Supporting Information

Glycoengineering of Antibody (Herceptin) Through Yeast Expression and *in vitro* Enzymatic Glycosylation

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SI Materials and Methods

Materials

All nucleotides and chemicals were purchased from Sigma-Aldrich (St. Louis, MO). Restriction endonucleases and T4 DNA ligase were from NEB (Beverly, MA), primers were from Mission Biotech (Taiwan), and Ni-NTA Agarose were from Qiagen (Santa Clarita, CA). Plasmids pPICZαA, pET28a, *Pichia pastoris X33* strains, and *Escherichia coli* stains TOP10 or DH5α used for recombinant DNA work were from Life Technologies CO., Ltd (Grand Island, NY). The Pichia GlycoSwitch® strains SuperMan5 and SuperMan5 (his-) were from BioGrammatics, Inc (Carlsbad, California). α2,6-Sialylglycopeptide was from Fushimi Pharmaceutical (Kagawa, Japan).

Construction of antibody expression vectors

Sequences of anti-HER2 were based on Canadian patent CA 2103059. All genes were obtained via cDNA synthesis and were ligated into the modified pPICZαA vector with N-terminal α-factor for protein secretion. The plasmids with correct sequences were transformed into DH5α competent cells by chemical transformation. Single colonies were picked and inoculated into TB medium with Zeocin antibiotics overnight, and the cell cultures were used for large amount plasmid DNA extraction.

Site-directed mutagenesis

The site-specific mutagenesis of all double-stranded plasmid DNA was employed Q5® Site-Diredcted Mutagenesis Kit (NEB, Beverly, MA) and transformed into DH5α competent cells by chemical transformation. Single colonies were picked and inoculated into TB medium with Kanamycin antibiotics overnight, and the cell cultures were used for plasmid DNA extraction and DNA sequencing.

Expression of enzymes

All endoglycosidases (ENGases) genes were obtained via PCR from genomic DNA or cDNA library, and the PCR products were ligated into the expression vector pET28a with N- or C-terminal His-tag modification for further purification. All plasmid DNAs were purified and transfected into *BL21* (DE3) chemically competent *E. coli* cells. Single colony was grown overnight at 37°C in TB medium with kanamycin antibiotics, diluted to 1:100 in fresh TB medium with kanamycin and grown for 4 hours to mid-exponential phase. The expression of protein ENGases was induced by 0.1mM IPTG at 16°C for 24 hours. The cells were harvested and suspended with an extraction buffer (50mM sodium phosphate, 300mM sodium chloride, and 10mM imidazole, pH7.0), then purified by using microfluidizer M-110L (Hyland Scientific, WA) and Ni-NTA agarose beads (GE Healthcare, MA). The

protein was eluted in the same buffer containing a higher concentration of imidazole (250mM) and the concentration was determined by Qubit Protein Quantitation (Invitrogen, CA).

Transformation of antibody expression plasmids into *P. pastoris*

The plasmid DNA was linearized with *SacI*, and then transformed into *P. pastoris X33* competent cells using *Pichia* EasyComp™ Kit (Invitrogen, CA). Single colonies were picked and confirmed by using QuickExtract™ DNA Extraction Solution (Epicentre Tech Corp, WI) and PCR.

Transformation of antibody expression plasmids into GlycoSwitch® *strains*

GlycoSwitch® strain SuperMan$_5$ was used as the host strain for expression of trastuzumab. The core GlycoSwitch® strain has a central glycan processing enzyme (OCH1) disrupted to prevent glycan elongation, and express a foreign mannosidase to trim existing glycans to a more uniform Man5 structure. White single colonies were picked and confirmed by using QuickExtract™ DNA Extraction Solution (Epicentre Tech Corp, WI) and PCR.

Fermentation conditions

Correct transformants were inoculated in buffered glycerol-complex medium (BMGY) and grown at 30°C for 48 hours followed by a 24-96 hour induction in buffer methanol-complex medium (BMMY) at 23°C with shaking.

Antibody purification

The antibody was captured by affinity chromatography from the supernatant medium of fermentations using a HiTrap Protein A FF resin and Protein L resin (GE Healthcare, MA). The resin was equilibrated with PBS pH7.4 and the supernatant medium was adjusted at

the same pH value. The column was washed with at least 5 column volumes of the same buffer and the antibody was eluted with 100mM of Glycin-HCl, pH2.3. The eluted protein was neutralized immediately with 1M of Tris-HCl to pH7.0.

SDS-PAGE detection of *E. coli* expressed ENGases and glycoengineered Herceptin antibodies

All the SDS-PAGE analyses were performed with NuPAGE® Novex® 4-12% Bis-Tris gel (Invitrogen, MA) in MOPS buffer either with or without DTT addition in samples.

Preparation of mono-GlcNAc-Trastuzumab

Recombinant trastuzumab with high-mannose type glycans in a PBS (1X, pH7.0) was incubated with EndoH or selected endoglycosidases (trastuzumab : endoglycosidase = 20 : 1 in weight ratio) at 37°C for 20 hours. LC-MS and SDS-PAGE analysis indicated the complete cleavage of the *N*-glycans on the heavy chain. The reaction mixtures were subjected to affinity chromatography on a column of protein A-agarose resin (1mL; GE Healthcare) pre-equilibrated with a sodium phosphate buffer (20mM, pH7.0). Then the column was washed with a sodium phosphate buffer (20mM, pH7.0, 10mL). The bound IgG was eluted with glycin-HCl buffer (50mM, pH2.3, 10mL), and the elution fractions were immediately neutralized with Tris-HCl buffer (1M, pH8.0). The antibody fractions were combined and concentrated by centrifugal filtration (Amicon Ultra centrifugal filter, Millipore, Billerica, MA) to give mono-GlcNAc trastuzumab. The product was trypsinized, and the glycopeptides, TKPREEQYNSTYR and EEQYNSTYR were analyzed using nanospray LC/MS to confirm the glycosylation patterns.

