Supporting Information for

Highly predictive reprogramming of tRNA modifications is linked to selective expression of codon-biased genes

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Figure S1: Cytotoxicity dose-response curves. *S. cerevisiae* cultures were exposed to H_2O_2 , TBHP, ONOO⁻, γ -Rad, MMS, EMS, IMS, MNNG, and NMU, and cell survival quantified by a colony-forming assay

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Table S1. Toxicant-induced changes in tRNA modifications

Table S2. Average fold-change values for toxicant-induced changes in tRNA modifications

Table S3: MMS treatment induces up- or down-regulation of 694 of 2381 proteins independent of changes in mRNA levels – *provided as a separate Excel file*.

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Supporting References

Figure S1: Cytotoxicity dose-response curves. *S. cerevisiae* cultures were exposed to H₂O₂, TBHP, ONOO⁻, γ -Rad, MMS, EMS, IMS, MNNG, and NMU, and cell survival quantified by a colony-forming assay. Data represents mean \pm SD for three biological replicates. Doses producing 80% lethality (LD₈₀) were chosen for studying stress-induced changes in tRNA modification patterns.



Figure S2. Hierarchical clustering analysis of stress-induced changes in tRNA modification levels. The fold-change values from **Table S1** for tRNA modifications (rows) from cells exposed to LD_{80} doses of the 9 toxicants (columns) were analyzed by hierarchical clustering using the centroid linkage algorithm in Cluster 3.0 following log₂-transformation of the fold-change data and the heat map representation was produced using Java Treeview, as described elsewhere.¹ The three boxes below the heat map define color-coding used to identify the three treatment conditions: unexposed control, alkylating agent and oxidizing agent.



MMS – Methyl methanesulfonate EMS – Ethyl methanesulfonate IMS – Isopropyl methanesulfonate MNNG - *N*-methyl-*N*'-nitro-*N*-nitrosoguanidine NMU - *N*-nitro-*N*-methylurea <u>Oxidizing agents</u> H₂O₂ – Hydrogen peroxide ONOO – Peroxynitrite GAMMA – γ-radiation TBHP – *t*-butyl hydroperoxide **Figure S3.** Loss of Trm140 confers sensitivity to the S_N^2 alkylating agents, MMS and EMS. Cultures of wild-type and $\Delta trm140$ mutant *S. cerevisiae* were exposed to an LD₈₀ dose of an alkylating agent determined for wild-type cells and cell survival was quantified by a colony-forming assay. Data represent mean ±SD for three biological replicates, with differences between wild-type and mutant cells determined by Student's t-test.



Figure S4: An analysis of statistically significant use of ACC and ACT codons in genes in Gene Ontology categories reveals highly significant enrichment of translation functions in genes upregulated by MMS and in intermediary metabolism categories in genes down-regulated by MMS.



Table S1. Toxicant-induced changes in tRNA modifications. Data represent fold-change values for 5 biological replicates of yeast exposed to LD_{80} doses of 9 toxicants (1 h, 37 °C). Each of the 5 datasets is derived from exposure to 9 different toxicants and 3 unexposed controls. Fold-change data were calculated by dividing the MS signal intensity for each ribonucleoside in each exposure sample by the average MS signal intensity for the 3 unexposed controls.

