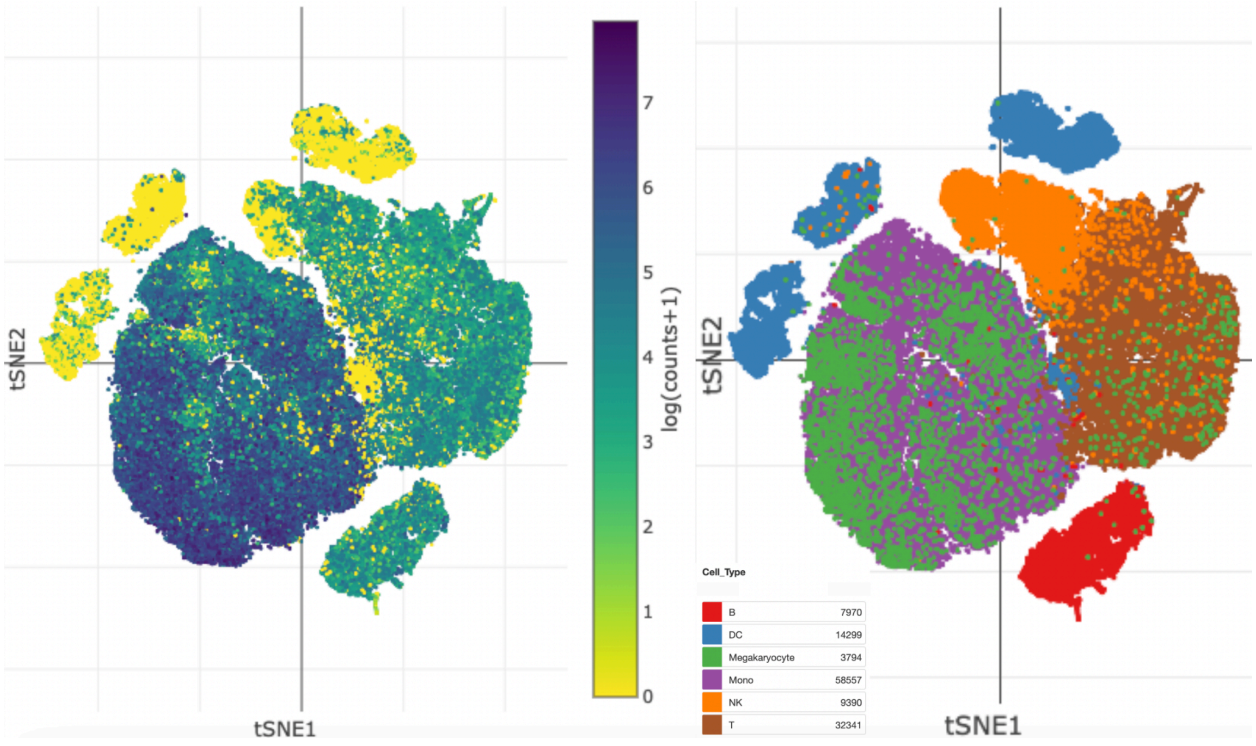
To further validate the biological specificity of the calgranulin gene cluster (Cluster~3: S100A8, S100A9, and S100A12), we investigated their expression patterns at single-cell resolution using publicly available transcriptomic data from the Single Cell Portal study SCP548 singlecellportal. This dataset comprises 126,351 peripheral blood mononuclear cells (PBMCs), profiled via 10x Genomics 3' v2 technology, and includes samples from 29 patients with sepsis and 36 healthy controls. Cells were classified into canonical immune subsets, including B cells, T cells, natural killer (NK) cells, dendritic cells (DCs), monocytes, and megakaryocytes.

While neutrophils—the principal source of calgranulins—are absent in PBMC preparations due to their fragility and density, monocytes represent an alternative and biologically relevant compartment for examining inflammatory gene signatures. Monocytes have been widely implicated in the dysregulated immune response characteristic of early sepsis, and their ability to express S100-family genes has been previously demonstrated.

Spatial distribution of calgranulin expression

To provide an overview of the transcriptional landscape, we visualized calgranulin expression across the entire dataset using t-distributed stochastic neighbor embedding (t-SNE). In the figure, the left panel displays log-normalized, median expression of the three calgranulin genes across all cells, while the right panel shows the corresponding cell type annotations.



