Electronic Supplementary Material (ESI) for Molecular Omics.

Supplementary Material

Supplementary Script S1. reformat_peptide3.py. Round up RT and rearrange the datatable into RT vs windows matrix.

Supplementary Tables

Supplementary Table S1. Example of details from a DIALib Y ion library formatted for use in Peakview. One Q1 value (out of 34) is depicted as an example, corresponding to the *m/z* window 400-425 *m/z* (average = 412.5 *m/z*). This example includes four theoretical Y ions (Q3s) calculated for the Gas1 N^{40}GS peptide FFYSNNGSQFYIR (from a maximum of 6). The protein_name and the Uniprot_ID have the same identifier. The isotype column is left blank. Stripped sequence is the amino acid sequence of the peptide without modifications. The Modification_sequence is the amino acid sequence incorporating a modification in [], in this case it contains the Q1. Prec_z and Frg_z indicate the charge state of the precursor and fragment ions, respectively. Prec_z is arbitrarily always set as 2. Frg_type can be *b*, *y*, and Y. Frg_nr is the fragment ion number. The values for RT_detected and iRT are the same, and in this example they are set to 12 min. Score is set to 0, decoy as FALSE, Prec_y as 0, confidence as 0.99, Shared as FALSE, N as 1; and the remaining columns are left blank.

Q1	Q3	RT-detected	Protein_name	isotype	Relative-intensity	Stripped_sequence	Modification_sequence	Prec_z	Frg_type	Frg_z	Frg_nr	iRT	Uniprot_id	score	decoy	Prec_y	confidence	shared	N	rank	mods	nterm	cterm
412.5	**821.8861**	**12**	**GAS1-N40GS**		**1**	FFYSNNGSQFYIR	FFYSNN[412]GSQFYIR	**2**	**Y**	**2**	**1**	**12**	**GAS1-N40GS**	**0**	**FALSE**	**0**	**0.99**	**FALSE**	**1**				
412.5	**923.4258**	**12**	**GAS1-N40GS**		**1**	FFYSNNGSQFYIR	FFYSNN[412]GSQFYIR	**2**	**Y**	**2**	**2**	**12**	**GAS1-N40GS**	**0**	**FALSE**	**0**	**0.99**	**FALSE**	**1**				
412.5	**1024.9654**	**12**	**GAS1-N40GS**		**1**	FFYSNNGSQFYIR	FFYSNN[412]GSQFYIR	**2**	**Y**	**2**	**3**	**12**	**GAS1-N40GS**	**0**	**FALSE**	**0**	**0.99**	**FALSE**	**1**				
412.5	**1642.7649**	**12**	**GAS1-N40GS**		**1**	FFYSNNGSQFYIR	FFYSNN[412]GSQFYIR	**2**	**Y**	**1**	**1**	**12**	**GAS1-N40GS**	**0**	**FALSE**	**0**	**0.99**	**FALSE**	**1**				

Supplementary Table S2. Select IgG glycopeptides identified by Byonic and detected by DIA using a DIALib Y ion library.

Byonic							**Y ion DIALib**				
Glycans NHFAGNa	Glycan type	Modification Type(s)	Observed m/z	z	Protein Name	Scan Time	Peptide	Precursor MZ	Precursor Charge	Observed RT	Normalized intensity
HexNAc(4)Hex(4)Fuc(1)	G1F	N[+1607]	932.696	3	>sp|P01857|IGHG1	7.1108	EEQYN[7.937]STYR	937	2	7.1428	0.0294
HexNAc(5)Hex(3)Fuc(1)	G0FN/B	N[+1648]	946.371	3	>sp|P01857|IGHG1	7.1453	EEQYN[7.937]STYR	937	2	7.1428	0.0294
HexNAc(4)Hex(5)Fuc(1)	G2F	N[+1769]	986.718	3	>sp|P01857|IGHG1	7.094	EEQYN[7.987]STYR	987	2	7.1552	0.0222
HexNAc(5)Hex(4)Fuc(1)	G1FN/B	N[+1810]	1000.388	3	>sp|P01857|IGHG1	7.1511	EEQYN[7.1012]STYR	1012	2	7.1524	0.0208
HexNAc(4)Hex(5)Fuc(1)NeuAc(1)	G2FS	N[+2060]	1083.742	3	>sp|P01857|IGHG1	7.2462	EEQYN[7.1087]STYR	1087	2	7.2472	0.0070
HexNAc(4)Hex(3)Fuc(1)	G0F	N[+1445]	868.017	3	>sp|P01859|IGHG2	8.3287	EEQFN[8.862]STFR	862	2	ND	ND
HexNAc(4)Hex(4)Fuc(1)	G1F	N[+1607]	922.041	3	>sp|P01859|IGHG2	8.3881	EEQFN[8.912]STFR	912	2	8.3572	0.0720
HexNAc(5)Hex(3)Fuc(1)	G0FN/B	N[+1648]	935.708	3	>sp|P01859|IGHG2	8.4446	EEQFN[8.937]STFR	937	2	ND	ND
HexNAc(4)Hex(5)Fuc(1)	G2F	N[+1769]	976.053	3	>sp|P01859|IGHG2	8.3563	EEQFN[8.987]STFR	987	2	8.3200	0.0444
HexNAc(5)Hex(4)Fuc(1)	G1FN/B	N[+1810]	989.732	3	>sp|P01859|IGHG2	8.3321	EEQFN[8.987]STFR	987	2	8.3200	0.0444
HexNAc(4)Hex(4)Fuc(1)NeuAc(1)	G1FS	N[+1898]	1019.060	3	>sp|P01859|IGHG2	8.8296	EEQFN[9.1012]STFR	1012	2	8.9125	0.0155
HexNAc(4)Hex(5)Fuc(1)NeuAc(1)	G2FS	N[+2060]	1073.072	3	>sp|P01859|IGHG2	8.8313	EEQFN[9.1062]STFR	1062	2	8.8259	0.0155