Set 1												
Ribonuc-	Ctrl	Ctrl	Ctrl	EMS	γ-Rad	H_2O_2	IMS	MMS	MNNG	NMU	ONOO	TBHP
leoside	1-1	1-2	1-3	1	1	1	1	1	1	1	1	1
D	1.023	1.003	0.973	1.298	1.098	1.048	1.103	0.996	1.030	1.071	1.073	1.092
Y	1.011	1.001	0.988	1.225	1.092	1.035	1.079	0.972	1.024	1.054	1.085	1.082
ncm ⁵ U	1.034	1.055	0.911	1.354	1.557	1.412	1.018	0.927	1.075	0.987	1.365	1.481
Ι	1.042	0.942	1.015	0.842	1.018	0.965	1.091	0.985	1.012	1.074	1.002	1.022
m ⁵ U	1.054	0.959	0.988	1.158	1.043	1.007	1.078	0.989	1.029	1.028	1.040	1.032
Gm	0.964	1.000	1.036	0.803	1.031	1.009	1.556	1.136	1.285	1.120	1.026	1.055
Um	1.012	0.977	1.011	1.278	1.025	0.891	1.262	0.977	1.104	1.188	1.604	1.257
m ⁵ C	1.034	0.971	0.995	1.112	1.243	1.282	1.094	1.021	1.045	1.055	1.313	1.294
m ³ C	1.019	1.004	0.976	1.703	1.105	1.043	1.190	1.515	1.088	1.157	1.074	1.083
Cm	1.028	0.950	1.022	0.828	1.048	1.207	1.099	0.993	0.996	1.063	1.073	1.319
mcm ⁵ U	1.026	0.968	1.006	1.125	1.068	1.083	1.158	1.052	1.074	1.164	1.043	1.128
m ¹ G	0.974	1.008	1.018	0.853	1.059	1.020	1.095	1.074	0.969	1.040	1.041	1.075
m ² G	0.962	0.992	1.046	0.718	1.035	1.012	1.183	1.098	1.074	1.159	0.994	1.044
ac ⁴ C	1.022	0.991	0.988	1.312	1.085	1.024	1.061	0.956	0.973	0.969	1.113	1.086
t ⁶ A	0.970	1.016	1.014	0.929	1.044	1.171	1.164	1.056	1.075	1.138	1.000	1.064
mcm ⁵ s ² U	1.008	1.005	0.987	1.053	1.045	1.043	1.054	1.031	1.051	1.043	1.014	1.094
m ⁷ G	1.065	0.985	0.949	1.214	1.105	1.034	1.098	1.433	0.900	1.289	1.106	1.087
m ¹ I	0.958	1.003	1.039	0.670	1.065	0.791	0.988	0.902	0.838	0.882	1.027	1.075
Am	1.008	0.991	1.001	0.989	1.063	0.972	1.666	0.982	1.526	1.604	1.619	1.065
m ² ₂ G	0.961	1.003	1.035	0.708	1.349	1.440	1.159	1.005	1.047	1.126	0.997	1.042
i6A	1.044	0.991	0.966	1.429	1.125	1.059	1.122	1.012	1.010	1.053	1.130	1.145
yW	1.456	0.712	0.832	1.005	0.743	0.715	1.313	0.612	1.429	1.244	1.624	0.720
m ¹ A	1.013	1.007	0.979	1.106	1.086	1.046	1.103	1.002	0.998	1.072	1.071	1.082

Set 2												
Ribonuc-	Ctrl	Ctrl	Ctrl	EMS	γ-Rad	H_2O_2	IMS	MMS	MNNG	NMU	ONOO	TBHP
leoside	2-1	2-2	2-3	2	2	2	2	2	2	2	2	2
D	1.016	1.065	0.918	1.065	1.128	1.093	1.249	1.131	1.023	1.031	1.016	1.011
Y	1.098	1.006	0.895	0.941	1.074	1.082	1.133	1.083	0.953	0.953	1.063	0.987
ncm ⁵ U	0.982	0.897	1.121	0.765	1.071	1.274	0.990	0.949	0.836	1.057	1.250	1.112
Ι	1.111	0.972	0.917	0.994	1.034	1.002	1.186	1.052	0.899	0.947	1.006	0.932
m ⁵ U	1.089	0.997	0.914	1.049	1.129	1.072	1.241	1.097	0.995	1.041	1.052	1.099
Gm	0.945	1.063	0.992	1.184	1.037	1.062	1.768	1.149	1.250	1.029	0.922	0.981
Um	1.089	0.988	0.924	0.814	0.704	1.129	0.985	0.823	0.868	0.997	1.142	0.834
m ⁵ C	1.029	1.033	0.937	0.933	1.319	1.366	1.121	0.958	0.870	0.942	1.226	1.224
m ³ C	0.993	1.052	0.954	1.608	1.028	1.056	1.375	1.763	1.141	1.194	0.962	1.015
Cm	1.052	1.028	0.920	0.915	1.015	1.218	1.182	1.027	0.850	0.894	0.910	1.227
mcm ⁵ U	0.969	1.051	0.979	1.207	1.113	1.043	0.986	1.103	0.973	1.320	0.985	0.960
m ¹ G	0.971	1.079	0.950	1.045	1.084	1.067	1.206	1.092	0.958	0.995	0.972	1.014
m ² G	0.960	1.063	0.977	1.081	1.085	1.070	1.347	1.211	1.063	1.107	0.914	1.017

