Supplemental file

for

AKKERMANSIA MUCINIPHILA USES HUMAN MILK OLIGOSACCHARIDES TO THRIVE IN THE EARLY CONDITIONS *IN VITRO*

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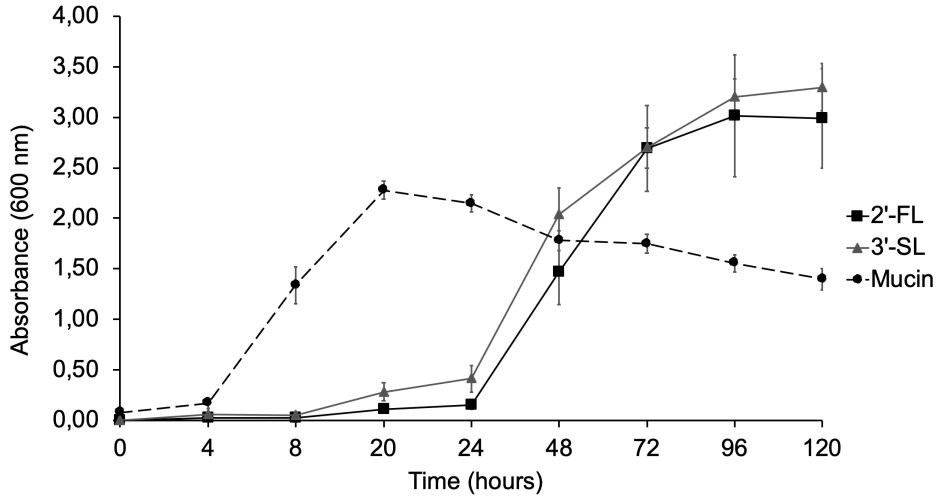
Supplementary Table 1: The primers used for transformation of Amuc_0369, Amuc_2136, Amuc_0010, Amuc_0771, Amuc_1686 and vector into *E. coli* BL21 Rosetta, including 6xHis-Tag on C-terminus. His-tags are given in bold.

Protein name	Protein	Forward primer (5'-3')	Reverse primer (5'-3')
β-hexosaminidase	Amuc_0369	GGATCCCAATTGGGAGCTCATGGAACAAATCATCCCGAAACC	TTA**GTGGTGATGGTG**ATGATGTGGCGTTTCAAAATACACTGTC
β-hexosaminidase	Amuc_2136	GGATCCCAATTGGGAGCTCATGAAGCAGATTGCGGATTCC	TTA**GTGGTGATGGTG**ATGATGCAACGGCTGTACGTTCAT
α-fucosidase	Amuc_0010	GGATCCCAATTGGGAGCTCATGCAGTCCGCCACTAAAATC	CTA**GTGGTGATGGTG**ATGATGTTTGCTCAGTTTGATGACGG
β-galactosidase	Amuc_0771	GGATCCCAATTGGGAGCTCATGGCATTGGCATGGGC	TTA**GTGGTGATGGTG**ATGATGCCACTGGTTGTCCAGGTTTTT
β-galactosidase	Amuc_1686	GGATCCCAATTGGGAGCTCATGCCCATGCCTTTGC	TTA**GTGGTGATGGTG**ATGATGCTTGGCAGGCTTGAACG
Backbone (pcdf-1b)	-	**CATCATCACCATCACCAC**TAGCTCGAGTCTGGTAAAGAAACCG	GAGCTCCCAATTGGGATCC
	-	**CATCATCACCATCACCAC**TAACTCGAGTCTGGTAAAGAAACCG	
	-	**CATCATCACCATCACCAC**TGACTCGAGTCTGGTAAAGAAACCG	

Supplementary Table 2: Fermentation products and sugar utilisation of *A. muciniphila* grown on human milk and mucin.

Mucin							
Time (hours)	Acetate (mM)	Propionate (mM)	1,2-propanediol (mM)	Succinate (mM)	Lactose (mM)	Glucose (mM)	Galactose (mM)
0	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.03 ± 0.06	0.04 ± 0.10	0.04 ± 0.10
4	0.94 ± 1.06	0.27 ± 0.30	0.00 ± 0.00	0.24 ± 0.12	0.00 ± 0.00	0.13 ± 0.20	0.13 ± 0.20
15	16.53 ± 4.55	11.72 ± 2.70	0.45 ± 0.60	0.74 ± 0.58	0.00 ± 0.00	0.08 ± 0.13	0.08 ± 0.13
18	17.17 ± 3.65	12.98 ± 1.93	0.40 ± 0.48	0.18 ± 0.42	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
24	17.21 ± 4.76	12.66 ± 2.26	0.50 ± 0.80	0.15 ± 0.38	0.02 ± 0.06	0.14 ± 0.22	0.14 ± 0.22
48	17.05 ± 4.14	12.55 ± 1.91	0.72 ± 1.00	0.14 ± 0.34	0.00 ± 0.00	0.09 ± 0.15	0.09 ± 0.15
72	16.82 ± 4.32	12.56 ± 1.99	0.57 ± 0.81	0.00 ± 0.00	0.00 ± 0.00	0.13 ± 0.21	0.13 ± 0.21
96	17.33 ± 4.53	12.75 ± 2.01	0.58 ± 0.80	0.00 ± 0.00	0.00 ± 0.00	0.11 ± 0.18	0.11 ± 0.18
120	17.55 ± 3.78	13.08 ± 1.72	0.59 ± 0.79	0.00 ± 0.00	0.00 ± 0.00	0.07 ± 0.11	0.07 ± 0.11
Human Milk							
Time (hours)	Acetate (mM)	Propionate (mM)	1,2-propanediol (mM)	Succinate (mM)	Lactose (mM)	Glucose (mM)	Galactose (mM)
0	0.00 ± 0.00	0.00 ± 0.00	0.90 ± 1.43	0.00 ± 0.00	21.85 ± 3.75	0.78 ± 0.48	0.01 ± 0.03
4	0.01 ± 0.03	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	20.69 ± 3.31	0.83 ± 0.76	0.07 ± 0.16
15	1.00 ± 0.68	1.61 ± 1.71	0.00 ± 0.00	0.16 ± 0.40	20.78 ± 1.37	0.79 ± 0.31	0.40 ± 0.13
18	1.46 ± 1.02	1.42 ± 1.00	0.00 ± 0.00	0.39 ± 0.61	20.23 ± 2.21	0.88 ± 0.25	0.54 ± 0.18
24	2.09 ± 1.49	2.08 ± 1.23	0.00 ± 0.00	0.56 ± 0.60	18.58 ± 2.82	1.34 ± 0.58	0.94 ± 0.48
48	4.29 ± 2.44	4.39 ± 2.65	0.00 ± 0.00	2.05 ± 1.41	14.13 ± 1.40	7.31 ± 2.93	4.44 ± 2.44
72	9.23 ± 2.19	9.55 ± 1.79	0.00 ± 0.00	8.14 ± 2.00	5.52 ± 1.22	11.00 ± 1.76	6.41 ± 1.30
96	12.15 ± 2.15	12.28 ± 1.79	0.03 ± 0.06	9.87 ± 0.96	0.59 ± 0.69	11.87 ± 2.43	7.10 ± 1.50
120	12.05 ± 2.28	13.10 ± 2.40	0.04 ± 0.11	10.51 ± 0.77	0.30 ± 0.25	13.19 ± 2.59	8.12 ± 1.40



