



IDENTIFYING VSV GENES THAT SHORTEN UNTRANSLATED REGIONS OF MRNA AND INDUCE IMMUNE RESPONSES

TEAM PROJECT

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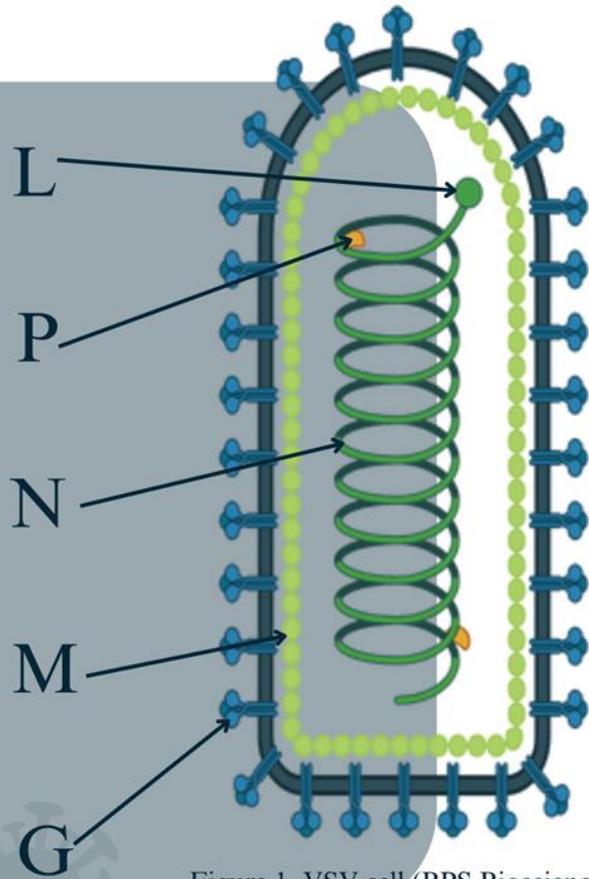


Figure 1. VSV cell (BPS Bioscience)

VESICULAR STOMATITIS VIRUS (VSV)

- Negative-sense RNA virus
- 5 structural proteins

VSV'S USES

- Primarily affects livestock and hoofed animals
- Minimal effects on human health
- Used in vaccine testing and research
- Reduces human 3' UTR length



3' UTRS

- Untranslated section of mRNA
- Length determined by APA
- Proximal PAS usage shortens
- Distal PAS usage lengthens
- Lengthening → folding over → dsRNA

