Quantitative Profiling of N-linked Glycosylation Machinery in Yeast *Saccharomyces cerevisiae*

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Supplementary Information

Supplementary Table 1. Yeast strains used in this study. Related to Experimental Procedures.

Name	Genotype	Source
WT	*MATα his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0 Δarg4Δ0*	This study
Δ*ost3*	*MATα his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0 Δarg4Δ0 Δost3::LEU2MX6*	This study
Δ*ost3*+p*OST6*	*MATα his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0 Δarg4Δ0 Δost3::LEU2MX6 + pOST3*	This study
Δ*ost6*	*MATα his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0 Δarg4Δ0 Δost6::LEU2MX6*	This study
Δ*ost6*+p*OST3*	*MATα his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0 Δarg4Δ0 Δost6::LEU2MX6 + pOST6*	This study
Δ*alg9*	*MATα his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0 Δarg4Δ0 Δalg9::NATNT2*	This study
Δ*alg3*	*MATα his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0 Δarg4Δ0 Δalg3::NATNT2*	This study

Supplementary Table 2. Plasmids used in this study. Related to Experimental Procedures.

Name	Gene	Souce
pOST3	*OST3*	(Schwarz et al., 2005)
pOST6	*OST6*	(Knauer and Lehle, 1999)

Supplementary Table 3. PRM MS assay for quantitative profiling of N-linked glycosylation machinery in yeast.
(Available as Excel file)

Supplementary Table 4. The list of all glycosylation sites identified after ZIC-HILIC enrichment.
(Available as Excel file)

Supplementary Table 5. PRM MS assay for quantitive profiling of mevalonate, ergosterol and dolichol pathways.
(Available as Excel file)

Supplementary Table 6. Site-specific N-glycosylation occupancy analysis of various OST mutant strains and Δ*alg9* strain compared to wild type cells. Data for Figure 2 and 3.

