

Supplementary Table 1. Primers used in this study.

Name	Sequence 5'→3'
LmSTT3A_for	AACACCAGAACTTAGTTTCGACGGATTCTAGAACTAGTGGATCCCCCGG GATGCCGGCCAAGAACCAGCA
LmSTT3A/B_rev	TCAGGAACATCGTATGGGTACGCCTTGTGGTGCGTCTCCT
LmSTT3A/B_HA	TCGATCAGATGGACAAGCACAAGCAGCACAAGGAGACGCACCACAAGG CGTACCCATACGATGTTCTCTGA
LmSTT3B_for	AACACCAGAACTTAGTTTCGACGGATTCTAGAACTAGTGGATCCCCCGG GATGCTGCTCTTGTTCCTCTC
LmSTT3C_for	AACACCAGAACTTAGTTTCGACGGATTCTAGAACTAGTGGATCCCCCGG GATGCCCTCGCAAACCTCGTAG
LmSTT3C_rev	TCAGGAACATCGTATGGGTAGCGCCTGCTCCCGCTCCAAG
LmSTT3C_HA	ACTTCAAGGACCTTCTTGATCCCAGAACGACTTGGAGCGGGAGCAGGCG CTACCCATACGATGTTCTCTGA
LmSTT3D_for	AACACCAGAACTTAGTTTCGACGGATTCTAGAACTAGTGGATCCCCCGG GATGGGCAAGCGGAAGGGAAA
LmSTT3D_rev	TCAGGAACATCGTATGGGTACCGTCGGTCTCTCGCTTTCAC
LmSTT3D_HA	CGTACCAGGAGGAGTACATGCGCCGGATGCGTGAAAGCGAGAACCGAC GGTACCCATACGATGTTCTCTGA
CYC1_HA	GCGTGACATAACTAATTACATGACTCGAGGTCGACGGTATCGATAAGCT TGCACTGAGCAGCGTAATCTG

Supplementary Table 2. Yeast strains used in this study.

Strain	Genotype	Reference
YG543	<i>MATa ade2 leu2 ura3 his3 lys2 stt3-7</i>	(Spirig <i>et al.</i> , 1997)
SS328	<i>MATα ade2-101 his3D200 lys2-801 ura3-52</i>	(Vijayraghavan <i>et al.</i> , 1989)
BY4741	<i>MATa his3delta1 leu2delta0 met15delta0 ura3delta0</i>	Euroscarf
BY4742	<i>MATα his3delta1 leu2delta0 met15delta0 ura3delta0</i>	Euroscarf
Y03108	<i>MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 ALG3::kanMX4</i>	Euroscarf
Y01993	<i>MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 ALG9::kanMX4</i>	Euroscarf
Y05405	<i>MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 ALG12::kanMX4</i>	Euroscarf
Y24390	<i>MAT a/α his3Δ1/his3Δ1 leu2Δ0/leu2Δ0 lys2Δ0/LYS2 MET15/met15Δ0 ura3Δ0/ura3Δ0 STT3::kanMX4/STT3</i>	Euroscarf
YBS10	<i>MATα his3Δ1 leu2Δ0 lys2Δ0 met15Δ0 ura3Δ0 STT3::kanMX4 pSTT3</i>	this study
YBS11	<i>MAT a his3Δ1 leu2Δ0 lys2Δ0 met15Δ0 ura3Δ0 STT3::kanMX4 pSTT3</i>	this study
Y20242	<i>MAT a/α his3Δ1/his3Δ1 leu2Δ0/leu2Δ0 lys2Δ0/LYS2 MET15/met15Δ0 ura3Δ0/ura3Δ0 WBP1::kanMX4/WBP1</i>	Euroscarf
Y26770	<i>MAT a/α his3Δ1/his3Δ1 leu2Δ0/leu2Δ0 lys2Δ0/LYS2 MET15/met15Δ0 ura3Δ0/ura3Δ0 OST1::kanMX4/OST1</i>	Euroscarf
Y22359	<i>MAT a/α his3Δ1/his3Δ1 leu2Δ0/leu2Δ0 lys2Δ0/LYS2 MET15/met15Δ0 ura3Δ0/ura3Δ0 OST2::kanMX4/OST2</i>	Euroscarf
Y20730	<i>MAT a/α his3Δ1/his3Δ1 leu2Δ0/leu2Δ0 lys2Δ0/LYS2 MET15/met15Δ0 ura3Δ0/ura3Δ0 SWP1::kanMX4/SWP1</i>	Euroscarf
YG2052	<i>MAT a his3Δ1 leu2Δ0 ura3Δ0 STT3::kanMX4 pLmSTT3A</i>	this study
YG2054	<i>MAT a his3Δ1 leu2Δ0 ura3Δ0 STT3::kanMX4 pLmSTT3B</i>	this study
YG2057	<i>MATα his3Δ1 leu2Δ0 ura3Δ0 STT3::kanMX4 pLmSTT3D</i>	this study
YG2058	<i>MATa his3Δ1 leu2Δ0 ura3Δ0 STT3::kanMX4 pLmSTT3D</i>	this study
YG2072	<i>MATα his3Δ1 leu2Δ0 ura3Δ0 WBP1::kanMX4 pLmSTT3D</i>	this study
YG2073	<i>MATα his3Δ1 leu2Δ0 ura3Δ0 OST1::kanMX4 pLmSTT3D</i>	this study
YG2075	<i>MATα his3Δ1 leu2Δ0 ura3Δ0 OST2::kanMX4 pLmSTT3D</i>	this study
YG2080	<i>MATα his3Δ1 leu2Δ0 ura3Δ0 SWP1::kanMX4 pLmSTT3D</i>	this study
YG2082	<i>MATa his3Δ1 leu2Δ0 ura3Δ0 STT3::kanMX4 ALG12::kanMX4 pLmSTT3D</i>	this study
YG2084	<i>MATα his3Δ1 leu2Δ0 ura3Δ0 STT3::kanMX4 WBP1::kanMX4 pLmSTT3D</i>	this study
YG2099	<i>MATa his3Δ1 leu2Δ0 ura3Δ0 OST1::kanMX4 OST2::kanMX4 pLmSTT3D</i>	this study

