Aim 1 Analysis: Characterizing The Dynamics of our Three Populations Using Transcriptomic Analysis

In this notebook, we will be completing our analysis of Days 7 - Day 13 of the CD34+ Cells in LEM.

The Research Questions of this Aim are:

1. Are There Unique Regions in Each of the 3 Populations of the GeneSpace?.
2. What is the genetic makeup of regions unique to a given population. Does this have functional implications?
3. Is There Statistically Significant Evidence of Dendritic Cell Commitment Among These Populations?
4. Is There Indications of Mast Cell Lineage Among These Populations?
5. Is There Statistically Significant Evidence of T-cell Activation Among Any Population?

Pre-Processing Workflow

Here, we begin by loading in necessary libraries, cleaning our data up to remove outliers from the UMAP space, and visualizing the general UMAP results.

Let's begin with loading in the necessary libraries. The comments indicate what each library allows us to do in this workflow.

In [1]:
```
# Loading Libraries

library(BiocSingular) # We need this to use the BioConductor libraries that work on
library(SingleCellExperiment) # We need this to use the SingleCellExperiment data s
library(ggplot2) # we need this to make ggplot visualizations #nolint
library(tidyr) # we need this to manipulate data #nolint
library(dplyr) # we need this to manipulate data #nolint
library(patchwork) # to display plots side by side. #nolint
library(ggforce) # Allows me to display circles on ggplots. #nolint
library(limma) # helps with differential expression analysis #nolint
library(IRdisplay) # lets me display JPEGs in the notebook #nolint
library(org.Hs.eg.db) # lets me do gene annotation #nolint
library(clusterProfiler) # lets me do gene set enrichment analysis #nolint

# library(scuttle)
# library(scran)
# library(scater)
```

```r
# library(scDblFinder)
# library(DropletUtils)
# library(DropletTestFiles)
# library(uwot)
# library(rtracklayer)
# library(PCAtools)
# library(celldex)
# library(SingleR)
# library(batchelor)
# library(bluster)
```

```
Loading required package: SummarizedExperiment

Loading required package: MatrixGenerics

Loading required package: matrixStats

Loading required package: MatrixGenerics

Loading required package: matrixStats


Attaching package: 'MatrixGenerics'


The following objects are masked from 'package:matrixStats':

    colAlls, colAnyNAs, colAnys, colAvgsPerRowSet, colCollapse,
    colCounts, colCummaxs, colCummins, colCumprods, colCumsums,
    colDiffs, colIQRDiffs, colIQRs, colLogSumExps, colMadDiffs,
    colMads, colMaxs, colMeans2, colMedians, colMins, colOrderStats,
    colProds, colQuantiles, colRanges, colRanks, colSdDiffs, colSds,
    colSums2, colTabulates, colVarDiffs, colVars, colWeightedMads,
    colWeightedMeans, colWeightedMedians, colWeightedSds,
    colWeightedVars, rowAlls, rowAnyNAs, rowAnys, rowAvgsPerColSet,
    rowCollapse, rowCounts, rowCummaxs, rowCummins, rowCumprods,
    rowCumsums, rowDiffs, rowIQRDiffs, rowIQRs, rowLogSumExps,
    rowMadDiffs, rowMads, rowMaxs, rowMeans2, rowMedians, rowMins,
    rowOrderStats, rowProds, rowQuantiles, rowRanges, rowRanks,
    rowSdDiffs, rowSds, rowSums2, rowTabulates, rowVarDiffs, rowVars,
    rowWeightedMads, rowWeightedMeans, rowWeightedMedians,
    rowWeightedSds, rowWeightedVars


Loading required package: GenomicRanges

Loading required package: stats4

Loading required package: BiocGenerics


Attaching package: 'BiocGenerics'


The following objects are masked from 'package:stats':

    IQR, mad, sd, var, xtabs


The following objects are masked from 'package:base':

    anyDuplicated, aperm, append, as.data.frame, basename, cbind,
    colnames, dirname, do.call, duplicated, eval, evalq, Filter, Find,
    get, grep, grepl, intersect, is.unsorted, lapply, Map, mapply,
    match, mget, order, paste, pmax, pmax.int, pmin, pmin.int,
    Position, rank, rbind, Reduce, rownames, sapply, saveRDS, setdiff,
    table, tapply, union, unique, unsplit, which.max, which.min
```

```
Loading required package: S4Vectors


Attaching package: 'S4Vectors'


The following object is masked from 'package:utils':

    findMatches


The following objects are masked from 'package:base':

    expand.grid, I, unname


Loading required package: IRanges


Attaching package: 'IRanges'


The following object is masked from 'package:grDevices':

    windows


Loading required package: GenomeInfoDb

Loading required package: Biobase

Welcome to Bioconductor

    Vignettes contain introductory material; view with
    'browseVignettes()'. To cite Bioconductor, see
    'citation("Biobase")', and for packages 'citation("pkgname")'.



Attaching package: 'Biobase'


The following object is masked from 'package:MatrixGenerics':

    rowMedians


The following objects are masked from 'package:matrixStats':

    anyMissing, rowMedians



Attaching package: 'tidyr'
```

```

The following object is masked from 'package:S4Vectors':

    expand


Attaching package: 'dplyr'

The following object is masked from 'package:Biobase':

    combine

The following objects are masked from 'package:GenomicRanges':

    intersect, setdiff, union

The following object is masked from 'package:GenomeInfoDb':

    intersect

The following objects are masked from 'package:IRanges':

    collapse, desc, intersect, setdiff, slice, union

The following objects are masked from 'package:S4Vectors':

    first, intersect, rename, setdiff, setequal, union

The following objects are masked from 'package:BiocGenerics':

    combine, intersect, setdiff, union

The following object is masked from 'package:matrixStats':

    count

The following objects are masked from 'package:stats':

    filter, lag

The following objects are masked from 'package:base':

    intersect, setdiff, setequal, union
```

```
Attaching package: 'limma'


The following object is masked from 'package:BiocGenerics':

    plotMA


Loading required package: AnnotationDbi


Attaching package: 'AnnotationDbi'


The following object is masked from 'package:dplyr':

    select





clusterProfiler v4.14.4 Learn more at https://yulab-smu.top/contribution-knowledge-m
ining/

Please cite:

T Wu, E Hu, S Xu, M Chen, P Guo, Z Dai, T Feng, L Zhou, W Tang, L Zhan,
X Fu, S Liu, X Bo, and G Yu. clusterProfiler 4.0: A universal
enrichment tool for interpreting omics data. The Innovation. 2021,
2(3):100141


Attaching package: 'clusterProfiler'


The following object is masked from 'package:AnnotationDbi':

    select


The following object is masked from 'package:IRanges':

    slice


The following object is masked from 'package:S4Vectors':

    rename


The following object is masked from 'package:stats':

    filter
```

Based on the process outlined in `d0_Analysis.ipynb`, I've created SingleCell Objects containing eexperimental data from the d7-d13 timepoints.

In [2]:
```
load("data/phenotype_with_ID.RData")
load("data/merge2.RData")
```

We have now loaded pre-processed merge2 data, and the associated phenotypes. Next, I will create an index linking cells to their phenotype. This will allow me to connect them to their flow_cytometry populations.

In [3]:
```
pheno.d7 <- rep("CD34+CD45RA-CLEC12A-", 3039)
names(pheno.d7) <- colnames(merge2)[1:3039]

pheno.merge2 <- c(pheno.d7, pheno.d10, pheno.d13)
```

The next step would be to complete PCA on our top genes. This has already be done, we simply need to access it using the `reducedDimNames` command.

In [4]:
```
# PCA has already been done on the top genes
reducedDimNames(merge2)
```

'PCA.cc' · 'UMAP.cc' · 'PCA.5k' · 'PCA.nocc' · 'UMAP.nocc' · 'TSNE.nocc' · 'TSNE.5k' · 'PCA' · 'TSNE'

Ok nice - next we add phenotype metadata

In [5]:
```
# Add phenotypes as a column in colData
colData(merge2)$Phenotype <- pheno.merge2
```

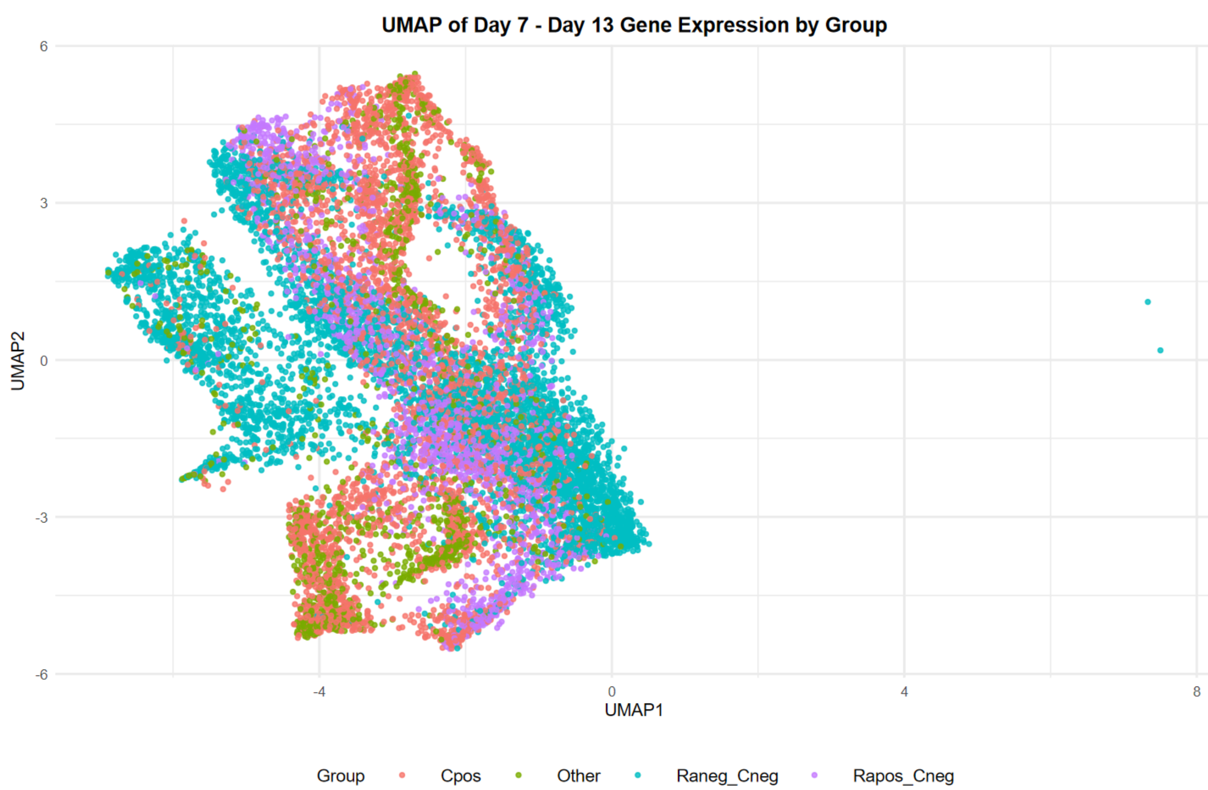
As we can see, there's too many phenotypes present here. Lets break the data into our 3 populations of interest, and ignore everything else as an `Other` category, to better understand our question.

In [6]:
```
# Establishing Population Groups

# Define phenotype groups
phenotype_groups <- list(
  Raneg_Cneg = c("CD34+CD45RA-CLEC12A-", "CD34-CD45RA-CLEC12A-"), # Ra-C-
  Rapos_Cneg = c("CD34+CD45RA+CLEC12A-", "CD34-CD45RA+CLEC12A-"), # Ra+C-
  Cpos = c("CD34-CD45RA-CLEC12A+", "CD34+CD45RA-CLEC12A+", "CD34+CD45RA+CLEC12A+",
  Other = c("CD10+", "CD14CD15+") # Pro -B #Pro-NM #FW Gating from a flow cytometer
)

# Assign group labels to phenotypes
group_labels <- sapply(pheno.merge2, function(phenotype) {
  group <- names(phenotype_groups)[sapply(phenotype_groups, function(g) phenotype %
  if (length(group) > 0) group else "Other"
})
```

```
# Add group labels to colData of the SCE object
colData(merge2)$Group <- group_labels
```

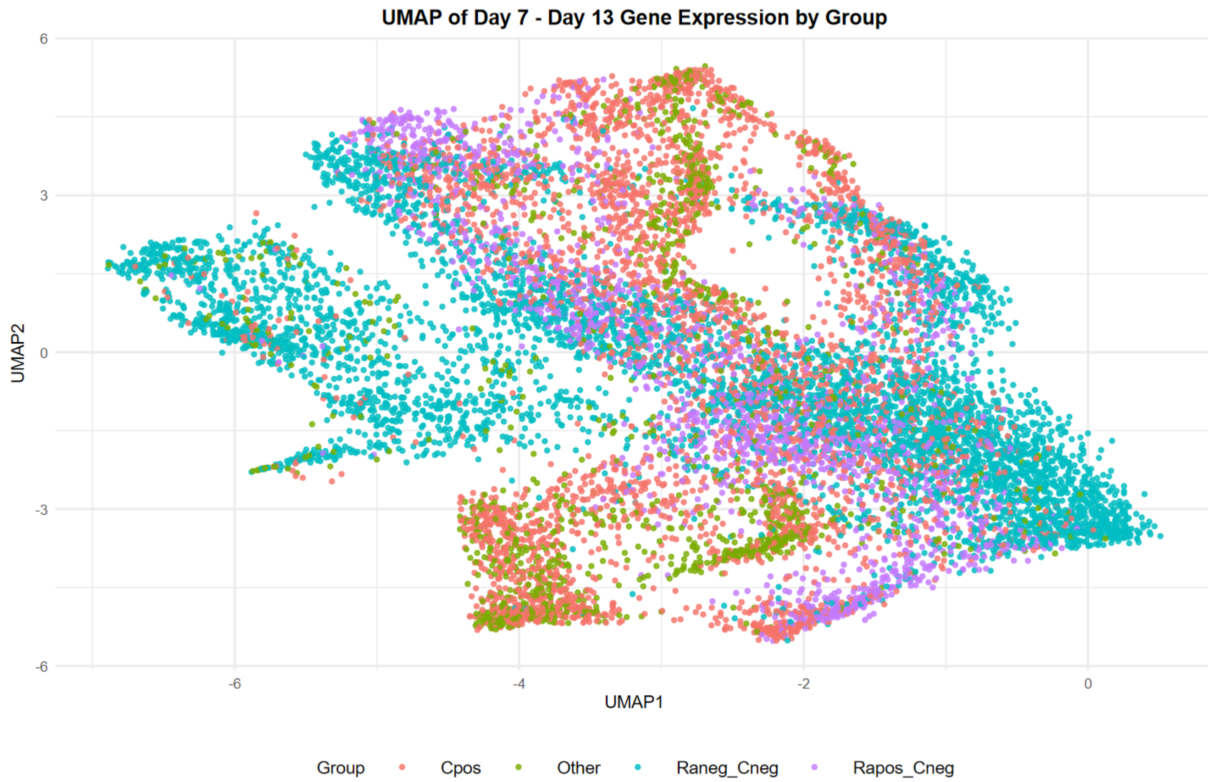
Nice! We can now begin visualizing the UMAP using the `ggplot` package.