ac ⁴ C	1.013	1.050	0.936	1.000	1.068	1.040	1.160	1.059	0.929	0.988	0.984	0.979
t ⁶ A	0.996	1.036	0.968	1.132	1.040	1.910	1.296	1.215	1.001	1.129	0.955	1.031
mcm ⁵ s ² U	0.986	1.079	0.935	1.102	1.005	1.448	1.701	1.259	0.946	1.053	1.175	1.166
m ⁷ G	1.004	1.050	0.946	1.487	1.073	1.076	1.285	1.802	0.999	1.461	0.979	1.021
m ¹ I	0.958	1.151	0.891	0.787	0.988	0.676	0.949	1.022	0.717	0.934	0.916	0.827
Am	0.934	1.076	0.990	1.056	1.045	1.036	1.526	1.157	1.618	1.611	1.530	0.980
m ² ₂ G	0.960	1.052	0.988	1.114	1.581	1.757	1.294	1.157	1.044	1.085	0.948	1.035
i6A	0.914	1.104	0.982	1.157	1.067	1.250	1.349	1.151	1.036	1.155	1.008	1.029
yW	1.037	0.988	0.975	0.802	1.026	1.170	0.738	0.378	0.627	0.674	1.214	0.929
m ¹ A	0.984	1.084	0.931	1.084	1.096	1.092	1.318	1.163	1.010	1.070	0.986	1.022
						Set 3						
Ribonuc-	Ctrl	Ctrl	Ctrl	EMS	γ-Rad	H_2O_2	IMS	MMS	MNNG	NMU	ONOO	TBHF
leosides	3-1	3-2	3-3	3	3	3	3	3	3	3	3	3
D	1.069	0.975	0.956	1.026	0.898	0.963	1.084	0.998	1.072	1.040	0.841	0.875
Y	1.084	0.943	0.974	1.044	0.892	0.930	1.069	0.976	1.134	1.012	0.888	0.880
ncm ⁵ U	0.982	1.112	0.905	1.165	1.086	0.951	1.095	1.014	1.118	1.138	0.817	1.035
Ι	1.062	0.940	0.998	0.989	0.877	0.971	0.996	0.925	1.034	0.941	0.885	0.802
m ⁵ U	0.989	1.015	0.996	1.007	0.935	0.976	1.154	1.058	1.125	0.999	0.994	0.994
Gm	0.965	1.043	0.991	1.268	0.991	0.994	1.681	0.950	1.307	1.039	0.955	0.960
Um	1.085	0.816	1.100	0.675	1.494	0.642	1.288	0.812	1.110	1.114	1.524	1.097
m ⁵ C	0.982	1.029	0.989	1.069	1.257	1.310	1.095	1.064	1.122	1.038	1.268	1.229
m ³ C	0.962	1.041	0.997	1.752	0.970	0.984	1.300	1.493	1.218	1.142	0.948	0.963
Cm	1.011	0.986	1.003	0.967	0.933	1.103	1.009	0.923	0.992	0.944	0.883	1.138
mcm ⁵ U	0.798	1.164	1.038	1.529	1.211	1.348	1.408	1.284	1.480	1.241	1.188	1.247
m ¹ G	1.000	1.028	0.972	0.988	0.957	0.952	1.033	0.962	1.045	0.972	0.874	0.908
m ² G	1.001	1.011	0.988	1.179	0.992	0.991	1.176	1.064	1.209	1.141	0.961	0.974
ac ⁴ C	1.069	1.025	0.906	1.066	0.961	0.946	1.071	0.970	1.043	1.016	1.016	0.888
t ⁶ A	0.956	1.029	1.015	1.180	0.998	1.586	1.165	1.115	1.164	1.113	1.022	0.990
mcm ⁵ s ² U	0.840	1.059	1.100	1.267	1.209	1.341	1.392	1.462	1.550	1.065	1.249	1.155
m ⁷ G	1.006	1.011	0.983	1.454	0.909	0.940	1.116	1.812	1.190	1.505	0.872	0.893
$m^{1}I$	1.103	0.972	0.925	0.885	0.861	0.813	0.764	0.679	0.852	0.777	0.879	0.669
Am	0.992	1.025	0.983	1.103	0.995	0.991	1.640	1.005	1.725	1.663	1.396	0.956
$m^2 G$	0.976	1.044	0.980	1.143	1.445	1.449	1.168	1.061	1.162	1.066	0.987	0.991
i6A	0.957	1.061	0.981	1.258	1.339	1.181	1.291	1.091	1.092	1.042	1.172	1.117
yW	1.103	1.002	0.894	0.506	0.977	0.912	0.558	0.512	0.631	0.588	0.829	0.772
m ¹ A	0.999	1.035	0.967	1.113	1.003	0.995	1.140	1.051	1.217	1.092	0.997	0.950
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Set 4												
Ribonuc-	Ctrl	Ctrl	Ctrl	EMS	γ-Rad	H_2O_2	IMS	MMS	MNNG	NMU	ONOO	TBHP
leosides	4-1	4-2	4-3	4	4	4	4	4	4	4	4	4
D	0.976	1.011	1.013	1.092	1.038	1.176	1.230	1.104	1.074	1.095	1.056	1.165
Y	0.973	1.016	1.011	1.117	1.033	1.273	1.259	1.063	1.097	1.084	1.089	1.166
ncm ⁵ U	1.050	0.995	0.954	1.032	1.042	1.440	1.100	0.898	0.939	1.128	1.187	1.198
Ι	0.995	1.055	0.950	1.176	1.098	1.267	1.197	1.085	1.051	1.187	1.145	1.223
m ⁵ U	0.686	1.362	0.952	0.918	0.732	0.891	1.036	0.976	1.052	1.124	1.474	0.909
Gm	0.993	1.008	0.999	1.237	1.036	1.069	1.519	1.072	1.264	1.121	1.047	1.091
Um	0.960	1.032	1.007	1.190	1.106	0.958	1.290	1.052	1.265	1.275	1.572	1.412
m ⁵ C	0.984	0.998	1.017	1.101	1.169	1.614	1.172	1.031	1.074	1.206	1.360	1.391

