# SUPPLEMENTARY INFORMATION

Structural basis of high-mannose mammalian N-glycan processing by human gut *Bacteroides*.

Trastoy *et al*.

## SUPPLEMENTARY TABLES

## Supplementary Table 1. Data collection and refinement statistics

	EndoBT-3987 <sub>WT</sub>	EndoBT-3987 <sub>D312A/E314L</sub> - Man <sub>9</sub> GlcNAc <sub>2</sub> Asn	EndoBT-3987 <sub>WT</sub> - Man <sub>5</sub> GlcNAc	EndoBT-3987 <sub>WT</sub> - Man <sub>9</sub> GlcNAc-1	EndoBT-3987 <sub>WT</sub> - Man <sub>9</sub> GlcNAc-2	
PDB code	6T8I	6TCV	бтсм	6T8K	6T8L	
Beamline	BL13-XALOC	I24 (DLS)	BL13-XALOC	I03 (DLS)	I03 (DLS)	
Wavelength (Å)	0.9791	0.9791	0.9791	0.9762	0.9762	
Resolution range (Å)	46.42-1.4	28.87- 1.31	20 -1.6	29.28- 2.0	19.18 -1.7 (1.761 - 1.7)	
Space group	P 3 <sub>1</sub> 2 1	R 3 :H	P 21 21 2	P 1	P 21 21 21	
Unit cell	74.6, 90, 74.6, 90, 133.5, 120	115.5, 115.5, 97.4, 90, 90, 120	76.4, 133.6, 49.7, 90, 90, 90	46.9, 59.6, 76.1, 97.3, 90.1, 92.3	49.4, 76.3, 116.7, 90, 90, 90	
Total reflections	831752 (82754)	566888 (46520)	434692 (44512)	96359 (9648)	323857 (32614)	
Unique reflections	85310 (8417)	115141 (10639)	66165 (6469)	53616 (3567)	49301 (4854)	
Multiplicity	9.8 (9.9)	4.9 (4.4)	6.6 (6.9)	1.8 (1.8)	6.6 (6.7)	
Completeness (%)	99.9 (99.9)	99.10 (92.02)	98.10 (97.51)	93.27 (64.38)	99.96 (99.98)	
Mean I/sigma(I)	24.5 (3.7)	10.75 (1.33)	15.42 (2.82)	9.89 (3.75)	16.46 (3.44)	
Wilson B-factor	16.6	15.8	17.95	19.0	15.2	
R-merge	0.044 (0.528)	0.068(0.659)	0.0784 (0.653)	0.054 (0.217)	0.080 (0.482)	
CC1/2	1 (0.942)	0.998 (0.671)	0.998 (0.895)	0.991 (0.775)	0.999 (0.903)	
CC*	1 (0.985)	0.999 (0.896)	1 (0.972)	0.998 (0.935)	0.999 (0.903)	
Reflections used in refinement	85229 (8415)	115139 (10638)	66157 (6469)	51477 (3558)	49298 (4853)	
Reflections used for R-free	4246 (393)	5621 (484)	3309 (324)	2419 (178)	2502 (235)	
R-work	0.16 (0.19)	0.1587 (0.23)	0.1727 (0.24)	0.1618 (0.19)	0.15 (0.18)	
R-free	0.18 (0.21)	0.1772 (0.24)	0.1893 (0.28)	0.1965 (0.25)	0.18 (0.26)	
Number of non-H atoms	3692	3511	3734	7533	3837	
Macromolecules	3409	3110	3356	6796	3347	
Ligands	12	133	78	230	115	
Protein residues	434	397	428	507	432	
RMS(bonds, Å)	0.005	0.014	0.016	0.01	0.018	
RMS(angles, °)	0.82	1.37	1.71	1.49	1.8	
Ramachandran favored (%)	97.9	97.4	97.4	97.6	97.9	
Ramachandran allowed (%)	2.1	2.6	2.6	2.3	2.1	
Ramachandran outliers (%)	0	0.0	0.00	0.00	0.00	
Rotamer outliers (%)	0.5	0.9	1.1	0.8	0.6	
Clashscore	0.7	1.7	2.1	1.6	0.7	
Average B-factor (Å <sup>2</sup> )	22.6	21.1	23.1	21.4	18.4	
Macromolecules	22.9	20.4	22.4	21.0	17.3	
Ligands	23.8	21.6	23.6	23.6	25.3	
Solvent	31.1	28.2	31.4	24.8	26.8	

