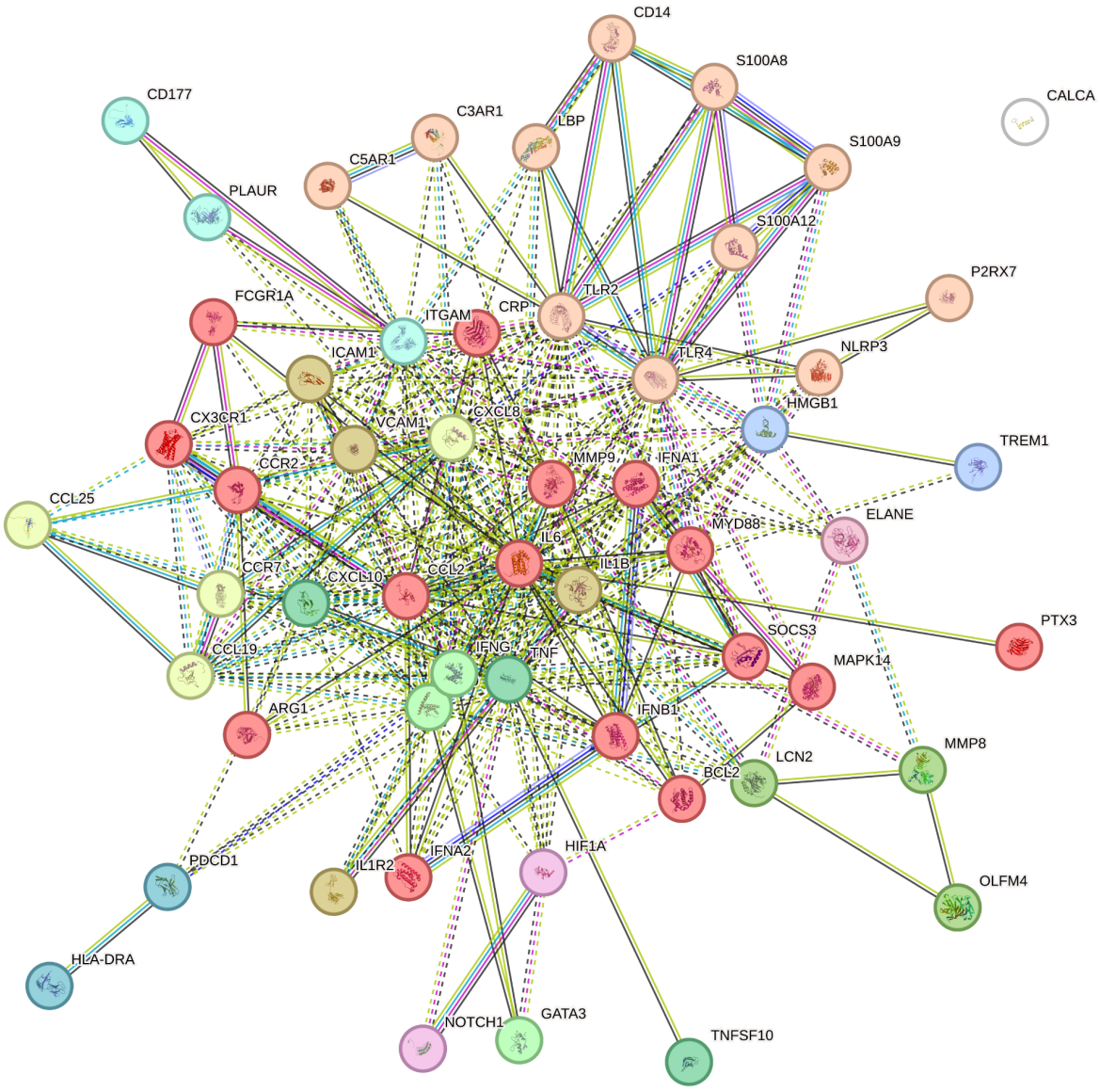
Protein-Protein interaction network by STRING database

The 55-genes panel:



Our STRING network visualization for the selected 55-gene sepsis panel shows a highly interconnected functional protein–protein interaction (PPI) map, constructed using the full STRING network at a high confidence threshold (0.700).

