Supplementary material for the manuscript: “omicsNPC: applying the Non-Parametric Combination methodology to the integrative analysis of heterogeneous omics data”

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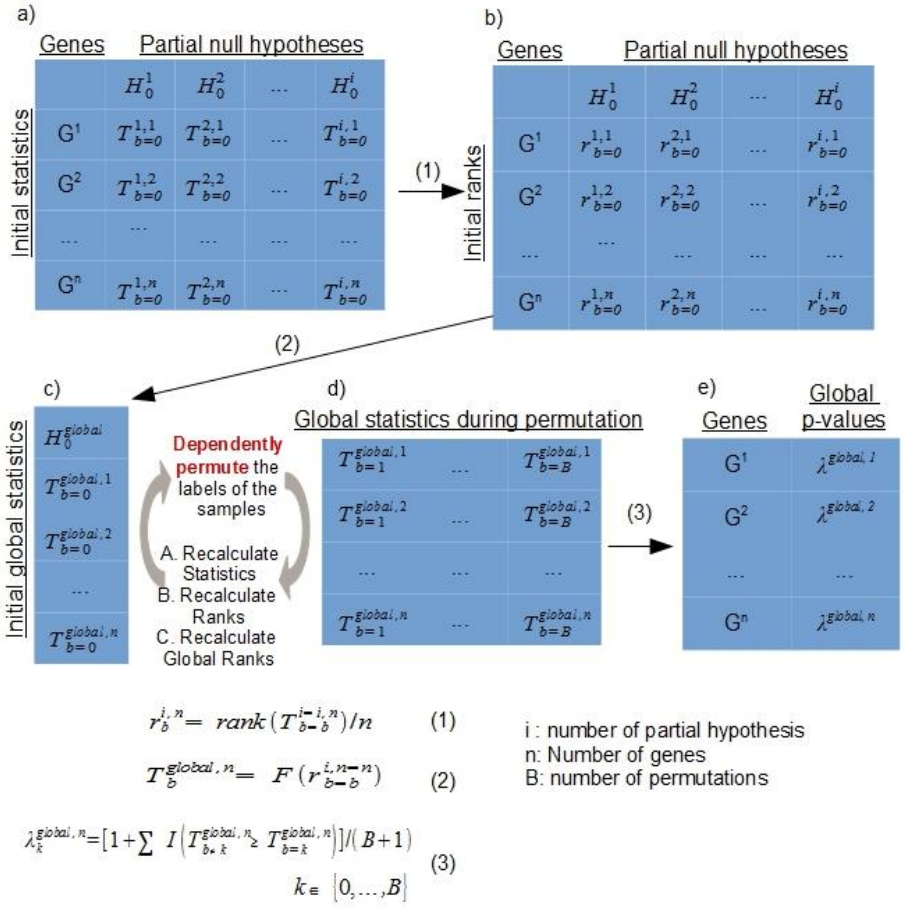
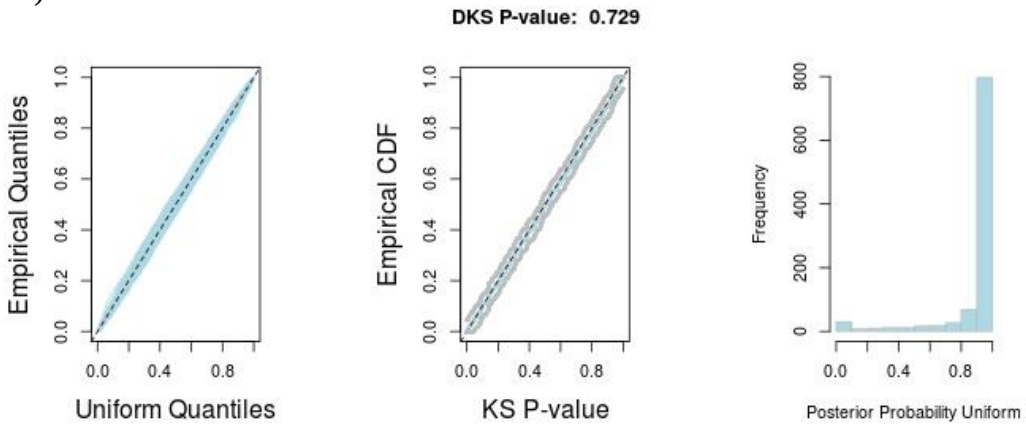


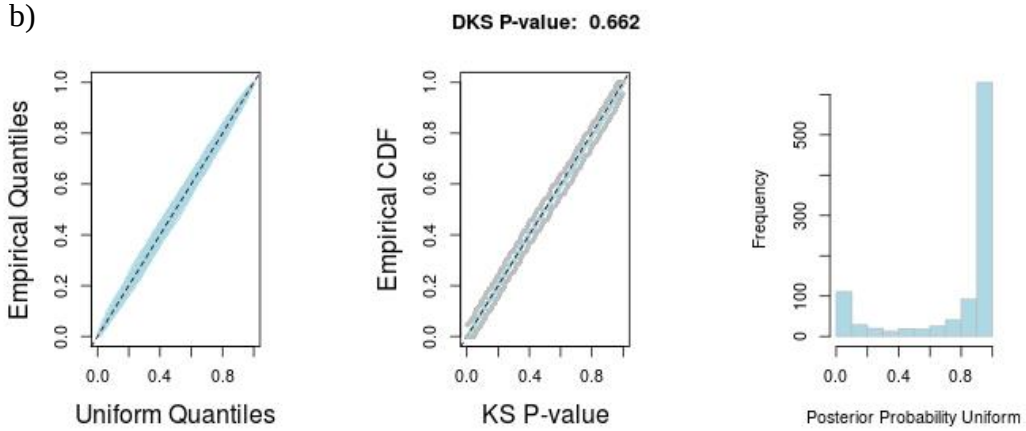
Fig A: Employing ranks in omicsNPC framework. a) Divide the question of interest, global null hypothesis, to a set of i partial null hypotheses. For each partial null hypothesis, select a test statistic sensitive to the alternative and for each gene n calculate its value in the observed data, $T_{b=0}^{i,n}$. Each column is filled with i's partial hypothesis statistics. b) For each column the statistics are ranked in the range of 0 to 1 from the least to the more extreme. c) For each gene, the ranks that belong to the same row are combined through a function F(), like the sum or the product, to calculate the global statistic on the observed data, $T_{b=0}^{globaln}$. d) The empirical distribution of the global statistic is generated through permutation. Steps A to C are repeated B times by dependently permuting the labels of the samples. e) Finally, for each gene a global p-value is calculated by employing function (3), which compares the value of the global statistic on the observed data against its empirical distribution.

a)



