

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

i 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?

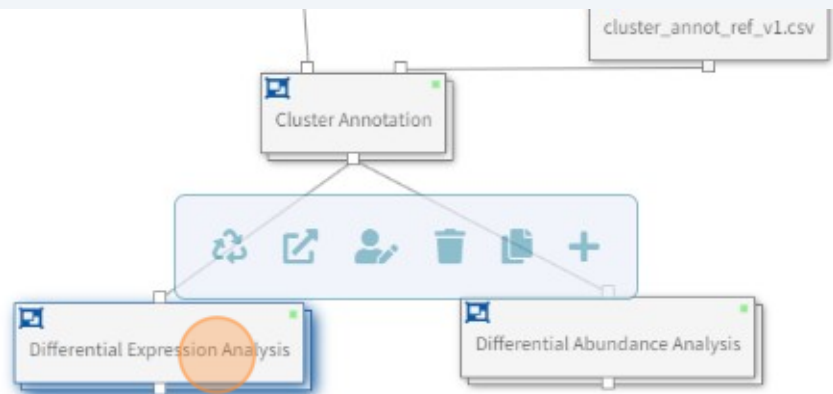
Did you notice there an median expression value for each cluster-marker-condition combination?



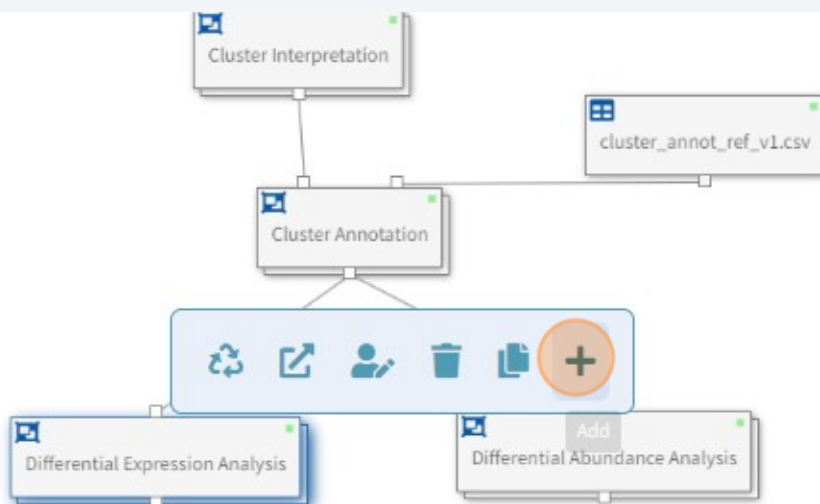
4

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

5 Click here.



6 Click here.



7 Add a Data Step

The screenshot shows the 'Add' step selection interface. On the left is a sidebar with a home icon and a breadcrumb 'LevelUpW'. Below it are 'Save' and 'Run All' buttons. The main area is titled 'Add' and has tabs for 'Step', 'Operator', 'Operator Library', 'Installed Apps', and 'App Library'. The 'Step' tab is active, showing a search bar and three options: 'Data step data' (Perform computation on user defined projection), 'Multi data step data' (Perform computation on user defined projection), and 'Join leftTable' (Join two data sets). The 'Data step data' option is highlighted with an orange circle.

