

SUPPLEMENTARY INFORMATION

Optimization of Statistical Methods Impact on Quantitative Proteomics Data

Anna Pursiheimo^{1,2,‡}, Anni P. Vehmas^{1,‡}, Saira Afzal^{1,‡}, Tomi Suomi^{1,3}, Thaman Chand¹, Leena Strauss⁴,
Matti Poutanen⁴, Anne Rokka¹, Garry L. Corthals^{1,5} *, Laura L. Elo^{1,2} *

¹ Turku Centre for Biotechnology, University of Turku and Åbo Akademi University, Tykistökatu 6, FI-20520 Turku, Finland

² Department of Mathematics and Statistics, University of Turku, FI-20014 Turku, Finland

³ Department of Information Technology, University of Turku, FI-20014 Turku, Finland

⁴ Department of Physiology and Turku Center for Disease Modeling, Institute of Biomedicine, University of Turku, Kiinamylynkatu 10, FI-20520 Turku, Finland

⁵ Van't Hoff Institute for Molecular Sciences, University of Amsterdam, P.O. Box 94157, 1090 GD Amsterdam, The Netherlands

[‡] Equal contribution

* To whom correspondence should be addressed:

Laura L. Elo, Tel. +358 2 333 8009, Fax +358 2 251 8808; Email: laliel@utu.fi

Garry Corthals, Tel. +31205255406; Email: corthals@uva.nl

Supplementary Table 1. Sensitivity and specificity of the statistical methods in the yeast spiked data.

DataSet	SAM			t-test			LIMMA			RP			ROTS			MSstats		
	Ups ^a	Non-Ups ^b	AUC ^c	Ups	Non-Ups	AUC	Ups	Non-Ups	AUC	Ups	Non-Ups	AUC	Ups	Non-Ups	AUC	Ups	Non-Ups	AUC
2vs4	0	5	0,503	0	0	0,676	0	0	0,758	1	4	0,887	0	0	0,753	30	400	0,606
25vs50	40	85	0,899	0	0	0,877	0	0	0,929	1	15	0,914	1	8	0,933	46	494	0,836
10vs25	45	351	0,954	37	28	0,949	43	66	0,964	9	12	0,957	43	71	0,975	46	507	0,930
4vs10	45	347	0,936	1	29	0,876	44	133	0,938	31	22	0,973	44	118	0,977	46	574	0,905
2vs10	45	362	0,924	42	83	0,975	45	145	0,986	36	25	0,976	45	127	0,991	46	561	0,904
10vs50	45	564	0,944	43	134	0,957	45	249	0,976	19	30	0,947	45	318	0,981	46	605	0,917
4vs25	46	687	0,889	46	419	0,883	46	472	0,944	36	34	0,968	45	190	0,989	46	719	0,810
2vs25	46	674	0,851	46	437	0,986	46	487	0,992	35	36	0,966	46	487	0,993	46	721	0,810
4vs50	46	744	0,751	46	537	0,876	46	590	0,941	35	37	0,968	46	453	0,985	46	768	0,769
2vs50	46	762	0,751	46	558	0,994	46	596	0,998	35	33	0,967	46	530	0,996	46	742	0,778
Median	45	463	0,894	42	108	0,916	45	197	0,954	33	27	0,967	45	158	0,983	46	590	0,823

^a The number of detected UPS1 proteins (true positives).

The total number of UPS1 proteins in the evaluation was 46.

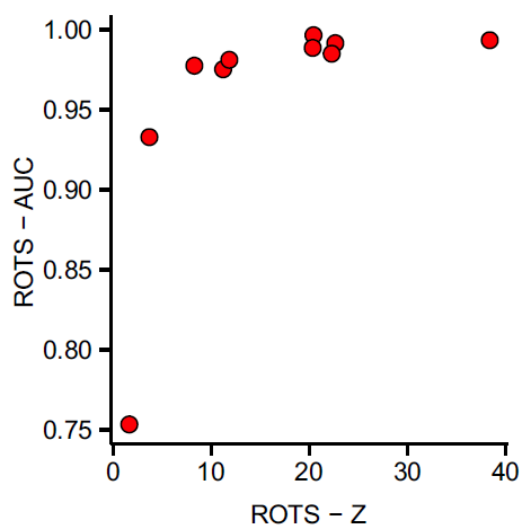
^b The number of detected non-UPS1 proteins (false positives).

The total number of non-UPS1 proteins in the evaluation was 898.

^c The observed area under the receiver operating characteristic curve.

Supplementary Table 2. The optimized ROTS parameters and the observed reproducibility values in the spiked yeast comparisons.

DataSet	α_0^a	α_1^a	k^b	R^c	Z^c	AUC ^d
2vs4	0,02	1	250	0,56	1,66	0,753
25vs50	0,22	1	180	0,72	3,70	0,933
10vs25	0,32	1	40	0,87	11,21	0,975
4vs10	0,36	1	110	0,85	8,28	0,977
2vs10	0,38	1	40	0,94	22,63	0,991
10vs50	0,28	1	60	0,87	11,84	0,981
4vs25	0,80	1	55	0,95	20,34	0,989
2vs25	0,17	1	45	0,96	38,36	0,993
4vs50	0,42	1	80	0,94	22,27	0,985
2vs50	0,30	1	55	0,95	20,41	0,996



^a The optimized parameters that specify the ROTS test statistic.

