Dynamic evolution of the macaque pulmonary proteome response to highly pathogenic avian influenza and Spanish Flu influenza infections

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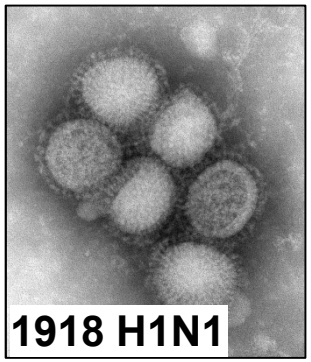
Influenza virus

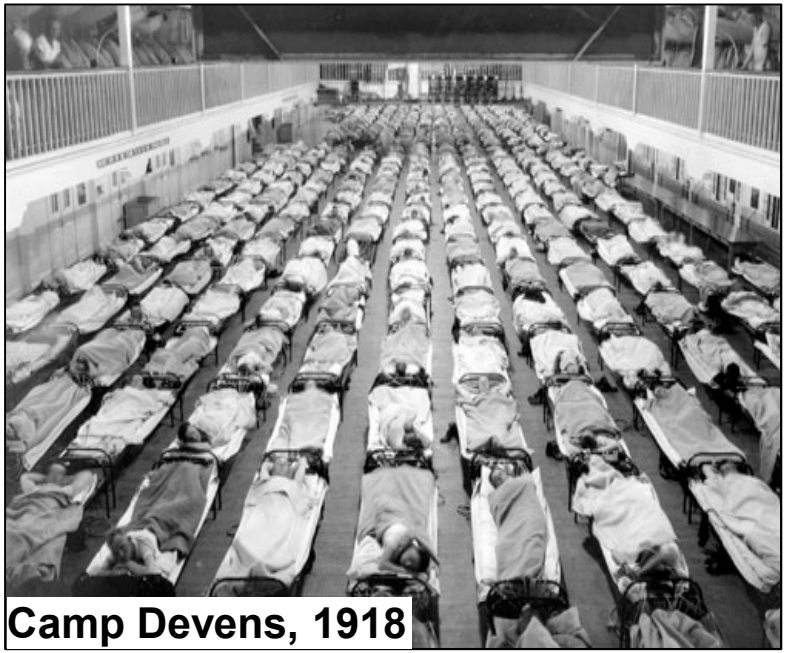
Pandemics

Name	Virus	Mortality Rate	Deaths
1. Spanish Flu	1918 H1N1	~2.5%	~50 million
2. Asian Flu	1957 H2N2	<0.1%	~2 million
3. Hong Kong Flu	1968 H3N2	<0.1%	~1 million
4. Swine Flu	2009 H1N1	<1%	~14,000

As of Jan 18, 2010
ECDC



1918 H1N1



Camp Devens, 1918

References: CDC, WHO

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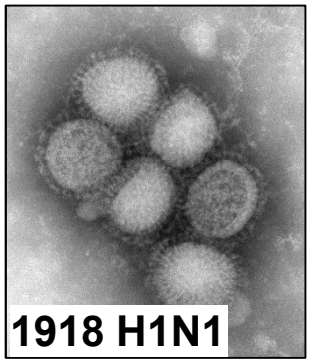
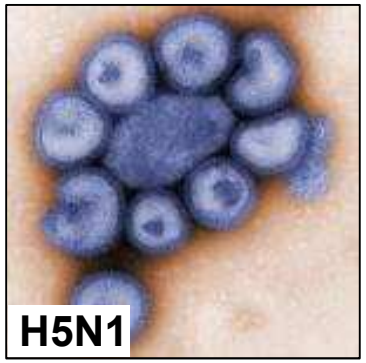
Outbreaks

Highly Pathogenic Avian Influenza (**HPAI**)	H5N1	**~60%**	492

As of Mar. 30, 2010





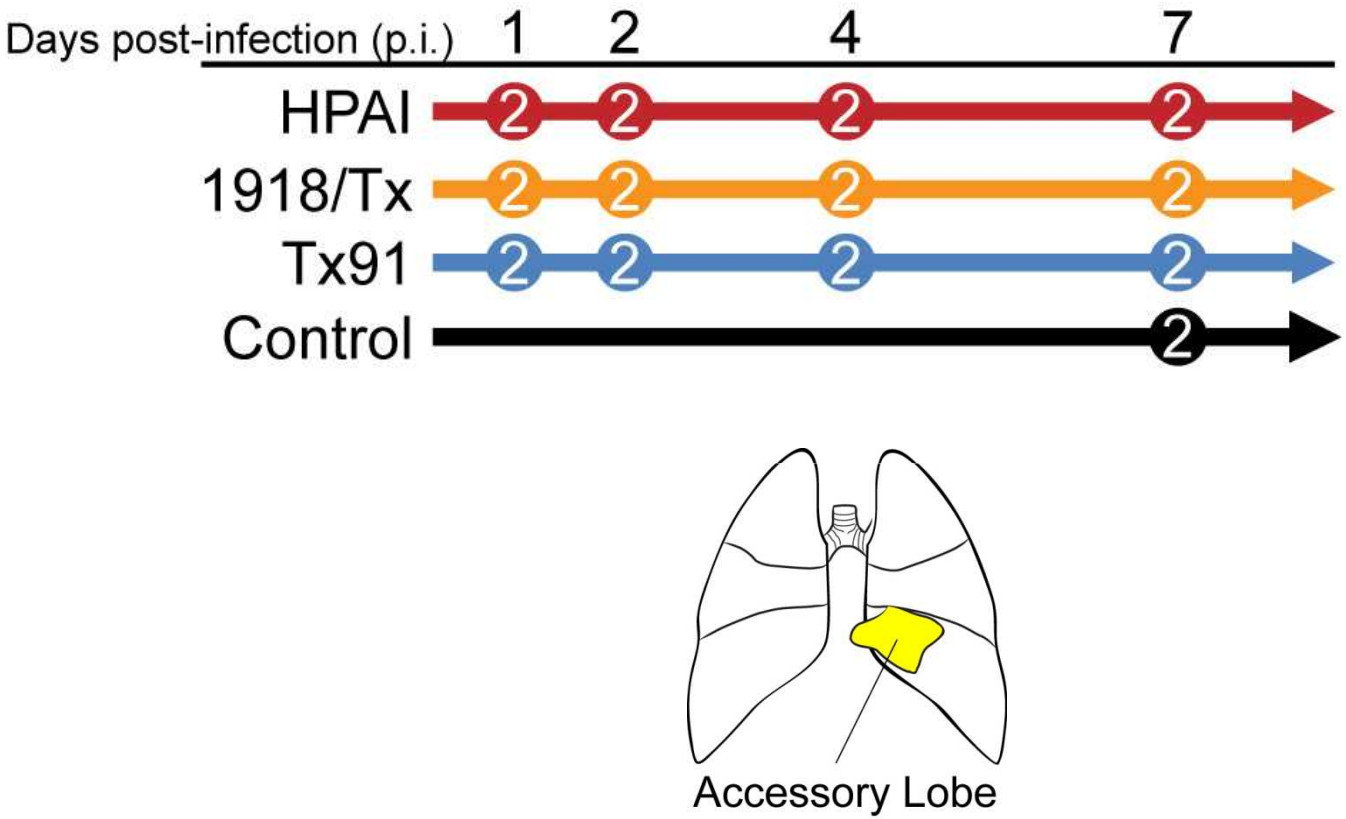
References: CDC, WHO

Presentation Outline

1. Preliminary Proteome screen of lung tissue from influenza-infected macaques.
   - **HPAI** High Virulence
   - **1918/Tx** Intermediate Virulence
   - **Tx91** Low Virulence

2. Identify proteins that increased in abundance during virus infection and directly correlated with observed disease progression in macaques.

Highest Pathology Observed During HPAI Infection



Baskin *et al.* PNAS (2009)

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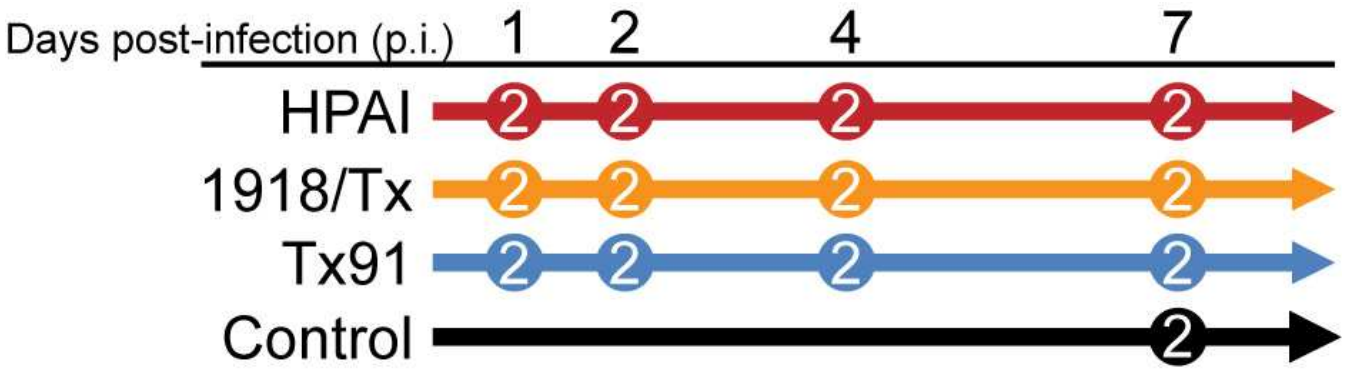

