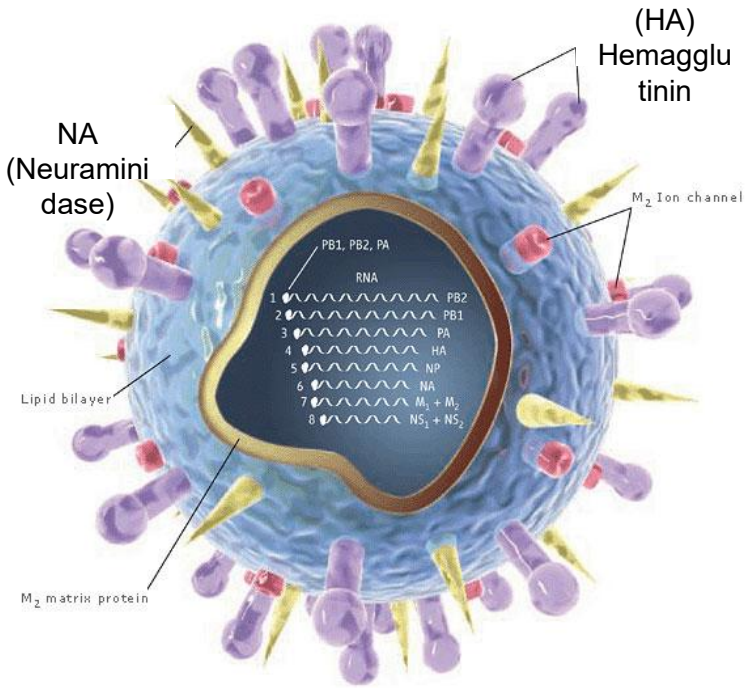
# Viral Infections

Unit 3

Paul Thomas
pthomas@fredhutch.org
Vaccine and Infectious Disease Division
Fred Hutchinson Cancer Center

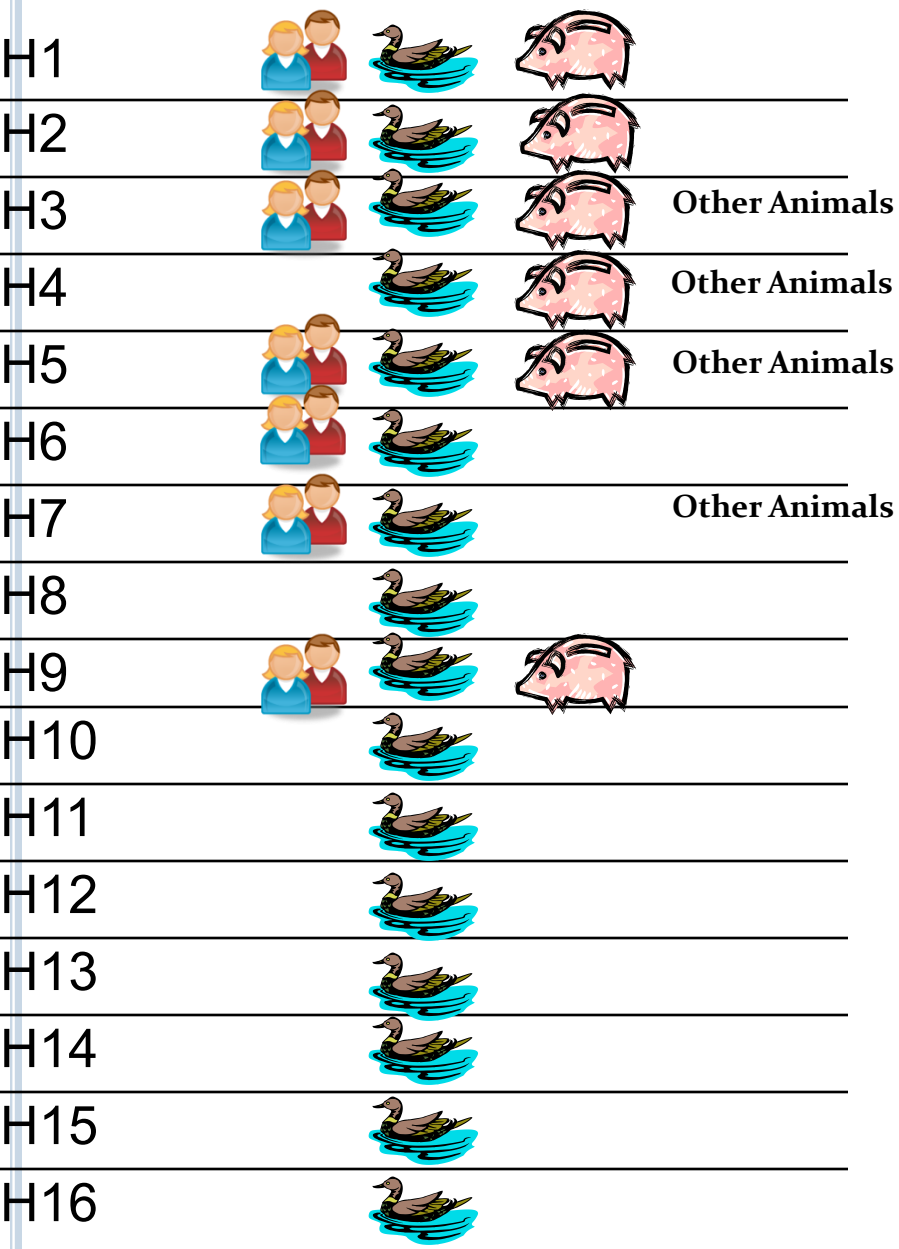
# Influenza A Virus

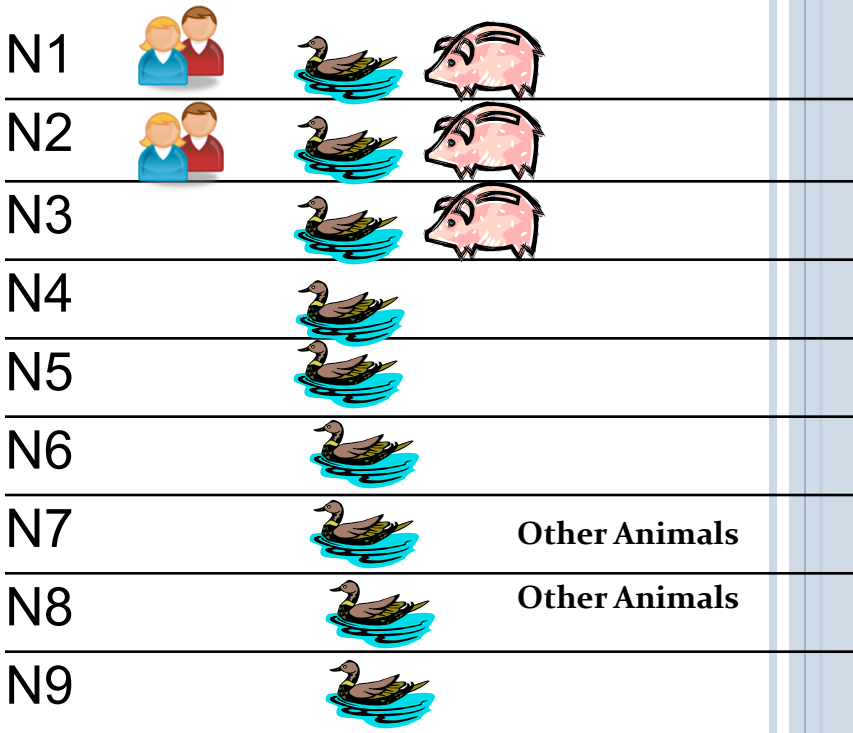
- Negative sense, segmented RNA virus
- *Orthomyxoviridae*
- Eight genes, 11 proteins (three alternate reading frames)
- Two non-structural proteins (NS1 and PB1-F2)
- Surface proteins HA and NA determine serotype



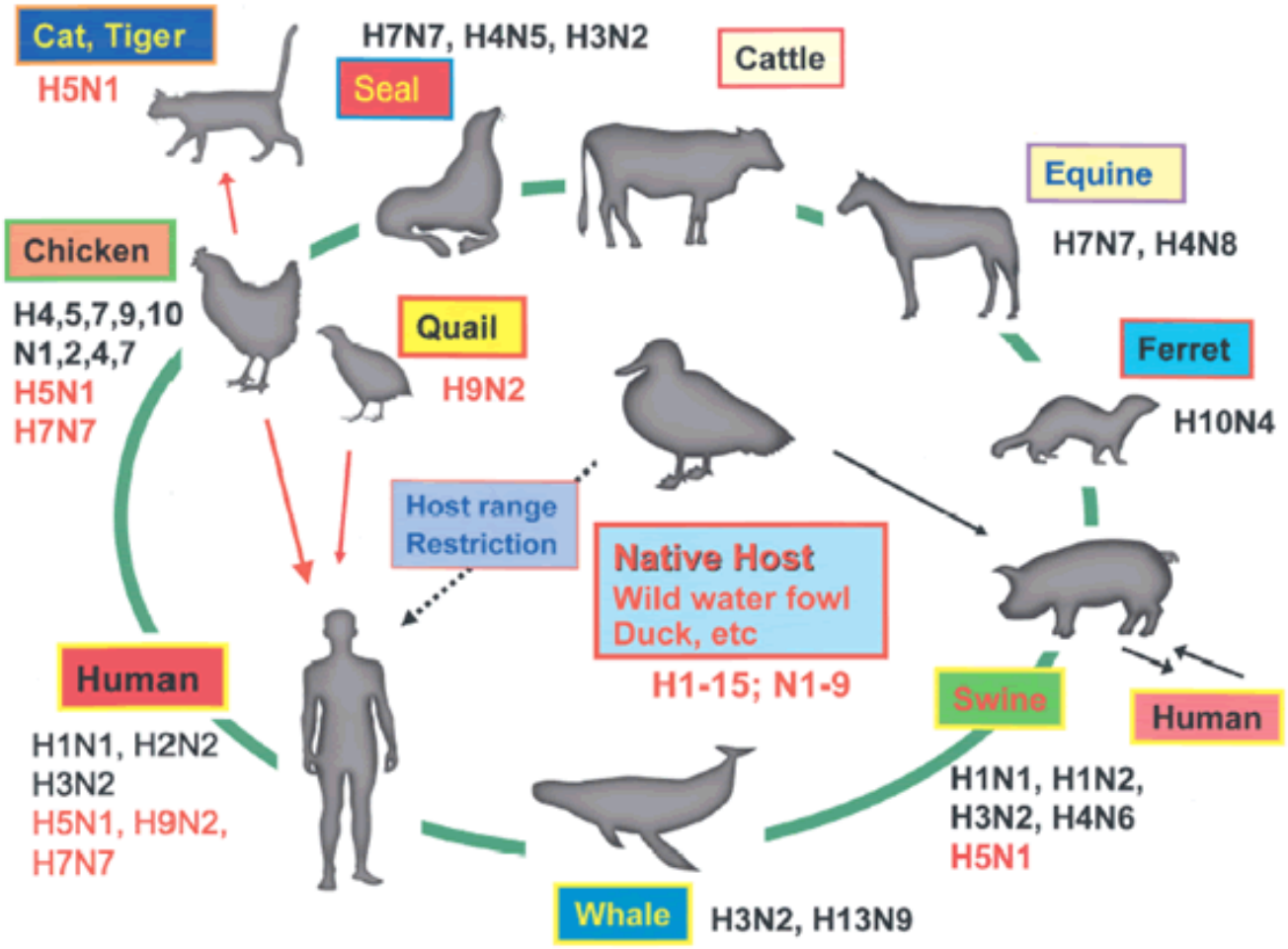

Modified from: Kaiser. *Science* 2006, 312:380-382.

# Influenza A HA and NA Subtypes

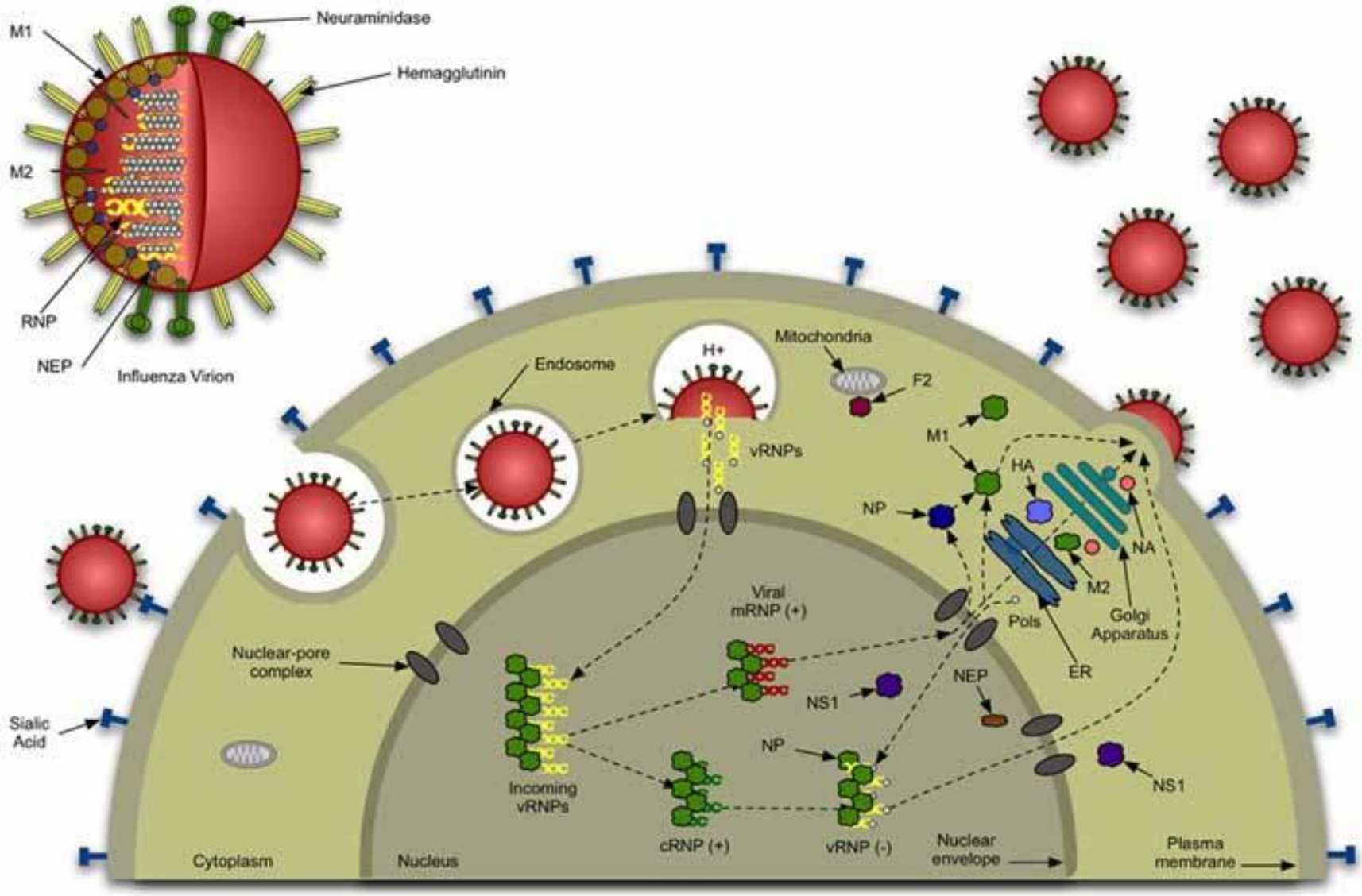


# Diverse Host tropism allows restriction and recombination

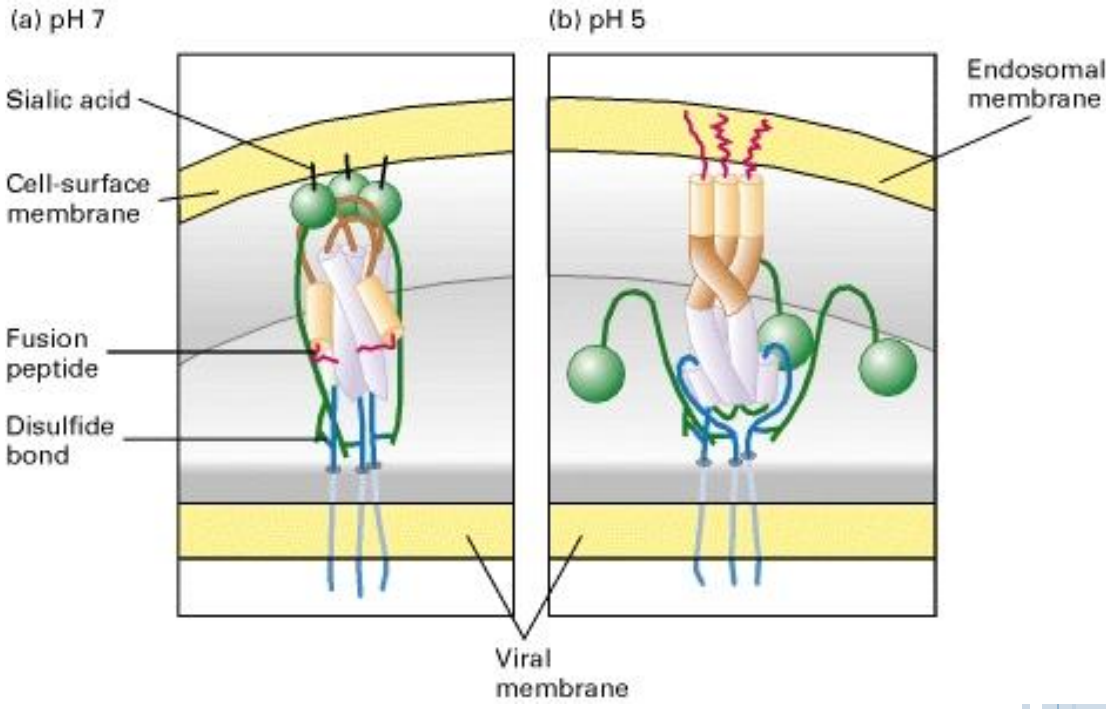


# Influenza Life cycle



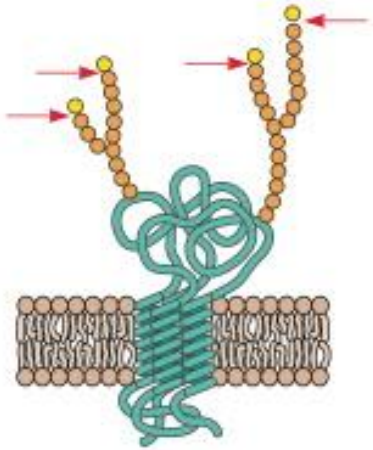
# HA is required for Cell Entry

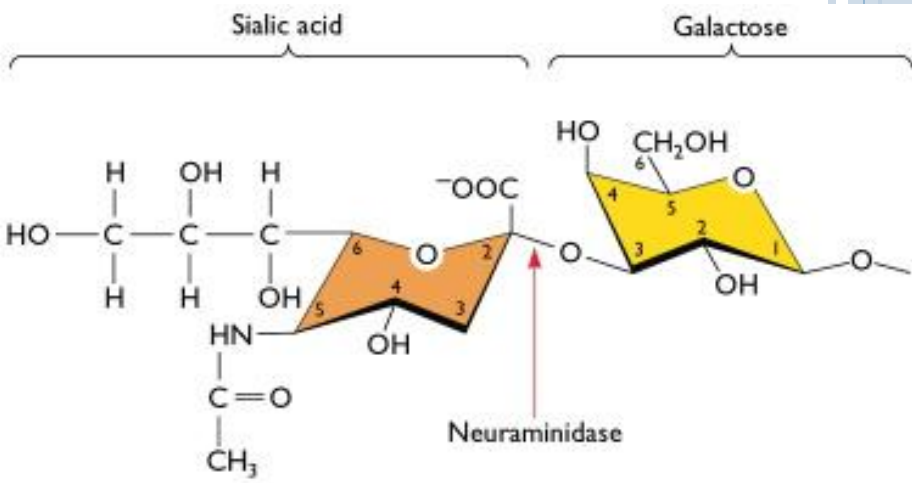
- HA binding to sialic acid on the surface of cells mediates initial attachment
- Virus is endocytosed, where the endosome is acidified
- This triggers a conformational change in the virus, resulting in membrane fusion
- For HA to be active, it needs to be cleaved by a protease into two pieces—this protease is generally restricted to the respiratory epithelium



