Supplemental Information

Indoleacrylic acid produced by commensal *Peptostreptococcus* species suppresses inflammation

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Supplemental Figure Legends

Figure S1. Genes involved in microbial mucin utilization in 90 HMP subjects. Related to Figure 1, Table 1, and Table S1.

(A) HMP subject IDs used for analysis.

(B) Heat map showing RPKM of the most abundant genes, shown as UniProt IDs, including cleavage enzymes (black) and transporters (red) found in 90 fecal metagenomic samples from HMP subjects. Asterisks indicate fucosidases or fucose transporter genes. See Supplemental Table 1 for gene list. Red branch in column dendrogram indicates distinct HMP subject.

Figure S2. Gene expression profiling and bacterial abundance after repeated bacterial gavage. Related to Figure 3.

Mice were orally gavaged with PBS, *A. muciniphila*, *C. butyricum*, or *P. russellii* every other day for 2 weeks and the distal large intestine was harvested for analysis.

(A) Quantitative RT-PCR showing expression of genes regulating goblet cell differentiation, *Agr2* (***P* = 0.0021), stem cell differentiation into secretory cell lineages, *Spedf* (***P* = 0.0048), *Atoh1*, *Gfi1* (**P* = 0.0347), *Hes1* (**P* = 0.0118), and stem cell differentiation into endocrine cells, Ngn3.

(B) Quantitative RT-PCR showing expression of goblet cell-encoded glycosyltransferase enzymes *Fut2* (**P* = 0.0382), *St6gal1* (***P* = 0.0024), and *B3gnt6* in the distal colon, n = 9-10 per group. Data pooled from 2 independent experiments.

(C) Quantitative RT-PCR showing the abundance of a *P. russellii* species-specific gene (HD superfamily hydrolase, copies per 10 ng total DNA) and total bacterial (16S rRNA copies per 10 ng DNA) in feces from PBS or *P. russellii*-treated mice, n = 9-10 per group (**P* = 0.0218). Data pooled

from 2 independent experiments.

Figure S3. Ion chromatograms and CID-MS spectra for identification of IPA, IA, and HPPA. Related to Figure 4.

(A-C) MS QTOF analysis of culture supernatant from *Peptostreptococcus* species shows the production of IPA (A), IA (B), and HPPA (C). Ion chromatograms extracted at *m/z* and retention time of IPA (*m/z* 190.0873), IA (*m/z* 188.0711), and HPPA (*m/z* 167.0711) are shown. Red, *P. anaerobius*; black, *P. russellii*; blue, *P. stomatis*. Data representative of 3 experiments.

(D-F), CID-MS spectra of IPA (D), IA (E), and HPPA (F).

Figure S4. Effects of IPA, IA, and HPPA on the colonic spheroid co-culture system and macrophages. Related to Figure 4.

(A) Representative light phase images of spheroids treated with 100 μM of each metabolite for 72 h compared to 0.1% DMSO control, scale bars = 200μm.

(B) Quantitative RT-PCR showing expression of glycosyltransferase enzymes β galactoside α-2,6 sialyltransferase 1 (*St6gal1; *P* = 0.0376), and the core 1 synthase, glycoprotein-N-acetylgalactosamine 3-beta-galactosyltransferase, 1 (*C1galt1; *P* = 0.0477), relative to *Actb* and compared to 0.1% DMSO control. Data pooled from 2 independent experiments using 4-5 independent colonic spheroid lines. Significance determined using one-way ANOVA with Dunnett post-test and expressed as mean + SEM.

(C) Representative light phase images of spheroids cultured for 2 days in the presence of 100 μM metabolite followed by stimulation for 20 h with 20 ng/mL LPS. Rows represent increasing magnification (5X and 40X), scale bars = 200μm. Arrows point to macrophages with extended pseudopods.

(D) IL-10 (***P = 0.0006; **P = 0.003), TNF (*P = 0.0384), and IL-6 (*P = 0.0183; ***P = 0.0004) production after 20 ng/mL LPS stimulation for 24 h in co-culture of large intestinal spheroids and BMDMs pre-treated for 48 h with 100 μM of each metabolite or 0.1% DMSO control. Data are pooled from 2 independent experiments using 4-5 independent colonic spheroid lines. Significance determined using one-way ANOVA with Dunnett post-test and expressed as mean + SEM.

