Electronic Supplementary Material (ESI) for Food & Function.
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Supplementary materials

Table S1 Effect of the experimental treatments on jejunal gene expression. Values are expressed compared to a reference sample of the control treatment. The average values of the treatments are expressed without the logarithmic transformation used for the statistical analysis.

Main Effects	OPN -		OPN +						
	SYN -	SYN +	SYN -	SYN +		*p-value*			
Genes	**CTR**	**SYN**	**OPN**	**CON**	**RSE**	**SYN**	**OPN**	**Interaction**	**Function**
ALPI	1.140[b]	2.204[a]	1.859[a]	2.091[a]	1.588	0.001*	0.084	0.027*	Enzyme hormone
ANPEP	1.146	1.159	1.088	1.172	1.354	0.710	0.784	0.811	Enzyme hormone
CCK	2.07	2.252	1.823	2.655	1.581	0.025*	0.897	0.257	Enzyme hormone
CCL20	0.361	0.661	1.049	0.801	2.737	0.198	0.266	0.866	Immune response
CLDN1	2.022	1.369	1.532	1.265	2.427	0.150	0.779	0.422	Barrier function
CLDN15	0.918	0.992	0.949	1.066	1.388	0.298	0.418	0.968	Barrier function
CLDN4	1.188	1.179	1.141	1.171	1.848	0.958	0.800	0.844	Barrier function
CRHR1	1.839	1.549	1.423	1.638	2.408	0.360	0.688	0.318	Stress
CXCL2	1.286	0.543	0.586	0.829	2.194	0.066	0.307	0.075	Immune response
DAO1	0.913	1.164	1.098	1.132	1.450	0.175	0.342	0.169	Enzyme hormone
FAXDC2	1.465	2.899	2.1	2.525	2.530	0.091	0.662	0.182	Immune response
GBP1	1.415	0.781	0.9	0.9	2.289	0.045*	0.347	0.217	Immune response
GCG	0.924	0.891	1.13	1.129	1.468	0.883	0.040*	0.900	Enzyme hormone
GPX2	1.826	1.433	1.868	1.433	2.094	0.091	0.846	0.957	Enzyme hormone
HNMT	1.954	1.563	1.844	1.724	1.451	0.312	0.956	0.250	Enzyme hormone
HSD11B1	2.3	1.525	1.675	1.872	1.721	0.383	0.631	0.059	Stress
HSPA4	1.458	1.25	1.266	1.33	1.456	0.555	0.674	0.207	Immune response
HSPB1	1.616	1.494	1.476	1.62	1.462	0.865	0.961	0.469	Immune response
IDO1	1.364[a]	0.284[b]	0.458[ab]	0.466[ab]	5.198	0.024*	0.372	0.035*	Enzyme hormone
IFNG	3.15	1.002	1.151	1.618	4.136	0.082	0.902	0.145	Immune response
IFNGR1	1.356	1.455	1.406	1.506	1.361	0.373	0.662	0.973	Immune response
IGF1R	1.382	1.112	1.212	1.343	1.437	0.300	0.687	0.202	Enzyme hormone
IL10	3.011	2.181	2.509	1.927	1.918	0.148	0.654	0.547	Immune response
IL17A	0.773	0.663	0.615	0.921	2.484	0.885	0.519	0.608	Immune response
IL1beta	1.276[a]	0.406[b]	0.393[b]	0.560[b]	2.389	0.036*	0.053	0.027*	Immune response
IL22	1.076	0.614	0.612	1.047	2.664	0.549	0.462	0.166	Immune response
IL6	2.899	1.103	1.379	1.356	2.304	0.050	0.356	0.103	Immune response
IL8	0.874	0.548	0.63	0.724	1.698	0.220	0.633	0.092	Immune response
MUC13	0.815	1.116	0.988	0.871	1.506	0.492	0.605	0.015*	Barrier function
MUC2	1.299	1.008	1.094	1.291	1.385	0.447	0.877	0.040*	Barrier function
NR3C1	1.344	1.423	1.264	1.42	1.266	0.192	0.783	0.729	Stress
OCLN	1.202	1.571	1.383	1.441	1.302	0.041*	0.815	0.212	Barrier function
PPARGC1alfa	0.967	1.058	0.785	0.827	1.550	0.400	0.253	0.664	Barrier function
PYY	0.993	1.166	1.014	1.172	1.526	0.194	0.806	0.945	Enzyme hormone
REG3G	5.741	6.127	6.78	7.876	6.391	0.390	0.168	0.463	Immune response
SI	2.286[b]	4.729[a]	2.771[ab]	3.569[ab]	1.884	0.004*	0.840	0.026*	Enzyme hormone
SLC11A2	1.503	1.307	1.409	1.285	1.281	0.096	0.702	0.874	Nutrient transport
SLC13A1	0.666[b]	1.086[a]	0.934[ab]	1.113[a]	2.255	0.026*	0.072	0.073	Nutrient transport
SLC15A1	1.632[b]	2.440[a]	1.893[ab]	2.171[ab]	1.490	0.010*	0.731	0.166	Nutrient transport

