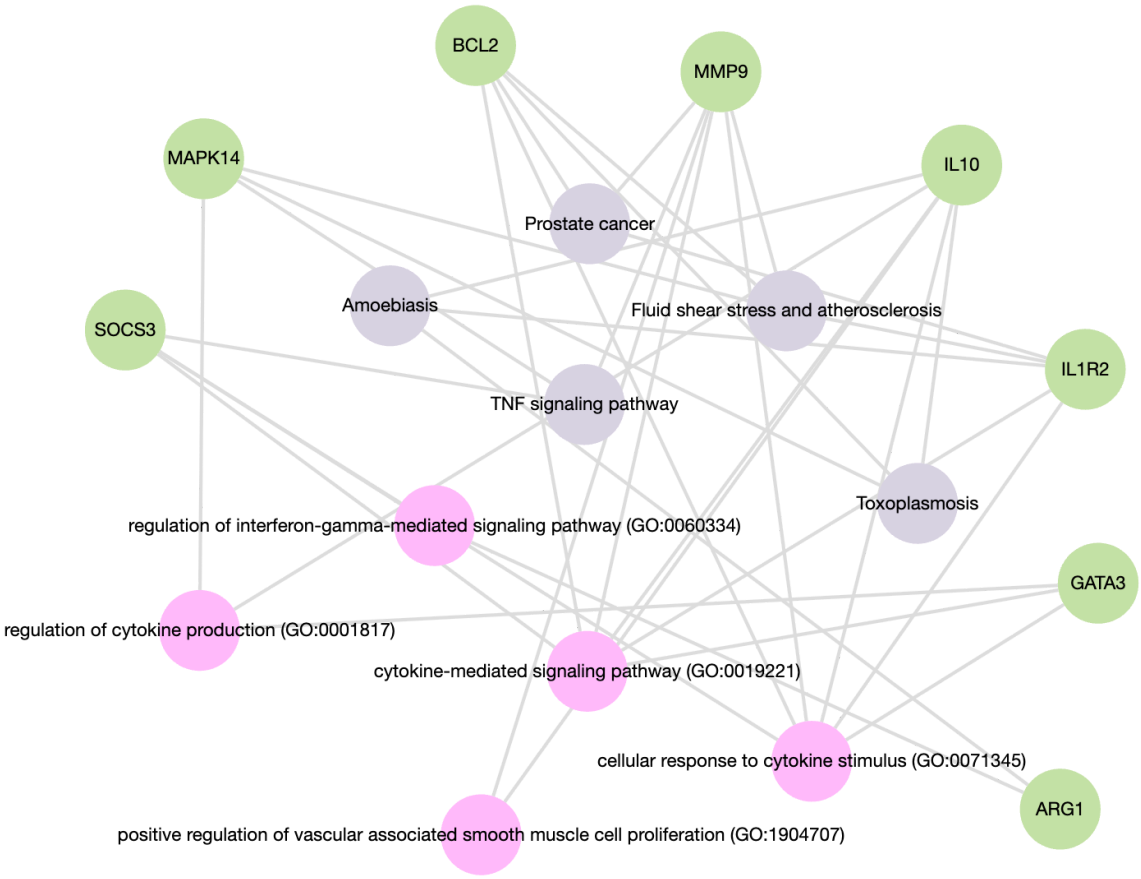
1. Biological Network Interpretation – Enrichr KG for Cluster 1 Genes

Node and Edge Meanings:

- 🟢 **Green nodes** = Genes
- 🟣 **Pink nodes** = Significantly enriched biological processes (GO terms)
- 🟠 **Orange nodes** = Mouse Phenotype (MP) terms (MGI Phenotypes)
- ⚪**Grey node** = KEGG signaling pathway (IL-17 signaling)
- ➖ **Edges** = Functional associations between genes and enriched biological terms (indicating that the gene is annotated to that GO/KEGG term)



Functional Themes:

1. Cytokine Signaling and Immune Modulation

All the following GO terms relate to **immune regulation and cytokine activity**:

- *Cytokine-mediated signaling pathway (GO:0019221)*
- *Regulation of cytokine production (GO:0001817)*
- *Cellular response to cytokine stimulus (GO:0071345)*
- *Regulation of interferon-gamma-mediated signaling pathway (GO:0060334)*

→ These highlight the cluster's role in **immune response suppression/modulation**, especially via **IL-10**, **SOCS3**, and **ARG1**, which are key in **dampening inflammation** in sepsis.

2. Immune Cell Differentiation and Anti-inflammatory Activity

- This suggests strong **immunosuppressive signaling**, likely involved in late or severe sepsis.

