyses showing enrichment of hepatocyte-associated metabolic pathways in microwell-cultured dBDOs, including glycosphingolipid biosynthesis, fructose and mannose metabolism, bile acid and bile salt transport, drug metabolism by cytochrome P450, primary bile acid biosynthesis, and drug metabolism by other enzymes.

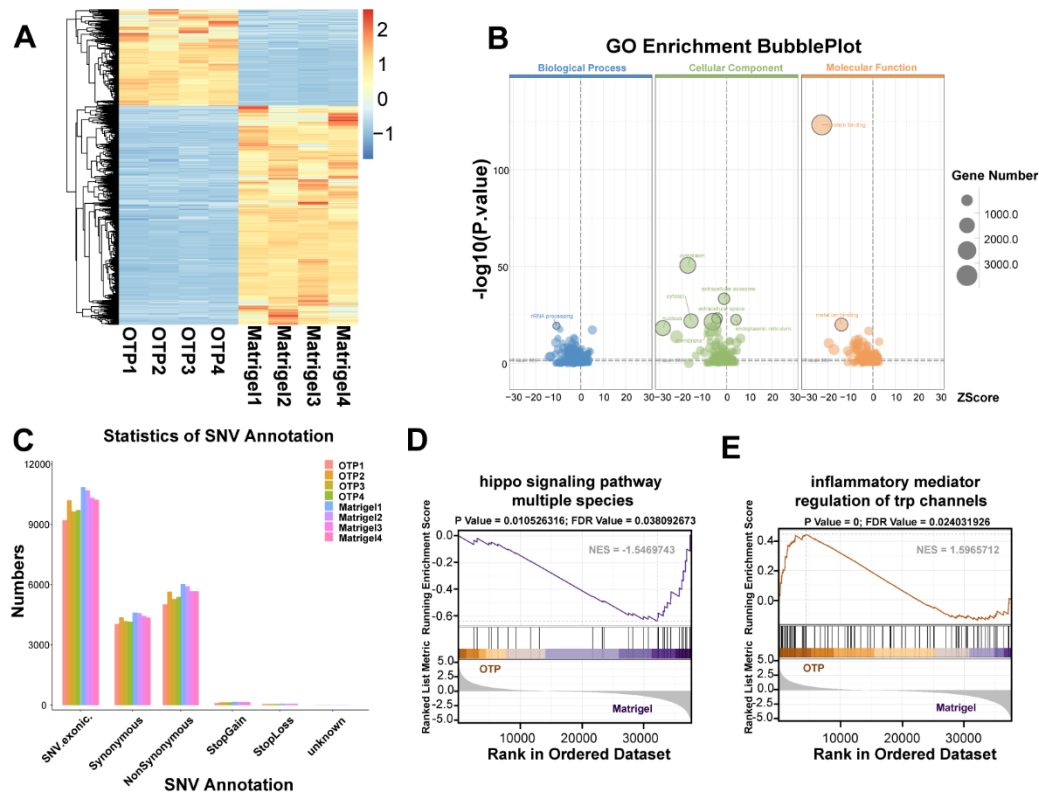


Figure S4. Supplementary transcriptomic analyses of OTP-associated dBDOs.

(A) Heatmap of differentially expressed genes between OTP-associated dBDOs (OTP1–OTP4) and Matrigel-cultured dBDOs (Matrigel1–Matrigel4). (B) Gene Ontology enrichment analysis of differentially expressed genes, highlighting remodeling of membrane-, cytoskeleton-, extracellular-, and metal ion-binding–related terms in OTP-associated dBDOs. (C) Statistics of single-nucleotide variant (SNV) annotation showing broadly comparable mutational category distributions between OTP-associated and Matrigel-cultured dBDOs. (D, E) Additional gene set enrichment analyses showing modulation of Hippo signaling and inflammatory mediator regulation of TRP channels in OTP-associated dBDOs.

