# Pathway analysis - Fig5

Contents

Objective

The idea of this document is to draft what we need to do to integrate the GSEA pathway analysis with the diff. expression analysis we did with DESeq2. We propose the following pipeline:

1. We fit a model with a formula of the type `Count_ij ~ Treat_i + Cell_j + Interaction_ij`

2. For every gene, test an hypothesis if there is a treatment effect in the cell specific expression.

3. For every gene, summarize the TPM level of each treatment, cell and interaction.

4. For every cell, take the genes that are differentially expressed and are among the top K most expressed genes for both treatments (the assumption here is to avoid cases where the `log2FC` value is extreme due to a very low quantity of reads in either treatment).

5. Using the t.stat values as a signal-2-noise metric, do a pathway analysis with GSEA.

TPM summary

We ranked three summary metrics by its decreasing order: `mean`, `median` and the third quartile `q75`. In the figure below, we can see that regardless of the metric we pick, we are going to observe several genes with a low amount of reads without the treatment but well expressed with treatment. There is a clear trend in the TPM levels for both treatment, and the gene surrounding the blue line are the more likely to be differentially expressed (not exactly a neighborhood around the line but the data cloud surrounding it).
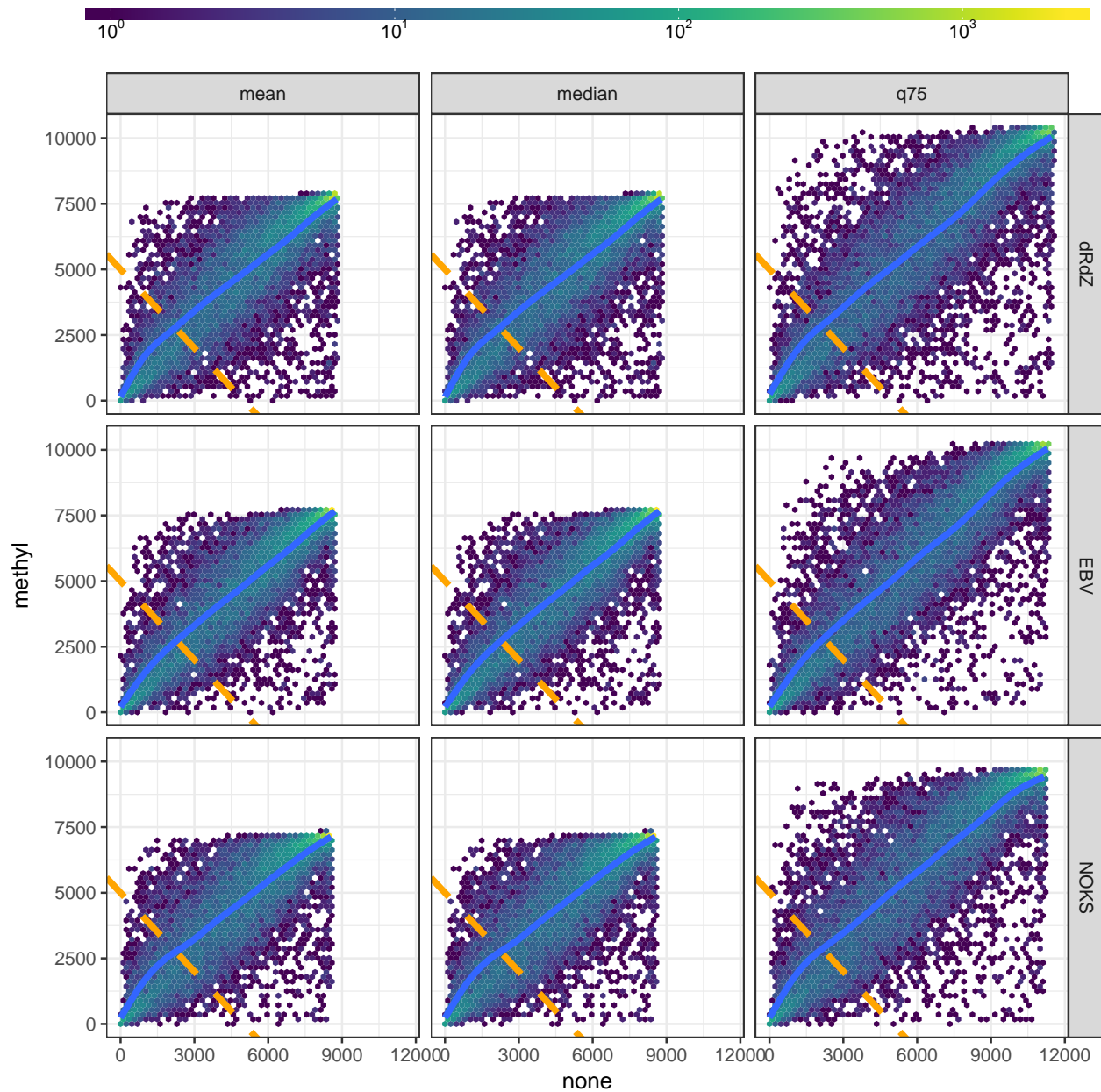
Two ideas:

a. Take the genes that are below the orange line. Those are the genes with the highest TPM, and we can expect for those differentially expressed genes to be part of the cell footprint.

b. Take the genes in the data cloud around the blue line. The genes that are differentially expressed are not going to ocurr due to the read imbalance in the treatment (i.e. the bias we had been dealing with), but the genes that are on the top of the line are more likely to not be important, as those are genes with the lowest TPM.

I am going to focus on **a** as it seems more conservative and try the mean ranking, as the median appears to shrink everything and the quantile seems to behave similarly to the mean.