Disease and Pathway Associations

The grey nodes represent disease or pathway relevance:

- *TNF signaling pathway*
- *Toxoplasmosis*, *Amoebiasis*
- *Fluid shear stress and atherosclerosis*
- *Prostate cancer*

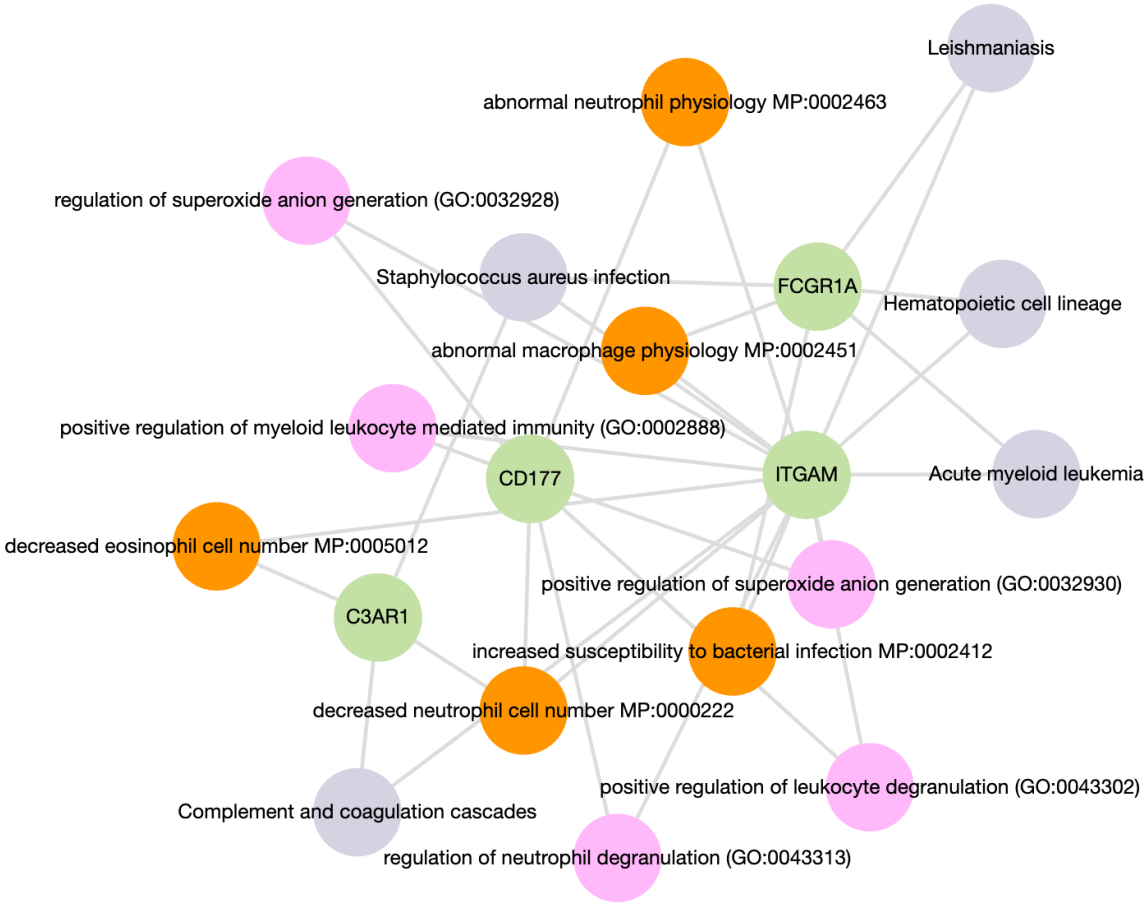
→ These links highlight **inflammatory conditions, infections**, and **vascular stress**, which are highly relevant in sepsis pathophysiology.

Functional Summary

Cluster 1 genes form a tightly connected immune-regulatory module. Enrichment analysis indicates strong associations with cytokine signaling (especially IL-10 and TNF pathways),

immunosuppression, and inflammation resolution. With ARG1 included, the cluster emphasizes the presence of **anti-inflammatory feedback mechanisms** often observed in **late-stage or immune-paralyzed sepsis**.

2. Biological Network Interpretation – Enrichr KG for Cluster 2 Genes



Key Biological Themes

1. Neutrophil-Mediated Immunity & Degranulation

- Positive regulation of myeloid leukocyte mediated immunity (GO:0002888)
- Positive regulation of leukocyte degranulation (GO:0043302)

- Regulation of neutrophil degranulation (GO:0043313)
  → These highlight the central role of these genes in activating and releasing neutrophil granules containing antimicrobial molecules — a core sepsis defense mechanism.

