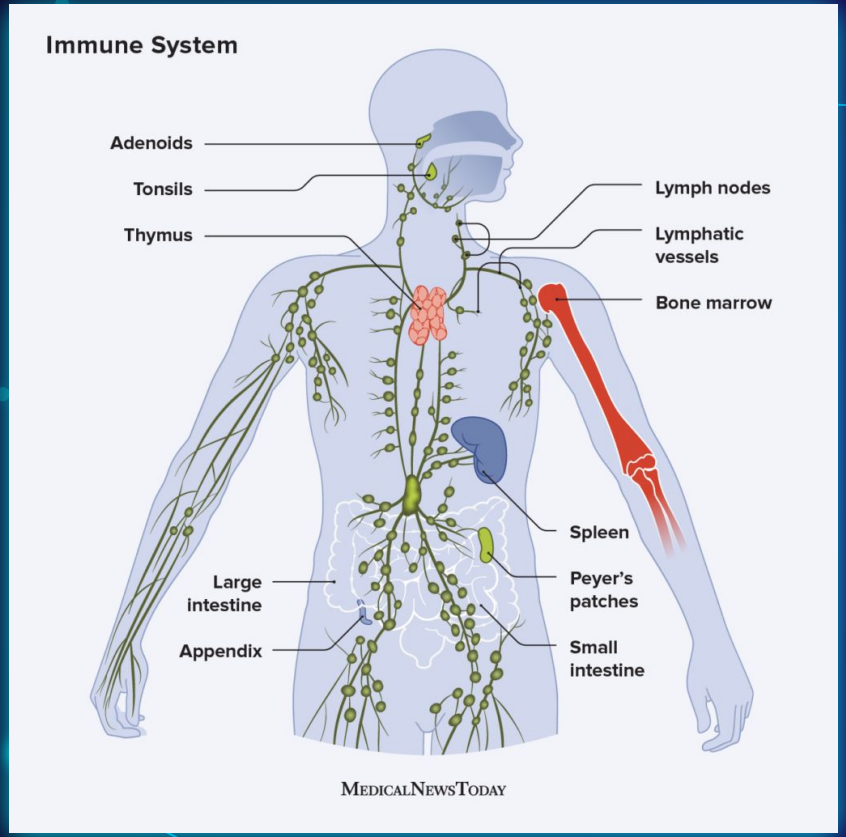


# What is NOs role in detecting diseases?

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# Study Objective and Hypothesis

NO production via NOS2 significantly shapes the IFN-γ-induced transcriptomic and metabolic reprogramming in macrophages, especially influencing NAD+ metabolism and immune signaling networks.
To dissect NO's contribution to gene expression changes and metabolic adaptation in IFN-gamma-activated macrophages and determine whether these NO-dependent changes point to therapeutic regulatory nodes.