Differentially expresed genes under this filter
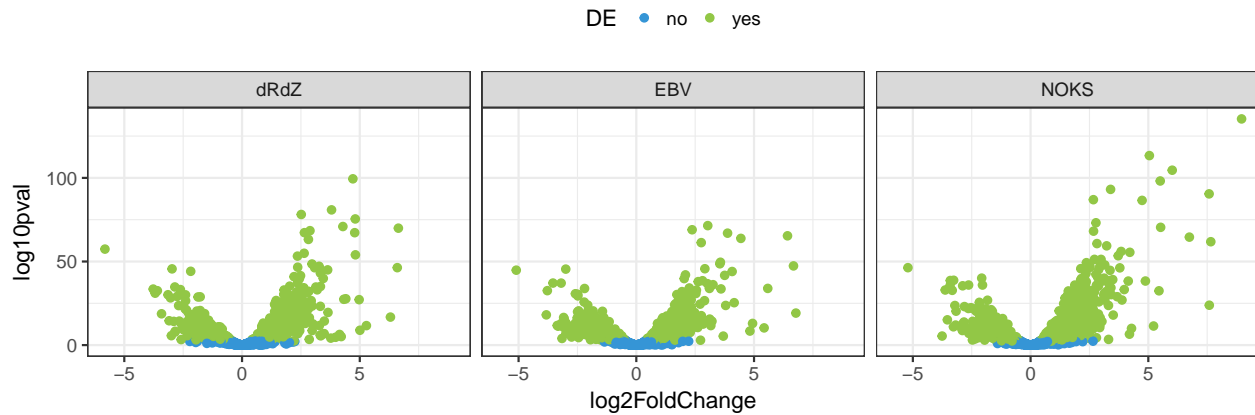
The plot above is a bit missleading because, even though we are filtering tons of genes it is not noticeable in the plot. So, we have in total:

```
## # A tibble: 3 x 3
##   cell      no   yes
##   <chr> <int> <int>
## 1 dRdZ   3095  1772
## 2 EBV    3384  1567
## 3 NOKS   2695  2322
```

GSEA analysis

There are two alternatives to do the GSEA analysis:
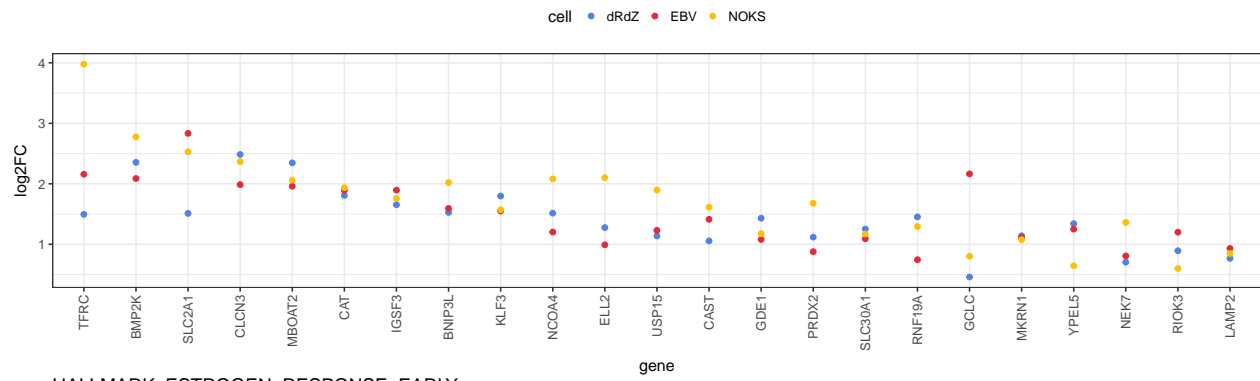
i. Use all the genes in the list
ii. Use only the differentially expressed genes