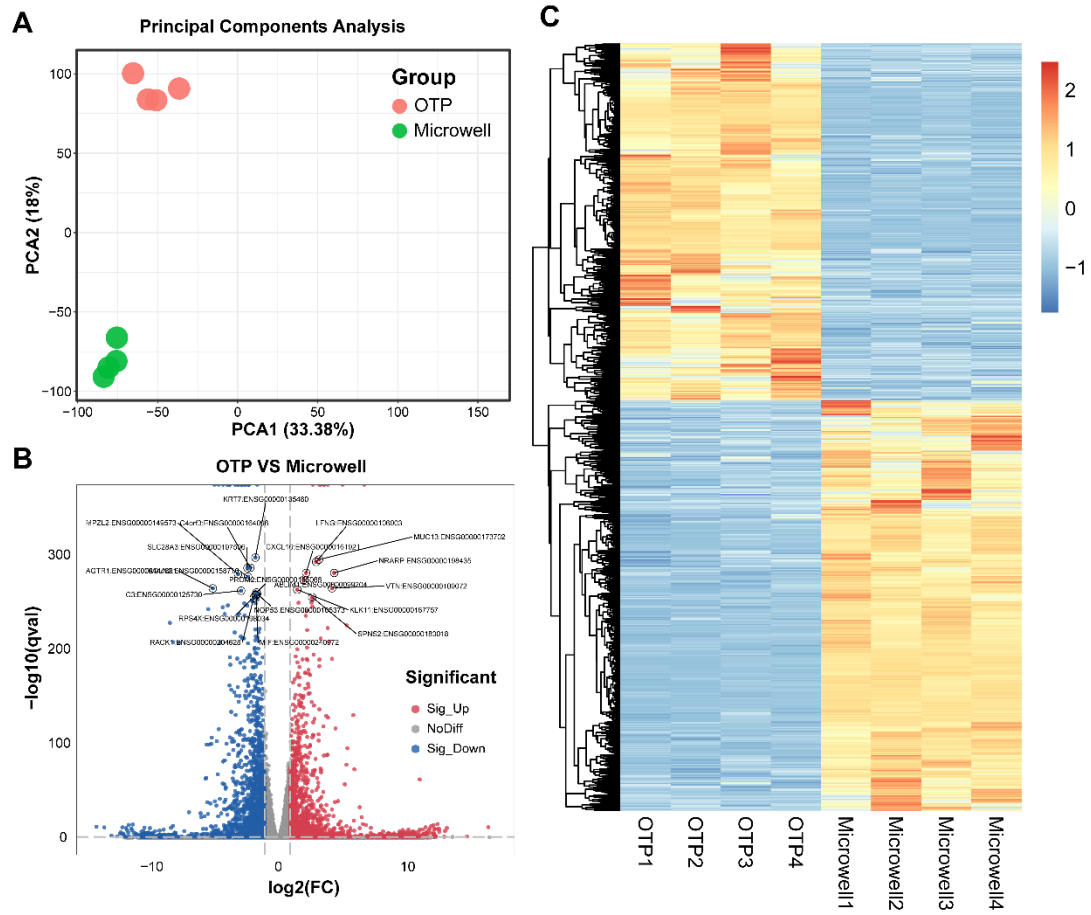
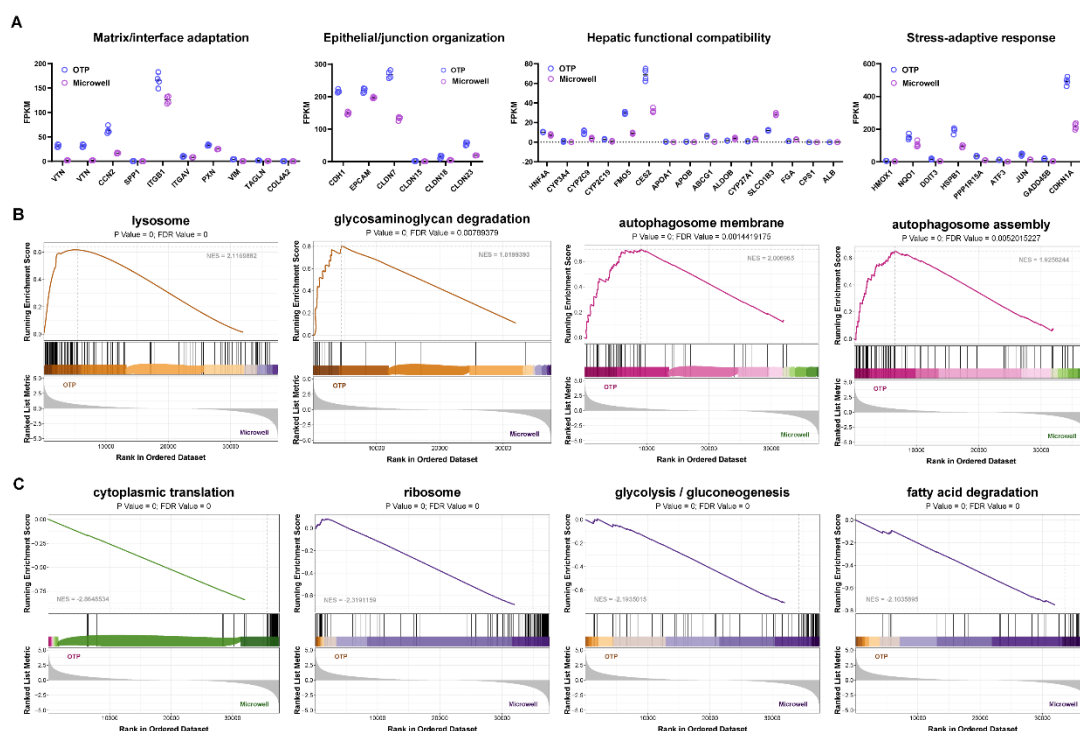


