Supplementary Information for:

Integrity of glycosylation processing of a glycan-depleted trimeric HIV-1 immunogen targeting key B-cell lineages

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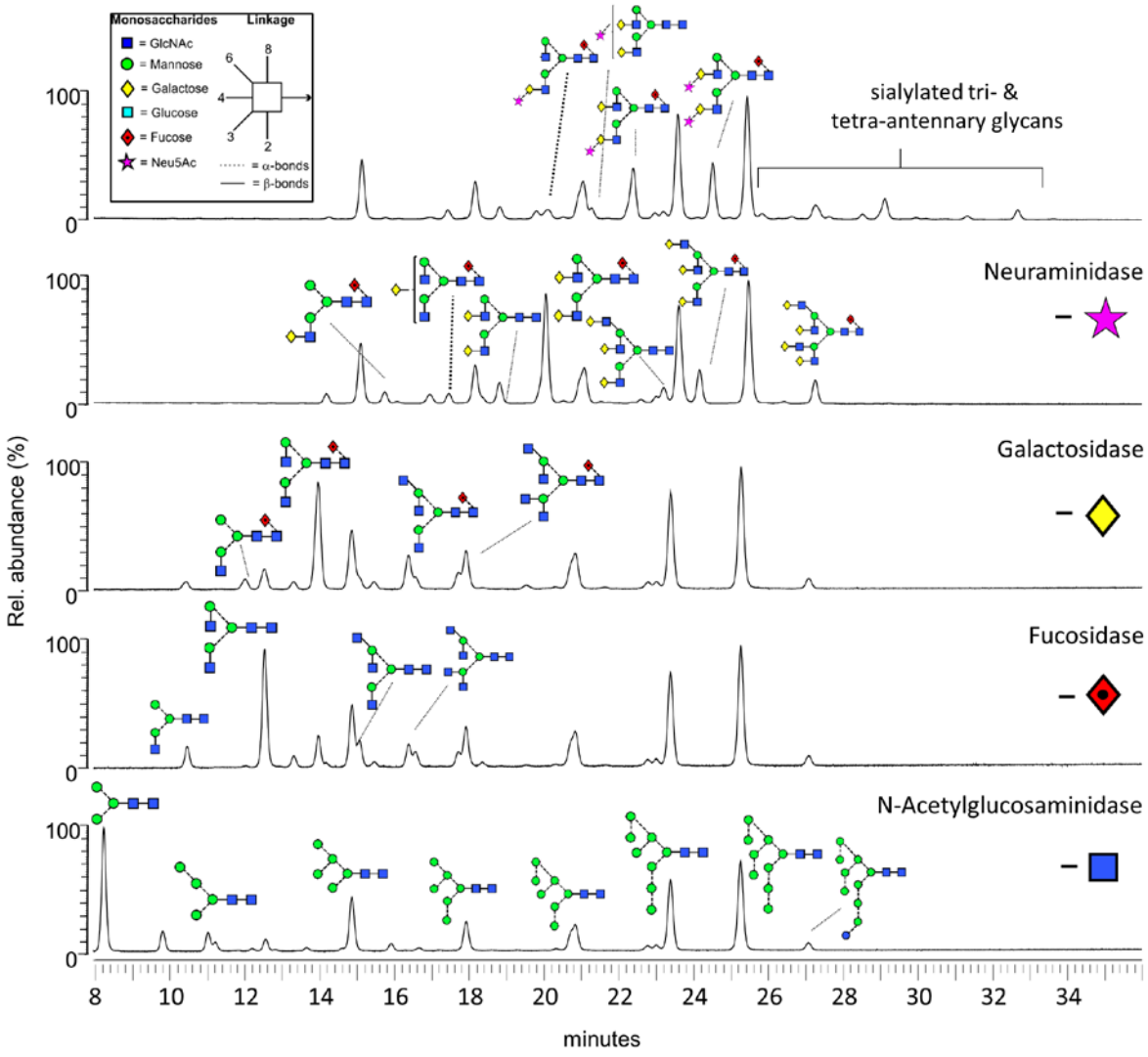

Figure S1. Glycan sequencing of BG505 SOSIP.v4.1-GT1 trimers by exoglycosidase digestion, related to Figure 2. Peaks were assigned by a sequential enzymatic digestion of 2-AA labelled glycans with a panel of exoglycosidases, followed by HILIC-UPLC analysis. The top panel shows the undigested glycan profile of BG505 SOSIP.v4.1-GT1 trimers produced in CHO cells. The profiles below represent digestions with the following exoglycosidases: Neuraminidase from *Clostridium perfringens*, β1,4-galactosidase from *Streptococcus pneumonia*, α-L-fucosidase from bovine kidney and β-*N*-acetylglucosaminidase from *S. pneumonia*. The HILIC-UPLC spectra of BG505 SOSIP.v4.1-GT1 looks highly similar to a previously published exoglycosidase array of BG505 SOSIP.664 produced in CHO cells (1).

Table S1. Overview of the thermostability of BG505 SOSIP trimers, related to Figure 1.

Construct	Cell line	Purification	T_m (°C)	Reference
SOSIP.664	HEK 293T, Transient	2G12+SEC	68.1	(2)
SOSIP.664	HEK 293T, stable	2G12+SEC	66.9	(3)
SOSIP.664	CHO, stable	2G12+SEC	66.3	(3)
SOSIP.v4.1	HEK 293F, transient	2G12+SEC	69.5	(4)
SOSIP.v4.1	CHO, stable	2G12+SEC	68.7	this paper
SOSIP.v4.1-GT1	HEK 293T, transient	PGT145+SEC	67.7	(5)
SOSIP.v4.1-GT1	CHO, stable	2G12+SEC	67.5	this paper

Table S2. Relative abundances of oligomannose-type glycan on BG505 SOSIP trimers, related to Figure 2. Abundances (as percentages of total glycans) of oligomannose-type glycans $Man_{5-9}GlcNAc_2$ (Man5-9), calculated after digestion of released glycans with Endoglycosidase H.

	Man5	Man6	Man7	Man8	Man9	Total
SOSIP.v4.1-GT1 gp140	8	6	8	17	18	57
SOSIP.664 gp140	5	7	8	15	23	59
SOSIP.v4.1-GT1 gp120	9	6	9	19	26	69
SOSIP.664 gp120	6	7	8	18	29	68
SOSIP.v4.1-GT1 gp41	2	1	1	0	0	4
SOSIP.664 gp41	1	6	4	1	10	12

Table S3. Library of glycan structures identified on BG505 SOSIP.v4.1-GT1 and SOSIP.664 trimers, related to Figure 3. The structures are represented as in the legend to Figure 3, using the Oxford glycan nomenclature (Oxford) as previously described (6). Da: Dalton; MW: Molecular weight; Calc: Calculated. Table S3 is available on-line as a separate file.

Table S4. N-linked glycopeptide compositions of trypsin- and chymotrypsin-digested BG505 SOSIP.v4.1-GT1 and SOSIP.664 trimers identified by LC-ESI MS, related to Figure 4. (A) SOSIP.v4.1-GT1 peak list; (B) SOSIP.664 peak list (in a separate Excel sheet). Site: N-glycosylation site; XIC: Extracted ion chromatogram; Exp.: Experimental determined mass (shown as a range when different charge states and/or different scans were recorded); Calc.: Calculated mass. All cysteines are carbamidomethylated. Lower case letters in the sequence indicate the positions of the modifications. Table A contains data from two analytical replicates per digest. Table S4 is available on-line as a separate file.

Supplementary References

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