Figure S2

A

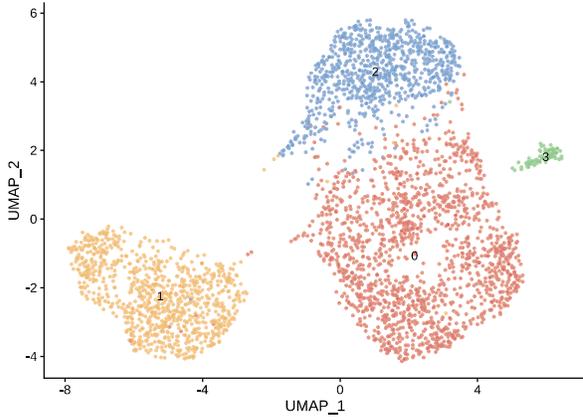### Figure 1 OS-17 by Clusters
Seurat-assigned clusters (optimized)

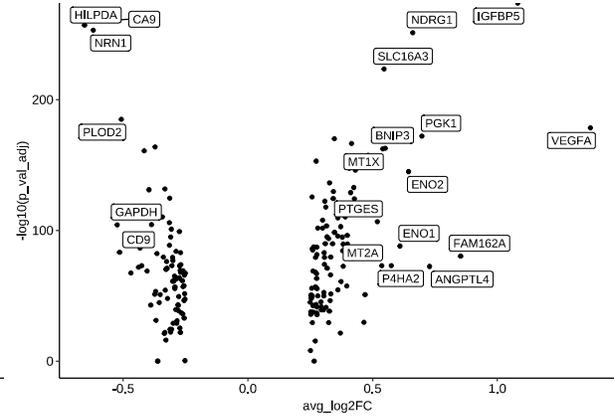### Cluster 0 Genes
Figure 1 OS-17 method

Figure 1 OS-17 Cluster 0
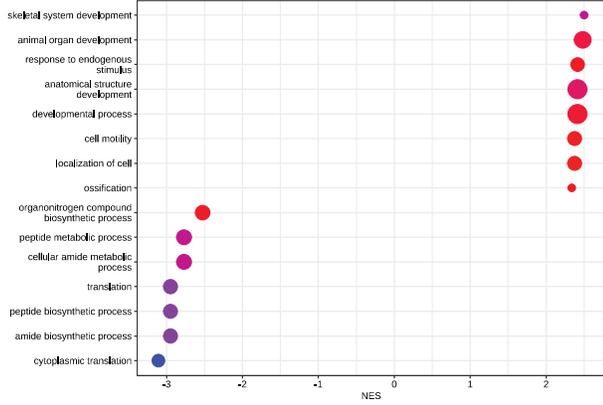GO-BP Gene Sets

Figure 1 OS-17 Cluster 0
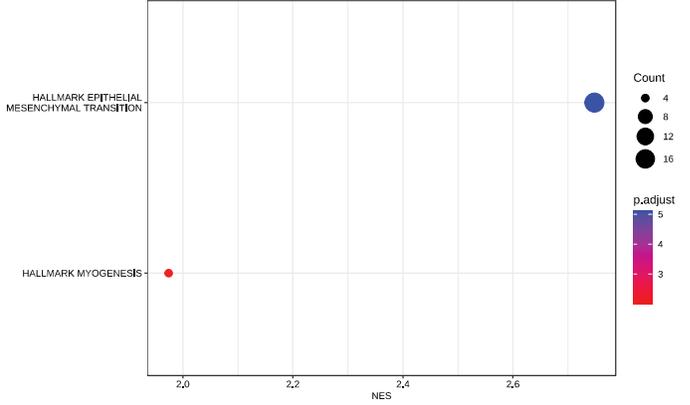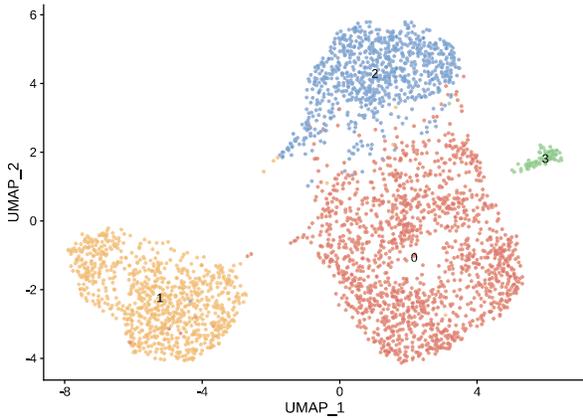Hallmark Gene Sets

Figure 1 OS-17 by Clusters
Seurat-assigned clusters (optimized)
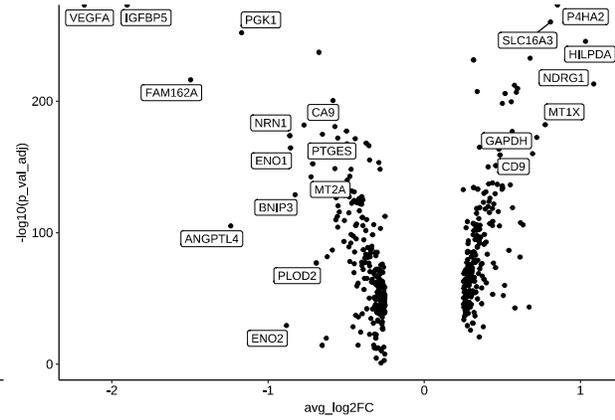

Cluster 1 Genes
Figure 1 OS-17 method

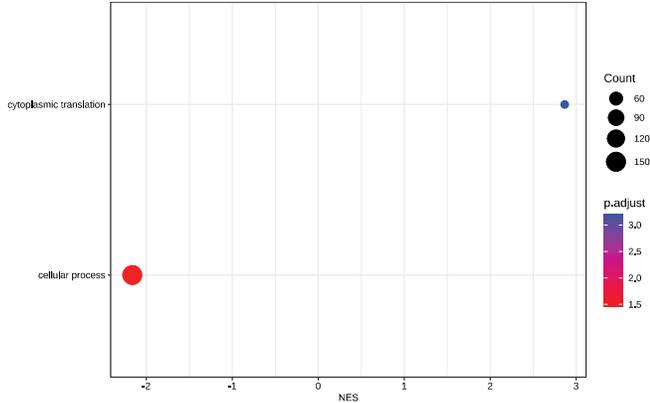

Figure 1 OS-17 Cluster 1
GO-BP Gene Sets

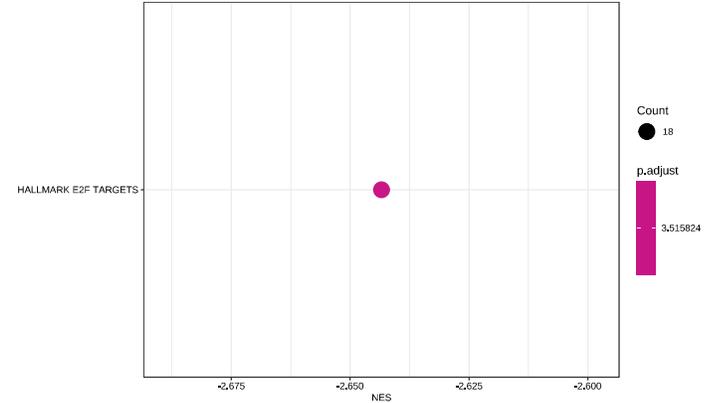### Figure 1 OS-17 Cluster 1
Hallmark Gene Sets

Figure S2 continued

Figure 1 OS-17 by Clusters
Seurat-assigned clusters (optimized)

Cluster 2 Genes
Figure 1 OS-17 method

Figure 1 OS-17 Cluster 2
GO-BP Gene Sets

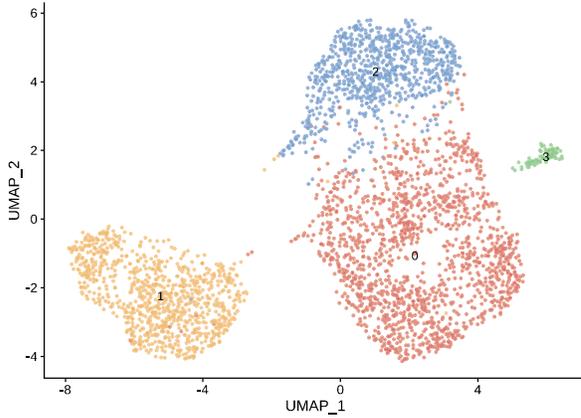### Figure 1 OS-17 Cluster 2
Hallmark Gene Sets

Figure 1 OS-17 by Clusters
Seurat-assigned clusters (optimized)

Cluster 3 Genes
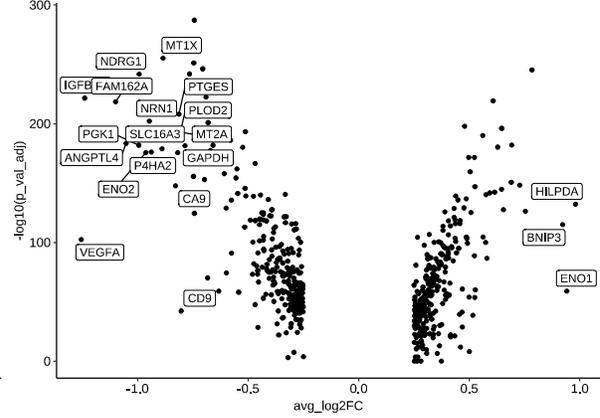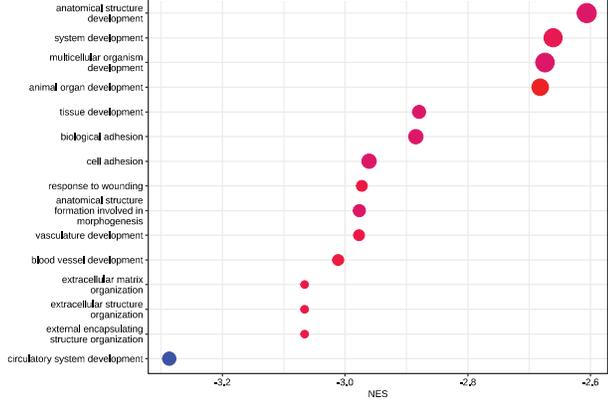Figure 1 OS-17 method

Figure 1 OS-17 Cluster 3
GO-BP Gene Sets

Figure 1 OS-17 Cluster 3
Hallmark Gene Sets
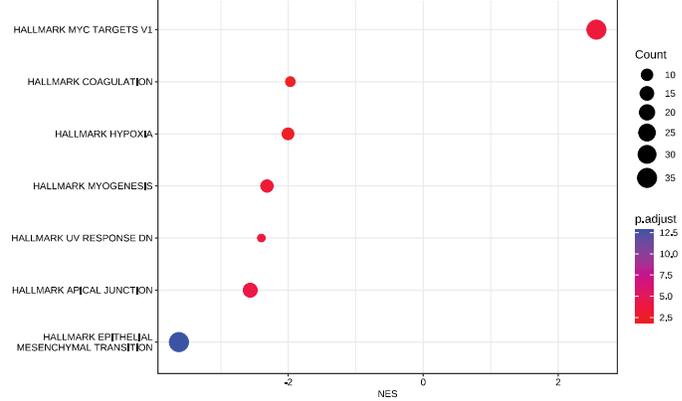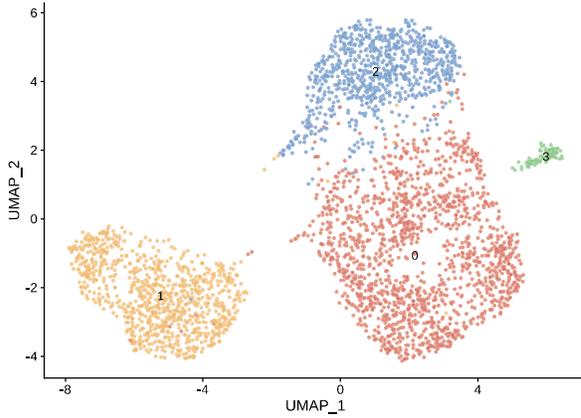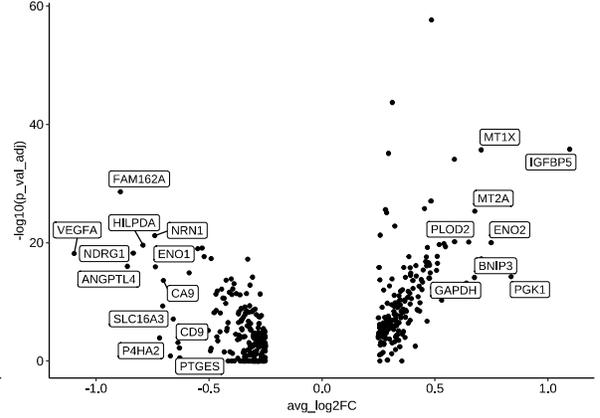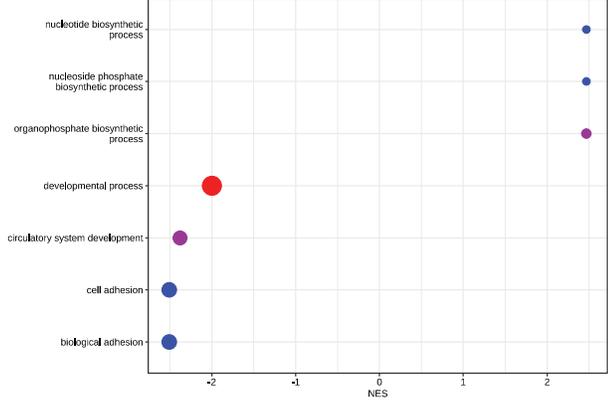
Figure S2 continued

B

Figure 1 NCH-OS-7 by Clusters
Seurat-assigned clusters (optimized)

