Supplementary files

Paired organoids from primary gastric cancer and lymphatic metastasis are useful for personalized medicine

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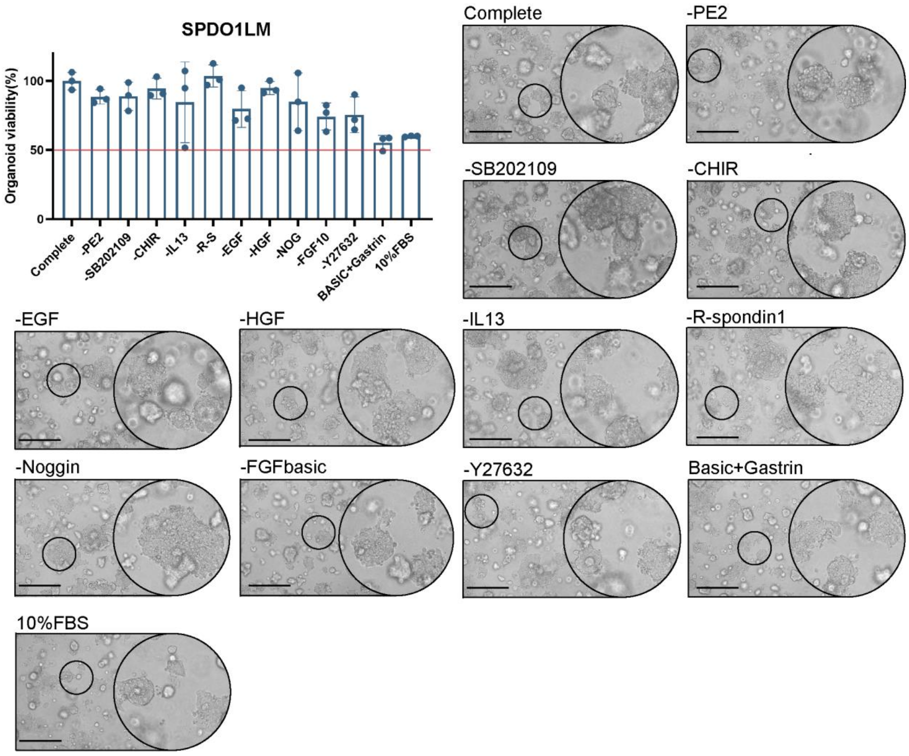

Figure S1. Analyzing culture medium for SPDO1LM organoid line. We deleted the components of culture medium of SPDO1LM line according to complete medium, PE2 minus, SB202109 minus, CHIR-99021 minus, IL13 minus, R-spondin 1 minus, EGF minus, HGF minus, noggin minus, FGF-10 minus, Y27632 minus, basic medium with gastrin, and 10% FBS only step-wisely. SPDO1LM organoid grows well in the medium removing related cytokines or small compounds. The cellular vitality of organoids could reach over 50% of the complete medium in the medium covering 10% FBS only, and continuously passaged. Light field, scale bar = 200 μm.