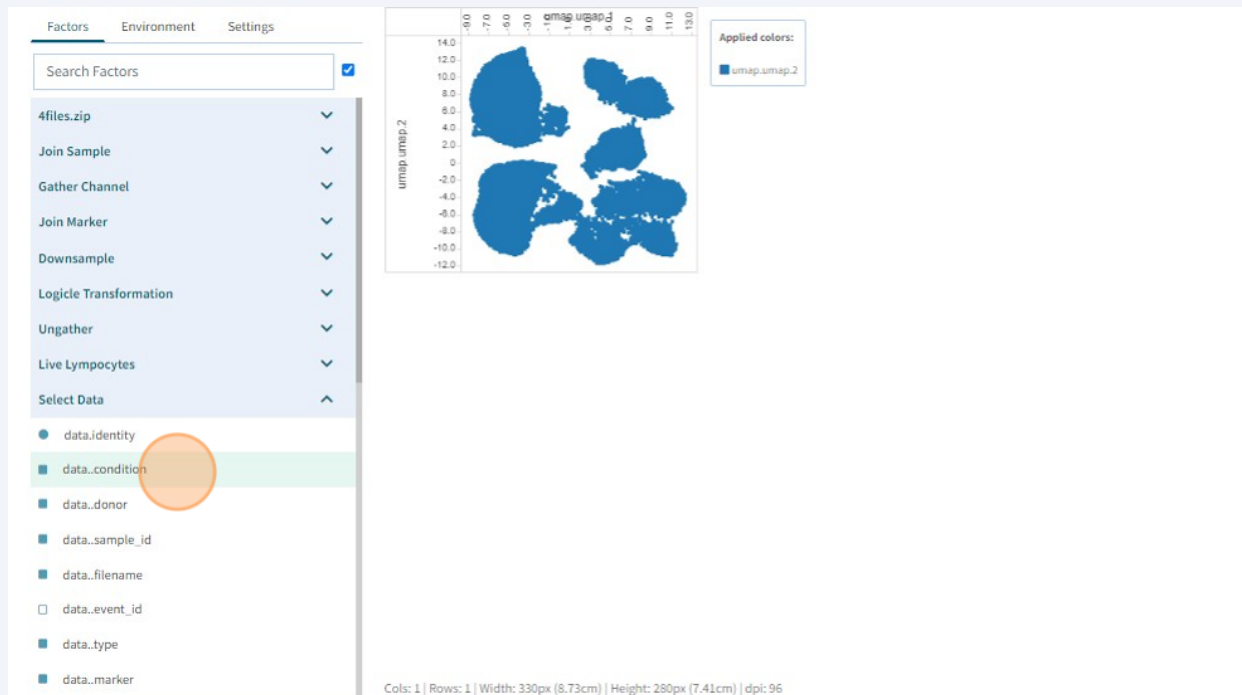
8 Clear the projection

The screenshot shows the 'Clear the projection' interface. At the top is a search bar labeled 'Search Tercen' and buttons for 'Learn' and 'Explore'. Below is a toolbar with icons for 'Colors', 'Labels', 'Error Bar', 'Clear' (highlighted with an orange circle), 'Legend', and 'Download'. The main area displays a table with columns 'c2' and 'c3'. The table has 7 rows, with the first row containing headers and the subsequent rows containing data points represented by small blue squares. A tooltip titled 'Applied colors:' is visible, showing a blue square and the text 'diffDS.logFC'.