Represents **diverse and functionally interconnected** components of sepsis biology. Shows **tight co-regulation and pathway interdependence**, validating it as biologically meaningful.

Our MCL clustering results for the 55-gene STRING network effectively break the interaction network into 12 biologically meaningful modules.

Clusters

color	cluster Id	gene count	description
	Cluster 1	16	— 1. Regulation of type 2 immune response 2. Hepatitis B
	Cluster 2	11	Regulation of TLR by endogenous ligand
	Cluster 3	4	— 1. African trypanosomiasis 2. Interleukin-10 signaling
	Cluster 4	4	— 1. Chemokine receptors bind chemokines 2. Positive regulation of neutrophil chemotaxis
	Cluster 5	3	Mixed, incl. Specific granule lumen, and Mononeuritis multiplex
	Cluster 6	3	Inflammatory bowel disease
	Cluster 7	3	Tumour necrosis factor family.
	Cluster 8	3	Positive regulation of neutrophil degranulation
	Cluster 9	2	PD-1 signaling
	Cluster 10	2	HMGB1, TREM1
	Cluster 11	2	Positive regulation of transcription from RNA polymerase II promoter in response to...
	Cluster 12	1	ELANE

Cluster 1 (16 genes) – Core Immunoregulation and Antiviral Response

- **Description**:
  - "Regulation of type 2 immune response"
  - "Hepatitis B"

This large central cluster represents broad immunoregulatory pathways, including cytokines, and immune checkpoints. Its enrichment in *type 2 immunity* and *viral pathways* may reflect immune modulation or tolerance mechanisms active during late-stage or chronic sepsis.

Biological interpretation:

This cluster includes genes central to:

- Pro-inflammatory cytokine signaling (such as, IL6, MYD88, SOCS3)
- Acute phase response (such as, CRP, PTX3)
- Interferon-mediated antiviral defense (such as, IFNA1, IFNA2, IFNB1)
- Leukocyte migration and chemotaxis (such as, CCL2, CCR2, CX3CR1)
- Apoptosis and cell survival (such as, BCL2, MAPK14)
- Extracellular matrix remodeling (such as, MMP9)

- Macrophage and neutrophil activation (such as, ARG1, FCGR1A)

Many of these genes are upregulated in early sepsis, reflecting the hyperinflammatory phase, with some (such as, ARG1, SOCS3, BCL2) also marking transition toward immune suppression.

🟠 Cluster 2 (11 genes) – Innate Immune Sensors

- **Description**:
    - "Regulation of TLR by endogenous ligand"

This group includes TLR2, TLR4, CD14, S100A8/9/12, NLRP3, P2RX7, LBP and C3/5AR1. Detection and amplification of danger signals through Toll-like receptors (TLRs), inflammasomes, and complement–immune crosstalk.

🧬 Biological interpretation:

This cluster represents the **first-line sensing and amplification arm** of the innate immune response in sepsis:

- TLR2/4 and CD14 form the core bacterial sensor complex.
- Calgranulins (S100A8/A9/A12) act as DAMPs, amplifying inflammation via TLR4 and RAGE.
- NLRP3 and P2RX7 link cellular stress or ATP release to inflammasome activation.
- Complement receptors (C3AR1, C5AR1) link pathogen sensing to immune effector activation.
- LBP enhances TLR4 signaling in the presence of LPS.

This is a prototypical hyperinflammatory sepsis module, explaining systemic cytokine release, endothelial activation, and immune cell recruitment.

Cluster 2 (Innate Immune Sensors) grouped 11 genes involved in pathogen recognition and endogenous danger signal amplification, including TLR2, TLR4, CD14, NLRP3, and S100A8/9/12. These genes function as pattern recognition receptors (PRRs) or DAMPs that initiate and amplify inflammatory signaling through the NF-κB and inflammasome pathways. Complement receptors C3AR1 and C5AR1 further integrate immune sensing with effector recruitment. The enrichment for "Regulation of TLR by endogenous ligand" highlights this cluster's central role in hyperinflammatory signaling during sepsis.

