

# RNA-seq Differential Expression Analysis

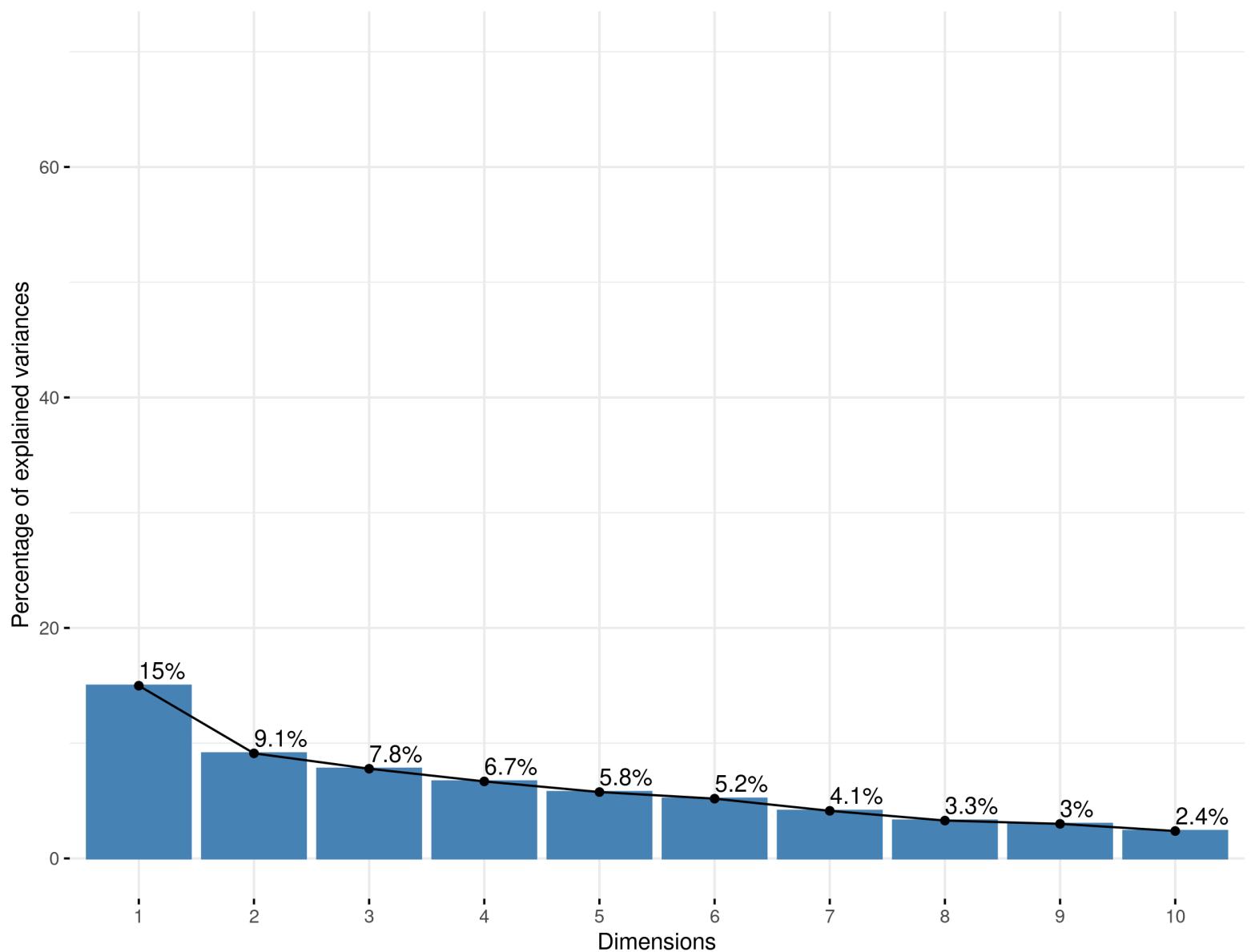
RNA-seq differential expression analysis was performed to compare the transcriptomic profiles between experimental (treatment with HDAC inhibitors) and control (baseline) groups. The differential expression analysis was conducted to identify genes with statistically significant changes in expression levels. This approach allowed for the detection of both up-regulated and down-regulated genes, providing insights into potential biological pathways affected under the experimental conditions.

## Single-sample Gene Set Enrichment Analysis

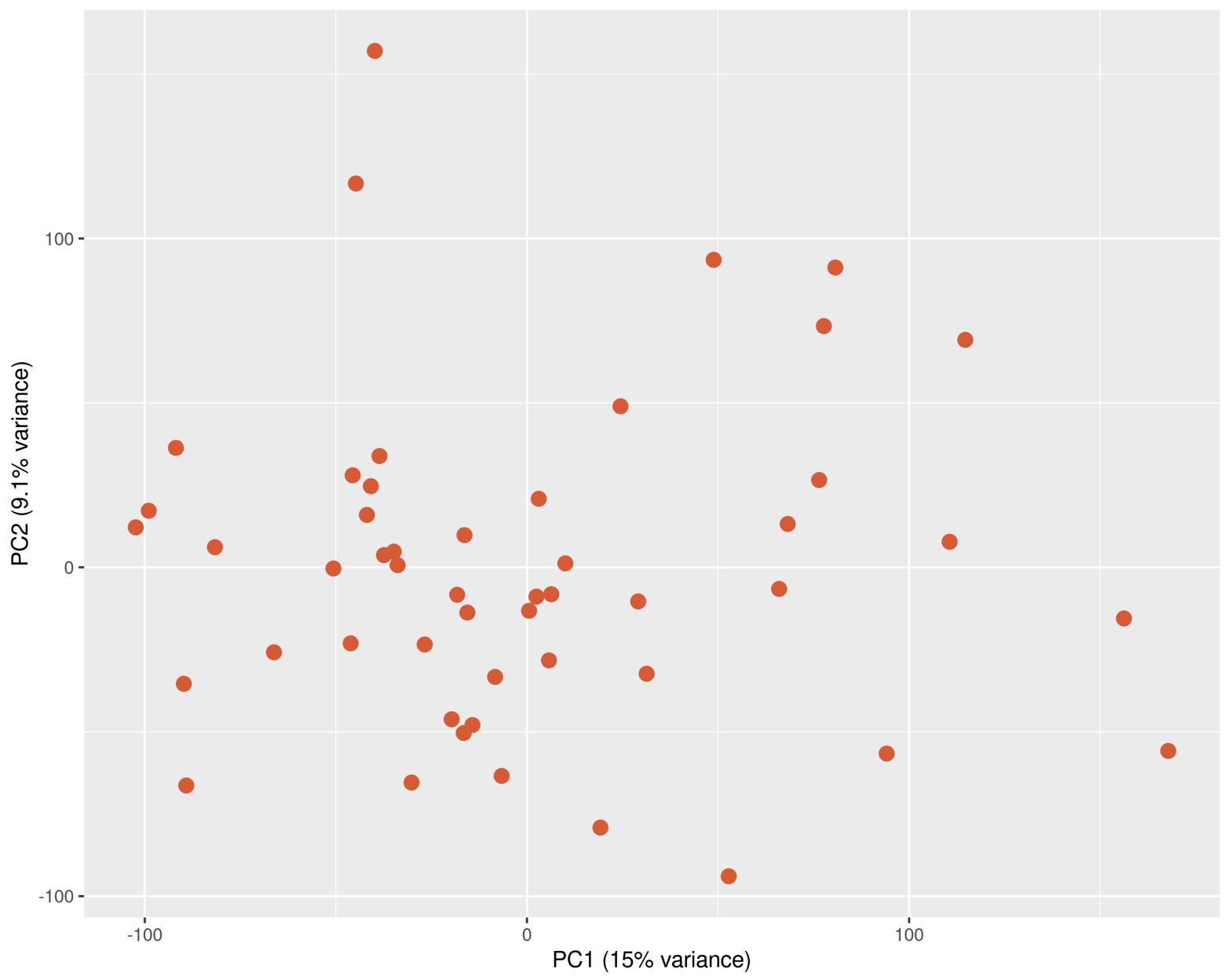
A total of 9 single-sample Gene Set Enrichment Analyses (ssGSEA) were performed for each of the 50 biological samples, utilizing distinct gene sets from the Molecular Signatures Database (MSigDB). The specific gene sets used are detailed in the table below.

MSigDB Collection	Title	Description
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C7	Immunologic Signature Gene Sets	Gene sets that represent cell states and perturbations within the immune system
C8	Cell Type Signature Gene Sets	Gene sets that contain curated cluster markers for cell types identified in single-cell sequencing studies of human tissue
H	Hallmark Gene Sets	Hallmark gene sets summarize and represent specific well-defined biological states or processes and display coherent expression

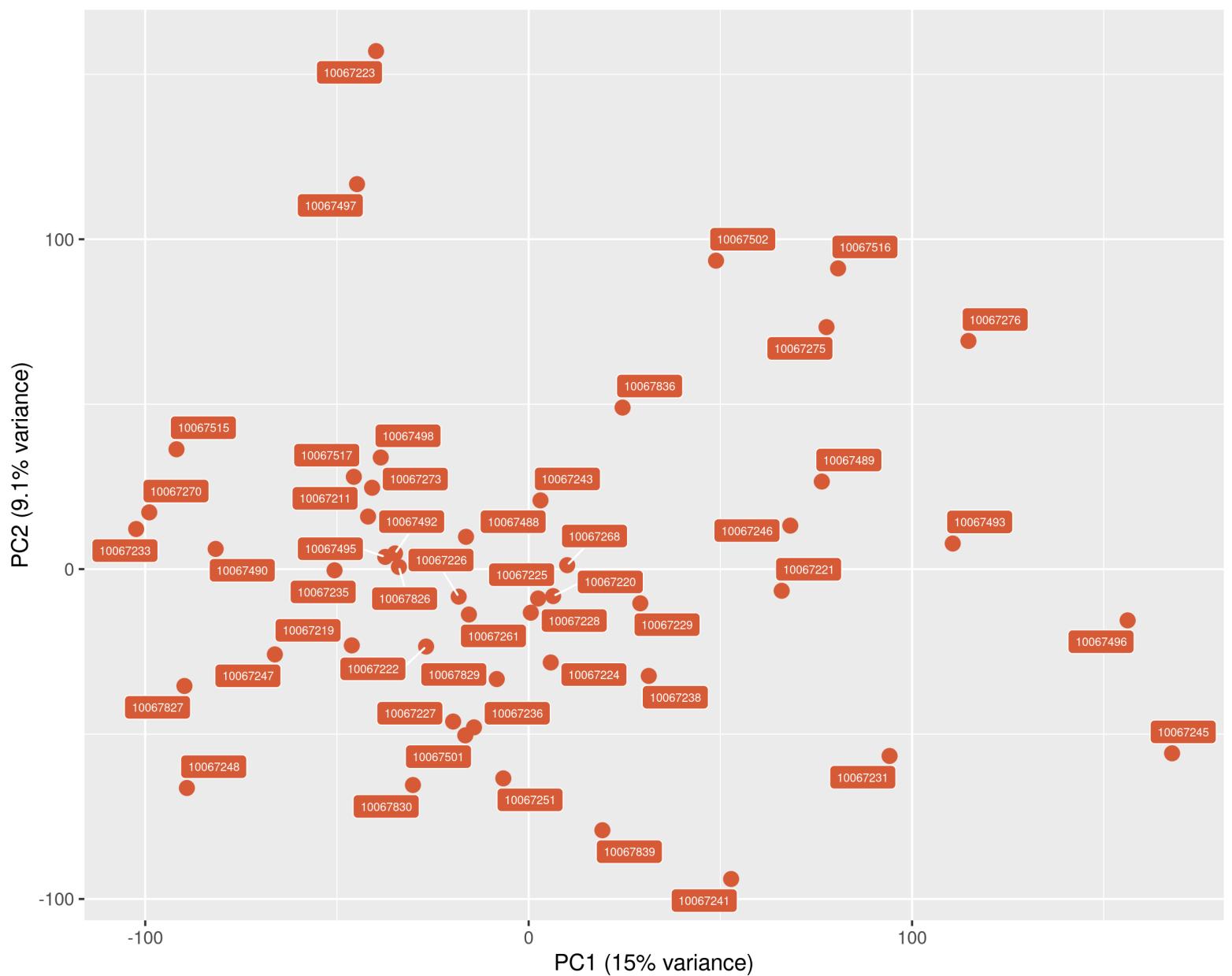
## Exploratory Analysis

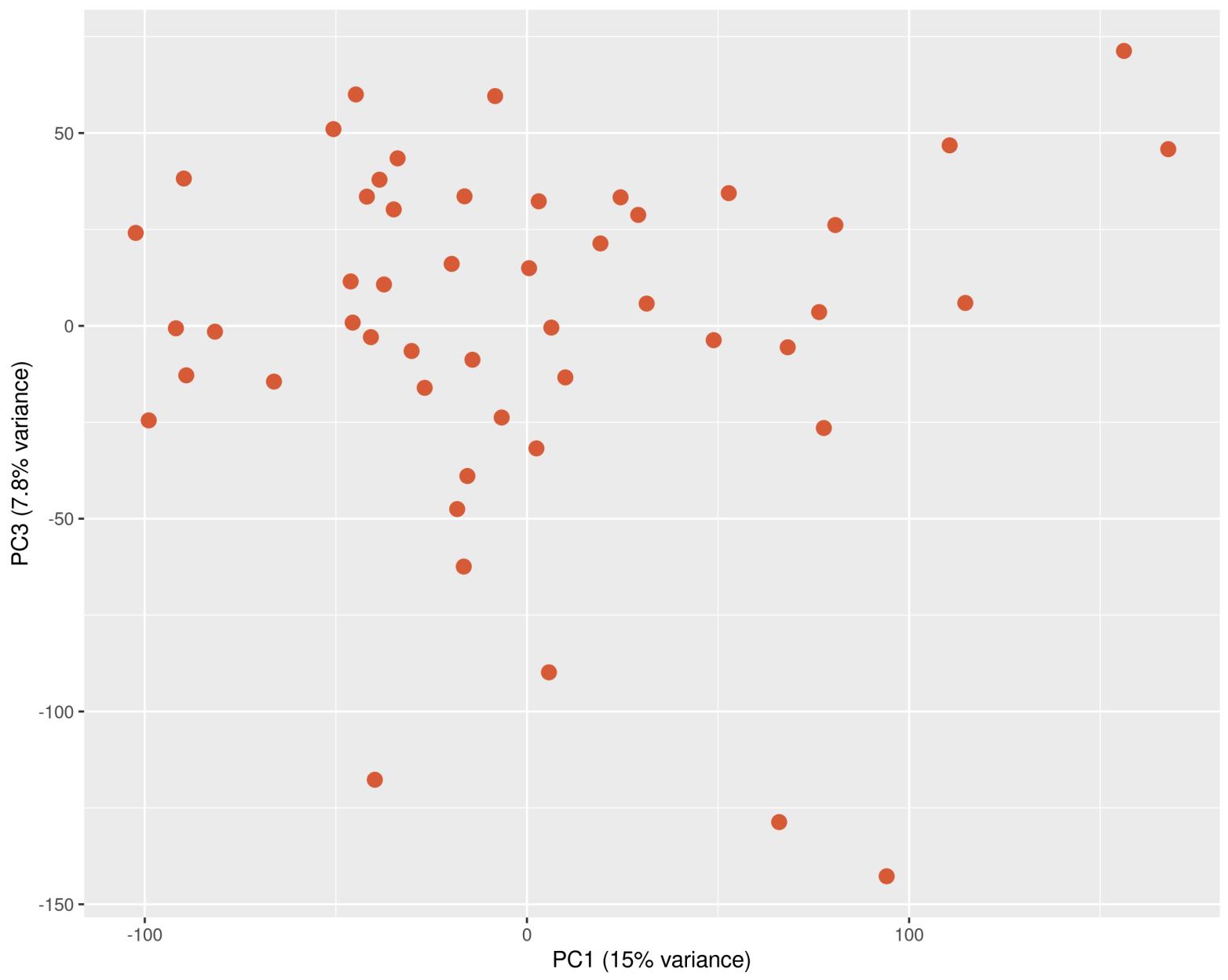


Percentage of variance explained by the first 10 Principal Components



PCA plot: Visualizing Sample Variability based on overall gene expression profiles. First and Second Principal Components are shown