Figure S5. Scaffold association induces a transcriptional state distinct from microwell aggregation.

(A) PCA of OTP-associated dBDOs and PDMS microwell-cultured dBDOs. (B) Volcano plot showing differentially expressed genes between OTP-associated dBDOs and microwell-cultured dBDOs. (C) Heatmap of differentially expressed genes.



(A) Curated expression analysis of genes related to matrix/interface adaptation, epithelial organization, hepatocyte-associated features, and stress-adaptive remodeling. (B) GSEA showing positively enriched lysosome- and autophagy-related programs in OTP-associated dBDOs. (C) GSEA showing negatively enriched ribosome/translation and selected metabolic pathways. NES, normalized enrichment score.

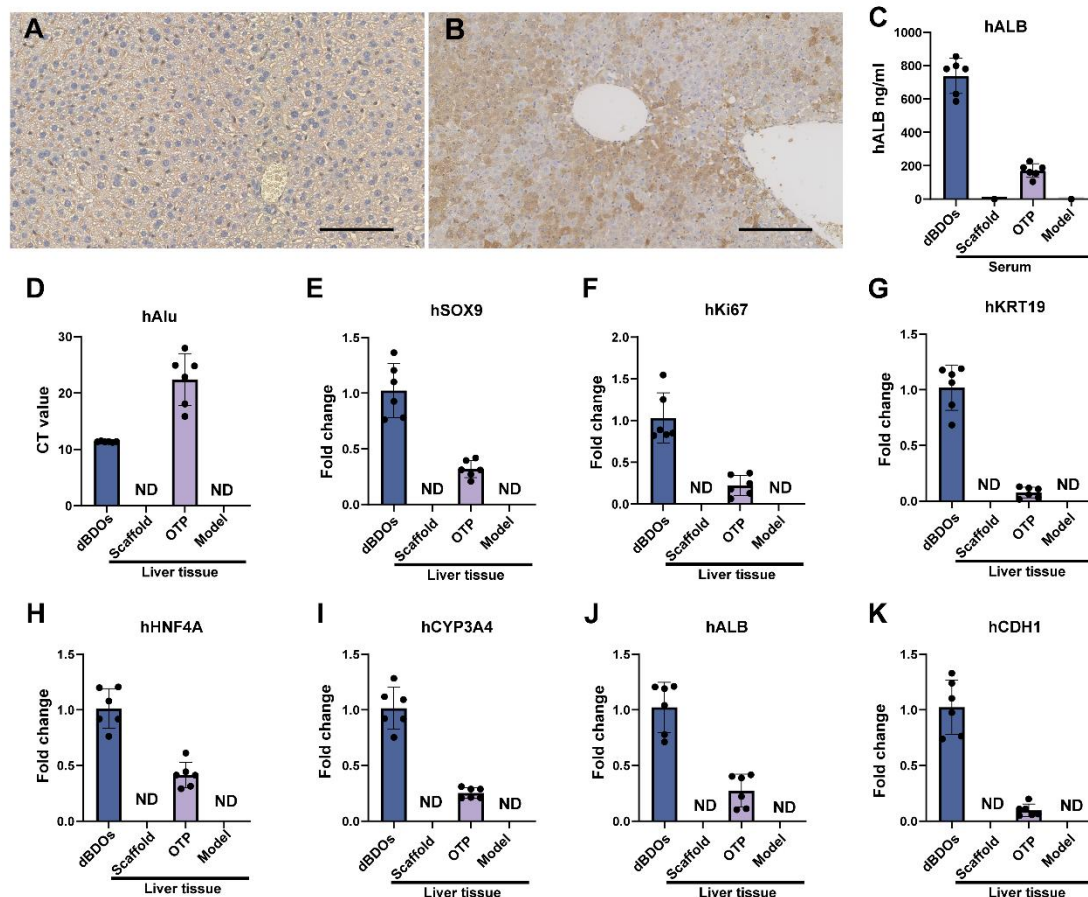


Figure S7. Donor-derived signals and human lineage-associated transcripts in OTP-treated mice at Day 14.

(A) Immunohistochemical staining of human albumin (hALB) in liver tissue from scaffold-only mice at Day 14, showing no evident hALB-positive signal. (B) hALB staining in liver tissue from OTP-treated mice at Day 14, showing sparse hALB-positive foci near the OTP placement region. Scale bar, 200 μ m. (C) Serum human albumin concentrations at Day 14 in the indicated groups; dBDO culture supernatant was included as an assay-positive reference. n = 6 per group. (D) Human-specific Alu DNA qPCR in liver tissue from the indicated groups. (E–K) Human-specific qPCR analysis of lineage- and state-associated transcripts in liver tissue from the indicated groups, including hSOX9 (E), hKi67 (F), hKRT19 (G), hHNF4A (H), hCYP3A4 (I), hALB (J), and hCDH1 (K). dBDOs served as a positive donor-cell reference. Scaffold-only and model liver tissues served as negative controls. n = 6 per group. ND, not detected.

