

Supplementary Information for

Systematic evaluation of colorectal cancer organoid system by single-cell RNA-Seq analysis

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Additional file 1: Supplementary Figures

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Fig. S2 Tumor organoid cells and normal organoid cells that cultured in conditioned medium express different WNT signaling pathway target genes.

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Fig. S4 Copy number variations of patient #1, #5 and #6 that were inferred by scRNA-seq data.

Fig. S5 Whole exome sequencing of organoids that cultured in chemical-defined and conditioned media.

Fig. S6 *In vitro* cultured organoids can maintain *in vivo* point mutations and CNVs of cancer cells.

Fig. S7 Cultured organoids tend to enrich for specific molecular features.

Fig. S8 Copy number variations of patient #4 and #7 that were inferred by scRNA-Seq data.

Additional file 2: Table. S1 Basic information of patients we collected, including pathological information, sequencing technology and culture information.

Additional file 3: Table. S2 UMAP clustering result of all single cells we analyzed.

Additional file 4: Table. S3 DEGs of *in vivo* normal and tumor epithelial cells and basic information of some DEGs and marker genes

Additional file 5: Table. S4 Summary table for genomic and epigenomic sequencing data, including WGS, WES and PBAT (DNA methylome) data

Fig S1

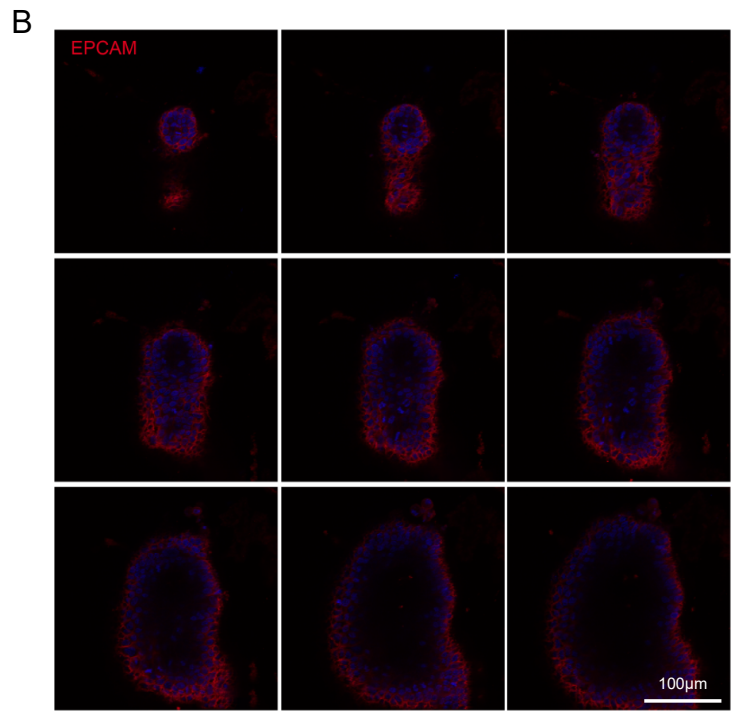
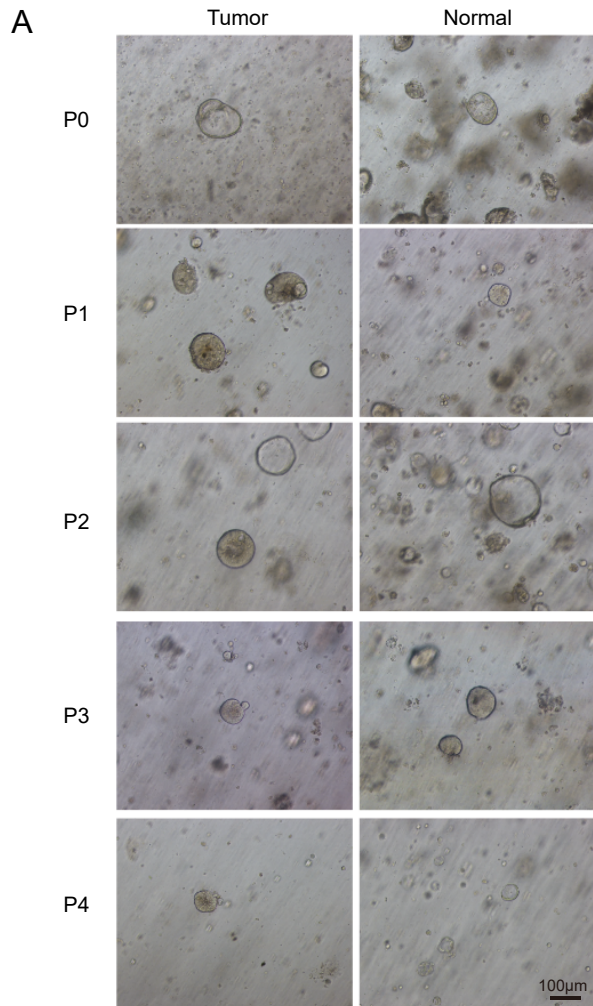


Fig. S1 Overview of sample collection and establishment of colorectal cancer organoid cultures.

- (A) Representative bright-field images of tumor- and paired adjacent normal tissue-derived organoids of the patient from different passages. All scale bars, 100 μm . "P" stands for passage.
- (B) Whole-mount immunofluorescent staining of EPCAM on normal tissue-derived organoids. Series of Z-axis optical sections are shown from left to right and from up to down. All scale bar, 100 μm .

