Pentosan polysulfate alleviates interstitial cystitis/bladder pain syndrome by modulating bile acid metabolism and activating the TGR5 receptor through gut microbiota regulation

Supplementary Files

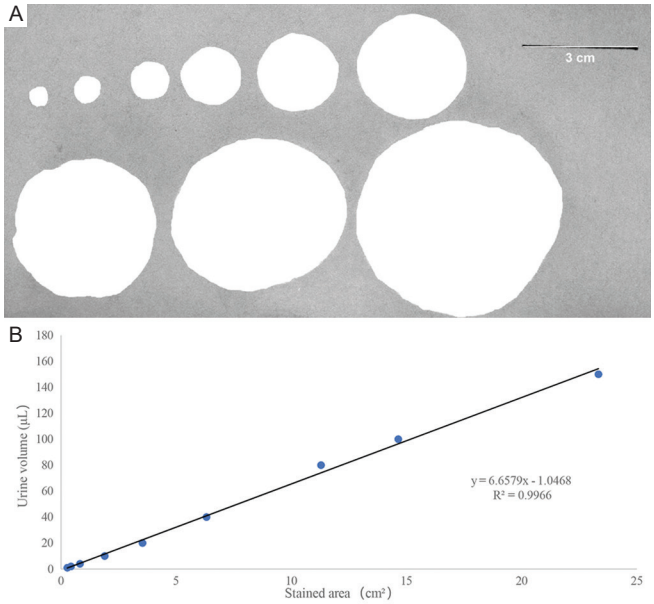

Figure S1. Standard curve of voiding spot on paper (VSOP). (A) Urine samples were collected to construct a standard curve and were dispensed onto filter paper in various volumes (1, 2, 4, 10, 20, 40, 80, 100, and 150 μL). (B) The formula y = 6.6579x-1.0468 (R^2 = 0.9966) was utilized to calculate individual void areas on the filter paper.

