

completeOlink - Manual

Here is an easy, step-wise guide to the use of completeOlink webserver for **OLINK** proteomics data analysis

A. Data upload:

Olink View
Outlier Detection
Filter Data
Statistics
Post-hoc Statistics
Pathway Enrichment
Additional Visualization

Data table: raw
Data table: meta
Data table: complete
Data table: panel metrics
Data table: group-wise panel metrics
About/ Citation

Show 10 entries
Search:

SampleID	Index	OlinkID	UniProt	Assay	MissingFreq	Panel_Version	PlatID	QC
All		A	A		All	All	All	A
1 A1	1	OID01216	O00533	CHL1	0.01875	v.1201	Example_Data_1_CAM.csv	Pas
2 A2	2	OID01216	O00533	CHL1	0.01875	v.1201	Example_Data_1_CAM.csv	Pas
3 A3	3	OID01216	O00533	CHL1	0.01875	v.1201	Example_Data_1_CAM.csv	Pas
4 A4	4	OID01216	O00533	CHL1	0.01875	v.1201	Example_Data_1_CAM.csv	Pas
5 A5	5	OID01216	O00533	CHL1	0.01875	v.1201	Example_Data_1_CAM.csv	Pas
6 A6	6	OID01216	O00533	CHL1	0.01875	v.1201	Example_Data_1_CAM.csv	Pas
7 A7	7	OID01216	O00533	CHL1	0.01875	v.1201	Example_Data_1_CAM.csv	Pas
8 A8	8	OID01216	O00533	CHL1	0.01875	v.1201	Example_Data_1_CAM.csv	Pas

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(a) Demo data upload

Olink View
Outlier Detection
Filter Data
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Data table: raw
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Show 10 entries
Search:

SampleID	Index	Sample_Type	OlinkID	UniProt	Assay	MissingFreq	Panel	Panel_Version	PlatID
All		All	A	A		All	All	All	All
1 Sample Control1	4	CONTROL	OID00471	P10145	IL8	0	Inflammation	v.3023	13627181
2 Sample Control1	4	CONTROL	OID00472	P15692	VEGFA	0	Inflammation	v.3023	13627181
3 Sample Control1	4	CONTROL	OID05124	P01732	CD8A	0	Inflammation	v.3023	13627181
4 Sample Control1	4	CONTROL	OID00474	P80098	MCP-3	0.46875	Inflammation	v.3023	13627181
5 Sample Control1	4	CONTROL	OID00475	P39905	GDNF	0	Inflammation	v.3023	13627181
6 Sample Control1	4	CONTROL	OID00476	Q9H5V8	CDCP1	0	Inflammation	v.3023	13627181
7 Sample Control1	4	CONTROL	OID00477	Q9BZW8	CD244	0	Inflammation	v.3023	13627181
8 Sample Control1	4	CONTROL	OID00478	P13232	IL7	0	Inflammation	v.3023	13627181

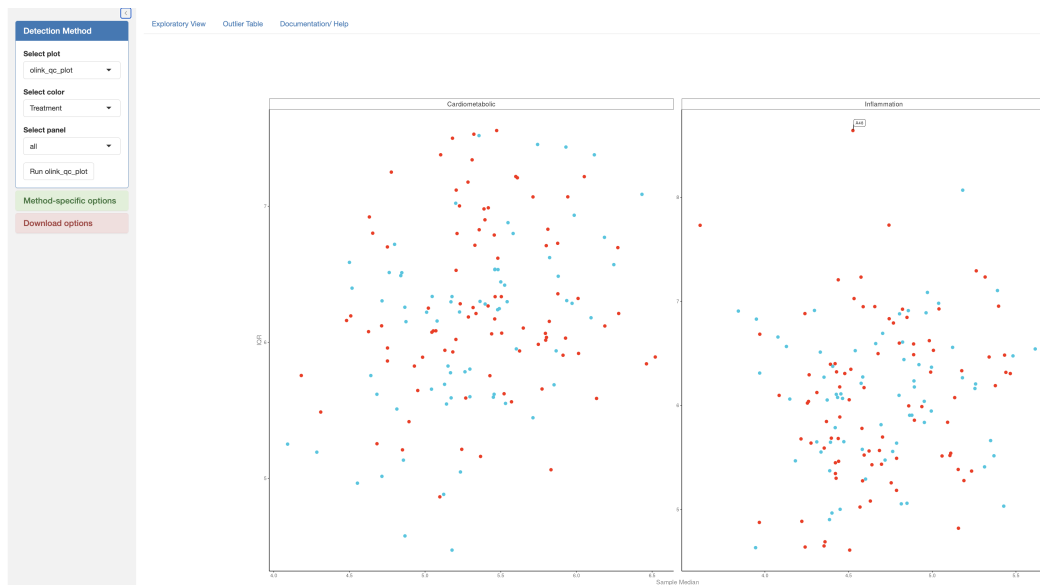
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(b) User data upload

