1. Organoid basal medium

Basal medium for culture: 2 mM Glutamax, 10 mM HEPES, 100 U/mL penicillin, and 100 μg/mL streptomycin in advanced DMEM/F12

Basal medium for fine-needle aspiration specimens: 2 mM Gluta MAX, 10 mM HEPES, 100 U/mL penicillin, 100 U/mL hygromycin B, and 100 μg/mL streptomycin in advanced DMEM/F12

2. Organoid culture medium

Complete medium: 50 % (v/v) with Wnt-3A, R-spondin 1, and mNoggin conditioned medium, human epidermal growth factor 50 ng/mL, human fibroblast growth factor-10 100 ng/mL, nicotinamide 10 mM, 500 nM A83-01, 1× B27 supplement, N-acetylcysteine 1.25 mM, 10% fetal bovine serum (FBS), and human gastrin I 0.01 μM in 50% basal culture medium.

Conditioned medium: Recombinant Wnt-3A, Noggin or R-spondin1 can be substituted with conditioned medium from L-WRN (ATCC® CRL-3276™) cell line. Conditioned media: L-WRN (ATCC® CRL-3276™) cell line was cultured in DMEM supplemented with 10% FBS, 1% penicillin and streptomycin; and when the cell is 70% confluent, suction all the media, and replace them with advanced DMEM (10% FBS, 1% penicillin and streptomycin). After washing with the media, new advanced DMEM (10% FBS, 1% penicillin and streptomycin) was added, and cultured 2 to 3 days to obtain conditioned media. After repeating 2 to 3 times, collect and filter them.

