**Use of the “LabeledVolcanoPlot” DSP DA plugin**

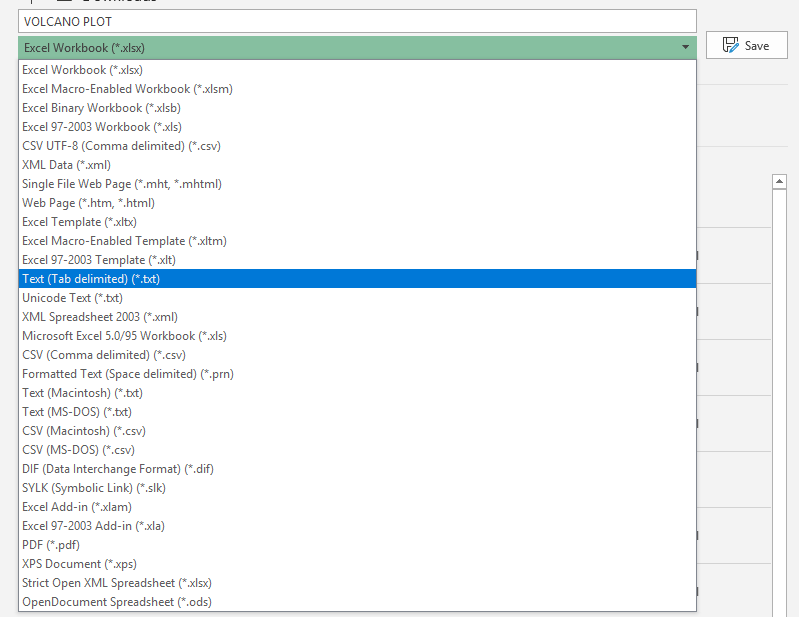
This vignette is a guide to running the LabeledVolcanoPlot DSP DA plugin and interpreting the resulting plots.

**Intended use**

This plug-in was designed for data from the GeoMx high-plex RNA assays, such as the CTA and WTA. It creates publication ready labeled volcano plots based on user inputs and statistical test results.

**Running the plugin**

The LabeledVolcanoPlot plugin requires an extra file input from DSP DA. After running a statistical test and creating a volcano plot in DSP DA, the results file should be Exported as an .xlsx file.



This file must be changed to a \t delimited file .txt before running the plugin. The script will not run if this is not changed. To do this open the Exported VOLCANO PLOT.xlsx file in excel and click Save As, change the format to Text (Tab delimited) (\*.txt).

In addition, the plugin accepts 17 “arguments” that you can set by modifying the top of the plugin’s code. Instructions for how to use these arguments are in-line in the plugin’s R code.

Briefly, the arguments are:

1. *de\_results\_filename:* The \t delimited file of the volcano plot results from DSP DA. This is the name of whatever \t file you’ve uploaded to the DSP DA.
2. *output\_format*: Desired output format for the volcano plot figure.
3. *plot\_title*: Title for figure
4. *negative\_label*: Matching negative x-axis label to the volcano plot in DSP DA; these labels are not transferred to results file so must be user added
5. *positive\_label*: Matching positive x-axis label to the volcano plot in DSP DA; these labels are not transferred to results file so must be user added
6. *show\_legend:* Should a color legend be shown
7. *n\_genes:* Number of top genes by pvalue/fdr to label on figure
8. *gene\_list*: Specified genes that will be labeled no matter what on figure. Default labeling method over n\_genes.
9. *pval\_thresh*: P-value threshold on y-axis; Default over fdr\_thresh
10. *fdr\_thresh:* False discovery rate threshold on y-axis
11. *fc\_thresh:* Fold change cutoff on x-axis.
12. *label\_fc:* Should genes below the FC threshold be labeled if they are also above the significance threshold
13. *thresh\_lines:* Should the threshold lines be shown on figure
14. *font\_size:* Font size on figure
15. *label\_size:* Size of font for the gene labels
16. *font\_family:* Font family for all text on figure
17. *plot\_width:* Width of saved figure in inches
18. *plot\_height:* Height of saved figure in inches
19. *default\_color:* Color of points not in target group or above significance threshold
20. *fc\_color:* Color of points below fc\_thresh but above significance threshold(s); change to same as default to not call out these targets
21. *target\_groups:* Specific target groups to be colored in plot. All genes in given target\_group are colored no matter where they are in the figure. If no group is given, targets are colored if they are above pval/fdr threshold.
22. *color\_options:* List of colors to use in figure. Must have at least the number of target\_groups.

**Interpretation of results**

The LabeledVolcanoPlot plugin outputs a typical volcano plot figure with fold change on the x-axis and pvalue or FDR on the y-axis for each target. A table of labeled genes in the figure is also output.

*Example figures with different input arguments.*

**n\_genes = 25**

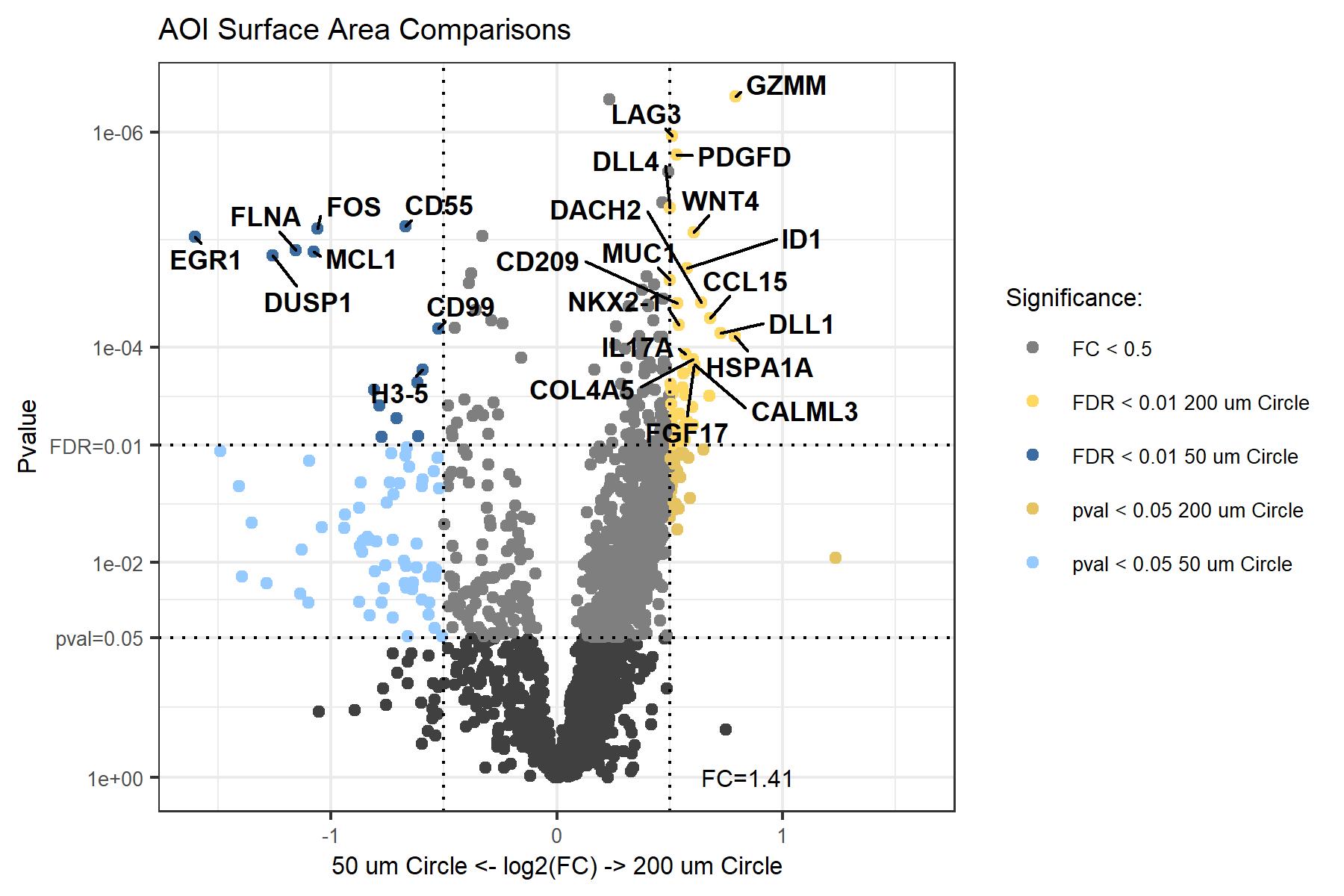
**fdr\_thresh = 0.01**

**pval\_thresh = 0.05**

**fc\_thresh = 0.55**

**label\_fc = FALSE**

**target\_groups = NULL**



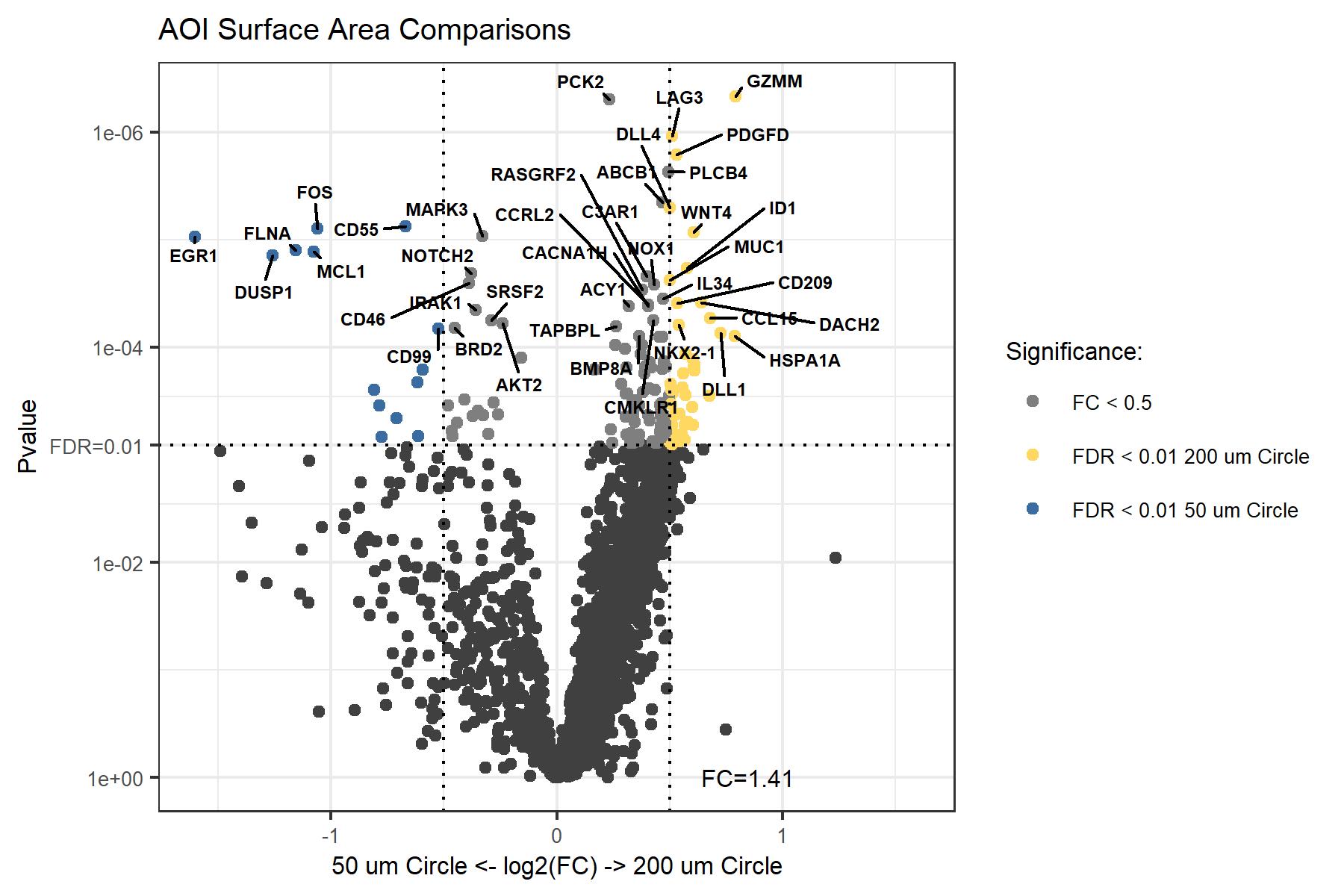
**n\_genes = 40**

**fdr\_thresh = 0.01**

**fc\_thresh = 0.5**

**label\_fc = TRUE**

**target\_groups = NULL**



**gene\_list = c("IL2RG", "GLUL", "SPIB", "C2")**

**target\_groups = c("Hemostasis", "DNA Repair")**

**default\_color = “grey80”**

**pval\_thresh = 0.05**

**fc\_thresh = 0.5**

**fdr\_thresh = 0.01**

