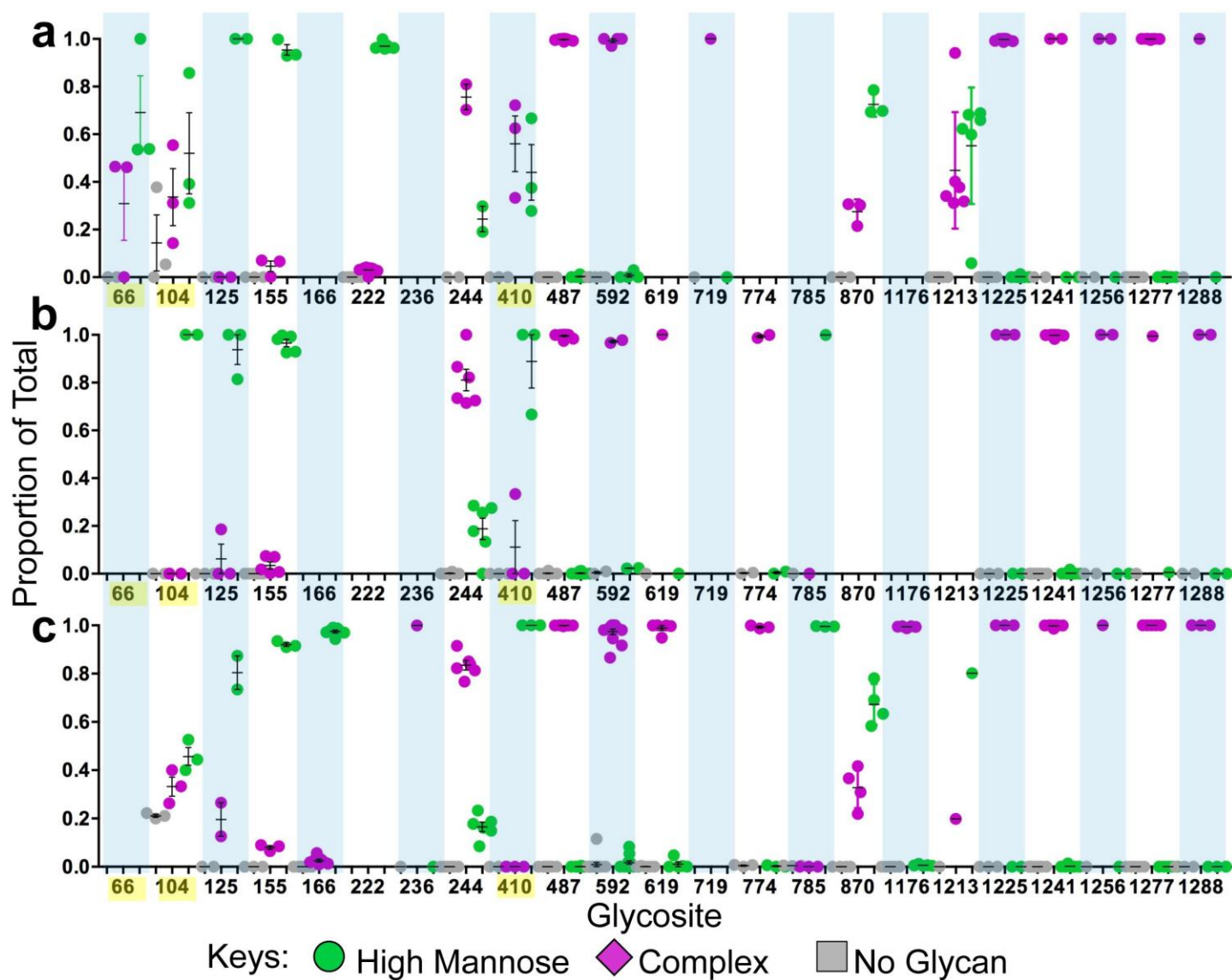


Supplementary Figure 1

Identification of peptides with multiple glycosylation sites with the protocol.

MS/MS spectra and fragment assignment of the (a) di-glycosylated, (b) tri-glycosylated, and (c) tetra-glycosylated peptides derived from BG505 SOSIP.664.



Supplementary Figure 2

Scatter plot of site-specific N-glycan processing of MERS-CoV S protein with a breakdown on the used proteases.

(a) Triple digestion, (b) the combination of trypsin and chymotrypsin, (c) chymotrypsin. The proportions of high mannose and complex type glycans at those glycosites highlighted in yellow were assigned based on the proportion of spectra hits since peak area did not reach the threshold. Mean \pm SEM were plotted.

Supplementary Information for

Global site-specific analysis of glycoprotein N-glycan processing

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Supplementary Table 1

Identification of the peptide glycosite N155 on MERS-CoV S protein by using the protocol.

Glycosite	Peptides identified
155	A.FMLGSSVGN#F.S
	A.FMLGSSVGN#FSDGK.M
	A.FMLGSSVGN#FSDGKM.G
	F.MLGSSVGN#F.S
	F.MLGSSVGN#FSDGK.M
	F.MLGSSVGN#FSDGKM.G
	F.MLGSSVGN#FSDGKMG.R
	F.MLGSSVGN#FSDGKMGRF.F
	F.MLGSSVGN#FSDGKMGRFF.N
	G.SSVGN#FSDGK.M
	G.SSVGN#FSDGKMG.R
	G.SSVGN#FSDGKMGR.F
	K.IYPAFMLGSSVGN#F.S
	K.IYPAFMLGSSVGN#FSDGK.M
	K.IYPAFMLGSSVGN#FSDGKMG.R
	L.GSSVGN#FSDGK.M
	L.GSSVGN#FSDGKM.G
	L.GSSVGN#FSDGKMG.R
	L.GSSVGN#FSDGKMGRFF.N
	M.LGSSVGN#FSDGK.M
	M.LGSSVGN#FSDGKM.G
	M.LGSSVGN#FSDGKMG.R
	M.LGSSVGN#FSDGKMGR.F
	M.LGSSVGN#FSDGKMGRF.F
	M.LGSSVGN#FSDGKMGRFF.N
	R.KIYPAFMLGSSVGN#F.S
	R.KIYPAFMLGSSVGN#FSDGK.M
	S.SVGN#FSDGK.M
	S.SVGN#FSDGKMG.R
	S.SVGN#FSDGKMGRFF.N

Supplementary Table 2

The number of MS/MS spectra that could be detected from each glycosylation site of invertase produced by the yeast *S. cerevisiae*.

Glycosite	N+0	N+3	N+203	Sum
23	267	0	1045	1312
64	81	0	538	619
97	166	0	2423	2589
111	1957	20	2963	4940
118	123	0	4305	4428
165	68	0	1471	1539
266	81	32	987	1100
275	1061	33	449	1543
356	480	1	1526	2007
369	26	0	371	397
384	24	0	2391	2415
398	17	0	1515	1532
512	813	0	10546	11359

Supplementary Table 3

The number of MS/MS spectra that could be detected from each glycosylation site of alpha-1-acid glycoprotein from bovine serum.

Glycosite	N+0	N+3	N+203	Sum
34	0	322	20	342
57	20	145	4	169
94	1	78	1	80
104	0	190	0	190
136	0	52	2	54

Supplementary Table 4

Percentages of spectra hits with non-enzymatic deamidation in total of all spectra hits identified.

Protein	N+1	Total	Percentage
invertase	2898	64010	4.53%
alpha-1-acid glycoprotein	89	5995	1.48%

Supplementary Table 5

Percentages of spectra hits with ^{18}O -incorporation into the C-termini of peptides in total of all spectra hits identified.

Protein	^{18}O -incorporation	Total	Percentage
invertase	224	64010	0.35%
alpha-1-acid glycoprotein	261	5995	4.35%