

Figure S1 The establishment of gut-on-chips based on IBAC M1. (A) The morphology on day 4 and 10 after seeding above or beneath membrane (bar=200 μm); (B) Image tight junction Occludin (green), ZO-1 (orange) and nuclei (blue) of gut membrane on day 14 and 21 after seeding above membrane (10X) (bar=100 μm); (C) Tight junction ZO-1, Occludin and nuclei (blue) staining on day 14; (D) Transporter PGP and MRP2 distribution on day 21; (E) Signals of intestinal Villin (red), and DAPI (blue) in gut-on-chip on day 21.

Figure S2 Profile of 3D liver equivalents. (A-B) Diameter (A) and cell number (B) on day 4, 7, 10, and 14 (n=60); (C) Live or dead staining and change of dead/live signal area ratio; (D) HE and PAS staining of day 7 and 10; (E-F) Secretion of liver function biomarker ALB per organoid and ALB per cell (E) and injury biomarker AST, ALT, and LDH (F) on day 4, 7, 10, and 14 (n=3). (G) Immunofluorescence staining on day 5 about enzyme CYP3A4 (green), transporter MRP2 (yellow), PGP (red), and DAPI (blue). Data were shown in mean \pm SD. (bar=100 μm), *, $P < 0.05$, **, $P < 0.01$.

Figure S3 Detection of cell viability, and organic function after gut-liver platform integration. (A-C) The level of UREA (A), ALT (B), and LDH (C) in the liver chamber of gut-2D liver-on-chips and gut-3D liver-on-chips; Data are mean \pm SD, n=3. (D) Signals of intestinal Occludin (green), ZO-1 (orange), and DAPI (blue) in gut-on-chip, gut-2D liver-on-chip and gut-3D liver-on-chip on day 21 (bar=100 μm).

Figure S4 Expression of structure protein, enzyme, and transporters of intestinal equivalents and liver equivalents. (A) The expression of structure protein, enzyme, and transporters of intestinal equivalents in gut-on-chip (single) and gut-3D liver-on-chips (co-culture); (B) The expression of structure protein, enzyme, and transporters of liver equivalents in gut-3D liver-on-chips (co-culture), 3D liver-on-chips (single), and U-bottom plates (static). Data are in mean \pm SD, n=3. *, $P < 0.05$, **, $P < 0.01$.

Figure S5 Intestinal toxic effect after a single dose APAP administration for 48 h. Toxic determination at endpoint after a single dose administration. Rho123, Mito-Tracker, CellROX, and cleaved Caspase-3 signal was determined in the experiment. (bar=500 μm)

Figure S6 APAP toxicity in ABT pretreated spheroids. (A) Activity of CYP3A4 enzyme after ABT pretreated for 1 h; (B) Cell viability of APAP after ABT interfered; (C) Toxicity of APAP indicated by fluorescence signals after ABT interfered (bar=100 μm). n=3, data were shown in mean \pm SD.

In gut-liver-on-chips, ABT was pretreated for 1 h before APAP given to the co-cultured system (Figure 6A). Concentration of APAP in liver chamber was supervised and there was no significant difference in APAP exposure after ABT disturbed (Figure 6B). Even if no significant difference was observed in AST change fold after ABT disturbed (Figure 6C), ALT change fold and ALB levels were slightly ameliorated (Figure 6D-E)

after ABT pretreatment. Fluorescent staining suggests mitigated liver injury after ABT pretreated (Figure 6F). PI, CellROX and H2AFX signals were slightly decreased after ABT treatment in ABT+APAP i.v. group compared with APAP i.v. group, while ameliorated TMRE and cleaved caspase-3 signals can also be observed. The change fold of CellROX, TMRE and cleaved caspase-3 slightly decreased compared with that without ABT treatment (Figure 6G-I). These results indicate the ABT alleviates liver injury induced by APAP without relation to APAP concentration.

Figure S7 Change of inhibition with drug concentration in 2D HepG2, 3D liver spheroid, and 2D Caco-2. (A) Cyclosporine; (B) Emodin; (C) Berberine. n=3, data were shown in mean \pm SD.

