

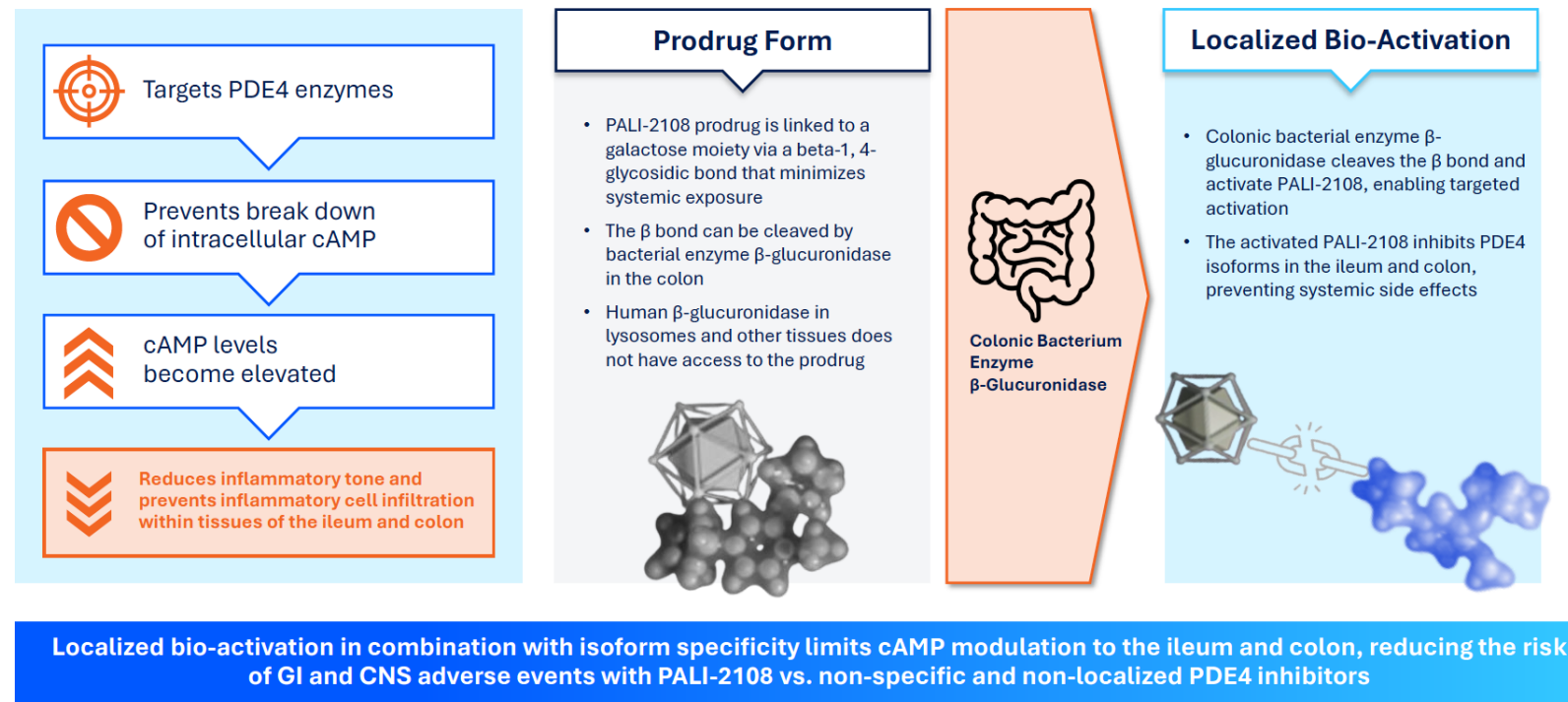
# Development of Mucosal Transcriptomic Signatures to Predict Response to the Microbiome-Activated Phosphodiesterase (PDE)-4 Inhibitor PALI-2108 in Ulcerative Colitis



