

Supplementary Material for "PolySTest: Robust statistical testing of proteomics data with missing values improves detection of biologically relevant features"

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	<i>Unified test</i>	<i>LIMMA</i>	<i>Miss test</i>	<i>Rank products</i>	<i>Permutation test</i>	<i>t-test</i>
3-species mixture FDR 0.01						
<i>TP</i>	2329	<b>2597</b>	175	13	0	1926
<i>FP (tFDR)</i>	64 (0.03)	124 (0.05)	2 (0.01)	1 (0.07)	0	76 (0.04)
3-species mixture FDR 0.05						
<i>TP</i>	2677	<b>2872</b>	814	232	2262	2582
<i>FP (tFDR)</i>	150 (0.05)	379 (0.12)	36 (0.04)	5 (0.02)	191 (0.08)	295 (0.1)
2-species mixture FDR 0.01 (condition 2 vs. 1)						
<i>TP</i>	<b>192</b>	186	21	45	0	80
<i>FP (tFDR)</i>	5 (0.03)	7 (0.04)	1 (0.05)	0 (0)	0 (0)	0 (0)
2-species mixture FDR 0.01 (condition 3 vs. 1)						
<i>TP</i>	<b>207</b>	198	31	61	0	139
<i>FP (tFDR)</i>	10 (0.05)	10 (0.05)	1 (0.03)	3 (0.05)	0 (0)	2 (0.01)
2-species mixture FDR 0.01 (condition 4 vs. 1)						
<i>TP</i>	<b>218</b>	206	41	67	152	165
<i>FP (tFDR)</i>	20 (0.08)	25 (0.1)	0 (0)	1 (0.01)	64 (0.3)	10 (0.06)

Table S1: Comparison of true and false positives within statistical tests for all ground truth data sets. The 2-species data set been normalized using the cyclic loess normalization by LIMMA to decrease effects from bias towards one side. TP: true positives, FP: false positives, tFDR: true FDR.

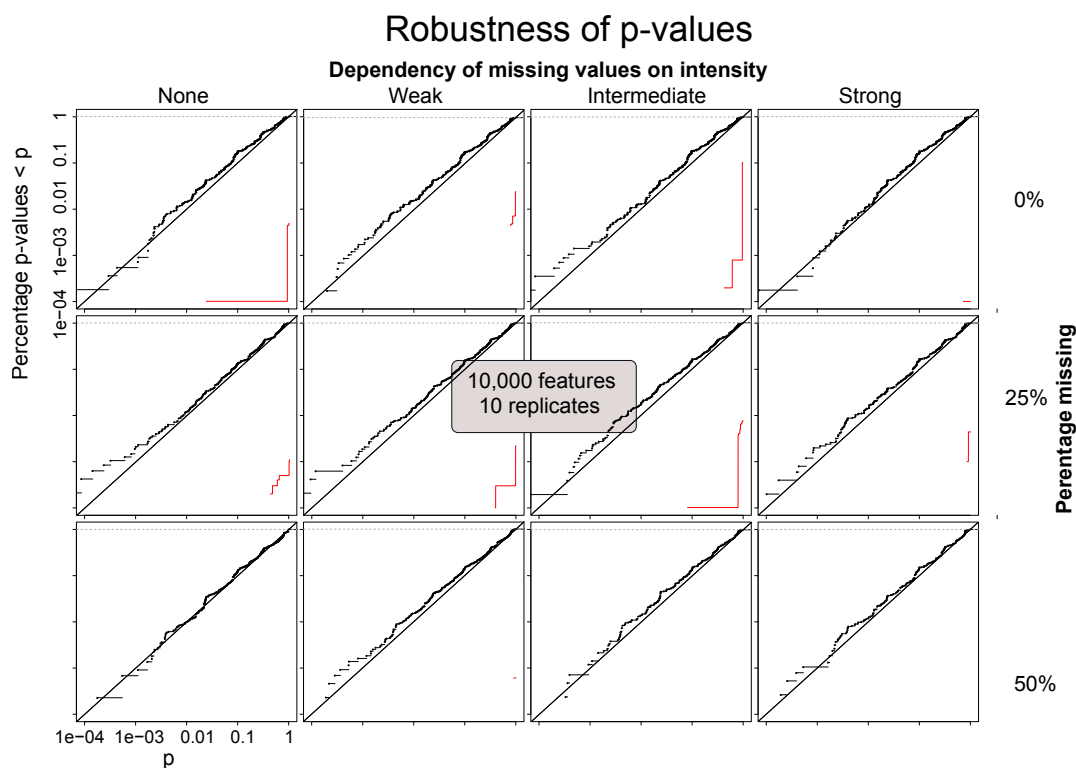


Figure S1: Cumulative distribution of p-values for different amounts and different value-dependent missing values. A linear increase corresponds to an uniform distribution of p-values which is valid down to very low p-values. The red lines denote the fraction of features with an FDR below the give  $p$ .

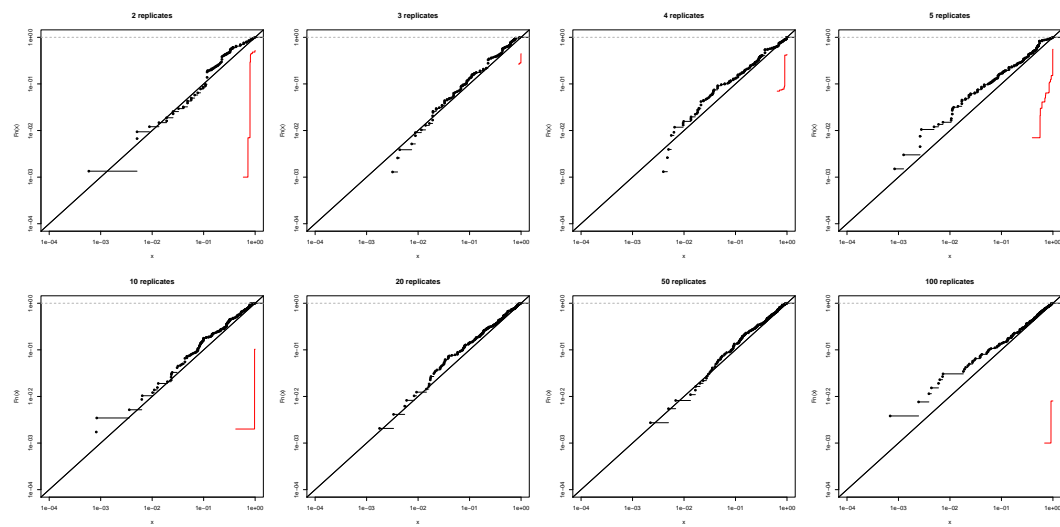


Figure S2: Cumulative distribution of p-values from Miss test for purely random artificial data consisting of 1,000 features.