# Neuraminidase acts to cleave the sialic acid receptors from the cell surface

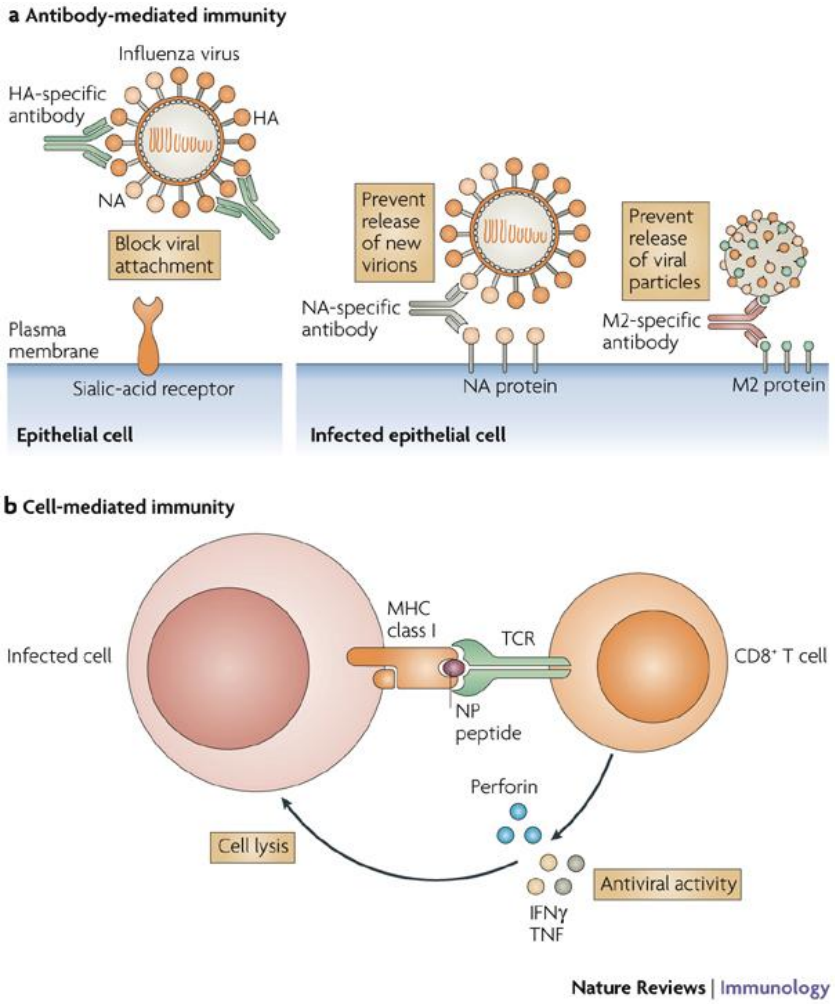
- IAV must balance the binding and entry activity of HA with the sialic acid cleavage activity of NA so that virus efficiently enters and buds from the cell surface—thus HA and NA are often "matched" for activity





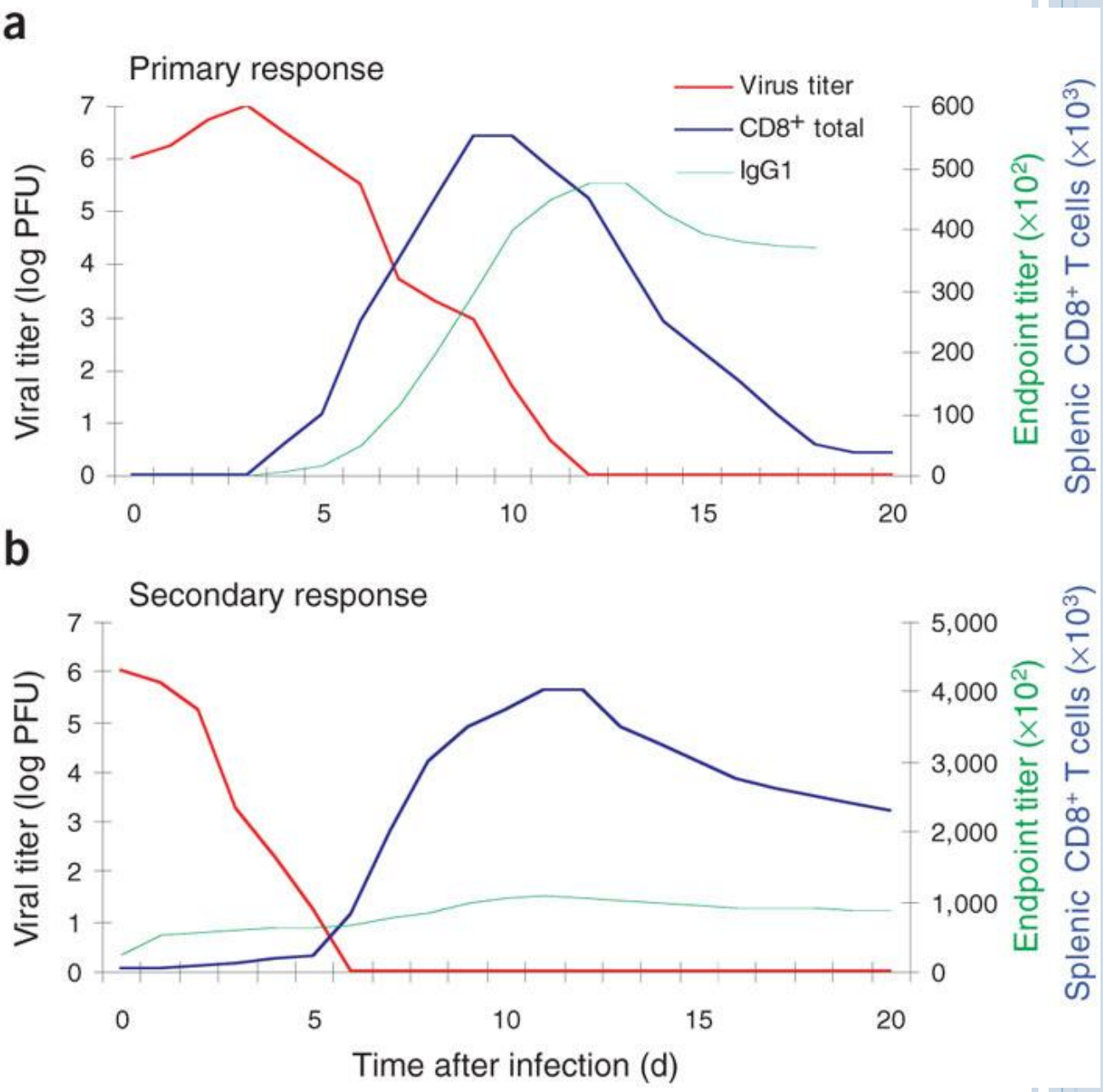
# Immune Mechanisms of Protection

- Antibody mediated immunity exerts the most pressure on the virus, leading to seasonal antigenic drift and pandemic strains of antigenic shift
- Internal proteins are relatively conserved allowing heterologous cellular protection
- Mutation of dominant CD8 epitopes over time suggests that CTLs provide immunological pressure

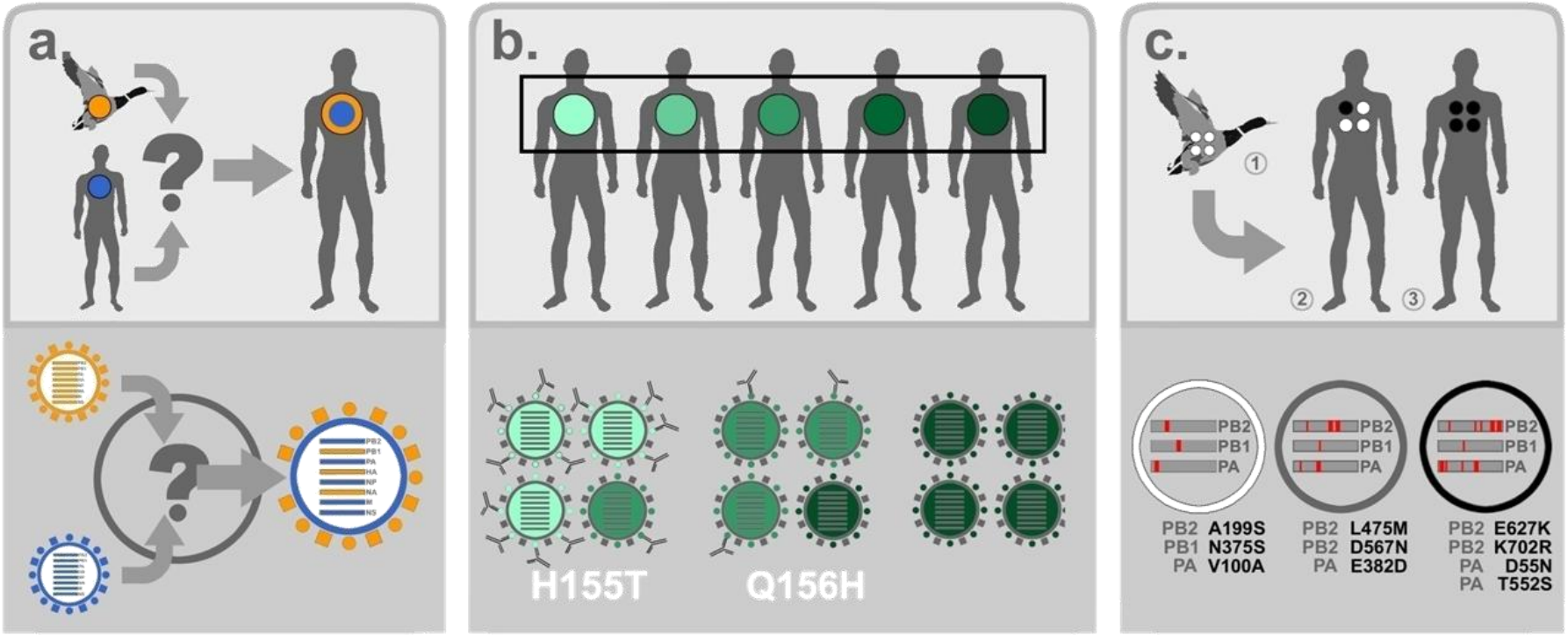


# Immune Course of influenza infection

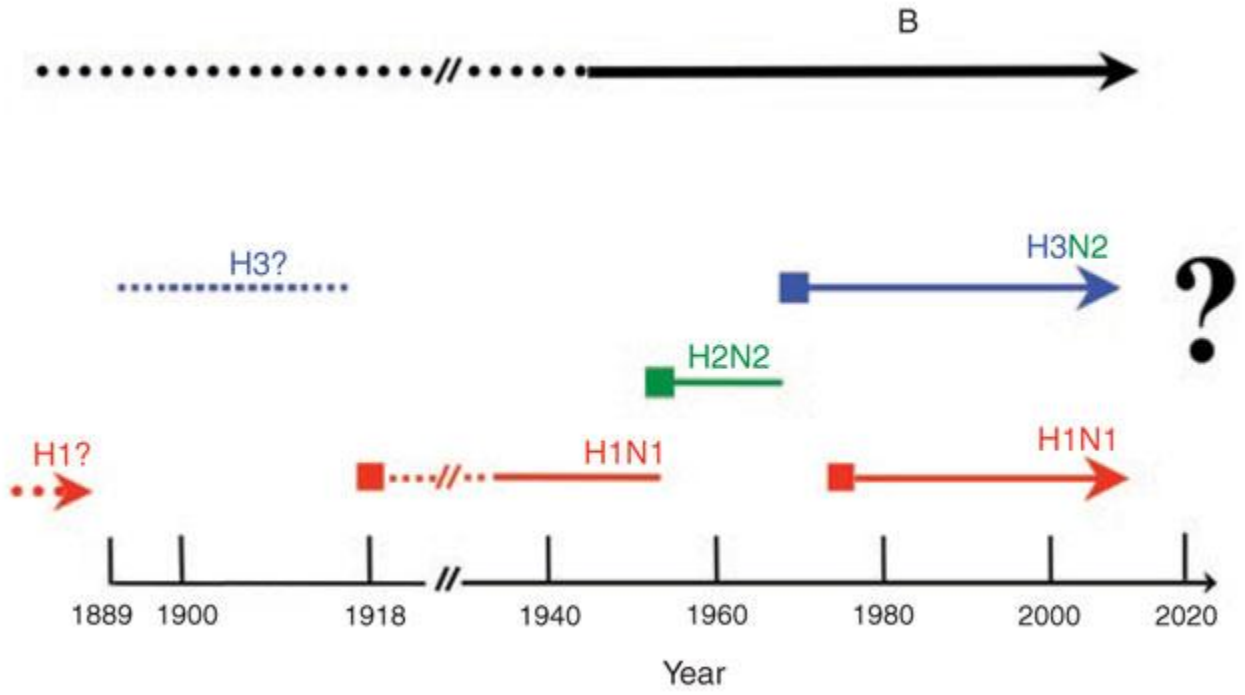
- Influenza is initially controlled by antibody and CD8+ T cells
- Secondary infection with heterologous virus is cleared with CD8+ T cell activity much more rapidly
- Homologous infection can be prevented by antibody (sterilizing immunity)



# Influenza evolution

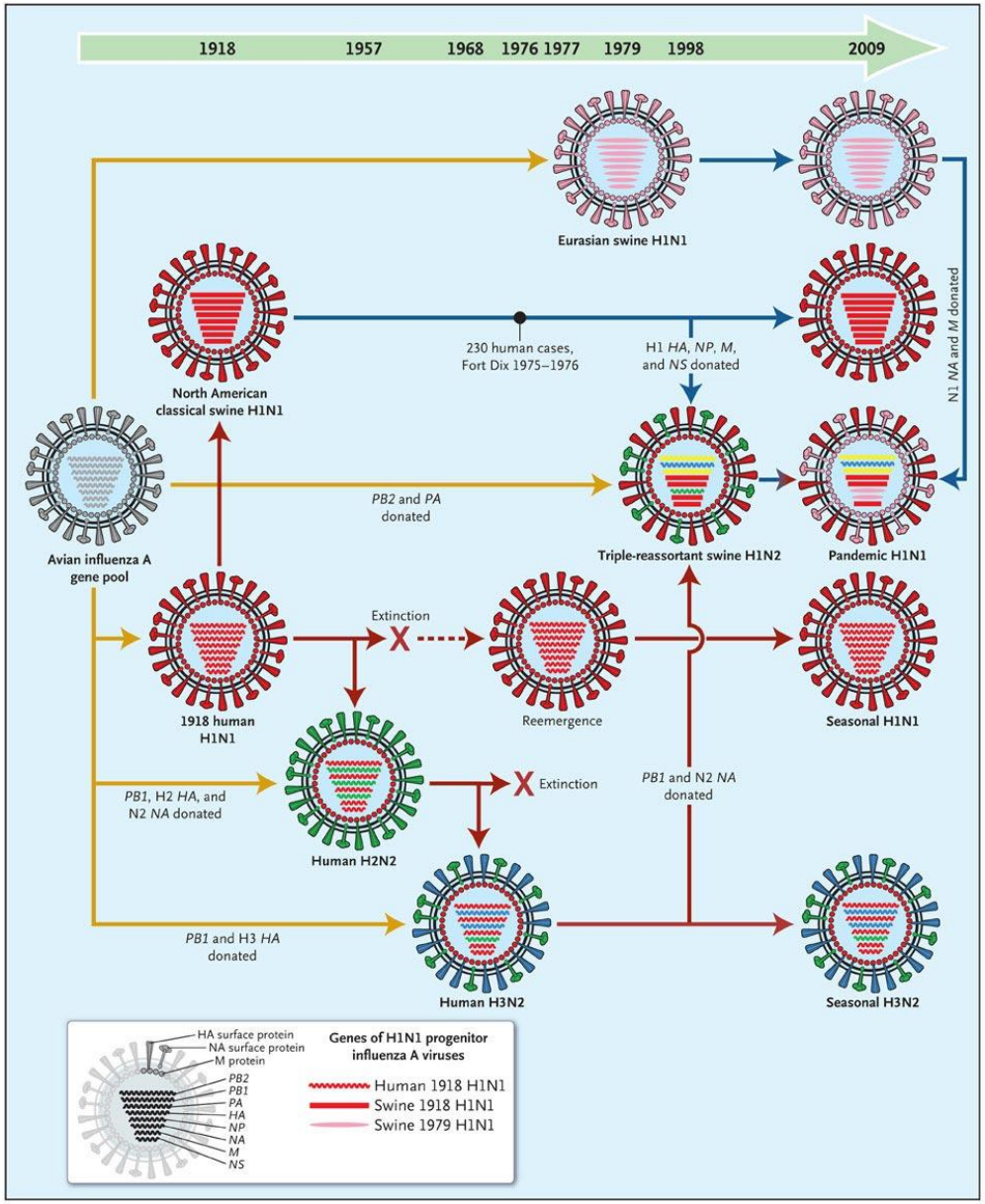


# Human influenza pandemics

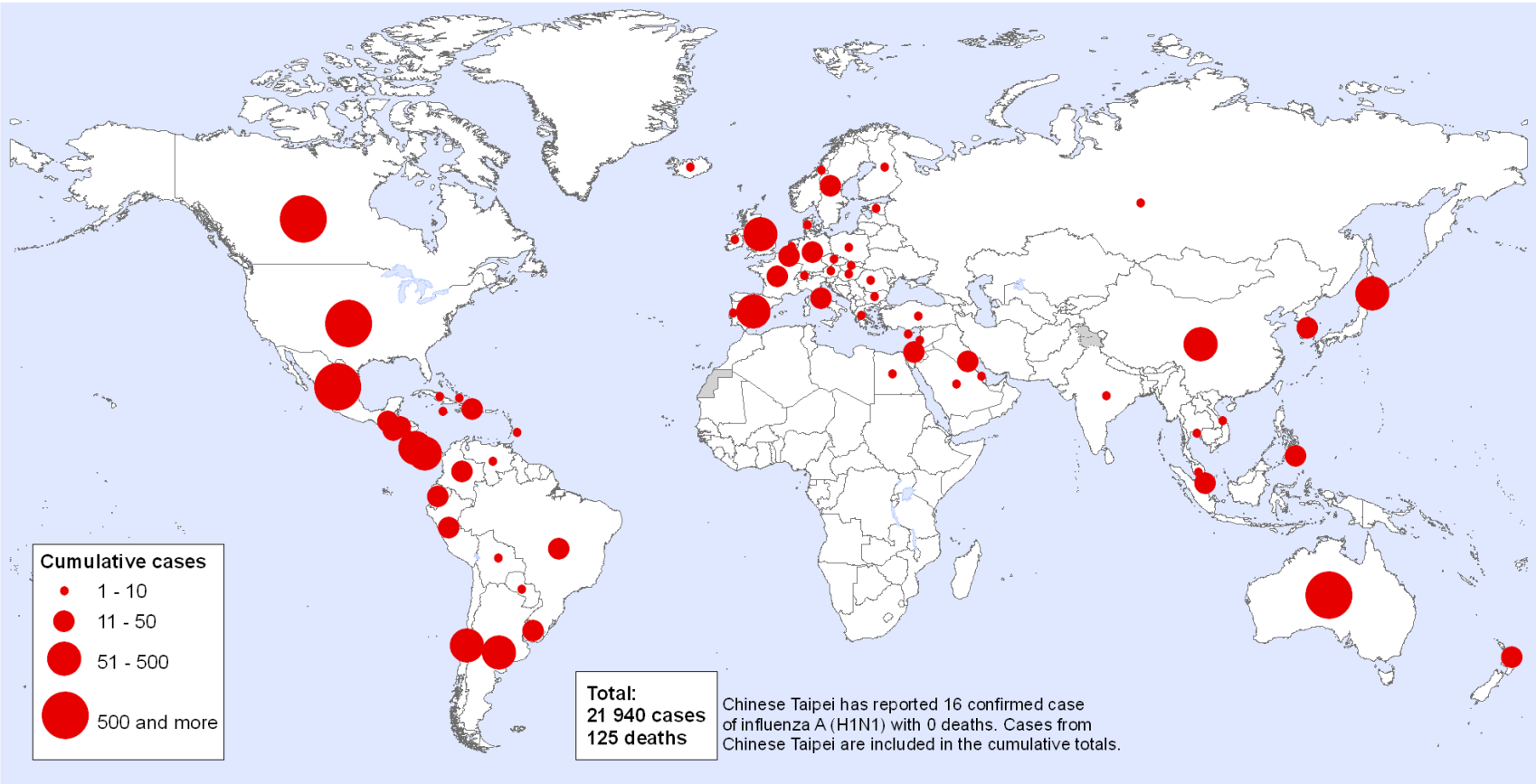


# Evolution of Human Influenza from 1918

- All current human influenza is majority-derived from the 1918 pandemic
- Distinct reservoirs have allowed evolution to occur with varying pressures, providing diverse sources for new gene introductions into the human pool



