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The Critical Role of CD38 in Regulating Natural Killer Cell Function in Pediatric Sepsis

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Abstract

Objective

To investigate the potential role of CD38 in regulating natural killer (NK) cell immune function in pediatric sepsis and to explore the mechanisms by which CD38 contributes to NK-cell dysfunction under septic conditions.

Methods

Publicly available single-cell RNA sequencing (scRNA-seq) datasets from pediatric sepsis patients and pediatric healthy controls were analyzed to characterize NK-cell heterogeneity and CD38-associated transcriptional features. After quality control, 23,774 cells and 32,738 genes were retained for downstream analysis. NK-cell subsets were identified based on canonical marker genes. Differential expression analysis, Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analyses, Monocle2 pseudotime trajectory analysis, and UCell pathway activity scoring were performed to compare CD38^high and CD38^low NK-cell subsets. In vitro, CD38 was silenced in NK-92 cells using siRNA. Quantitative real-time PCR (qRT-PCR) was used to assess the expression of CD38 and NK effector-related genes, including FGFBP2 and GZMB. Confocal microscopy was used to evaluate cell morphology. Flow cytometry was performed to detect intracellular PRF1 expression and CD107a surface exposure, and Western blotting was used to measure FGFBP2 protein expression.

Results

The NK-cell landscape in pediatric sepsis was less diverse than that in healthy controls, and the number of NK cells was reduced in sepsis samples. CD38 expression in NK cells was significantly lower in pediatric sepsis patients than in healthy controls, although CD38^high NK cells represented the predominant subset overall. Transcriptomic analysis revealed marked differences between CD38^high and CD38^low NK cells. The CD38^high subset exhibited enrichment in ribonucleoprotein complex biogenesis, cytoplasmic translation, non-coding RNA processing, ribosome-related pathways, and viral infection-associated pathways. Compared with NK cells from sepsis patients, NK cells from healthy controls expressed higher levels of cytokine/chemokine genes, including IFNG, TNF, CSF2, CCL3, CCL5, and XCL1. In addition, CD38^high NK cells showed significantly increased expression of activating receptor genes (NCR1, NCR2, KLRD1, FCGR3A) and cytotoxic effector genes (GZMA, PRF1, GZMH, GZMK, GNLY). Pseudotime analysis indicated that CD38^high and CD38^low NK cells did not occupy distinct differentiation states, suggesting that CD38-related differences primarily reflected functional status rather than developmental trajectory. UCell scoring further demonstrated that CD38^high NK cells exhibited significantly higher activities in cytotoxicity, interferon response, inflammatory response, metabolism, interferon-γ response, and glycolysis pathways. In vitro, siRNA-mediated CD38 knockdown in NK-92 cells significantly reduced CD38 mRNA expression, downregulated FGFBP2 and GZMB

transcripts, decreased intracellular PRF1 levels, impaired CD107a degranulation, and reduced FGFBP2 protein expression, while causing no obvious morphological abnormalities.

Conclusion

CD38 is closely associated with the activated and cytotoxic phenotype of pediatric NK cells. Downregulation of CD38 in pediatric sepsis is linked to impaired NK-cell effector function and may contribute to sepsis-associated immunosuppression. These findings suggest that CD38 may serve as a potential molecular target for understanding and modulating immune dysregulation in pediatric sepsis.

1 Introduction

Sepsis is a common clinical syndrome in pediatric critical care medicine and is associated with substantial mortality. Its defining feature is a dysregulated host response to infection that ultimately results in life-threatening organ dysfunction [1]. Because the pediatric immune system is still undergoing maturation, both innate and adaptive immune responses show pronounced age dependence. Accordingly, the immunopathogenesis of pediatric sepsis differs from that in adults and often manifests as a complex disequilibrium in which hyperinflammation and immunosuppression coexist [2,3].Natural killer (NK) cells are key effector cells of the innate immune system. Early in infection, NK cells contribute to pathogen clearance and inflammatory regulation via cytotoxic activity and cytokine secretion [4,5]. During the progression of sepsis, however, NK cells may exhibit reduced abundance, impaired effector function, and increased expression of exhaustion-associated phenotypes, suggesting an important role in sepsis-associated immunoparalysis [6–9].

CD38 is a multifunctional transmembrane molecule broadly expressed on the surface of diverse immune cell types and possesses receptor, adhesion, and enzymatic activities [10,11]. In NK cells, CD38 is constitutively expressed and can be functionally coupled to activating receptors such as CD16, thereby influencing signal transduction, cytotoxicity, and cytokine production [12]. Nevertheless, during the unique developmental stage of pediatric immunity, the expression landscape of CD38 on NK cells and its regulatory effects on immune function in sepsis state remain insufficiently defined.

Therefore, using single-cell transcriptomic datasets from pediatric sepsis patients and healthy controls, together with in vitro functional assays in NK-92 cells, we sought to investigate potential mechanisms by which CD38 regulates NK-cell immune function in children. Our findings are intended to provide a theoretical basis for elucidating immune dysregulation in pediatric sepsis and for identifying potential therapeutic targets.