UniProt ID	Protein	Glycosylation Site	Δost3	Δost3+pOST6	Δost6	Δost6+pOST3	Δalg9
P00729	*CPY*	N124	19.29 ± 3.68	29.27 ± 0.04	94.29 ± 6.26	100.11 ± 5.69	33.04 ± 13.09
P00729	*CPY*	N479	89.9 ± 11.06	94.18 ± 3.23	105.43 ± 4.56	107.89 ± 0.44	21.53 ± 10.06
P12684	**HMG2**	N150	3.3 ± 2.05	33.37 ± 9.94	73.01 ± 8.4	75.67 ± 9.36	37.76 ± 0.01
P17967	**PDI**	N117	13.45 ± 4.35	20.06 ± 1.7	95.36 ± 1.03	99.2 ± 9.31	46.8 ± 4.13
P17967	**PDI**	N155	72.91 ± 6.02	83.64 ± 4.43	111.08 ± 6.57	95.22 ± 9.47	69.62 ± 14.49
P17967	**PDI**	N174	76.59 ± 11.17	82.93 ± 6.44	108.09 ± 6.67	97.56 ± 4.56	94.09 ± 1.13
P17967	**PDI**	N425	5.39 ± 5.68	46.64 ± 3.46	107.44 ± 3.23	90.73 ± 6.88	21.81 ± 5.41
P17967	**PDI**	N82	67.5 ± 6.95	69.86 ± 2.07	72.49 ± 4.31	107.95 ± 3.31	30.51 ± 10.22
P22146	GAS1	N40	83.44 ± 5.59	96.7 ± 3.14	84.82 ± 1.79	103.73 ± 1.01	97.9 ± 7.68
P23797	**GPI12**	N110	11.51 ± 2.15	13.95 ± 0.53	104.43 ± 4.92	89.25 ± 7.49	3.86 ± 2.74
P27810	**KTR1**	N120	53.26 ± 4.43	53.45 ± 1.16	92.77 ± 0.99	98.32 ± 3.21	93.22 ± 9.81
P27825	**CNE1**	N416	N/A	27.5 ± 2.09	85.43 ± 1.25	86.67 ± 11.83	41.97 ± 14.87
P31382	**PMT2**	N403	4.77 ± 4.48	11.83 ± 0.38	91.64 ± 0.5	96.97 ± 9.5	65.35 ± 3.45
P32353	ERG3	N40	48.01 ± 8.81	102.21 ± 3.06	109.13 ± 5.35	95.6 ± 3.31	99.64 ± 7.85
P32623	*CRH2*	N233/N237	41.08 ± 13.57	N/A	102.13 ± 7.52	0 ± 0	N/A
P32623	*CRH2*	N310	50.57 ± 6.28	68.22 ± 4.06	105.93 ± 2.15	90.61 ± 7.87	55.3 ± 9.11
P33302	PDR5	N734	39.71 ± 8.12	94.65 ± 4.88	87.12 ± 0.24	94.74 ± 6.4	27.33 ± 10.89
P33754	**SEC66**	N12	13.17 ± 0.48	59.13 ± 3.37	101.25 ± 1.68	85.88 ± 11.36	92.73 ± 8.93
P33767	*WBP1*	N332	24.9 ± 6.36	61.64 ± 6.42	103.42 ± 1.51	92.73 ± 1.94	65.08 ± 14.79
P33767	*WBP1*	N60	4.4 ± 3.94	32.54 ± 8.34	99.01 ± 0.57	90.83 ± 6.28	12.84 ± 5.05
P36016	**LHS1**	N458	0.38 ± 1.56	5.5 ± 4.91	84.83 ± 4.39	89.18 ± 11.22	4.73 ± 3.05
P36051	**MCD4**	N198	39.75 ± 16.9	85.29 ± 3.57	N/A	47.95 ± 8.33	N/A
P36051	**MCD4**	N90	87.95 ± 9.28	106.62 ± 1.66	98.43 ± 0.12	105.68 ± 4.93	100.98 ± 8.24
P36091	DCW1	N203	87.52 ± 11.14	86.9 ± 4.86	99.08 ± 3.96	86 ± 1.58	84.68 ± 11.98
P37302	**APE3**	N150	52.91 ± 11.54	81.13 ± 4.36	84.99 ± 7.04	90.64 ± 11.42	52.53 ± 8.71
P37302	**APE3**	N162	107.35 ± 6.74	98.5 ± 10.61	103.27 ± 9.71	95.19 ± 8.87	29.59 ± 10.22
P37302	**APE3**	N85	47.72 ± 1.83	65.85 ± 6.25	109.52 ± 2.65	93.52 ± 4.38	102.58 ± 11.08
P37302	**APE3**	N96	82.18 ± 8.16	104.05 ± 2.42	59.81 ± 7.83	93.82 ± 6.01	5.02 ± 4.67
P38244	**PFF1**	N121	19.74 ± 5.78	73.63 ± 3.1	100.54 ± 13.07	103.76 ± 7.58	16.6 ± 3.61
P38248	*ECM33*	N304	10.34 ± 7.84	63.23 ± 4.48	108.15 ± 6.07	87.17 ± 1.07	55.37 ± 16.56
P38843	CHS7	N31	34.83 ± 4.32	104.88 ± 1.61	98.51 ± 1.01	99.97 ± 8.25	87.25 ± 6.8
P38875	GPI16	N184	6.87 ± 1.76	99.3 ± 9.93	97.36 ± 6.52	104.43 ± 3.53	95.52 ± 9.03
P38993	**FET3**	N244	8.69 ± 2.29	66.67 ± 1.51	79.97 ± 7.76	103.74 ± 12.75	40.52 ± 9.09
P38993	**FET3**	N359	64.51 ± 4.08	94.36 ± 4.94	72.94 ± 12.37	100.55 ± 10.6	72.7 ± 13.97
P39007	STT3	N539	66.68 ± 11.55	102.21 ± 5.5	89.18 ± 7.88	103.61 ± 4.28	95.22 ± 10.08
P39105	PLB1	N215	93.52 ± 4.38	90.36 ± 5.46	78.04 ± 7.36	93.1 ± 6.83	73.29 ± 6.06
P39105	PLB1	N489	76.92 ± 2.9	101.69 ± 3.65	93.94 ± 3.43	81.76 ± 1.73	92.46 ± 7.03
P40345	PDAT	N439	103.31 ± 0.13	98.13 ± 1.84	93.1 ± 6.83	88.05 ± 9.77	N/A
P40533	TED1	N266	34.18 ± 3.81	78.45 ± 1.32	87.97 ± 1.07	94.34 ± 0.36	60.37 ± 6.62
P40557	EPS1	N264	7.53 ± 3.1	108.27 ± 8.03	87.91 ± 3.6	90.04 ± 8.2	85.27 ± 6.25
P40557	EPS1	N299	40.37 ± 19	90.71 ± 9.52	75.86 ± 15.22	85.34 ± 3.58	56.07 ± 0.13
P41543	*OST1*	N217	22.19 ± 5.98	59.19 ± 0.88	98.58 ± 6.44	104.58 ± 6.74	N/A
P43561	FET5	N24	63.27 ± 16.13	78.26 ± 9.3	94.58 ± 2.7	89.3 ± 0.25	25.95 ± 7.39
P43561	FET5	N364	66.96 ± 0.37	97.89 ± 7.22	103.5 ± 3.96	96.16 ± 6.1	88.24 ± 12.25
P43611	**OSW7**	N297	0.13 ± 7.23	24.45 ± 9.38	103.23 ± 1.55	99.58 ± 4.67	39.4 ± 6.82
P46982	MNN5	N136	29.66 ± 7	93.32 ± 5.1	89.4 ± 5.08	92.41 ± 10.92	21.9 ± 0.98
P46992	YJR1	N219	50.14 ± 8.37	87.88 ± 1.37	89.87 ± 7.09	95.07 ± 4.66	44.3 ± 10.17
P52911	EXG2	N157	12.88 ± 1.05	80.59 ± 10.41	102.84 ± 4.52	100.04 ± 7.43	50.23 ± 12.71
P52911	EXG2	N50	35.06 ± 6.25	106.87 ± 5.12	88.11 ± 9.93	85.14 ± 5.77	78.55 ± 14.79
P53379	MKC7	N286	28.41 ± 11.05	96.74 ± 6.56	88.69 ± 13.07	96.01 ± 1.36	68.19 ± 10.36
P54003	SUR7	N47	28.16 ± 5.8	96.54 ± 3.85	88.39 ± 3.1	98.93 ± 1.42	53.97 ± 4.47
Q03103	**ERO1**	N458	58.06 ± 13.22	48.29 ± 4.07	74.07 ± 9.39	94.94 ± 2.27	41.26 ± 6.33
Q03281	HEH2	N520	27.3 ± 12.33	101.28 ± 3.38	96.09 ± 7.03	83.06 ± 10.2	8.12 ± 3.54
Q03674	PLB2	N193	2.11 ± 0.27	105.66 ± 4.32	76.22 ± 0.19	102.12 ± 0.84	N/A
Q03674	PLB2	N217	33.69 ± 7.84	85.64 ± 9.61	102.54 ± 7.69	98.66 ± 4.15	97.06 ± 6.47
Q03691	ROT1	N139	75.96 ± 15	82.94 ± 1.65	106.94 ± 1.91	104.16 ± 0.57	101.63 ± 4.83
Q06689	**YL413**	N429	10.52 ± 2.79	25.63 ± 14.09	105.59 ± 2.77	90.98 ± 11.19	40.78 ± 13.13
Q06689	**YL413**	N49	74.13 ± 11.88	92.56 ± 3.09	112.57 ± 12.49	91.95 ± 8.07	N/A
Q07830	**GPI13**	N411	22.18 ± 8.07	71.24 ± 6.77	96.53 ± 14.64	94.37 ± 5.33	79.6 ± 7.76
Q12465	**RAX2**	N640	51.16 ± 0.74	92.91 ± 6.76	94.94 ± 2.27	95.91 ± 10.74	32.91 ± 9.91
Q12465	**RAX2**	N677	3.73 ± 2.18	101.59 ± 1.19	106.04 ± 6.39	98.63 ± 6.26	29.25 ± 7.96
Q12465	**RAX2**	N88	1.44 ± 0.24	63.72 ± 12.38	96.74 ± 6.56	94.26 ± 6.12	N/A

Bold and italic - Ost3p substrates. Italic - novel Ost3 substrates.

Supplementary Table 7. Protein levels of glycoproteins in various OST mutant strains and Δ*alg9* strain compared to wild type cells. Data for Figure 5B.

UniProt ID	Protein	Δost3	Δost3+pOST6	Δost6	Δost6+pOST3	Δalg9
P00729	CPY	123.44 ± 7.14	93.91 ± 1.76	85.59 ± 8.94	109.36 ± 14.09	95.59 ± 10.99
P12684	HMG2	120.25 ± 1.71	88.6 ± 8.98	124.2 ± 18.72	158.37 ± 17.55	141.5 ± 16.93
P17967	PDI	182.48 ± 26.59	121.57 ± 9.45	91.2 ± 7.34	118.45 ± 9.76	164.86 ± 5.39
P22146	GAS1	55.04 ± 7.93	80.67 ± 1.8	93.4 ± 12.41	85.6 ± 2.67	114.75 ± 3.58
P23797	GPI12	84.9 ± 1.88	99.18 ± 7.71	85.39 ± 10	99.01 ± 7.47	99.27 ± 5.53
P27810	KTR1	130.97 ± 5.58	99.22 ± 0.5	119.8 ± 6	90.17 ± 5.43	110.93 ± 12.84
P27825	CNE1	N/A	217.01 ± 12.78	68.77 ± 0.45	111.94 ± 21.65	125.67 ± 15.71
P31382	PMT2	128.04 ± 9.51	106.33 ± 2.18	96.38 ± 5.46	99.32 ± 5.15	143.82 ± 10.64
P32353	ERG3	104.47 ± 9.87	110.61 ± 9.13	85.79 ± 4.45	105.3 ± 19.91	498.58 ± 67.42
P32623	CRH2	79.04 ± 1.08	96.3 ± 2.81	90.48 ± 6.3	123.64 ± 13.24	143.27 ± 14.61
P33302	PDR5	70.8 ± 4.22	92.75 ± 4.94	51.16 ± 3.76	92.6 ± 7.63	67.92 ± 6.8
P33754	SEC66	114.55 ± 4.35	102.87 ± 0.56	92.7 ± 8.84	94.46 ± 3.79	123.3 ± 18.98
P33767	WBP1	106.87 ± 7.81	105.13 ± 5.96	99.99 ± 8.85	98.57 ± 5.1	108.65 ± 5.29
P36016	LHS1	193.98 ± 16.49	121.16 ± 7.75	161.95 ± 39.58	122.5 ± 9.18	140.02 ± 7.5
P36051	MCD4	172.67 ± 1.55	88.52 ± 6.38	97.81 ± 0.36	105.07 ± 1.26	129.16 ± 4.98
P36091	DCW1	108.61 ± 26.26	89 ± 0.56	109.7 ± 4.2	91.73 ± 9.65	173.78 ± 17.35
P37302	APE3	88.38 ± 18.93	97.32 ± 14.92	83.88 ± 6.31	103.98 ± 6.31	86.58 ± 13.3
P38244	PFF1	65.06 ± 13.84	95.42 ± 11.24	97.27 ± 8.36	84.28 ± 6.19	105.28 ± 12.64
P38248	ECM33	66.16 ± 13.1	80.4 ± 3.99	70.91 ± 3.59	93.68 ± 8.47	131.78 ± 8.31
P38843	CHS7	95.76 ± 2.17	121.37 ± 9.43	91.85 ± 5.2	117.97 ± 8.35	170.38 ± 18.28
P38875	GPI16	105.61 ± 4.13	107.53 ± 2.55	N/A	104.17 ± 7.08	99.25 ± 16.36
P38993	FET3	70.65 ± 4.64	76.43 ± 8.74	61.09 ± 8.11	76.5 ± 6.24	111.72 ± 14.39
P39007	STT3	96.63 ± 8.97	99.91 ± 2.23	98.51 ± 6.21	97.44 ± 4.81	109.56 ± 18.32
P39105	PLB1	66.03 ± 8.86	55.34 ± 0.34	85.22 ± 5.29	122.38 ± 11.25	100.69 ± 8.04
P40345	PDAT	82.04 ± 5.24	94.83 ± 7.31	N/A	80.26 ± 4.47	96.34 ± 11.97
P40533	TED1	100.29 ± 15.62	90.77 ± 7.12	87.23 ± 7.12	91.79 ± 10.22	99.41 ± 5.26
P40557	EPS1	152.92 ± 26.91	87.06 ± 1.17	100.04 ± 11.86	89.92 ± 12.69	171.64 ± 17.28
P41543	OST1	109.59 ± 7.89	107.89 ± 3.71	103.7 ± 3.12	109.41 ± 5.65	98.51 ± 7.57
P43561	FET5	51.68 ± 16.13	85.01 ± 5.37	75.45 ± 0.91	102.93 ± 9.23	95.38 ± 14.97
P43611	OSW7	107.6 ± 15.79	82.79 ± 5.7	76.03 ± 0.41	65.74 ± 16.86	117.24 ± 8.98
P46982	MNN5	79.93 ± 6.04	84.56 ± 0.83	108.41 ± 16.77	90.21 ± 6.67	172.91 ± 26.82
P46992	YJR1	116.54 ± 8.14	105.36 ± 0.8	91.81 ± 4.08	113.65 ± 4.19	92.74 ± 8.75
P52911	EXG2	95.66 ± 10.65	85.09 ± 6.27	96.63 ± 6.16	104.43 ± 4.38	103.77 ± 11.87
P53379	MKC7	70.21 ± 5.56	92.72 ± 1.76	90.13 ± 7.89	93.14 ± 9.59	127.83 ± 7.15
P54003	SUR7	95.44 ± 3.04	87.64 ± 5.8	87.62 ± 8.67	84.71 ± 4.01	114.09 ± 18.67
Q03103	ERO1	216.12 ± 23.43	127.29 ± 2.12	104.01 ± 7.87	117.14 ± 4.44	169.75 ± 10.81
Q03281	HEH2	119.35 ± 3.68	95.9 ± 9.41	85.36 ± 8.13	86.45 ± 0.05	124.06 ± 13.24
Q03674	PLB2	59.09 ± 12.06	77.46 ± 6.42	94.82 ± 8.56	128.16 ± 12.06	108.76 ± 5.22
Q03691	ROT1	208.75 ± 54.58	102.99 ± 10.04	108.83 ± 3.22	120.52 ± 0.18	253.45 ± 28.11
Q06689	YL413	60 ± 6.16	101.91 ± 2.62	80.25 ± 5.64	94.06 ± 10.68	89.49 ± 9.57
Q07830	GPI13	140.93 ± 21.19	93.74 ± 3.4	102.16 ± 6.34	98.09 ± 4.3	109.92 ± 7.42
Q12465	RAX2	90.71 ± 6.22	91.81 ± 8.65	81.17 ± 3.25	94.58 ± 10.37	161.21 ± 9.23

Supplementary Table 8. Protein levels of different mevalonate, ergosterol and dolichol pathway proteins in strains deleted of *ALG3* or *ALG9* gene, or treated with sterol inhibitor miconazole. Data for Figure 5C.

UniProt ID	Protein	Δ*alg3*	Δ*alg9*	miconazole
P41338	ERG10	96.38 ± 7.34	89.06 ± 4.96	180.77 ± 0.75
P12683	HMG1	169.9 ± 1.34	177.27 ± 5.73	N/A
P12684	HMG2	160.84 ± 2.05	167.73 ± 2.38	86.34 ± 9.44
P07277	ERG12	114.77 ± 4.36	158.25 ± 0.63	144.2 ± 3.99
P32377	ERG19	90.48 ± 1.87	106.74 ± 11.52	144.51 ± 12.79
P08524	ERG20	124.35 ± 2.06	111.88 ± 10.25	125.67 ± 4.87
P29704	ERG9	100.18 ± 9.74	97.34 ± 3.22	157.37 ± 8.93
P32476	ERG1	259.71 ± 47.38	140.98 ± 5.53	102 ± 35.18
P38604	ERG7	131.31 ± 12.47	100.18 ± 2.36	147.58 ± 0.03
P10614	ERG11	482.55 ± 62.26	383.42 ± 23.58	571.84 ± 17.43
P53045	ERG25	471.15 ± 94.77	399.78 ± 10.2	404.82 ± 49.68
P53199	ERG26	97.68 ± 5.76	94.57 ± 6.31	175.91 ± 6.89
Q12452	ERG27	106.5 ± 10.12	103.92 ± 5.58	188.25 ± 4.68
P25087	ERG6	98.46 ± 11.23	95.85 ± 2.43	190.6 ± 5.05
P32352	ERG2	105.56 ± 14.6	110.93 ± 14.97	211.25 ± 5.04
P32353	ERG3	422.12 ± 41.84	434.27 ± 30.95	502.72 ± 21.75
P54781	ERG5	301.25 ± 43.12	232.19 ± 15.94	420.38 ± 52.99
P25340	ERG4	94.81 ± 9.23	94.53 ± 5.75	180.77 ± 0.75
P35196	RER2	100.92 ± 11.4	100.32 ± 6.39	92.23 ± 3.85
Q12063	NUS1	151.58 ± 23.59	142.06 ± 0.21	127.52 ± 5.66

References to Supplementary Information

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