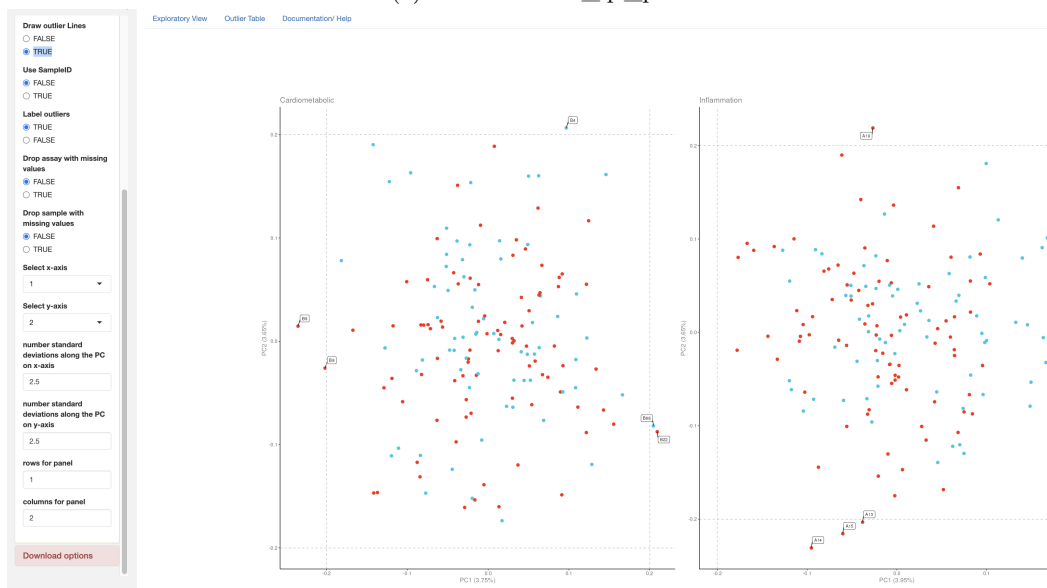
Figure 1: Olink View tab

The **Olink View** tab allows data upload. User can optionally use the demo data by selecting “Yes” for “Use demo data”. Figure 1 depicts both scenarios.

User needs to upload the csv file (the CSV file is characterized by the presence of “;” as a delimiter instead of comma) and a sample manifest file (regular CSV file) where information about each unique “SampleID” in the npx raw data file is available. SampleID (s) are required to be character strings.



(a) method: olink_qc_plot



(b) method: olink_pca_plot

Figure 2: Outlier Detection tab (demo data; color: Treatment)

B. Outlier detection: Three outlier detection methods are available with control over parameters (optional) for accurate detection. The user can choose any of the three methods to find outliers or this step can be entirely skipped. See Figure 2. Note: If this step is used the outliers that are found in the most recent run/execution, are propagated to the next analysis step. Although, the user can choose to use the original data instead of the filtered data at every subsequent step. Results of **olink_umap_plot** can change in each run because of the inherent stochasticity of UMAP method.

