

## Supplemental Figure S1:

### 1.1. Cell viability

Relative cell viability was measured using the cell counting kit 8 (CCK-8) assay. Caco-2 cells were dispensed in 96-well plates at  $7 \times 10^3$  cells per well. After cell adherence, cells were administered with different dose of Ancientino (0, 12.5, 25, 50, 100  $\mu\text{g/mL}$ ) for 24 h. Then CCK-8 solution was added to each well and incubated at  $37^\circ\text{C}$  for 4 h. The absorbance was measured at 450 nm to calculate the relative cell viability.

To investigate the anti-colitis activity of Ancientino *in vitro*, Caco-2 cells were selected for research. The cells were treated for 24 h with different dose of Ancientino (0, 12.5, 25, 50, 100  $\mu\text{g/mL}$ ) and the relative viability of the cells was measured. As shown in Fig S1, Ancientino-treated groups exhibited no significant difference on the relative cell viability compared to the control group. The results demonstrated that Ancientino has no apparent cytotoxicity to the Caco-2 cells. Based on these preliminary data 25, 50, 100  $\mu\text{g/mL}$  were selected in the succedent experiments.

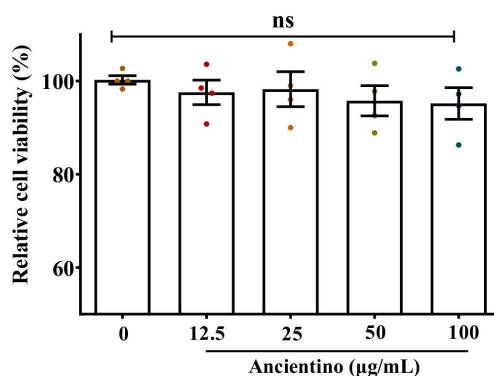


Figure S1. Cell viability. Cell viability is expressed as a percentage relative to control (Ancientino, 0  $\mu\text{g/mL}$ ). Every dot in the figure represents an independent experiment, and different colors represent different groups. Data are presented as the mean  $\pm$  SEM, with data obtained from 4 independent experiments. ns indicates  $p > 0.05$ .