Leigh syndrome: A factory for making influenza?

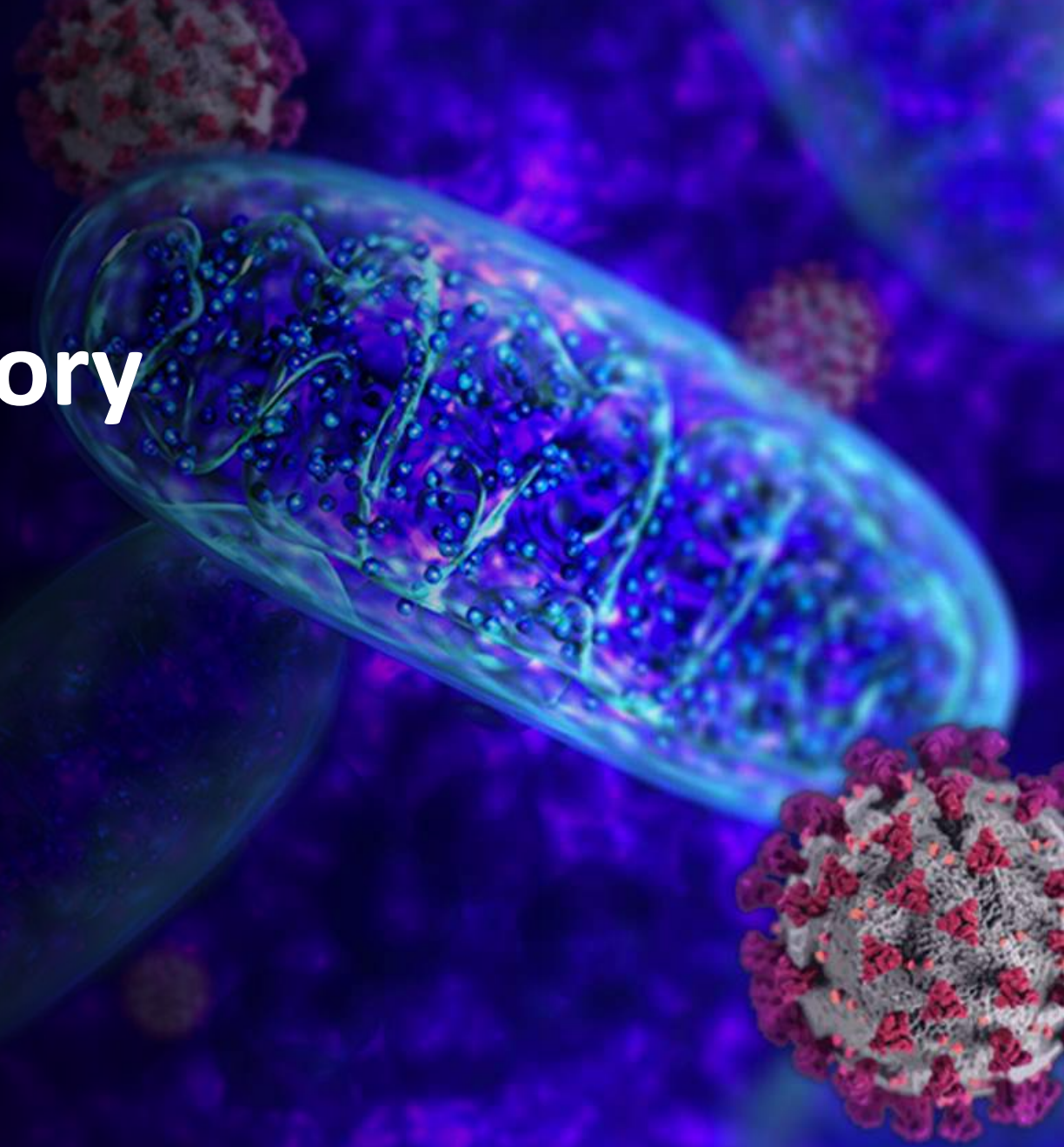
Peter J. McGuire MS, MBBCh

Investigator, Metabolic Medicine Branch

Head, Metabolism, Infection and Immunity Section

Monthly MitoExpert Series

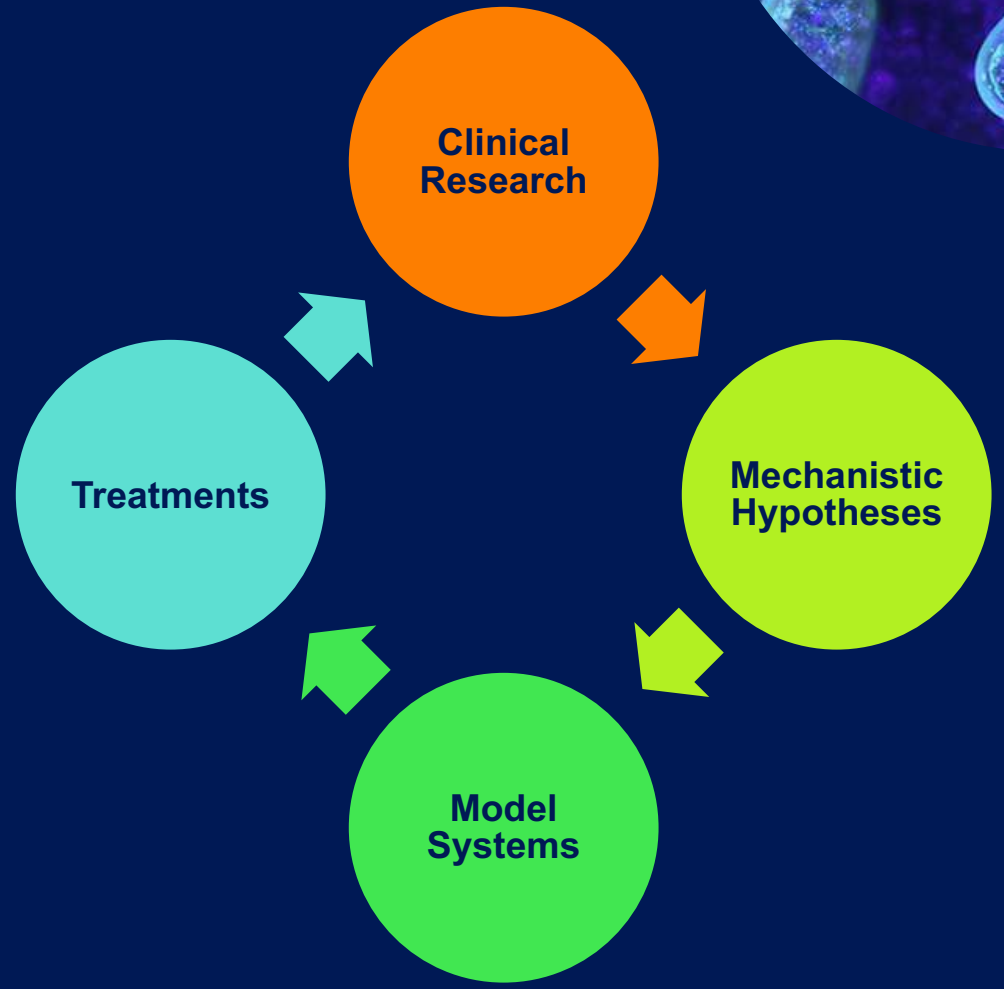
February 2, 2024



NIH National Human Genome Research Institute

MINI Section Mission

"The Metabolism, Infection and Immunity (MINI) Section aims to define the risk factors and mechanisms involved in infection-related clinical decline in children with mitochondrial disease."



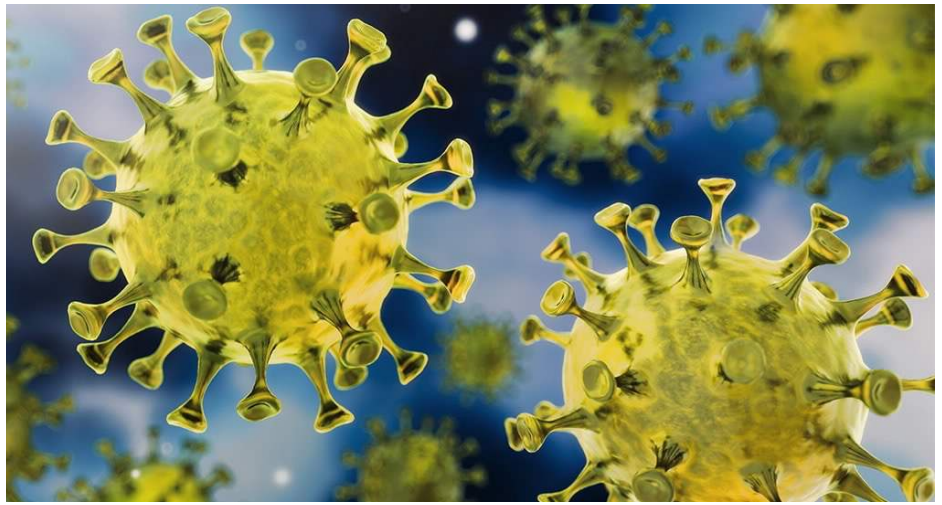
NIH NHGRI MINI

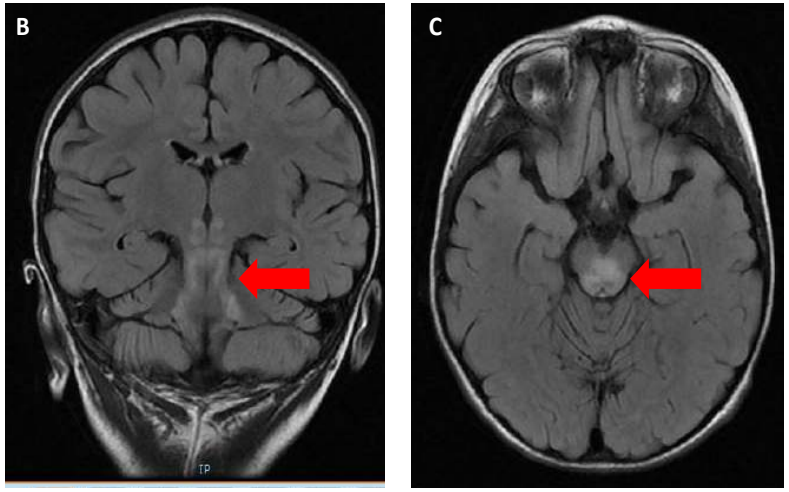
NIH MINI Study: Metabolism, Infection and Immunity (NCT01780168)

- Natural history study of infection and immunity in children with MD
  - ✓ Infection history
  - ✓ Immune function
  - ✓ Disability

Viral infection and mitochondrial disease (MtD)

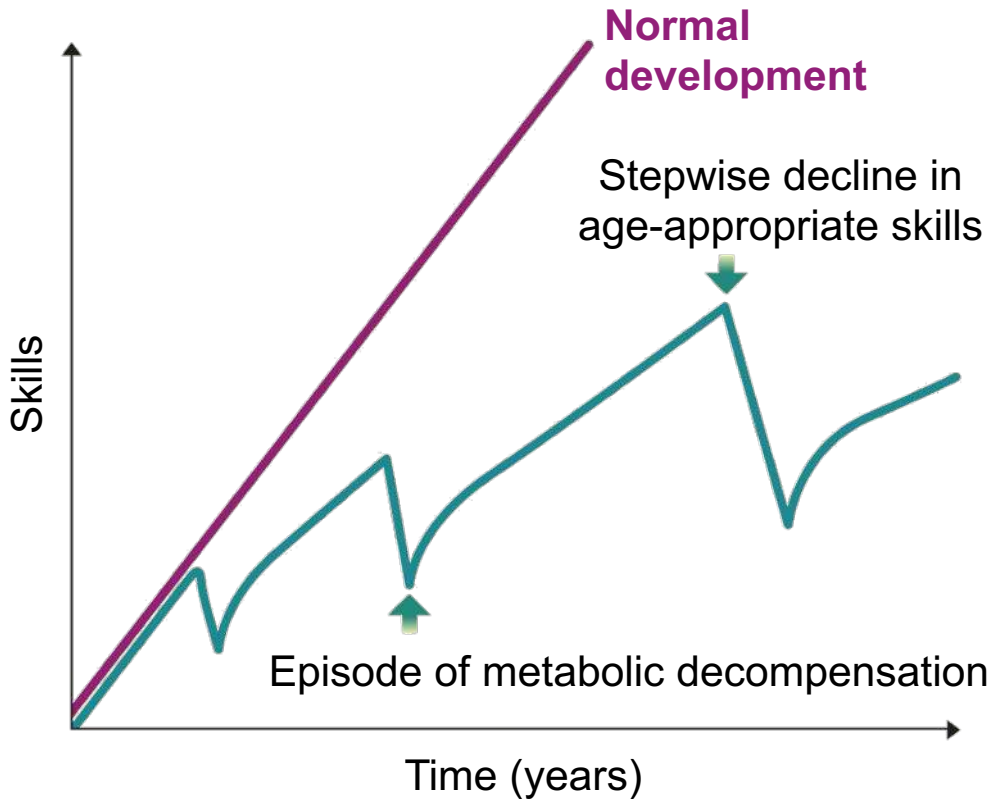
- Up to 80% of children with MtD experience recurrent infections, mostly respiratory. (Tarasenko et al., 2017)
- May cause metabolic decompensation
- Intercurrent infection is a leading cause of episodic neurodegeneration in MtD. (Edmonds et al., 2002)





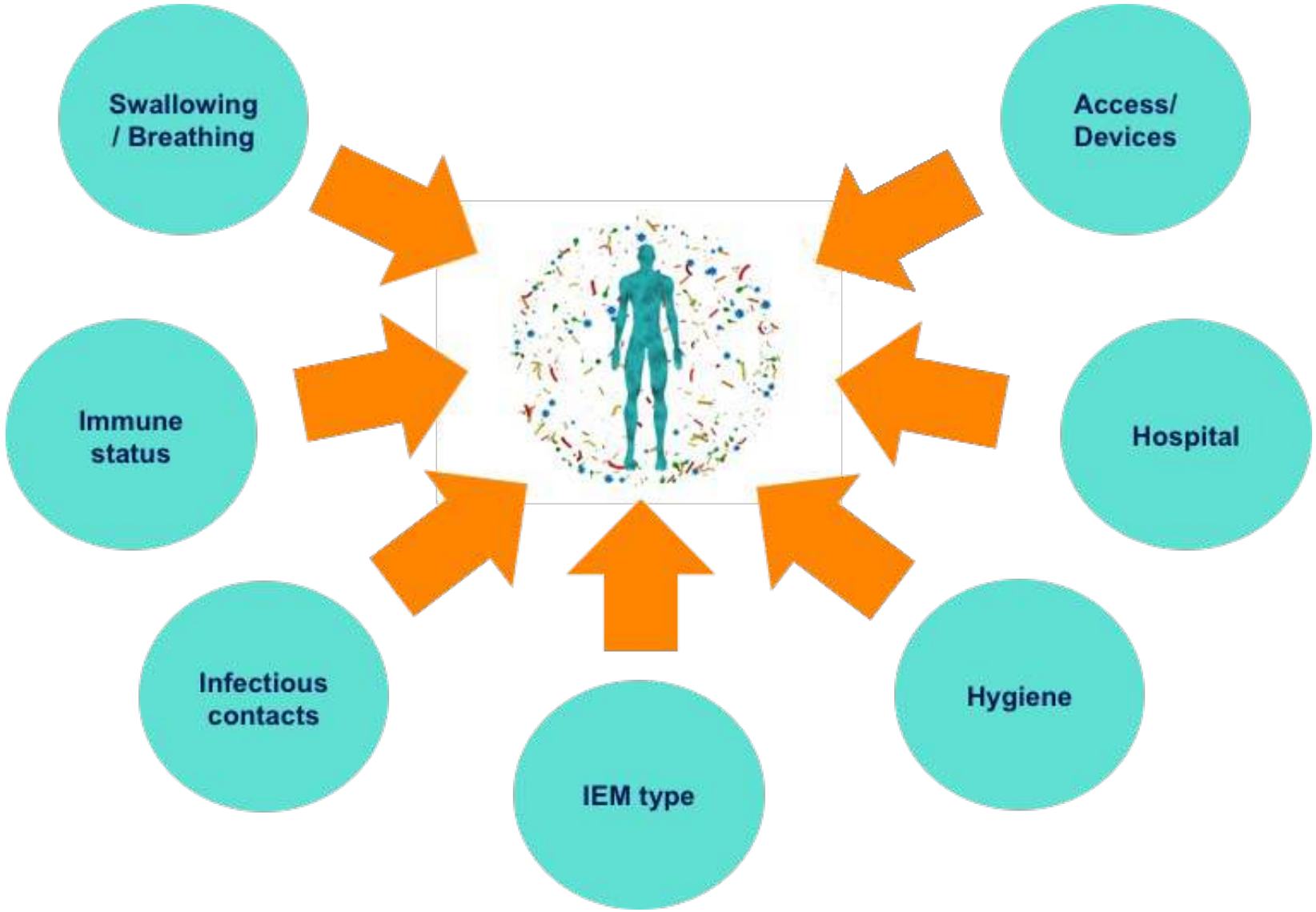
MtD and infection

- Intercurrent viral infection is a leading cause of **metabolic decompensation** in MtD patients
  - Life-threatening bioenergetic/organ failure
- Such systemic perturbations **exacerbate disease progression**