Supplementary Figure 1: Growth curve of *A. muciniphila* grown in mucin (black dotted line), 2'-FL (black line), 3'-SL (grey line) measured by optical density at 600 nm.

Supplementary Table 3: a) Carbon balance of *Akkermansia muciniphila* on HMOs and GlcNAc after 48 hours of fermentation, b) Production of sugars and metabolites of *A. muciniphila* grown in 2'-FL (top) and 3'-SL (bottom).

a)

		Substrates		Products											Carbon recovery (%)	
HMO	Time point (h)	2'-fucosyllactose	GlcNAc	2'-fucosyllactose	GlcNac	GalNac	Fucose	Lactose	1,2-propanediol	Glucose	Galactose	Acetate	Propionate	Lactate	Average	SD
2'-fucosyllactose	48	81.58	89.26	81.58	89.26	2.79	9.24	31.42	2.05	4.55	4.88	31.75	28.33	0.00	86.58	3.45
HMO	Time point (h)	3'-sialyllactose	GlcNAc	3'-sialyllactose	GlcNac	GalNac	Fucose	Lactose	Neuraminic Acid	Glucose	Galactose	Acetate	Propionate	Lactate	Average	SD
3'-sialyllactose	48	172.66	60.82	172.66	60.82	2.90	0.20	8.59	47.76	2.48	2.81	42.82	35.02	0.00	82.79	9.10

b)

2'-FL											
Time (hours)	Acetate (mM)	Propionate (mM)	1,2-propanediol (mM)	GalNAc (mM)	GlcNAc (mM)	Succinate (mM)	Galactose (mM)	Glucose (mM)	Lactose (mM)	2'-Fucosyllactose (mM)	Fucose (mM)
0	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.29 ± 0.04	22.27 ± 1.76	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	8.29 ± 0.33	0.00 ± 0.00
4	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.63 ± 0.14	23.30 ± 1.86	0.00 ± 0.00	0.43 ± 0.61	0.00 ± 0.00	0.00 ± 0.00	8.81 ± 0.42	0.00 ± 0.00
8	0.22 ± 0.29	0.00 ± 0.00	0.00 ± 0.00	0.76 ± 0.14	23.67 ± 2.20	0.00 ± 0.00	0.57 ± 0.81	0.00 ± 0.00	0.00 ± 0.00	8.95 ± 0.52	0.00 ± 0.00
20	2.06 ± 0.08	3.19 ± 0.21	0.00 ± 0.00	0.46 ± 0.32	20.73 ± 1.75	0.00 ± 0.00	0.02 ± 0.03	0.00 ± 0.00	0.00 ± 0.00	7.92 ± 0.51	0.04 ± 0.05
24	4.50 ± 3.32	3.20 ± 1.05	0.00 ± 0.00	0.47 ± 0.22	18.79 ± 3.95	0.00 ± 0.00	0.33 ± 0.47	0.00 ± 0.00	0.00 ± 0.00	7.24 ± 1.61	0.64 ± 0.63
48	15.88 ± 3.52	9.44 ± 2.15	0.68 ± 0.26	0.35 ± 0.10	11.16 ± 0.36	0.00 ± 0.00	0.81 ± 0.26	0.76 ± 0.16	2.62 ± 0.72	4.53 ± 0.06	1.54 ± 0.18
72	26.27 ± 6.89	18.01 ± 5.04	3.22 ± 1.33	0.26 ± 0.10	1.00 ± 0.21	0.00 ± 0.00	0.38 ± 0.18	0.49 ± 0.05	3.00 ± 0.30	0.61 ± 0.72	0.33 ± 0.17
96	33.46 ± 1.38	24.78 ± 0.59	5.24 ± 0.28	0.00 ± 0.00	0.07 ± 0.10	0.00 ± 0.00	0.55 ± 0.38	0.58 ± 0.32	1.96 ± 0.69	0.09 ± 0.09	0.45 ± 0.48
120	35.82 ± 0.34	27.52 ± 0.63	5.96 ± 0.42	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.46 ± 0.49	0.46 ± 0.40	0.77 ± 0.97	0.10 ± 0.06	0.51 ± 0.35