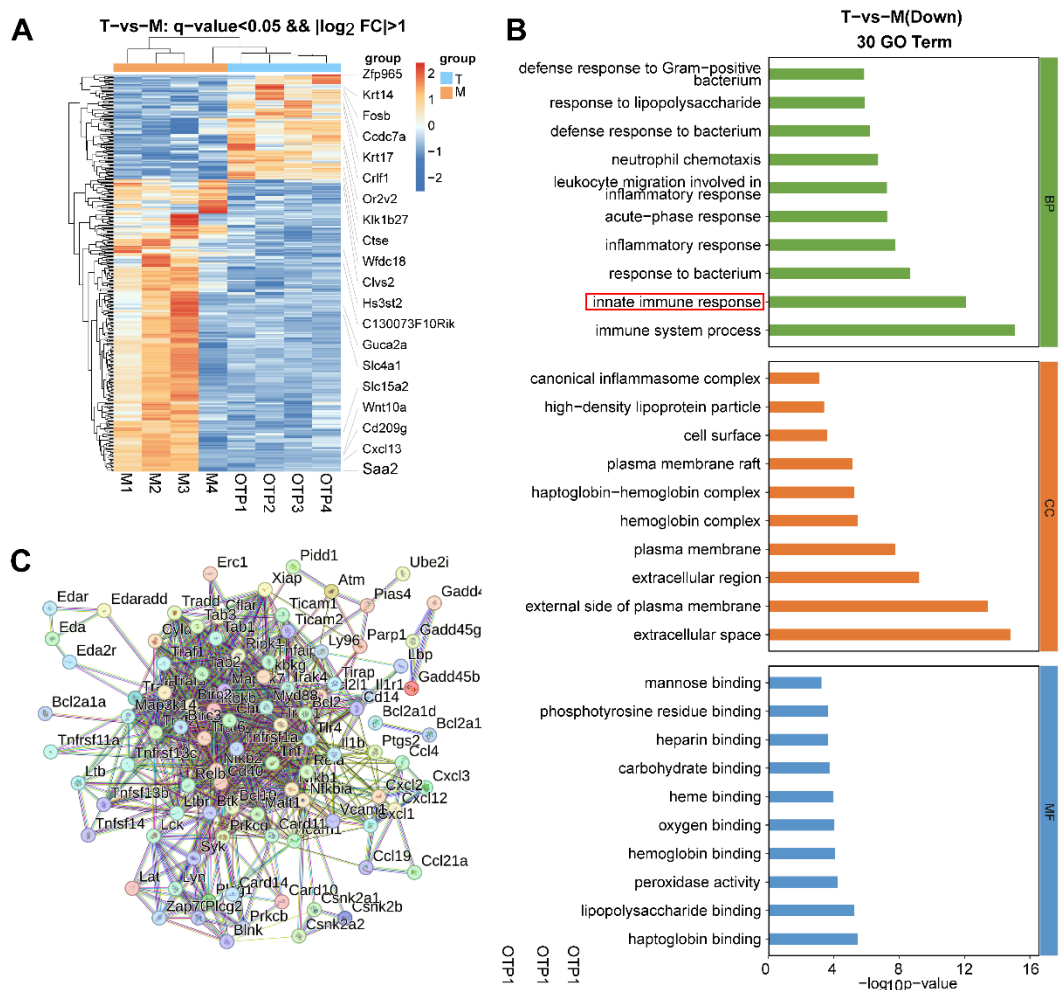


Figure S8. Supplementary bulk transcriptomic analyses show reduced inflammation-associated programs in OTP-treated tissue at Day 3.

(A) Heatmap of differentially expressed genes between OTP-treated and model tissue samples collected from the patch-placement region or the anatomically corresponding region at Day 3. (B) Gene Ontology enrichment analysis of genes downregulated in OTP-treated tissue, showing enrichment of innate immune response, inflammatory response, leukocyte migration, and related immune-associated terms. (C) Protein-protein interaction network of