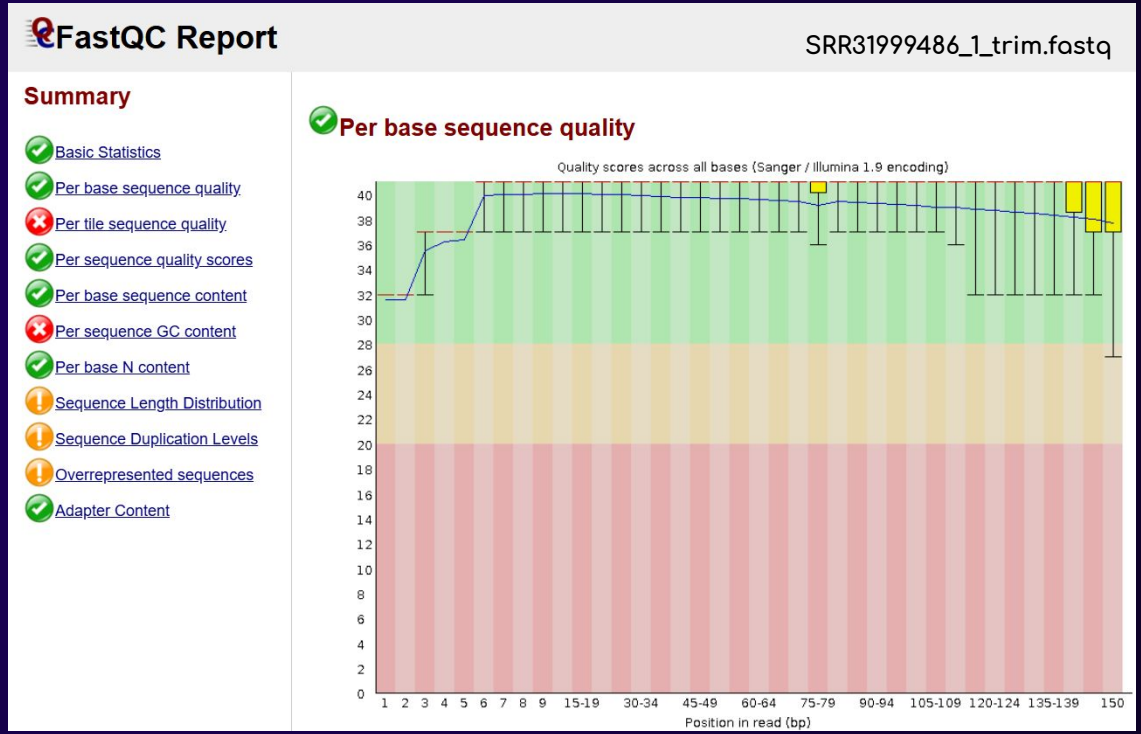
Question: How does NO influence gene expression in IFN-γ-activated macrophages?

**You can visit their article and view their findings at:**

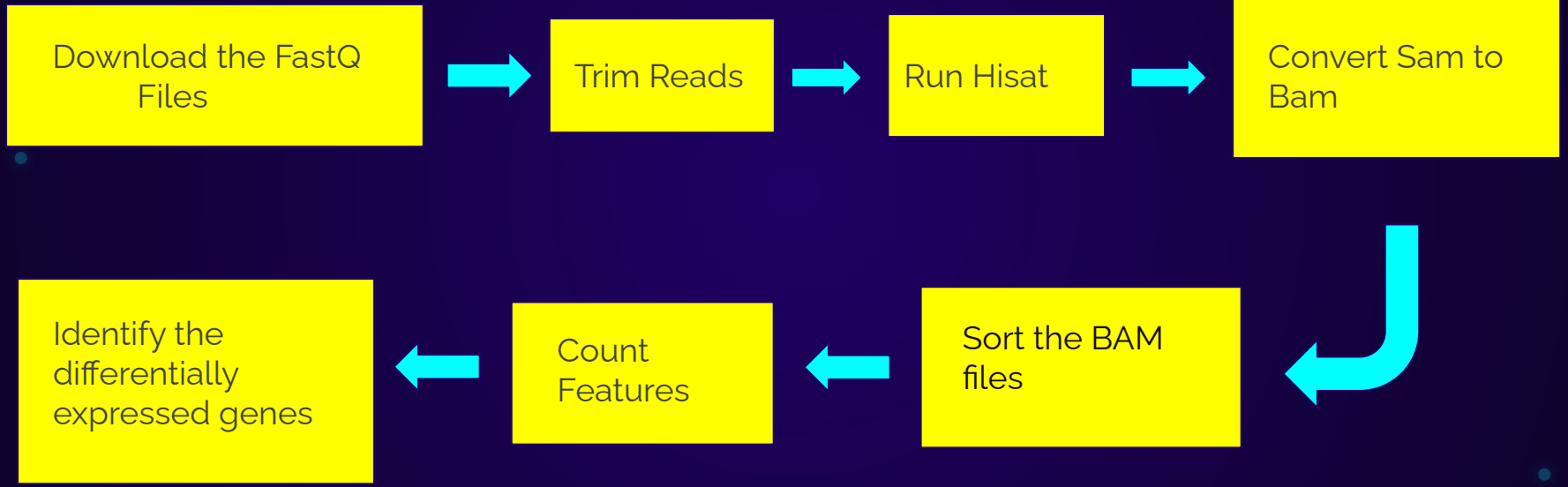
**https://www.nature.com/articles/s41467-021-21407-w#data-availability**

