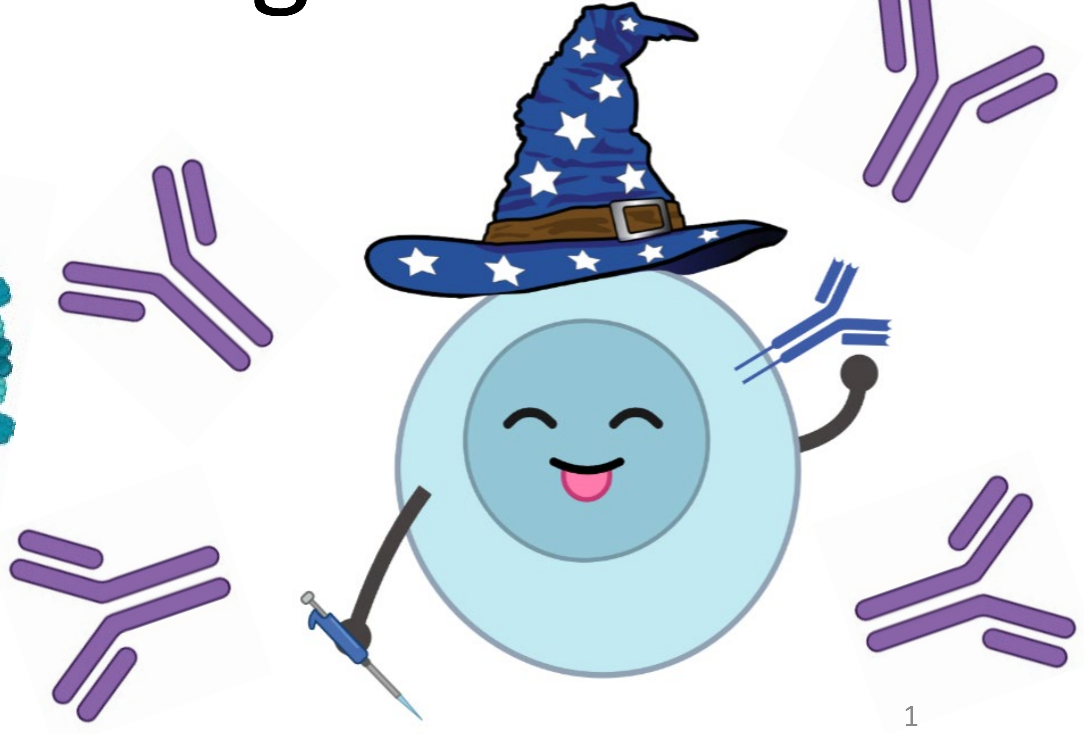
Impact of Host and Vaccine Characteristics on Immune Responses Following Influenza Vaccination

Dissertation proposal by

W. Zane Billings

2023-04-06

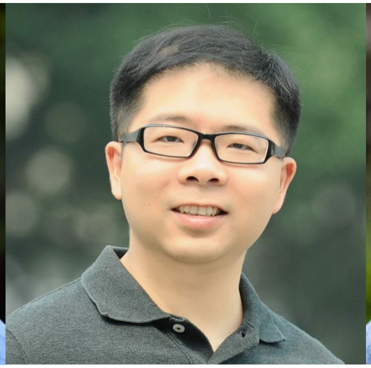
Contact info: https://wzbillings.com/



Acknowledgements

Committee


Andreas Handel


Ye Shen

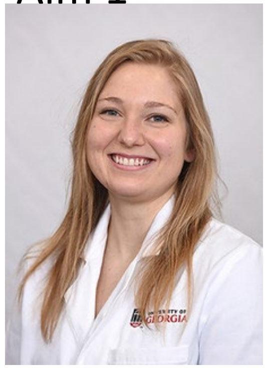

Amy Winter


Natalie Dean

Money

UNIVERSITY OF GEORGIA
Graduate School

Aim 1


Amanda Skarlupka

Aim 2


Yang Ge


Tzu-Chun Chu


Veronika Zarnitsyna

Aim 3


Savannah Hammerton


Jessica Knight

- UGA Ross Lab (Ted Ross, Michael Carlock)
- Everyone who told Andreas whether they thought what I was doing was interesting or not

- UGA Infectious Disease Interest Group
- IMAG-MSM Immunology Subgroup
- CIVIC, CIVR-HRP, CEIRR, CIDER, etc.
- Biorender.com

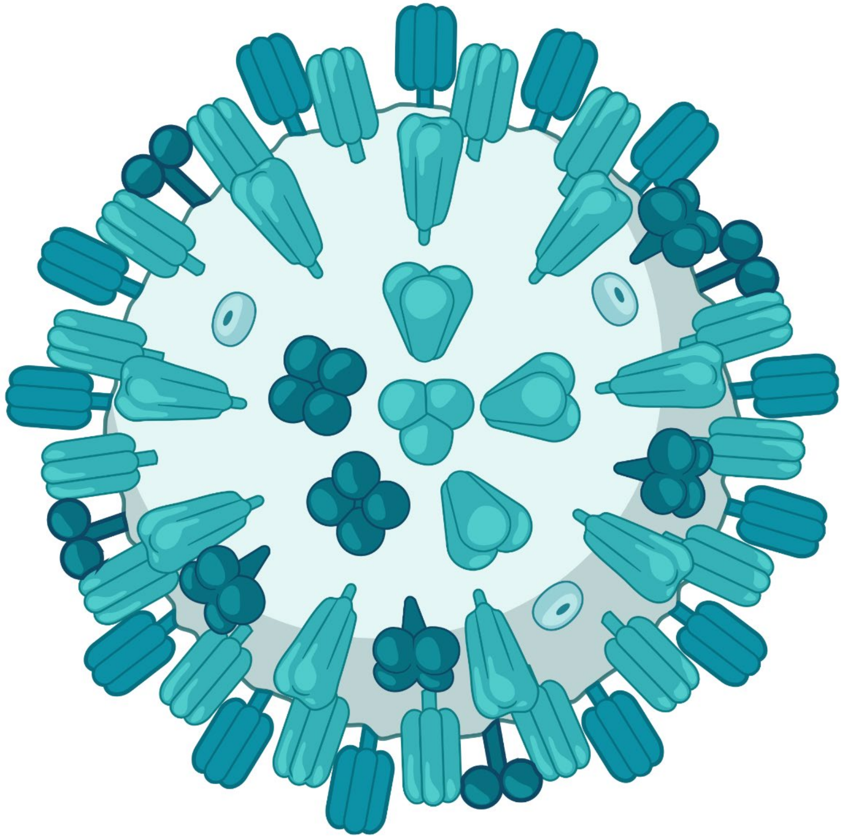
Specific aims

1. Develop metrics for the quantification of the total immune response to an influenza vaccine, incorporating both magnitude and breadth.
2. Quantify the role of pre-vaccination titer, prior vaccinations, vaccine dose, and antigenic distance on individual vaccine response.
3. Explore how age and vaccine dose interact to affect the antibody response.

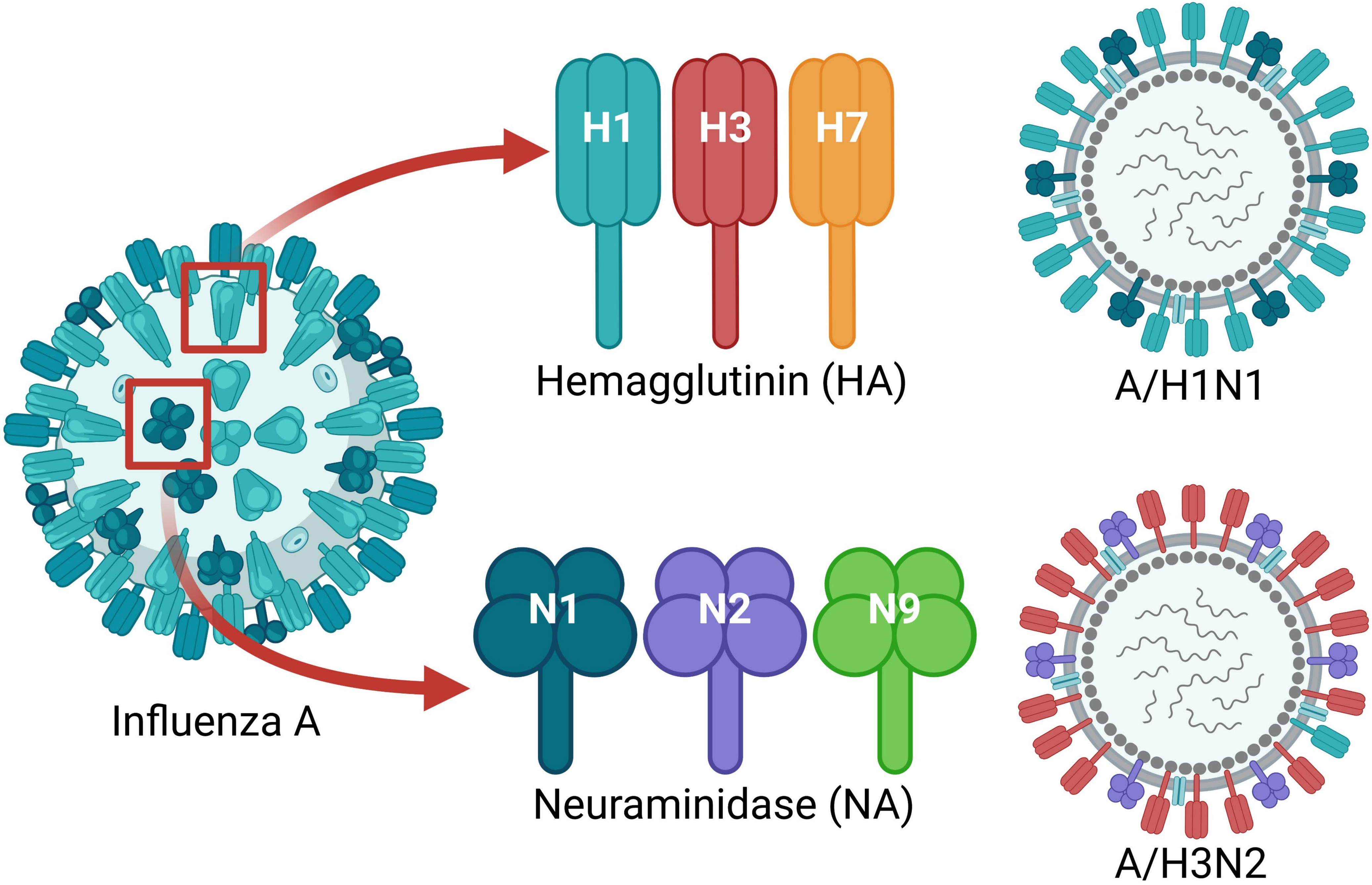
Overview

- General background: motivation, terminology, how do we study this?
- Data description
- Aim-specific background, preliminary data, and proposed study

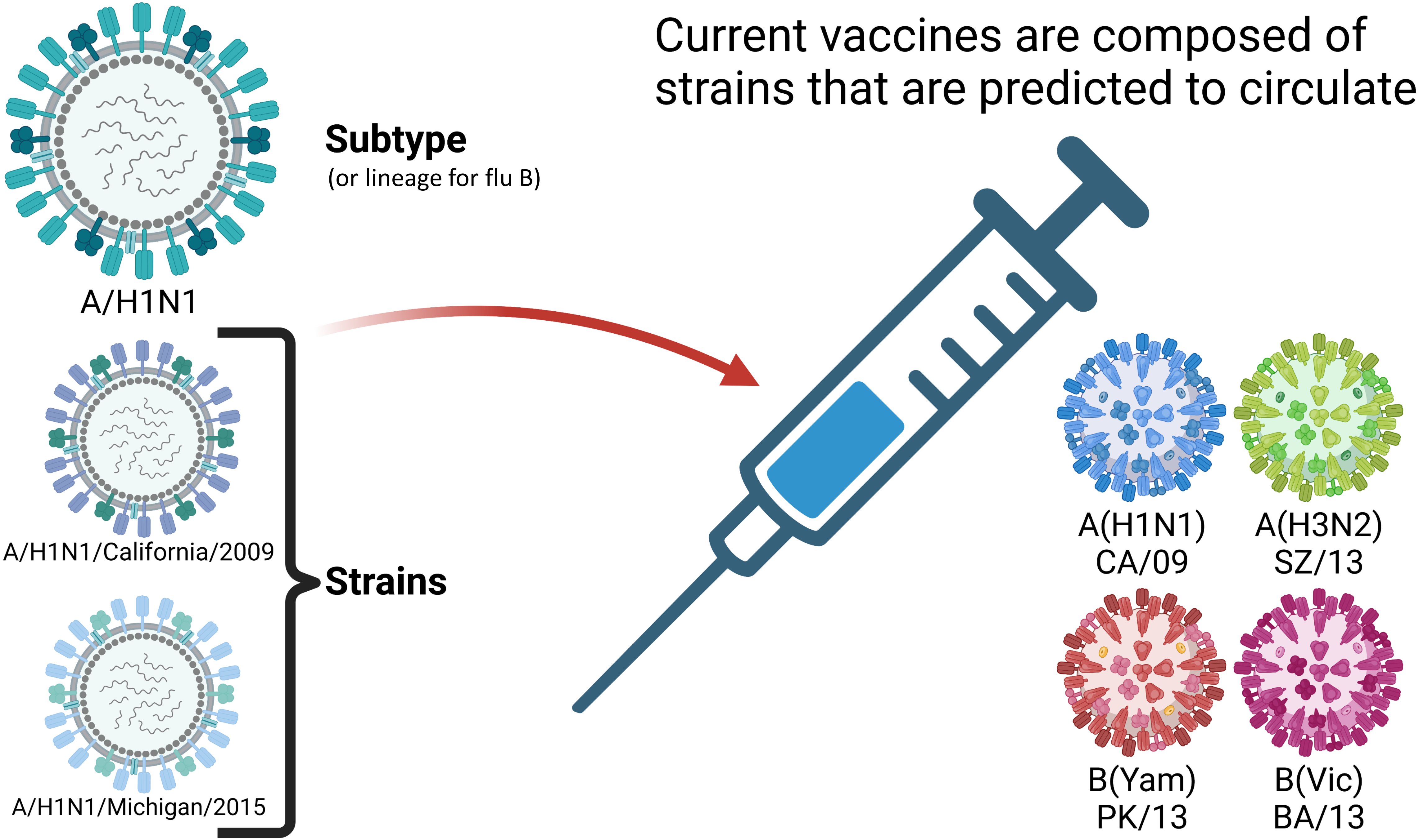
Influenza viruses

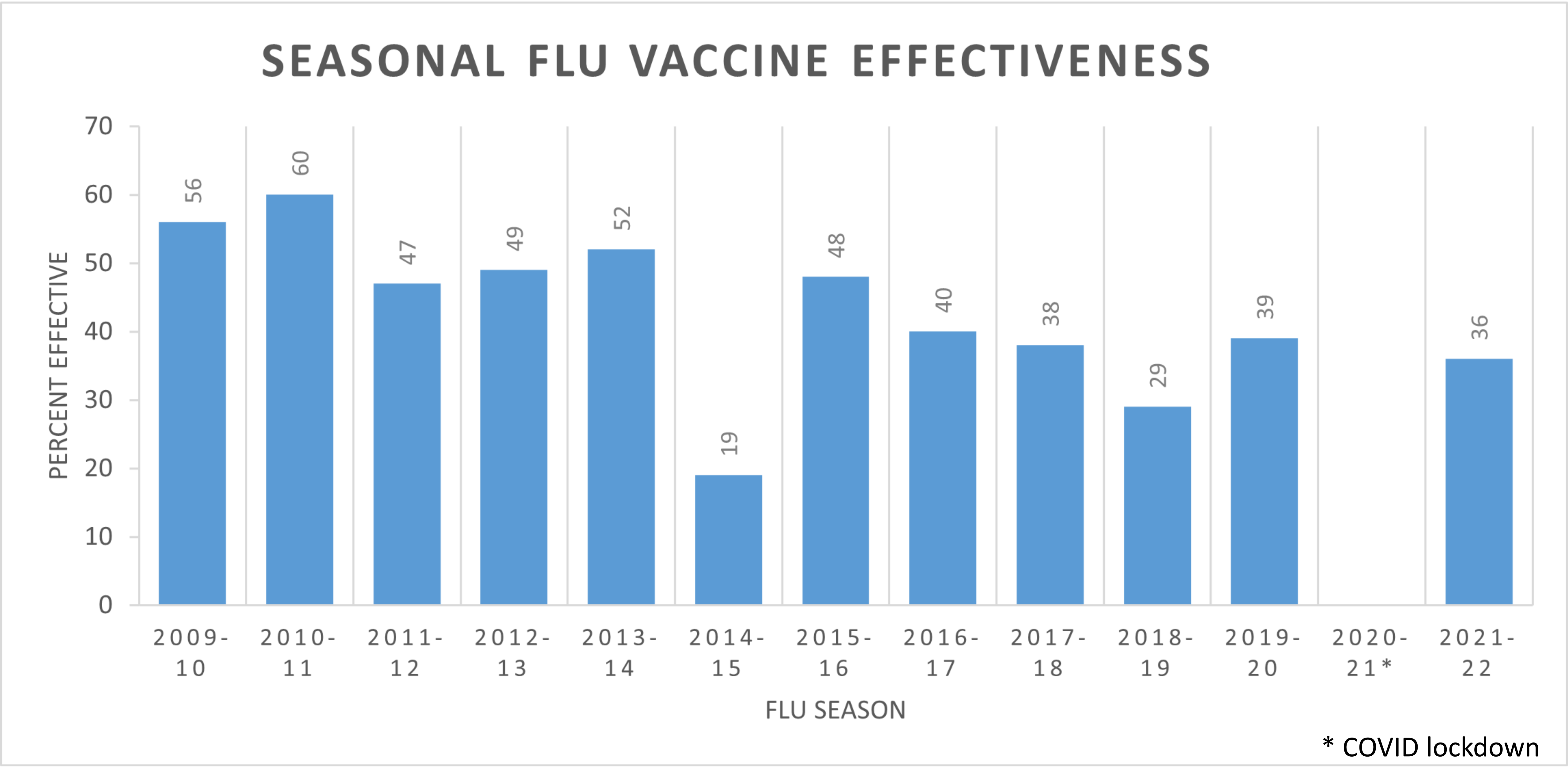


- (-)SSRNA virus
- Segmented genome
- Flu A and B are distinct genera that circulate in humans and cause seasonal epidemics
- A has a natural animal reservoir in wild waterfowl and can infect domestic poultry and livestock.

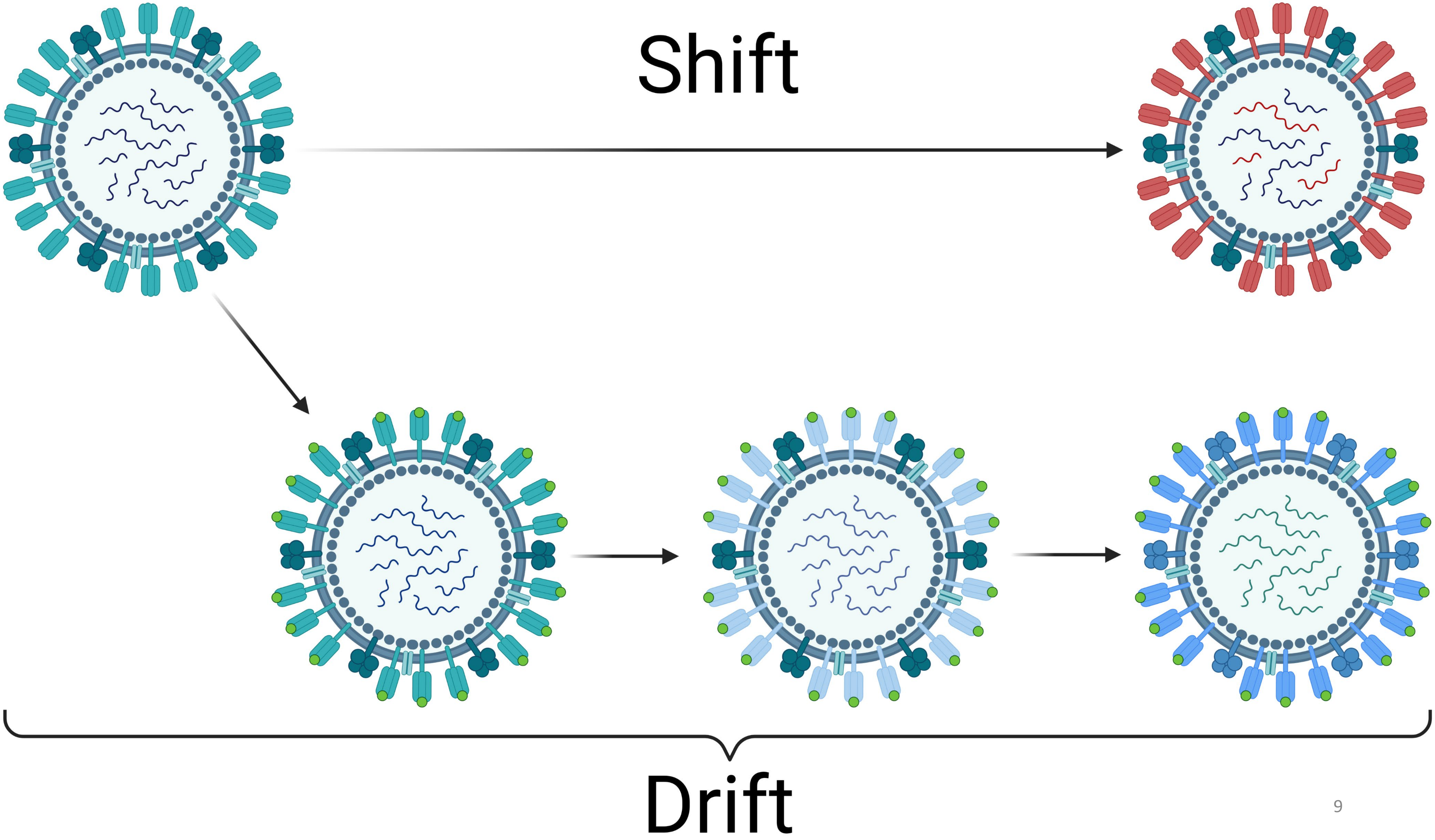

H1
H3
H7
Hemagglutinin (HA)
A/H1N1
N1
N2
N9
Neuraminidase (NA)
Influenza A
A/H3N2

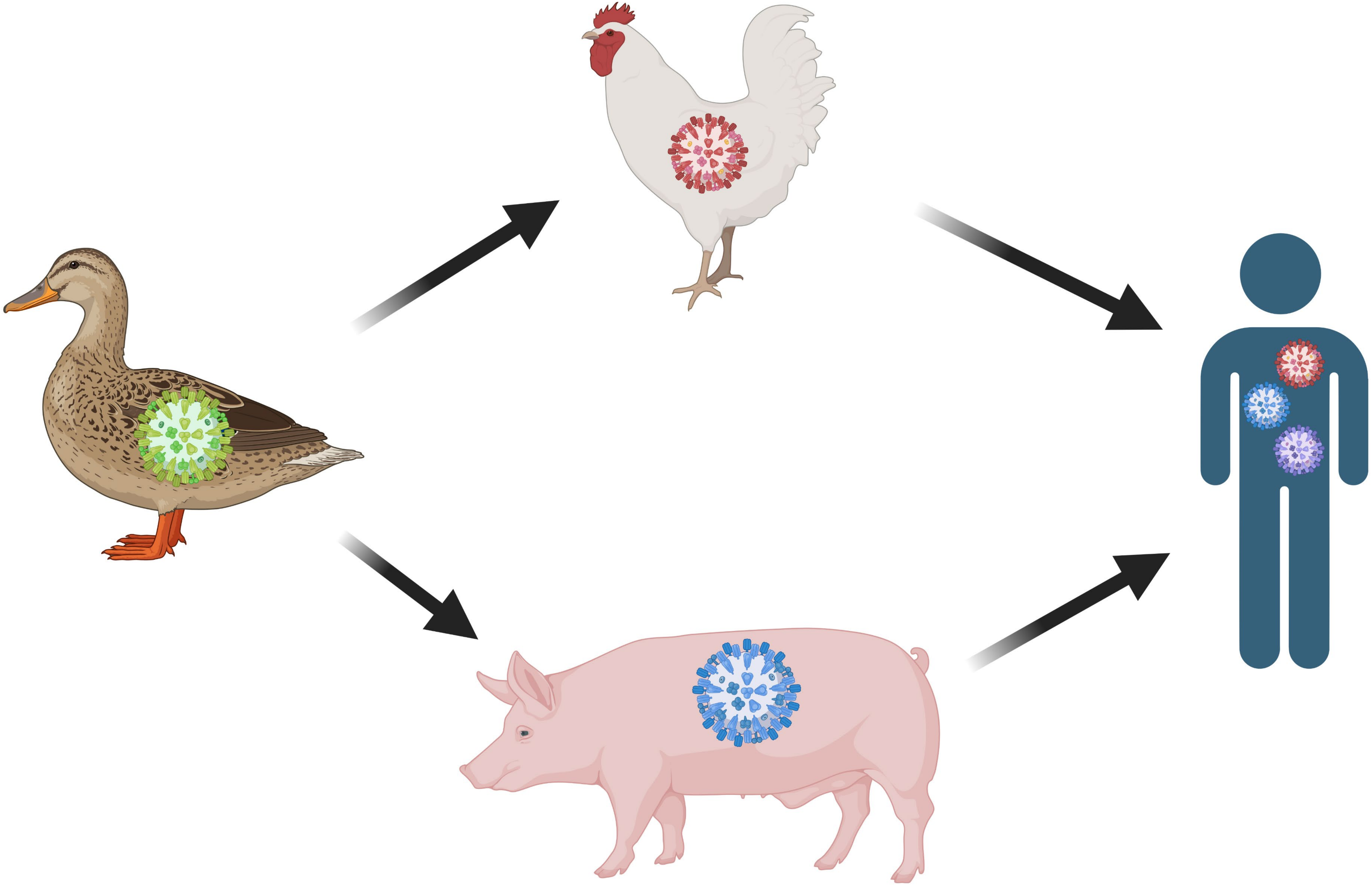
Current vaccines are composed of strains that are predicted to circulate.