Statistics for the highest-resolution shell are shown in parentheses

Supplementary Table 2. Prime	r sequences used in EndoBT-	-3987 cloning experiments
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EndoBT Mutant	Forward Primer(5'-3')	Reverse Primer (5'-3')
Y49A	ctttccatcacgcagagctgcattattttcataaatgccactgtaggc	gcctacagtggcatttatgaaaataatgcagctctgcgtgatggaaag
Y69A	tactgtagtagcagcagtctcaccgtgcaattcgacaacc	ggttgtcgaattgcacggtgagactgctgctactacagta
Y95A	tgcgccttattatacgtttccaaagcagcagcatcaattttcacttttgc	gcaaaagtgaaaattgatgctgctgctttggaaacgtataataaggcgca
Y99A	caaaatctgtattatgcgccttattagccgtttccaaataagcagcatcaattt	aaattgatgctgcttatttggaaacggctaataaggcgcataatacagattttg
H103A	cggatacaatgcaaaatctgtattagccgccttattatacgtttccaaataa	ttatttggaaacgtataataaggcggctaatacagattttgcattgtatccg
F107A	agteetgeggatacaatgeageatetgtattatgegeettattatae	gtataataaggcgcataatacagatgctgcattgtatccgcaggact
E200A	taatgcaaggttatttgttctttgcagtaaatgacgtaaatccgctg	cagcggatttacgtcatttactgcaaagaacaaataaccttgcatta
N202A	ttcagcggatttacgtcagctacttcaaagaacaaataaccttgcattacac	gtgtaatgcaaggttatttgttctttgaagtagctgacgtaaatccgctgaa
D203A	gttatttgttctttgaagtaaatgccgtaaatccgctgaatactctttc	gaaagagtattcagcggatttacggcatttacttcaaagaacaaataac
N208A	attetetaattggaaagaagagtagccagcggatttacgtcatttacttca	tgaagtaaatgacgtaaatccgctggctactctttctttc
N230A	atgttgttgtactgtttgctgctgctatcaactatgatgctgaagcag	ctgcttcagcatcatagttgatagcagcagcaaacagtacaacaacat
N245A	ctgtacattcggggcacattgtacacgaggacgtcctgc	gcaggacgtcctcgtgtacaatgtgccccgaatgtacag
H277A	tgccaagcetgtaatgtcagcgttaccaagcagtcetaac	gttaggactgcttggtaacgctgacattacaggcttggca
Y339A	ctgtttggtttcagcaccagacgagctgcagcagcc	ggctgctgcagctcgtctgtgtgctgaaaccaaacag
N403A	actaccgccaccacctaaggcgaattccattgaaataccgga	tccggtatttcaatggaattcgccttaggtggtggcggtagt
S432A	catatttagccggagctggagcaaatcccataaaccatccat	atggatggtttatgggatttgctccagctccggctaaatatg

