**Analysis 1: Macrophages M2 polarization**

**Cluster\_1**

Enrichment Result of Cluster\_1 (LLM Summary)

Cytoskeletal Dynamics and Vesicle-Linked Adhesion Signaling

**Pathway Analysis:**

This module encompasses pathways associated with cytoskeletal organization, cell adhesion, and vesicle-mediated signaling, integrating mechanical and secretory processes that regulate cellular interaction with the extracellular environment. The enrichment of “stress fiber,” “contractile actin filament bundle,” and “focal adhesion” highlights actin cytoskeleton remodeling and its coupling to integrin-based adhesion complexes, which are fundamental for cell motility, morphology, and mechanotransduction. “Small GTPase-mediated signal transduction” underscores the involvement of Rho family GTPases such as RhoA, Rac1, and Cdc42 in controlling actin polymerization, adhesion turnover, and vesicle trafficking, thereby coordinating cellular responses to environmental cues.

The inclusion of “cell-substrate junction” and related vesicle lumen terms (“vesicle lumen,” “secretory granule lumen,” “ficolin-1-rich granule lumen”) suggests an additional layer of regulation through vesicular transport and secretion. These vesicles likely carry adhesion molecules, proteases, and immune mediators, supporting processes such as cell migration, extracellular matrix remodeling, and innate immune modulation. The mention of “ficolin-1-rich granule” is particularly noteworthy, linking cytoskeletal and secretory machinery to immune granules found in myeloid cells, where actin remodeling underlies exocytosis and pathogen response.

Altogether, this module represents the dynamic interface between cytoskeletal contractility, adhesion signaling, and vesicle-mediated secretion, contributing to cellular adhesion, migration, and immune effector activity.

Representative module name: Cytoskeletal Dynamics and Vesicle-Associated Adhesion Signaling.

**Gene Analysis:**

The listed genes constitute a regulatory network centered on actin cytoskeleton organization, adhesion signaling, and vesicle-mediated trafficking that collectively govern cellular motility, morphology, and adaptive responses to environmental stimuli. Core cytoskeletal genes including ACTB, ACTG1, ACTN1, MYL9, and FLNB highlight the structural and contractile framework that maintains cell shape and drives migration through stress fiber formation and focal adhesion dynamics. Regulators such as CDC42, RHOF, ARHGAPs, ARHGEFs, and GEF-GAP adaptors modulate small GTPase activity, coordinating actin polymerization and vesicle motility. Integrins (ITGA3, ITGA4, ITGAX) and focal adhesion components (FN1, VCL, PTK2) physically couple cytoskeletal tension with extracellular matrix signaling, facilitating mechanotransduction and dynamic adhesion turnover. Vesicle trafficking and endocytosis-related genes (PICALM, AP2A2, DENND4B/C, PI4KA, RAB38) link actin rearrangement to membrane remodeling and cargo sorting, while lysosomal and proteolytic factors (CTSA, CTSC, CTSZ, CTSB, ASAH1) contribute to matrix degradation and immune effector functions through secretory vesicle release. The inclusion of APP, BACE1, and LGALS3 suggests connections to endosomal physiology and immune modulation, especially within myeloid cells. Key adaptor and scaffolding proteins such as PDLIM2, LIMA1, CYFIP1, and EPS8 integrate adhesion and actin dynamics with signal transduction, enabling cross-talk between mechanical stimuli and kinase pathways including PDPK1 and MAPKAP1. Overall, the gene ensemble represents a mechanochemically responsive network that synchronizes adhesion, cytoskeletal rearrangement, and vesicular trafficking to mediate cell migration, phagocytosis, and tissue remodeling. Notably, regulators of Rho-family signaling (CDC42, TIAM1, and ARHGEFs) and adhesion kinases (PTK2, FN1, VCL) emerge as pivotal nodes linking structure and signaling in this integrative cytoskeletal-adhesion interface.

**Cluster\_2**

Enrichment Result of Cluster\_2 (LLM Summary)

Clathrin-Mediated Endocytosis and Vesicle Recycling

**Pathway Analysis:**

This module centers on clathrin-mediated endocytosis and vesicle recycling, highlighting processes that regulate membrane trafficking, receptor turnover, and synaptic function. The enrichment of “clathrin-coated pit,” “clathrin coat,” and “clathrin coat of coated pit” identifies the structural basis of vesicle formation, where clathrin triskelions assemble into coated pits to mediate the internalization of membrane receptors, transporters, and signaling molecules. The presence of “clathrin-dependent endocytosis” underscores its essential role in the selective uptake of cargo, enabling cells to modulate surface receptor density and maintain signaling homeostasis.