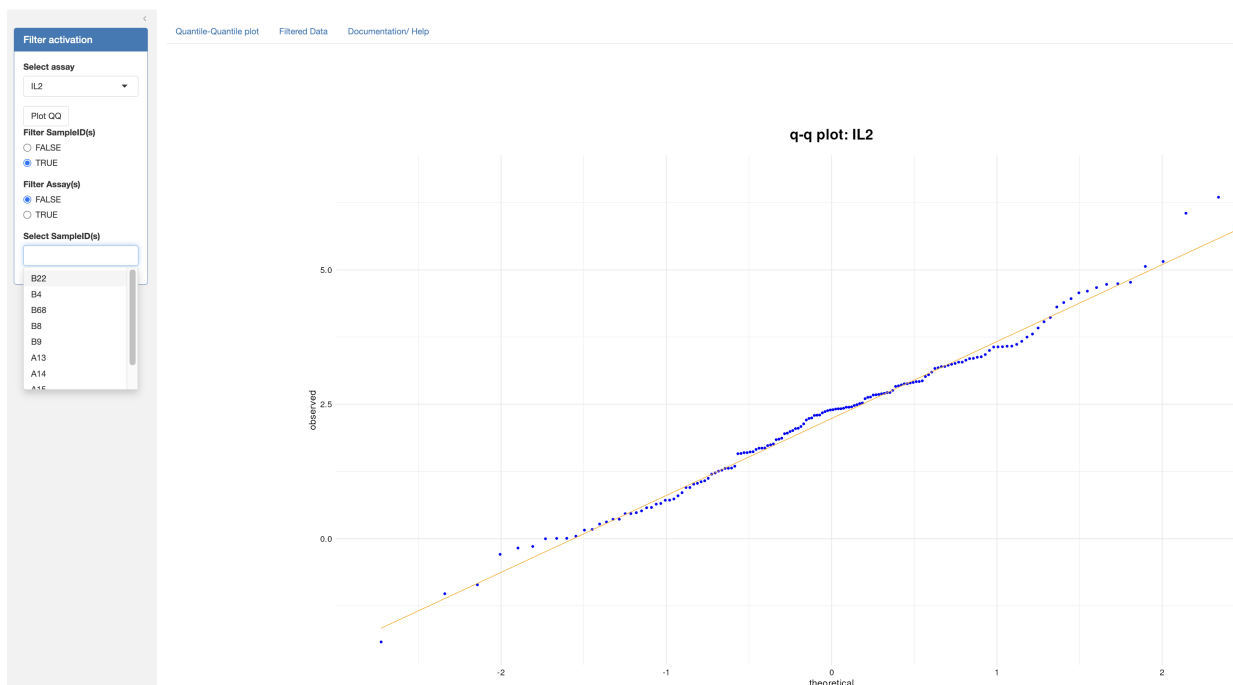


Figure 3: Filter Data tab (demo data)

C. Filter Data: While the filtering step is optional, the user has the choice of removing samples (outliers from outlier detection step B) as well as assays (having non-parametric or non-standard distribution) before further analysis. See Figure 3. Qualitative feature of each assay is captured using quartile-quartile plot, a standard way to detect if there are departures from normal distribution (non-parametric distribution).

D. Statistics: Statistical analysis methods offered in the application can be broadly grouped as “parametric” and “non-parametric” statistics. Parametric statistical tests can be further grouped as tests where “Outcome factor with exactly two levels” and “Outcome factor with greater than two levels”. The former comprises of t-test (olink_ttest) [See Figure 4.] whereas the latter includes analysis of variance (anova; olink_anova)[See Figure 5.] and linear mixed effects model (lmer; olink_lmer).

The corresponding “non-parametric” test for “Outcome factor with exactly two levels” is the Mann-Whitney U test (olink_wilcox), while for “Outcome factor with greater than two levels” the tests are Kruskal-Wallis test/ Friedman test (olink_one_non_parametric) and two-way ordinal analysis of variance (olink_ordinalRegression). Both olink_ttest and olink_wilcox results can be used to generate volcano plots corresponding to the test outcome. In addition to statistical tests, this section allows user to generate heatmaps for a choice of grouping variable. Clustering can be invoked both on rows and/or columns, and the user can choose to use only significant assays obtained in the most recently run/executed statistical test.

E. Post-hoc Statistics: All statistical tests in the group “Outcome factor with greater than two levels”[See Figure 6] irrespective of distribution, require post hoc analysis before meaningful results are generated. All active (chosen) functions have their corresponding usage information available in a tab labelled “Documentation/ Help”.

F. Pathway Enrichment: Following t-test or Mann-Whitney U test or the appropriate post-hoc analysis, the user has the option of performing pathway enrichment using over-representation analysis (ORA) or gene set enrichment analysis (GSEA)[See Figure 8].

G. Additional Visualization: Lastly, significant assays can be visualized using boxplots and the p-value significance added to the plot in case t-test were previously performed. Download handlers have been implemented for easy download of all result tables. Figures generated during analysis can be customized for size and downloaded in three file formats.

