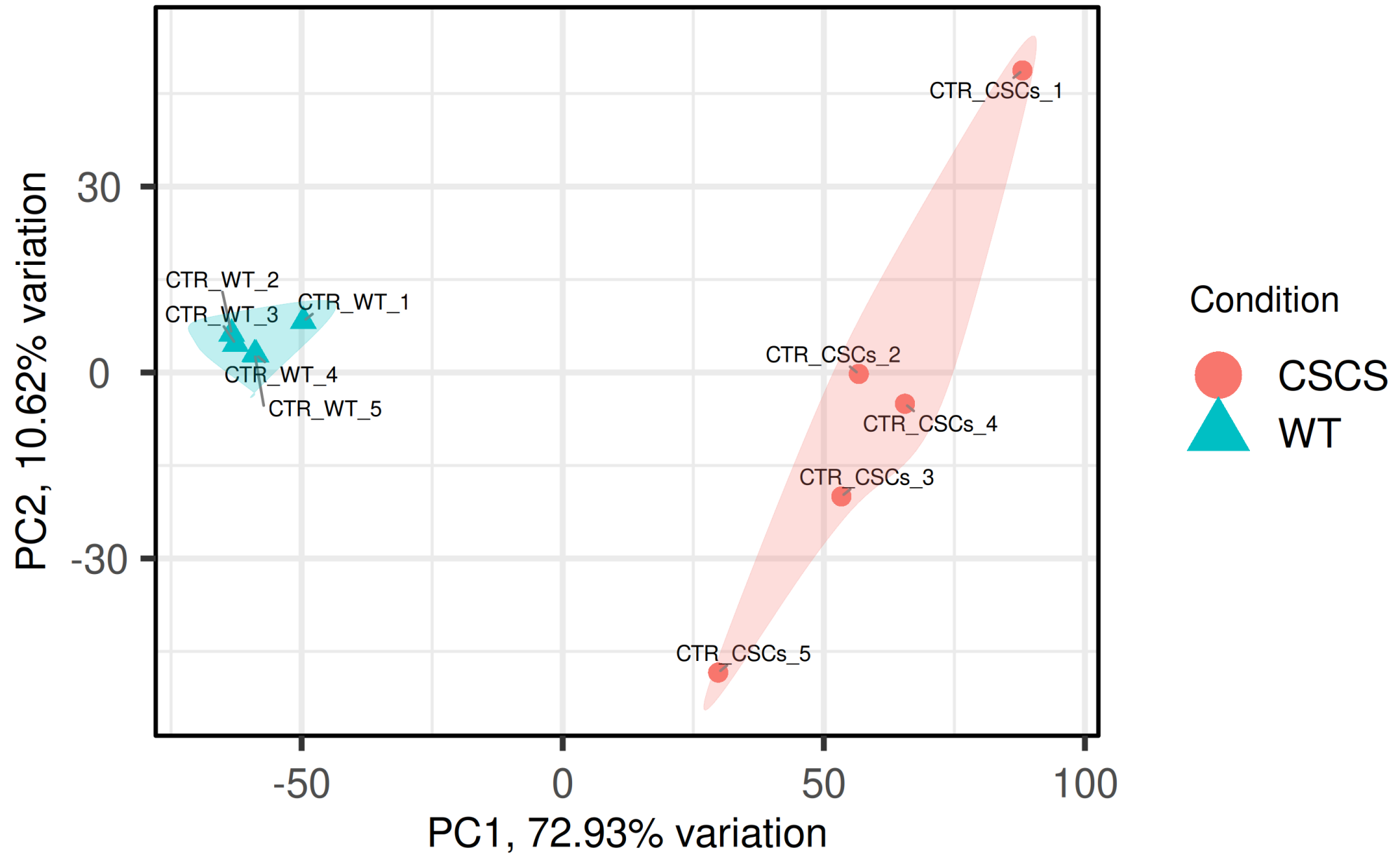


Supplementary Data S1. Integrated multi-omics profiling of colorectal CSCs derived from HCT116 cells compared with WT HCT116 cells. Comprehensive transcriptomic, proteomic, metabolomic and lipidomic analyses are shown. The dataset includes PCA to assess sample clustering and separation between conditions, hierarchical clustering and heatmaps of the most variable or differentially regulated features, and differential expression analyses visualized by volcano plots. Lists of the top 25 upregulated and downregulated genes, proteins, metabolites and lipid species are also provided. For transcriptomic datasets, differential expression analyses were performed using fold-change and statistical significance thresholds (fold change ≥ 1.5 and false discovery rate (FDR) ≤ 0.05). For proteomics, metabolomic and lipidomic analyses, significantly altered metabolites and lipid species were identified using fold change ≥ 1.5 and P value ≤ 0.05 . PCA plots include PERMANOVA statistics to evaluate group separation. 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. In addition, ORA was used to identify biological processes and pathways significantly enriched among genes or proteins increased or decreased in CSCs relative to WT cells. For metabolomic datasets, pathway enrichment was assessed using QEA, and ORA was used to identify metabolite sets or pathways enriched among metabolites altered between conditions. For lipidomic datasets, ORA was performed to identify enriched lipid-associated pathways or annotations among lipid species altered between conditions. 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 associated with CSCs or WT cells. Together, these analyses provide an integrated multi-omics characterization of molecular differences between colorectal cancer stem cells and their parental HCT116 wild-type counterparts.

CSCs vs WT

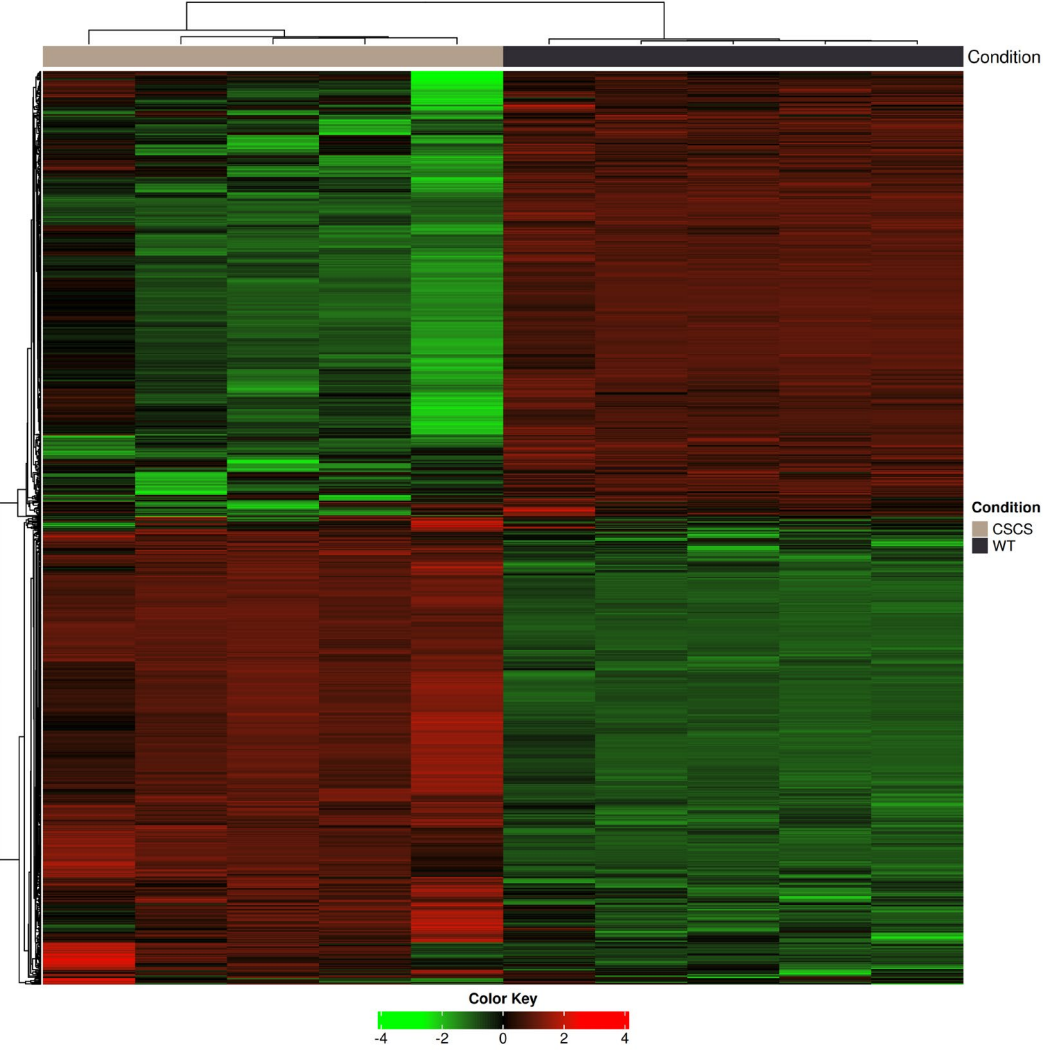
RNA Seq

CSCs vs WT

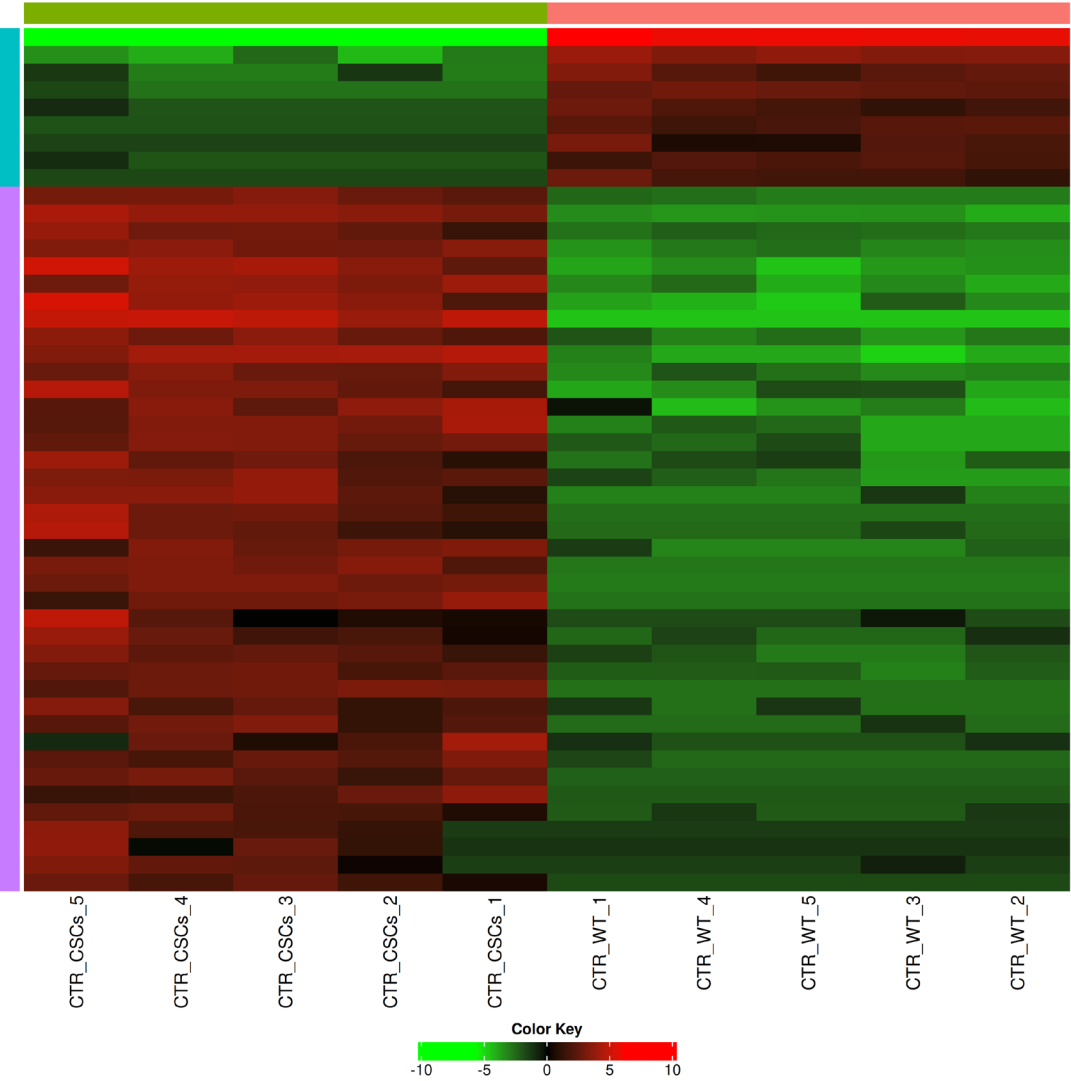


CSCs vs WT

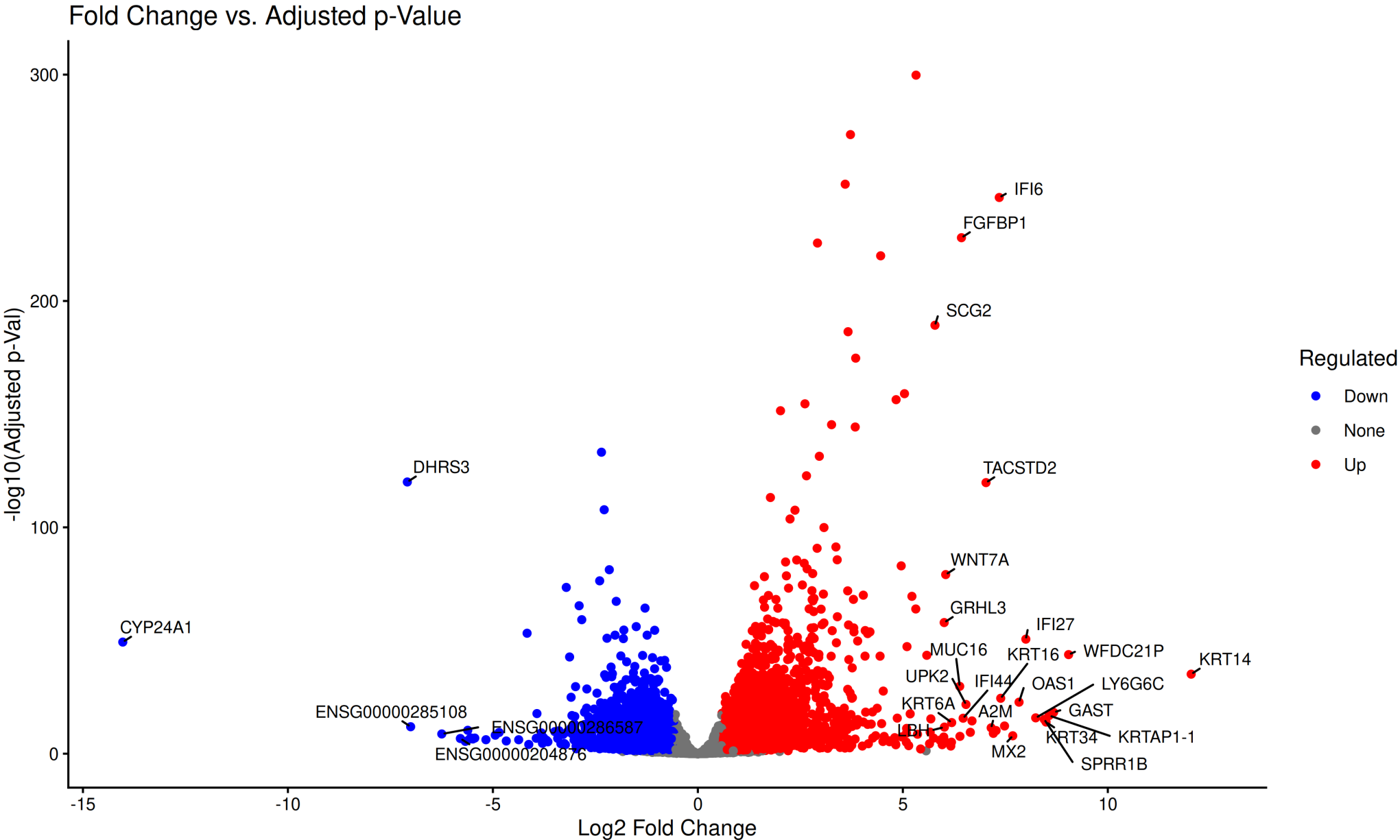
Hierarchical clustering of the top 1000 most variable genes



Heatmap of the top 50 DEGs



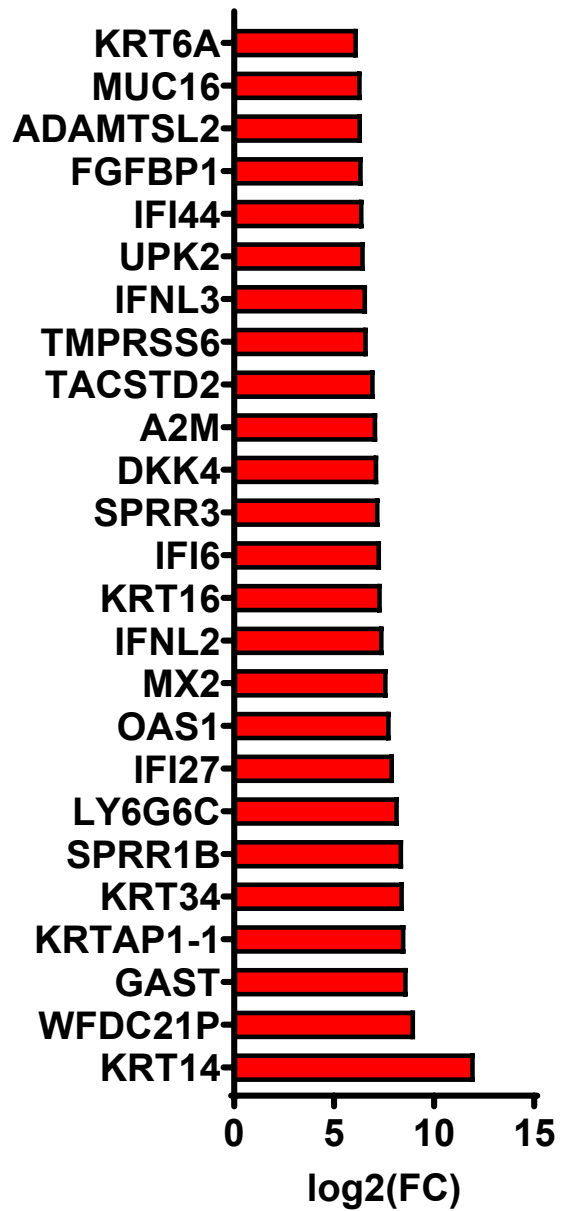
CSCs vs WT



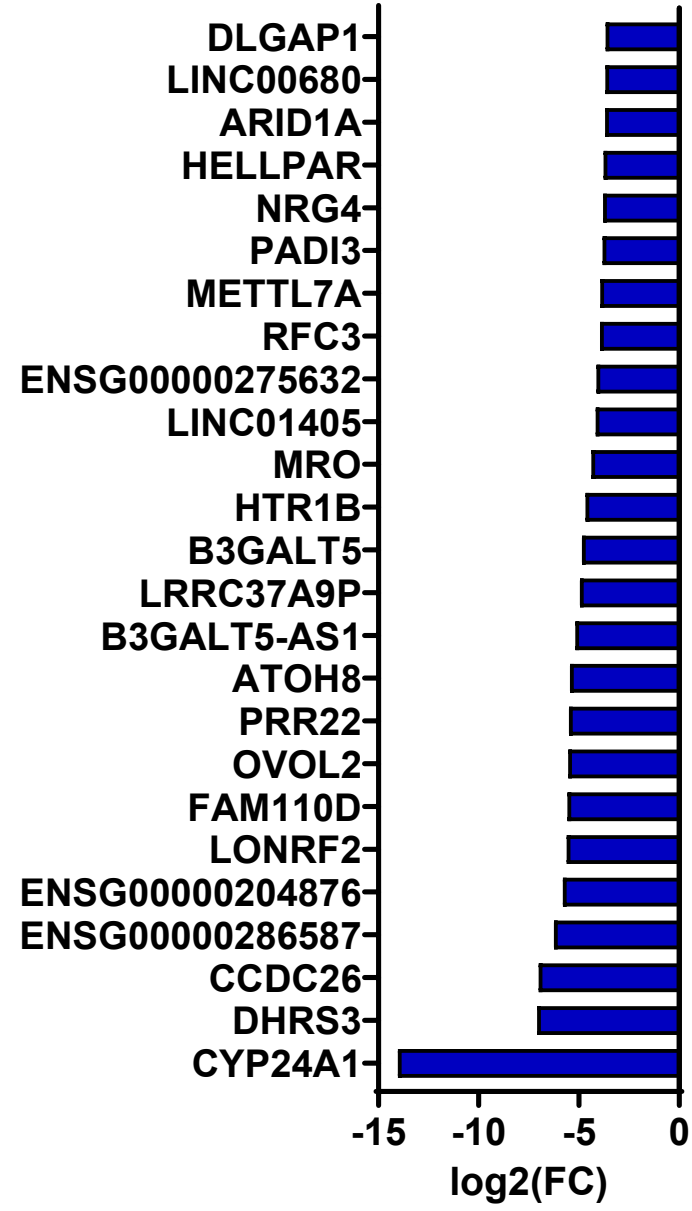
Fold change (FC) threshold: 1.5
FDR cutoff: 0.05