(E) Quantitative RT-PCR showing expression of AhR and PXR target genes, *Cyp1a1* and *Ugt1a1*, respectively, relative to *Actb* and compared to LPS-stimulated 0.1% DMSO control. Data pooled from 2 independent experiments using 4-5 independent colonic spheroid lines. Significance determined using one-way ANOVA with Dunnett post-test and expressed as mean + SEM.

Figure S5. Relative abundance of mucin utilization genes and *fld* genes in PRISM cohort. Related to Figure 5.

(A) Quantitative RT-PCR showing expression of NRF2 target genes heme oxygenase 1 (*Hmox1;* ****P = 0.0001; *P = 0.0154), NADPH:quinone dehydrogenase 1 (*Nqo1;* ****P = 0.0001), glutamate-cysteine ligase, modifier subunit (*Gclm;* ****P = 0.0001), and glutamate-cysteine ligase, catalytic subunit (*Gclc*; ****P = 0.0001), relative to *Actb* and compared to LPS-stimulated 0.1% DMSO control. Data pooled from 2 independent experiments using 4-5 independent colonic spheroid lines. Significance determined using one-way ANOVA with Dunnett post-test and expressed as mean + SEM

(B) Relative activation of NRF2 by 300 μM IPA, 300 μM IA (**** P = 0.0001), and 12.5 μM tBHQ (**** P = 0.0001) after overnight stimulation with 20 ng/mL LPS of RAW264.7 cells transduced with a NRF2-luciferase reporter. Data representative of 2 independent experiments; significance determined using one-way ANOVA and expressed as mean + SEM.

(C) Increased abundance of an α-L-fucosidase (homologous to one encoded by *R. gnavus*) and a

peptidase E (homologous to one encoded by *Citrobacter koseri*) in CD patients compared to control individuals.

(D) Significantly increased abundance of monosaccharide transporter genes in CD patients compared to control individuals.

(E) RPKM of *fld* genes (color coded for different genes) in the PRISM cohort. Rows indicate different individuals.

Figure S1

A

HMP subject ID		
SRS011061	SRS015264	SRS018351
SRS011084	SRS015369	SRS018427
SRS011134	SRS015431	SRS018575
SRS011239	SRS015578	SRS018656
SRS011271	SRS015663	SRS018817
SRS011302	SRS015782	SRS019267
SRS011405	SRS015890	SRS019381
SRS011452	SRS015960	SRS019397
SRS011529	SRS016018	SRS019601
SRS011586	SRS016056	SRS019968
SRS012273	SRS016095	SRS020233
SRS012902	SRS016203	SRS020328
SRS013158	SRS016267	SRS020869
SRS013215	SRS016335	SRS022137
SRS013476	SRS016495	SRS022524
SRS013521	SRS016517	SRS022713
SRS013687	SRS016585	SRS023346
SRS013800	SRS016753	SRS043001
SRS013951	SRS016954	SRS045004
SRS014235	SRS016989	SRS045713
SRS014287	SRS017103	SRS048164
SRS014313	SRS017191	SRS049712
SRS014459	SRS017247	SRS049900
SRS014613	SRS017307	SRS049959
SRS014683	SRS017433	SRS050752
SRS014923	SRS017521	SRS052697
SRS014979	SRS017701	SRS053335
SRS015133	SRS017821	SRS053398
SRS015190	SRS018133	SRS054590
SRS015217	SRS018313	SRS054956