Fig. S2

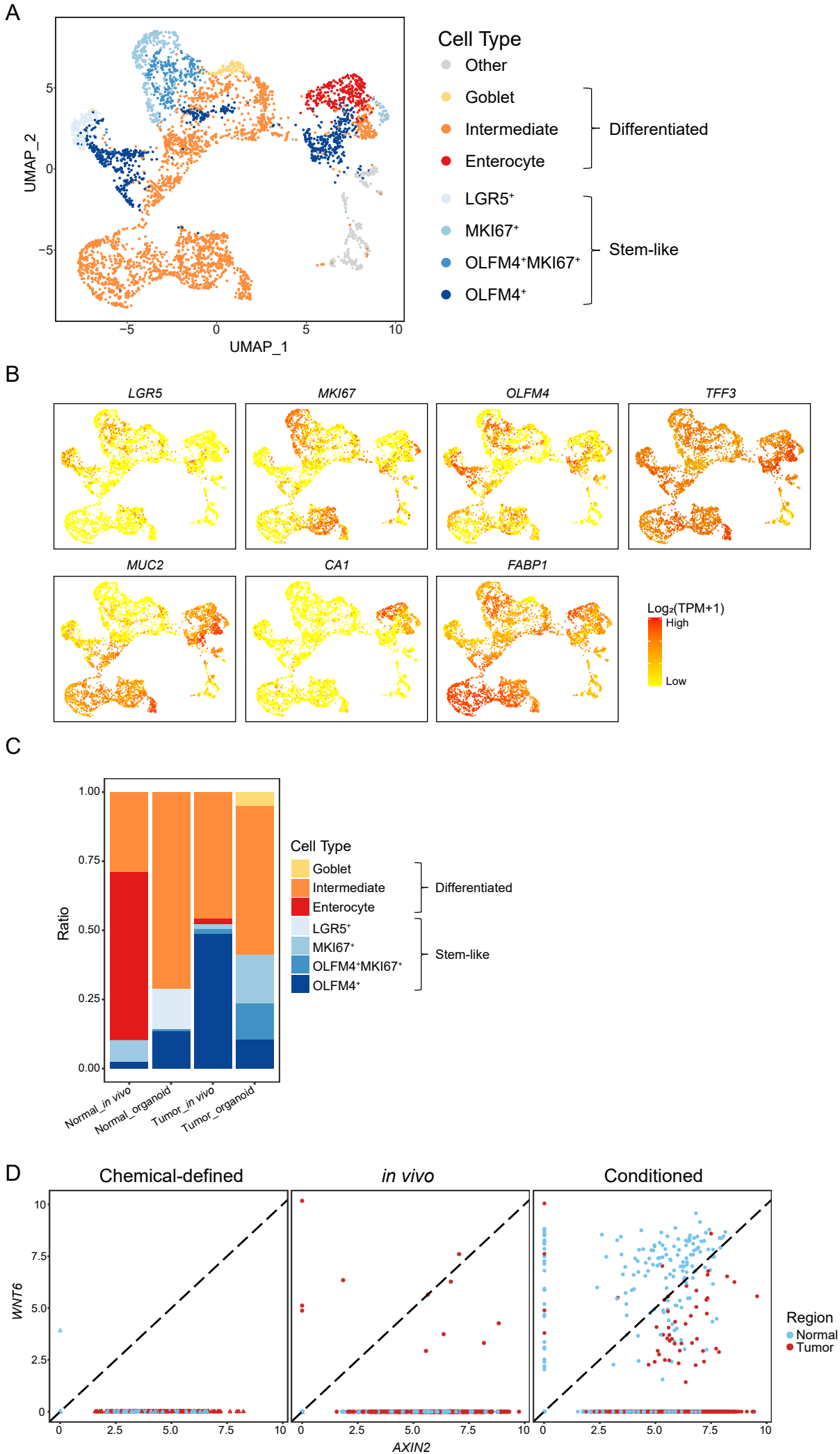


Fig. S2 Tumor organoid cells and normal organoid cells that were cultured in conditioned medium express different WNT signaling pathway target genes.

- (A) UMAP clustering of cells and colors represent epithelial subtypes.
- (B) Expression patterns of intestinal cell type markers were projected onto the UMAP plot. The colors from yellow to red represent expression levels from low to high. *OLFM4* and *LGR5*, intestinal stem/pluripotent cell markers; *MKI67*, cell proliferative marker; *CA1* and *FABP1*, enterocyte markers; *MUC2* and *TFF3*, goblet cell markers. Basic information of these cell type markers can be found in Supplementary Table S3.
- (C) Bar plot showing the distribution of epithelial subtypes in different conditions.
- (D) Scatterplot showing the expression patterns of the WNT signaling pathway target genes *WNT6* and *ANXIN2* in each individual cell. Different colors represent cells collected from normal or tumor regions.