#### **SUPPLEMENTARY NOTE 1**

#### The catalytic cycle of EndoBT-3987

We are now able to visualize two steps of the catalytic cycle of EndoBT-3987: (i) the binding of the N-linked Man<sub>9</sub>GlcNAc<sub>2</sub>Asn substrate to the active site of the enzyme and (ii) the binding of the glycan products, Man<sub>5</sub>GlcNAc and Man<sub>9</sub>GlcNAc, before being released from the active site (SI Fig. 3a). The proposed substrate-mediated catalytic mechanism has been extensively studied for chitinases (EC 3.2.1.14) of the GH18 family, and involves the participation of the 2-acetamide group of the substrate GlcNAc (-1).<sup>1-5</sup> In the first step, the N-linked HM glycan substrate binds to the active site generating the distortion of the GlcNAc (-1) residue from a more energetic favorable chair  ${}^{1}C_{4}$  conformation, to a skew boat  ${}^{1}S_{5}$  conformation. The E314 residue acts in this step as an acid and protonates the glycosidic bond. The D312 residue makes a hydrogen bond with the nitrogen of the C2-acetamide group of GlcNAc (-1). Thus, the carbonyl group attacks the anomeric carbon of GlcNAc (-1) leading to the formation of an oxazolinium intermediate.<sup>1,2,6,7</sup> Recently, QM/MM metadynamics simulations on chitinase B from Serratia marcescens (SmChiB) suggests that this reaction intermediate is a neutral oxazoline with a oxazolinium ion formed towards the reaction products.<sup>8</sup> In the EndoBT-3987<sub>D312A/E314L</sub>-Man<sub>9</sub>GlcNAc<sub>2</sub>Asn substrate complex, GlcNAc (-1) is in a skew boat  ${}^{1}S_{5}$ conformation but the C2-acetamide group is orientated towards A312, stabilized by hydrogen bonds between the nitrogen of this group and Y380, and it is not able to reach the anomeric carbon. The oxygen of the acetamide group requires D312 to rotate 180 ° and produce the first step of the reaction.<sup>2</sup> In the second step, E314 acts as a base and deprotonates a water molecule that attacks the anomeric carbon of GlcNAc (-1), breaking the oxazoline ring and regenerating the hemiacetal sugar with retention of anomeric configuration (SI Fig. 3b;<sup>1</sup>). The skew-boat conformation  ${}^{1}S_{5}$  is also observed in other x-ray crystal structures of chitinases in complex with the glycan substrate where the acid/base glutamic acid residue is mutated by a leucine of GH18 family. <sup>9,10</sup> However, GlcNAc (-1) adopts a boat conformation <sup>1,4</sup>*B* in x-ray crystal structures of chitinases in which the acid/base residue is mutated by glutamine (SI Fig. 3c). <sup>1,11</sup> We have mutated the acid/base residue by a hydrophobic residue in order to completely abolish the activity of the enzyme, because by working with other GH18 ENGases we have observed some residual hydrolytic that we would like to avoid in our co-crystallization experiments. Enzymes from the GH84 and GH20 families also show a substrate mediated mechanism. In the crystal structures of enzyme-substrates complexes from GH84 and GH20 families, GlcNAc (-1) adopts <sup>1,4</sup>B/<sup>4</sup>E<sup>12</sup> and <sup>1</sup>S<sub>3</sub><sup>13</sup> conformations, respectively. It is worth noting that, in the enzyme-Man<sub>9</sub>GlcNAc structures, GlcNAc (-1) displays a <sup>1,4</sup>B conformation, as observed in other chitinases.<sup>14–17</sup> However, GlcNAc (-1) shows a <sup>1</sup>S<sub>5</sub> conformation in the EndoBT-3987<sub>WT</sub>-Man<sub>5</sub>GlcNAc product, GlcNAc (-1) also displayed a <sup>1</sup>S<sub>5</sub> conformation<sup>14</sup>, a conformation also proposed by QM/MM metadynamics simulations on *Sm*ChiB<sup>8</sup>. The boat conformation <sup>1,4</sup>B and the skew boat <sup>1</sup>S<sub>5</sub> conformation are close in the conformational landscape for pyranoses.<sup>19</sup>

This mechanism requires the highly conserved  $D_1XD_2XE$  catalytic motif in the family that performs the double displacement reaction. The role of  $D_1$  is to provide a negative charge that keeps D2(D312)-E(E314) protonated<sup>1,4</sup>. It has been shown that the entrance of the substrate produces the rotation of  $D_2$ , stabilized by  $D_1$  in the unliganded enzyme, to form hydrogen bonds with E. In EndoBT-3987  $D_1$  is substituted by an asparagine, and we did not observe any conformational changes in D312 from the unliganded to the enzyme-product structures. However, in both unliganded X-ray crystal structures of EndoBT<sub>WT</sub> (pdb codes 6T8I and 3POH), the conformation of the acid/base residue E314 is facing Y315, making hydrogen bonds with a water molecule and too far away from D312 to make hydrogen bonds (D312<sub>OD1</sub>-E314<sub>OE2</sub> = 4.52 Å). This conformation is different in the enzyme-product complex structures where E314 is at a distance that can interact with D312 (D312<sub>OD1</sub>-E314<sub>OE2</sub> = 2.55 Å) by hydrogen bonds. E314 is also closer to the O1 of GlcNAc (-1) (Glu<sub>OE2</sub>-GlcNAc(-1)<sub>O1</sub> = 2.47 Å) that is protonated in the first reaction event in the enzyme-product complex structures. Thus, this might be the active conformation of E314 in the wild type enzyme-substrate complex in order to form hydrogen bonds with D312<sup>1,8</sup>, protonate O4 of GlcNAc (+1), and later deprotonate a water molecule that produces the second nucleophilic attack on the anomeric carbon. In addition, the conformation of F353 changes when the substrate enters the binding site in order to accommodate GlcNAc (-1) and GlcNAc (+1). This conformation remains after Asn-GlcNAc (+1) is released.

