

Figure S1. Composition of CRC-PDOs. Fluorescence microscopy images for goblet cells (Muc2⁺, red), entero-endocrine cells (ChgA⁺, red), enterocytes (VL1⁺, red) and proliferating cells (Ki-67, red). Counterstain, DAPI (blue) and epithelial, E-cadherin (green). Scale bars, 100 μ m. CRC, colorectal cancer; PDO, patient-derived organoid; Muc2, mucin 2; ChgA, chromogranin A.

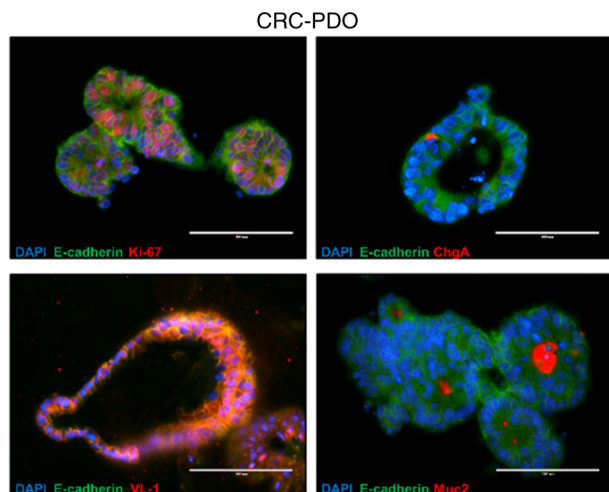


Figure S2. Immunohistochemical analysis of organoids and corresponding samples. H&E staining and CK19, CK20 and CDX2 immunostaining of CRC-PDO #3. Magnifications at 40x. Scale bar, 50 μ m. H&E, hematoxylin and eosin; CK, cytokeratin; CRC, colorectal cancer; PDO, patient-derived organoid.

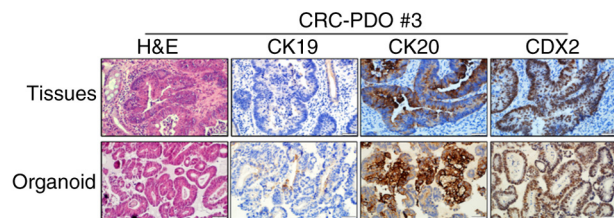


Figure S3. Dose-dependent effect of butyrate on CRC-PDOs. (A) Morphology of CRC-PDO #3 treated with butyrate of the indicated concentrations after 3 days. Scale bar, 200 μ m. (B) Relative CRC-PDO #3 size 3 days after treatment with butyrate of the indicated concentrations (n=3). (C) MTT cell viability assay of organoids described in B (n=3). Data are presented as mean \pm standard error of the mean. *P<0.05, **P<0.01. CRC, colorectal cancer; PDO, patient-derived organoid.

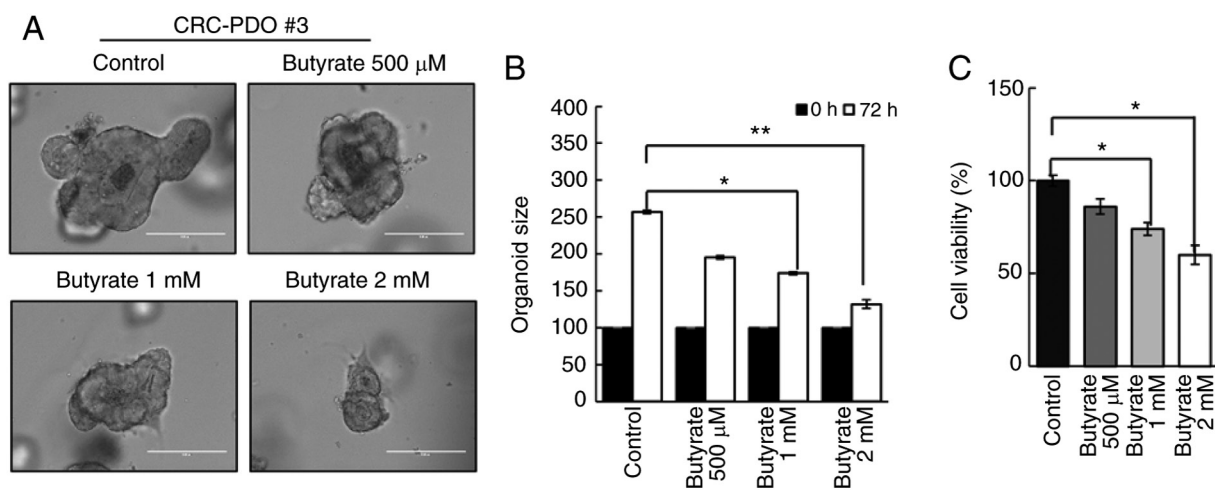


Figure S4. Expression of Ki-67 and γ -H2AX in CRC-PDOs treated with butyrate and radiation. (A) CRC-PDO #7 was treated with radiation and/or butyrate and analyzed by reverse transcription-quantitative PCR for Ki-67 after 72 h. The expression level of the target mRNA was normalized to that of GAPDH mRNA (n=3). Data are presented as mean \pm standard error of the mean. *P<0.05, **P<0.01. (B) Protein expression of γ -H2AX after RT, with or without butyrate, in the CRC-PDOs. β -actin served as a loading control. CRC, colorectal cancer; PDO, patient-derived organoid; RT, radiation treatment; IR, irradiation.