Cluster 0 Genes
Figure 1 NCH-OS-7 method

Figure 1 NCH-OS-7 Cluster 0
GO-BP Gene Sets

Figure 1 NCH-OS-7 Cluster 0
Hallmark Gene Sets
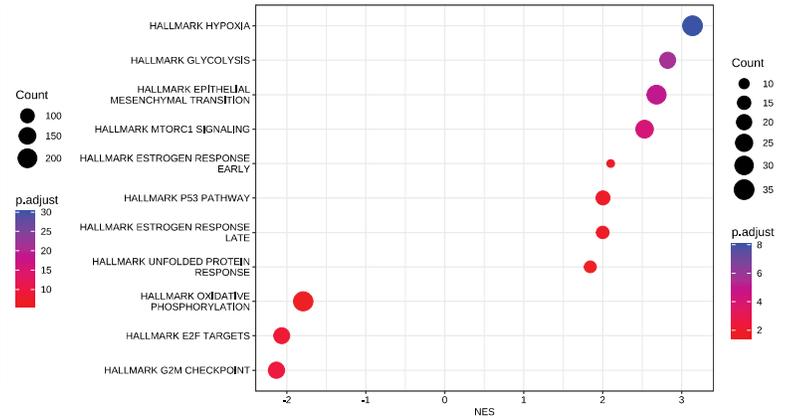
Figure 1 NCH-OS-7 by Clusters
Seurat-assigned clusters (optimized)

Cluster 1 Genes
Figure 1 NCH-OS-7 method

Figure 1 NCH-OS-7 Cluster 1
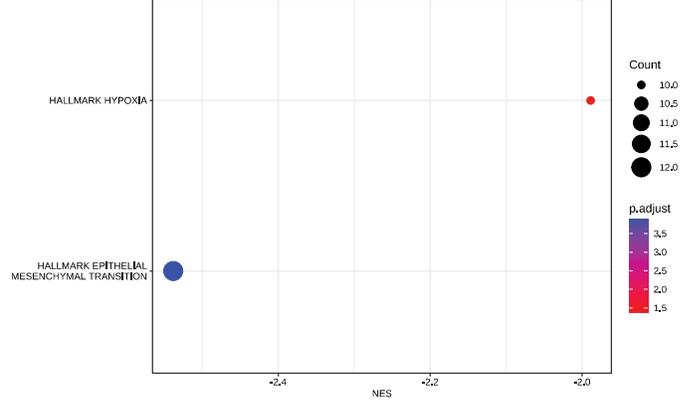GO-BP Gene Sets

Figure 1 NCH-OS-7 Cluster 1
Hallmark Gene Sets

Figure S2 continued

Figure 1 NCH-OS-7 by Clusters
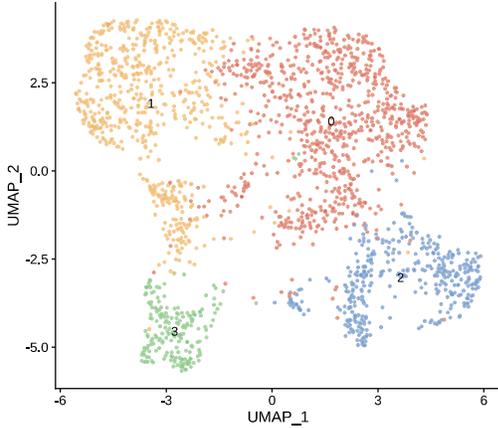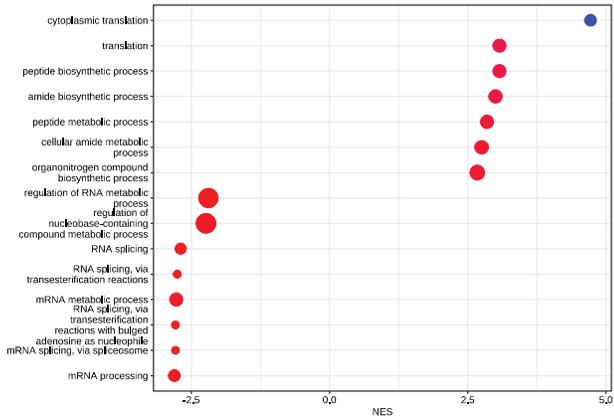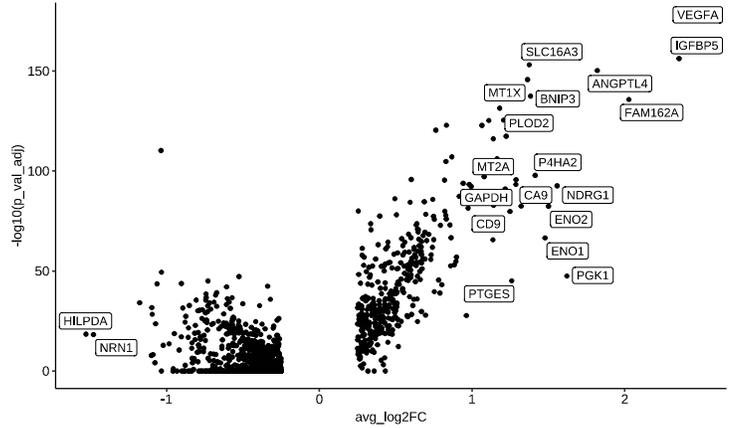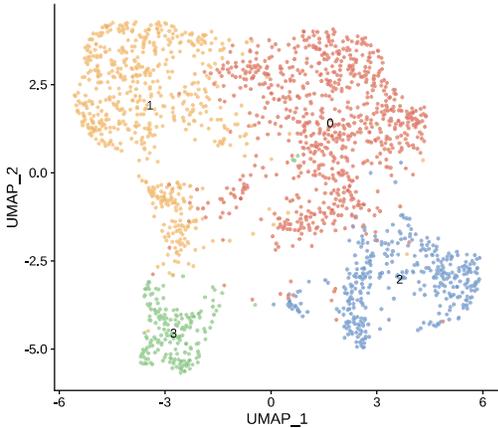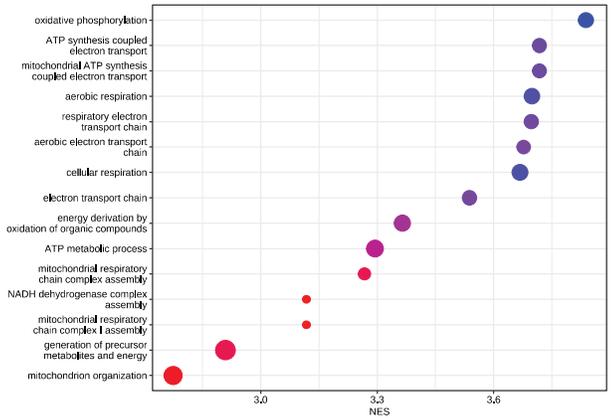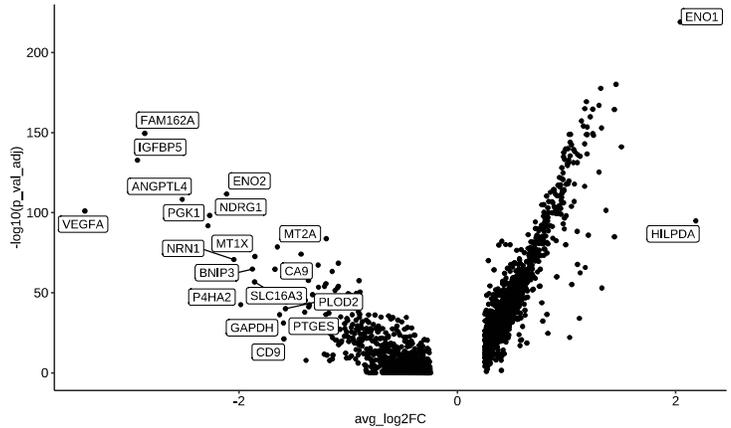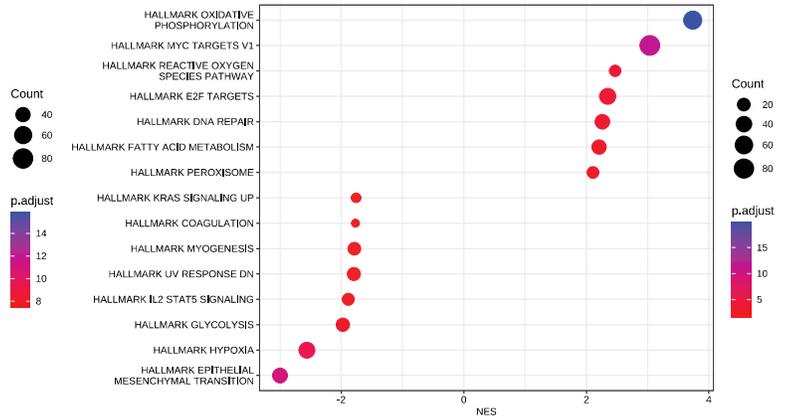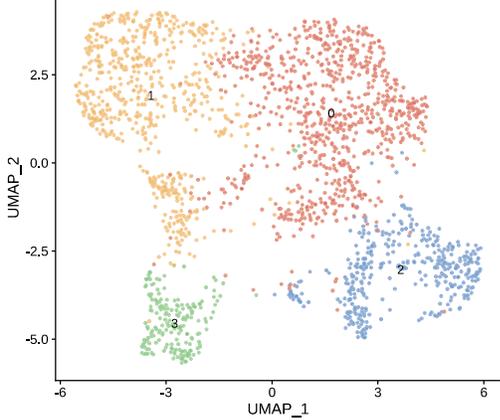Seurat-assigned clusters (optimized)
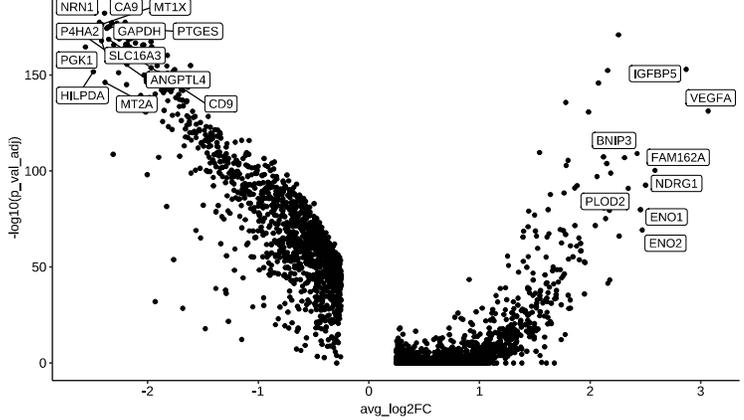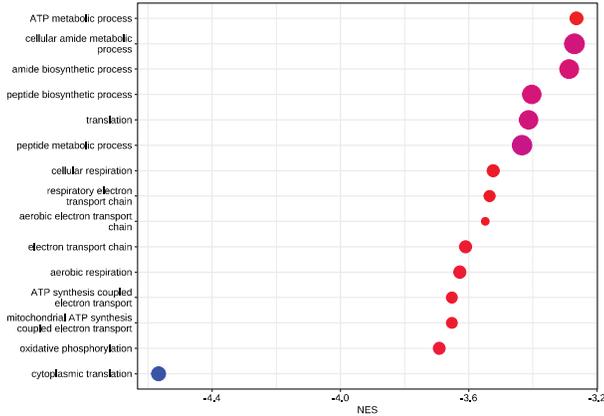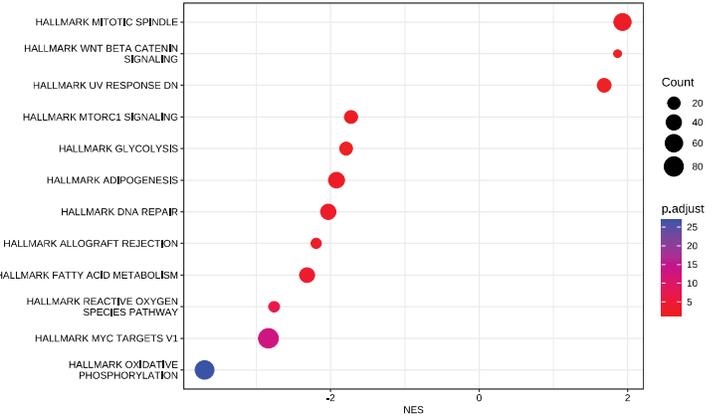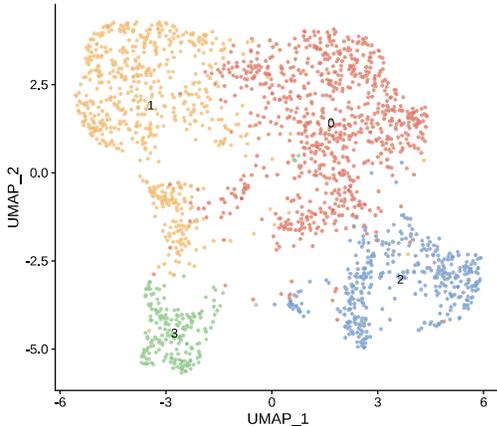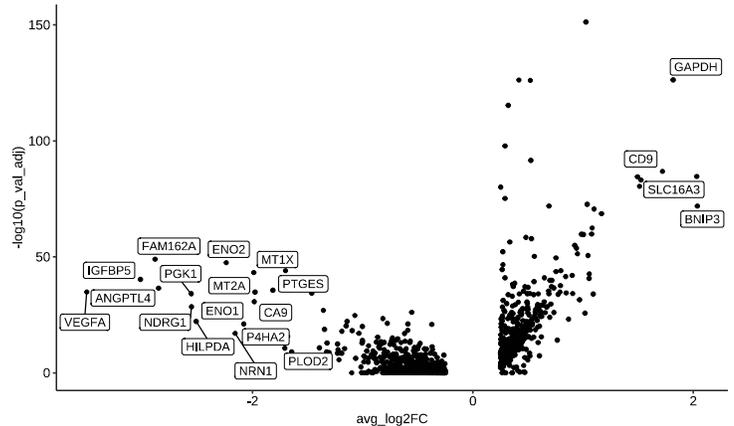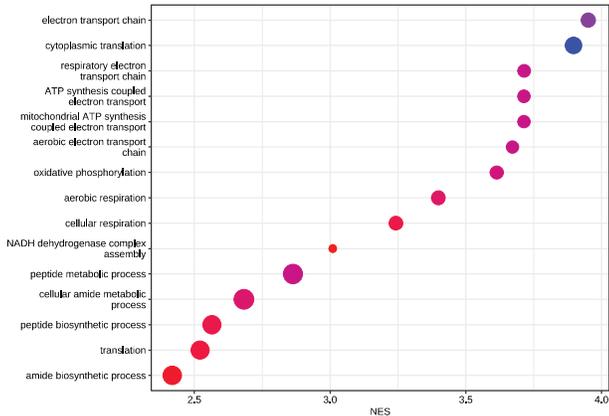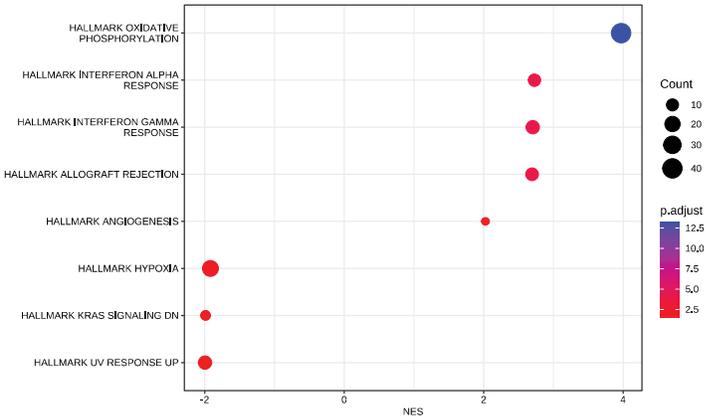
Cluster 2 Genes
Figure 1 NCH-OS-7 method