3'-SL										
Time (hours)	Acetate (mM)	Propionate (mM)	GalNAc (mM)	GlcNAc (mM)	Succinate (mM)	Galactose (mM)	Glucose (mM)	Lactose (mM)	3'-Sialyllactose (mM)	Neuraminic acid (mM)
0	0.00 ± 0.00	0.00 ± 0.00	0.38 ± 0.40	24.31 ± 2.16	0.00 ± 0.00	0.02 ± 0.03	0.00 ± 0.00	0.11 ± 0.04	11.09 ± 2.14	0.38 ± 0.66
4	0.00 ± 0.00	0.00 ± 0.00	0.31 ± 0.00	24.66 ± 0.04	0.00 ± 1.71	0.12 ± 0.00	0.00 ± 0.19	0.10 ± 0.00	11.37 ± 2.24	0.44 ± 0.76
8	0.70 ± 0.07	0.00 ± 0.07	0.32 ± 0.00	23.77 ± 0.04	0.00 ± 1.99	0.10 ± 0.00	0.00 ± 0.18	0.07 ± 0.00	10.96 ± 2.00	0.41 ± 0.70
20	3.77 ± 0.95	2.58 ± 0.95	0.32 ± 0.00	21.33 ± 0.02	0.00 ± 1.26	0.00 ± 0.00	0.00 ± 0.00	0.26 ± 0.00	10.70 ± 2.05	1.82 ± 0.48
24	4.68 ± 1.39	2.65 ± 1.39	0.10 ± 0.00	21.10 ± 0.18	0.00 ± 1.63	0.00 ± 0.00	0.00 ± 0.00	0.20 ± 0.00	10.79 ± 1.87	1.46 ± 0.47
48	21.41 ± 3.62	11.67 ± 3.62	0.36 ± 0.00	7.60 ± 0.03	0.00 ± 1.71	0.47 ± 0.00	0.41 ± 0.16	0.72 ± 0.06	7.51 ± 1.85	4.34 ± 2.60
72	31.44 ± 2.20	19.35 ± 2.20	0.52 ± 0.00	2.10 ± 0.14	0.00 ± 0.45	1.17 ± 0.00	0.97 ± 0.22	3.39 ± 0.13	1.11 ± 0.98	12.73 ± 2.06
96	33.96 ± 3.41	22.79 ± 3.41	0.49 ± 0.00	0.54 ± 0.52	0.00 ± 0.54	1.26 ± 0.00	0.84 ± 0.29	2.18 ± 0.23	0.00 ± 0.00	11.89 ± 3.03
120	36.55 ± 2.82	25.67 ± 2.82	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.51 ± 0.00	0.20 ± 0.30	0.57 ± 0.34	0.00 ± 0.00	10.97 ± 2.54

Supplementary Table 4: Abundance of *A. muciniphila* enzymes involved in carbohydrate metabolism with their corresponding KEGG identifier (KO ID). The average of Log10 transformed LFQ values is shown. Colouring is based on abundance from the most abundant (red) to medium abundant (orange) to least abundant (green).