All genes
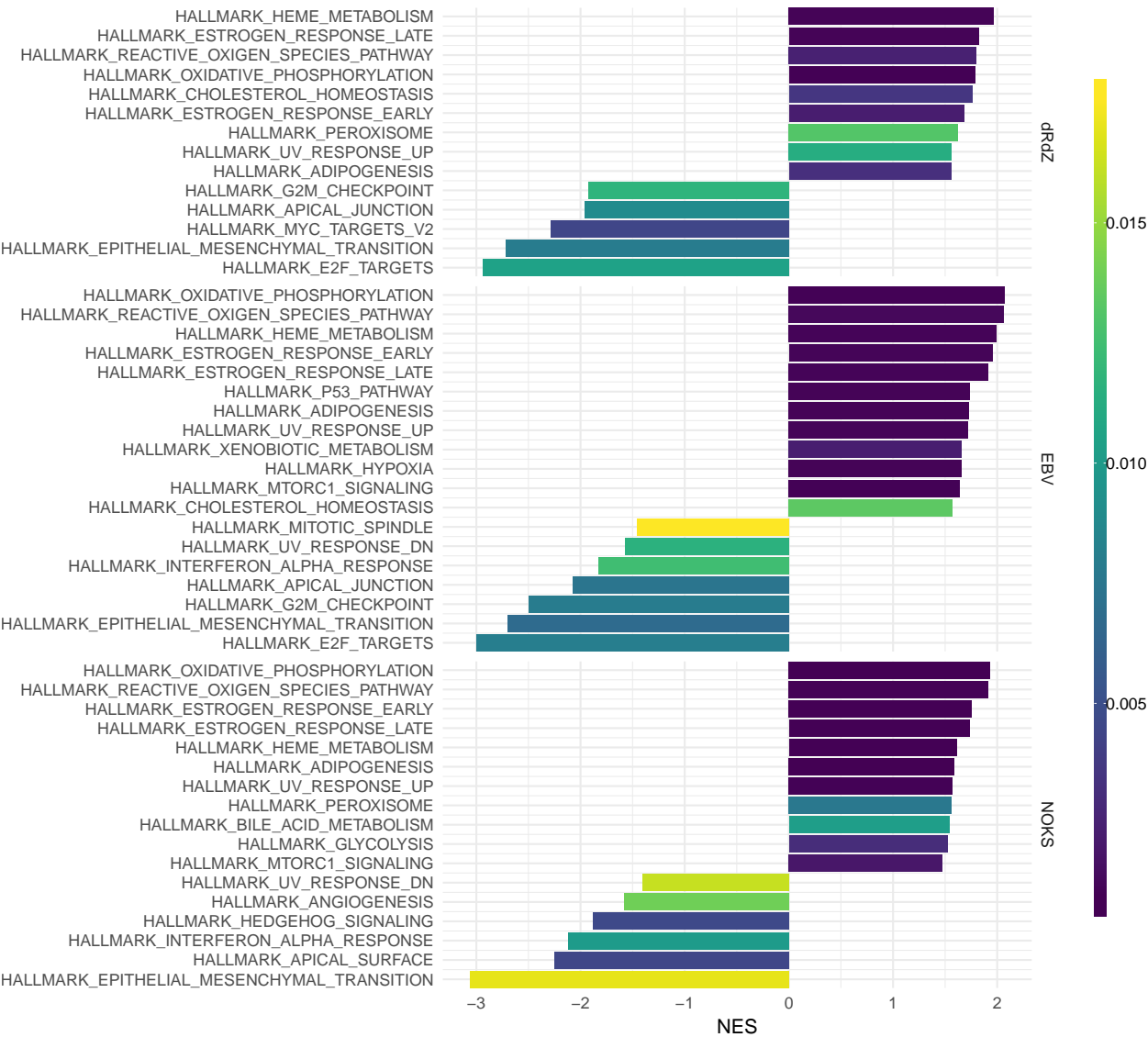
Only differentially expressed genes

Further exploration

A lot of the pathways coincide, hence I am going to focus on the ones obtained when using the DE genes explicitly.

