

# Nanostring Analysis for Dataset X v3.0

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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 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	RNAAdvance	P0010	None	IFNy	200µL
## 7	RNAAdvance	P0010	None	IgG	200µL
## 8	RNAlater	P0010	large fragments	None	500µL
## 9	RNAlater	P0010	None	None	500µL
## 10	RNAAdvance	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?

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x

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

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x
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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

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How many genes represent each function type Information?

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Cell.Type	n
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	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

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Cellular.Stress	n
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-	723
+	31

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Complement.System	n
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-	720
+	34

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Death.Receptor.Signaling	n
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-	736
+	18

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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

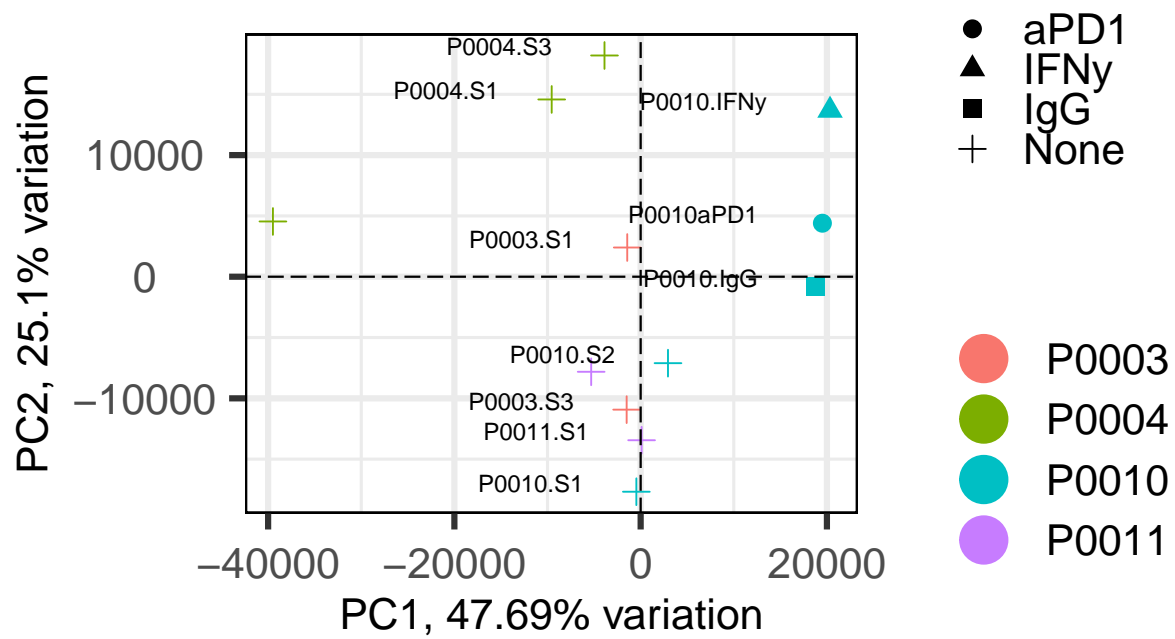
Can we group the samples?

