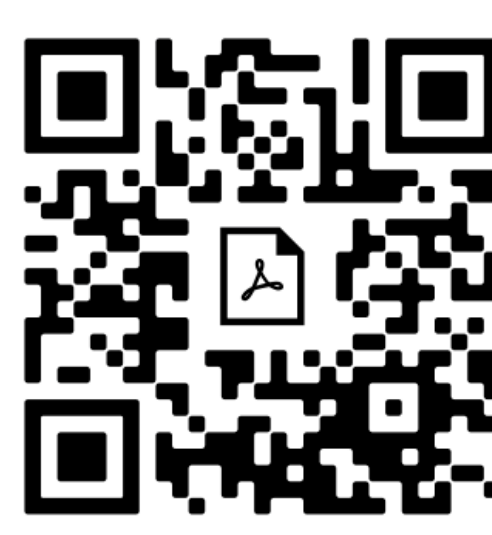




INTRODUCTION

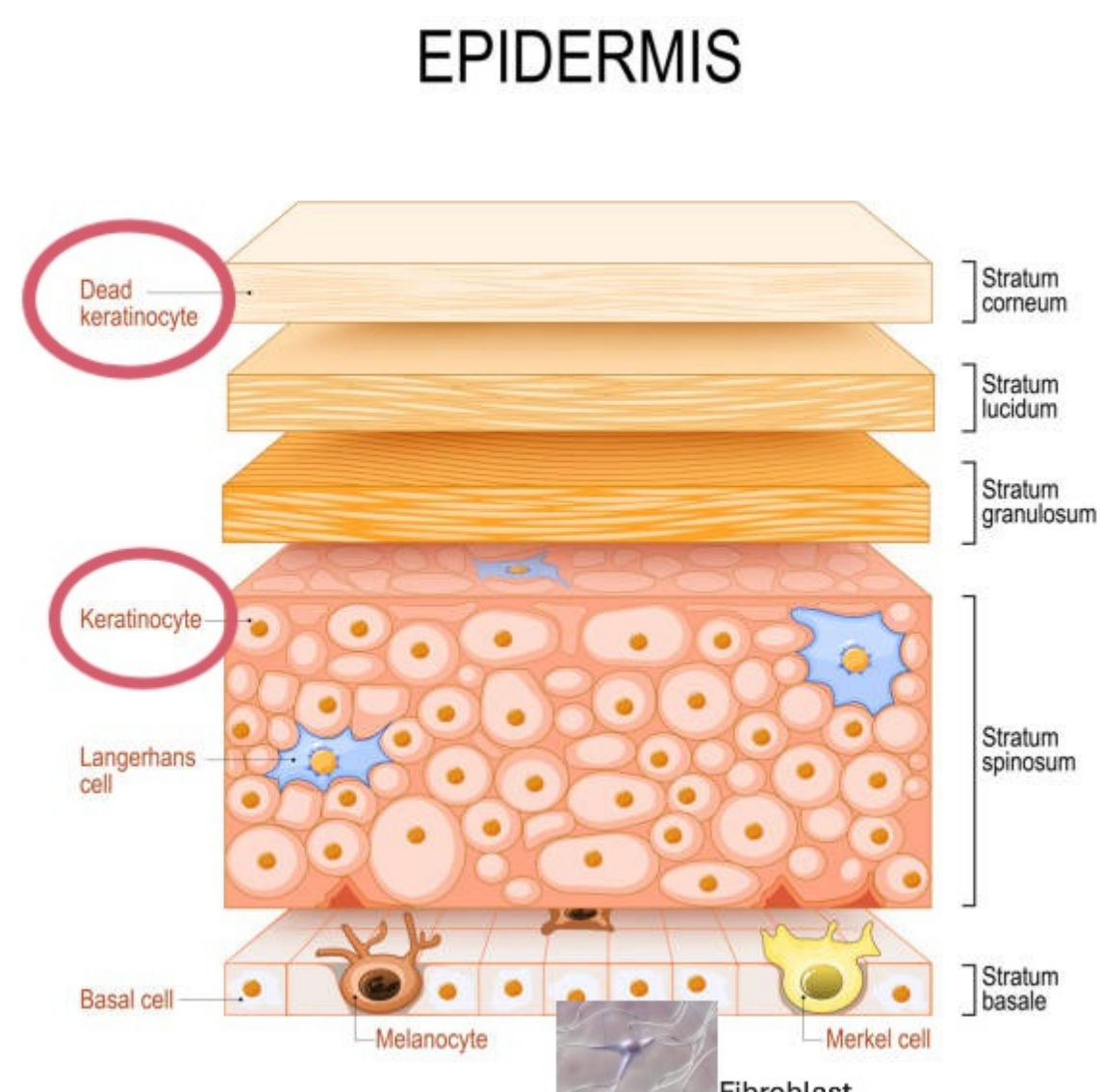


Figure 1. Structure of the human epidermis and localization of major skin cell types.

- Senescence is a stable growth arrest with heterogenous transcriptomes
- Dataset retrieved from Hernandez et al., 2017 where fibroblasts, keratinocytes and melanocytes were profiled at different timepoints post irradiation
- **Aim:** to identify potential senescence biomarkers in keratinocytes.

METHODS

- **Data & design:** RNA-seq raw counts from Hernandez et al., 2017, 6 reps per group, 78 in total
- **Pre-processing:**
 - removed lowly expressed genes (CPM ≥ 1 in ≥ 3 libraries)
 - normalisation (edgeR, TMM)
- **Model & differential expression:** GLM and QLF --> due to the negative binomial distribution of the dataset
- **Software**
 - R (v4.x), packages: edgeR, limma, pheatmap, ggplot2, dplyr, biomaRt, ggrepel

