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# **Defining the Role of** Powassan Virus in **Evading Host Antiviral** Immunity

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## BACKGROUND

Powassan Virus (POWV) is an emerging neurotropic flavivirus transmitted to humans through the bite of an infected tick. Currently, there is no specific antiviral treatment nor approved vaccine for POWV. During infection, many interferonindependent host proteins and pathways sense and respond to viral infection. Flaviviruses have evolved multiple mechanisms to counteract host antiviral programs, often with individual viral proteins mediating this antagonism. However, it has not been determined if these mechanisms are conserved across diverse flaviviruses.

## OBJECTIVES

- Identify the antiviral factors in the Central Nervous System (CNS) controlling POWV infection.
- Identify POWV proteins that antagonize expression of Type I interferons and ISGs.

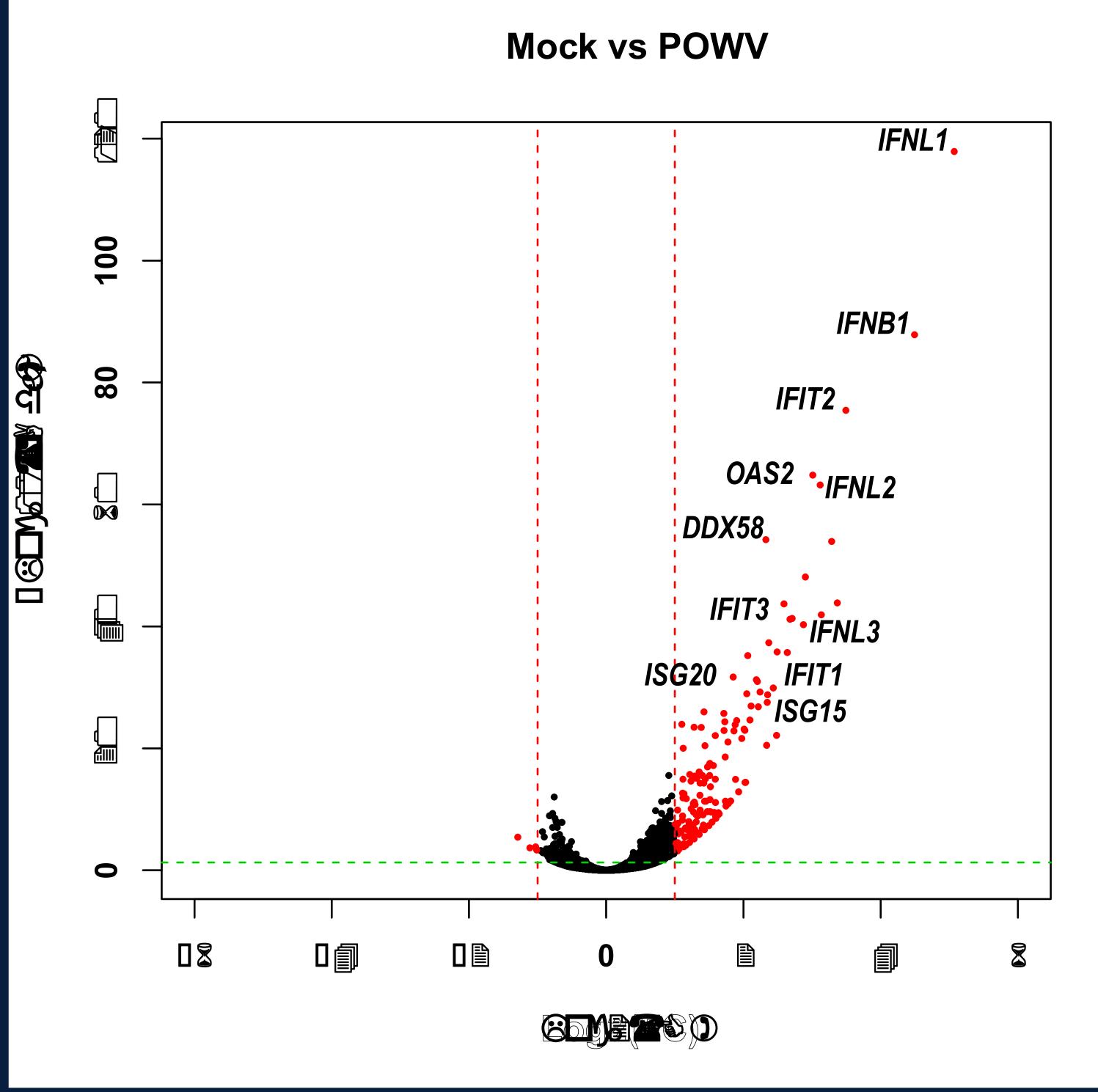
## METHODS

- Evaluate POWV infection levels in CNS cell lines.
- Infection of Human microglial cell line (HMC3) with POWV for 24 and 48 h.
- Transcriptomic analysis from HMC3 cells infected with POWV for 48 h.
- Firefly assay with luciferase reporter vector IFNβ (Type I IFN) and POWV protein expression vector.





