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

```
##    RNA.Solution Tumor          Fraction Treatment Amount.of.Material.Provide
## 1      RNAlater P0003   large fragments      None                        500µL
## 2      RNAlater P0003 single cell flow       None                        500µL
## 3      RNAlater P0004   large fragments      None                        500µL
## 4      RNAlater P0004              None      None                        500µL
## 5      RNAlater P0004 single cell flow       None                        500µL
## 6     RNAdvance P0010              None      IFNy                        200µL
## 7     RNAdvance P0010              None       IgG                        200µL
## 8      RNAlater P0010   large fragments      None                        500µL
## 9      RNAlater P0010              None      None                        500µL
## 10    RNAdvance P0010              None      aPD1                        200µL
## 11     RNAlater P0011   large fragments      None                        500µL
## 12     RNAlater P0011              None      None                        500µL
```

What is being quantified?

What Gene functions are being quantified?

x
Gene
Cell.Type
Adaptive.Immunity
Apoptosis
Cell.Cycle
Cellular.Stress
Complement.System

x
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
RNAdvance	3
RNAlater	9

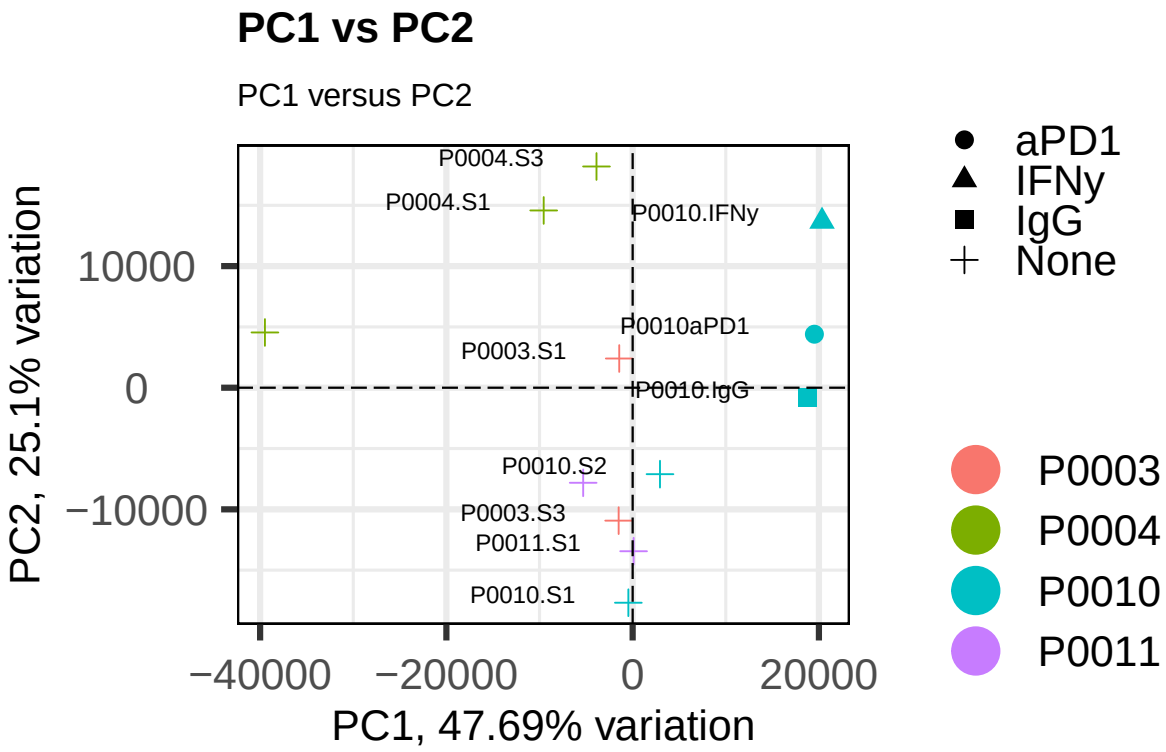
Tumor	n
P0003	2
P0004	3
P0010	5
P0011	2

Treatment	n
aPD1	1
IFNy	1
IgG	1
None	9

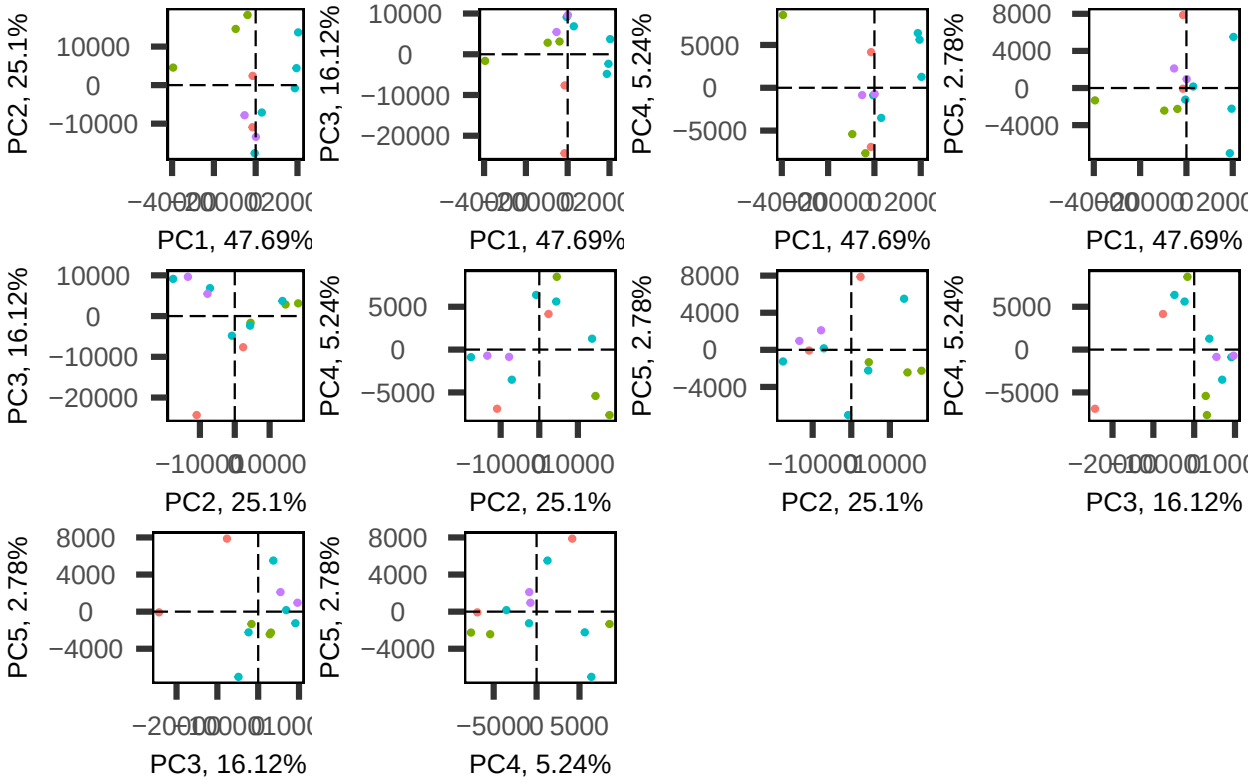
Can we group the samples?

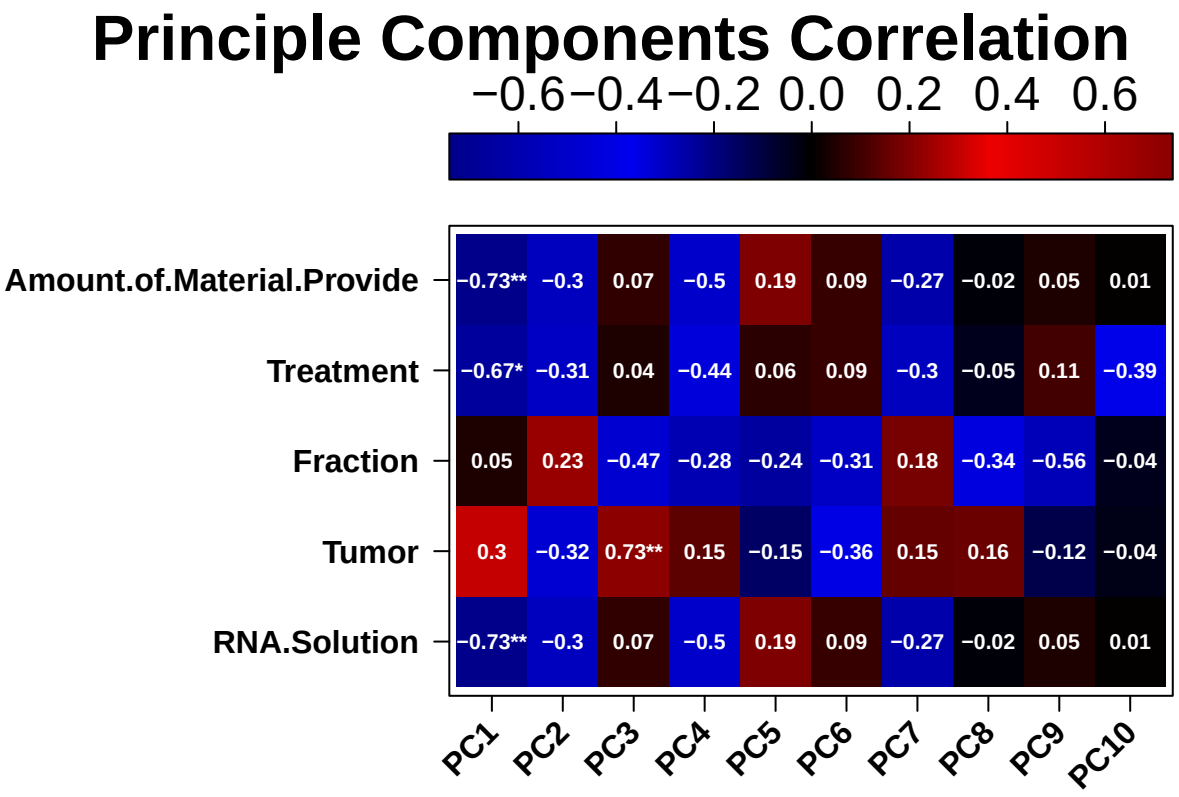
PCA plots give an idea what similiarties are to be expected from the samples that are in it.

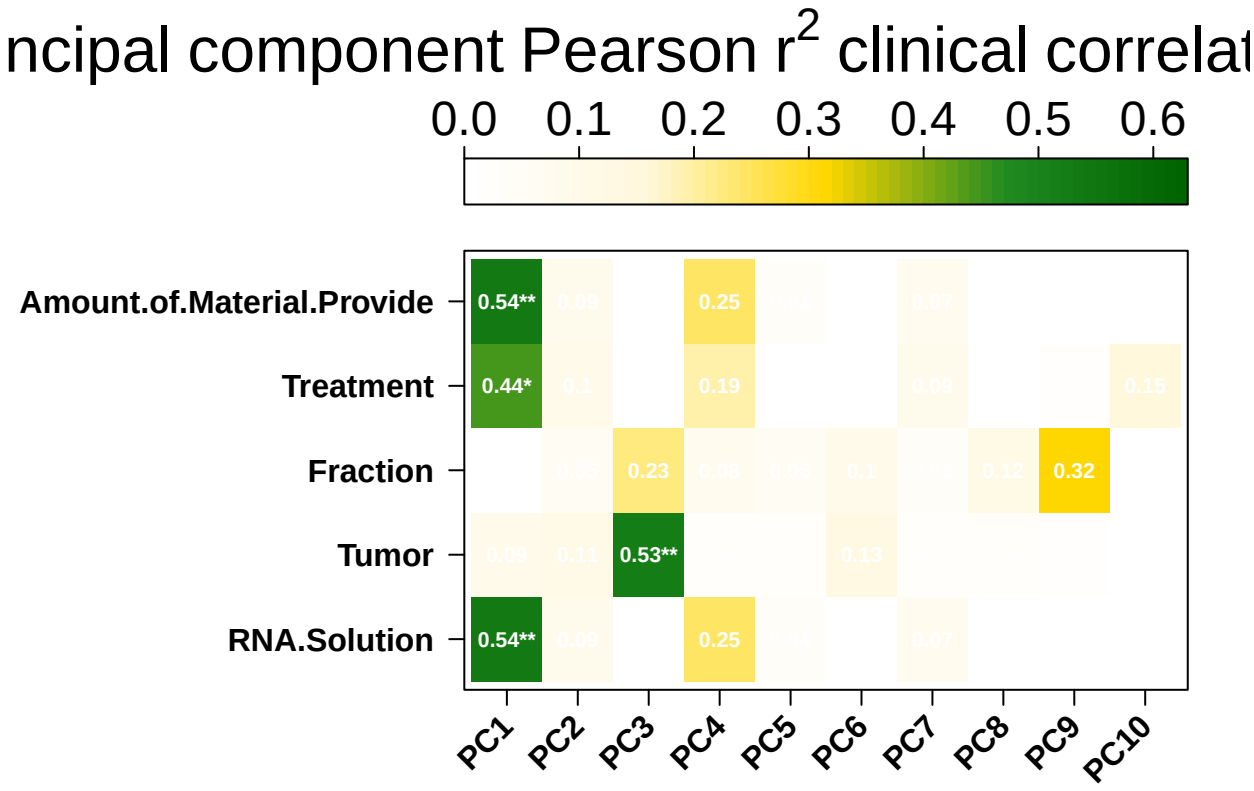
PCA - Calculates varability across entire dataset for each gene - Groups components - Using these components it allows to check for bias and grouping within samples