SLC16A1	1.398	1.187	1.137	1.204	1.457	0.674	0.252	0.283	Nutrient transport
SLC30A1	1.498	1.508	1.466	1.636	1.372	0.408	0.737	0.467	Nutrient transport
SLC39A4	1.24	1.753	1.5	1.529	1.448	0.146	0.566	0.072	Nutrient transport
SLC5A1	1.694^{b}	2.791^{a}	1.844^{ab}	2.349^{ab}	1.668	0.005*	0.909	0.215	Nutrient transport
SLC7A8	1.077	2.604	2.375	3.175	3.187	0.043*	0.296	0.082	Nutrient transport
SOD2 m	1.321	0.96	1.07	1.058	1.402	0.103	0.423	0.121	Enzyme hormone
TFF3	1.313	1.124	1.368	1.327	1.512	0.836	0.682	0.564	Barrier function
TGFbeta1	1.550^{a}	0.904^{b}	0.916^{b}	1.105^{ab}	1.583	0.108	0.163	0.010*	Barrier function
TLR2	0.817	0.431	0.635	0.651	2.178	0.073	0.911	0.171	Immune response
TLR4	2.542^{a}	1.297^{b}	1.577^{ab}	1.620^{ab}	1.755	0.068	0.465	0.017*	Immune response
TNFalfa	1.796	1.04	1.146	1.238	1.884	0.137	0.292	0.166	Immune response
ZO1	1.323	1.109	1.196	1.239	1.351	0.261	0.823	0.265	Barrier function

CTR: no additives in the diet; SYN: prebiotic+probiotic additives in the diet; OPN: osteopontin in the diet; CON: symbiotic + osteopontin in the diet. n=8. Gene abbreviations detailed in Supplementary materials (Table S2). *Indicates $p < 0.05$ and statistical difference present. ^{a,b} Indicate statistically significant differences between means. SEM, standard error of the mean.

Table S2 List of 56 genes (abbreviation, name and function group) used for gene expression analysis using Open Array Real-Time PCR custom designed.

Genes	Name	Function group
ALPI	Intestinal alkaline phosphatase	Enzyme hormone
ANPEP	Alanyl aminopeptidase membrane	Enzyme hormone
CCK	Cholecystokinin	Enzyme hormone
CCL20	Chemokine (C-C motif) ligand 20	Immune response
CLDN1	Claudin-1	Barrier function
CLDN15	Claudin-15	Barrier function
CLDN4	Claudin-4	Barrier function
CRHR1	Corticotropin releasing hormone receptor 1	Stress
CXCL2	Chemokine (C-X-C motif) ligand 2	Immune response
DAO1	Diamine oxidase	Enzyme hormone
FAXDC2	Fatty acid hydrolase domain containing 2	Immune response
GBP1	Guanylate binding protein 1	Immune response
GCG	Glucagon	Enzyme hormone
GPX2	Glutathione peroxidase 2	Enzyme hormone
HNMT	Histamine N-methyltransferase	Enzyme hormone
HSD11B1	Hydroxysteroid (11-beta) dehydrogenase 1	Stress
HSPA4	Heat shock protein 70	Immune response
HSPB1	heat shock protein 27	Immune response
IDO1	Indoleamine 2,3-dioxygenase	Enzyme hormone
IFNG	Interferon gamma	Immune response
IFNGR1	Interferon gamma receptor 1	Immune response
IGF1R	Insulin-like growth factor 1 receptor	Enzyme hormone
IL10	Interleukin 10	Immune response
IL17A	Interleukin 17 A	Immune response
IL1beta	Interleukin 1 beta	Immune response
IL22	Interleukin 22	Immune response
IL6	Interleukin 6	Immune response
IL8	Interleukin 8	Immune response
MUC13	Mucin 13	Barrier function
MUC2	Mucin 2	Barrier function
NR3C1	Glucocorticoid receptor	Stress
OCLN	Ocludin	Barrier function
PPARGC1alfa	Peroxisome proliferative activated receptor gamma, coactivator 1 alpha	Immune response
PYY	Peptide tyrosine tyrosine	Enzyme hormone
REG3G	Regenerating-islet derived protein 3 gamma	Immune response
SI	Sucrase-isomaltase	Enzyme hormone

SLC11A2	Solute carrier family 11 (proton-coupled divalent metal ion transporter) member 2	Nutrient transport
SLC13A1	Solute carrier family 13 (sodium/sulfate symporters) member 1	Nutrient transport
SLC15A1	Solute carrier family 15 (oligopeptide transporter) member 1	Nutrient transport
SLC16A1	Monocarboxylate transporter 1	Nutrient transport
SLC30A1	Solute carrier family 30 (zinc transporter) member 1	Nutrient transport
SLC39A4	Solute carrier family 39 (zinc transporter) member 4	Nutrient transport
SLC5A1	Solute carrier family 5 (sodium/glucose cotransporter) member 1	Nutrient transport
SLC7A8	Solute carrier family 7 (amino acid transporter light chain, L System) member 8	Nutrient transport
SOD2	Superoxide dismutase	Enzyme hormone
TFF3	Trefoil factor 3	Barrier function
TGFbeta1	Transforming growth factor beta 1	Immune response
TLR2	Toll-like receptor 2	Immune response
TLR4	Toll-like receptor 4	Immune response
TNFalfa	Tumor necrosis factor alpha	Immune response
ZO1	Zonula occludens 1	Barrier function

