Investigating the Impact of Radiation Therapy on CAR T-Cell Efficacy: Insights from RNA-Seq Analysis

An RNA-Seq Analysis of Tumor Microenvironment and Immune Activation in Response to Radiation

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Introduction

Objective: Understand the impact of low-dose radiation on CAR T-cell efficacy using RNA-seq data from tumors and lymph nodes.

Dataset: GEO accession GSE281695 (mouse model of CD19+ lymphoma).

- Investigates the effects of low-dose radiation therapy (RT) on CAR T-cell efficacy.
- Focuses on gene expression changes in irradiated (IR) vs. non-irradiated (Non-IR) tumors and lymph nodes.

Non- irradiated

7 days vs 24 hours

Irradiated

7 days vs 24 hours

Objective

- How does RT affect gene expression?
- What pathways and immune responses are activated?
- How do gene expression changes evolve over time (24 hours vs. 7 days)?

- Link to human precision medicine:
  - **Relevance**: Insights into immune pathways and tumor responses can inform personalized strategies to enhance CAR T-cell therapy efficacy in humans.
  - **Potential Impact**: Identify biomarkers for patient stratification, optimize radiation dosing for individual patients, and improve outcomes in CD19+ hematologic malignancies.

Methods

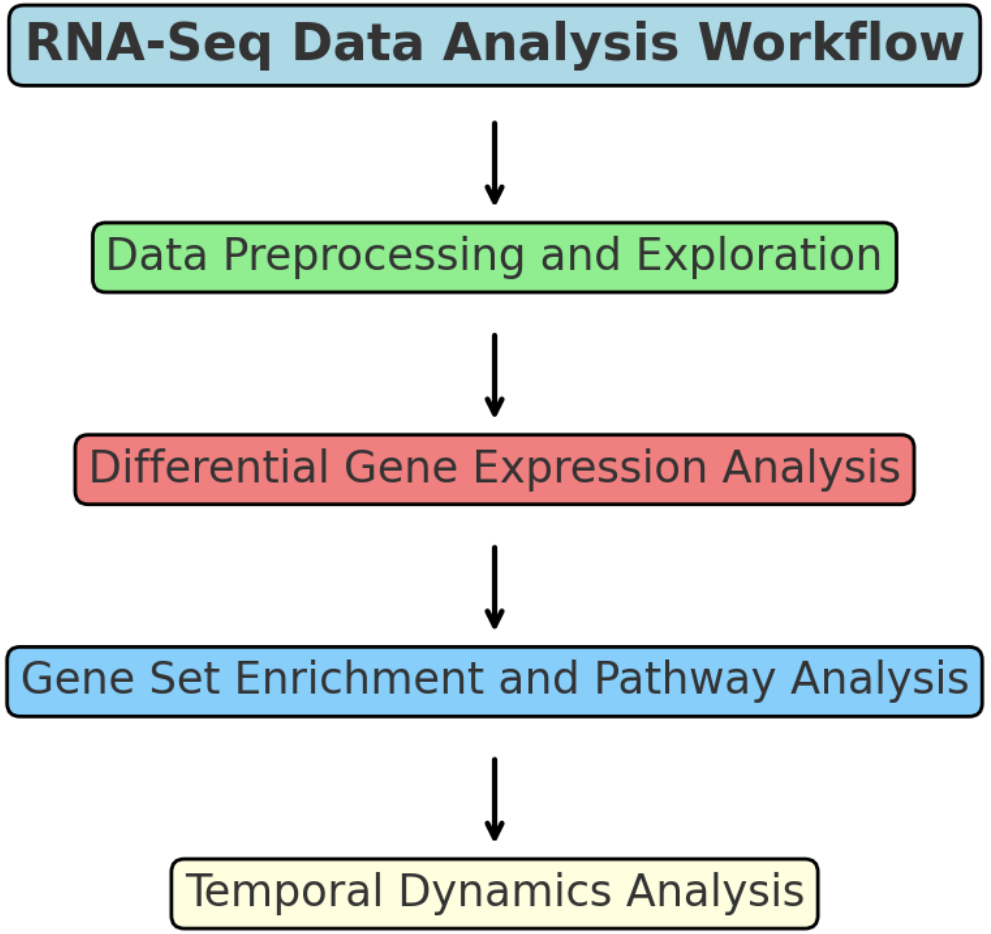
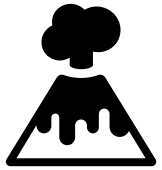