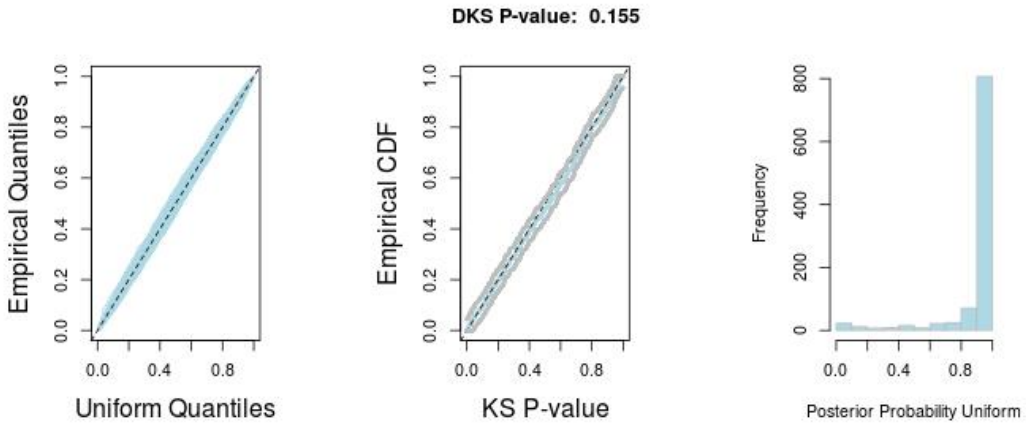
omicsNPC - Fisher

b)



omicsNPC - Liptak

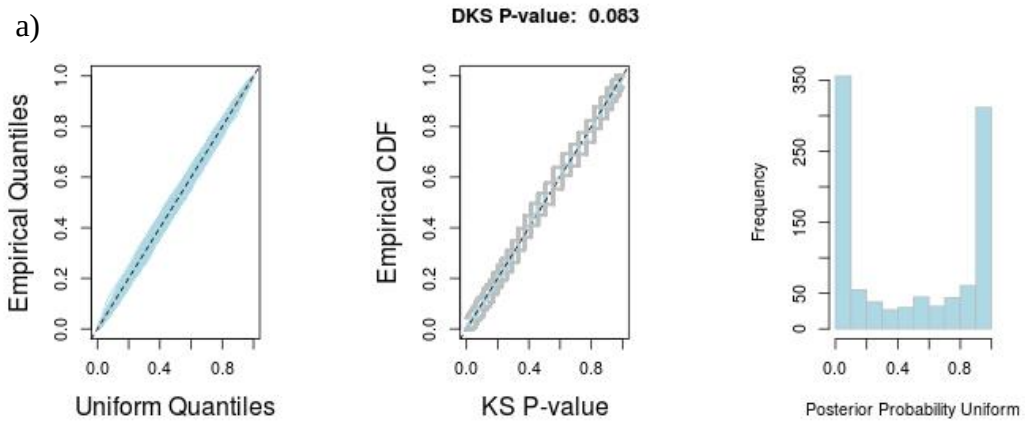
c)



omicsNPC - Tippett

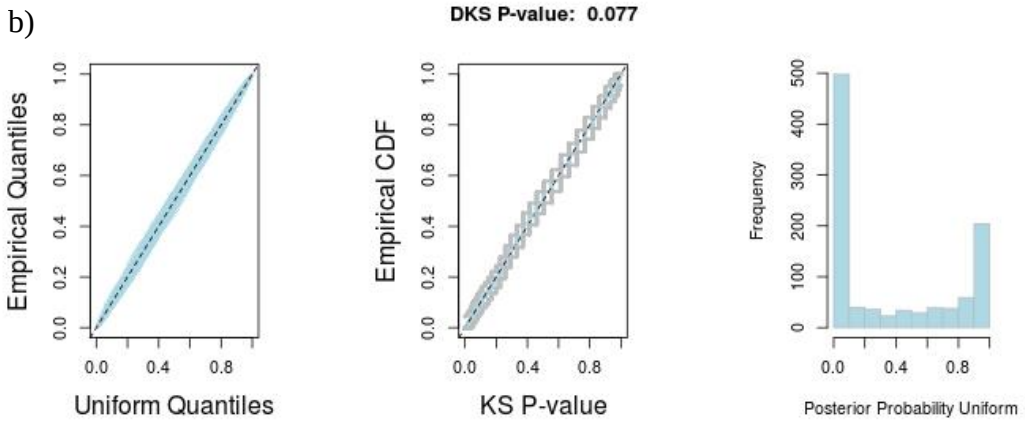
Fig B: JNC's diagnostics plots on uncorrelated samples employing omicsNPC. Two uncorrelated microarray datasets were simulated and analysed using omicsNPC. a) Fisher combining function was employed. The diagnostic plots are, from left to right: (i) a quantile-quantile (Q-Q) plot of the p-values for each study versus the uniform quantiles. The lines should be close to the 45º diagonal; lines below the diagonal indicate optimist results, lines above the diagonal conservatives ones; (ii) the empirical distribution function of the first level KS test p-values (blue) with confidence bands (grey), again the line should fall along the 45º line; and (iii) a histogram of the posterior probabilities that each set of p-values is uniform, these values should be near one. b) Liptak combining function was employed. The order of diagnostic plots is the same as a). c) Tippett combining function was employed. The order of diagnostic plots is the same as a). In all cases omicsNPC produced calibrated p-values. Q-Q plots' lines fall on the 45º line, the empirical distribution function of the first level KS test fallen also on the 45º line, the histogram of the posterior probabilities was near one and the dks p-values were not significant, as expected.

a)



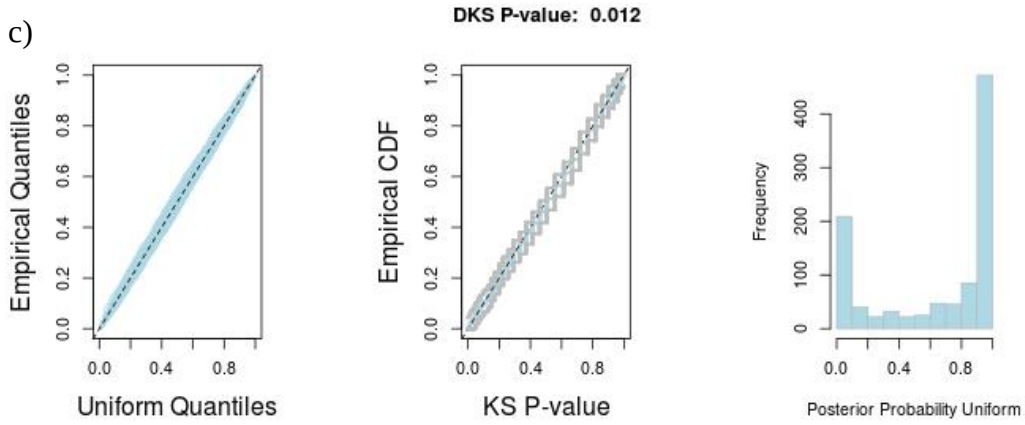
$NPC^{no\text{-}correction}$ - Fisher

b)