B

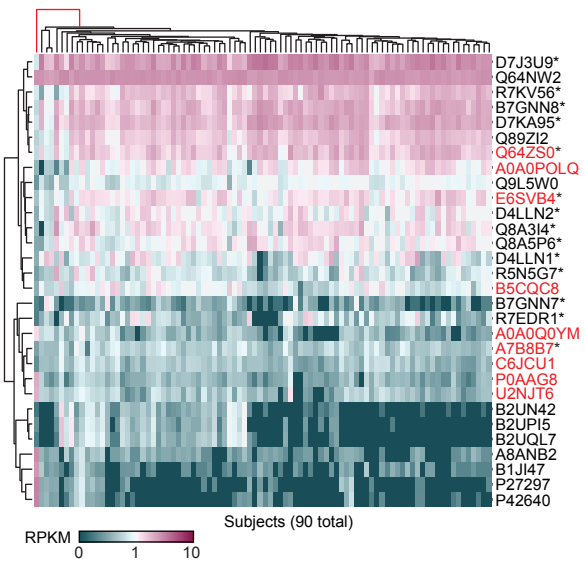

Figure S2

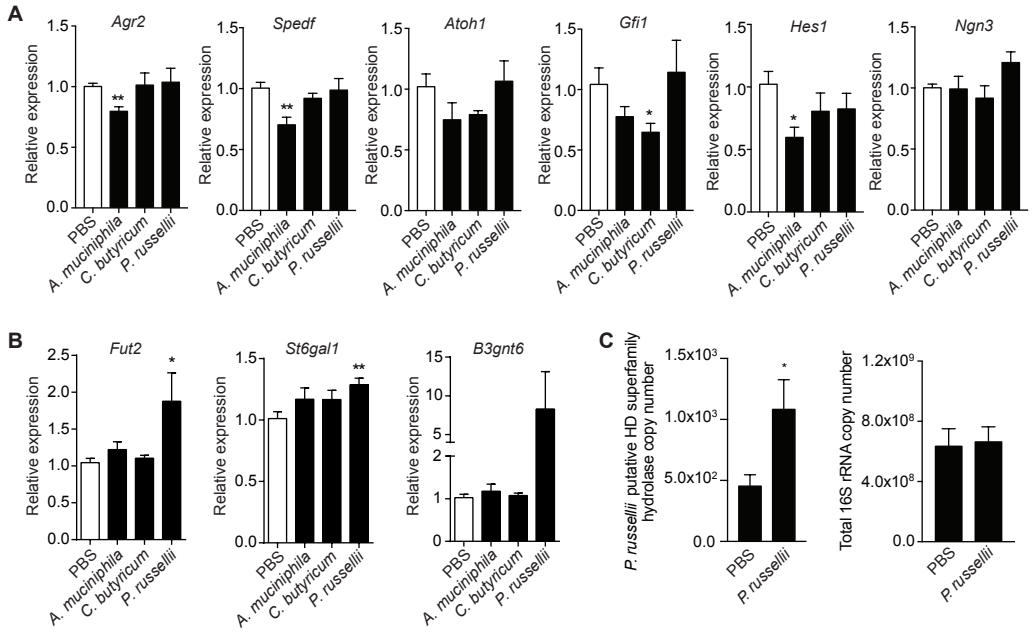
A
Agr2
Spedf
Atoh1
Gfi1
Hes1
Ngn3
Relative expression
PBS
A. muciniphila
C. butyricum
P. russellii
B
Fut2
St6gal1
B3gnt6
C
P. russellii putative HD superfamily hydrolase copy number
Total 16S rRNA copy number