Tarasenko TN et al. (2017) *Mol Genet Metab*.; Gordon-Lipkin EM et al. (2022) *Clin Transl Med.*; Jameson et al. (2011) *Paediatr Child Health*

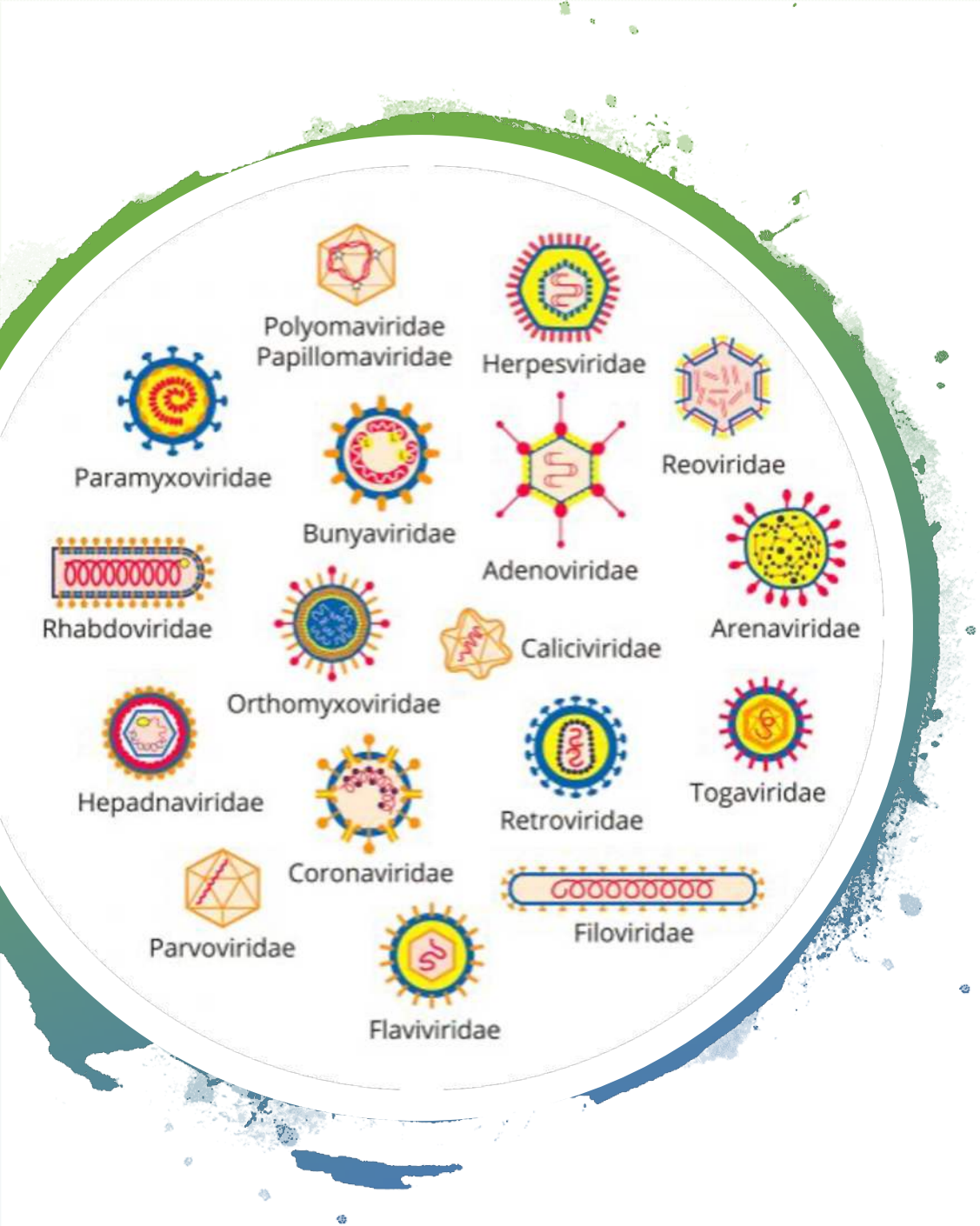
Risk factors for adverse outcomes due to infection



Viral exposures via the AntiViral Antibody Response Deconvolution Algorithm (Monaco et al., bioRxiv, 2018)

	Fam	MtD		Shared
Virus	Number (%)		P-value	
Enterovirus B	9/39 (23%)	9/17 (53%)	0.06	5/17 (29%)
Rhinovirus A	12/39 (31%)	9/17 (53%)	0.14	3/17 (18%)
Enterovirus C	3/39 (7.7%)	8/17 (47%)	**0.002**	4/17 (24%)
Rhinovirus B	7/39 (18%)	5/17 (29%)	0.48	1/17 (5.9%)
Enterovirus A	4/39 (10%)	4/17 (24%)	0.23	2/17 (12%)
Influenza B virus	6/39 (15%)	4/17 (24%)	0.47	1/17 (5.9%)
Respiratory syncytial virus	6/39 (15%)	4/17 (24%)	0.47	3/17 (18%)
Enterovirus D	3/39 (7.7%)	3/17 (18%)	0.35	1/17 (5.9%)
Human mastadenovirus D	0/39 (0.0%)	3/17 (18%)	**0.02**	3/17 (18%)
SARS related coronavirus	3/39 (7.7%)	3/17 (18%)	0.35	1/17 (5.9%)

Gordon-Lipkin et al., *Clin Trans Med*, 2022



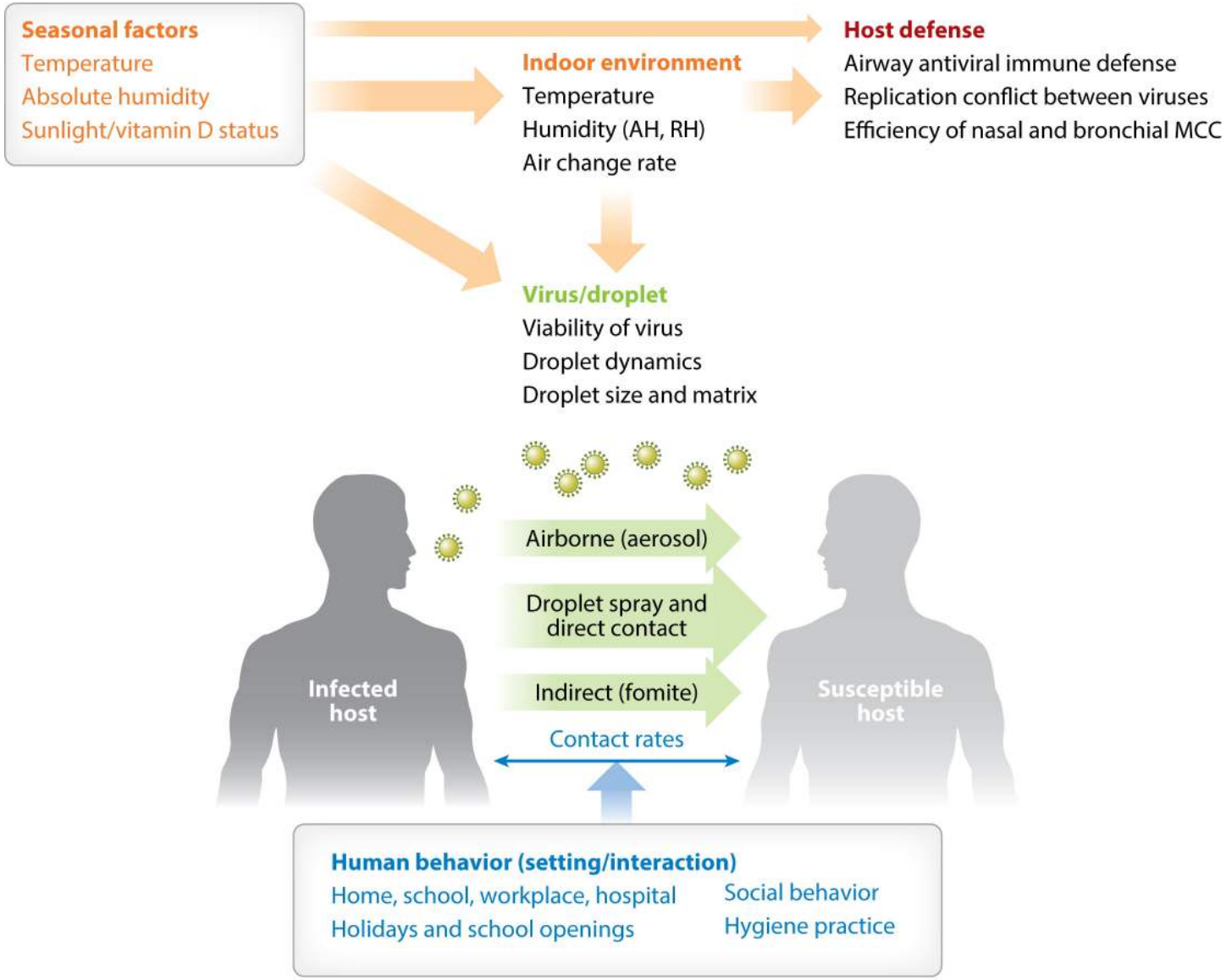
What are viruses?

- Submicroscopic infectious agents
- Infect all life forms
- Needs a living cell to replicate
- Core material of DNA or RNA

How are viruses transmitted?

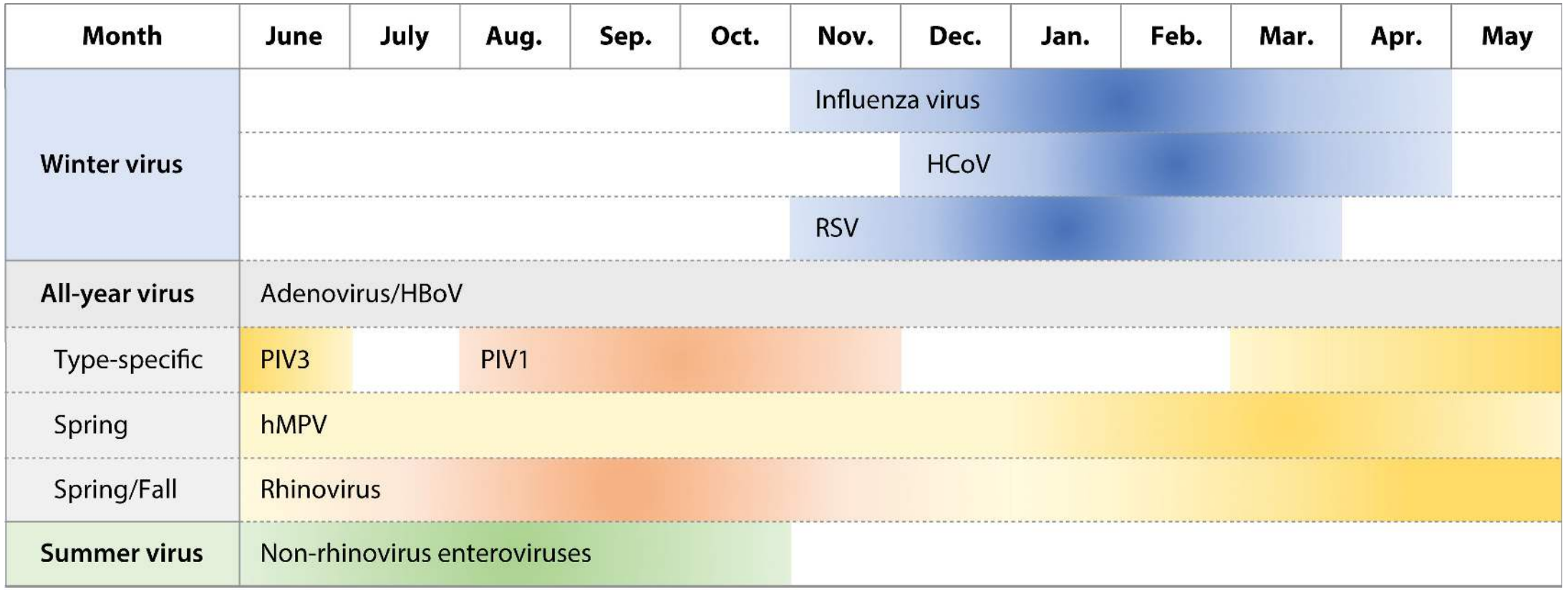
- Touch – e.g. SARS-Co-V2
- Respiratory droplets – e.g. SARS-Co-V2, influenza
- Direct contact – e.g. Epstein-Barr virus, human papilloma virus
- Blood – e.g. HIV, Hepatitis C, Ebola
- Contaminated food or water – e.g. Noroviruses
- Insects – e.g. Zika (mosquitoes)
- Childbirth – e.g. cytomegalovirus

Factors that affect virus transmission



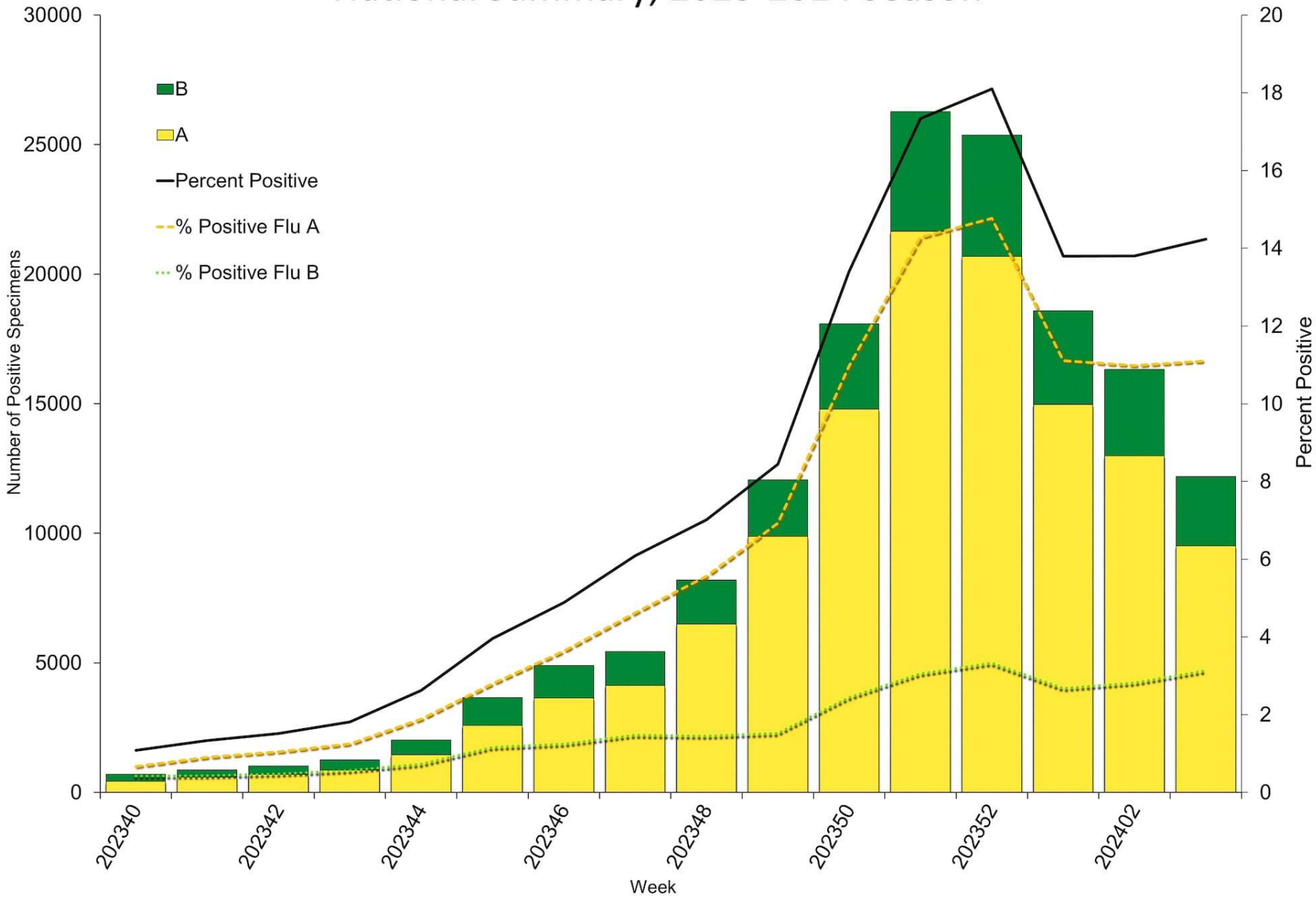
Moriyama M, et al. 2020.
Annu. Rev. Virol. 7:83–101