Fig. S3

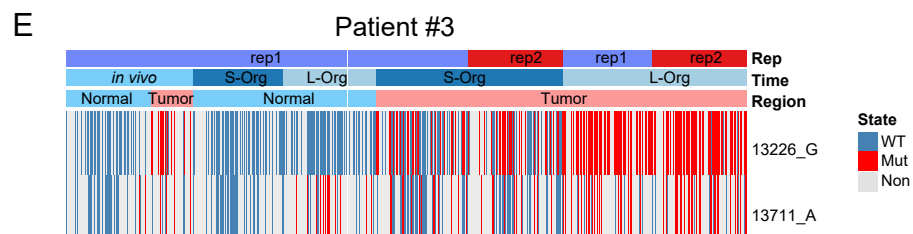
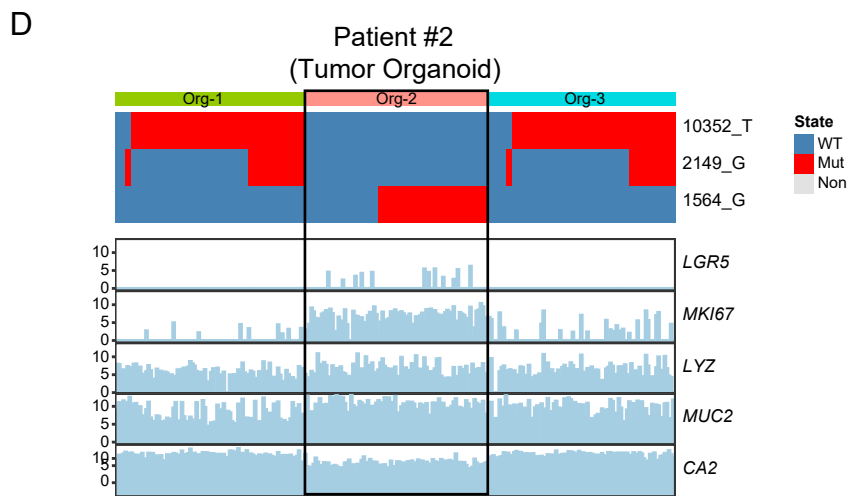
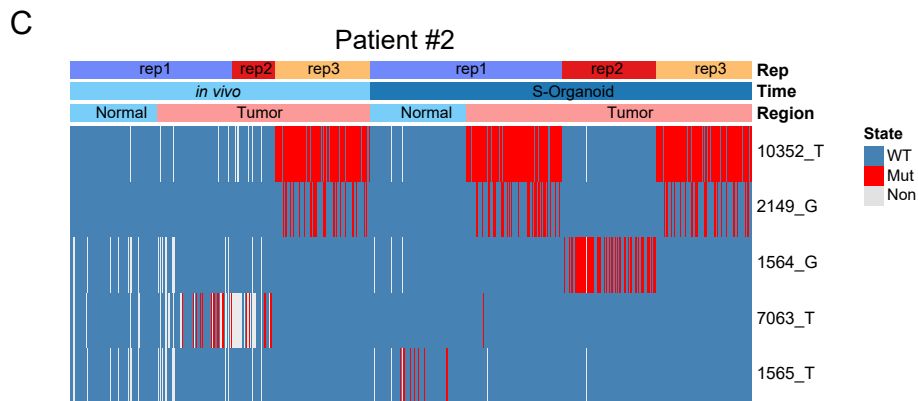
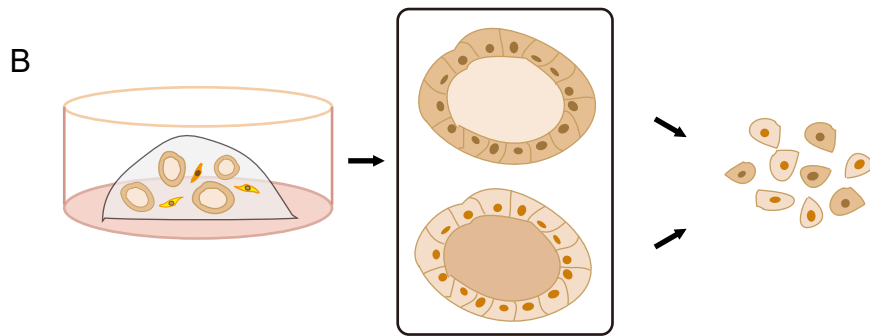
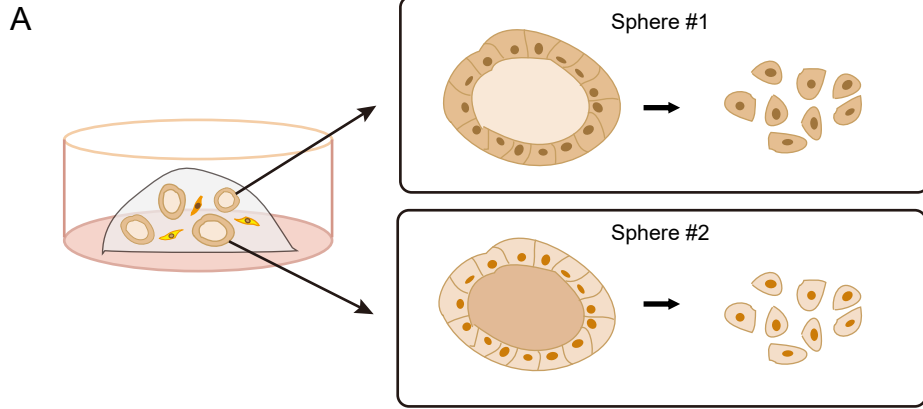
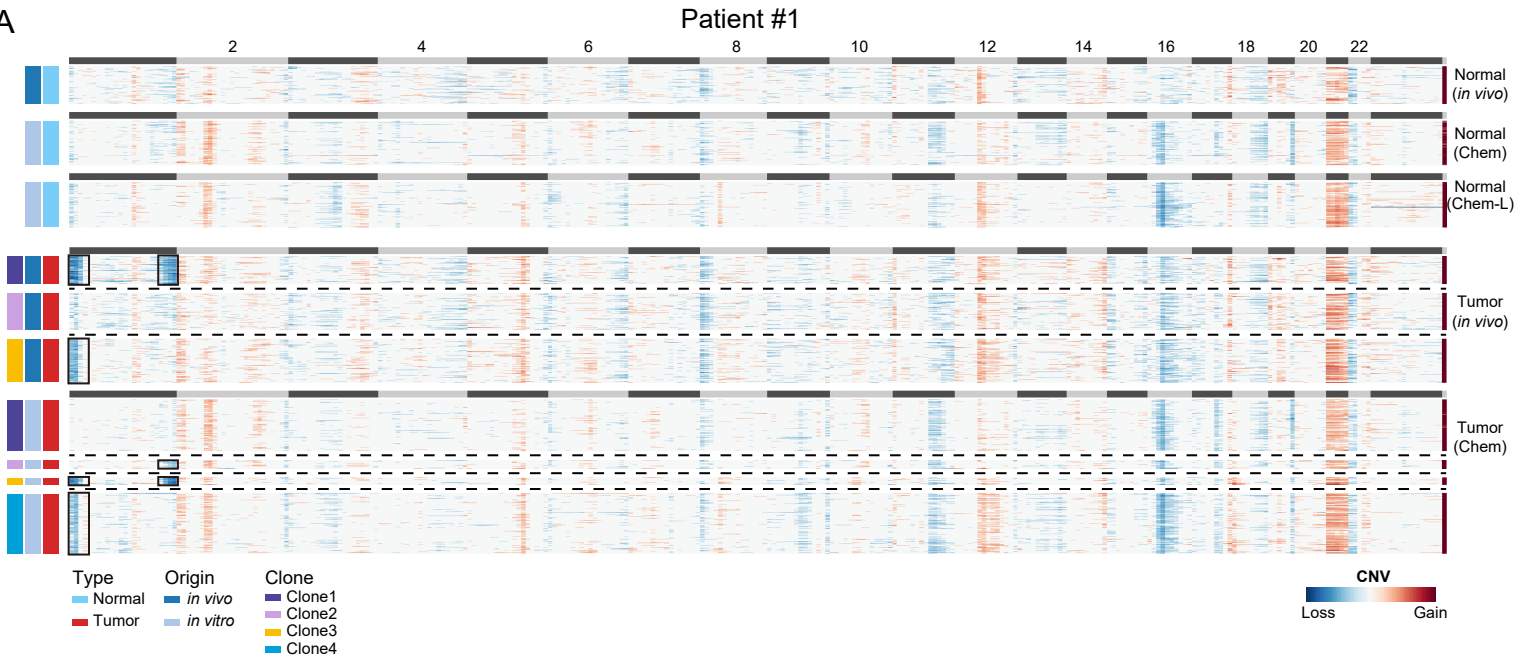


Fig. S3 Mitochondrial mutations of patients #2 and #3.

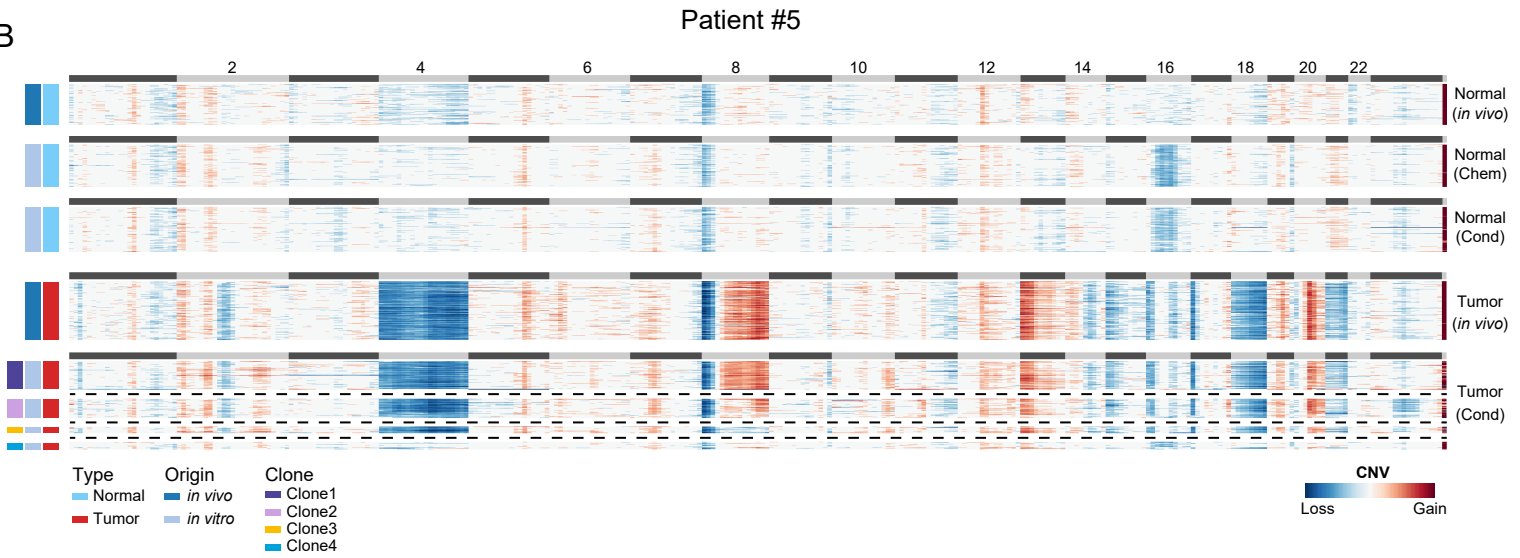
- (A) Schematic diagram showing single cell picking strategies of patient #1 and #2 for *in vitro* cultured organoids.
- (B) Schematic diagram showing single cell picking strategies of other patients for *in vitro* cultured organoids.
- (C) Heatmap showing mitochondrial mutation of patient #2 (P2). Red represents mutant site, blue represents wild type and grey represents site with reads lower than 9. Chem, chemical-defined medium; Cond, conditioned medium.
- (D) Heatmap showing the mitochondrial mutations in tumor organoid cells of patient #2 that cultured in chemical-defined medium. Cells of three single organoid spheres were showed. Bar plot showing the corresponding expression levels of intestinal cell type markers. *OLFM4*, pluripotent cell marker; *MKI67*, cell proliferation marker; *LYZ*, Paneth cell marker; *MUC2*, goblet cell marker; *CA2*, enterocyte marker
- (E) Heatmap showing mitochondrial mutation of patient #3. Red represents mutant site, blue represents wild type and grey represents site with reads lower than 9. Chem, chemical-defined medium; Cond, conditioned medium.