^b The optimized top list size.

^c The observed reproducibility value R and the corresponding reproducibility Z -score.

^d The observed area under the receiver operating characteristic curve.

Supplementary Table 3. Median sensitivity and specificity of the five statistical methods in the yeast spiked data using different normalization approaches.

Method	SAM		t-test		LIMMA		RP		ROTS	
	Ups ^a	Non-Ups ^b	Ups	Non-Ups	Ups	Non-Ups	Ups	Non-Ups	Ups	Non-Ups
Median	45	463	42	108	45	197	33	27	45	158
Progenesis	45	575	44	592	45	707	33	28	44	735
None	45	621	16	701	42	827	33	28	42	793

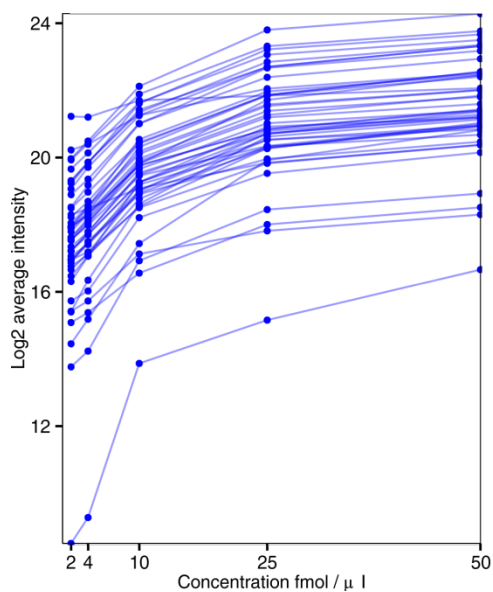
^a The number of detected UPS1 proteins (true positives).

The total number of UPS1 proteins in the evaluation was 46.

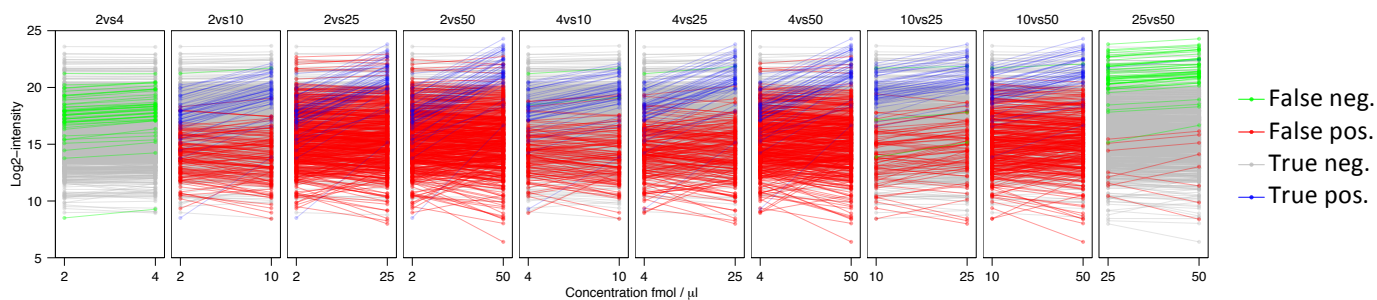
^b The number of detected non-UPS1 proteins (false positives).

The total number of non-UPS1 proteins in the evaluation was 898.