# Coding and Analysis Pipeline

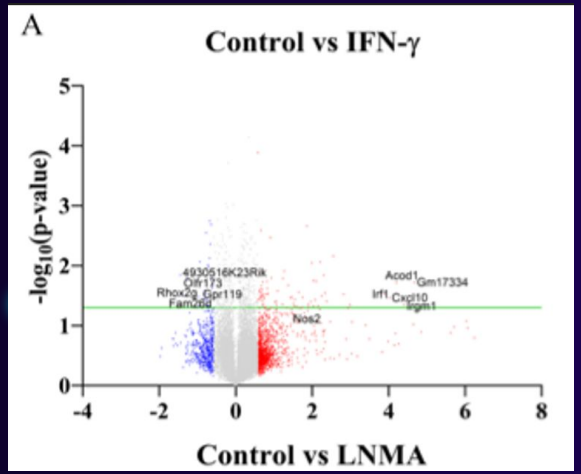
RNA-seq data were quality-checked (FastQC), trimmed (fastqc), and aligned to the mouse genome. FeatureCounts was used to quantify gene expression.

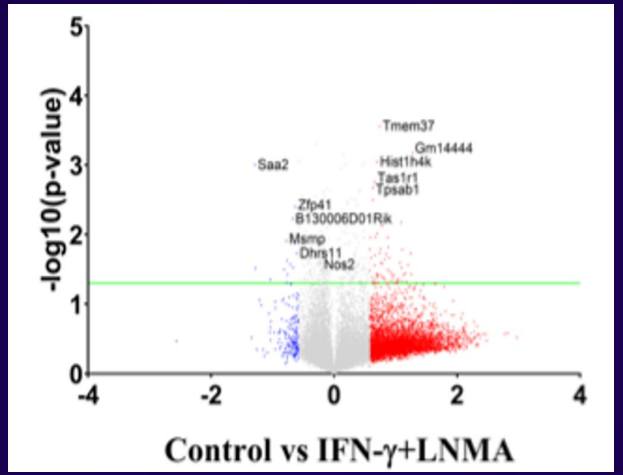


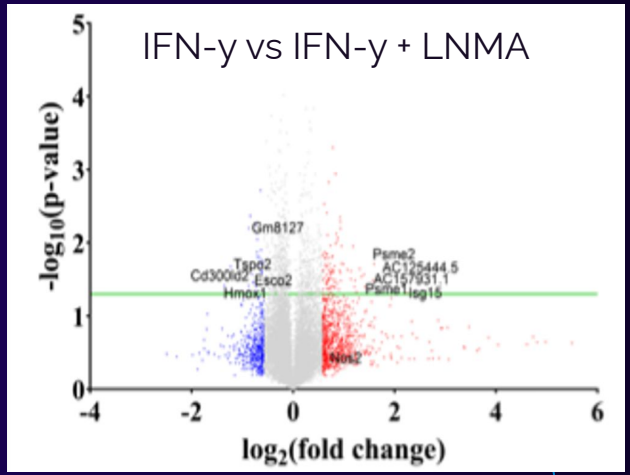
# RNA-seq Analysis Pipeline: IFN-γ and NO Transcriptome Profiling



# Differential Expression Analysis: Impact of IFN-γ and NO on Gene Regulation




**Nos2** is strongly upregulated.

Downregulated genes: **Gpr119**, **Rhox2g**, **Fam26d**.

Indicates a **broad transcriptional shift** toward an **inflammatory activation state**.

Many genes (**Tmem37**, **Gm14444**, **Hist1h4k**, **Tpsab1**) still **upregulated**.

**Nos2** remains expressed despite NOS inhibition.

Suggests part of the IFN-γ response is **NO-independent**, while some genes are **modulated via NO**.

**Nos2** is not significantly different → NO **production inhibited**, but **transcription persists**.

**HmoX1**, **Cd300ld2**, **Esco2** → **downregulated** with LNMA (NO-dependent).

**Psme2**, **Psme1** → **upregulated** with NO, possibly linked to **proteasome/immune regulation**.

# Methods

RAW 264.7 macrophages from a mouse genome was treated in three independent experiments in 4 conditions: untreated, IFN-Gamma treated, LNMA treated, and IFN-gamma plus LNMA treated.