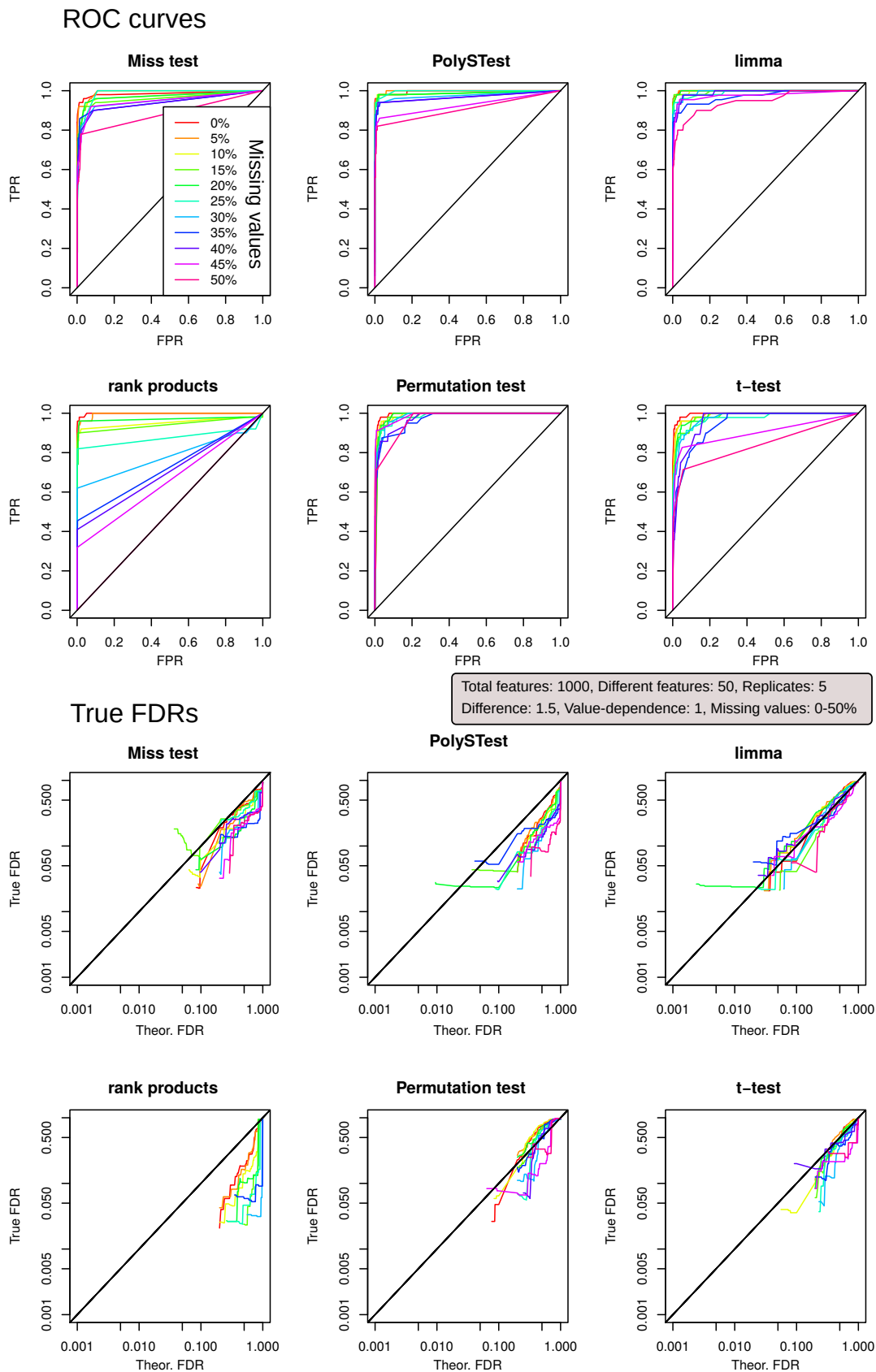


Figure S3: ROC curves and comparison of estimated and real FDR for the data sets from Fig. 2C when additionally comparing different percentages of missing values.