HALLMARK_HEME_METABOLISM

HALLMARK_ESTROGEN_RESPONSE_EARLY
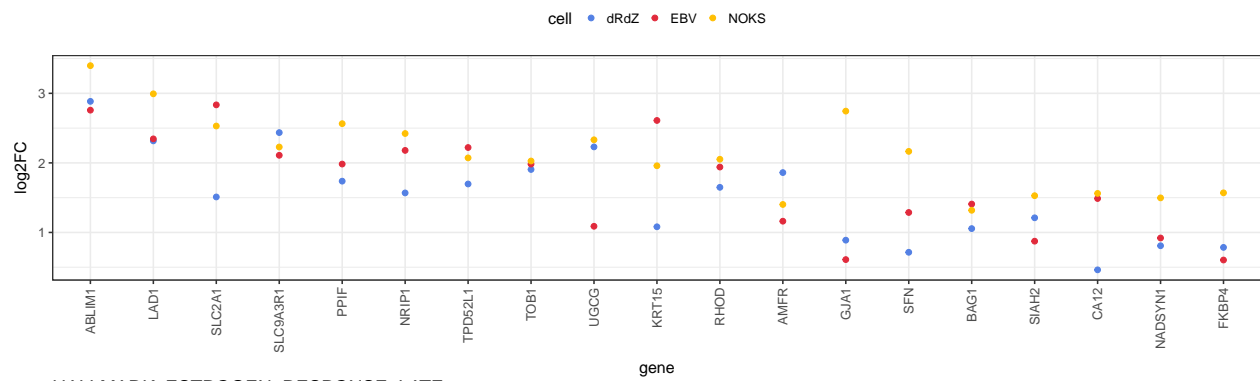
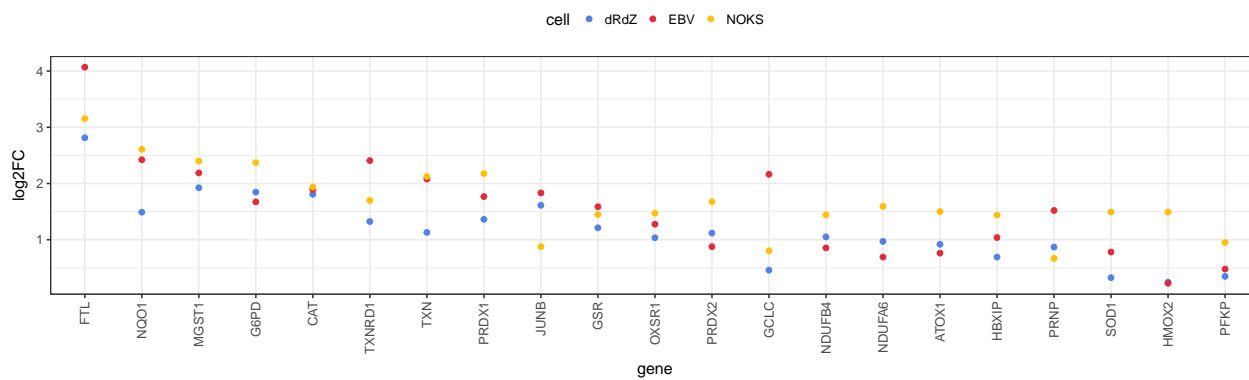# HALLMARK_ESTROGEN_RESPONSE_LATE
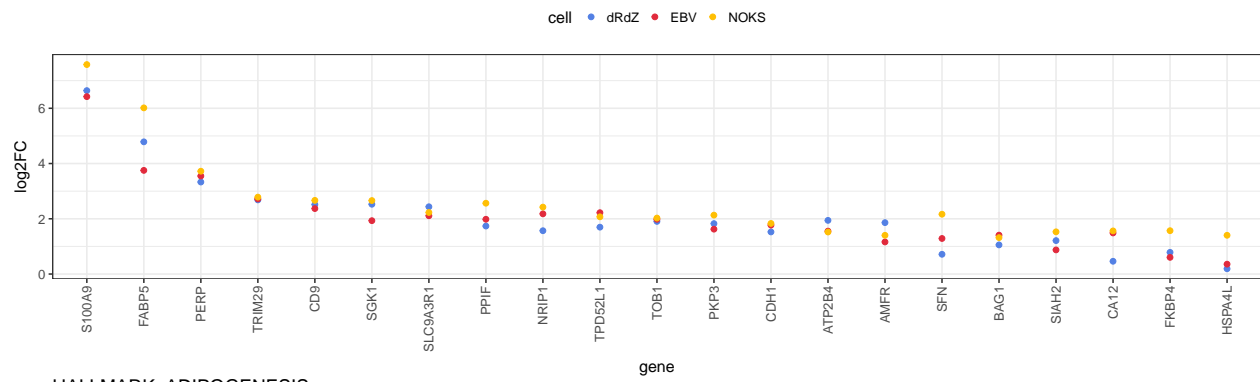
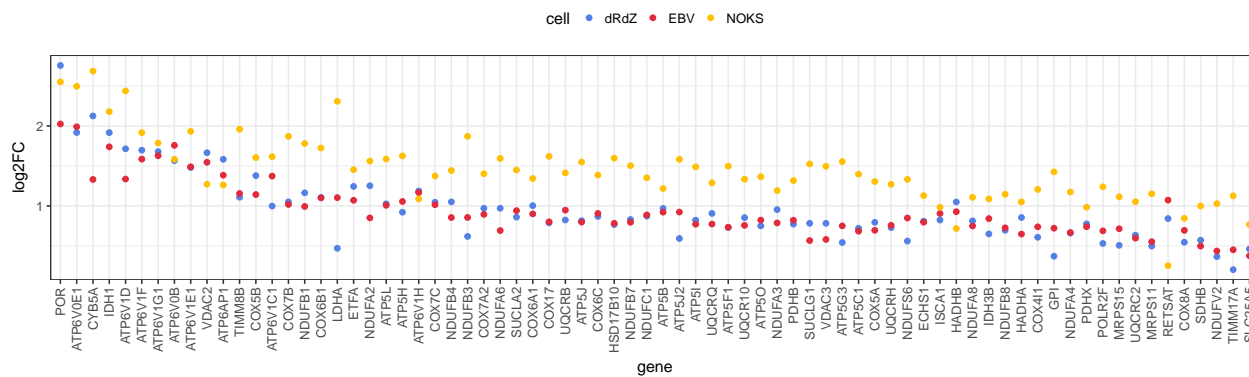# HALLMARK_ADIPOGENESIS