Transglycosylation of mono-GlcNAc trastuzumab (rHer-G) with SGP

α2,6-Sialylglycopeptide (SGP, 300mM) was added to the mixture of an Endo-S2 WT and mono-GlcNAc trastuzumab in TAPS buffer (pH7.4). Trastuzumab-GlcNAc : Endo-S2 WT : sialylglycopeptide (α2,6) = 14.4 : 270 : 1 (by weight ratio). The solution was incubated for 15 minutes to 15 hours at 37°C without shaking. Transglycosylation efficiency was evaluated by SDS-PAGE analysis. The ratio of protein bands on SDS-PAGE was calculated by ImageQuant TL (GE Healthcare, MA) software.

Western blot

Proteins subjected to SDS-PAGE were transferred to PVDF membrane using iBlot® Module (Invitrogen, CA, USA). Unoccupied protein binding sites on the PVDF membrane were saturated by incubating with 3% skim milk (in 1XTBS) for one hour. Full-length and truncated forms of trastuzumab protein were detected using HRP-conjugated anti-human IgG antibodies. Briefly, membranes were incubated for one hour with HRP-conjugated anti-human IgG antibody (1/5000 dilution) followed by triplicate washing by TBST buffer, and the bound antibody was detected using ECL plus western blot detection kit.

Analysis of glycopeptides using LC-MS/MS

The glycopeptides with or without endoglycosidase treatment were analyzed by high resolution and high mass accuracy nanoflow LC-MS/MS. Glycopeptide samples were injected at 10 μL/min into a precolumn (150 μm I.D. × 30 mm, 5 μm, 200 Å) and then separated in a reverse phase C18 nano-column (75 μm I.D. × 200 mm, 3 μm, 200 Å) for analysis in an LTQ Orbitrap XL ETD mass spectrometer (Thermo Fisher Scientific) equipped with a nanoelectrospry ion source (New Objective). Separation was performed at

300 nL/min using 0.1% formic acid in water as mobile phase A and 0.1% formic acid in 80% acetonitrile as mobile phase B. Survey full scan MS spectra (from m/z 320 to 2000) were acquired in the orbitrap with a mass resolution of 30,000 at m/z 400. The three most intense ions were sequentially isolated for HCD (Resolution 7500) and the electrospray voltage was maintained at 1.8 kV with the capillary temperature set at 200°C. The MS spectra were acquired for composition assignment and quantitation of glycopeptides. Assignment of glycopeptides was based on matching the measured masses with the database, which combined predicted masses of tryptic peptides (by Protein Digest Simulator Basic) and *N*- and *O*-linked glycans (from the Consortium of Functional Glycomics), as well as the retention time of the glycopeptides by in-house software. Each assigned glycopeptide was confirmed by the appearance of glycan fragments in its MS/MS spectra.

FcγRIIIA analyte biotinylation

Recombinant Human Fc gamma RIIIA/CD16a Protein (FcγRIIIA) (R&D Systems, MN) analyte was biotinylated by using EZ-Link Sulfo-NHS-LC-LC-Biotin (sulfosuccinimidyl-6-[biotinamido]-6-hexanamido hexanoate) Kit (Invitrogen, CA, USA) as instructed by the manufacturer, and dialyzed against PBS (pH7.0) and concentrated with Vivaspin 0.5 columns from Sartorius Stedim (1000 MWCO PES).

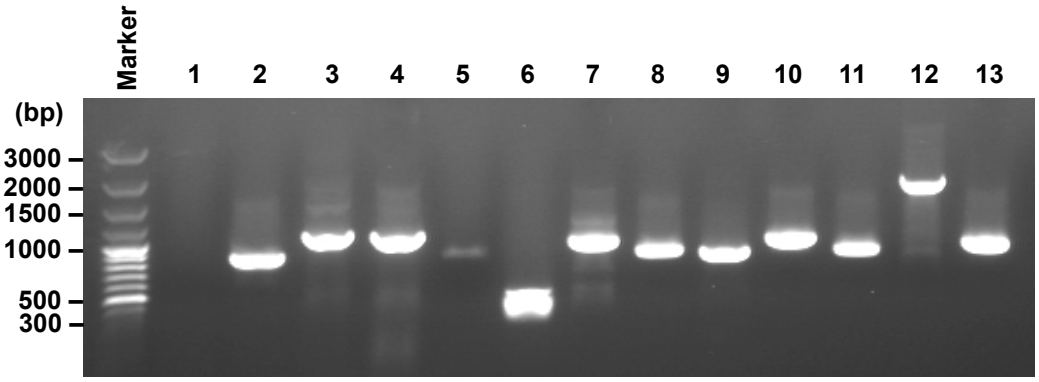
Biolayer interferometry (BLI) analysis

BLI experiments were performed on an Octet® HTX System (FortéBio®) at 30°C with an agitation speed of 1000 rpm. Streptavidin coated biosensors (PALL/FortéBio®, 18-5021)

were loaded with biotinylated recombinant human FcγRIIIA (2.5μg/ml) in PBS buffer pH7.0 for 300 seconds. Immobilization levels were between 0.6-1.0 nm. For association (for k_{on}) monitoring, IgG samples were diluted with PBS buffer pH7.0 to suitable concentrations (50, 100, 200, 400nM for Herceptin®; 1, 5, 10, 20nM for rHer-M5 and rHer-SCT) and transferred to a solid-black 96 well plate (Greiner Bio-One, 655900). IgG samples were allowed to bind to FcγRIIIA loaded biosensors for 360 seconds (association phase). The dissociation phase (for k_{off}) was recorded for 300 seconds. The sensorgrams were measured and referenced against the buffer reference signal by the Data Analysis Software 9.0.0.10 (FortéBio®). The sensorgrams with the concentrations signals of each association and dissociation phase were combined and processed with referencing for background subtraction and fitted to a 1:1 binding model.

