

IPA 7.1 Facilitates Generation of New Hypotheses and Provides Additional Insights into Ebola-modulated Immune Response Mechanisms

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Abstract

Ebola pathogenesis and the resulting host immune response are not well understood. Last year, Hartman et al. (1) published a paper that used Ingenuity Pathways Analysis (IPA) to examine the relevant signaling pathways triggered upon infection with Ebola virus containing a mutation in the VP35 protein (R312A). Our main questions were whether analysis of this dataset using new content and tools in IPA could generate additional insights and develop new hypotheses for further exploration. In addition to analyzing the associated Canonical Pathways, we also explored alternative workflows within IPA to facilitate data interpretation. The Pathway Building tools in IPA uncovered genes that are upstream of the VP35/R312A-upregulated gene set that are potentially involved in the immune response mechanism, but that have not been studied in immune cells. Since the VP35 mutation restores IRF3 activity, we explored IRF3-regulated genes and the cellular functions and pathways that are significantly impacted. Core Analysis in IPA exposed new immune-specific Canonical Pathways that contributed additional insight into the data. Taken together, within four hours, analysis in IPA 7.1 unveiled additional insights and hypotheses of these data.

Introduction

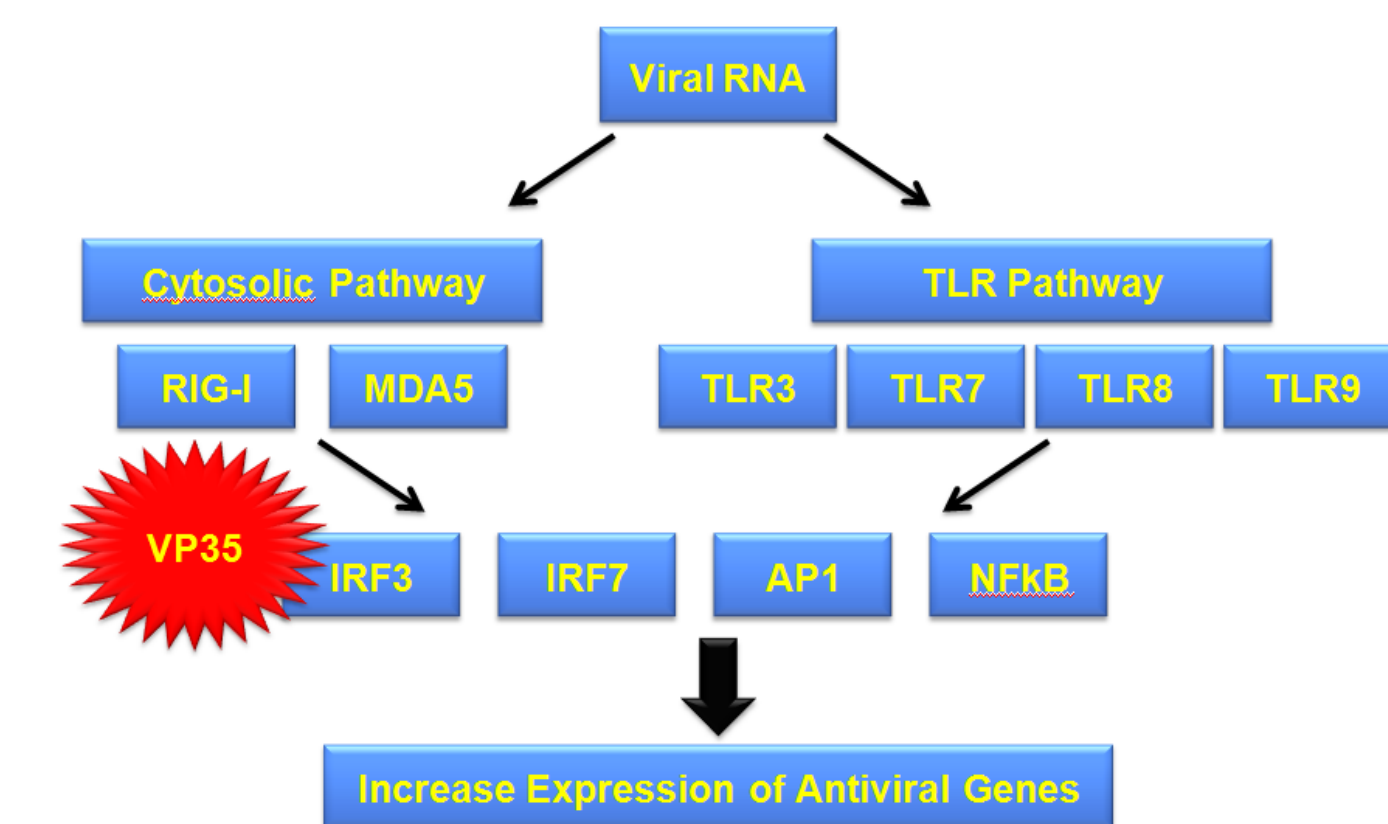


Figure 1. Key Players in the Anti-Viral Response. Both the Cytosolic and TLR pathways increase expression of antiviral genes. VP35 inhibits activation of IRF-3, thus contributing to a decreased immune response in infected individuals. VP35 also plays an essential role in virus transcription and replication because it functions as a cofactor for the viral polymerase and it is an integral member of the ribonucleoprotein complex. Additionally, VP35 serves as an RNA-silencing suppressor.

Materials and Methods

A list of upregulated genes from HepG2 cells infected with Ebola virus VP35/R312A was obtained from Hartman et al. (1) and used as a dataset for a Core Analysis in IPA.

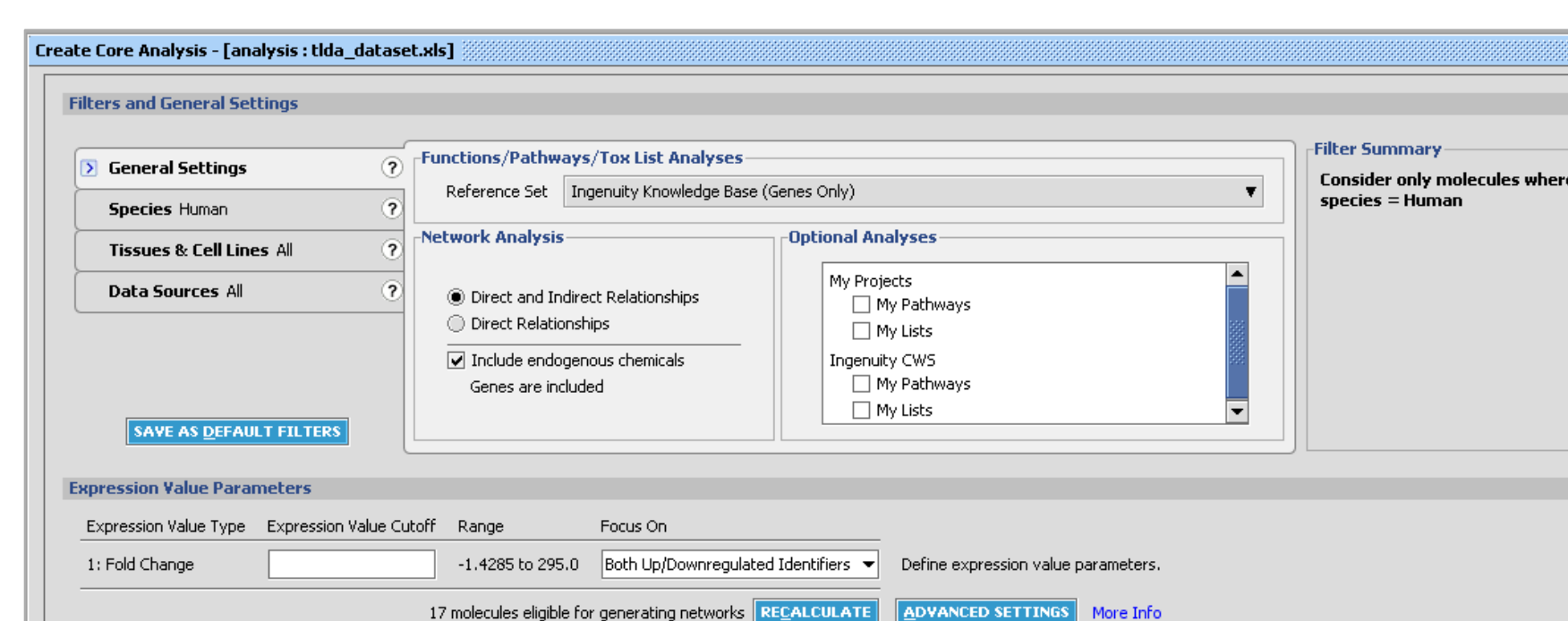


Figure 2. Analysis Settings in IPA.