2 Materials and Methods

2.1 Curation and annotation of single-cell sequencing datasets

(1) Data sources
Publicly available single-cell RNA-sequencing (scRNA-seq) datasets from pediatric sepsis patients and pediatric healthy controls were used:

GEO: https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE151263

Zenodo: https://zenodo.org/records/5524378#.YUzcFy1h3GJ

(2) Data preprocessing and quality control
Quality control criteria were as follows: 200–5000 detected genes per cell (nFeature_RNA) and mitochondrial gene proportion (percent.mt) 10%.

Standard analyses were performed using Seurat (v3.1.2): NormalizeData for normalization; FindVariableFeatures for selection of the top 2000 highly variable genes; ScaleData for scaling; and RunPCA for dimensionality reduction. Harmony was applied for batch effect correction, followed by FindNeighbors and FindClusters for clustering (resolution = 2.0). Two-dimensional visualization was performed using UMAP (RunUMAP).

(3) Cell subcluster annotation
Differentially expressed genes (DEGs) were identified using FindAllMarkers (Wilcoxon rank-sum test) and cell types were annotated based on established marker genes. NK cells were defined by high expression of canonical cytotoxicity-related genes, including NCAM1, FCGR3A, KLRF1, KLRD1, KLRC1, KLRK1, NKG7, and GNLY.

(4) Pseudotime analysis
Pseudotime trajectories of NK cells were inferred using Monocle2. A CellDataSet object was constructed using highly variable genes, followed by dimensionality reduction with ICA. Trajectories were then learned with the DDRTree algorithm, and pseudotime was calculated to compare the distribution of CD38^high and CD38^low subpopulations. Differentially expressed genes (DEGs) along the pseudotime trajectory were identified at an FDR < 0.05.

(5) Pathway activity scoring (UCell)
UCell was applied to compute single-cell gene-set activity scores for predefined signatures (cytotoxicity, interferon response, inflammatory response, metabolism, interferon-γ response, glycolysis, etc.), enabling comparisons of pathway activities between CD38^high and CD38^low NK cells.

2.2 In vitro experimental validation

(1) qRT-PCR to assess the effect of siRNA-mediated CD38 knockdown on gene expression
Total RNA was extracted from control and CD38-knockdown NK-92 cells using TRIzol, and RNA purity was assessed (A260/A280 ≈ 2.0). cDNA was synthesized using HiScript III and subjected to qPCR using the following reaction system: 2× ChamQ Universal SYBR qPCR Master Mix (10 μL), forward and reverse primers (0.4 μL each), cDNA (2μL), and nuclease-free water to 20 μL. Each sample was analyzed in three

technical replicates and melting curves were generated. GAPDH served as the internal reference, and relative expression was calculated using the 2^−ΔΔCt method. Data are presented as mean ± SD (≥ 3 independent experiments).

(2) Confocal microscopy for cell morphology

NK-92 cells were collected 48 h after treatment and washed with PBS. Cells were then fixed with 4% paraformaldehyde for 15 min at room temperature. After washing, the cells were settled onto poly-L-lysine-coated glass slides or cytospun onto slides. The slides were washed with PBS, and nuclei were stained with DAPI for 15 min in the dark. Coverslips were mounted using an anti-fade mounting medium. Images were acquired using a laser scanning confocal microscope.

(3) Flow cytometry
Cells were stained for surface and intracellular markers using anti-CD56/APC, anti-LAMP-1 (CD107a)/PE, and anti-PRF1/FITC. Data were acquired on a FACSCanto II flow cytometer and analyzed using FlowJo.

(4) Western blotting
Total protein was extracted and quantified by BCA assay. Equal amounts of protein (30μg) were separated by SDS-PAGE and transferred to PVDF membranes. After blocking with 5% BSA, membranes were incubated with primary antibodies at 4°C overnight and with secondary antibodies at room temperature for 1 h. Signals were detected using an Odyssey imaging system and quantified by densitometry.

2.3 Statistical analysis

Statistical analyses were performed using GraphPad Prism 8.0. Quantitative data are expressed as mean ± SD. Two-group comparisons were conducted using a two-tailed unpaired t-test. Multi-group comparisons were performed using one-way ANOVA with Dunnett's or Tukey's multiple-comparison tests. $P < 0.05$ was considered statistically significant.

3 Results

3.1 Construction of a single-cell atlas and identification of NK subpopulations in pediatric sepsis and healthy controls

We analyzed scRNA-seq data from pediatric sepsis patients (n = 5) and pediatric healthy controls (pHCs; n = 76). After quality control, 23,774 cells and 32,738 genes were retained for downstream analyses. NK-cell subpopulations were identified and annotated based on marker genes (Figure 1a), and differences in NK-cell composition across disease states were systematically evaluated.