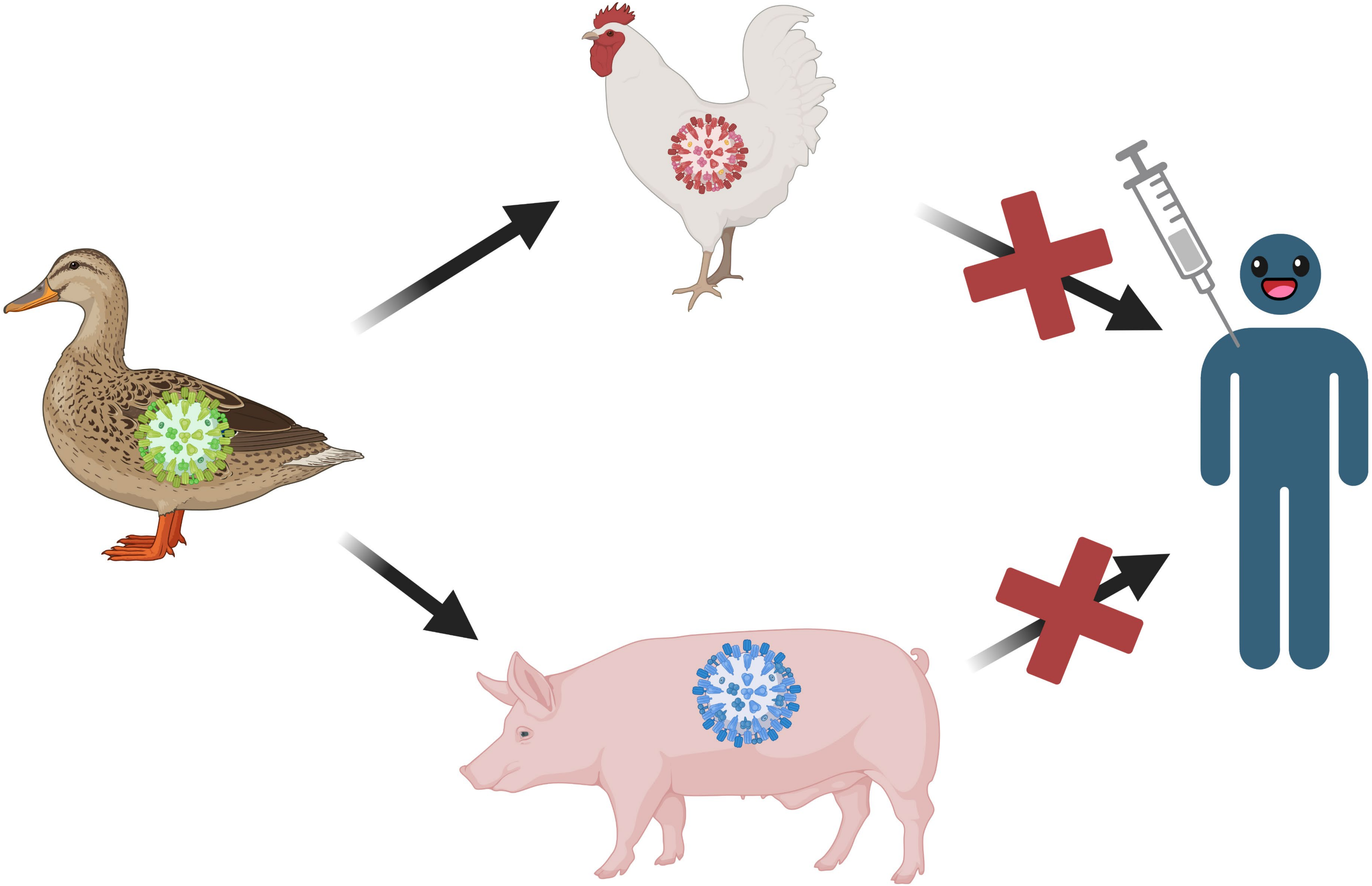




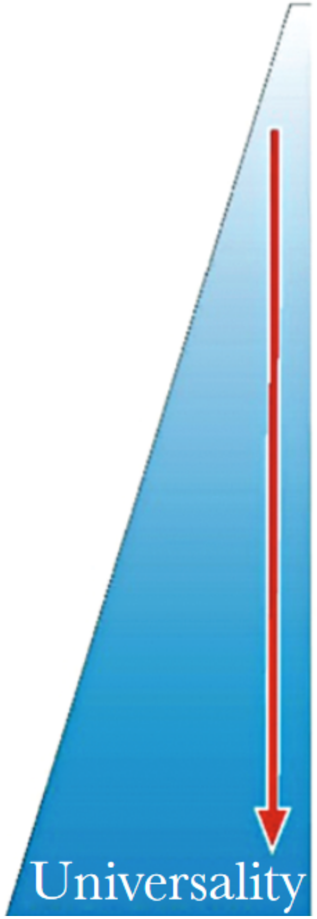
CDC: https://www.cdc.gov/flu/vaccines-work/effectiveness-studies.htm


Shift
Drift



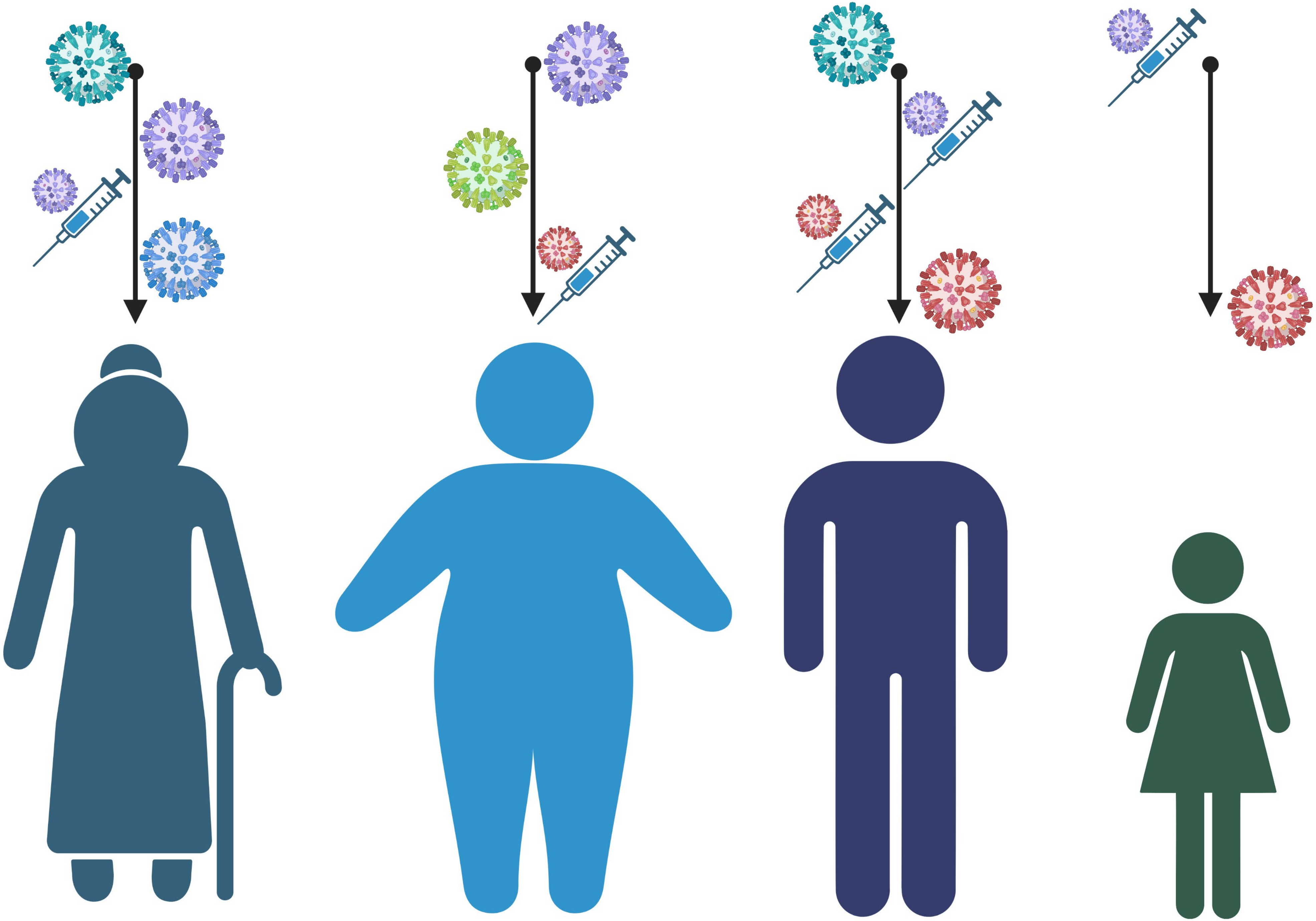


A **universal** vaccine could solve both problems!



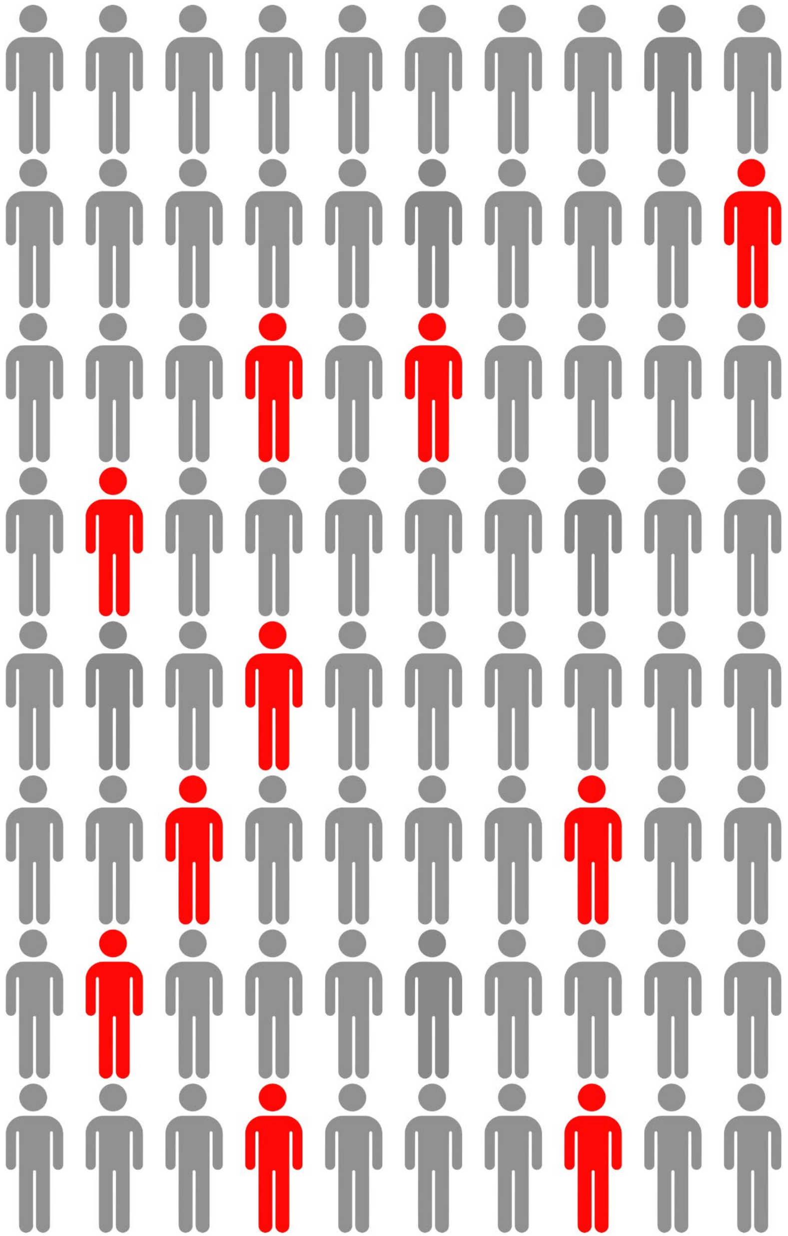
Vaccine	Coverage
Strain specific	Current circulating strains
Subtype specific	All strains within a single HA subtype (eg, H1)
Multisubtype	Multiple HA subtypes within single group (eg, H1/H5/H9)
Pan-group	Covering all group 1 or 2 influenza A viruses
Universal	All influenza A viruses (with or without influenza B viruses)