“Synaptic vesicle recycling” and “presynaptic endocytosis” indicate specialized forms of this process in neurons, where rapid clathrin-dependent retrieval of synaptic vesicles is critical for sustaining neurotransmission and synaptic plasticity. This coupling of endocytosis and exocytosis ensures efficient neurotransmitter release during repetitive neuronal activity. These pathways are also vital in other cell types for receptor-mediated signaling, nutrient uptake, and antigen presentation, linking them to developmental, metabolic, and immune regulatory functions.

Dysregulation of clathrin-mediated trafficking contributes to a wide range of pathologies, including neurodegenerative diseases, defective synaptic signaling, and altered receptor dynamics in cancer and metabolic disorders. Collectively, this module defines a core membrane trafficking network centered on clathrin-dependent vesicle formation and recycling, establishing a fundamental mechanism for maintaining cellular communication and membrane composition.

Representative module name: Clathrin-Mediated Endocytosis and Vesicle Recycling Network.

**Gene Analysis:**

The listed genes collectively form a functional network governing clathrin-mediated endocytosis, vesicle trafficking, and receptor recycling, essential processes for maintaining membrane composition, signaling homeostasis, and synaptic activity. Central components such as AP1 and AP2 adaptor complex subunits (AP1B1, AP1S1, AP2A2, AP2M1, AP2S1) and clathrin light chain (CLTB) facilitate coated pit formation and cargo selection, driving the internalization of receptor complexes like TFRC (transferrin receptor) and LRP1. Early endocytic regulators FCHO1, FCHO2, and DAB2 initiate clathrin coat assembly, while phosphoinositide-modifying enzymes such as OCRL, SYNJ1, SYNJ2, and PICALM fine-tune vesicle maturation and uncoating via lipid composition remodeling. Actin cytoskeletal elements (ACTB, ACTG1) and associated kinases (AAK1, BMP2K) orchestrate membrane deformation and vesicle scission, linking cytoskeletal tension to endocytic dynamics. GIT1, GIT2, and CAPN2 connect membrane trafficking to focal adhesion remodeling and signal transduction, integrating endocytosis with cellular motility and receptor turnover. APP and CYFIP1 highlight neuronal and synaptic associations, where endocytic recycling sustains neurotransmitter release and receptor availability critical for synaptic plasticity. Chaperones such as HSPD1 and scaffolding elements like CALM2 ensure conformational flexibility and regulation of membrane curvature during vesicle formation. Together, these genes delineate a conserved vesicle transport network that coordinates receptor internalization, cargo sorting, and recycling across diverse cellular contexts. Notably, key regulatory hubs including PICALM, SYNJ1, and LRP1 align this module with neurodegenerative pathways, where disrupted clathrin-dependent endocytosis contributes to impaired membrane turnover and pathological protein aggregation.

Cluster\_3

Enrichment Result of Cluster\_3 (LLM Summary)

Lysosomal Lipid Metabolism and Phosphoinositide Biosynthesis

**Pathway Analysis:**

This module encompasses phospholipid and glycerolipid metabolism within lysosomal and vacuolar compartments, reflecting the integration of membrane lipid synthesis, remodeling, and turnover essential for cellular homeostasis and signaling. The enrichment of pathways such as “phospholipid metabolic process,” “phosphatidylinositol biosynthetic process,” and “glycerophospholipid metabolic process” highlights lipid biosynthesis as a key driver of membrane integrity, vesicular trafficking, and signal transduction. Phosphoinositides, in particular, serve as molecular regulators of membrane curvature and recruit proteins involved in endocytosis, autophagy, and cytoskeletal organization.

The association of “vacuolar lumen” and “lysosomal lumen” with these lipid processes points to active lipid degradation and recycling within acidic compartments, consistent with lysosomal hydrolysis of membrane-derived lipids and subsequent reuse of lipid precursors for membrane biogenesis or energy storage. “Glycerolipid biosynthetic process” and “glycerolipid metabolic process” illustrate the anabolic arm of this network, generating key structural and signaling lipids such as phosphatidylcholine, phosphatidylethanolamine, and phosphatidylinositol that sustain membrane dynamics and organelle function. Lysosomal phospholipid metabolism is also crucial for cellular lipid sensing and nutrient signaling through the mTOR pathway.

Collectively, this module defines the molecular machinery linking lipid anabolism and catabolism to membrane renewal and signaling, with implications in metabolic regulation, autophagy, and lysosomal storage disorders.