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## Introduction and Aim

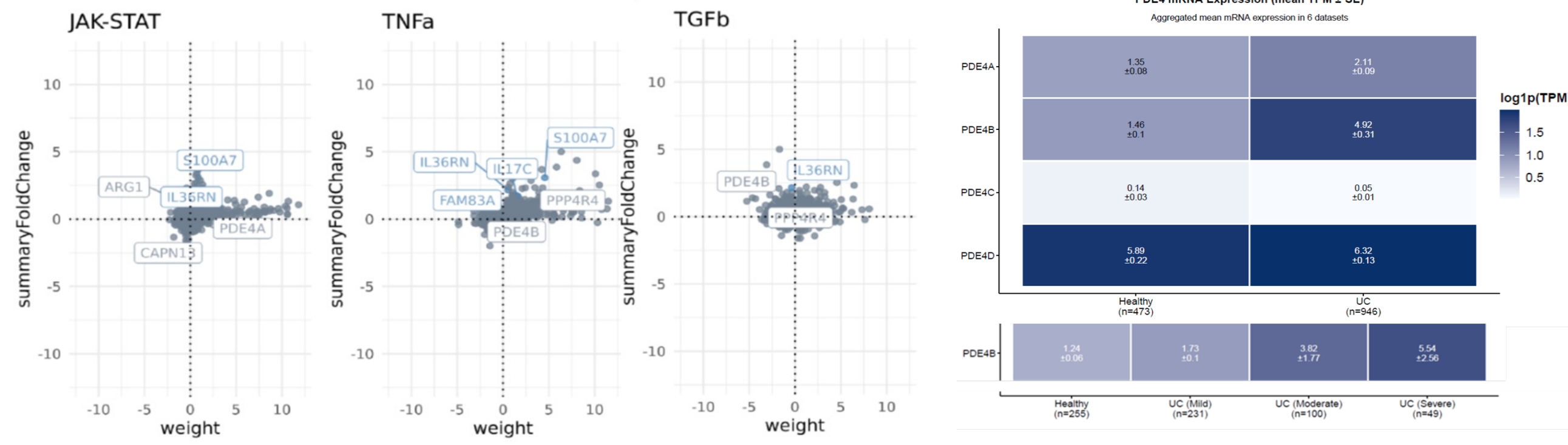
- Ulcerative colitis (UC) is a chronic, relapsing inflammatory bowel disease (IBD) with substantial clinical heterogeneity. Treatment response varies considerably among patients, and validated predictive biomarkers capable of identifying responders before treatment initiation remain unavailable, resulting in prolonged trial-and-error treatment selection.
- Phosphodiesterase-4 (PDE4) inhibition increases intracellular cyclic adenosine monophosphate (cAMP) levels, suppressing multiple pro-inflammatory signaling pathways and immune cell activation. Transcriptomic changes associated with PDE4/cAMP signaling may provide a mechanistically informed approach for predicting therapeutic response.
- PALI-2108 is a novel, once-daily oral prodrug engineered for delayed ileocolonic release and microbiome-mediated conversion to the active metabolite, PALI-0008, enabling high local drug exposure at sites of intestinal inflammation while minimizing systemic exposure.



- We developed a mechanistically anchored transcriptomic biomarker signature integrating PDE4/cAMP pathway biology and evaluated its ability to predict therapeutic response to PALI-2108 in patients with UC.