m ³ C	0.948	1.027	1.025	1.546	0.993	1.002	1.252	1.657	1.188	1.223	0.995	1.071
Cm	0.959	0.982	1.059	1.043	0.954	1.386	1.137	1.028	1.099	1.164	1.082	1.384
mcm ⁵ U	0.914	1.034	1.052	1.204	1.093	1.061	1.224	1.217	1.132	1.200	1.039	1.130
m ¹ G	0.979	1.006	1.015	1.029	1.085	1.127	1.059	1.006	1.023	1.062	1.058	1.158
m ² G	0.990	1.012	0.998	1.080	1.048	1.141	1.167	1.046	1.086	1.138	1.063	1.114
ac ⁴ C	0.972	1.015	1.013	1.072	1.044	1.351	1.133	0.985	1.026	1.184	1.163	1.166
t ⁶ A	1.395	0.845	0.760	0.803	0.936	1.377	0.819	0.814	0.933	0.819	0.952	0.682
mcm ⁵ s ² U	0.954	1.027	1.020	1.014	0.959	0.822	1.023	0.999	1.085	1.208	0.878	0.825
m ⁷ G	0.896	1.027	1.077	1.454	0.888	1.084	1.189	1.720	1.141	1.635	0.987	1.082
m ¹ I	0.967	1.003	1.030	0.935	1.022	0.775	0.951	0.955	0.913	0.911	0.982	0.926
Am	1.095	0.979	0.926	0.977	1.017	0.941	1.380	0.992	1.640	1.550	1.612	0.926
m ² ₂ G	1.143	0.957	0.900	0.977	1.364	1.516	1.019	0.924	1.012	1.009	1.117	0.925
i6A	1.442	0.794	0.765	0.870	1.024	1.601	0.914	0.720	0.784	0.991	1.544	1.205
yW	1.056	1.038	0.906	0.764	1.103	1.268	0.622	0.644	0.604	0.599	1.158	1.088
m ¹ A	0.968	1.022	1.009	1.119	1.040	1.053	1.174	1.116	1.078	1.156	1.011	1.090

