

Nanostring Analysis for Dataset X v2.0

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Guiding Questions

Which immune cell type (and other) to compare with what we learned from flow cytometry?

Did PD1 elicit any changes in expression profile compared with IgG?

What can we learn about IFN γ treatment.immune cells activated?

What are the characteristics of the samples?

Basic Setup

##	Sample.Name	RNA.Solution	Tumor	Fraction	Treatment
## 1	P0003.S1	RNA Later	P0003	large fragments	None
## 2	P0003.S3	RNA Later	P0003	single cell flow	None
## 3	P0004.S1	RNA Later	P0004	large fragments	None
## 4	P0004.S2	RNA Later	P0004	None	None
## 5	P0004.S3	RNA Later	P0004	single cell flow	None
## 6	P0010.IFN γ	RNA Later	P0010	None	IFN γ
## 7	P0010.IgG	RNA Later	P0010	None	IgG
## 8	P0010.S1	RNA Later	P0010	large fragments	None
## 9	P0010.S2	RNA Advance	P0010	None	None
## 10	P0010.aPD1	RNA Advance	P0010	None	aPD1
## 11	P0011.S1	RNA Advance	P0011	large fragments	None
## 12	P0011.S2	RNA Advance	P0011	None	None

What is being quantified?

Number of Nanostring Controls

What Gene functions are being quantified?

x

Gene

Cell.Type

Adaptive.Immunity

Apoptosis

Cell.Cycle

Cellular.Stress

Complement.System

Death.Receptor.Signaling

Extracellular.matrix.organization

Fc.Receptor.Signaling

x
Innate.Immunity
Interferon.Signaling
Interleukin.Signaling
NF.kB
MAPK.Signaling
Metabolism
TLR.Signaling
VEGF.Signaling
Wnt.Signaling

How many genes represent each function type Information?

Cell.Type	n
	712
B-cells	4
CD45	1
CD8 T cells	2
Cytotoxic cells	8
DC	4
Exhausted CD8	4
Macrophages	2
Mast cells	2
Neutrophils	3
NK CD56dim cells	3
NK cells	2
T-cells	5
Th1 cells	1
Treg	1

Cellular.Stress	n
-	723
+	31

Complement.System	n
-	720
+	34

Death.Receptor.Signaling	n
-	736
+	18

Extracellular.matrix.organization	n
-	714

Extracellular.matrix.organization	n
+	40

Fc.Receptor.Signaling	n
-	681
+	73

Innate.Immunity	n
-	733
+	21

Interferon.Signaling	n
-	664
+	90

Interleukin.Signaling	n
-	631
+	123

NF.kB	n
-	709
+	45

MAPK.Signaling	n
-	689
+	65

Metabolism	n
-	712
+	42

TLR.Signaling	n
-	686
+	68

VEGF.Signaling	n
-	698

VEGF.Signaling	n
+	56

Wnt.Signaling	n
-	734
+	20

What comparisons can be made?

Fraction	n
large fragments	4
None	6
single cell flow	2

RNA.Solution	n
RNA Later	8
RNAdvance	4

Tumor	n
P0003	2
P0004	3
P0010	5
P0011	2

Treatment	n
aPD1	1
IFNy	1
IgG	1
None	9

Can we group the samples?

PCA plots give an idea what similarities are to be expected from the samples that are in it.

PCA - Calculates variability across entire dataset for each gene - Groups components - Using these components it allows to check for bias and grouping within samples