## PDE4 Is Enriched in Inflammatory and Fibrotic Pathways and Overexpressed in UC and CD Patient Tissues

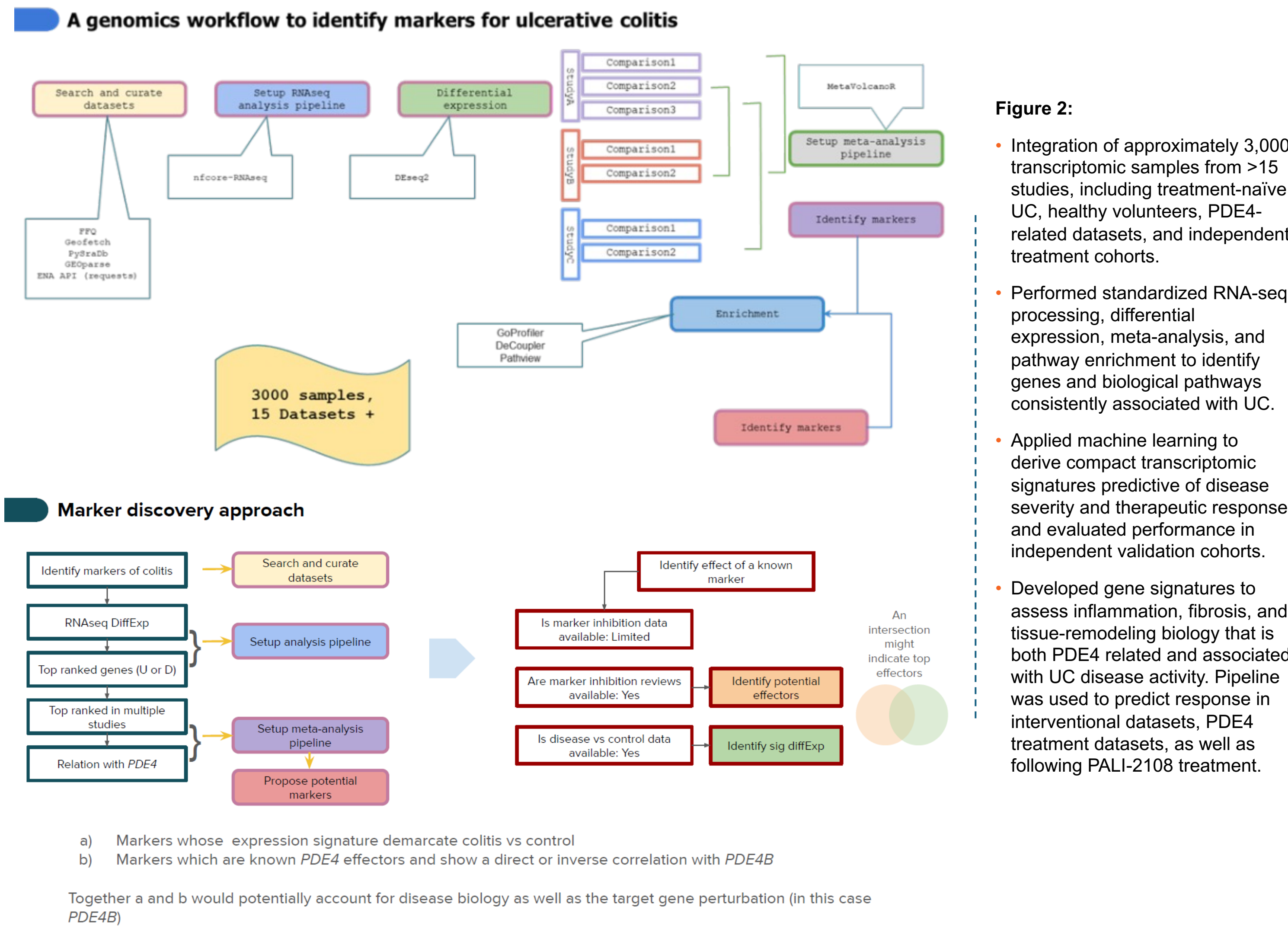
**Figure 1:** (left) Volcano Plots of RNAseq biomarker data demonstrate enriched PDE4 expression in multiple inflammatory and fibrotic pathways in UC and CD patient tissue biopsy samples.



## Methods

A multi-stage biomarker framework was applied using publicly available and proprietary RNA sequencing datasets (see **Figure 2**). The publicly available discovery cohort included n=1751 (UC (N=1338); controls (N=413)). Differential expression analyses compared case vs control, inflammatory vs non-inflammatory, and severe vs mild disease states. Genes selection was based on differential expression ( $P \leq 0.05$ ,  $|\log FC| \geq 0.6$ ), reproducibility, and biological relevance. A random-effects meta-analysis was used to identify robust markers, enhanced using correlation of known PDE4 effectors with PDE4B expression and seven PDE4 inhibition datasets. Final gene signatures were achieved by integrating PDE4B-correlated effectors with meta-analysis-derived markers. Machine learning-based weighted scoring classified responder phenotypes, with a composite disease activity score (Pscore-Nscore), where Pscore reflects upregulated PDE4B-associated genes and Nscore reflects inverse/downregulated genes.

## Integrative Transcriptomic Meta-Analysis Workflow for Biomarker Discovery and Fibrosis Pathway Assessment in UC and PDE4 Inhibition Studies



## Transcriptomic Biomarker Signatures Demonstrate Superior Separation Using Biomarker Score Compared to Selecting Based on PDE4 Expression Alone

