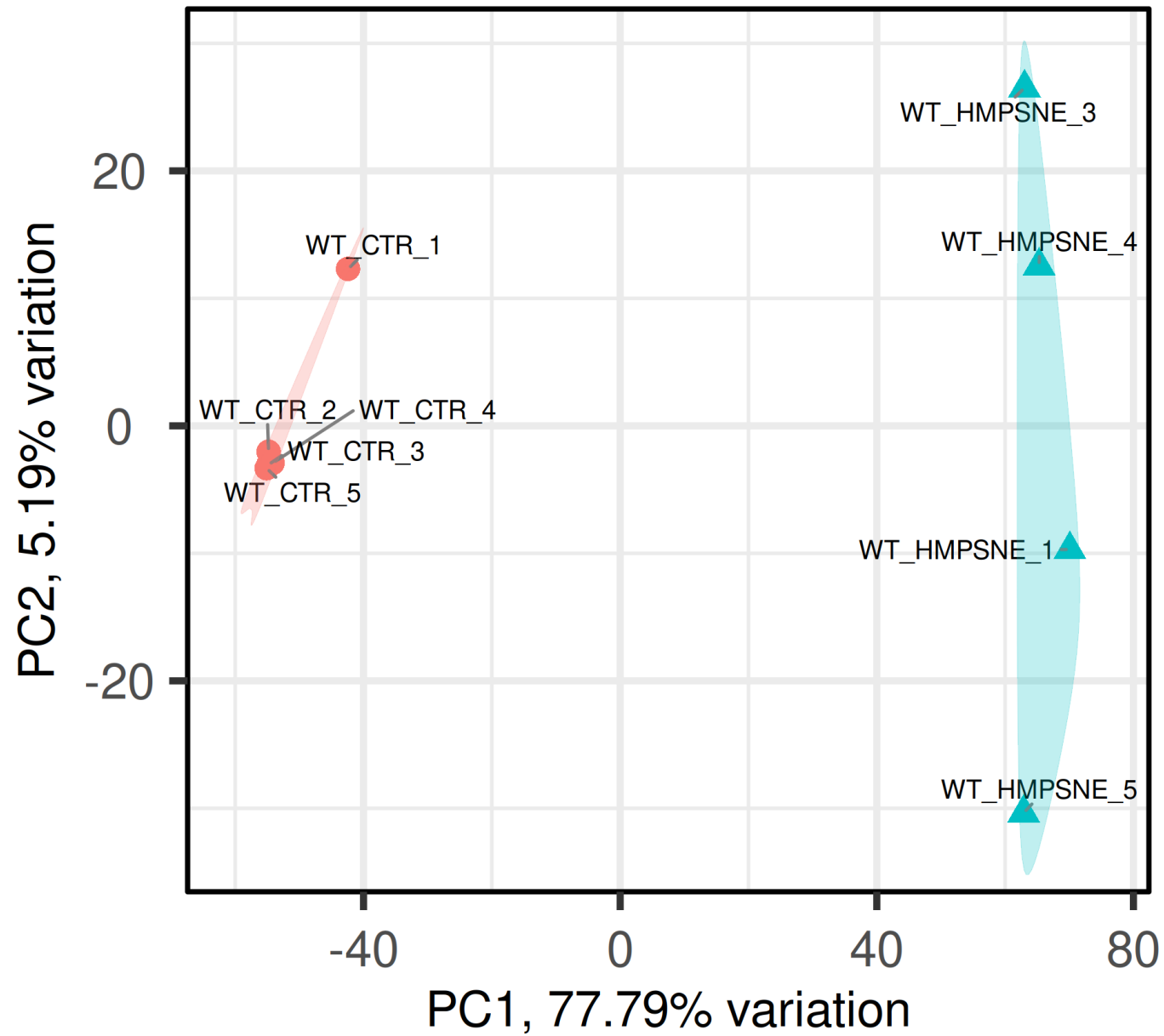


Supplementary Data S3. Multi-omics profiling of pharmacological 3-MST inhibition in WT HCT116 cells. Integrated transcriptomic, proteomic, metabolomic and lipidomic analyses comparing WT HCT116 cells treated with the 3-MST inhibitor HMPSNE and untreated WT HCT116 control cells (WT_CTR) are shown. The dataset includes PCA, hierarchical clustering, heatmaps of the most variable or differentially regulated features, volcano plots of differential expression, and lists of the top 25 upregulated and downregulated genes, proteins, metabolites and lipid species. Differential expression analyses were performed using a fold-change threshold ≥ 1.5 with statistical significance defined as false discovery rate (FDR) ≤ 0.05 for transcriptomic datasets and $P \leq 0.05$ for proteomic, metabolomic and lipidomic datasets. Functional interpretation of differential features was performed using pathway enrichment analyses. For transcriptomic and proteomic datasets, GSEA was conducted using GO biological process and cellular component annotations, as well as KEGG, Reactome and Hallmark gene sets. ORA was additionally performed to identify pathways and biological processes significantly enriched among features increased or decreased following HMPSNE treatment. For metabolomic datasets, pathway enrichment was evaluated using QEA to identify metabolic pathways significantly altered following pharmacological inhibition of 3-MST. ORA was also applied to determine metabolite pathways significantly associated with treatment. For lipidomic datasets, enrichment of lipid pathways was assessed using ORA. Lipid ontology enrichment was further evaluated using the LION (Lipid Ontology) framework in both ranking and target-list modes to identify lipid structural and functional categories associated with HMPSNE treatment. Together, these analyses provide a comprehensive multi-omics characterization of molecular changes induced by pharmacological inhibition of 3-MST in HCT116 wild-type colorectal cancer cells.

