Research Square

Preprints are preliminary reports that have not undergone peer review.
They should not be considered conclusive, used to inform clinical practice,
or referenced by the media as validated information.

Transcriptomic stratification value of a dual-axis myeloid imbalance framework in sepsis: an integrative study based on a discovery cohort and external validation cohorts

Qinyuan Du
Affiliated Hospital of Shandong University of Traditional Chinese Medicine
Congcong Qin
Shandong University of Traditional Chinese Medicine
Guochen Li
Affiliated Hospital of Shandong University of Traditional Chinese Medicine
Qianyu Bi
Affiliated Hospital of Shandong University of Traditional Chinese Medicine
Hao Hao
Affiliated Hospital of Shandong University of Traditional Chinese Medicine
Feihu Zhang
Affiliated Hospital of Shandong University of Traditional Chinese Medicine
Ba Batejin
Inner Mongolia International Mongolian Medicine Hospital
Shuanglin Zhang
Inner Mongolia International Mongolian Medicine Hospital
Baogenna Bao
Inner Mongolia International Mongolian Medicine Hospital
Meiyu Bao
Inner Mongolia International Mongolian Medicine Hospital
Li Kong
konglizy2@163.com

Affiliated Hospital of Shandong University of Traditional Chinese Medicine

Research Article

Keywords: sepsis, host-response heterogeneity, transcriptomic stratification, myeloid imbalance, antigen presentation, interferon/stress response, external validation

Posted Date: April 7th, 2026

DOI: https://doi.org/10.21203/rs.3.rs-9236704/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License. Read Full License

Additional Declarations: No competing interests reported.

Abstract

Background

Sepsis is a life-threatening syndrome characterized by infection-induced dysregulation of the host response and subsequent organ dysfunction, with marked clinical heterogeneity. Conventional stratification approaches based on single inflammatory markers or clinical severity scores often fail to robustly capture host-response states. Recent studies using public transcriptomic cohorts have suggested that reproducible immune phenotypes and myeloid functional remodeling exist in sepsis; however, substantial variability remains across studies in phenotype definitions, gene-set composition, and clinical interpretability. Identifying a stratification framework from public transcriptomic data that is both biologically interpretable and reproducible across cohorts remains a key challenge in precision stratification of sepsis. In this study, we constructed a dual-axis myeloid imbalance framework composed of an antigen-presentation-associated myeloid program and an interferon/stress-responsive myeloid program using public whole-blood and peripheral blood mononuclear cell transcriptomic cohorts, and evaluated its value for outcome stratification and disease-state discrimination through module-level, gene-level, and cross-cohort validation.

Methods

Three public transcriptomic cohorts were included. GSE65682 served as the discovery cohort for comparing host transcriptomic differences between 28-day ICU survivors and non-survivors. GSE57065 served as the external validation cohort for comparing septic patients with healthy controls and for assessing early temporal dynamics at 0, 24, and 48 hours. GSE48080 was analyzed as a PBMC-based supplementary cohort providing orthogonal supportive evidence. Two core modules were constructed using fixed representative gene sets: the antigen-presentation-associated myeloid program (MS2_APC) and the IFN/stress-responsive myeloid program (MS3_IFN_stress). A dual-axis index was then derived to quantify the degree of imbalance between the two axes. Based on this framework, we performed phase-space distribution analysis, module distribution comparisons, cross-cohort effect-size integration, representative gene-expression heatmaps, focused volcano plots, dual-axis gene bubble heatmaps, and ROC analyses.

Results

In GSE65682, 28-day ICU non-survivors showed significantly lower MS2_APC and significantly higher dual-axis index than survivors, whereas MS3_IFN_stress alone did not reach statistical significance, indicating that outcome-related signals were primarily

characterized by suppression of the APC-related axis and greater dual-axis imbalance. The phase-space plot further showed that non-survivors clustered more toward a relatively low-APC, highly imbalanced region defined by MS2_APC and MS3_IFN_stress. External validation in GSE57065 demonstrated that septic patients, compared with healthy controls, had significantly reduced MS2_APC and markedly increased MS3_IFN_stress and dual-axis index, supporting stable disease-state relevance of this framework. Cross-cohort effect integration further indicated that reduction of MS2_APC and elevation of the dual-axis index were more consistent across cohorts and comparison settings, whereas GSE48080 provided only trend-level PBMC support with limited statistical strength. Gene-level analyses showed that APC-related representative genes exhibited more coherent negative shifts across both discovery and validation cohorts, whereas IFN/stress-related genes displayed more dispersed patterns. ROC analysis suggested that the dual-axis index had limited discriminatory performance for outcome stratification in GSE65682 but strong discrimination between septic and healthy states in GSE57065.

Conclusions

We propose and validate a dual-axis myeloid imbalance framework in sepsis. This framework was associated with adverse outcomes in the discovery cohort, showed clear disease-state relevance in an external whole-blood cohort, and further suggested at the gene level that APC attenuation is more reproducible across cohorts than isolated IFN/stress-related variation. This framework provides a transcriptomic perspective for understanding host-response heterogeneity in sepsis, although its clinical predictive performance and bedside applicability still require validation in independent clinical cohorts.

1 Introduction

Sepsis remains a core syndrome in critical care medicine with persistently high mortality and substantial healthcare resource utilization. Its pathobiology is not merely a linear escalation of infectious burden, but rather the consequence of complex interactions among pathogen-related stimuli, host immune responses, microcirculatory dysfunction, metabolic reprogramming, and organ failure.[1] For decades, clinical evaluation of sepsis has largely relied on evidence of infection, inflammatory biomarkers, lactate levels, and severity scores such as the Sequential Organ Failure Assessment (SOFA) and Acute Physiology and Chronic Health Evaluation II (APACHE II). However, these measures have limited ability to characterize host-response heterogeneity, particularly in identifying patient subgroups with distinct states of immune dysregulation.[1–3] Accordingly, establishing a host-response framework in sepsis that is both biologically interpretable and clinically relevant has become an important direction in precision critical care research.

Public transcriptomic studies have substantially advanced the current understanding of host-response heterogeneity in sepsis. Previous work has shown that patients with sepsis exhibit marked transcriptomic divergence in immune activation, myeloid-cell remodeling, antigen-presentation capacity, interferon signaling, and metabolic stress responses.[4–6] In particular, at the whole-blood transcriptomic level, myeloid immune pathways may provide a more stable reflection of host-response states than isolated inflammatory mediators. Nevertheless, two major limitations remain. First, the definitions of "phenotypes," "endotypes," and "host-response subtypes" vary considerably across studies, resulting in unclear module boundaries and limited comparability between cohorts.[4–6] Second, although many transcriptomic stratification studies have identified meaningful biological signals, they often lack sufficiently clear cross-cohort validation and gene-level interpretation, which hinders the development of reusable and transferable frameworks.[4–6]

Against this background, we proposed a dual-axis myeloid imbalance framework centered on myeloid functional remodeling. The first axis, designated MS2_APC, represents an antigen-presentation-associated myeloid program enriched for MHC class II, antigen-processing, and APC-like immune features. The second axis, designated MS3_IFN_stress, represents an IFN/stress-responsive myeloid program characterized by interferon-stimulated genes, stress-response signatures, and antiviral-like signaling. On this basis, we further derived a dual-axis index to summarize the degree of imbalance between the two axes. We hypothesized that, in sepsis, the most stable and reproducible abnormality is not isolated IFN/stress upregulation, but rather suppression of APC-like myeloid function and the resulting dual-axis imbalance.[5–7]

To test this hypothesis, we performed stratification analyses using three public transcriptomic cohorts. GSE65682 served as the discovery cohort and focused on 28-day ICU outcome-associated stratification. GSE57065 served as the external validation cohort and was primarily used to evaluate the disease-state discriminatory value of the framework in sepsis. GSE48080 was included as a PBMC-based supplementary cohort to provide supportive evidence under a different sample background. Through module-level, gene-level, and cross-cohort integrative analyses, we sought to address three questions: first, whether the dual-axis framework could identify outcome-associated signals in the discovery cohort; second, whether the framework could be reproduced in an independent external cohort and display clear disease-state relevance; and third, whether APC-related programs exhibited greater consistency and reproducibility than IFN/stress-related programs at the gene level. Addressing these questions may provide a more robust framework for transcriptomic stratification of host-response heterogeneity in sepsis.[5,6,8,9]

2 Materials and Methods

2.1 Study design overview

This study was an integrative analysis based on public transcriptomic databases and was organized across three levels: discovery, external validation, and supplementary support. First, GSE65682 was used as the discovery cohort to identify dual-axis module changes associated with adverse outcomes by comparing 28-day ICU survivors and non-survivors. Second, GSE57065 was used as the external validation cohort to evaluate the ability of the dual-axis framework to distinguish disease states in an independent whole-blood sepsis cohort and to explore its temporal stability within the early time window of 0, 24, and 48 hours. Third, GSE48080 was included as a PBMC-based supplementary cohort to provide orthogonal support under a different blood-compartment background. The overall analytical workflow included construction of module scores based on fixed gene sets, module-level comparisons and phase-space visualization, cross-cohort effect-size integration, gene-level differential-expression analysis, representative gene-expression pattern visualization, and evaluation of the discriminatory performance of the dual-axis index.[5,6,8–10]

2.2 Data sources and cohort composition

All public transcriptomic datasets analyzed in this study were retrieved from the Gene Expression Omnibus database and included GSE65682, GSE57065, and GSE48080.[8,9] Data were downloaded and curated on February 15, 2026. GSE65682 is a whole-blood transcriptomic cohort comprising 802 samples and was used as the discovery cohort. GSE57065 is an independent whole-blood transcriptomic cohort comprising 107 samples and was used as the external validation cohort. GSE48080 is a PBMC-based cohort comprising 23 samples and was used for supplementary support. The core characteristics of these three cohorts are summarized in Table 1, whereas detailed grouping information, temporal structure, and additional annotations are provided in Supplementary Table S1.

Cohort	Sample source	Total samples (n)	Primary grouping	Role in study	Main contribution
GSE65682	Whole blood	802	28-day ICU outcome	Discovery cohort for outcome stratification	Framework discovery and outcome association
GSE57065	Whole blood	107	Healthy vs septic shock/patient	External validation cohort (disease-state validation)	Biological relevance and external disease-state support
GSE48080	PBMC	23	Healthy / septic survivor / septic non-survivor	PBMC supplementary cohort	Orthogonal supplementary support

2.3 Data preprocessing and sample matching

Expression matrices from all cohorts were processed using a unified preprocessing workflow. For data generated from different platforms, probe-to-gene mapping or harmonization of gene annotations was first performed according to the corresponding platform annotation files, followed by construction of gene-level expression matrices. For probes mapped to the same gene symbol, a representative expression value was retained according to predefined preprocessing rules. Sample metadata were matched to the expression matrix columns using GEO accession identifiers, and sample IDs were further standardized by removing quotation marks, spaces, and other formatting inconsistencies to ensure consistency across analytical files. For the module score tables and metadata tables, samples were further filtered according to grouping variables, and only those eligible for the corresponding comparative analyses were retained. In GSE65682, the outcome_group was defined as ICU_Survivor_28d and ICU_NonSurvivor_28d; in GSE57065, the disease_group was defined as Healthy_Control and Septic_Shock_or_Patient, while time_group was retained for time-stratified analyses; in GSE48080, the disease_outcome_group was defined as Healthy_Control, Septic_Survivor, and Septic_NonSurvivor. All analyses were conducted using successfully matched samples with complete grouping information.

