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Supplemental Information

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with Inter-organoid Homogeneity
and Inter-patient Heterogeneity**

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Supplementary Information

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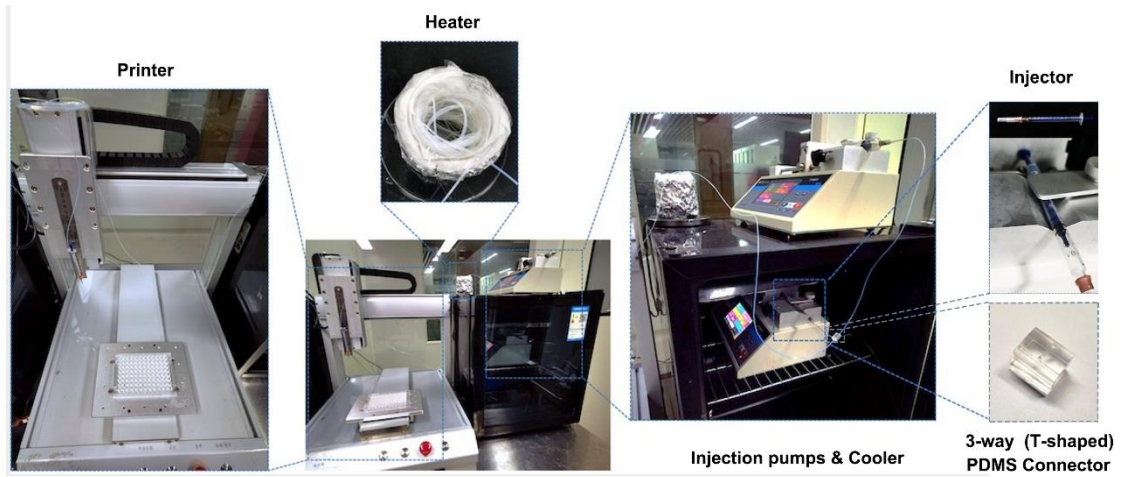


Figure S1. The automated organoid system setup. Related to Figure 1.