🟡 Cluster 3 (4 genes) – IL-10 Anti-inflammatory Pathway

- **Description**:
    - "African trypanosomiasis"
    - "Interleukin-10 signaling"

This smaller cluster (ICAM1, VCAM1, IL1B and IL1R2) is centered on immune suppression, This cluster integrates **pro- and anti-inflammatory cytokine signaling** (IL1B, IL1R2) with **endothelial activation and leukocyte adhesion** (ICAM1, VCAM1). It reflects regulatory control over immune responses and immune–vascular crosstalk.

🧬 **Biological Interpretation**

- Cytokine regulation: IL1B promotes inflammation; IL1R2 inhibits it by acting as a "decoy" receptor, part of a negative feedback loop often seen in immune resolution or immune dysregulation phases of sepsis.
- Endothelial activation: ICAM1 and VCAM1 expression increases in response to cytokines like IL1B and TNFα, facilitating leukocyte adhesion and transmigration — key steps in immune cell infiltration during systemic inflammation.

Cluster 3 (4 genes) encompassed IL1B, IL1R2, ICAM1, and VCAM1, highlighting a regulatory module that integrates inflammatory cytokine signaling with endothelial adhesion. IL1B acts as a potent pro-inflammatory mediator, while IL1R2 serves as a decoy receptor to attenuate its activity. ICAM1 and VCAM1 reflect endothelial activation, promoting immune cell recruitment and vascular inflammation. Together, this cluster likely represents a key interface between immune modulation and vascular dysfunction during sepsis progression.

🟡 **Cluster 4 (4 genes) – Chemokine Axis**

- **Description**:
    - "Chemokine receptors bind chemokines"
    - "Positive regulation of neutrophil chemotaxis"

This cluster includes CCL19, CCL25, CCR7, CXCL8. This cluster defines a chemokine signaling module orchestrating: Directional migration (chemotaxis) of immune cells (especially T cells, DCs, and neutrophils), Leukocyte trafficking between tissues and secondary lymphoid organs and amplification of inflammation via recruitment of effector cells to infection sites.

🧬 **Biological Interpretation:**

1. Innate immunity via CXCL8 — a key driver of neutrophil chemotaxis, inflammation, and tissue infiltration.
2. Adaptive immunity via CCL19–CCR7 and CCL25, which guide T-cell and dendritic cell migration between lymphoid and mucosal tissues.

Cluster 4 grouped four chemokines and receptors (CXCL8, CCL19, CCL25, CCR7) involved in the regulation of immune cell trafficking. CXCL8 promotes neutrophil migration to infected tissues, while the CCL19–CCR7 and CCL25 axes support adaptive immune cell homing and mucosal

immune responses. The enrichment for “chemokine receptor binding” and “positive regulation of neutrophil chemotaxis” indicates this cluster’s central role in coordinating immune cell mobilization during sepsis.

🟢 Cluster 5 (3 genes) – Granule and Antimicrobial Defense

- **Description**:
    - "Mixed; incl. Specific granule lumen and Mononeuritis multiplex"

It includes LCN2, OLFM4, and MMP8 related neutrophil granule proteins with antimicrobial activity and potential vascular effects.Specialized neutrophil effector proteins stored in specific granules, released during infection to perform direct antimicrobial activity, modulate the immune response, and contribute to tissue injury in sepsis.

🧬 Biological Interpretation

- Direct antimicrobial activity (LCN2, OLFM4),
- Tissue remodeling and damage (MMP8),
- Innate immune amplification (LCN2 via TLR modulation).

This cluster reflects the **late phase of neutrophil activation**, where degranulation releases powerful enzymes and antimicrobial factors. While protective in controlled infections, this response can contribute to **collateral tissue damage** and **multi-organ failure** in septic patients. Cluster 5 grouped LCN2, OLFM4, and MMP8 — genes encoding proteins stored in neutrophil specific granules and rapidly released during degranulation. These molecules contribute to direct antimicrobial defense (LCN2, OLFM4) and extracellular matrix remodeling (MMP8). The enrichment in “specific granule lumen” highlights this cluster’s role in neutrophil effector function during sepsis, with potential links to tissue injury and disease severity.