The IFN-y induces activation of NO synthesis and LNMA is a pan-NOS inhibitor which suppresses NO synthesis.

This design allowed them to distinguish NO-dependent effects and RNA-seq was conducted 6 hours post-stimulation to capture early gene expression changes using the Griess assay measuring Nitric levels

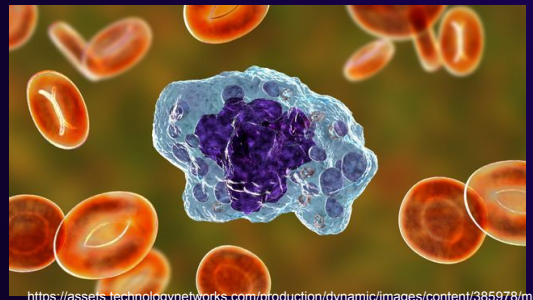
In parallel, the measured lactate production, NADS levels, and cell survival to assess functional metabolic outcomes using colorimetric and enzymatic lactate assay measuring Lactate levels

# Macrophages

Macrophages are the white blood cells produced by the innate immune system and these cells target and engulf foreign substances, dead cells, also including cancerous cells.

Method: they ingest the undesirable cell and then releases enzymes which then break down the cell

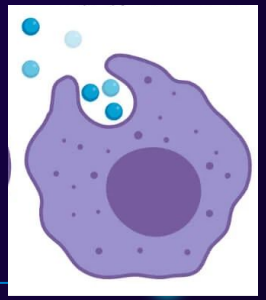
They also play a huge part in homeostasis, specifically cleaning up the dead cells



Computer depiction of a macrophage surrounded by red blood cells

https://assets.technologynetworks.com/production/dynamic/images/content/385978/monocytes-and-macrophages-macrophage-and-monocyte-function-origin-and-related-conditions-385978-640x360.jpg

An illustration of a macrophage ingesting foreign substances

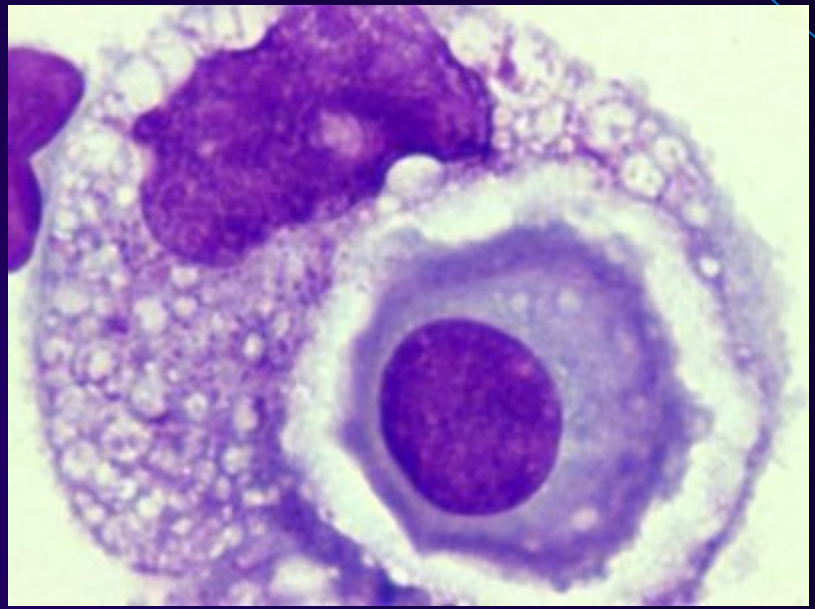


https://microbenotes.com/wp-content/uploads/2021/04/Macrophages.jpeg

# *What is IFN-gamma?*

Interferon-gamma is a protein released by animal cells in response to a virus. As a key immune cytokine (immune secretion):
-Inhibits virus replication,
-defends against pathogens,
-regulates inflammatory processes,
-provides tumor immunity
**-Induces nitric oxide (NO) production via (iNOS)**