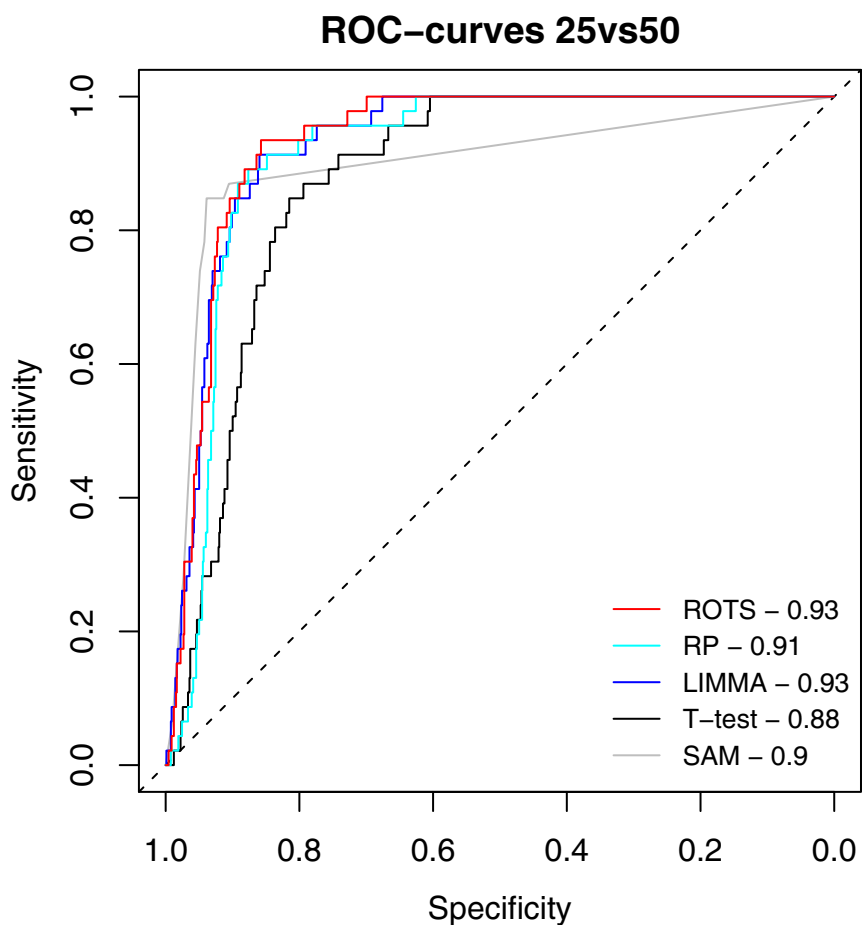
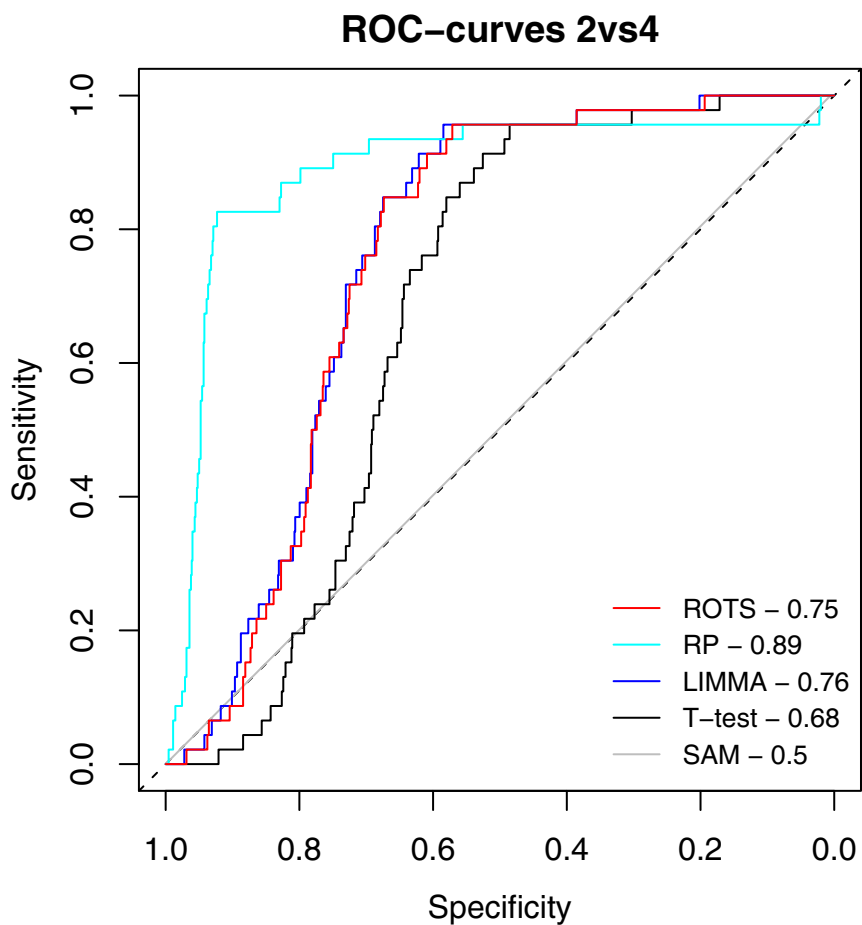
Supplementary Figure 1. The average log₂ replicate intensity of the 46 UPS1 proteins as a function of different concentrations of the UPS1 proteins in the yeast spiked data.



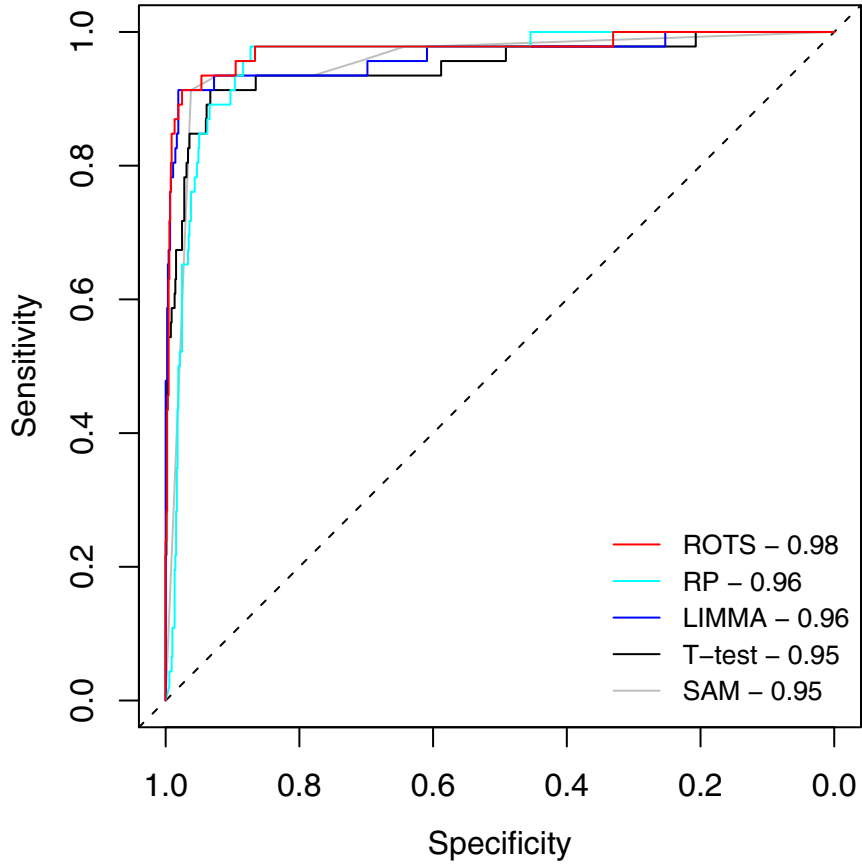
Supplementary Figure 2. The average log₂ replicate intensity of the ROTS detections from all the comparisons. Red and blue lines indicate the proteins regarded as significant by ROTS. Blue lines are true positives and red lines are false positives. Gray and green lines indicate the proteins regarded as non-significant by ROTS. Gray lines are true negatives and green lines are false negatives.