WT-HMPSNE vs WT-CTR

RNA Seq

WT - HMPSNE



Condition



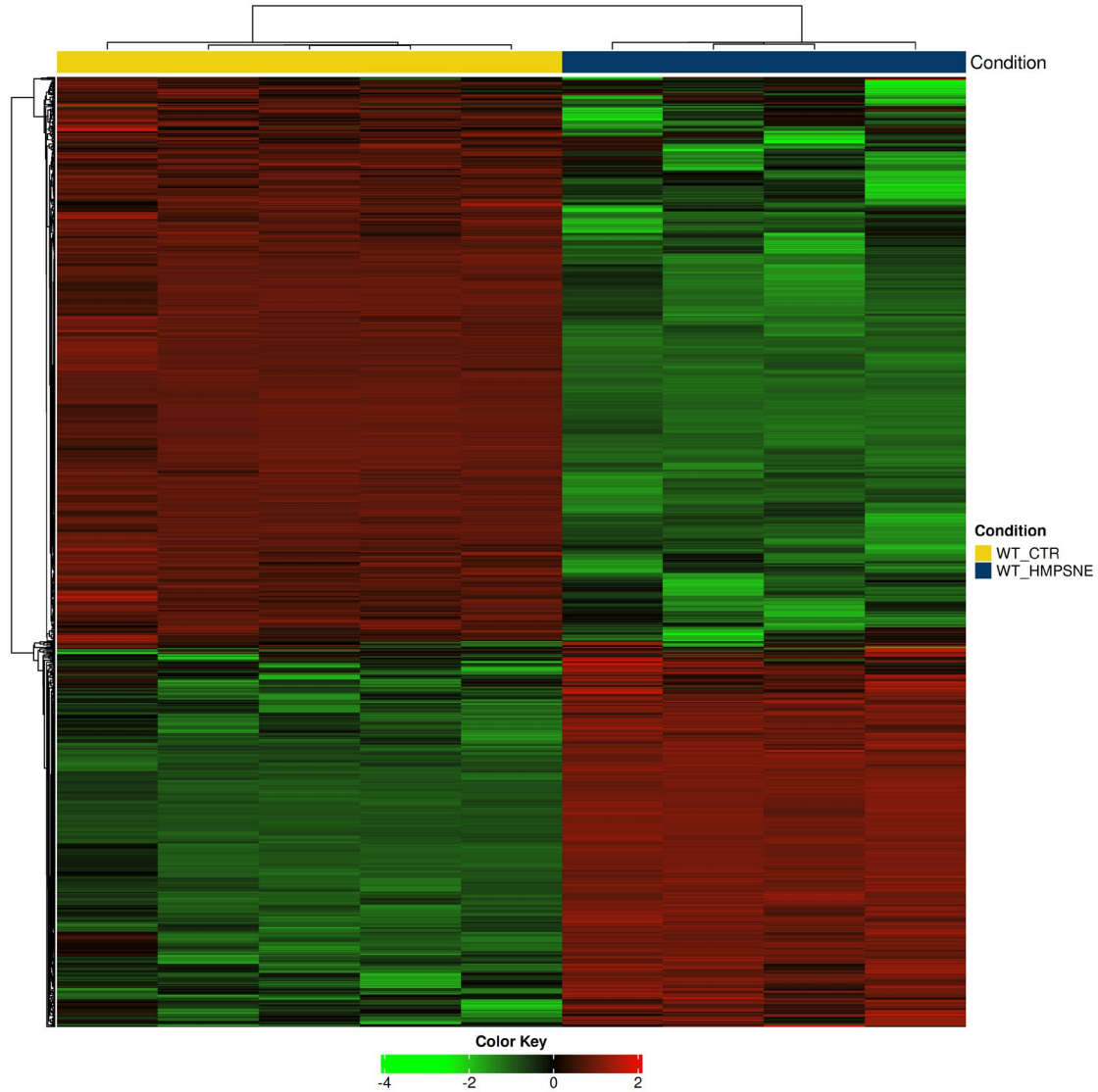
WT_CTRL



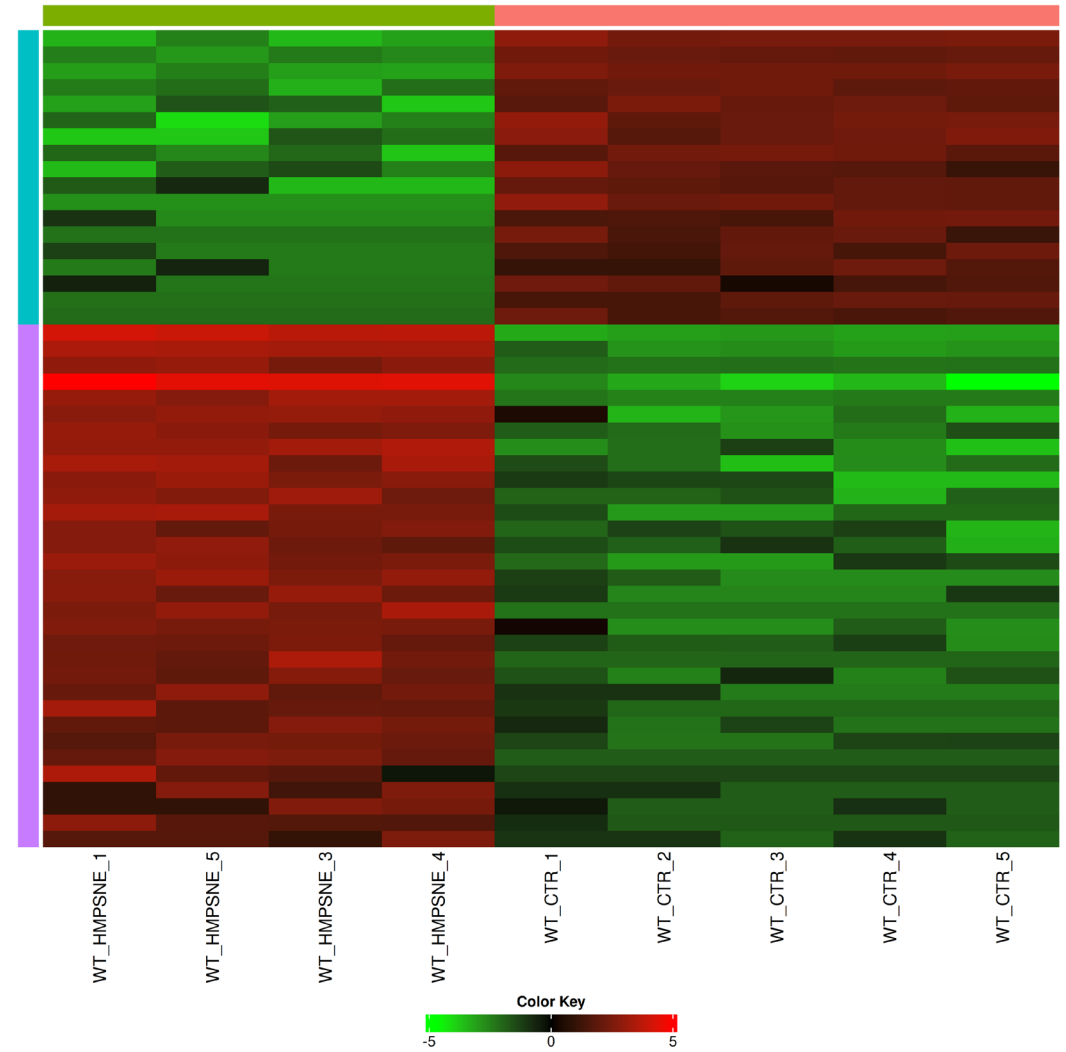
WT_HMPSNE

WT – HMPSNE

Hierarchical clustering of the top 1000 most variable genes

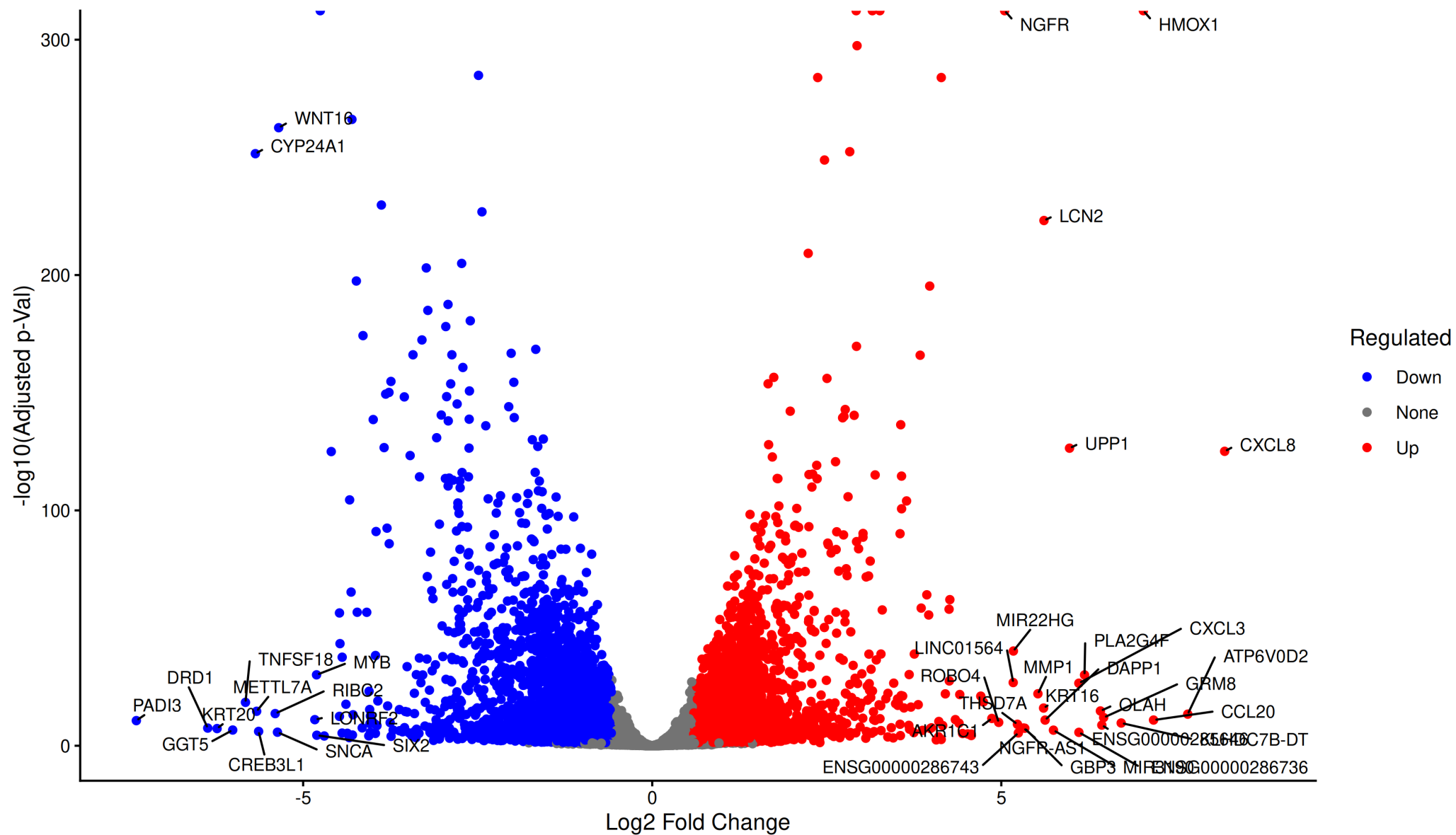


Heatmap of the top 50 DEGs



WT – HMPSNE

Fold Change vs. Adjusted p-Value

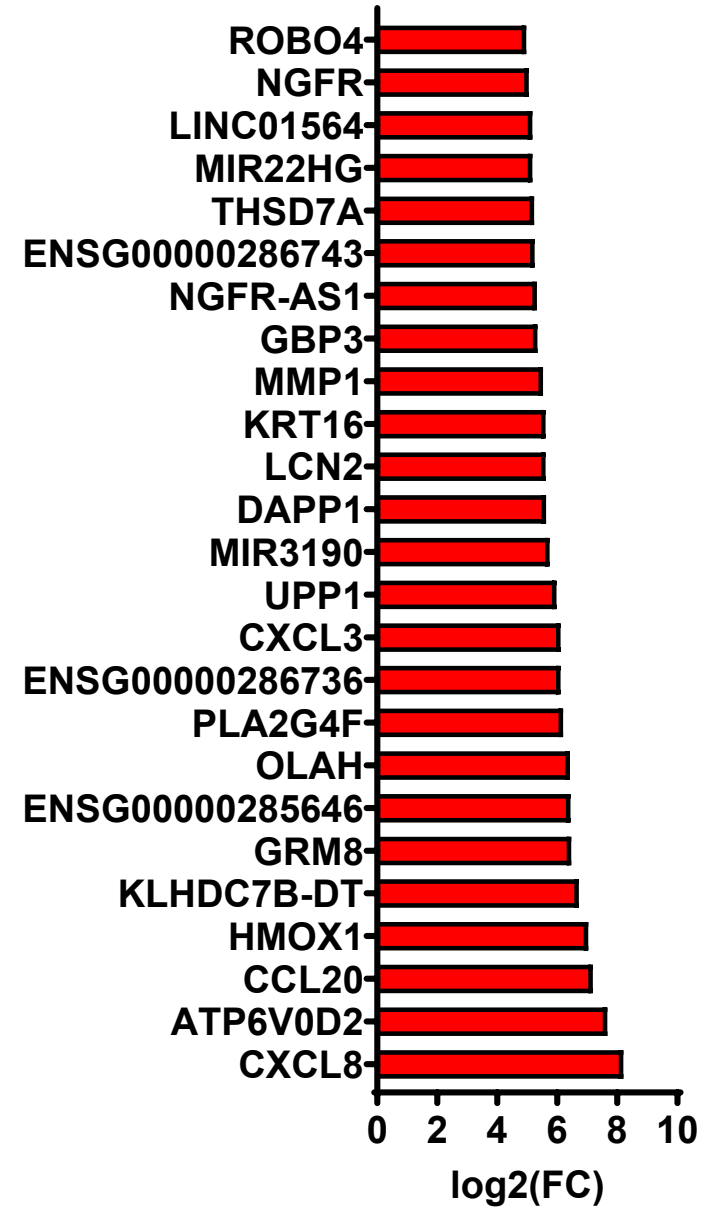


Fold change (FC) threshold: 1.5

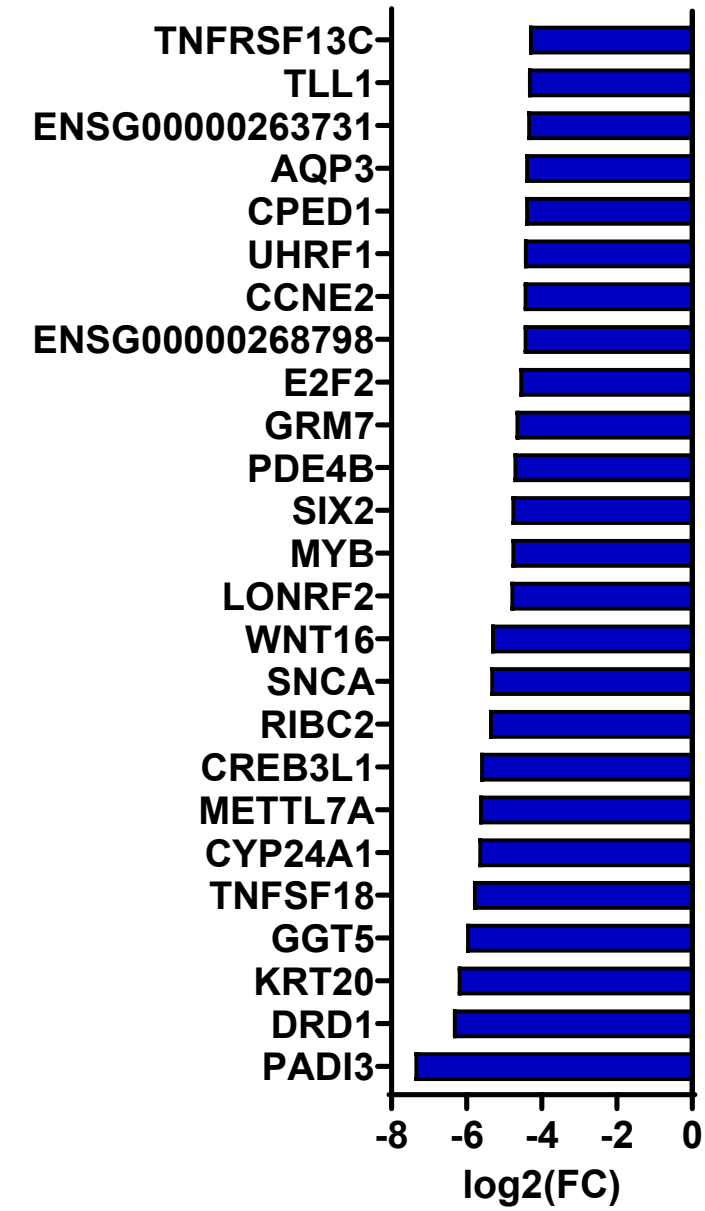
FDR cutoff: 0.05

WT – HMPSNE

Top 25_Upregulated genes in treated WT cells

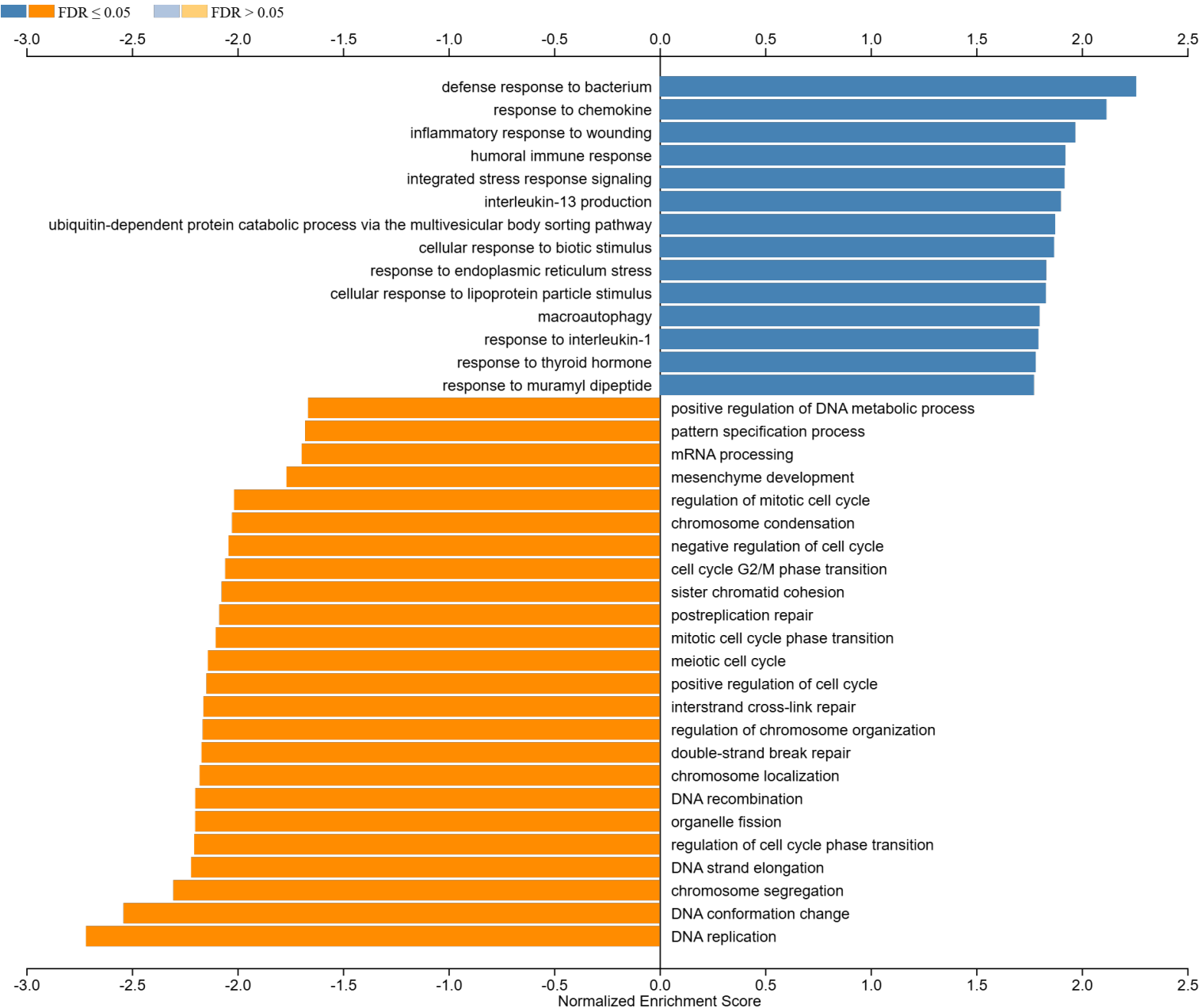


Top 25_Downregulated genes in treated WT cells



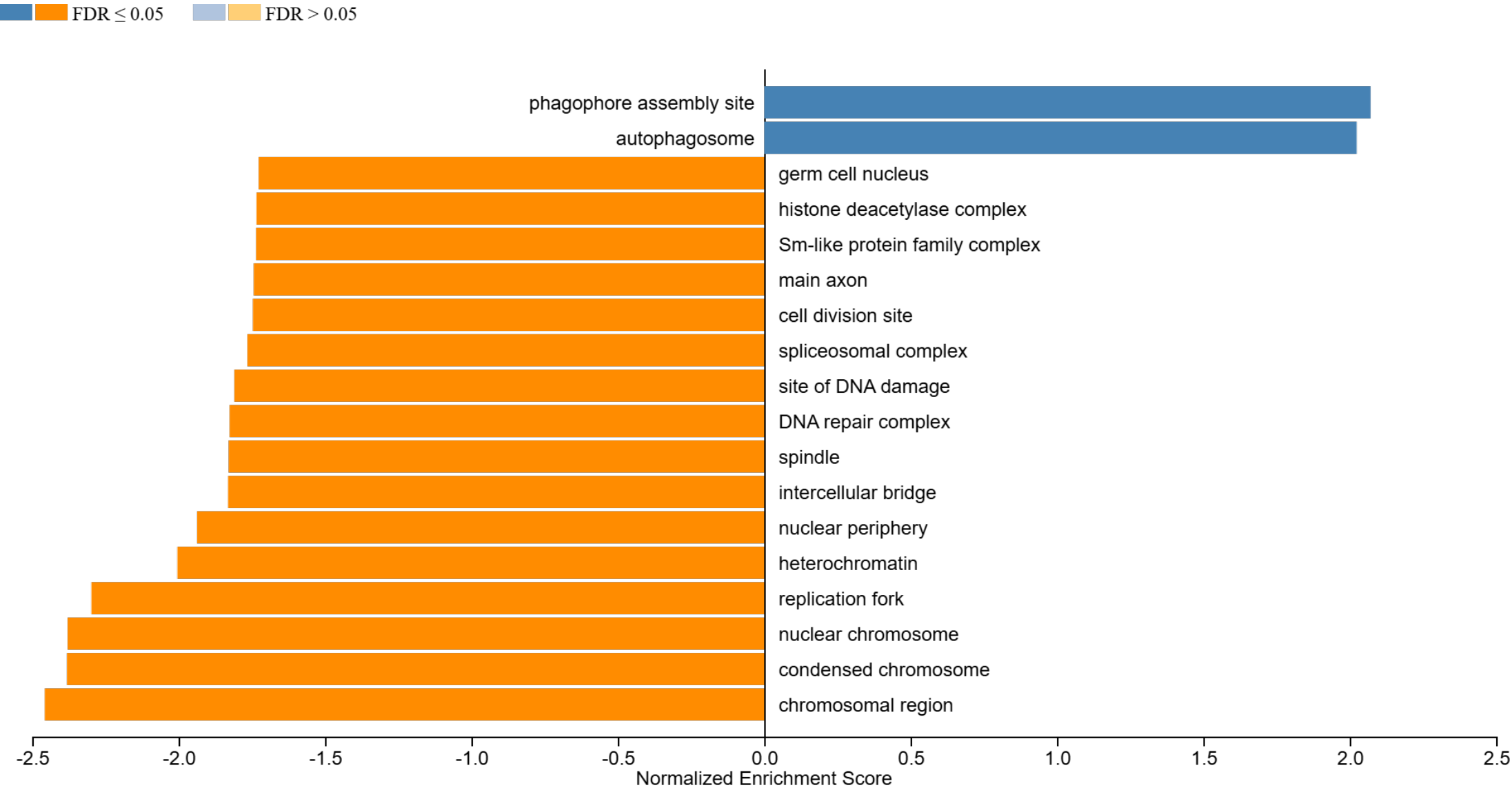
WT – HMPSNE

GSEA (Gene Set Enrichment Analysis) - geneontology_Biological_Process



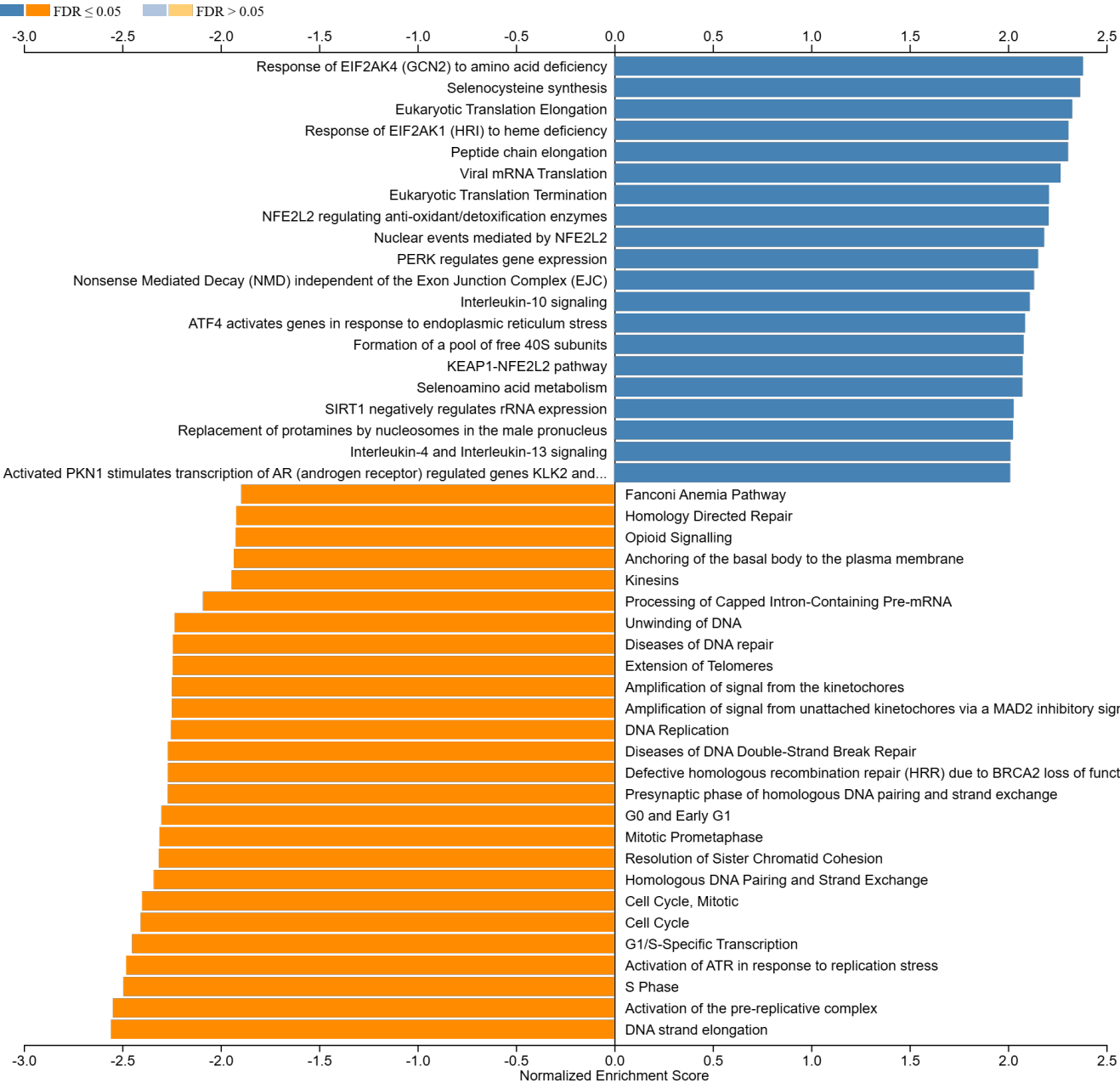
WT – HMPSNE

GSEA (Gene Set Enrichment Analysis) - geneontology_Cellular_Component



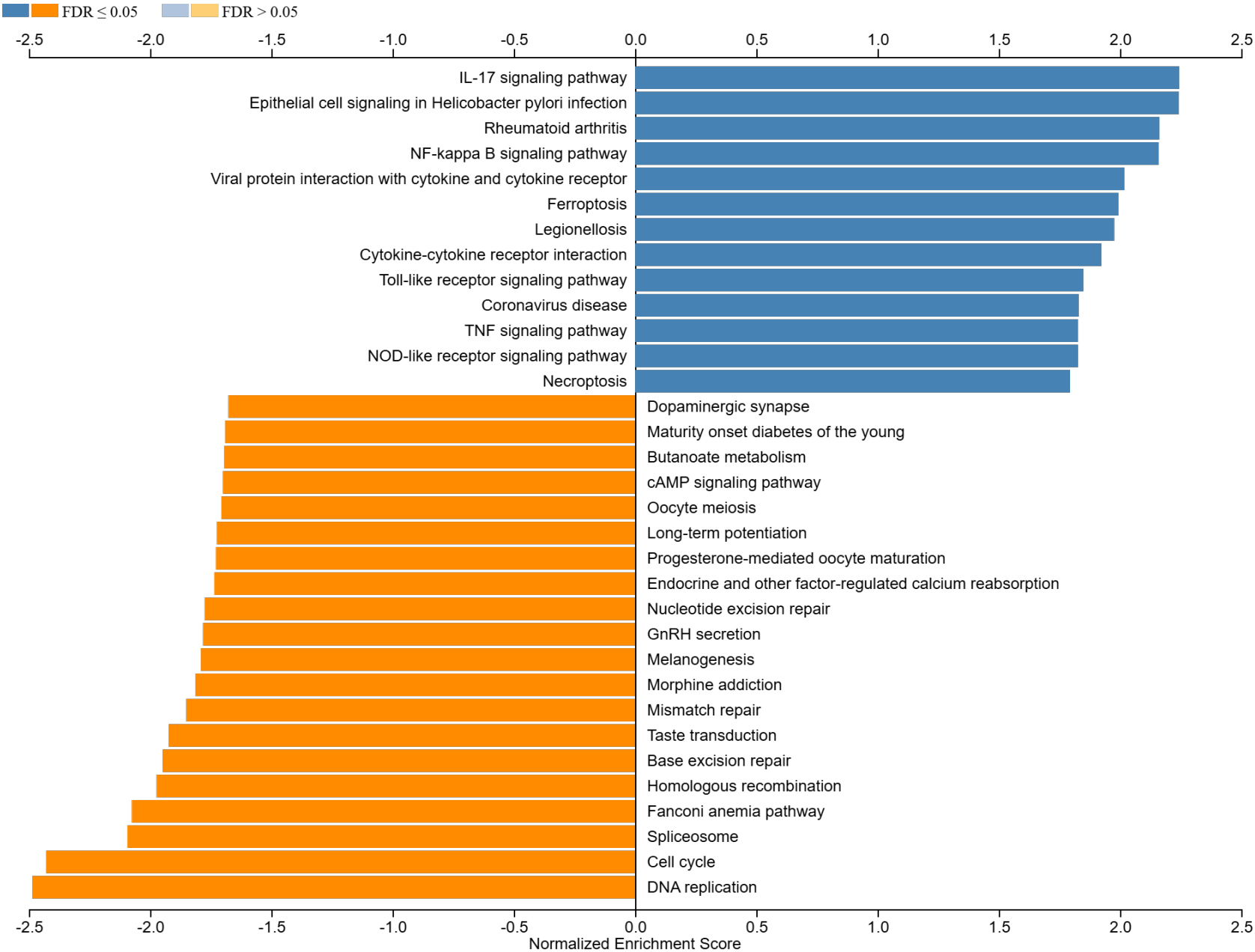
WT – HMPSNE

GSEA (Gene Set Enrichment Analysis) - pathway_Reactome



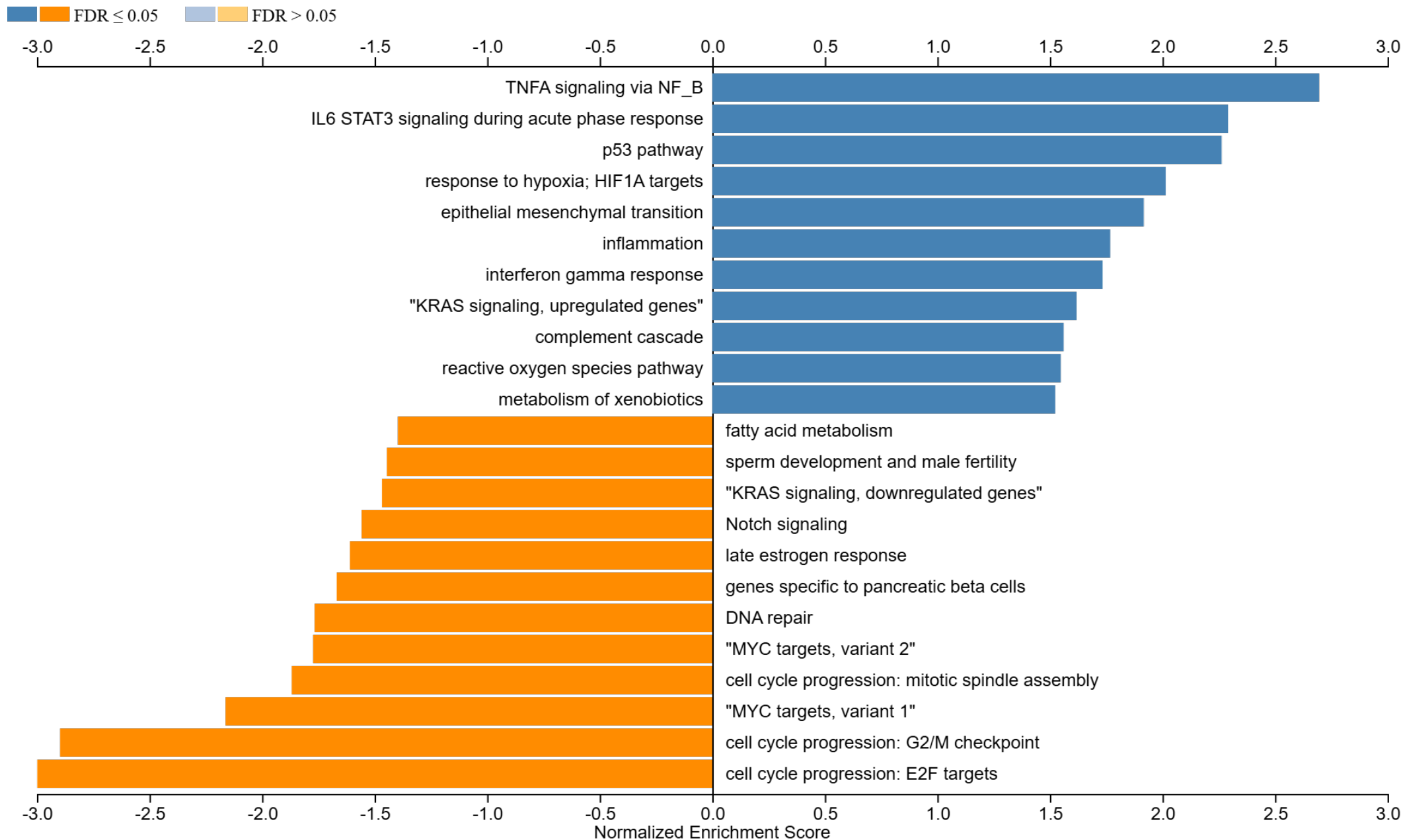
WT – HMPSNE

GSEA (Gene Set Enrichment Analysis) - pathway_KEGG



WT – HMPSNE

GSEA (Gene Set Enrichment Analysis) - community-contributed_Hallmark50

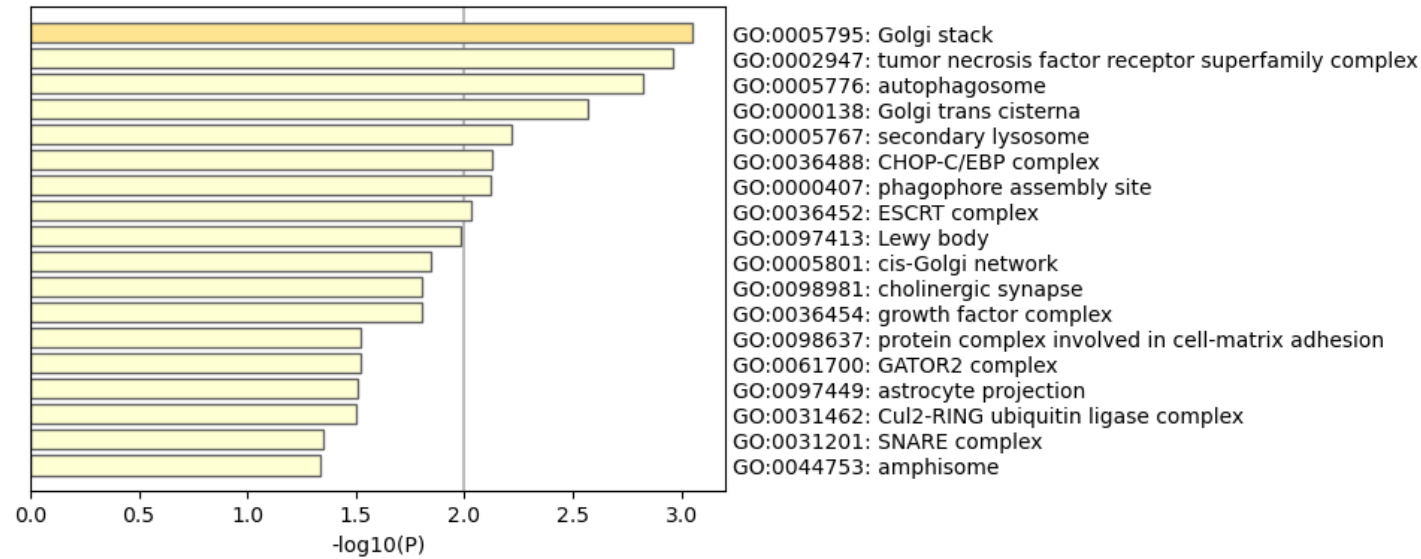


WT – HMPSNE

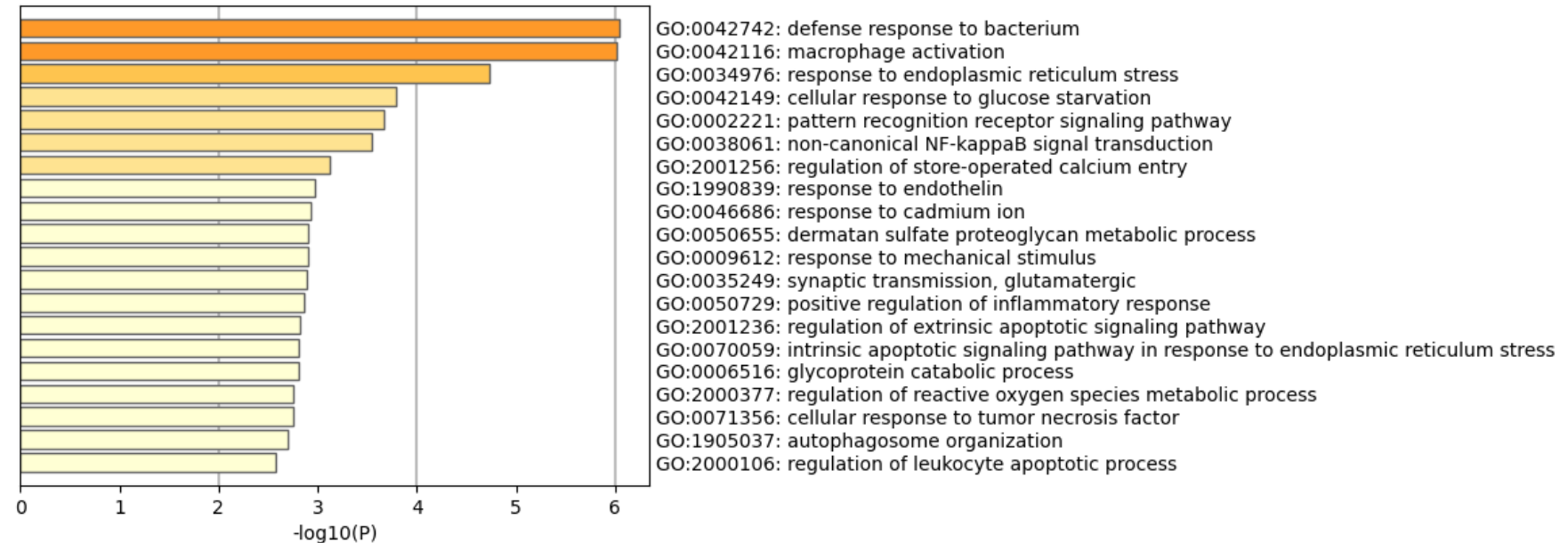
ORA (Over-Representation Analysis)

Activation in treated WT cells

GO Cellular Components



GO Biological Process

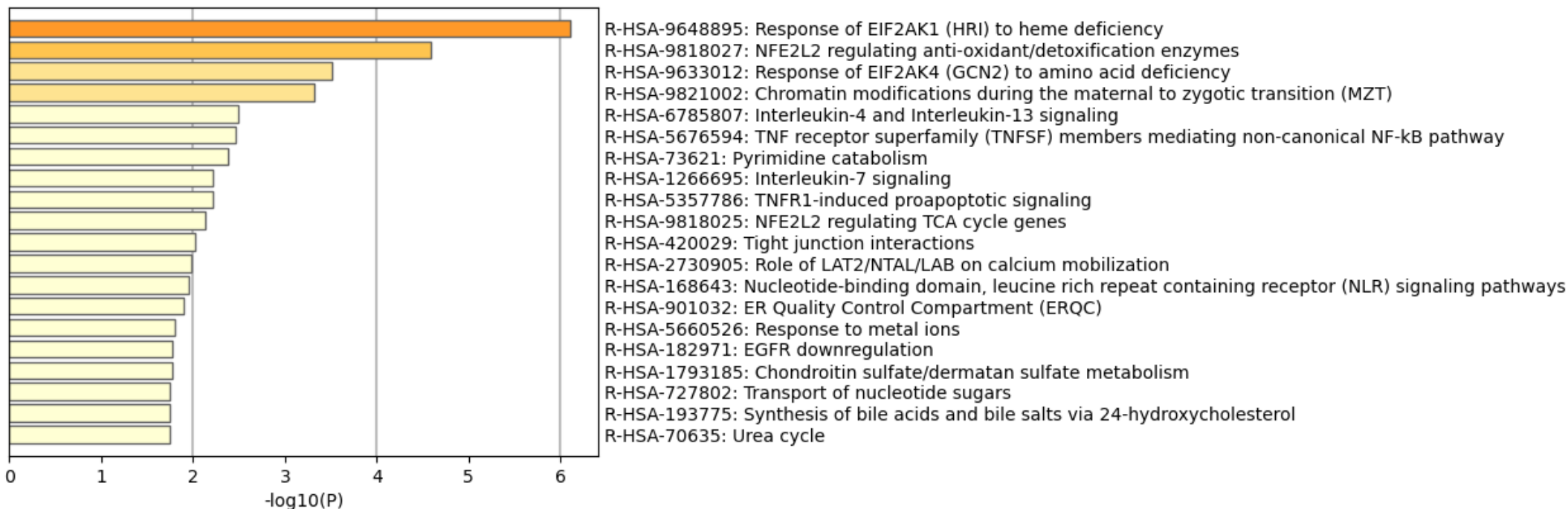


WT – HMPSNE

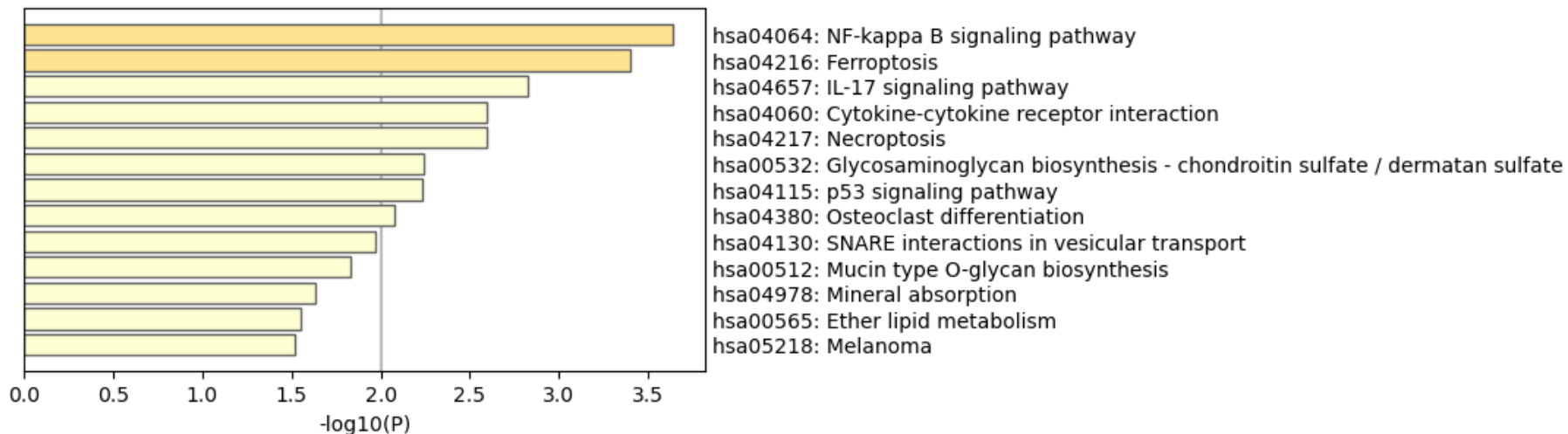
ORA (Over-Representation Analysis)

