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Comprehensive Data Analysis Report

Sample Report

Company Name
Address 1
Address 2

Date

RNA was isolated from 24 mouse tumors and run on the PanCancer IO 360 Mouse Panel. The panel analyzes the expression of 770 genes that are vital components involved in the complex interplay between the tumor, microenvironment and immune response in cancer. Comparisons were completed as requested.

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**Each report includes up to 4 comparisons. This sample report is only showing the data for one comparison (untreated vs treated).*

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Sample Key

Lane	Nanostring File Name	Description	Treatment Group	RNA (ng/ul)
1	RCC file name	Tube name	Untreated	119.2
2	RCC file name	Tube name	Untreated	23.4
3	RCC file name	Tube name	Untreated	324.3
4	RCC file name	Tube name	Treated	132.5
5	RCC file name	Tube name	Treated	24.6
6	RCC file name	Tube name	Treated	234.7
7	RCC file name	Tube name	A	534.3
8	RCC file name	Tube name	A	634.4
9	RCC file name	Tube name	A	45.6
10	RCC file name	Tube name	B	10.3
11	RCC file name	Tube name	B	222.4
12	RCC file name	Tube name	B	239
13	RCC file name	Tube name	Untreated	22.1
14	RCC file name	Tube name	Untreated	67.4
15	RCC file name	Tube name	Untreated	75.0
16	RCC file name	Tube name	Treated	24.6
17	RCC file name	Tube name	Treated	234.7
18	RCC file name	Tube name	Treated	534.3
19	RCC file name	Tube name	A	634.4
20	RCC file name	Tube name	A	88.0
21	RCC file name	Tube name	A	41.1
22	RCC file name	Tube name	B	15.5
23	RCC file name	Tube name	B	62.3
24	RCC file name	Tube name	B	55.6

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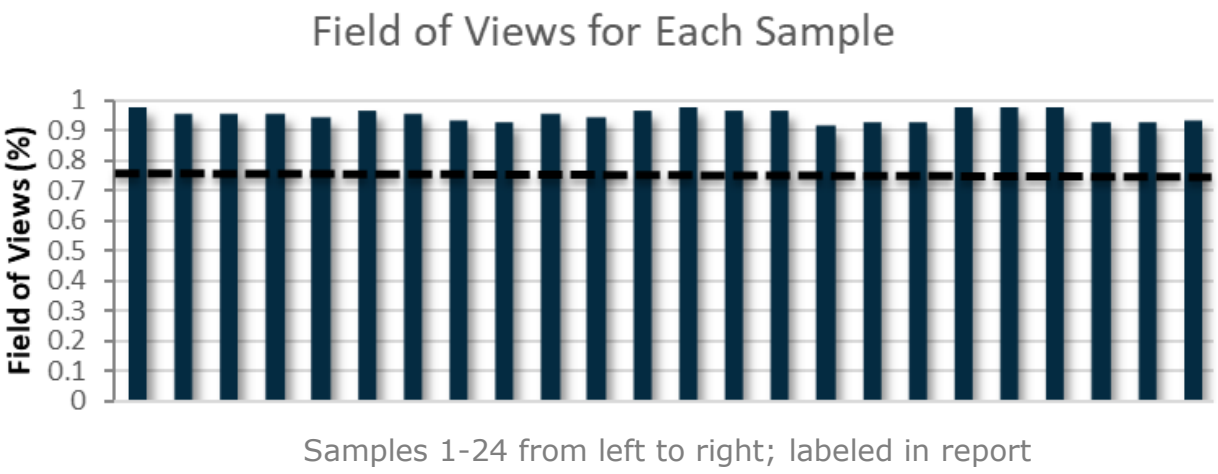
Quality Control Analysis

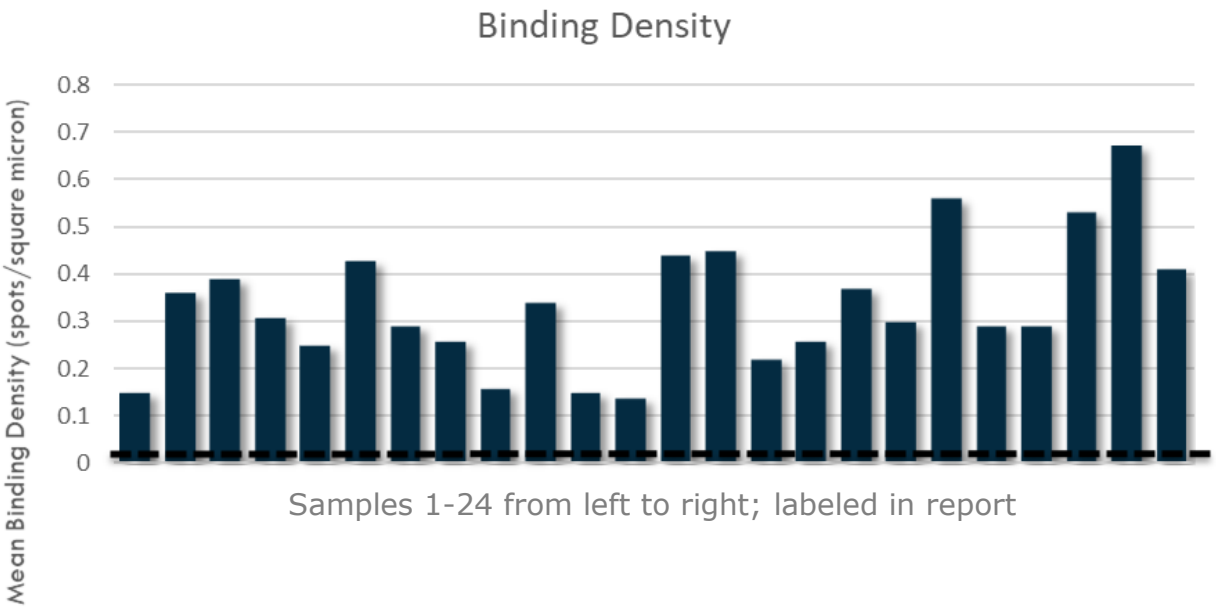
Field of Views

Imaging QC refers to the percentage of FOVs successfully counted by a digital Analyzer scan. Consistently reduced percentages can be indicative of an issue associated with the nCounter instrumentation. 75% is the Canopy Biosciences FOV cutoff for quality control.



Binding Density

The mean binding density is measured in spots per square micron. Acceptable probe count measurements are between 0.05 and 2.25 spots per square micron. When too many probes are present, the Analyzer may not distinguish each individual probe accurately.