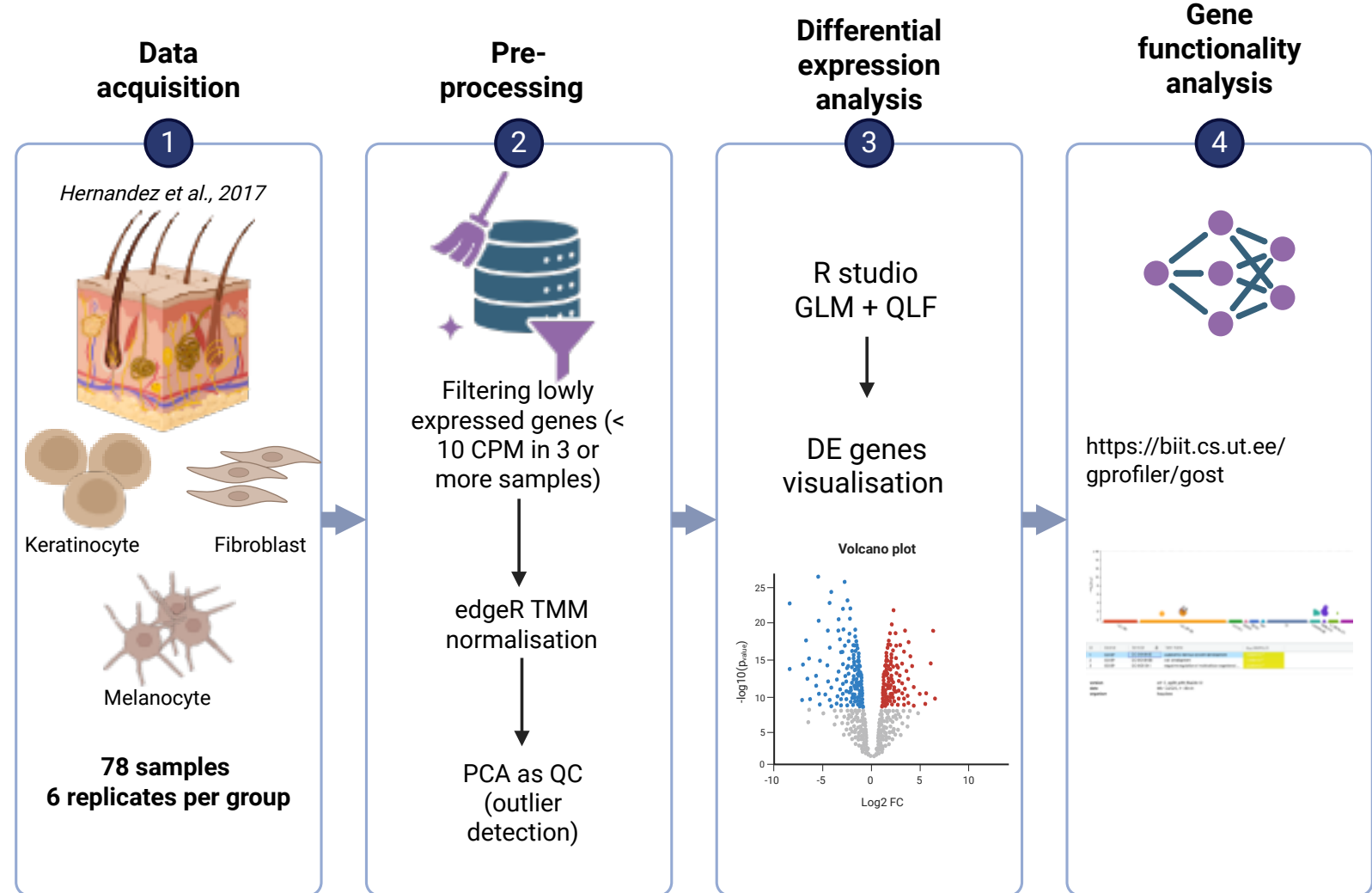


Figure 2. bioinformatics pipeline of our case study (created in biorender).

CONCLUSION

PTCHD4 can possibly be used as a biomarker