

Supporting Information

Colon-available Mango (Poly)phenols Exhibit Mitigating Effects on the Intestinal Barrier Function in Human Intestinal Cell Monolayers Under Inflammatory Conditions

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Table S1. Age of the six ileostomy participants in the study.

Volunteer ID	Age
S02	34
S04	65
S05	56
S07	42
S08	33
S10	53

Table S2. Primers used for studies of gene expression.

Gene	Forward	Reverse	T (°C)	Amplicon Size (bp)	Amplification efficiency (%)
Interleukin-8	5' ACT GAG AGT GAT TGA GAG TGG AC 3'	5' AAC CCT CTG CAC CCA GTT TTC 3'	59 °C	127	105 ± 5
Nitric oxide synthase 2	5' TCT GTT CAA GAC CAA ATT CCA CC 3'	5' CGG GGA CTC ATT CTG CTG C 3'	58 °C	151	110 ± 8
Mucin 2	5' GAG GGC AGA ACC CGA AAC C 3'	5' GGC GAA GTT GTA GTC GCA GAG 3'	59 °C	117	101 ± 5
Ribosomal Protein L15	5' GCA GCC ATC AGG TAA GCC AAG 3'	5' AGC GGA CCC TCA GAA GAA AGC 3'	57 °C	100	104 ± 5

Table S3. (Poly)phenols presented in ileal fluid from six subjects pre- (0 h) and post-mango intake (4-8 h). Data is expressed in nmol as mean value \pm SD.

	S02		S04		S05		S07		S08		S10	
	0 h	4-8 h	0 h	4-8 h	0 h	4-8 h	0 h	4-8 h	0 h	4-8 h	0 h	4-8 h
<i>Cinnamic acids</i>												
4'-Hydroxy-3'-methoxycinnamic acid	8 \pm 0.2	65 \pm 0.3*	865 \pm 50	37 \pm 2	257 \pm 4	41 \pm 2	339 \pm 20	15 \pm 0.1	135 \pm 4	16 \pm 0.1	118 \pm 6	122 \pm 4
3'-Methoxycinnamic acid- <i>O</i> -hexoside	12 \pm 0.4	3 \pm 1	<LQ	<LQ	96 \pm 24	<LQ	136 \pm 3	4 \pm 0.3	<LQ	<LQ	7 \pm 2	46 \pm 2 *
4'-Hydroxy-3',5'-dimethoxycinnamic acid	2.4 \pm 0.1	46 \pm 1*	165 \pm 3	11 \pm 0.2	116 \pm 12	119 \pm 1	35 \pm 1	3.7 \pm 0.1	74 \pm 3	188 \pm 3 *	44 \pm 5	368 \pm 2 *
3,5-dimethoxycinnamic acid hexoside 1	0.9 \pm 0.1	<LQ	<LQ	<LQ	<LQ	<LQ	0.9 \pm 0.1	9 \pm 2 *	<LQ	<LQ	2 \pm 1	15 \pm 0 *
3',4'-Dihydroxycinnamic acid	16 \pm 1	5 \pm 0.5	118 \pm 2	26 \pm 0.4	2.8 \pm 0.3	2 \pm 0.1	11 \pm 0.2	3.5 \pm 0.4	82 \pm 1	16 \pm 1	38 \pm 2	9.1 \pm 0.4
3'-Methoxycinnamic acid-4'-glucuronide	13 \pm 1	9 \pm 0.8	247 \pm 66	<LQ	<LQ	6.7 \pm 0.1*	<LQ	7.9 \pm 0.3 *	<LQ	17 \pm 2 *	5 \pm 0.6	11 \pm 0.3 *
4'-Hydroxycinnamic acid-3'-sulfate	67 \pm 2	10 \pm 0.3	<LQ	16 \pm 0.4*	<LQ	1.2 \pm 0.1*	<LQ	15 \pm 3 *	<LQ	55 \pm 0 *	1.5 \pm 0.1	9.9 \pm 0.3 *
3'-Methoxycinnamic acid-4'-sulfate	1299 \pm 49	962 \pm 119	2027 \pm 739	731 \pm 4	<LQ	22 \pm 0.7*	1207 \pm 136	3068 \pm 115 *	4702 \pm 498	1926 \pm 17	2958 \pm 363	975 \pm 25
Total cinnamic acids	1418\pm54	1100 \pm 123	3422 \pm 860	821 \pm 7	472 \pm 40	192 \pm 4	1729\pm160	3126 \pm 121 *	4993 \pm 506	2218 \pm 23	3173 \pm 380	1555 \pm 34
<i>Phenylpropanoic acids</i>												
3-(4'-Hydroxy-3'-methoxyphenyl)propanoic acid	22 \pm 1	<LQ	<LQ	62 \pm 1 *	6089 \pm 872	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	16 \pm 0.1
3-(3'-Hydroxy-4'-methoxyphenyl)propanoic acid	<LQ	<LQ	<LQ	17 \pm 0.4 *	<LQ	35 \pm 1 *	<LQ	<LQ	<LQ	14 \pm 0.3	<LQ	25 \pm 2 *
3-(3'-Hydroxyphenyl)propanoic acid-4'-glucuronide	652 \pm 12	559 \pm 39 *	<LQ	<LQ	<LQ	57 \pm 2 *	104 \pm 32	379 \pm 40 *	<LQ	205 \pm 4 *	<LQ	286 \pm 9 *