2.4 Construction of the dual-axis framework

A fixed gene-set strategy was used to construct the dual-axis framework. The MS2_APC module was designed to represent an antigen-presentation-associated myeloid program and included representative genes such as HLA-DRA, HLA-DRB1, HLA-DPA1, HLA-DPB1, HLA-DMA, HLA-DMB, CD74, CTSS, FCER1G, LST1, C1QA, C1QB, C1QC, FCGR3A, and IFI30, primarily reflecting MHC class II-mediated antigen presentation, antigen

processing, and APC-like myeloid functions. The MS3_IFN_stress module was designed to represent an interferon/stress-responsive myeloid program and included representative genes such as IFIT1, IFIT2, IFIT3, ISG15, MX1, MX2, OAS1, OAS2, OAS3, BST2, XAF1, IRF7, STAT1, and CXCL10, mainly reflecting IFN-stimulated responses, antiviral-like programs, and stress-related signaling pathways. Table 2 presents the representative genes of the dual-axis framework and their major functional interpretations, while the full gene annotations are provided in Supplementary Table S2.

Table 2
Representative genes of the dual-axis framework Note: Only representative genes and their major biological interpretations are shown in the main text. Full gene annotations are provided in Supplementary Table S2.

Module	Representative gene	Major functional category	Biological interpretation
MS2_APC	CD74	MHC-II invariant chain	APC-like / antigen-presentation-associated myeloid program
MS2_APC	HLA-DRA	Antigen presentation	APC-like / antigen-presentation-associated myeloid program
MS2_APC	HLA-DRB1	Antigen presentation	APC-like / antigen-presentation-associated myeloid program
MS2_APC	HLA-DPA1	Antigen presentation	APC-like / antigen-presentation-associated myeloid program
MS2_APC	CTSS	Antigen processing	APC-like / antigen-presentation-associated myeloid program
MS2_APC	IFI30	Antigen processing	APC-like / antigen-presentation-associated myeloid program
MS2_APC	FCER1G	Myeloid immune activation	APC-like / antigen-presentation-associated myeloid program
MS2_APC	LST1	Myeloid activation marker	APC-like / antigen-presentation-associated myeloid program
MS3_IFN_stress	IFIT1	Interferon-stimulated response	IFN/stress-responsive myeloid program
MS3_IFN_stress	IFIT2	Interferon-stimulated response	IFN/stress-responsive myeloid program
MS3_IFN_stress	IFIT3	Interferon-stimulated response	IFN/stress-responsive myeloid program
MS3_IFN_stress	ISG15	Interferon-stimulated response	IFN/stress-responsive myeloid program
MS3_IFN_stress	MX1	Antiviral response	IFN/stress-responsive myeloid program

Module	Representative gene	Major functional category	Biological interpretation
MS3_IFN_stress	MX2	Antiviral response	IFN/stress-responsive myeloid program
MS3_IFN_stress	OAS1	Antiviral response	IFN/stress-responsive myeloid program
MS3_IFN_stress	IRF7	IFN regulatory signaling	IFN/stress-responsive myeloid program

For each sample, the expression values of genes within each module were first extracted from the normalized expression matrix and then summarized at the gene-set level to generate a module score, representing the relative activity of the corresponding myeloid functional axis in that sample. The same fixed gene sets and the same scoring strategy were applied across all cohorts to ensure consistency in cross-cohort comparisons. A dual-axis index was further derived to quantify the overall imbalance between the IFN/stress-related axis and the APC-related axis. Higher values of this index indicate that a given sample is shifted toward a more imbalanced state characterized by relatively low APC and relatively high IFN/stress signaling. The primary purpose of this index was to quantify the relative displacement between the two axes rather than to replace either individual module.

2.5 Differential expression and gene-level analyses

To place the dual-axis framework within the broader transcriptomic landscape, differential expression analyses were performed in GSE65682 for ICU non-survivors versus survivors and in GSE57065 for septic patients versus healthy controls. For each gene, the between-group mean difference, log2 fold change, and nominal P value were calculated, followed by multiple-testing correction using the Benjamini–Hochberg method to obtain the false discovery rate. Volcano plots preferentially highlighted genes related to the dual-axis modules to avoid overinterpreting the figures as simple rankings of differentially expressed genes. In addition, to summarize cross-cohort consistency at the gene-set level, we generated a dual-axis-focused volcano plot, a dual-axis gene bubble heatmap, and a representative gene-expression heatmap. In the gene bubble heatmap, bubble color represents log2 fold change and bubble size represents −log10(P), thereby simultaneously reflecting directionality and statistical strength. The representative gene-expression heatmap was constructed using row-wise Z-score normalization to visualize sample-level expression patterns.

2.6 Statistical analysis

Between-group comparisons of continuous variables were performed using the two-sided Mann–Whitney U test, whereas comparisons among multiple groups were performed using

the Kruskal–Wallis test. In cross-cohort analyses, effect direction and magnitude were primarily summarized using Cohen’s d with approximate 95% confidence intervals. The discriminatory performance of the dual-axis index was assessed using receiver operating characteristic curve analysis, including its ability to distinguish ICU survivors from non-survivors in GSE65682 and septic patients from healthy controls in GSE57065. The area under the curve was calculated, and the optimal cutoff value was determined using the Youden index, with the corresponding sensitivity and specificity also reported. All data processing, statistical analyses, and visualizations were performed in the Python environment, mainly using the pandas, numpy, scipy, matplotlib, and scikit-learn packages. Because this study used only de-identified transcriptomic data from public databases and involved no new patient recruitment or intervention, no additional ethics approval was required.

3 Results

3.1 Identification of an outcome-associated dual-axis myeloid imbalance pattern in the GSE65682 discovery cohort

In the GSE65682 discovery cohort, phase-space analysis of the dual-axis framework showed that 28-day ICU survivors and non-survivors were not separated along a single dimension but instead exhibited an overall positional shift within the two-dimensional module space defined by MS2_APC and MS3_IFN_stress. Compared with survivors, the centroid of the non-survivor group was shifted more toward a low-MS2_APC region, suggesting that outcome-associated host-response remodeling was primarily characterized by an overall imbalance under suppression of the APC-related axis.

Comparison of module distributions showed that MS2_APC was significantly lower in non-survivors, whereas the dual-axis index was significantly higher in non-survivors. Although MS3_IFN_stress showed an increasing trend, the between-group difference did not reach statistical significance. These findings indicate that outcome-associated differences in the discovery cohort were mainly driven by downregulation of the APC-related axis together with increased overall dual-axis imbalance, rather than by isolated elevation of IFN/stress signaling.

Further comparison of the distributions of the three core metrics between survivors and non-survivors showed that MS2_APC module scores were significantly lower in non-survivors, whereas the dual-axis index was significantly higher. By contrast, although MS3_IFN_stress showed a modest upward trend, the between-group difference did not reach statistical significance. These findings suggest that, in the discovery cohort, signals

more directly associated with adverse outcomes were not simply attributable to enhanced IFN/stress signaling, but rather to persistent downregulation of the APC-like myeloid program and the resulting aggravation of dual-axis imbalance. In other words, non-survivors were not merely characterized by “greater inflammation” or “stronger stress responses,” but were more likely to be in an abnormal state marked by impaired antigen-presentation capacity and reduced coordination of myeloid immune function. Consistent with the phase-space distribution, reduced MS2_APC and elevated dual-axis index pointed in the same direction and jointly defined the overall shift of non-survivors toward a “low-APC/high-imbalance” region. In contrast, changes in MS3_IFN_stress appeared more similar to an accompanying signal, with greater inter-sample variability and substantial overlap between groups, indicating limited stability of this axis when used alone for outcome stratification. Therefore, the findings from the discovery cohort support the interpretation that, in host-response remodeling associated with adverse outcomes in sepsis, suppression of APC-related myeloid function represents the more central abnormality, while the dual-axis index further amplifies the overall imbalance associated with this abnormal state.

ROC analysis showed that the dual-axis index had only moderate-to-low discriminatory performance for distinguishing ICU survivors from non-survivors in GSE65682, suggesting that this index is better interpreted as a host-response framework signal rather than as a stand-alone high-performance prognostic marker.

The kernel density distributions showed that the curve for non-survivors was shifted overall to the right relative to that for survivors, although substantial overlap remained between the two groups, suggesting that the dual-axis index is more informative for capturing risk shifts at the population level than for defining a clear boundary at the individual level.

Overall, GSE65682 provided the discovery basis for this study, indicating that adverse outcomes in sepsis were mainly associated with downregulation of the APC-related myeloid program and increased dual-axis imbalance, whereas changes in the IFN/stress axis alone showed relatively limited statistical stability at the discovery stage.

3.2 External validation in GSE57065 supports clear disease-state relevance of the dual-axis framework

In the independent whole-blood cohort GSE57065, we first compared dual-axis-related module scores between healthy controls and septic patients. The results showed that, compared with healthy controls, septic patients exhibited a marked reduction in MS2_APC, whereas MS3_IFN_stress and the dual-axis index were significantly increased. In contrast to the discovery cohort, the external validation cohort showed stronger signals and more consistent directions, suggesting that the dual-axis framework had substantially

greater discriminatory value at the disease-state level than as an isolated predictor of outcome. Specifically, the reduction in MS2_APC suggested more pronounced suppression of antigen-presentation-associated myeloid function in septic patients, whereas the increase in MS3_IFN_stress reflected a more prominent background of interferon/stress activation. Together, these changes shifted the dual-axis index upward in the sepsis group and produced clearer group separation. Compared with the relatively modest outcome-associated signal observed in GSE65682, the disease-state signal in GSE57065 was supported not only by stronger statistical evidence but also by highly concordant directions across all three core metrics, indicating that the dual-axis framework should first be regarded as a stratification tool for characterizing host-response states in sepsis and only secondarily as a potential outcome-related marker in specific settings. These findings provide critical external evidence for the subsequent cross-cohort integrative analysis and further support APC attenuation and dual-axis imbalance as more stable host-response features.

ROC analysis further supported this finding. In GSE57065, the dual-axis index showed excellent discriminatory performance for distinguishing septic patients from healthy controls, with an AUC approaching 1.0, indicating highly robust statistical separation in the disease-state discrimination setting.

This suggests that the dual-axis framework not only captures host-response differences at the module level, but also effectively reflects the overall transcriptomic shift between healthy and septic states at the composite-index level. However, this does not imply that the index is already ready for direct translation into a bedside diagnostic tool, because the current evidence is still based on retrospective analyses of public cohorts, and the comparison between healthy controls and septic patients is less clinically complex than real-world ICU scenarios. A more appropriate interpretation is that the dual-axis index demonstrates strong capacity for identifying host-response states in the external validation cohort, thereby supporting the biological plausibility of this framework as a transcriptomic stratification approach in sepsis, rather than simply equating it with a mature clinical diagnostic marker.