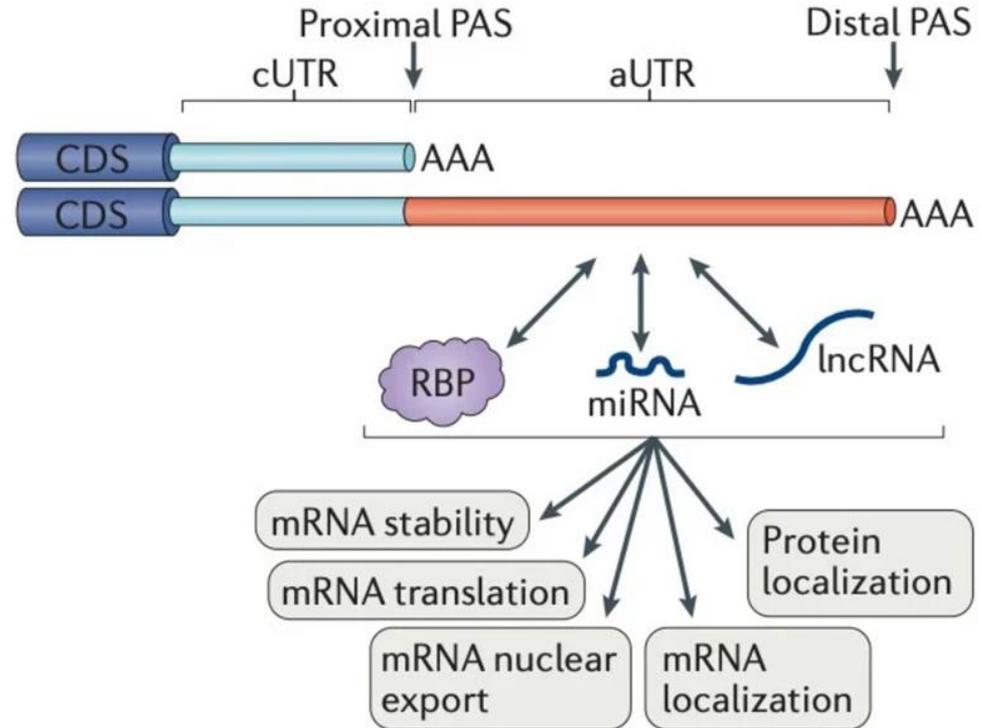


Figure 2. Alternative Polyadenylation (APA) mechanism
(Tian and Manley)

DOUBLE STRANDED RNA INDUCES IMMUNE RESPONSE ...