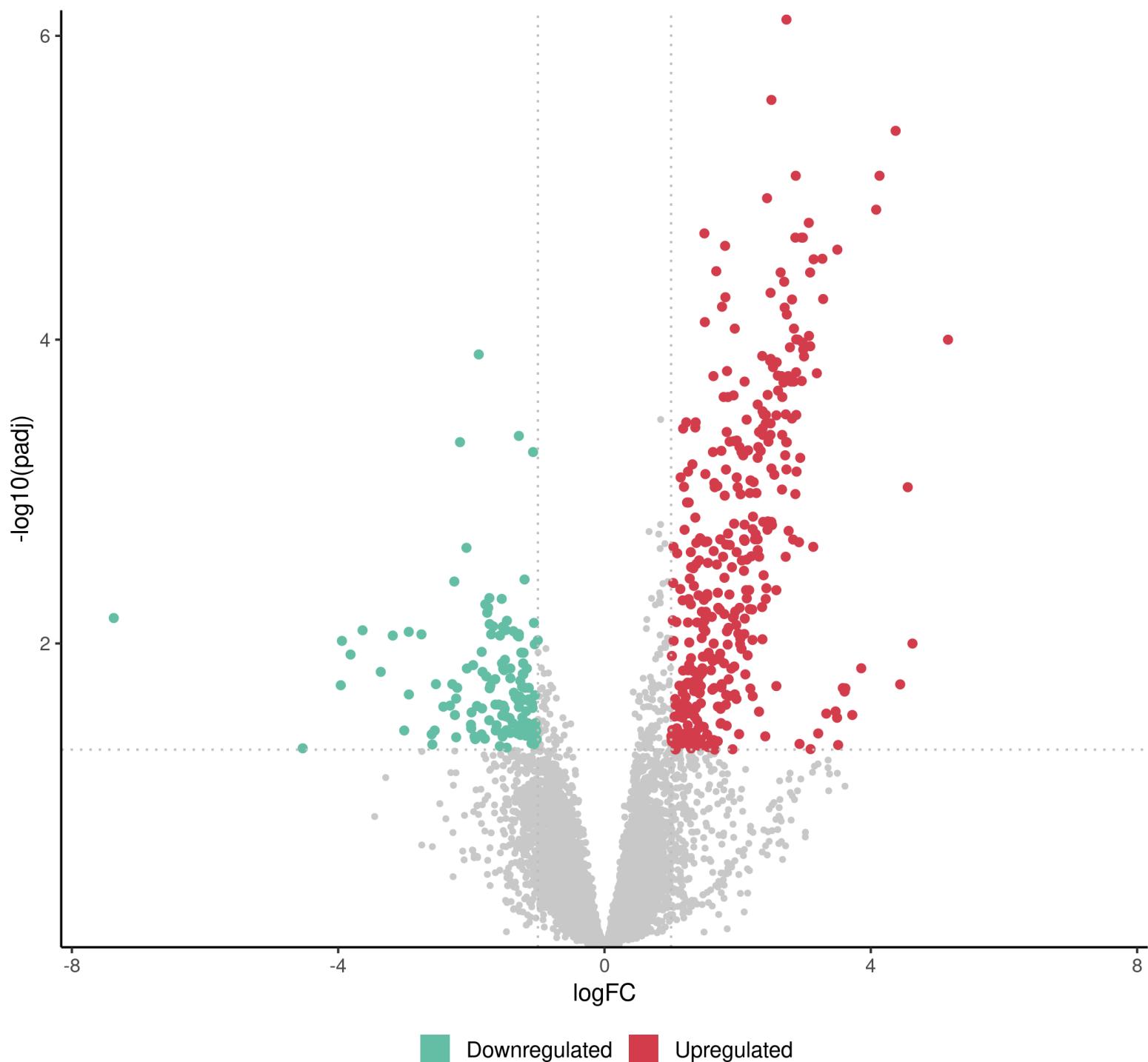


PCA Plot: Visualizing Sample Variability based on overall gene expression profiles. First and Third Principal Components are shown

# Differential Expression Analysis

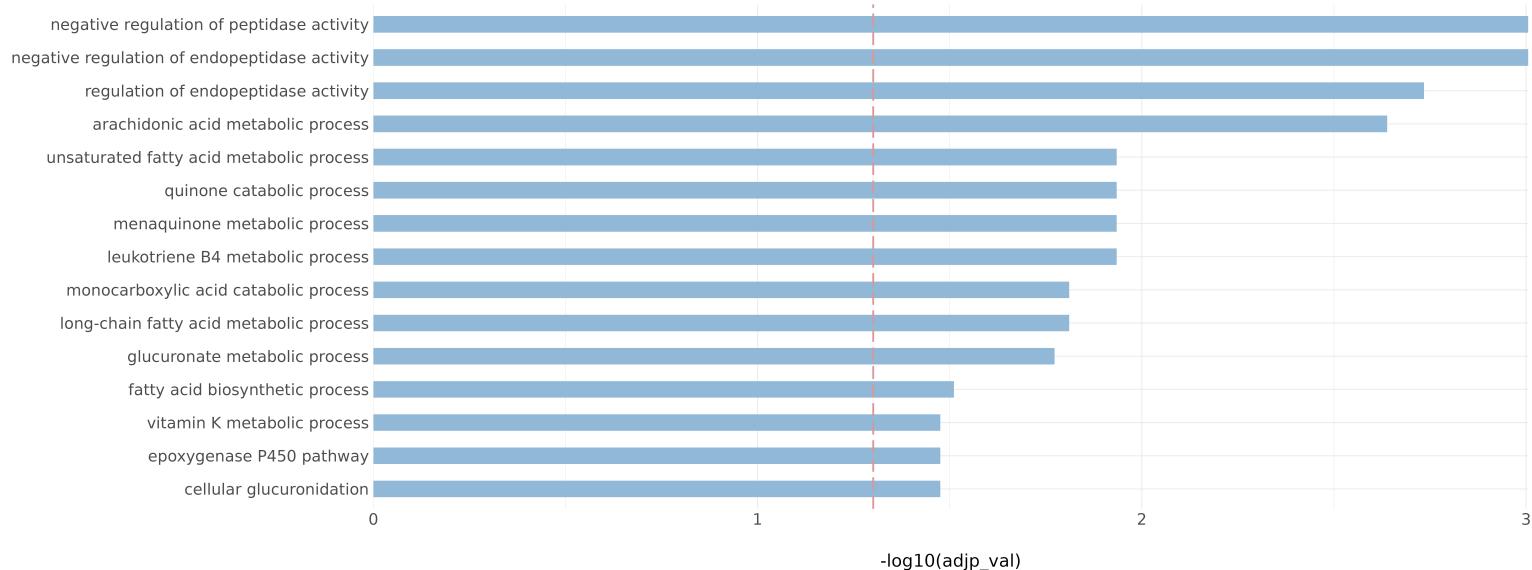
## Volcano Plot for Baseline vs C3D1

$|\log FC| > 1$  &  $\text{padj} < 0.05$  &  $\text{TPM} > 1$



Volcano Plot: Points above the adjusted p-value significance threshold (horizontal line) and beyond the log2 fold change cutoff (vertical lines) represent differentially expressed genes.

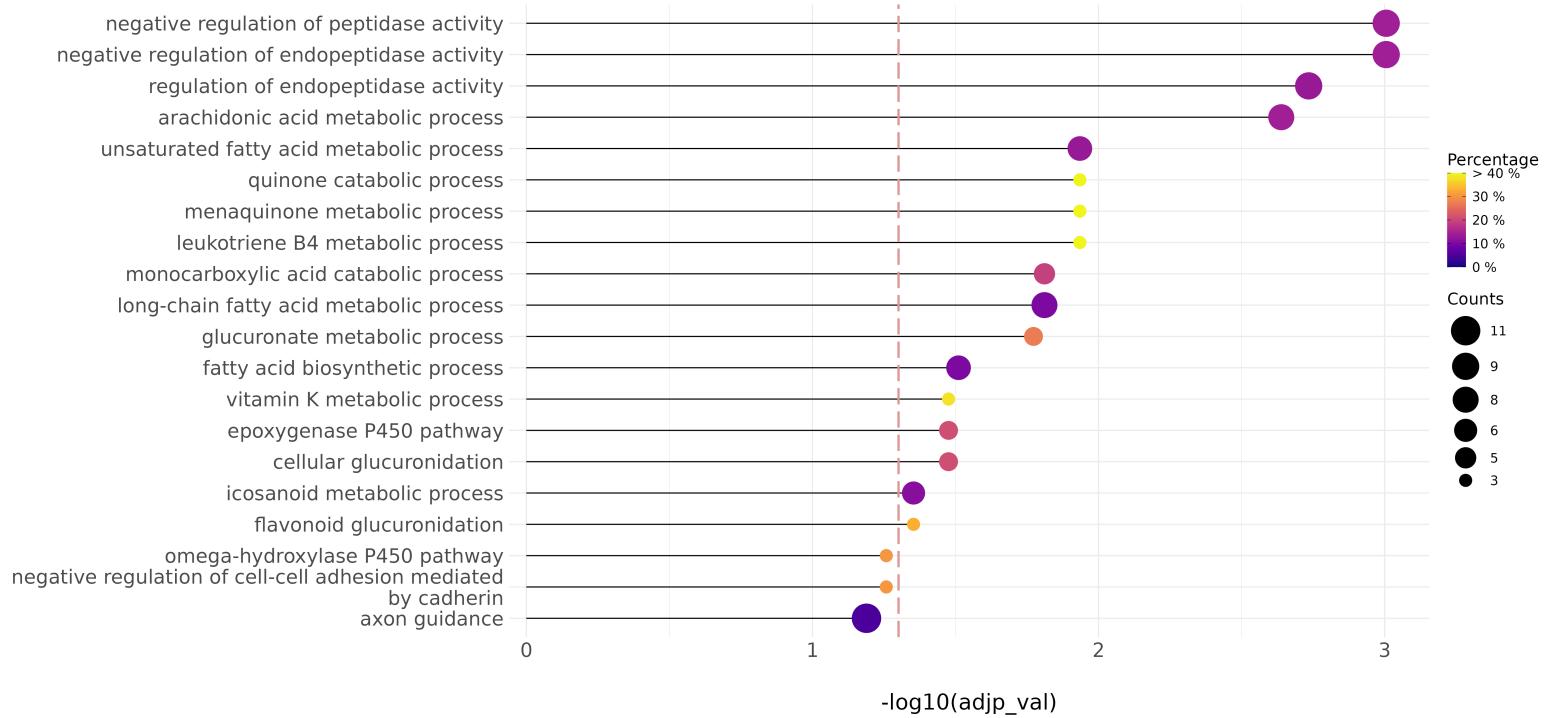
### GO Biological Process 2021 for Down Regulated Genes Baseline vs C3D1



Pathway Enrichment Analysis Bar Plot of the top 20 enriched **Biological Processes** detected during Gene Ontology (GO) analysis of **downregulated** differentially expressed genes. The length of each bar refers to the statistical significance ( $-\log_{10}$  p-value), indicating the strength of association between the gene set and each process.

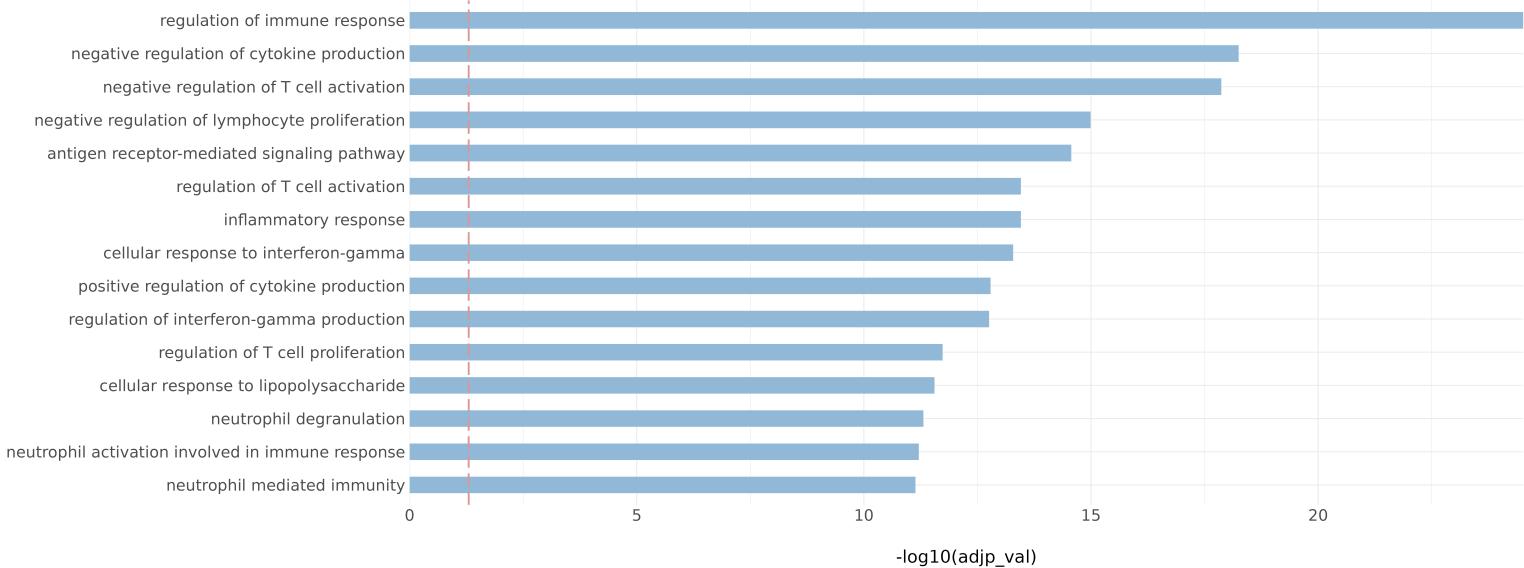
# GO Biological Process 2021 for Down Regulated Genes

## Baseline vs C3D1



Pathway Enrichment Analysis Lollipop Chart of the top 20 enriched **Biological Processes** detected during Gene Ontology (GO) analysis of **downregulated** differentially expressed genes. Circle size refers to the number of DEGs associated with each GO term, while the color gradient reflects the percentage of downregulated genes within the total gene set for each term

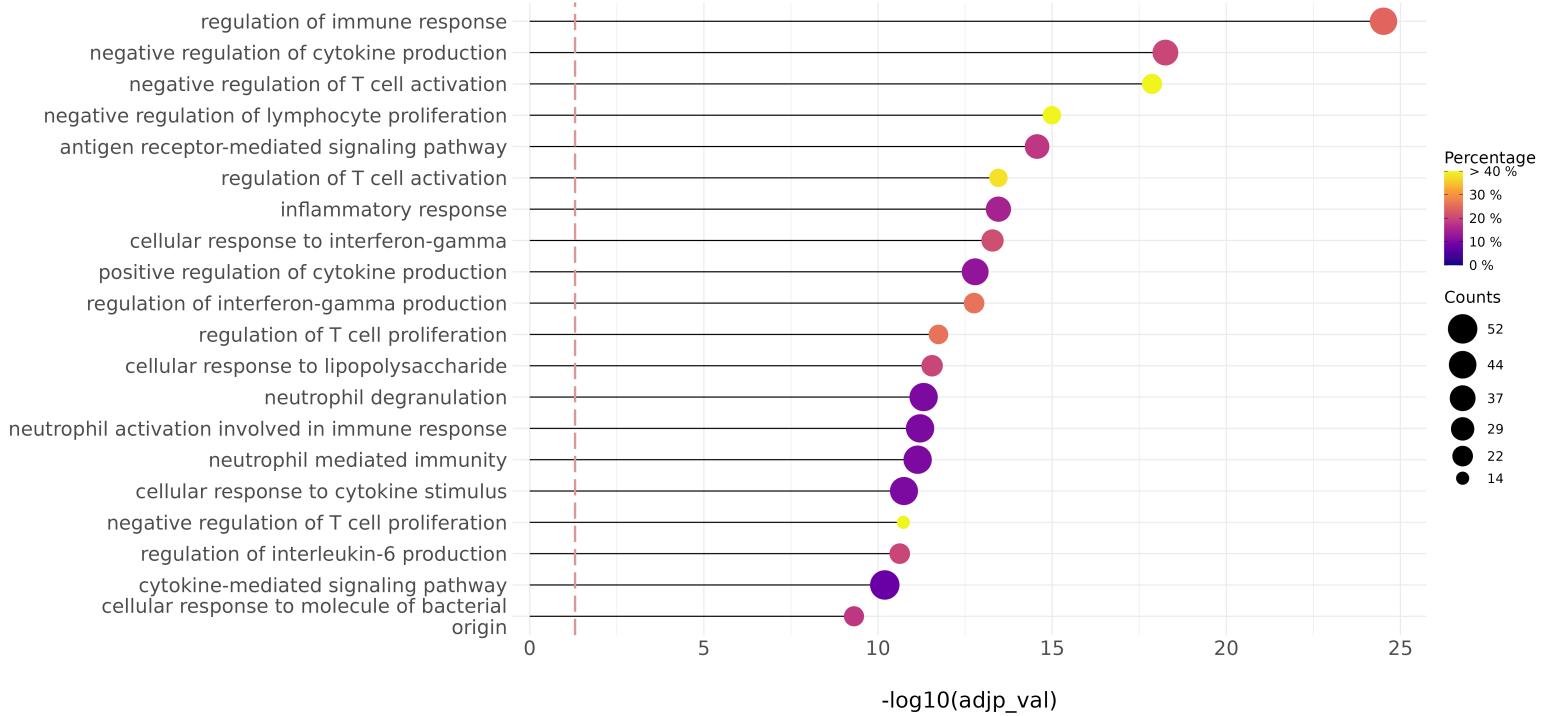
### GO Biological Process 2021 for Up Regulated Genes Baseline vs C3D1



Bar Plot of the top 20 enriched **Biological Processes** detected during Gene Ontology (GO) analysis of **upregulated** differentially expressed genes.