🟢 Cluster 6 (3 genes) – Mucosal Inflammation

- **Description**:
    - "Inflammatory bowel disease"

Despite the IBD label for (GATA3, IFNG and IL10) This cluster contains master regulators of

adaptive immunity, representing a balance between proinflammatory Th1 responses (IFNG), anti-inflammatory signaling (IL10), and Th2 differentiation (GATA3).

🧬 Biological Interpretation

- IFNG drives early Th1-mediated immune activation and pathogen clearance.
- IL10 acts as a brake, limiting damage from excessive inflammation.
- GATA3 promotes Th2 differentiation, often associated with the immunosuppressive phase of sepsis.

Cluster 6 grouped three immune modulators: IFNG, IL10, and GATA3. These genes represent the cytokine balance between proinflammatory Th1 signaling (IFNG), anti-inflammatory resolution (IL10), and Th2 polarization (GATA3). Their co-regulation suggests a key role in adaptive immune modulation and immune exhaustion in later stages of sepsis. Although STRING annotated this cluster under “Inflammatory bowel disease,” the core biology reflects phase-specific immune reprogramming seen in systemic inflammation.

🟢 **Cluster 7 (3 genes) – TNF Pathway**

- **Description**:
    - "Tumor necrosis factor family"

This cluster is driven by TNF, TNFSF10, and CXCL10. A focused cytokine module involving TNF superfamily signaling, apoptotic signaling (via TNFSF10), and chemokine amplification (via CXCL10), representing a critical axis in inflammatory amplification, cell death, and immune cell recruitment.

🧬 **Biological Interpretation**

- Cytokine storm initiation (TNF),
- Immune effector cell recruitment (CXCL10), and
- Apoptotic pathway activation (TNFSF10).

Cluster 7 consisted of TNF, TNFSF10, and CXCL10, reflecting a focused pro-inflammatory and pro-apoptotic signaling axis. TNF is a key driver of systemic inflammation in sepsis, while TNFSF10 (TRAIL) mediates immune-triggered apoptosis. CXCL10, an interferon-induced chemokine, promotes T-cell recruitment and amplifies the cytokine response. This cluster likely contributes to both the inflammatory cascade and immune-mediated tissue damage observed in severe sepsis.

🔵 Cluster 8 (3 genes) – Neutrophil Degranulation

- **Description**:
    - "Positive regulation of neutrophil degranulation"

It includes CD177, ITGAM, PLAUR. This cluster consists of membrane-associated molecules that regulate neutrophil adhesion, activation, and degranulation, key mechanisms in pathogen clearance and inflammatory tissue damage during sepsis.

🧬 Biological Interpretation

- Neutrophil-specific markers of maturation and activation,
- Central to adhesion to the endothelium, transmigration, and release of granule contents,
- Linked to vascular injury and amplification of local inflammation in sepsis.

This cluster likely captures a key effector module of sepsis pathophysiology — activated neutrophils damaging tissue while trying to clear infection.

Cluster 8 grouped CD177, ITGAM, and PLAUR — key surface proteins involved in neutrophil adhesion and degranulation. These genes reflect neutrophil activation, migration, and effector function, processes that contribute to both pathogen clearance and tissue injury in sepsis. The STRING enrichment for “positive regulation of neutrophil degranulation” highlights this cluster’s role in executing and regulating innate immune effector responses.

🔵 Cluster 9 (2 genes) – Immune Checkpoint Signaling

- **Description**:
    - "PD-1 signaling"

Includes PDCD1 (PD-1) and HLA-DRA components. This cluster captures a key immune checkpoint axis involving PD-1–mediated suppression of T-cell responses and MHC class II antigen presentation, both of which are strongly dysregulated in the immunosuppressive phase of sepsis.