ND = not detected

Supplementary Table S3. DIALib Y ion library for the eight cell wall glycopeptides with fixed RT at 12 min.

Supplementary Table S4. Peakview output of mesurement of ions and peptides using the Y ion library for the eight cell wall glycopeptides.

Supplementary Table S5. DIALib *by*, *by*+Y, stop*by*, and stop*by*+Y ion libraries for the eight cell wall glycopeptides with fixed RT at 12 min.

Supplementary Table S6. DIALib *by* ion library for the eight cell wall glycopeptides using published manually curated RTs [5].

Supplementary Table S7. DIALib stop*by* ion library for the eight cell wall glycopeptides using published manually curated RTs [5].

Supplementary Table S8. DIALib Y ion library for IgG glycopeptides across the full range of retention times and *m/z* windows.

Supplementary Table S9. Byonic search results for IgG *N*-glycopeptides.

Supplementary Table S10. ProteinPilot search results for cell wall glycopeptides from wild type yeast with potential *O*-glycosylation.

Supplementary Table S11. Byonic search results for cell wall glycopeptides from wild type yeast with potential *O*-glycosylation.

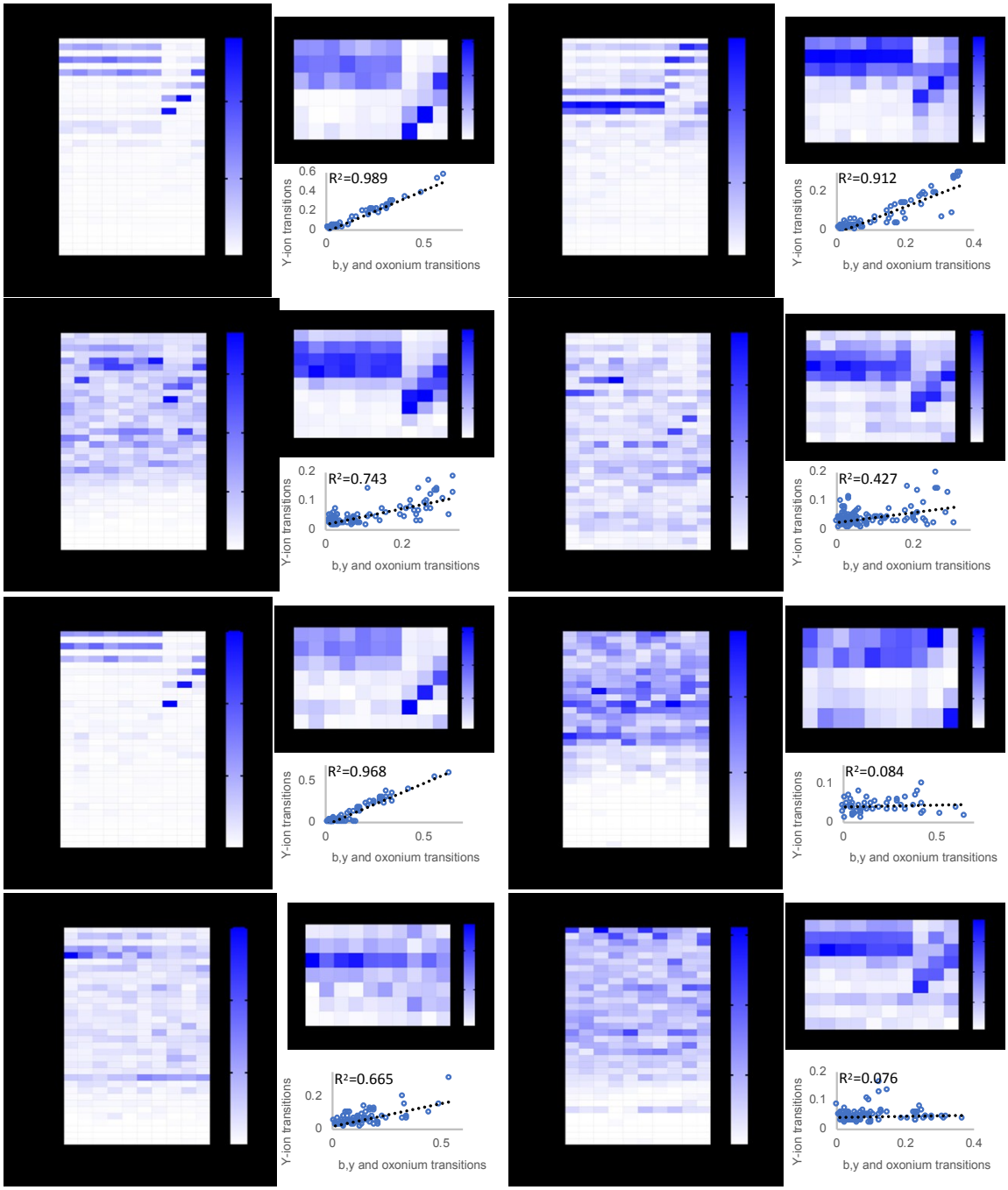
Supplementary Table S12. DIALib Y ion library for the Hsp150 cell wall T^{318}SGTLEMNLK glycopeptide across the full range of retention times and *m/z* windows.