# **Transcriptomic analysis of POWV-infected** HMC3 cell lines revealed significant induction of Type I interferons, Type III interferons and several IFN-stimulated genes (ISGs) that have not been previously associated with antiviral activity against POWV. See Table 1.



**Figure 3.** Induction of host genes in HMC3 cells in response to POWV infection. The Log<sub>2</sub> fold change of infected vs. uninfected gene expression is shown on the x-axis. The  $-Log_{10}$  of the adjusted p-value for expression of each gene is shown on the y-axis. Genes that are significantly regulated are indicated in red. Upregulated genes of interest are labeled.

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#### RESULTS

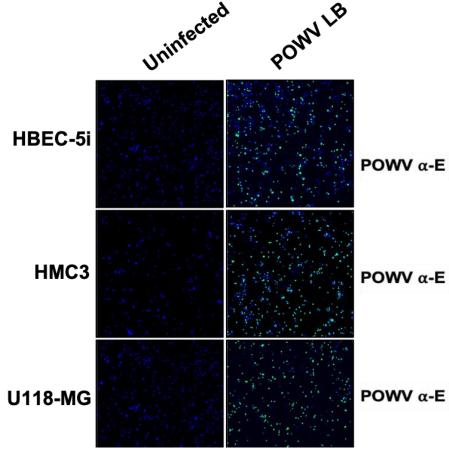


Figure 1. Infection of

indicated cell lines with

POWV LB. Cells were

infected for 48 hours. POW

infection was detected via

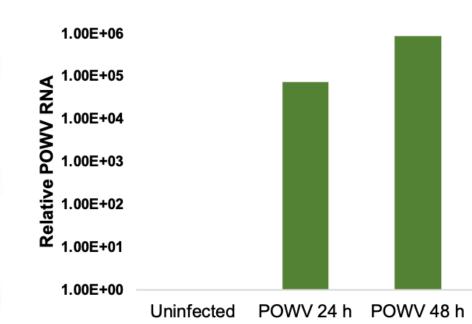
immunofluorescence using

POWV E protein. The cell

nuclei were stained with

Hoechst.

an antibody recognizing the



HMC3 Cell line

Figure 2. Infection of HMC3 cell lines with POWV. Relative POWV quantified by gRT-PCR is indicated on the y-axis. Cells were infected for 24 and 48 hours. POWV RNA was normalized to 18S rRNA. Also shown is an uninfected control

<u>Gene</u>	<u>Log 2-fold change</u>	Adjusted p-value
IFNL1	5.072360524	1.31E-118
IFNL2	3.367760191	1.35E-44
IF127	2.030740493	4.07E-15
IFIT2	3.49398642	3.66E-76
IFNB1	4.492852713	1.42E-88
IFNL3	2.482924943	7.25E-23
ISG20	2.203603061	1.10E-31
HERC5	2.90330216	8.08E-49
IFI6	2.639223283	1.91E-36
ISG15	2.351900211	1.65E-29
IFIT1	2.875465832	5.29E-41
IFIT3	2.711212948	4.91E-42
BST2	2.024979807	3.96E-15
0462	3 110012573	6 155-61

**Table 1.** Upregulated genes in response to POWV in HMC3
 cells. Upregulated ISGs of interest are highlighted in blue.

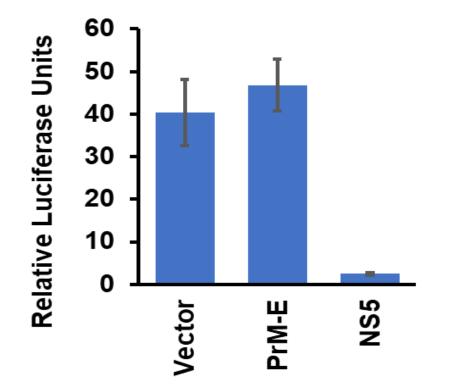


Figure 4. Plasmids expressing POWV LB prM-E, NS5, or an empty vector control were co-transfected into HMC3 cells with a reporter plasmid expressing Firefly luciferase under the control of the IFNβ promoter.

#### **FUTURE GOALS**

- Test the antiviral activity of identified upregulated ISGs from our RNA-seq dataset and characterize the restriction mechanisms of these antiviral host factors.
- Identify POWV proteins that antagonize expression of Type I and ISGs and determine the mechanism of inhibition of IFN signaling.

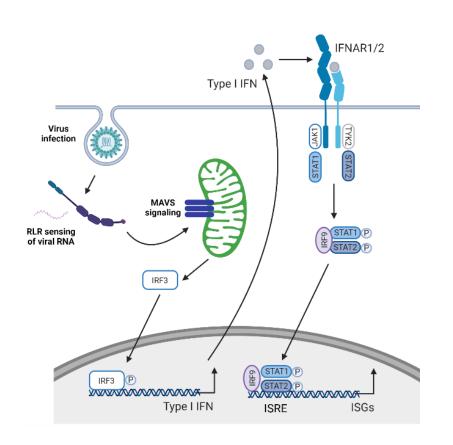


Figure 5. Schematic of Type I interferon signaling pathway.