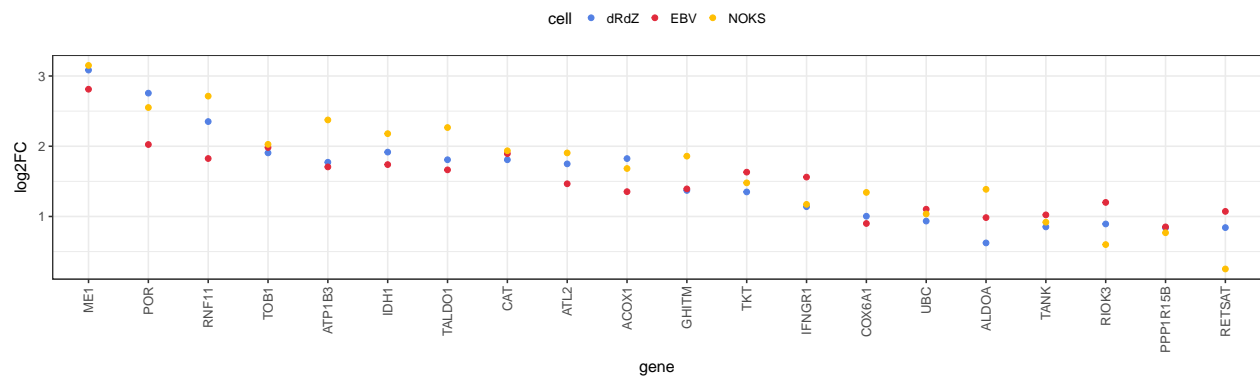
HALLMARK_XENOBIOTIC_METABOLISM
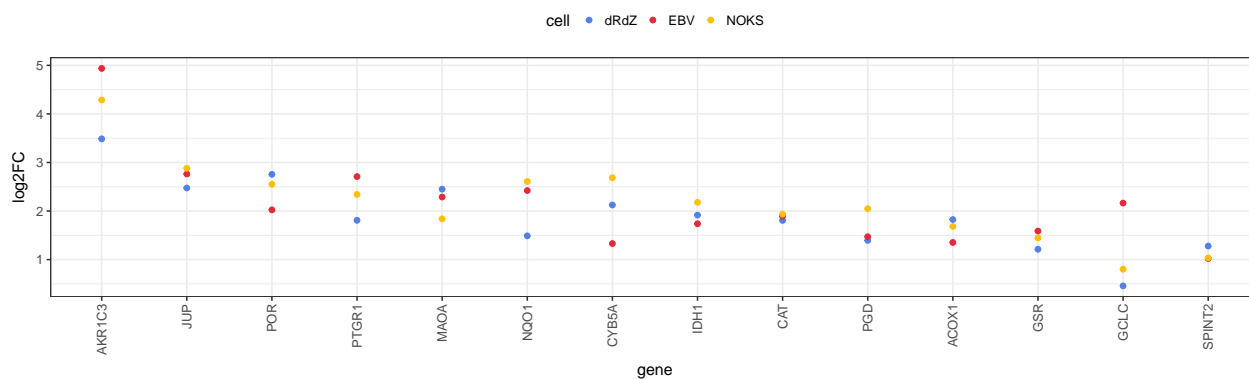
HALLMARK_UV_RESPONSE_UP
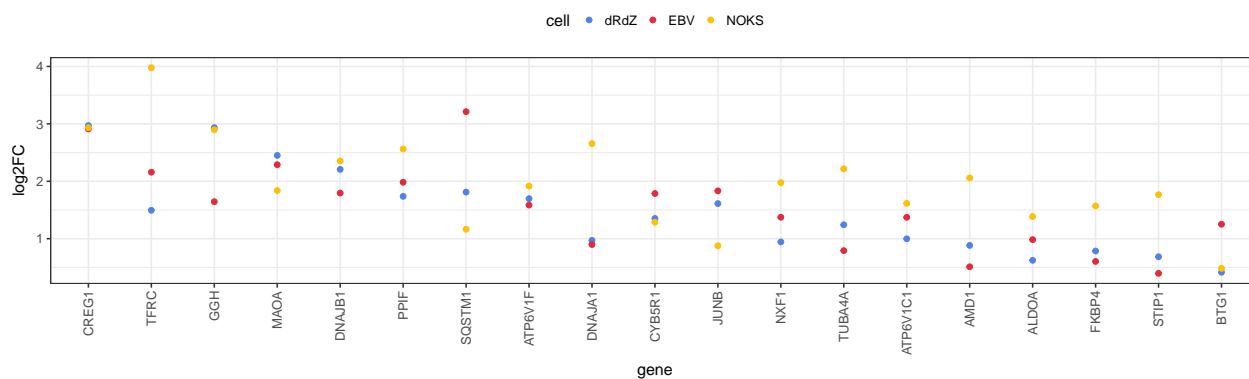
HALLMARK_REACTIVE_OXIGEN_SPECIES_PATHWAY