In the time-course analysis, we further examined changes in module scores among septic patients in GSE57065 at 0, 24, and 48 hours. The results showed that neither MS2_APC, MS3_IFN_stress, nor the dual-axis index exhibited a clear time-dependent stratification pattern within this early observation window, and neither the overall comparisons nor the pairwise comparisons between time points reached statistical significance. These findings suggest that, in the current cohort and at the present sampling density, the dual-axis framework primarily captures cross-sectional features of the overall host-response state in sepsis rather than rapid dynamic fluctuations occurring over the first several tens of hours. In other words, this framework appears to be better suited for identifying whether

a patient is in a “sepsis-related imbalance state” than for reflecting subtle temporal changes within a short time window. This observation also indicates that further evaluation of its value for dynamic monitoring will require validation in longitudinal cohorts with higher temporal resolution or larger sample sizes.

Therefore, the main contribution of GSE57065 lies in providing relatively clear and directionally consistent validation of the dual-axis framework at the disease-state level in an independent whole-blood cohort. Compared with the discovery cohort, the signals in this cohort were stronger and the group separation was clearer, further reinforcing the stability of APC attenuation and dual-axis imbalance, while also indicating that the external reproducibility of this framework for disease-state discrimination is superior to its stand-alone discriminatory performance for outcome prediction.

3.3 Cross-cohort integrative analyses indicate that APC attenuation is the more stable signal

To compare the direction and magnitude of each module across different cohorts and endpoints at an overall level, we constructed a cross-cohort forest plot and a module-effect bubble plot. The forest plot showed that, across both the discovery and validation cohorts, reduction of MS2_APC and elevation of the dual-axis index displayed more stable consistency, whereas MS3_IFN_stress varied more substantially in magnitude across different cohorts and comparison settings. Specifically, in the comparison between non-survivors and survivors in GSE65682, MS2_APC showed a decreasing trend while the dual-axis index showed an increasing trend, forming the most central result pattern at the discovery stage. In the comparison between septic patients and healthy controls in GSE57065, this pattern was further strengthened and accompanied by a more evident increase in MS3_IFN_stress, indicating that disease-state comparisons produced a stronger overall separation effect than outcome comparisons. By contrast, in GSE48080, although the directions of some metrics were generally consistent with those observed in the other cohorts in both the sepsis-versus-healthy and non-survivor-versus-survivor comparisons, the statistical strength was limited, suggesting that this cohort is better interpreted as supplementary evidence rather than as primary validation evidence. The module-effect bubble plot further compressed different cohorts, comparisons, and metrics into a unified two-dimensional framework and showed that MS2_APC and the dual-axis index not only exhibited more stable directions across most key comparisons, but also had more concentrated significance levels and effect sizes. Although MS3_IFN_stress showed an increase in some comparisons, its cross-cohort consistency was clearly weaker than that of the other two metrics. Taken together, these cross-cohort integrative analyses do not support the interpretation that the IFN/stress axis represents the most stable dominant signal; rather, they suggest that APC attenuation and the corresponding

increase in dual-axis imbalance are the more reliable and reproducible features of host-response remodeling in sepsis.

The module-effect bubble plot further condensed the four key comparisons into a single two-dimensional panel, allowing direct side-by-side comparison of the direction and magnitude of the signals across different cohorts, endpoints, and modules. The results showed that, in the outcome comparison in GSE65682 and the disease-state comparison in GSE57065, both MS2_APC and the dual-axis index exhibited clearer effect directions and stronger statistical support, indicating that these two metrics showed relatively good stability at both the discovery and validation levels. By contrast, although MS3_IFN_stress showed an upward trend in some comparisons, its statistical significance and consistency were weaker than those of the other two metrics. In the two comparisons in GSE48080, the direction of change of some metrics was consistent with the main analyses, but the bubbles were generally smaller and the statistical support was limited, indicating that this cohort provided trend-level supplementary evidence rather than strong validation. Overall, this plot further reinforced a central conclusion: the more stable and reproducible host-response abnormality in sepsis is primarily characterized by reduction of the APC-like myeloid program together with increased dual-axis imbalance.

Table 3 summarizes the main results of the core dual-axis-related metrics across key cohort comparisons. In GSE65682, the significant changes were mainly characterized by reduced MS2_APC and an increased dual-axis index. In GSE57065, reduced MS2_APC, increased MS3_IFN_stress, and an increased dual-axis index were all supported. Overall, GSE48080 provided only trend-level supplementary evidence. Detailed statistical results are presented in Supplementary Table S3.

Table 3
Main results of the dual-axis-related metrics across key cohort comparisons Note: Only the main results of the core dual-axis-related metrics are shown in the main text. Detailed statistical results are provided in Supplementary Table S3.

Cohort	Comparison	Metric	Direction of change	Cohen's d	P value
GSE65682	Non-survivor vs Survivor	MS2_APC	Lower in non-survivors	-0.41	< 0.001
GSE65682	Non-survivor vs Survivor	MS3_IFN_stress	Trend toward lower levels in non-survivors	-0.16	0.113
GSE65682	Non-survivor vs Survivor	Dual-axis index	Higher in non-survivors	0.24	0.027
GSE57065	Septic vs Healthy	MS2_APC	Lower in septic patients	-2.89	< 0.001
GSE57065	Septic vs Healthy	MS3_IFN_stress	Higher in septic patients	0.46	0.004
GSE57065	Septic vs Healthy	Dual-axis index	Higher in septic patients	3.29	< 0.001
GSE48080	Sepsis vs Healthy	MS2_APC	Trend toward lower levels in sepsis	-0.58	0.23
GSE48080	Sepsis vs Healthy	MS3_IFN_stress	Trend toward higher levels in sepsis	0.42	0.635
GSE48080	Sepsis vs Healthy	Dual-axis index	Trend toward higher levels in sepsis	1.35	0.06
GSE48080	Non-survivor vs Survivor	MS2_APC	Trend toward lower levels in non-survivors	-0.32	0.734
GSE48080	Non-survivor vs Survivor	MS3_IFN_stress	Trend toward lower levels in non-survivors	-0.49	0.385
GSE48080	Non-survivor vs Survivor	Dual-axis index	Trend toward lower levels in non-survivors	-0.14	0.623

Taken together, these findings support the conclusion that, within the current analytical framework, reduction of the APC-like program and dual-axis imbalance show greater cross-cohort consistency than isolated changes in the IFN/stress axis, and are therefore more suitable as central anchors for host-response stratification in sepsis. Specifically, both reduced MS2_APC and elevated dual-axis index exhibited more stable directional

patterns and reproducibility not only in the outcome comparison within the discovery cohort but also in the disease-state comparison within the external validation cohort. By contrast, although MS3_IFN_stress may retain biological relevance in certain settings, its magnitude of change and statistical stability were comparatively limited. These results suggest that the key imbalance in sepsis is not merely enhanced stress or interferon signaling per se, but rather a form of structured immune remodeling arising in the context of impaired APC-related myeloid function. This interpretation not only provides cross-cohort support for the dual-axis framework, but also offers a clearer direction for subsequent mechanistic studies and clinical stratification validation centered on APC attenuation.

3.4 Gene-level analyses place the dual-axis framework within a broader transcriptomic context

To determine whether the dual-axis framework was reflected only at the level of module scores or was also supported by a broader gene-level background, we performed volcano plot analyses in both GSE65682 and GSE57065. The results showed that, although a differential expression background was present in the outcome comparison in GSE65682, the overall number of significant genes and the signal intensity were relatively limited, suggesting that outcome-related transcriptomic remodeling was characterized more by directional shifts than by extensive large-scale reprogramming. In contrast, the disease-state comparison in GSE57065 showed a stronger and more widespread transcriptomic shift, with not only a markedly greater number of significantly differentially expressed genes but also clearer stratification of both upregulated and downregulated genes, indicating that host-response perturbation at the disease-state level was more pronounced than the within-sepsis differences associated with outcome. The difference in comparison strength was also consistent with the module-level results described above: GSE65682 was more suitable for identifying directional signals of APC attenuation and dual-axis imbalance, whereas GSE57065 was more appropriate for validating the reproducibility of this framework in disease-state discrimination in sepsis. Therefore, the significance of the volcano plot analysis did not lie merely in ranking the most significantly differentially expressed genes, but rather in showing, from the perspective of the global transcriptomic background, that both the discovery and validation cohorts exhibited expression shifts supportive of the dual-axis framework, although the former was characterized by a milder outcome-related signal and the latter by a stronger disease-state-related remodeling pattern.

In the focused volcano plots, we further highlighted the genes belonging to the dual-axis gene sets to determine whether this framework could exhibit a structured rather than random differential expression pattern within the global transcriptomic background. The results showed that MS2_APC-related genes were more likely to display directionally

concordant shifts in both key comparisons, particularly in the comparison between septic patients and healthy controls in GSE57065, where they were more concentrated overall in the negative direction. This finding suggests that suppression of the APC-like myeloid program was not driven by a few isolated genes, but more likely reflected coordinated downregulation of a functionally related group of genes. By contrast, although some MS3_IFN_stress-related genes showed significant changes in both cohorts, and their upward trend was more apparent in GSE57065, their spatial distribution was relatively dispersed. They neither showed the same degree of concentrated shift as the APC-related axis nor exhibited equally stable consistency between the discovery and validation settings. These results indicate that the IFN/stress axis may represent a more variable signal influenced by disease context, inter-individual differences, and the magnitude of stress responses, whereas the APC-related axis is more consistent with a reproducible core imbalance pattern in sepsis. In other words, the focused volcano plots do not simply show “which group of genes is more significant,” but rather demonstrate that the two functional modules differ fundamentally in how they are organized within the background of global transcriptomic differences: MS2_APC is more concentrated and directionally coherent, whereas MS3_IFN_stress is more dispersed and heterogeneous. This provides a direct gene-level explanation for the module-level findings.

Based on the fixed gene sets listed in Table 2, we further constructed a cross-cohort dual-axis gene bubble heatmap that integrated the direction and statistical strength of representative gene changes across the two core comparisons in the discovery and validation settings. The results showed that MS2_APC-related genes were consistently shifted in the negative direction in both GSE65682 and GSE57065, and that multiple representative genes exhibited concordant directions together with relatively strong statistical support in both comparisons. This indicates that downregulation of the APC-related program was not confined to a single cohort, but instead represented a reproducible shared feature across cohorts. In contrast, although MS3_IFN_stress-related genes showed stronger positive signals in the validation cohort, the magnitude of change was weaker in the discovery cohort, and the statistical strength of some genes was unstable. As a result, the overall cross-cohort consistency of this module was clearly inferior to that of the APC module. The importance of this figure lies in its ability to elevate gene-level differences from “single-cohort observations” to “cross-cohort comparisons,” thereby further demonstrating that APC attenuation is not an incidental local phenomenon but a key basis for the stability of the dual-axis framework. Taken together, the focused volcano plots and the bubble heatmap indicate that the dual-axis framework is able to generate a relatively clear module-level and cross-cohort conclusion primarily because the APC-related gene set shows stronger directional concordance and reproducibility, whereas IFN/stress-related genes more often reflect background amplification or accompanying changes.

To further examine sample-level expression patterns, we generated a heatmap of representative dual-axis genes in GSE65682. The results showed that APC-related genes, including LST1, CTSS, IFI30, CST3, CD74, HLA-DRB1, HLA-DMA, and HLA-DPA1, were overall lower in non-survivors, and this decrease was not confined to a few isolated samples but instead appeared as a relatively consistent downward shift at the group level. In contrast, although IFN/stress-related genes such as MX2, BST2, GBP2, IRF7, XAF1, IFIT2, GBP1, and OAS1 showed higher expression in some non-survivors, their distribution was more heterogeneous, with substantially greater variation across samples. This heatmap suggests that the main outcome-associated feature was more consistent with coordinated downregulation of a set of APC-related genes rather than stable, synchronous upregulation of a set of IFN/stress-related genes. In other words, the expression pattern reflected by the APC-related axis was more concentrated and coherent, whereas the IFN/stress axis more strongly represented an accompanying heterogeneous response.