Fig. S4

A



B



C

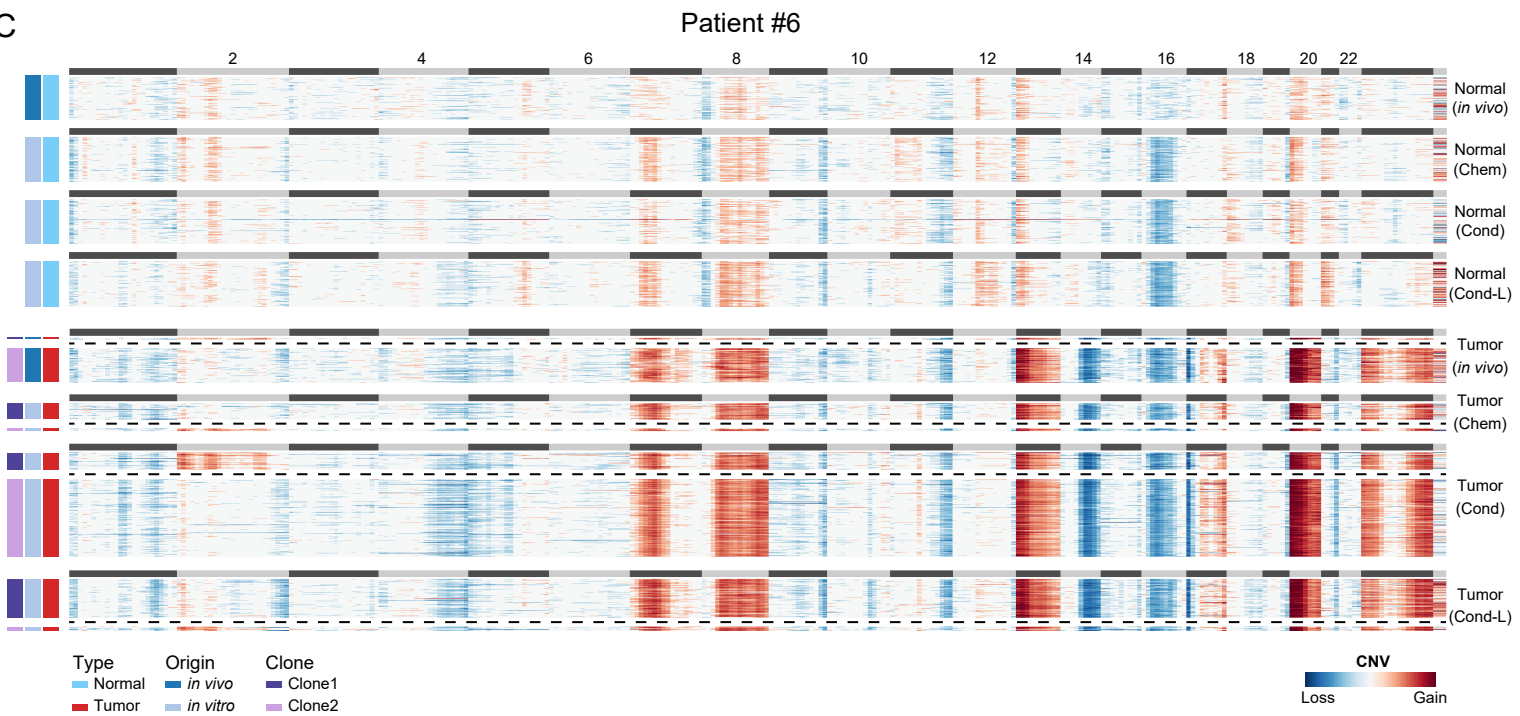


Fig. S4 Copy number variations of patient #1, #5 and #6 that were inferred by scRNA-Seq data.

- (A) CNV patterns of *in vivo* and *in vitro* cultured tumor cells of patient #1 (P1). Blue, deletion. White, normal. Red, amplification. There were three and four subclones were identified for tumor cells *in vivo* and *in vitro* respectively. We clustered the cells based on their CNV patterns, and we defined cells possessing different CNVs as distinct clones.
- (B) CNV patterns of *in vivo* and *in vitro* cultured tumor cells of patient #5 (P5). Blue, deletion. White, normal. Red, amplification. There were four subclones were identified for tumor cells *in vitro*.
- (C) CNV patterns of *in vivo* and *in vitro* cultured tumor cells of patient #6 (P6). Blue, deletion. White, normal. Red, amplification. Two subclones were identified for both *in vivo* and *in vitro* tumor cells.