References and Acknowledgement

1. "Whole-Genome Expression Profiling Reveals That Inhibition of Host Innate Immune Response Pathways by Ebola Virus Can Be Reversed by a Single Amino Acid Change in the VP35 Protein," A.L. Hartman, L. Ling, S.T. Nichol, M.L. Hibberd. *Journal of Virology*, June 2008, p. 5348-5358.

The authors would like to acknowledge that the datasets used in this poster are publicly available and are obtainable from the reference cited here in this poster.

Results

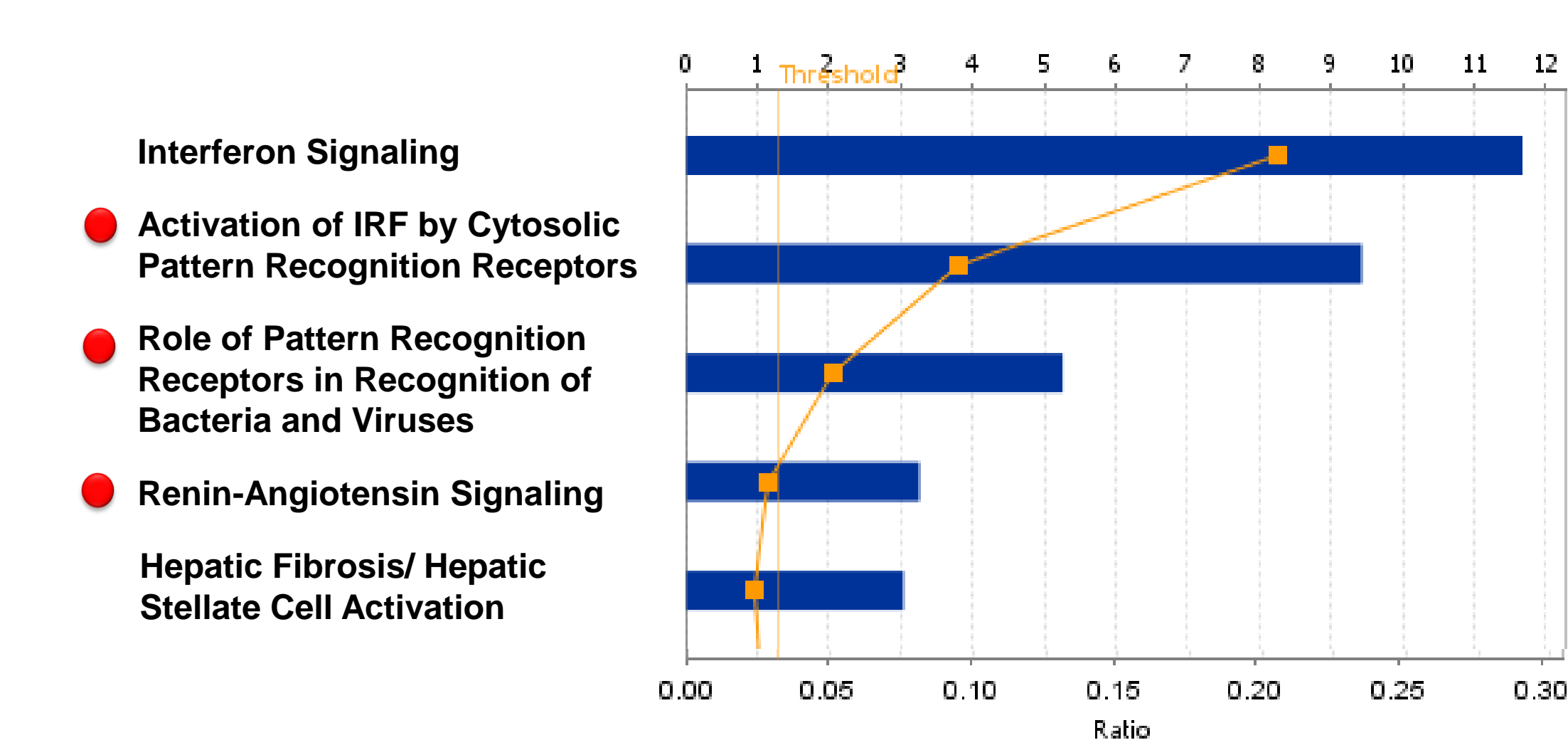


Figure 3. Canonical Pathways in IPA are indicative of Viral Infection, Inflammation, Liver Damage and Immune Response Pathways. Blue bars indicate significance. Orange line indicates ratio. Pathways marked with ● are new since IPA 7 or 7.1.

