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Supplemental information

Personalized drug screening using patient-derived organoid and its clinical relevance in gastric cancer

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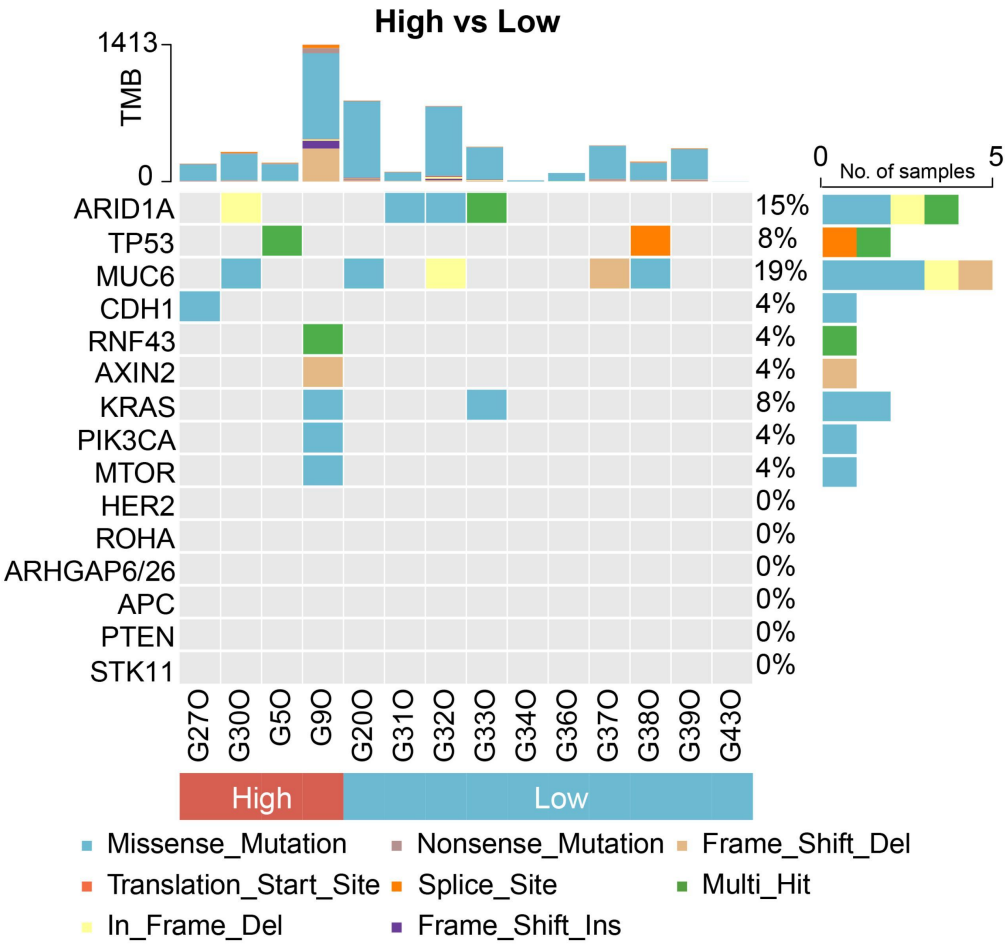


Figure S1. Establishment of patient-derived GC organoid biobank, related to Figure 1. The mutation landscape of high growth rate organoids (n=4) and low growth rate organoids (n=10).