Activation in treated WT cells

Reactome Gene Sets



KEGG Pathway

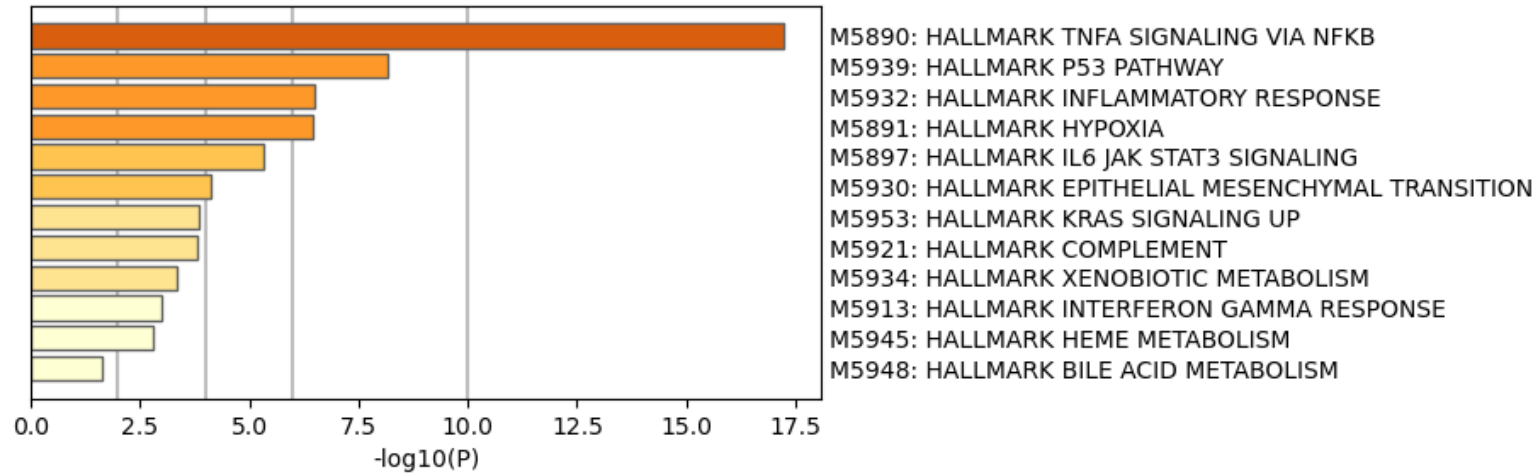


WT – HMPSNE

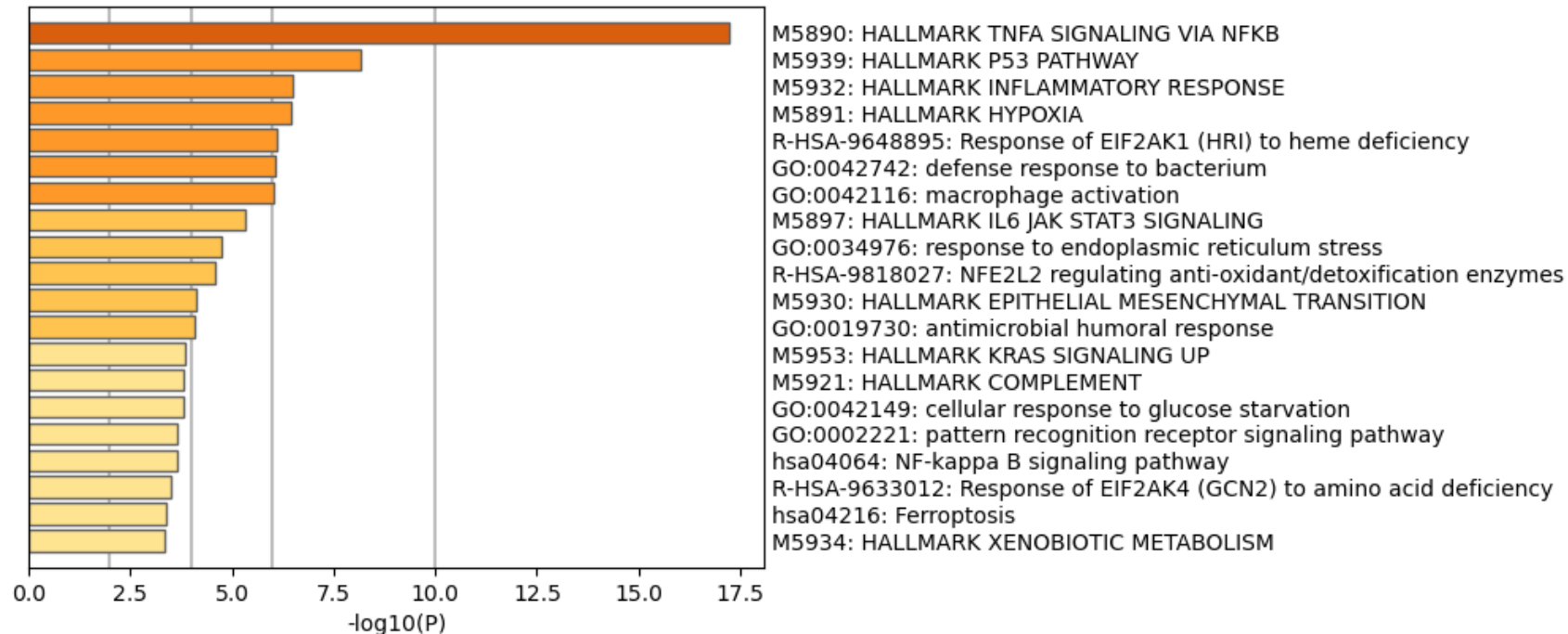
ORA (Over-Representation Analysis)

Activation in treated WT cells

Hallmark Gene Sets



Combined GO

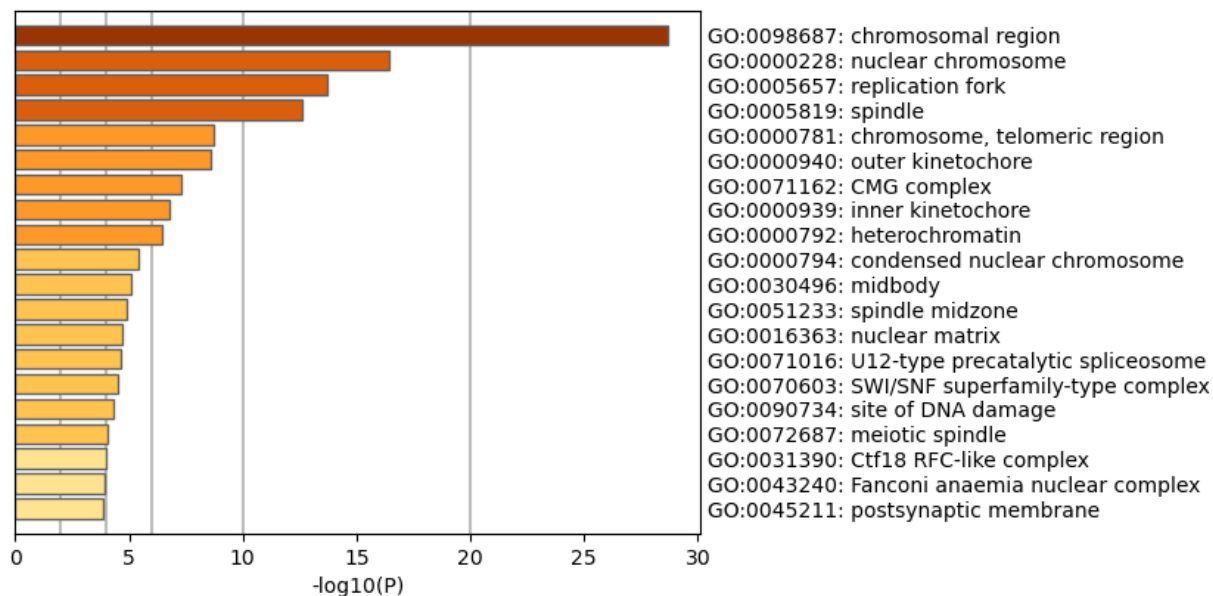


WT – HMPSNE

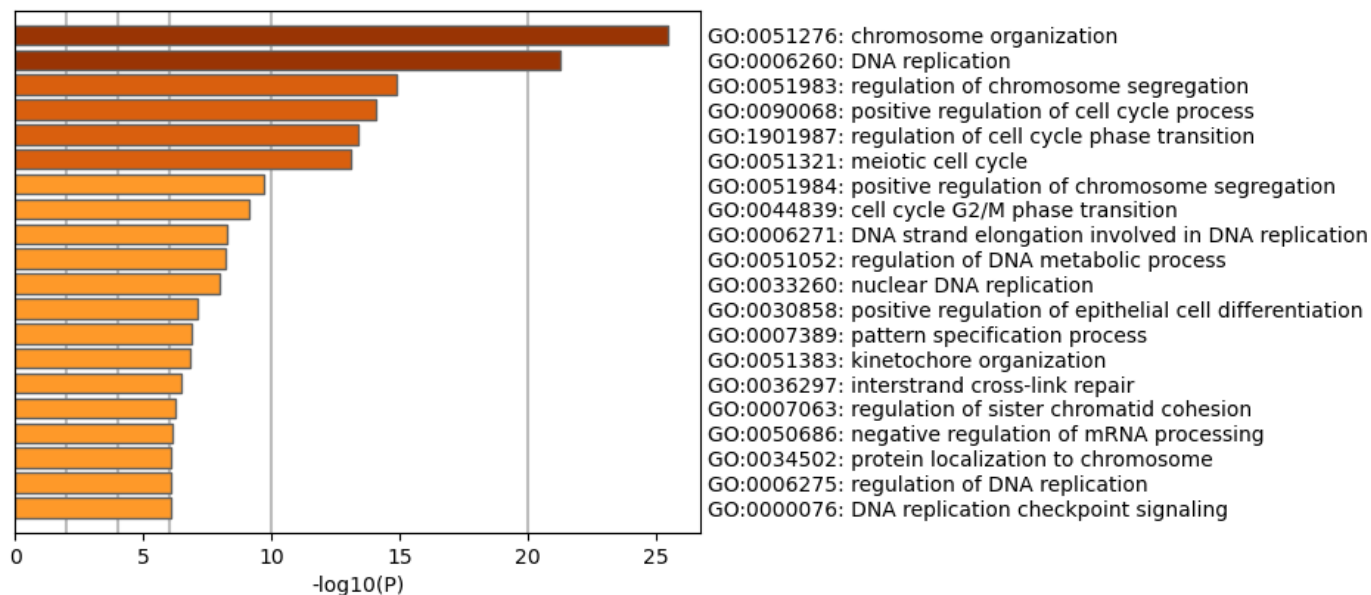
ORA (Over-Representation Analysis)

Inhibition in treated WT cells

GO Cellular Components



GO Biological Process

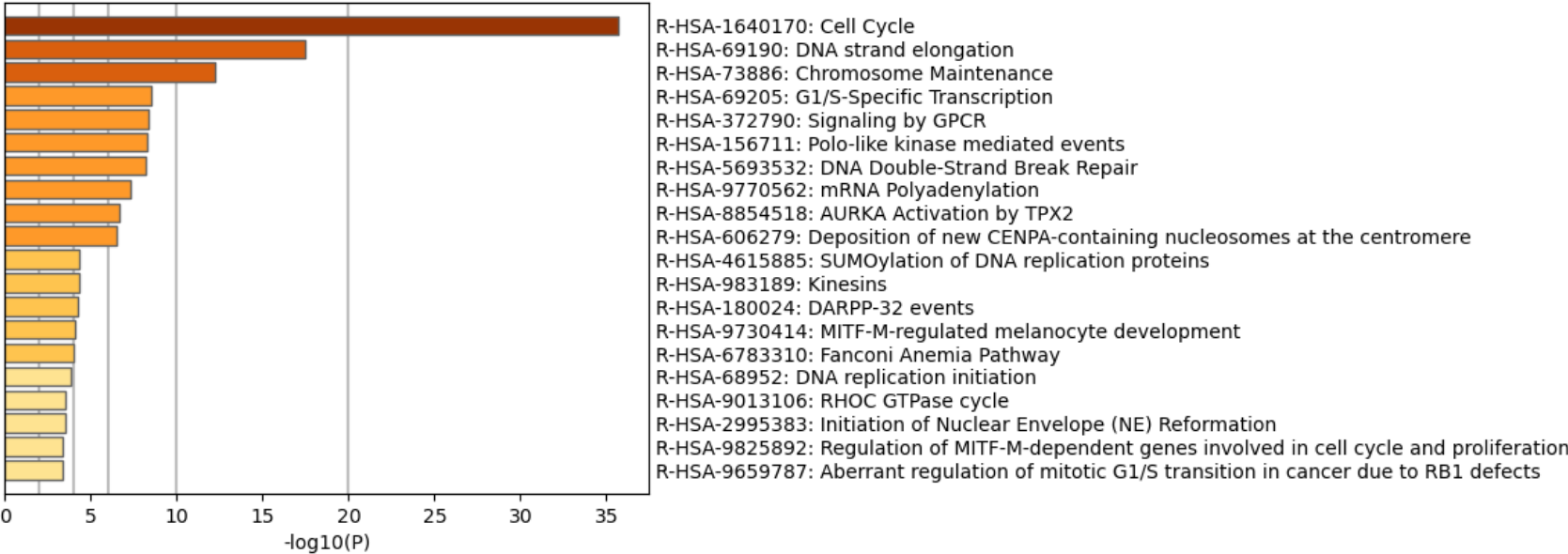


WT – HMPSNE

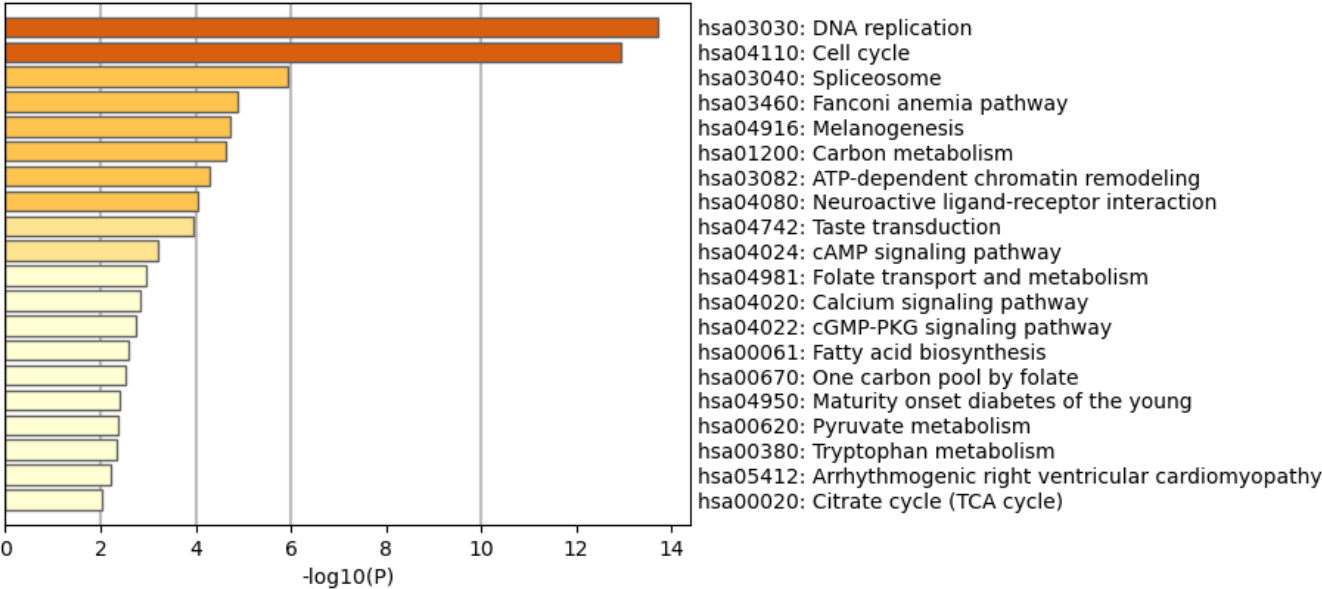
ORA (Over-Representation Analysis)

Inhibition in treated WT cells

Reactome Gene Sets



KEGG Pathway

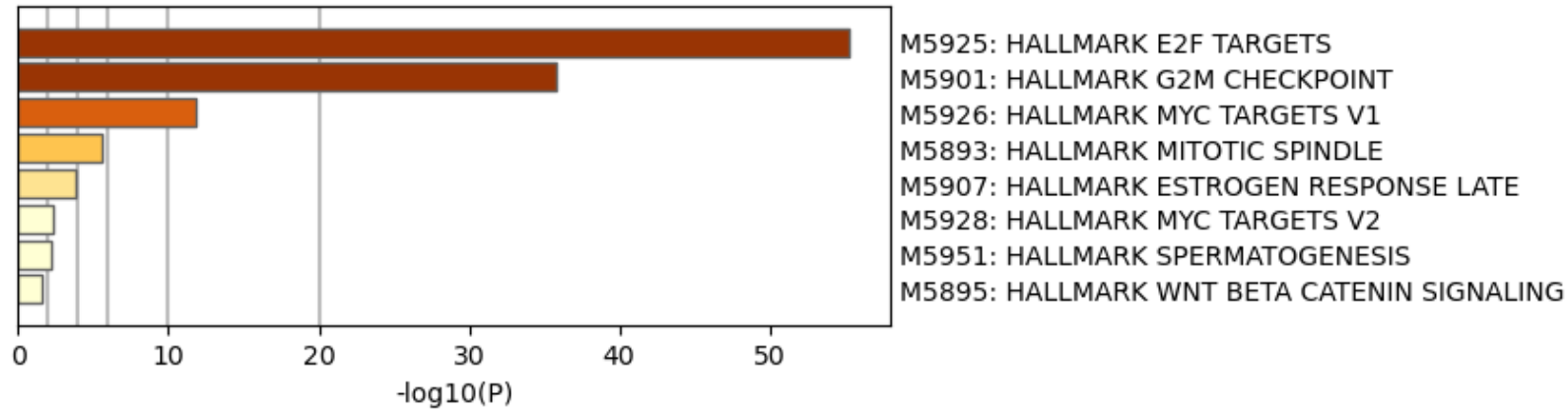


WT – HMPSNE

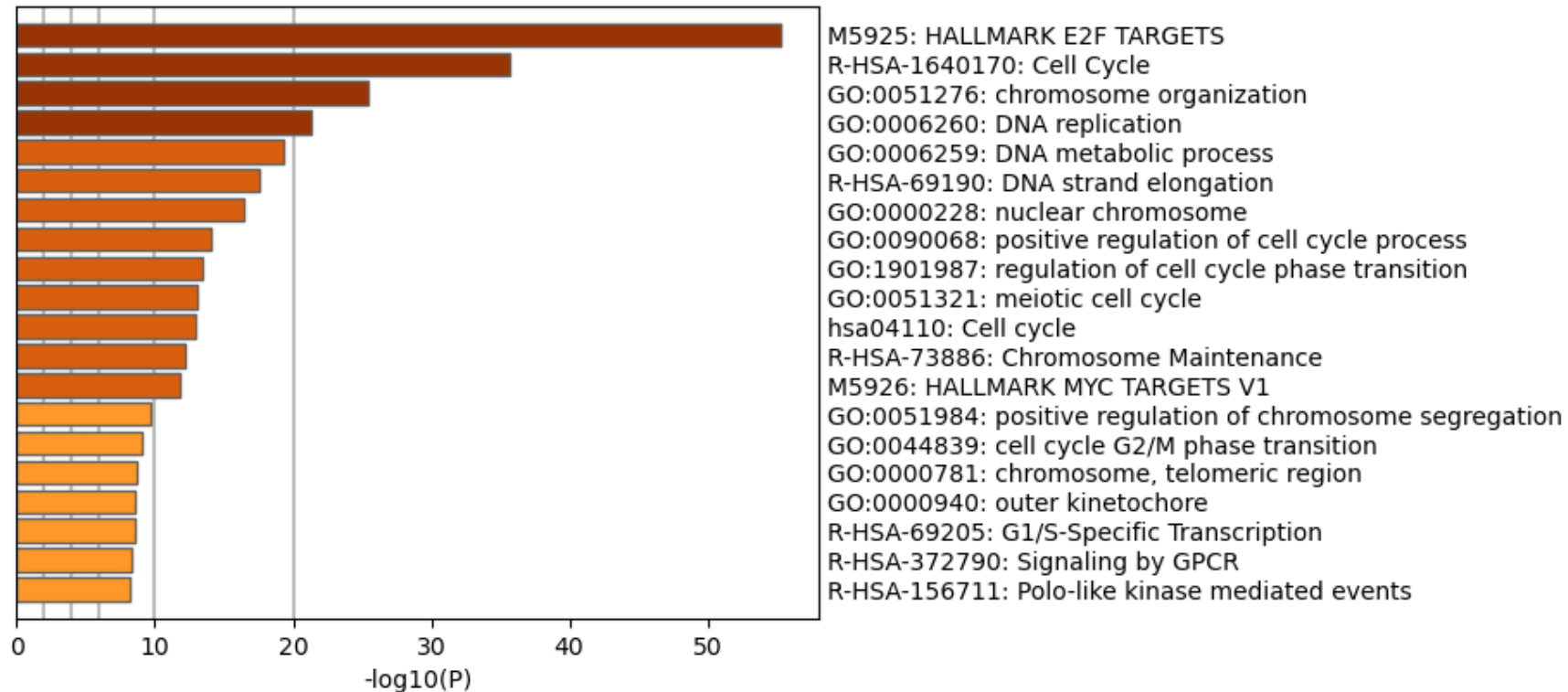
ORA (Over-Representation Analysis)

