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MDPI

SUPPLEMENTARY MATERIAL

S1 Ussing Chamber protocol

Esophageal biopsies were mounted in Ussing Chambers with a 9-mm opening, that was reduced to fit biopsy specimens to an area of 1.76 mm^2 using a technique previously described [47]. Both compartments were filled with 1.5 ml 10 mmol/l glucose KRB solution. The chambers were kept at 37 °C and continuously oxygenated and circulated by gas flow (95% O_2/5% CO_2) during the whole experiment. A four-electrode system was used, as described previously [48]. Ag/Cl electrodes with 3M NaCl / 2% agar bridges were used for measurement of transepithelial potential difference (PD) and current (I) monitoring. Every minute, direct pulses of 1.5, -1.5, 3.-3 and 0 μA, with duration of 235 ms were calculated. Every minute the TEER was obtained according to Ohm's law from the slope of the I-U line.

S2 TRPV1 and tight junction gene transcription

Gene transcription of 18S was used as reference. Total RNA was isolated from snap-frozen biopsies using Trizol (Invitrogen, Carlsbad, CA, USA), followed by a cleaning step with the RNeasy Mini kit (Qiagen, Austin,TX, USA). Quantification of the purified RNA was carried out using the NanoDrop spectrophotometer (Nano- Drop Technologies, Wilmington, DE, USA). C-DNA was synthesized using the iScript cDNA Syntheses Kit (Bio-Rad, Hercules, CA, USA). PCR amplifications were run on the MyIQ real-time PCR detection system (Bio-Rad, Hercules, CA, USA). PCRs were performed with 5 μL of

cDNA template diluted to a concentration of 4 ng/mL, 12.5 μL iQ Sybr Green supermix (Bio-Rad), 1 μL forward and reversed primers (10 μmol/L) and 5.5 μL of sterile water. Cycling conditions were as follows: 3 min at 95 °C for denaturation of cDNA followed by 40 amplification cycles of 10 s at 95 °C and annealing for 45 s at 60 °C.

The following primers were used:

Name	**Forward / Reverse Primer Name**	**Sequence**
18SRNA	18SrRNA1F	5′-GTAACCCGTTGAACCCCATT-3′
	18SrRNA1R	5′-CCATCCAATCGGTAGTAGCG-3′
ZO-1	ZO-1 F	5′-AGGGGCAGTGGTGGTTTTCTGTTCTTTC-3′
	ZO-1 R	5′-GCAGAGGTCAAAGTTCAAGGCTCAAGAGG-3′
OCLN	OCLNf1	5′-TCAGGGAATATCCACCTATCACTTCAG-3′
	OCLNr1	5′-CATCAGCAGCAGCCATGTACTCTTCAC-3′
CLDN-4	CLDN4f1	5′-ACAGACAAGCCTTACTCC-3′
	CLDN4r1	5′-GGAAGAACAAAGCAGAG-3′
CLDN-1	CLDN1_f	5'-GGGCTGCAGCTGTTGGGCTT -3'
	CLDN1_r	5' GGGTTGCTTGCAATGTGCTGCT-3'
E-cadherin	E-cadh_f	5'-CACCTGGAGAGAGGCCGCGT-3'
	E-cadh_r	5'-AACGGAGGCCTGATGGGGCG-3'
TRPV-1	TRPVf1	TCTCACCTACATCCTCCTGCTCAA
	TRPVr1	TTGCTCTCCTGTGCGATCTTGT