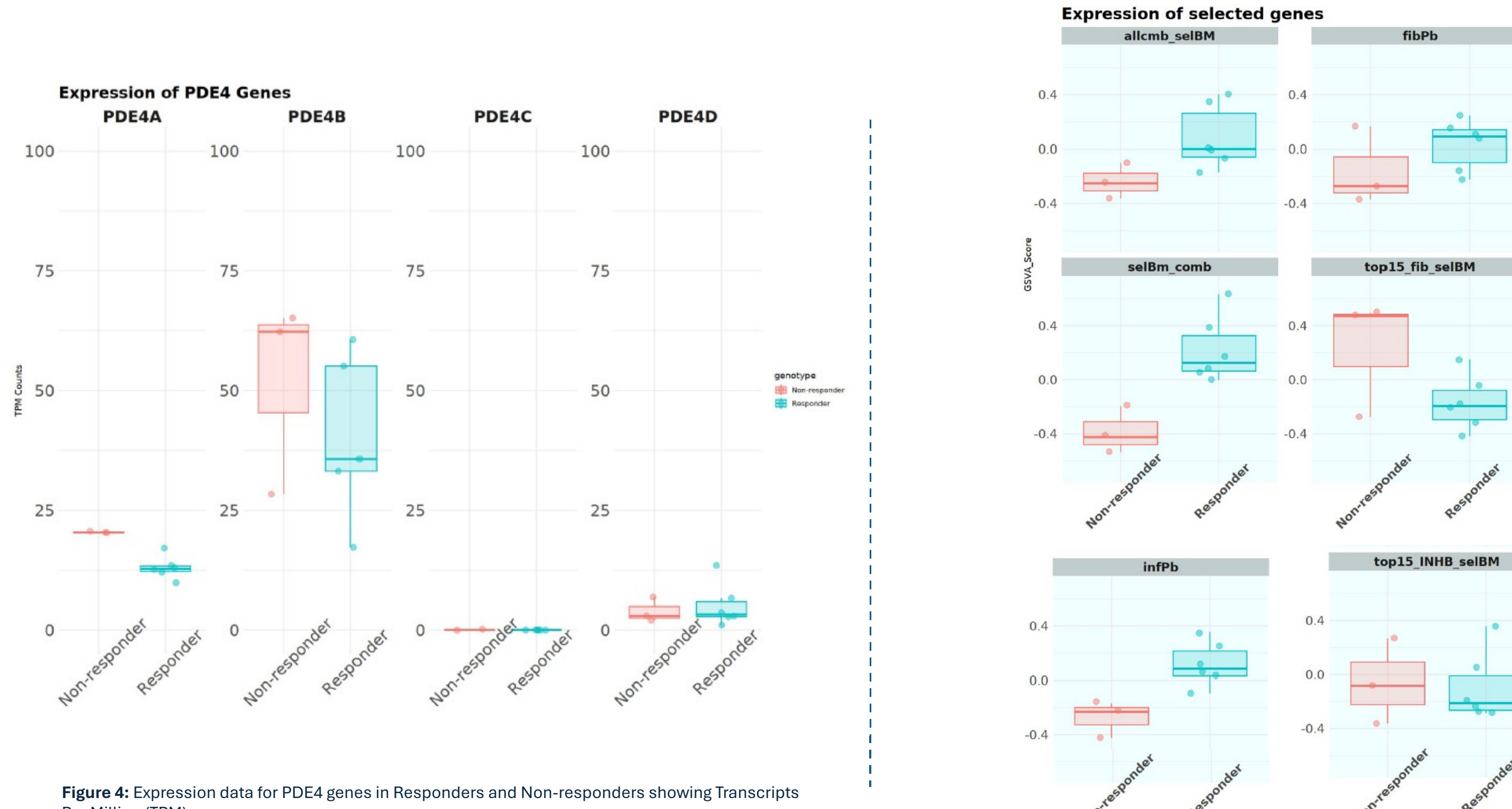


**Figure 4:** Expression data for PDE4 genes in Responders and Non-responders showing Transcripts Per Million (TPM).

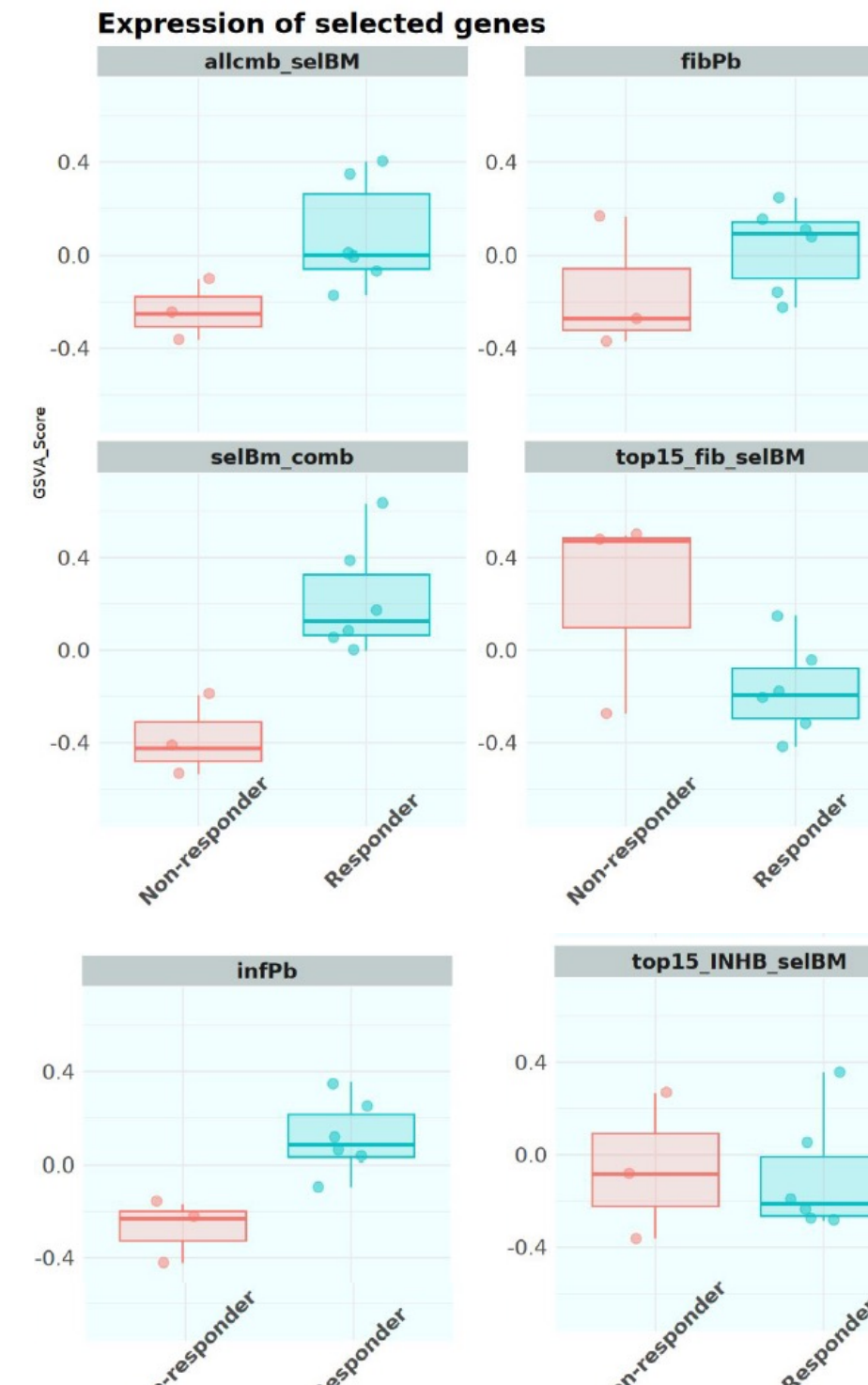


**Figure 5:** Group expression score data for groups of high performing biomarkers associated with inflammation and fibrosis and under evaluation as part of a Disease Activity Score (DAS) index for PDE4 related genes and those correlated with UC and CD disease activity