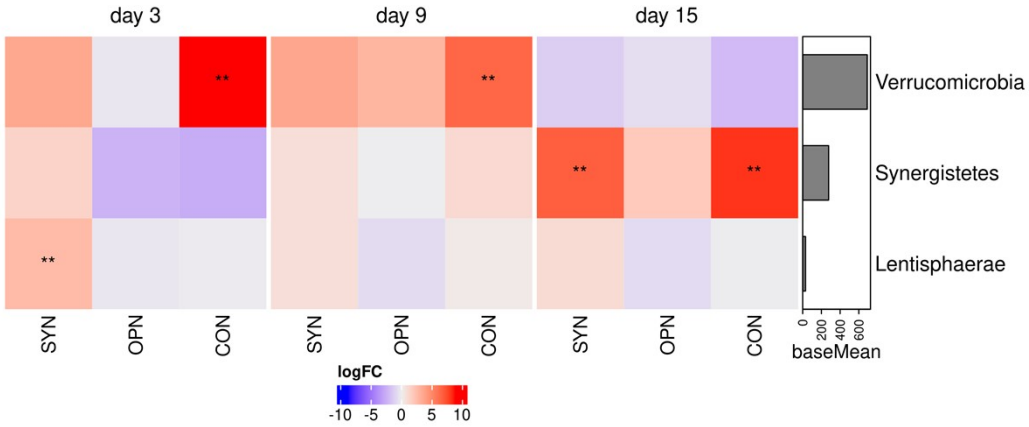

Figure S1 Heatmap of the impact of experimental treatments on bacterial phyla observed in faecal samples on various sampling days (3, 9 and 15). CTR: no additives in the diet; SYN: prebiotic + probiotic additives in the diet; OPN: osteopontin in the diet; CON: synbiotic + osteopontin in the diet. n = 8. * Indicates $p < 0.1$; ** indicates $p < 0.05$.

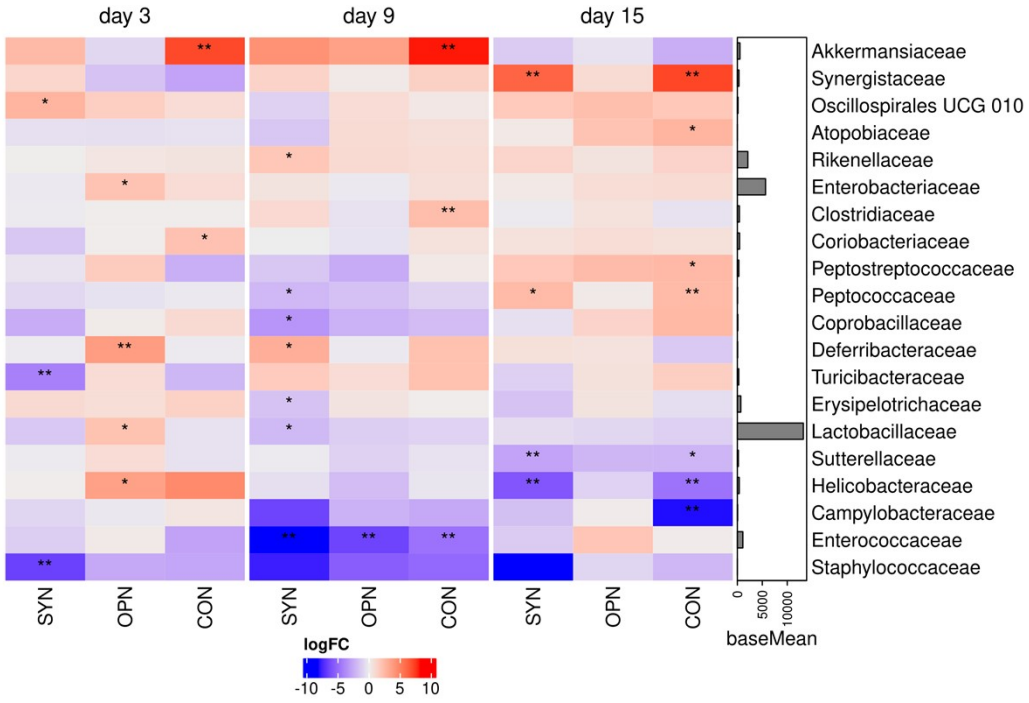

Figure S2 Heatmap of the impact of experimental treatments on bacterial families observed in faecal samples on various sampling days (3, 9 and 15). CTR: no additives in the diet; SYN: prebiotic + probiotic additives in the diet; OPN: osteopontin in the diet; CON: synbiotic + osteopontin in the diet. n = 8. * Indicates $p < 0.1$; ** indicates $p < 0.05$.