Fig. S5

A

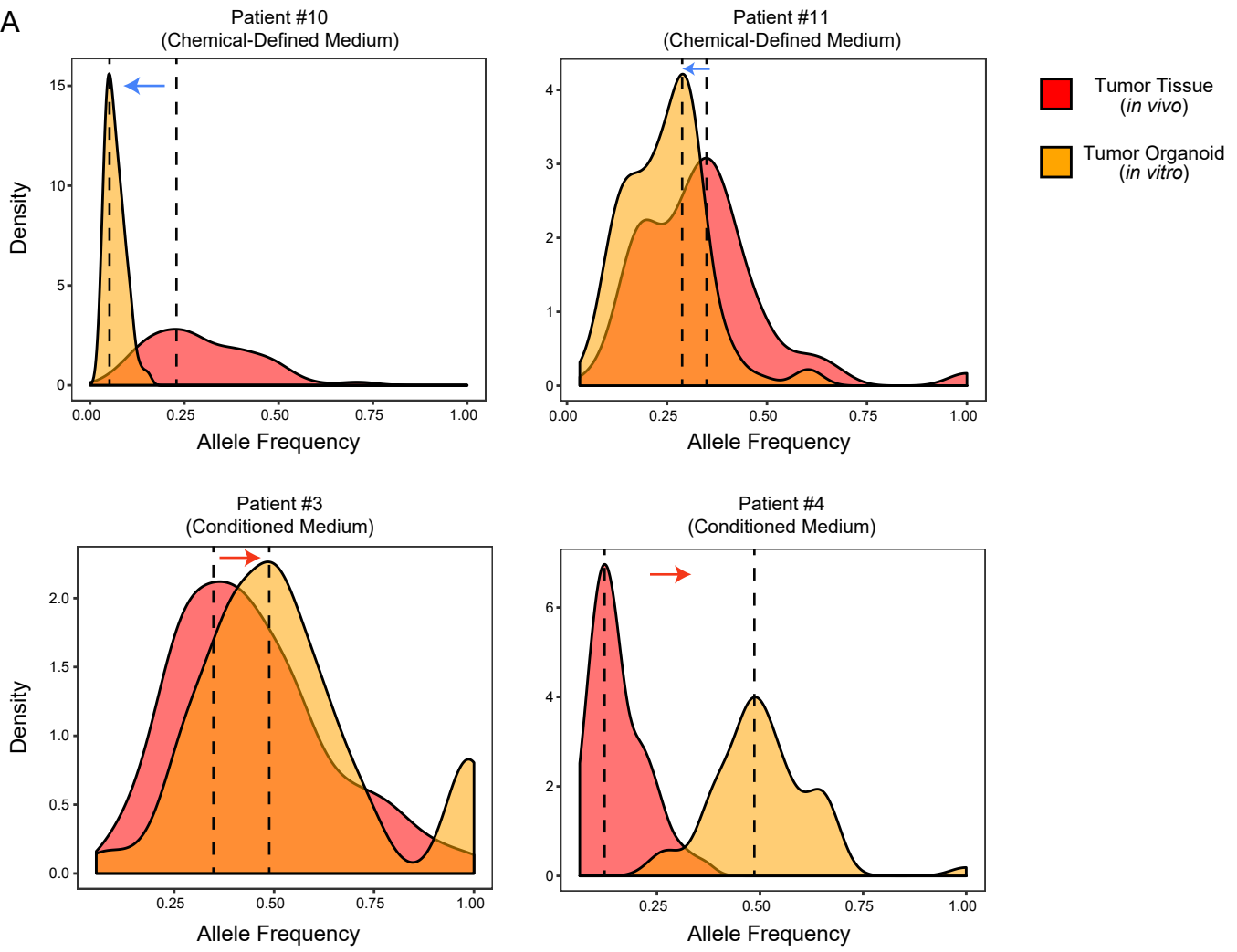
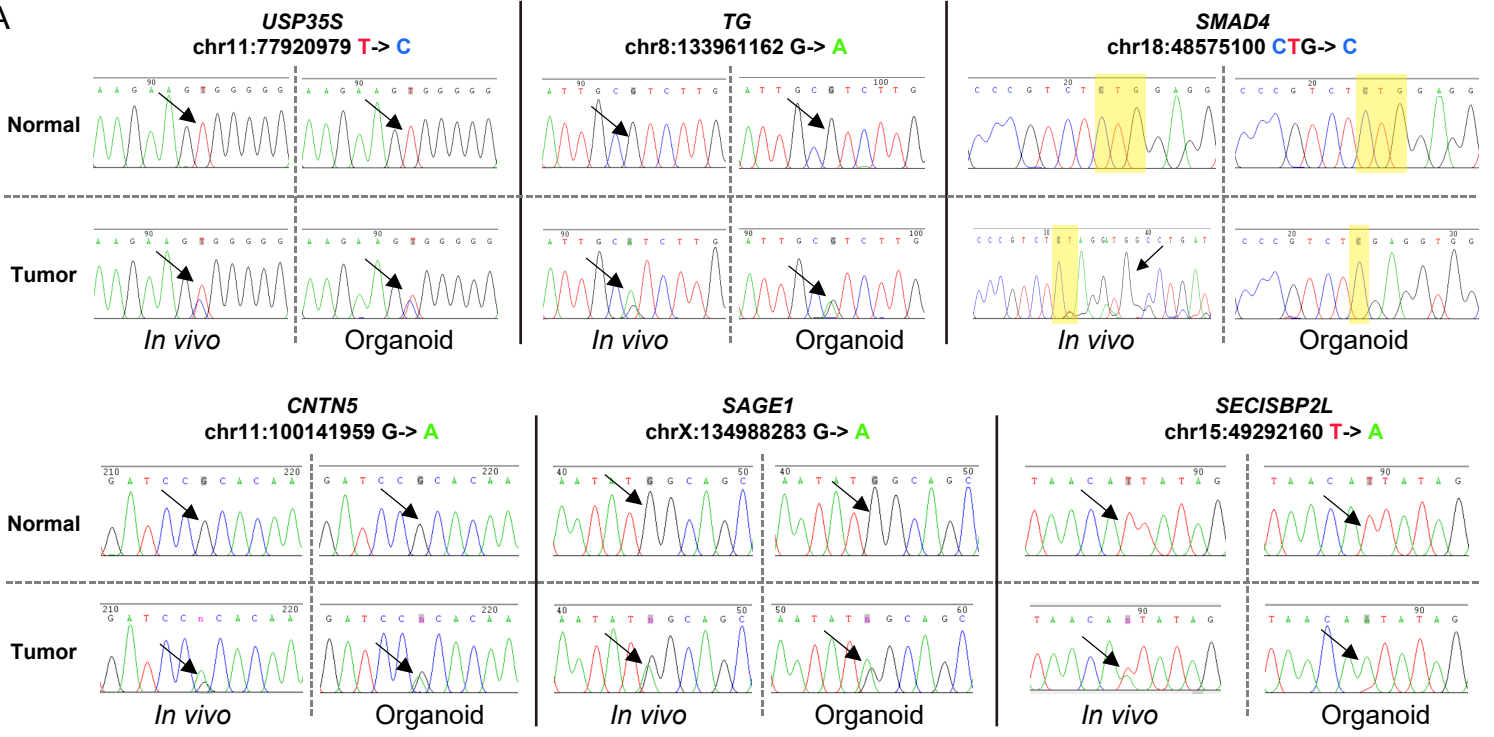


Fig. S5 Whole exome sequencing of organoids that were cultured in chemical-defined and conditioned media.

(A) Density plot showing the allele frequency of somatic mutations that shared by in vivo and in vitro tumor cells. Tumor organoids of patient #10 and #11 (P10 and P10) were cultured in chemical-defined medium, while tumor organoids of patient #3 and #4 were cultured in conditioned medium.