🧠 Biological Interpretation

This cluster reflects adaptive immune suppression through two coordinated mechanisms:

- PD-1 overexpression: leads to functional exhaustion of T cells, reducing cytokine production and cytotoxic activity.
- Downregulation or dysfunction of HLA-DRA: impairs antigen presentation to CD4+ T cells, limiting immune activation.

Together, these mechanisms contribute to the “immune exhaustion” and secondary infection risk seen in prolonged or late-stage sepsis.

Cluster 9 grouped PDCD1 (PD-1) and HLA-DRA, reflecting adaptive immune suppression through immune checkpoint signaling and impaired antigen presentation. PD-1 is a key regulator of T-cell exhaustion, while HLA-DRA represents MHC class II–dependent antigen presentation. This cluster aligns with sepsis-induced immunoparalysis and highlights mechanisms of host vulnerability during the late phase of disease.

🔵 Cluster 10 (2 genes) – Alarmin-Driven Innate Activation

- **Description**:
    - "HMGB1, TREM1"

This cluster captures danger-associated molecular pattern (DAMP) signaling and innate immune amplification, centered on HMGB1 as a DAMP and TREM1 as an inflammation enhancer.

🧠 Biological Interpretation

- HMGB1 is released during necrosis or cellular stress and binds to TLR4 and RAGE, promoting broad pro-inflammatory signaling.
- TREM1 further amplifies signals from TLR pathways, exacerbating cytokine release and leukocyte activation.

Together, these genes fuel systemic inflammation, endothelial dysfunction, and organ damage, making them central to late sepsis pathogenesis.

Cluster 10 included HMGB1 and TREM1, representing a danger signal–amplifying module in the innate immune response. HMGB1 functions as a prototypical DAMP, activating TLR and RAGE signaling, while TREM1 acts as an amplifier of inflammatory cytokine production. This cluster reflects a key axis in sepsis-related cytokine storm and inflammatory tissue injury.

🟣 Cluster 11 (2 genes) – Transcriptional Activation

- **Description**:

- "Positive regulation of transcription from RNA polymerase II promoter in response to hypoxia"

This includes signaling regulators like NOTCH1 and HIF1A, This cluster highlights a hypoxia-responsive transcriptional module that: Regulates adaptive responses to inflammatory or ischemic stress. Controls genes involved in angiogenesis, metabolism, cell survival, and immune modulation and is key during tissue hypoxia, a common condition in septic organs.

🧬 **Biological Interpretation**

- HIF1A activates genes for metabolic shift (glycolysis), angiogenesis, and immune signaling under low oxygen.
- NOTCH1 interacts with NF-κB and HIF1 pathways to modulate T-cell differentiation, macrophage polarization, and cell survival.

Together, these genes form a non-canonical transcriptional axis shaping the immune response under pathophysiological stress — especially endothelial activation, organ dysfunction, and immunomodulation in sepsis.

Cluster 11 grouped NOTCH1 and HIF1A, two stress-induced transcriptional regulators that modulate gene expression under hypoxic and inflammatory conditions. HIF1A orchestrates metabolic and inflammatory responses to tissue hypoxia, while NOTCH1 influences immune cell fate and transcriptional reprogramming. Their co-expression suggests a shared role in adapting immune and vascular responses to sepsis-induced oxygen deprivation.

🔴 **Cluster 12 (1 gene) – Isolated Neutrophil Enzyme**

- **Description**:
    - "ELANE"

Though not highly connected, ELANE (neutrophil elastase) is critical in NET formation and tissue damage in sepsis.