Erbelding et al, JID, 2018



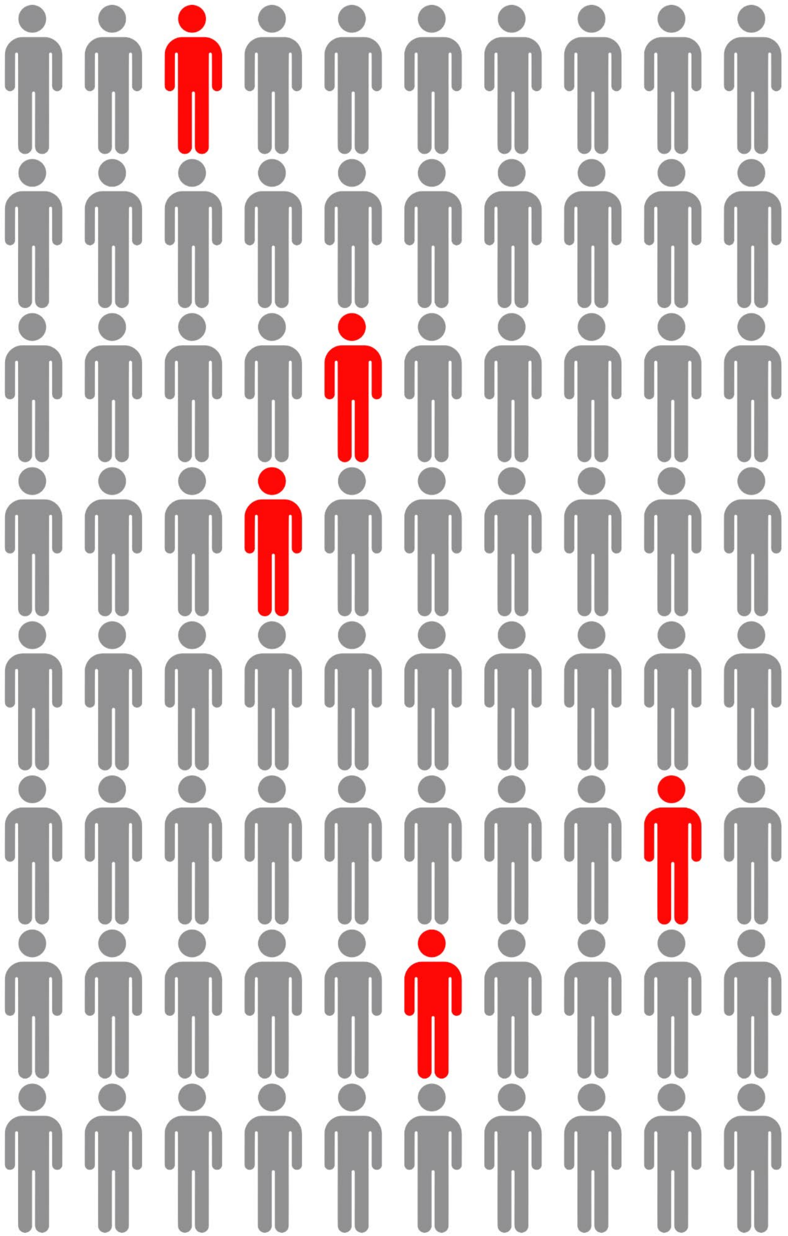
Placebo

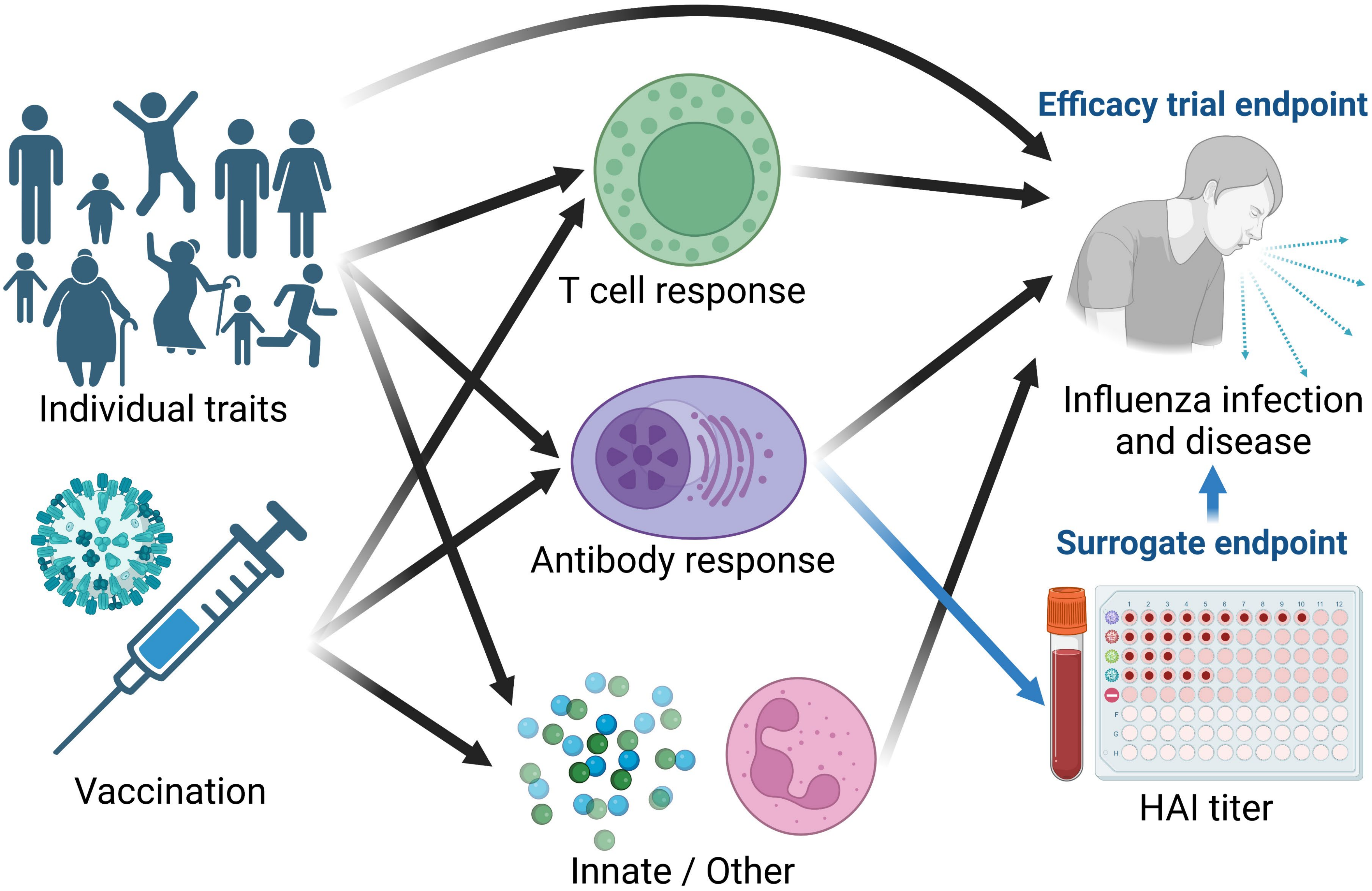
10/80 = 12.5% risk

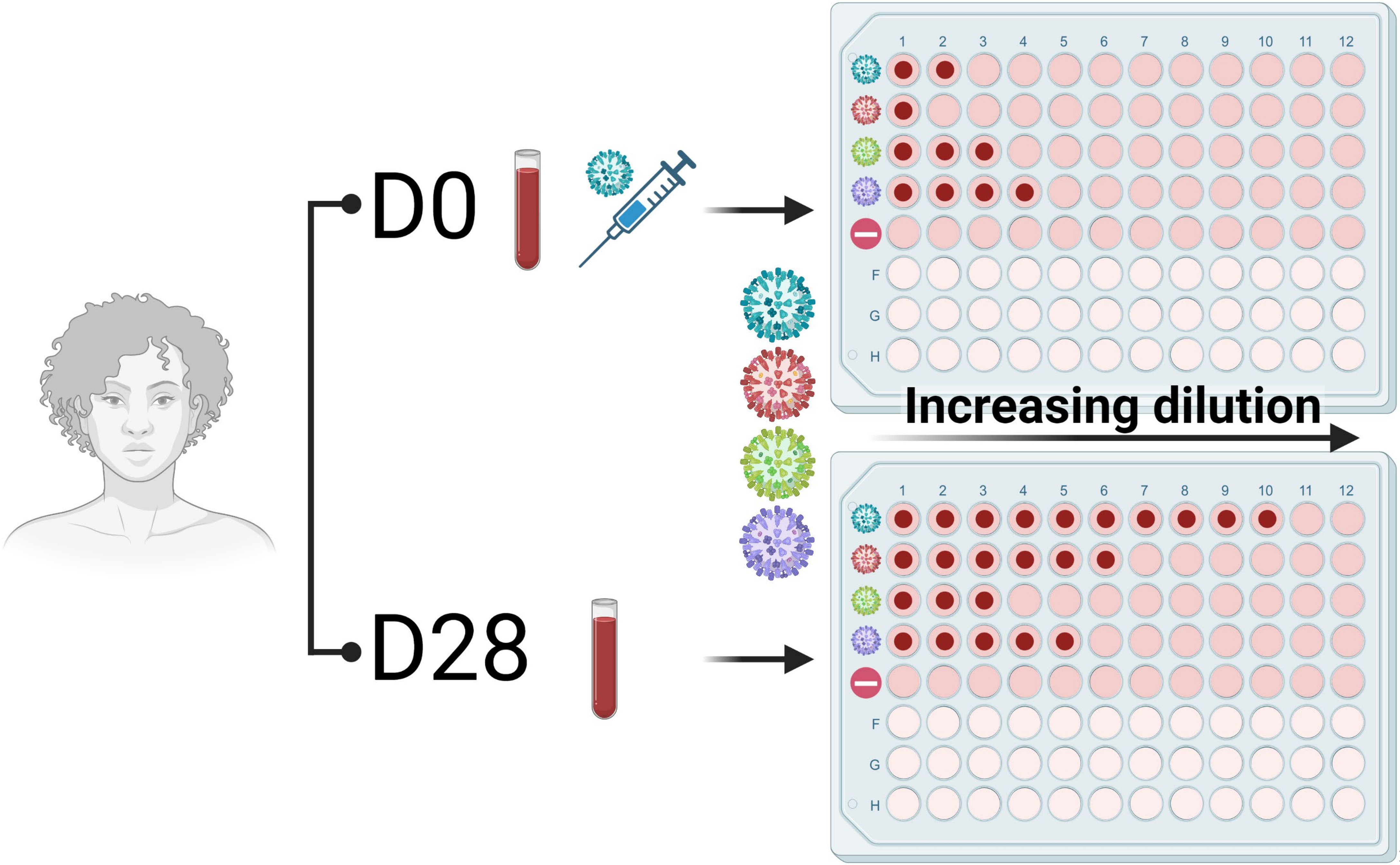


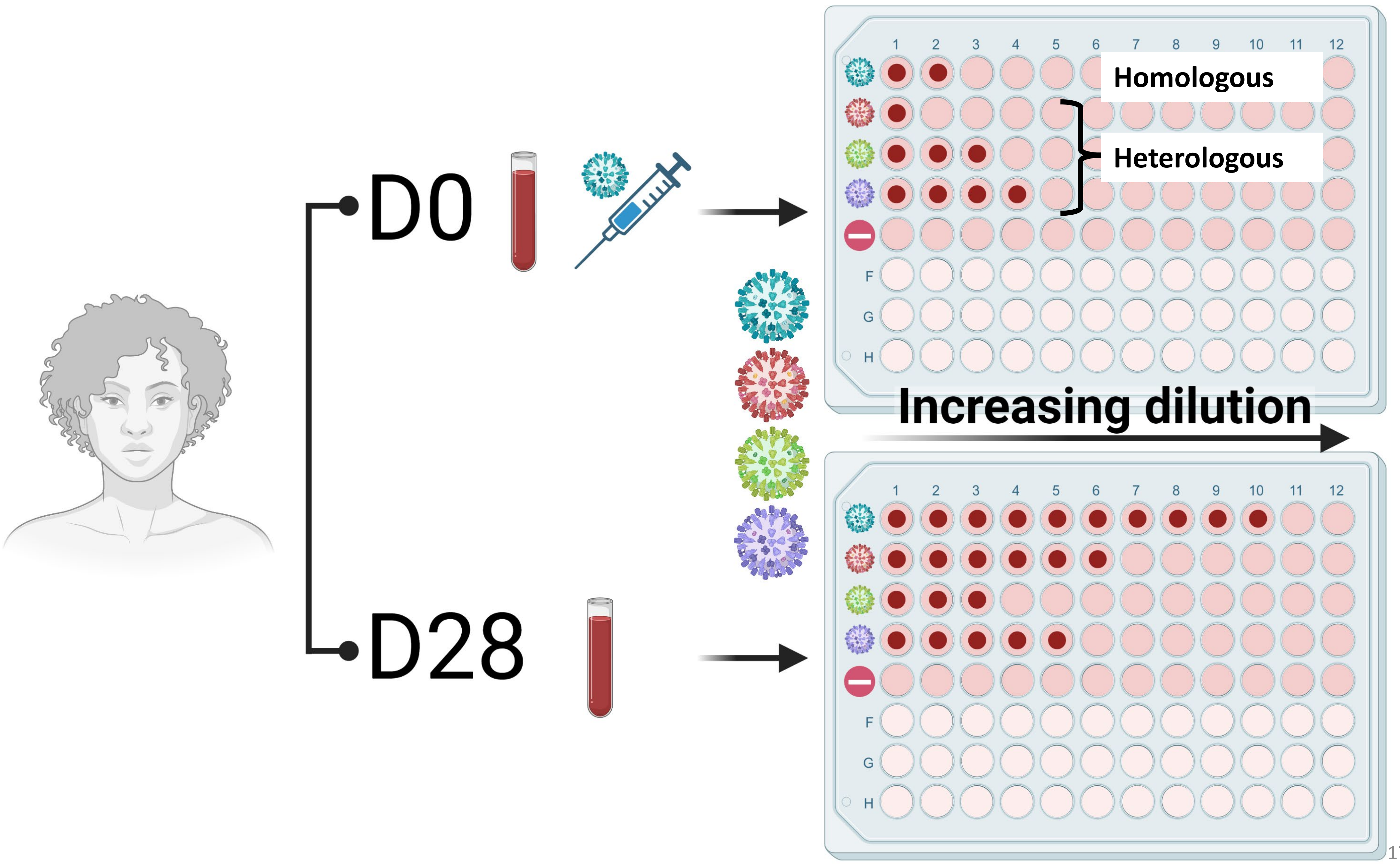
Vaccine

5/80 = 6.25% risk




Efficacy trial endpoint
T cell response
Individual traits
Influenza infection and disease
Antibody response
Surrogate endpoint
Vaccination
HAI titer
Innate / Other


D0
D28
Increasing dilution
1 2 3 4 5 6 7 8 9 10 11 12
F
G
H
1 2 3 4 5 6 7 8 9 10 11 12
F
G
H


D0
D28
Homologous
Heterologous
Increasing dilution
1 2 3 4 5 6 7 8 9 10 11 12
F
G
H

How do we evaluate these vaccines?

1. **Magnitude:** the response to the homologous strain.
2. **Breadth:** responses to heterologous strains.
3. **Overall strength:** can we combine magnitude and breadth into one measurement of “goodness”?

Data description

Data description: UGAFluVac

- Run by Ted Ross, currently housed at UGA
- 2013-2016 in Stuart, FL and Pittsburgh, PA
- January 2017 – Present in Athens, GA
- Prospective open cohort design with prevaccination and postvaccination serum samples tested against a wide homologous panel
- Participants received either Fluzone or Fluzone HD (if ≥65)

Data description: RocFluVac

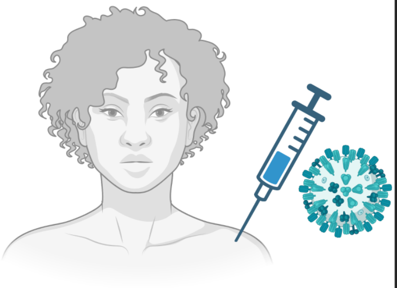
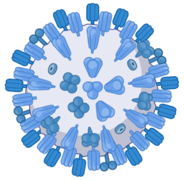
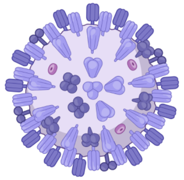
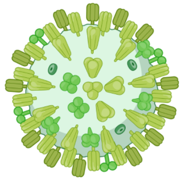
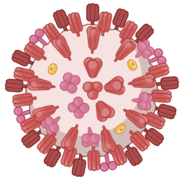
- Run by Andrea Sant and Angela Branche, currently at the University of Rochester
- Longitudinal data from 2015 – 2019
- HAI measurements to select strains pre- and post-vaccination, plus additional assays (ELISA, FRNT, T cells)
- Participants (18-49) received Fluzone, Fluzone HD, Flucelvax, or Flublok

Aims

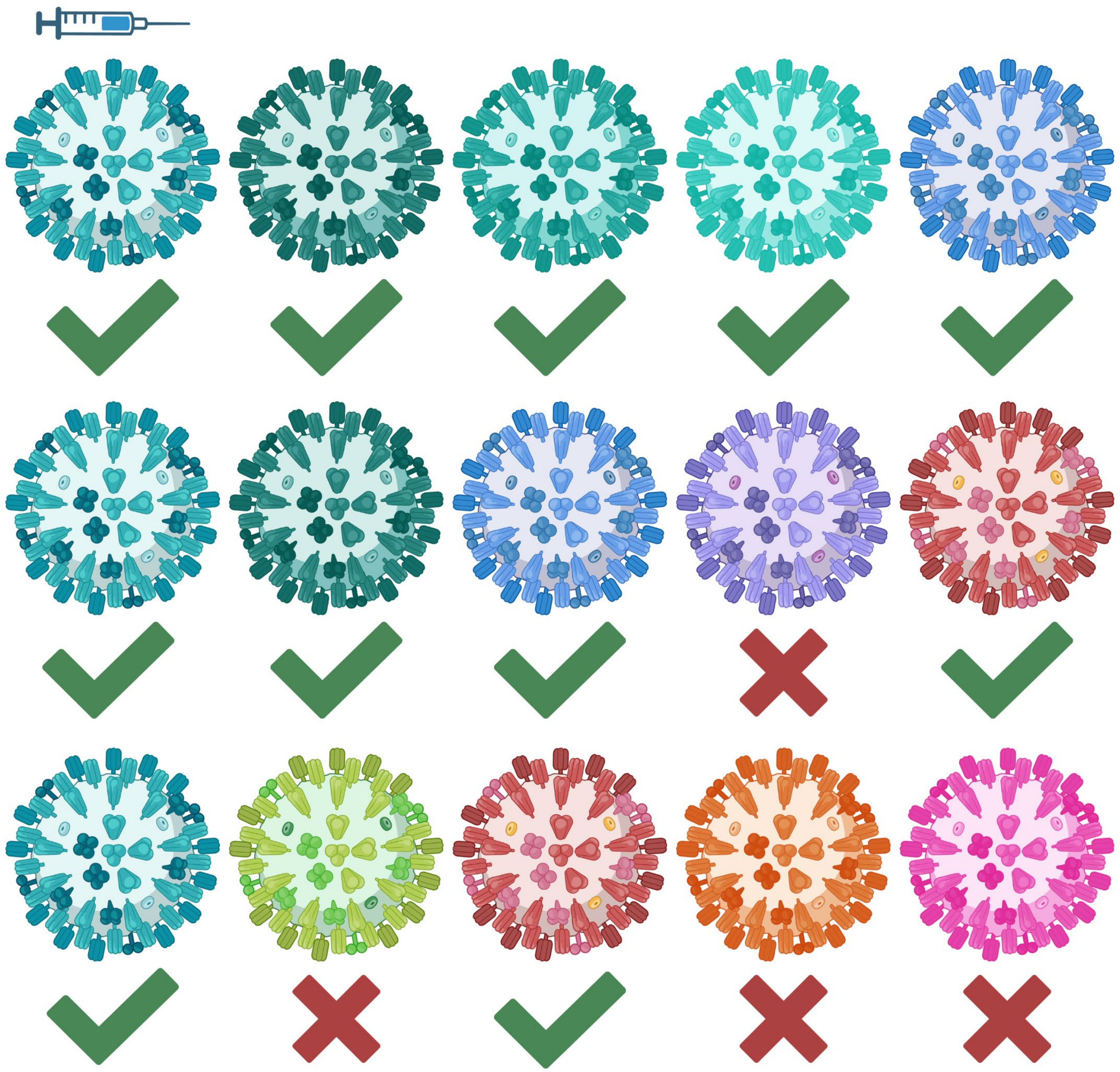
Specific aims

1. Develop metrics for the quantification of the total immune response to an influenza vaccine, incorporating both magnitude and breadth.
2. Quantify the role of pre-vaccination titer, prior vaccinations, vaccine dose, and antigenic distance on individual vaccine response.
3. Explore how age and vaccine dose interact to affect the antibody response.

Aim 1: Develop metrics for the quantification of the total immune response to an influenza vaccine, incorporating both strength and breadth.

	Seroconverted	HAI titer	
	✓	160	← **Magnitude**
	✓	80	
	✗	10	
	✗	20	
	✓	40	
Overall	60%	40	← **Breadth**

Lab Panel



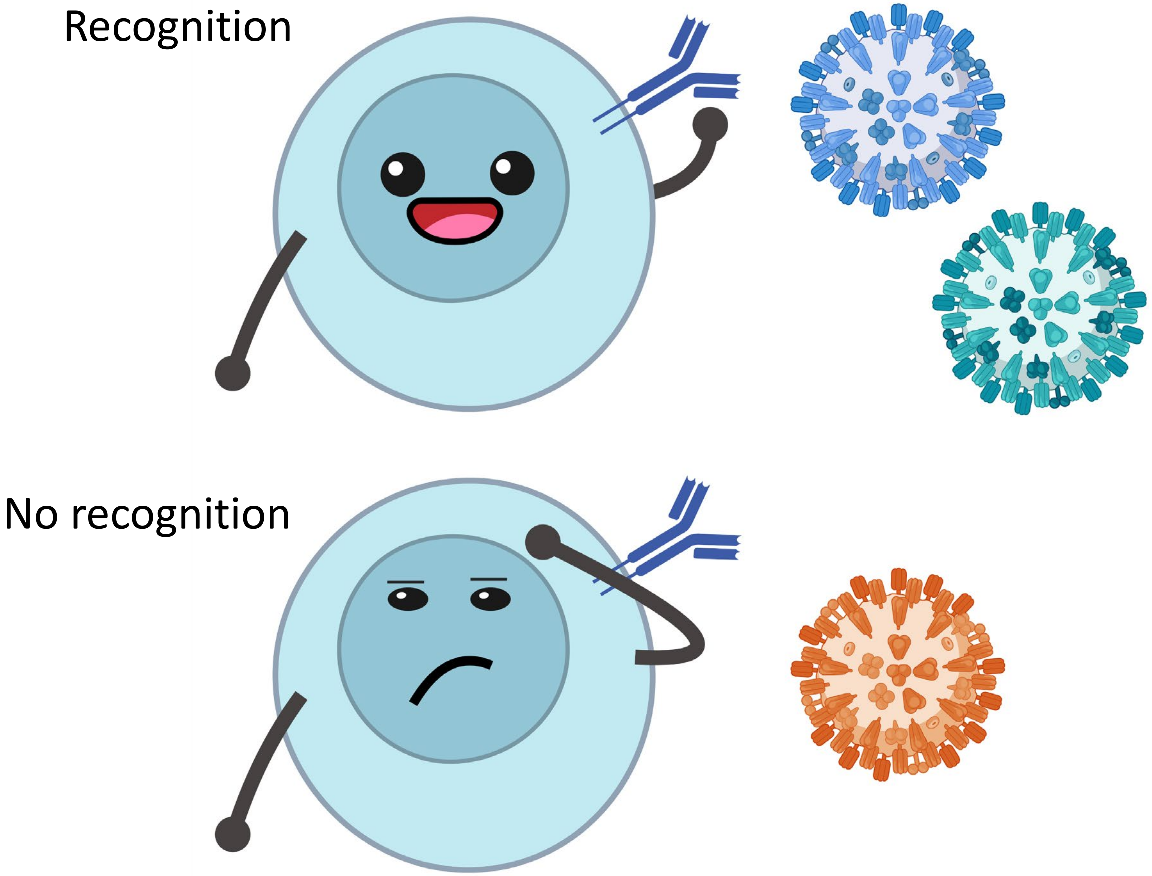
Observed % Seroconverted

100%

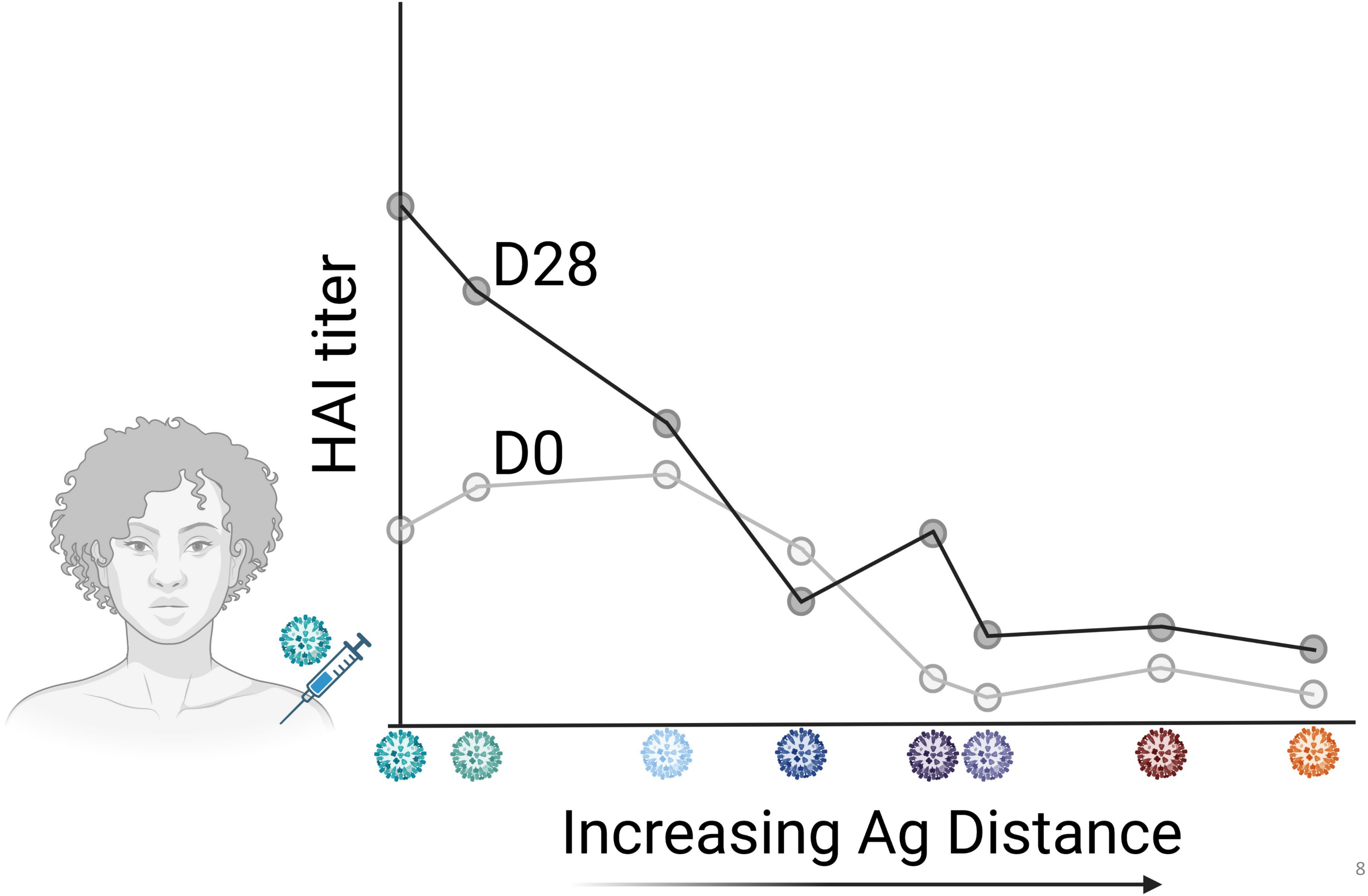
80%

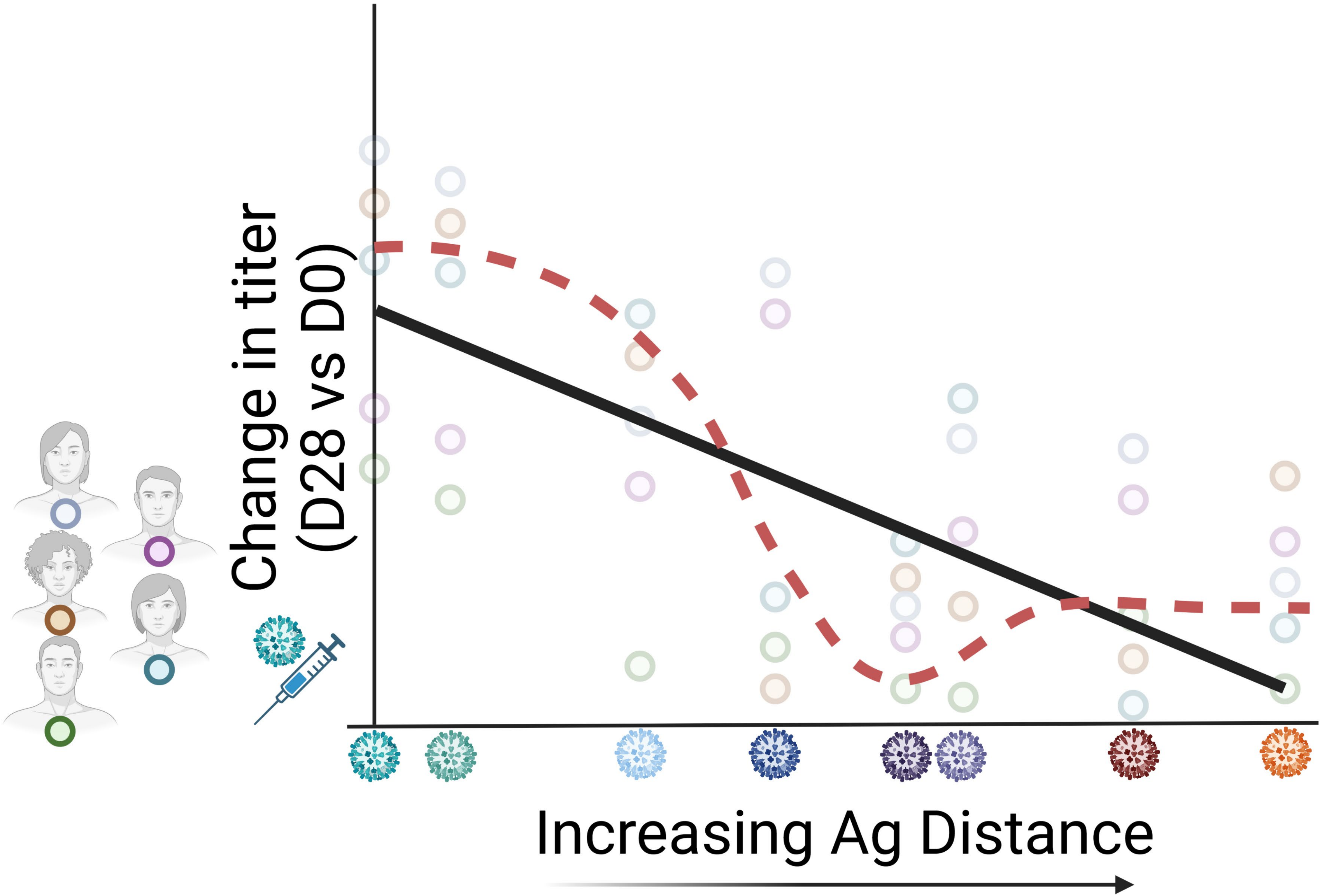
40%

Antigenic distance:
how different are two strains?

