

Fig 1. The effects of different doses of short-chain fatty acids (SCFAs) on prevention of IL-1 β -induced IL-8 induction in H4 cells.

A	Con	IL-1 β	Ace 5mM-IL-1 β	Ace 10mM-IL-1 β	Ace 20mM-IL-1 β	Ace 30mM-IL-1 β
IL8(pg/mg protein)	52.825	1453.376	965.813	791.379	258.939	76.022
	55.575	1211.505	944.436	817.771	271.825	81.368
	60.497	1475.066	950.636	795.051	308.373	95.882
Average	56.299	1379.982	953.628	801.400	279.712	84.424
SD	3.887	146.308	10.998	14.296	25.644	10.277
B	Con	IL-1 β	Pro 5mM-IL-1 β	Pro 10mM-IL-1 β	Pro 20mM-IL-1 β	Pro 30mM-IL-1 β
IL8(pg/mg protein)	52.825	1453.376	705.500	748.230	496.256	382.263
	55.575	1211.505	638.961	674.413	535.368	441.531
	60.497	1475.066	820.252	599.458	458.374	449.388
Average	56.299	1379.982	721.571	674.034	496.666	424.394
SD	3.887	146.308	91.708	74.387	38.498	36.697
C	Con	IL-1 β	Buty 5mM-IL-1 β	Buty 10mM-IL-1 β	Buty 20mM-IL-1 β	Buty 30mM-IL-1 β
IL8(pg/mg protein)	52.825	1453.376	1075.495	621.582	729.811	742.070
	55.575	1211.505	1213.957	442.900	666.235	682.192
	60.497	1475.066	1393.093	991.664	642.199	658.468
Average	56.299	1379.982	1227.515	685.382	679.415	694.243
SD	3.887	146.308	159.232	279.890	45.268	43.084

Fig 2. SCFA inhibited IL-1 β induced IL-8 secretion in H4 organoids and fetal human intestinal organoids.

A	Con	IL-1 β	Ace-IL-1 β	Pro-IL-1 β	Buty-IL-1 β
IL8(pg/mg protein)	3877.257	9801.792	5787.86	3931.875	5470.684
	3791.802	9906.25	5534.542	4660.213	5457.261
	3209.186	7542.541	4679.091	5392.119	5049.431
Average	3626.082	9083.528	5333.831	4661.402	5325.792
SD	363.562	1335.555	580.996	730.123	239.430
B	Con	IL-1 β	Ace-IL-1 β	Pro-IL-1 β	Buty-IL-1 β
IL8(pg/mg protein)	348.719	451.427	396.819	275.035	296.982
	348.557	486.971	427.190	263.215	325.936
	376.641	578.029	385.319	244.607	269.337
Average	357.972	505.476	403.110	260.952	297.418
SD	16.167	65.298	21.632	15.340	28.302
C	Con	IL-1 β	Ace-IL-1 β	Pro-IL-1 β	Buty-IL-1 β
IL8(pg/mg protein)	928.992	2479.371	1094.093	1180.704	1490.306
	1050.068	1916.334	1106.584	1052.224	1447.706
	1031.451	1966.013	1094.093	948.612	1246.876
Average	1003.504	2120.573	1098.257	1060.513	1394.963
SD	65.197	311.720	7.212	116.268	130.004

Fig 3. The anti-inflammatory effects of SCFAs on immature enterocytes in ex vivo.

A	Con	IL-1β	Ace-IL-1β	Pro-IL-1β	Buty-IL-1β
MIP2	28.932	388.940	37.909	62.260	33.761
(pg/mg protein)	22.033	322.404	49.815	43.465	53.640
	51.375	354.497	77.106	63.148	83.612
	63.766	357.779	96.218	63.468	82.878
	98.350	260.631	70.979	82.729	75.646
	113.639	361.347	80.471	86.741	65.649
Average	63.016	340.933	68.750	66.968	65.865
SD	36.847	44.679	21.337	15.753	19.392
B	Con	IL-1β	Ace-IL-1β	Pro-IL-1β	Buty-IL-1β
MIP2	31.001	349.426	68.460	79.776	80.605
(pg/mg protein)	29.272	304.590	70.904	78.532	77.645
	32.411	281.947	68.277	74.888	66.943
	27.835	272.426	64.093	62.249	47.281
	31.772	255.415	67.033	85.176	47.761
	19.533	274.758	65.687	60.516	48.153
Average	28.637	289.760	67.409	73.523	61.398
SD	4.766	33.305	2.374	9.981	15.648

Fig 4. SCFAs inhibit IL-1 β induced histone deacetylases 3 (HDAC3) and HDAC5 mRNA expression in immature enterocytes.

A	Con	Ace	Pro	Buty	IL-1β	Ace-IL-1β	Pro-IL-1β	Buty-IL-1β
H4-HDAC3 mRNA fold	0.992	0.757	1.924	1.940	8.360	3.001	3.079	0.748
(Normalized to GAPDH)	0.906	1.342	2.107	1.562	11.349	3.970	4.423	0.872
	1.112	1.137	1.801	1.661	9.457	3.034	3.535	1.035
Average	1.004	1.079	1.944	1.721	9.722	3.335	3.679	0.885
SD	0.103	0.297	0.154	0.196	1.512	0.550	0.683	0.144
B	Con	Ace	Pro	Buty	IL-1β	Ace-IL-1β	Pro-IL-1β	Buty-IL-1β
H4-HDAC5 mRNA fold	1.004	1.398	1.662	1.897	10.986	0.462	0.742	0.443
(Normalized to GAPDH)	0.919	1.867	1.651	1.310	12.553	0.480	0.747	0.654
	1.083	1.666	1.544	1.058	12.466	0.349	0.929	0.471
Average	1.002	1.644	1.619	1.422	12.002	0.430	0.806	0.523
SD	0.082	0.236	0.065	0.430	0.881	0.071	0.107	0.114

Fig 5. SCFAs inhibit IL-1 β induced HDACs protein expression in immature enterocytes.

HDACs Protein (Luminescent intensity)	Con	Ace	Pro	Buty	IL-1 β	Ace-IL-1 β	Pro-IL-1 β	Buty-IL-1 β
	7416.0	7647.0	3633.0	3241.0	19115.0	3852.0	2469.0	3241.0
	6771.0	8255.0	4877.0	3063.0	20670.0	3707.0	2997.0	3063.0
	7594.0	6207.0	4013.0	2997.0	16651.0	4334.0	1189.0	4013.0
Average	7260.3	7369.7	4174.3	3100.3	18812.0	3964.3	2218.3	3439.0
SD	433.0	1051.8	637.5	126.2	2026.6	328.2	929.7	505.0

Fig 6. Both HDAC3 and HDAC5 are required for mediating IL-1 β induced IL-8 secretion in immature enterocytes *in vitro* and *ex vivo*

A	Con	IL-1 β	TSA-IL-1 β	LMK-IL-1 β
IL8(pg/mg protein)	68.618	1453.376	825.419	919.872
	69.027	1211.505	802.803	866.237
	74.170	1475.066	766.930	545.120
Average	70.605	1379.982	798.384	777.076
SD	3.094	146.308	29.494	202.662
B	Con	IL-1 β	TSA-IL-1 β	LMK-IL-1 β
MIP2 (pg/mg protein)	46.896	160.702	88.374	35.974
	37.205	164.465	71.539	33.517
	29.076	160.975	58.002	34.400
	51.375	151.262	92.871	53.418
	63.766	147.962	55.967	52.571
	29.076	156.541	60.842	57.285
Average	42.899	156.984	71.266	44.528
SD	12.502	5.774	14.596	10.029
C	Con	IL-1 β	TSA-IL-1 β	LMK-IL-1 β
MIP2 (pg/mg protein)	31.001	349.426	61.155	69.647
	29.272	304.590	50.734	70.888
	32.411	272.896	50.949	68.390
	35.023	281.946	67.238	69.647
	45.206	264.577	66.912	70.888
	44.090	222.465	75.584	68.390
Average	36.167	282.650	62.095	69.642
SD	6.843	42.391	9.857	1.117

Fig 7. SCFAs increase G protein-coupled receptors 109A (GPR109A) mRNA expression in response to IL-1 β stimulation in human immature enterocytes

	Con	Ace	Pro	Buty	IL-1 β	Ace-IL-1 β	Pro-IL-1 β	Buty-IL-1 β
109A mRNA fold	1.16	2.88	2.94	4.16	2.39	16.46	10.97	10.01
(Normalized to GAPDH)	0.86	3.28	4.06	4.92	1.89	19.90	15.47	14.65
	1.00	3.14	4.54	4.29	2.31	22.15	10.86	15.75
Average	1.01	3.10	3.85	4.46	2.20	19.50	12.43	13.47
SD	0.152	0.202	0.821	0.409	0.267	2.866	2.631	3.047

Fig 8. 109A is required for SCFAs anti IL-1 β induced IL8 induction in H4 cells

	Con	IL-1 β	0.2mM MB Ace-IL-1 β	0.2mM MB Pro-IL-1 β	0.2mM MB Buty -IL-1 β	Ace-IL-1 β	Pro-IL-1 β	Buty-IL-1 β
IL8								
(pg/ mg protein)	18.959	919.264	864.756	873.847	1440.781	585.205	396.026	547.326
	14.809	1371.54	904.844	945.696	916.526	642.623	459.94	684.226
	12.869	871.819	885.766	994.976	1324.138	627.615	336.368	426.11
Average	15.546	1054.208	885.122	938.173	1227.148	618.481	397.445	552.554
SD	3.111	275.840	20.052	60.914	275.256	29.779	61.798	129.137

Supporting information

S1 Fig. SCFAs inhibited IL-1 β induced IL8 induction in Caco2 cells but not by inhibition of HDACs activity

A	Con	IL-1β	Ace-IL-1β	Pro-IL-1β	Buty-IL-1β
IL8(pg/mg protein) (Caco2)	23.521	483.126	97.785	70.813	64.532
	31.709	456.819	70.778	80.659	69.341
	28.072	449.254	57.274	60.260	61.358
Average	27.767	463.067	75.279	70.577	65.077
SD	4.102	17.779	20.627	10.201	4.019
B	Con	IL-1β	Ace-IL-1β	Pro-IL-1β	Buty-IL-1β
Caco2 -HDAC3 mRNA fold	1.067	0.717	1.008	0.929	1.138
(Normalized to GAPDH)	0.744	1.021	0.857	0.598	1.411
	0.794	0.831	1.095	0.802	1.440
Average	0.868	0.856	0.987	0.777	1.330
SD	0.174	0.153	0.120	0.167	0.166
C	Con	IL-1β	Ace-IL-1β	Pro-IL-1β	Buty-IL-1β
Caco2-HDAC5 mRNA fold	1.029	0.626	0.668	0.996	0.890
(Normalized to GAPDH)	0.870	0.601	0.815	0.646	0.984
	1.117	0.944	0.878	0.762	1.325
Average	1.005	0.724	0.787	0.801	1.066
SD	0.125	0.191	0.108	0.178	0.229