Supplementary Table 3. Peptides containing N-glycosylation sequons identified by mass spectrometry.

Glycosylation site	Observed (m/z)	Observed (Da)	Predicted (Da)	Δ mass (Da)	Score	Expect	Rank	Sequence
ECM33_57*	976.9972	1951.9798	1951.9772	0.0025	38	0.047	1	L.DKISGCSTIVGNLTIT G.D
ECM33_83*	1064.5200	3190.5382	3190.5405	-0.0023	50	0.0031	1	G.DLGSAALASIQEIDG SLTIFNSSSLSSFSA.D
EXG2_50*	848.4091	1694.8036	1694.8039	-0.0004	44	0.01	1	K.FASYANDTITVK.G
GAS1_149**	1404.3256	4209.9550	4209.9543	0.0007	70	2.00E-05	1	K.TVVDTFANYTNVLG FFAGNEVTNNYTNTD ASAFVK.A
GAS3_269*	578.7707	1155.5268	1155.5295	-0.0028	30	0.018	1	Y.DKLNSTFE.D
PLB2_47*	848.4067	1694.7988	1694.7999	-0.0011	72	1.60E-05	1	R.NASGLSTAETDWLK. K
PLB2_193*	962.4853	1922.9561	1922.9585	-0.0024	65	8.8e-05	1	K.SIVNPGGSNLTYTIE R.W
PRY3_101*	884.4063	2650.1971	2650.1976	-0.0005	55	0.00058	1	K.YNYSNPGFSESTGHF TQVVK.S
SAG1_248*	723.3017	1444.5889	1444.5936	-0.0047	49	0.00087	1	N.DWWFPQSYN.D
SAG1_79*	1135.5703	2269.1260	2269.1325	-0.0065	104	9.60E-09	1	K.LLNSSQTATISLADG TEAFK.C
SAG1_79	1034.0333	2066.0520	2066.0531	-0.0011	133	1.20E-11	1	K.LLNSSQTATISLADG TEAFK.C
YJR1_99	918.4402	1834.8658	1834.8639	0.0020	89	2.9e-07	1	K.QFAFYTSPGFTVNNS R.S

* GlcNAc-modified asparagine. All methionines are oxidized and all cysteines are alkylated. Peptides previously identified (Schulz *et al*, manuscript submitted) are not detailed.

Supplementary Table 4. Relative glycosylation site occupancy in different yeast strains.