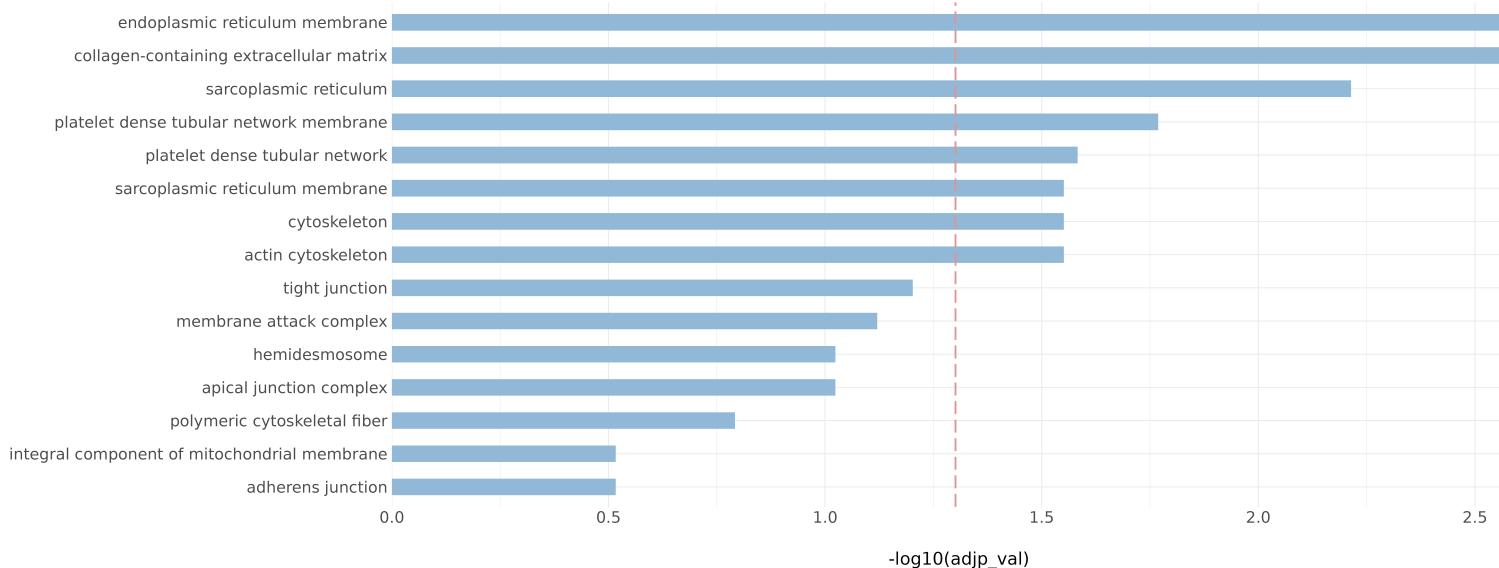
# GO Biological Process 2021 for Up Regulated Genes

## Baseline vs C3D1



Bar Plot and Lollipop Chart of the top 20 enriched **Biological Processes** detected during Gene Ontology (GO) analysis of **upregulated** differentially expressed genes.

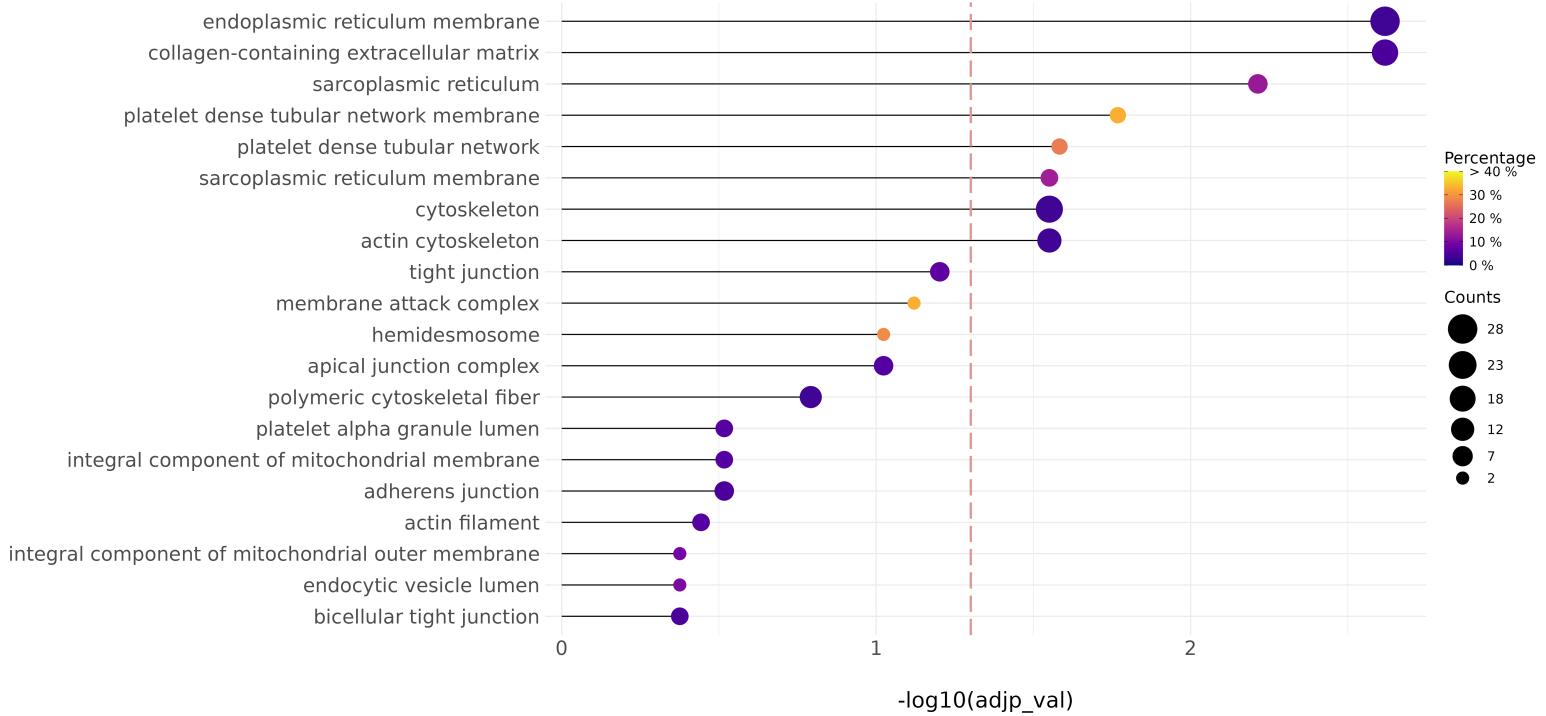
GO Cellular Component 2021 for Down Regulated Genes  
Baseline vs C3D1



Bar Plot of the top 20 enriched **Cellular Component** detected during Gene Ontology (GO) analysis of **downregulated** differentially expressed genes.

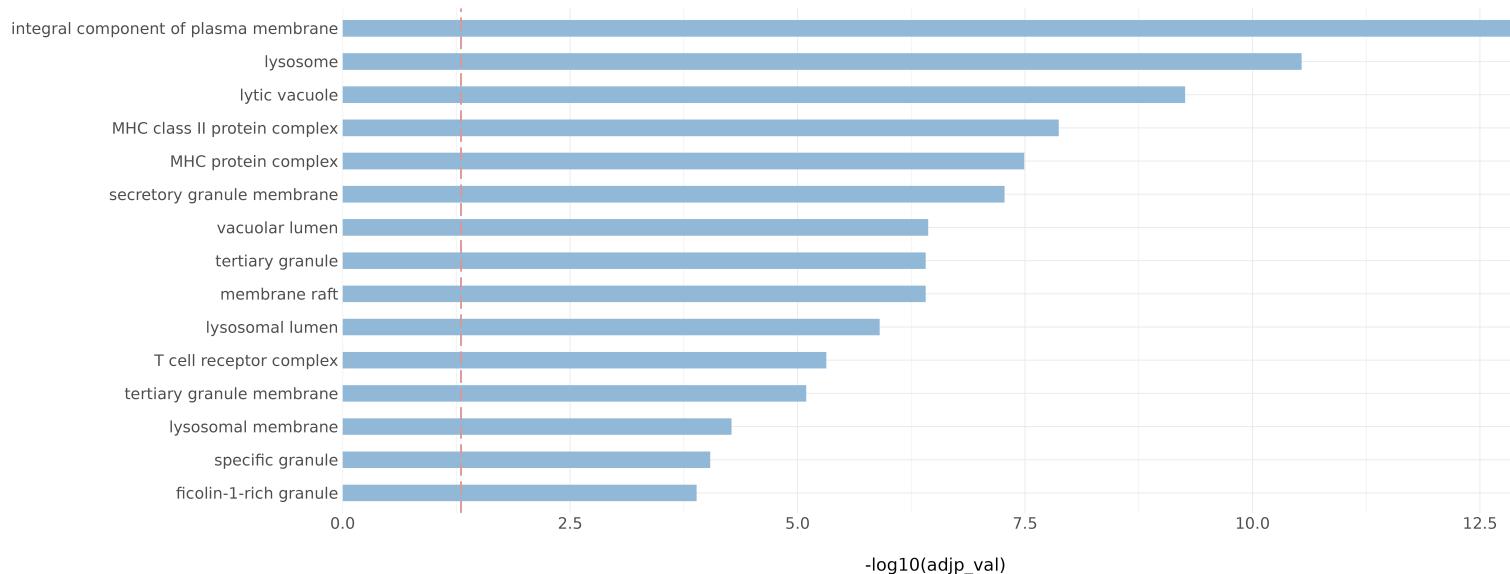
# GO Cellular Component 2021 for Down Regulated Genes

Baseline vs C3D1



Lollipop Chart of the top 20 enriched **Cellular Component** detected during Gene Ontology (GO) analysis of **downregulated** differentially expressed genes.

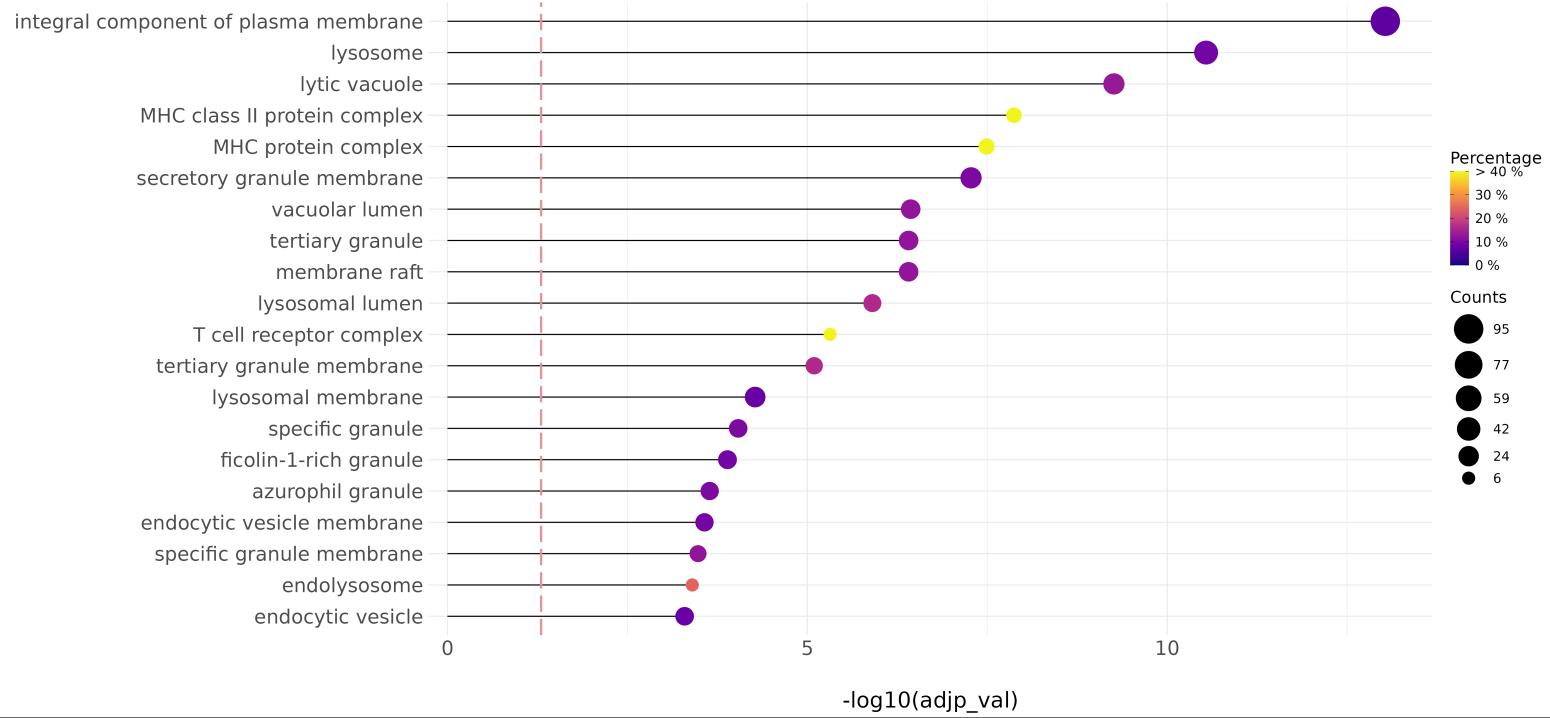
GO Cellular Component 2021 for Up Regulated Genes  
Baseline vs C3D1



Bar Plot of the top 20 enriched **Cellular Component** detected during Gene Ontology (GO) analysis of **upregulated** differentially expressed genes.

# GO Cellular Component 2021 for Up Regulated Genes

## Baseline vs C3D1



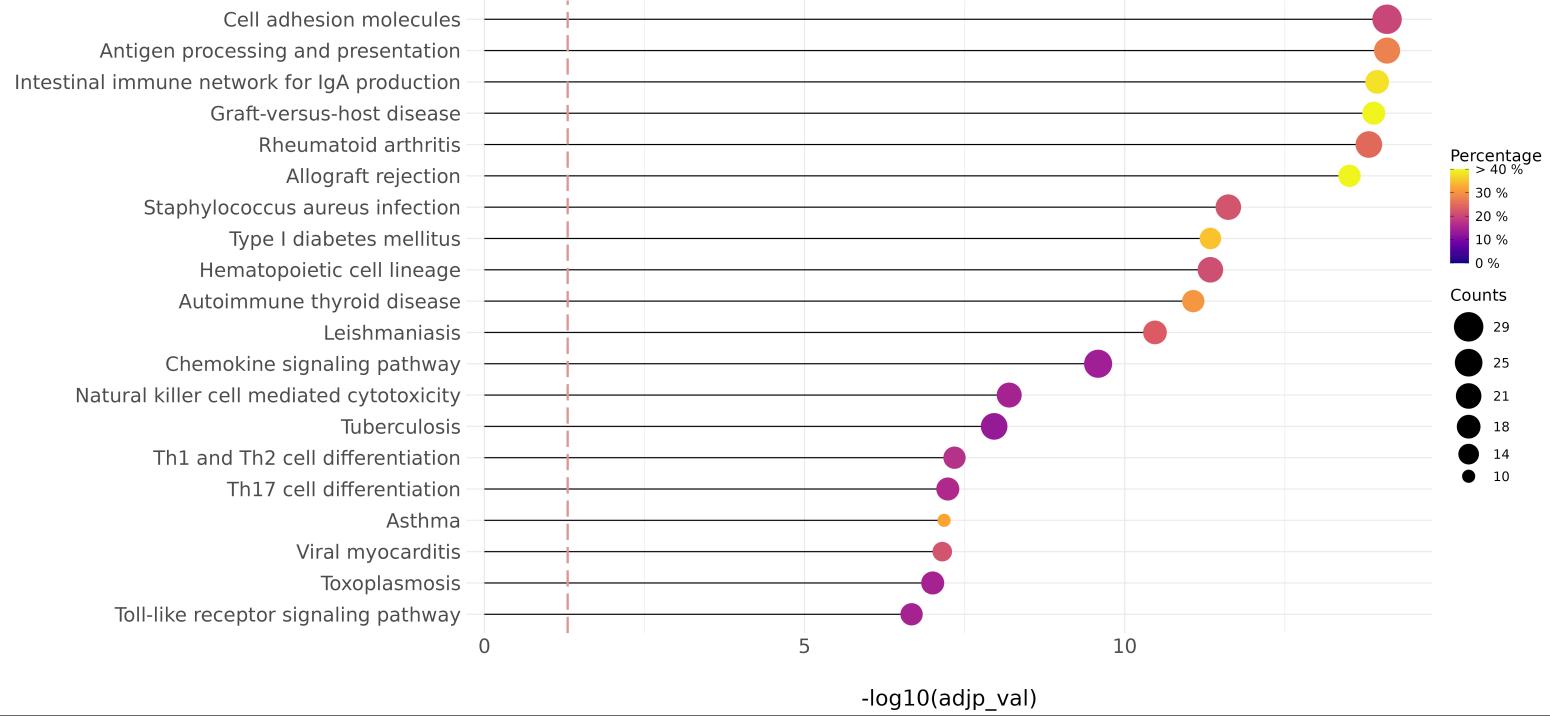
Lollipop Chart of the top 20 enriched **Cellular Component** detected during Gene Ontology (GO) analysis of **upregulated** differentially expressed genes.