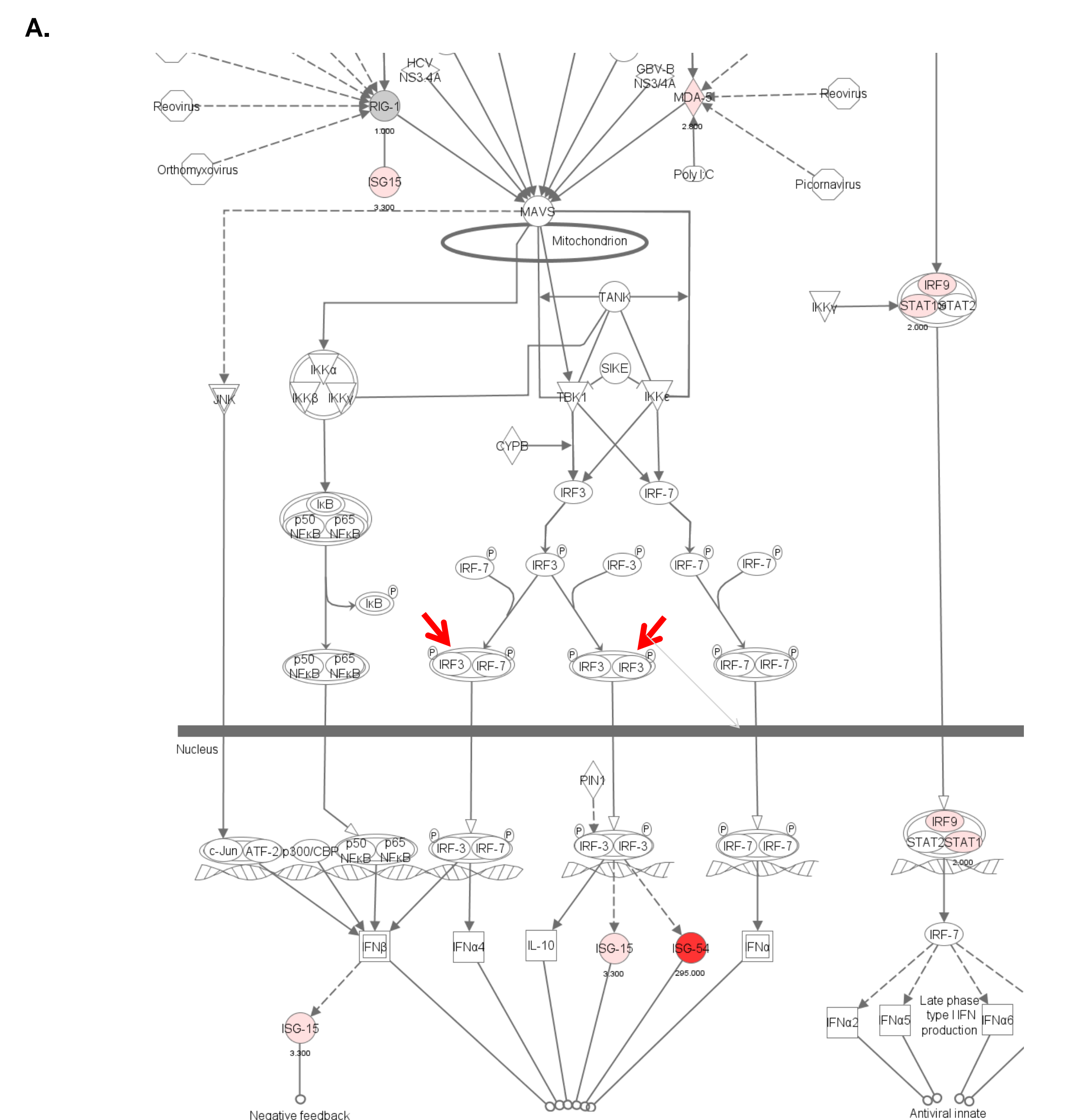


Figure 4. Activation of IRF by Cytosolic Pattern Recognition Receptors. A) Only a portion of the full Canonical Pathway is displayed. This pathway became available in IPA 7. The affected genes were unique to the VP35/R312A-infected cells and are colored red/ pink. Ratio 6/63. Significance = 3.7x10⁻¹⁰. Red arrows show where VP35 protein would affect this pathway. B) Abbreviated Canonical Pathway description for this pathway. Descriptions assist in familiarizing non-experts to the details of specific Canonical Pathways. Canonical Pathway descriptions became available in IPA 7.

