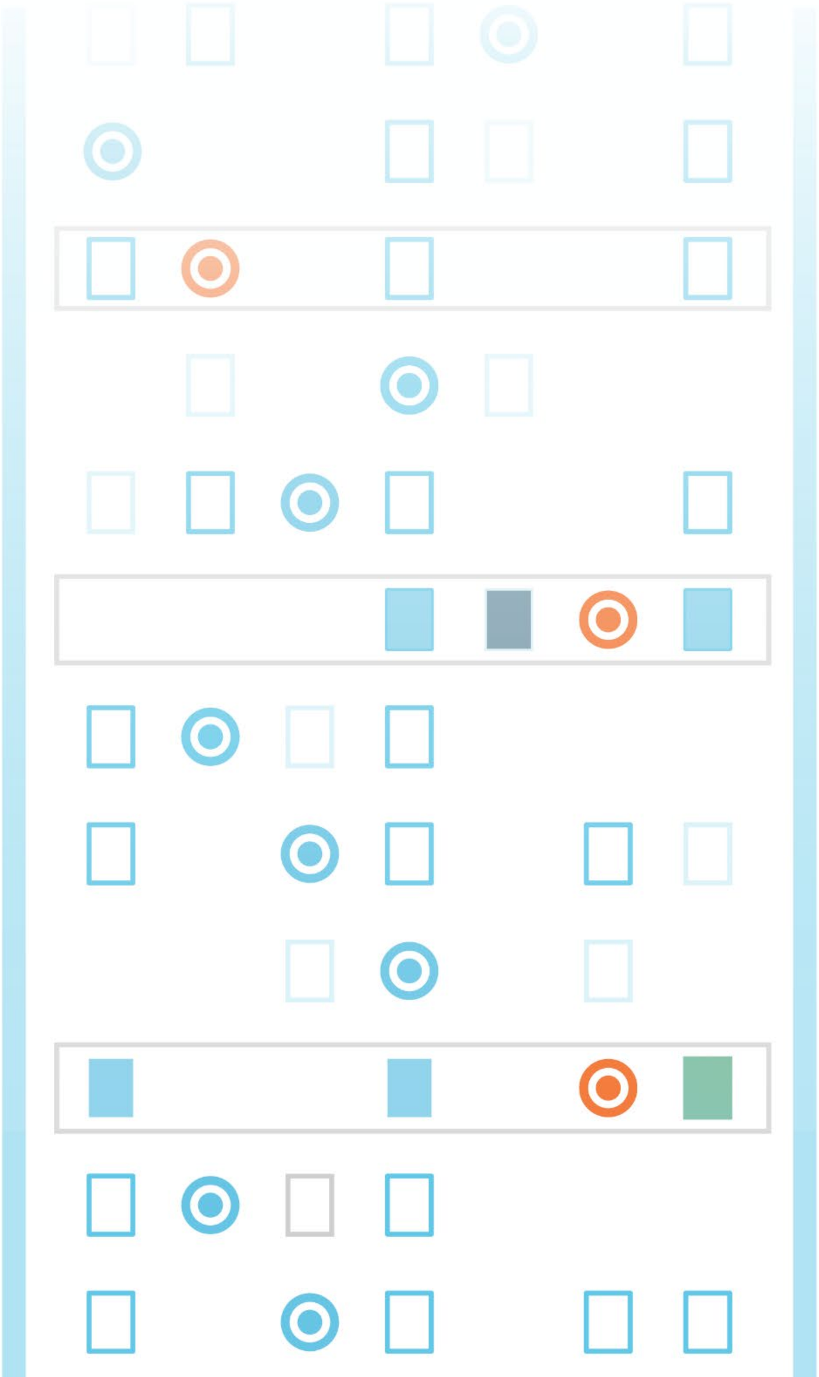
IsoSpeak Guide: Generating and Exporting Reports with IsoSpeak

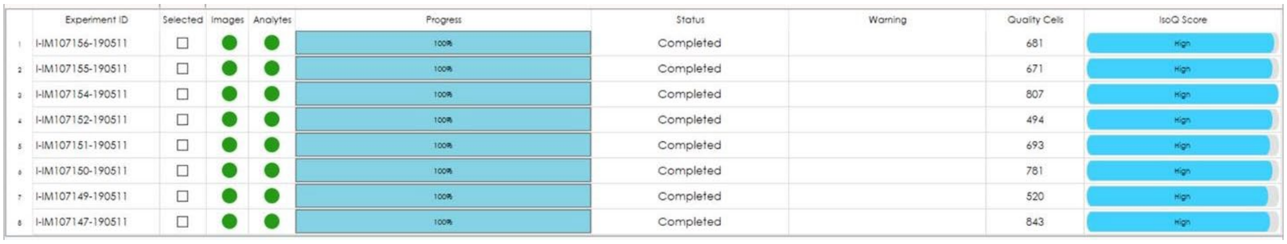


Rev 1.5

1. Pre-Report:

Single-Cell IsoQ Score:

The IsoQ Score is an automated quality metric for your samples generated by the IsoSpeak. Samples are analyzed for cell quality during this phase and given an IsoQ Score. All samples should have a blue IsoQ Score indicating that the sample quality passes, and the data can proceed through the automated IsoSpeak analysis.



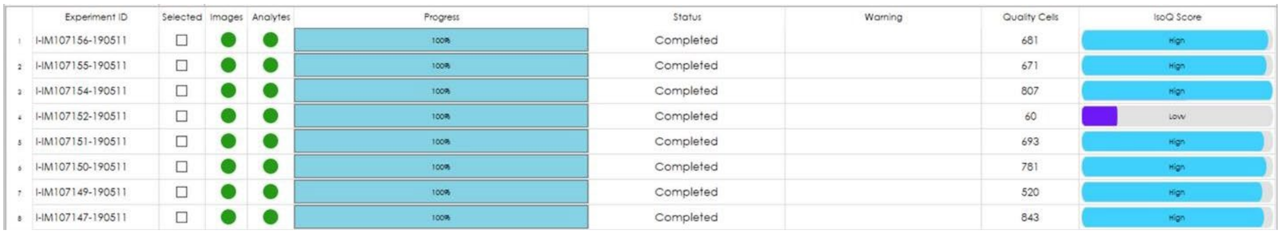
	Experiment ID	Selected	Images	Analytes	Progress	Status	Warning	Quality Cells	IsoQ Score
1	I-IM107156-190511	☐	●	●	100%	Completed		681	High
2	I-IM107155-190511	☐	●	●	100%	Completed		671	High
3	I-IM107154-190511	☐	●	●	100%	Completed		807	High
4	I-IM107152-190511	☐	●	●	100%	Completed		494	High
5	I-IM107151-190511	☐	●	●	100%	Completed		693	High
6	I-IM107150-190511	☐	●	●	100%	Completed		781	High
7	I-IM107149-190511	☐	●	●	100%	Completed		520	High
8	I-IM107147-190511	☐	●	●	100%	Completed		843	High

Troubleshooting:

The IsoQ score should be used to judge the success of sample analysis of a given condition. The metric takes into account a combination of sample-related elements required to meet specifications, per IsoPlexis protocols, to successfully gather single-cell proteomic data. The components of the IsoQ score correlate to effective sample staining, stimulation, loading, viability, and other sample characteristics.

Similar samples falling under the same experimental condition should generally be grouped together when using the IsoQ score to evaluate sample quality and resulting data quality.

If purple IsoQ scores occur, it is likely that for any number of reasons certain sample requirements were not met, causing a failure to obtain accurate, single-cell proteomic data across these samples. This is an indication that the condition had notable issues which will likely require contacting IsoPlexis customer support to assist with troubleshooting, and replicating this subset of the run to confirm accuracy of results.