### SUPPLEMENTARY FIGURES

	b	
GDDLEVGKNIDESAYSGIYENNAYLRDGKSNLVSKVVELHGETYATTVKMGLSKTPNTATSAKV	250	-
KIDAAYLETYNKAHNTDFALYPQDLVTFANEGILTVNANTKSAEVEMTIRAGEGLQEDKTYAIP	150	-
VAISDQSSDITIKDEDAKHCIYLVKDMRNAGDAYKGEGVMQGYLFFEVNDVNPLNTLSFQLENG	100 75	E
KLLWDVVVLFAANINYDAEAGRPRVQCNPNVQYLLDNNETLLQPLRRRGVKVLLGLLGNHDITG	50	
LAQLSEQGAKDFAREVAQYCKAYNLDGVNY <mark>D</mark> D <mark>E</mark> YSNSPDLSNPSLTNPSTAAAARLCYETKQAM	37	
PDKLVTVFDWGQMYGVATVDGVDAKEWIDIVVANYGSAAYPIGQMTKKQCSGISMEFNLGGGGS	25 20	=
LSASKAQSMIDGGYGWFMGFAPSPAKYGSVFSRLQGGGEVLYGSNVAAPTIFYKKNDPTPYKYP	15	
DDL	10	

Supplementary Figure 1 | Recombinant production of EndoBT-3987. a The recombinant EndoBT-3987 construct (residues 27-476) highlighted in yellow. Residues not included in the sequences are highlighted in oranges. Catalytic residues are highlight in green. To obtain catalytic inactive EndoBT D312 and E314 were mutated by alanine and leucine, respectively.
b SDS-PAGE showing purified EndoBT-3987. Source data is provided as a 'Source\_data\_file\_Supplementary\_Fig\_1b'.



Supplementary Figure 2 | Electron density map of the refined EndoBT-3987 X-ray crystal structures. Stereo view of the final electron density maps (2mFo-DFc contoured at  $1\sigma$ ) corresponding to the EndoBT-3987<sub>WT</sub> (a), EndoBT-3987<sub>D312A/E314L</sub>-Man<sub>9</sub>GlcNAc<sub>2</sub>Asn (b), EndoBT-3987<sub>WT</sub>-Man<sub>5</sub>GlcNAc (c), EndoBT-3987<sub>WT</sub>-Man<sub>9</sub>GlcNAc-1 (d), and EndoBT-3987<sub>WT</sub>-Man<sub>9</sub>GlcNAc-2 (e) structures.



Supplementary Figure 3 | The catalytic mechanism of EndoBT-3987. a Catalytic site of EndoBT-3987 in the BT-3987-unliganded (left), EndoBT-3987<sub>D312A/E314L</sub>-Man<sub>9</sub>GlcNAc<sub>2</sub>Asn (center) and EndoBT-3987<sub>WT</sub>-Man<sub>9</sub>GlcNAc-2 (right) crystal structures. In the first step, the Nlinked HM glycan substrate binds to the active site generating the distortion of the GlcNAc (-1) residue from a more energetic favorable chair  ${}^{1}C_{4}$  conformation, to a skew boat  ${}^{1}S_{5}$ conformation. The E314 residue acts in this step as an acid and protonates the glycosidic bond. The D312 residue makes a hydrogen bond with the nitrogen of the C2-acetamide group of GlcNAc (-1). Thus, the carbonyl group attacks the anomeric carbon of GlcNAc (-1) leading to the formation of an oxazolinium intermediate. In the second step, E314 acts as a base and deprotonates a water molecule that attacks the anomeric carbon of GlcNAc (-1), breaking the oxazoline ring and regenerating the hemiacetal sugar with retention of anomeric configuration. **b** Chemical structure and symbol representation of Asn-Man<sub>9</sub> (upper panel) and proposed catalytic mechanism for EndoBT-3987. c x-ray crystal structure Chitinase B from Serratia marcescense inactive mutant E144Q in complex with N-acetylglucosamine-pentamer (GH18 chitinase) (pdb code: 1E6N). d EndoA from Arthrobacter protophormiae in complex with Man3GlcNAc-thiazoline (GH85 ENGase) (pdb code: 3FHQ).