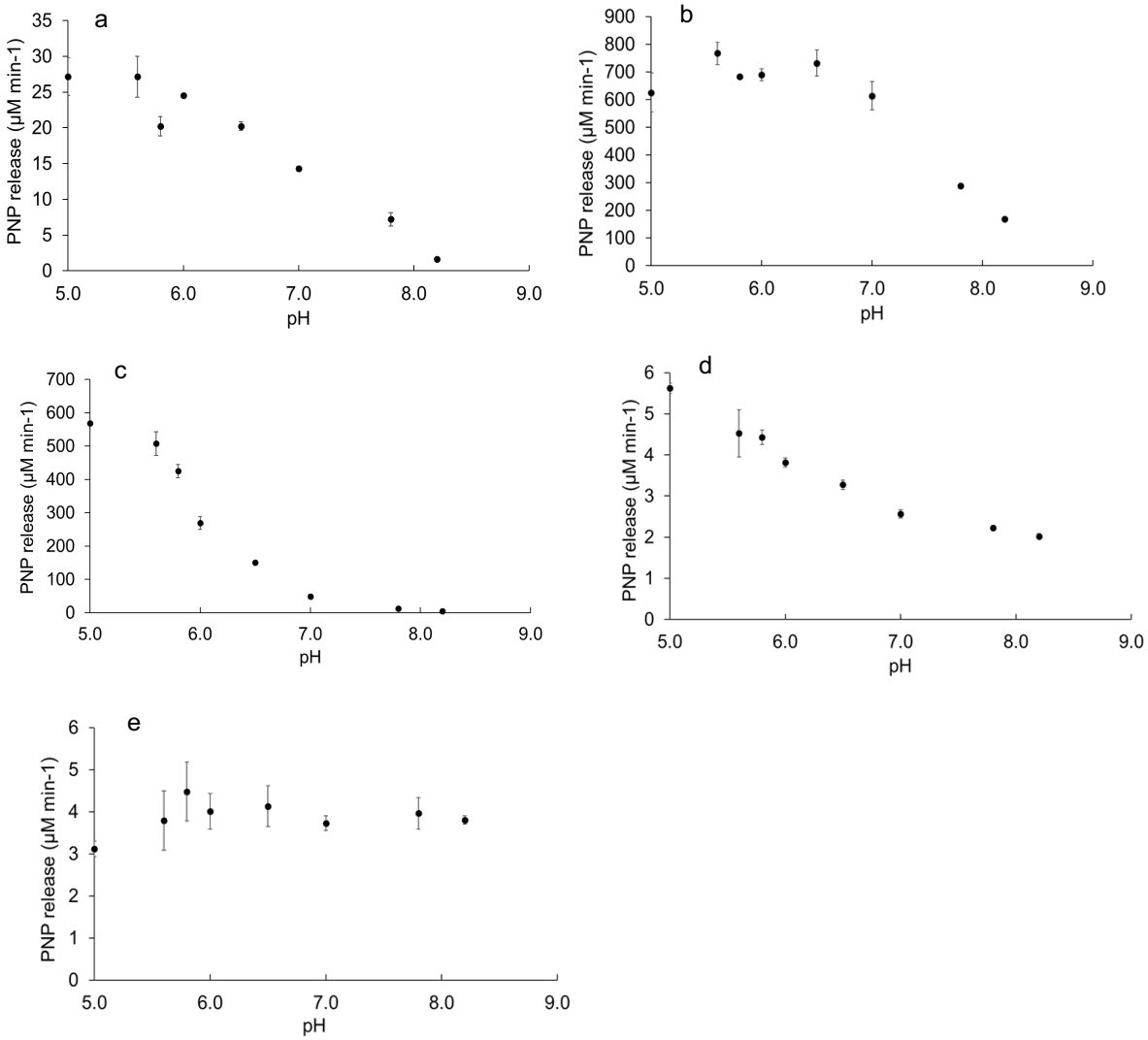
Locus tag	Protein	Average_LFQ_Human Milk	KO ID	Pathway
Amuc_1210	Phosphoenolpyruvate carboxykinase [GTP]	9.03	ko00010;ko00020;ko00620	Glycolysis/Gluconeogenesis; Citrate Cycle (TCA cycle); Pyruvate metabolism
Amuc_1417	Glyceraldehyde-3-phosphate dehydrogenase, type I	8.95	ko00010	Glycolysis/Gluconeogenesis
Amuc_1832	L-fucose isomerase	8.61	ko00051	Fructose and mannose metabolism
Amuc_1630	Serine hydroxymethyltransferase	8.47		
Amuc_0309	2,3-bisphosphoglycerate-independent phosphoglycerate mutase	8.09	ko00010;ko00680	Glycolysis/Gluconeogenesis;Methane metabolism
Amuc_1418	Phosphoglycerate kinase	8.07	ko00010	Glycolysis/Gluconeogenesis
Amuc_1975	Glucose-6-phosphate isomerase	7.95	ko00010;ko00030;ko00500;ko00520	Glycolysis/Gluconeogenesis; Pentose phosphate pathway; Starch and sucrose metabolism; Amino sugar and nucleotide sugar metabolism
Amuc_0721	Fructose-bisphosphate aldolase, class II	7.85	ko00010;ko00030;ko00051;ko00680	Glycolysis/Gluconeogenesis; Pentose phosphate pathway; Fructose and mannose metabolism; Methane metabolism
Amuc_1413	Capsular exopolysaccharide family	7.76		
Amuc_1194	Enolase 2	7.71	ko00010;ko00680;ko03018	Glycolysis/Gluconeogenesis;Methane metabolism;RNA degradation
Amuc_1125	UDP-glucose 4-epimerase	7.56	ko00052;ko00520	Galactose metabolism; Amino sugar and nucleotide sugar metabolism
Amuc_0844	Enolase 1	7.30	ko00010;ko00680;ko03018	Glycolysis/Gluconeogenesis;Methane metabolism;RNA degradation
Amuc_1481	6-phosphofructokinase	7.15	ko00010;ko00030;ko00051;ko00052;ko00680	Glycolysis/Gluconeogenesis; Pentose phosphate pathway; Fructose and mannose metabolism; Amino sugar and nucleotide sugar metabolism; Methane metabolism
Amuc_0660	UDP-N-acetylmuramate/alanine ligase	7.08		
Amuc_1471	Ribose-phosphate pyrophosphokinase	7.01	ko00030;ko00230	Pentose phosphate pathway; Purine metabolism
Amuc_0653	UDP-N-acetylmuramyl-tripeptide synthetase	7.00	ko00300;ko00550	Lysine biosynthesis; Peptidoglycan biosynthesis
Amuc_0654	UDP-N-acetylmuramoyl-tripeptide--D-alanyl-D-alanine ligase	6.95	ko00300;ko00550	Lysine biosynthesis; Peptidoglycan biosynthesis
Amuc_1388	4-hydroxy-3-methylbut-2-en-1-yl diphosphate synthase	6.89	ko00900	Terpenoid backbone biosynthesis
Amuc_1822	Glucosamine-6-phosphate isomerase	6.79	ko00520	Amino sugar and nucleotide sugar metabolism
Amuc_1741	UTP--glucose-1-phosphate uridylyltransferase	6.72	ko00040;ko00052;ko00500;ko00520	Pentose and glucuronate interconversions; Galactose metabolism; Starch and sucrose metabolism; Amino sugar and nucleotide sugar metabolism
Amuc_0431	Pyruvate kinase	6.71	ko00010;ko00230;ko00620	Glycolysis/Gluconeogenesis; Purine metabolism; Pyruvate metabolism
Amuc_0562	Triosephosphate isomerase	6.62	ko00010;ko00051;ko00562	Glycolysis/Gluconeogenesis; Fructose and mannose metabolism; Inositol phosphate metabolism
Amuc_0772	Phosphoglycerate mutase 1 family	6.60		
Amuc_1249	GDP-mannose 4,6-dehydratase	6.59	ko00051;ko00520	Fructose and mannose metabolism; Amino sugar and nucleotide sugar metabolism
Amuc_0969	Galactokinase	6.54	ko00052;ko00520	Galactose metabolism; Amino sugar and nucleotide sugar metabolism
Amuc_0210	6-phosphofructokinase 1	6.09	ko00010;ko00030;ko00051;ko00052;ko00680	Glycolysis/Gluconeogenesis; Pentose phosphate pathway; Fructose and mannose metabolism; Amino sugar and nucleotide sugar metabolism; Methane metabolism
Amuc_1309	Aldose 1-epimerase	6.05	ko00010	Glycolysis/Gluconeogenesis
Amuc_0661	D-alanine--D-alanine ligase	6.04	ko00473;ko00550	D-Alanine metabolism; Peptidoglycan biosynthesis
Amuc_0676	2-dehydro-3-deoxyphosphooctonate aldolase	5.98	ko00540	Lipopolysaccharide biosynthesis
Amuc_1248	NAD-dependent epimerase/dehydratase	5.94	ko00051;ko00520	Fructose and mannose metabolism; Amino sugar and nucleotide sugar metabolism
Amuc_1671	Phosphofructokinase	5.92	ko00010;ko00030;ko00051;ko00052;ko00680	Glycolysis/Gluconeogenesis; Pentose phosphate pathway; Fructose and mannose metabolism; Amino sugar and nucleotide sugar metabolism; Methane metabolism
Amuc_0664	Cell wall hydrolase/autolysin	5.89		
Amuc_1192	Mannose-1-phosphate guanylyltransferase (GDP)	5.71	ko00051;ko00520	Fructose and mannose metabolism; Amino sugar and nucleotide sugar metabolism
Amuc_1435	Nucleotide sugar dehydrogenase	5.63	ko00040;ko00053;ko00500;ko00520	Pentose and glucuronate interconversions; Galactose metabolism; Starch and sucrose metabolism; Amino sugar and nucleotide sugar metabolism
Amuc_0097	ROK family protein	5.58	ko00010;ko00052;ko00500;ko00520;ko00521	Glycolysis/Gluconeogenesis; Galactose metabolism; Starch and sucrose metabolism; Amino sugar and nucleotide sugar metabolism; Streptomycin biosynthesis
Amuc_0550	3D domain protein	5.52		
Amuc_1895	UDP-N-acetylglucosamine 1-carboxyvinyltransferase	5.50	ko00520;ko00550	Amino sugar and nucleotide sugar metabolism; Peptidoglycan biosynthesis
Amuc_1830	L-fuculokinase	5.47	ko00051	Fructose and mannose metabolism
Amuc_0169	3-deoxy-manno-octulosonate cytidylyltransferase	5.00	ko00540	Lipopolysaccharide biosynthesis
Amuc_0208	Glucose-1-phosphate thymidylyltransferase	5.00	ko00521;ko00523	Streptomycin biosynthesis; Polyketide sugar unit biosynthesis
Amuc_0414	Phosphoheptose isomerase	5.00	ko00540	Lipopolysaccharide biosynthesis
Amuc_0656	UDP-N-acetylmuramoylalanine--D-glutamate ligase	5.00	ko00471;ko00550	D-Glutamine and D-glutamate metabolism; Peptidoglycan biosynthesis
Amuc_0681	UDP-N-acetylmuramate	5.00		
Amuc_0795	Glycerol-3-phosphate dehydrogenase [NAD(P)+]	5.00	ko00564	Glycerophospholipid metabolism
Amuc_1211	N-acetylmuramoyl-L-alanine amidase family 2	5.00		
Amuc_1541	Lipid A biosynthesis acyltransferase	5.00	ko00540	Lipopolysaccharide biosynthesis
Amuc_1646	4-hydroxy-3-methylbut-2-enyl diphosphate reductase	5.00	ko00900	Terpenoid backbone biosynthesis

Supplementary Table 5: All the differentially expressed *A. muciniphila* proteins found in Human milk compared to mucin cultures.

