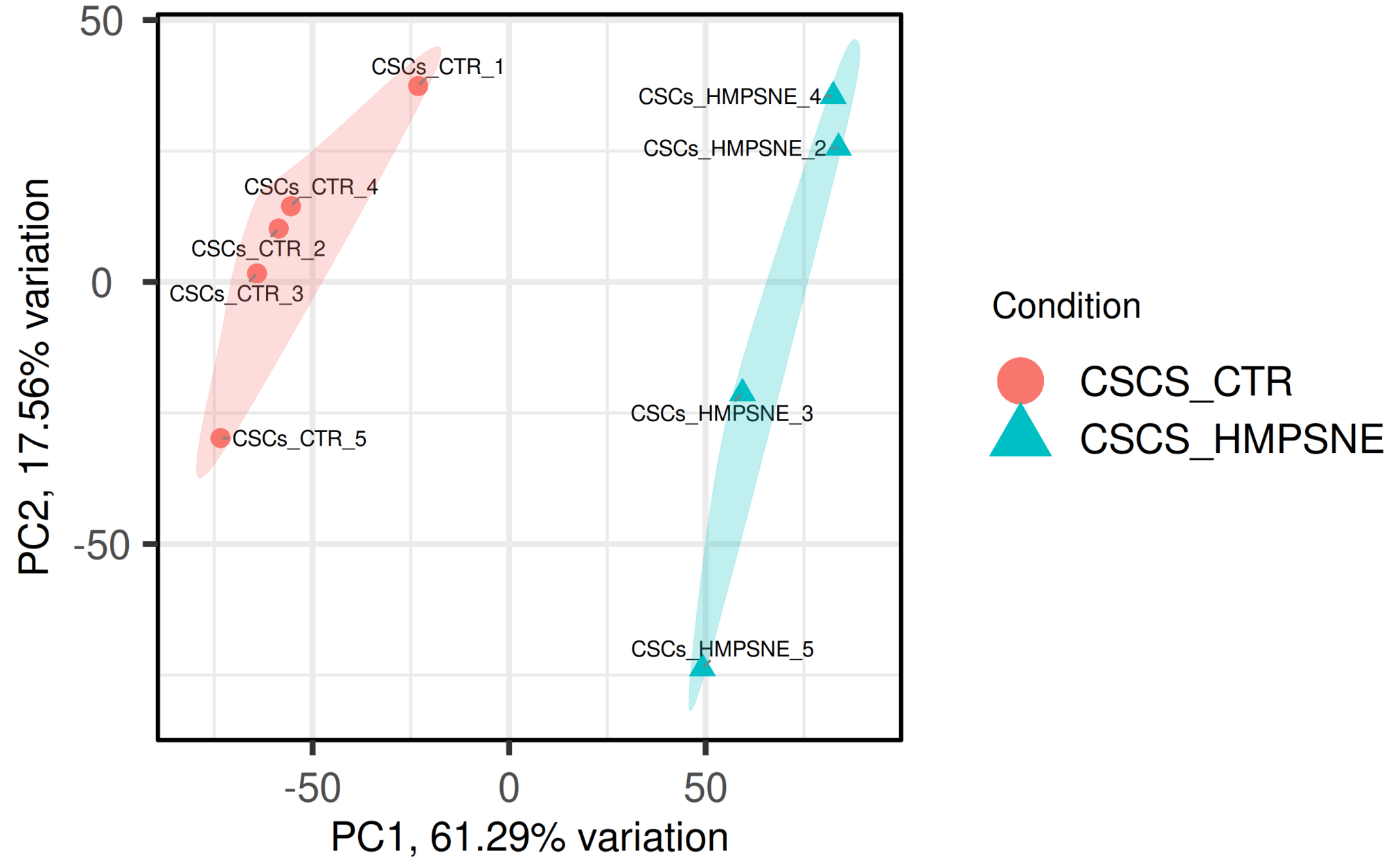


Supplementary Data S2. Multi-omics profiling of pharmacological 3-MST inhibition in colorectal cancer stem cells. Integrated transcriptomic, proteomic, metabolomic and lipidomic analyses comparing HCT116-derived CSCs treated with the 3-MST inhibitor HMPSNE vs. untreated CSC controls (CSCs_CTR) are shown. 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 and enrichment analyses appropriate for each omics layer. For transcriptomic and proteomic datasets, GSEA was performed using GO biological process and cellular component annotations as well as KEGG, Reactome and Hallmark gene sets. ORA was additionally used to identify pathways and biological processes significantly enriched among features increased or decreased following HMPSNE treatment. For metabolomic datasets, pathway enrichment was assessed using QEA to evaluate coordinated changes in metabolite sets across metabolic pathways. ORA was additionally applied to identify metabolite pathways significantly associated with HMPSNE treatment. For lipidomic datasets, enrichment of lipid pathways and lipid-associated annotations 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 structural and functional lipid categories altered following 3-MST inhibition. The dataset additionally includes integrated multi-omics comparisons across three experimental conditions (WT, CSCs_CTR and CSCs_HMPSNE), including PCA analyses and differential profiles across transcriptomic, proteomic, metabolomic and lipidomic datasets. Lipid structural properties, including fatty-acid chain length and unsaturation indices, were also analyzed to characterize lipid remodeling associated with CSC state and 3-MST inhibition. Together, these analyses provide a comprehensive multi-omics characterization of molecular changes induced by pharmacological inhibition of 3-MST in colorectal cancer stem cells.

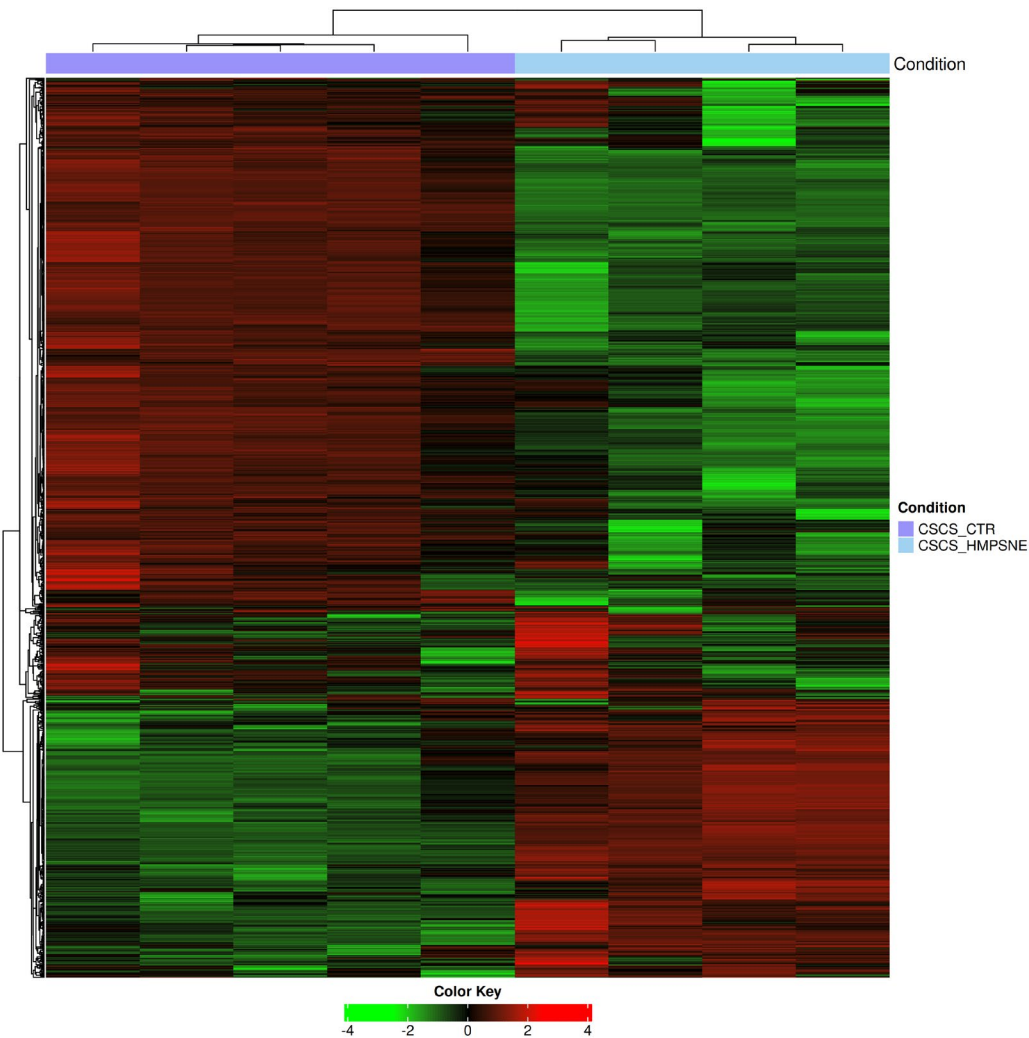
CSCs-HMPSNE vs CSCs-CTR RNA Seq

CSCs – HMPSNE

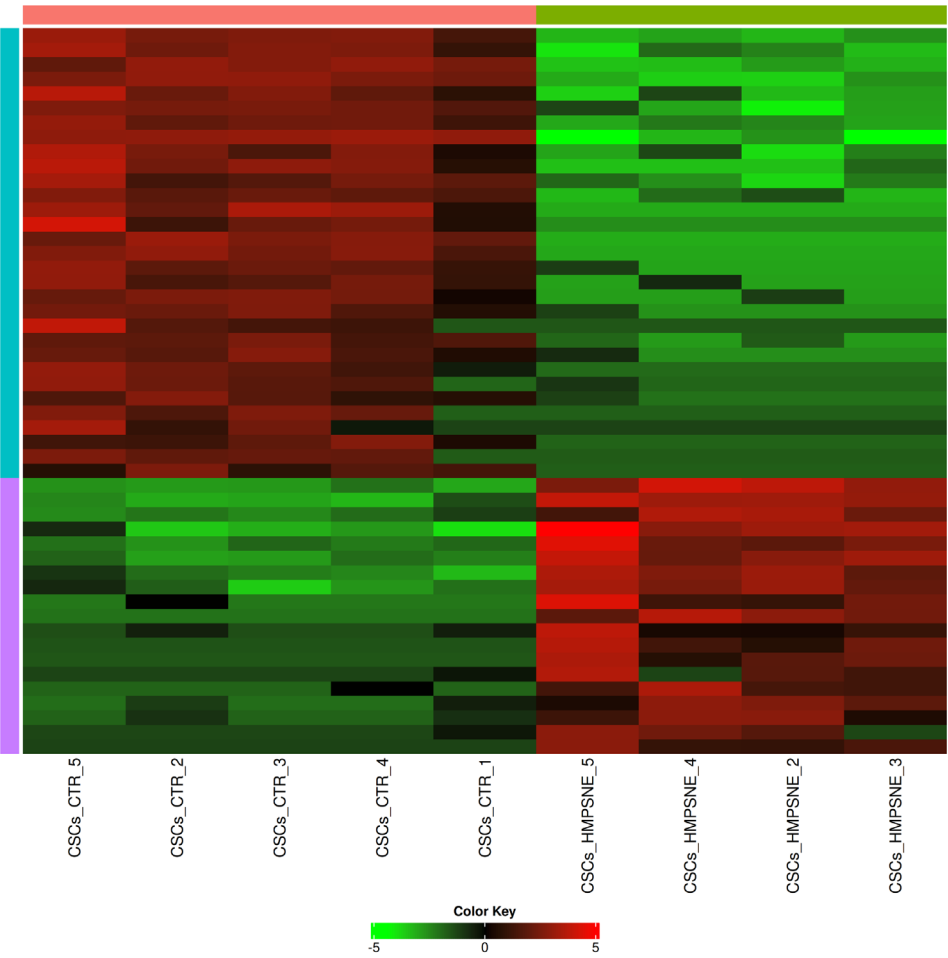


CSCs – HMPSNE

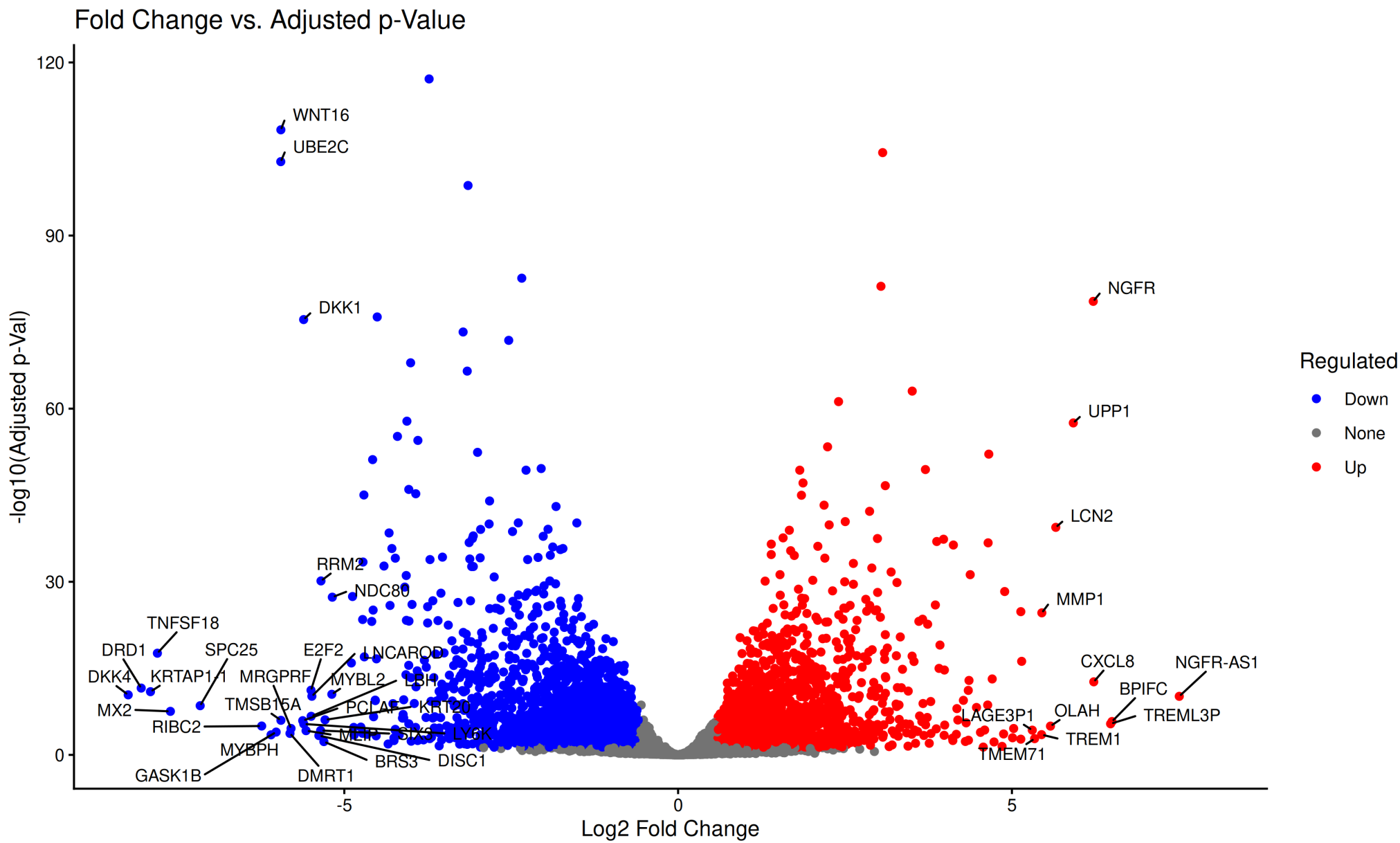
Hierarchical clustering of the top 1000 most variable genes



Heatmap of the top 50 DEGs



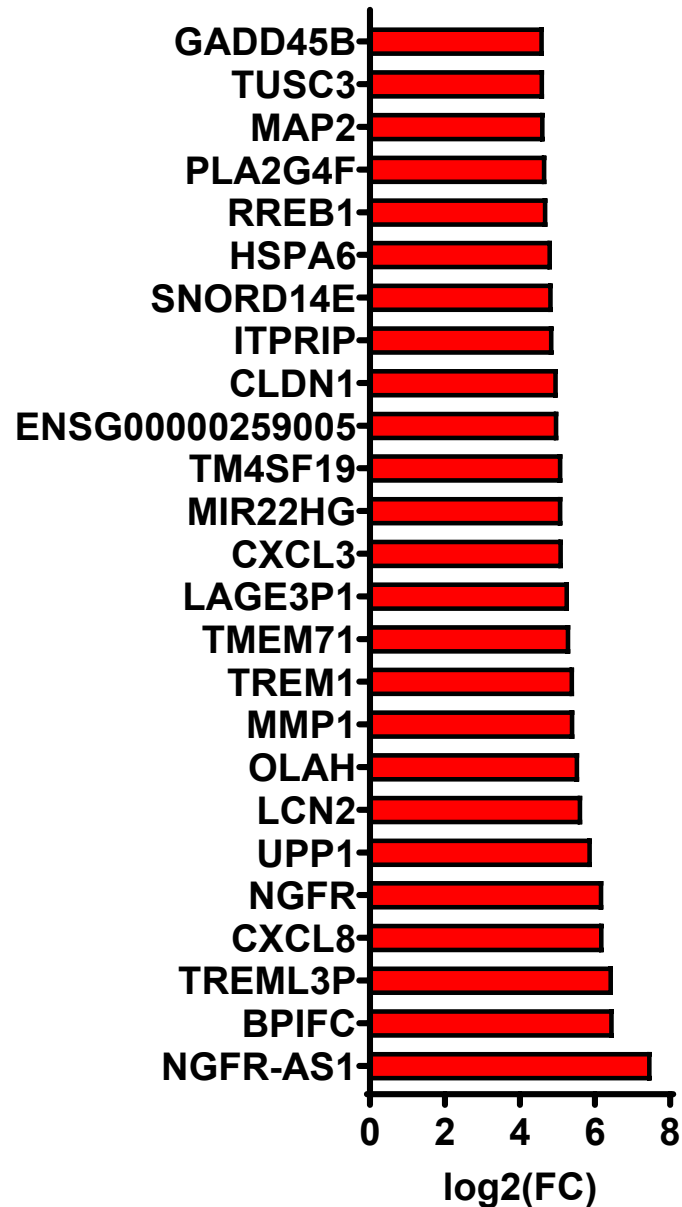
CSCs – HMPSNE



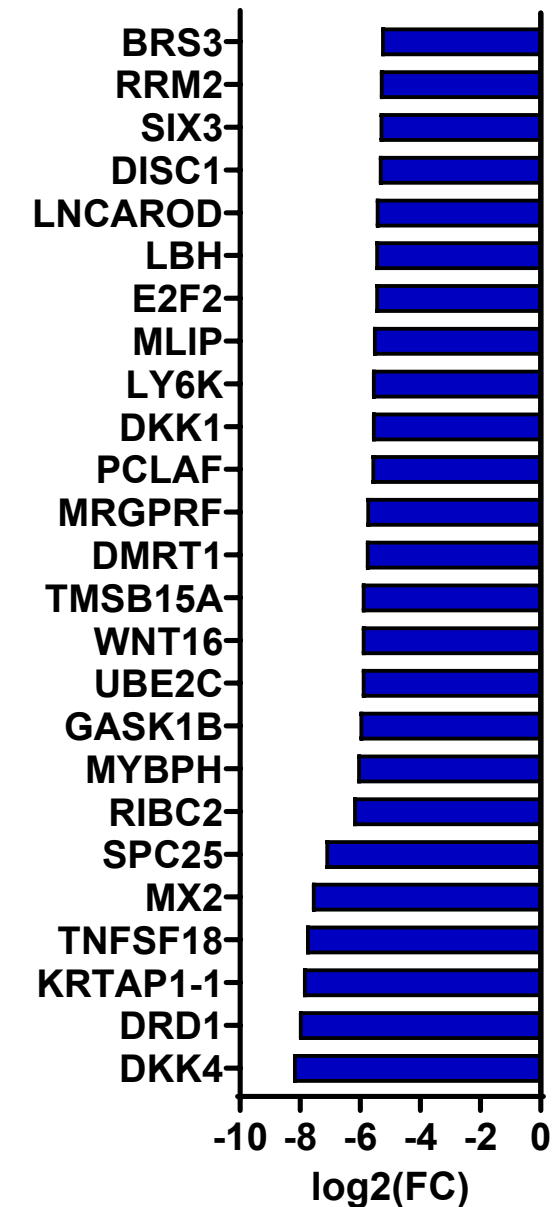
Fold change (FC) threshold: 1.5
FDR cutoff: 0.05

CSCs – HMPSNE

Top 25_Upregulated genes in treated CSCs

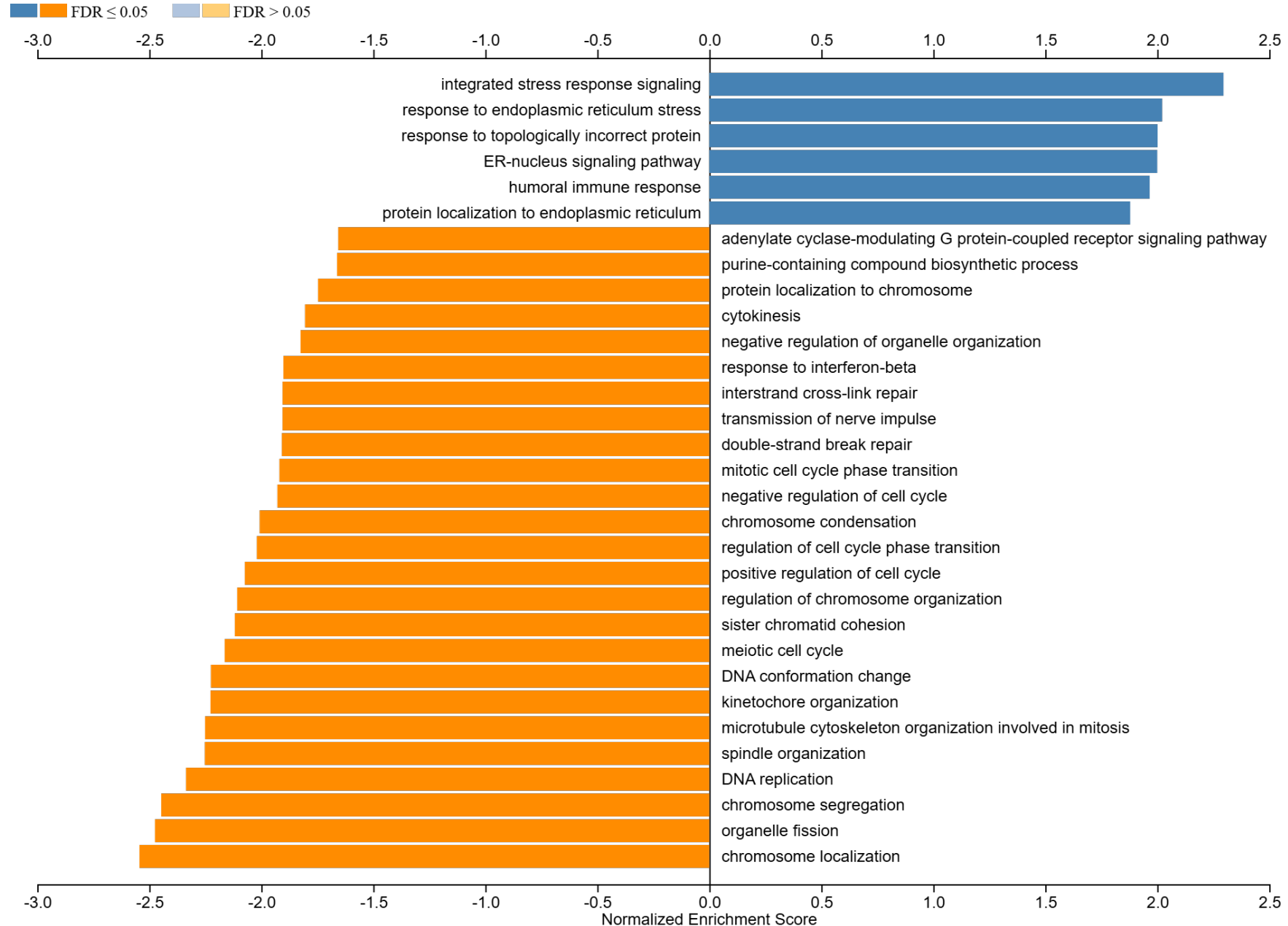


Top 25_Downregulated genes in treated CSCs



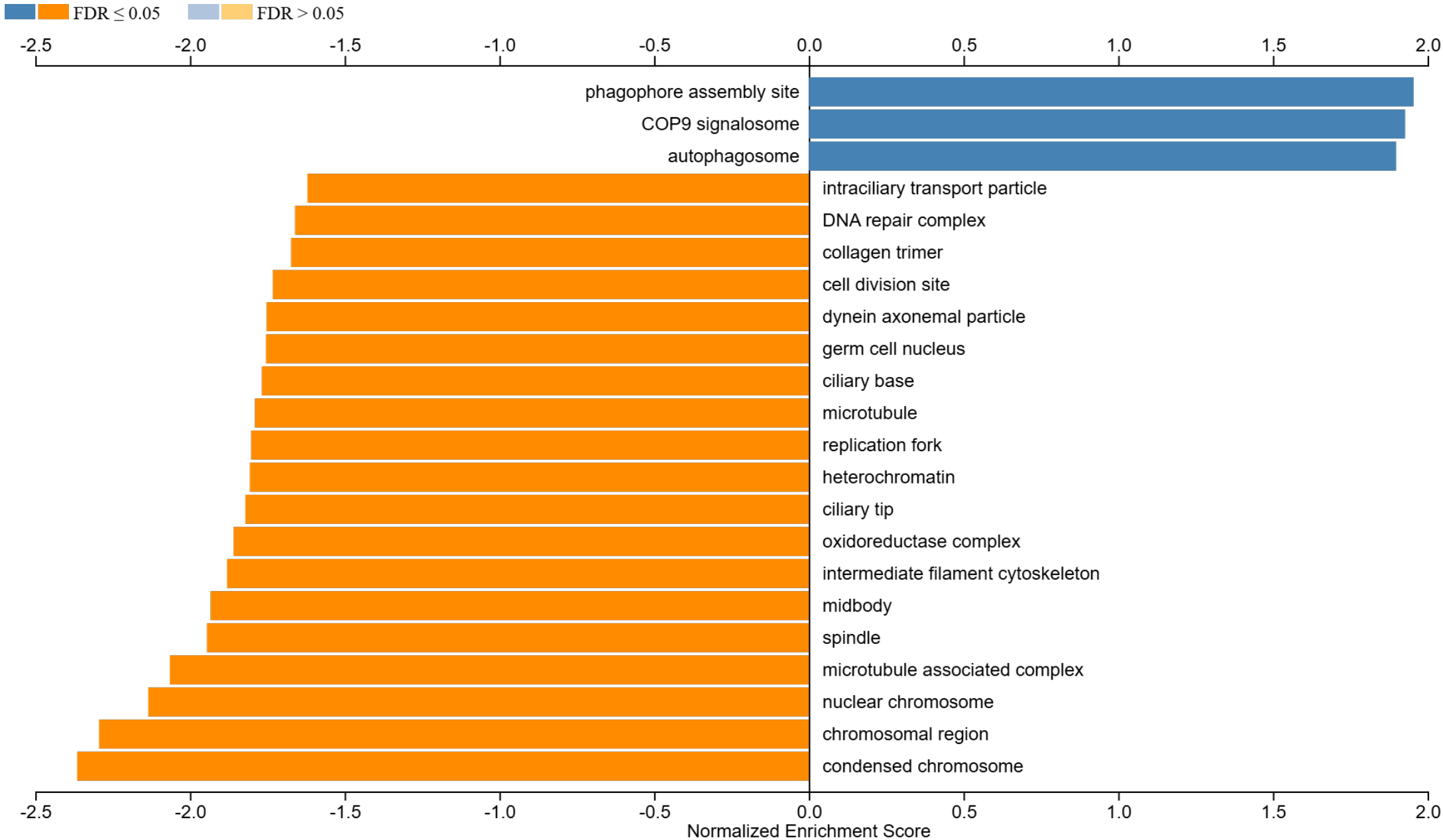
CSCs – HMPSNE

GSEA (Gene Set Enrichment Analysis) - geneontology_Biological_Process



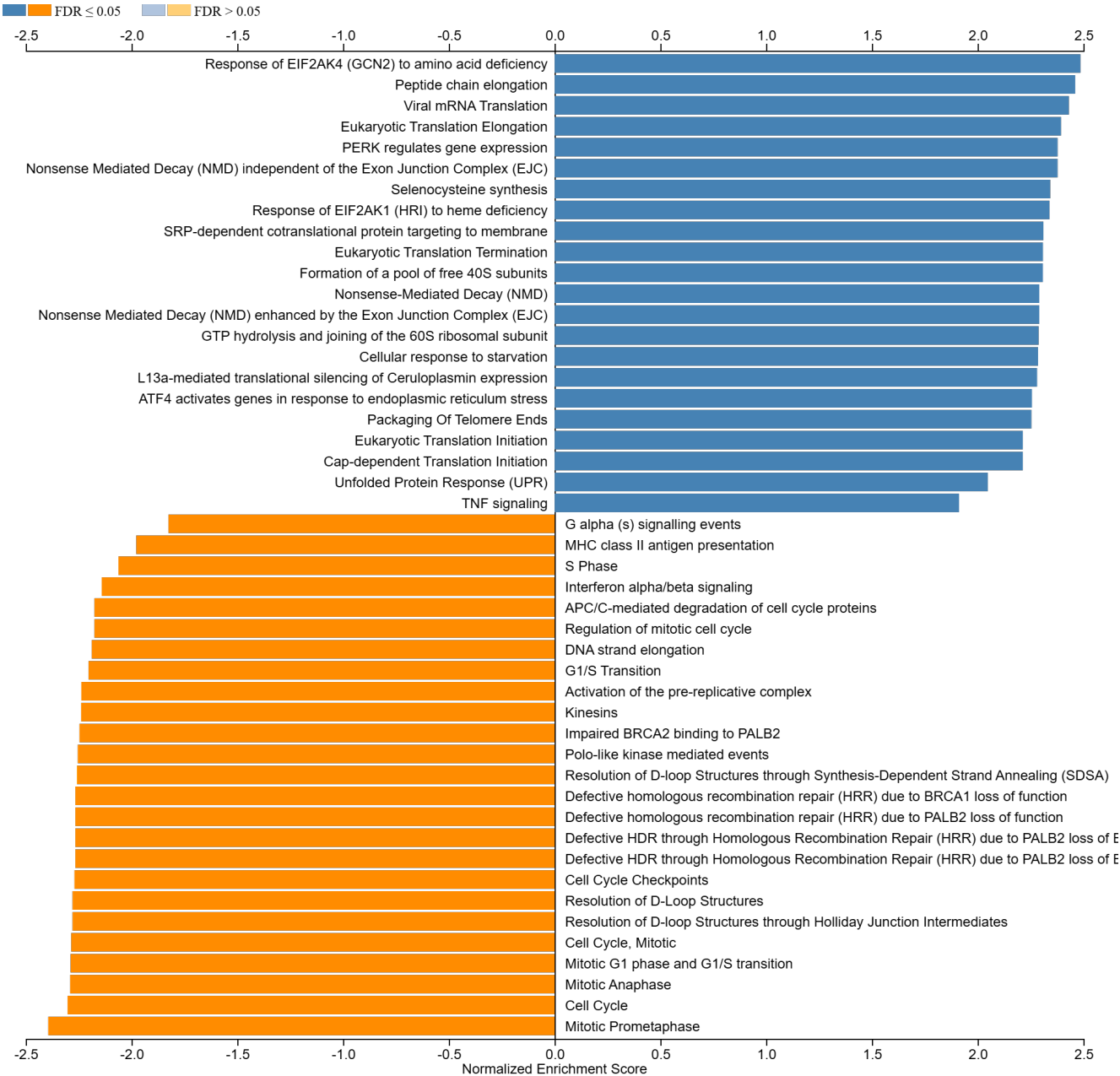
CSCs – HMPSNE

GSEA (Gene Set Enrichment Analysis) - geneontology_Cellular_Component



CSCs – HMPSNE

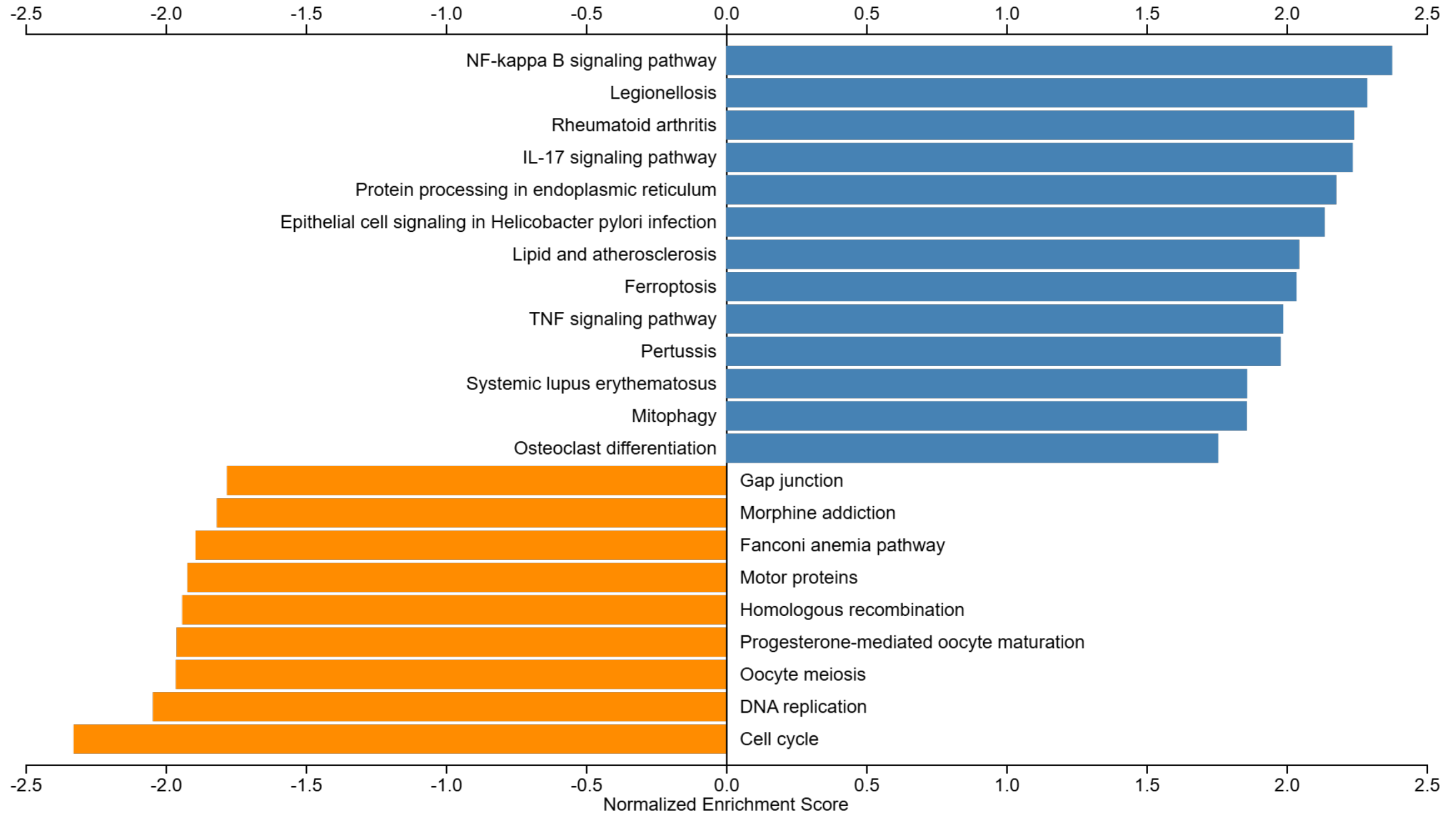
GSEA (Gene Set Enrichment Analysis) - pathway_Reactome