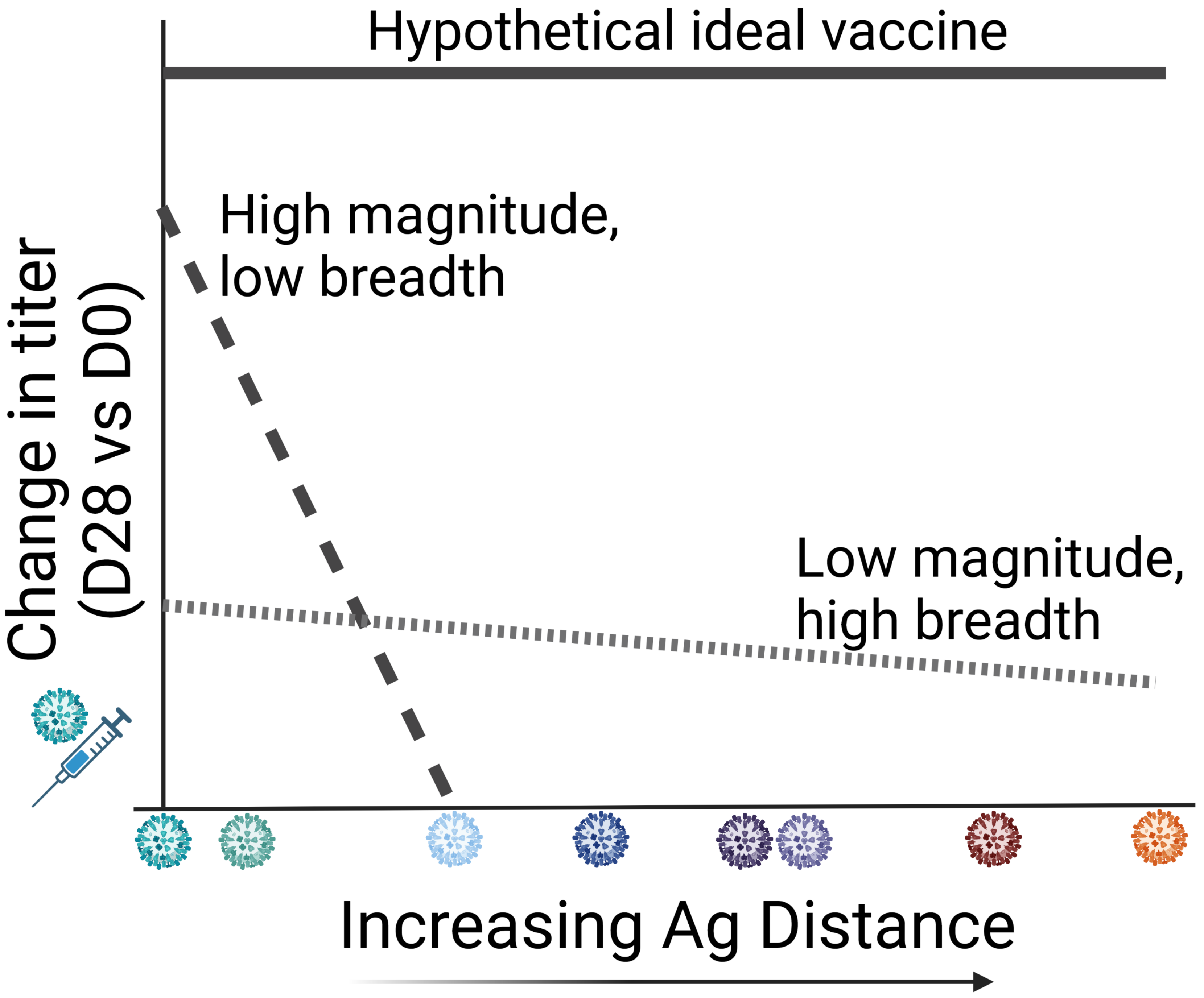


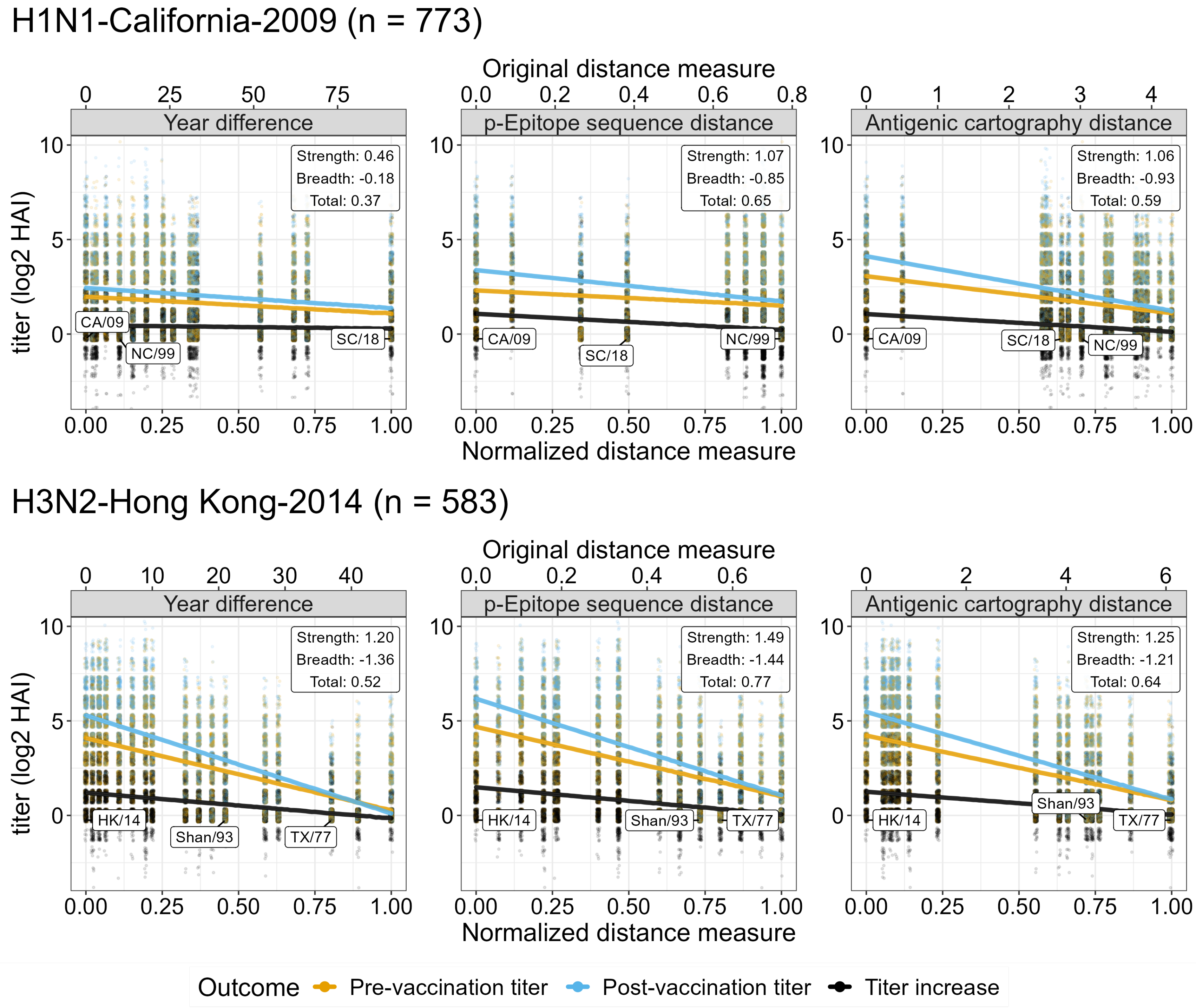
- **Temporal** method: based on years of strain isolation
- **Sequence** method: based on genetic or amino acid sequence differences
- **Antigenic** method: based on observed immune responses


HAI titer
D28
D0
Increasing Ag Distance



Change in titer
(D28 vs D0)
Increasing Ag Distance

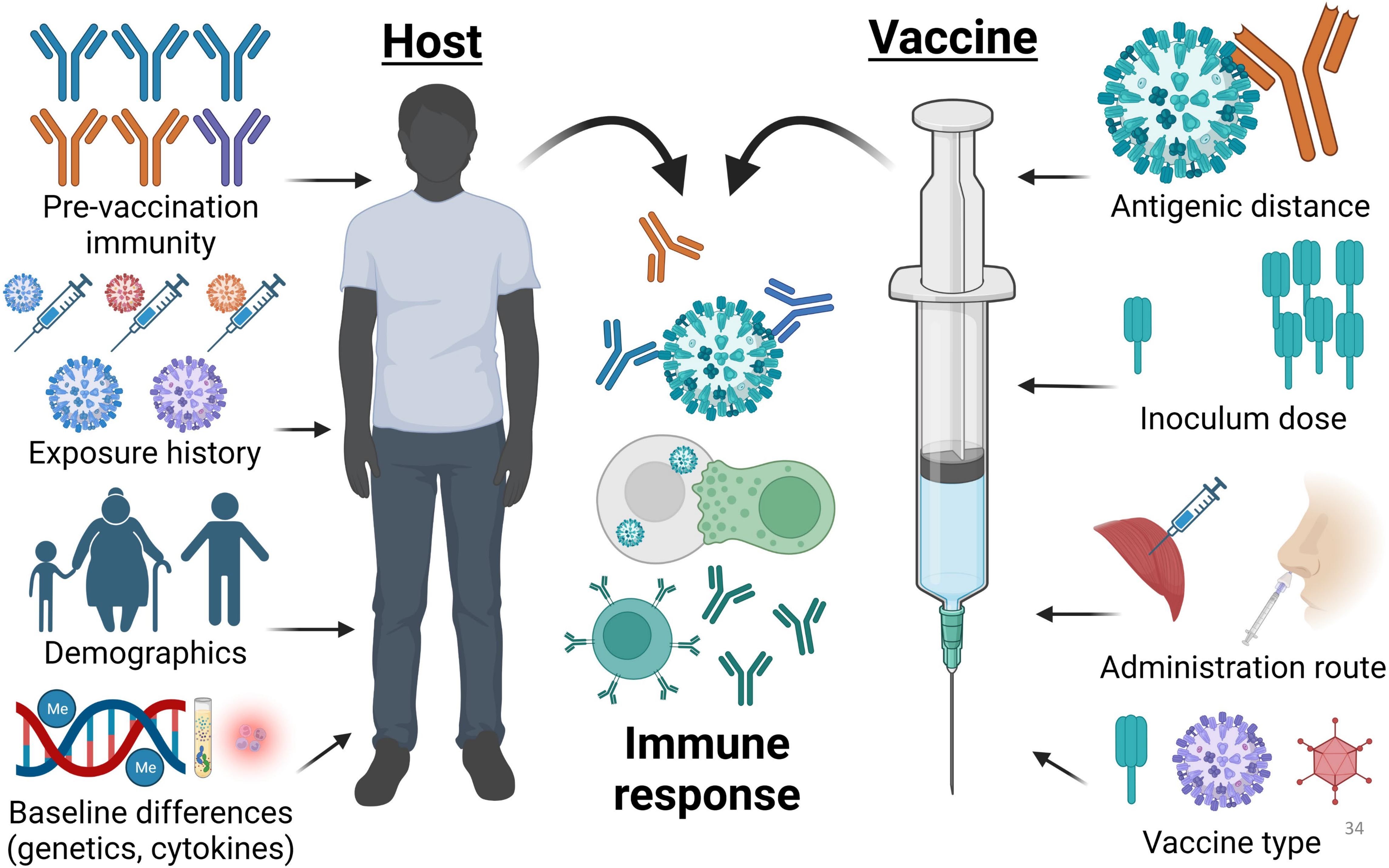

Hypothetical ideal vaccine
High magnitude, low breadth
Low magnitude, high breadth
Change in titer (D28 vs D0)
Increasing Ag Distance

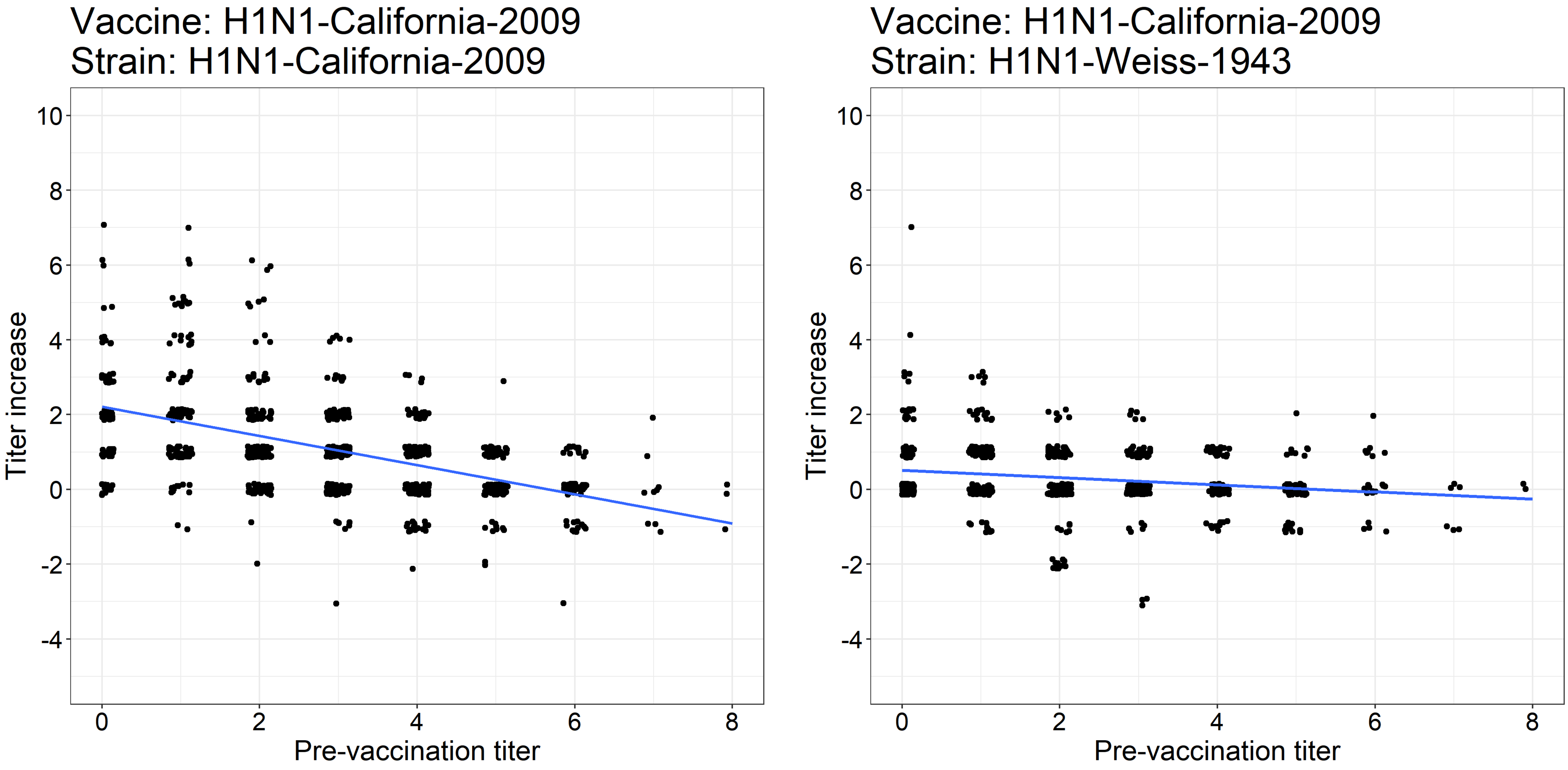

H1N1-California-2009 (n = 773)
Original distance measure
Year difference
p-Epitope sequence distance
Antigenic cartography distance
Strength: 0.46
Breadth: -0.18
Total: 0.37
Strength: 1.07
Breadth: -0.85
Total: 0.65
Strength: 1.06
Breadth: -0.93
Total: 0.59
CA/09
NC/99
SC/18
titer (log2 HAI)
Normalized distance measure
H3N2-Hong Kong-2014 (n = 583)
Original distance measure
Year difference
p-Epitope sequence distance
Antigenic cartography distance
Strength: 1.20
Breadth: -1.36
Total: 0.52
Strength: 1.49
Breadth: -1.44
Total: 0.77
Strength: 1.25
Breadth: -1.21
Total: 0.64
HK/14
Shan/93
TX/77
titer (log2 HAI)
Normalized distance measure
Outcome
Pre-vaccination titer
Post-vaccination titer
Titer increase

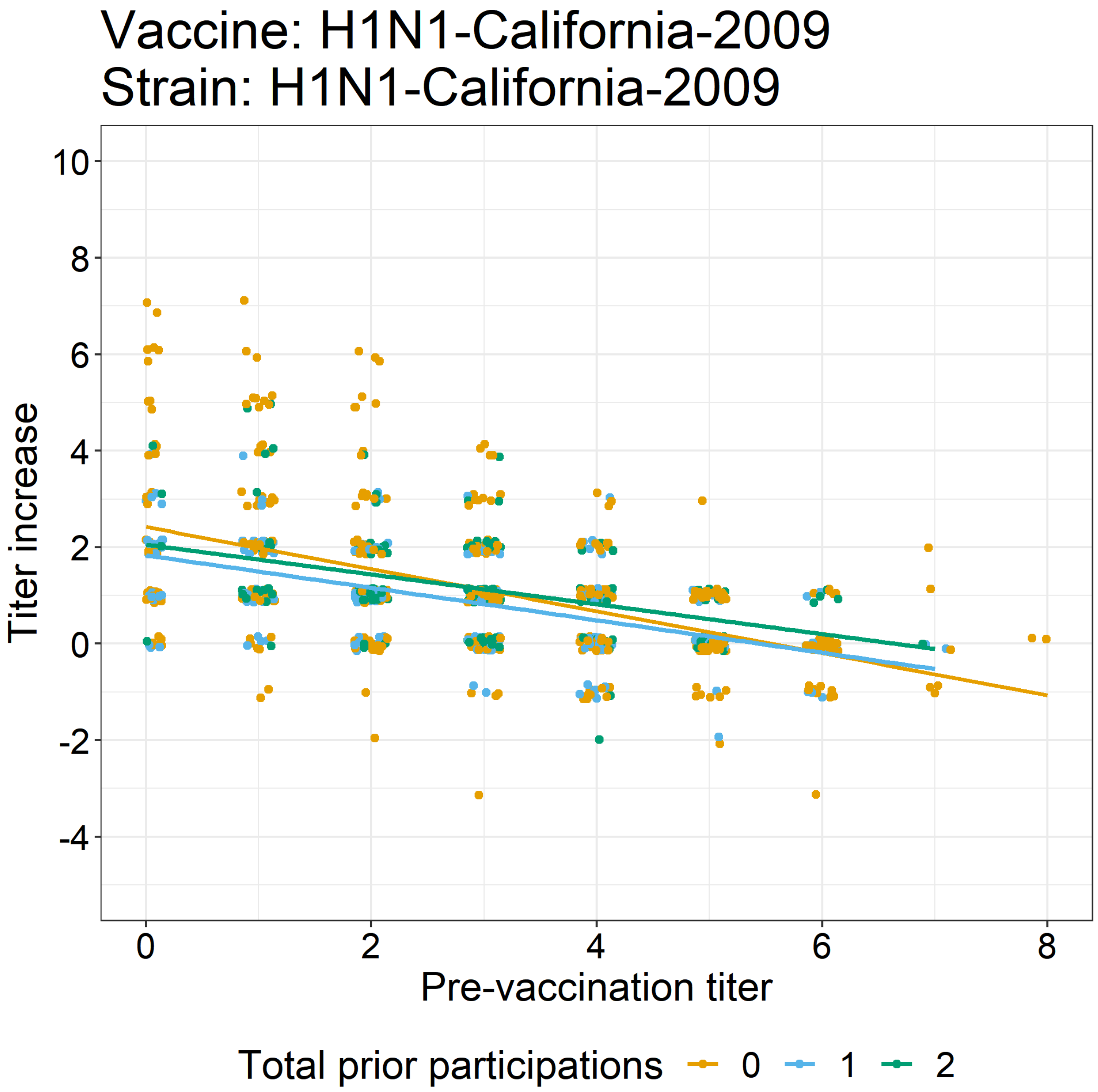
Proposed study

- Finalize linear regression models
- Compare linear and nonlinear statistical models.
- Explore potential weighting schemes for the overall response and how these interact with the distance measurement used.
- As a case study, compare Fluzone SD and HD.
- Compare **variance of metrics** by subsampling panels: take *k* of our measured strains at a time, and compute the metrics on this subsample. Repeat that a bunch of times.

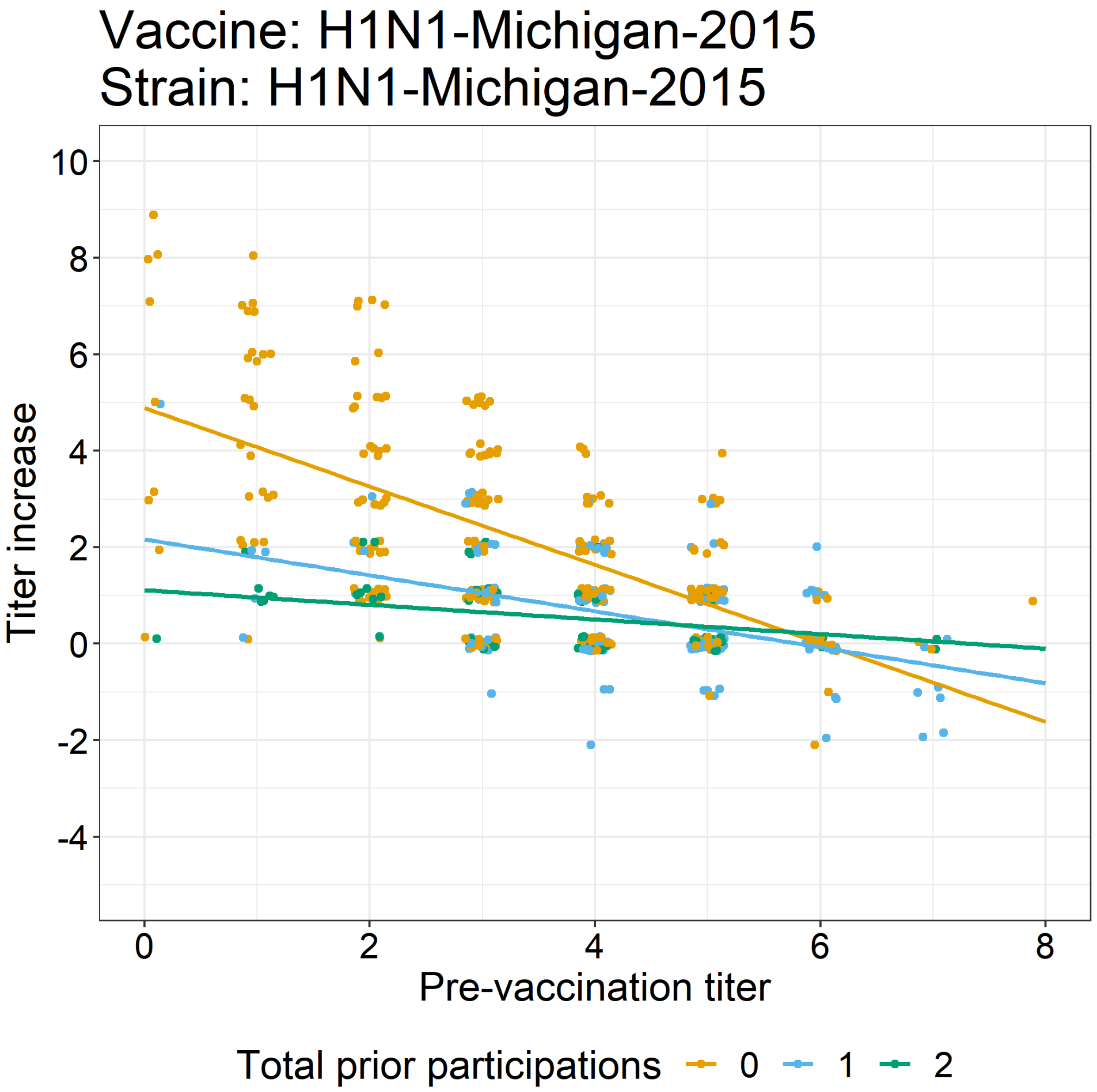
Aim 2: Quantify the role of pre-vaccination titer, prior vaccinations, vaccine dose, and antigenic distance on individual vaccine response.