2. Oxidative Burst & Host Defense

- Regulation of superoxide anion generation (GO:0032928)
  → These processes describe respiratory burst activity, a hallmark of activated neutrophils and macrophages during pathogen attack.

3. Host Susceptibility & Phenotypic Impacts

- **Mouse Phenotype Terms**:
  - Abnormal neutrophil physiology (MP:0002463)
  - Abnormal macrophage physiology (MP:0002451)
  - Decreased neutrophil/eosinophil cell number (MP:0002222, MP:0005012)
  - Increased susceptibility to bacterial infection (MP:0002412)
    → These MP terms support the functional importance of these genes in maintaining normal innate immune physiology and pathogen clearance.

Functional Summary

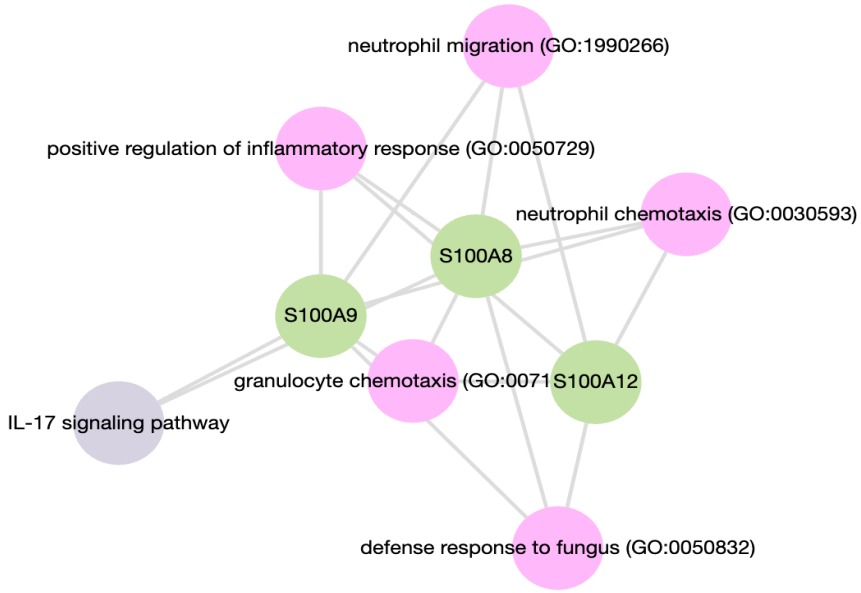
This network demonstrates that Cluster 2 genes form a tightly regulated module that:

- Supports **innate immune effector functions** (such as, oxidative burst, phagocytosis, degranulation)
- Is crucial for immune cell development and activation
- Has functional evidence from both human GO terms and murine knockout phenotypes
- Plays a key role in host-pathogen interactions, particularly in bacterial infection scenarios

These genes likely contribute to sepsis pathogenesis through impaired immune defense when dysregulated.

3. Biological Network Interpretation – Enrichr KG for Cluster 3 Genes

This graph presents an enriched knowledge graph linking three sepsis-related genes (S100A8, S100A9, S100A12) with their associated biological processes:



Biological Insights:

The network suggests that **S100A8, S100A9, and S100A12** are involved in a tightly linked inflammatory response module, specifically centered around **neutrophil function** and **innate immunity**.

1. Neutrophil Activation and Migration:

- **Neutrophil migration (GO:1990266)**
- **Neutrophil chemotaxis (GO:0030593)**
- **Granulocyte chemotaxis (GO:0071621)**

These processes highlight the role of this gene cluster in directing neutrophil movement toward infection or inflammatory sites , a hallmark of acute sepsis response.

2. Pro-inflammatory Signaling:

- **Positive regulation of inflammatory response (GO:0050729)**
  These genes amplify inflammation via cytokine induction and immune cell activation.

- **IL-17 signaling pathway (KEGG)**
  Links to IL-17-mediated signaling, which is essential for mobilizing neutrophils and coordinating early innate immune responses, especially at mucosal sites.

3. Antimicrobial and Host Defense:

- **Defense response to fungus (GO:0050832)**
  Suggests a role in nutritional immunity by sequestering metal ions (such as, zinc, manganese) that pathogens need to survive.
  This is consistent with the calprotectin complex formed by S100A8/A9.

Functional Summary:

This enrichment map indicates that the S100A gene cluster orchestrates a multifaceted neutrophil-driven defense program, comprising:

- Chemotaxis: Recruitment of neutrophils to infected/inflamed tissue
- Cytokine amplification: Enhancing immune activation via IL-17 signaling
- Antimicrobial activity: Fungal and bacterial growth suppression by metal sequestration
- Inflammatory regulation: Balancing the immune response intensity in sepsis