The presence of double stranded RNA (dsRNA) or double stranded DNA in the cytosol following infection by viruses or intracellular bacteria trigger the cytosolic PRR system. The key sensors of viral dsRNA are Retinoic-acid-inducible gene 1 (RIG-I) and /or Melanoma-differentiation-associated gene 5 (MDA5) proteins. The activated PRRs then interact with a mitochondrial adaptor protein -MAVS (mitochondrial antiviral signaling protein), which in turn results in the activation of TANK-binding kinase 1 (TBK1) and inhibitor of kappa B kinase epsilon (IKKε). The activated kinases induce the serine phosphorylation of IRF3 and IRF7, which results in their homo or heterodimerization. These dimers translocate to the nucleus, where they activate the transcription of early phase type 1 interferons (IFNα4 and IFNβ) as well as other protein involved in the innate antiviral response. The early phase type 1 interferons induce the later phase of type 1 IFNs (IFNα non-α4 and IFNβ) largely via IRF7 homodimers. The later phase of interferons further augments the innate immune response of the host. The adaptor protein MAVS, in addition to activating the IRF pathway also

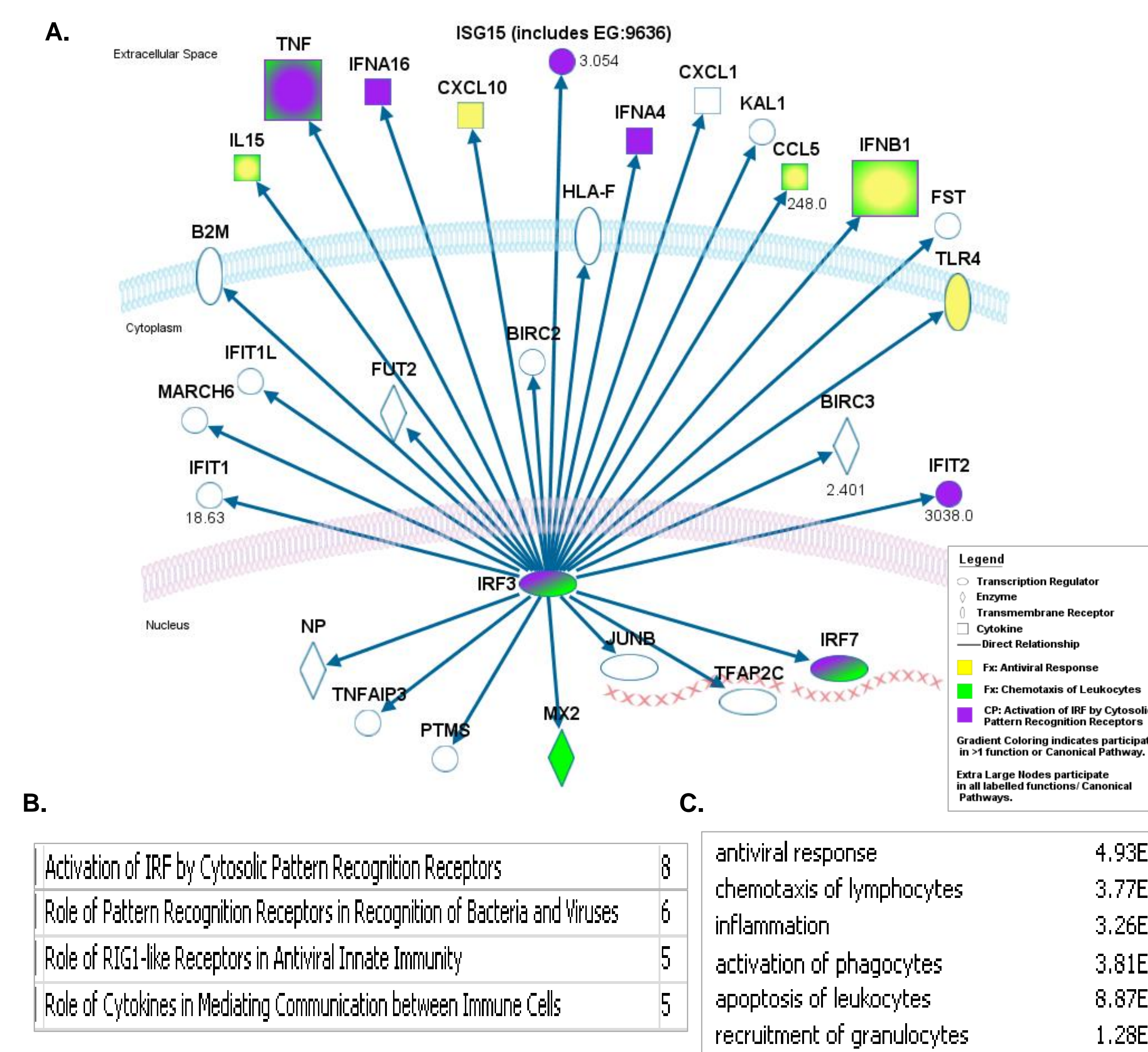


Figure 5. Path Designer Pathway of Downstream Neighbors of IRF3 and Associated Function and Canonical Pathways. A) Direct downstream neighbors of IRF3 were found by using Grow (direct interactions, molecule types = gene products, relationship types = transcription or expression). The expression levels of the genes that were measured in this dataset are indicated near their respective nodes. B) Some of the relevant Canonical Pathways involved in the IRF3 neighborhood. The number indicates the number of molecules from this pathway that are also members of the indicated Canonical Pathway. C) Functional Analysis of this pathway. Numbers indicate significance calculated with a right-tailed Fisher's Exact Test. Selected functions are shown. IPA assists in rapidly identifying and displaying the associated Functions and Pathways without having to do extensive literature research manually. The pathway is understandable to experts as well as non-experts.