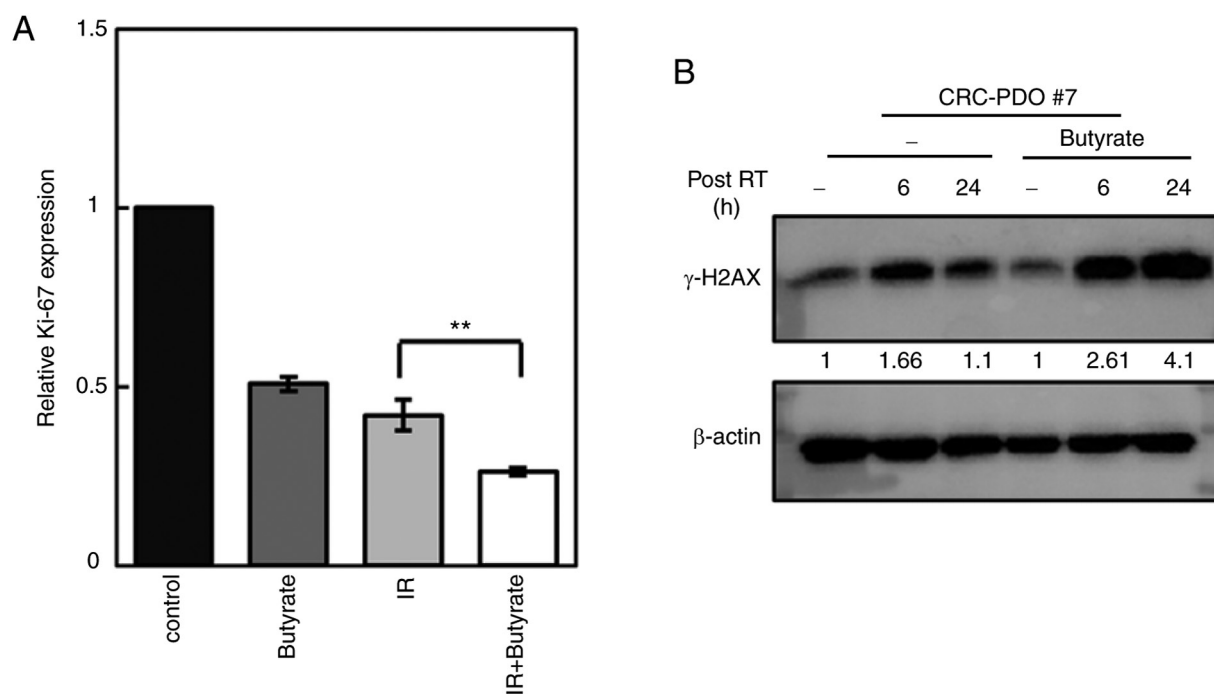


Table SI. Clinical characteristics of patients.

Patient ID	Age, years	Sex	(y)pTNM ^a	Stage	KRAS	NRAS	BRAF	MSI/MSS	Tumor location	Butyrate response
CRC-PDO #3	53	F	ypT3N1M0	III	Mt	Wt	Wt	MSS	Rectum	Response
CRC-PDO #5	85	F	ypT3N0M0	II	Wt	Wt	Wt	MSS	Rectum	Response
CRC-PDO #7	65	F	ypT3N2M0	III	Mt	Wt	Wt	MSS	Rectum	Response
CRC-PDO #8	48	M	ypT2N2M0	III	N/A	N/A	N/A	N/A	Rectum	Response
CRC-PDO #9	71	M	ypT2N0M0	I	Wt	Wt	Wt	MSS	Rectum	No response
CRC-PDO #11	53	M	ypT3N1M0	III	Wt	Wt	Wt	MSS	Rectum	No response
CRC-PDO #13	58	M	ypT2N1M0	III	Wt	Wt	Wt	MSS	Rectum	Response
CRC-PDO #33	59	F	pT3N0M0	II	Wt	Wt	Wt	MSS	Colon	No response

^aIndicates the TNM stage after preoperative therapy. All tumors were staged according to the 8th edition of the American Joint Committee on Cancer (23). F, female; M, male; Mt, mutation; Wt, wild type; MSI, microsatellite instability; MSS, microsatellite stable; CRC, colorectal cancer; PDO, patient-derived organoid; N/A, not available.

Table SII. Primer sequences.

Gene	Forward primer (5'-3')	Reverse primer (5'-3')
p21	TGTCCGTCAGAACCCATGC	AAAGTCGAAGTTCCATCGCTC
p57	GCGGCGATCAAGAAGCTGT	GCTTGGCGAAGAAATCGGAGA
Gadd45	TGCTGTGACAACGACATCAAC	GTGAGGGTTCGTGACCAGG
GAPDH	TGCACCACCAACTGCTTAGC	GGCATGGACTGTGGTCATGAG