Nuclear Myosin 1 regulates platelet activation and immune response in mice

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Abstract

Cellular differentiation involves a complex series of events associated with change in cellular shape, function and proliferative capacity. This process is mostly regulated by specific expression of multiple genes which guide the cell through the differentiation process but also ensure proper function of terminal cell types. Over the last decade, the role of cellular metabolism on maintaining pluripotency of stem cells and subsequent differentiation is getting more attention as there is a direct link between the metabolic status of cells and their differentiation potential. We have recently shown that deletion of Nuclear Myosin 1 (NM1) leads to a molecular switch from oxidative phosphorylation to glycolysis and subsequent tumorigenesis in mice. In the present study, we explored the role of NM1 during differentiation of hematopoietic progenitor stem cells to terminal blood and bone marrow stromal cells. Remarkably, we found that NM1 deletion leads to differential expression of genes associated with platelet activation, immune system response and osteoclast differentiation with glycolysis-dependent processes being upregulated while oxidative phosphorylation-dependent processes being generally suppressed in bone marrow tissue isolated from NM1 knock-out mice. The study provides novel insights into the underlying mechanisms of hematopoietic differentiation and suggests that NM1 is a potential therapeutic target for blood-related disorders.

Introduction

Nuclear Myosin 1 (NM1) belongs to the family of unconventional single-headed myosins present in both cytoplasm and cell nucleus. These proteins are involved in various processes such as regulation of the plasma membrane and actin cytoskeleton, exocytosis of vesicles, movements, repair of DNA damage foci and chromatin and transcription regulation. NM1 is the first myosin to be found in the cell nucleus and over the years several studies described its general roles and mechanism of function in Polymerase I, II, III transcription (Venit et al., 2021; Venit et al., 2020a). NM1 is one of the three known alternatively spliced isoform of the Myo1C gene exhibiting an extra 16 amino-acids stretch at its N-terminus. Although its function is not known, this unique N-terminal extension may be important to regulate actin- and ATP-binding properties providing some potential mechanical and functional advantages above other Myo1C isoforms. All Myo1C isoforms share the same nuclear localization signal and, in the cytoplasm, it has been shown that two main isoforms partially overlap in function (Dzijak et al., 2012; Venit et al., 2016). In the cell nucleus, NM1 has been shown to directly bind to the chromatin at transcription start site forming a complex with actin and Pol II, which is later on released and replaced by NM1 binding to the chromatin remodeling complex B-WHICH via direct interaction with the ATPase subunit SNF2H (Almuzzaini et al., 2015; Sarshad et al., 2013). This not only leads to chromatin remodeling around transcription start site but also to NM1 association with the histone-acetyl- and histone-methyl-transferases PCAF and Set1, respectively. They acetylate and methylate surrounding histones as a mark for active transcription and allow polymerase-mediated transcription. Recently, we reported that these mechanisms are important for numerous cellular functions, including the transcriptional response to DNA damage (Venit et al., 2020b). Mechanistically, we reported that NM1 is part of the DNA damage signaling cascade as it interacts with p53 to activate transcription of the cell cycle regulator p21 (Cdkn1A). Upon DNA damage, p53 binds to its responsive promoter upstream of the Cdkn1A gene regardless of NM1, however, its binding is not sufficient for effective transcription which happens only after NM1 binding to the Cdkn1A promoter and NM1-dependent acetylation and methylation of surrounding H3 histones. This allows for rapid p21 protein and cell cycle arrest until DNA damage is repaired, while cells missing nuclear myosin 1 accumulate DNA damage and are prone to apoptosis (Venit et al., 2020a; Venit et al., 2020b).

In a more recent study, we reported a novel role for NM1 as tumor suppressor and regulator of cellular metabolism. Results from this study show that NM1 is part of the nutrient-sensing PI3K/Akt/mTOR pathway, forming a positive feedback loop with mTOR. NM1 deletion leads to a suppression of PI3K/Akt/mTOR pathway and mitochondrial functions in energy metabolism. In addition, it directly affects expression of mitochondrial transcription factors TFAM and PGC1α. This negatively regulates

mitochondrial oxidative phosphorylation and leads to metabolic reprograming towards glycolysis, a hall mark of cancer cells. Indeed, NM1 knockout (KO) cells are able to form solid tumors in a nude mouse model even though they have suppressed the PI3K/Akt/mTOR signalling pathway suggesting that the metabolic switch towards aerobic glycolysis provides a sufficient signal for carcinogenesis (Venit et al., 2022). The metabolic switch from oxidative phosphorylation to glycolysis caused by NM1 deletion is not only affecting tumorigenic potential of the somatic cells but it may also affect pluripotent stem cells which under normal conditions keep the glycolytic type of metabolism and only change to oxidative phosphorylation during differentiation. To test whether NM1 has an effect on pluripotent stem cells and their differentiation potential, here we performed transcriptomic analysis of bone marrow tissue.

In general, the bone marrow contains hematopoietic lineages with progenitor hematopoietic stem cells (HSCs) giving rise to red blood cells, white blood cells and platelets. All other cell types are components of the bone marrow stroma and are involved in the formation of specific microenvironments regulating HSCs. The cell types present in bone marrow stroma include mesenchymal stem cells, fibroblasts, adipocytes, osteoblasts, osteoclasts and endothelial cells (Yu and Scadden, 2016). From the metabolic perspective, hematopoietic stem cells use anaerobic glycolysis as an only energy source and switch to oxidative phosphorylation only during differentiation (Morganti et al., 2022). Energy metabolism of fully differentiated hemopoietic cells depends on their function. Erythrocytes lose their nucleus and mitochondria during differentiation and therefore are fully dependent on glycolysis (Zhang et al., 2011), monocytes use oxidative phosphorylation as primary energy source. However, they switch to glycolysis upon activation to pro-inflammatory M1 macrophages and back to oxidative phosphorylation upon switching to anti-inflammatory M2 macrophages (O'Neill and Hardie, 2013; Shapiro et al., 2011). Similarly to monocytes, lymphocytes use oxidative phosphorylation in their quiescent state and switch to glycolytic metabolism only upon activation (Macintyre and Rathmell, 2013). In contrast, neutrophils use glycolysis and their limited mitochondria serve only for apoptotic signaling (Jeon et al., 2020). Finally, platelets or thrombocytes, which are produced by cytoplasmic fragmentation of megakaryocytes and are essential for blood coagulation on the site of injury use both oxidative phosphorylation and glycolysis to produce energy but their activation is dependent on aerobic glycolysis (Aibibula et al., 2018; Kulkarni et al., 2019). The bone marrow stromal cells follow similar metabolic patterns as hematopoietic lineages and mesenchymal stem cells use glycolysis as a primary source of metabolism (Palomaki et al., 2013). Differentiation of these cells to osteoblasts however is not associated with metabolic switch to oxidative phosphorylation and osteoblasts use glycolysis to produce ATP (Lee et al., 2017). In contrast, osteoclasts,

which have hematopoietic origin in monocytes use oxidative phosphorylation during differentiation and maturation and switch to glycolytic metabolism only after activation (Ahn et al., 2016; Lemma et al., 2016).

Here, we show evidence that in the bone marrow NM1 deletion leads to differential expression of genes associated with platelet activation and blood coagulation, immune system response and osteoclast differentiation. In the same tissue, we show that NM1 deletion also leads to upregulation of platelet activation gene programs which are known to be dependent on glycolysis. In contrast, lymphocyte and osteoclast differentiation gene programs which are dependent on oxidative phosphorylation are suppressed. We, therefore, propose that NM1 has a potential key role in hematopoietic progenitor stem cells differentiation to terminal blood and bone marrow stromal cells.

Material and Methods

Isolation of bone marrow from mice and isolation of RNA.

Wild type (WT) and NM1 KO C57/Bl6 mice (8 months old; 25-35 g in weight) were used for the analysis (Venit et al., 2013). The mice were housed in a pathogen-free sterile ventilation system supplied with sterile woodchip and *ad libitum* feed and water supply. Mice were kept in a room maintained at a temperature of 21-24 °C on a schedule of 12 h light/dark cycle. All animal experiments were performed after approval by the NYUAD-IACUC (Protocol 20-0004). Bone marrow was isolated according to a previously published protocol(Amend et al., 2016). In short, femurs and tibiae were taken from euthanized mice and cleaned from surrounding muscles, connective tissues and epiphysis parts of each long bone. All bones from single mice were placed in the 0.5ml tube with the hole on the bottom nested in the 1.5ml collection tube. Bones were spun down at 14000 x g in a microcentrifuge for 15 seconds to pellet the bone marrow at the bottom of the bigger tube. For each bone marrow sample, femurs and tibiae from single NM1 WT or KO mice were used. RNA was isolated by RNAzol according to the manufacturer's protocol (Merc) and final RNA concentration measured by Qubit RNA BR Assay Kit.