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

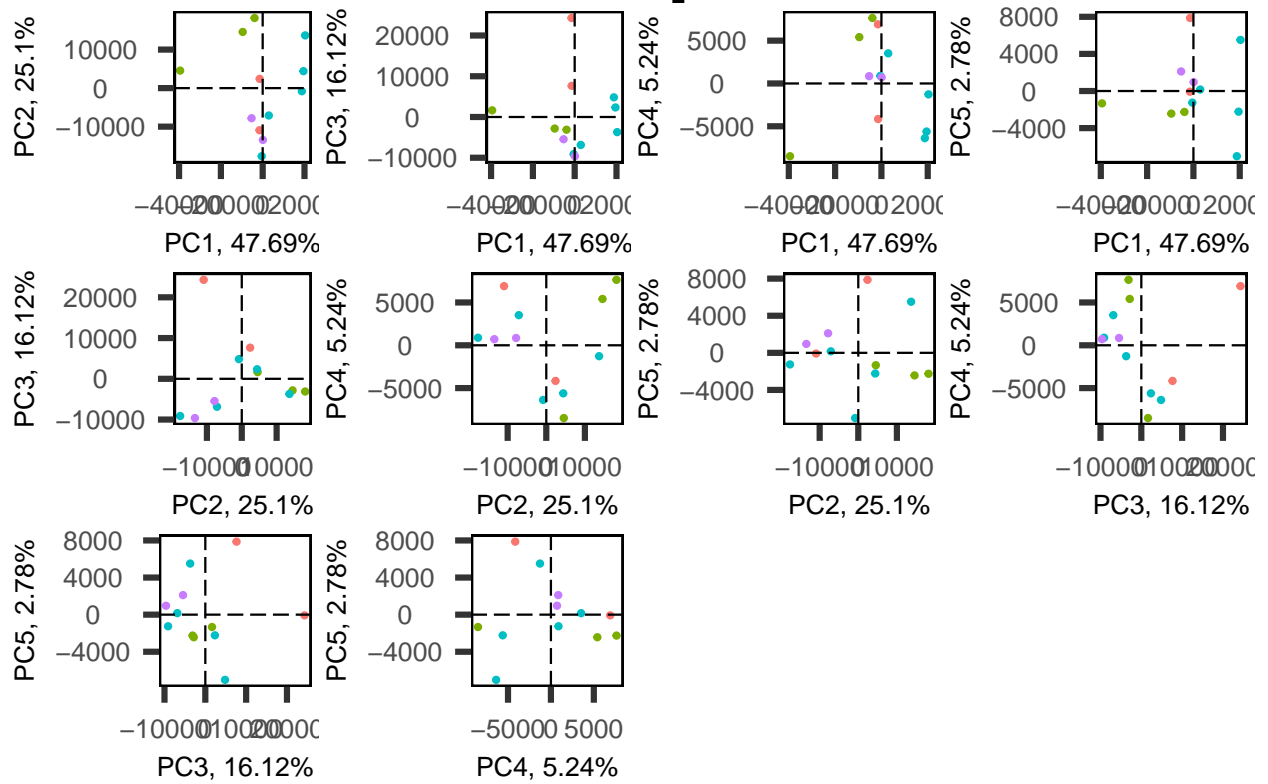
PCA - Calculates variability across entire dataset for each gene - Groups components - Using these components it allows to check for bias and grouping within samples