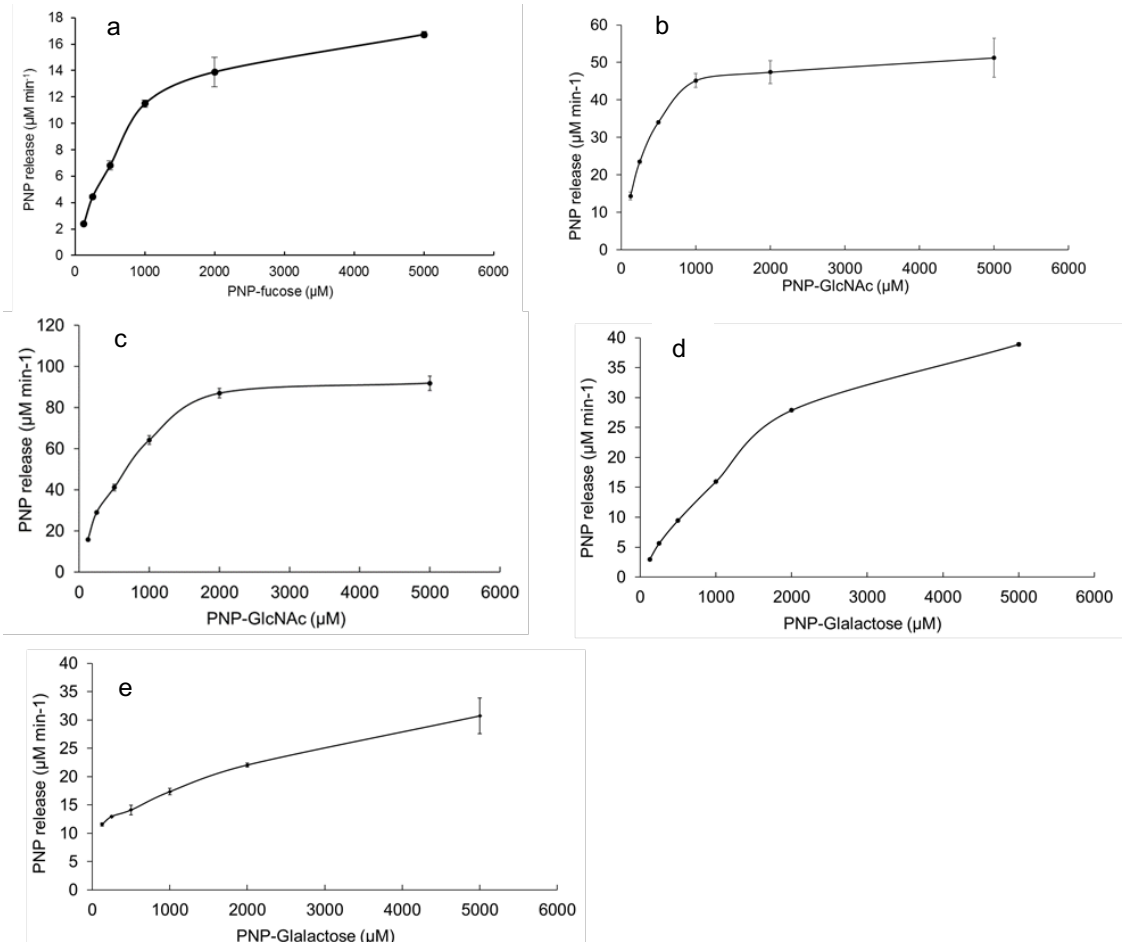
Gene names	Protein names	Log10 fold_change	*p*-value
Amuc_0037	Amino acid permease-associated region	0.65	0.00
Amuc_0172	Putative uncharacterized protein	0.22	0.00
Amuc_0201	Carboxyl transferase	0.27	0.00
Amuc_0335	ABC transporter related	0.51	0.01
Amuc_0372	Glutamate decarboxylase	0.67	0.00
Amuc_0419	General substrate transporter	0.45	0.00
Amuc_0468	Transcriptional regulator, DeoR family	0.42	0.03
Amuc_0541	Ribonuclease, Rne/Rng family	0.28	0.00
Amuc_0543	Putative uncharacterized protein	0.24	0.00
Amuc_0544	Tetratricopeptide TPR_2 repeat protein	0.14	0.01
Amuc_0627	Putative uncharacterized protein	0.41	0.01
Amuc_0670	Trypsin-like protein serine protease typically periplasmic contain C-terminal PDZ domain-like protein	0.29	0.01
Amuc_0687	Outer membrane autotransporter barrel domain protein	0.89	0.00
Amuc_0705	D-isomer specific 2-hydroxyacid dehydrogenase NAD-binding	0.23	0.02
Amuc_0714	UPF0365 protein	0.36	0.04
Amuc_0738	GMP synthase, large subunit	0.27	0.01
Amuc_0836	ATP-dependent chaperone ClpB	0.24	0.00
Amuc_0841	Argininosuccinate lyase	0.25	0.02
Amuc_0876	Heavy metal translocating P-type ATPase	0.18	0.04
Amuc_0955	Ribosome-binding factor A	0.47	0.02
Amuc_0970	SSS sodium solute transporter superfamily	0.23	0.01
Amuc_1033	Sulfatase	0.24	0.02
Amuc_1091	Cytochrome-c peroxidase	0.28	0.01
Amuc_1099	Putative uncharacterized protein	0.46	0.03
Amuc_1100	Putative uncharacterized protein	0.37	0.02
Amuc_1101	Cell division protein FtsA	0.43	0.00
Amuc_1184	Enolase 2	0.62	0.00
Amuc_1225	Inorganic diphosphatase	0.66	0.00
Amuc_1310	17 kDa surface antigen	0.62	0.00
Amuc_1408	60 kDa chaperonin	0.28	0.00
Amuc_1443	Putative uncharacterized protein	0.39	0.00
Amuc_1525	Putative uncharacterized protein	0.68	0.00
Amuc_1584	Type II secretion system protein	0.64	0.00
Amuc_1586	Type II secretion system protein E	0.59	0.00
Amuc_1656	Putative uncharacterized protein	0.52	0.00
Amuc_1689	Dihydrolipoyl dehydrogenase	0.44	0.03
Amuc_1695	Cytochrome bd ubiquinol oxidase subunit I	0.76	0.00
Amuc_1751	Glycoside hydrolase family 13 domain protein/ 1,4 -alpha-glucan branching enzyme	0.61	0.04
Amuc_1755	Sulfatase	1.02	0.01
Amuc_1776	TPR repeat-containing protein	0.93	0.00
Amuc_1782	Glutamyl-tRNA(Gln) amidotransferase subunit A	0.15	0.01
Amuc_1869	Glycosyl transferase group 1	0.74	0.00
Amuc_2001	Putative lipoprotein	0.36	0.02
Amuc_2034	Methylmalonyl-CoA carboxytransferase	0.12	0.02
Amuc_2043	RND efflux system, outer membrane lipoprotein, NodT family	0.45	0.01
Amuc_2127	Carbohydrate-selective porin OprB	0.53	0.00