$NPC^{no\text{-}correction}$ - Liptak

c)

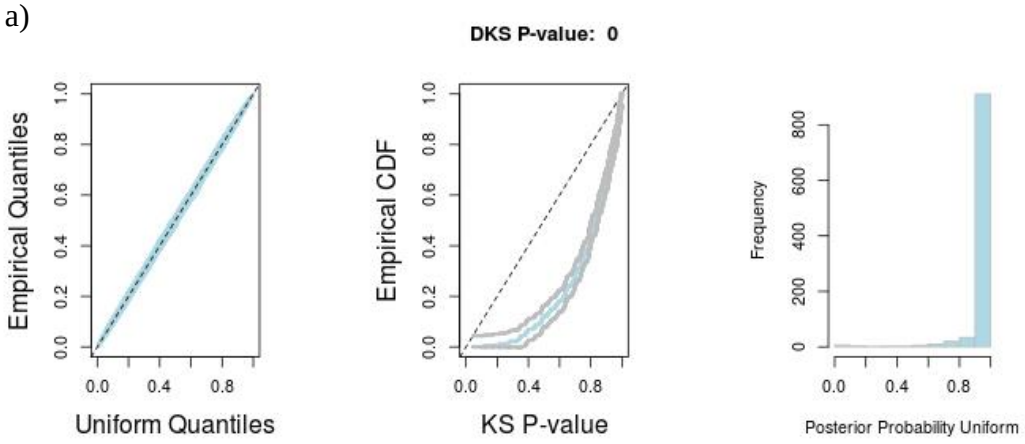


$NPC^{no\text{-}correction}$ - Tippett

Fig C: JNC's diagnostics plots on uncorrelated samples with $NPC^{no\text{-}correction}$. Details as in Fig B. In all cases $NPC^{no\text{-}correction}$ produced slightly un-calibrated p-values. Although Q-Q plots' lines fell on the 45º line and the empirical distribution function of the first level KS test fell also on the 45º line, the histogram of the

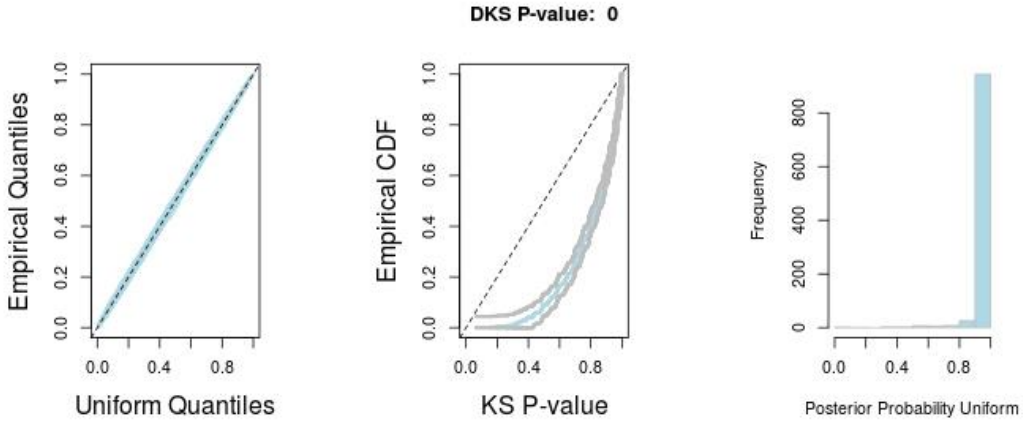
posterior probabilities was greatly deviated between zero and one and the dks p-values were one order of magnitude less than omicsNPC dks p-values. Last, Tippett dks p-value was statistical significant, 0.012.

a)



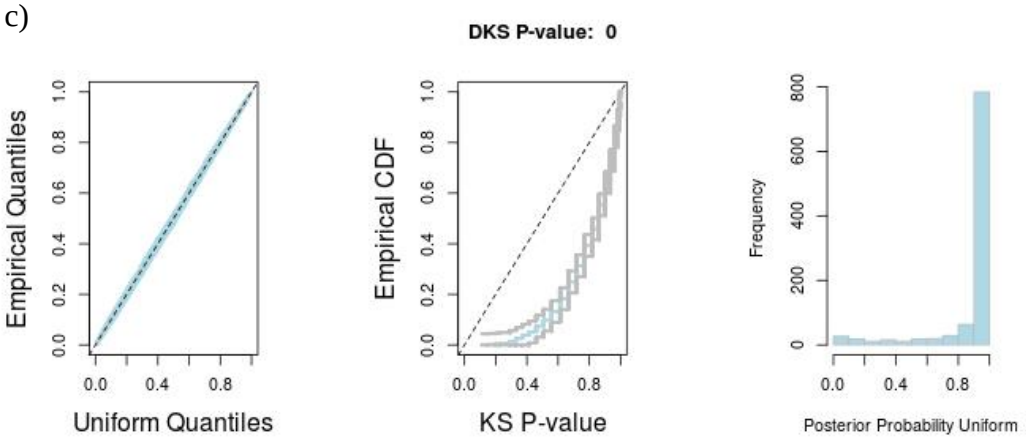
omicsNPC - RankSum

b)



omicsNPC - RankProd

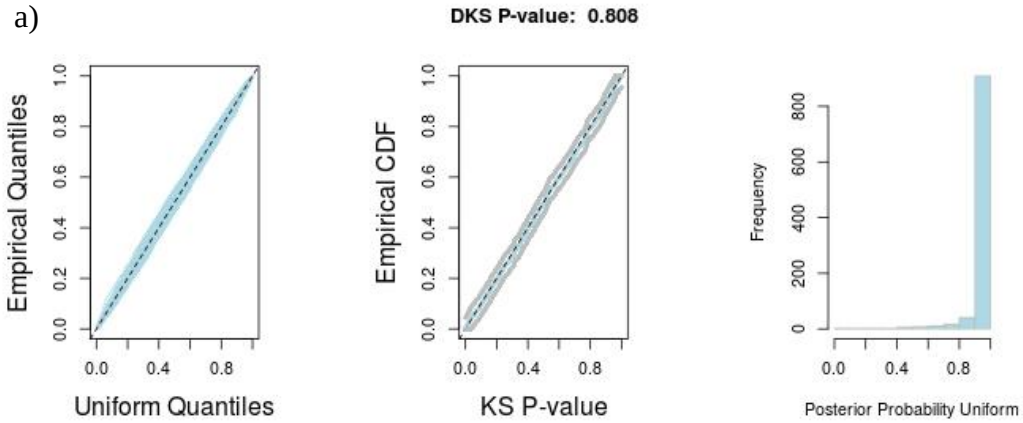
c)



RankProd

Fig D: JNC's diagnostics plots on uncorrelated samples employing omicsNPC$^{\text{RankSum}}$, omicsNPC$^{\text{RankProd}}$, RankProd. Details as in Fig B. In all cases highly significant dks test p-values are produced.