## PC1 vs PC2

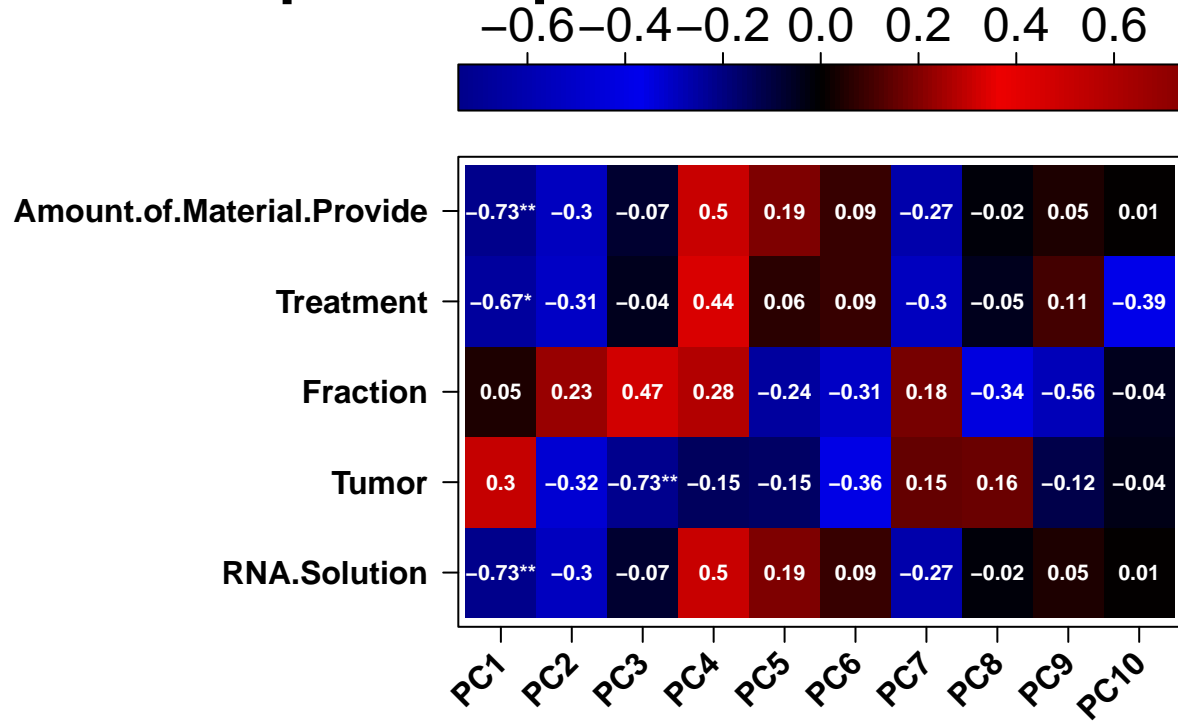
PC1 versus PC2



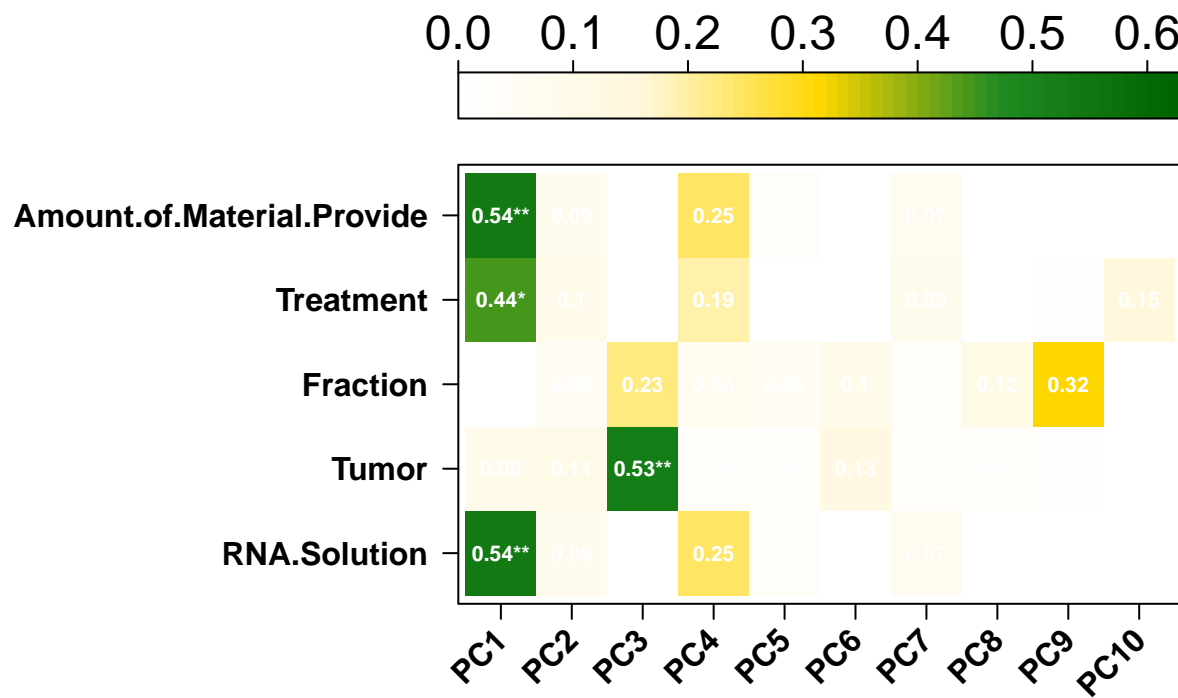
# Pairs plot



# Principle Components Correlation



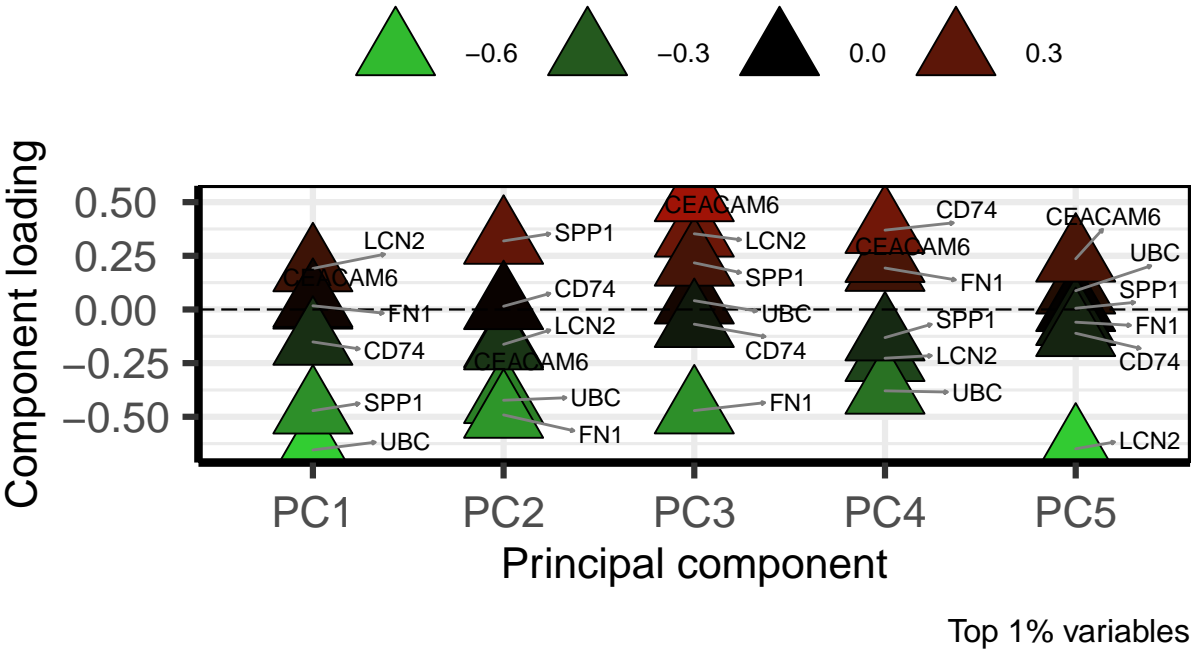
# Principal component Pearson $r^2$ clinical