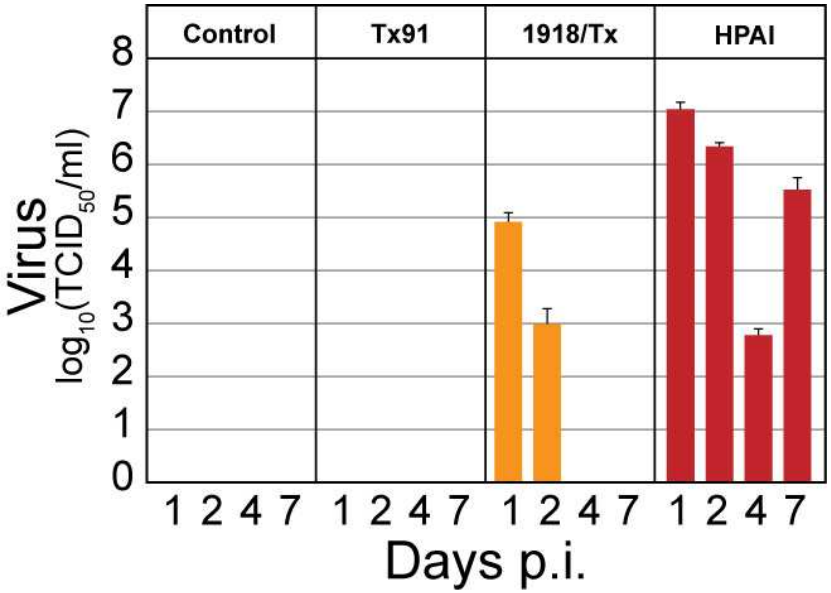

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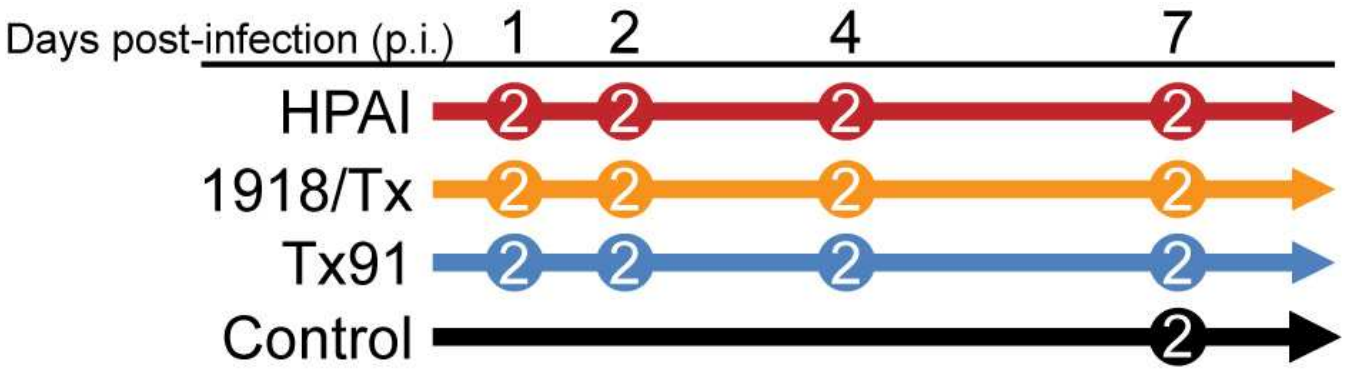