Fig. S6

A



B

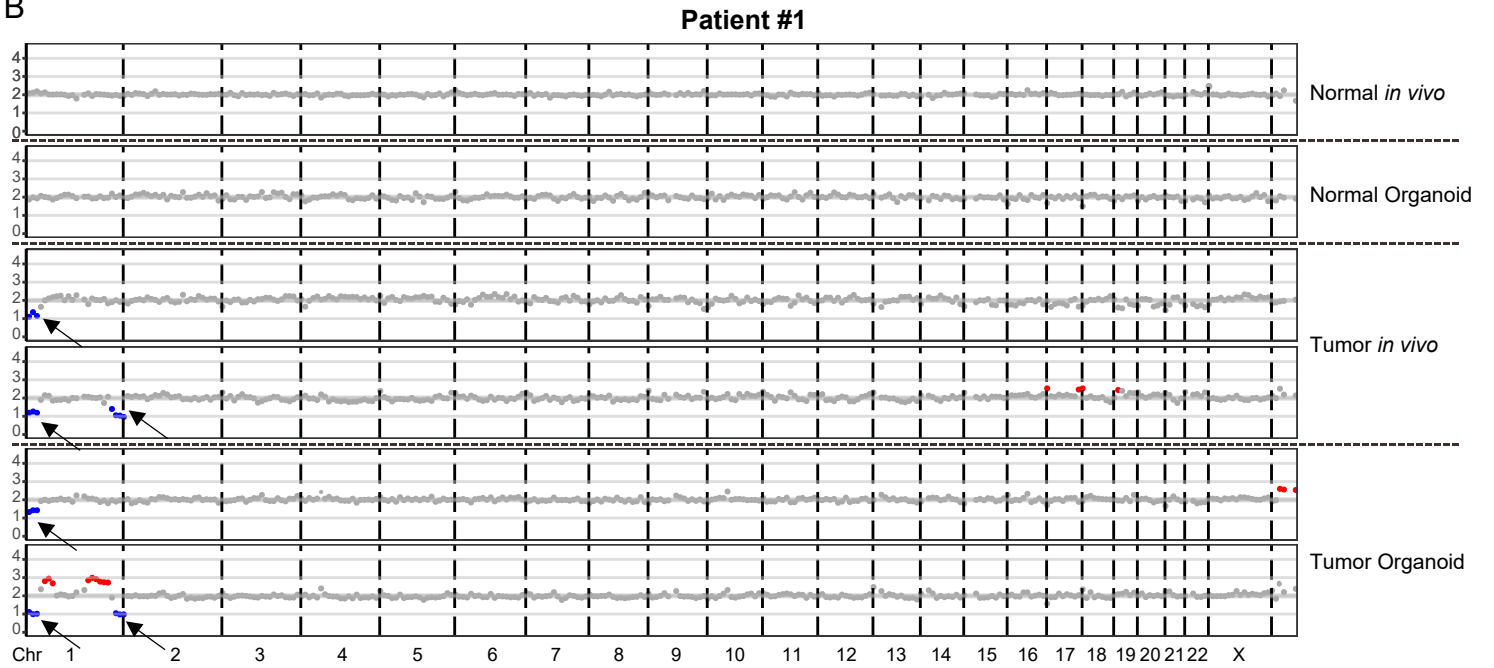


Fig. S6 *In vitro* cultured organoids can maintain *in vivo* point mutations and CNVs of cancer cells.

- (A) Point mutation verification of six mutation sites for patient #1 *in vivo* tissues and derived organoids by Sanger sequencing. Arrows indicate the sites of mutation.
- (B) Bulk whole genome sequencing (WGS) of *in vivo* and *in vitro* tumor cells and normal cells for patient #1. Blue, deletion. Red, amplification. Arrows indicate the CNV site.