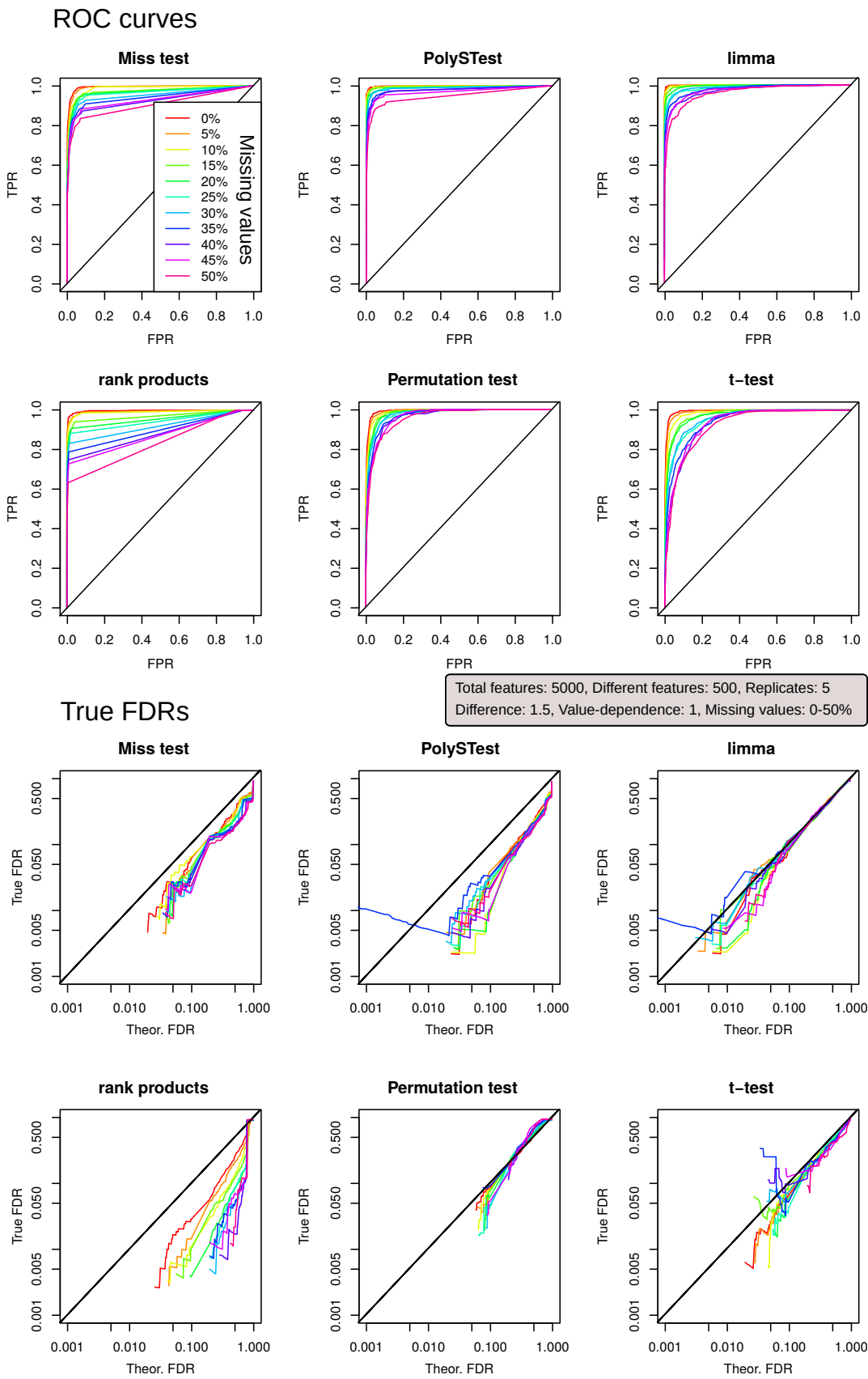


Figure S4: ROC curves and comparison of estimated and real FDR for data sets with similar parameters as in Fig. 2C when additionally comparing different percentages of missing values. The number of features was increased to 5000 to allow higher coverage of the over the FDR range.

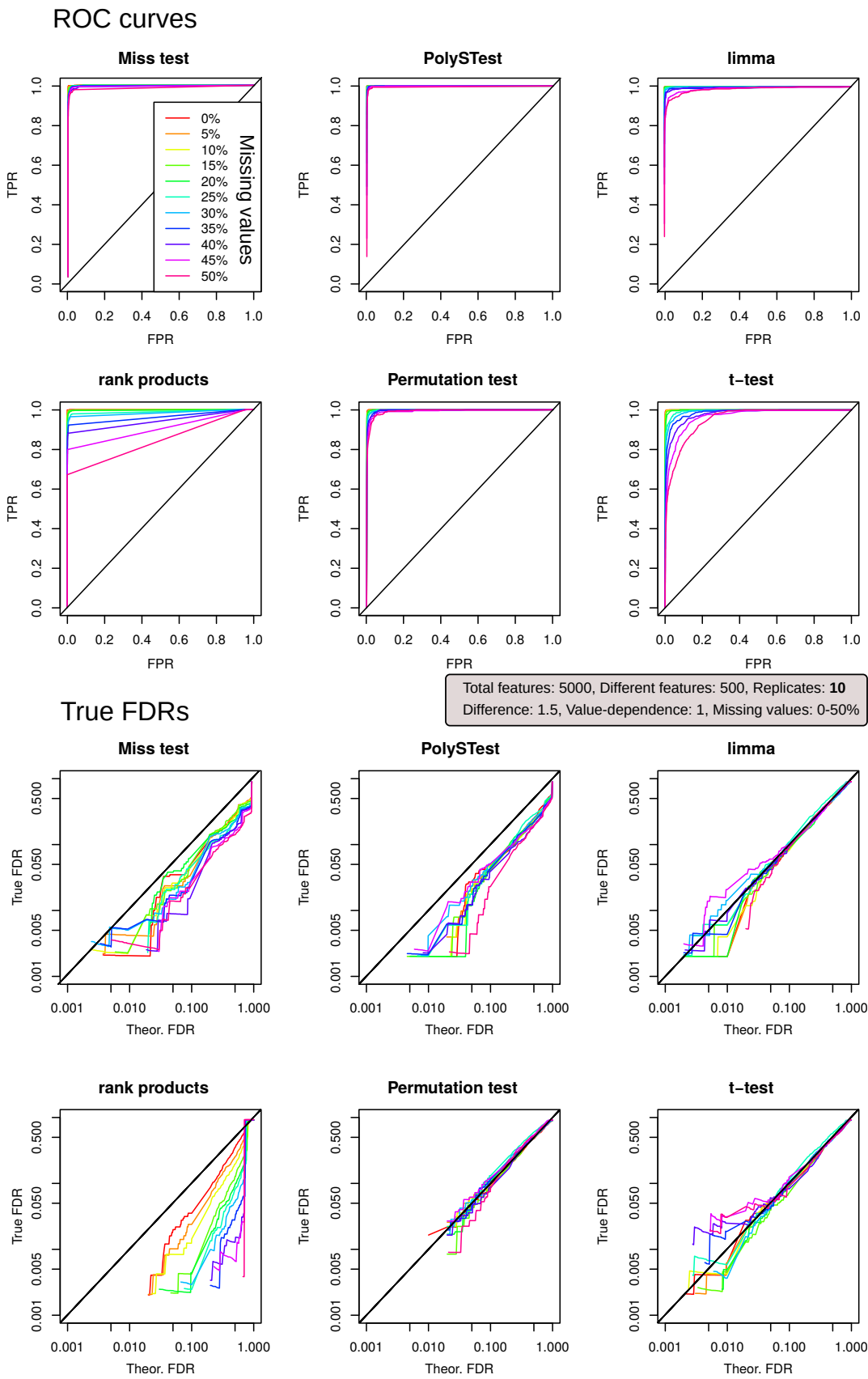


Figure S5: ROC curves and comparison of estimated and real FDR for data sets with similar parameters as in Fig. 2C when additionally comparing different percentages of missing values. The number of features was increased to 5000 to allow higher coverage of the over the FDR range.

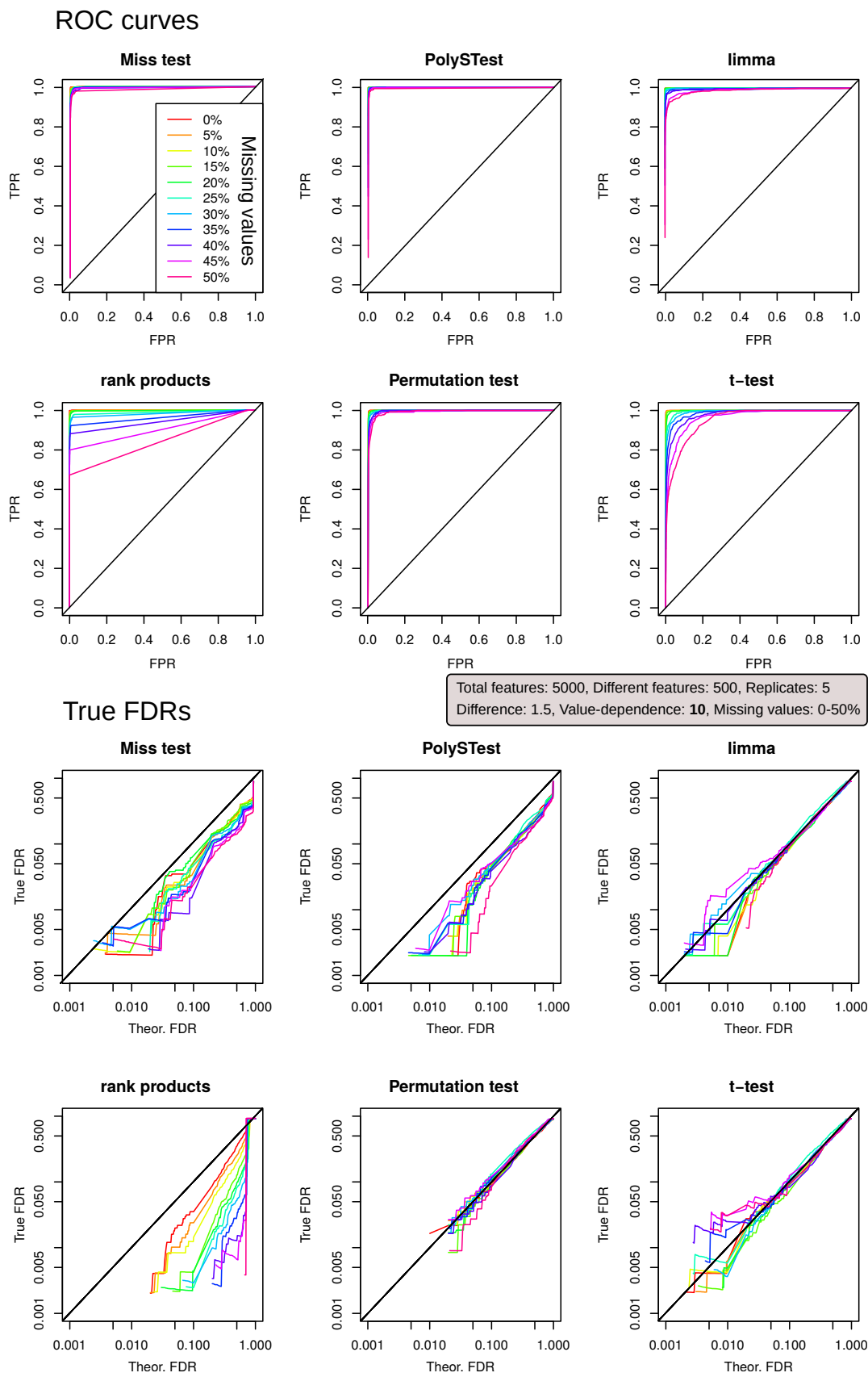


Figure S6: ROC curves and comparison of estimated and real FDR for data sets with similar parameters as in Fig. 2C when additionally comparing different percentages of missing values. The number of features was increased to 5000 to allow higher coverage of the over the FDR range.

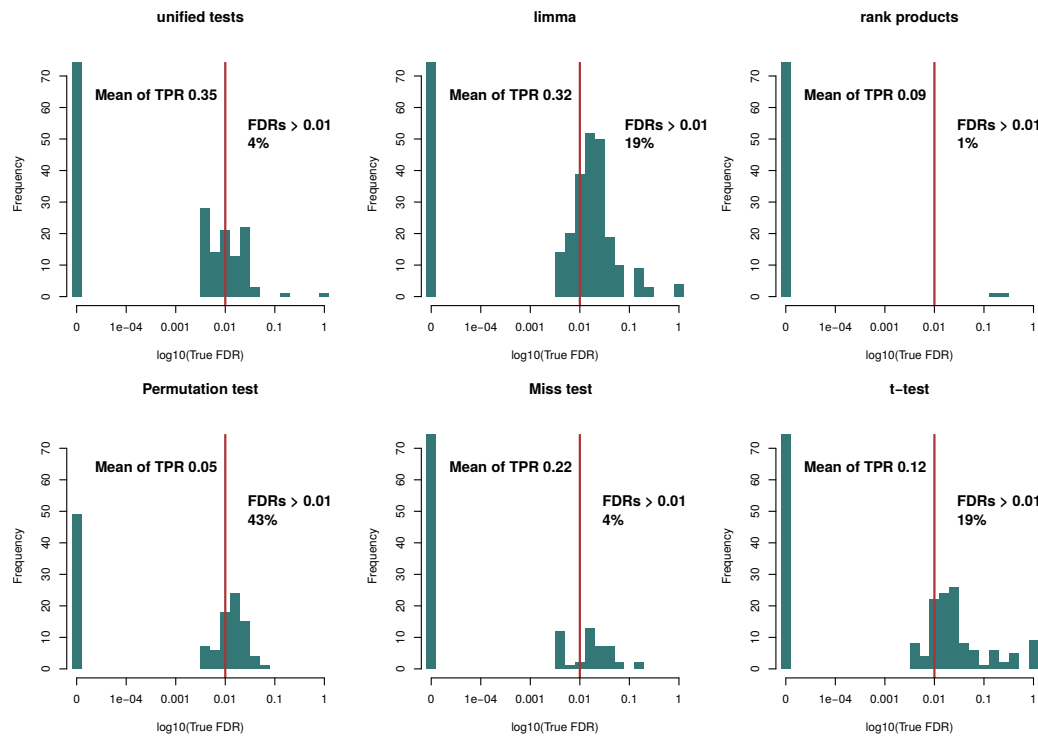


Figure S7: Distribution of true FDRs at a given FDR of 0.01 for 1,584 artificial data sets with 500 features spanning 3-10 replicates, 2-100 truly regulated proteins shifted by 1, 1.5, 2 and 5 from their original random value, and 0-100 value-dependence and missing values of 0%-50%.

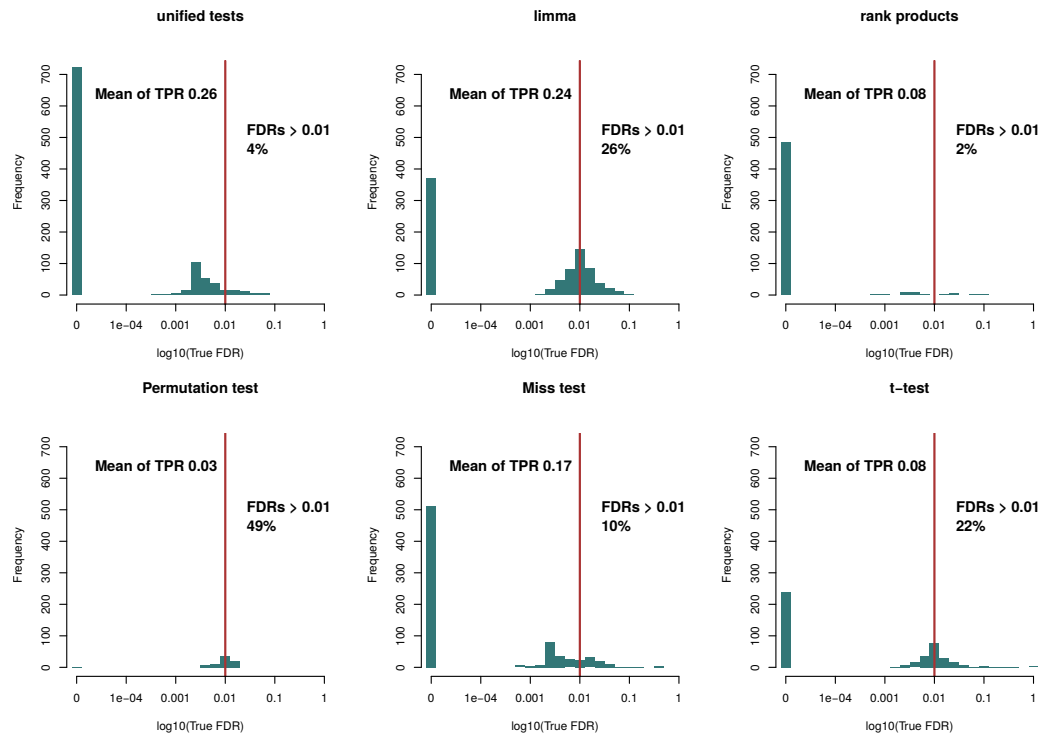


Figure S8: Distribution of true FDRs at a given FDR of 0.01 for 1,584 artificial data sets with 5,000 features spanning 3-10 replicates, 2-100 truly regulated proteins shifted by 1, 1.5, 2 and 5 from their original random value, and 0-100 value-dependence and missing values of 0%-50%.