Host
Vaccine
Pre-vaccination immunity
Exposure history
Demographics
Me
Me
Baseline differences (genetics, cytokines)
Immune response
Antigenic distance
Inoculum dose
Administration route
Vaccine type


Vaccine: H1N1-California-2009
Strain: H1N1-California-2009
Titer increase
Pre-vaccination titer
10
8
6
4
2
0
-2
-4
0
2
4
6
8
Vaccine: H1N1-California-2009
Strain: H1N1-Weiss-1943
Titer increase
Pre-vaccination titer



Vaccine: H1N1-California-2009
Strain: H1N1-California-2009
Titer increase
Pre-vaccination titer
Total prior participations
0
1
2



Vaccine: H1N1-Michigan-2015
Strain: H1N1-Michigan-2015
Titer increase
Pre-vaccination titer
Total prior participations
0
1
2

Thousands more exploratory plots (data not shown)

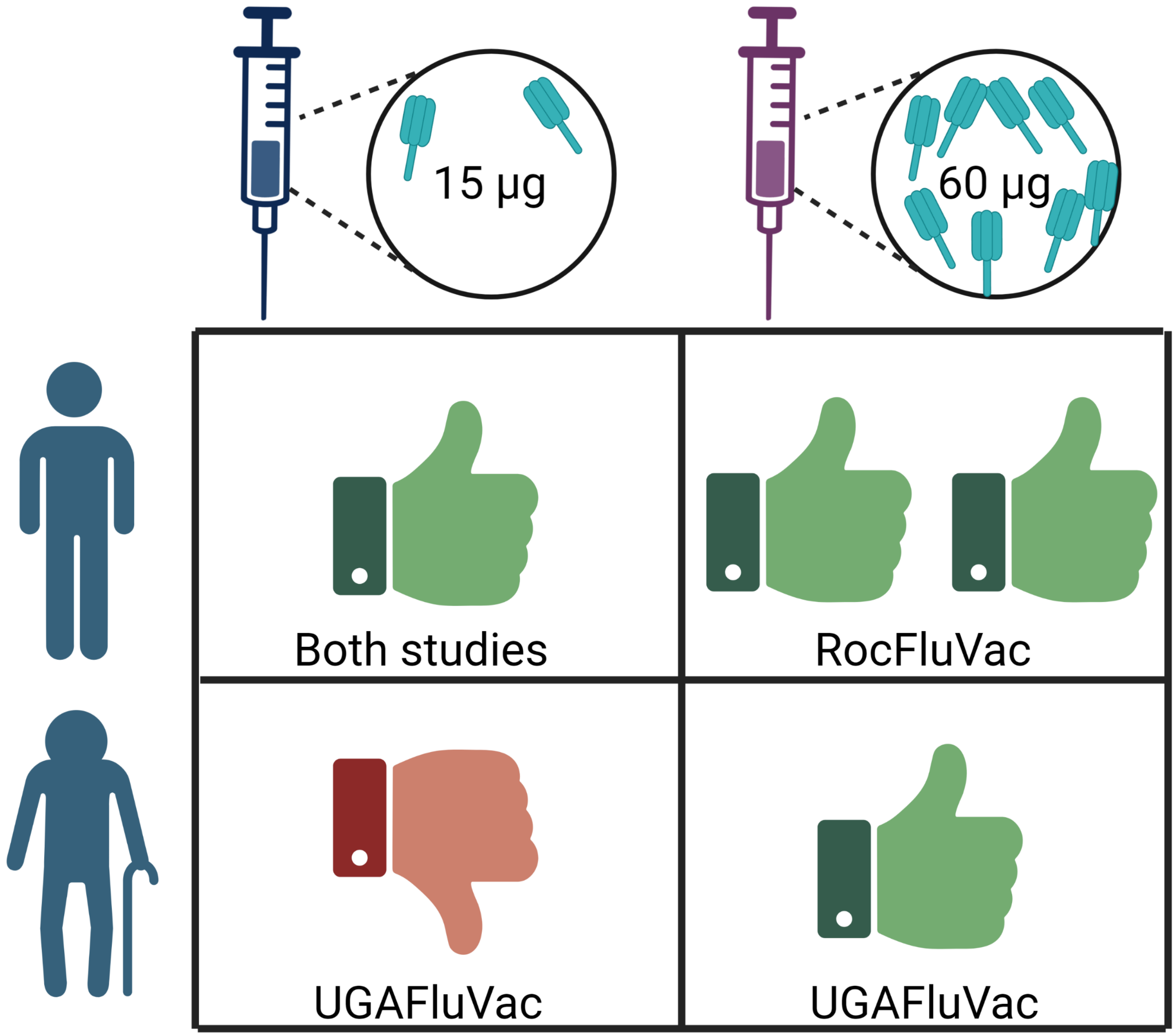
```
Zane@DESKTOP-J4M5FIT MINGW64 /d/Research/CIVIC-EDA/Figures (main)
$ find . -type f | wc -l
3391

Zane@DESKTOP-J4M5FIT MINGW64 /d/Research/CIVIC-EDA/Figures (main)
$ du -sh .
329M    .
```

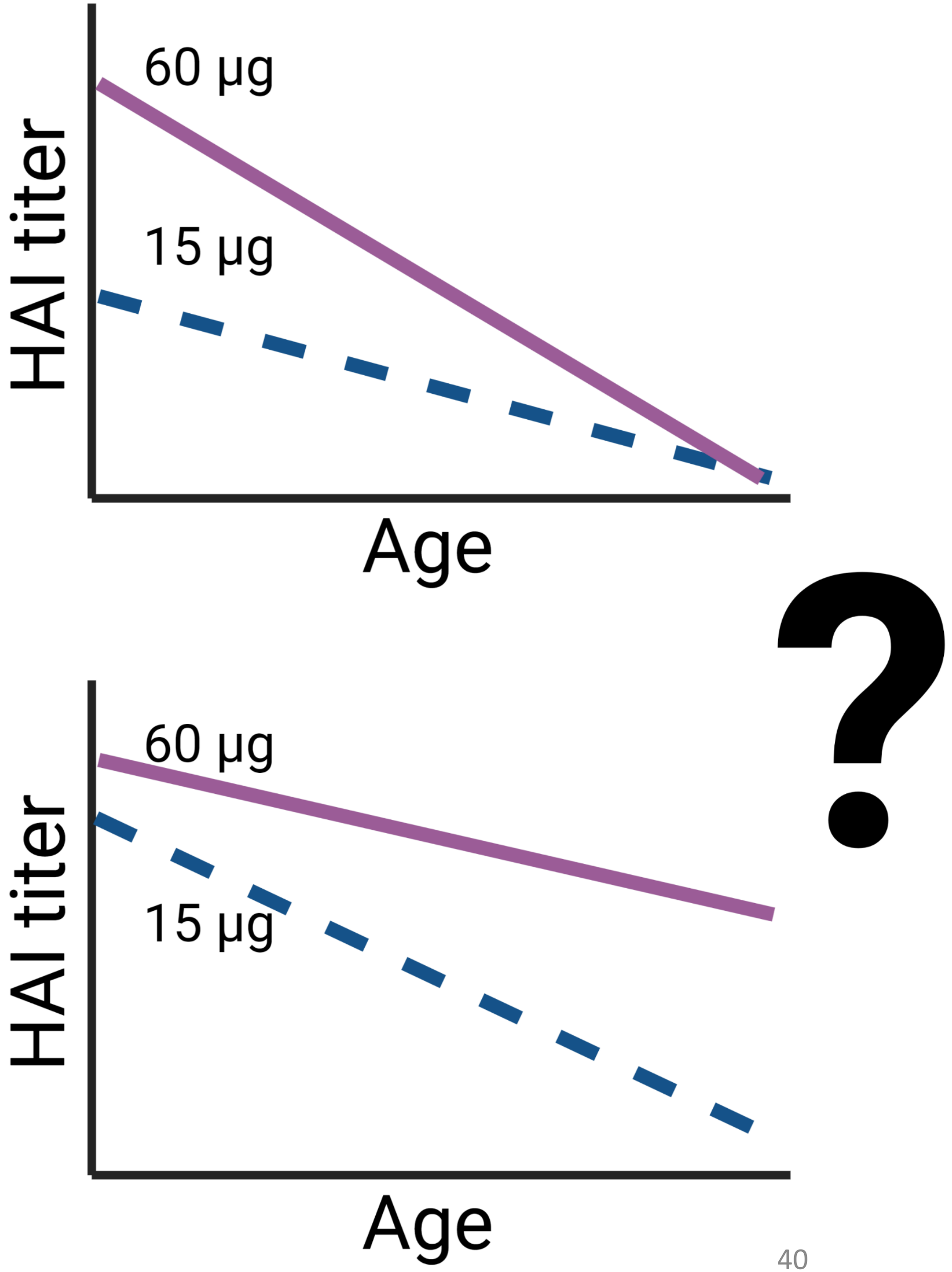
Proposed study

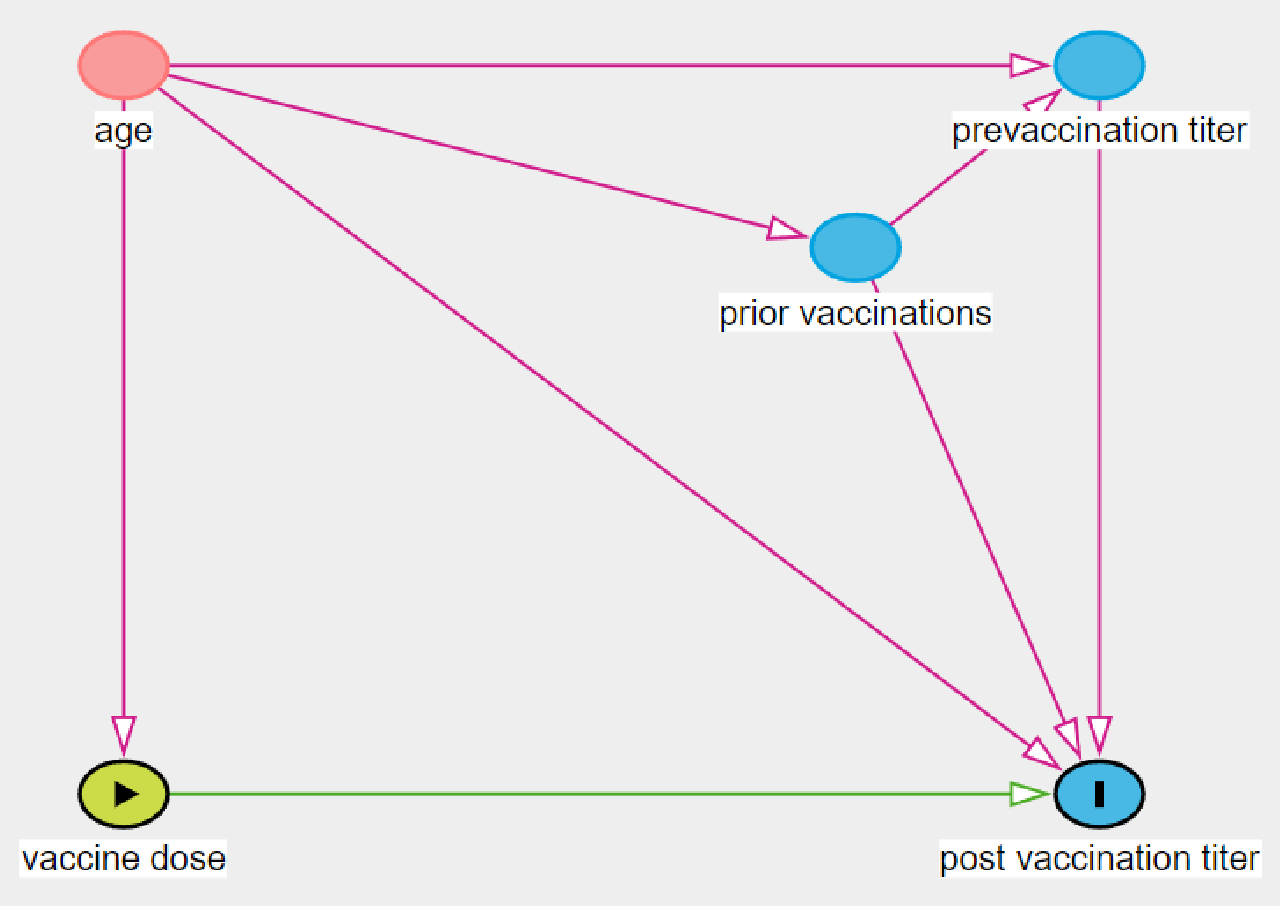
- We will first consider models for homologous responses only, and then we will expand our analysis to consider Ag distance.
- We can compare models with Ag distance to strain-specific models (including strain as a nominal variable).
- Modeling approaches:
  - Graphical causal modeling with DAG analysis (**causal approach**)
  - Bayesian hierarchical linear models (**inferential approach**)
  - Machine learning models like random forest (**predictive approach**)
  - Ordinary differential equation models (**mechanistic approach**)

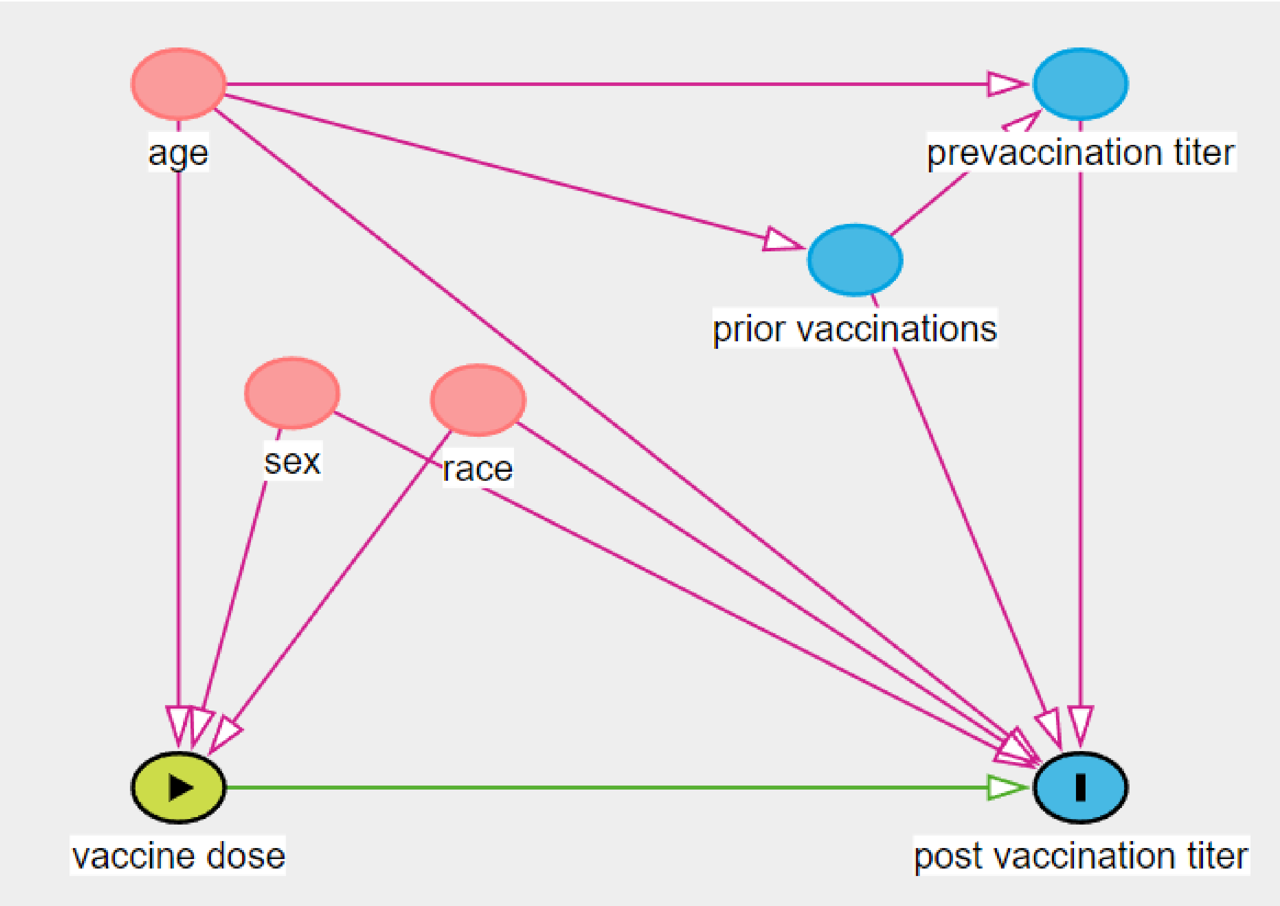
Aim 3: Explore how age and vaccine dose interact to affect the antibody response.



(Homologous response only!)







$$\text{Post-titer} \sim \mathcal{N}(\mu, \sigma)$$
$$\mu = \beta_1 \text{ dose} + \beta_2 \text{ age} + \gamma_{12} \text{ dose} \cdot \text{age}$$

$$\text{Post-titer} \sim \mathcal{N}(\mu, \sigma)$$
$$\mu = \beta_1 \text{ dose} + \beta_2 \text{ age} + \gamma_{12} \text{ dose} \cdot \text{age} + \beta_3 \text{ sex} + \beta_4 \text{ race}$$

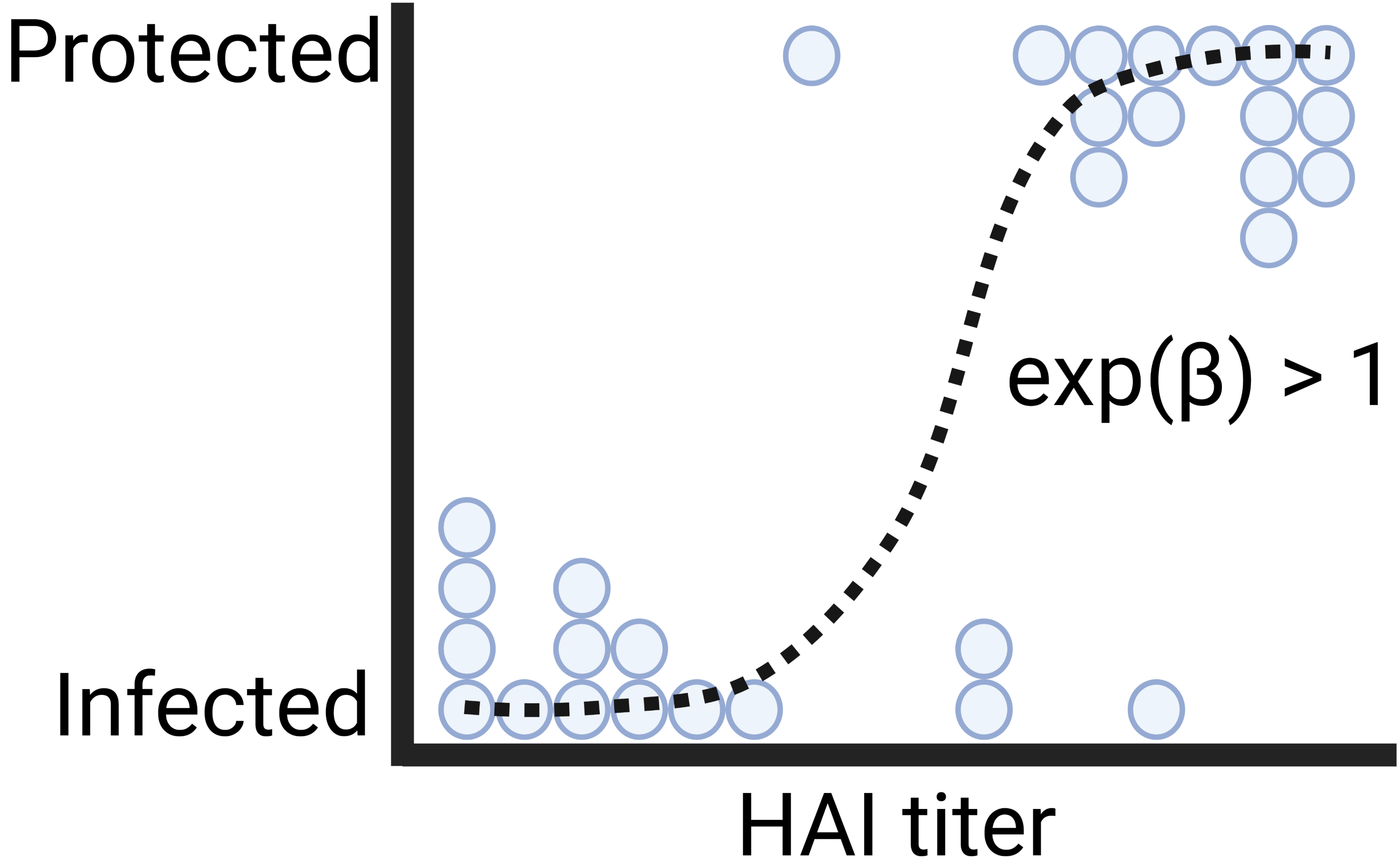
Proposed study

- Combine **UGAFluVac** data (HD in 65+) with **RocFluVac** data provided by Andrea Sant (HD in 18 – 49).
- DAG analysis
  - What do we adjust for to get an unbiased treatment effect?
  - Do our observed correlations match the implied correlations?
  - What other DAGs could show the same pattern?
- Causal estimation
  - Regression with robust SEs; analysis of unmeasured confounding
  - Targeted maximum likelihood estimation (TMLE) approach
  - Estimates on both subsets, as well as overall data

Timeline

Aim	Objectives	2023		2024			2025
		Summer	Fall	Spring	Summer	Fall	Spring
*	Obtain and prepare all data sources	■					
1	Regression analyses and modeling extensions	■	■	■			
1	Robustness and subsampling analysis		■	■			
2	Graphical causal modeling		■	■			
2	Machine learning modeling		■	■			
2	Hierarchical inferential modeling		■	■	■		
2	Mechanistic modeling			■	■	■	
3	Causal modeling and theoretical framework				■	■	
3	Formal statistical analysis					■	■
*	Final dissertation writing					■	■

Thank you!