downregulated genes, highlighting interconnected immune-, cytokine-, and chemokine-related nodes.

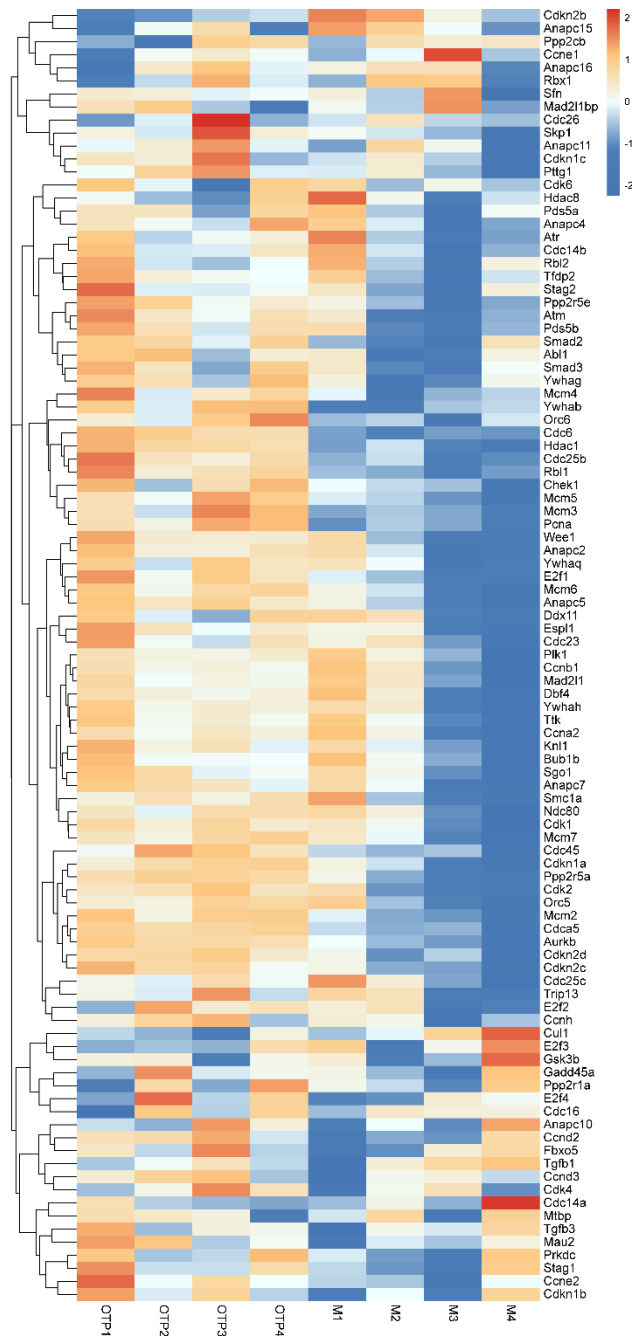


Figure S9. Supplementary heatmap of selected cell-cycle-associated transcripts in the patch-placement region of the remnant liver at Day 3. The expression of representative cell-cycle-associated transcripts in OTP-treated (OTP1–OTP4) and model (M1–M4) tissue samples collected from the patch-placement region or the anatomically corresponding region at Day 3 after hepatectomy, showing increased expression of selected cell-cycle-associated genes in OTP-treated samples.