Seasonality of respiratory viruses

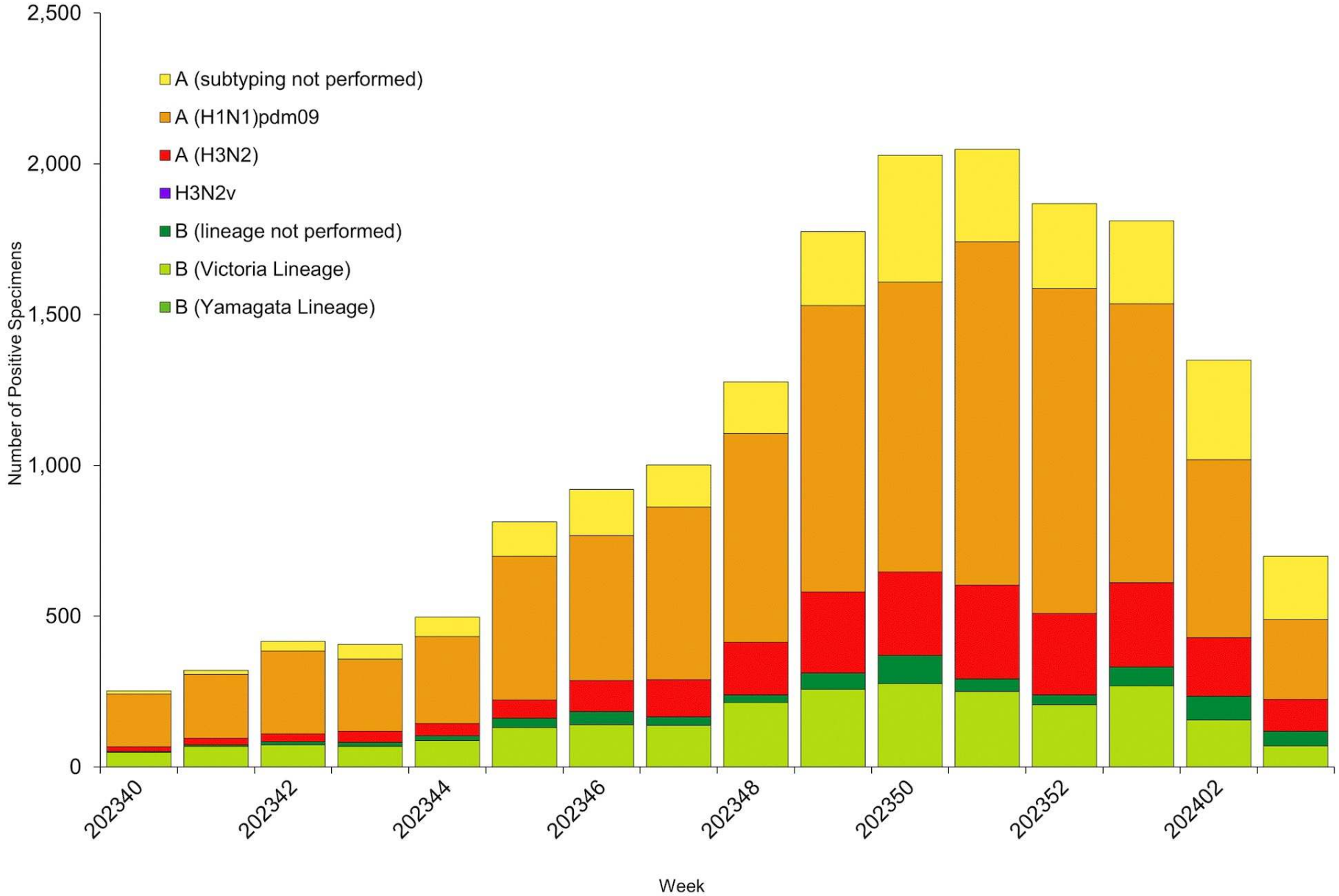


Moriyama M, et al. 2020.
Annu. Rev. Virol. 7:83–101

Influenza Positive Tests Reported to CDC by U.S. Clinical Laboratories, National Summary, 2023-2024 Season



Influenza Positive Tests Reported to CDC by U.S. Public Health Laboratories, National Summary, 2023-2024 Season

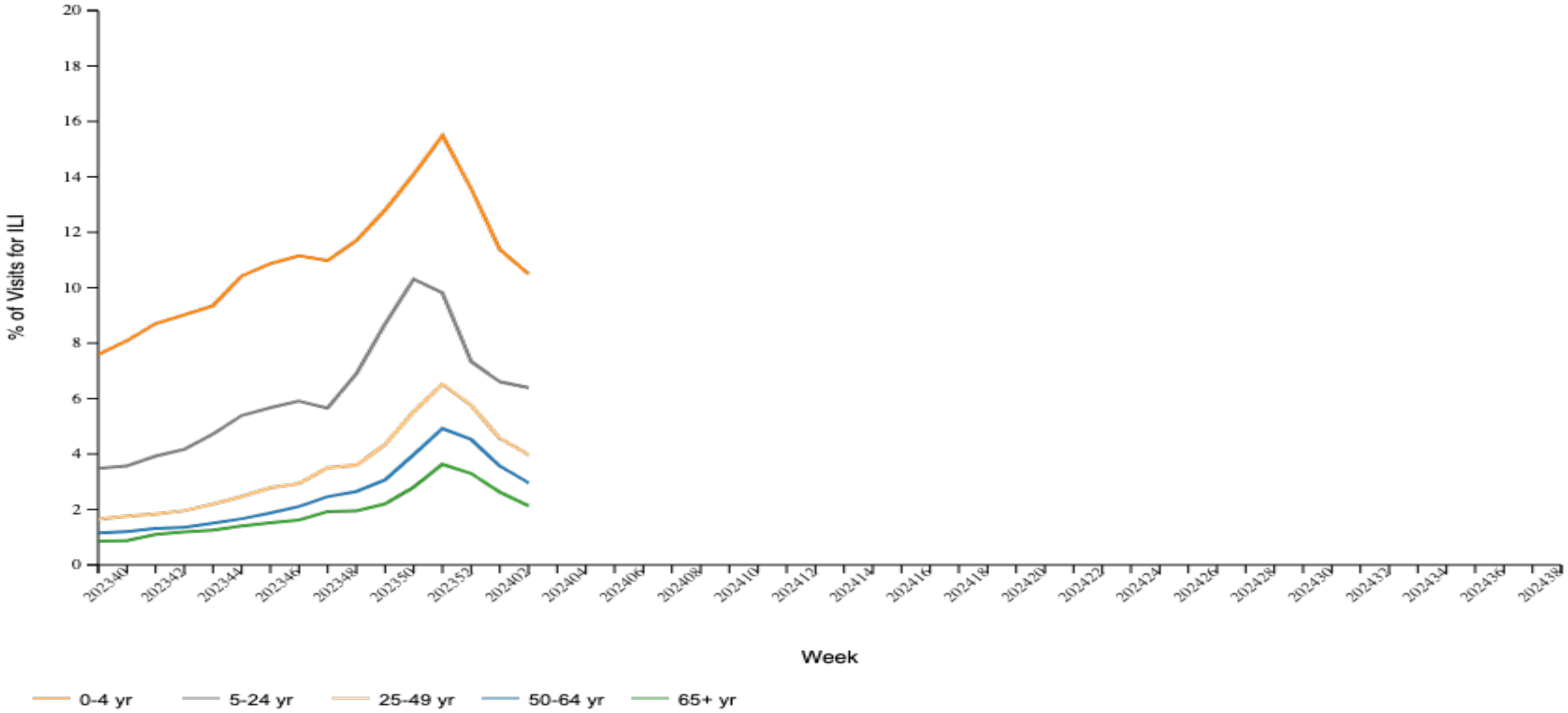


FLUVIEW interactive

Percent of Outpatient Visits for Respiratory Illness by Age Group

Reported by the U.S. Outpatient Influenza-like Illness Surveillance Network (ILINet)

Weekly National Summary, 2023-24 Influenza Season through the Week Ending January 20, 2024



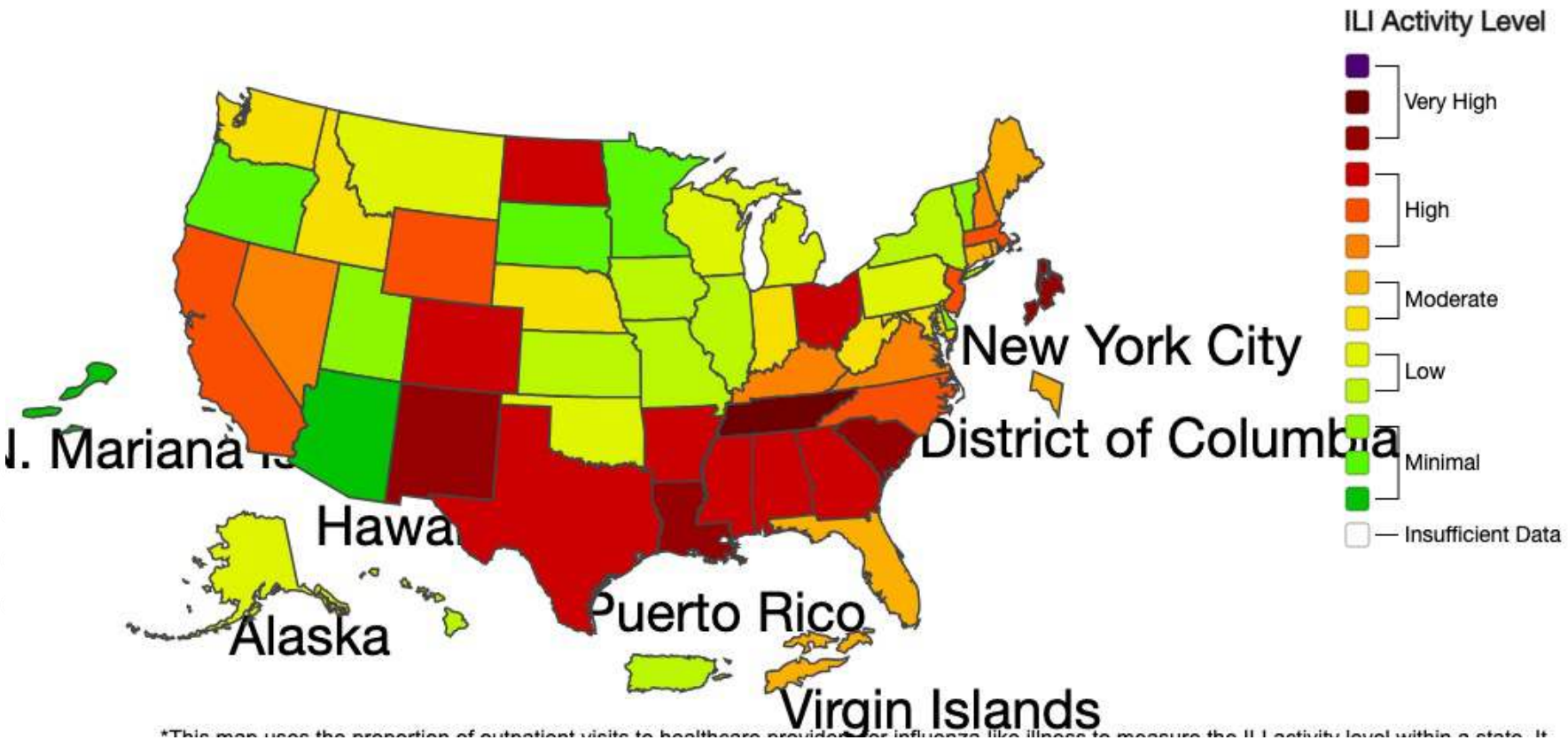
FLUVIEW interactive

A Weekly Influenza Surveillance Report Prepared by the Influenza Division

Outpatient Respiratory Illness Activity Map Determined by Data Reported to ILINet

This system monitors visits for respiratory illness that includes fever plus a cough or sore throat, also referred to as ILI, not laboratory confirmed influenza and may capture patient visits due to other respiratory pathogens that cause similar symptoms.