CSCs vs WT

Top 25_Upregulated genes in CSCs

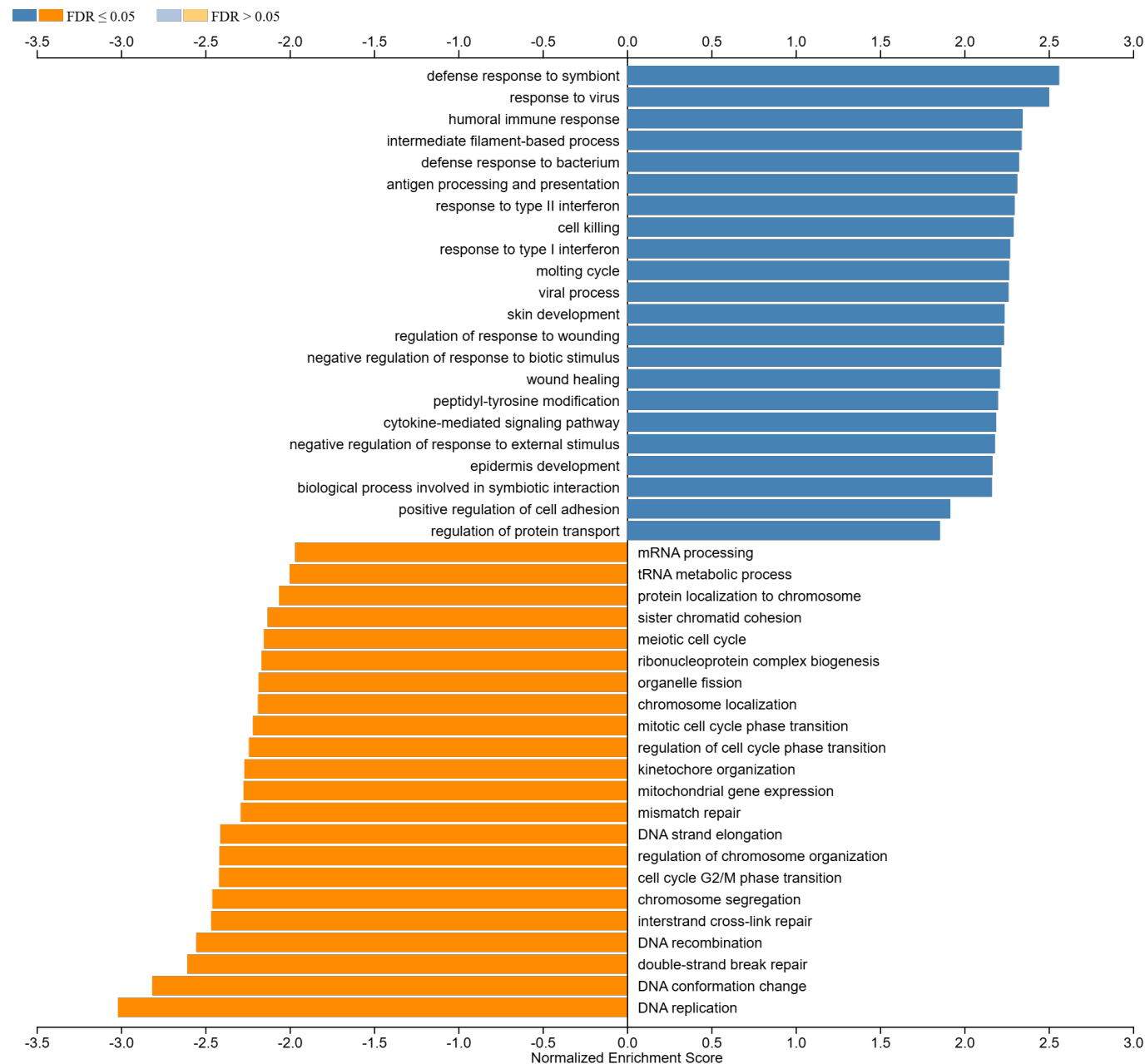


Top 25_Downregulated genes in CSCs

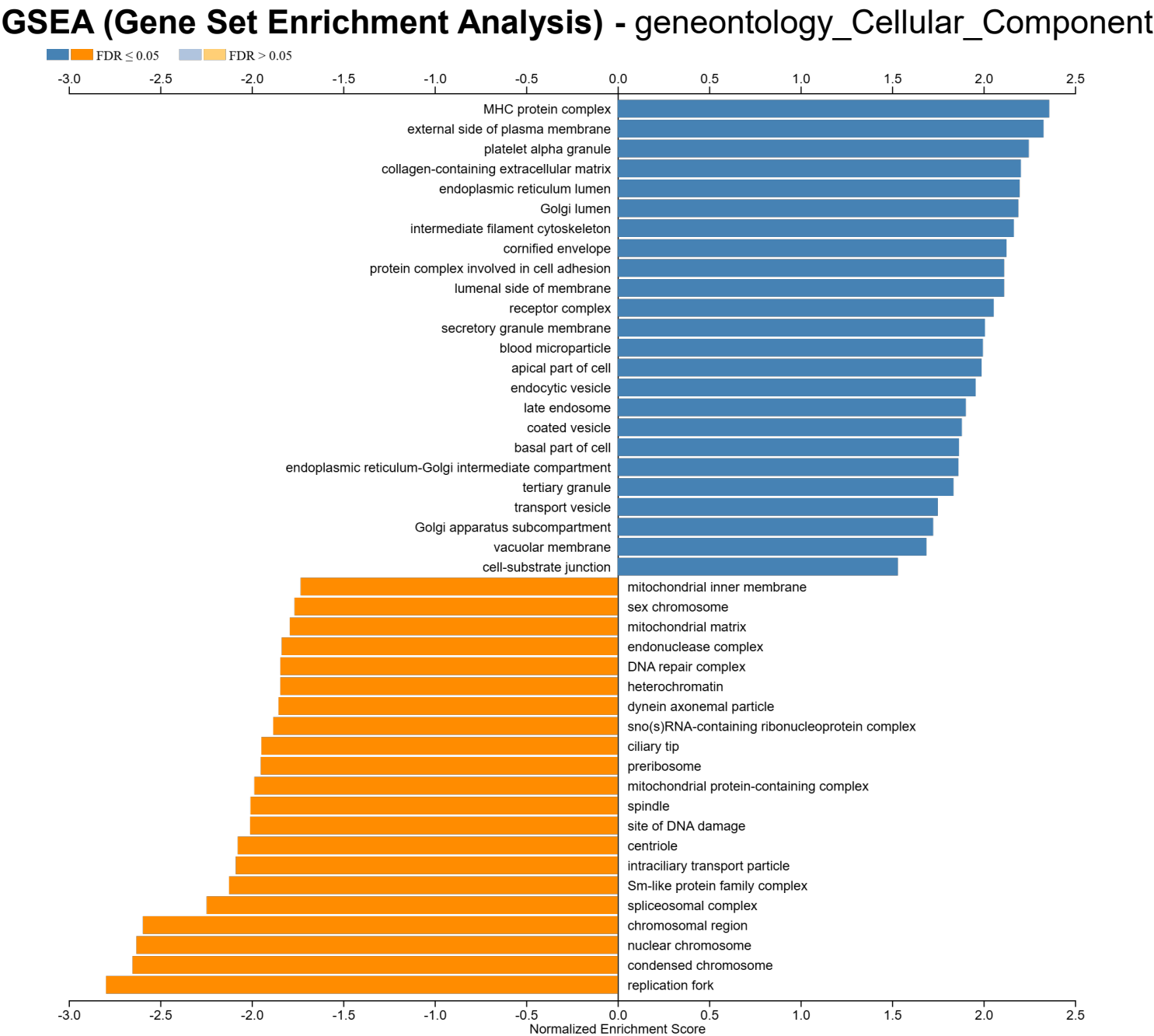


CSCs vs WT

GSEA (Gene Set Enrichment Analysis) - geneontology_Biological_Process

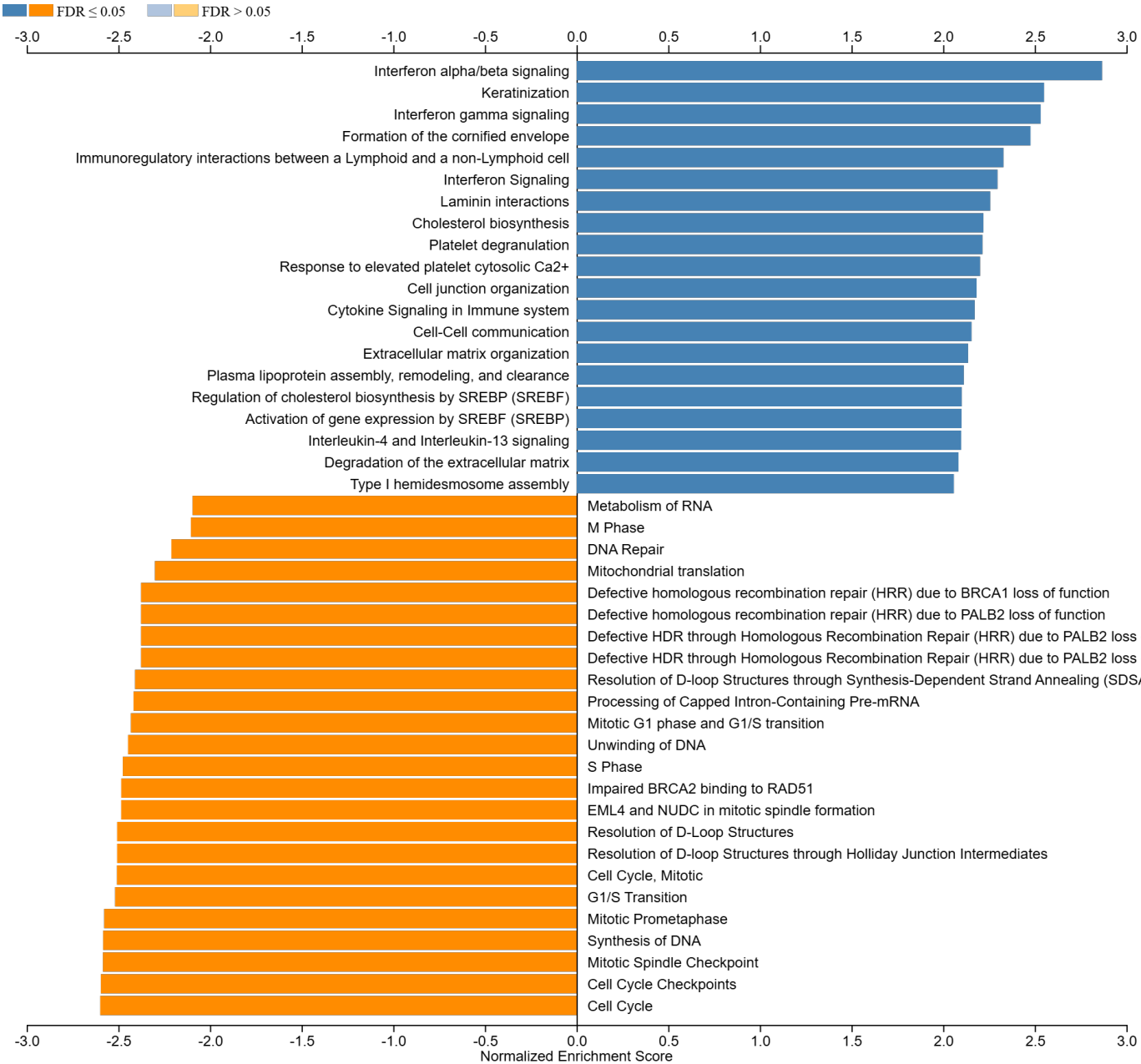


CSCs vs WT



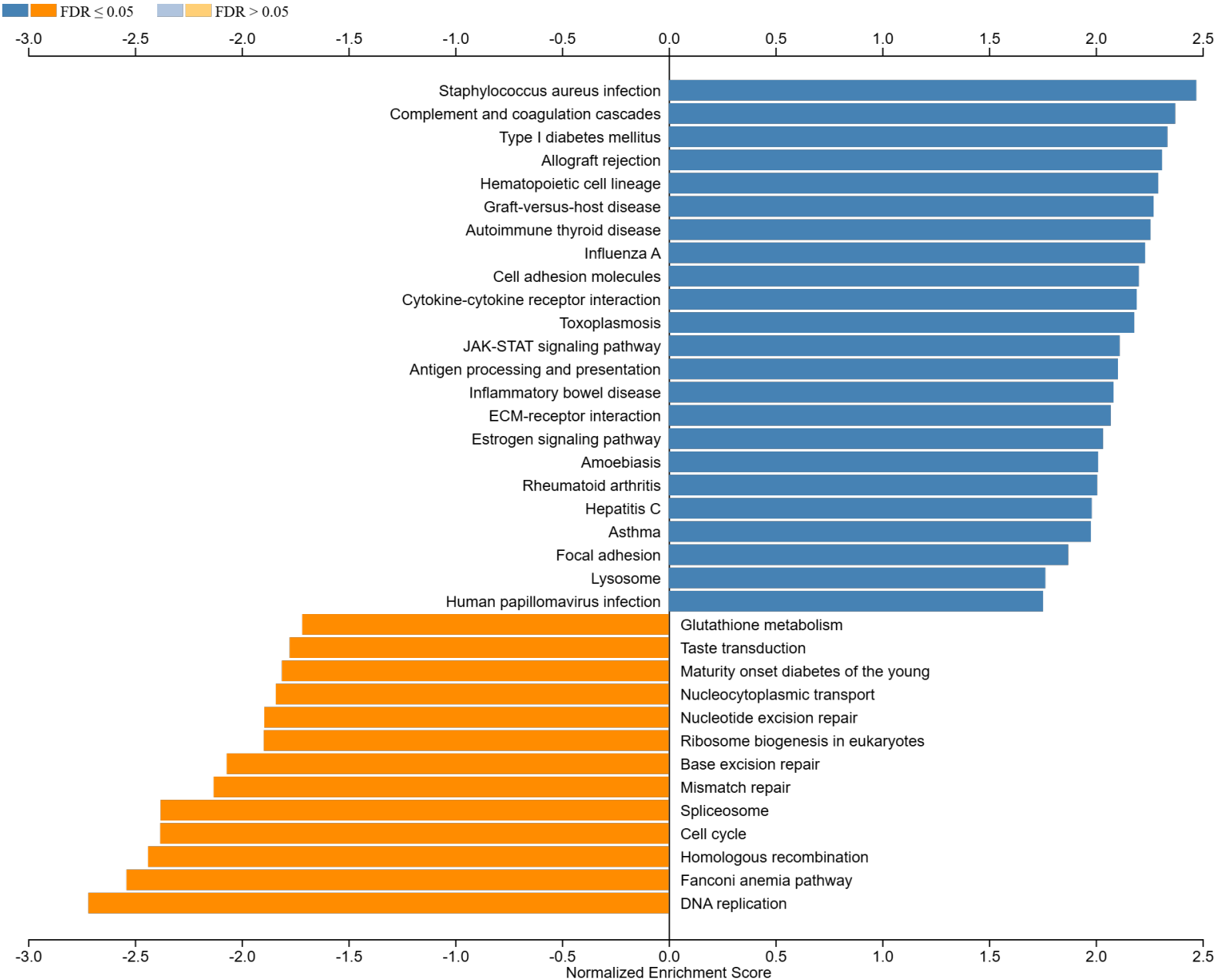
CSCs vs WT

GSEA (Gene Set Enrichment Analysis) - pathway_Reactome



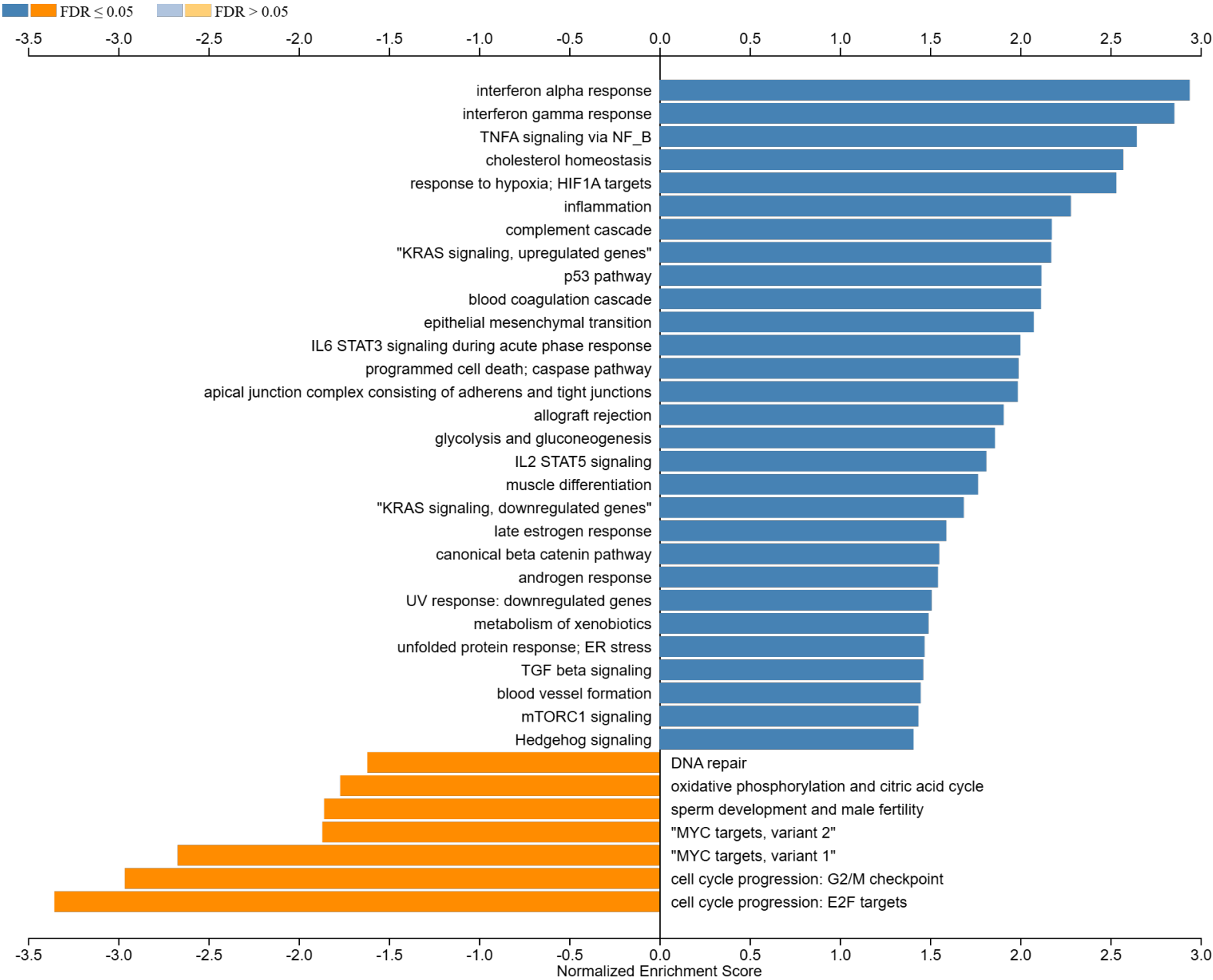
CSCs vs WT

GSEA (Gene Set Enrichment Analysis) - pathway_KEGG



CSCs vs WT

GSEA (Gene Set Enrichment Analysis) - Hallmark50

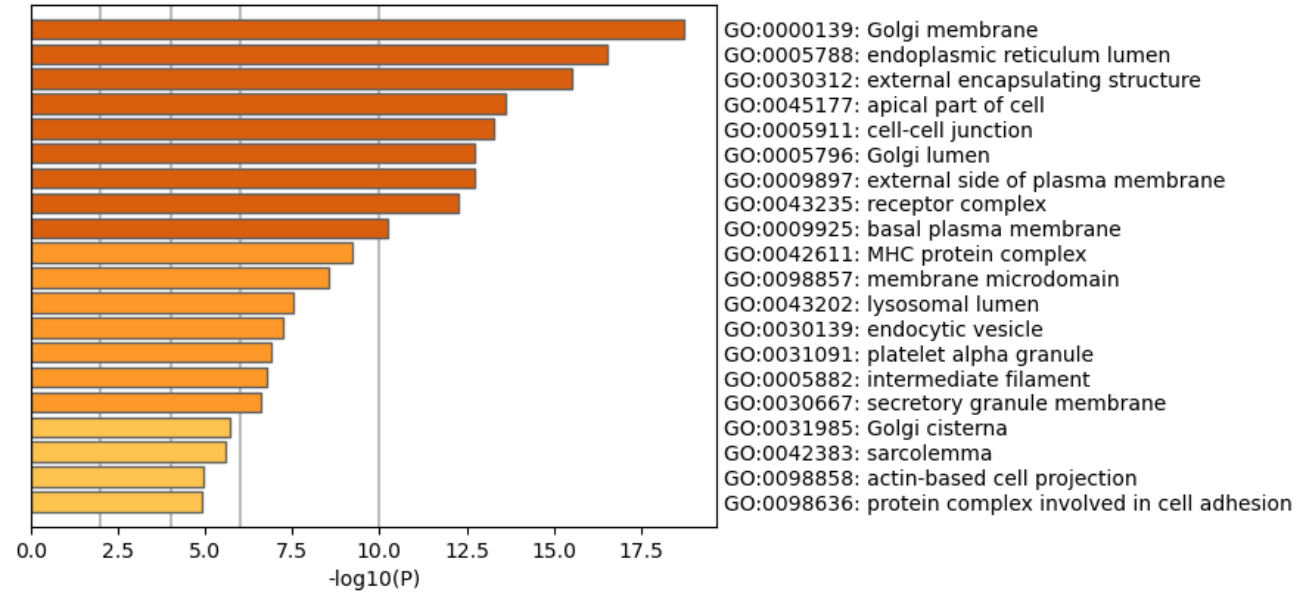


CSCs vs WT

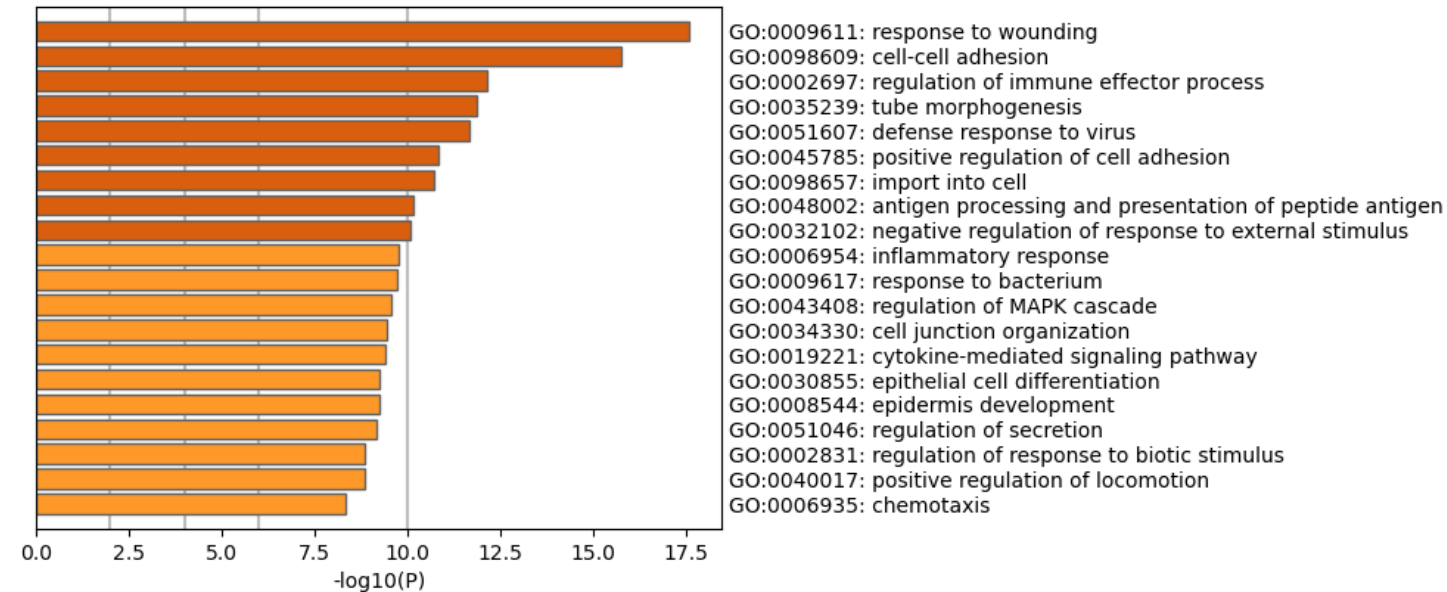
ORA (Over-Representation Analysis)

Activation in CSCs

GO Cellular Components



GO Biological Process

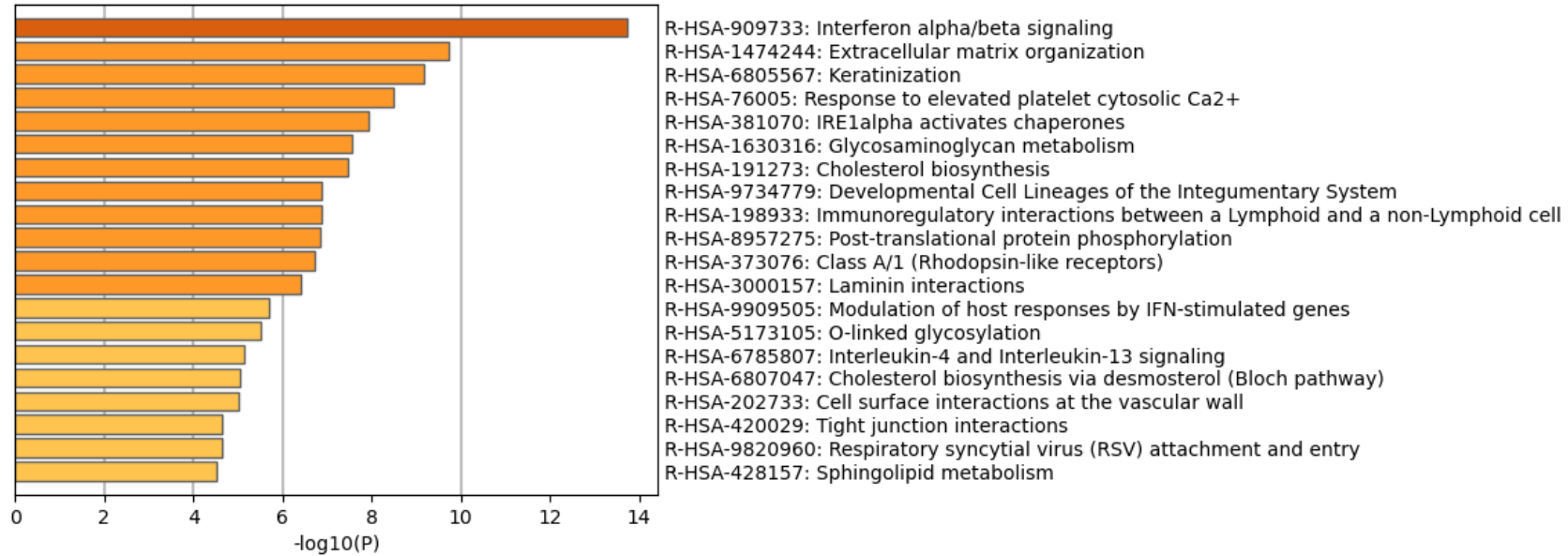


CSCs vs WT

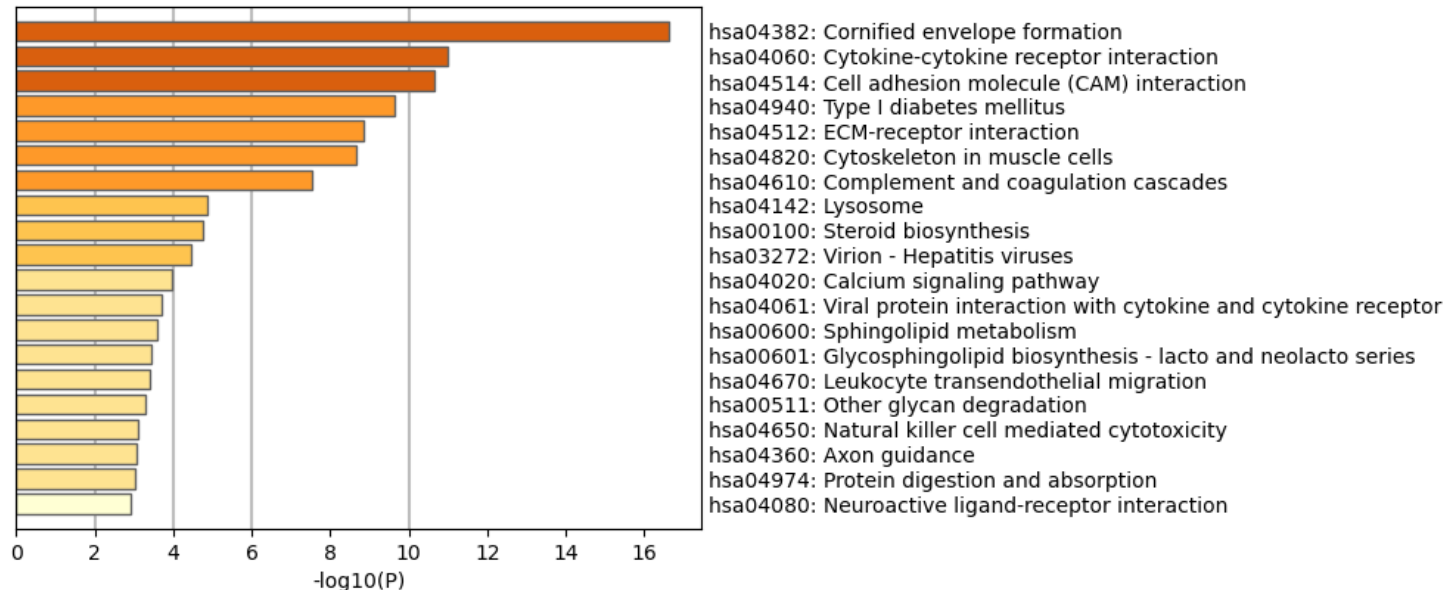
ORA (Over-Representation Analysis)