The number of NK cells in children with sepsis was significantly lower than that in the healthy control group. (Figure 1b). Across all samples, the proportion of CD38^high NK cells was higher than that of the

CD38^low subset (Figure 1d). Notably, CD38 expression in NK cells was significantly higher in healthy controls than in sepsis patients (Figure 1e). A DEG heatmap demonstrated marked transcriptional differences between CD38^high and CD38^low NK cells (Figure 1c). GO enrichment analysis indicated that the CD38^high subset was significantly enriched for ribonucleoprotein complex biogenesis, cytoplasmic translation, and non-coding RNA processing; KEGG analysis suggested enrichment in ribosome-related and viral infection-associated pathways (Figure 1f1–f2).

3.2 NK-cell subset imbalance in sepsis and CD38-specific low-expression features

To systematically evaluate the relationship between CD38 and NK-cell functional states, we analyzed gene sets spanning cytokines, chemokines, surface receptors, and cytotoxic effector molecules. Compared with the sepsis group, NK cells from healthy controls exhibited significantly higher expression of cytokine/chemokine genes including IFNG, TNF, CSF2, CCL3, CCL5, and XCL1 (Figure 2a).Further analyses showed that activating receptor genes such as NCR1, NCR2, KLRD1, and FCGR3A were markedly upregulated in the CD38^high NK subset (Figure 2b). Consistently, cytotoxic effector genes including GZMA, PRF1, GZMH, GZMK, and GNLY were coordinately elevated in CD38^high NK cells (Figure 2c), suggesting that CD38^high NK cells possess stronger activation and cytotoxic potential.

Pseudotime analysis indicated that CD38^high and CD38^low NK cells did not occupy distinct differentiation stages (Figure 2d), implying that CD38-associated differences more likely reflect functional states rather than differentiation trajectories. Concordantly, UCell scoring revealed significantly higher activities of cytotoxicity, interferon response, metabolism, inflammatory response, interferon-γ response, and glycolysis pathways in CD38^high NK cells (Figure 2e1–e6).

3.3 qRT-PCR analysis of the impact of CD38 levels on FGFBP2 and GZMB expression in NK-92 cells

CD38 was knocked down in NK-92 cells using siRNA, and qRT-PCR confirmed a significant reduction in CD38 mRNA (Figure 3a). Immunofluorescence imaging indicated no overt abnormalities in cell morphology or proliferation (Figure 3d1–d3). In contrast, FGFBP2 expression decreased by approximately 40-fold and GZMB expression decreased by approximately 2-fold following CD38 knockdown (Figure 3b–c).

3.5 CD38 knockdown impairs degranulation and cytotoxic mediator expression in NK-92 cells

Flow cytometry showed that intracellular PRF1 levels were significantly reduced in the CD38-knockdown group (Figure 4a-c). In parallel, surface exposure of the degranulation marker CD107a was decreased (Figure 4d-f), indicating that reduced CD38 expression suppresses NK-92 effector function.

3.6 Western blot validation of CD38-dependent regulation of FGFBP2 protein expression

At the protein level, we observed a clear and consistent trend: FGFBP2 protein expression was markedly reduced in CD38-knockdown NK-92 cells compared with controls (Figure 5). This finding aligns with our

qRT-PCR results and suggests that CD38 deficiency may influence the translational efficiency and/or stability of FGFBP2 via post-transcriptional regulatory mechanisms.

4 Discussion

The central pathophysiological hallmark of sepsis is an infection-triggered dysregulated host response leading to organ dysfunction [1]. Given that pediatric immunity is dynamically developing and exhibits age-dependent features in both innate and adaptive responses, pediatric sepsis often presents as a complex immune imbalance characterized by the coexistence of hyperinflammation and immunosuppression [2,3]. NK cells participate in early pathogen clearance and inflammatory regulation through perforin/granzyme release and secretion of cytokines such as IFN-γ and TNF [4,5]. During sepsis progression, NK cells may display reduced numbers, insufficient activation, diminished cytotoxicity, and increased exhaustion phenotypes, thereby contributing to sepsis-induced immunoparalysis [6–9]. Thus, identifying key molecular determinants of NK-cell dysfunction in pediatric sepsis is essential for understanding immune dysregulation and developing intervention strategies.

By integrating pediatric sepsis scRNA-seq data with NK-92 in vitro functional assays, our study systematically evaluated the relationship between CD38 and NK-cell functional states. We found that NK-cell subset composition was simplified in sepsis compared with healthy controls, and CD38 expression in NK cells was significantly higher in healthy controls than in sepsis patients, suggesting that pediatric sepsis may involve an NK-cell hypofunctional phenotype characterized by CD38 downregulation. Consistently, NK cells from healthy controls expressed higher levels of IFNG, TNF, CSF2, and chemokines such as CCL3, CCL5, and XCL1, suggesting reduced responsiveness of septic NK cells in inflammatory mediator production and immune recruitment—features consistent with sepsis-associated immunoparalysis [6–9].