Glycan Microarray on NHS-activated glass slides

The synthetic *N*-glycan ligands were printed on NHS-activated glass slides through amide bond formation, with 100 μM each of glycans. Each sample was printed with three replicates, and slide images were obtained from a fluorescence scan after incubation with DyLight649-conjugated donkey anti-human IgG antibody. The incubation time of ENGases (1mg/ml) for glycans cleavage was 16 hours at 30°C with shaking. Two human IgG antibodies, PG16 and 2G12 were used for binding to the α2,6-sialylated complex-type oligosaccharides and mannose type oligosaccharides, respectively.



Lane	Template (genomic DNA)	Primer pairs	Product (bp)
1	*rHer/ΔOCH1*	KanMX-Fw + KanMX-Rv	/
2	*rHer/ΔOCH1ΔALG1*	KanMX-Fw + KanMX-Rv	810
3	*rHer/ΔOCH1*	ALG1-Fw-2 + ALG1-Rv-2	1037
4	*rHer/ΔOCH1ΔALG1*	ALG1-Fw-2 + ALG1-Rv-2	929
5	*rHer/ΔOCH1ΔGTPase*	KanMX-Fw + KanMX-Rv	810
6	*rHer/ΔOCH1*	GTPase-Fw-2 + GTPase-Rv-2	339
7	*rHer/ΔOCH1ΔGTPase*	GTPase-Fw-2 + GTPase-Rv-2	932
8	*rHer/ΔOCH1ΔMNN9*	KanMX-Fw + KanMX-Rv	810
9	*rHer/ΔOCH1*	MNN9-Fw-2 + MNN9-Rv-2	783
10	*rHer/ΔOCH1ΔMNN9*	MNN9-Fw-2 + MNN9-Rv-2	934
11	*rHer/ΔOCH1ΔPMT5*	KanMX-Fw + KanMX-Rv	810
12	*rHer/ΔOCH1*	PMT5-Fw-2 + PMT5-Rv-2	1790
13	*rHer/ΔOCH1ΔPMT5*	PMT5-Fw-2 + PMT5-Rv-2	890

Fig. S1. Identification of knockout genes by using the polymerase chain reaction (PCR) and the gel electrophoresis. Primers and length of PCR products were listed below the gel picture.

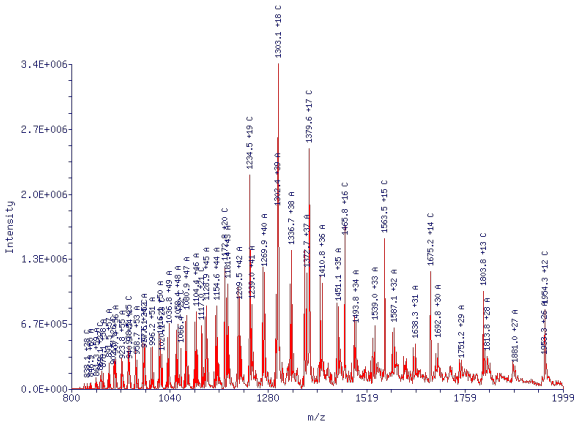
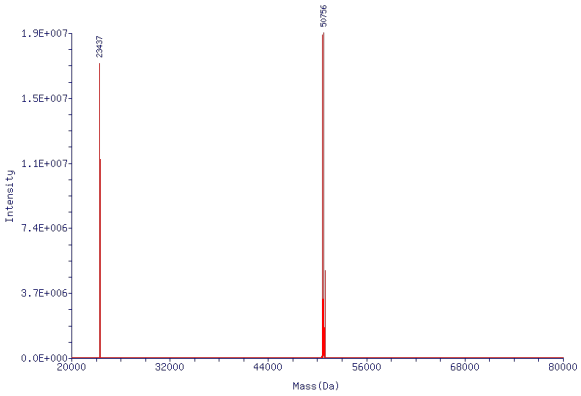
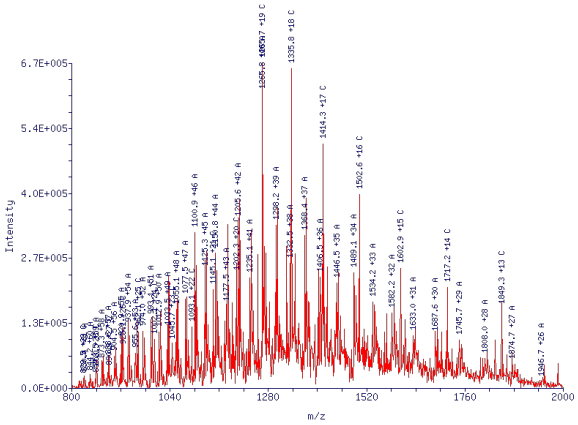
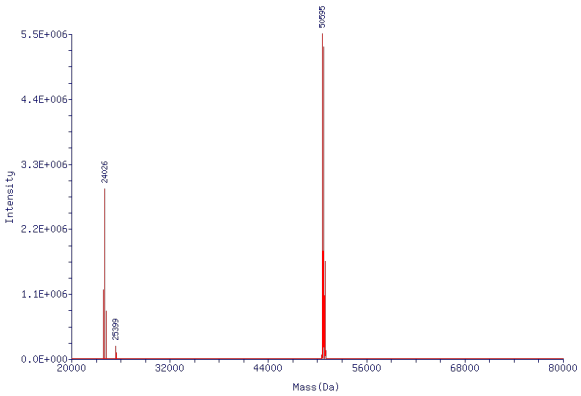
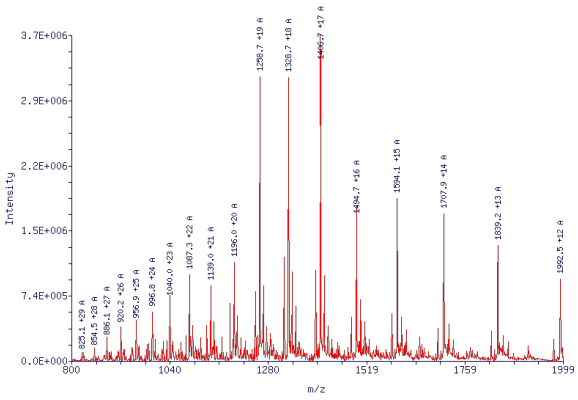
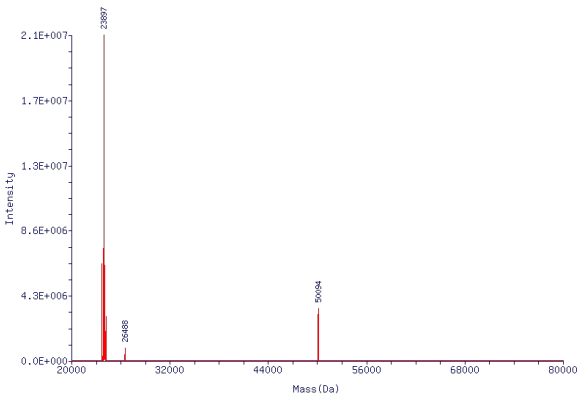
ESI-MS Spectrum	Deconvoluted Mass Spectrum
Herceptin 	**Herceptin**  Light chain: 23437 Da, Heavy chain: 50756 Da
rHer/X33 	**rHer/X33**  Light chain: 24026 Da, Heavy chain: 50595 Da
rHer/ΔOCH1 	**rHer/ΔOCH1**  Light chain: 23897 Da, Heavy chain: 50094 Da