Activation in CSCs

Reactome Gene Sets



KEGG Pathway

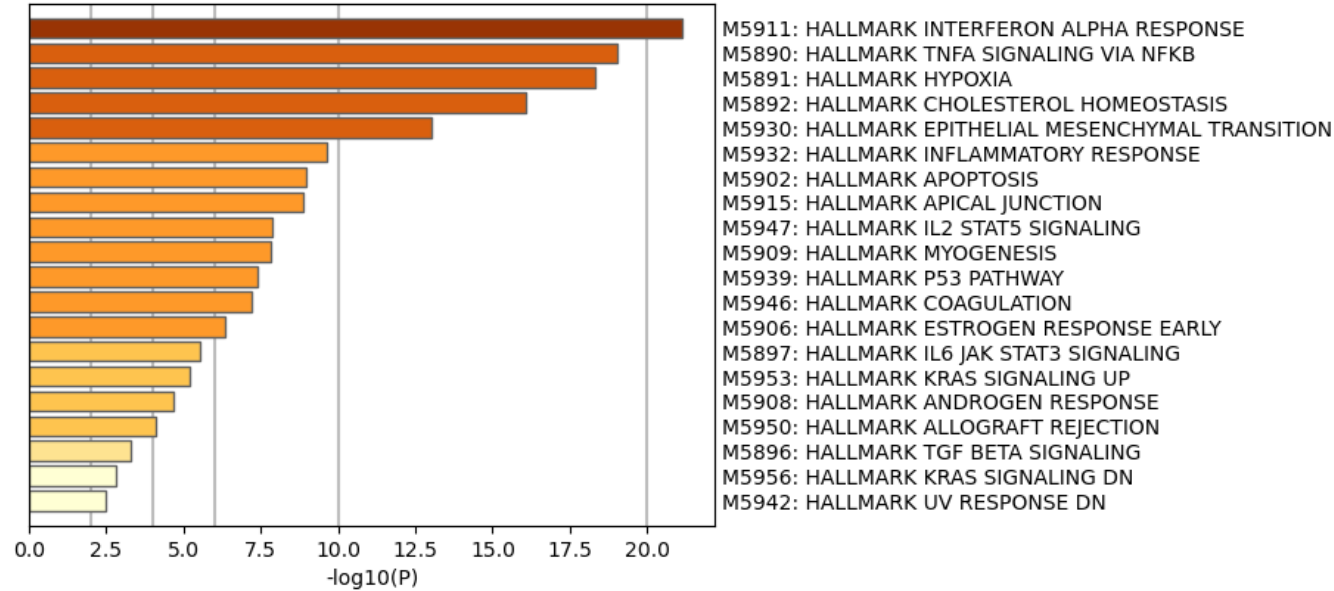


CSCs vs WT

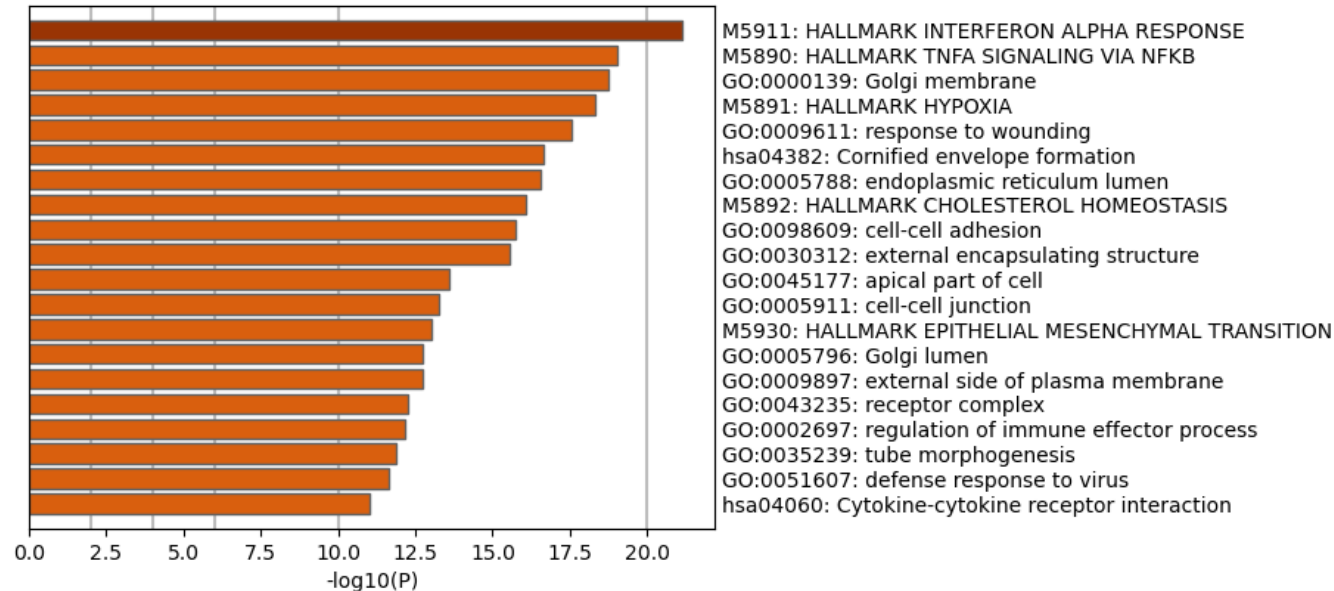
ORA (Over-Representation Analysis)

Activation in CSCs

Hallmark Gene Sets



Combined GO

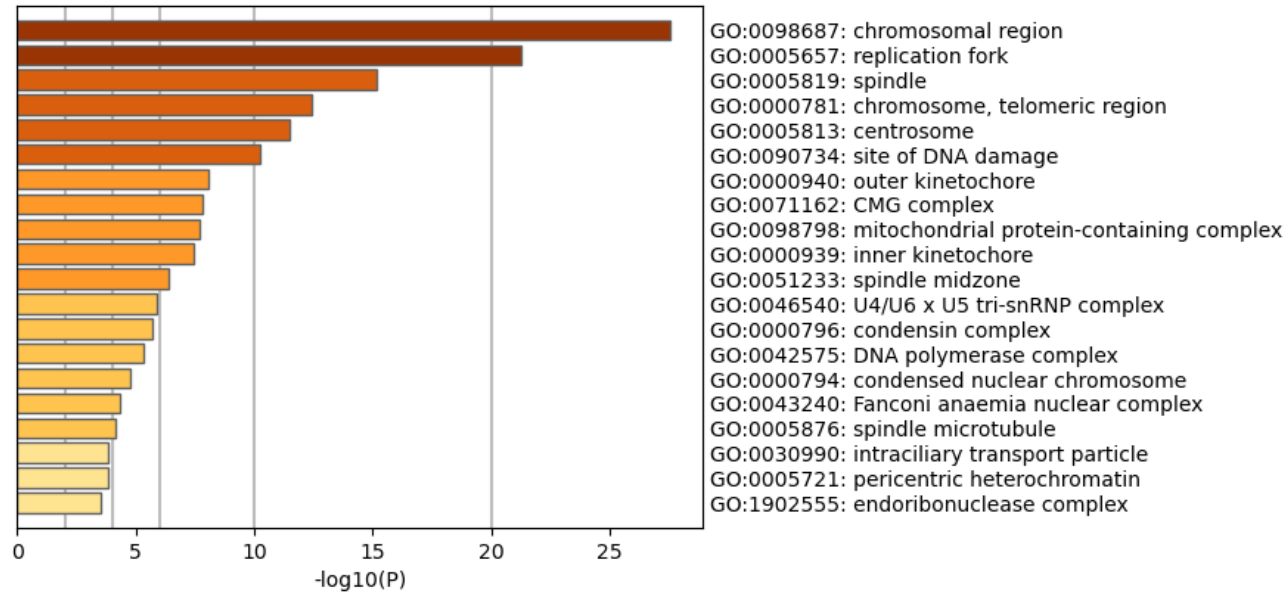


CSCs vs WT

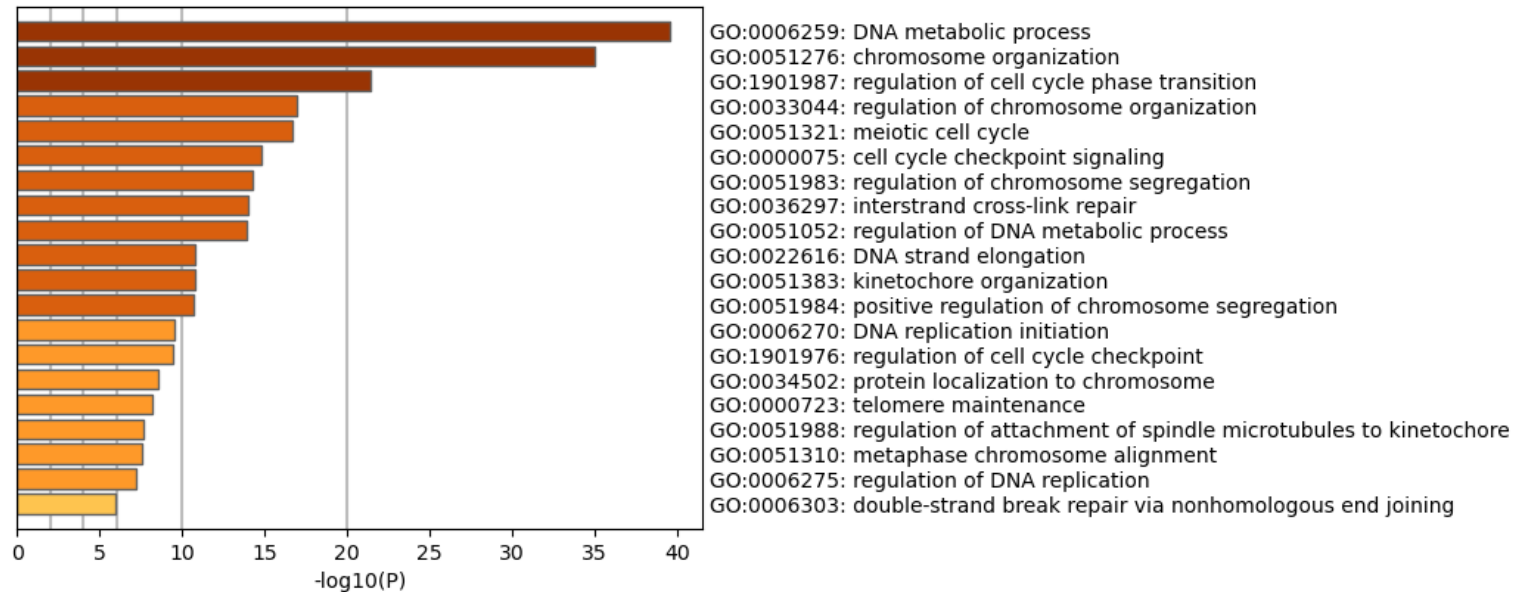
ORA (Over-Representation Analysis)

Inhibition in CSCs

GO Cellular Components



GO Biological Process

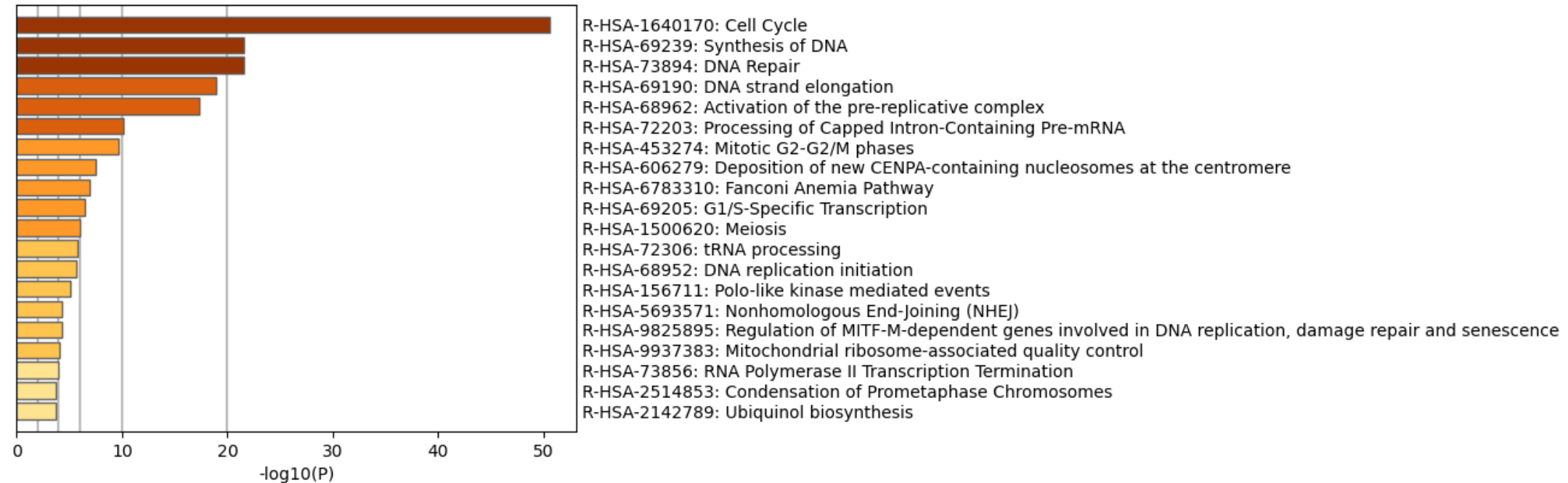


CSCs vs WT

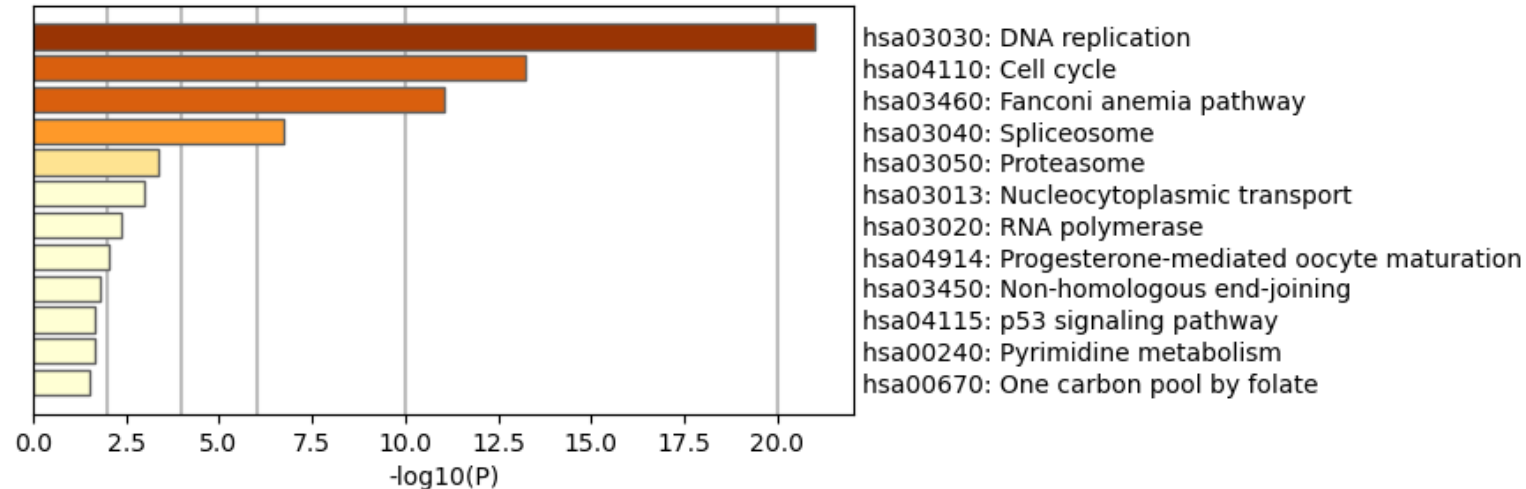
ORA (Over-Representation Analysis)

Inhibition in CSCs

Reactome Gene Sets



KEGG Pathway

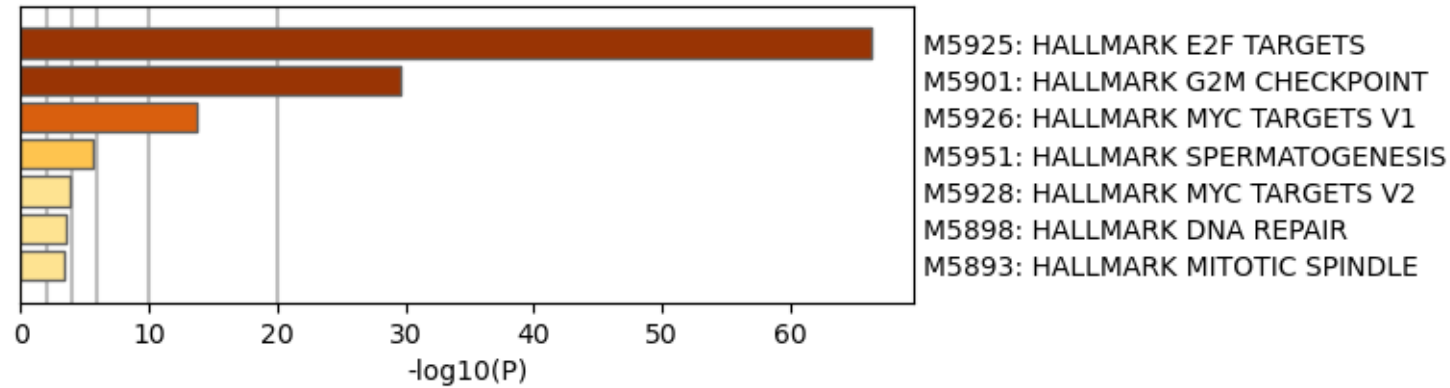


CSCs vs WT

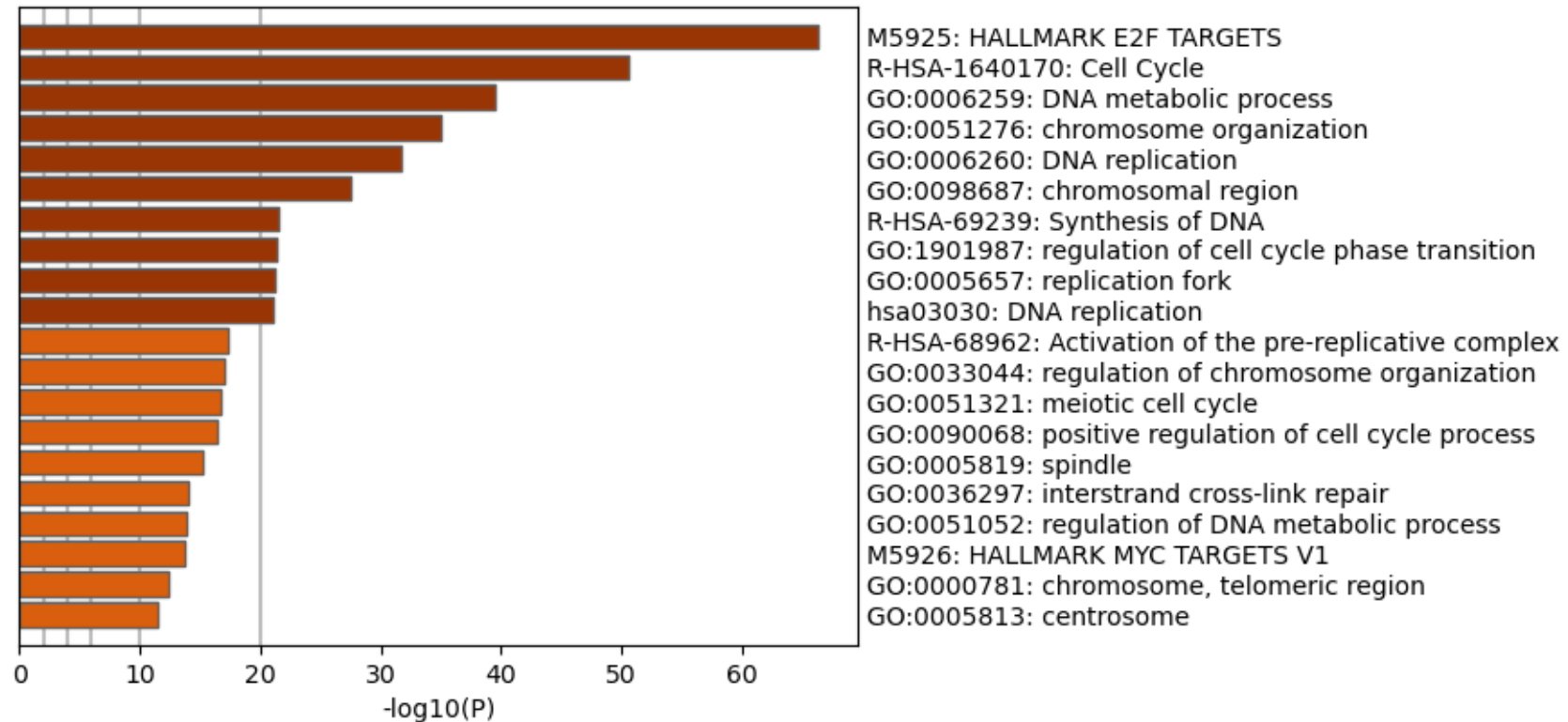
ORA (Over-Representation Analysis)