RNA-Seq library preparation, sequencing, and analysis

Three replicates were prepared for both WT and KO genotypes. 500ng of total RNA were used for RNA-Seq library preparation with the NEBNext Ultra II RNA Library Prep Illumina Kit (NEB) and sequenced with the NextSeq 500/550 sequencing platform (performed at the NYUAD Sequencing Center within the NYUAD Core Technology Platform). Subsequent analysis, including quality trimming, was executed using the BioSAILs workflow execution system. Trimmomatic (version 0.36) was used for quality trimming of the raw reads to get rid of low-quality bases, systematic base-calling errors and sequencing adapter contamination(Bolger et al., 2014). The quality of the sequenced reads pre/post quality trimming was assessed by FastQC and only the reads that passed quality trimming in pairs were selected for downstream analysis (https://www.bioinformatics.babraham.ac.uk/projects/fastqc/). The quality-trimmed RNA-Seq reads were aligned to the Mus musculus GRCm38 (mm10) genome using HISAT2 (version 2.0.4)(Kim et al., 2015). The conversion and sorting of SAM alignment files for each sequenced sample to BAM format were done by using SAMtools (version 0.1.19)(Li et al., 2009). The BAM alignment files were processed using HTseq-count, using the reference annotation file to produce raw counts for each sample. The raw counts were analyzed using the NASQAR online analysis tool (http://nasqar.abudhabi.nyu.edu/), to merge, normalize, and identify differentially expressed genes (DEG) as well as for production of PCA and

MA plots. DEG with log2(FC) ≥ 0.5 and adjusted p-value of <0.05 for upregulated genes, and log2(FC) ≤ −0.5 and adjusted p-value of <0.05 for downregulated genes between the NM1 WT and KO bone marrow samples were used for gene ontology (GO) enrichment analysis using DAVID Bioinformatics (https://david.ncifcrf.gov/)(Huang da et al., 2009). Bone marrow RNA-Seq data were deposited in the Gene Expression Omnibus (GEO) repository under accession number GSE224167. Gene ontology bar charts and gene expression heatmaps were produced in GraphPad Prism 8.3.0 software. The STRING database was used for production of interaction map based on textmixing, experimental data, co-expression and database search (Szklarczyk et al., 2021).

Results

Bone marrow isolated from NM1 WT and KO mice was used for RNA sequencing and differential gene expression. Results from Principal Component Analysis (PCA) (Figure 1A) and MA plot (Figure 1B) show that there are significant differences in gene expression profiles between WT and KO conditions, with 374 significantly up-regulated and 358 significantly down-regulated genes. Since these results suggest a potential regulatory function of NM1 in the bone marrow, we next performed gene ontology analysis of all differentially expressed genes to assess the biological processes and pathways specifically affected by NM1 deletion (Figure 2B). Among the most affected ones, we found biological processes associated with extracellular matrix and cell adhesion, immune system processes, platelet activation and coagulation, osteoclast differentiation, and regulation of PI3K-AKT, Protein kinase B (PKB), MAPK-ERK or Rap1 signaling pathways. To define which of these processes are activated or suppressed upon NM1 deletion, we performed gene ontology analysis with only up-regulated (Figure 2B) or only down-regulated (Figure 2C) genes. While the majority of aforementioned processes are activated upon NM1 deletion, osteoclast differentiation and processes related to immune system and immune response are suppressed in NM1 KO mice. This suggests that depending on the process, NM1 may have an antagonistic effect on expression of specific genes. All affected pathways are interconnected and have been shown to regulate hematopoiesis stem cell maintenance and lineage development. PI3K-AKT signaling is a pro-survival and pro-proliferative pathway which together with PKB signaling regulates proliferation and renewal of HSCs, contribute to their differentiation. If deregulated, PI3K-AKT signaling dramatically affects hematopoiesis leading to various hematologic malignancies (Polak and Buitenhuis, 2012; Ringshausen et al., 2002; Silva et al., 2008). Rap1 signaling pathway regulates extracellular matrix formation and cell adhesion and it is important for differentiation of megakaryocytes and platelets via MAPK-ERK pathways (Baumgartner et al., 2018; Geest and Coffer, 2009; Stork and Dillon, 2005), which is in agreement with observed gene ontology terms found in the upregulated genes related to extracellular matrix, cell adhesion and platelet activation (Figure 2B).

Next, we focused on differentially expressed genes associated with haemopoietic differentiation, platelet activation and blood coagulation. We plotted them as Venn diagram to identify common and specific genes for each GO term (Figure 3A) and then performed STRING analysis of these genes to produce a gene interaction map based on textmixing, experimental data, co-expression and database search (Szklarczyk et al., 2021) (Figure 3B). This analysis led to the discovery of two main gene clusters, a cluster of genes directly involved in platelet activation and blood coagulation processes and one for genes involved in cell signaling cascades. The first group contains collagen genes (Col1a1, Col1a2), which are recognized by von

Willbrand factor (Vwf) and genes expressing platelet glycoproteins (GP5, GP9, Gp1ba, GP1bb) which attach platelets to collagen network directly or via Vwf. Integrins (Itgb3, Itga2b, Itgb1, Itga6) present on the platelet plasma membrane then mediate platelet aggregation via binding to extracellular proteins such as fibrinogen to form blood clots (Dwivedi and Pomin, 2020). Several other factors regulate platelets activation, including platelet factor 4 (Pf4) released during platelet aggregation to neutralize the anticoagulant effect of heparin, Multimerin 1 (Mmrn1) which stabilizes factor V in platelets, and the CD9 antigen (Cd9) that controls platelet activation and aggregation via cell adhesion and others (Serpine2, Serping1, Thbd, Tfpi, G6b, F2Rl3, F2Rl2, Pros1). The second group of genes identified in the STRING analysis is related to different cellular signaling cascades having a role during cell adhesin, growth and proliferation. This group contains genes expressing Tyrosine-protein kinases (Mapk13, Mapk3, Src, Mertk), thyrosine-protein phosphatases (Ptprj), receptors and ligands for thyrosine kinases (Kitl, Axl, Gas6), and G-protein coupled signaling proteins (Adyc6, Adyc5, Il1A, Gnas, P2Ry1, Gucy1a3, Gucy1b3). All differentially expressed genes with their expression profiles are shown as heatmaps in descending order and organized based on their association with the KEGG pathway GO term "Platelet activation" (Figure 3C), the Biological process GO term "Platelet activation" (Figure 3D), the KEGG pathway GO term "Hematopoietic cell lineage" (Figure 3D) and the Biological process GO term "Blood coagulation" (Figure 3E).

We next performed String analysis of genes related to the gene ontology term "Immune response process" and found that they cluster into three distinguished groups (Figure 4A). The first group of genes is associated with innate immune system and defense response to viruses. Among them we found the interferon regulatory factor 7 (Ifn7), a key transcriptional regulator of type I interferon-dependent immune responses against DNA and RNA viruses. Compatible with the identification of Ifn7, in the same group we found the mitochondrial antiviral signaling protein (Mavs), a central factor in signal transduction upon viral infection, required for activation of transcription factors regulating interferon beta expression (Ren et al., 2020). Ifitm1 and Iftm3, interferon-induced transmembrane antiviral proteins which inhibits the entry of the viruses to the host cell cytoplasm, also cluster in the same group, similarly to Serine incorporator 5 (Serinc5), required to restrict incorporation of viral particles into cells. We also found interferon-induced genes such as Oas2 and Oas3, coding for 2'-5' oligoadenylatesynthetases 2 and 3, which are dsRNA-activated antiviral enzymes that degrade viral RNA (Choi et al., 2015) and Samhd1 a cellular nuclease with dNTPase activity that reduces cellular dNTP levels to protect cells from retroviral reverse transcription during viral replication as well as Z-DNA-binding protein 1 (Zbp1), a cytoplasmic DNA sensor that detects DNA of viral or bacterial origin. Finally, we identified Nlprp1a and Nlprp1b, two factors

that play a crucial role in the formation of inflammasome in response to pathogens and other damage associated signals.

In the second cluster there are proteins involved in intracellular signaling cascades and immune regulatory proteins, some of which were already described in platelet activation (Src, Axl, Cd4). In addition to those, we found hematopoietic tyrosine-protein kinase Hck which regulates phagocytosis and neutrophil, monocyte, macrophage and mast cell function during innate immune response. Protein kinase C theta (Prkcq) regulates T-cell activation, proliferation, differentiation and survival. Tyrosine protein-kinase Jak3 mediates essential signaling events in both innate and adaptive immunity and plays a crucial role during T-cell development. The other immune system receptors and regulators found in this clusters are TYRO protein tyrosine kinase-binding protein (Tyrobp), Tumor necrosis factor alpha-induced protein 8-like protein 2 (Tnfaip8l2), leukocyte immunoglobulin-like receptor subfamily B member 3 (Pirb), CMRF35-like molecule 5 (Cd300ld) and leukocyte specific transcript 1 (Lst1).