# Swine-Origin H1N1 Incidence






Data Source: World Health Organization
Map Production: Public Health Information and Geographic Information Systems (GIS)
World Health Organization

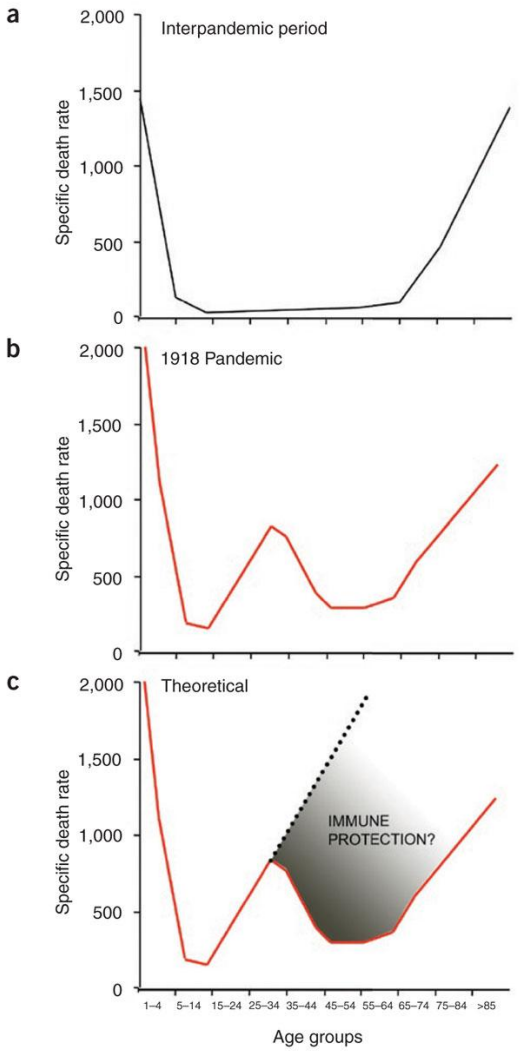
World Health Organization



*Map produced: 05 June 2009 08:10 GMT*

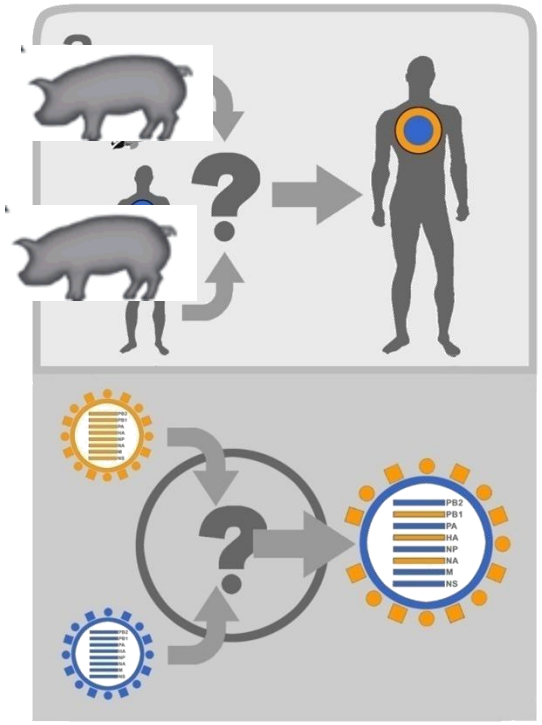
# 1918 (AND POSSIBLY SWORH1N1) MORTALITY CURVES SUGGEST PREVIOUS EXPOSURE

- The "U" shaped curve of regular influenza infection demonstrates the highest mortality among children (naïve) and the elderly (immunocomprimised)
- The 1918 pandemic had a "W" shaped curve, with a spike in deaths among young adults—immunopathology or prior protection for ~40 year olds?



# Predictions of the 2009/H1N1 pandemic

- The 2009 H1N1 pandemic emerged as a particularly novel threat: an antigenic shift event between two swine viruses, without the "human" virus component expected to be required
- The initial rapid spread bred fears of an equally high incidence of severe morbidity and mortality (~90,000 deaths in the US, ~1.8 million hospitalizations)



# Pre-Exisitng Cross-reactive Immunity to 2009/H1N1

The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

## Cross-Reactive Antibody Responses to the 2009 Pandemic H1N1 Influenza Virus

Kathy Hancock, Ph.D., Vic Veguilla, M.P.H., Xiuhua Lu, M.D., Weimin Zhong, Ph.D., Eboneé N. Butler, M.P.H., Hong Sun, M.D., Feng Liu, M.D., Ph.D., Libo Dong, M.D., Ph.D., Joshua R. DeVos, M.P.H., Paul M. Gargiullo, Ph.D., T. Lynnette Brammer, M.P.H., Nancy J. Cox, Ph.D., Terrence M. Tumpey, Ph.D., and Jacqueline M. Katz, Ph.D.

**Table 1. Cross-Reactive Microneutralization Antibody Response against Pandemic Influenza A (H1N1) Virus in Pediatric and Adult Recipients of Seasonal Trivalent Inactivated Influenza Vaccines.***

| Type of Vaccine, Influenza Season, and Influenza Virus Used in Assay | Age Group | No. of Subjects | Increase in Antibody Titer by a Factor of ≥4 | Geometric Mean Titer† | | Microneutralization Titer of ≥40 for Children or ≥160 for Adults‡ | |
|---|---|---|---|---|---|---|---|
| | | | | Before Vaccination (95% CI) | After Vaccination (95% CI) | Before Vaccination | After Vaccination |
| | | | % | | | % | |
| **Children** | | | | | | | |
| Trivalent inactivated influenza vaccine | | | | | | | |
| 2005–2007 | 6 mo to 9 yr | 33 | | | | | |
| Seasonal H1N1 | | | 67 | 26 (16–40) | 267 (171–418) | 45 | 94 |
| Pandemic H1N1 | | | 0 | 5 (5–6) | 6 (5–6) | 0 | 0 |
| 2007–2008 | 5 yr to 9 yr | 13 | | | | | |
| Seasonal H1N1 | | | 85 | 42 (22–80) | 575 (303–1093) | 54 | 100 |
| Pandemic H1N1 | | | 0 | 10 (7–15) | 12 (8–17) | 8 | 15 |
| 2008–2009 | 6 mo to 23 mo | 9 | | | | | |
| Seasonal H1N1 | | | 100 | 5 (4–7) | 285 (202–402) | 0 | 100 |
| Pandemic H1N1§ | | | 0 | 5 | 5 | 0 | 0 |
| Trivalent inactivated influenza vaccine with adjuvant | | | | | | | |
| 2008–2009 | 6 mo to 59 mo | 45¶ | | | | | |
| Seasonal H1N1 | | | 96 | 12 (8–18) | 193 (134–280) | 24 | 100 |
| Pandemic H1N1 | | | 2 | 6 (5–7) | 8 (7–9) | 0 | 4 |

# Table Continued

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| **Adults** | | | | | | | |
| Trivalent inactivated influenza vaccine | | | | | | | |
| 2007–2008 | 18 yr to 64 yr | 148 | | | | | |
| Seasonal H1N1 | | | 75 | 48 (40–58) | 598 (497–720) | 29 | 93 |
| Pandemic H1N1 | | | 22 | 25 (21–31) | 54 (44–65) | 7 | 25 |
| 2008–2009 | 18 yr to 40 yr | 83 | | | | | |
| Seasonal H1N1 | | | 78 | 29 (22–38) | 546 (418–713) | 20 | 88 |
| Pandemic H1N1 | | | 12 | 11 (9–14) | 21 (16–26) | 6 | 7 |
| **Older adults** | | | | | | | |
| Trivalent inactivated influenza vaccine | | | | | | | |
| 2007–2008 | ≥60 yr | 63 | | | | | |
| Seasonal H1N1 | | | 54 | 31 (22–42) | 143 (105–194) | 14 | 54 |
| Pandemic H1N1 | | | 5 | 92 (71–121) | 97 (74–127) | 33 | 43 |
| 2008–2009 | ≥60 yr | | | | | | |
| Seasonal H1N1 | | 49** | 18 | 22 (17–28) | 51 (39–66) | 6 | 14 |
| Pandemic H1N1 | | 50** | 0 | 47 (36–61) | 51 (39–65) | 8 | 8 |

# EARLY PANDEMIC H1N1: APRIL – JULY 2009

Table 2. Estimates of pandemic (H1N1) 2009–related cases and rates of illness and hospitalization by age distribution of confirmed case-patients, United States, April–July 2009

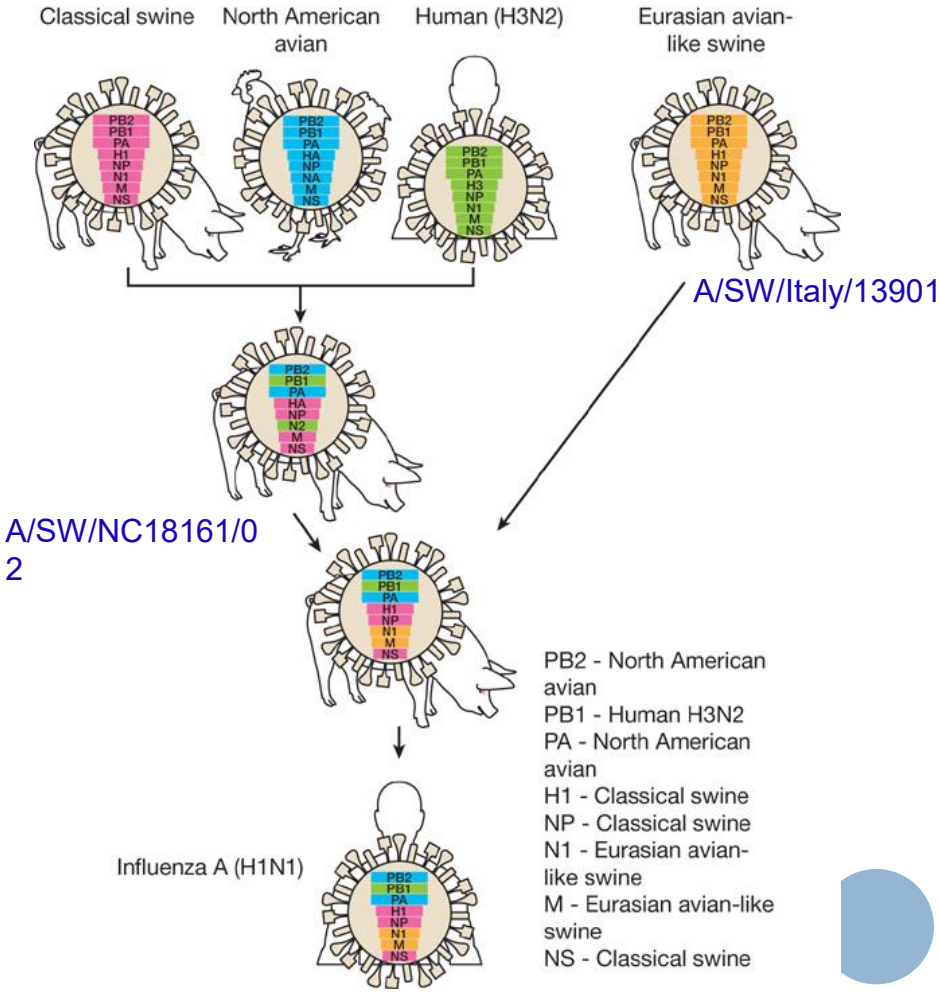
| | Estimated no. case-patients | | Estimated rate/100,000* | |
|---|---|---|---|---|
| Parameter | Median | 90% range | Median | 90% range |
| Total no. case-patients by age group, y† | 3,052,768 | 1,831,115–5,720,928 | 997 | 598–1,868 |
| 0–4 | 397,033 | 238,149–744,045 | 1,870 | 1,122–3,505 |
| 5–24 | 1,820,284 | 1,091,845–3,411,237 | 2,196 | 1,317–4,115 |
| 25–49 | 612,862 | 367,608–1,148,511 | 577 | 346–1,081 |
| 50–64 | 180,297 | 108,146–337,879 | 319 | 192–599 |
| ≥65 | 42,292 | 25,368–79,256 | 107 | 64–201 |
| No. hospitalized case-patients by age group, y | 13,764 | 9,278–21,305 | 4.5 | 3.0–7.0 |
| 0–4 | 2,768 | 1,866–4,285 | 13.0 | 8.8–20.2 |
| 5–24 | 4,991 | 3,364–7,725 | 6.0 | 4.1–9.3 |
| 25–49 | 3,440 | 2,319–5,324 | 3.2 | 2.2–5.0 |
| 50–64 | 1,912 | 1,289–2,959 | 3.4 | 2.3–5.2 |
| ≥65 | 654 | 441–1,012 | 1.7 | 1.1–2.6 |
| Multiplier | | | | |
| Hospitalized | 2.7 | 1.7–4.5 | – | – |
| Nonhospitalized | 79 | 47 –148 | – | – |
| Through May 12 | 33 | 23–49 | – | – |
| After May 12 | 84 | 50–163 | – | – |

*United States Population Estimates, 2009.
†Age distributions from line list and aggregate reports of laboratory-confirmed cases and hospitalizations to the Centers for Disease Control and Prevention through July 23, 3009.

Reed C, Angulo FJ, Swerdlow DL, Lipsitch M, Meltzer MI, Jernigan D, et al. Estimates of the prevalence of pandemic (H1N1) 2009, United States, April–July 2009. *Emerg Infect Dis.* **15** (12): 2004-7.

# 2009 Pandemic H1N1

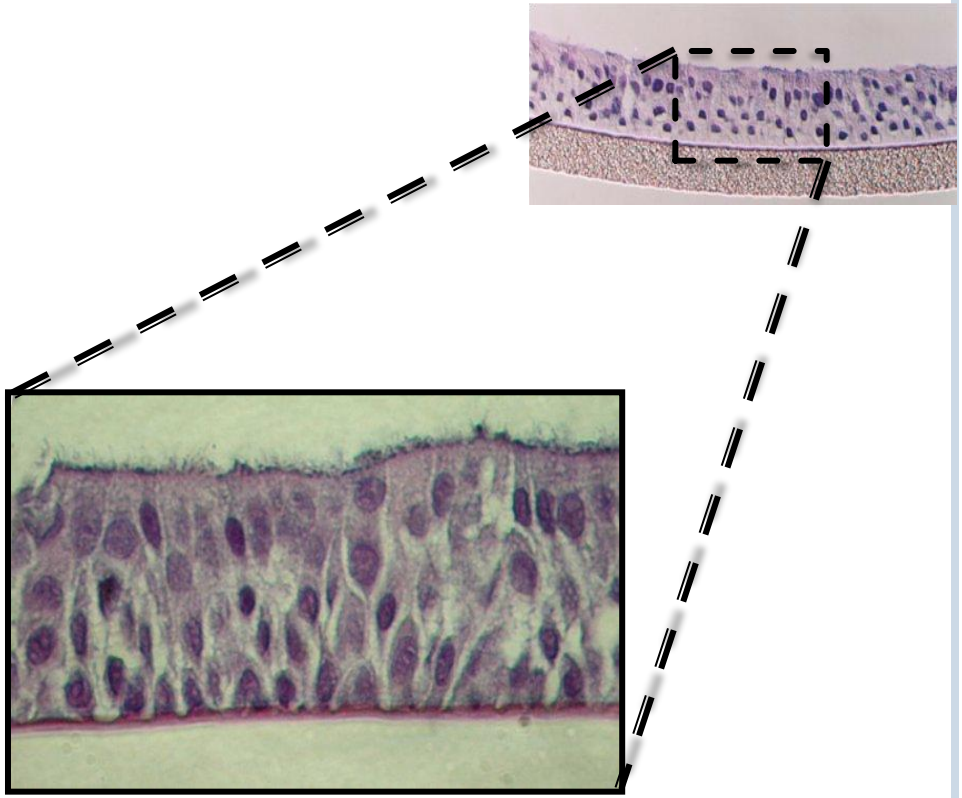
- 2009/H1N1 resulted from the recombination of two viruses (American and Eurasian Swine)
- The American Swine virus was itself a recombinant of three viruses that established itself in 1998
- These viruses are genetically distant from the human seasonal H1N1 (reference strain A/Brisbane/59/07)



# H1N1 Swine Flu studies: response in human cells

Measures:

- Infectivity and growth of virus ($TCID_{50}$, immunofluorescence)
- Secretion of inflammatory mediators from apical and basolateral surfaces (multiplexed immunoassay)
- Transcriptional response over the first 24 hours (Exon arrays, fluidigm analysis)
- Confirm results by “swapped viruses” made by reverse genetics