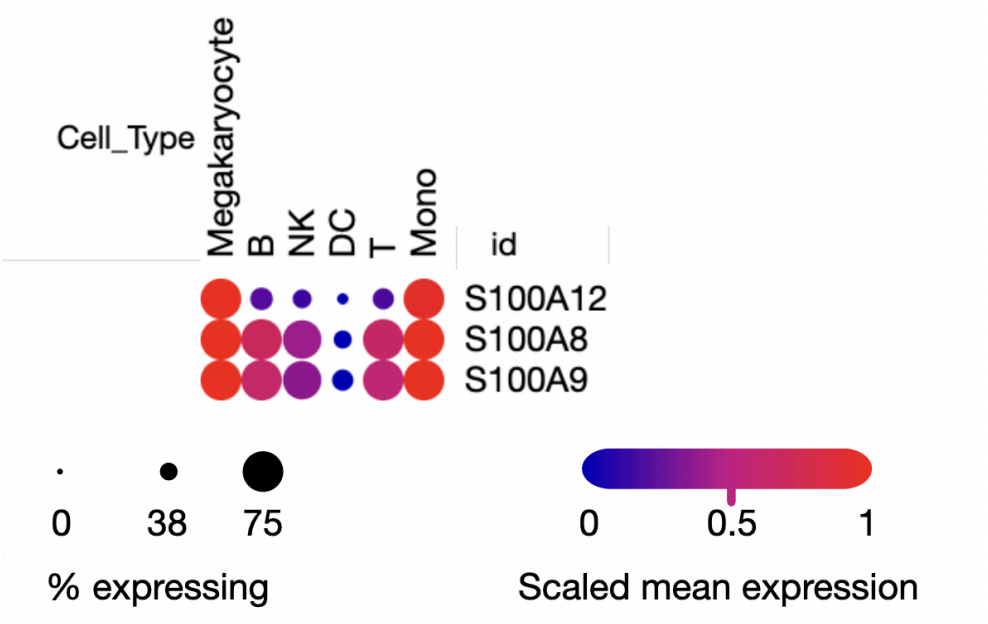
Regions of high expression (purple) aligned predominantly with monocytes and megakaryocytes, confirming these as the main contributors to calgranulin signal in the absence of neutrophils. This spatial view highlights that inflammatory gene expression is not randomly distributed but localized to biologically relevant myeloid compartments, reinforcing their importance in blood-based sepsis diagnostics.

Expression Across All PBMC Cell Types

While the t-SNE map provides a spatial view of overall calgranulin signal (based on aggregate median expression), the dot plot breaks down the contribution of each gene S100A8, S100A9, S100A12 across individual immune subsets, quantifying both expression intensity and prevalence.

As expected, the strongest and most widespread expression was observed in monocytes, where all three genes showed both high prevalence and high expression intensity. Notably, megakaryocytes also exhibited substantial expression across all calgranulin genes, with dot sizes and intensities comparable to monocytes. This highlights the emerging immunological role of megakaryocytes in inflammatory responses.

In contrast, other immune subsets—such as T cells, B cells, NK cells, and dendritic cells—showed minimal or inconsistent expression. Specifically, DCs displayed negligible expression of all three genes, while B cells and NK cells exhibited moderate prevalence with low to intermediate expression levels, depending on the specific gene. These findings confirm the myeloid-skewed expression profile of the calgranulin family.



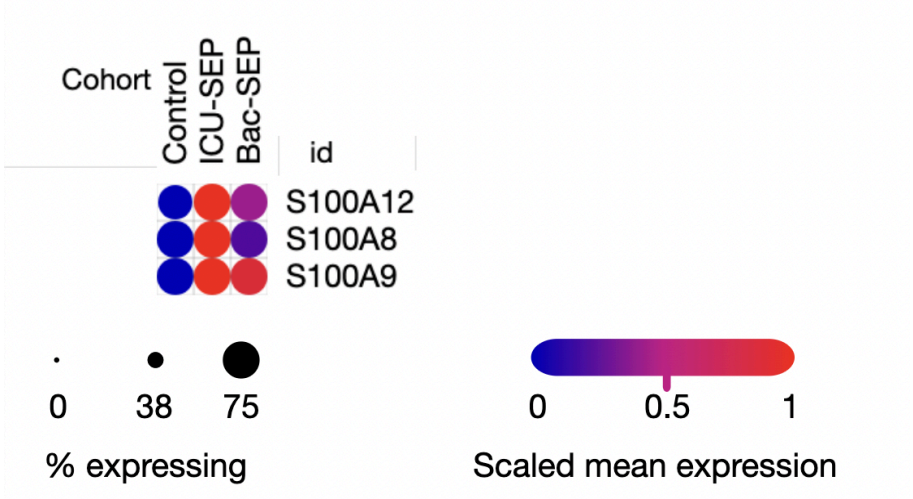
Monocytes, which are readily captured in PBMC datasets, are known to play pivotal roles in initiating and sustaining the inflammatory response during systemic infection. Megakaryocytes, while less studied in sepsis, have been increasingly recognized for their immunomodulatory functions, including cytokine production and interaction with innate immune cells. This cell type–resolved analysis confirms that calgranulin gene activity is largely confined to myeloid-derived populations within peripheral blood. Importantly, the signal is not exclusively tied to neutrophils—as previously assumed—but is also robustly present in cell types that are readily captured in PBMC assays, reinforcing the feasibility of using these genes for blood-based diagnostics.