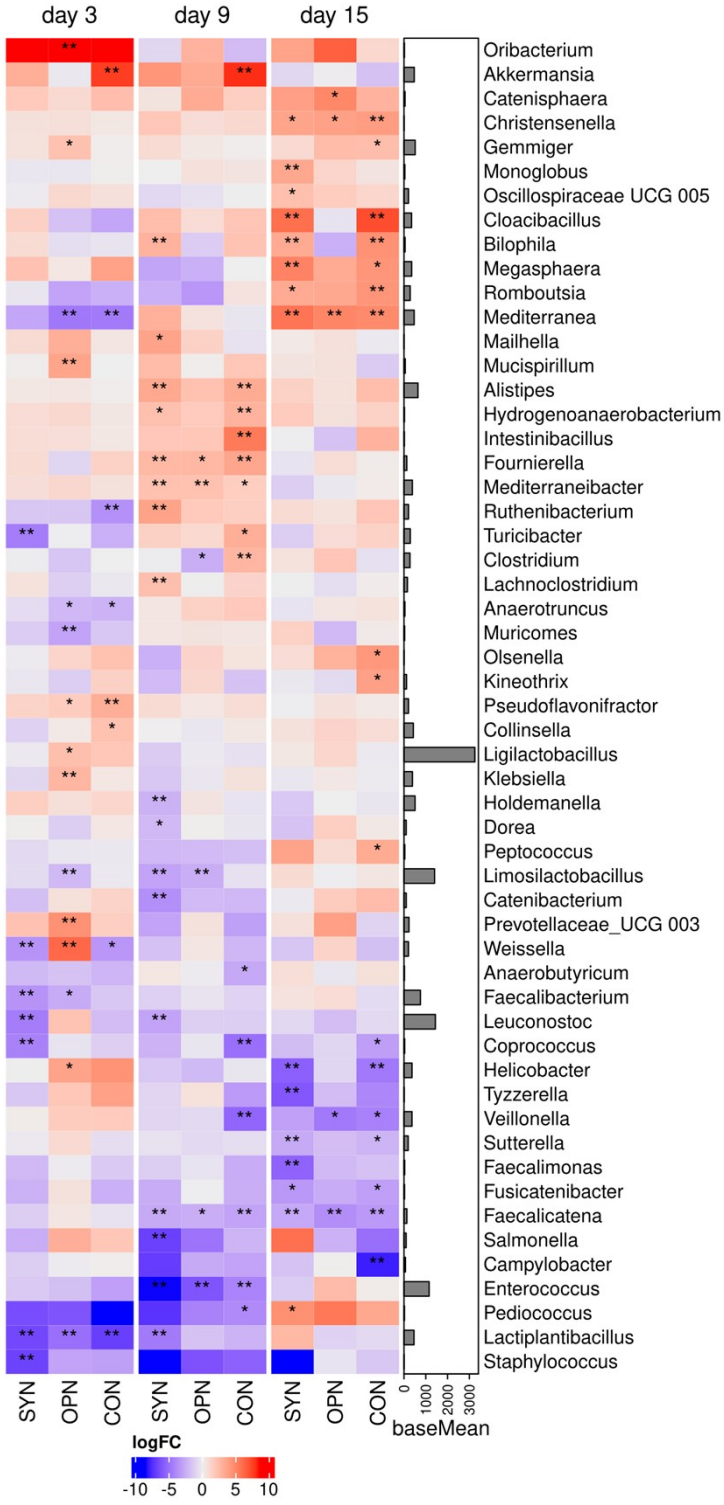

Figure S3 Heatmap of the impact of experimental treatments on bacterial genera observed in faecal samples on various sampling days (3, 9 and 15). CTR: no additives in the diet; SYN: prebiotic + probiotic additives in the diet; OPN: osteopontin in the diet; CON: synbiotic + osteopontin in the diet. n = 8. * Indicates $p < 0.1$; ** indicates $p < 0.05$.