**How does it do this?**
IFN-gamma is a macrophage-activating factor that stimulates macrophage cells to become more effective in eliminating pathogens by triggering extensive changes in gene expression

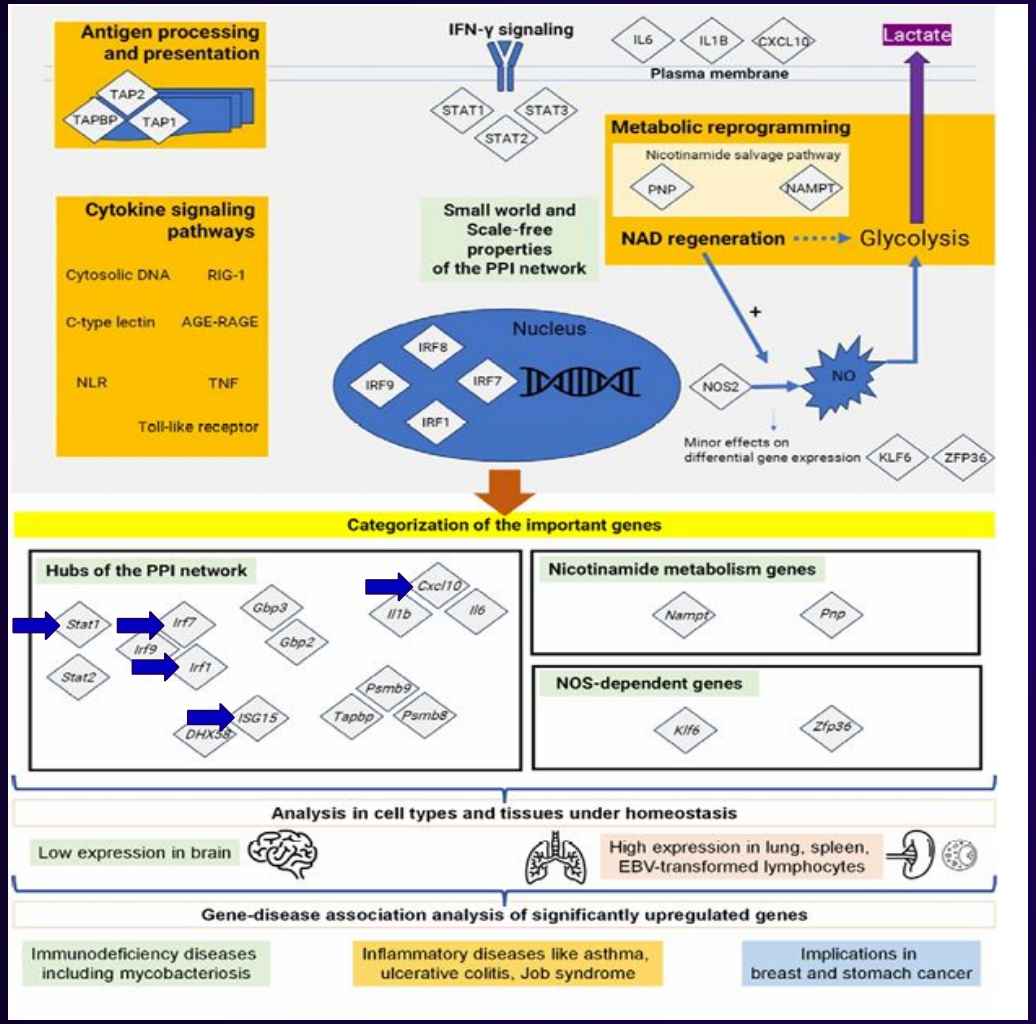


Abnormal plasma cell engulfed by a macrophage in pleural fluid

https://atlas.gechem.org/index.php?option=com_k2&view=item&id=1422:abnormall-plasma-cell-engulfed-by-a-macrophage-in-peural-fluid&lang=en

# The Role of Nitric Oxide (NO)

IFN-γ increases NO and lactate levels while reducing macrophage survival; blocking NO reverses these effects.
Most gene changes caused by IFN-γ don't need NO, but a few, like Klf6 and Zfp36, do.
IFN-γ also boosts $NAD^+$-producing genes, linking metabolism to immune activity.
Inhibiting this $NAD^+$ pathway lowers NO and improves survival, while restoring $NAD^+$ brings NO back.
Overall, NO connects IFN-γ signals, metabolism, and gene regulation into a coordinated immune response