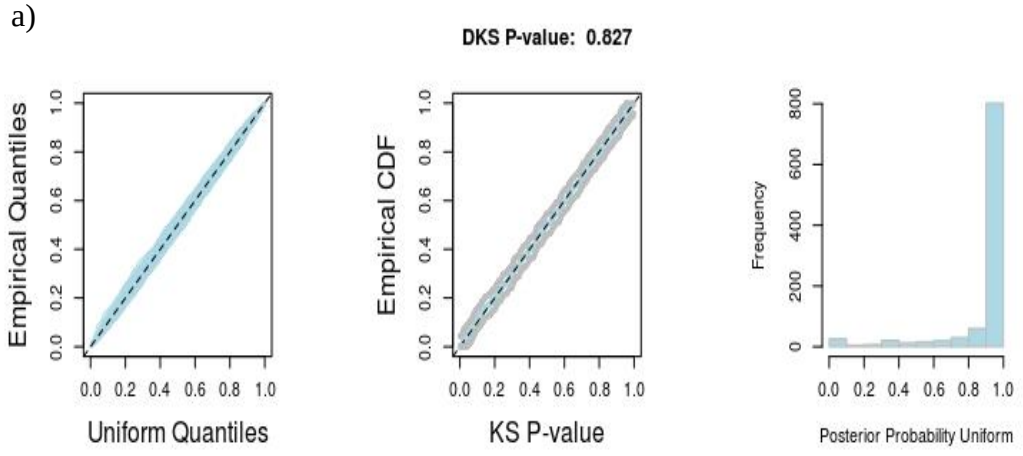
a)



CP – Combining P-values

Fig E: JNC's diagnostics plots on uncorrelated samples employing combining p-values (CP) approach. Details as in Fig B, analysed using CP. Combining p-values parametrically produced calibrated p-values on uncorrelated data. Q-Q plots' lines fall on the 45º line, the empirical distribution function of the first level KS test fallen also on the 45º line, the histogram of the posterior probabilities was near one and the dks p-values were not significant, as expected.

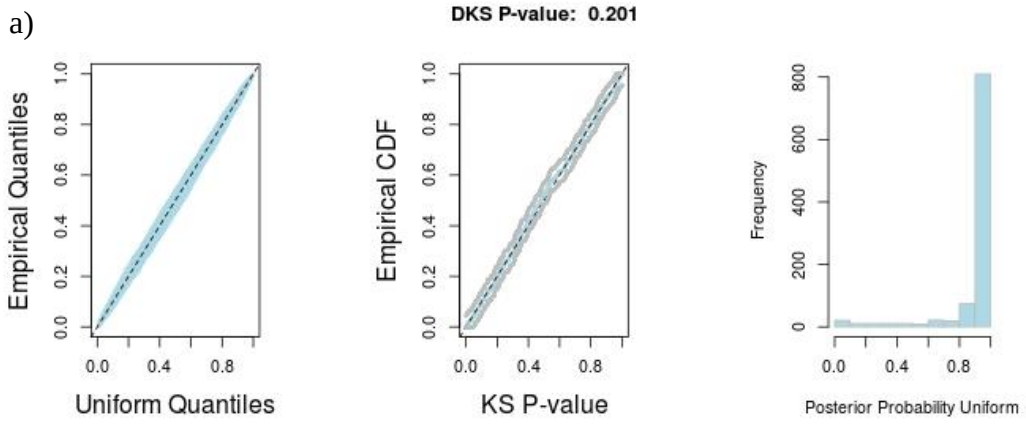
a)



Benjamini

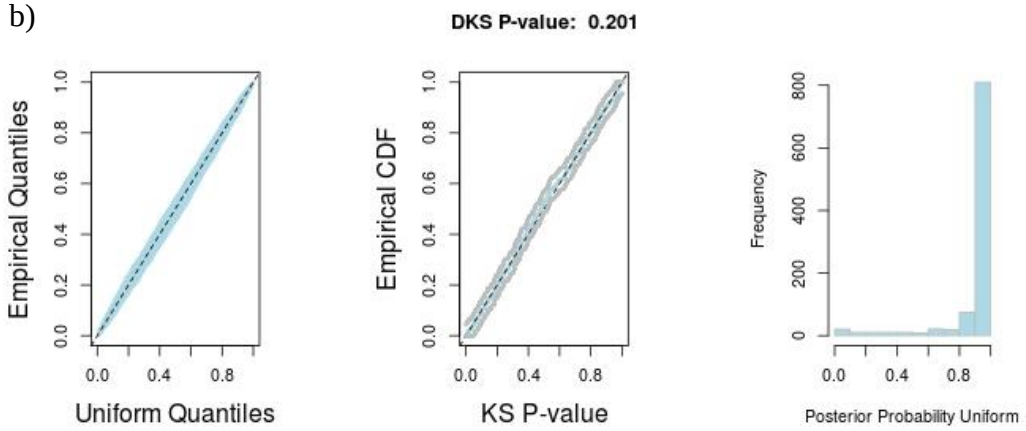
Fig F: JNC's diagnostics plots on uncorrelated samples employing Benjamini approach. Details as in Fig B. Benjamini produced calibrated p-values on uncorrelated samples: Q-Q plots' lines fall on the 45º line, the empirical distribution function of the first level KS test fallen also on the 45º line, the histogram of the posterior probabilities was near one and the dks p-values were not significant, as expected.

a)



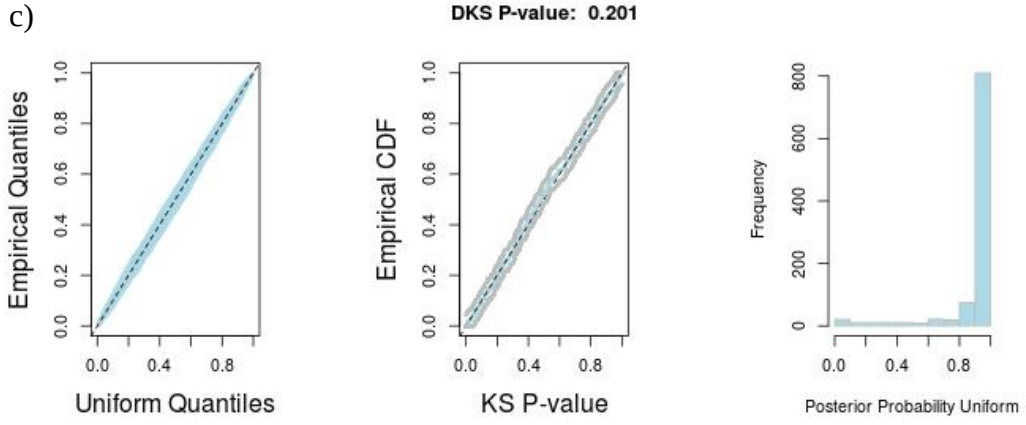
omicsNPC - Fisher

b)



omicsNPC - Liptak

c)