3. Organoid isolation and culture

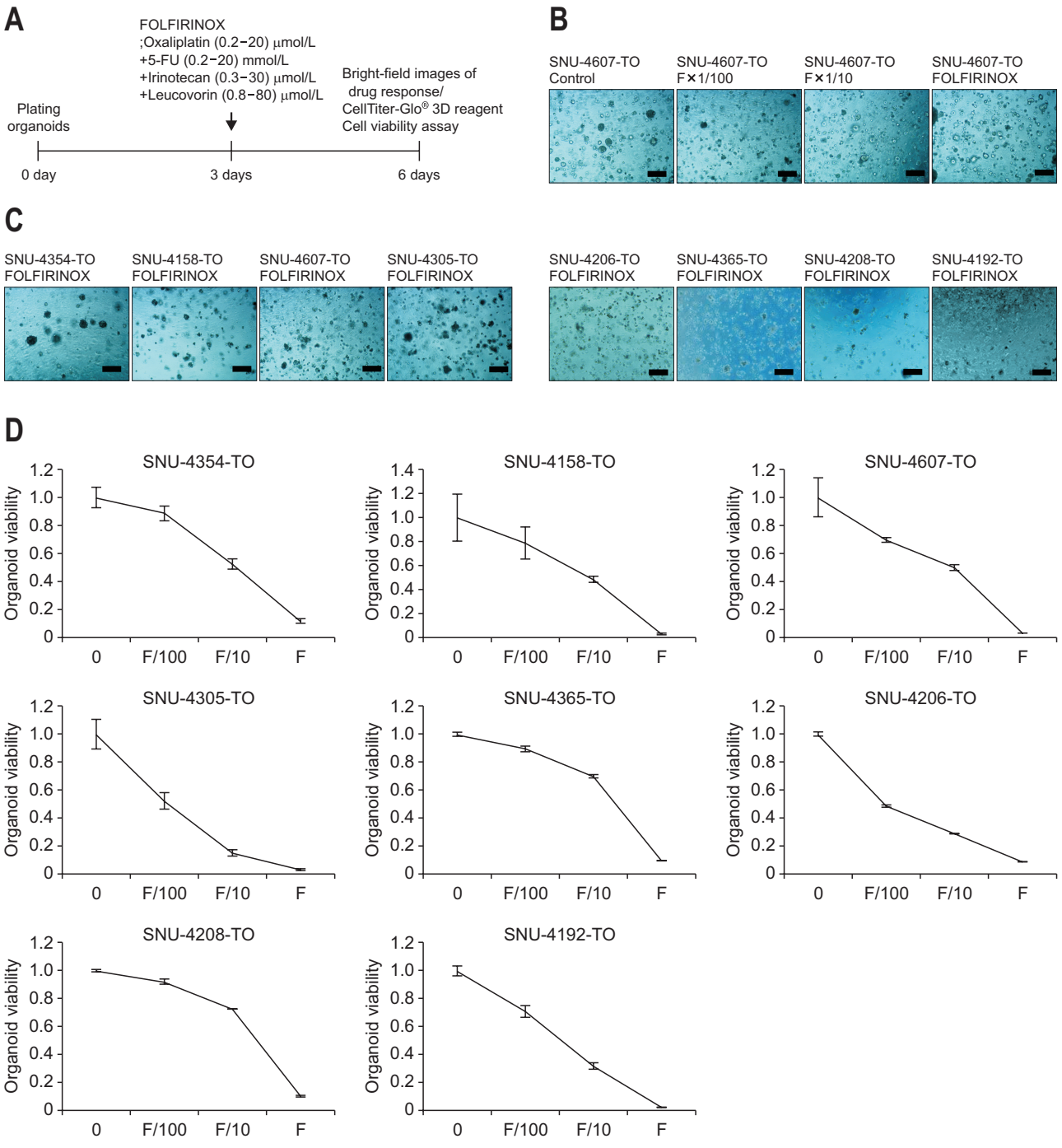
1. Within 24 hours, the biopsy samples were further processed to generate organoids.
2. Before isolation, thaw Matrigel in 4°C refrigerator, and place a 24-well plate in a CO_2 incubator (5% CO_2, 37°C) to pre-warm it.
3. Wash the biopsy samples with ice-cold Dulbecco's phosphate-buffered saline (DPBS) in 50 mL conical tube, until nearly all debris removed.
4. Cut the sample with fine scissors into pieces of less than 3 mm in size on a petri dish, and transfer the pieces into a 50 mL conical tube.
5. Add 30 mL of ice-cold DPBS, and wash the fragments 3 times. Gently mix the fragments, and after centrifuging the fragments for 3 minutes or less, discard the supernatant.
6. Allow the fragments to settle, discard the supernatant, and if the content has many red blood cells, add red cell lysis buffer.
7. Add 10 mL of ice-cold DPBS, gently mix the content, and examine one drop of the supernatant under a microscope, to determine whether it contains crypts.
8. To collect organoids fractions, centrifuge them at 1,200 rpm for 3 minutes at 4°C. Carefully discard the supernatant, without disturbing the pellet.
9. Resuspend the pellets in 1 mL of ice-cold DPBS, and transfer into a 1.5 mL Eppendorf tube .
10. Place 20 μL of the cell suspension in a petri dish, and count the crypts under a microscope. Calculate the total number of cells (counted cells ×50).
11. Add the appropriate volume of ice-cold DPBS to the tube (50 μL of Matrigel for 100 organoids).
12. Using a 200 μL pipette, resuspend the cell pellet with Matrigel (50 μL of Matrigel for 100 organoids).
13. Apply 50 μL of the suspension in Matrigel (containing 100 organoids) to the center of each well of a pre-warmed 24-well plate.
14. Place the plate in a CO_2 incubator (5% CO_2, 37°C) for 10 minutes, to allow complete polymerization of the Matrigel.
15. Add 500 μL of optimized complete medium to each well, and incubate at 37°C. To avoid anoikis, supplement the culture medium with 10 μM Y-27632 for the first 2 days.
16. Every 3 to 4 days after plating the organoids, replace the medium with 500 μL of the complete medium.
17. Examine the cultures daily, and passage the organoids when outgrowth is apparent. Organoids are generally passaged at a 1: (5–6) split ratio every 5 to 7 days, respectively.