Figure S3

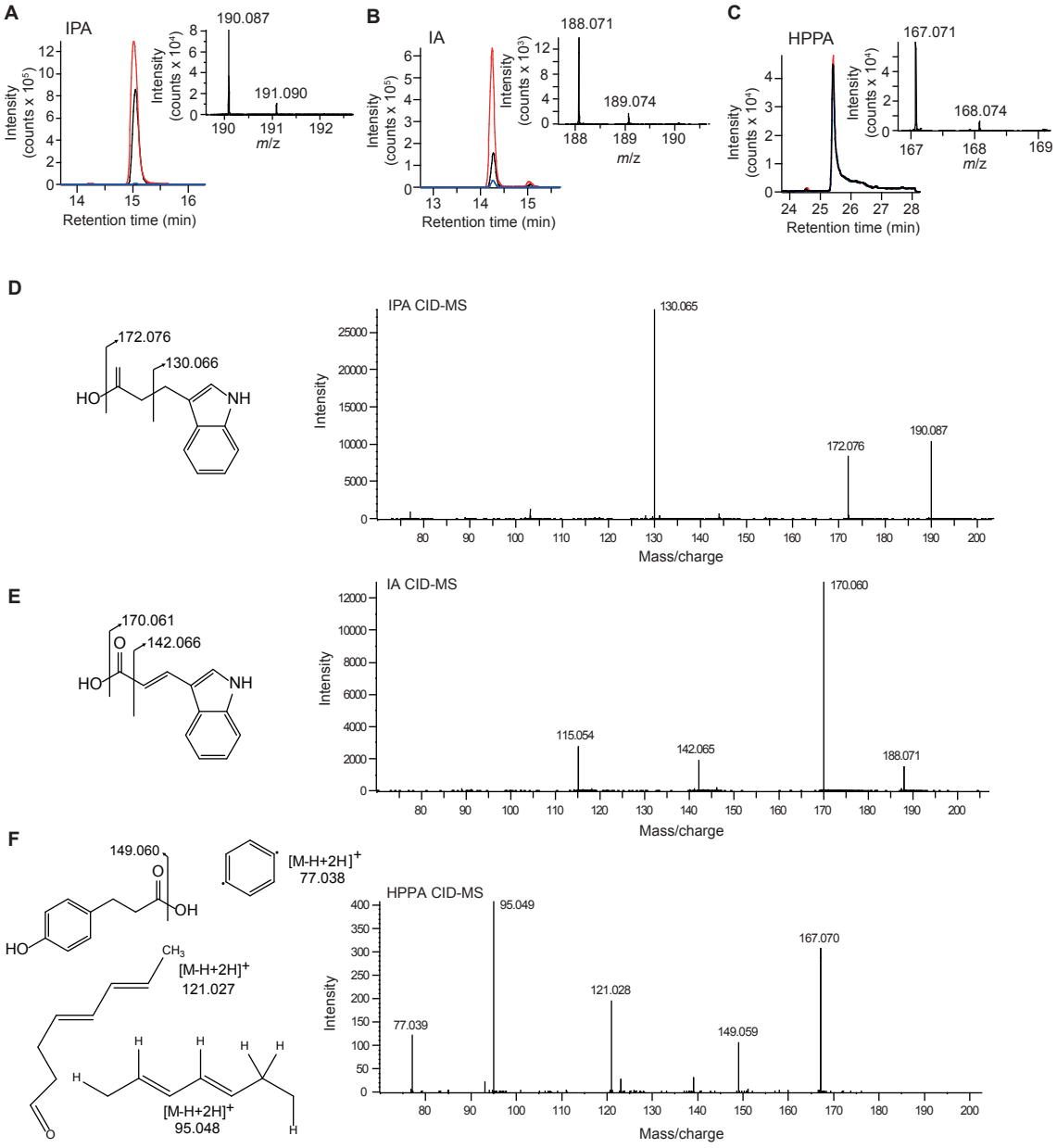
A
IPA
Intensity (counts x 10^5)
Retention time (min)
Intensity (counts x 10^4)
190.087
191.090
m/z
B
IA
Intensity (counts x 10^5)
Retention time (min)
Intensity (counts x 10^3)
188.071
189.074
m/z
C
HPPA
Intensity (counts x 10^4)
Retention time (min)
Intensity (counts x 10^4)
167.071
168.074
m/z
D
172.076
130.066
HO
NH
IPA CID-MS
Intensity
130.065
172.076
190.087
Mass/charge
E
170.061
O
142.066
HO
NH
IA CID-MS
Intensity
170.060
115.054
142.065
188.071
Mass/charge
F
149.060
O
OH
HO
[M-H+2H]+
77.038
CH3
[M-H+2H]+
121.027
H
H
H
H
H
[M-H+2H]+
95.048
O
H
HPPA CID-MS
Intensity
95.049
167.070
121.028
77.039
149.059
Mass/charge