Supplementary Table 6: Top 10 abundant *A. muciniphila* proteins found in human milk compared to 10 most significantly abundant in mucin cultures.

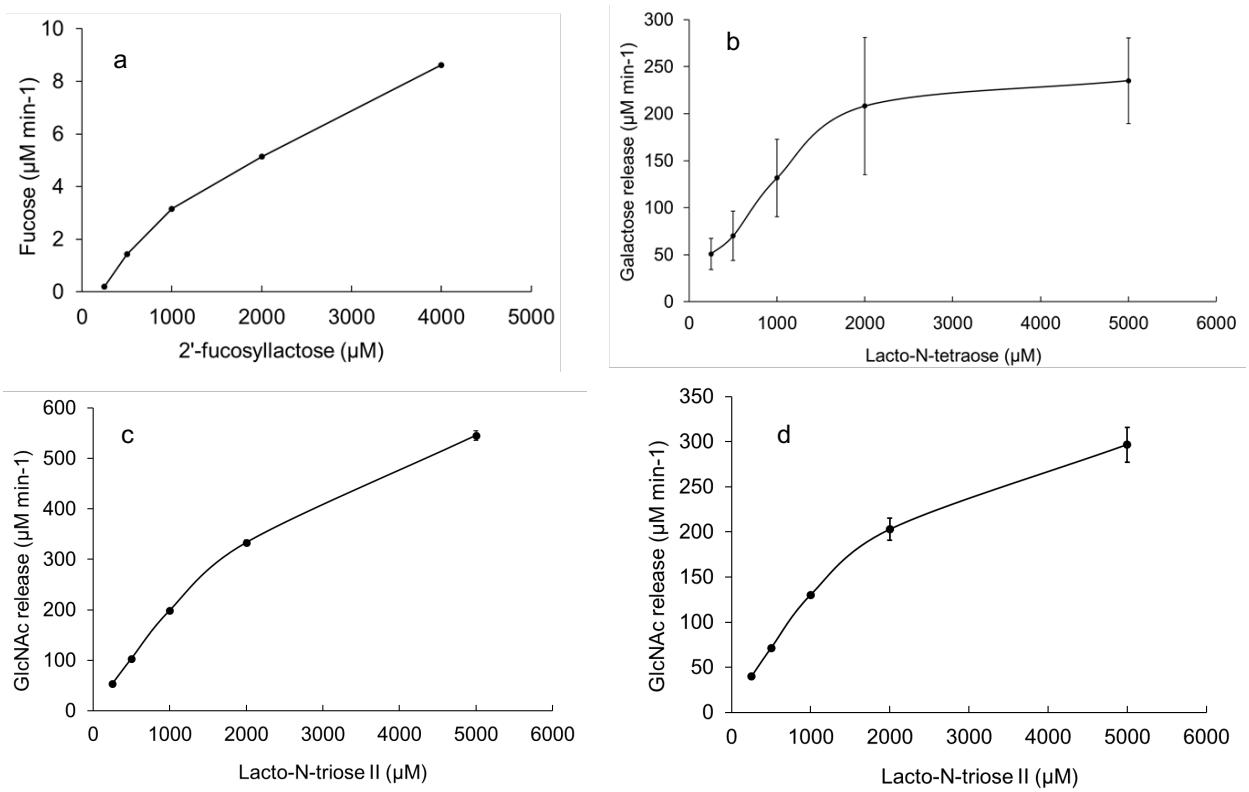
Environmental condition	Protein name	KO ID	Proteins	Kegg Brite Functional Category	Log10 Fold change	p-value
Human Milk	Amuc_1755	x	Sulfatase	x	1.015	0.01
	Amuc_1776	x	TPR repeat-containing protein	x	0.934	0.00
	Amuc_0687	x	Outer membrane autotransporter barrel domain protein	x	0.892	0.00
	Amuc_1695	K00425	Cytochrome bd ubiquinol oxidase subunit I	Energy metabolism	0.758	0.00
	Amuc_1869	x	Glycosyl transferase group 1	x	0.737	0.00
	Amuc_1525	x	Putative uncharacterised protein	x	0.681	0.00
	Amuc_0372	K01580	Glutamate decarboxylase	Carbohydrate metabolism, amino acid metabolism	0.668	0.00
	Amuc_1225	K15986	Inorganic diphosphatase	Energy metabolism	0.655	0.00
	Amuc_0037	K20265	Amino acid permease	Membrane transport	0.648	0.00
	Amuc_1584	K02653	Type II secretion system protein	Membrane transport	0.645	0.00
Mucin	Amuc_0557	K02954	30S ribosomal protein S14	Translation	-2.886	0.00
	Amuc_0243	x	Putative uncharacterized protein	x	-2.441	0.00
	Amuc_1476	K02990	30S ribosomal protein S6	Translation	-2.389	0.00
	Amuc_2106	K00029	Malate dehydrogenase (Oxaloacetate-decarboxylating) (NADP(+))	Carbohydrate metabolism	-2.254	0.00
	Amuc_1430	K02909	50S ribosomal protein L31	Translation	-2.183	0.03
	Amuc_1068	K00789	S-adenosylmethionine synthase	Amino acid metabolism	-2.182	0.00
	Amuc_1059	K07239	Acriflavin resistance protein	Cellular process and signalling	-2.174	0.00
	Amuc_1254	K03217	Membrane protein oxaA	Folding, sorting and degradation, membrane transport, translation	-2.072	0.02
	Amuc_0070	K01867	Tryptophanyl-tRNA synthetase	Translation, amino acid metabolism	-2.045	0.00
	Amuc_2099	x	Putative uncharacterized protein	x	-2.044	0.00