CSCs – HMPSNE

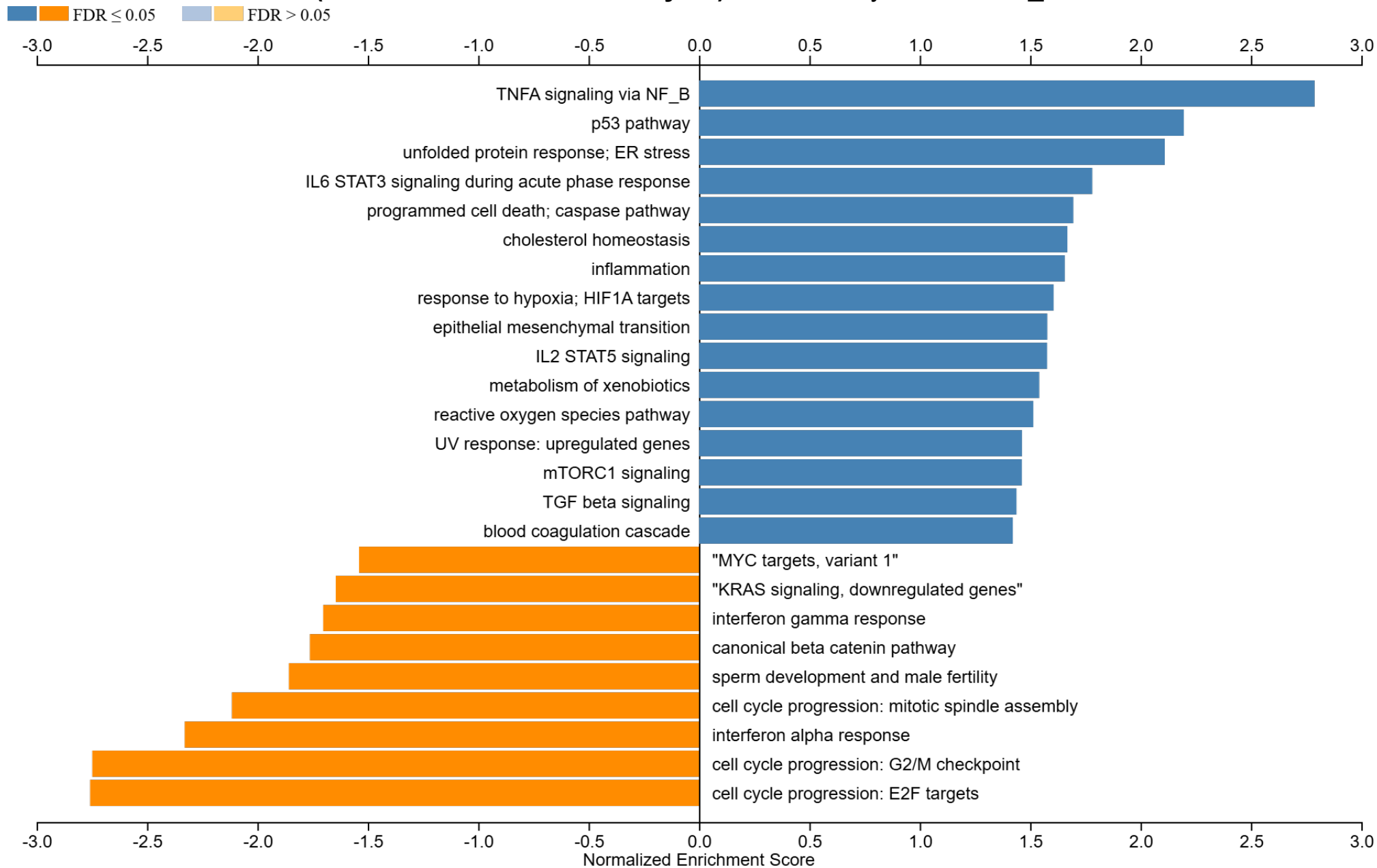
GSEA (Gene Set Enrichment Analysis) - pathway_KEGG

FDR ≤ 0.05 FDR > 0.05



CSCs – HMPSNE

GSEA (Gene Set Enrichment Analysis) - community-contributed_Hallmark50

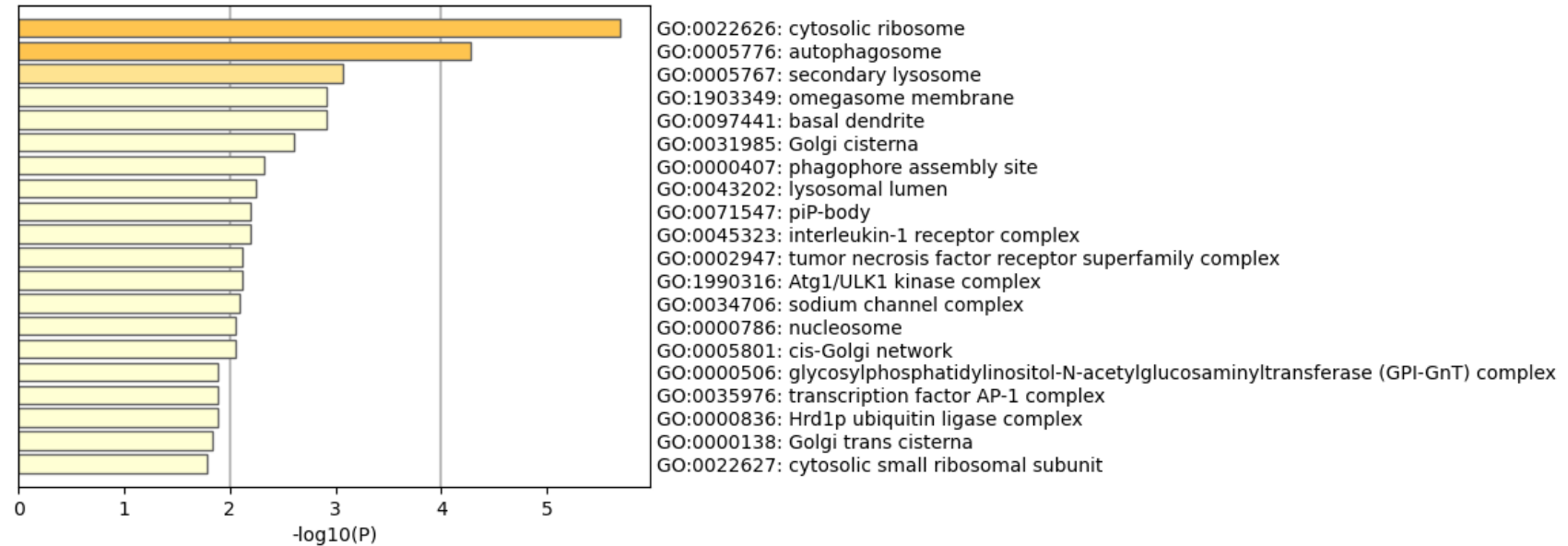


CSCs – HMPSNE

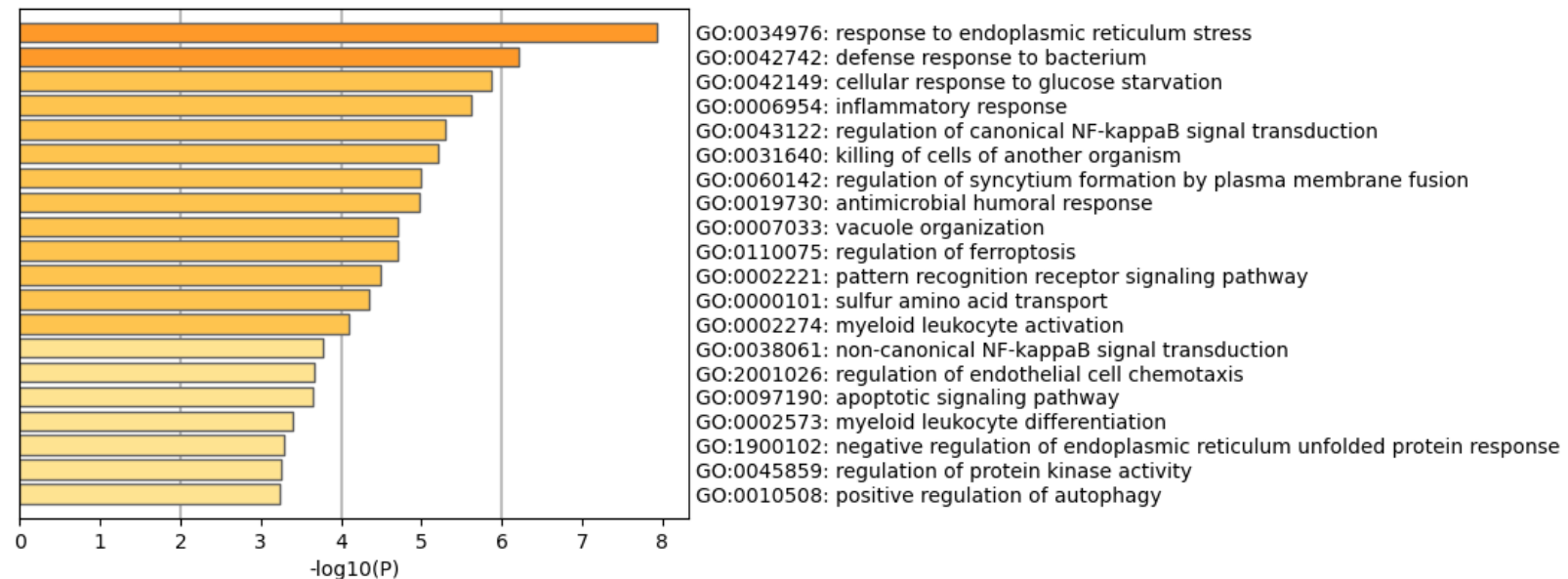
ORA (Over-Representation Analysis)

Activation in treated CSCs

GO Cellular Components



GO Biological Process

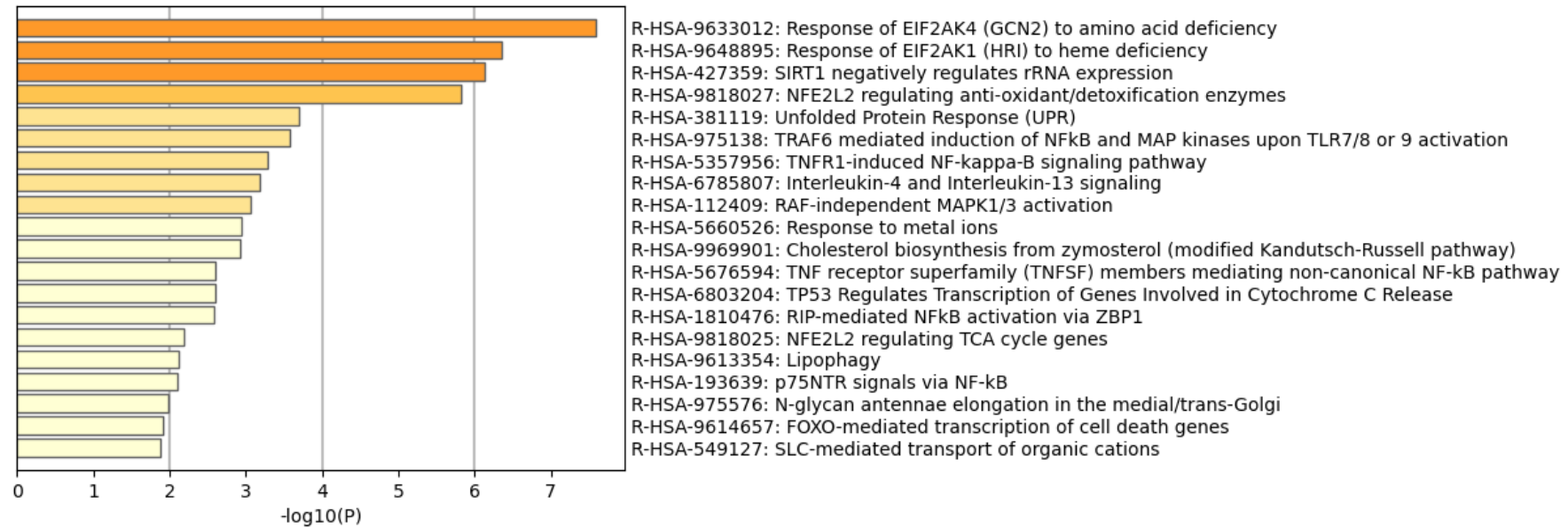


CSCs – HMPSNE

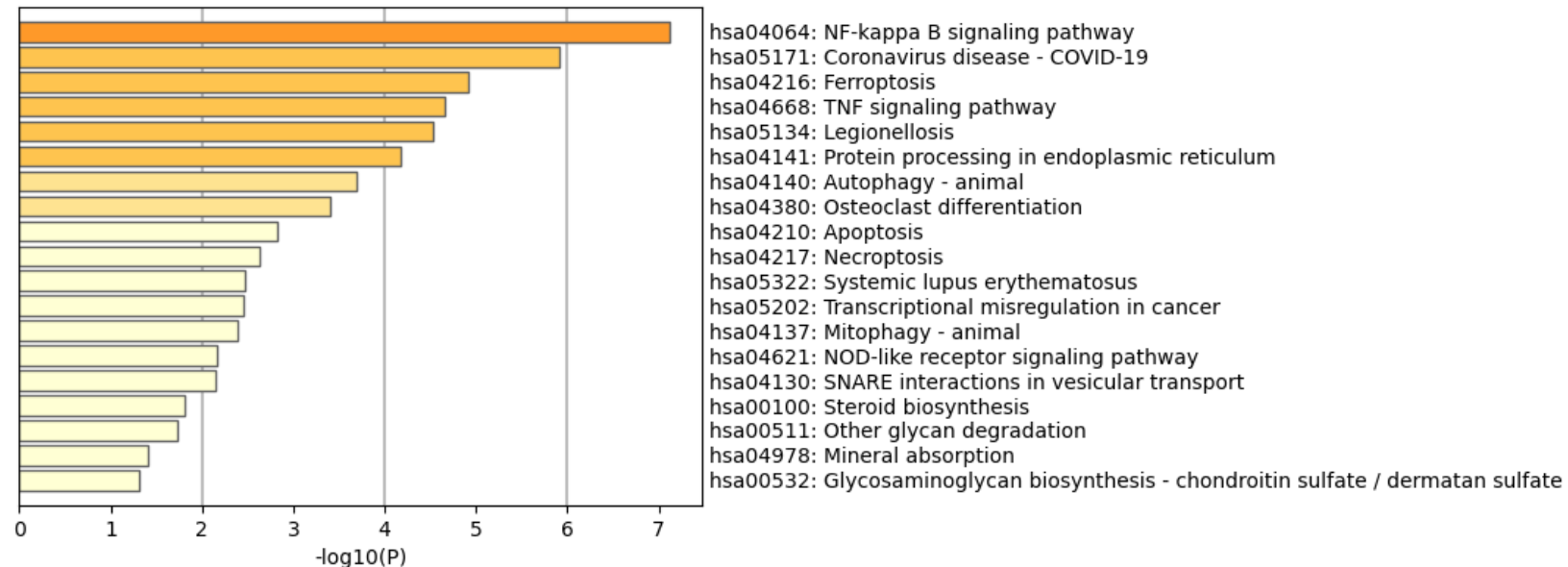
ORA (Over-Representation Analysis)

Activation in treated CSCs

Reactome Gene Sets



KEGG Pathway

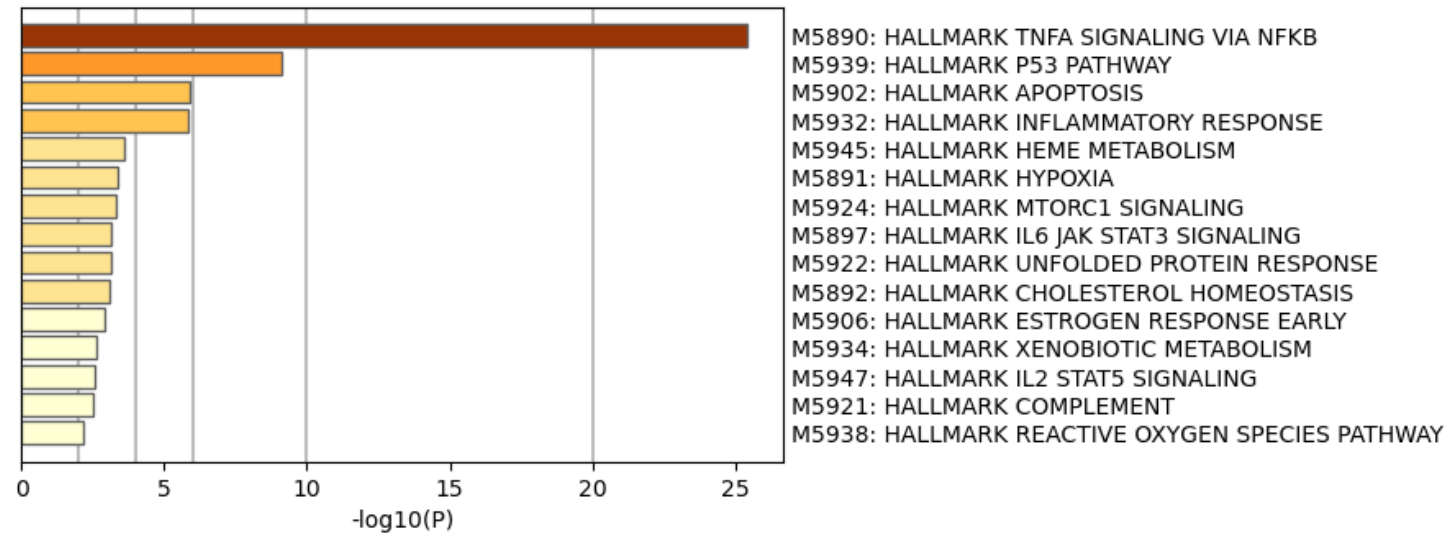


CSCs – HMPSNE

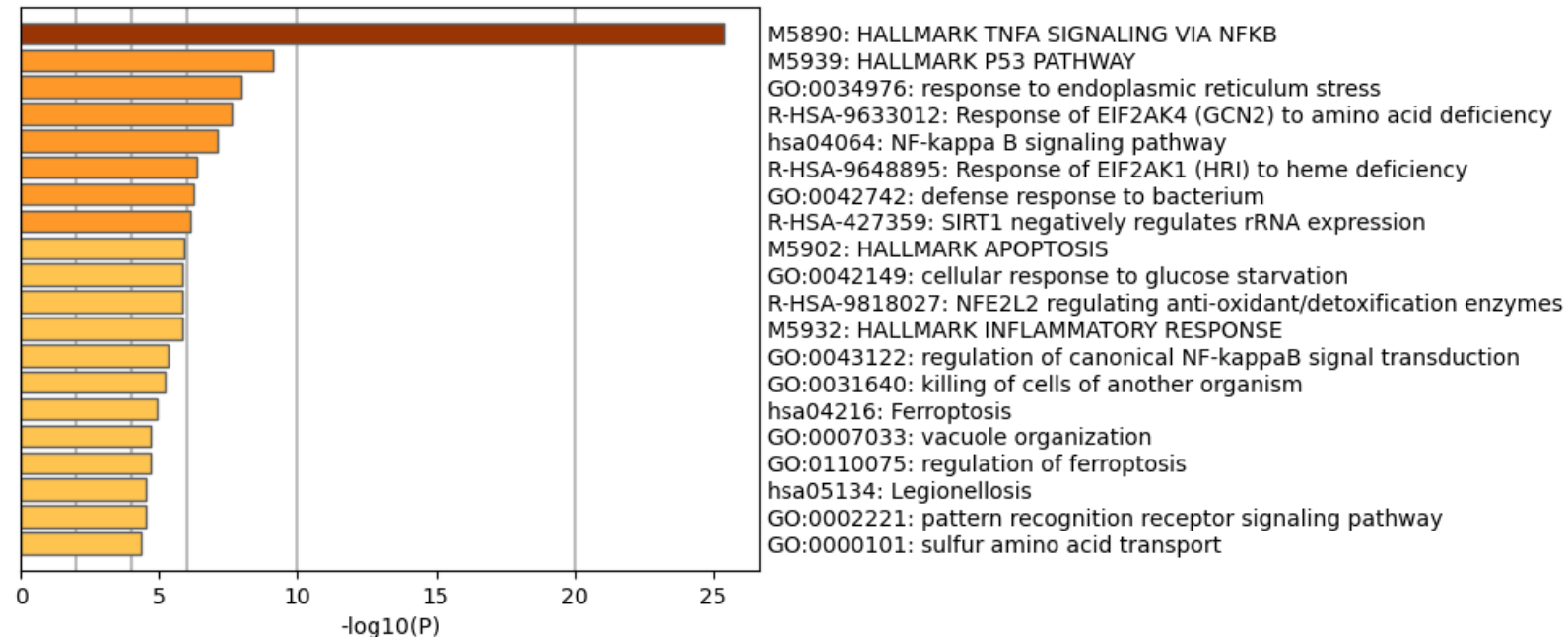
ORA (Over-Representation Analysis)

Activation in treated CSCs

Hallmark Gene Sets



Combined GO

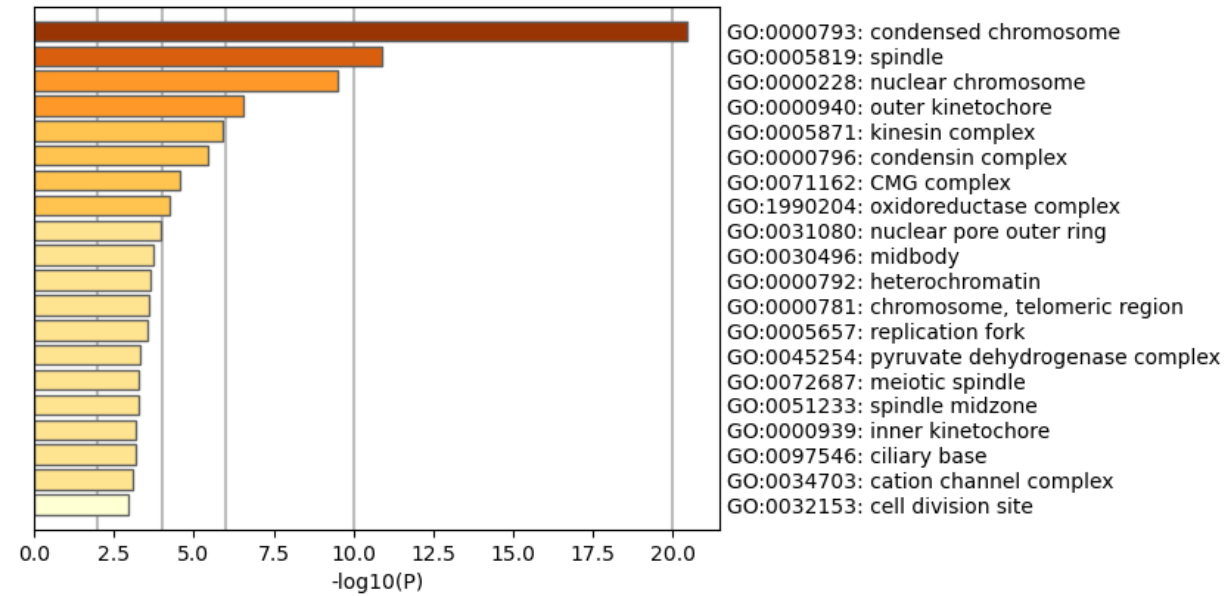


CSCs – HMPSNE

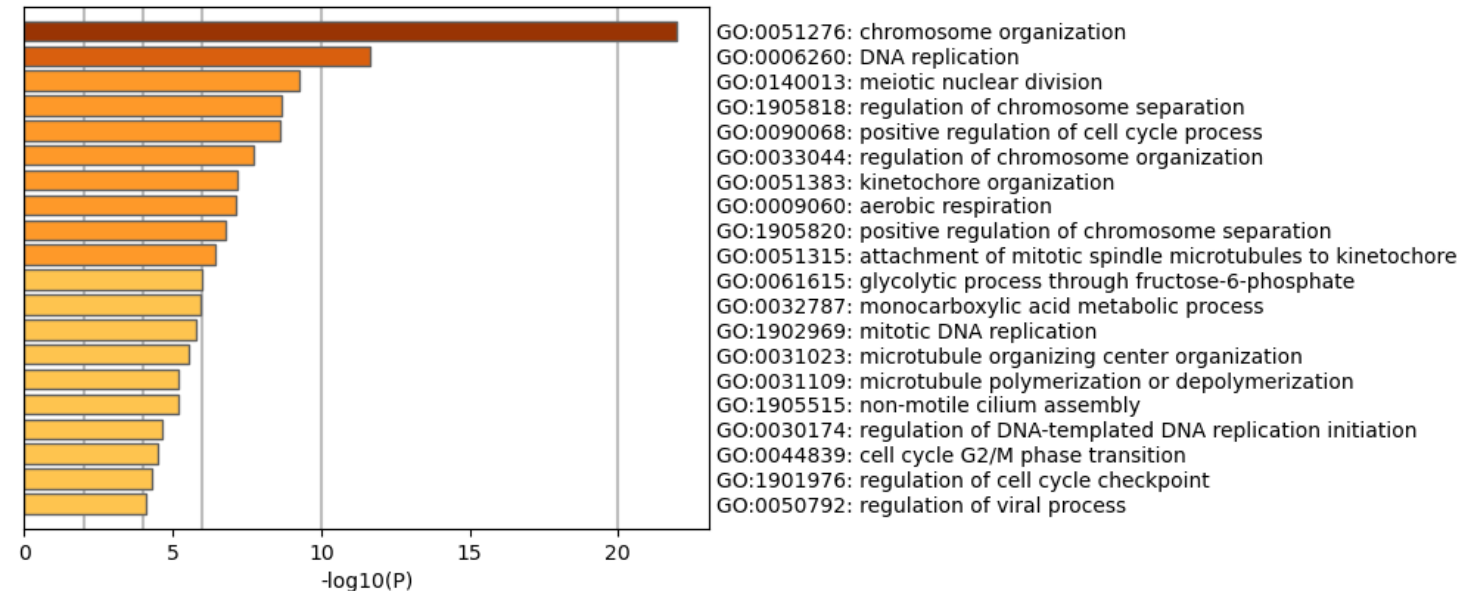
ORA (Over-Representation Analysis)

Inhibition in treated CSCs

GO Cellular Components



GO Biological Process

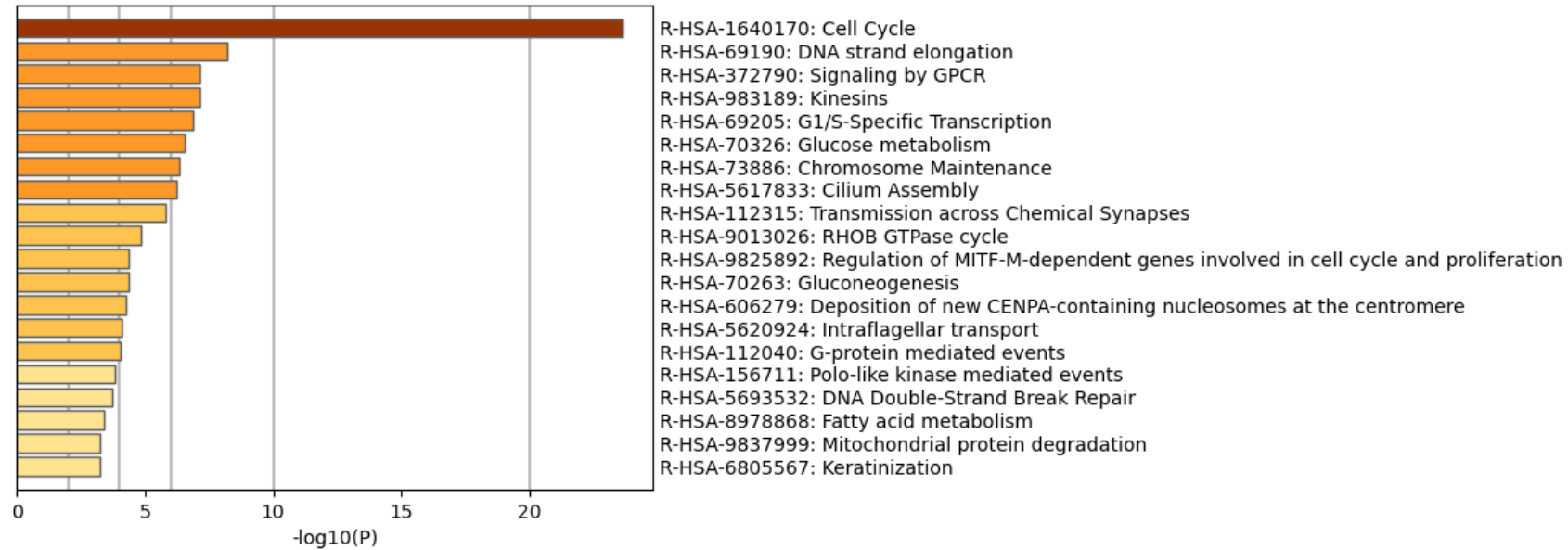


CSCs – HMPSNE

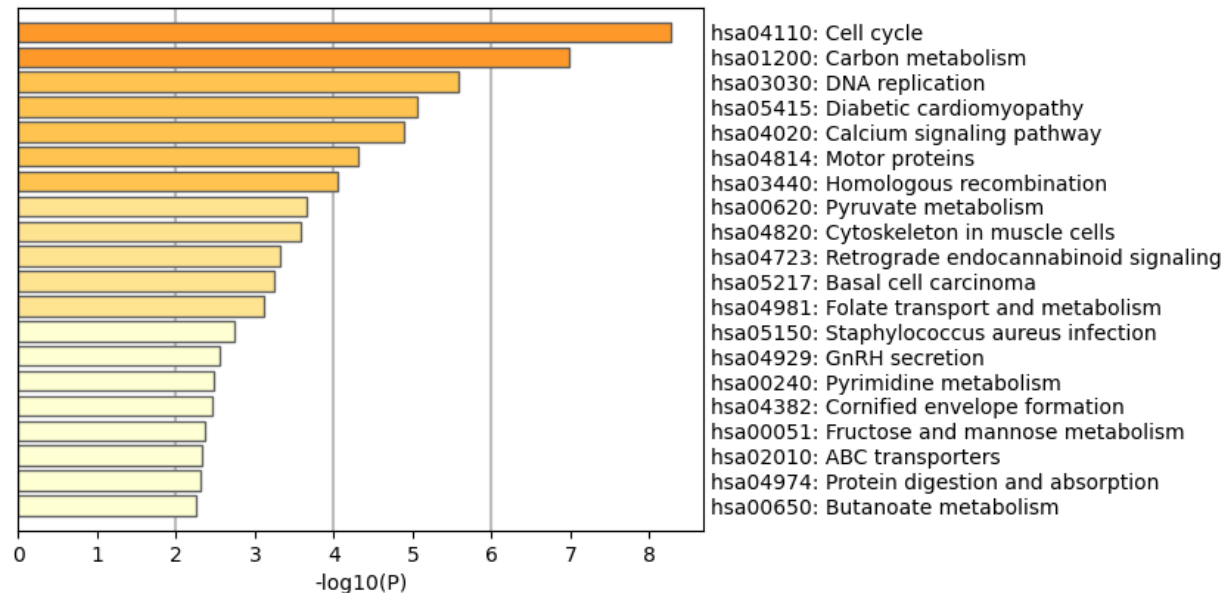
ORA (Over-Representation Analysis)

