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INTRODUCTION

BACKGROUND

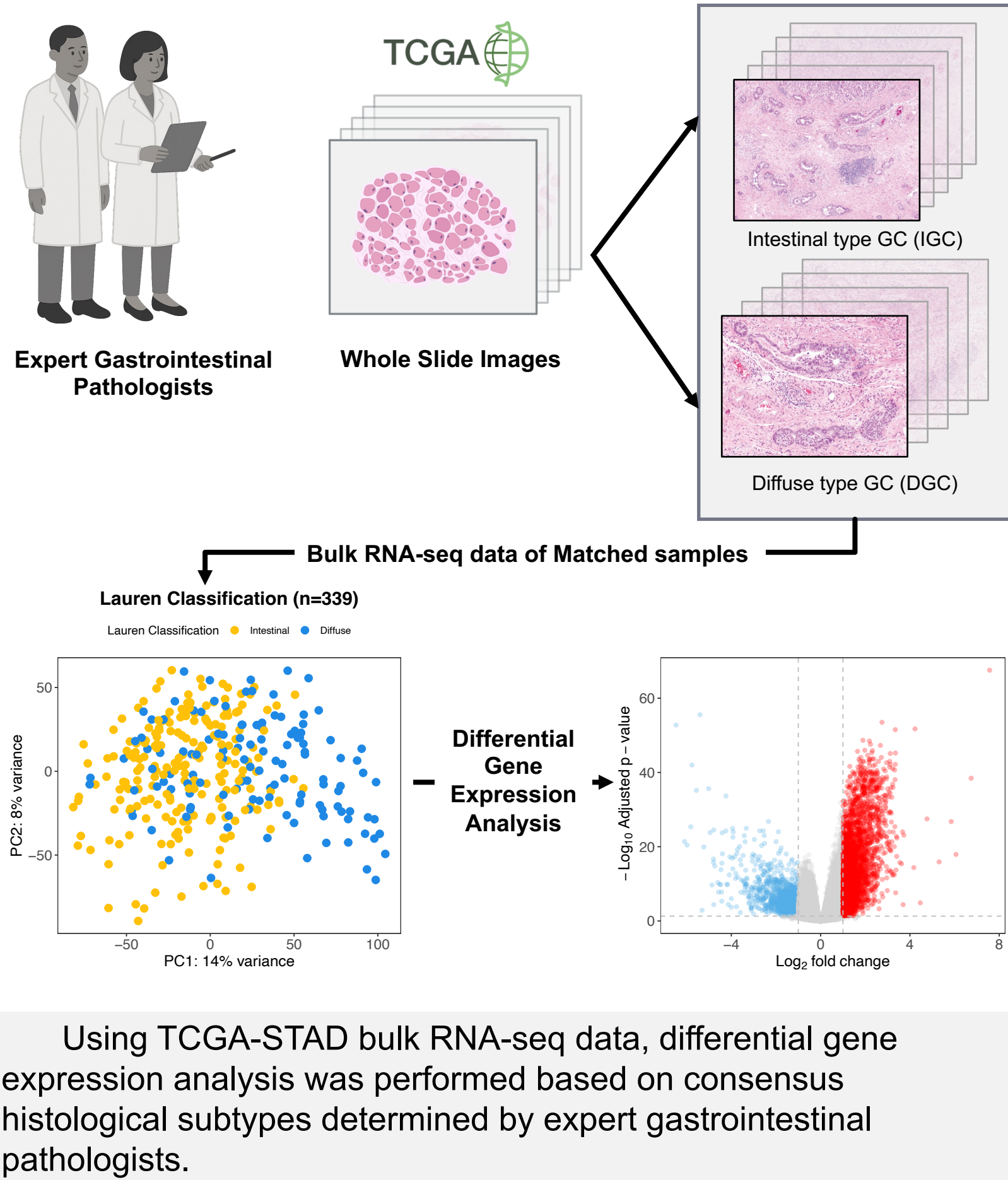
Advanced **gastric cancer (GC)** is one of the most challenging malignancies, with high mortality rates and a lack of effective treatment strategies. Histomorphological features, termed “morphologic phenotypes”, serve as predictors of therapeutic response-lymphoepithelial carcinoma demonstrates immunotherapy responsiveness, whereas **diffuse-type GC** exhibits resistance to both immunotherapy and targeted therapies, including HER2 and CLDN18 inhibitors.