2023-24 Influenza Season Week 3 ending Jan 20, 2024

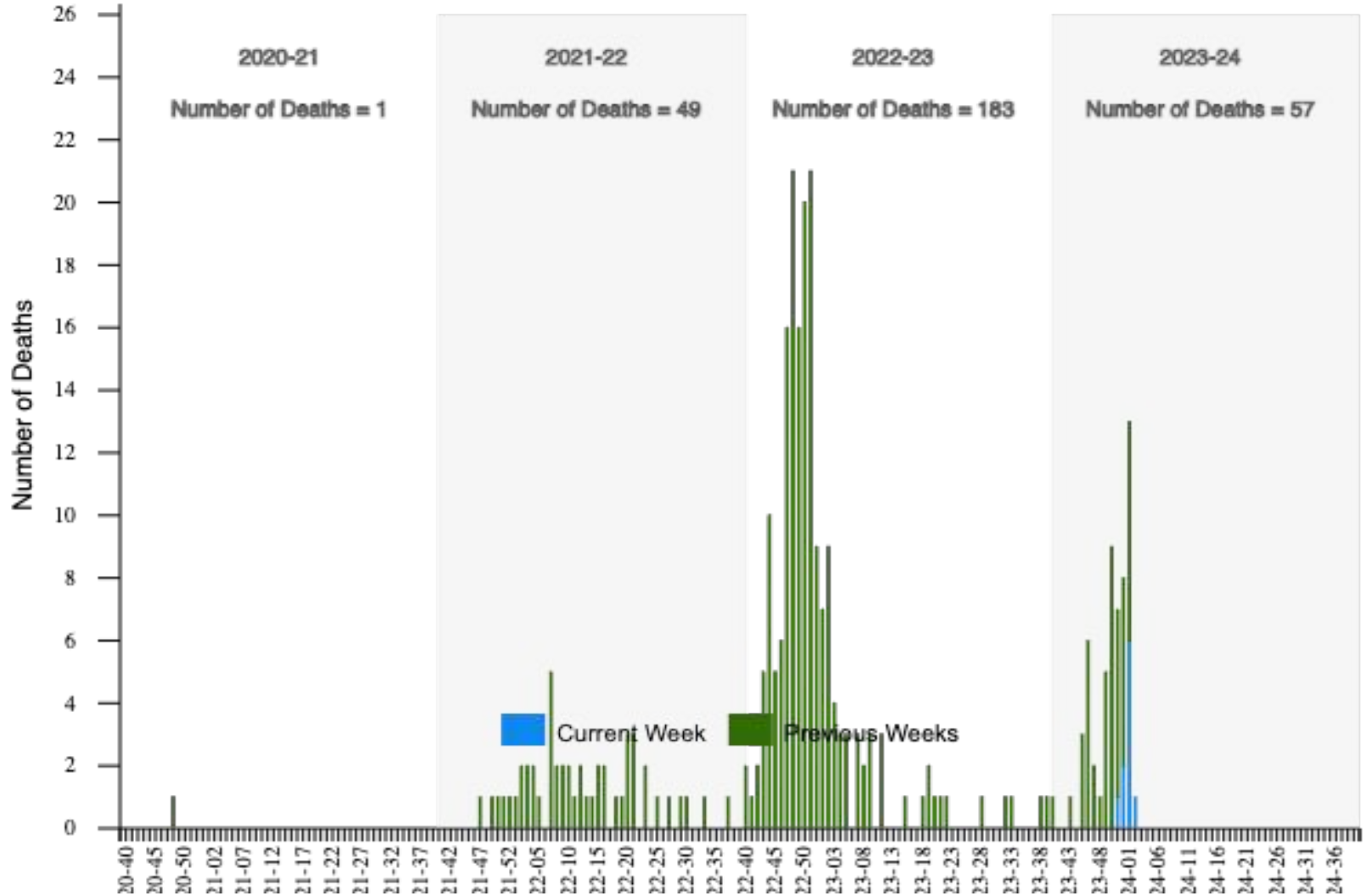


*This map uses the proportion of outpatient visits to healthcare providers for influenza-like illness to measure the ILI activity level within a state. It

FLUVIEW interactive

CDC

Influenza-Associated Pediatric Deaths
by Week of Death, 2020-21 season to 2023-24 season



NIH NHGRI


STOP THE
SPREAD
Take everyday preventive actions to help stop the spread of flu viruses!
#FIGHT FLU
CDC

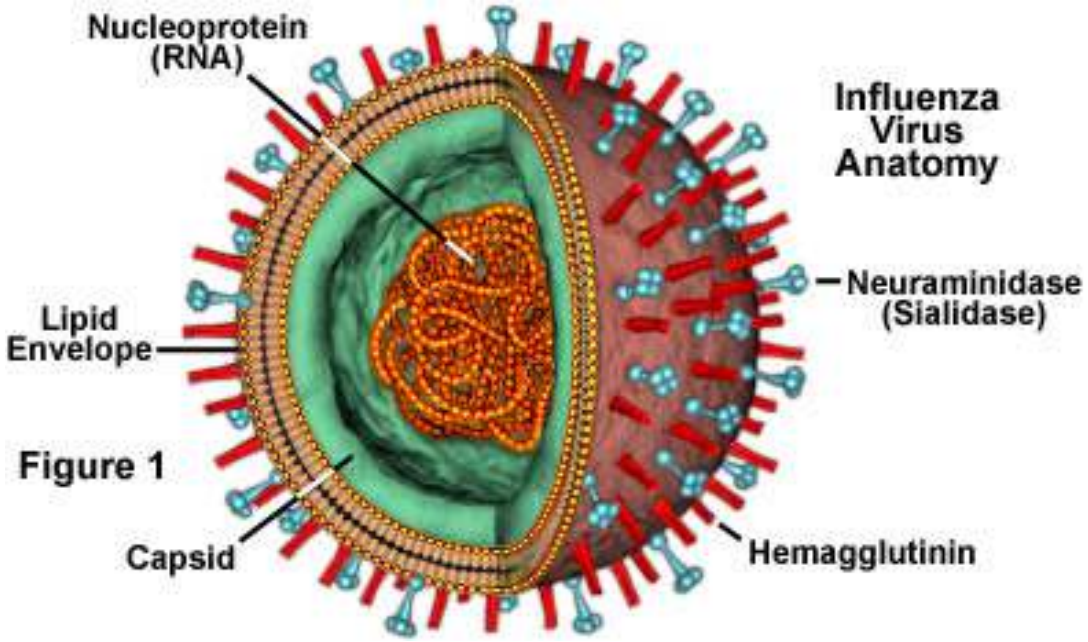

GET YOURSELF AND YOUR FAMILY
VACCINATED!
A yearly flu vaccine is the first and most important step in protecting against flu viruses.
#FIGHT FLU
CDC

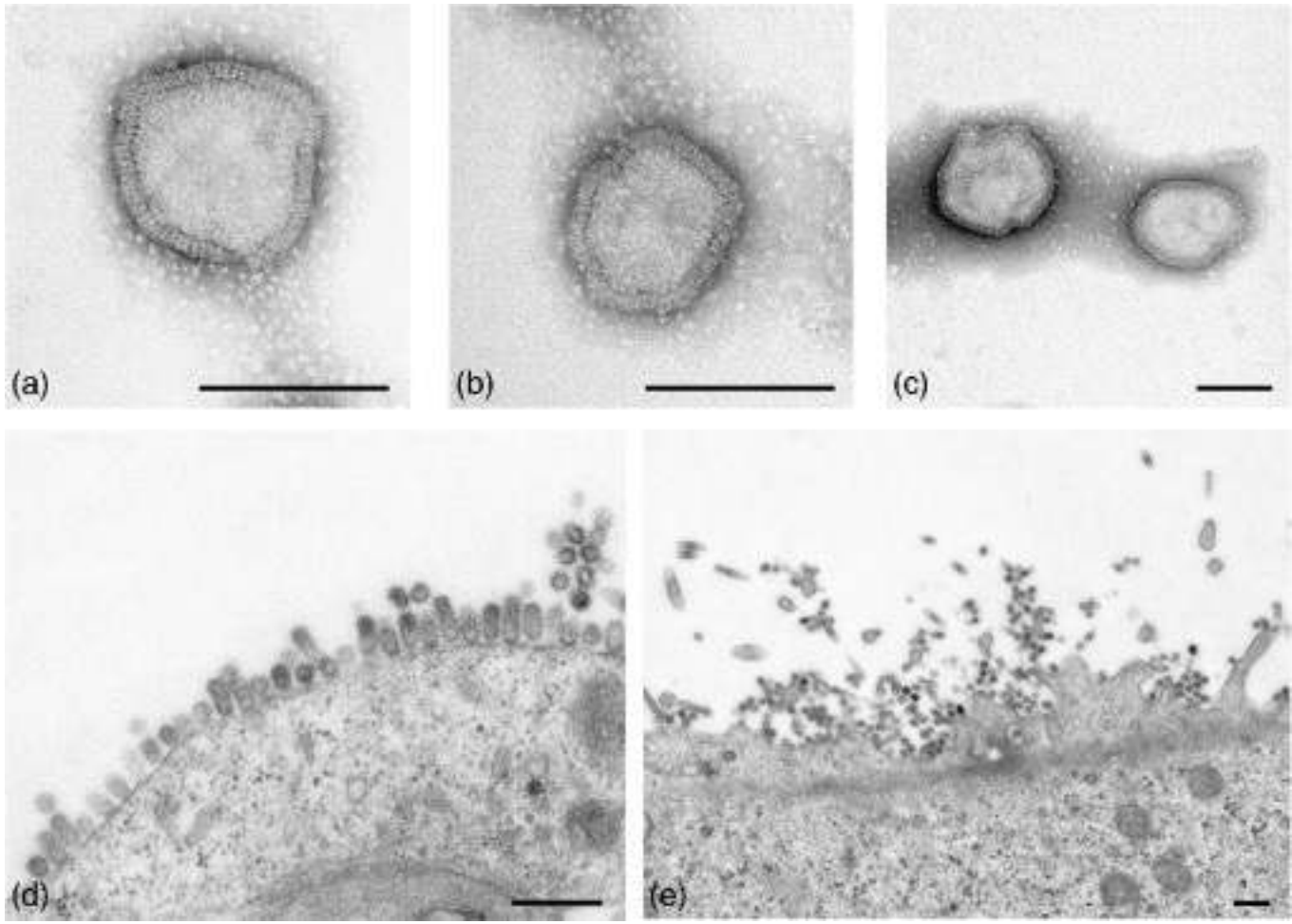

SICK WITH FLU? KNOW WHAT TO DO!
Influenza (or flu) is a contagious respiratory illness caused by flu viruses. Most people with flu have mild illness and do not need medical care or antiviral drugs. If you get flu symptoms, in most cases, you should stay home and avoid contact with others except to get medical care.
KNOW THE SYMPTOMS OF FLU
Flu viruses can cause mild to severe illness, and at times can lead to death. The flu is different from a cold. The flu usually comes on suddenly.
People who have flu often feel some or all of these symptoms: Fever* or feeling feverish/chills, cough, sore throat, runny or stuffy nose, muscle or body aches, headaches, fatigue (tiredness). Some people may have vomiting and diarrhea. This is more common in children.
* It's important to note that not everyone with flu will have a fever.
TAKE ANTIVIRAL DRUGS IF YOUR DOCTOR PRESCRIBES THEM!
Antiviral drugs can be used to treat flu illness. Antiviral drugs can make illness milder and shorten the time you are sick. They also can prevent serious flu complications.
CDC recommends that antiviral drugs be used early to treat people who are very sick with the flu and people who get flu symptoms who are at high risk of serious flu complications, either because of their age or because they have a high risk medical condition.
STAY HOME WHEN SICK
When you are sick, limit contact with others as much as possible. Remember to cover your nose and mouth with a tissue when you cough or sneeze, and throw tissues in the trash after you use them. Stay home for at least 24 hours after your fever is gone except to get medical care or for other necessities.
*Your fever should be gone for 24 hours without the use of a fever-reducing medicine before resuming normal activities.
www.cdc.gov/flu/takingcare.htm
#FIGHT FLU
CDC

Influenza

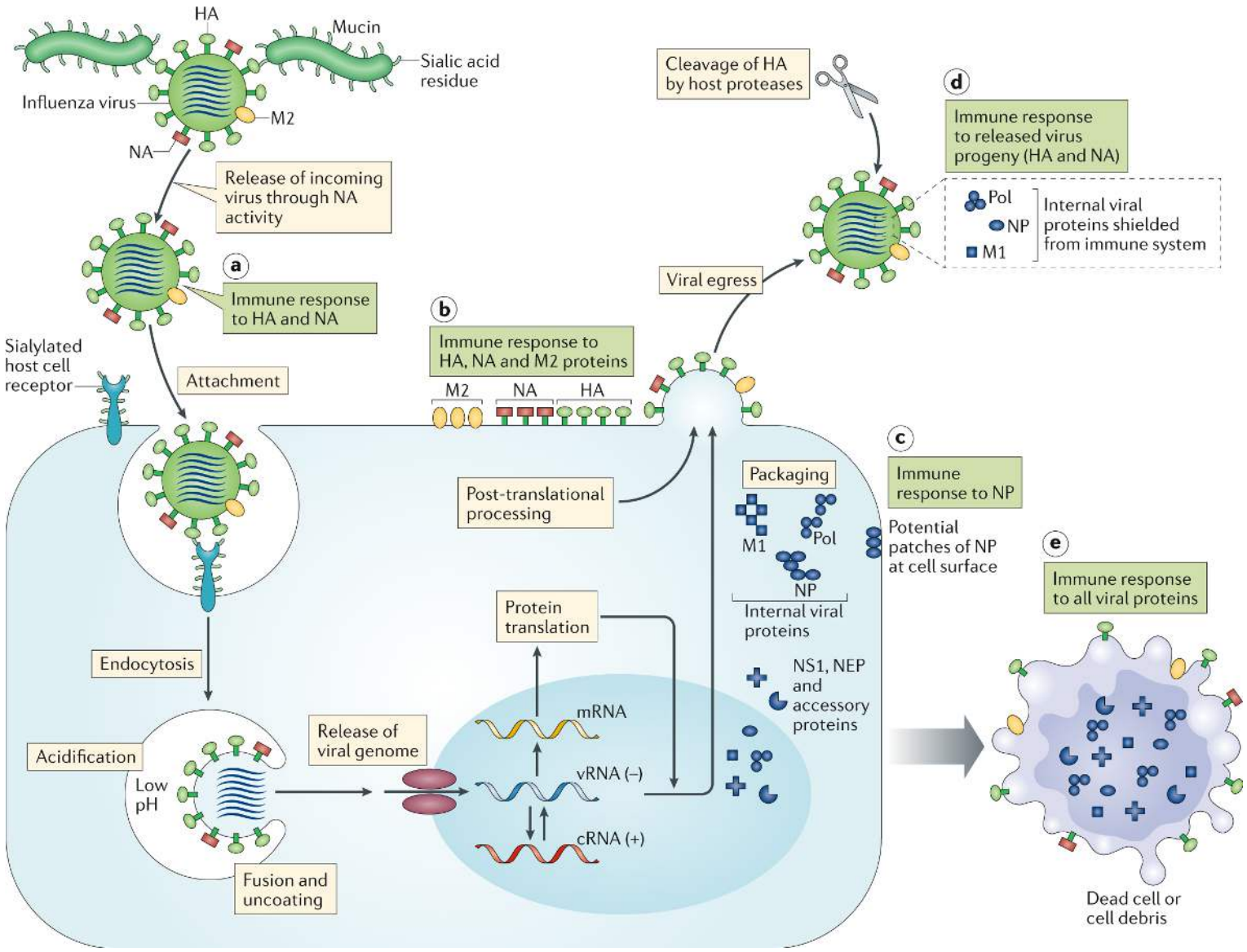
Influenza

- RNA virus
- Typed by surface proteins (e.g., H1N1, H3N2)
- Global public health burden
  - Pandemics
- Systemic inflammation → MtD disease exacerbation
- Exacerbates CNS disease in *Ndufs4*$^{-/-}$ mouse
  - ↑Seizures


Nucleoprotein
(RNA)
Influenza
Virus
Anatomy
Neuraminidase
(Sialidase)
Lipid
Envelope
Figure 1
Capsid
Hemagglutinin


(a)
(b)
(c)
(d)
(e)

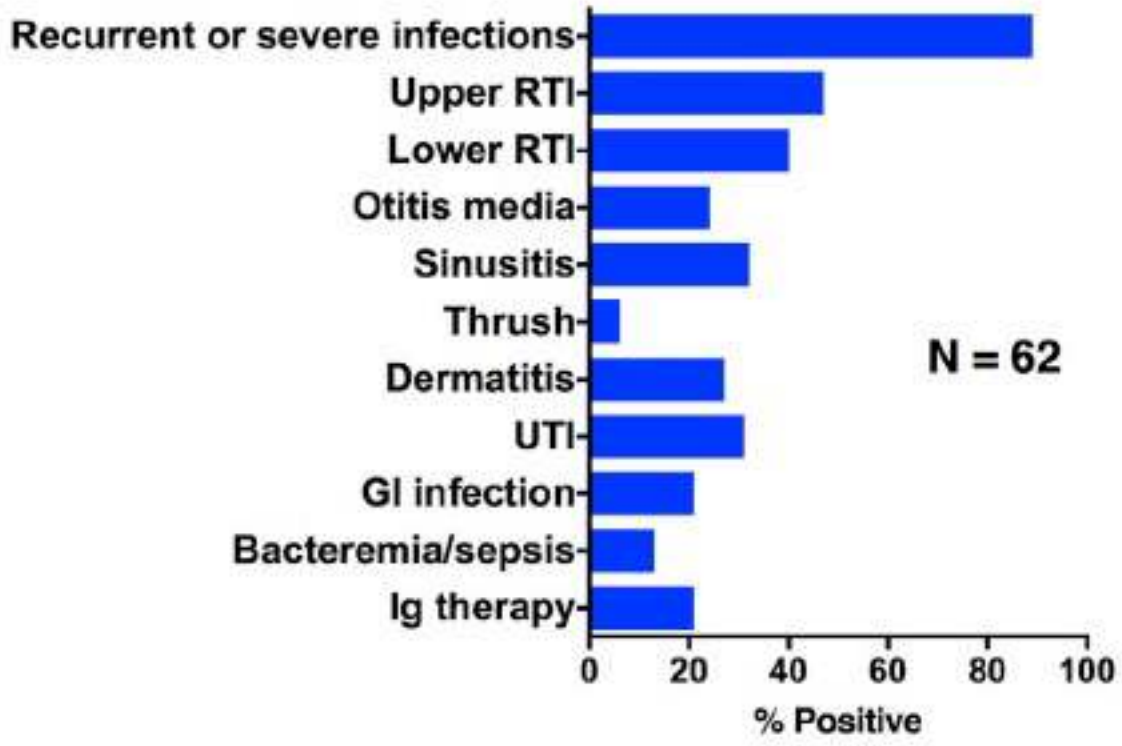
Infection cycle of influenza

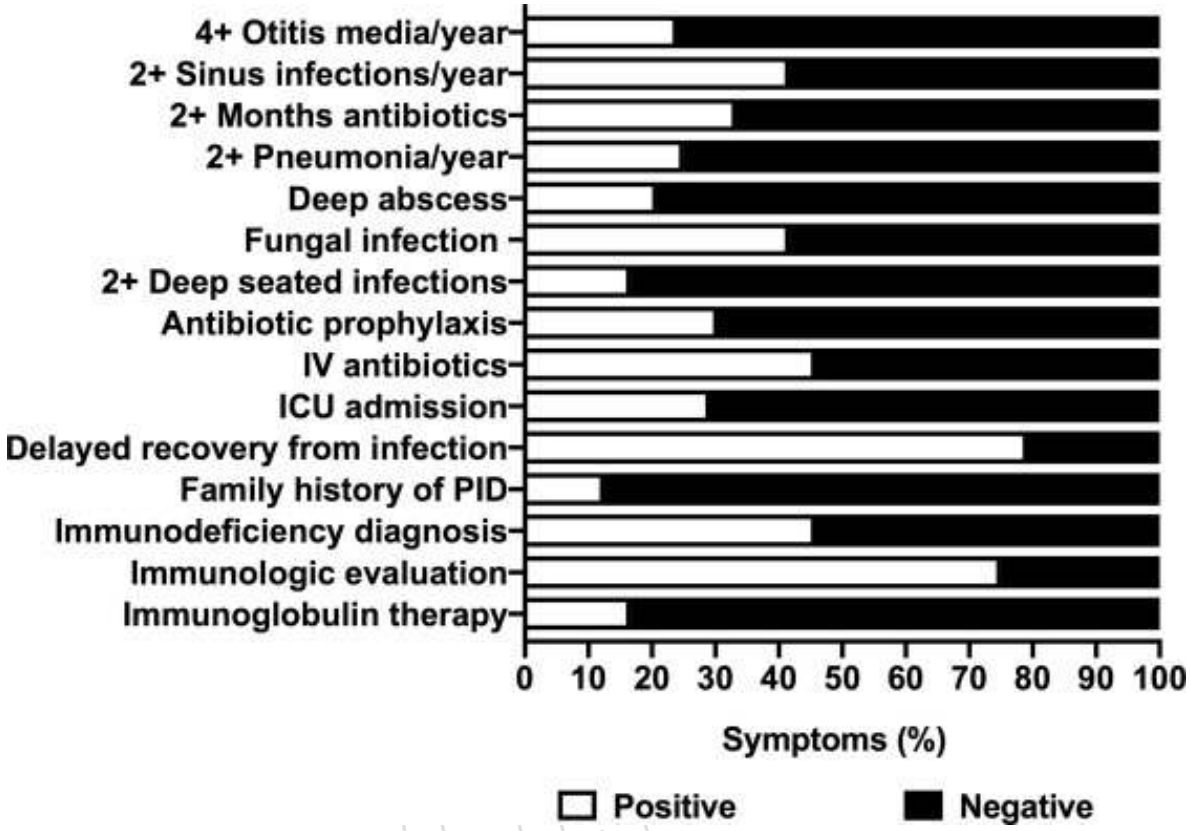


Krammer, *Nat Rev Immunol* (2019)

Do children with MtD get “sicker” during viral infection?

A

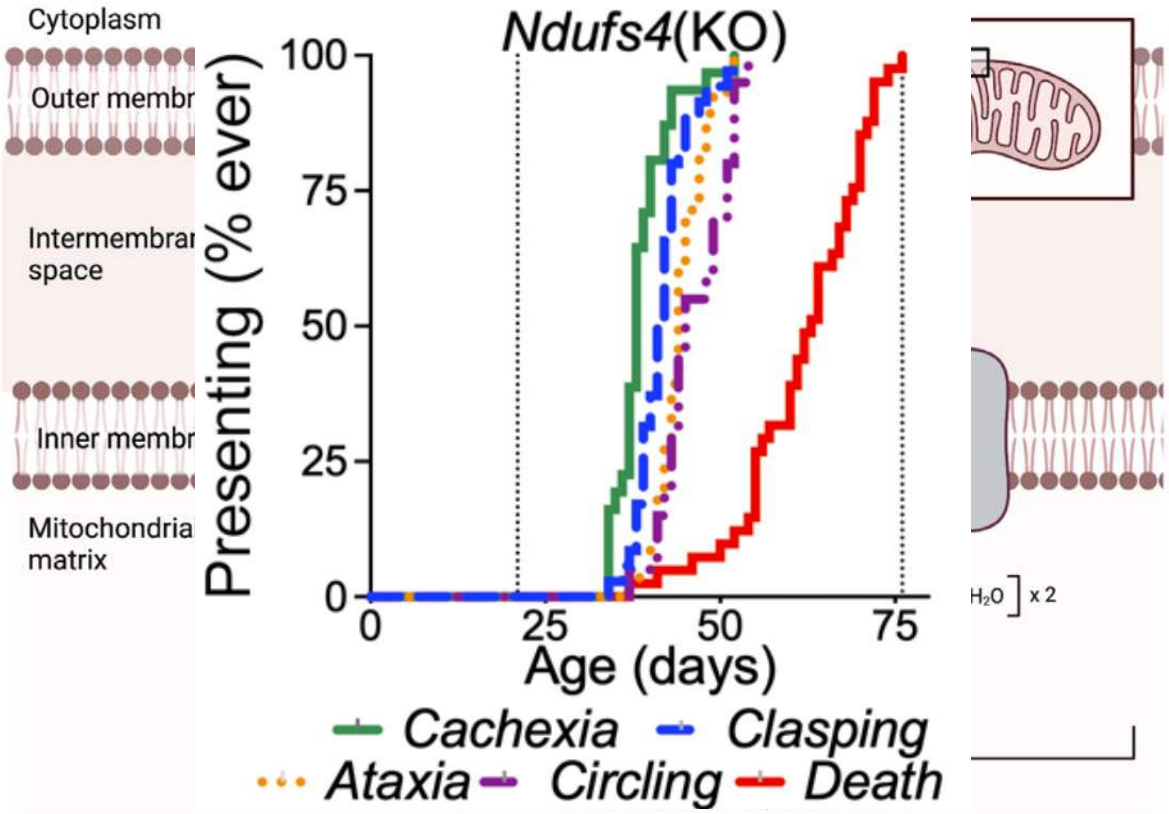

Recurrent or severe infections
Upper RTI
Lower RTI
Otitis media
Sinusitis
Thrush
Dermatitis
UTI
GI infection
Bacteremia/sepsis
Ig therapy
N = 62
0
20
40
60
80
100
% Positive


4+ Otitis media/year
2+ Sinus infections/year
2+ Months antibiotics
2+ Pneumonia/year
Deep abscess
Fungal infection
2+ Deep seated infections
Antibiotic prophylaxis
IV antibiotics
ICU admission
Delayed recovery from infection
Family history of PID
Immunodeficiency diagnosis
Immunologic evaluation
Immunoglobulin therapy
0
10
20
30
40
50
60
70
80
90
100
Symptoms (%)
Positive
Negative

NIH
NHGRI

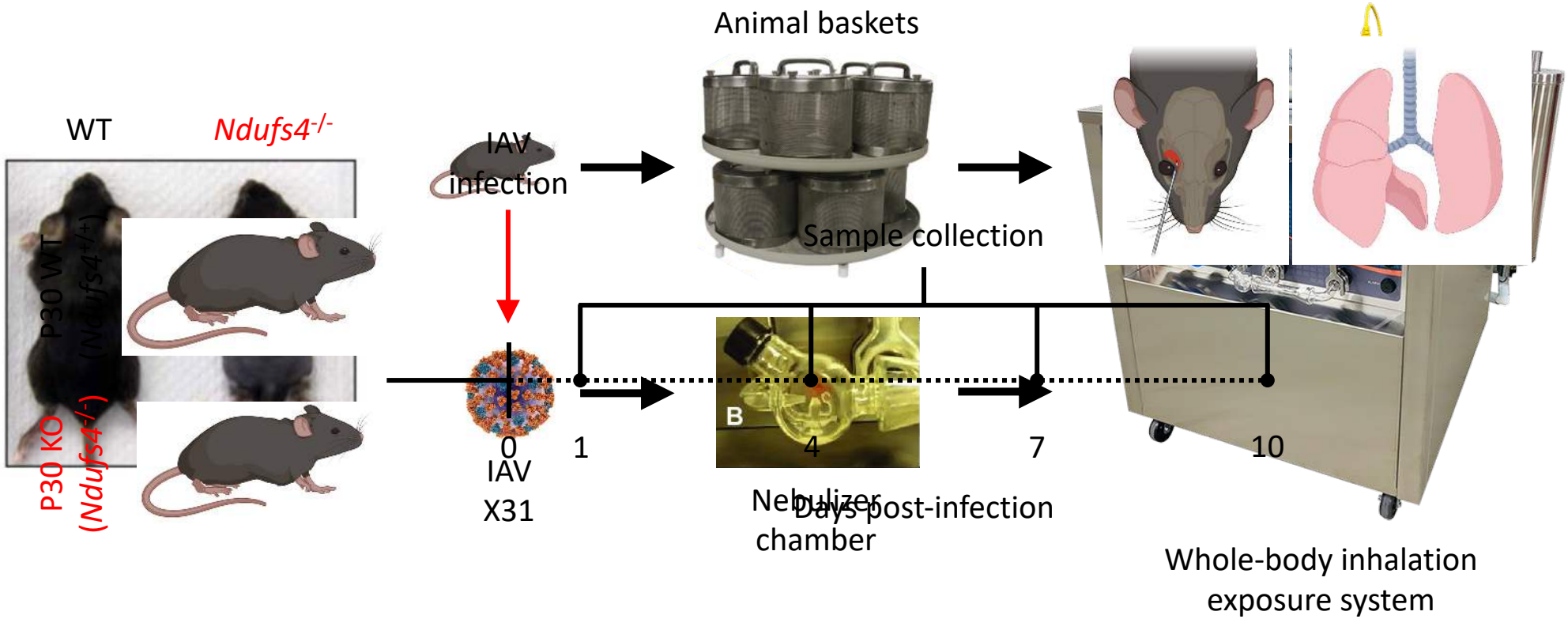
Ndufs4 KO mouse model of LS

- NADH:ubiquinone oxidoreductase core subunit S4 (Ndufs4)
- Homozygotes (*Ndufs4*$^{-/-}$) display **severe phenotype**
  - Transient alopecia (P16-35)
  - Natural death ~P60
- Recapitulate characteristics of the human MtD LS



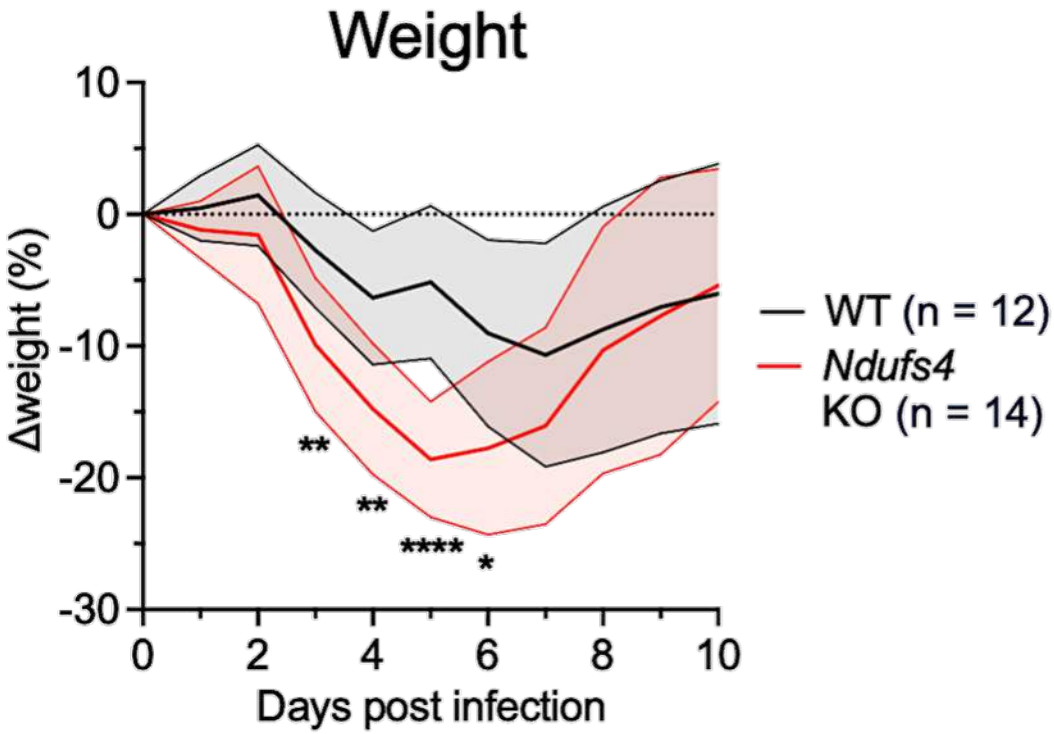
Kruse SE et al. (2008) *Cell Metab.*; Avrutsky MI et al. (2022) *Transl Vis Sci Technol.*; Stokes JC et al. (2022) *JCI Insight.*