Figure S4

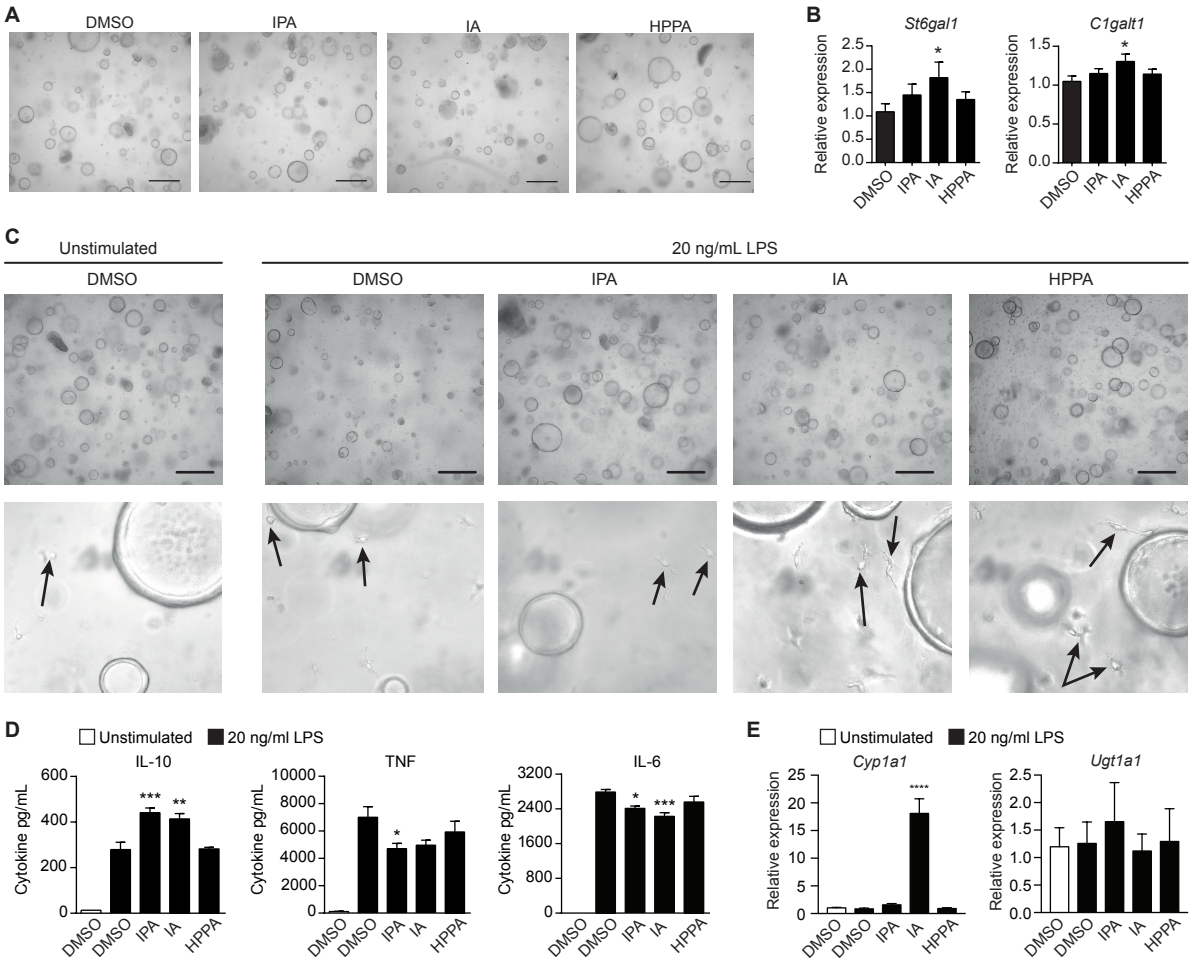
A
DMSO
IPA
IA
HPPA
B
St6gal1
C1galt1
Relative expression
DMSO
IPA
IA
HPPA
C
Unstimulated
20 ng/mL LPS
DMSO
DMSO
IPA
IA
HPPA
D
Unstimulated
20 ng/ml LPS
IL-10
TNF
IL-6
Cytokine pg/mL
E
Unstimulated
20 ng/ml LPS
Cyp1a1
Ugt1a1
Relative expression