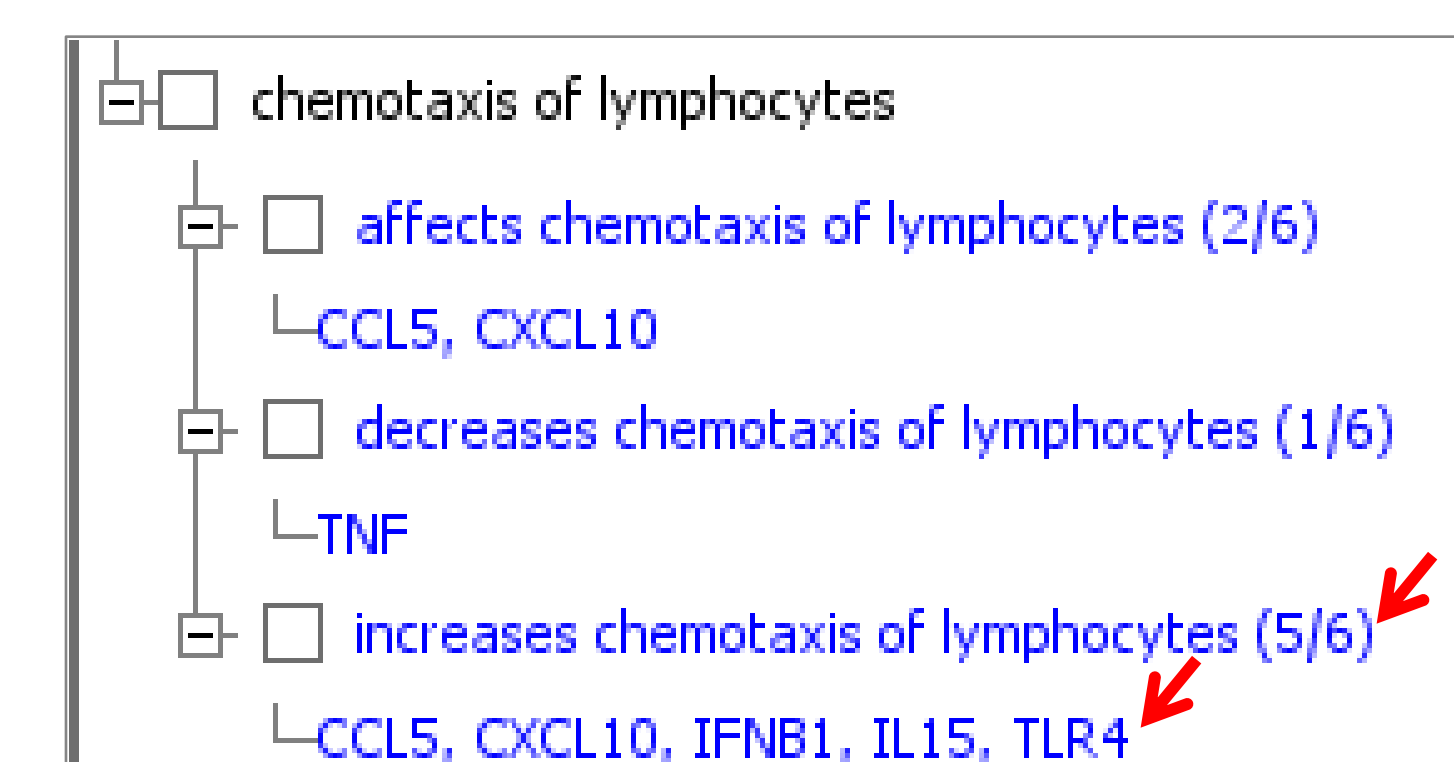


Figure 6. Effect on Function of IRF3 Downstream Neighbors. Effect on Function categorizes molecules into three effects: affects, increases, and decreases, which can facilitate drawing conclusions from the data. The supporting literature for the involvement of the group of molecules in the specific function can be found by clicking on the name of the function. Here we might expect that overall chemotaxis of lymphocytes is increased with activated IRF3 since each of these genes was upregulated in the experiment, and 5/6 of them are known to increase chemotaxis of lymphocytes.

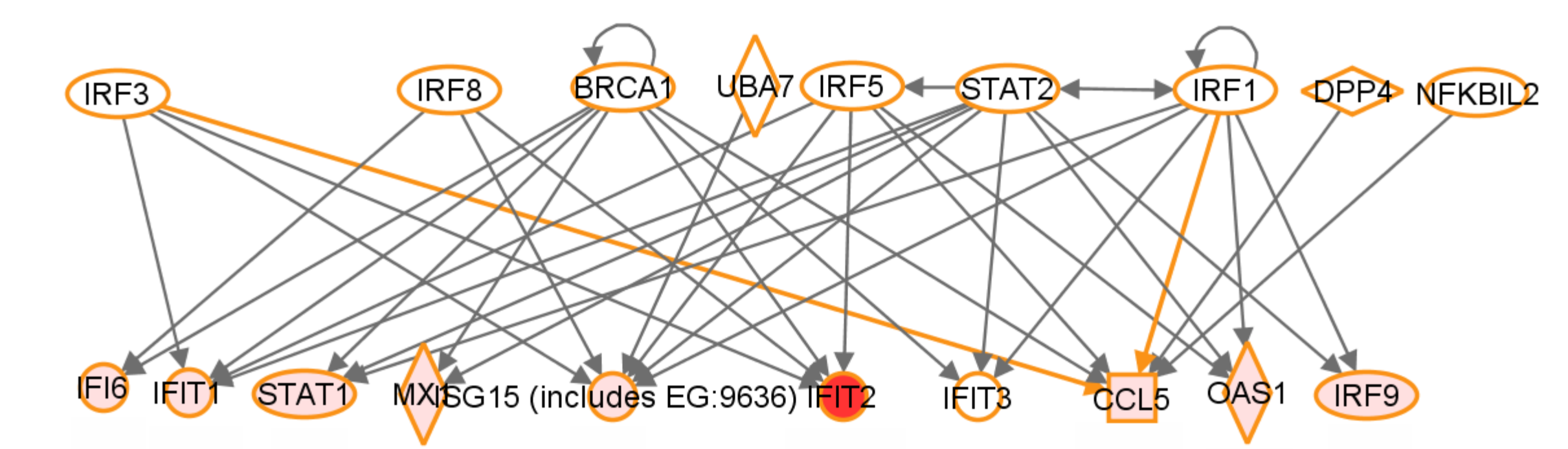


Figure 7. Upstream Regulators of Genes Upregulated with VP35-R312A infection. Grow was used to find the upstream genes that directly regulate the affected genes reported in (1). Grow settings: Upstream, Direct Interactions, Molecule type = gene products, Relationship type = activation, expression, inhibition, leads to, modification, phosphorylation, protein-DNA interactions, proteolysis, reaction, regulation of binding, transcription, or translation. Red/ pink nodes are from the original list of genes studied in (1). Orange highlighting indicates the expression or involvement in primary immune cells (B and T lymphocytes, dendritic cells, NK cells, macrophages, monocytes, neutrophils, and monocyte-derived macrophages). Many of the upstream genes target several down-stream genes, indicating possible regulatory mechanisms that could be used for follow-up studies. BRCA1 could be an interesting gene for possible follow-up studies because it is expressed in immune cells and is known to directly increase transcription of a number of immune-related genes in other tissues, but these interactions have not been confirmed in immune cells.

Conclusions

- IPA was able to:
 - Identify possible upstream regulators of VP35-regulated genes, including at least one unexpected possible regulator.
 - Uncover downstream neighbors of IRF3 along with their associated biological functions and Canonical Pathways.
 - Highlight new biologically relevant immunology and infectious disease-related Canonical Pathways with their associated descriptions.
 - Interpret a publically-available dataset in a short amount of time. The total time to run all analyses, create custom pathways, and analyze Canonical Pathway and Functional Analysis output was approximately 4 hours.