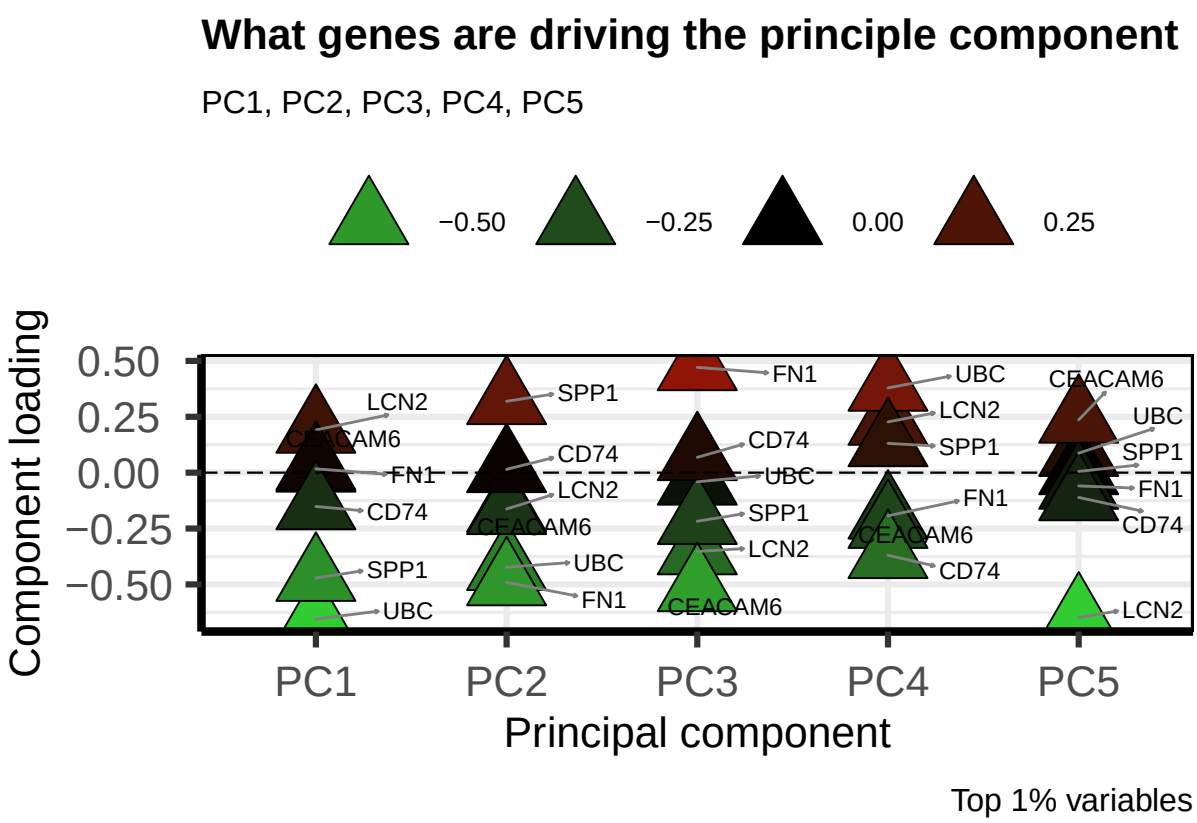

PC1 vs PC2
PC1 versus PC2
P0004.S3
P0004.S1
P0010.IFNy
P0010aPD1
P0003.S1
P0010.IgG
P0010.S2
P0003.S3
P0011.S1
P0010.S1
10000
0
−10000
PC2, 25.1% variation
−40000
−20000
0
20000
PC1, 47.69% variation
aPD1
IFNy
IgG
None
P0003
P0004
P0010
P0011

Pairs plot


PC2, 25.1%
PC3, 16.12%
PC4, 5.24%
PC5, 2.78%
PC1, 47.69%
PC2, 25.1%
PC3, 16.12%
PC4, 5.24%


Principle Components Correlation
−0.6 −0.4 −0.2 0.0 0.2 0.4 0.6
Amount.of.Material.Provide
Treatment
Fraction
Tumor
RNA.Solution
−0.73** −0.3 0.07 −0.5 0.19 0.09 −0.27 −0.02 0.05 0.01