The third cluster of genes derived from the STRING analysis relates to immune complement system to enhance the phagocytosis and clearance of microbes and damaged cells. It contains complement factors Cfd, C1s1, C1qc, C1rl and regulatory proteins Serping 1, which regulates activation of complement system by inhibiting C1 factor (Gorelik et al., 2017) and Ficolin-1 (Fcna), which is extracellular lectin functioning as a recognition receptor in innate immunity in the lectin complement system pathway (Matsushita et al., 2002). Genes classified under GO terms “Immune system process” are shown as a heatmap in descending order based on individual gene expression level (Figure 4B). In the same heatmap for differentially expressed genes under the GO term “Immune system process”, several genes involved in osteoblast differentiation can also be found. This is not surprising as osteoclast have hematopoietic origin in monocytes and are closely related to macrophages. While string analysis does not show any particular clustering of osteoblast differentiation-related genes (Figure 4C), gene expression heatmap suggests that majority of common genes between osteoclast and immune response GO terms are downregulated, osteoclast-specific genes Tnfsf11 and Acp5 required for osteoclast differentiation, activation and bone resorption are found among those genes that are upregulated (Figure 4D).

Although further mechanistic insights are needed, taken altogether, these results suggest that NM1 is a potentially novel regulator of hematopoietic progenitor stem cells differentiation to terminal blood and bone marrow stromal cells.

Discussion

After the cell nucleus, mitochondria are probably the second most studied cellular organelle due to their role as powerhouse producing energy via oxidative phosphorylation in healthy cells. In contrast to oxidative phosphorylation, aerobic glycolysis has long been considered a metabolic pathway typical of cancer cells or a consequence of aberrant mitochondrial function. However, discoveries over the last decade have shown that aerobic glycolysis is a full-featured pathway that does not only produce energy but also provides specific byproducts and signaling molecules or specific intracellular and extracellular conditions to many cell types. Moreover, there is a clear interplay between oxidative phosphorylation and aerobic glycolysis and the energy production pathways can change even in the same cell type based on the actual needs, cellular activity or differentiation status. The regulation of this metabolic switch is still poorly understood but it is getting gradual attention as it is associated with many metabolic disorders. Recently, we discovered that Nuclear Myosin 1 plays a critical role in this process and its mutation or deletion leads to a metabolic switch from oxidative phosphorylation to glycolysis and subsequent tumorigenesis in mice (Venit et al., 2022). Because of these observations, we hypothesized that NM1 may be a general factor regulating the switch between oxidative phosphorylation and glycolysis in multiple tissues such as the bone marrow.

The bone marrow is a very heterogenous tissue giving rise to several, mostly hematopoietic cell types with very specific functions and metabolic needs, and thus it is an interesting non-cancerous model to study whether NM1 depletion affects cell differentiation possibly by affecting the interplay between oxidative phosphorylation and glycolysis. Several studies have suggested an involvement of both actin and myosin in the regulation of the immune system (Record et al., 2021). In addition, initial phenotyping of our NM1 KO mice revealed decreased total number of red blood cells with higher corpuscular volume and hemoglobin content, and decreased bone mineral density suggesting that NM1 can play a role in the differentiation of cells in bone marrow (Venit et al., 2013). To answer this question, we performed transcriptomic analysis on the bone marrow tissue isolated from NM1 WT and KO mice. We found that genes related to platelet activation are generally upregulated, while genes associated with immune response and osteoclast differentiation are suppressed upon NM1 deletion. In contrast, gene programs involved in erythrocyte differentiation are not significantly affected. Although we do not have mechanistic insights at this stage, these results suggest that NM1 may not have a specific role in erythrocytes differentiation or there are compensatory mechanisms upon NM1 deletion. Such mechanisms are observed in the so-called “sports anemia” when trained athletes have decreased hematocrit but total mass of red blood cells and hemoglobin levels are higher (Mairbaurl, 2013). The lack of a dominant effect

on transcription profiles during erythropoiesis could also be due to the very metabolic setup of differentiated erythrocytes. Even though there is a short stage during differentiation which depends on oxidative phosphorylation, final red blood cells fully depend on glycolysis. As NM1 deletion pushes cells towards glycolytic metabolism, erythrocytes should not be affected as much since cells depend on oxidative phosphorylation. In accordance with it, osteoclasts which normally use oxidative phosphorylation as primary energy source and glycolysis only upon activation to increase local acidosis (Ahn et al., 2016), are transcriptionally suppressed in NM1 KO mice. The bone mineral density depends on the interplay between bone forming osteoblasts and bone resorbing osteoclasts. Even though we showed osteoclast differentiation to be suppressed, previous results show that NM1 KO mice have decreased bone mineral density (Venit et al., 2013). This could be due to hyperactivity of osteoclasts caused by increased glycolysis which leads to higher extracellular acidity and bone resorption as seen during osteoporosis (Ahn et al., 2016; Xue et al., 2016). As mentioned, genes related to immune system processes are also suppressed in NM1 KO mice. White blood cells differ heavily from each other in their mode of action and metabolic requirements and our transcriptomic analyses did not show any specific cell type to be affected in the absence of NM1. However, the majority of gene ontology terms are associated with immune response to virus infection and the primary white blood cells responsible for this action are lymphocytes. In comparison to neutrophils which are responsible for clearing bacterial infections and dependent on glycolysis, lymphocytes use oxidative phosphorylation to produce ATP and, therefore, are transcriptionally suppressed in NM1 KO mice. Finally, we found genes associated with platelet activation programs to be upregulated in NM1 KO bone marrow. This agrees with the literature, as glycolysis is required for successful platelet activation and NM1 push towards the aerobic glycolysis could promote this process. Under normal conditions, platelets are released to blood stream and activated only at the place of vessel wall injury, which leads to reorganization of their morphology and formation of platelet plug (Luu et al., 2018). Therefore, it would be interesting to study whether transcriptional activation of platelets already in bone marrow leads to their morphological changes and whether it affects the function of the bone marrow itself or number and functionality of platelets in the blood stream. Apart from their main role in blood coagulation, there is increasing evidence that platelets communicate with immune cells in inflammation processes, they are involved in osteoclast differentiation and cancer development and progression (Madel et al., 2019; Maitz et al., 2006; Stoiber and Assinger, 2020; Totani and Evangelista, 2010). Therefore, even if it is plausible that NM1 plays a role in the differentiation and activation of hematopoietic cell lineages via regulating their metabolic state at the transcriptional level, direct effect on the functioning of these specific cell types and their mutual

cooperation to keep body homeostasis remains to be elucidated and will be the objective of future studies.

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Author Contributions

TV and PP conceived the research and wrote the manuscript. TV performed all in vivo work in both wild type and NM1 KO mice, RNA-seq analyses and made the figures. PP supervised the research. All authors read and approved the manuscript.

Declaration of Interests

The authors declare no conflict of interests.

Figure legends

Figure 1. Deletion of NM1 leads to differential gene expression in mice bone marrow. (A) PCA plot showing the distribution of RNA-seq samples isolated from NM1 WT and KO bone marrow samples. **(B)** MA plot visualization of the differences in gene expression between experimental conditions as a function of log fold change versus the mean of normalized counts. Each dot represents single gene expression profile with only red marked genes to be significantly differentially expressed.

Figure 2. Gene ontology analysis of bone marrow samples isolated from NM1 WT and KO mice. (A) Gene ontology analysis based on all differentially expressed genes between experimental conditions shows biological process- and KEGG pathway-associated gene ontology terms plotted in descending order based on their significance. **(B)** Gene ontology analysis based on upregulated genes in NM1 KO samples. **(C)** Gene ontology analysis based on downregulated genes in NM1 KO samples.

Figure 3. Gene expression analysis of genes associated with platelet activation. (A) Venn diagram shows the intersection of differentially expressed genes associated with a. KEGG GO term "Platelet activation", b. Biological process GO term "Platelet activation", c. KEGG GO term "Haemopoietic cell lineage" and d. Biological GO term "Blood coagulation" **(B)** String analysis of all genes associated with aforementioned GO terms. **(C)** Heatmap of differentially expressed genes between NM1 WT and KO bone marrow samples associated with KEGG pathway GO term "Platelet activation". **(D)** Heatmap of differentially expressed genes between NM1 WT and KO bone marrow samples associated with Biological process GO term "Platelet activation". **(E)** Heatmap of differentially expressed genes between NM1 WT and KO bone marrow samples associated with KEGG pathway GO term "Hematopoietic cell lineage". **(F)** Heatmap of differentially expressed genes between NM1 WT and KO bone marrow samples associated with Biological process GO term "Blood coagulation".