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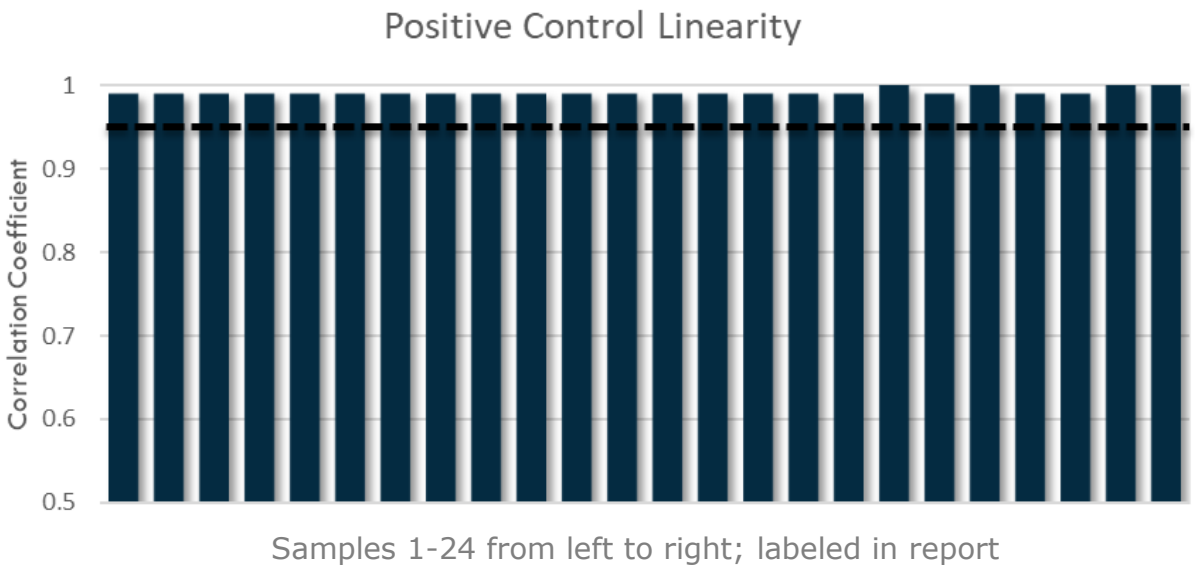
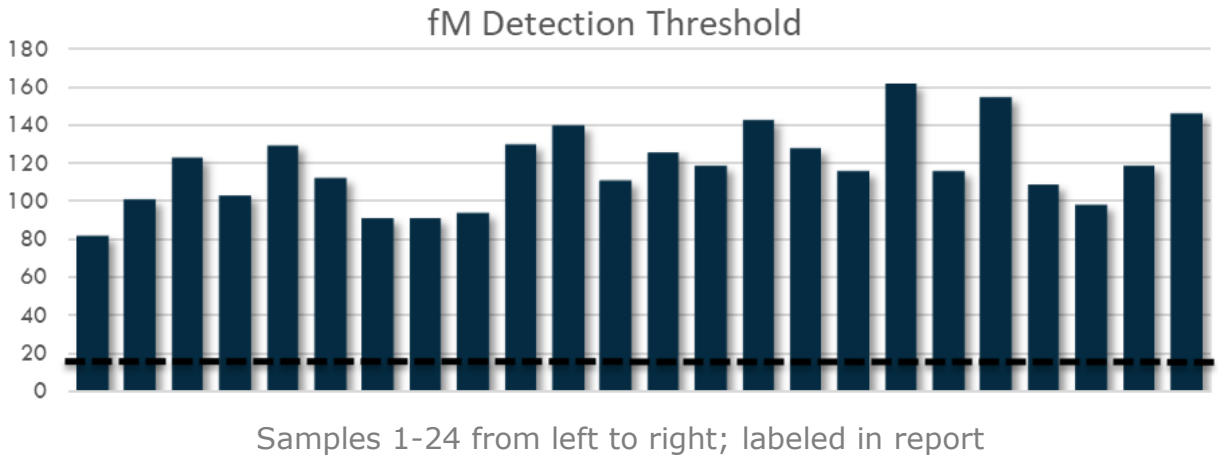
Positive Control Linearity

This assay contains a variety of positive control probes targeting molecules added during the production of the kit. Positive control linearity is a correlation analysis in log2 space between concentrations of added targets and the resulting counts. Low correlation values (below 0.95) may indicate an issue regarding hybridization.



fM Detection Threshold

fM detection threshold is a calculation of limit of detection based on positive and negative control probes. The 0.5 fM positive control probes must produce raw counts significantly higher than the mean of the negative control probes. Detection threshold below the minimum value indicates hybridization difficulties.



Controls

Positive controls

	Class Name	Gene Name	Accession #	Average Count	Median	%CV	StdDev
1	Positive	Pos_A	Accession #	124640.66	12440.76	0.13	4600.33
2	Positive	Pos_B	Accession #	64839.32	64779.32	0.15	1520.2
3	Positive	Pos_C	Accession #	2152.11	2144.11	0.11	332.4
4	Positive	Pos_D	Accession #	942.5	932.5	0.12	56.3
5	Positive	Pos_E	Accession #	523.4	511.4	0.12	44.3
6	Positive	Pos_F	Accession #	79.3	77.3	0.13	11.4

Negative controls

	Class Name	Gene Name	Accession #	Average Count	Median	StdDev
1	Negative	Neg_A	Accession #	13.16	13	3.34
2	Negative	Neg_B	Accession #	9.97	10	4.05
3	Negative	Neg_C	Accession #	8.54	8.5	3.22
4	Negative	Neg_D	Accession #	8.22	8.12	2.88
5	Negative	Neg_E	Accession #	11	11.1	3.12
6	Negative	Neg_F	Accession #	13.4	13	3.77

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Housekeeping Genes

	Class Name	Gene Name	Accession #	Average Count	Median	StdDev
1	Housekeeping	Gene1	Accession #	416.66	388.46	183
2	Housekeeping	Gene2	Accession #	39.32	33.32	12.2
3	Housekeeping	Gene3	Accession #	152.11	143.11	65.4
4	Housekeeping	Gene4	Accession #	42.5	41.5	19.3
5	Housekeeping	Gene5	Accession #	523.4	513.4	123.3
6	Housekeeping	Gene6	Accession #	124.66	104.9	33.33
7	Housekeeping	Gene7	Accession #	139.32	122.8	38.2
8	Housekeeping	Gene8	Accession #	215.11	201.21	66.4
9	Housekeeping	Gene9	Accession #	242.5	240.3	109.3
10	Housekeeping	Gene10	Accession #	323.4	312.4	122.3
11	Housekeeping	Gene11	Accession #	140.66	137.26	55.33
12	Housekeeping	Gene12	Accession #	339.32	320.32	108.2
13	Housekeeping	Gene13	Accession #	152.11	140.6	55.4
14	Housekeeping	Gene14	Accession #	42.5	37.3	19.3
15	Housekeeping	Gene15	Accession #	123.4	113.4	24.3
16	Housekeeping	Gene16	Accession #	152.11	132.9	43.4
17	Housekeeping	Gene17	Accession #	342.5	322.4	129.3
18	Housekeeping	Gene18	Accession #	223.4	218.4	72.3
19	Housekeeping	Gene19	Accession #	79.3	66.2	29.4
20	Housekeeping	Gene20	Accession #	66.4	54.7	18.3

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Normalization Factors

Normalization factors are listed below. A normalization factor above 10 indicates poor RNA quality or low input which may lead to inaccurate data. Any sample with a normalization factor above 10 will be removed from further analysis.

Description	RNA (ng/µl)	Normalization Factor
Tube name	43.3	4.99
Tube name	41.4	0.6
Tube name	123.5	0.8
Tube name	314.6	0.44
Tube name	89.7	0.86
Tube name	224.9	1.3
Tube name	353.4	2.09
Tube name	211	4.1
Tube name	65.7	2.5
Tube name	77.3	0.4
Tube name	45.9	0.9
Tube name	112.8	1.7
Tube name	243.5	1.12
Tube name	109.8	3.2
Tube name	77	0.4
Tube name	53.5	0.77
Tube name	77.5	5.8
Tube name	87.9	8.2
Tube name	132.2	2.4
Tube name	105.5	4.1