correlation





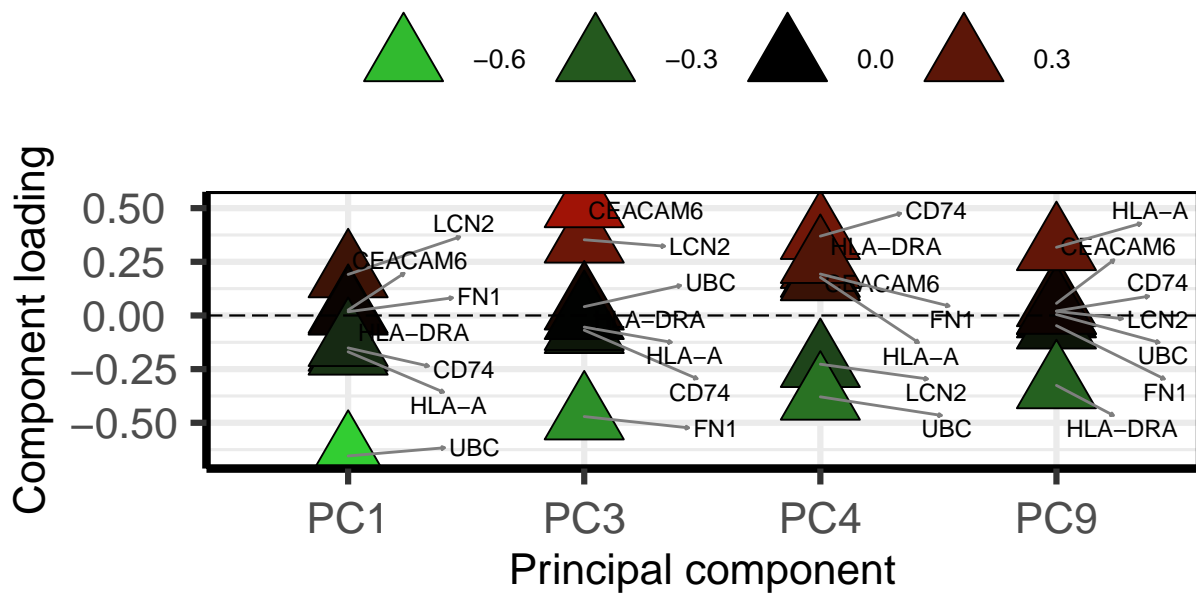
What genes are driving the principle component

PC1, PC2, PC3, PC4, PC5



## What genes are driving the principle component

Focus on Significant Components



## Component Comparison

PC1 versus PC3