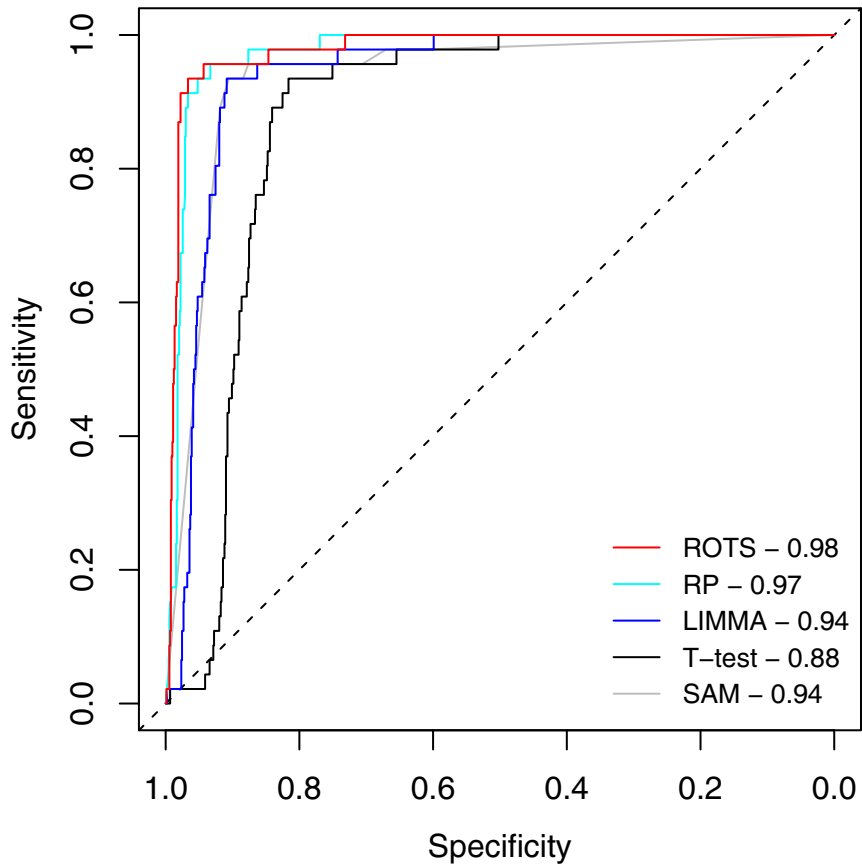
Supplementary Figure 3. Receiver operating characteristic (ROC) curves in the spiked yeast datasets together with the area under the curve (AUC) for each method.



