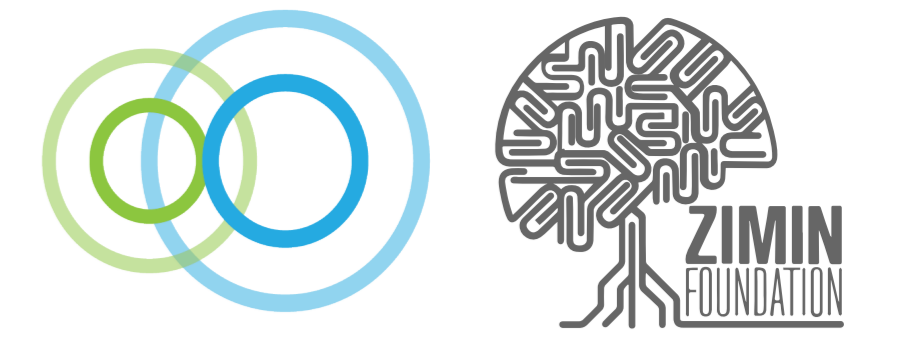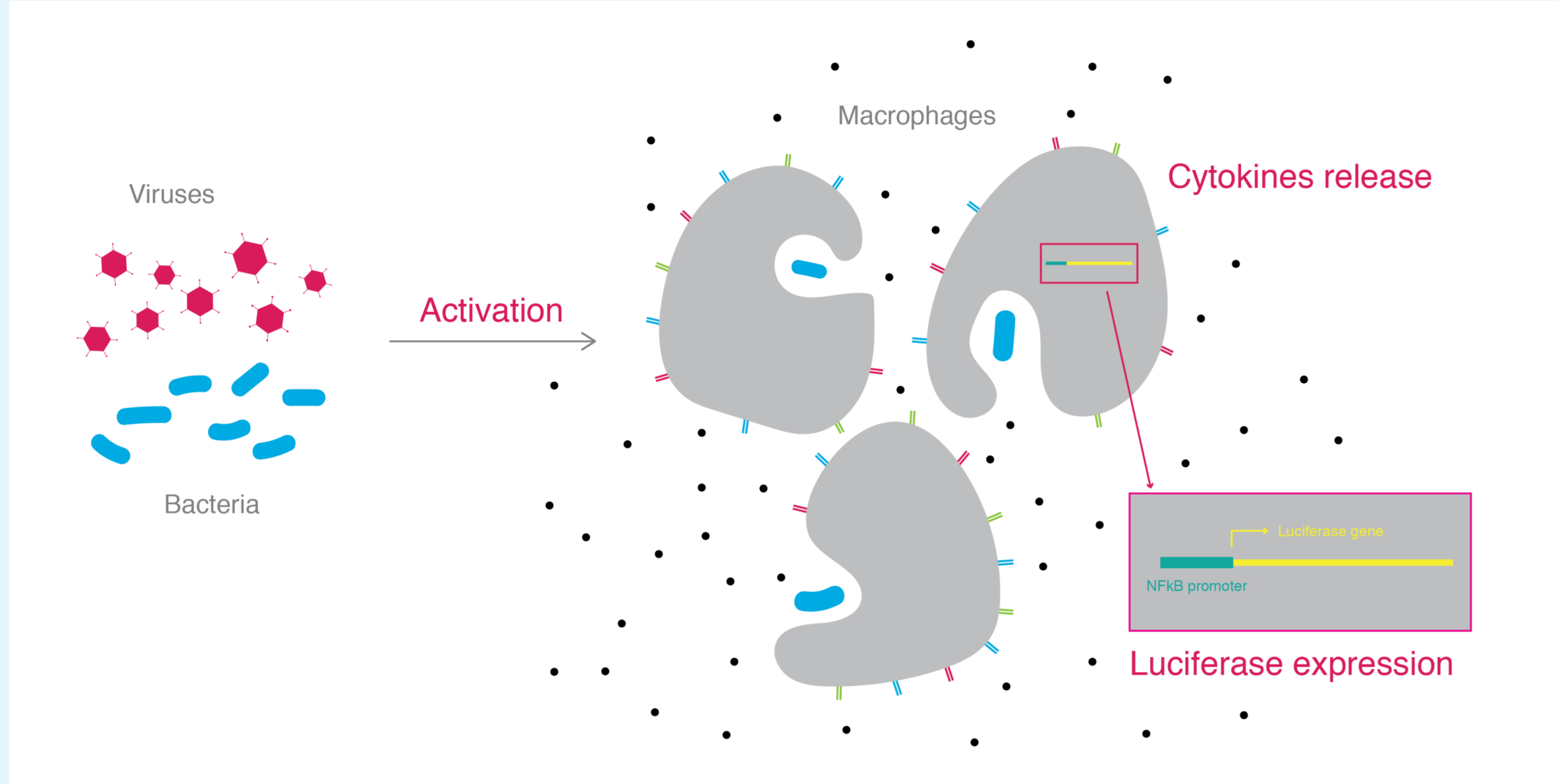# Analyzing the regulation of innate immune activation pathways associated with proinflammatory disease
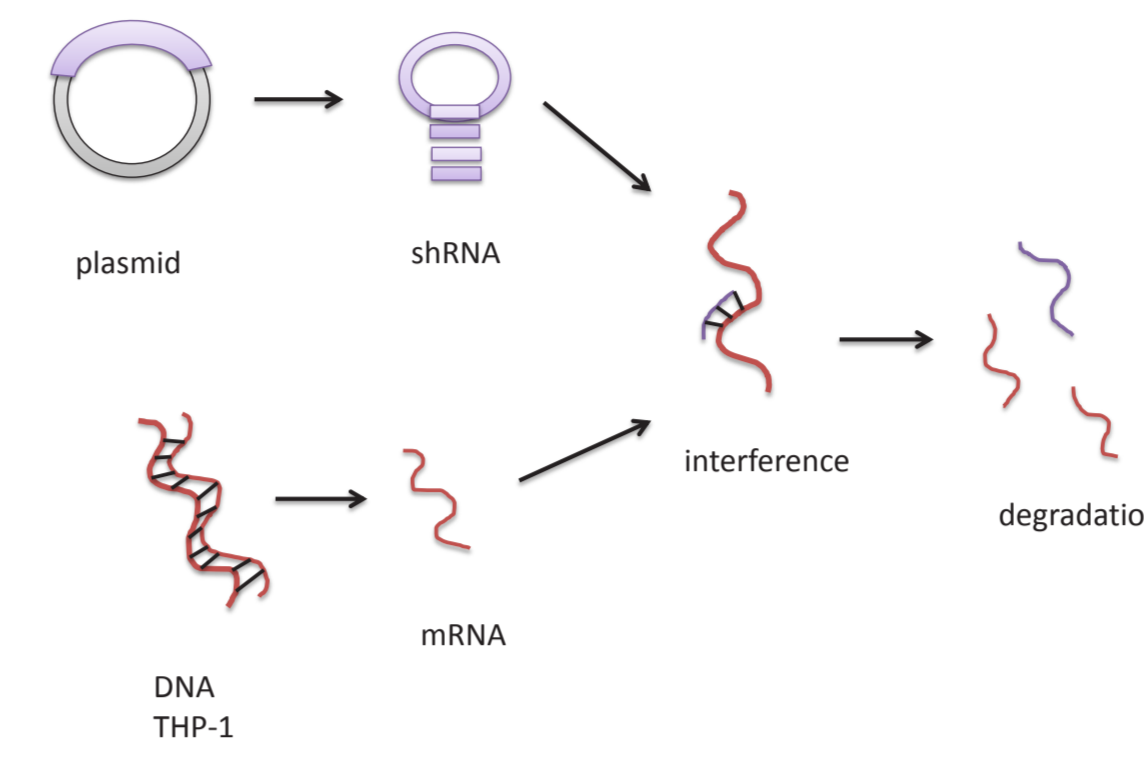
Victoria Borodina, Rita Gileva, Alba Guembe, Maria Dolgaya, Elizaveta Ignatyeva, Alexandra Kolodyazhnaya, Vera Sokolova, Marlen Toktomamatov, Maria Shubina, Natalia Markelova, Katya Putintseva, Elizaveta Leshchiner

