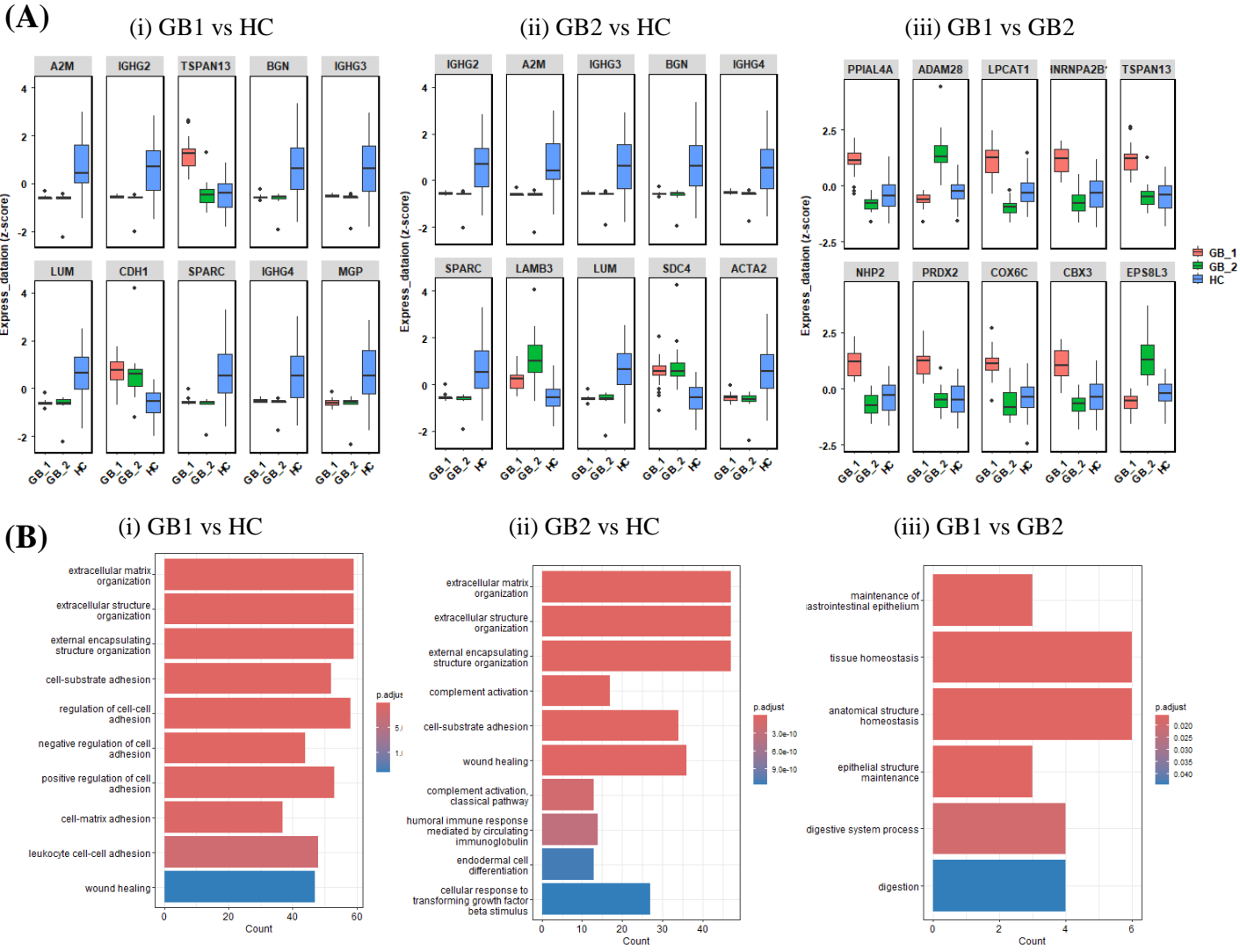


**Figure 1. Data Quality Assessment and Differential Expression Analysis (A vs B Analysis)**

- (A) PCA Plot.** Comparing healthy surrounding tissue (HC) with Glioblastoma tumors, including metastasis (GB1) and non-metastasis (GB2), across different factors. Showing clear separation along PC1 (9.07% variance) on the x-axis and PC2 on the y-axis (5.26% variance).
- (B) Survival Plot.** Comparing metastasis (GB1) and non-metastasis (GB2). GB1 shows significantly shorter survival compared to GB2 ( $p = 0.0076$ ).
- (C) MA Plot.** Showing the log2 fold change on the y-axis versus the mean expression (log10) on the x-axis for all genes between different conditions (GB1 vs HC, GB2 vs HC and GB1 vs GB2). Each point represents a gene, with red colour for upregulated, blue for downregulated and black for Non-significant.
- (D) Volcano Plot.** Showing the log2 fold change on the x-axis versus the significance ( $-\log_{10} p$ -value) on the y-axis for all genes between different conditions (GB1 vs HC, GB2 vs HC and GB1 vs GB2). Each point represents a gene, with red colour for upregulated, blue for downregulated and black for Non-significant. The top 5 upregulated genes and downregulated genes are labeled.