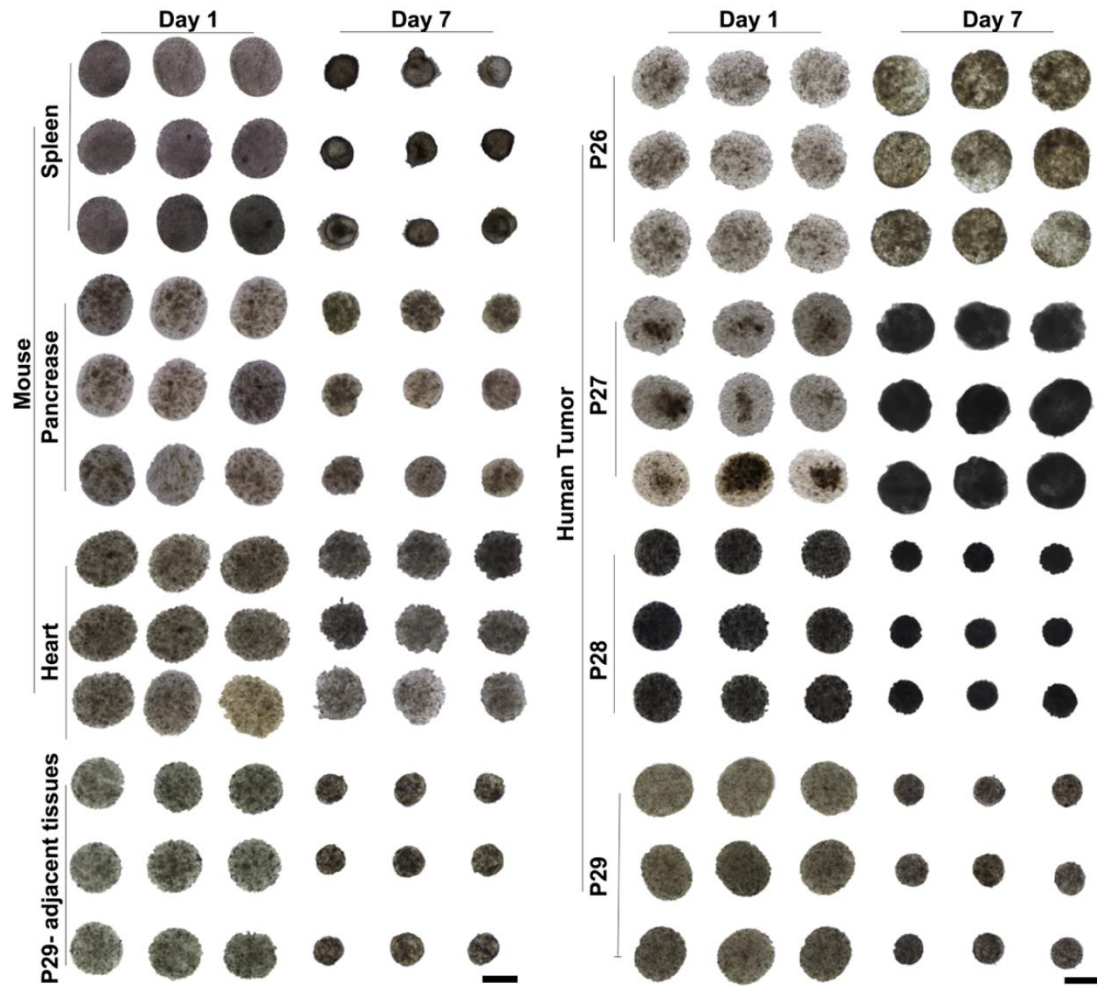


Figure S2. Additional organoid precursors and organoids from mouse organs and human tumors.

(A) Bright-field images of organoid precursors and organoids derived from mouse spleen, pancreas, heart and human tumor P29 adjacent (healthy) tissues acquired on day 1 and day 7; (B) Bright-field images of organoid precursors and organoids derived from human tumor P26, P27, P28, P29 acquired on day 1 and day 7. Scale bar in all panels: 400 μm . Related to Figure 2.

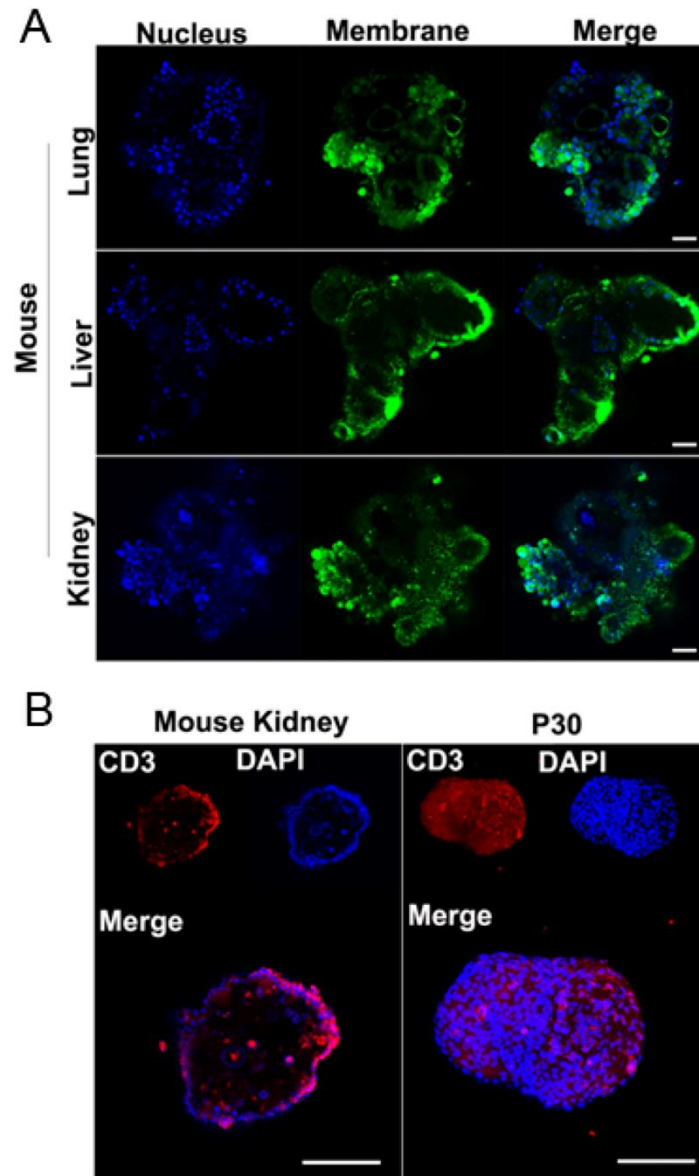


Figure S3. Organotypic constructs and immune microenvironment of multiple organoids. (A) Fluorescent images of organoids derived from mouse lung, liver and kidney, by staining the cell membrane with CMFDA and the nuclei with DAPI. Scale bar in all panels: 200 μm ; (B) Immunofluorescence staining for CD3 of the organoids derived from mouse kidney and P30 tumor, by using a Nikon A1R+ Laser scanning confocal microscopy. Scale bar in all panels: 300 μm . Related to Figure 3.