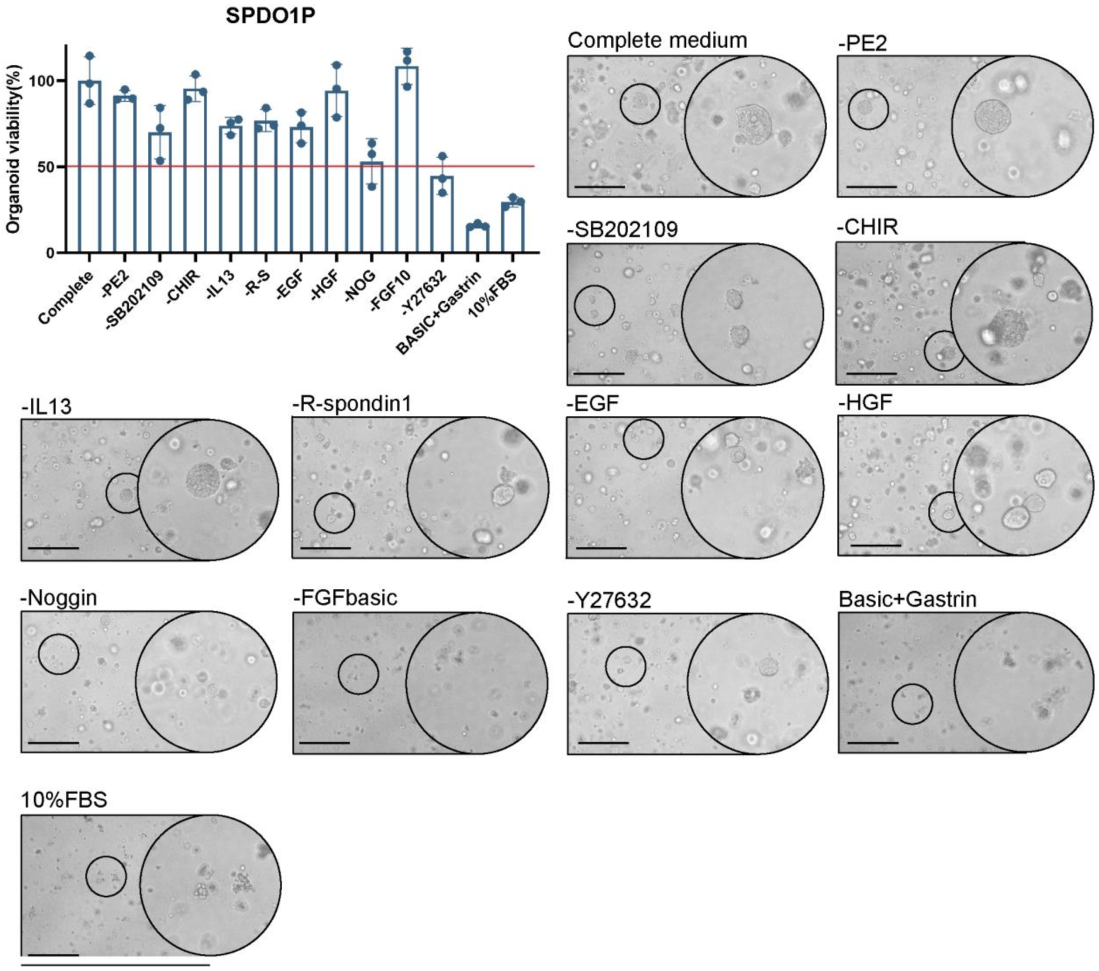

Figure S2. Analyzing culture medium for SPDO1P organoid line. We deleted the components of culture medium of SPDO1P line according to complete medium, PE2 minus, SB202109 minus, CHIR-99021 minus, IL13 minus, R-spondin1 minus, EGF minus, HGF minus, noggin minus, FGF-10 minus, Y27632 minus, basic medium with gastrin, and 10% FBS only step-wisely. After removing noggin and Y27632, the cellular vitality of SPDO1P line is significantly decreased. The SPDO1P line can not grow and passage in basic medium with gastrin or 10% FBS-DMEM only. Light field, scale bar = 200 μm.