CD38 is a multifunctional transmembrane molecule with both receptor and enzymatic activities and has been implicated in immune activation signaling and metabolic homeostasis [10,11]. In NK cells, CD38 is functionally coupled to CD16, implying a role in regulating NK activation and effector output [12]. In our study, CD38^high NK cells exhibited increased expression of activating receptors (NCR1, NCR2, KLRD1, FCGR3A) accompanied by coordinated upregulation of cytotoxic effectors (GZMA, PRF1, GNLY), indicating that high CD38 expression is closely associated with enhanced NK effector function. Pathway scoring further showed that CD38^high NK cells had higher activity in cytotoxicity, interferon/inflammatory responses, and metabolism/glycolysis. As effector activation is commonly coupled to metabolic reprogramming, enhanced glycolysis may provide energy to support effector molecule synthesis, vesicle trafficking, and degranulation, rendering these findings biologically plausible [15].

Pseudotime analyses showed no clear separation of CD38^high and CD38^low NK cells along differentiation stages, supporting the interpretation that CD38-associated differences predominantly reflect functional states rather than differentiation trajectories. In vitro experiments further supported a

positive regulatory role for CD38 in NK effector function: CD38 knockdown in NK-92 cells reduced FGFBP2 and GZMB transcription, decreased PRF1 protein abundance, and impaired CD107a degranulation, indicating that CD38 deficiency directly compromises the molecular reserves and functional output required for NK cytotoxicity. Taken together, our single-cell and in vitro results suggest that CD38 downregulation in pediatric sepsis may impair NK effector function by weakening activation signal integration and metabolic support, thereby contributing to immunosuppression.

Several limitations of this study should be noted. First, the scRNA-seq data were derived from public cohorts with a limited number of sepsis cases, and the imbalance between case and control sample sizes may affect robustness. Second, the in vitro validation was performed in the NK-92 cell line, necessitating further confirmation in primary pediatric NK cells and in vivo models. Third, the precise downstream pathways through which CD38 modulates NK-cell function (e.g., Ca^2+ signaling, NAD^+ metabolism, and broader immunometabolic networks) require further mechanistic investigation. Despite these limitations, our findings reveal a strong association between CD38 and NK-cell hyporesponsiveness in pediatric sepsis, supported by in vitro evidence, and indicate that CD38 may represent an important molecular node for understanding immune imbalance and for potential therapeutic intervention in pediatric sepsis.

Declarations

Funding

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Ethics approval and consent to participate

Not applicable.

Author Contribution

Ting Chen and Zhengjun Wang wrote the main manuscript text and prepared Figures 4–5. Cheng Huang prepared Figures 1–3. All authors reviewed the manuscript.

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Disclosure statement

No potential conflict of interest was reported by the author(s).