Fig S7

Patient #8

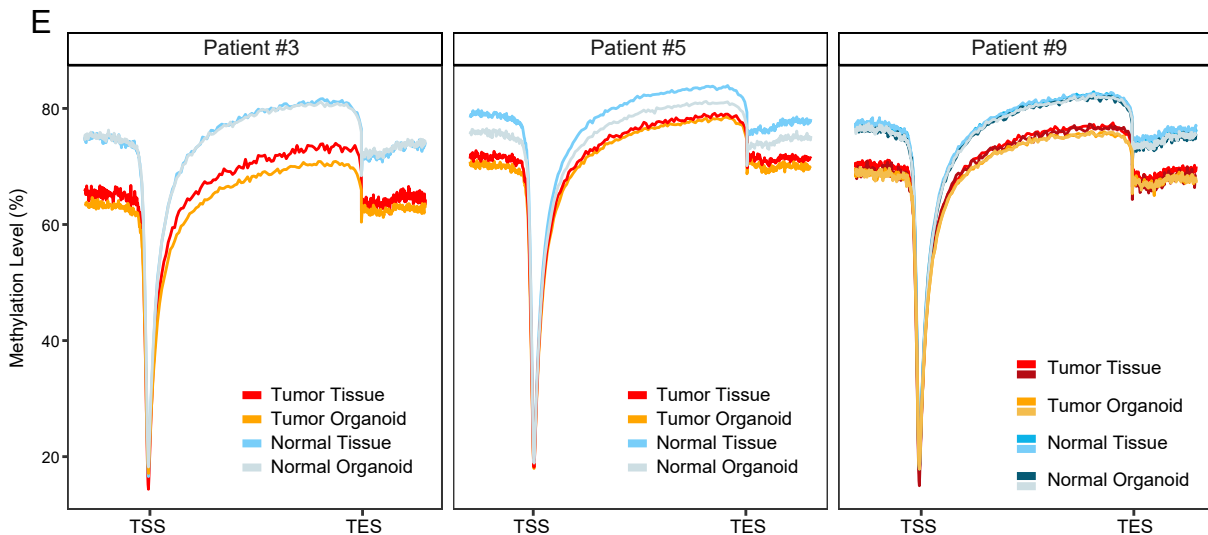
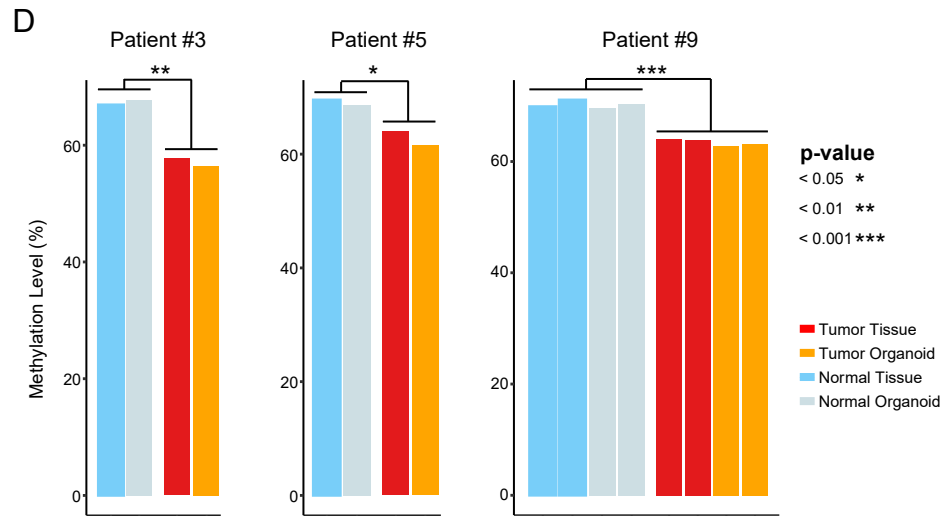
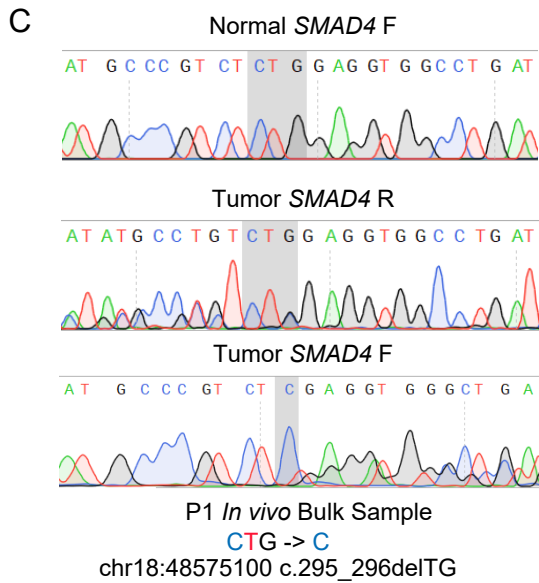
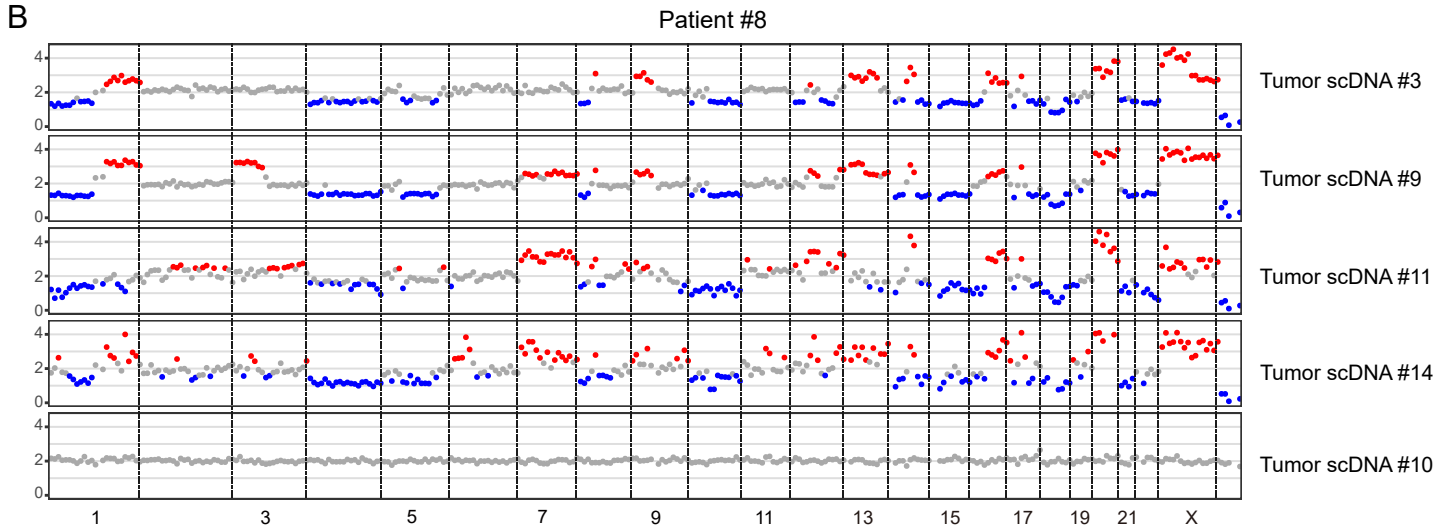
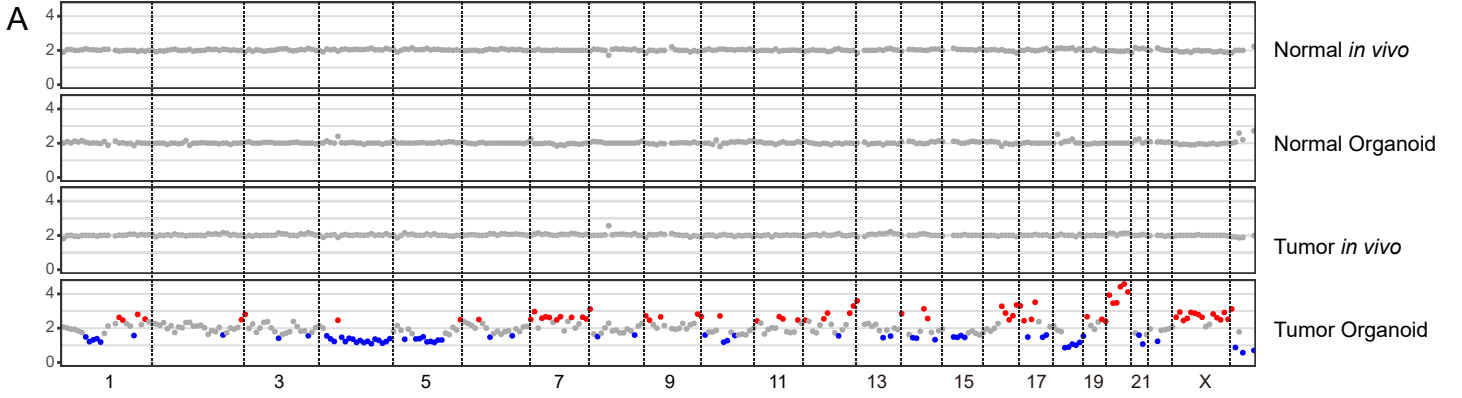


Fig. S7 Cultured organoids tend to enrich for specific molecular features.