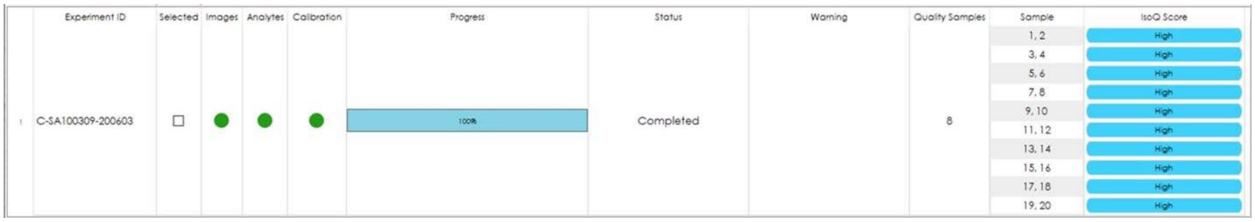
	Experiment ID	Selected	Images	Analytes	Progress	Status	Warning	Quality Cells	IsoQ Score
1	I-IM107156-190511	☐	●	●	100%	Completed		681	High
2	I-IM107155-190511	☐	●	●	100%	Completed		671	High
3	I-IM107154-190511	☐	●	●	100%	Completed		807	High
4	I-IM107152-190511	☐	●	●	100%	Completed		60	Low
5	I-IM107151-190511	☐	●	●	100%	Completed		693	High
6	I-IM107150-190511	☐	●	●	100%	Completed		781	High
7	I-IM107149-190511	☐	●	●	100%	Completed		520	High
8	I-IM107147-190511	☐	●	●	100%	Completed		843	High

As a general practice, IsoPlexis will assist with troubleshooting all runs with reported purple IsoQ scores. However, if for any reason you are uncertain or concerned about the quality of your data, IsoPlexis can assist in confirming its validity and accuracy.

Users should export a log of the IsoQ scores by going to **Export -> IsoQ Score Log** and send the log to IsoPlexis customer support for further assistance.

CodePlex IsoQ Score:

The IsoQ score for CodePlex samples is an automated quality metric for each of the samples processed through IsoSpeak. An IsoQ score is displayed for each sample in each CodePlex chip (including background samples), i.e. there will be a total of ten IsoQ scores generated by the software for every chip. Samples are analyzed for intra- and inter-well consistency and issues with analyte signal saturation during the data processing phase and given a cumulative IsoQ score. Blue IsoQ scores are normal and indicate high sample and data quality. The number of samples included in the final data is indicated in the Quality Samples field. This is typically eight samples, if this is how many non-background samples were originally loaded onto the chip.



	Experiment ID	Selected	Images	Analytes	Calibration	Progress	Status	Warning	Quality Samples	Sample	IsoQ Score
1	C-SA100309-200603	☐	●	●	●	100%	Completed		8	1, 2	High
										3, 4	High
										5, 6	High
										7, 8	High
										9, 10	High
										11, 12	High
										13, 14	High
										15, 16	High
										17, 18	High
										19, 20	High

Troubleshooting:

The IsoQ score is an indicator of the reliability and accuracy of the data from a particular sample. The components of the CodePlex IsoQ score correlate to a sample's intra- and inter-well consistency and any issues with analyte signal saturation. If a reported IsoQ score is purple, there may be an issue with one or both of these parameters. As a general practice, IsoPlexis will provide guided data analysis support to help troubleshoot all samples with reported purple IsoQ scores. However, if for any reason you are uncertain or concerned about the quality of your data, IsoPlexis will gladly assist in confirming its validity and accuracy. Users should export a log of the IsoQ scores by going to Export -> IsoQ Score Log and send the log to IsoPlexis customer support for further assistance.

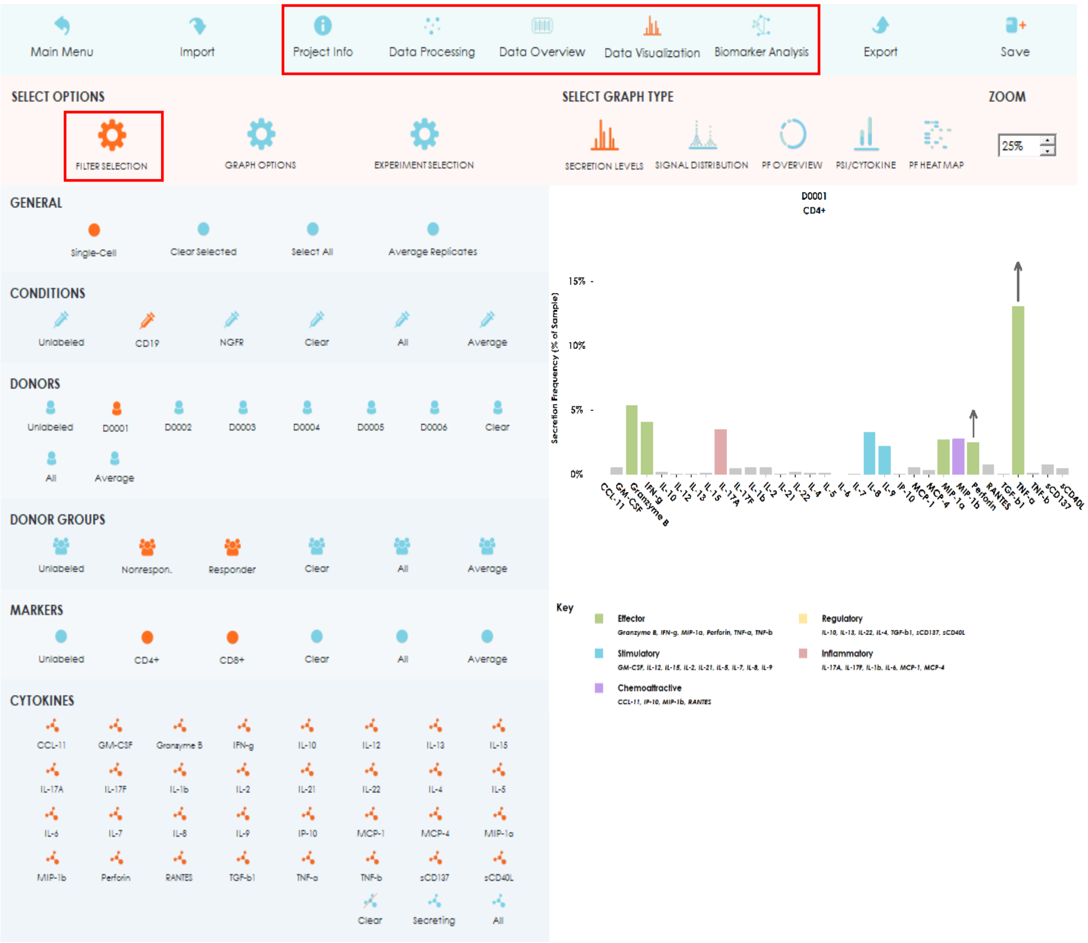
	Experiment ID	Selected	Images	Analytes	Calibration	Progress	Status	Warning	Quality Samples	Sample	IsoQ Score
1	C-SA100309-200603	☐	●	●	●	100%	Completed		8	1, 2	High
										3, 4	High
										5, 6	High
										7, 8	High
										9, 10	Low
										11, 12	High
										13, 14	High
										15, 16	High
										17, 18	High
										19, 20	High

2. Generating Reports: Annotation and Overview

IsoSpeak allows the user to explore their IsoCode data interactively at various levels of detail, from high level summary graphs to more comprehensive polyfunctional profiling.

Analytics Interface: Overview

The general interface for the bioinformatics suite in IsoSpeak is very similar whether you are in **Data Overview, Data Visualization, or Biomarker Analysis** mode. The **Main Toolbar** (top) lets you go to different program stages, as well as go back to the Main Menu.

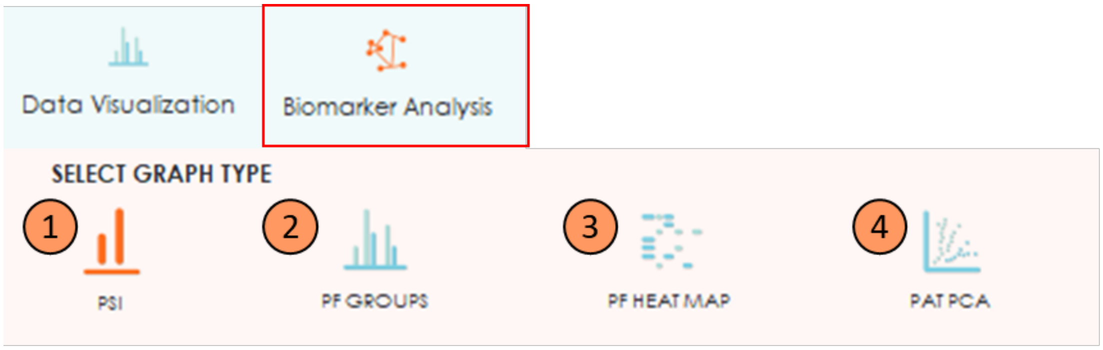


The **Filter Selection** (left) options allow you to select specific subsets of the data from the loaded experiment(s). You can filter the experiments based on stimulation conditions, donor IDs, donor groups, number of cells, cell markers, and cytokines. Adjusting the filters will automatically recreate the displayed graph or table, only showing the samples and sample subsets meeting the criteria of the selected filters.