Figure S8 Identify of emodin and its metabolites by LC-MS. (A-B) LC-MS detection of blank and emodin standard. (C) Emodin, emodin-*O*-glucuronide, and emodin-*O*-sulfate were eluted at 7.51, 5.46, and 6.48 min, respectively, in samples. (D-F) Mass spectra of emodin (D), emodin-*O*-glucuronide (E), and emodin-*O*-sulfate (F), respectively.

Figure S9 Influence of cyclosporine on oral administration of berberine. (A) Change of ALB level; (B) Change of TEER; (C) Level of berberine at the basolateral side of gut-liver-on-chips; (D) Level of berberine at the apical side of gut-liver-on-chips after 48 h. n=3, data were shown in mean \pm SD. *, $P < 0.05$

Figure S10 Identify of berberine by LC-MS. (A) LC-MS detection of berberine standard. Berberine was eluted at 4.97 min; (B) Mass spectra of berberine; (C) LC-MS detection of berberine in a sample.

Figure S11 Photos of the chip and the IBAC rocker. (A-C) Chip placed on the IBAC rocker; (D) Chamber and membrane layout

Table S1 UPLC gradient conditions for emodin, berberine, and metabolites analysis

Table S2 RT-qPCR primers

Table S3 Time-related APAP exposure in non-inoculate membrane, gut-on-chips, gut-liver-on-chips, and liver-on-chips (n=3)

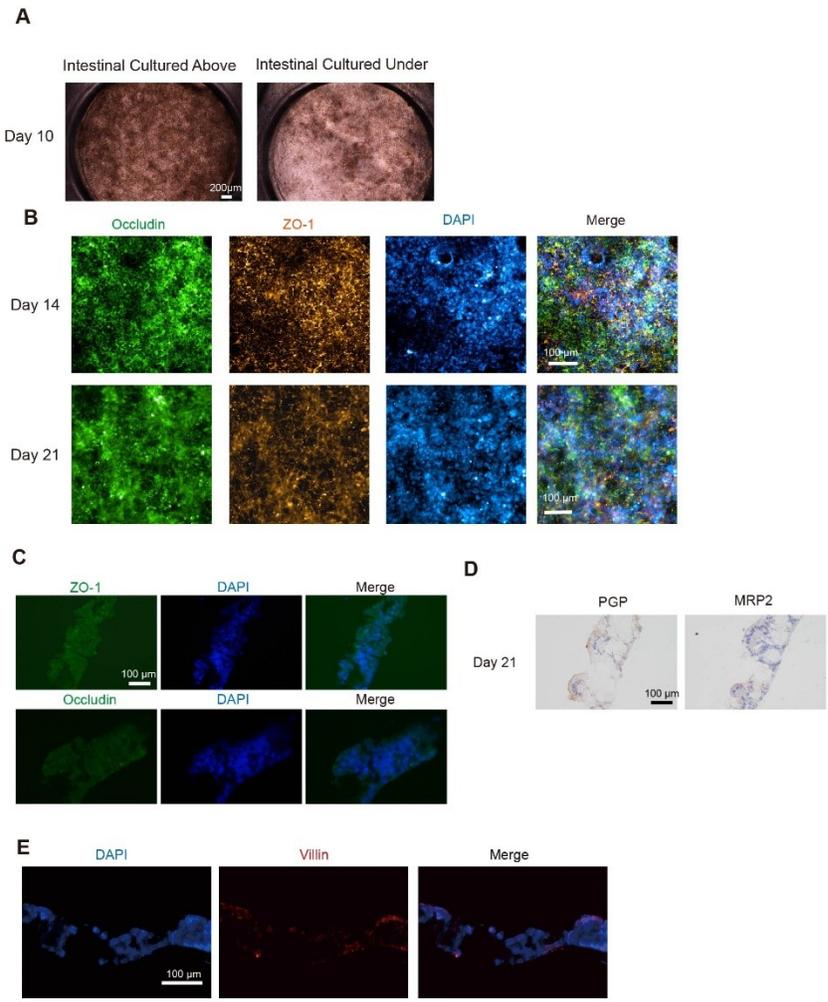


Figure s1

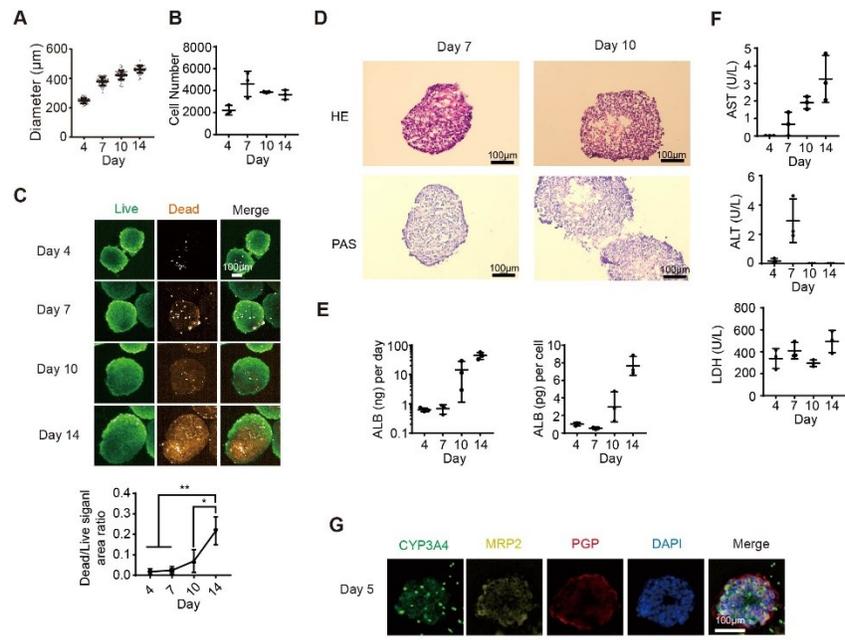


Figure s2

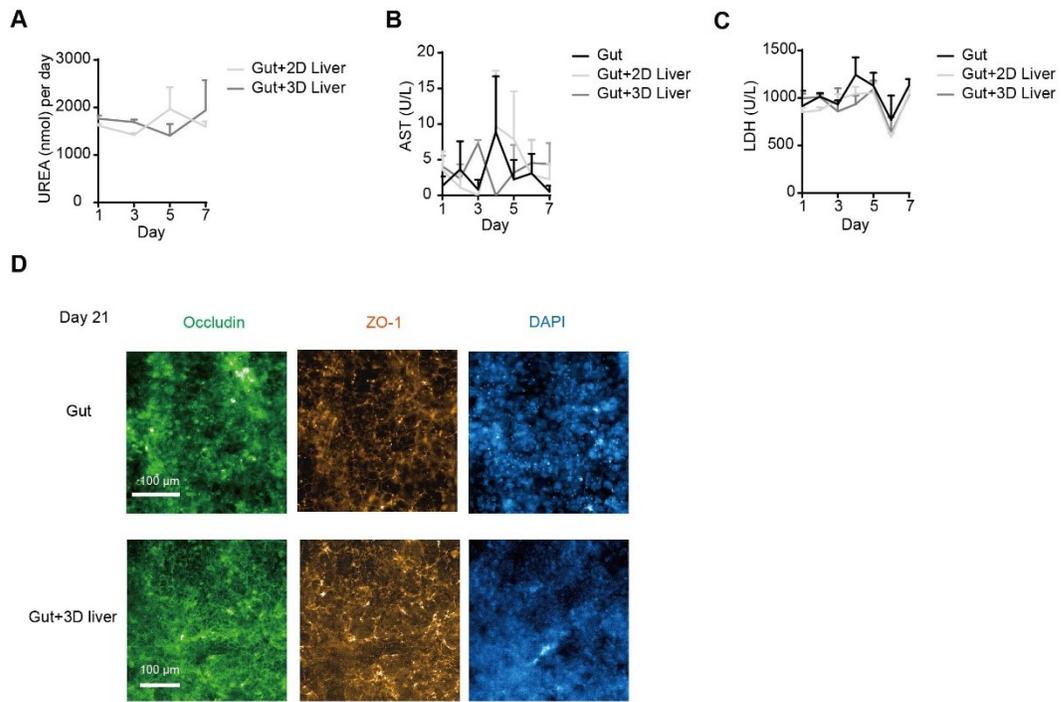