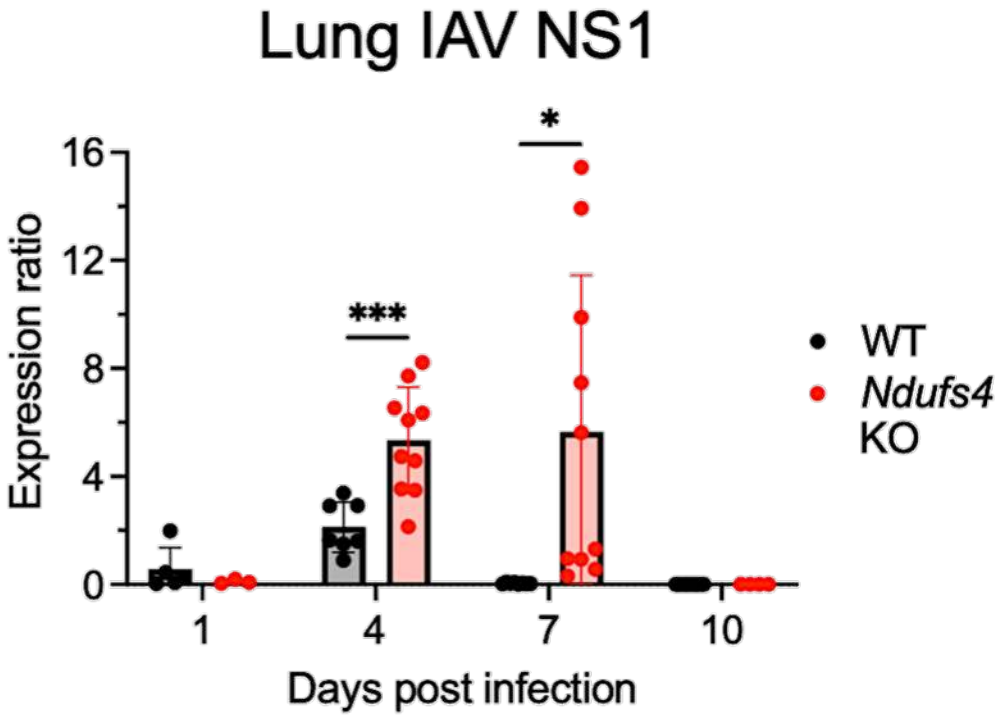
Influenza A virus (IAV) infection model



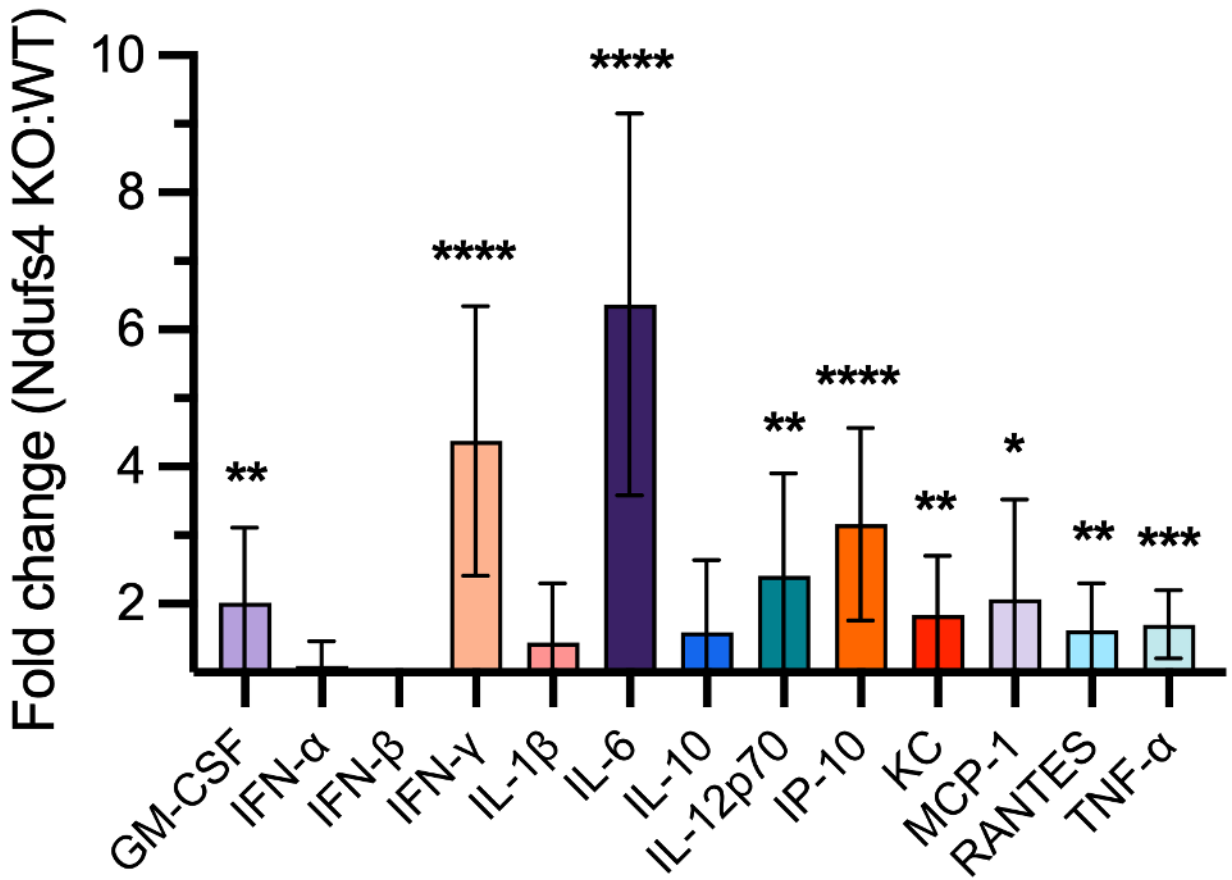
Jin Z et al. (2014) *Cell Metab*.

IAV-infected *Ndufs4* KO mice exhibit increased weight loss and lung viral load





Cytokine storm is prominent in IAV-infected *Ndufs4* KO mice



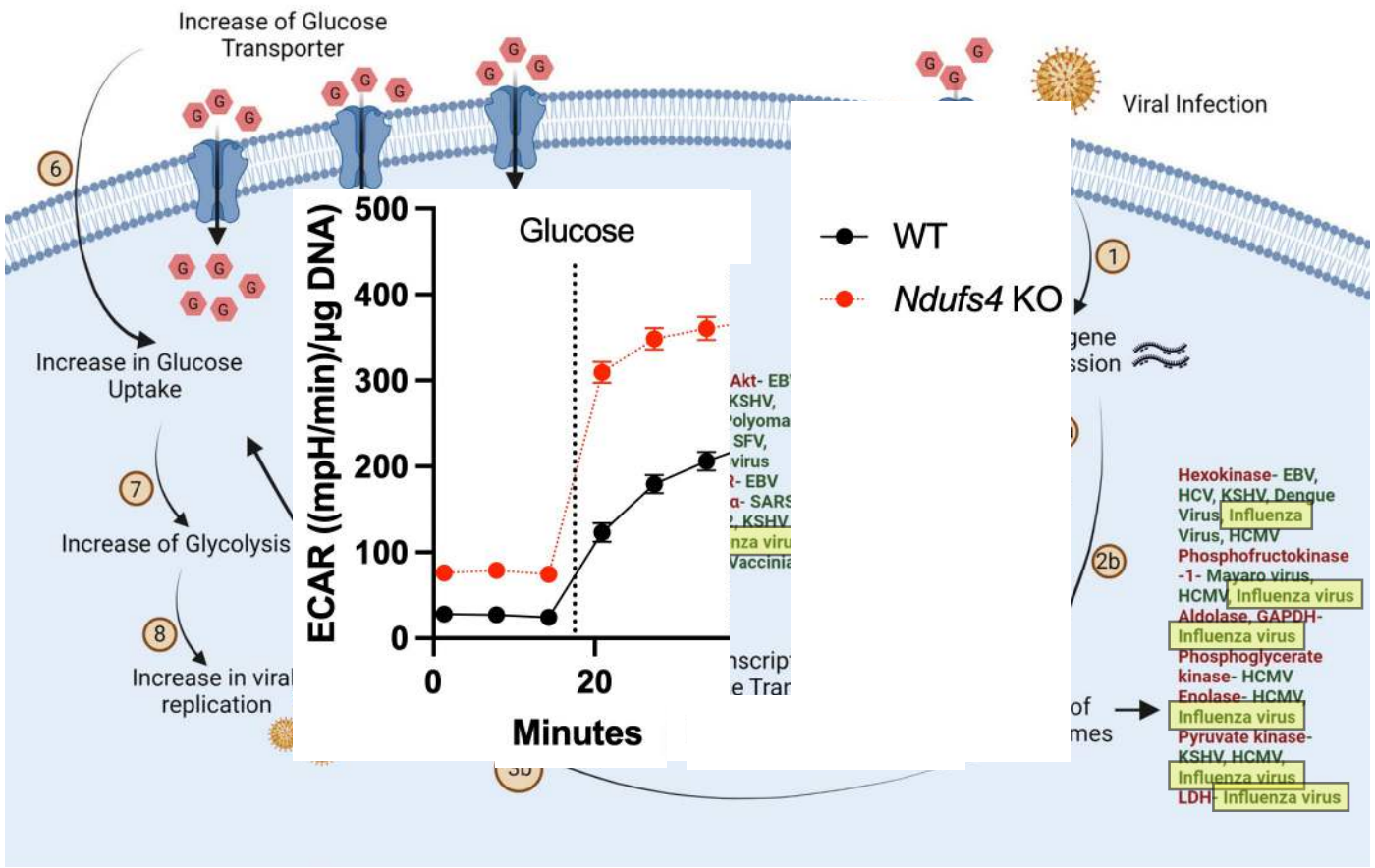
Yes, ***Ndufs4* KO mice do become "sicker"** during viral infection

What are the mechanisms of enhanced viral load?

Viruses induce metabolic reprogramming in infected host cells

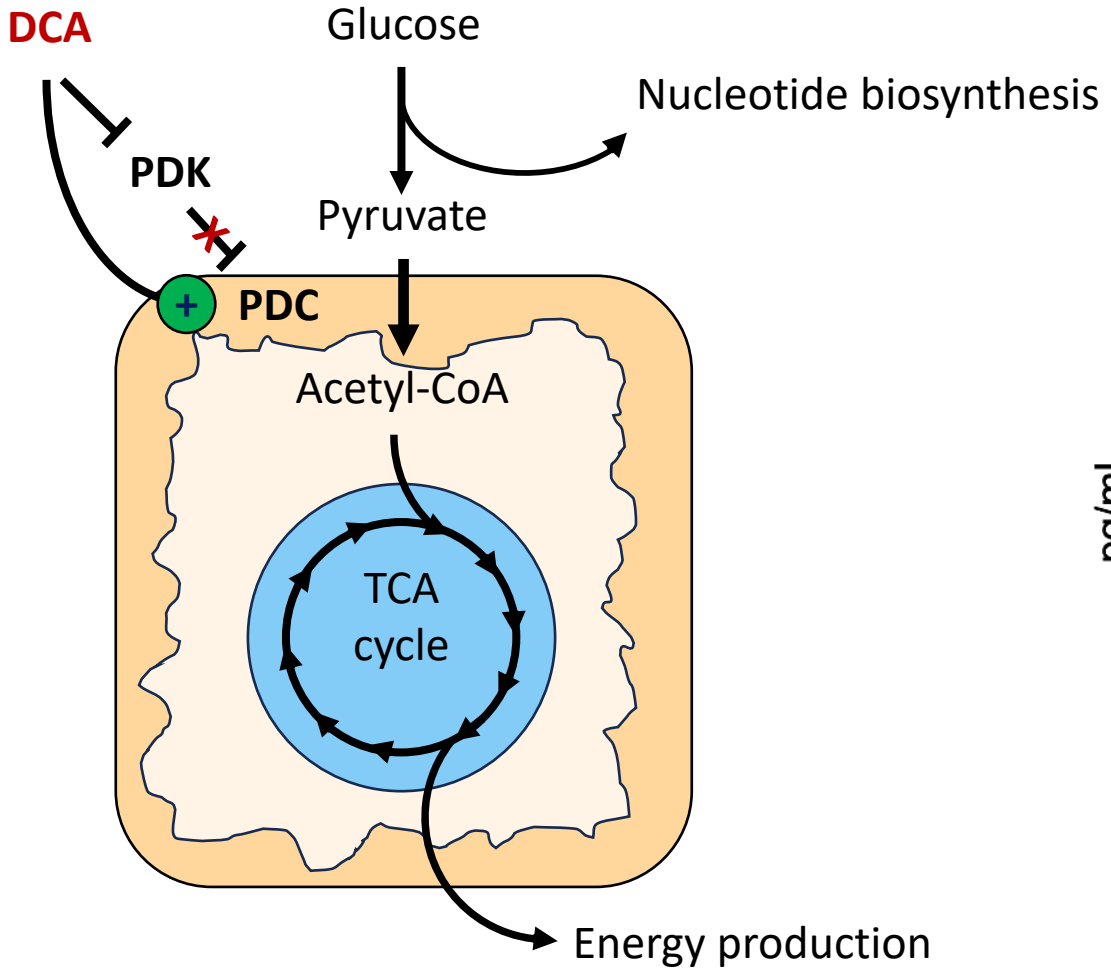
- **Enhanced glycolytic rate** in infected cells
  - Intrinsic host factor for **optimal viral replication**
- Shift host metabolic intermediates to nucleotide biosynthesis

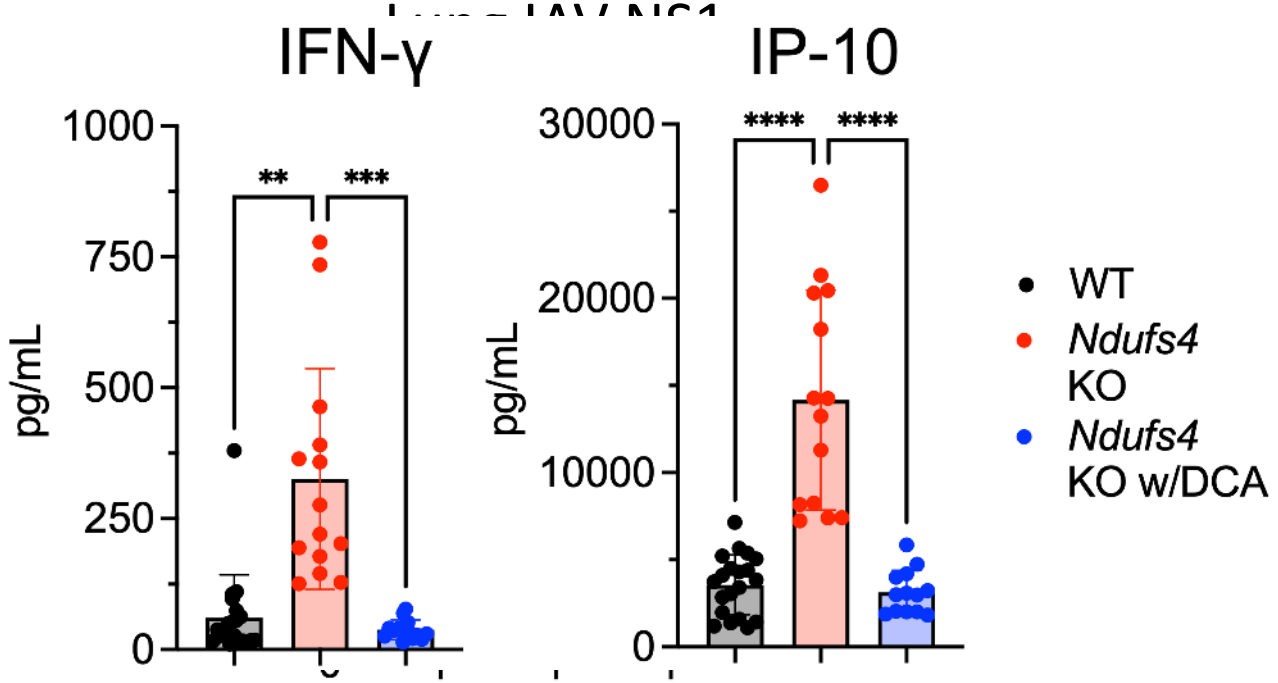
What treatment could limit viral replication?



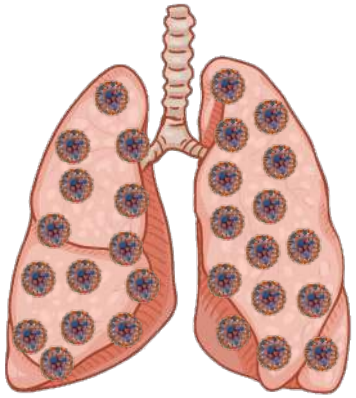
Goyal P et al. (2023) *Mol Cell Biochem*.