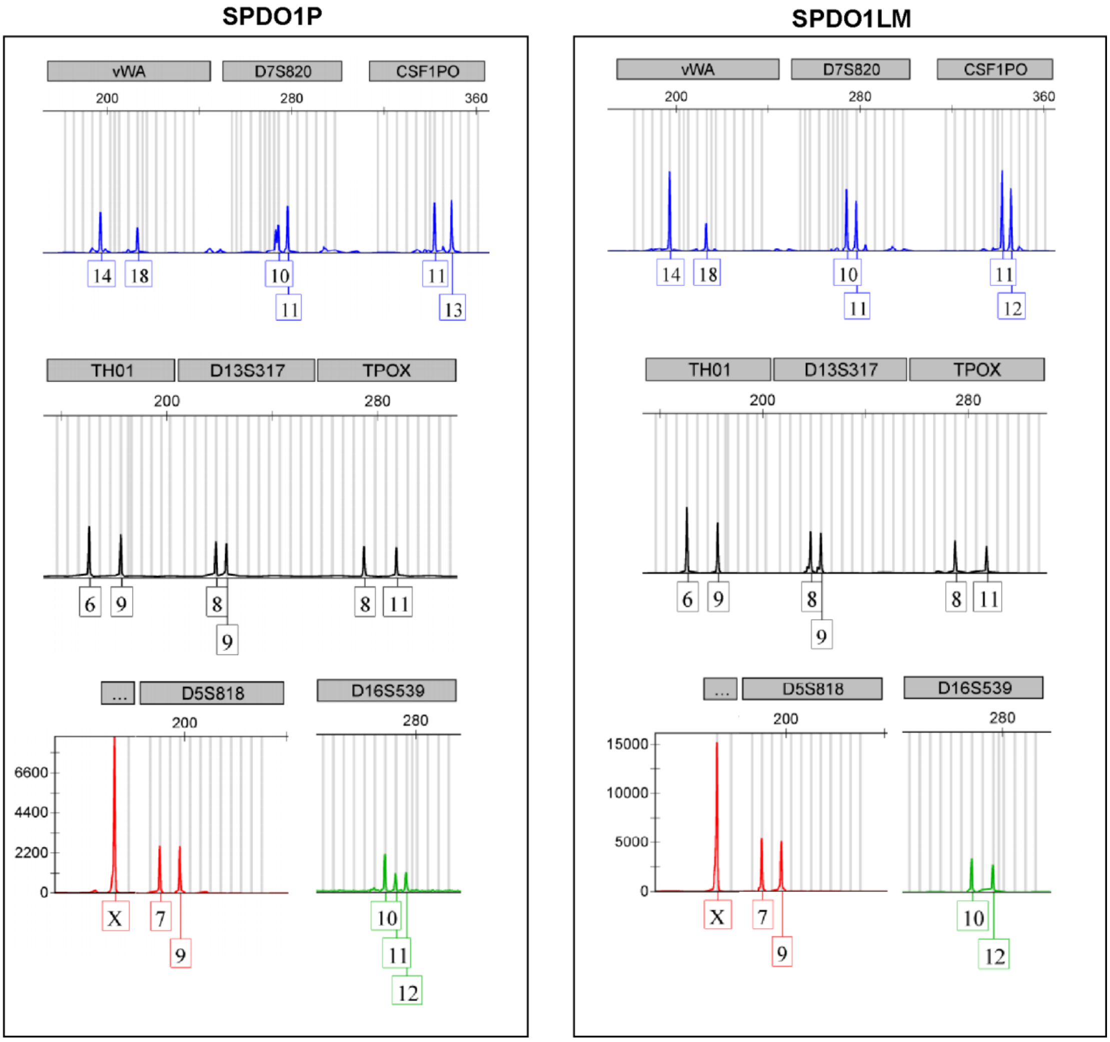

Figure S3. The chromatograms of STRs assay for SPDO1P and SPDO1LM. The two organoid lines show the similar peaks at vWA, D7S820, TH01, D13S317, TPOX and amelogenin loci. The peaks at CSF1PO and D16S539 loci show one allele shift.

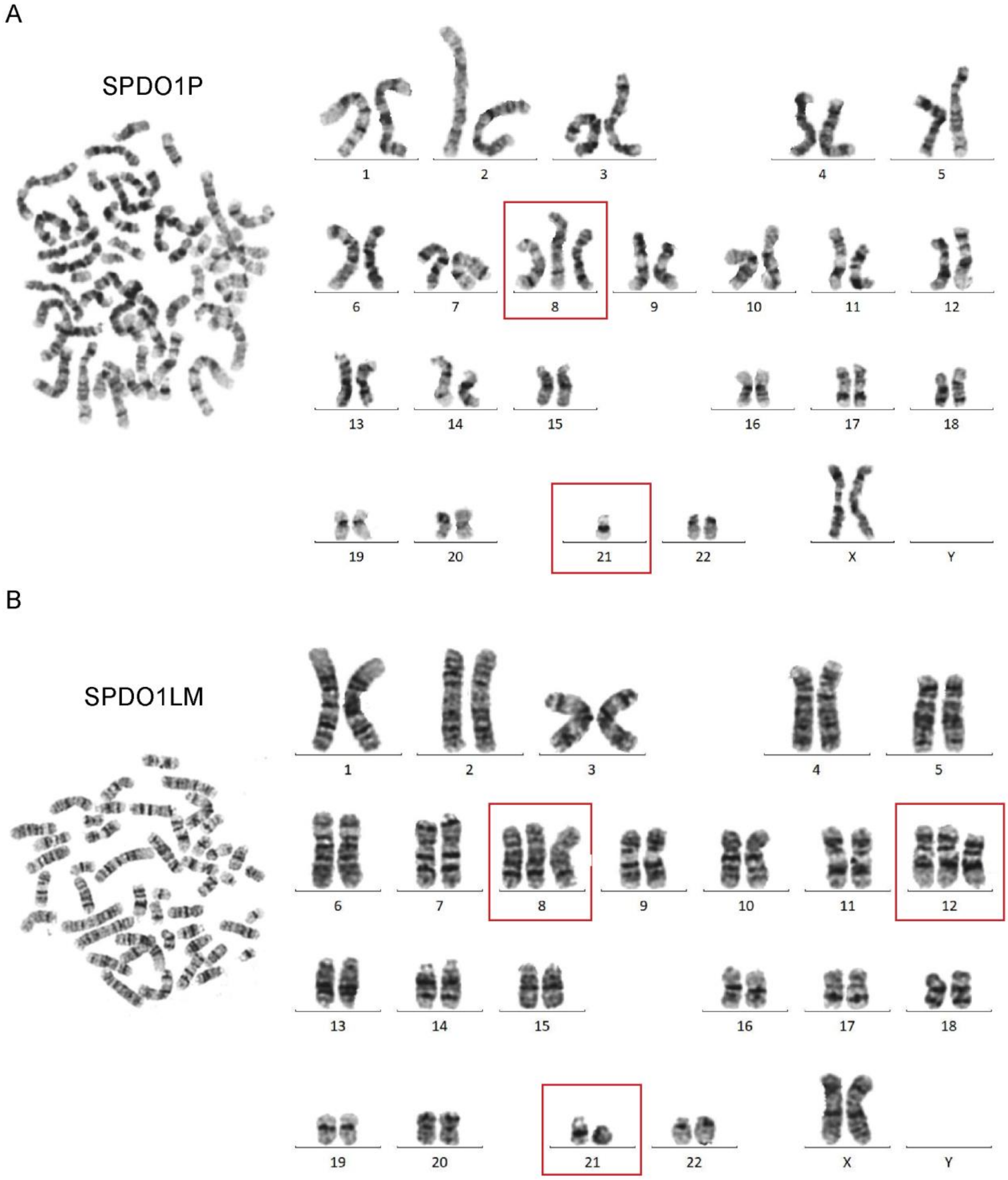

Figure S4. The karyotypes assay of chromosomes for SPDO1P and SPDO1LM. (A) The karyotype of SPDO1P shows 46 chromosomes with an additional chromosome 8 and a chromosome 21 lost. (B) The karyotype of SPDO1LM shows 48 chromosomes with an additional chromosome 8 and a chromosome 12, as well as a circular chromosome 21.