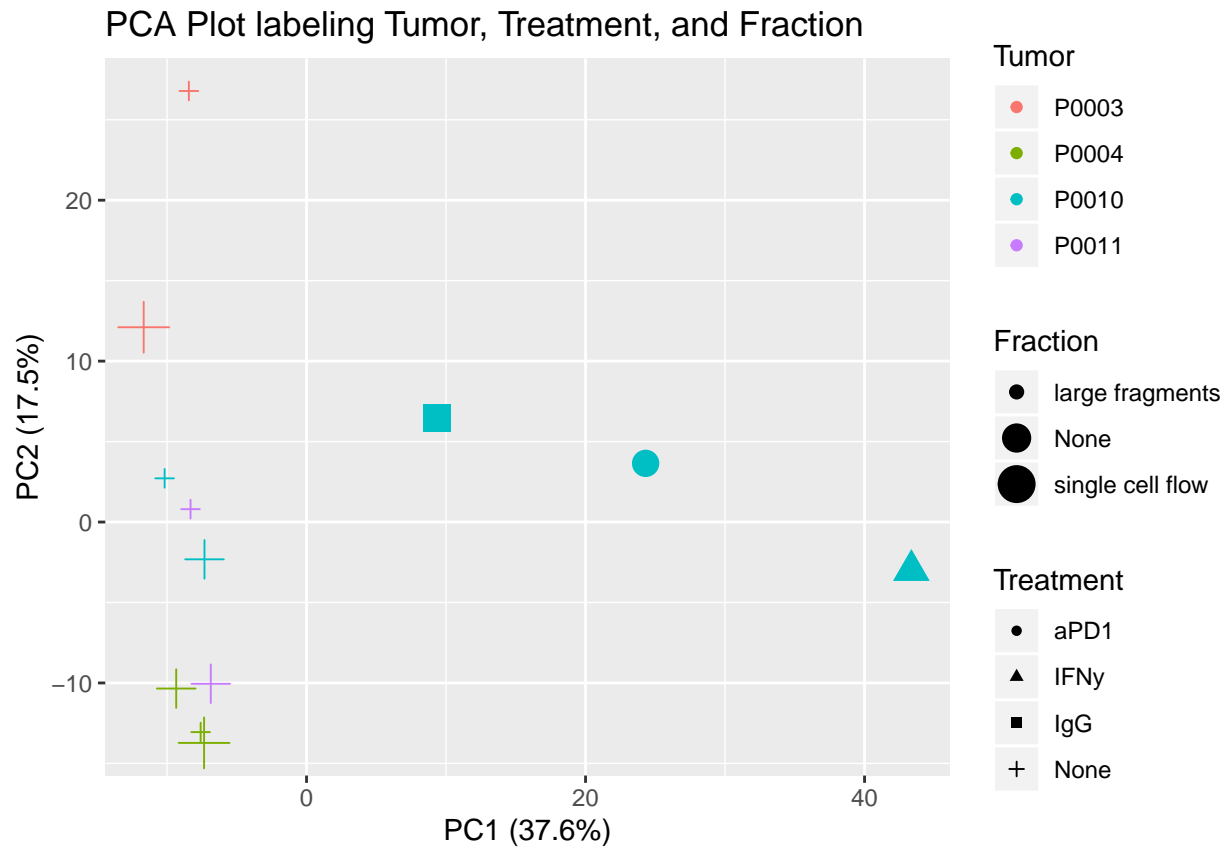
```
## Importance of components:
##          PC1      PC2      PC3      PC4      PC5      PC6
## Standard deviation 17.1568 11.7095 11.0791 8.76368 7.71650 5.47066
## Proportion of Variance 0.3755 0.1749 0.1566 0.09796 0.07595 0.03817
## Cumulative Proportion 0.3755 0.5503 0.7069 0.80487 0.88082 0.91899
```

```
##
##          PC7      PC8      PC9      PC10     PC11      PC12
## Standard deviation  4.79097 4.02371 3.49170 3.04994 1.69550 5.792e-15
## Proportion of Variance 0.02928 0.02065 0.01555 0.01186 0.00367 0.000e+00
## Cumulative Proportion 0.94827 0.96892 0.98447 0.99633 1.00000 1.000e+00
```