EpiAirway™, MatTek

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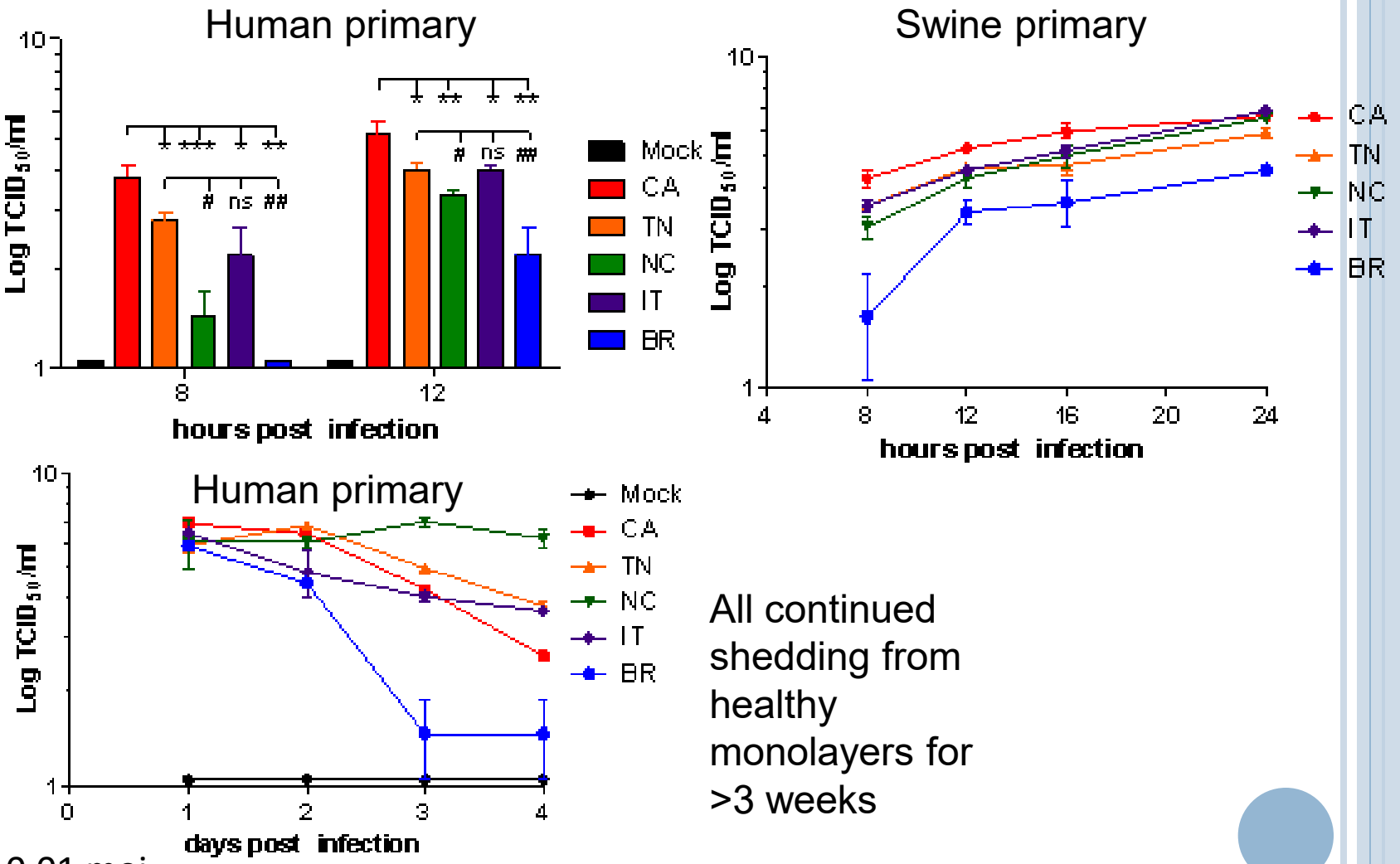
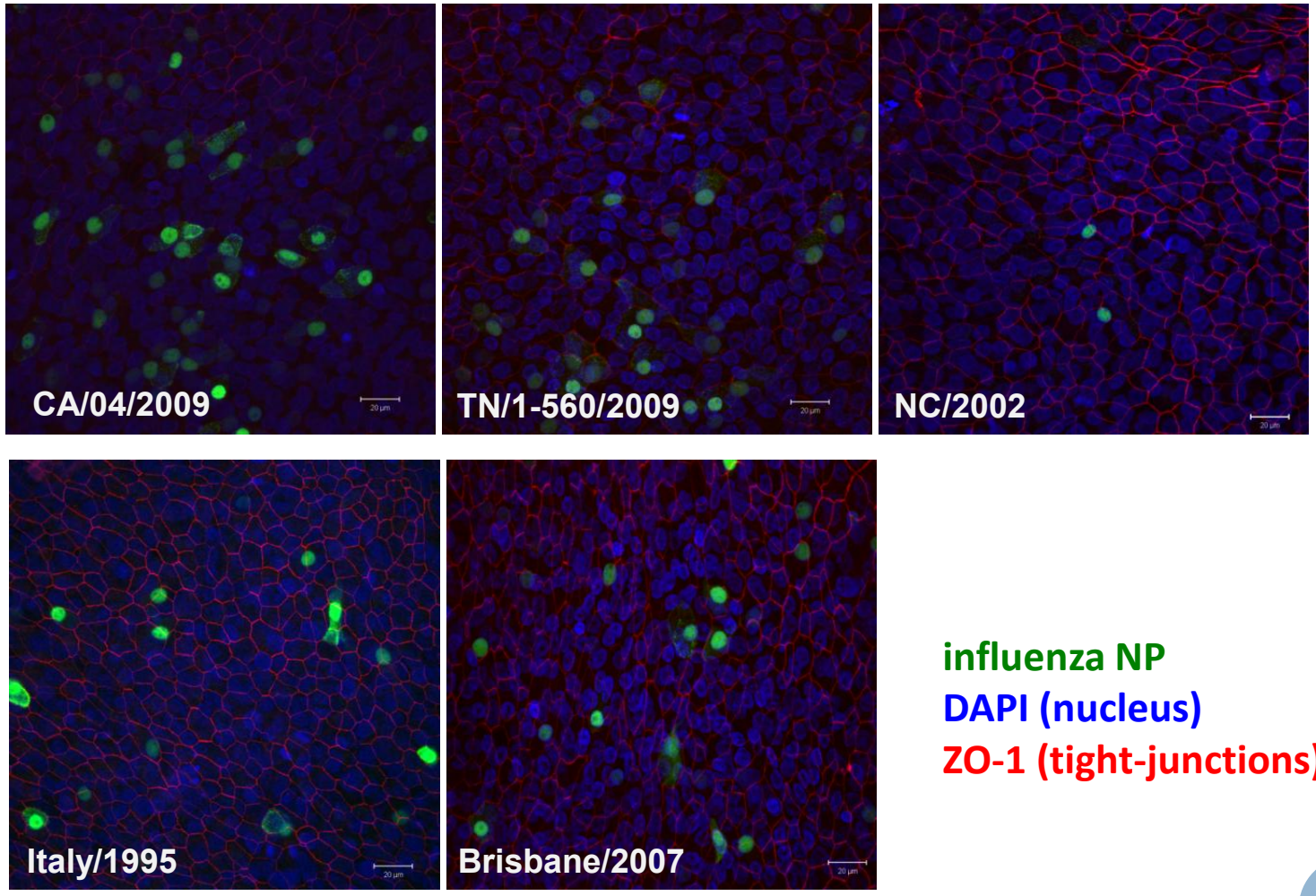
# VIRAL GROWTH KINETICS IN HAE CELLS




0.01 moi

All continued shedding from healthy monolayers for >3 weeks

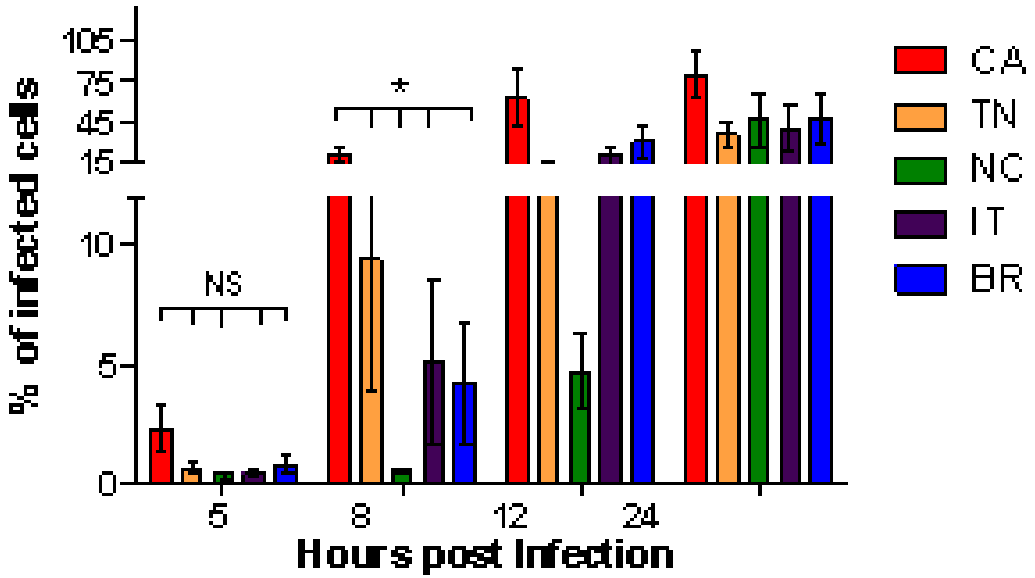
# Influenza NP detection in 3D HAE cultures viral growth kinetics in HAE cells




influenza NP
DAPI (nucleus)
ZO-1 (tight-junctions)

**8 hr post infection- 0.01 moi**

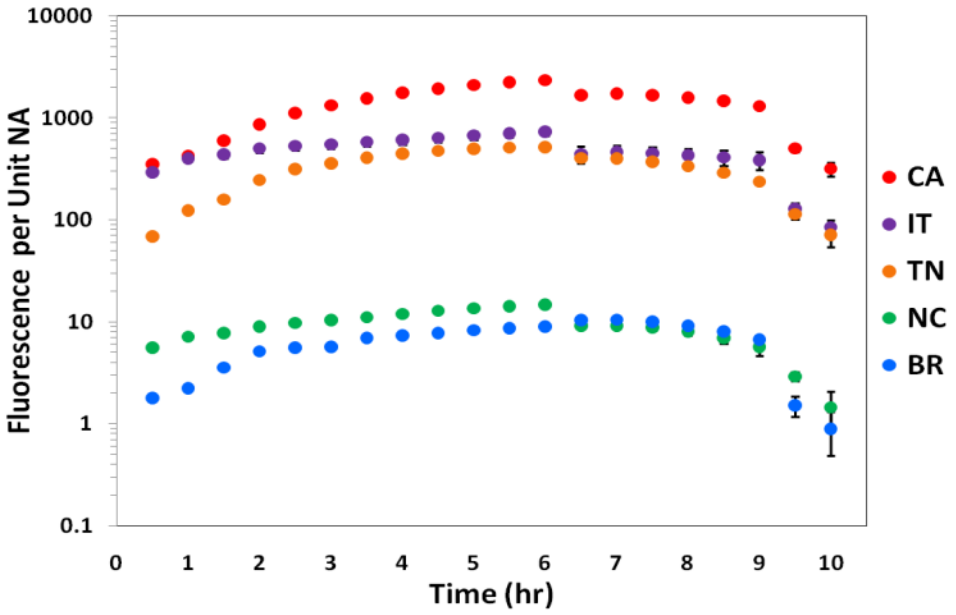
# More rapid colonization of culture by Pandemic and Esw virus




By 12 hours, pandemic strains and Italy have infected ~50%-75% of the culture

# Higher NA activity in Pandemic and ESw

- NA activity measured as ability to convert sialic acid containing substrate
- Results normalized to functional viral titer, so NA activity/infectious virion
- Higher NA activity may relate to ability of virus to spread efficiently



# GROWTH SUMMARY

- The pandemic virus acquired a rapid growth phenotype in human cells similar to the Esw virus
- This phenotype associates with both the NA and M of Esw virus
- The Esw virus transmits more efficiently in ferrets
- Titer and infected cell number can be de-coupled across infections/individuals

# ODE model of influenza infection—Andreas Handel, UGA

$$\frac{dU}{dt} = \lambda D - \frac{b}{1+s_1X}UV \qquad \text{uninfected cells}$$

$$\frac{dE}{dt} = \frac{b}{1+s_1X}UV - \frac{g}{1+s_3X}E \qquad \text{latent infected cells}$$

$$\frac{dI}{dt} = \frac{g}{1+s_3X}E - dI \qquad \text{productively infected cells}$$

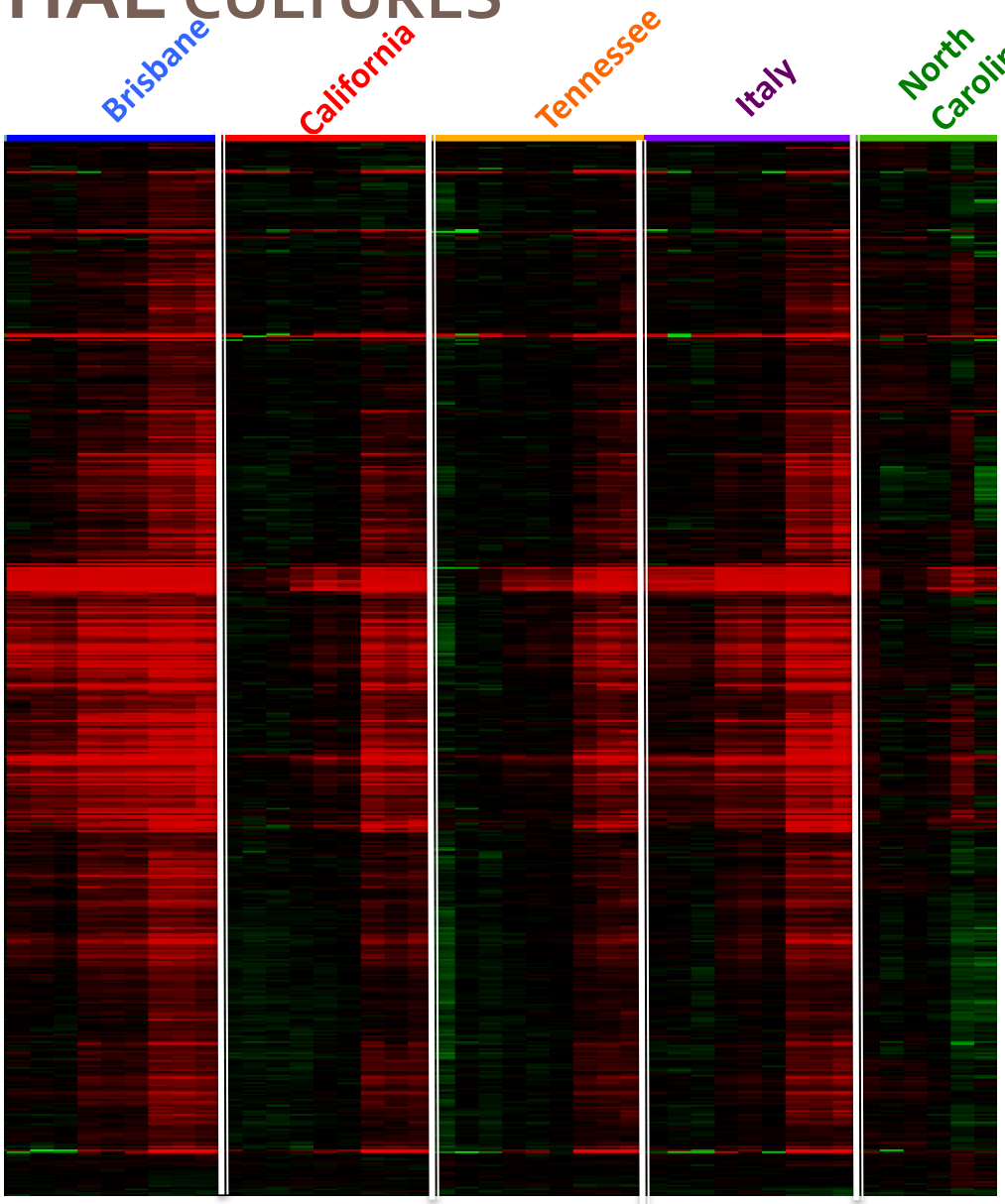
$$\frac{dD}{dt} = dI - \lambda D \qquad \text{dead cells}$$

$$\frac{dV}{dt} = \frac{p}{1+s_2X}I - cV - \gamma\frac{b}{1+s_1X}VU \qquad \text{free virus}$$

Why wasn't the Esw virus a pandemic?