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Figures

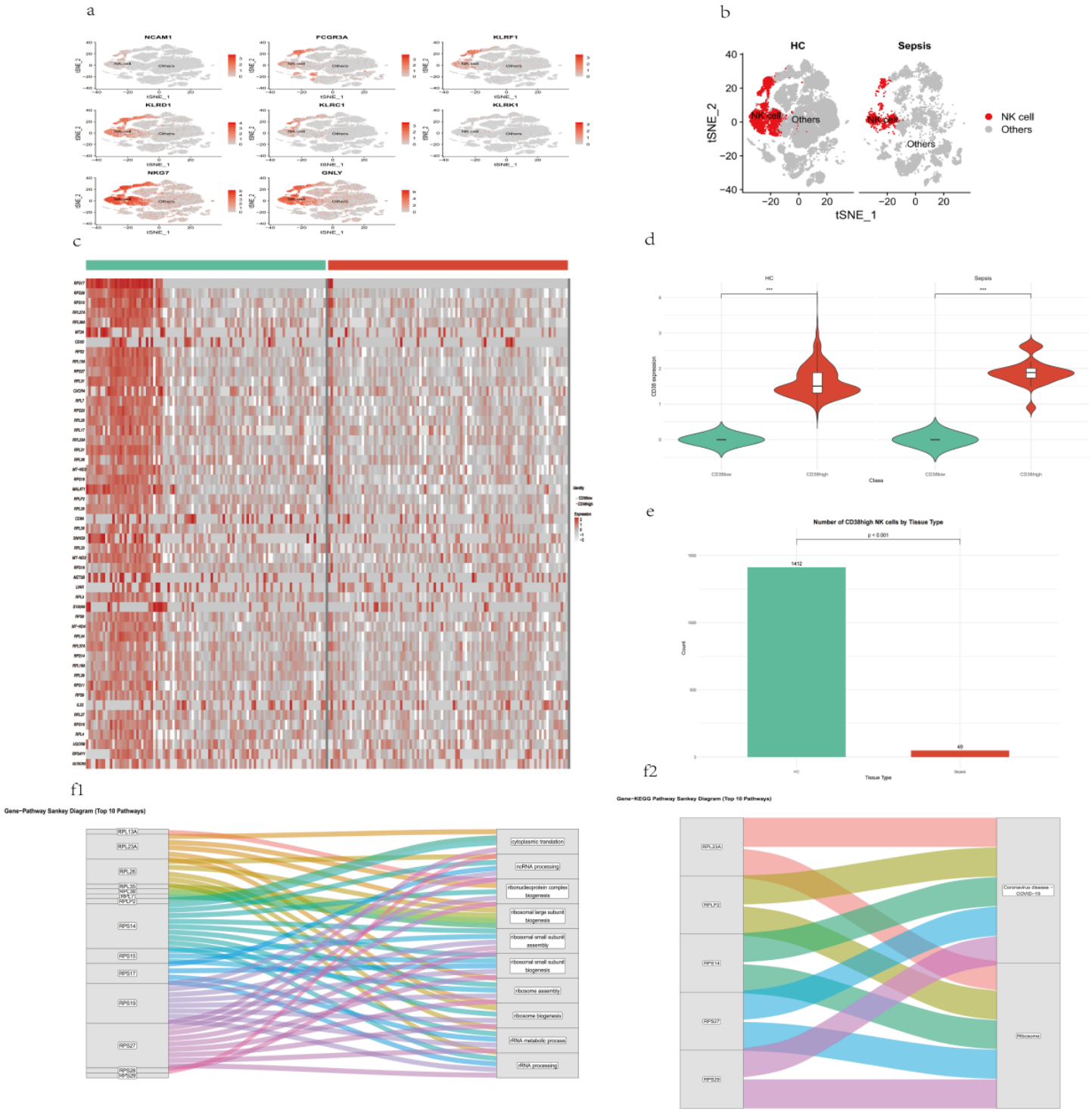


Figure 1

Single-cell transcriptomic atlas and functional characteristics of CD38-defined NK-cell subsets in peripheral blood from pediatric sepsis patients and healthy controls.(a) Unsupervised clustering and annotation of NK cells based on characteristic markers; a t-SNE plot shows canonical NK-cell markers. (b) Comparison of NK-cell subset composition between sepsis patients (n = 5) and pediatric healthy controls (n = 76), showing reduced subset diversity in the sepsis group.(c) Heatmap of DEGs distinguishing CD38^high and CD38^low NK-cell subsets.(d) Proportions of CD38^high and CD38^low NK

subsets across all samples, showing a higher fraction of CD38^high cells.(e) Violin plot comparing CD38 expression in NK cells between healthy controls and sepsis patients, indicating significantly higher CD38 expression in controls.(f1) GO (BP) enrichment of DEGs in CD38^high NK cells, highlighting ribonucleoprotein complex biogenesis, cytoplasmic translation, and non-coding RNA processing. (f2) KEGG enrichment of DEGs in CD38^high NK cells, highlighting COVID-19-related and ribosome pathways.

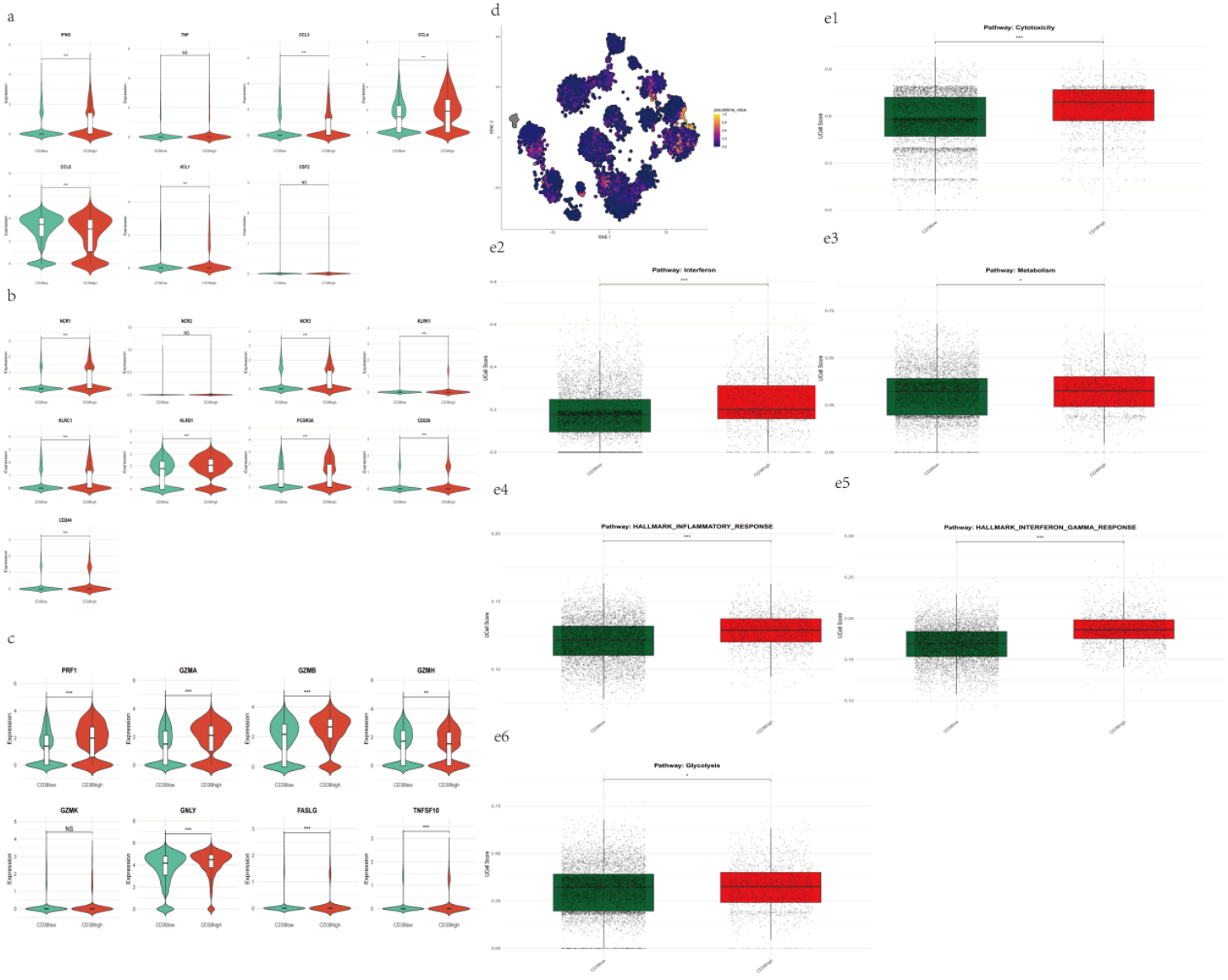


Figure 2

Association between CD38 expression and NK-cell functional states.(a) Heatmap of functional gene expression in NK cells from healthy controls versus sepsis patients. Cytokine/chemokine genes (including IFNG, TNF, CSF2, CCL3, CCL5, and XCL1) were significantly higher in healthy controls (DESeq2; adjusted $P < 0.05$).(b) Differential expression of surface receptor genes between CD38^high and CD38^low NK subsets. Activating receptors NCR1, NCR2, KLRD1, and FCGR3A were upregulated in CD38^high NK cells (two-tailed unpaired t-test; $*P < 0.05$, $**P < 0.01$, $***P < 0.001$; mean ± SD; ≥3 biological replicates).(c)Differential expression of cytotoxic effector genes between CD38^high and

CD38^low NK subsets. Key cytotoxic molecules GZMA, PRF1, GZMH, GZMK, and GNLY showed coordinated high expression in CD38^high NK cells (statistics as in b).(d) Pseudotime analysis showing distributions of CD38^high and CD38^low NK cells along the trajectory; no clear separation in differentiation stage was observed (Monocle2; no significant branching).(e1–e6) UCell pathway activity scores comparing CD38^high versus CD38^low NK cells: (e1) cytotoxicity, (e2) interferon response, (e3) metabolism, (e4) inflammatory response, (e5) interferon-γ response, and (e6) glycolysis; all were significantly elevated in CD38^high NK cells (Mann–Whitney U test; ***P < 0.01, ***P < 0.001*).