Supplementary Table S13. Peakview output of measurement of ions and peptides using the DIALib Y ion library from Supplementary Table S12.

Supplementary Table S14. DIALib oxonium ion library across the full range of retention times and *m/z* windows.

Supplementary Table S15. Peakview output of measurement of ions and peptides using the DIALib oxonium ion library from Supplementary Table S14.

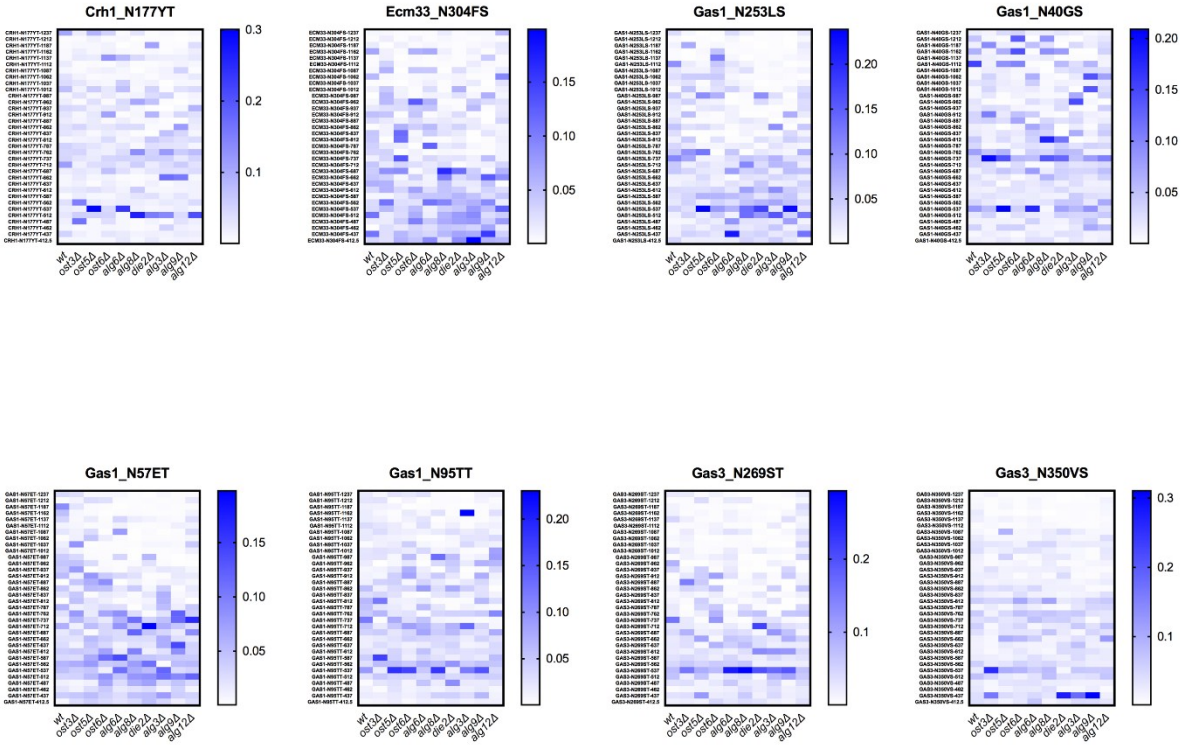
Supplementary Figures



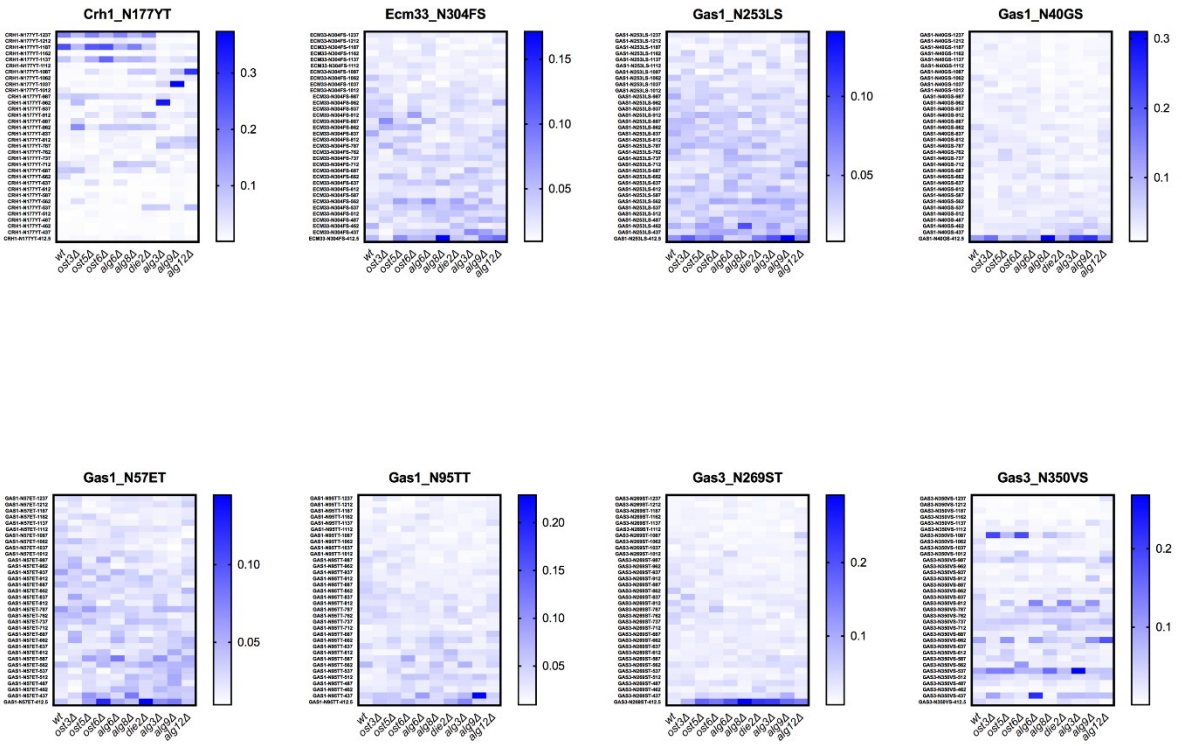
Supplementary Figure S1. DIALib Y ion measurement of site-specific glycan structural heterogeneity for eight cell wall yeast glycopeptides. Abundance of glycoforms of glycopeptides containing Gas1 N^{40}GS, Gas1 N^{95}TT, Gas1 N^{253}LS, Gas3 N^{350}VS, Crh1 N^{177}YT, Gas1 N^{57}ET, Ecm33 N^{304}FS, and Gas3 N^{269}ST measured using a DIALib Y ion library or a manually curated ion library [5] in wild type yeast and yeast with mutations in the *N*-glycan biosynthesis pathway.

Each square corresponds to the mean intensity of triplicate measurements. Also shown is the correlation between the data obtained using a DIALib Y ion library and a manually curated ion library [5] for each glycosylation site, with linear fit and R^2 values.