−0.67* −0.31 0.04 −0.44 0.06 0.09 −0.3 −0.05 0.11 −0.39
0.05 0.23 −0.47 −0.28 −0.24 −0.31 0.18 −0.34 −0.56 −0.04
0.3 −0.32 0.73** 0.15 −0.15 −0.36 0.15 0.16 −0.12 −0.04
−0.73** −0.3 0.07 −0.5 0.19 0.09 −0.27 −0.02 0.05 0.01
PC1 PC2 PC3 PC4 PC5 PC6 PC7 PC8 PC9 PC10

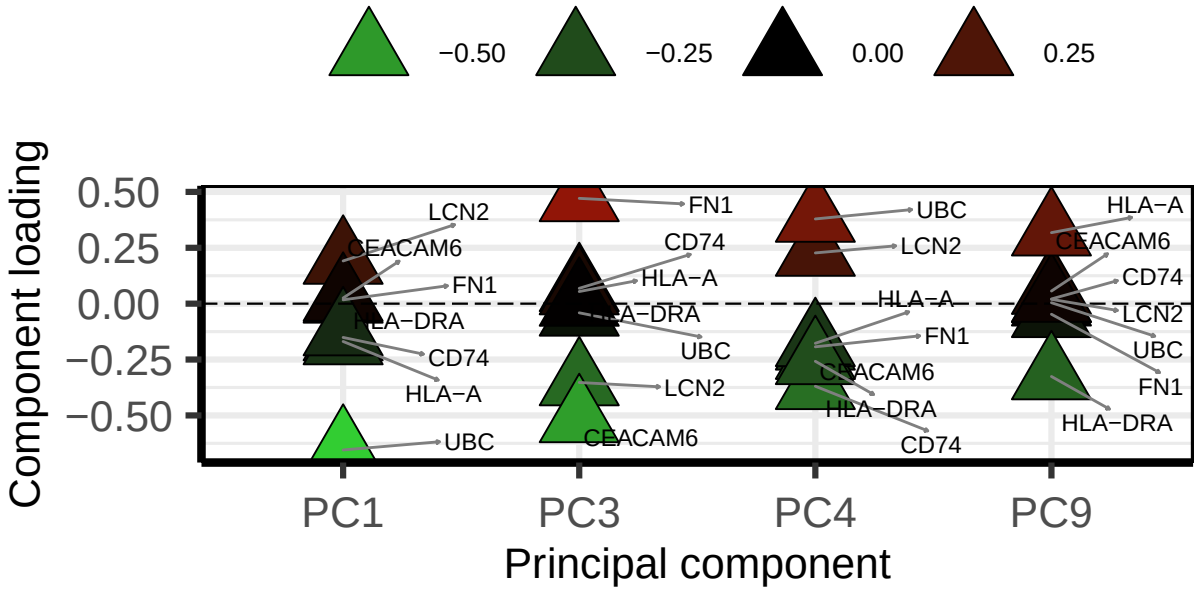

ncipal component Pearson r2 clinical correlat
0.0 0.1 0.2 0.3 0.4 0.5 0.6
Amount.of.Material.Provide
Treatment
Fraction
Tumor
RNA.Solution
0.54**
0.25
0.07
0.44*
0.19
0.09
0.15
0.23
0.12
0.32
0.53**
0.13
0.54**
0.25
0.07
PC1
PC2
PC3
PC4
PC5
PC6
PC7
PC8
PC9
PC10