In [7]:
```
options(repr.plot.width = 12, repr.plot.height = 8)

umap_df <- reducedDim(merge2, "UMAP.cc") %>%
  as.data.frame() %>%
  mutate(Group = merge2$Group)


gg_umap <- ggplot(umap_df, aes(x = UMAP1, y = UMAP2, color = Group)) +
  geom_point(alpha = 0.8, size = 1) +
  labs(
    title = "UMAP of Day 7 - Day 13 Gene Expression by Group",
    x = "UMAP1",
    y = "UMAP2",
    color = "Group"
  ) +
  theme_minimal(base_size = 12) +
  theme(
    plot.title = element_text(hjust = 0.5, face = "bold"),
    legend.position = "bottom",
    legend.text = element_text(size = 12), # Increase legend text size
    legend.key.size = unit(1.5, "cm") # Increase legend color box size
  )

# Print the UMAP plot
print(gg_umap)
```



It appears that we have to outliers, with a value exceedin `UMAP1 = 4` on the x-axis. Lets get rid of these, and then continue with our analysis.

In [8]:
```
# Identify non-outlier cells
valid_cells <- which(reducedDim(merge2, "UMAP.cc")[, 1] < 4) # Filtering UMAP1 < 4

# Subset SingleCellExperiment object to keep only valid cells
merge2_clean <- merge2[, valid_cells]

# Confirming we get the sampe plot
umap_df <- reducedDim(merge2_clean, "UMAP.cc") %>%
  as.data.frame() %>%
  mutate(Group = merge2_clean$Group)


gg_umap <- ggplot(umap_df, aes(x = UMAP1, y = UMAP2, color = Group)) +
  geom_point(alpha = 0.8, size = 1) +
  labs(
    title = "UMAP of Day 7 - Day 13 Gene Expression by Group",
    x = "UMAP1",
    y = "UMAP2",
    color = "Group"
  ) +
  theme_minimal(base_size = 12) +
  theme(
    plot.title = element_text(hjust = 0.5, face = "bold"),
    legend.position = "bottom",
    legend.text = element_text(size = 12), # Increase legend text size
    legend.key.size = unit(1.5, "cm") # Increase legend color box size
  )
gg_umap
```



Next, lets add a column delineating which day each cell belongs to, so that we may analyze the differences in the three populations between each time point as well.

In [9]:
```
# Extract the day information from cell names
colData(merge2_clean)$Day <- gsub(".*Day_([0-9]+).*", "\\1", rownames(colData(merge
# Convert to a factor (optional, for better categorical handling)
colData(merge2_clean)$Day <- factor(colData(merge2_clean)$Day, levels = sort(unique

colnames(colData(merge2_clean))
```

'Sample' · 'Barcode' · 'sum' · 'detected' · 'subsets_Mito_sum' · 'subsets_Mito_detected' · 'subsets_Mito_percent' · 'altexps_Antibody Capture_sum' · 'altexps_Antibody Capture_detected' · 'altexps_Antibody Capture_percent' · 'total' · 'sizeFactor' · 'label' · 'scDblFinder.cluster' · 'scDblFinder.class' · 'scDblFinder.score' · 'scDblFinder.weighted' · 'scDblFinder.difficulty' · 'scDblFinder.cxds_score' · 'scDblFinder.mostLikelyOrigin' · 'scDblFinder.originAmbiguous' · 'batch' · 'Phenotype' · 'Group' · 'Day'

Analysis Workflow

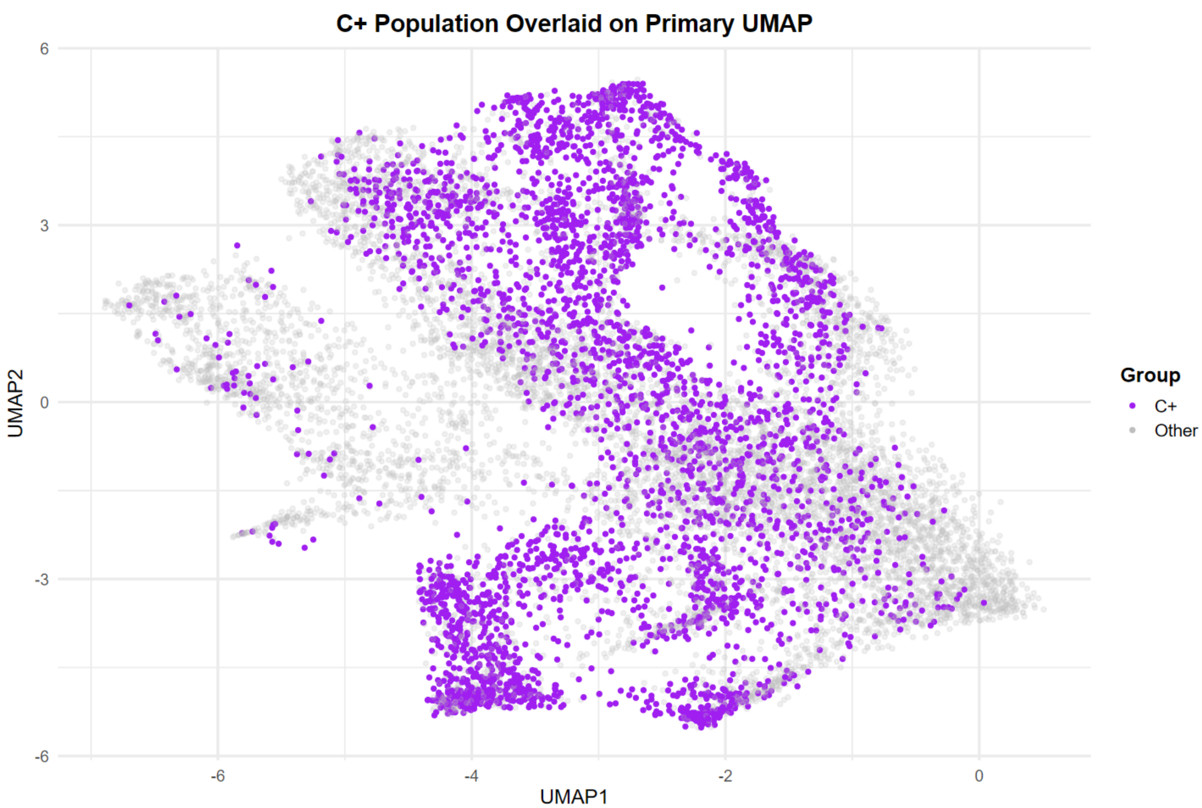
RQ 1: Are There Unique Regions in Each of the 3 Populations of the GeneSpace?

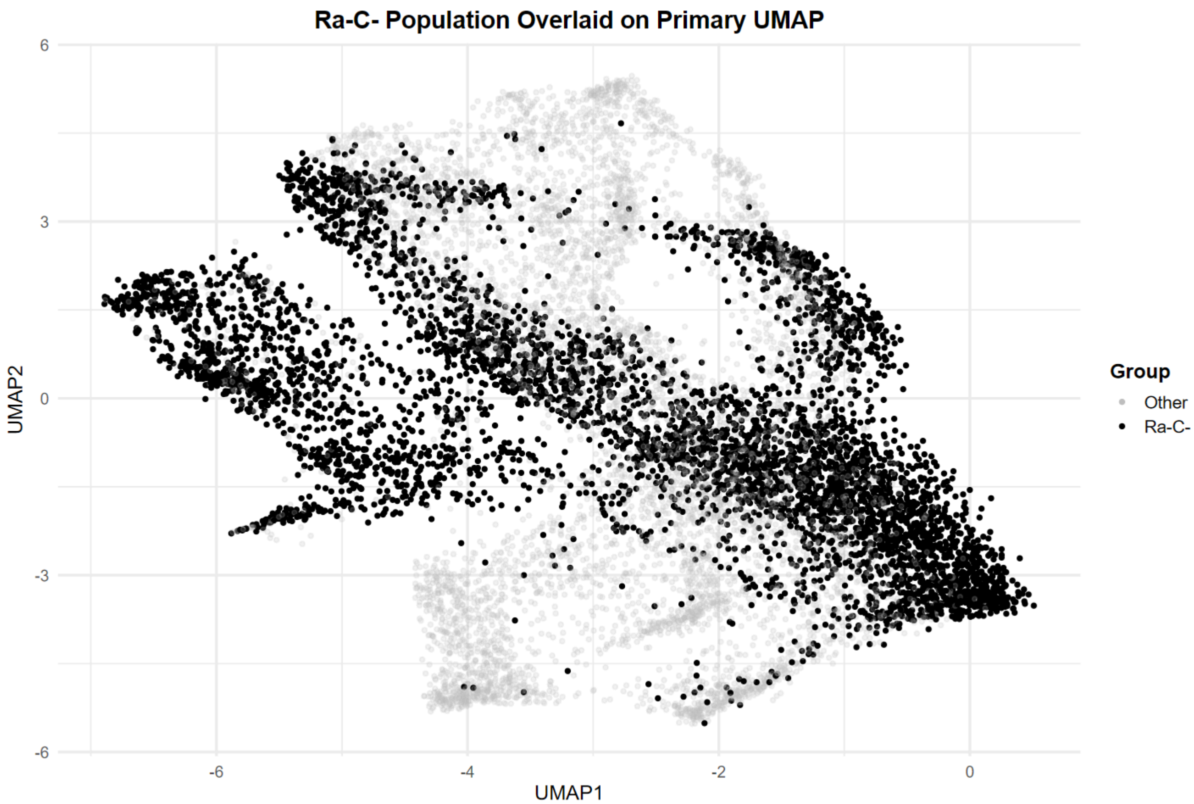
In this section, we will be looking at how the three populations are similiar, and how they are different, at a genespace level. The goal is to especially establish the changes in cell fate differences in the 3 populations.

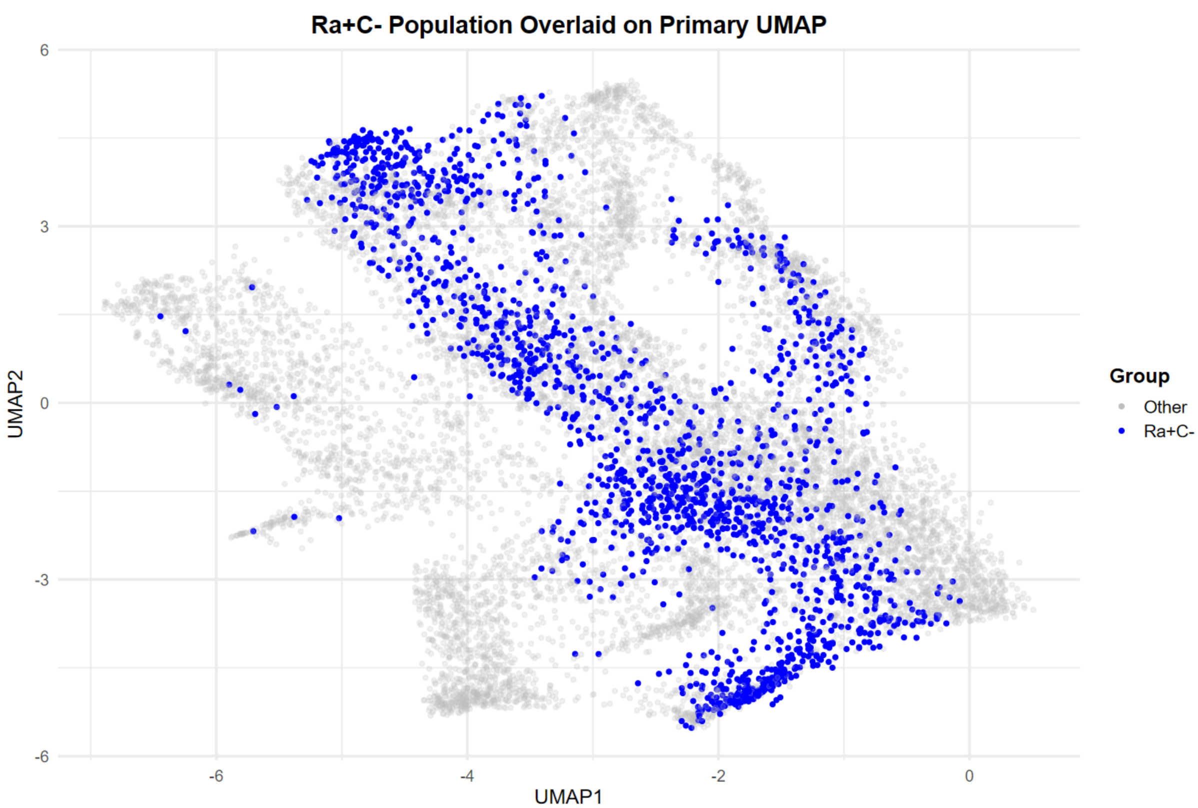
Lets begin by visualizing the 3 populations in the genespace, and look for qualitativie difference and similiarities in their population spread.