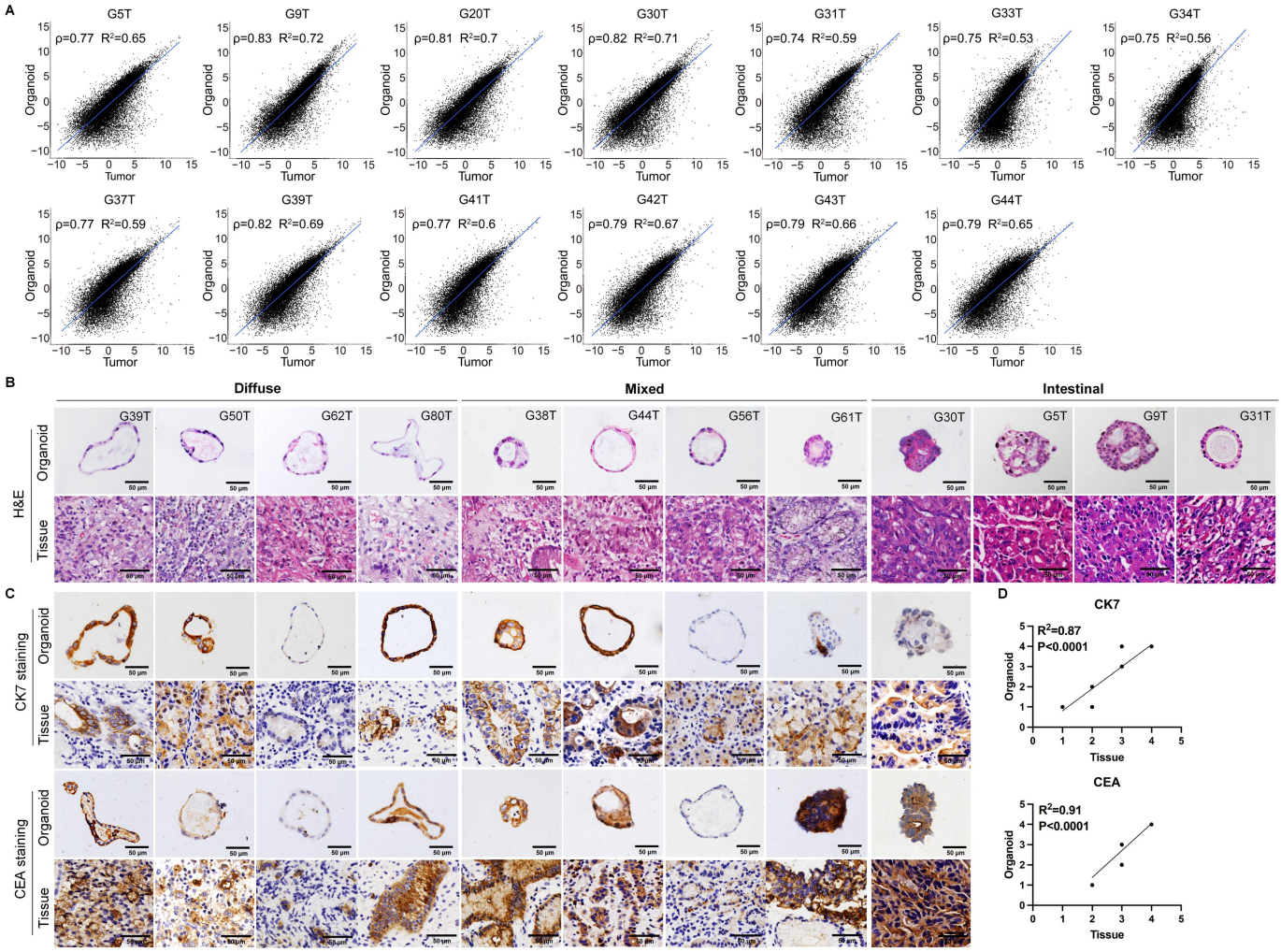


Figure S2. Transcriptome analysis and histopathologic characteristics between GC organoids and their derived primary GCs, related to Figure 2. (A) Comparison of the gene expression between tumor organoids and their corresponding paired tissue. Each dot represents the log2 FPKM value of a transcript. Correlation is indicated as Spearman correlation coefficient (ρ), R square (R^2). **(B)** Representative images of hematoxylin and eosin staining of GC organoids and matched primary GC tumor tissues. **(C)** Immunohistochemistry of GC protein markers CK7 and CEA in organoids and their corresponding primary GC tumor tissues. Scale bar, 50 μm. **(D)** Scatterplot showing the correlation of CK7 and CEA in organoids and tissue (n=17). Correlation is indicated as Pearson R^2 value.

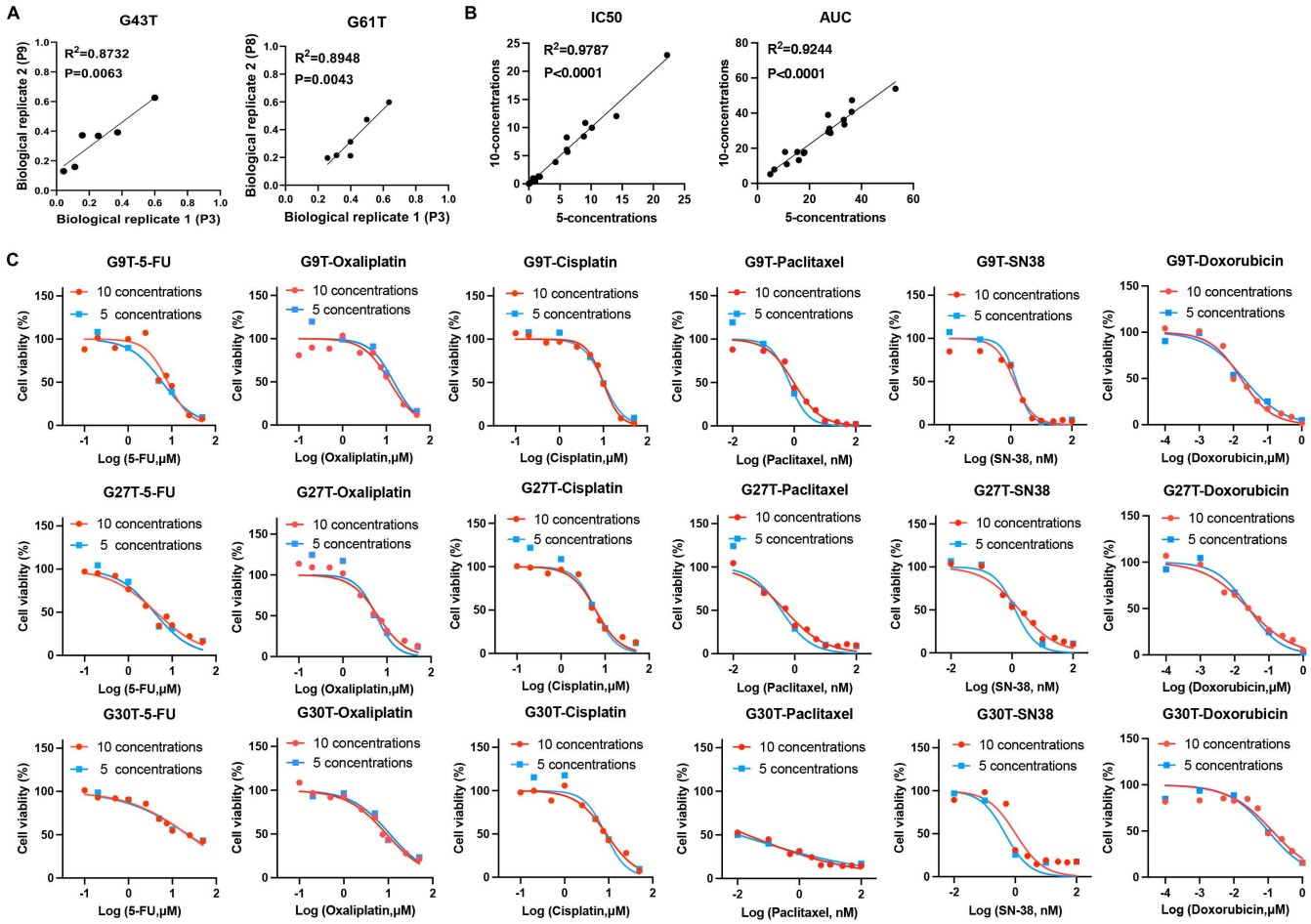


Figure S3. Drug screening of GC patient-derived organoids to chemotherapeutics, related to Figure 3. (A) Scatterplot showing the correlation between the 1-AUC values in two independent runs of drug screening (n = 3). Correlation is indicated as Pearson R^2 value. **(B)** Scatterplot showing the correlation between the 1-AUC values in two drug concentrations (10 concentrations and 5 concentrations) (n = 18). Correlation is indicated as Pearson R^2 value. **(C)** Dose response curves showing the chemosensitivity of 3 GC organoids to 6 conventional chemotherapeutics (5-FU, oxaliplatin, cisplatin, paclitaxel, SN-38, doxorubicin) in two drug concentrations (n = 3) (10 concentrations and 5 concentrations).