Introduction

Innate immune cells are the first line of organisms' protection against pathogen invasion. Innate immune cells also effectively transmit the information about pathogens' composition and quantity. In the present work, we studied innate immunity and its mechanisms with special interest in autoimmune diseases (Crohn's disease in particular, which is hypothesized to have a significant bacterial immunity component). We used human monocytes THP-1 as a model system and utilized modern methods such as RNA interference, ELISA, transfection, luminescence-based reporter to measure the level of activation and viability of immune cells. The first aim of our work was to understand which pathogens and pathogen-associated molecular patterns (PAMPs) induce potent immune response, and which immune receptors are present on THP-1 cells. The second aim was to determine which genes and pathways are responsible for immune activation by switching off certain genes-candidates by using RNA-interference.

Part I

Innate immune cells (monocytes, THP-1) that were engineered to contain a Luciferase reporter for immune activation were counted, plated and stimulated with various stimuli: LPS, WGP, Zymosan, Pam3Cys, PolyI:C, R848, MDP. The same procedure was followed for already differentiated immune cells (macrophages) to measure cytokine production. The different immune reagents derived mainly from bacterial, fungal and viral structures and represent pathogen-associated molecular patterns (PAMPs). PAMPs bind to certain cellular receptors, which then activate immune pathways within the cell and result in the release of cytokines, small proteins responsible for further immune signaling. To analyse and compare the immune activation among the different reagents we first used luminescence screening for NF-kB transcription factor-driven Luciferase expression in THP-1 cells.