Representative module name: Lysosomal Lipid Metabolism and Phosphoinositide Biosynthesis Network.

**Gene Analysis:**

The listed genes form a cohesive network regulating lipid metabolism, lysosomal function, and membrane remodeling, which are crucial for maintaining cellular homeostasis, energy balance, and signaling. Core phospholipid synthesis enzymes such as AGPAT2, AGPAT4, CDIPT, LPCAT1, and LPCAT4 contribute to the generation of glycerophospholipids and phosphatidylinositols, sustaining membrane structure and signaling platforms. Complementary lipid-degrading enzymes including ASAH1, LIPA, CTSA, CTSC, CTSS, GALC, GLB1, HEXB, MGLL, and PLA2G15 mediate lysosomal and cytoplasmic lipid catabolism, enabling the recycling of lipid intermediates and preventing toxic lipid accumulation. Genes linked to lysosomal organization and trafficking, such as BLOC1S6, NPC2, GRN, HGSNAT, LGMN, and RAB38, highlight the importance of vesicular transport and degradation systems in regulating lipid turnover. Phosphoinositide metabolism is supported by PI4KA, SYNJ1, SYNJ2, and PIK3R1/5, which generate signaling lipids that coordinate trafficking, endocytosis, and autophagy. Lipid-binding and transport proteins such as OSBPL5, OSBPL8, and FABP5 facilitate lipid distribution among organelles, integrating metabolic and signaling functions. Notably, the presence of PDGFB and STK11IP links lipid metabolic pathways to nutrient sensing and mTOR regulation, reflecting crosstalk between lipid status and growth control. Dysfunction in multiple members of this network is implicated in lysosomal storage diseases, neurodegeneration, and metabolic disorders, underscoring the critical integration of lipid synthesis, degradation, and trafficking. Collectively, this gene set defines a coordinated lysosomal–phosphoinositide signaling system that maintains organelle integrity, membrane renewal, and cellular metabolic flexibility.

**Cluster\_4**

Enrichment Result of Cluster\_4 (LLM Summary)

Mitochondrial Energy Metabolism and Respiratory Chain Assembly

**Pathway Analysis:**

This module represents a core metabolic network centered on mitochondrial organization, oxidative phosphorylation, and energy metabolism. The enrichment of pathways such as “generation of precursor metabolites and energy,” “cellular respiration,” and “oxidoreductase activity, acting on NAD(P)H” highlights a system devoted to ATP production through electron transport and oxidative phosphorylation. The inclusion of “mitochondrial respiratory chain complex I assembly” and “NADH dehydrogenase complex assembly” indicates a focus on the biogenesis and maintenance of electron transport chain components essential for mitochondrial redox balance and energy transduction. These processes ensure the efficient oxidation of NADH and FADH2, coupling electron transfer to proton gradient generation, which drives ATP synthase activity and supports cellular energetics.

“Purine nucleotide metabolic process” further links energy generation to nucleotide synthesis and cofactor metabolism, reflecting the integrated control of bioenergetic and biosynthetic demands, particularly in proliferative and metabolically active cells. “Mitochondrion organization” underscores the structural coordination of mitochondrial morphology, fission, and fusion necessary for optimal respiratory efficiency and reactive oxygen species management. Disruption of these pathways contributes to mitochondrial dysfunction, impaired ATP production, and oxidative stress, processes implicated in aging, neurodegeneration, and metabolic disorders.

Collectively, this module defines the molecular framework underlying mitochondrial respiration, redox homeostasis, and the coupling of energy generation with nucleotide metabolism, ensuring efficient cellular adaptation to metabolic and energetic demands.

Representative module name: Mitochondrial Energy Metabolism and Respiratory Chain Assembly.

**Gene Analysis:**

The listed genes compose a versatile metabolic and mitochondrial network responsible for energy production, redox homeostasis, and structural maintenance of the organelle. Multiple components of the electron transport chain, including NDUFA, NDUFB, COX, SDHC, UQCRC, and ATPAF1, underscore a strong focus on oxidative phosphorylation and mitochondrial respiratory efficiency, linking NADH oxidation to ATP generation. Supporting cofactors such as SIRT3, SIRT5, and NNT regulate oxidative metabolism and detoxification of mitochondrial reactive oxygen species, preserving redox balance during high metabolic demand. Mitochondrial organization and dynamics are facilitated by FIS1, MIEF2, OXA1L, SAMM50, and TOMM22, coordinating protein import, fission, and cristae formation, while chaperones HSPD1 and HSPA8 maintain proteostasis to sustain oxidative function. Metabolic enzymes such as ACO2, HADHA, HADHB, MDH1, and LDHB connect the tricarboxylic acid cycle and fatty acid oxidation to energy conversion, whereas G6PD, ME3, and SHPK contribute to NAD(P)H production and biosynthetic capacity. Genes involved in nucleotide and carbohydrate metabolism, including PRPS1, PRPSAP2, PFKFB2, PYGL, and AMPD2, ensure integrated control of bioenergetic flux and precursor supply. Regulatory proteins such as PPARG, EPAS1, NR4A3, and BCL2L1 indicate dynamic adaptation to oxygen levels, energy stress, and apoptosis, linking mitochondrial metabolism to cellular survival pathways. Defects in several of these genes, including NDUFAF5, BCS1L, and SDHC, are associated with mitochondrial disorders, underscoring the network’s clinical relevance. Overall, this module represents a central bioenergetic machinery coupling metabolic substrate utilization, redox regulation, and mitochondrial biogenesis. The most noteworthy nodes—SIRT3, NDUFAF5, and PPARG—integrate respiration with metabolic and stress-responsive pathways.

**Cluster\_5**

Enrichment Result of Cluster\_5 (LLM Summary)

Central Carbon and Small Molecule Catabolism

**Pathway Analysis:**

This module encompasses fundamental metabolic and catabolic processes involved in the breakdown and utilization of carbohydrates, organic acids, and sulfur-containing compounds, reflecting a central role in cellular energy metabolism and biosynthetic regulation. The enrichment of pathways such as “carbohydrate catabolic process,” “organic acid catabolic process,” and “small molecule catabolic process” indicates coordinated degradation of nutrient-derived substrates into metabolic intermediates that feed into the tricarboxylic acid cycle and associated redox reactions. “Oxidoreductase activity, acting on the CH-OH group of donors, NAD or NADP as acceptor” underscores the importance of dehydrogenase-dependent redox conversions in sustaining NADH and NADPH pools, which drive both energy production and anabolic reactions.

The inclusion of “sulfur compound metabolic process” suggests integration of redox-active sulfur biochemistry, supporting antioxidant defense, amino acid metabolism, and cofactor biosynthesis. “Pyridine-containing compound metabolic process” links this network to the synthesis and recycling of NAD(P)+ cofactors, which are essential for maintaining redox balance and metabolic plasticity. The presence of “carboxylic acid binding” and “amide metabolic process” emphasizes enzymatic diversity supporting substrate specificity across a range of metabolic reactions. Collectively, these pathways represent a flexible metabolic framework that enables cells to adapt their catabolic activity to nutrient availability and energy demand.

This system is fundamental for cellular homeostasis and energy balance, with dysregulation contributing to metabolic diseases, oxidative stress, and mitochondrial dysfunction.

Representative module name: Central Carbon and Small Molecule Catabolism Network.

**Gene Analysis:**

The listed genes collectively define a versatile metabolic network coordinating carbohydrate, lipid, amino acid, and redox metabolism with organelle function and signaling. Enzymes such as ACAA2, ACAT1, HADHA, HADHB, MDH1, LDHB, and SUCLA2 link glycolysis, fatty acid β-oxidation, and the tricarboxylic acid cycle to energy production and NADH/NADPH regeneration, central to cellular bioenergetic balance. NAD(P)-dependent dehydrogenases including G6PD, NNT, ME3, and BDH1/2 support redox homeostasis and biosynthesis, while sulfur- and nitrogen-related metabolism genes such as CBS, ETHE1, SCLY, and SUOX reflect engagement of sulfur compound turnover and antioxidant mechanisms. Amino acid and nucleotide metabolism nodes including ALDH4A1, DPYD, PDK2/4, and PGM2 ensure dynamic metabolic adaptation to nutrient and oxygen availability, modulated by regulators such as PPARG, FOXK2, and SIRT3 that integrate metabolic sensing with transcriptional control and mitochondrial function. Lysosomal hydrolases (CTSZ, GALC, HEXB, LIPA, HGSNAT) and lipid-associated proteins (DGAT1, FAR2, FABP4/5, CERK) emphasize substrate catabolism and lipid trafficking, interfacing with endomembrane systems. Components such as PICALM, APP, and TREM2 suggest cross-talk between metabolism, endosomal signaling, and immune response, consistent with metabolic reprogramming in macrophages and glia. Collectively, this gene set delineates a central metabolic hub that balances energy generation, redox maintenance, and biomolecule recycling to support cellular resilience and signaling. The most noteworthy nodes, including PPARG, G6PD, SIRT3, and HADHA, highlight a network linking mitochondrial metabolism with oxidative stress control and nutrient-responsive regulation, central to homeostasis and metabolic disease adaptation.

**Cluster\_6**

Enrichment Result of Cluster\_6 (LLM Summary)

Integrated Translational and Organelle Stress Response

**Pathway Analysis:**

This module highlights fundamental processes related to translation and ribosome organization across cytoplasmic and mitochondrial compartments, integrated with cellular responses to environmental and redox stress. Core pathways such as “cytoplasmic translation,” “mitochondrial translation,” “ribosome,” and “ribosomal subunit” reflect active protein synthesis machinery responsible for generating structural, enzymatic, and regulatory proteins under basal and adaptive conditions. The enrichment of “structural constituent of ribosome” underscores the importance of ribosomal proteins as essential architectural and functional elements of translation complexes. The inclusion of “mitochondrial protein-containing complex” and “endoplasmic reticulum protein-containing complex” emphasizes compartment-specific translation and post-translational processing, linking cytoplasmic translation to organelle biogenesis and inter-organelle communication.

The presence of “4 iron, 4 sulfur cluster binding” connects protein synthesis to redox-sensitive processes, as these cofactors are critical for electron transport, metabolic enzyme function, and oxidative stress regulation. Additionally, “response to radiation” and “response to light stimulus” suggest that translational or mitochondrial adaptations may occur in response to environmental cues, reflecting protective mechanisms against cellular damage. Together, these associations indicate that translational regulation and organelle coordination are vital not only for maintaining proteostasis but also for adapting to oxidative, metabolic, and environmental challenges.

Collectively, the module represents an integrative translation-centered network that couples ribosomal biogenesis with mitochondrial and endoplasmic reticulum functions to sustain protein synthesis and stress resilience.

Representative module name: Integrated Translational and Organelle Stress Response Network.

**Gene Analysis:**

The listed genes form an extensive network coupling translational regulation and ribosome function with mitochondrial organization, proteostasis, and adaptive stress responses. Core translation factors, including EIF2D, EIF2S1, EIF3 complex subunits, EIF4A1, and multiple ribosomal proteins (RPL and RPS families), underscore the centrality of cytoplasmic and mitochondrial protein synthesis in sustaining cellular metabolism and growth. Mitochondrial translation and respiratory integrity are further supported by MRPL and MRPS subunits, LRPPRC, OXA1L, TIMM23, TOMM22, and SAMM50, which coordinate mitochondrial ribosome activity with protein import and oxidative phosphorylation. The integration of endoplasmic reticulum (EMC3, EMC6, SEC61A1, SSR4, STT3A) and quality control factors (SEL1L, NEMF) reflects post-translational modification and folding under proteostatic load. Oxidative and genotoxic stress response genes such as CAT, APEX1, ERCC1/2, and GADD45A highlight mechanisms that protect against reactive oxygen species and DNA damage during high translational demand. The presence of DNA repair (MSH6, POLD1, PRKDC) and ubiquitin-regulatory components (BRCC3, USP47) further links protein synthesis to genome stability maintenance. RBM3 and TP53INP1 reflect translational reprogramming under stress, enabling selective mRNA translation and cell survival. Additionally, NFU1, NUBP1, and NUBP2 connect translation to 4Fe–4S cluster assembly, crucial for electron transport and DNA repair. Notably, regulators such as YY1 and DNMT3A tie ribosomal biogenesis and mitochondrial activity to epigenetic and transcriptional control. Collectively, this gene set defines a coordinated translational–mitochondrial axis that maintains proteome integrity and energetics while supporting cellular adaptation to oxidative, DNA, and metabolic stress.

**Cluster\_7**

Enrichment Result of Cluster\_7 (LLM Summary)

Lysosomal and Pigment Organelle Organization

**Pathway Analysis:**

This module reflects processes related to endolysosomal and pigment vesicle organization, highlighting specialized membrane-bound compartments involved in degradation, trafficking, and cellular pigmentation. The enrichment of “lysosomal membrane,” “vacuolar membrane,” and “lytic vacuole membrane” underscores a network of organelles that mediate intracellular digestion, recycling of macromolecules, and maintenance of metabolic and redox balance. Lysosomal membranes serve as crucial interfaces for transporters, ion channels, and signaling complexes that regulate autophagy, metabolite exchange, and cell survival under stress conditions.