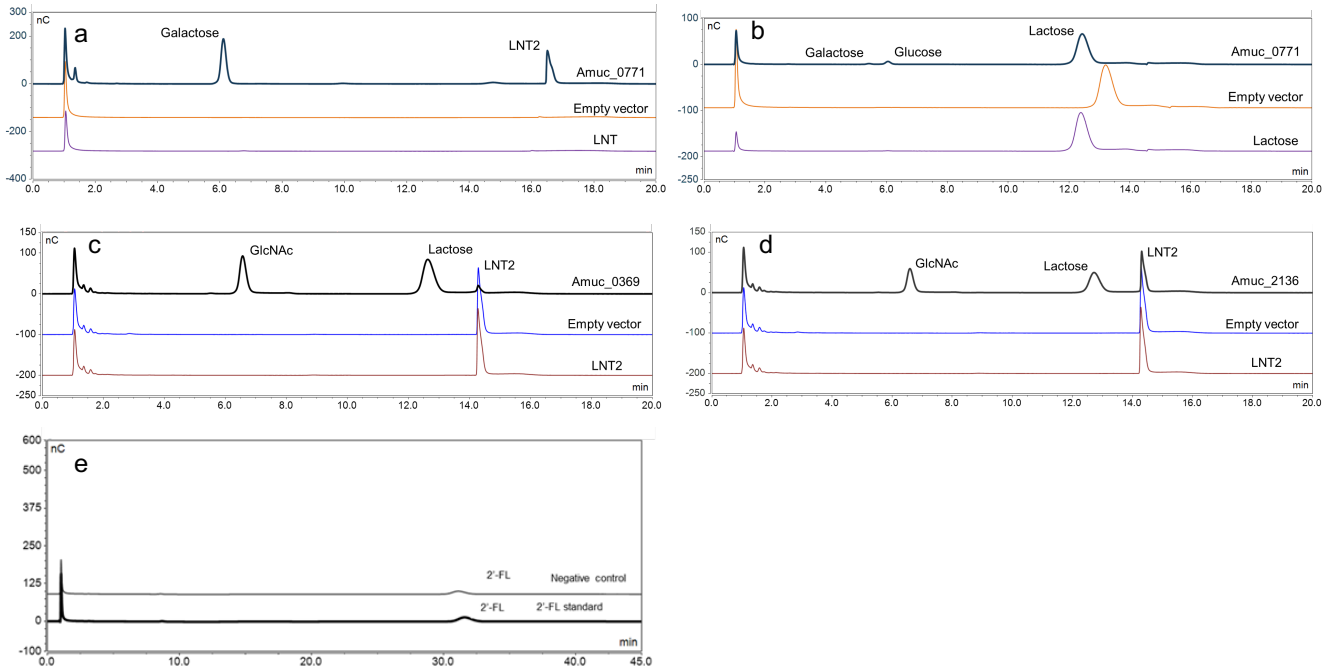
Supplementary Figure 2: pH dependent of *A. muciniphila* GHs using pNP substrates. a) Amuc_0010 and PNP-fucose, b) Amuc_0369 and GlcNAc-PNP, c) Amuc_2136 and GlcNAc-PNP, d) Amuc_0771 and PNPG, e) Amuc_1686 and PNPG



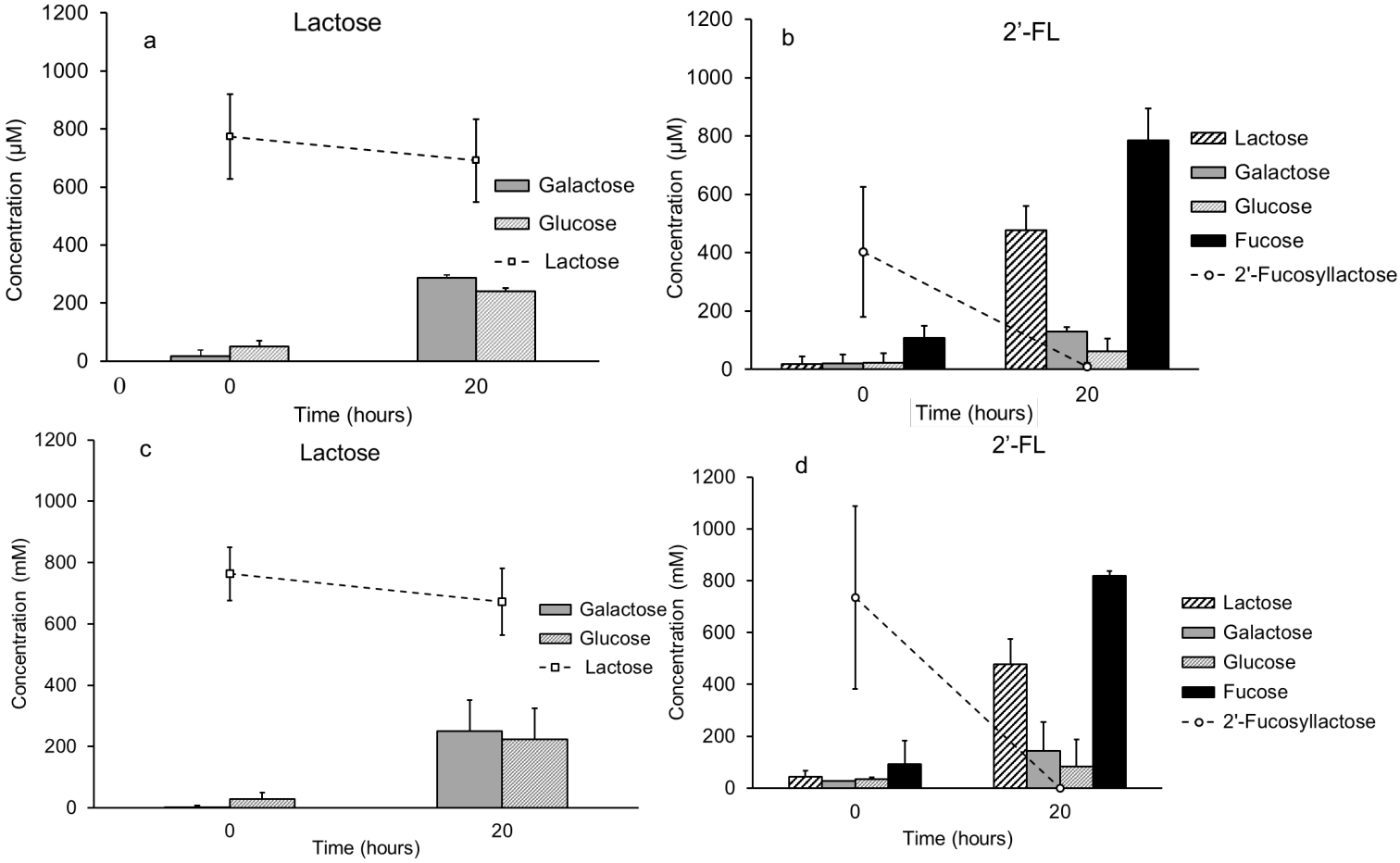
Supplementary Figure 3: Kinetic analysis of *A. muciniphila* GHs against PNP substrates. a) Amuc_0010 and PNP-fucose, b) Amuc_0369 and GlcNAc-PNP, c) Amuc_2136 and GlcNAc-PNP, d) Amuc_0771 and PNPG, e) Amuc_1686 and PNPG



