

Nanostring Analysis for Dataset X

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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 IFNg 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.IFNy	RNA Later	P0010	None	IFNy
## 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

x

Cellular.Stress
Complement.System
Death.Receptor.Signaling
Extracellular.matrix.organization
Fc.Receptor.Signaling
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
+	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
+	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

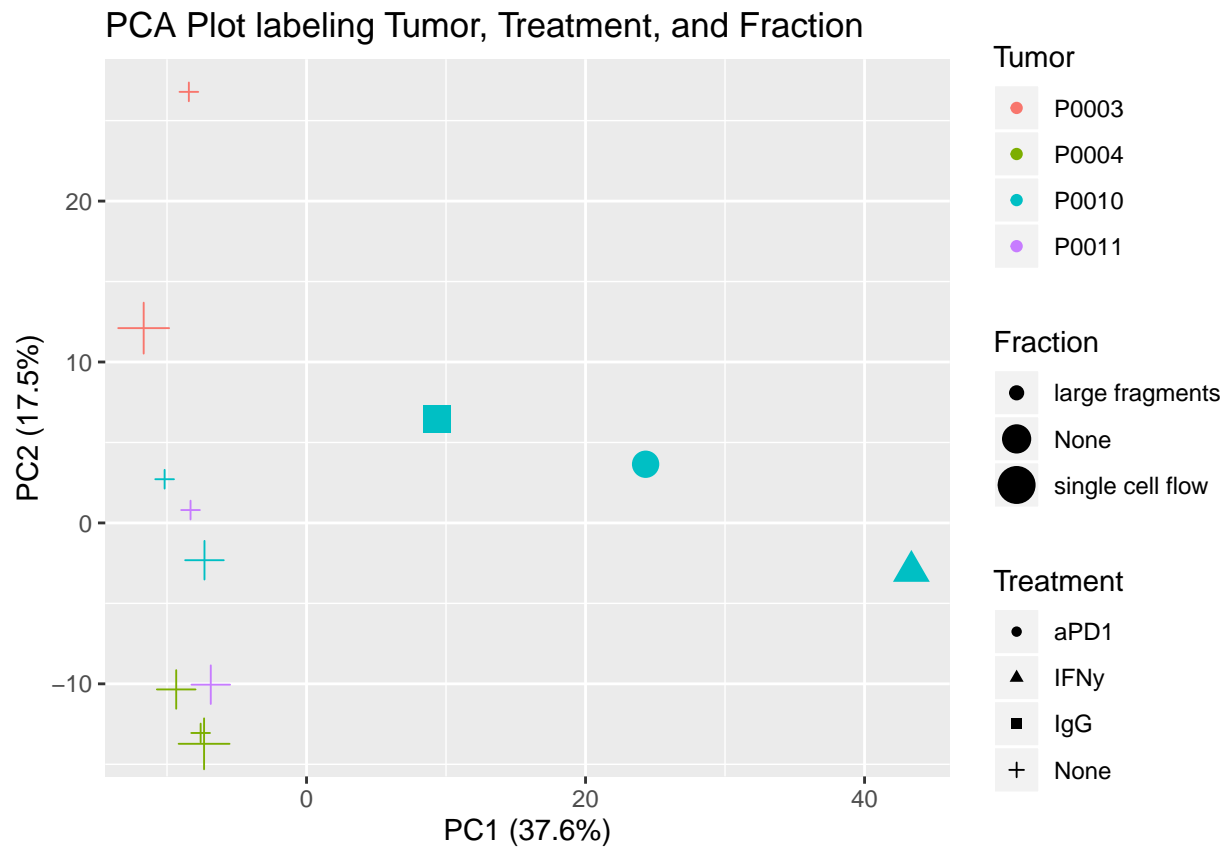
```
## Importance of components:
##          PC1      PC2      PC3      PC4      PC5      PC6      PC7
## Standard deviation  17.1568 11.7095 11.0791 8.76368 7.71650 5.47066 4.79097
## Proportion of Variance 0.3755 0.1749 0.1566 0.09796 0.07595 0.03817 0.02928
```

```
## Cumulative Proportion  0.3755  0.5503  0.7069  0.80487  0.88082  0.91899  0.94827
##                        PC8    PC9    PC10    PC11    PC12
## Standard deviation    4.02371  3.49170  3.04994  1.69550  5.792e-15
## Proportion of Variance 0.02065  0.01555  0.01186  0.00367  0.000e+00
## Cumulative Proportion  0.96892  0.98447  0.99633  1.00000  1.000e+00
```

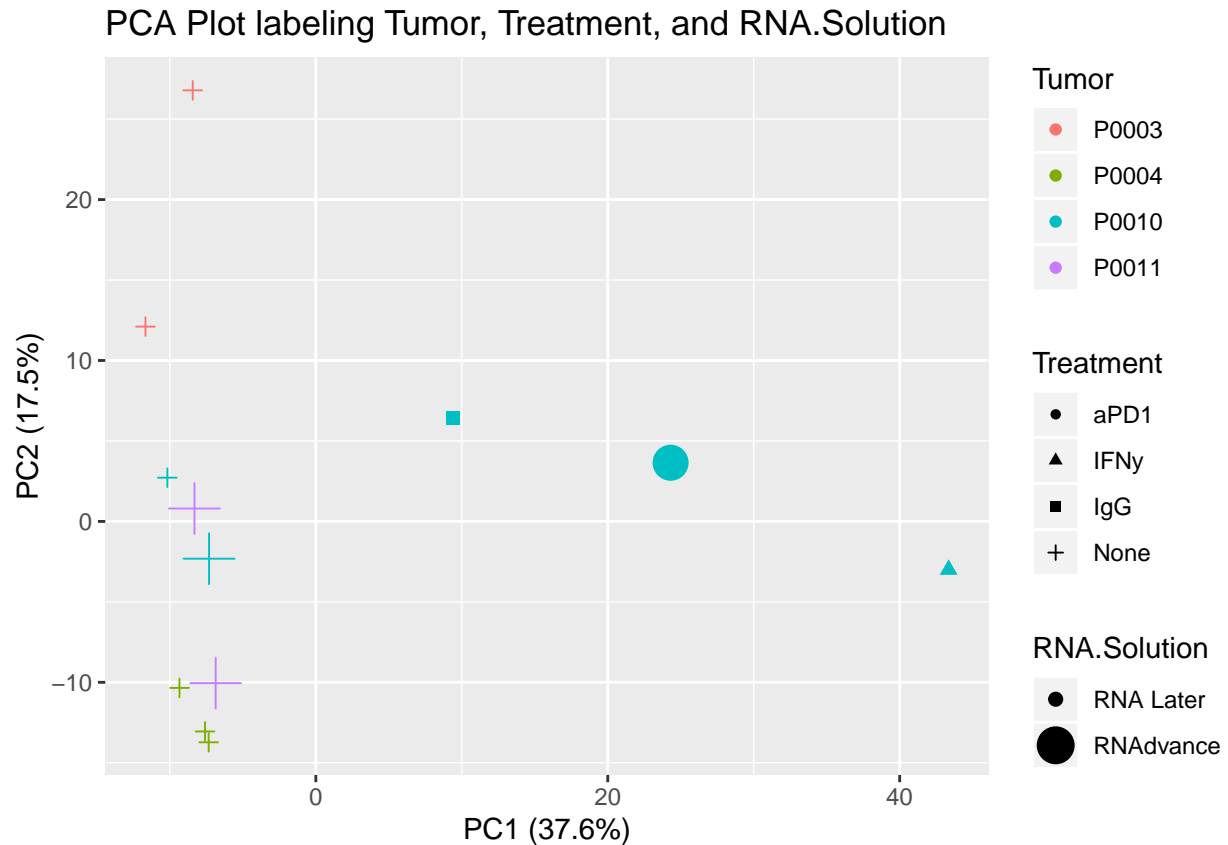
```
## Joining, by = "Sample.Name"
```

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?

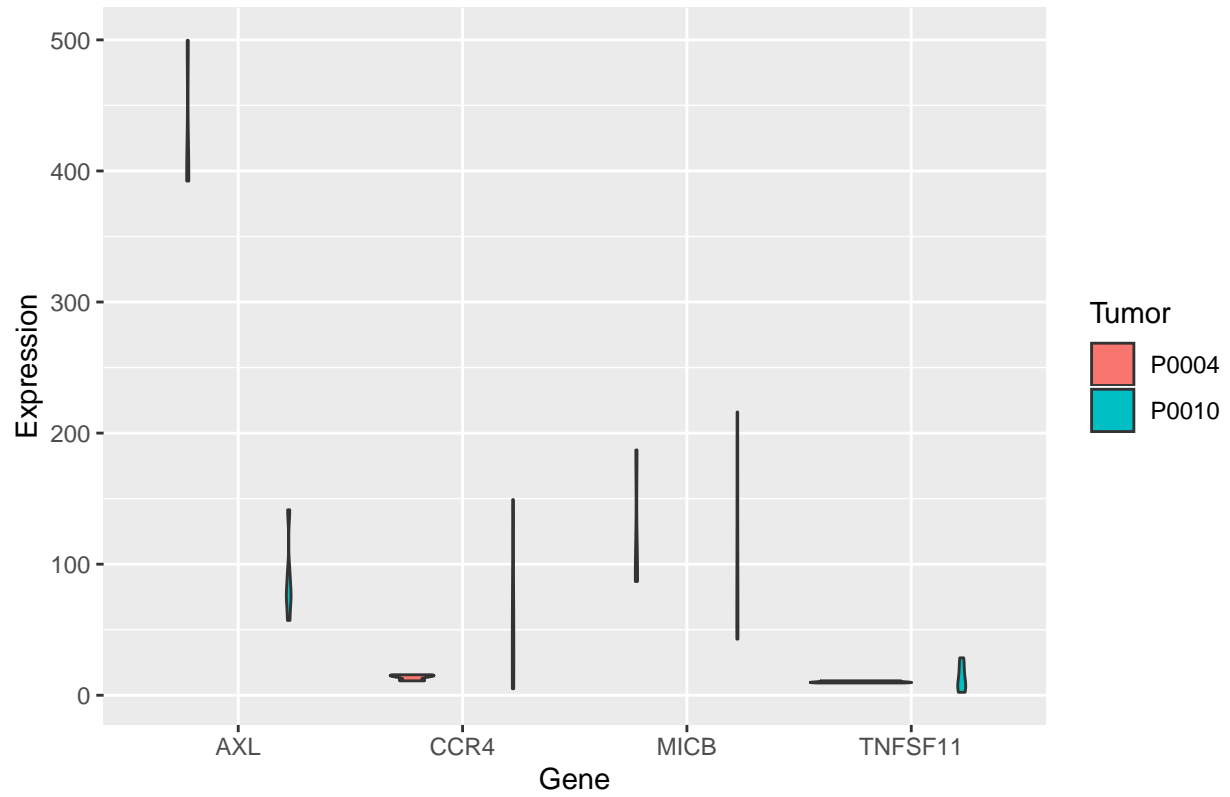
What can we learn about IFNγ treatment.immune cells activated? Which immune cell type (and other) to compare with what we learned from flow cytometry? PD1 elicited response?

*Missing Flow Cytometry data

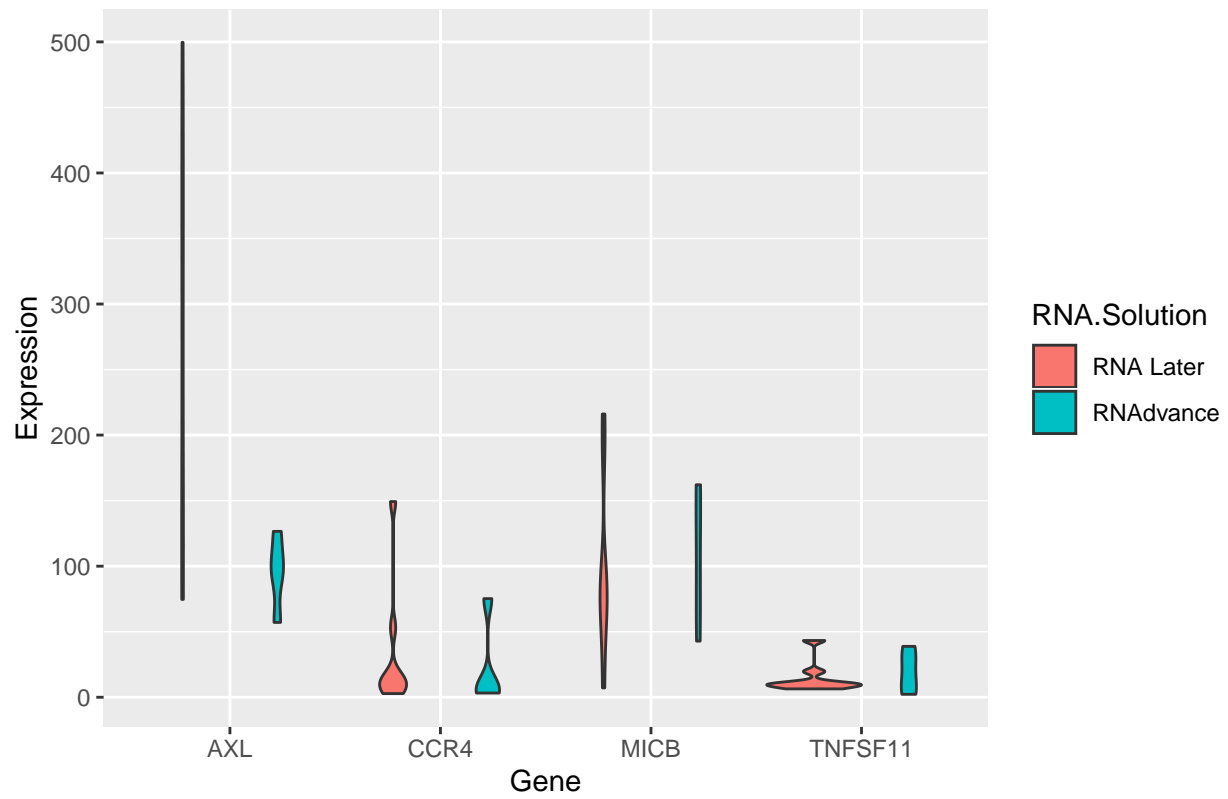
```
## Joining, by = "Sample.Name"
```

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

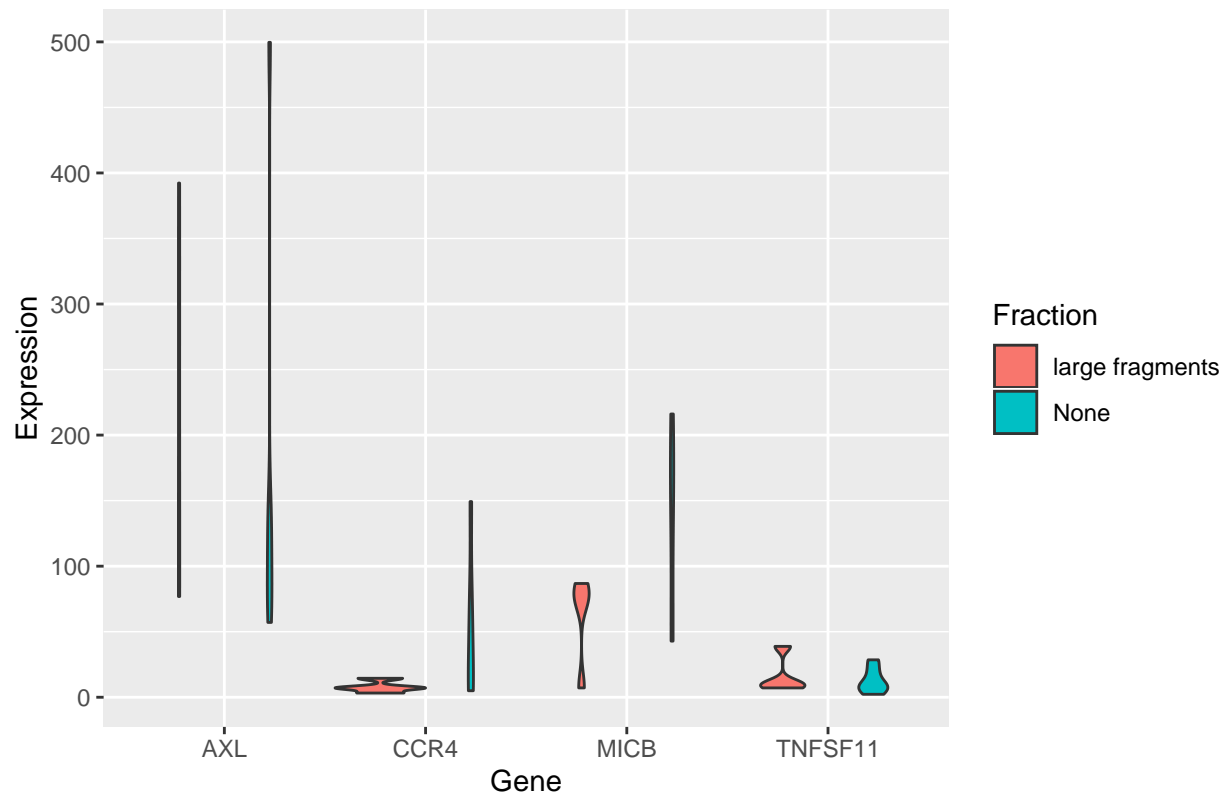
Is there a relationship for Tumor?

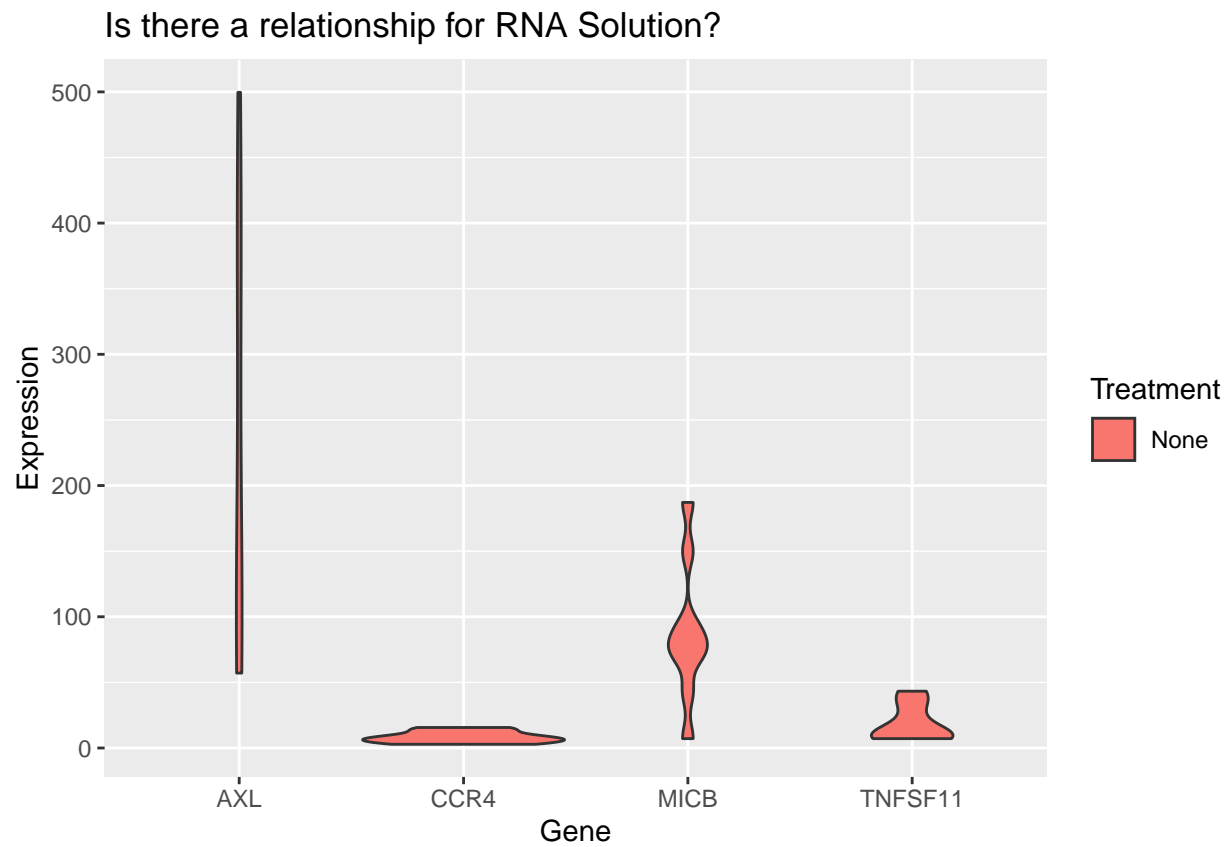


Is there a relationship for RNA Solution?



Is there a relationship for Tumor?





Heatmaps of Each Immune Cell Type

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