c2	c3
■	■
■	■
■	■
■	■
■	■
■	■

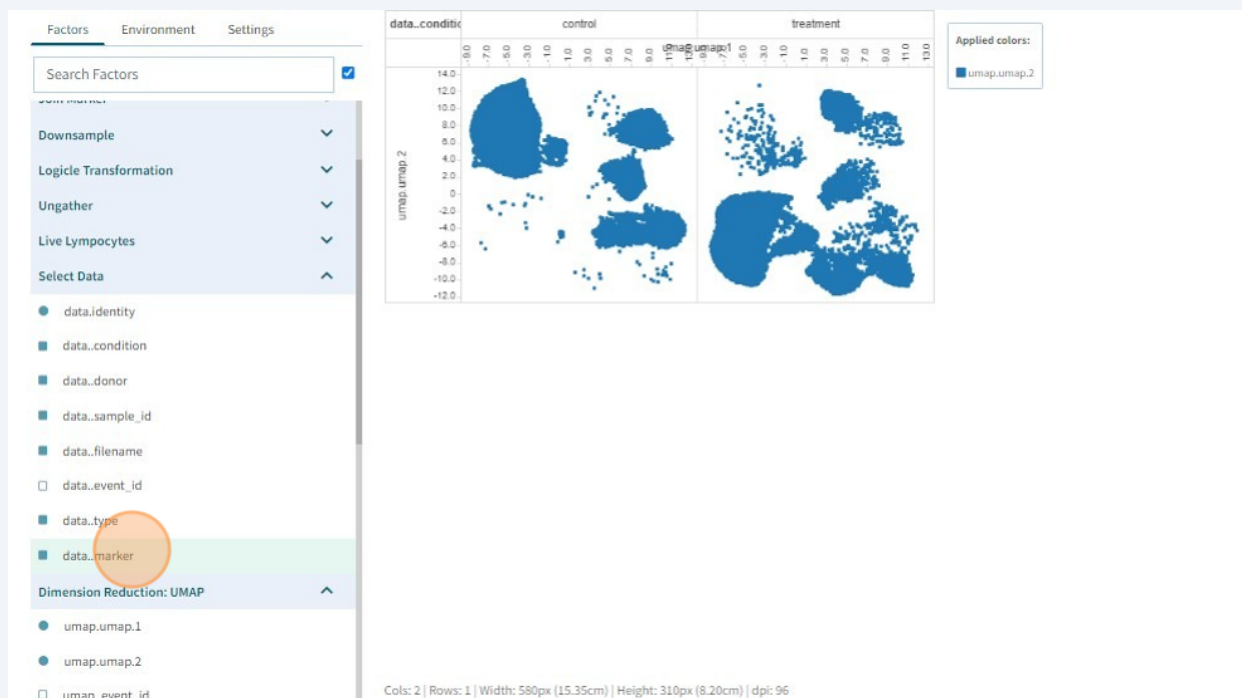
9

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