Fig. S2. A comparison of ESI-MS and deconvoluted mass within reduced Herceptin, rHer-X33, and rHer-M5 (=rHer/ΔOCH1).

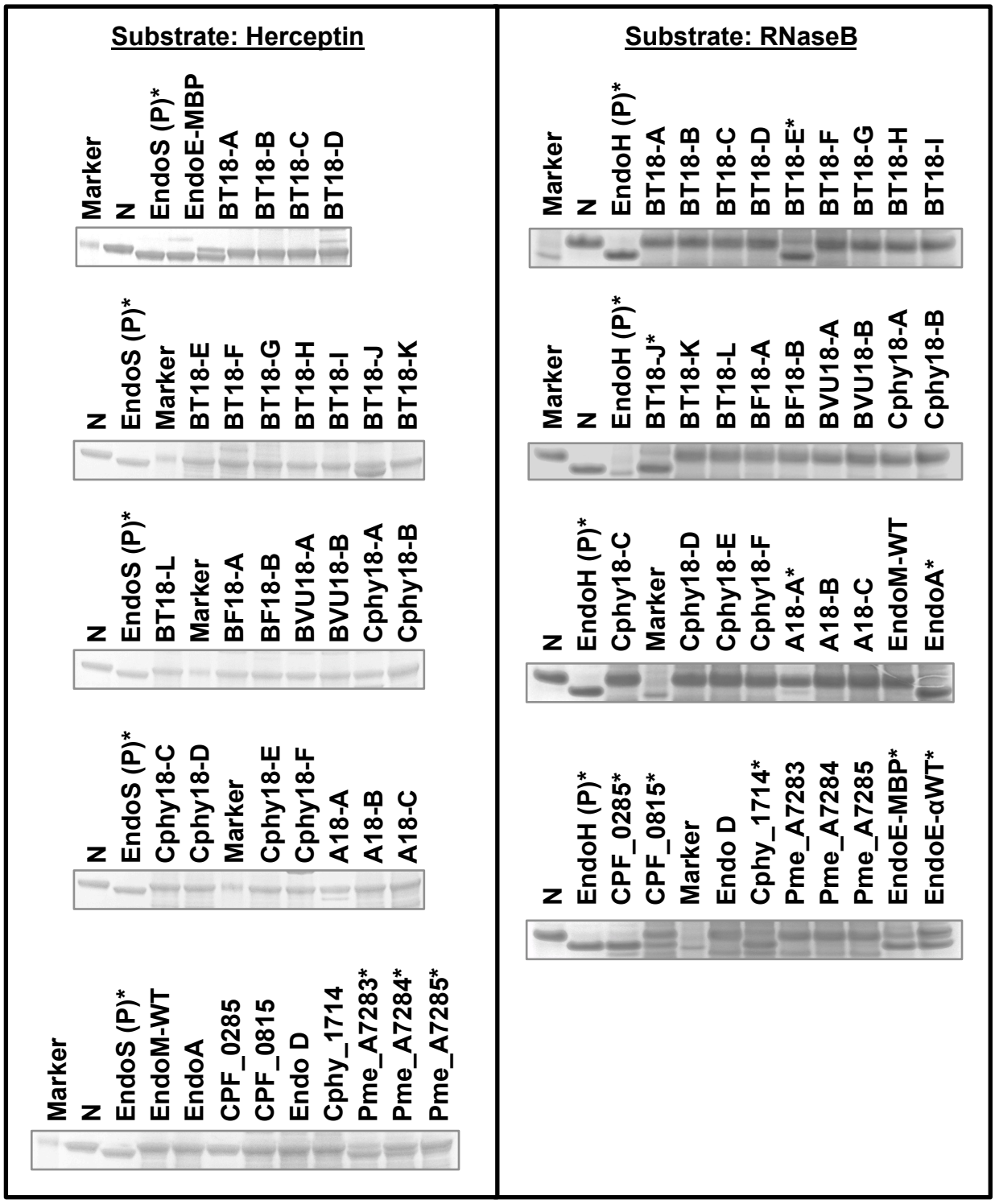
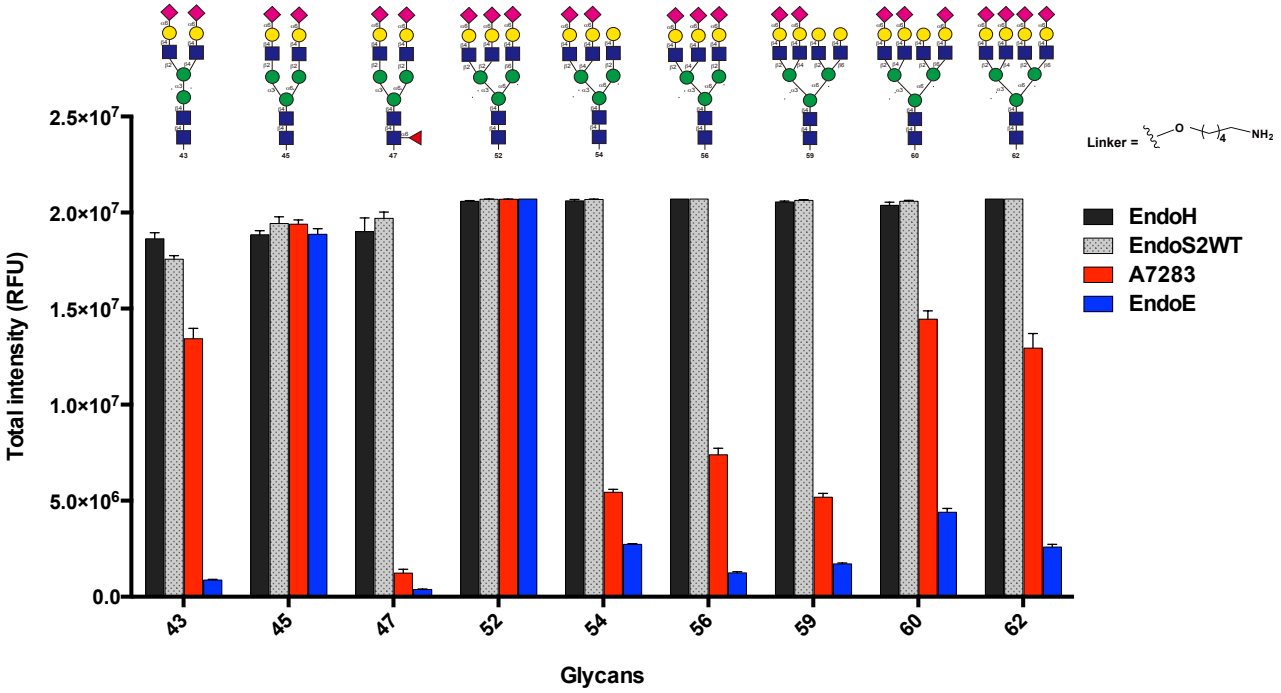