Figure 4. Gene expression analysis of genes associated with immune system response and osteoclast differentiation. (A) Heatmap of differentially expressed genes between NM1 WT and KO bone marrow samples associated with Biological process GO term "Immune system process". **(B)** Heatmap of differentially expressed genes between NM1 WT and KO bone marrow samples associated with KEGG pathway GO term "Osteoclast differentiation".

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Figure 1

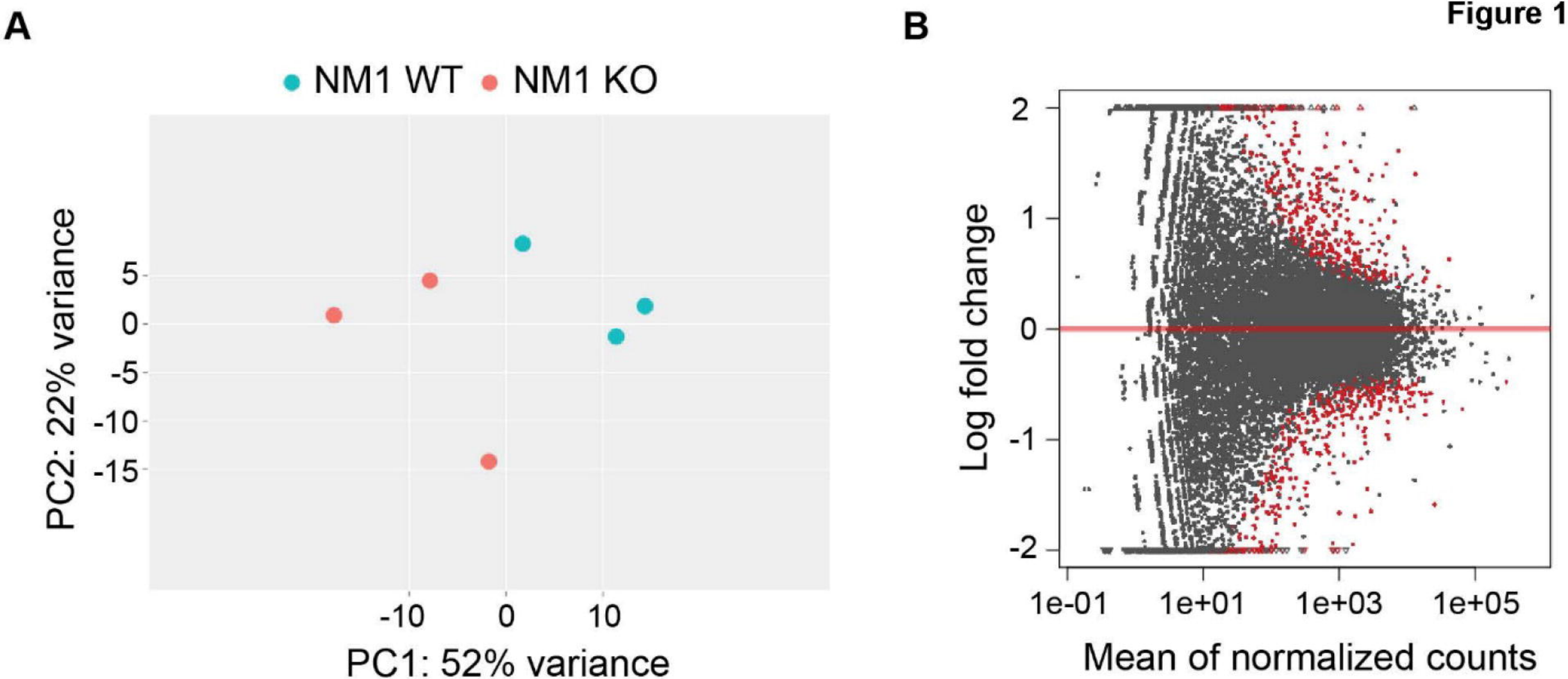

A
NM1 WT
NM1 KO
PC2: 22% variance
5
0
-5
-10
-15
-10
0
10
PC1: 52% variance
B
Log fold change
2
1
0
-1
-2
1e-01
1e+01
1e+03
1e+05
Mean of normalized counts