The inclusion of “melanosome” and “pigment granule” extends the functional landscape of this module to encompass pigment synthesis, storage, and distribution—processes that mirror lysosomal lineage specialization. Melanosomes, as derivatives of the endolysosomal system, share trafficking machinery and functional overlap with lysosomal compartments but are tailored for the production and transfer of melanin, contributing to pigmentation, photoprotection, and immune responses. “Golgi apparatus subcompartment” links this module to upstream trafficking pathways, reflecting the biosynthetic and sorting routes that supply lysosomes and pigment granules with essential enzymes and structural components.

Together, these pathways describe a coordinated endomembrane network that integrates degradative, synthetic, and secretory functions. Dysregulation of this system is implicated in lysosomal storage disorders, pigmentation defects, and neurodegenerative diseases due to impaired vesicular transport or degradation.

Representative module name: Lysosomal and Pigment Organelle Membrane Organization Network.

**Gene Analysis:**

The listed genes form a coherent lysosomal and endomembrane trafficking network that integrates degradation, recycling, and pigment granule organization, supporting cellular homeostasis and specialized secretory functions. Core lysosomal and vacuolar components such as ATP6AP2, CTSA, CTNS, HGSNAT, and TMEM175 sustain acidification, enzymatic activity, and metabolite exchange required for macromolecule turnover and nutrient recycling. Vesicular trafficking genes including RAB5B, RAB11A, RAB38, GGA2, AP1B1, AP2A2, and STX10 coordinate endocytosis, vesicle sorting, and delivery of cargo between Golgi, lysosomes, and pigment organelles. Structural and regulatory proteins such as CHMP1A, CHMP2A, and VPS53 denote involvement of the ESCRT and TRAPPIII complexes, crucial for vesicle scission, autophagic flux, and membrane remodeling. Pigment-related genes like MFSD12, MREG, and RAB38 highlight specialization toward melanosome formation and melanin transport, representing lysosomal lineage organelles adapted for pigmentation and photoprotection. The presence of autophagy-related factors (ATG4B, GABARAP, GABARAPL1, SPNS1) and signaling modulators (RRAGD, RHEB, STK11IP) connects lysosomal catabolism to the mTOR nutrient-sensing pathway, linking organelle function with energy and stress responses. Additionally, membrane enzymes such as LPCAT1, LYPLA2, and SULF2 regulate lipid remodeling and glycosaminoglycan processing, essential for vesicular dynamics and receptor recycling. Dysregulation of these systems contributes to lysosomal storage disorders, pigmentary abnormalities, and neurodegenerative diseases. Collectively, this gene set defines an endolysosomal signaling and transport framework integrating autophagy, cargo recycling, and pigment granule biogenesis to maintain cellular metabolic and structural balance. The most noteworthy elements include RAB38, ATP6AP2, and MFSD12, which exemplify the convergence of lysosomal function and pigment vesicle specialization.

**Cluster\_8**

Enrichment Result of Cluster\_8 (LLM Summary)

Lymphocyte Activation and Differentiation in Adaptive Immunity

**Pathway Analysis:**

This module is centered on the activation and differentiation of immune cells, particularly lymphocytes, reflecting the central processes that drive adaptive immune responses. The enrichment of “cell activation involved in immune response,” “leukocyte activation involved in immune response,” and “positive regulation of leukocyte activation” underscores pathways that regulate the transition of resting immune cells into active effector states following antigen recognition or cytokine stimulation. These processes involve signaling cascades mediated by antigen receptors, co-stimulatory molecules, and transcriptional regulators that coordinate cytokine production, proliferation, and effector function.

The inclusion of “lymphocyte differentiation,” “B cell activation,” and “B cell differentiation” indicates engagement of adaptive immune mechanisms underlying antibody production and immunologic memory. B cell activation drives clonal expansion and plasma cell differentiation, essential for humoral immunity, while coordinated leukocyte activation promotes T and B cell cross-talk, antigen presentation, and cytokine networking. “Positive regulation of cell activation” integrates these events into a broader context of immune amplification, ensuring a robust response to pathogens while maintaining fine-tuned control to prevent excessive inflammation.

Together, these pathways delineate a dynamic immune activation network linking cellular differentiation to effector function, central to host defense and immunological adaptation. Dysregulation in such pathways can lead to immune deficiency, autoimmunity, or chronic inflammation.

Representative module name: Lymphocyte Activation and Differentiation Network for Adaptive Immunity.