Method

Use filtered data

☒ FALSE
☐ TRUE

Select panel

all

Select variable

Treatment

Select test

olink_test

Parameters

P-value cutoff

0.05

P-value column to use

Adjusted_pval

Pair-id

☐ TRUE
☒ FALSE

Run olink test

Plot

Heatmap

Download Options

Statistical test result

Statistical test plot

Statistical test log

Documentation/ Help

Heatmap

ComplexHeatmap Documentation/ Help

Inner visualization

Copy CSV Excel Show All 10 entries

Search

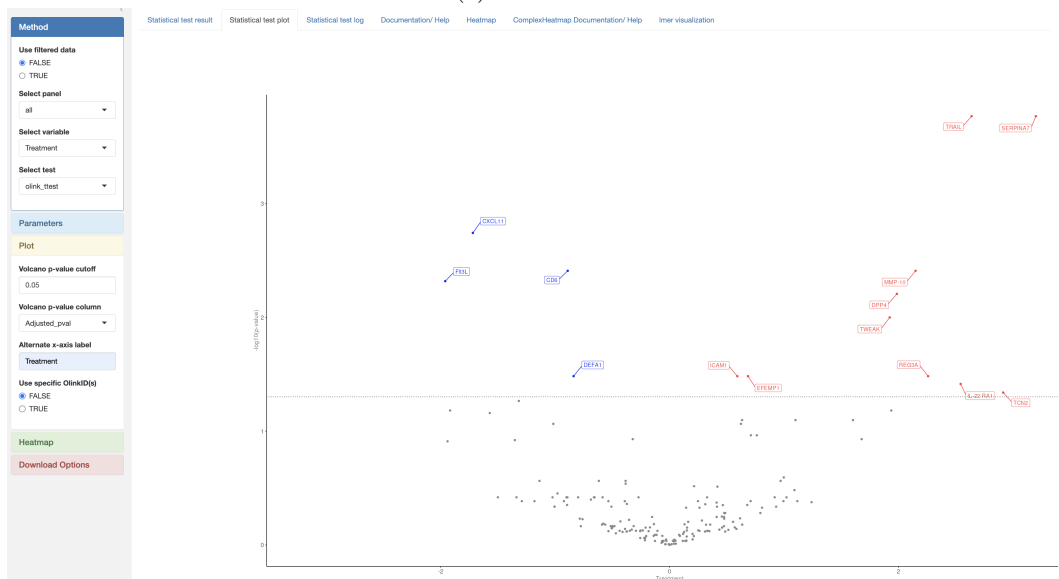
olink test

	Assay	OlinkID	UniProt	Panel	estimate	Untreated	Treated	statistic	p-value	parameter	conf.low	conf.high	method	alternative	Adjusted_pval	Threshold
	A	P	A	All	All	All	A	A	All	All	All	All	A	All	All	All
1	TRAIL	OID00488	P50591	Olink Inflammation	2.639	10.04	7.4	4.97	0.00001851103913620632	145.745	1.59	3.689	Welch Two Sample t-test	two.sided	0.0001710813215703923	Up
2	SERPINA7	OID01232	P05543	Olink Cardiometabolic	3.201	12.26	9.059	4.979	0.0000185957958286872	139.422	1.93	4.472	Welch Two Sample t-test	two.sided	0.0001710813215703923	Up
3	CXCL11	OID00486	O14625	Olink Inflammation	-1.719	3.897	5.616	-4.31	0.0002957411100113864	148.867	-2.507	-0.931	Welch Two Sample t-test	two.sided	0.00181387890809837	Down
4	MMP-10	OID00527	P08238	Olink Inflammation	2.15	11.348	9.188	4.055	0.00008482225458731057	133.107	1.101	3.199	Welch Two Sample t-test	two.sided	0.003903180191504221	Up
5	CD6	OID00499	Q6WUJ7	Olink Inflammation	-0.89	1.844	2.734	-3.989	0.0001060646791169625	141.105	-1.331	-0.449	Welch Two Sample t-test	two.sided	0.003903180191504221	Down
6	IFI3L	OID00533	P49771	Olink Inflammation	-1.96	4.171	6.131	-3.879	0.0001568019151478983	148.828	-2.959	-0.962	Welch Two Sample t-test	two.sided	0.004812272064535549	Down
7	DPP4	OID01266	P27487	Olink Cardiometabolic	1.987	6.221	4.235	3.766	0.0002367703108137154	151.567	0.844	3.029	Welch Two Sample t-test	two.sided	0.00622367674138091	Up
8	TWEAK	OID00555	O43508	Olink Inflammation	1.924	10.056	8.132	3.595	0.0004357291155828183	153.654	0.867	2.981	Welch Two Sample t-test	two.sided	0.01002176905840482	Up
9	EFEMP1	OID01281	Q12805	Olink Cardiometabolic	0.686	2.775	2.09	3.201	0.001664181597611961	153.746	0.293	1.109	Welch Two Sample t-test	two.sided	0.03290615774754727	Up
10	REG3A	OID01280	Q06141	Olink Cardiometabolic	2.259	9.486	7.227	3.149	0.001999788833208386	142.217	0.841	3.678	Welch Two Sample t-test	two.sided	0.03290615774754727	Up