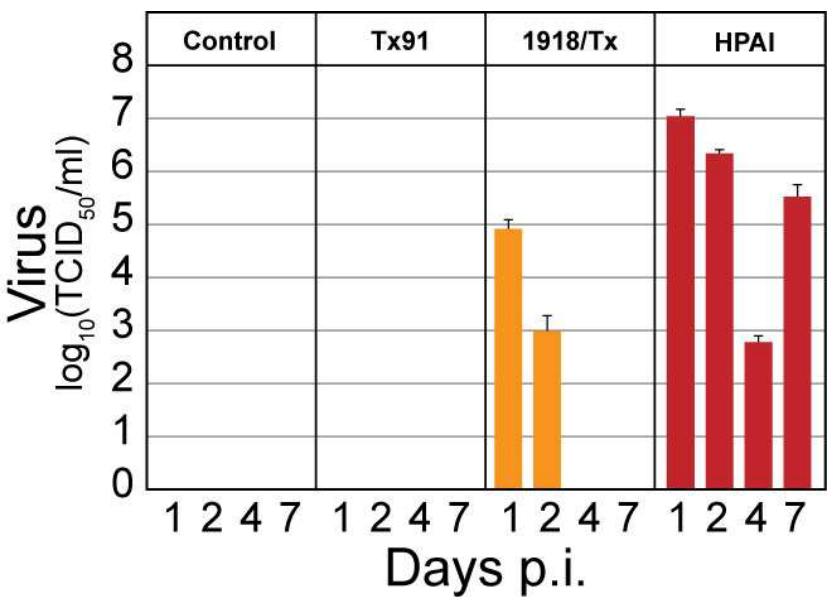

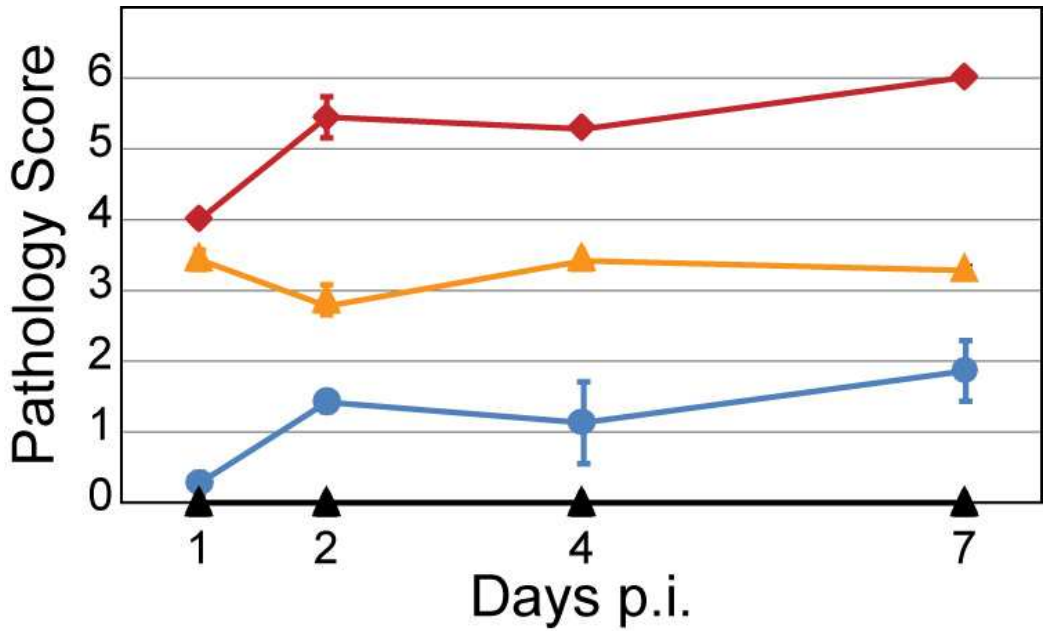

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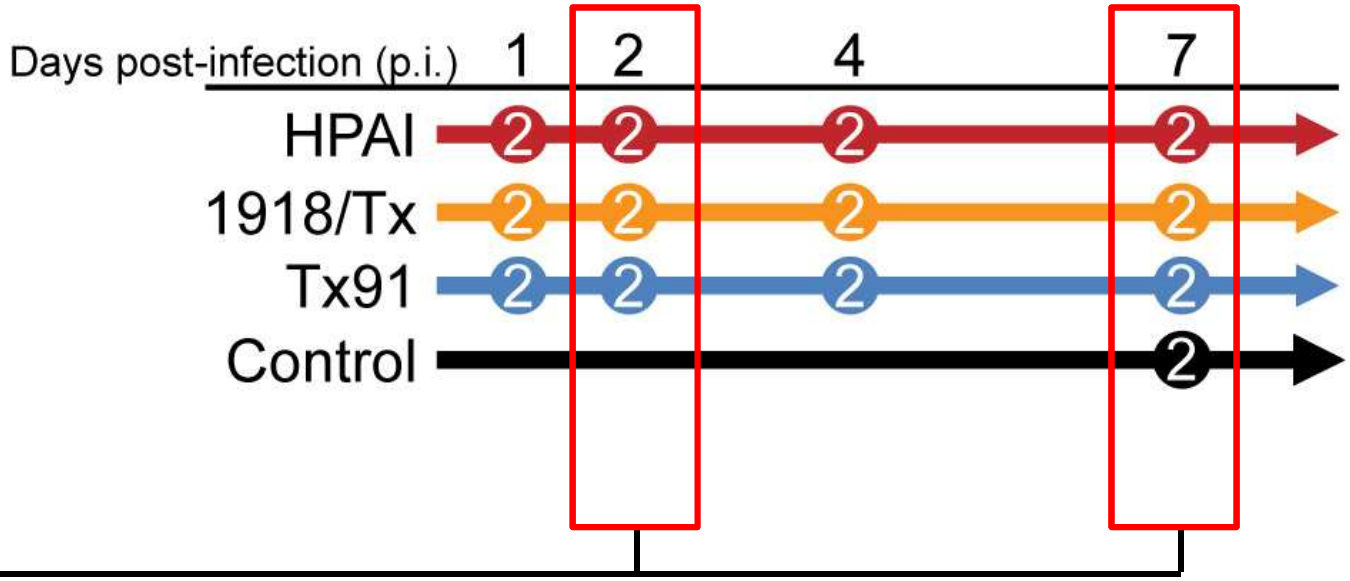
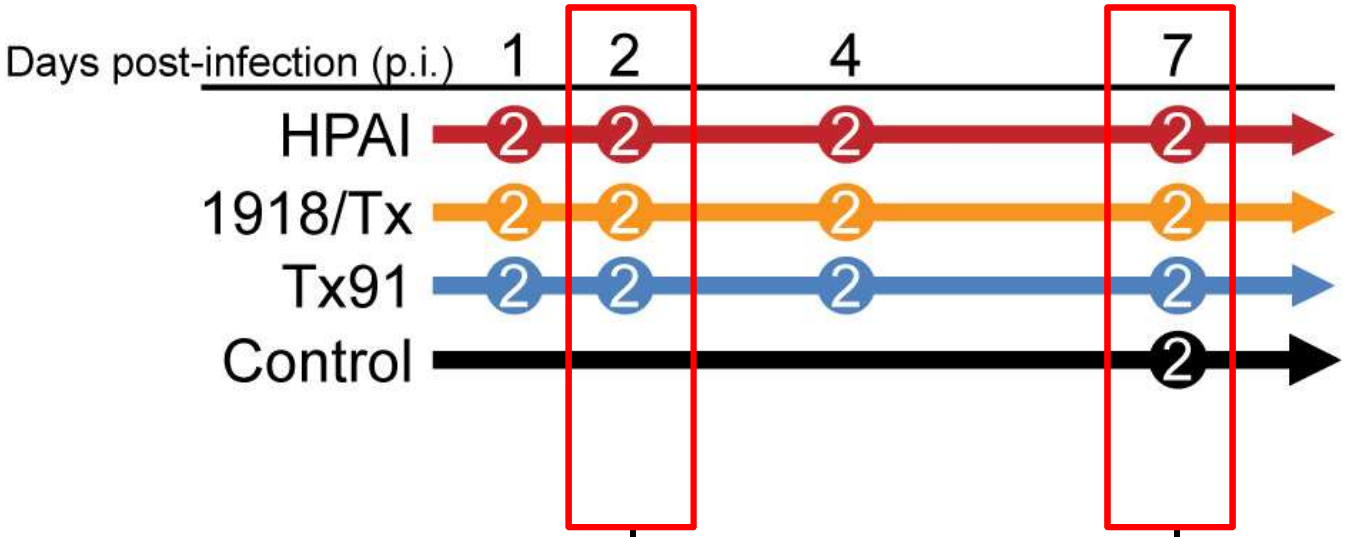
Samples Selected for Proteome Analysis



1 **Collect proteins from lung tissue**

2 **Identify Tryptic Peptides**

Viral Proteins Observed Exclusively During Infection



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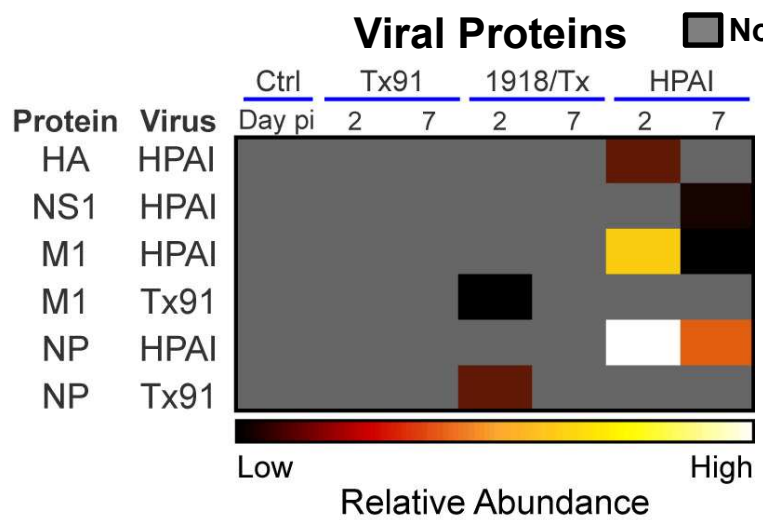
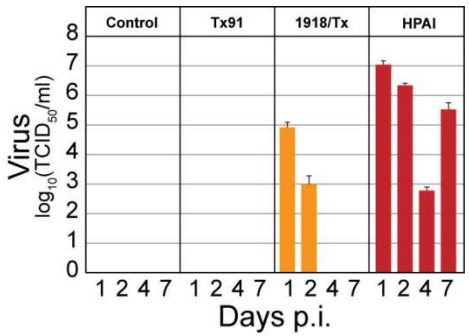
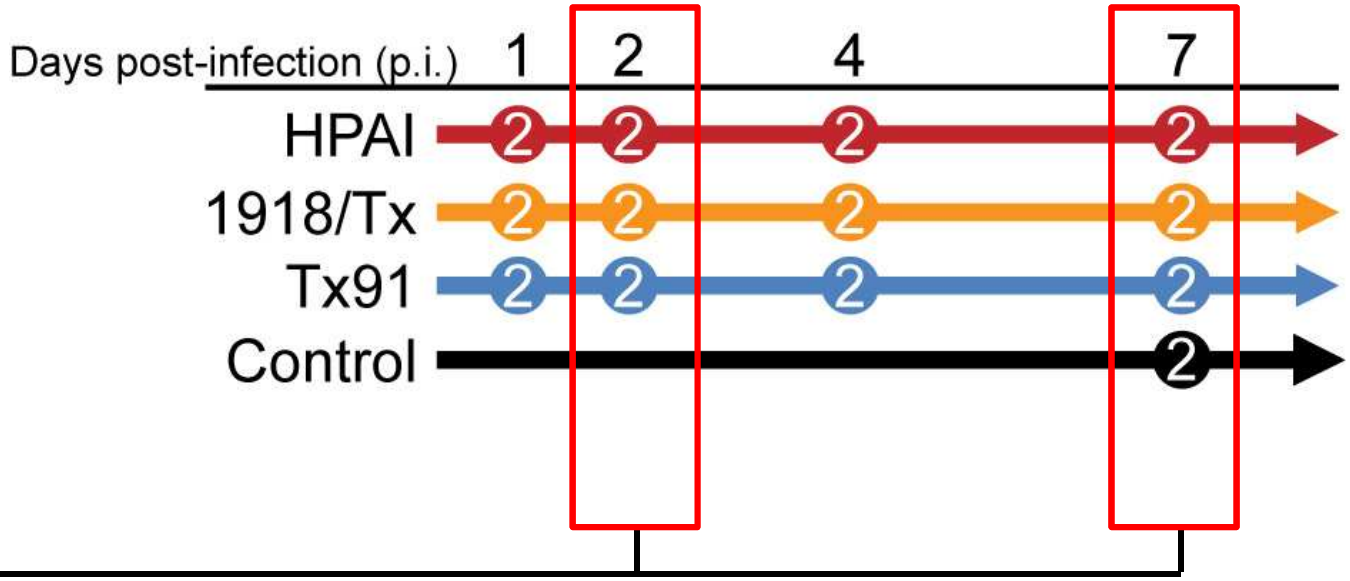