Despite these associations, **comprehensive molecular understanding** based on meticulous morphological assessments remains elusive. Herein, we aim to identify robust biomarkers and elucidate the molecular mechanisms underlying GC phenotypes, providing a more precise reflection of the spatial phenotype of GC.

OBJECTIVE

Identifying the molecular mechanisms of gastric cancer (GC) subtypes through TCGA-STAD bulk RNA-seq analysis. Unlike previous TCGA classifications, our study is based on a **unified pathological consensus** from expert pathologists within the same institution, ensuring greater accuracy and reproducibility.

METHODS



4,342 Differentially Expressed Genes (DEGs) Between DGC and IGC

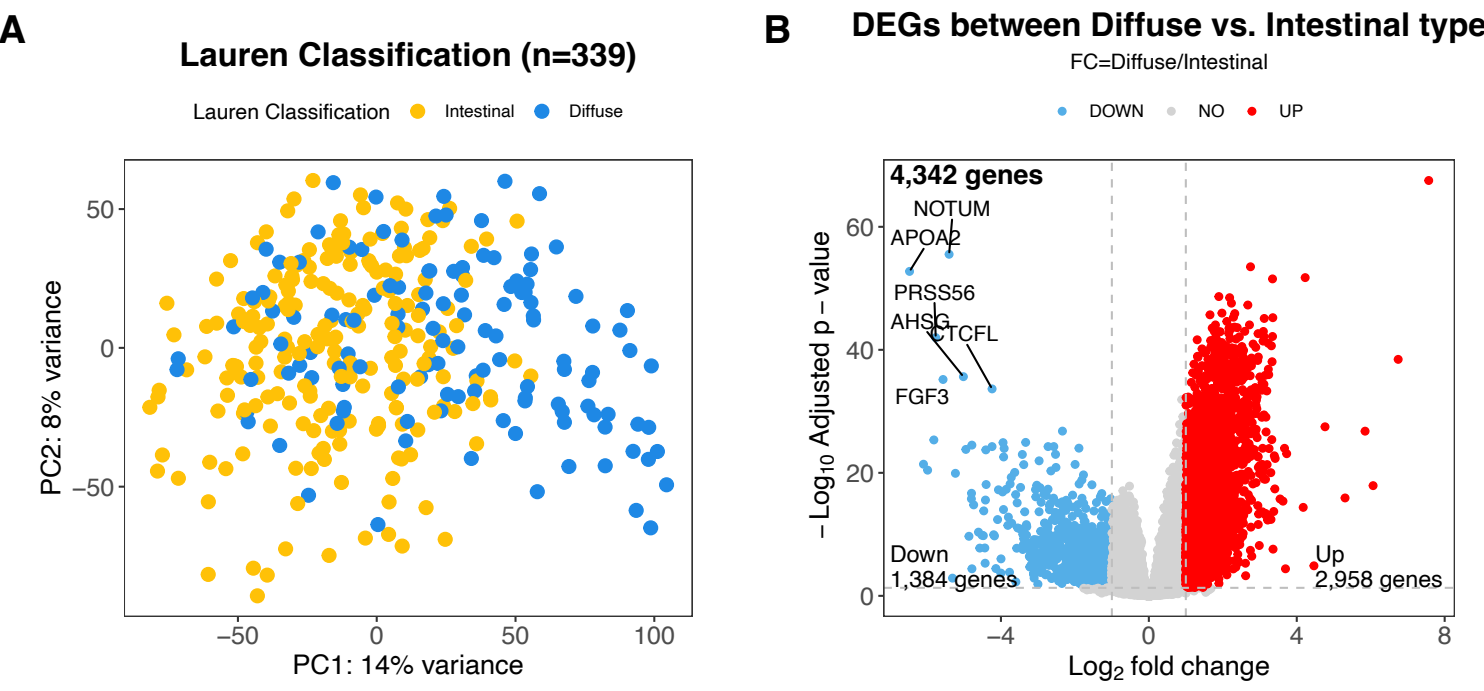
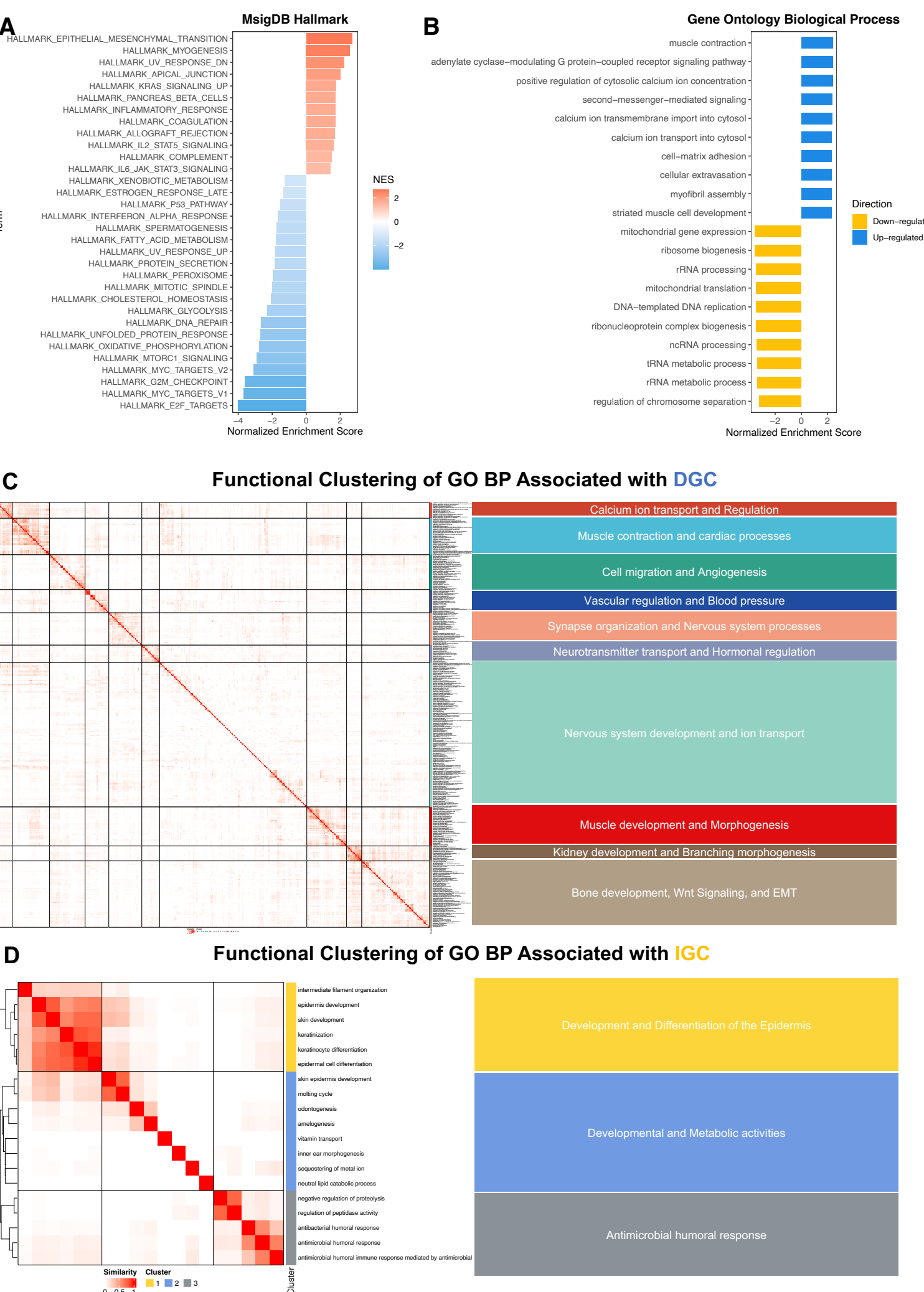