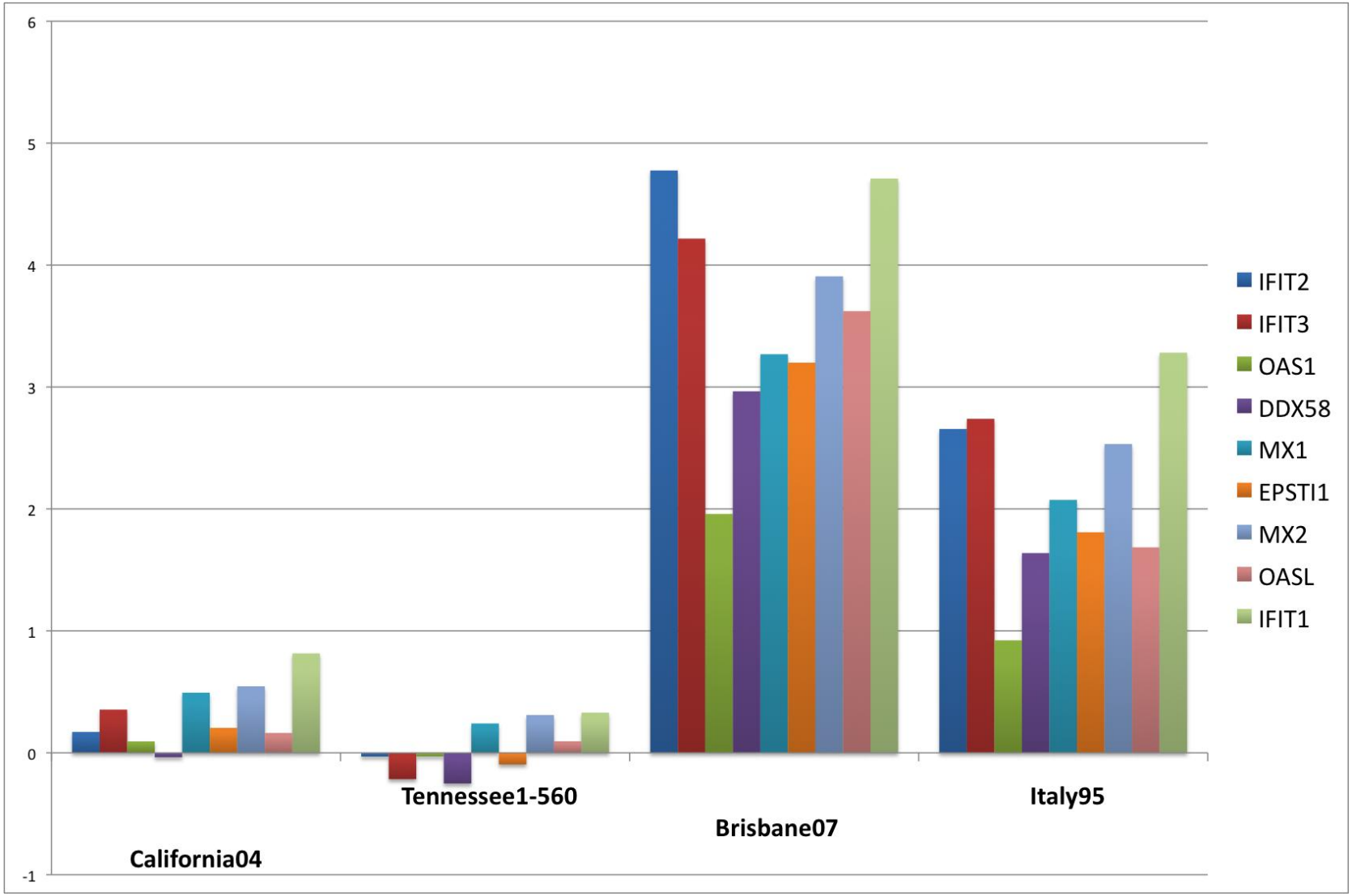
# Transcriptome analysis of pandemic virus infected HAE cultures

mRNA expression in hAE cultures infected at MOI=0.01



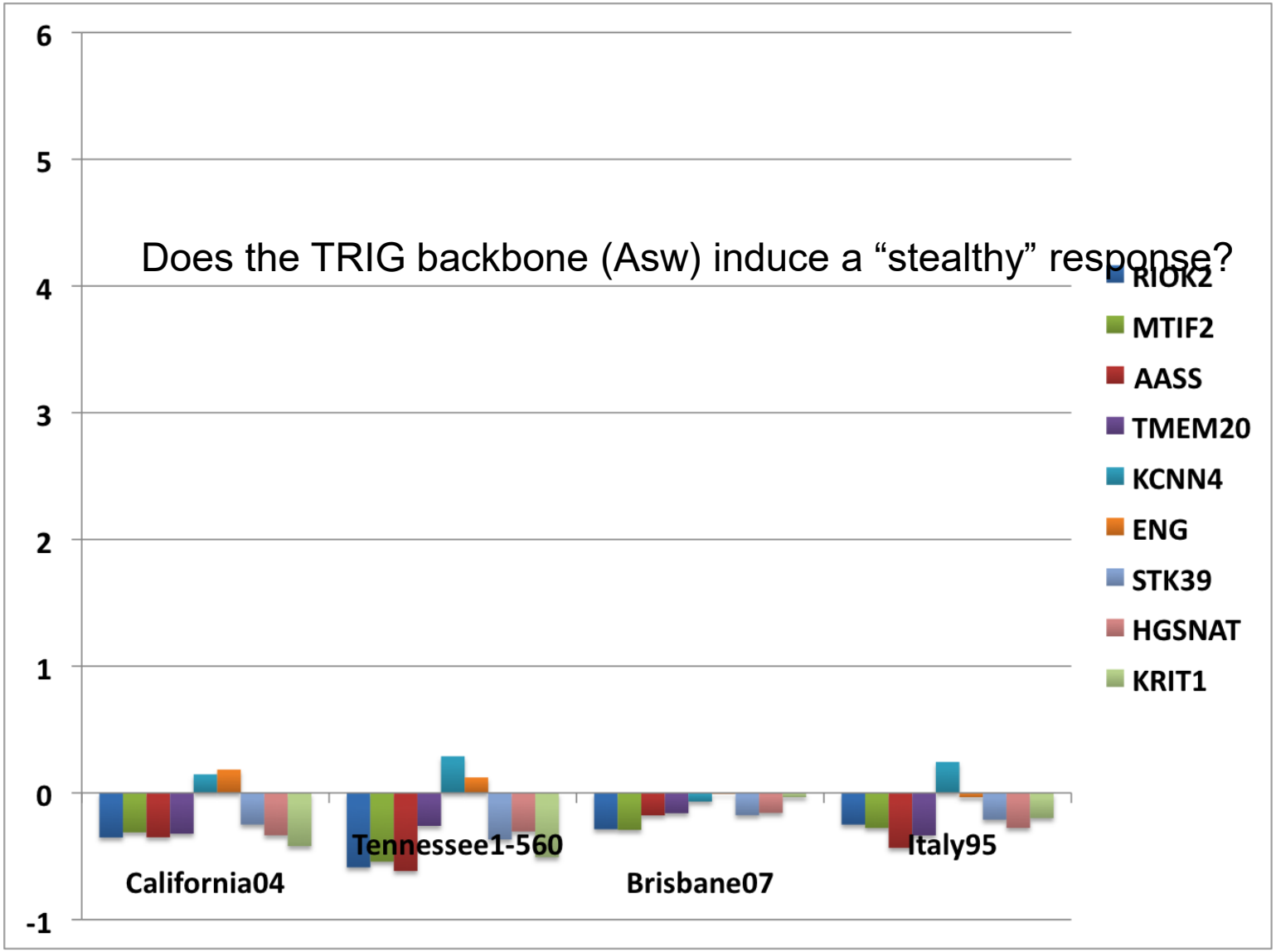

BIC applied to k-means clustering:
2 clusters
271 upregulated in all
24 downregulated or differential

# Top 9 most significant differentially expressed genes 12 hours post-infection with A/Brisbane/59/2007(H1N1)



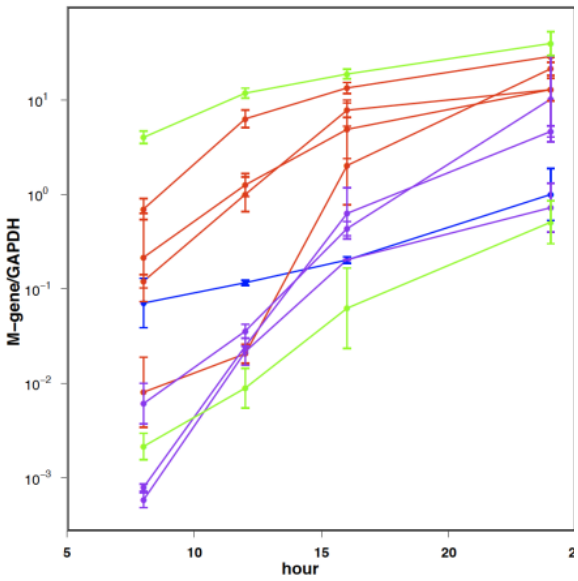

A

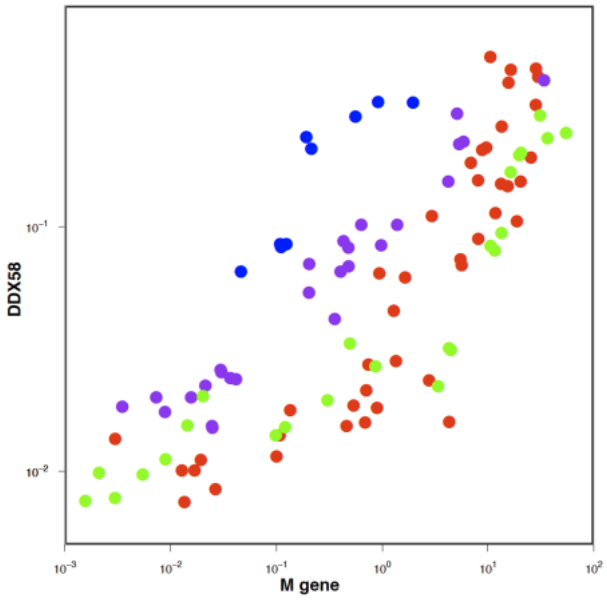
# Top 9 most significant differentially expressed genes at 12 hours post-infection with A/California/04/2009(H1N1)

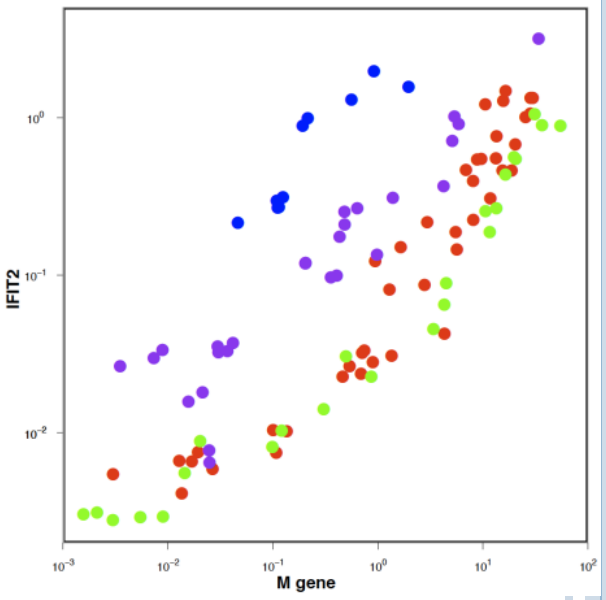



A

# Host response as a function of virus




Fluidigm Real Time PCR from primary human cell infections (2 donors)

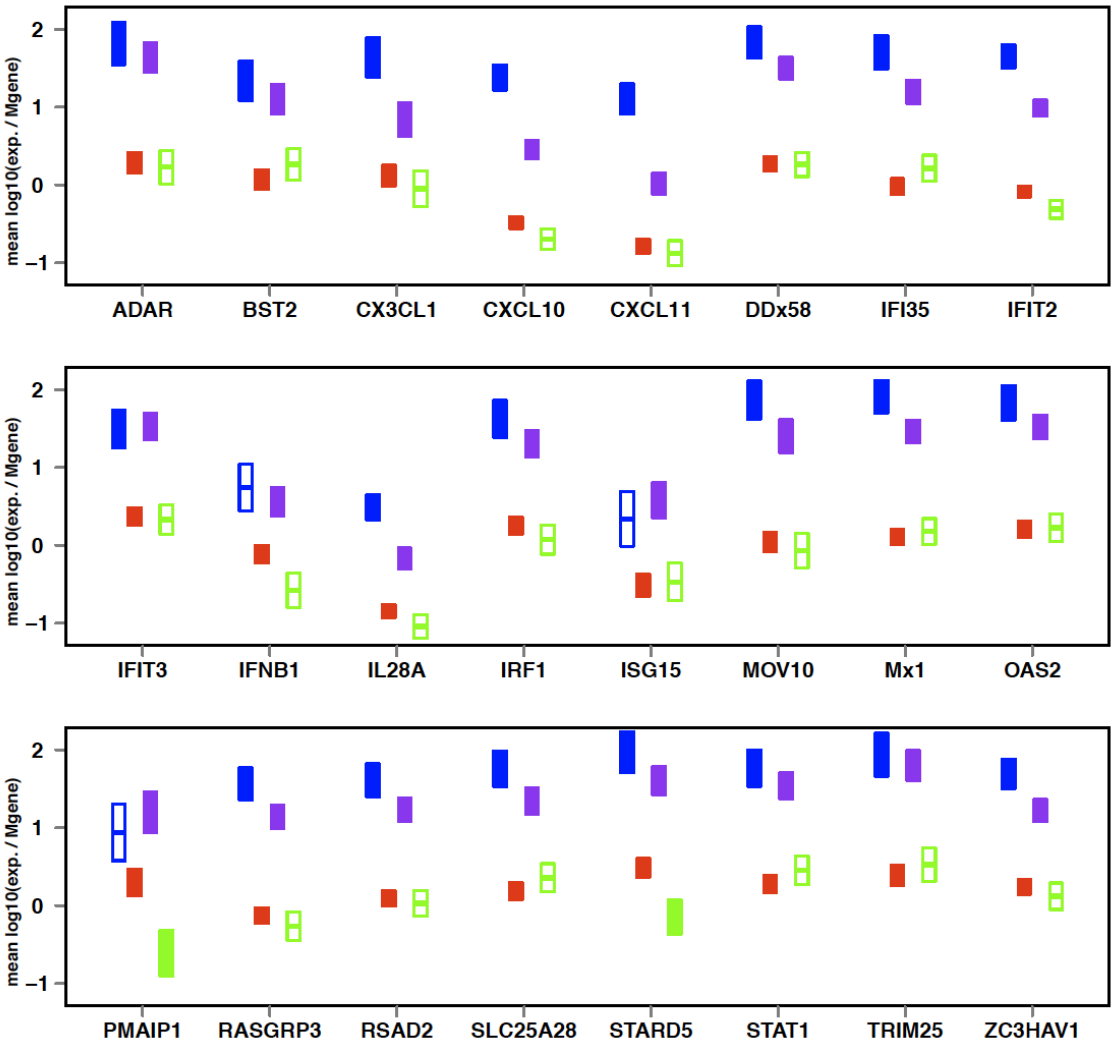
Brisbane California Italy North Carolina

# HOST RESPONSE AS A FUNCTION OF VIRUS II

Brisbane California

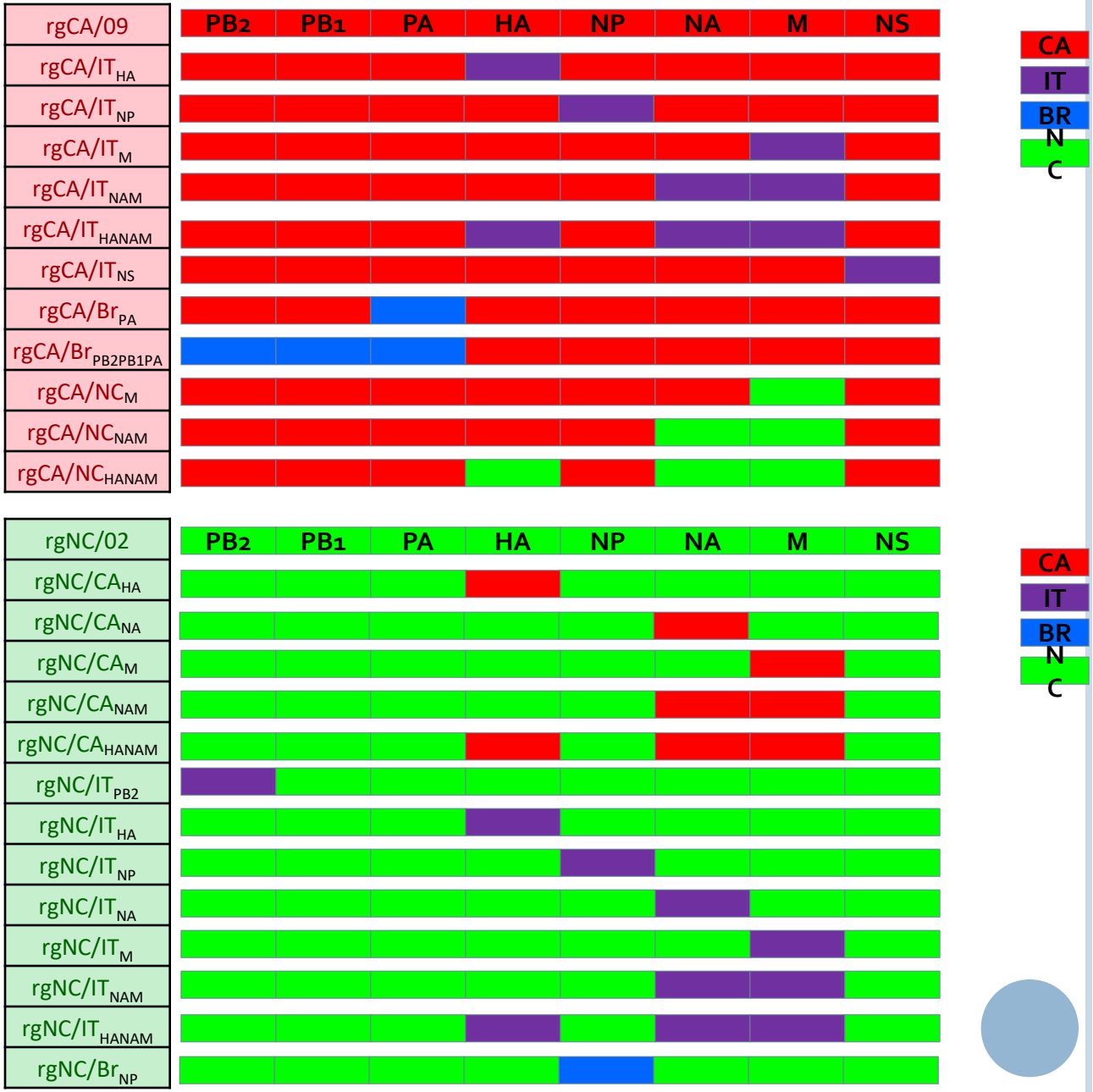
Italy North Carolina

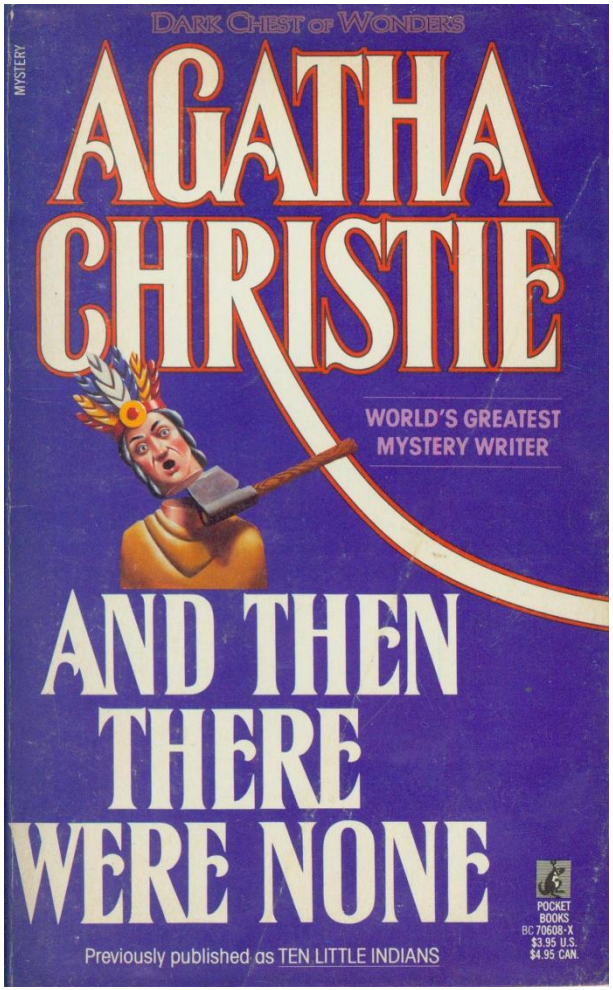
$$\frac{expression - expression_{mock}}{\max(expression)}$$
$$Mgene$$



# Swaps

What's the mechanistic basis of the stealthy (or noisy) phenotype?