1. Library capture

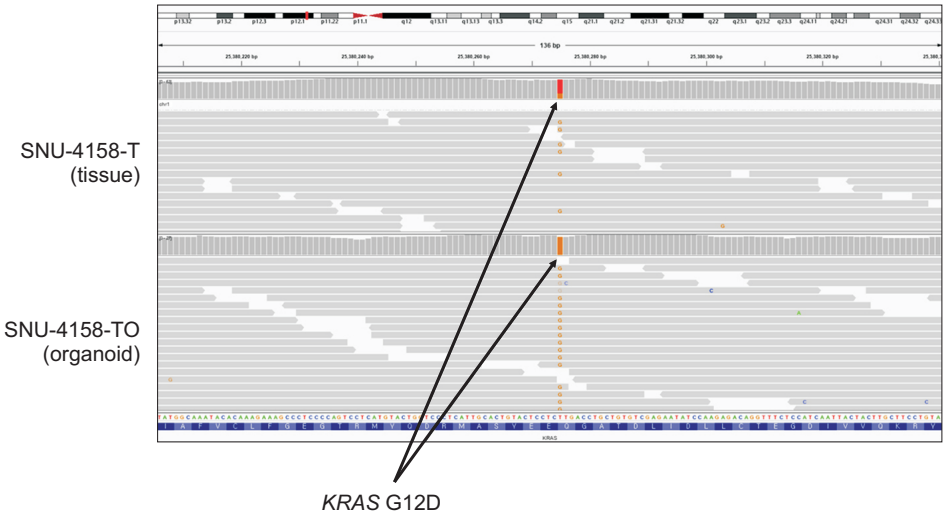
SureSelect sequencing libraries were prepared according to the manufacturer's instructions (Agilent SureSelect all Exon kit 50 Mb), using the Bravo automated liquid handler. Three μg of genomic DNA in 120 mL EB buffer was fragmented to a median size of 150 bp using the Covaris-S2 instrument (Covaris, Woburn, MA, USA) with the following settings: duty cycle 10%, intensity 5, cycles per burst 200, and mode frequency sweeping for 360 seconds at 4℃. The fragmentation efficiency was evaluated by capillary electrophoresis on DNA1000 chips (Bioanalyzer, Agilent, Santa Clara, CA, USA). Sequencing adapters were ligated on the DNA fragments, following the manufacturer's protocol (Agilent). The adapter-ligated DNA was amplified by polymerase chain reaction (PCR). The quality of the PCR products was assessed by capillary electrophoresis (Bioanalyzer, Agilent). SureSelect hyb #1, #2, #3, and #4 reagents (Agilent) were mixed to prepare the hybridization buffer. The amplified DNA fragments were concentrated 500 ng in 3.4 μL. SureSelect block #1, #2, and #3 reagents (Agilent) were added to the 500 ng of DNA. The hybridization buffer and the DNA blocker mix were incubated for 5 minutes at 95℃, and then for 10 minutes at 65℃ in a thermal cycler. RNase block (Agilent) was added to the SureSelect oligo capture library (Agilent). The capture library was incubated for 2 minutes at 65℃. First the hybridization buffer, and then the DNA blocker mix were added to the capture library, and the mixture was incubated for 24 hours at 65℃ in a thermal cycler. Fifty μL of streptavidin-coated Dynal MyOne Streptavidin T1 (Invitrogen, Waltham, MA, USA) were washed three times with 200 mL SureSelect binding buffer (Agilent), and resuspended in 200 mL of the binding buffer. The hybridization mixture was added to the bead suspension, and incubated for 30 minutes at room temperature with mixing. The beads were washed with 500 mL SureSelect wash buffer #1 (Agilent) for 15 minutes at room temperature, and 3 times with 500 mL SureSelect wash buffer #2 (Agilent) for 10 minutes at 65℃. DNA was eluted with 50 mL SureSelect elution buffer (Agilent) for 10 minutes at room temperature. Fifty mL of SureSelect neutralization buffer (Agilent) was added to the eluted DNA. The reaction product was purified with the AM-Pure XP beads (Beckman, Brea, CA, USA). The captured library was amplified to add index tags using Herculase II Fusion DNA Polymerase (Finnzymes, Espoo, Finland). The quality of the amplified libraries was verified by capillary electrophoresis (Bioanalyzer, Agilent). After QPCR using SYBR Green PCR Master Mix (Applied Biosystems, Waltham, MA, USA), six libraries that index tagged in equimolar amounts in the pool were combined. Cluster generation occurred in the flow cell on the cBot automated cluster generation system (Illumina, San Diego, CA, USA).

2. Analysis of captured reads

The flow cell was loaded on HISEQ 2500 sequencing system (Illumina), and sequencing performed with 2× 100 bp read length. Those paired-end sequences were firstly mapped to the human genome, where the reference sequence was UCSC assembly hg19 (original GRCh37 from NCBI, February 2009), using the mapping program BWA (version 0.7.12), and a mapping result file was generated in BAM format using BWA-MEM. Then, Picard-tools (version 1.130) was applied, in order to remove PCR duplicates. The local realignment process was performed to locally realign reads with BAM files. By using Genome Analysis Toolkit, base quality score recalibration and local realignment around indels were performed. Haplotype Caller of GATK was used for variant genotyping for each sample based on the BAM file previously generated (SNP and short indels candidates were detected). Those variants are annotated by SnpEff v4.1g, to vcf file format, filtering with dbSNP for the version of 142. Then, SnpEff was applied to filter additional databases, including ESP6500, ClinVar, and dbNSFP 2.9. Nonreference discordance rate (NDR) was calculated for the genotype difference in percentage of the biallelic SNP position where both two results have variation. NDR of less than 1% means that two samples are equal to each other; on the other hand, NDR of about 30% means that they are relatively different from each other.