**Gene Analysis:**

The listed genes constitute a functional network underlying immune cell activation, differentiation, and adaptive immune regulation, integrating signal transduction, transcriptional control, and vesicular trafficking. Central immune regulators such as CD79A, CD86, and FCGR2B mark B cell receptor and co-stimulatory signaling pathways essential for antigen recognition and humoral activation, while ITGA4, ITGAM, and SPN contribute to adhesion and migration processes that coordinate immune cell communication within tissues. Transcriptional controllers including SPI1, IKZF1, NFATC3, TCF3, MAFB, and RARA orchestrate lineage specification and effector differentiation in lymphoid and myeloid lineages, linking extracellular signaling to the development of adaptive immune capacity. PI3K-Akt pathway mediators (PIK3R1, PIK3R6, PDPK1) and PLCG2 translate receptor-derived cues into downstream cytokine and proliferation responses, while PRKDC and DCLRE1C underscore genomic stability roles vital for V(D)J recombination and antigen receptor diversity. Components of vesicular trafficking such as VAMP7, APBB1IP, and TBC1D10C regulate receptor recycling and immunological synapse function, maintaining efficient immune signaling. The presence of TREM2, CD300A, and TNFSF13 reflects immunomodulatory and checkpoint functions balancing activation with tolerance. Additionally, transcriptional and epigenetic regulators such as YY1, SMARCC1, and KAT2A highlight chromatin remodeling as a mechanism supporting sustained immune gene expression during activation. The inclusion of stress and chaperone proteins like HSPD1 and HSPH1 emphasizes cellular resilience under inflammatory conditions. Collectively, this gene set delineates a transcriptionally and spatially coordinated program that drives lymphocyte activation, effector differentiation, and immune memory formation. Among key nodes, SPI1, PI3K signaling components, and co-stimulatory receptors emerge as principal integrators of adaptive immune response regulation.

**Cluster\_9**

Enrichment Result of Cluster\_9 (LLM Summary)

Lipid-Associated Trafficking and Regulatory Signaling

**Pathway Analysis:**

This module integrates processes related to lipid binding, intracellular trafficking, xenobiotic response, and post-transcriptional regulation, highlighting mechanisms that coordinate membrane dynamics with gene regulatory control. The enrichment of “phospholipid binding” and “phosphatidylinositol binding” indicates the involvement of lipid-binding and signaling proteins that interact with phosphoinositides, a key class of membrane lipids regulating vesicular transport, cytoskeletal organization, and signal transduction. The presence of “early endosome” and “protein targeting” further underscores the importance of vesicle-mediated trafficking and sorting, essential for receptor recycling, protein localization, and cellular detoxification.

“Response to xenobiotic stimulus” connects these processes to metabolic adaptation and defense against foreign compounds, emphasizing the interplay between endosomal transport and detoxification machinery. The enrichment of “transferase complex, transferring phosphorus-containing groups” implicates kinases and related enzymes that phosphorylate lipids and proteins, linking phosphoinositide signaling to intracellular communication and stress responses. Interestingly, “negative regulation of miRNA transcription” and “negative regulation of miRNA metabolic process” suggest coupling between membrane-associated signaling and transcriptional or post-transcriptional control, potentially through feedback mechanisms involving lipid-sensitive signaling cascades that modulate miRNA pathways.

Collectively, this module represents an integrated system in which lipid signaling and endosomal trafficking converge with gene regulatory networks to orchestrate cellular responses to environmental and metabolic challenges.

Representative module name: Lipid-Associated Trafficking and Regulatory Signaling Network.