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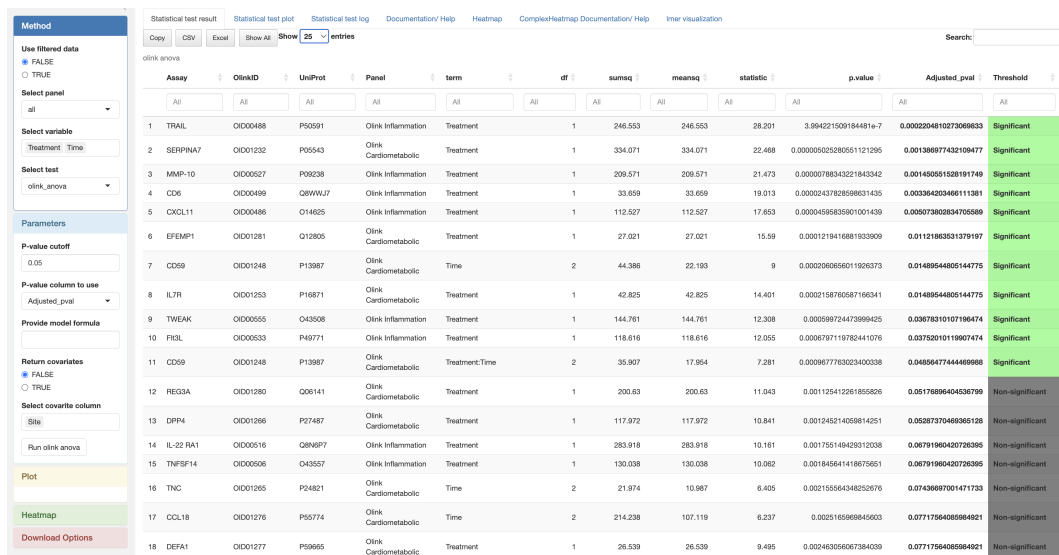
Previous 1 2 3 4 5 ... 19 Next

(a) t-test result

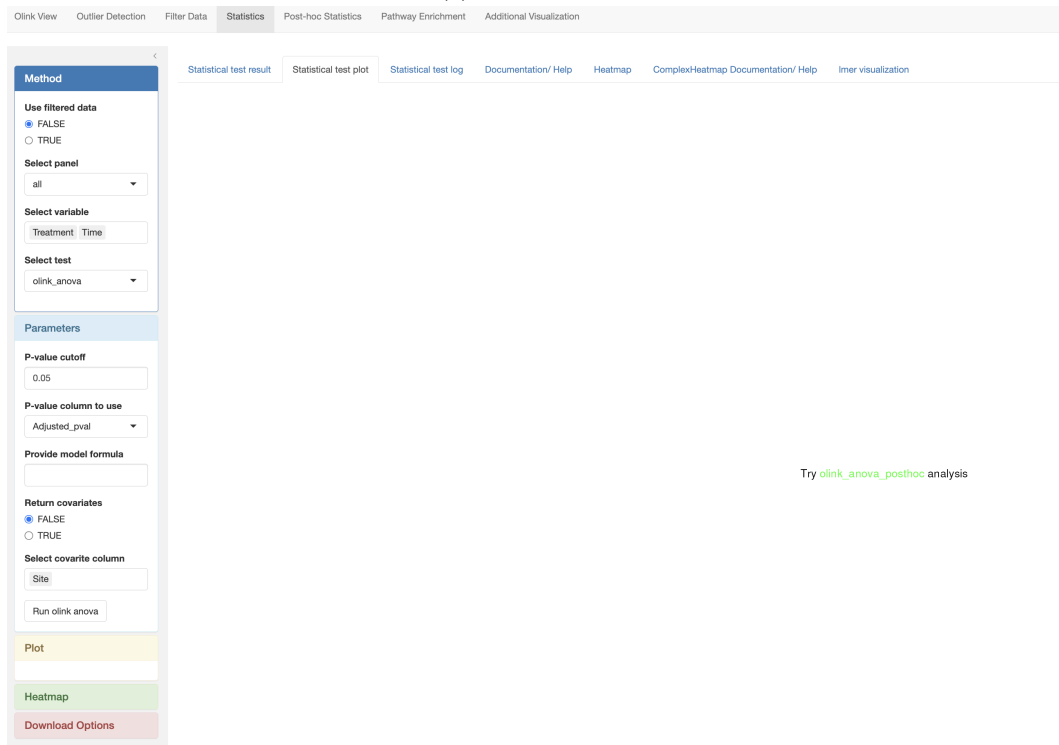


(b) t-test plot

Figure 4: Statistics tab (two-level variable)