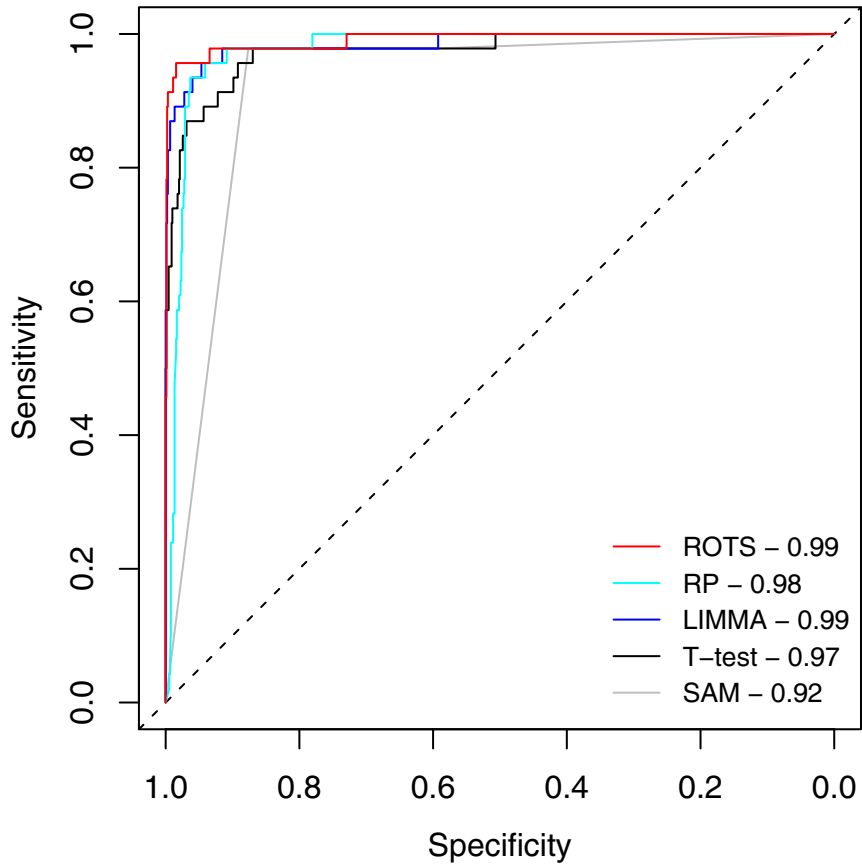
ROC-curves 10vs25



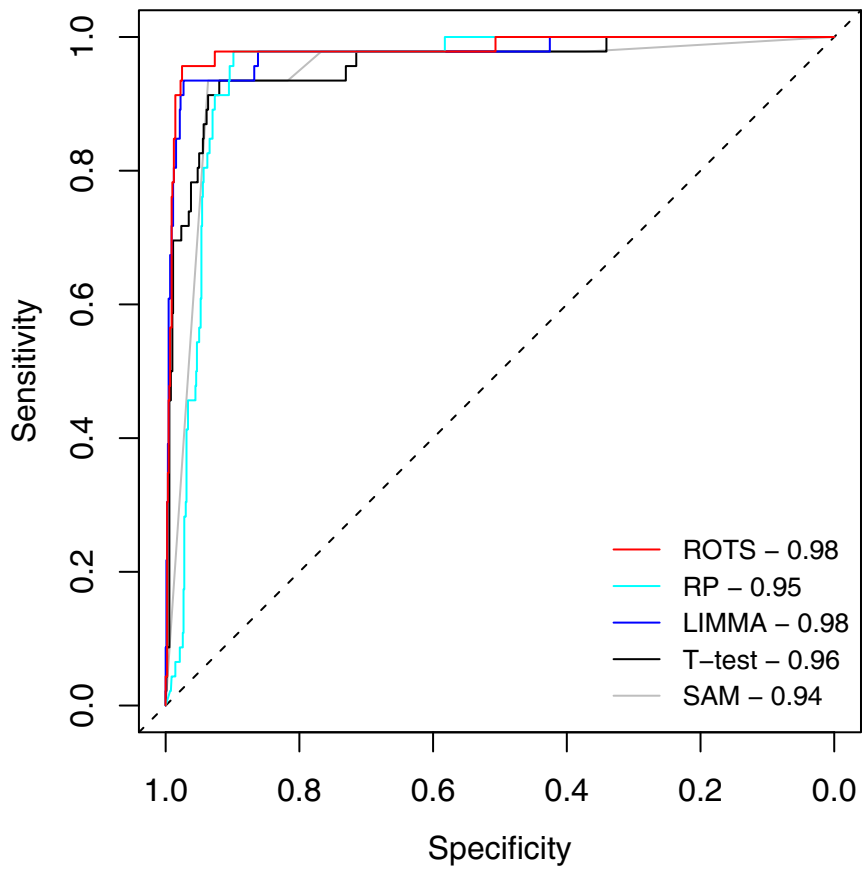
ROC-curves 4vs10



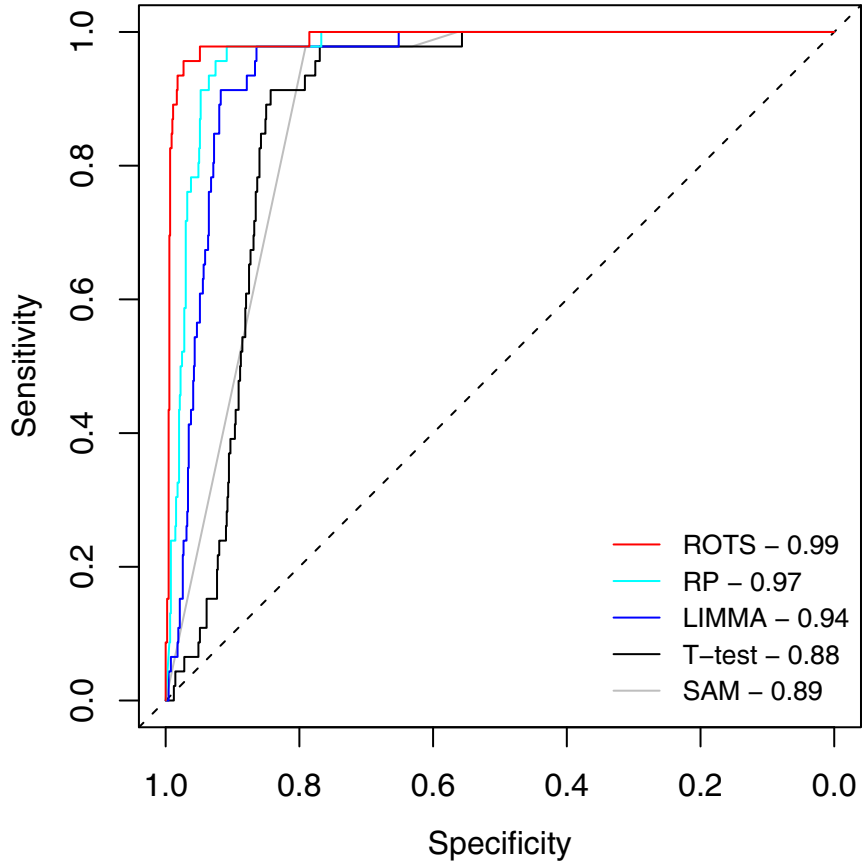
ROC-curves 2vs10



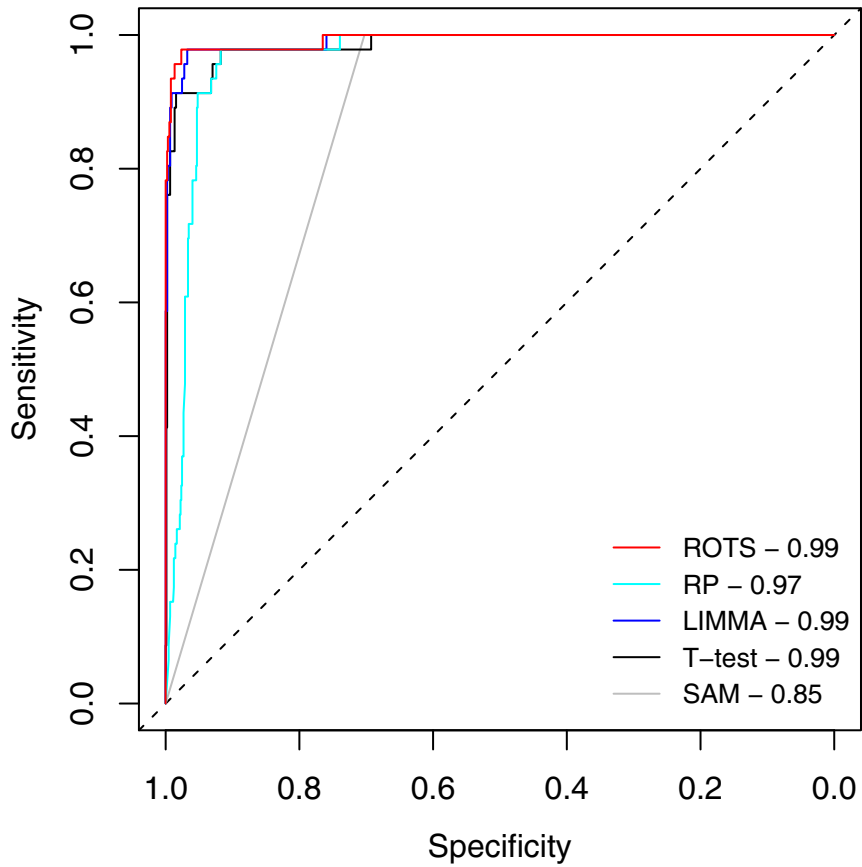
ROC-curves 10vs50



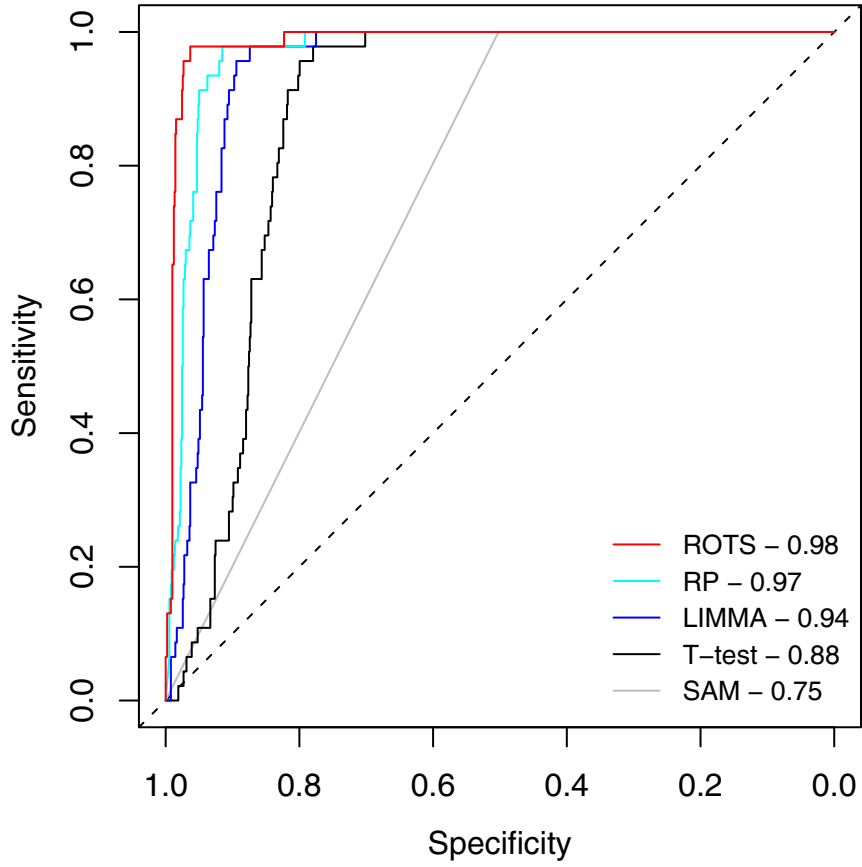
ROC-curves 4vs25



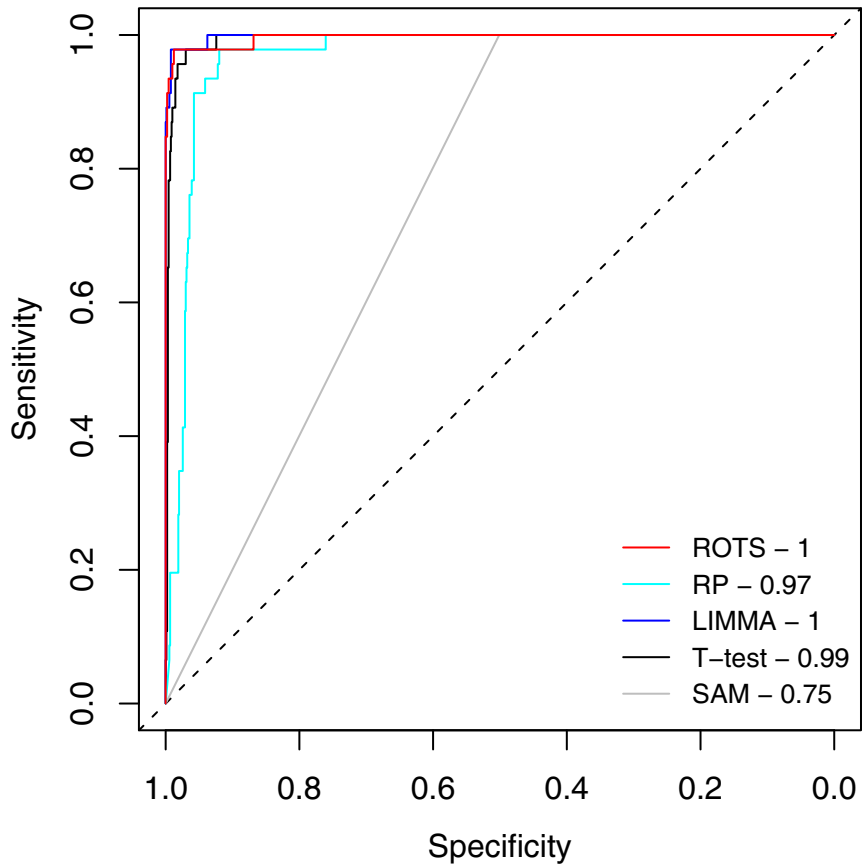
ROC-curves 2vs25



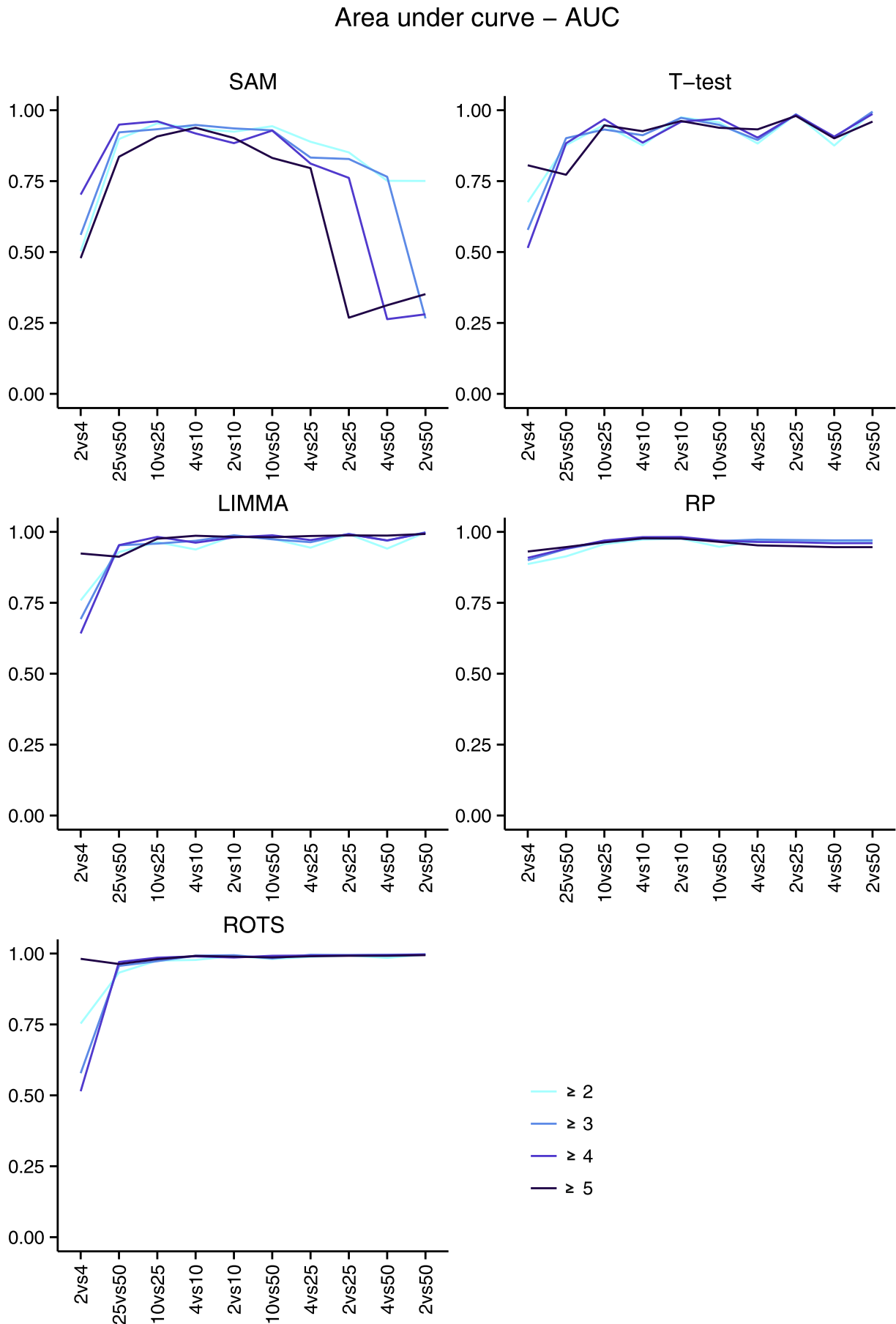