Figure S5

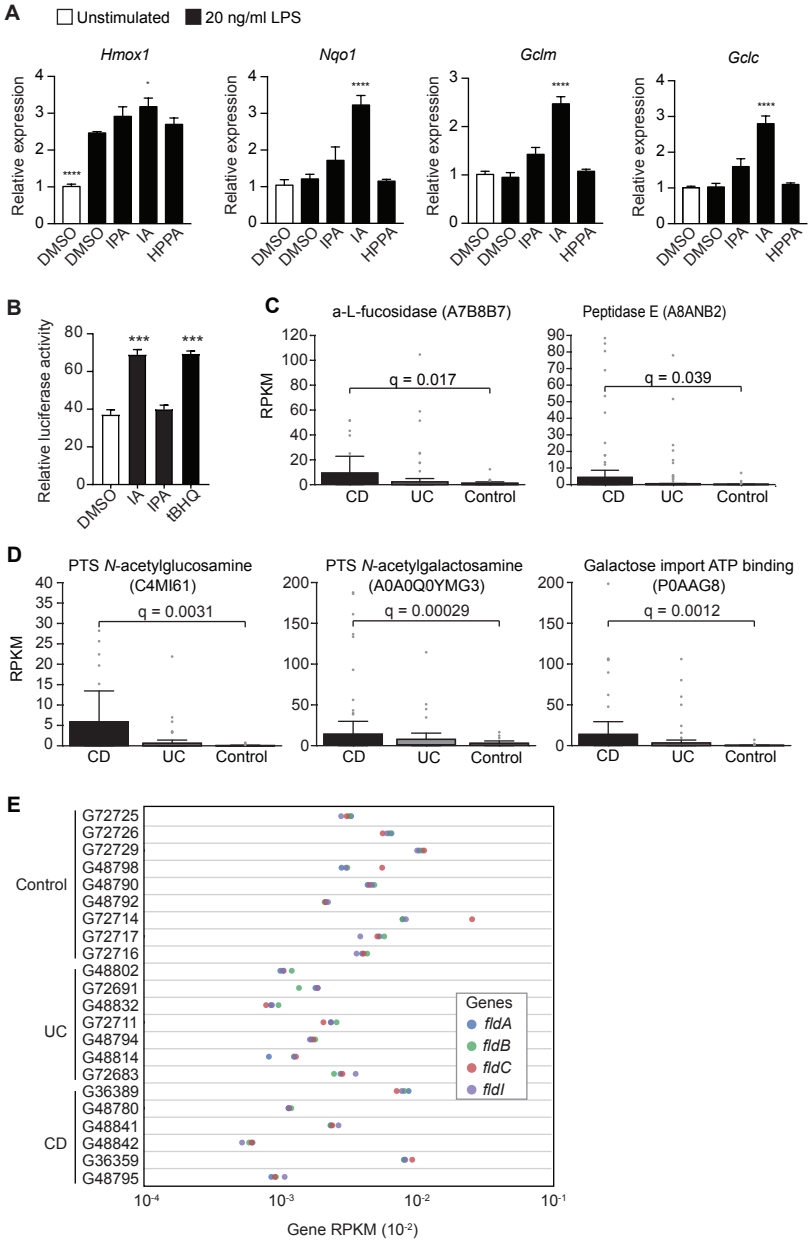
A
Unstimulated
20 ng/ml LPS
Hmox1
Nqo1
Gclm
Gclc
Relative expression
DMSO
DMSO
IPA
IA
HPPA
B
Relative luciferase activity
DMSO
IA
IPA
tBHQ
C
a-L-fucosidase (A7B8B7)
Peptidase E (A8ANB2)
RPKM
q = 0.017
q = 0.039
CD
UC
Control
D
PTS N-acetylglucosamine (C4MI61)
PTS N-acetylgalactosamine (A0A0Q0YMG3)
Galactose import ATP binding (P0AAG8)
q = 0.0031
q = 0.00029
q = 0.0012
E
Control
UC
CD
G72725
G72726
G72729
G48798
G48790
G48792
G72714
G72717
G72716
G48802
G72691
G48832
G72711
G48794
G48814
G72683
G36389
G48780
G48841
G48842
G36359
G48795
Genes
fldA
fldB
fldC
fldI
Gene RPKM (10-2)

Table S1. Seed genes used for computational analyses including transporter genes and cleavage genes. Related to Figure 1.