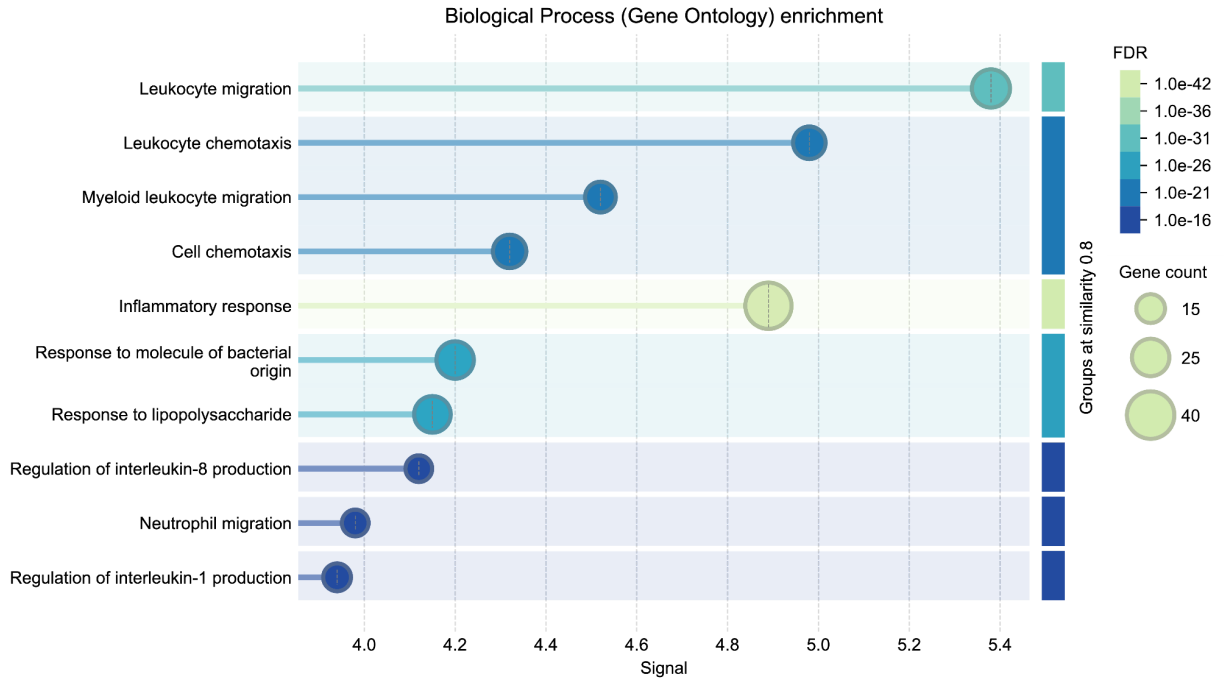
🧬 **Biological Interpretation**

- A front-line antimicrobial effector, degrading bacterial proteins directly.
- A driver of tissue damage, especially in lungs and vasculature, when overactive.
- A modulator of inflammation, by processing cytokines and chemokines.

Cluster 12, containing only ELANE, reflects neutrophil granule–associated protease activity. ELANE (neutrophil elastase) is released during neutrophil activation and contributes to both antimicrobial defense and tissue damage. Its unique clustering likely reflects the absence of direct interactions in the STRING network, but biologically, it represents a key effector in sepsis-associated neutrophil pathology.

STRING GO Biological Process (BP) enrichment

This is a **STRING GO Biological Process enrichment bubble plot**, showing which immune related processes are most significantly enriched in our 55-gene panel.



Our gene signature is highly enriched for immune cell trafficking and innate immune responses, particularly:

- **Neutrophil and myeloid migration**
- **Chemotaxis**
- **TLR and LPS-driven signaling**
- **IL-1 and IL-8 cytokine regulation**