Inhibition in treated CSCs

Reactome Gene Sets



KEGG Pathway

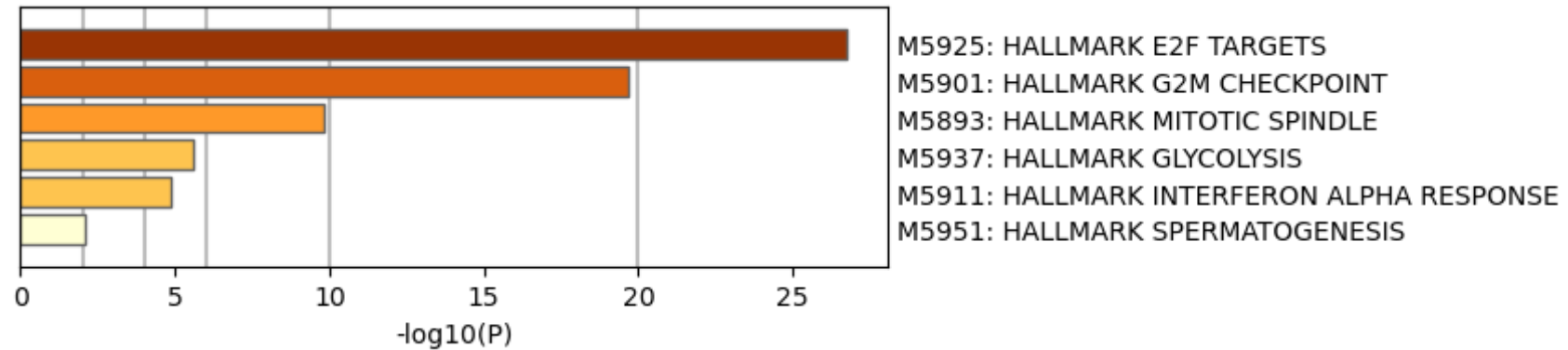


CSCs – HMPSNE

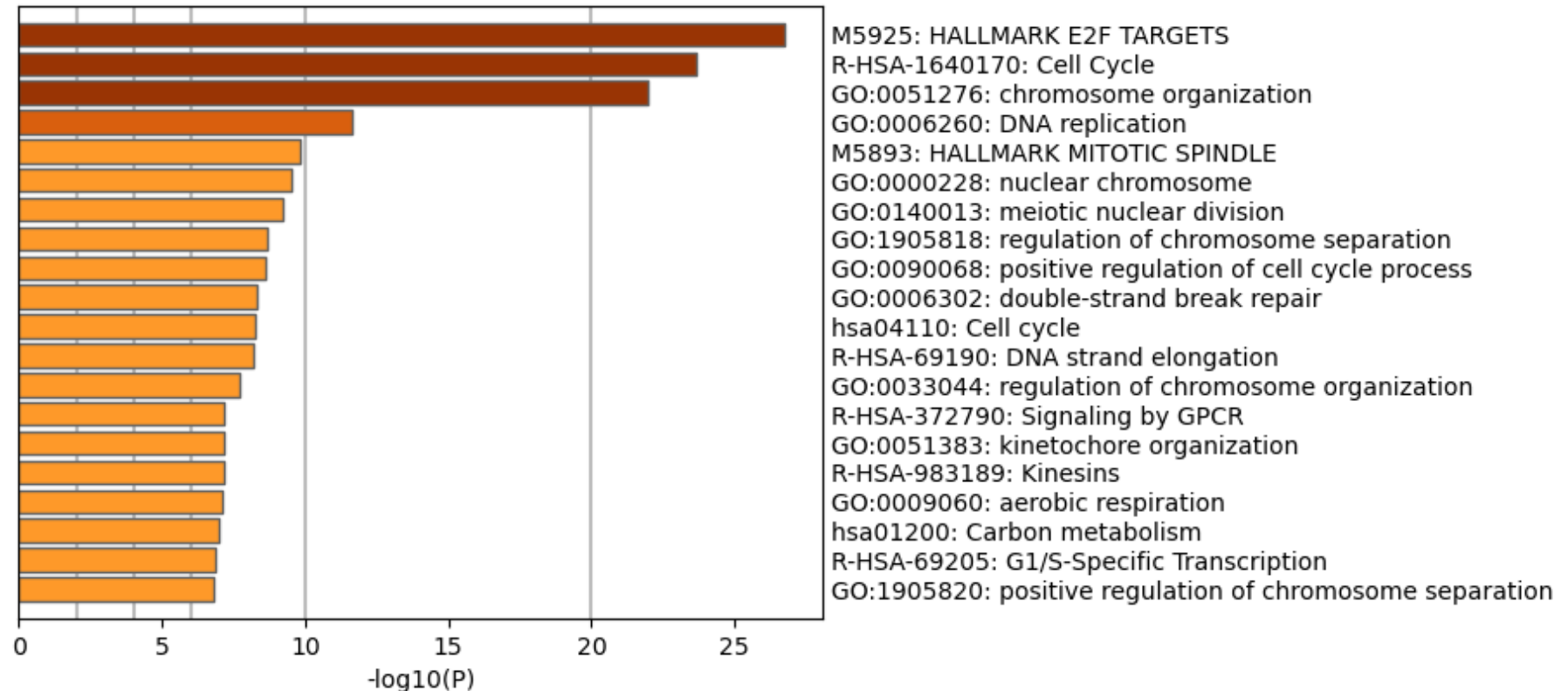
ORA (Over-Representation Analysis)

Inhibition in treated CSCs

Hallmark Gene Sets

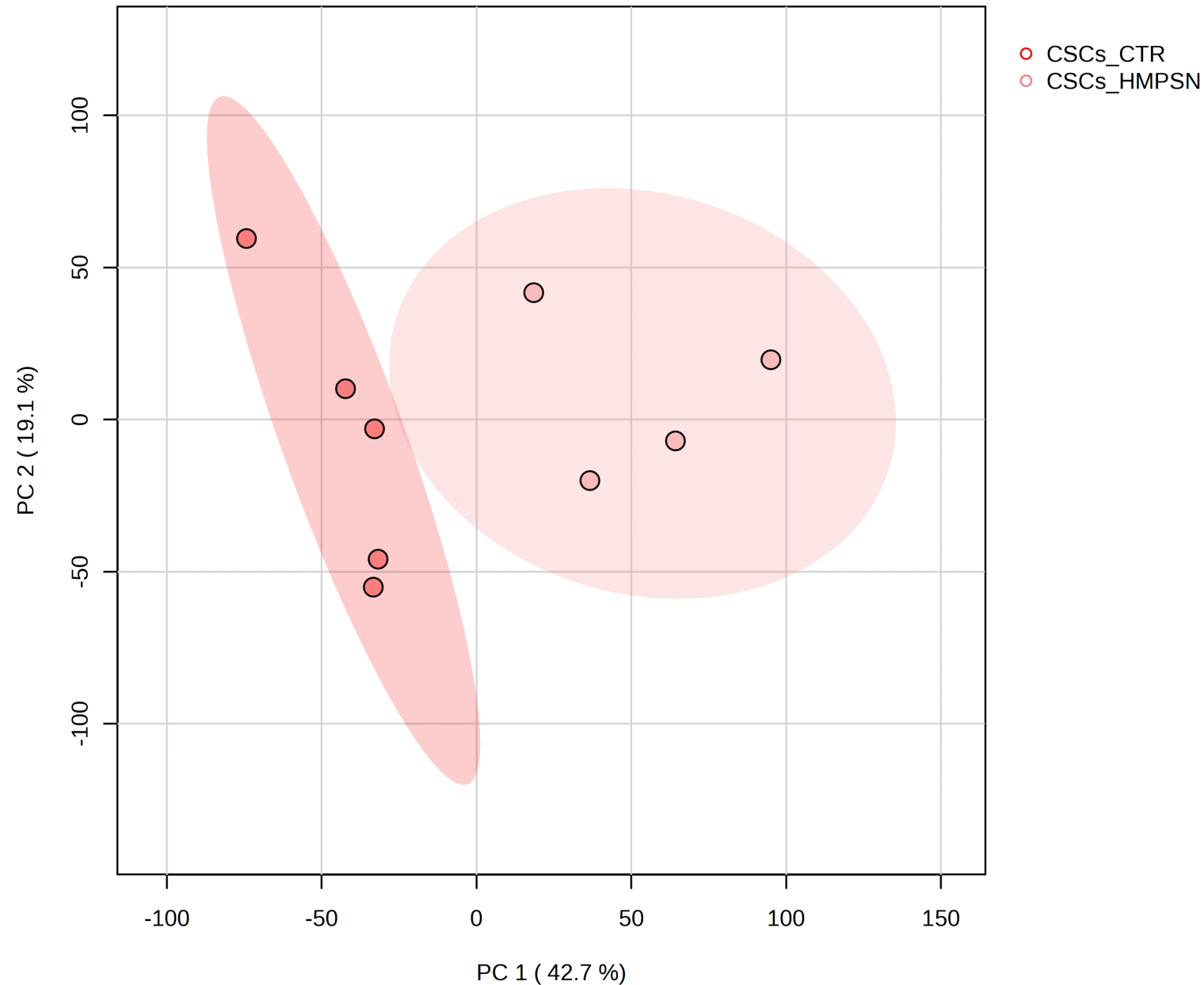


Combined GO

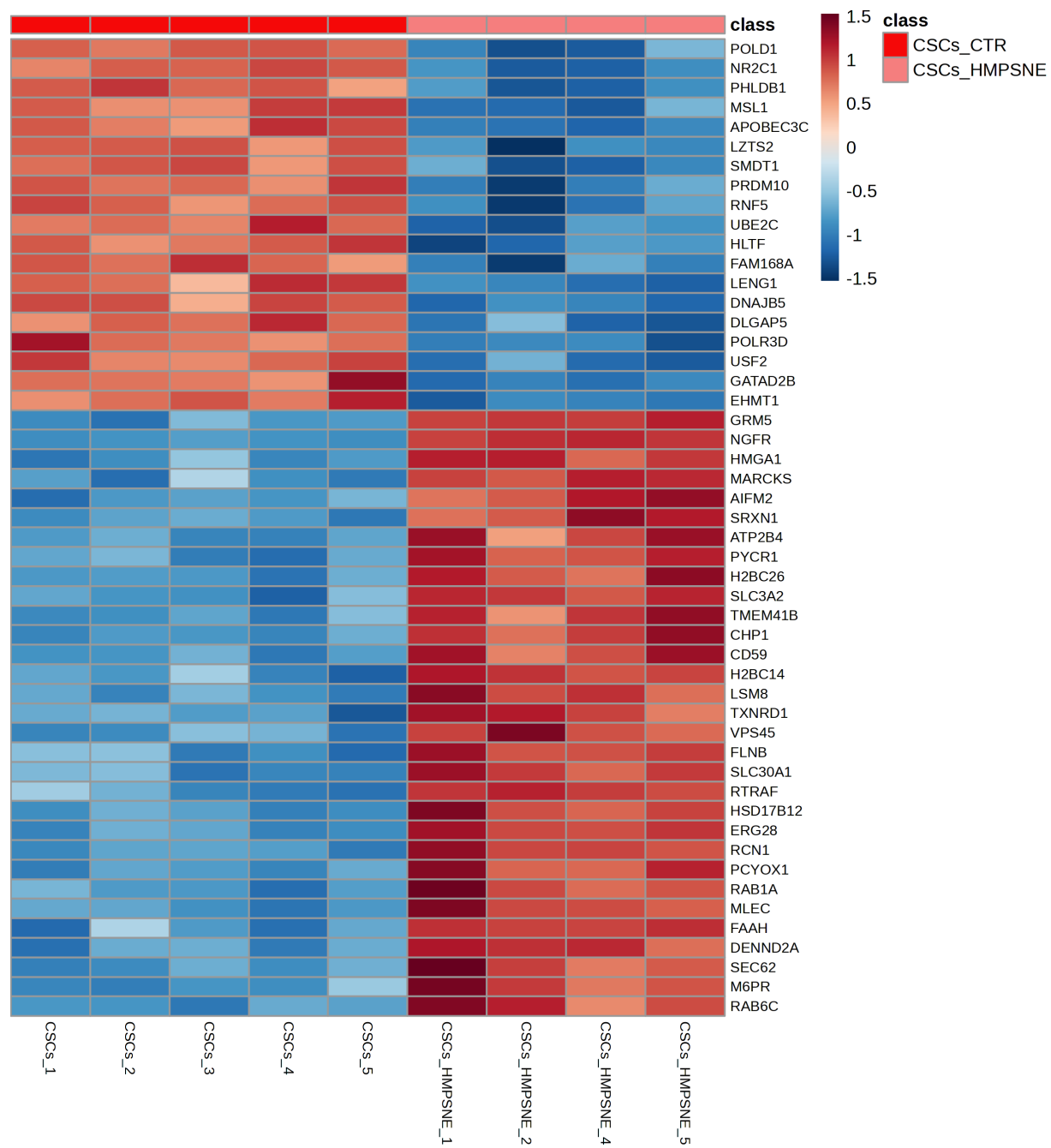


CSCs-HMPSNE vs CSCs-CTR Proteomics

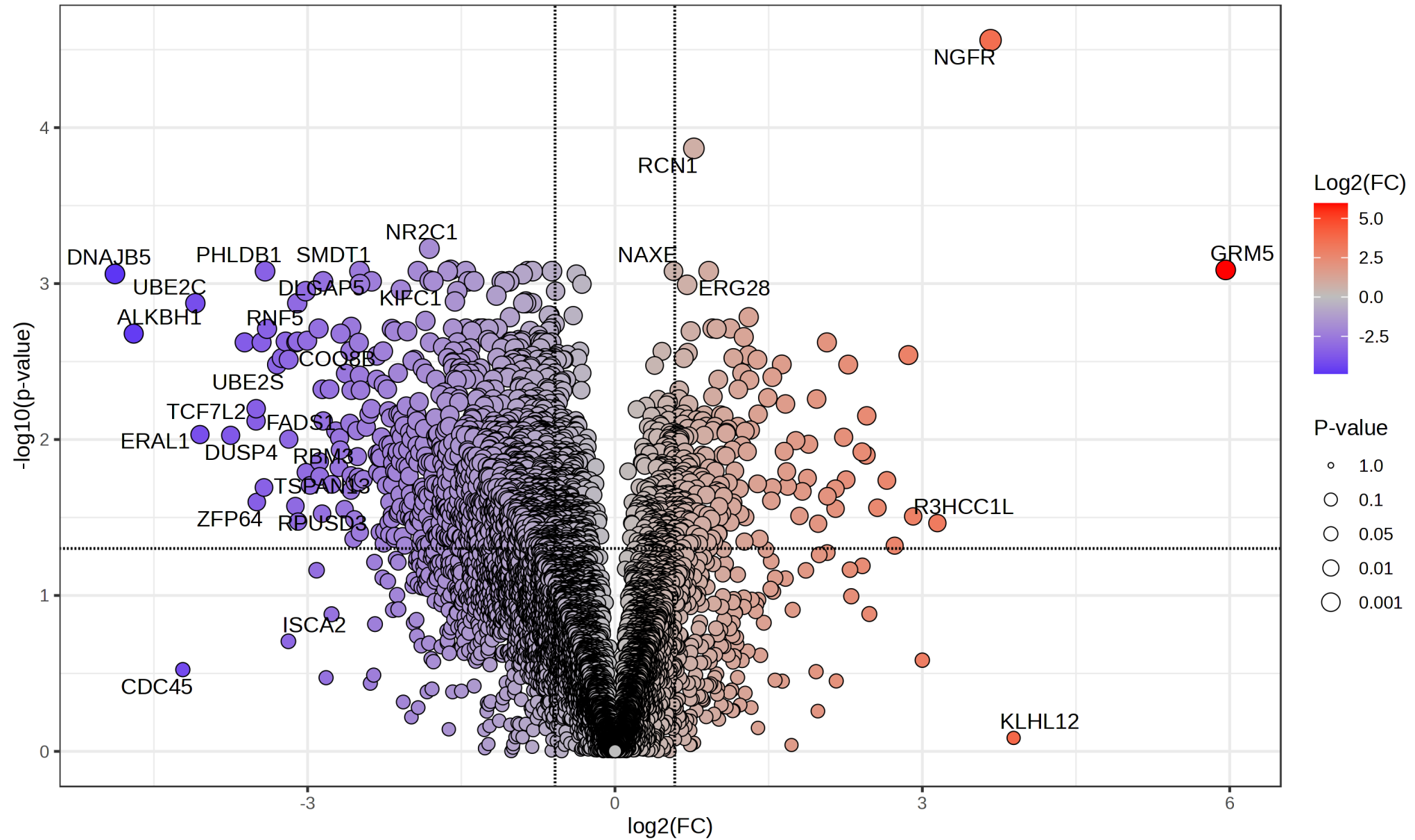
CSCs – HMPSNE



CSCs – HMPSNE



CSCs – HMPSNE

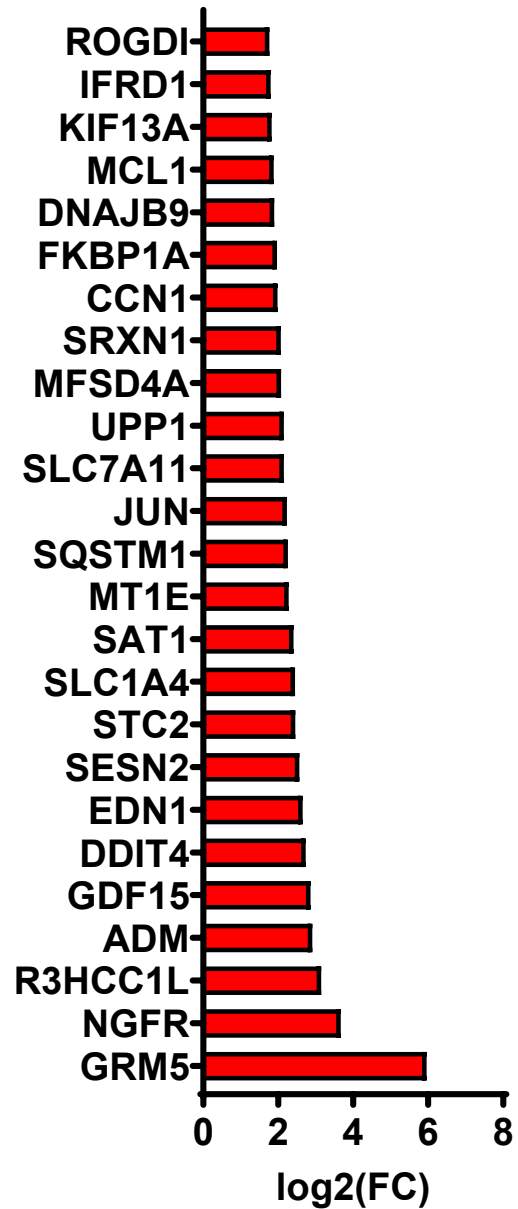


Fold change (FC) threshold: 1.5

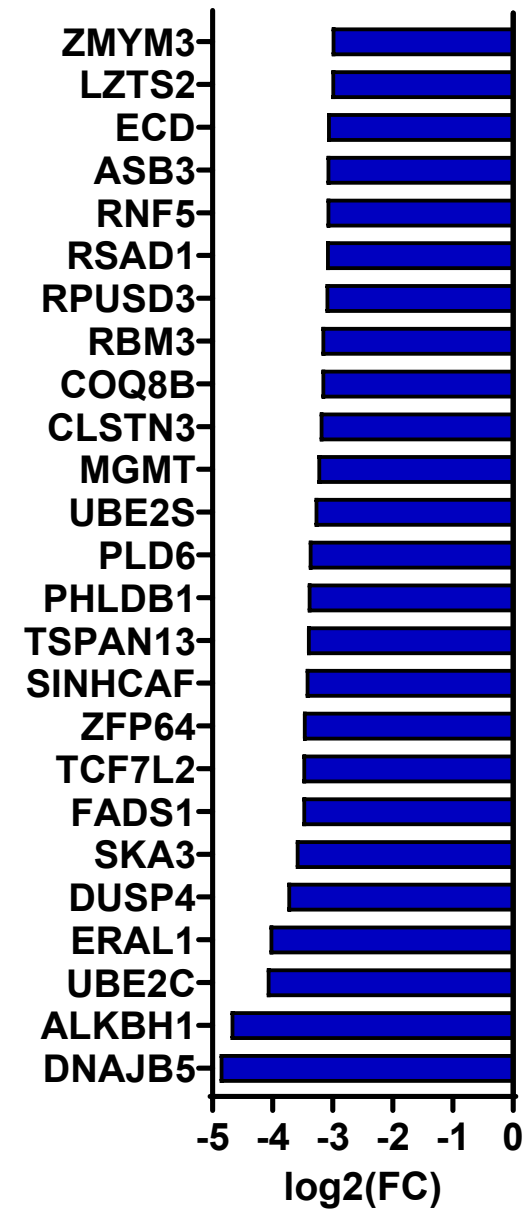
P-value threshold: 0.05

CSCs – HMPSNE

Top 25_Upregulated proteins in treated CSCs



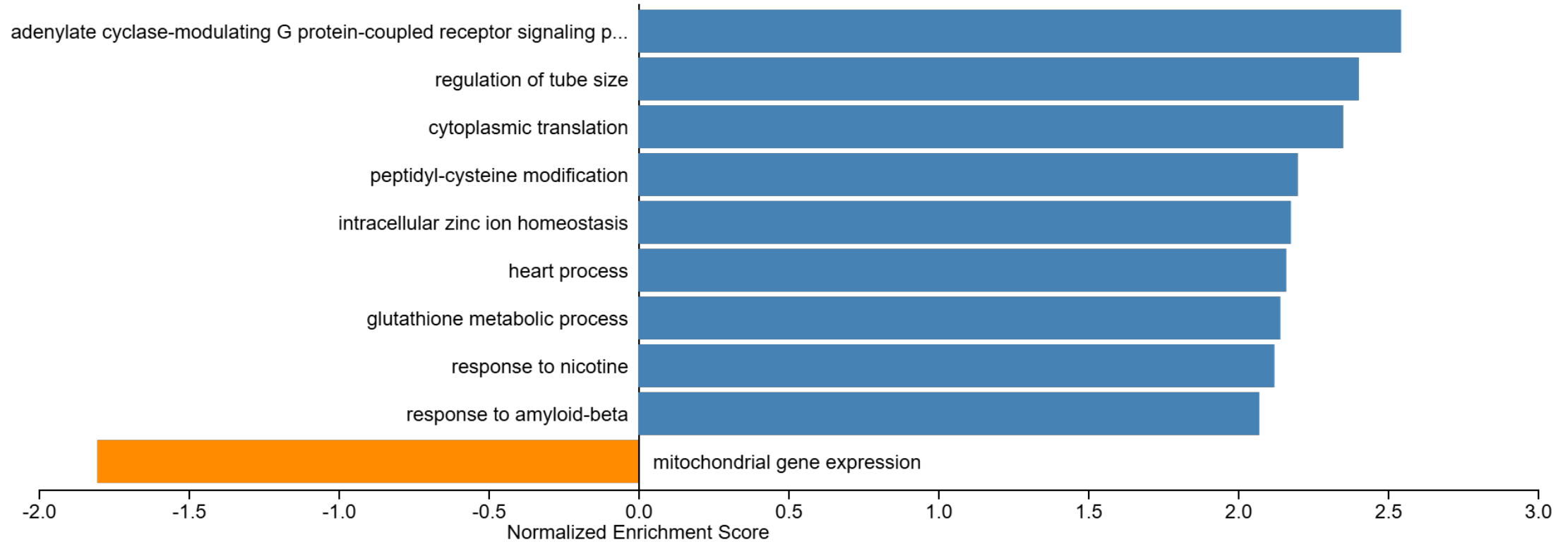
Top 25_Downregulated proteins in treated CSCs



CSCs – HMPSNE

GSEA (Gene Set Enrichment Analysis) - geneontology_Biological_Process

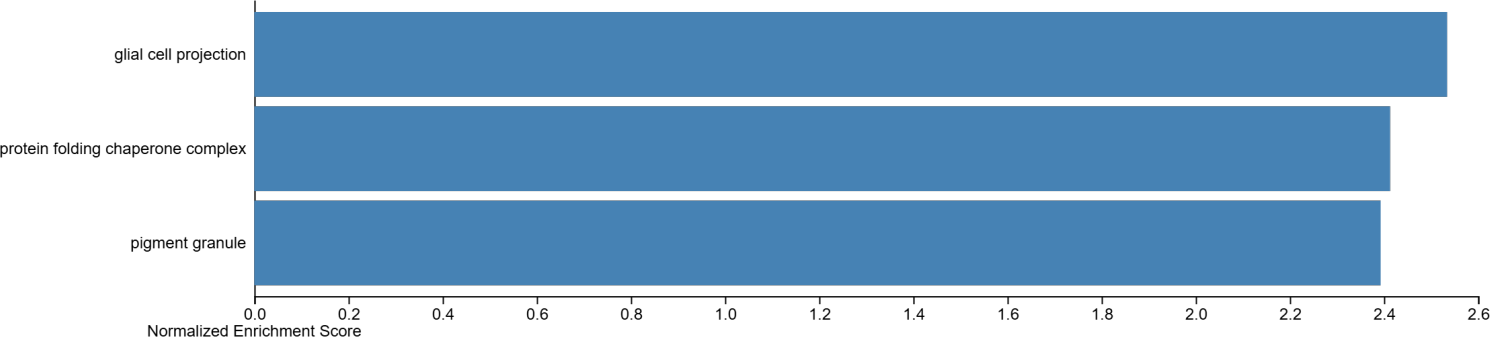
■ FDR ≤ 0.05 ■ FDR > 0.05



CSCs – HMPSNE

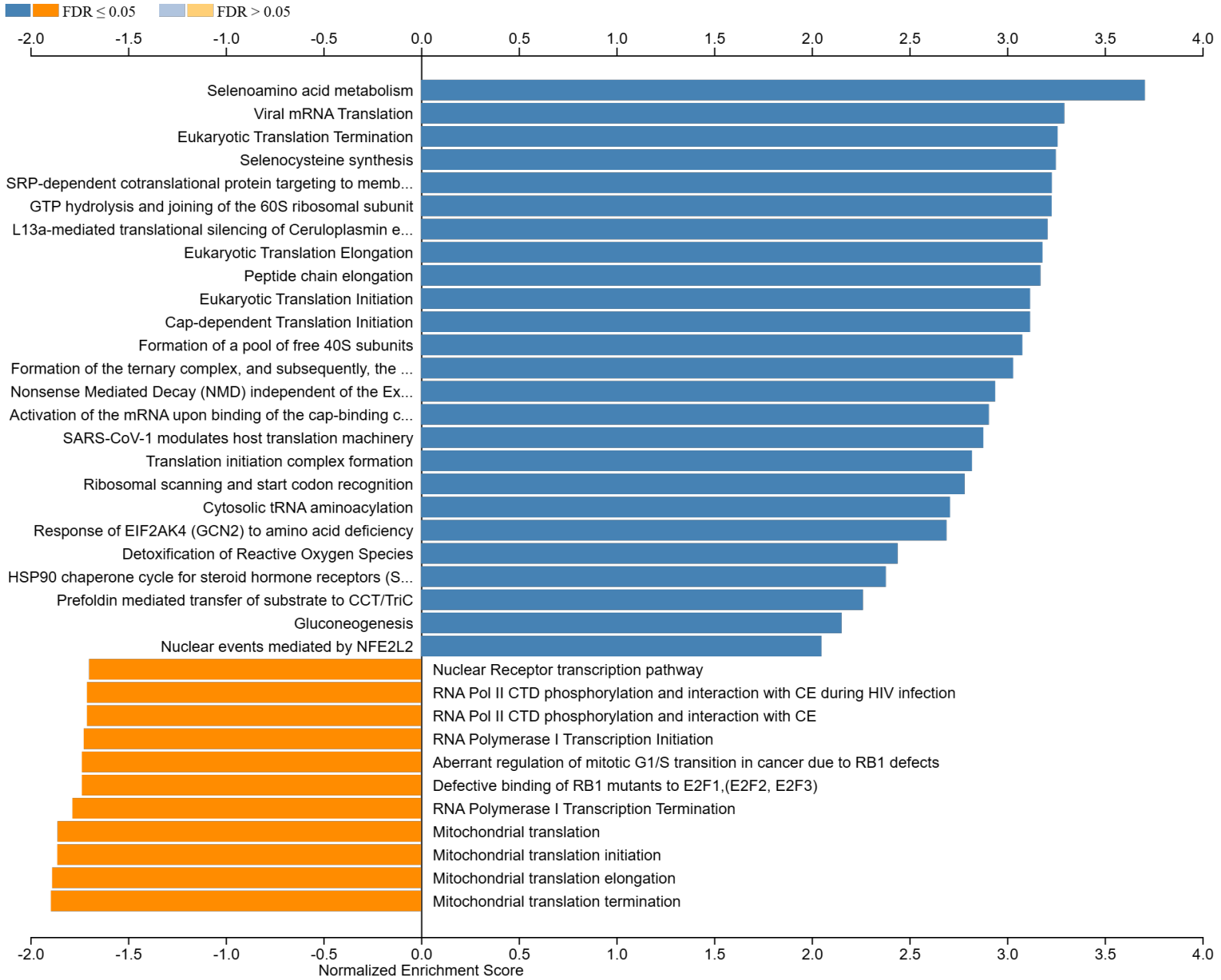
GSEA (Gene Set Enrichment Analysis) - geneontology_Cellular_Component

FDR ≤ 0.05 FDR > 0.05



CSCs – HMPSNE

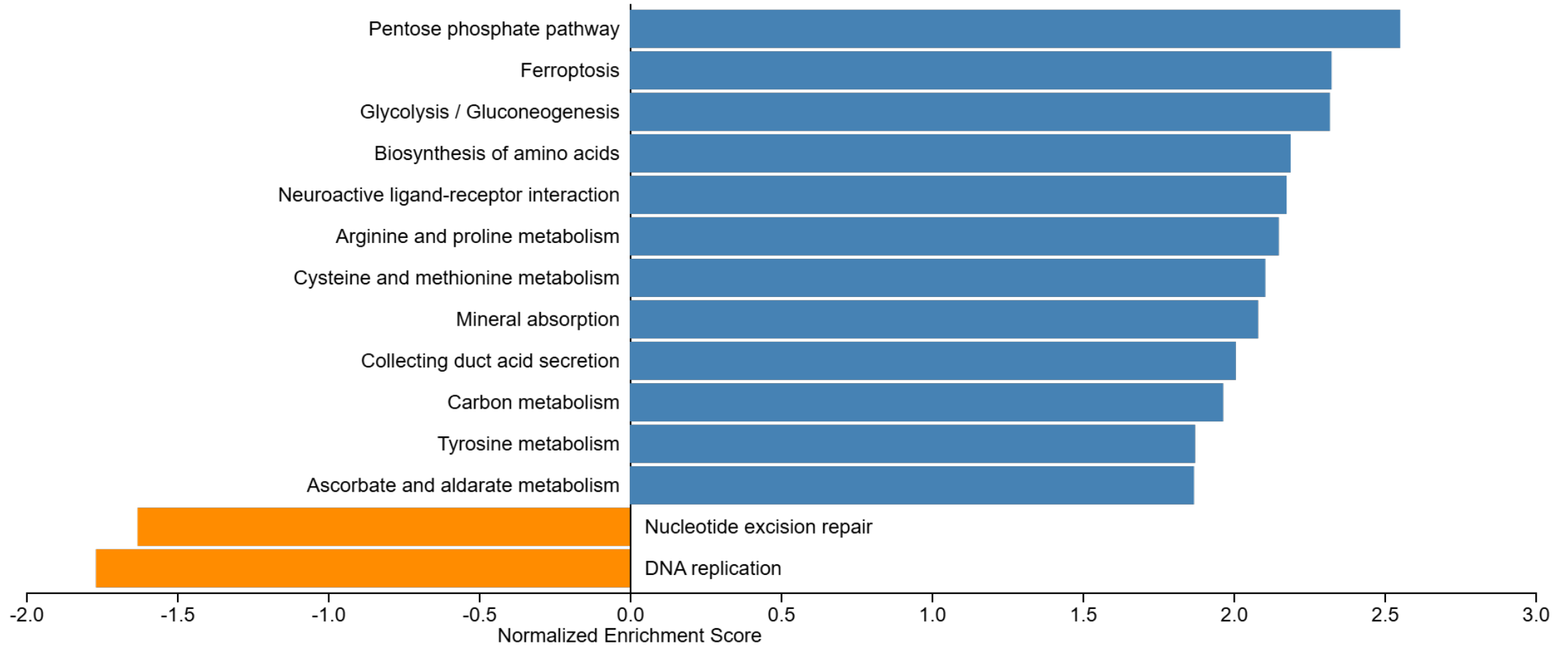
GSEA (Gene Set Enrichment Analysis) - pathway_Reactome



CSCs – HMPSNE

GSEA (Gene Set Enrichment Analysis) - pathway_KEGG

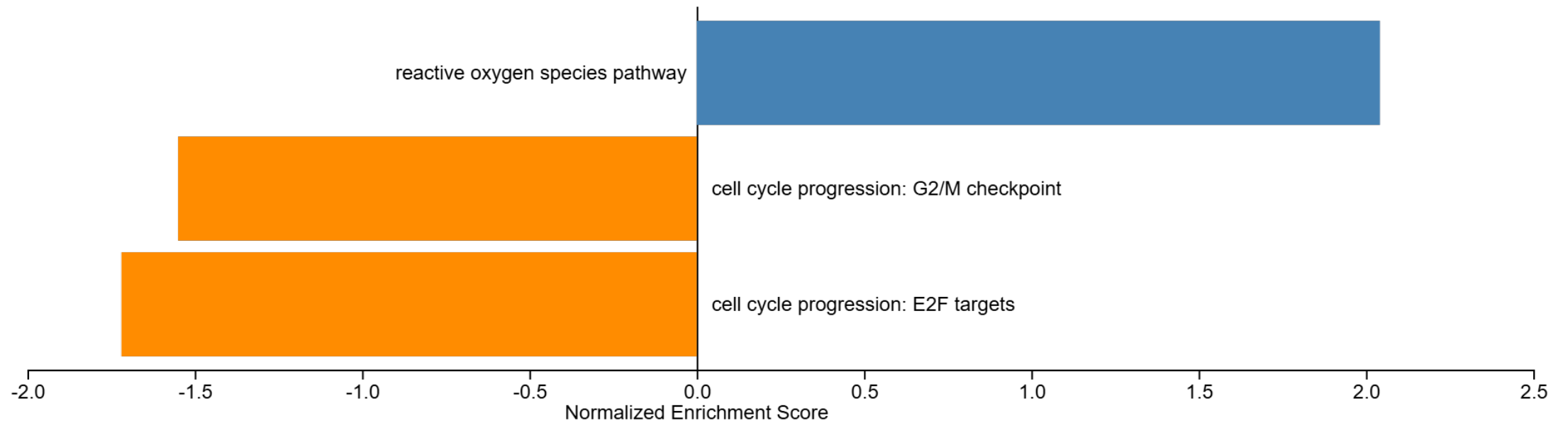
■ FDR ≤ 0.05 ■ FDR > 0.05



CSCs – HMPSNE

GSEA (Gene Set Enrichment Analysis) - community-contributed_Hallmark50

■ FDR ≤ 0.05 ■ FDR > 0.05

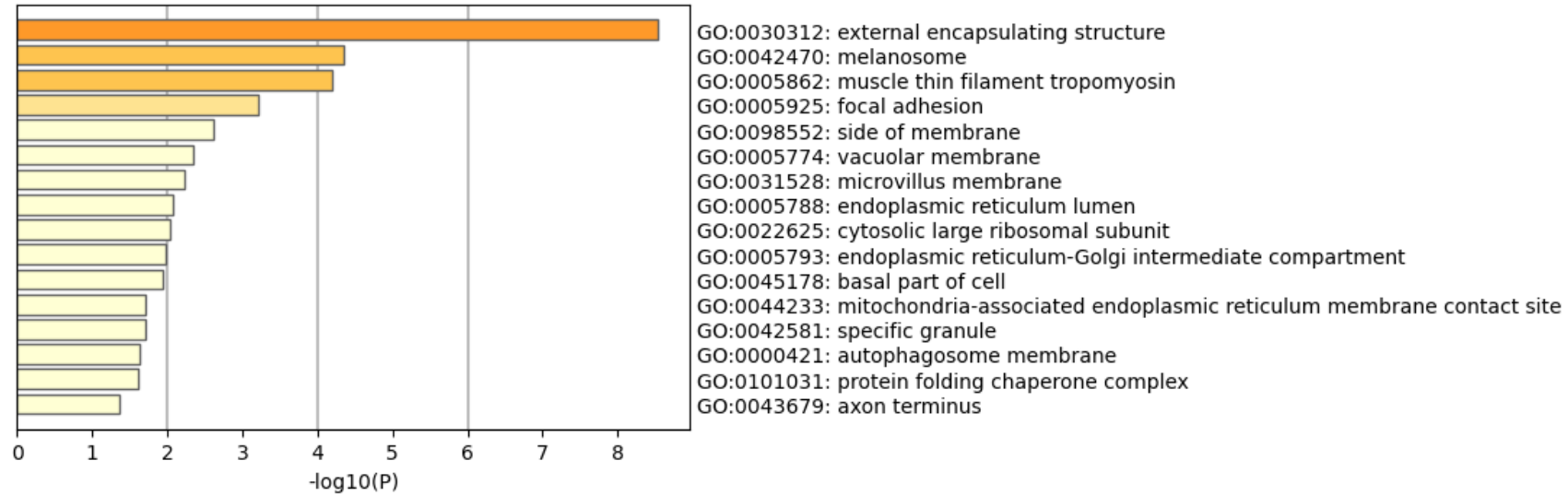


CSCs – HMPSNE

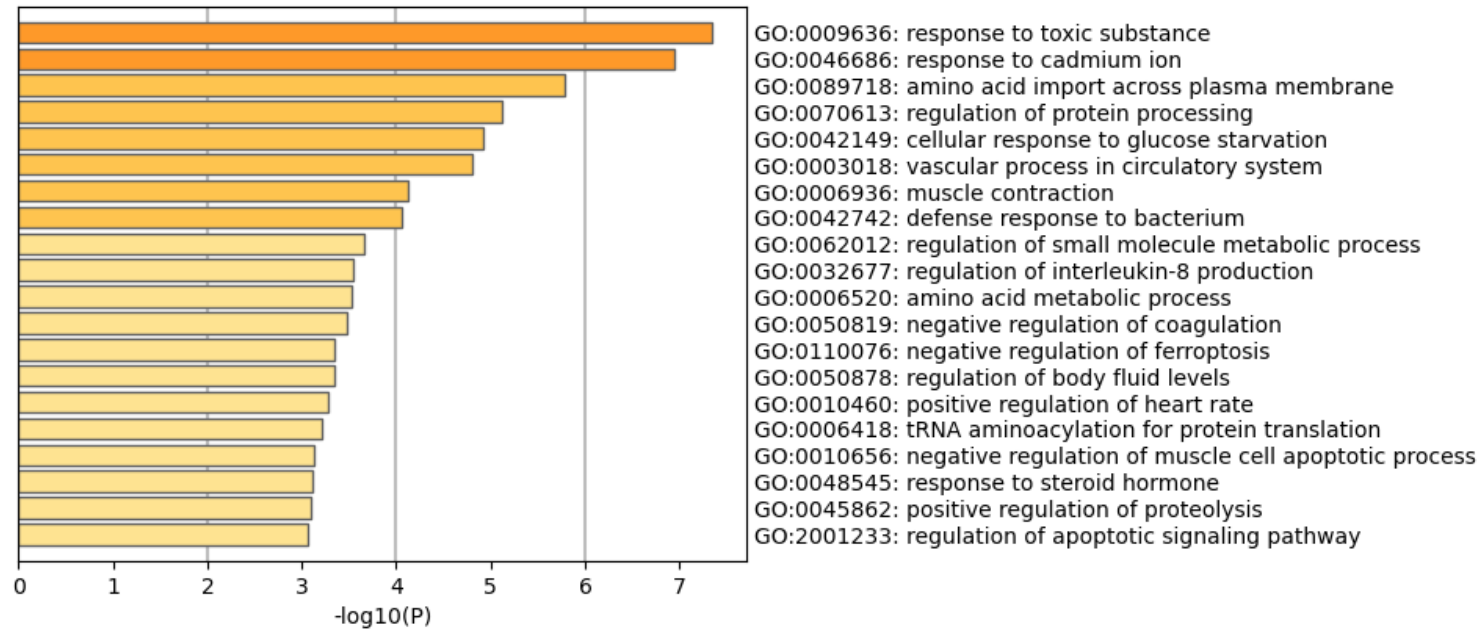
ORA (Over-Representation Analysis)

Activation in treated CSCs

GO Cellular Components



GO Biological Process

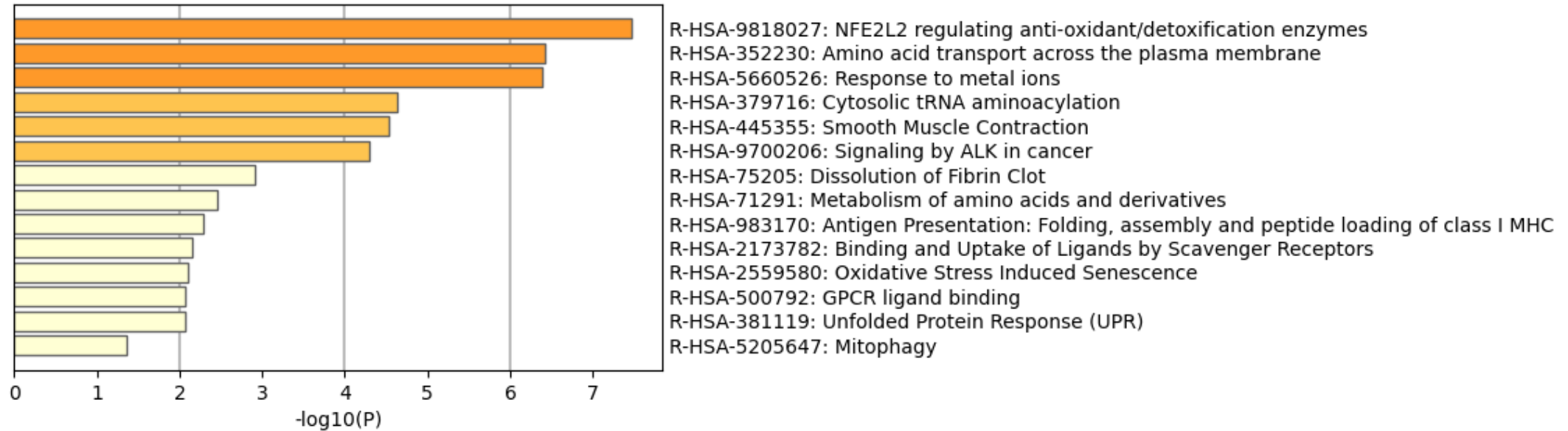


CSCs – HMPSNE

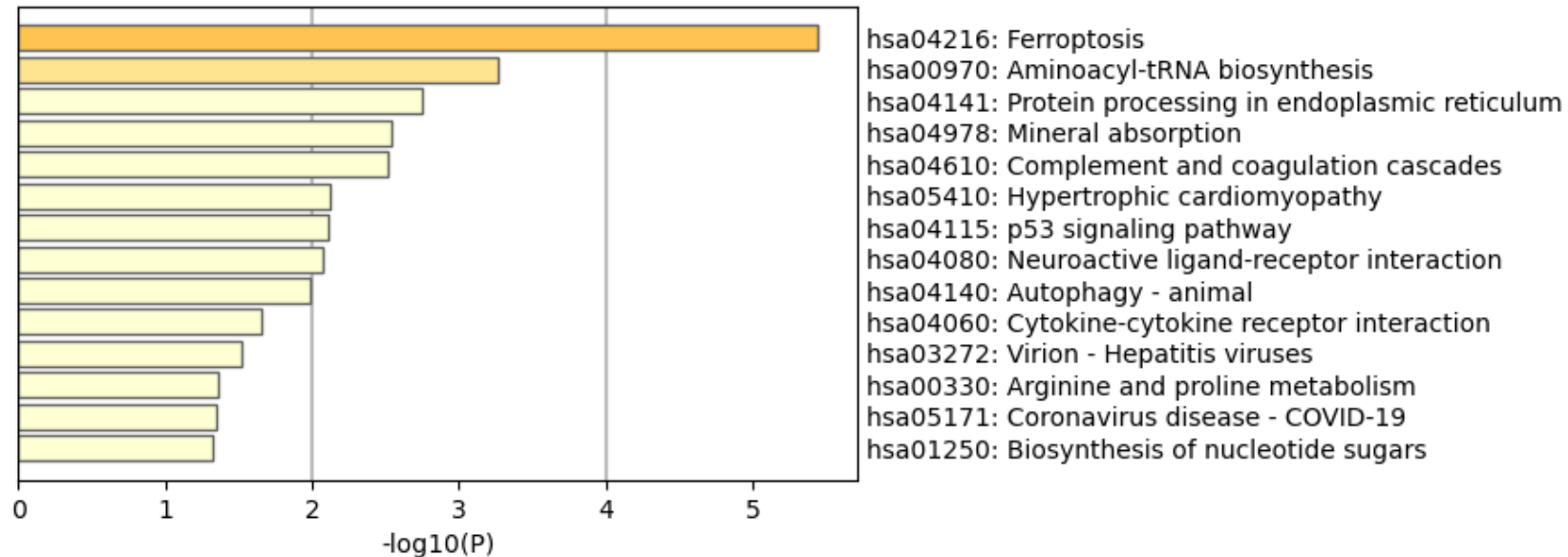
ORA (Over-Representation Analysis)

Activation in treated CSCs

Reactome Gene Sets



KEGG Pathway

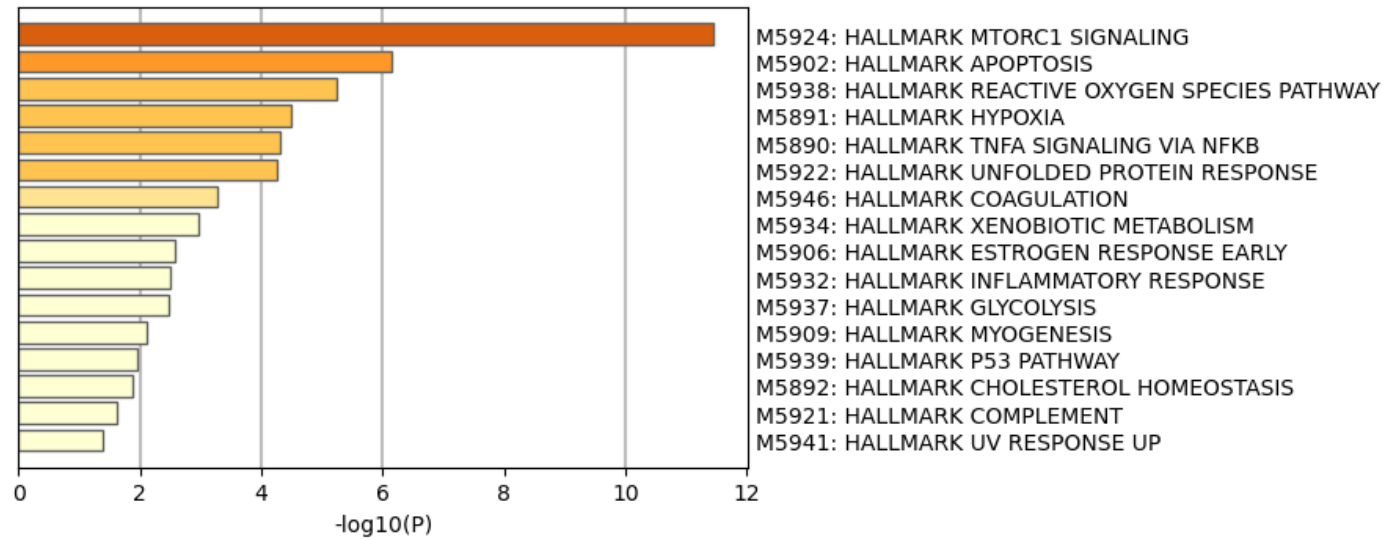


CSCs – HMPSNE

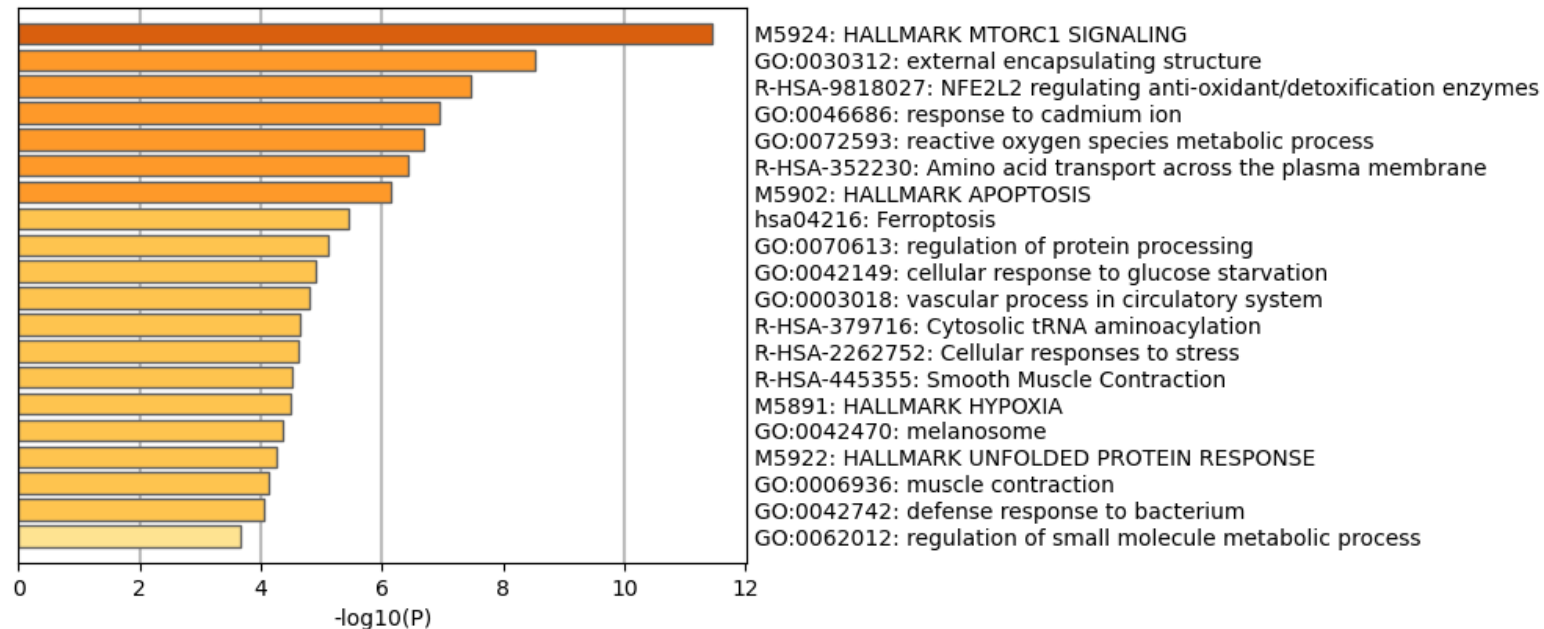
ORA (Over-Representation Analysis)

Activation in treated CSCs

Hallmark Gene Sets



Combined GO

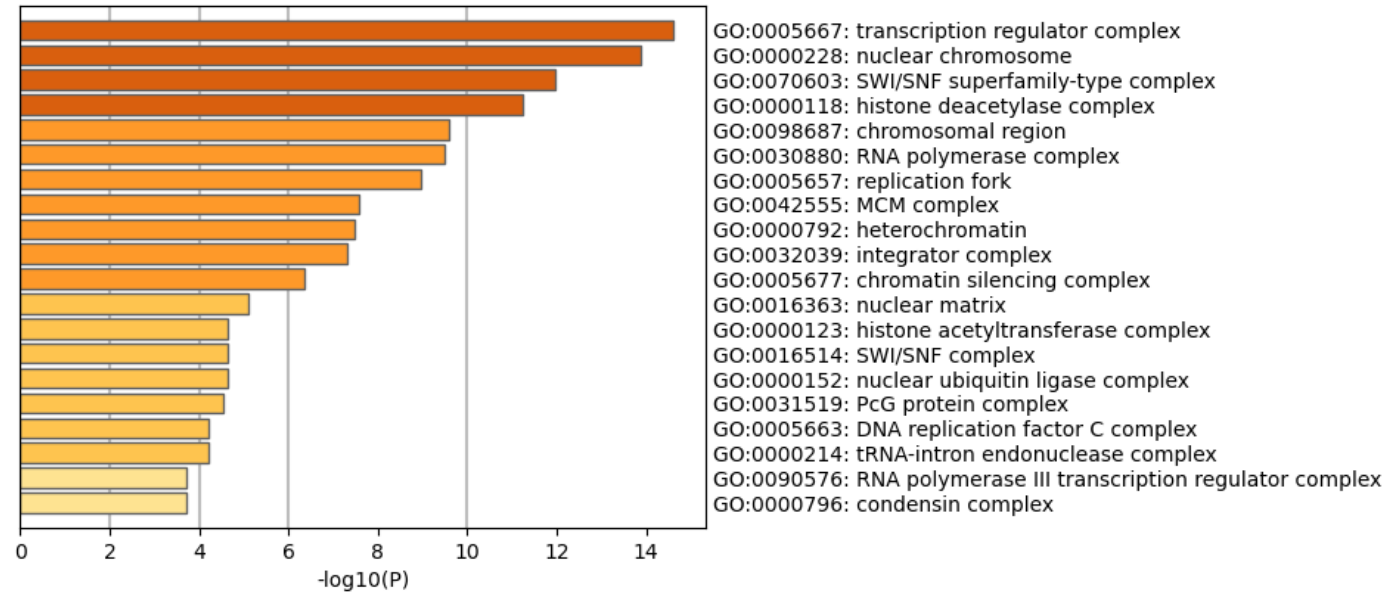


CSCs – HMPSNE

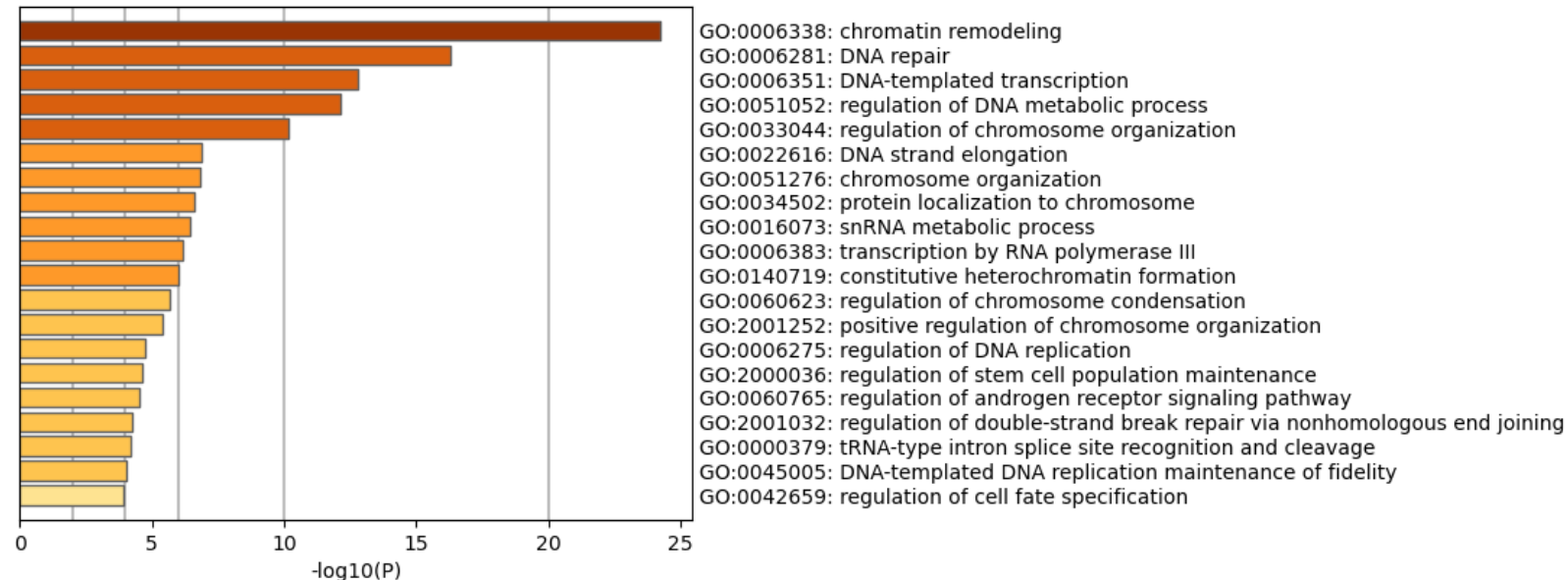
ORA (Over-Representation Analysis)

Inhibition in treated CSCs

GO Cellular Components



GO Biological Process

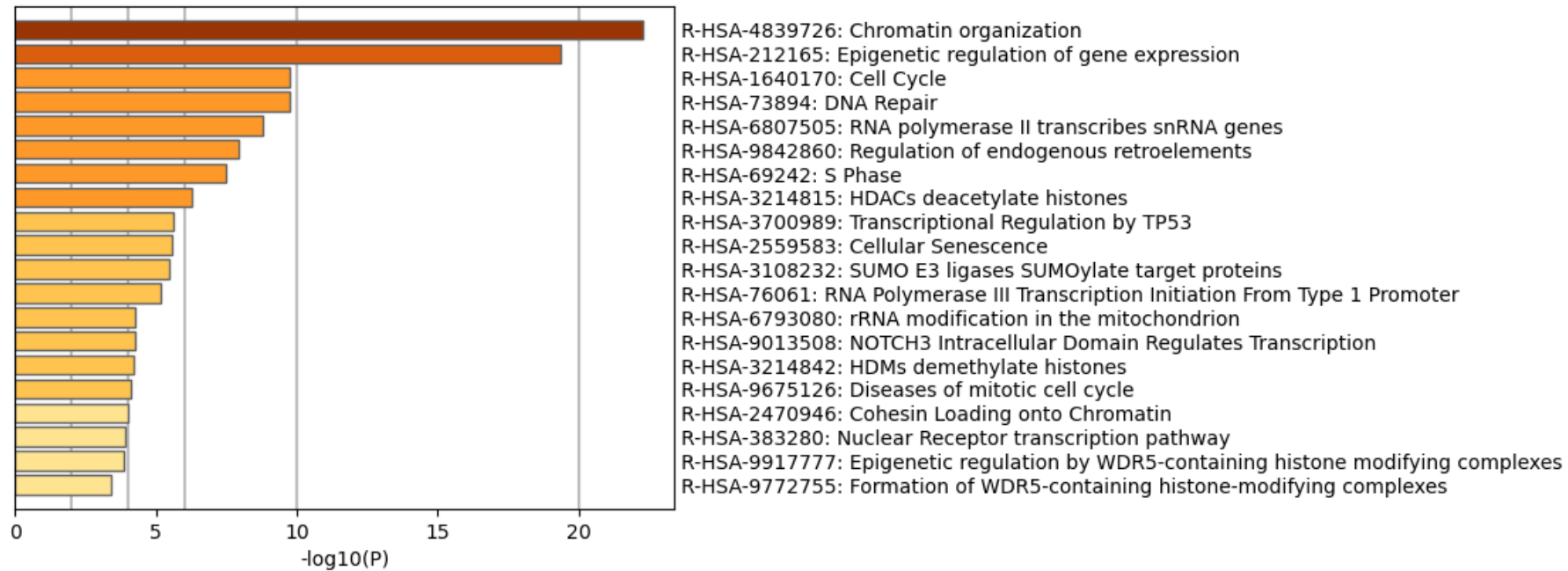


CSCs – HMPSNE

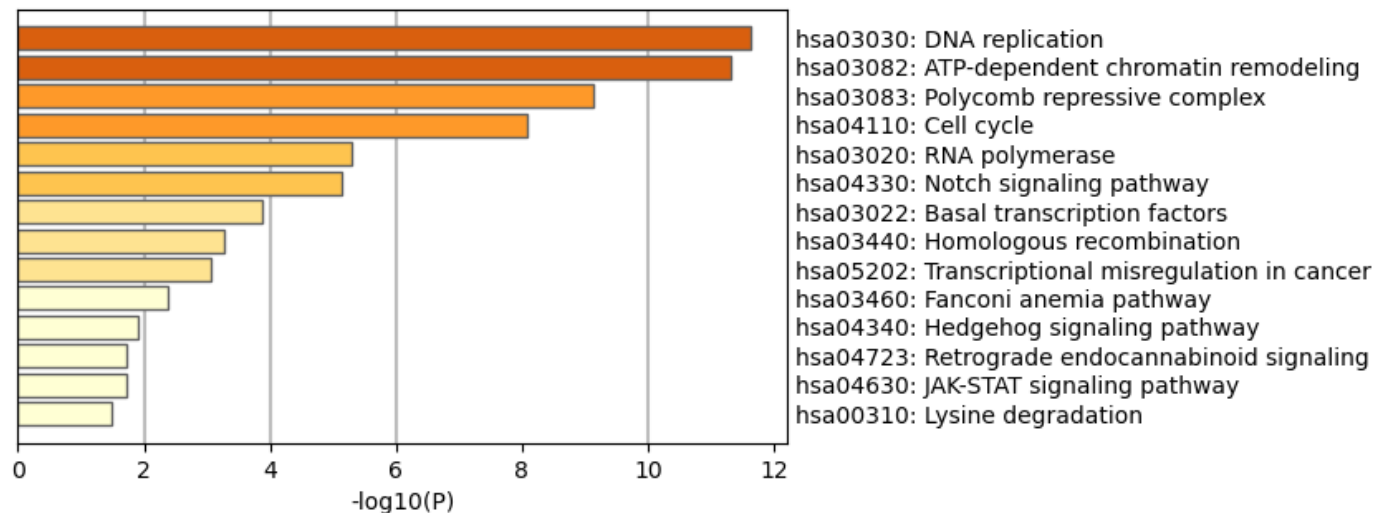
ORA (Over-Representation Analysis)

Inhibition in treated CSCs

Reactome Gene Sets



KEGG Pathway

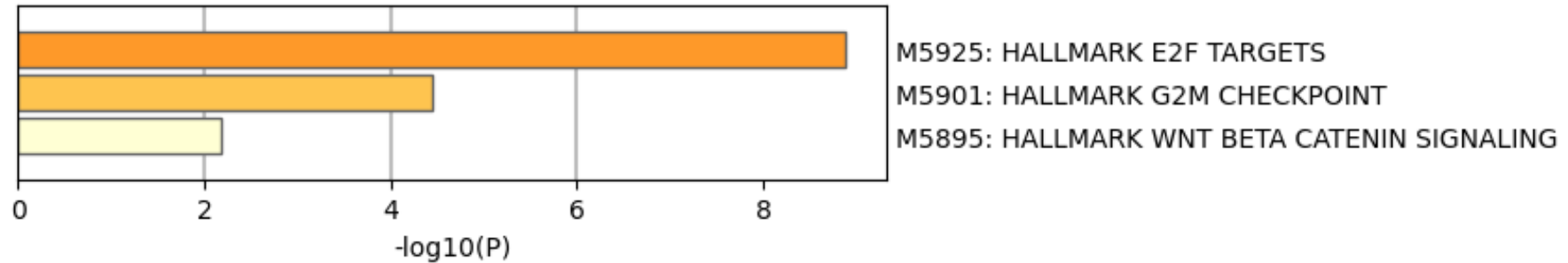


CSCs – HMPSNE

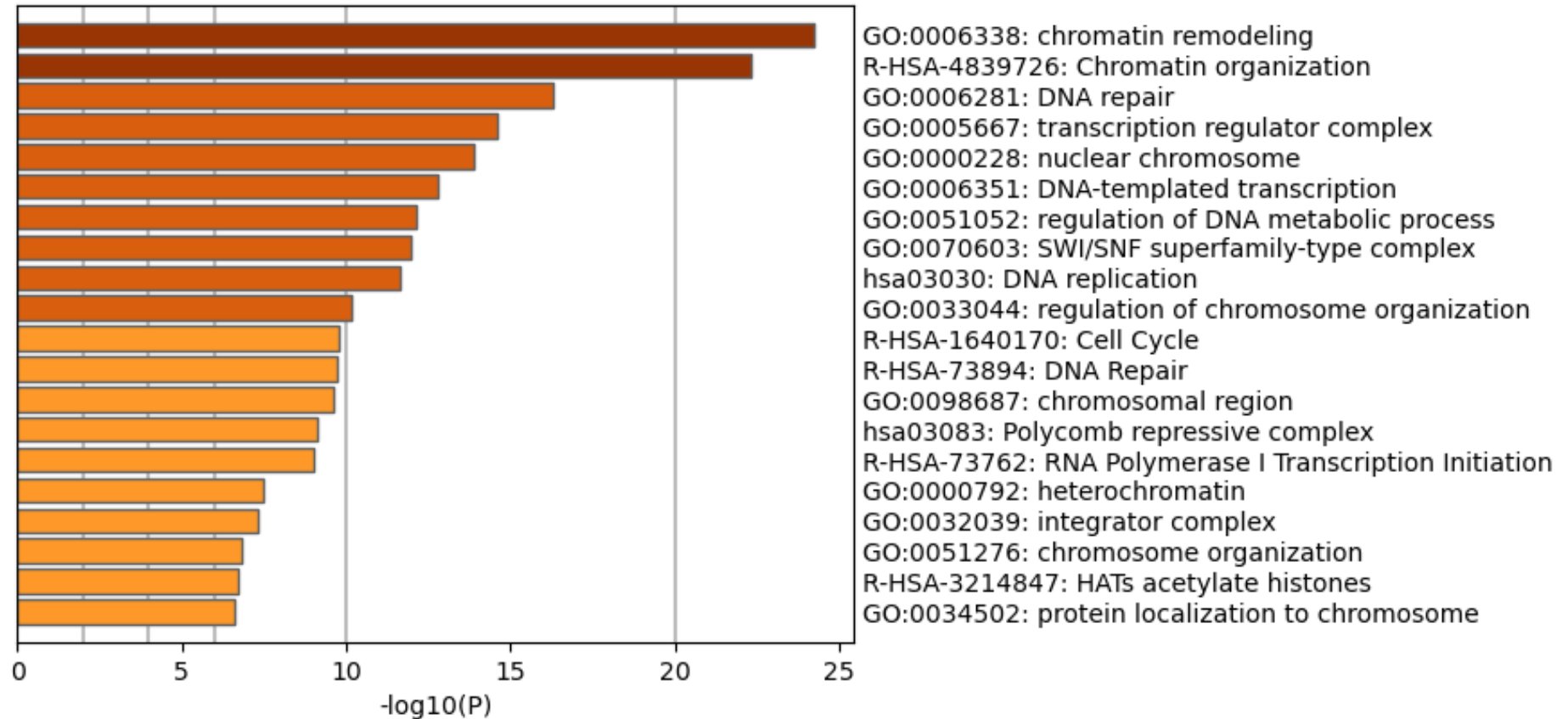
ORA (Over-Representation Analysis)

Inhibition in treated CSCs

Hallmark Gene Sets

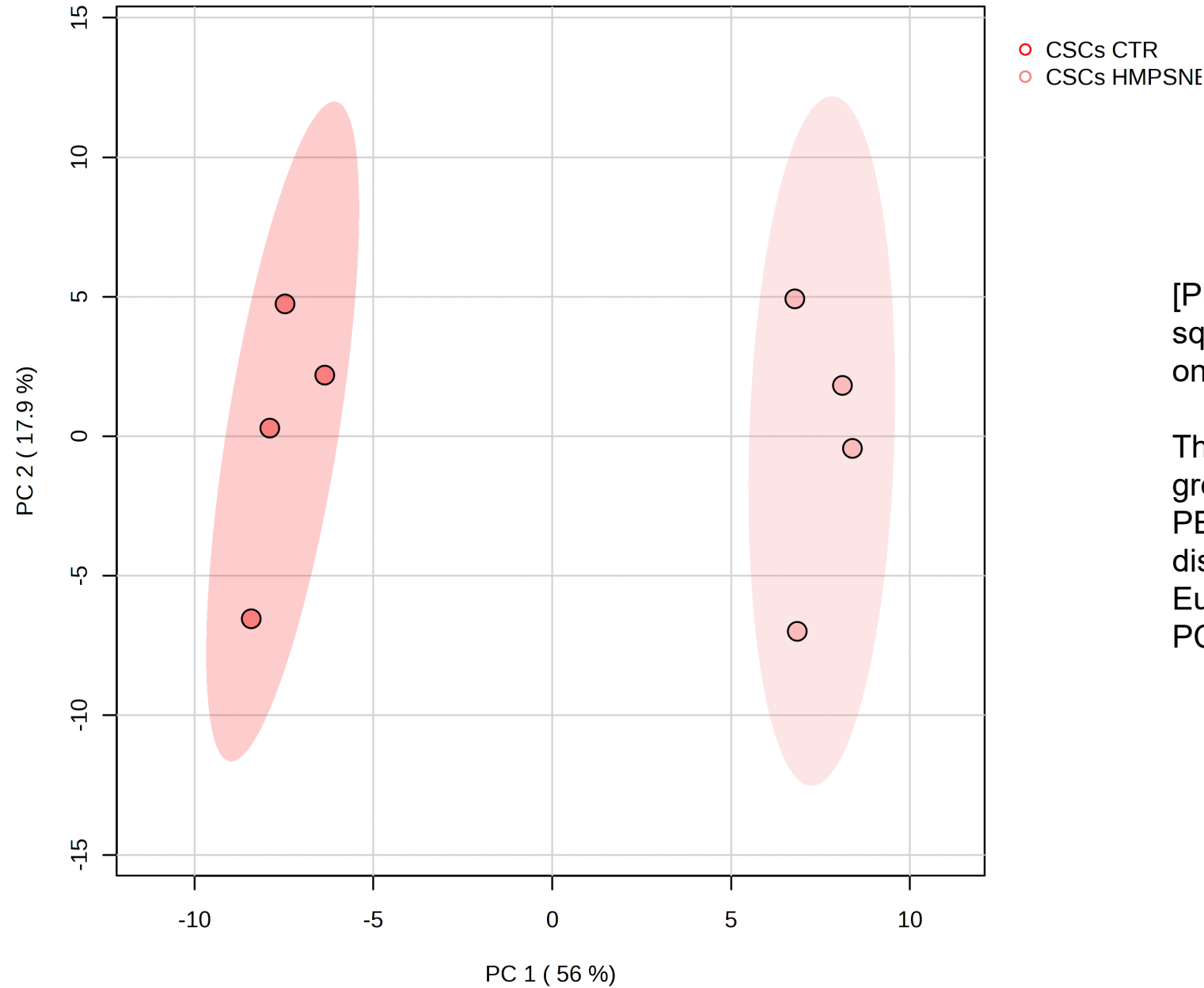


Combined GO



CSCs-HMPSNE vs CSCs-CTR Metabolomics

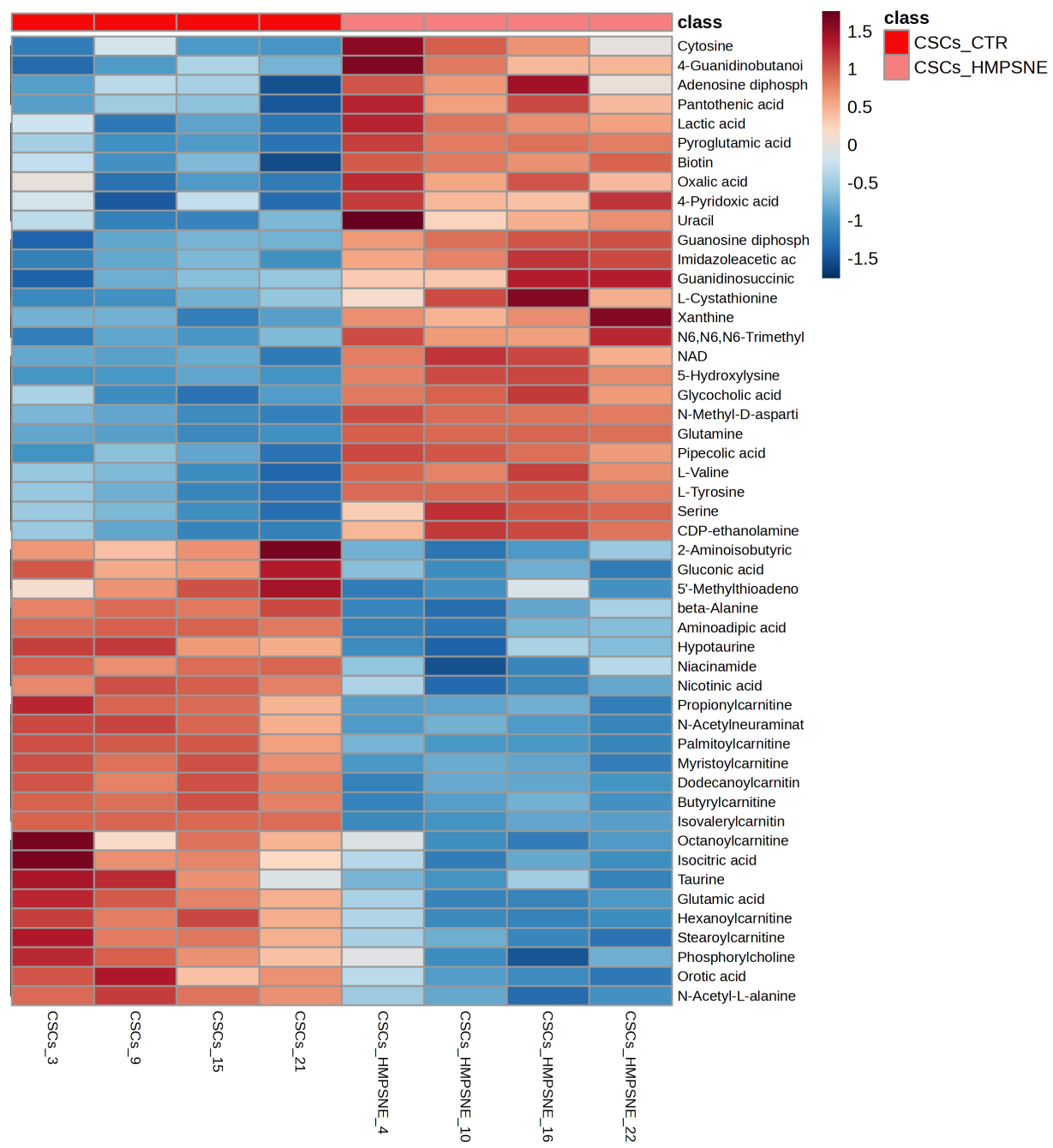
CSCs – HMPSNE



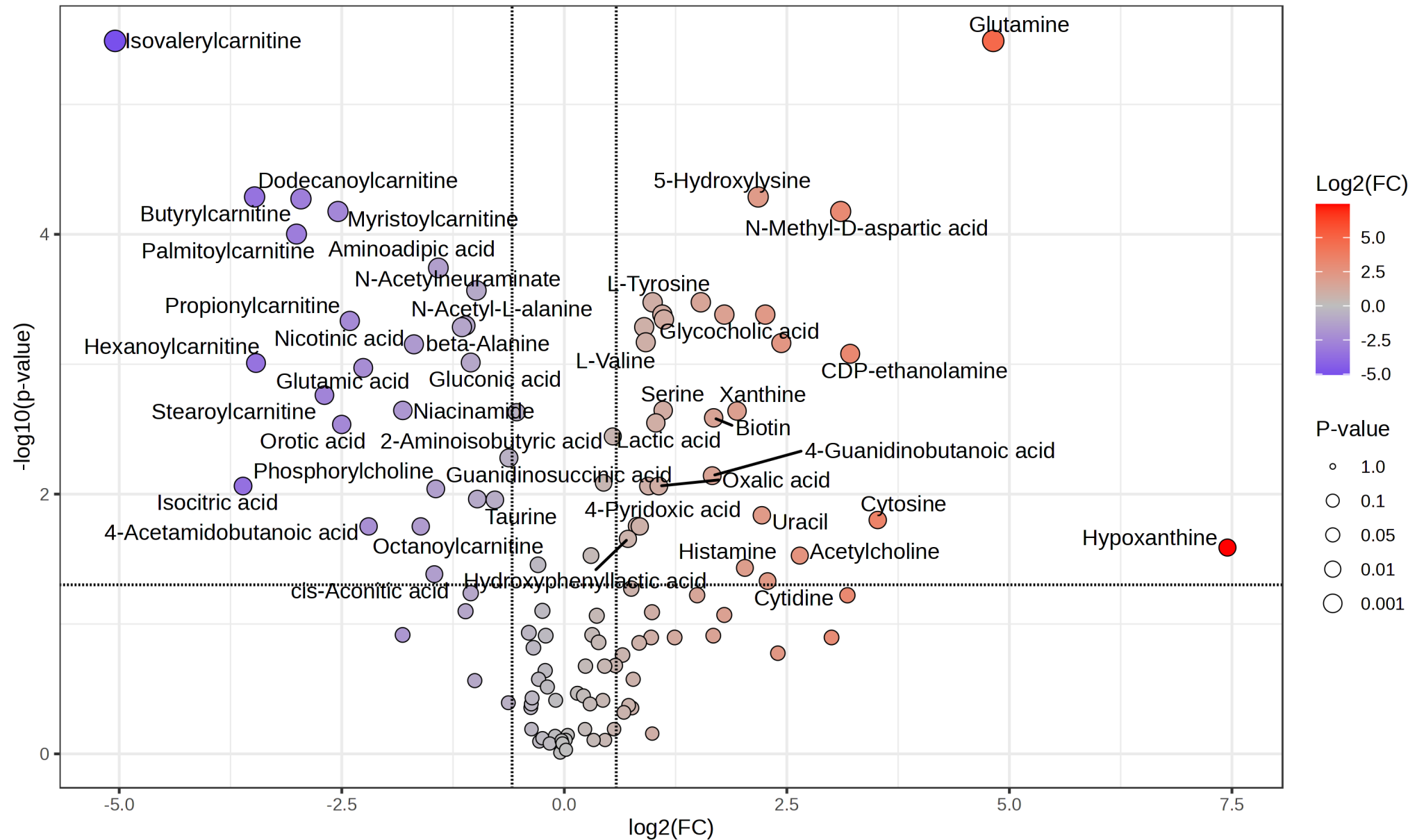
[PERMANOVA] F-value: 18.051; R-squared: 0.75053; p-value (based on 999 permutations): 0.024

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.

CSCs – HMPSNE



CSCs – HMPSNE

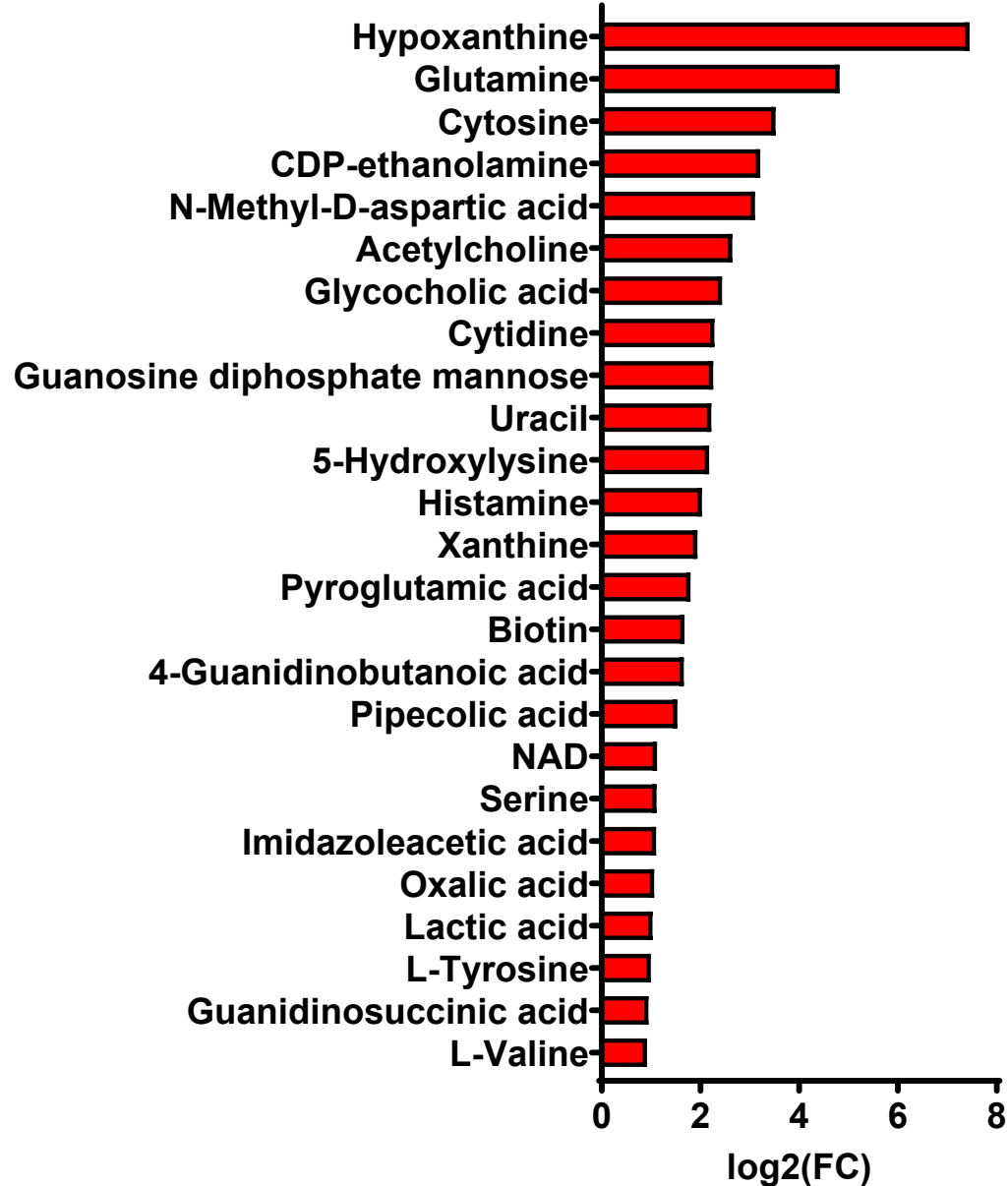


Fold change (FC) threshold: 1.5

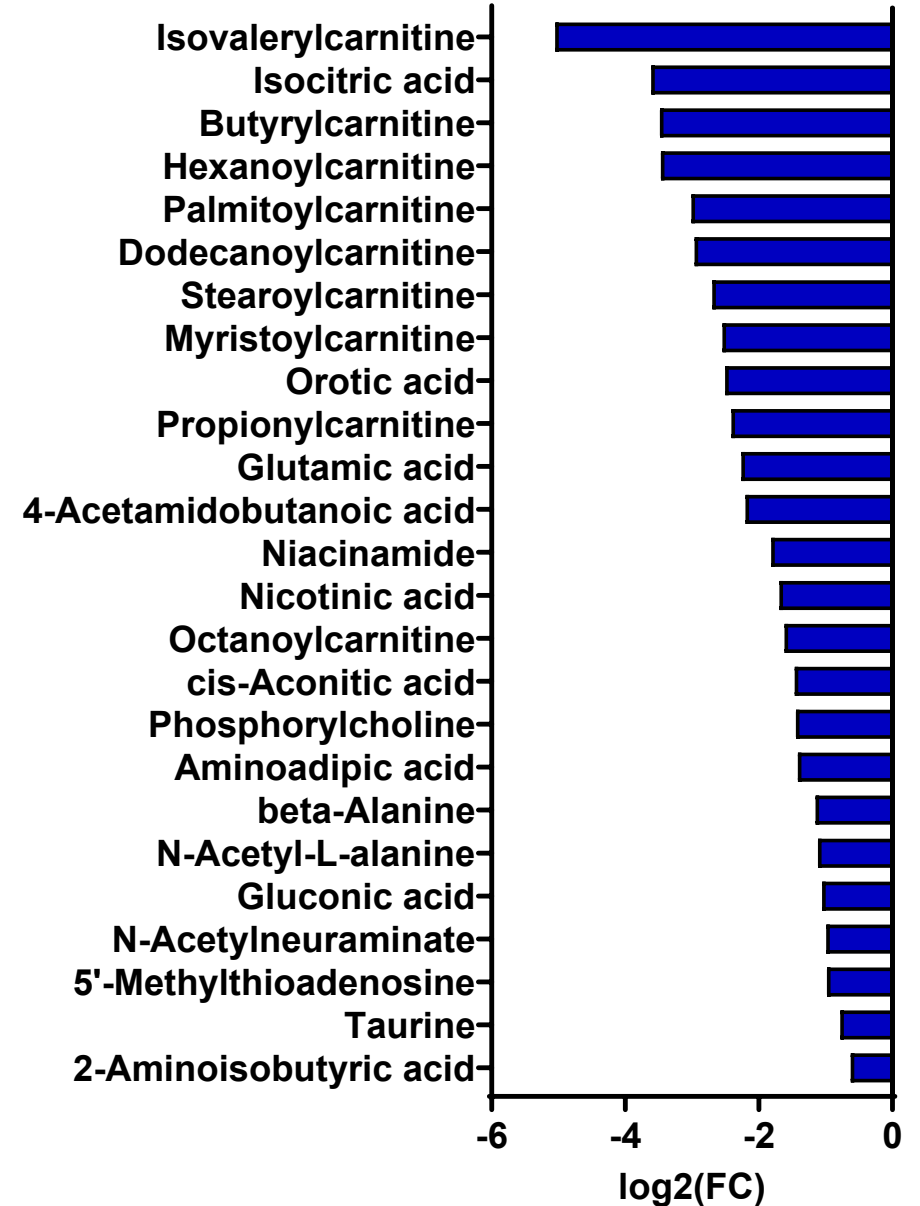
P-value threshold: 0.05

CSCs – HMPSNE

Top 25_Increased metabolites in treated cells



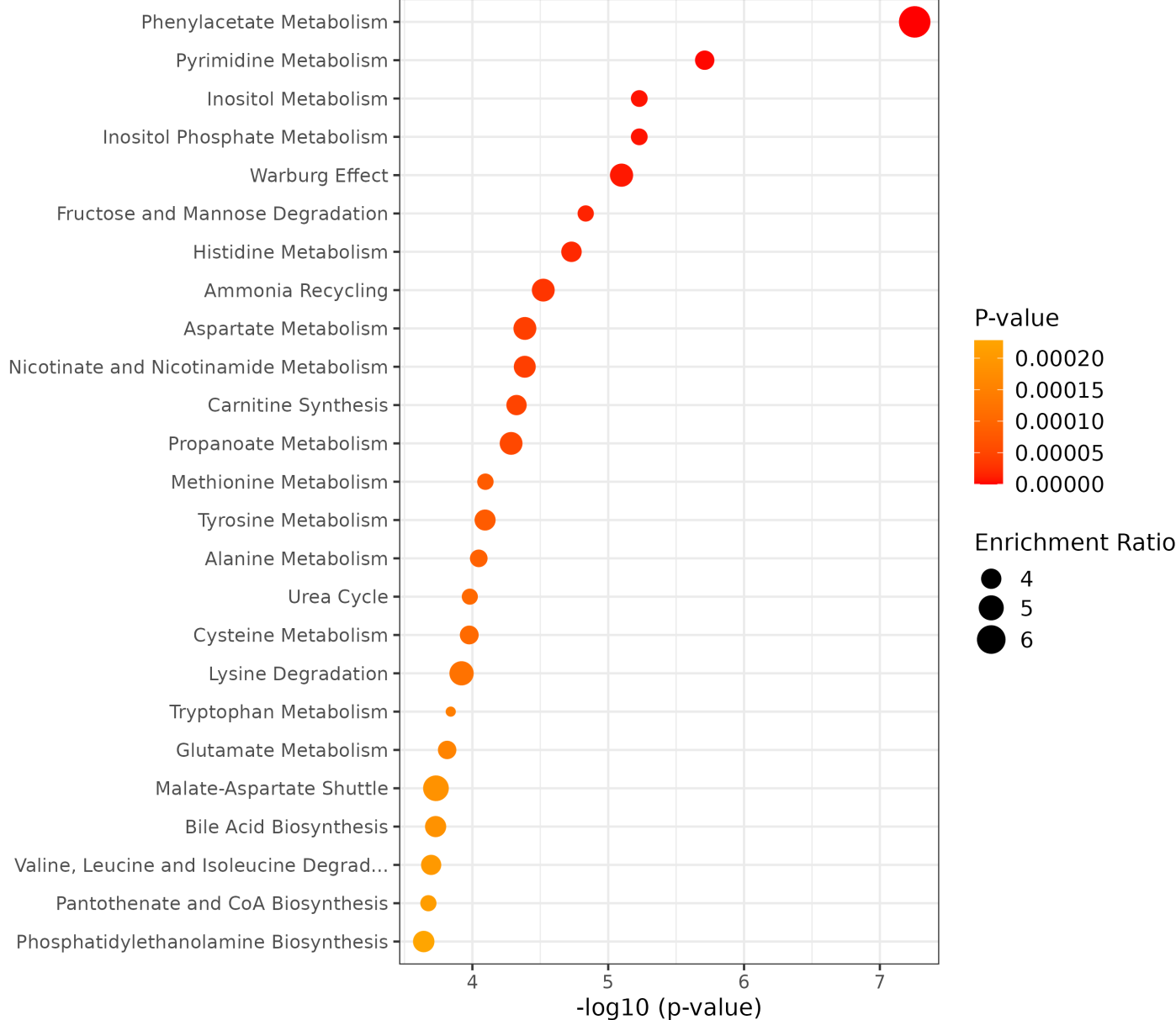
Top 25_Decreased metabolites in treated cells



CSCs – HMPSNE

Quantitative Enrichment Analysis (QEA)

Overview of Enriched Metabolite Sets (Top 25)

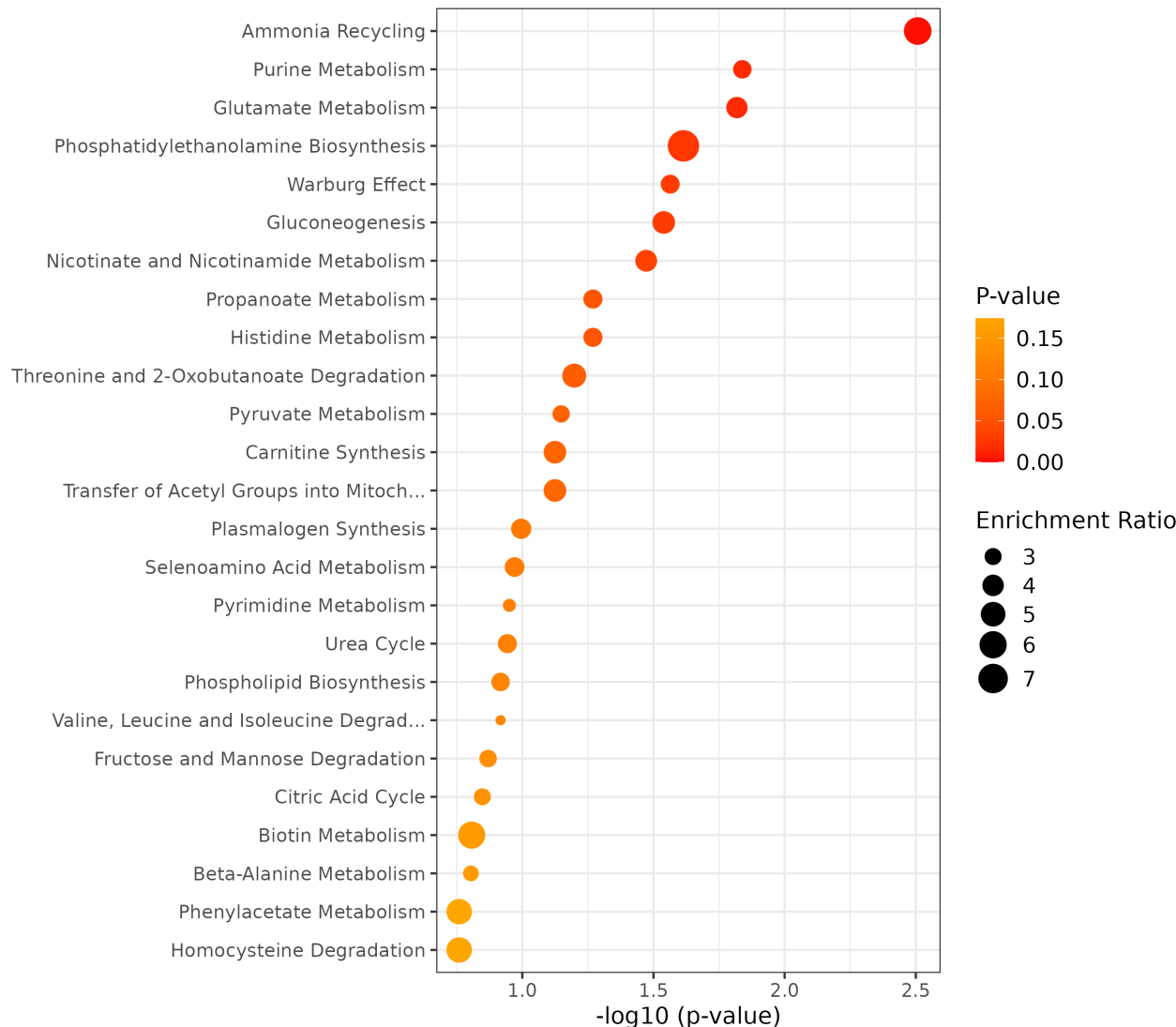


CSCs – HMPSNE

Over-Representation Analysis (ORA)

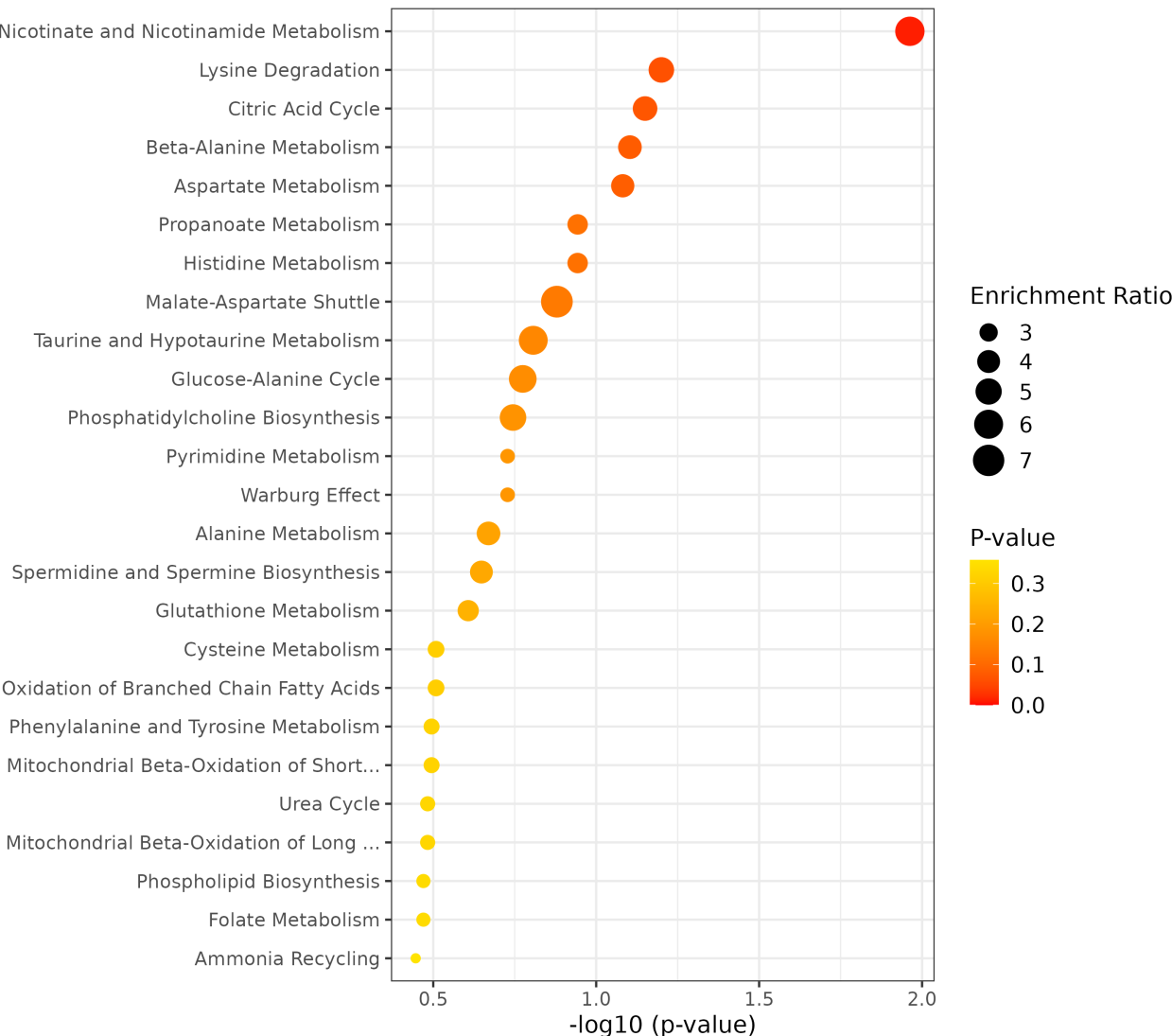
Activation in treated cells

Overview of Enriched Metabolite Sets (Top 25)



Inhibition in treated cells

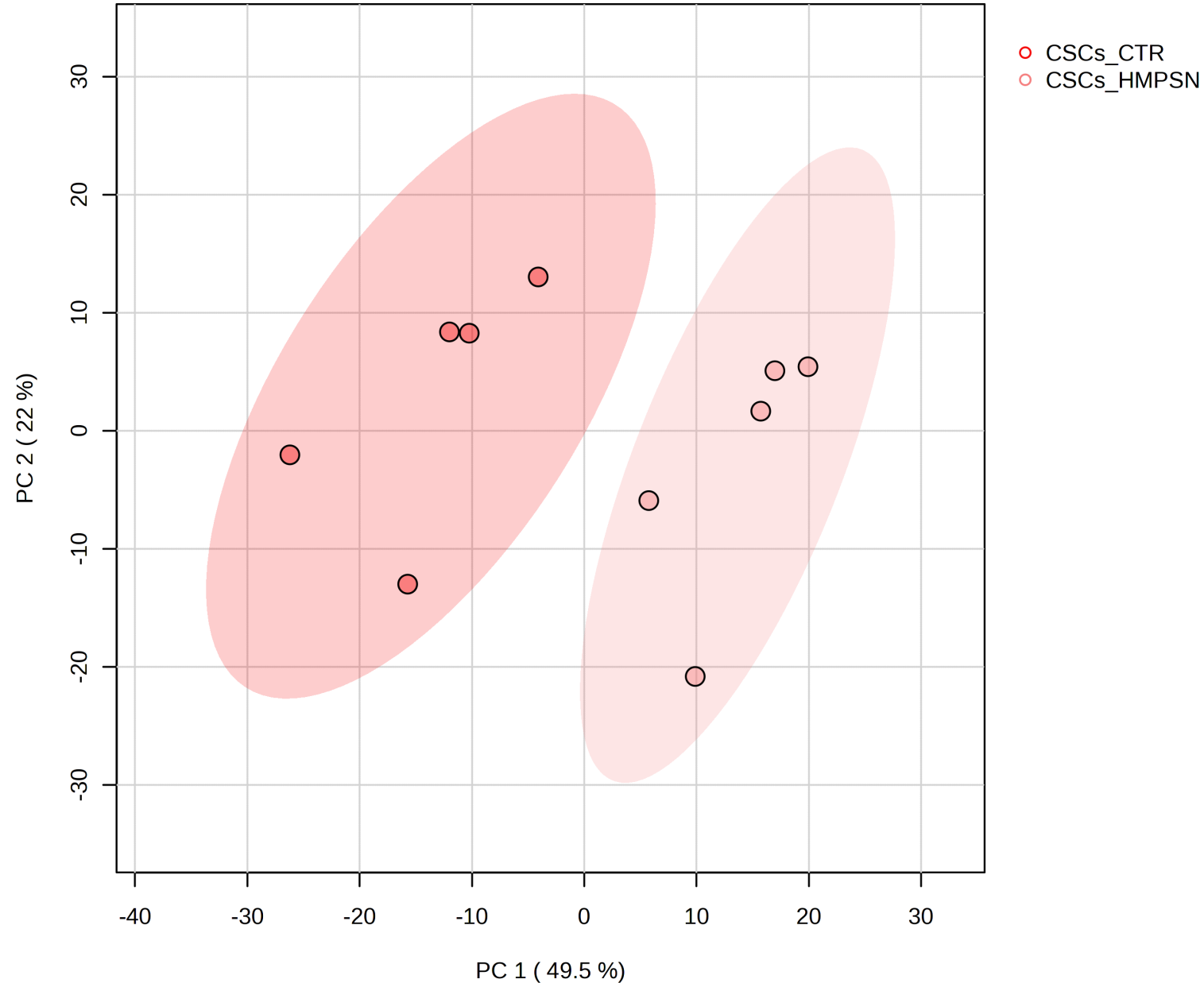
Overview of Enriched Metabolite Sets (Top 25)



CSCs-HMPSNE vs CSCs-CTR

Lipidomics

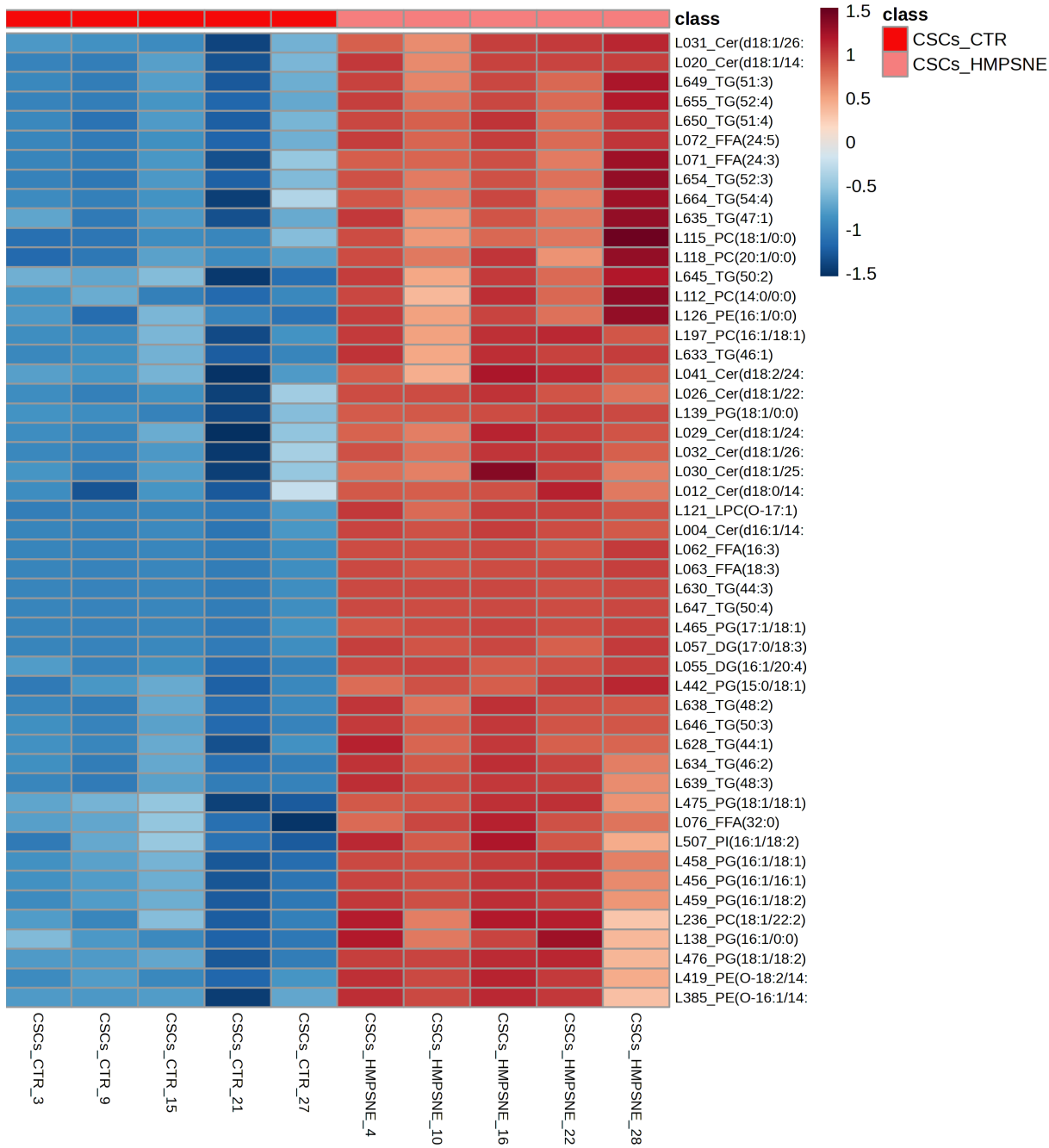
CSCs – HMPSNE



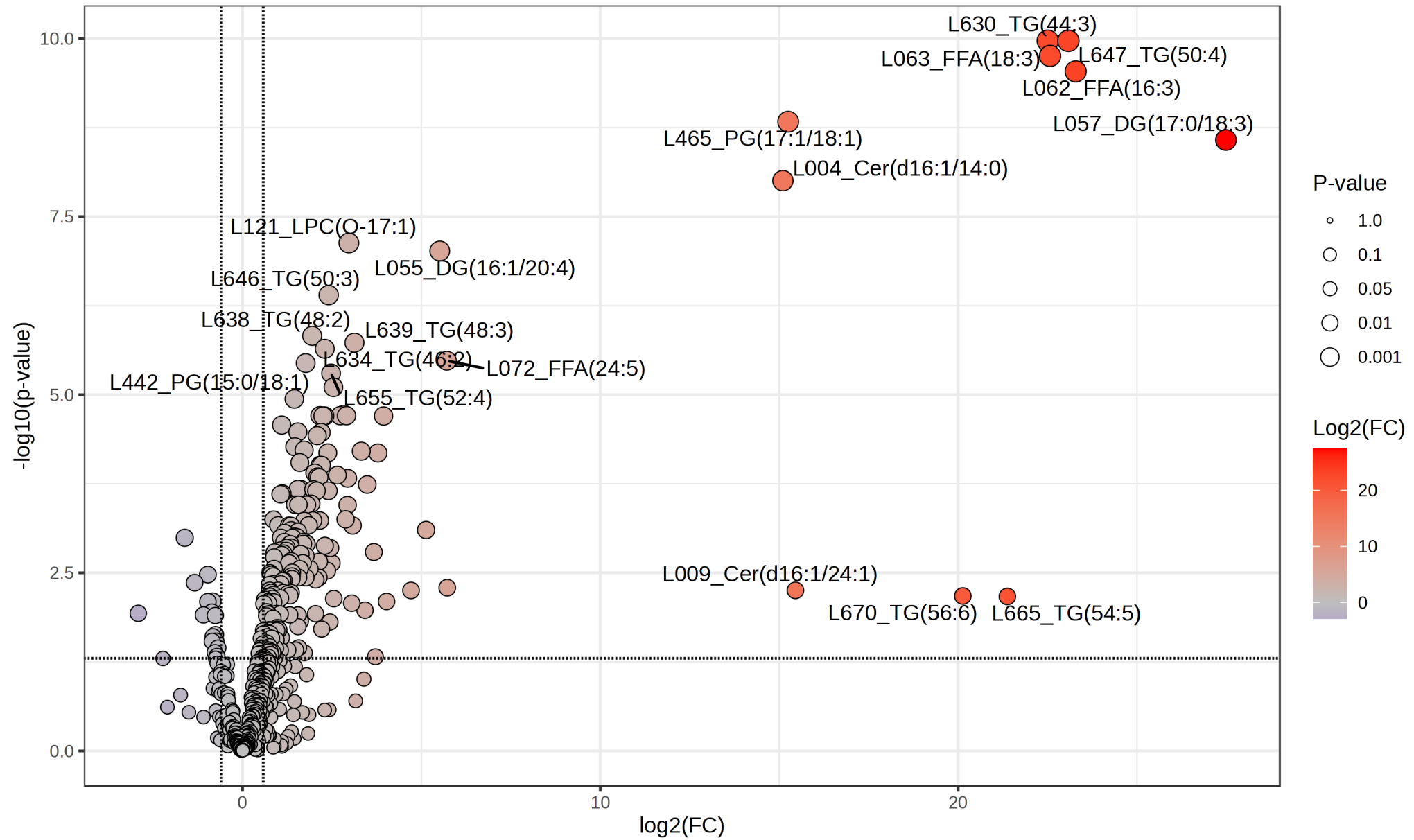
[PERMANOVA] F-value: 11.801;
R-squared: 0.59599; p-value
(based on 999 permutations):
0.008

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.

CSCs – HMPSNE



CSCs – HMPSNE

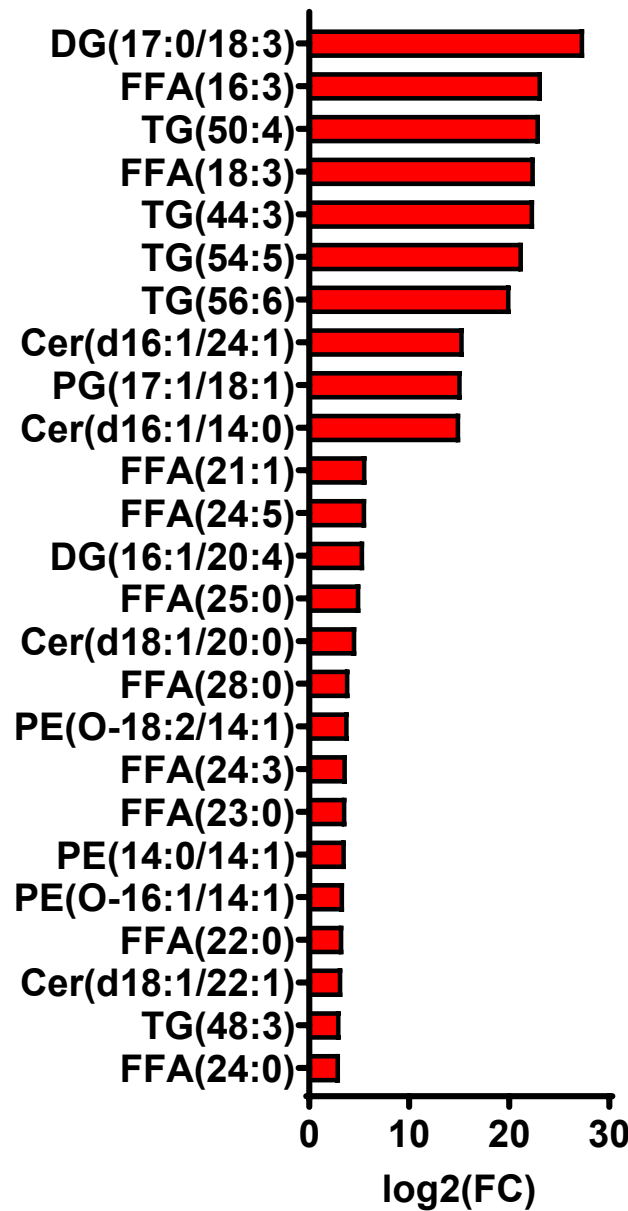


Fold change (FC) threshold: 1.5

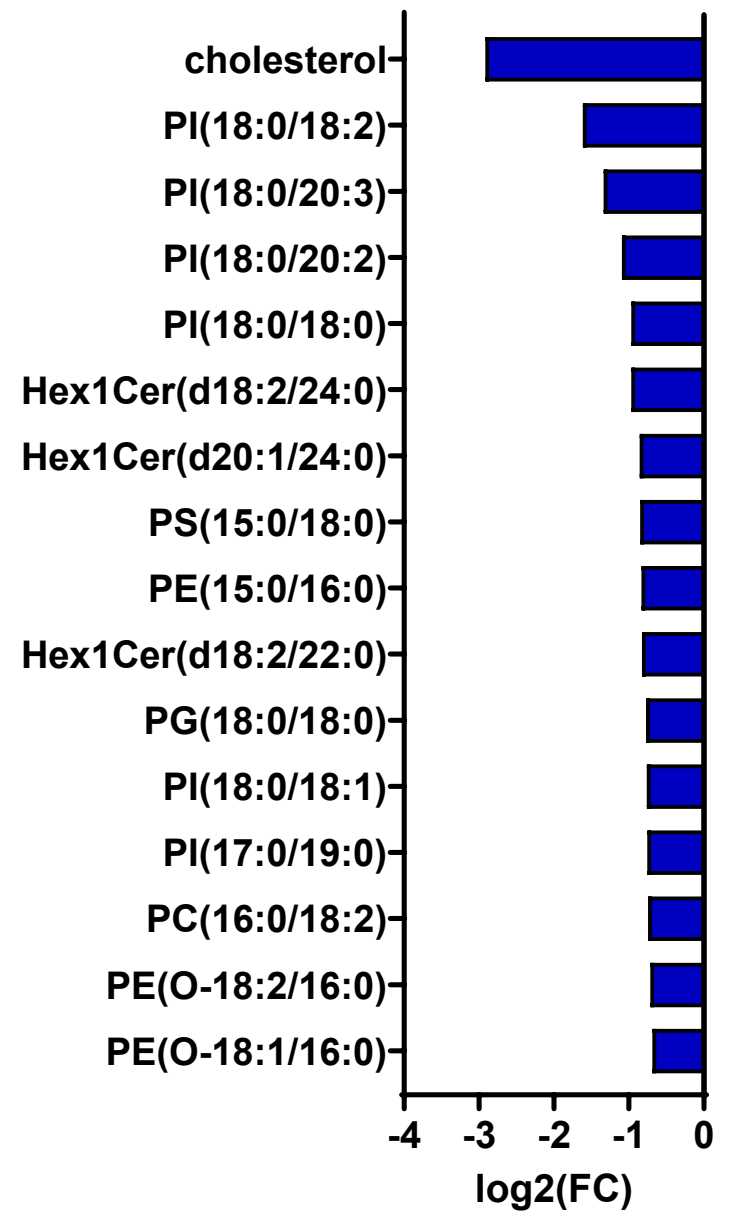
P-value threshold: 0.05

CSCs – HMPSNE

Top 25_Increased lipids in treated CSCs



Top 25_Decreased lipids in treated CSCs

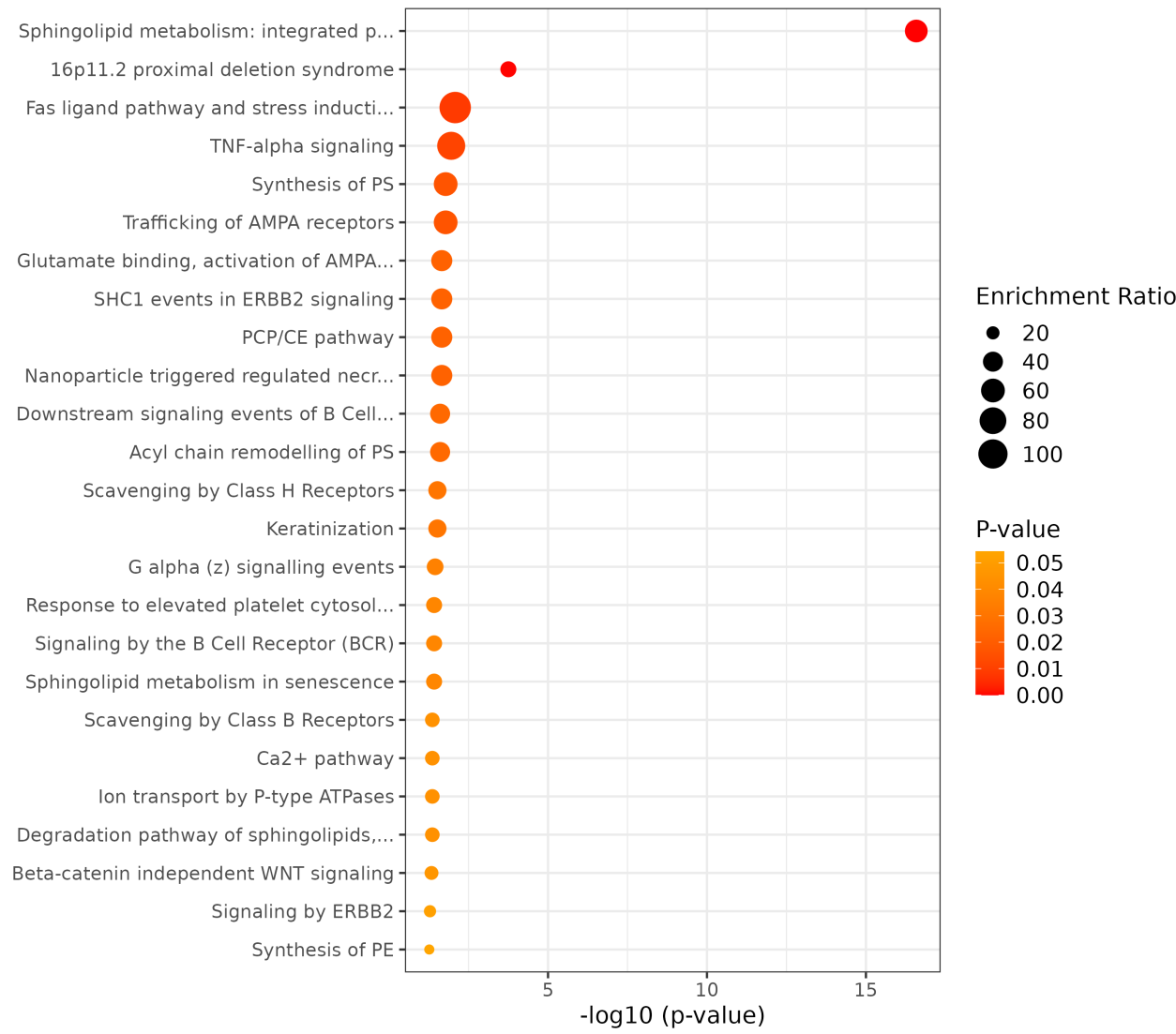


CSCs – HMPSNE

Over-Representation Analysis (ORA)

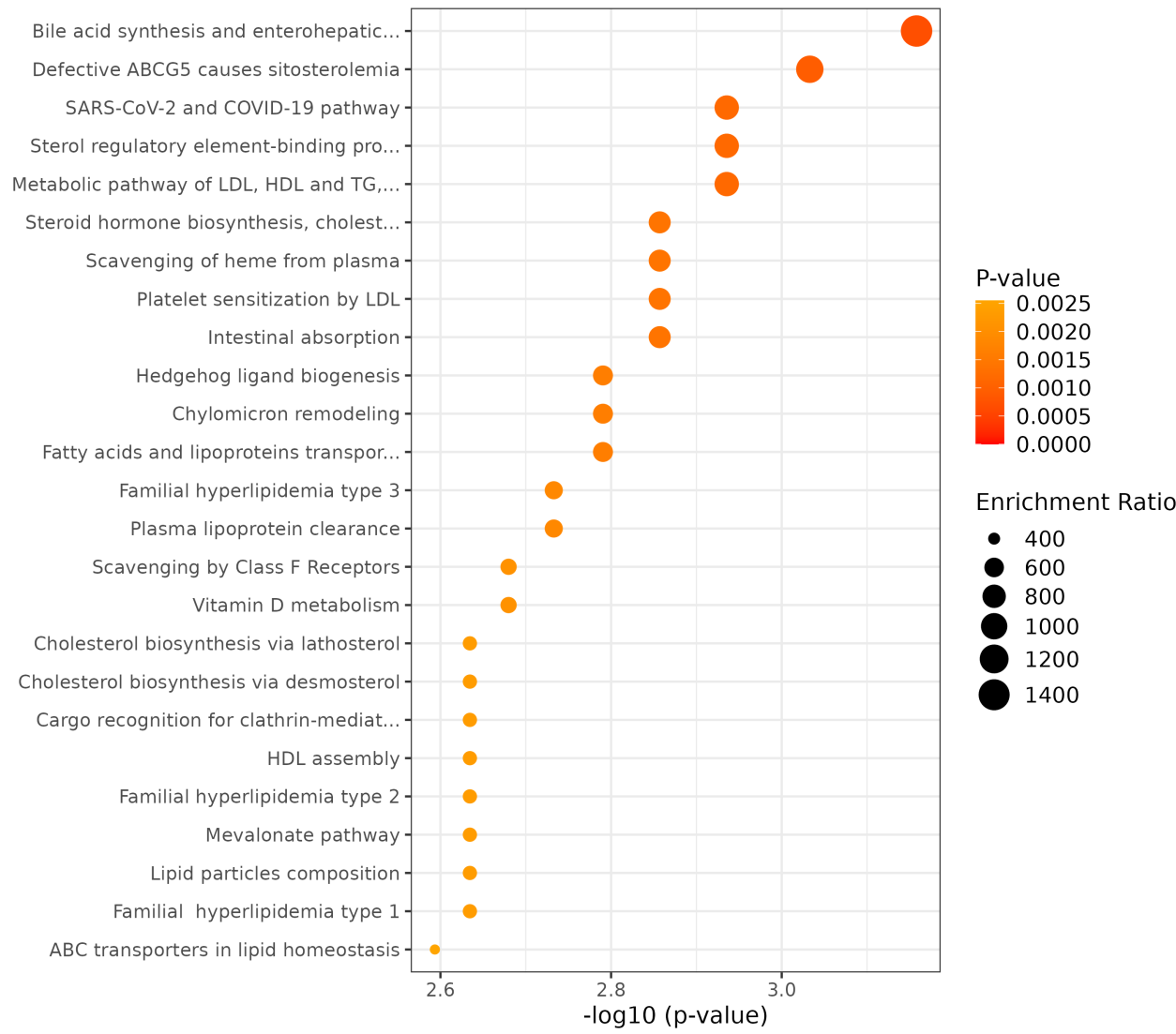
Activation in treated CSCs

Overview of Enriched Metabolite Sets (Top 25)



Inhibition in treated CSCs

Overview of Enriched Metabolite Sets (Top 25)



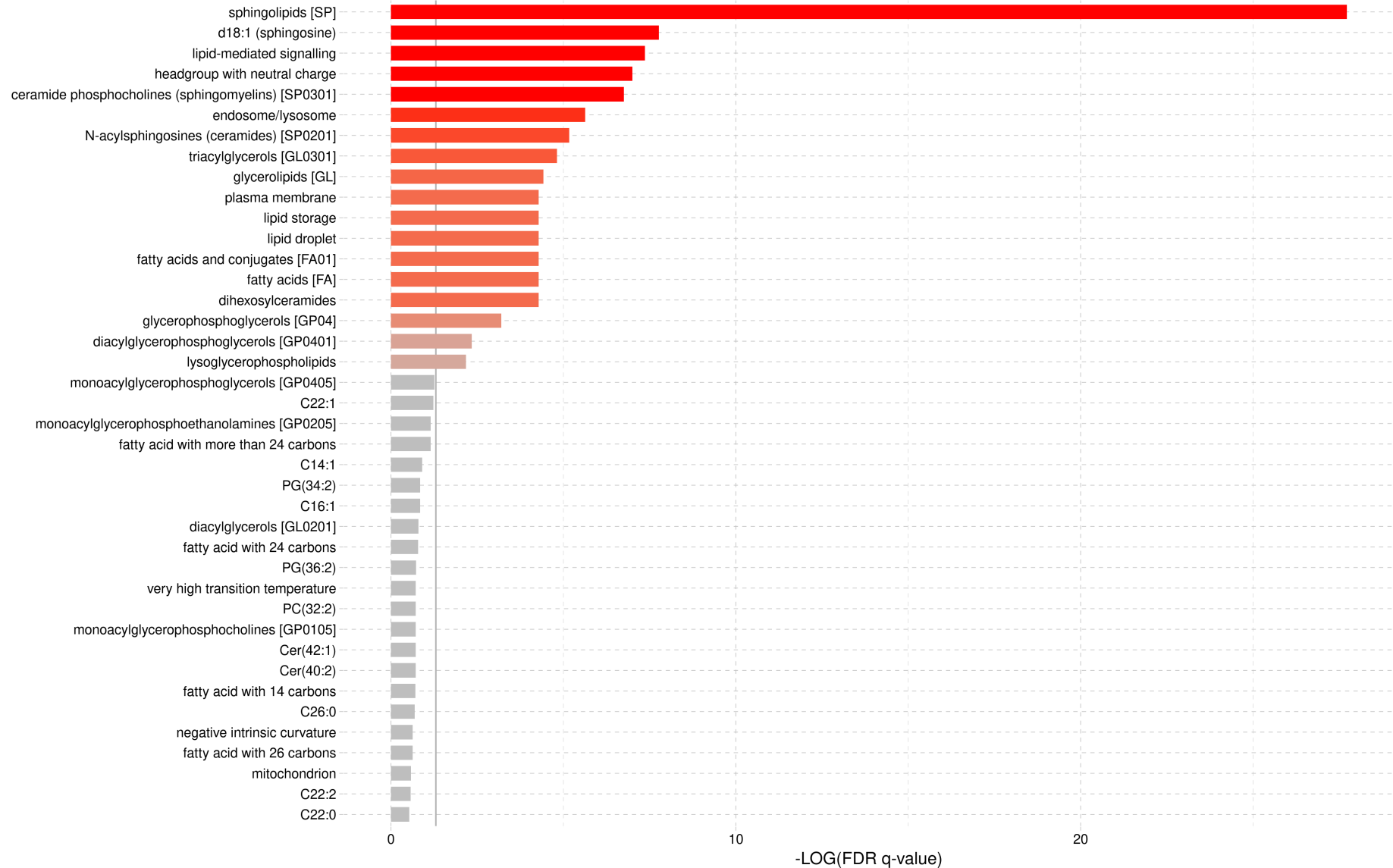
CSCs – HMPSNE

LION enrichment analysis – *ranking mode*

Increased in treated CSCs

LION enrichment analysis *ranking mode*

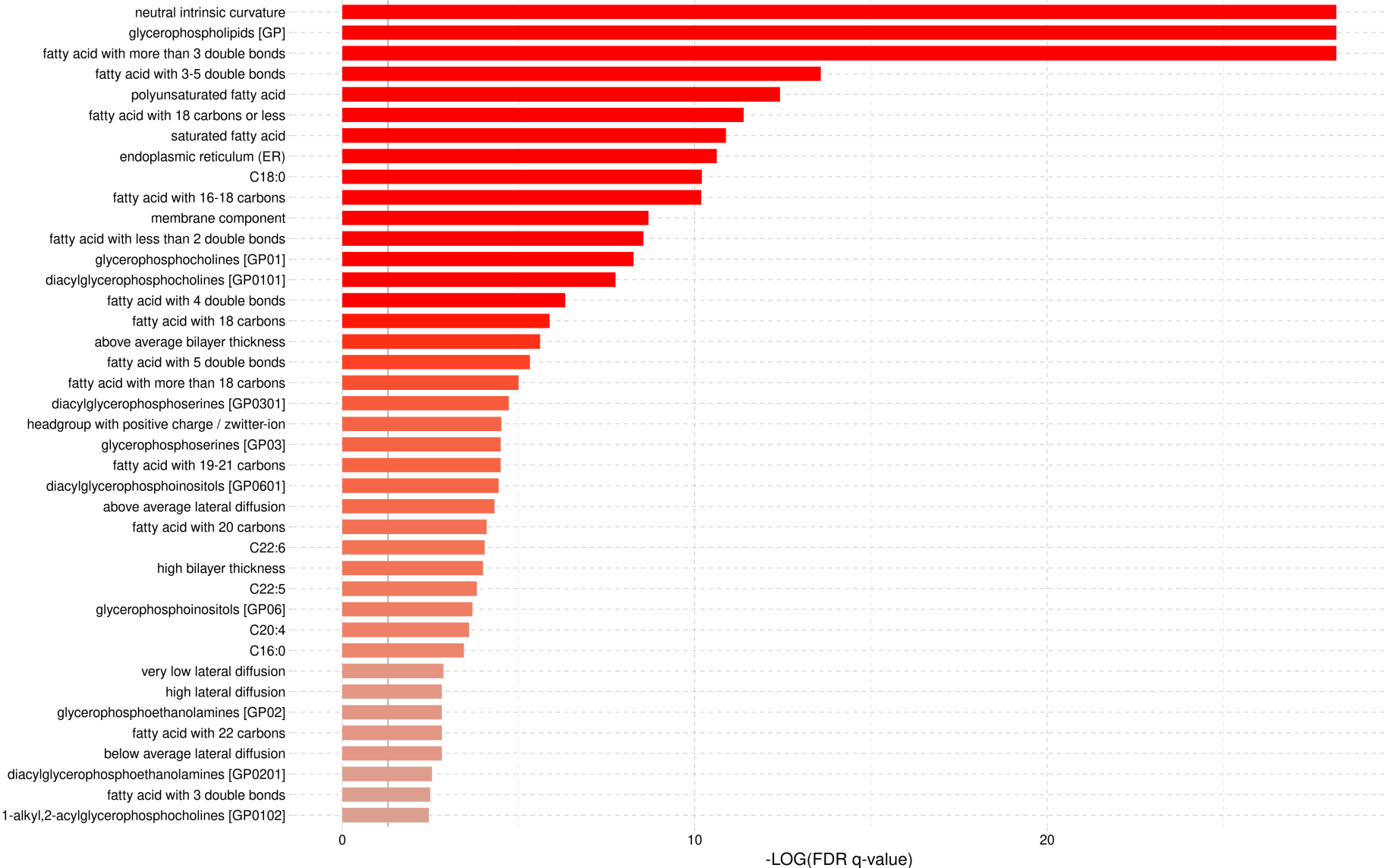
CSCs_HMPSNE vs. CSCs_CTR



CSCs – HMPSNE
LION enrichment analysis – *ranking mode*
Decreased in treated CSCs

LION enrichment analysis *ranking mode*

CSCs_CTRL vs. CSCs_HMPSNE

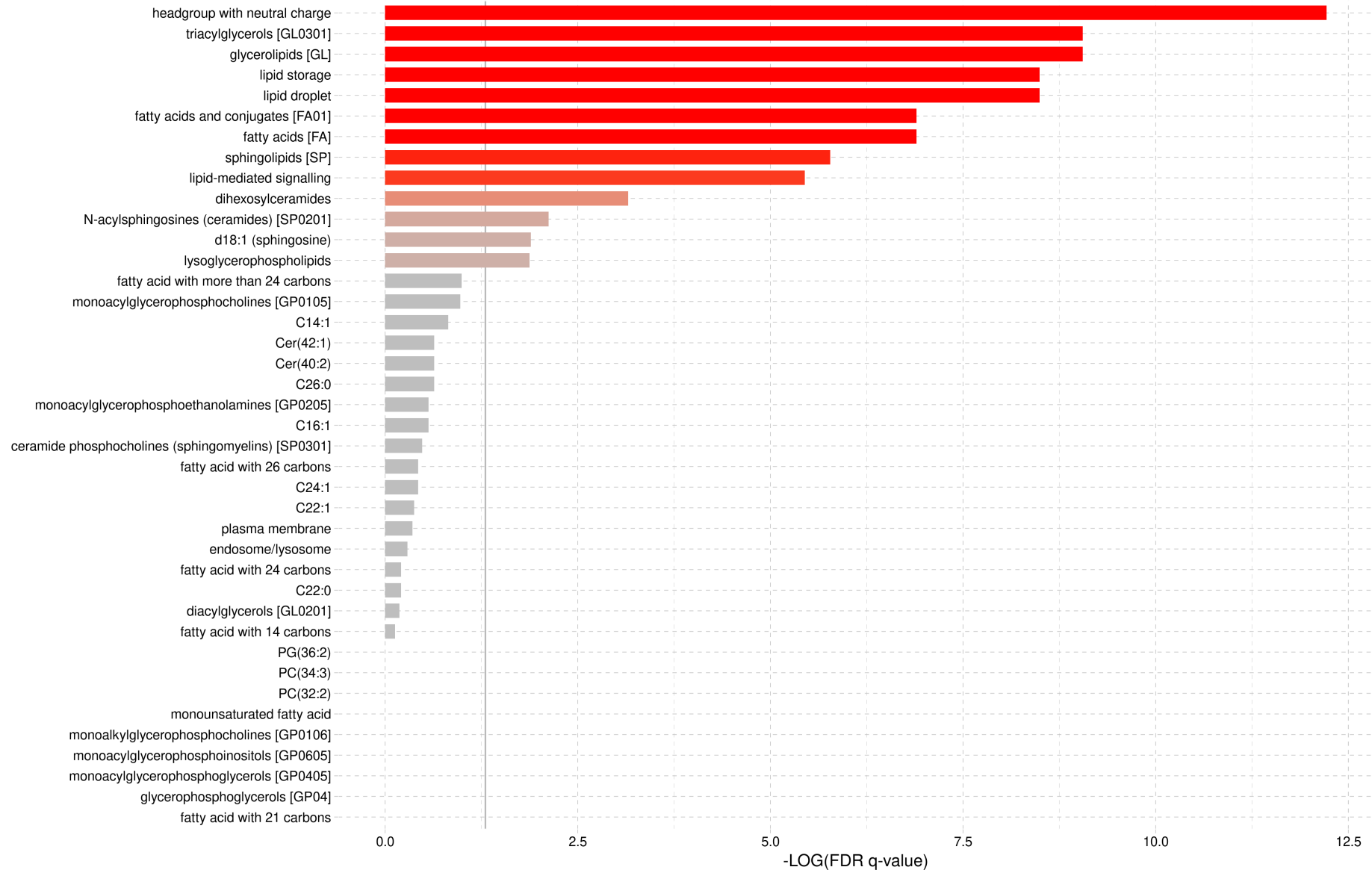


CSCs – HMPSNE

LION enrichment analysis - *target-list mode*

Increased in treated CSCs

LION enrichment analysis *target-list mode*

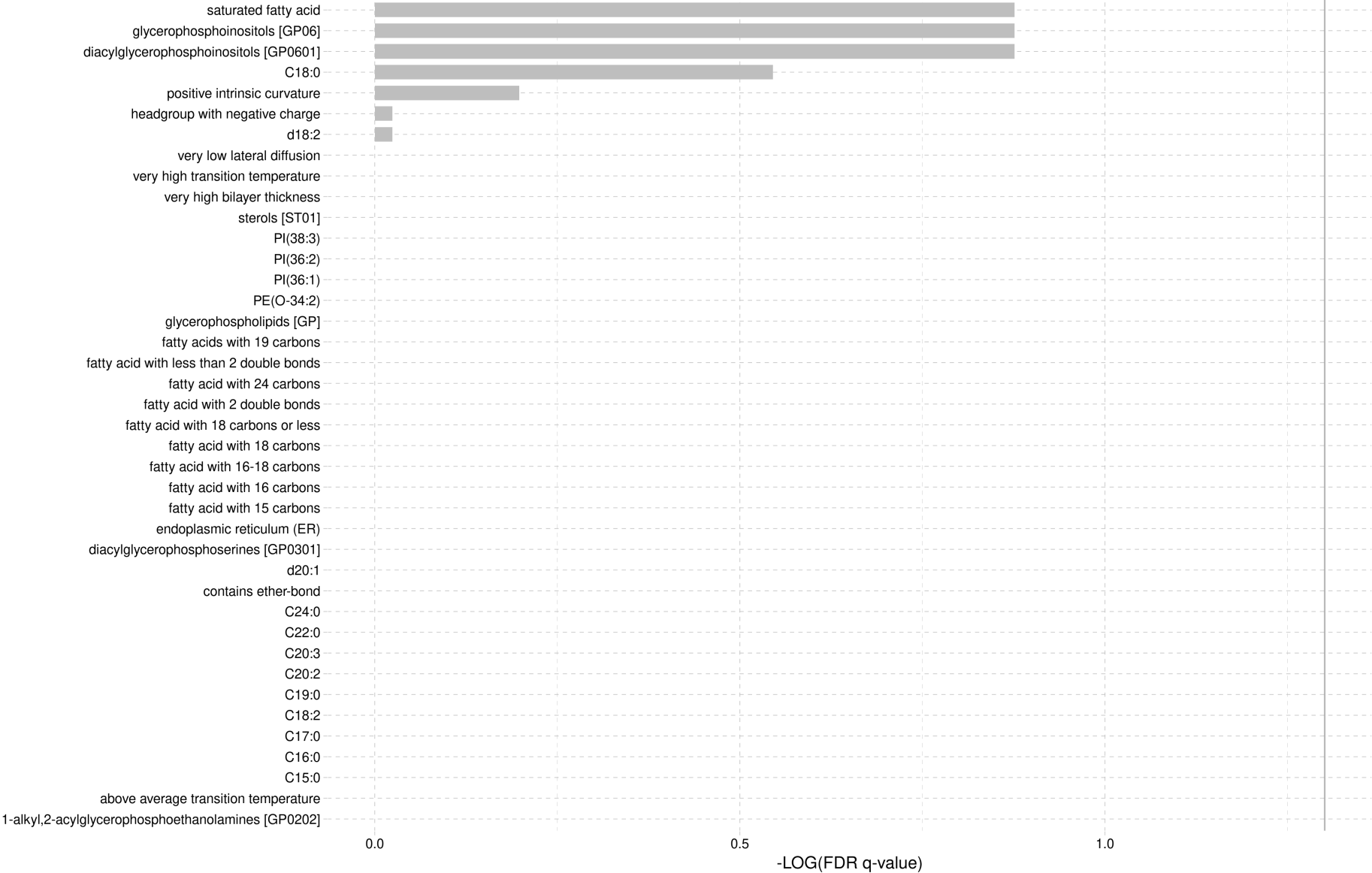


CSCs – HMPSNE

LION enrichment analysis - *target-list mode*

Decreased in treated CSCs

LION enrichment analysis *target-list mode*

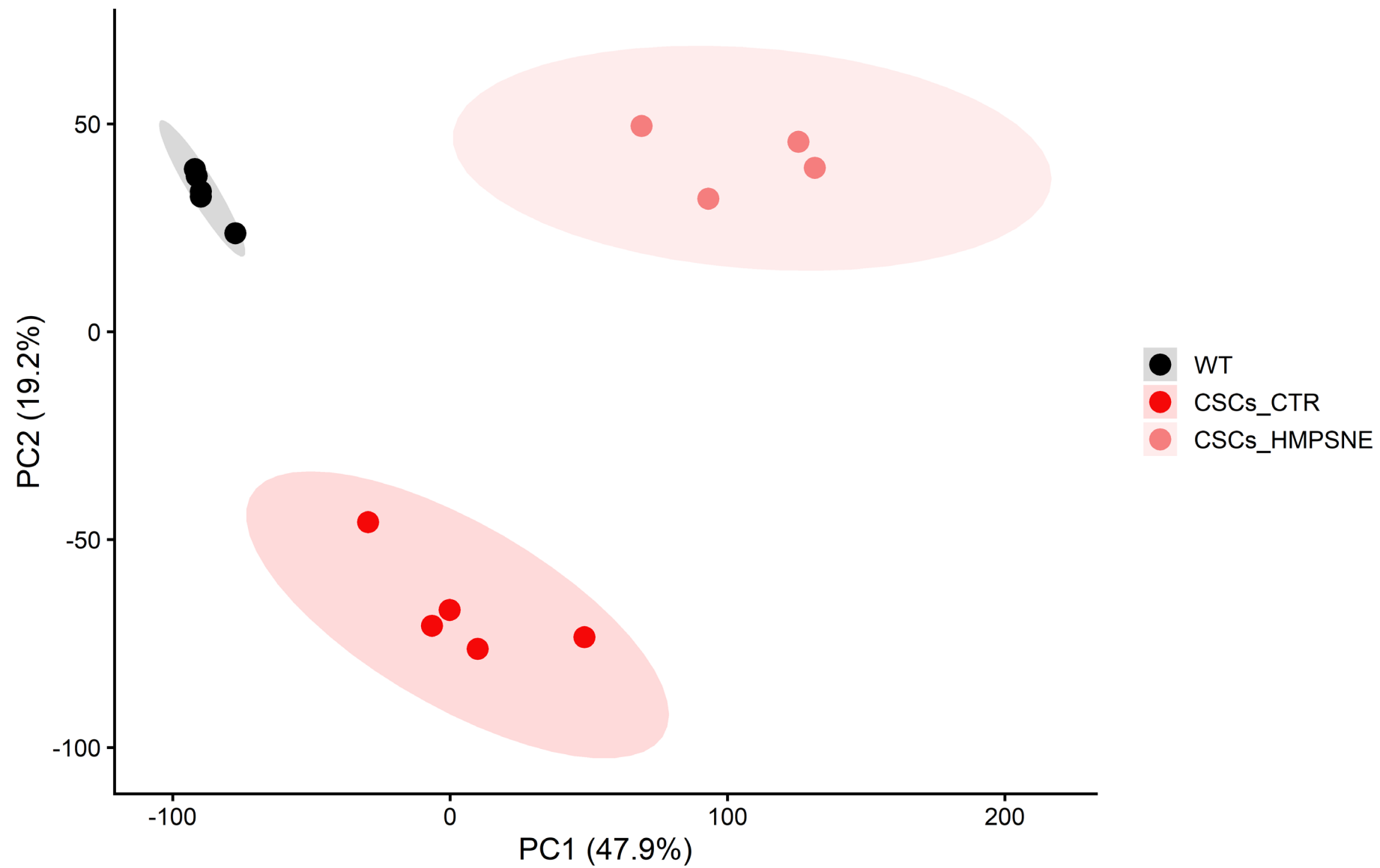


WT vs CSCs-CTR vs CSCs-HMPSNE

RNA Seq

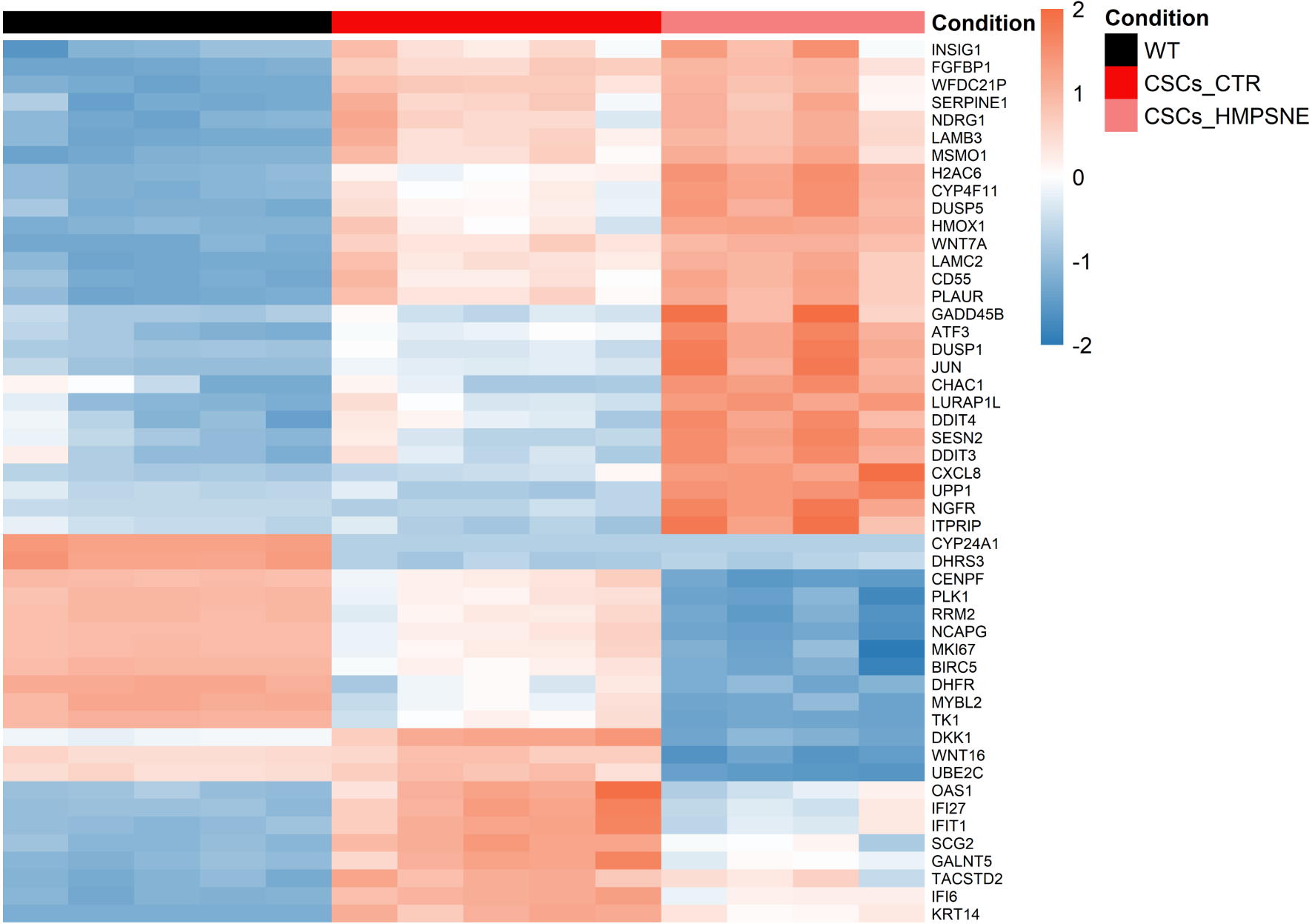
WT vs CSCs-CTR vs CSCs-HMPSNE

PCA – RNA-seq



WT vs CSCs-CTR vs CSCs-HMPSNE

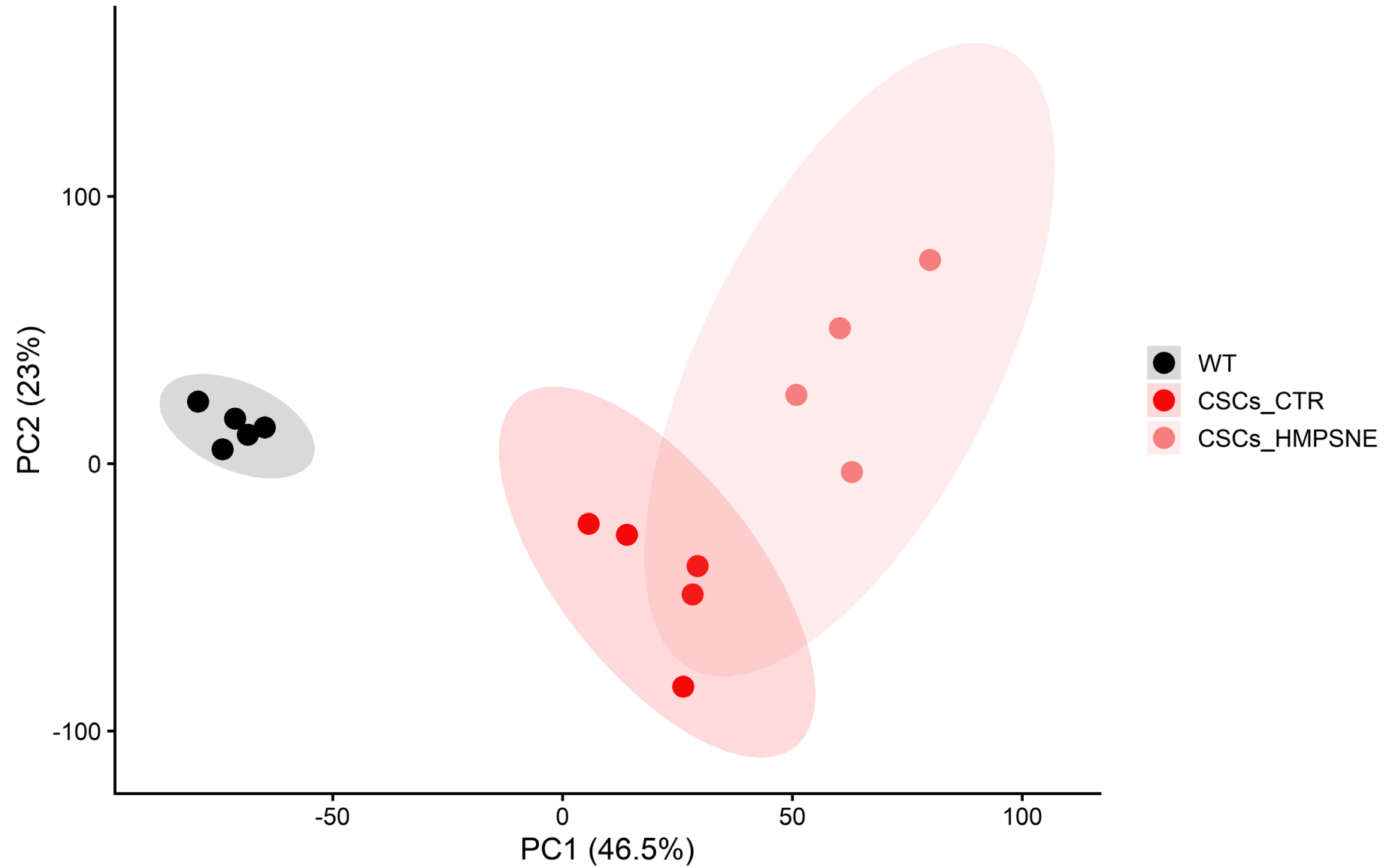
Top 50 Variable Genes



WT vs CSCs-CTR vs CSCs-HMPSNE Proteomics

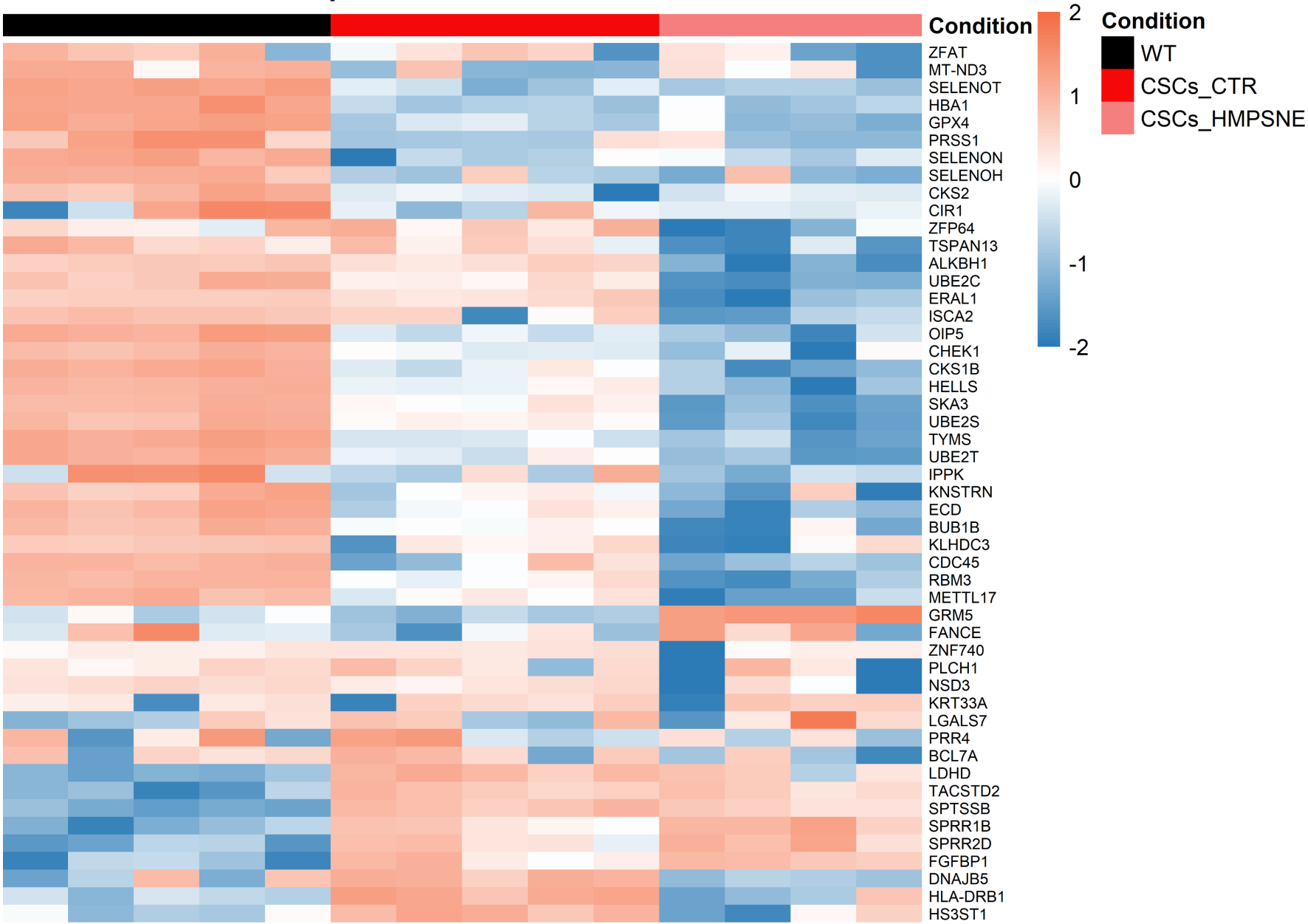
WT vs CSCs-CTR vs CSCs-HMPSNE

PCA – Proteomics



WT vs CSCs-CTR vs CSCs-HMPSNE

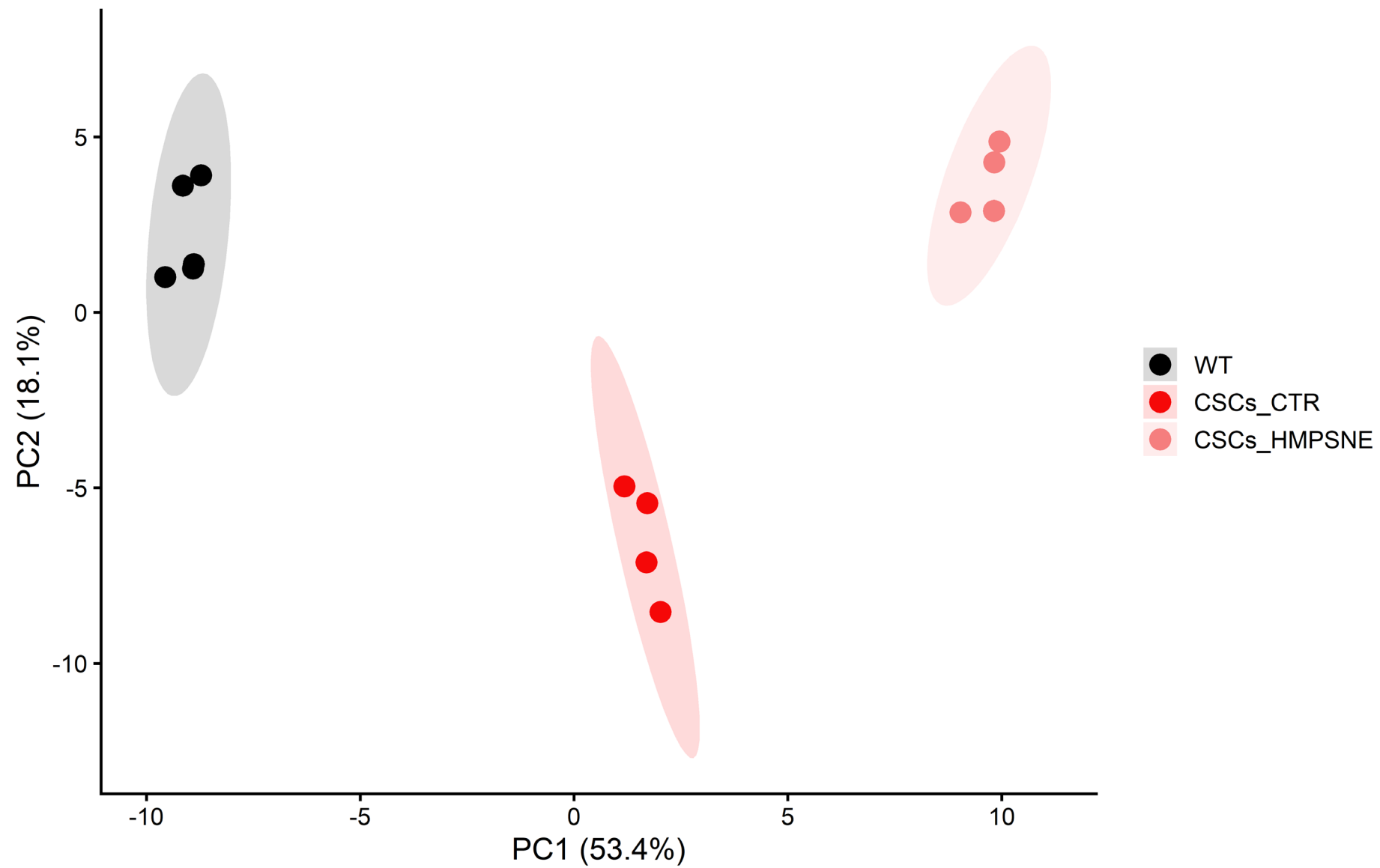
Top 50 Variable Proteins



WT vs CSCs-CTR vs CSCs-HMPSNE Metabolomics

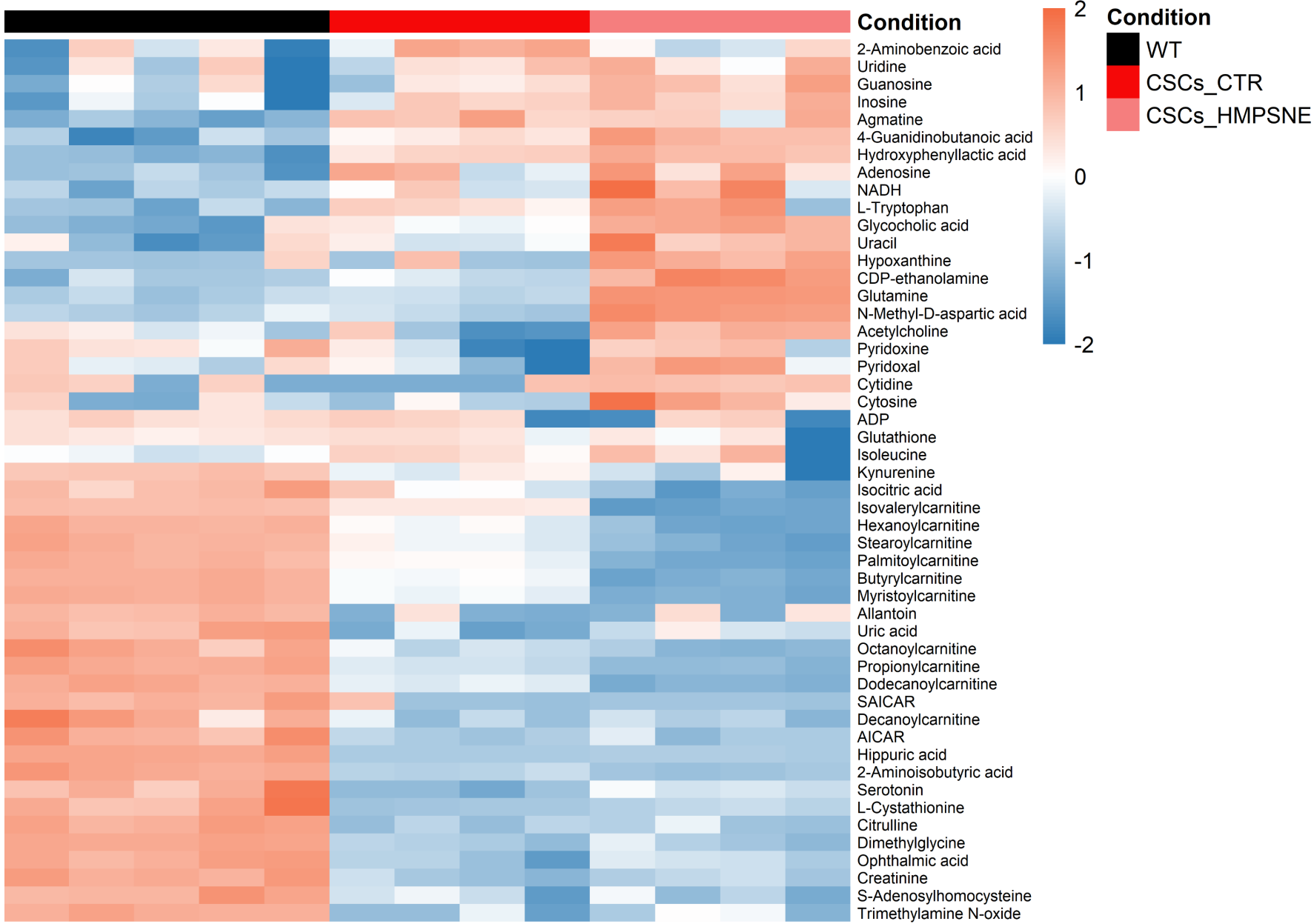
WT vs CSCs-CTR vs CSCs-HMPSNE

PCA – Metabolomics



WT vs CSCs-CTR vs CSCs-HMPSNE

