A04 - Differential Expression Analysis

1 Open the workflow called "Differential Analysis"

2 The diffcyt algorithm works in either two modes.

- differential expression
- differential abundance

We will focus on the differential expression mode for this guide.

3 The Differential expression block has already been executed.

Double click on the block to explore the two prepared views

One is a "Heatmap LFC" the other is the "Median by Condition Plot"

We will open both

Discuss! the Heatmap LFC

What do you think we are looking at here?

Did you notice there is an LFC value for each cluster-marker combination?

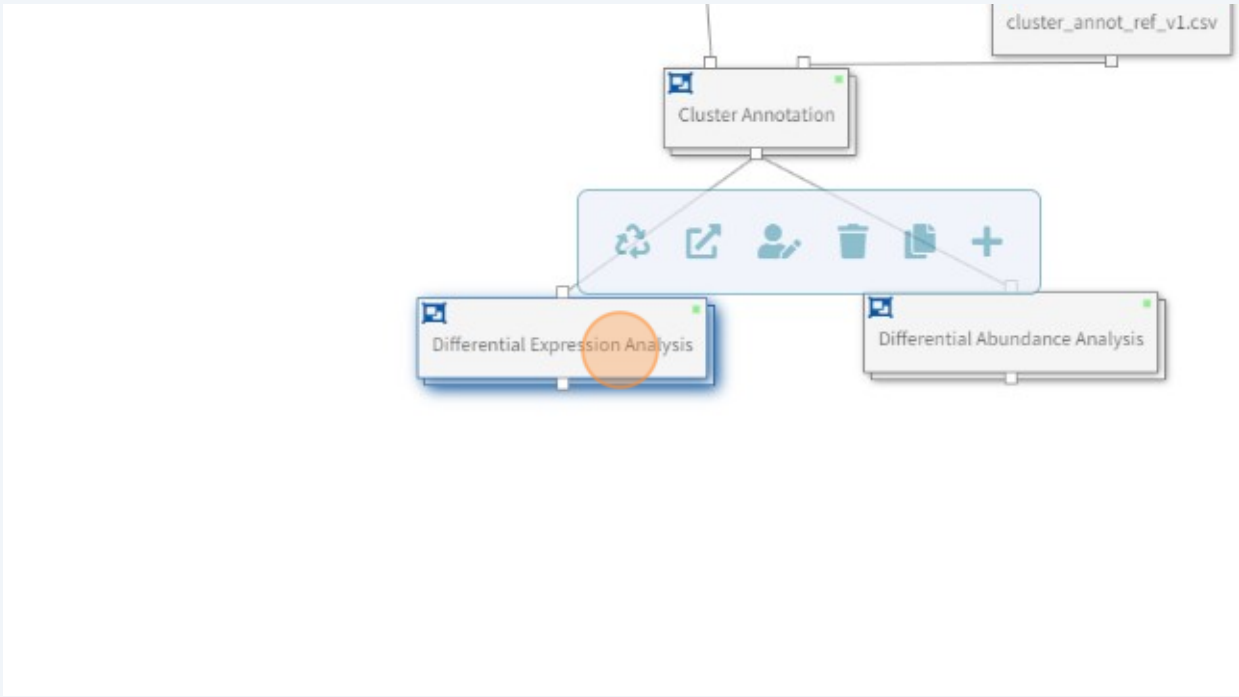
Discuss!

What do you think we are looking at here?

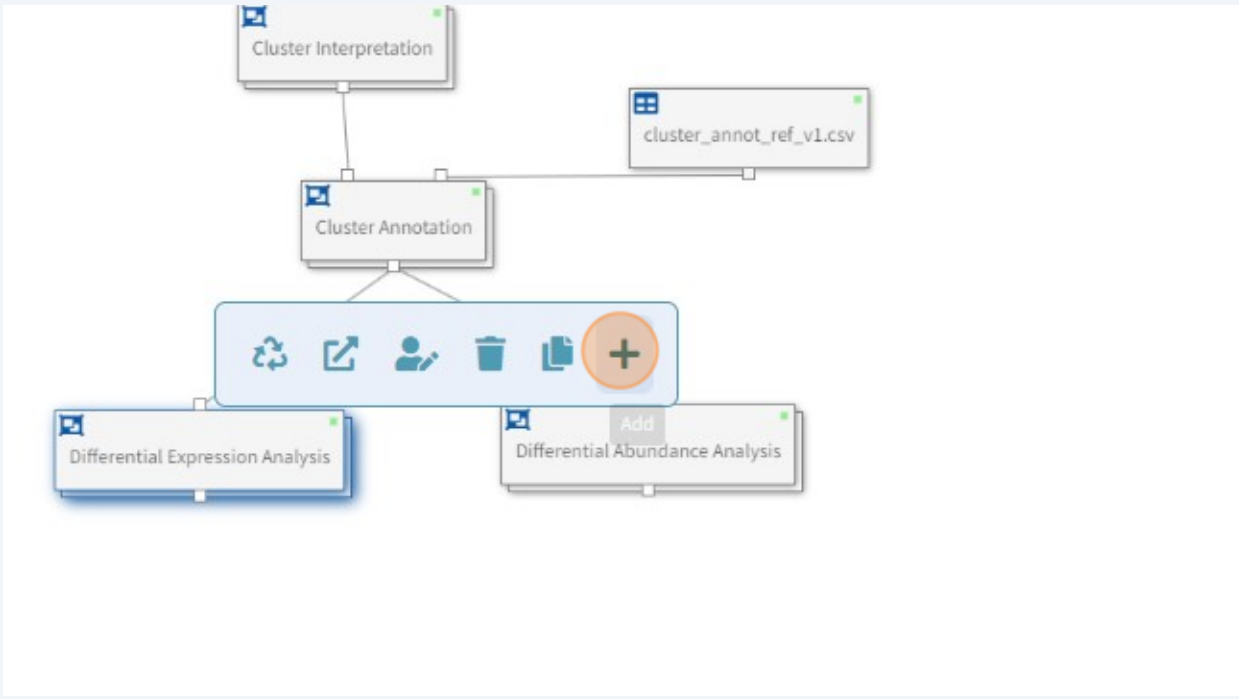
Did you notice there an median expression value for each cluster-marker-condition combination?

4 We will now use the results of diffcyt algorithm to create useful plots.

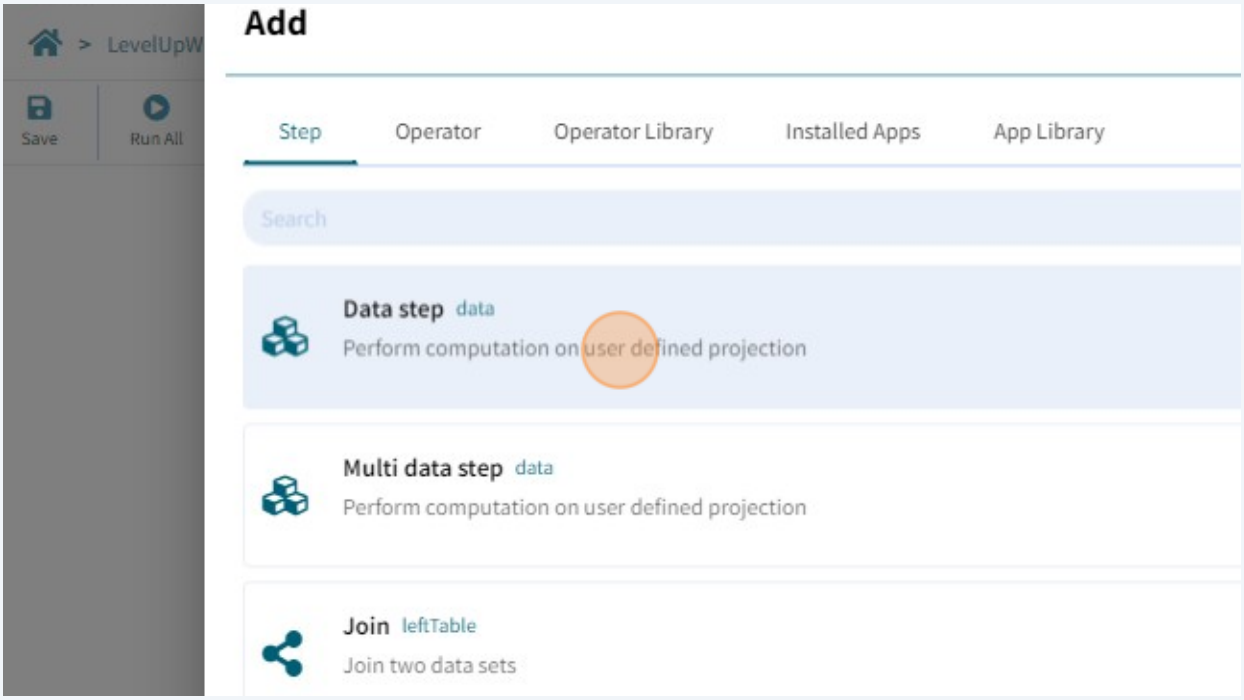
5 Click here.



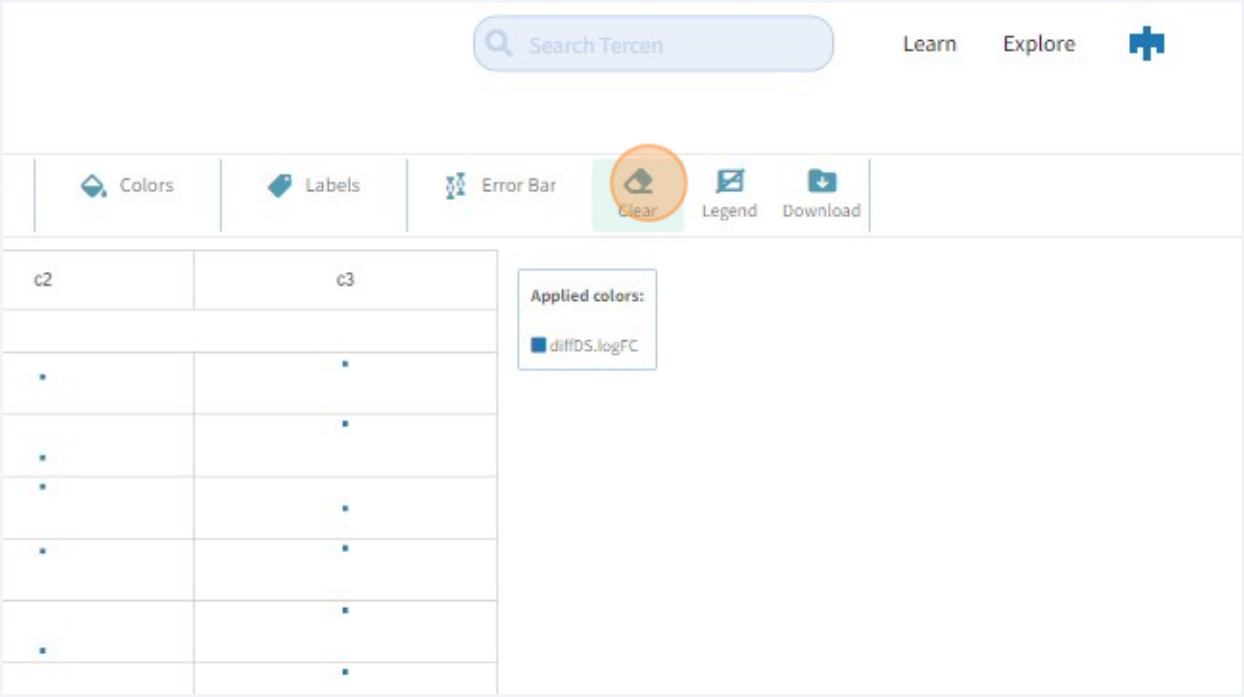
6 Click here.



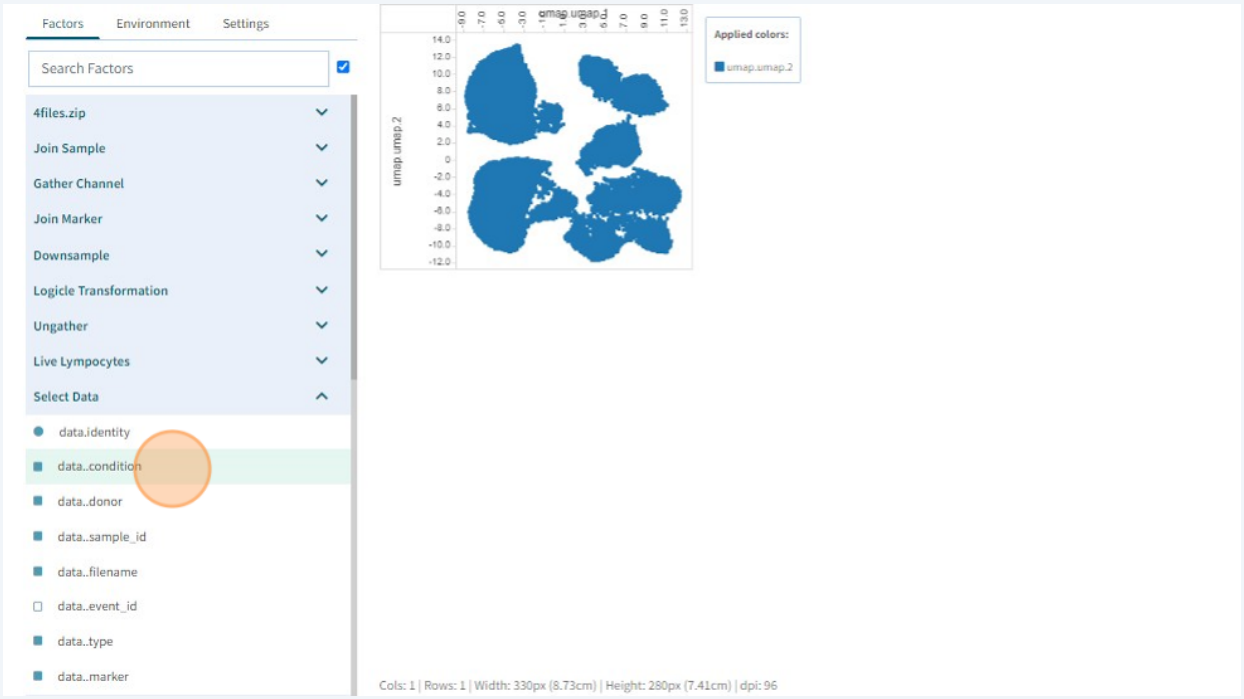
7 Add a Data Step



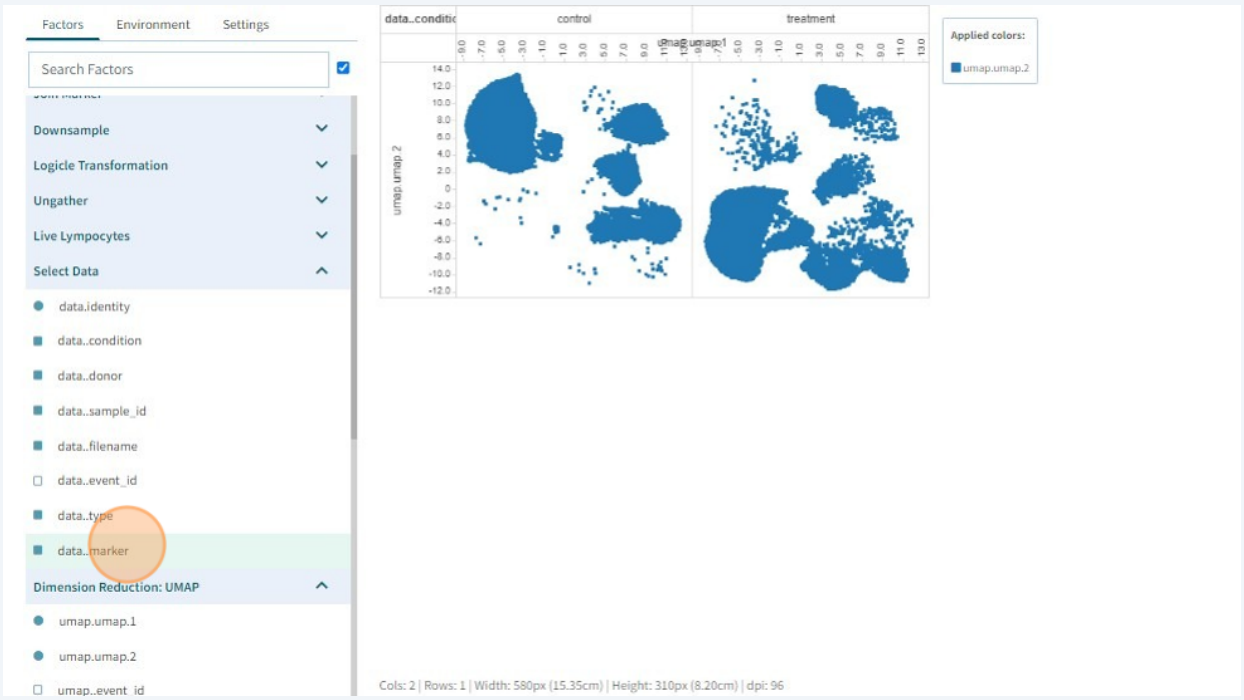
8 Clear the projection



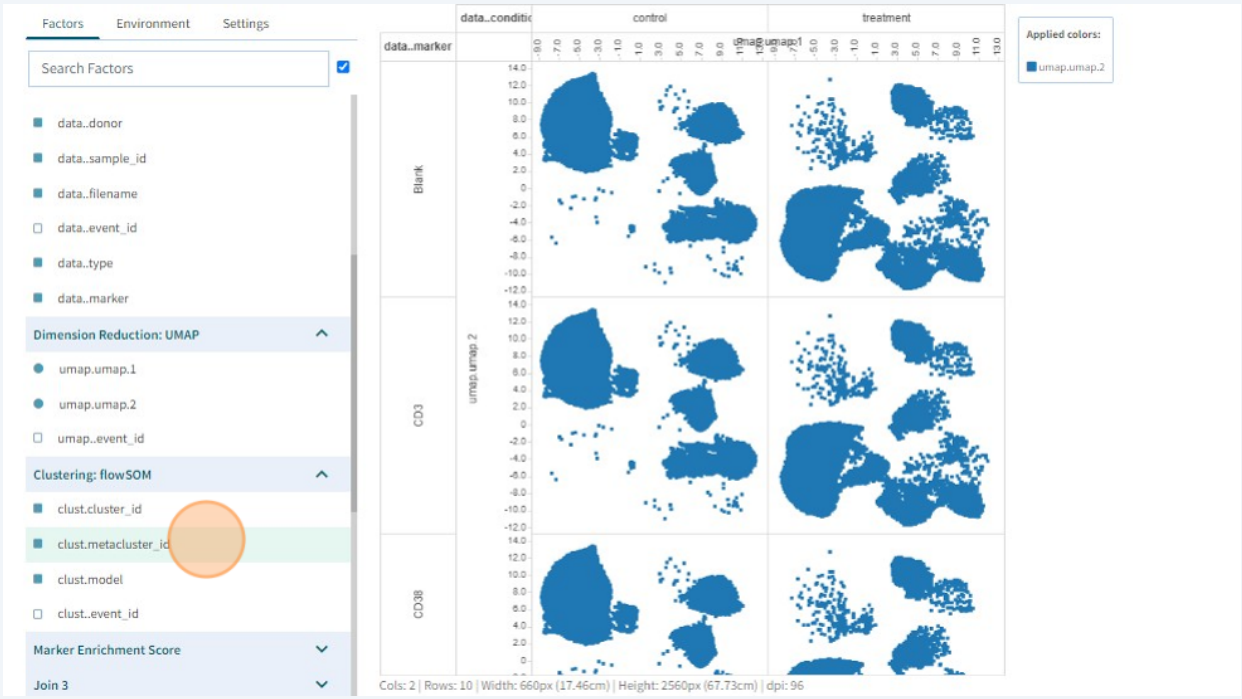
9 Create a umap1 vs. umap2 plot and separate by "data.condition" by putting it on the columns and thereby splitting the umap into two.



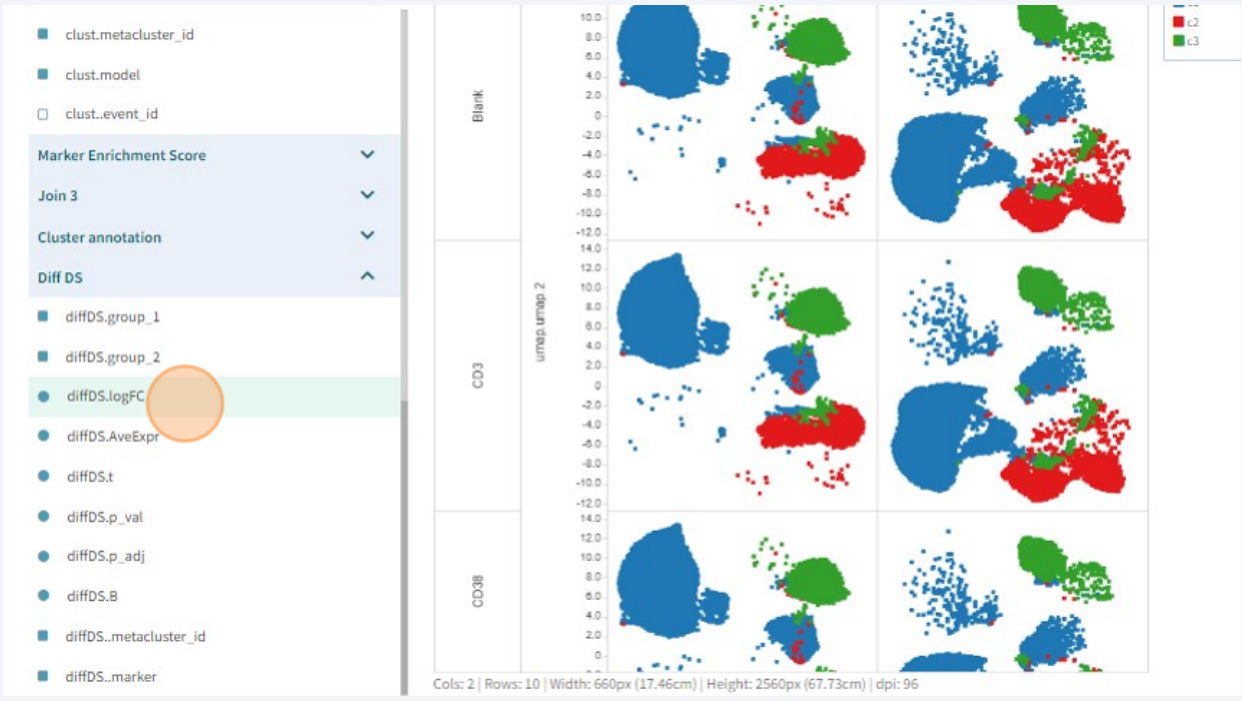
10 We will add the data.marker information to the plot by putting it on the rows



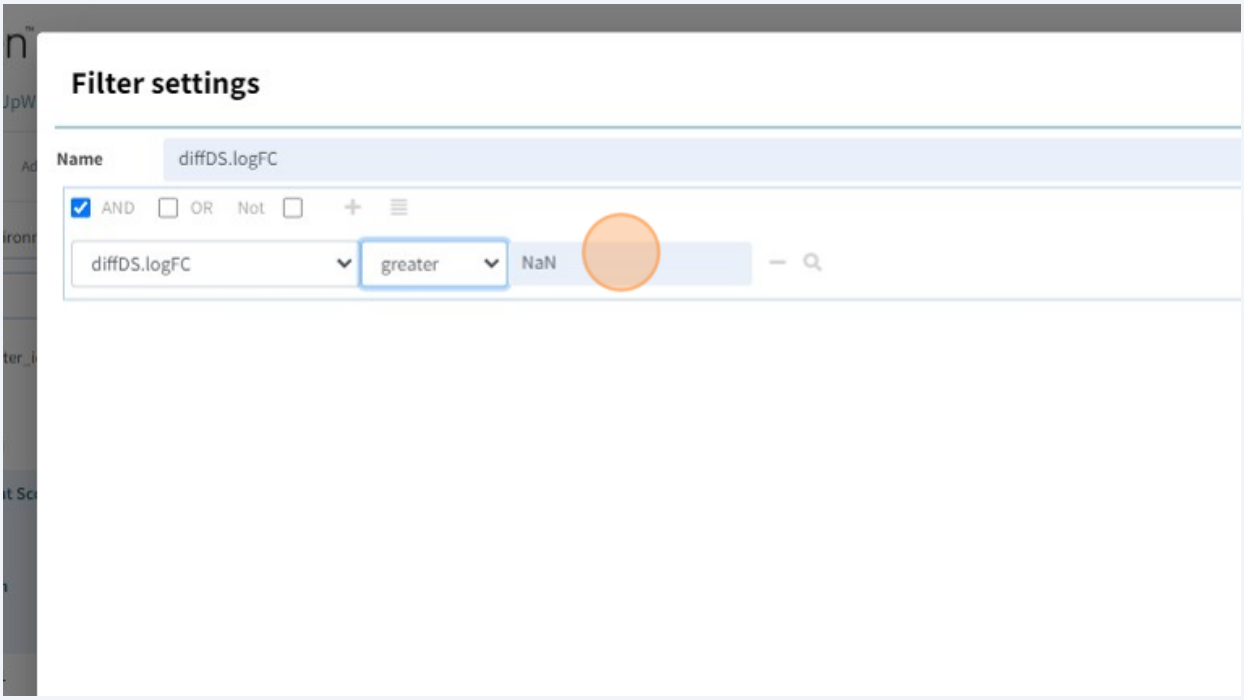
11 We will color by the factor called "clust.metacluster_id"