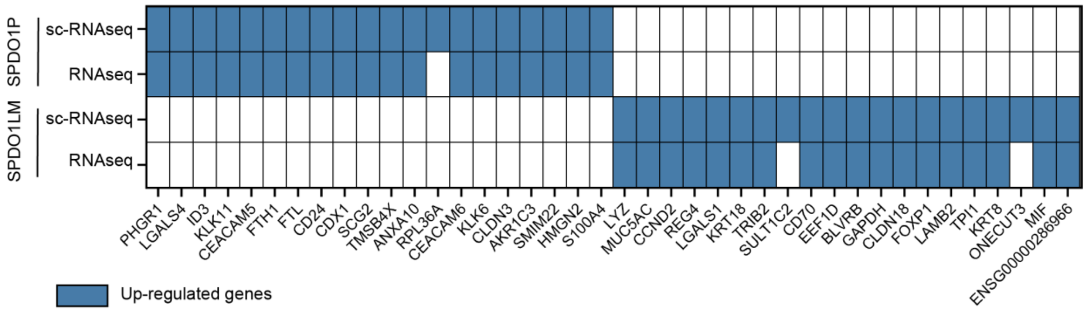

Figure S5. **Heatmap of the top 20 upregulated genes in scRNA-seq and RNA-seq for the two organoids**. The 95% genes (19/20, except RPL36A) are consistently upregulated in SPDO1P. The 90% genes (18/20, except SULT1C2 and ONECUT3) are consistently upregulated in SPDO1LM.