Protected
Infected
exp(β) > 1
HAI titer

Calculating Ag distance

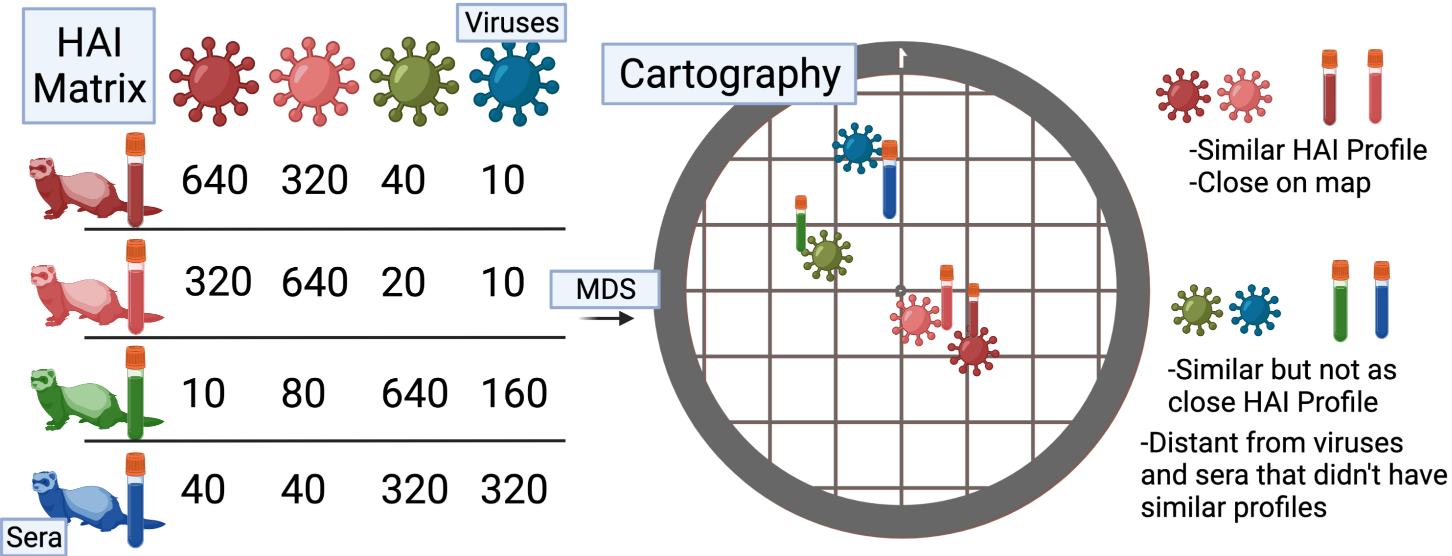
Temporal method

A/H3N2/Aichi/2/1968

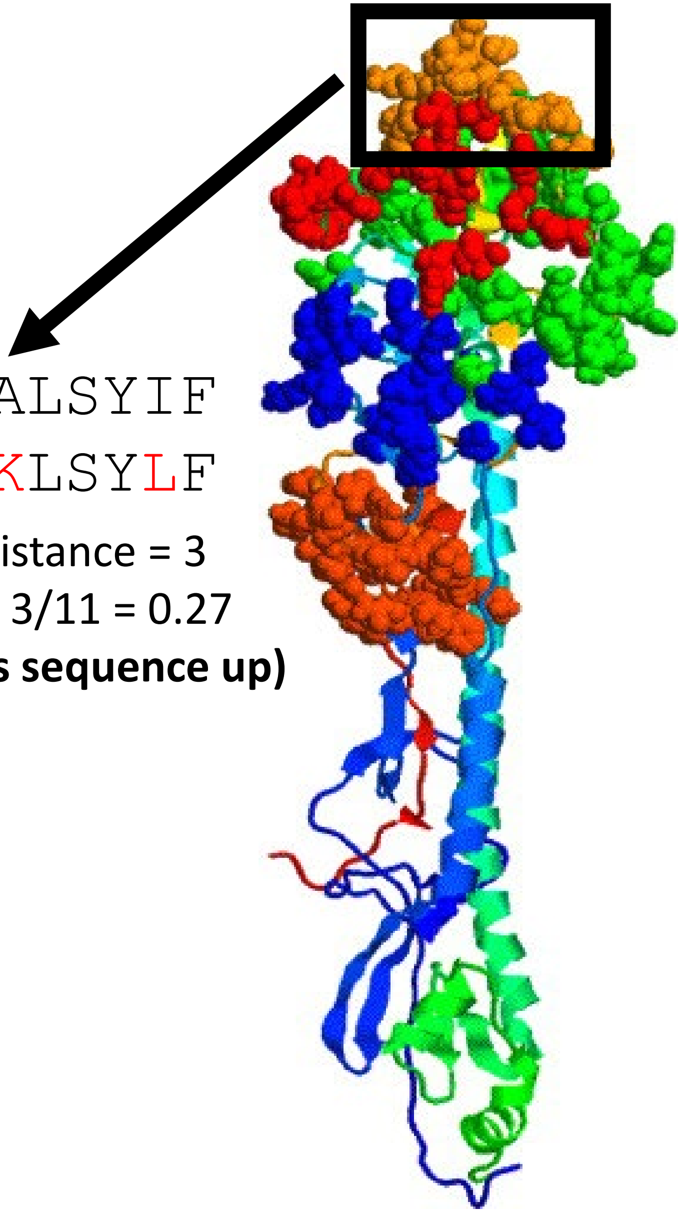
A/H3N2/Kansas/14/2017

|2017 – 1968| = 49

Antigenic cartography method



(dominant) *p*-Epitope method



MKTIIALSYIF

MLTIIKLSYLF

Hamming distance = 3

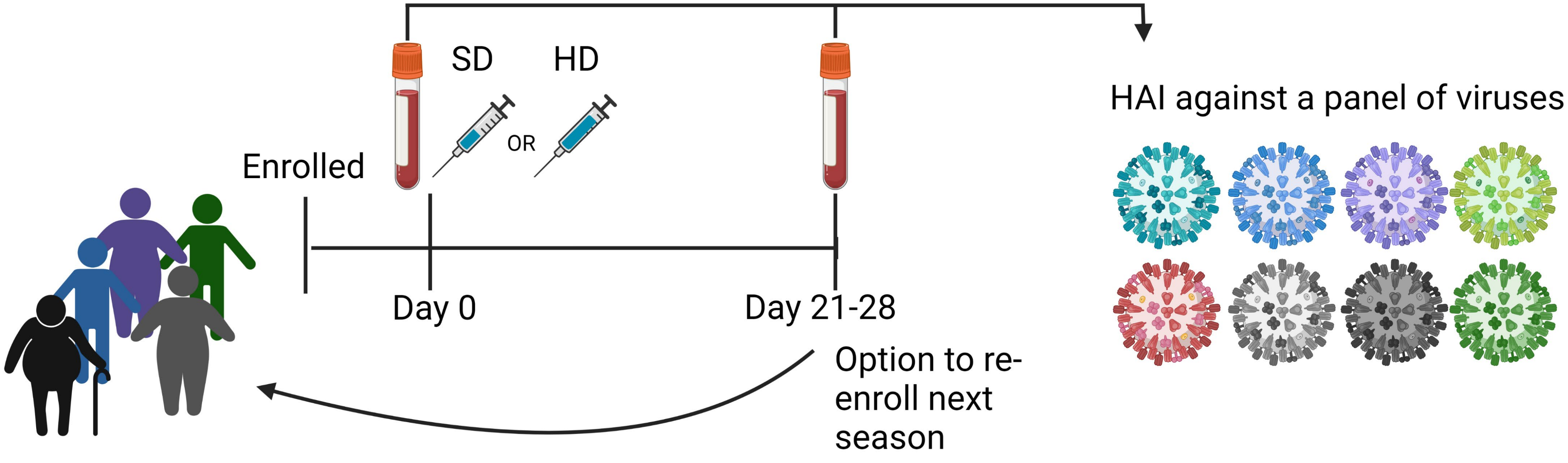
p-Epitope = 3/11 = 0.27

(I made this sequence up)

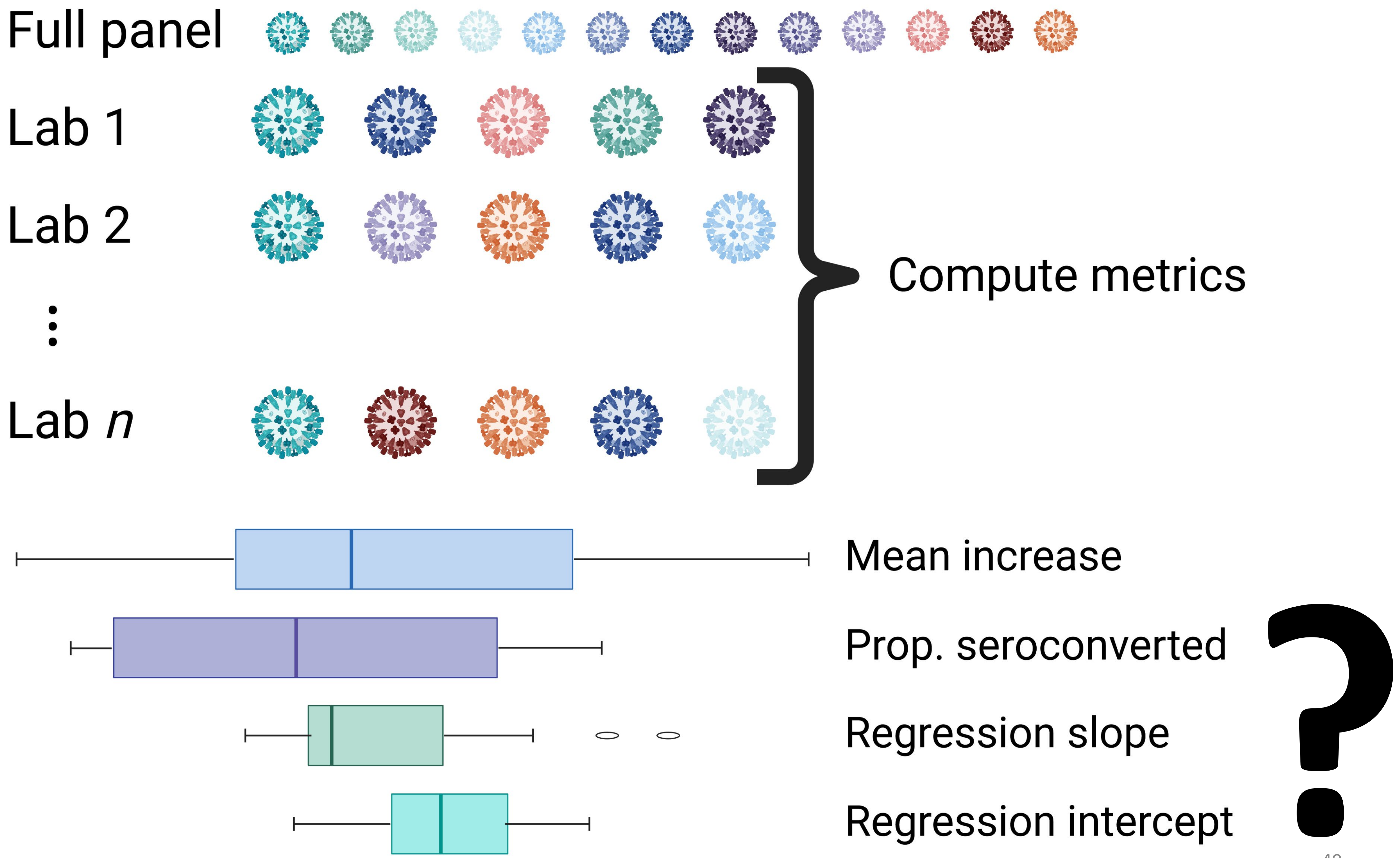
Protein structure from Gupta, Earl, and Deem. Vaccine 2006.
Cartography figure made by Amanda Skarlupka.

Influenza Season	**2016-2017**	**2017-2018**	**2018-2019**	**2019-2020**	**2020-2021**	**2021-2022**	**2022-2023**
Vaccine Strains **Strain change** *excluded from High-Dose	H1N1: CA/09 **H3N2: HK/14** B-Vic: BR/08 B-Yam: PH/13*	**H1N1: MI/15** H3N2: HK/14 B-Vic: BR/08 B-Yam: PH/13*	H1N1: MI/15 **H3N2: SG/16** **B-Vic: CO/17** B-Yam: PH/13*	**H1N1: BR/18** **H3N2: KS/17** B-Vic: CO/17 B-Yam: PH/13*	**H1N1: GD/19** **H3N2: HK/19** **B-Vic: WA/19** B-Yam: PH/13	**H1N1: VIC/19** **H3N2: TAS/20** B-Vic: WA/19 B-Yam: PH/13	H1N1: VIC/19 **H3N2: DAR/21** **B-Vic: AUS/21** B-Yam: PH/13
Cohort Identifiers • UGA = Fluzone • FLU = Fluzone Children/Teens • FB = FluBlok • FM = FluMist • FMC = FluMist Children/Teens • FC = Flucelvax • FC1C = Flucelvax Children/Teens	UGA1 18-74 y.o. n=148	UGA2 18-83 y.o. n=199 FLU1 12-17 y.o. n=72	UGA3 18-80 y.o. n=100 FLU2 12-17 y.o. n=150 FM1 18-49 y.o. n=96	UGA4 18-85 y.o. n=371 FLU3 11-17 y.o. n=99	UGA5 18-85 y.o. n=270 FLU4 12-17 y.o. n=69	UGA6 18+ y.o. n=248 FLU5 10-17 y.o. n=89 FB1 18+ y.o. n=75	UGA7 18+ y.o. Target: n=250 FLU5 8-17 y.o. Target: n=100 FB2 18+ y.o. Target: n=50 FM2 + FMC2 8+ y.o. Target: n=50 / 50 FC1 + FC1C 8+ y.o. Target: n=50+
Timepoints & Samples collected	Timepoints: D0, D7, D21 Serum: D0, D21 PBMC: all timepoints	Timepoints: D0, D3, D7, D21 Serum: D0, D3, D21 PBMC: all timepoints RNA PAXgene: D3 *FLU1: serum-only	Timepoints: D0, D7, D28 Serum: D0, D28 PBMC: all timepoints *FLU2: serum-only	Timepoints: D0, D3, D7, D28, D90 Serum: D0, D3, D28 PBMC: all timepoints Plasma: all timepoints RNA PAXgene: all timepoints Urine: all timepoints *FLU3: no D3	Timepoints: D0, D7, D28, D60 Serum: D0, D28, D60 PBMC: all timepoints Plasma: all timepoints RNA PAXgene: D0 Urine: all timepoints	Timepoints: D0, D3, D7, D28 Serum: D0, D28 PBMC: D0, D7, D28 Plasma: D0, D7, D28 RNA PAXgene: D0 Urine: all timepoints	Timepoints: D0, D3, D7, D28, D90 Serum: D0, D7, D28, D90 PBMC: D0, D7, D28, D90 Plasma: D0, D7, D28, D90 RNA PAXgene: D0 Saliva & Urine: all timepoints

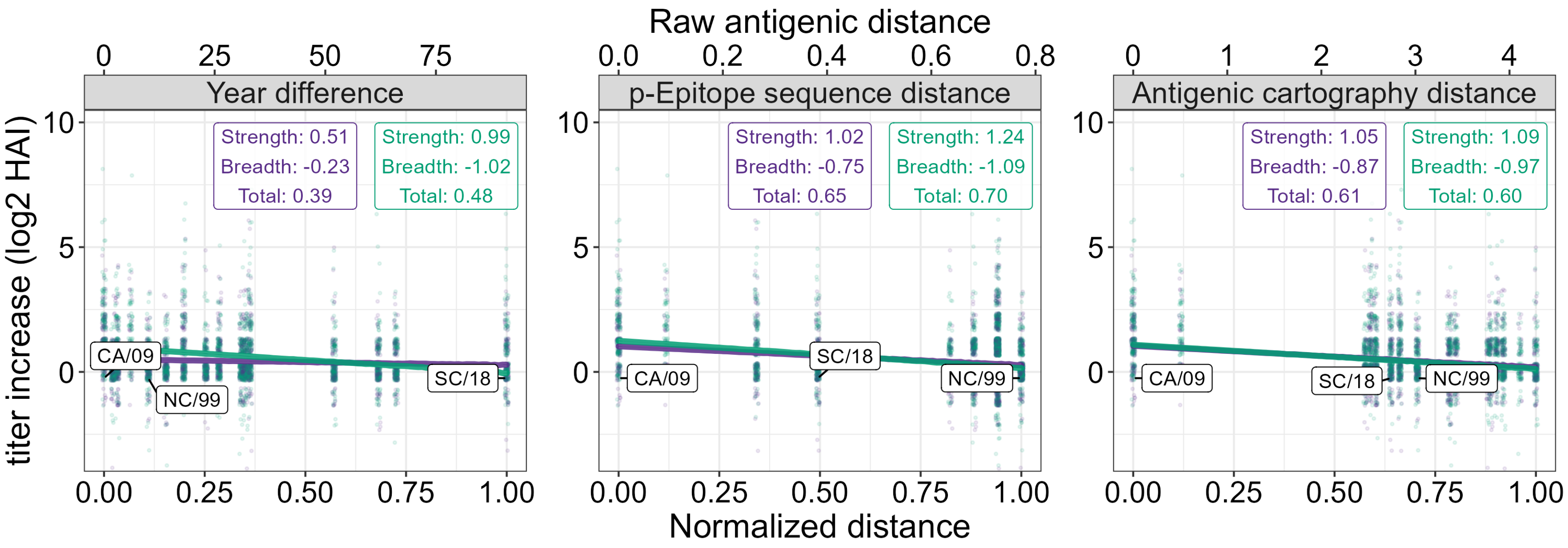
“**UGAFluVac**”: this, plus a similar study from 2013 – 2016 also by Ted Ross



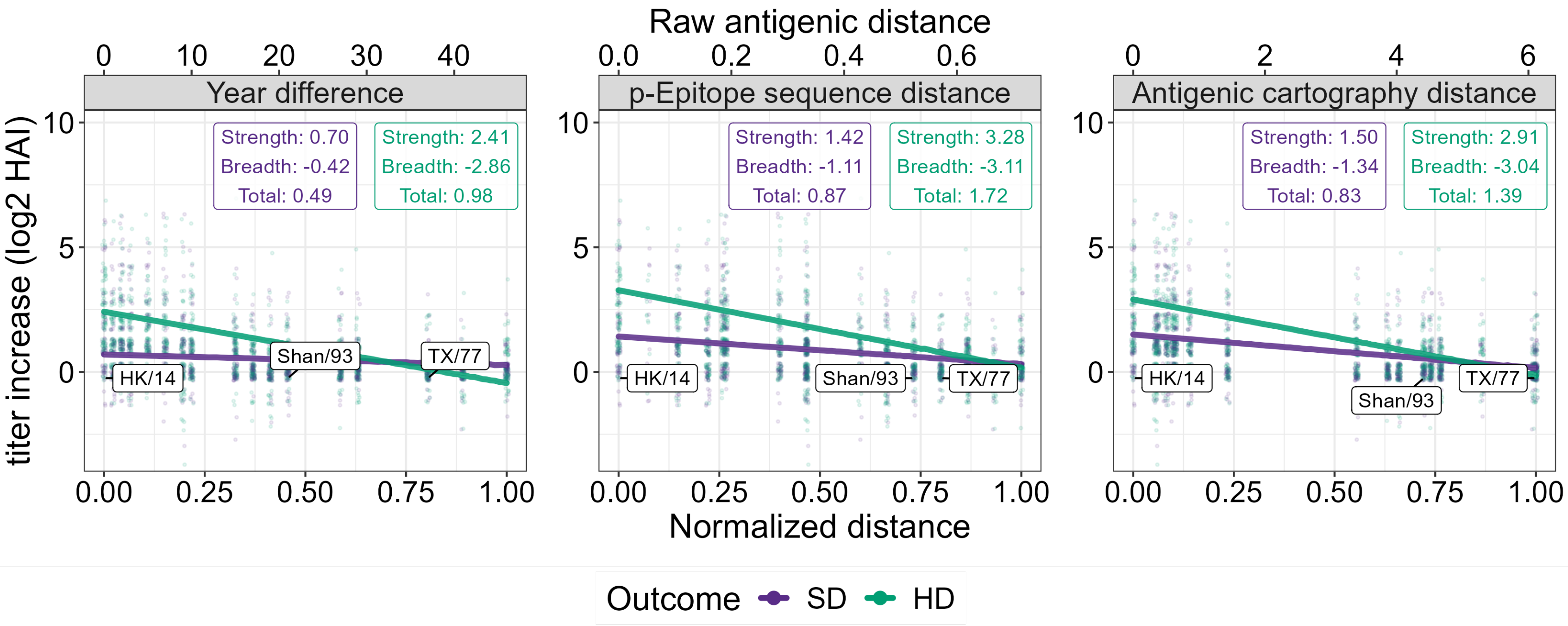
UGAFluVac conceptual figure. The design of **RocFluVac** was similar, but with emphasis on diverse immunological measurements rather than heterologous HAI panels. (Figure made by Amanda Skarlupka.)

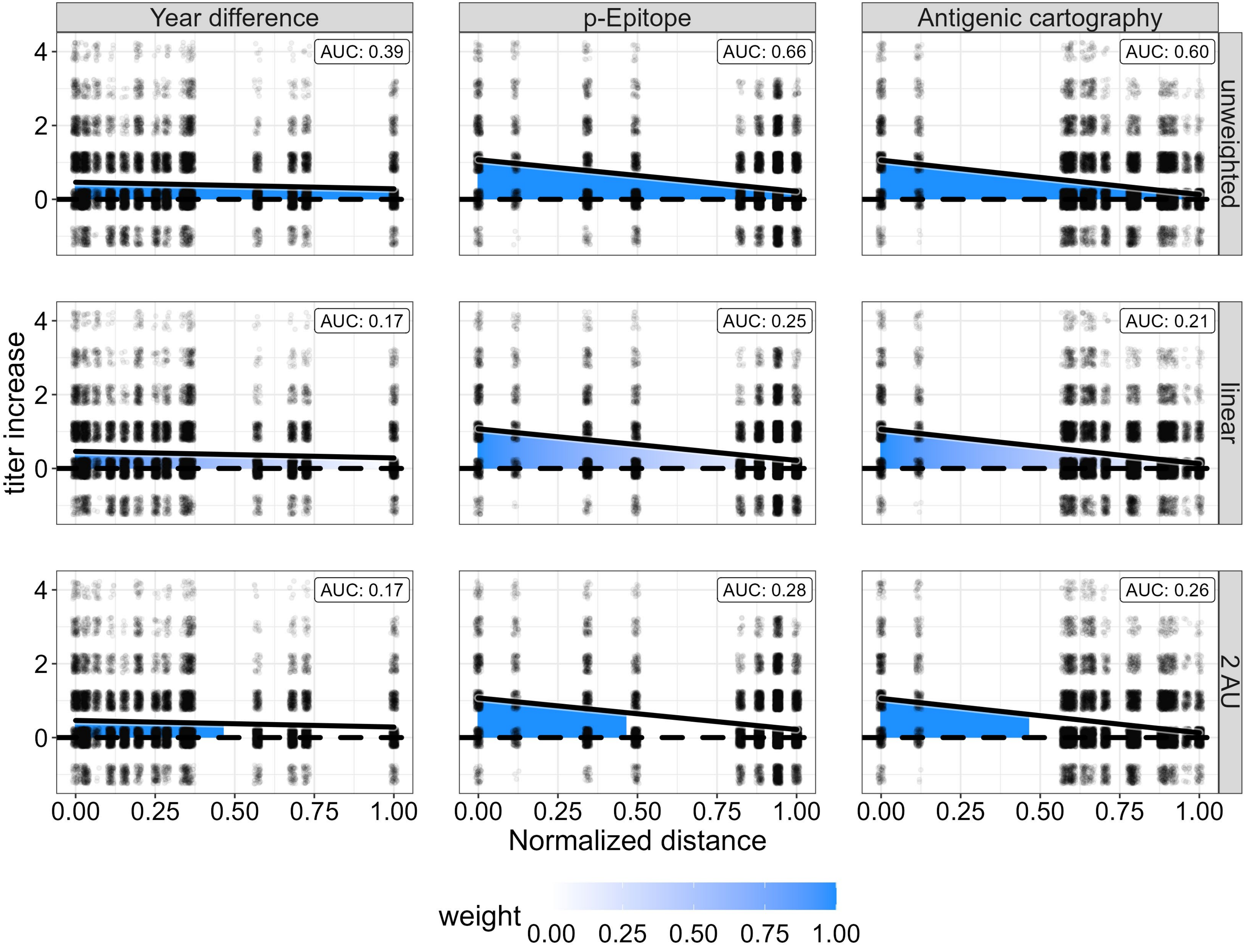

Full panel
Lab 1
Lab 2
⋮
Lab *n*
Compute metrics
Mean increase
Prop. seroconverted
Regression slope
Regression intercept
?