for senescence in keratinocytes

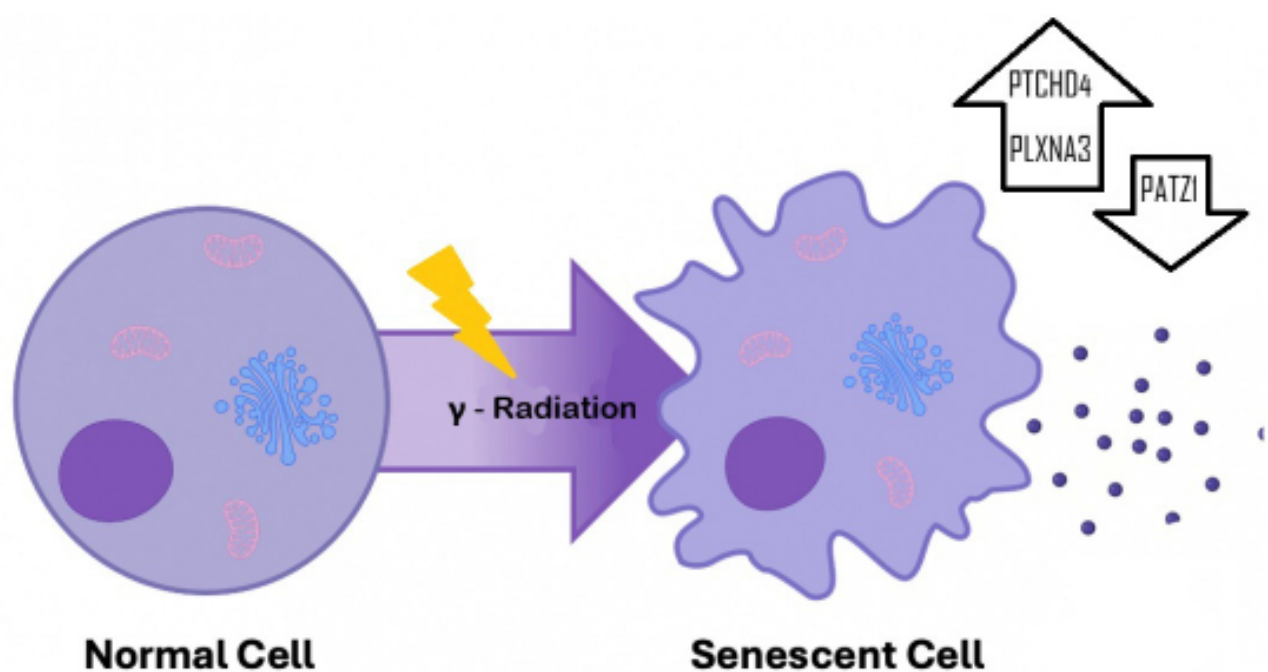


Figure 3. Induction of cellular senescence by γ -radiation and associated gene expression changes.