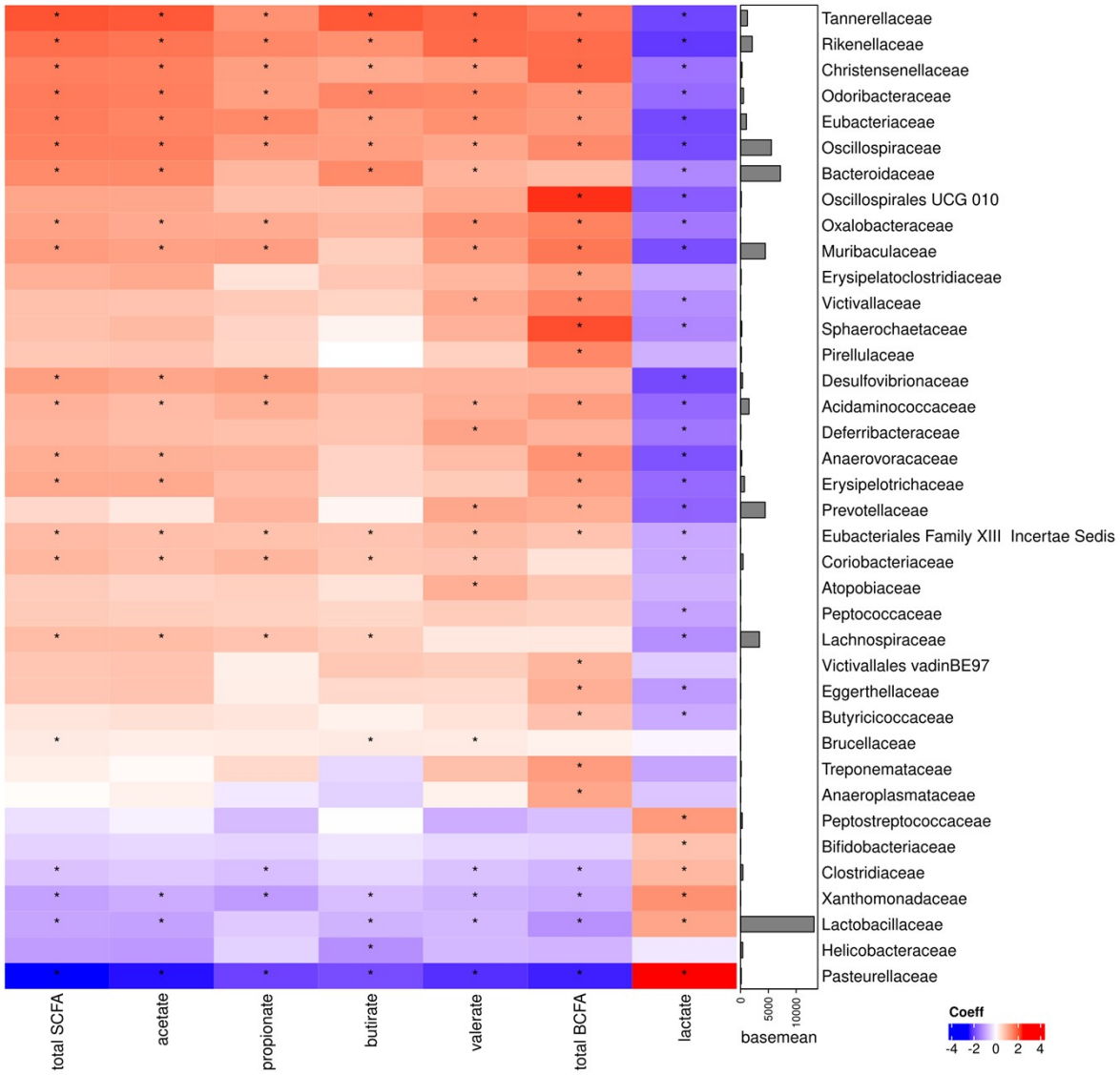

Figure S4 Heatmap of correlations between colonic Short-Chain Fatty Acids (SCFA) concentration and bacterial families detected on faecal samples from day 15. BCFA, Branched-chain fatty acids. *Indicates $p < 0.05$ and statistical difference present.