Immune Stimulants	Receptor
LPS	TLR 2,4
WGP	Dectin 1
Zymosan	TLR 2, Dectin 1
Pam3Cys	TLR 1, 2
PolyI:C	TLR3
R848	TLR 7, 8
MDP	NOD 2
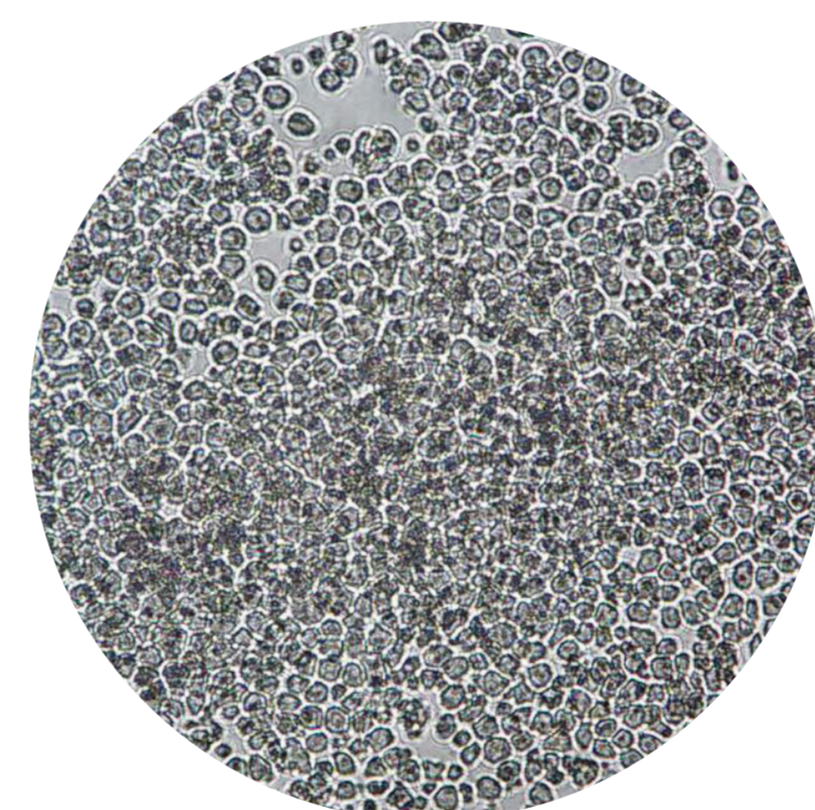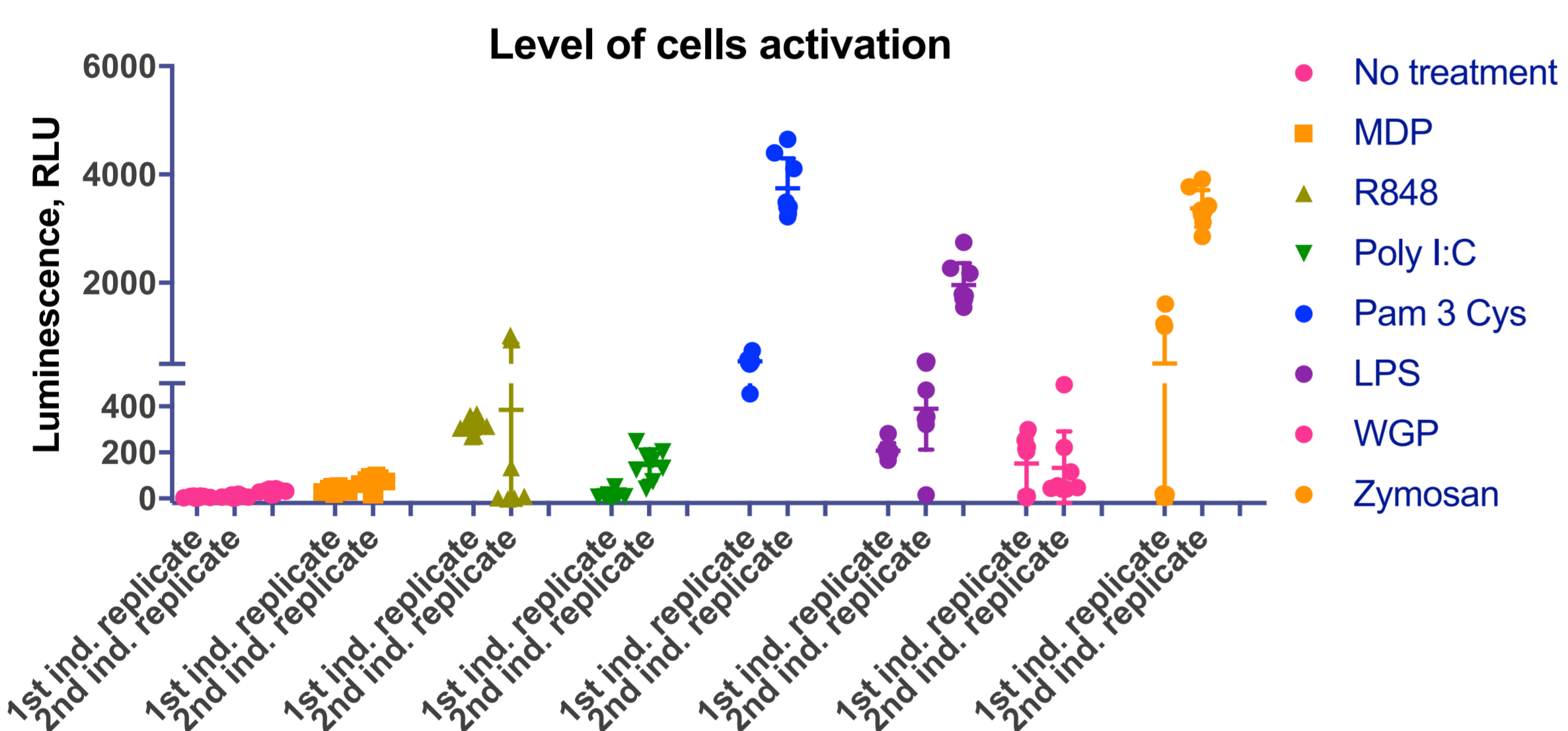
Figure 1

ELISA (Enzyme-linked ImmunoSorbent Assay) measures the concentration of a specific molecule (for example, a certain protein) in a sample. We used ELISA to determine the levels of IL-6 Cytokine produced from differentiated THP-1 cells in response to immune stimuli. IL-6 is one of the major pro-inflammatory cytokines deregulated in autoimmune diseases, such as Crohn's.