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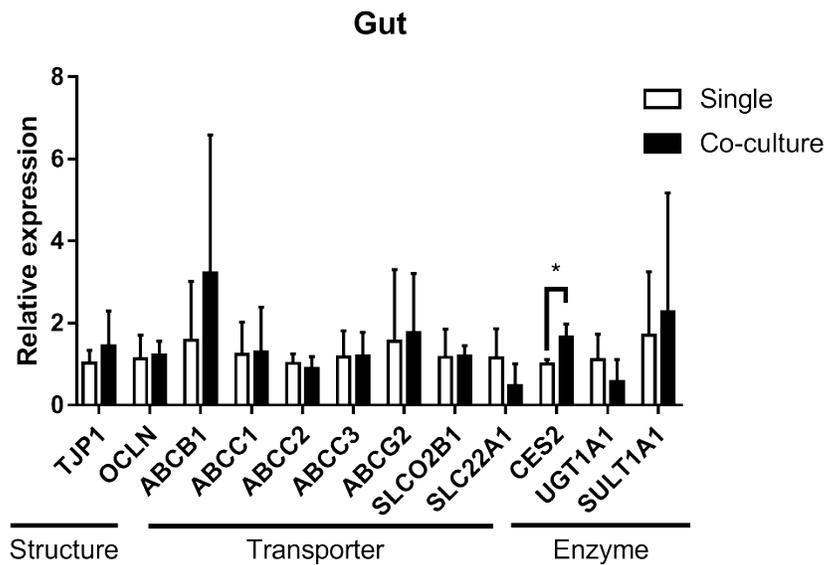
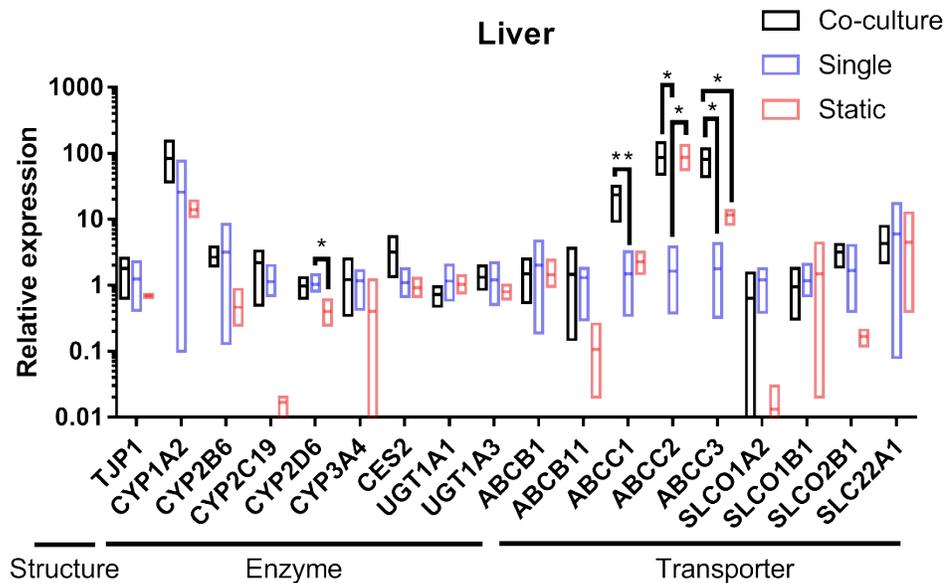
A**B**