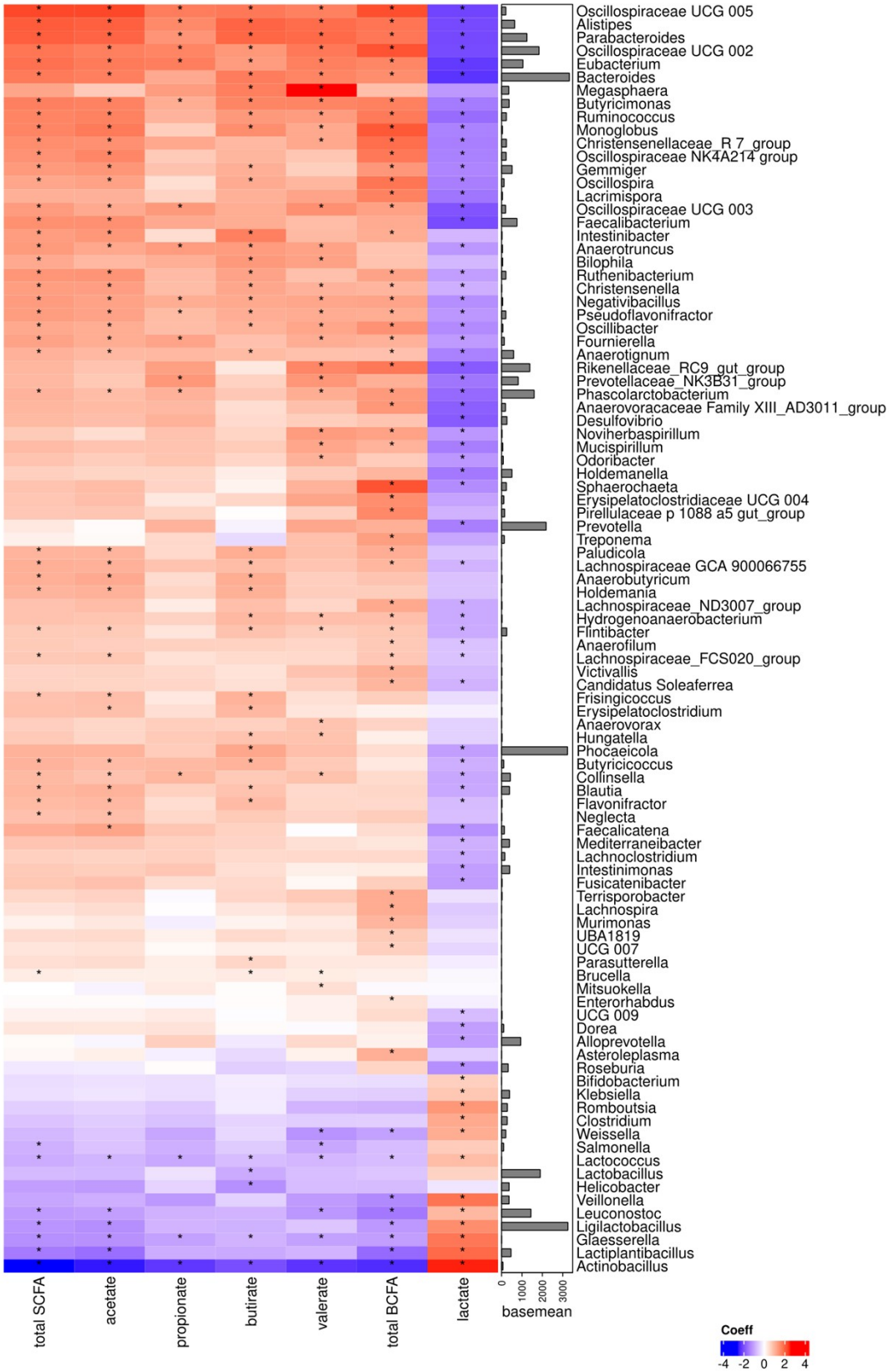

Figure S5 Heatmap of correlations between colonic Short-Chain Fatty Acids (SCFA) concentration and bacterial genera detected on faecal samples from day 15. BCFA, Branched-chain fatty acids. *Indicates $p < 0.05$ and statistical difference present.