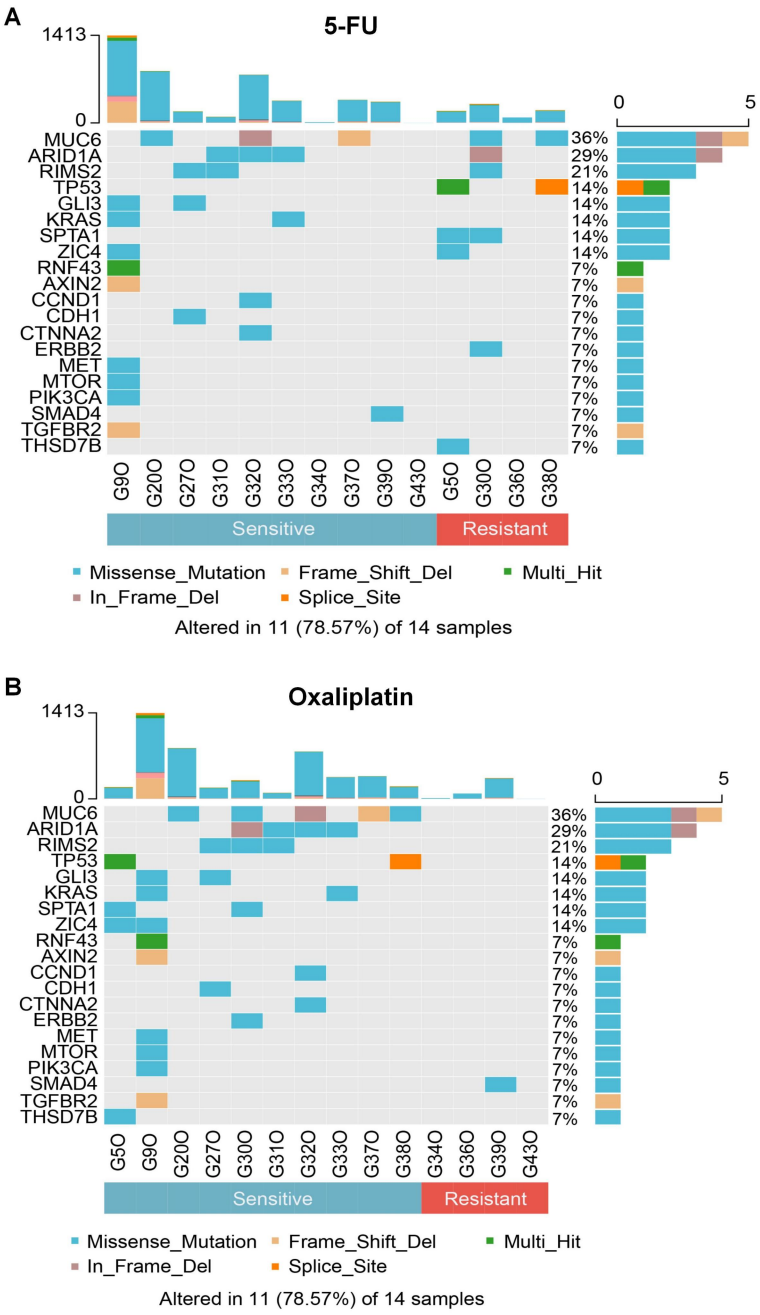


Figure S4. The mutation landscape of GC patient-derived organoids in response to chemotherapeutics, related to Figure 4. (A) The mutation landscape of 5-FU sensitive organoids (n = 10) and 5-FU resistant organoids (n = 4). The top 20 most frequently occurred mutations are displayed. **(B)** The mutation landscape of oxaliplatin sensitive organoids (n = 10) and oxaliplatin resistant organoids (n = 4).