Inhibition in treated WT cells

Hallmark Gene Sets

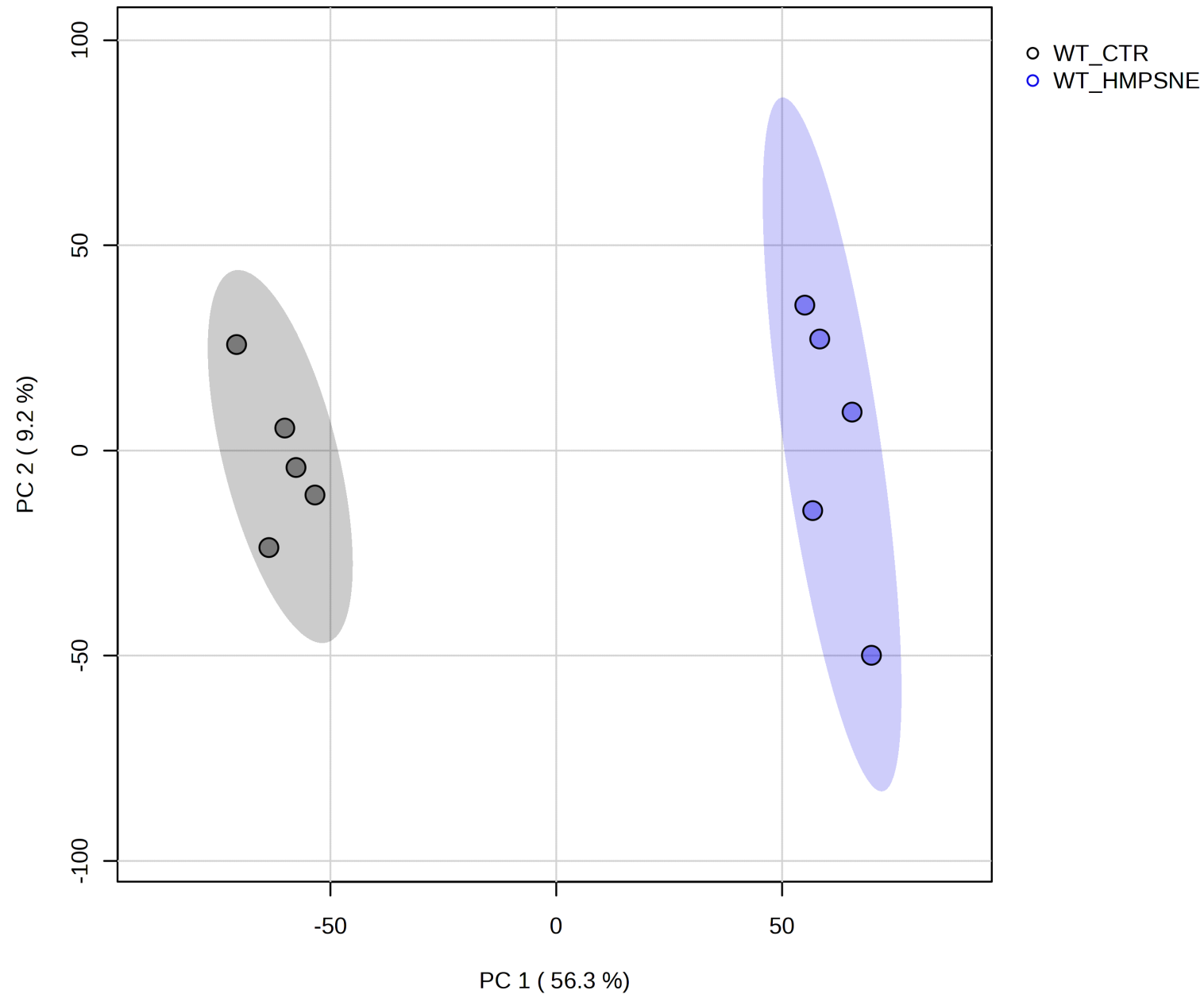


Combined GO

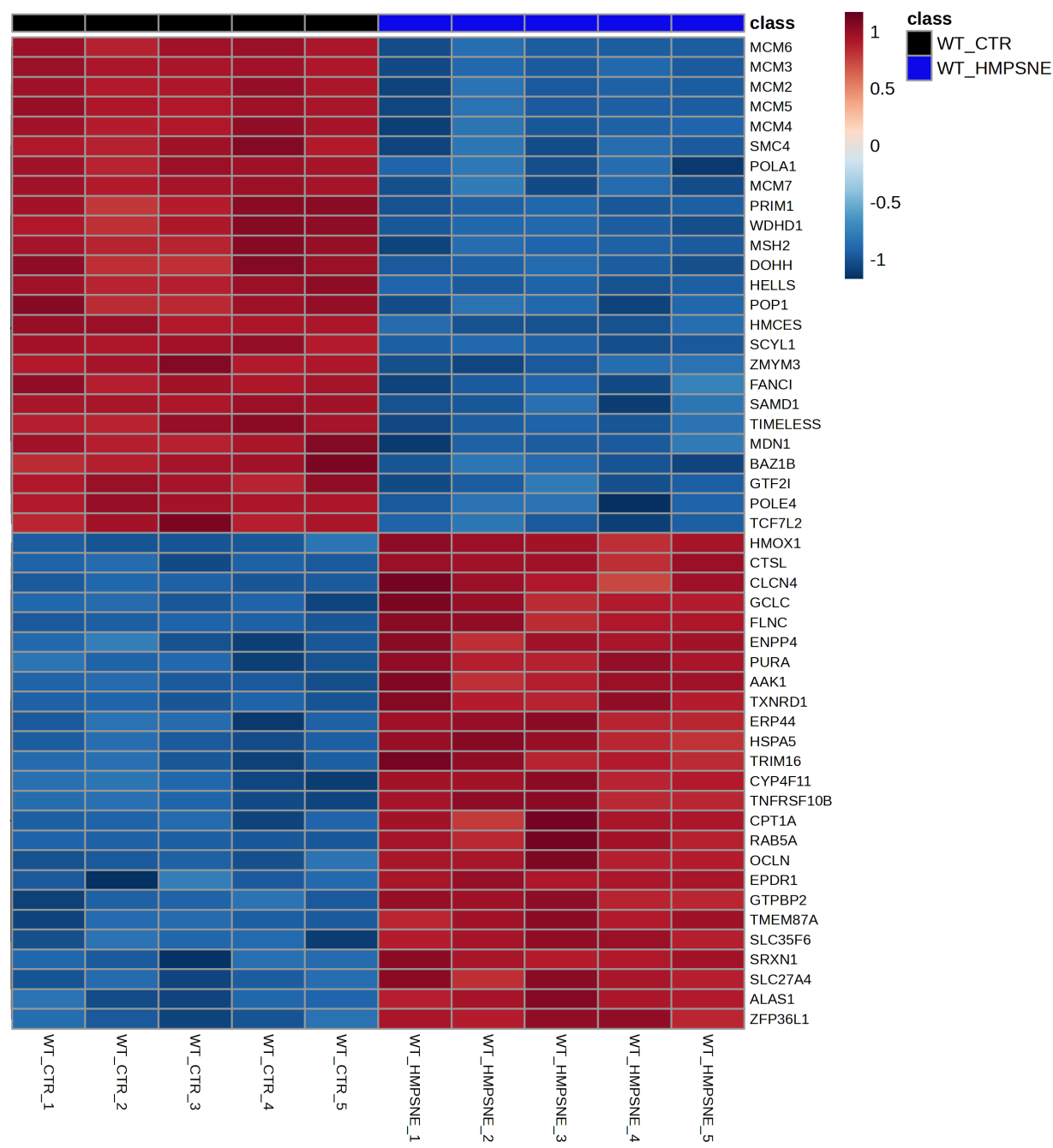


WT-HMPSNE vs WT-CTR Proteomics

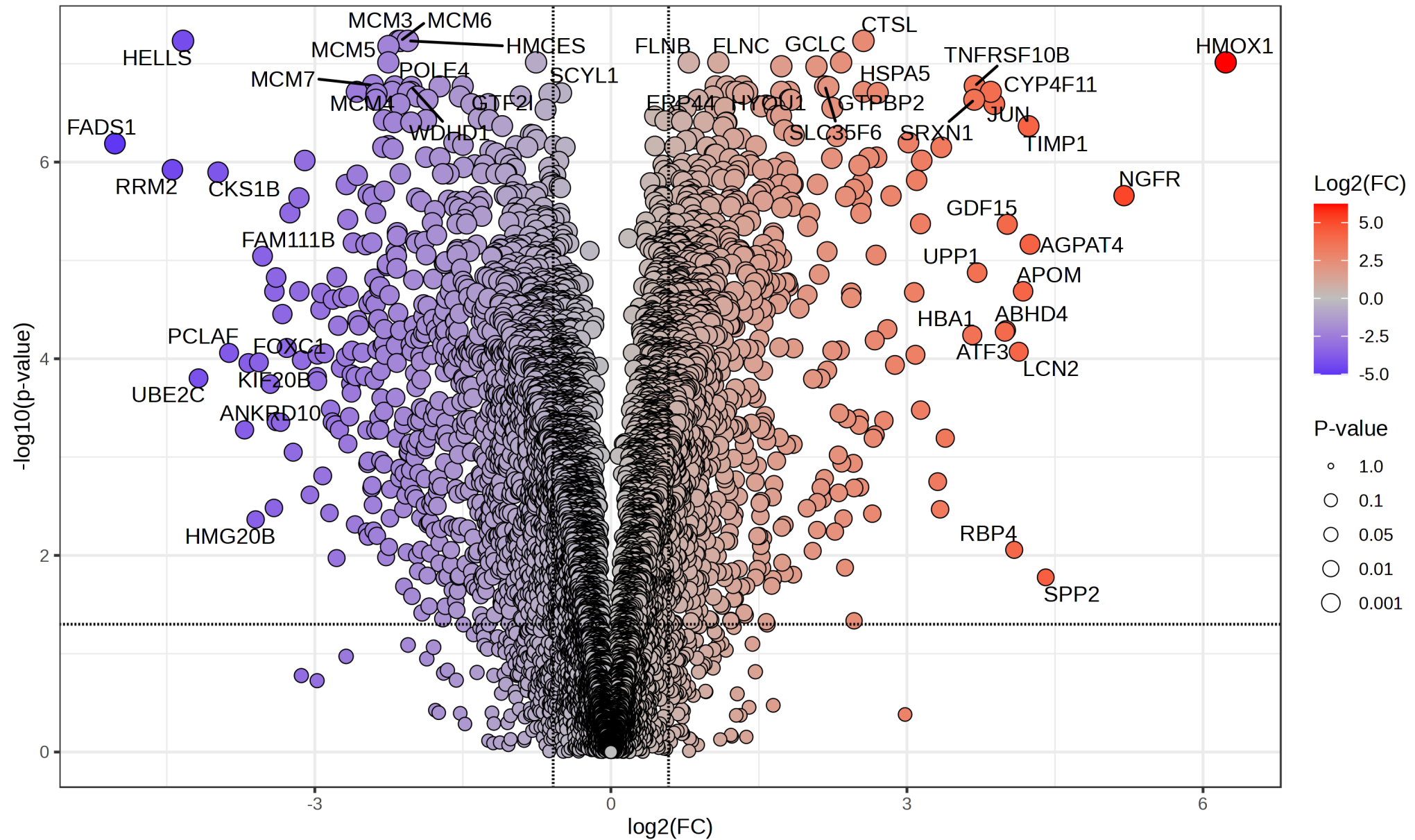
WT – HMPSNE



WT – HMPSNE



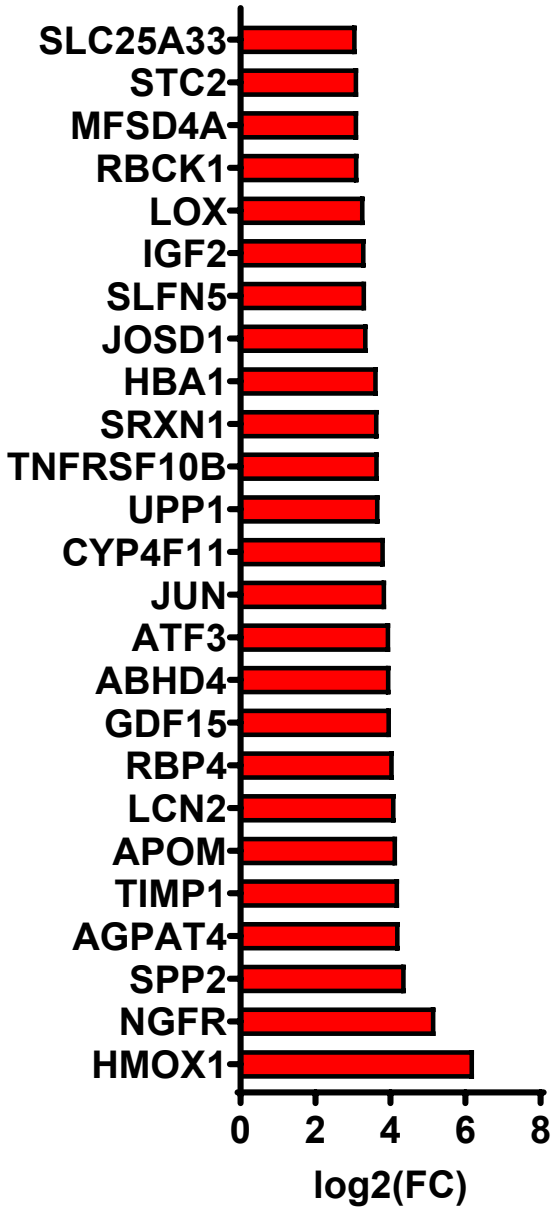
WT – HMPSNE



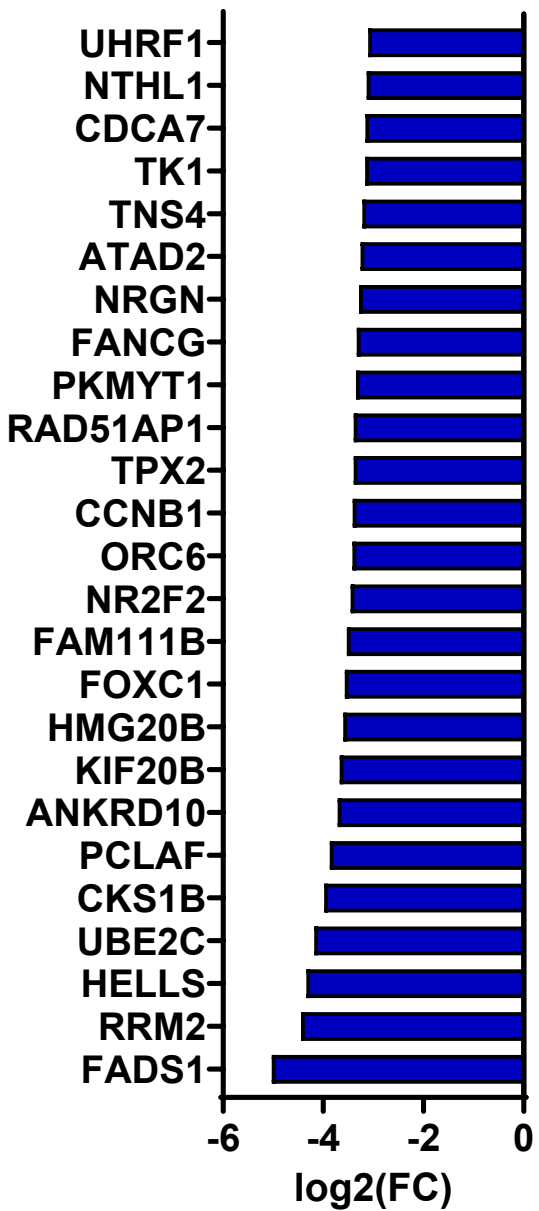
Fold change (FC) threshold: 1.5
P-value threshold: 0.05

WT – HMPSNE

Top 25_Upregulated proteins in treated WT cells

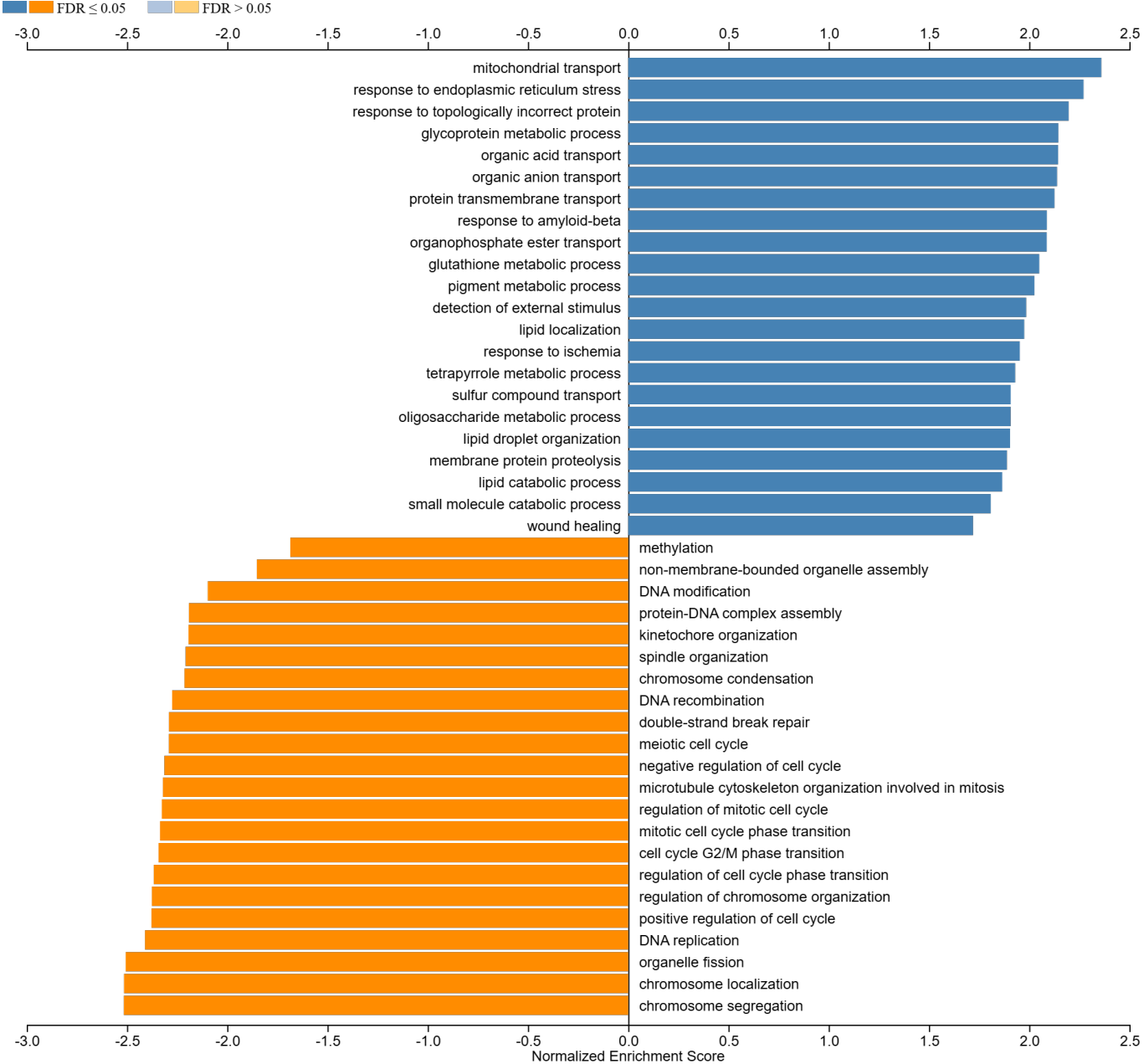


Top 25_Downregulated proteins in treated WT cells



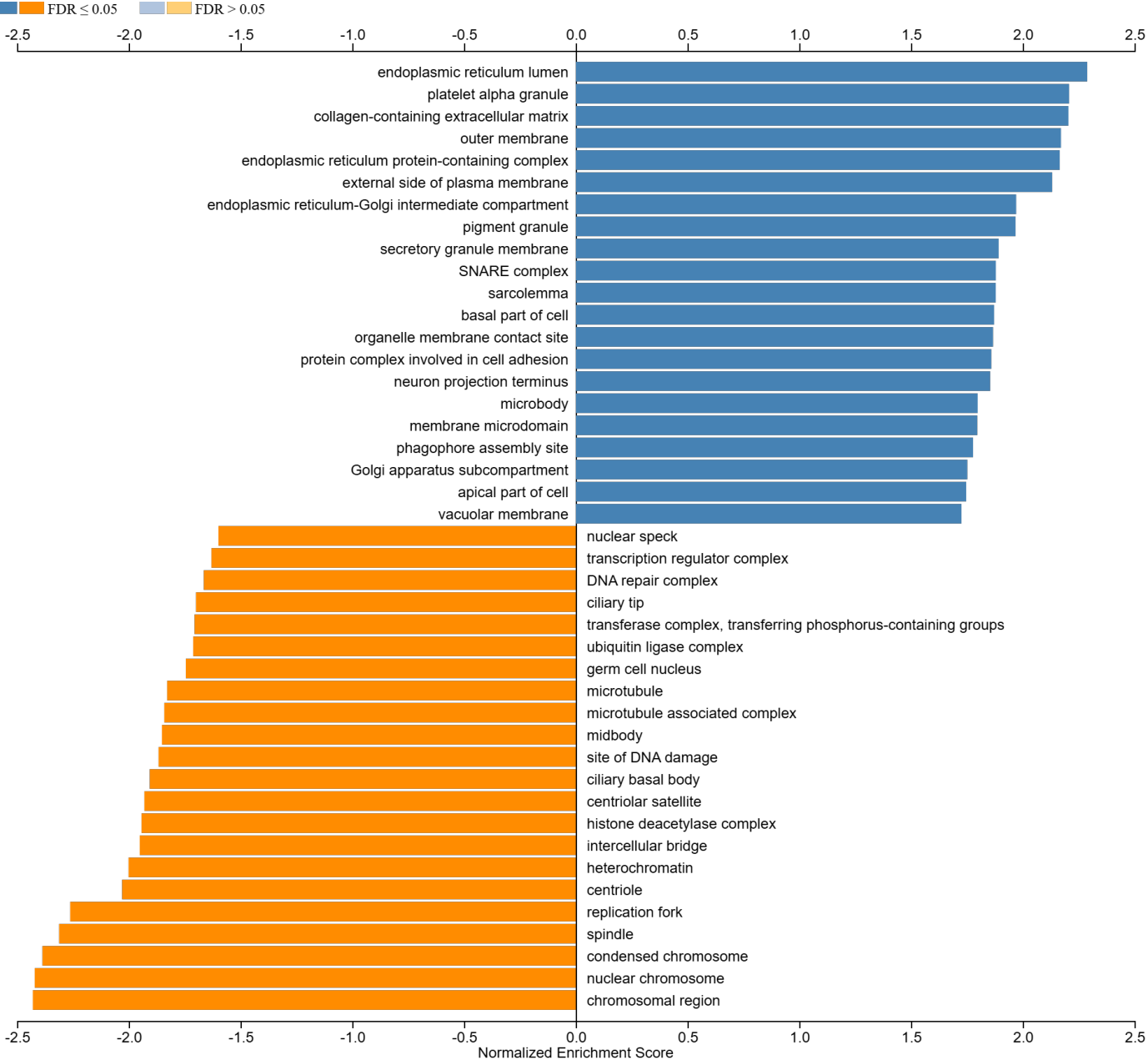
WT – HMPSNE

GSEA (Gene Set Enrichment Analysis) - geneontology_Biological_Process



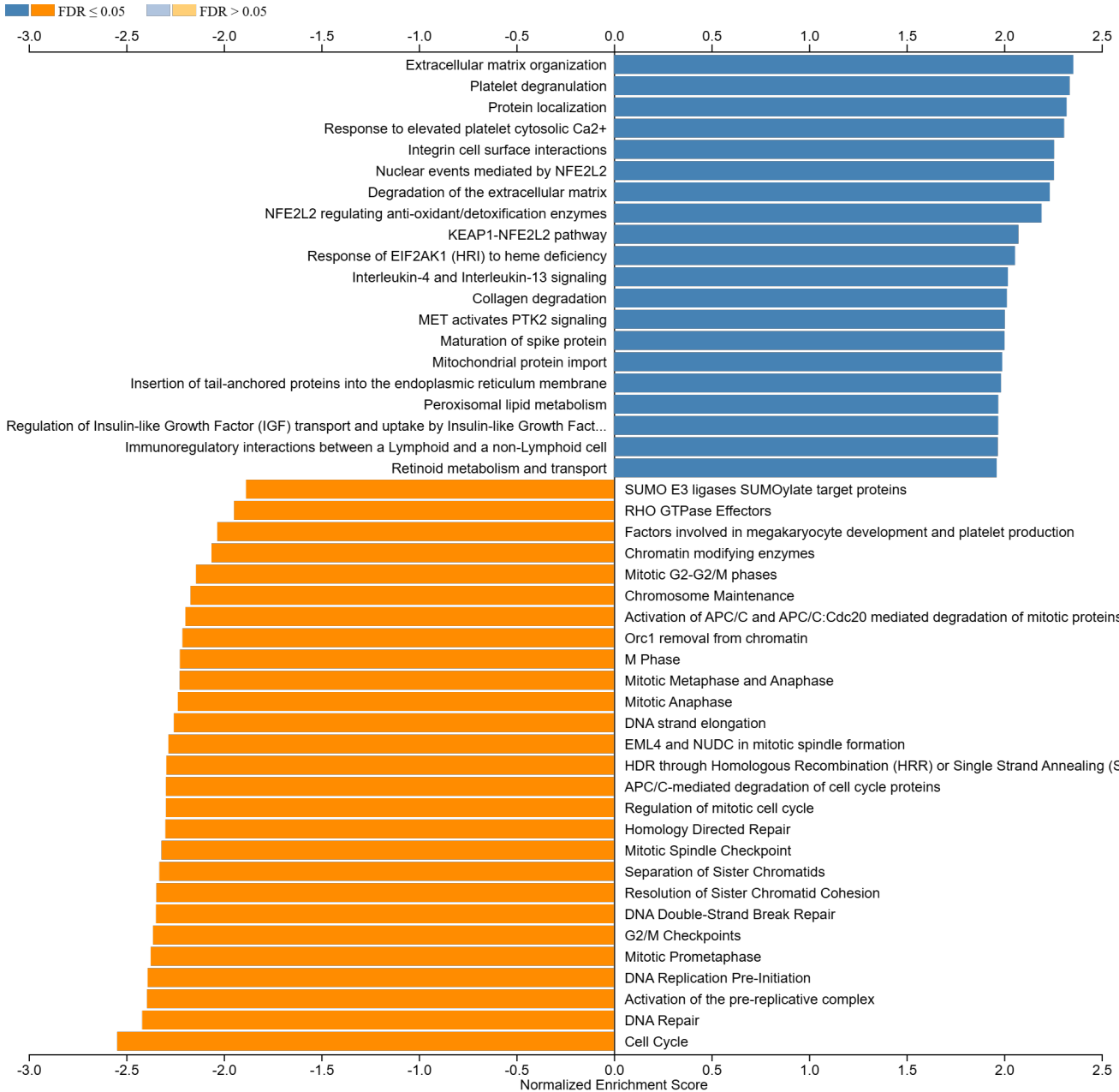
WT – HMPSNE

GSEA (Gene Set Enrichment Analysis) - geneontology_Cellular_Component



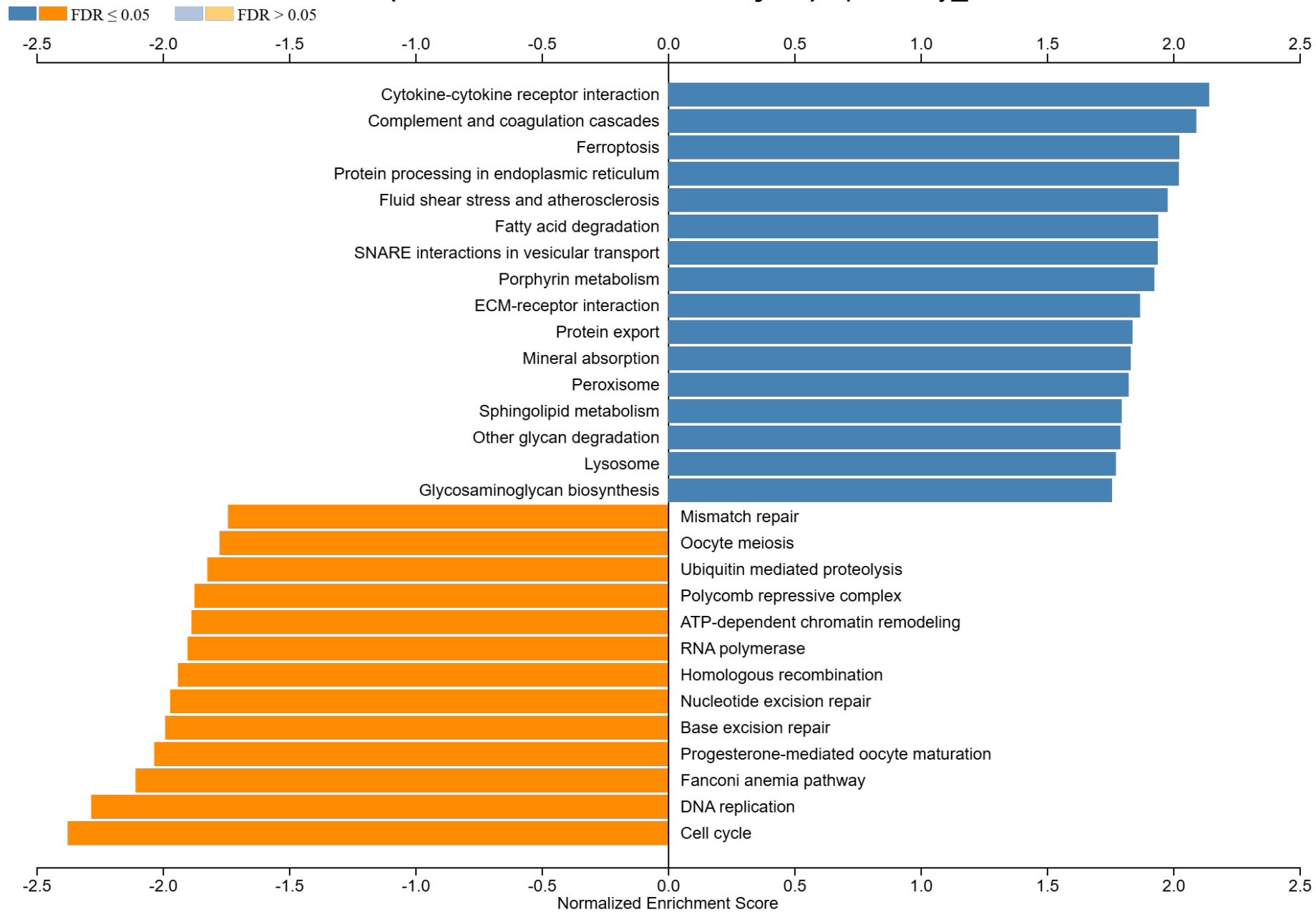
WT – HMPSNE

GSEA (Gene Set Enrichment Analysis) - pathway_Reactome



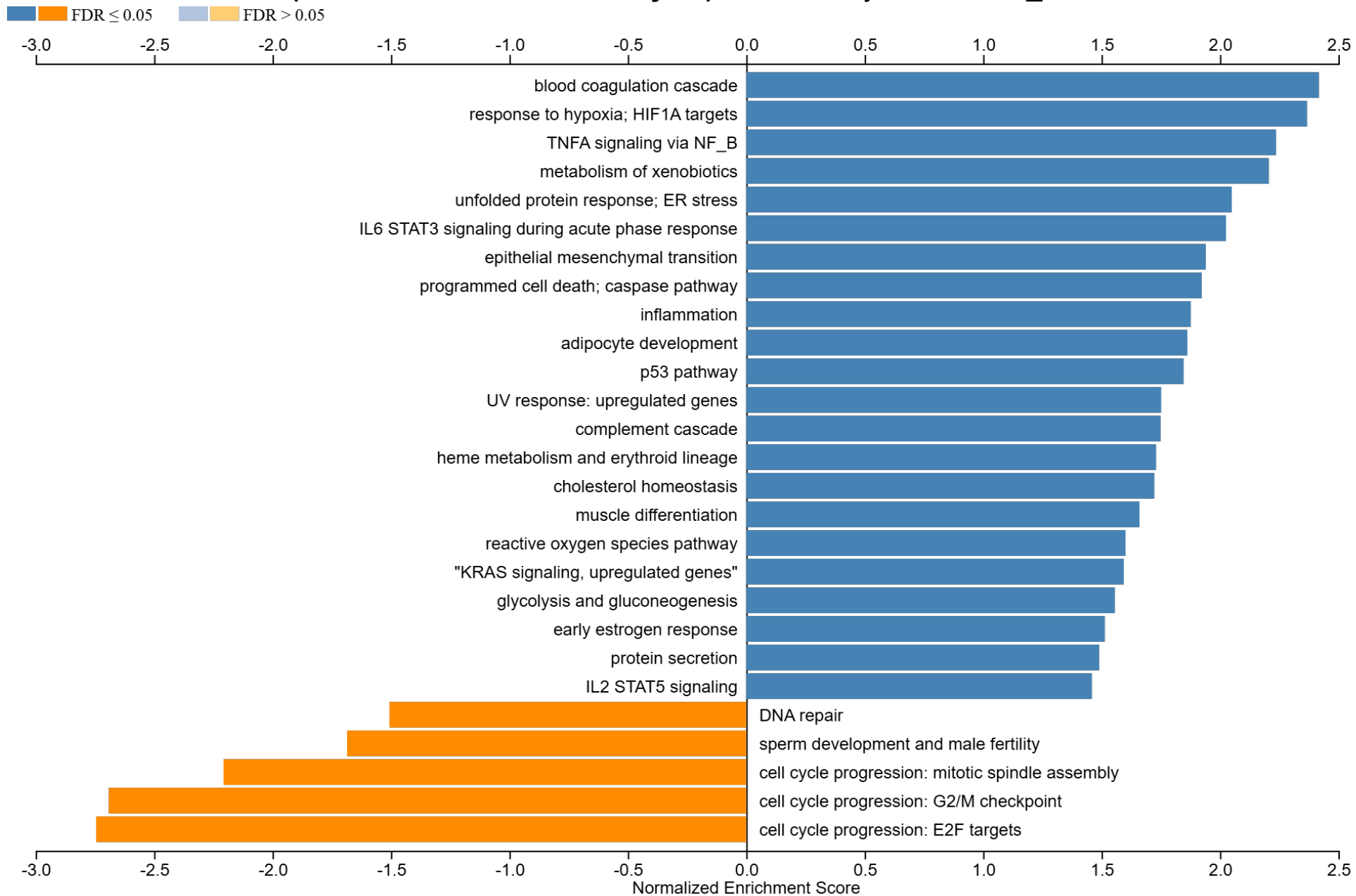
WT – HMPSNE

GSEA (Gene Set Enrichment Analysis) - pathway_KEGG



WT – HMPSNE

GSEA (Gene Set Enrichment Analysis) - community-contributed_Hallmark50

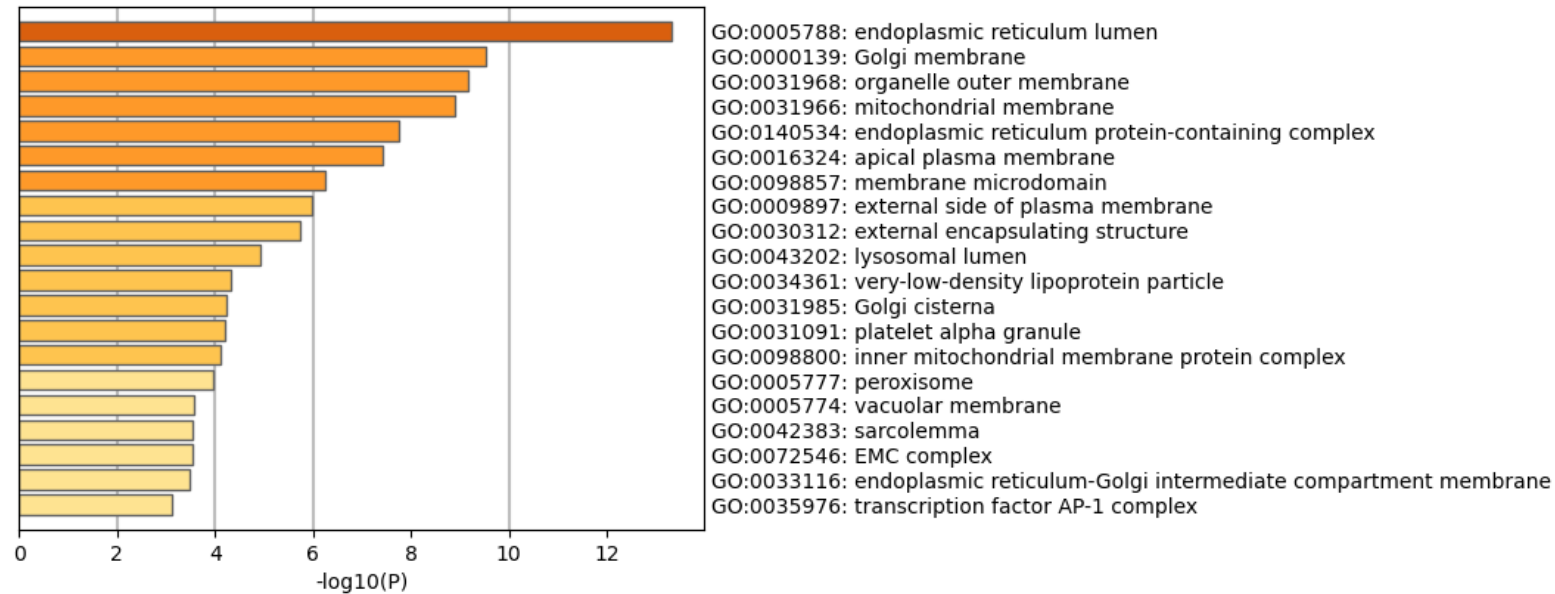


WT – HMPSNE

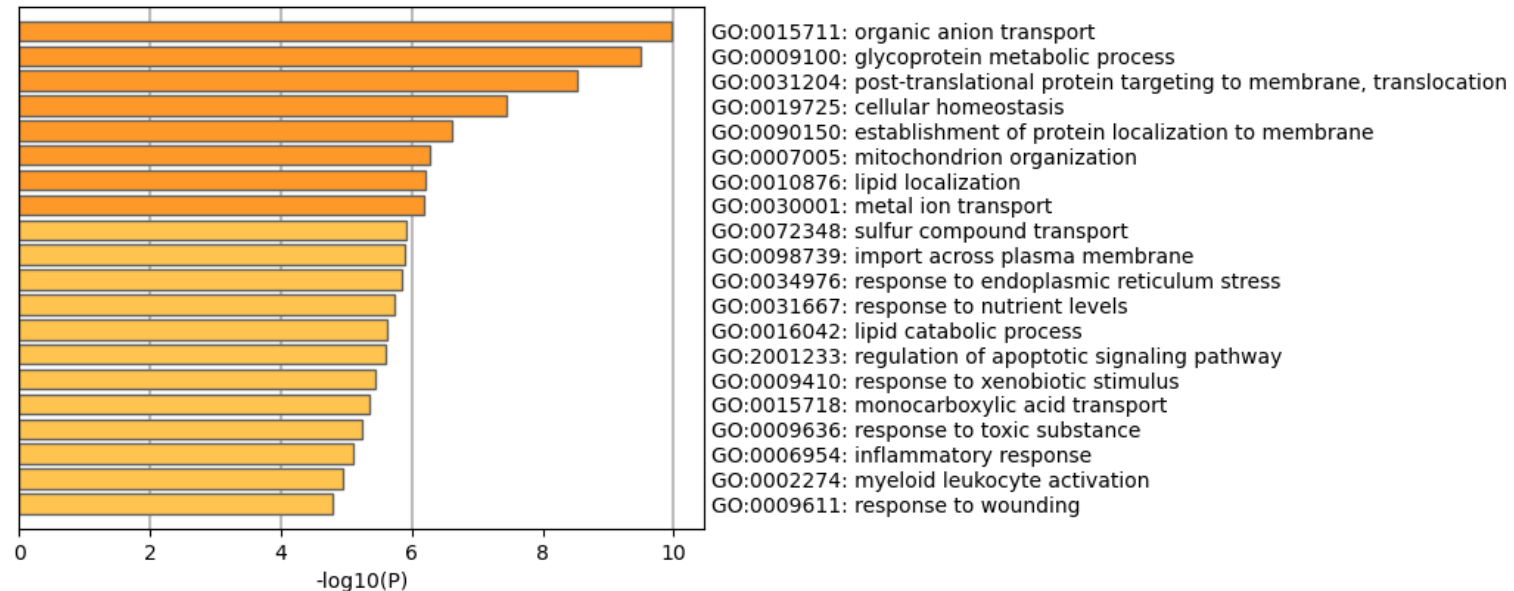
ORA (Over-Representation Analysis)

Activation in treated WT cells

GO Cellular Components



GO Biological Process

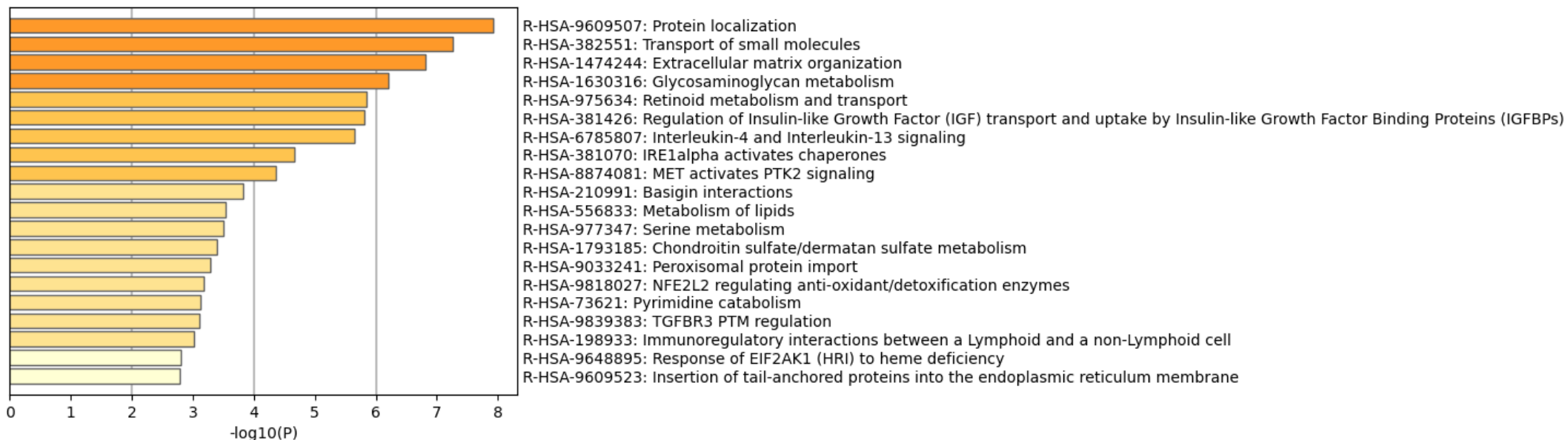


WT – HMPSNE

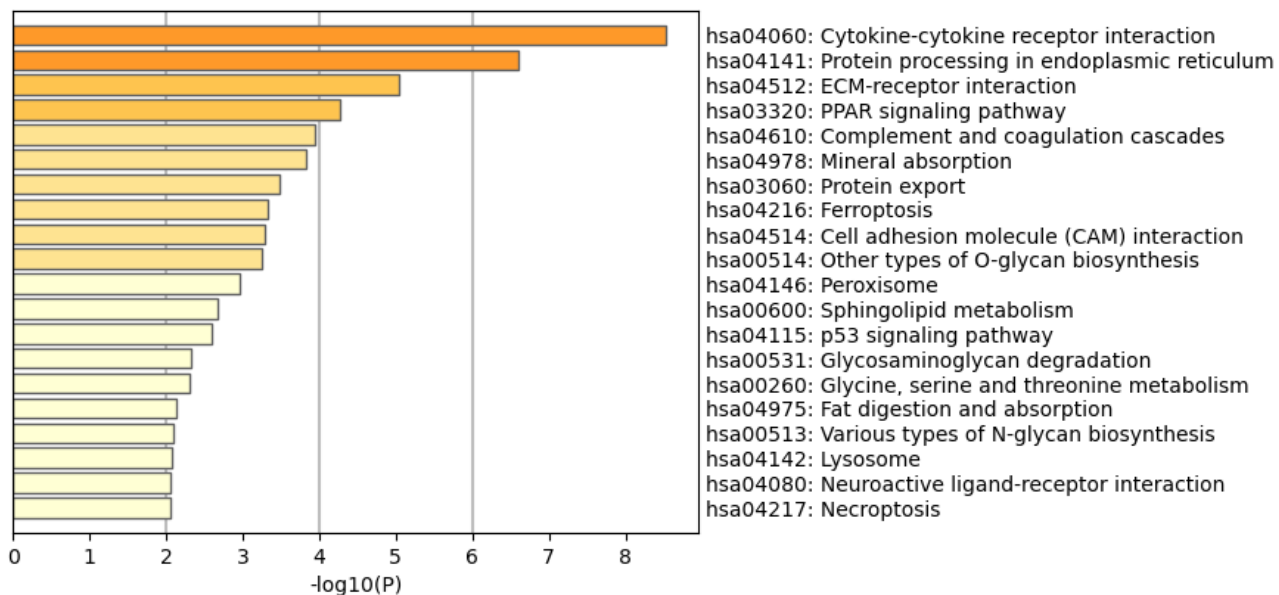
ORA (Over-Representation Analysis)

Activation in treated WT cells

Reactome Gene Sets



KEGG Pathway

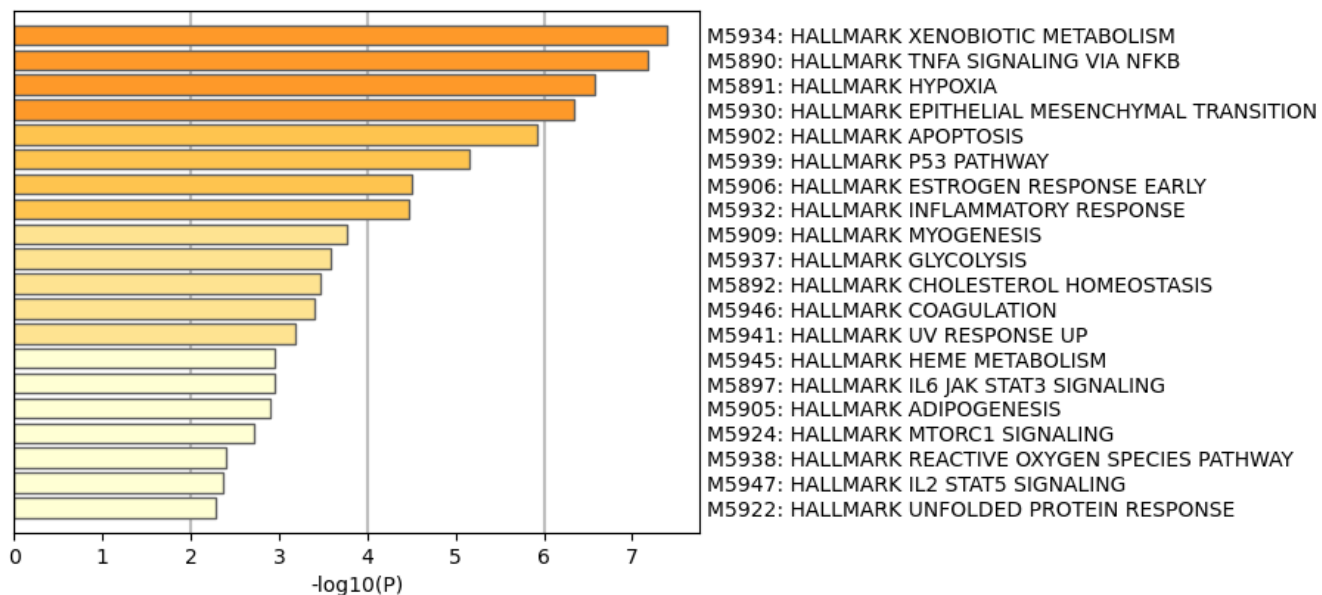


WT – HMPSNE

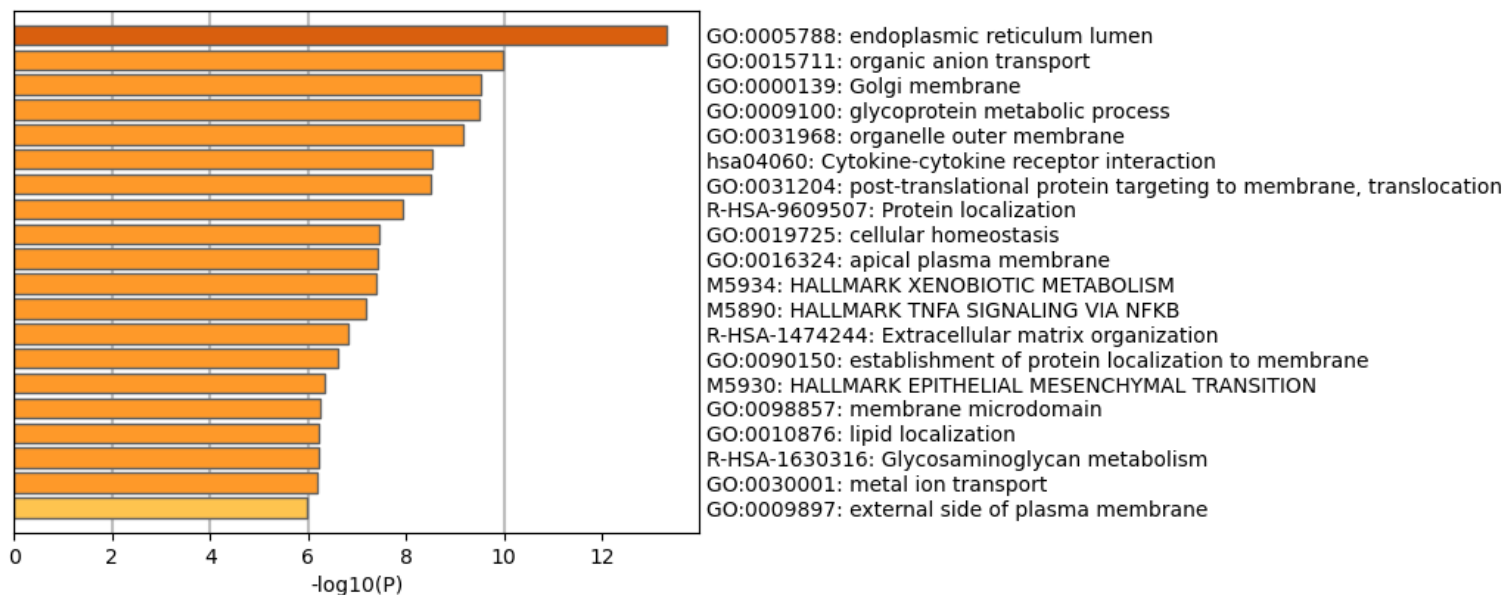
ORA (Over-Representation Analysis)

Activation in treated WT cells

Hallmark Gene Sets



Combined GO

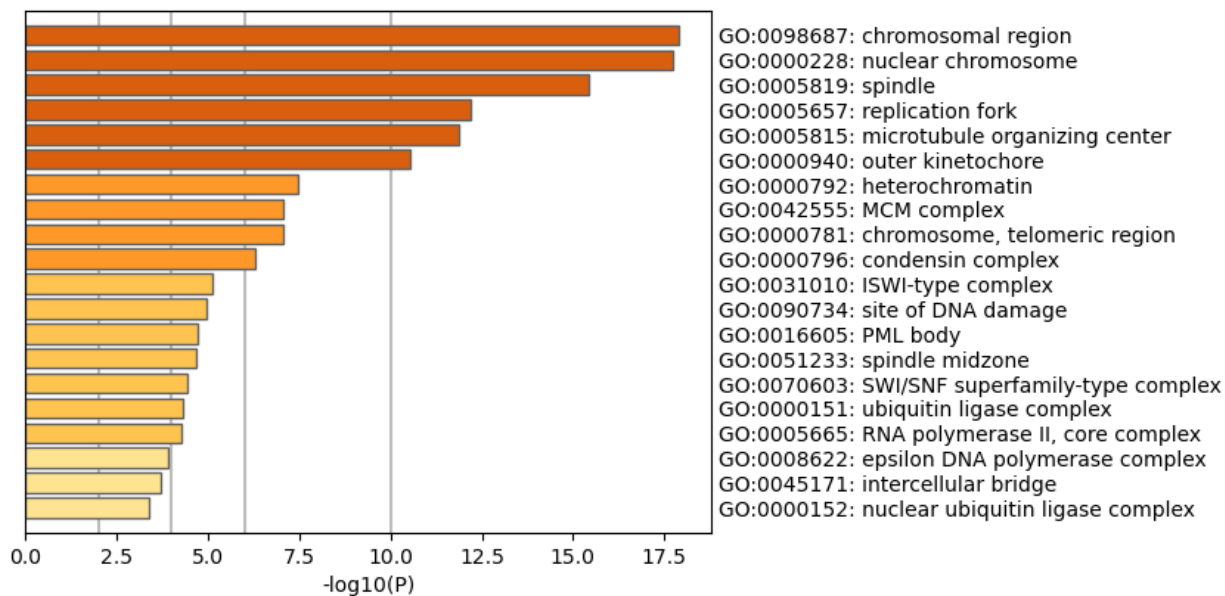


WT – HMPSNE

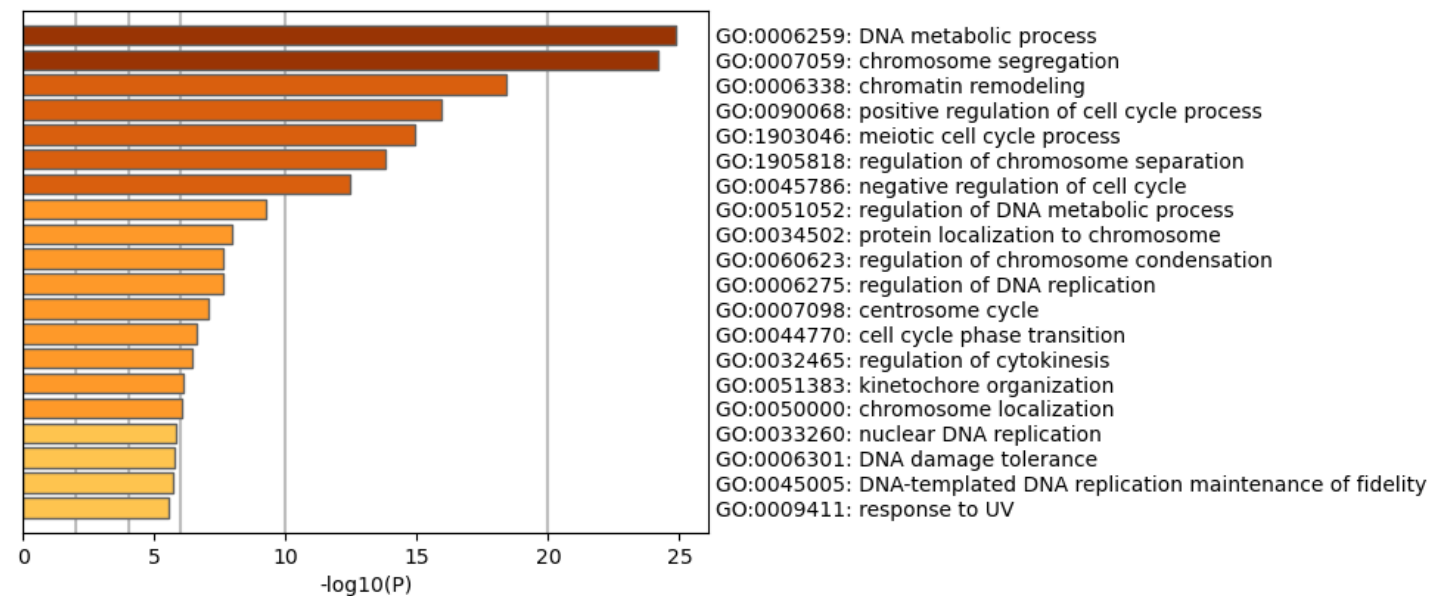
ORA (Over-Representation Analysis)

Inhibition in treated WT cells

GO Cellular Components



GO Biological Process

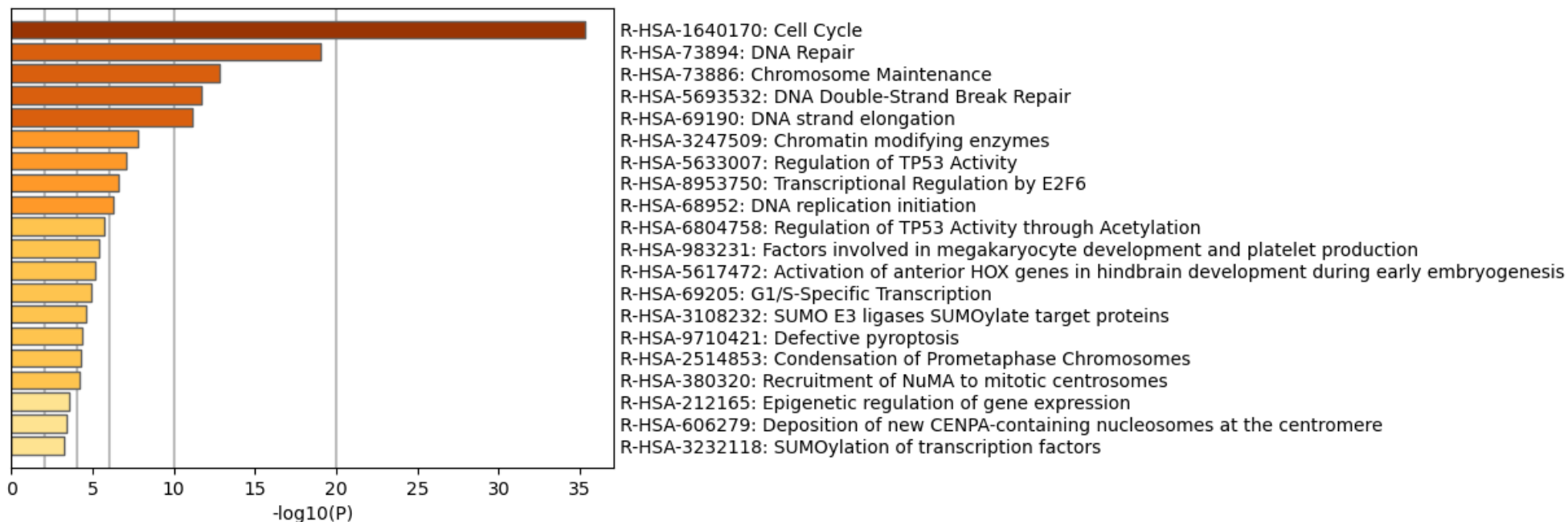


WT – HMPSNE

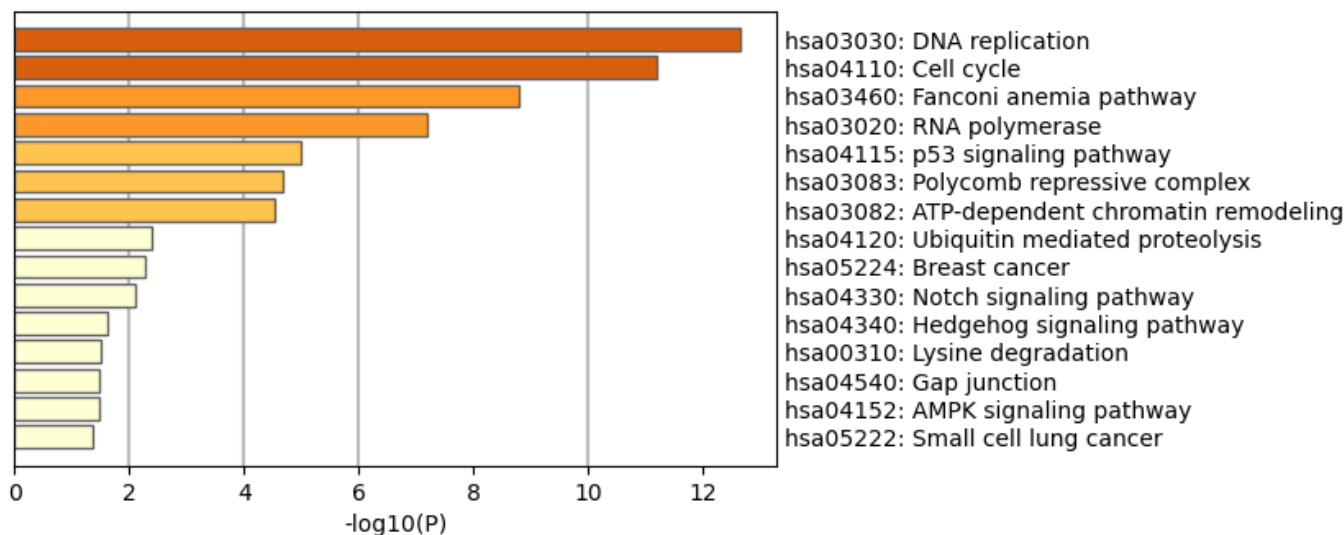
ORA (Over-Representation Analysis)

Inhibition in treated WT cells

Reactome Gene Sets



KEGG Pathway

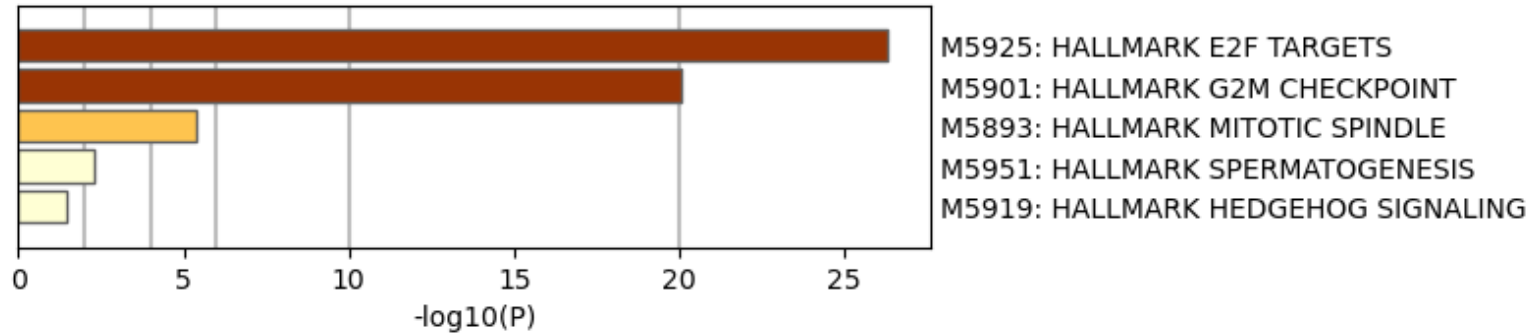


WT – HMPSNE

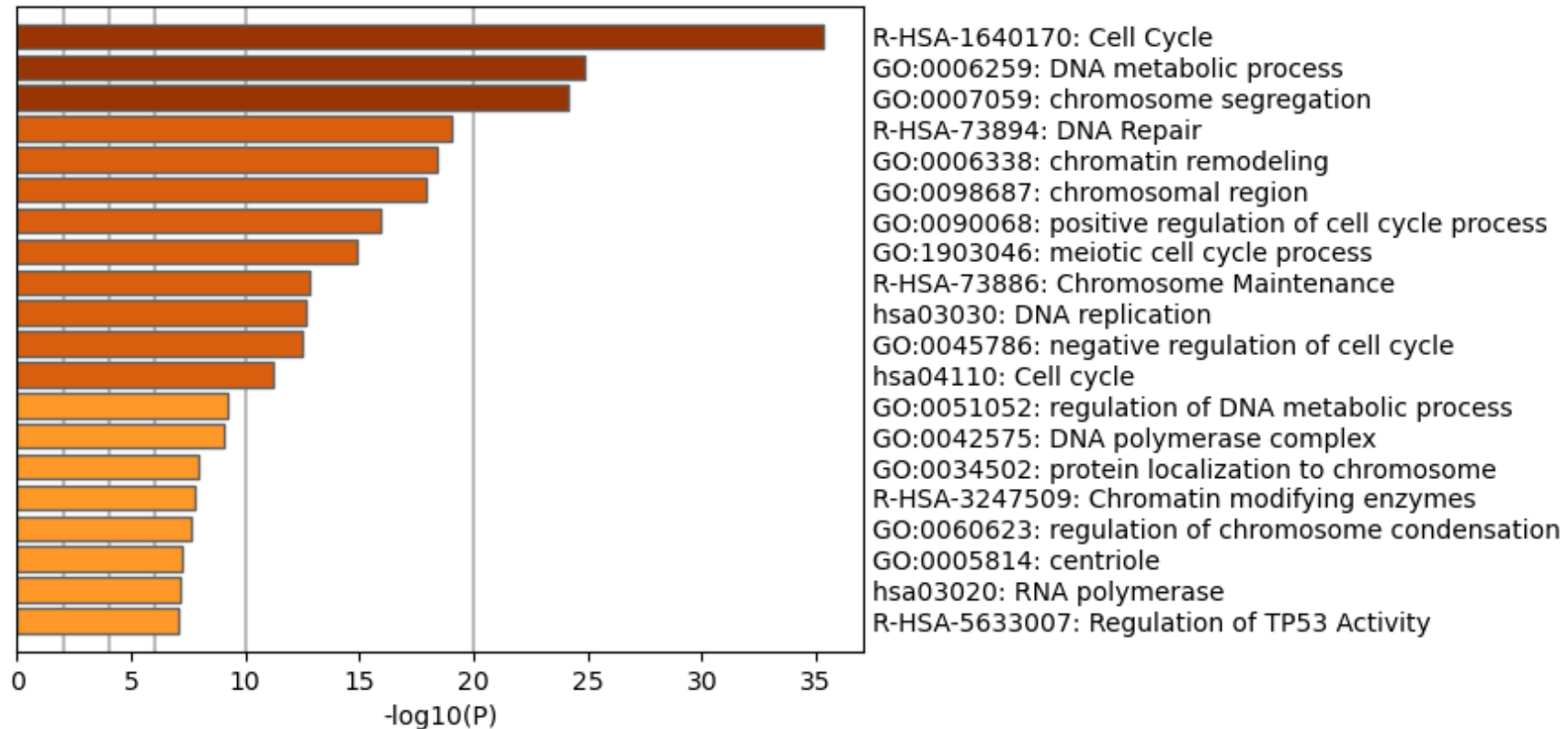
ORA (Over-Representation Analysis)

Inhibition in treated WT cells

Hallmark Gene Sets

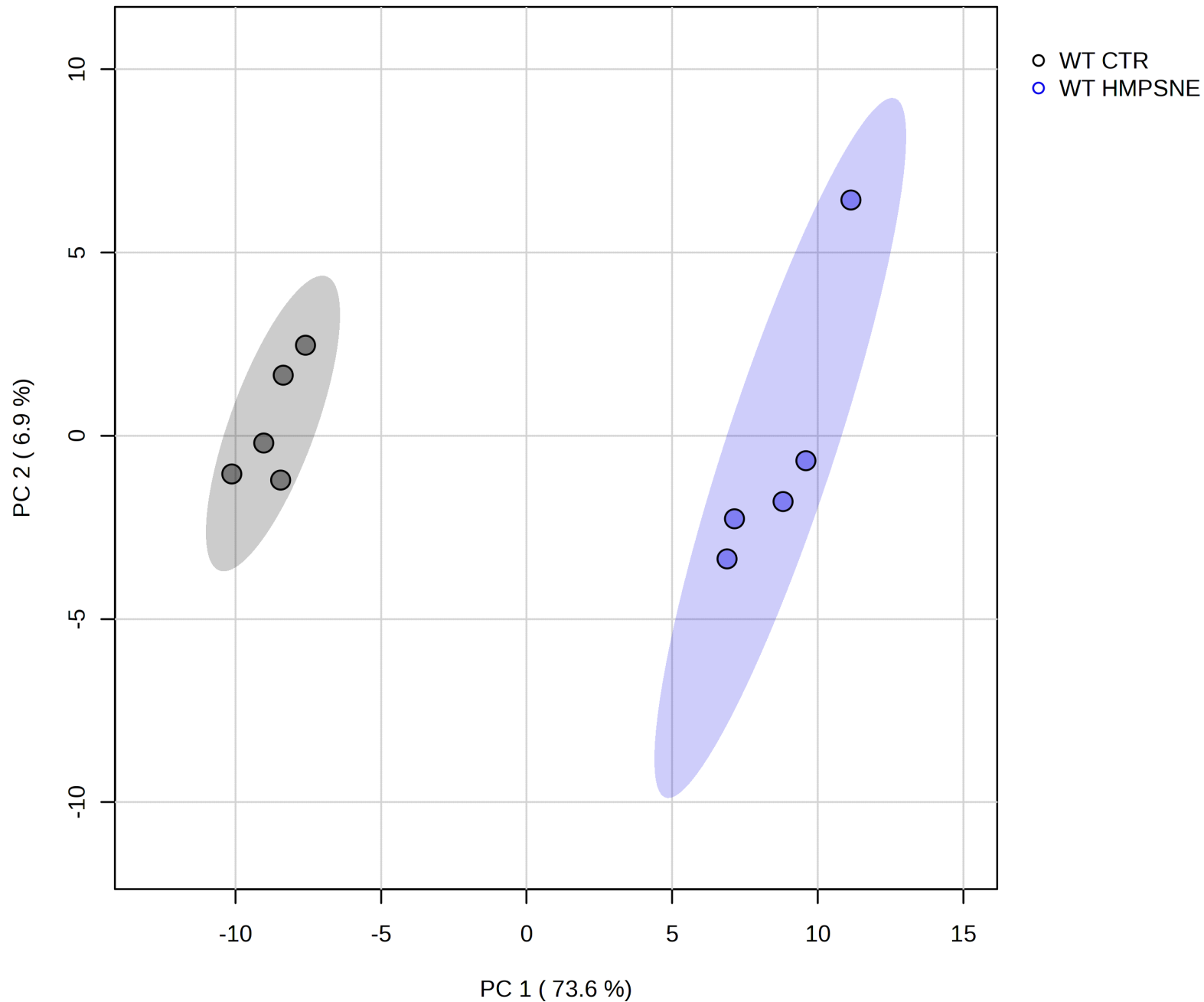


Combined GO



WT-HMPSNE vs WT-CTR Metabolomics

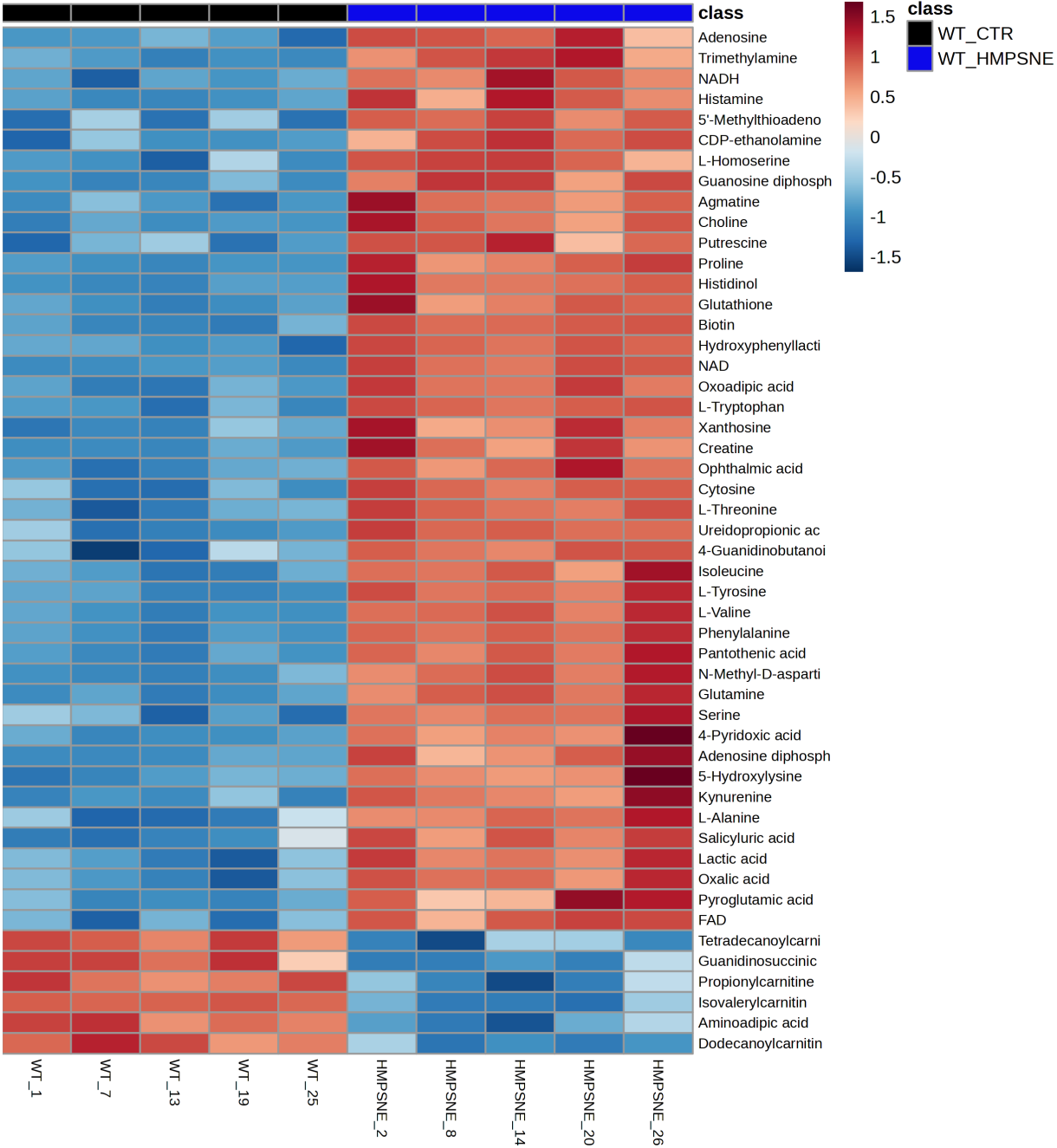
WT – HMPSNE



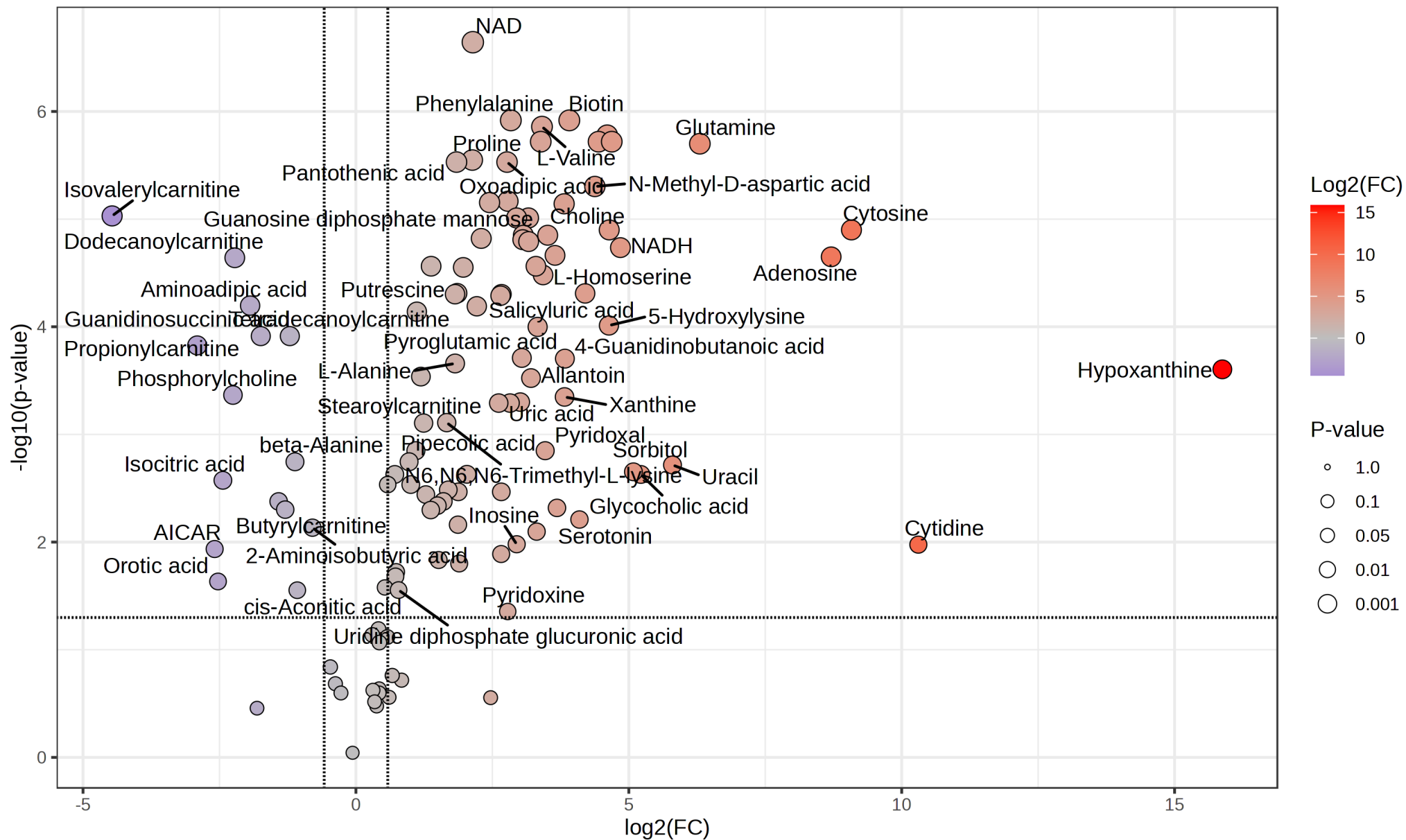
[PERMANOVA] F-value: 69.204; R-squared: 0.89638; p-value (based on 999 permutations): 0.011

The statistical significances of the group patterns are evaluated using PERMANOVA. Please note the distributions are computed using the Euclidean distance based on the PCs in the current display.

WT – HMPSNE



WT – HMPSNE

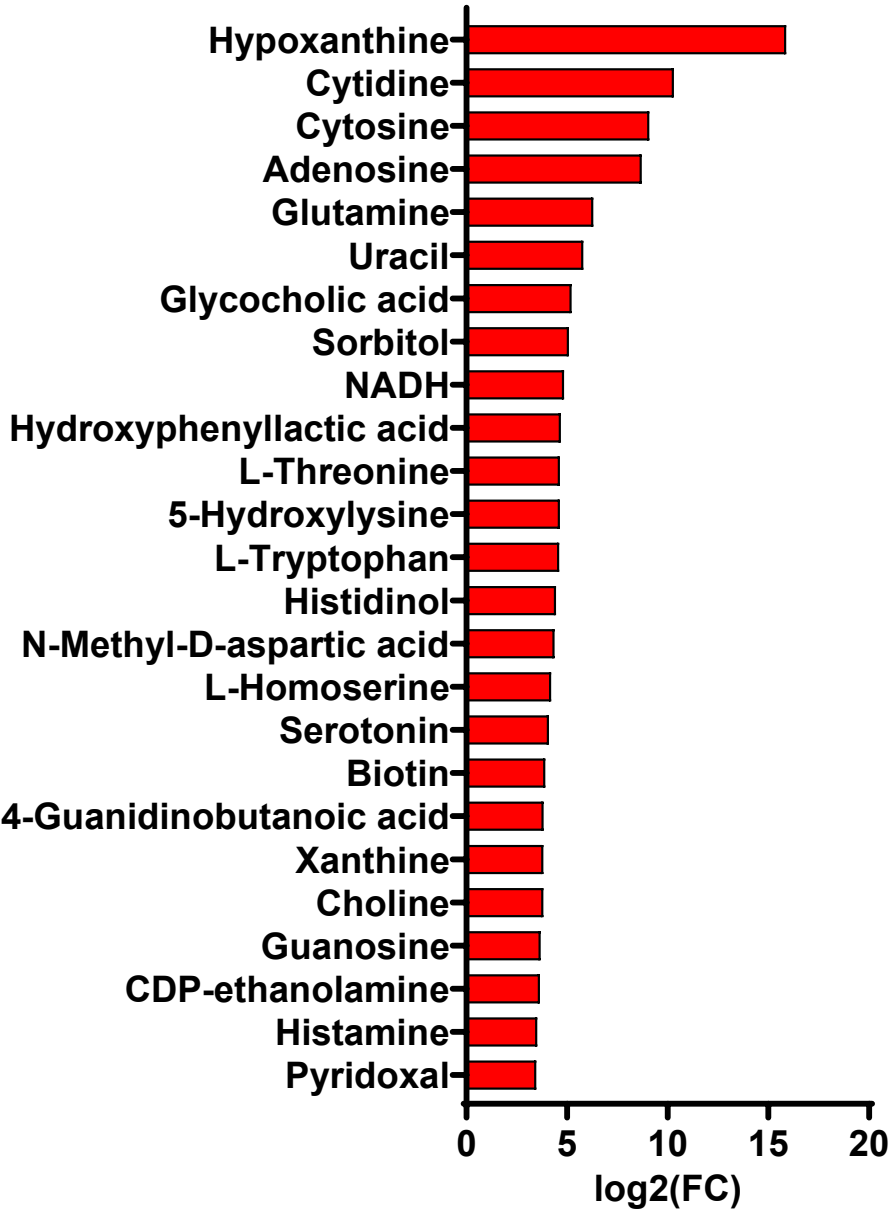


Fold change (FC) threshold: 1.5

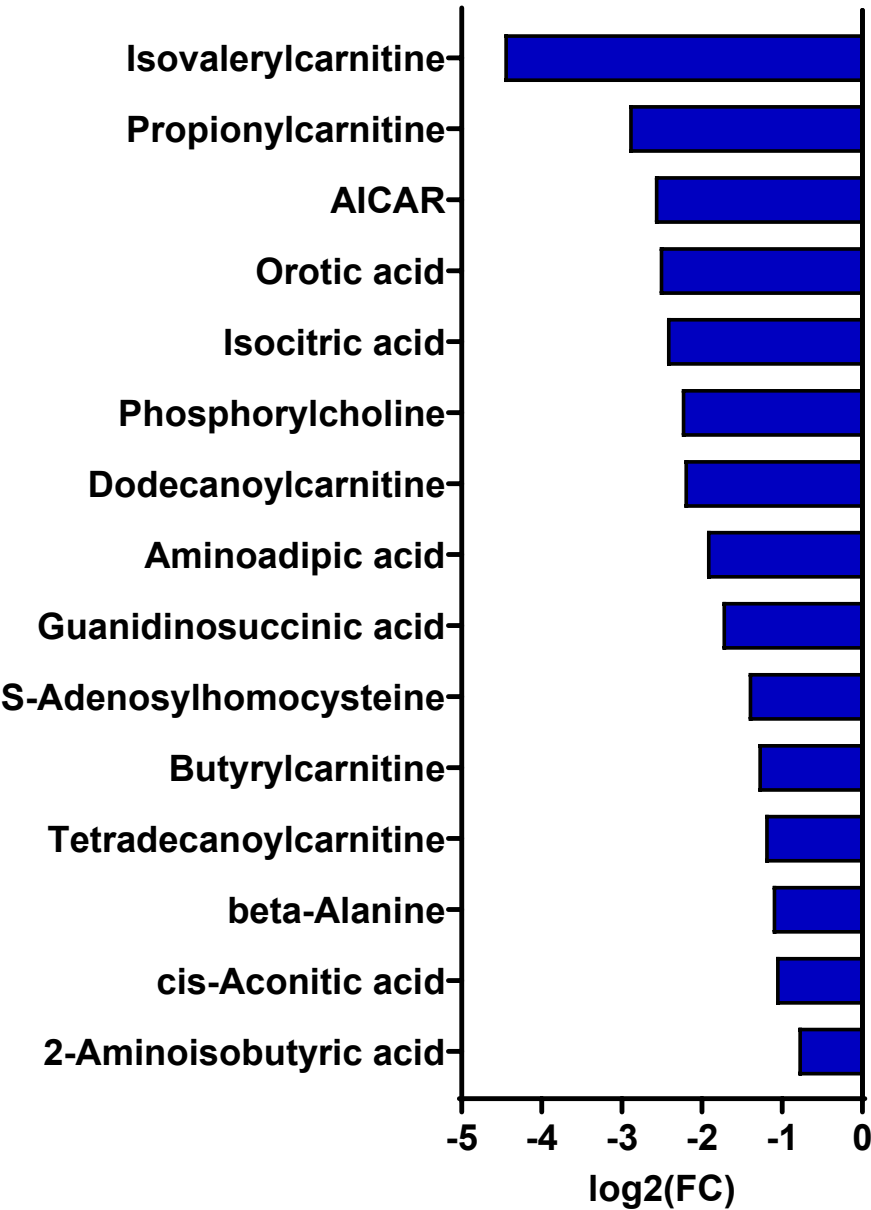
P-value threshold: 0.05

WT – HMPSNE

Top 25_Increased metabolites in treated cells



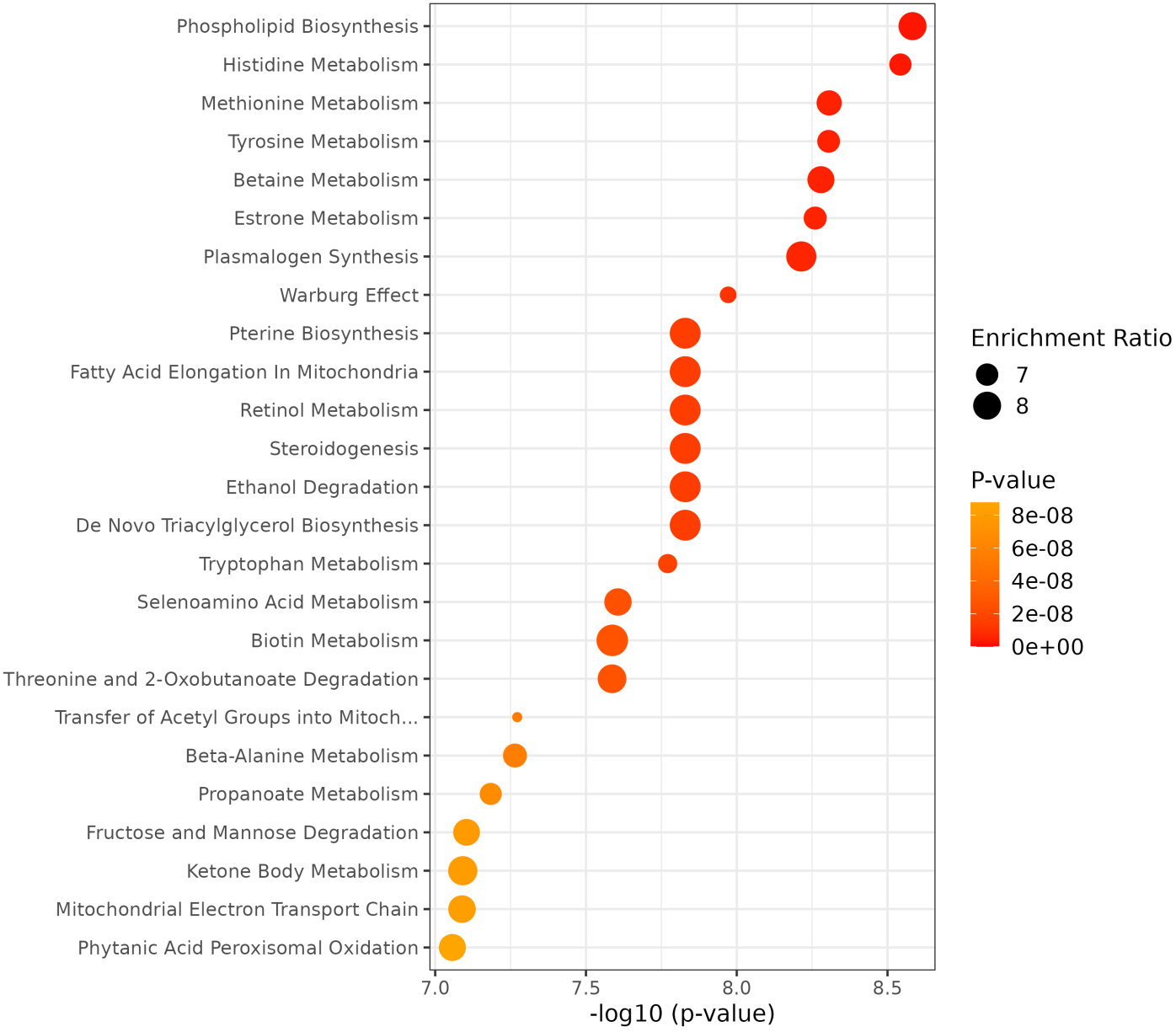
Top 25_Decreased metabolites in treated cells



WT – HMPSNE

Quantitative Enrichment Analysis (QEA)

Overview of Enriched Metabolite Sets (Top 25)

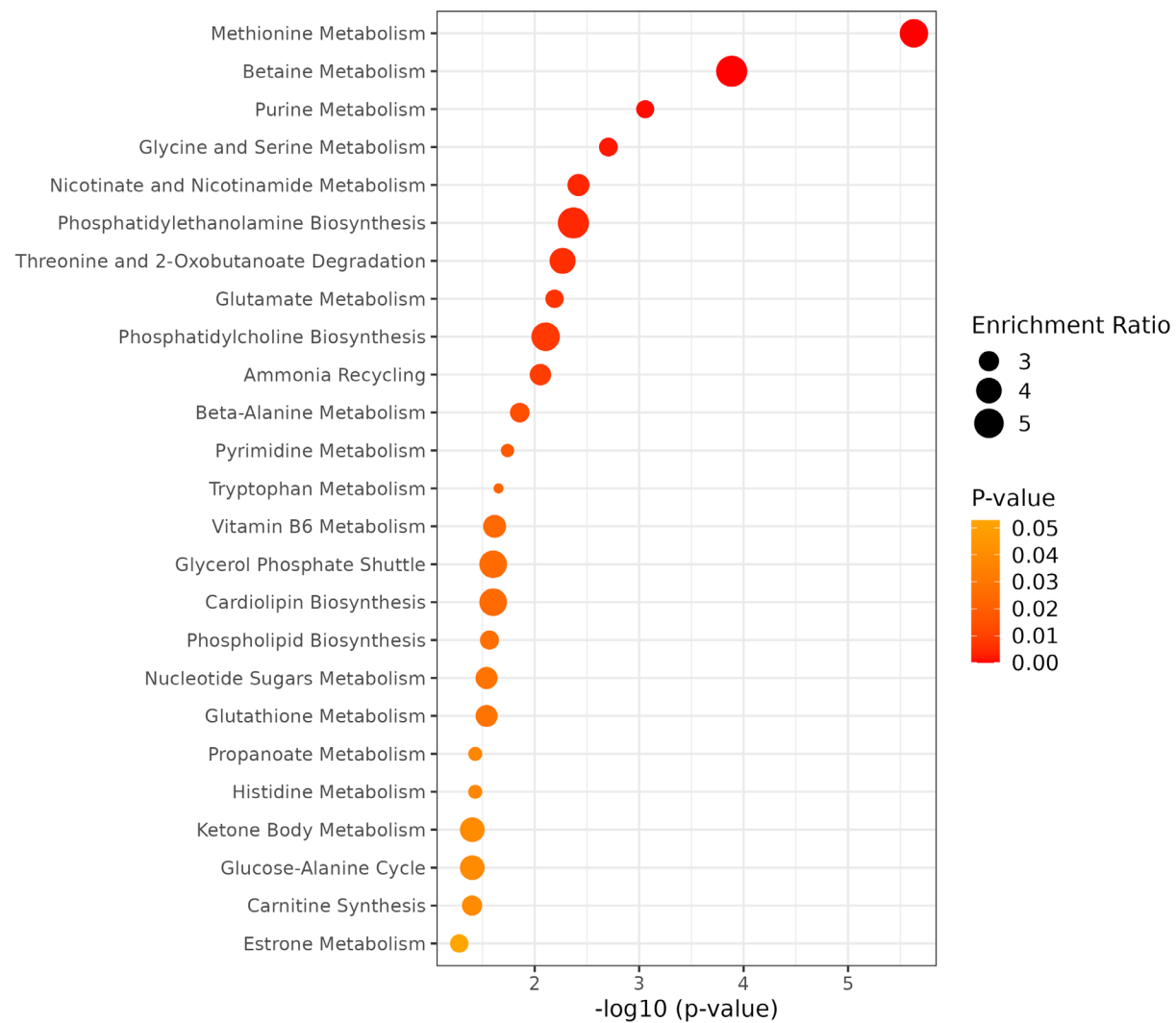


WT – HMPSNE

Over-Representation Analysis (ORA)

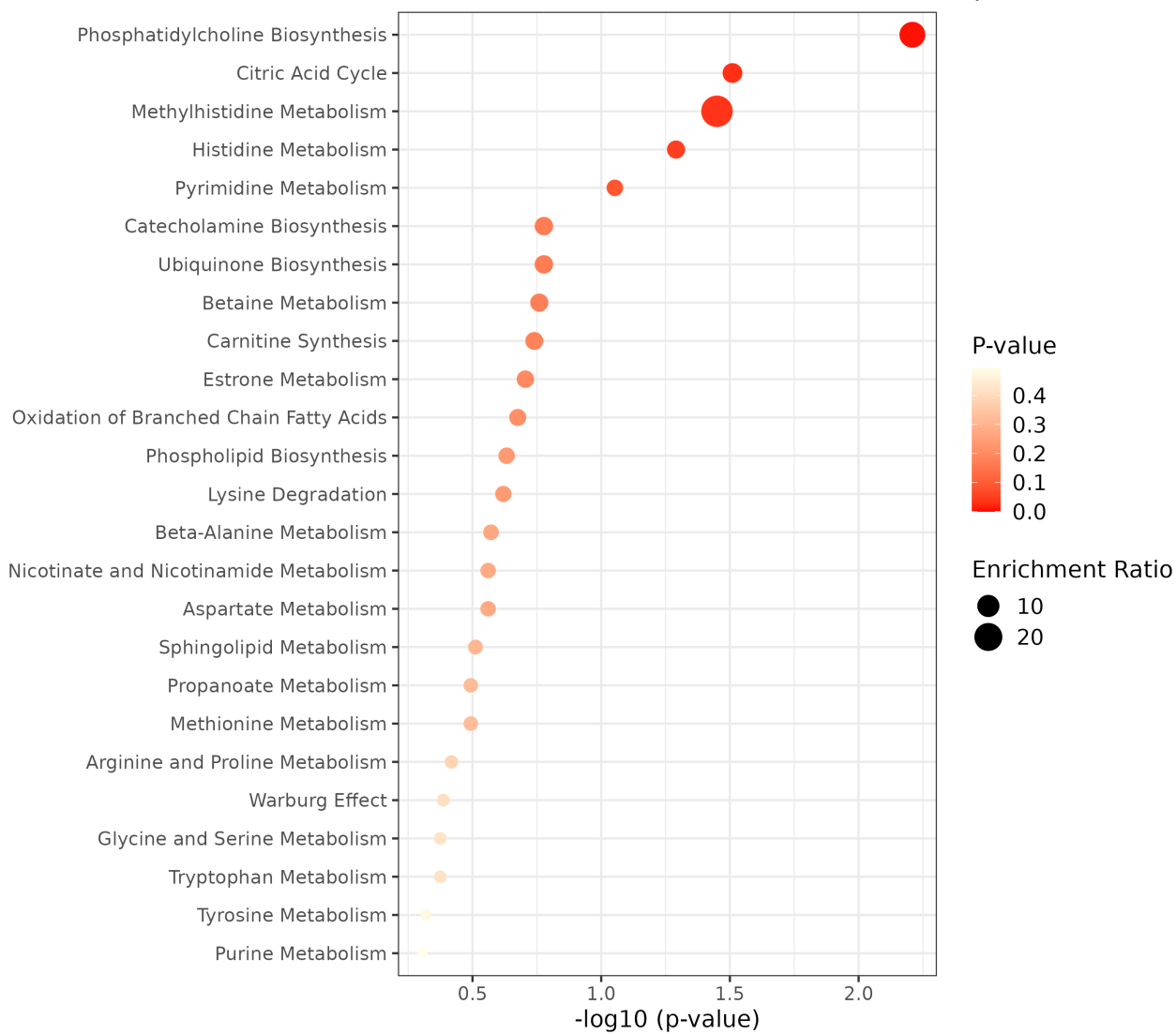
Activation in treated cells

Overview of Enriched Metabolite Sets (Top 25)



Inhibition in treated cells

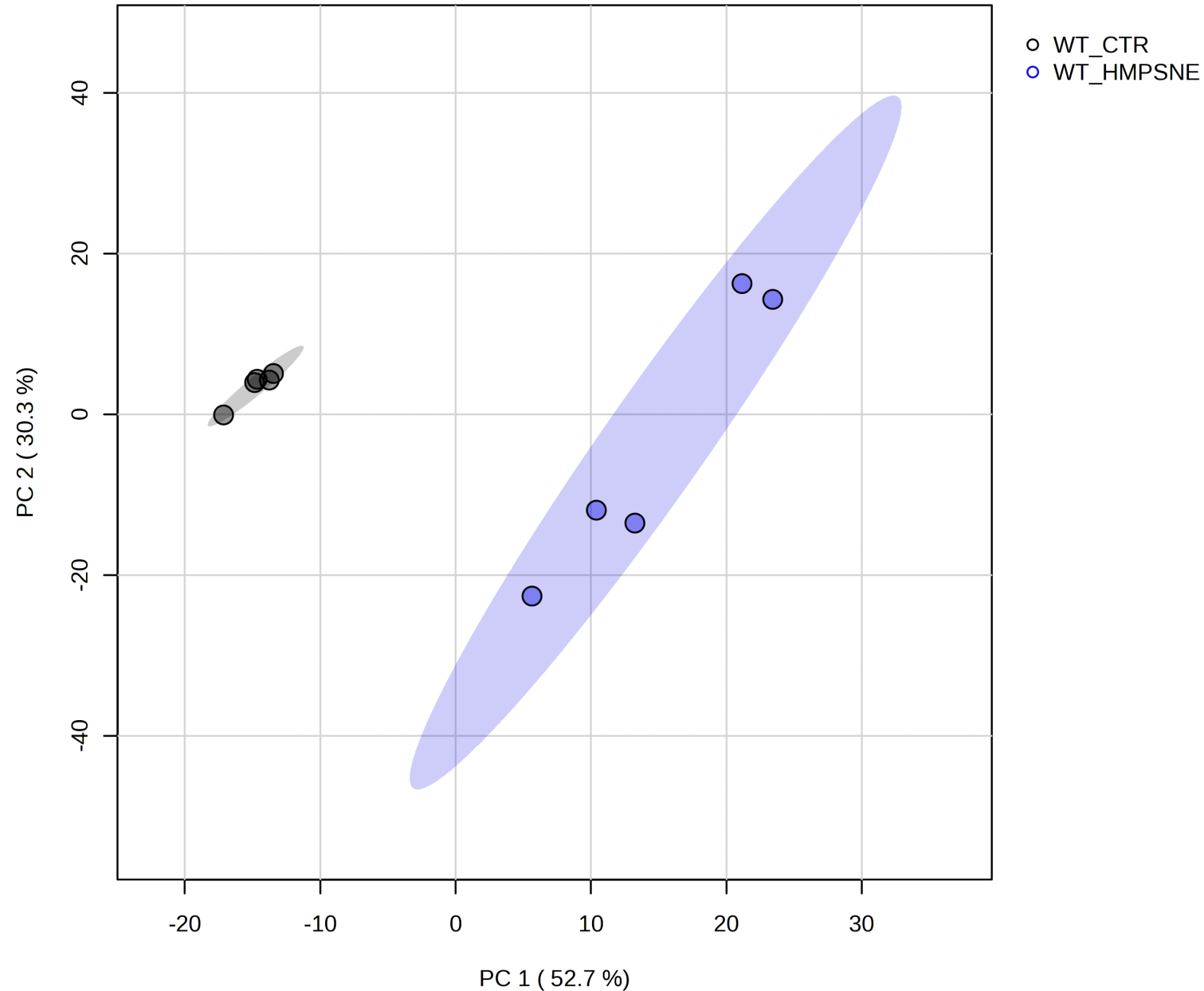
Overview of Enriched Metabolite Sets (Top 25)



WT-HMPSNE vs WT-CTR

Lipidomics

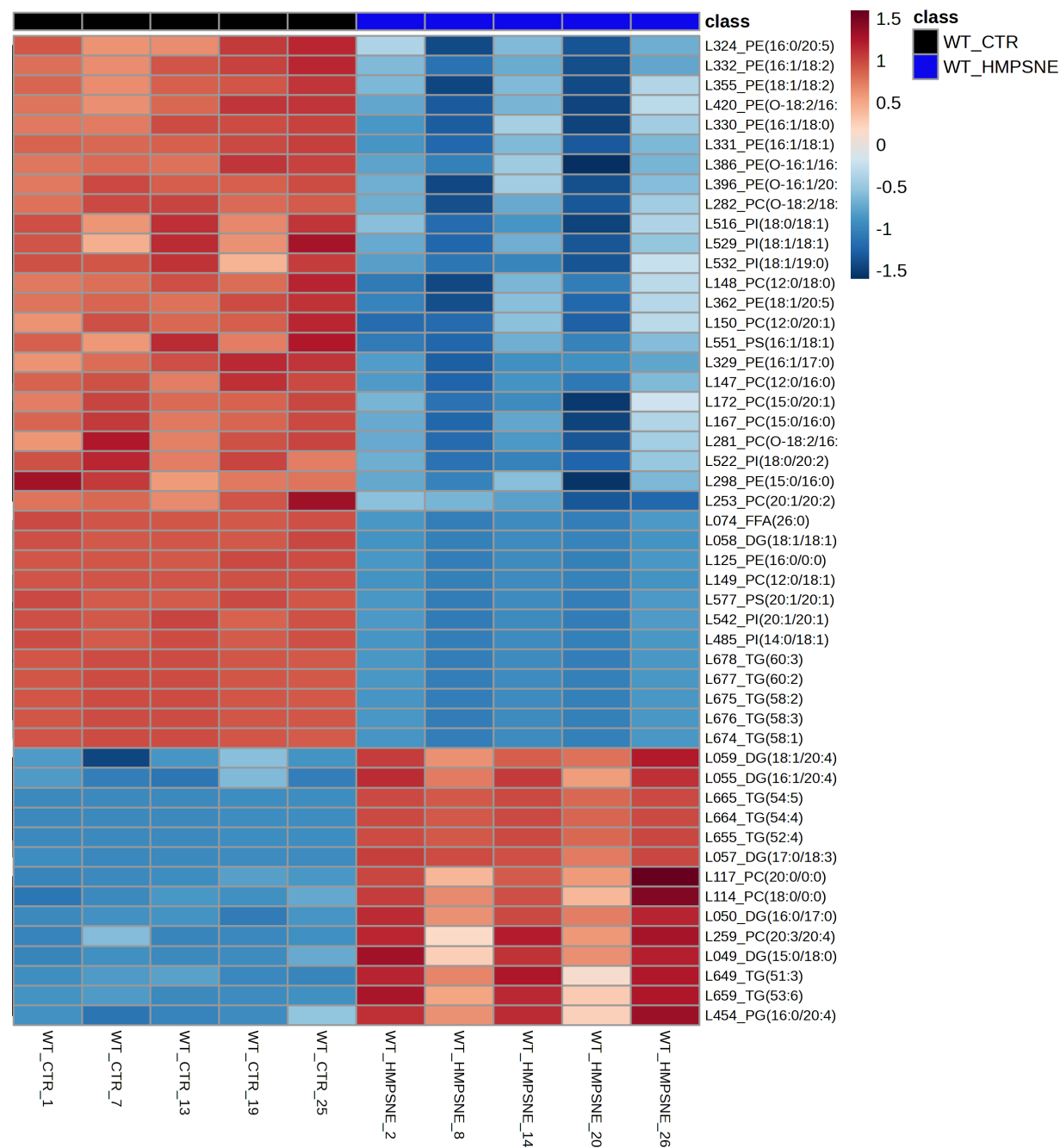
WT – HMPSNE



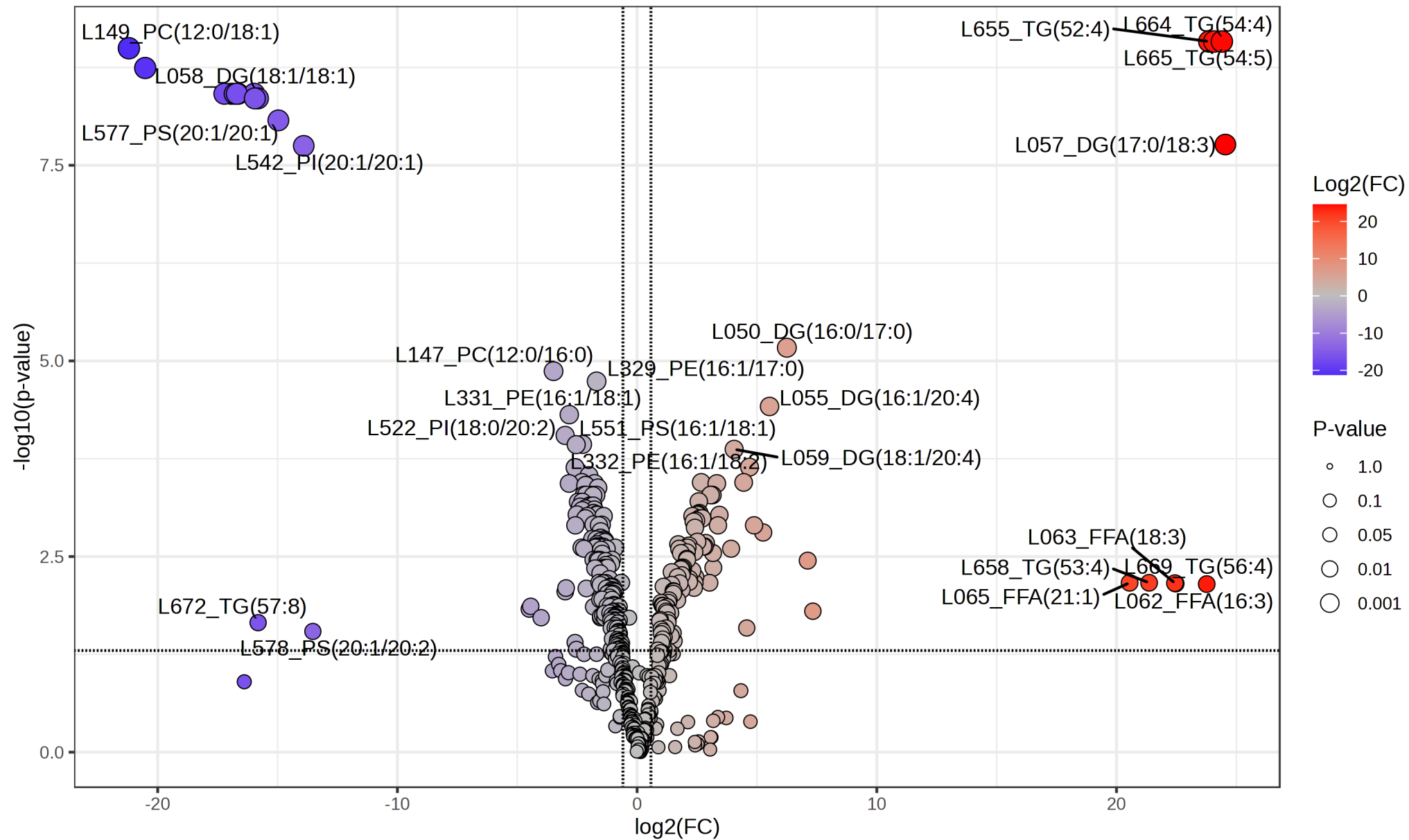
[PERMANOVA] F-value: 12.37; R-squared: 0.60726; p-value (based on 999 permutations): 0.012

The statistical significances of the group patterns are evaluated using PERMANOVA. Please note the distributions are computed using the Euclidean distance based on the PCs in the current display.

