

Please place files signature, exclusion.signature, and average.signature.exclusion in a known location.

Open a terminal window with the the directory where the scripts are located as working directory.

Please run the scripts as follows:

1. Script to calculate Dysfunction, Exclusion and TIDE scores. Input should be expression (FPKM  $\log_2(x+1)$  - average) data in matrix format as in the attached file format\_examples>colon.expression.

`./dysfunction_exclusion_patient_original_signatures.R` [path to file signature] [path to file exclusion.signature] [path to file average.signature.exclusion] [path to gene expression file] [path to output file with name of output file]

2. Scripts to generate plots

`./anti-correlation_plot.R` [path to result of dysfunction\_exclusion\_patient\_original\_signatures.R] [path to output file containing the plot with name of file]

`./average_correlation_exclusion_plot.` [path to output of dysfunction\_exclusion\_patient\_original\_signatures.R] [path to output file containing the plot with f name of file]

`./TAM_correlation_exclusion_plot.R` [path to result of dysfunction\_exclusion\_patient\_original\_signatures.R] [path to output file containing the plot with name of file]

`./CAF_correlation_exclusion_plot.R` [path to result of dysfunction\_exclusion\_patient\_original\_signatures.R] [path to output file containing the plot with name of file]

`./MDSC_correlation_exclusion_plot.R` [path to result of dysfunction\_exclusion\_patient\_original\_signatures.R] [path to output file containing the plot with name of file]

2. Scripts to check gene expression and correlations with Dysfunction and Exclusion. Input file should be gene expression centered in csv format as in the attached file format\_examples>expression\_centered.csv.

`./gene_expression_plot.R` [path to gene expression file in csv format] [list of genes to check separated by commas] [path to output containing expressions of CTL genes with filename] [path to the directory where plots should be saved]

Examples:

`./dysfunction_exclusion_patient_original_signatures.R /Users/mesm/Desktop/`

signature /Users/mesm/Desktop/exclusion.signature /Users/mesm/Desktop/  
average.signature.exclusion /Users/mesm/Desktop/colon.expression /Users/mesm/  
Desktop/colon\_results.csv

/anti-correlation\_plot.R /Users/mesm/Desktop/colon\_results.csv /Users/mesm/  
Desktop/colon\_correlation\_dysfunction\_exclusion\_plot.png

./average\_correlation\_exclusion\_plot.R /Users/mesm/Desktop/colon\_results.csv /  
Users/mesm/Desktop/colon\_Average\_correlation\_CTL\_exclusion\_plot.png

./TAM\_correlation\_exclusion\_plot.R /Users/mesm/Desktop/colon\_results.csv /  
Users/mesm/Desktop/colon\_TAM\_correlation\_CTL\_exclusion\_plot.png

./CAF\_correlation\_exclusion\_plot.R /Users/mesm/Desktop/colon\_results.csv /  
Users/mesm/Desktop/colon\_CAF\_correlation\_CTL\_exclusion\_plot.png

./MDSC\_correlation\_exclusion\_plot.R /Users/mesm/Desktop/colon\_results.csv /  
Users/mesm/Desktop/colon\_MDSC\_correlation\_CTL\_exclusion\_plot.png