Subset of Samples Selected for Proteome Analysis



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Unique Proteins
4,276

6-Fold Difference

258
Decrease

412
Increase

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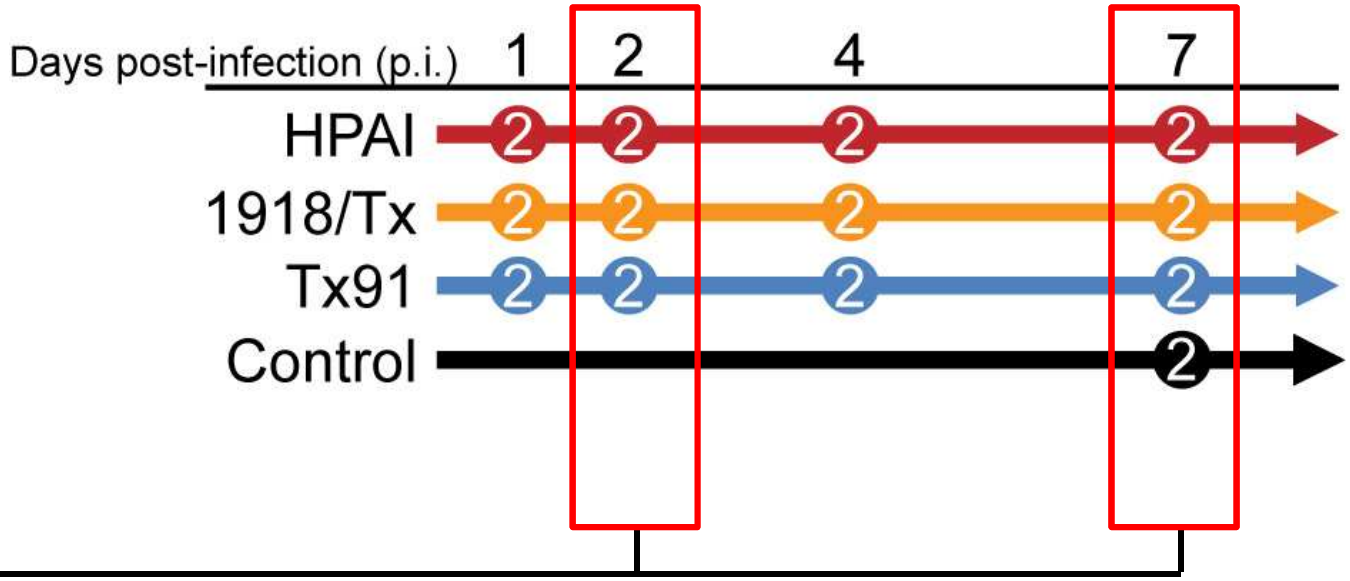

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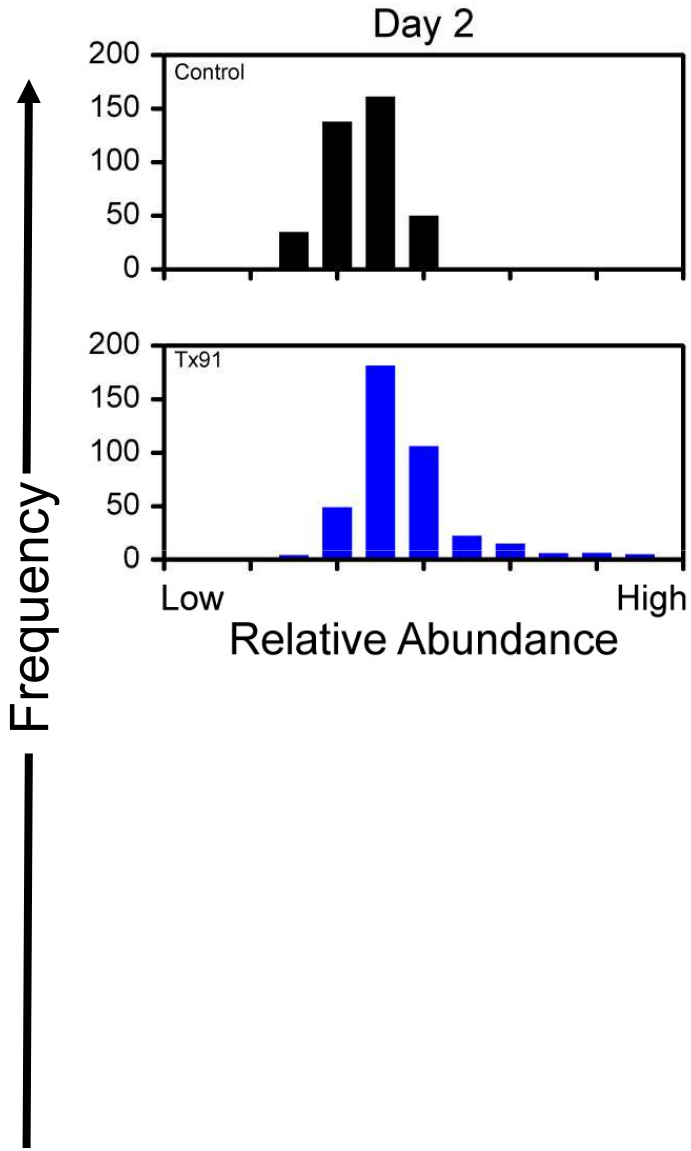
Tx91 Produces a Shift in Protein Abundance

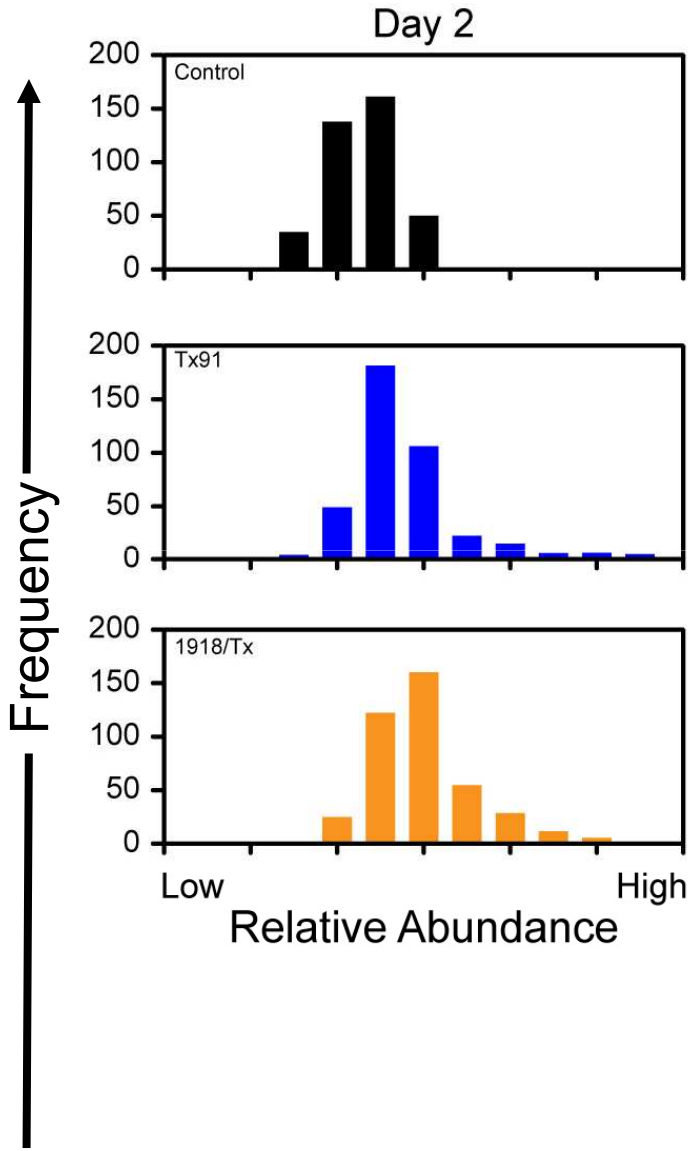


412 Proteins
Day 2

QUESTIONS

1. Do all viruses produce a shift to the right?
2. Shift same magnitude?
3. Similar distribution profiles?

1918/Tx Produces Similar Shift in Protein Abundance



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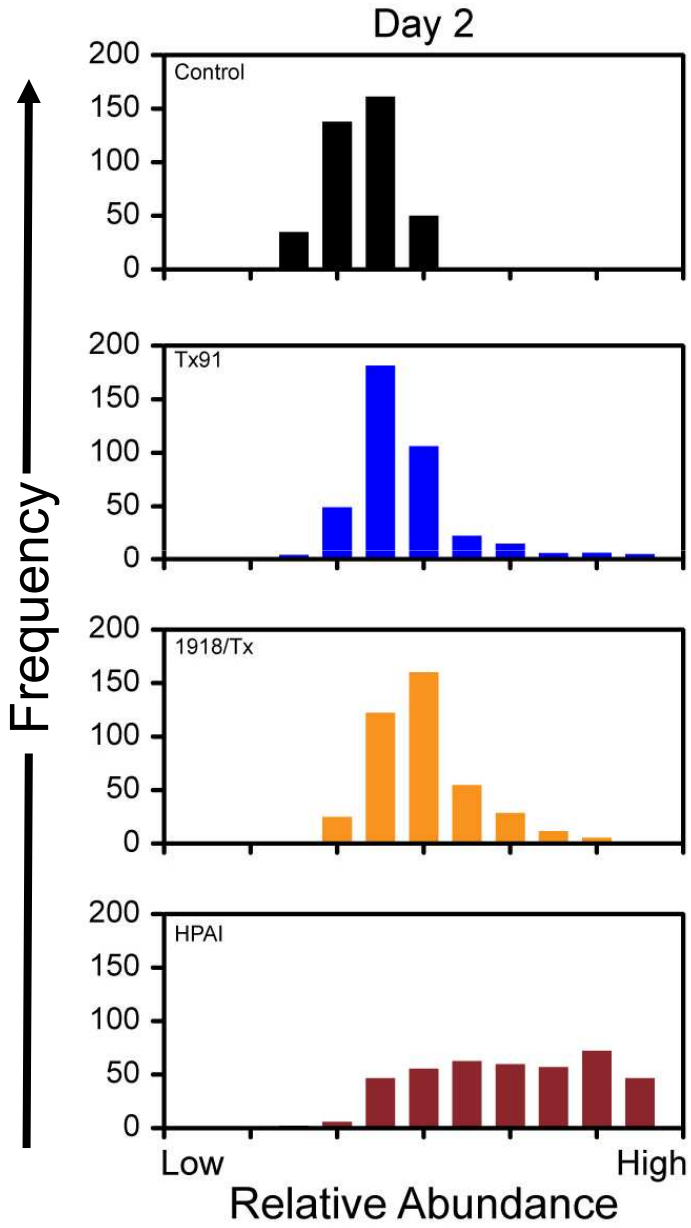
Exaggerated Shift Observed during HPAI Infection