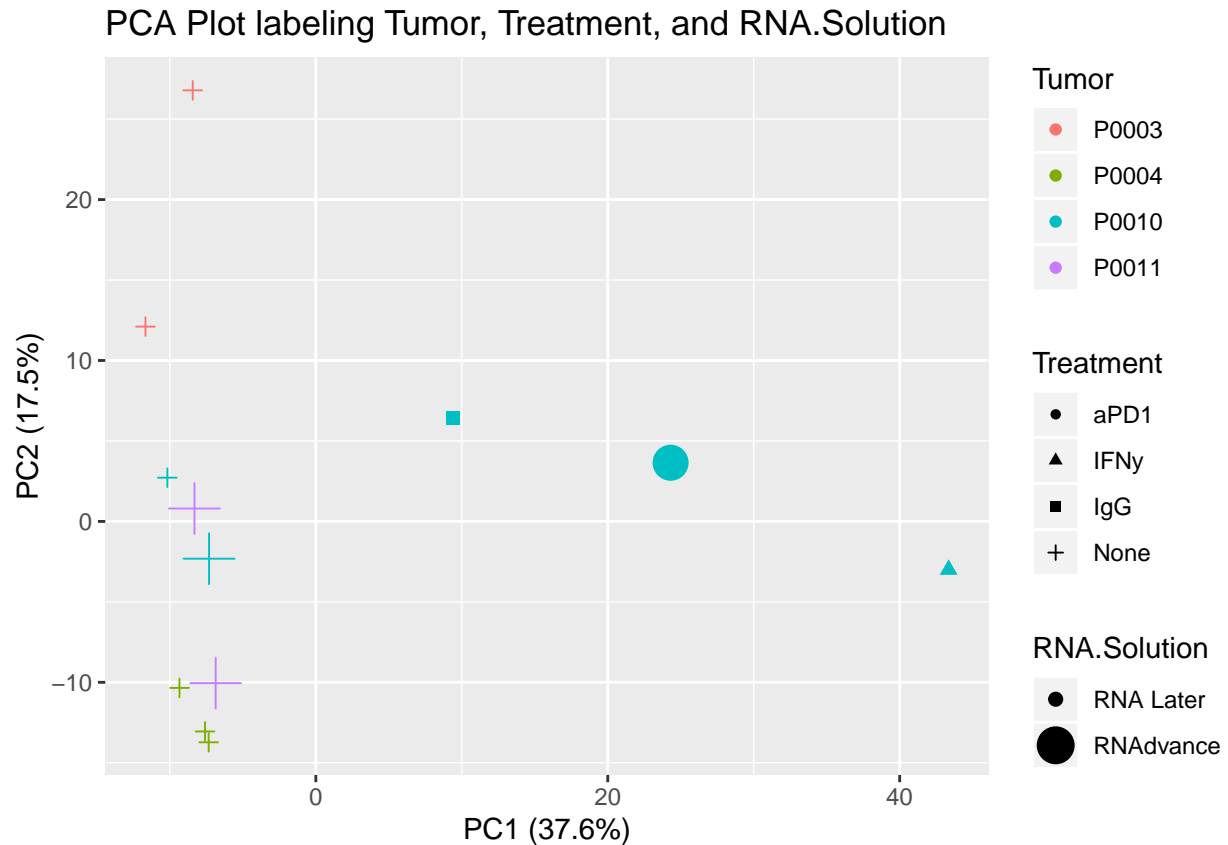
```
## Joining, by = "Sample.Name"
```

Samples Separate by treatment

```
## Warning: Using size for a discrete variable is not advised.
```



```
## Warning: Using size for a discrete variable is not advised.
```



Immune Cell Type Composition?