Set 5												
Ribonuc-	Ctrl	Ctrl	Ctrl	EMS	γ-Rad	H_2O_2	IMS	MMS	MNNG	NMU	ONOO	TBHP
leosides	5-1	5-2	5-3	5	5	5	5	5	5	5	5	5
D	1.156	0.948	0.896	0.994	0.946	1.065	1.077	0.945	0.929	0.950	1.008	1.008
Y	1.171	0.939	0.890	1.005	0.993	1.160	1.071	0.938	0.938	0.938	1.084	1.090
ncm ⁵ U	0.992	0.995	1.013	1.018	1.056	1.377	1.109	0.947	1.050	1.138	1.178	1.089
Ι	1.248	0.885	0.868	0.977	0.979	1.200	1.078	0.897	1.028	1.049	1.159	1.094
m ⁵ U	1.016	0.976	1.008	1.487	0.968	1.148	1.144	1.044	1.044	1.095	0.975	1.056
Gm	1.102	0.953	0.945	1.108	0.960	0.969	1.415	0.968	1.153	0.987	0.952	0.962
Um	0.868	0.998	1.134	1.083	0.945	0.902	1.377	1.020	1.264	1.197	0.682	1.196
m ⁵ C	1.117	0.934	0.948	1.028	1.081	1.474	1.098	0.935	0.997	1.072	1.278	1.229
m ³ C	1.115	0.946	0.939	1.358	0.956	1.025	1.118	1.482	1.009	1.046	0.954	0.926
Cm	1.080	0.956	0.964	0.980	0.966	1.230	1.049	0.949	0.969	1.004	0.999	1.248
mcm ⁵ U	0.912	1.011	1.077	1.057	0.927	0.970	1.152	1.130	1.221	1.107	0.857	0.932
m ¹ G	1.085	0.963	0.952	0.927	0.993	1.044	0.963	0.901	0.881	0.901	0.989	0.999
m ² G	1.089	0.955	0.957	1.017	0.974	1.030	1.079	0.989	1.023	1.036	0.968	0.992
ac ⁴ C	1.092	0.955	0.953	0.912	0.941	0.895	0.985	0.937	0.926	0.922	0.920	0.941
t ⁶ A	1.101	0.944	0.955	0.811	0.982	0.827	0.814	0.823	0.789	0.777	0.944	0.879
mcm ⁵ s ² U	0.526	1.167	1.306	1.234	0.929	0.748	1.245	1.112	1.406	1.309	0.846	1.035
m ⁷ G	1.048	0.991	0.961	1.364	0.991	1.058	1.058	1.479	0.949	1.368	0.987	1.016
m ¹ I	1.102	0.944	0.954	0.835	0.947	0.696	0.851	0.820	0.806	0.799	0.921	0.803
Am	1.034	0.978	0.989	1.004	0.977	0.961	1.451	1.002	1.638	1.593	1.557	0.959
m ² ₂ G	0.985	0.992	1.023	1.058	1.341	1.616	1.105	1.003	1.105	1.136	1.140	1.112
i6A	0.946	1.001	1.053	1.097	1.109	1.695	1.183	0.965	1.112	1.244	1.581	1.207
yW	1.102	0.932	0.965	0.662	1.046	1.121	0.598	0.629	0.590	0.586	1.057	0.995
m ¹ A	0.894	1.071	1.035	1.005	1.075	1.016	0.986	0.978	0.996	1.035	1.021	0.965

Table S2. Average fold-change values for toxicant-induced changes in tRNA modifications. Data in the table were derived by calculating the average value for the fold-change data in **Table S1** for each toxicant (LD₈₀ dose). Those data deemed to be significantly different from the average value for the unexposed controls (**Table S1**) by Student's t-test (p < 0.05) are highlighted in yellow.

		Oxidizir	ng agent	ts	Alkylating agents						
Nucleoside	TBHP	γ-Rad	H_2O_2	ONOO ⁻	MMS	MNNG	NMU	IMS	EMS		
D	1.030	1.021	1.069	0.999	1.035	1.026	1.037	1.149	1.095		
Y	1.041	1.017	1.096	1.042	1.007	1.029	1.008	1.122	1.066		
ncm ⁵ U	1.183	1.162	1.291	1.159	0.947	1.004	1.090	1.062	1.067		
Ι	1.015	1.001	1.081	1.039	0.989	1.005	1.039	1.110	0.996		
m ⁵ U	1.018	0.961	1.019	1.107	1.033	1.049	1.057	1.131	1.124		
Gm	1.010	1.011	1.021	0.981	1.055	1.252	1.059	1.588	1.120		
Um	1.159	1.055	0.905	1.305	0.937	1.122	1.154	1.240	1.008		
m ⁵ C	1.273	1.214	1.409	1.289	1.002	1.021	1.063	1.116	1.049		
m ³ C	1.012	1.010	1.022	0.987	1.582	1.129	1.152	1.247	1.593		
Cm	1.263	0.983	1.229	0.989	0.984	0.981	1.014	1.095	0.947		
mcm ⁵ U	1.079	1.082	1.101	1.022	1.157	1.176	1.207	1.185	1.224		
m ¹ G	1.031	1.036	1.042	0.987	1.007	0.975	0.994	1.071	0.969		
m ² G	1.028	1.027	1.049	0.980	1.081	1.091	1.116	1.191	1.015		
ac ⁴ C	1.012	1.020	1.051	1.039	0.981	0.979	1.016	1.082	1.072		
t ⁶ A	0.929	1.000	1.374	0.975	1.005	0.992	0.995	1.051	0.971		
mcm ⁵ s ² U	1.055	1.029	1.080	1.032	1.173	1.208	1.135	1.283	1.134		
m ⁷ G	1.020	0.993	1.038	0.986	1.649	1.036	1.452	1.149	1.395		
m ¹ I	0.860	0.977	0.750	0.945	0.876	0.825	0.860	0.901	0.822		
Am	0.977	1.019	0.980	1.543	1.027	1.629	1.604	1.533	1.026		
$m^2 G$	1.021	1.416	1.556	1.038	1.030	1.074	1.084	1.149	1.000		
i6A	1.140	1.133	1.357	1.287	0.988	1.007	1.097	1.172	1.162		
yW	0.901	0.979	1.037	1.176	0.555	0.776	0.738	0.766	0.748		
m ¹ A	1.022	1.060	1.040	1.017	1.062	1.060	1.085	1.144	1.085		