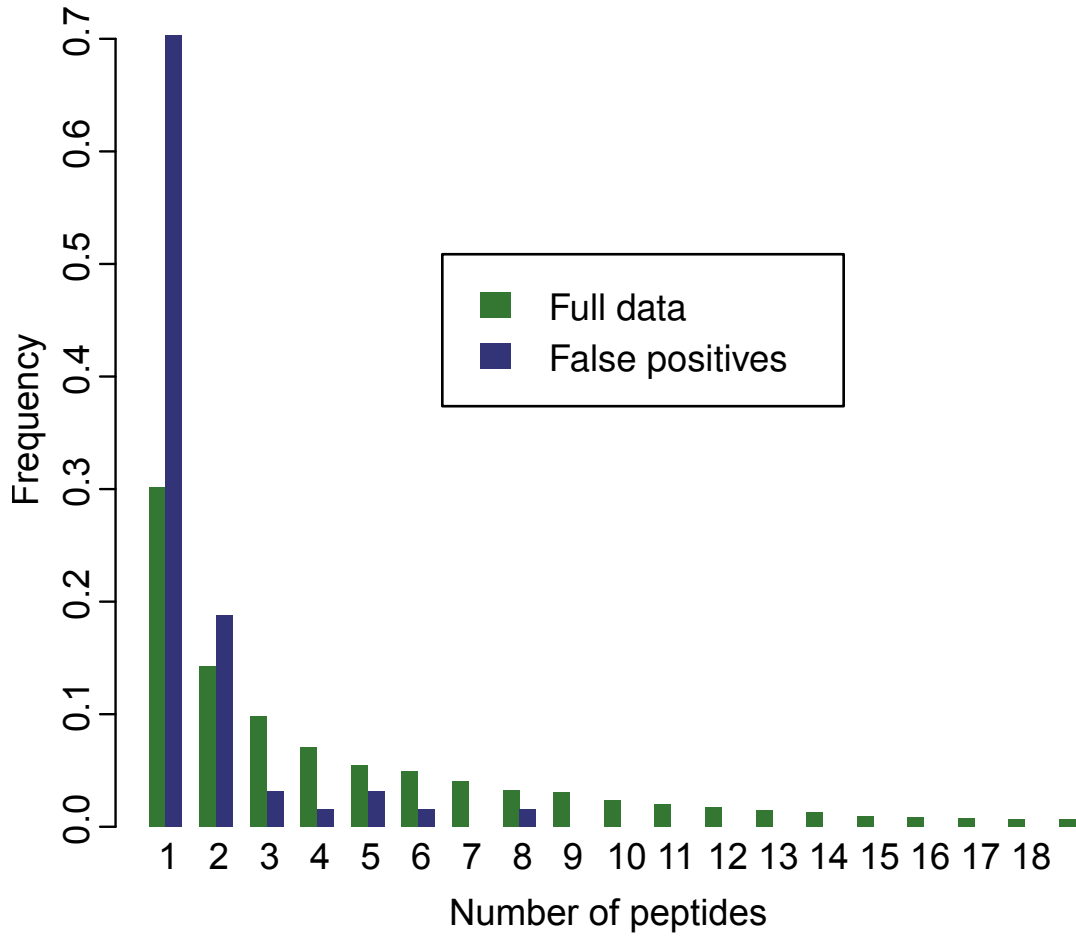


Figure S9: False positives are mostly proteins derived from low peptide numbers compared to the full data set, indicating that they, at least partially, might in fact be wrongly identified true positives.

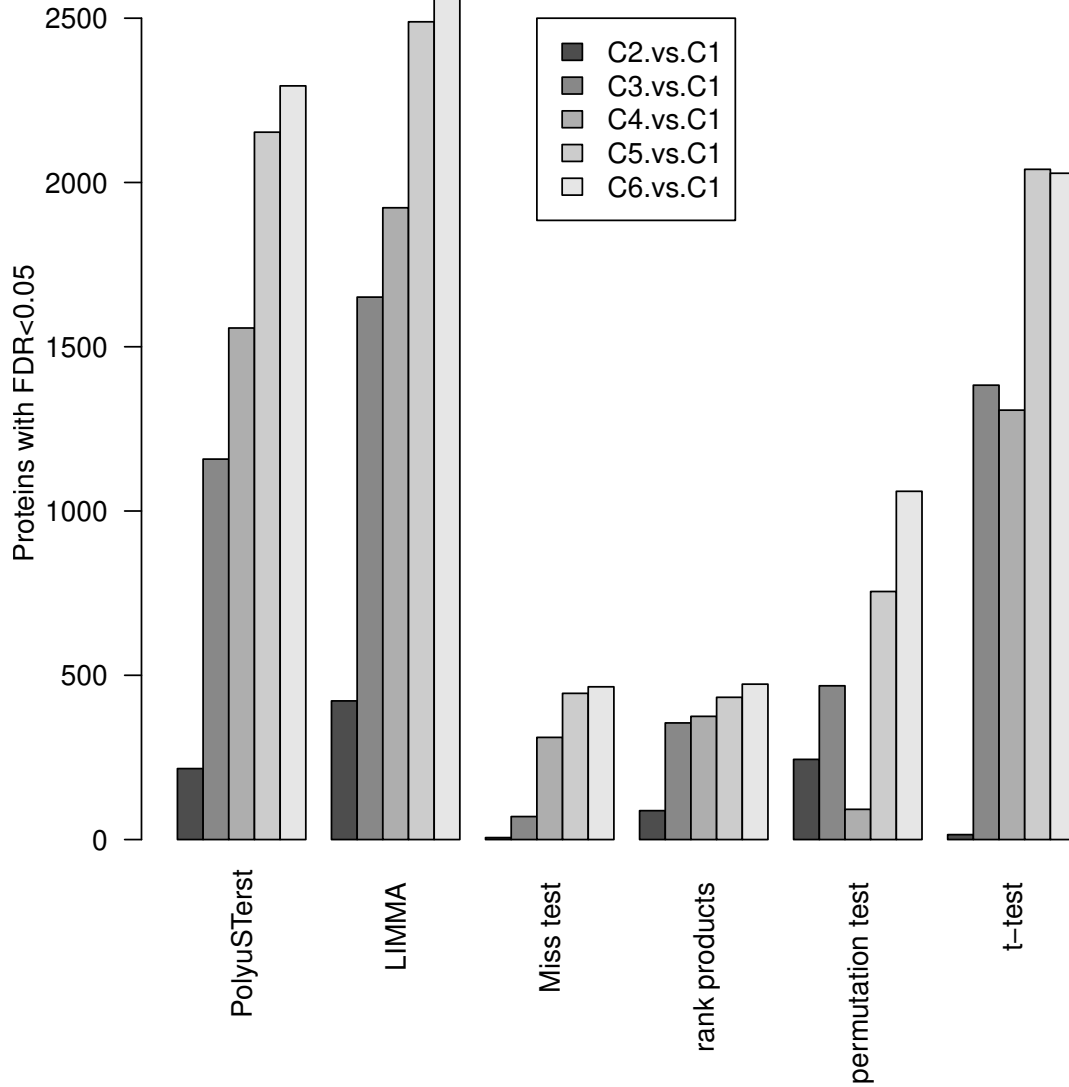


Figure S10: Number of differentially regulated proteins at FDR < 0.05 for the different statistical tests and comparisons of different muscle differentiation states versus stem cells.