Figure 1. (A) PCA plot of the 339 samples (125 DGC and 214 IGC) after the batch correction with SVA (Surrogate Variable Analysis). **(B)** A total of **4,342 DEGs** were identified. 2,958 genes were significantly upregulated in DGC, whereas 1,384 genes were more highly expressed in IGC.

Distinct Oncogenic Pathways in DGC and IGC



RESULTS

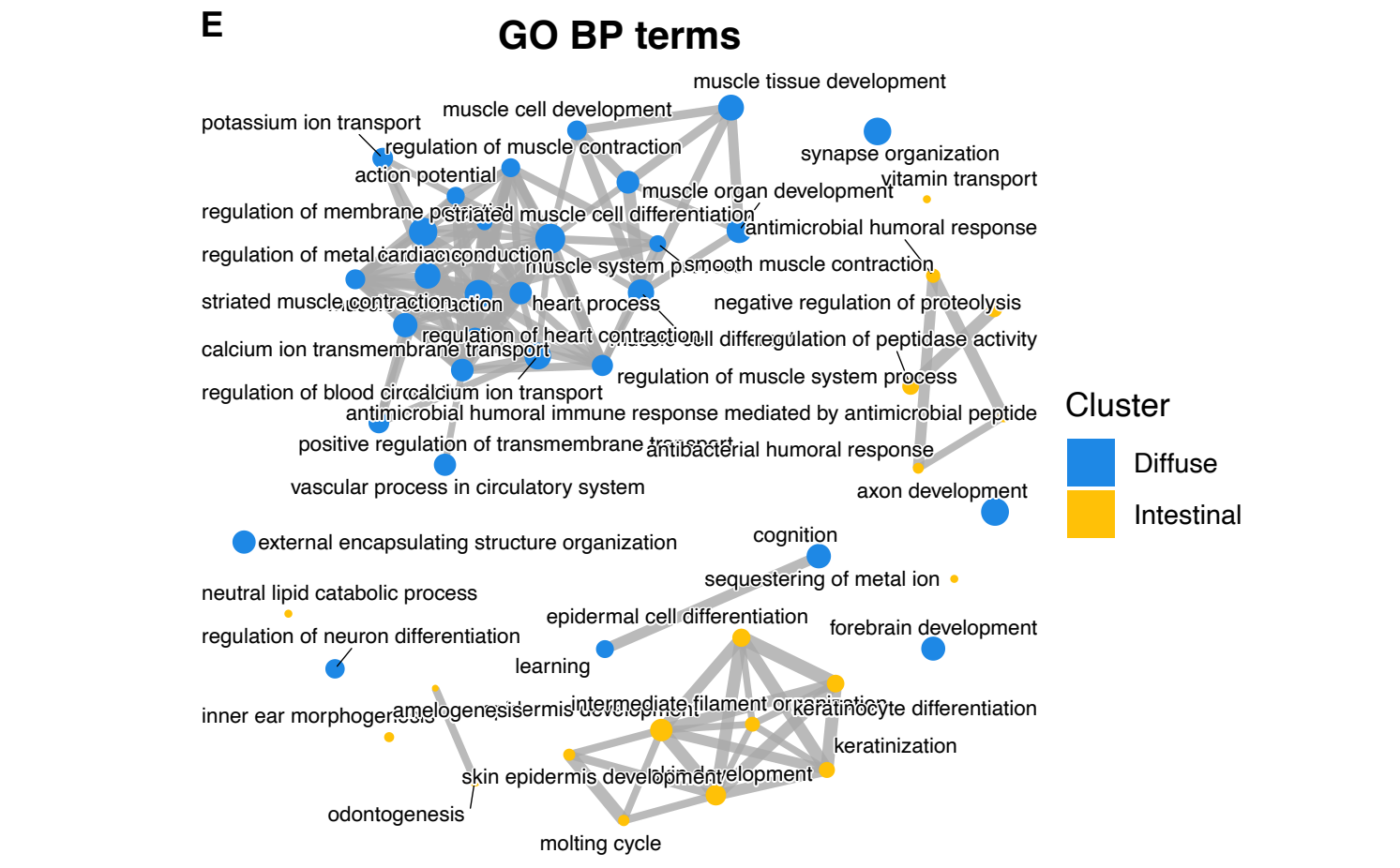


Figure 2. Gene Set Enrichment Analysis (GSEA) revealed significantly enriched terms in DGC (NES>0) and IGC (NES<0) based on **(A)** MSigDB Hallmark and **(B)** Gene Ontology Biological Process (GO BP). The top10 GO BP terms for each subtype are presented. **(C)** Clustering of GO BP terms. Similarity-based clustering of GO BP terms significantly enriched by GSEA and Over-Representation Analysis (ORA), highlighting functional modules relevant to **(C)** DGC and **(D)** IGC. **(E)** Clustering of GO BP terms

Significant enrichment of pathways associated with epithelial-mesenchymal transition (**EMT**), **myogenesis**, **angiogenesis**, **neurogenesis**, and **calcium signaling regulation** in **DGC**. In contrast, **IGC** showed enrichment in **gastric epithelial differentiation markers**, **DNA repair processes**, and **immune response pathways**, suggesting that these two histological subtypes follow **distinct oncogenic trajectories**.

PKD2-Mediated Calcium Signaling Promotes EMT via SNAIL, ZEB, and TWIST Pathways

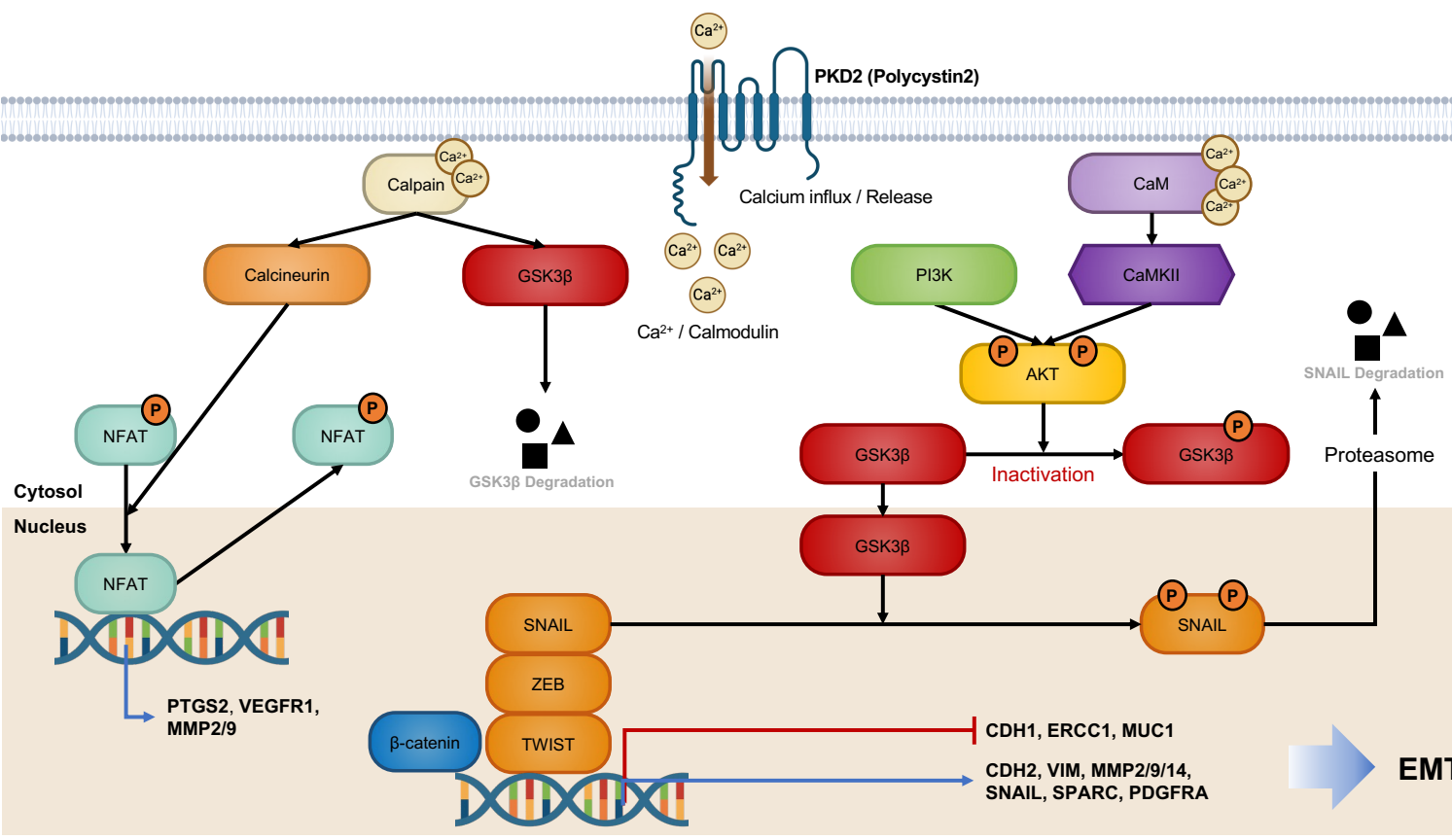


Figure 3. Schematic representation of epithelial-to-mesenchymal transition (EMT) regulation via PKD2-mediated calcium signaling. Activation of PKD2 (Polycystin-2) at the plasma membrane leads to intracellular Ca²⁺ influx, which triggers multiple downstream pathways.

M2 Macrophage and CAF Enrichment in the Tumor Microenvironment of DGC

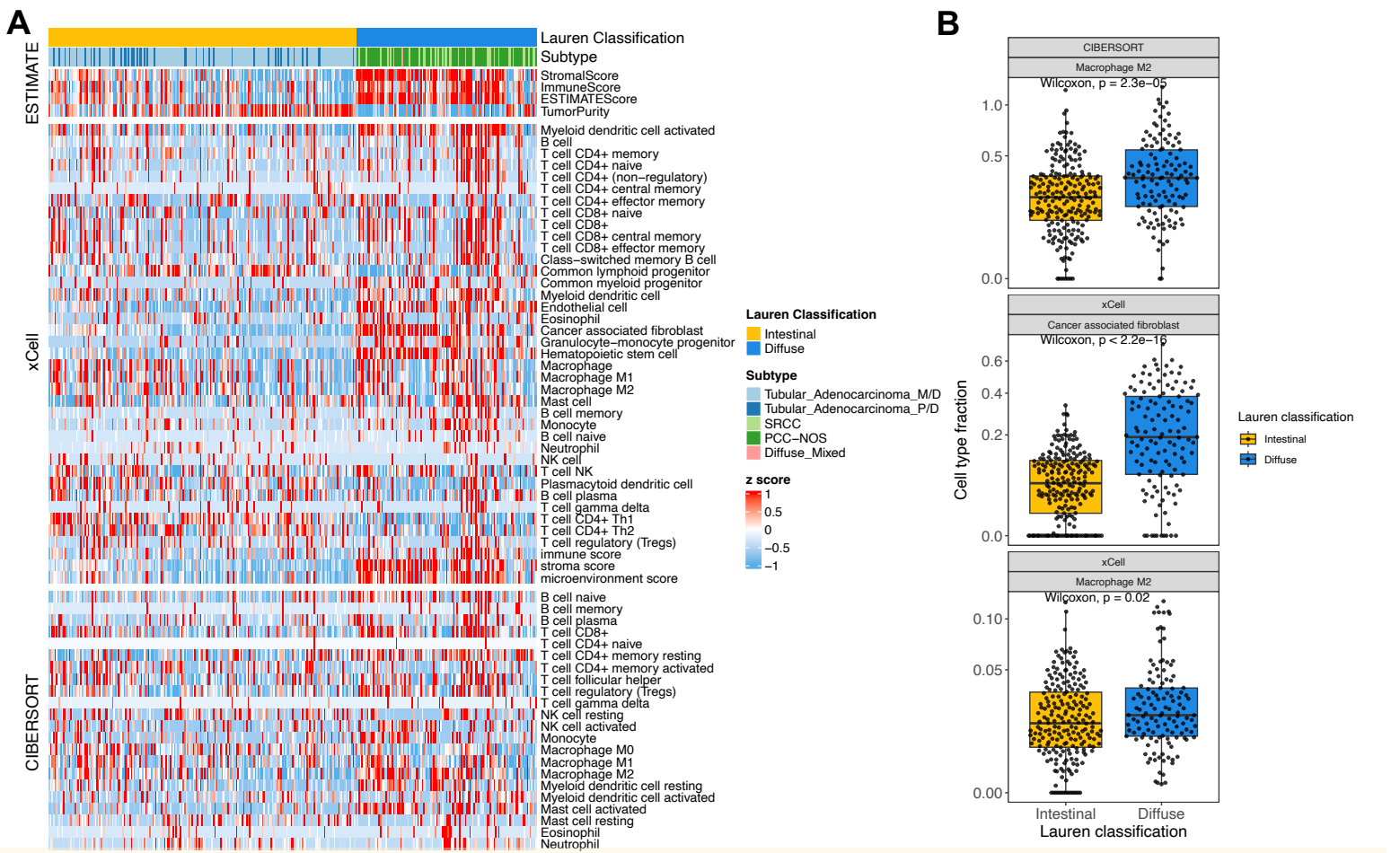


Figure 4. (A) Heatmap showing inferred cell type proportions and microenvironment scores across TCGA-STAD samples, stratified by Lauren classification. Cell type proportions were estimated using the ESTIMATE, xCell, and CIBERSORT algorithms. Each column represents an individual sample. **(B)** Box plots highlighting statistically significant differences in inferred cell type fractions between IGC and DGC, with increased infiltration of **M2 macrophages** and **CAFs** observed in DGC.

CONCLUSION

Our integrative analysis of TCGA-STAD bulk RNA-seq data, supported by expert pathological classification, revealed distinct molecular programs between diffuse-type and intestinal-type gastric cancers.

- Diffuse-type GC (DGC) exhibited enrichment in pathways related to **EMT**, **myogenesis**, **angiogenesis**, **neurogenesis**, and **calcium signaling**, along with **increased infiltration of M2 macrophages and CAFs**, suggesting a more invasive and immune-modulating microenvironment.
- In contrast, **intestinal-type GC (IGC)** was enriched in **epithelial differentiation**, **DNA repair**, and **immune activation pathways**.

These findings highlight divergent **oncogenic mechanisms** and **tumor microenvironmental landscapes** across GC subtypes, offering potential insights for subtype-specific therapeutic strategies.

REFERENCES

Iyer, Pritish et al. “Diffuse gastric cancer: histologic, molecular, and genetic basis of disease.” *Translational gastroenterology and hepatology* vol. 5 52. 5 Oct. 2020