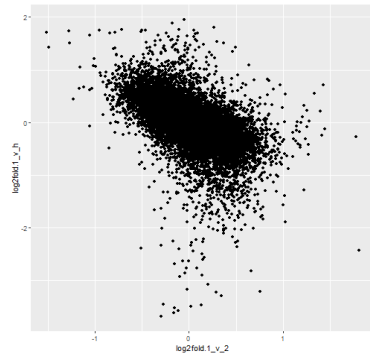
**Figure 2. Gene Expression Analysis and Pathway Analysis (A vs B Analysis)**

**(A) Boxplot of The Top 10 Most Significantly Differentially Expressed Genes.** Comparing between different conditions (GB1 vs. HC, GB2 vs. HC and GB1 vs. GB2).

**(B) Pathway Enrichment Bar Chart.** Showing the significantly enriched pathways identified from the analysis and colored by adjusted p-value. Comparing between different conditions (GB1 vs. HC, GB2 vs. HC and GB1 vs. GB2).

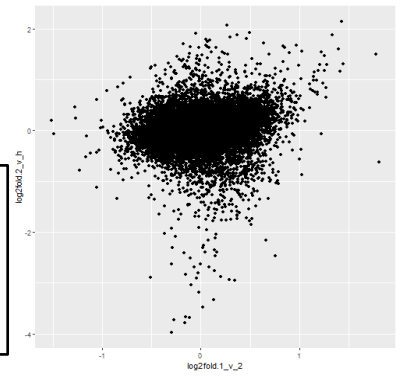
(A) (i)  
GB1 and HC  
vs.  
GB1 and GB2

Cor: -0.5644693  
P value: <2.2e-16  
95 percent confidence  
interval:  
-0.5741632 -0.5546173



(ii)  
GB2 and HC  
vs.  
GB1 and GB2

Cor: 0.171  
P value: < 2.2e-16  
95 percent confidence  
interval:  
0.1570766, 0.1849218



(B) (i)  
GB1 and HC  
vs.  
GB1 and GB2



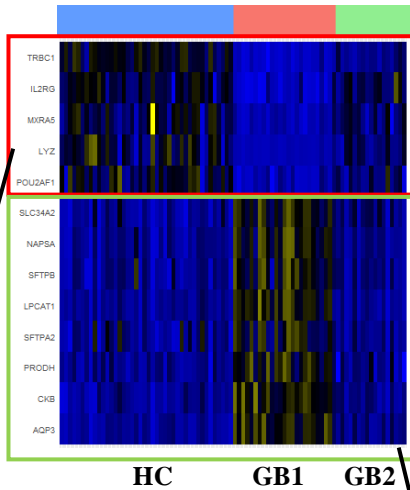
**Both Significant Genes:** AQP3, CKB, IL2RG, LPCAT1, LYZ, MXRA5, NAPSA, POU2AF1, PRODH, SFTPA2, SFTPB, SLC34A2, TRBC1  
**P value:** 1.243443e-09