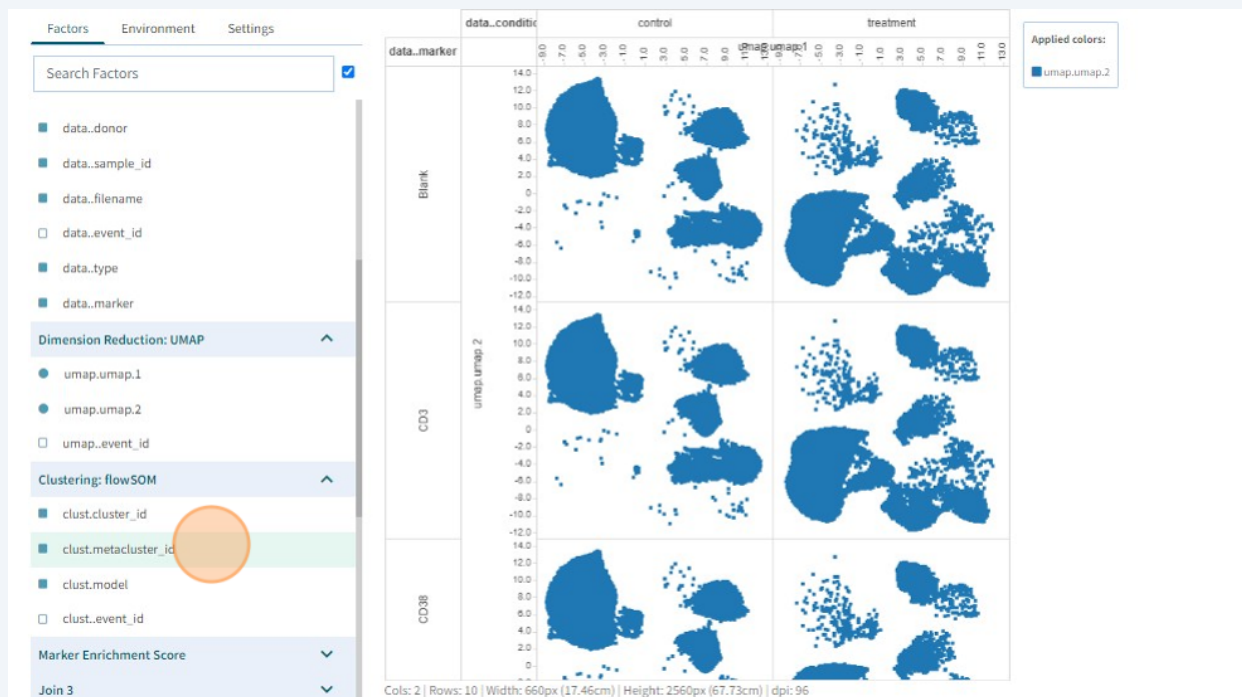


10

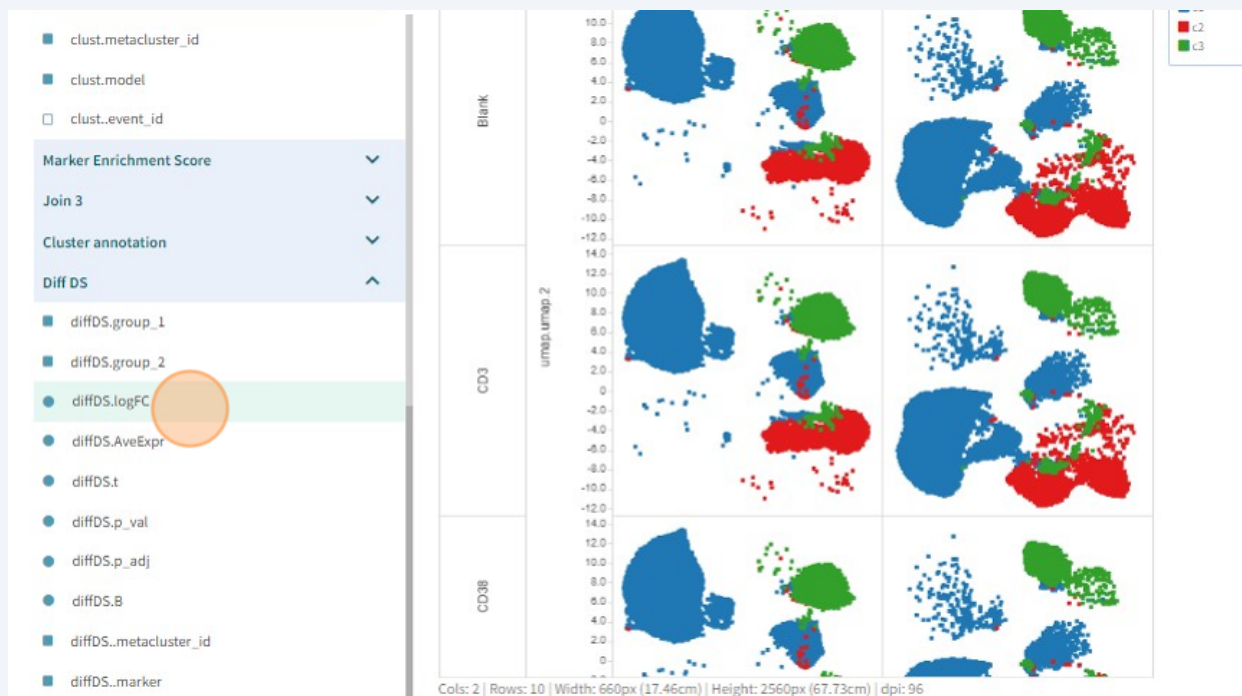
We will add the data.marker information to the plot by putting it on the rows