Fig. S3. Screening of useful ENGases which were expressed in *E. coli* by using commercial Herceptin (with complex type glycans on the Fc region, in the left panel) and RNaseB (with high mannose type glycans, in the right panel) as the substrates. Effective ENGase were labeled with the star symbol that was presented band-shifted on the SDS-PAGE.

(a)



(b)

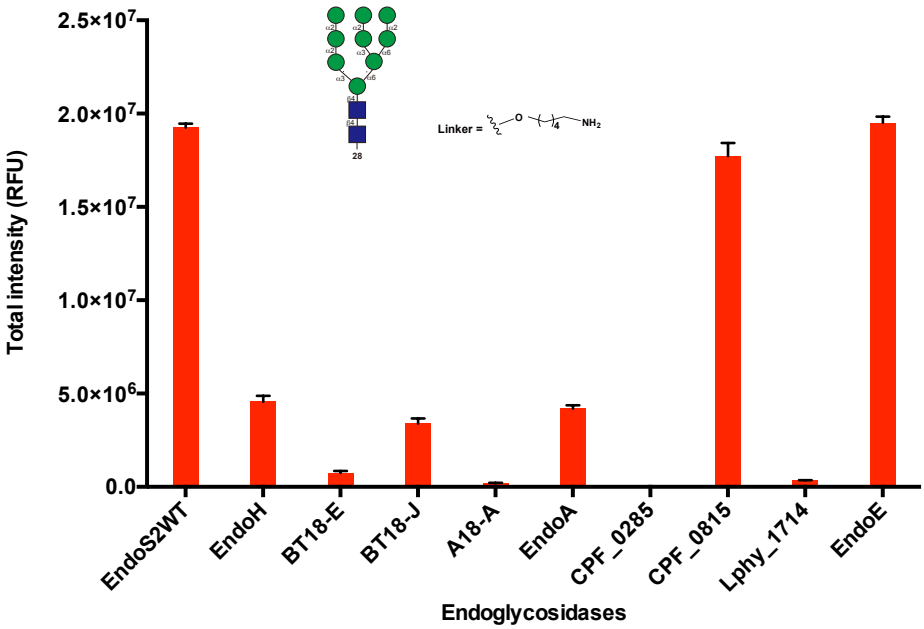
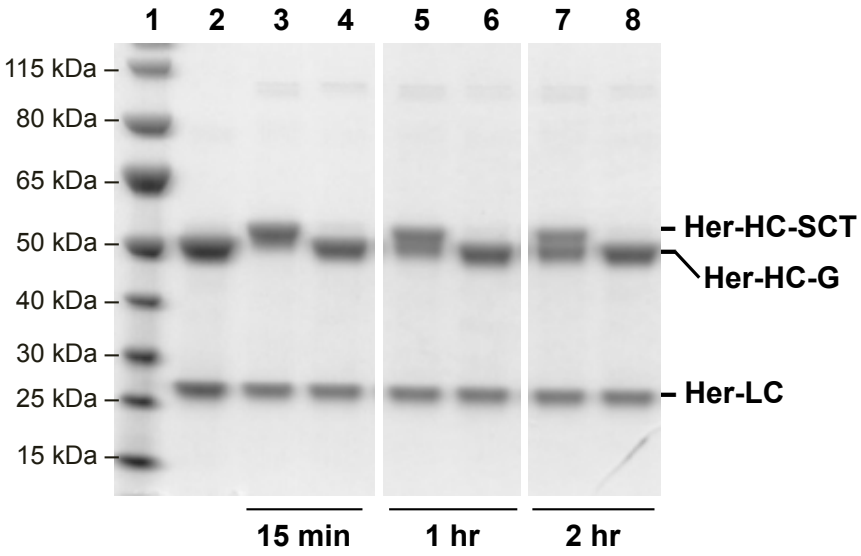


Fig. S4. Characterization of ENGases which catalyzed the hydrolysis of multi-antennary complex type and mannose type *N*-glycans on the array with OC_5NH_2 linker. (a) Binding of PG16 antibody (an antibody which recognizes glycans with terminal sialic acids) to a panel of arrayed glycans after treatment with various ENGases. (b) Binding of 2G12 antibody (an antibody which recognize Man9 glycans) to the Man9-GlcNAc-GlcNAc-OC_5NH_2-linked array after treatment with various ENGases. The mean signal intensities (RFU) and standard errors calculated for three independent replicates on the array are shown.



Lane 1: Marker
Lane 2: rHer-GlcNAc
Lane 3: rHer-GlcNAc + oxazoline-SCT + Endo-S2 D184Q_ 15 min
Lane 4: rHer-GlcNAc + oxazoline-SCT + Endo-S2 WT_ 15 min
Lane 5: rHer-GlcNAc + oxazoline-SCT + Endo-S2 D184Q_ 1 hr
Lane 6: rHer-GlcNAc + oxazoline-SCT + Endo-S2 WT_ 1 hr
Lane 7: rHer-GlcNAc + oxazoline-SCT + Endo-S2 D184Q_ 2 hr
Lane 8: rHer-GlcNAc + oxazoline-SCT + Endo-S2 WT_ 2 hr

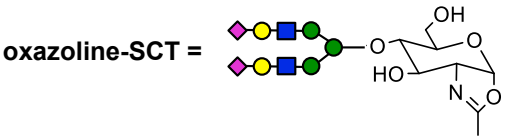
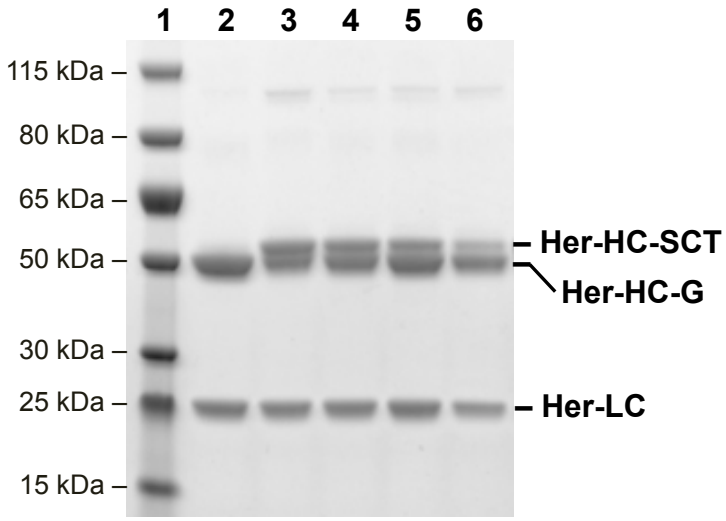


Fig. S5. Endo-S2 WT and Endo-S2 D184Q catalyzed transglycosylation of trastuzumab by using oxazoline-SCT as the glycan donor. rHer-GlcNAc : oxazoline-SCT : Endo-S2 WT/Endo-S2 D184Q = 20 : 10 : 1 (by weight ratio). The reaction was incubated at 37°C with shaking, and characterized by SDS-PAGE analysis. Abbreviations: rHer-GlcNAc: trastuzumab with GlcNAc on Fc-Asn297, SCT: α2,6-sialylated biantennary complex type glycan, HC: heavy chain, LC: light chain, G: GlcNAc.



Lane 1: Marker
Lane 2: rHer-GlcNAc
Lane 3: rHer-GlcNAc + oxazoline-SCT + Endo-S2 D184Q_ 1 hr
Lane 4: rHer-GlcNAc + oxazoline-SCT + Endo-S2 D184Q_ 2 hr
Lane 5: rHer-GlcNAc + oxazoline-SCT + Endo-S2 D184Q_ 3 hr
Lane 6: rHer-GlcNAc + oxazoline-SCT + Endo-S2 D184Q_ 16 hr

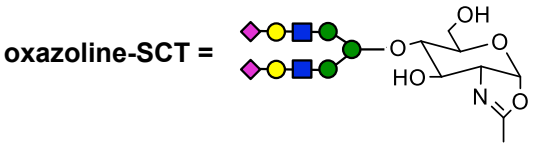
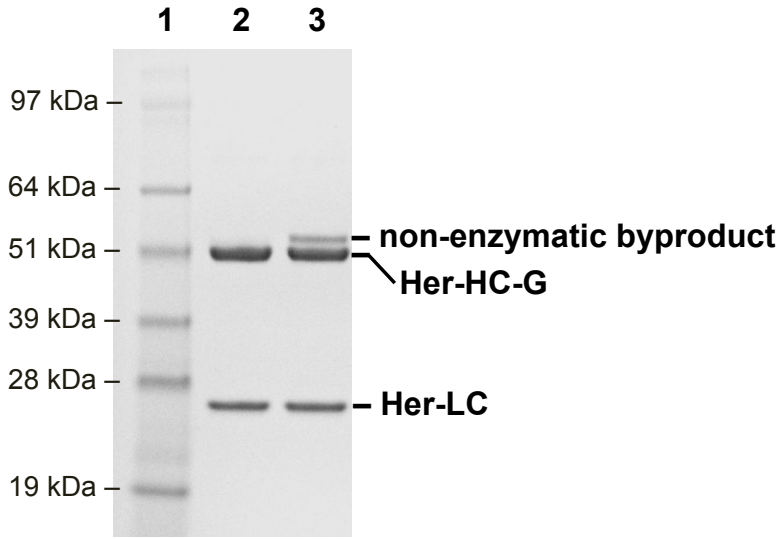