Table S3: MMS treatment induces up- or down-regulation of 694 of 2381 proteins independent of changes in mRNA levels. *PROVIDED AS A SEPARATE EXCEL DOCUMENT*.

			0/	Memorane ana ceu wali proteins
Gene	# Amino	# The	% The	Gene function
OFD1	acids	Inr	1 hr	Cellerell metric OFD1
SEDI	1529	422	29.2	Cell wall protein SEDI
FLOI	1538	432	28.1	Flocculation protein FLOI
FLO9	1323	356	26.9	Flocculation protein FLO9
DAN4	1162	312	26.9	Cell wall protein DAN4
SVS1	261	65	24.9	Protein SVS1 (VO_4^{-5}) resistance, cell wall protein) ²
MUC1	1368	338	24.7	Flocculation protein FLO11
FLO5	1076	257	23.9	Flocculation protein FLO5
SRL1	211	49	23.2	Cell wall protein SRL1
AGA1	726	156	21.5	A-agglutinin cell wall anchorage subunit
FIT3	205	44	21.5	Facilitator of iron transport 3 (outer cell wall)
WSC4	606	125	20.6	Cell wall integrity and stress response component 4
FIT2	154	30	19.5	Facilitator of iron transport 2 (outer cell wall)
FIT1	529	103	19.5	Facilitator of iron transport 1 (outer cell wall)
CCW12	134	26	19.4	Covalently-linked cell wall protein 12
PRY3	882	160	18.1	Cell wall protein PRY3
FIG2	1610	291	18.1	Factor-induced gene 2 protein (cell wall integrity)
HSP150	388	65	16.8	Cell wall mannoprotein HSP150
FLO10	1170	194	16.6	Flocculation protein FLO10
DSE2	326	54	16.6	Protein DSE2 (GPI-anchored cell wall protein)
SPI1	149	24	16.1	Uncharacterized cell wall protein SPI1
KRE9	277	42	15.2	Cell wall synthesis protein KRE9
SCW11	543	80	14.7	Cell wall glycanase SCW11 ³
WSC3	557	82	14.7	Cell wall integrity and stress response component 3
PIR1	342	50	14.6	Cell wall mannoprotein PIR1
CTS1	563	81	14.4	Endochitinase (outer cell wall)
COX14	71	10	14.1	Cytochrome c oxidase assembly protein COX14 (mitochondrial membrane)
DAN1	299	41	137	Cell wall protein DAN1
SAG1	651	89	13.7	Alpha-agglutinin (cell surface glycoprotein)
KNH1	269	36	13.4	Cell wall synthesis protein KNH1
PRM7	116	15	12.9	Pheromone-regulated protein PRM7 (membrane protein)
AGA2	88	11	12.5	A-agglutinin-binding subunit
SOM1	75	9	12.0	Protein SOM1 (mitochondrial inner membrane)
CRH1	508	60	11.8	Probable glycosidase CRH1 (GPI-anchored cell wall protein)
HKR1	1803	213	11.0	Signaling mucin HKR1 (plasma membrane)
SLG1	379	44	11.6	Protein SI G1 (cell membrane, stress response signaling)
PAU6	121	14	11.6	Seringuperin-6 (stress response endoplasmic reticulum)
ECM19	113	13	11.0	Protein FCM19 (mitochondrial membrane)
SUN4	421	48	11.5	Septation protein SUN4 (cell wall septation)
FRG28	149	17	11.4	Ergosterol biosynthetic protein 28 (endonlasmic reticulum scaffold protein)
NUP/2	<u>1</u> +) <u>/</u> 21	<u> </u>	11.4	Nucleonorin NIIP42
MKC7	507	67	11.4	Aspartia protainase MKC7 (GPL anchorad call wall protain)
ATD15	67	7	11.2	ATD synthese subunit angilon, mitachandrial membrana
AIPIS	03	/	11.1	A 1 r synthase subunit epsiton, mitochondrial membrane