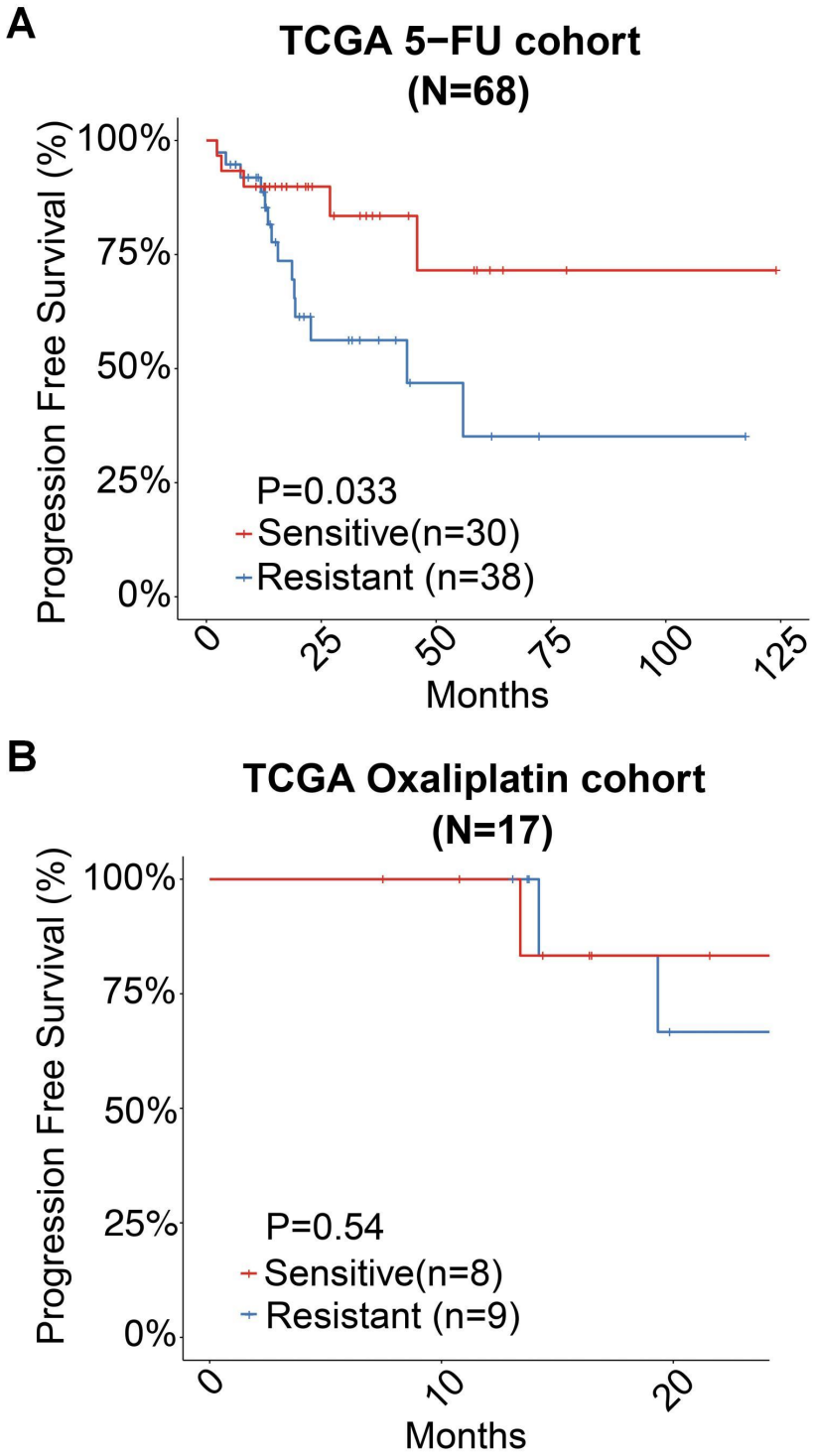


Figure S5. Gene expression biomarkers to distinguish sensitive and resistant responses of GC patients chemosensitivity, related to Figure 5. (A) Progression free survival rate based on the gene signature of 5-FU in TCGA cohort (n = 68). **(B)** Progression free survival rate based on the gene signature of oxaliplatin in TCGA cohort (n = 17).

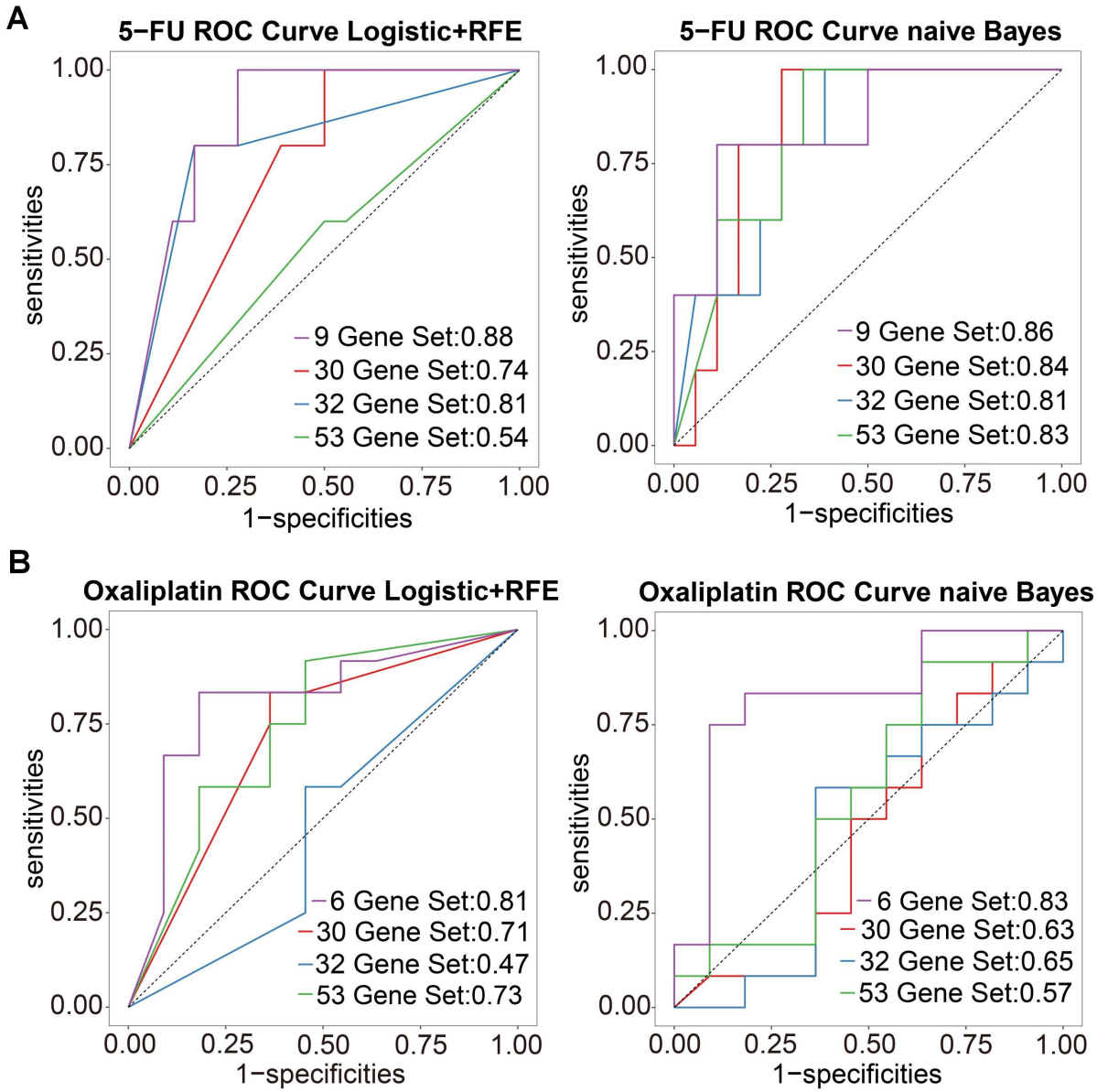


Figure S6. Gene expression biomarkers to distinguish sensitive and resistant responses of GC patients chemosensitivity, related to Figure 5. (A-B) ROC analysis to discriminate our 5-FU and oxaliplatin gene expression biomarker panel with other panels from published studies, such as 30-gene signature, 53-gene signature, 32-gene signature in our dataset based on the logistic model (left) or the naive Bayes model (right), respectively.