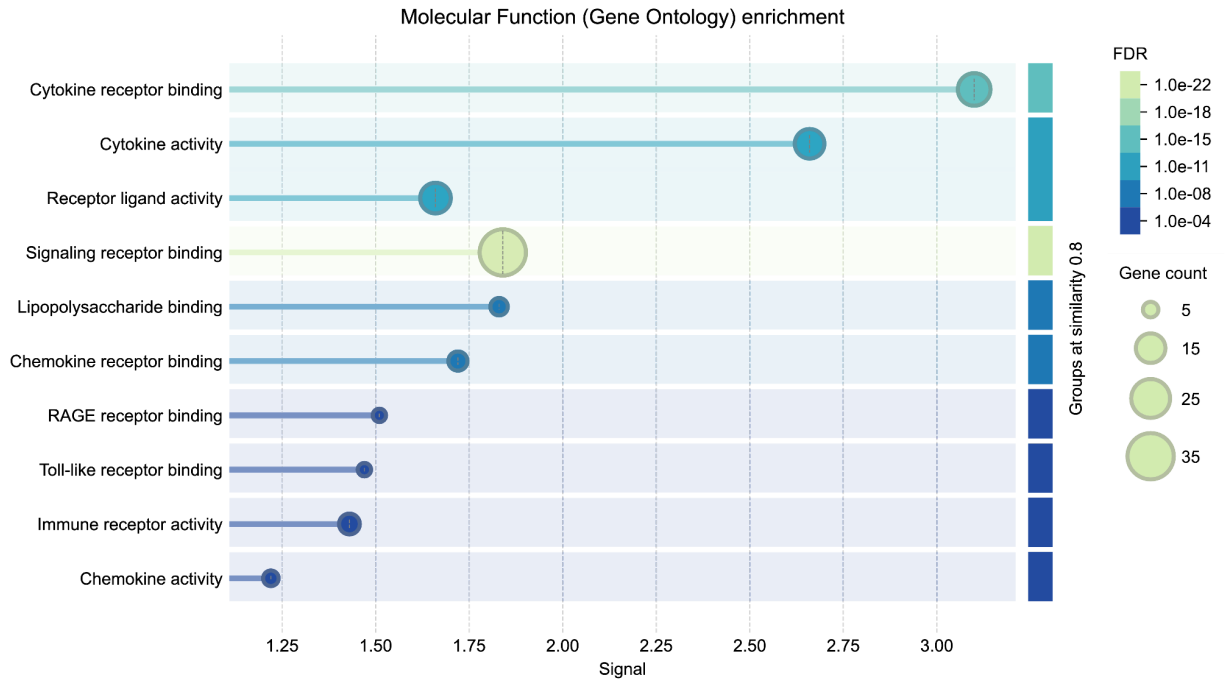
This aligns extremely well with what we biologically expect from **sepsis pathogenesis**:

- **Early hyperinflammation** → cytokine storm, neutrophil infiltration
- **Pattern recognition** → LPS and bacterial ligands triggering immune activation
- **Granule release and degranulation** → MMPs, ELANE, OLFM4
- **DAMP/PAMP signaling** → TLRs, S100s, HMGB1, PLAUR

Gene Ontology enrichment analysis of the final gene panel revealed highly significant overrepresentation of immune migration and innate sensing pathways. Top terms included “leukocyte migration” (FDR < 1e-31), “leukocyte chemotaxis,” and “response to lipopolysaccharide,” reflecting the strong involvement of myeloid cell trafficking and pattern recognition in sepsis pathophysiology. Additional enrichment for “regulation of interleukin-1 and -8 production” underscores the panel’s relevance to cytokine-driven inflammation and neutrophil activation.

STRING molecular function (MF) enrichment

This is a **STRING molecular function enrichment plot** (based on Gene Ontology: Molecular Function terms) and it gives us valuable insight into **what types of biochemical activities** our diagnostic genes are involved in.