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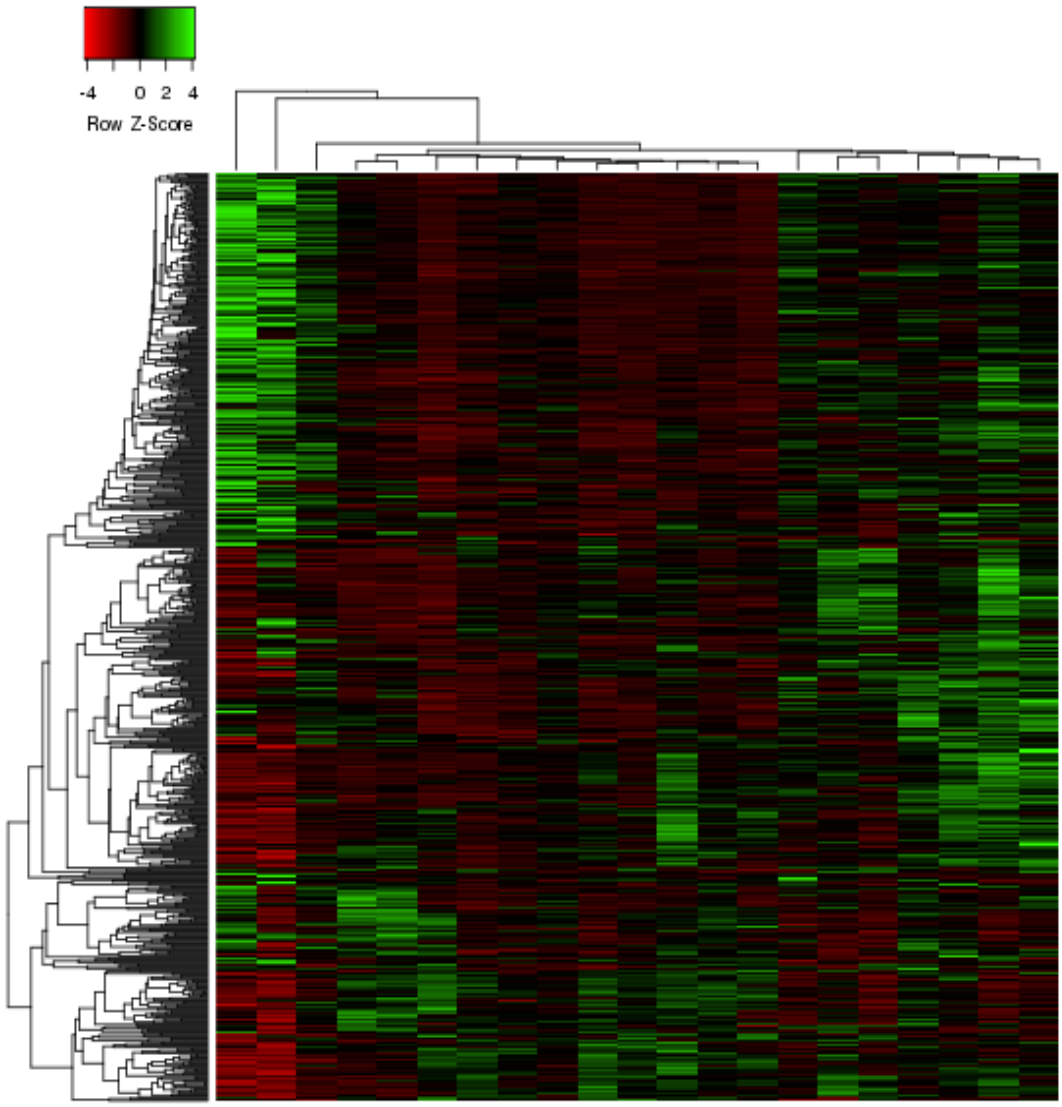
Data Analysis – all samples

Heat Map

Unbiased clustering was performed to generate a heat map analysis of the normalized samples. The average linkage clustering method and the Spearman Rank Correlation distance measurement method were employed to generate the data.



Samples 1-24 from left to right; labeled in report

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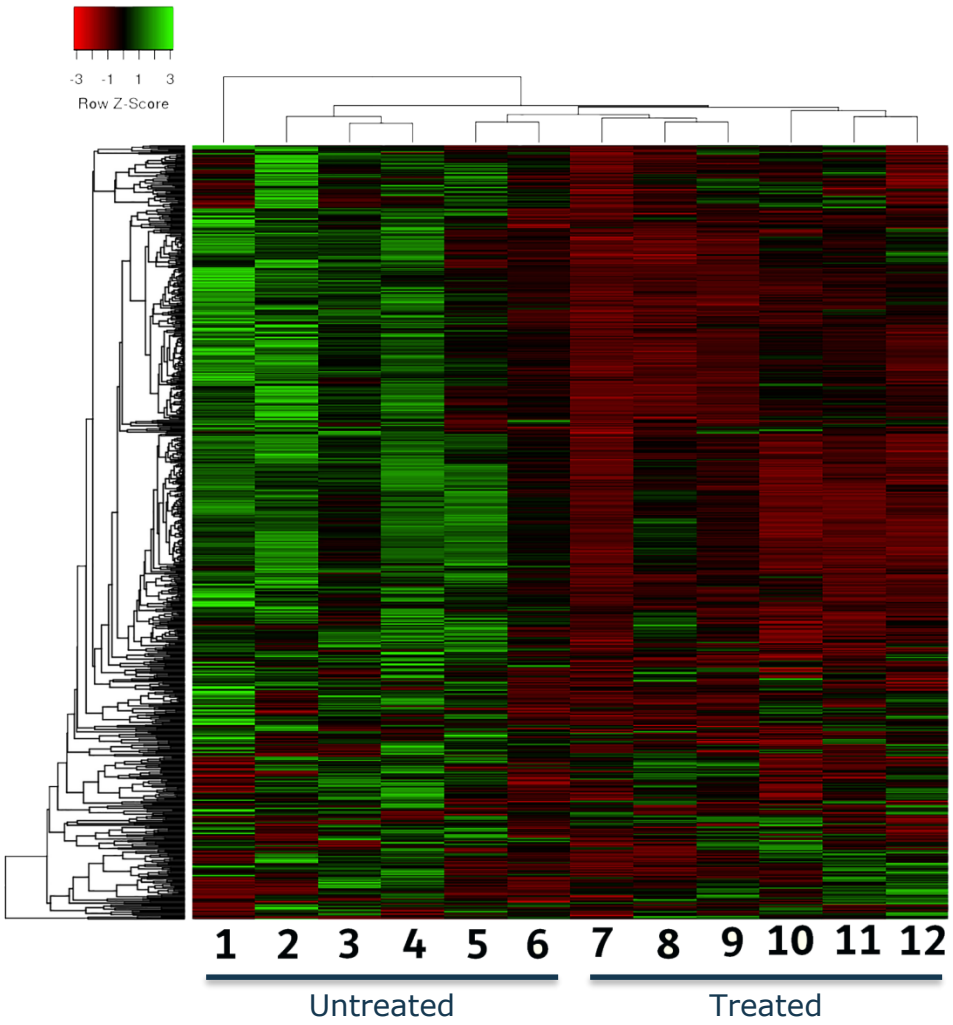
Data Analysis*
Untreated vs Treated

**Each report includes up to 4 comparisons. This sample report is only showing the data for one comparison (untreated vs treated).*

CANOPY BIOSCIENCES

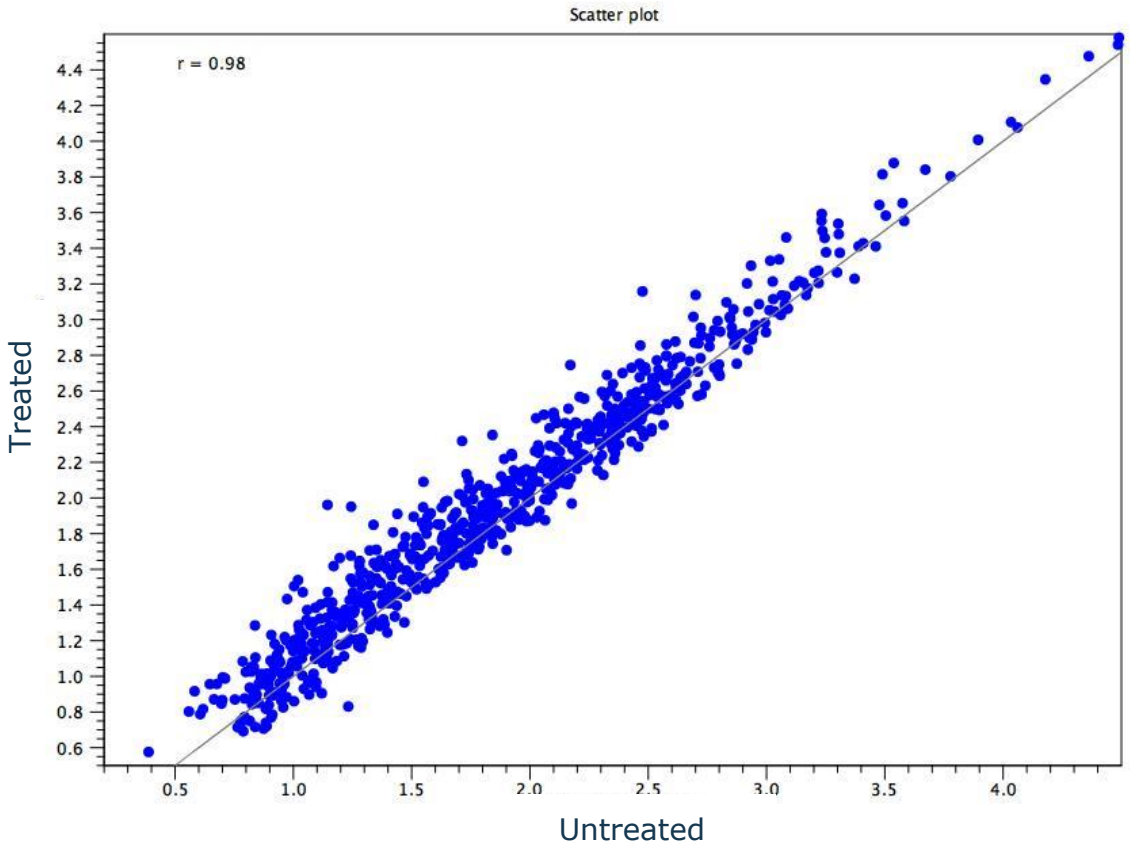
Untreated vs Treated Heat Map

Unbiased clustering was performed to generate a heat map analysis of the samples. The average linkage clustering method and the Spearman Rank Correlation distance measurement method were employed to generate the data.



