Bioinformatic analysis

FastQs were aligned to GRCh38 using nf-core/rnaseq 3.0. Bam files were aligned to TxDb.Hsapiens.UCSC.hg38.knownGene using GenomicAlignments::summarizeOverlaps4 to produce raw gene counts. DEGs were found using DESeq25 subsetting to stem cells (d4WENR) and differentiated cells (d4ENR) and comparing CH-223191 and DMSO treated organoids and controlling for replicate. Reported DE genes have p-value <0.05.

Principal component analysis figures were made with vanceStabilizingTransformation and plotPCA functions of DESeq2 and plotted with ggplot2.

Values in RPM\_LFC\_violins were computed from reads per million and plotted using ggplot2, plotting pseudo log2 fold change (adding 0.5) of RPM scores for the CH-223191 treated case over the DMSO treated case.

The heatmap showing selected MYC target genes was generated using Morpheus (https://software.broadinstitute.org/morpheus).

Comparison with publicly available data sets

Single cell data, epi\_raw\_counts02.h5ad, was downloaded from https://www.gutcellatlas.org/ and subsequent single-cell analysis was performed using Seurat7 on healthy adult cells from the large intestine. For each cell type the marker genes were computed using FindMarkers restricting to adjusted p-value <0.05. Gene set enrichment significance of these sets was determined using fgsea comparing these with the log2 fold enrichment in the DESeq2 analysis comparing d4ENR CH-223191 treated to differentiated DMSO treated organoids.

Seurat objects for inflamed, non-inflamed and healthy control colon samples were kindly provided by the Simmons lab8 and merged. Differential gene expression between inflamed cells and healthy controls and between non-inflamed cells and healthy controls was determined by subsetting to epithelial cells and using FindMarkers to find DE genes with padj < 0.05.

DEGs were compared to the d4ENR DEGs identified by RNAseq treated with CH-223191 vs DMSO, ranked by log2(FC). Gene Set Enrichment Analysis was performed using GESA v.4.2.2 (software.broadinstitute.org/gsea/index.jsp). The ranked DEG list from the RNAseq data was used to perform a correlation with the dataset of Dotti et al.9 comparing to UC vs. control patient organoids.

Transcriptional regulators upstream of DEGs were predicted using Ingenuity Pathway Analysis restricting to a p-value of <0.05, predicted to be activated or inhibited.