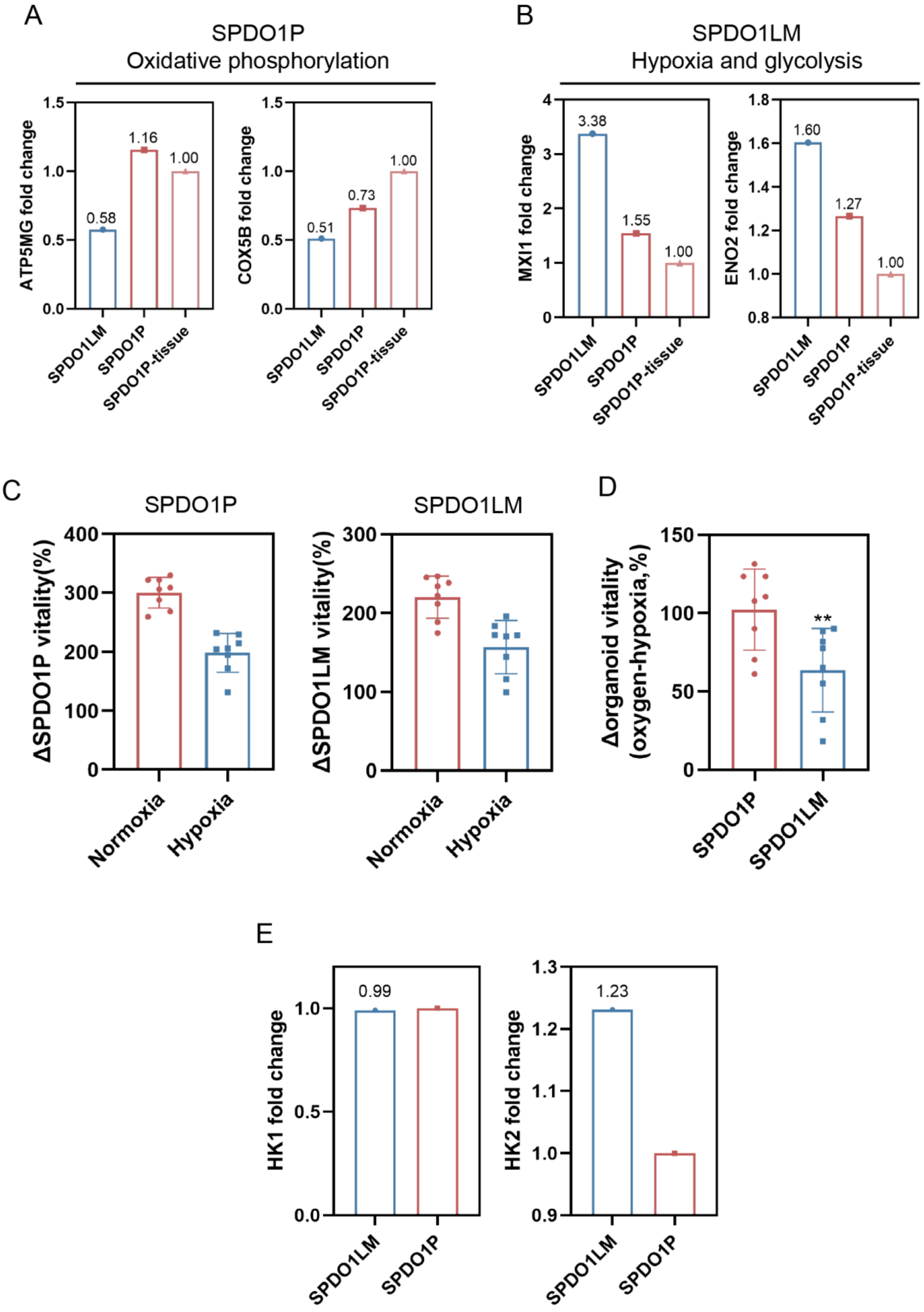

Figure S6. **Examination of oxidative phosphorylation related genes and glycolysis-related genes.** (A) The fold change of ATP5MG and COX5B involved in oxidative phosphorylation on SPDO1LM, SPDO1P and SPDO1P-tissue. (B) The fold change of MXI1 and ENO2 involved in glycolysis on SPDO1LM, SPDO1P and SPDO1P-tissue. (C) △ SPDO1P vitality and △ SPDO1LM vitality in normoxic and hypoxic condition. (D) The △ organoid vitality of SPDO1P and SPDO1LM in normoxic or hypoxic condition. (E) The fold change of gene expression levels of HK1 and HK2 in SPDO1LM and SPDO1P.

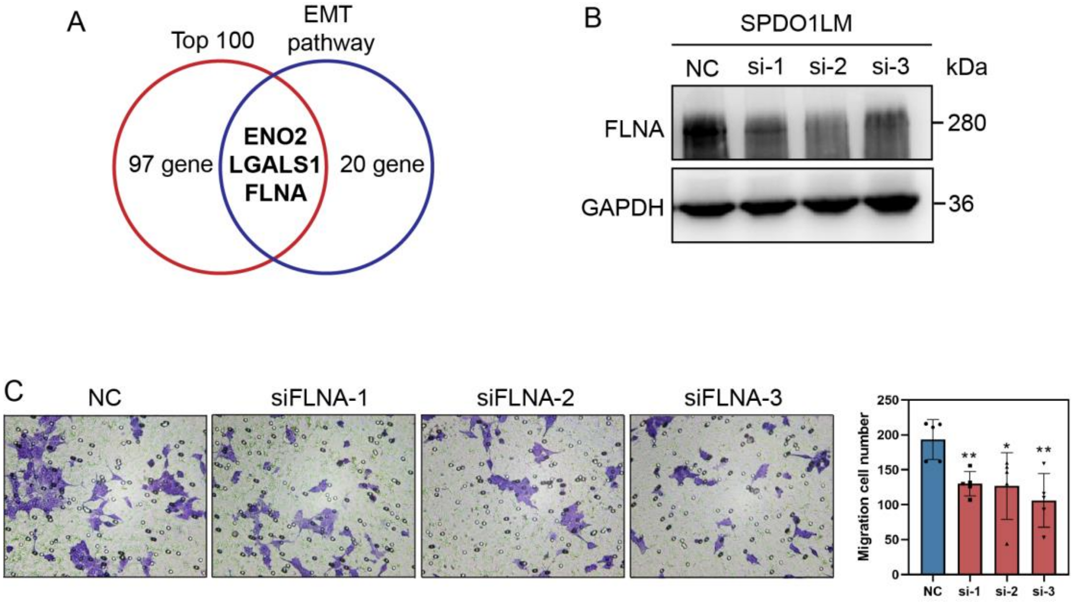

Figure S7. Biological function analysis of FLNA. (A) In SPDO1LM, the top 100 upregulation genes intersected with genes of EMT pathway, including ENO2, LGALS1 and FLNA. (B) FLNA knockdown is analyzed by western blot. (C) 2D migration assay upon FLNA knockdown.

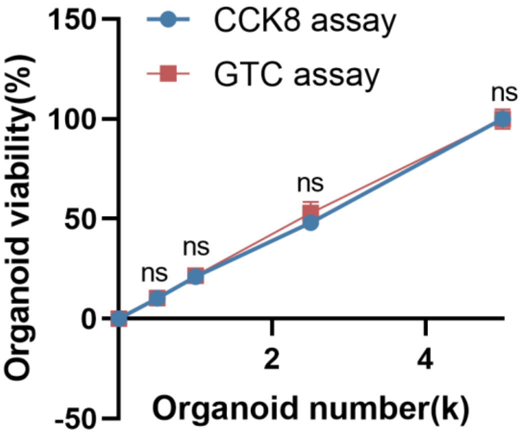

Figure S8. Comparison of organoid viability and organoid numbers between CCK8 and GTC 3D assays. There was no statistical difference between CCK8 and GTC 3D methods.