(a) ANOVA result



(b) ANOVA plot

Figure 5: Statistics tab (>two-level variable)

Link View Outlier Detection Filter Data Statistics **Post-hoc Statistics** Pathway Enrichment Additional Visualization

Posthoc Statistical test result Posthoc Statistical test log Documentation/ Help

Copy CSV Excel Show All Show 25 entries Search:

olink_anova_posthoc

Assay OlinkID UniProt Panel term contrast estimate conf.low conf.high Adjusted_pval Threshold

1	SERPINA7	OID01232	P05543	Olink Cardiometabolic	Treatment:Time	Treated Week 12 - Untreated Week 6	-5.795	-8.959	-2.632	0	Significant
2	TRAIL	OID00488	P05091	Olink Inflammation	Treatment:Time	Untreated Baseline - Treated Week 12	4.239	1.813	6.665	0	Significant
3	SERPINA7	OID01232	P05543	Olink Cardiometabolic	Treatment:Time	Treated Week 12 - Untreated Week 12	-5.346	-8.509	-2.183	0	Significant
4	TRAIL	OID00488	P05091	Olink Inflammation	Treatment:Time	Treated Week 12 - Untreated Week 12	-4.041	-6.466	-1.615	0	Significant
5	TRAIL	OID00488	P05091	Olink Inflammation	Treatment:Time	Treated Week 12 - Untreated Week 6	-3.853	-6.279	-1.428	0	Significant
6	CD6	OID00499	Q8WWJ7	Olink Inflammation	Treatment:Time	Treated Baseline - Untreated Week 6	1.645	0.554	2.737	0	Significant
7	SERPINA7	OID01232	P05543	Olink Cardiometabolic	Treatment:Time	Untreated Baseline - Treated Week 12	4.765	1.602	7.929	0	Significant
8	CD6	OID00499	Q8WWJ7	Olink Inflammation	Treatment:Time	Treated Week 12 - Untreated Week 6	1.621	0.529	2.712	0	Significant
9	MMP-10	OID00527	P08238	Olink Inflammation	Treatment:Time	Treated Baseline - Untreated Baseline	-3.633	-6.196	-1.07	0.001	Significant
10	PSL	OID00533	P49771	Olink Inflammation	Treatment:Time	Treated Baseline - Untreated Week 12	3.363	0.79	5.936	0.003	Significant
11	MMP-10	OID00527	P08238	Olink Inflammation	Treatment:Time	Untreated Baseline - Treated Week 6	3.074	0.511	5.636	0.009	Significant
12	MMP-10	OID00527	P08238	Olink Inflammation	Treatment:Time	Treated Baseline - Untreated Week 6	-2.972	-5.535	-0.409	0.013	Significant
13	CXCL11	OID00486	O14625	Olink Inflammation	Treatment:Time	Treated Week 6 - Untreated Week 6	2.355	0.284	4.426	0.016	Significant
14	SERPINA7	OID01232	P05543	Olink Cardiometabolic	Treatment:Time	Treated Baseline - Treated Week 12	3.687	0.403	6.971	0.018	Significant
15	MMP-10	OID00527	P08238	Olink Inflammation	Treatment:Time	Treated Baseline - Untreated Week 12	-2.861	-5.424	-0.298	0.019	Significant

(a) different effect & filter term

Link View Outlier Detection Filter Data Statistics **Post-hoc Statistics** Pathway Enrichment Additional Visualization

Posthoc Statistical test result Posthoc Statistical test log Documentation/ Help

Copy CSV Excel Show All Show 10 entries Search:

olink_anova_posthoc

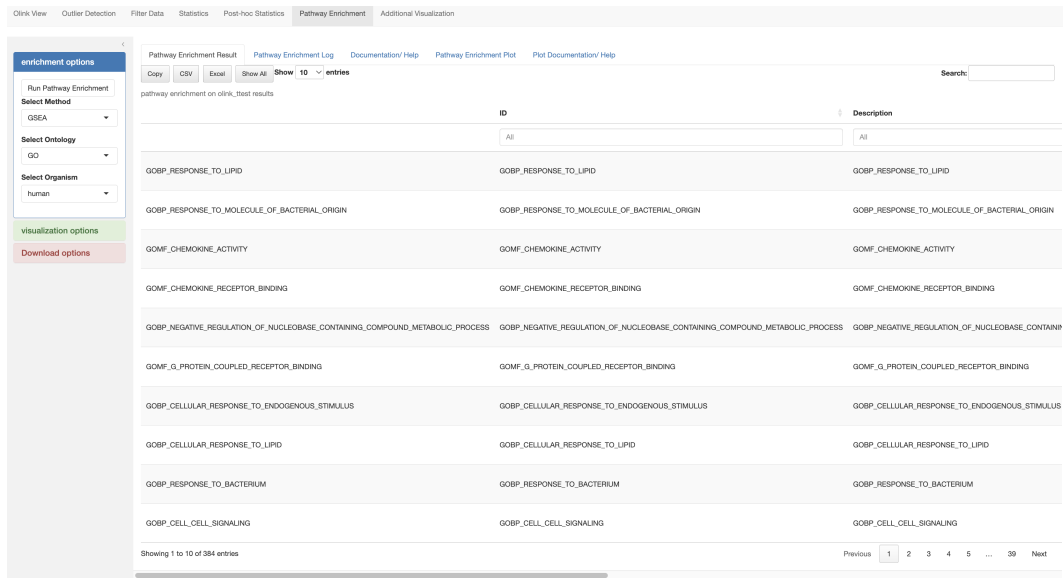
Assay OlinkID UniProt Panel term contrast estimate conf.low conf.high Adjusted_pval Threshold

1	CD59	OID01248	P13987	Olink Cardiometabolic	Treatment:Time	Treated Week 12 - Treated Week 6	-2.395	-3.733	-1.058	0	Significant
2	CD59	OID01248	P13987	Olink Cardiometabolic	Treatment:Time	Untreated Week 12 - Treated Week 6	-1.665	-2.954	-0.377	0.004	Significant
3	CD59	OID01248	P13987	Olink Cardiometabolic	Treatment:Time	Treated Baseline - Treated Week 6	-1.605	-2.943	-0.268	0.009	Significant
4	CD59	OID01248	P13987	Olink Cardiometabolic	Treatment:Time	Treated Week 6 - Untreated Week 6	1.43	0.142	2.718	0.02	Significant
5	CD59	OID01248	P13987	Olink Cardiometabolic	Treatment:Time	Untreated Baseline - Treated Week 12	1.277	-0.012	2.565	0.054	Non-significant
6	CD59	OID01248	P13987	Olink Cardiometabolic	Treatment:Time	Untreated Baseline - Treated Week 6	-1.119	-2.407	0.17	0.129	Non-significant
7	CD59	OID01248	P13987	Olink Cardiometabolic	Treatment:Time	Treated Week 12 - Untreated Week 6	-0.965	-2.253	0.323	0.261	Non-significant
8	CD59	OID01248	P13987	Olink Cardiometabolic	Treatment:Time	Treated Baseline - Treated Week 12	0.79	-0.547	2.127	0.53	Non-significant
9	CD59	OID01248	P13987	Olink Cardiometabolic	Treatment:Time	Untreated Week 12 - Untreated Week 12	-0.73	-2.018	0.558	0.576	Non-significant
10	CD59	OID01248	P13987	Olink Cardiometabolic	Treatment:Time	Untreated Baseline - Untreated Week 12	0.547	-0.644	1.738	0.77	Non-significant

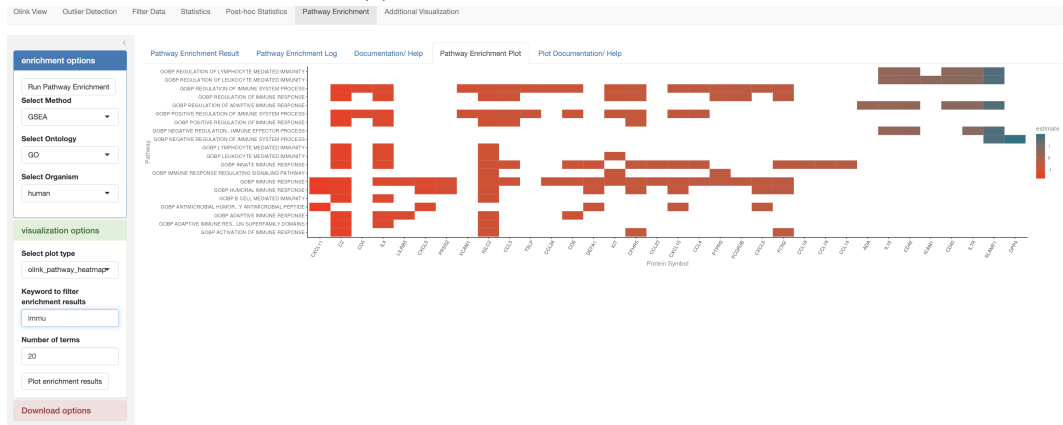
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(b) same effect & filter term