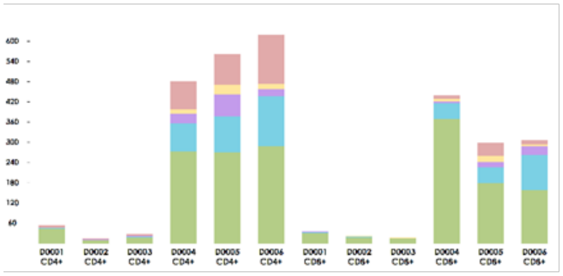
Analytics Interface: Biomarker Analysis

Biomarker analysis allows you to create an end-to-end data report.

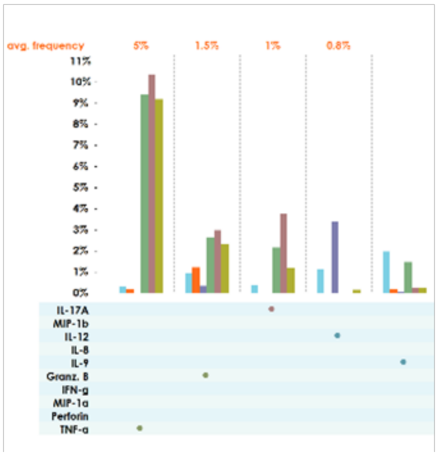
Below are types of data visualizations within the Biomarker Analysis tab:



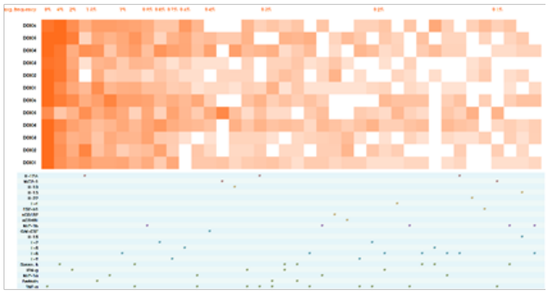
① Polyfunctional Strength Index



② Polyfunctional Groups



③ Polyfunctional Overview

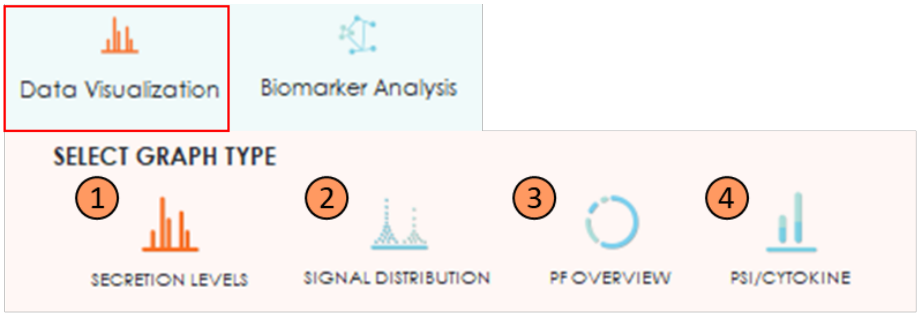


④ PAT PCA

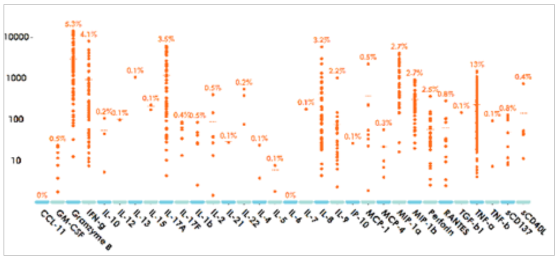
Analytics Interface: Data Visualization

Data visualization provides alternative visualizations to couple with your biomarker report.

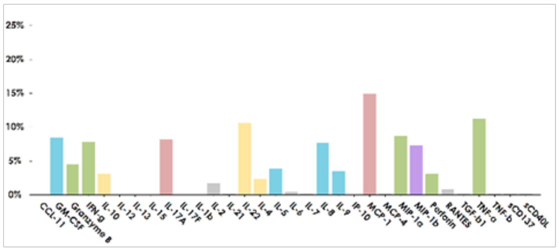
Below are types of data visualizations within the Data Visualization tab:



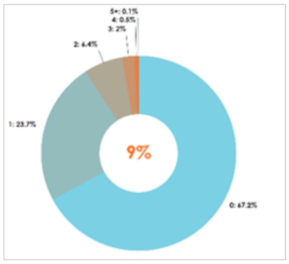
① **Cytokine Signal Intensity**



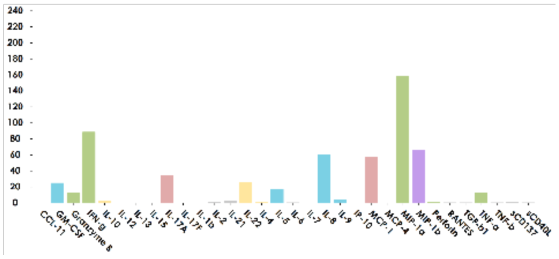
② **Cytokine Secretion Frequency**



③ **Polyfunctional Overview**



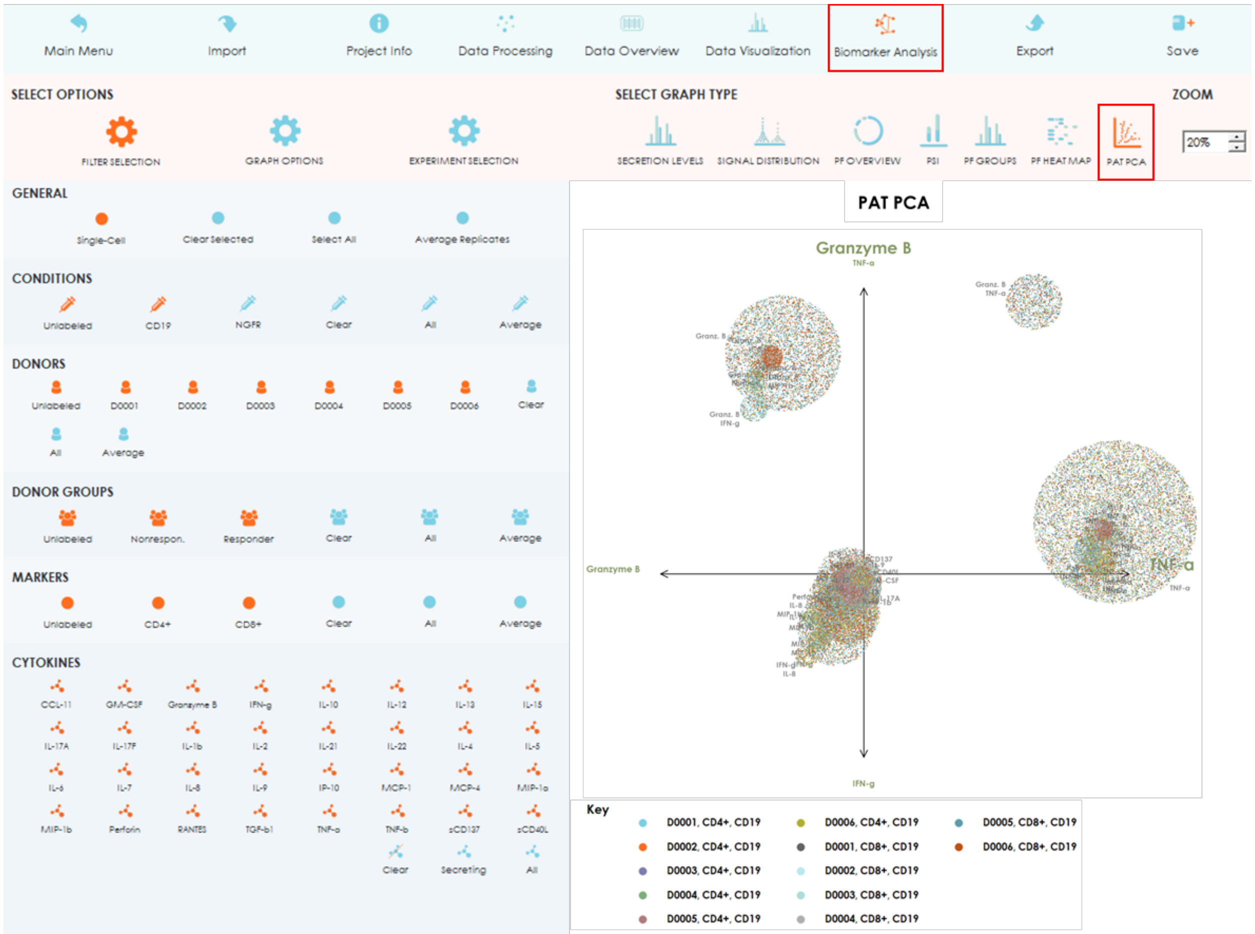
④ **Contribution to Overall PSI**



3. Assembling a Data Report: Step-by-step instructions

Data reports can be easily created by filtering, selecting, exporting using the method described in section 4. Exporting Data.

Single-Cell PAT-PCA



Method:

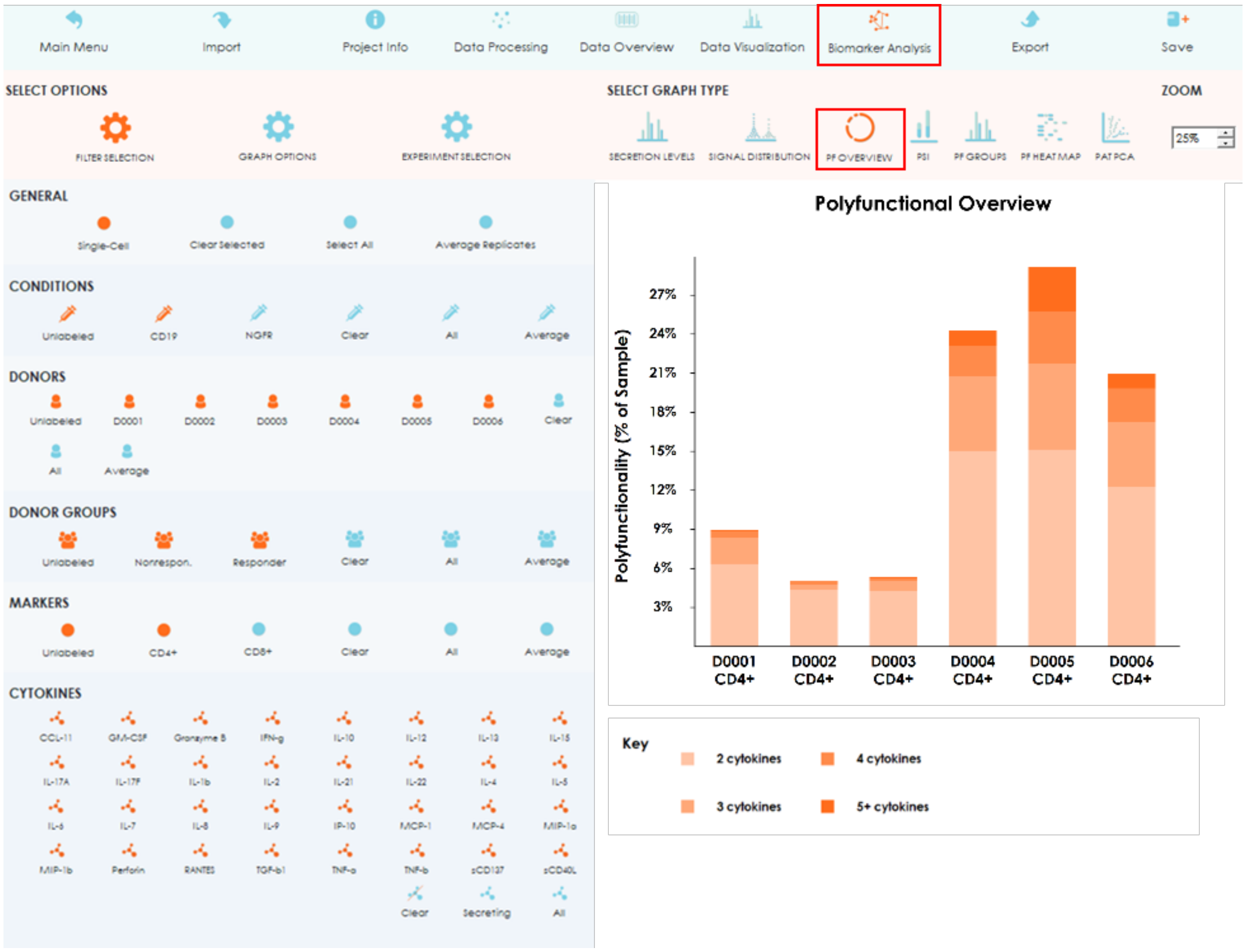
1. Select **Biomarker Analysis** in the **Main Toolbar** (top)
2. Select the **Conditions, Donors, Donor Groups, Markers,** and **Cytokines** you want to analyze
3. Select **PF Heat Map** under **Select Graph Type**
4. **Export** using the export options described in section 4. Exporting Data

Single-Cell Polyfunctional Heatmap

Method:

1. Select **Biomarker Analysis** in the **Main Toolbar** (top)
2. Select the **Conditions, Donors, Donor Groups, Markers,** and **Cytokines** you want to analyze
3. Select **PAT PCA** under **Select Graph Type**
4. **Export** using the export options described in section 4. Exporting Data

Single-Cell Polyfunctional Overview



Method:

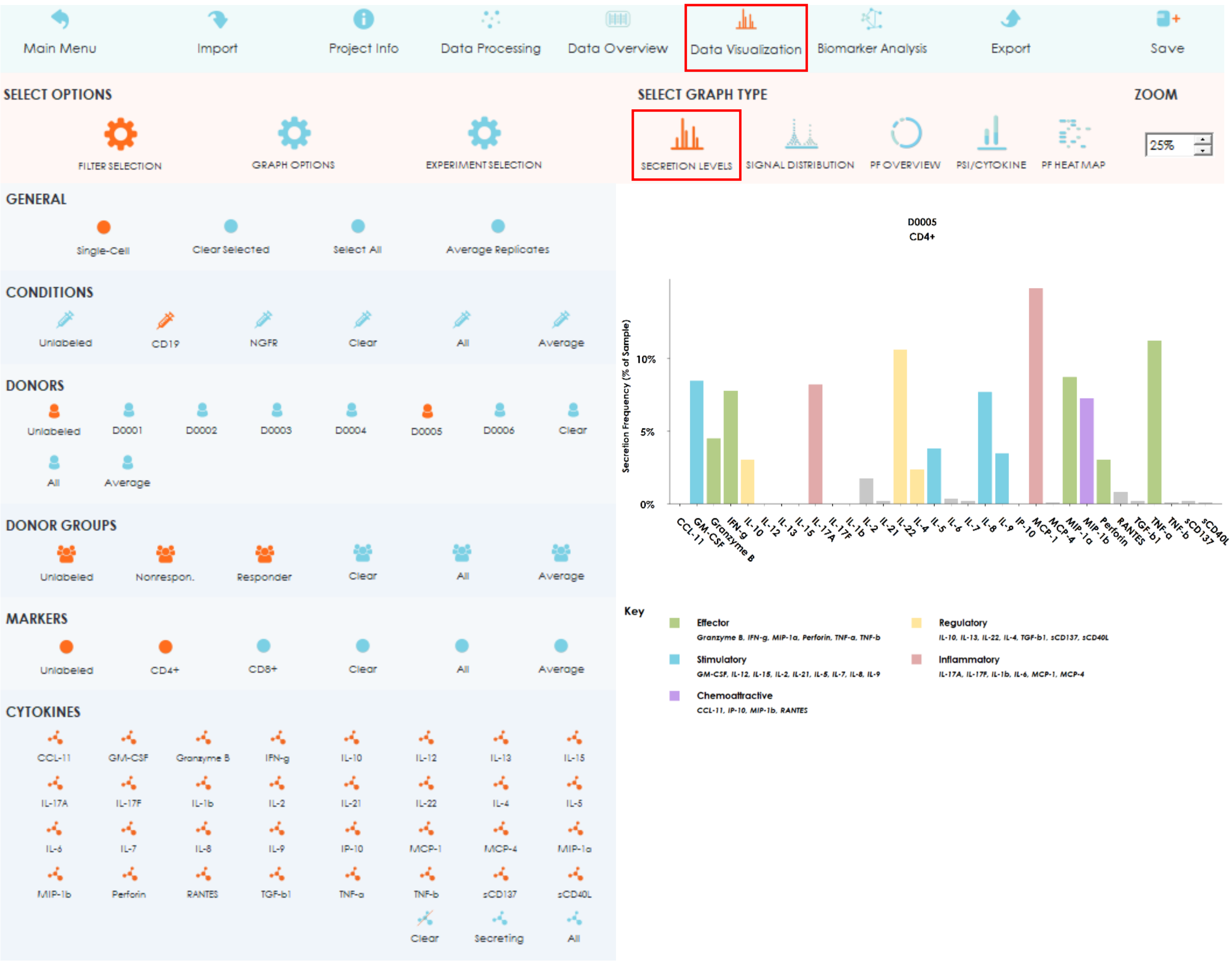
1. Select **Biomarker Analysis** in the **Main Toolbar** (top)
2. Select the **Conditions, Donors, Donor Groups, Markers,** and **Cytokines** you want to analyze
3. Select the **PF Overview** under **Select Graph Type**
4. **Export** using the export options described in section 4. Exporting Data

Single-Cell Polyfunctional Strength Index (PSI)

Method:

1. Select **Biomarker Analysis** in the **Main Toolbar** (top)
2. Select the **Conditions, Donors, Donor Groups, Markers,** and **Cytokines** you want to analyze
3. Select the **PSI** under **Select Graph Type**
4. **Export** using the export options described in section 4. Exporting Data

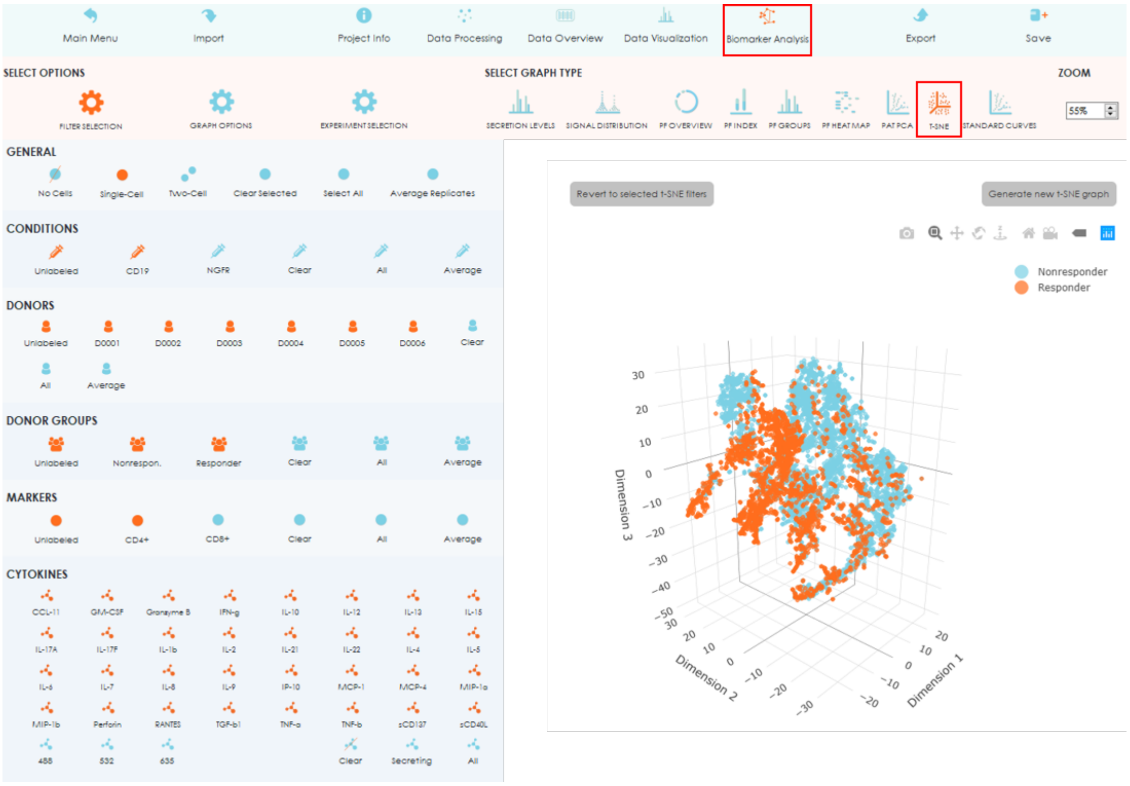
Single-Cell Polyfunctional Strength Index (PSI) Individual Contribution

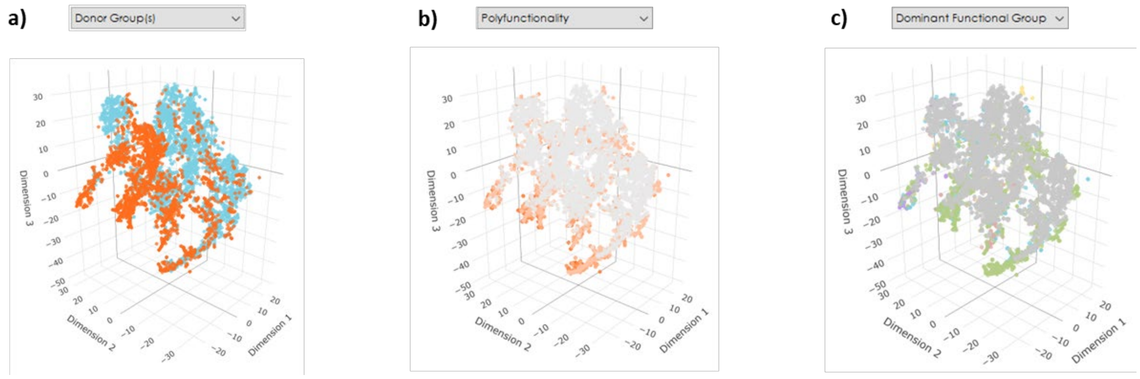


Method:

1. Select **Data Visualization** in the **Main Toolbar** (top)
2. Select the **Conditions, Donors, Donor Groups, Markers,** and **Cytokines** you want to analyze
3. Select the **Secretion Levels** under **Select Graph Type**
4. **Export** using the export options described in section 4. Exporting Data

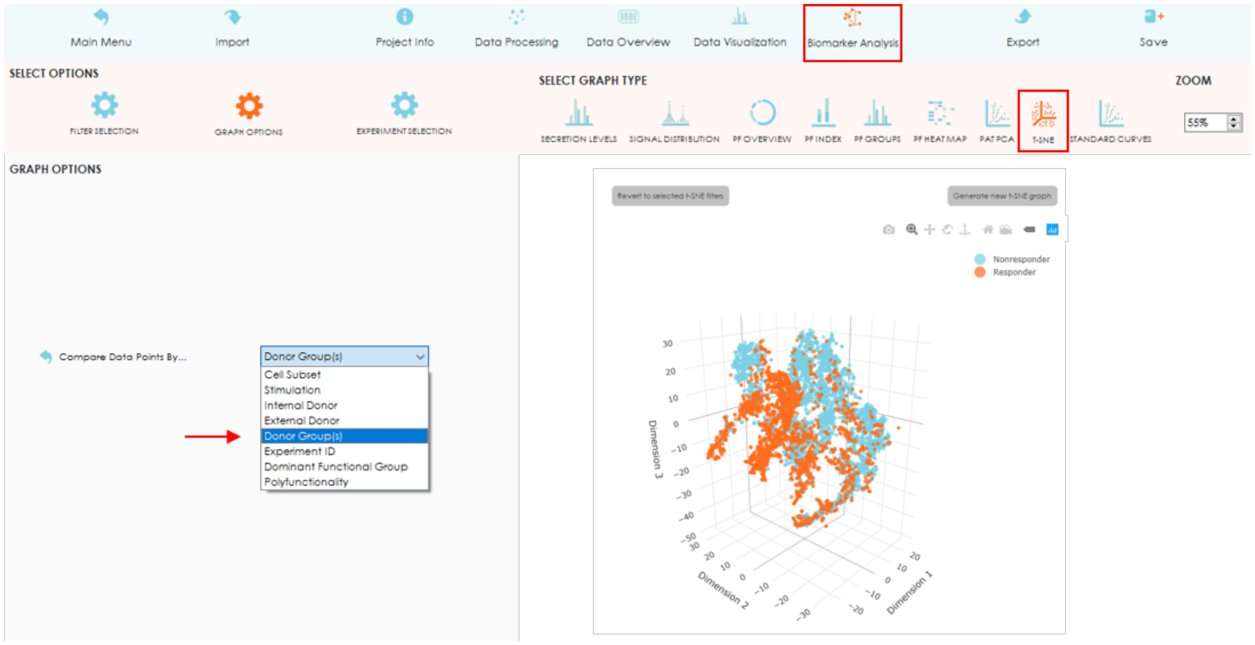
Single-Cell Cytokine Mapping 3D tSNE





The single cell cytokine mapping tSNE provides a 3D visualization of single-cell functional cytokines and a high dimensional mapping of the unique polyfunctional cell subsets. For example, the three tSNE data point comparisons above include a) Donor Groups, b) Polyfunctionality, and c) Dominant Functional Groups. Donor Group cell mapping allows users to stratify data points from samples by responders (orange) verses non-responders (blue). The Polyfunctionality cell mapping displays data points based on the degree of polyfunctionality of the samples (darker orange indicates a higher number of non-redundant cytokines secreted per single-cell). Users can hover over individual data points to get a detailed readout of the given data point including cytokine secretions, cell subsets, and additional information based on your selected filters. The Dominant Functional Group mapping displays a color-coded visualization of data points based on the cytokine profile being secreted. These include inflammatory, regulatory, chemoattractive, stimulatory and effector cytokine profiles. Nonsecretors are displayed in grey.

Single-Cell Cytokine Mapping 3D tSNE Options



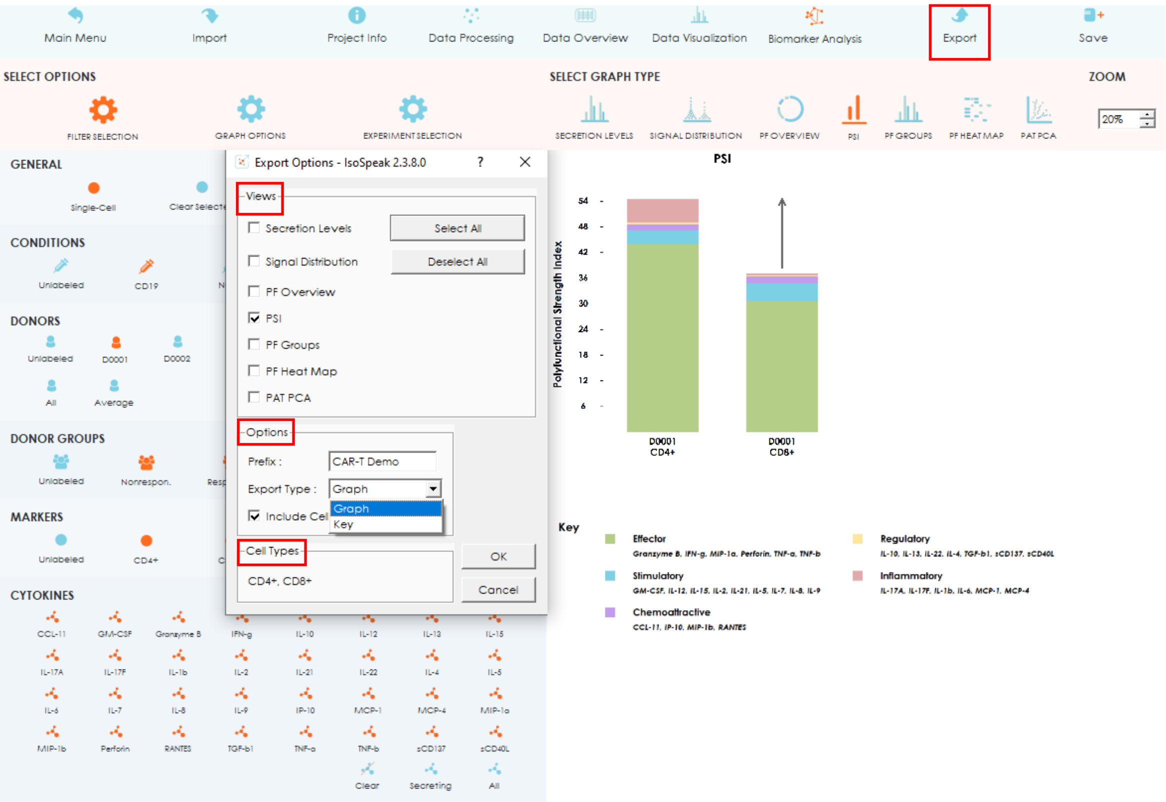
Compare Data Points By... Donor Group(s)

Method:

1. Select **Biomarker Analysis** in the **Main Toolbar** (top)
2. Select the **Conditions, Donors, Donor Groups, Markers,** and **Cytokines** you want to analyze
3. Select the **T-SNE** under **Select Graph Type**
4. Select **Generate t-SNE Graph** button at the top right to create a t-SNE visualization. Click yes to confirm
5. To change the visualization's displayed data points, select the drop-down menu under graph options

4. Exporting Data

To export any data or visualization from the **Data Overview**, **Data Visualization** or **Biomarker Analysis** modules, click on the **Export** button. You will see an **Export Options** dialog pop up, with the selected option matching the currently visible graph/table.



Export Options - IsoSpeak 2.3.8.0

Views
☐ Secretion Levels
☐ Signal Distribution
☐ PF Overview
☑ PSI
☐ PF Groups
☐ PF Heat Map
☐ PAT PCA

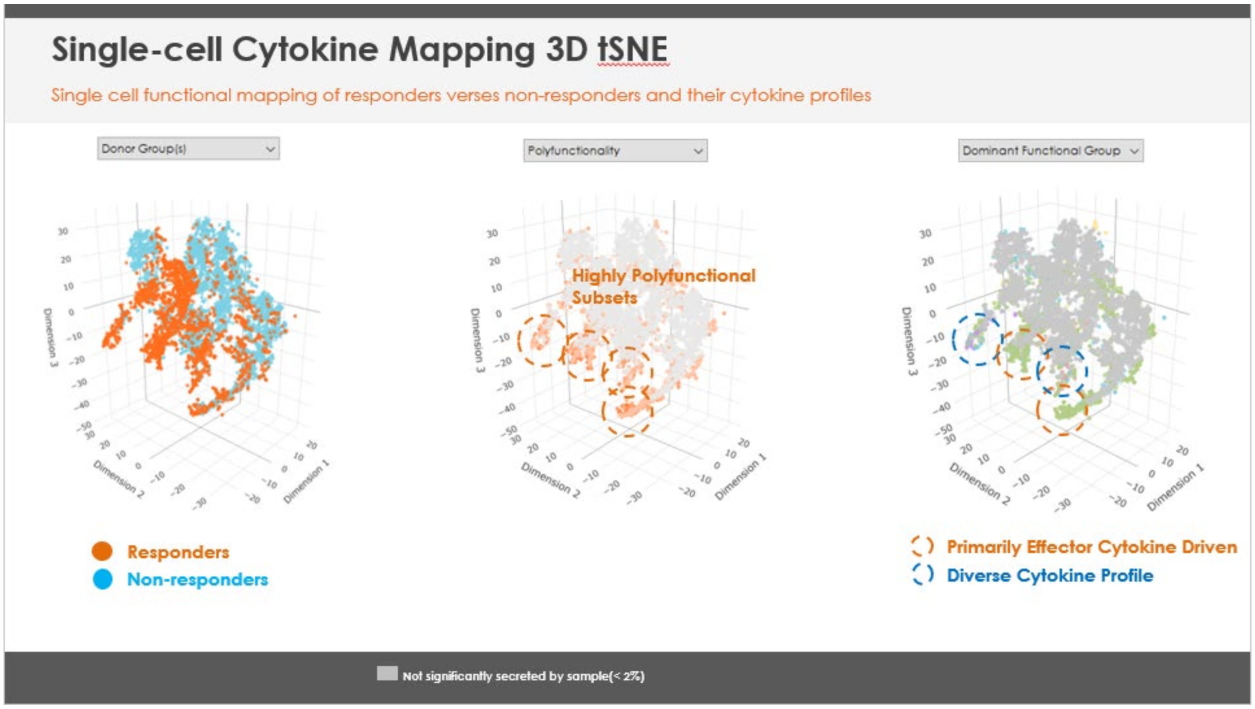
Options
Prefix : CAR-T Demo
Export Type : Graph
☑ Include Cel

Cell Types
CD4+, CD8+

The **Export Options** dialog has three sections. **Views** allows user to select one or all available tables/graphs to a filed based on the selection

Views contains all the different available table or graph types. Users can check any of the items to be exported at the same time. IsoSpeak will export each selected table or graph to a file based on your selection in the Options area. Options allow you to define the prefix and export type of each graph/table, and whether or not the analyzed cell types are included in the filenames. The default filename prefix is the project name. Cell types are included by default in the filenames". Under export type, users can choose between "CSV" or "Text" when exporting tables, and "Graph" or "Key" when exporting graphs. Cell Types displays the cell samples currently being viewed.

5. Example Biomarker Report



Single-cell Cytokine Mapping 3D tSNE

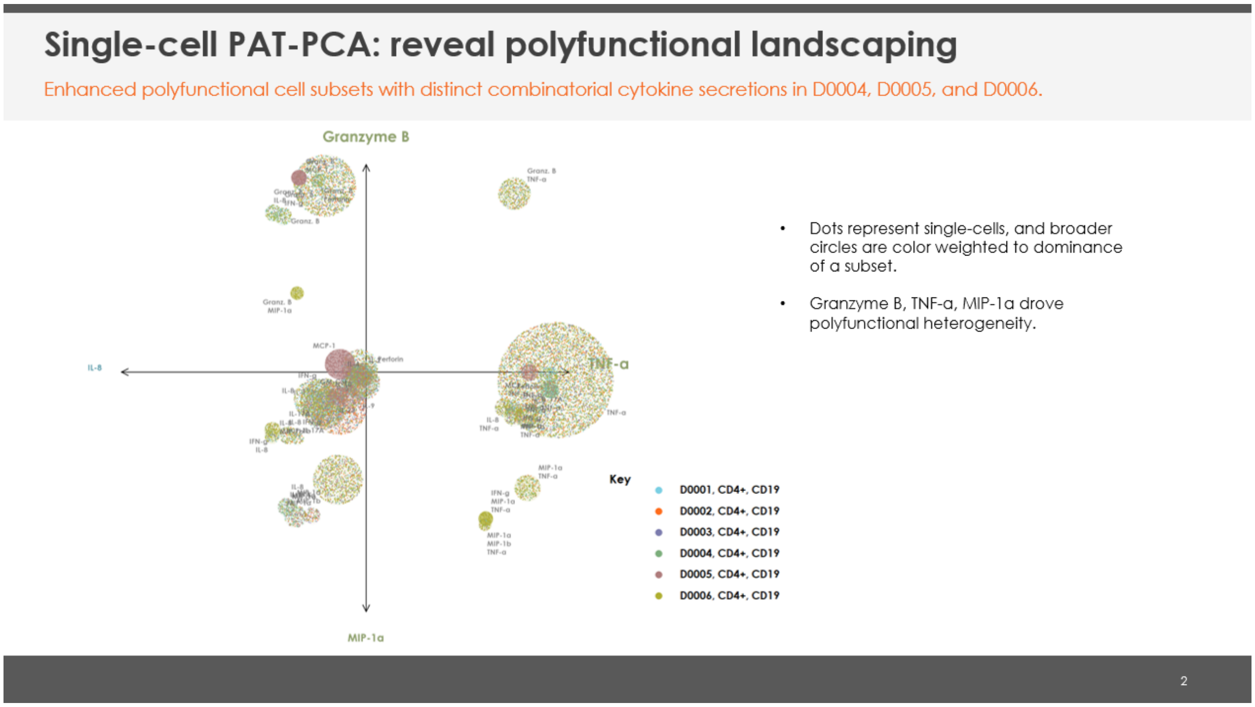
Single cell functional mapping of responders verses non-responders and their cytokine profiles

- ● **Responders**
- ● **Non-responders**

Primarily Effector Cytokine Driven

Diverse Cytokine Profile

▨ Not significantly secreted by sample(< 2%)



Single-cell PAT-PCA: reveal polyfunctional landscaping

Enhanced polyfunctional cell subsets with distinct combinatorial cytokine secretions in D0004, D0005, and D0006.

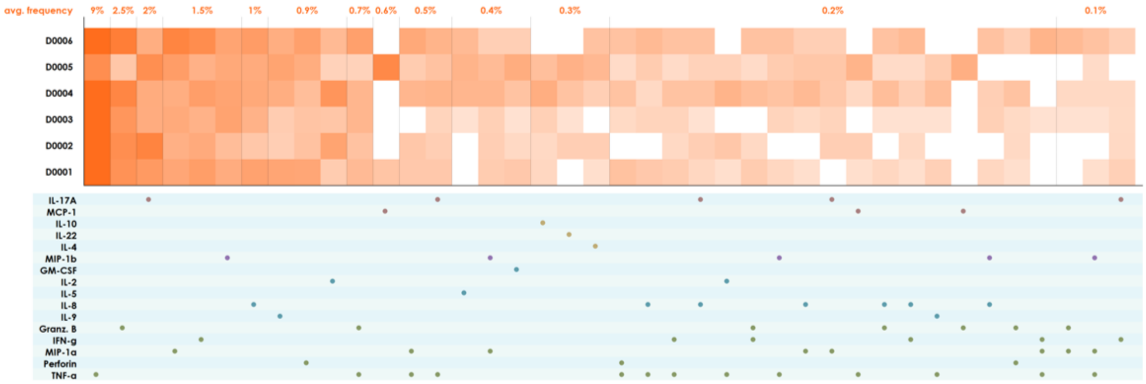
- Dots represent single-cells, and broader circles are color weighted to dominance of a subset.
- Granzyme B, TNF-a, MIP-1a drove polyfunctional heterogeneity.

Key

- ● D0001, CD4+, CD19
- ● D0002, CD4+, CD19
- ● D0003, CD4+, CD19
- ● D0004, CD4+, CD19
- ● D0005, CD4+, CD19
- ● D0006, CD4+, CD19

Single-Cell Functional Subsets (Heatmap)

Enhanced polyfunctional cell subsets with distinct combinatorial cytokine secretions in D0004, D0005, and D0006.

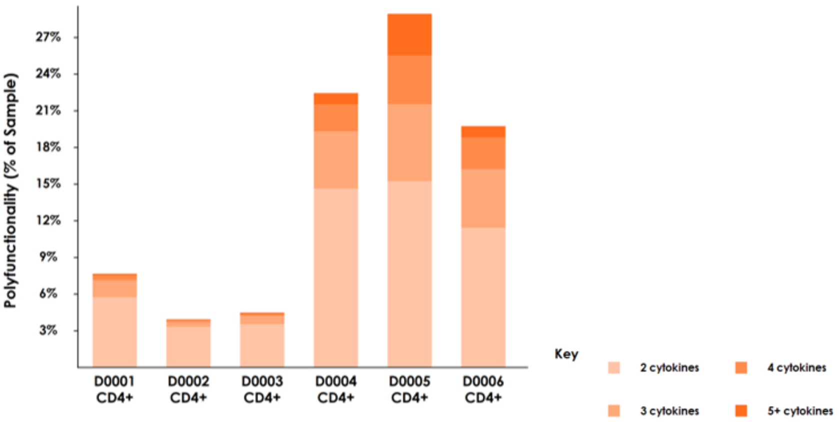


color indicates percentage of sample secreting the specified combination of cytokines:

■ 0.1% ■ 0.5% ■ 1% ■ 2.5% ■ 5%

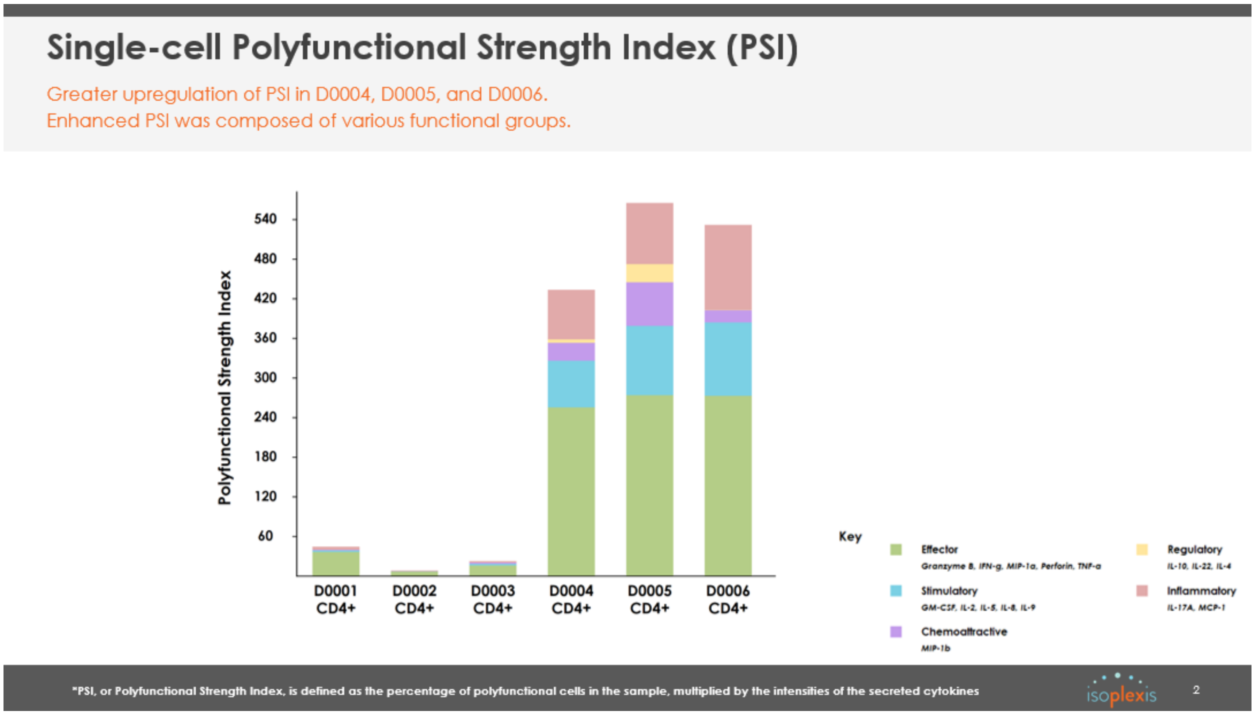
Single-cell Polyfunctionality Overview

Greater upregulation of polyfunctionality in D0004, D0005 and D0006.



* Polyfunctionality is defined as 2+ cytokines secreted per cell

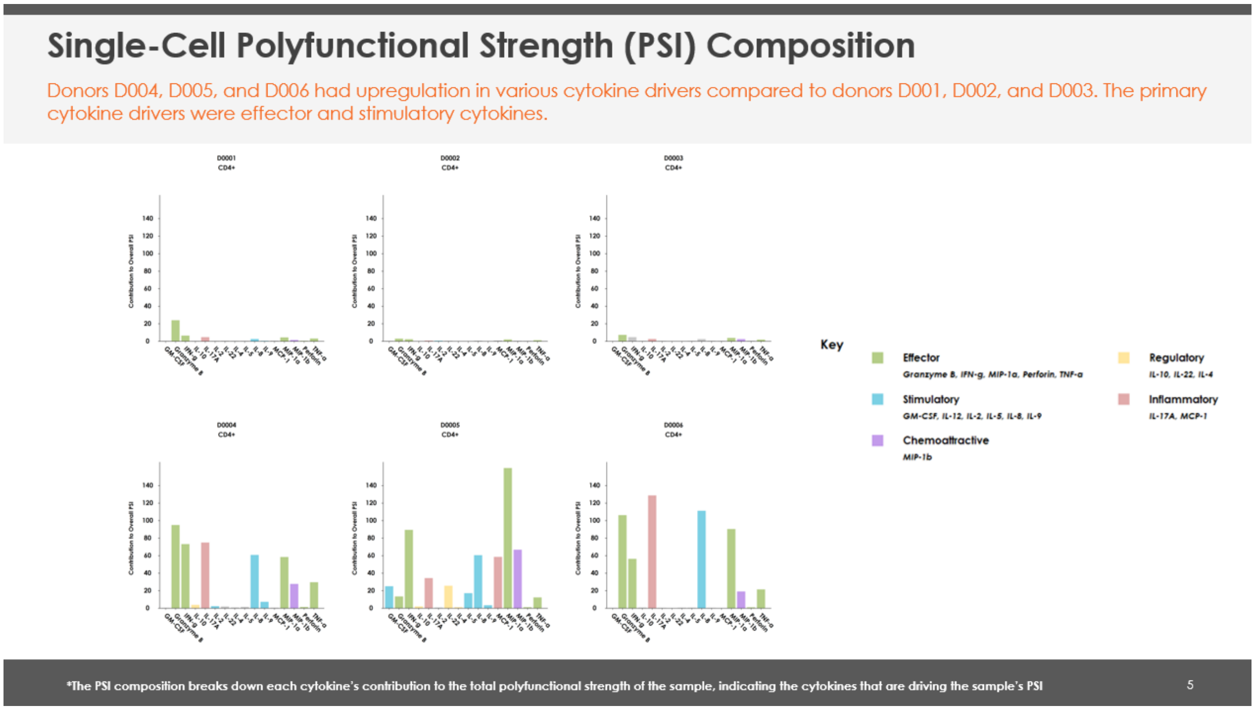
isoplexis



Single-cell Polyfunctional Strength Index (PSI)

Greater upregulation of PSI in D0004, D0005, and D0006.
Enhanced PSI was composed of various functional groups.

*PSI, or Polyfunctional Strength Index, is defined as the percentage of polyfunctional cells in the sample, multiplied by the intensities of the secreted cytokines



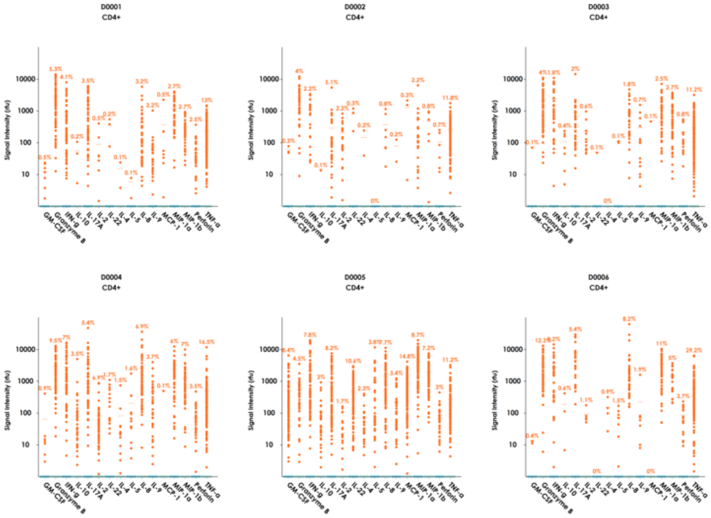
Single-Cell Polyfunctional Strength (PSI) Composition

Donors D004, D005, and D006 had upregulation in various cytokine drivers compared to donors D001, D002, and D003. The primary cytokine drivers were effector and stimulatory cytokines.

*The PSI composition breaks down each cytokine's contribution to the total polyfunctional strength of the sample, indicating the cytokines that are driving the sample's PSI

Single-cell Cytokine Signal Distribution

An increase of various protein secretion intensity in D0004, D0005 and D0006.



Key

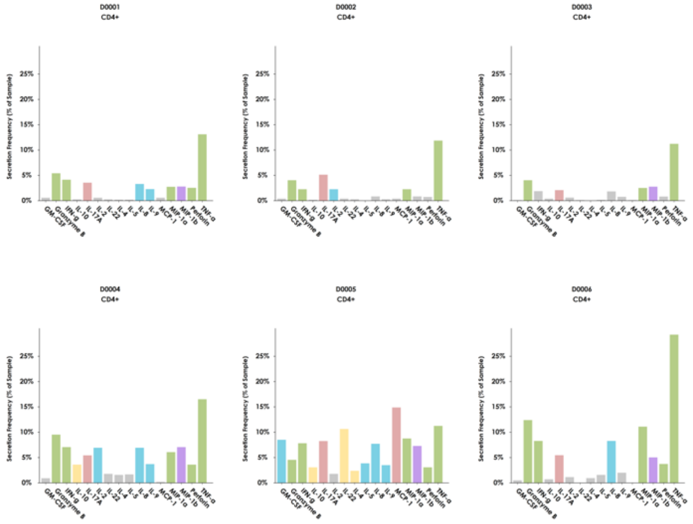
— Secretion threshold, defined per cytokine as avg(zero-cell signal) + 3 stdev(zero-cell signal)

● Signal > threshold (cytokine is being secreted by cell)

5% Percentage of cells secreting corresponding cytokine

Single-cell Cytokine Secretions

An increase of various protein secretions in D0004, D0005 and D0006.



Key

🟩 Effector
Granzyme B, IFN-g, MIP-1a, Perforin, TNF-a

🟦 Stimulatory
GM-CSF, IL-2, IL-5, IL-8, IL-9

🟪 Chemoattractive
MIP-1b

🟨 Regulatory
IL-10, IL-22, IL-4

🟥 Inflammatory
IL-17A, MCP-1

⬜ Not significantly secreted by sample(< 2%)