3-(4'-Hydroxyphenyl)propanoic acid-3'-glucuronide	384 ± 35	547 ± 21	7961 ± 130	493 ± 29	<LQ	323 ± 15 *	527 ± 96	3402 ± 109 *	<LQ	1146 ± 94 *	<LQ	10735 ± 124
3-(4'-Methoxyphenyl)propanoic acid-3'-glucuronide	20 ± 1	32 ± 10	102 ± 1	99 ± 7	65 ± 4	48 ± 2	11 ± 4	<LQ	111 ± 5	<LQ	114 ± 14	<LQ
3-(3'-Hydroxyphenyl)propanoic acid-4'-sulfate	<LQ	<LQ	<LQ	<LQ	<LQ	117 ± 12 *	194 ± 42	215 ± 50	<LQ	511 ± 25 *	<LQ	330 ± 30
3-(4'-Hydroxyphenyl)propanoic acid-3'-sulfate	2248 ± 143	629 ± 27	<LQ	4630 ± 164 *	7139 ± 2740	73 ± 0.1	2391 ± 467	228 ± 20	2689 ± 545	636 ± 16	1601 ± 289	<LQ
Total phenylpropanoic acids	3326 ± 192	1767 ± 97	8063 ± 131	5301 ± 202	13293 ± 3615	653 ± 32	3227 ± 641	4224 ± 220	2800 ± 550	2512 ± 140	1715 ± 303	11392 ± 165
<i>Phenylacetic acids</i>												
Phenylacetic acid	214 ± 2	<LQ	1416 ± 10	<LQ	<LQ	<LQ	<LQ	<LQ	6123 ± 217	<LQ	5734 ± 31	<LQ
3'-Hydroxyphenylacetic acid-4'-sulfate	<LQ	1554 ± 15 *	<LQ	<LQ	415 ± 6	143 ± 1	<LQ	902 ± 14 *	<LQ	626 ± 9 *	2904 ± 51	1643 ± 15
3'-Methoxyphenylacetic acid-4'-sulfate	1626 ± 156	530 ± 75	<LQ	<LQ	<LQ	144 ± 9 *	1562 ± 1	577 ± 136	<LQ	<LQ	1396 ± 313	<LQ
4'-Methoxyphenylacetic acid-3'-sulfate	206 ± 14	210 ± 56	<LQ	<LQ	115 ± 4	37 ± 1	150 ± 5	140 ± 30	<LQ	176 ± 6 *	1167 ± 419	776 ± 93
Total phenylacetic acids	2046 ± 171	2294 ± 146	1416 ± 10	<LQ	530 ± 10	324 ± 11	1712 ± 6	1619 ± 180	6123 ± 217	802 ± 15	11201 ± 814	2419 ± 108
<i>Benzoic acids</i>												
3,4,5-Trihydroxybenzoic acid	1.6 ± 0.1	685 ± 31 *	13 ± 9	3052 ± 92 *	7.5 ± 0.4	2459 ± 79 *	15 ± 1	5592 ± 952 *	<LQ	2562 ± 52 *	0.8 ± 0.1	1765 ± 200 *
Trihydroxybenzoic acid	<LQ	<LQ	1.2 ± 0.1	160 ± 1 *	0.5 ± 0.1	<LQ	0.8 ± 0.2	21 ± 3 *	2.8 ± 0.1	<LQ	<LQ	<LQ
3,4-dihydroxy-5-(3,4,5-trihydroxybenzoyloxy)benzoic acid	<LQ	11 ± 2 *	<LQ	4.3 ± 0.1 *	<LQ	3 ± 5 *	<LQ	21 ± 1 *	<LQ	34 ± 1 *	<LQ	10 ± 1 *
3,5-Dihydroxy-4-methoxybenzoic acid	<LQ	<LQ	<LQ	4.8 ± 0.2 *	<LQ	46 ± 1 *	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ
Dihydroxybenzoic acid-sulfate 2	0.1 ± 0.1	307 ± 7 *	<LQ	42 ± 1 *	<LQ	71 ± 1 *	<LQ	646 ± 17 *	<LQ	196 ± 0.5 *	<LQ	193 ± 1 *
3-Hydroxy-4-methoxybenzoic acid-5-sulfate	<LQ	<LQ	<LQ	896 ± 17 *	<LQ	137 ± 2 *	<LQ	2454 ± 28 *	<LQ	467 ± 4 *	<LQ	1336 ± 1261

5-Hydroxy-3-methoxybenzoic acid-4-sulfate	<LQ	489 ± 5 *	<LQ	23 ± 1 *	<LQ	34 ± 0.4 *	<LQ	271 ± 5 *	<LQ	49 ± 2 *	<LQ	60 ± 2 *
3,4-Dihydroxybenzoic acid	28 ± 3	<LQ	504 ± 47	918 ± 41 *	<LQ	137 ± 14 *	<LQ	403 ± 52 *	125 ± 5	1750 ± 19 *	202 ± 29	515 ± 56 *
4-Hydroxybenzoic acid	11 ± 1	<LQ	296 ± 6	53 ± 3	85 ± 3	86 ± 2	41 ± 1	<LQ	<LQ	<LQ	<LQ	58 ± 4 *
Hydroxybenzoic acid-hexoside 1	71 ± 4	87 ± 13	42 ± 30	267 ± 17 *	<LQ	3 ± 1	7 ± 5	245 ± 27 *	37 ± 3	638 ± 23 *	<LQ	340 ± 64 *
3-Hydroxybenzoic acid-4-sulfate	64 ± 7	<LQ	50 ± 17	<LQ	<LQ	<LQ	<LQ	254 ± 8 *	<LQ	556 ± 13 *	1116 ± 59	380 ± 27 *
Total benzoic acids	176 ± 15	1579 ± 59 *	906 ± 109	5420 ± 173 *	93 ± 3	2976 ± 105 *	64 ± 7	9653 ± 1093 *	165 ± 9	6252 ± 114 *	1319 ± 88	4657 ± 1613 *
<i>Hydroxybenzenes</i>												
1,2,3-Trihydroxybenzene	7.6 ± 0.1	61 ± 0.6 *	33 ± 10	352 ± 33 *	24 ± 6	495 ± 27 *	4.4 ± 0.2	45 ± 1 *	<LQ	132 ± 7 *	34 ± 3	12 ± 6
Methylbenzene-sulfate 1	0.3 ± 0.1	<LQ	1.6 ± 0.2	<LQ	<LQ	<LQ	4.8 ± 1	<LQ	6.4 ± 0.3	<LQ	<LQ	<LQ
Methylbenzene-sulfate 2	13 ± 0.2	60 ± 0.1 *	14 ± 8	0.3 ± 0.1	<LQ	<LQ	26 ± 4	1.6 ± 0.1	110 ± 2	1.1 ± 0.1	131 ± 1	11 ± 0.1
Dihydroxybenzene-sulfate 3	<LQ	1.8 ± 0.4	<LQ	22 ± 1	<LQ	91 ± 1	<LQ	4 ± 3	<LQ	46 ± 1	<LQ	22 ± 0.1
Hydroxy-methylbenzene-sulfate 1	6.3 ± 0.1	9.5 ± 0.2 *	<LQ	2.6 ± 0.2 *	8.2 ± 0.5	<LQ	<LQ	3.6 ± 0.1 *	0.8 ± 0.1	74 ± 8	92 ± 1	6 ± 2
Hydroxy-methylbenzene-sulfate 2	<LQ	11 ± 0.5 *	<LQ	0.7 ± 0.1 *	4.4 ± 0.1	0.7 ± 0.1	<LQ	2 ± 0.1 *	<LQ	82 ± 8	5.7 ± 0.1	28 ± 4
Total hydroxybenzenes	27 ± 0.4	143 ± 2 *	49 ± 19	378 ± 34 *	37 ± 6	587 ± 28 *	35 ± 5	56 ± 4 *	117 ± 3	335 ± 25	263 ± 5	79 ± 12
<i>Galloyl derivatives</i>												
Mono-galloyl-glucoside 1	<LQ	6 ± 4	<LQ	9 ± 0.2 *	<LQ	16 ± 0.1 *	<LQ	68 ± 1 *	<LQ	6.1 ± 0.1 *	<LQ	<LQ
Mono-galloyl-glucoside 2	<LQ	1.9 ± 0.9	<LQ	<LQ	<LQ	<LQ	<LQ	5.3 ± 0.3 *	<LQ	8 ± 0.5 *	<LQ	32 ± 1 *
Tetra-galloyl-glucoside 1	<LQ	0.1 ± 0.1	<LQ	0.7 ± 0.1 *	<LQ	0.4 ± 0.1 *	<LQ	4.4 ± 0.2 *	<LQ	3.4 ± 0.2 *	<LQ	0.4 ± 0.1 *
Tetra-galloyl-glucoside 2	<LQ	6 ± 1	<LQ	3.8 ± 0.3 *	<LQ	1.8 ± 0.2 *	<LQ	15 ± 2 *	<LQ	9.2 ± 0.1 *	<LQ	1.3 ± 0.1 *
Penta-galloyl-glucoside	<LQ	1.5 ± 0.3 *	<LQ	3.9 ± 0.4 *	<LQ	1.9 ± 0.1 *	<LQ	14 ± 0.3 *	<LQ	5.3 ± 0.2 *	<LQ	1.8 ± 0.1 *
Total galloyl derivatives	<LQ	15 ± 8 *	<LQ	17 ± 1 *	<LQ	20 ± 0.5 *	<LQ	106 ± 5 *	<LQ	31.9 ± 1 *	<LQ	36 ± 1.5 *
Total (poly)phenols	6993±432	6798±435	13856±1129	11937±417	14425±3674	4752±180	6867±819	18784±1623 *	14198±1285	12151±318	17671±1590	20138±1933

S02 is volunteer 2; S04 is volunteer 4; S05 is volunteer 5; S07 is volunteer 7; S08 is volunteer 8; and S10 is volunteer 10.

* 4-8 h post-mango intake was significantly higher than pre-mango intake (p < 0.05)

Table S4. UHPLC–HRMS based identifications of (poly)phenols compounds and related compounds in ileal fluid collected 0 and 4-8 h after mango puree consumption. RT, retention time; [M-H]- Exp, experimental exact mass; Δ , mass error.

RT (min)	Compounds	Chemical Structure	[M-H]- Exp. (m/z)	Δ (ppm)	MSIMI ^a	Quantified as
<i>Cinnamic acids</i>						
11.5	4'-Hydroxy-3'-methoxycinnamic acid	C ₁₀ H ₉ O ₄	193.0495	2.72	1	Standard
8.6	3'-Methoxycinnamic acid- <i>O</i> -hexoside	C ₁₆ H ₁₉ O ₉	355.1023	1.98	2	4'-Hydroxy-3'-methoxycinnamic acid
9.4	3',4'-Dihydroxycinnamic acid	C ₉ H ₇ O ₄	179.0338	-0.59	1	Standard
11.5	4'-Hydroxy-3',5'-dimethoxycinnamic acid	C ₁₁ H ₁₁ O ₅	223.0600	-0.05	1	Standard
8.6	3,5-Dimethoxycinnamic acid hexoside 1	C ₁₇ H ₂₁ O ₁₀	385.1129	2.58	2	4'-Hydroxy-3',5'- dimethoxycinnamic acid
8.6	3'-Methoxycinnamic acid-4'-glucuronide	C ₁₆ H ₁₇ O ₁₀	369.0816	2.76	1	Standard
8.8	4'-Hydroxycinnamic acid-3'-sulfate	C ₉ H ₇ O ₇ S	258.9907	1.40	2	3'-Methoxycinnamic acid-4'-sulfate
9.0	3'-Methoxycinnamic acid-4'-sulfate	C ₁₀ H ₉ O ₇ S	273.0063	1.66	1	Standard
<i>Phenylpropanoic acids</i>						
11.1	3-(4'-Hydroxy-3'-methoxyphenyl)propanoic acid	C ₁₀ H ₁₁ O ₄	195.0652	3.46	1	Standard
11.6	3-(3'-Hydroxy-4'-methoxyphenyl)propanoic acid	C ₁₀ H ₁₁ O ₄	195.0652	-1.30	1	Standard
7.1	3-(3'-Hydroxyphenyl)propanoic acid-4'-glucuronide	C ₁₅ H ₁₇ O ₁₀	357.0816	3.10	2	3-(4'-Methoxyphenyl)propanoic acid-3'-glucuronide
8.1	3-(4'-Hydroxyphenyl)propanoic acid-3'-glucuronide	C ₁₅ H ₁₇ O ₁₀	357.0816	2.60	2	3-(4'-Methoxyphenyl)propanoic acid-3'-glucuronide

9.7	3-(4'-Methoxyphenyl)propanoic acid-3'-glucuronide	C ₁₆ H ₁₉ O ₁₀	371.0973	1.61	1	Standard
7.9	3-(3'-Hydroxyphenyl)propanoic acid-4'-sulfate	C ₉ H ₉ O ₇ S	261.0063	1.27	2	3-(4'-Hydroxyphenyl)propanoic acid-3'-sulfate
8.4	3-(4'-Hydroxyphenyl)propanoic acid-3'-sulfate	C ₉ H ₉ O ₇ S	261.0063	1.62	1	Standard

Phenylacetic acids

9.4	Phenylacetic acid	C ₈ H ₇ O ₂	135.0440	-0.71	1	Standard
7.3	3'-Hydroxyphenylacetic acid-4'-sulfate	C ₈ H ₇ O ₆ S	230.9957	0.03	2	3-(4'-Hydroxyphenyl)propanoic acid-3'-sulfate
6.6	3'-Methoxyphenylacetic acid-4'-sulfate	C ₉ H ₉ O ₇ S	261.0063	1.39	2	3-(4'-Hydroxyphenyl)propanoic acid-3'-sulfate
7.3	4'-Methoxyphenylacetic acid-3'-sulfate	C ₉ H ₉ O ₇ S	261.0063	1.50	2	3-(4'-Hydroxyphenyl)propanoic acid-3'-sulfate

Benzoic acids

3.2	3,4,5-Trihydroxybenzoic acid	C ₇ H ₅ O ₅	169.0131	-0.40	1	Standard
2.4	Trihydroxybenzoic acid	C ₇ H ₅ O ₅	169.0131	0.49	2	3,4,5-Trihydroxybenzoic acid
8.7	3,4-Dihydroxy-5-(3,4,5-trihydroxybenzoyloxy)benzoic acid	C ₁₄ H ₉ O ₉	321.0241	1.10	2	3,4,5-Trihydroxybenzoic acid
4.5	Dihydroxybenzoic acid-sulfate 2	C ₇ H ₅ O ₈ S	248.9705	0.75	2	3-Hydroxy-4-methoxybenzoic acid-5-sulfate
8.0	3,5-Dihydroxy-4-methoxybenzoic acid	C ₈ H ₇ O ₅	183.0287	0.44	1	Standard
8.4	4-Hydroxybenzoic acid	C ₇ H ₅ O ₃	137.0233	-0.66	1	Standard
6.7	3,4-Dihydroxybenzoic acid	C ₇ H ₅ O ₄	153.0182	0.29	1	Standard
4.5	3-Hydroxybenzoic acid-4-sulfate	C ₇ H ₅ O ₇ S	232.975	0.78	2	3-Hydroxy-4-methoxybenzoic acid-5-sulfate
6.9	3-Hydroxy-4-methoxybenzoic acid-5-sulfate	C ₈ H ₇ O ₈ S	262.9856	2.27	1	Standard
7.2	5-Hydroxy-3-methoxybenzoic acid-4-sulfate	C ₈ H ₇ O ₈ S	262.9856	1.32	2	3-Hydroxy-4-methoxybenzoic acid-5-sulfate
4.7	Hydroxybenzoic acid hexoside 1	C ₁₃ H ₁₅ O ₈	299.0767	3.76	2	3,4-Dihydroxybenzoic acid

Hydroxybenzenes

3.5	1,2,3-Trihydroxybenzene	C ₆ H ₅ O ₃	125.0233	-2.72	1	Standard
8.2	Methylbenzene-sulfate 1	C ₇ H ₇ O ₅ S	203.0002	3.00	2	1-hydroxybenzene-2-sulfate
9.4	Methylbenzene-sulfate 2	C ₇ H ₇ O ₅ S	203.0002	1.97	2	1-hydroxybenzene-2-sulfate
4.9	Dihydroxybenzene-sulfate 3	C ₆ H ₅ O ₆ S	204.9801	-0.07	2	1-hydroxybenzene-2-sulfate
3.4	Hydroxy-methylbenzene-sulfate 1	C ₇ H ₇ O ₆ S	218.9957	2.17	2	1-hydroxybenzene-2-sulfate
4.2	Hydroxy-methylbenzene-sulfate 2	C ₇ H ₇ O ₆ S	218.9957	2.85	2	1-hydroxybenzene-2-sulfate
<i>Galloyl derivatives</i>						
2.6	Mono-galloyl glucoside 1	C ₁₃ H ₁₅ O ₁₀	331.0659	1.27	2	Penta-galloyl glucoside
6.6	Mono-galloyl glucoside 2	C ₁₃ H ₁₅ O ₁₀	331.0659	1.31	2	Penta-galloyl glucoside
9.9	Tetra-galloyl glucoside 1	C ₃₄ H ₂₇ O ₂₂	787.0994	2.04	2	Penta-galloyl glucoside
10.7	Tetra-galloyl glucoside 2	C ₃₄ H ₂₇ O ₂₂	787.0994	2.04	2	Penta-galloyl glucoside
11.2	Penta-galloyl glucoside	C ₄₁ H ₃₁ O ₂₆	939.1098	1.87	1	Standard

^a Metabolite Standards Initiative Metabolite Identification (MSIMI) levels (Sumner *et al.*, 2007). Reference compounds were available for all compounds identified at MSIMI level 1.

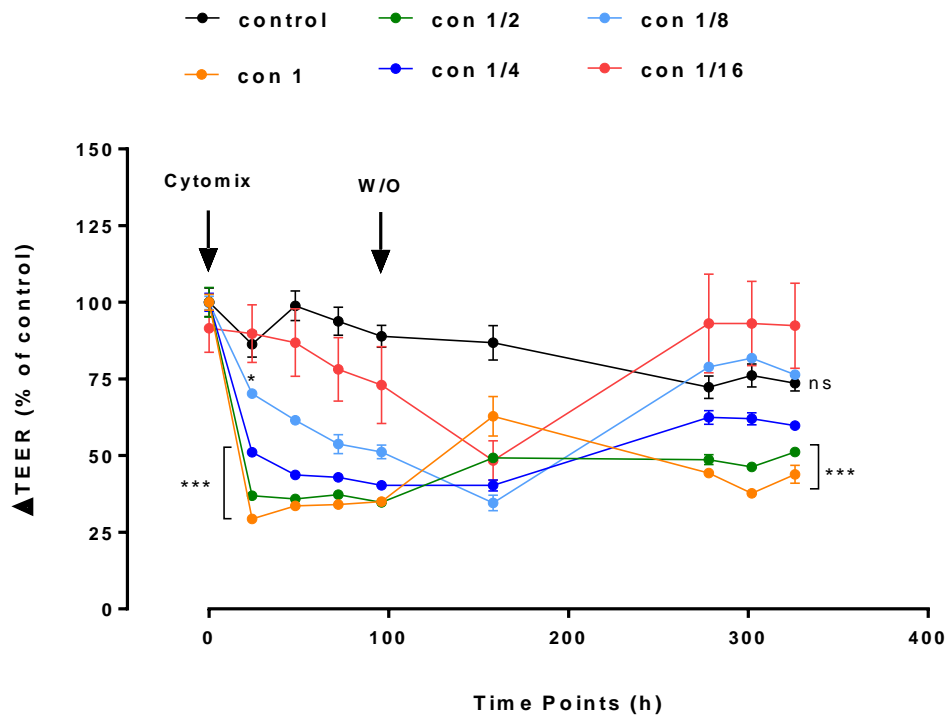


Figure S1. Effect of cytomix on trans epithelial electric resistance (TEER) in polarized Caco-2/HT29-MTX co-culture. After 21 days of culture in trans well insert, serial dilutions of the concentration 1 (TNF- α 100 ng/mL, IFN- γ 50 ng/mL, LPS 10 μ g/mL) of the cytomix mixture in DMEM were tested to monitor the minimal effective doses on the epithelial barrier function. After 96 h of incubation, the basolateral medium was replaced with fresh medium w/o cytomix. TEER was determined every 24 h and expressed as changes relative to cells cultured in the absence of cytomix (Control). Two-way ANOVA with a Bonferroni's multiple comparisons; * $p < 0.05$, *** $p < 0.001$ vs control at the same time point.

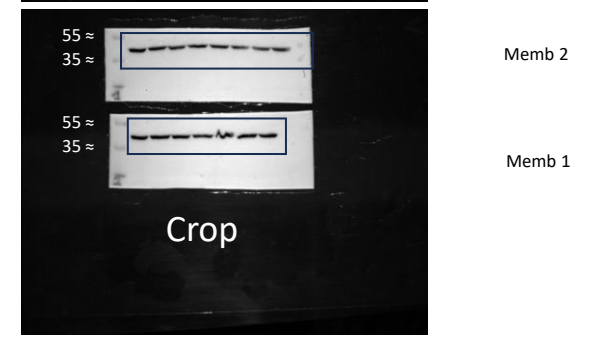
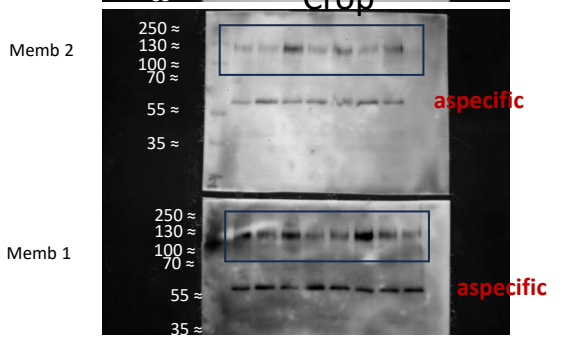
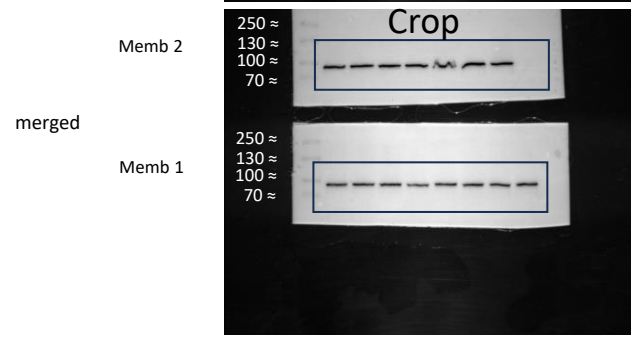
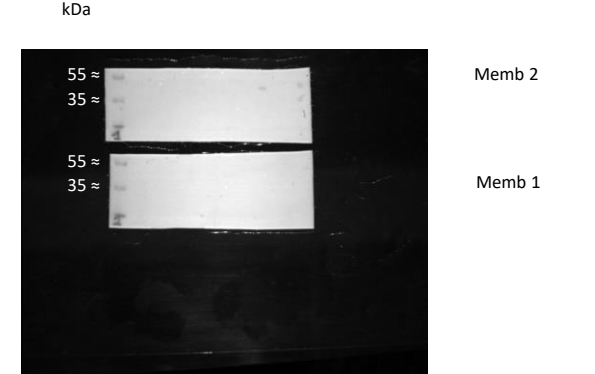
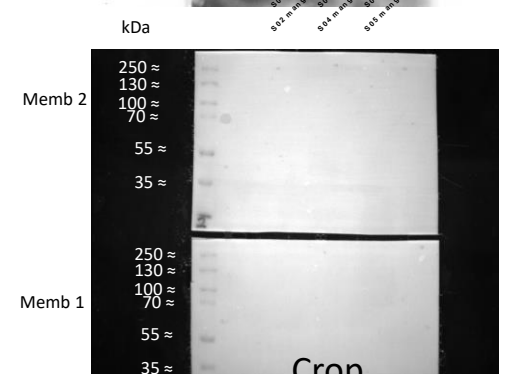
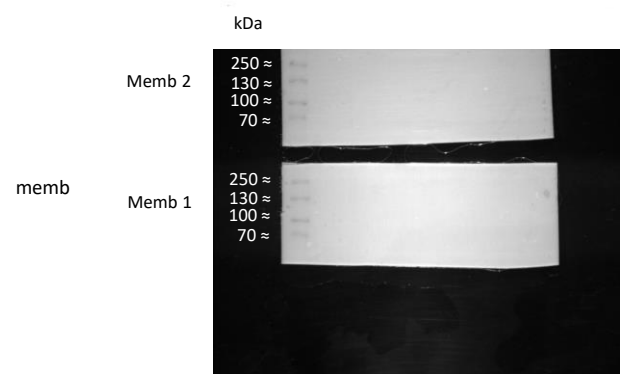
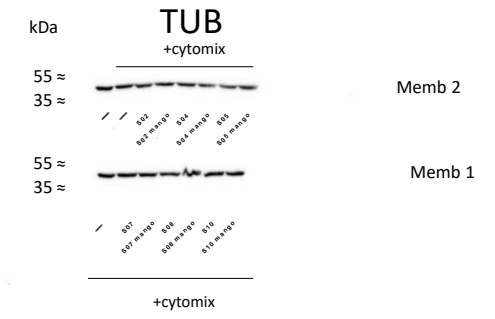
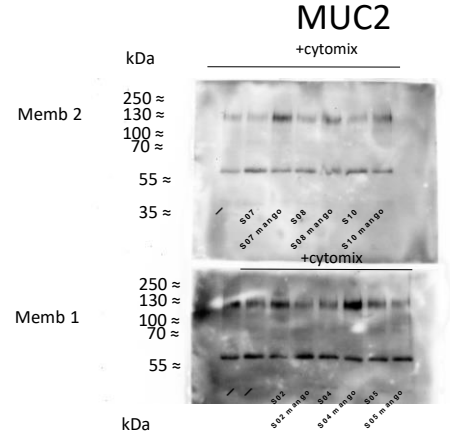
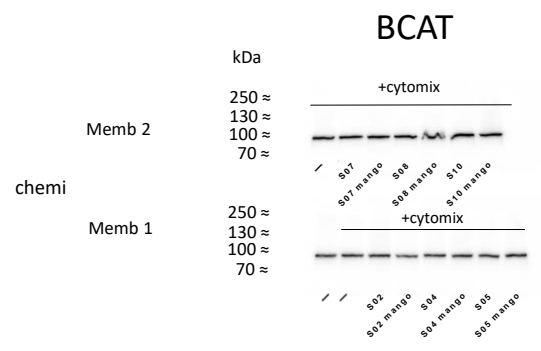


Figure S2. MUC2 and BCAT expression profile in Caco-2/HT29 monolayers. The images report the original results concerning the expression of BCAT, MUC2 and that of the housekeeping Tubulin. In upper, middle and bottom panels line are reported chemiluminescence, acquisition of membranes with white light and both merged signals, respectively. The exposure times for chemiluminescence were automatically selected by the iBright FL1500 automated system software (Life Technology, Thermo Fisher, Milan, Italy) to avoid saturation of the signals. For the complete description of the protocol followed see M&M.