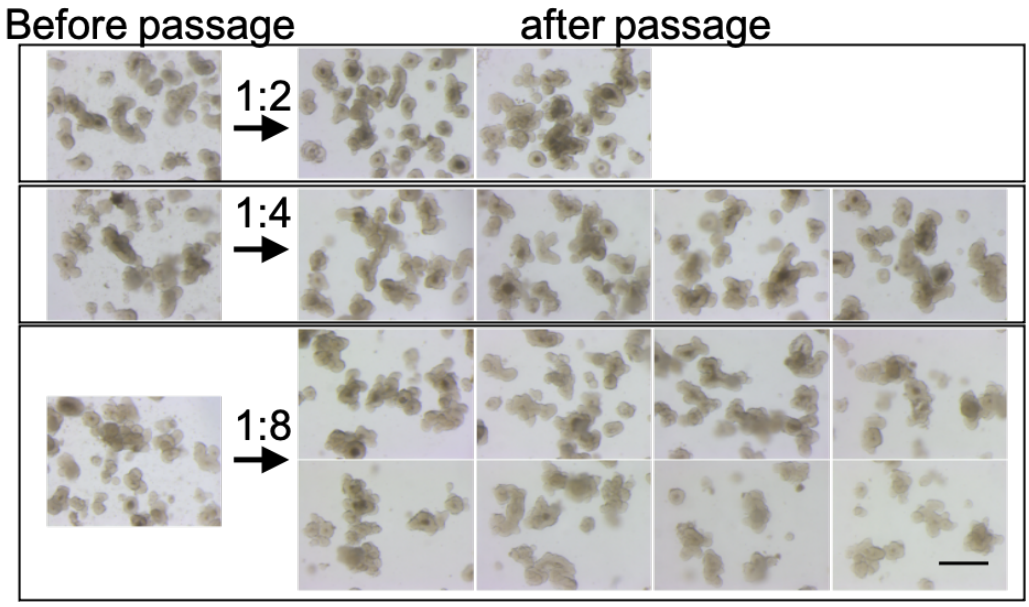


Figure S1 Morphology of PDOs before and after the passage.

PDOs (HCT64-1T) were mechanically disrupted by pipetting, splited in the indicated ratio, and cultured in ENR medium. Images were collected at four days after plating using stereomicroscope. (Bar = 400 μM)

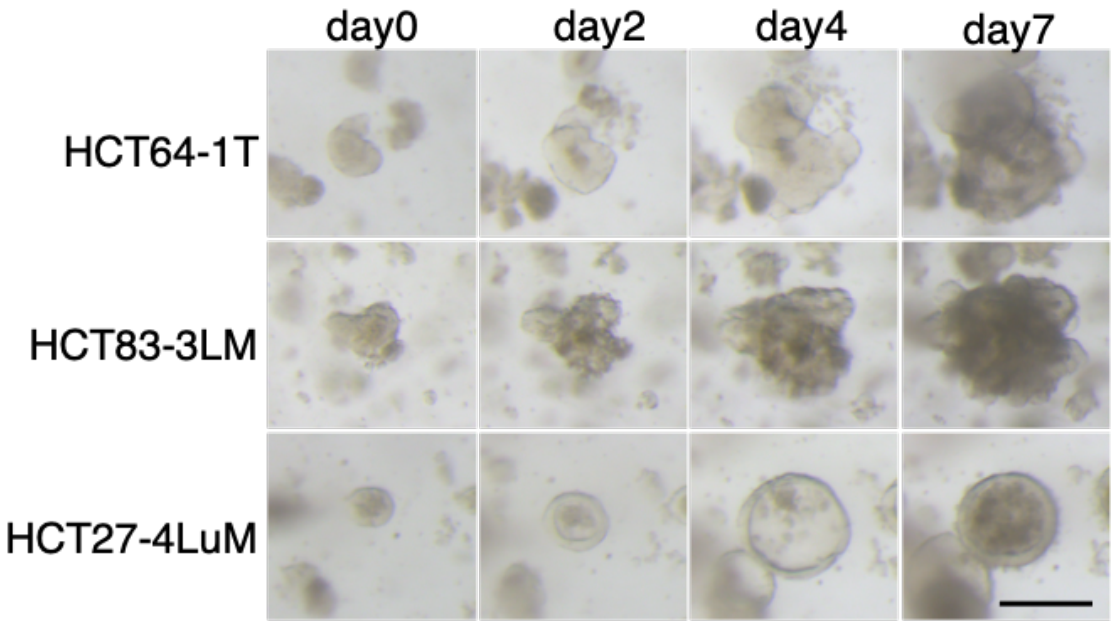


Figure S2. Morphological change during organoid growth.

Organoids were broken into small pieces by pipetting, expanded 1:4 in Matrigel and cultured in ENR medium (day0). Medium was changed every 3-4 days. To examine the morphology of organoids during the culture, each organoid was followed by identifying their location manually. Three PDOs representing type 0 (HCT64-1T), type 1 (HCT83-3LM) and type 5 (HCT27-4LuM) were shown. Image was taken at indicated days. Bar = 200μm