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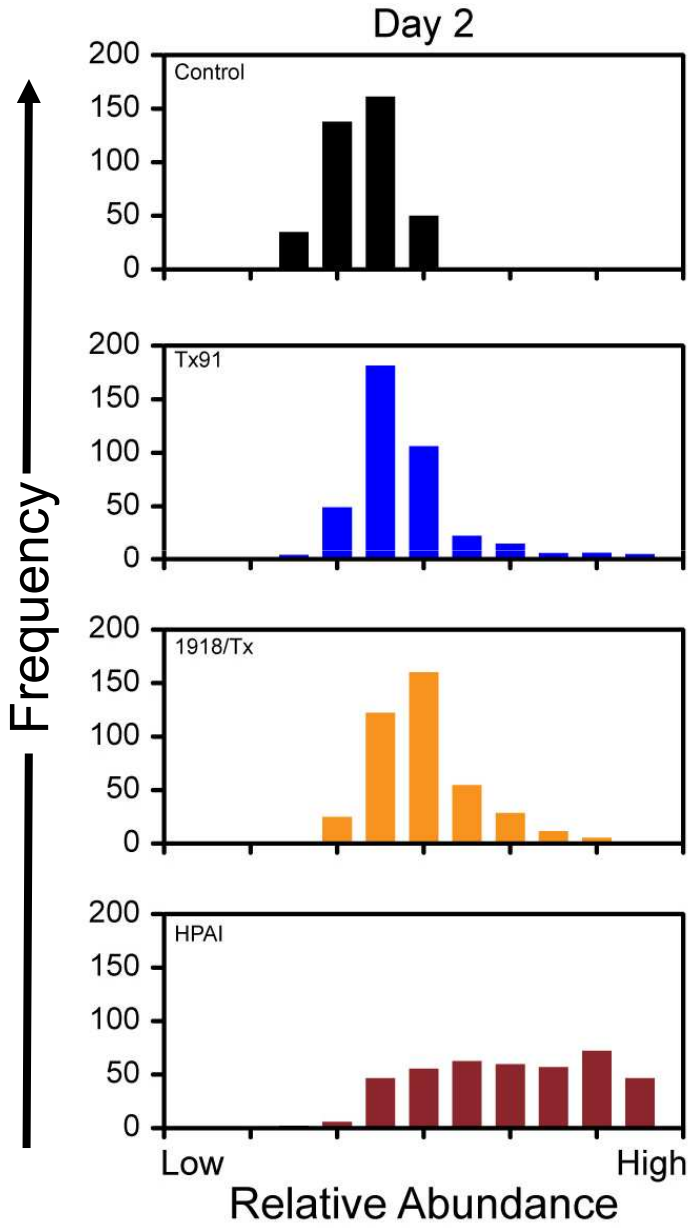
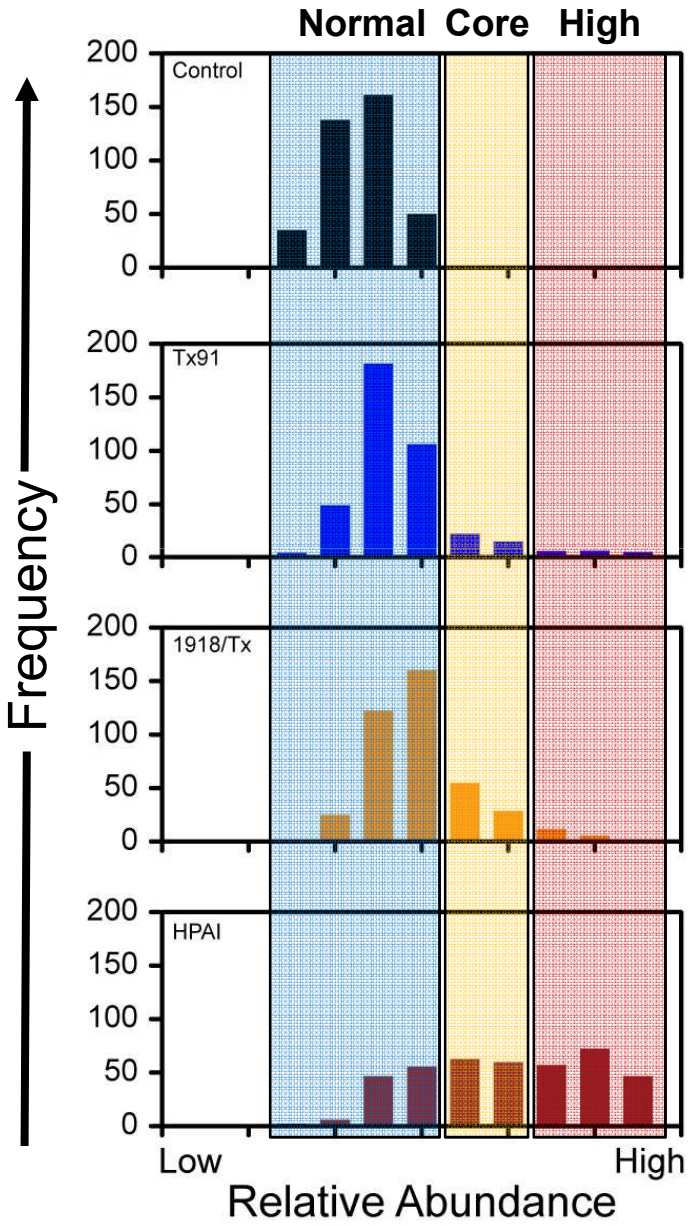


412 Proteins
Day 2

QUESTIONS

1. Do all viruses produce a shift to the right?
   -YES
2. Shift same magnitude?
   -NO
3. Similar distribution profiles?
   -NO

Distinct Expression Profiles Correlate to Viral Virulence



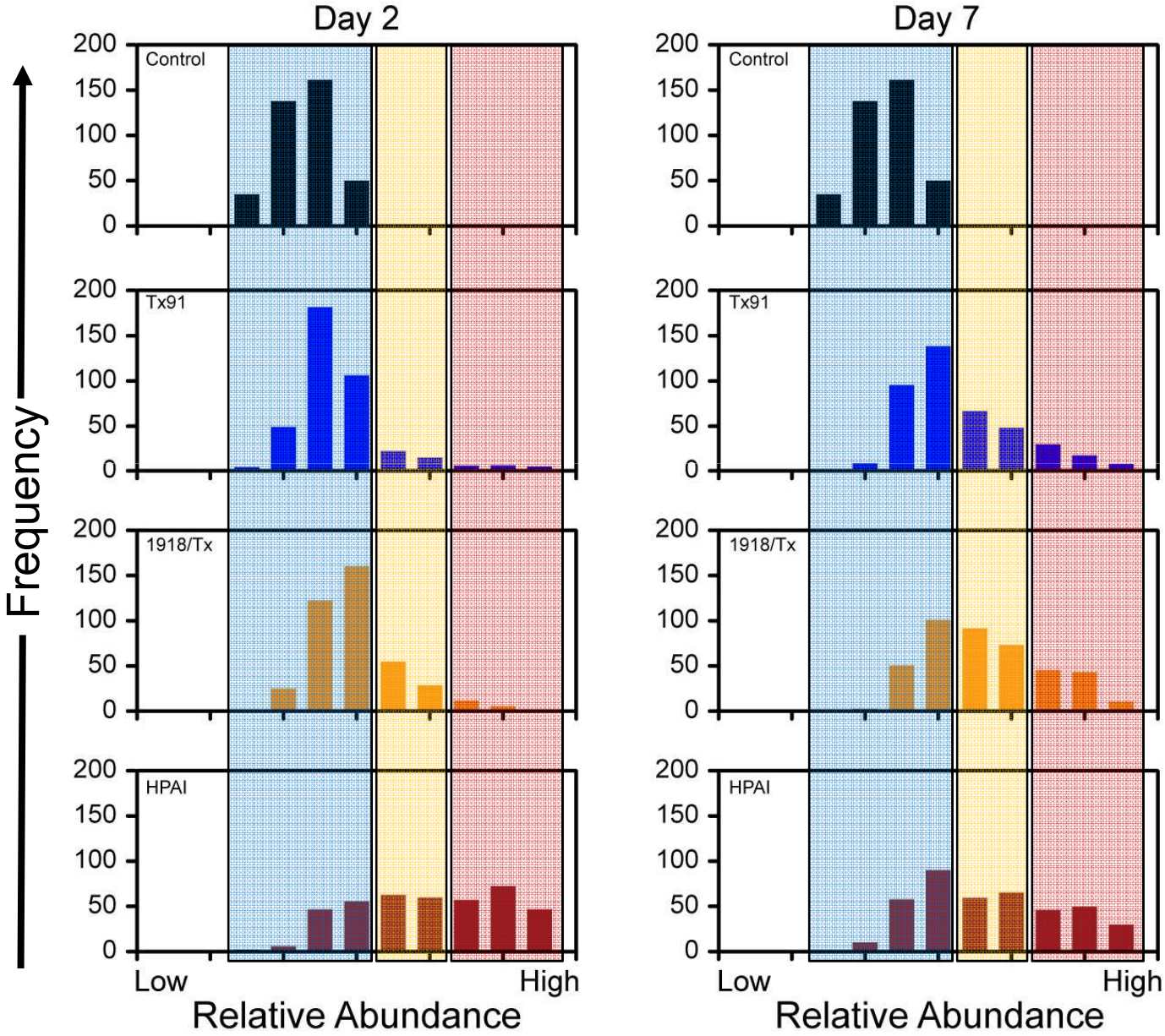
412 Proteins
Day 2

NORMAL: Healthy levels of abundance.