In [10]:
```
options(repr.plot.width = 12, repr.plot.height = 8)

gg_AllDay_AllPop <- gg_umap

gg_Cpos <- ggplot(umap_df, aes(x = UMAP1, y = UMAP2)) +
  geom_point(aes(color = ifelse(Group == "Cpos", "C+", "Other")),
    alpha = ifelse(umap_df$Group == "Cpos", 1, 0.2), size = 1
  ) +
  scale_color_manual(
    values = c("C+" = "purple", "Other" = "gray"),
    name = "Group"
  ) +
  labs(
    title = "C+ Population Overlaid on Primary UMAP",
    x = "UMAP1",
    y = "UMAP2"
  ) +
  theme_minimal(base_size = 14) +
  theme(
    plot.title = element_text(hjust = 0.5, face = "bold"),
    legend.position = "right",
    legend.text = element_text(size = 12),
    legend.title = element_text(size = 14, face = "bold")
  )

gg_RanegCneg <- ggplot(umap_df, aes(x = UMAP1, y = UMAP2)) +
  geom_point(aes(color = ifelse(Group == "Raneg_Cneg", "Ra-C-", "Other")),
    alpha = ifelse(umap_df$Group == "Raneg_Cneg", 1, 0.2), size = 1
  ) +
  scale_color_manual(
    values = c("Ra-C-" = "black", "Other" = "gray"),
    name = "Group"
  ) +
  labs(
    title = "Ra-C- Population Overlaid on Primary UMAP",
    x = "UMAP1",
    y = "UMAP2"
  ) +
  theme_minimal(base_size = 14) +
  theme(
    plot.title = element_text(hjust = 0.5, face = "bold"),
    legend.position = "right",
    legend.text = element_text(size = 12),
    legend.title = element_text(size = 14, face = "bold")
  ) #+
# scale_x_continuous(breaks = seq(-7, 1, by = 1)) + # More x-axis ticks
# scale_y_continuous(breaks = seq(-7, 6, by = 1)) # More y-axis ticks


gg_RaposCneg <- ggplot(umap_df, aes(x = UMAP1, y = UMAP2)) +
  geom_point(aes(color = ifelse(Group == "Rapos_Cneg", "Ra+C-", "Other")),
    alpha = ifelse(umap_df$Group == "Rapos_Cneg", 1, 0.2), size = 1
```

```
  ) +
  scale_color_manual(
    values = c("Ra+C-" = "blue", "Other" = "gray"),
    name = "Group"
  ) +
  labs(
    title = "Ra+C- Population Overlaid on Primary UMAP",
    x = "UMAP1",
    y = "UMAP2"
  ) +
  theme_minimal(base_size = 14) +
  theme(
    plot.title = element_text(hjust = 0.5, face = "bold"),
    legend.position = "right",
    legend.text = element_text(size = 12),
    legend.title = element_text(size = 14, face = "bold")
  )


# Print the UMAP plot
print(gg_Cpos)
print(gg_RanegCneg)
print(gg_RaposCneg)
```



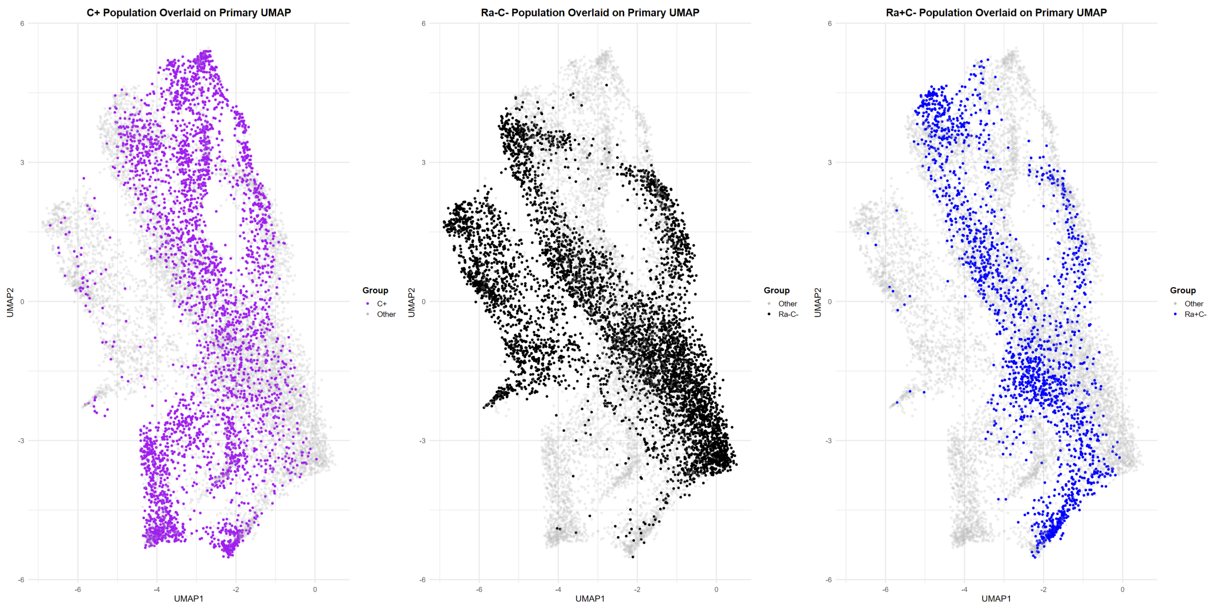




Nice! Lets now create a faceted visualization, showing the distribution of the 3 populations overlaid in the same UMAP.

In [11]:
```
# Makes the plot nice and wide.
options(repr.plot.width = 28, repr.plot.height = 14)
```

```r
# Combine two plots side by side
gg_combined <- gg_Cpos + gg_RanegCneg + gg_RaposCneg + plot_layout(ncol = 3)

# Print the combined plot
print(gg_combined)
```



Very interesting, what are some take-aways we can see from this distribution. Perhaps we can manually subset groups and complete DGE on them, to look for more pronounced differences between groups:

1. All groups have some homogeneity along the diagonal, which is their primary region of overlap.

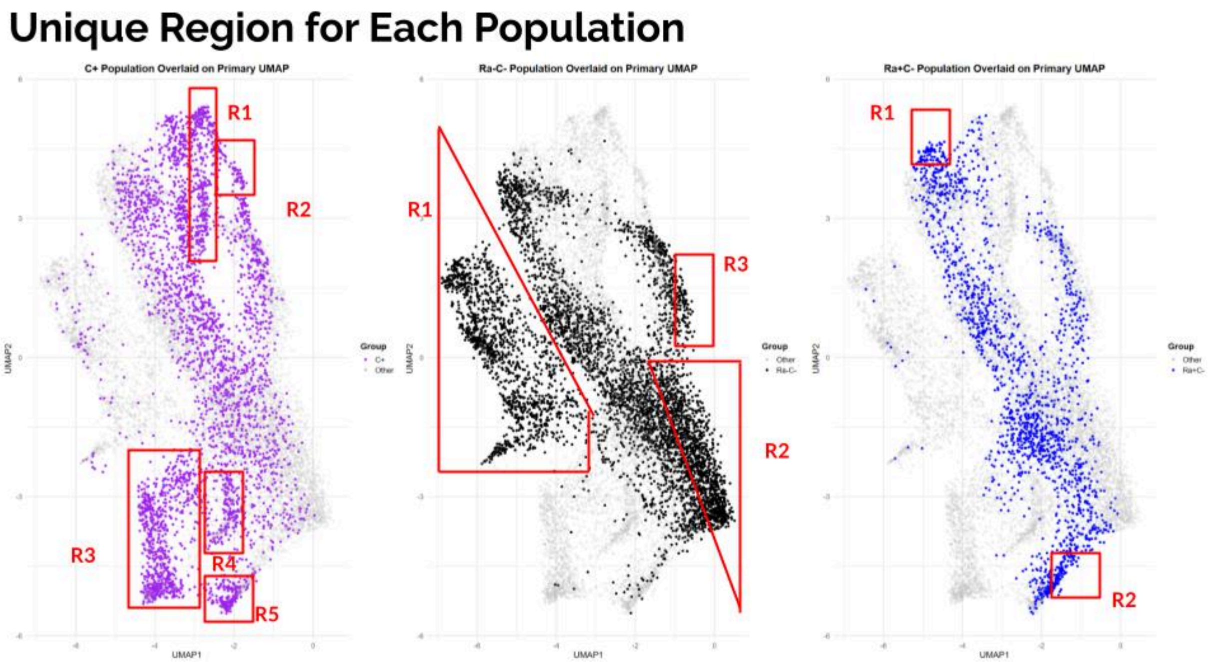
2. At the bottom left, the island is primarily C+ cells. That area is dominated by the C+ cells, and nothing else. Hence, it appears to be a near homogenous C+ population that exists there, within this dataset. Similiarly, the peak at the top of the plot is also dominated by C+ cells, and could be another subset to look at when completing DGE.

3. The larger island, on a diagonal from -4 to -7, seems to be primarily occuped by the Ra-C- population. Additionally, the bottom right peak also seems to be enriched for this population.

4. The small long island at the bottom is very enriched for Ra+C- cells, but the lower half has some overlap with C+ population. Additionally, there is a small enrichment in the top left, which is unique to the population, but it is limited.

Heres's the regions of interest highlighted with circles:

In [12]:
```r
display_jpeg(file = "img/3Unique.jpg")
```



RQ2 : What is the Genetic Makeup of Regions Unique to Each Population. Does it Have Functional Implications?

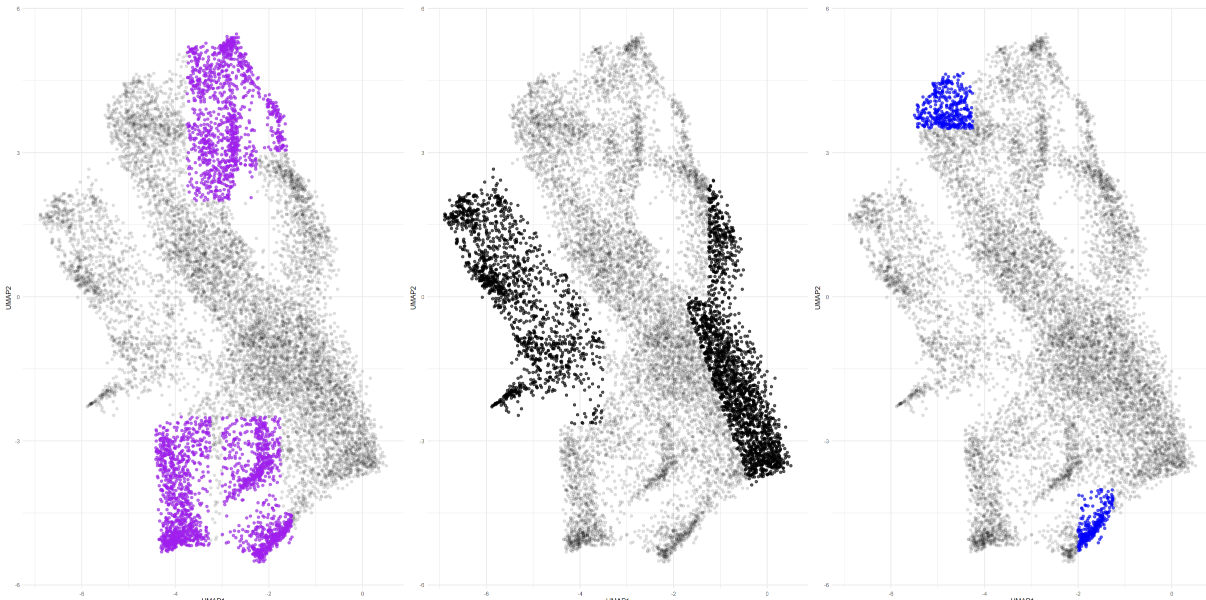
Now that we have identified these unique regions, lets take a look at what genes are over-expressed in this locations, compared to the rest of the UMAP. This will give some hints into what is functionally unique about these cells within each population - perhaps pointing to the existance of a subcluster/subpopulation with a linked fate.