Taken together, the gene-level analyses indicate that the dual-axis framework is not an abstract scoring system detached from the global expression background, but rather a structured transcriptomic pattern supported by fixed representative gene sets. Across the focused volcano plots, the cross-cohort gene bubble heatmap, and the representative gene-expression heatmap, APC-related genes consistently exhibited a more stable and coherent negative shift, whereas IFN/stress-related genes showed more dispersed and heterogeneous patterns of change. These findings further indicate that APC attenuation represents the more central and reproducible biological anchor of the dual-axis framework.

3.5 GSE48080 provides supplementary PBMC-level support rather than primary external validation

As a PBMC-based cohort, GSE48080 provided supplementary evidence for evaluating the transferability of the dual-axis framework across different blood-component backgrounds. Comparisons among healthy controls, septic survivors, and septic non-survivors showed that MS2_APC, MS3_IFN_stress, and the dual-axis index all displayed certain directional differences, but the overall statistical separation was limited, particularly with respect to outcome stratification within the septic subgroups. Specifically, some degree of distributional shift could be observed in the combined comparison between healthy and septic samples, but the statistical significance was weaker than that observed in the whole-blood cohorts. In the comparison between survivors and non-survivors, the three metrics showed even greater overlap, indicating limited stability of outcome-related signals in the PBMC background.

This result indicates that the dual-axis framework does not manifest with equal strength across all sample backgrounds. In the PBMC setting, it primarily provides directional

support rather than evidence strong enough to serve as the main external validation. Given the relatively small sample size, the more restricted cellular composition, and the inherent differences between PBMC and whole blood with respect to key myeloid components, GSE48080 is best interpreted as providing orthogonal supplementary evidence. These findings suggest that the framework retains a certain degree of biological relevance across different peripheral immune backgrounds, but its statistical strength and clinical generalizability are insufficient to replace the core validation provided by GSE57065. Therefore, within the overall evidence structure of this study, GSE57065 was designated as the primary external validation cohort, whereas GSE48080 was regarded as supplementary supportive evidence.

4 Discussion

Using public transcriptomic cohorts, this study proposed and validated a dual-axis myeloid imbalance framework in sepsis. Through discovery-stage analysis, external validation, gene-level investigation, and cross-cohort integration, we established an evidence framework spanning from modules to genes and from single-cohort findings to multi-cohort reproducibility. Overall, the results indicate that host-response heterogeneity in sepsis can be characterized by two interrelated but asymmetrical myeloid programs: one representing an antigen-presentation-associated program centered on MHC class II, antigen processing, and APC-like functions, and the other representing an IFN/stress-related program characterized by interferon-stimulated genes and stress-response signatures. More importantly, these two programs do not show equal stability, and suppression of the APC-like program demonstrates greater consistency across cohorts, comparisons, and analytical levels.

One of the most important findings of this study is that, in the GSE65682 discovery cohort, adverse outcomes were more clearly associated with reduced MS2_APC and increased dual-axis imbalance than with isolated enhancement of MS3_IFN_stress. Traditionally, studies of host responses in sepsis have tended to emphasize increased inflammation or upregulation of IFN-related pathways. However, our findings suggest that, in outcome-associated comparisons, the more stable abnormality may not be inflammation per se, but rather the decline in APC-like myeloid function. In other words, the IFN/stress axis may reflect a more dynamic and heterogeneous activation background, whereas reduction of the APC axis may more closely represent the core of persistent immune remodeling.

This conclusion was further strengthened in the GSE57065 external validation cohort. Compared with the relatively modest outcome-related signal observed in the discovery cohort, the disease-state comparison in GSE57065 showed a more pronounced reduction in MS2_APC and a stronger increase in the dual-axis index, accompanied by a clearer elevation of MS3_IFN_stress. This suggests that the dual-axis framework has a clearer

expression profile for disease-state discrimination than for outcome prediction. In other words, the framework should first be understood as a host-response stratification signal, and only secondarily as a potential prognostic tool in specific contexts. This distinction is critical to the positioning of the present study: we do not present the dual-axis index as a stand-alone high-performance predictor, but rather as a biologically meaningful host-response stratification framework.

At the gene level, our analyses further demonstrated the reproducibility of APC attenuation. Across the focused volcano plots, the cross-cohort gene bubble heatmap, and the representative gene-expression heatmap, APC-related genes consistently showed directionally concordant shifts in both the discovery and validation settings. By contrast, although IFN/stress-related genes exhibited significant changes in some comparisons, their overall direction and magnitude were more dispersed. This suggests that, if a more robust myeloid host-response anchor is to be identified in sepsis, suppression of the APC-like program may deserve priority over isolated IFN/stress-related changes.

Our observation of a decline in the APC-like program is consistent with the immune-suppressive features described in previous studies of sepsis host-response stratification. Multiple transcriptomic studies have shown that one of the key differences among endotypes or host-response subtypes lies in the degree of impairment in antigen presentation, monocyte activation, and MHC class II-related pathways. In particular, reduced monocyte HLA-DR has long been regarded as an important marker of sepsis-associated immunosuppression and increased susceptibility to secondary infection. The MS2_APC module defined in the present study provides a more systematic, transcriptome-level modular representation of this APC-like myeloid functional axis. Compared with conventional single-marker approaches, this module is not restricted to HLA-DR alone, but integrates multiple genes involved in antigen processing and presentation, including HLA-DRA, HLA-DRB1, HLA-DPA1, CD74, CTSS, and IFI30, thereby more stably reflecting global impairment of the APC program.

On the other hand, previous endotype studies in sepsis have also emphasized the importance of IFN-related signaling, inflammatory amplification, and stress-pathway activation. However, these signals are often more susceptible to variation according to infection site, pathogen type, sampling time, and baseline patient status. In our study, although MS3_IFN_stress-related genes showed increased expression in the validation cohort, their cross-cohort consistency was clearly weaker than that of the APC-related program. This suggests that, in studies of host-response heterogeneity, the IFN/stress axis may represent an important but more variable background signal, whereas APC attenuation may constitute the more reproducible core anchor. Unlike previous studies that aimed to define novel endotypes, our study did not attempt to rename or reclassify sepsis subtypes. Instead, we used a dual-axis framework to compress transcriptomic

heterogeneity, thereby preserving biological interpretability while improving cross-cohort transferability and stability.

From a clinical perspective, the framework proposed in this study has three main implications. First, it provides a more compact transcriptomic coordinate system for understanding host-response heterogeneity in sepsis, thereby avoiding oversimplification into a dichotomy of “high inflammation” versus “low inflammation.” Second, it offers a fixed modular basis for horizontal comparisons across public cohorts, helping to reduce the inconsistency that arises when different studies use non-overlapping signature definitions. Third, it suggests that future higher-level stratification studies or bedside translational efforts should prioritize the APC-related axis, rather than focusing predominantly on the more variable IFN/stress axis.

This study also has several limitations. First, all analyses were based on public transcriptomic cohorts, and no independent prospective clinical cohort was available for external clinical validation. Therefore, the current findings are better regarded as hypothesis-generating and framework-validating rather than immediately translatable into a clinical tool. Second, GSE48080 had a relatively small sample size, and the PBMC background differs inherently from whole blood; accordingly, its results should be interpreted only as supplementary support. Third, the ROC performance of the dual-axis index for outcome discrimination in GSE65682 was limited, indicating that the index is currently better suited as a structured host-response signal than as an independent prognostic classifier. Fourth, although this study provides mechanistic clues at both the gene and module levels, the causal role of APC-related pathways has not yet been tested using independent experimental data or functional studies. Future validation in independent clinical samples, prospective cohorts, and mechanistic experiments will be essential to strengthen the translational value of this framework.

Overall, the significance of this study lies not in proposing a “universal predictive score,” but in establishing a more robust host-response framework for sepsis based on public transcriptomic data and showing through cross-cohort analyses that, among myeloid imbalance-related signals, APC attenuation may represent a more stable and reproducible anchor than IFN/stress-related variation. This conclusion may offer both methodological and conceptual value for future studies on sepsis stratification, immune-state monitoring, and candidate target discovery.

5 Conclusions

Based on public whole-blood and PBMC transcriptomic cohorts, this study constructed and validated a dual-axis myeloid imbalance framework in sepsis. The results showed that this framework was associated with adverse outcomes in the discovery cohort, demonstrated

clear disease-state relevance in an independent external whole-blood cohort, and further suggested at the gene level that downregulation of the APC-like myeloid program is more stable and reproducible than IFN/stress-related variation. The dual-axis framework provides a biologically interpretable transcriptomic perspective for understanding host-response heterogeneity in sepsis, although its clinical predictive utility and bedside applicability still require further validation in independent clinical cohorts.

Declarations

Funding

This study was supported by the National Natural Science Foundation of China (Grant No. 82204876).

Author Contributions

Qinyuan Du and Congcong Qin contributed equally to this work. Li Kong conceived the study, designed the overall manuscript framework, supervised the academic content, and approved the final version of the manuscript. Qinyuan Du contributed to study conception, data curation, statistical analysis, figure preparation, and drafting of the manuscript. Congcong Qin contributed to study design, literature review, interpretation of the results, and manuscript revision. Guochen Li and Qianyu Bi participated in data preprocessing, result verification, and figure organization. Hao Hao and Feihu Zhang participated in study discussion, interpretation of the findings, and academic revision of the manuscript. Batejin, Shuanglin Zhang, Baogenna Bao, and Meiyu Bao participated in data organization, discussion of the results, and manuscript revision. All authors reviewed and approved the final version of the manuscript and take responsibility for the authenticity, accuracy, and integrity of the work.

Competing Interests

The authors declare that they have no competing interests.

Data Availability

The transcriptomic datasets used in this study were obtained from publicly available databases and can be accessed through the corresponding accession numbers, including GSE65682, GSE57065, and GSE48080. The curated data, figure outputs, and analysis code generated during the current study are available from the corresponding author on reasonable request.

References

1. Singer M, Deutschman CS, Seymour CW, Shankar-Hari M, Annane D, Bauer M, et al. The third international consensus definitions for sepsis and septic shock (Sepsis-3). JAMA. 2016;315(8):801–810.
2. Vincent JL, Moreno R, Takala J, Willatts S, De Mendonça A, Bruining H, et al. The SOFA (Sepsis-related Organ Failure Assessment) score to describe organ dysfunction/failure. Intensive Care Med. 1996;22(7):707–710.
3. Knaus WA, Draper EA, Wagner DP, Zimmerman JE. APACHE II: a severity of disease classification system. Crit Care Med. 1985;13(10):818–829.
4. Davenport EE, Burnham KL, Radhakrishnan J, Humburg P, Hutton P, Mills TC, et al. Genomic landscape of the individual host response and outcomes in sepsis: a prospective cohort study. Lancet Respir Med. 2016;4(4):259–271.
5. Scicluna BP, van Vught LA, Zwinderman AH, Wiewel MA, Davenport EE, Burnham KL, et al. Classification of patients with sepsis according to blood genomic endotype: a prospective cohort study. Lancet Respir Med. 2017;5(10):816–826.
6. Scicluna BP, Cano-Gamez K, Rademaker E, Butler JM, van Vught LA, Zaal E, et al. A consensus blood transcriptomic framework for sepsis. Nat Med. 2025;31(12):4119–4130.
7. Joshi I, Carney WP, Rock EP. Utility of monocyte HLA-DR and rationale for therapeutic GM-CSF in sepsis immunoparalysis. Front Immunol. 2023;14:1130214.

Figures

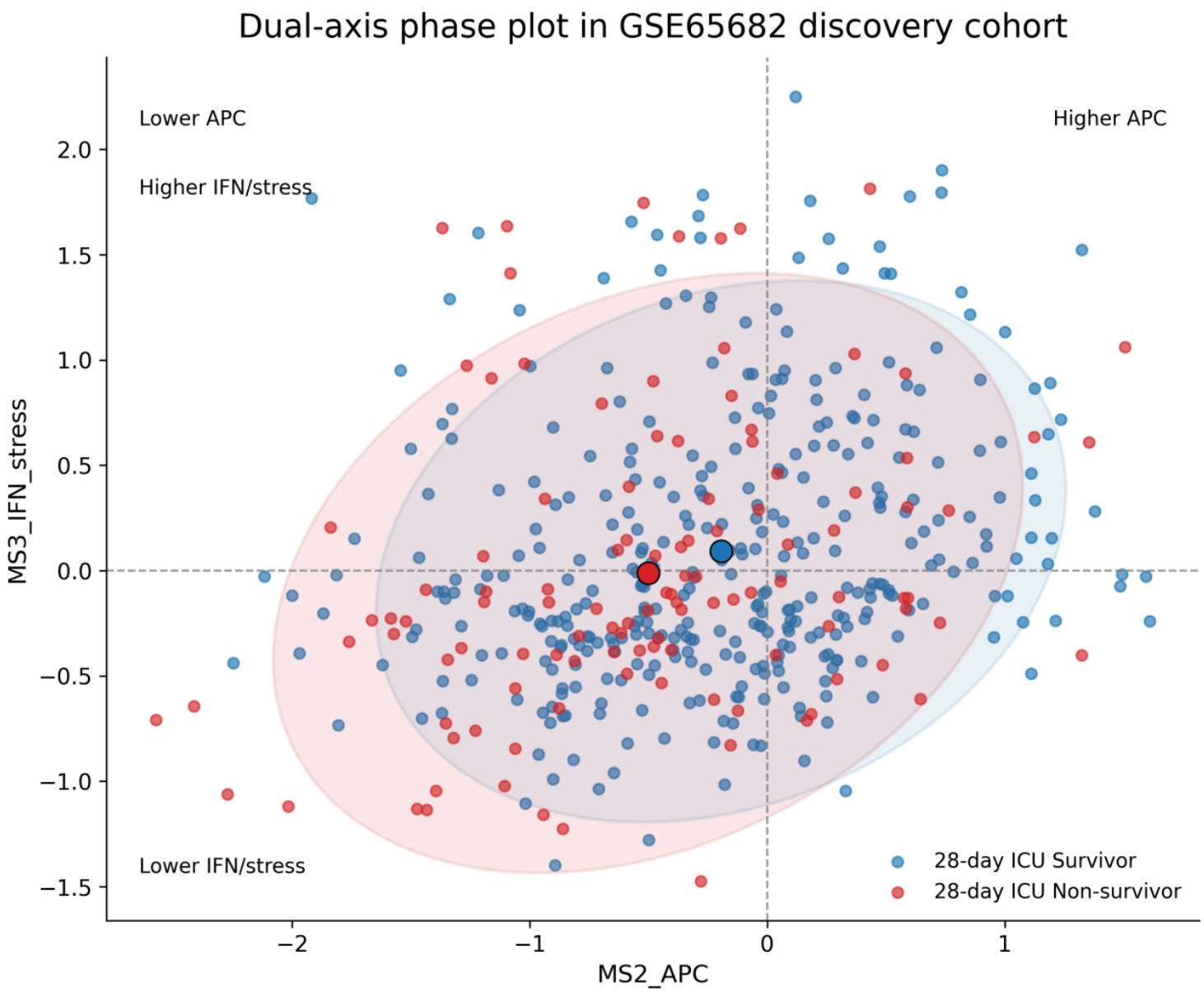


Figure 1

Dual-axis phase-space distribution in the GSE65682 discovery cohort

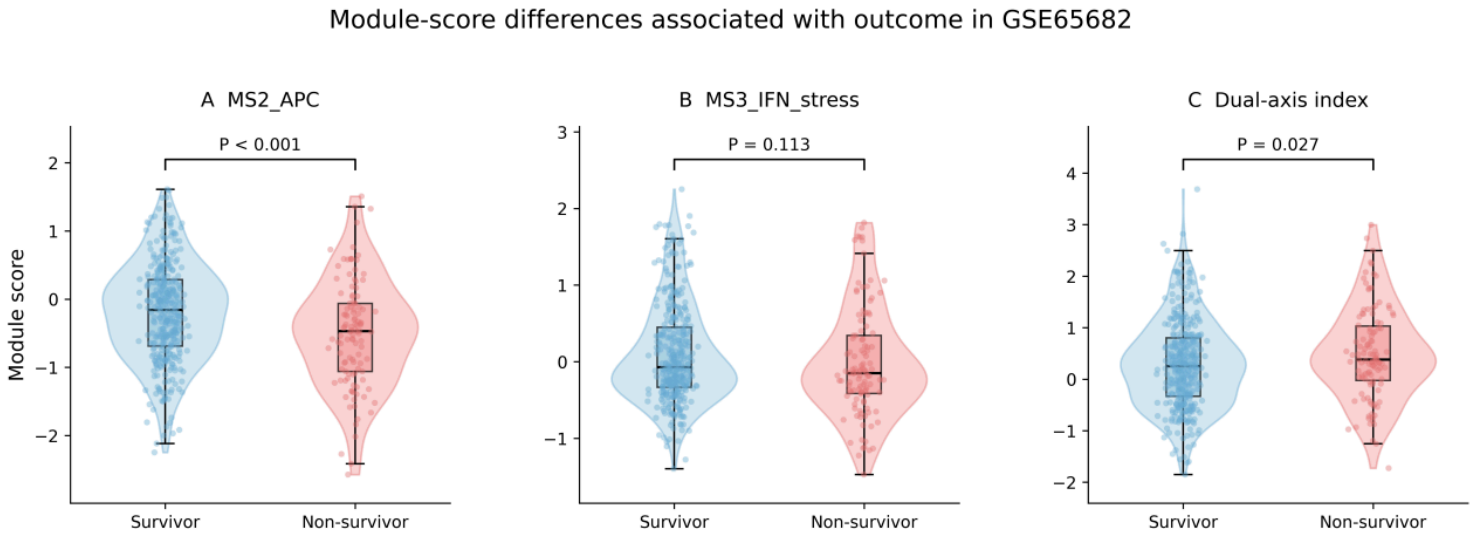


Figure 2

Outcome-associated differences in module scores in the GSE65682 discovery cohort

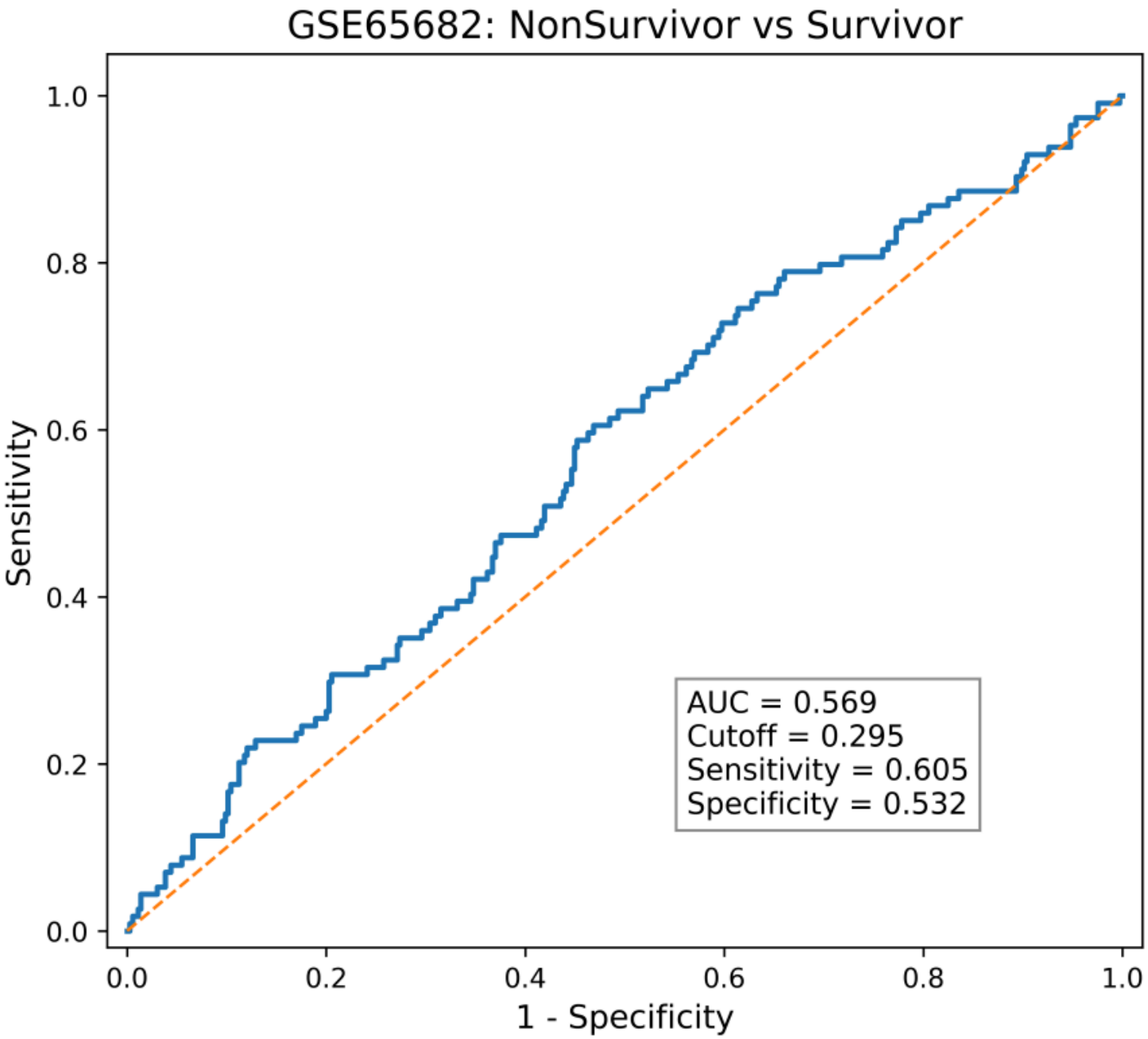


Figure 3

ROC performance of the dual-axis index for outcome stratification in the GSE65682 cohort

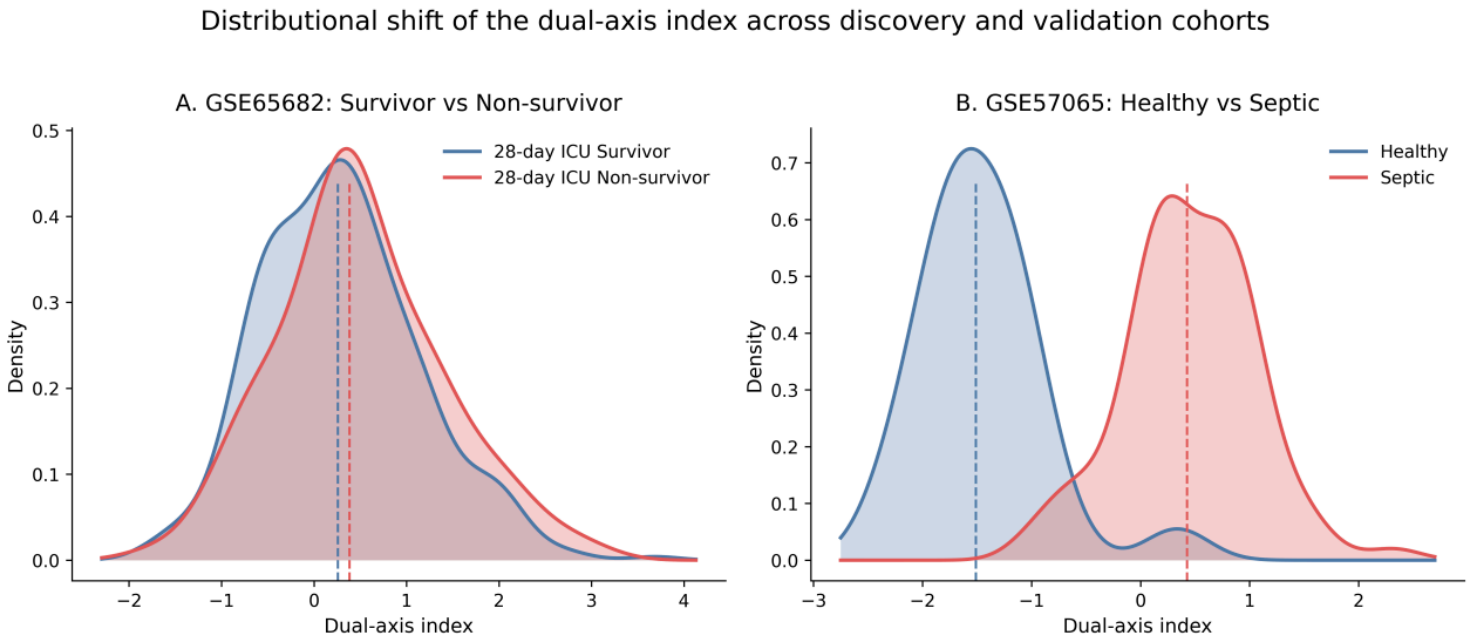


Figure 4

Distributional shift of the dual-axis index in the discovery and validation settings

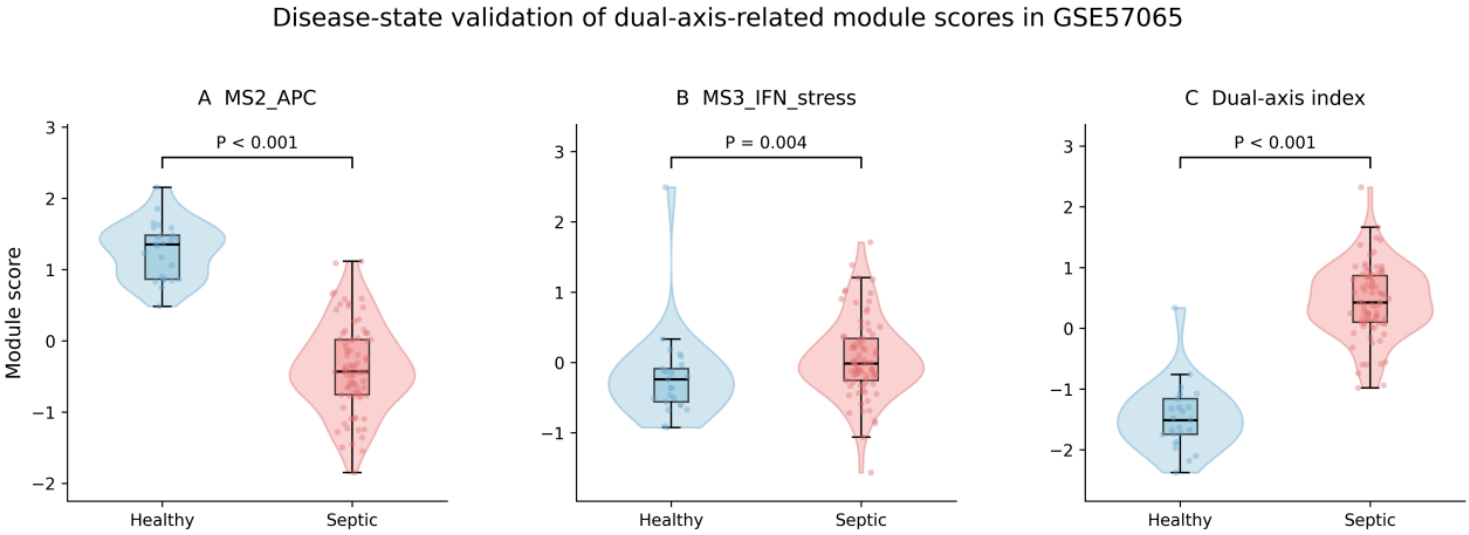


Figure 5

Disease-state validation of dual-axis-related module scores in the GSE57065 cohort

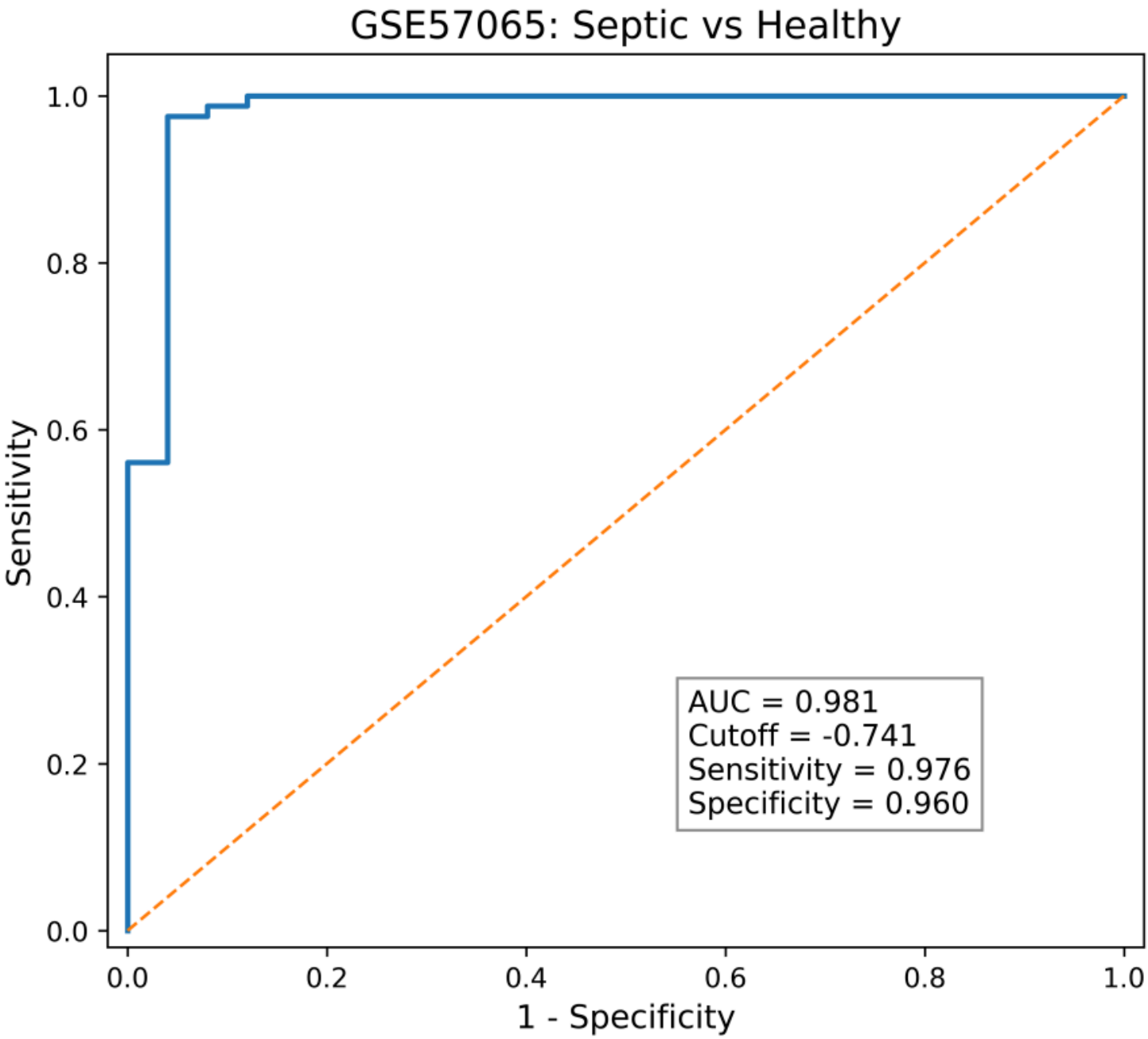


Figure 6

ROC performance of the dual-axis index for disease-state discrimination in the GSE57065 cohort

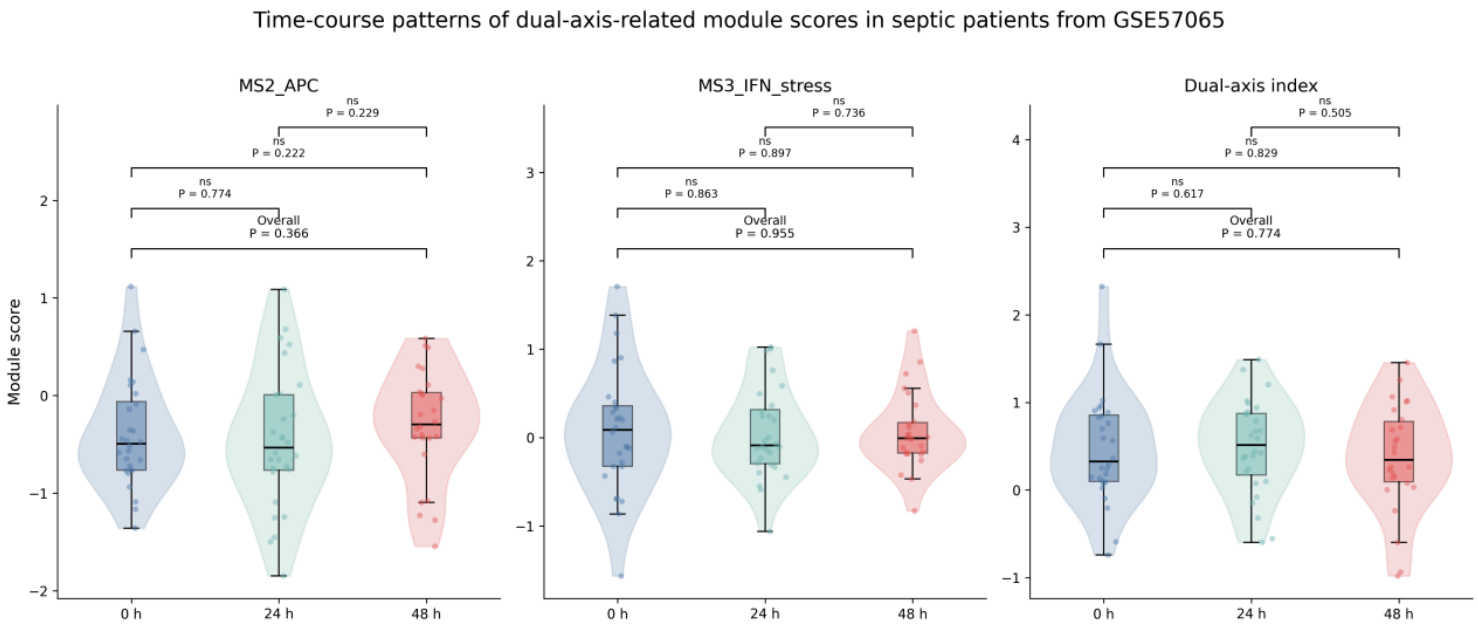


Figure 7

Temporal patterns of dual-axis-related module scores in septic patients from GSE57065