Supplementary Figure 4 | The product Man<sub>5</sub> glycan binding site. a Surface representation with annotated domains and GH loops of the EndoBT-3987<sub>WT</sub>- Man<sub>5</sub>GlcNAc crystal structure. **b** Two views of the electron density of Man<sub>5</sub>GlcNAc product shown at 1.0  $\sigma$  r.m.s deviation. **c** Two views of the key residues of EndoBT-3987 interacting with Man<sub>5</sub>GlcNAc substrate are coloured in orange. The catalytic residues are coloured in yellow (D312 and E314). **d** Two views of superposition of EndoBT-3987<sub>WT</sub>- Man<sub>5</sub>GlcNAc product (green), EndoBT-3987<sub>WT</sub>-Man<sub>9</sub>GlcNAc-1 product (yellow), and EndoBT-3987<sub>WT</sub>- Man<sub>9</sub>GlcNAc-2 crystal structures (orange).



Supplementary Figure 5 | Structural basis of EndoBT-3987 specificity for high mannose type *N*-glycans. a Superimposition of EndoBT-3987<sub>WT</sub> crystal structure and the CT-type glycan product from the EndoS2-CT crystal structure (pdb code: 6MDV). b Superimposition of EndoT crystal structure (pdb code: 4AC1) and the Man<sub>9</sub>GlcNAc product form the EndoBT-3987<sub>WT</sub>- Man<sub>9</sub>GlcNAc-2 crystal structure.