Untreated vs Treated Scatter Plot

A scatter plot was generated using the averages of the sample groups. This is intended to give an overview of how two groups correlate based on the correlation coefficient, which is provided on each graph.



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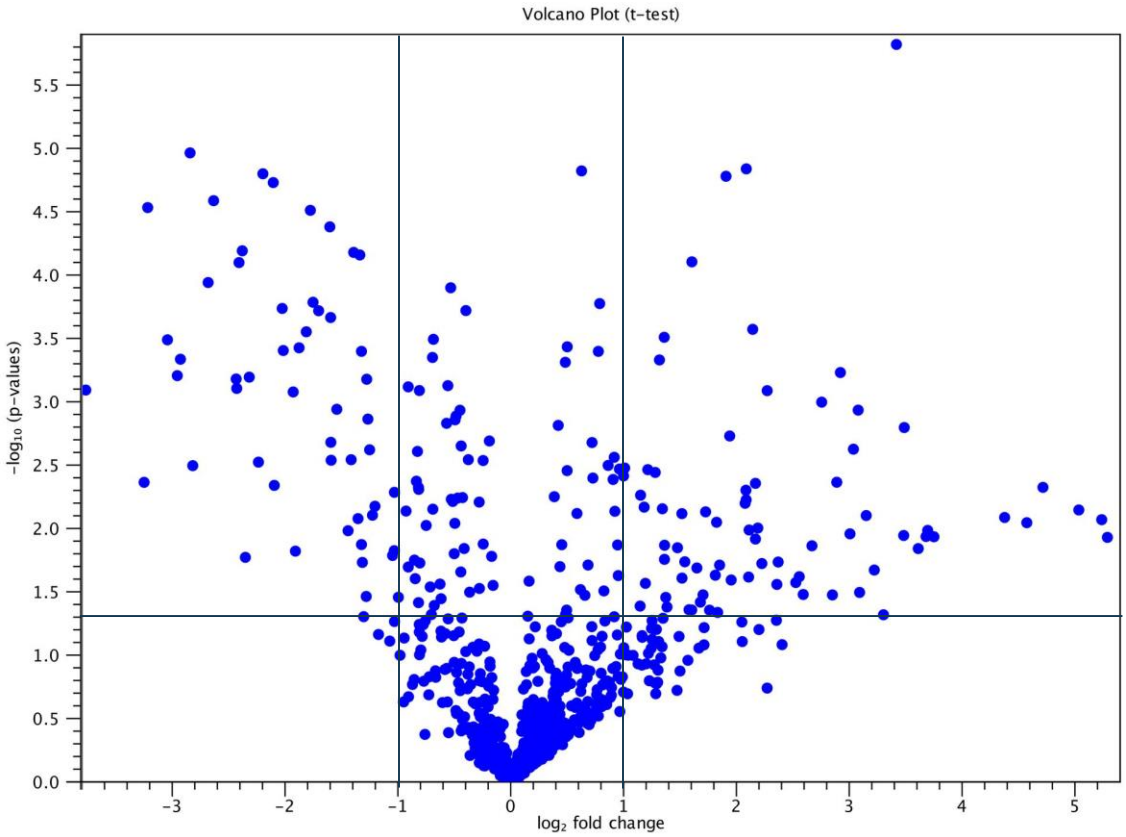
Untreated vs Treated
Volcano Plot

A volcano plot was generated using the Gaussian Statistical Analysis (t-test). Volcano plots show both fold change and p-value. The log2 fold change is plotted on the x-axis and the negative log10 p-value is plotted on the y-axis. Genes in the upper left box have a fold change of ≤-2 and a p-value of ≤0.05. Genes in the upper right box have a fold change of ≥2 and a p-value of ≤0.05. The top 25 genes with the greatest statistically differential expression are identified in the gene table on the following slide.



Untreated vs Treated
Top Differentially Expressed Genes

The top 25 genes with the greatest statistically differential expression are shown below. If fewer than 25 genes are shown, then there were less than 25 genes with a statistically significant p-value ($p \leq 0.05$). Fold changes and p-values for all genes are reported in an accompanying excel file.

Gene	Fold Change	p-value
Gene 1	9.12	0.0121
Gene 2	7.06	0.0027
Gene 3	6.67	0.0123
Gene 4	5.00	0.0122
Gene 5	4.98	0.0314
Gene 6	4.21	0.0011
Gene 7	3.11	0.0037
Gene 8	3.01	0.0066
Gene 9	2.43	0.0333
Gene 10	2.31	0.0015
Gene 11	2.29	0.0044
Gene 12	2.23	0.0225
Gene 13	2.22	0.0022
Gene 14	2.20	0.0037
Gene 15	2.17	0.0228
Gene 16	2.16	0.0113
Gene 17	2.11	0.0023
Gene 18	2.09	0.0018
Gene 19	-2.06	0.0030
Gene 20	1.99	2E-06
Gene 21	1.96	0.0318
Gene 22	1.88	0.0016
Gene 23	1.88	0.0148
Gene 24	1.78	0.0043
Gene 25	1.77	0.0305

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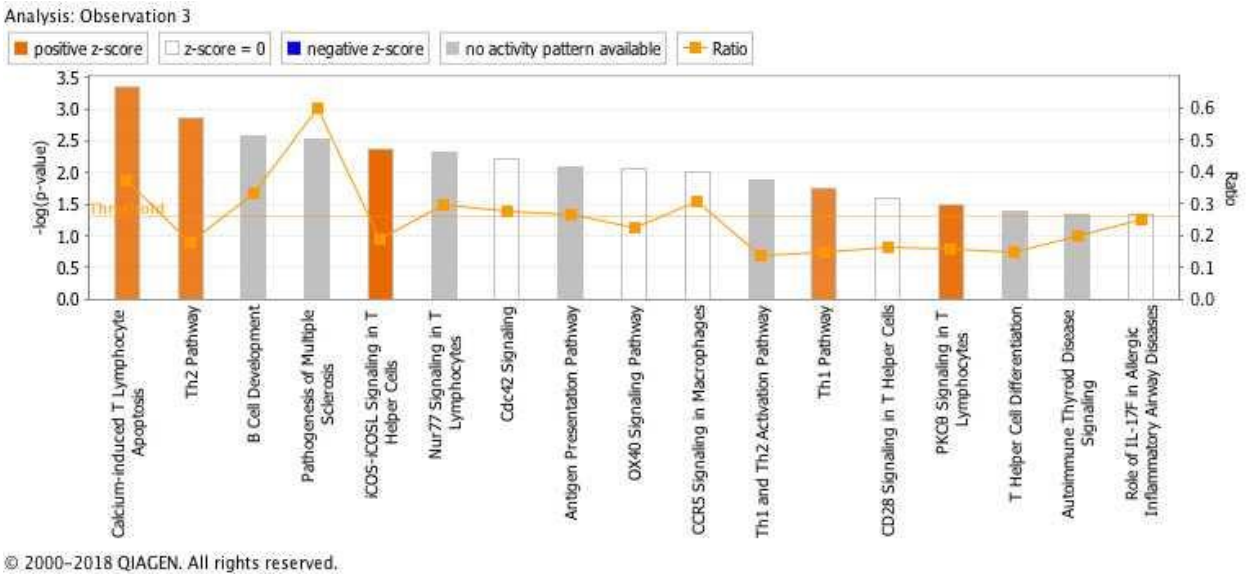
Untreated vs Treated Pathway Mapping