DCA treatment abrogates IAV replication and cytokine storm in *Ndufs4* KO mice

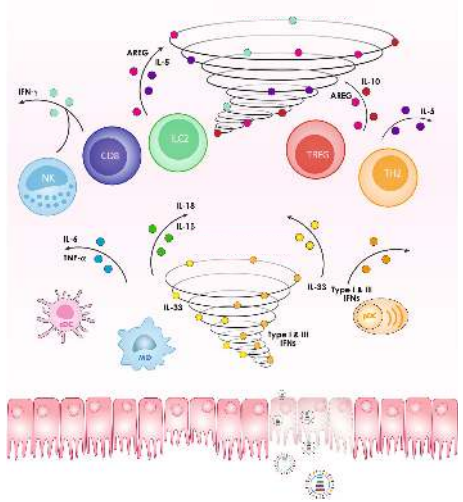




In vivo summary



↑ lung viral load



Cytokine storm

*Lower threshold in MtD

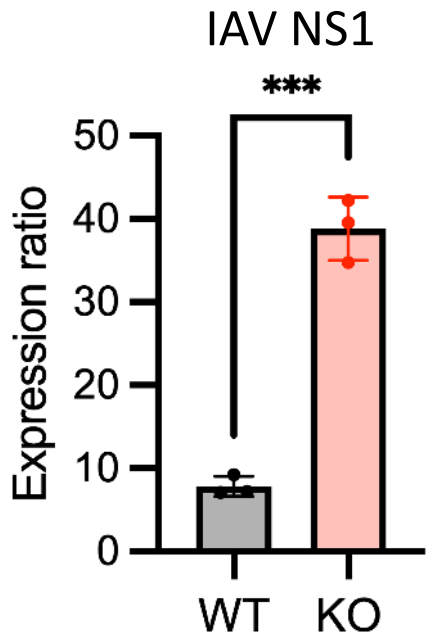
Metabolic decompensation

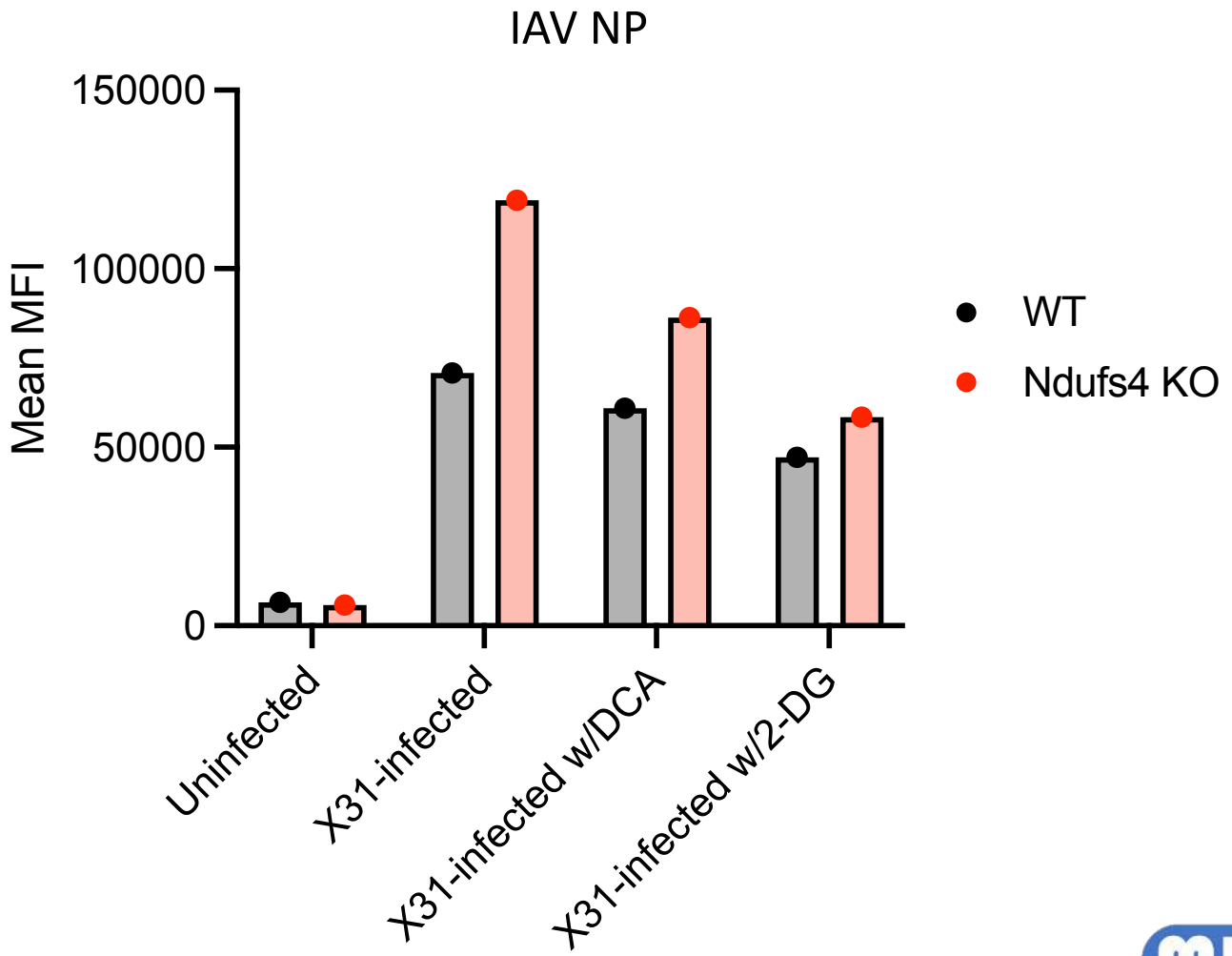
Guo et al. (2017) *Semin Immunopathol*.; Fajgenbaum DC et al. (2020) *N Engl J Med*.; *Jestin M et al. (2020) *Mol Metab*.

How can ↑glycolysis facilitate increased viral load ?

IAV-infected *Ndufs4* KO LET1 epithelial cells demonstrate increased viral load

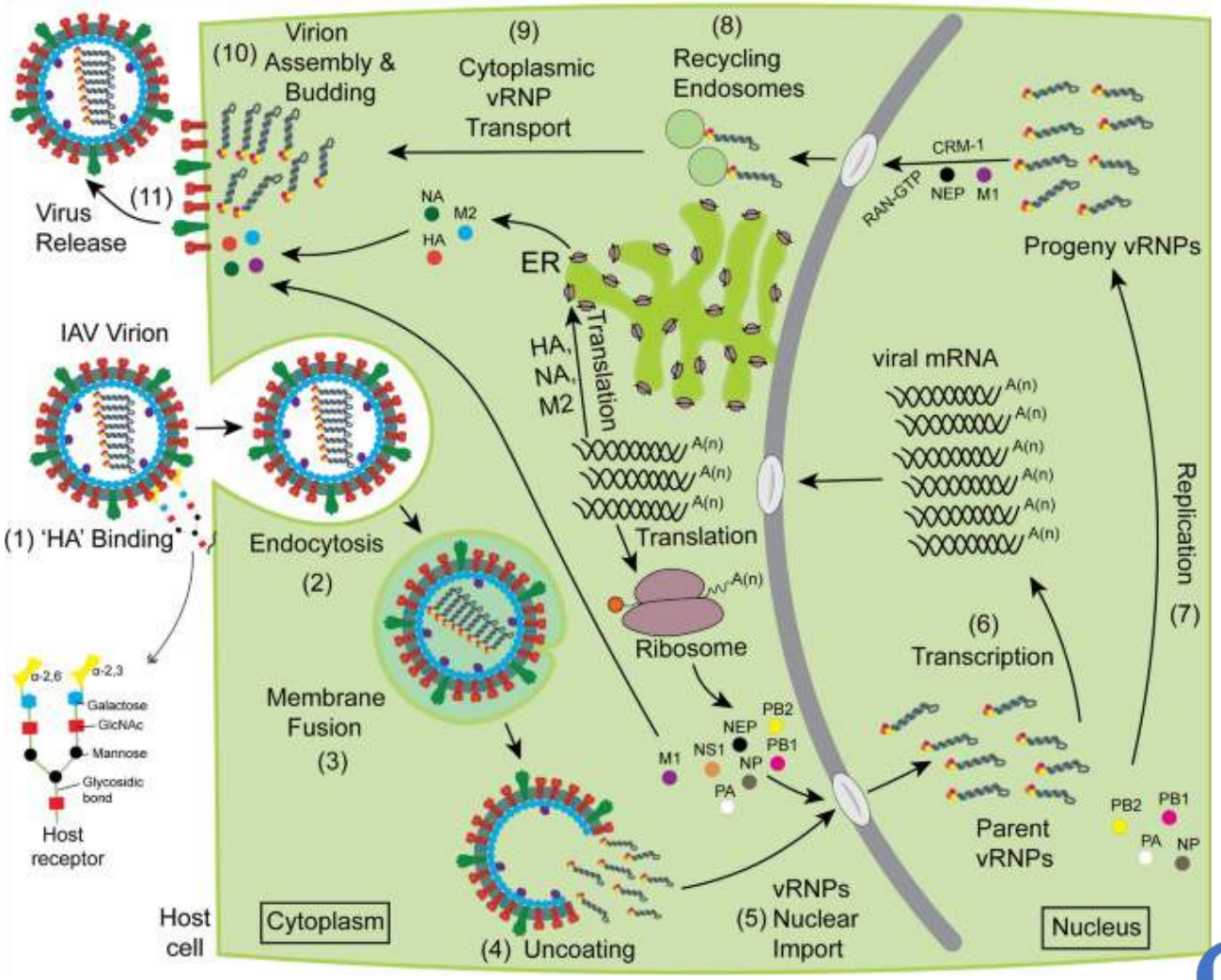
- LET1 cell line
  - Murine lung epithelial type I





Overview of the IAV life cycle

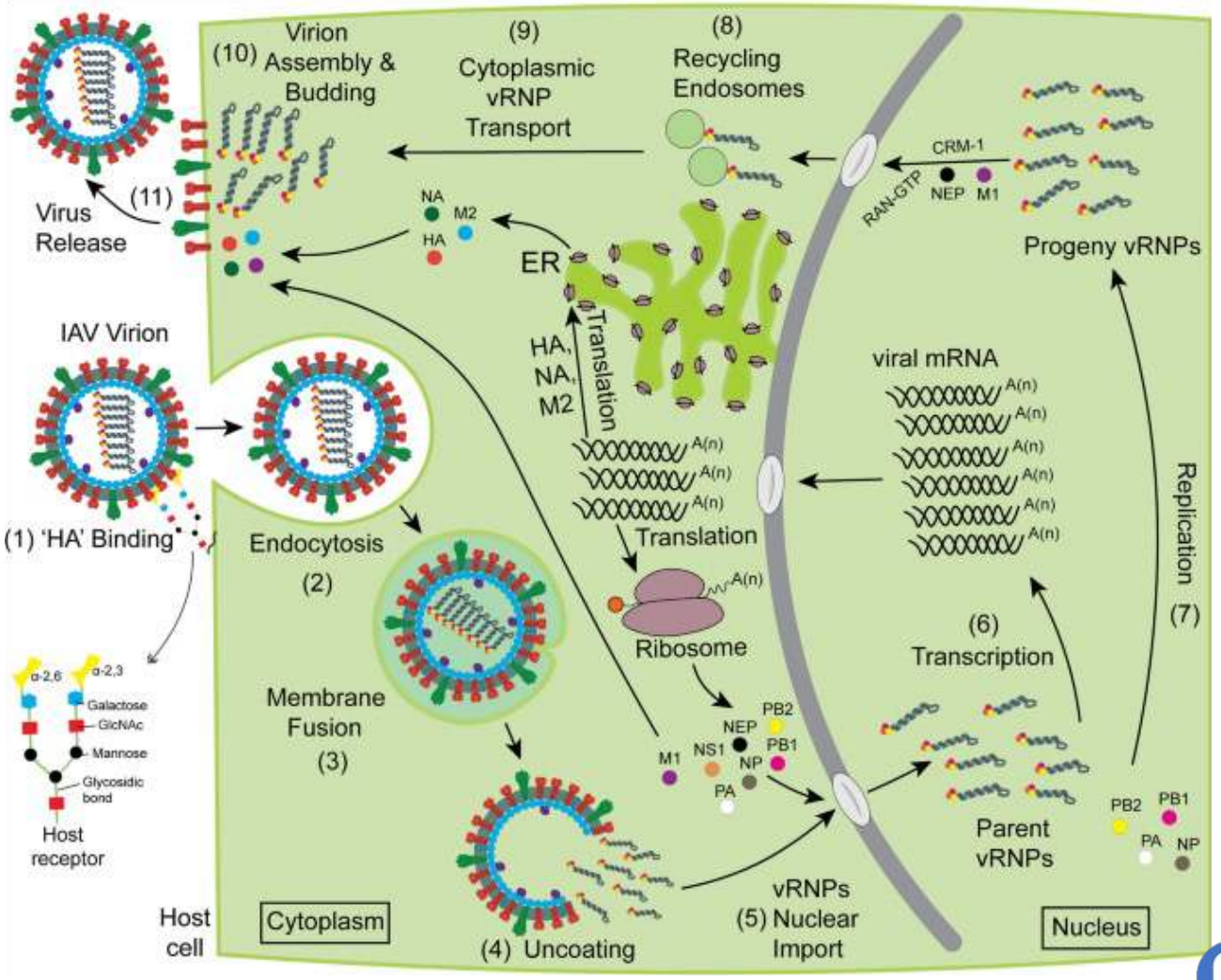
- Viral life cycle
  - (1) Attachment
  - (2 - 4) Entry/fusion
  - (5) Nuclear import
  - (6) Transcription
  - (7) Replication
  - (8 - 10) Virion assembly
  - (11) Virion release



Chauhan RP and Gordon ML. (2022) *Virus Genes*

Overview of the IAV life cycle

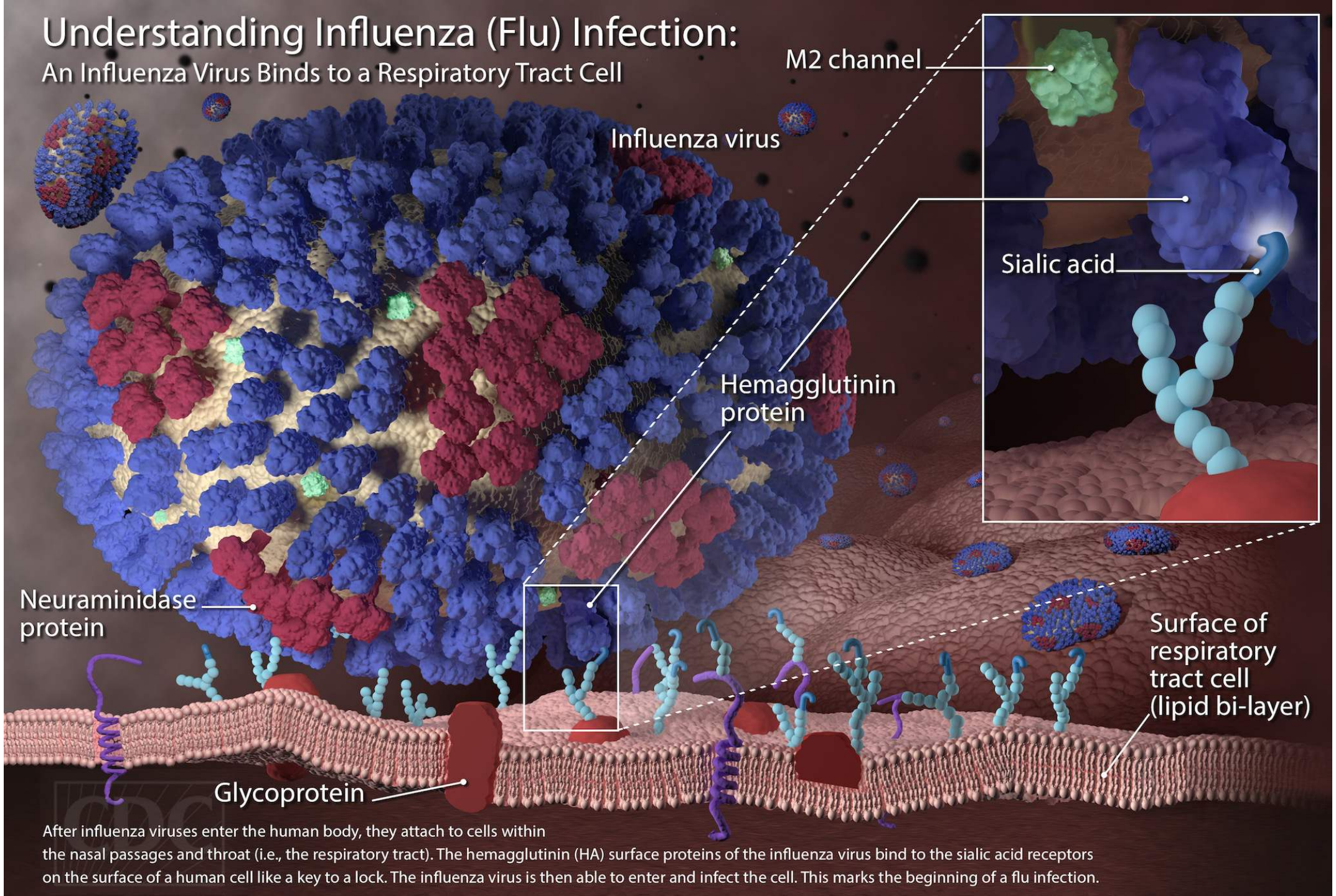
- Viral life cycle
  - ***(1) Attachment**
  - (2 - 4) Entry/fusion
  - (5) Nuclear import
  - ***(6) Transcription**
  - ***(7) Replication**
  - (8 - 10) Virion assembly
  - ***(11) Virion release**