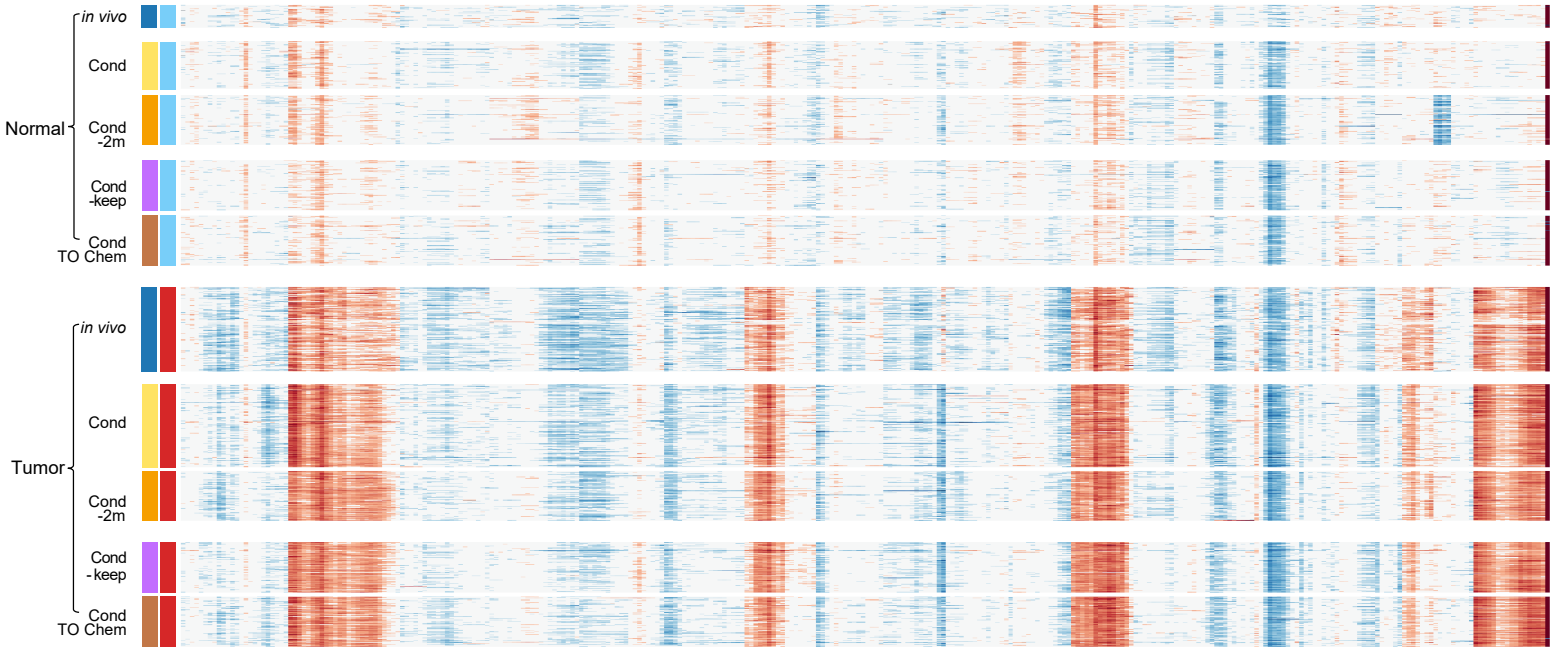
- (A) CNVs of patient #8 at bulk levels for magnetic-activated cell sorting (MACS)-enriched EPCAM⁺ tumor epithelial cells and adjacent normal cells as well as derived organoid cultures.
- (B) CNVs of patient #8 at the single-cell level for tumor epithelial cells *in vivo*. In total, we sequenced 20 cells and other cells we not showed exhibited similar pattern as cell #10.
- (C) Point mutation verification of *SMAD4* for patient #1 *in vivo* tissues and derived organoids by Sanger sequencing.
- (D) Bar plot showing the global DNA methylation ratio of *in vivo* and *in vitro* cultured tumor cells and normal cells. All organoids of these patients were constructed in conditioned medium.
- (E) Line plot showing the DNA methylation ration around transcriptional start site (TSS), gene body and transcriptional end site (TES). The x axis represents the relative position of genes and y axis represents the average DNA methylation levels. Different color represents cells with different origins. It reflects that the organoids cultured *in vitro* maintained the global DNA methylation signatures of corresponding cells *in vivo*. *In vivo* normal tissues and *in vitro* normal tissue-derived organoids have similar DNA methylation profiles, and the methylation levels are higher than both *in vivo* tumor cells and *in vitro* tumor-derived organoids as expected.

Fig. S8

A

Patient #4

Medium 1 3 5 7 9 11 13 15 17 19 21 X



B

Patient #7

Medium 1 3 5 7 9 11 13 15 17 19 21 X

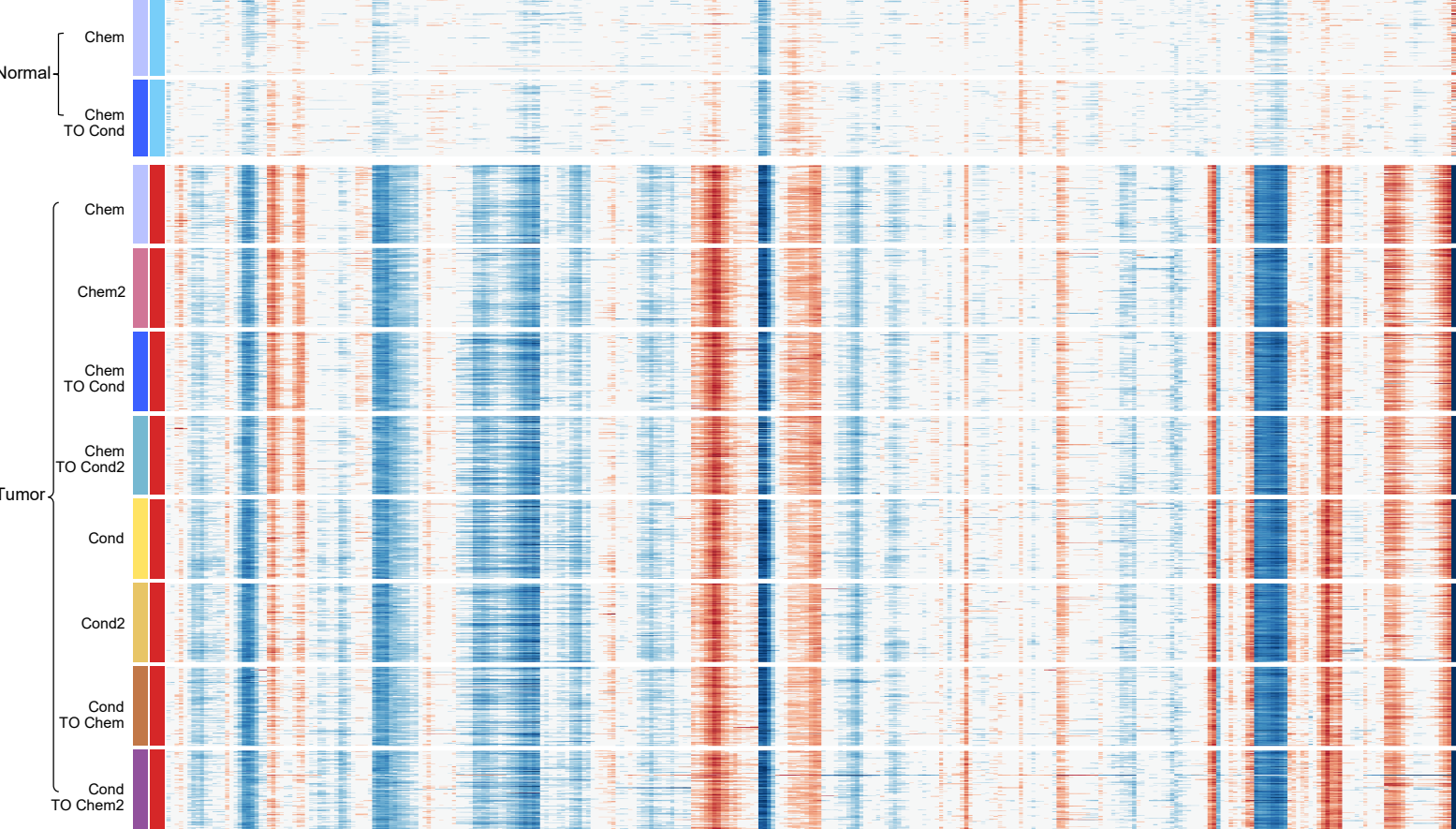


Fig. S8 Copy number variations of patient #4 and #7 that were inferred by scRNA-Seq data.

- (A) CNV patterns of *in vivo* and *in vitro* cultured tumor cells and normal cells of patient #4. Blue, deletion. White, normal. Red, amplification.
- (B) CNV patterns of *in vivo* and *in vitro* cultured tumor cells and normal cells of patient #7. Blue, deletion. White, normal. Red, amplification.