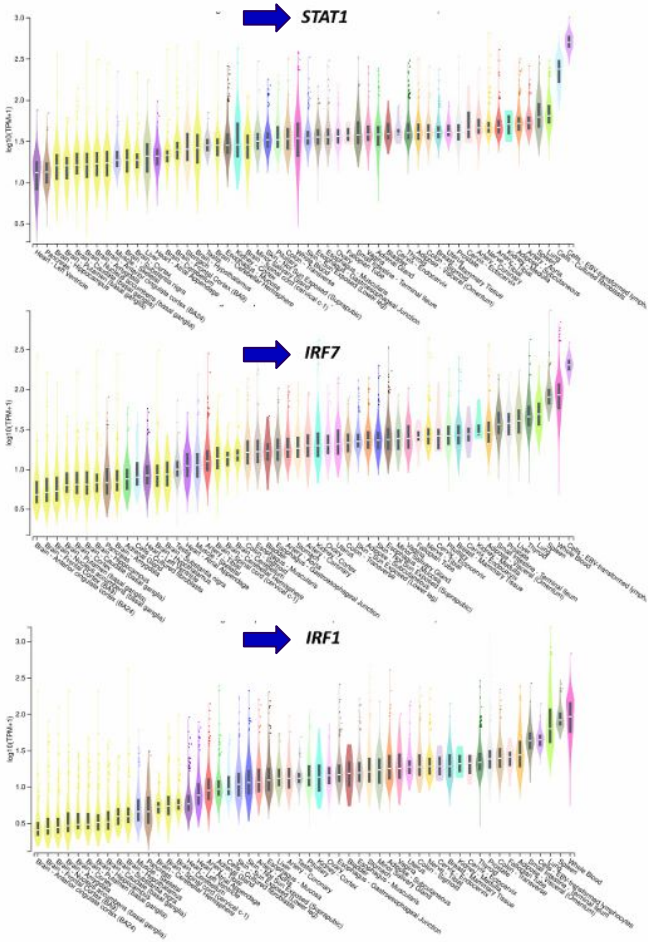



This diagram captures the entire story of the study in one image:

It starts with **IFN-γ activating macrophages**, which triggers **nitric oxide (NO) production**. NO fuels **metabolic reprogramming**, boosting NAD+ production and glycolysis—resulting in **lactate increase** seen in the results.

In the center, we see the top 5 most connected protein hubs in the PPI network—leading powerful immune responses and controlling gene expression.

Down below, genes like **KLF6 and ZFP36**, which were selectively influenced by NO, show how nitric oxide affects a focused part of the transcriptome.




## *Implications*

Now let's look at 3 of those hubs in the PPI network which were the most differentially expressed. Their extensive connections with other proteins make them control switches for immune responses.

**STAT1** helps cells respond to interferons like IFN-γ by turning on other immune genes.

**IRF7** is important in fighting viruses—mainly in lymphoid tissues.

**IRF1** is a master regulator. It can turn on or off lots of genes involved in immunity and even tumor defense.

What's exciting is that these genes weren't just highly expressed—they were the hubs in the signaling network that we saw in the previous slide. This means they're central players, coordinating many other responses triggered by IFN-γ."

These hub genes play a big role in shaping the immune landscape. These genes were not only upregulated but were also central in the structure of the IFN-γ response, as seen in the protein interaction maps.

# So...What is NOs role in detecting diseases?

Nitric oxide (NO) is like a molecular alarm bell—its presence reveals how immune cells, like macrophages, are responding to infections and inflammation. In this study, NO helped uncover key gene expression changes linked to diseases such as cancer, asthma, and autoimmune disorders. By tracking NO levels and the genes it affects, scientists can better understand how cells behave during disease. This makes NO a powerful biomarker for detecting and even predicting disease activity. In short, nitric oxide doesn’t just fight infection—it also helps reveal the body’s hidden battles.

# THANK YOU

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