Fig. S6. Endo-S2 D184Q catalyzed transglycosylation of trastuzumab using oxazoline-SCT as the glycan donor with increasing time. rHer-GlcNAc : oxazoline-SCT : Endo-S2 D184Q = 20 : 10 : 1 (by weight ratio). The reaction was incubated at 37°C with shaking, and characterized by SDS-PAGE analysis. Abbreviations: rHer-GlcNAc: trastuzumab with GlcNAc on Fc-Asn297, SCT: α2,6-sialylated biantennary complex type glycan, HC: heavy chain, LC: light chain, G: GlcNAc.



Lane 1: Marker
Lane 2: rHer-GlcNAc
Lane 3: rHer-GlcNAc + oxazoline-SCT

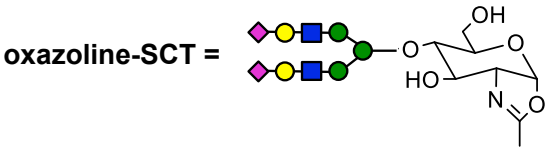


Fig. S7. Non-enzymatic byproducts on trastuzumab resulted from using oxazoline-SCT as the glycan donor. rHer-GlcNAc : oxazoline-SCT = 20 : 10 (by weight ratio). The reaction was incubated at 37°C for 1 hour, and characterized by SDS-PAGE analysis. Abbreviations: rHer-GlcNAc: trastuzumab with GlcNAc on Fc-Asn297; SCT: α2,6-sialylated biantennary complex type glycan; HC: heavy chain; LC: light chain; G: GlcNAc.

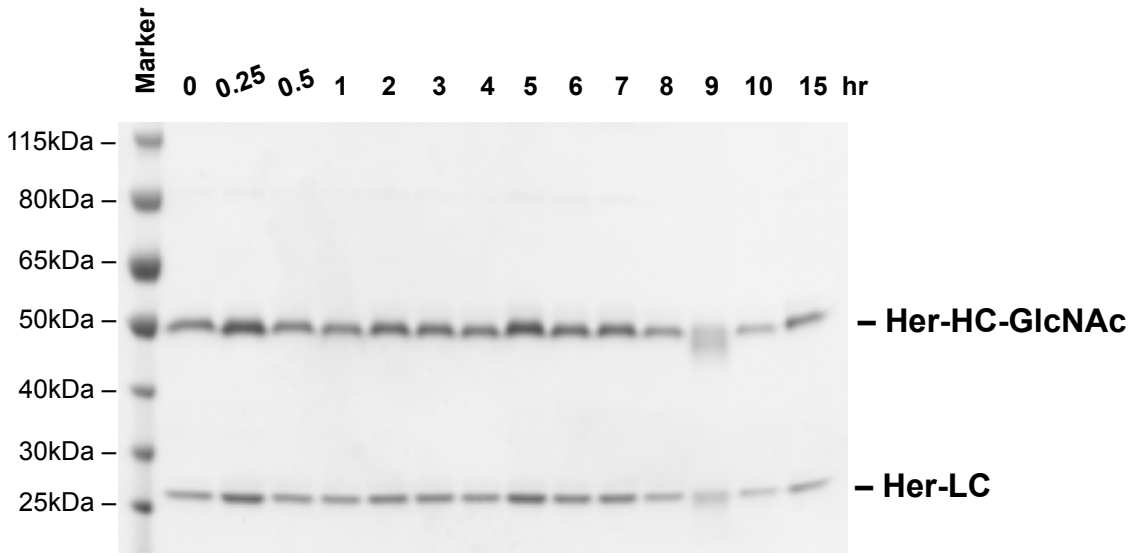


Fig. S8. Profiling of non-enzymatic glycosylation of trastuzumab using SGP. Trastuzumab-GlcNAc : SGP = 14.4 : 1 (by weight ratio). The final concentration of SGP is 300mM. Transglycosylation efficiency was evaluated by SDS-PAGE analysis. Abbreviations: Her: trastuzumab; HC: heavy chain; LC: light chain; SGP: α2,6-sialylated biantennary complex type glycopeptide.

Table S1. Oligonucleotide primers used for the amplification of the whole *OCH1*, *ALG1*, *GTPase*, *PMT5*, and *MNN9* locus from *P. pastoris* genomic DNA, for amplification of fragments to assemble KanMX integration cassette.