RESULTS

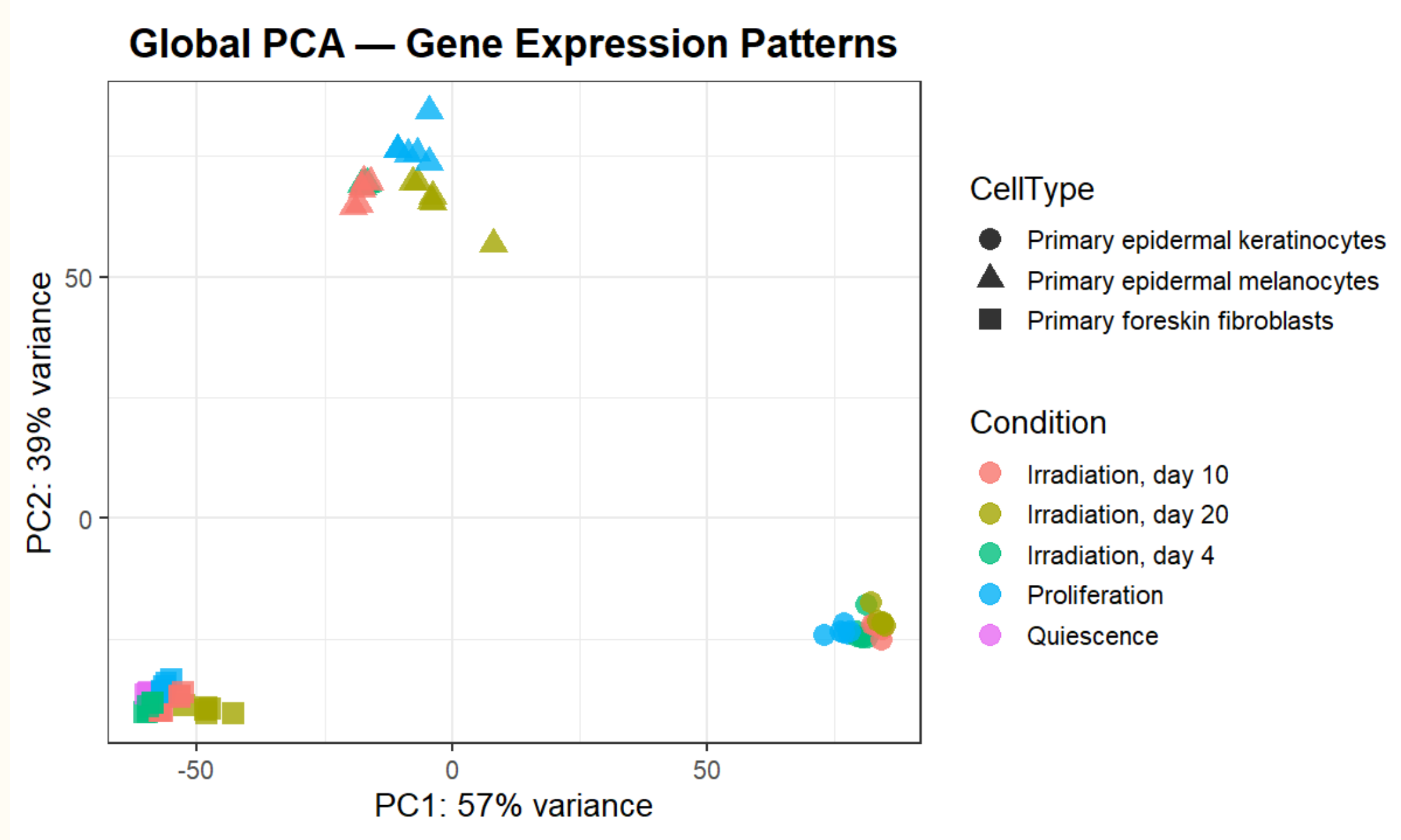


Figure 4. Principal Component Analysis (PCA) of global gene expression patterns across primary skin cell types and experimental conditions. PCA was performed on normalized gene expression values to visualize overall transcriptional relationships between samples. Each point represents one biological sample, colored by experimental condition and shaped by cell type. The first two principal components (PC1 and PC2) explain 57% and 39% of the total variance, respectively. Distinct clustering indicates that both cell type and irradiation time contribute strongly to transcriptional variation.

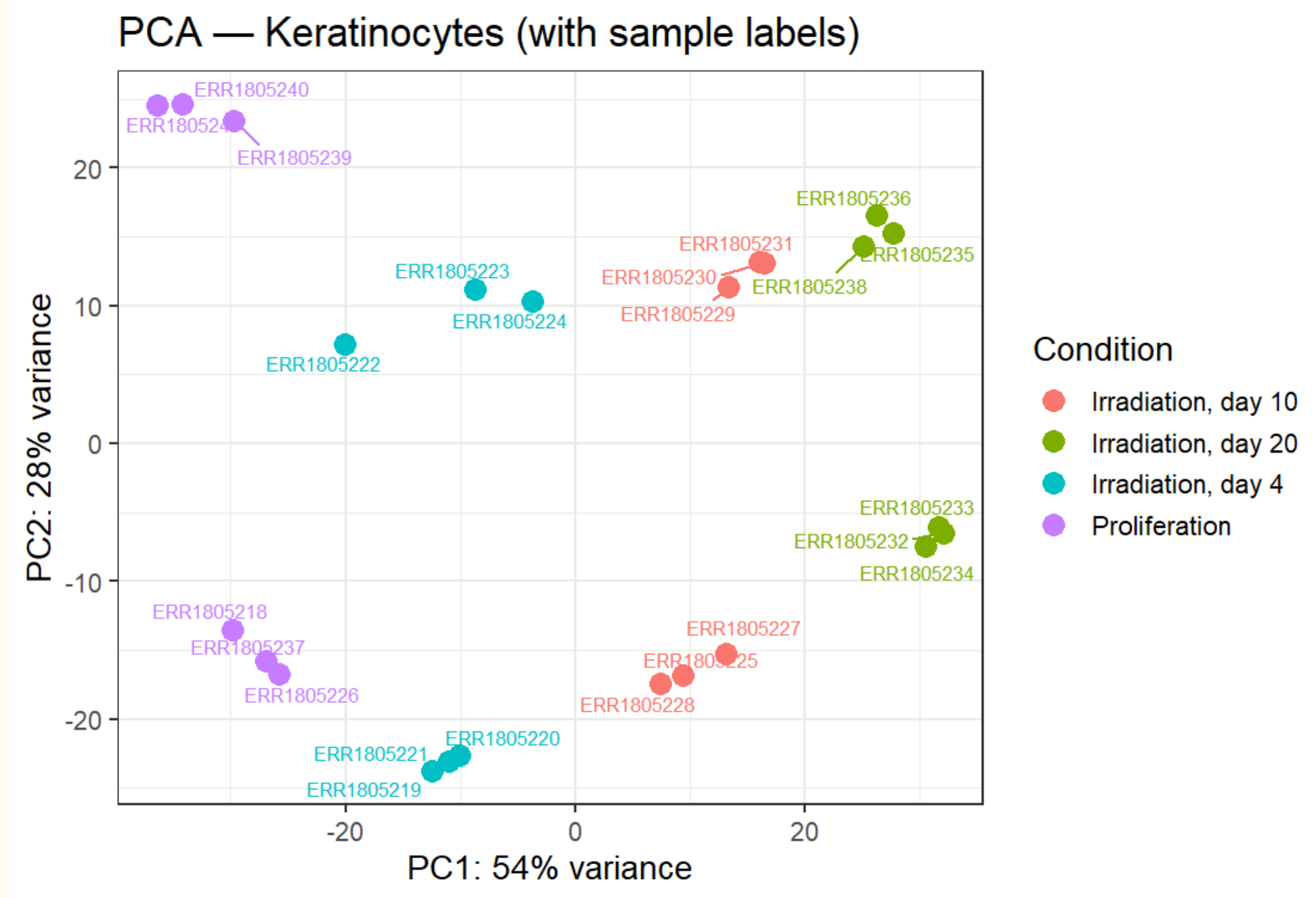


Figure 5. Temporal changes in keratinocyte transcription after irradiation. PCA shows a temporal trajectory of keratinocyte transcription. Proliferating cells cluster at one extreme, Day 4–10 samples occupy intermediate positions with mixed proliferative and stress-response genes, and Day 20 samples form a distinct cluster, reflecting stable senescence. Clusters are divided into two groups, likely due to batch effects arising from technical variation.