Figure 2

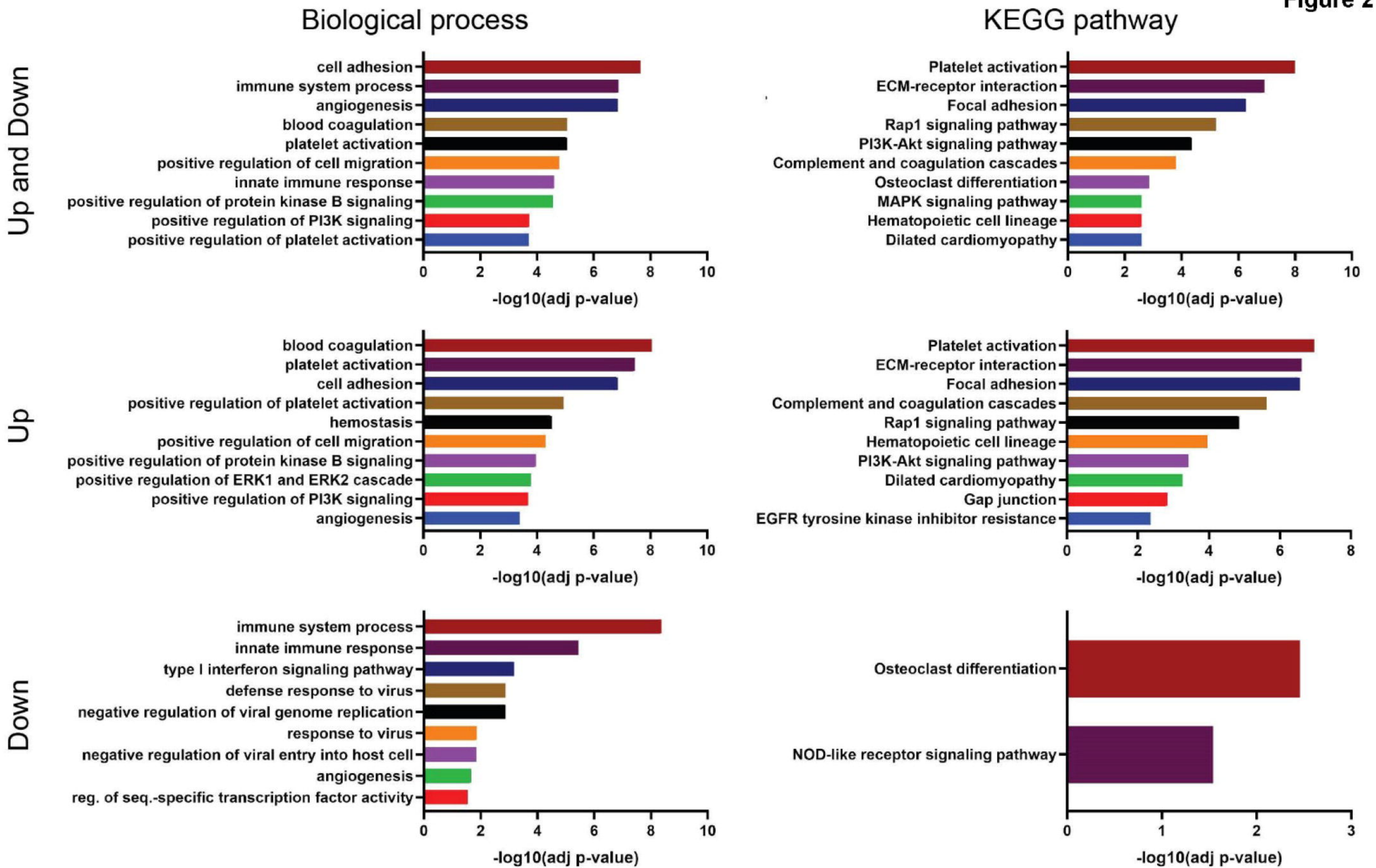
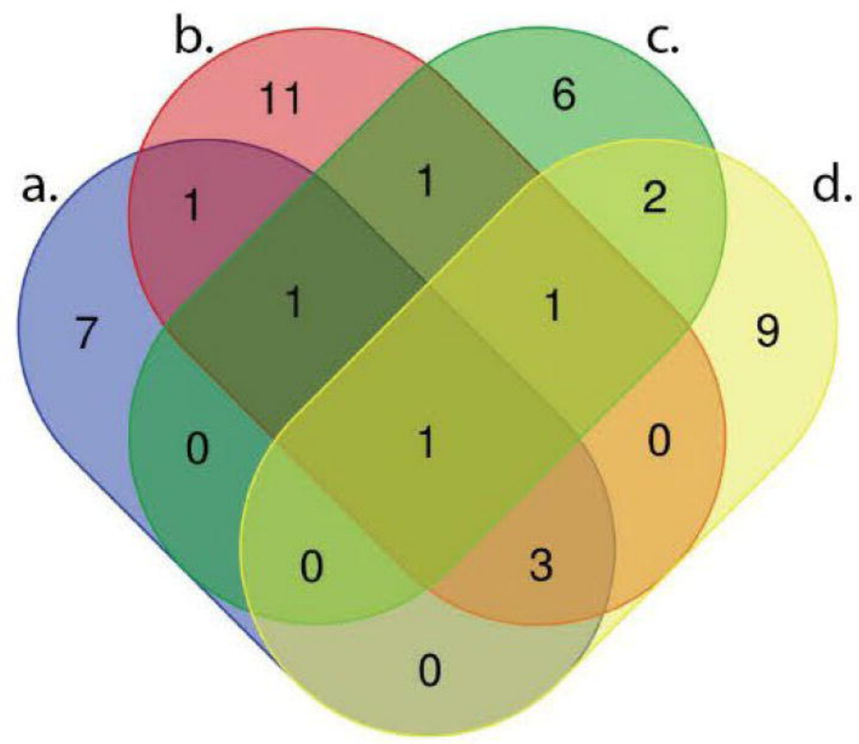
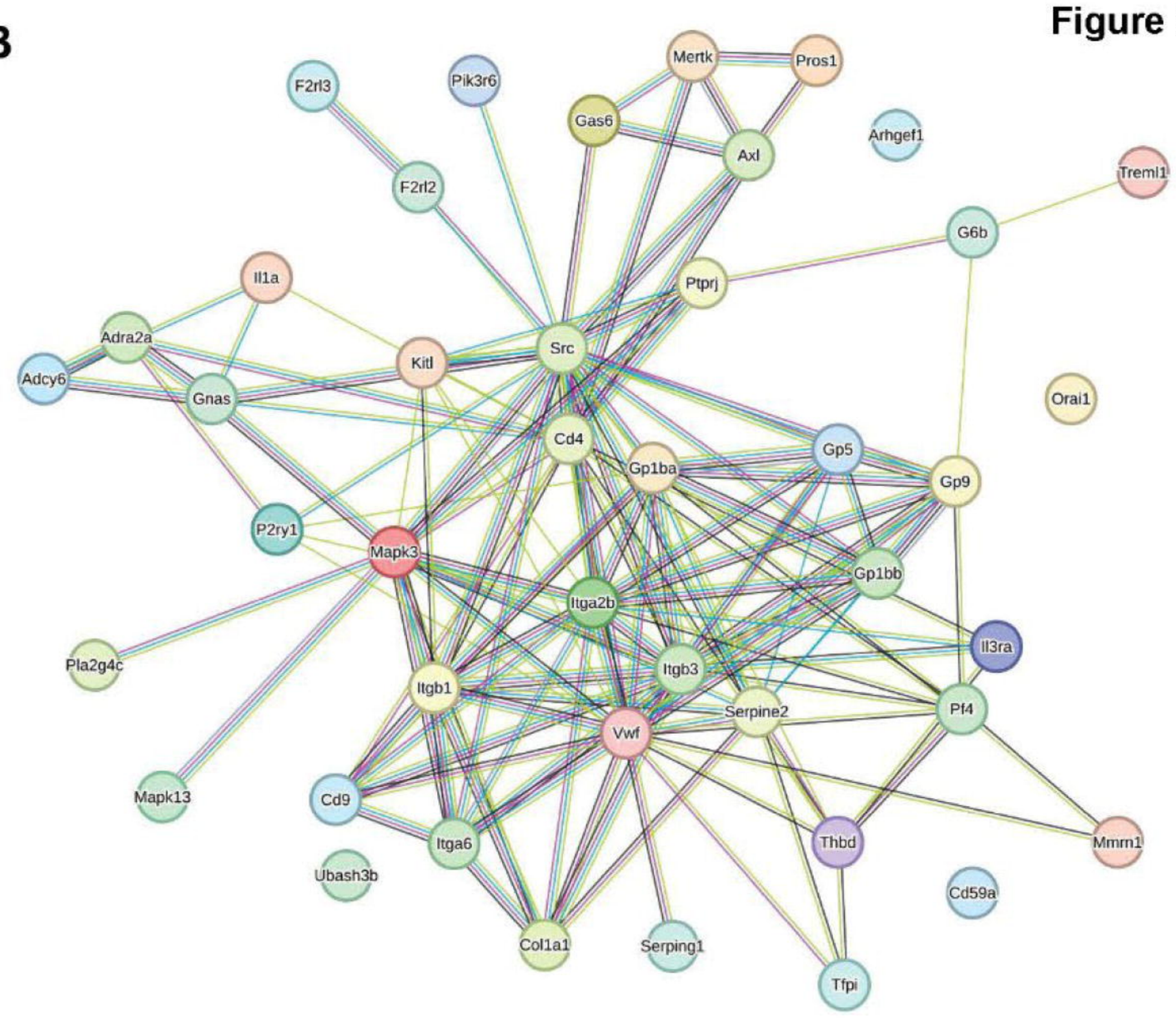

Biological process
KEGG pathway
Up and Down
cell adhesion
immune system process
angiogenesis
blood coagulation
platelet activation
positive regulation of cell migration
innate immune response
positive regulation of protein kinase B signaling
positive regulation of PI3K signaling
positive regulation of platelet activation
0 2 4 6 8 10
-log10(adj p-value)
Platelet activation
ECM-receptor interaction
Focal adhesion
Rap1 signaling pathway
PI3K-Akt signaling pathway
Complement and coagulation cascades
Osteoclast differentiation
MAPK signaling pathway
Hematopoietic cell lineage
Dilated cardiomyopathy
0 2 4 6 8 10
-log10(adj p-value)
Up
blood coagulation
platelet activation
cell adhesion
positive regulation of platelet activation
hemostasis
positive regulation of cell migration
positive regulation of protein kinase B signaling
positive regulation of ERK1 and ERK2 cascade
positive regulation of PI3K signaling
angiogenesis
0 2 4 6 8 10
-log10(adj p-value)
Platelet activation
ECM-receptor interaction
Focal adhesion
Complement and coagulation cascades
Rap1 signaling pathway
Hematopoietic cell lineage
PI3K-Akt signaling pathway
Dilated cardiomyopathy
Gap junction
EGFR tyrosine kinase inhibitor resistance
0 2 4 6 8
-log10(adj p-value)
Down
immune system process
innate immune response
type I interferon signaling pathway
defense response to virus
negative regulation of viral genome replication
response to virus
negative regulation of viral entry into host cell
angiogenesis
reg. of seq.-specific transcription factor activity
0 2 4 6 8 10
-log10(adj p-value)
Osteoclast differentiation
NOD-like receptor signaling pathway
0 1 2 3
-log10(adj p-value)