Primer name	Sequence (5' to 3')
OCH1-Fw	ATGGCGAAGGCAGATGGCAG
OCH1-Rv	TTAGTCCTTCCAACTTCCTTCA
ALG1-Fw-1	ATGTCTCAGTTGAAGGAAGG
ALG1-Rv-1	CTAAGTCAACAAACTACCTA
ALG1-Fw-2	AGCCGTGATCCTGGTTATGGGAGA
ALG1-Rv-2	TGACATGTTCCTTAATGAGCTCGGTA
GTPase-Fw-1	ATGAGTTCCAACAAGCAAATTACAG
GTPase-Rv-1	CTAACATTGACAGGTTGATTCCG
GTPase-Fw-2	CACAATTGGAGATAAGACCATCAA
GTPase-Rv-2	AACAATAGACCTTCATCATCCGCC
PMT5-Fw-1	ATGACTCCTGAGATATTTGGTC
PMT5-Rv-1	CTACTGAAATTGATCGCAAGGG
PMT5-Fw-2	GGCTTTGTTTCTTGCGTTCACA
PMT5-Rv-2	AAGATGGAGCGAAATGGAATA
MNN9-Fw-1	ATGTTGGTGCCCAATAAAAAC
MNN9-Rv-1	CTACTCGTTATAGTGGTAAA
MNN9-Fw-2	CTATTGTCAAGCAAAATCCCTCC
MNN9-Rv-2	ACCTCCAACACCATCCAGTTGCA
OCH1-KanMX-Fw	GAAGACAATTATGGGTAAGGAAAAGACTCACGTTTCGAGG
OCH1-KanMX-Rv	CTTTCTCTTTTAACATCTCTCCTTAGAAAAACTCATC
ALG1-KanMX-Fw	CGTTGTCTTTCATGGGTAAGGAAAAGACTCACGTTTCGAGGC
ALG1-KanMX-Rv	ATCAAACTTCAAAGAAGCCACTTAGAAAAACTCATCGAGCATCA
GTPase-KanMX-Fw	CACTTGCTCATGGGTAAGGAAAAGACTCACGTTTCGAGGC
GTPase-KanMX-Rv	TCTGACCCTCTTGTACACTGTTAGAAAAACTCATCGAGCATCA
PMT5-KanMX-Fw	TCACAGTGGCCGTGAGATTCTTCAATATTATGGGTAAGGAA
PMT5-KanMX-Rv	CCAACCGACAAATTAGAAAAACTCATCGAGCATCAAATGA
MNN9-KanMX-Fw	ATGAACTTACATGGGTAAGGAAAAGACTCACGTTTCGAGG
MNN9-KanMX-Rv	CTGTACTCAAATCTCCATGTGGTTAGAAAAACTCATCGAGCA
KanMX-Fw	ATGGGTAAGGAAAAGACTCAC
KanMX-Rv	TTAGAAAAACTCATCGAGCATC

Table S2. ENGases for protein *N*-glycans characterized in this study.

No.	Abbreviation	Organisms	GenBank	CAZy family
1	BT18-A [BT1038]	*Bacteroides thetaiotaomicro*n VPI-5482	AAO76145.1	GH18
2	BT18-B [BT1044]	*Bacteroides thetaiotaomicro*n VPI-5482	AAO76151.1	GH18
3	BT18-C [BT1048]	*Bacteroides thetaiotaomicro*n VPI-5482	AAO76155.1	GH18
4	BT18-D [BT1284]	*Bacteroides thetaiotaomicro*n VPI-5482	AAO76391.1	GH18
5	BT18-E [BT1285]	*Bacteroides thetaiotaomicro*n VPI-5482	AAO76392.1	GH18
6	BT18-F [BT1632]	*Bacteroides thetaiotaomicro*n VPI-5482	AAO76739.1	GH18
7	BT18-G [BT2825]	*Bacteroides thetaiotaomicro*n VPI-5482	AAO77931.1	GH18
8	BT18-H [BT3050]	*Bacteroides thetaiotaomicro*n VPI-5482	AAO78156.1	GH18
9	BT18-I [BT3753]	*Bacteroides thetaiotaomicro*n VPI-5482	AAO78858.1	GH18
10	BT18-J [BT3987]	*Bacteroides thetaiotaomicro*n VPI-5482	AAO79092.1	GH18
11	BT18-K [BT4406]	*Bacteroides thetaiotaomicro*n VPI-5482	AAO79511.1	GH18
12	BT18-L [BT4709]	*Bacteroides thetaiotaomicro*n VPI-5482	AAO79814.1	GH18
13	BF18-A [BF1312]	*Bacteroides fragilis* NCTC 9343	CAH07029.1	GH18
14	BF18-B [BF3414]	*Bacteroides fragilis* NCTC 9343	CAH09105.1	GH18
15	BVU18-A [BVU0617]	*Bacteroides vulgatus* ATCC 8482	ABR38325.1	GH18
16	BVU18-B [BVU1273]	*Bacteroides vulgatus* ATCC 8482	ABR38963.1	GH18
17	Cphy18-A [Cphy_1652]	*Clostridium phytofermentans* ISDg	ABX42024.1	GH18
18	Cphy18-B [Cphy_1799]	*Clostridium phytofermentans* ISDg	ABX42168.1	GH18
19	Cphy18-C [Cphy_1800]	*Clostridium phytofermentans* ISDg	ABX42169.1	GH18
20	Cphy18-D [Cphy_2210]	*Clostridium phytofermentans* ISDg	ABX42576.1	GH18
21	Cphy18-E [Cphy_2572]	*Clostridium phytofermentans* ISDg	ABX42933.1	GH18
22	Cphy18-F [Cphy_3479]	*Clostridium phytofermentans* ISDg	ABX44096.1	GH18
23	A18-A [A6286]	*Prevotella melaninogenica* ATCC 25845	ADK96185.1	GH18
24	A18-B [A7010]	*Prevotella melaninogenica* ATCC 25845	ADK96847.1	GH18
25	A18-C [A7292]	*Prevotella melaninogenica* ATCC 25845	ADK97249.1	GH18
26	EndoM	*Mucor hiemalis*	BAB43869.1	GH85
27	EndoA	*Arthrobacter protophormiae* AKU 0647	AAD10851.1	GH85
28	CPF_0285	*Clostridium perfringens* ATCC 13124	ABG82341.1	GH85
29	CPF_0815	*Clostridium perfringens* ATCC 13124	ABG82927.1	GH85
30	EndoD	*Streptococcus pneumoniae*	BAB62042.1	GH85
31	Lphy_1714	*Lachnoclostridium phytofermentans*	ABX42086.1	GH85
32	Pme_A7283	*Prevotella melaninogenica* ATCC 25845	ADK97378.1	GH85
33	Pme_A7284	*Prevotella melaninogenica* ATCC 25845	ADK97201.1	GH85
34	Pme_A7285	*Prevotella melaninogenica* ATCC 25845	ADK97032.1	GH85
35	EndoE	*Enterococcus faecalis* V583	AAO79989.1	GH18/20