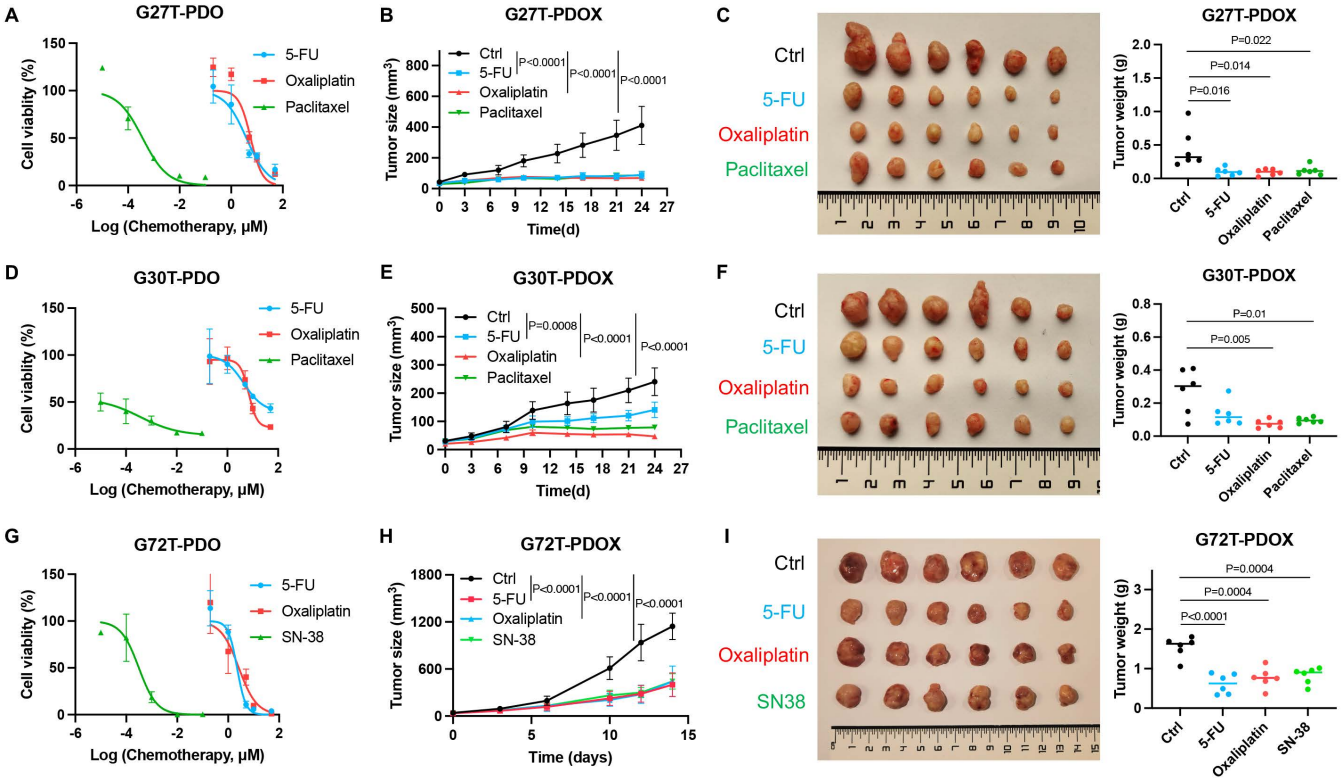


Figure S7. Drug response status observed in GC patient-derived organoids is validated in PDOXs in mice, related to Figure 6. (A) Dose-response curves of 5-FU (AUC = 28.71%, IC50 = 3.89 μM), oxaliplatin (AUC = 29.79%, IC50 = 7.26 μM) and paclitaxel (AUC = 10.89%， IC50 = 0.38 nM) in G27T-PDO in vitro (n = 3). **(B)** Mean tumor size (mm^3) of G27T-PDOX with 5-FU, oxaliplatin, paclitaxel, or vehicle control over time (n = 6 per group) (mean ± SEM). **(C)** Images of G27T-PDOX tumors and tumor weight at sacrifice. **(D)** Dose-response curves of 5-FU (AUC = 53.91%, IC50 = 17.15 μM), oxaliplatin (AUC = 40.78%, IC50 = 10.83 μM) and paclitaxel (AUC = 17.86%， IC50 = 0.02 nM) in G30T-PDO in vitro (n = 3). **(E)** Mean tumor size (mm^3) of G30T-PDOX with 5-FU, oxaliplatin, paclitaxel, or vehicle control over time (n = 6 per group) (mean ± SEM). **(F)** Images of G30T-PDOX tumors and tumor weight at sacrifice. **(G)** Dose-response curves of 5-FU (AUC = 9.70%, IC50 = 1.77 μM), oxaliplatin (AUC = 12.45%, IC50 = 2.42 μM) and SN-38 (AUC = 1.52%, IC50 = 0.16 nM) in G72T-PDO in vitro (n = 3). **(H)** Mean tumor size (mm^3) of G72T-PDOX with 5-FU, oxaliplatin, SN-38, or vehicle control over time (n = 6 per group) (mean ± SD). **(I)** Images of G72T-PDOX tumors and tumor weight at sacrifice.