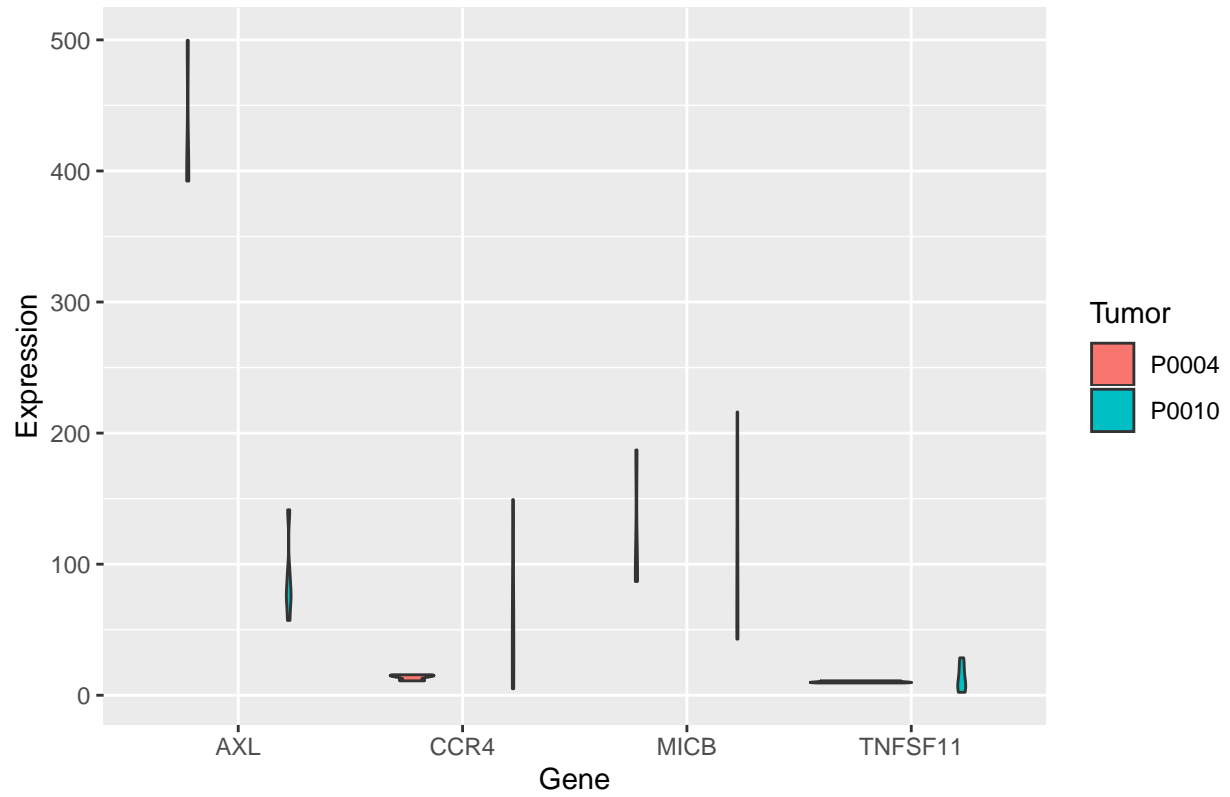
What can we learn about IFNγ treatment.immune cells activated? Which immune cell type (and other) to compare with what we learned from flow cytometry? PD1 elicited response?

*Missing Flow Cytometry data

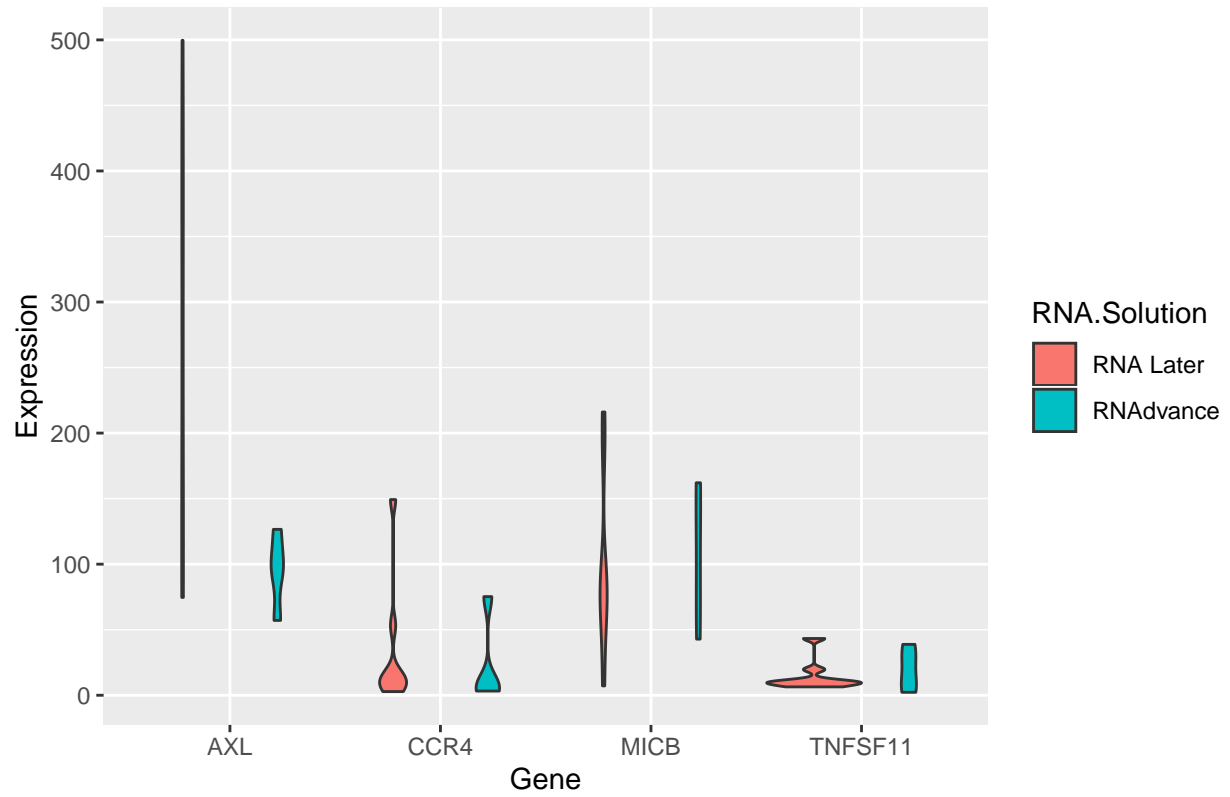
```
## Joining, by = "Sample.Name"
```

```
## Warning: Column `Sample.Name` joining character vector and factor, coercing
## into character vector
```