Supplementary Fig. 1. (A) Protocol for drug test on organoids. Each organoid was plated on 24 well (1.0×10^5/well), cultured for 3 days, and FOLFIRINOX (oxaliplatin, 5-FU, irinotecan, and leucovorin) was treated to the complete culture medium. After 3 days of drug treatment, cell viability was measured by CellTiter®–Glo cell viability assay. (B) Dose-response representative images of organoid treated by FOLFIRINOX regimen. All of the experiments were carried out in triplicate, and data are represented as mean±SD. Scale bars, 400 μm. (C) Bright-field images of eight representative organoids treated by FOLFIRINOX. Scale bars, 400 μm. (D) FOLFIRINOX dose-response curves of eight organoids (X-axis, FOLFIRINOX concentration; Y-axis, luminescence that represents cell viability, $0<Y\leq1$). All of the experiments were carried out in triplicate, and data are represented as mean±SD.



Supplementary Fig. 2. *KRAS* mutation in tissue and organoid.

Supplementary Table 1. Baseline Characteristics of Eight Patients Who Were Selected for Drug Response Assay

Characteristics	Data
Sex, male/female	1/7
Age, mean±SD, yr	58.9±9.7
Size, mean±SD, cm	4.1±1.7
Location	
Head/uncinate	3
Body	2
Tail	3
Needle, 19G/22G	5/3
Locally advanced, cancer stage III	3
Metastatic, cancer stage IV	5
Metastasis site	
Liver	3
Lung	2
Peritoneum	2

G, gauge.

Supplementary Table 2. NDR between Biopsy Tissues and Organoids (SNU4158-T, Biopsy Tissue; SNU-4158-TO, Organoid)

	SNU-4158-T	SNU-4208-T	SNU-4309-T	SNU-4354-T	SNU-4365-T	SNU-4425-T	SNU-4607-T	SNU-3947-T	SNU-4607-TO	SNU-3947-TO	SNU-4208-TO	SNU-4158-TO	SNU-4309-TO	SNU-4365-TO	SNU-4354-TO	SNU-4425-TO
SNU-4158-T		28.71	25.65	29.50	29.49	29.17	29.51	29.04	30.15	29.69	29.47	5.27*	28.69	30.37	30.12	29.95
SNU-4208-T			29.20	27.82	29.66	29.69	29.25	29.77	30.20	30.27	7.15*	29.39	29.32	30.08	28.59	30.11
SNU-4309-T				29.47	29.25	29.32	29.33	29.82	29.97	30.21	30.27	29.13	3.16*	29.31	29.64	29.60
SNU-4354-T					29.81	29.56	29.44	29.35	30.18	30.70	29.24	30.15	29.77	30.77	4.74*	30.51
SNU-4365-T						28.98	29.00	29.44	29.78	30.24	31.02	30.42	29.53	5.38*	30.66	30.16
SNU-4425-T							28.44	28.98	29.57	29.91	30.24	29.66	29.36	29.77	30.01	5.69*
SNU-4607-T								29.32	5.80*	29.80	29.72	29.79	29.48	29.07	29.63	28.45
SNU-3947-T									29.89	6.58*	31.04	30.04	30.08	30.08	29.97	29.75
SNU-4607-TO										29.17	29.98	29.24	30.11	29.63	29.52	28.42
SNU-3947-TO											28.41	28.43	29.95	28.87	29.28	28.36
SNU-4208-TO												26.97	29.91	30.22	27.79	28.29
SNU-4158-TO													28.43	29.62	29.14	28.98
SNU-4309-TO														29.60	30.10	29.79
SNU-4365-TO															28.92	29.58
SNU-4354-TO																28.58

*Biopsy and organoid pair of same patient.

Supplementary Table 3. Degree of Agreement between AUC and (PFS or OS) on Chemotherapeutic Agents (Spearman's Rho (ρ); 8 Organoids)

	5-FU (8)	Irinotecan (8)	Oxaliplatin (8)	FOLFIRINOX (stage III)	FOLFIRINOX (stage IV)	FOLFIRINOX (8)
ρPFS	0.24	0.55	0.17	1.0	0.6	0.29
ρOS	0.12	0.45	0.21	0.5	0.7	0.48

AUC, area under the curve; PFS, progression-free survival; OS, overall survival; FOLFIRINOX, oxaliplatin, 5-FU, irinotecan, and leucovorin.
Cancer stage III: SNU-4354-PO, SNU-4206-PO, and SNU-4305-PO. Cancer stage IV: SNU-4365-PO, SNU-4158-PO, SNU-4607-PO, SNU-4208-PO, and SNU-4192-PO.