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

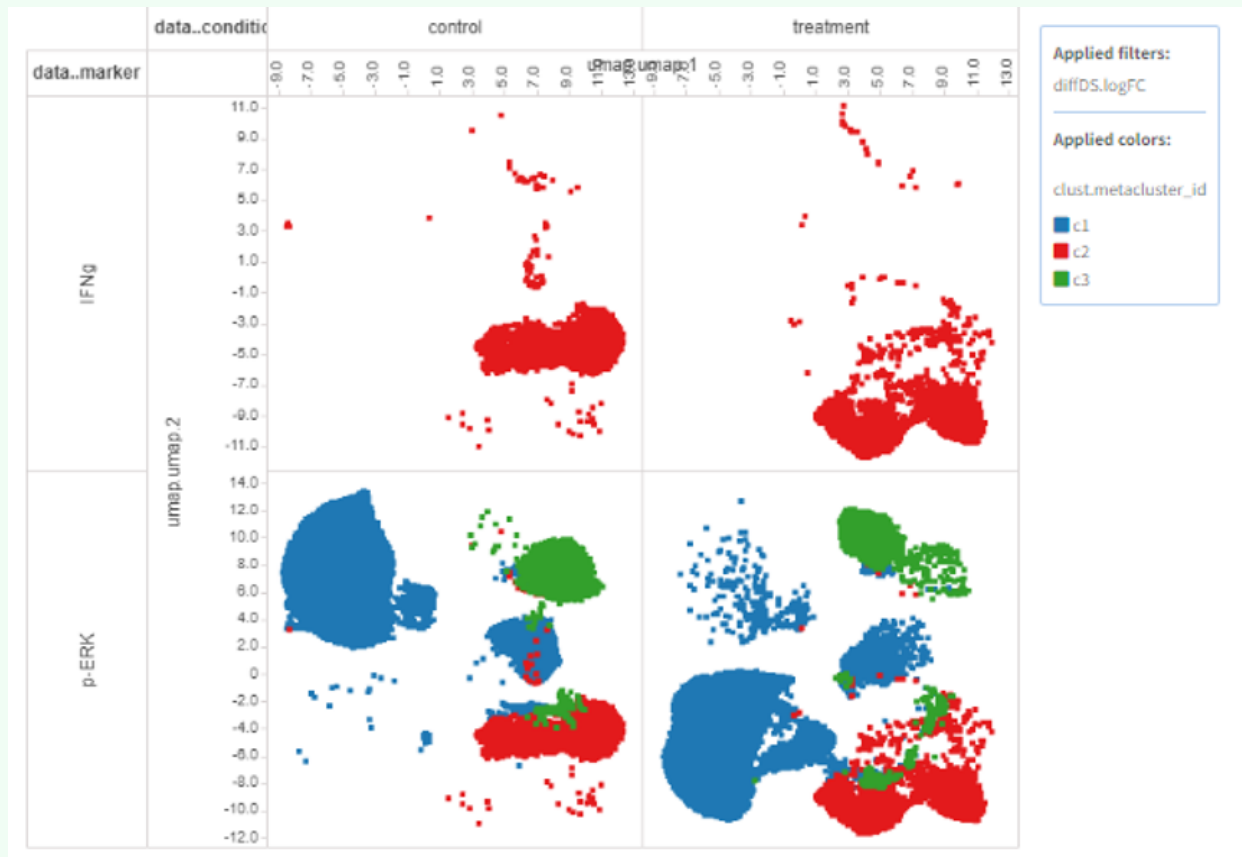
We want LFC greater than "0.5" (i.e. expression who have increased by more than 41% by the stimulation treatment)

The screenshot shows a web-based filter settings interface. At the top, the title "Filter settings" is displayed. Below it, a section labeled "Name" contains the text "diffDS.logFC". Underneath, there are logical operators: ☒ AND, ☐ OR, and ☐ Not. To the right of these are a plus sign and a menu icon. Below the operators, there is a filter rule entry. It consists of a dropdown menu showing "diffDS.logFC", followed by another dropdown menu showing "greater", and then a text input field containing "NaN". To the right of the "NaN" field is a blue circular button with a minus sign and a search icon. The entire interface is set against a light gray background.