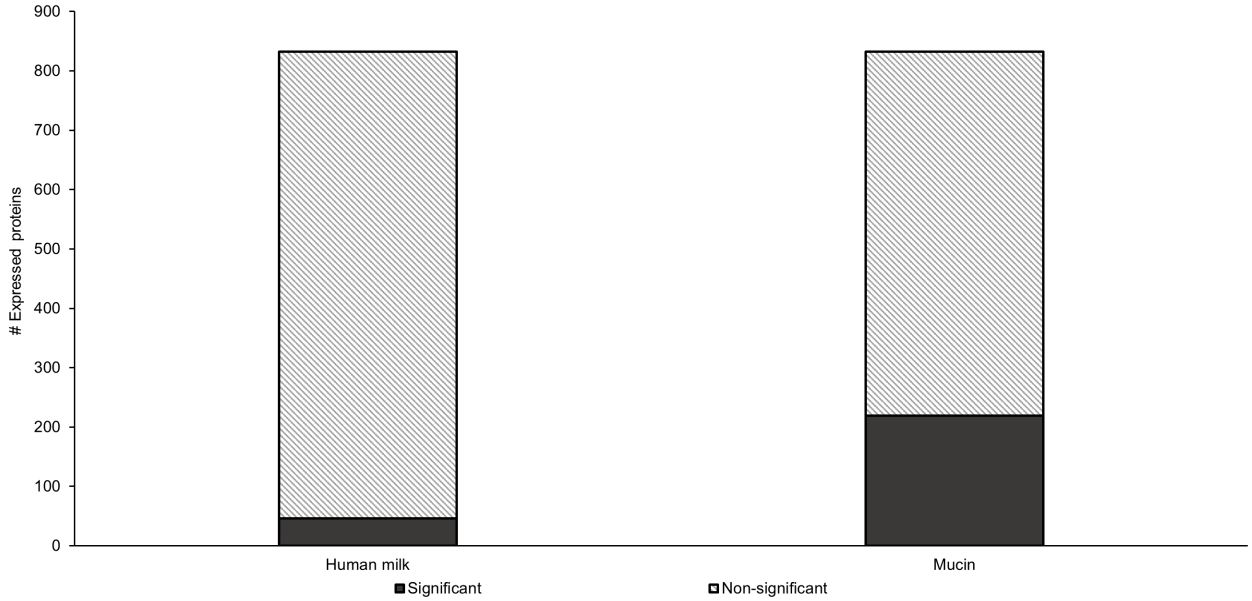
Supplementary Figure 4: Kinetic analysis of *A. muciniphila* GHs against HMOs. a) Amuc_0010 and 2'-fucosyllactose, b) Amuc_0771 and Lacto-N-tetraose, c) Amuc_0369 and Lacto-N-triose II, d) Amuc_2136 and Lacto-N-triose II.



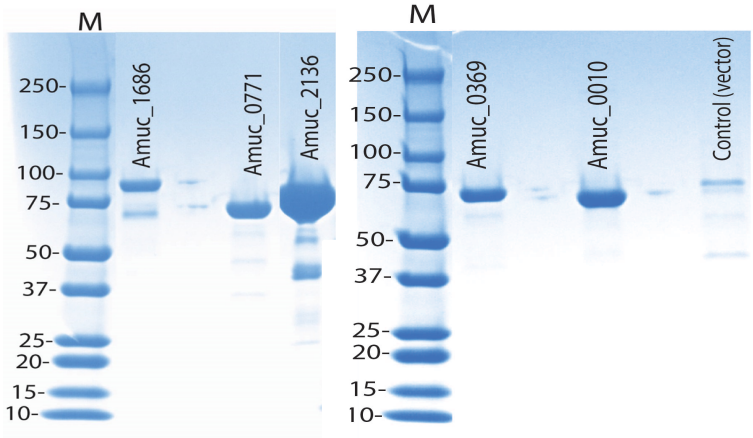
Supplementary Figure 5: Purified *E. coli* BL21 Rosetta strain (Empty vector) specificity against HMOs and lactose. a) Lacto-N-tetraose, b) lactose, c) and d) Lacto-N-triose II, e) 2'-fucosyllactose



Supplementary Figure 6: Enzymatic activity of cell lysates of *A. muciniphila* against lactose and 2'-FL. a,b) human milk cell lysate and c,d) mucin cell lysate.



Supplementary Figure 7: Indication of the amount of significant and non-significant proteins expressed by *A. muciniphila* either in human milk or mucin condition.



Supplementary Figure 8: SDS-PAGE analysis of the purified recombinant *A. muciniphila* GHs. M- protein marker.