A - ROI outline

B - Original Image

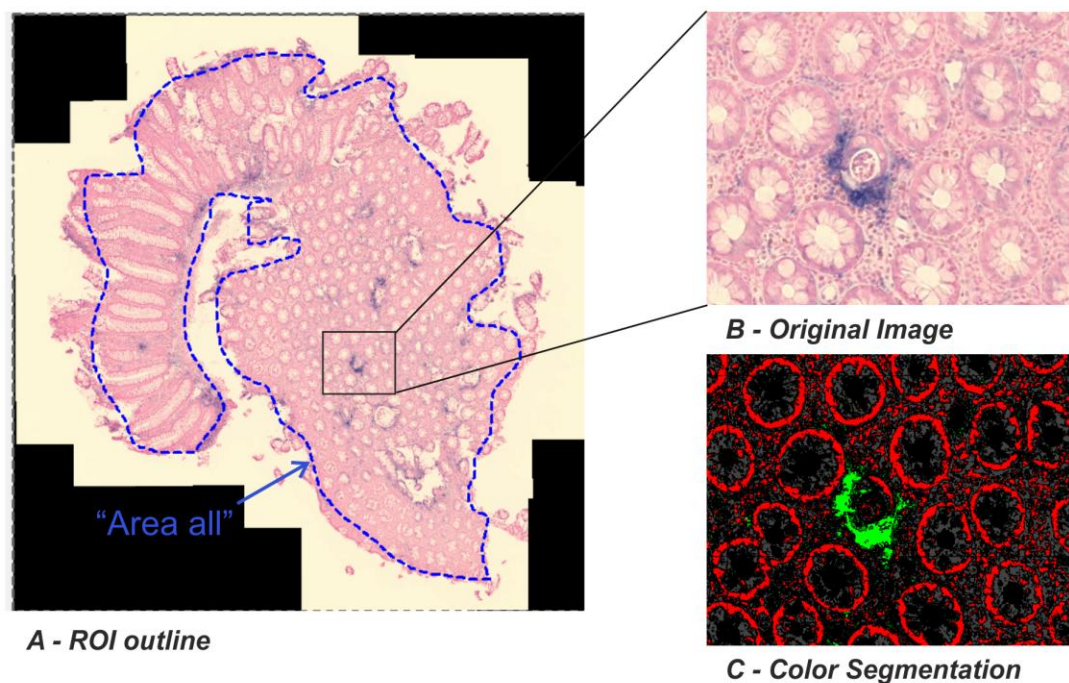*C - Color Segmentation*

Color classes
- 🟥 Red
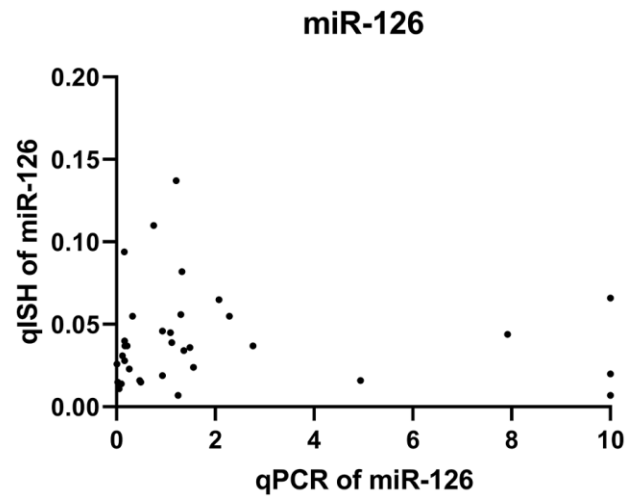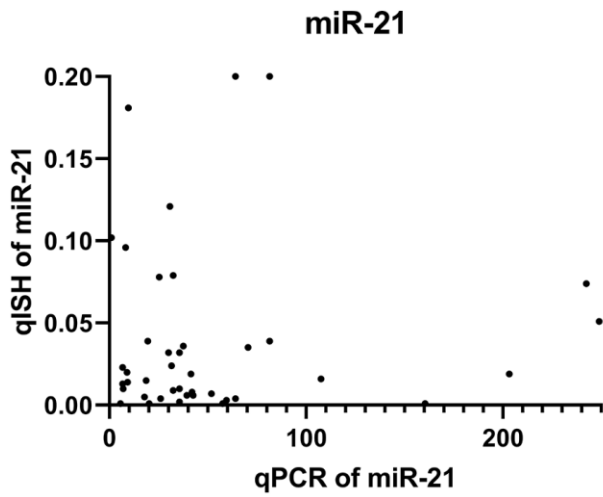- 🟩 Blue intense
- 🟩 Blue weak
- ⬜ Connective tissue
- ⬛ Background

Variables
TR: Total Red
Bi: Blue intense
Bw: Blue weak
Ct: Connective tissue
TT: Total Tissue

Calculations
$TT = TR + Bi + Bw + Ct$
miRNA fraction = Bi/TT

Supplementary Figure 1. Example of the image analysis for ISH using VisiomorphDP pixel classification of miR-21 stained section. ROI was selected manually for all the sections without any artefacts. miR-21 staining (blue) and Nuclear Fast Red background stain (red) in A and B. Pixel classification is shown in C. ISH, in situ hybridization. ROI, region of interest.

Supplementary Figure 2. The relationship between quantitative image analysis of situ hybridization (qISH) and reverse transcription quantitative polymerase chain reaction (RT-qPCR) quantification of miR-2 and miR-126 in the rectal mucosa of UC patients.