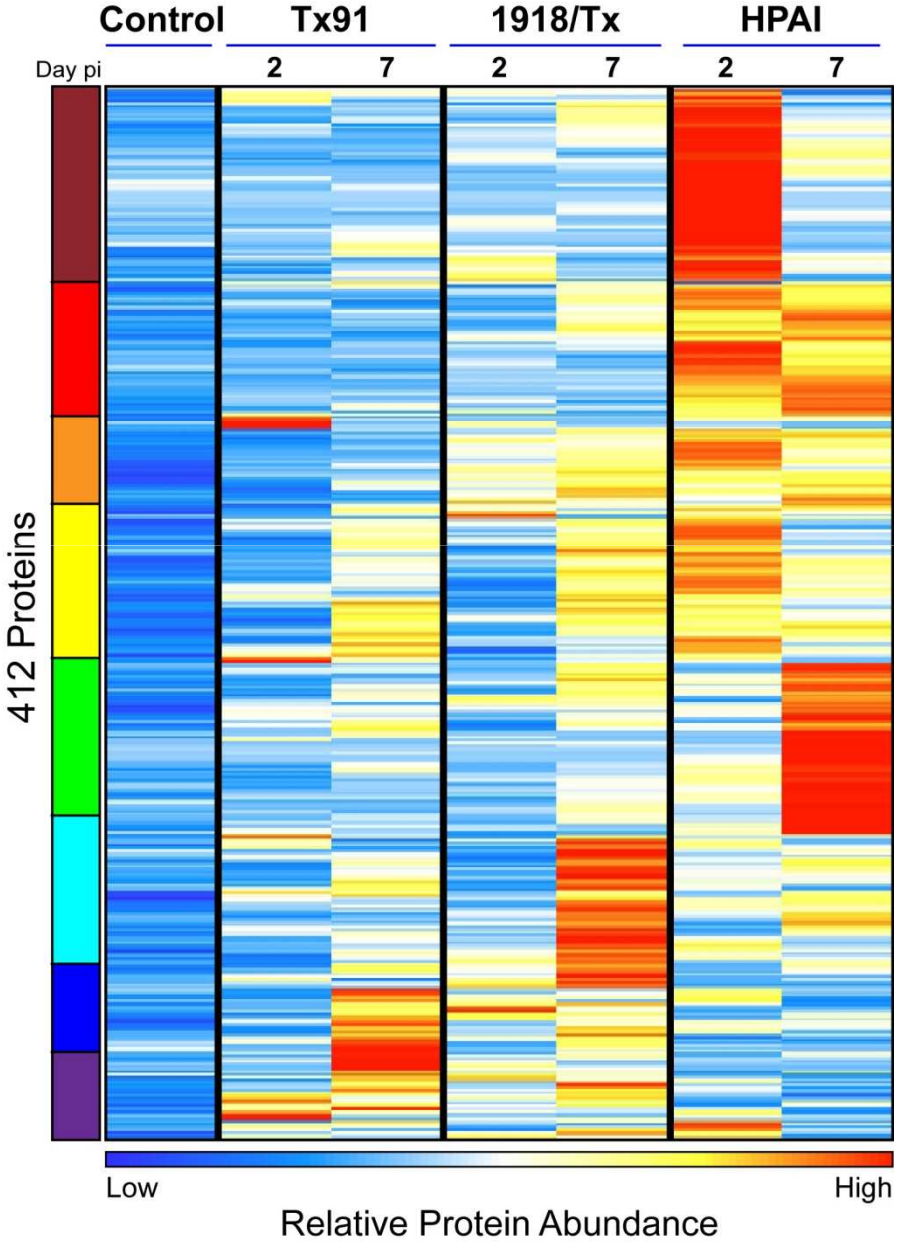
CORE: General response to viral infection

HIGH: Exaggerated response, may contribute to disease

Late Shift Towards “High Response” by 1918/Tx



8 Distinct Response Proteome Profiles to Virus



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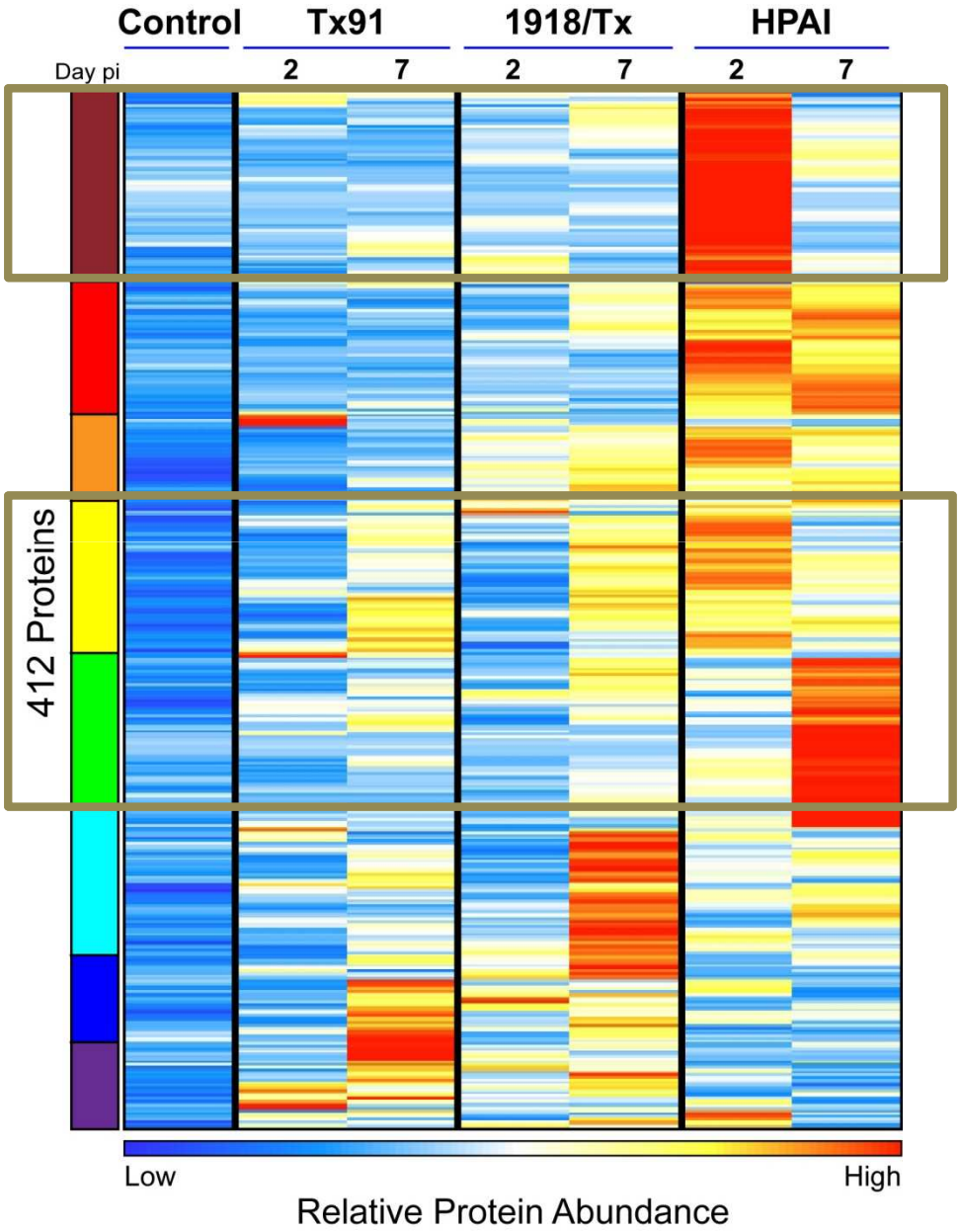