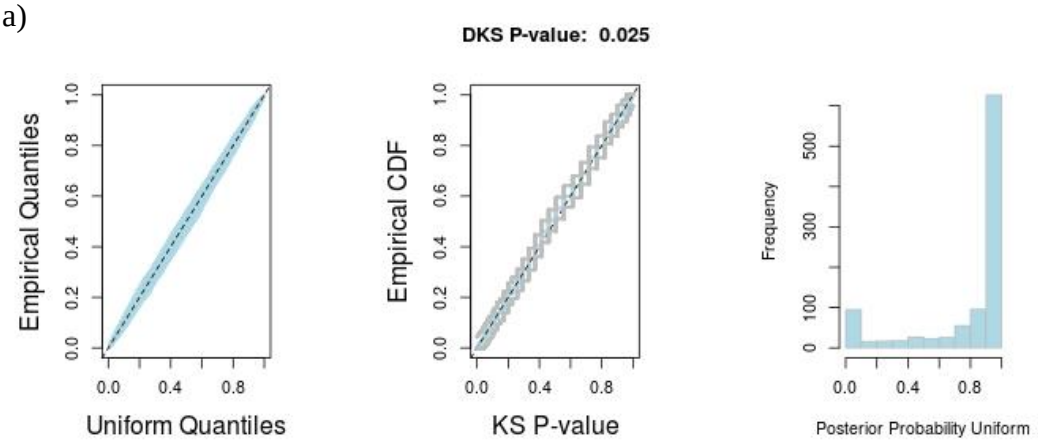


omicsNPC - Tippett

Fig G: JNC's diagnostics plots on correlated samples employing omicsNPC. Two perfectly correlated microarray datasets were simulated and analysed using omicsNPC. a) Fisher combining function was employed. The diagnostic plots are, from left to right: (i) a quantile-quantile (Q-Q) plot of the p-values for each study

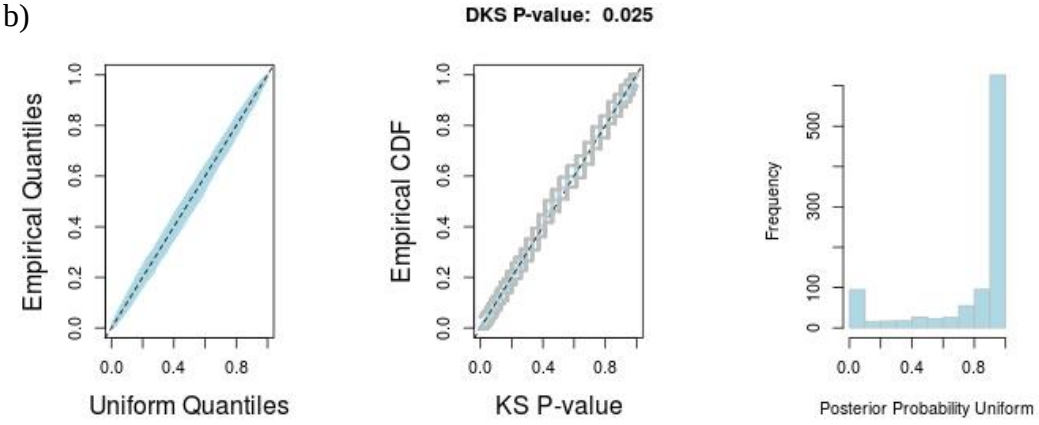
versus the uniform quantiles, these lines should be close to the 45º diagonal; lines below the diagonal indicate optimist results, lines above the diagonal conservatives ones; (ii) the empirical distribution function of the first level KS test p-values (blue) with confidence bands (grey), again the line should fall along the 45º line; (iii) a histogram of the posterior probabilities that each set of p-values is uniform these values should be near one. b) Liptak combining function. The order of diagnostic plots is the same as a). c) Tippett combining function. The order of diagnostic plots is the same as a). In all cases omicsNPC produced calibrated p-values. Q-Q plots' lines fall on the 45º line, the empirical distribution function of the first level KS test fallen also on the 45º line, the histogram of the posterior probabilities was near one and the dks p-values were not significant, as expected.

a)



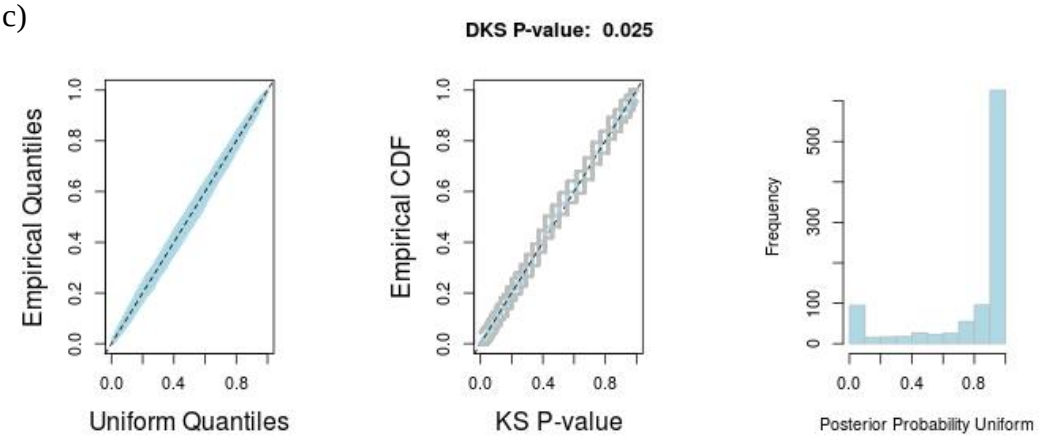
$NPC^{no\text{-}correction}$ - Fisher

b)



$NPC^{no\text{-}correction}$ - Liptak

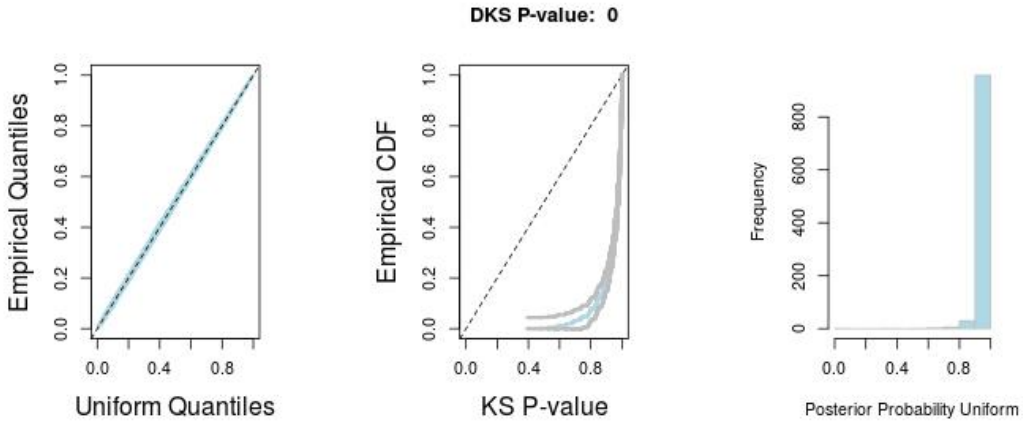
c)