To begin, we must modify our `Merg2` sce object to only contain cells that belong to these populations. Lets do that below:

In [13]:
```
options(repr.plot.width = 12, repr.plot.height = 8)

## Segmenting Unique CPos Cells
CPos_R1_pooled <- rownames(subset(umap_df, UMAP1 > -3.75 & UMAP1 < -2.25 &
  UMAP2 > 2 & UMAP2 < 6)) # nolint Square region R1
CPos_R2_pooled <- rownames(subset(umap_df, UMAP1 > -2.5 & UMAP1 < -1.5 &
  UMAP2 > 3 & UMAP2 < 5)) # nolint Square region R2
CPos_R3_pooled <- rownames(subset(umap_df, UMAP1 > -4.5 & UMAP1 < -3.25 &
  UMAP2 > -5.5 & UMAP2 < -2.5)) # nolint Square region R3
CPos_R4_pooled <- rownames(subset(umap_df, UMAP1 < -1.75 & UMAP1 > -3 &
  -4.5 < UMAP2 & UMAP2 < -2.5)) # nolint Square region R4
CPos_R5_pooled <- rownames(subset(umap_df, UMAP1 > -3 & UMAP1 < -1.5 &
  -5.75 < UMAP2 & UMAP2 < -4.5)) # nolint Square region R5

# Combine all unique C+ Cells into one vector
Cpos_unique_pooled <- unique(c(
  CPos_R1_pooled, CPos_R2_pooled,
  CPos_R3_pooled, CPos_R4_pooled, CPos_R5_pooled
))

# Subset the SCE object to retain only cells in any of the selected squares
```

```r
merge2_CPosUnique <- merge2_clean[, Cpos_unique_pooled]

# Visualizing the unique C+ cells
Cpos_subset_plot <- ggplot(umap_df, aes(x = UMAP1, y = UMAP2)) +
  geom_point(alpha = 0.1) + # Lightly plot all cells
  geom_point(data = umap_df[Cpos_unique_pooled, ], color = "purple", alpha = 0.6) +
  theme_minimal()



## Segmenting Unique Ra-C- Cells
RanegCneg_R1_pooled <- rownames(subset(umap_df, UMAP1 > -7 & UMAP1 < -3.5 &
  UMAP2 > -2.65 & UMAP2 < (-1.5 * UMAP1 + (-6.0)))) # nolint Square region R1
RanegCneg_R2_pooled <- rownames(subset(
  umap_df,
  UMAP2 < 0 & UMAP2 > (-2.8 * UMAP1 + (-5.0))
)) # nolint Square region R2
RanegCneg_R3_pooled <- rownames(subset(umap_df, UMAP1 > -1.25 & UMAP1 < 0.5 &
  UMAP2 > 0 & UMAP2 < 3)) # nolint Square region R3

# Combine all unique Ra-C- Cells into one vector
RanegCneg_unique_pooled <- unique(c(
  RanegCneg_R1_pooled, RanegCneg_R2_pooled,
  RanegCneg_R3_pooled
))

# Subset the SCE object to retain only cells in any of the selected squares
merge2_RanegCnegUnique <- merge2_clean[, RanegCneg_unique_pooled]

# Visualizing the unique Ra-C- cells
Raneg_Cneg_subset_plot <- ggplot(umap_df, aes(x = UMAP1, y = UMAP2)) +
  geom_point(alpha = 0.1) + # Lightly plot all cells
  geom_point(data = umap_df[RanegCneg_unique_pooled, ], color = "black", alpha = 0.
  theme_minimal()



## Segmenting Unique Ra+C- Cells
RaposCneg_R1_pooled <- rownames(subset(umap_df, UMAP1 > -5.5 & UMAP1 < -4.25 &
  UMAP2 > 3.5 & UMAP2 < 5.5)) # nolint Square region R1
RaposCneg_R2_pooled <- rownames(subset(umap_df, UMAP1 > -2 & UMAP1 < -1.25 &
  UMAP2 > -5.5 & UMAP2 < -4)) # nolint Square region R1

# Combine all unique Ra+C- Cells into one vector
RaposCneg_unique_pooled <- unique(c(
  RaposCneg_R1_pooled, RaposCneg_R2_pooled
))

# Subset the SCE object to retain only cells in any of the selected squares
merge2_RaposCnegUnique <- merge2_clean[, RaposCneg_unique_pooled]

# Visualizing the unique Ra+C- cells
Rapos_Cneg_subset_plot <- ggplot(umap_df, aes(x = UMAP1, y = UMAP2)) +
  geom_point(alpha = 0.1) + # Lightly plot all cells
  geom_point(data = umap_df[RaposCneg_unique_pooled, ], color = "blue", alpha = 0.6
  theme_minimal()
```

```r
# Plotting 3 Subsets side by side
options(repr.plot.width = 28, repr.plot.height = 14)

# Combine two plots side by side
gg_uniquePops_combined <- Cpos_subset_plot + Raneg_Cneg_subset_plot + Rapos_Cneg_su

# Print the combined plot
print(gg_uniquePops_combined)
```



In [14]:
```r
# Initialize the Region column with a default value of "None"
colData(merge2_clean)$Region <- "None"

# Combine all Regions cell names into a single vector
all_R1_cells <- unique(c(RaposCneg_R1_pooled, RanegCneg_R1_pooled, CPos_R1_pooled))
all_R2_cells <- unique(c(RaposCneg_R2_pooled, RanegCneg_R2_pooled, CPos_R2_pooled))
all_R3_cells <- unique(c(RanegCneg_R3_pooled, CPos_R3_pooled))
all_R4_cells <- unique(CPos_R4_pooled)
all_R5_cells <- unique(CPos_R5_pooled)


# Ensure that we're only using valid cell names
valid_R1_cells <- intersect(all_R1_cells, colnames(merge2_clean))
valid_R2_cells <- intersect(all_R2_cells, colnames(merge2_clean))
valid_R3_cells <- intersect(all_R3_cells, colnames(merge2_clean))
valid_R4_cells <- intersect(all_R4_cells, colnames(merge2_clean))
valid_R5_cells <- intersect(all_R5_cells, colnames(merge2_clean))


# Assign "R1" to all valid R1 cells in the Region column
colData(merge2_clean)$Region[colnames(merge2_clean) %in% valid_R1_cells] <- "R1"
colData(merge2_clean)$Region[colnames(merge2_clean) %in% valid_R2_cells] <- "R2"
colData(merge2_clean)$Region[colnames(merge2_clean) %in% valid_R3_cells] <- "R3"
colData(merge2_clean)$Region[colnames(merge2_clean) %in% valid_R4_cells] <- "R4"
colData(merge2_clean)$Region[colnames(merge2_clean) %in% valid_R5_cells] <- "R5"
```

```
# Verify the result
table(colData(merge2_clean)$Region)
```

```
None   R1   R2   R3   R4   R5
5125 2882 1961 1316  511  384
```

Additionally, we will repeatedly be comparing different regions and different populations against each other. Instead of writing the same script over and over again, I have defined the function `perform_dge` to do this work for me in one location, saving space in this notebook.

Here is some documentation regarding this function:

Arugments: The function has the capacity to take up to 5 arguments at maximum, and 3 arugments at minimum:

- `sce_object` : An sce object (in this analysis, merge2_clean) containing the experimental CITE-Seq data.
- `target_pop` : One of the populations you want to compare.
- `target_region` : The region in the target population that you want to compare
- `comp_pop` : *Optional* - the other population you want to compare to.
- `comp_region` : *Optional* - theo ther population's region you want to compare to.

Note: Only the first 3 arguments are necessary. If only these are passed, the function will compare the target region to the remaining cells within the target population.

Finally, the function will return a list containing the **top genes** that are differentially expressed, and **top CD marker associated genes** that are differentially expresseed.

As a reminder, here is a table containing all the populations and their regions of interest, as defined above:

Population | Regions | Description| ---------------|-------------|------------------| "Raneg_Cneg"| "R1" , "R2" , "R3"| CD45Ra-Clec12A- population "Rapos_Cneg"| "R1" , "R2" | CD45Ra+Clec12A- population "Cpos" | "R1", "R2", "R3", "R4", "R5" | Clec12A+ population

In [15]:
```
perform_dge <- function(sce_object, target_pop, target_region, comp_pop = NULL, com
  # Identify target cells
  target_cells <- colnames(sce_object)[colData(sce_object)$Group == target_pop]
  sce_target <- sce_object[, target_cells]

  # Identify cells within the target region
  target_region_cells <- colnames(sce_target)[colData(sce_target)$Region == target_

  # Determine comparison group
  if (!is.null(comp_pop) && !is.null(comp_region)) {
    comp_cells <- colnames(sce_object)[colData(sce_object)$Group == comp_pop]
    sce_comp <- sce_object[, comp_cells]
    comp_region_cells <- colnames(sce_comp)[colData(sce_comp)$Region == comp_region
    comparison_cells <- c(target_region_cells, comp_region_cells)
    colData(sce_target)$ComparisonGroup <- ifelse(colnames(sce_target) %in% target_
```

```
      target_region, "Other"
    )
  } else {
    colData(sce_target)$ComparisonGroup <- ifelse(colnames(sce_target) %in% target_
      target_region, "Other"
    )
  }

  # Extract logcounts assay
  expr_matrix <- assay(sce_target, "logcounts")

  # Create a design matrix
  design <- model.matrix(~ 0 + colData(sce_target)$ComparisonGroup)
  colnames(design) <- levels(factor(colData(sce_target)$ComparisonGroup))

  # Fit the linear model
  fit <- lmFit(expr_matrix, design)

  # Define the contrast
  contrast <- makeContrasts(contrasts = paste0(target_region, "-Other"), levels = d
  fit2 <- contrasts.fit(fit, contrast)
  fit2 <- eBayes(fit2)


  # Extract top genes
  top_genes <- topTable(fit2, coef = 1, number = Inf, ) %>% # since I am always onl
    filter(P.Value < 0.05) %>% # Filter by p-value
    arrange(desc(abs(logFC))) # Sort by Log-Fold Change

  # Extract top CD genes
  cd_genes <- top_genes[grep("CD", top_genes$ID), ]
  excluded_patterns <- "CDK|CDC|PCD|LCD|CDR|NCD|CCD|TCD|OCD|3CD|DCD|KCD"
  cd_genes <- cd_genes[!grepl(excluded_patterns, cd_genes$ID), ]

  # Return the results as a list
  return(list(top_genes = as.data.frame(top_genes), top_cd_genes = as.data.frame(cd
}
```

Characterizing Region 1 (R1) of the Ra+C- Population

In this section we will be answering the question `What makes the Ra+C- Region 1 (R1) cells different from the remaining Ra+C- cells?` . We shall do so by completing DGE, and then supplementing it with a manual scrape of the top genes, and a automatic `Gene Ontology` (GO) analaysis. Lets begin with the workflow below:

In [16]:
```
raPosCneg_R1_comparison <-
    perform_dge(sce_object = merge2_clean, target_pop = "Raneg_Cneg", target_region

# Print the top Genes differentially expressed in this region
head(raPosCneg_R1_comparison$top_genes, 20)

# Print the top Cluster Differentiating Genes differentially expressed in this regi
head(raPosCneg_R1_comparison$top_cd_genes, 20)
```

```
Warning message in asMethod(object):
"sparse->dense coercion: allocating vector of size 1.5 GiB"
Warning message:
"Zero sample variances detected, have been offset away from zero"
```

A data.frame: 20 × 7

	ID	logFC	AveExpr	t	P.Value	adj.P.Val	B
	<chr>	<dbl>	<dbl>	<dbl>	<dbl>	<dbl>	<dbl>
1	HBD	2.600558	0.9332628	60.29083	0.000000e+00	0.000000e+00	1381.5478
2	STXBP5	2.387527	1.9199339	69.60401	0.000000e+00	0.000000e+00	1722.4838
3	ITGA2B	2.281450	0.7618509	92.47893	0.000000e+00	0.000000e+00	2561.5289
4	LTBP1	2.165247	0.6594841	65.40463	0.000000e+00	0.000000e+00	1567.9860
5	GP1BB	2.099730	0.6598842	72.72827	0.000000e+00	0.000000e+00	1837.8255
6	RAP1B	1.979228	2.1936284	70.07873	0.000000e+00	0.000000e+00	1739.9956
7	ABCC4	1.925287	1.0928290	72.21383	0.000000e+00	0.000000e+00	1818.8220
8	SPINK2	-1.924872	1.7426931	-69.27794	0.000000e+00	0.000000e+00	1710.4599
9	PLCB1	-1.875570	1.8908794	-59.28000	0.000000e+00	0.000000e+00	1345.0226
10	RNF220	-1.828865	2.5707110	-57.12300	0.000000e+00	0.000000e+00	1267.5479
11	SLC24A3	1.793877	0.6780904	57.21880	0.000000e+00	0.000000e+00	1270.9741
12	MED12L	1.747457	0.8803669	63.72476	0.000000e+00	0.000000e+00	1506.4814
13	C1QTNF4	-1.742253	1.5501189	-60.94412	0.000000e+00	0.000000e+00	1405.2200
14	PLXDC2	1.705843	0.9520478	58.30154	0.000000e+00	0.000000e+00	1309.7954
15	SH3BGRL3	1.699835	2.6590816	58.64659	0.000000e+00	0.000000e+00	1322.2032
16	ATP8B4	-1.679106	1.6559922	-52.94559	0.000000e+00	0.000000e+00	1119.7040
17	NKAIN2	-1.664888	2.7157017	-37.30191	1.739818e-271	3.557491e-269	608.1647
18	RAB27B	1.629171	0.9554767	58.10343	0.000000e+00	0.000000e+00	1302.6790
19	UBE2C	1.595314	0.9767416	53.48448	0.000000e+00	0.000000e+00	1138.5884
20	LAT	1.566811	0.4916719	72.43056	0.000000e+00	0.000000e+00	1826.8277