Top 50 Variable Metabolites

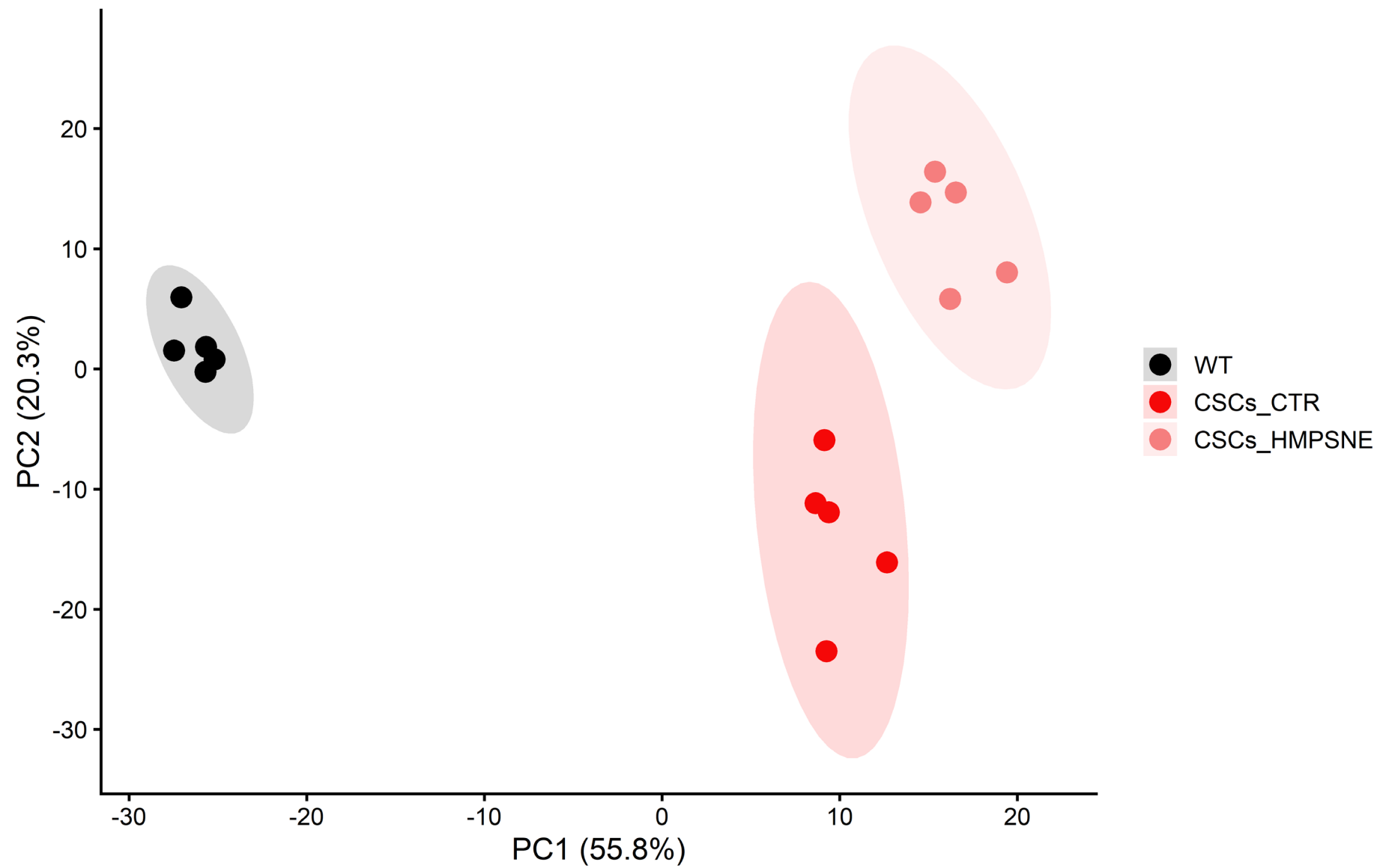


WT vs CSCs-CTR vs CSCs-HMPSNE

Lipidomics

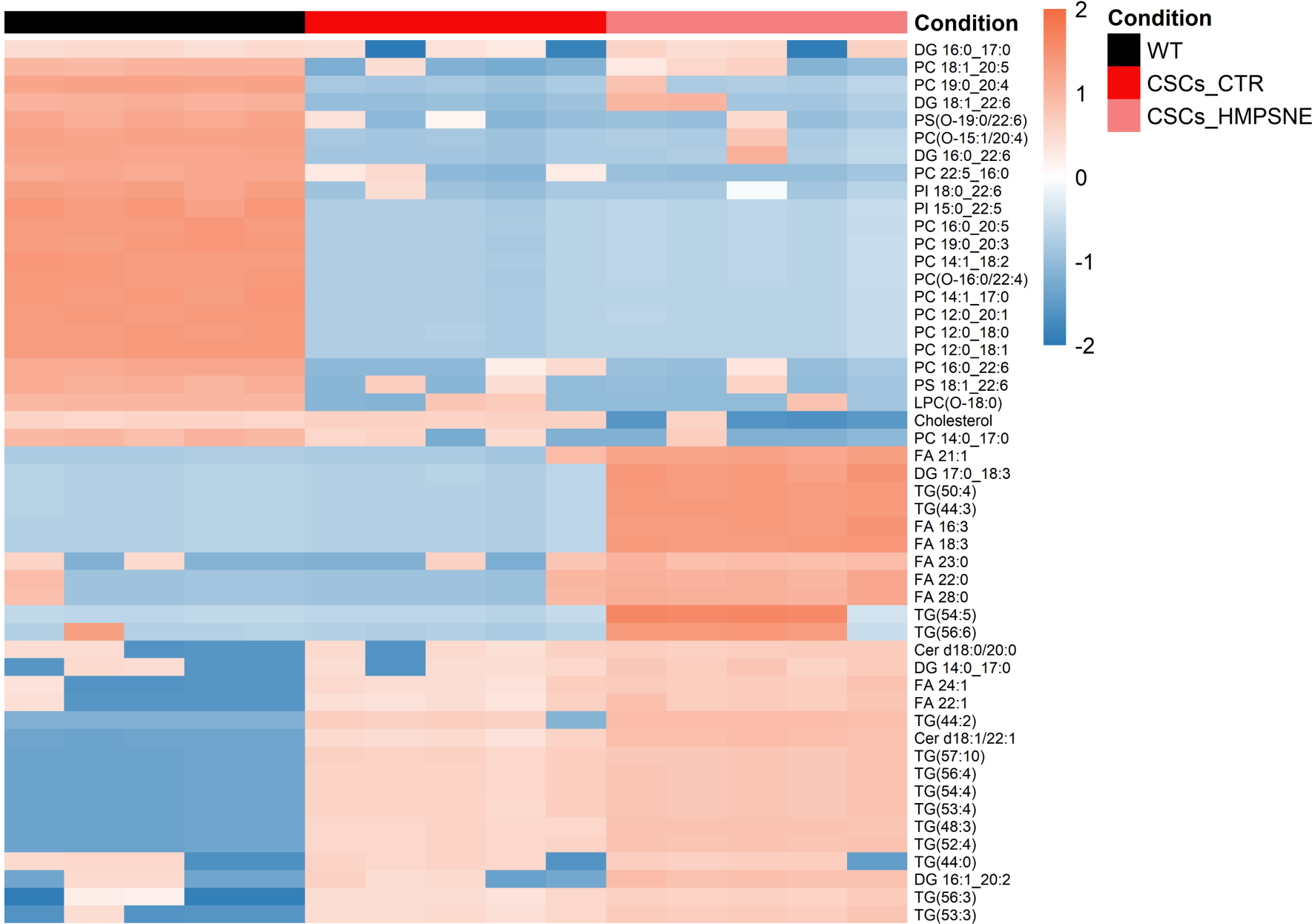
WT vs CSCs-CTR vs CSCs-HMPSNE

PCA – Lipidomics



WT vs CSCs-CTR vs CSCs-HMPSNE

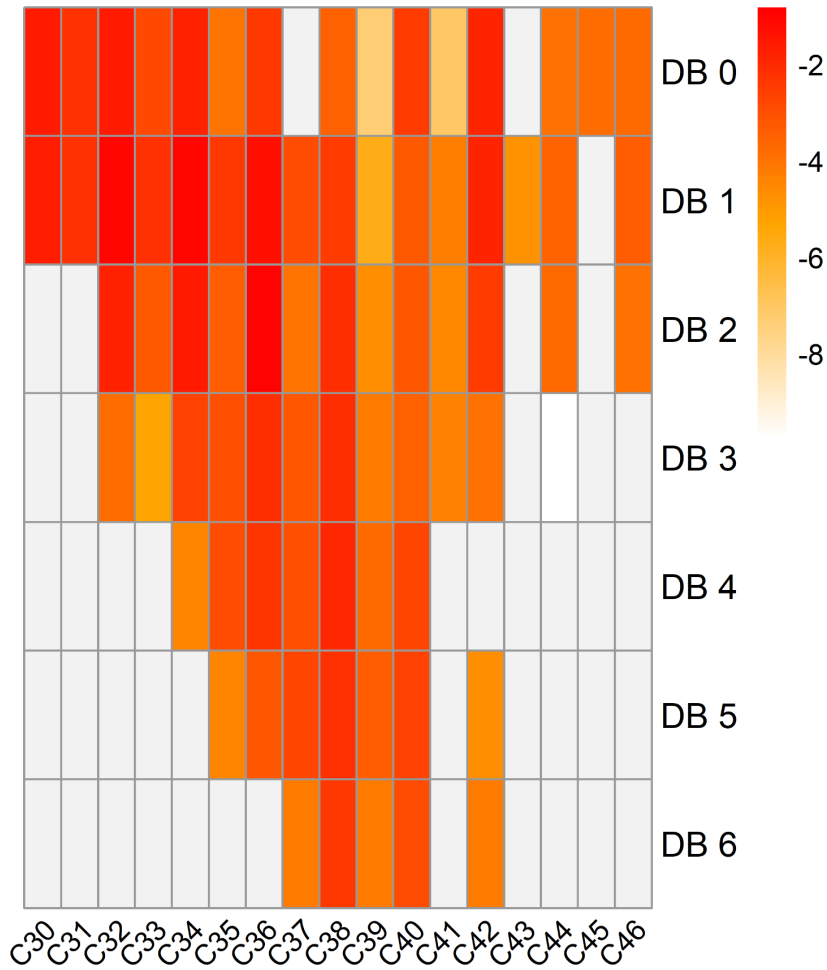
Top 50 Variable Lipids



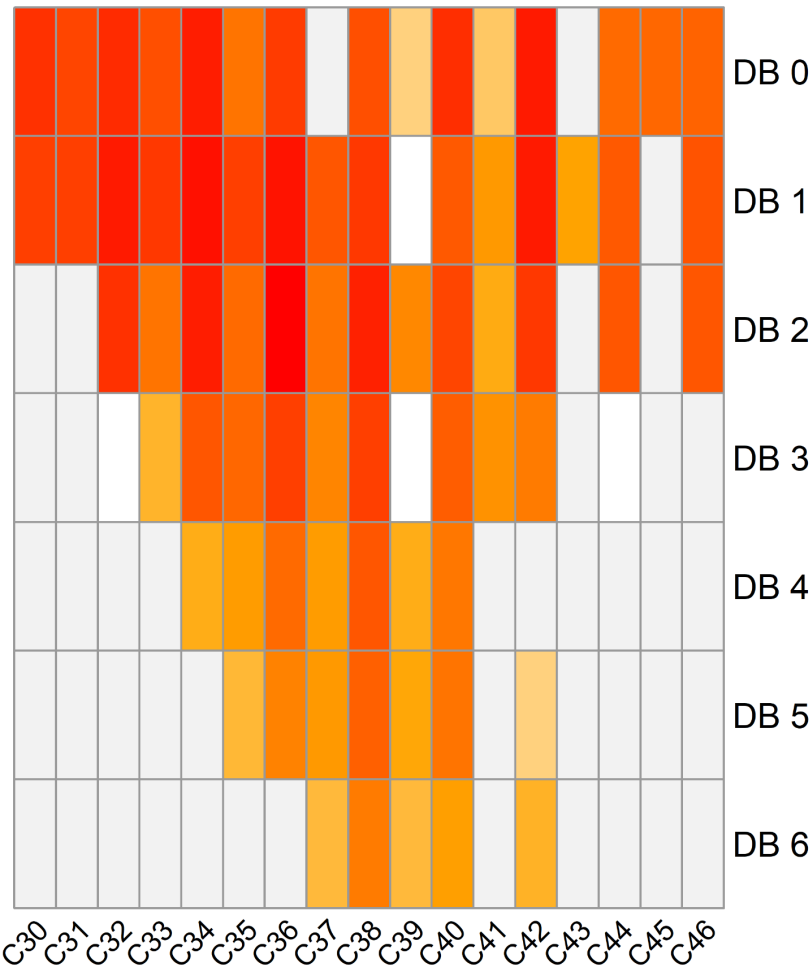
WT vs CSCs-CTR vs CSCs-HMPSNE

FA_chainlength_unsaturation

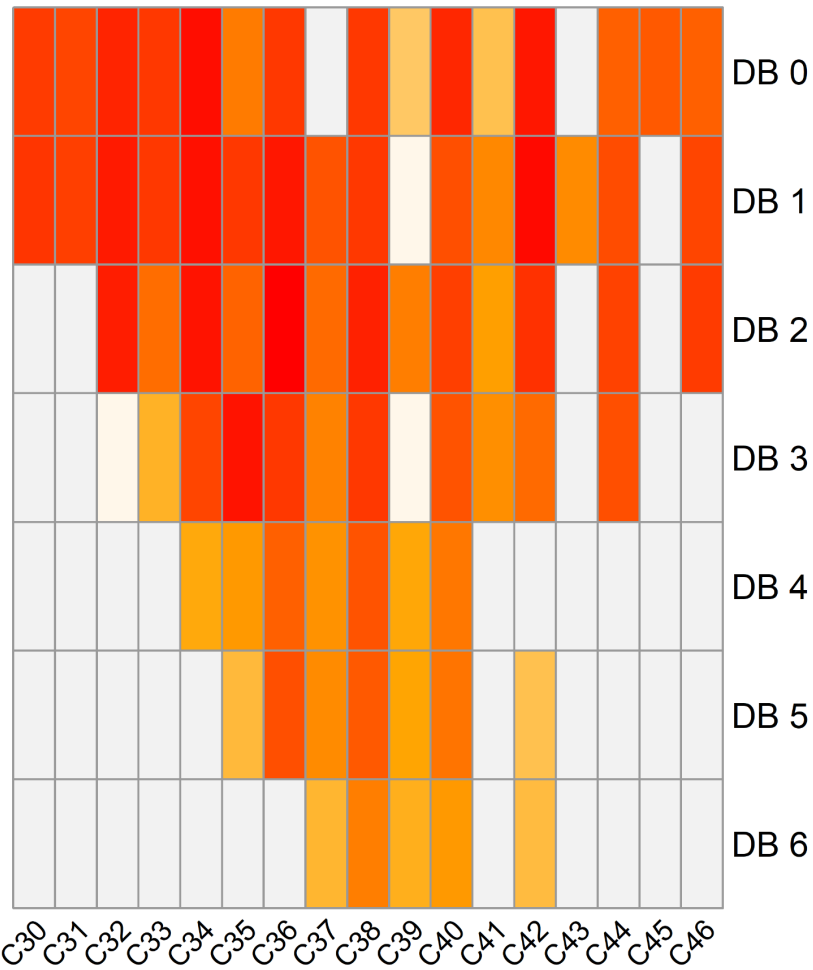
WT



CSCs_CTR

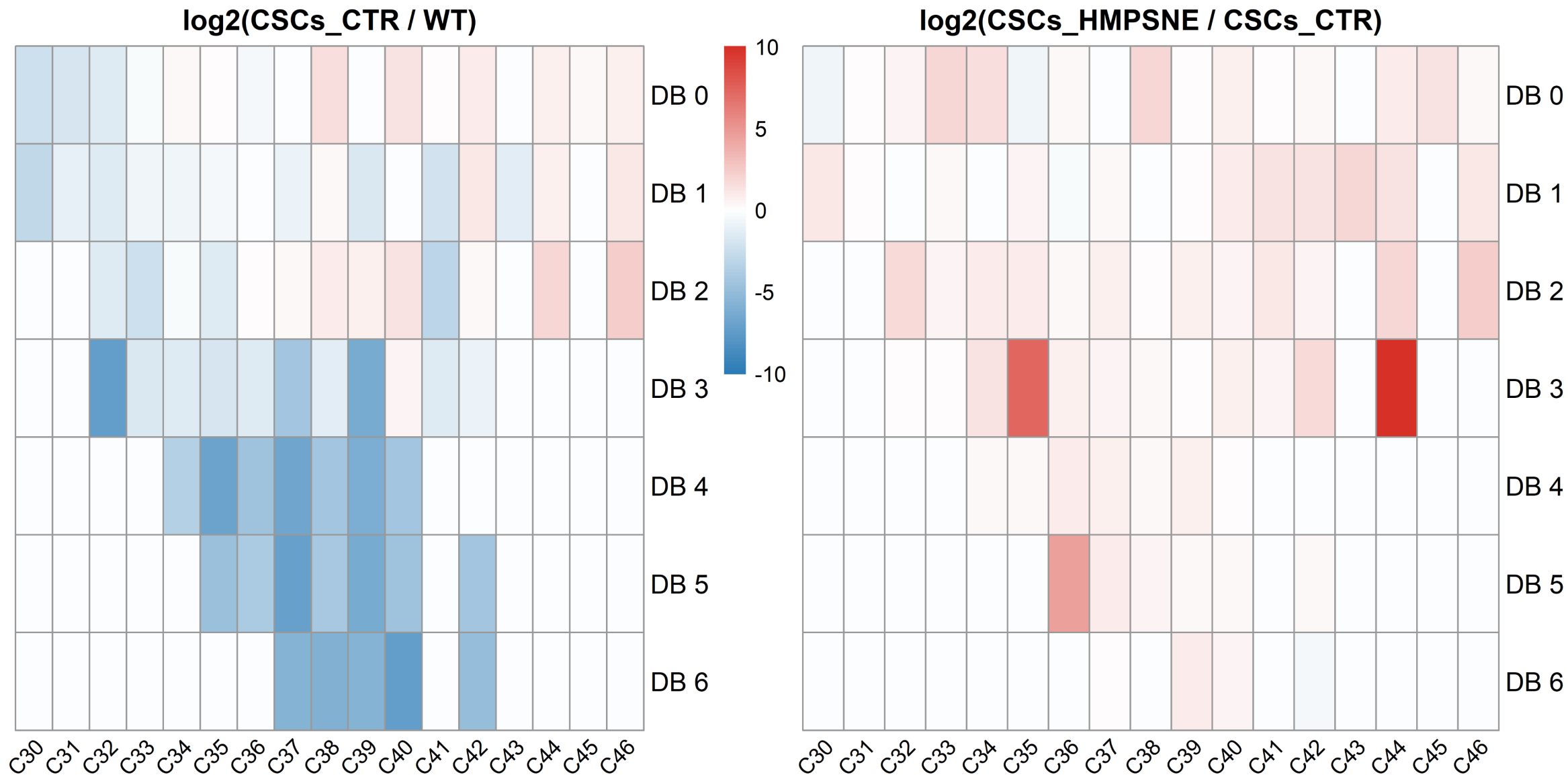


CSCs_HMPSNE



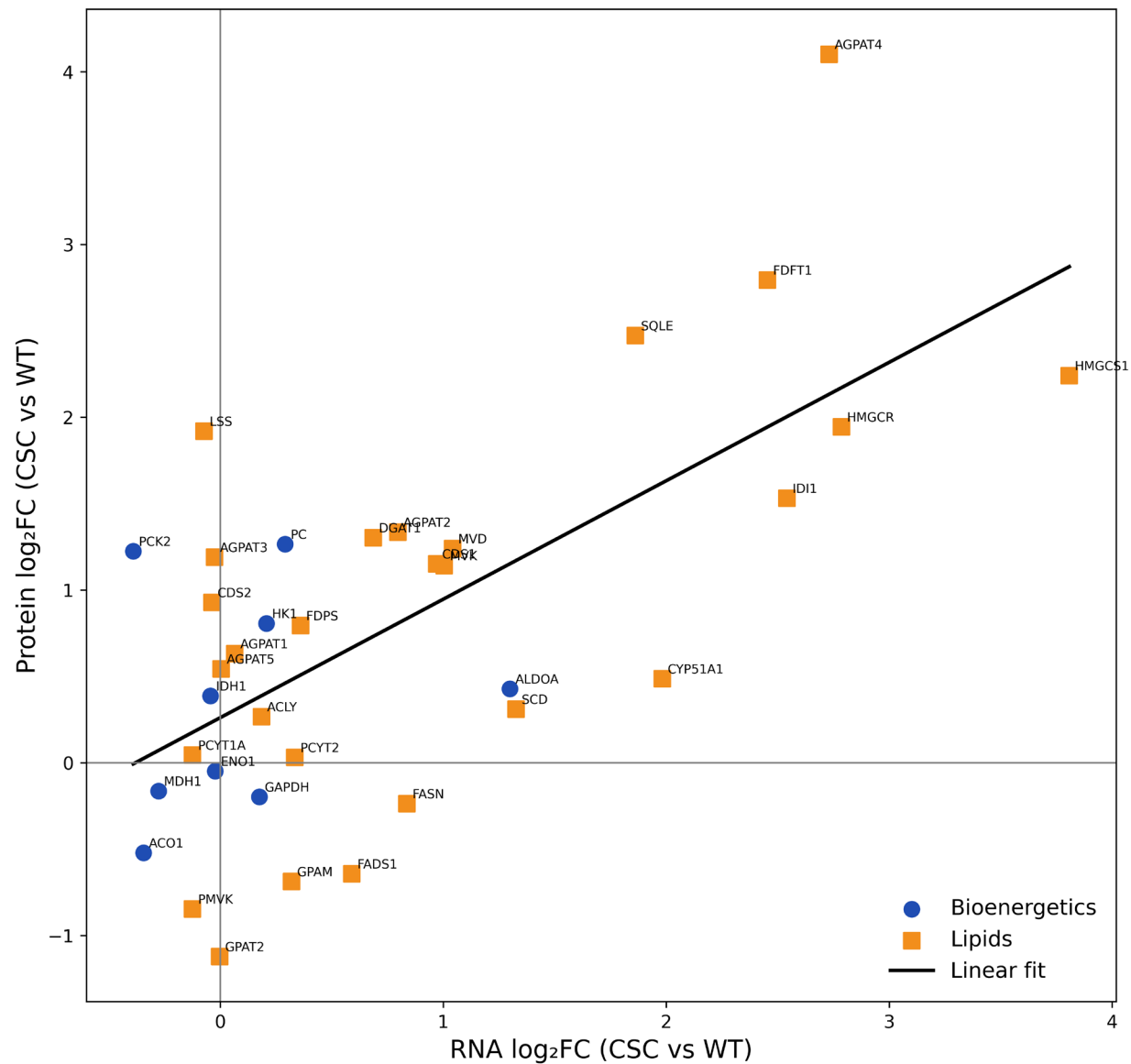
WT vs CSCs-CTR vs CSCs-HMPSNE

FA_chainlength_unsaturation_FC

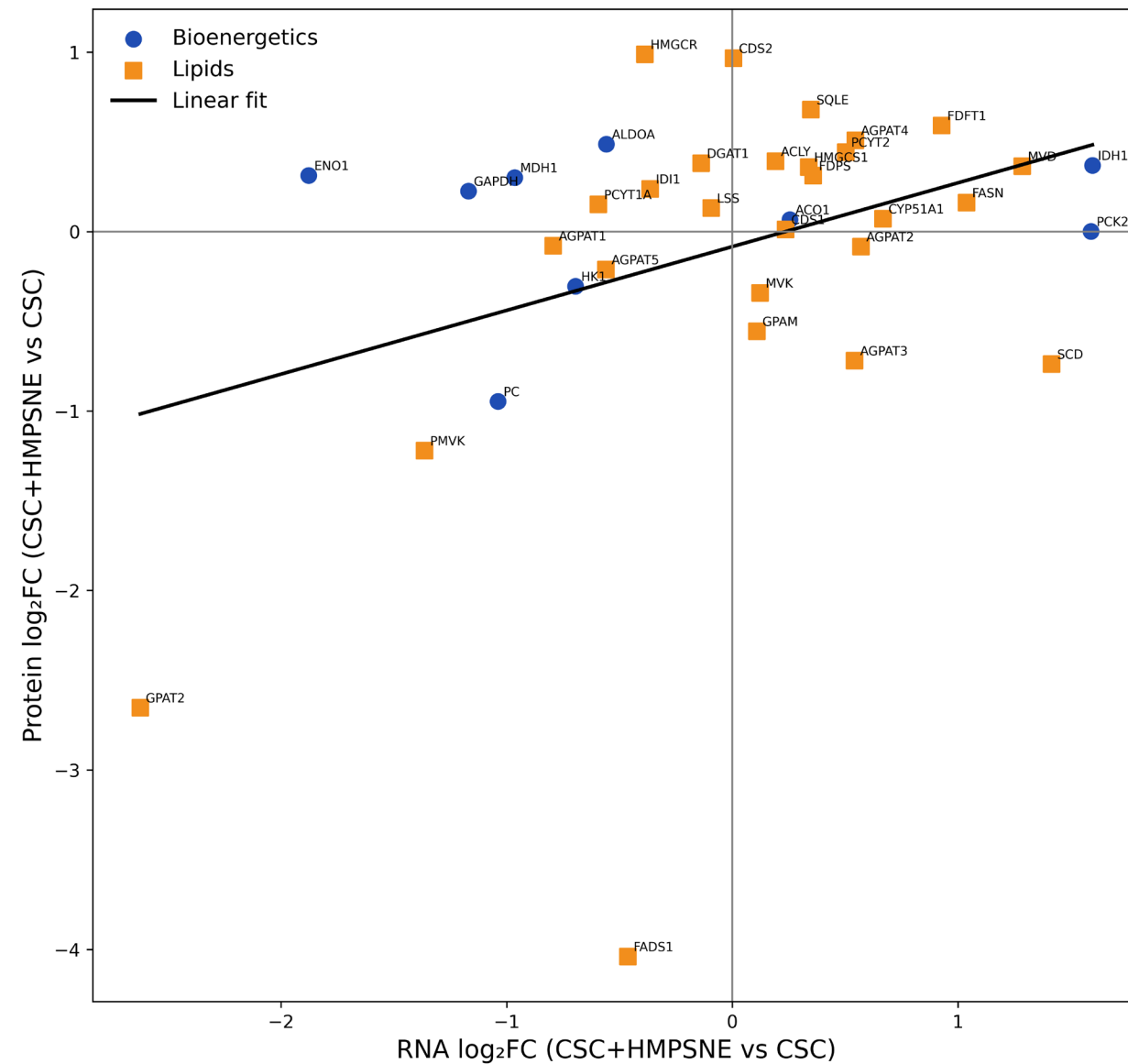


WT vs CSCs-CTR vs CSCs-HMPSNE

RNA-Protein correlation (metabolic enzymes, CSC vs WT)
Pearson $r = 0.65$

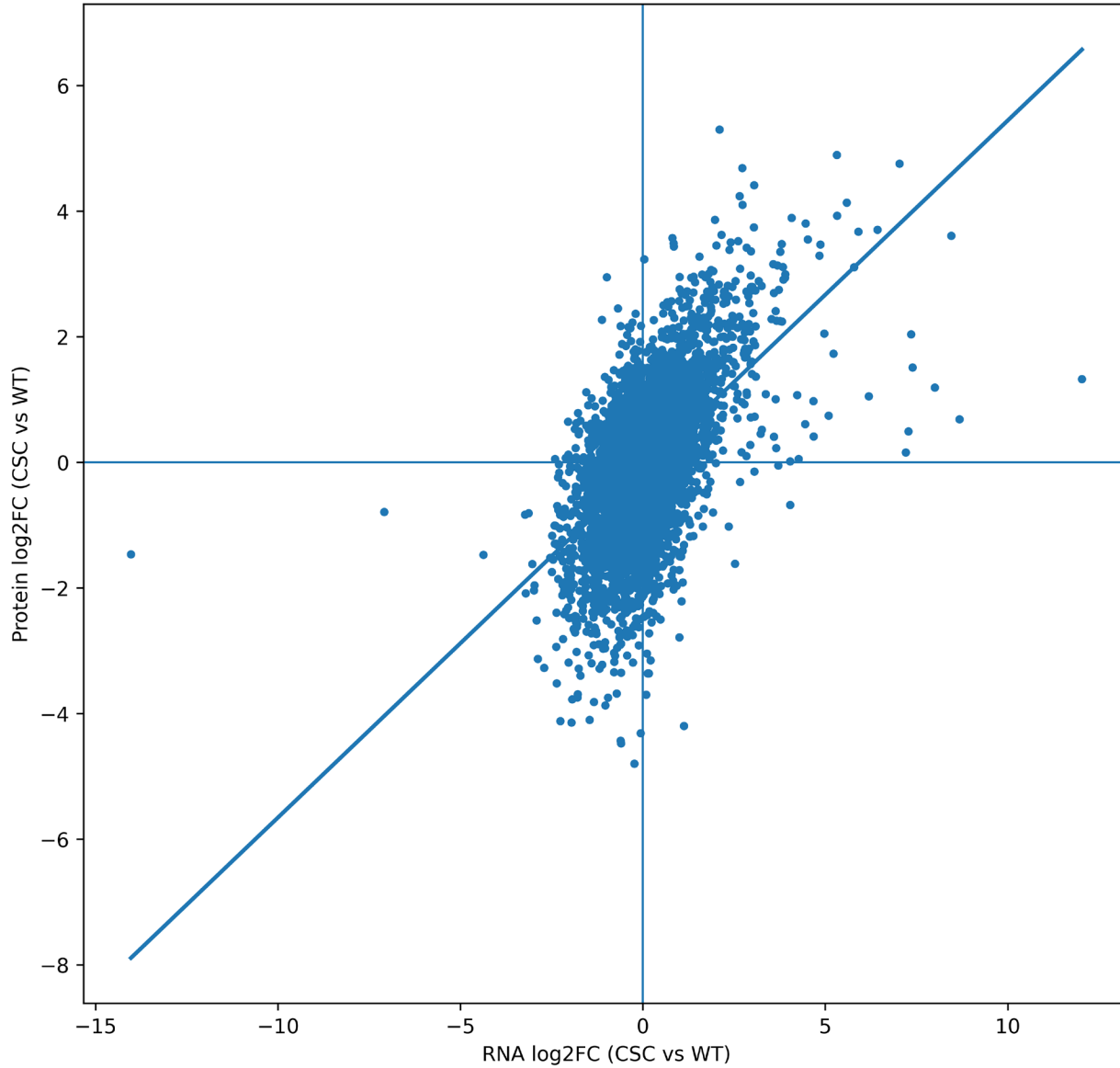


RNA-Protein correlation (metabolic enzymes, CSC+HMPSNE vs CSC)
Pearson $r = 0.35$



WT vs CSCs-CTR vs CSCs-HMPSNE

RNA-Protein correlation (all genes, CSC vs WT)
Pearson $r = 0.54$



RNA-Protein correlation (all genes, CSC+HMPSNE vs CSC)
Pearson $r = 0.26$