Labeled genes (left): NRN1, CA9, MT1X, P4HA2, GAPDH, PTGES, PGK1, SLC16A3, HILPDA, ANGPTL4, MT2A, CD9

Labeled genes (right): IGFBP5, VEGFA, BNIP3, FAM162A, NDRG1, PLOD2, ENO1, ENO2

Figure 1 NCH-OS-7 Cluster 2
GO-BP Gene Sets

- ATP metabolic process
- cellular amide metabolic process
- amide biosynthetic process
- peptide biosynthetic process
- translation
- peptide metabolic process
- cellular respiration
- respiratory electron transport chain
- aerobic electron transport chain
- electron transport chain
- aerobic respiration
- ATP synthesis coupled electron transport
- mitochondrial ATP synthesis coupled electron transport
- oxidative phosphorylation
- cytoplasmic translation

Figure 1 NCH-OS-7 Cluster 2
Hallmark Gene Sets

- HALLMARK MITOTIC SPINDLE
- HALLMARK WNT BETA CATENIN SIGNALING
- HALLMARK UV RESPONSE DN
- HALLMARK MTORC1 SIGNALING
- HALLMARK GLYCOLYSIS
- HALLMARK ADIPOGENESIS
- HALLMARK DNA REPAIR
- HALLMARK ALLOGRAFT REJECTION
- HALLMARK FATTY ACID METABOLISM
- HALLMARK REACTIVE OXYGEN SPECIES PATHWAY
- HALLMARK MYC TARGETS V1
- HALLMARK OXIDATIVE PHOSPHORYLATION

Figure 1 NCH-OS-7 by Clusters
Seurat-assigned clusters (optimized)

Cluster 3 Genes
Figure 1 NCH-OS-7 method

Labeled genes (right): GAPDH, CD9, SLC16A3, BNIP3

Labeled genes (left): FAM162A, ENO2, MT1X, IGFBP5, PGK1, MT2A, PTGES, ANGPTL4, ENO1, CA9, VEGFA, NDRG1, P4HA2, HILPDA, PLOD2, NRN1

Figure 1 NCH-OS-7 Cluster 3
GO-BP Gene Sets

- electron transport chain
- cytoplasmic translation
- respiratory electron transport chain
- ATP synthesis coupled electron transport
- mitochondrial ATP synthesis coupled electron transport
- aerobic electron transport chain
- oxidative phosphorylation
- aerobic respiration
- cellular respiration
- NADH dehydrogenase complex assembly
- peptide metabolic process
- cellular amide metabolic process
- peptide biosynthetic process
- translation
- amide biosynthetic process

Figure 1 NCH-OS-7 Cluster 3
Hallmark Gene Sets

- HALLMARK OXIDATIVE PHOSPHORYLATION
- HALLMARK INTERFERON ALPHA RESPONSE
- HALLMARK INTERFERON GAMMA RESPONSE
- HALLMARK ALLOGRAFT REJECTION
- HALLMARK ANGIOGENESIS
- HALLMARK HYPOXIA
- HALLMARK KRAS SIGNALING DN
- HALLMARK UV RESPONSE UP

Figure S2 continued

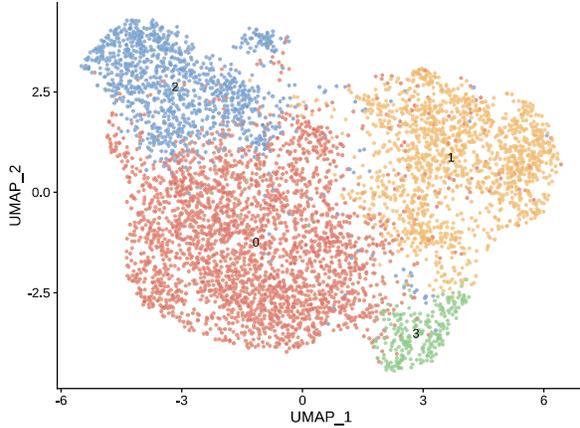# Figure S2 continued

Figure 3 OS-17 by Clusters
Seurat-assigned clusters (optimized)

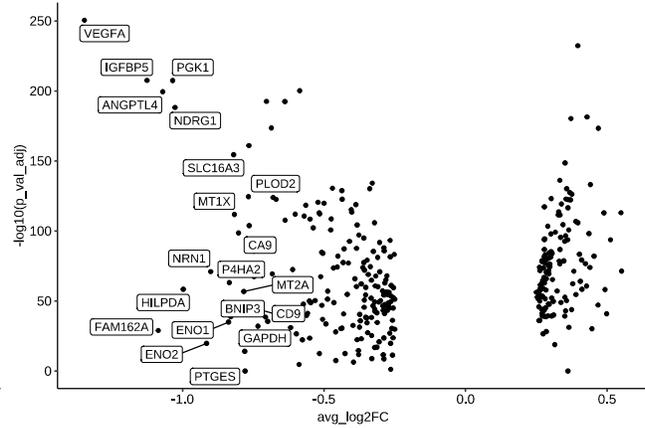Cluster 2 Genes
Figure 3 OS-17 method
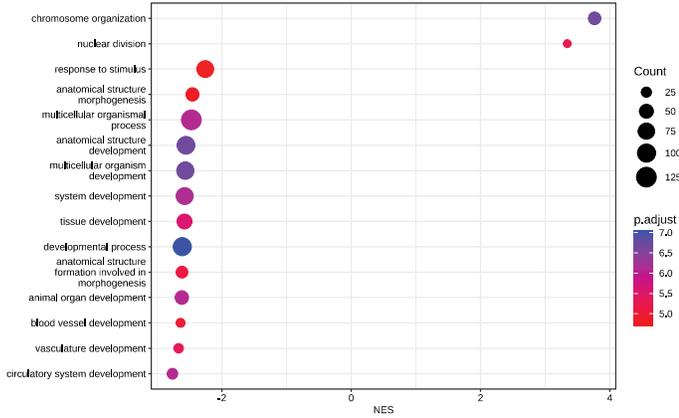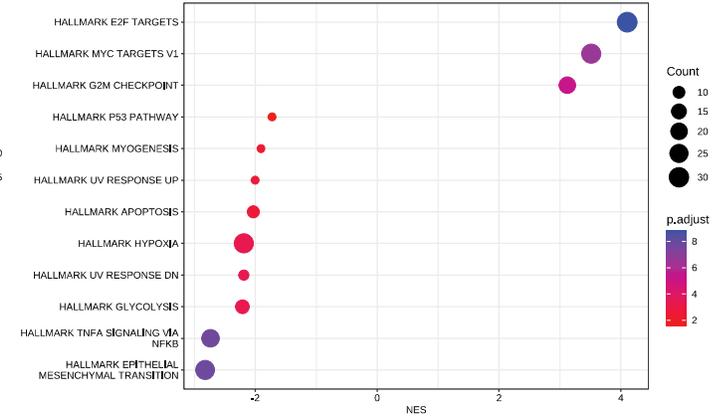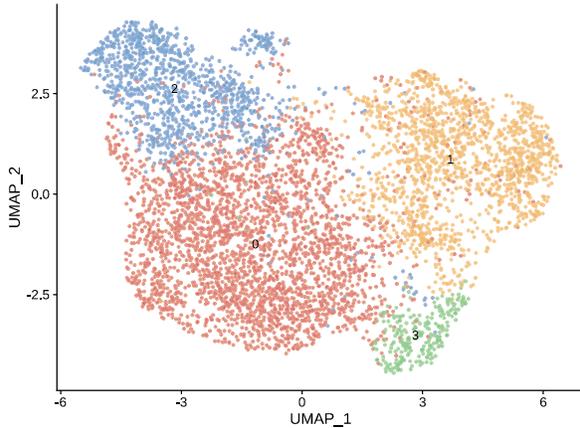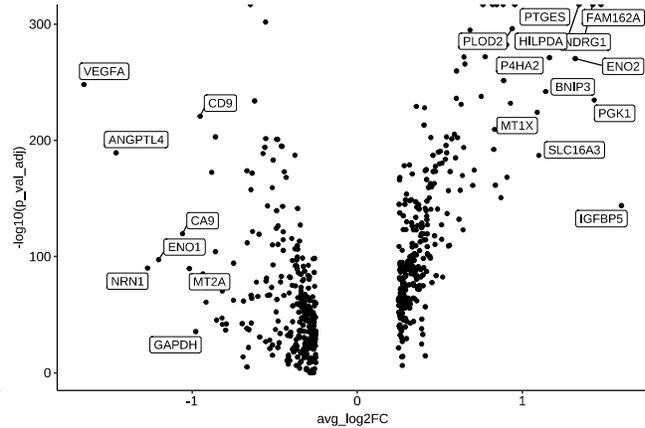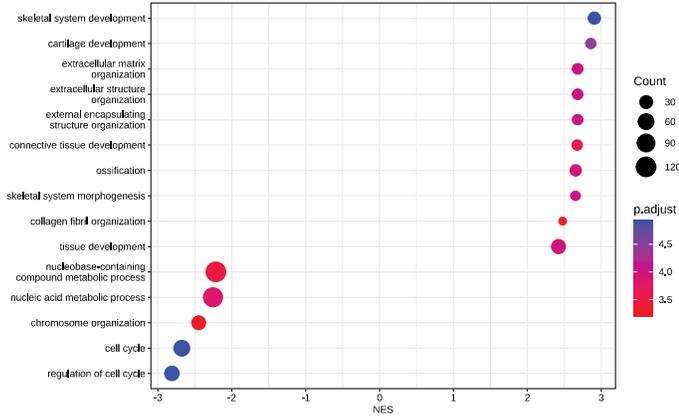

Figure 3 OS-17 Cluster 2
GO-BP Gene Sets

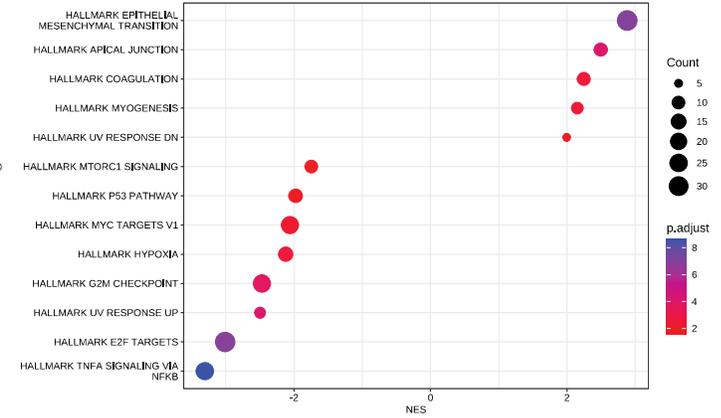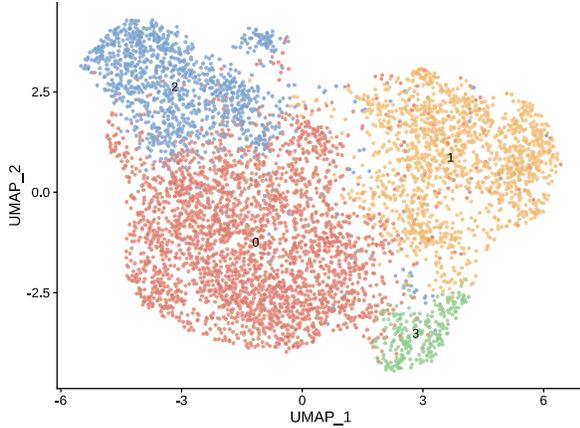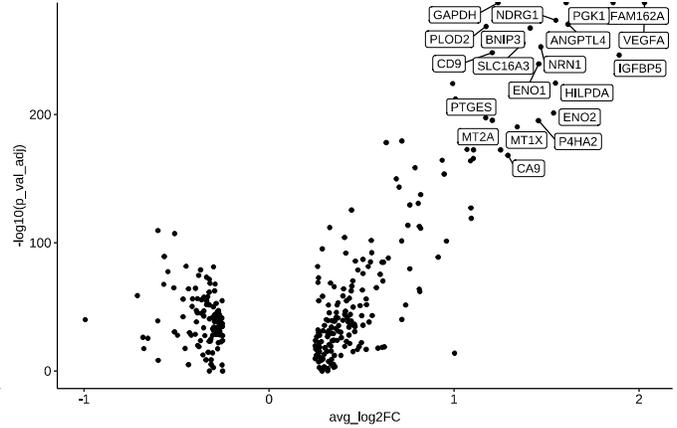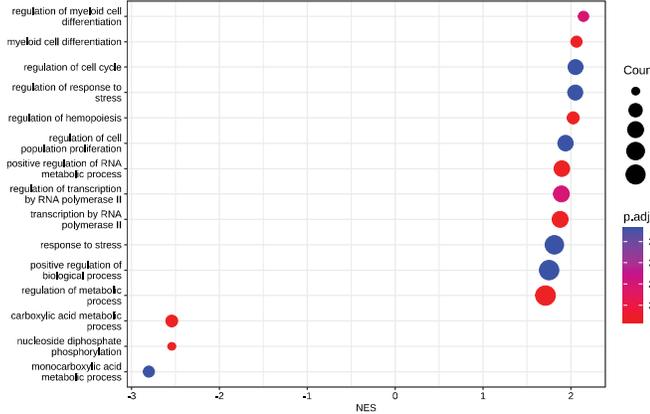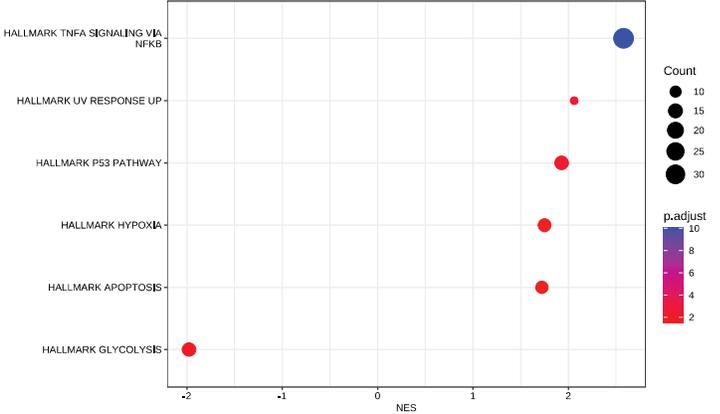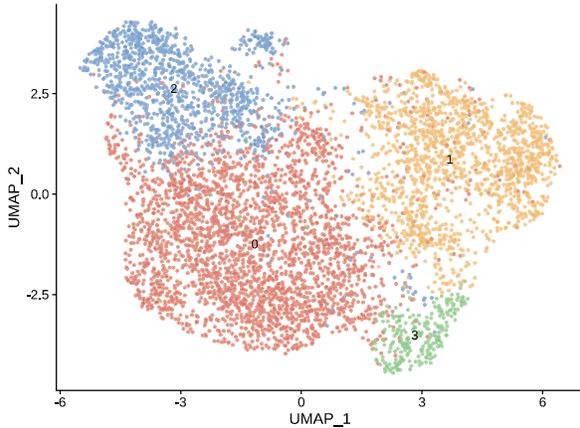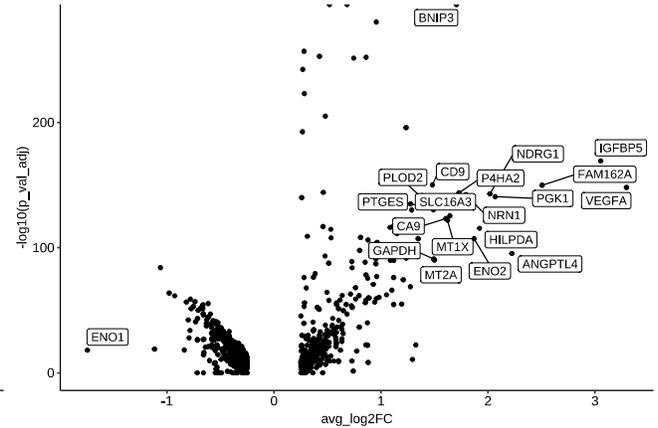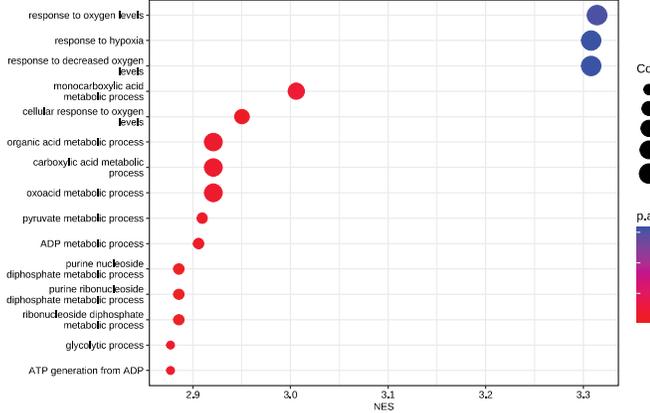Figure 3 OS-17 Cluster 2
Hallmark Gene Sets