A data.frame: 20 × 7

	ID	logFC	AveExpr	t	P.Value	adj.P.Val	B
	<chr>	<dbl>	<dbl>	<dbl>	<dbl>	<dbl>	<dbl>
33	CD74	-1.3886235	2.2728535	-44.70930	0.000000e+00	0.000000e+00	840.1121
95	CD99	-1.0149487	1.4580142	-43.25054	0.000000e+00	0.000000e+00	792.7357
99	CD84	0.9984581	0.4955969	53.73067	0.000000e+00	0.000000e+00	1147.2357
107	CD48	-0.9684666	0.8941089	-44.67238	0.000000e+00	0.000000e+00	838.9039
126	CD63	0.9135788	2.2570242	37.46660	1.289011e-273	2.759011e-271	613.0672
142	CD36	0.8744668	0.3246596	36.77724	9.774527e-265	1.873076e-262	592.6316
143	CD34	-0.8677303	1.3150180	-35.24866	2.094257e-245	3.376736e-243	548.1467
177	CD44	-0.8126885	1.2309836	-32.72168	1.334583e-214	1.732166e-212	477.2655
195	CD55	0.7716636	0.5686104	39.33549	3.607161e-298	9.105220e-296	669.5748
275	CD52	-0.6601983	1.5512427	-23.33758	6.625781e-115	3.587429e-113	247.9604
318	CD53	-0.6224523	0.8918330	-28.84401	2.102008e-170	1.904346e-168	375.5861
529	CD164	-0.4921140	1.8707070	-20.19441	1.613185e-87	6.567762e-86	185.0219
552	CD200	-0.4793880	0.4247843	-28.94135	1.828026e-171	1.681095e-169	378.0259
659	BICD1	-0.4351051	1.0190811	-19.46995	1.103106e-81	4.153786e-80	171.6179
839	CD69	0.3802578	0.6807524	16.06343	9.060894e-57	2.367150e-55	114.4202
847	CD37	-0.3784251	1.4375666	-16.07689	7.365320e-57	1.926934e-55	114.6266
970	CD9	0.3453646	0.1117994	24.97147	2.049375e-130	1.293262e-128	283.6182
992	CD226	0.3390867	0.1304901	27.69840	4.042864e-158	3.302966e-156	347.3312
1017	CD82	0.3335424	0.5675827	17.01665	2.590232e-63	7.512289e-62	129.4362
1041	CD302	-0.3262979	0.5838855	-18.37965	2.984599e-73	1.005887e-71	152.2521

Characterizing Region 2 (R2) of the Ra+C- Population

In this section we will be answering the question `What makes the Ra+C- Region 2 (R2) cells different from the remaining Ra+C- cells?` . We shall do so by completing DGE, and then supplementing it with a manual scrape of the top genes, and a automatic `Gene Ontology` (GO) analaysis. Note that this is a repetition of the previous workflow:

In [17]:
```
raPosCneg_R2_comparison <-
    perform_dge(sce_object = merge2_clean, target_pop = "Raneg_Cneg", target_region

# Print the top Genes differentially expressed in this region
head(raPosCneg_R2_comparison$top_genes, 20)
```

```
# Print the top Cluster Differentiating Genes differentially expressed in this regi
head(raPosCneg_R2_comparison$top_cd_genes, 20)
```

```
Warning message in asMethod(object):
"sparse->dense coercion: allocating vector of size 1.5 GiB"
Warning message:
"Zero sample variances detected, have been offset away from zero"
```

A data.frame: 20 × 7

	ID	logFC	AveExpr	t	P.Value	adj.P.Val	B
	<chr>	<dbl>	<dbl>	<dbl>	<dbl>	<dbl>	<dbl>
1	NKAIN2	1.6173566	2.7157017	35.88455	2.191341e-253	2.673509e-249	566.5133
2	HIST1H4C	-1.3112881	2.2538890	-28.05665	6.383785e-162	3.894215e-159	356.0775
3	HMGB2	-1.2863952	2.6003865	-38.38509	1.345418e-285	2.462181e-281	640.6437
4	TOP2A	-1.1748550	1.2552635	-34.55373	8.701558e-237	3.981071e-233	528.3167
5	UBE2C	-1.1563485	0.9767416	-34.60196	2.213043e-237	1.157137e-233	529.6850
6	TUBB4B	-1.1293153	1.5703254	-35.09399	1.772643e-243	1.297610e-239	543.7134
7	HBD	-1.1049158	0.9332628	-20.55624	1.679011e-90	2.301628e-88	191.8769
8	HLA-DRA	1.0408271	2.3423770	26.37118	2.330121e-144	9.072847e-142	315.6858
9	MSI2	1.0300816	2.5742865	30.68656	6.450141e-191	1.026442e-187	422.7771
10	RNF220	1.0277936	2.5707110	26.97889	1.350358e-150	6.101786e-148	330.0294
11	UBE2S	-1.0216306	1.3800974	-33.73812	8.204519e-227	2.144954e-223	505.3652
12	SPINK2	1.0212230	1.7426931	28.70188	7.356749e-169	5.609675e-166	372.0369
13	HIST1H1B	-1.0188295	1.0765159	-29.62785	5.103361e-179	5.336803e-176	395.4053
14	HOPX	1.0046959	1.1811817	35.59054	1.100540e-249	1.007022e-245	557.9968
15	MKI67	-0.9951843	1.0553881	-34.98354	4.186815e-242	2.554027e-238	540.5534
16	CENPF	-0.9712544	1.3730378	-29.45835	3.845265e-177	3.703698e-174	391.0874
17	CD74	0.9527469	2.2728535	28.00636	2.192806e-161	1.294498e-158	354.8448
18	PLCB1	0.9493143	1.8908794	24.61417	5.956410e-127	1.639177e-124	275.6575
19	RRM2	-0.9471306	0.8967019	-38.44819	1.993591e-286	7.296741e-282	642.5521
20	NRIP1	0.9264414	2.4574898	29.47771	2.349684e-177	2.324345e-174	391.5795

A data.frame: 20 × 7

	ID	logFC	AveExpr	t	P.Value	adj.P.Val	B
	<chr>	<dbl>	<dbl>	<dbl>	<dbl>	<dbl>	<dbl>
17	CD74	0.9527469	2.2728535	28.006355	2.192806e-161	1.294498e-158	354.84484
45	CD52	0.7753846	1.5512427	27.875007	5.459927e-160	3.122481e-157	351.63352
64	CD99	0.6737835	1.4580142	26.238619	5.168612e-143	1.950272e-140	312.59046
100	CD48	0.5969166	0.8941089	24.800467	9.422292e-129	2.715475e-126	279.79801
136	CD34	0.5490061	1.3150180	20.871016	3.923522e-93	5.544588e-91	197.92294
138	CD37	0.5443091	1.4375666	23.676609	4.705785e-118	1.118419e-115	255.20109
195	CD44	0.4687010	1.2309836	17.705505	2.986698e-68	2.739753e-66	140.77385
315	CD84	-0.4014853	0.4955969	-17.942543	5.442702e-70	5.201262e-68	144.76716
316	CD53	0.4005713	0.8918330	17.744817	1.542205e-68	1.432646e-66	141.43284
389	CD63	-0.3767893	2.2570242	-13.996693	9.252102e-44	4.594792e-42	84.59377
394	CD36	-0.3754074	0.3246596	-14.368022	5.617585e-46	2.988506e-44	89.67381
520	CD200	0.3326701	0.4247843	19.285397	3.159699e-80	3.648207e-78	168.27364
523	CD109	0.3322049	0.8986139	14.935910	1.805819e-49	1.059211e-47	97.68097
570	CD55	-0.3204002	0.5686104	-14.666228	8.527844e-48	4.824229e-46	93.84272
727	CD164	0.2829355	1.8707070	11.305824	2.595364e-29	7.704212e-28	51.52489
842	SCD	-0.2633034	0.6314002	-13.023544	3.319280e-38	1.372757e-36	71.87000
1027	CD79B	0.2341851	0.3376392	15.483915	5.876647e-53	3.780161e-51	105.67854
1441	C2CD2	0.1882607	0.4018778	11.341493	1.743786e-29	5.240091e-28	51.91960
1479	HACD3	-0.1857094	1.1536948	-8.458455	3.440544e-17	5.721298e-16	23.88617
1711	CD38	-0.1694150	0.7399566	-7.333030	2.578988e-13	3.285539e-12	15.09935

Characterizing Region 1 (R1) of the C+ Population

In this section we will be answering the question `What makes the C+ Region 1 (R1) cells different from the remaining C+ cells?` . We shall do so by completing DGE, and then supplementing it with a manual scrape of the top genes. Note that this is a repetition of the previous workflow:

In [18]:
```
Cpos_R1_comparison <-
    perform_dge(sce_object = merge2_clean, target_pop = "Cpos", target_region = "R1

# Print the top Genes differentially expressed in this region
head(Cpos_R1_comparison$top_genes, 20)

# Print the top Cluster Differentiating Genes differentially expressed in this regi
head(Cpos_R1_comparison$top_cd_genes, 20)
```

```
Warning message:
"Zero sample variances detected, have been offset away from zero"
```

A data.frame: 20 × 7

	ID	logFC	AveExpr	t	P.Value	adj.P.Val	B
	<chr>	<dbl>	<dbl>	<dbl>	<dbl>	<dbl>	<dbl>
1	TOP2A	1.943276	1.3769076	45.74262	0.000000e+00	0.000000e+00	814.3644
2	HIST1H4C	1.925317	2.3573200	30.62303	2.306751e-183	1.918850e-180	405.4065
3	MKI67	1.817081	1.2989187	49.55594	0.000000e+00	0.000000e+00	926.0785
4	UBE2C	1.698571	0.9020936	46.68556	0.000000e+00	0.000000e+00	841.7910
5	HMGB2	1.661068	2.7763606	33.39165	2.178373e-213	2.847523e-210	474.4930
6	CENPF	1.462420	1.3749132	33.77545	1.161869e-217	1.575020e-214	484.3259
7	ASPM	1.425954	1.0068074	40.02830	1.822738e-290	8.339254e-287	651.8870
8	NUSAP1	1.408244	1.0801089	44.33975	0.000000e+00	0.000000e+00	773.8441
9	UBE2S	1.311736	1.3815108	34.03413	1.481856e-220	2.086054e-217	490.9864
10	HIST1H1B	1.299407	1.0095044	31.22222	9.759089e-190	9.399801e-187	420.0710
11	CENPE	1.273867	0.8845728	36.49646	1.193035e-248	3.358945e-245	555.6415
12	HMMR	1.245465	0.7621866	40.38427	9.113318e-295	4.765093e-291	661.7871
13	TUBB4B	1.237407	1.5383101	29.03084	8.702895e-167	5.491977e-164	367.2696
14	CDK1	1.234474	0.7712793	43.13791	0.000000e+00	0.000000e+00	739.4298
15	KPNA2	1.178221	1.0672469	34.18255	3.199156e-222	4.878847e-219	494.8198
16	TUBB	1.146956	2.8305532	27.33934	8.741665e-150	4.637010e-147	328.1595
17	TPX2	1.140368	0.9513845	35.00740	1.517589e-231	2.923436e-228	516.2768
18	KIF11	1.110784	0.7894748	41.02054	1.722484e-302	1.050744e-298	679.5653
19	SMC4	1.108021	1.6302565	31.62733	4.365967e-194	4.699963e-191	430.0783
20	SAMHD1	1.067395	2.0192066	16.11328	1.804498e-56	3.057705e-54	113.7024

A data.frame: 20 × 7

	ID	logFC	AveExpr	t	P.Value	adj.P.Val	B
	<chr>	<dbl>	<dbl>	<dbl>	<dbl>	<dbl>	<dbl>
287	CD34	-0.3903839	0.53207044	-12.983288	1.042245e-37	1.053790e-35	70.6924335
319	CD74	0.3669891	3.26015404	6.774873	1.447153e-11	3.894651e-10	11.0997499
495	CD52	-0.2986400	0.73682286	-8.457527	3.906943e-17	1.623133e-15	23.7144037
938	CDYL	0.2070244	1.07175479	6.374878	2.061648e-10	4.944847e-09	8.5001428
961	CD36	0.2044258	0.56109847	5.454332	5.244542e-08	9.282180e-07	3.1063561
1057	CD1D	0.1933482	0.35725796	7.229902	5.866953e-13	1.799969e-11	14.2443287
1135	CD2AP	0.1831994	1.01727529	5.433625	5.885873e-08	1.035218e-06	2.9945094
1168	CD37	-0.1793484	1.44754954	-5.119618	3.220804e-07	5.101603e-06	1.3498041
1189	CD84	0.1769242	0.39274125	7.846612	5.581901e-15	1.997098e-13	18.8226322
1257	CD1C	0.1695980	0.47744823	4.079163	4.617056e-05	5.027934e-04	-3.4088474
1258	CD53	0.1695372	1.29404331	4.877085	1.122918e-06	1.628365e-05	0.1455097
1285	SCD	0.1679191	0.65393698	6.177727	7.222710e-10	1.627823e-08	7.2757348
1502	CD96	-0.1498861	0.31276717	-6.038967	1.707456e-09	3.680482e-08	6.4365504
1562	CD226	0.1455231	0.12519030	9.265590	3.237663e-20	1.630009e-18	30.7248689
1670	CD86	0.1390596	0.41886951	4.933793	8.427443e-07	1.248797e-05	0.4219298
1690	CD180	0.1379317	0.34619455	6.177165	7.248197e-10	1.632562e-08	7.2722971
1880	HACD4	0.1278411	0.64434172	4.995050	6.159714e-07	9.331610e-06	0.7240626
1970	CD9	0.1240892	0.05155304	9.578395	1.766450e-21	9.535964e-20	33.6024257

	ID	logFC	AveExpr	t	P.Value	adj.P.Val	B
	<chr>	<dbl>	<dbl>	<dbl>	<dbl>	<dbl>	<dbl>
2001	CDV3	-0.1222131	1.35935331	-4.080951	4.581838e-05	4.997016e-04	-3.4015833
2132	CD79B	-0.1168537	0.21083310	-7.258916	4.750914e-13	1.471135e-11	14.4515760

Characterizing Region 2 (R2) of the C+ Population

In this section we will be answering the question `What makes the C+ Region 2 (R2) cells different from the remaining C+ cells?` . We shall do so by completing DGE, and then supplementing it with a manual scrape of the top genes. Note that this is a repetition of the previous workflow:

In [19]:

```
Cpos_R2_comparison <-
    perform_dge(sce_object = merge2_clean, target_pop = "Cpos", target_region = "R2

# Print the top Genes differentially expressed in this region
head(Cpos_R2_comparison$top_genes, 20)

# Print the top Cluster Differentiating Genes differentially expressed in this regi
head(Cpos_R2_comparison$top_cd_genes, 20)
```

```
Warning message:
"Zero sample variances detected, have been offset away from zero"
```

A data.frame: 20 × 7

	ID	logFC	AveExpr	t	P.Value	adj.P.Val	B
	<chr>	<dbl>	<dbl>	<dbl>	<dbl>	<dbl>	<dbl>
1	HIST1H4C	-1.2749375	2.3573200	-11.906050	4.331929e-32	8.750025e-28	58.269990
2	AFF3	-0.8891336	2.3235517	-9.048177	2.318076e-19	5.921326e-16	29.215236
3	HDAC9	-0.8515274	2.6566573	-10.257135	2.358134e-24	2.157752e-20	40.594449
4	HIST1H1B	-0.8467840	1.0095044	-11.897492	4.781304e-32	8.750025e-28	58.171945
5	MPO	-0.7439102	1.9990494	-5.437782	5.751320e-08	1.079508e-05	3.453652
6	HIST1H1D	-0.6271537	1.2377228	-9.333600	1.733501e-20	7.049763e-17	31.779463
7	RABGAP1L	-0.6140369	1.8144553	-9.360959	1.346667e-20	6.161169e-17	32.029248
8	RUNX2	-0.5982653	1.9477654	-6.855253	8.332255e-12	4.356698e-09	12.077480
9	BCL2	-0.5901856	1.9437992	-7.473339	9.747972e-14	8.108762e-11	16.444884
10	UBE2E2	-0.5896008	1.7408516	-9.484982	4.250084e-21	2.592622e-17	33.170273
11	WWOX	-0.5687601	1.8820647	-7.626114	3.071738e-14	2.810717e-11	17.580742
12	AUTS2	-0.5674274	1.8597347	-8.427869	5.011197e-17	6.793142e-14	23.905475
13	HIST1H1C	-0.5566508	0.8933185	-10.046564	1.922543e-23	1.407340e-19	38.515301
14	PRDX1	0.5466051	2.4794835	8.242752	2.326274e-16	2.778513e-13	22.391076
15	SFMBT2	-0.5393858	1.7277444	-7.182869	8.245688e-13	5.803854e-10	14.346801
16	PTTG1	0.5344782	1.1813705	9.150839	9.200361e-20	2.806187e-16	30.128793
17	DIAPH3	-0.5253225	1.0074927	-9.194185	6.209996e-20	2.066292e-16	30.517470
18	FCHSD2	-0.5165822	2.3827329	-7.594183	3.917356e-14	3.497052e-11	17.341499
19	DIAPH2	-0.5058354	1.7573645	-8.630524	8.998986e-18	1.646859e-14	25.600407
20	LINC01572	-0.5037277	1.1909193	-8.434224	4.751244e-17	6.688472e-14	23.958039

A data.frame: 20 × 7

	ID	logFC	AveExpr	t	P.Value	adj.P.Val	B
	<chr>	<dbl>	<dbl>	<dbl>	<dbl>	<dbl>	<dbl>
133	CD44	-0.28755418	1.66439588	-5.597538	2.335505e-08	5.058095e-06	4.32802737
151	CD52	0.27580557	0.73682286	5.015646	5.539139e-07	7.481108e-05	1.26319967
190	CD63	0.25708566	1.89852791	4.735291	2.271162e-06	2.490101e-04	-0.09512028
195	CD38	-0.25596597	1.04718520	-4.828010	1.436003e-06	1.684588e-04	0.34556992
206	CD34	0.25273801	0.53207044	5.330375	1.039926e-07	1.829919e-05	2.87979671
230	CD99	0.23948973	1.17319929	5.063733	4.316057e-07	6.122946e-05	1.50392221
336	CDYL	-0.21229202	1.07175479	-4.211074	2.603367e-05	1.745162e-03	-2.42775880
512	CD59	0.17919633	0.45740367	5.222297	1.866608e-07	3.009680e-05	2.31373576
652	CD81	0.16235847	1.13598289	3.510907	4.520228e-04	1.603148e-02	-5.12067109
714	CDV3	0.15469354	1.35935331	3.335462	8.601467e-04	2.281321e-02	-5.71947848
897	BICD1	-0.13941746	0.94689170	-2.968668	3.010523e-03	5.910957e-02	-6.87278265
939	C2CD5	-0.13669178	0.55794857	-3.662291	2.535397e-04	1.026074e-02	-4.57948931
1148	CD84	-0.12110820	0.39274125	-3.447401	5.724499e-04	1.708058e-02	-5.34094334
1281	CD4	-0.11476129	0.28359894	-3.038748	2.392579e-03	4.969965e-02	-6.66274769
1623	CD1D	0.09991974	0.35725796	2.399103	1.648548e-02	1.966064e-01	-8.39878191
1661	CD58	0.09834087	0.46969995	2.775606	5.538164e-03	9.264276e-02	-7.42617491
2099	CD79B	0.08226754	0.21083310	3.283514	1.034948e-03	2.614225e-02	-5.89092936
2198	TBCD	-0.07854332	0.45369966	-2.372639	1.771344e-02	2.060806e-01	-8.46184204

	ID	logFC	AveExpr	t	P.Value	adj.P.Val	B
	<chr>	<dbl>	<dbl>	<dbl>	<dbl>	<dbl>	<dbl>
2545	CD99L2	-0.06261173	0.21312940	-2.487192	1.292024e-02	1.661608e-01	-8.18386370
2918	CD200R1	-0.04557340	0.09994318	-2.020258	4.343008e-02	3.701012e-01	-9.23500483

Characterizing Region 3 (R3) of the C+ Population

In this section we will be answering the question `What makes the C+ Region 2 (R2) cells different from the remaining C+ cells?` . We shall do so by completing DGE, and then supplementing it with a manual scrape of the top genes. Note that this is a repetition of the previous workflow:

In [20]:
```
Cpos_R3_comparison <-
    perform_dge(sce_object = merge2_clean, target_pop = "Cpos", target_region = "R3

# Print the top Genes differentially expressed in this region
head(Cpos_R3_comparison$top_genes, 20)

# Print the top Cluster Differentiating Genes differentially expressed in this regi
head(Cpos_R3_comparison$top_cd_genes, 20)
```

```
Warning message:
"Zero sample variances detected, have been offset away from zero"
```

A data.frame: 20 × 7

	ID	logFC	AveExpr	t	P.Value	adj.P.Val	B
	<chr>	<dbl>	<dbl>	<dbl>	<dbl>	<dbl>	<dbl>
1	CST3	2.844294	2.1637611	39.57353	5.421427e-285	9.921483e-281	639.3532
2	S100A6	2.082420	1.6373426	38.37640	1.051391e-270	1.282732e-266	606.4672
3	S100A10	2.045921	1.0808110	38.29754	9.047193e-270	8.278407e-266	604.3157
4	LYZ	2.007371	1.5582085	26.15018	3.107731e-138	2.146152e-135	301.6571
5	SAMHD1	1.901196	2.0192066	28.80481	1.773546e-164	1.803154e-161	362.0221
6	S100A4	1.889538	3.3488066	36.22702	1.572152e-245	8.220334e-242	548.5259
7	HLA-DRA	1.708710	3.2477191	30.21503	4.584386e-179	6.453581e-176	395.5820
8	VIM	1.691064	3.3620246	31.65851	2.013729e-194	3.879185e-191	430.9163
9	HLA-DPA1	1.674286	2.1864050	32.17635	4.959687e-200	1.008497e-196	443.8214
10	HLA-DPB1	1.604700	1.9964030	30.14867	2.276424e-178	3.085904e-175	393.9807
11	SLC8A1	1.603701	0.8390865	35.40450	4.499459e-236	1.829830e-232	526.7621
12	MPO	-1.565697	1.9990494	-17.19247	9.564979e-64	1.321086e-61	130.4648
13	COTL1	1.554372	1.9108972	33.24414	9.408496e-212	2.459717e-208	470.7944
14	HLA-DRB1	1.507356	2.4250991	29.08263	2.564059e-167	2.760209e-164	368.5552
15	S100A11	1.502511	1.8005538	32.69560	1.050033e-205	2.260721e-202	456.8780
16	ANXA2	1.477705	1.0167411	33.83245	2.681142e-218	8.177706e-215	485.8561
17	CD74	1.467318	3.2601540	27.78502	3.352864e-154	2.921861e-151	338.3823
18	PLXDC2	1.466705	0.9928553	33.11150	2.759646e-210	6.733719e-207	467.4179
19	RTN1	1.419965	0.4684784	40.34251	2.916659e-294	1.067526e-289	660.6889
20	KCNQ5	-1.417320	2.3019023	-22.74562	3.613062e-107	1.520019e-104	230.2260

A data.frame: 20 × 7

	ID	logFC	AveExpr	t	P.Value	adj.P.Val	B
	<chr>	<dbl>	<dbl>	<dbl>	<dbl>	<dbl>	<dbl>
17	CD74	1.4673176	3.26015404	27.785018	3.352864e-154	2.921861e-151	338.38232
39	CD1C	1.1770754	0.47744823	29.475448	2.316241e-171	2.568992e-168	377.85896
58	CD36	0.9897158	0.56109847	27.015999	1.306573e-146	1.062708e-143	320.92243
106	CD48	0.8113073	1.29148444	23.129594	1.627070e-110	7.352146e-108	237.91910
111	CD86	0.7988978	0.41886951	29.480308	2.063198e-171	2.359847e-168	377.97455
159	CD1D	0.6891653	0.35725796	26.143265	3.619463e-138	2.453259e-135	301.50488
278	CD37	0.5373237	1.44754954	14.747038	7.626808e-48	6.583698e-46	93.98203
309	CD34	-0.5176654	0.53207044	-16.343751	5.449568e-58	6.312014e-56	117.25487
323	CD4	0.5098941	0.28359894	20.678192	6.938321e-90	1.853646e-87	190.50256
449	CD302	0.4410703	1.10428854	13.827663	2.035842e-42	1.484340e-40	81.54418
550	CD63	0.3935811	1.89852791	10.644307	4.481249e-26	1.665159e-24	44.15140
602	CD38	-0.3743976	1.04718520	-10.359351	8.392601e-25	2.914398e-23	41.24643
631	CD53	0.3648039	1.29404331	9.935960	5.695404e-23	1.815832e-21	37.06760
684	HACD3	-0.3519215	0.92054313	-11.397410	1.366389e-29	6.010961e-28	52.18403
909	CD96	-0.2985389	0.31276717	-11.416678	1.103400e-29	4.889289e-28	52.39626
986	HACD4	0.2869140	0.64434172	10.632637	5.059863e-26	1.874454e-24	44.03097
1029	CD1E	0.2804092	0.10755063	15.812364	1.633185e-54	1.712785e-52	109.27794
1186	CD200R1	0.2585260	0.09994318	17.283109	2.246267e-64	3.211547e-62	131.90922

	ID	logFC	AveExpr	t	P.Value	adj.P.Val	B
	<chr>	<dbl>	<dbl>	<dbl>	<dbl>	<dbl>	<dbl>
1197	CD68	0.2573989	0.18316388	12.989085	9.695052e-38	6.014383e-36	70.82912
1531	CD99	-0.2208288	1.17319929	-6.790842	1.297458e-11	1.922601e-10	11.27148

Characterizing Region 4 (R4) of the C+ Population

In this section we will be answering the question `What makes the C+ Region 4 (R4) cells different from the remaining C+ cells?` . We shall do so by completing DGE, and then supplementing it with a manual scrape of the top genes. Note that this is a repetition of the previous workflow:

In [21]:
```
Cpos_R4_comparison <-
    perform_dge(sce_object = merge2_clean, target_pop = "Cpos", target_region = "R4

# Print the top Genes differentially expressed in this region
head(Cpos_R4_comparison$top_genes, 20)

# Print the top Cluster Differentiating Genes differentially expressed in this regi
head(Cpos_R4_comparison$top_cd_genes, 20)

# print it as csv
```

```
Warning message:
"Zero sample variances detected, have been offset away from zero"
```

A data.frame: 20 × 7

	ID	logFC	AveExpr	t	P.Value	adj.P.Val	B
	<chr>	<dbl>	<dbl>	<dbl>	<dbl>	<dbl>	<dbl>
1	MPO	2.8475984	1.9990494	19.302327	4.221984e-79	1.716987e-75	166.21781
2	PRTN3	2.3095339	0.4257153	26.606883	1.250049e-142	2.287651e-138	312.25834
3	AZU1	1.7662224	0.2779040	30.812220	2.281937e-185	8.352117e-181	410.57526
4	LRMDA	1.5977458	2.2763890	16.870219	1.567365e-61	3.824475e-58	125.87388
5	FNDC3B	1.3983028	1.2114140	19.801434	6.052030e-83	3.164433e-79	175.04751
6	SERPINB1	1.3156648	1.9682292	16.123110	1.555673e-56	3.163289e-53	114.40711
7	CST3	-1.2797978	2.1637611	-9.201877	5.790519e-20	7.623697e-18	30.70691
8	HIST1H4C	-1.2525414	2.3573200	-10.322318	1.221591e-24	2.470246e-22	41.36658
9	LYST	1.1831815	1.1446418	16.114164	1.780624e-56	3.430138e-53	114.27253
10	PRSS57	1.1804039	1.0461957	15.444283	3.656454e-52	5.818690e-49	104.37935
11	TUBB4B	-1.1790977	1.5383101	-14.695370	1.568426e-47	2.207921e-44	93.75628
12	MKI67	-1.1584932	1.2989187	-14.368750	1.420513e-45	1.856865e-42	89.26997
13	TOP2A	-1.1223199	1.3769076	-12.301026	4.243544e-34	2.098891e-31	62.98637
14	TUBA1B	-1.0974248	3.9090800	-12.982430	1.053470e-37	7.010557e-35	71.23871
15	S100A4	-1.0880403	3.3488066	-11.111343	3.140980e-28	8.530052e-26	49.56462
16	HMGB2	-1.0364533	2.7763606	-10.599882	7.110184e-26	1.626499e-23	44.18586
17	SRGN	0.9886033	1.9498024	13.199777	6.853482e-39	5.119272e-36	73.95629
18	HIST1H1B	-0.9691742	1.0095044	-12.080453	5.714418e-33	2.550652e-30	60.40262
19	S100A6	-0.9655470	1.6373426	-9.281096	2.808909e-20	3.969455e-18	31.42235
20	S100A10	-0.9496436	1.0808110	-9.277406	2.905548e-20	4.074558e-18	31.38889