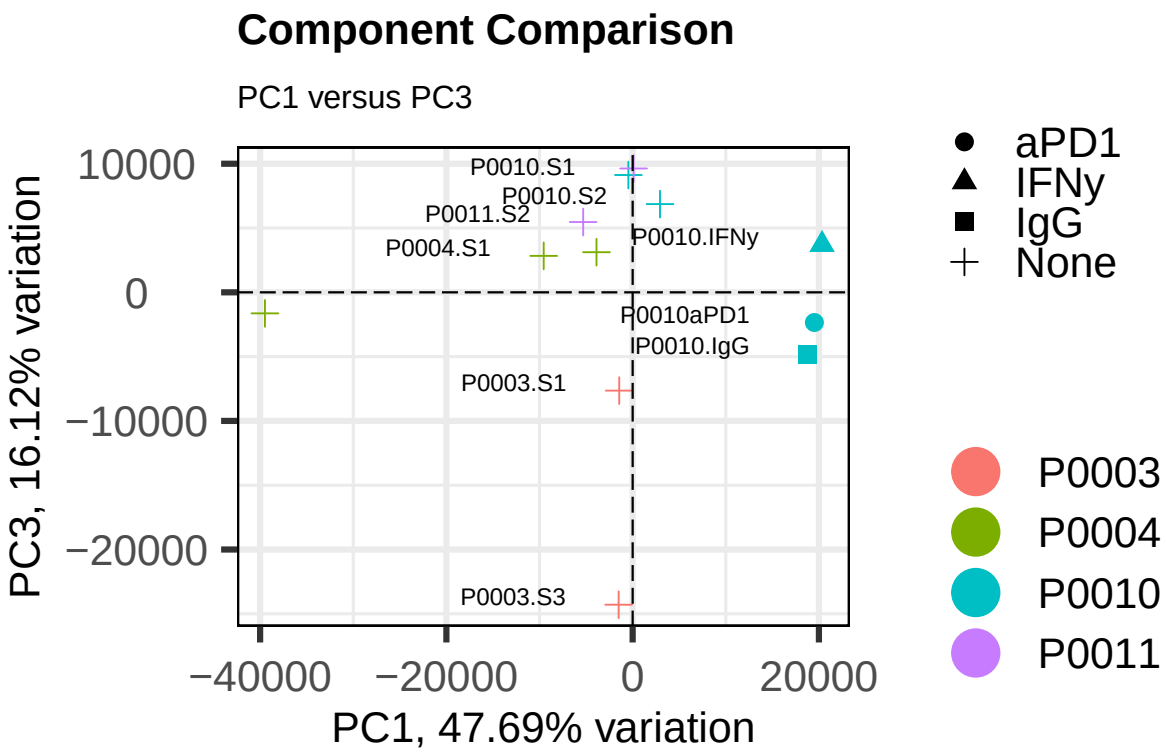


Top 1% variables

What genes are driving the principle component

Focus on Significant Components



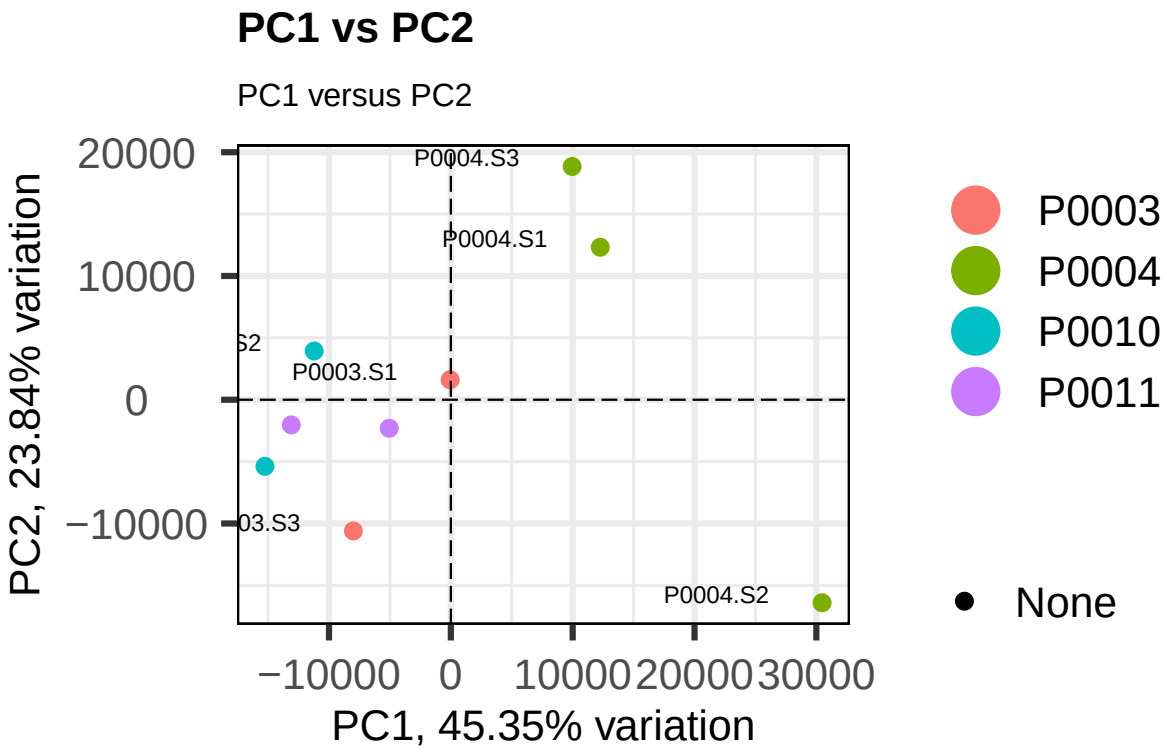


Can we group the samples without treatment samples?

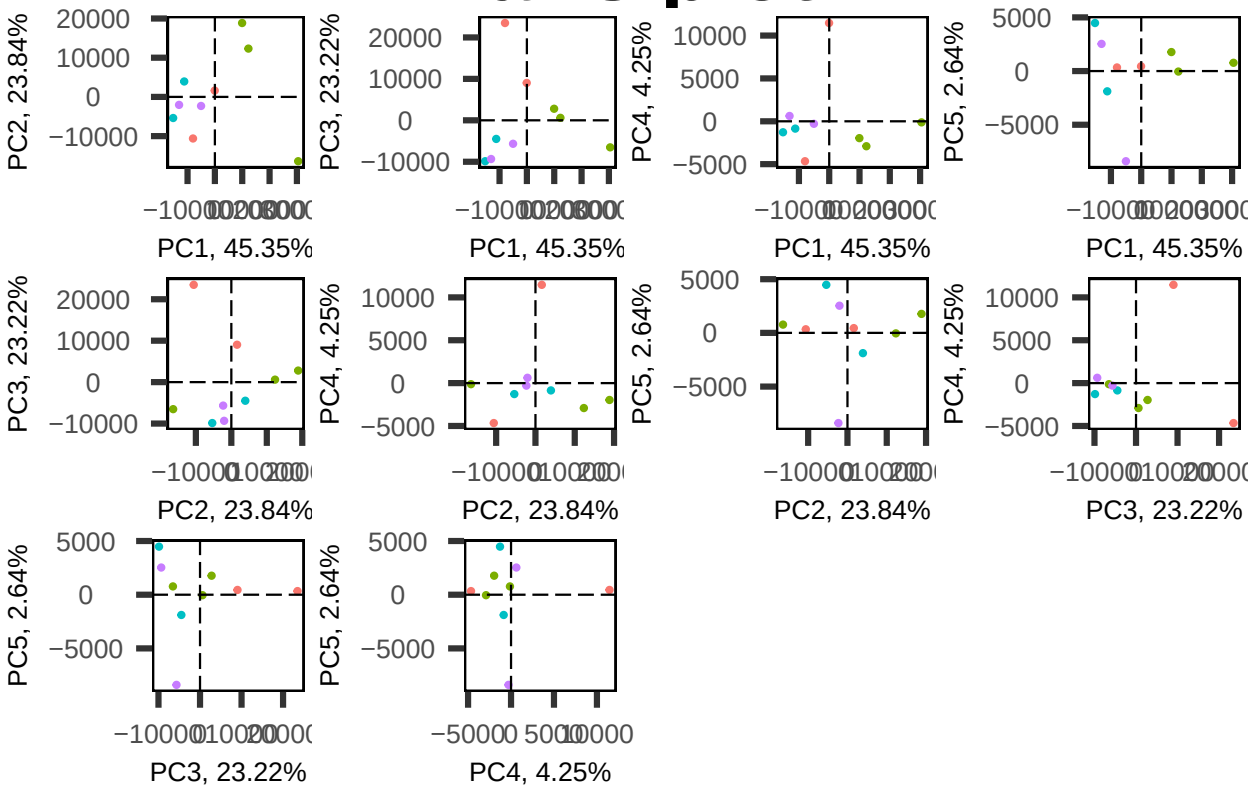
One of the strongest indicators for how RNA expression changed was due to treatment / day effect. What are the factors if these samples are excluded from the analysis?

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

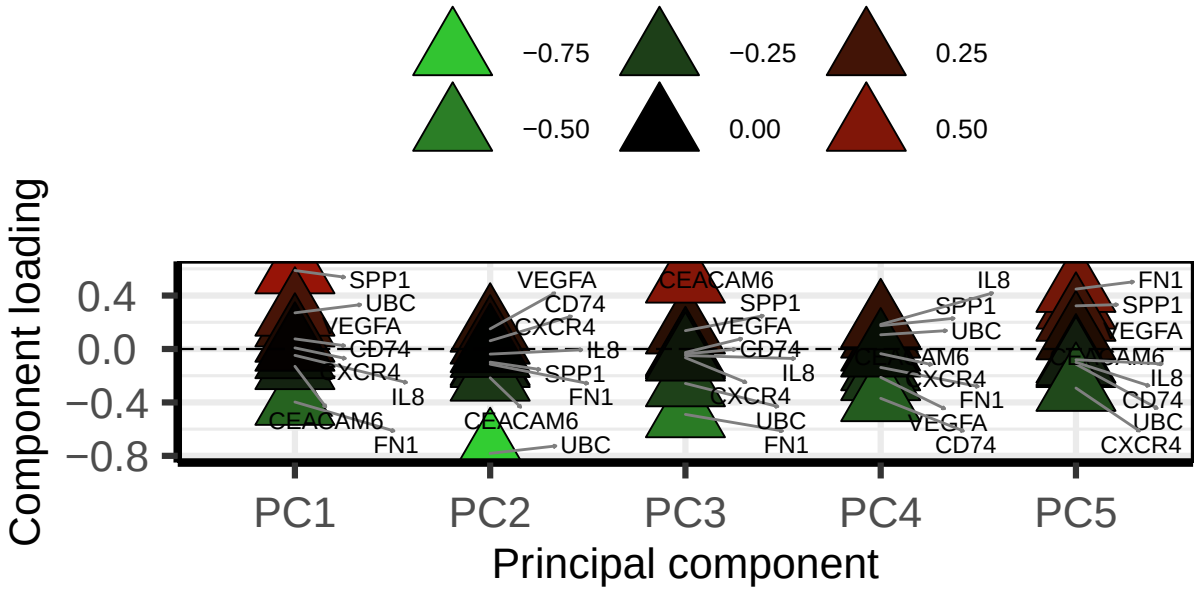

PC1 vs PC2
PC1 versus PC2
20000
10000
0
−10000
PC2, 23.84% variation
P0004.S3
P0004.S1
S2
P0003.S1
03.S3
P0004.S2
−10000
0
10000
20000
30000
PC1, 45.35% variation
P0003
P0004
P0010
P0011
None

Pairs plot


PC2, 23.84%
PC3, 23.22%
PC4, 4.25%
PC5, 2.64%
PC1, 45.35%
PC2, 23.84%
PC3, 23.22%
PC4, 4.25%

What genes are driving the principle component

PC1, PC2, PC3, PC4, PC5



Top 1% variables