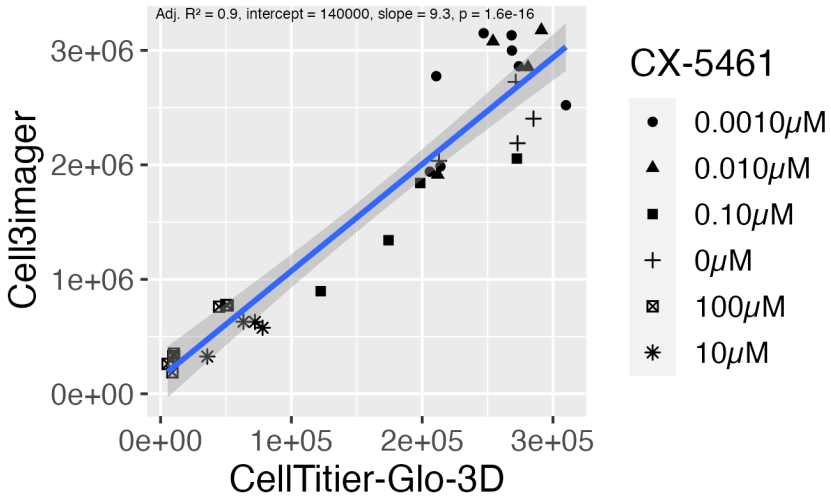
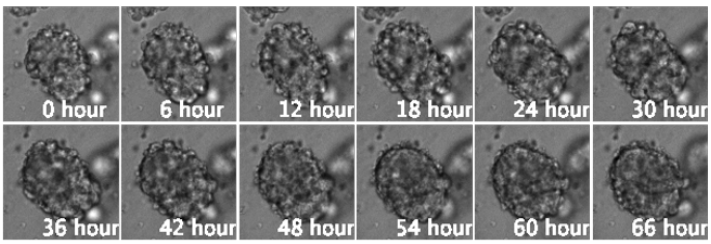


Figure S3 The correlation of cell viabilities as measured by Cell3imager and CellTitier Glo-3D.

PDO (HCT64-1T) was cultured in 96 well dishes with the indicated concentrations of CX-5461 for 10 days. Cell Viability was assessed by imaging using Cell3imager and by quantifying ATP using CellTiterGlo-3D. Linear regression line and confidence interval were shown in blue and gray, respectively.

A



B

PDOs	0 hour	6 hour	12 hour	18 hour	24 hour	30 hour	36 hour	42 hour	48 hour	54 hour	60 hour	66 hour
1	type1	type1	type1	type1	type1	type1	type1	type1	type1	type1	type1	type1
2	type1	type1	type1	type1	type1	type1	type1	type1	type1	type1	type1	type1
3	type1	type1	type1	type1	type1	type1	type1	type1	type1	type1	type1	type1
4	type1	type1	type1	type1	type1	type1	type1	type1	type1	type1	type1	type1
5	type0	type0	type0	type0	type0	type0	type0	type0	type0	type0	type0	type0
6	type0	type0	type0	type0	type0	type0	type0	type0	type0	type0	type0	type0
7	type0	type0	type0	type0	type0	type0	type0	type0	type0	type0	type0	type0
8	type0	type2	type2	type2	type0	type0	type0	type3	type3	type3	type3	type3
9	type0	type0	type0	type0	type0	type0	type0	type0	type0	type0	type0	type0
10	type0	type0	type0	type0	type0	type0	type0	type0	type0	type0	type0	type0
11	type1	type1	type1	type1	type1	type1	type1	type1	type1	type1	type1	type1
12	type0	type0	type0	type0	type0	type0	type0	type0	type0	type0	type0	type0
13	type0	type0	type0	type0	type0	type0	type0	type0	type0	type0	type0	type0
14	type0	type0	type0	type0	type0	type0	type0	type0	type0	type0	type0	type0
15	type0	type0	type0	type0	type0	type1	type1	type1	type1	type1	type1	type1
16	type0	type0	type0	type0	type0	type0	type0	type0	type0	type0	type0	type0
17	type0	type0	type0	type0	type0	type0	type0	type0	type0	type0	type0	type0
18	type0	type0	type0	type0	type0	type0	type0	type0	type0	type0	type0	type0
19	type2	type2	type2	type2	type2	type2	type2	type2	type2	type2	type2	type2
20	type0	type0	type0	type0	type0	type0	type0	type0	type0	type0	type0	type0
21	type0	type0	type0	type0	type0	type0	type0	type0	type0	type0	type0	type0

Figure S4 Analysis of time-lapse imaging of PDOs.

(A) PDOs were cultured in Matrigel, and images were taken from 4 days to 7 days after expansion. Single organoid images were manually cropped and used for classification. (B) Morphological type of 21 organoids in each time point was determined by three referees and the summary was shown.

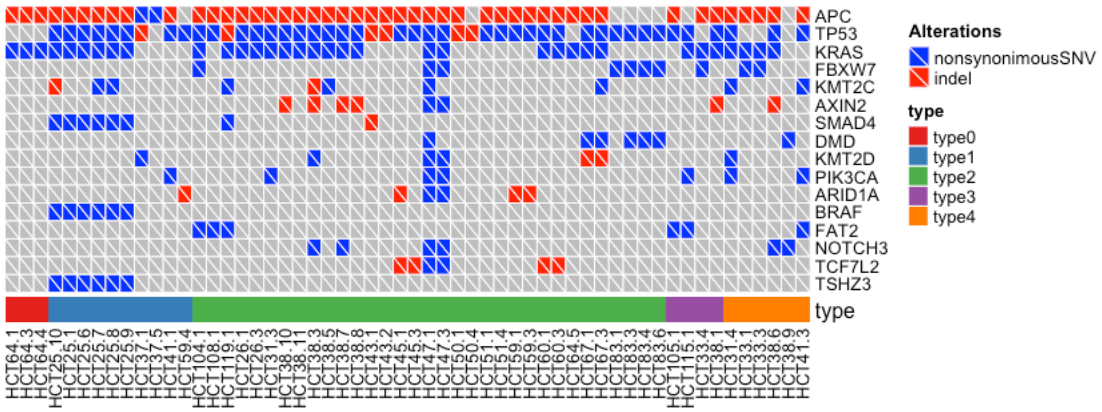


Figure S5. Mutation profile of PDOs.

Somatic mutations detected in multiple organoids were shown.