It is important to note that the displayed dot plot reflects aggregated expression across all cells from both sepsis patients and healthy controls, and does not separately contrast expression between conditions. Nevertheless, the presence of strong calgranulin expression in monocytes and megakaryocytes highlights their baseline expression potential in these immune subsets, which are accessible in blood-based diagnostic assays.

Monocyte-Specific Expression Stratified by Clinical Condition

To investigate condition-specific regulation, we next focused on monocytes and stratified calgranulin expression levels by clinical cohort. The cohorts included healthy controls, patients with sepsis admitted to the ICU (ICU-SEP), and patients with microbiologically confirmed bacterial sepsis (Bac-SEP). As shown in Figure, the percentage of monocytes expressing each gene (indicated by dot size) remained relatively stable across conditions, suggesting that calgranulin expression is widespread in this cell type regardless of clinical state.

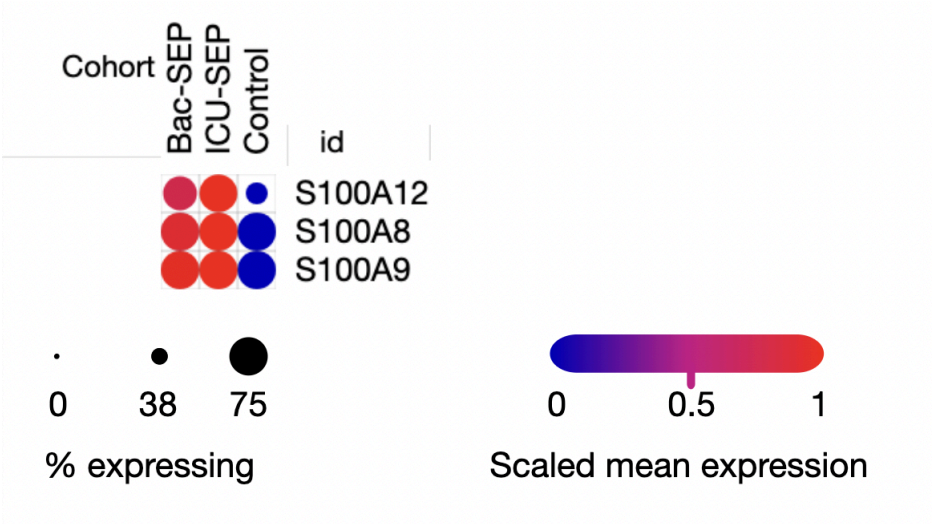
However, the color scale—which reflects scaled mean expression—revealed marked increases in expression intensity for both S100A12 and S100A9 in ICU-SEP and Bac-SEP patients compared to controls. A more modest upregulation was also observed for S100A9, particularly in the Bac-SEP group. These findings indicate that while calgranulin genes are constitutively expressed in monocytes, their transcriptional activity is amplified under septic conditions.



These findings indicate that monocytes expressing calgranulin genes exhibit heightened transcriptional activity in sepsis, with consistent upregulation observed across both ICU-admitted and bacteremia-confirmed patient groups. This reinforces the role of monocytes as key amplifiers of the systemic inflammatory response in sepsis and enhances their potential utility as diagnostic biomarkers detectable through peripheral immune profiling.

Global expression patterns stratified by condition

To obtain a broader view of calgranulin gene activity in circulating immune compartments, we analyzed expression across all PBMC cell types stratified by clinical condition (Control, ICU-SEP, and Bac-SEP), as shown in Figure. This visualization offers insight into how both the breadth (percentage of cells expressing) and intensity (scaled mean expression) of calgranulin transcription shift in disease.



Most notably, the expression of all three calgranulin genes was visibly elevated in ICU-SEP and Bac-SEP patients compared to healthy controls. These results provide further evidence that dysregulated calgranulin transcription is a hallmark of systemic inflammatory activation in sepsis, observable at the single-cell level in circulating immune compartments.

Interestingly, the expression levels of all three calgranulin genes—S100A8, S100A9, and S100A12—appeared slightly higher in the ICU-SEP group compared to the Bac-SEP group. This subtle elevation may indicate a more pronounced or sustained inflammatory response among patients admitted to intensive care, who often present with greater clinical severity and multi-organ dysfunction. It is also possible that ICU-SEP patients were sampled at a different phase of the immune trajectory—such as a later or more amplified stage of systemic inflammation—compared to those with microbiologically confirmed bacterial sepsis. Moreover,

while Bac-SEP represents a well-defined etiological subset, ICU-SEP includes patients with more heterogeneous or unresolved infectious causes, which may contribute to variability in transcriptional responses. These observations suggest that calgranulin gene expression may be sensitive not only to the presence of sepsis, but also to its clinical context and severity. However, further investigation using time-resolved and larger-cohort datasets is needed to clarify the biological underpinnings of these cohort-specific differences.