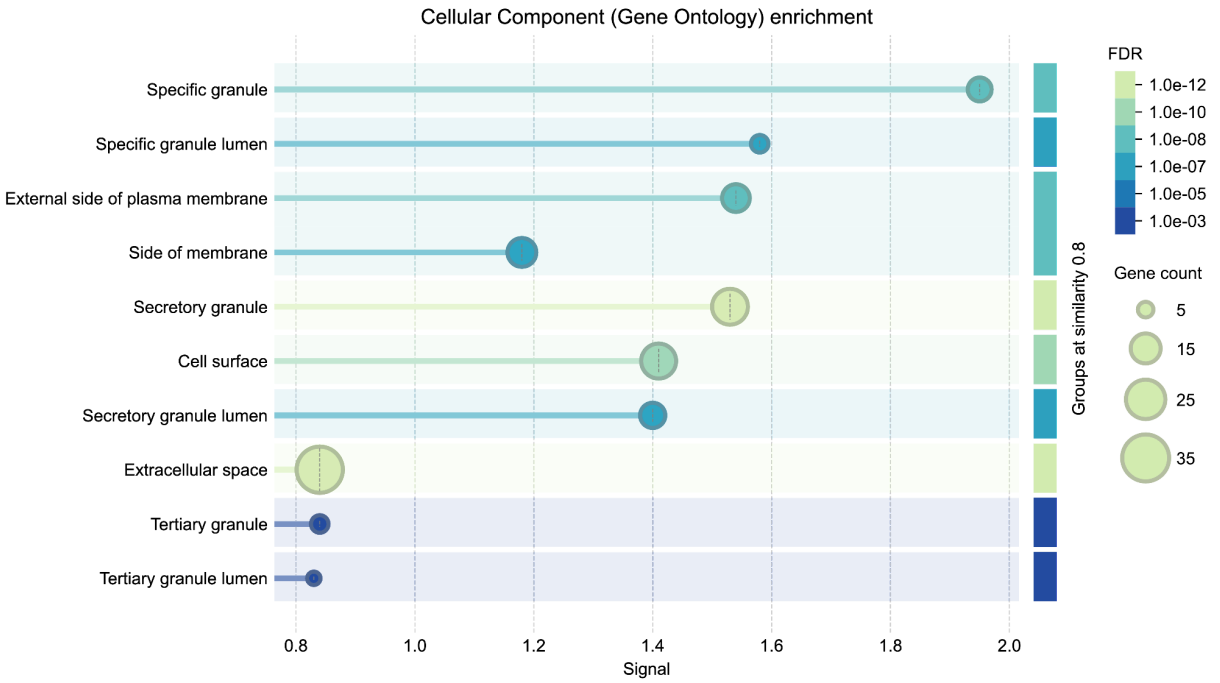
Our panel of genes is:

- **Cytokine-heavy**: Many genes are either cytokines or bind to cytokine receptors.
- **Involved in receptor–ligand signaling**: A large proportion regulate or participate in **cell communication** during immune responses.
- **Highly enriched for innate sensing**: TLRs, LPS-binding, and RAGE pathways are well represented.
- **Focused on immune activation and communication**, consistent with early sepsis pathophysiology (hyperinflammation and immune amplification).

Molecular function enrichment revealed that the diagnostic gene panel is significantly enriched in cytokine-related activities, including “cytokine receptor binding” (FDR < 1e−15), “cytokine activity,” and “receptor ligand activity.” Additional enrichment for “lipopolysaccharide binding,” “Toll-like receptor binding,” and “RAGE receptor binding” supports the panel’s role in innate immune recognition and inflammatory amplification. These functions align with the early-phase pathophysiology of sepsis and highlight key signaling nodes within the diagnostic signature.

STRING GO Cellular Component (CC) enrichment

This STRING chart shows **GO Cellular Component enrichment**, which tells us **where in or on the cell** our sepsis signature genes tend to function or localize.



Our gene panel is **heavily enriched in neutrophil-specific granules**, secretory pathways, and immune-active membrane regions:

- **Granule localization (specific, secretory, tertiary)**: confirms that many of our genes (such as, MMP8, MMP9, ELANE, OLFM4, LCN2) are stored in granules and released during neutrophil degranulation, a hallmark of hyperinflammation in sepsis.
- **Cell surface / membrane localization**: includes receptors like TLRs, CD14, PDCD1, TREM1, CCR2, indicating cell–cell communication, activation, and immune checkpoint signaling.
- **Extracellular space**: includes cytokines, chemokines, and DAMPs like S100A8/9/12, IL6, and HMGB1, emphasizing their role in paracrine immune signaling.

Cellular component enrichment revealed strong localization to neutrophil granules, membranes, and secreted compartments. “Specific granules” and “secretory granules” were top-ranked (FDR < 1e−7), highlighting the importance of granule-stored inflammatory mediators such as MMP8, OLFM4, and LCN2. Enrichment on the “external side of the plasma membrane” and “extracellular space” underscores the role of immune signaling molecules and cell-surface receptors in driving systemic inflammation in sepsis.