Figure 3

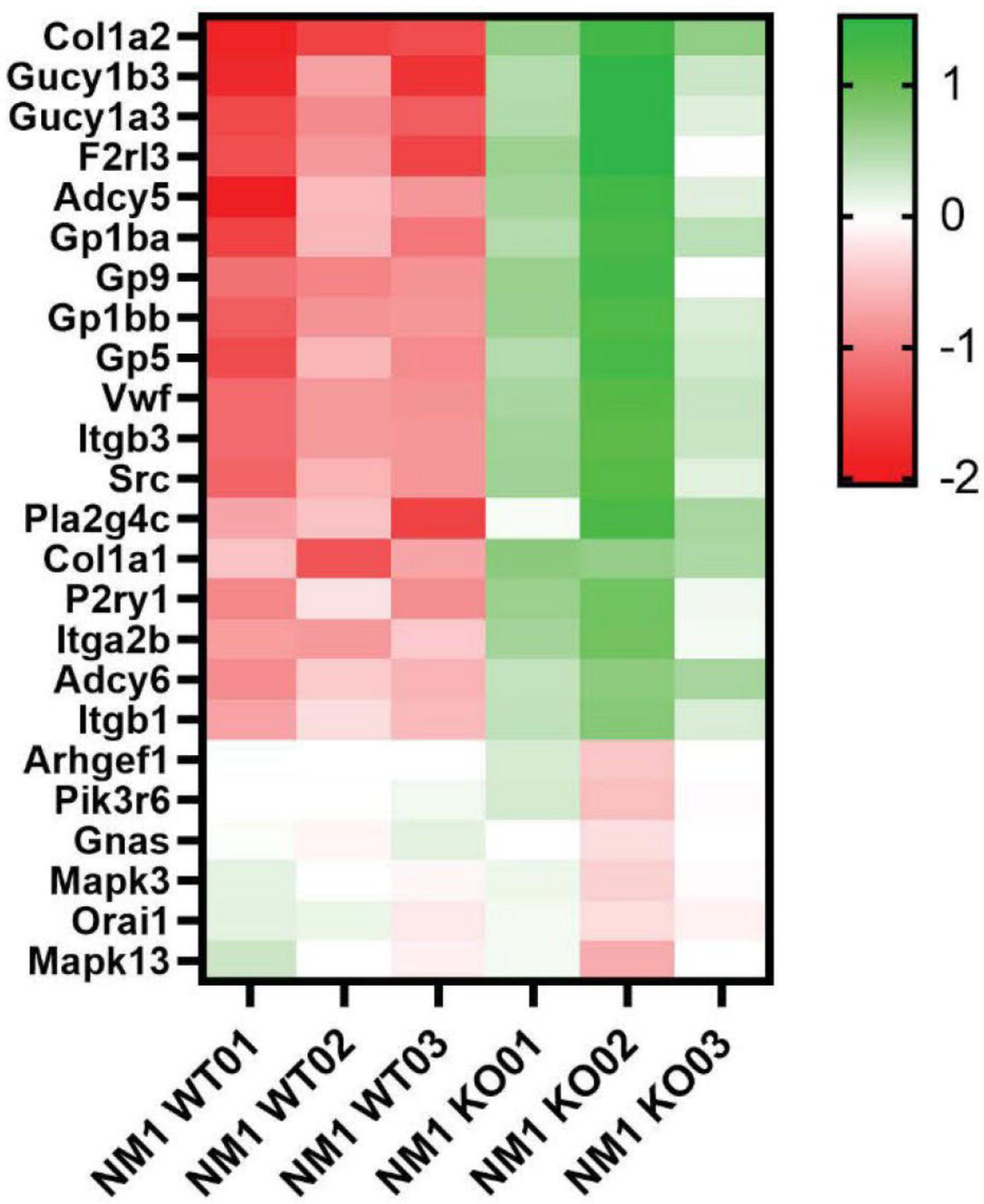
A



B



C



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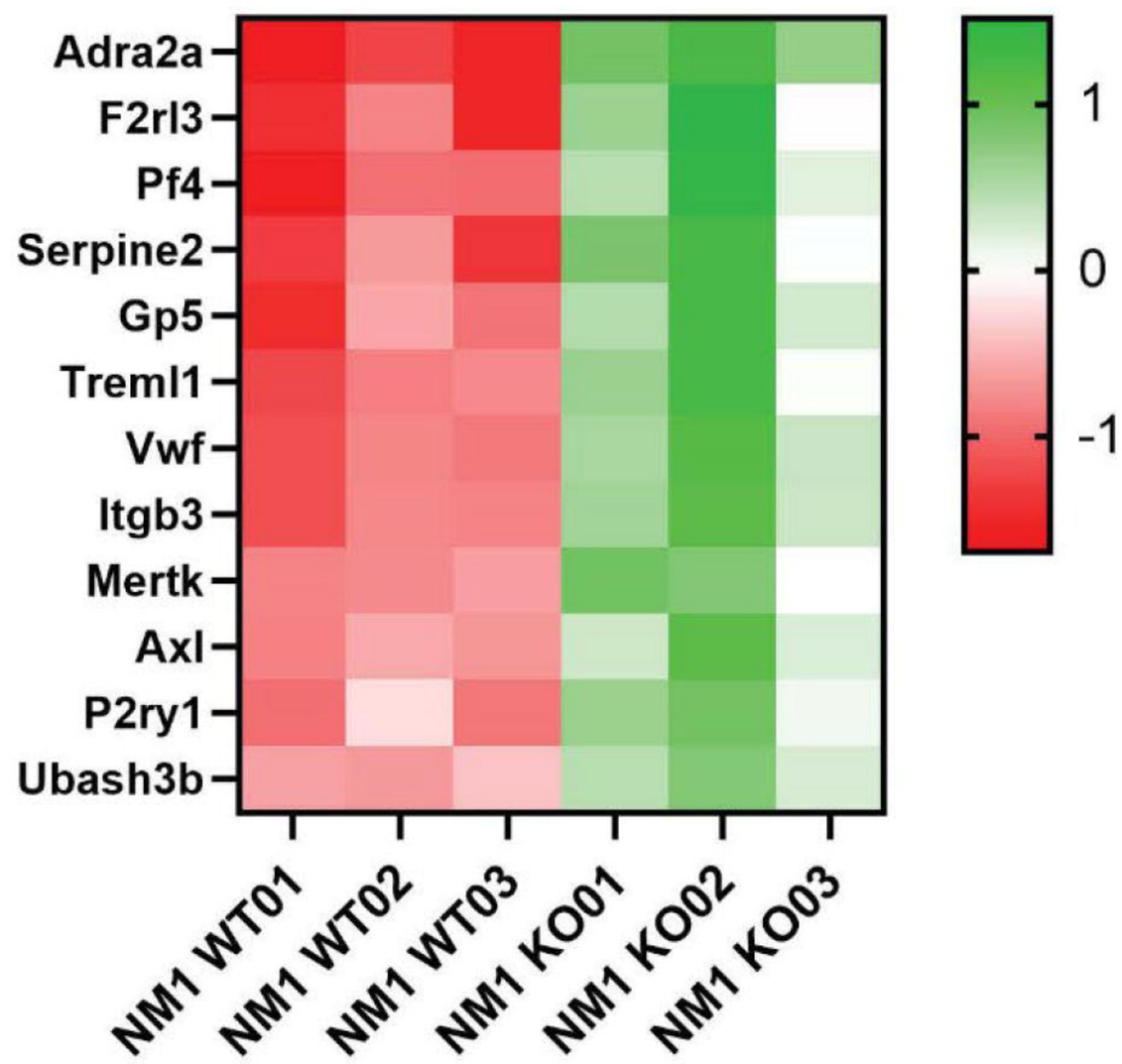

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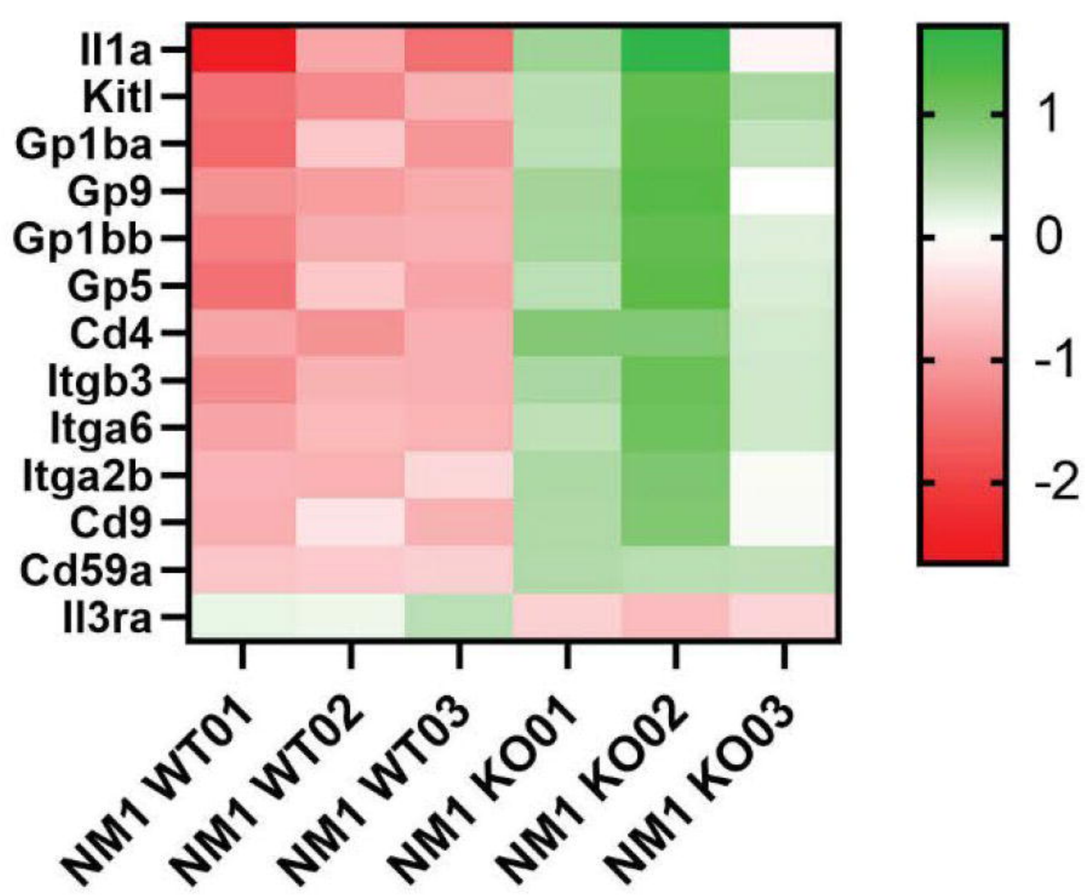