Inhibition in CSCs

Hallmark Gene Sets

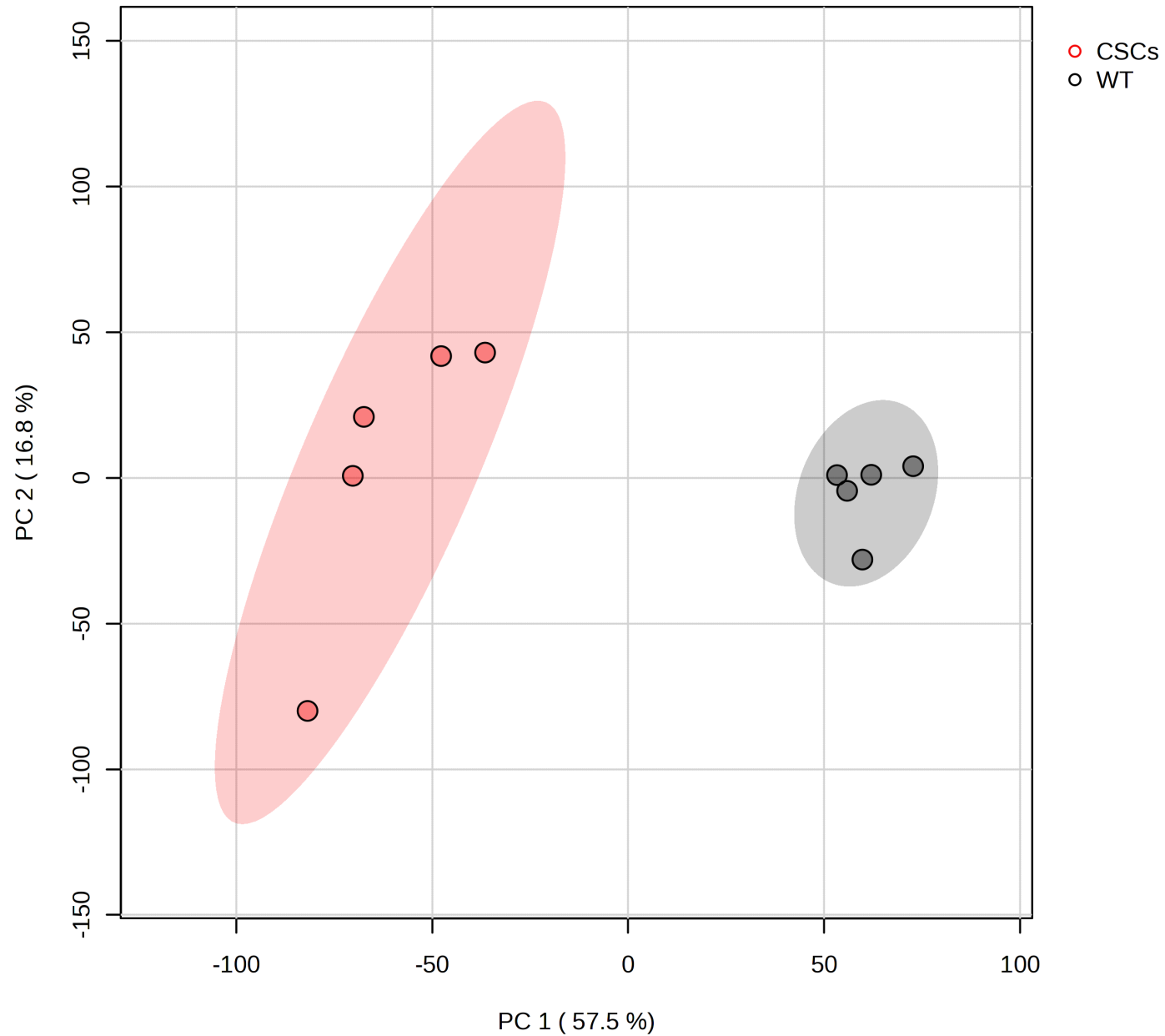


Combined GO



CSCs vs WT Proteomics

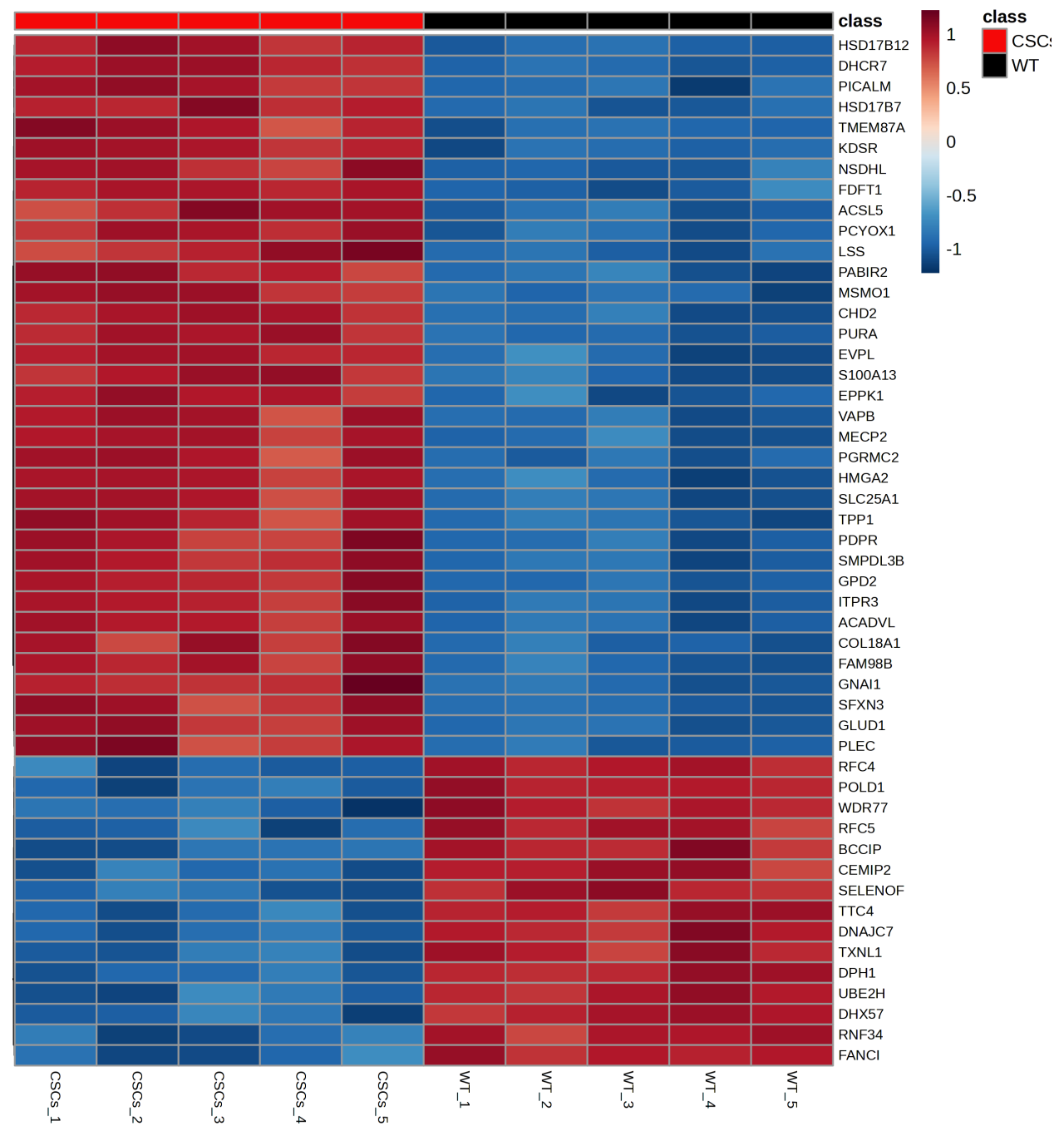
CSCs vs WT



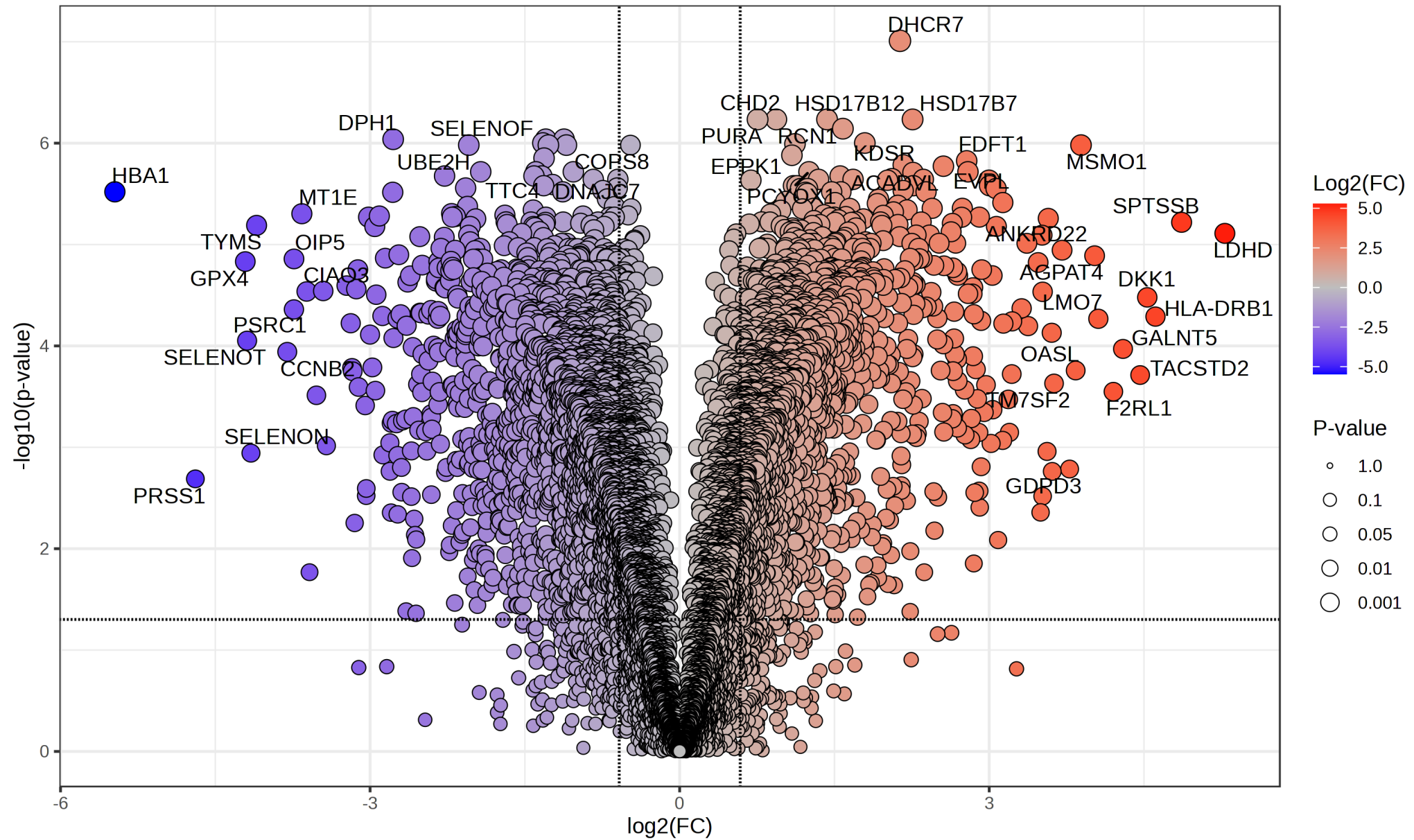
[PERMANOVA] F-value: 23.709; R-squared: 0.74771; p-value (based on 999 permutations): 0.01