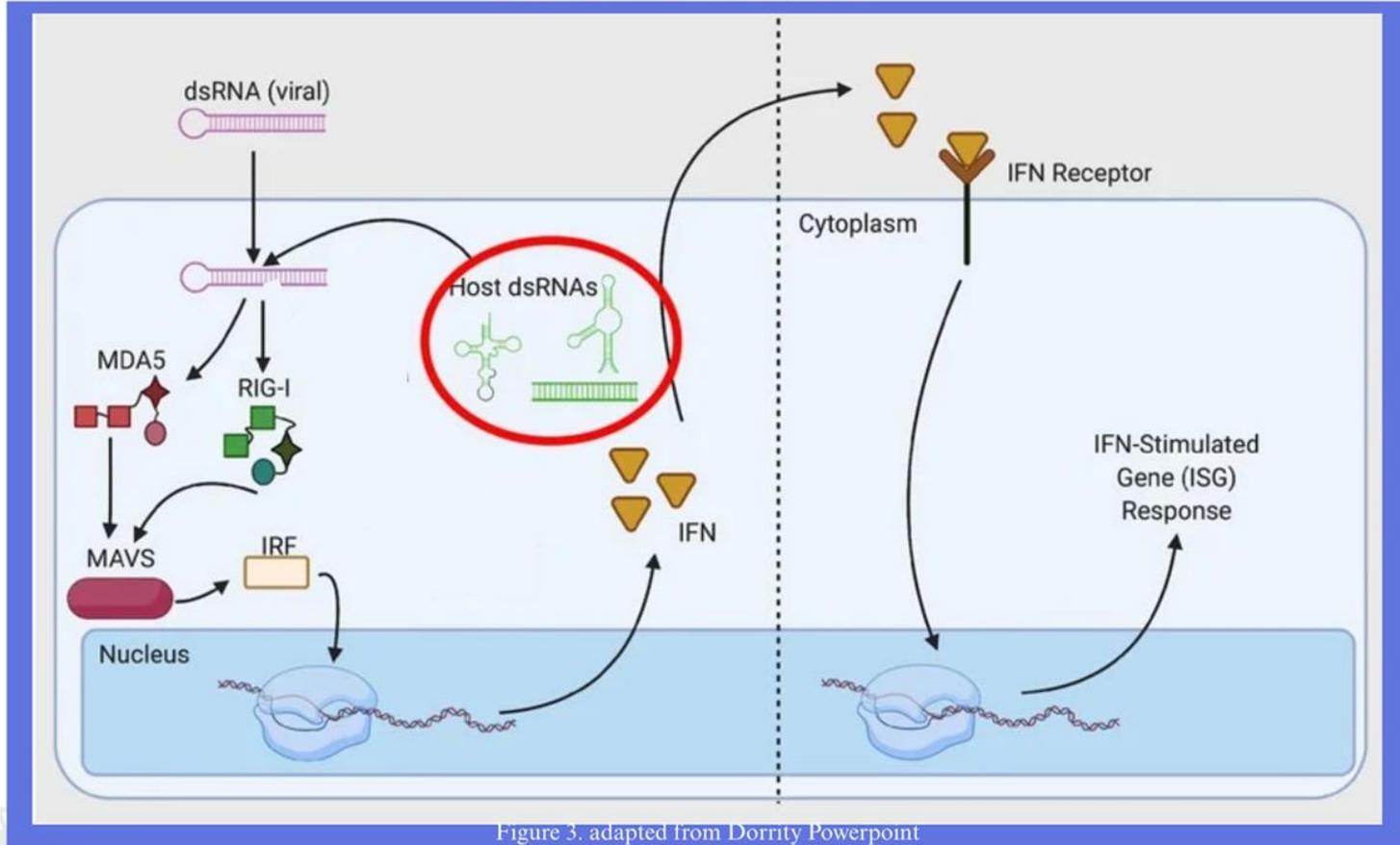


Figure 3. adapted from Dorriy Powerpoint

VIRUSES

- Effect on 3'UTR is context dependent
- Many Viruses alter 3'UTR length
 - Herpes Simplex Virus
 - La Crosse Virus
 - Influenza A





GOAL: FINDING VSV GENE THAT SHORTENS UTRS

TRANSFECTION OF THP-1 CELLS