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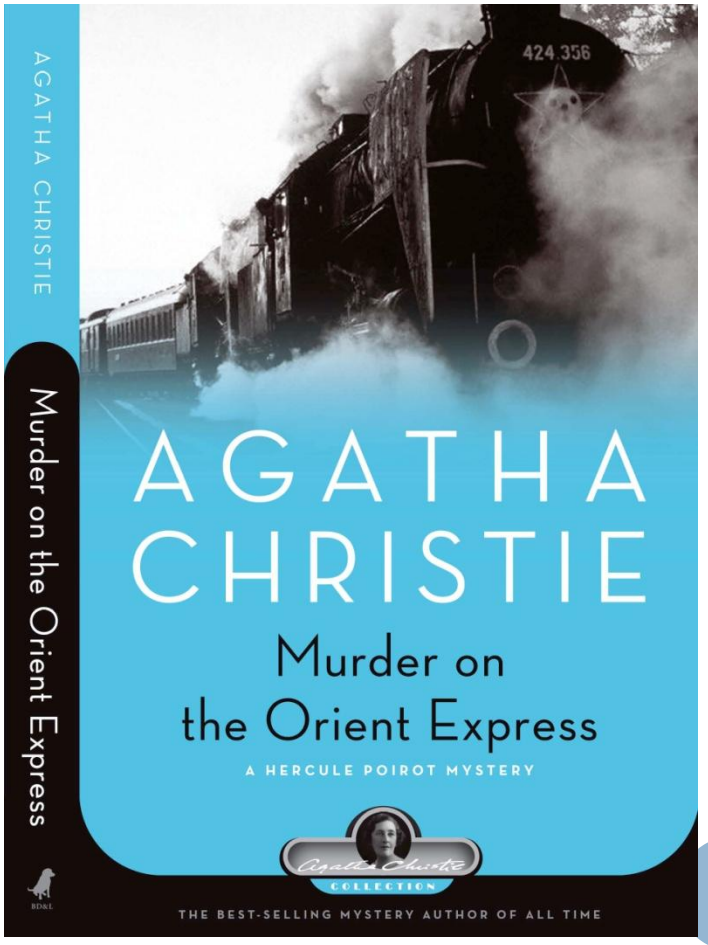
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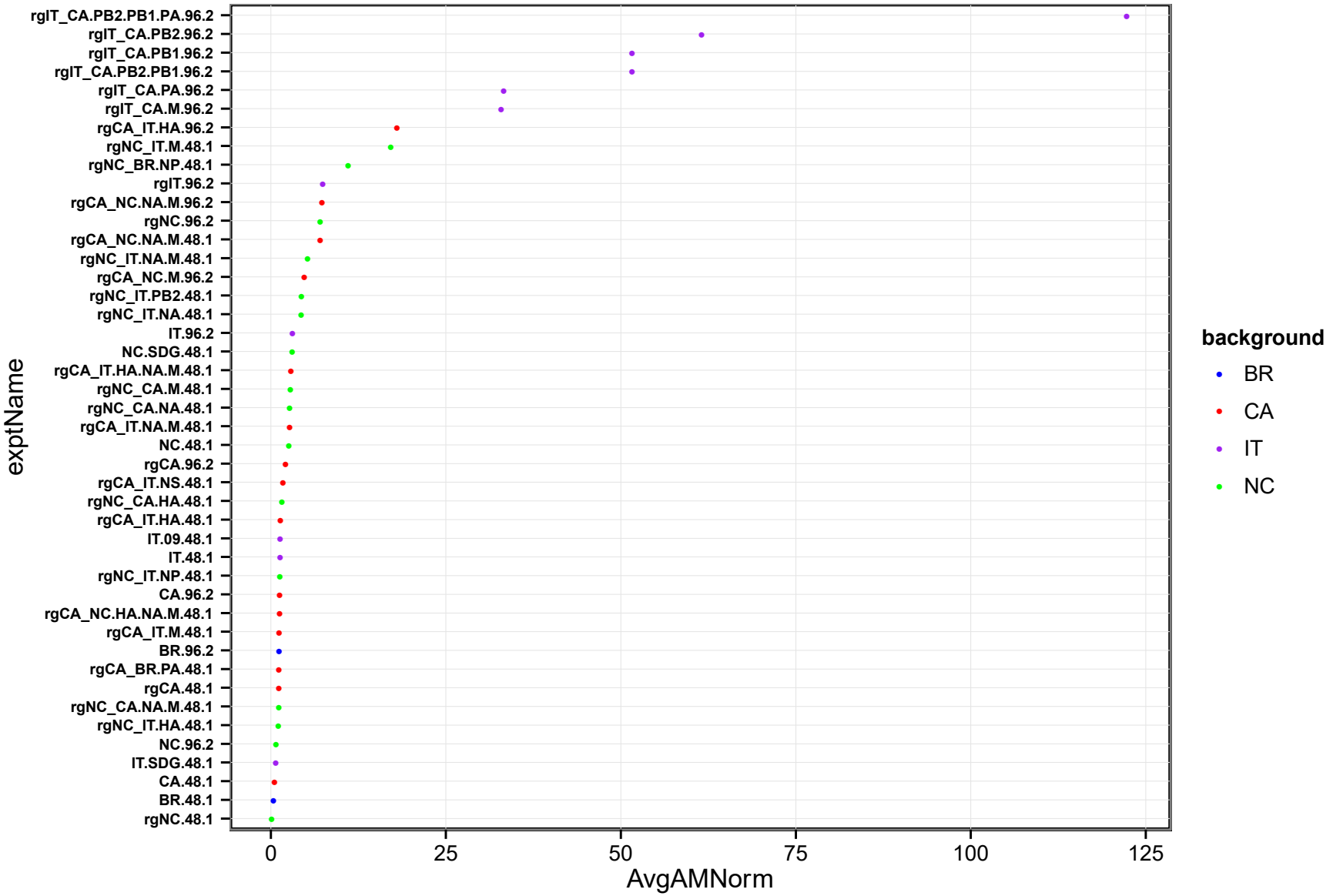
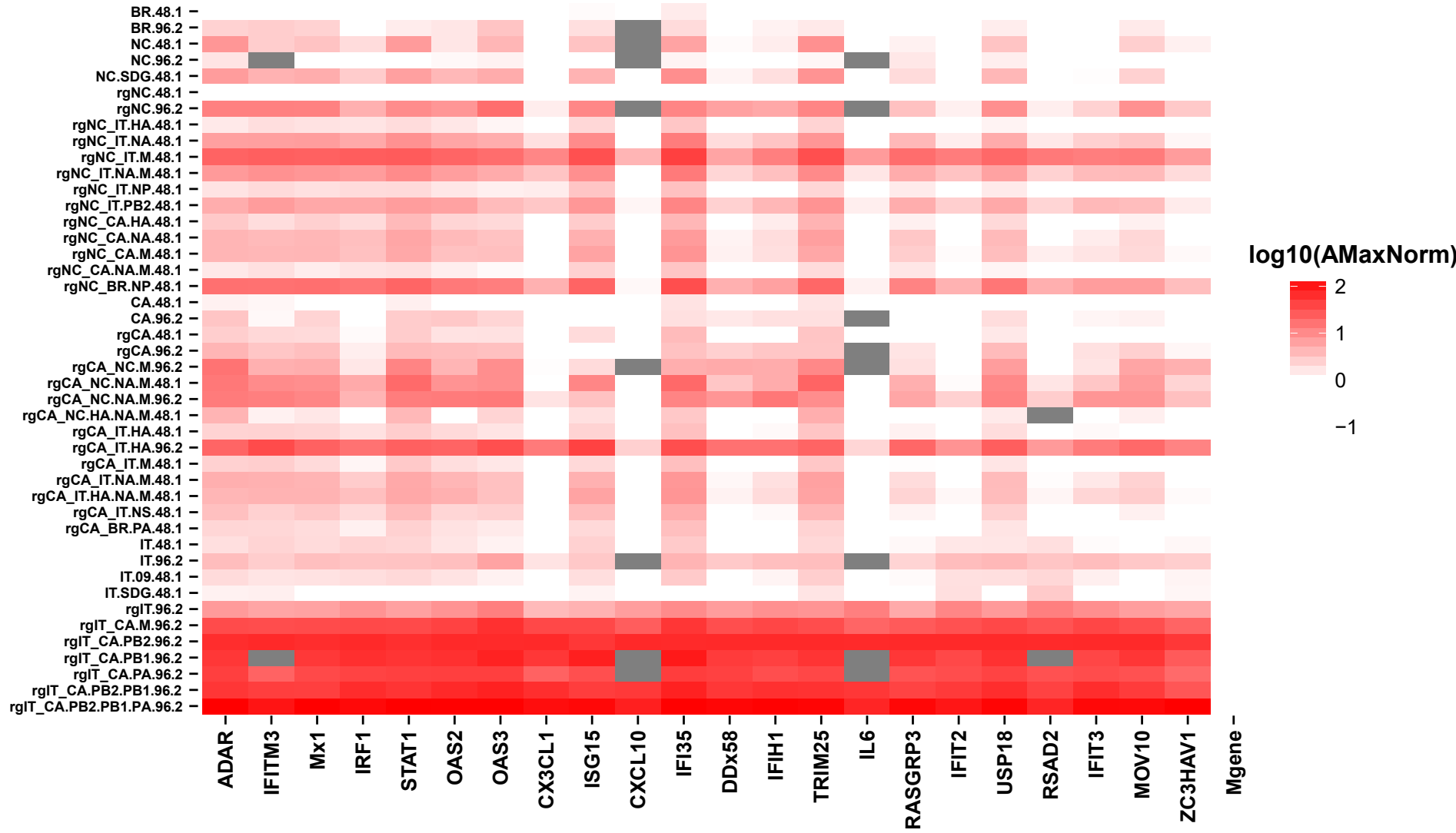
Murder on the Orient Express
A HERCULE POIROT MYSTERY
COLLECTION
THE BEST-SELLING MYSTERY AUTHOR OF ALL TIME

# Average amplitude across all genes normalized to M-gene



# Amplitude (“A”) normalized to M-gene



# The pandemic strain is efficient and stealthy

- Rapid + stealthy growth = Pandemic

Morbidity and Mortality Weekly Report

**Limited Human-to-Human Transmission of Novel Influenza A (H3N2) Virus — Iowa, November 2011**

- The set of genes induced by diverse viruses is largely equivalent in the first 24 hours— “the flu program”
- The pandemic strategy is distinct from the well-adapted human seasonal virus
- Kinetic differences in the first ~18 hours of infection are critical to the quality and quantity of the later response
- The stealthy phenotype ismediated by contriubtions of the P-gene complex, with potential roles for NP and NS

# ODE MODEL OF INFLUENZA INFECTION

$$\frac{dU}{dt} = \lambda D - \frac{b}{1+s_1X}UV \qquad \text{uninfected cells}$$

$$\frac{dE}{dt} = \frac{b}{1+s_1X}UV - \frac{g}{1+s_3X}E \qquad \text{latent infected cells}$$

$$\frac{dI}{dt} = \frac{g}{1+s_3X}E - dI \qquad \text{productively infected cells}$$

$$\frac{dD}{dt} = dI - \lambda D \qquad \text{dead cells}$$

$$\frac{dV}{dt} = \frac{p}{1+s_2X}I - cV - \gamma\frac{b}{1+s_1X}VU \qquad \text{free virus}$$

$$\frac{dX}{dt} = wI - \delta X \qquad \text{innate immune response (IFN)}$$

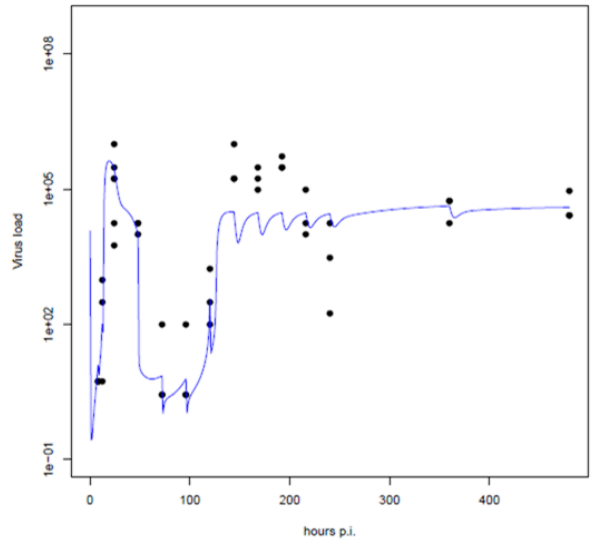
# AICc VALUES OF 8 DIFFERENT MODELS

1. No IR and no cell-regrowth
2. No IR, with cell-regrowth
3. With IR reducing virus production, no cell-regrowth
4. With IR reducing infection rate, no cell-regrowth
5. With IR prolonging latency, no cell-regrowth
6. With IR reducing virus production, with cell-regrowth
7. With IR reducing infection rate, with cell-regrowth
8. With IR prolonging latency, with cell-regrowth

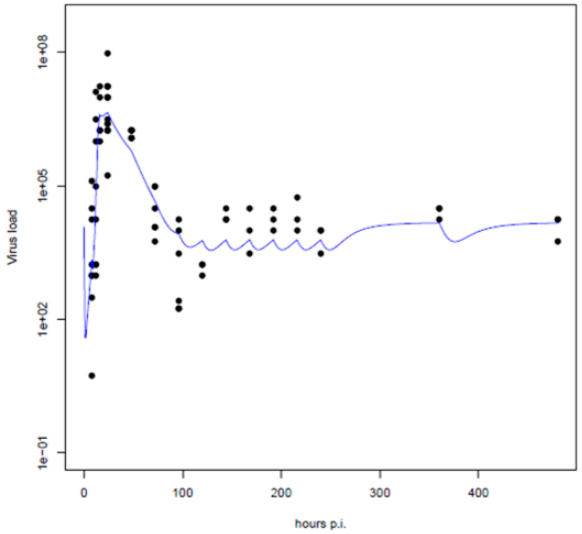
| Model | BB | CA | IT | NC |
|---|---|---|---|---|
| 1 | 54.5 | 54.7 | 33.1 | 28.2 |
| 2 | 48.8 | -22.6 | 0.8 | 28.5 |
| 3 | 52.8 | 24.8 | 17.0 | 30.3 |
| 4 | 59.9 | 33.2 | 38.3 | 33.6 |
| 5 | 53.2 | 32.1 | 24.6 | 31.7 |
| 6 | -11.6 | -17.6 | -11.1 | 33.2 |
| 7 | 54.5 | -17.7 | 6.1 | 29.3 |
| 8 | 56.1 | -17.3 | 6.2 | 34.3 |