		β <sub>2</sub>	- β <sub>4</sub>	α,	β <sub>s</sub>	β	β,	
$\begin{array}{c} \texttt{PT3987} \\ \texttt{HYPREF1057} & 0.0960 \\ \texttt{P5641} & 0.1340 \\ \texttt{P577} & 0.1592 \\ \texttt{BN777} & 0.1592 \\ \texttt{BN777} & 0.0240 \\ \texttt{B5616} & 0.5150 \\ \texttt{B5616} & 0.5150 \\ \texttt{B7676} & \texttt{B5616} \\ \texttt{B7676} & \texttt{B7676} \\ \texttt{B76766} & \texttt{B7676} \\ \texttt{B7676} & \texttt$	$     \begin{array}{r}       61 \\       62 \\       145 \\       232 \\       56 \\       56 \\       61 \\       59 \\       59 \\     \end{array} $	VUGLEG. — ETXATTVKMO LVDLYKNEBHSYTTD RIE VIGLES. — DKUGTEVFFR ATKTET. — ELSVKVKVA ATKTET. — SVKVKVA PAGAB. — SVKKTVFT DSLFS. — DKGATDFYVM DSLFPN. — EKGSIDFYVM DSLFPN. — EKGSIDFYVM SIGLRN. — SVETELFIK SVGFRD. — AGSTLFYLM NVGFRD. — AGSTLFYLM NVGFRD. — AGDHIO	$\begin{array}{c} SKTPNTA & - \texttt{TSAKVKI}\\ SKTVLSK & - \texttt{NSTKVS}\\ TKAPOKG & - \texttt{VDVEEPV}\\ DAVSS & - \texttt{VEVEPV}\\ DAVSSD & - \texttt{VEVEPV}\\ DAVSSD & - \texttt{VEVEPV}\\ DAVSSD & - \texttt{VEVEPV}\\ EKAAKD & - \texttt{VEVEPV\\ SKAAKDVE & - \texttt{TKVVV}\\ SOATTA & - \texttt{DSTTVV}\\ SKETVMP & - \texttt{VEAPV}\\ KETVKSD & - CSTVVV\\ KTAKLASECTVTVS\\ KTTAKLASECTVTVS\\ KTTAKLVSEATVTSV\\ \end{array}$	DA YUM TYYKA HIND DA YA YUM TYYKA HIND DA YA YUM A HIND DOY METYI A HIND DOY METYI JIM KOD DOY METYI JIM KOD DOY METYI JIM KOD DOY METYI JIM KOD DOY METYI DI SID DOY METHI DI S	- PALYBODUWEPANE - PELYBOUWEBANGA - PELYBONWEPADE E AVLYBKANWEPADE E AVLYBKANWEPADE E AVLYBKANWEPADE - VLYBKEAVET PON - VLYBEELVETAON - VLYBEE	CILTUNANTKS GVVA IDAGLKS GVI LIAPDEIR GKIVIKAGORE GLIVIKAGORE GVVTIPAGETS ATVKT-GET-K ATVA-GEV-K GVVTIPAGETS ATVKT-GET-K GVVALGEV-K GRVAVGSGRDI GIIKLAKGSEQ GVIKLAAGSVV GTINLKKSEQ	- ABUGUST RAGE SVOUGVELAAGS SVOUGVELAAGS SABUEWILAAS- - ASUEVILTPSA - ASUEVILTPSA SSAVEVILTPSA SSAVEVILTPSA SSAVEVILTSAN SSAVEVISTSA SSAVEVISTSA SSAVEVISTSA SSALKEYSSA SALKEYSSA SAMKUSTSSC SJDMKUSTSSC SJDMKUSTSSC SJDMKUSTSSC	GIQEDK EDMIEKDK EIQEKDK GISLGS GISLGS GISLGS DISLEYVT QLEKNG EIKENG DINAED DINAED TISPKK SISPTK SISTDK
		β <sub>s</sub>	β <sub>9</sub>	β <sub>10</sub>			β <sub>11</sub>	β.,
$ \begin{array}{r} \text{BT3987} \\ \text{HMPREF1057} & -00960 \\ \text{B5G41} & -01340 \\ \text{B5777} & -01592 \\ \text{BN777} & -00240 \\ \text{B5G16} & -05150 \\ \text{DWW03} & -09645 \\ \text{HMPREF0663} & -10414 \\ \text{HMPREF0663} & -10414 \\ \text{HMPREF1057} & -02916 \\ \text{JCM6292} & -370 \\ \text{HMPREF91057} & 02916 \\ \text{JCM6292} & -370 \\ \text{HMPREF9456} & -02551 \\ \text{JCM10512} & 5006 \\ \text{HMPREF9455} & -00111 \\ \end{array} $	148 153 151 234 319 322 146 143 143 149 147 133 147	YAR UVASDO-SSDITIKD TYLDYKSSO-NGLETNE TYLDYKSSO AVLDYKUPY-QNAAVKO- AYLDYKYVA-SDOVVKO- TYMEPILVAK-SDOVAVQN TYMEPILVAK-SGOVAVQN TYMEPILVAGSAQASA VYAFFIKYGGAAQSSA TYVEVSAGT-SGNEKSA- TYVEVSAGT-SGNEKLAK- STVEPIRMAYT-SGDFKLAK- STVEPIRMAYT-SGDFKLAK- STVEPIRMAYT-SGDFKLAK- STVEPIRMAYT-SGDFKLAK-	DARHCIILVKDMRNAG HISHCVILTDKSCWI ABCRVNAIVRK ADCRVNAIVRK ADCRVNAIVRK EAECVVNIVRK ADCRVNAIVRK EAEVVPIVSRI EAEVVPIVSRI EAEVVPIVSRI EAEFILSKNP DKGEFVLVRVISKNP DKGEFVLVRVISKNP EDETRLIVKDITKP EDETRLIVKDIGLP ADQTRNIFKKDIGLP ADQTRNIFVKDIGIP	