Pathway analysis was performed on genes with a p-value of ≤ 0.05 using Ingenuity Pathway Analysis (IPA) software. The height of the bars indicates the significance of the overlap of the genes in the NanoString panel with the pathway database. Significance values are calculated using the Fisher's right tailed exact test, and the –log(p-value) is displayed on the y-axis. The taller the bar, the more significant the overlap of the NanoString dataset with the pathway. Orange bars mean the pathway is activated, blue bars mean the pathway is inhibited, and grey bars mean no pattern was detected. The orange points connected by a thin orange line represent the Ratio. The Ratio is calculated as # of genes in a given pathway that meet the criteria cutoff divided by the total # of genes that make up that pathway. The threshold line corresponds to a p-value of 0.05. The y-axis represents a –log p value resulting in a 1.3 threshold value. Bars that are above this threshold line are indicated to be significantly enriched in the analysis. The most significant pathways are highlighted on the following slides. You can perform additional pathway analysis on your data by using the IPA software. Your data has been uploaded into the IPA server and Canopy Biosciences provides a 30 day access period.

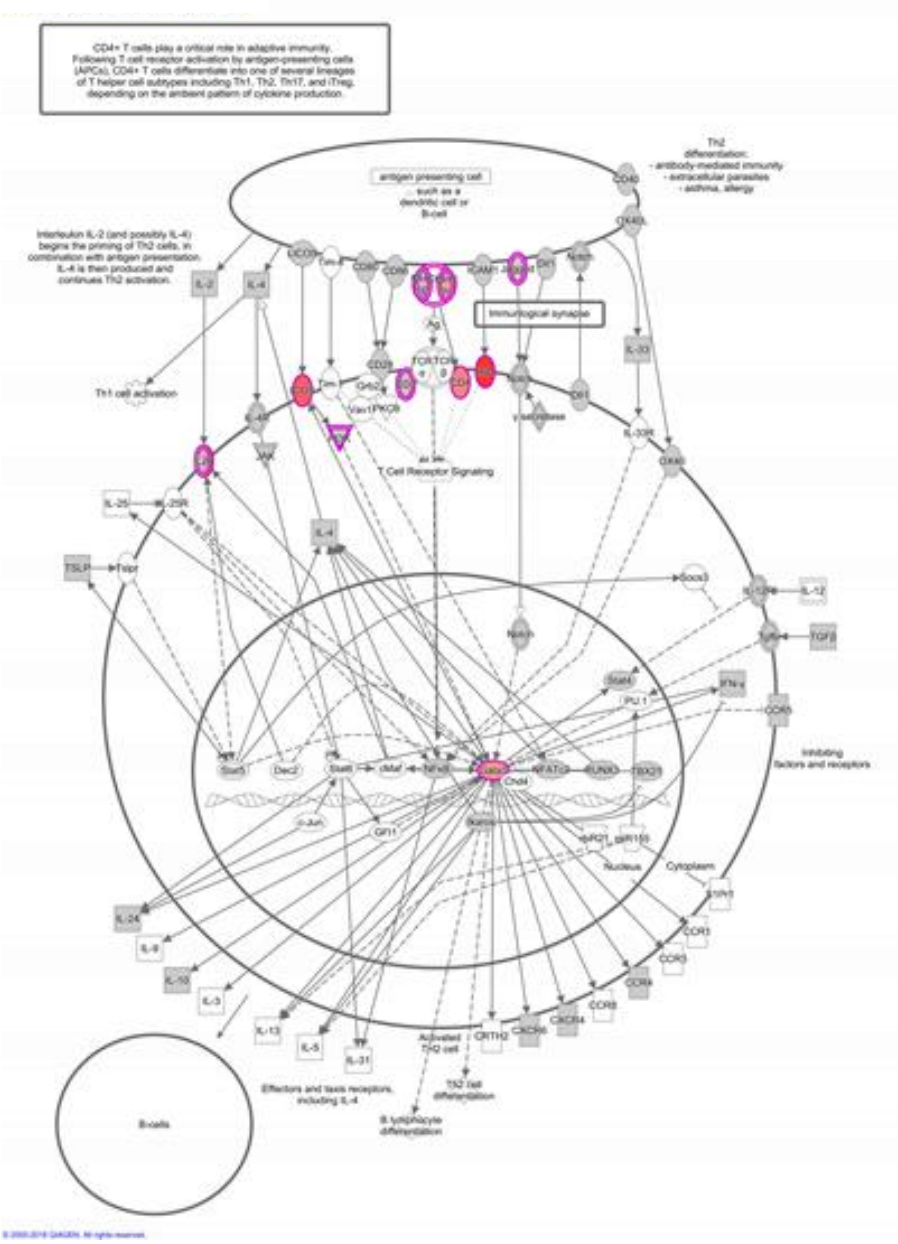


Please note that the pathways that follow may not necessarily correlate to this summary figure as this is a sample report.

Untreated vs Treated Pathway Mapping

Genes that are up-regulated are shown in red. Genes that are down-regulated are shown in green. Genes that are not significantly differentially expressed are shown in grey. Genes that are not in the dataset are shown in white.

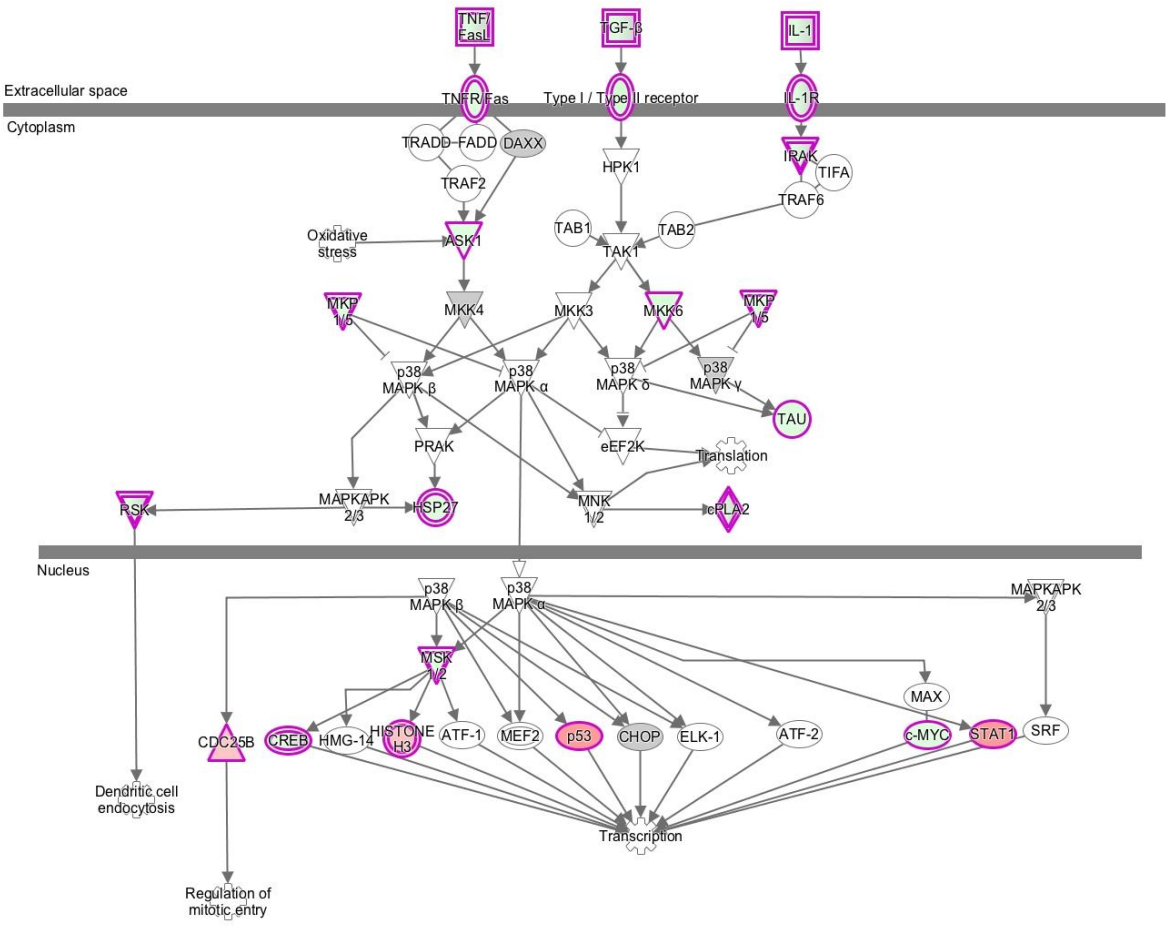
Th2 Pathway



Untreated vs Treated Pathway Mapping

Genes that are up-regulated are shown in red. Genes that are down-regulated are shown in green. Genes that are not significantly differentially expressed are shown in grey. Genes that are not in the dataset are shown in white.