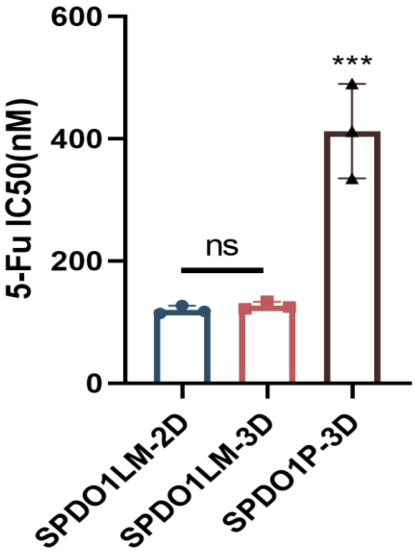

Figure S9. Examination of 5-Fu IC50 for SPDO1LM-2D, SPDO1LM-3D, and SPDO1P-3D. There is no significant difference of the IC50 between the SPDO1LM-2D (119.70±5.61 nM) and SPDO1LM-3D SPDO1LM-3D (126.67±5.33 nM) . However, both SPDO1LM-2D and SPDO1LM-3D are sensitive to 5-Fu than SPDO1P line (412.47±61.20 nM).

Table S1. Components of complete medium, SPDO1P advanced medium, and SPDO1LM advanced medium

	Component	Concentration	Cat.	Supplier
		Complete medium		
Basic	Advanced DMEM/F12		12634010	Thermo Fisher Scientific
	HEPES	10mM	15630130	Sigma-Aldrich
	B27	1x	17504044	Thermo Fisher Scientific
	N2 additive	1x	17502048	Thermo Fisher Scientific
	GlutaMAX	1x	35050061	Thermo Fisher Scientific
	Nicotinamide	4mM	N0636	Sigma-Aldrich
	N-Acetyl-L-cysteine	1 μM	A7250-10G	Sigma-Aldrich
	Gastrin	10 nM	G9145	Sigma-Aldrich
Antibiotic	Primocin	1x	ant-pm-1	InvivoGen
	Penicillin-streptomycin	1x	15070063	Thermo Fisher Scientific
	Mycoplasma removal reagent	1x	40607ES03	GASEN
Cytokines	EGF	50 ng/ml	AF-100-15	PeproTech
	Noggin	100 ng/ml	250-38	PeproTech
	R-Spondin1	200 ng/ml	120-38	PeproTech
	FGF-basic	10 ng/ml	100-18B	PeproTech
	Prostaglandin E2	100nM	2296	Tocris Bioscience
	Y-27632	10 μM	Y0503	Sigma-Aldrich
	SB202190	5 μM	S7067	Sigma-Aldrich
	HGF	20ng/ml	100-39	PeproTech
	CHIR-99021	100nM	SML1046	Sigma-Aldrich
	Human IL-13	20ng/ml	200-13	PeproTech
		SPDO1P advanced medium		
Basic	Advanced DMEM/F12		12634010	Thermo Fisher Scientific
	HEPES	10mM	15630130	Sigma-Aldrich
	B27	1x	17504044	Thermo Fisher Scientific
	N2 additive	1x	17502048	Thermo Fisher Scientific
	GlutaMAX	1x	35050061	Thermo Fisher Scientific
	Nicotinamide	4mM	N0636	Sigma-Aldrich
	N-Acetyl-L-cysteine	1 μM	A7250-10G	Sigma-Aldrich
	Gastrin	10 nM	G9145	Sigma-Aldrich
Antibiotic	Primocin	1x	ant-pm-1	InvivoGen
	Penicillin-streptomycin	1x	15070063	Thermo Fisher Scientific
Cytokines	EGF	50 ng/ml	AF-100-15	PeproTech
	Noggin	100 ng/ml	250-38	PeproTech
	R-Spondin1	200 ng/ml	120-38	PeproTech
	Y-27632	10 μM	Y0503	Sigma-Aldrich
	SB202190	5 μM	S7067	Sigma-Aldrich

SPDO1LM advanced medium				
Basic	Advanced DMEM/F12		12634010	Thermo Fisher Scientific
	FBS	0.1	10100147	Gibco
Antibiotic	Primocin	1x	ant-pm-1	InvivoGen
	Penicillin-streptomycin	1x	15070063	Thermo Fisher Scientific

Table S2. Basic information of the drug sensitive analysis

Drugs	**Cat.**	**Supplier**
5-Fluorouracil	M2289	AbMole
Oxaliplatin	MB1174	Meilunbio
Paclitaxel	M1970	AbMole