Figure 1: RNA-Seq analysis workflow

Methods

1. Data Preprocessing and Exploration

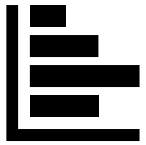
- Import data, verify sample groups, and inspect scaling (raw vs. normalized counts).
- Visualize with PCA plot of the raw data.
- Tools: readxl and ggplot2



2. Differential Gene Expression Analysis

- Identify differentially expressed genes (DEGs) for key comparisons (e.g., IR vs. Non-IR, time points).
- Tools:DESeq2, ggplot2

Methods



3. Gene Set Enrichment and Pathway Analysis

- Analyze biological processes and immune-related pathways using DEGs.
- Perform GO, KEGG, and curated immune pathway enrichment analyses.
- Tools: fgsea and MSigDB.

4. Temporal Dynamics Analysis

- Compare gene expression changes at 24 hours vs. 7 days.
- Cluster DEGs and create time-series visualizations for dynamic changes.
- Tools: ImpulseDE2, ComplexHeatmap.

Results: Data Preprocessing and Exploration

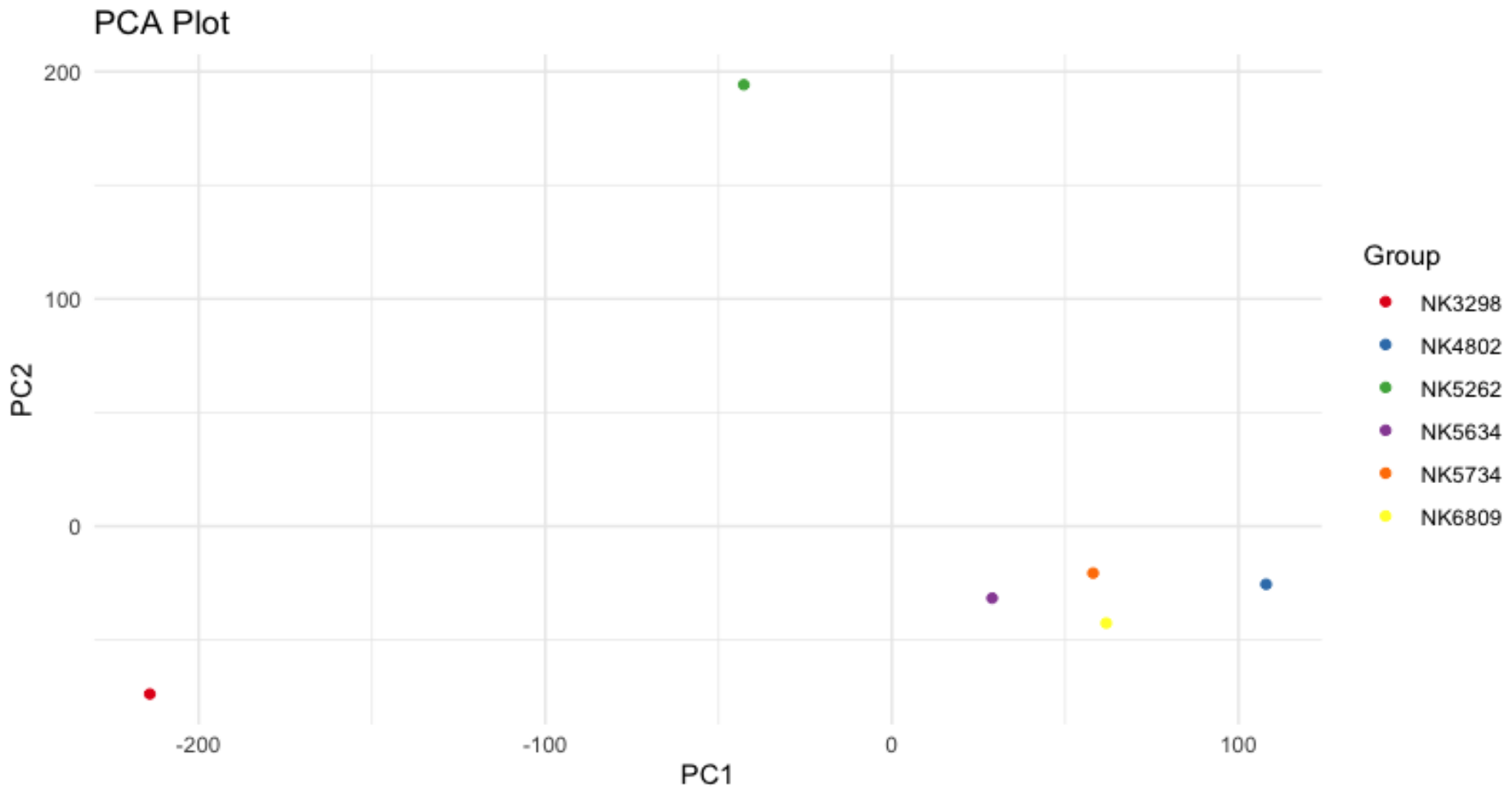
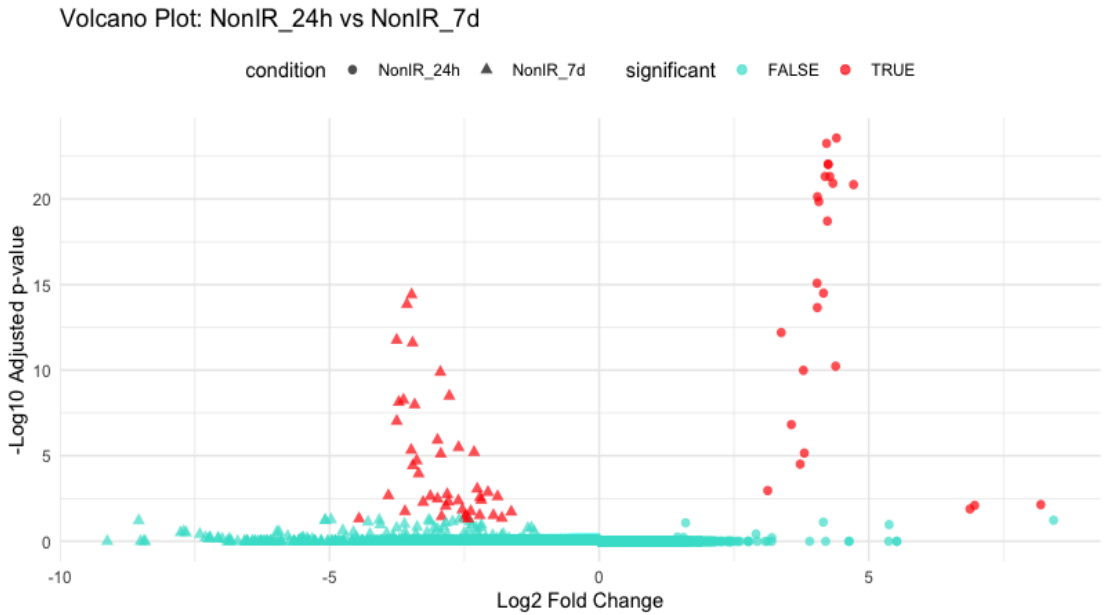
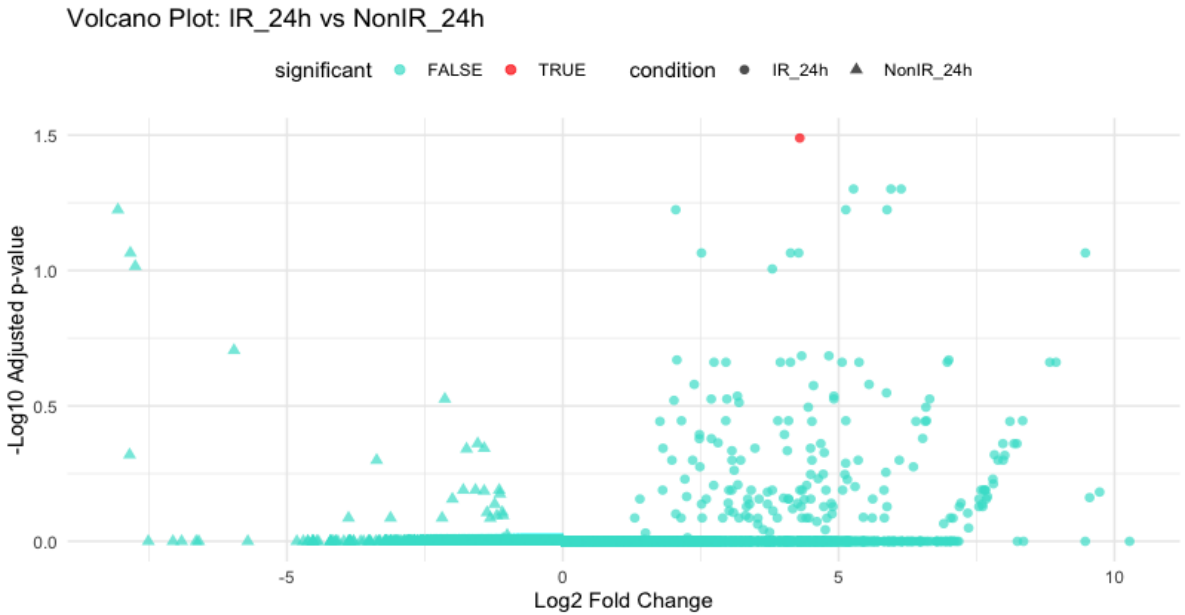