Figure 3 OS-17 by Clusters
Seurat-assigned clusters (optimized)

Cluster 3 Genes
Figure 3 OS-17 method

Figure 3 OS-17 Cluster 3
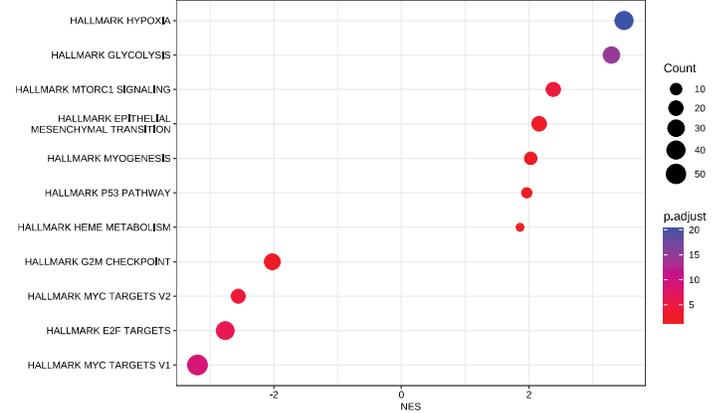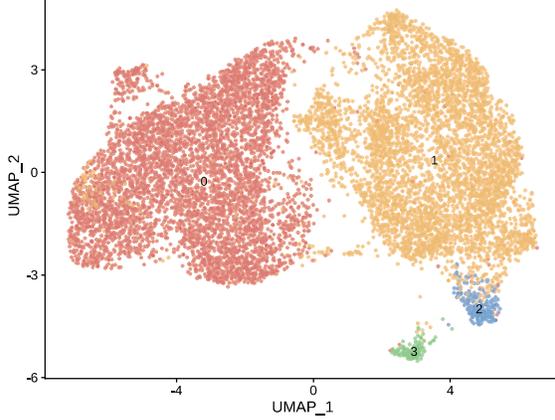GO-BP Gene Sets

Figure 3 OS-17 Cluster 3
Hallmark Gene Sets

Figure S2 continued

D

Figure 3 143B by Clusters
Seurat-assigned clusters (optimized)

Cluster 0 Genes
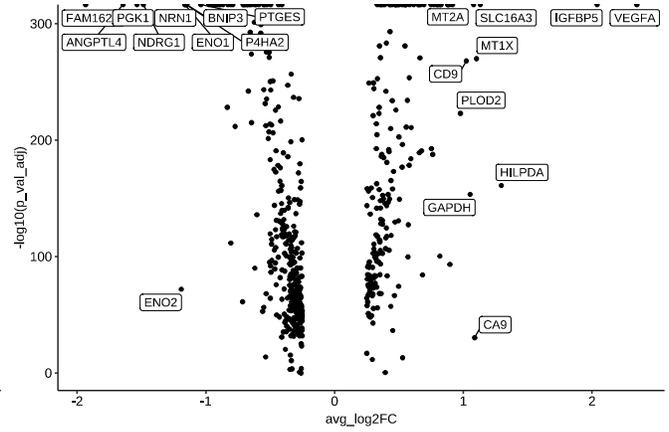Figure 3 143B method

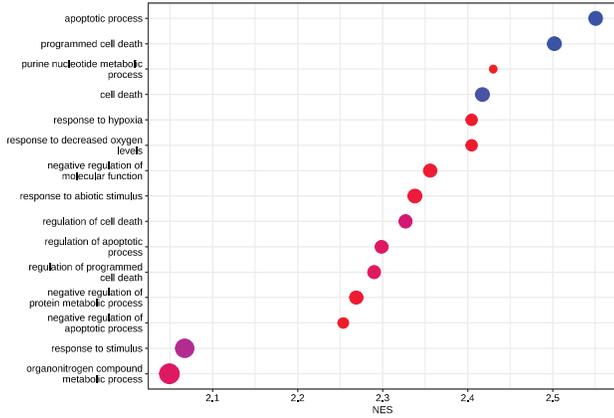Figure 3 143B Cluster 0
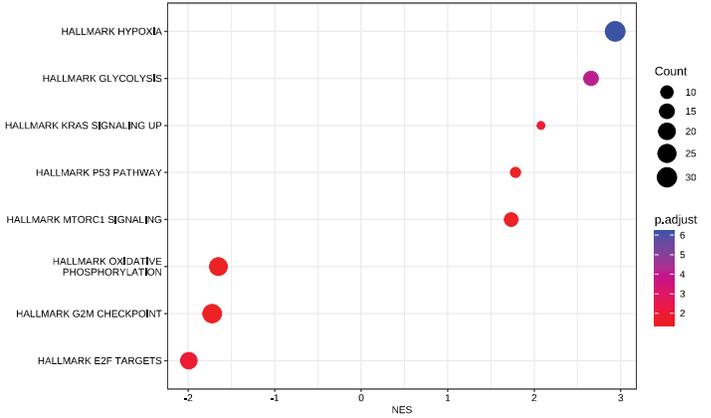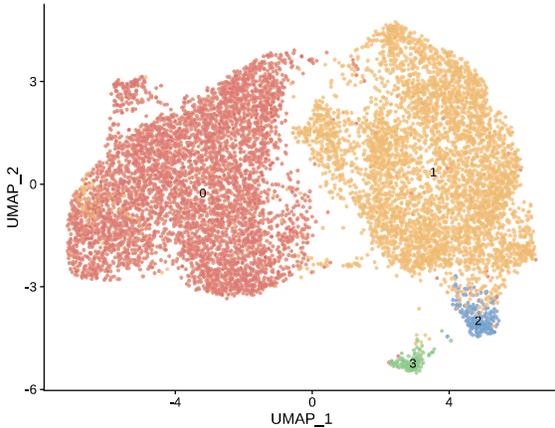GO-BP Gene Sets

Figure 3 143B Cluster 0
Hallmark Gene Sets

Figure 3 143B by Clusters
Seurat-assigned clusters (optimized)

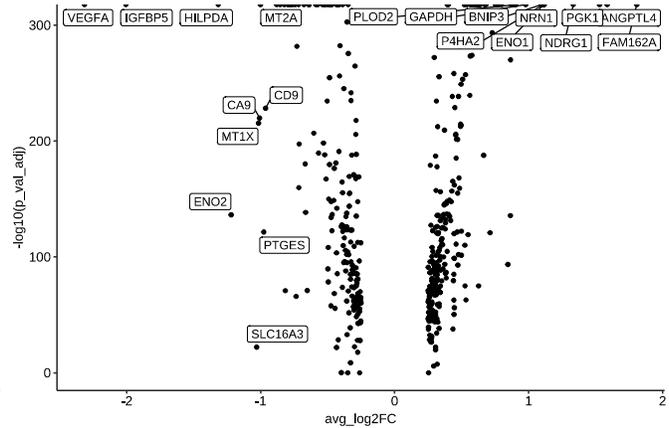Cluster 1 Genes
Figure 3 143B method

Figure 3 143B Cluster 1
GO-BP Gene Sets

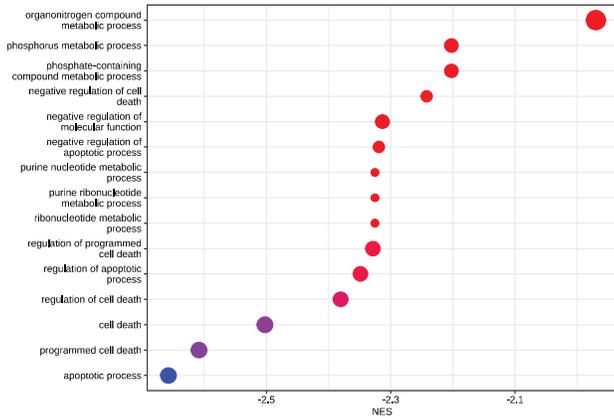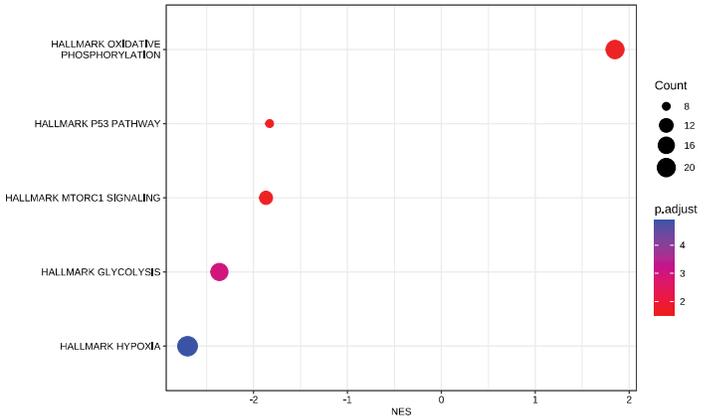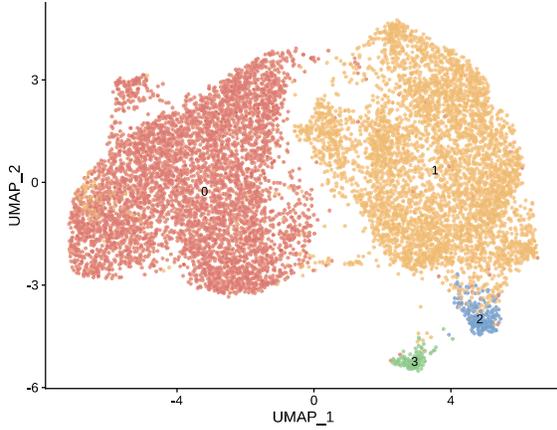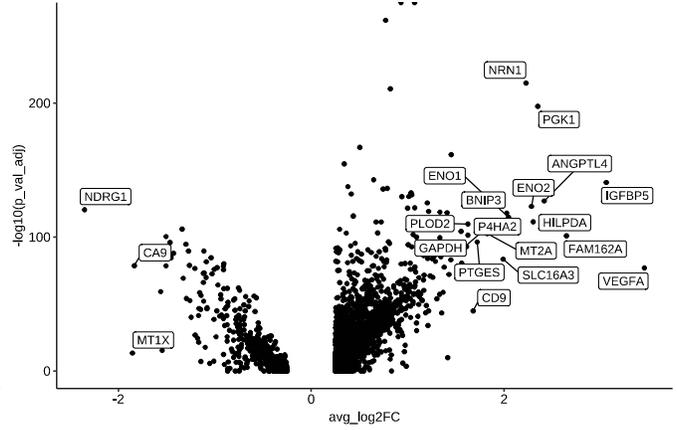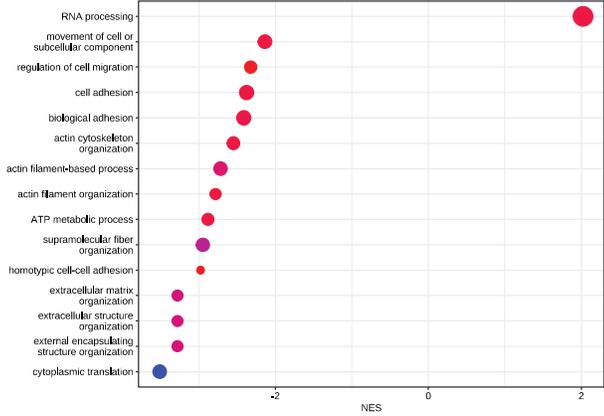Figure 3 143B Cluster 1
Hallmark Gene Sets