WT – HMPSNE



WT – HMPSNE

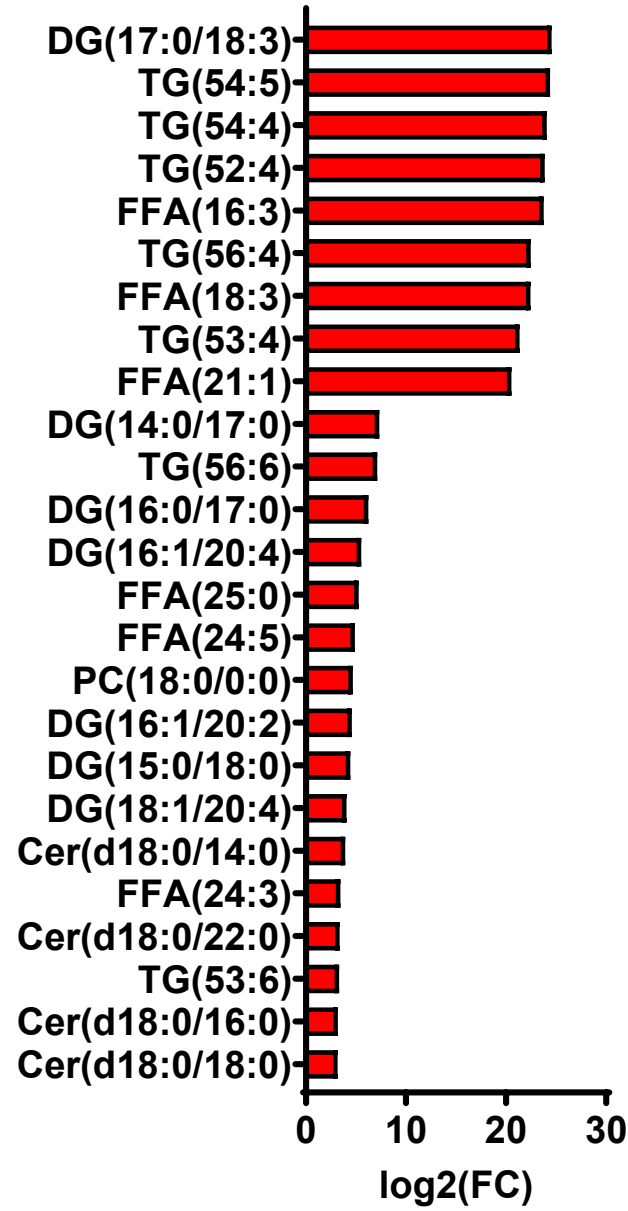


Fold change (FC) threshold: 1.5

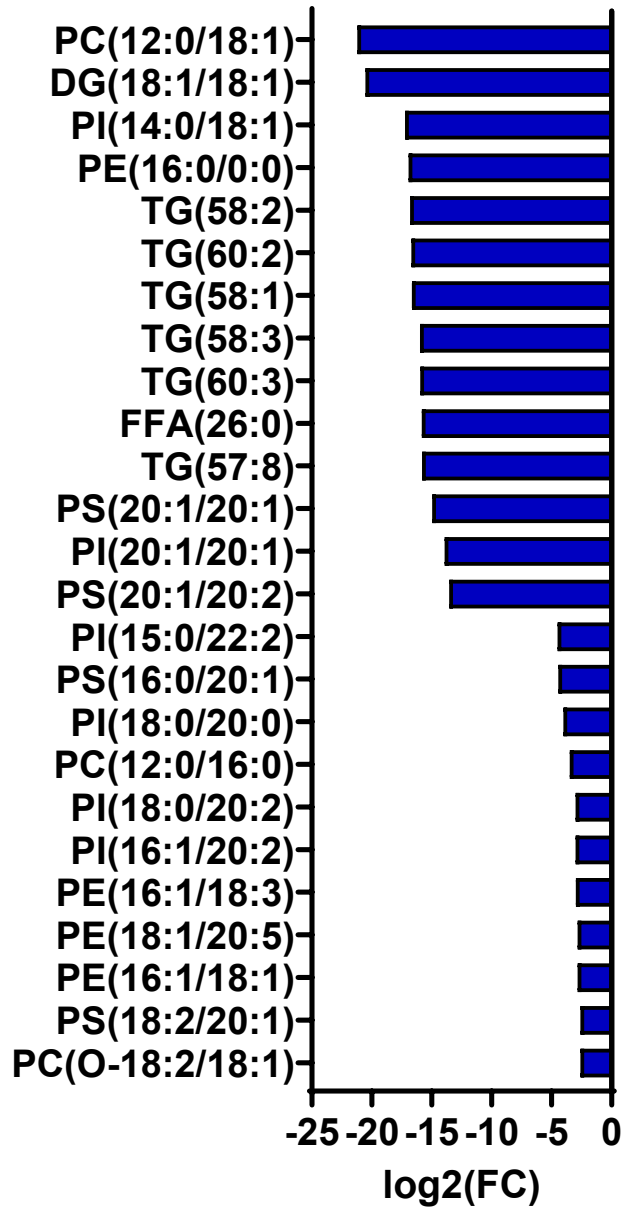
P-value threshold: 0.05

WT – HMPSNE

Top 25_Increased lipids in treated WT cells



Top 25_Decreased lipids in treated WT cells

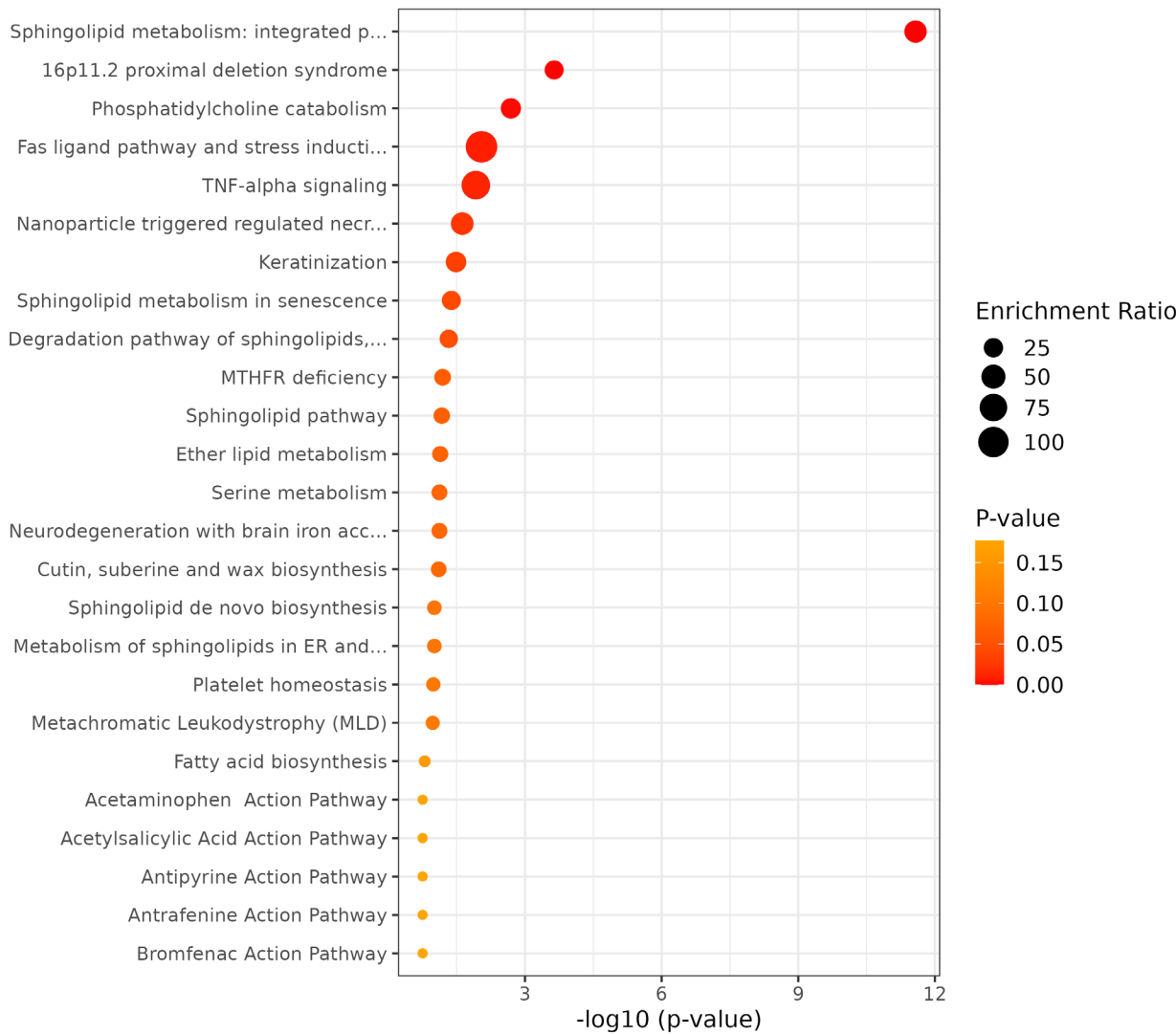


WT – HMPSNE

Over-Representation Analysis (ORA)

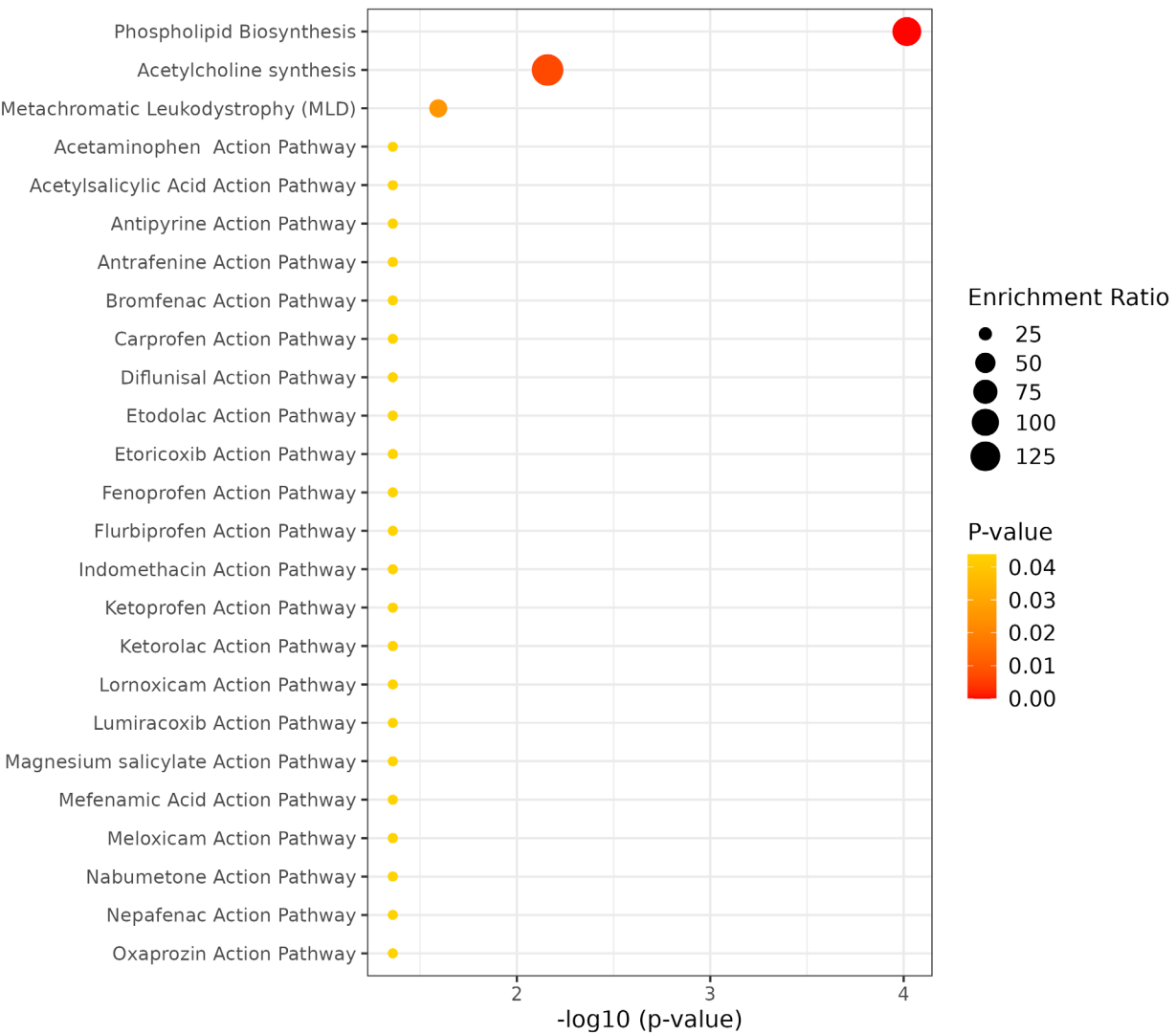
Activation in treated WT cells

Overview of Enriched Metabolite Sets (Top 25)



Inhibition in treated WT cells

Overview of Enriched Metabolite Sets (Top 25)



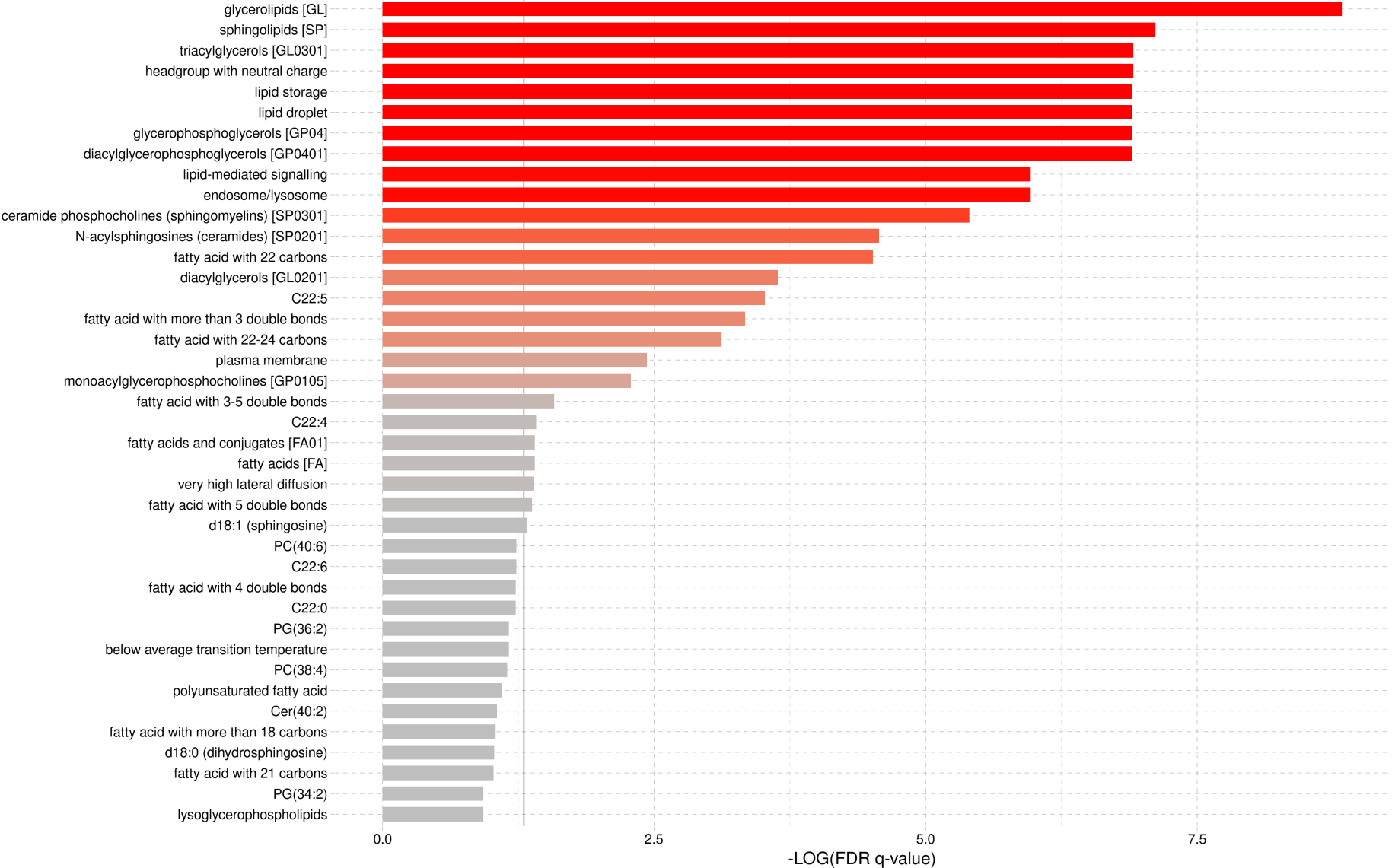
WT – HMPSNE

LION enrichment analysis – *ranking mode*

Increased in treated WT cells

LION enrichment analysis *ranking mode*

WT_HMPSNE vs. WT_CTR



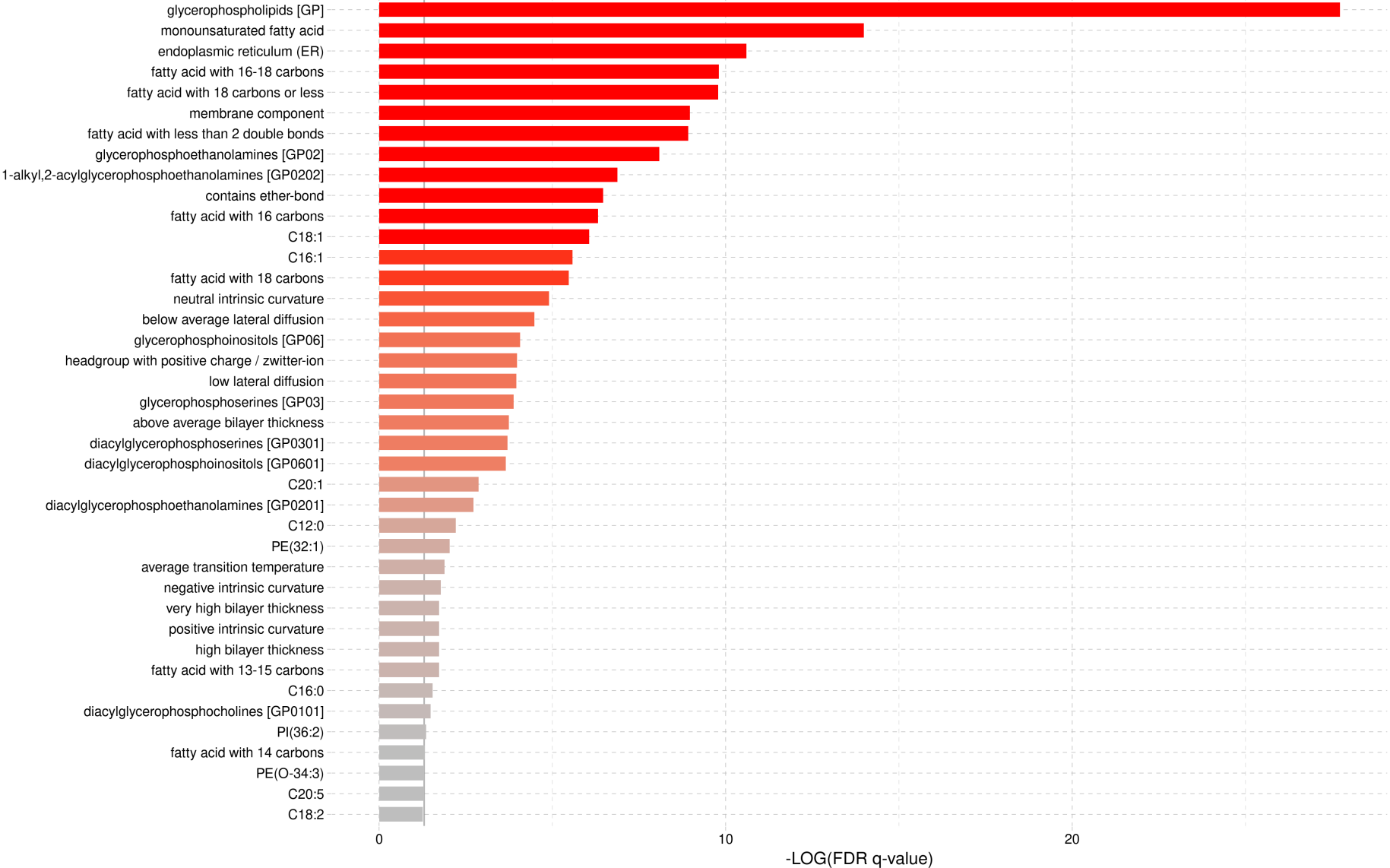
WT – HMPSNE

LION enrichment analysis – *ranking mode*

Decreased in treated WT cells

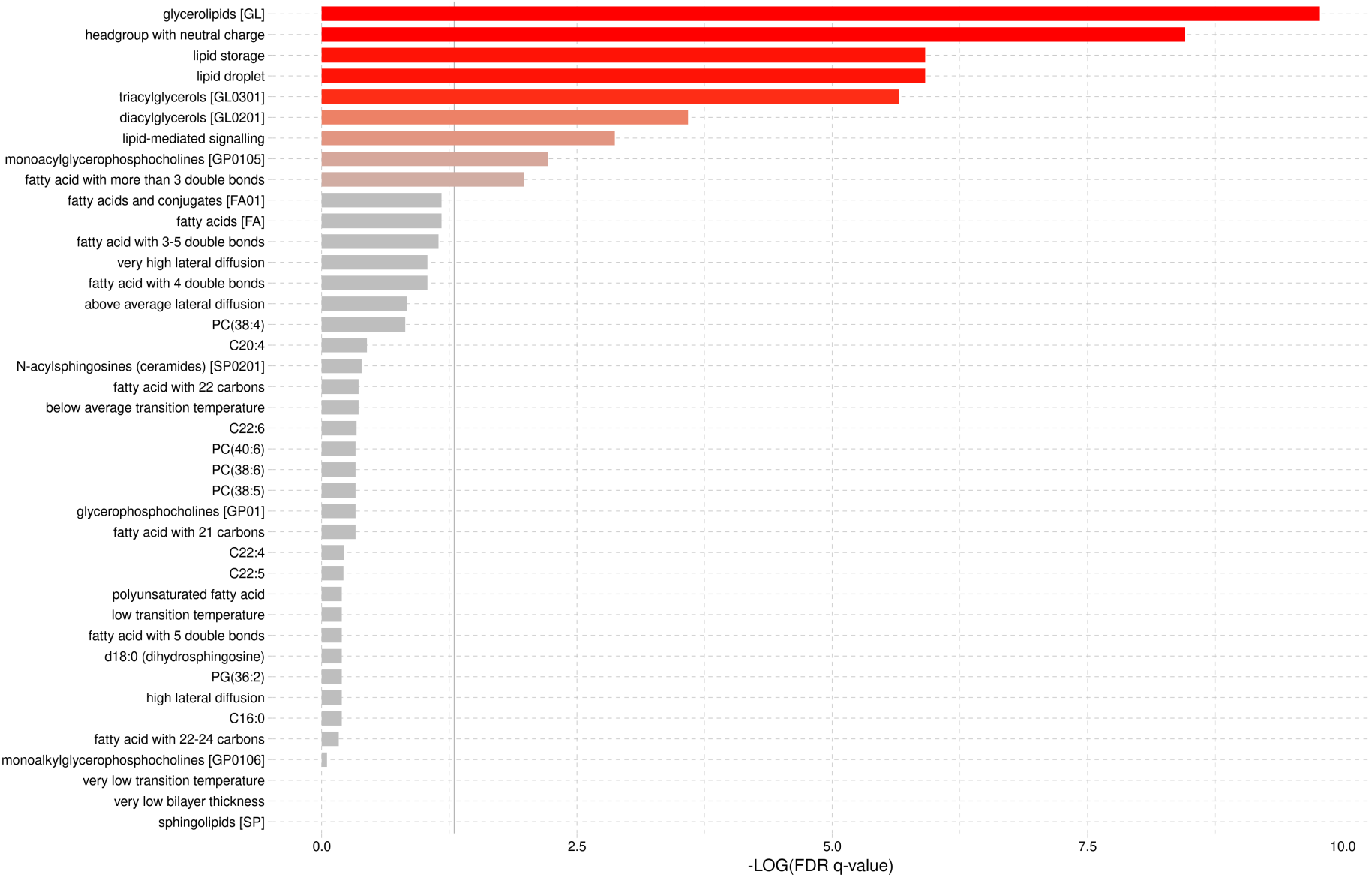
LION enrichment analysis *ranking mode*

WT_CTRL vs. WT_HMPSNE



WT – HMPSNE
LION enrichment analysis - *target-list mode*
Increased in treated WT cells

LION enrichment analysis *target-list mode*



WT – HMPSNE

LION enrichment analysis - *target-list mode*

Decreased in treated WT cells

LION enrichment analysis *target-list mode*