HALLMARK_OXIDATIVE_PHOSPHORYLATION

7

HALLMARK_G2M_CHECKPOINT
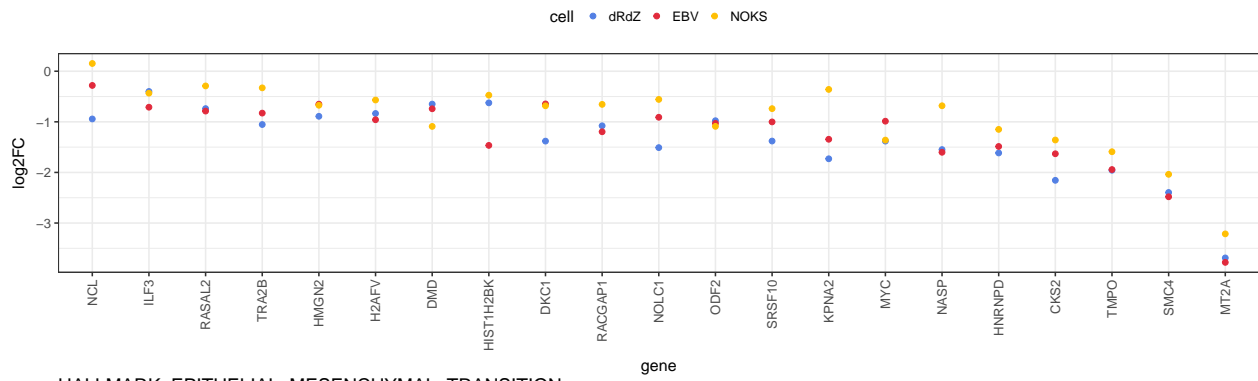
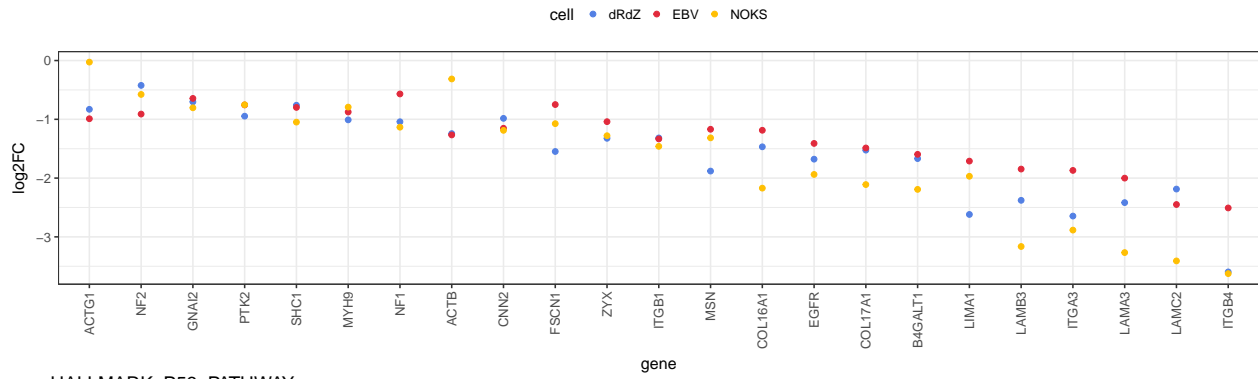## HALLMARK_EPITHELIAL_MESENCHYMAL_TRANSITION
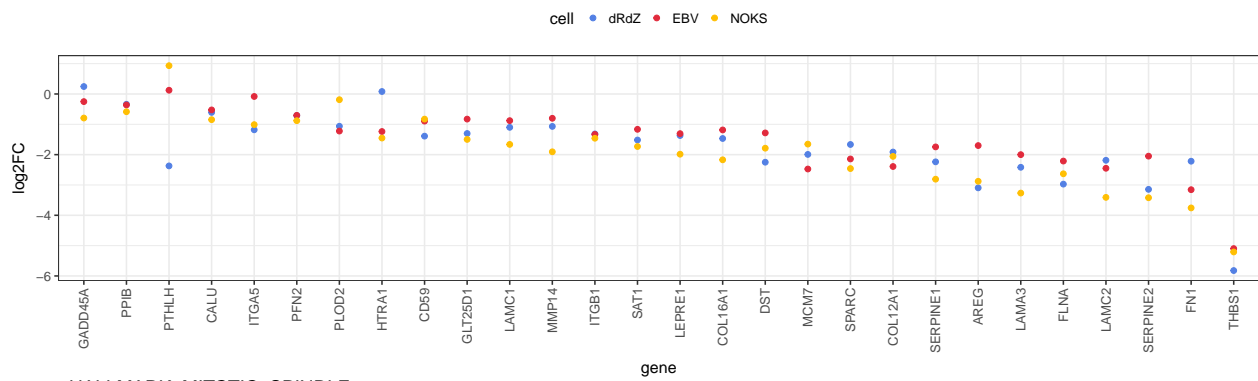
HALLMARK_MITOTIC_SPINDLE
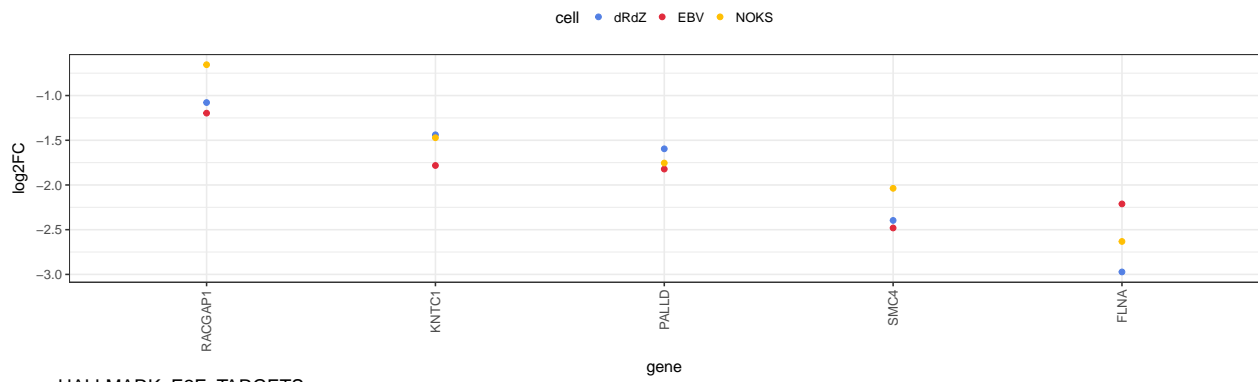
HALLMARK_E2F_TARGETS
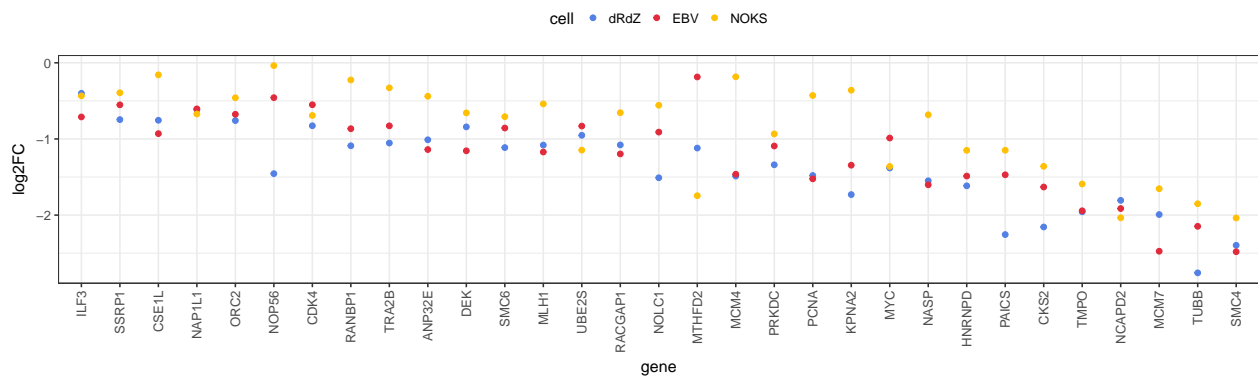
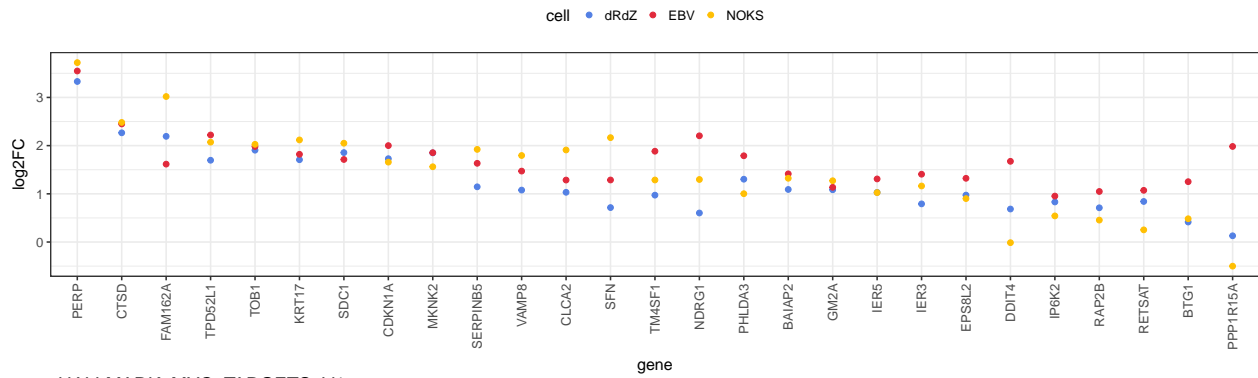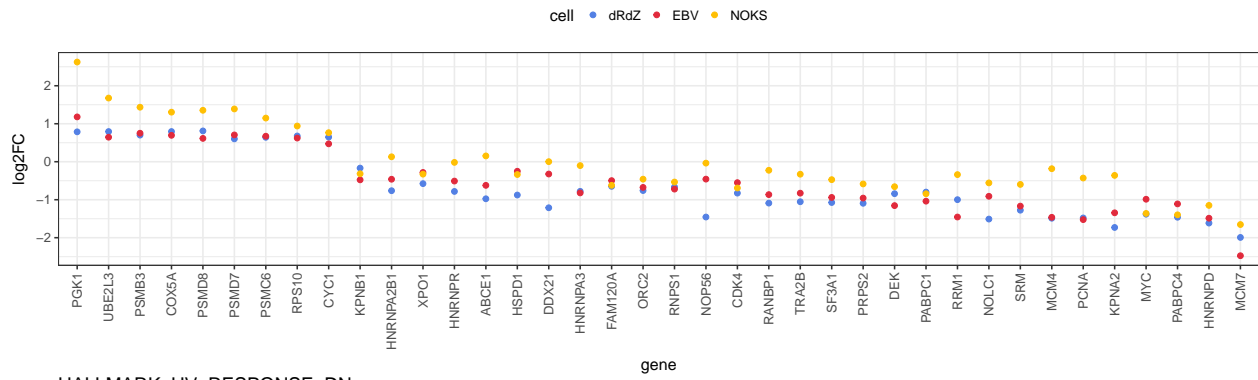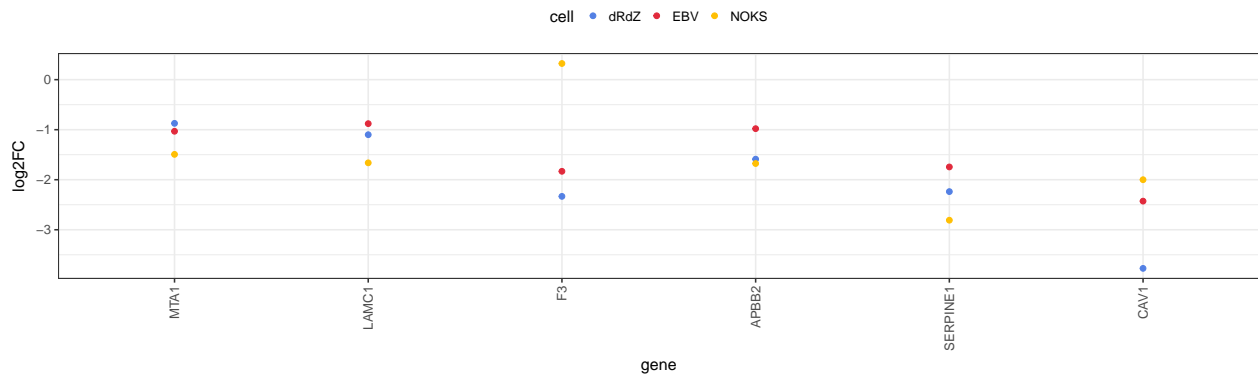HALLMARK_APICAL_JUNCTION