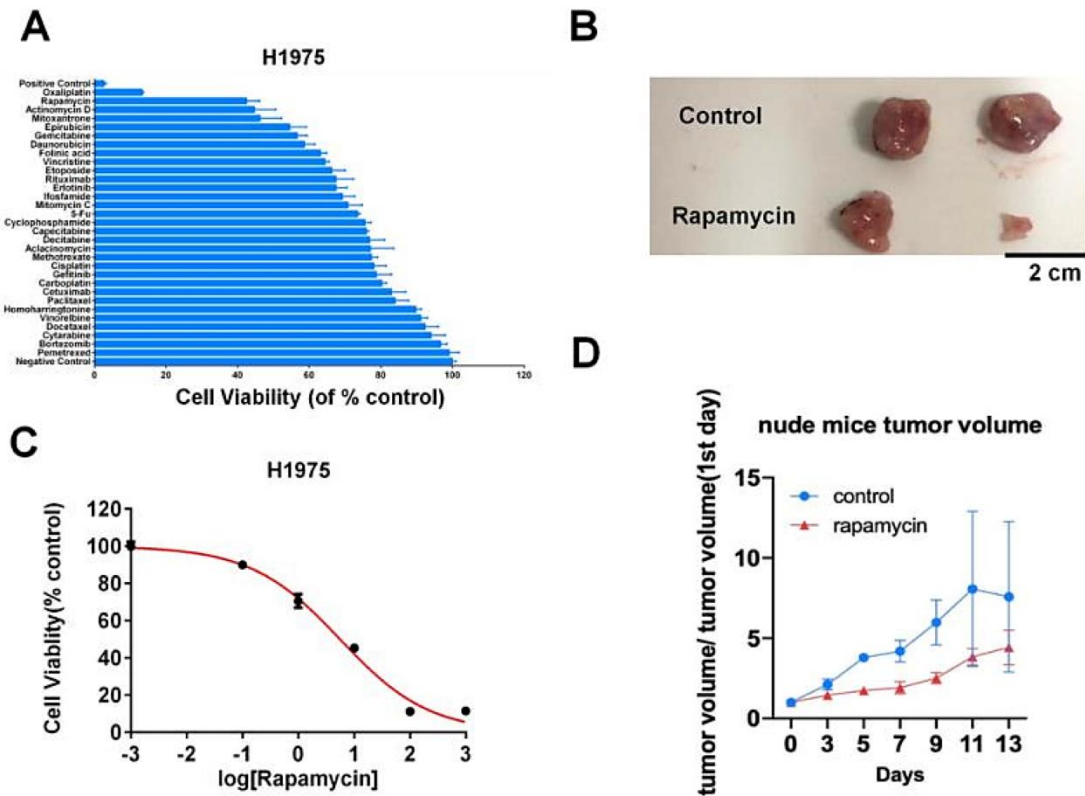


Figure S5. Validation of organoid screening using xenograft mice. (A) H1975 organoid cell viability quantification by conditioning organoids in single drugs at 10 μ M for 2 days ($n = 3$); (B) xenograft outcome by dosing the mice with Rapamycin for 13 days ($n = 2$), Scale bar, 2 cm; (C) the dose-response curve of H1975 organoid to Rapamycin for 2 days ($n = 3$); (D) the measure of tumor growth over 13 days by dosing the mice with Rapamycin at 2.0 mg/kg ($n = 2$). Related to Figure 5.

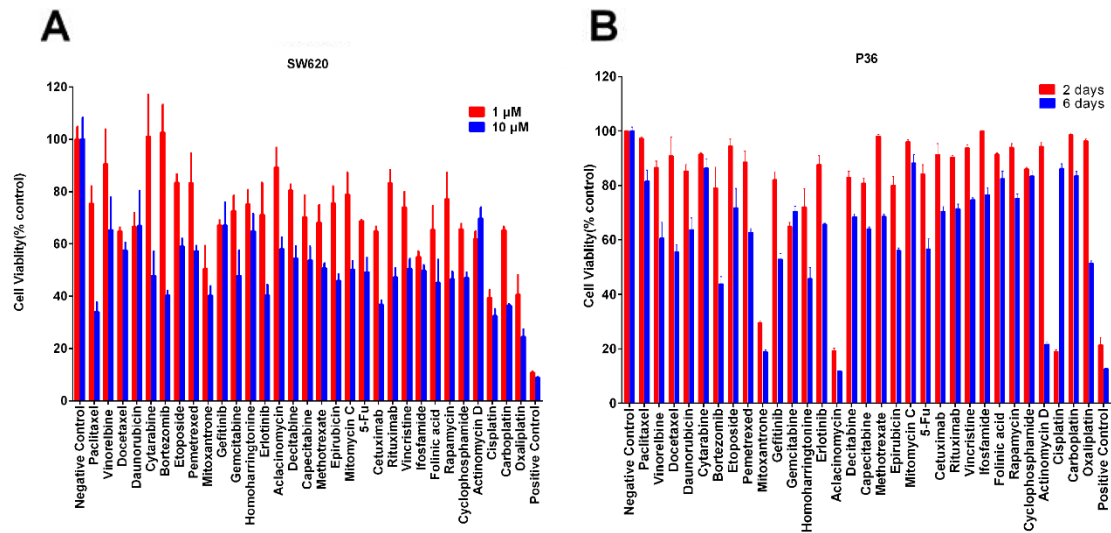


Figure S6. Drugs screening by organoids in different days and different concentration. (A)

Comparison of the drug screening outcome of P36 organoids (D7), evaluated after being cultured on drug conditions for 2 days and 6 days, respectively (n = 3). (B) Cell viability of SW620 organoids cultured in different single drugs at 1 μ M or 10 μ M for 2 days, as the prescreening for single drug recommendation.

The SW620 cells were initially loaded in Matrigel at 2×10^7 cells per mL (n = 3). Related to Figure 5.

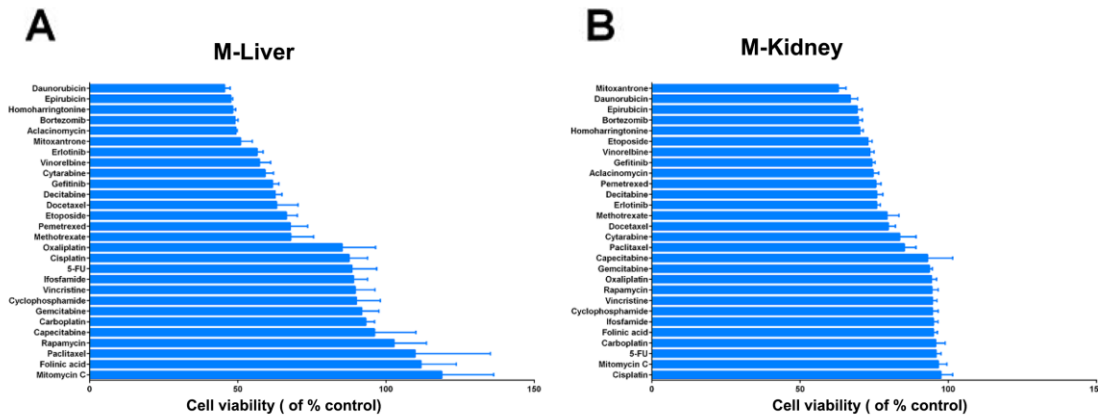


Figure S7. Detailed screening outcome of the anti-tumor drug hepatotoxicity and nephrotoxicity on mouse liver (A) and kidney (B) organoids (n = 4). Related to Figure 7.

Table S1. Comparison of traditional organoid using 96-well plate and a droplet organoid. Scale bar:

50 μm . Related to Figure 5.

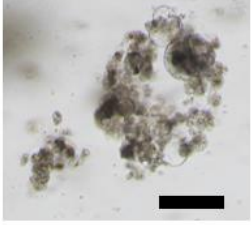
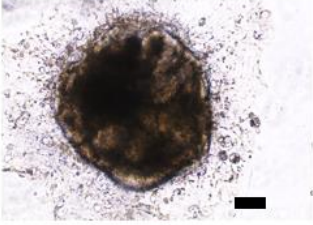
	Traditional organoids, 2 weeks	Droplet organoids, 1 week
Organoid portrait		
Matrigel use per well	30 μl	~ 0.1 μl

Table S2. Clinical characteristics of the patients in this study. Related to Figure 5 and 6.

	Histopathology	Tumor Stage	Clinical Treatment	Chemotherapy Cycle	Clinical Response
P1	Lung squamous carcinoma	T2aN0M0, Phase IB	No Chemotherapy	NA	NA
P2	hepatocellular carcinoma	T3N0M0, Phase IIIA	No Chemotherapy	NA	NA
P3	thymoma	T1N0M0, Phase I	No Chemotherapy	NA	NA
P4	Esophagus small cell carcinoma	T3N2M0, Phase IIIB	Etoposide	3	SD
P5	Gastric adenocarcinoma	T3N0M1, Phase IV	5-FU + Oxaliplatin	1	SD
P6	Gastric adenocarcinoma	T3N1M0, Phase IIB	Oxaliplatin	1	SD
P7	Esophageal squamous cell carcinoma	T3N2M0, Phase IIIB	Docetaxel	2	SD
P8	Gastric adenocarcinoma	T3N3aM0, Phase IIIB	Oxaliplatin	3	SD
P9	Colonic adenocarcinoma	T3N1M1b, Phase IVB	5-FU + Folinic acid + Oxaliplatin + Capecitabine	5	SD
P10	Colonic adenocarcinoma	T3N1M0, Phase IIIB	Oxaliplatin, Oxaliplatin + Capecitabine	2	SD
P11	Rectal adenocarcinoma	T3N0M0, Phase IIA	Capecitabine	2	SD
P12	Colonic adenocarcinoma	T3N2M0, Phase IIIB	5-FU + Oxaliplatin	2	SD
P13	Rectal adenocarcinoma	T3N2M0, Phase IIIB	5-FU + Oxaliplatin	1	SD
P14	Rectal adenocarcinoma	T3N0M0, Phase IIIB	Oxaliplatin + Capecitabine	4	SD
P15	Colonic adenocarcinoma	T3N0M0, Phase IIA	Oxaliplatin + Capecitabine	3	SD
P16	Colonic adenocarcinoma	T3N1M1, Phase IVB	Oxaliplatin + Capecitabine	3	SD
P17	Pulmonary adenocarcinoma	T2AN0M0, Phase IB	Cisplatin + Pemetrexed (disodium)	4	SD
P18	Rectal adenocarcinoma	T2N0M0, Phase I	Capecitabine + Oxaliplatin	4	SD
P19	Gastric adenocarcinoma	T3N2M1, Phase IV	Docetaxel	1	PD
P20	Hepatic adenocarcinoma	T1N0M0, Phase I	Gemcitabine	1	PD
P21	Colonic adenocarcinoma	T3N0M0, Phase IIA	Capecitabine + Oxaliplatin	3	SD

P22	Colonic adenocarcinoma	T3N2M0, Phase IIIB	Oxaliplatin	5	SD
P23	Rectal adenocarcinoma	T3N0M0, Phase IIA	Oxaliplatin + Capecitabine	3	SD
P24	Colonic adenocarcinoma	T3N0M0, Phase IIA	Oxaliplatin + Capecitabine	3	SD
P25	Gastric adenocarcinoma	T3N2M0, Phase IIIA	No Chemotherapy	NA	NA
P26	Urothelial carcinoma	T1N0M0, Phase I	No Chemotherapy	NA	NA
P27	Colonic adenocarcinoma	T3N0M0, Phase IIA	No Chemotherapy	NA	NA
P28	Pulmonary adenocarcinoma	T3N0M0, Phase IIB	No Chemotherapy	NA	NA
P29	Pulmonary adenocarcinoma	T2BN0M0, Phase IIA	No Chemotherapy	NA	NA
P30	Rectal adenocarcinoma	T3N1M0, Phase IIIB	No Chemotherapy	NA	NA
P31	Rectal adenocarcinoma	T3N0M0, Phase IIA	No Chemotherapy	NA	NA
P33	Rectal adenocarcinoma	T3N0M0, Phase I	No Chemotherapy	NA	NA
P34	Colonic adenocarcinoma	T3N0M0, Phase IIA	No Chemotherapy	NA	NA
P35	Colonic adenocarcinoma	T2N0M0, Phase I	No Chemotherapy	NA	NA
P36	Colonic adenocarcinoma	T3N1M0, Phase IIIB	No Chemotherapy	NA	NA
P37	Rectal adenocarcinoma	T3N0M0, Phase I	No Chemotherapy	NA	NA
P38	Rectal adenocarcinoma	T3N0M0, Phase I	No Chemotherapy	NA	NA