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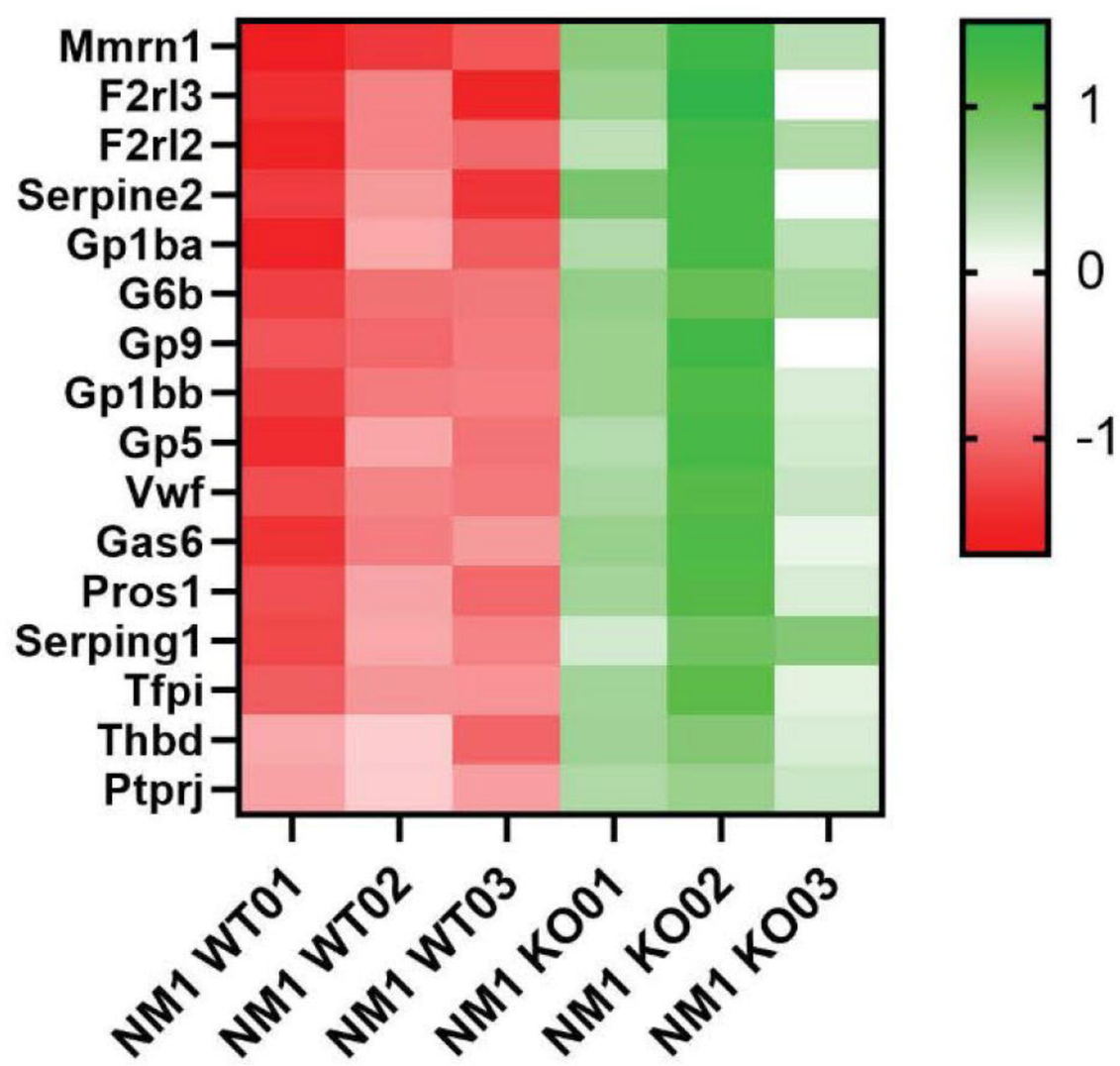
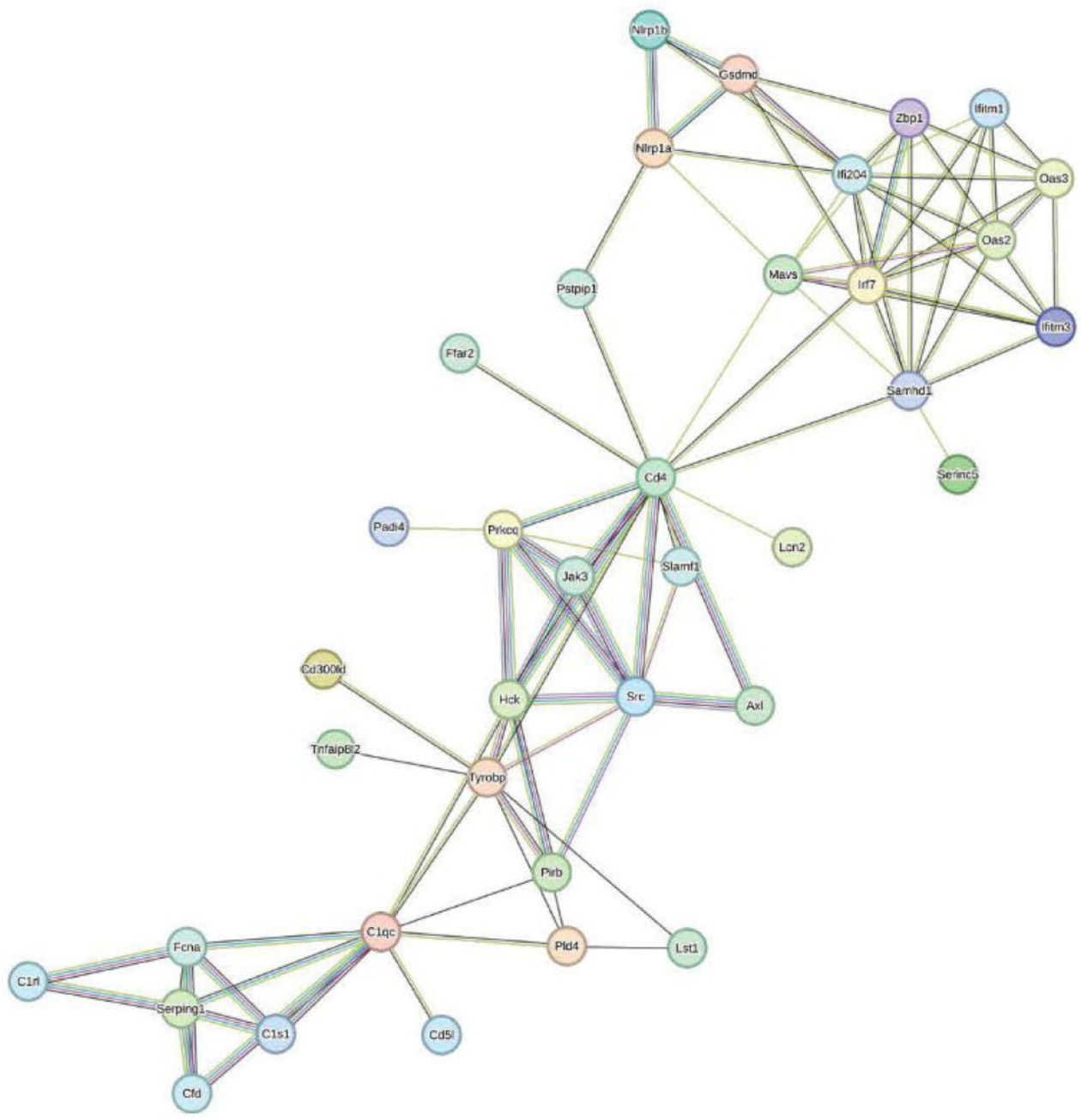


