

Prolonged culturing of colonic epithelial organoids derived from healthy individuals and ulcerative colitis patients results in the decrease of LINE-1 methylation level

Inciuraite Ruta^{1†}, Steponaitiene Ruta^{1†}, Raudze Odeta¹, Kulokiene Ugne¹, Kiudelis Vytautas², Lukosevicius Rokas¹, Ugenskiene Rasa³, Adamonis Kestutis², Kiudelis Gediminas^{1,2}, Jonaitis Laimas Virginijus^{1,2}, Kupcinskas Juozas^{1,2}, Skieceviciene Jurgita^{1*}

¹ Institute for Digestive Research, Academy of Medicine, Lithuanian University of Health Sciences, A. Mickeviciaus st. 9, 44307, Kaunas, Lithuania

² Department of Gastroenterology, Academy of Medicine, Lithuanian University of Health Sciences, A. Mickeviciaus st. 9, 44307, Kaunas, Lithuania

³ Department of Genetics and Molecular Medicine, Academy of Medicine, Lithuanian University of Health Sciences, A. Mickeviciaus st. 9, 44307, Kaunas, Lithuania

[†]These authors contributed equally to this work

*Corresponding author: Jurgita Skieceviciene, PhD, Institute for Digestive Research, Academy of Medicine, Lithuanian University of Health Sciences, Mickeviciaus st. 9, 44307, Kaunas, Lithuania. Email: jurgita.skieceviciene@lsmuni.lt, phone: (370 37) 327236.

A

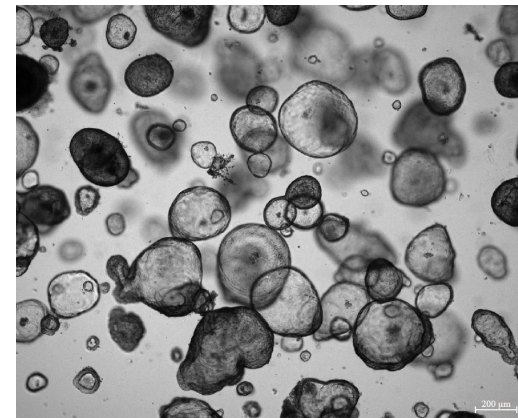
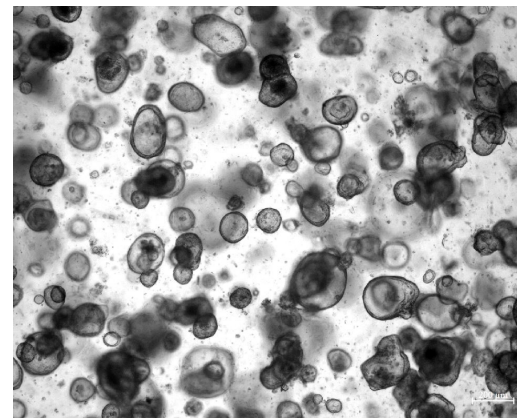
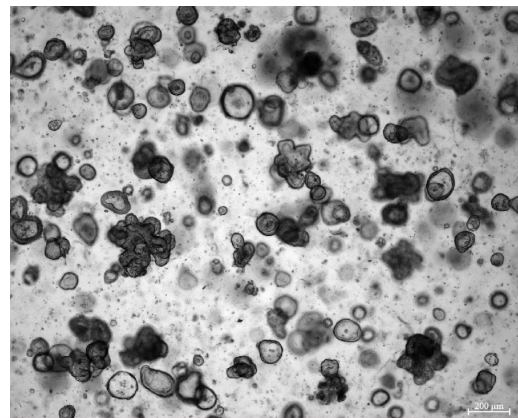
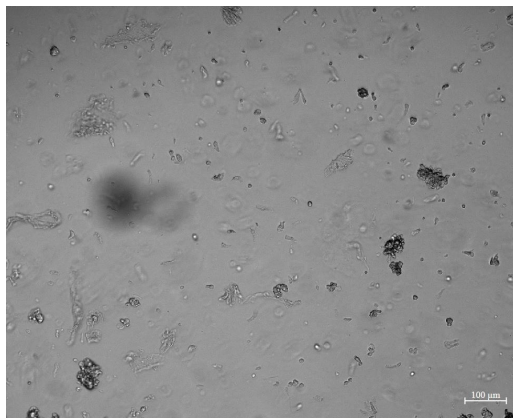
Crypts

Primary organoids_P0

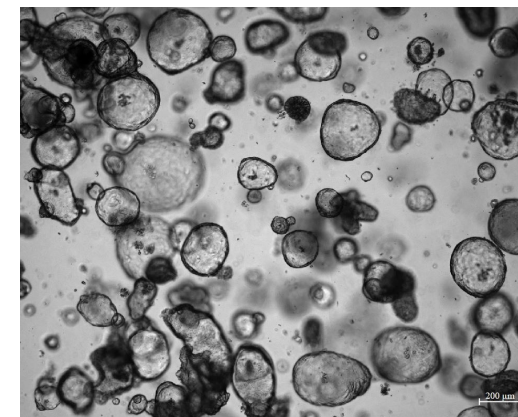
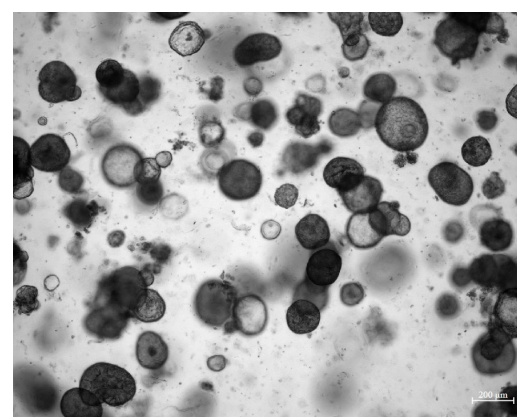
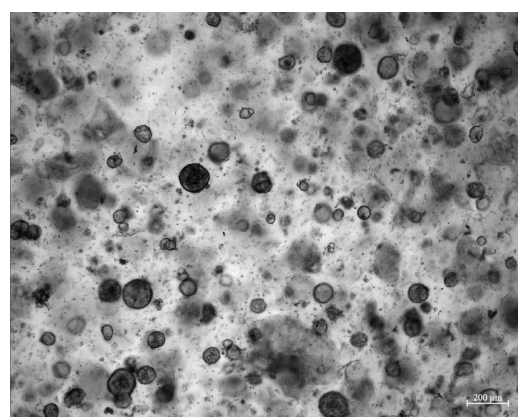
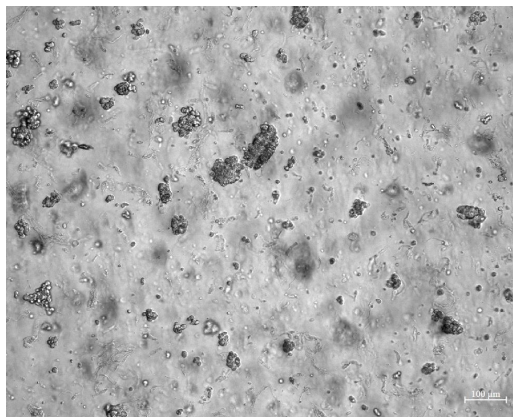
Organoids_P1

Organoids_P5

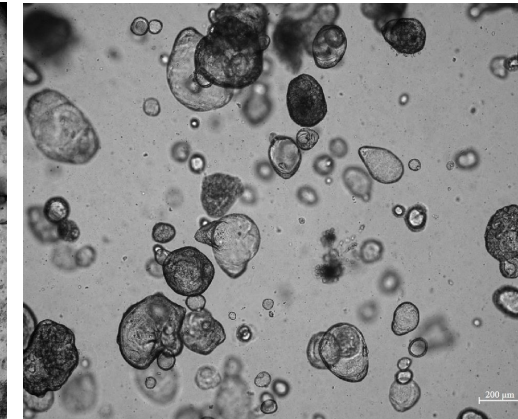
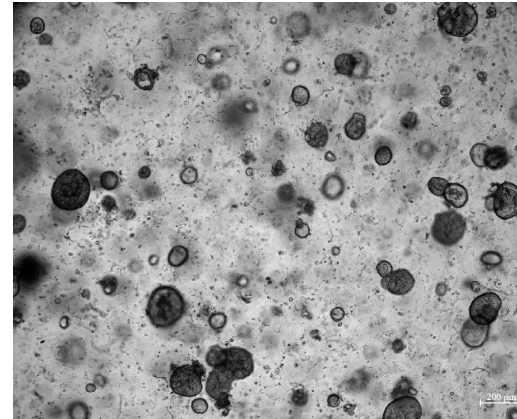
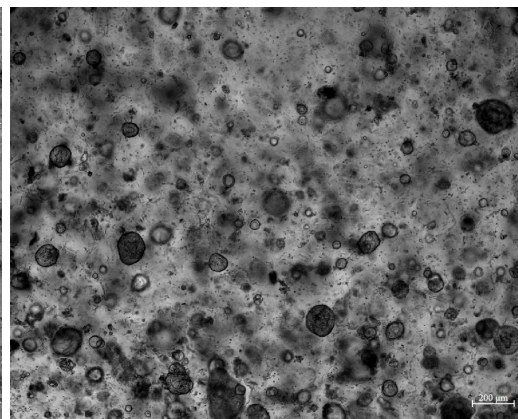
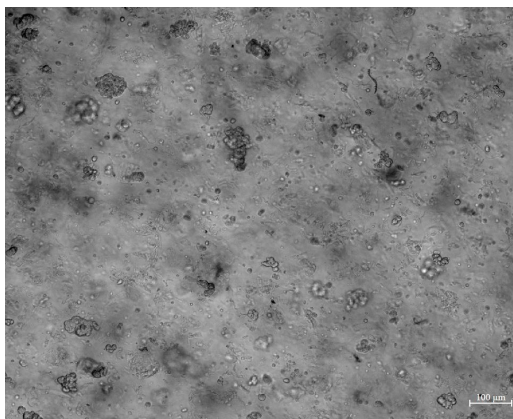
CON



qUC



aUC

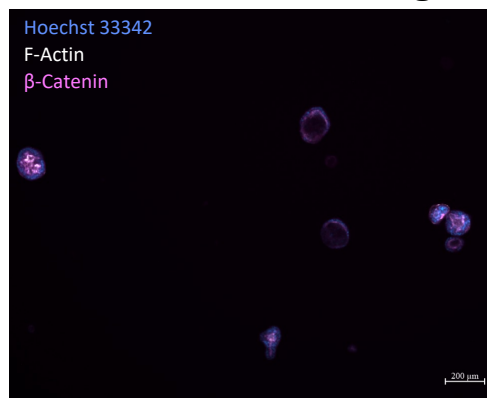
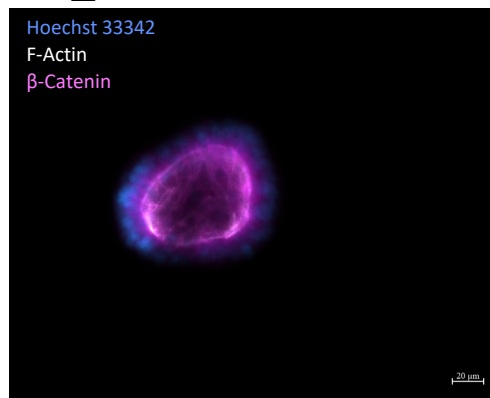
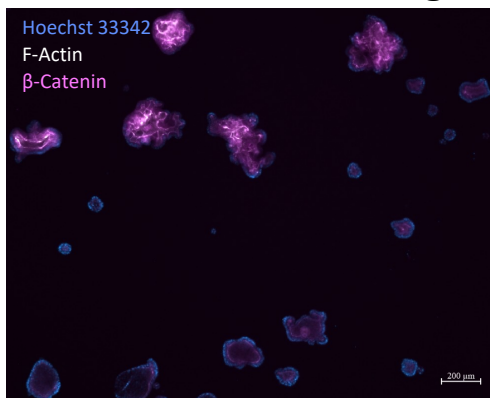


B

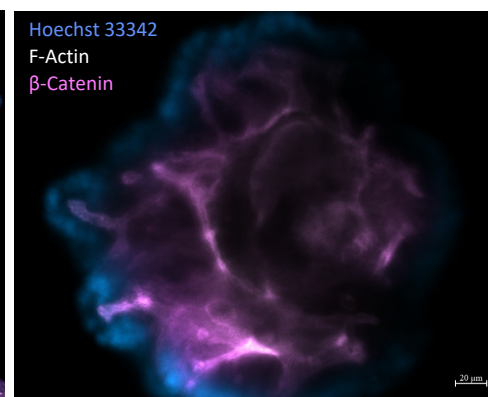
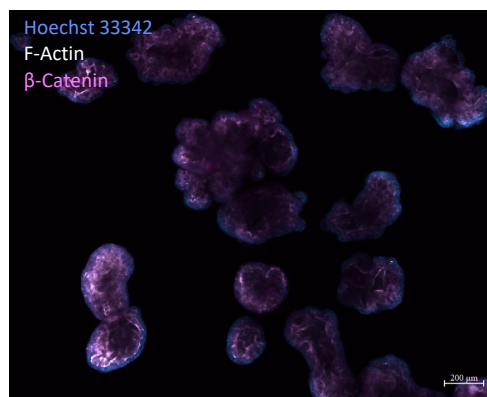
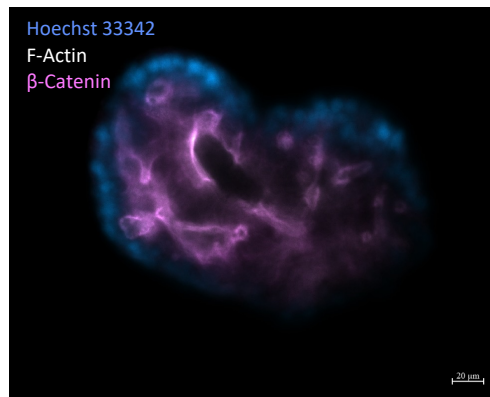
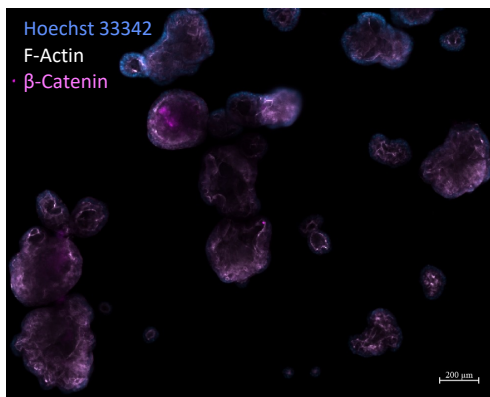
Organoids_P1

Organoids_P5

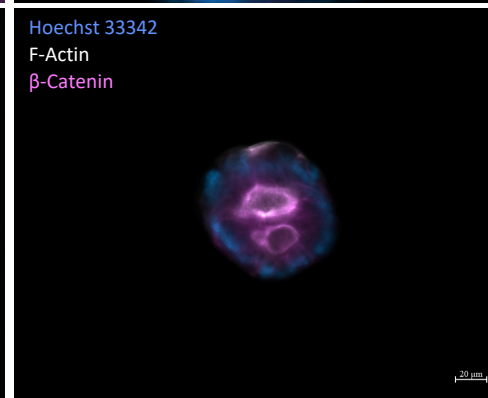
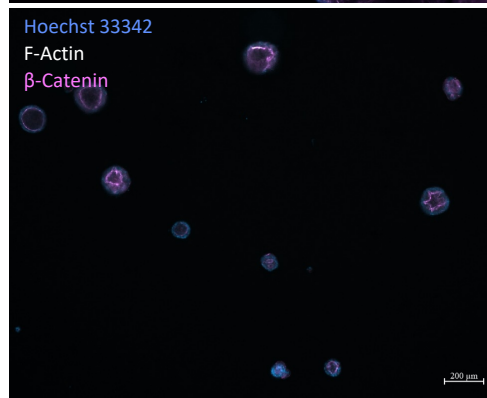
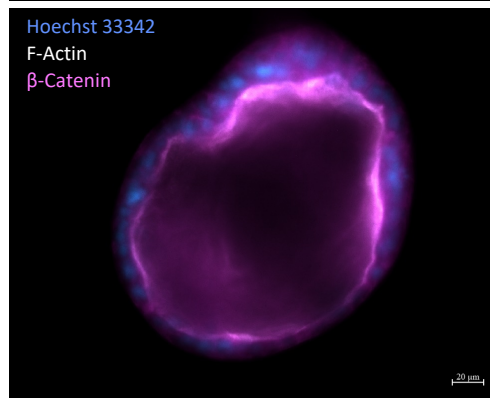
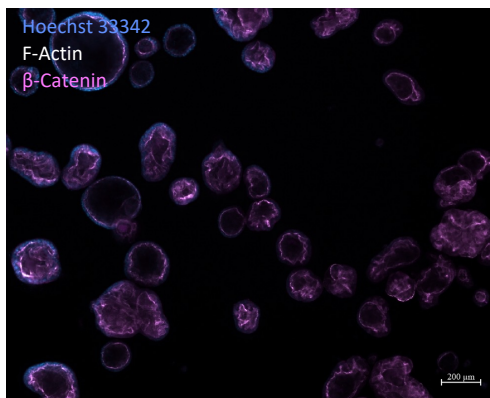
CON



qUC



aUC

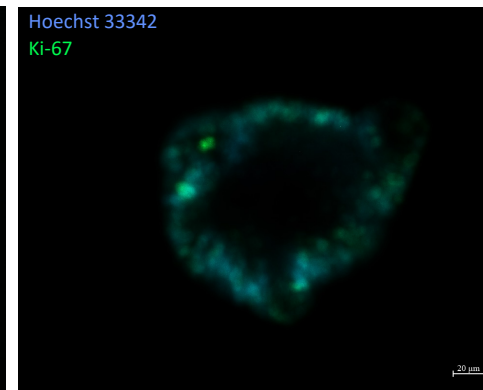
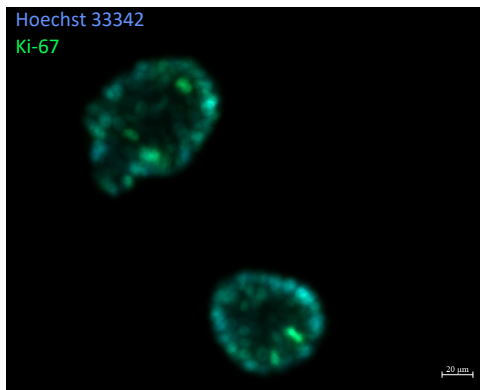
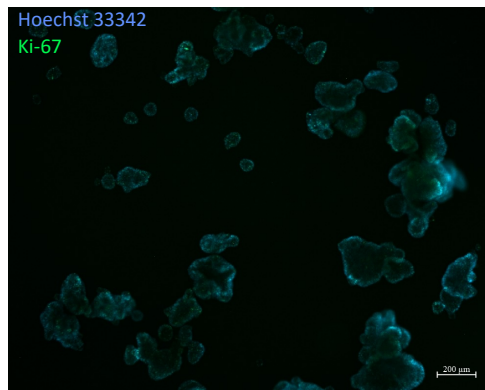


C

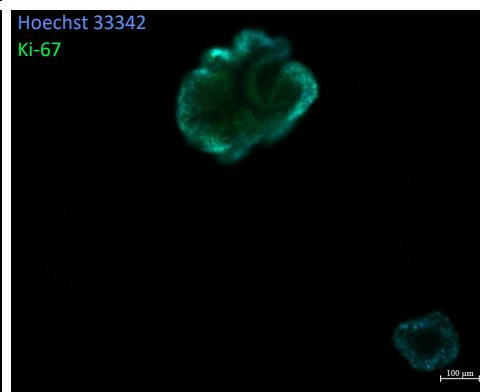
Organoids_P1

Organoids_P5

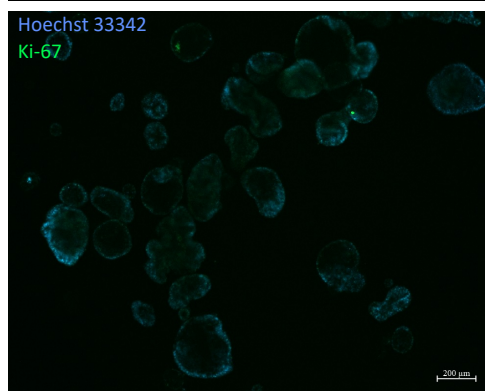
CON



qUC



aUC

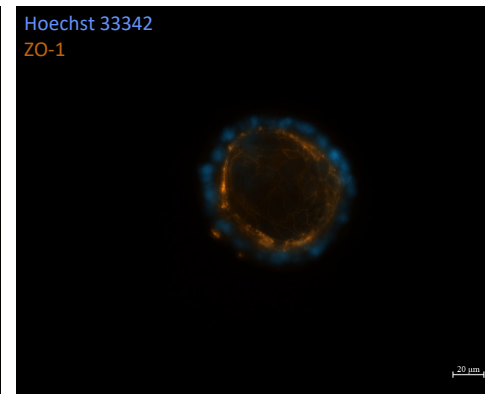
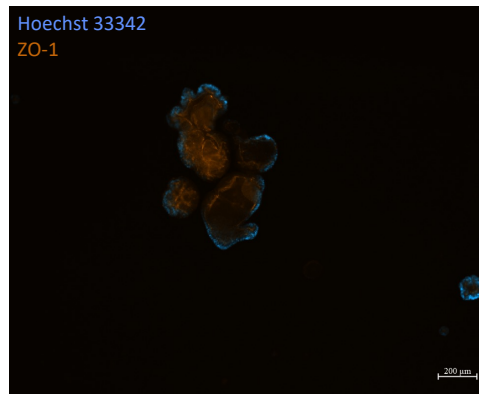
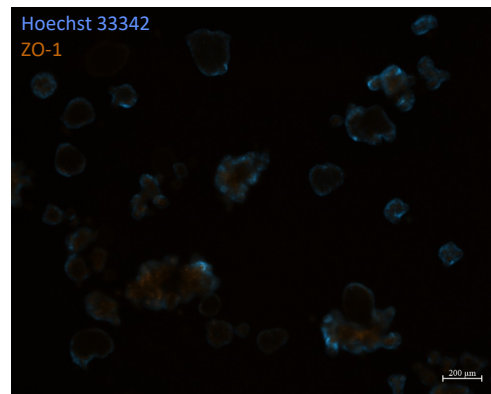


D

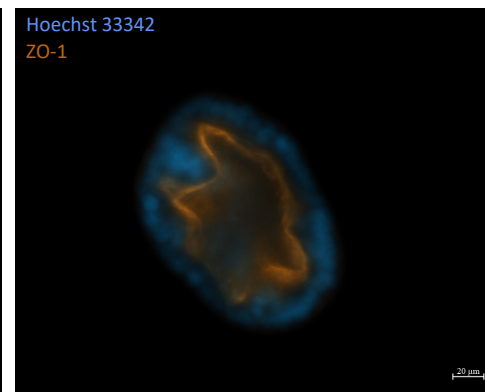
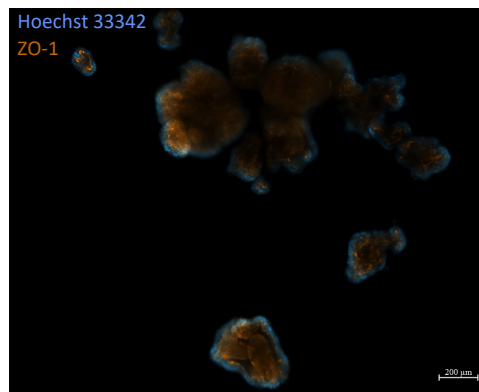
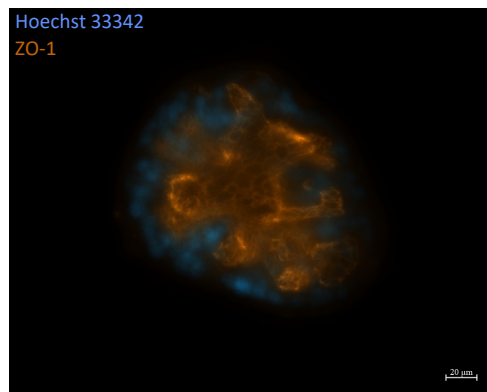
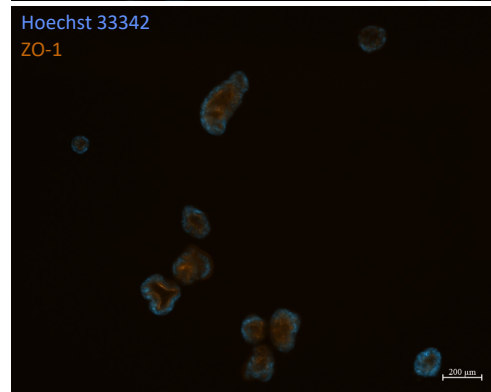
Organoids_P1

Organoids_P5

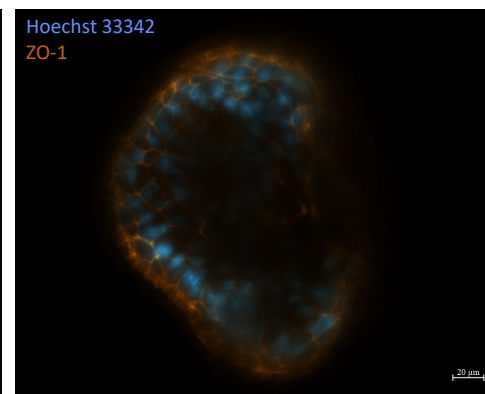
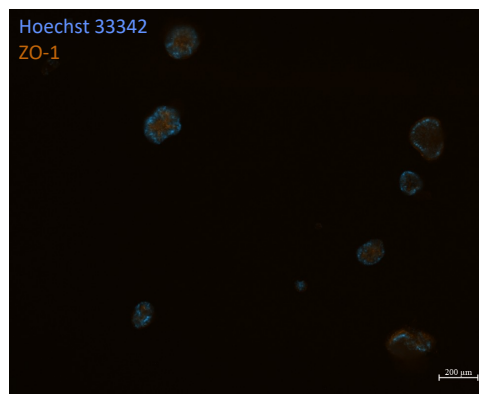
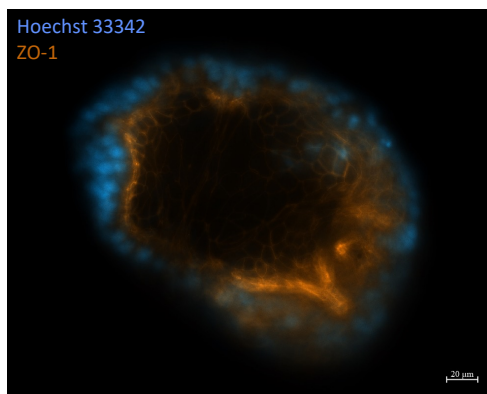
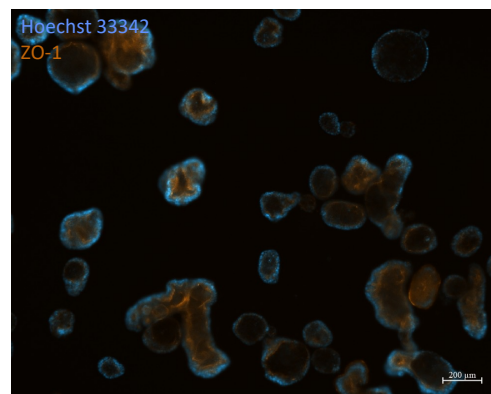
CON



qUC



aUC

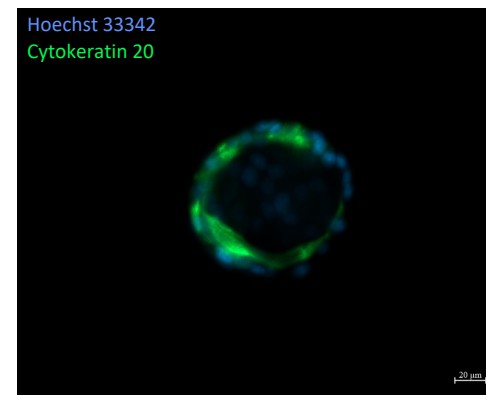
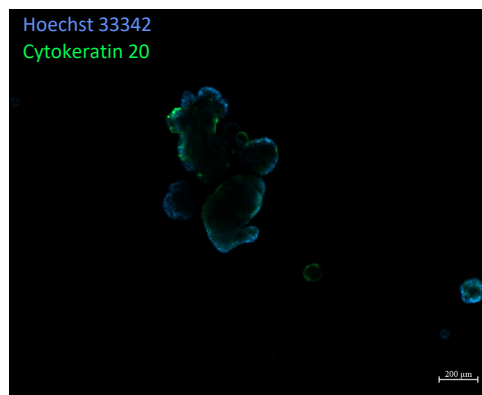
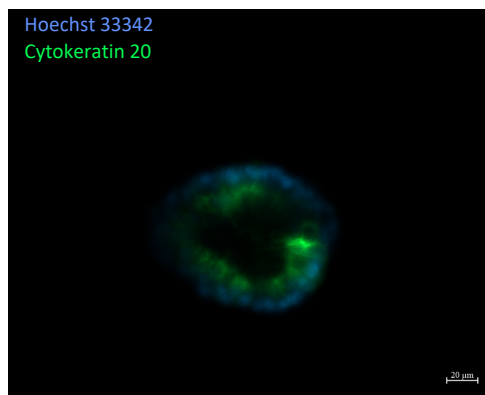
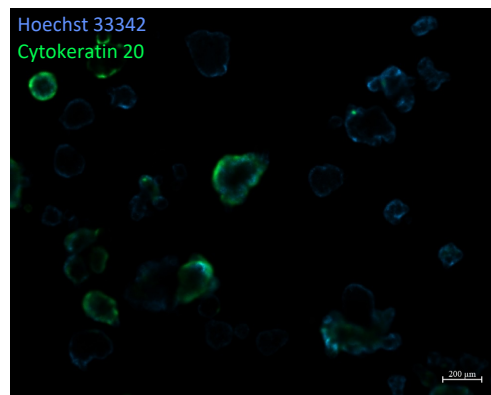


E

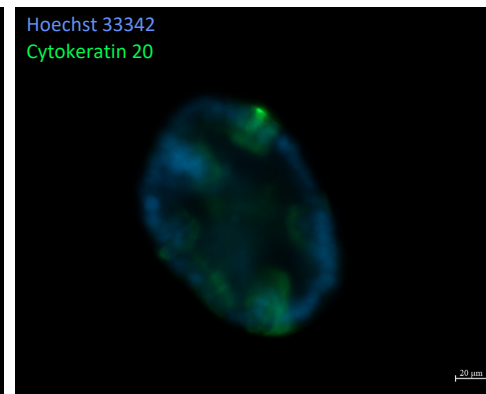
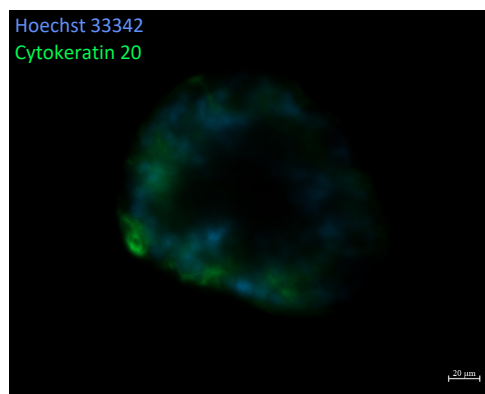
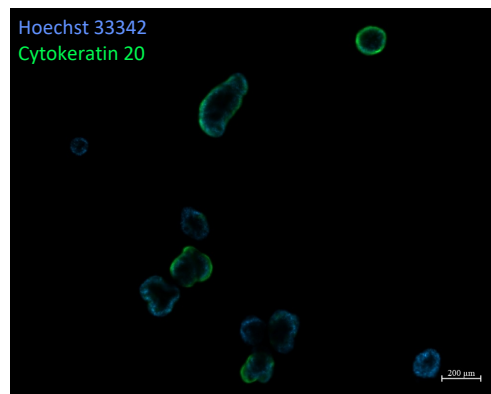
Organoids_P1

Organoids_P5

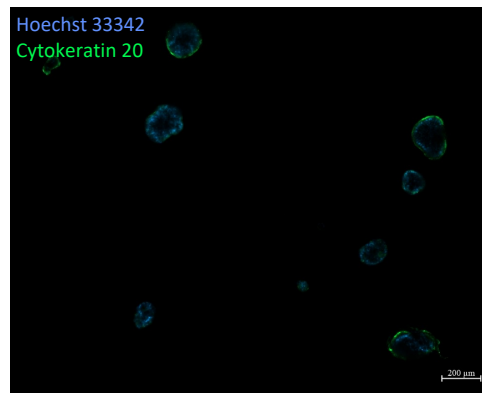
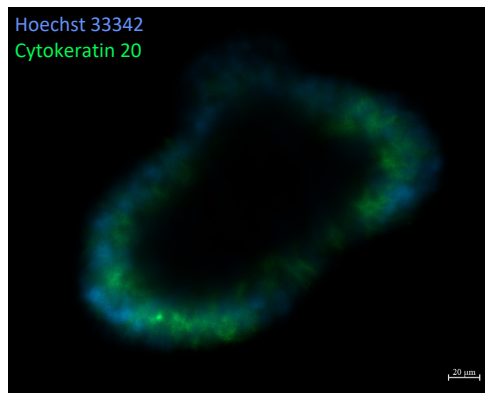
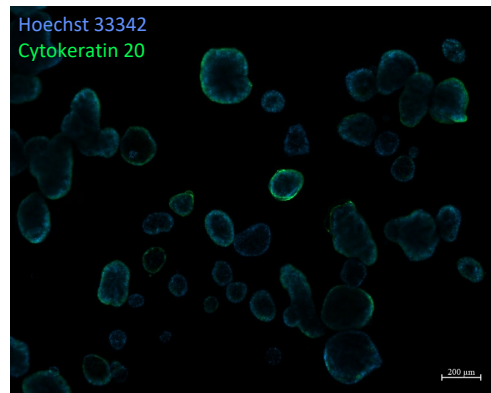
CON



qUC



aUC

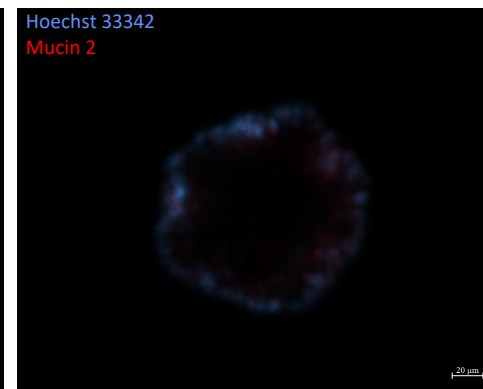
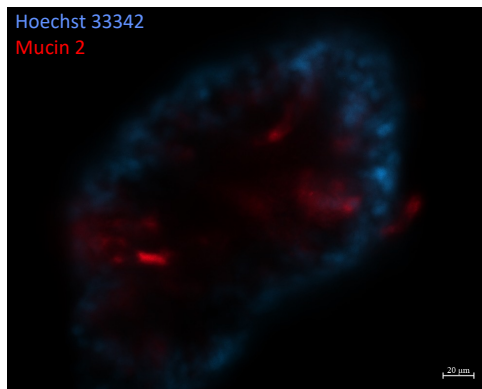
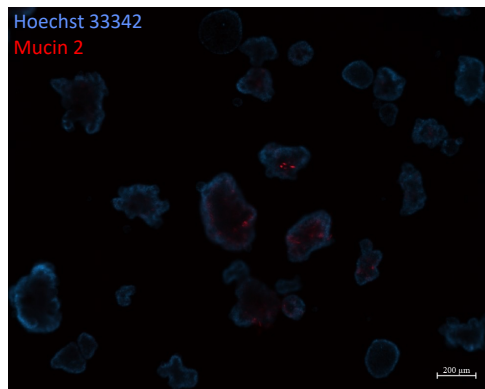


F

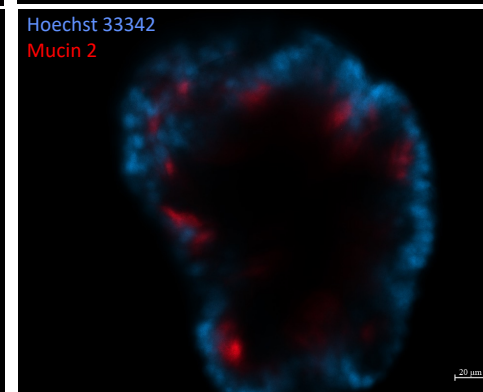
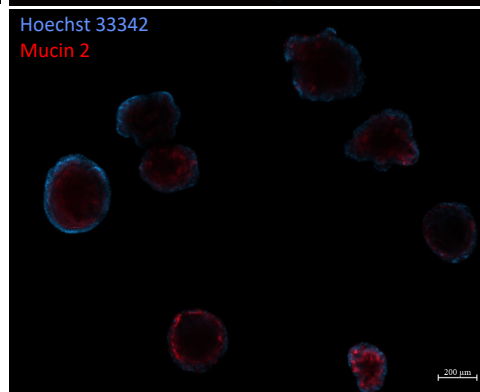
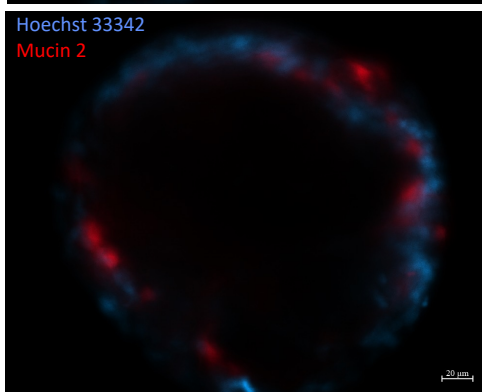
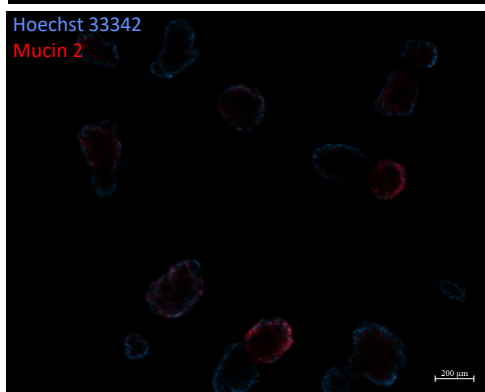
Organoids_P1

Organoids_P5

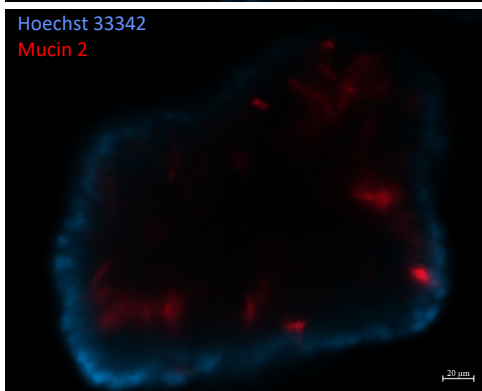
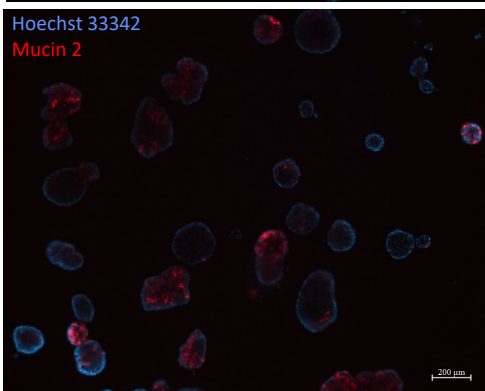
CON



qUC



aUC

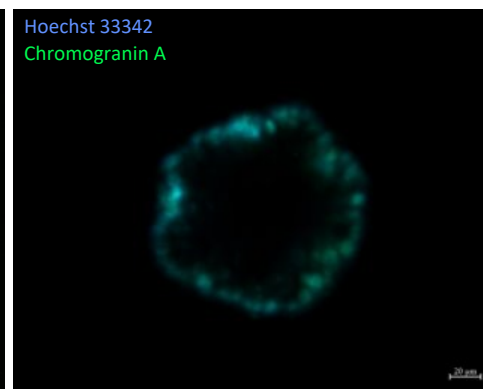
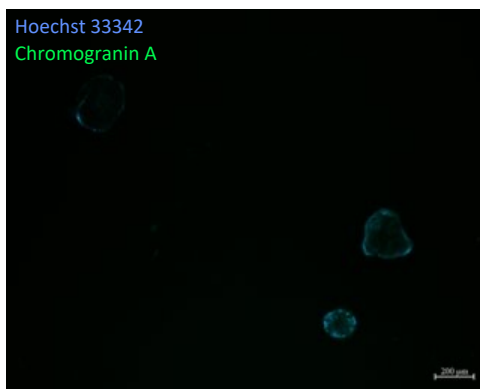
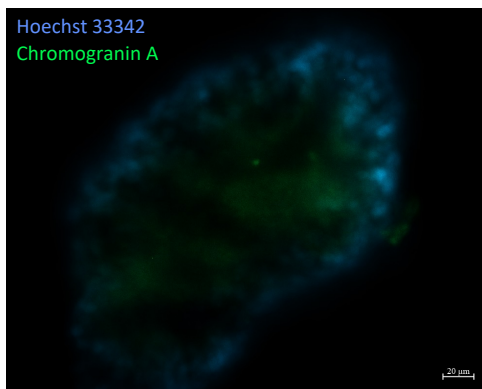
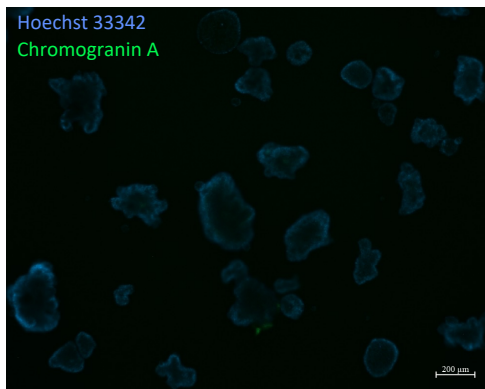


G

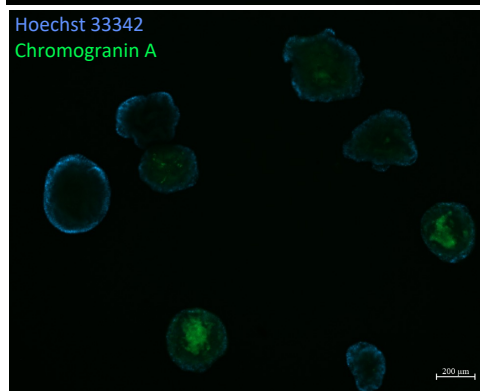
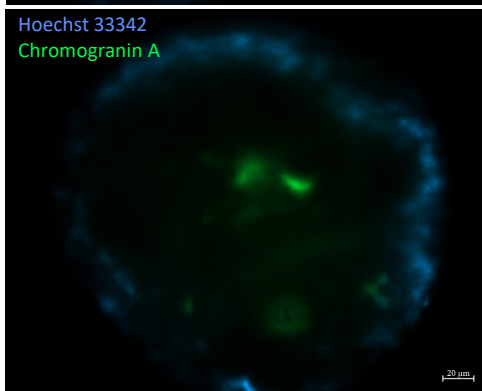
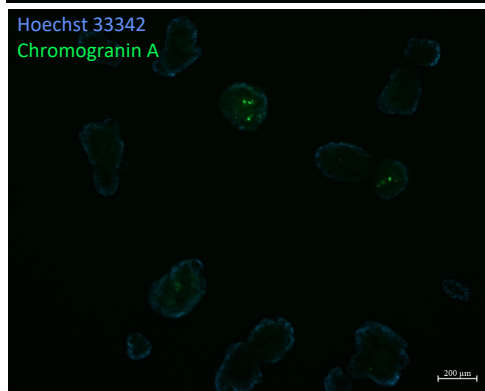
Organoids_P1

Organoids_P5

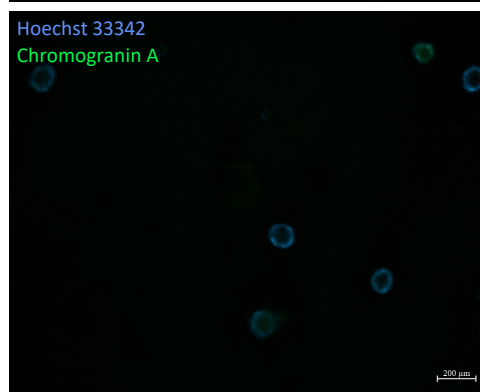
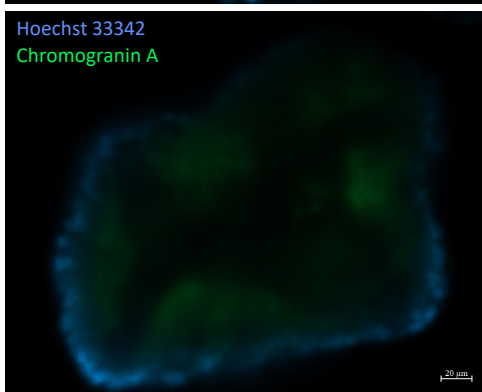
CON



qUC



aUC



Supplementary Fig. 1. The dynamics of formation and structural rearrangements of UC patient- and control individual-derived human colon organoids during long-term culturing. All images were acquired with ZEISS Axio Observer 7 inverted fluorescence microscope using 5x, 10x and 40x objectives, and analyzed by ZEISS ZEN 3.1 (blue edition) software. **(A)** Representation of structural transition from freshly isolated UC patient and control individual colon crypts on left panel to early-passage (P0 and P1) cystic organoids in middle panels to late-passage (P5) cystic organoids on right panel. **(B-G)** Immunofluorescence microscopy pictures of early-passage (P1) and late-passage (P5) colon organoids showing consistent organoid structure and cellular composition. Hoechst 33342 (blue) was used in all cases as a counterstain for cell nuclei. **(B)** Proper epithelial cell monolayer polarity is defined by β -catenin (pink) labeling stained basolateral side and F-actin (Phalloidin) (white) labeling stained apical side. **(C)** Areas of proliferation are identified by Ki67 (green) expressing proliferating cells. **(D)** Epithelial barrier integrity is defined by detection of tight junction protein ZO-1 (orange). **(E)** Absorptive colonocytes are defined by positive Cytokeratin 20 (green) staining. **(F)** Mucin-producing Goblet cells are identified by positive Mucin 2 (red) staining. **(G)** Hormone-producing enteroendocrine cells are defined by positive Chromogranin A (green) staining.

CON – control, qUC – quiescent ulcerative colitis, aUC – active ulcerative colitis.