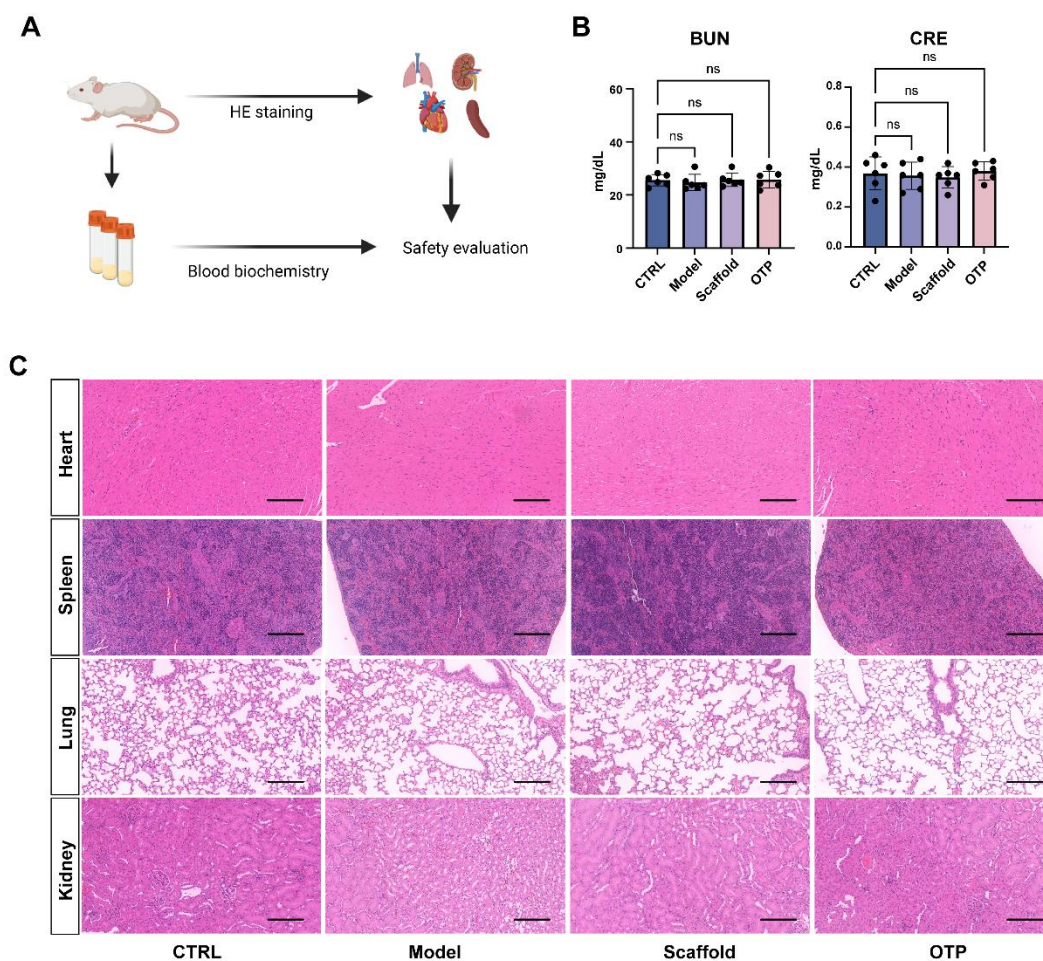


Figure S10. Fourteen-day tolerability assessment after OTP implantation.

(A) Schematic illustration of the in vivo tolerability evaluation protocol performed 14 days after OTP placement on the remnant liver surface in FRG mice. (B) Renal function markers BUN and creatinine (CRE) levels in Control, Model, Scaffold, and OTP groups at day 14. $n = 6$ per group. One-way ANOVA. Data are presented as mean $\pm$ SEM. ns, $P > 0.05$. (C) H&E staining of heart, spleen, lung, and kidney sections from mice in the Control, Model, Scaffold, and OTP groups on day 14. Scale bar, 100 μm . ns, not significant.