12 We will create a filter based on the factor called "diffDS.logFC" (i.e., the LFC)

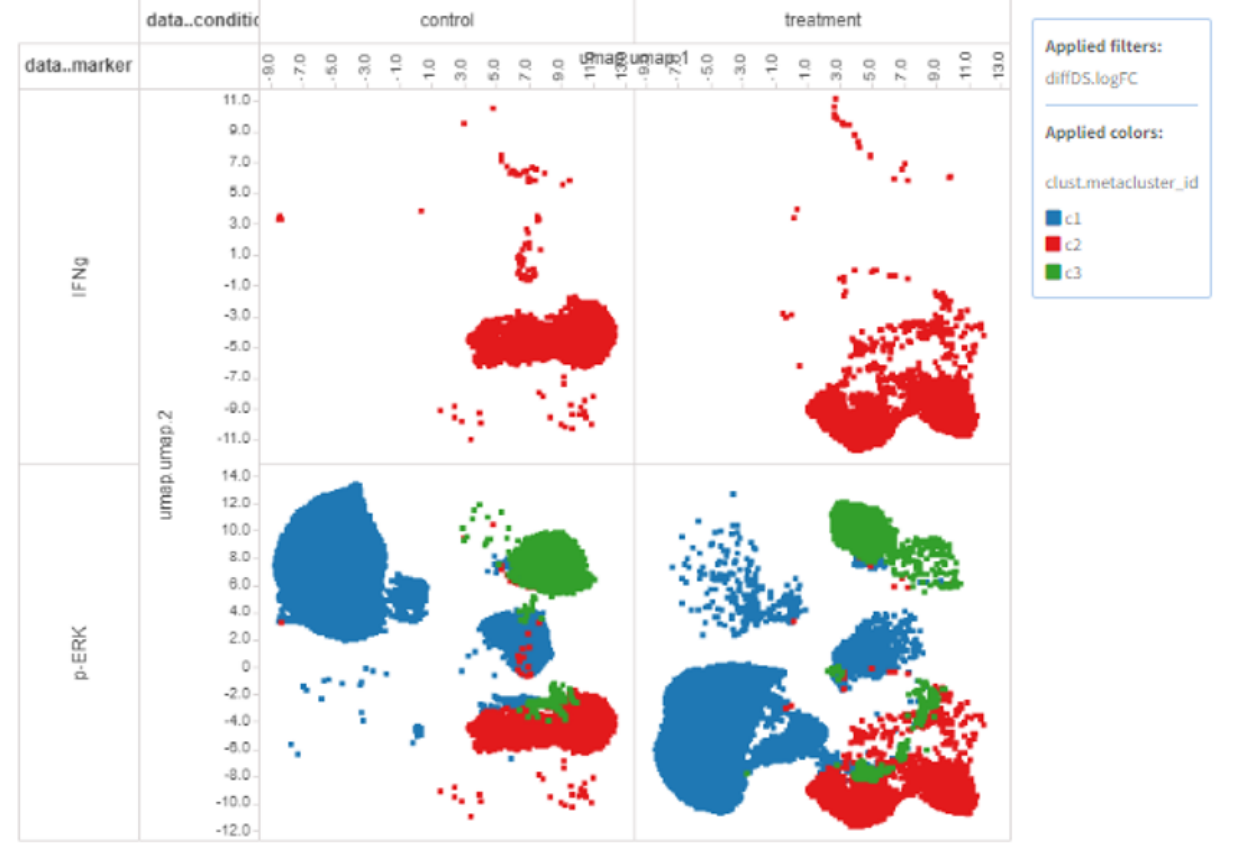


13 We want LFC greater than "0.5" (i.e. expression who have increased by more than 41% by the stimulation treatment)

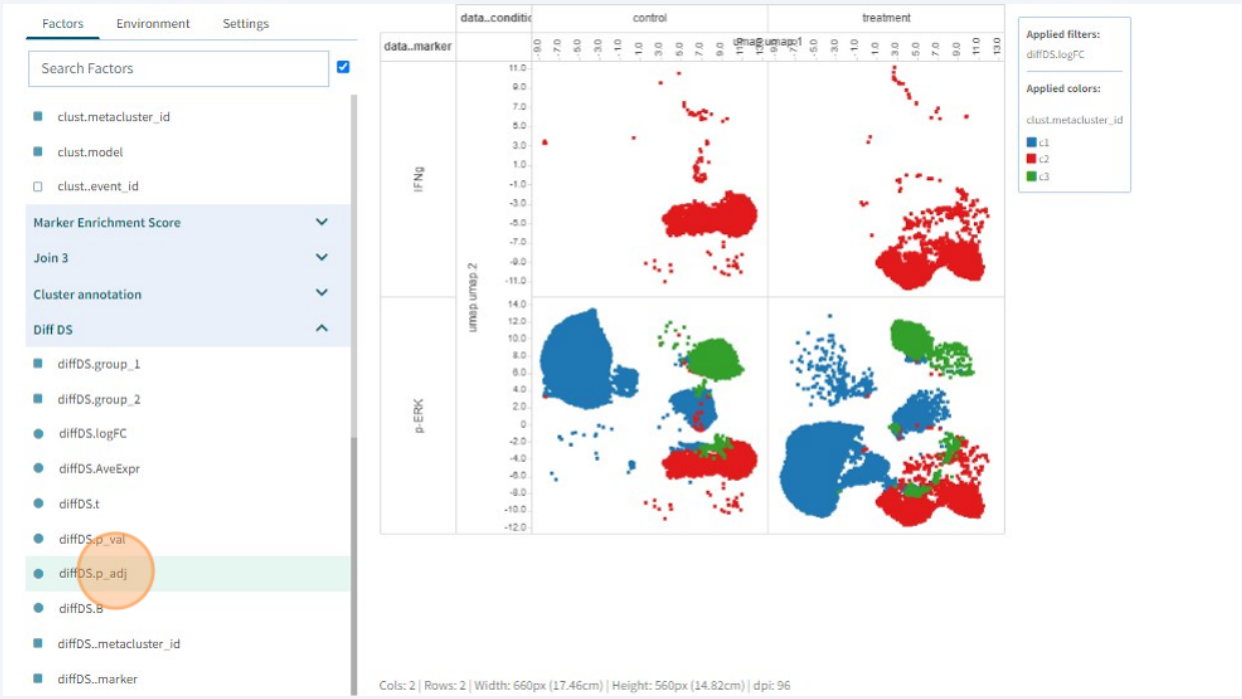


Discuss!

Why is there only two markers showing?