Figure 4

A



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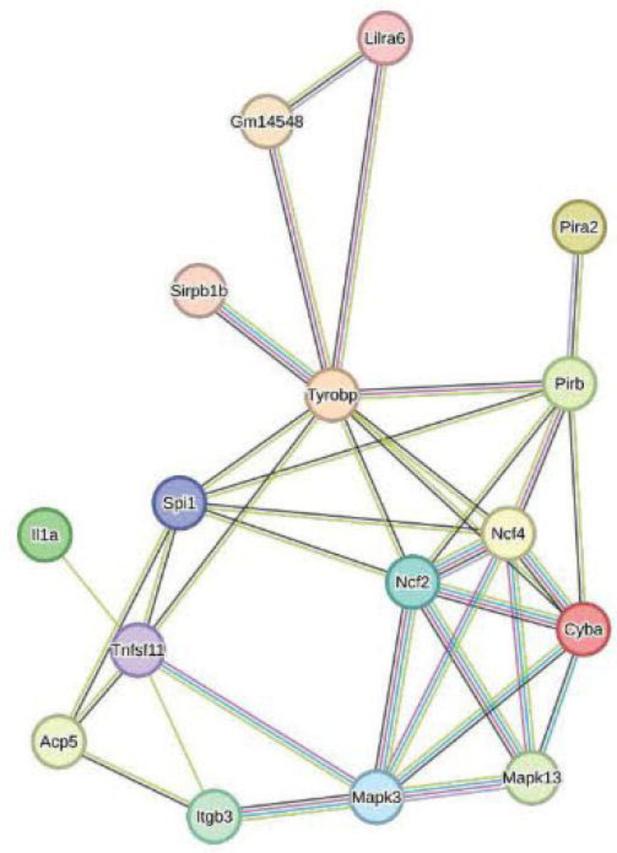

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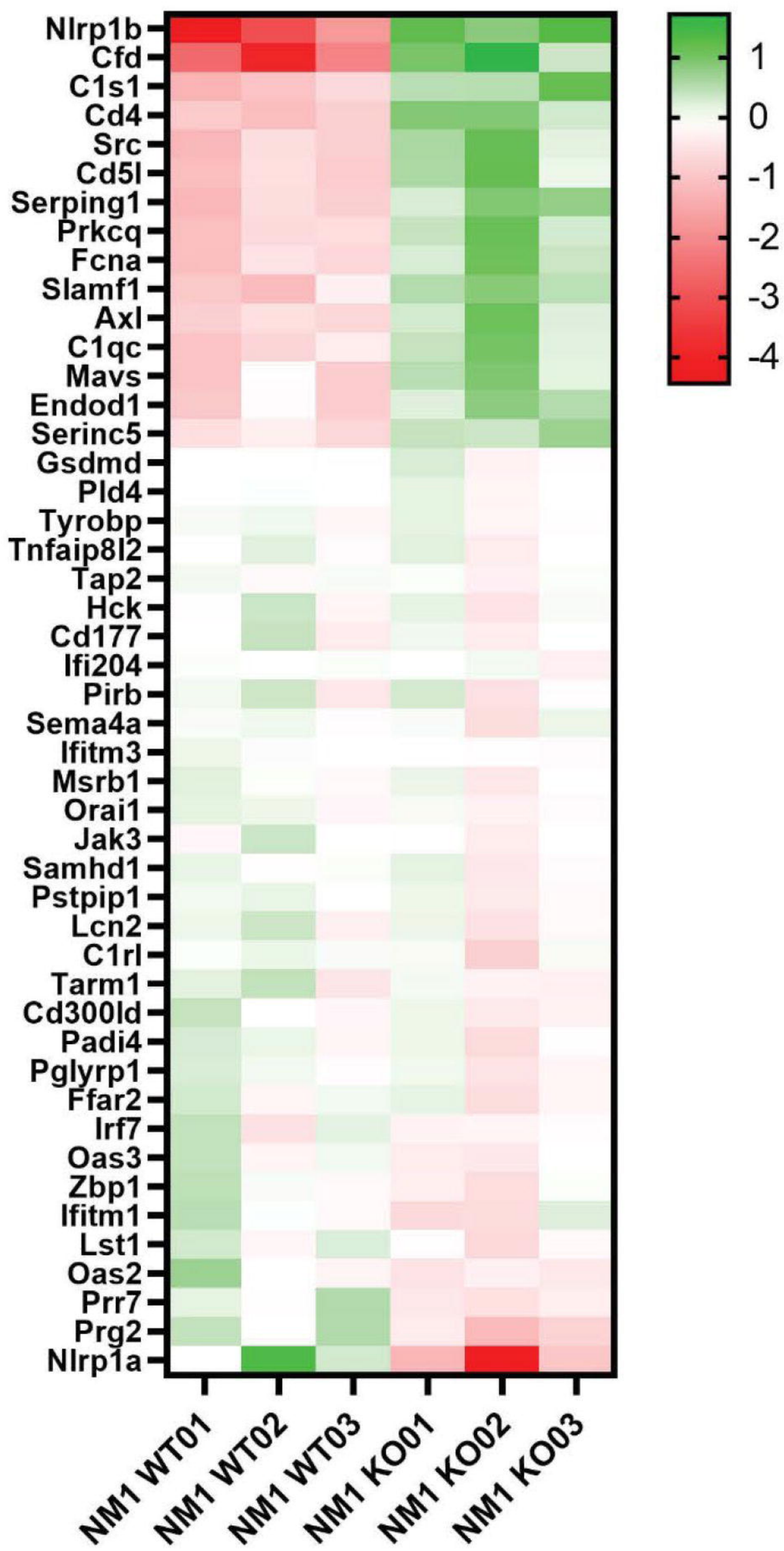
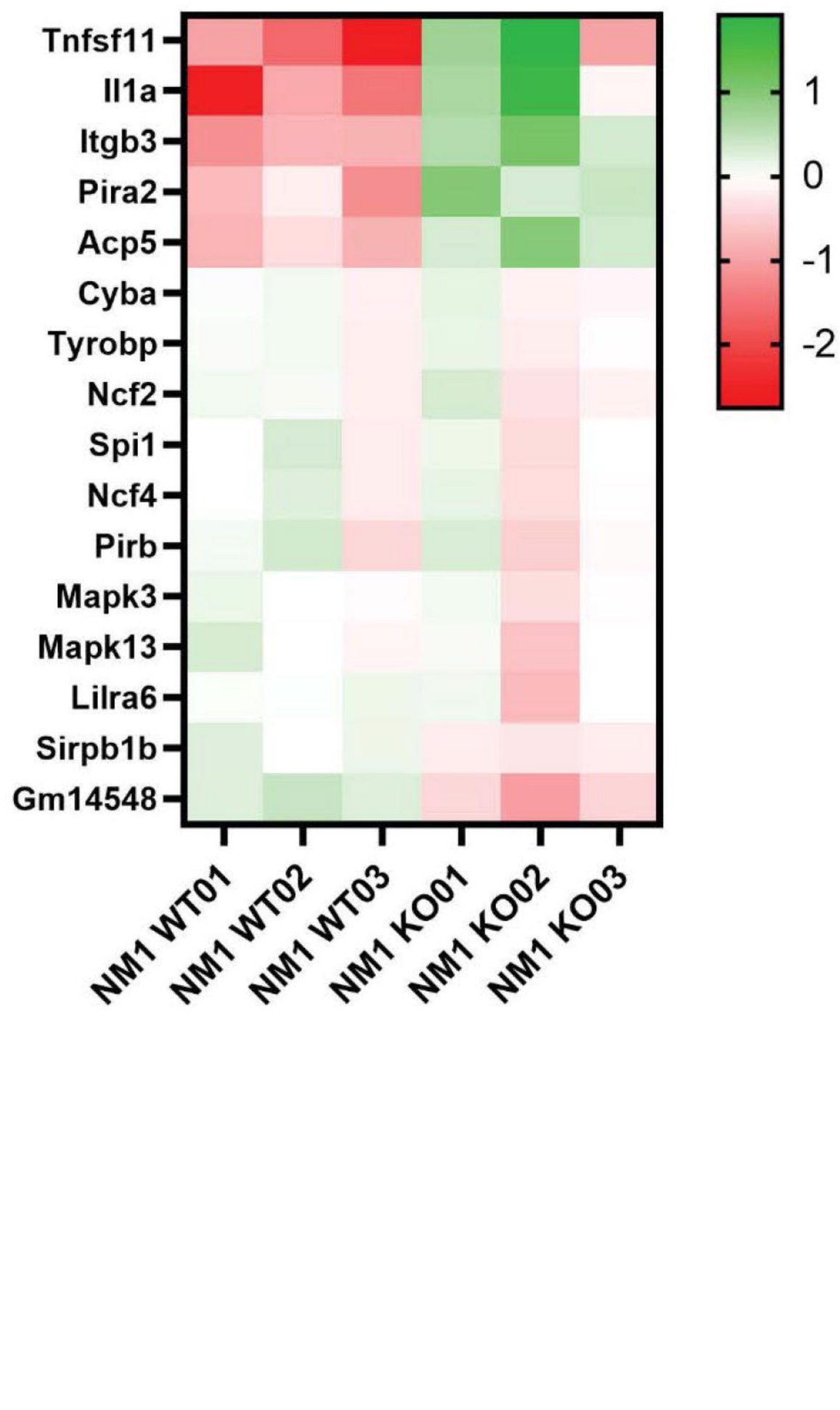
D