$NPC^{no\text{-}correction}$ - Tippett

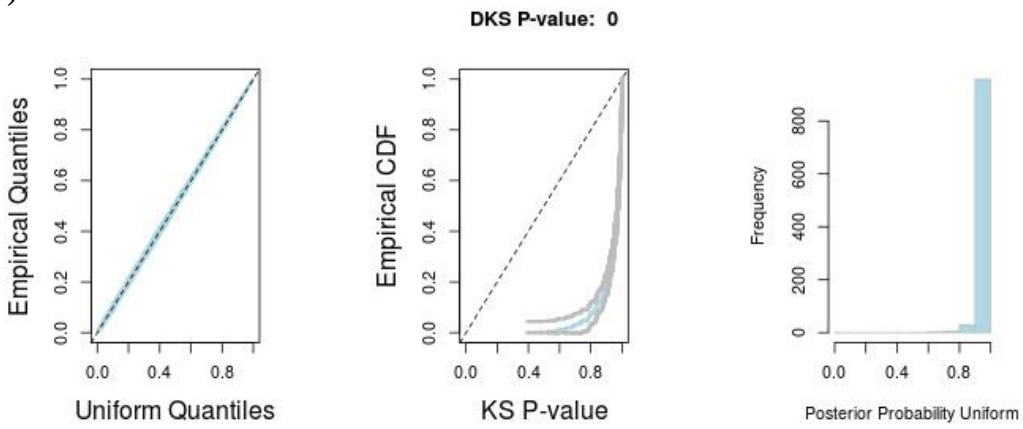
Fig H: JNC's diagnostics plots on correlated samples employing NPC$^{\text{no-correction}}$. Details as in Fig G. In all cases NPC produced slightly un-calibrated p-values. Although Q-Q plots' lines fall on the 45º line and the empirical distribution function of the first level KS test fallen also on the 45º line, the histogram of the posterior probabilities was sightly deviated between zero and one. Last, all dks p-values were statistical significant and one order of magnitude less than omicsNPC dks p-values.

a)



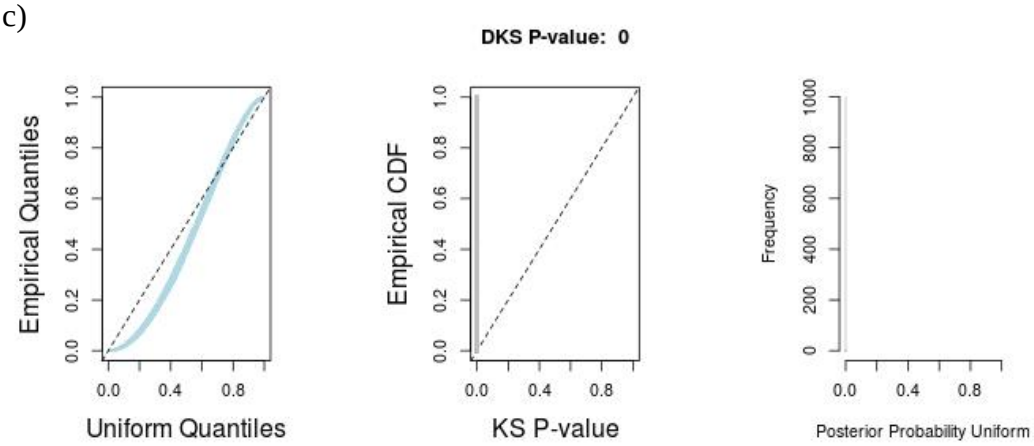
omicsNPC - RankSum

b)



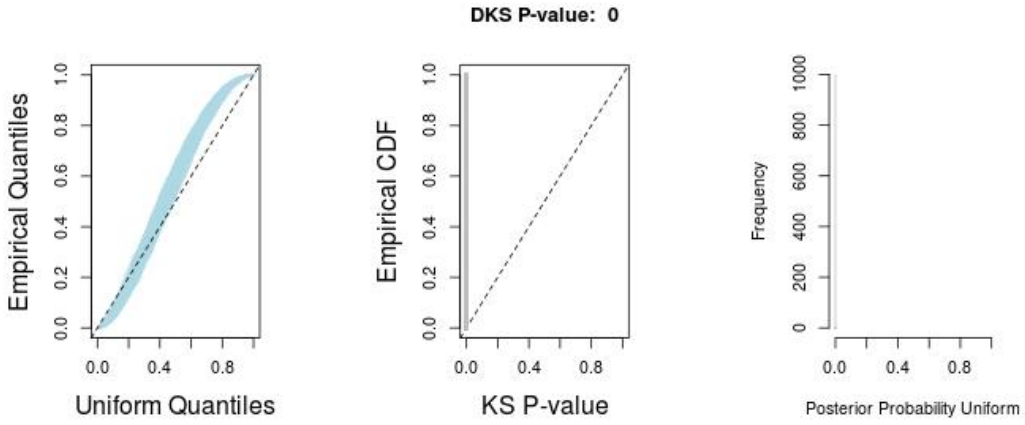
omicsNPC - RankProd

c)



RankProd

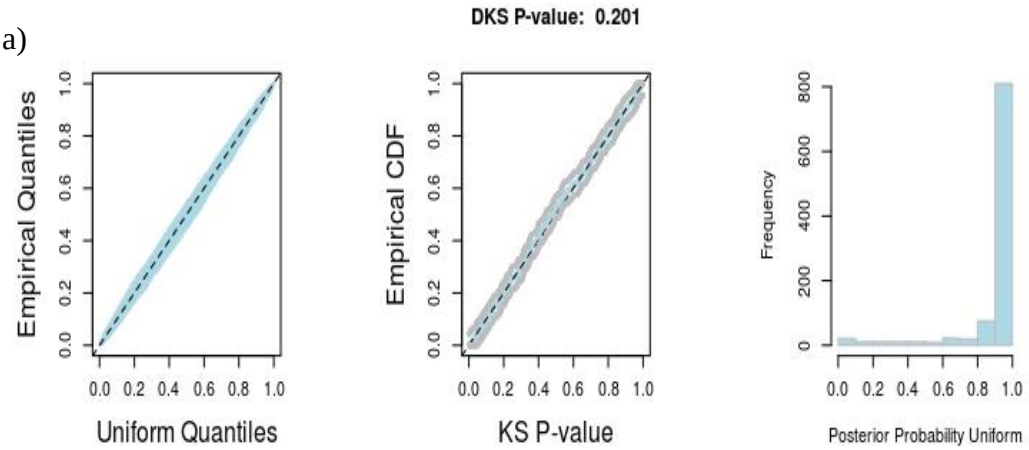
Fig I: JNC's diagnostics plots on correlated samples employing rank approaches.
Details as in Fig G. In all cases the dks p-value is highly significant.