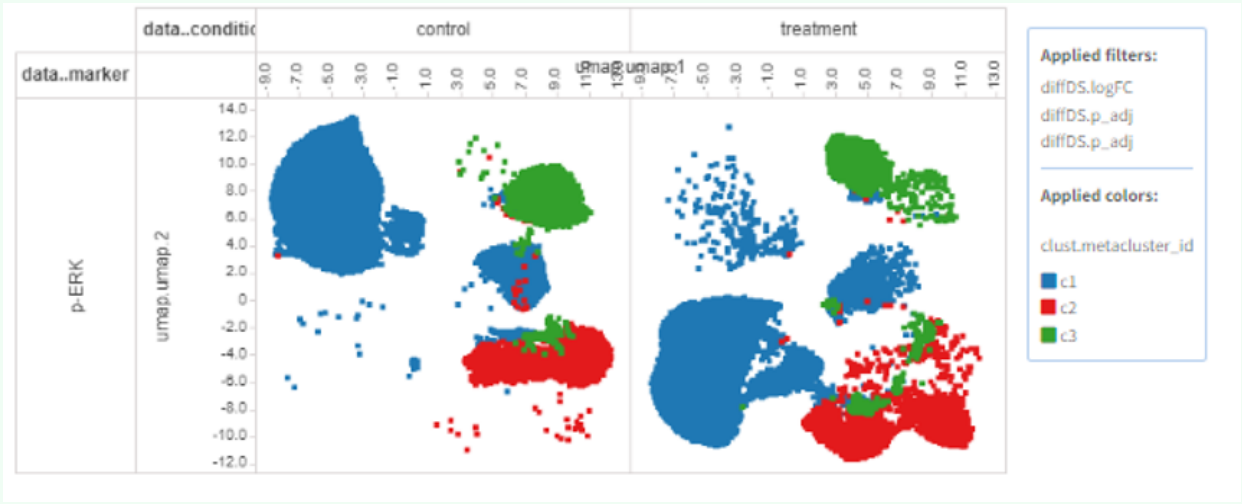


14 Add another filter using the factor "diffDS.p_adj" and set the value to be less than 0.05



Discuss!

Why is there only one marker showing?



15 Well done, you have explored diffcyt in the mode "expression"

We will now briefly look at diffcyt using its alternative mode "abuncance"

16 In the same workflow go to the double box called "Differential Abundance Analysis".

Double-click on this double box, and you will see two prepared views

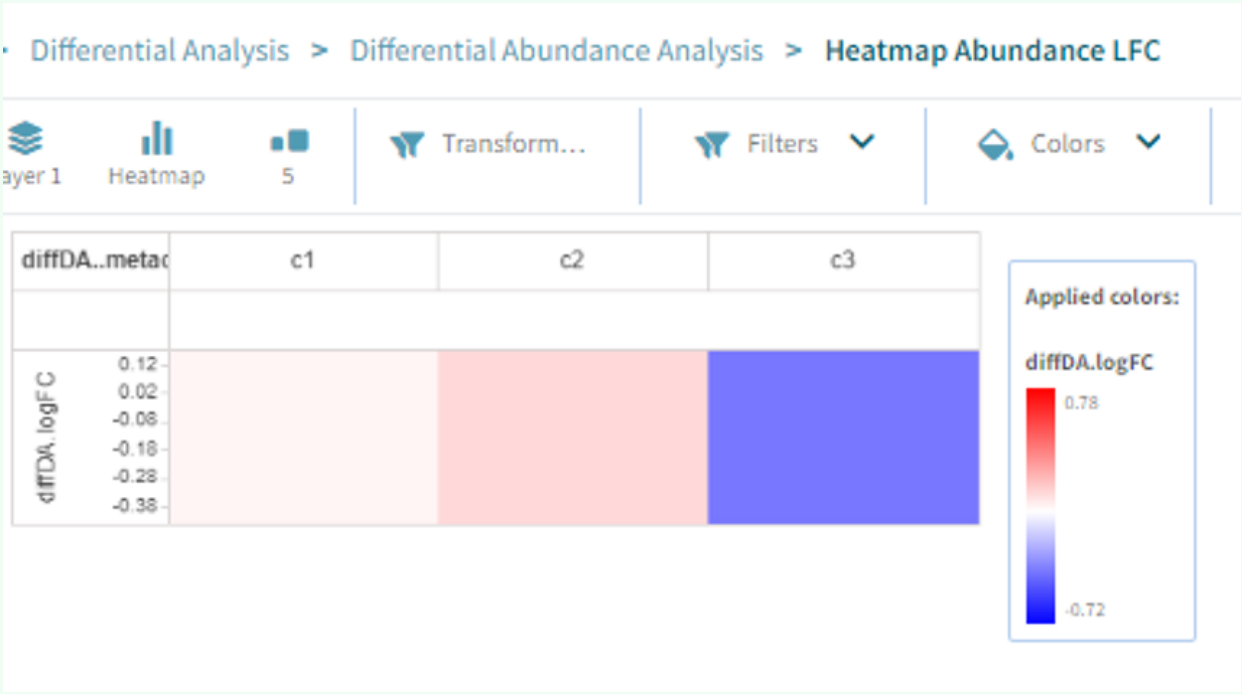
- "Heatmap Abundance LFC"
- "Abundance plot"

We will discuss both views.

Discuss!

Where are the markers missing in the Heatmap Abundance LFC?

Which clusters increased/decreased with the stimulation



Discuss!

Why are the bars the same for each marker?

What does this plot want to protect you from?

Hint: Noise