Platform Extensions for Biodefense (Research In Progress)

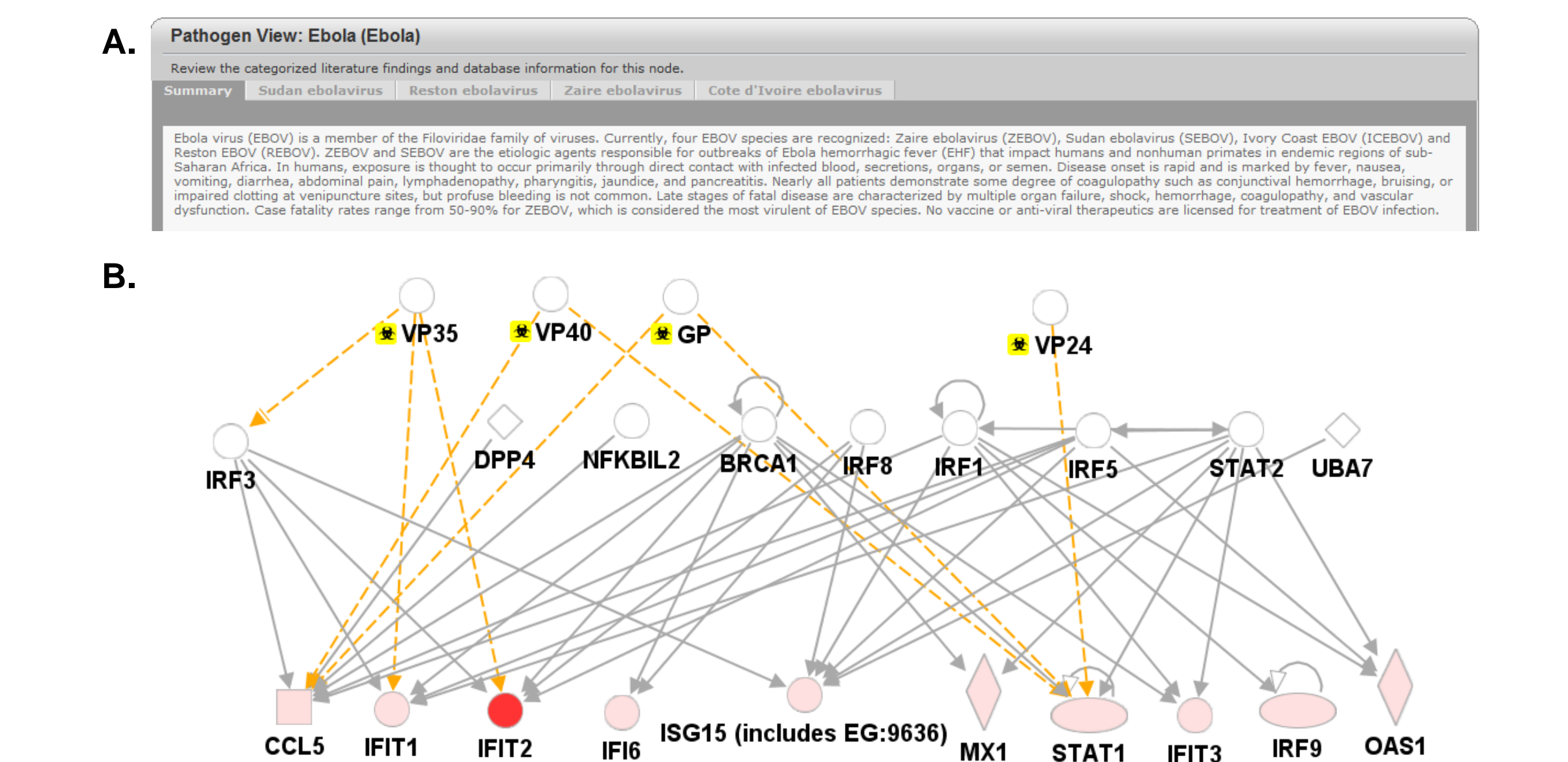


Figure 8. Pathogen Ingenuity Pathways Analysis (PIPA) is a prototype version of IPA that is current research at Ingenuity Systems. A) A Portion of Pathogen View from PIPA. PIPA contains new content on several pathogens. Pathogen View contains summary information on the pathogen as well as links to findings and the supporting literature. B) PIPA was used to add known interactions of Ebola virus proteins with the host proteins. Molecules indicated with the ● are from the Ebola virus genome. Orange lines are specific to pathogen-host interactions.

For further information

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