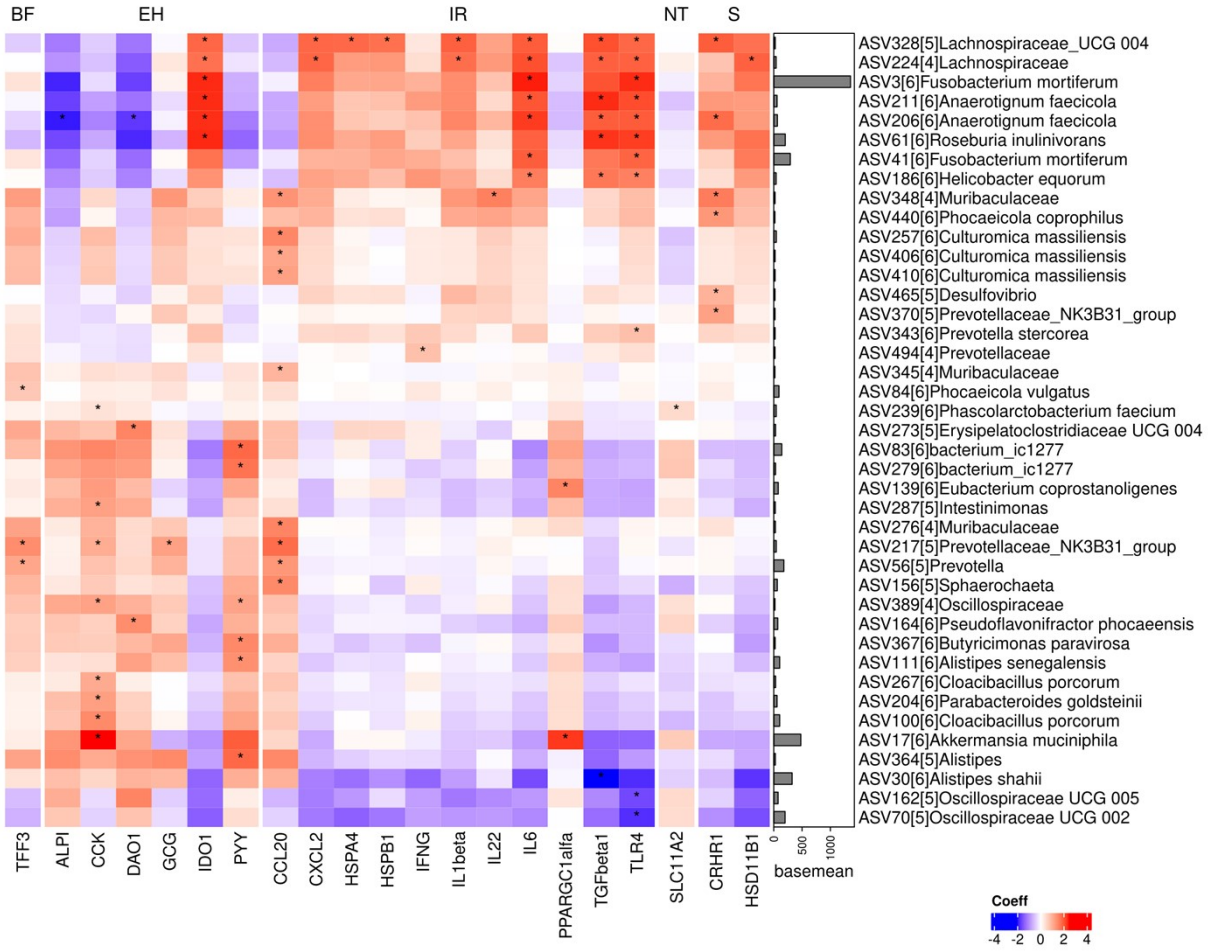

Figure S6 Heatmap of correlations between jejunal gene expression and ASVs detected on faecal samples from day 15. *Indicates $p < 0.05$ and statistical difference present.

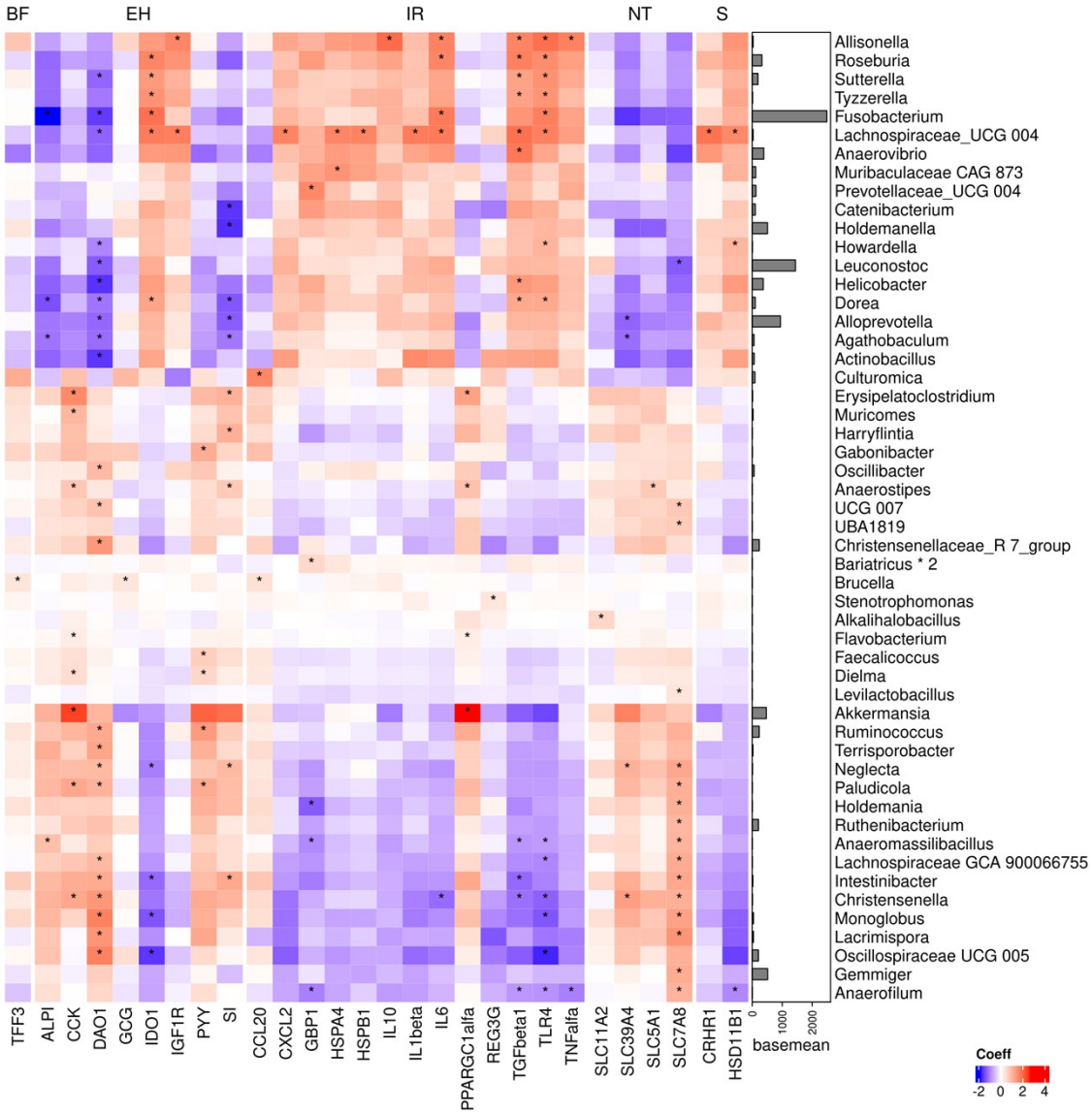

Figure S7 Heatmap of correlations between jejunal gene expression and bacterial genera detected on faecal samples from day 15. *Indicates $p < 0.05$ and statistical difference present.