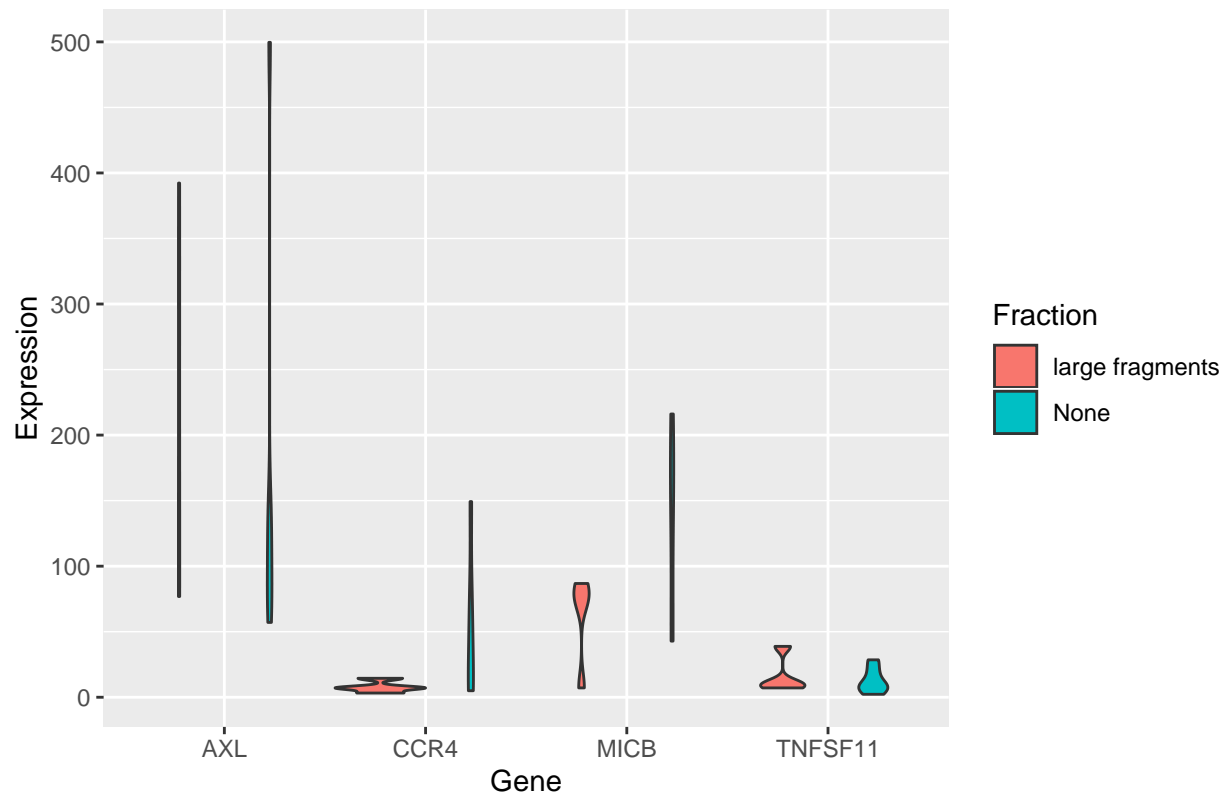
Is there a relationship for Tumor?