Table S3. Gene mutations of SPDO1P, SPDO1LM and parental tissues

SPDO1P				SPDO1LM			
Parental tissue		Organoid line		Parental tissue		Organoid line	
KALRN	Missense_Mutation	MYH9	Missense_Mutation	MAP3K13	Missense_Mutation	KALRN	Missense_Mutation
PIK3C2B	Missense_Mutation	RAB11FIP5	Missense_Mutation	LAMA2	Missense_Mutation	TAOK2	Missense_Mutation
BCL6	Missense_Mutation	SETD1B	Missense_Mutation	ARHGAP35	Missense_Mutation	EFTUD2	Missense_Mutation
BRAF	Missense_Mutation	LAMA2	Missense_Mutation	IL21R	Missense_Mutation	FAT3	Missense_Mutation
SETD1B	Missense_Mutation	TFE3	Missense_Mutation	SRRM2	Missense_Mutation	LAMA2	Missense_Mutation
LAMA2	Missense_Mutation	MYO5A	Missense_Mutation	EPHA7	Missense_Mutation	DCTN1	Missense_Mutation
ANK1	Missense_Mutation	LARP4B	Missense_Mutation	ROBO2	Missense_Mutation	IL21R	Missense_Mutation
GNAI1	Missense_Mutation	MAP4	Missense_Mutation	VHL	Missense_Mutation	ARHGEF2	Missense_Mutation
STAG2	Missense_Mutation	IL21R	Missense_Mutation	KAT6B	Missense_Mutation	SRRM2	Missense_Mutation
IL21R	Missense_Mutation	ERBB2	Missense_Mutation	FAT3	Missense_Mutation	EPHA7	Missense_Mutation
JMJD1C	Missense_Mutation	PTPRF	Missense_Mutation	SETD1B	Missense_Mutation	MYO5A	Missense_Mutation
SRRM2	Missense_Mutation	FAT4	Missense_Mutation	PRPF8	Missense_Mutation	FAT4	Missense_Mutation
EPHA7	Missense_Mutation	FGFR2	Missense_Mutation	ERBB3	Missense_Mutation	BRD4	Missense_Mutation
MYO5A	Missense_Mutation	CLASP2	Missense_Mutation	LZTR1	Missense_Mutation	KAT6B	Missense_Mutation
PTPRC	Splice_Site	KAT6B	Missense_Mutation	ZNF703	Missense_Mutation	SETD1B	Missense_Mutation
BCL9	Missense_Mutation	EP300	Missense_Mutation	GOT2	Missense_Mutation	PRPF8	Missense_Mutation
FAT4	Missense_Mutation	PRPF8	Missense_Mutation	LRP1B	Missense_Mutation	ERBB3	Missense_Mutation
BRD4	Missense_Mutation	ERBB3	Missense_Mutation	CALR	Missense_Mutation	AKT1	Missense_Mutation
KAT6B	Missense_Mutation	PIP5K1A	Splice_Site	COL1A1	Missense_Mutation	LRP1B	Missense_Mutation
CIC	Frame_Shift_Ins	CACNA1D	Missense_Mutation	SYNE1	Nonsense_Mutation	EXT2	Missense_Mutation
POLD1	Missense_Mutation	CEP89	Missense_Mutation	EPPK1	Missense_Mutation	ZNF703	Missense_Mutation
ACSL6	Missense_Mutation	LRP1B	Missense_Mutation	NCKIPSD	Missense_Mutation	CEP89	Missense_Mutation
ERBB3	Missense_Mutation	PLXNB2	Missense_Mutation	DLG1	Missense_Mutation	MGAM	Missense_Mutation
AKT1	Missense_Mutation	MUC16	Missense_Mutation	FGFR2	Missense_Mutation	ARID1A	Missense_Mutation
CCAR1	Missense_Mutation	SRGAP3	Missense_Mutation	MYCBP2	Missense_Mutation	UHRF1BP1L	Missense_Mutation
ZNF703	Missense_Mutation	DLG1	Missense_Mutation	ZMYM2	Missense_Mutation	MUC16	Missense_Mutation
CEP89	Missense_Mutation	BRAF	Missense_Mutation	RASGRP1	Missense_Mutation	SYNE1	Nonsense_Mutation
LRP1B	Missense_Mutation	PIM1	Missense_Mutation	MAGI2	Missense_Mutation	DLG1	Missense_Mutation
LTBP1	Missense_Mutation	TLX3	Missense_Mutation	LRRK2	Nonsense_Mutation	MN1	Missense_Mutation
SYNE1	Nonsense_Mutation	NSD1	Missense_Mutation	SVEP1	Missense_Mutation	SMARCB1	Missense_Mutation
TET2	Missense_Mutation	AKT1	Missense_Mutation	EPHB6	Missense_Mutation	FGFR2	Missense_Mutation
EPPK1	Missense_Mutation	MYH10	Missense_Mutation	CEP89	Missense_Mutation	MYCBP2	Missense_Mutation
DLG1	Missense_Mutation	MAGI2	Missense_Mutation	EPHA1	Missense_Mutation	GOLGA5	Missense_Mutation
MUC16	Missense_Mutation	DNMT1	Missense_Mutation	ITGA9	Missense_Mutation	MAGI2	Missense_Mutation
SMARCB1	Missense_Mutation	SVEP1	Missense_Mutation	PAX3	Missense_Mutation	DMD	Missense_Mutation
FGFR2	Missense_Mutation	SMCHD1	Missense_Mutation	MYH10	Missense_Mutation	LRRK2	Nonsense_Mutation
MYCBP2	Missense_Mutation	POLE	Missense_Mutation	CTCF	Nonsense_Mutation	SVEP1	Missense_Mutation
GOLGA5	Missense_Mutation	TSHR	Missense_Mutation	NOTCH2	Missense_Mutation	CRTC1	Missense_Mutation
MAGI2	Missense_Mutation	SPEG	Missense_Mutation	PREX2	Missense_Mutation	TTK	Missense_Mutation
SVEP1	Missense_Mutation	EPHA1	Missense_Mutation	TRIM7	Missense_Mutation	SPEG	Missense_Mutation