Plasmid

Cell Membrane

Escape to Nucleus

Endocytosis

Endosome

Lipid Bubble

Positive Control:

- IAV-NS1

Negative controls:

- Mock
- Unstimulated

Five VSV genes

MEASURING RNA WITH QPCR

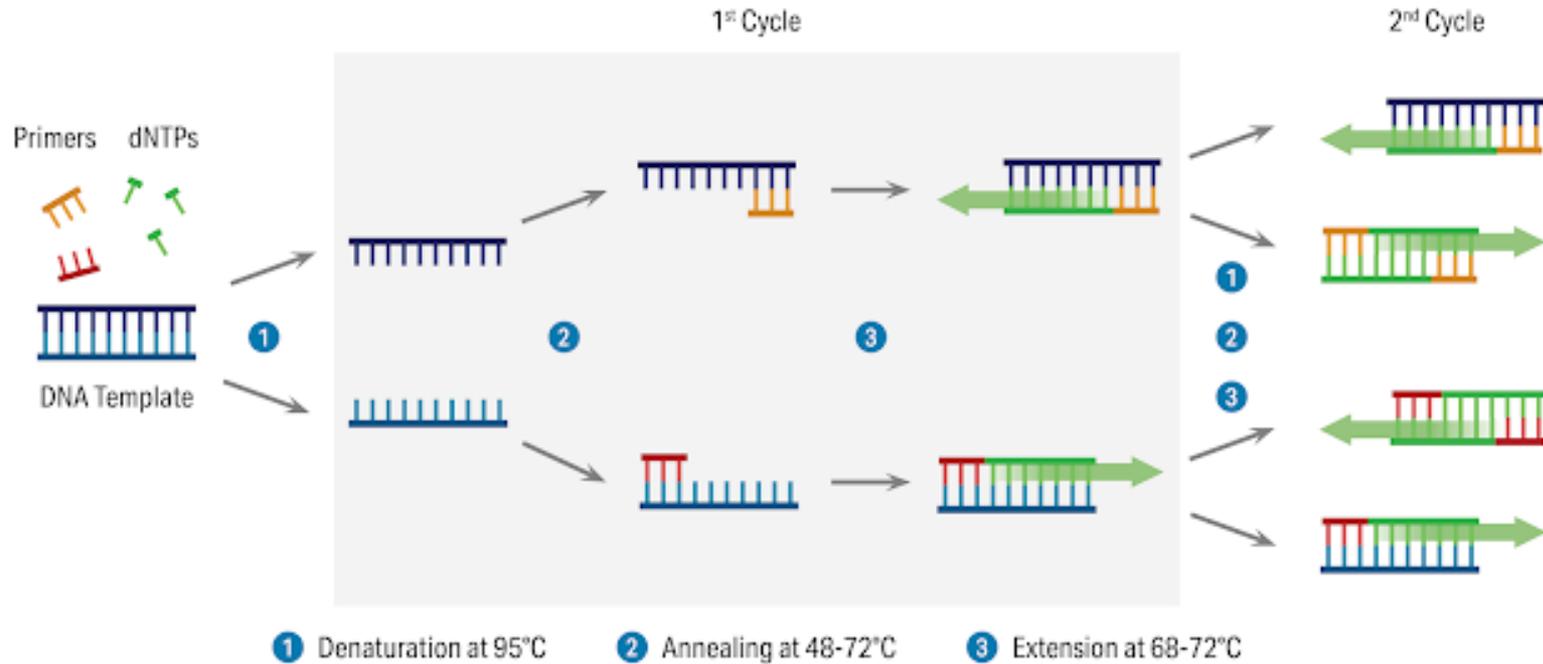
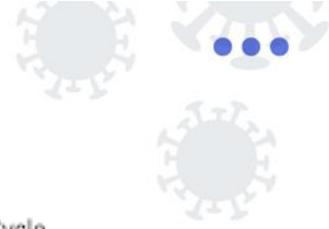


Figure 5. qPCR by AAT Bioquest

Primers: 18S, IFN β , PKR ORF and UL, SMC1A ORF and UL, Viral Gene

ORF/UL RATIO

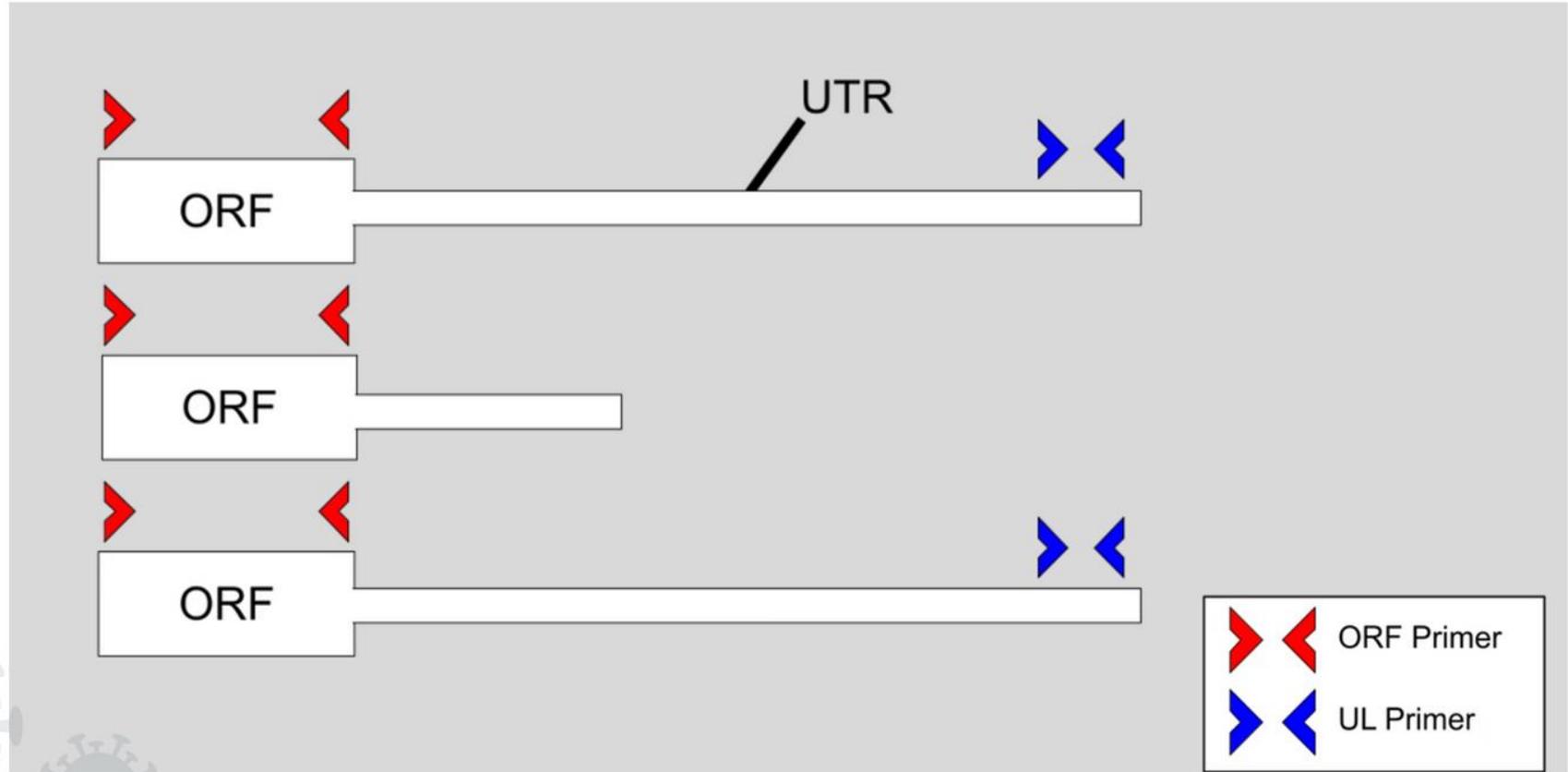


Figure 6. ORF compared to UL

VSV-G AND L SHORTEN LENGTH OF UTR IN PKR

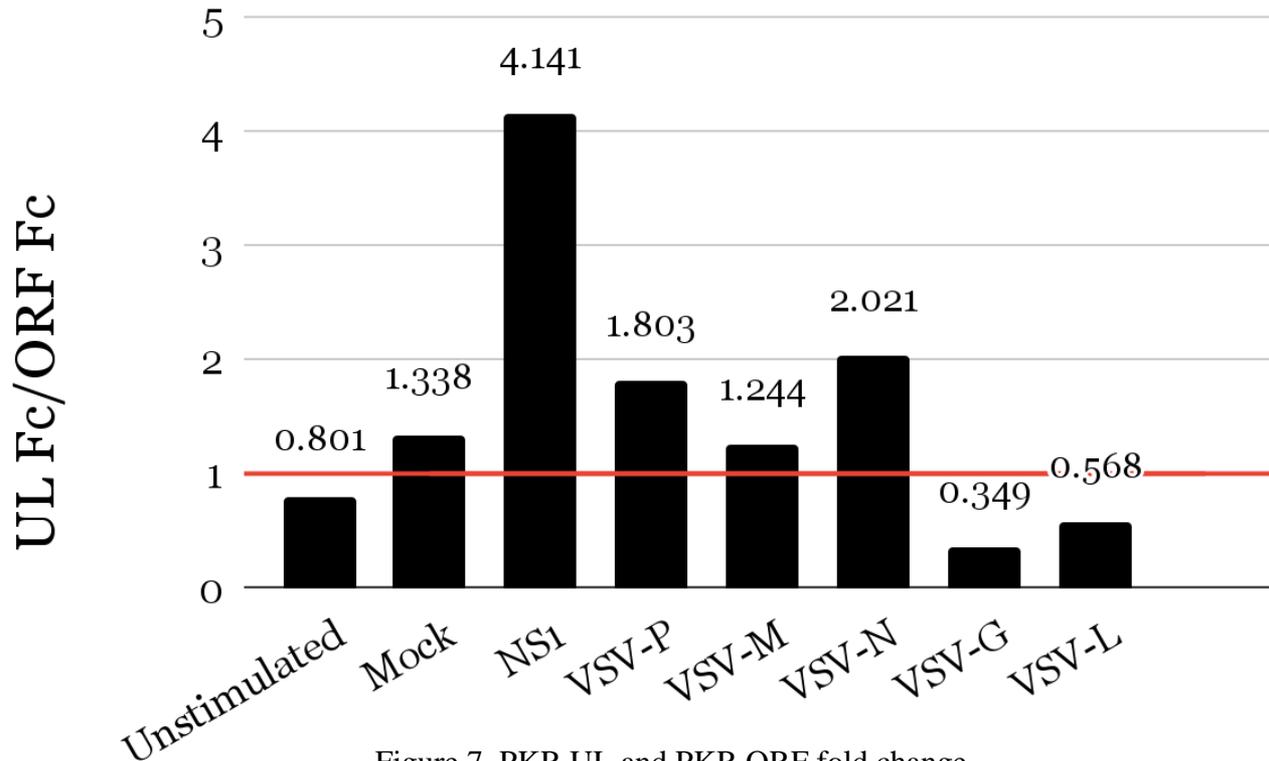
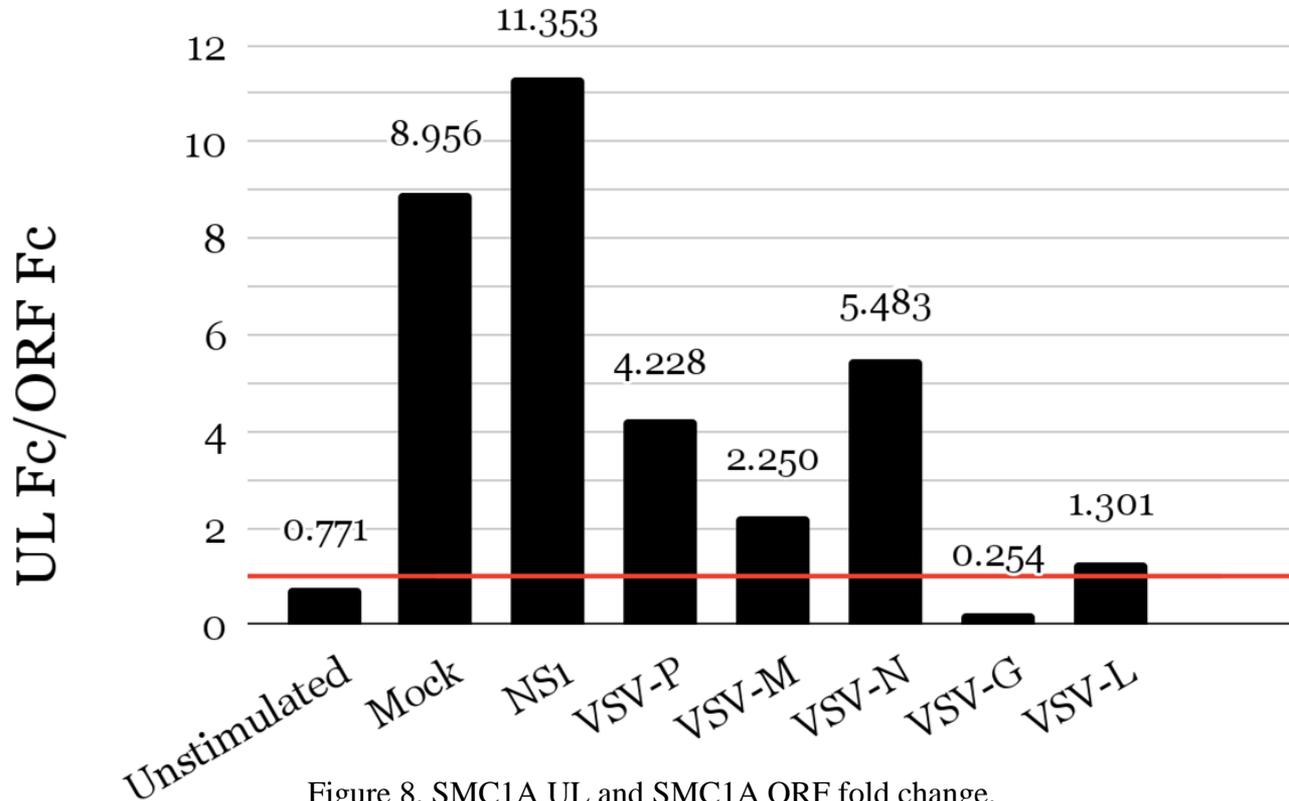


Figure 7. PKR UL and PKR ORF fold change.

VSV-G SHORTENS THE LENGTH OF UTR ••

IN SMC1A



VSV-G LOWERS IFN β EXPRESSION

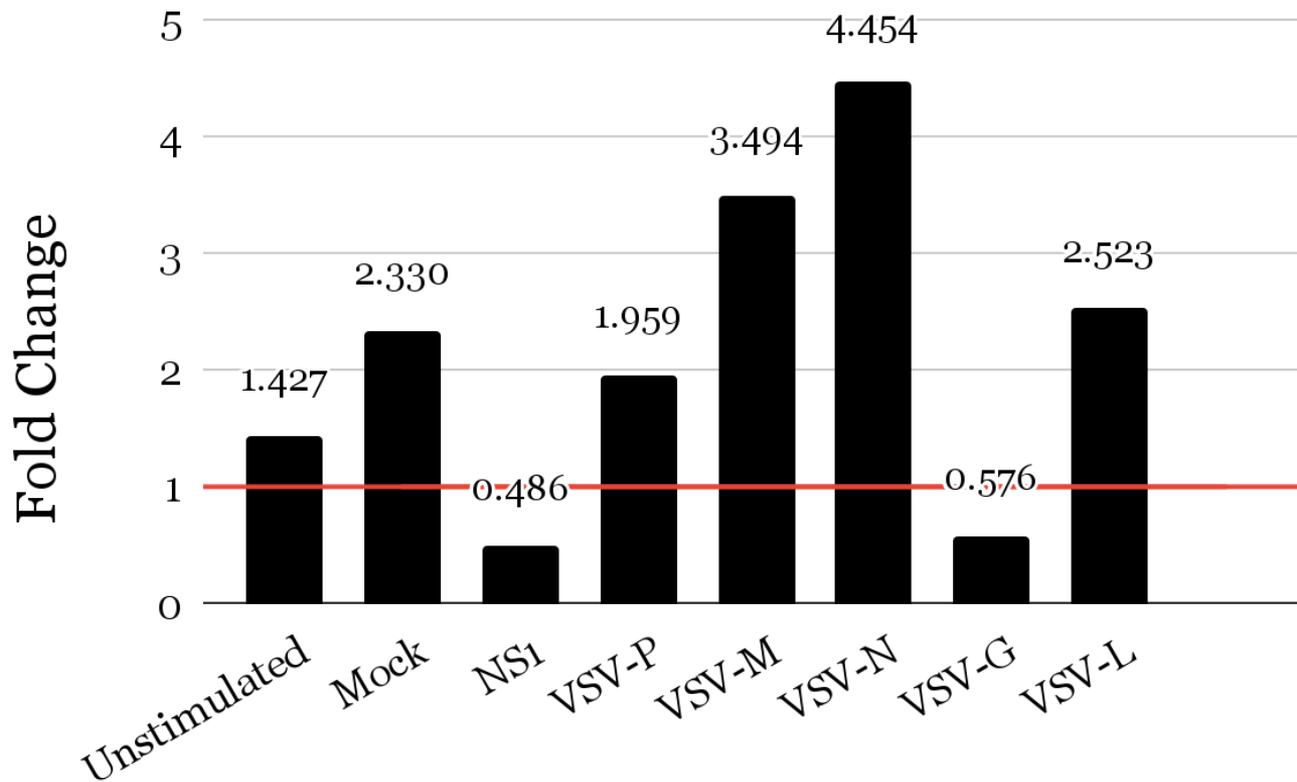
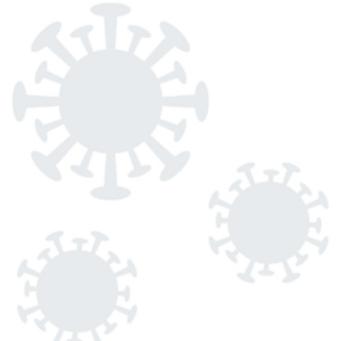


Figure 9. IFN Fold Change

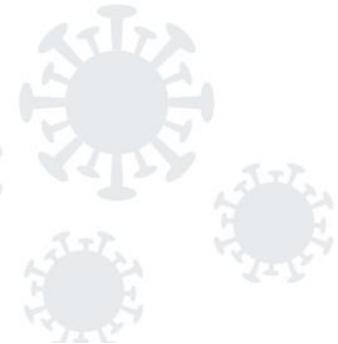
SUMMARY

- VSV-G potentially shortens 3'UTRs
- VSV-G suppresses IFN β



CURRENT IMPLICATIONS

- Change of vaccine platforms/pseudo-typing
 - Vaccine must induce immune response
- VSV-G used in gene therapy
- Common experimental protein
 - Needs more research to confirm findings



FUTURE DIRECTIONS

- Separating the G gene
 - Finding the amino acid sequence
- VSV-G in other organisms
 - Test in non-human cell lines
- Finding relationship between shortening of UTRs and immune response

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