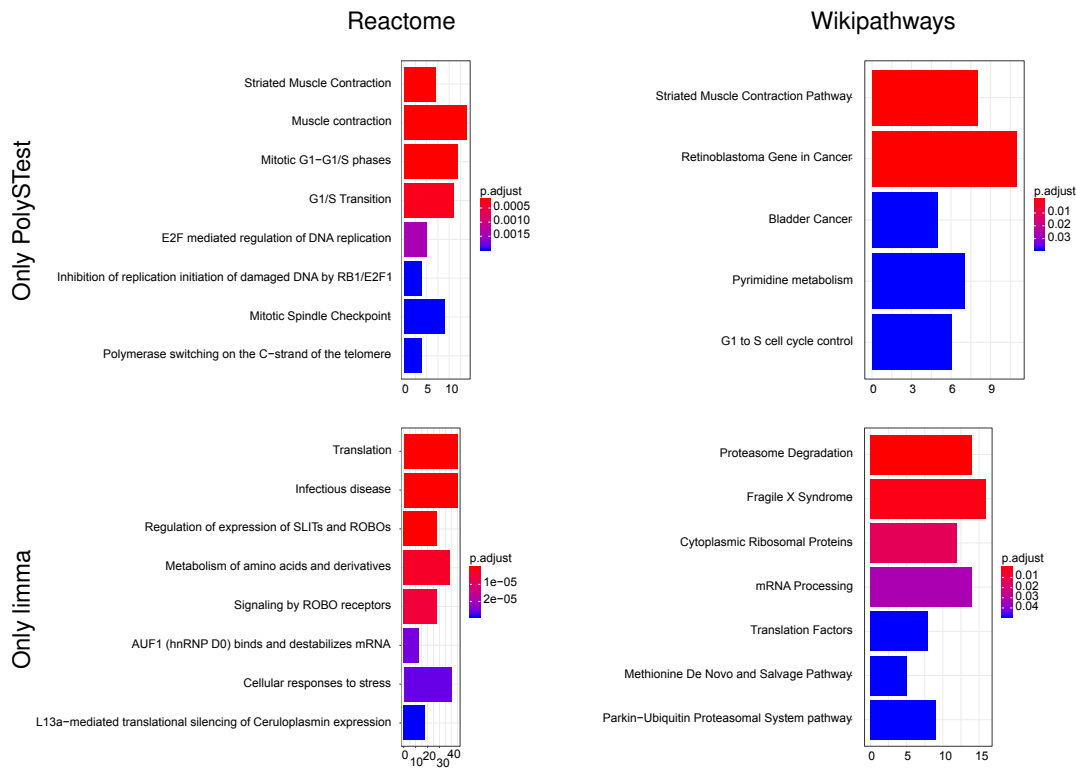


Figure S11: Result from pathway enrichment analysis (Reactome and Wikipathways) for proteins uniquely identified by PolySTest (upper panels) and LIMMA (lower panels). FDR threshold: 0.05.

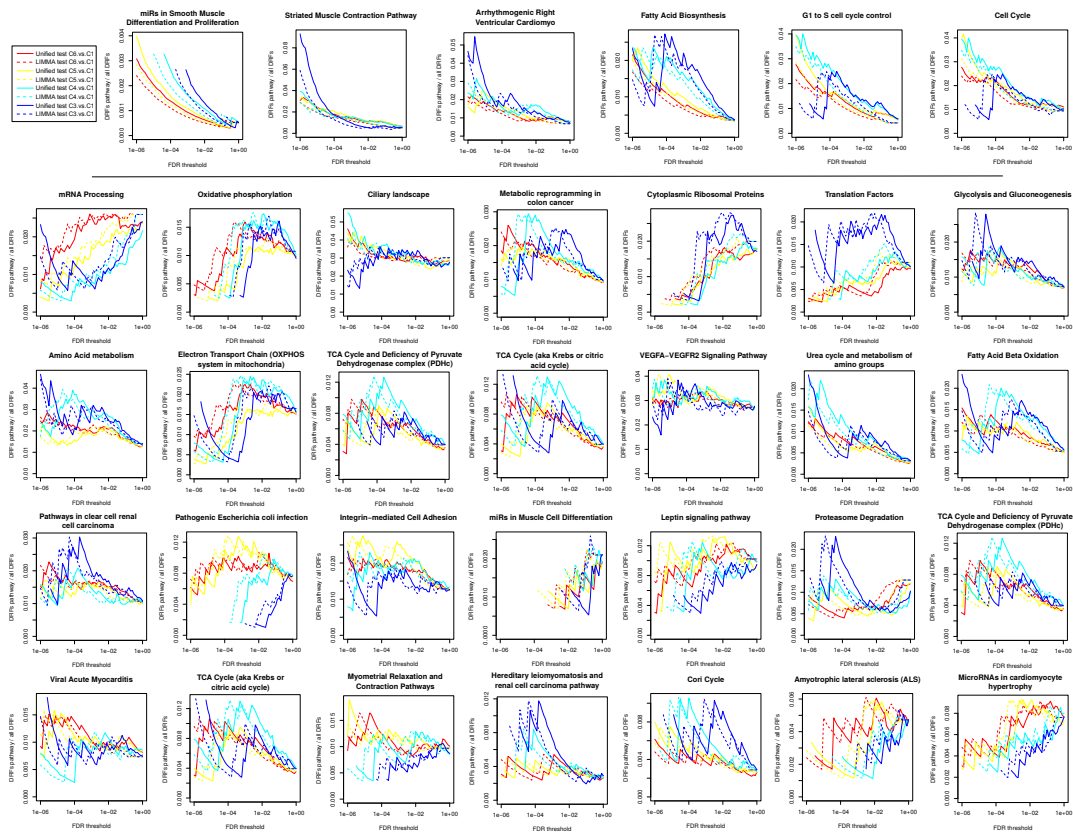


Figure S12: Gene-background ratios of DRFs for most enriched pathways and pathways related to muscle cells and cell cycle. The gene groups were taken from WikiPathways.