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 vs WT



CSCs vs WT

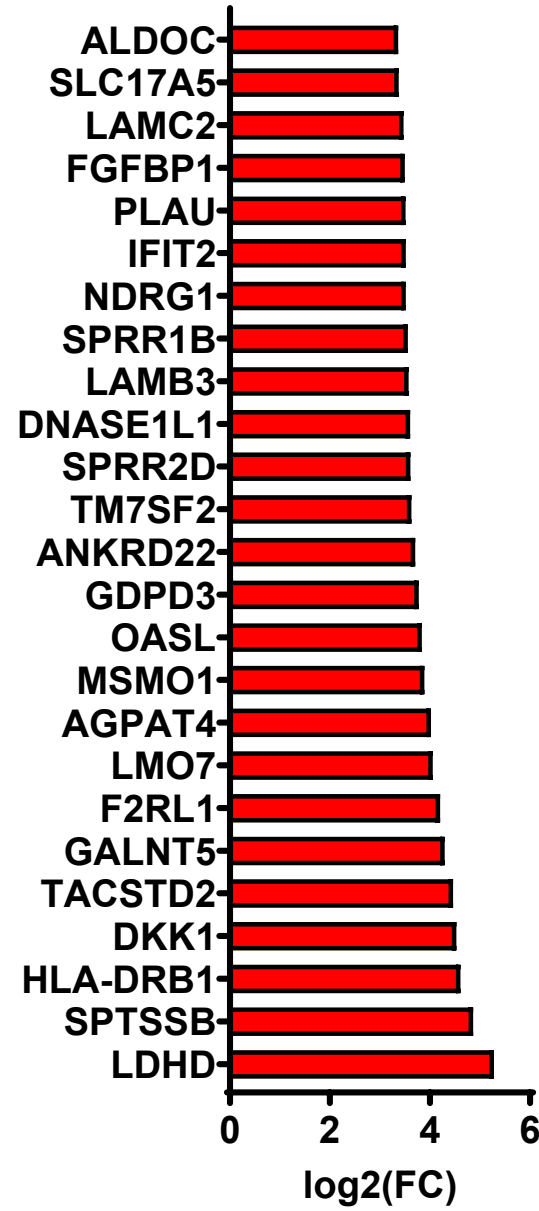


Fold change (FC) threshold: 1.5

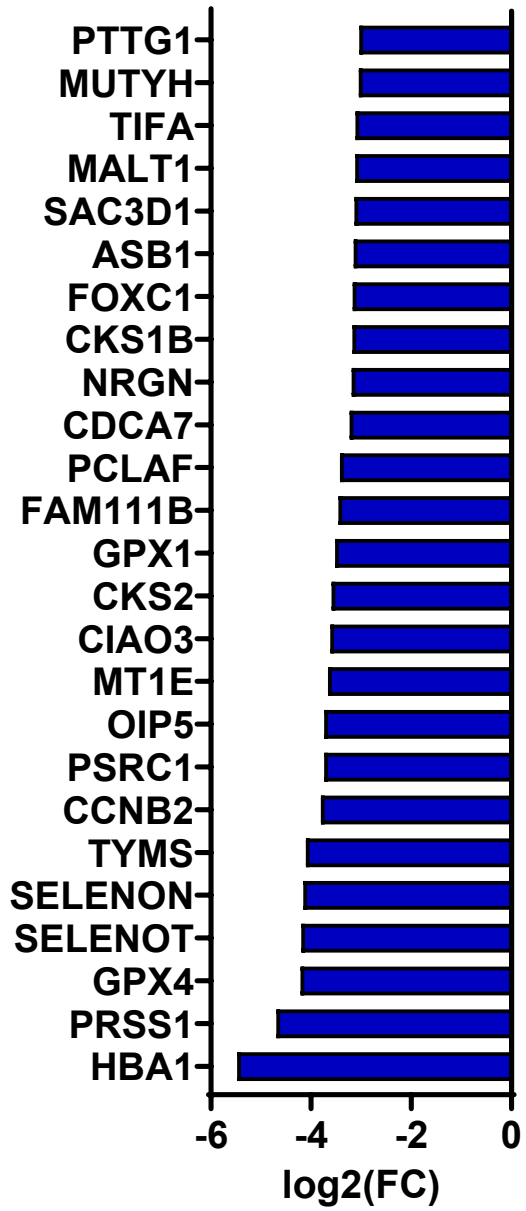
P-value threshold: 0.05

CSCs vs WT

Top 25_Upregulated proteins in CSCs

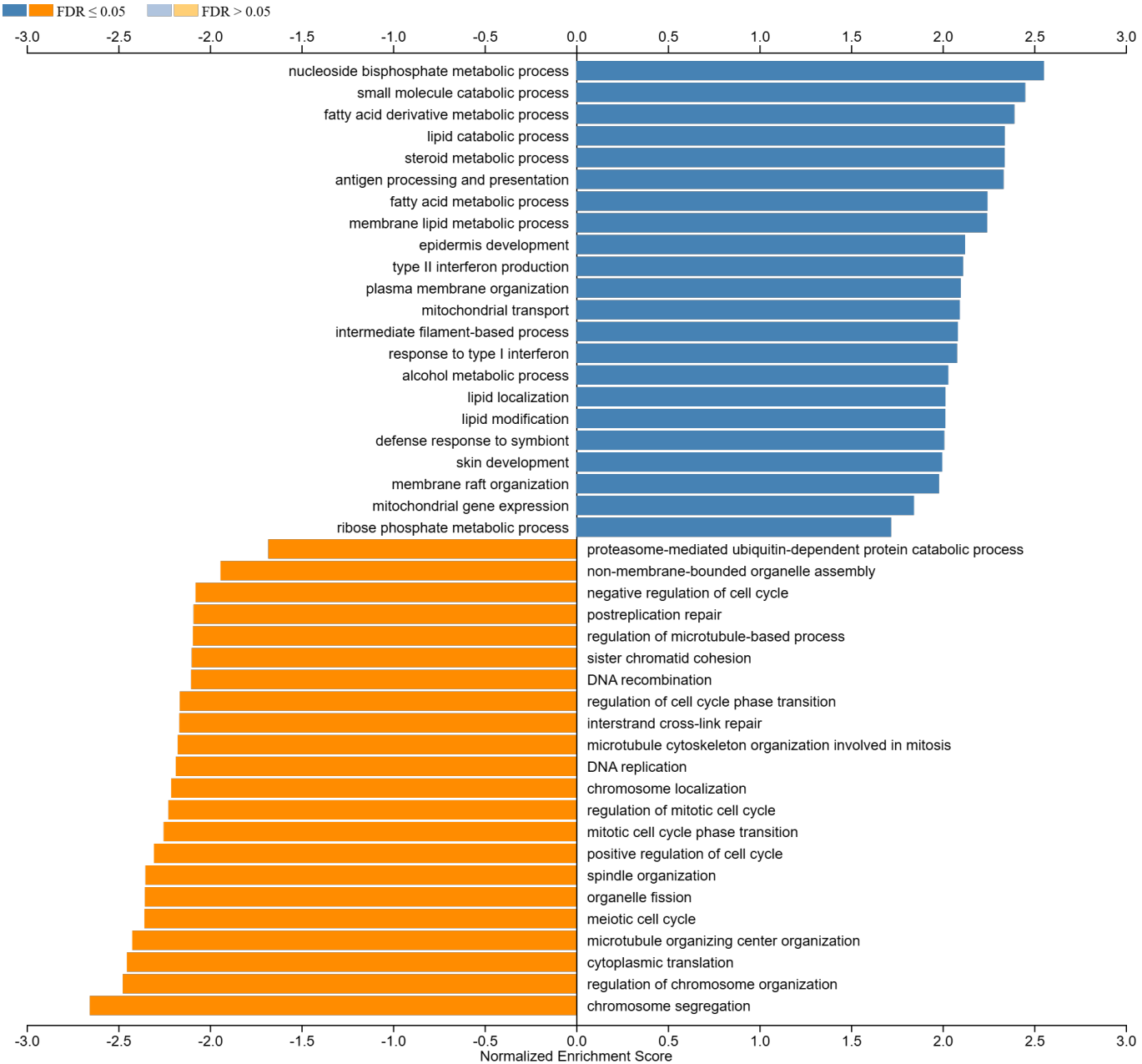


Top 25_Downregulated proteins in CSCs



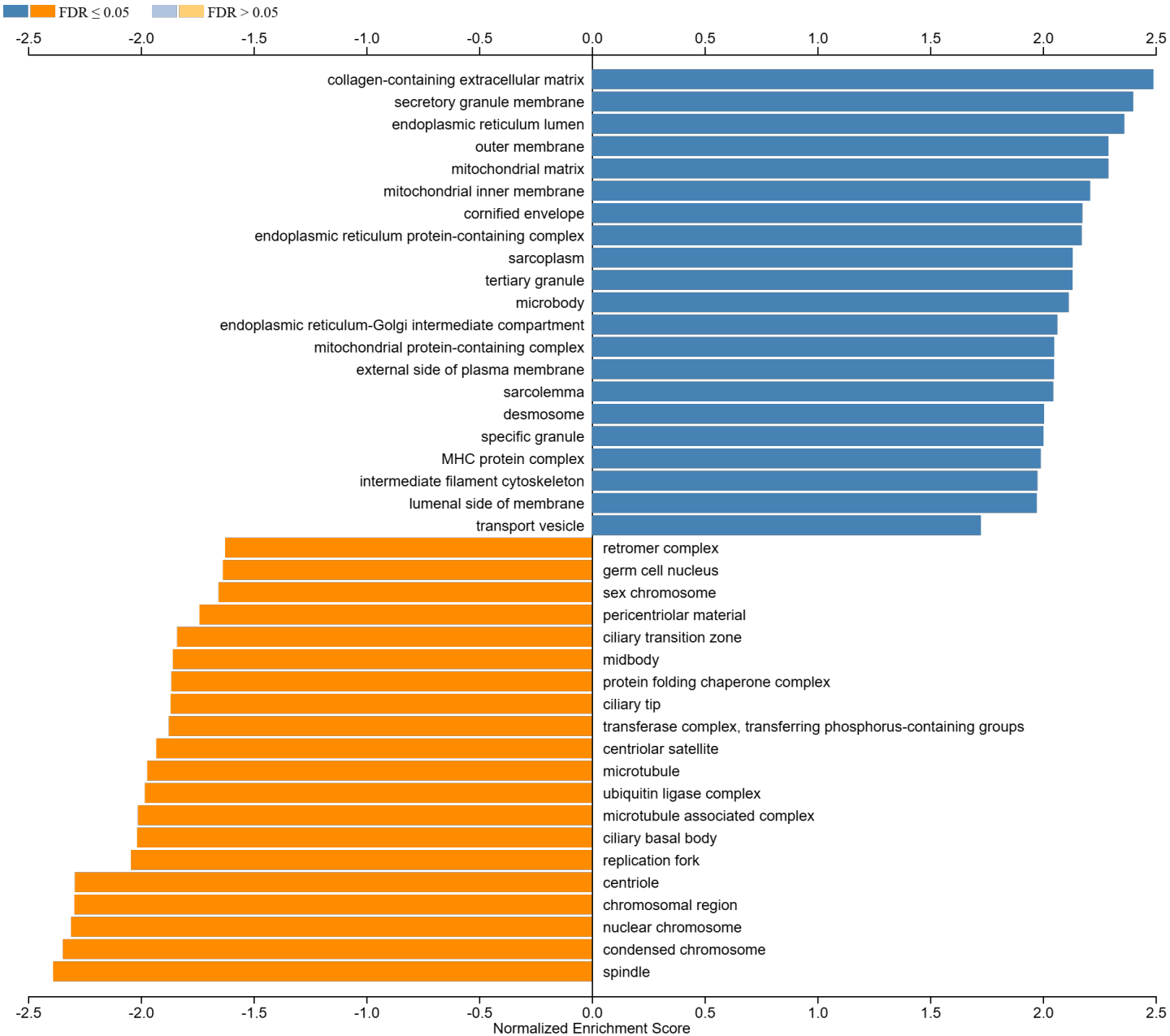
CSCs vs WT

GSEA (Gene Set Enrichment Analysis) - geneontology_Biological_Process



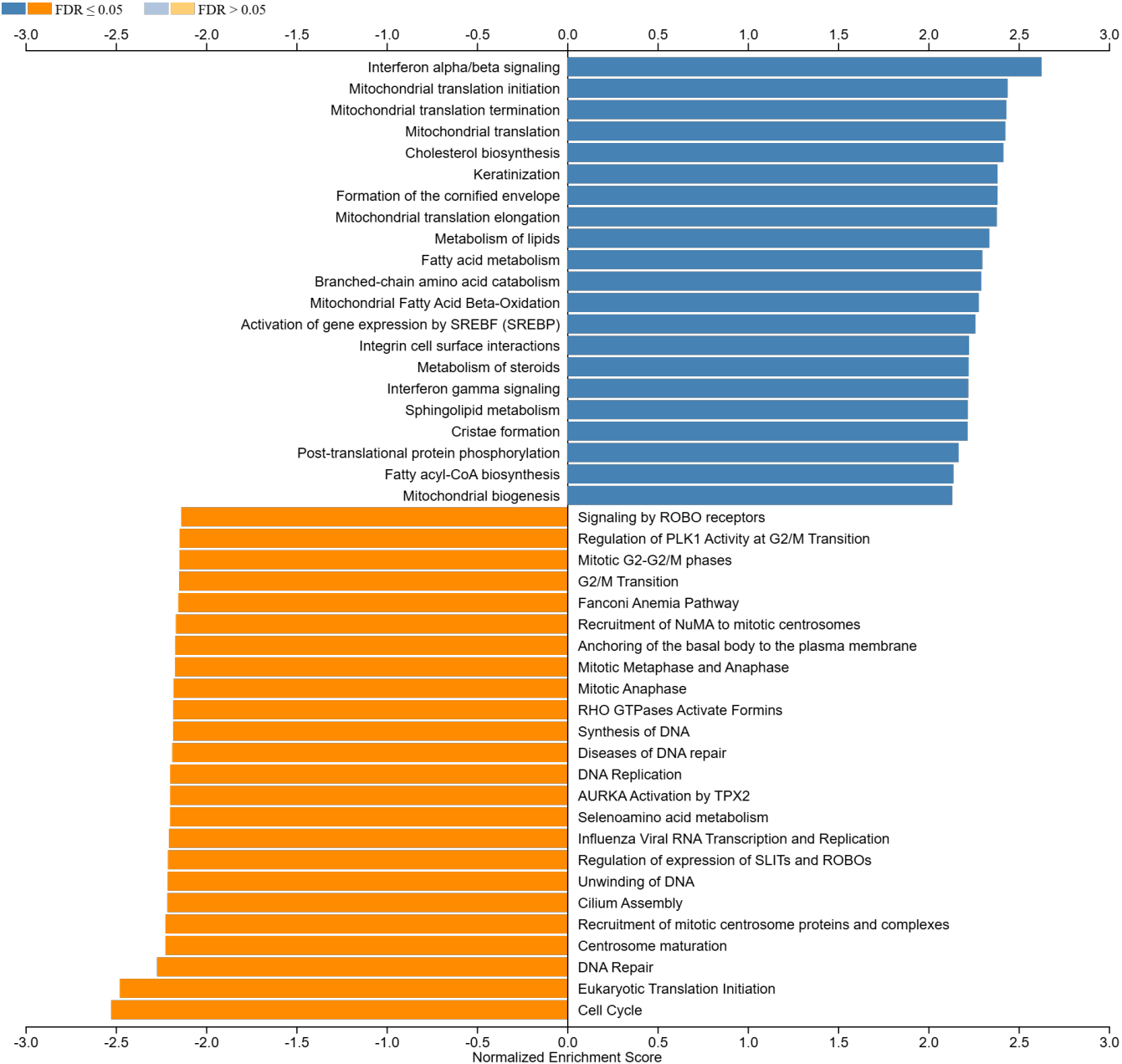
CSCs vs WT

GSEA (Gene Set Enrichment Analysis) - geneontology_Cellular_Component



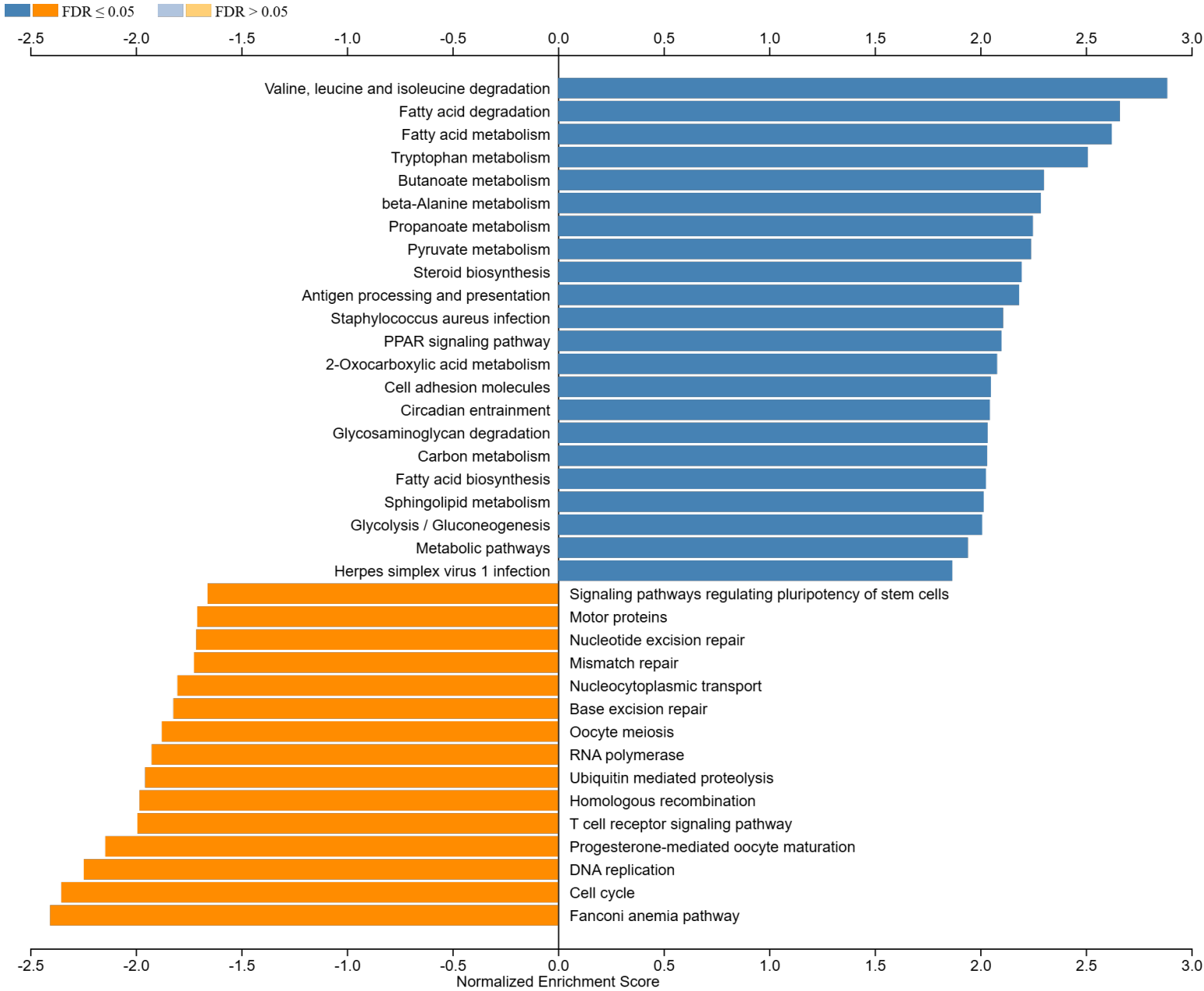
CSCs vs WT

GSEA (Gene Set Enrichment Analysis) - pathway_Reactome



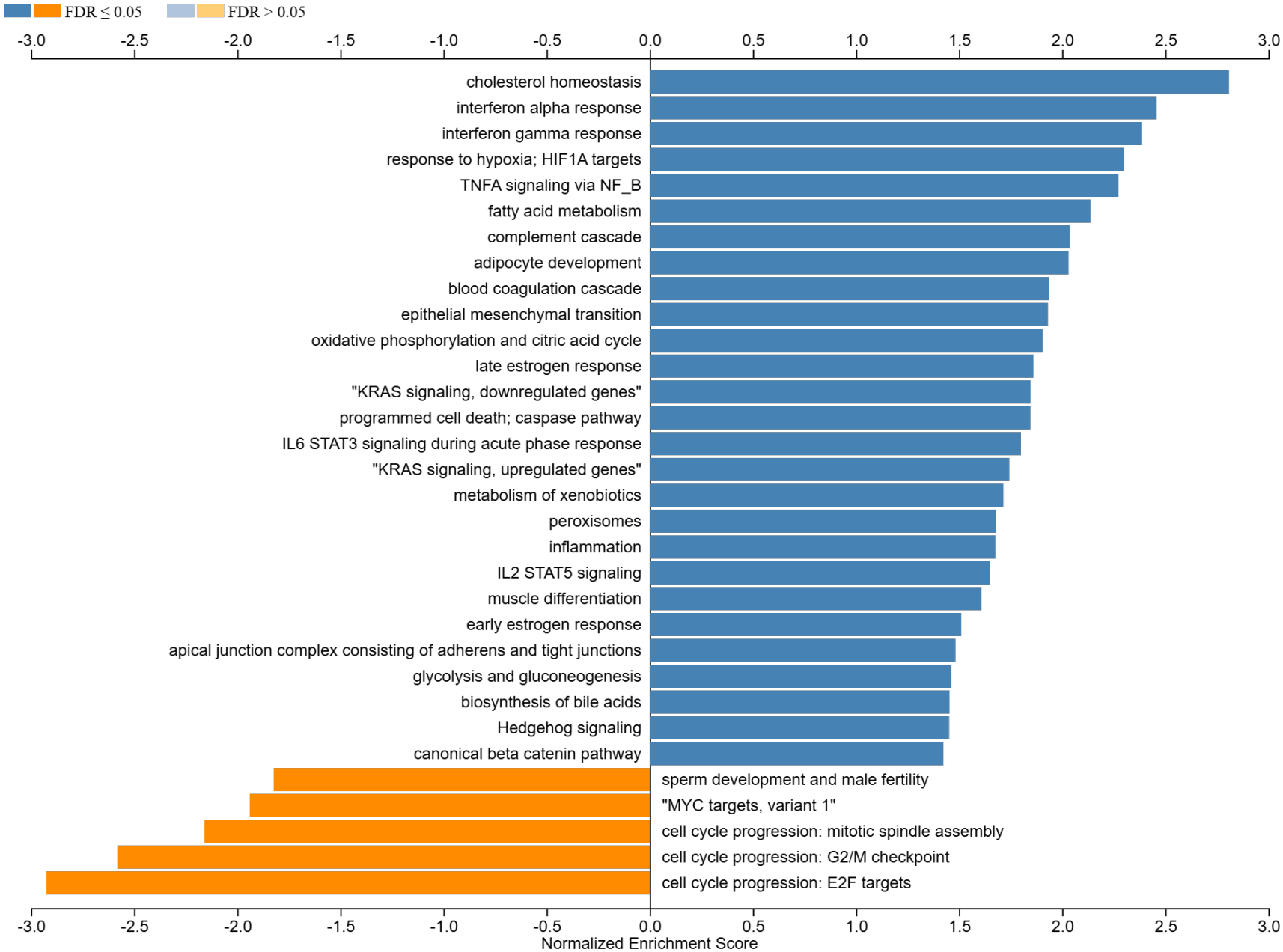
CSCs vs WT

GSEA (Gene Set Enrichment Analysis) - pathway_KEGG



CSCs vs WT

GSEA (Gene Set Enrichment Analysis) - Hallmark50

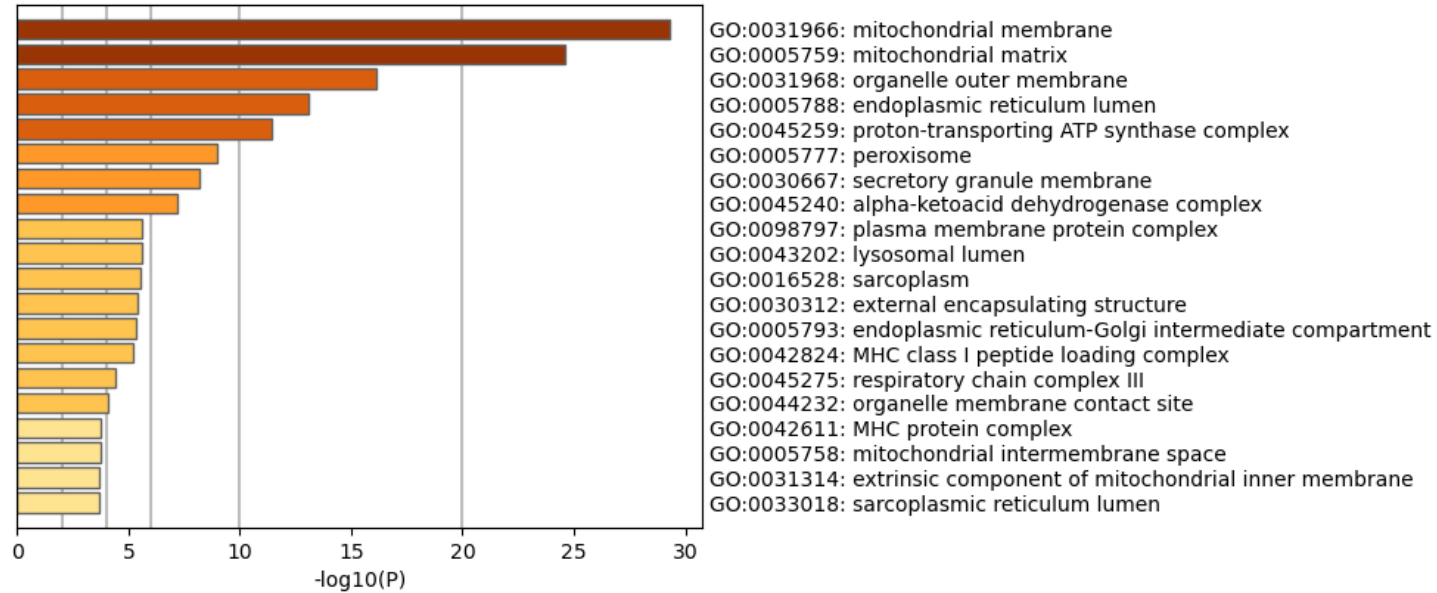


CSCs vs WT

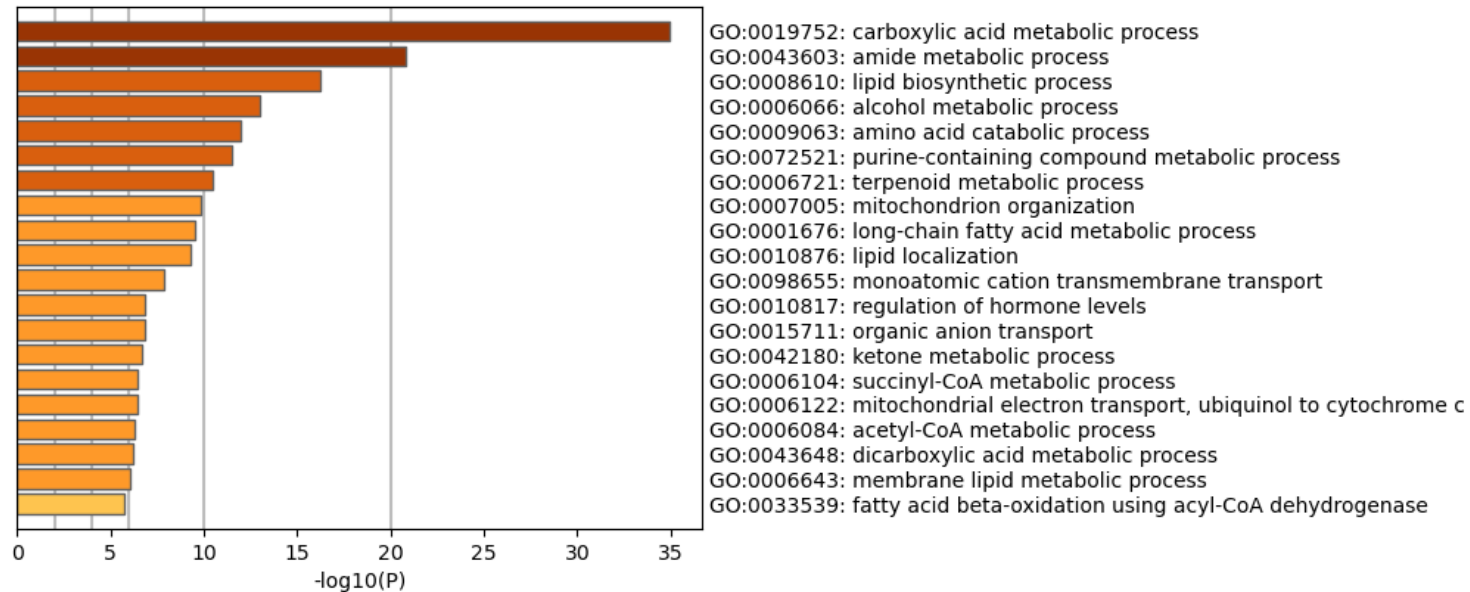
ORA (Over-Representation Analysis)

Activation in CSCs

GO Cellular Components



GO Biological Process

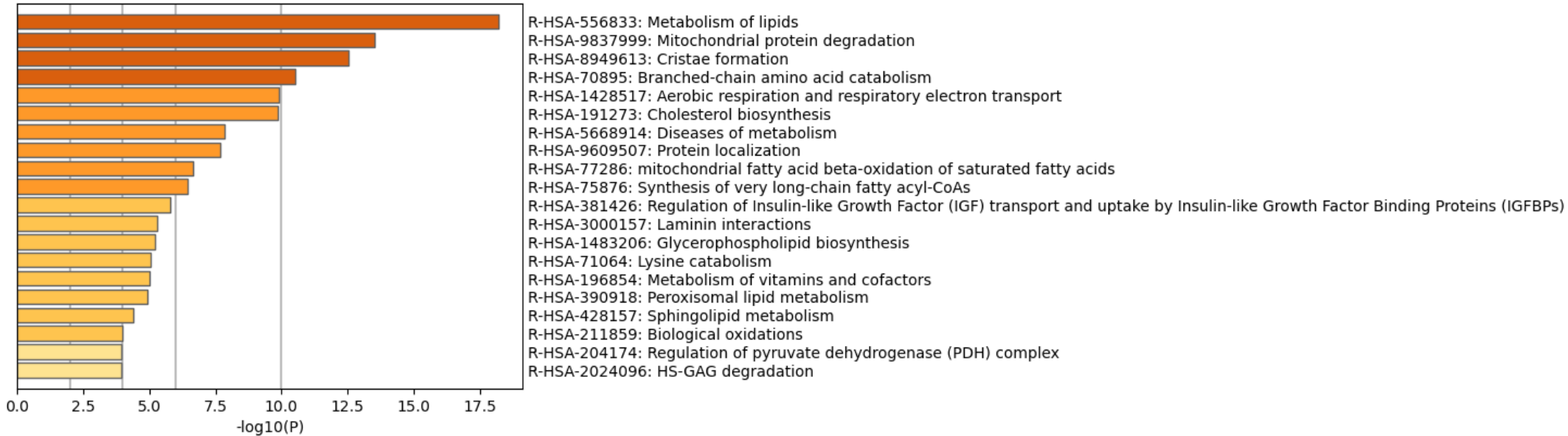


CSCs vs WT

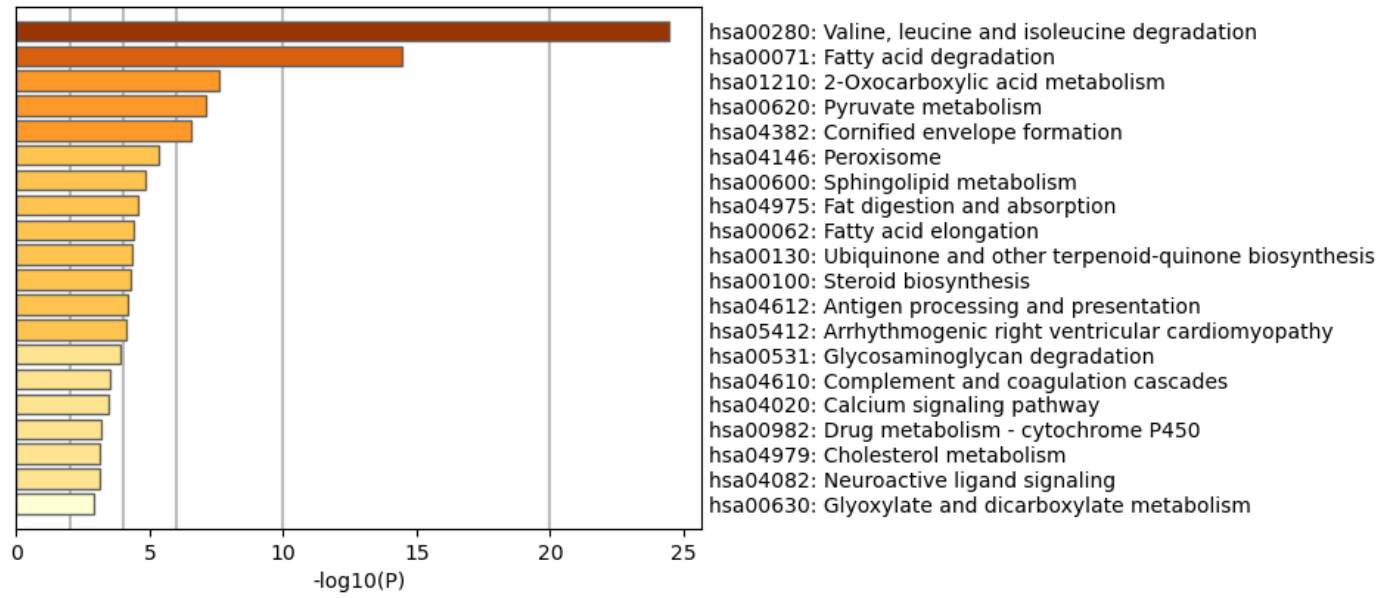
ORA (Over-Representation Analysis)

Activation in CSCs

Reactome Gene Sets



KEGG Pathway

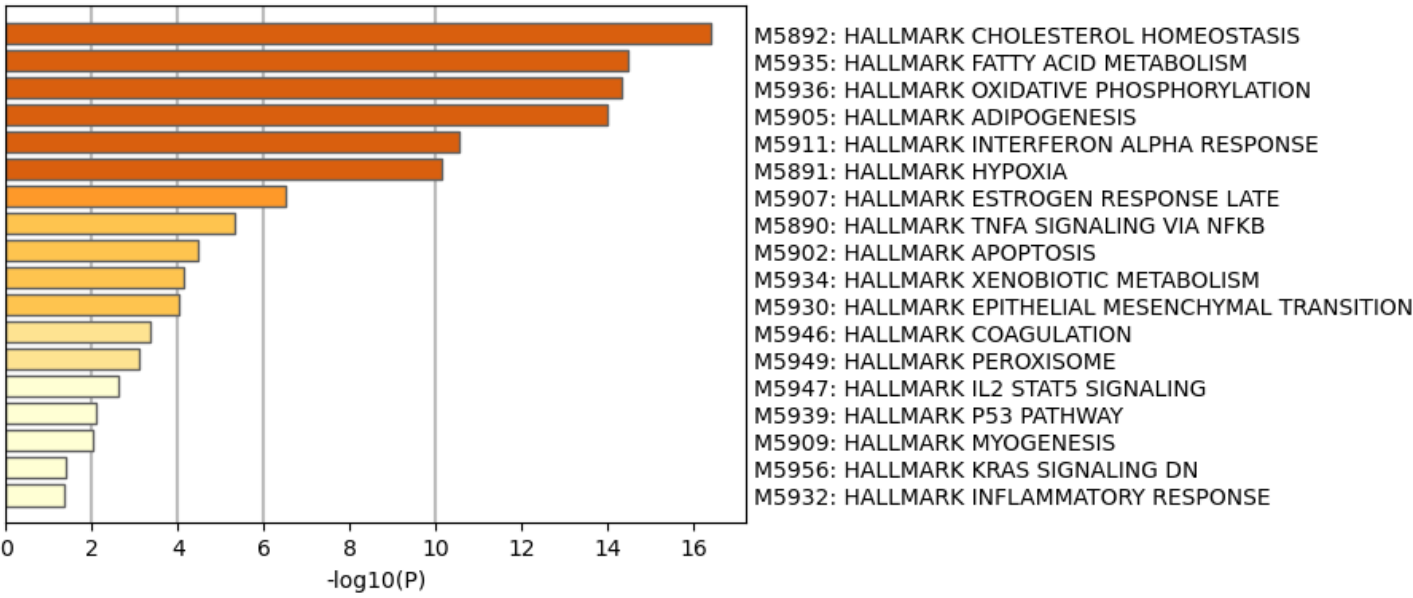


CSCs vs WT

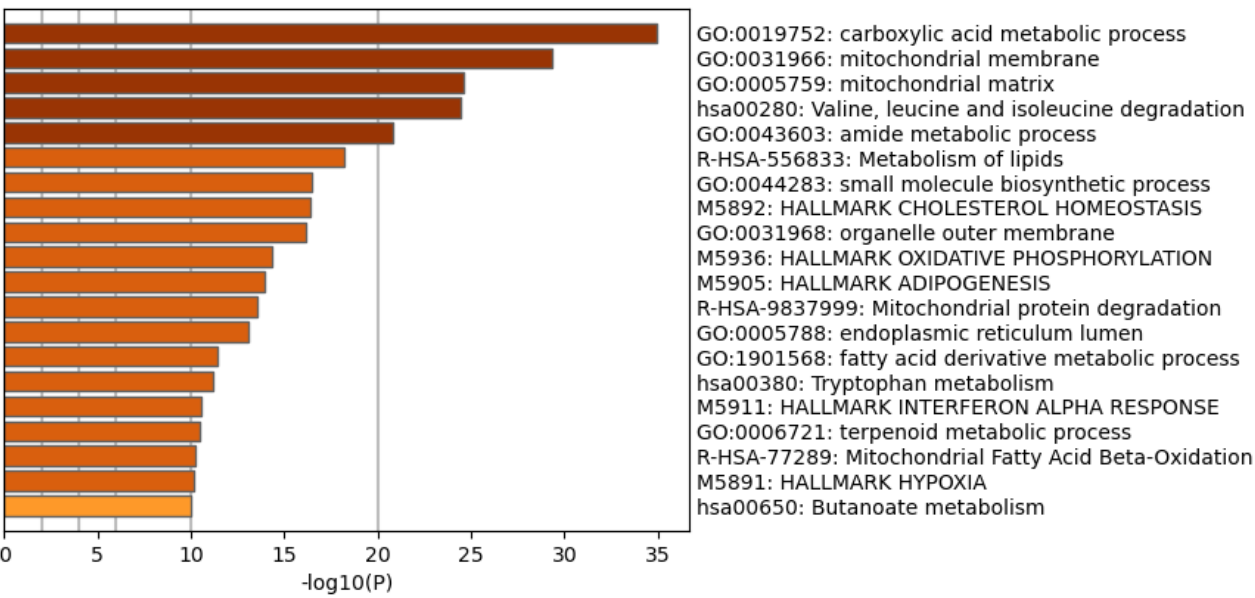
ORA (Over-Representation Analysis)

Activation in CSCs

Hallmark Gene Sets



Combined GO

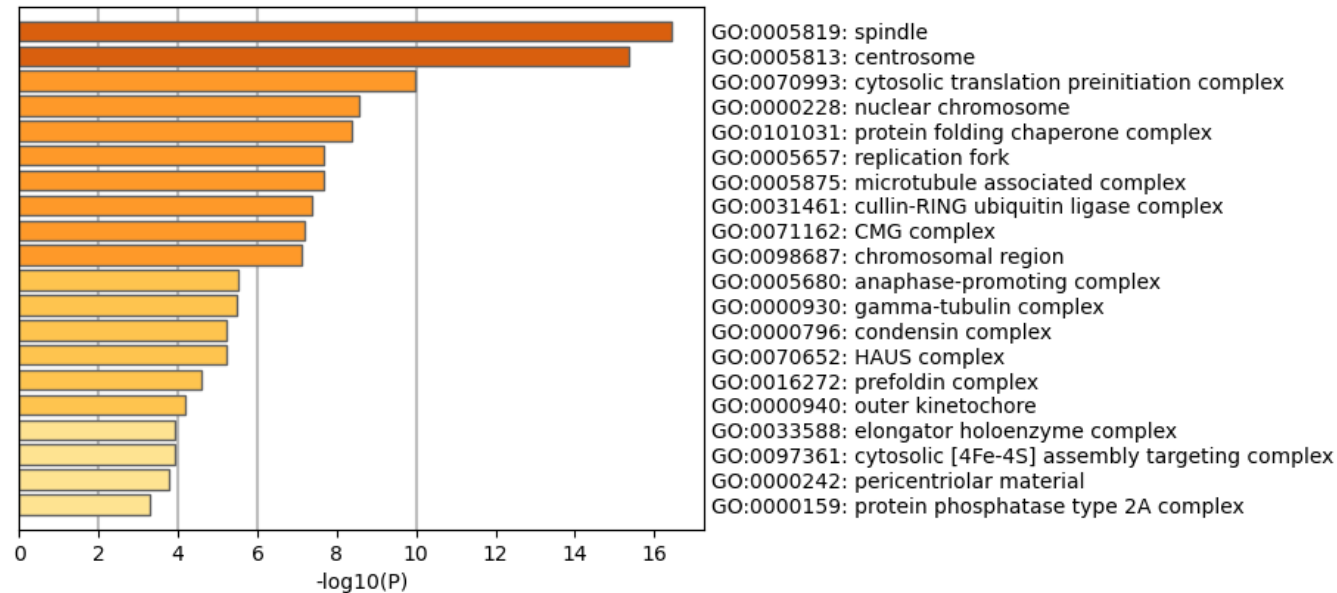


CSCs vs WT

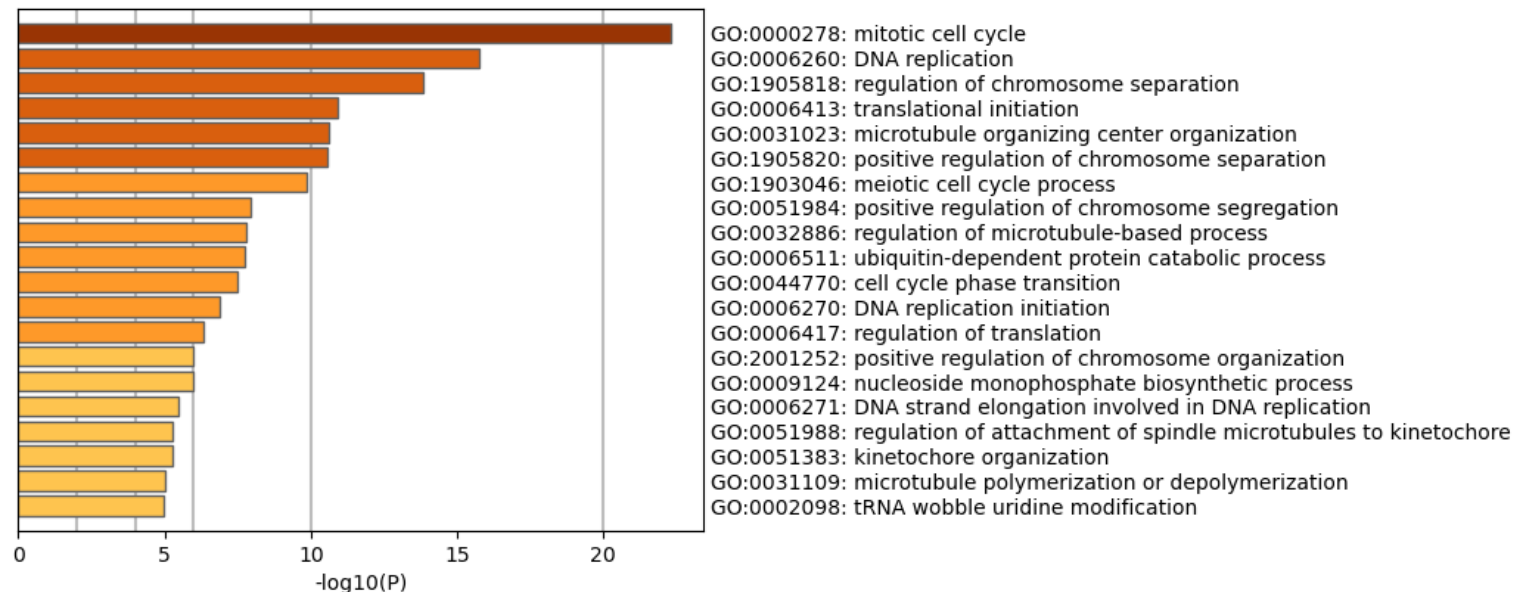
ORA (Over-Representation Analysis)