Peptides	WT+vec.	$\Delta stt3$ +D	$\Delta wbp1$ +D	$\Delta ost1$ +D	$\Delta ost1\Delta ost2$ +D	$\Delta stt3\Delta wbp1$ +D*	$\Delta ost2$ +D*	$\Delta swp1$ +D*
CRH2_28	1.00±0.00	1.00±0.00	1.00±0.00	1.00±0.00	1.00±0.00	1.00	1.00	1.00
CRH2_96	1.00±0.00	0.99±0.02	0.94±0.08	1.00±0.00	1.00±0.00	1.00	1.00	1.00
CRH2_233	1.00±0.00	0.96±0.04	0.95±0.04	0.94±0.06	0.98±0.03	0.98	1.00	1.00
CRH2_310	1.00±0.00	1.00±0.00	1.00±0.00	1.00±0.00	1.00±0.00	1.00	1.00	1.00
ECM33_83	1.00±0.00	1.00±0.00	1.00±0.00	1.00±0.00	1.00±0.00	1.00	1.00	1.00
ECM33_197	1.00±0.00	0.70±0.01	0.56±0.15	0.60±0.02	0.60±0.03	0.64	0.67	0.47
ECM33_210	1.00±0.00	0.96±0.02	0.94±0.04	0.99±0.02	0.97±0.01	0.99	0.97	0.97
ECM33_268	1.00±0.00	1.00±0.00	1.00±0.00	1.00±0.00	1.00±0.00	1.00	1.00	1.00
ECM33_329	1.00±0.00	0.67±0.06	0.60±0.13	0.76±0.01	0.75±0.02	0.68	0.73	0.74
GAS1_40	1.00±0.00	0.98±0.02	0.97±0.02	0.95±0.02	0.94±0.02	0.95	1.00	1.00
GAS1_57	1.00±0.00	0.97±0.03	0.97±0.06	0.98±0.02	1.00±0.00	0.86	1.00	1.00
GAS1_95	1.00±0.00	1.00±0.00	1.00±0.01	1.00±0.00	1.00±0.00	1.00	1.00	1.00
GAS1_149	1.00±0.00	1.00±0.00	1.00±0.00	1.00±0.00	1.00±0.00	0.97	1.00	1.00
GAS1_253	0.99±0.01	1.00±0.00	1.00±0.00	1.00±0.00	1.00±0.00	1.00	1.00	1.00
GAS3_201	1.00±0.00	1.00±0.00	1.00±0.00	1.00±0.00	1.00±0.00	1.00	1.00	1.00
GAS3_269	1.00±0.00	1.00±0.00	1.00±0.00	0.99±0.02	1.00±0.00	1.00	1.00	1.00
GAS3_350	1.00±0.00	1.00±0.00	1.00±0.00	1.00±0.00	1.00±0.00	1.00	1.00	1.00
GAS5_24	1.00±0.00	1.00±0.00	0.99±0.01	1.00±0.00	0.99±0.01	1.00	0.97	0.99
GAS5_60	1.00±0.00	1.00±0.00	1.00±0.00	1.00±0.00	1.00±0.00	1.00	1.00	1.00
GAS5_344	1.00±0.00	1.00±0.00	1.00±0.00	1.00±0.00	1.00±0.00	1.00	1.00	1.00
SAG1_79	0.72±0.04	1.00±0.00	0.00±0.00	1.00±0.00	1.00±0.00	0.99	0.98	1.00
SAG1_248	1.00±0.00	1.00±0.00	0.99±0.01	1.00±0.00	1.00±0.00	1.00	1.00	1.00
CRH1_177	1.00±0.00	1.00±0.00	0.98±0.03	0.98±0.03	1.00±0.00	1.00	0.96	0.97
PLB2_47	1.00±0.00	1.00±0.00	1.00±0.00	1.00±0.00	1.00±0.00	1.00	1.00	1.00
PRY3_101	1.00±0.00	1.00±0.00	1.00±0.00	1.00±0.00	1.00±0.00	1.00	1.00	1.00
CCW14_87	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00	0.00	0.00
CWP1_45	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00	0.00	0.00
TOS1_417	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00	0.00	0.00

The relative occupancy of a site given at the left is shown for wild-type strain (BY4742) with empty vector, and strains expressing LmSTT3D: $\Delta stt3$ (YG2057), $\Delta wbp1$ (YG2072), $\Delta ost1$ (YG2073), $\Delta ost1\Delta ost2$ (YG2099), $\Delta stt3\Delta wbp1$ (YG2084), $\Delta ost2$ (YG2075), and $\Delta swp1$ (YG2080). Values are given for the sites indicated and represent the mean of three independent measurements.* Single measurement.

Spirig, U., Glavas, M., Bodmer, D., Reiss, G., Burda, P., Lippuner, V., te Heesen, S., and Aebi, M. (1997). The STT3 protein is a component of the yeast oligosaccharyltransferase complex. *Mol. Gen. Genet.* 256, 628-637.

Vijayraghavan, U., Company, M., and Abelson, J. (1989). Isolation and characterization of pre-mRNA splicing mutants of *Saccharomyces cerevisiae*. *Genes Dev* 3, 1206-1216.