A tool to determine and visualize differentially regulated features using multiple approaches

**Data input**

**Data layout**

First column for quantification:

Replicates are grouped

Number of replicates:

Number of conditions:

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**Statistical testing**

▶ Run analysis

Paired tests?

Comparison 1:  vs

Comparison 2:  vs

Comparison 3:  vs

Add new comparison

Help (click on the right to see the help page) +

**Data details**

Number of features: 1224  
 Number of data columns in file: 12  
 Percentage of missing values: 21.77  
 Condition 1: HF.Rep.1, HF.Rep.2, HF.Rep.3  
 Condition 2: TTA.Rep.1, TTA.Rep.2, TTA.Rep.3  
 Condition 3: FO.Rep.1, FO.Rep.2, FO.Rep.3  
 Condition 4: TTA.FO.Rep.1, TTA.FO.Rep.2, TTA.FO.Rep.3

Show  entries Search:

Feature	Quantitative data columns							
	C1 Rep 1	C2 Rep 1	C3 Rep 1	C4 Rep 1	C1 Rep 2	C2 Rep 2	C3 Rep 2	C4 R
	HF.Rep.1	TTA.Rep.1	FO.Rep.1	TTA.FO.Rep.1	HF.Rep.2	TTA.Rep.2	FO.Rep.2	TTA.FO
All	All	All	All	All	All	All	All	All
A0JPQ8	-0.0277967998	-0.0270594235	-0.3002030066	0.2644644037				
A1L108	0.0481386393	-0.1690967953	-0.0692033187	0.2354957748				
A2VCW9	0.4615707061	-0.7500572739	0.4215763157	0.0808386809	0.4649272631	-0.1667008773	0.3211096844	-0.066
A7VJC2	-0.019566896	-0.1218971684	-0.0671155127	0.2170181238	0.0227086242	0.0179514949	-0.0686570265	-0.00:
A7VJC2;P04256;Q6URK4	0.2936658772	0.0532100156	-0.2935421529	-0.0533337399				
A9UMV8	-0.0535483953	0.0789590533	-0.0569234692	0.0340262478	0.0410262671	-0.0568309877	0.0029697172	-0.05:
A9UMV8;P0C0S7					-0.2098372243	0.1134569958	0.4665244279	-0.30:
B0BN93	0.0055282924	0.0462658885	-0.1580272153	0.0109022652	-0.235274093	-0.152115903	0.2409092493	0.11:
B0BNA5	0.2461735023	-0.6908962234	-0.0803437293	0.5745540557				
B0BNC9	0.471875814	0.5664259417	0.1403550607	-0.9869140657	0.380020444	-1.4131803545	0.2443289577	0.22:

Showing 1 to 10 of 1,224 entries Previous  2 3 4 5 ... 123 Next

Figure S13: Screenshot of the PolySTest web service. Upload of quantitative proteomics data and setting of experimental design.

PolySTest
?

A tool to determine and visualize differentially regulated features using multiple approaches

Data input

Data layout

First column for quantification

Replicates are grouped

Number of replicates

Number of conditions

Statistical testing

▶ Run analysis

Paired tests?

Comparison 1:

C2 vs C1

Comparison 2:

C3 vs C1

Comparison 3:

C4 vs C1

Add new comparison

Help (click on the right to see the help page)

Data details

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Condition 2: TTA.Rep.1, TTA.Rep.2, TTA.Rep.3

Condition 3: FO.Rep.1, FO.Rep.2, FO.Rep.3

Condition 4: TTA.FO.Rep.1, TTA.FO.Rep.2, TTA.FO.Rep.3

Thresholds

To select feature groups and adapt visualization groups below.

FDR threshold

0.1

lower log-ratio threshold: -0.4257

upper log-ratio threshold: 0.4257

Select all features filtered by FDR and fold-change

Select tests and comparison

Further criteria for feature selection.

Which statistical test(s)?

unified tests

limma

Miss test

rank products

permutation test

t-test

Which comparison(s)?

C2 vs C1

C3 vs C1

C4 vs C1

Select features

Number selected features: 390

Select all shown features

Select all (filtered) features

Clear selected features in table

Download results

Column visibility
Copy
Print
Show 5 entries
Search:

Feature	Log-ratios			unified				li
	C2 vs C1	C3 vs C1	C4 vs C1	C2 vs C1	C3 vs C1	C4 vs C1	C2 vs C1	
A0JPQ8	0.00074	-0.27	0.29	1	0.799662444408487	0.309234136632215	0.368850796111752	0.2665
A1L108	-0.22	-0.12	0.19	0.471714443689806	1	0.497686571439312	0.157238147896602	0.4317
A2VCW9	-0.83	-0.26	-0.42	0.00448434877433635	0.794434510823065	0.107390951715546	0.00112108719358409	0.2122
ATVJC2	-0.073	-0.094	-0.021	0.852963067754386	1	1	0.213240766938596	0.3419
ATVJC2;P04256;Q6URK4	-0.24	-0.59	-0.35	0.424884825931975	0.316487064755491	0.245933633167548	0.141628275310658	0.105

Showing 1 to 5 of 1,224 entries Previous 1 2 3 4 ... 245 Next

Details on tests and feature (e.g. proteins) expression changes (max. 30 with lowest unified FDRs shown)

Scale features to mean

Features

- P59863
- P59862
- P59861
- Q52046
- Q52045
- Q52044
- P18488
- P59299
- Q52047
- Q52048
- Q71488
- P07569
- P52791
- Q54831
- P59571
- P11602
- Q52047
- P11602
- P11774
- P21773;P27871
- P59297
- Q54830
- P59573
- Q52044
- Q52045
- P11602
- P11603
- P11604
- P07872
- P11605
- P11606
- P11607

Feature expression over conditions

Row 1: unified tests  
 Row 2: limma  
 Row 3: Miss test  
 Row 4: rank products  
 Row 5: permutation test  
 Row 6: t-test

Download as pdf

Co-expression patterns and significance

Scale features to mean

Row annotation  
 <-1  
 <-0.05  
 <-0.01  
 <-0.001

13