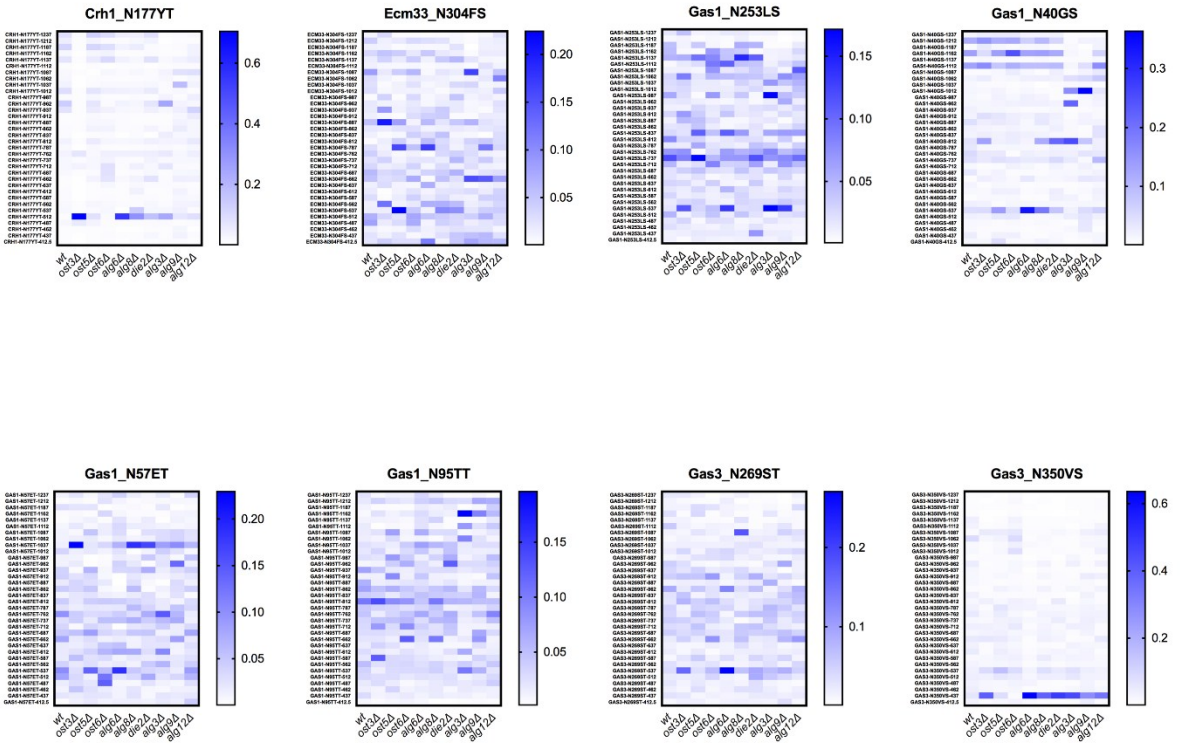
by library



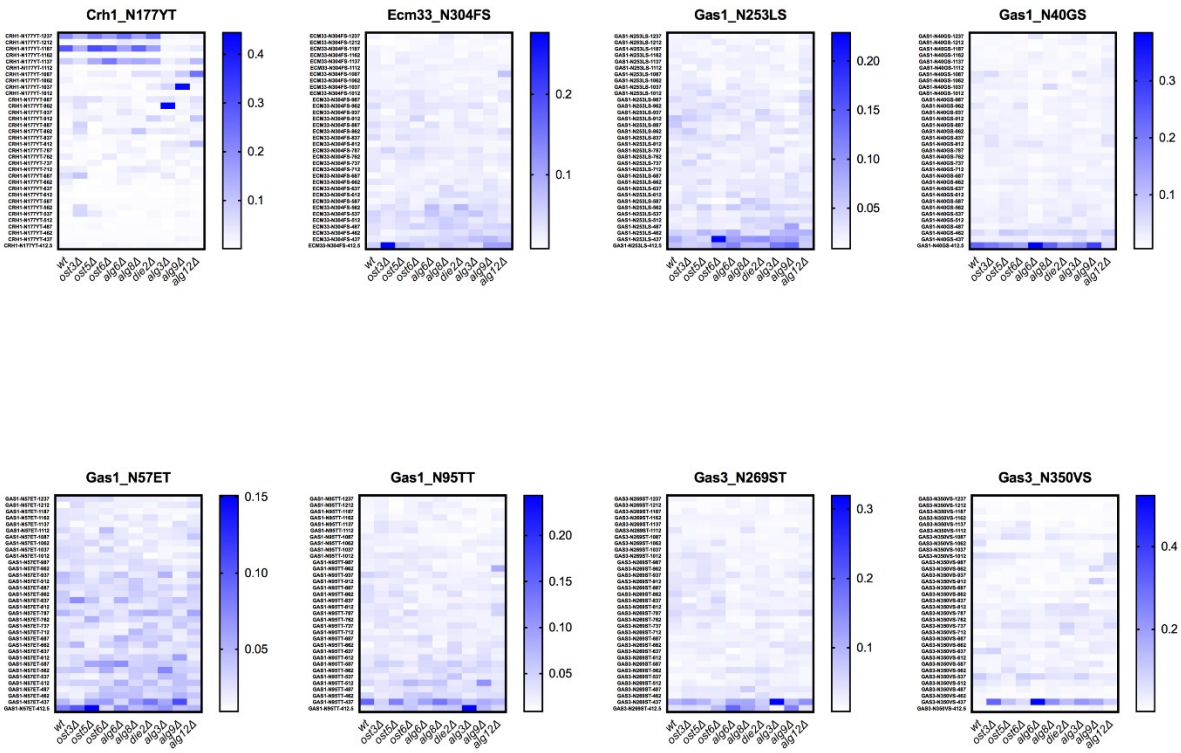
by+Y library



stop*by* library

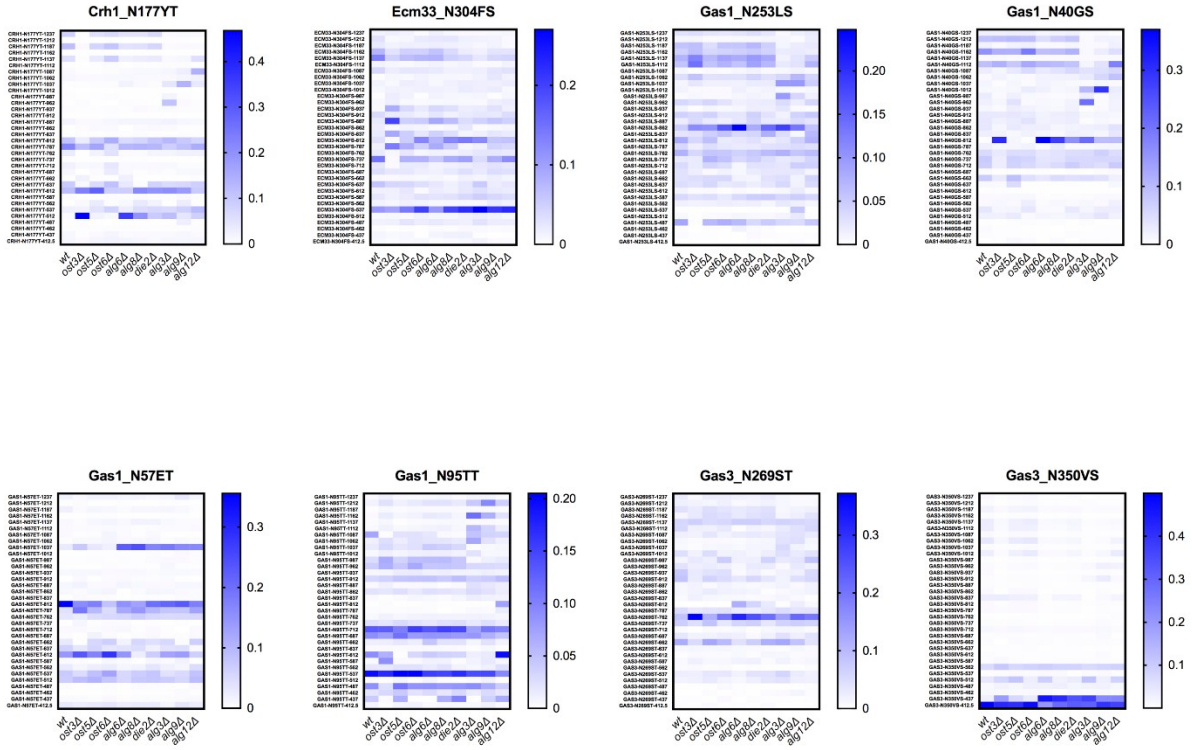


stop*by*+Y library

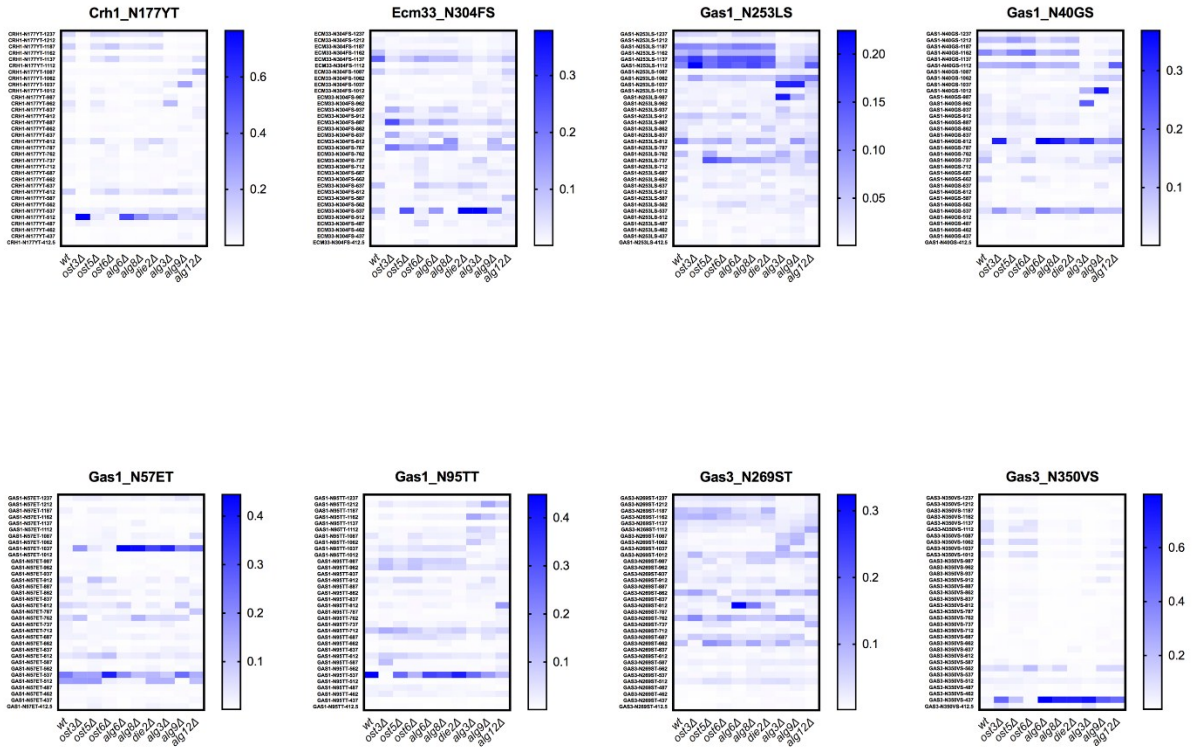


Supplementary Figure S2. Theoretical DIALib *by*, *by*Y, stop*by*, and stop*by*Y ion libraries underperform compared to the DIALib Y ion library without optimization of peptide retention times. Abundance of glycoforms of eight cell wall glycopeptides measured using DIALib *by*, *by*Y, stop*by*, and stop*by*Y ion libraries in wild type yeast and yeast with mutations in the *N*-glycan biosynthesis pathway. Each square corresponds to the mean intensity of triplicates.