Bar Plot of the top 20 enriched terms detected during KEGG analysis of **upregulated** differentially expressed genes.

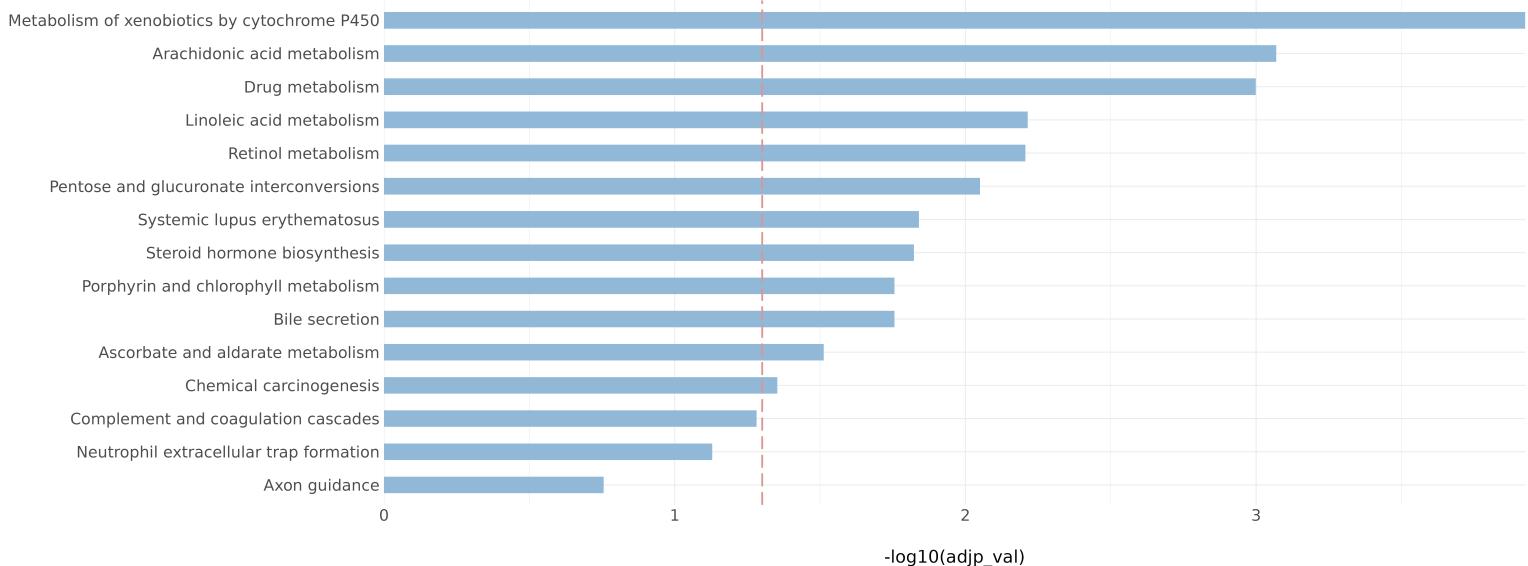
# KEGG 2021 Human for Up Regulated Genes

## Baseline vs C3D1



Lollipop Chart of the top 20 enriched terms detected during KEGG analysis of **upregulated** differentially expressed genes.

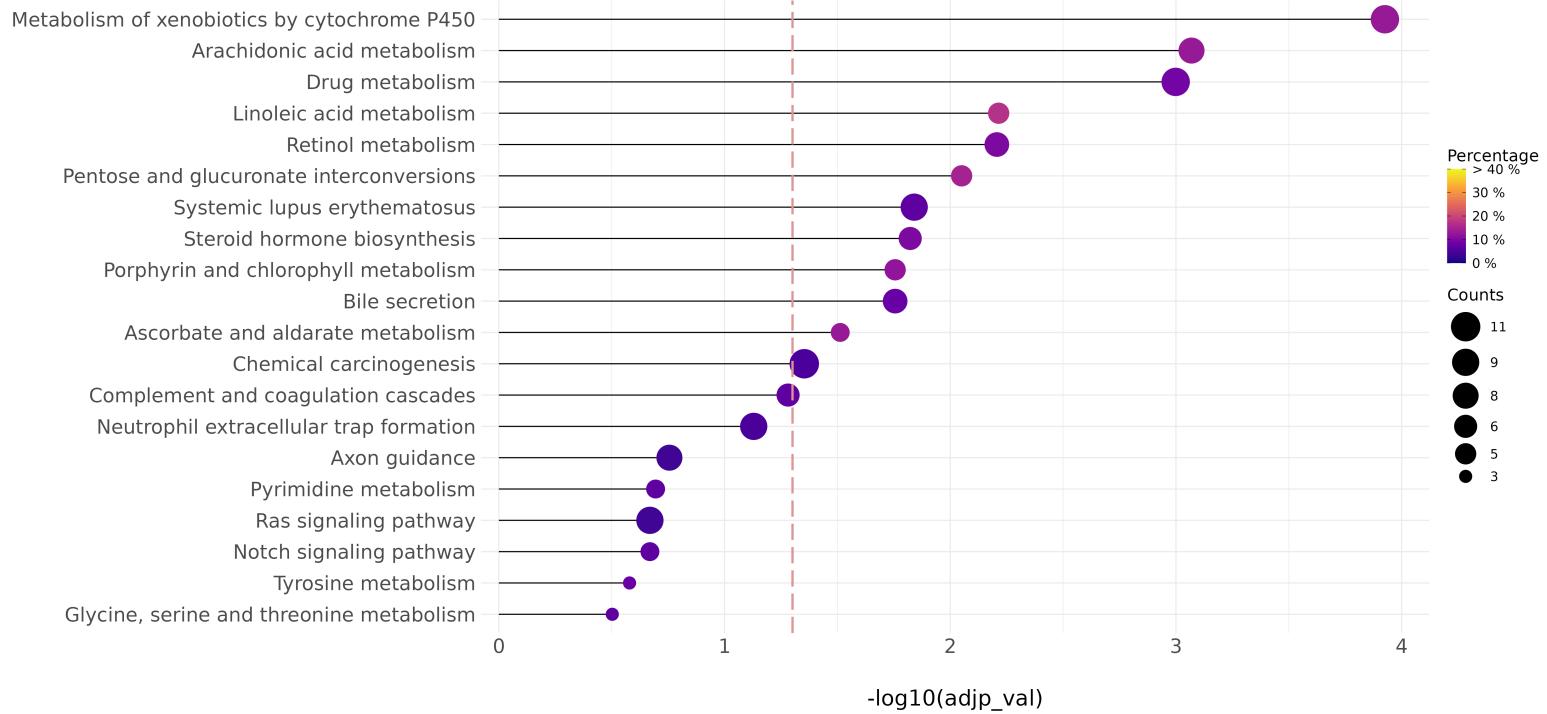
KEGG 2021 Human for Down Regulated Genes  
Baseline vs C3D1



Bar Plot of the top 20 enriched terms detected during KEGG analysis of **downregulated** differentially expressed genes.

# KEGG 2021 Human for Down Regulated Genes

## Baseline vs C3D1



Lollipop Chart of the top 20 enriched terms detected during KEGG analysis of **downregulated** differentially expressed genes.

# Single-sample Gene Set Enrichment Analysis

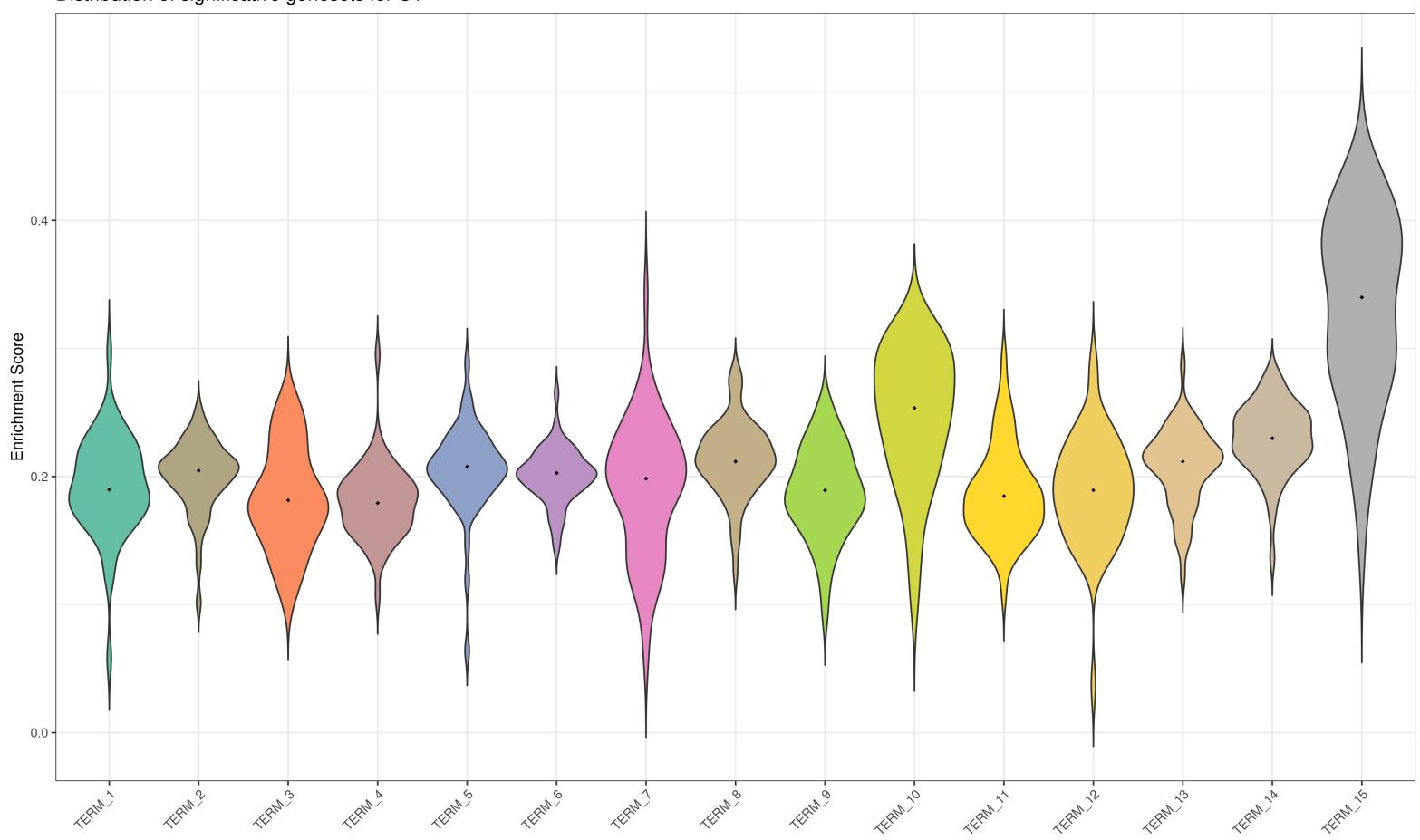
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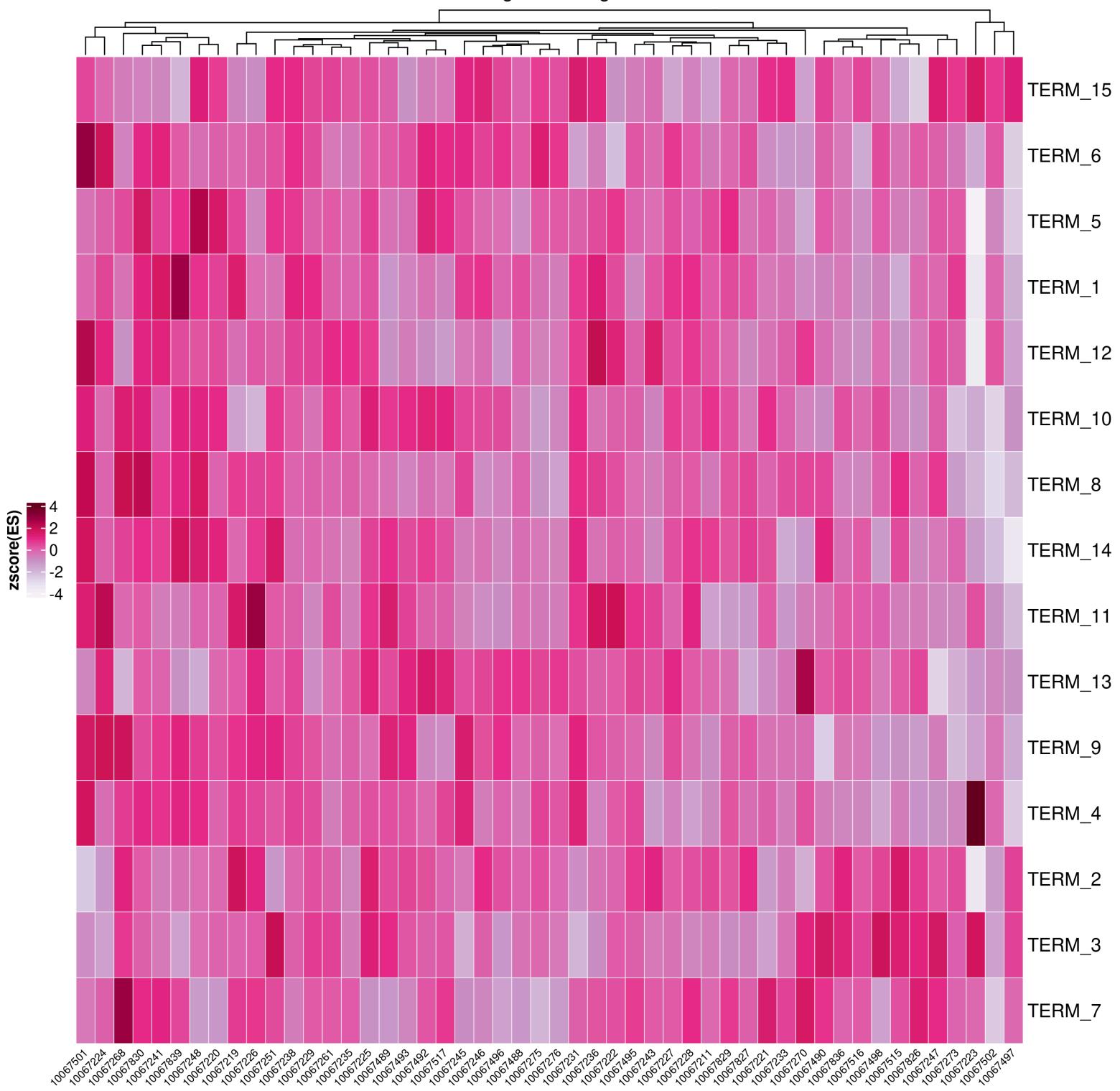
## C1: Positional Gene Sets

Term	Term ID
chr1p22	TERM_1
chr1p35	TERM_2
chr1q22	TERM_3
chr12p11	TERM_4
chr14q22	TERM_5
chr15q22	TERM_6
chr16q11	TERM_7
chr2p15	TERM_8
chr2p22	TERM_9
chr3q12	TERM_10
chr3q23	TERM_11
chr4q12	TERM_12
chr8p11	TERM_13
chr8q22	TERM_14
MT	TERM_15