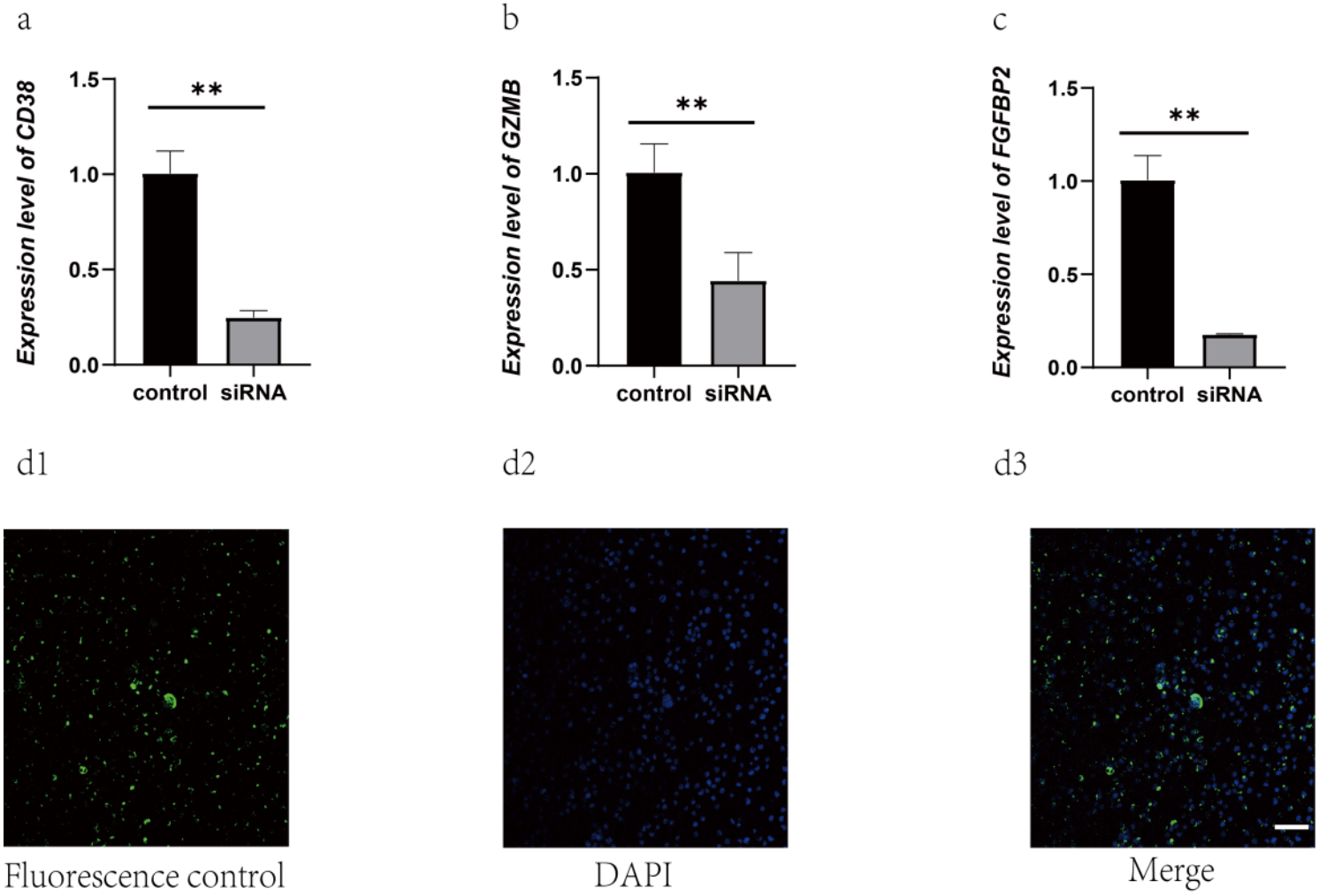


Figure 3

Regulation of effector-related gene expression by CD38 knockdown in NK-92 cells.(a) Validation of CD38 knockdown efficiency by qRT-PCR. CD38 mRNA levels were significantly reduced in the CD38-knockdown group compared with controls (***P < 0.01*; two-tailed unpaired t-test), confirming successful establishment of a CD38-knockdown NK-92 cell model.(b-c) qRT-PCR analysis of FGFBP2 and GZMB mRNA levels in control versus CD38-knockdown cells. FGFBP2 decreased ~40-fold and GZMB decreased ~2-fold after CD38 knockdown (***P < 0.01*), indicating a positive regulatory role of CD38 on these effector genes.(d1-d3) Confocal microscopy assessment suggests no significant impact of CD38 knockdown on NK-92 cell proliferation.