**Gene Analysis:**

The listed genes form a diverse regulatory network linking lipid signaling, endosomal trafficking, and stress-responsive transcriptional control, collectively maintaining membrane dynamics, redox balance, and metabolic adaptation. Key endomembrane components such as AP1B1, AP3M2, CHMP1A, CHMP2A, SNX family members, and RAB5B/C regulate vesicle formation, sorting, and receptor trafficking between the plasma membrane, Golgi, and endosomes. Lipid-modifying enzymes including AGPAT2, DAGLA, PLA2G15, LIPA, and OSBPL family members govern phospholipid metabolism and phosphoinositide-mediated signaling, central to membrane curvature, cargo specificity, and cellular homeostasis. Regulatory adaptors (OCRL, PICALM, FCHO2, VPS53, BBS5) contribute to clathrin-dependent endocytosis and lysosomal function, linking trafficking to degradation pathways. Kinases and phosphatases such as PIK3R1/5/6, PRKACA, PRKAR1B, MAPKAP1, and PPM1F highlight active signal relay between lipid microdomains and downstream metabolic circuits. Antioxidant and detoxification genes including CAT, GSTM2, and TXNRD2 connect redox state regulation to stress tolerance, whereas transcriptional and epigenetic modulators such as YY1, NCOR2, DNMT3A, REST, and CREB1 link lipid signaling to gene expression and chromatin dynamics. The presence of autophagy-related genes (ATG13, GABARAPL1, GABARAP) and mitochondrial-associated components (HADHA, MIEF2, SAMM50, TIMM23) underscores cross-communication between trafficking, metabolism, and organelle quality control. Immune and inflammatory genes including CD300A, TREM2, NFATC3, and PYCARD connect lipid-mediated signaling to innate immune modulation and phagosome maturation. Collectively, this network integrates phosphoinositide metabolism, endosomal sorting, oxidative metabolism, and transcriptional regulation to mediate adaptive responses to metabolic and xenobiotic stress. The most functionally central components, including PIK3R1, OCRL, and YY1, bridge membrane dynamics with metabolic and gene regulatory homeostasis.

**Cluster\_10**

Enrichment Result of Cluster\_10 (LLM Summary)

Myeloid Cell Activation and Phagocytic Membrane Remodeling

**Pathway Analysis:**

This module highlights a coordinated program governing myeloid cell activation, phagocytosis, and membrane remodeling processes essential for innate immune defense and cellular homeostasis. The enrichment of “myeloid leukocyte activation” and “phagocytosis, engulfment” emphasizes the roles of macrophages, neutrophils, and dendritic cells in pathogen recognition, uptake, and clearance. These processes rely on receptor-mediated signaling and cytoskeletal rearrangements that facilitate target binding, membrane deformation, and engulfment of microbes or apoptotic cells. “Membrane invagination” reflects the mechanical and molecular events underlying the formation of phagosomes and endocytic vesicles, driven by actin remodeling and regulated by small GTPases such as Rac1 and Cdc42, which coordinate signaling from pattern recognition receptors and integrins.

The inclusion of “regulation of protein localization to membrane” underscores the spatial control of key signaling molecules and receptors required for immune activation, including the trafficking of components such as Fc receptors, toll-like receptors, and phosphoinositide kinases to specific membrane domains. This precise localization enables the integration of signaling cascades controlling cytokine production, oxidative burst, and vesicular fusion with lysosomes, processes essential for antimicrobial activity and immune regulation.

Overall, this module represents the cellular machinery coupling membrane dynamics, receptor signaling, and vesicular trafficking to myeloid cell activation and phagocytic function, processes fundamental to innate immunity, tissue remodeling, and inflammation resolution.

Representative module name: Myeloid Activation and Phagocytic Membrane Remodeling Network.

**Gene Analysis:**

The listed genes form a functional network centered on myeloid cell activation, phagocytosis, and vesicular membrane remodeling, supporting the cellular and molecular mechanisms essential for innate immune defense and tissue homeostasis. Core genes such as ITGAM, FCGR2B, and TREM2 encode key surface receptors that mediate pathogen recognition, complement binding, and phagocytic uptake, while adaptors such as ELMO1, FARP1, and RAP1A coordinate actin cytoskeletal rearrangements crucial for membrane extension and phagosome formation. Effector molecules like CTSC, GRN, and LGALS3, along with lysosomal and endosomal regulators including GABARAP, PICALM, and VAMP7, reflect the importance of vesicle trafficking and fusion in phagolysosome maturation and degradation of internalized material. Regulation of receptor sorting and endocytosis is further supported by AP2M1, FCHO2, and BIN2, highlighting clathrin-mediated processes that sustain receptor turnover and signaling. Myeloid transcriptional and signaling regulators—SPI1, CEBPA, NR4A3, and PDPK1—govern activation programs and coordinate responses to external cues such as IFNG through IFNGR1/2 and TGF-β signaling via TGFBR2. The inclusion of apoptotic and redox-regulatory genes, including BCL2L1 and TREX1, suggests tight coupling between phagocytosis, inflammatory resolution, and cell survival. Collectively, these genes represent a phagocytic signaling axis that integrates membrane remodeling, endocytic trafficking, and transcriptional feedback to enable efficient pathogen clearance, immune regulation, and tissue repair. Notably, TREM2, FCGR2B, and ITGAM serve as central nodes linking recognition, engulfment, and immunometabolic adaptation in myeloid cells.