Distribution of significative genesets for C1



Distribution of significative genesets for C1

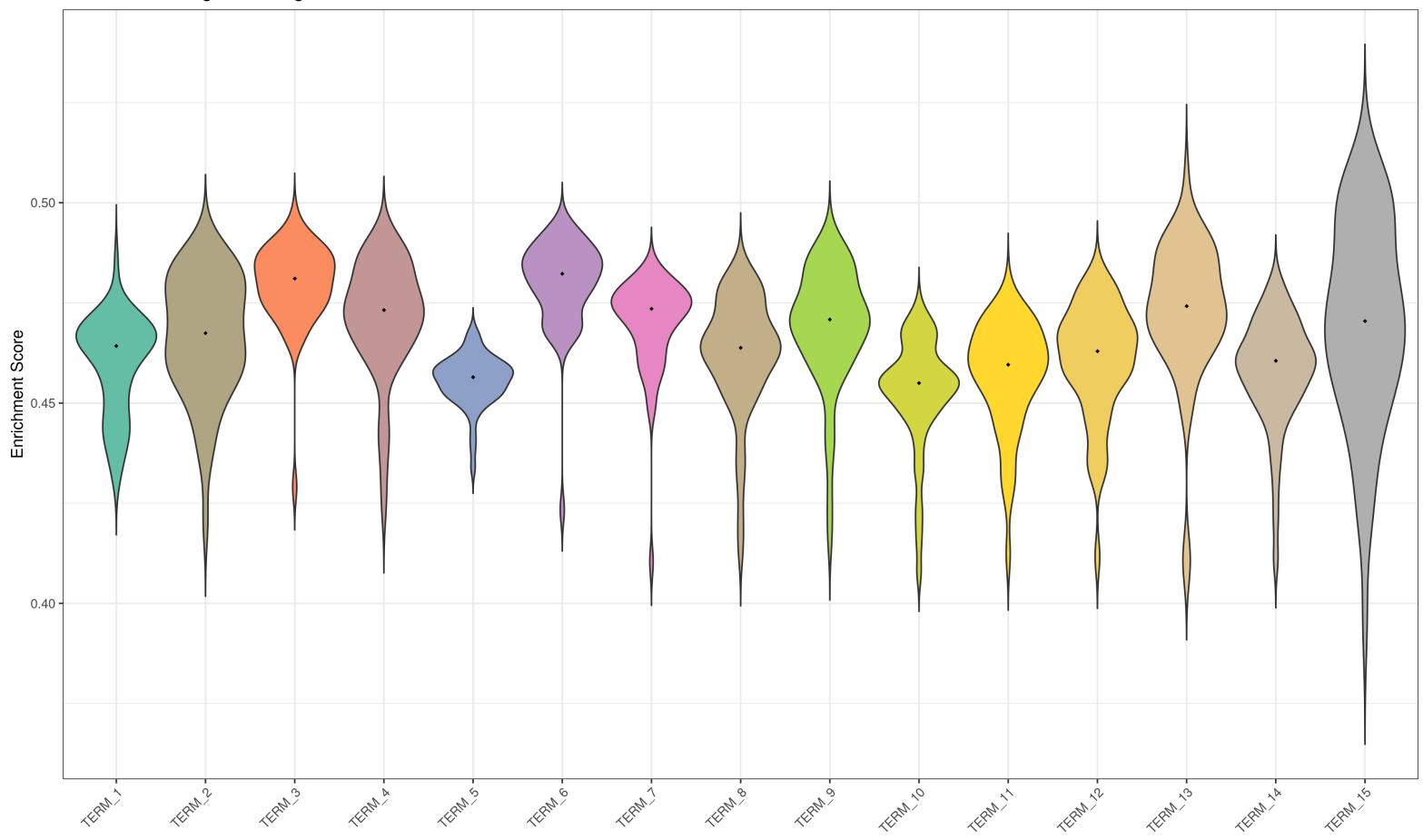


# Single-sample Gene Set Enrichment Analysis

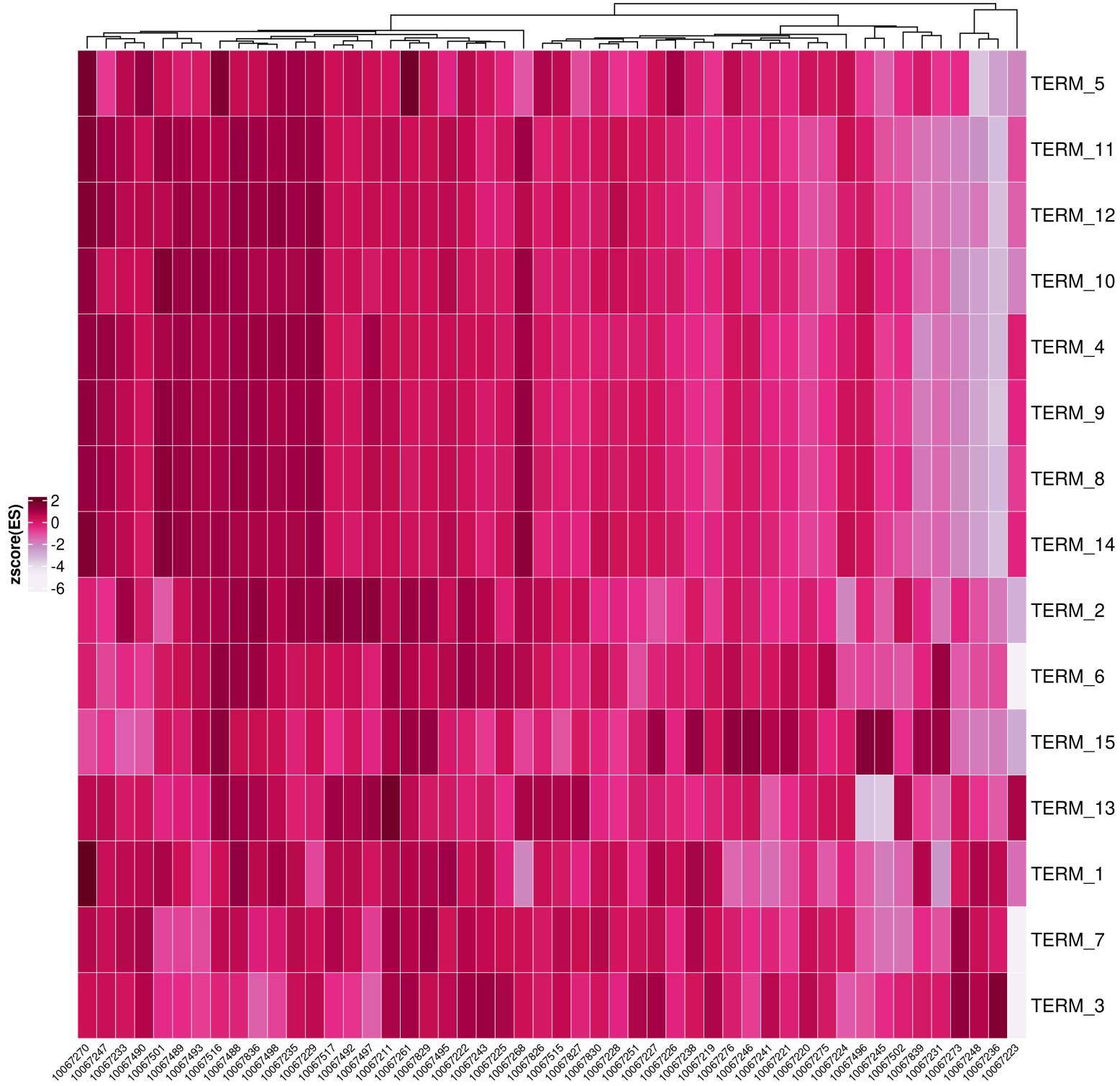
## C2: Curated Gene Sets

Term	Term ID
VISALA_RESPONSE_TO_HEAT_SHOCK_AND_AGING_DN	TERM_1
MIKHAYLOVA_OXIDATIVE_STRESS_RESPONSE_VIA_VHL_UP	TERM_2
BIOCARTA_IRES_PATHWAY	TERM_3
WP_CYTOPLASMIC_RIBOSOMAL_PROTEINS	TERM_4
TIEN_INTESTINE_PROBIOTICS_6HR_UP	TERM_5
NGO_MALIGNANT_GLIOMA_1P_LOH	TERM_6
BIOCARTA{EIF}_PATHWAY	TERM_7
KEGG_RIBOSOME	TERM_8
REACTOME_EUKARYOTIC_TRANSLATION_ELONGATION	TERM_9
REACTOME_SRP_DEPENDENT_COTRANSLATIONAL_PROTEIN_TARGETING_TO_MEMBRANE	TERM_10
REACTOME_EUKARYOTIC_TRANSLATION_INITIATION	TERM_11
REACTOME_ACTIVATION_OF_THE_MRNA_UPON_BINDING_OF_THE_CAP_BINDING_COMPLEX_AND_EIFS_AND_SUBSEQUENT_BINDING_TO_43S	TERM_12
REACTOME_INLA_MEDIATED_ENTRY_OF_LISTERIA_MONOCYTOGENES INTO_HOST_CELLS	TERM_13
REACTOME_RESPONSE_OF{EIF2AK4}_GCN2_TO_AMINO_ACID_DEFICIENCY	TERM_14
REACTOME_MODULATION_BY_MTB_OF_HOST_IMMUNE_SYSTEM	TERM_15

Distribution of significative genesets for C2



## Distribution of significative genesets for C2

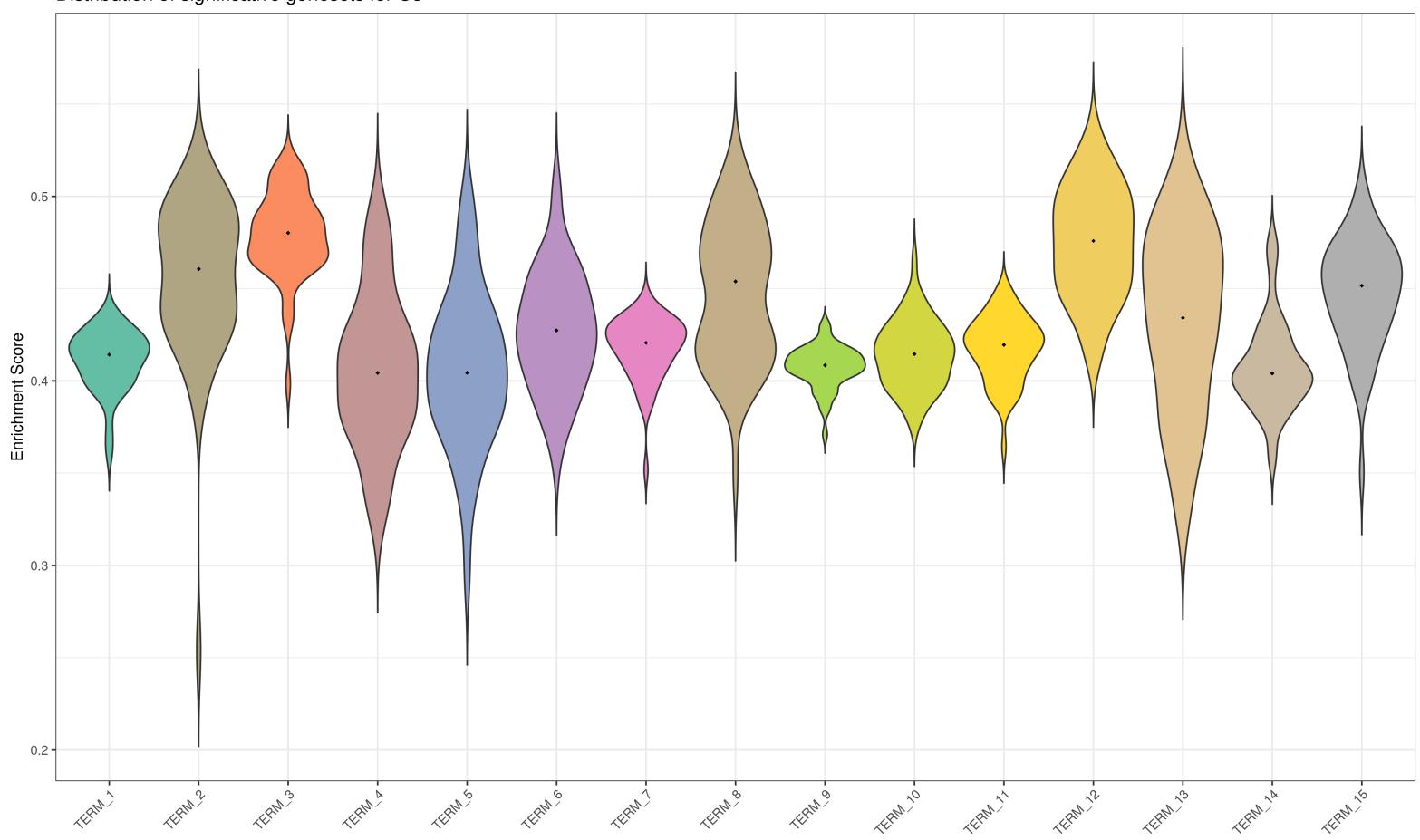


# Single-sample Gene Set Enrichment Analysis

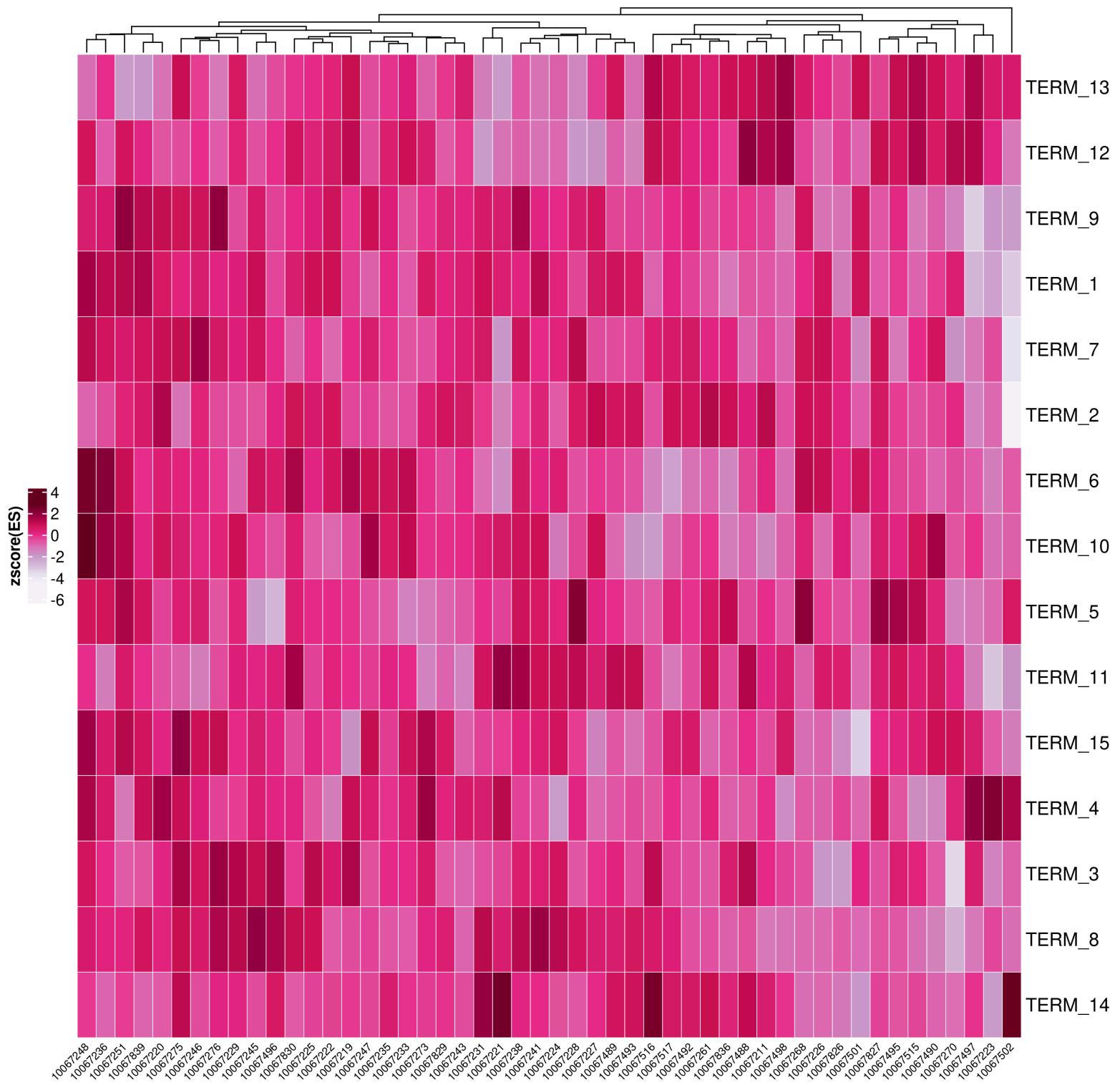
## C3: Regulatory Target Gene Sets

Term	Term ID
MIR548AY_3P	TERM_1
MIR4442	TERM_2
MIR1180_3P	TERM_3
MIR3186_5P	TERM_4
MIR101_2_5P	TERM_5
MIR105_3P	TERM_6
AACGGTT_MIR451	TERM_7
CGTCTTA_MIR208	TERM_8
AAGGGAT_MIR188	TERM_9
GTAGGCA_MIR189	TERM_10
ZNF19_TARGET_GENES	TERM_11
MDM2_TARGET_GENES	TERM_12
MAP2K1_TARGET_GENES	TERM_13
ATM_TARGET_GENES	TERM_14
RBM17_TARGET_GENES	TERM_15

