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    RNAdvance P0010              None      None
## 10   P0010aPD1    RNAdvance P0010              None      aPD1
## 11    P0011.S1    RNAdvance P0011   large fragments      None
## 12    P0011.S2    RNAdvance 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

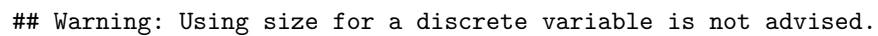| Treatment	n
aPD1	1
IFNy	1
IgG	1
None	9

Can we group the samples?

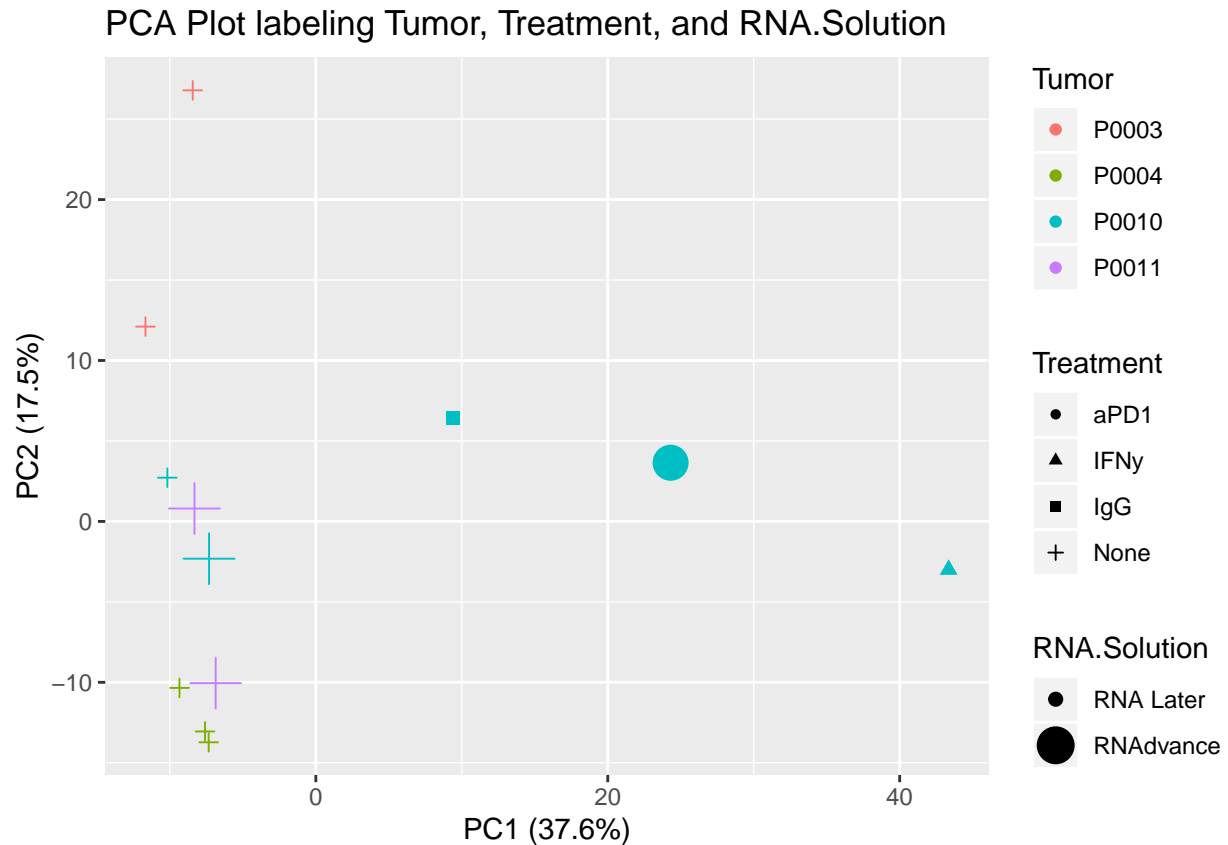PCA plots give an idea what similiarties are to be expected from the samples that are in it.

PCA - Calculates varability 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.
```

PCA Plot labeling Tumor, Treatment, and RNA.Solution


Immune Cell Type Composition?

What can we learn about IFNg treatment.immune cells activated? Which immune cell type (and other) to compare with what we learned from flow cytometry? PD1 elicit response?
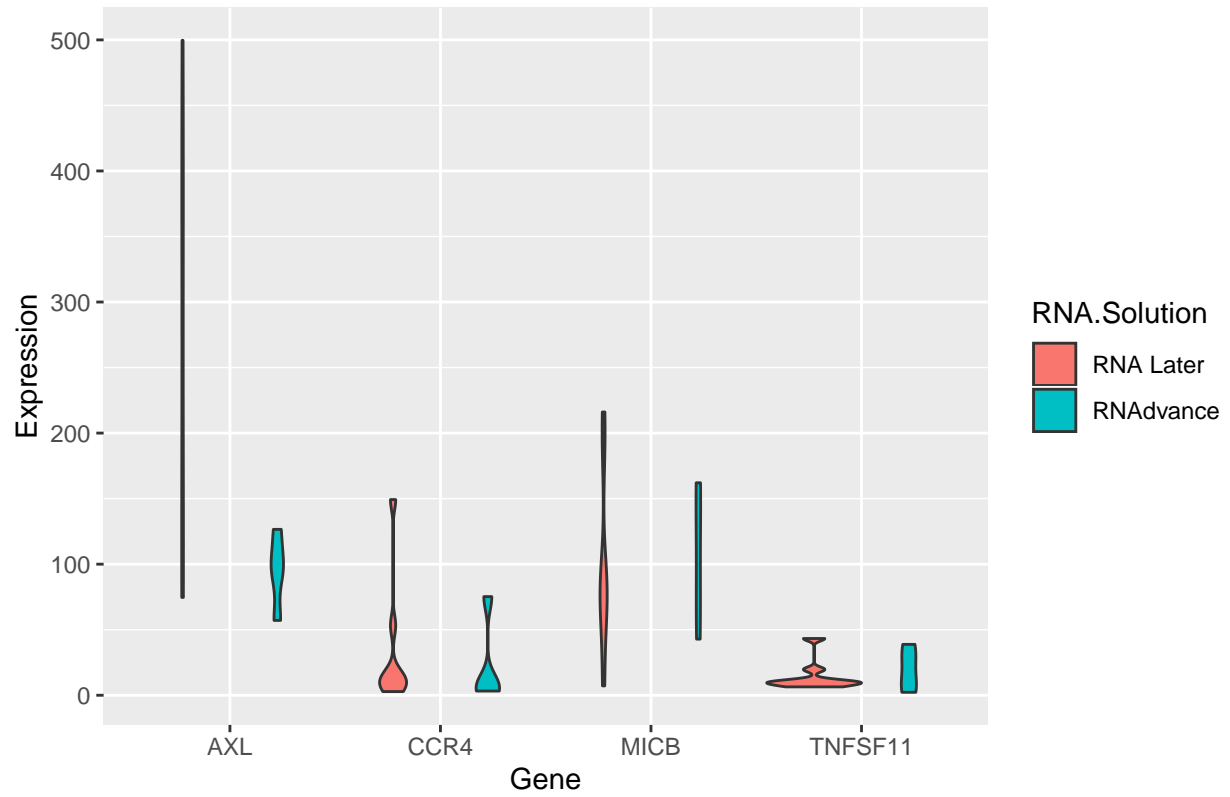
*Missing Flow Cytometry data

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

## Warning: Column `Sample.Name` joining character vector and factor, coercing
## into character vector
```
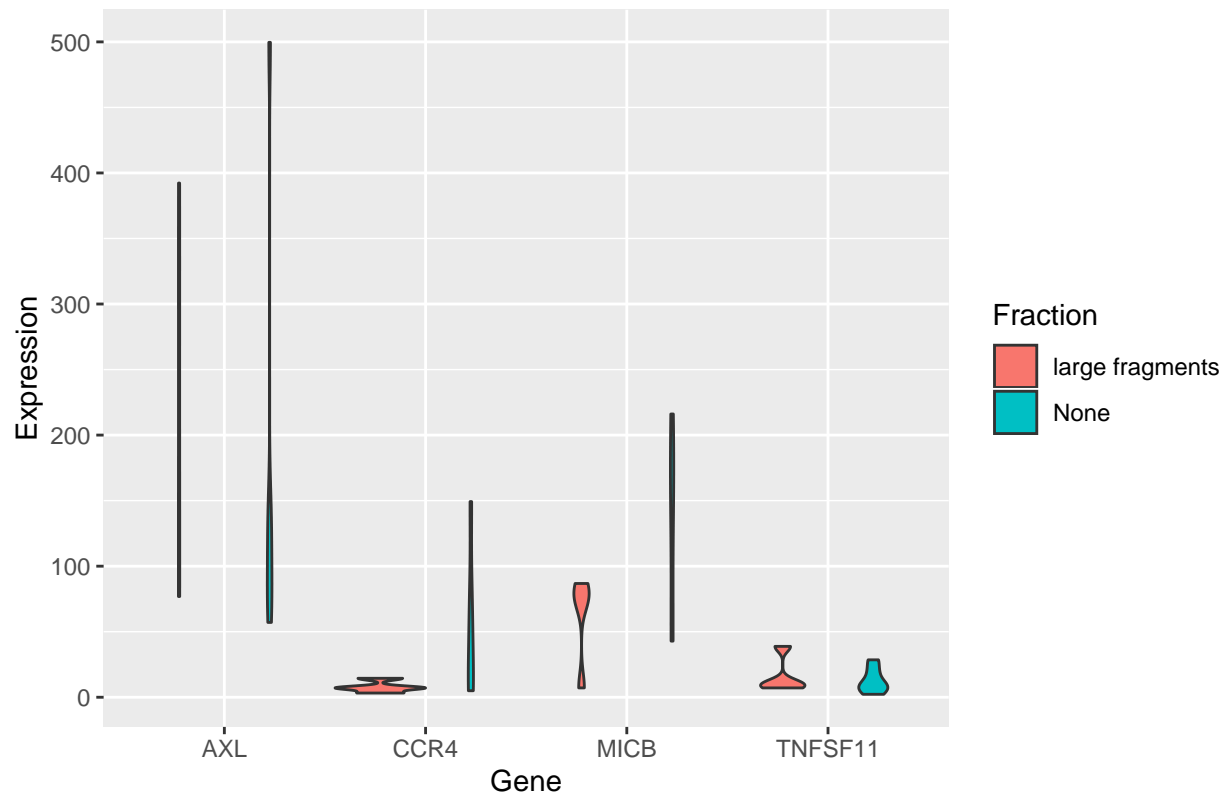
Is there a relationship for Tumor?
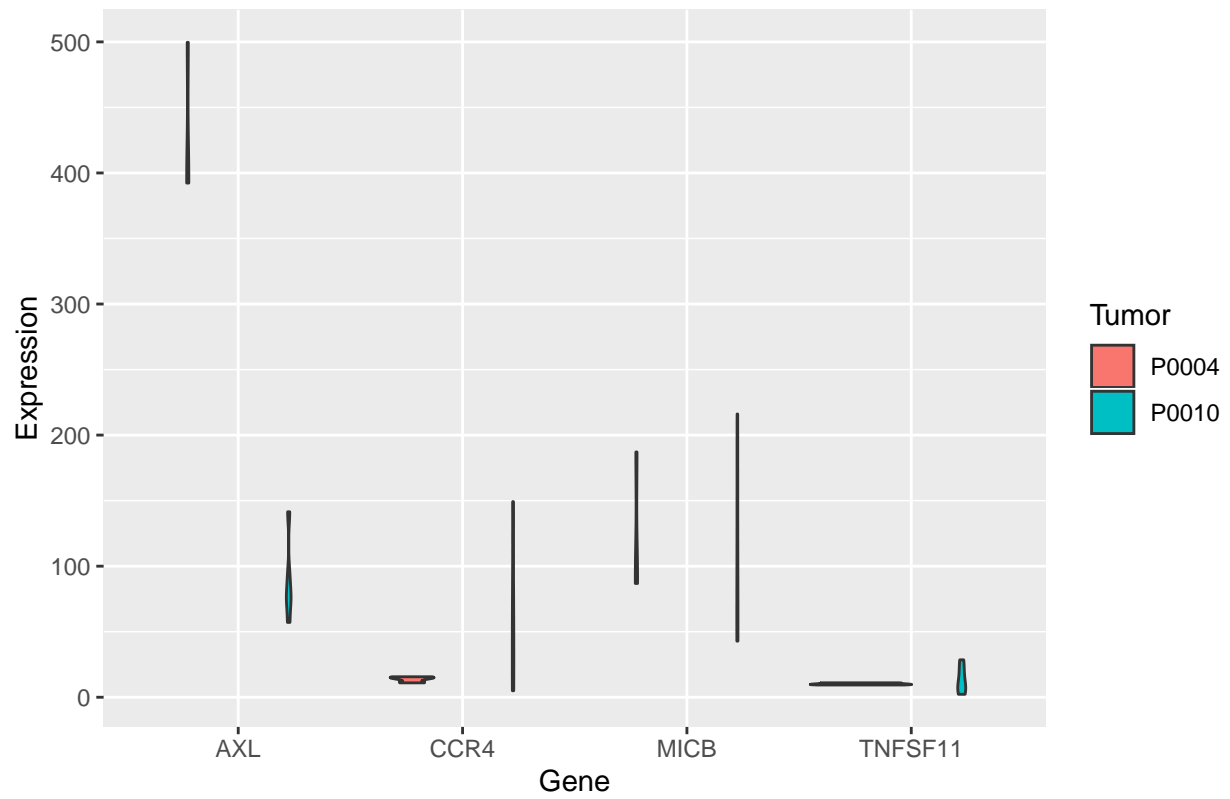
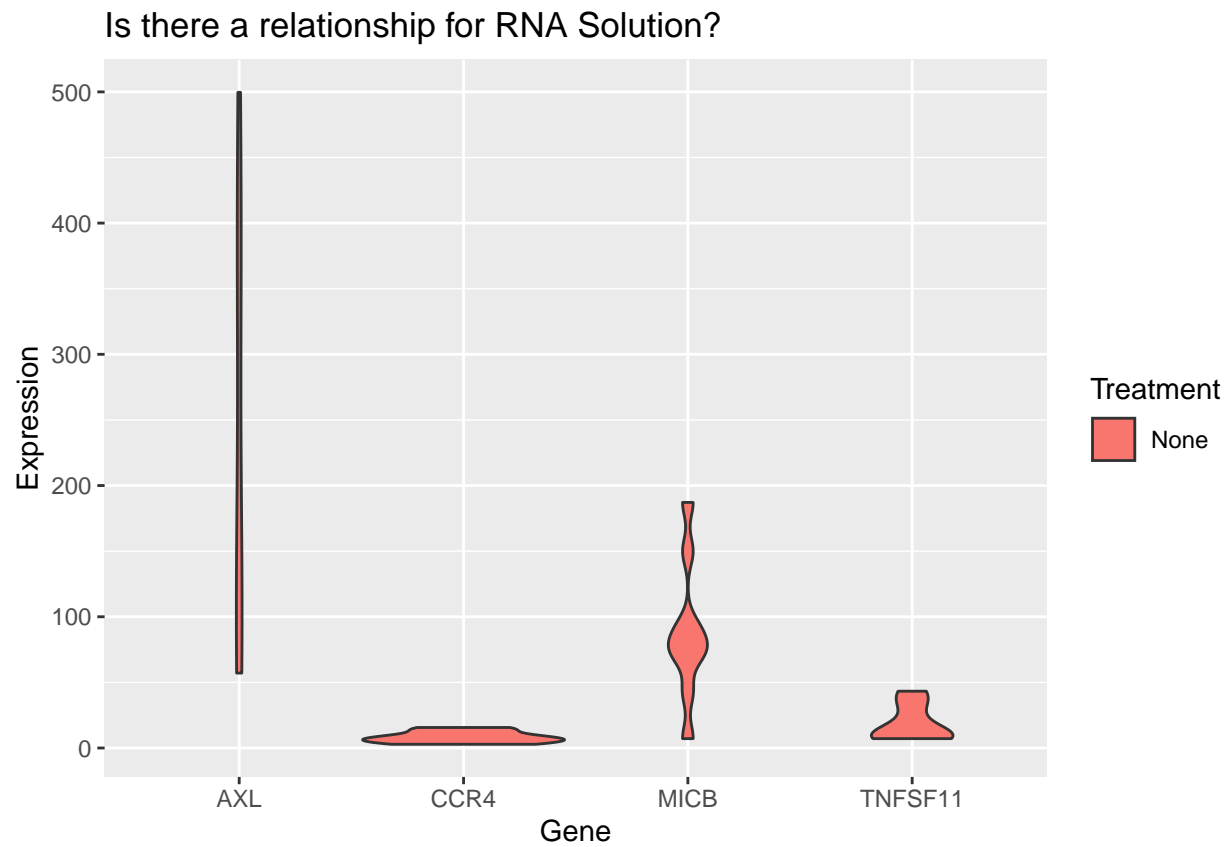Is there a relationship for RNA Solution?

Is there a relationship for Tumor?

Is there a relationship for RNA Solution?

Heatmaps of Each Immune Cell Type

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

B−cells

B−cells

Exhausted CD8

Exhausted CD8

T−cells

T−cells

CD8 T cells

CD8 T cells

Neutrophils

Neutrophils

Cytotoxic cells

Cytotoxic cells

NK CD56dim cells

NK CD56dim cells

Mast cells

MIF TTK

Treatment
Fraction
Tumor
RNA.Solution

P0004.S3
P0003.S3
P0010.IgG
P0010.IFNy
P0003.S1
P0010aPD1
P0004.S2
P0010.S1
P0010.S2
P0011.S2
P0004.S1
P0011.S1

2500
2000
1500
1000
500

Treatment
aPD1
IFNy
IgG
None

Fraction
large fragments
None
single cell flow

Tumor
P0003
P0004
P0010
P0011

Mast cells

NK cells