# SUPPLEMENTAL MATERIAL

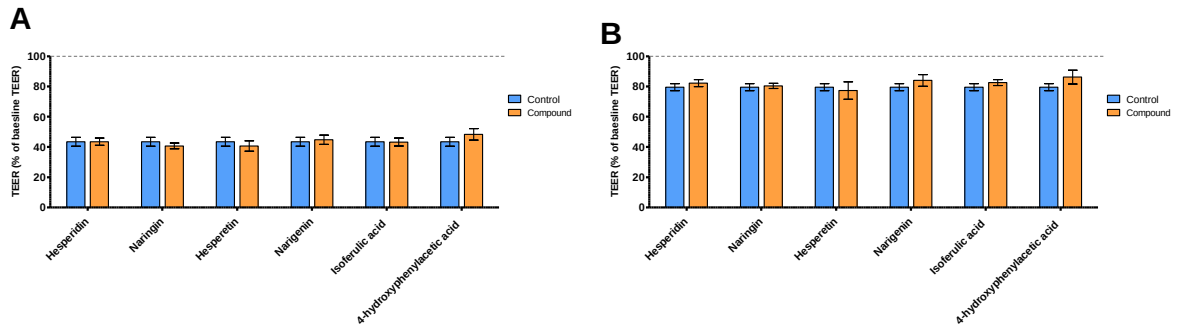


**Figure s1. The effects of citrus flavonoids, their aglycones and their metabolites on transepithelial electrical resistance (TEER) of Caco-2 monolayers after 24 hours (a) and after 30 hours (b) of co-culture with PMA-simulated THP-1 cells.** At baseline, Caco-2 cells cultured in transwell inserts for 14 days were placed in co-culture with PMA-stimulated THP-1-Blue™ NF-κB cells. Caco-2 cells were then incubated with either the test compounds (50 µM) or culture medium containing 0.1% DMSO as vehicle control (medium + DMSO control) added to the apical compartment. After 24 hours of incubation, THP-1 cells were incubated with 500 ng/ml LPS in the basolateral compartment for an additional 6 hours. TEER was measured at baseline, after 24 hours and after 30 hours of incubation. Values are represented as mean percentage from the baseline value ± SEM. No significant differences were observed between the test compounds and the medium + DMSO control.

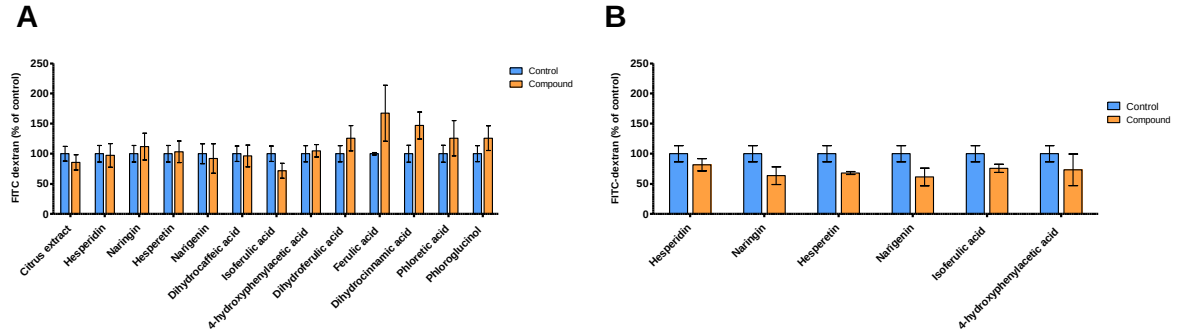


**Figure s2. The effects of citrus flavonoids, their aglycones and their metabolites on fluorescein isothiocyanate–dextran 4kDa (FITC-D4) permeation of Caco-2 monolayers in co-culture with PMA-simulated THP-1 cells at concentrations of 100 µM (a) or 50 µM (b).** Caco-2 cells cultured in transwell inserts for 14 days were placed in co-culture with PMA-simulated THP-1-Blue™ NF-κB cells. Caco-2 cells were then incubated with either culture medium containing 0.1% DMSO (medium + DMSO control) or test compounds (100 µM or 50 µM) added to the apical compartment. FITC-D4 was added apically to the caco-2 cells after 30 hours of incubation. FITC-D4 concentrations were measured in the basolateral compartment after 1 hour of incubation. Values are represented as mean ± SEM. No significant differences were observed between the test compounds and the medium + DMSO control.

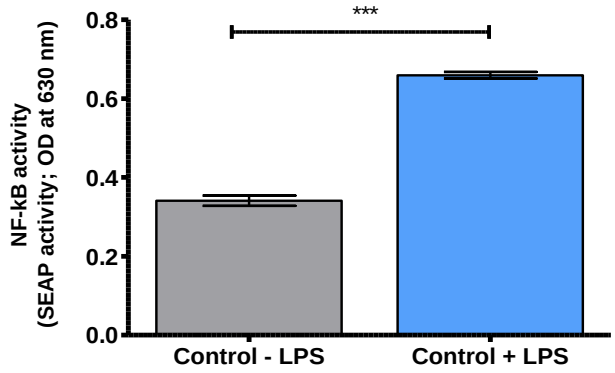


**Figure s3. Basolateral secretion of NF-κB-inducible SEAP by PMA-simulated THP-1 cells in co-culture with Caco-2 cells alone and after stimulation with LPS.** At baseline, Caco-2 cells were placed in co-culture with PMA-stimulated THP-1-Blue™ NF-κB cells. Caco-2 cells were then incubated with culture medium containing 0.1% DMSO as vehicle control (medium + DMSO control) added to the apical compartment. After 24 hours of incubation, THP-1 cells were incubated with culture medium containing 500 ng/ml LPS (control + LPS) or culture medium alone (control – LPS) in the basolateral compartment for an additional 6 hours. Levels of NF-κB-inducible SEAP were measured in basolateral medium after 30 hours of incubation. Values are represented as mean ± SEM. * represents significant difference (*: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$).

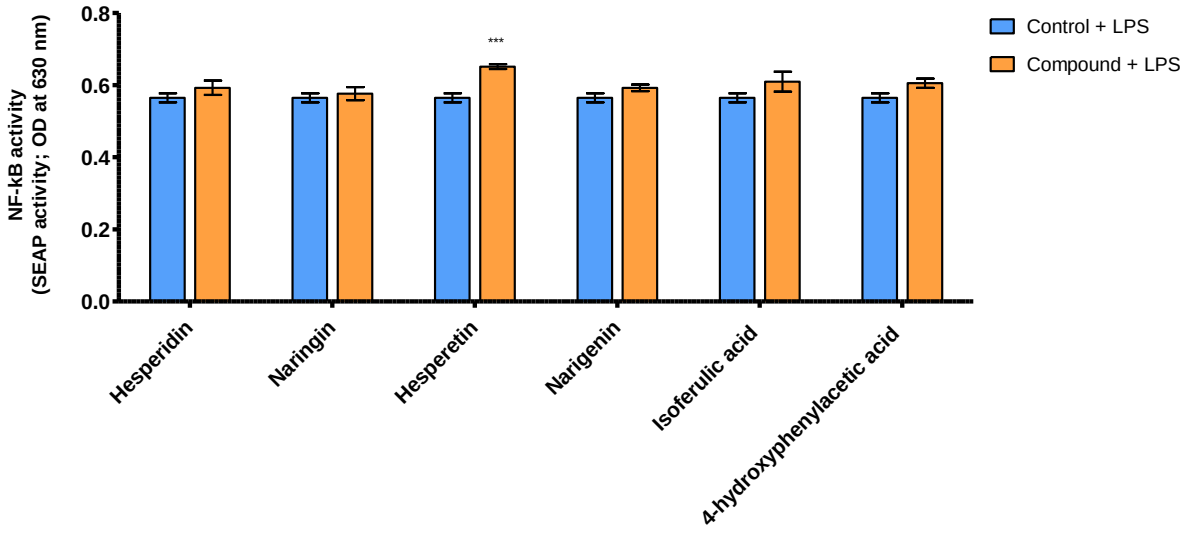


**Figure s4. The effects of citrus flavonoids, their aglycones and their metabolites on basolateral secretion of NF-κB-inducible SEAP after LPS stimulation by PMA-simulated THP-1 cells in co-culture with Caco-2 cells.** At baseline, Caco-2 cells were placed in co-culture with PMA-stimulated THP-1-Blue™ NF-κB cells. Caco-2 cells were then incubated with either the test compounds (50 µM) or culture medium containing 0.1% DMSO as vehicle control (medium + DMSO control) added to the apical compartment. After 24 hours of incubation, THP-1 cells were incubated with 500 ng/ml LPS in the basolateral compartment for an additional 6 hours. Levels of NF-κB-inducible SEAP were measured in basolateral medium after 30 hours of incubation. Values are represented as mean ± SEM. * represents significant difference from medium + DMSO control (*: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$).

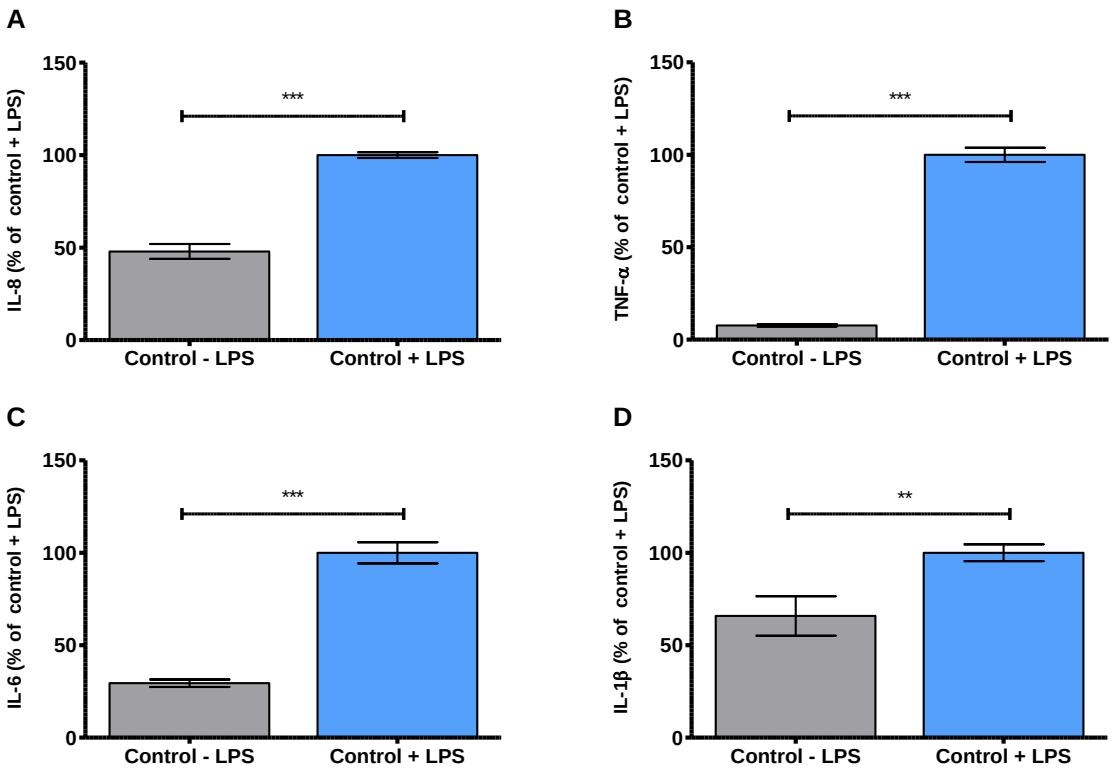


**Figure s5. Basolateral secretion of IL-8 (a), TNF-α (b), IL-6 (c) and IL-1β (d) by PMA-simulated THP-1 cells in co-culture with Caco-2 cells alone and after stimulation with LPS.** At baseline, Caco-2 cells were place in co-culture with PMA-stimulated THP-1-Blue™ NF-κB cells. Caco-2 cells were then incubated with culture medium containing 0.1% DMSO as vehicle control (medium + DMSO control) added to the apical compartment. After 24 hours of incubation, THP-1 cells were incubated with culture medium containing 500 ng/ml LPS (control + LPS) or culture medium alone (control – LPS) in the basolateral compartment for an additional 6 hours. Cytokine levels were measured in basolateral medium after 30 hours of incubation. Values are represented as mean ± SEM. * represents significant difference (*: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$).

**Table s1.** Sequences of the primers used for qPCR analysis

| Gene | Forward | Reverse |
|---|---|---|
| 18S RNA | GTA ACC CGT TGA ACC CCA TT | CCA TCC AAT CGG TAG TAG CG |
| ZO-1 | AGG GGC AGT GGT GGT TTT CTG TTC TTT C | GCA GAG GTC AAA GTT CAA GGC TCA AGA GG |
| OCCLUDIN | TCA GGG AAT ATC CAC CTA TCA CTT CAG | CAT CAG CAG CAG CCA TGT ACT CTT CAC |
| CLDN-2 | AAC TAC TAC GAT GCC TAC C | GAA CTC ACT CTT GAC TTT GG |
| CLDN-3 | TTC ATC GGC AGC AAC ATC ATC | CGC CTG AAG GTC CTG TGG |
| CLDN-4 | ACA GAC AAG CCT TAC TCC | GGA AGA ACA AAG CAG AGA G |
| E-Cadherin | CAC CTG GAG AGA GGC CGC GT | AAC GGA GGC CTG ATG GGG CG |
| MLCK | GCC TGA CCA CGA ATA TAA GTT | GCT CC TTC TCA TCA TCA TCT G |

18S RNA: 18S ribosomal RNA; ZO-1: Zona Occludens-1; CLDN: Claudin; MLCK: myosin light chain kinase.