## Results

### Transcriptomic Biomarker Signature Demonstrates Predictive Performance for Treatment Response in UC Patients

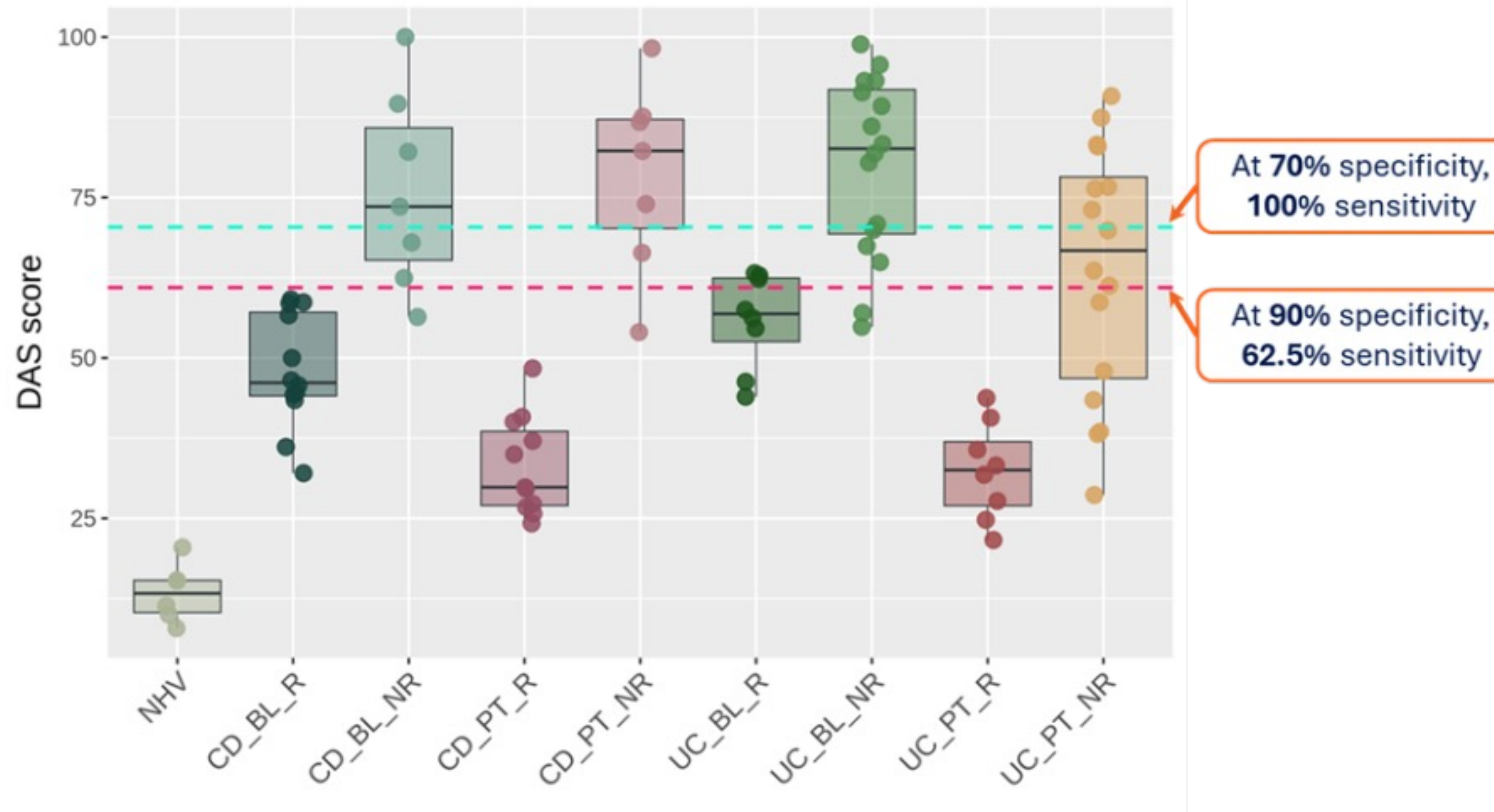


**Figure 3:** The model demonstrated an AUC of approximately 0.67. Optimized gene sets achieved 100% sensitivity at 70% specificity (blue dashed line) and 62.5% sensitivity at 90% specificity (red dashed line), representing candidate thresholds for responder classification using the DAS RNA-seq assay. AUC, area under the curve; BL, baseline; CD, Crohn's disease; DAS, disease activity score; NHV, normal healthy volunteers; NR, non-responders; PT, post-treatment; R, responders; UC, ulcerative colitis.

## Summary

- We identified a mechanistically anchored PDE4/cAMP-based transcriptomic biomarker signature in UC patients with distinct likelihoods of therapeutic response, supporting a biologically informed approach to patient stratification.
- The biomarker demonstrated reproducible predictive performance across independent UC datasets, with optimized gene sets providing 100% sensitivity at 70% specificity and 62.5% sensitivity at 90% specificity for responder classification.
- Predictive performance was driven primarily by inflammatory signaling pathways, with strong concordance between gene-level expression and pathway-level activity, supporting the biological relevance of the signature.
- PDE4 pathway modulation was associated with reduced fibrosis-related transcriptional activity, suggesting that PALI-2108 may influence both inflammatory and tissue-remodeling pathways while supporting the utility of transcriptomic biomarkers for predicting treatment response.
- The developed transcriptomic signature may enable stratification of UC patients by predicting response to PDE4 inhibition and associated inflammatory/fibrotic pathways, supporting biomarker-guided development of PALI-2108. Prospective validation is ongoing.