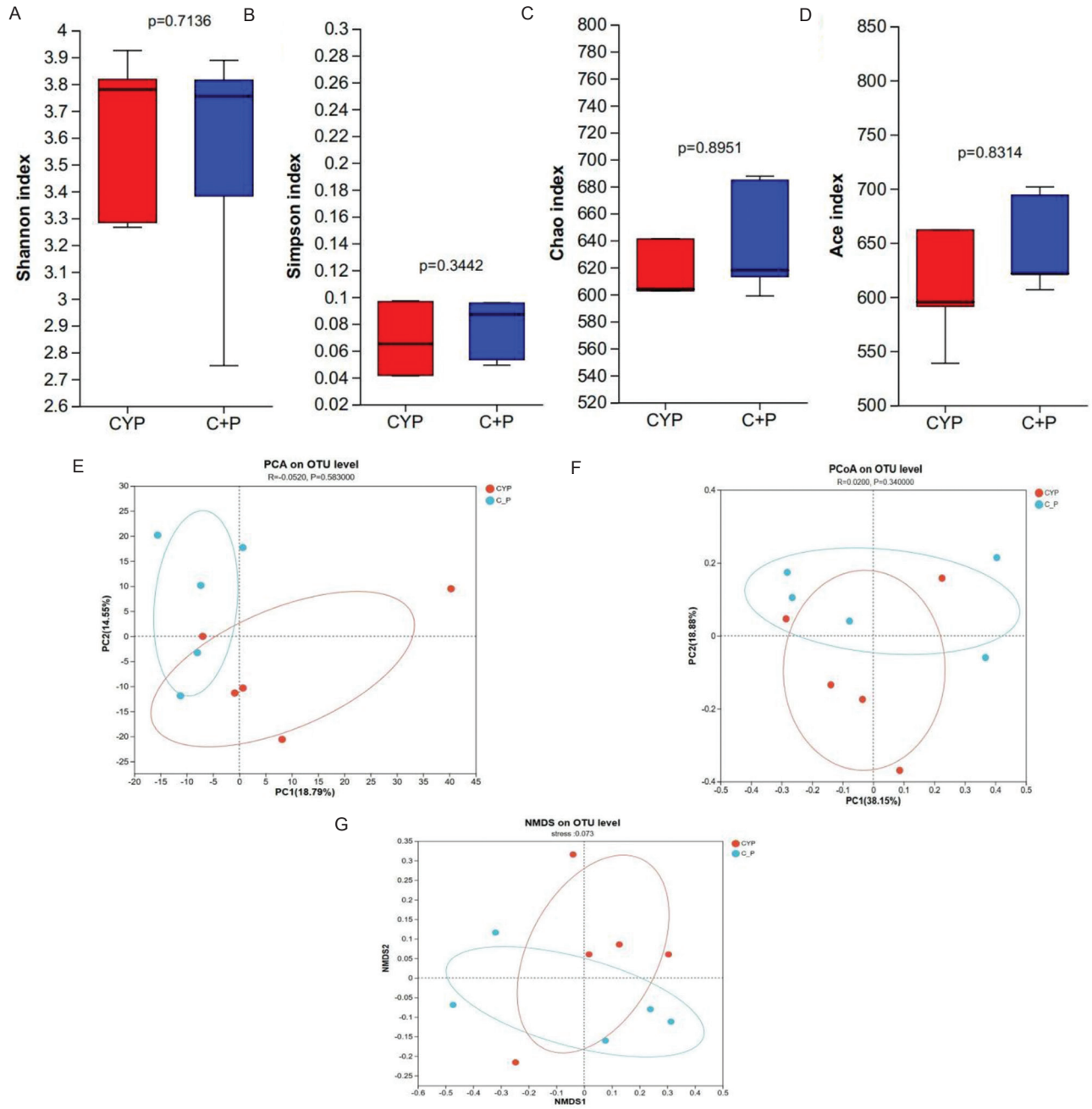

Figure S2. Analysis of microbial diversity through 16S rDNA sequencing. Microbial α-diversity was assessed using (A) Shannon index, (B) Simpson index, (C) Chao1 index, and (D) Ace index. Microbial β-diversity was evaluated through (E) principal component analysis (PCA), (F) principal coordinates analysis (PCoA), and (G) non-metric multidimensional scaling (NMDS).
Abbreviations: C + P: Cyclophosphamide + Pentosan polysulfate; CYP: Cyclophosphamide; OTU: Operational taxonomic unit.

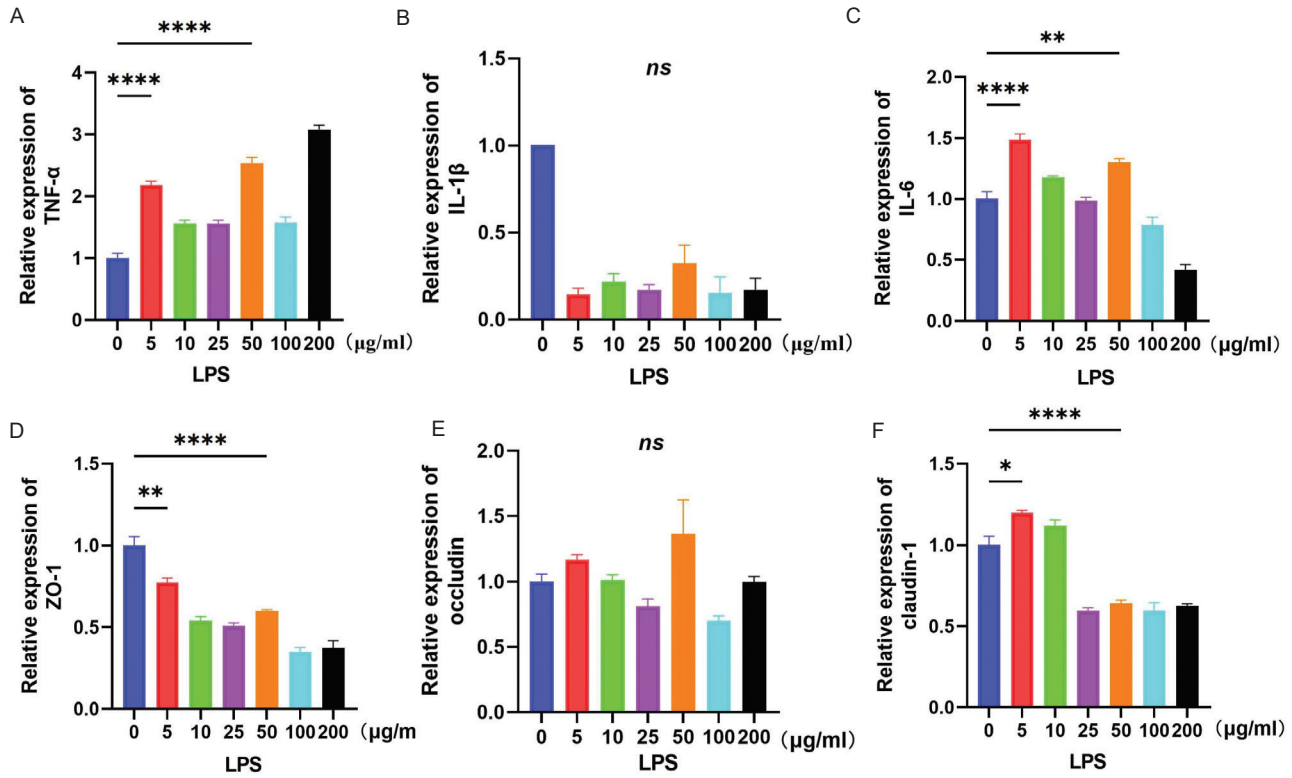

Figure S3. Successful establishment of an IC/BPS cell model in SV-HUC-1 cells induced by lipopolysaccharide. (A–C) Changes in mRNA levels of inflammatory cytokines TNF-α, IL-1β, and IL-6 after incubation with different concentrations of LPS for 24 h in SV-HUC-1 cells, n = 3. (D–F) Changes in mRNA levels of bladder epithelial barrier tight junction proteins ZO-1, occludin, and claudin-1 after incubation with different concentrations of LPS for 24 h in SV-HUC-1 cells, n = 3.

Note: Results are presented as mean ± SEM, with statistical significance indicated by $*p < 0.05$, $**p < 0.01$, $****p < 0.0001$ (one-way analysis of variance).

Abbreviations: LPS: Lipopolysaccharide; ns: Not significant.

Table S1. The primary sequences of primers for quantitative PCR in mouse

Mus-gene	Forward primer (5' – 3')	Reverse primer (5' – 3')
β-actin	AGAGCTACGAGCTGCCTGAC	AGCACTGTGTTGGCGTACAG
Tnfa	AGGGTCTGGGCCATAGAACT	CCACCACGCTCTTCTGTCTAC
Il6	CAGGCAGGCAGTATCACTCA	AGCTCATATGGGTCCGACAG
Zo1	AGAGACAAGATGTCCGCCAG	TGCAATTCCAAATCCAAACC
Claudin-1	GCCATCTACGAGGGACTGTG	CCCCAGCAGGATGCCAATTA
Occludin	ACTCCTCCAATGGCAAAGTG	CCCCACCTGTCGTGTAGTCT

Abbreviation: PCR: Polymerase chain reaction

Table S2. The primary sequences of primers for quantitative PCR in cell

Homo-gene	Forward primer (5' – 3')	Reverse primer (5' – 3')
GAPDH	GAGTCAACGGATTTGGTCGT	TTGATTTTGGAGGGATCTCG
TNFA	TCCTTCAGACACCCTCAACC	AGGCCCCAGTTTGAATTCTT
IL1B	GCTGAGGAAGATGCTGGTTC	TCCATATCCTGTCCCTGGAG
IL6	AGGAGACTTGCCTGGTGAAA	CAGGGGTGGTTATTGCATCT
ZO1	TGAGGCAGCTCACATAATGC	GGTCTCTGCTGGCTTGTTTC
Claudin-1	GCCGTTGGCATGAAGTGTATG	GCCAGTGAAGAGAGCCTGAC
Occludin	CCTTCACCCCCATCTGACTA	GCAGGTGCTCTTTTTGAAGG
GPBAR1	CTGCCTCCTCGTCTACTTGG	GTAGGGGGCTGGGAAGATAG
FXR	ATCAAAGGGGATGAGCTGTG	CAGCCAACATTCCCATCTCT
VDR	GACGCCCACCATAAGACCTA	AGATTGGAGAAGCTGGACGA
CAR	TAATGCGCTGACTTGTGAGG	TCATGCCAGCATCTAAGCAC
PXR	CAAGGCTACGCTGACAATCA	CAGGGCTACATTTCCCAAAA
GR	ATAGCATGGGAGCTGGATTG	CCATGTGTTTTCATGGCTTG
A5B1	ACTCAAGCAAAAGGGAGCAA	TGCAAGCCTGTTGTATCAGC

Abbreviation: PCR: Polymerase chain reaction

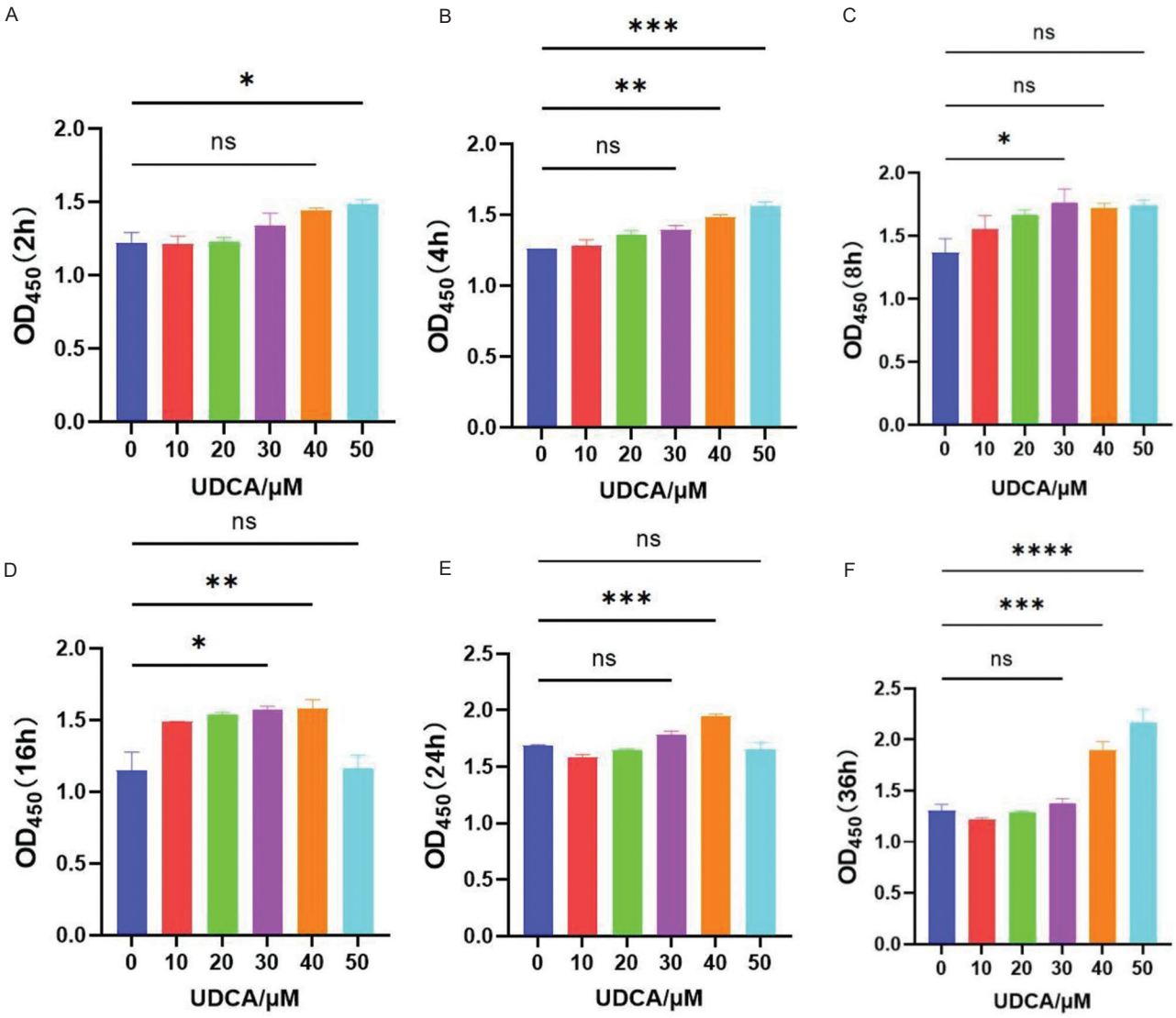

Figure S4. The toxic effects of ursodeoxycholic acid on SV-HUC-1 cells at different concentrations using CCK8. Optical density (OD) values of SV-HUC-1 cells treated with UDCA at concentrations of 10 μM, 20 μM, 30 μM, 40 μM, and 50μM for durations of (A) 2 h, (B) 4 h, (C) 8 h, (D) 16 h, (E) 24 h, and (F) 32 h.

Note: Results are presented as mean ± SE, with statistical significance indicated by *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001 (one-way analysis of variance).

Abbreviations: ns: Not significant; UDCA: Ursodeoxycholic acid.