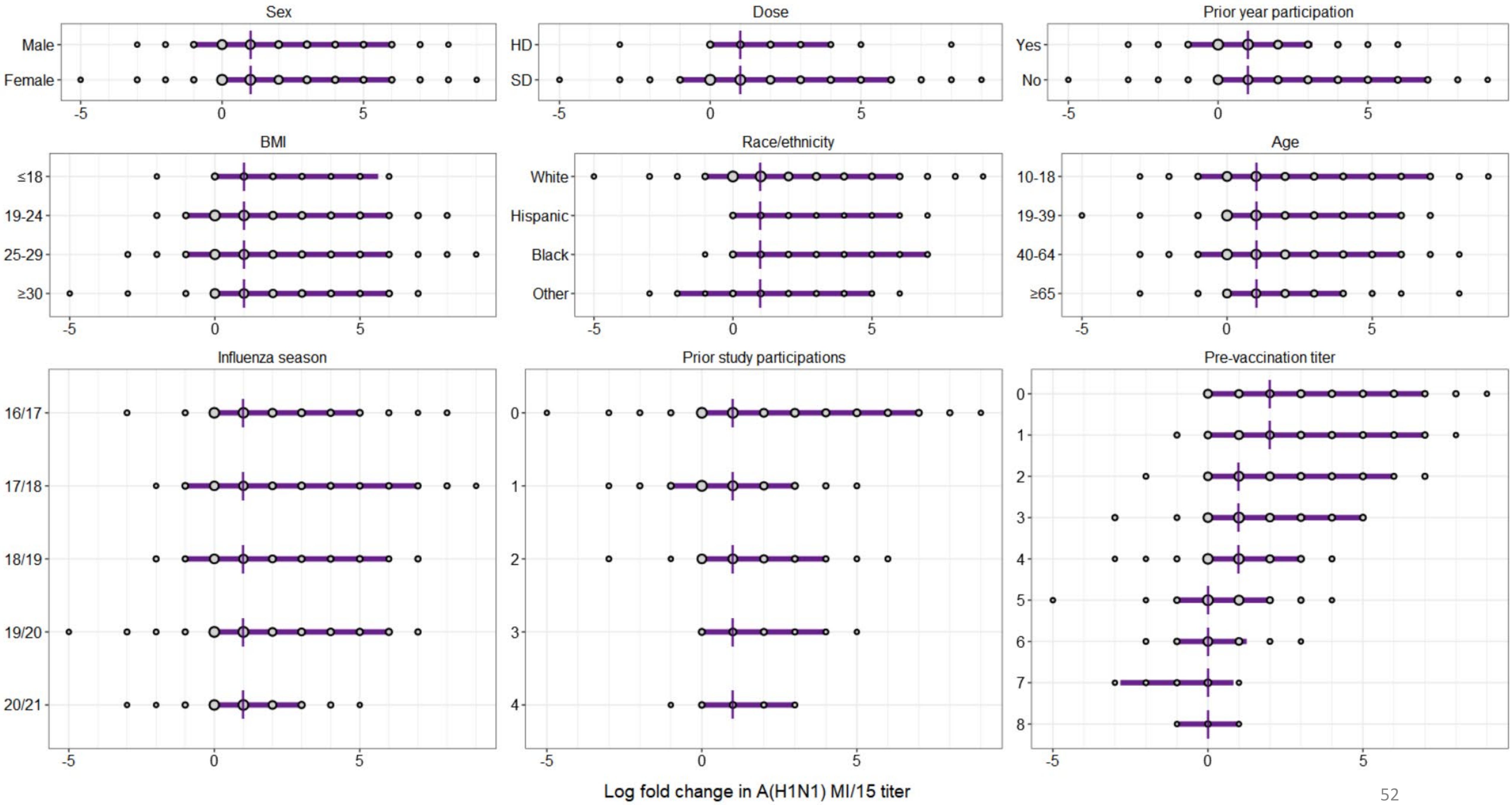
H1N1-California-2009 (SD = 127; HD = 174)

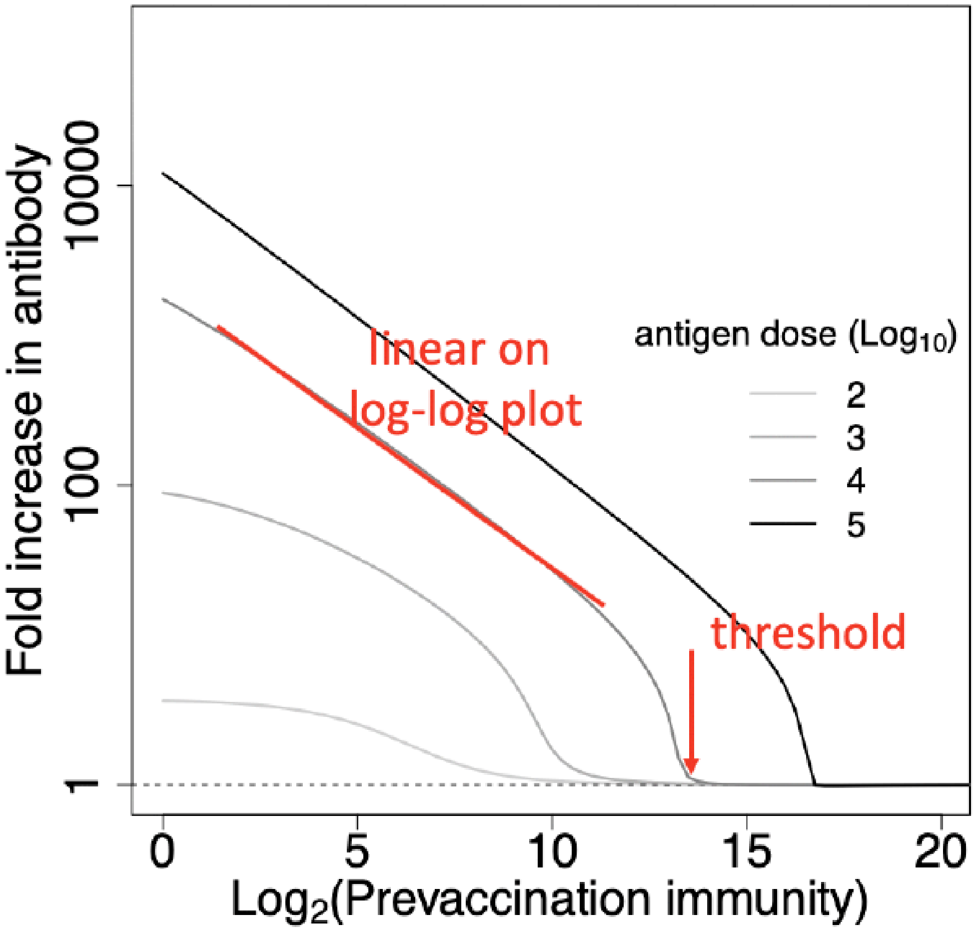


H3N2-Hong Kong-2014 (SD = 56; HD = 93)




Year difference
p-Epitope
Antigenic cartography
AUC: 0.39
AUC: 0.66
AUC: 0.60
AUC: 0.17
AUC: 0.25
AUC: 0.21
AUC: 0.17
AUC: 0.28
AUC: 0.26
unweighted
linear
2 AU
titer increase
Normalized distance
weight
0.00 0.25 0.50 0.75 1.00


Sex
Male
Female
Dose
HD
SD
Prior year participation
Yes
No
BMI
≤18
19-24
25-29
≥30
Race/ethnicity
White
Hispanic
Black
Other
Age
10-18
19-39
40-64
≥65
Influenza season
16/17
17/18
18/19
19/20
20/21
Prior study participations
Pre-vaccination titer
Log fold change in A(H1N1) MI/15 titer



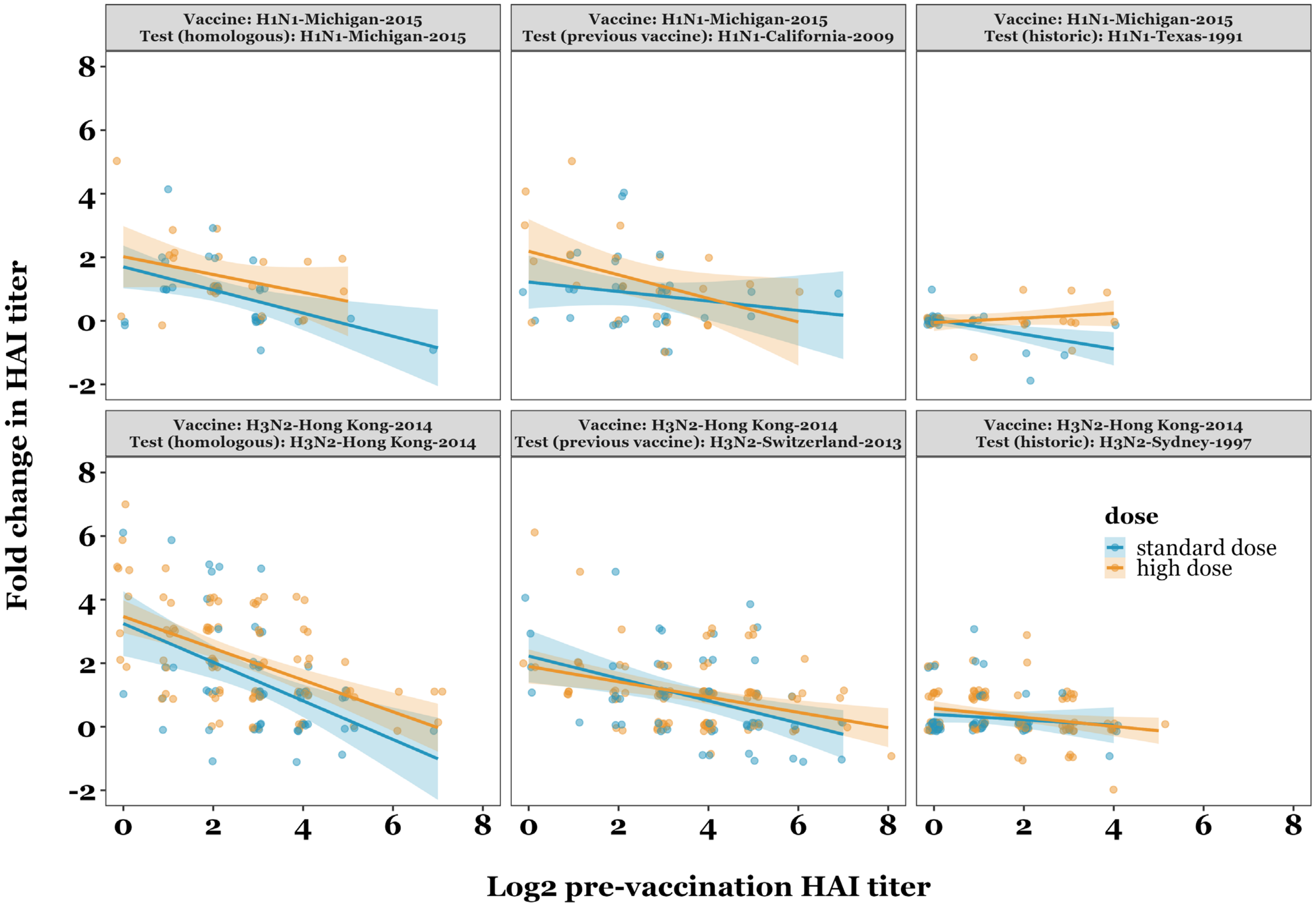
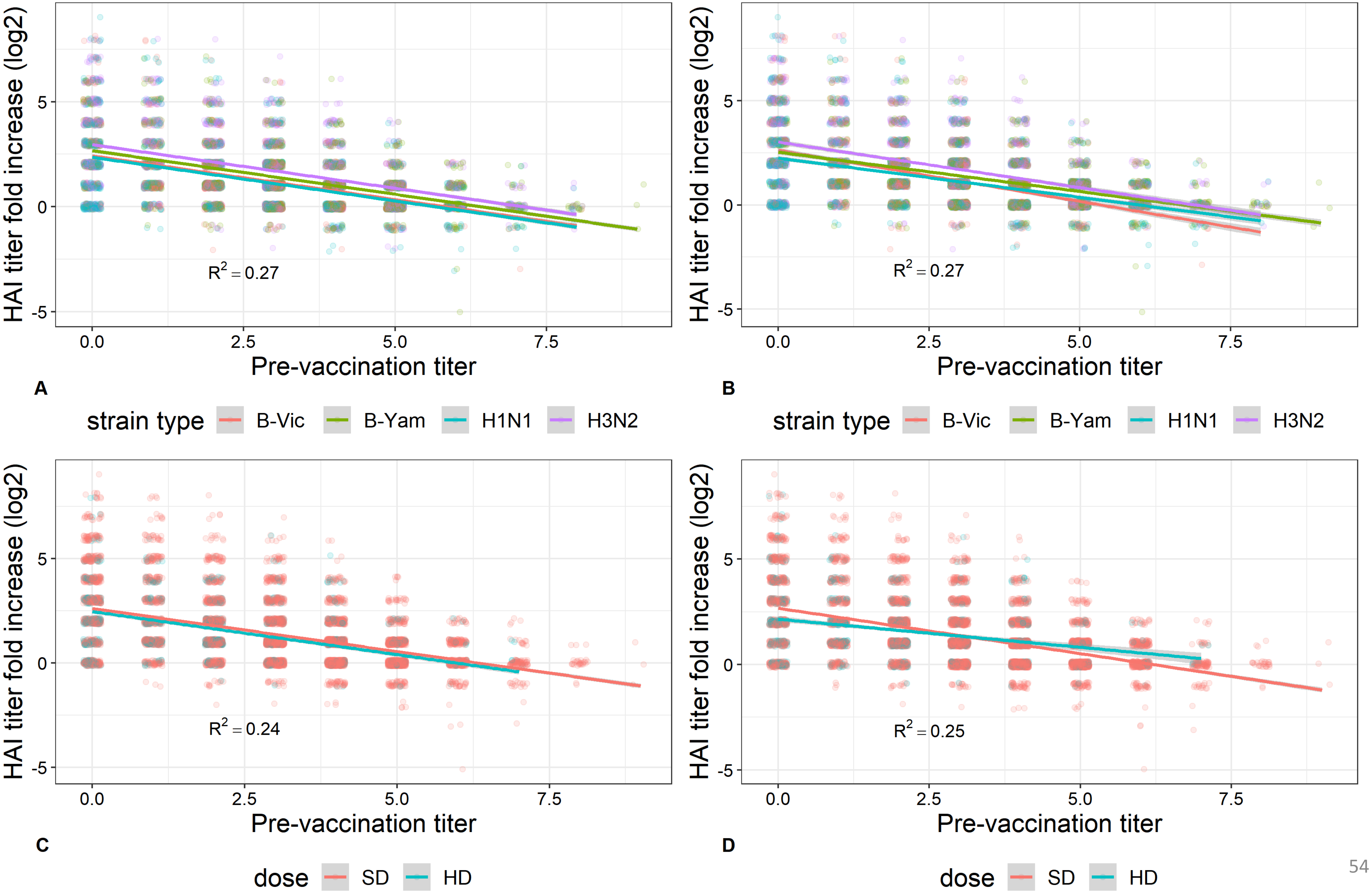
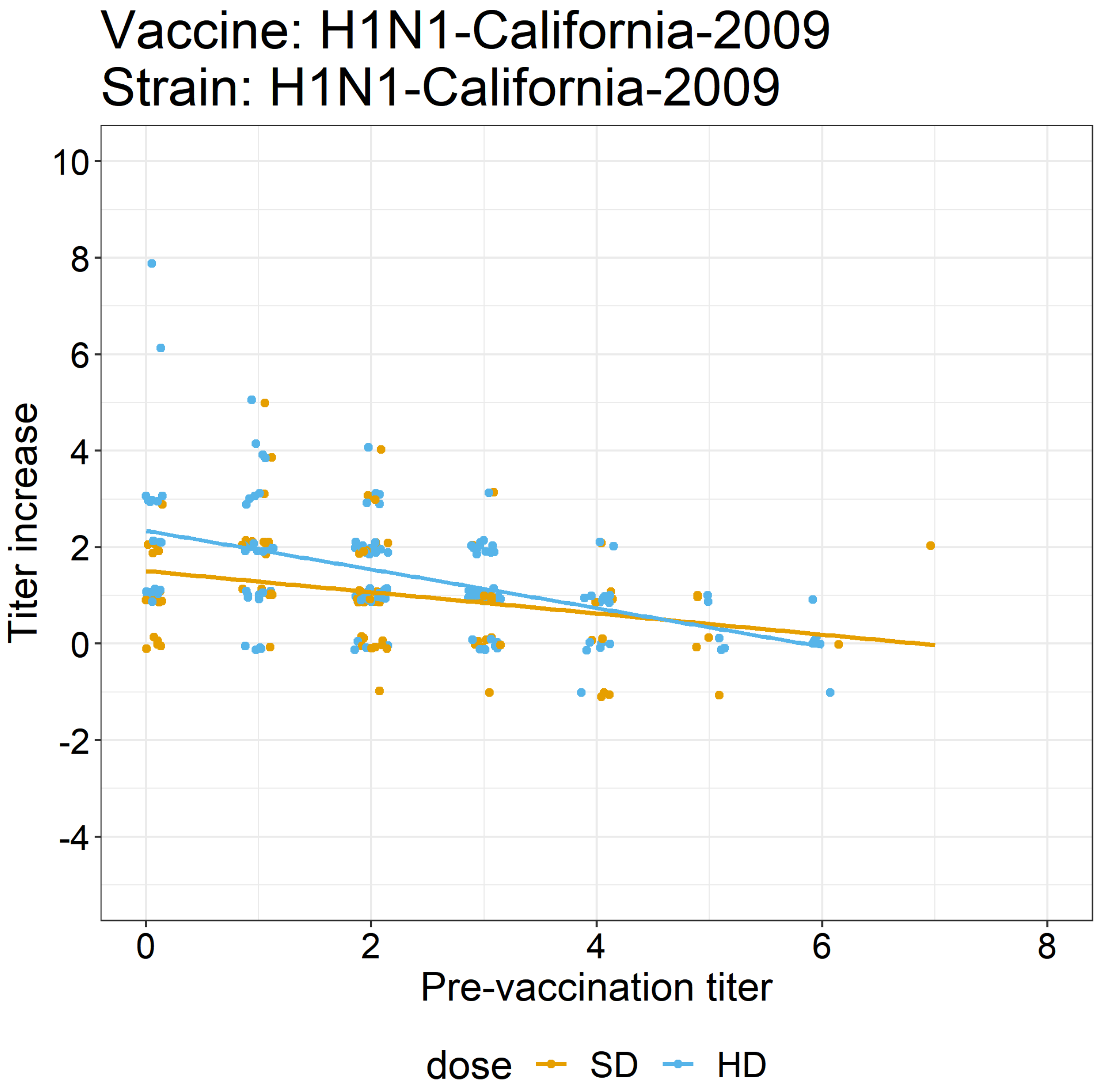
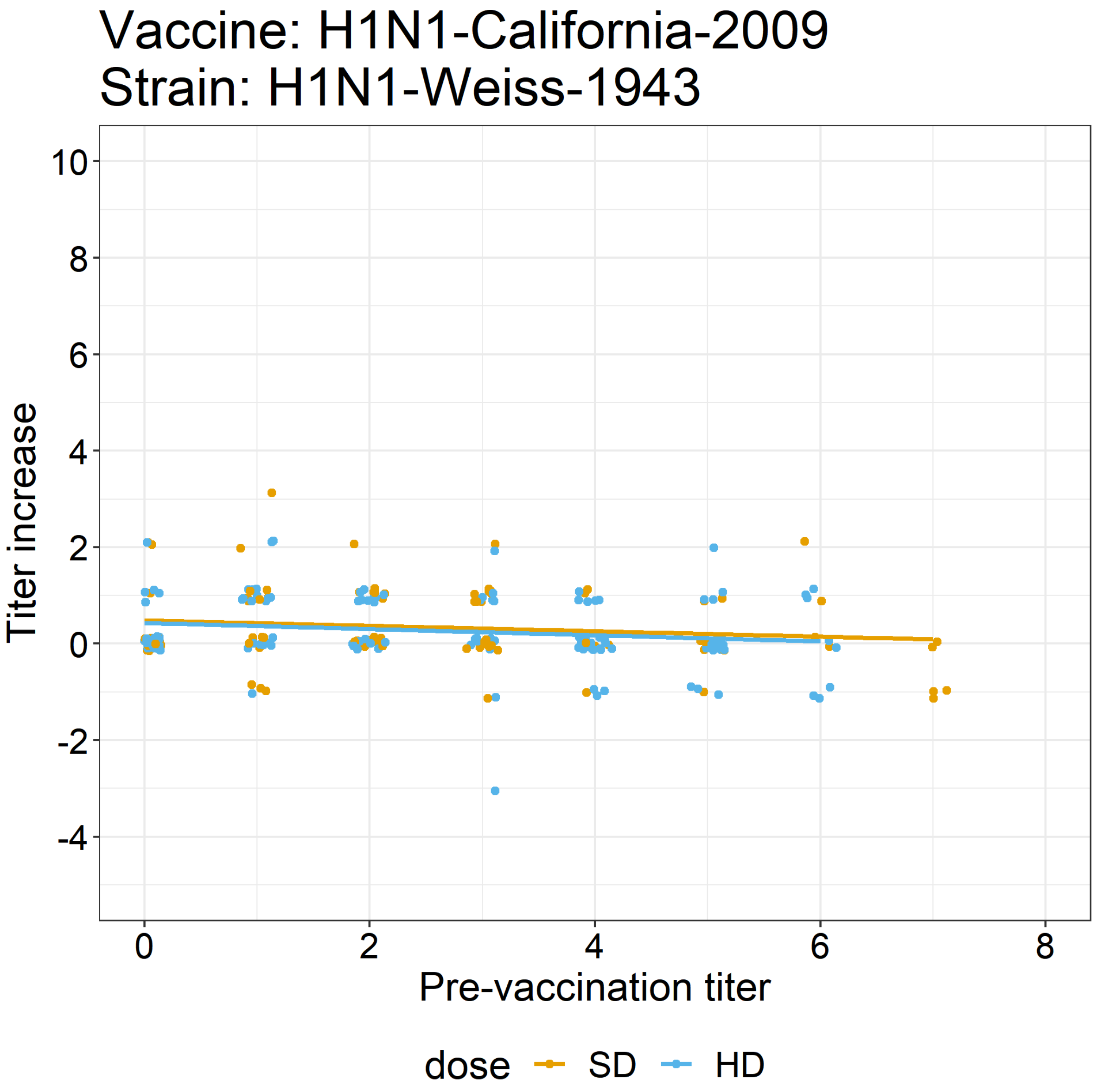
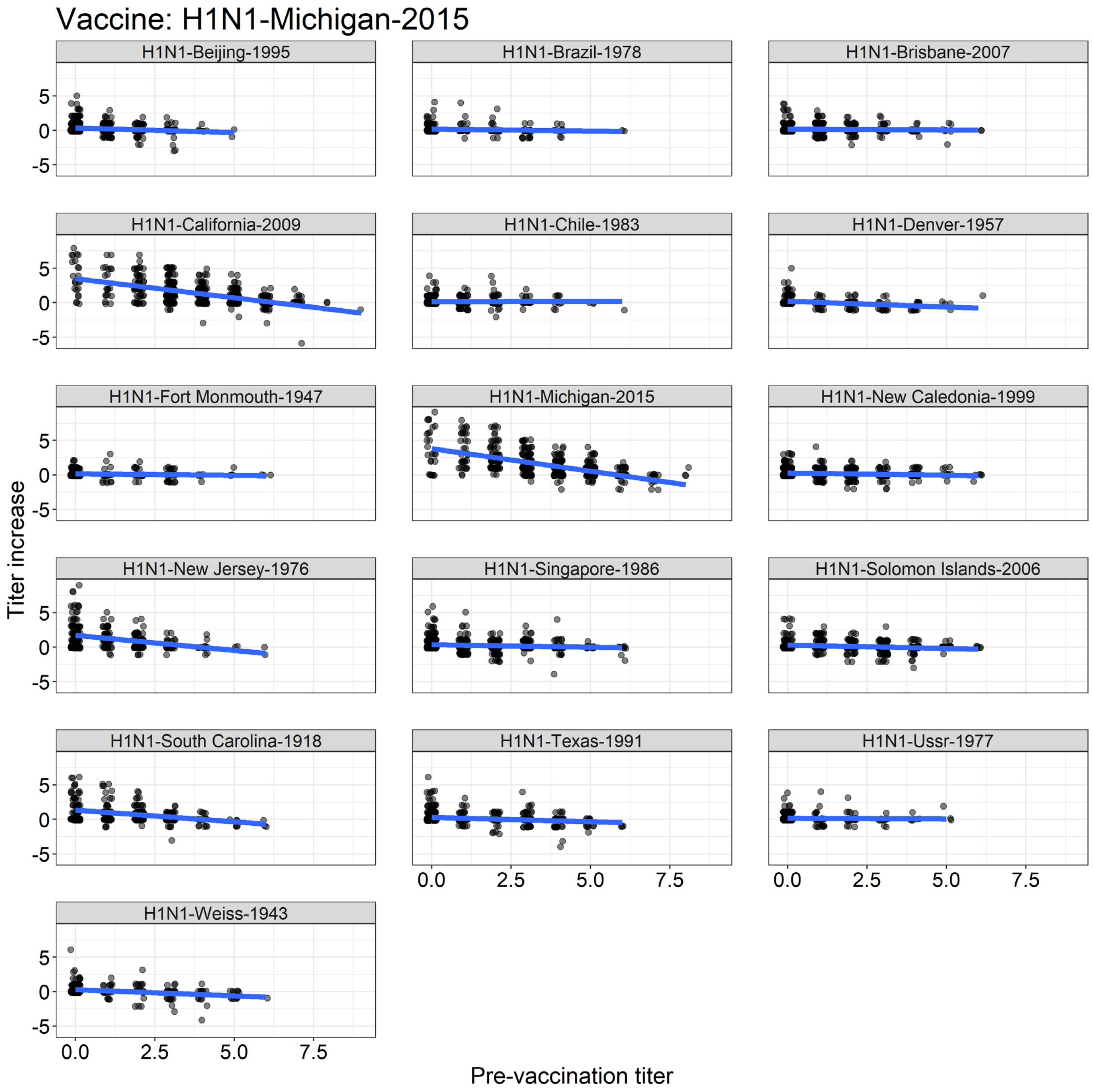