Transiently HIGH Responders

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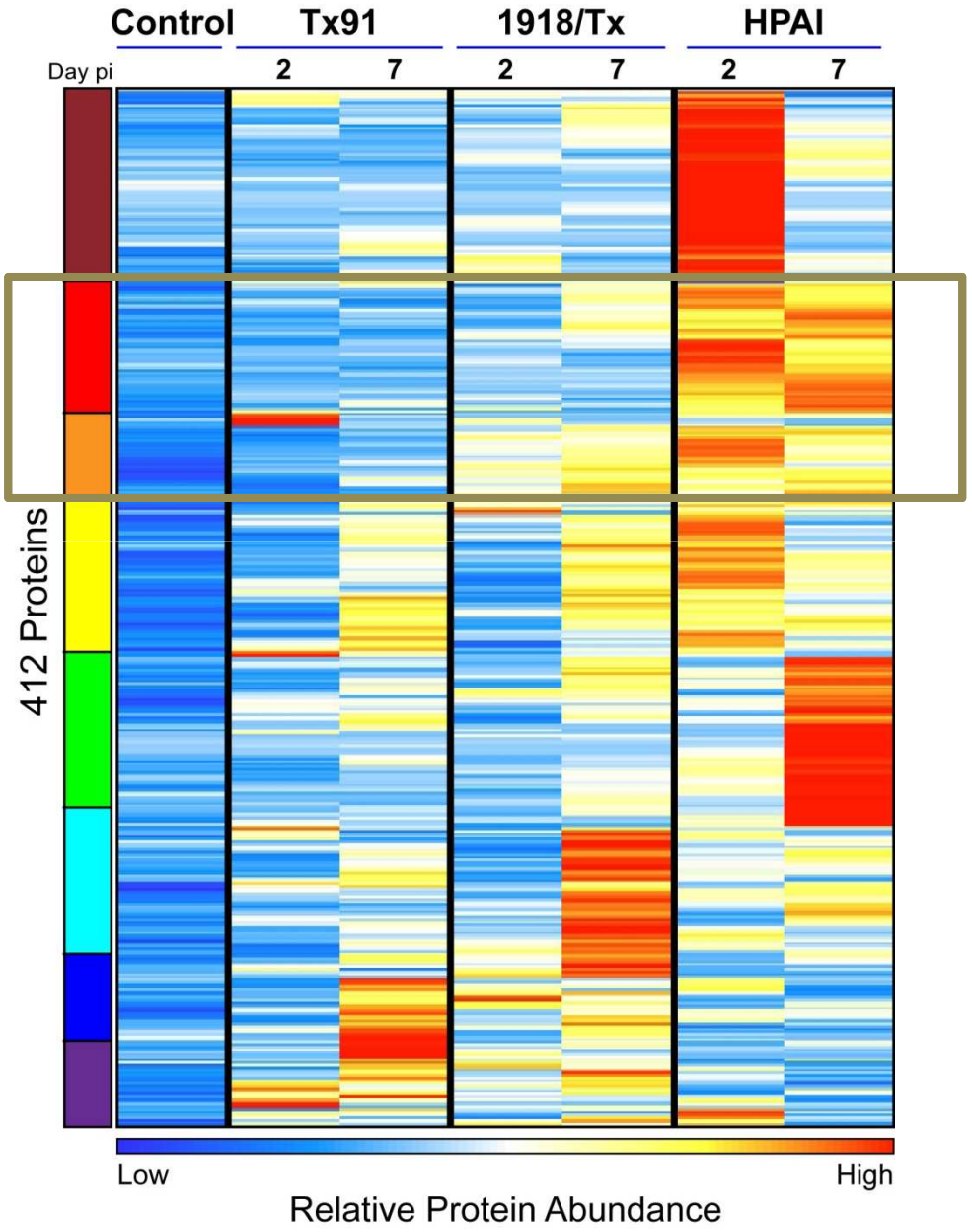
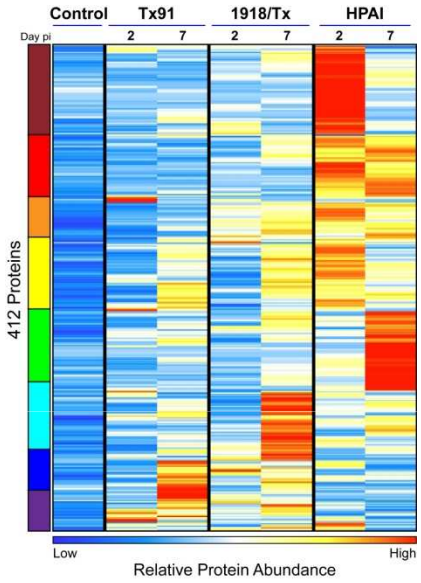
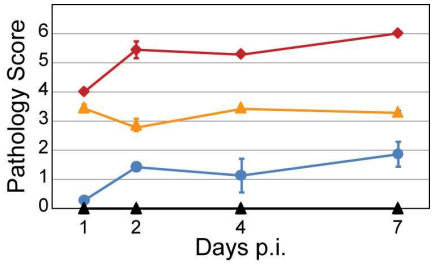
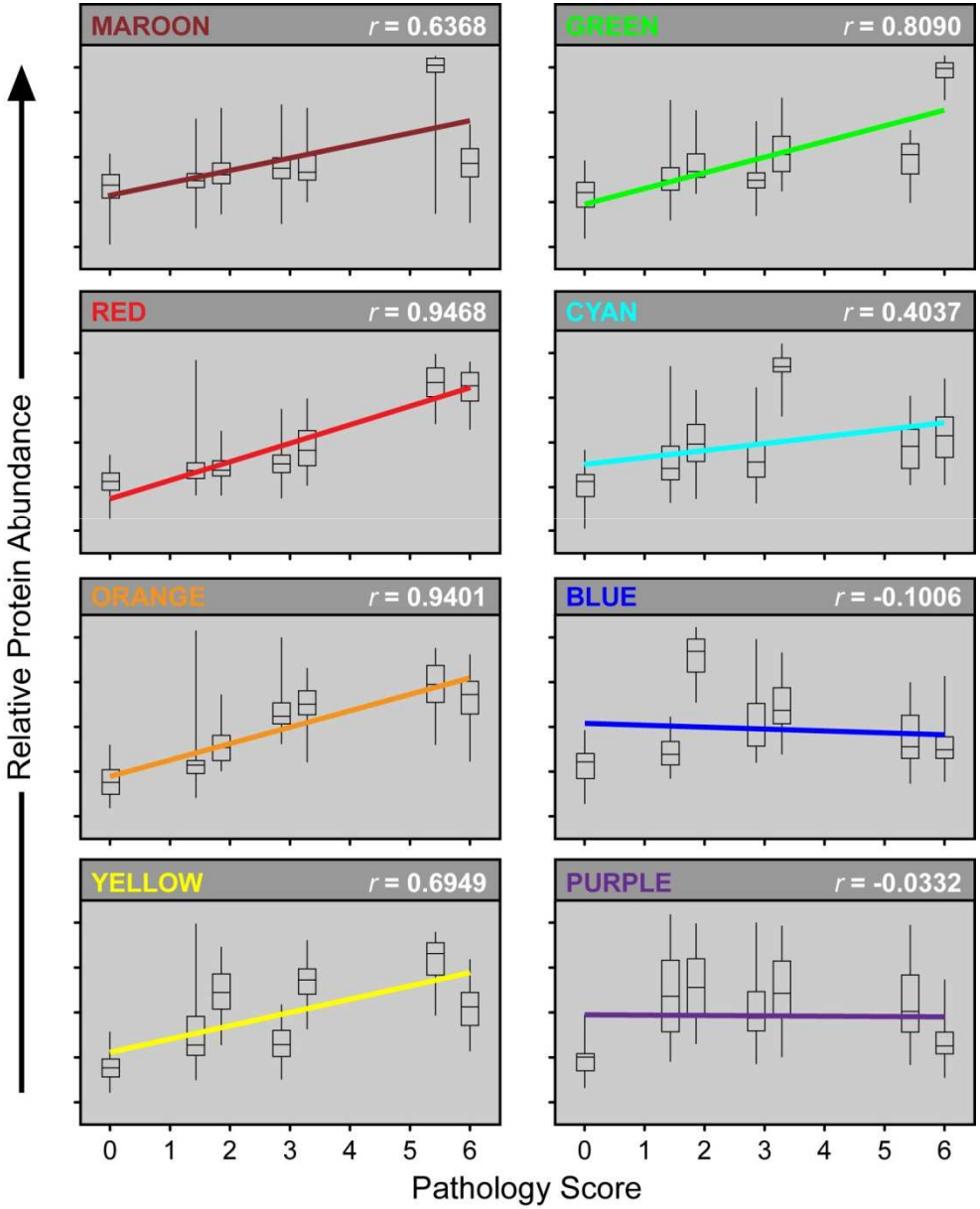