Transporter genes

Gene ID	Description	Organism
A0A015XFR8	H+ symporter permease	*Bacteroides fragilis*
Q64ZS0	L-fucose permease	*Bacteroides fragilis*
E6SVB4	L-fucose transporter	*Bacteroides helcogenes*
R5TBE0	Transporter putative fucose permease	*Clostridium hathewayi*
A0A0H2YS94	L-fucose:H+ symporter permease	*Clostridium perfringens*
A0A0C7TE87	L-fucose permease	*Clostridium sordellii*
A0A0D6CKJ0	Putative sialic acid ABC transporter permease component	*Bifidobacterium breve*
D4BLW0	ABC transporter, ATP-binding protein	*Bifidobacterium breve*
A0A0N9KQA4	Putative sialic acid transporter	*Clostridium perfringens*
A0A0F0CAG7	Sialic acid TRAP transporter permease protein SiaT	*Clostridium sp. FS41*
P0AAG8	Galactose/methyl galactoside import ATP-binding protein MglA	*Escherichia coli*
C6JCU1	Galactose/methyl galactoside import ATP-binding protein MglA	*Ruminococcus sp. 5_1_39BFAA*
A0A0Q0YMG3	PTS N-acetylgalactosamine transporter subunit IID	*Clostridium butyricum*
A0A099I9R0	PTS acetylgalactosamine transporter subunit IID	*Clostridium innocuum*
A0A0P0LQS4	N-acetylglucosamine related transporter, NagX	*Bacteroides vulgatus*
A0A0H2NL40	PTS N-acetylglucosamine transporter subunit IIABC	*Bifidobacterium bifidum*
A0A0M0AEZ4	PTS N-acetylglucosamine transporter subunit IIABC	*Clostridium botulinum*
C4IM61	Pts system, N-acetylglucosamine-specific iibc component	*Clostridium butyricum*
A0A099I8F5	PTS N-acetylglucosamine transporter subunit IIBC	*Clostridium innocuum*
B1BM12	PTS system, N-acetylglucosamine-specific IIBC component	*Clostridium perfringens*
A0A0H2YS35	PTS system, N-acetylglucosamine-specific IIBC component	*Clostridium perfringens*
A0A0C7R356	Pts system	*Clostridium sordellii*
B5CQC8	PTS system, N-acetylglucosamine-specific IIBC component	*Ruminococcus lactaris*
A0A0L0LQG2	PTS mannose transporter subunit IIABC	*Bifidobacterium breve*
A0A099I4W2	PTS mannose transporter subunit IID	*Clostridium innocuum*
U2NJT6	PTS system mannose-specific transporter subunit IID	*Clostridium intestinale*
A0A0D8VYR1	PTS mannose transporter subunit IIA	*Escherichia coli*