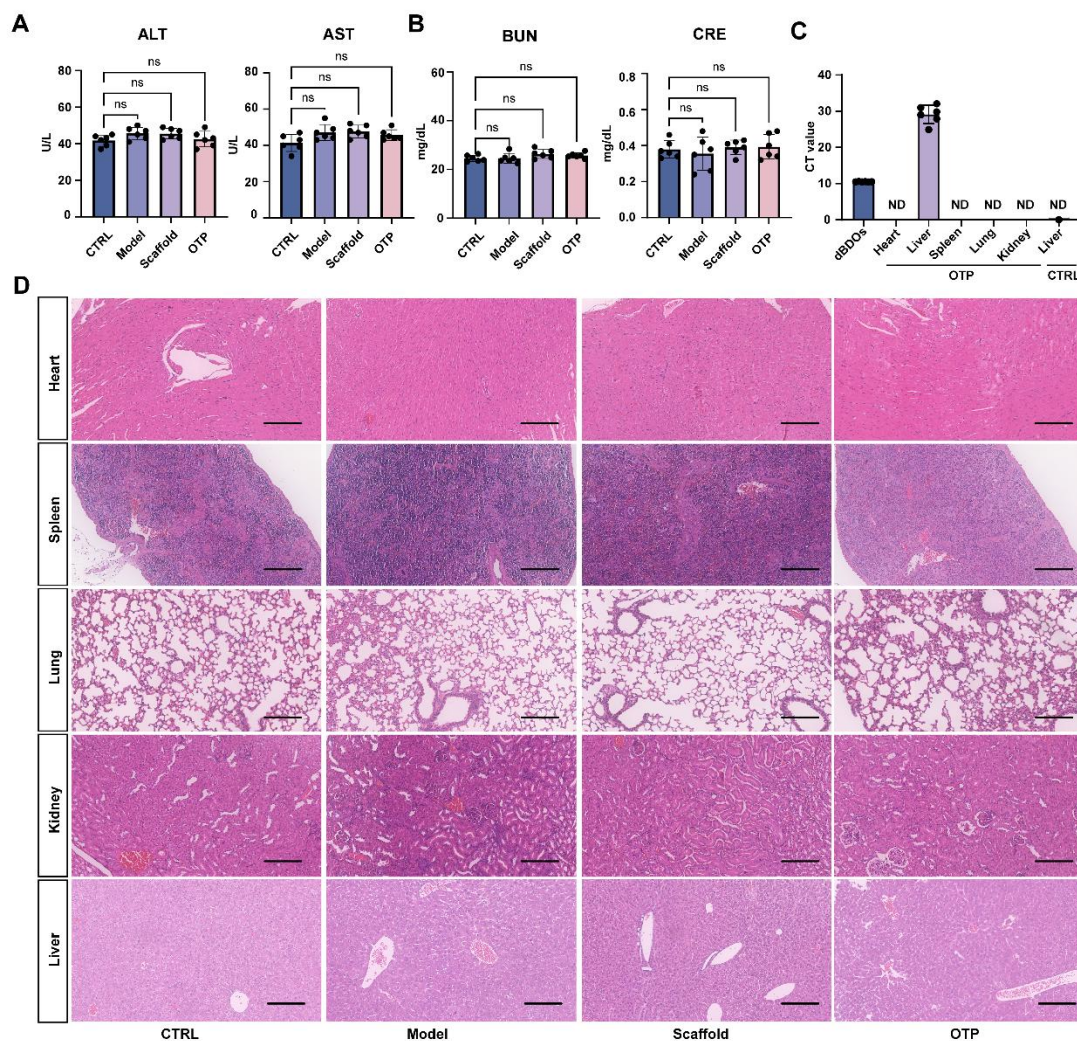


Figure S11. Two-month safety and biodistribution assessment after OTP implantation.

(A) Serum ALT and AST levels at 2 months after surgery in control, model, scaffold-only, and OTP-treated mice. $n = 6$ per group. One-way ANOVA. Data are presented as mean $\pm$ SEM. (B) Serum BUN and creatinine levels at 2 months after surgery in the indicated groups. $n = 6$ per group. One-way ANOVA. Data are presented as mean $\pm$ SEM. (C) Human-specific Alu DNA qPCR analysis of major organs at 2 months after OTP implantation. dbDOs served as a positive human donor-cell reference, and control mouse liver served as a negative control. Ct values are shown. ND, not detected. (D) Representative H&E staining of heart, spleen, lung, kidney and liver at 2 months after surgery in control, model, scaffold-only, and OTP-treated mice. No obvious histopathological abnormalities or ectopic tissue-like lesions were observed in the examined organs. Scale bar, 100 μ m. ns, not significant.