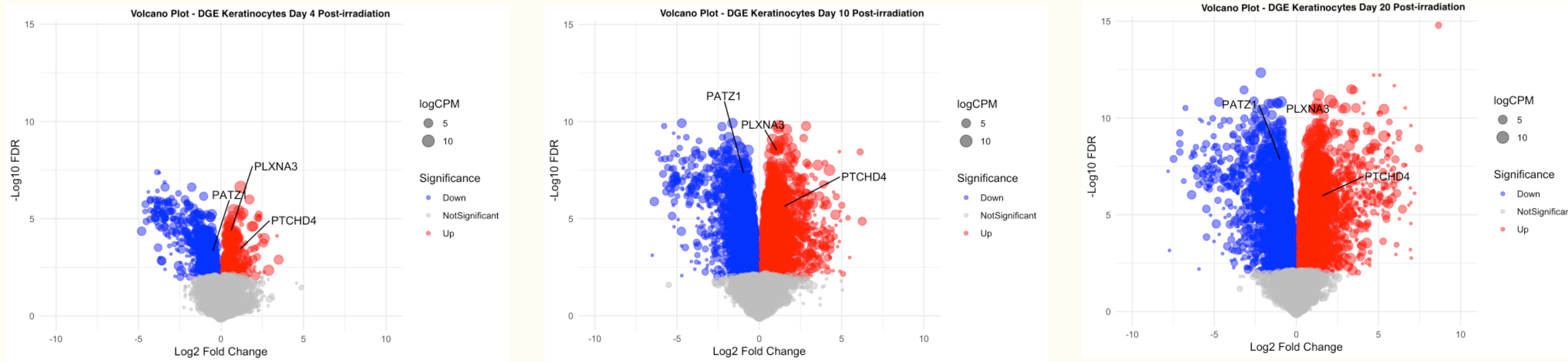


Figure 6. Volcano Plot of differential gene expression in keratinocytes at Day 4, 10 and 20 post-irradiation. The plot shows Log₂ Fold Change (x-axis) versus –Log₁₀ FDR (y-axis), representing the magnitude and significance of expression changes relative to Day 1 (proliferation). Red and blue points indicate significantly upregulated and downregulated genes, respectively, while grey points represent non-significant changes. Circle size represents gene expression level shown as logCPM.

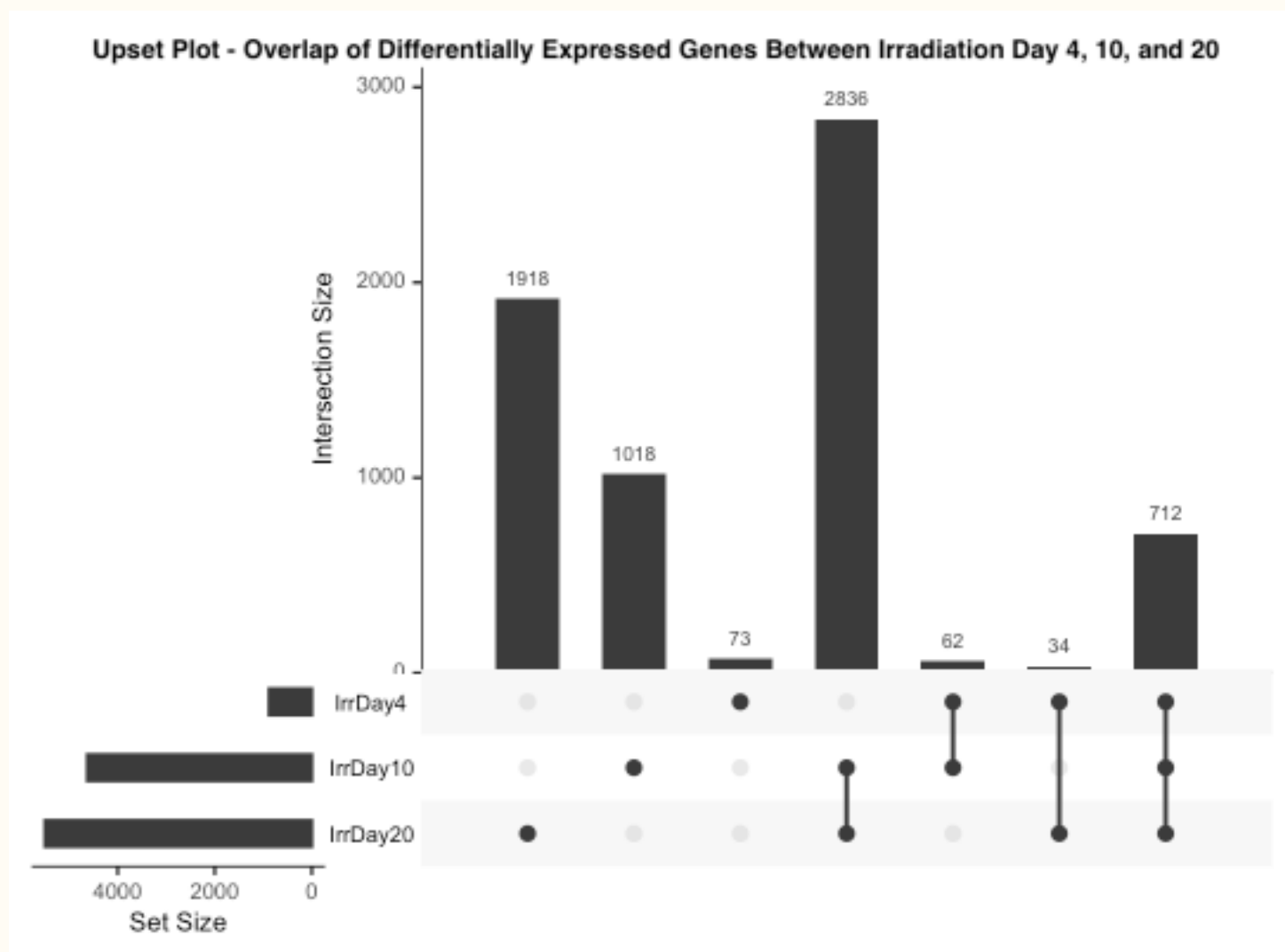


Figure 7. Upset Plot depicting the number of overlapping significantly differentially expressed genes across irradiation timepoints in keratinocytes. For selecting significantly expressed genes the FDR cut-off is 0.01.

- Differential gene expression increases with time post-irradiation
- PATZ1, PLXNA3, PTCHD4, are significantly differentially expressed, increasing in significance while maintaining similar expression levels
- These genes have been shown to be involved in senescence-linked pathways, supporting their role in inducing a senescence state in keratinocytes in response to radiation
- Days 10 and 20 post-irradiation show the most shared significantly differentially expressed genes
- Day 4 is the closest to proliferation state, shown by the low number of differentially expressed genes compared to the reference point

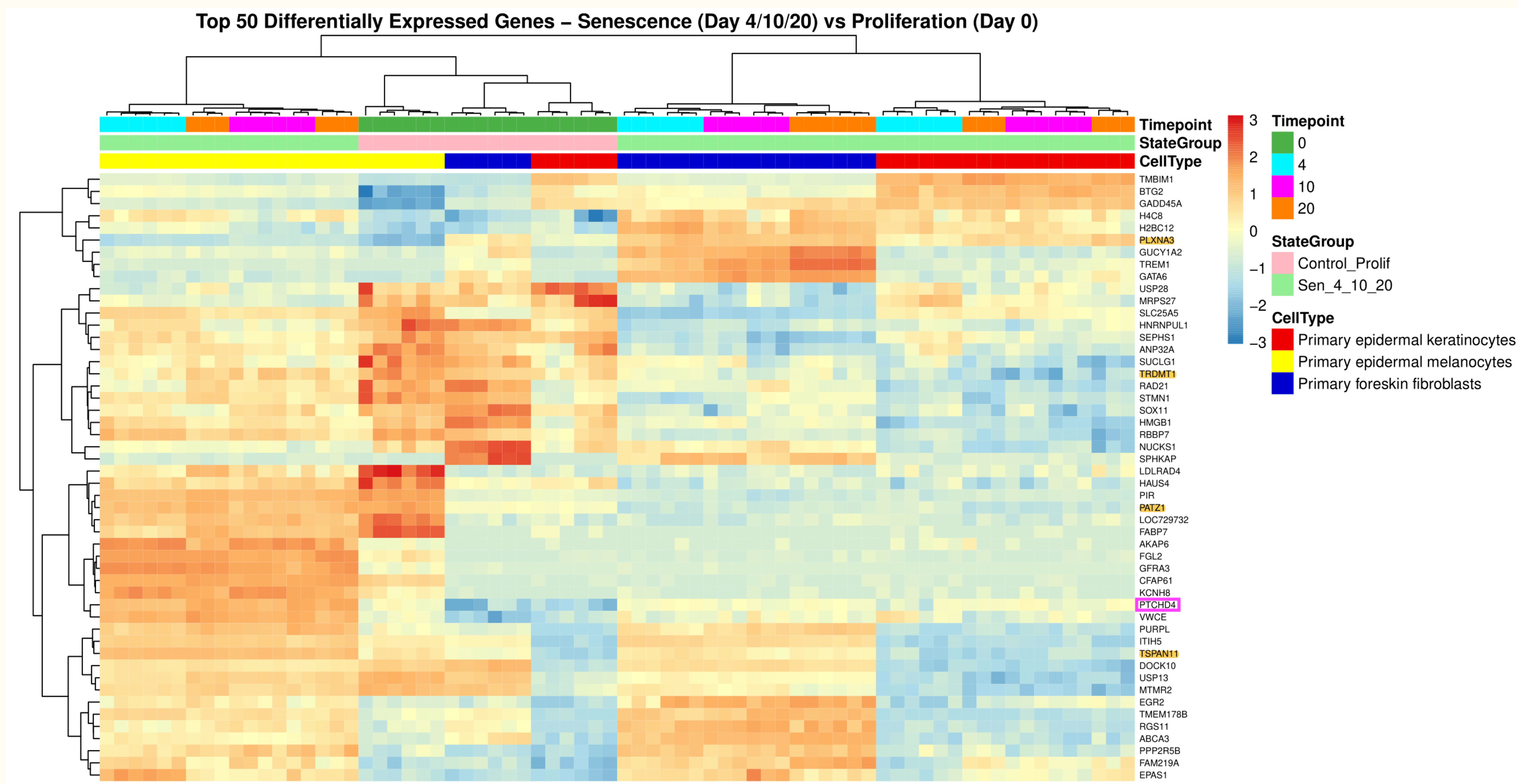


Figure 8. Heatmap of the top 50 significantly DE genes across the 3 different cell types (Melanocytes, Fibroblasts, Keratinocytes) in irradiated against proliferation state. Columns are clustered and annotated. Genes are shown in the rows, and columns are samples. Few notable senescence core genes are PATZ1, PLXNA3, PTCHD4, TRDMT1, TSPAN11. Z-score scaling: comparing the differences of each gene expression from its mean across all the samples.