P38 MAPK Signaling



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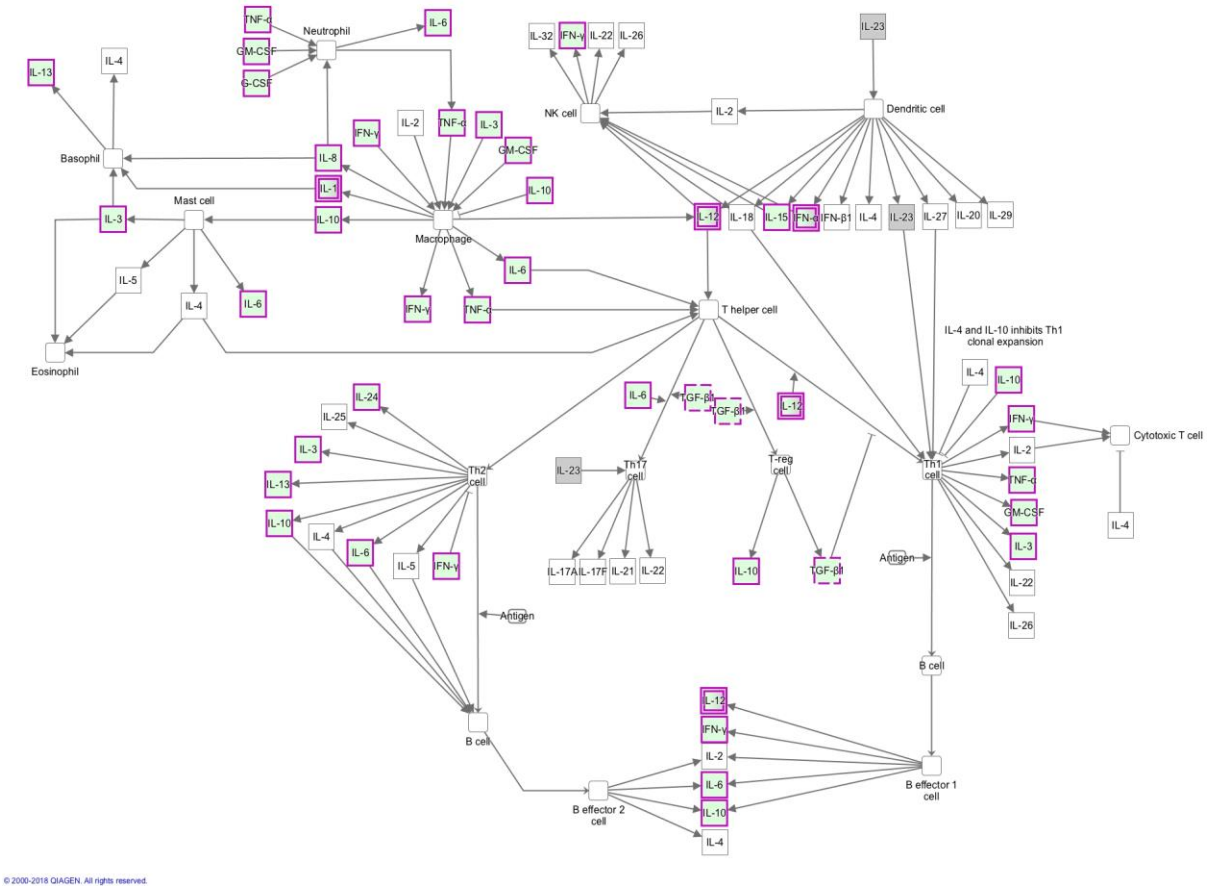
CANOPY BIOSCIENCES

Untreated vs Treated Pathway Mapping

Genes that are up-regulated are shown in red. Genes that are down-regulated are shown in green. Genes that are not significantly differentially expressed are shown in grey. Genes that are not in the dataset are shown in white.

Role of Cytokines in Mediating Communication between Immune Cells



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Untreated vs Treated Immune Cell Profiling QC Values

The QC value is a p-value resulting from testing the null hypothesis that a given gene signature used for cell type profiling exhibits no greater cell type-specific behavior than a randomly selected gene signature of similar size. The p-value for each cell type is given in a table as shown below. The p-value is not applicable (n/a) for cell types in which the gene signature is a single gene. It is important to keep in mind that cell type abundance data with a p-value above a certain threshold may still be relevant. Also, note that these p-values are not an indication of significance between the two groups; the p-values generated by comparing the two groups are shown on the figures that follow.

Cell Type	QC p-value
B cells	0.02
CD8 T cells	0.04
CD45	n/a
Cytotoxic cells	0.09
DCs	0.16
Exhausted CD8	0
Macrophages	0.12
Mast cells	0.07
Neutrophils	0.02
NK CD56dim cells	0.3
NK cells	0.12
T cells	0
Th1 cells	n/a
Tregs	n/a

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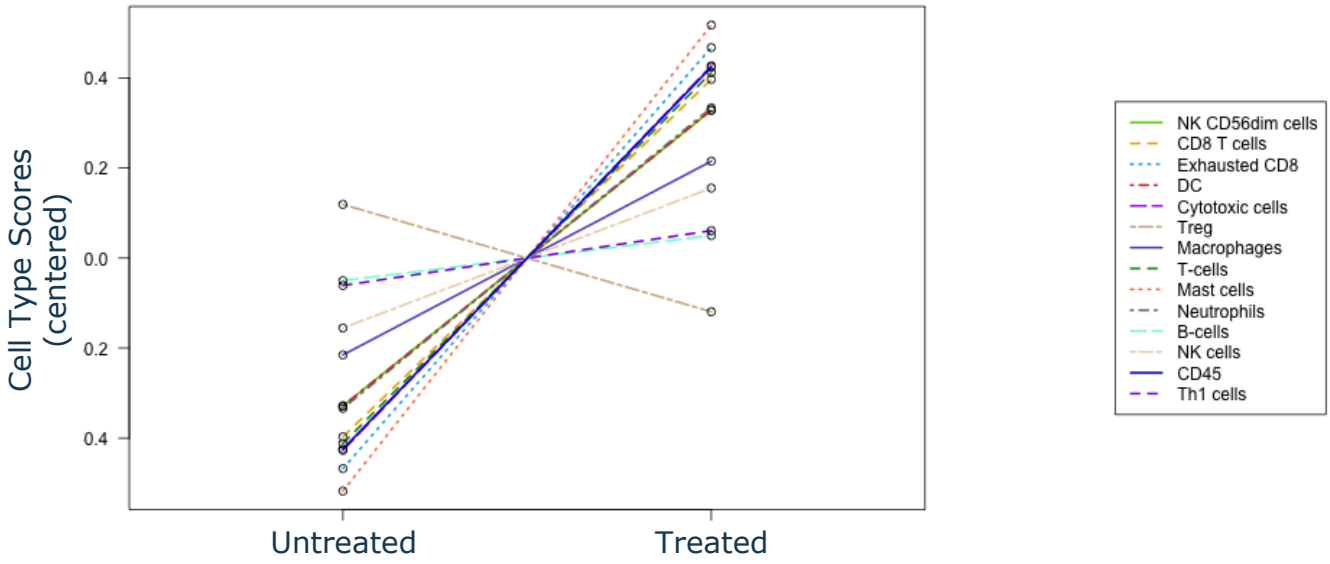
Untreated vs Treated
Immune Cell Profiling
Cell Type Score Summary

Below is a summary of the cell type scores. These scores are calculated using the gene signatures derived by Danaher *et al*. 2017. Individual cell abundance scores are available on subsequent pages.