Distribution of significative genesets for C3



## Distribution of significative genesets for C3

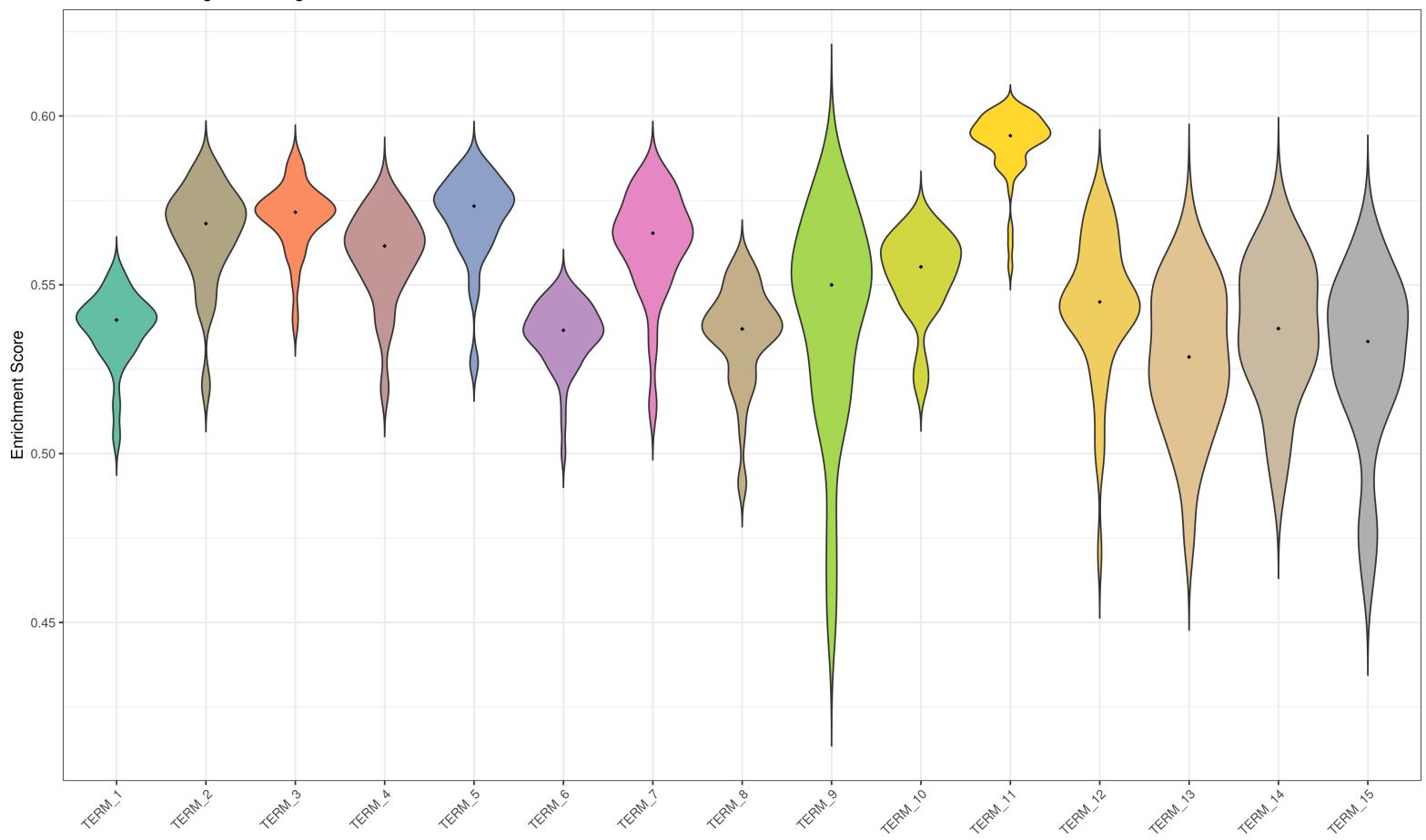


# Single-sample Gene Set Enrichment Analysis

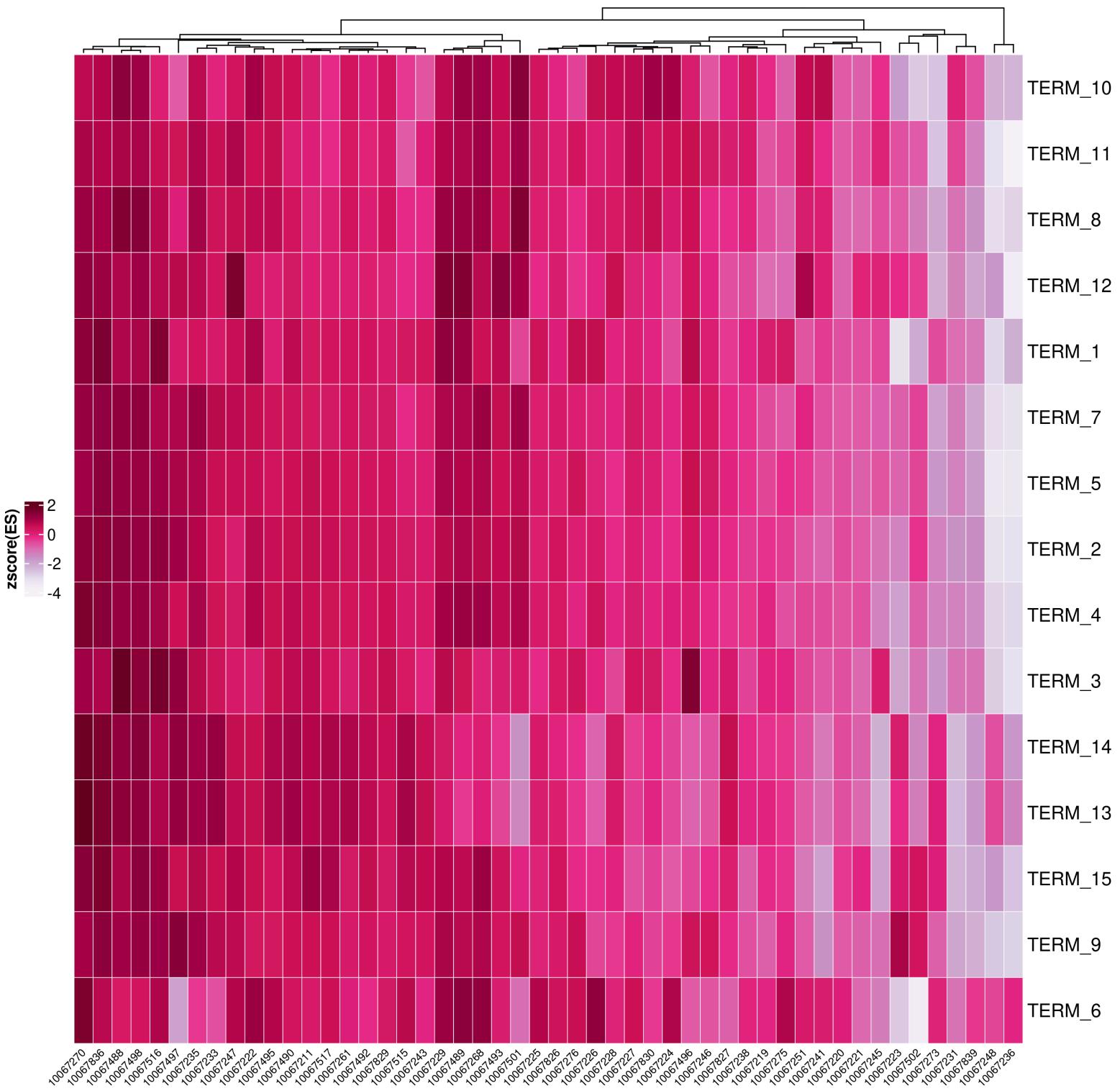
## C4: Computational Gene Sets

Term	Term ID
MORF_CCNI	TERM_1
MORF_ACTG1	TERM_2
MORF_JUND	TERM_3
MORF_NPM1	TERM_4
MORF_TPT1	TERM_5
GCM_RAD21	TERM_6
GCM_TPT1	TERM_7
GNF2{EIF3S6}	TERM_8
GNF2_GLTSCR2	TERM_9
GNF2_ST13	TERM_10
GNF2_TPT1	TERM_11
MODULE_29	TERM_12
MODULE_81	TERM_13
MODULE_115	TERM_14
MODULE_150	TERM_15

Distribution of significative genesets for C4



Distribution of significative genesets for C4

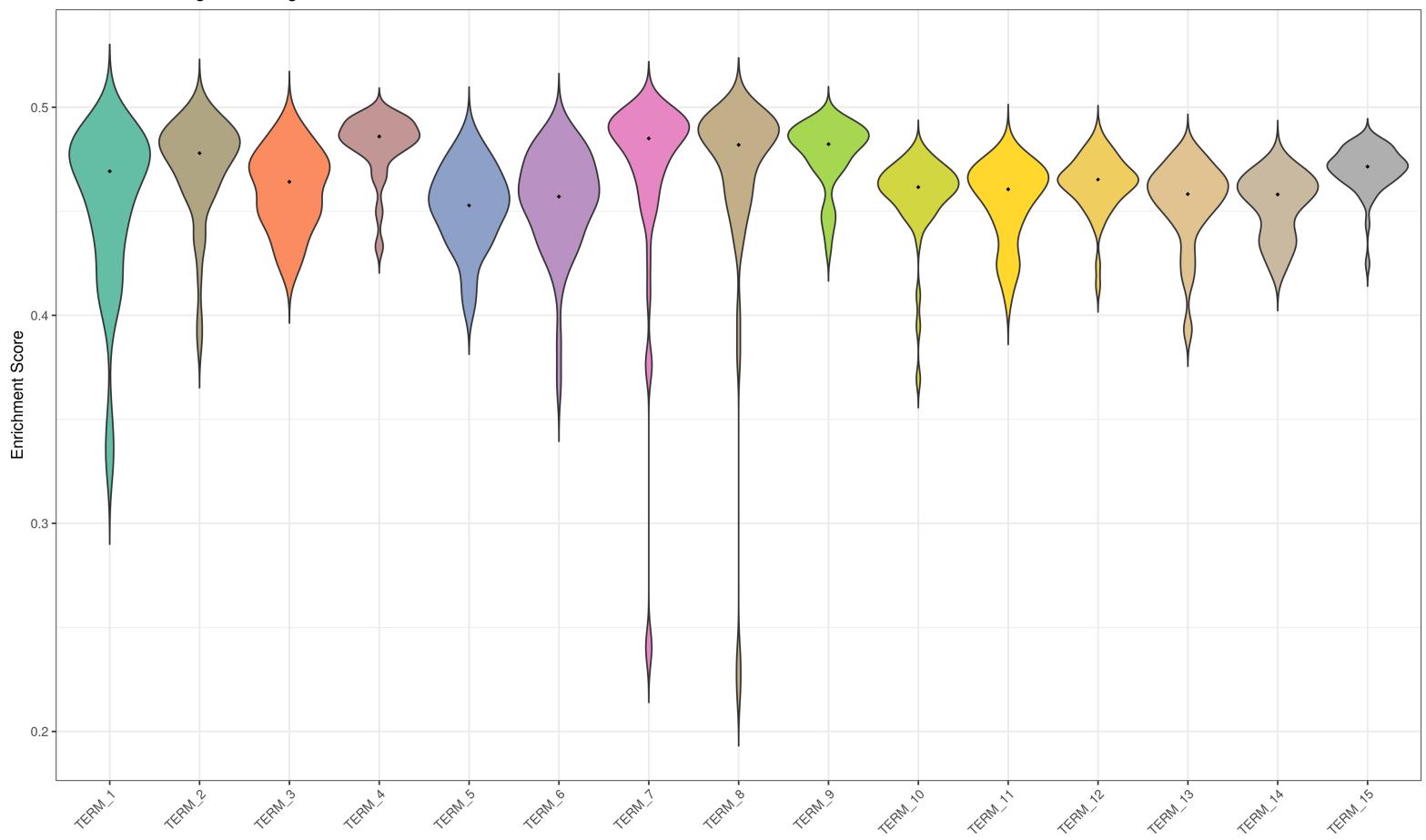


# Single-sample Gene Set Enrichment Analysis

## C5: Ontology Gene Sets

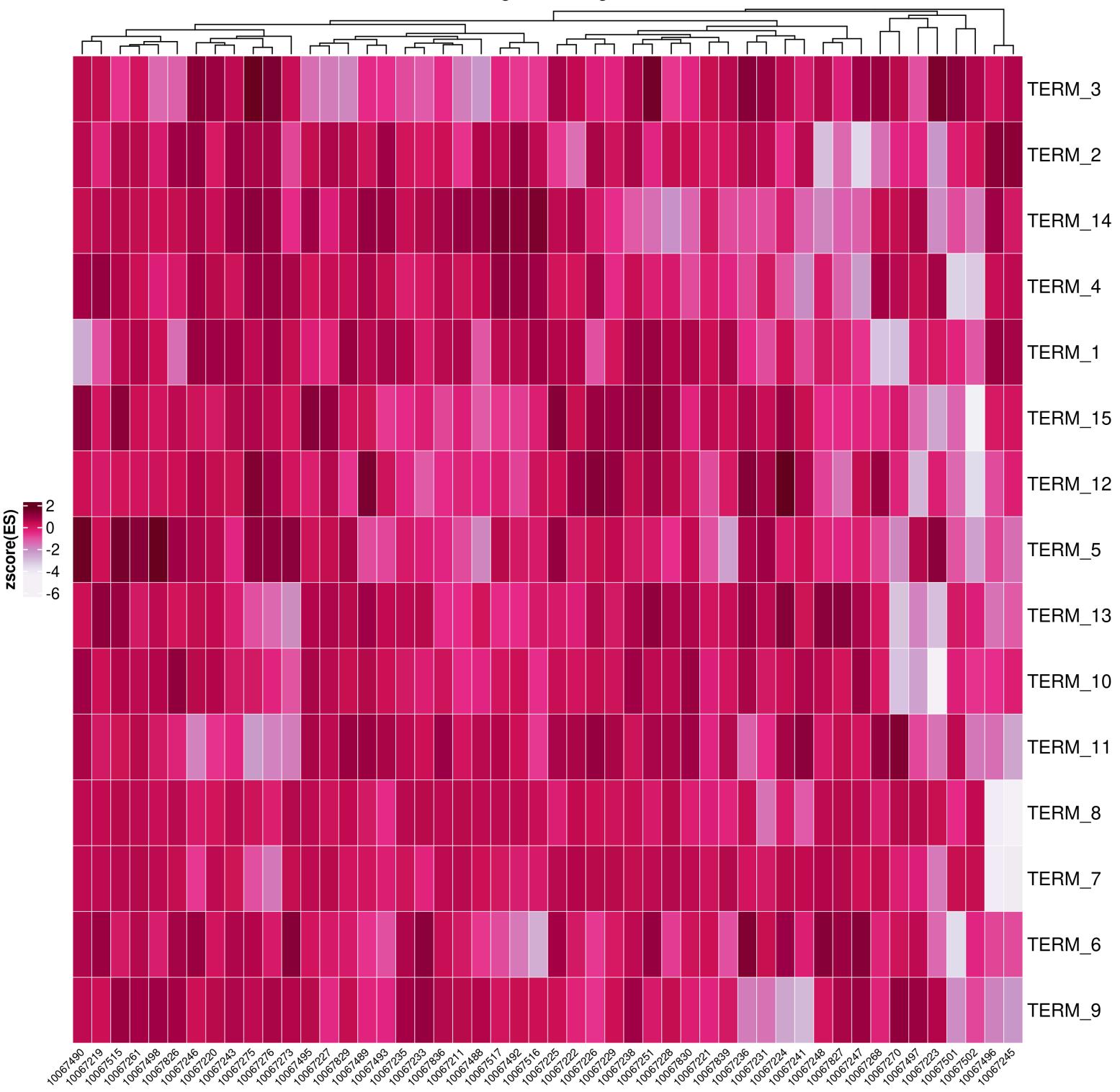
Term	Term ID
HP_UTERINE_PROLAPSE	TERM_1
HP_LOSS_OF_VOICE	TERM_2
HP_GLOMUS_JUGULAR_TUMOR	TERM_3
HP_INAPPROPRIATE_SEXUAL_BEHAVIOR	TERM_4
HP_HYPEREXTENSIBILITY_AT_ELBOW	TERM_5
HP_OPSCLONUST	TERM_6
GOBP_POSITIVE_REGULATION_OF_TRANSMISSION_OF_NERVE_IMPULSE	TERM_7
GOBP_RESPONSE_TO_INDOLE_3_METHANOL	TERM_8
GOBP_PROTEIN_LOCALIZATION_TO_BICELLULAR_TIGHT_JUNCTION	TERM_9
GOBP_REGULATION_OF_ENDOPLASMIC_RETICULUM_TUBULAR_NETWORK_ORGANIZATION	TERM_10
GOBP_POSITIVE_REGULATION_OF_ESTABLISHMENT_OF_PROTEIN_LOCALIZATION_TO_TELOMERE	TERM_11
GOCC_PARASPECKLES	TERM_12
GOCC_ENDOPLASMIC_RETICULUM_TUBULAR_NETWORK_MEMBRANE	TERM_13
GOMF_ADENYLATE_CYCLASE_ACTIVATOR_ACTIVITY	TERM_14
GOMF_RNA_STRAND_ANNEALING_ACTIVITY	TERM_15