# Fits for Model 6—IR reduces virus production and cells regrow

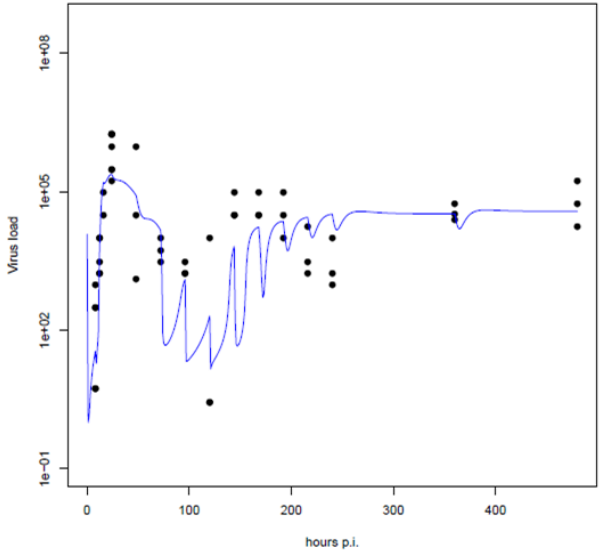
BR



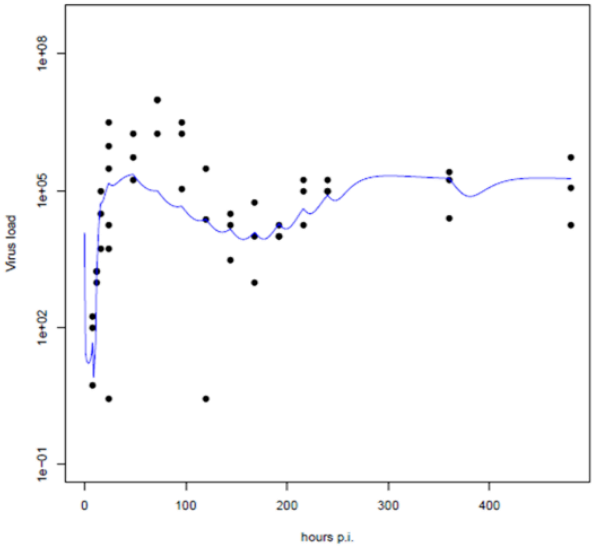

CA



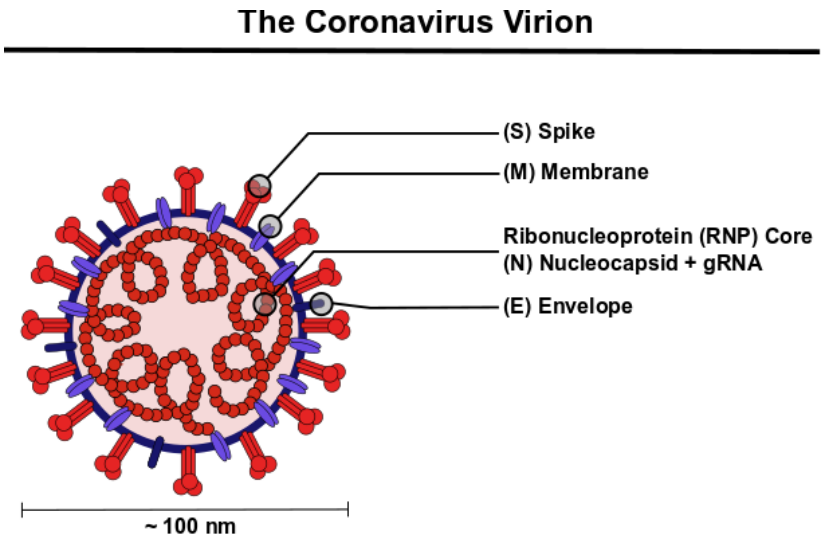

IT



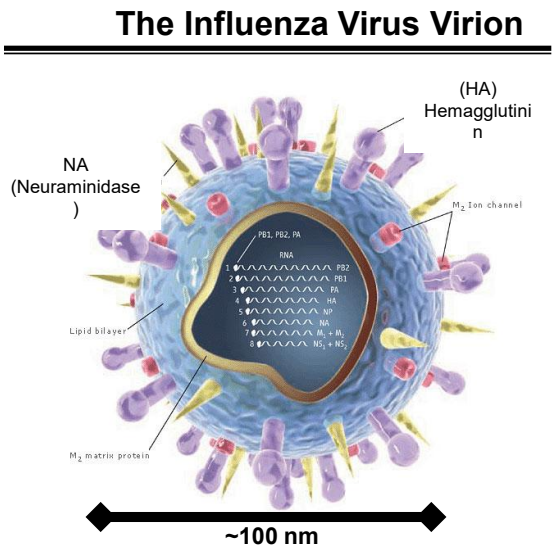

NC



# SARS-CoV-2 vs. Influenza virus



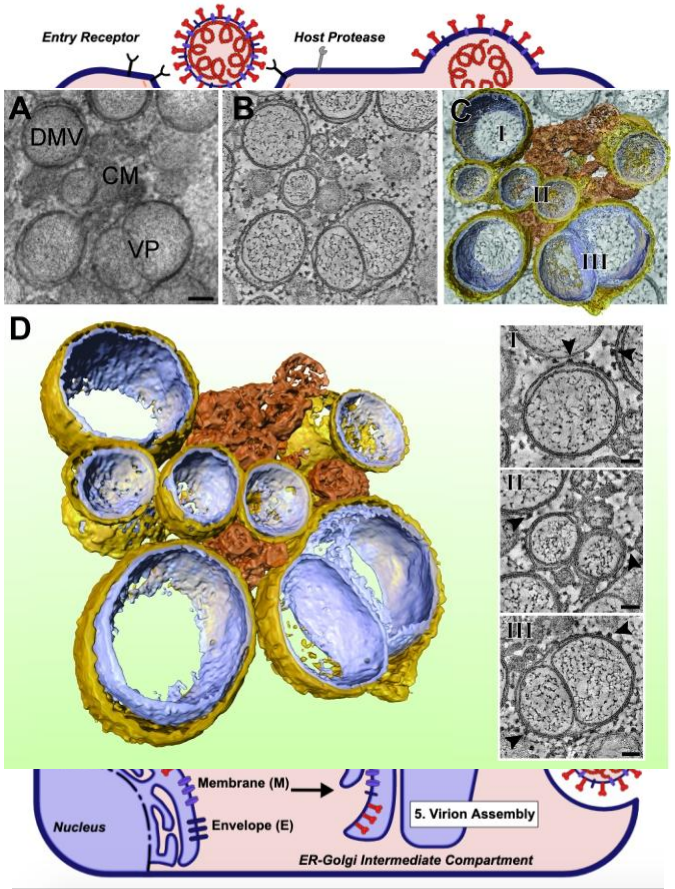

(+) ssRNA genome ~28-32 Kb
29 proteins



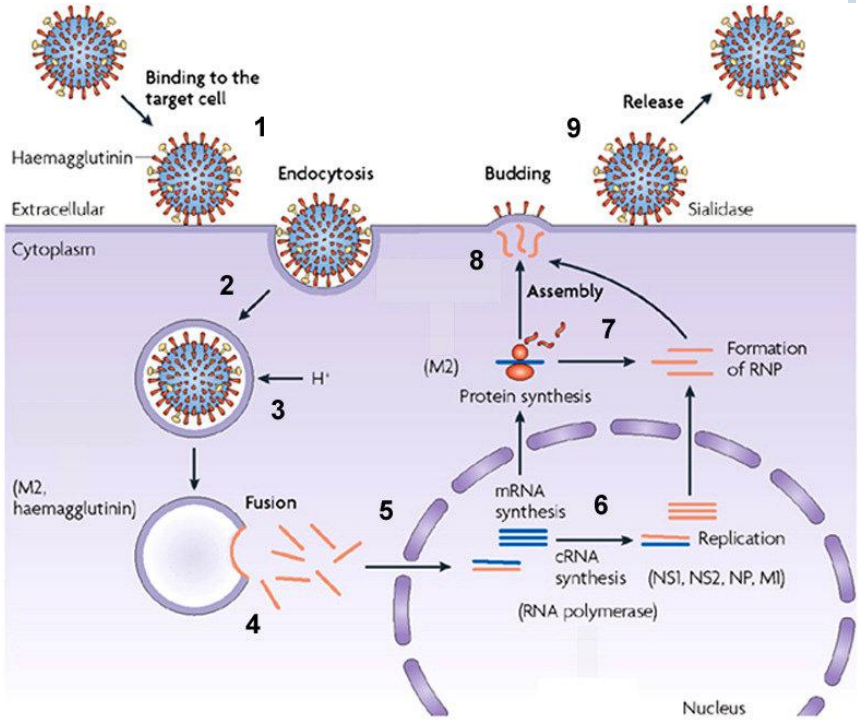

(-) segmented ssRNA genome ~28-32 Kb
~14 Kb, 10-14 proteins

# Coronavirus and influenza virus replication cycles

## Coronavirus

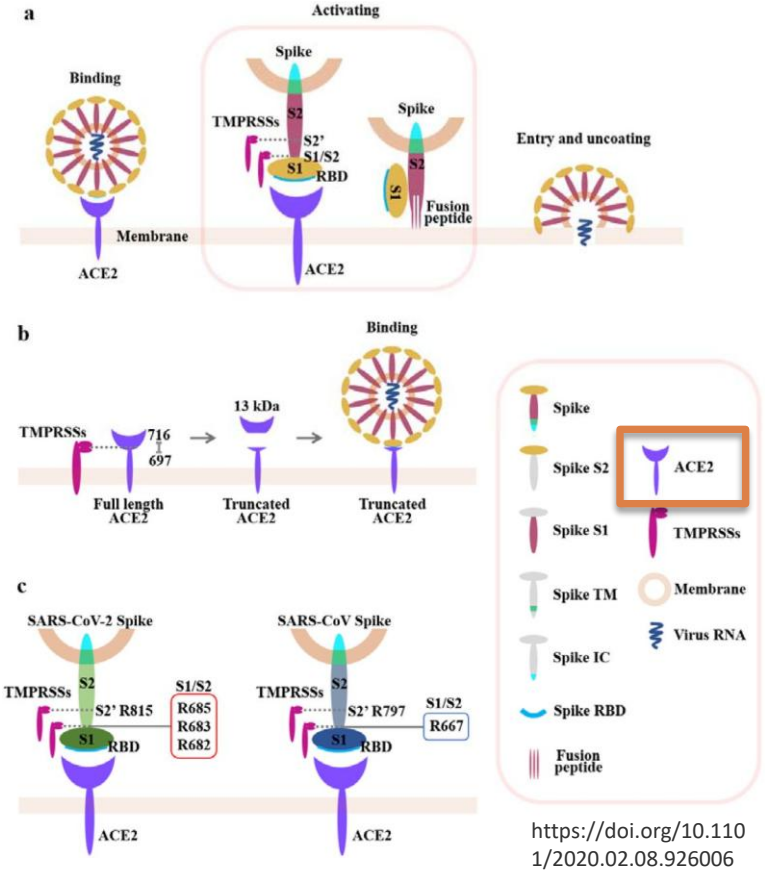



## Influenza virus



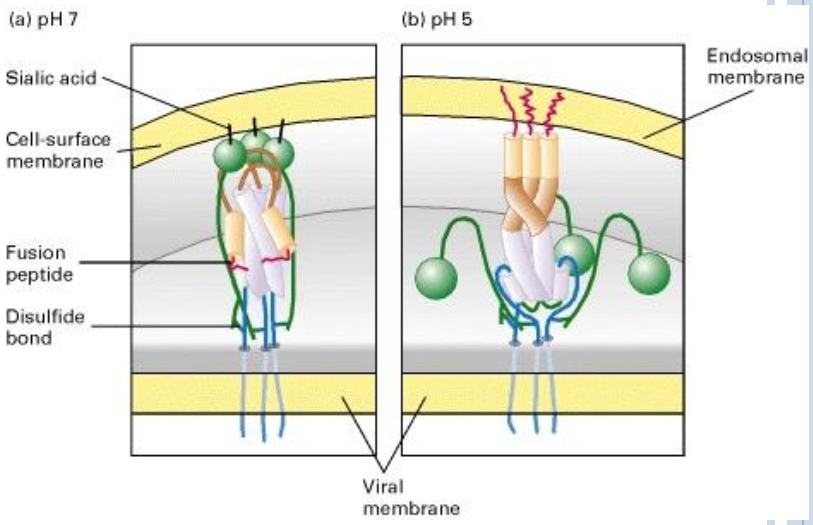
# Distinct receptor binding features of SARS vs. influenza viruses

## Coronavirus



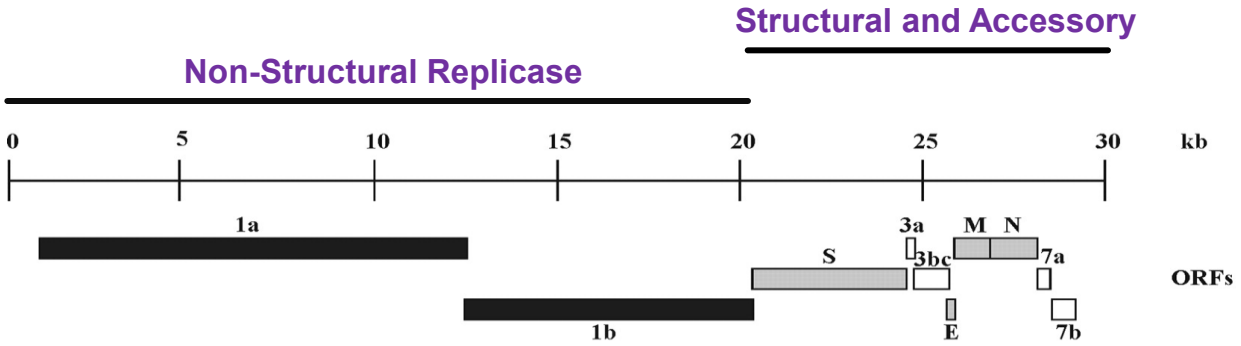

https://doi.org/10.1101/2020.02.08.926006

## Influenza virus




Influenza HA binds to sialic acid residues on diverse surface proteins

# Coronavirus Genome Encodes Several IFN Antagonists




**1. Non-Structural Proteins (nsp1-16)**

Conserved across CoVs

Various, required functions

IFN antagonists: nsp1, PLP2 (nsp3)

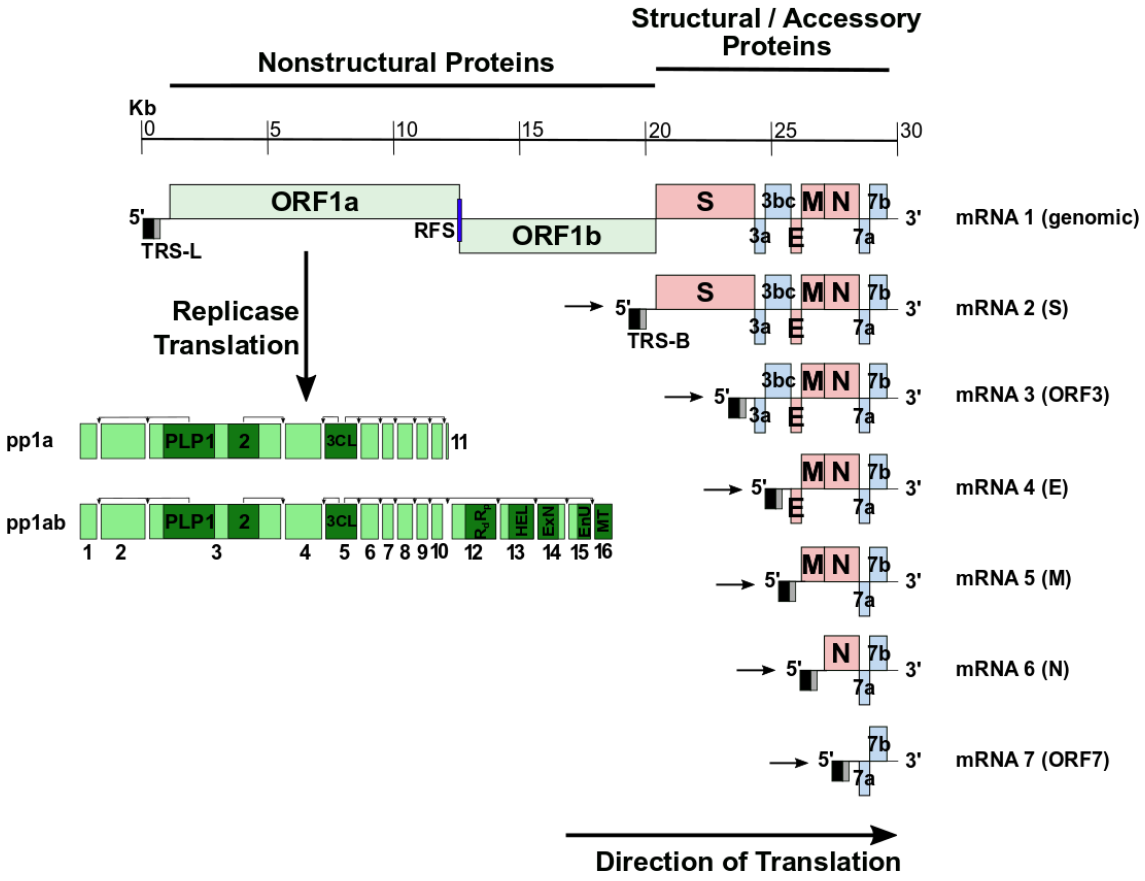
**2. Accessory Proteins**

Unique to subfamilies and species

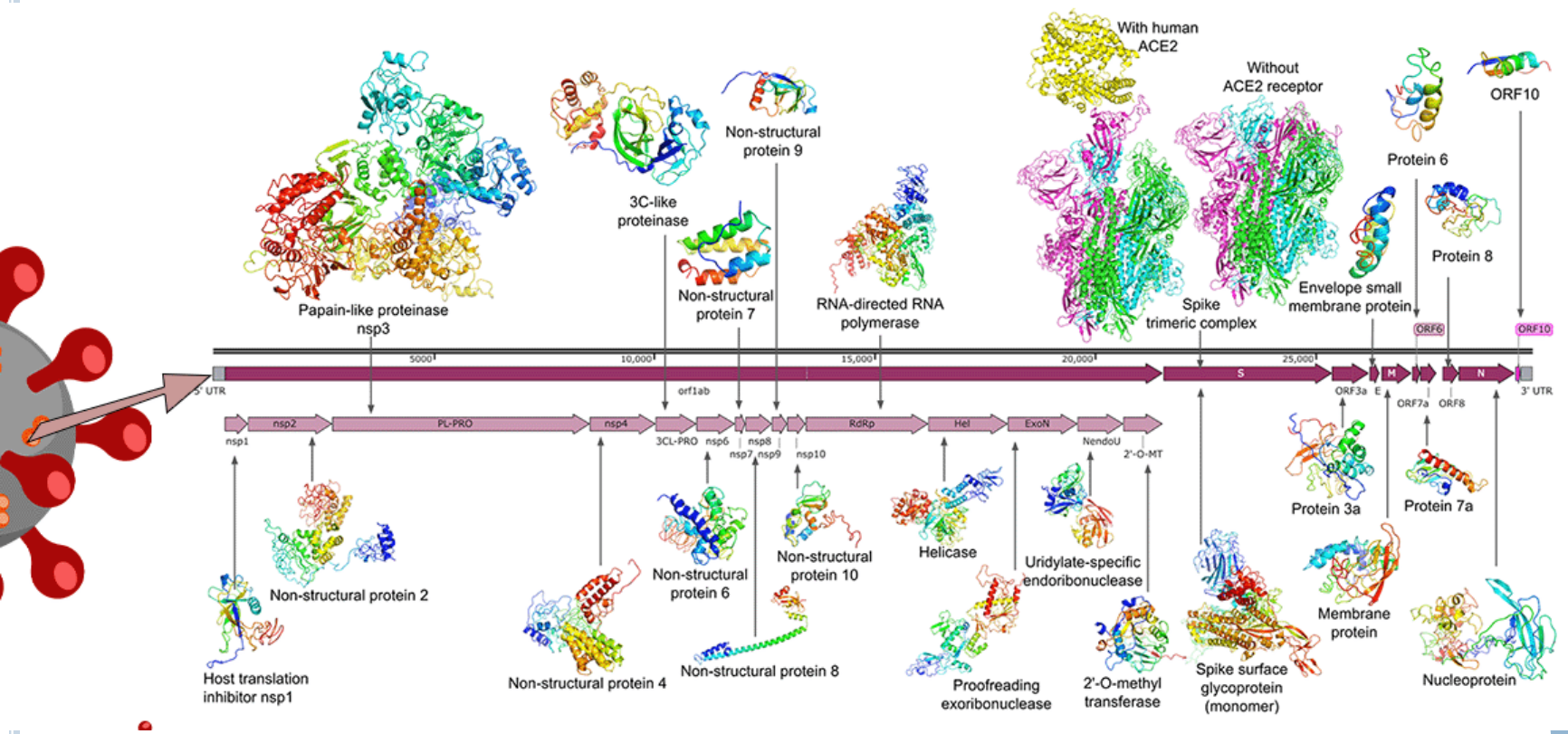
Function dispensable for replication

Encode virulence factors

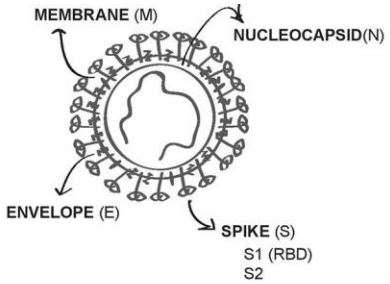
# Coronavirus Genome Structure and Duplication