CP – Combining P-values

Fig J: JNC's diagnostics plots on correlated samples employing combining p-values (CP) approaches. Details as in Fig G. The dks p-value is highly significant, and the KS p-values are clearly shifted towards zero.

a)



Benjamini

Fig K: JNC's diagnostics plots on correlated samples employing Benjamini approach. Details as in Fig F. The Benjamini method produced calibrated p-values: Q-Q plots' lines fall on the 45º line, the empirical distribution function of the first level KS test fallen also on the 45º line, the histogram of the posterior probabilities was near one and the dks p-values were not significant, as expected.

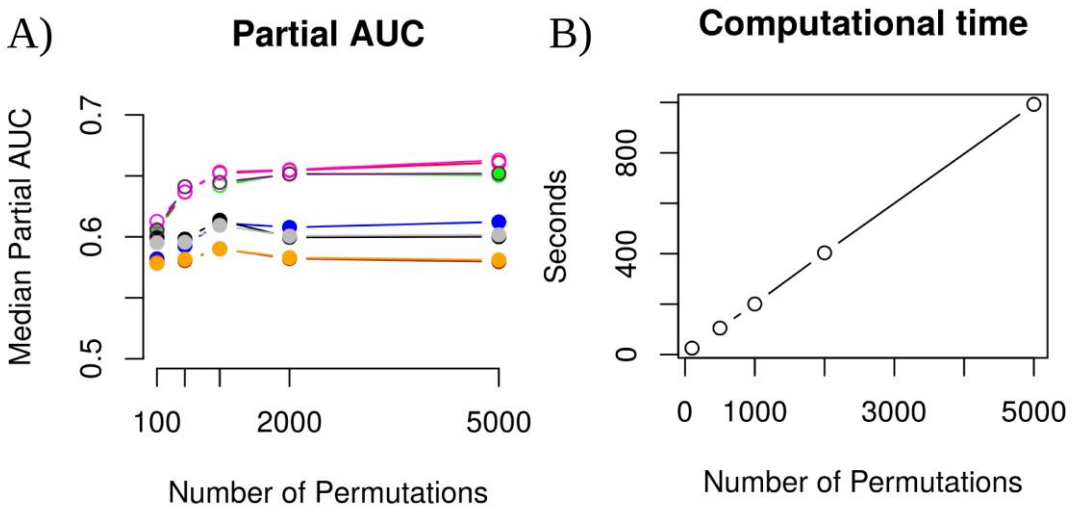


Fig L: Performance and computational time in relation to the number of permutations. A) The figure illustrates the median pAUCs of each algorithm in the Different Modalities scenario, when all genes were taken into account. Each line corresponds to a specific method, which analysed the data in combination. Specifically, red – omicsNPC$^{\text{Fisher}}$, green – omicsNPC$^{\text{Tippett}}$, blue – omicsNPC$^{\text{Liptak}}$, dark magenta – Benjamini, black omicsNPC$^{\text{RankSum}}$, brown – omicsNPC$^{\text{RankProd}}$, orange – RankProd, magenta – CP, grey – RankSum_scores. The x axis represents the number of permutations and the y axis the median pAUCs. Filled circles suggest that the observed differences from the best method were statistical significant at level 0.05. B) The figure illustrates the computational time of omicsNPC in relation with the number of permutations. As evident the computational time has a linear relation to the number of permutations.

Table A: median partial AUCs and significance for increasing sample size and genes (not) deregulated over all data modalities (Different Modalities scenario).

	pAUC, s= 4	Significance	pAUC, s= 6	Significance	pAUC, s= 8	Significance	pAUC, s=10	Significance
voom+limma	0.614	0	0.687	0	0.621	0	0.638	0
Limma	0.572	0	0.592	0	0.611	0	0.598	0
Chi-square test	0.597	0	0.556	0	0.576	0	0.551	0
Wilcoxon rank sum	0.558	0	0.604	0	0.587	0	0.555	0
OmicsNPC-Fisher	0.668	0	0.756	0.114	0.715	0.081	0.7	0.277
OmicsNPC-Liptak	0.66	0	0.702	0	0.69	0	0.673	0
OmicsNPC-Tippett	0.608	0	0.705	0	0.647	0	0.666	0
OmicsNPC-RankSum	0.707	0.052	0.73	0	0.707	0.005	0.678	0
OmicsNPC-RankProd	0.673	0	0.691	0	0.675	0	0.647	0
RankProd	0.679	0	0.692	0	0.676	0	0.646	0
CP	**0.723**	**1**	**0.77**	**1**	**0.724**	**1**	**0.71**	**1**
Benjamini, u=1	0.627	0	0.714	0	0.652	0	0.666	0
RankSum Scores	0.708	0.174	0.735	0	0.709	0.008	0.678	0

Table legend: the table illustrates the median pAUCs of each algorithm in the Different Modalities scenario, on genes that have the same behavior across all modalities, i.e., they are either deregulated in all datasets or in none of them. Each row corresponds to a specific method. The first four rows report the results for each data modality analysed in isolation, whereas the remaining rows report the results obtained from the integrative analysis methods. Labels "pAUCs, s=X" represents the median values for X samples. Significance columns show the p-values from the Wilcoxon two sided tests between each method and the best one. The best performing method for each configuration is reported in bold.

Table B: median partial AUCs and significance for increasing sample size and all genes (Different Modalities scenario).

	pAUC, s= 4	Significance	pAUC, s= 6	Significance	pAUC, s= 8	Significance	pAUC, s= 10	Significance
OmicsNPC-Fisher	0.614	0	0.68	0.461	0.656	0.499	0.647	0.583
OmicsNPC-Liptak	0.59	0	0.62	0	0.607	0	0.603	0
OmicsNPC-Tippett	0.592	0	0.682	0.242	0.639	0	0.651	1
OmicsNPC-RankSum	0.617	0	0.631	0	0.612	0	0.603	0
OmicsNPC-RankProd	0.594	0	0.61	0	0.593	0	0.584	0
RankProd	0.599	0	0.611	0	0.593	0	0.585	0
CP	**0.638**	**1**	**0.688**	**1**	**0.657**	**1**	0.65	0.883
Benjamini, u=1	0.6	0	0.684	0.429	0.642	0	**0.652**	**1**
RankSum Scores	0.618	0	0.633	0	0.614	0	0.601	0

Table legend: the table illustrates the median pAUCs of each algorithm in the Different Modalities scenario, when all genes were taken into account. Each row corresponds to a specific method. The column "pAUCs, s=X" represents the median values, X equals the number of samples. Significance columns show the p-values from the Wilcoxon two sided tests between each method and the best one. The best performing method for each configuration is reported in bold.