Cleavage genes

Gene ID	Description	Organism
Q89ZI2	O-GlcNAcase BT_4395	*Bacteroides thetaiotaomicron*
P0CB39	Phosphoethanolamine transferase EptC	*Escherichia coli*
Q64NW2	ATP-dependent Clp protease proteolytic subunit	*Bacteroides fragilis*
Q0TR53	O-GlcNAcase NagJ	*Clostridium perfringens*
Q97QL9	6-phospho-beta-galactosidase 2	*Streptococcus pneumoniae*
Q6RUF5	Blood-group-substance endo-1,4-beta-galactosidase	*Clostridium perfringens*
P29768	Sialidase	*Salmonella typhimurium*
B2UQL7	Glycosyl hydrolase family 109 protein 2	*Akkermansia muciniphila*
P0C6E9	Sialidase	*Vibrio cholerae*
P27297	Protein bax	*Escherichia coli*
Q8XM24	Probable alpha-N-acetylglucosaminidase	*Clostridium perfringens*
Q8A3I4	Alpha-L-fucosidase	*Bacteroides thetaiotaomicron*
A5F7A4	Sialidase	*Vibrio cholerae*
B2UL12	Alpha-1,3-galactosidase A	*Akkermansia muciniphila*
A8ANB2	Peptidase E	*Citrobacter koseri*
Q8A5P6	Putative lipoprotein	*Bacteroides thetaiotaomicron*
B3TLD6	Lacto-N-biosidase	*Bifidobacterium bifidum*
B7GNN8	Alpha-1,3/4-fucosidase, putative	*Bifidobacterium longum subsp.*
Q3T552	Endo-alpha-N-acetylgalactosaminidase	*Bifidobacterium longum subsp.*
Q8XMG4	Exo-alpha-sialidase	*Clostridium perfringens*
B7GUN8	Beta-galactosidase	*Bifidobacterium longum subsp.*
Q8XMJ5	Uncharacterized protein	*Clostridium perfringens*
B7GNN6	Glycoside hydrolase family 2, TIM barrel	*Bifidobacterium longum subsp.*
D7KA95	Alpha-L-fucosidase 1	*Bacteroides sp. 3_1_23*
Q0ZI53	Beta-galactosidase	*Bifidobacterium bifidum*
B7GNN7	Uncharacterized protein	*Bifidobacterium longum subsp.*
A5A2I5	Beta-galactosidase	*Bifidobacterium bifidum*
Q0ZII7	Beta-galactosidase	*Bifidobacterium bifidum*
B5UB72	Endo-alpha-N-acetylgalactosaminidase (Fragment)	*Enterococcus faecalis*
R7H0Q2	Alpha-L-fucosidase	*Ruminococcus sp. CAG:90*
A4K5H9	Beta-galactosidase BbgIII	*Bifidobacterium bifidum*
B7GN40	Alpha-L-fucosidase	*Bifidobacterium longum subsp.*
B7GUD7	Beta-galactosidase	*Bifidobacterium longum subsp.*
B7GTT5	Alpha-L-fucosidase	*Bifidobacterium longum subsp.*
A7B557	BNR/Asp-box repeat protein	*Ruminococcus gnavus*
A7B8B7	Alpha-L-fucosidase	*Ruminococcus gnavus*
B7GNW4	Beta-galactosidase	*Bifidobacterium longum subsp.*
D7J3U9	Alpha-L-fucosidase 2	*Bacteroides sp. D22*
B2UPI5	Exo-alpha-sialidase	*Akkermansia muciniphila*
B2UN42	Exo-alpha-sialidase	*Akkermansia muciniphila*
Q9L5W0	Mucin-desulfating sulfatase MdsA	*Prevotella sp. RS2*
Q5MAH5	Mucin-desulfating glycosidase	*Prevotella sp. RS2*
D4LLN2	Alpha-L-fucosidase	*Ruminococcus sp. SR1/5*
D4LLN1	Alpha-L-fucosidase	*Ruminococcus sp. SR1/5*
R7KV56	Alpha-L-fucosidase	*Bacteroides thetaiotaomicron*
R5N5G7	Alpha-L-fucosidase	*Ruminococcus sp. CAG:17*
R5MZG0	Glycoside hydrolase family 29 (Alpha-L-fucosidase)	*Ruminococcus sp. CAG:17*
R7EDR1	Alpha-L-fucosidase	*Roseburia sp. CAG:471*
P77848	Sialidase (Neuraminidase)	*Clostridium tertium*
O82882	Metalloprotease StcE	*Escherichia coli*
Q48259	Zinc metalloprotease (Fragment)	*Helicobacter pylori*
Q7BS42	Serine protease pic autotransporter	*Escherichia coli*
Q8CWC7	Serine protease pic autotransporter	*Escherichia coli*
Q8CWC7	Serine protease pic autotransporter	*Escherichia coli*
Q57450	Enterotoxin 1	*Shigella flexneri*
B1JI47	Beta-hexosaminidase	*Yersinia pseudotuberculosis*
Q7CPC0	Phosphoethanolamine transferase CptA	*Salmonella typhimurium*
Q7A1I3	Lipoteichoic acid synthase	*Staphylococcus aureus*
P42640	Putative phosphoethanolamine transferase YhbX	*Escherichia coli*
P39349	Putative phosphoethanolamine transferase YjgX	*Escherichia coli*

Table S2. Oligonucleotides used for gene expression studies.

Gene ID	Source	Assay ID
Gapdh	Invitrogen	Mm99999915
Actb	Invitrogen	Mm02619580
Ki67	Invitrogen	Mm01278617
Muc2	Invitrogen	Mm01276696
IL10	Invitrogen	Mm01288386
IL22	Invitrogen	Mm01226722
Tnf	Invitrogen	Mm00443258
Fut2	Invitrogen	Mm00490152
B3gnt6	Invitrogen	Mm04204642
C1galt1	Invitrogen	Mm01167001
St6gal1	Invitrogen	Mm00486119
Spedf	Invitrogen	Mm00600221
Cyp1a1	Invitrogen	Mm00487218
Ugt1a1	Invitrogen	Mm02603337
Abcb1b (Mdr-1)	Invitrogen	Mm00440736
Hmox1	Invitrogen	Mm00516005
Gclm	Invitrogen	Mm00514996
Gclc	Invitrogen	Mm00802655
Nqo1	Invitrogen	Mm01253561