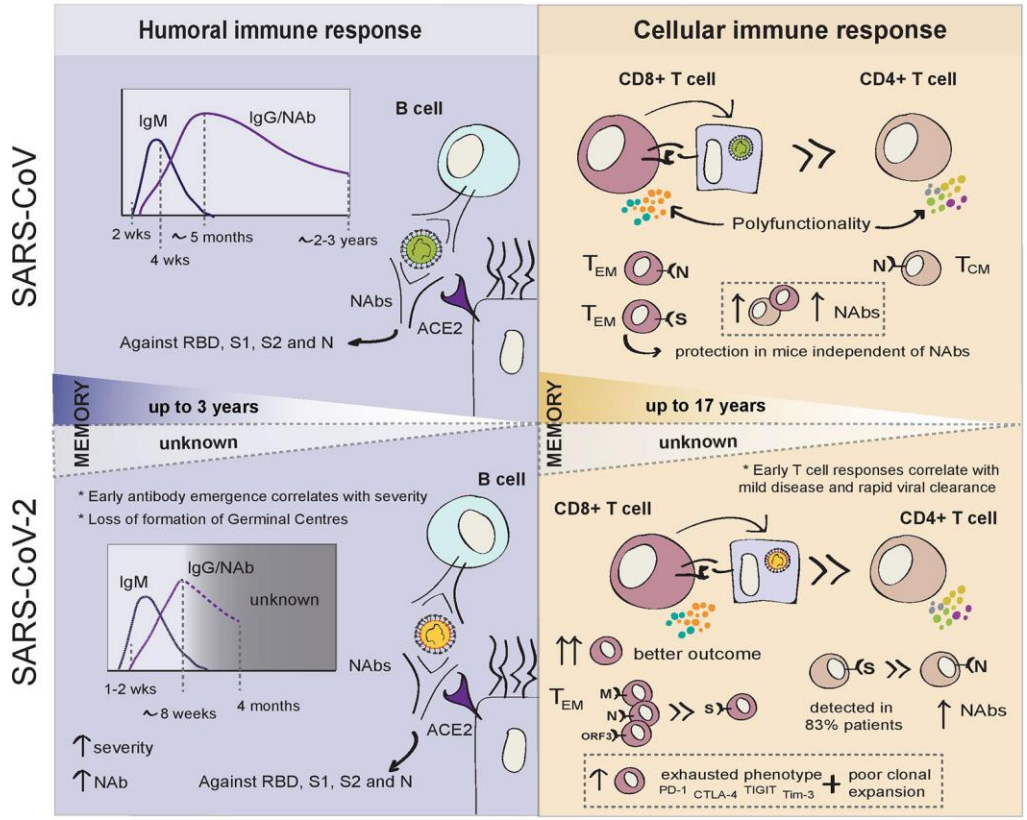


# Large SARS-Cov-2 proteome contains many immunomodulatory non-structural proteins



# Protective Immunity against SARS-CoV-2




https://www.frontiersin.org/files/Articles/571481/

# SARS-CoV-2 vs. Influenza Virus Summary

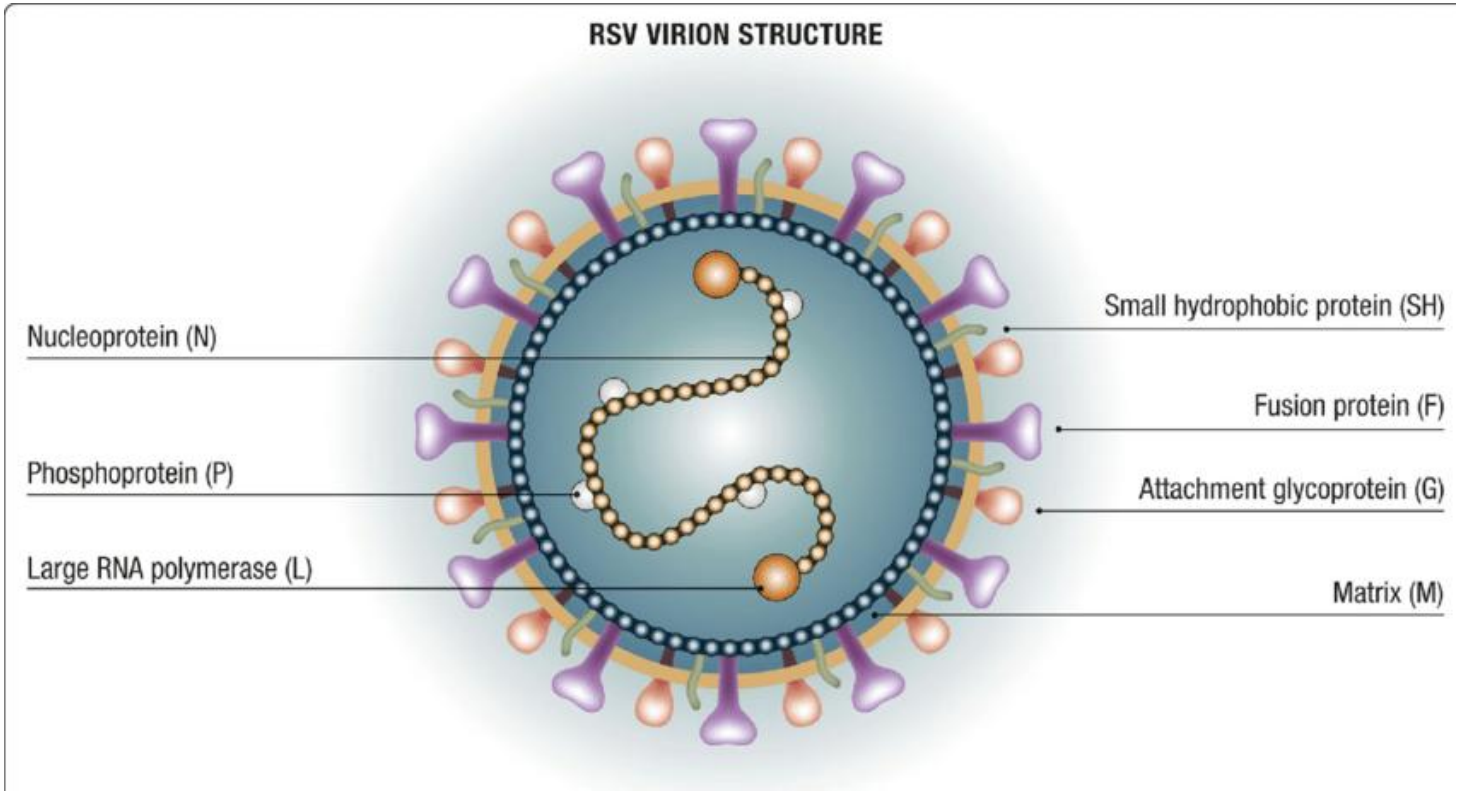
## SARS-CoV-2

- RNA virus (+ sense)
- Single segment
- Large genome
- Multiple immune antagonists
- Specific receptor (ACE2)

## Influenza virus

- RNA virus (- sense)
- 8 segments
- Much smaller genome (than CoV)
- Single immune antagonist (ds RNA sequestration)
- Non-specific receptor

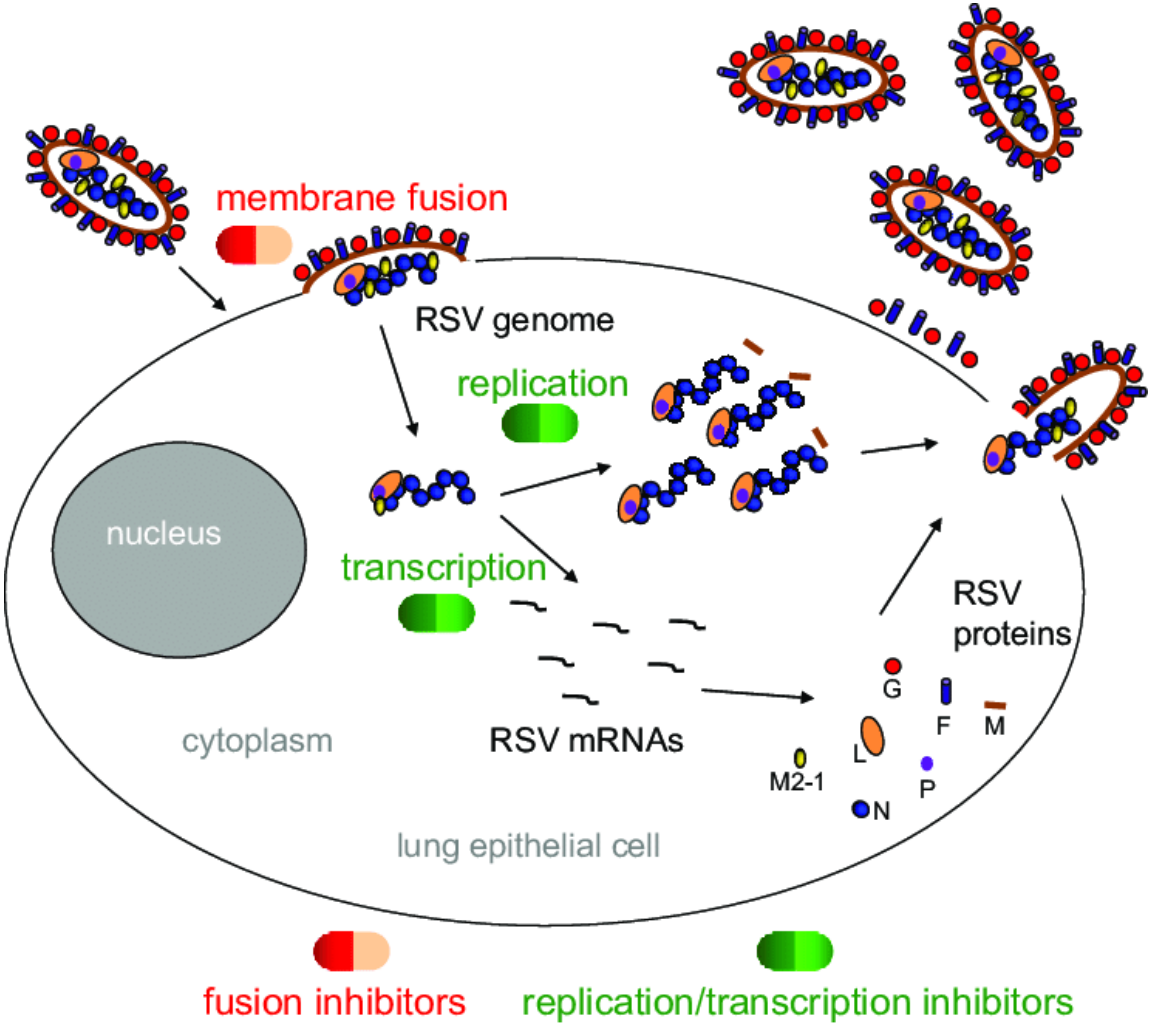
# RSV Virion Structure



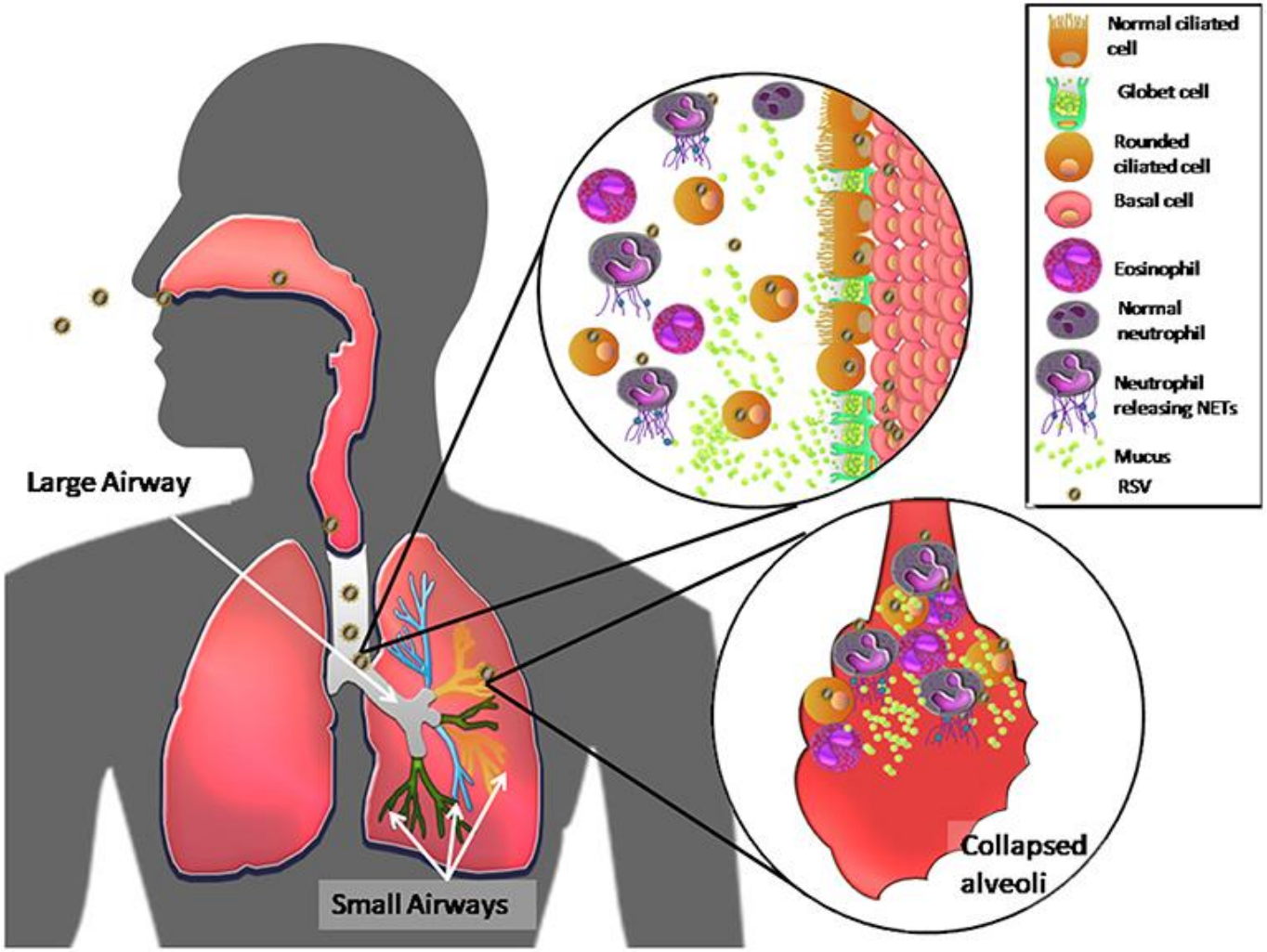

Epidemiology and prevention of respiratory syncytial virus infections in children in Italy. Italian Journal of Pediatrics. 47. 198. 10.1186/s13052-021-01148-8.

New antiviral approaches for respiratory syncytial virus and other mononegaviruses: Inhibiting the RNA polymerase

# RSV Replication



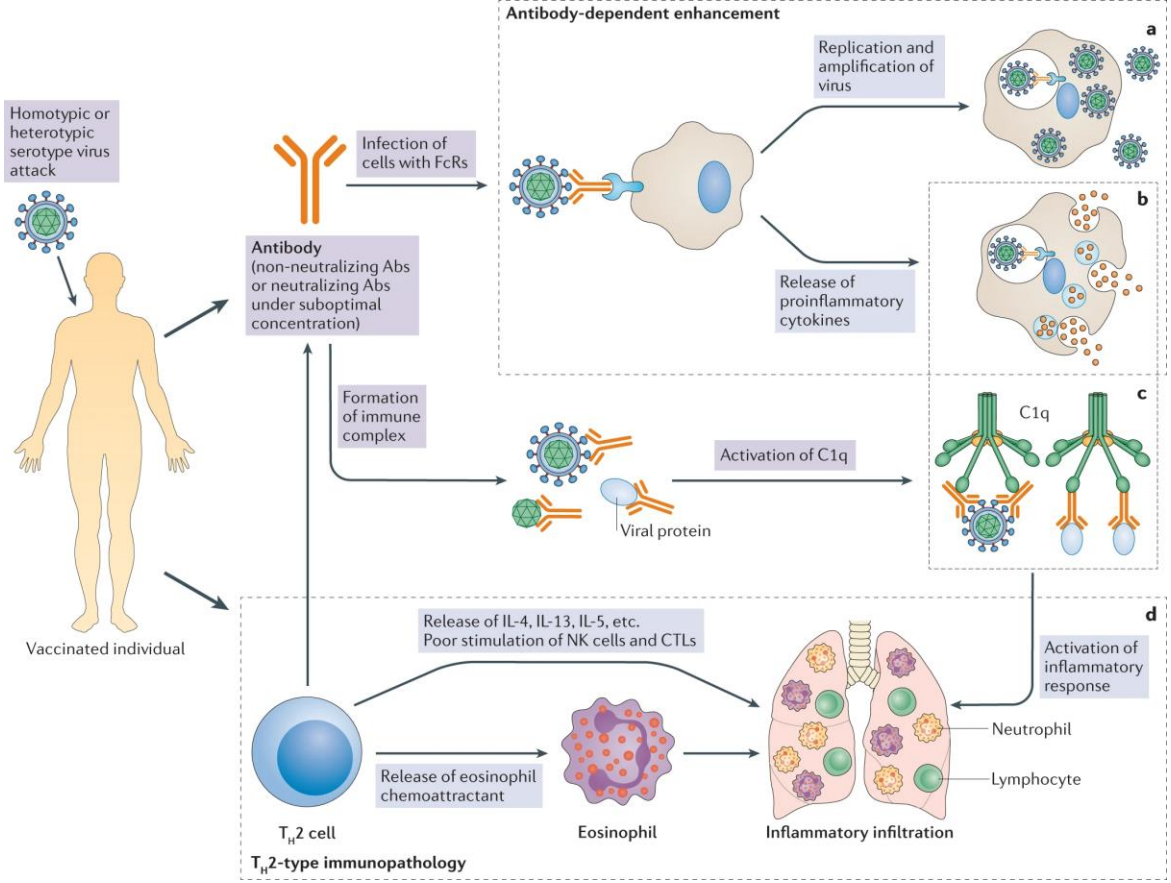
# Unique Features of RSV pathogenesis




https://www.frontiersin.org/files/Articles/450448

# RSV Vaccination Failure

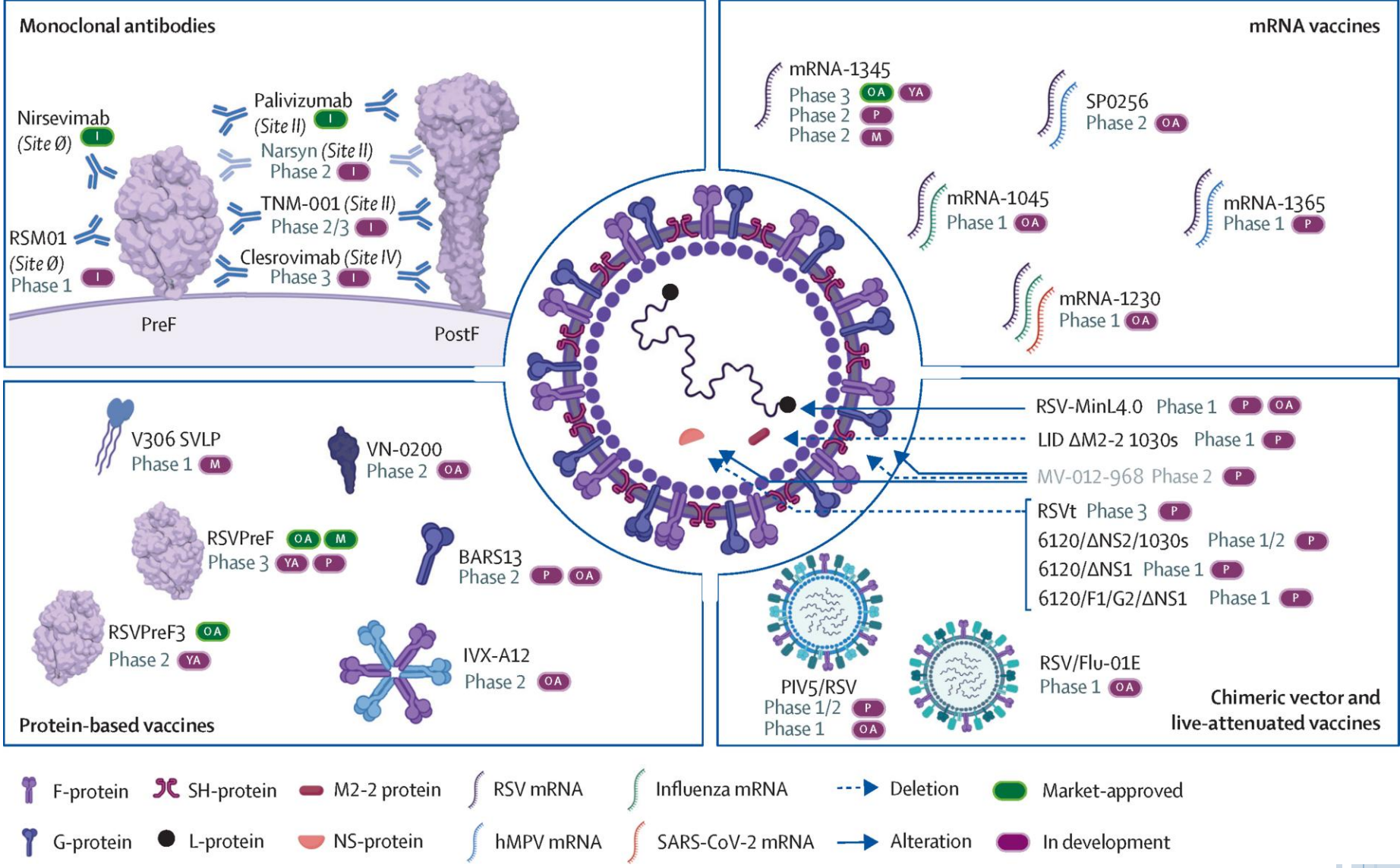
1960 era vaccine
80% of children suffered severe disease after infection
Two deaths




Published: 14 December 2008

## Lack of antibody affinity maturation due to poor Toll-like receptor stimulation leads to enhanced respiratory syncytial virus disease

Maria Florencia Delgado, Silvina Coviello, A Clara Monsalvo, Guillermina A Melendi, Johanna Zea Hernandez, Juan P Batalle, Leandro Diaz, Alfonsina Trento, Herng-Yu Chang, Wayne Mitzner, Jeffrey Ravetch, José A Melero, Pablo M Irusta & Fernando P Polack




- New vaccines and monoclonals approved for older adults and pregnant women (vaccines) and infants (monoclonals)