MDN1	Missense_Mutation	LRRK2	Nonsense_Mutation	NAV1	Missense_Mutation	EPHA1	Missense_Mutation
SPEG	Missense_Mutation	PIK3C2B	Missense_Mutation	MUC16	Missense_Mutation	TCF4	Missense_Mutation
EPHA1	Missense_Mutation	PAX3	Missense_Mutation	PTPRT	Missense_Mutation	CSMD3	Missense_Mutation
LRRK2	Nonsense_Mutation	IRS2	Missense_Mutation	FAT4	Missense_Mutation	KDR	Missense_Mutation
TCF4	Missense_Mutation	CTCF	Nonsense_Mutation	ZNF132	Missense_Mutation	PAX3	Missense_Mutation
CLASP2	Missense_Mutation	COL18A1	Missense_Mutation	TSC2	Missense_Mutation	CTCF	Nonsense_Mutation
PAX3	Missense_Mutation	CHD8	Missense_Mutation	FLNA	Missense_Mutation	SETBP1	Missense_Mutation
CTCF	Nonsense_Mutation	PREX2	Missense_Mutation	SPEG	Missense_Mutation	PREX2	Missense_Mutation
ZNF292	Missense_Mutation	SYNE1	Nonsense_Mutation	CACNA1D	Missense_Mutation	HCFC1	Missense_Mutation
PREX2	Missense_Mutation	HCFC1	Missense_Mutation	CIC	Frame_Shift_Ins	TOP3A	Missense_Mutation
SETBP1	Missense_Mutation	WASF3	Missense_Mutation	CDKN1B	Frame_Shift_Del	KIAA1109	Missense_Mutation
TOP3A	Missense_Mutation	KRAS	Missense_Mutation	KMT2B	Frame_Shift_Ins	POLD1	Missense_Mutation
MGAM	Missense_Mutation	SMC1A	Missense_Mutation			KRAS	Missense_Mutation
KRAS	Missense_Mutation	PTPRT	Missense_Mutation			CASP9	Missense_Mutation
CASP9	Missense_Mutation	NOTCH2	Missense_Mutation			PTPRT	Missense_Mutation
PTPRT	Missense_Mutation	JAK3	Missense_Mutation			CACNA1D	Missense_Mutation
DNMT3A	Missense_Mutation	NFATC4	Missense_Mutation			ZNF132	Missense_Mutation
ZNF132	Missense_Mutation	ZNF132	Missense_Mutation			FLNA	Missense_Mutation
ARHGAP35	Missense_Mutation	CASP8	Missense_Mutation			EPPK1	Missense_Mutation
FLNA	Missense_Mutation	PIK3CA	Missense_Mutation			FAT1	Missense_Mutation
FAT3	Missense_Mutation	FAT1	Missense_Mutation			CIC	Frame_Shift_Ins
FAT1	Missense_Mutation	CIC	Frame_Shift_Ins			CDKN1B	Frame_Shift_Del
CACNA1D	Missense_Mutation					KMT2B	Frame_Shift_Ins
PRPF8	Missense_Mutation						

Table S4. Upregulated pathways of SPDO1LM and SPDO1P by GSEA

Upregulated pathways in SPDO1LM line		
Pathways	**NES**	***P* value**
HYPOXIA	1.908967871	2.19E-05
EPITHELIAL_MESENCHYMAL_TRANSITION	1.863697467	0.00038268
GLYCOLYSIS	1.706351405	0.000707662
COAGULATION	1.696646134	0.002850029
APOPTOSIS	1.689650696	0.001798553
ANGIOGENESIS	1.624695307	0.02353202
IL2_STAT5_SIGNALING	1.608993477	0.003016902
PI3K_AKT_MTOR_SIGNALING	1.580752483	0.011457187
P53_PATHWAY	1.580626188	0.003155236
Upregulated pathways in SPDO1P line		
Pathways	**NES**	***P* value**
OXIDATIVE_PHOSPHORYLATION	2.193680594	1.32E-10
MYC_TARGETS_V1	1.811704218	4.47E-06
E2F_TARGETS	1.748947836	8.51E-05
TGF_BETA_SIGNALING	1.594527816	0.009737311
DNA_REPAIR	1.46178747	0.00836816
G2M_CHECKPOINT	1.437581198	0.011323814
INTERFERON_GAMMA_RESPONSE	1.403246896	0.020619045