Inhibition in CSCs

GO Cellular Components



GO Biological Process

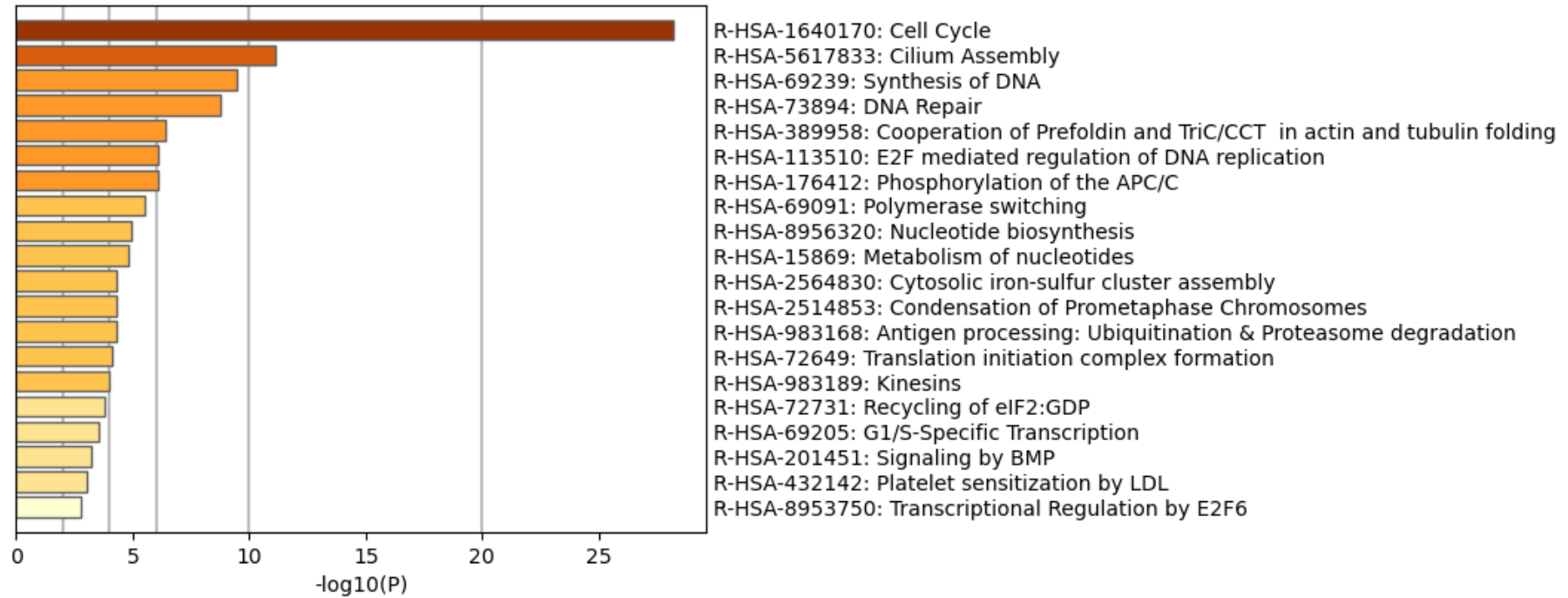


CSCs vs WT

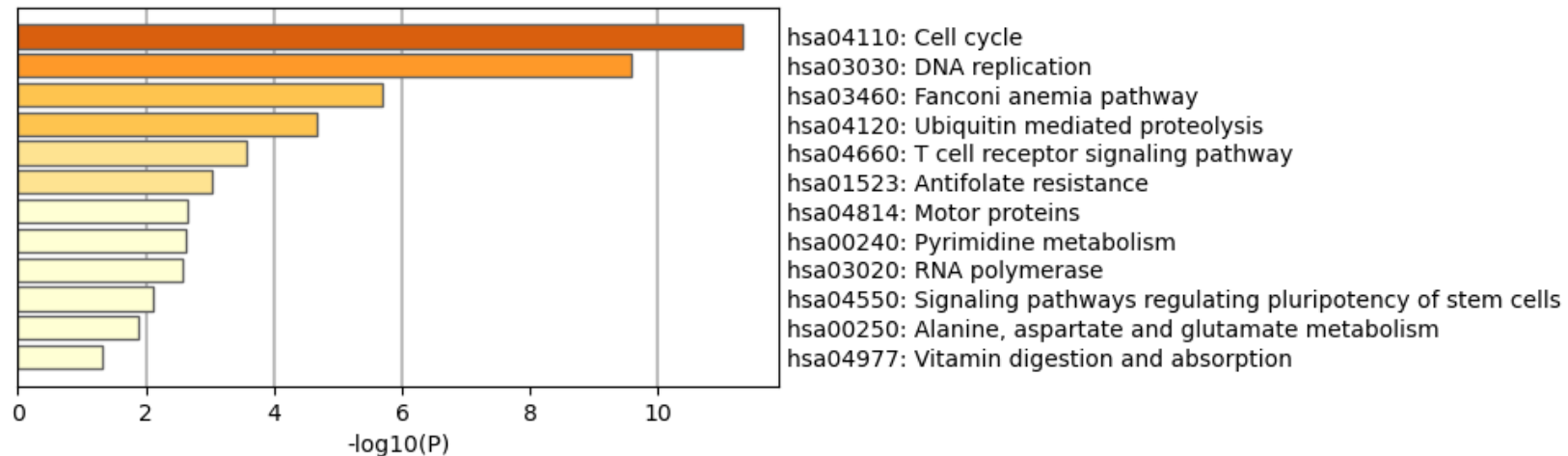
ORA (Over-Representation Analysis)

Inhibition in CSCs

Reactome Gene Sets



KEGG Pathway

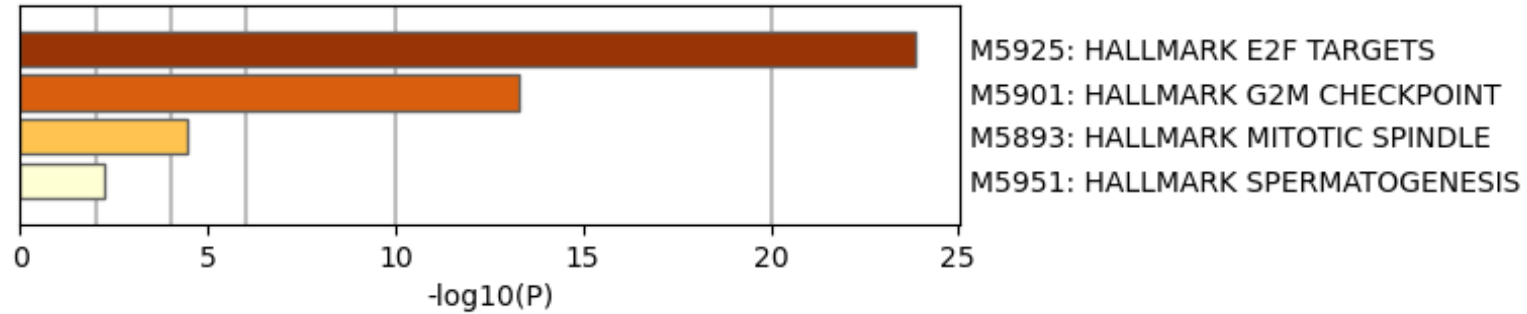


CSCs vs WT

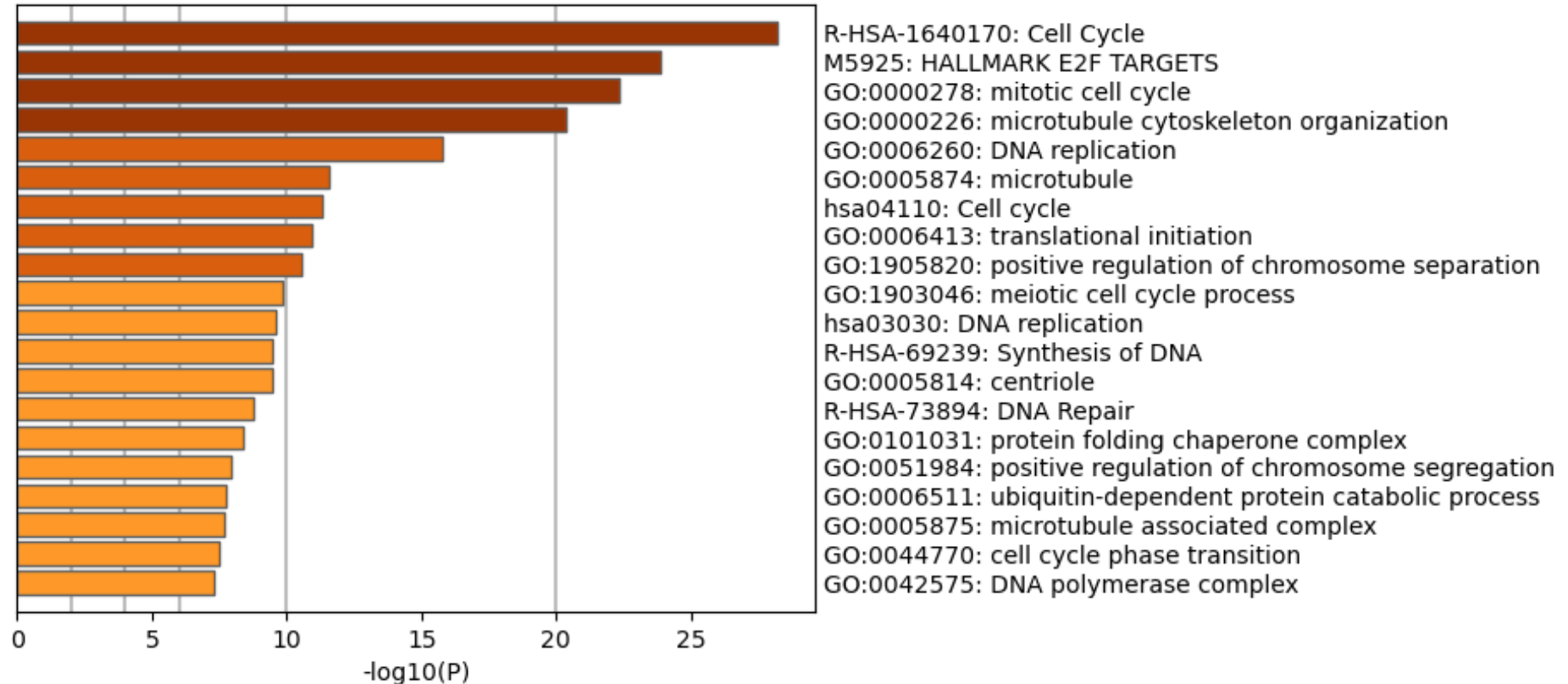
ORA (Over-Representation Analysis)

Inhibition in CSCs

Hallmark Gene Sets

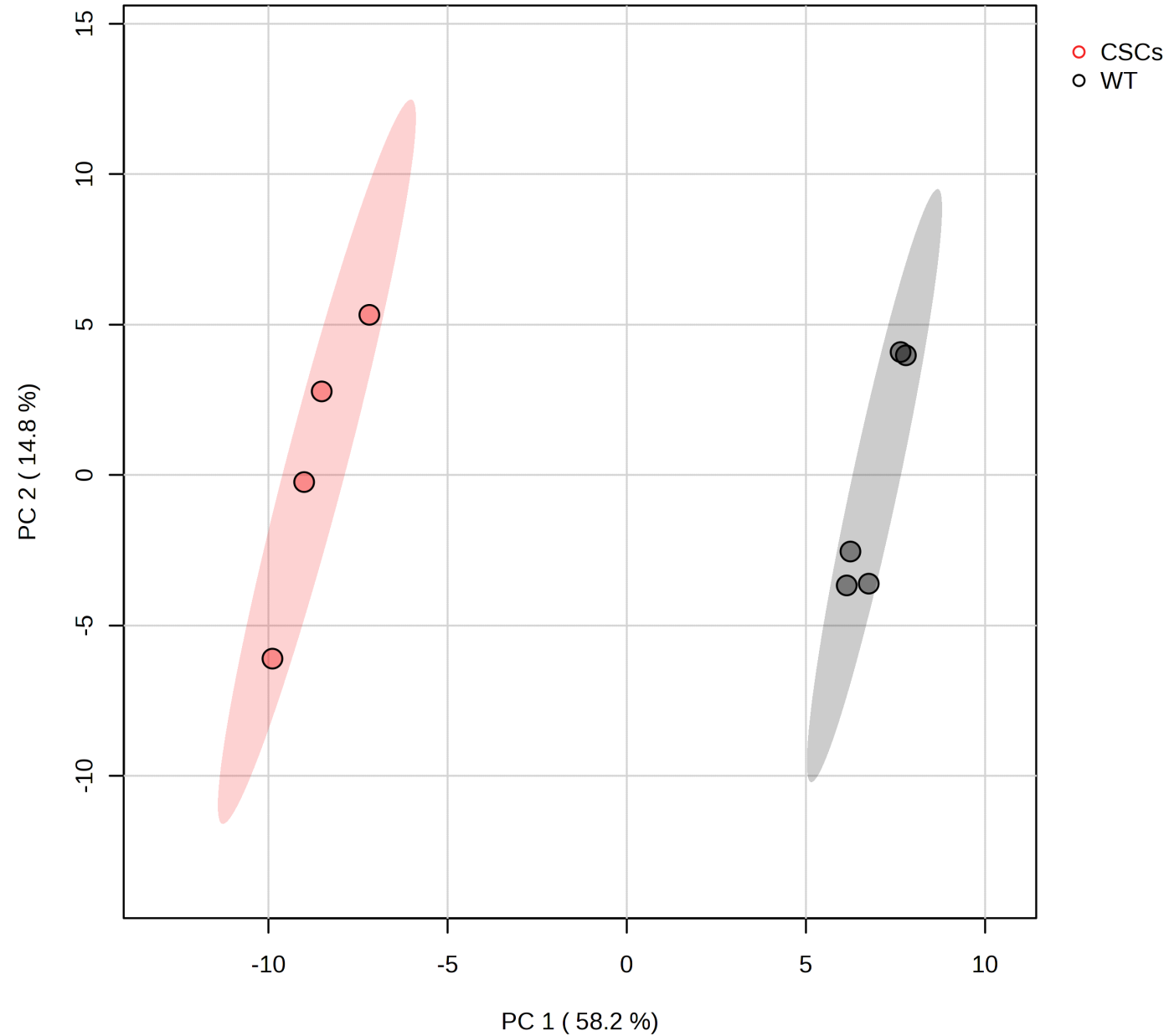


Combined GO



CSCs vs WT Metabolomics

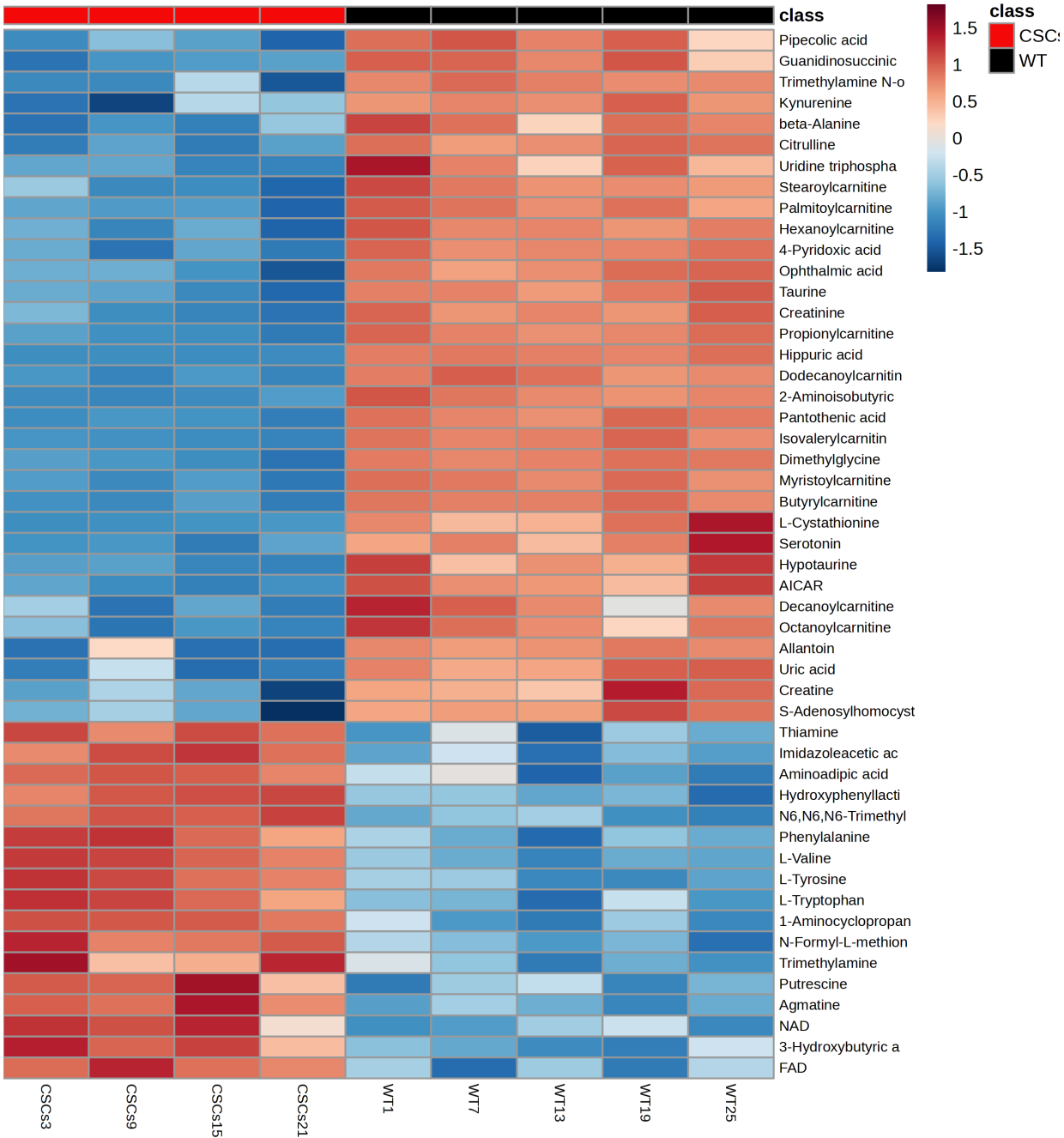
CSCs vs WT



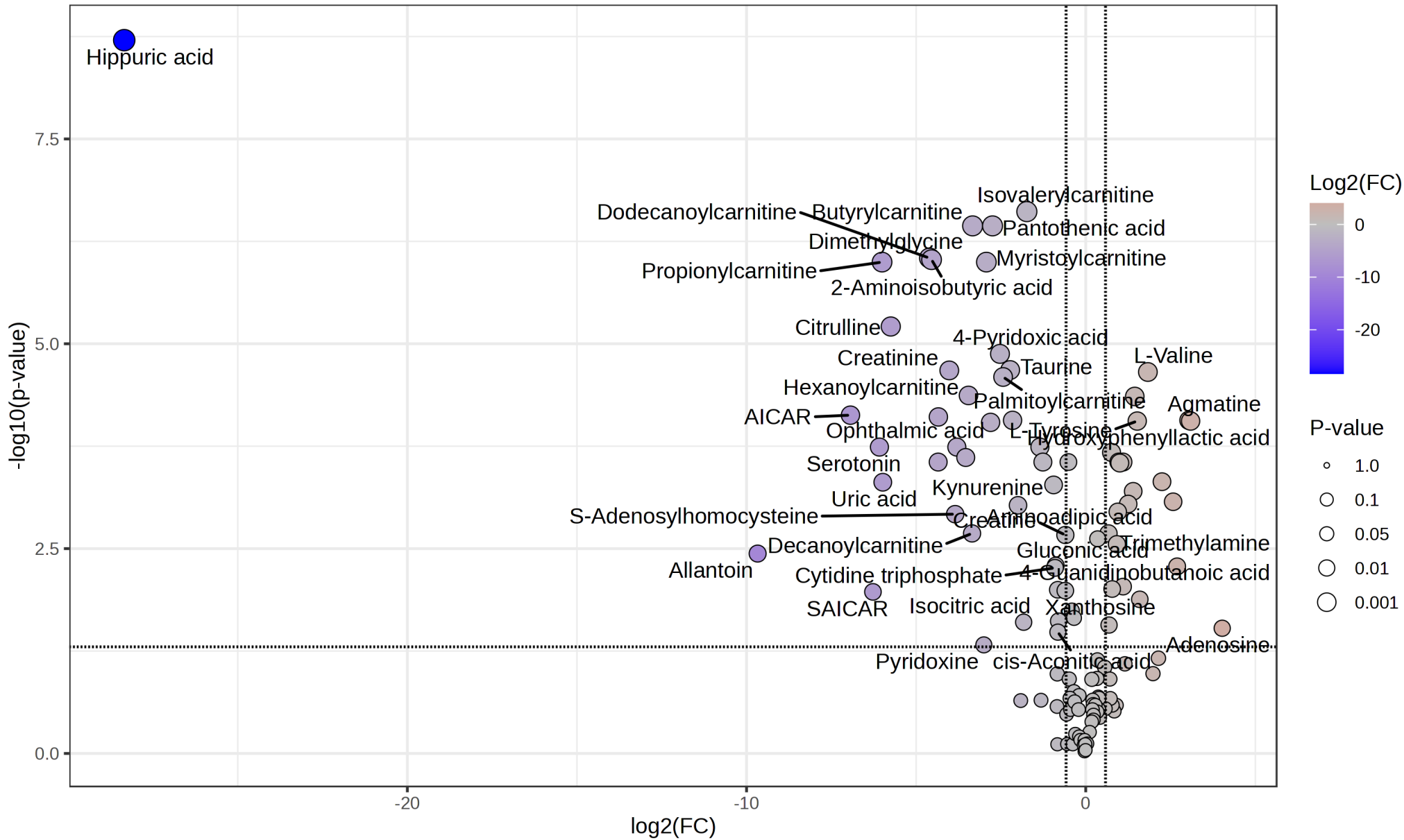
[PERMANOVA] F-value: 26.273; R-squared: 0.78962; p-value (based on 999 permutations): 0.01

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 vs WT



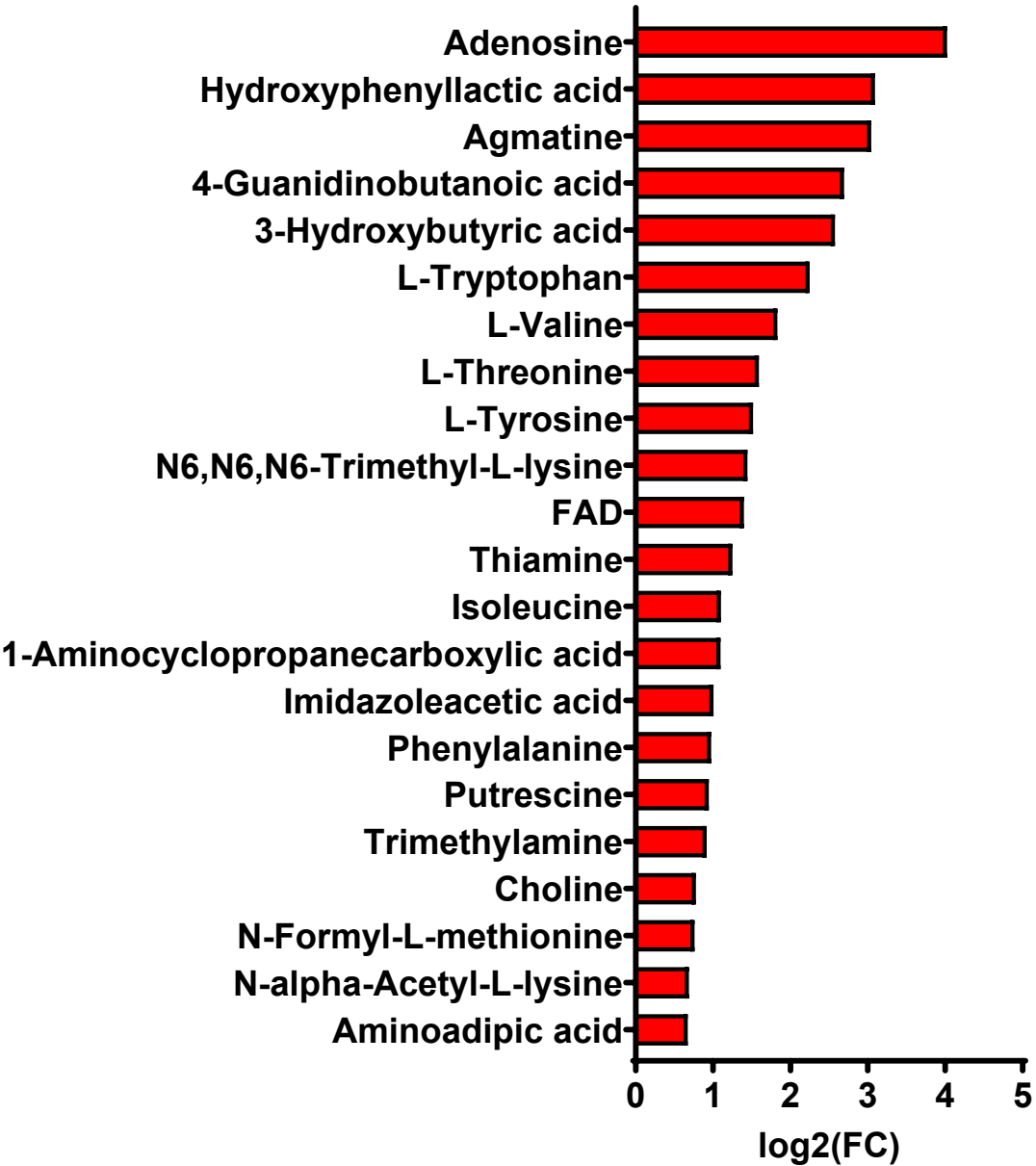
CSCs vs WT



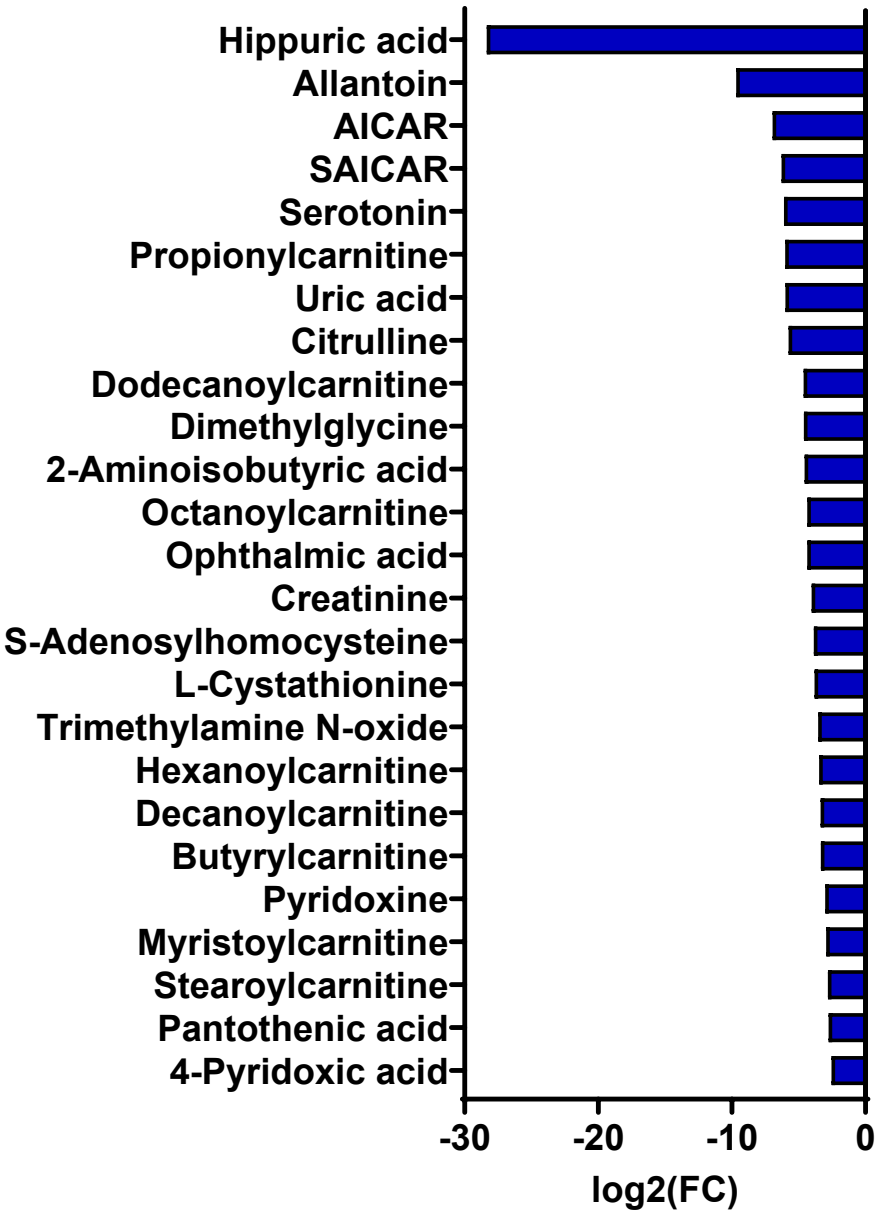
Fold change (FC) threshold: 1.5
P-value threshold: 0.05

CSCs vs WT

Top 25_Increased metabolites in CSCs



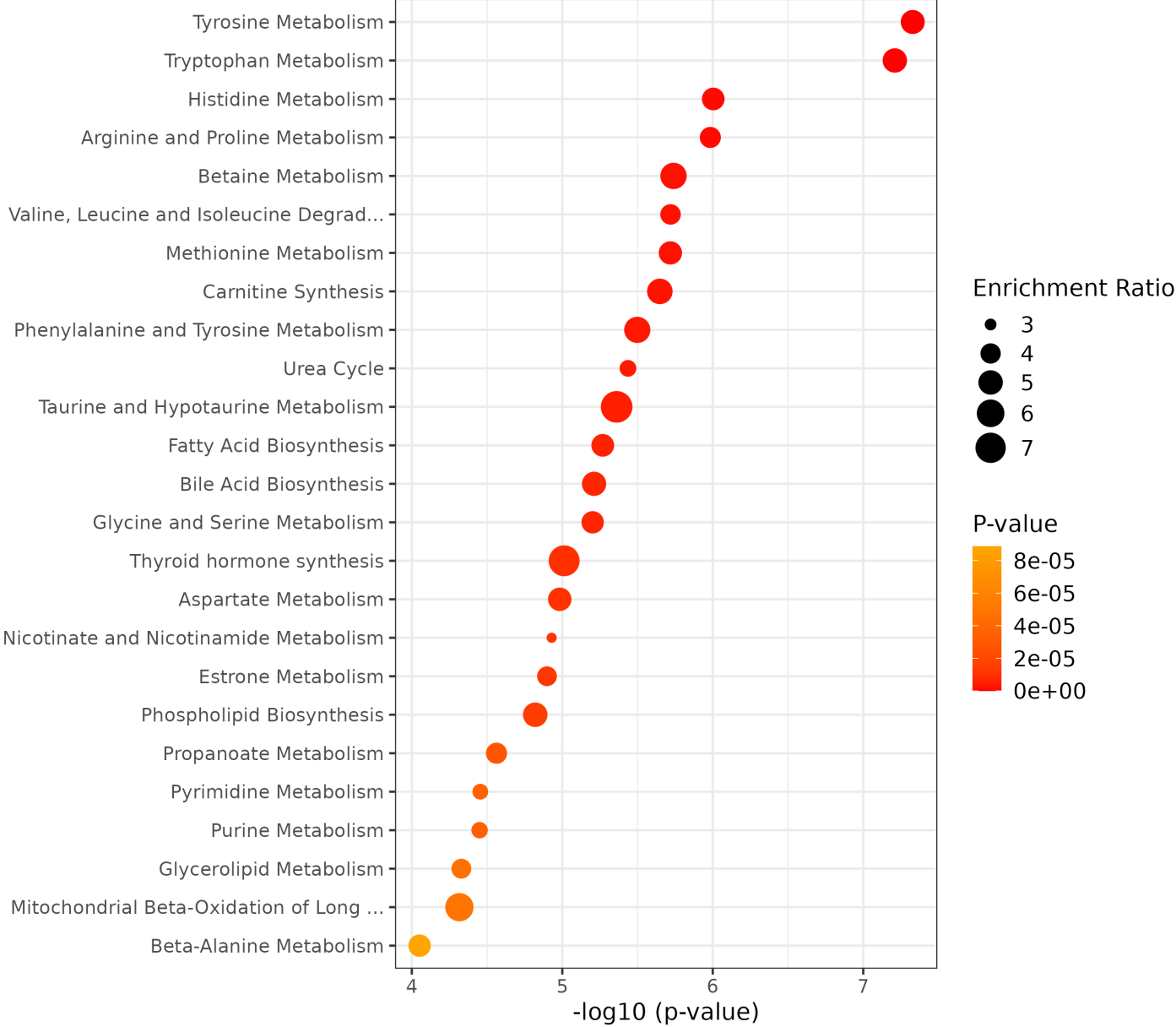
Top 25_Decreased metabolites in CSCs



CSCs vs WT

Quantitative Enrichment Analysis (QEA)

Overview of Enriched Metabolite Sets (Top 25)

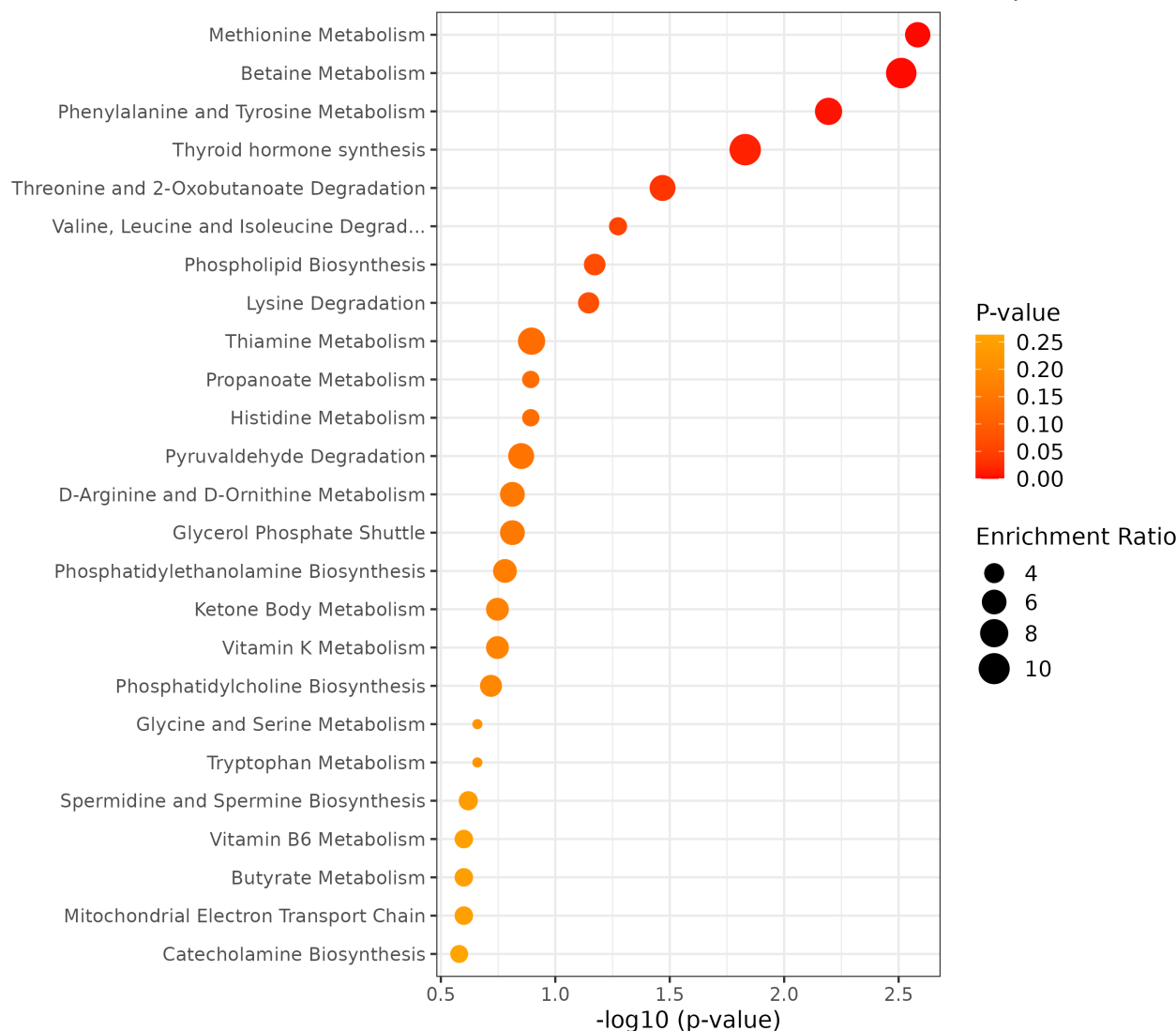


CSCs vs WT

Over-Representation Analysis (ORA)

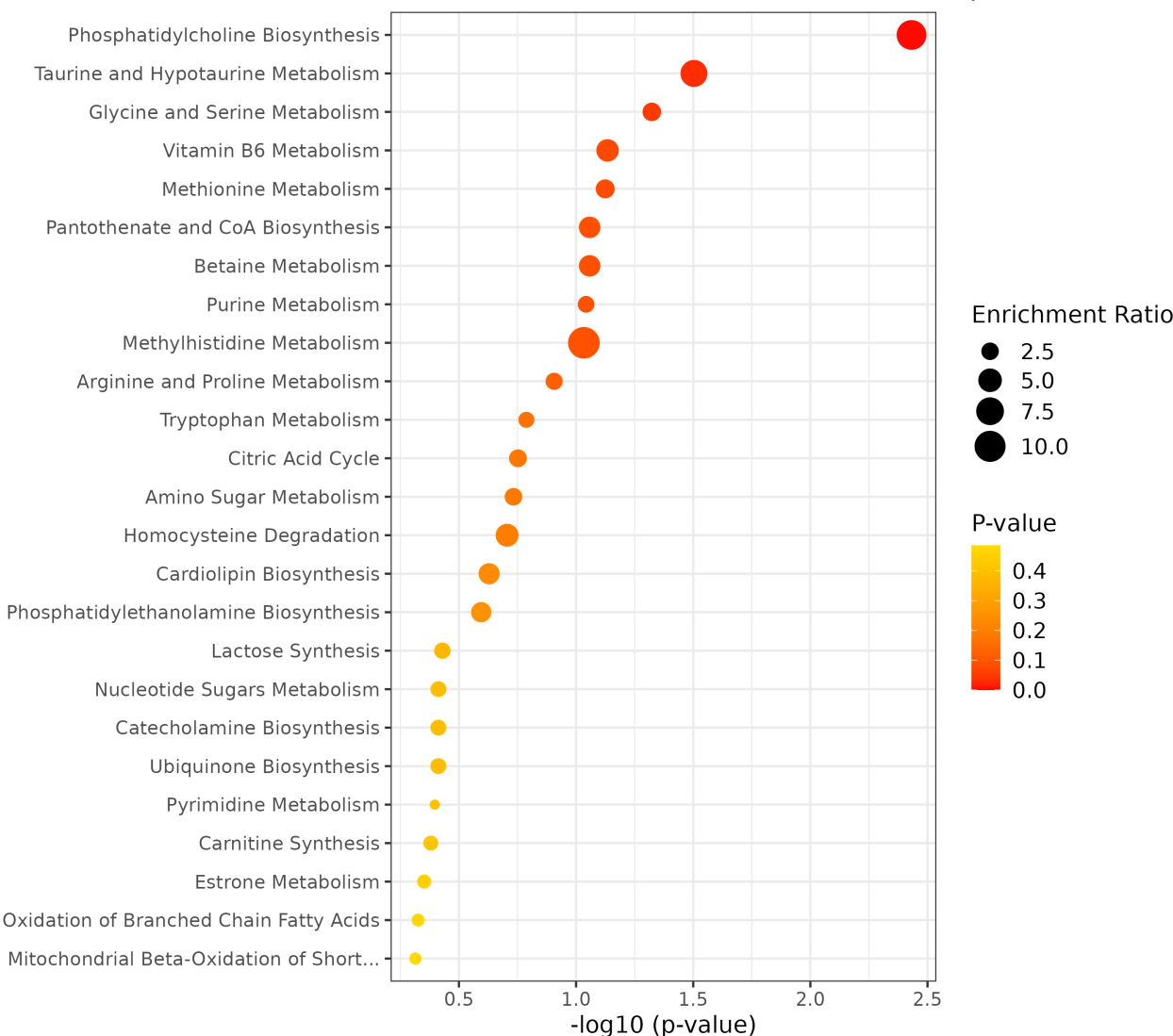
Activation in CSCs

Overview of Enriched Metabolite Sets (Top 25)



Inhibition in CSCs

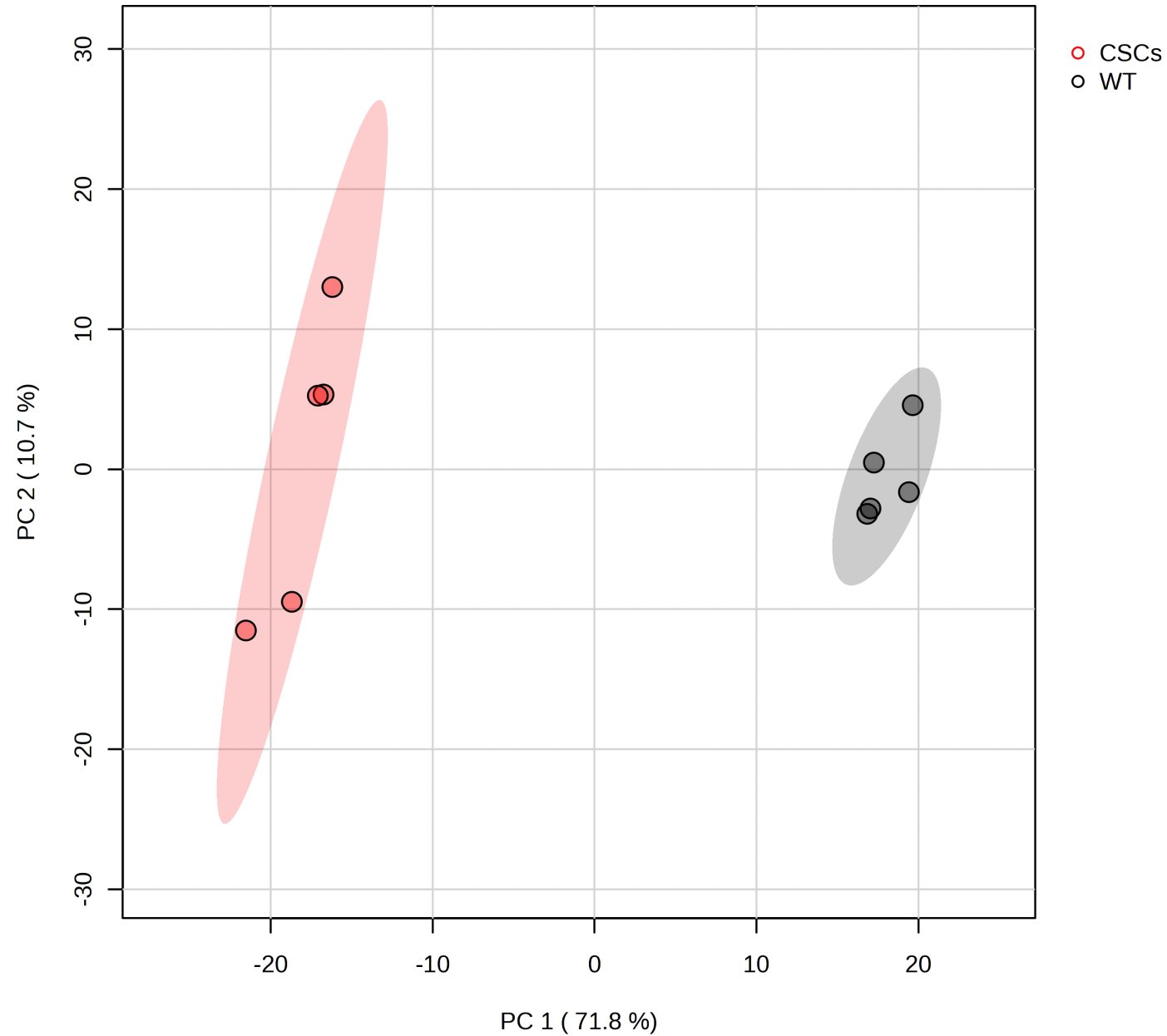
Overview of Enriched Metabolite Sets (Top 25)



CSCs vs WT

Lipidomics

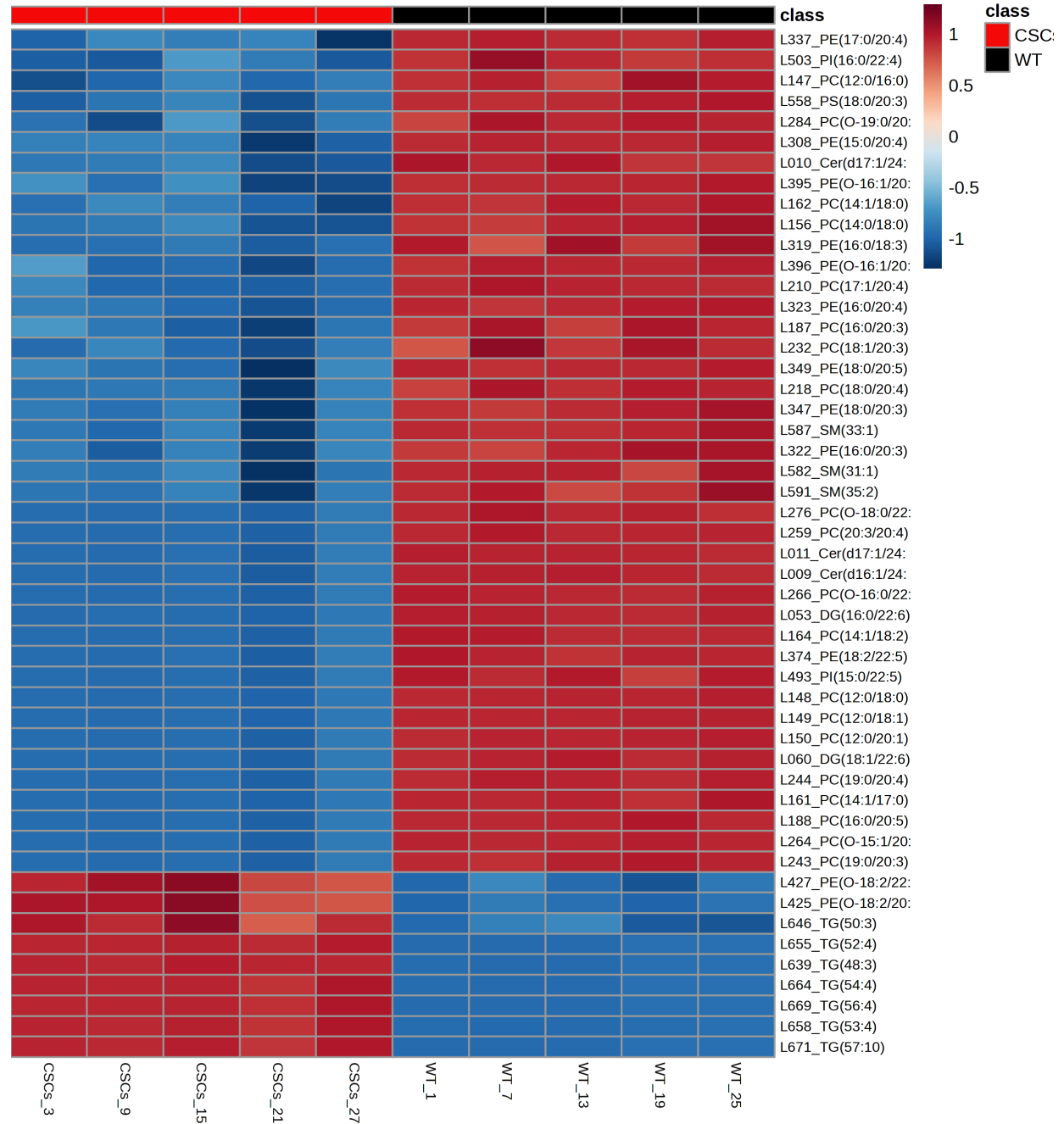
CSCs vs WT



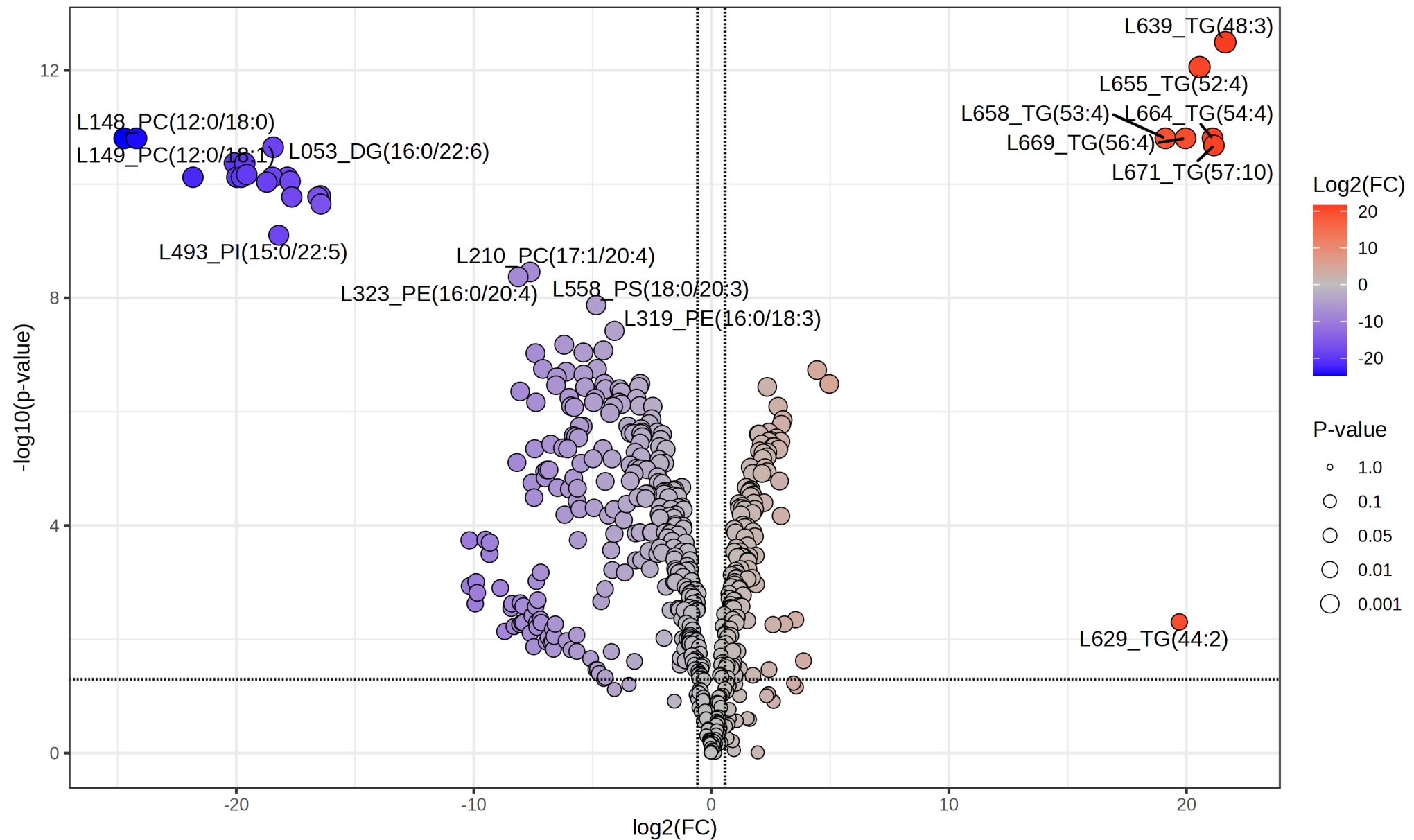
[PERMANOVA] F-value: 50.832; R-squared: 0.86402; p-value (based on 999 permutations): 0.008