Chauhan RP and Gordon ML. (2022) *Virus Genes*

Understanding Influenza (Flu) Infection:

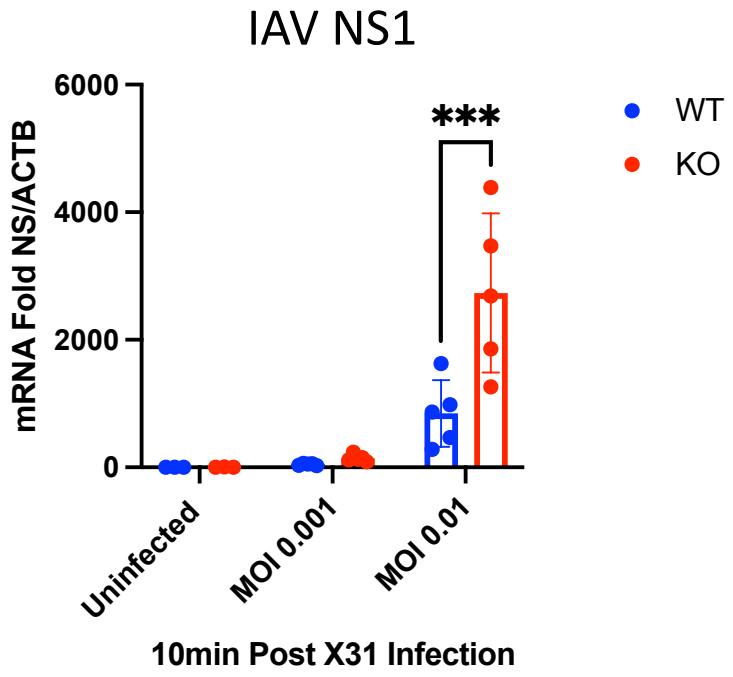
An Influenza Virus Binds to a Respiratory Tract Cell

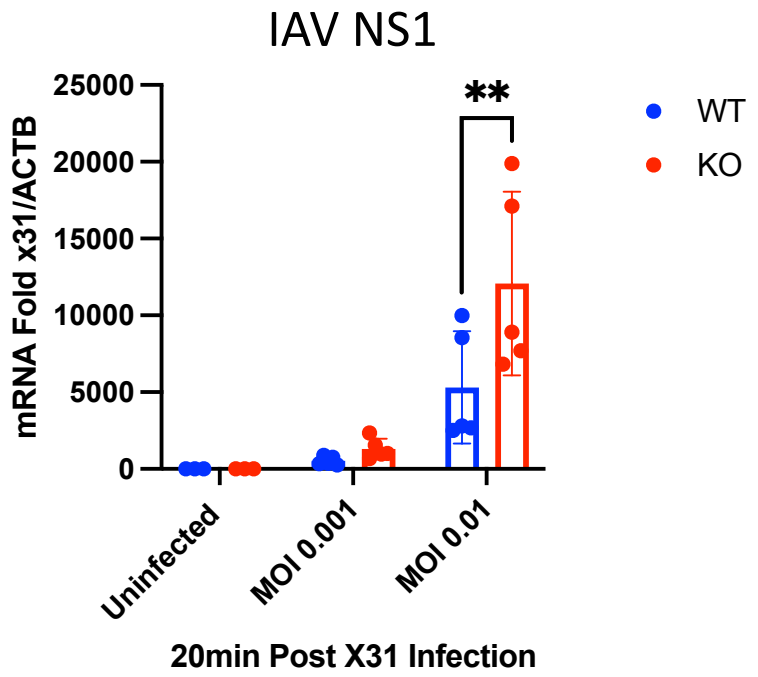


After influenza viruses enter the human body, they attach to cells within the nasal passages and throat (i.e., the respiratory tract). The hemagglutinin (HA) surface proteins of the influenza virus bind to the sialic acid receptors on the surface of a human cell like a key to a lock. The influenza virus is then able to enter and infect the cell. This marks the beginning of a flu infection.

Preliminary data suggests enhanced IAV attachment in *Ndufs4* KO LET1

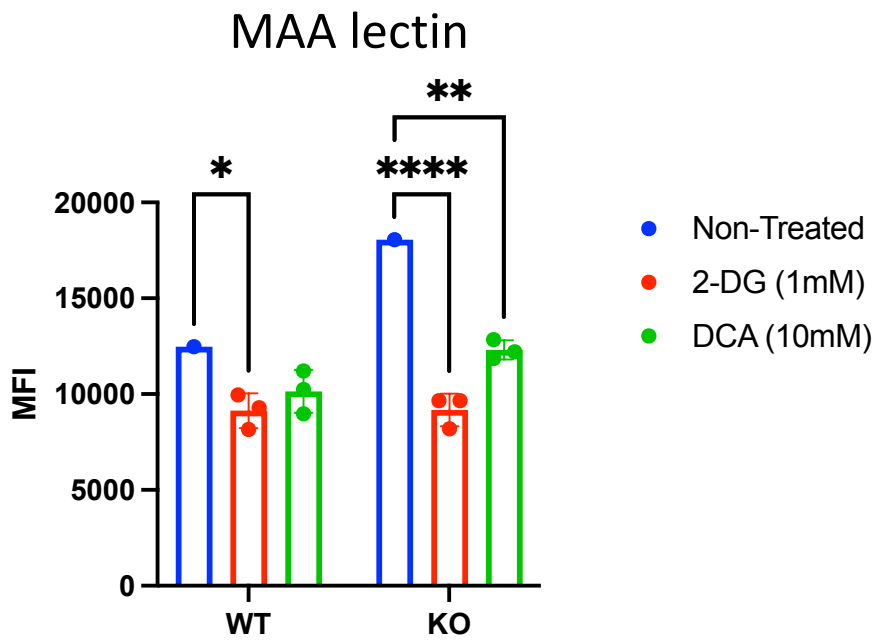
- IAV HA binding/attachment
  - Occurs within **~10 min**
  - Nuclear import within ~1 hr





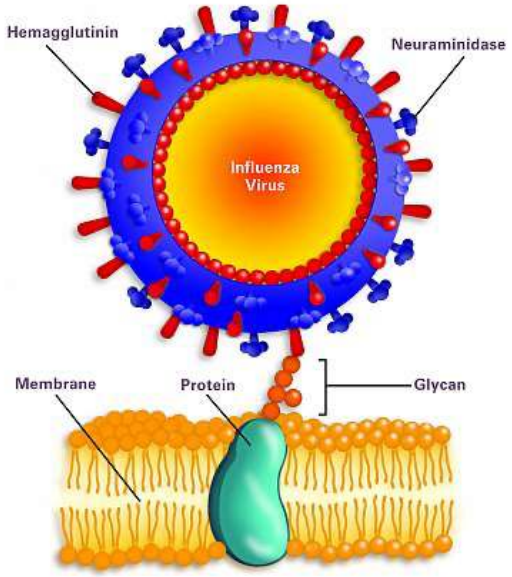
Ndufs4 KO LET1 epithelial cells demonstrate increased sialylation

- IAV attachment is in large part dependent upon α2,3-sialylation of cellular glycans
- **Increase in SNA lectin binding** to *Ndufs4* KO LET1
  - Global sialylation
- **Increase in MAA lectin binding** to *Ndufs4* KO LET1
  - Specific α2,3-sialylation



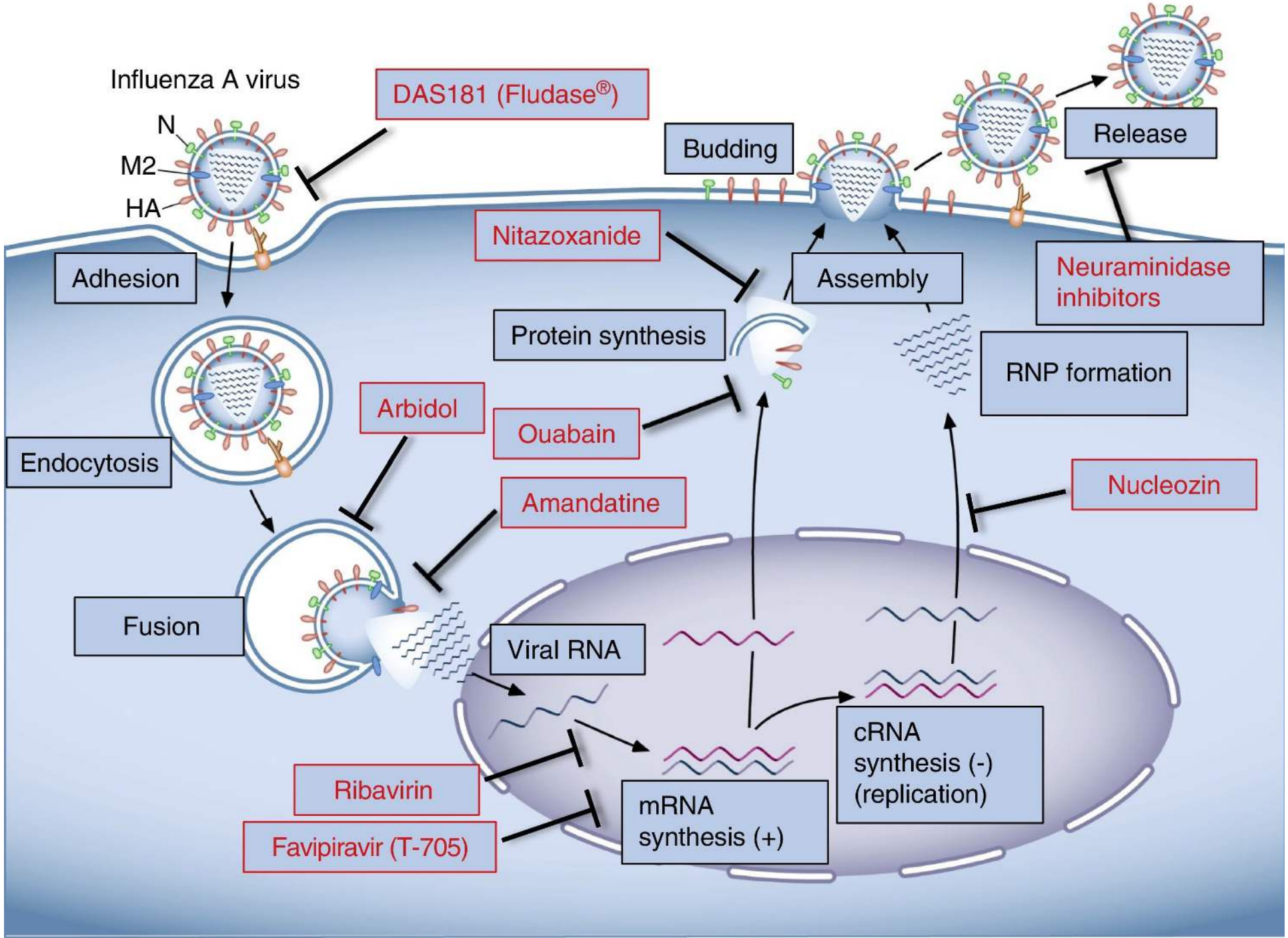
In vitro mechanism summary (thus far)

- **Enhanced IAV binding/ attachment** appears to be a contributing factor to viral infection advantage in *Ndufs4* KO model
- Literature suggests that **enhanced glycolysis** contributes to increased viral transcription and replication



What if X31 IAV has a "compounded" infection advantage in *Ndufs4* KO?

https://www.nih.gov/news-events/nih-research-matters/sugars-cell-surface-are-key-flu-infections


Influenza A virus
N
M2
HA
DAS181 (Fludase®)
Budding
Release
Nitazoxanide
Adhesion
Assembly
Neuraminidase inhibitors
Protein synthesis
RNP formation
Arbidol
Ouabain
Endocytosis
Nucleozin
Amandatine
Fusion
Viral RNA
cRNA synthesis (-) (replication)
Ribavirin
mRNA synthesis (+)
Favipiravir (T-705)

Tamiflu is FDA approved for use against influenza A&B*

*Tamiflu has been studied only in strains of influenza that were circulating at the time of the clinical studies conducted to support FDA approval

Tamiflu is the only prescription oral antiviral medicine approved to treat a wide range of ages, from patients

TWO WEEKS OF AGE TO THOSE 65+

ACCORDING TO THE CDC,

CLINICAL STUDIES OF VIRUSES CAUSING FLU DURING THE LAST THREE SEASONS (2010-11, 2011-12, 2012-13) HAVE SHOWN

LOW RATES OF RESISTANCE TO TAMIFLU*

*These low rates of resistance do not imply that the use of Tamiflu will have a positive outcome for any particular patient.

AVAILABLE IN CAPSULES AND A LIQUID FORMULA (ORAL SUSPENSION)

TAMIFLU HELPS BLOCK THE VIRUS' ABILITY TO REPLICATE IN THE BODY

SEE A DOCTOR QUICKLY.
TREATMENT SHOULD BEGIN WITHIN

2 DAYS (48 HRS)

OF FLU SYMPTOM ONSET

Summary

- Influenza remains a major threat to children with MtD
- Mouse model of Leigh syndrome gets sicker during infection
- Lungs from Leigh syndrome mice contains higher viral loads
- Respiratory epithelial cells with MtD may be “stickier” for influenzas virus (more receptors)
- Tamiflu can help interupt the viral life cycle and reduce morbidity

Thank you

Peter McGuire, MS, MBBCh
Eliza Gordon-Lipkin MD
Tatiana Tarasenko, MD, PhD
Shannon Kruk, BSN, RN
Amanda Fuchs, PhD
Jose-Luis Marin Franco PhD
Emily Warren, PhD
Bharati Singh, PhD
Payal Banerjee, MSc
Elizabeth Thompson, BS
Jillian Jetmore, BS
Melissa Schlein, BS

NIBIB
Kaitlyn Sadtler, PhD
Heather Kalish, PhD

FDA
Surrender Khurana, PhD

NHGRI Animal Core
Tannia Clark, DVM

NHGRI Flow Cytometry Core
Stacie Anderson, BS
Martha Kirby, BS

Children's Hospital of Philadelphia
Marni Falk, MD
Amy Goldstein, MD
Zarazuela Zolkipli-Cunningham, MBChB
Rebecca Ganetzky, MD

Children's National Medical Center
Andrea Gropman, MD

Seattle Children's Hospital
Russ Saneto, DO, PhD

Lurie Children's Hospital
Carlos E. Prada, MD

Texas Children's Hospital
Fernando Scaglia, MD

UT- Houston
Mary Kay Koenig, MD
Susan Pacheco, MD

Children's Hospital of Akron
Bruce Cohen, MD

Children's Hospital of Orange County
Jose Abdenur, MD

Cleveland Clinic
Sumit Parikh, MD

Children's Hospital of Colorado
Austin Larsen, MD

Columbia University
Michio Hirano, MD

Mount Sinai
Kimihiko Oishi, MD

VMP Genetics
Fran Kendall, MD