Figure 2: PCA plot of the raw data

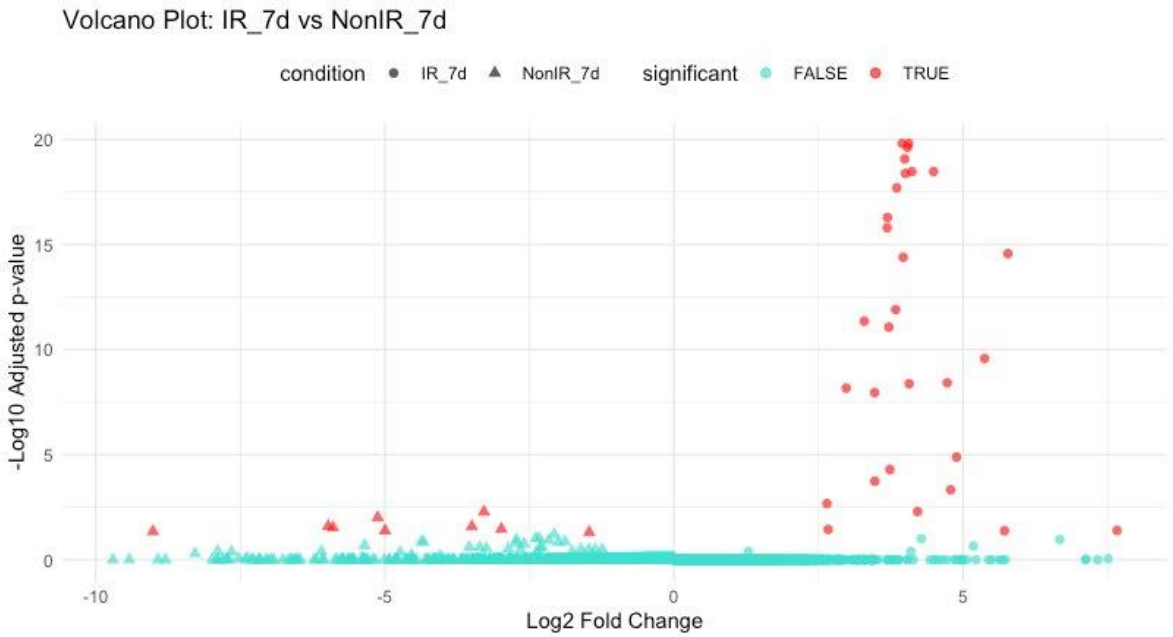
Results: DGE

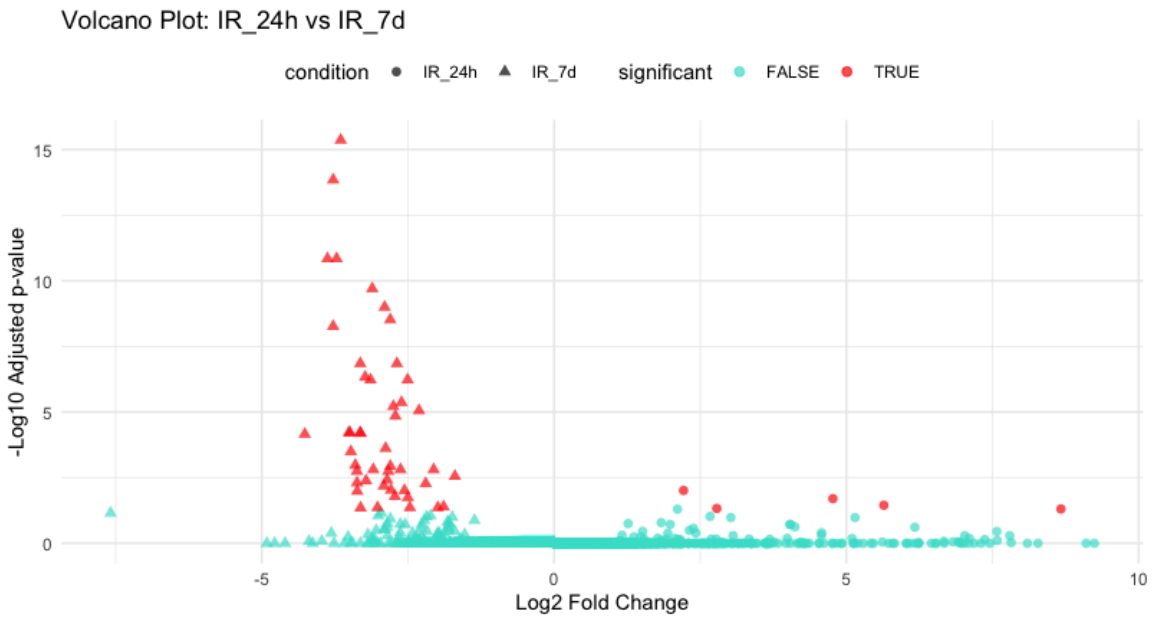




Figures 3,4: Volcano plots displaying significant DEGs (adjusted p-value < 0.05, fold change > 2) between IR and Non-IR tumors at 24 hours and 7 days post-radiation.

Results: DGE





Figures 5,6: Volcano plots displaying significant DEGs (adjusted p-value < 0.05, fold change > 2) between IR and Non-IR tumors at 24 hours and 7 days post-radiation.