A data.frame: 20 × 7

	ID	logFC	AveExpr	t	P.Value	adj.P.Val	B
	<chr>	<dbl>	<dbl>	<dbl>	<dbl>	<dbl>	<dbl>
63	CD74	-0.7150619	3.2601540	-7.575058	4.529492e-14	3.475554e-12	17.3189563
277	CD52	-0.4388130	0.7368229	-7.099802	1.496502e-12	9.694419e-11	13.8819585
311	CD164	-0.4225014	1.4962513	-7.157294	9.913394e-13	6.609110e-11	14.2862342
339	CD1C	-0.4094522	0.4774482	-5.654342	1.685336e-08	6.432221e-07	4.7652160
357	CD36	-0.4036869	0.5610985	-6.178264	7.198422e-10	3.322440e-08	7.8360348
397	CD53	-0.3805762	1.2940433	-6.286188	3.640248e-10	1.767065e-08	8.5016877
564	CD44	0.3305364	1.6643959	5.705780	1.250929e-08	4.844003e-07	5.0548880
672	CD84	0.3025318	0.3927413	7.684825	1.959387e-14	1.579637e-12	18.1434574
678	CD38	0.3009633	1.0471852	5.034617	5.021197e-07	1.451665e-05	1.4781942
749	CD1D	-0.2890853	0.3572580	-6.181621	7.048548e-10	3.265619e-08	7.8565685
882	CD96	0.2637392	0.3127672	6.088744	1.256668e-09	5.568439e-08	7.2924556
1035	CD81	-0.2430534	1.1359829	-4.666111	3.180509e-06	7.719483e-05	-0.2981373
1157	HACD3	-0.2283692	0.9205431	-4.454647	8.656844e-06	1.928479e-04	-1.2572510
1160	CD63	-0.2279814	1.8985279	-3.718789	2.032093e-04	3.019758e-03	-4.2514083
1189	CD86	-0.2250331	0.4188695	-4.572334	4.984328e-06	1.156826e-04	-0.7288949
1254	CD48	-0.2156195	1.2914844	-3.513896	4.469848e-04	5.934019e-03	-4.9899092
1445	CD302	0.1958048	1.1042885	3.664205	2.516585e-04	3.608634e-03	-4.4522048
1657	CD300A	-0.1778407	0.3300785	-4.886472	1.071039e-06	2.910252e-05	0.7480713
1892	CD47	-0.1623991	1.1277816	-3.178094	1.494940e-03	1.674305e-02	-6.1103392
2162	CD34	-0.1471191	0.5320704	-2.743194	6.114397e-03	5.341123e-02	-7.3951513

Characterizing Region 5 (R5) of the C+ Population

In this section we will be answering the question `What makes the C+ Region 5 (R5) cells different from the remaining C+ cells?` . We shall do so by completing DGE, and then supplementing it with a manual scrape of the top genes. Note that this is a repetition of the previous workflow:

In [22]:
```
Cpos_R5_comparison <-
    perform_dge(sce_object = merge2_clean, target_pop = "Cpos", target_region = "R5

# Print the top Genes differentially expressed in this region
head(Cpos_R5_comparison$top_genes, 20)
```

```r
# Print the top Cluster Differentiating Genes differentially expressed in this regi
head(Cpos_R5_comparison$top_cd_genes, 20)
```

```
Warning message:
"Zero sample variances detected, have been offset away from zero"
```

A data.frame: 20 × 7

	ID	logFC	AveExpr	t	P.Value	adj.P.Val	B
	<chr>	<dbl>	<dbl>	<dbl>	<dbl>	<dbl>	<dbl>
1	LTB	3.106609	1.2740898	28.62571	1.176798e-162	1.305211e-159	358.3944
2	TUBA1B	-2.921061	3.9090800	-36.86598	6.033597e-253	3.154796e-249	566.1566
3	TCF4	2.816225	1.7692575	25.91093	5.949752e-136	4.032720e-133	296.9723
4	JCHAIN	2.804626	0.4055437	44.25181	0.000000e+00	0.000000e+00	771.9434
5	H2AFZ	-2.686776	3.7948555	-34.87649	4.659122e-230	1.894762e-226	513.4821
6	LINC01478	2.435756	0.2620662	42.21482	4.191758e-317	7.671126e-313	713.8319
7	HMGB2	-2.414741	2.7763606	-24.42964	3.783900e-122	2.036684e-119	265.2299
8	CCDC50	2.359768	1.1358201	27.21492	1.465646e-148	1.277240e-145	325.9713
9	CUX2	2.315946	0.4353410	39.75071	4.023346e-287	3.681462e-283	644.8176
10	UGCG	2.248940	0.7868915	27.39955	2.226463e-150	1.987580e-147	330.1539
11	AC023590.1	2.203652	0.4591193	31.18060	2.719170e-189	4.739254e-186	419.6750
12	NIBAN3	2.148335	0.4199959	33.66580	1.943175e-216	5.080153e-213	482.1385
13	LINC01374	2.103218	0.3242030	38.96610	1.014193e-277	6.186746e-274	623.1778
14	RABGAP1L	2.059722	1.8144553	28.29074	2.874816e-159	2.843814e-156	350.6009
15	TPM2	2.043972	0.2870637	39.14803	6.806123e-280	4.982218e-276	628.1799
16	KCNQ5	-2.031652	2.3019023	-18.12351	2.430731e-70	5.264331e-68	146.1699
17	CARD11	1.994982	0.6294967	30.34581	1.937617e-180	2.836749e-177	399.3065
18	IRF8	1.985258	1.2900046	20.16680	8.289671e-86	2.638350e-83	181.6977
19	FCHSD2	1.973939	2.3827329	25.85706	1.933508e-135	1.263720e-132	295.7951
20	LRMDA	-1.914075	2.2763890	-18.99255	9.357970e-77	2.429156e-74	160.9015

A data.frame: 20 × 7

	ID	logFC	AveExpr	t	P.Value	adj.P.Val	B
	<chr>	<dbl>	<dbl>	<dbl>	<dbl>	<dbl>	<dbl>
62	CD74	1.4602567	3.2601540	14.705108	1.369381e-47	1.369418e-45	93.963105
164	CD2AP	1.0363949	1.0172753	16.961146	3.749002e-62	6.323375e-60	127.371493
228	CD164	0.9516593	1.4962513	15.378149	9.554399e-52	1.128066e-49	103.494256
281	CD37	0.8932082	1.4475495	13.904418	7.371545e-43	6.131952e-41	83.118993
351	CD63	-0.8293675	1.8985279	-12.850532	5.421698e-37	3.562649e-35	69.681283
359	CDYL	0.8201088	1.0717548	13.735999	6.804267e-42	5.497637e-40	80.907333
439	CD53	0.7633746	1.2940433	11.897958	4.755664e-32	2.497304e-30	58.369259
556	HACD3	-0.6845285	0.9205431	-12.665119	5.287620e-36	3.331018e-34	67.416855
731	CD81	-0.5988106	1.1359829	-10.839106	5.796802e-27	2.326412e-25	46.743790
788	CD38	-0.5814162	1.0471852	-9.123921	1.173382e-19	3.193083e-18	30.080301
797	CDT1	-0.5783227	0.6152767	-12.768688	1.487135e-36	9.599759e-35	68.678018
854	CD34	-0.5545999	0.5320704	-9.739828	3.801655e-22	1.191305e-20	35.751696
1091	CD4	0.4853419	0.2835989	10.759487	1.342812e-26	5.313326e-25	45.910539
1186	SCD	-0.4639307	0.6539370	-9.158297	8.599748e-20	2.350705e-18	30.387537
1470	CD36	0.4080784	0.5610985	5.809341	6.812367e-09	8.050999e-08	5.717626
1497	CD33	-0.4029567	0.5386984	-8.939800	6.084250e-19	1.574891e-17	28.453509
1522	CD48	-0.3963579	1.2914844	-6.031739	1.784833e-09	2.239516e-08	7.022078
1733	HACD1	-0.3655580	0.3836370	-9.496355	3.820853e-21	1.126890e-19	33.467614
1832	CD59	-0.3523438	0.4574037	-8.526151	2.189492e-17	5.166834e-16	24.914636
1835	CD180	0.3519795	0.3461945	8.443808	4.384175e-17	1.013678e-15	24.229380

Characterizing Region 1 (R1) of the Ra-C- Population

In this section we will be answering the question `What makes the Ra-C- Region 1 (R1) cells different from the remaining Ra-C- cells?` . We shall do so by completing DGE, and then supplementing it with a manual scrape of the top genes. Note that this is a repetition of the previous workflow:

In [23]:
```
Raneg_Cneg_R1_comparison <-
    perform_dge(sce_object = merge2_clean, target_pop = "Raneg_Cneg", target_region

# Print the top Genes differentially expressed in this region
head(Raneg_Cneg_R1_comparison$top_genes, 20)
```

```
# Print the top Cluster Differentiating Genes differentially expressed in this regi
head(Raneg_Cneg_R1_comparison$top_cd_genes, 20)

write.csv(Raneg_Cneg_R1_comparison$top_genes, "topGenes_Region1.csv")
write.csv(Raneg_Cneg_R1_comparison$top_cd_genes, "topCD_Region1.csv")
```

```
Warning message in asMethod(object):
"sparse->dense coercion: allocating vector of size 1.5 GiB"
Warning message:
"Zero sample variances detected, have been offset away from zero"
```

A data.frame: 20 × 7

	ID	logFC	AveExpr	t	P.Value	adj.P.Val	B
	<chr>	<dbl>	<dbl>	<dbl>	<dbl>	<dbl>	<dbl>
1	HBD	2.600558	0.9332628	60.29083	0.000000e+00	0.000000e+00	1381.5478
2	STXBP5	2.387527	1.9199339	69.60401	0.000000e+00	0.000000e+00	1722.4838
3	ITGA2B	2.281450	0.7618509	92.47893	0.000000e+00	0.000000e+00	2561.5289
4	LTBP1	2.165247	0.6594841	65.40463	0.000000e+00	0.000000e+00	1567.9860
5	GP1BB	2.099730	0.6598842	72.72827	0.000000e+00	0.000000e+00	1837.8255
6	RAP1B	1.979228	2.1936284	70.07873	0.000000e+00	0.000000e+00	1739.9956
7	ABCC4	1.925287	1.0928290	72.21383	0.000000e+00	0.000000e+00	1818.8220
8	SPINK2	-1.924872	1.7426931	-69.27794	0.000000e+00	0.000000e+00	1710.4599
9	PLCB1	-1.875570	1.8908794	-59.28000	0.000000e+00	0.000000e+00	1345.0226
10	RNF220	-1.828865	2.5707110	-57.12300	0.000000e+00	0.000000e+00	1267.5479
11	SLC24A3	1.793877	0.6780904	57.21880	0.000000e+00	0.000000e+00	1270.9741
12	MED12L	1.747457	0.8803669	63.72476	0.000000e+00	0.000000e+00	1506.4814
13	C1QTNF4	-1.742253	1.5501189	-60.94412	0.000000e+00	0.000000e+00	1405.2200
14	PLXDC2	1.705843	0.9520478	58.30154	0.000000e+00	0.000000e+00	1309.7954
15	SH3BGRL3	1.699835	2.6590816	58.64659	0.000000e+00	0.000000e+00	1322.2032
16	ATP8B4	-1.679106	1.6559922	-52.94559	0.000000e+00	0.000000e+00	1119.7040
17	NKAIN2	-1.664888	2.7157017	-37.30191	1.739818e-271	3.557491e-269	608.1647
18	RAB27B	1.629171	0.9554767	58.10343	0.000000e+00	0.000000e+00	1302.6790
19	UBE2C	1.595314	0.9767416	53.48448	0.000000e+00	0.000000e+00	1138.5884
20	LAT	1.566811	0.4916719	72.43056	0.000000e+00	0.000000e+00	1826.8277