Table C: median partial AUCs and significance for different number of modalities and genes (not) deregulated over all data modalities (Different Modalities scenario).

	pAUC, m = 2	Significance	pAUC, m = 3	Significance	pAUC, m = 4	Significance
OmicsNPC-Fisher	0.663	0.277	0.675	0.659	0.696	0.355
OmicsNPC-Liptak	0.651	0.001	0.638	0	0.663	0
OmicsNPC-Tippett	0.659	0.017	0.645	0	0.651	0
OmicsNPC-RankSum	0.656	0.017	0.638	0	0.676	0
OmicsNPC-RankProd	0.644	0	0.619	0	0.646	0
RankProd	0.644	0	0.621	0	0.646	0
CP	**0.668**	**1**	**0.681**	**1**	**0.699**	**1**
Benjamini, u=1	0.659	0.018	0.646	0.001	0.656	0
RankSum Scores	0.651	0.001	0.638	0	0.672	0

Table legend: the table illustrates the median pAUCs of each algorithm in the Different Modalities scenario, on genes that have the same behavior across all modalities, i.e., they are either deregulated in all datasets or in none of them. Each row corresponds to a specific method. The column "pAUCs, m = X" represents the median values, X equals the number of modalities used in the analysis. Significance columns show the p-values from the Wilcoxon two sided tests between each method and the best one. The best performing method for each configuration is reported in bold.

Table D: median partial AUCs and significance for different number of modalities and all genes (Different Modalities scenario).

	pAUC, m = 2	Significance	pAUC, m = 3	Significance	pAUC, m = 4	Significance
OmicsNPC-Fisher	0.654	0.547	0.654	0.841	0.645	1
OmicsNPC-Liptak	0.63	0	0.61	0	0.599	0
OmicsNPC-Tippett	0.652	0.149	0.645	0.211	0.644	0.841
OmicsNPC-RankSum	0.634	0	0.609	0	0.604	0
OmicsNPC-RankProd	0.624	0	0.595	0	0.585	0
RankProd	0.625	0	0.597	0	0.585	0
CP	**0.654**	**1**	**0.656**	**1**	0.643	0.989
Benjamini, u=1	0.652	0.183	0.646	0.231	**0.646**	**1**
RankSum Scores	0.627	0	0.608	0	0.602	0

Table legend: the table illustrates the median pAUCs of each algorithm in the Different Modalities scenario, when all genes were taken into account. Each row corresponds to a specific method. The column "pAUCs, m = X" represents the median values, X equals the number of modalities used in the analysis. Significance columns show the p-values from the Wilcoxon two sided tests between each method and the best one. The best performing method for each configuration is reported in bold.

Table E: median pAUCs and significance for increasing number of permutations (Different Modalities scenario).

	pAUC perm= 100	Significance	pAUC, perm = 500	Significance	pAUC, perm = 1000	Significance	pAUC, perm= 2000	Significance	pAUC, perm= 5000	Significance
OmicsNPC-Fisher	0.605	0.063	0.637	0.698	0.652	0.659	0.654	0.779	0.661	0.883
OmicsNPC-Liptak	0.582	0	0.593	0	0.611	0	0.608	0	0.612	0
OmicsNPC-Tippett	0.604	0.038	**0.641**	**1**	0.642	0.134	0.652	0.231	0.651	0.049
OmicsNPC-RankSum	0.599	0.001	0.598	0	0.614	0	0.6	0	0.6	0
OmicsNPC-RankProd	0.579	0	0.58	0	0.59	0	0.582	0	0.58	0
RankProd	0.578	0	0.582	0	0.59	0	0.583	0	0.581	0
CP	**0.613**	**1**	0.637	1	**0.653**	**1**	**0.655**	**1**	**0.663**	**1**
Benjamini, u=1	0.606	0.121	0.641	0.925	0.645	0.201	0.651	0.301	0.652	0.076
RankSum Scores	0.595	0	0.596	0	0.609	0	0.601	0	0.602	0

Table legend: the table illustrates the median pAUCs of each algorithm in the Different Modalities scenario, when all genes were taken into account. Each row corresponds to a specific method. The column "pAUCs, perm = X" represents the median values, X equals the number of permutations used in the analysis. Significance columns show the p-values from the Wilcoxon two sided tests between each method and the best one. The best performing method for each configuration is reported in bold.

Table F: median pAUCs and significance (Correlated Modalities scenario).

	pAUC, cor=0.6	Signifi-cance	pAUC, cor=0.7	Signifi-cance	pAUC, cor=0.8	Signifi-cance	pAUC, cor=0.9	Signifi-cance	pAUC, cor=1	Signifi-cance
Single dataset 1 – Limma	0.595	0	0.596	0	0.592	0	0.595	0	0.597	0
Single dataset 2 – Limma	0.593	0	0.596	0	0.601	0	0.604	0	0.598	0
OmicsNPC-Fisher	**0.647**	**1**	0.648	0.678	0.647	0.968	**0.649**	**1**	**0.651**	**1**
OmicsNPC-Liptak	0.646	1	**0.649**	**1**	**0.648**	**1**	0.649	0.779	0.651	0.602
OmicsNPC-Tippett	0.619	0	0.622	0	0.62	0	0.626	0	0.623	0
OmicsNPC-RankSum	0.62	0	0.627	0	0.625	0	0.631	0	0.633	0
OmicsNPC-RankProd	0.618	0	0.626	0	0.622	0	0.631	0	0.632	0
RankProd	0.584	0	0.576	0	0.558	0	0.548	0	0.534	0
CP	0.614	0	0.605	0	0.593	0	0.586	0	0.579	0
Benjamini, u=1	0.625	0	0.633	0	0.633	0.015	0.645	0.094	0.627	0
RankSum Scores	0.587	0	0.576	0	0.558	0	0.55	0	0.537	0

Table legend: the table illustrates the median pAUCs of each algorithm in the correlated modalities scenario. Each row corresponds to a specific method. The column "pAUCs, cor = X" represents the median values, X represents the level of correlation introduced in the data. Significance columns show the p-values from the Wilcoxon two sided tests between each method and the best one. The best performing method for each configuration is reported in bold.

Table G. Differentially Expressed Genes in the BRCA data.

FDR	RNAseq	RNAseqV2	Exp-Gene	Fisher	Liptak	Tippett
0.01	5205	5178	3756	5523	5284	5823
0.03	6437	6406	4936	6890	6633	7195
0.05	7116	7091	5674	7721	7429	8027
0.07	7594	7596	6236	8312	8012	8603
0.1	8153	8155	6851	8982	8656	9270
0.11	8331	8332	7048	9192	8857	9448

Table legend: results according to different single-dataset analyses or omicsNPC with different combining functions (columns) and various FDR thresholds in the range [0.1 – 0.11] (rows). Combining data types with omicsNPC leads to a higher number of finding, independently by the threshold and combining function used.