Figure s4

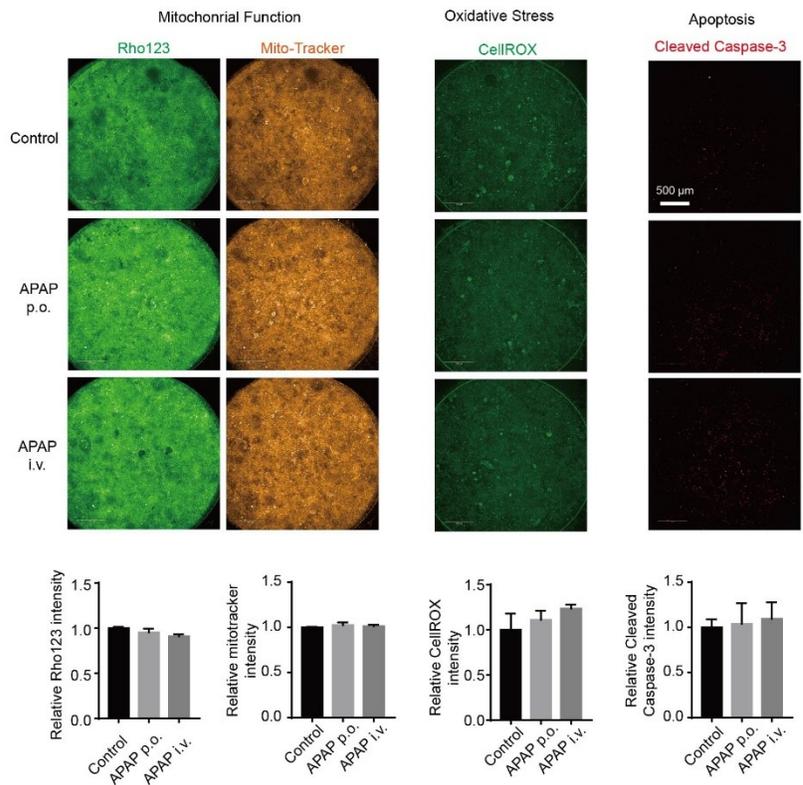


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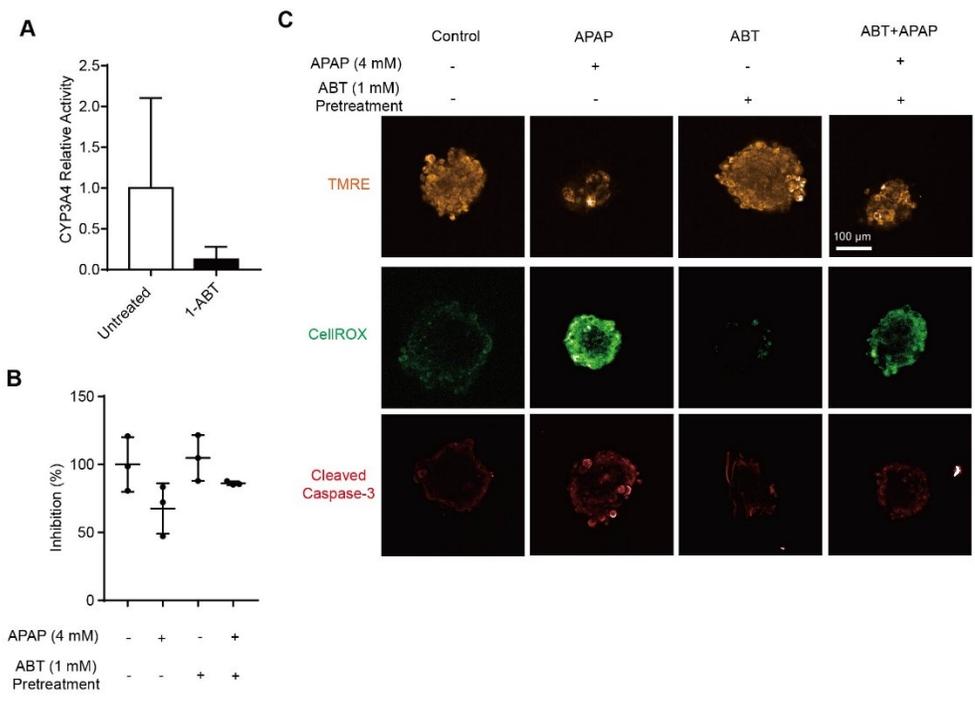


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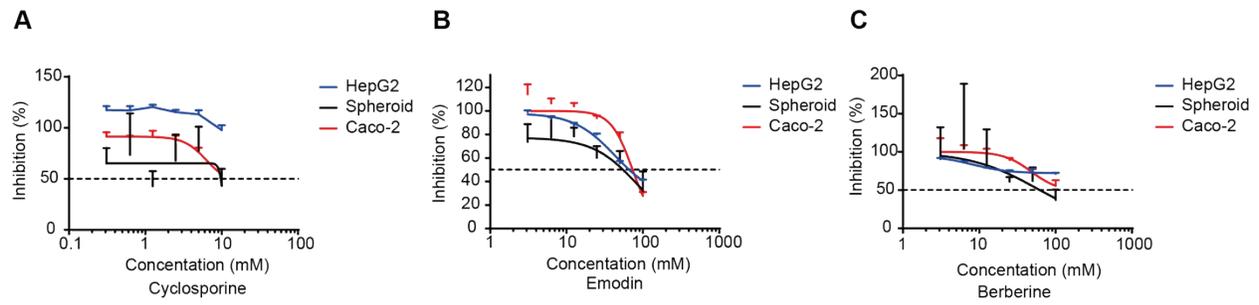


Figure s7

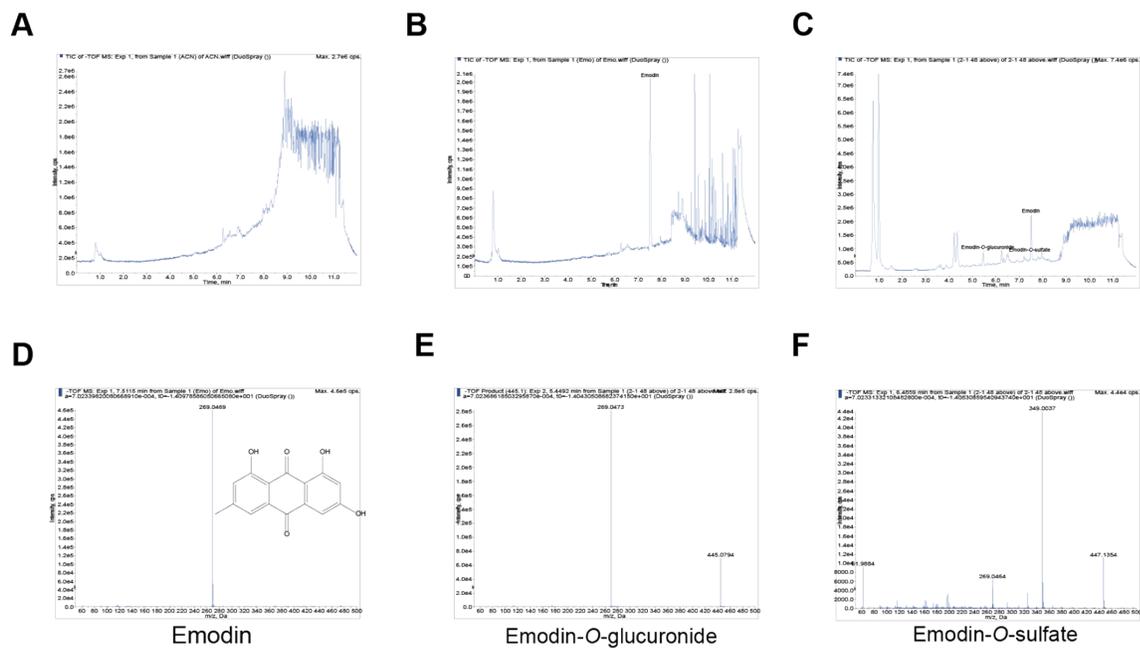


Figure s8

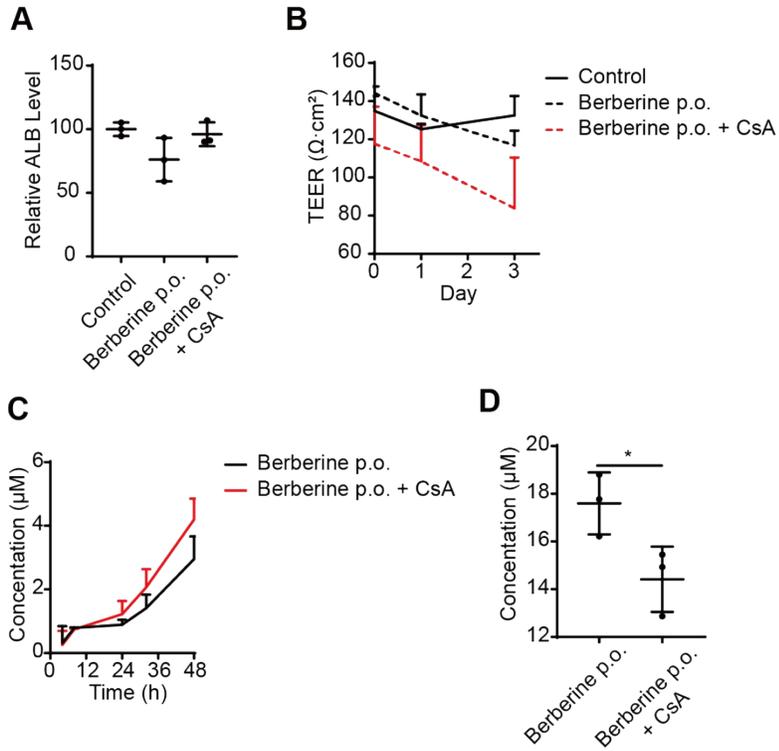


Figure s9

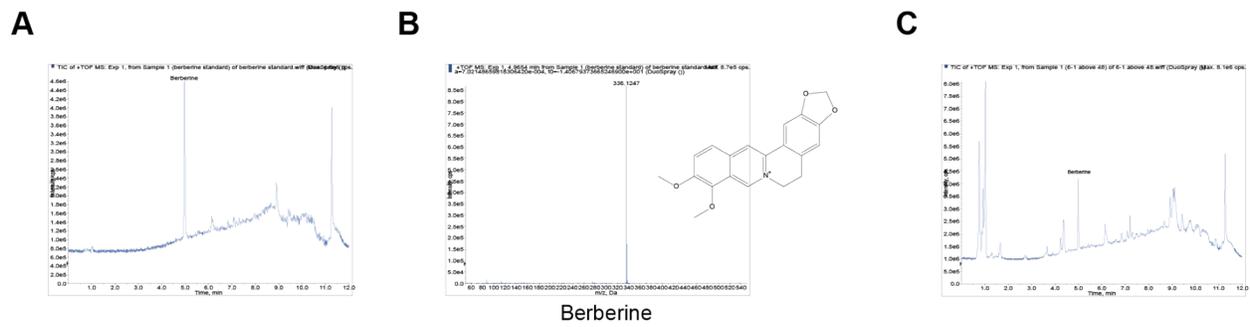
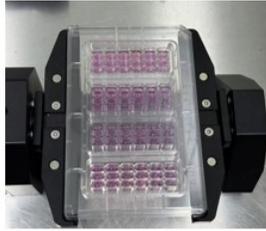


Figure s10

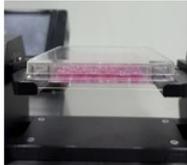
A



B



C



D

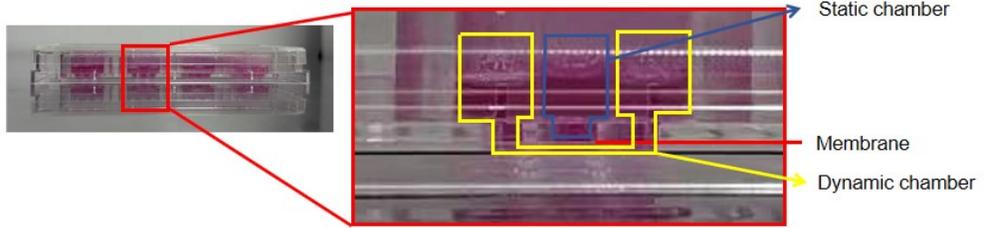


Figure s11