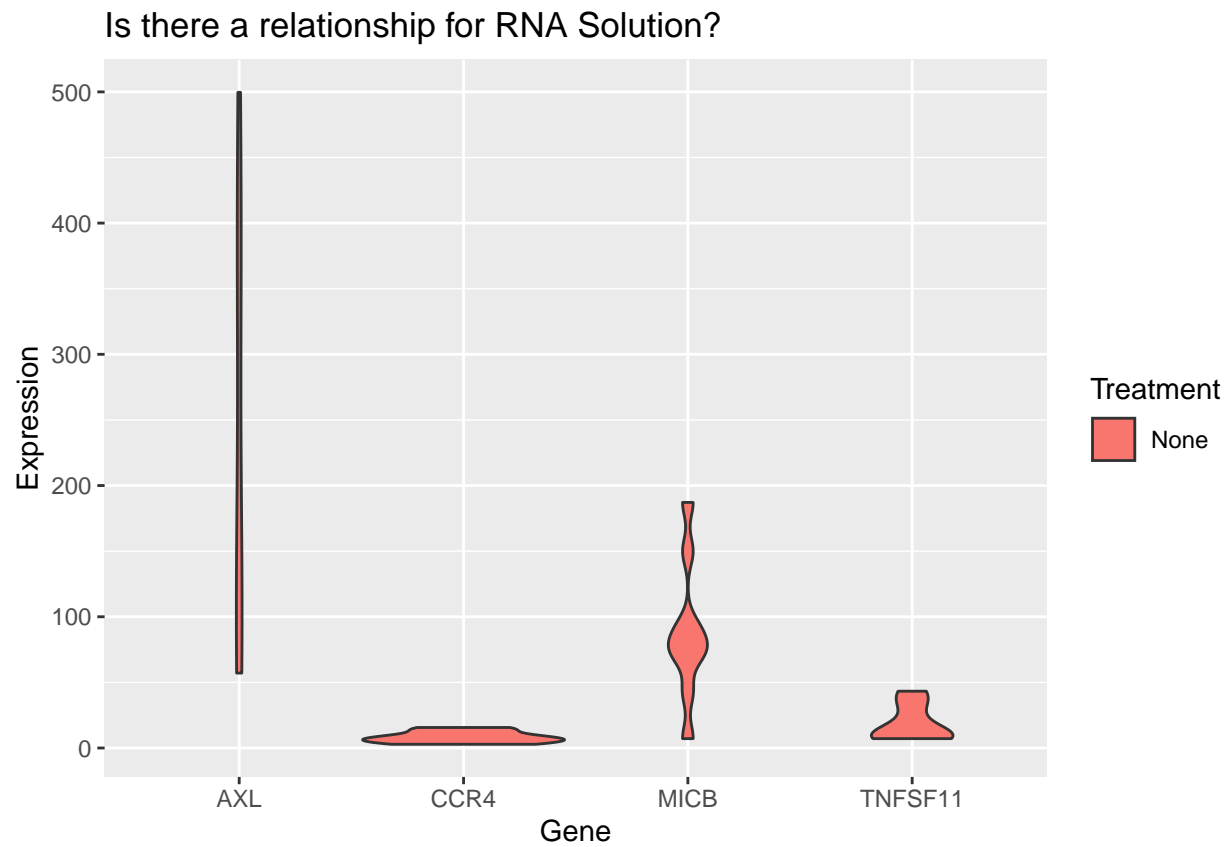


Is there a relationship for RNA Solution?



Is there a relationship for Tumor?





Heatmaps of Each Immune Cell Type

```
## Joining, by = "Sample.Name"
```