Figure 6: Post-hoc Statistics tab (>two-level variable)

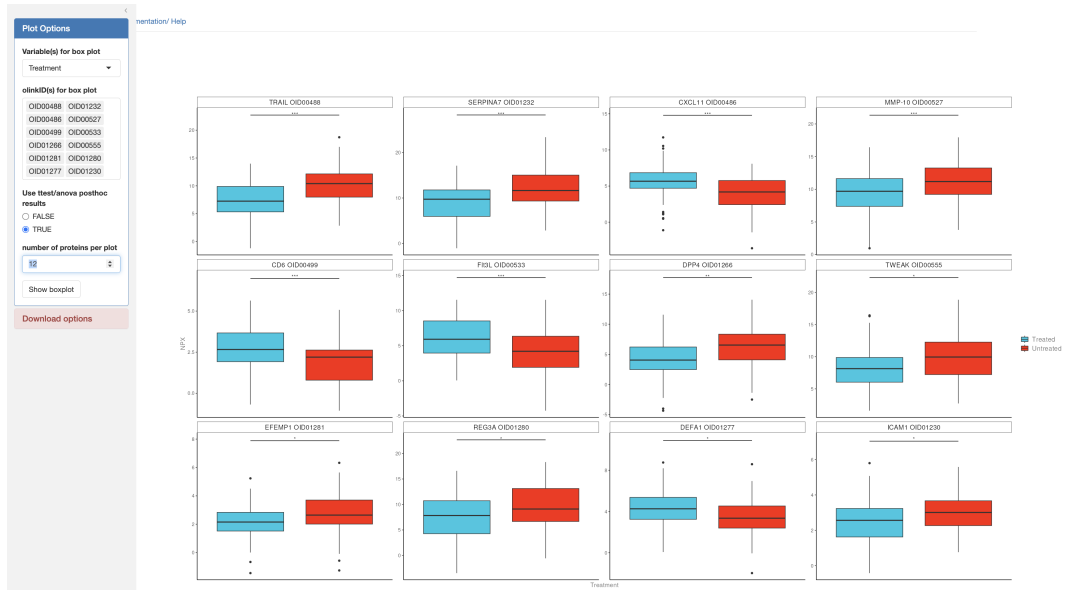


(a) Gene Set Enrichment Results

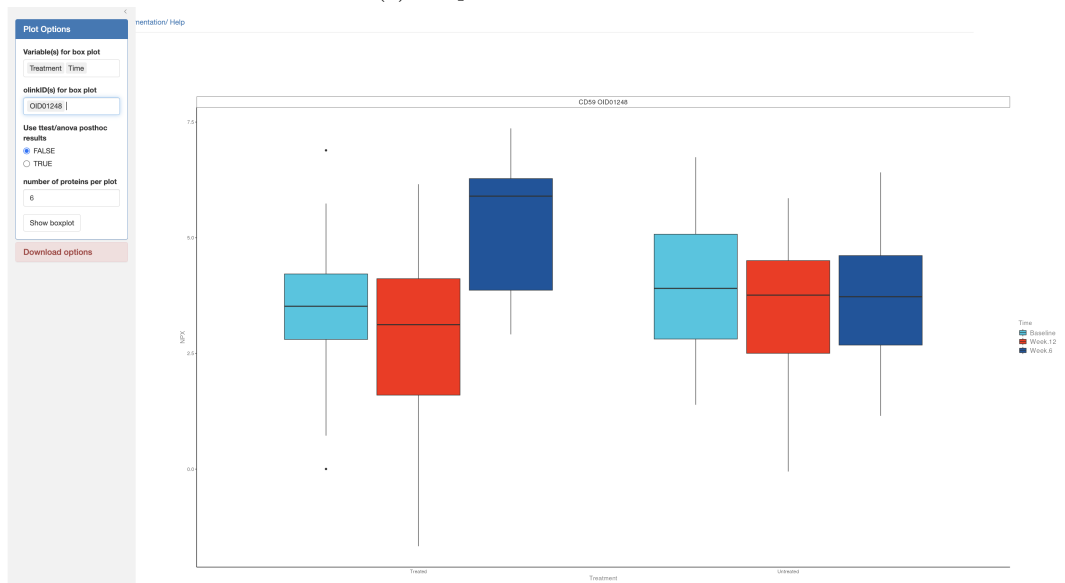


(b) Gene Set Enrichment Heatmap

Figure 7: Pathway Enrichment tab (demo data t-test results)



(a) Boxplot with t-test results



(b) Boxplot with ANOVA results

Figure 8: Additional Visualization tab (demo data)