Persistently HIGH Responders

Proteome Subsets Directly Correlate with Disease Progression







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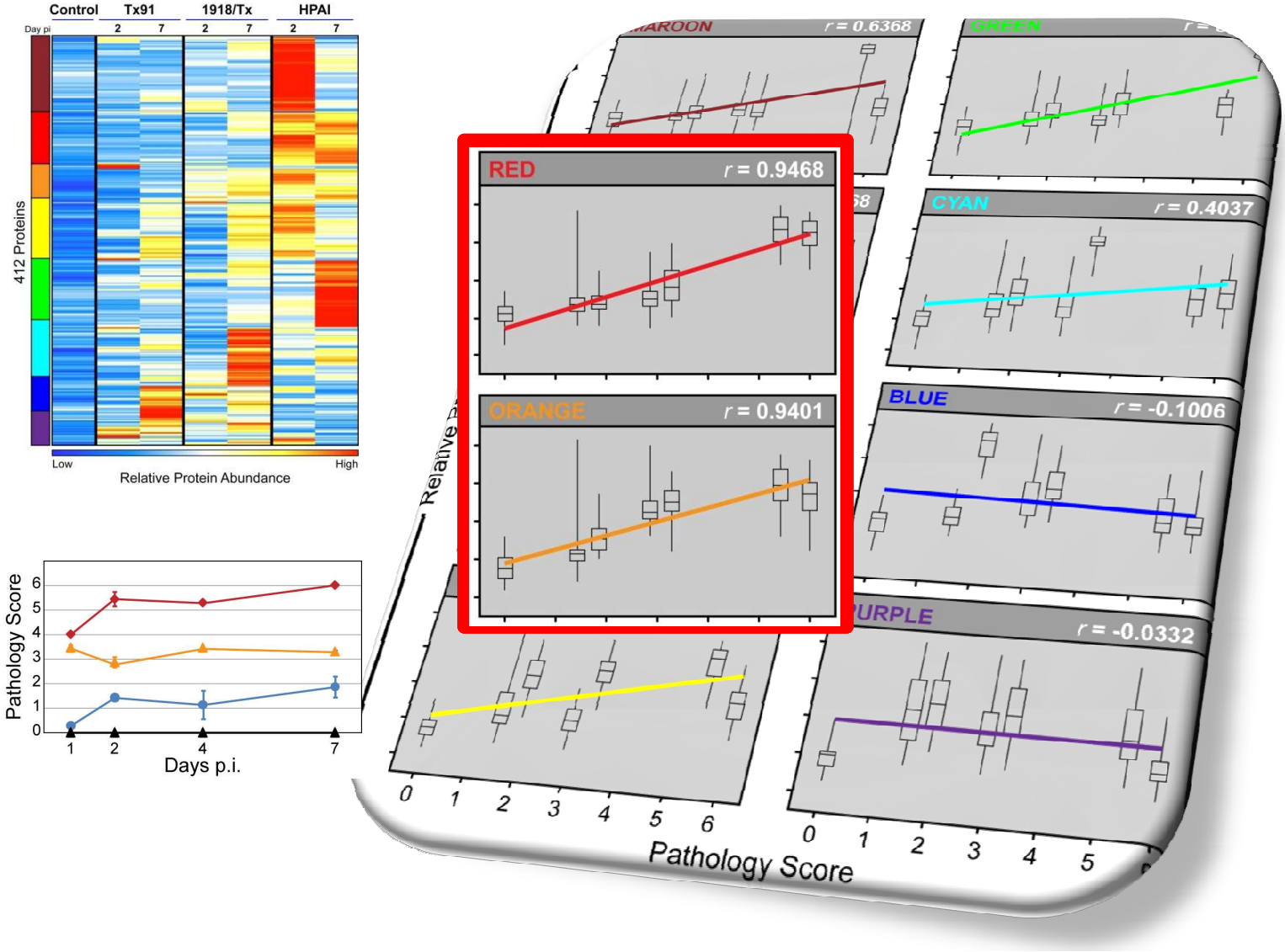
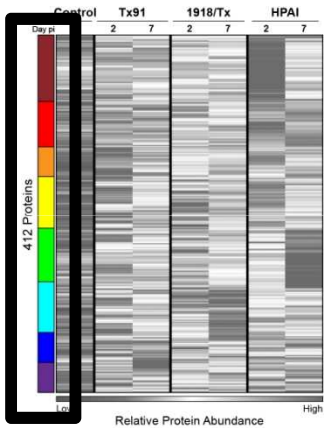


Influenza Viral Infection Triggers Antiviral RNA Receptors and IFN-Stimulated Genes & Proteins

Proteins are color-coded based on clusters



EXTRACELLULAR

CRP

IFN-α/β

IL-1β

CASP1

VIRAL RNA

TLR2

FCGR3A

CD163

ITGAL

TLR

TICAM2

TOLLIP

P58IPK

STAT1

STAT2

Multivesicular Body

Antiviral IFN-Stimulated RNA Receptors

RIG-I ISG20 OAS1 OAS3 PKR

MDA5 DHX58 OAS2 OASL ADAR

SYK

DEFA5 GZMB

PTX3

PRKCB

MAVS

DAG

KRAS

Endosome

TLR7 TLR8

TOLLIP

PLCG1

MITOCHONDRIA

CMPK2

Antiviral IFN-Stimulated Proteins

ISG15 IDO IFI44 MX1

IFIT1 IFIT2 IFIT3 MX2

CYTOPLASM

PARP14

IFN-α

IFN-β

NUCLEUS

HIST

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ISG

Inflammatory Cytokines

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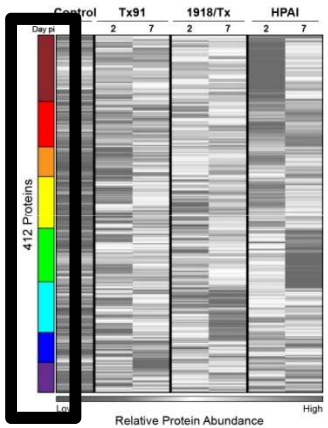

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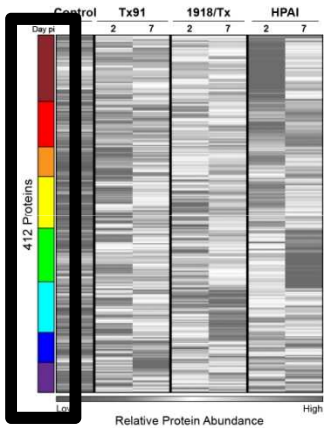

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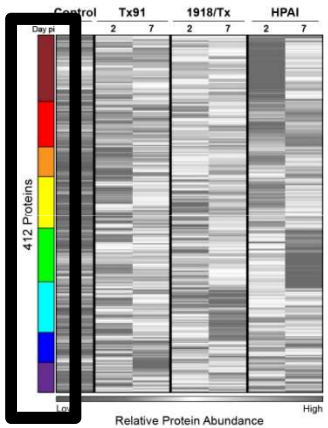

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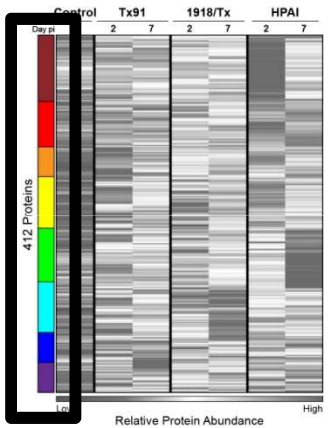

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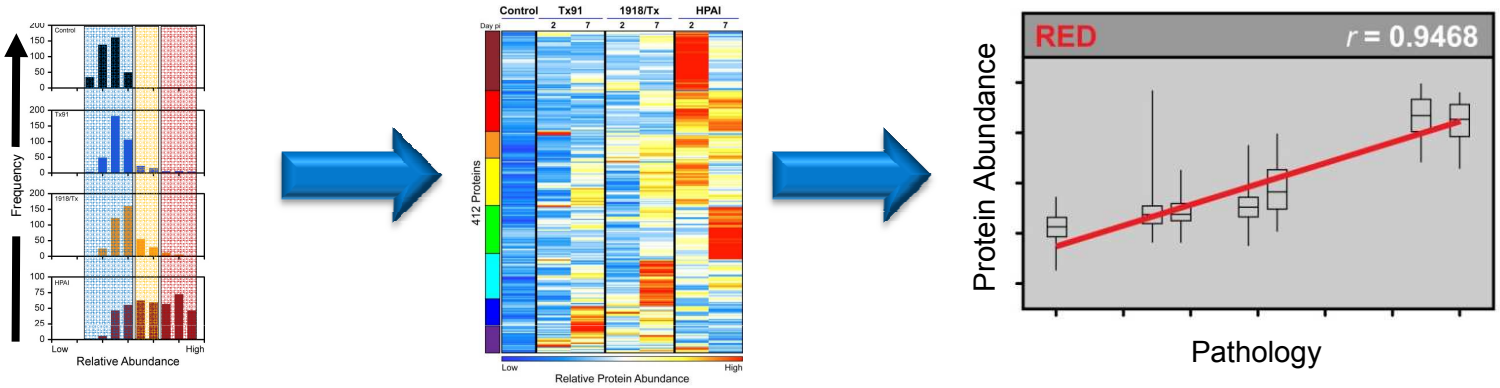
Summary

- We distinguished “Core” (general) protein responders to virus from a group of “High” responders that potentially contribute to disease progression.



- “High responders” that directly correlate with disease progression are antiviral proteins involved in innate immune defense, including MX1, PKR, and OAS.

- Future studies will focus on determining how virus replicates in the presence of these proinflammatory, antiviral proteins?

Acknowledgements

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