 Table S4: Proteins with threonine content >10% are membrane- and cell wall-related proteins.

 Mambrane and cell wall proteins.

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CIS3	228	25	11	Cell wall mannoprotein CIS3
NUP1	1077	118	11	Nucleoporin NUP1
YPS1	570	62	10.9	Aspartic proteinase 3 (GPI-anchored plasma memb. protein)
DAN3	121	13	10.7	Cell wall protein DAN3
TIR4	488	52	10.7	Cell wall protein TIR4
SPS100	327	35	10.7	Sporulation-specific wall maturation protein
PAU1	121	13	10.7	Seripauperin-1/14 (stress response, endoplasmic reticulum)
PAU4	121	13	10.7	Seripauperin-4 (stress response, endoplasmic reticulum)
QRI5	112	12	10.7	Mitochondrial mRNA-processing protein COX24 (inner membrane)
NUP57	542	58	10.7	Nucleoporin NUP57
PAU2	121	13	10.7	Seripauperin-2 (stress response, endoplasmic reticulum)
DAN2	125	13	10.4	Cell wall protein DAN2
STE2	432	45	10.4	Pheromone alpha factor receptor
PAU3	125	13	10.4	Seripauperin-3 (stress response, endoplasmic reticulum)
SCW10	390	40	10.3	Probable family 17 glucosidase SCW10 (cell wall glycanase)
MFA2	39	4	10.3	Mating hormone A-factor 2 (cell membrane protein)
YPS3	509	52	10.2	Aspartic proteinase yapsin-3 (GPI-anchored plasma membrane protein)
PRM2	657	67	10.2	Pheromone-regulated membrane protein 3
UTR2	468	47	10	Probable glycosidase CRH2 (GPI-anchored cell wall protein)

]	Proteins	with t	hreon	ine content >10%, but not related to the membrane or cell wall
Gene	# Amino acids	# Thr	% Thr	Gene function
DDR48	395	105	26.6	Stress protein DDR48
BSC1	329	76	23.1	Bypass of stop codon protein 1
ARG80	178	24	13.5	Arginine metabolism regulation protein I
RPA14	138	18	13.0	DNA-directed RNA polymerase I subunit RPA14
MSB2	1307	165	12.6	Protein MSB2
MRPL49	162	20	12.3	54S ribosomal protein L49, mitochondrial
RPS27A	83	10	12.0	40S ribosomal protein S27-A
RPS27B	83	10	12.0	40S ribosomal protein S27-B
YPS5	166	20	12.0	Putative uncharacterized protein YGL259W
PBP4	186	22	11.8	Protein PBP4 (cytoplasmic stress granule)
ABM1	124	14	11.3	Aberrant microtubules protein 1
RNH203	111	12	10.8	Ribonuclease H2 subunit C
WHI5	296	32	10.8	G1-specific transcriptional repressor WHI5
RPL22A	122	13	10.7	60S ribosomal protein L22-A
SRB6	122	13	10.7	Mediator of RNA polymerase II transcription subunit 22
BAR1	588	62	10.5	Barrierpepsin
CTK3	297	31	10.4	CTD kinase subunit gamma
MED8	224	23	10.3	Mediator of RNA polymerase II transcription subunit 8
TAF14	245	25	10.2	Transcription initiation factor TFIID subunit 14
MED6	296	30	10.1	Mediator of RNA polymerase II transcription subunit 6
RPL37B	89	9	10.1	60S ribosomal protein L37-B
TUP1	714	72	10.1	General transcriptional corepressor TUP1
NRM1	250	25	10.0	Transcription factor NRM1

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