# Distribution of significative genesets for C5



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## Distribution of significative genesets for C5

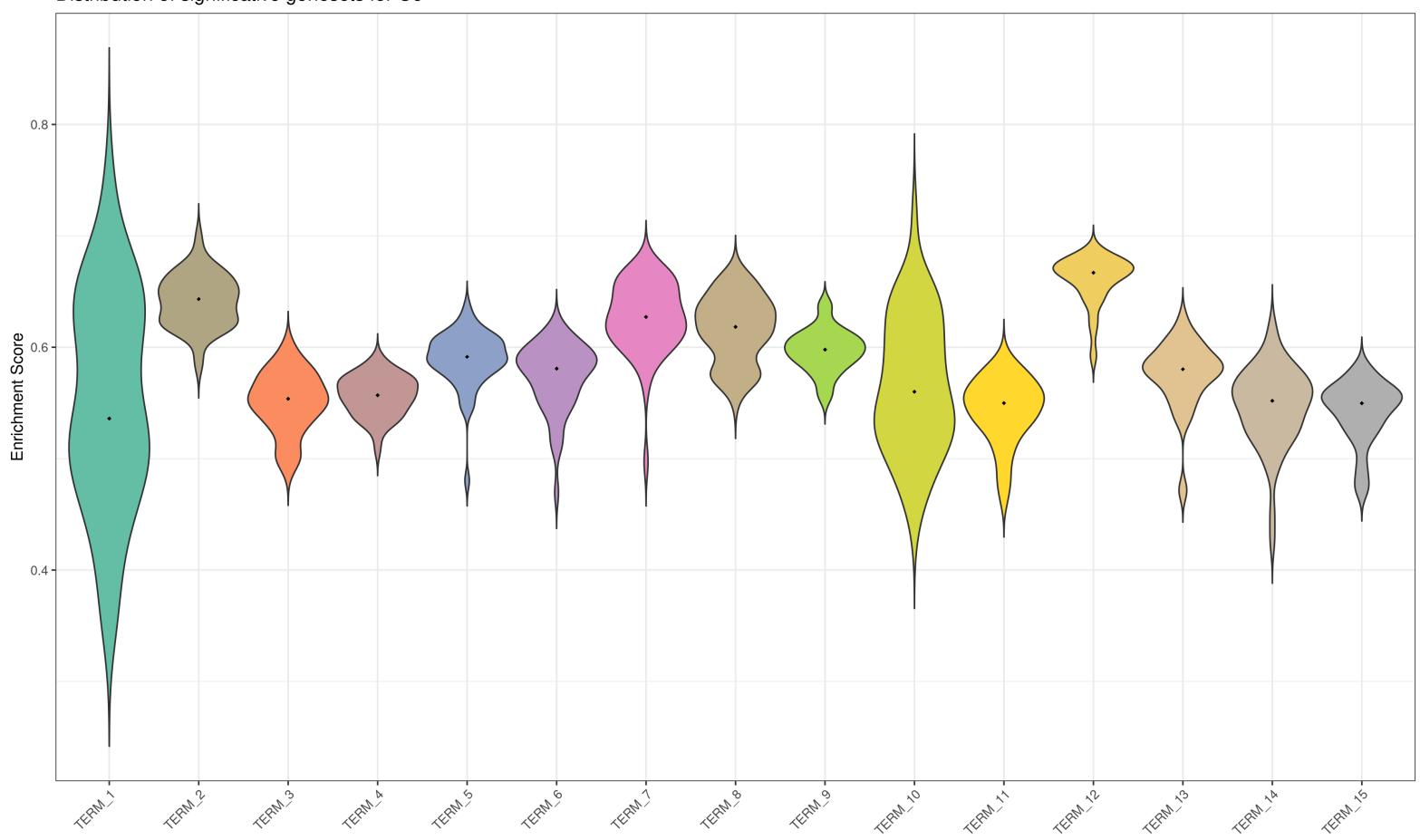


# Single-sample Gene Set Enrichment Analysis

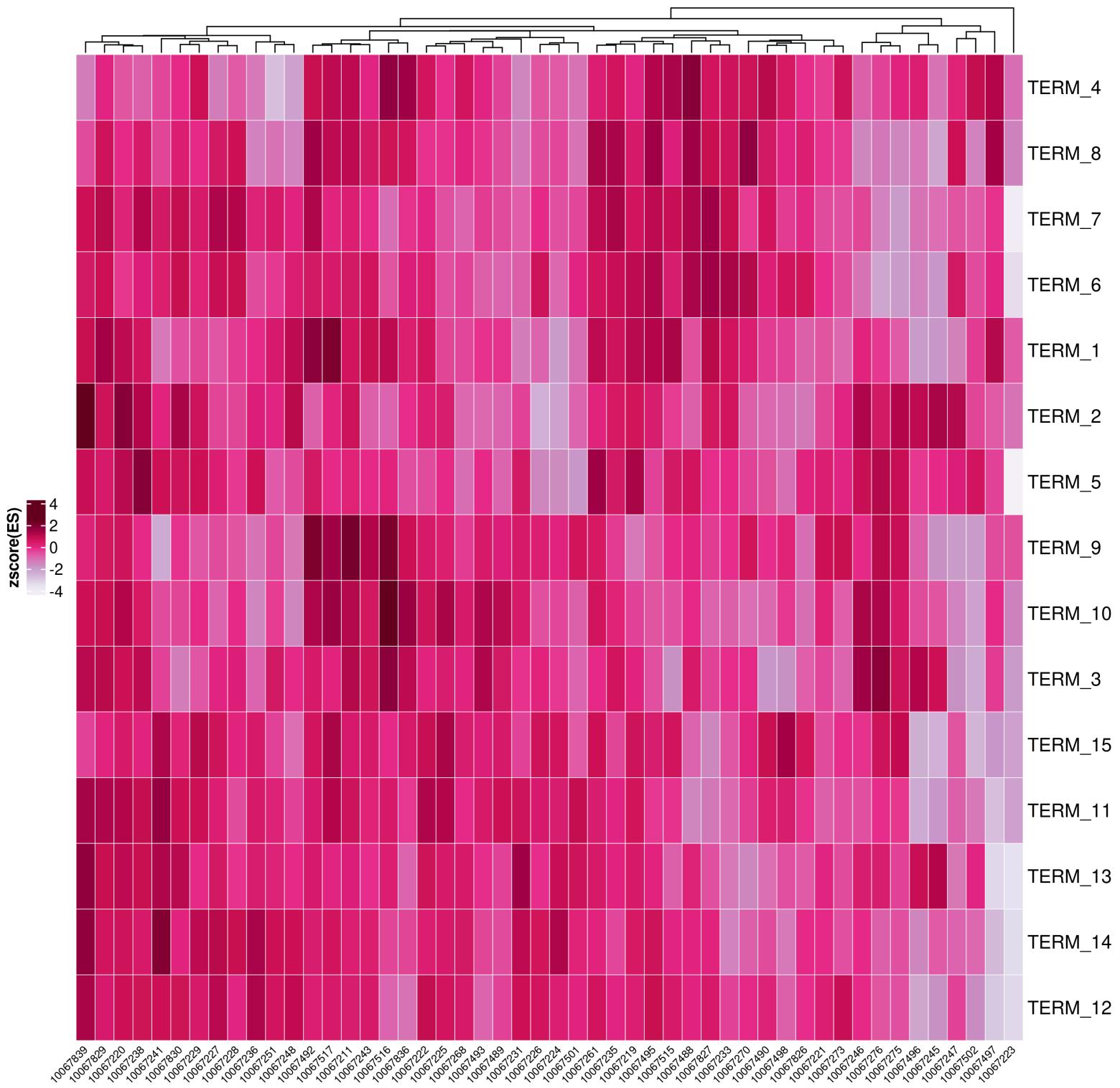
## C6: Oncogenic Signature Gene Sets

Term	Term ID
HINATA_NFKB_MATRIX	TERM_1
BCAT_BILD_ET_AL_DN	TERM_2
CAMP_UP.V1_DN	TERM_3
CAMP_UP.V1_UP	TERM_4
EIF4E_DN	TERM_5
RB_P107_DN.V1_DN	TERM_6
RB_P130_DN.V1_DN	TERM_7
SIRNA{EIF4GI}_UP	TERM_8
TBK1.DN.48HRS_UP	TERM_9
CORDENONSI_YAP_CONSERVED_SIGNATURE	TERM_10
VEGF_A_UP.V1_DN	TERM_11
TBK1.DF_DN	TERM_12
PGF_UP.V1_UP	TERM_13
ERBB2_UP.V1_DN	TERM_14
GCNP_SHH_UP_LATE.V1_UP	TERM_15

Distribution of significative genesets for C6



Distribution of significative genesets for C6

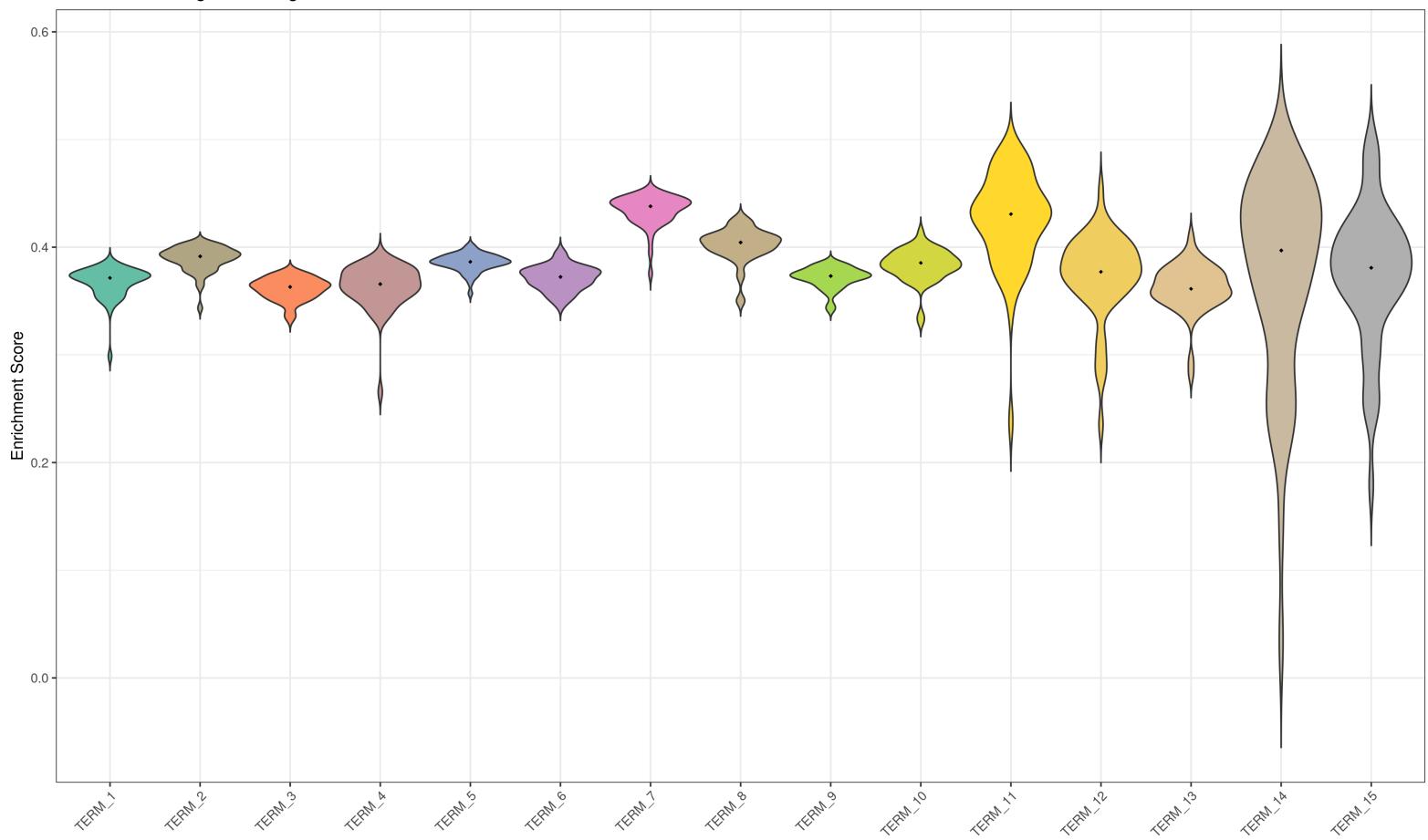


# Single-sample Gene Set Enrichment Analysis

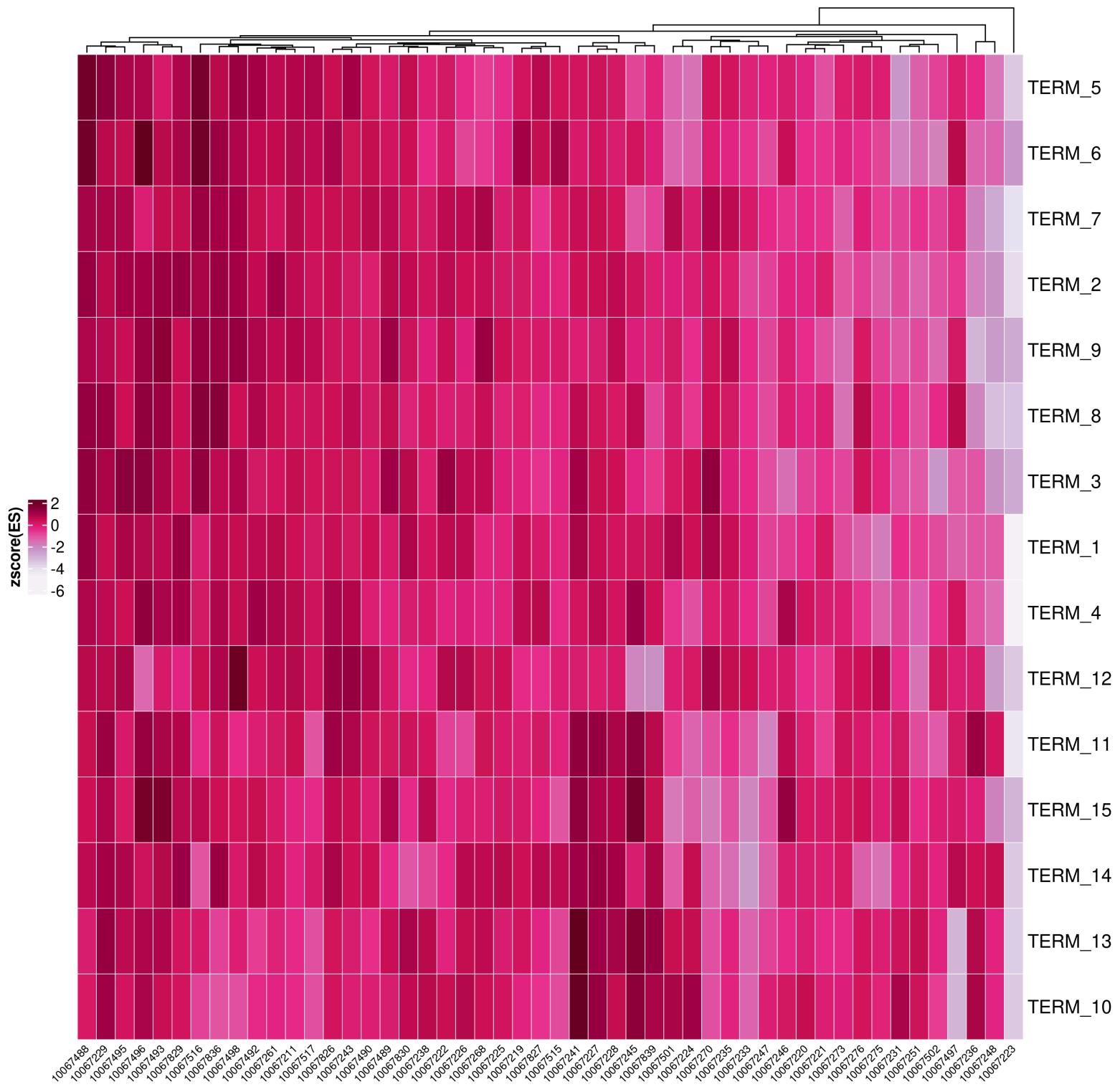
## C7: Immunologic Signature Gene Sets

Term	Term ID
GSE22886_NEUTROPHIL_VS_DC_DN	TERM_1
GSE22886_NEUTROPHIL_VS_MONOCYTE_DN	TERM_2
GSE22886_NAIVE_TCELL_VS_NEUTROPHIL_UP	TERM_3
GSE22886_NAIVE_CD4_TCELL_VS_DC_DN	TERM_4
GSE9006_HEALTHY_VS_TYPE_1_DIABETES_PBMC_1MONTH_POST_DX_UP	TERM_5
GSE9006_HEALTHY_VS_TYPE_1_DIABETES_PBMC_4MONTH_POST_DX_UP	TERM_6
GSE2405_0H_VS_9H_A_PHAGOCYTOPHILUM_STIM_NEUTROPHIL_DN	TERM_7
GSE2405_0H_VS_24H_A_PHAGOCYTOPHILUM_STIM_NEUTROPHIL_UP	TERM_8
GSE41978_ID2_KO_VS_ID2_KO_AND_BIM_KO_KLRG1_LOW_EFFECTOR_CD8_TCELL_DN	TERM_9
THAKAR_PBMC_INACTIVATED_INFLUENZA AGE_21_30YO_NONRESPONDER_7DY_UP	TERM_10
THAKAR_PBMC_INACTIVATED_INFLUENZA AGE_70PLS_VS_21_30YO_0DY_UP	TERM_11
BUCASAS_PBMC_FLUARIX_FLUVIRIN_CAUCASIAN_MALE AGE_18_40YO_LOW_RESPONDERS_1DY _POSITIVE_PREDICTIVE_OF_TITER	TERM_12
ERWIN_COHEN_BLOOD_LIVE_VACCINE_TC_83 AGE_23_48YO_VACCINATED_VS_CONTROL_14DY_UP	TERM_13
COLE_BLOOD_FLUMIST_QUADRIVALENT AGE_03_17YO_7DY_UP	TERM_14
BUCASAS_PBMC_FLUARIX_FLUVIRIN_CAUCASIAN_MALE AGE_18_40YO_HIGH_RESPONDERS_1DY _3DY_POSITIVE_PREDICTIVE_OF_TITER	TERM_15