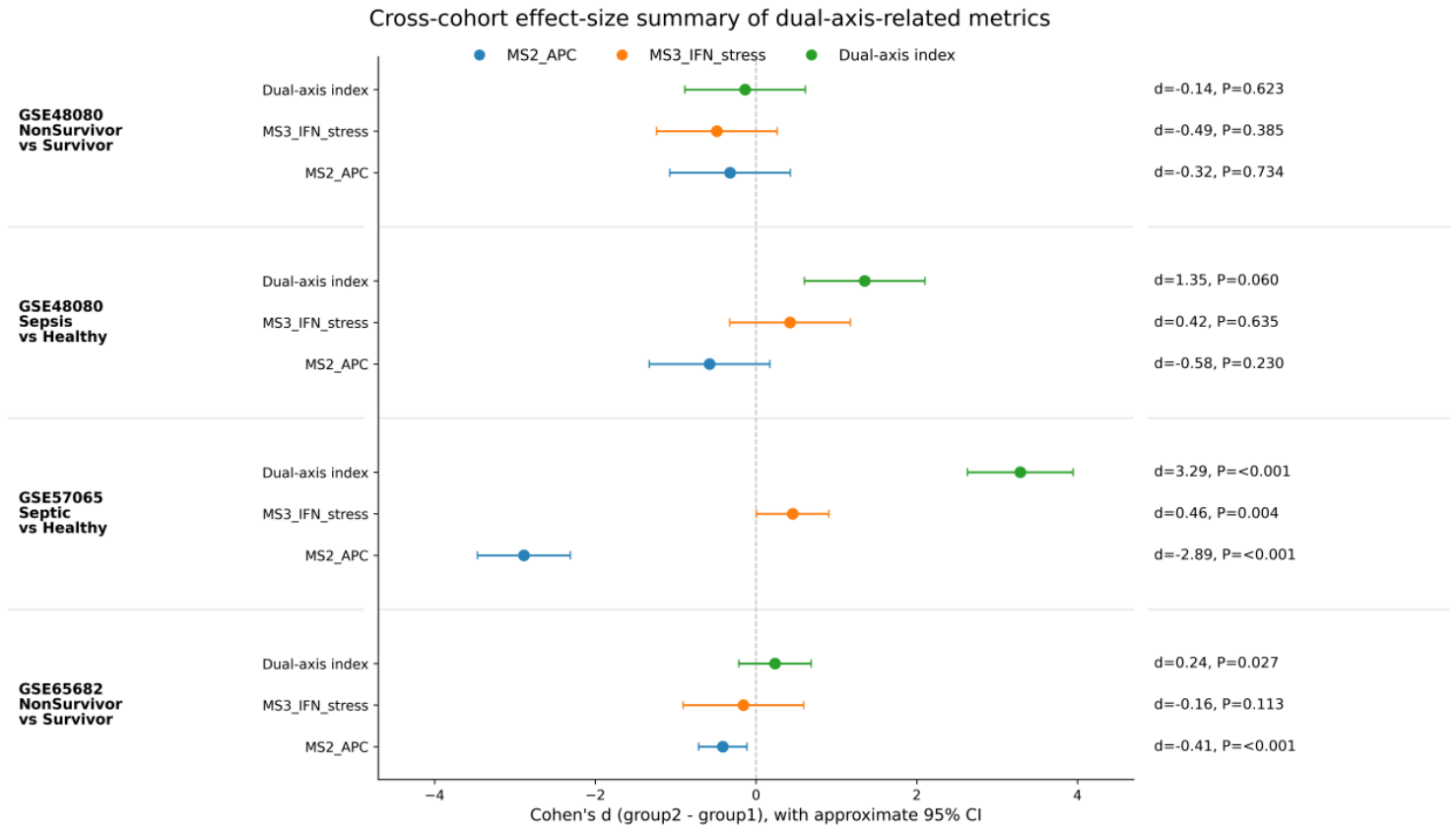


Figure 8

Cross-cohort summary of effect sizes for the dual-axis framework across different cohorts

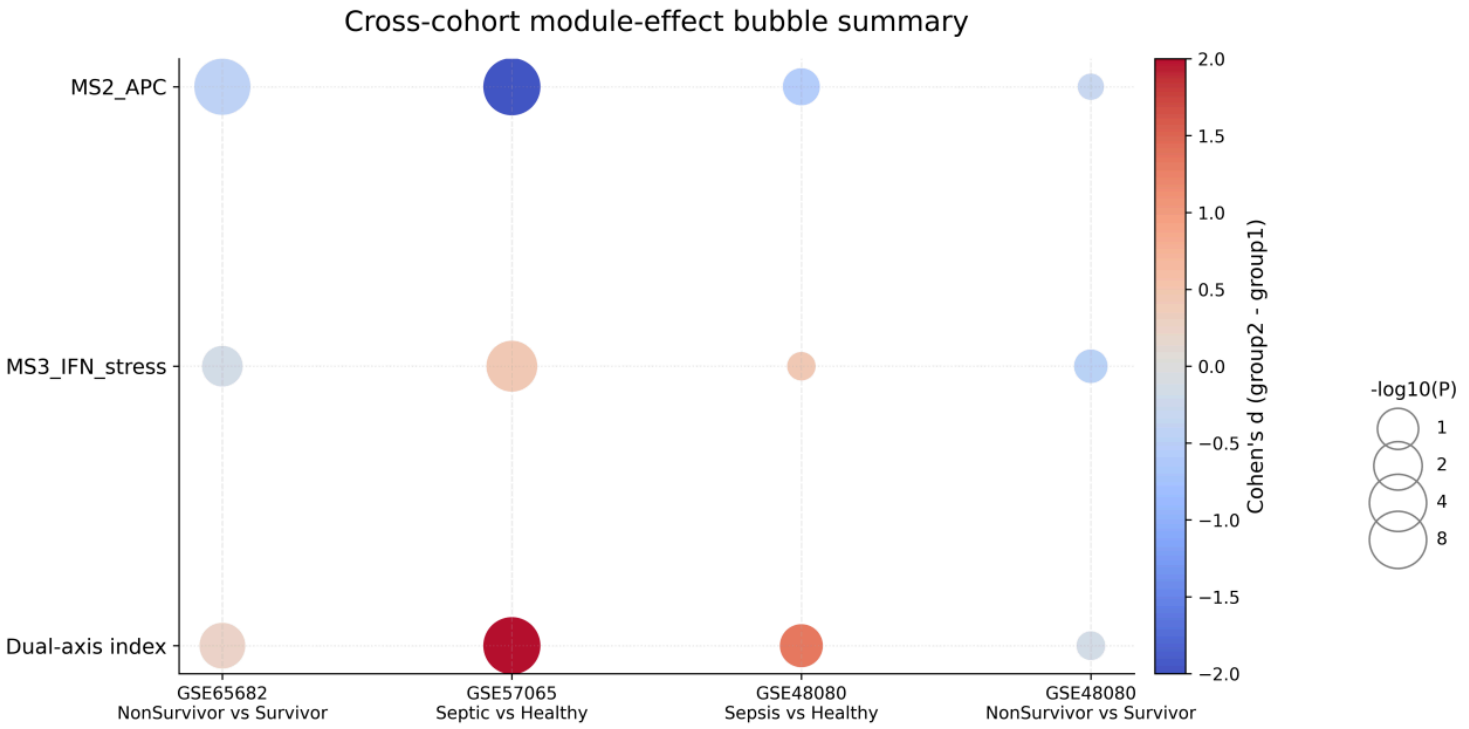


Figure 9

Cross-cohort bubble summary of effect sizes for dual-axis-related modules across different cohorts and comparisons