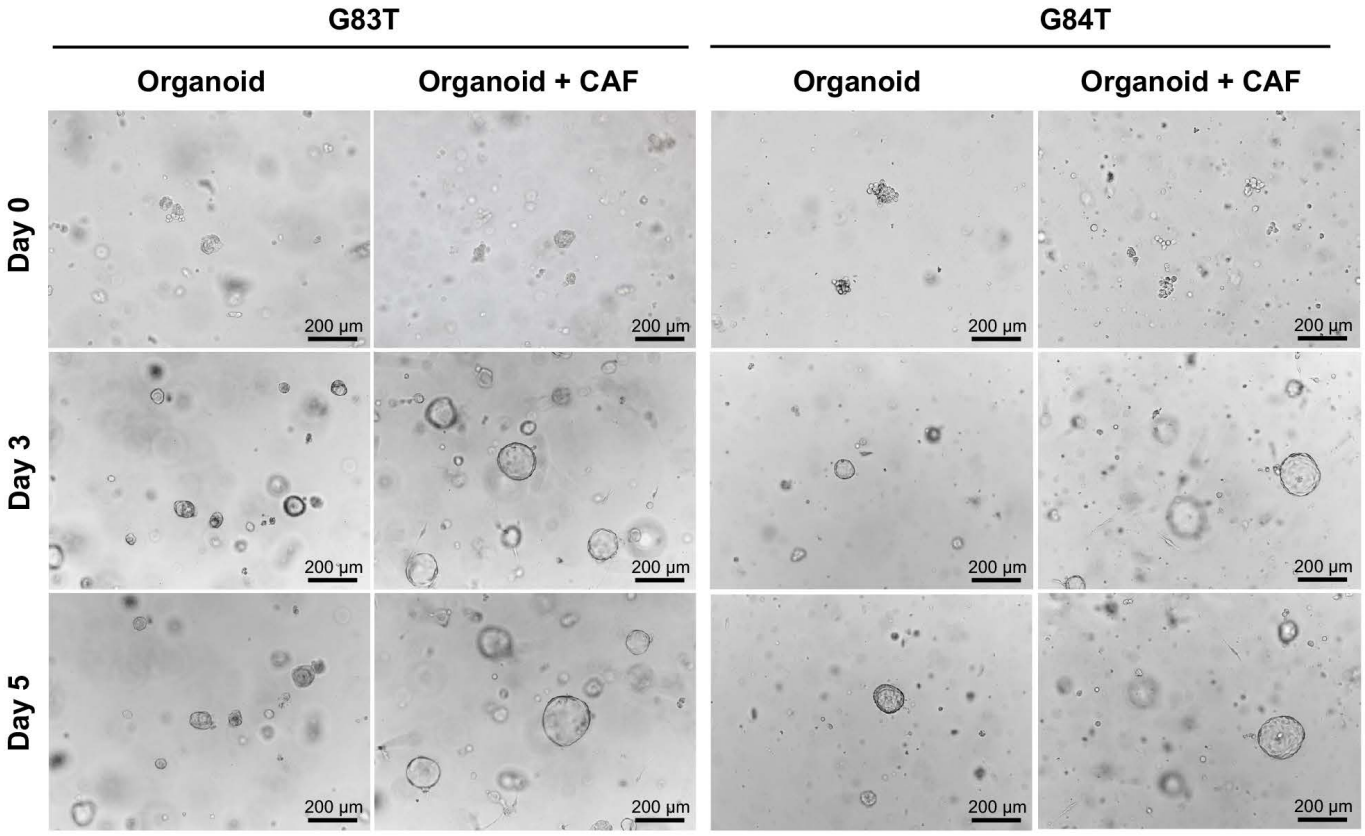


Figure S8. The organoid model as a platform to investigate interaction between cells of the tumor microenvironment and tumor cells, related to Figure 7. Brightfield images and growth profile of organoids in the presence or absence of CAFs in co-culture on day 0, day 3 and day 5. Scale bar = 200 µm.