Figure S2 continued

Figure 3 143B by Clusters
Seurat-assigned clusters (optimized)

Cluster 2 Genes
Figure 3 143B method

Figure 3 143B Cluster 2
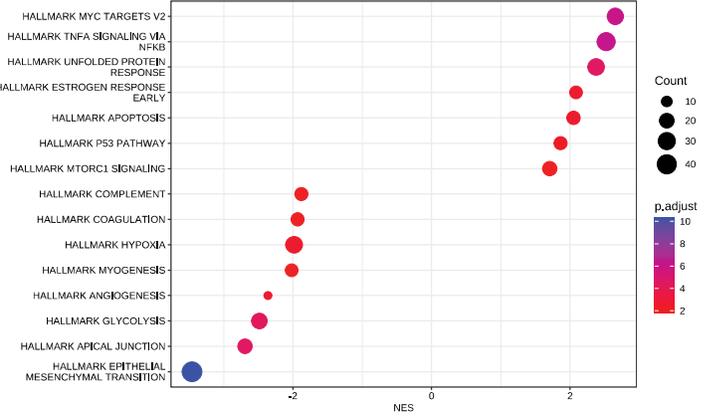GO-BP Gene Sets

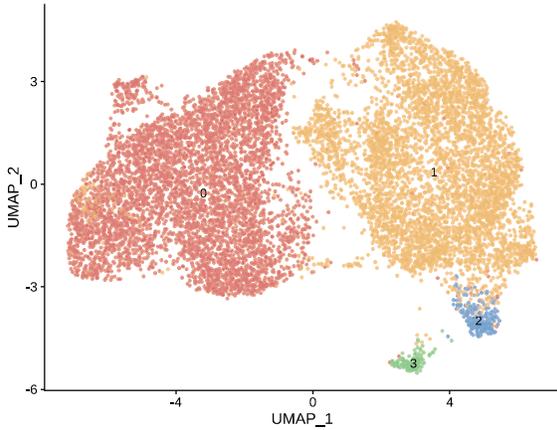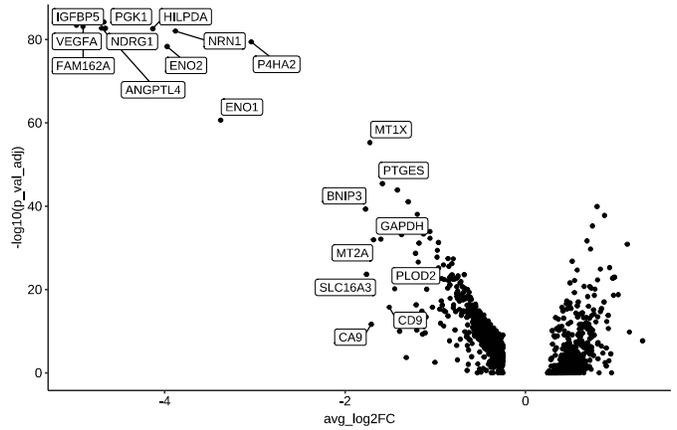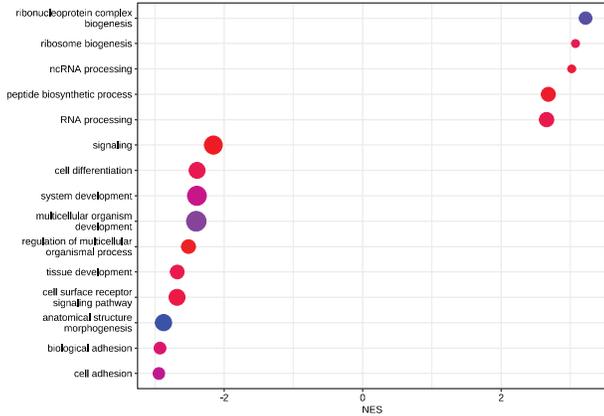Figure 3 143B Cluster 2
Hallmark Gene Sets

Figure 3 143B by Clusters
Seurat-assigned clusters (optimized)

Cluster 3 Genes
Figure 3 143B method

Figure 3 143B Cluster 3
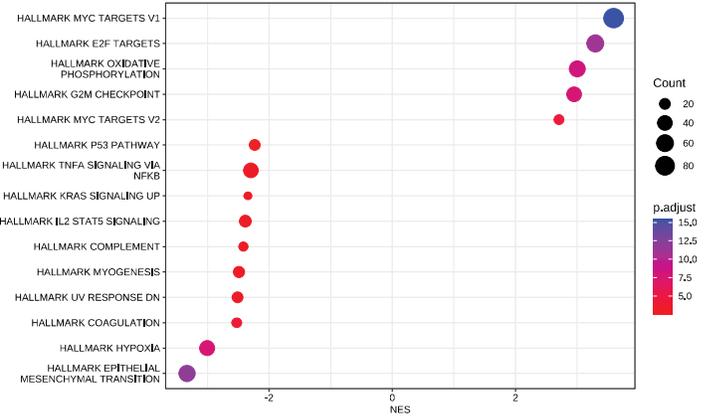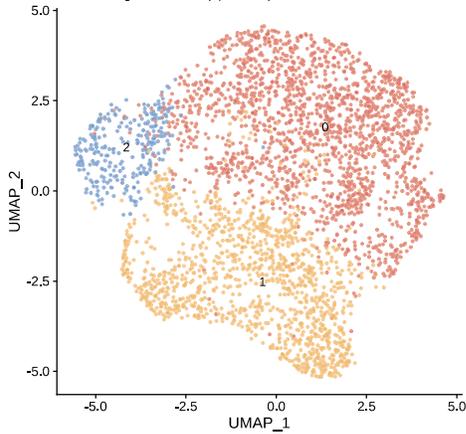GO-BP Gene Sets

Figure 3 143B Cluster 3
Hallmark Gene Sets

Figure S2 continued

E

Figure 3 NCH-OS-2 by Clusters
Seurat-assigned clusters (optimized)

Cluster 0 Genes
Figure 3 NCH-OS-2 method

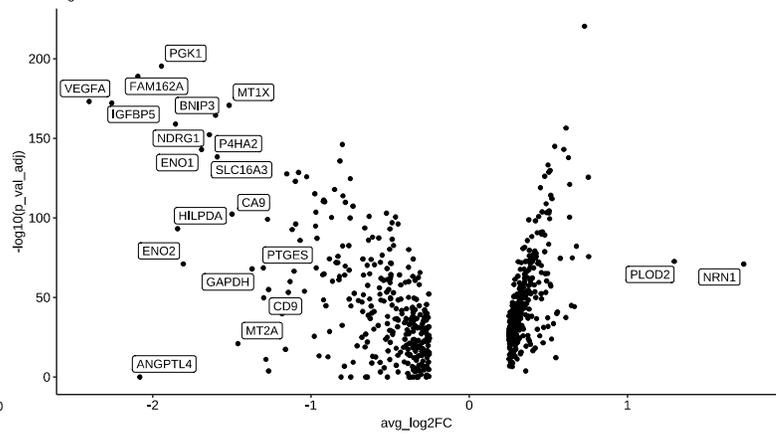### Figure 3 NCH-OS-2 Cluster 0
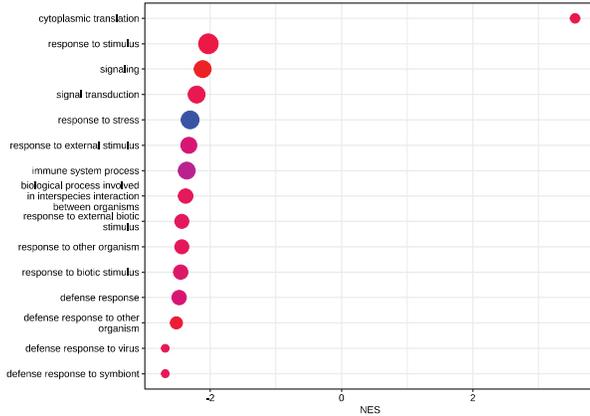GO-BP Gene Sets

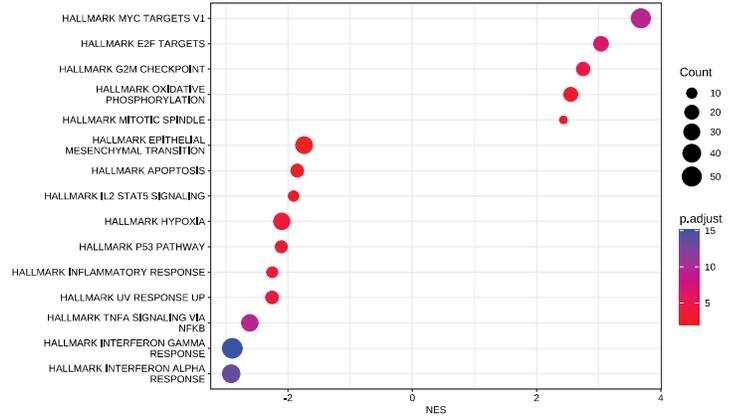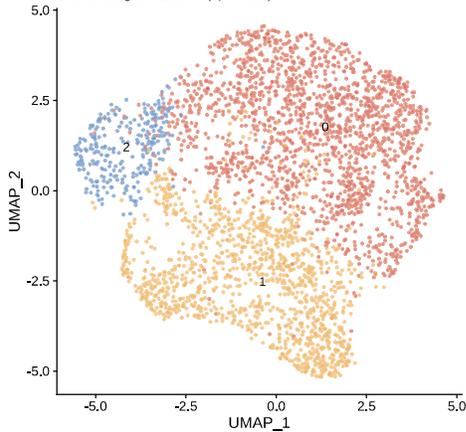### Figure 3 NCH-OS-2 Cluster 0
Hallmark Gene Sets

Figure 3 NCH-OS-2 by Clusters
Seurat-assigned clusters (optimized)
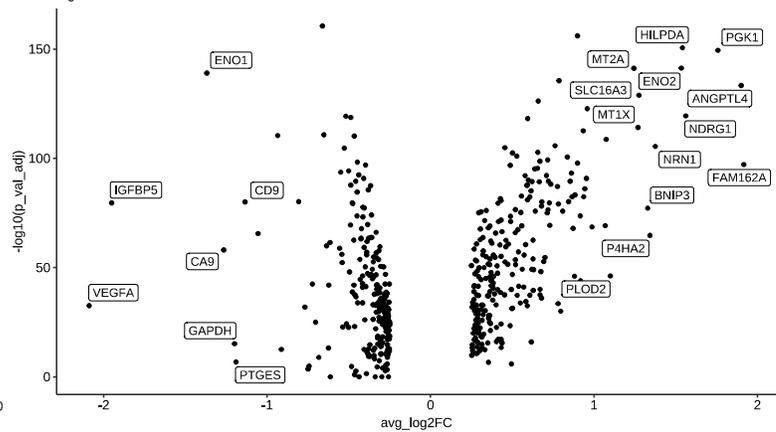
Cluster 1 Genes
Figure 3 NCH-OS-2 method

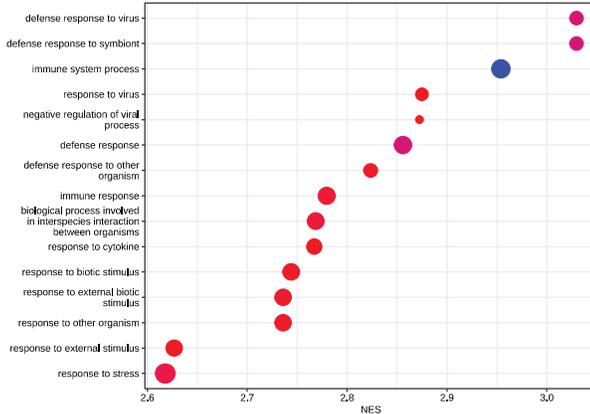### Figure 3 NCH-OS-2 Cluster 1
GO-BP Gene Sets

Figure 3 NCH-OS-2 Cluster 1
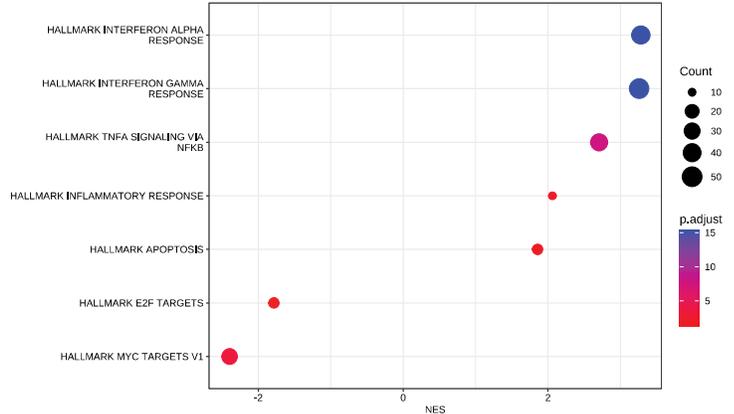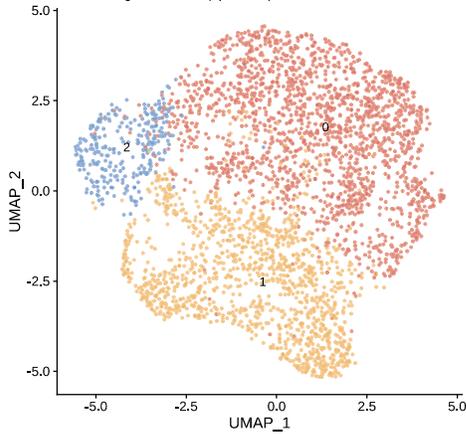Hallmark Gene Sets

Figure S2 continued

Figure 3 NCH-OS-2 by Clusters
Seurat-assigned clusters (optimized)

Cluster 2 Genes
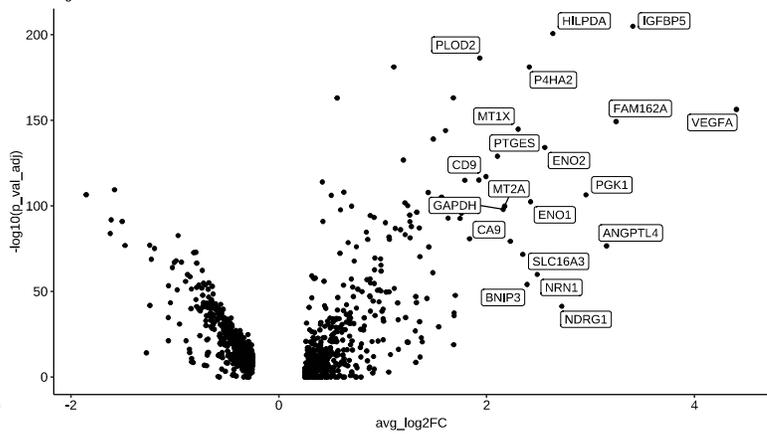Figure 3 NCH-OS-2 method

Figure 3 NCH-OS-2 Cluster 2
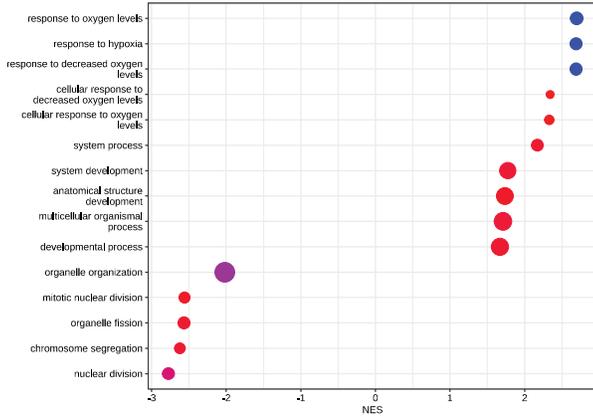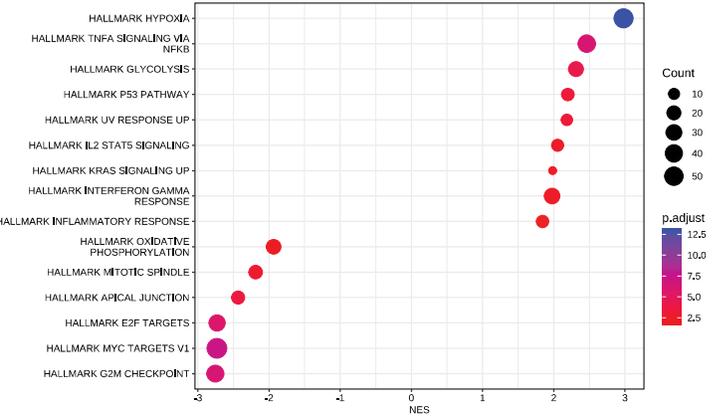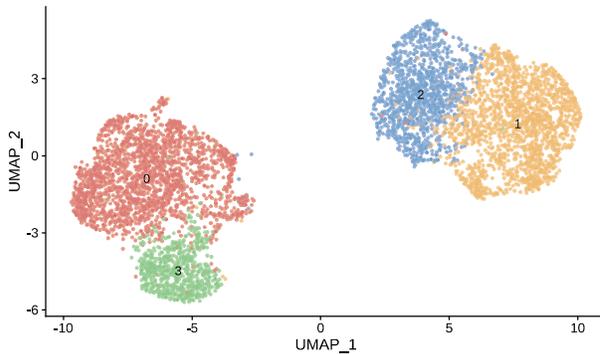GO-BP Gene Sets

Figure 3 NCH-OS-2 Cluster 2
Hallmark Gene Sets

F

Figure 3 NCH-OS-7 by Clusters
Seurat-assigned clusters (optimized)

Cluster 0 Genes
Figure 3 NCH-OS-7 method

Figure 3 NCH-OS-7 Cluster 0
GO-BP Gene Sets

Figure 3 NCH-OS-7 Cluster 0
Hallmark Gene Sets

Figure S2 continued

Figure 3 NCH-OS-7 by Clusters
Seurat-assigned clusters (optimized)

Cluster 1 Genes
Figure 3 NCH-OS-7 method

Figure 3 NCH-OS-7 Cluster 1
GO-BP Gene Sets

Figure 3 NCH-OS-7 Cluster 1
Hallmark Gene Sets

Figure 3 NCH-OS-7 by Clusters
Seurat-assigned clusters (optimized)

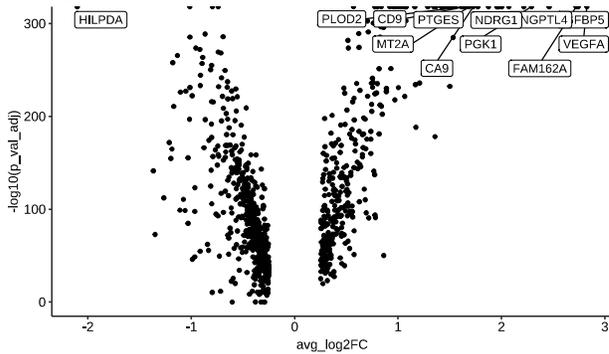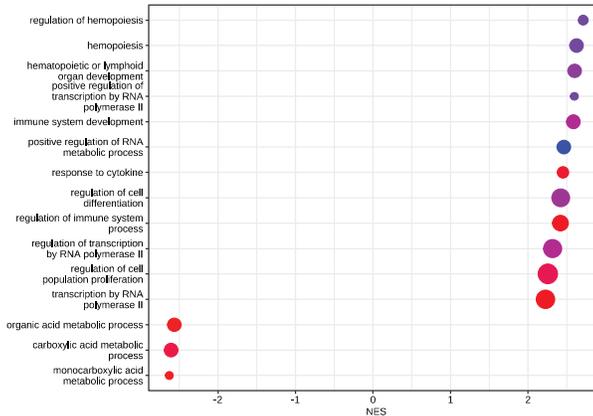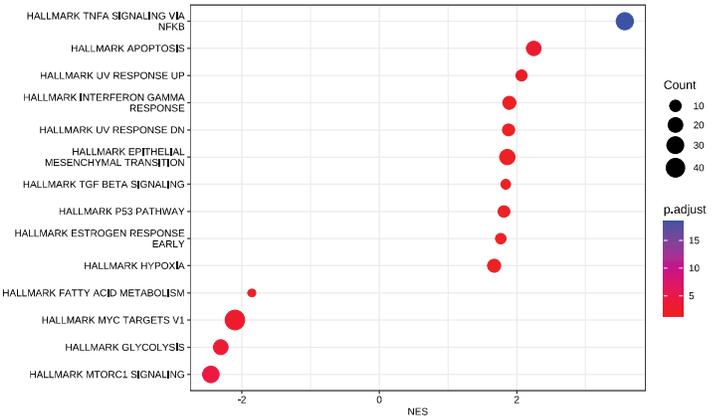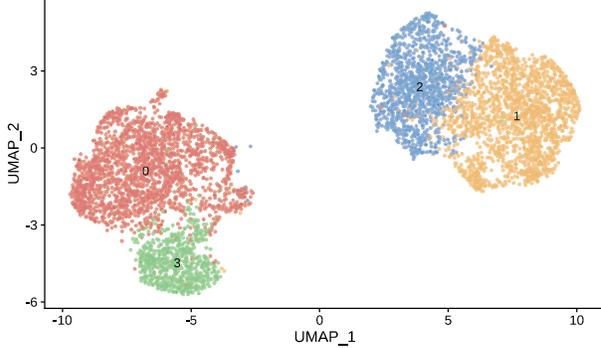## Cluster 2 Genes
Figure 3 NCH-OS-7 method

Figure 3 NCH-OS-7 Cluster 2
GO-BP Gene Sets

Figure 3 NCH-OS-7 Cluster 2
Hallmark Gene Sets

Figure S2 continued

Figure 3 NCH-OS-7 by Clusters
Seurat-assigned clusters (optimized)
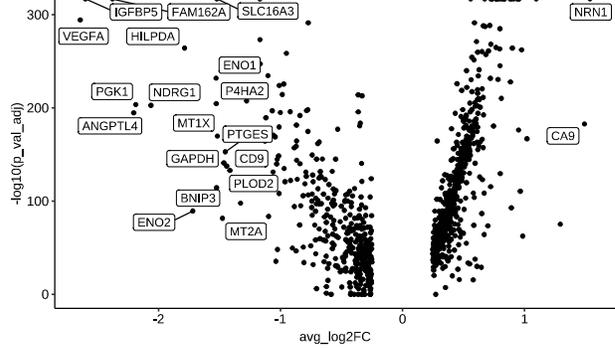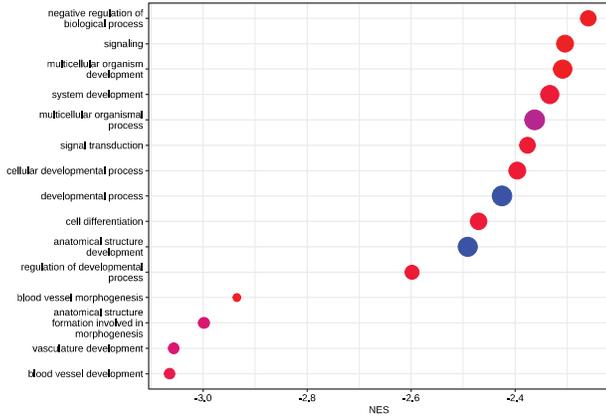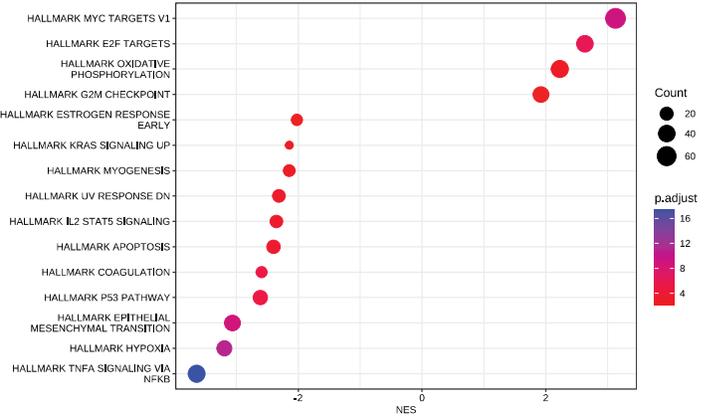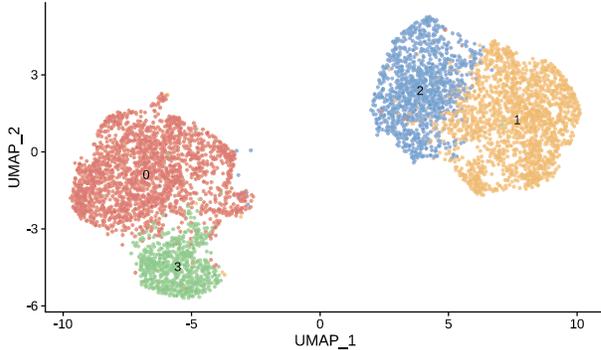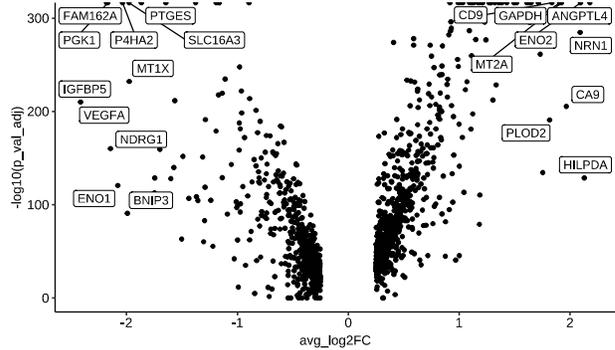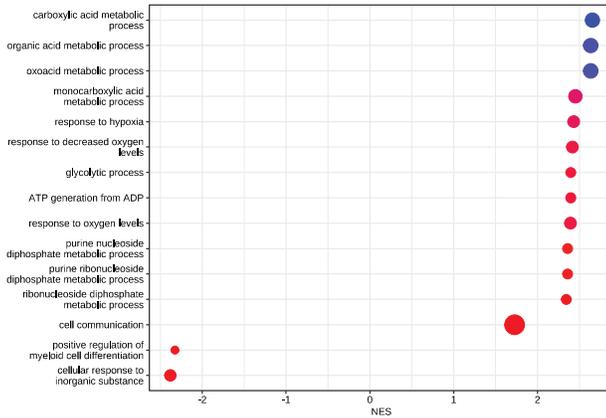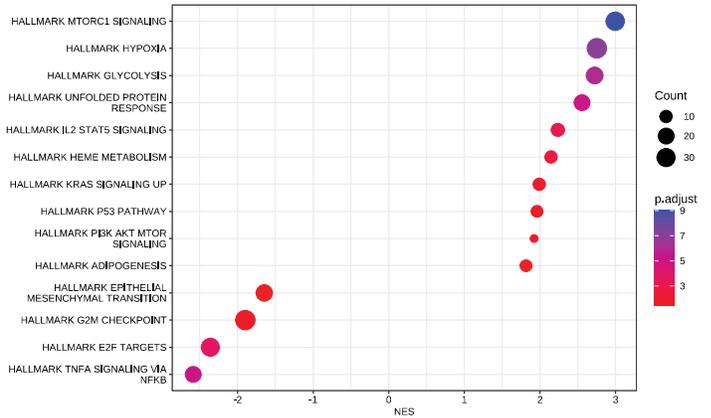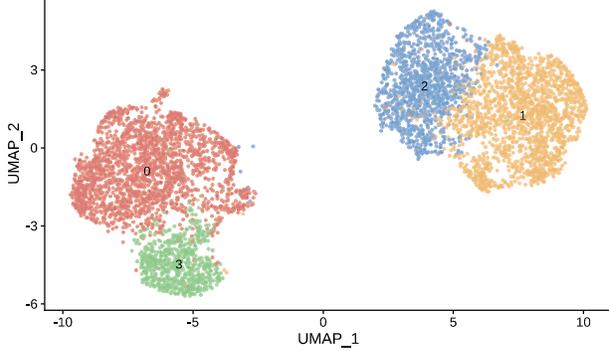

Cluster 3 Genes
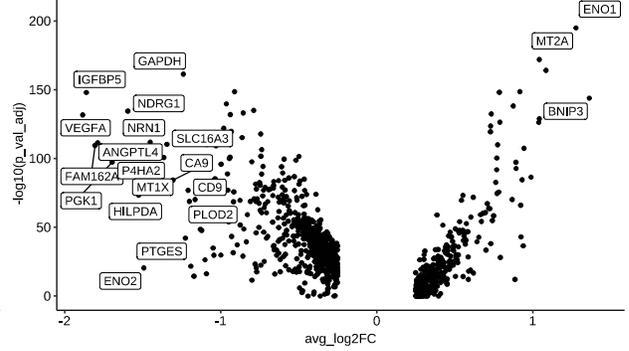Figure 3 NCH-OS-7 method

Figure 3 NCH-OS-7 Cluster 3
GO-BP Gene Sets

Figure 3 NCH-OS-7 Cluster 3
Hallmark Gene Sets

G

Figure 5 OS-17 by Clusters
Seurat-assigned clusters (optimized)

Cluster 0 Genes
Figure 5 OS-17 method

Figure 5 OS-17 Cluster 0
GO-BP Gene Sets

Figure 5 OS-17 Cluster 0
Hallmark Gene Sets

Figure S2 continued

Figure 5 OS-17 by Clusters
Seurat-assigned clusters (optimized)

Cluster 1 Genes
Figure 5 OS-17 method

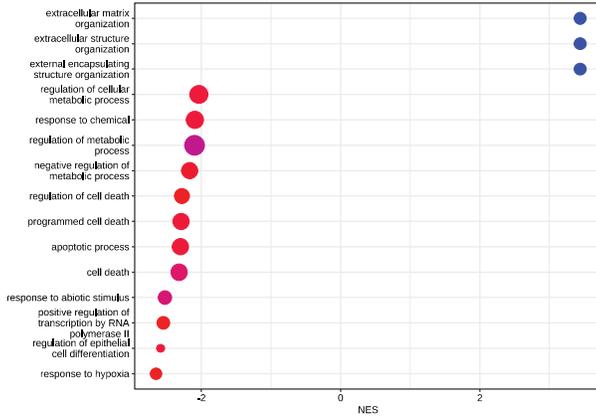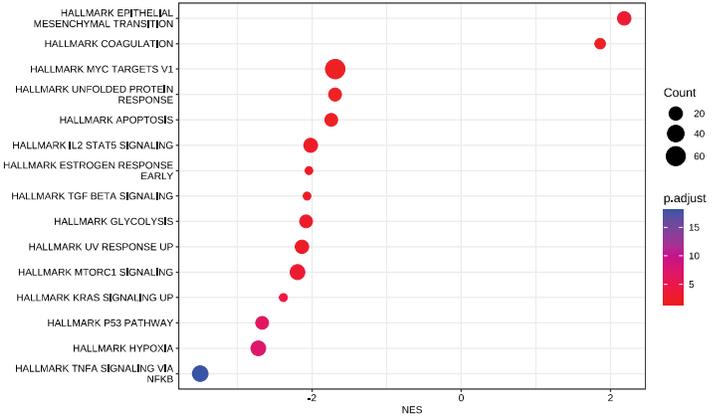### Figure 5 OS-17 Cluster 1
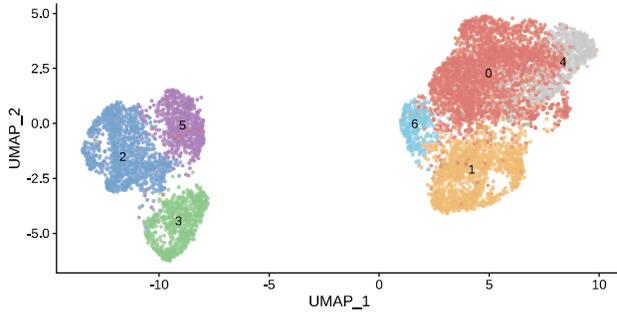GO-BP Gene Sets

Figure 5 OS-17 Cluster 1
Hallmark Gene Sets

Figure 5 OS-17 by Clusters
Seurat-assigned clusters (optimized)

Cluster 2 Genes
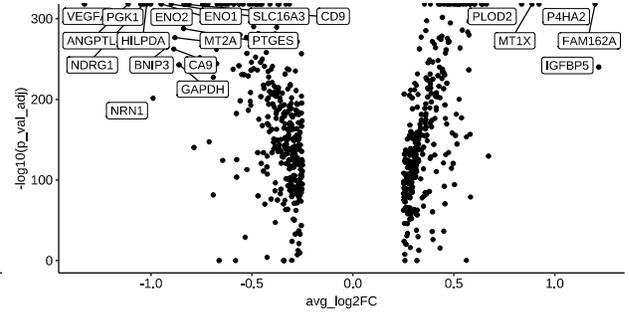Figure 5 OS-17 method
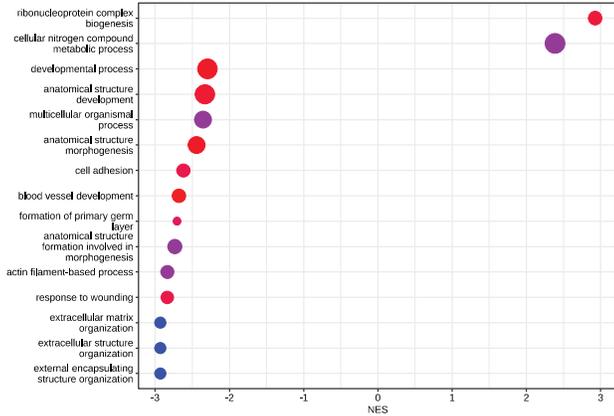

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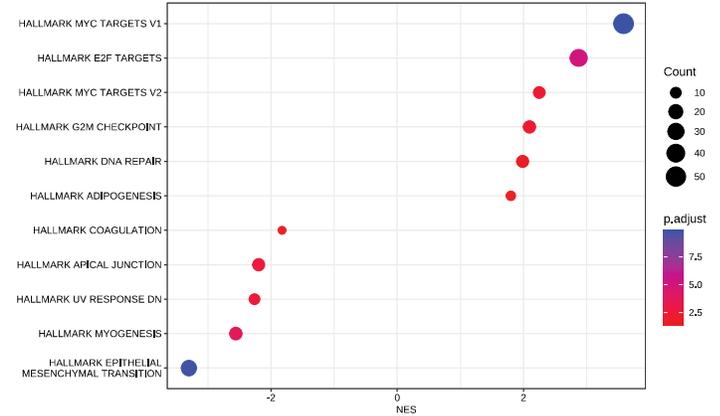GO-BP Gene Sets

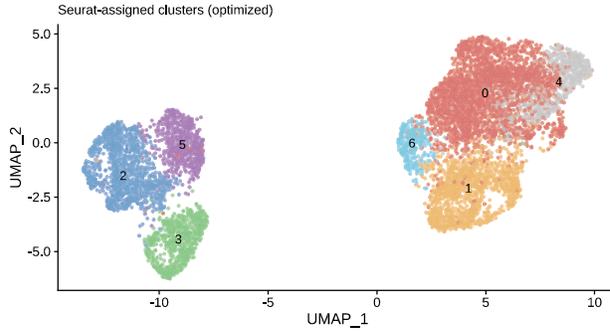### Figure 5 OS-17 Cluster 2
Hallmark Gene Sets
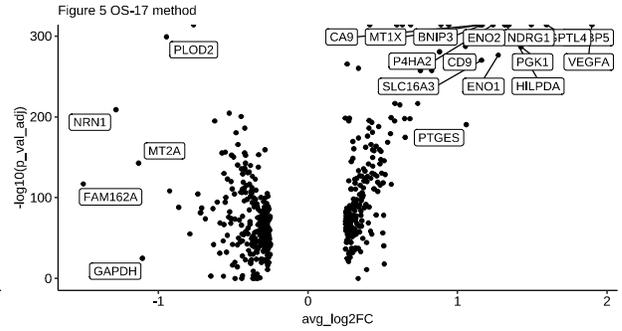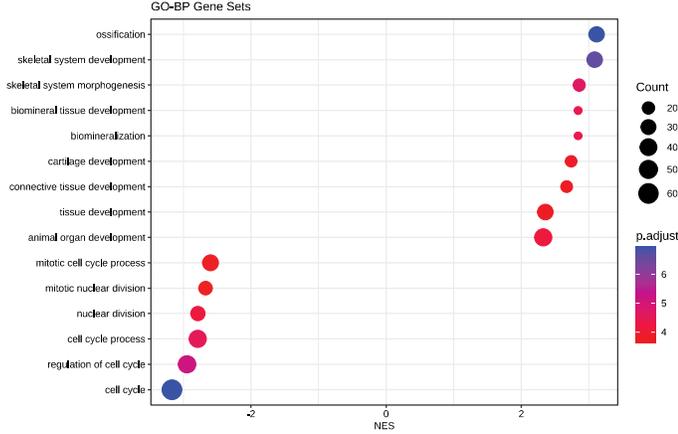
Figure S2 continued

Figure 5 OS-17 by Clusters
Seurat-assigned clusters (optimized)
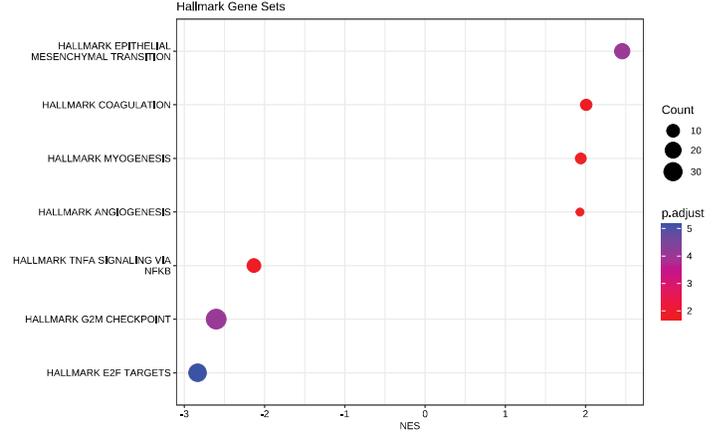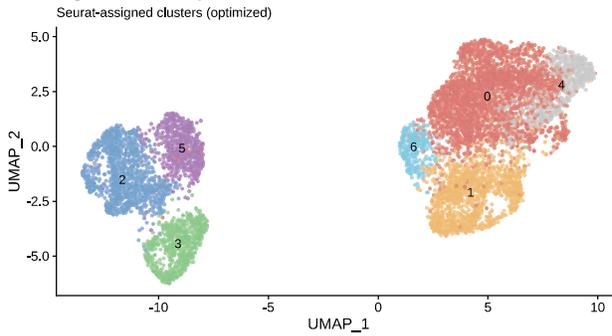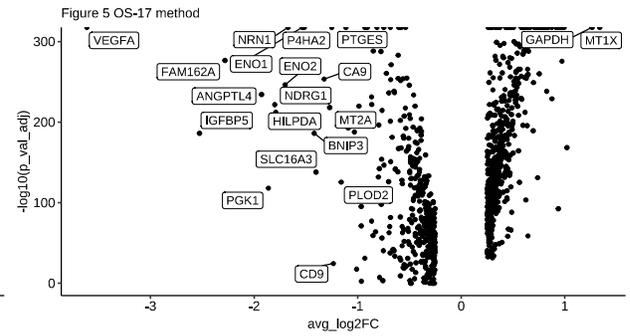
Cluster 3 Genes
Figure 5 OS-17 method
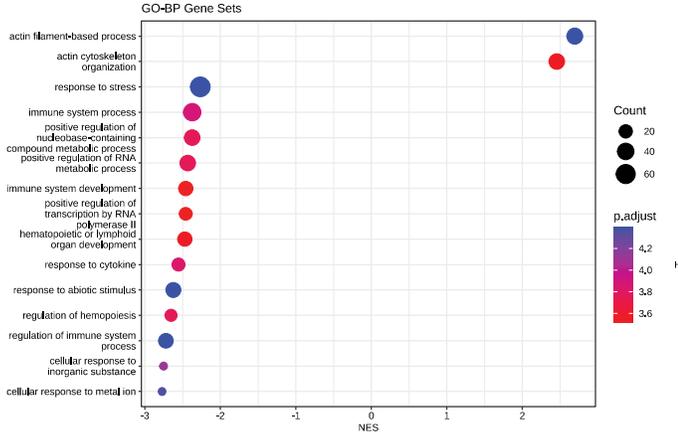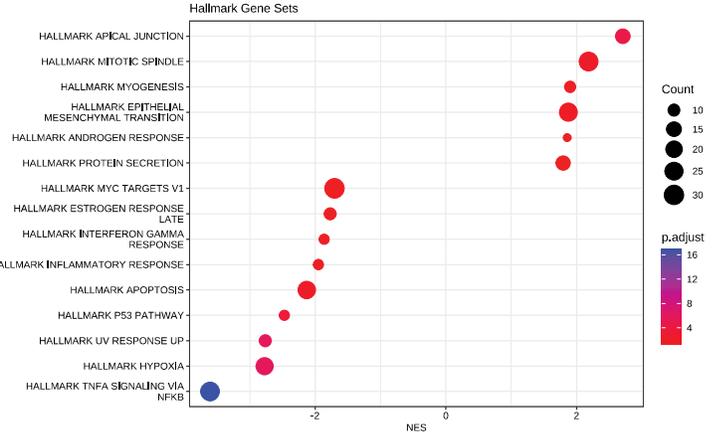
Figure 5 OS-17 Cluster 3
GO-BP Gene Sets
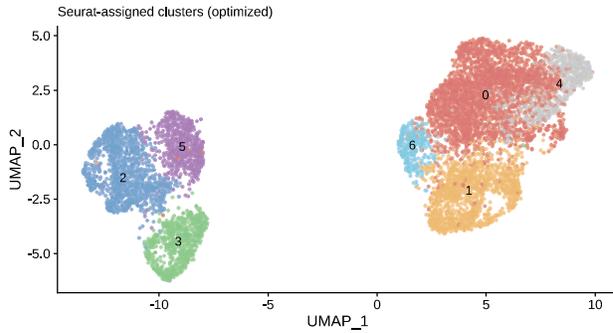
Figure 5 OS-17 Cluster 3
Hallmark Gene Sets
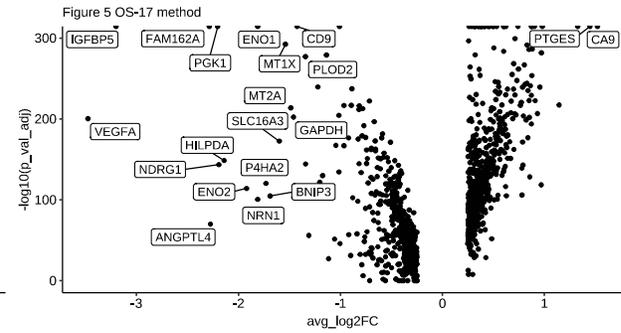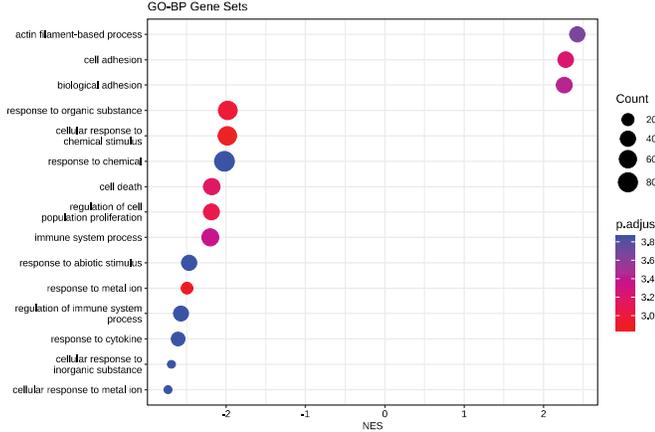
Figure 5 OS-17 by Clusters
Seurat-assigned clusters (optimized)
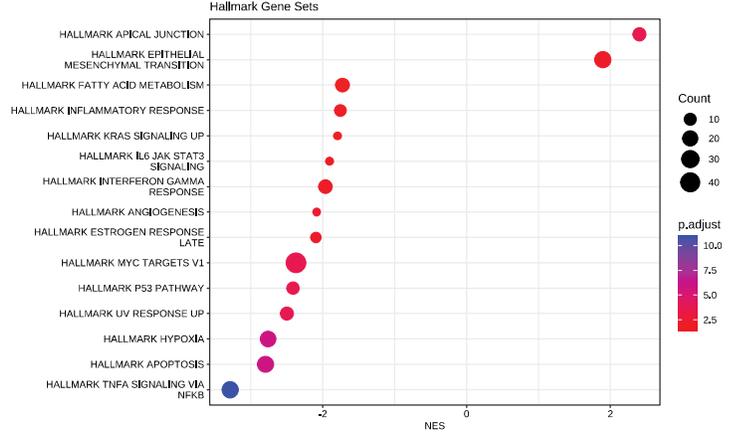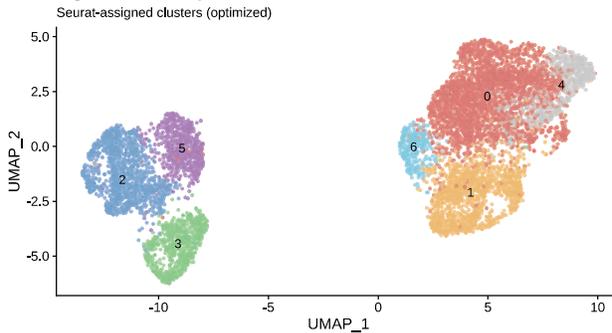
Cluster 4 Genes
Figure 5 OS-17 method