A data.frame: 20 × 7

	ID	logFC	AveExpr	t	P.Value	adj.P.Val	B
	<chr>	<dbl>	<dbl>	<dbl>	<dbl>	<dbl>	<dbl>
33	CD74	-1.3886235	2.2728535	-44.70930	0.000000e+00	0.000000e+00	840.1121
95	CD99	-1.0149487	1.4580142	-43.25054	0.000000e+00	0.000000e+00	792.7357
99	CD84	0.9984581	0.4955969	53.73067	0.000000e+00	0.000000e+00	1147.2357
107	CD48	-0.9684666	0.8941089	-44.67238	0.000000e+00	0.000000e+00	838.9039
126	CD63	0.9135788	2.2570242	37.46660	1.289011e-273	2.759011e-271	613.0672
142	CD36	0.8744668	0.3246596	36.77724	9.774527e-265	1.873076e-262	592.6316
143	CD34	-0.8677303	1.3150180	-35.24866	2.094257e-245	3.376736e-243	548.1467
177	CD44	-0.8126885	1.2309836	-32.72168	1.334583e-214	1.732166e-212	477.2655
195	CD55	0.7716636	0.5686104	39.33549	3.607161e-298	9.105220e-296	669.5748
275	CD52	-0.6601983	1.5512427	-23.33758	6.625781e-115	3.587429e-113	247.9604
318	CD53	-0.6224523	0.8918330	-28.84401	2.102008e-170	1.904346e-168	375.5861
529	CD164	-0.4921140	1.8707070	-20.19441	1.613185e-87	6.567762e-86	185.0219
552	CD200	-0.4793880	0.4247843	-28.94135	1.828026e-171	1.681095e-169	378.0259
659	BICD1	-0.4351051	1.0190811	-19.46995	1.103106e-81	4.153786e-80	171.6179
839	CD69	0.3802578	0.6807524	16.06343	9.060894e-57	2.367150e-55	114.4202
847	CD37	-0.3784251	1.4375666	-16.07689	7.365320e-57	1.926934e-55	114.6266
970	CD9	0.3453646	0.1117994	24.97147	2.049375e-130	1.293262e-128	283.6182
992	CD226	0.3390867	0.1304901	27.69840	4.042864e-158	3.302966e-156	347.3312
1017	CD82	0.3335424	0.5675827	17.01665	2.590232e-63	7.512289e-62	129.4362
1041	CD302	-0.3262979	0.5838855	-18.37965	2.984599e-73	1.005887e-71	152.2521

Characterizing Region 2 (R2) of the Ra-C- Population

In this section we will be answering the question `What makes the Ra-C- Region 2 (R2) cells different from the remaining Ra-C- cells?` . We shall do so by completing DGE, and then supplementing it with a manual scrape of the top genes. Note that this is a repetition of the previous workflow:

In [24]:
```
Raneg_Cneg_R2_comparison <-
    perform_dge(sce_object = merge2_clean, target_pop = "Raneg_Cneg", target_region

# Print the top Genes differentially expressed in this region
head(Raneg_Cneg_R2_comparison$top_genes, 20)
```

```r
# Print the top Cluster Differentiating Genes differentially expressed in this regi
head(Raneg_Cneg_R2_comparison$top_cd_genes, 20)
```

```
Warning message in asMethod(object):
"sparse->dense coercion: allocating vector of size 1.5 GiB"
Warning message:
"Zero sample variances detected, have been offset away from zero"
```

A data.frame: 20 × 7

	ID	logFC	AveExpr	t	P.Value	adj.P.Val	B
	<chr>	<dbl>	<dbl>	<dbl>	<dbl>	<dbl>	<dbl>
1	NKAIN2	1.6173566	2.7157017	35.88455	2.191341e-253	2.673509e-249	566.5133
2	HIST1H4C	-1.3112881	2.2538890	-28.05665	6.383785e-162	3.894215e-159	356.0775
3	HMGB2	-1.2863952	2.6003865	-38.38509	1.345418e-285	2.462181e-281	640.6437
4	TOP2A	-1.1748550	1.2552635	-34.55373	8.701558e-237	3.981071e-233	528.3167
5	UBE2C	-1.1563485	0.9767416	-34.60196	2.213043e-237	1.157137e-233	529.6850
6	TUBB4B	-1.1293153	1.5703254	-35.09399	1.772643e-243	1.297610e-239	543.7134
7	HBD	-1.1049158	0.9332628	-20.55624	1.679011e-90	2.301628e-88	191.8769
8	HLA-DRA	1.0408271	2.3423770	26.37118	2.330121e-144	9.072847e-142	315.6858
9	MSI2	1.0300816	2.5742865	30.68656	6.450141e-191	1.026442e-187	422.7771
10	RNF220	1.0277936	2.5707110	26.97889	1.350358e-150	6.101786e-148	330.0294
11	UBE2S	-1.0216306	1.3800974	-33.73812	8.204519e-227	2.144954e-223	505.3652
12	SPINK2	1.0212230	1.7426931	28.70188	7.356749e-169	5.609675e-166	372.0369
13	HIST1H1B	-1.0188295	1.0765159	-29.62785	5.103361e-179	5.336803e-176	395.4053
14	HOPX	1.0046959	1.1811817	35.59054	1.100540e-249	1.007022e-245	557.9968
15	MKI67	-0.9951843	1.0553881	-34.98354	4.186815e-242	2.554027e-238	540.5534
16	CENPF	-0.9712544	1.3730378	-29.45835	3.845265e-177	3.703698e-174	391.0874
17	CD74	0.9527469	2.2728535	28.00636	2.192806e-161	1.294498e-158	354.8448
18	PLCB1	0.9493143	1.8908794	24.61417	5.956410e-127	1.639177e-124	275.6575
19	RRM2	-0.9471306	0.8967019	-38.44819	1.993591e-286	7.296741e-282	642.5521
20	NRIP1	0.9264414	2.4574898	29.47771	2.349684e-177	2.324345e-174	391.5795

A data.frame: 20 × 7

	ID	logFC	AveExpr	t	P.Value	adj.P.Val	B
	<chr>	<dbl>	<dbl>	<dbl>	<dbl>	<dbl>	<dbl>
17	CD74	0.9527469	2.2728535	28.006355	2.192806e-161	1.294498e-158	354.84484
45	CD52	0.7753846	1.5512427	27.875007	5.459927e-160	3.122481e-157	351.63352
64	CD99	0.6737835	1.4580142	26.238619	5.168612e-143	1.950272e-140	312.59046
100	CD48	0.5969166	0.8941089	24.800467	9.422292e-129	2.715475e-126	279.79801
136	CD34	0.5490061	1.3150180	20.871016	3.923522e-93	5.544588e-91	197.92294
138	CD37	0.5443091	1.4375666	23.676609	4.705785e-118	1.118419e-115	255.20109
195	CD44	0.4687010	1.2309836	17.705505	2.986698e-68	2.739753e-66	140.77385
315	CD84	-0.4014853	0.4955969	-17.942543	5.442702e-70	5.201262e-68	144.76716
316	CD53	0.4005713	0.8918330	17.744817	1.542205e-68	1.432646e-66	141.43284
389	CD63	-0.3767893	2.2570242	-13.996693	9.252102e-44	4.594792e-42	84.59377
394	CD36	-0.3754074	0.3246596	-14.368022	5.617585e-46	2.988506e-44	89.67381
520	CD200	0.3326701	0.4247843	19.285397	3.159699e-80	3.648207e-78	168.27364
523	CD109	0.3322049	0.8986139	14.935910	1.805819e-49	1.059211e-47	97.68097
570	CD55	-0.3204002	0.5686104	-14.666228	8.527844e-48	4.824229e-46	93.84272
727	CD164	0.2829355	1.8707070	11.305824	2.595364e-29	7.704212e-28	51.52489
842	SCD	-0.2633034	0.6314002	-13.023544	3.319280e-38	1.372757e-36	71.87000
1027	CD79B	0.2341851	0.3376392	15.483915	5.876647e-53	3.780161e-51	105.67854
1441	C2CD2	0.1882607	0.4018778	11.341493	1.743786e-29	5.240091e-28	51.91960
1479	HACD3	-0.1857094	1.1536948	-8.458455	3.440544e-17	5.721298e-16	23.88617
1711	CD38	-0.1694150	0.7399566	-7.333030	2.578988e-13	3.285539e-12	15.09935

Characterizing Region 1 (R3) of the Ra-C- Population

In this section we will be answering the question `What makes the Ra-C- Region 3 (R3) cells different from the remaining Ra-C- cells?` . We shall do so by completing DGE, and then supplementing it with a manual scrape of the top genes. Note that this is a repetition of the previous workflow:

In [25]:
```
Raneg_Cneg_R3_comparison <-
    perform_dge(sce_object = merge2_clean, target_pop = "Raneg_Cneg", target_region

# Print the top Genes differentially expressed in this region
head(Raneg_Cneg_R3_comparison$top_genes, 20)

# Print the top Cluster Differentiating Genes differentially expressed in this regi
head(Raneg_Cneg_R3_comparison$top_cd_genes, 20)
```

```
Warning message in asMethod(object):
"sparse->dense coercion: allocating vector of size 1.5 GiB"
Warning message:
"Zero sample variances detected, have been offset away from zero"
```

A data.frame: 20 × 7

	ID	logFC	AveExpr	t	P.Value	adj.P.Val	B
	<chr>	<dbl>	<dbl>	<dbl>	<dbl>	<dbl>	<dbl>
1	FHIT	-1.4255407	1.9786639	-19.698355	1.670403e-83	3.056922e-79	176.56335
2	WWOX	-1.3788230	2.2088429	-18.252876	2.677836e-72	1.960230e-68	150.82943
3	NKAIN2	-1.3215391	2.7157017	-12.467633	3.368158e-35	4.109265e-32	65.75139
4	RAD51B	-1.3124129	1.8692422	-21.187735	8.163596e-96	2.987958e-91	204.84806
5	IMMP2L	-1.2600372	1.9230808	-19.037507	2.739828e-78	2.507012e-74	164.58520
6	LRMDA	-1.1954152	2.1615629	-17.548987	4.097231e-67	2.499379e-63	138.92597
7	SMYD3	-1.1305355	2.3841554	-19.496263	6.822155e-82	8.323256e-78	172.86257
8	MALAT1	-1.0991620	5.8601224	-17.163512	2.372408e-64	9.648056e-61	132.58439
9	STXBP5	-1.0934012	1.9199339	-10.908477	2.011331e-27	1.840419e-24	47.97152
10	HMGB2	1.0885756	2.6003865	13.663350	8.139813e-42	1.354206e-38	80.90205
11	PTTG1	1.0686504	1.1477022	17.426922	3.113348e-66	1.627881e-62	136.90420
12	ZBTB20	-1.0380624	1.4574735	-15.686479	2.825661e-54	7.955540e-51	109.46464
13	LRBA	-1.0210120	2.6127848	-17.302823	2.415707e-65	1.105216e-61	134.86168
14	MIR924HG	-0.9673563	1.6771880	-12.422634	5.826470e-35	6.879181e-32	65.20672
15	INPP4B	-0.9477302	1.7127337	-13.294340	1.029226e-39	1.506828e-36	76.08759
16	SNHG3	0.9329528	1.3746169	16.643203	1.040811e-60	3.463157e-57	124.22549
17	EIF4G3	-0.8830465	1.8522299	-15.116481	1.318710e-50	3.217741e-47	101.05004
18	HIST1H4C	-0.8687174	2.2538890	-8.148676	4.522432e-16	1.190831e-13	22.10884
19	HBD	-0.8567509	0.9332628	-7.191580	7.267651e-13	1.047257e-10	14.84506
20	AC008014.1	-0.8518681	1.1810701	-12.807045	5.085818e-37	6.648073e-34	69.91945

A data.frame: 20 × 7

	ID	logFC	AveExpr	t	P.Value	adj.P.Val	B
	<chr>	<dbl>	<dbl>	<dbl>	<dbl>	<dbl>	<dbl>
126	CDV3	0.4613797	1.2229999	9.938467	4.442713e-23	2.665701e-20	38.0565977
247	CD84	-0.3533260	0.4955969	-7.186041	7.565594e-13	1.085915e-10	14.8056108
348	CD164	0.3068021	1.8707070	5.665244	1.543257e-08	9.336323e-07	5.1061422
405	CD36	-0.2896939	0.3246596	-5.085077	3.797517e-07	1.676634e-05	2.0035589
421	CD82	-0.2824216	0.5675827	-6.556442	6.018063e-11	5.985519e-09	10.5158853
508	CD63	-0.2598229	2.2570242	-4.428176	9.688833e-06	2.674366e-04	-1.1086749
513	CD74	0.2591115	2.2728535	3.323134	8.959829e-04	1.289562e-02	-5.3802127
571	CD48	0.2480557	0.8941089	4.562841	5.157164e-06	1.517342e-04	-0.5054093
577	HACD3	0.2477402	1.1536948	5.238582	1.678891e-07	8.128185e-06	2.7922191
816	CD37	-0.2100434	1.4375666	-4.061703	4.939885e-05	1.101796e-03	-2.6594795
940	CD44	0.1974135	1.2309836	3.383897	7.196813e-04	1.079551e-02	-5.1768245
956	CDT1	-0.1961757	0.7378909	-4.642021	3.530970e-06	1.131519e-04	-0.1423327
1012	CD34	0.1893594	1.3150180	3.232176	1.235812e-03	1.669593e-02	-5.6778086
1022	CD38	-0.1886832	0.7399566	-3.793207	1.503217e-04	2.855478e-03	-3.7111988
1040	CD99	0.1868761	1.4580142	3.199527	1.384411e-03	1.840568e-02	-5.7826245
1207	CD55	-0.1723566	0.5686104	-3.611420	3.072609e-04	5.290830e-03	-4.3826659
1280	CD58	-0.1668748	0.5847240	-4.324458	1.556313e-05	4.052797e-04	-1.5610683
1339	BICD1	-0.1613899	1.0190811	-3.251286	1.155817e-03	1.577332e-02	-5.6159664
1683	CD2AP	0.1395765	0.9274637	3.125345	1.785289e-03	2.247794e-02	-6.0168337
1713	CD47	-0.1384016	1.2640750	-2.926076	3.446824e-03	3.835731e-02	-6.6188598

Since it would get very unwieldy if I was to characterize and interpret these populations here, I have done so in an external slide deck, which can be found using this link:

https://docs.google.com/presentation/d/1Jd8VXRSZ-4r_1EfP6tuxpk56tDw5Yt-8lMsy-ucJkSI/edit?usp=sharing

However, I did relabel the populations according to their subsets. Here's the final result: