

Supplementary material

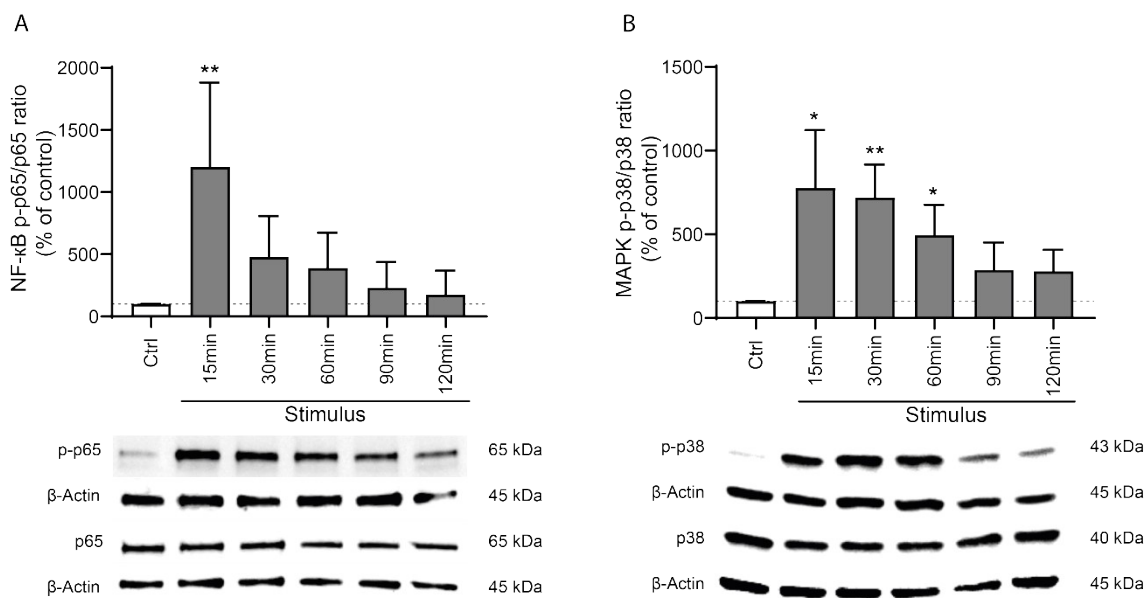


Figure S1. Determination of the incubation period with the pro-inflammatory cocktail to evaluate the NF-κB and MAPK signaling pathways in the triple co-culture. A) NF-κB p65 subunit and B) MAPK p38 phosphorylation ratios assessed by Western blot after treatment of cells with the pro-inflammatory cocktail (10 μg/mL LPS in the apical compartment; 25 ng/mL IL-1β and 50 ng/mL TNF-α in the basolateral compartment) for incubation periods ranging from 15 to 120min. All results are presented as a percentage of the unstimulated control and were obtained from at least three independent biological replicates. *p<0.05, **p<0.01 relative to the unstimulated control.

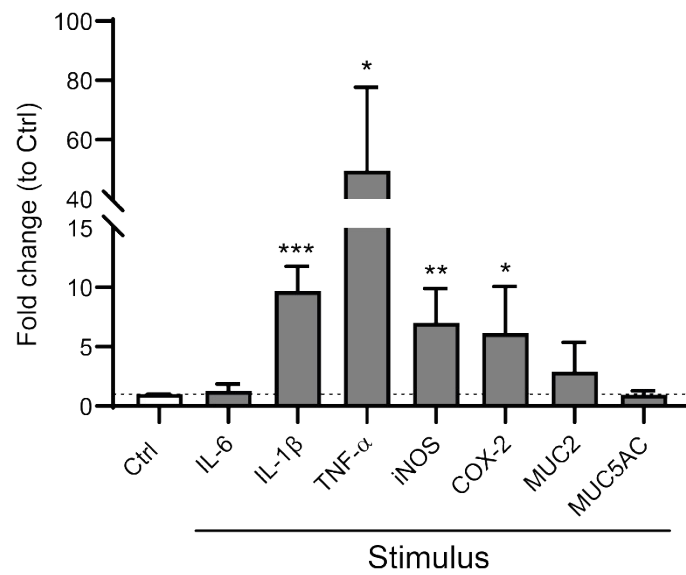


Figure S2. Expression levels of genes related to inflammation, including pro-inflammatory cytokines, inducible enzymes, and mucins following the incubation of the triple co-culture with the pro-inflammatory cocktail (10 $\mu\text{g}/\text{mL}$ LPS in the apical compartment; 25 ng/mL IL-1 β and 50 ng/mL TNF- α in the basolateral compartment) for 3h, assessed by RT-PCR. All results are presented as a percentage of the unstimulated control and were obtained from at least three independent biological replicates. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ relative to the unstimulated control.

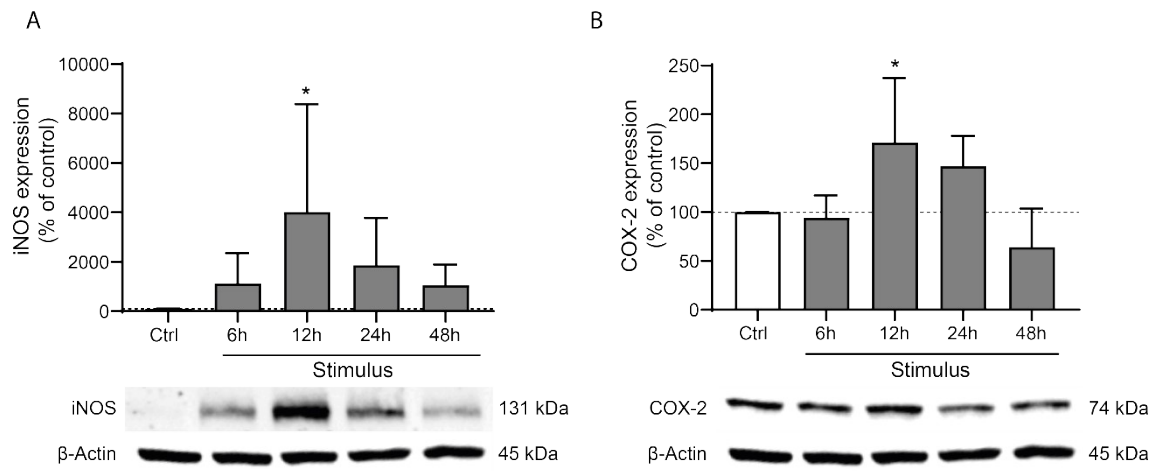


Figure S3. Determination of the incubation period with the pro-inflammatory cocktail to evaluate the protein expression levels of inducible enzymes in the triple co-culture. A) iNOS and B) COX-2 protein expressions assessed by Western blot after treatment of cells with the pro-inflammatory cocktail stimulus (10 $\mu\text{g}/\text{mL}$ LPS in the apical compartment; 25 ng/mL IL-1 β and 50 ng/mL TNF- α in the basolateral compartment) for incubation periods ranging from 6 to 48h. All results are presented as a percentage of the unstimulated control and were obtained from at least three independent biological replicates. * $p < 0.05$ relative to the unstimulated control.

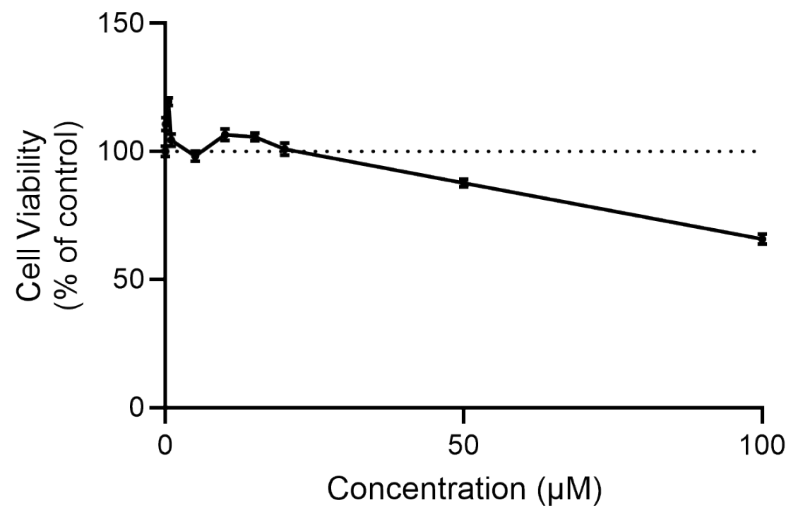


Figure S4. Cytotoxic assessment of 11β,13-dihydrolactucin (0.1–100 µM) in human colon-derived CCD18-Co myofibroblasts by metabolic reduction of MTT after 24-hours of incubation.