Results: Gene Set Enrichment and Pathway Analysis

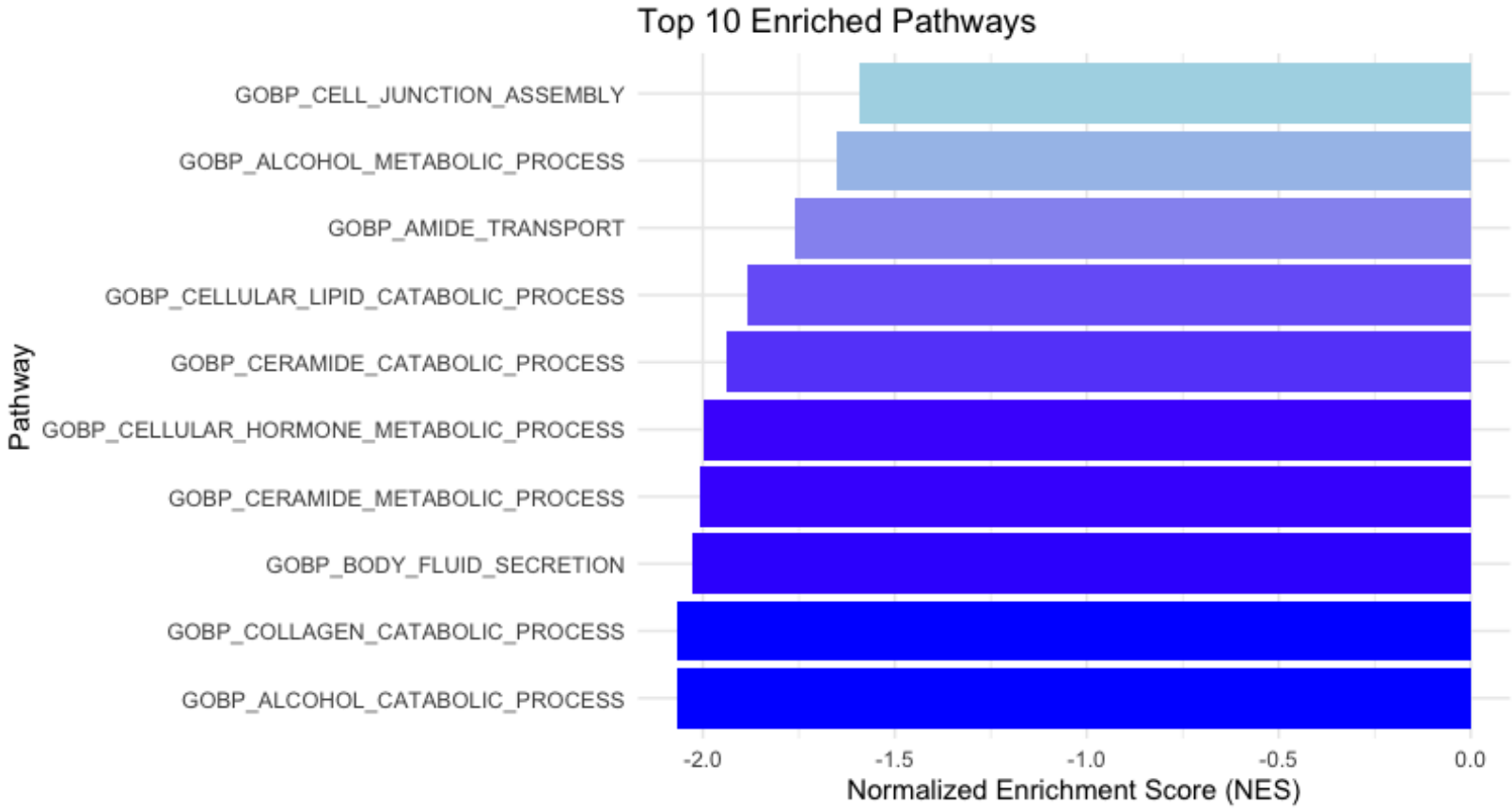


Figure 7: Bar plot showing enriched pathways derived from significant DEGs

Results: Temporal Dynamics Analysis

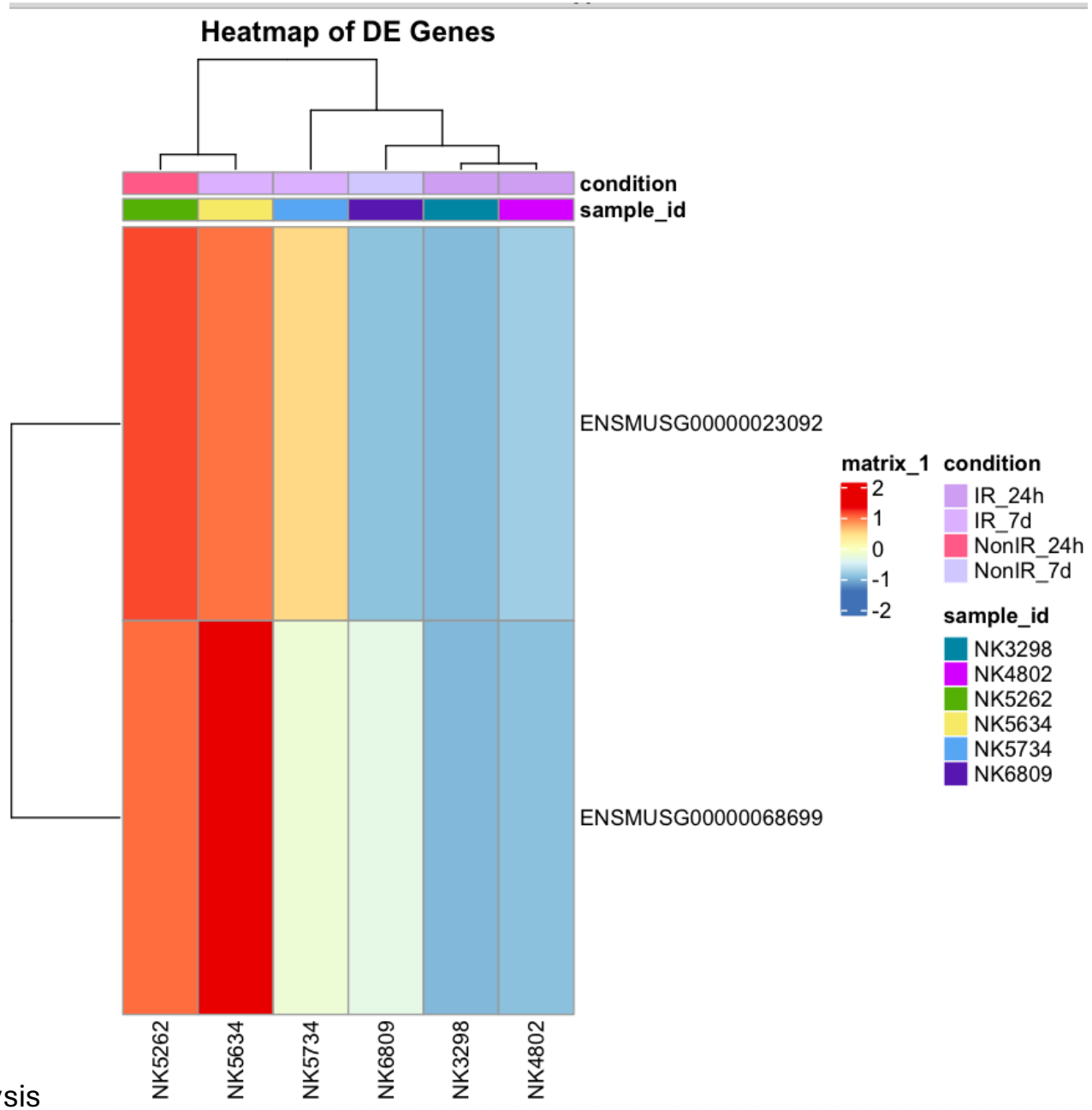


Figure 8: Heatmap of Time Differential Gene Analysis

Discussion

Key Insights:

- **Delayed Effects of Radiation:**
  - Strongest transcriptional changes occur 7 days post-radiation, suggesting that radiation takes time to fully affect the tumor.
  - Minimal changes at 24h suggest that radiation slowly reshapes the tumor microenvironment.
- **Pathway Implications:**
  - **Lipid metabolism** (e.g., ceramide pathways): May support tumor cell death.
  - **Structural remodeling** (e.g., collagen catabolism): Could make it easier for immune cells to reach and attack the tumor.

Discussion

Clinical Recommendations:

- Radiation should be used selectively for patients whose treatment plans can benefit from the gradual changes in immune activation and tumor remodeling.
- Combining radiation with therapies targeting metabolic pathways could improve outcomes.
- These findings provide a foundation for tailoring radiation therapy to optimize CAR T-cell efficacy in cancer treatment.

Conclusion

Next Steps for Research:

- Validate enriched pathways in human datasets.
- Optimize timing of CAR T-cell infusion to align with the development of radiation-induced changes at 7 days.
- Explore functional roles of pathways to identify new therapeutic targets.

Final Takeaway:

- Radiation has a delayed but potentially beneficial effect on the tumor microenvironment.
- While not a one-size-fits-all preparation method, it offers insights for precision medicine treatments.
- Further studies are needed to confirm findings and optimize its integration into CAR T-cell therapy protocols for human cancers.