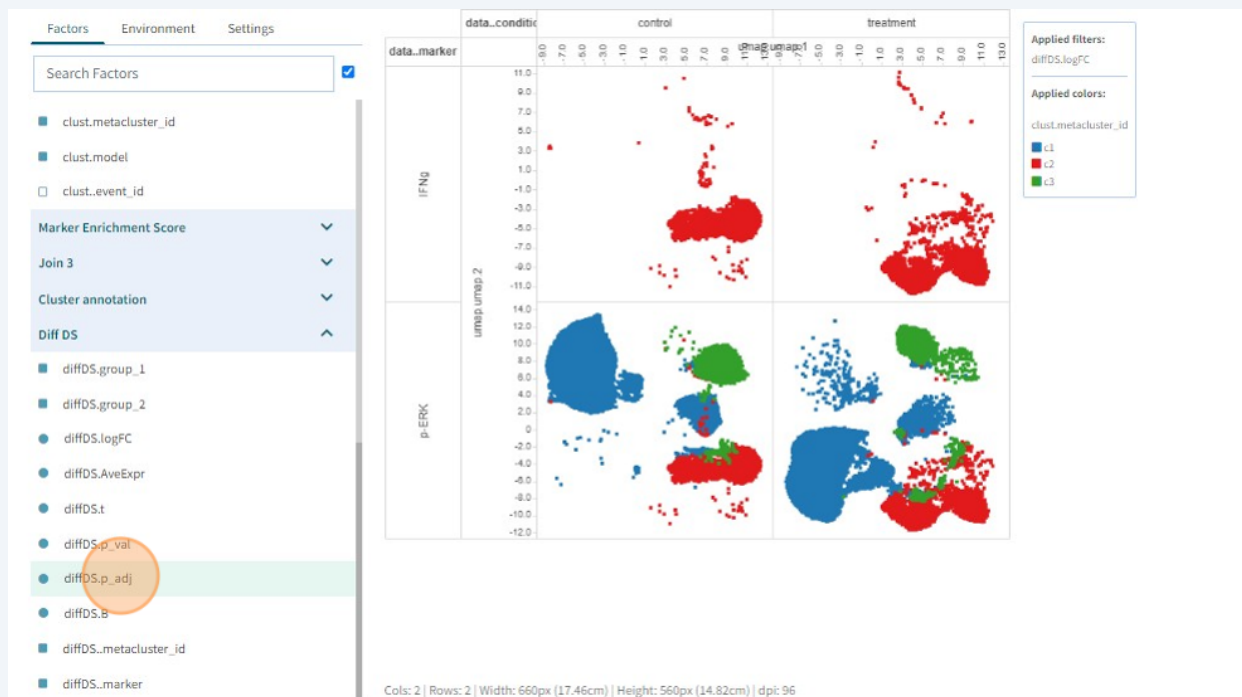
Discuss!

Why is there only two markers showing?