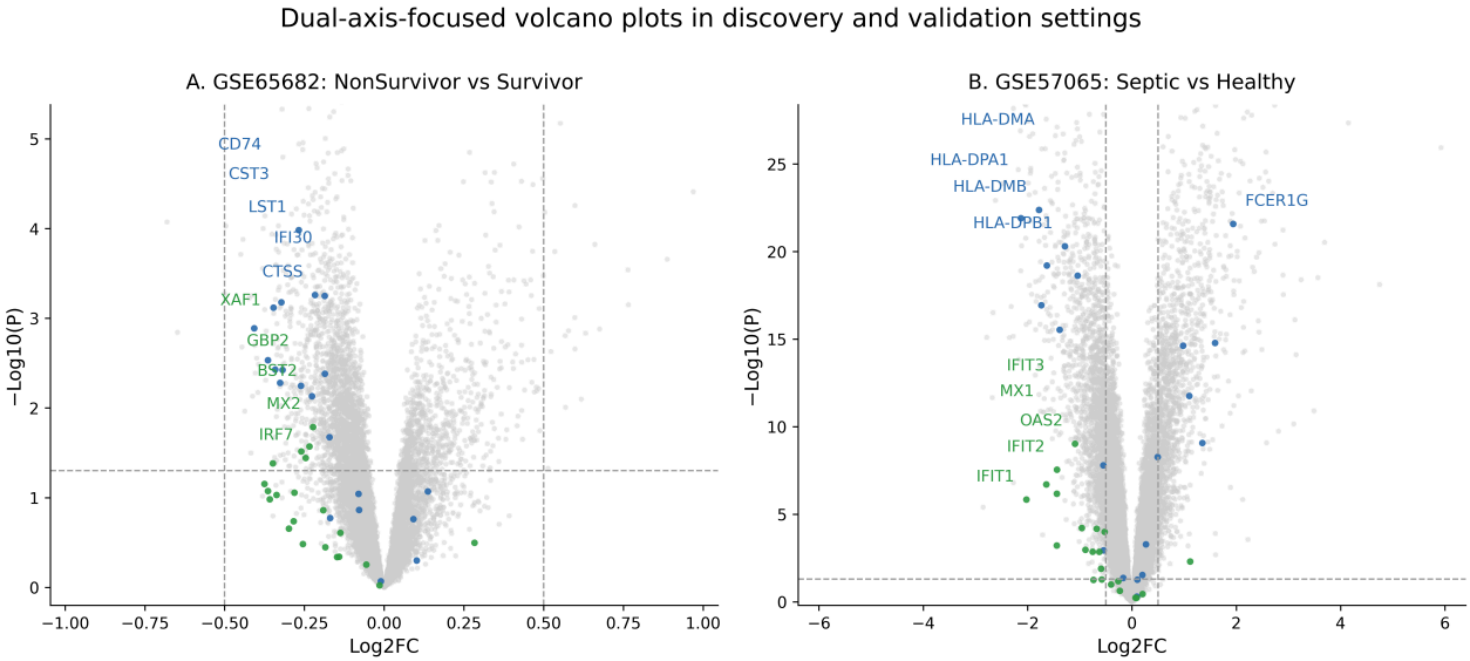


Figure 10

Dual-axis-focused volcano plots in the discovery and validation cohorts

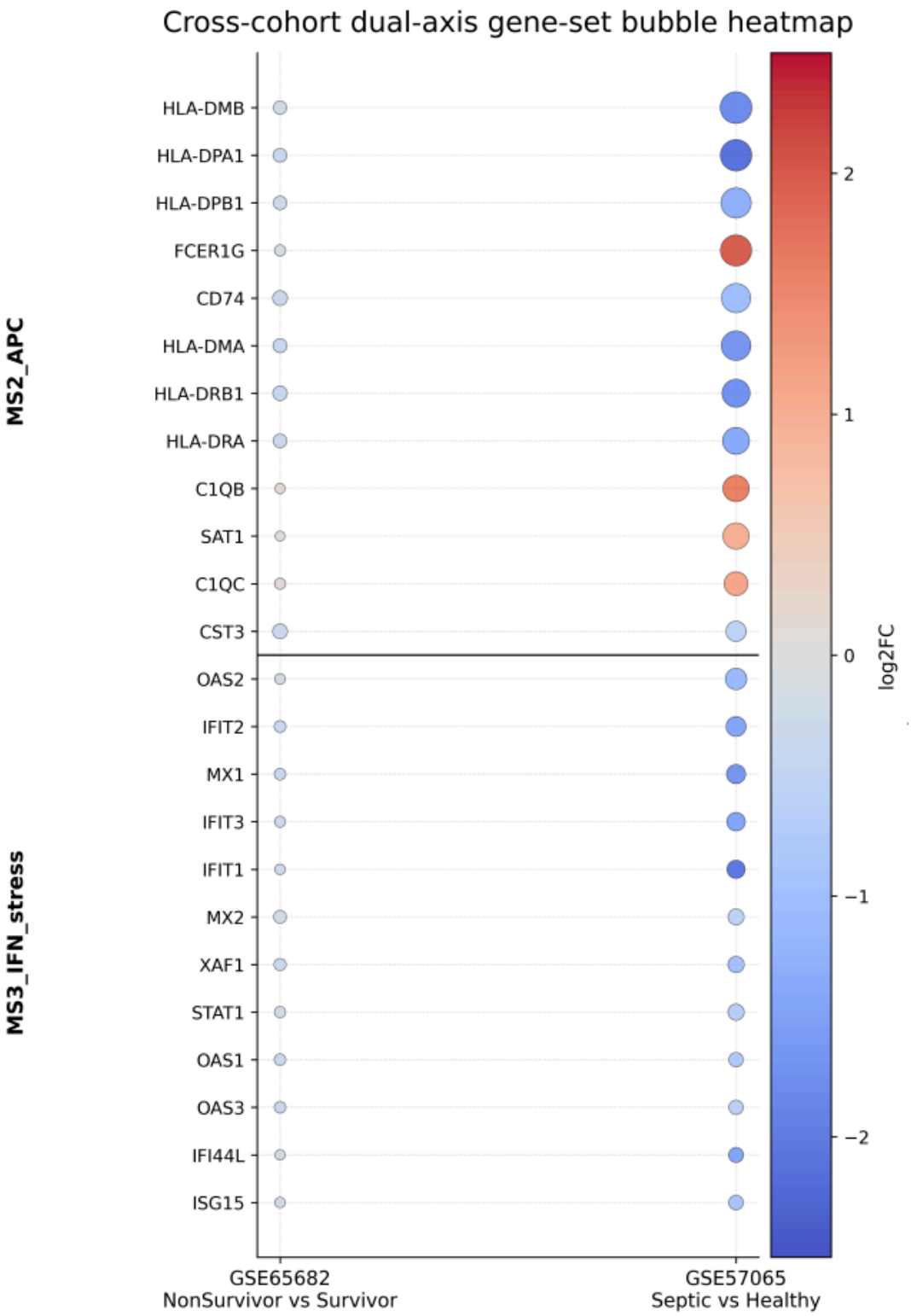


Figure 11

Cross-cohort bubble heatmap of dual-axis gene sets in the discovery and validation cohorts

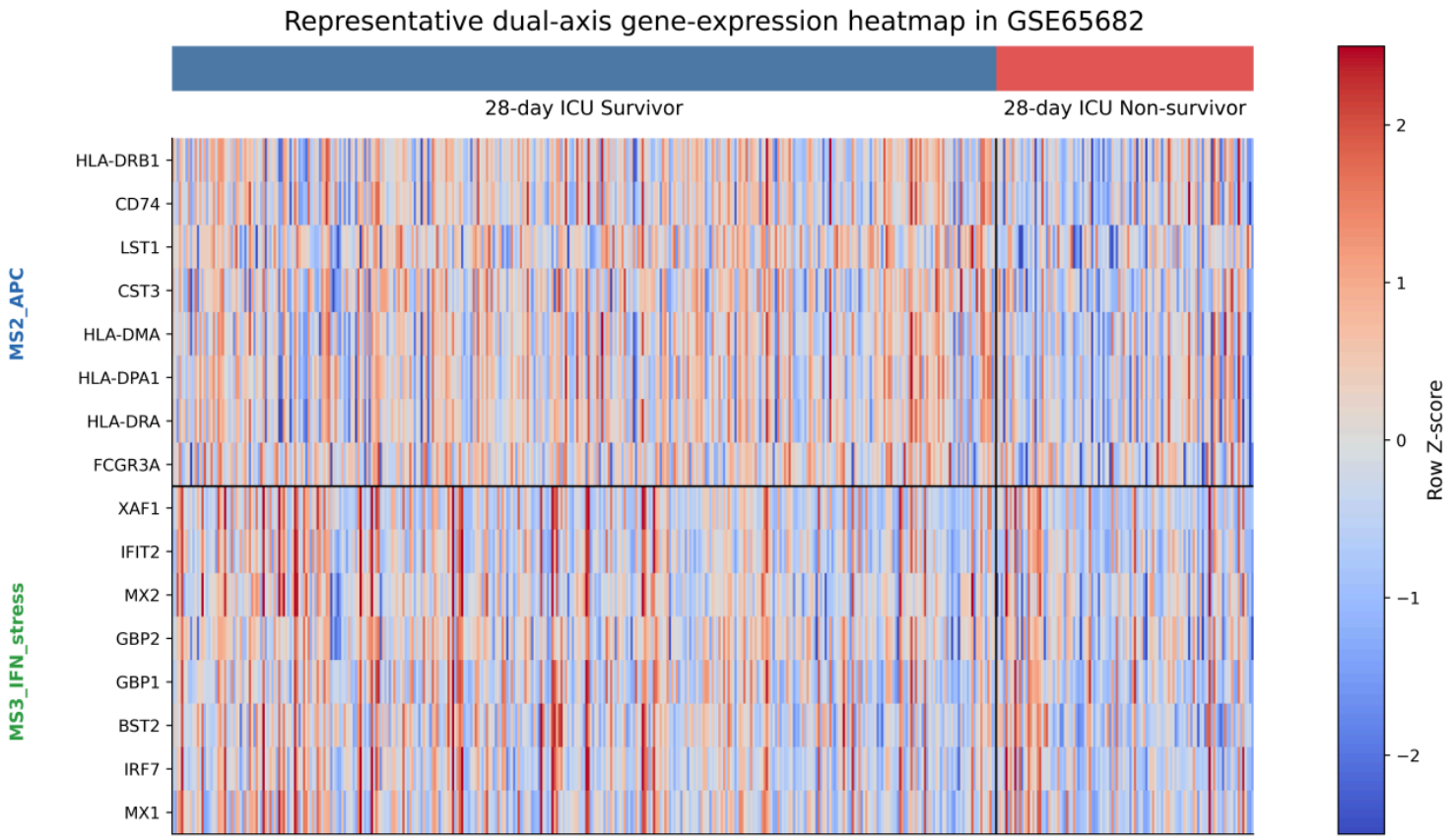


Figure 12

Heatmap of representative dual-axis genes in the GSE65682 discovery cohort

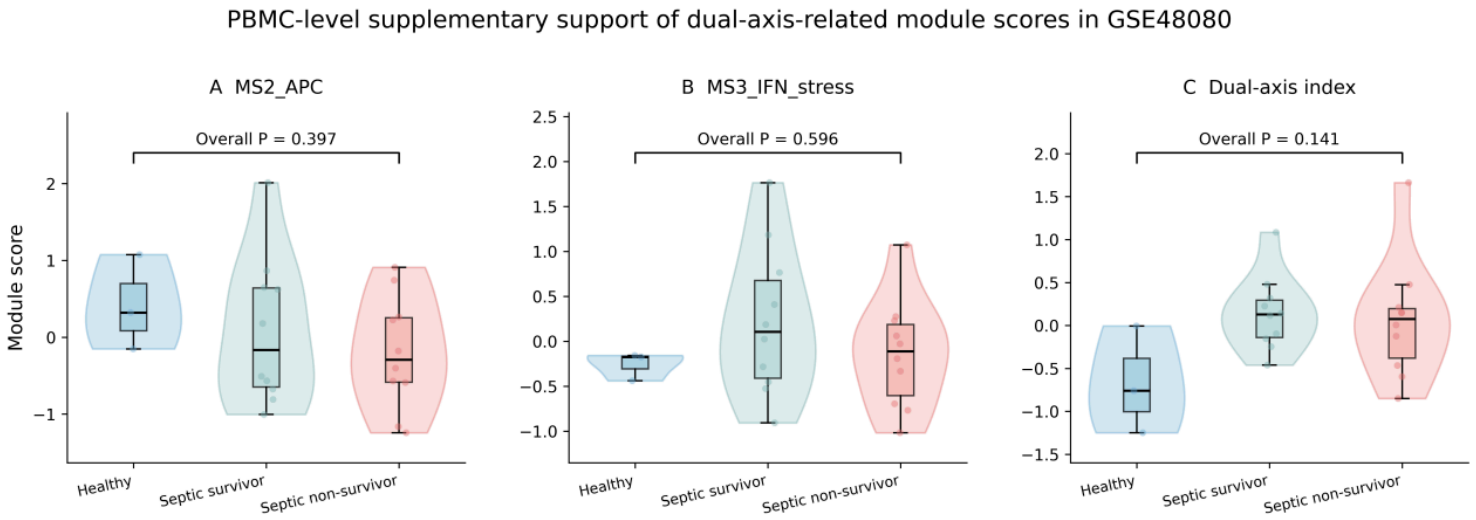


Figure 13

PBMC-based support for dual-axis-related module scores in the GSE48080 cohort

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Table1cohortcharacteristics.xlsx