

## **Supplementary data**

### **Supplementary experimental procedure**

#### **Cell death rate**

Caco-2 cells were plated at a density of  $1 \times 10^4$  cells in a 96-well culture plate at 37°C in 5% CO<sub>2</sub>. Heat-treated OLL2712 were added at 100 µg/mL and cultured for 24 h. The cells were washed twice with Hanks' balanced salt solution, and stained with propidium iodide (PI) and Hoechst33342 for 30 min at 37°C. The cells were examined under a fluorescence microscope (Keyence, BZ-X810). PI and Hoechst 33342 were detected using TRITC (excitation: 545/25 nm, emission: 605/70 nm, Keyence) and DAPI filters (excitation: 360/40 nm, emission: 460/50 nm, Keyence), respectively. The cell death rate was calculated as the percentage of PI-positive cells among all cells.

#### **Lactate dehydrogenase (LDH) assay**

Cell viability was assessed by measuring the release of LDH using Cytotoxicity LDH Assay Kit-WST (Dojindo Laboratories) according to the manufacturer's instructions.

**Table S1. Primer sequences in real-time polymerase chain reaction (PCR) used in this study**

<i>Gene</i>	Sequences (5'→3')	
<i>GAPDH</i>	Takara, HA067812	
<i>CLDN1</i> [56]	Forward	GTGCGATATTTCTTCTTGCAGG
	Reverse	TTCGTACCTGGCATTGACTGG
<i>FIIIR</i>	Forward	ACCTTCTTGCCAACTGGTATCA
	Reverse	AGCACGATGAGCTTGACCTTG
<i>MYD88</i>	Forward	GGCTGCTCTCAACATGCGA
	Reverse	CTGTGTCCGCACGTTCAAGA
<i>OCN</i> [57]	Forward	TCAGGGAATATCCACCTATCACTTCAG
	Reverse	CATCAGCAGCAGCCATGTACTCTTCAC
<i>TJP1</i>	Forward	ACCAGTAAGTCGTCCTGATCC
	Reverse	TCGGCCAAATCTTCTCACTCC
<i>CLDN4</i> [56]	Forward	GGCTGCTTTGCTGCAACTGTC
	Reverse	GAGCCGTGGCACCTTACACG

Not referenced PCR primer sequences were obtained from Primerbank

(<https://pga.mgh.harvard.edu/primerbank/>) [58].

### Supplementary References

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57. Braniste, V.; Leveque, M.; Buisson-Brenac, C.; Bueno, L.; Fioramonti, J.; Houdeau, E. Oestradiol decreases colonic permeability through oestrogen receptor beta-mediated up-regulation of occludin and junctional adhesion molecule-A in epithelial cells. *J. Physiol.* 2009, 587, 3317–3328. <https://doi.org/10.1113/jphysiol.2009.169300>.
58. Spandidos, A.; Wang, X.; Wang, H.; Seed, B. PrimerBank: a resource of human

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