Figure 2

Part II

After that, we have transfected plasmids individually into THP-1 cells, where shRNAs were designed to block the mRNA of the selected gene. If Luciferase activation was altered after the gene knockdown and immunostimulation, we concluded that this particular gene was responsible for the immune activation pathway. To control for cell viability, we used Resazurin stain.
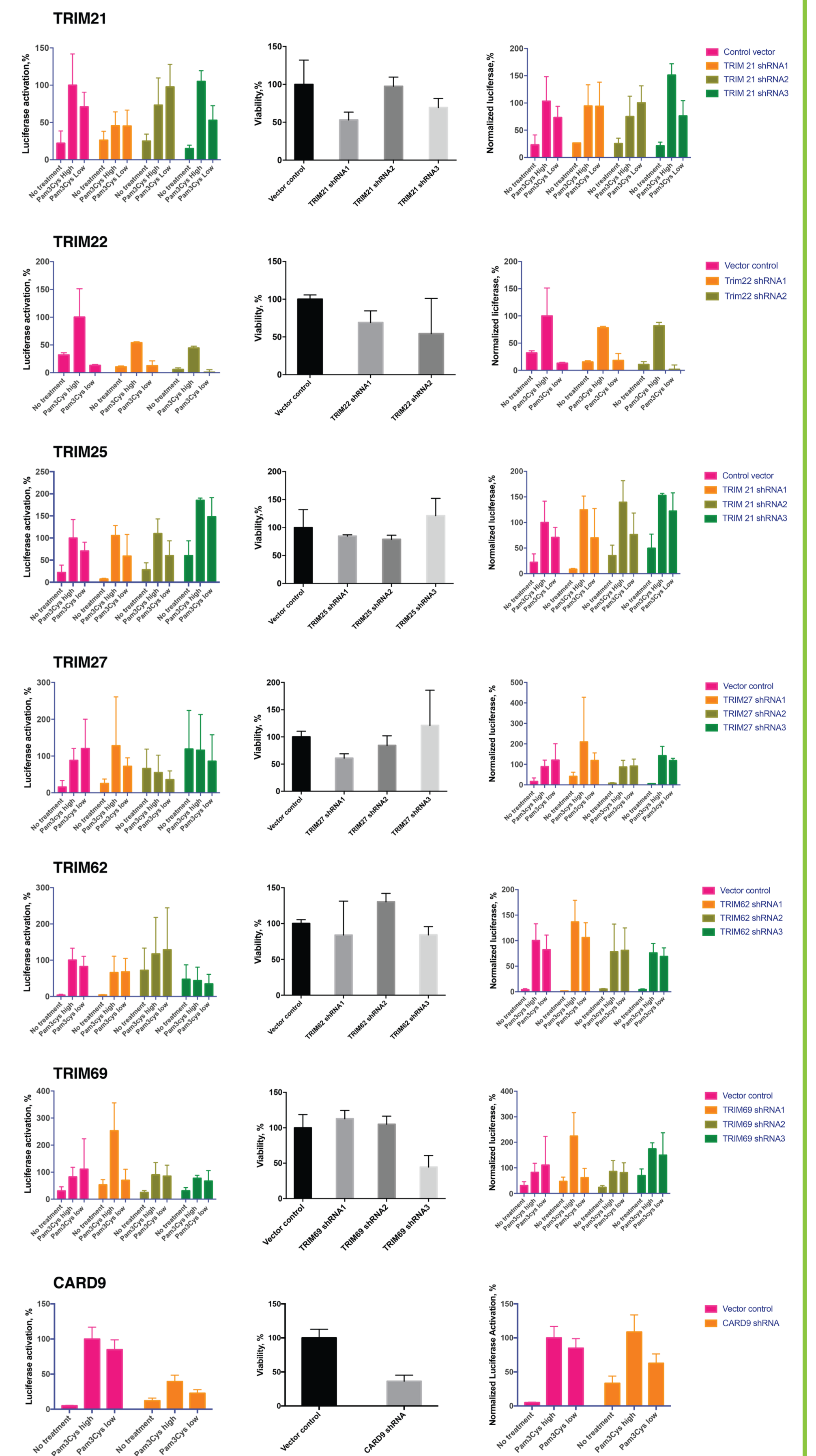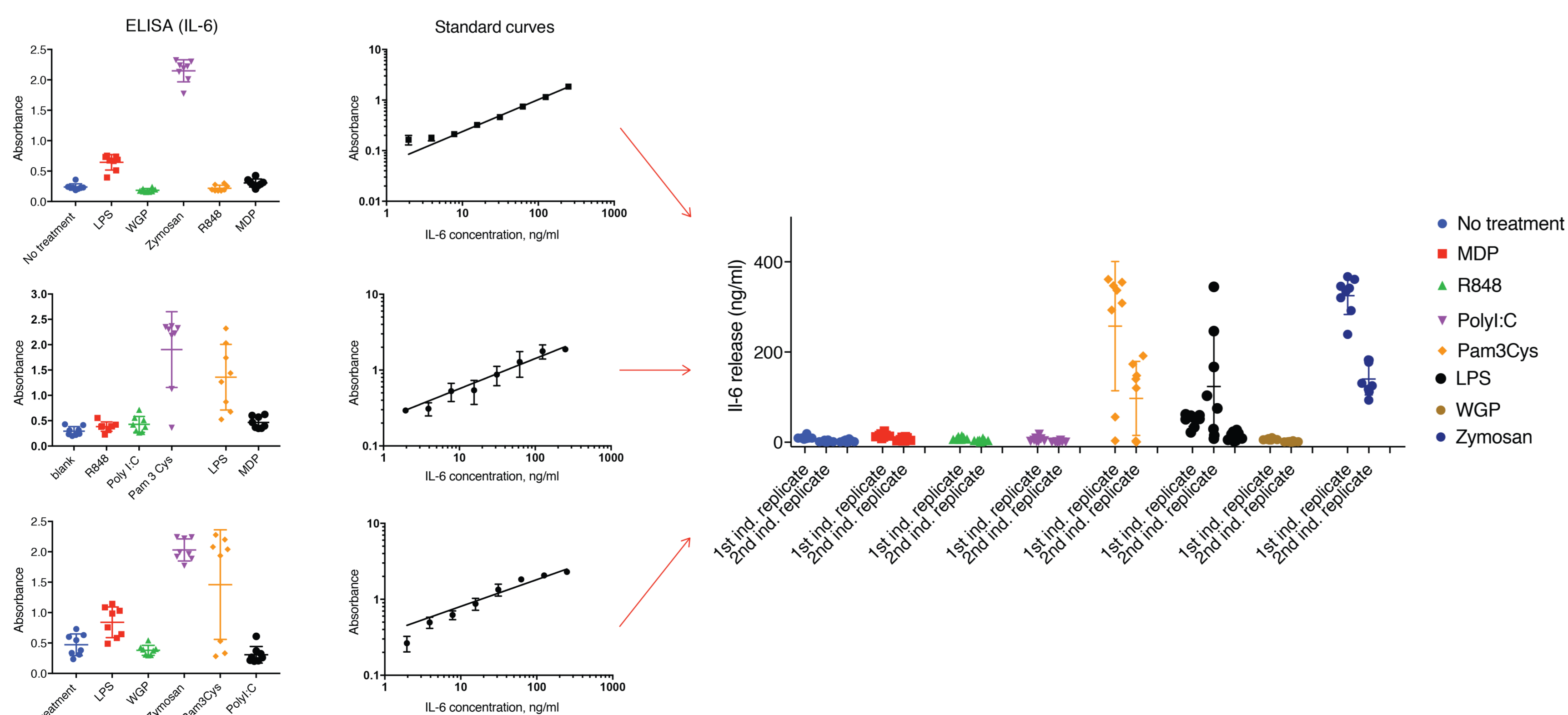
Among the immune stimuli, we selected Pam3Cys to determine the genes responsible for the immune activation in this pathway. Our list of candidate genes included members of TRIM family of proteins (TRIM21, TRIM22, TRIM25, TRIM27, TRIM62, TRIM69, CARD9). For each gene, we have used 3 shRNAs to increase the probability of specific gene knockdown. We have used E.Coli DH5a bacteria to amplify and then purify plasmid to use it in the mammalian cells.

Figure 3

Conclusions

1. We detected transcriptional activation and pro-inflammatory cytokine release (IL-6) in human monocytes (THP-1) in response to immune stimulation. Pam3Cys, a bacterial lipopeptide, was the most effective immune stimulus.
2. Based on the results of RNA interference experiments, we can preliminarily conclude that certain genes (such as TRIM69 and TRIM22) may regulate immune activation pathways. Our findings warrant further investigation.

Supplementary figure 1: