**Organoid extraction (water-soluble metabolites and lipids)**

The organoid samples were homogenized with Mixer Mill (Retsch) and ceramic beads at maximum frequency for 2 minutes to 4 minutes in pre-cooled racks after adding ice-cold methanol/$H_2O$ (4:1, v/v, 500 µL per 40 mg tissue) with internal standards (4 µM of lamivudine, D4-glutaric acid, and D8-phenylalanine, 16 µl Splash Lipidomix per 40 mg tissue). 500 µL of homogenate was then collected and extracted by applying 60 µL 0.2 M HCl, 200 µL chloroform, 200 µL chloroform, and 200 µL $H_2O$ consecutively with vortex. The extracts were spun down at 16000 g for 10 minutes, and the upper phase (water-soluble metabolites) was evaporated for 30 minutes at 35°C under nitrogen and dried in SpeedVac (Eppendorf) at 15 °C overnight. The lower phase (lipids) was evaporated to dryness at 45 °C under nitrogen. The interphase was used to determine the protein concentration with the BCA assay. Samples were stored at -80 °C.

**Culture medium extraction for water-soluble metabolites**

The water-soluble metabolites in the culture medium were extracted with RP18 SPE columns (Merck). Briefly, 50 µL medium was mixed with 50 µL $H_2O$ and 400 µL methanol/acetonitrile (5/3, v/v) containing internal standards (4 µM D4-glutaric acid, D8-phenylalanine), vortexed and ultrasound for 3 minutes. The supernatants were then filtered through the RP18 SPE columns (activated by elution of 1 mL acetonitrile and equilibrated by elution 1 mL methanol/acetonitrile/$H_2O$ (5/3/2, v/v/v) before usage) after centrifugation (5 minutes, 16000 g, 4 °C). The eluents were collected and mixed with 400 µL of methanol/acetonitrile/$H_2O$ (5/3/2, v/v/v). The mixtures were vortexed, ultrasound, centrifuged, and filtered as before and the eluent was collected and evaporated in SpeedVac overnight at 15 °C. Samples were stored at -80 °C.

**Culture medium extraction for lipids**

The lipids in the culture medium were extracted with methanol and chloroform. Briefly, 200 µL medium sample was mixed with 800 µL methanol containing internal standards (6 µL Splash Lipidomix). 120 µL 0.2 M HCl, 400 µL chloroform, 400 µL chloroform, and 400 µL $H_2O$ were added to the mix consecutively and vortexed. The lower phase of the spun-down samples was collected with a 200 µL micro syringe (Hamilton) and evaporated to dryness at 45 °C under nitrogen. Samples were stored at -80 °C.

**LC-MS analysis of water-soluble metabolites**

Water-soluble metabolites from organoid and culture medium samples were dissolved in 200 µl 5 mM ammonium acetate (in 75% acetonitrile (v/v)) before loading to LC/MS. LC-MS analysis was performed on an Ultimate 3000 HPLC system (Thermo Fisher Scientific) coupled

with a Q Exactive Plus MS (Thermo Fisher Scientific) in both ESI positive and negative mode. The analytical gradients were carried out using an Accucore 150-Amide-HILIC column (2.6 µm, 2.1 mm x 100 mm, Thermo Fisher Scientific) with solvent A (5 mM ammonium acetate in 5% acetonitrile) and solvent B (5 mM ammonium acetate in 95% acetonitrile). 3 µl sample was applied to the Amide- HILIC column at 30°C, and the analytical gradient lasted 20 minutes. During this time, 98% of solvent B was applied for 1 minute, followed by a linear decrease to 40% within 5 minutes and maintained for 13 minutes before returning to 98% in 1 minute and appended with a 5-minute equilibration step. The flow rate was maintained at 350 µL/min. The eluents were analyzed with MS in ESI positive/negative mode with ddMS2. The full scan at 70k resolution (69-1000 m/z scan range, 1e6 AGC-Target, 50 ms maximum Injection Time (maxIT)) was followed by a ddMS2 at 17.5k resolution (1e5 AGC target, 50 ms maxIT, 1 loop count, 0.1 s to 10 s apex trigger, 2e3 minimum AGC target, 20 s dynamic exclusion). The HESI source parameters were set as 30 sheath gas flow rate, 10 auxiliary gas flow rate, 0 sweep gas flow rate, spray voltage 3.6 kV (2.5 kV for negative mode), 320 °C capillary temperature, and the heater temperature of auxiliary gas was 120 °C. The annotation of the metabolites was performed using the El-Maven software (Elucidata, https://www.elucidata.io/el-maven) with an offset of ± 15ppm.

**LC-MS/MS analysis of lipids**

The lipids from the organoid and culture medium samples were dissolved in 100 µl of isopropylalcohol (iPrOH) before loading. The analytical gradients were carried out using an Accucore C8 column (2.6 µm, 2.1 mm x 50 mm, Thermo Fisher Scientific) with solvent A (acetonitrile/$H_2O$/formic acid (10/89.9/0.1, v/v/v)) and solvent B (acetonitrile/iPrOH/$H_2O$/formic acid (45/45/9.9/0.1, v/v/v/v)). 3 µl sample was applied to the C8 column at 40°C, and the analytical gradient lasted for 35 minutes. During this time, 20% of solvent B was applied for 2 minutes, followed by a linear increase to 99.5% within 5 minutes and maintained for 27 minutes before returning to 20% in 1 minute and appended with a 5-minute equilibration step. The flow rate was maintained at 350 µL/min. The full scan and ddMS2 parameters were the same as the analysis of the water-soluble metabolites, except the scan range were adjusted to 200-1000 m/z. The HESI source parameters were also adapted with a 3-sweep gas flow rate and a 3.2 kV spray voltage (3.0 kV for negative mode). Peaks corresponding to the calculated lipid masses (± 5 ppm) were integrated using El-Maven software.