ROC-curves 4vs50



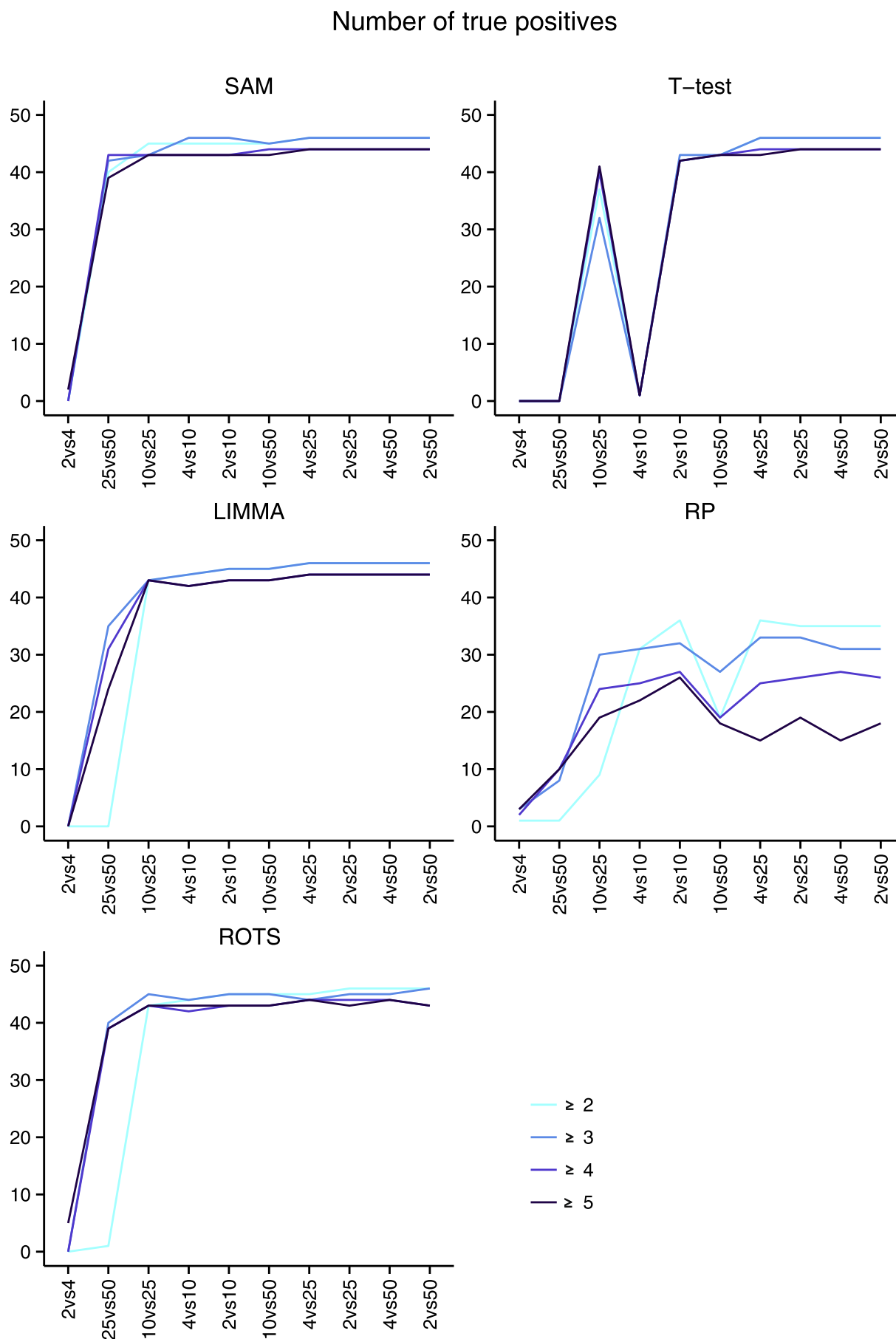
ROC-curves 2vs50



Supplementary Figure 4. Effect of the number of peptides used for quantitation on the ROC performance of the statistical tests in the spiked yeast data. The area under the curve (AUC) values are shown for the five statistical methods tested (the separate plots) when the minimum of the number of quantified peptides per protein varied between two and five (the different colors).



Supplementary Figure 5. Effect of the number of peptides used for quantitation on the number of UPS1 proteins detected (true positives) in the spiked yeast data. A separate plot is shown for each of the five statistical methods tested when the minimum of the number of quantified peptides per protein varied between two and five (the different colors).



Supplementary Figure 6. Effect of the number of peptides used for quantitation on the number of yeast proteins detected (false positives) in the spiked yeast data. A separate plot is shown for each of the five statistical methods tested when the minimum of the number of quantified peptides per protein varied between two and five (the different colors).