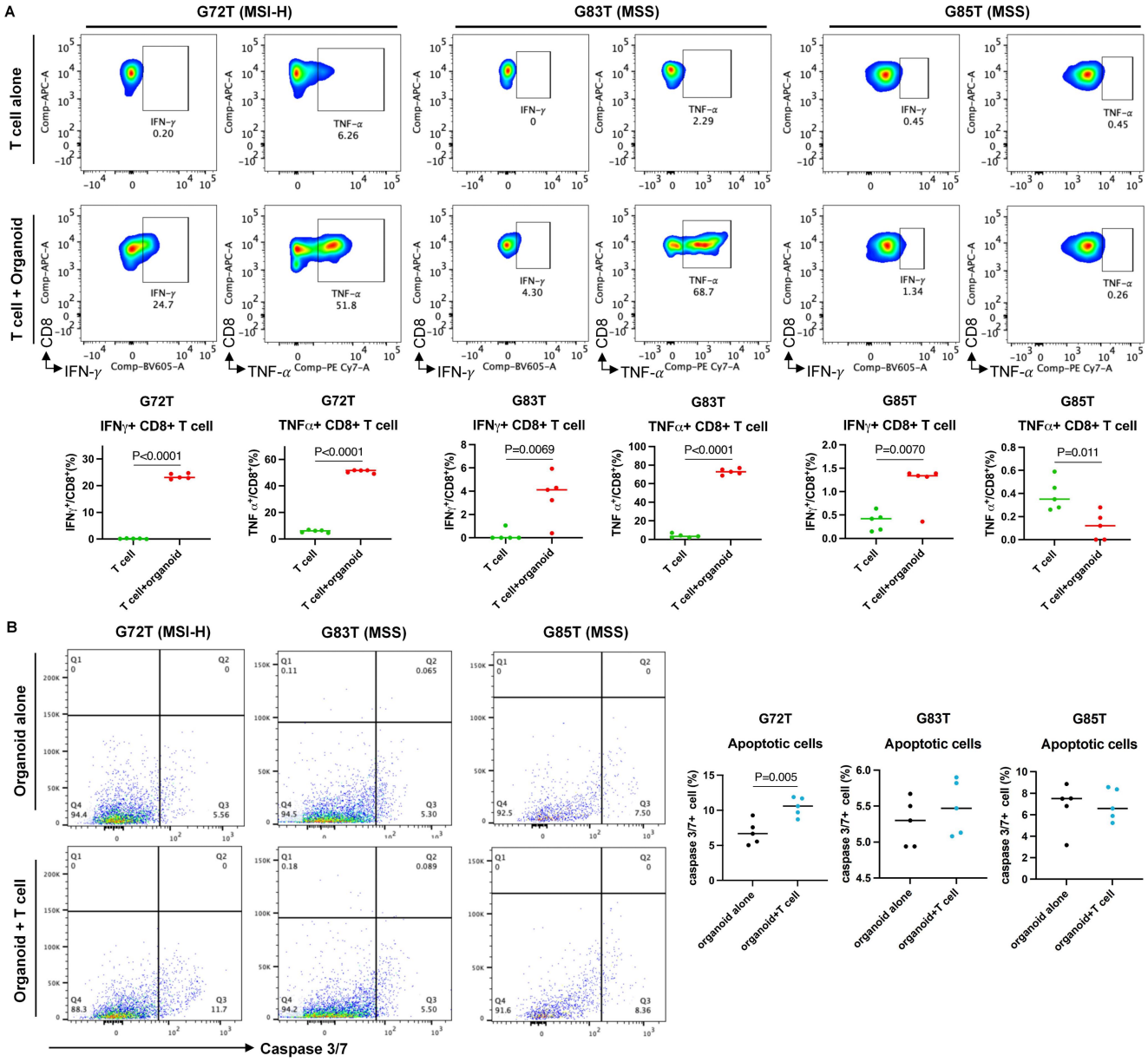


Figure S9. The organoid model as a platform to investigate interaction between cells of the tumor microenvironment and tumor cells, related to Figure 7. (A) Upper panel: Representative flow cytometry plots gated on CD8+ T cells tested for reactivity against organoids after 2 weeks of co-culture with autologous tumor organoids. Lower panel: Quantification of IFN-γ and TNF-α production of CD8+ T cells stimulated with autologous tumor organoids (n = 5). Error bars represent SD of technical replicates. **(B)** Left panel: representative flow cytometry plots gated on organoids with or without autologous T cell coculture tested for apoptosis. Right panel: quantification of caspase 3/7+ organoids at 18 h (n = 5).

Table S2. Statistical analysis of clinicopathologic characteristics of patients, related to Figure 1.

Variable	High Growth Rate (n=4)	%	Low Growth Rate (n=13)	%	*P* value
Mean age (y ± SD)	59.8 ±18.7		58.0 ±13.6		0.87
Sex					0.261
M	4	33.3	8	66.7	
F	0	0	5	100	
Tumor size					0.63
< 3 cm	0	0	4	100	
3-5 cm	2	25	6	75	
> 5 cm	2	40	3	60	
Differentiation					0.118
Poor	0	0	8	100	
Moderate/Poor	3	42.9	4	57.1	
Moderate	1	50	1	50	
TNM _ stage					0.519
I/II	0	0	4	100	
III/IV	4	30.7	9	69.3	

Table S3. Drug screening of GC patient-derived organoids to chemotherapeutics, related to Figures 3 and 6.

	AUC	G5T	G9T	G20T	G23T	G24T	G27T	G28T	G30T	G31T	G32T	G33T	G34T	G36T	G37T
	5-FU	68.61	31.13	18.38	34.01	22.46	28.71	46.39	53.91	16.10	21.38	30.39	39.12	56.24	15.51
	Oxaliplatin	17.53	47.37	27.77	41.74	48.19	29.79	97.62	40.78	28.87	26.05	38.22	52.55	76.36	30.33
	Cisplatin	25.75	38.98	32.67	31.75	36.94	30.03	81.12	36.18	47.17	23.54	33.65	47.53	58.56	29.23
	Paclitaxel	4.32	5.26	8.07	14.94	1.70	10.89	50.71	17.86	8.12	9.02	3.28	6.47	3.94	3.17
	SN-38	5.45	7.92	7.86	19.65	10.38	13.20	62.53	17.69	11.81	10.33	11.20	21.30	30.11	6.79
	Doxorubicin	12.40	25.52	55.73	17.25	24.28	17.23	19.08	35.88	18.81	10.33	11.20	21.30	34.50	14.07
Replicate 1															
	IC50	G5T	G9T	G20T	G23T	G24T	G27T	G28T	G30T	G31T	G32T	G33T	G34T	G36T	G37T
	5-FU (μM)	39.22	6.14	2.44	6.11	3.71	3.89	14.95	17.15	0.86	1.88	5.01	9.79	25.12	0.77
	Oxaliplatin (μM)	4.13	17.53	9.26	13.63	16.10	7.26	126.00	10.83	4.00	4.36	9.68	22.05	57.08	6.39
	Cisplatin (μM)	6.79	12.10	8.75	8.55	11.24	7.45	73.54	10.09	10.07	5.95	9.36	18.11	29.34	6.38
	Paclitaxel (nM)	0.03	0.71	0.01	0.27	0.01	0.38	0.94	0.02	0.03	0.09	0.02	0.06	0.07	0.01
	SN-38 (nM)	2.19	1.77	0.59	1.68	0.47	1.37	70.83	0.47	0.89	0.80	1.67	4.04	8.17	0.50
	Doxorubicin (μM)	0.011	0.070	0.335	0.003	0.021	0.026	0.011	0.099	0.017	0.008	0.017	0.040	0.086	0.007

	AUC	G38T	G39T	G41T	G42T	G43T	G44T	G47T	G48T	G50T	G55T	G56T	G58T	G59T	G61T
	5-FU	61.64	30.73	23.80	15.57	35.60	30.15	23.52	45.35	14.87	36.10	22.08	50.31	8.73	49.79
	Oxaliplatin	40.92	60.94	51.13	46.37	59.70	59.12	29.63	59.26	19.84	66.03	46.95	52.75	49.35	77.68
	Cisplatin	18.75	43.58	27.71	22.60	25.37	37.68	14.87	20.20	7.93	35.06	24.16	23.92	6.98	48.13
	Paclitaxel	5.90	5.62	1.41	3.18	4.31	7.57	8.89	43.92	0.49	15.81	3.83	23.26	1.61	29.75
	SN-38	13.54	17.18	10.60	8.05	11.54	21.73	16.04	46.67	4.97	22.25	10.38	20.61	5.23	40.46
	Doxorubicin	14.04	16.15	12.87	6.10	17.13	20.64	5.92	34.21	6.19	19.69	14.54	24.65	9.44	40.59
Replicate 1															
	IC50	G38T	G39T	G41T	G42T	G43T	G44T	G47T	G48T	G50T	G55T	G56T	G58T	G59T	G61T
	5-FU (μM)	29.09	7.47	4.29	2.16	8.50	5.70	1.48	9.38	1.96	7.37	4.03	15.81	0.47	16.20
	Oxaliplatin (μM)	12.67	30.61	19.73	15.44	32.62	30.63	5.75	24.48	3.05	32.16	18.07	19.09	18.54	101.30
	Cisplatin (μM)	3.94	14.87	9.41	6.62	6.31	15.67	3.36	4.03	2.22	15.93	7.74	9.34	2.12	26.97
	Paclitaxel (nM)	0.03	0.05	0.04	0.01	0.13	0.12	0.02	2.03	0.01	3.31	1.14	4.83	0.23	2.99
	SN-38 (nM)	2.06	1.46	1.82	0.78	1.39	5.17	1.30	1.17	1.20	2.55	2.06	6.41	0.60	9.37
	Doxorubicin (μM)	0.003	0.009	0.009	0.002	0.008	0.032	0.001	0.074	0.004	0.026	0.024	0.030	0.022	0.139

	AUC	G62T	G63T	G65T	G72T	G73T	G74T	G78T	G80T	G82T	G83T	G84T	G86T	G87T
Replicate 1	5-FU	30.65	60.50	44.26	9.70	27.03	31.27	24.40	10.86	37.86	52.57	50.03	31.53	57.48
	Oxaliplatin	56.34	85.97	98.74	12.45	56.74	60.00	33.67	15.47	53.77	70.73	54.37	69.65	61.66
	Cisplatin	28.79	33.37	25.69	9.99	25.27	23.50	21.44	15.75	45.97	41.44	72.19	22.84	17.67
	Paclitaxel	7.25	12.90	5.34	3.85	9.63	20.31	15.18	5.85	9.03	28.36	36.53	7.77	31.49
	SN-38	15.92	36.11	8.99	1.52	11.55	24.12	13.48	12.43	14.77	52.26	32.02	18.13	46.44
	Doxorubicin	21.59	25.04	17.53	1.88	19.32	12.50	13.32	10.61	26.85	51.37	18.02	18.82	39.62
	IC50	G62T	G63T	G65T	G72T	G73T	G74T	G78T	G80T	G82T	G83T	G84T	G86T	G87T
	5-FU (μM)	3.37	30.34	15.03	1.77	3.26	5.57	3.84	2.49	9.55	20.18	9.20	7.04	21.17
	Oxaliplatin (μM)	23.31	92.76	133.60	2.42	24.48	33.39	6.44	2.08	24.03	57.09	19.84	38.26	24.12
	Cisplatin (μM)	8.84	18.46	6.79	3.44	7.16	8.32	3.48	1.02	16.02	11.84	36.46	8.44	3.81
	Paclitaxel (nM)	0.76	1.89	1.11	1.58	3.97	0.41	1.08	0.06	2.04	5.76	4.84	2.92	7.96
	SN-38 (nM)	1.07	11.98	2.87	0.16	1.19	3.03	0.35	1.15	2.59	30.93	1.26	7.32	12.80
	Doxorubicin (μM)	0.034	0.067	0.030	0.003	0.042	0.006	0.006	0.001	0.053	0.245	0.031	0.053	0.150

	AUC	G5T	G33T	G34T	G43T	G61T	G63T
Replicate 2	5-FU	70.43	39.54	36.48	39.16	47.33	42.28
	Oxaliplatin	11.39	52.83	57.98	62.56	59.82	59.46
	Cisplatin	20.30	59.64	60.58	36.86	31.31	16.64
	Paclitaxel	4.98	11.35	10.49	12.95	19.64	9.24
	SN-38	2.77	16.71	22.60	15.83	21.52	18.02
	Doxorubicin	3.06	29.37	28.17	37.15	21.21	10.75

		G9T	G32T	G36T	G39T	G41T	G43T	G59T	G62T	G20T	G30T	G50T	G56T
Combined therapy	AUC	40.78	26.71	43.24	43.70	27.15	35.26	23.14	30.05	17.90	36.40	18.77	32.11
	IC50 (μM)	15.87	2.93	10.55	8.04	4.11	7.26	3.60	3.87	2.97	11.34	3.47	5.06

Table S4. Drug responses in organoid corresponding follow-up gastric cancer patients, related to Figures 6

Number	UICC Tumor stage	Metastasis	Therapy prior to derivation	Postoperative chemotherapy	Clinical response	Organoid treatment		Organoid response
						AUC (%)	IC50 (μM)	
G9T	IIIB	None	None	Hyperthermic intraperitoneal chemotherapy (Oxaliplatin+5-FU) *3	No recurrence (49 months)	40.78	15.87	sensitive
G32T	IIIB	None	None	SOX (S-1+Oxaliplatin) *7	No recurrence (35 months)	26.71	2.93	sensitive
G36T	IIIB	None	None	FOLFOX (Oxaliplatin+5-FU) *7	No recurrence (34 months)	43.24	10.55	sensitive
G39T	IIIB	None	None	Hyperthermic intraperitoneal chemotherapy (Oxaliplatin+5-FU) *4	Death (RFS 10 months)	43.7	8.04	sensitive
G41T	IIIB	None	none	Capeox (Capecitabine +Oxaliplatin) *7	No recurrence (33 months)	27.15	4.11	sensitive
G43T	IIIB	None	None	SOX (S-1+Oxaliplatin) *4	No recurrence (33 months)	35.26	7.261	sensitive
G59T	IIIA	None	None	Capeox (Capecitabine + Oxaliplatin) *6	No recurrence (29 months)	23.14	3.599	sensitive
G62T	IIIC	None	None	SOX (S-1+Oxaliplatin) *5	No recurrence (29 months)	30.05	3.866	sensitive

G20T	IV	Omentum and lymphatic metastasis	None	Capeox (Capecitabine + Oxaliplatin) *7	Omentum and lymphatic metastasis (PFS 11 months)	17.89	2.974	sensitive
G30T	IV	Liver	None	SOX (S-1+Oxaliplatin) *5	No recurrence (36 months)	36.39	11.34	sensitive
G50T	IV	Peritoneal implantation metastasis	None	SOX (S-1+Oxaliplatin) *7+PD-1	Death (PFS 25 months)	18.77	3.472	sensitive
G56T	IV	Ovary	Hyperthermic intraperitoneal chemotherapy (Paclitaxel +Cisplatin) *3	SOX (S-1+Oxaliplatin) *10	Peritoneal implantation metastasis (PFS 10 months)	32.11	5.063	sensitive