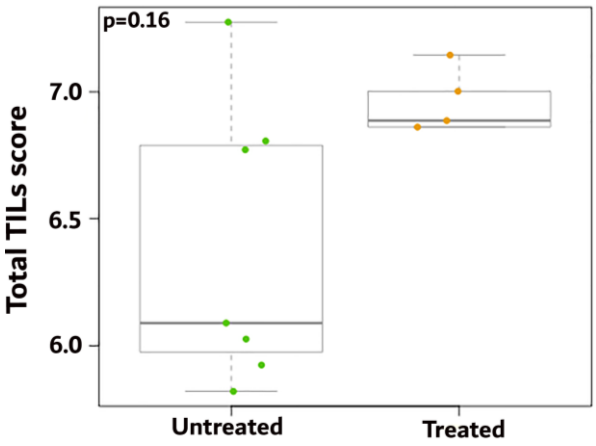


Please note that the individual cell type abundance scores that follow may not necessarily correlate to this summary figure as this is a sample report.

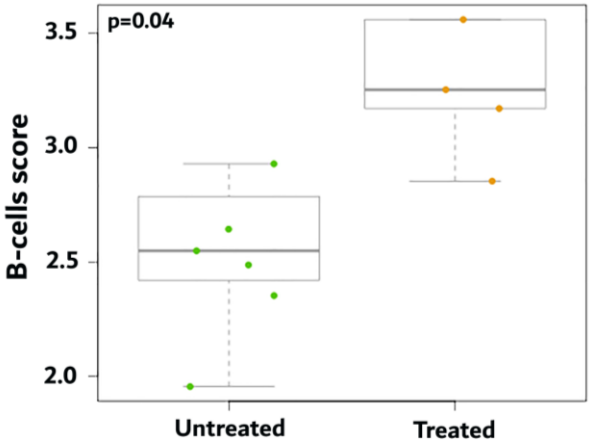
Untreated vs Treated
Immune Cell Profiling
Total TILs and Individual Cell Type Scores

Total TILs (top left) and raw cell type abundance graphs are shown below and on the following slides. Abundance estimates are given on the log2 scale, so a unit increase in score corresponds to a doubling of a cell type's abundance. These scores do not support claims about whether one cell type is more abundant than another. Rather, they permit claims that a cell type is more abundant in one group than in another. All p-values were generated using a Student's t-test.

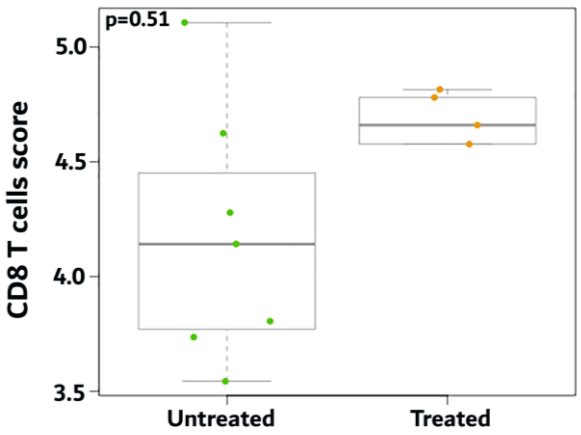
Total TILs



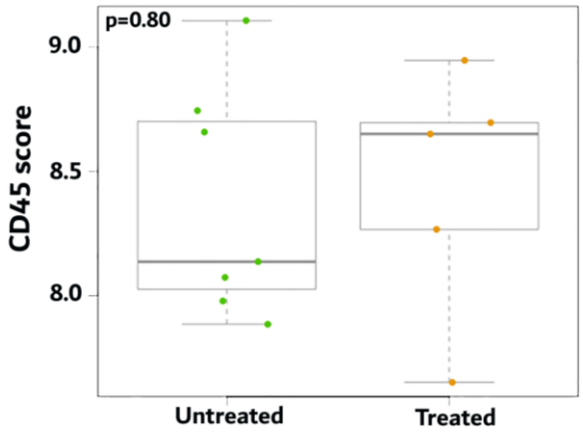
B Cells



CD8 T Cells



CD45



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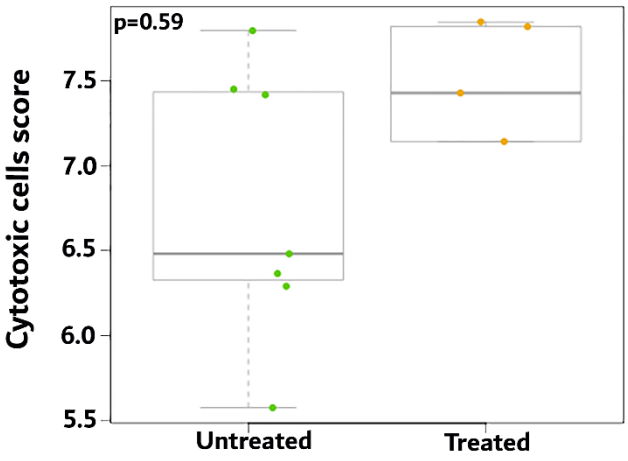
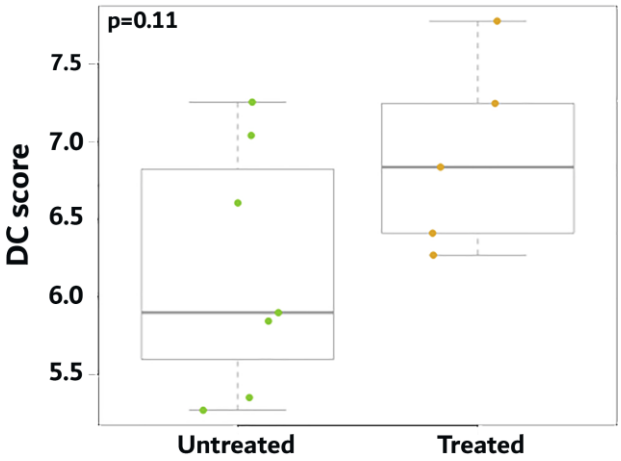
Untreated vs Treated
Immune Cell Profiling
Individual Cell Type Scores

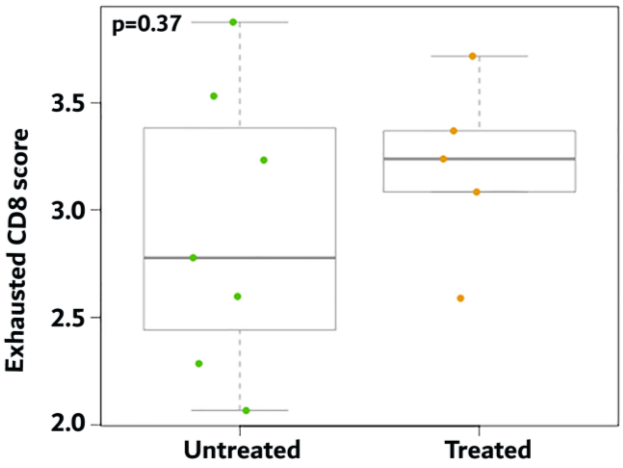
Cytotoxic Cells



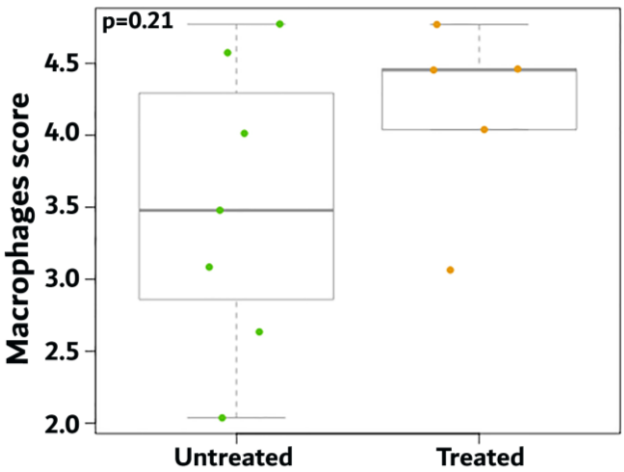
Dendritic Cells



Exhausted CD8



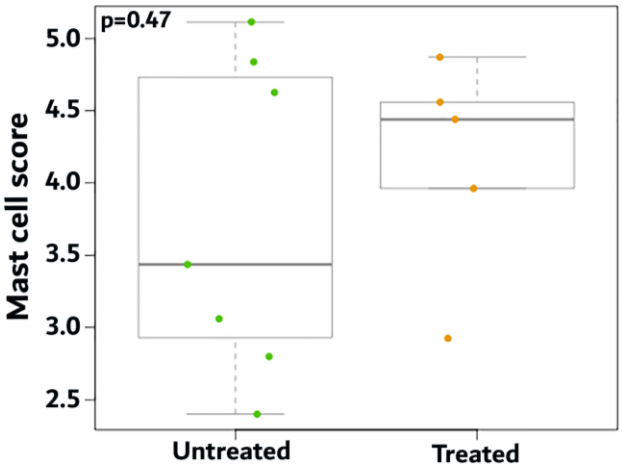
Macrophages



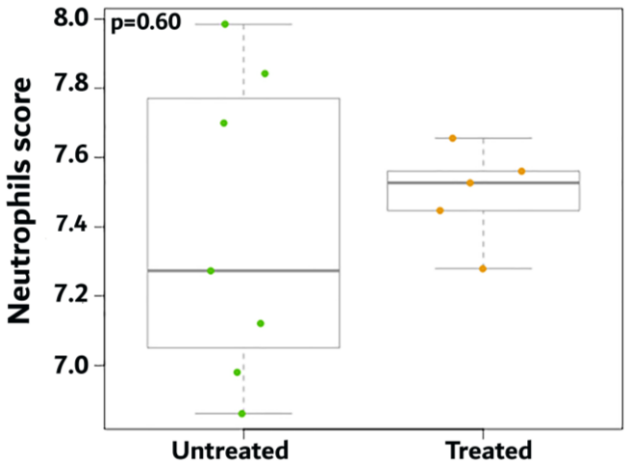
CANOPY BIOSCIENCES

Untreated vs Treated
Immune Cell Profiling
Individual Cell Type Scores

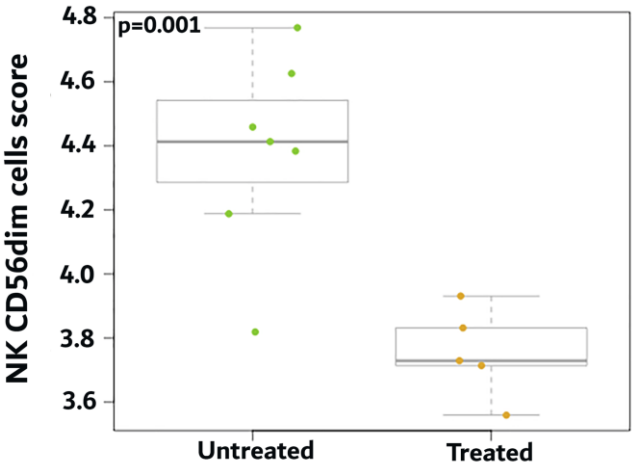
Mast Cells



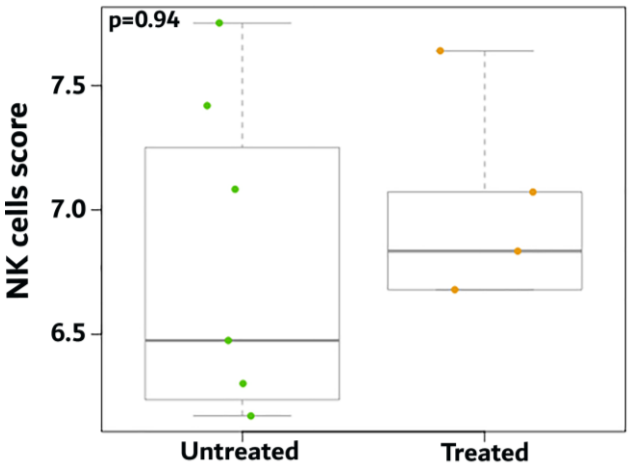
Neutrophils



NK CD56dim Cells



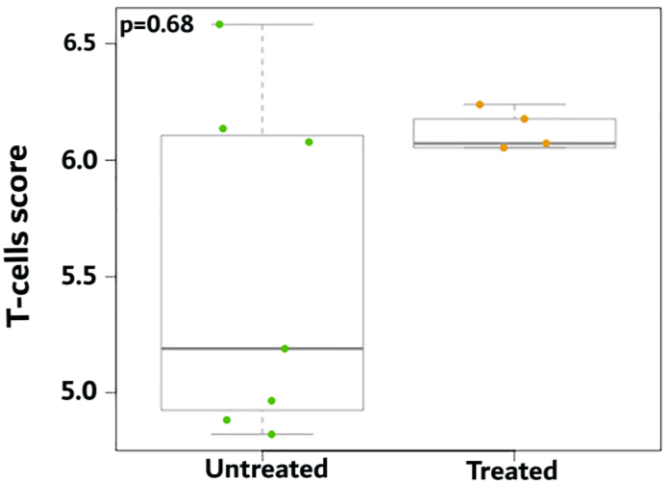
NK Cells



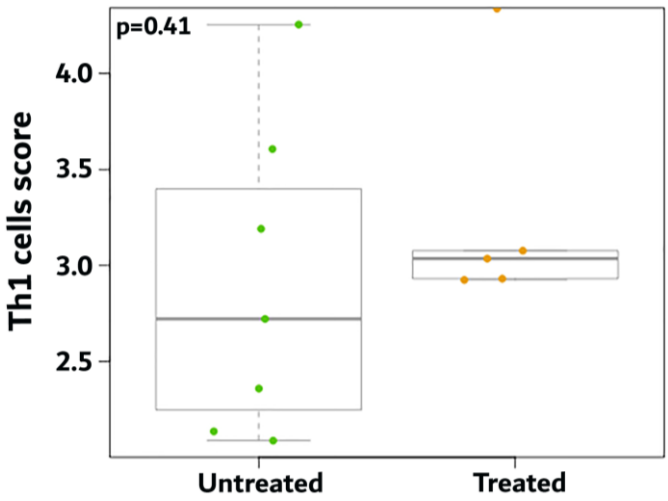
CANOPY BIOSCIENCES

Untreated vs Treated
Immune Cell Profiling
Individual Cell Type Scores

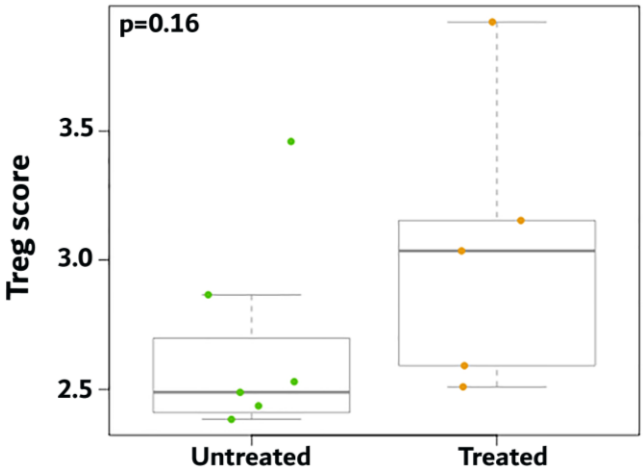
T Cells



Th1 Cells



Treg



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Recommendations & Contact Information

Recommendations

- Recommendation #1
- Recommendation #2

Contact Information

- For questions on the report please contact info@canopybiosciences
- For additional analysis please contact sales@canopybiosciences for a quote

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