### Distribution of significative genesets for C7



## Distribution of significative genesets for C7

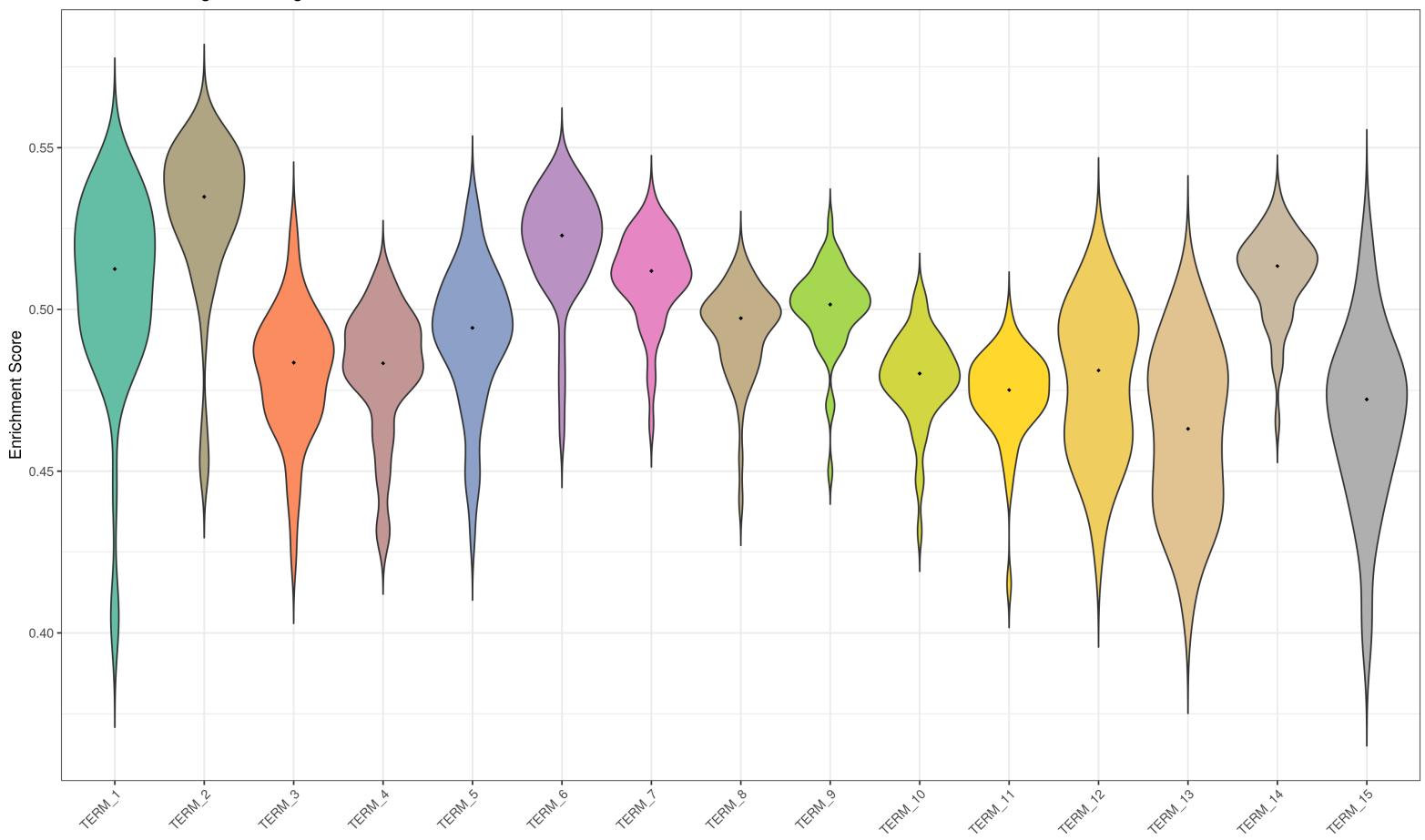


# Single-sample Gene Set Enrichment Analysis

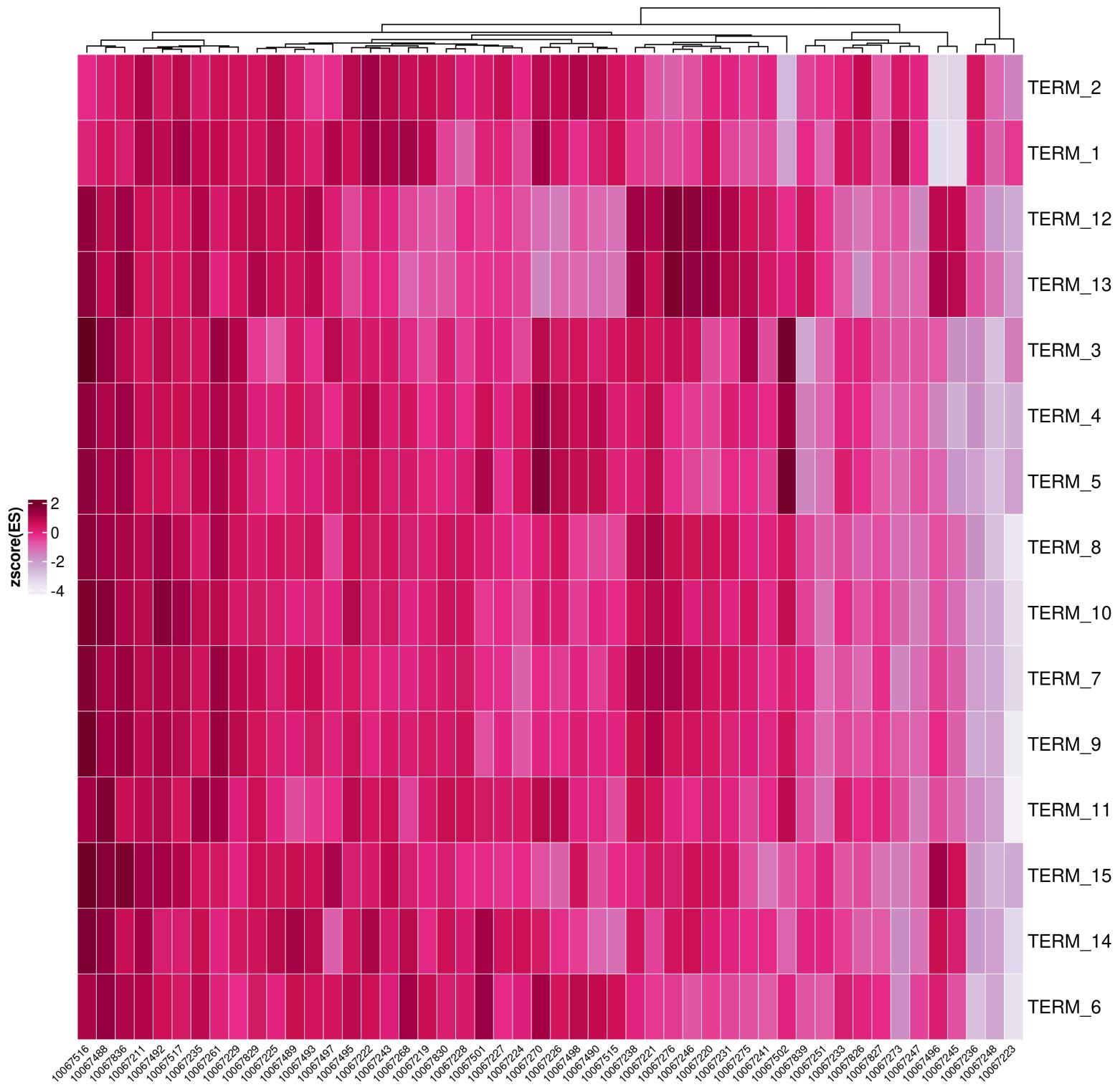
## C8: Cell Type Signature Gene Sets

Term	Term ID
BUSSLINGER_ESOPHAGEAL_PROLIFERATING_BASAL_CELLS	TERM_1
BUSSLINGER_ESOPHAGEAL_EARLY_SUPRABASAL_CELLS	TERM_2
BUSSLINGER_GASTRIC_PPP1R1B_POSITIVE_CELLS	TERM_3
BUSSLINGER_DUODENAL_DIFFERENTIATING_STEM_CELLS	TERM_4
BUSSLINGER_DUODENAL_TRANSIT_AMPLIFYING_CELLS	TERM_5
MANNO_MIDBRAIN_NEUROTYPES_BASAL	TERM_6
LAKE_ADULT_KIDNEY_C8_DESCENDING_THIN_LIMB	TERM_7
LAKE_ADULT_KIDNEY_C9_THIN_ASCENDING_LIMB	TERM_8
LAKE_ADULT_KIDNEY_C10_THIN_ASCENDING_LIMB	TERM_9
LAKE_ADULT_KIDNEY_C18_COLLECTING_DUCT_PRINCIPAL_CELLS_MEDULLA	TERM_10
LAKE_ADULT_KIDNEY_C19_COLLECTING_DUCT_INTERCALATED_CELLS_TYPE_A_MEDULLA	TERM_11
LAKE_ADULT_KIDNEY_C23_ENDOTHELIAL_CELLS_AVR	TERM_12
LAKE_ADULT_KIDNEY_C24_ENDOTHELIAL_CELLS_AEA_AND_DVR	TERM_13
FAN_OVARY_CL0_XBP1_SELK_HIGH_STROMAL_CELL	TERM_14
FAN_OVARY_CL2_PUTATIVE_EARLY_ATRETIQUE_FOLLICLE_THECAL_CELL_1	TERM_15

Distribution of significative genesets for C8



## Distribution of significative genesets for C8

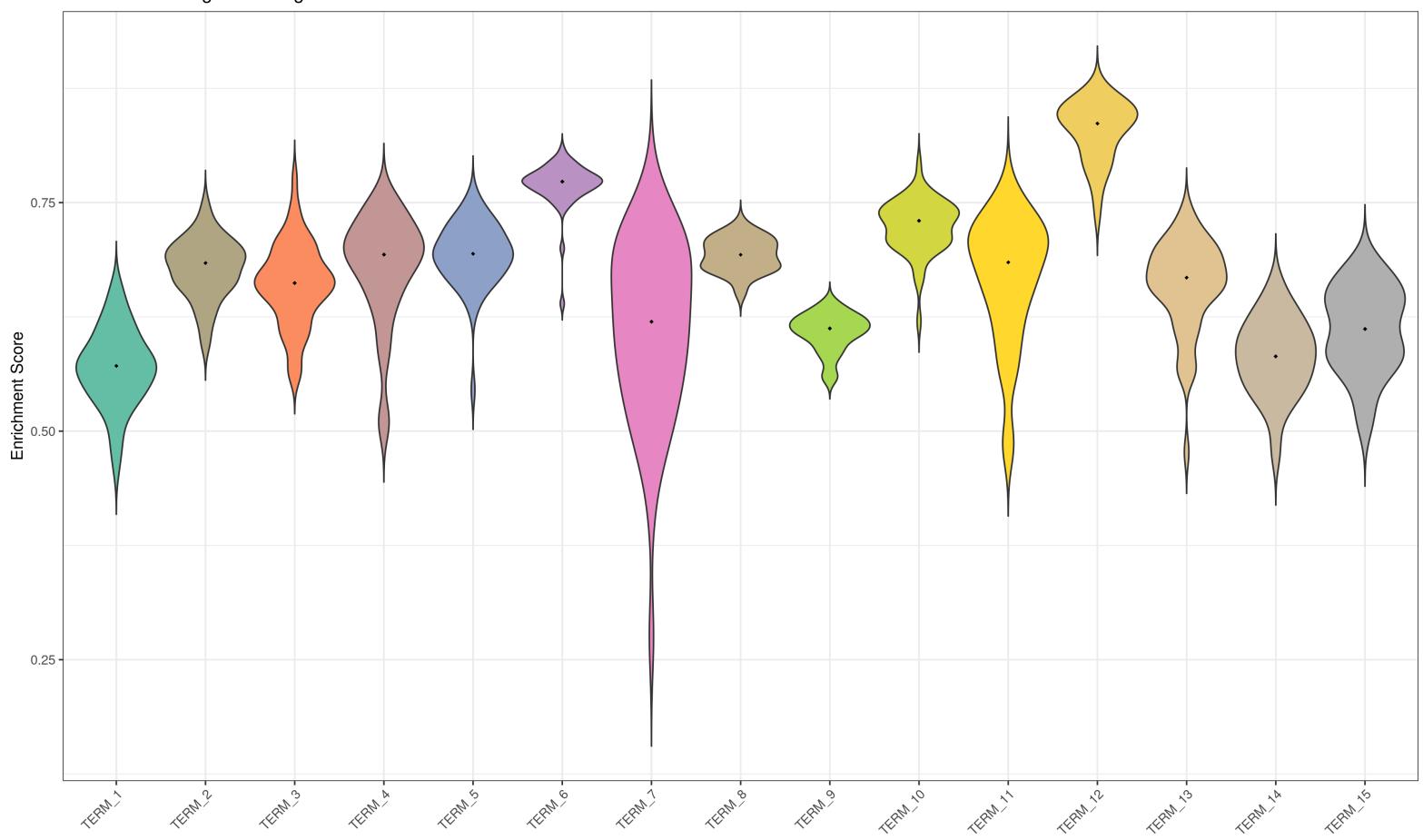


# Single-sample Gene Set Enrichment Analysis

## H: Hallmark Gene Sets

Term	Term ID
HALLMARK_CHOLESTEROL_HOMEOSTASIS	TERM_1
HALLMARK_MITOTIC_SPINDLE	TERM_2
HALLMARK_TGF_BETA_SIGNALING	TERM_3
HALLMARK_G2M_CHECKPOINT	TERM_4
HALLMARK_ANDROGEN_RESPONSE	TERM_5
HALLMARK_PROTEIN_SECRETION	TERM_6
HALLMARK_INTERFERON_ALPHA_RESPONSE	TERM_7
HALLMARK_UNFOLDED_PROTEIN_RESPONSE	TERM_8
HALLMARK_PI3K_AKT_MTOR_SIGNALING	TERM_9
HALLMARK_MTORC1_SIGNALING	TERM_10
HALLMARK_E2F_TARGETS	TERM_11
HALLMARK_MYC_TARGETS_V1	TERM_12
HALLMARK_OXIDATIVE_PHOSPHORYLATION	TERM_13
HALLMARK_REACTIVE_OXYGEN_SPECIES_PATHWAY	TERM_14
HALLMARK_UV_RESPONSE_DN TERM_15	TERM_15

Distribution of significative genesets for H



Distribution of significative genesets for H