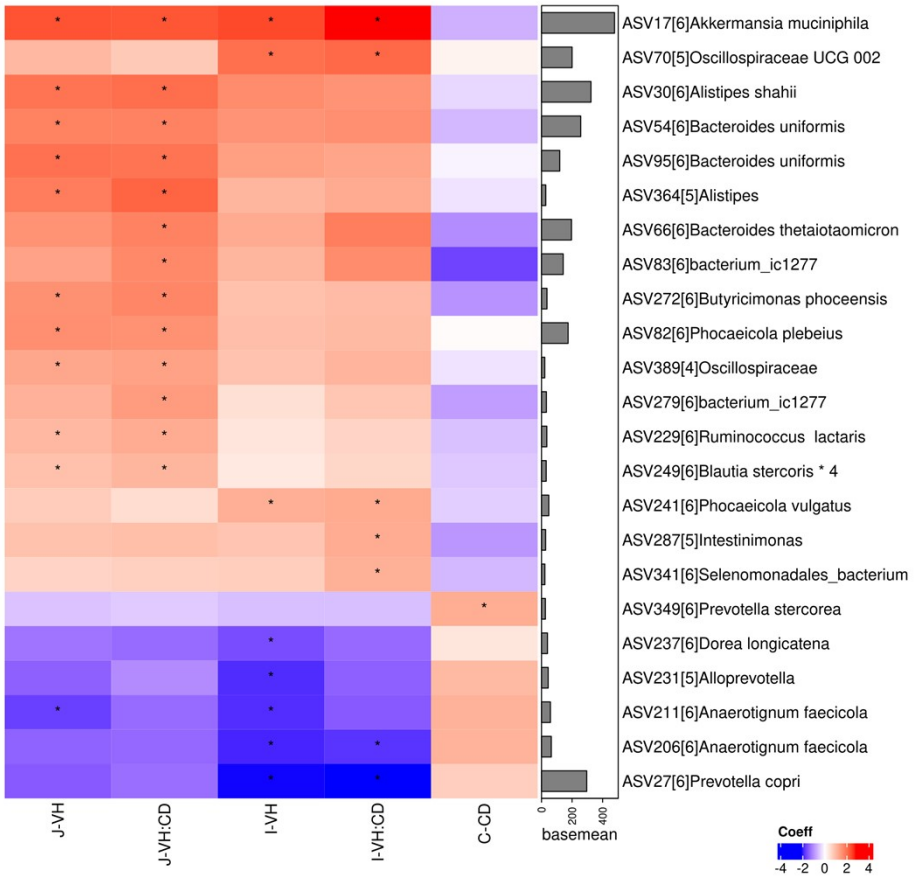

Figure S8 Heatmap of correlations between histology (jejunal, ileal and colonic) and ASVs detected on faecal samples from day 15. J=jejunal, I=ileal, C=colonic. VH=villus height; CD=crypt depth; VH.CD=ratio villus height:crypt depth. *Indicates $p < 0.05$ and statistical difference present.

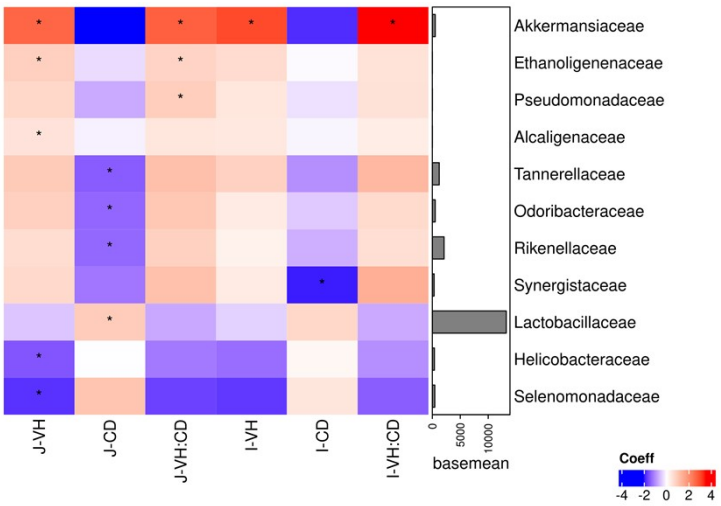

Figure S9 Heatmap of correlations between histology (jejunal and ileal) and bacterial families detected on faecal samples from day 15. J=jejunum, I=ileum. VH=villus height; CD=crypt depth; VH.CD=ratio villus height:crypt depth. *Indicates $p < 0.05$ and statistical difference present.