DAY KG G G V M (0 Y L) CD K G P D A V K N V ( ) K G P D A V K N V ( ) N P K Q I [ N V Y ) S K K I N I ( ) G S K K I N I ( ) G S K K I N I ( ) G H K N G Q V I ( ) G H K S G I ( ) I ( ) C H K S G I ( ) I ( ) C H K S G I ( ) I ( ) C H K S G I ( ) I ( ) C H K S G I ( ) I ( ) C H K S G I ( ) I ( ) C C K Y V D G A P G V W F S C C T K Y I D G A P G V W F S C	P BEVNDUNPLNAES PEVNDUNPLNAES Y EVNDCNPLNAUE Y EVNDCNPLNAUE Y EVNDCNPLNAIE Y EVNDCNPLNAIE Y EVNDCNPLNAIE Y EVNDCNPLNAIE Y EVNDCNPLNAIE Y EVNDCNPLNAIE Y EVNDCNPLNAIE Y EVNDCNPLNAE Y EVNDCNPLNAE Y EVNDCNPLNAE CMEVNDAPLWNLG CMEVNDTNPLNNLG CMEVNDTNPLNNLG CMEVNDTNPLNNLG CMEVNDTNPLNNLG CMEVNDTNPLNNLG CMEVNDTNPLNNLG CMEVNDTNPLNNLG CMEVNDTNPLNNLG CMEVNDTNPLNNLG CMEVNDTNPLNNLG CMEVNDTNPLNNLG CMEVNDTNPLNNLG Y PLNNLG Y PLN Y PLN	$\begin{array}{llllllllllllllllllllllllllllllllllll$	VVU DJAANIND D VVU DJAANIND D DJL DJSANINN NJL JJAANINN AV LJAANINN AV LJAANINN AV JJACINN OVI DJSCINNN OVI DJSCINNN OVI DJSCINNN VVI DJSCINNN VVI DJSCINNN VI DJSCINNN VVI DJSCINNN VVI DJSCINNN VVI DJSCINNN VI DJSCINN VI DJSCINNN VI D	BACRPRVQ VECRPVLY BTGRVVVLA AEDVVVLA AEDVVVLA SKQKVYMH SKQKVYMH BTGEVYNY BTGEVYNY BTGEVYNY BTGEVYNY BTGRVYIF BTGRVVIF BTGRVYIF BTGRVYIF
		α,	β <sub>14</sub>	α4	() β <sub>15</sub>	$\rightarrow$		α
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Supplementary Figure 6 | Sequence alignment of EndoBT-3987 with homologues found in the *Bacteroidetes* phylum. Comparison of BT3987 from *B. thetaiotaomicron VPI-5482* (Q8A0N4, Uniprot code), HMPREF1057\_00960 from *B. finegoldii* CL09T03C10 (K5CRT6, Uniprot code, Identity: 49.3%), B5G41\_01340 from *Alistipes onderdonkii* (A0A1Y3R5N0, Uniprot code, Identity: 46.8%), BN777\_01592 from *Bacteroides sp. CAG:770* (R6TYV2, Uniprot code, Identity: 46.3%), BN702\_00240 from *Bacteroides sp. CAG:545* (R5SGN4, Uniprot code), B5G16\_05150 from Alistipes sp. An66 (A0A1Y3VE51, Uniprot code, Identity: 39.9%), DWW03\_09645 from *Alistipes sp. AF14-19* (A0A374A074, Uniprot code, Identity: 38.6%), HMPREF0663\_10414 from *Prevotella oralis ATCC 33269* (E7RMR4, Uniprot code, Identity: 35.9%), HMPREF0659\_A6286 from *Prevotella melaninogenica strain ATCC 25845* (D9RSV7, Uniprot code, Identity: 35.9%), HMPREF9135 1107 from *Prevotella baroniae*  *F0067* (U2QH48, Uniprot code, Identity: 35.6%), Bcop\_1060 from *B. coprosuis DSM 18011* (F3ZTN6, Uniprot code, Identity: 36.5%), HMPREF1057\_02916 from *B. finegoldii CL09T03C10* (K5CJA5, Uniprot code, Identity: 36%), JCM6292\_370 from *B. pyogenes JCM* 6292 (W4P4D8, Uniprot code, Identity: 39.1%), HMPREF9456\_02551 from *Dysgonomonas* mossii DSM 22836 (F8X2F1, Uniprot code, Identity: 34.7%), CM10512\_5006 from *Bacteroides reticulotermitis JCM 10512* (W4V122, Uniprot code, Identity: 34.1%), HMPREF9455\_00111 from *Dysgonomonas gadei ATCC BAA-286* (F5ISP4, Uniprot code, Identity: 34.7%).



Supplementary Figure 7 | Biochemical Synthesis of  $Man_5GlcNAc_2Asn$  and  $Man_9GlcNAc_2Asn$ . a Scheme showing the chemical synthesis pathway of  $Man_5GlcNAc_2Asn$  and  $Man_9GlcNAc_2Asn$ . b ESI Mass Spectrum of purified  $Man_5GlcNAc_2Asn$  and  $Man_9GlcNAc_2Asn$ .

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