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 vs WT



CSCs vs WT

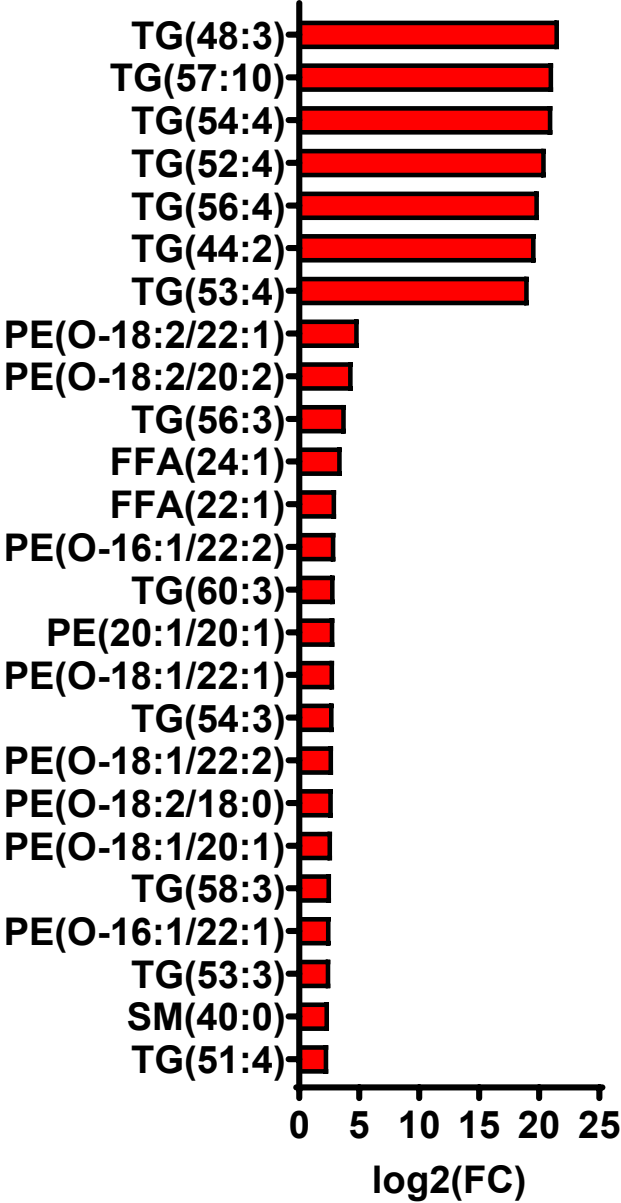


Fold change (FC) threshold: 1.5

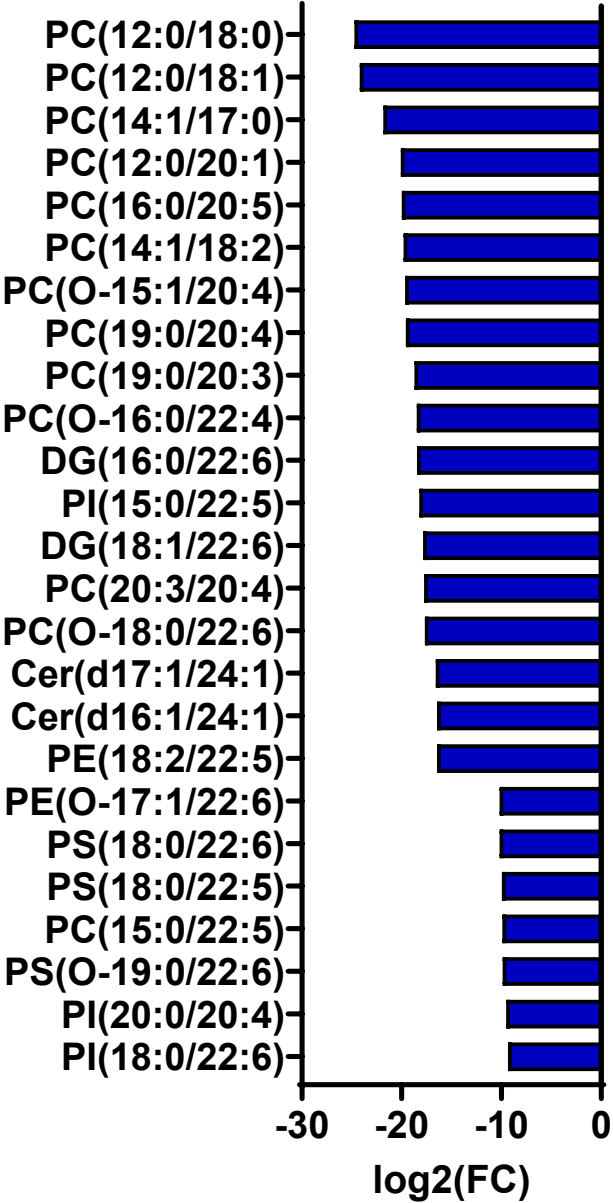
P-value threshold: 0.05

CSCs vs WT

Top 25_Increased lipids in CSCs



Top 25_Decreased lipids in CSCs

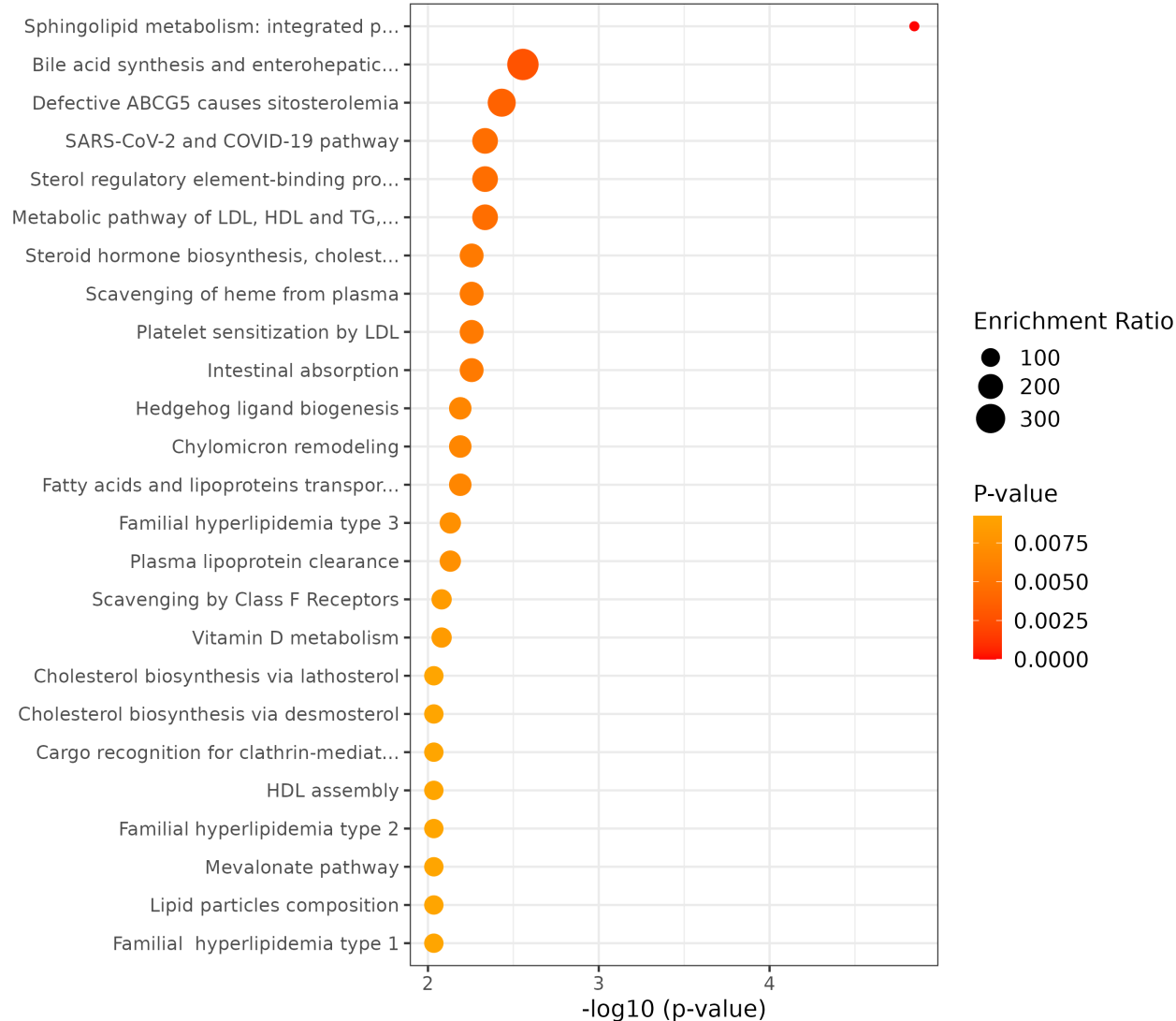


CSCs vs WT

Over-Representation Analysis (ORA)

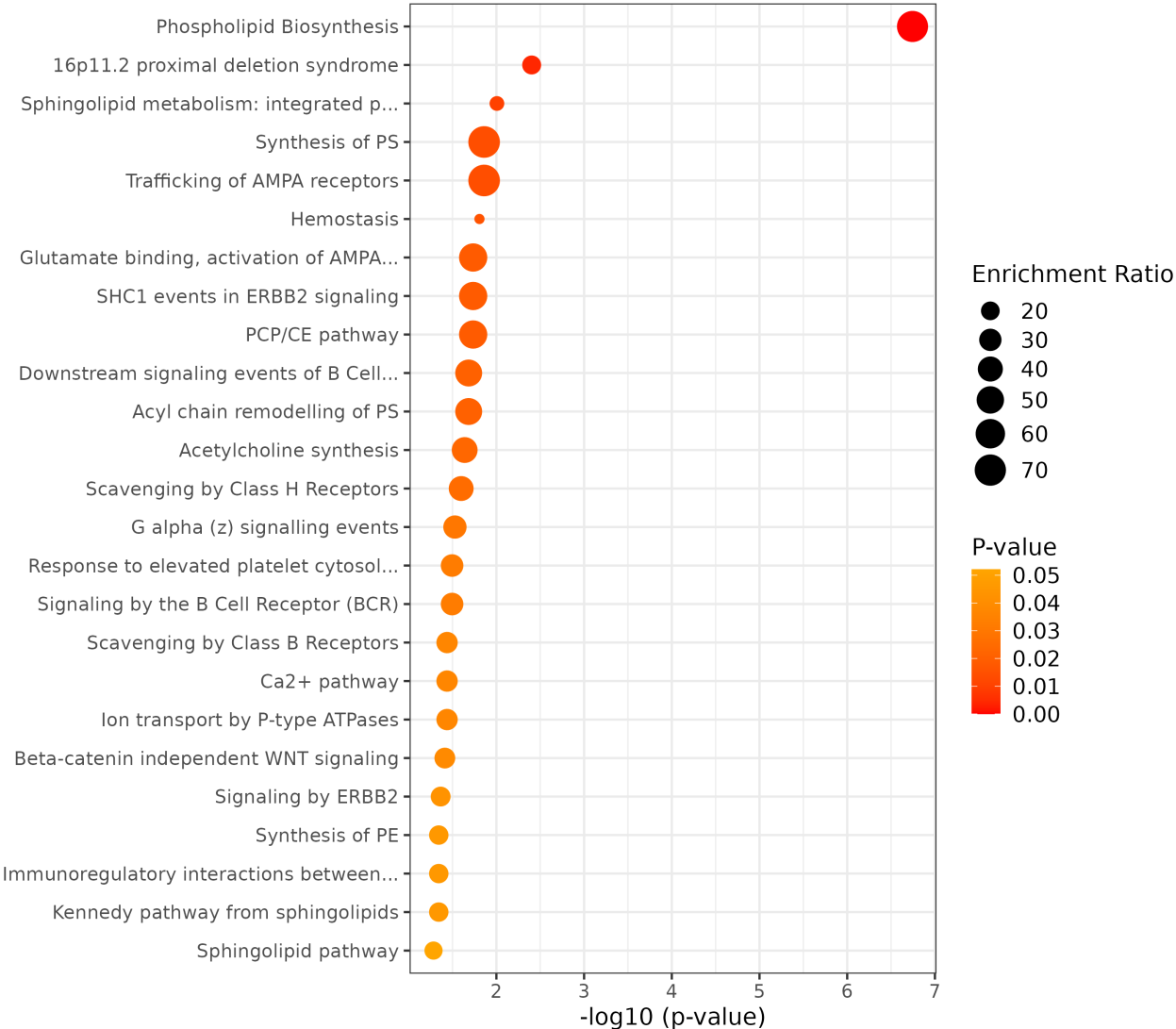
Activation in CSCs

Overview of Enriched Metabolite Sets (Top 25)



Inhibition in CSCs

Overview of Enriched Metabolite Sets (Top 25)



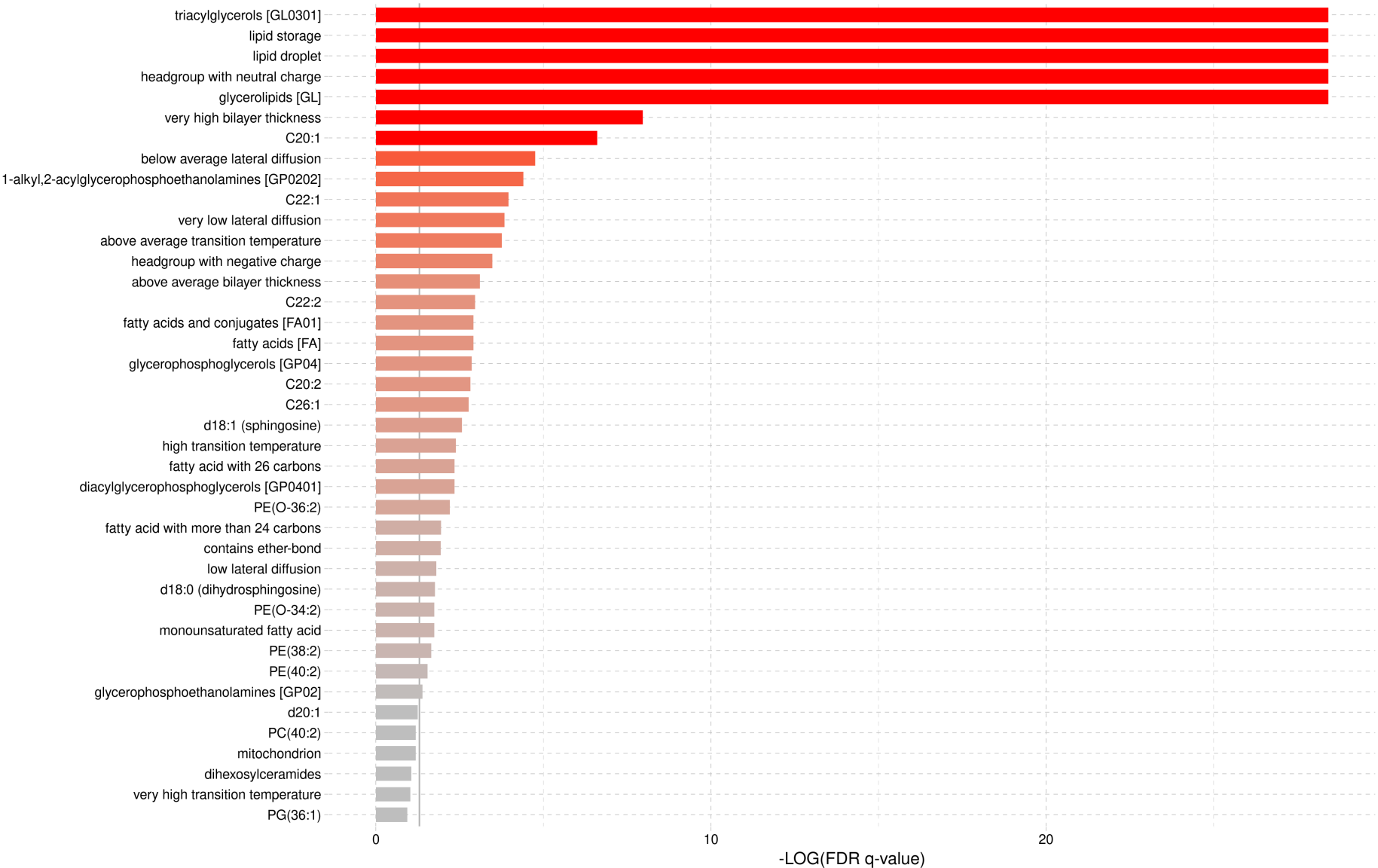
CSCs vs WT

LION enrichment analysis – *ranking mode*

Increased in CSCs

LION enrichment analysis *ranking mode*

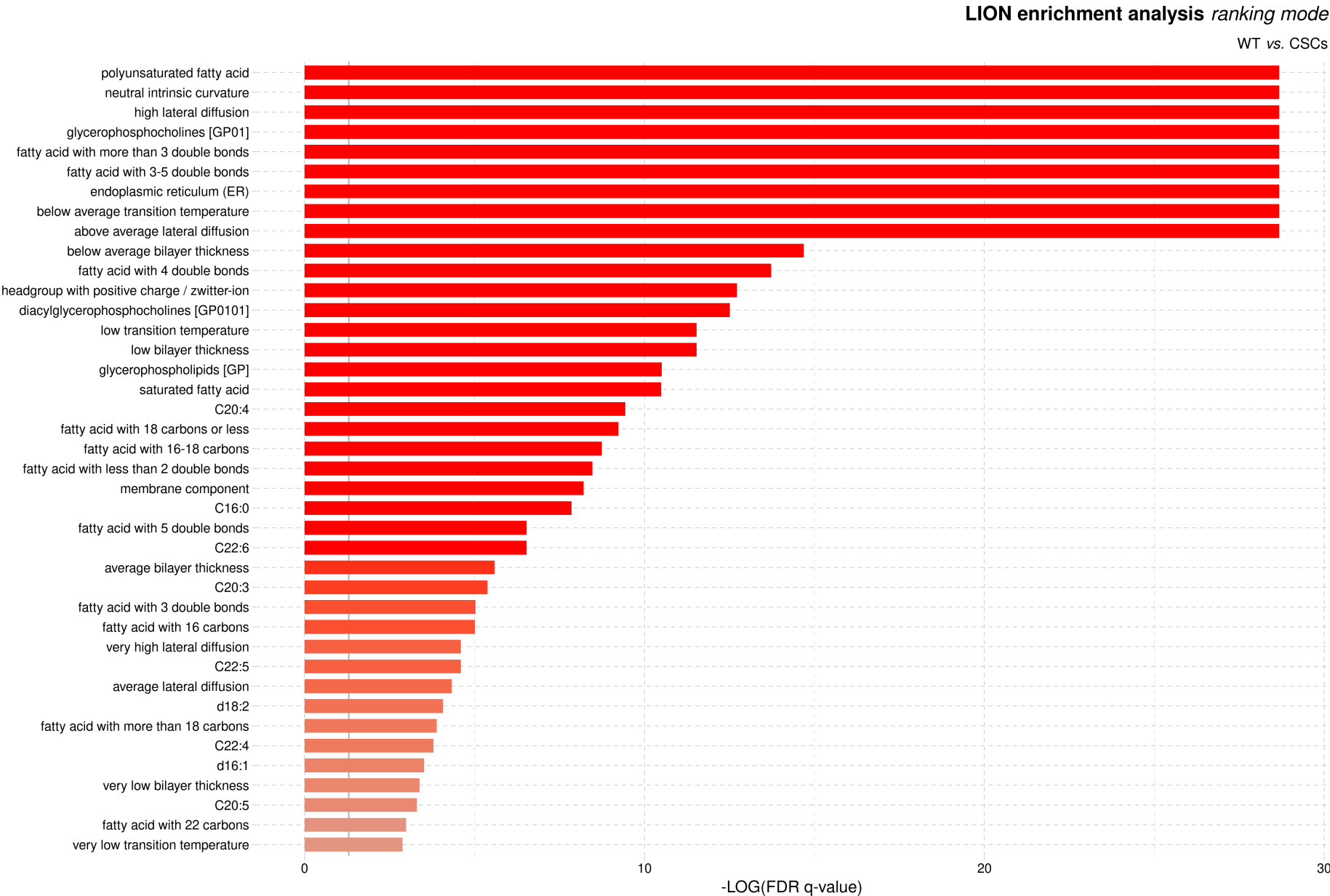
CSCs vs. WT



CSCs vs WT

LION enrichment analysis – *ranking mode*

Decreased in CSCs

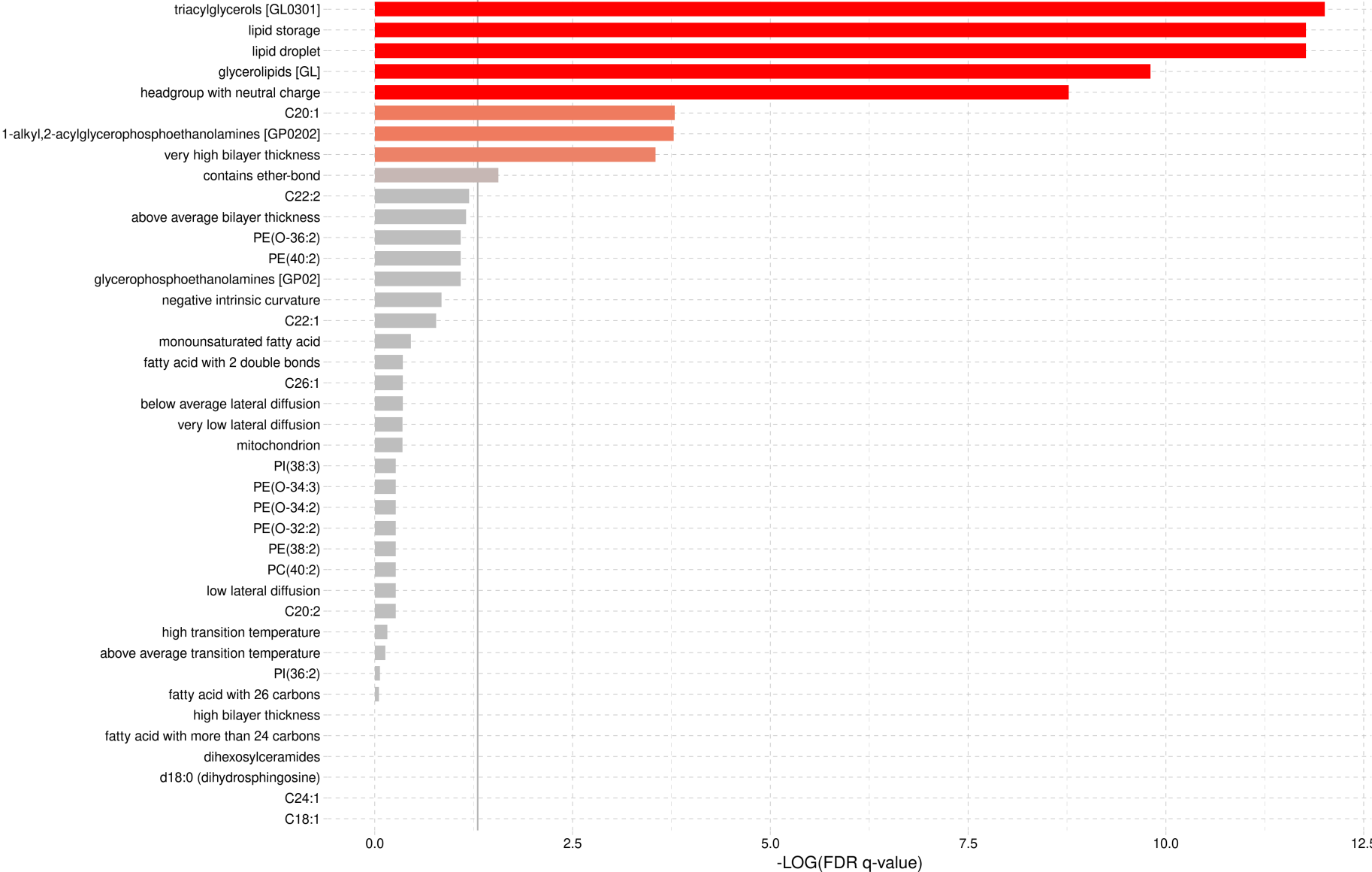


CSCs vs WT

LION enrichment analysis - *target-list mode*

Increased in CSCs

LION enrichment analysis *target-list mode*



CSCs vs WT

LION enrichment analysis - *target-list mode*

Decreased in CSCs

LION enrichment analysis *target-list mode*