(ii)  
GB2 and HC  
vs.  
GB1 and GB2

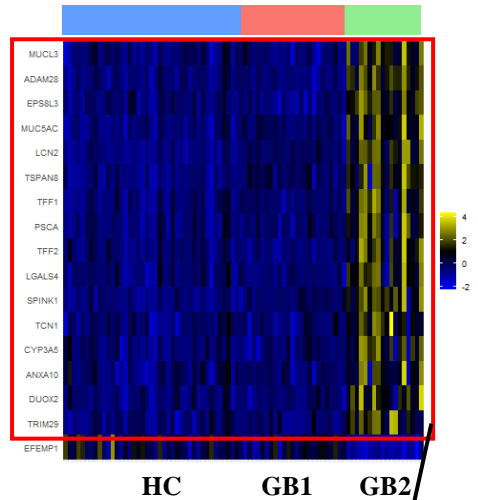


**Both Significant Genes:** ADAM28, ANXA10, CYP3A5, DUOX2, EFEMP1, EPS8L3, LCN2, LGALS4, MUC5AC, MUCL3, PSCA, SPINK1, TCN1, TFF1, TFF2, TRIM29, TSPAN8  
**P value:** 2.816184e-17

(C) (i)  
GB1 and HC  
vs.  
GB1 and GB2

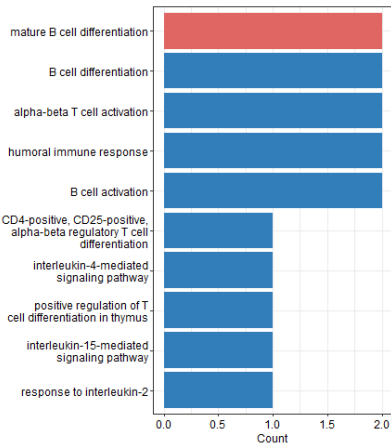


(ii)  
GB2 and HC  
vs.  
GB1 and GB2

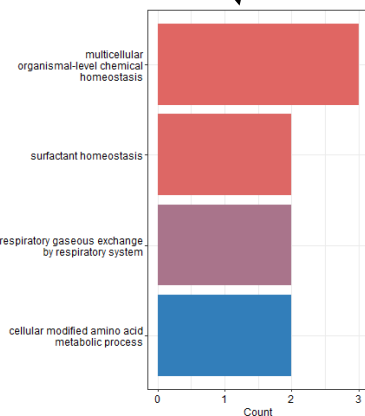


(D)

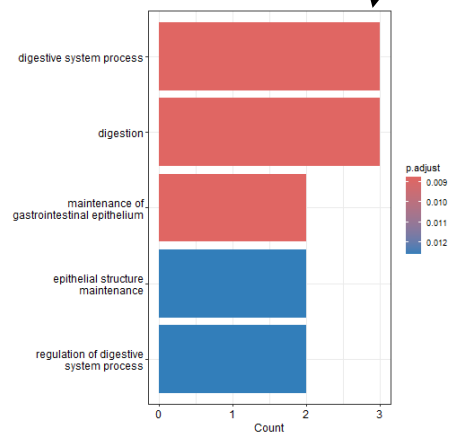
(i)



(ii)



(iii)



### **Figure 3. Sample Distribution and Gene Expression Analysis ([A vs B]n Analysis)**

**(Figures are in the previous page)**

- (A) Venn Diagram, Both Significant Genes and P value.** Showing whether the significant genes in one comparison are shared with those in another (GB1 vs. HC compared with GB1 vs. GB2, GB2 vs. HC compared with GB1 vs. GB2), revealing similarities or differences in gene changes. (GB1 vs. HC compared with GB1 vs. GB2, GB2 vs. HC compared with GB1 vs. GB2).
- (B) Fold vs Fold Scatterplots and Correlation Results.** Showing all genes change in strength and direction across two comparisons and their correlation, helping to see if the changes are similar. (GB1 vs. HC compared with GB1 vs. GB2, GB2 vs. HC compared with GB1 vs. GB2).
- (C) Heatmap of Exogenous Protein binding Pathway Gene Expression.** Showing the expression levels of all potentially interesting genes across all samples, revealing patterns and how they relate to each group. (GB1 vs. HC compared with GB1 vs. GB2, GB2 vs. HC compared with GB1 vs. GB2).
- (D) Pathway Enrichment Bar Chart.** Showing the significantly enriched pathways identified from the analysis and colored by adjusted p-value.