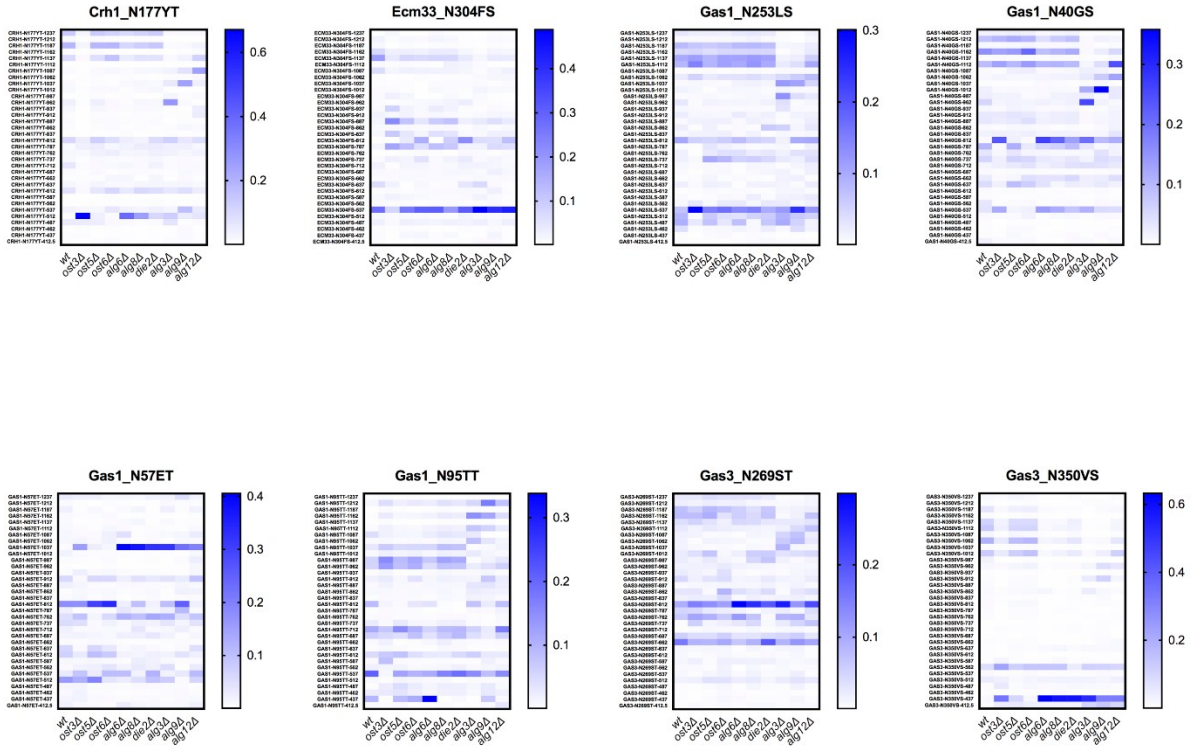
by library



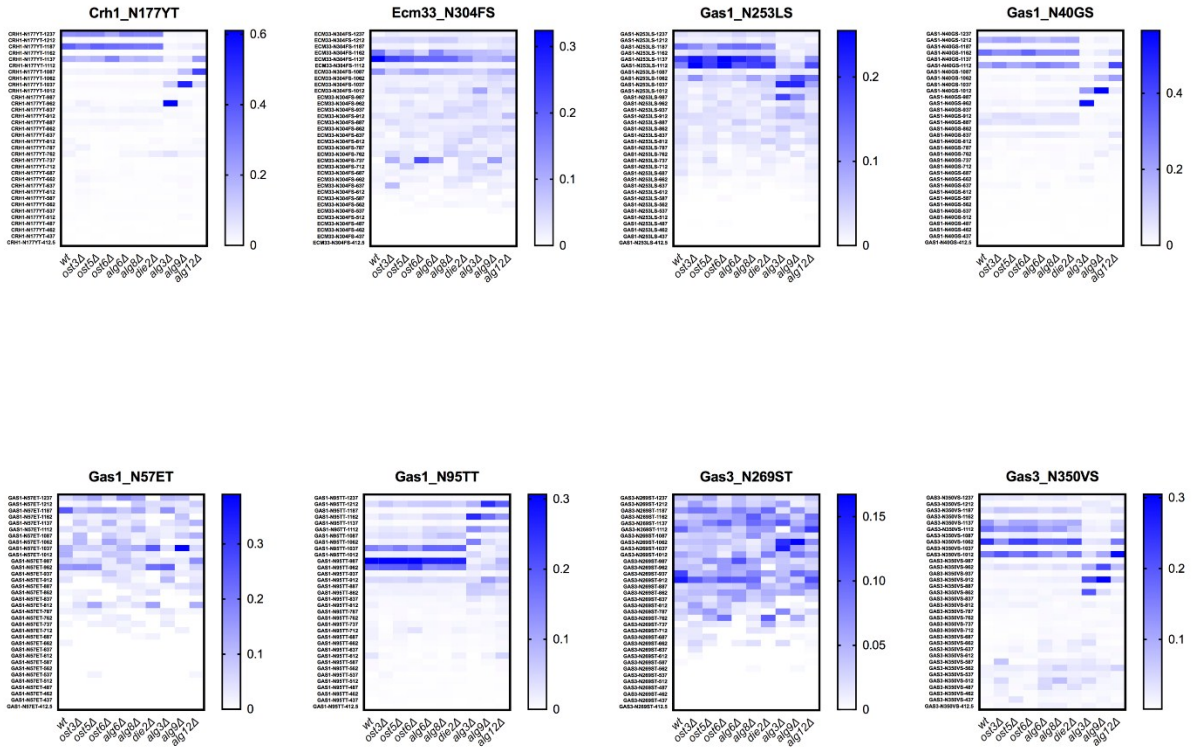
stop*by* library



stop*by*+Y library



Y library



Supplementary Figure S3. Optimized retention times improve peak picking for DIALib libraries. Abundance of glycoforms of all eight glycopeptides measured using the *by*, stop*by*, stop*by*+Y, and Y DIALib libraries and manually curated library incorporating experimental peptide retention times [5], measured in wild type yeast and in yeast with mutations in the *N*-glycan biosynthesis pathway.