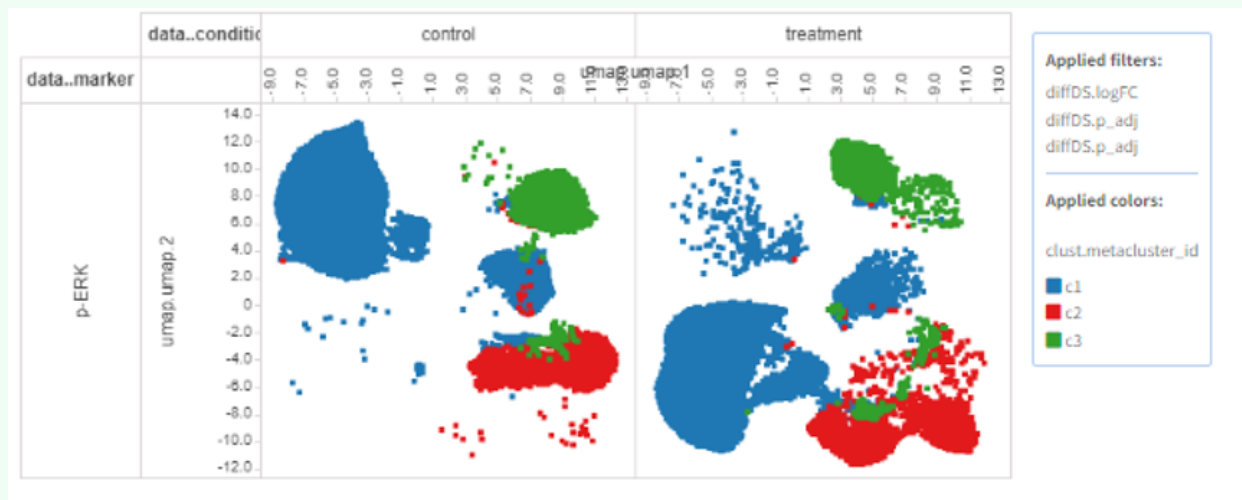
14

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 "abundance"

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"

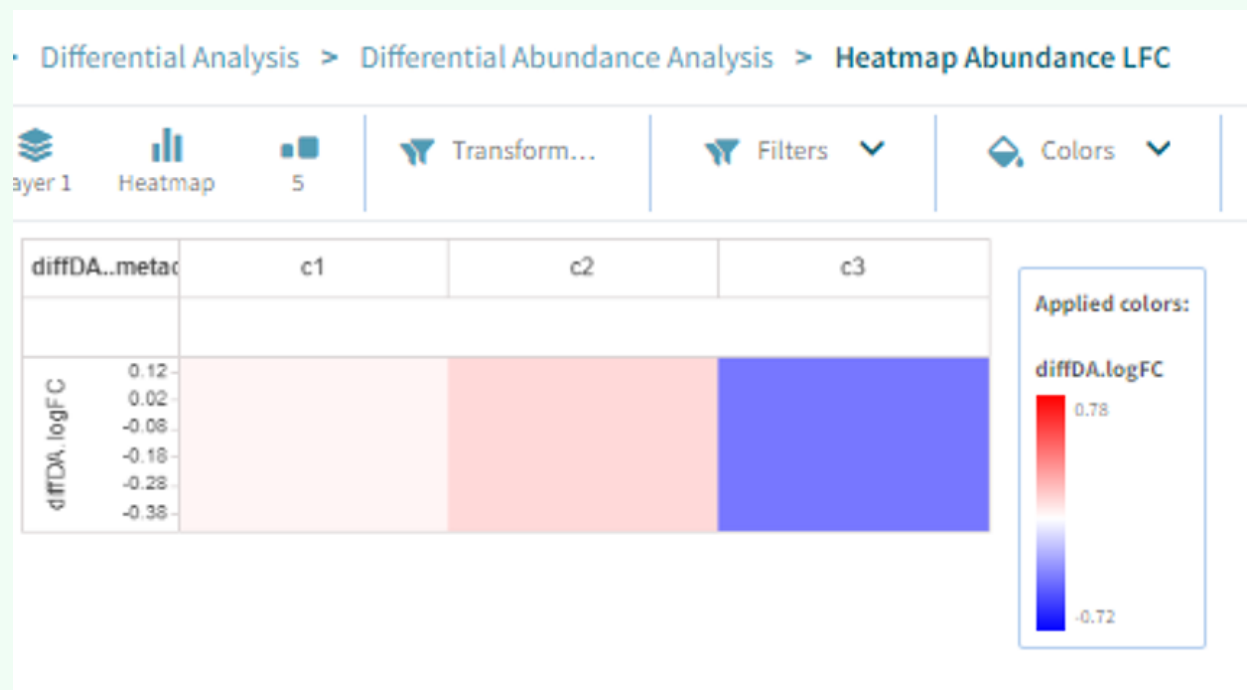
We will discuss both views.



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