Figure 5 OS-17 Cluster 4
GO-BP Gene Sets

Figure 5 OS-17 Cluster 4
Hallmark Gene Sets
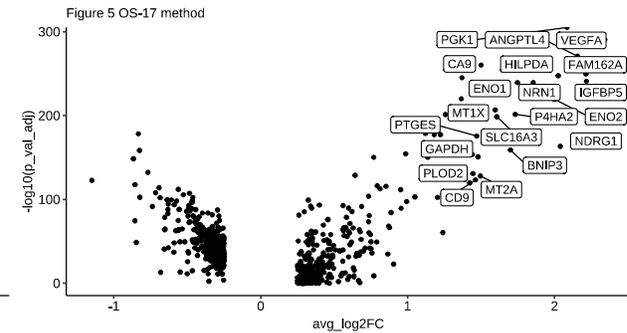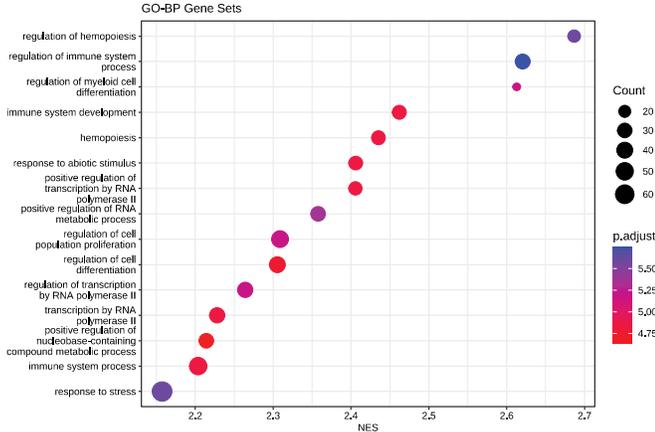
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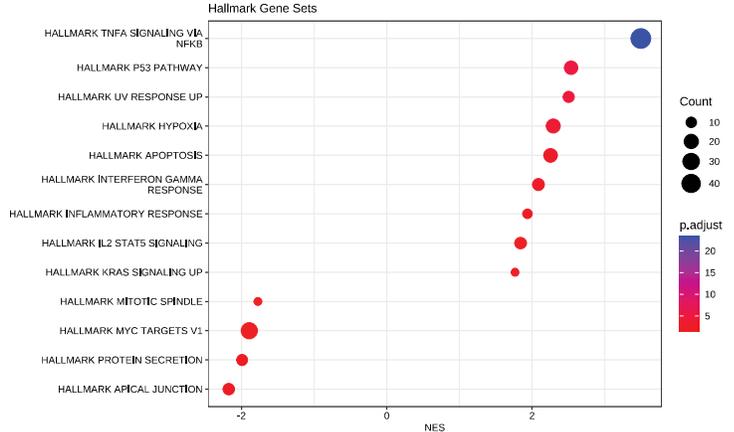
Figure 5 OS-17 by Clusters
Seurat-assigned clusters (optimized)
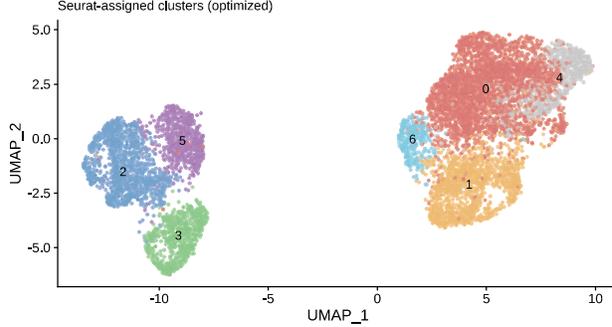
Cluster 5 Genes
Figure 5 OS-17 method

Figure 5 OS-17 Cluster 5
GO-BP Gene Sets

Figure 5 OS-17 Cluster 5
Hallmark Gene Sets
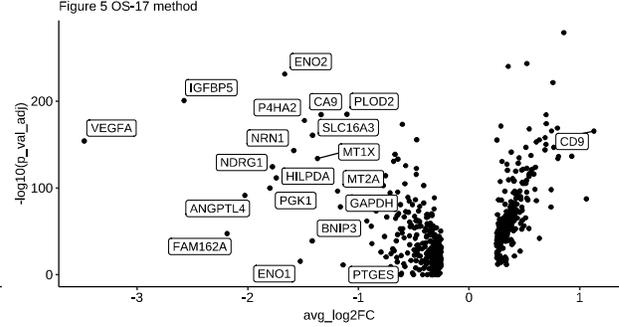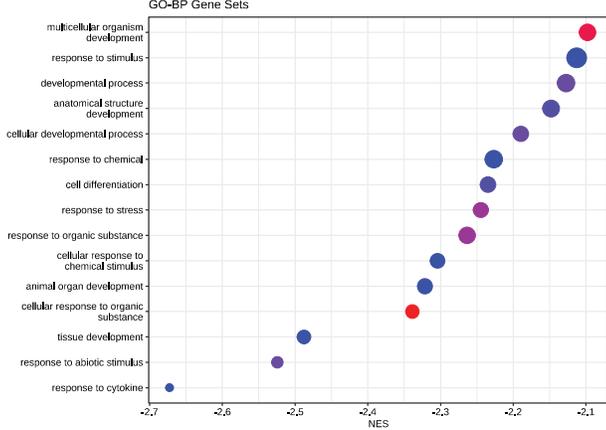
Figure 5 OS-17 by Clusters
Seurat-assigned clusters (optimized)
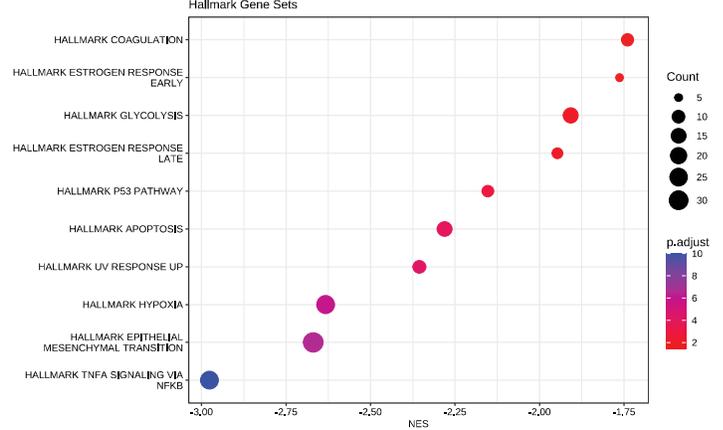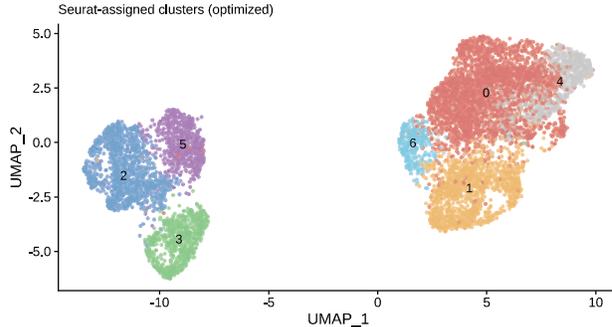
Cluster 6 Genes
Figure 5 OS-17 method

Figure 5 OS-17 Cluster 6
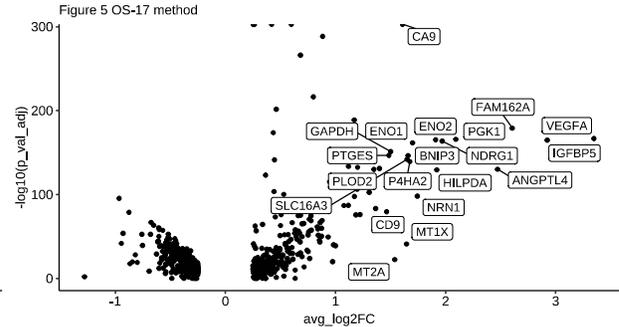GO-BP Gene Sets

Figure 5 OS-17 Cluster 6
Hallmark Gene Sets
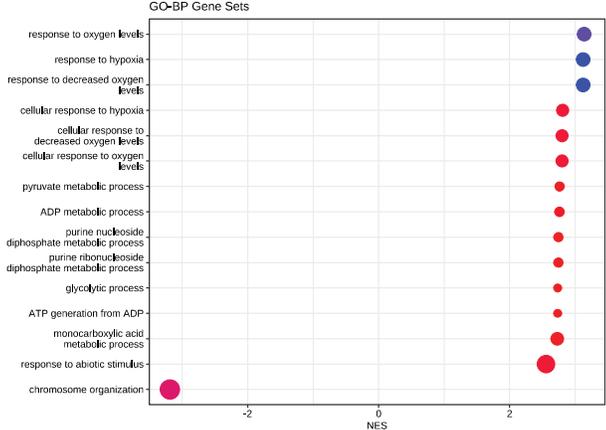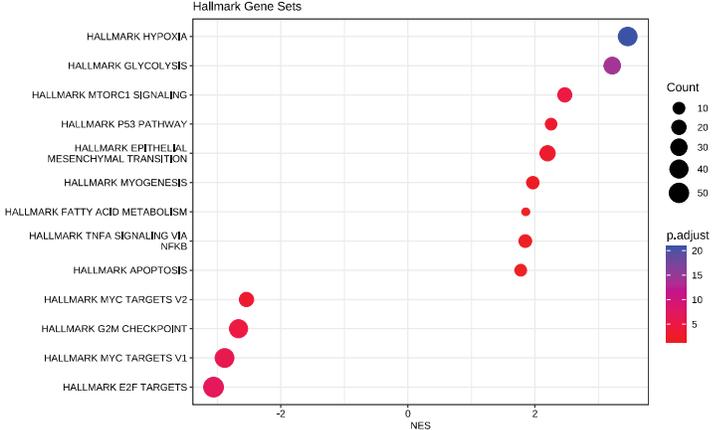
Figure S2 continued

Figure S2. In-depth analysis of clusters utilized in analyses. A) OS-17 cell culture and B) NCH-OS-2 flank cluster analysis (Figure 1). C-F) Merged tibia and lung cluster analysis for OS-17, 143B, NCH-OS-2, and NCH-OS-7, respectively (Figure 3). G) Lineage-tagged OS-17 cell culture, tibia, and lung cluster analysis (Figure 5). The upper left of each analysis displays all clusters for the dataset. The upper right shows a volcano plot analysis, where the top differentially expressed genes for the cluster of interest, compared to all others in the dataset, are shown. DotPlots on the bottom left depict the significantly upregulated GO BioProcesses and GO MolFunction gene lists in the cluster of interest from the differentially expressed gene list for the cluster. Bar plot on the bottom left shows upregulated msigdb Hallmark pathways from the differentially expressed gene list for the cluster. The Wilcoxon rank sum test, or "wilcox" statistical method, was used to generate differentially expressed gene lists.