Supplementary files:

Figure S1: **Preliminary experiments for determination culture conditions**. **A) Description of differentiation approaches used in our study.** \* previously described growth condition ([Ferraretto et al., 2018](#_ENREF_7)). **B) Proliferation of HT-29 and Caco2 cell lines after sodium butyrate treatment**. The proliferation activity of the HT-29 and Caco2 cells ws significantly decreased after NaBt treatment. The decrease in cell proliferation was more apparent in the Caco2 cell line, especially at concentrations of 10mM: at this concentration, a large number of floating cells was observed. Because of this, the concentration of 10mM NaBt was not used for the following experiments. Results are showed as mean ± SD (n = 9). Results were evaluated by t-test. Statistically different results in comparison to control cells are marked by \* P ≤ 0.05, \*\* P ≤ 0.01, \*\*\* P ≤ 0.001. **C)** **Measurement of alkaline phosphatase activity**. The ALP activity of NaBt-differentiated HT-29 and Caco2 cells (model 1 and 2) increased both dose- and time-dependent manner during 48 and 72 h in comparison to control cells. In case the spontaneously differentiated Caco2 cells (model 3), the time-dependent increase in ALP activity was also observed. The highest increase of ALP activity was detected after 72h of treatment with 5mM NaBt for both cell lines and in day 14 in postconfluent growth. The biggest increase in ALP activity was observed for NaBt-differentiated Caco2 cells (model 2), followed by spontaneously differentiated Caco2 (model 3) and in HT-29 (model 1). The relative ALP activity was enhanced 5-fold (model 1), 24-fold (model 2), and 8-fold (model 3) in comparison to controls. Results are showed as mean ± SD (n = 6). \* P ≤ 0.05, \*\* P ≤ 0.01, \*\*\* P ≤ 0.001. **Based on obtained results, 5mM concentration of sodium NaBt incubated for 72h for HT-29 and Caco2 and 14 days of postconfluent growth of Caco2 was set up as an appropriate treatment for the following experiment.**