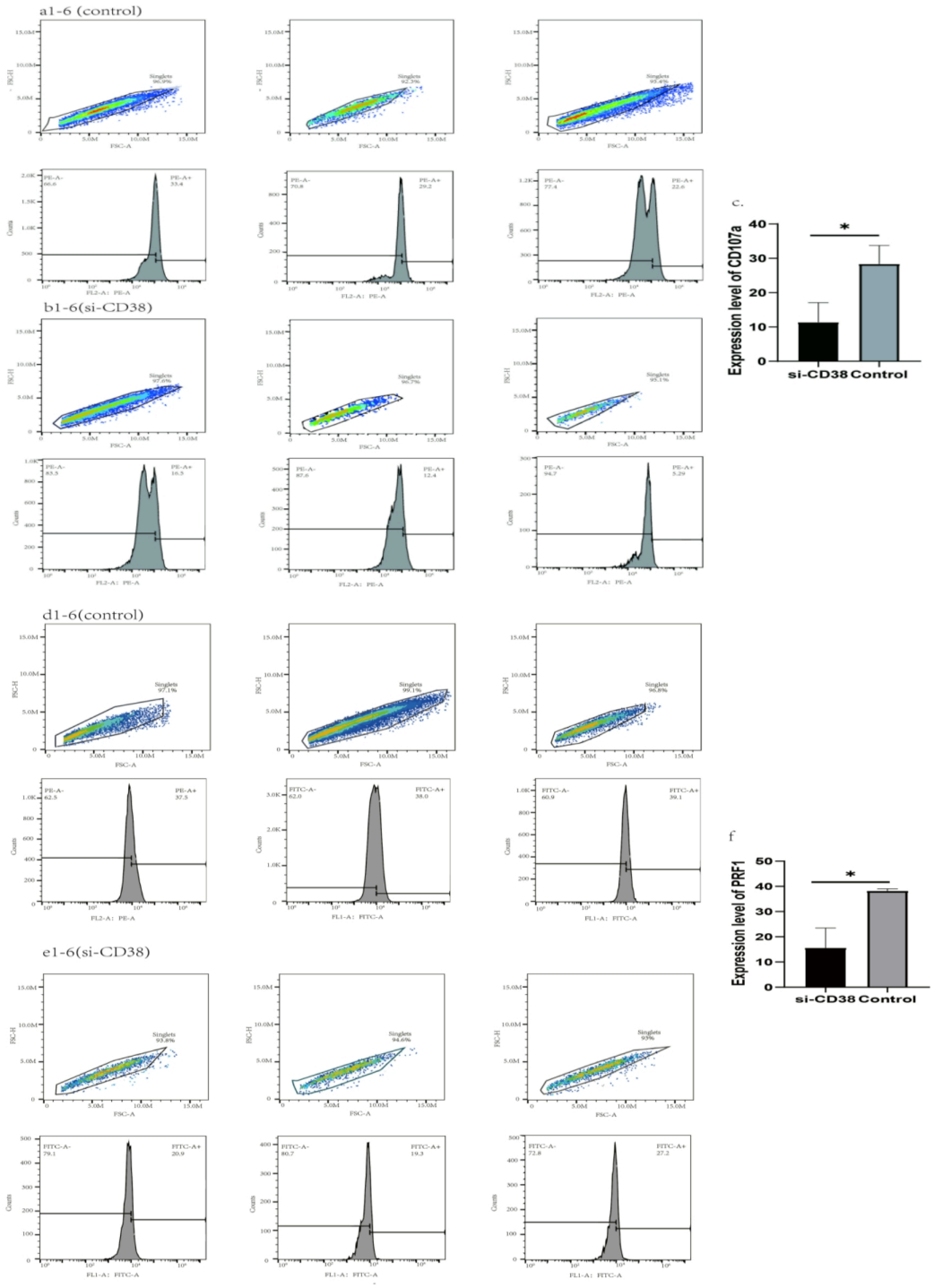


Figure 4

Flow cytometry confirms that CD38 knockdown inhibits degranulation and cytotoxic function in NK-92 cells.(a-c) Representative flow plots and quantitative comparison of PRF1 expression. (a1–a3) Representative dot plots/histograms of PRF1 staining in CD38-knockdown NK-92 cells; (b1–b3) corresponding plots for controls. (c)Quantification of PRF1 positivity and/or mean fluorescence intensity (MFI), showing significant downregulation of PRF1 protein after CD38 knockdown.(d–f) Representative

flow plots and quantitative comparison of CD107a expression. (d1–d3) Representative dot plots/histograms of CD107a staining in CD38-knockdown NK-92 cells; (e1–e3) corresponding plots for controls. (f) Quantification of CD107a positivity and/or MFI, indicating significantly impaired degranulation after CD38 knockdown.
All flow cytometry experiments included ≥ 3 biological replicates; data are presented as mean ± SD. Two-group comparisons were performed using a two-tailed unpaired t-test; significance threshold: **P < 0.01*. For immunofluorescence quantification, ≥ 8000 cells were counted per group and compared using the same t-test.

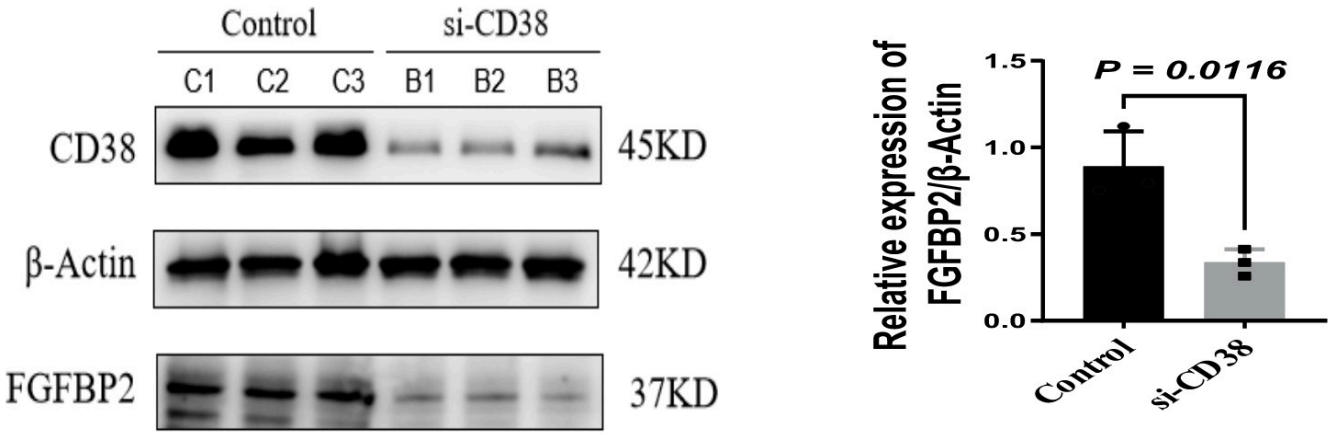


Figure 5

CD38 knockdown reduces FGFBP2 protein expression in NK-92 cells.(a1) Representative western blot of FGFBP2 in NK-92 cells transfected with CD38-targeting siRNA versus control siRNA; GAPDH served as the loading control.(a2) Densitometric quantification of FGFBP2 bands from at least three independent experiments (mean ± SD).