HALLMARK_P53_PATHWAY

HALLMARK_MYC_TARGETS_V1

HALLMARK_UV_RESPONSE_DN

HALLMARK_INTERFERON_ALPHA_RESPONSE

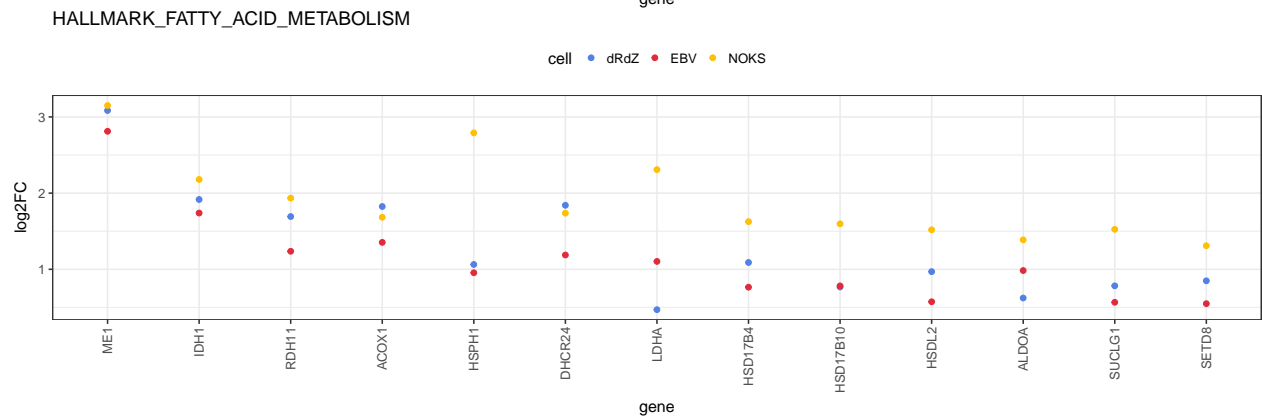## HALLMARK_MTORC1_SIGNALING

HALLMARK_GLYCOLYSIS

HALLMARK_FATTY_ACID_METABOLISM
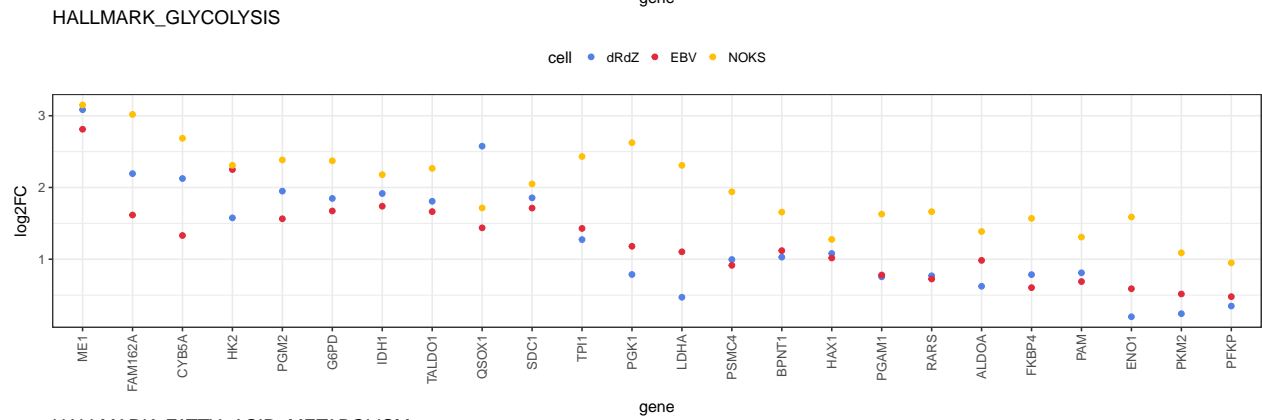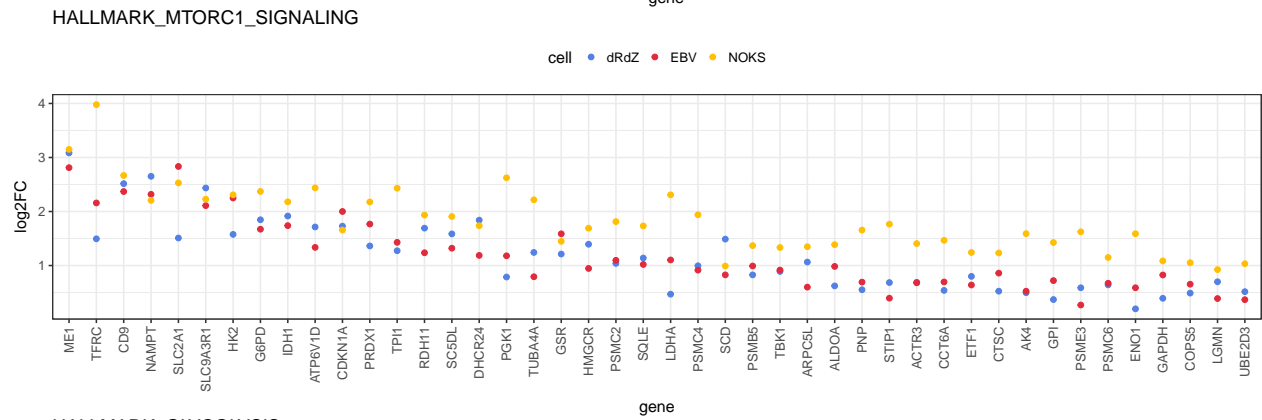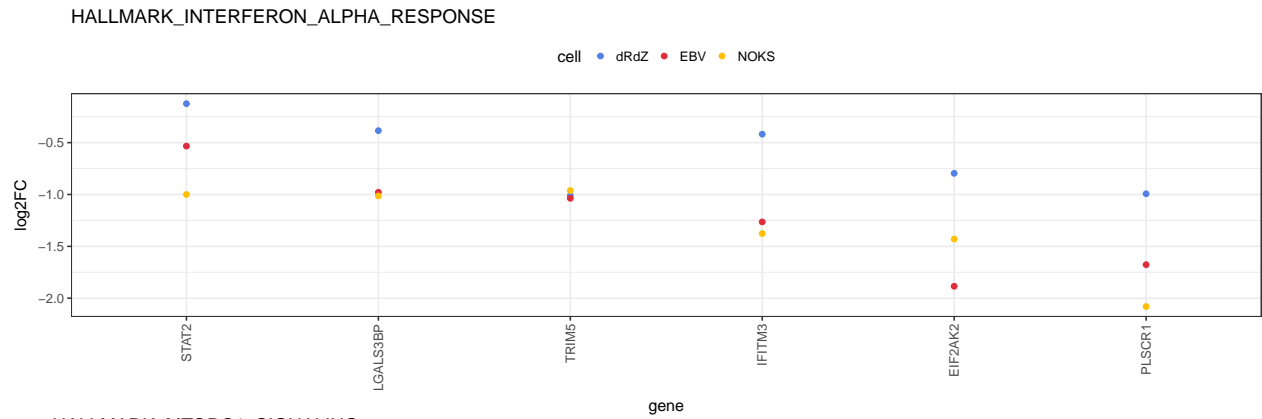
HALLMARK_PEROXISOME

HALLMARK_MYC_TARGETS_V2