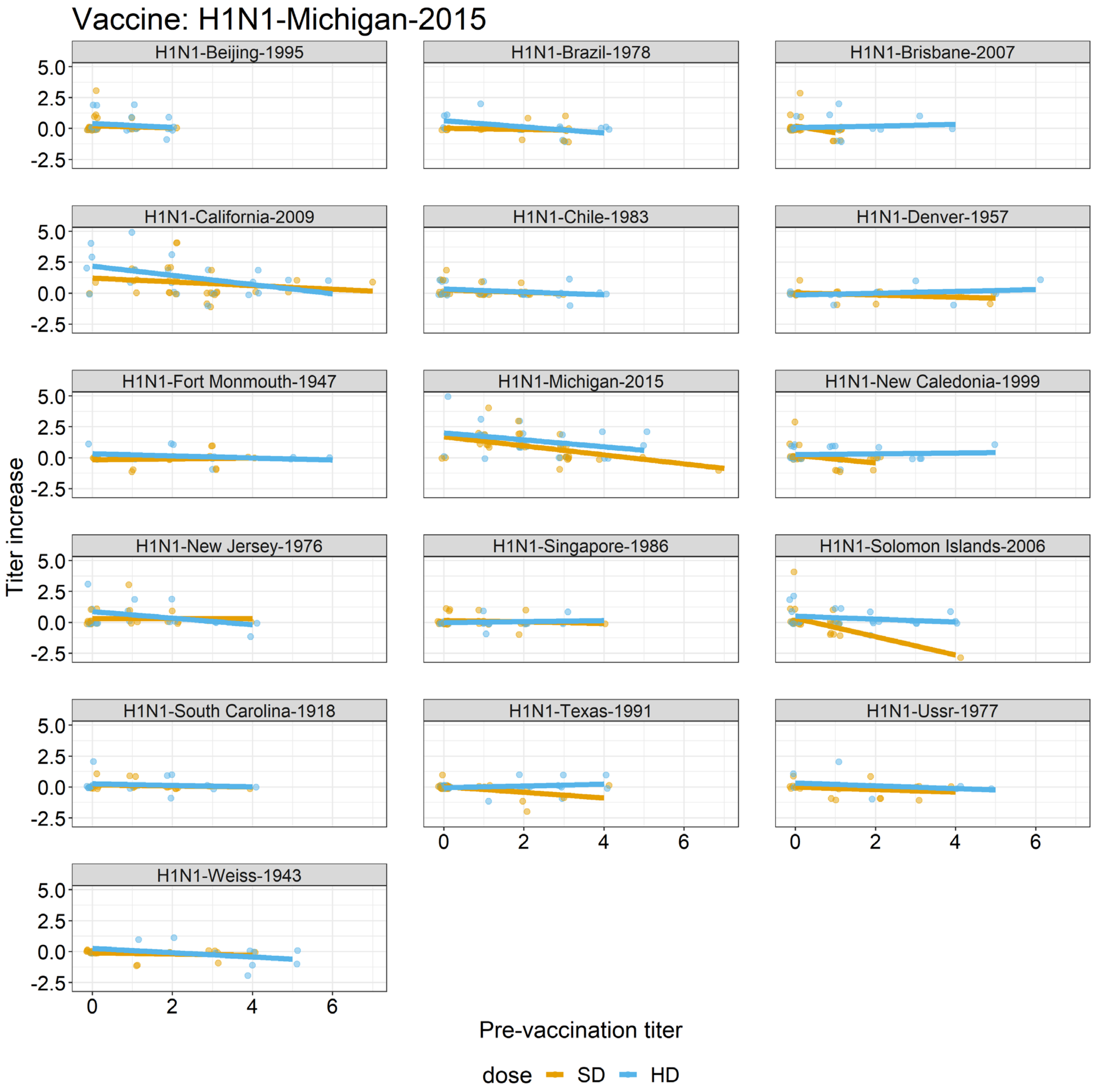
Fig from Zarnitsyna VI et al, PLoS Pathog, 2016.


A
HAI titer fold increase (log2)
Pre-vaccination titer
$R^2 = 0.27$
strain type B-Vic B-Yam H1N1 H3N2
B
HAI titer fold increase (log2)
Pre-vaccination titer
$R^2 = 0.27$
strain type B-Vic B-Yam H1N1 H3N2
C
HAI titer fold increase (log2)
Pre-vaccination titer
$R^2 = 0.24$
dose SD HD
D
HAI titer fold increase (log2)
Pre-vaccination titer
$R^2 = 0.25$
dose SD HD


Vaccine: H1N1-California-2009
Strain: H1N1-California-2009
Titer increase
Pre-vaccination titer
dose SD HD

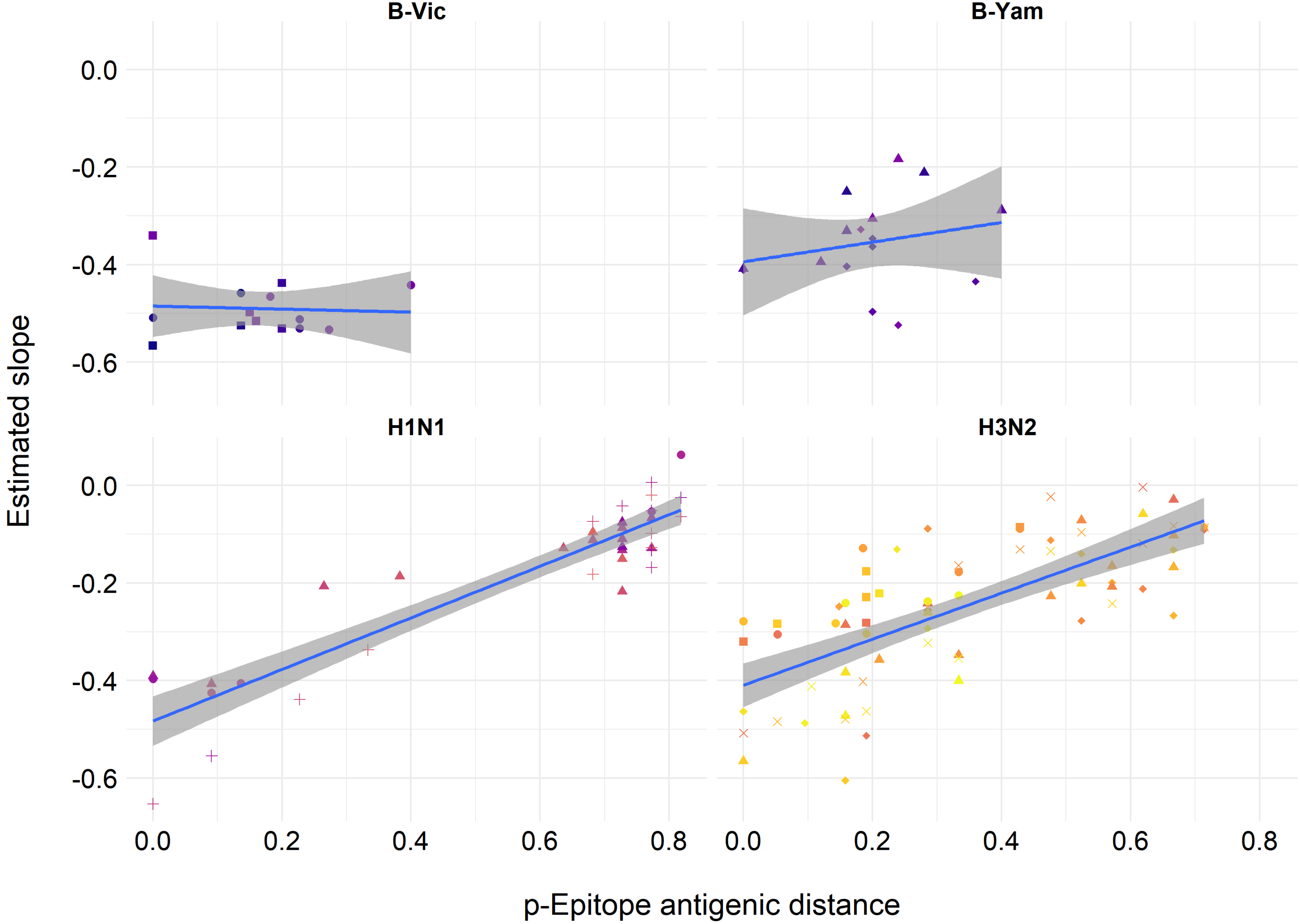

Vaccine: H1N1-California-2009
Strain: H1N1-Weiss-1943
Titer increase
Pre-vaccination titer
dose SD HD

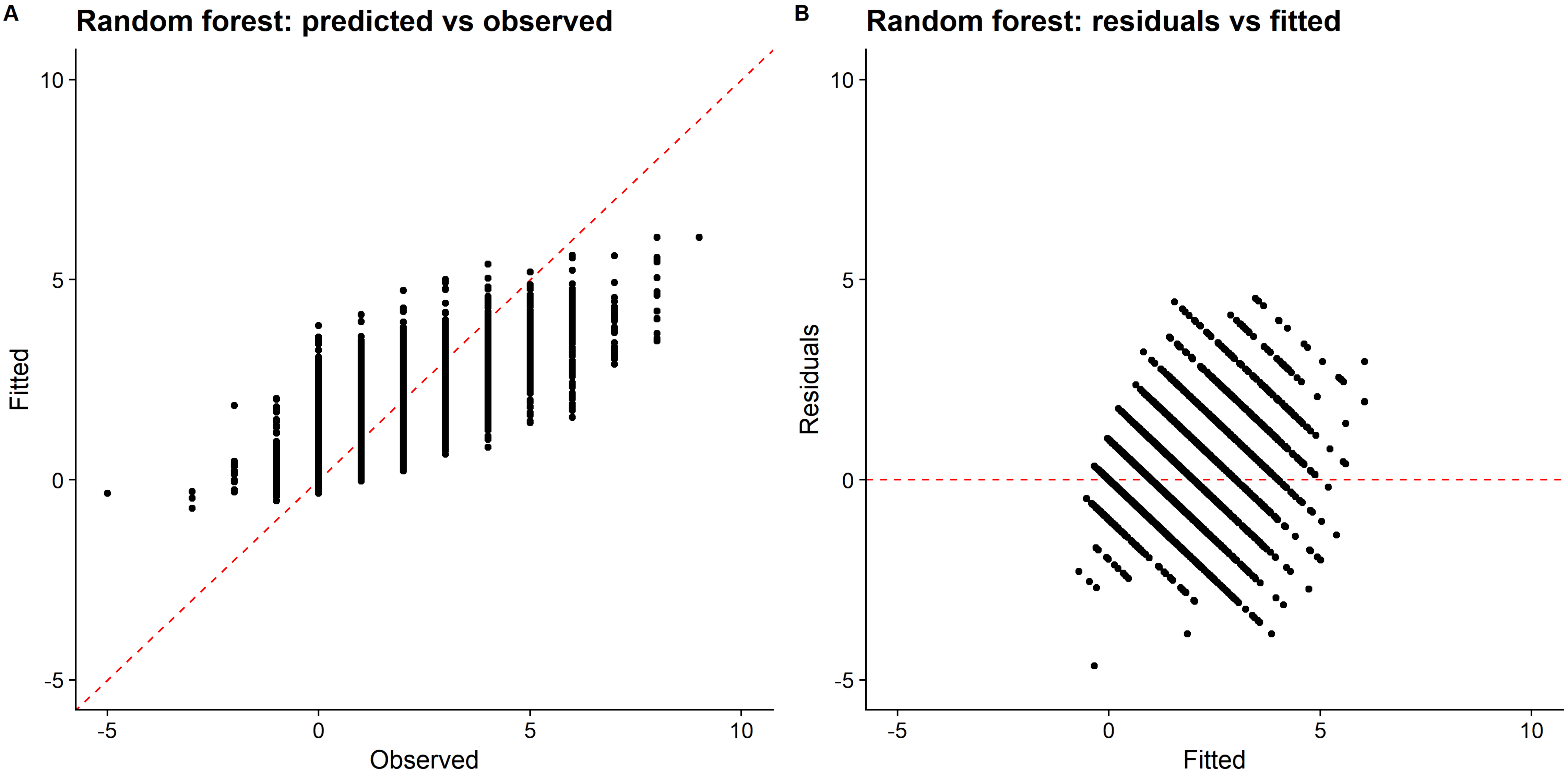

Vaccine: H1N1-Michigan-2015
H1N1-Beijing-1995
H1N1-Brazil-1978
H1N1-Brisbane-2007
H1N1-California-2009
H1N1-Chile-1983
H1N1-Denver-1957
H1N1-Fort Monmouth-1947
H1N1-Michigan-2015
H1N1-New Caledonia-1999
H1N1-New Jersey-1976
H1N1-Singapore-1986
H1N1-Solomon Islands-2006
H1N1-South Carolina-1918
H1N1-Texas-1991
H1N1-Ussr-1977
H1N1-Weiss-1943
Titer increase
Pre-vaccination titer

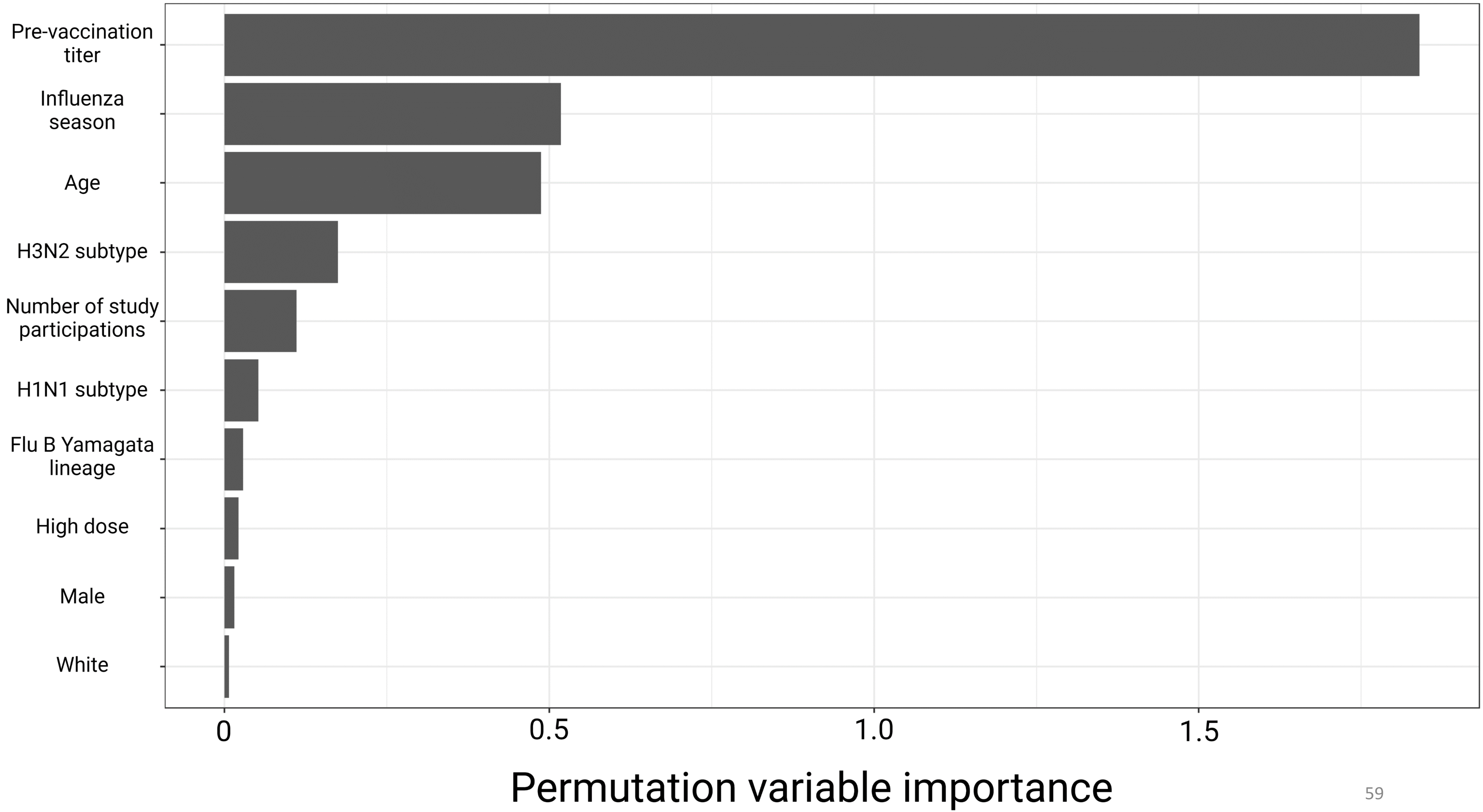

Vaccine: H1N1-Michigan-2015
H1N1-Beijing-1995
H1N1-Brazil-1978
H1N1-Brisbane-2007
H1N1-California-2009
H1N1-Chile-1983
H1N1-Denver-1957
H1N1-Fort Monmouth-1947
H1N1-Michigan-2015
H1N1-New Caledonia-1999
H1N1-New Jersey-1976
H1N1-Singapore-1986
H1N1-Solomon Islands-2006
H1N1-South Carolina-1918
H1N1-Texas-1991
H1N1-Ussr-1977
H1N1-Weiss-1943
Titer increase
Pre-vaccination titer
dose SD HD

All vaccine/assay strain combinations

Colors represent assay strains,shapes represent vaccine strains

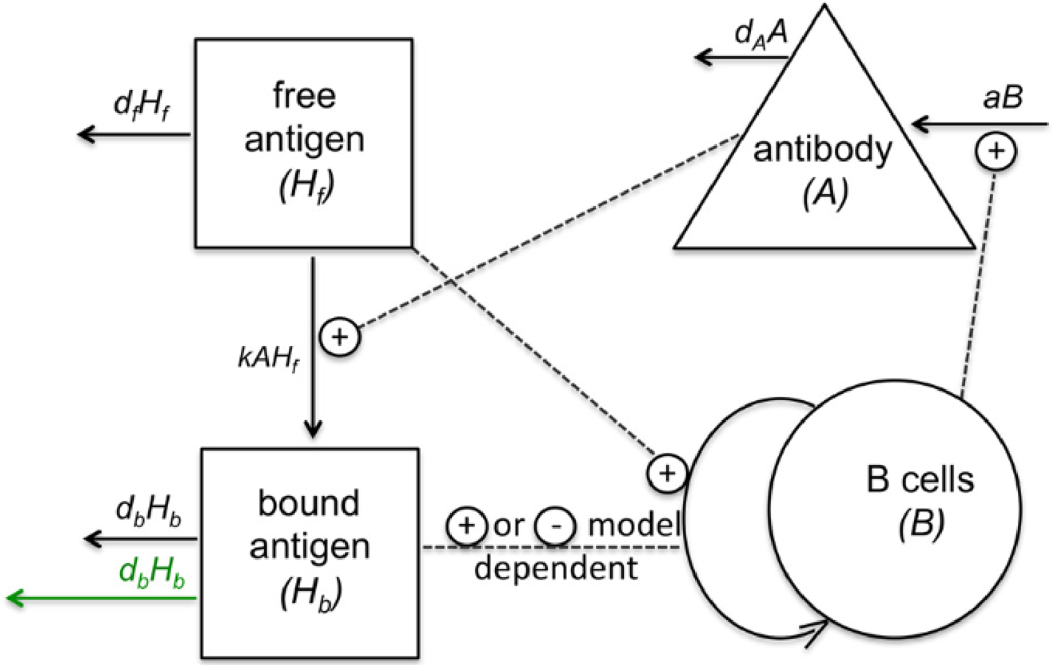



A
Random forest: predicted vs observed
Fitted
Observed
10
5
0
-5
-5
0
5
10
B
Random forest: residuals vs fitted
Residuals
Fitted
10
5
0
-5
-5
0
5
10


Pre-vaccination titer
Influenza season
Age
H3N2 subtype
Number of study participations
H1N1 subtype
Flu B Yamagata lineage
High dose
Male
White
0
0.5
1.0
1.5
Permutation variable importance

A. Models schematic and equations

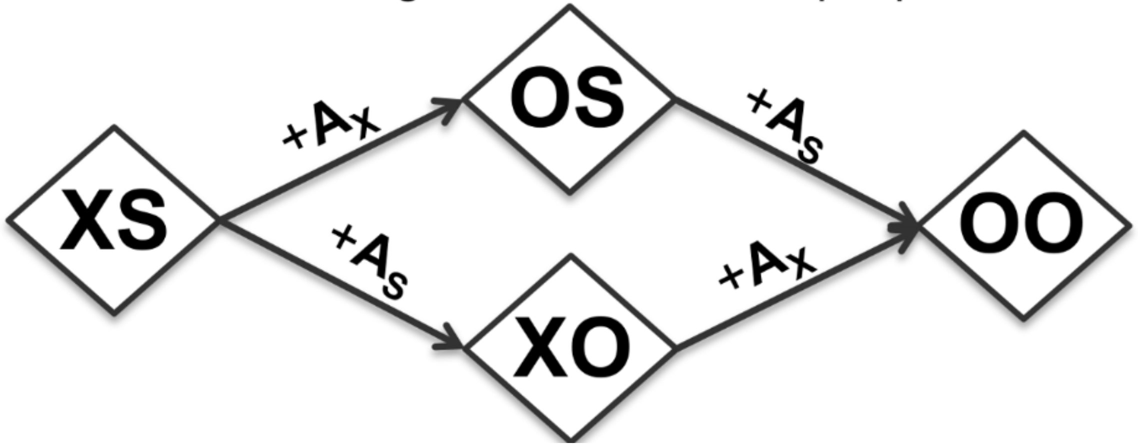
Basic model with antigen clearance (ACM)
or Fc-mediated inhibition (FIM) or epitope masking (EMM)



$$
\begin{aligned}
\text{(free antigen)} \quad \frac{dH_f}{dt} &= -kAH_f - d_f H_f \\
\text{(bound antigen)} \quad \frac{dH_b}{dt} &= kAH_f - d_b H_b \\
\text{(B cells)} \quad \frac{dB}{dt} &= sB\left(\frac{H_f + \delta H_b}{\phi + H_f + \delta H_b}\right)\left(\frac{1}{1+\alpha H_b}\right) \\
\text{(antibodies)} \quad \frac{dA}{dt} &= aB - kAH_f - d_A A
\end{aligned}
$$

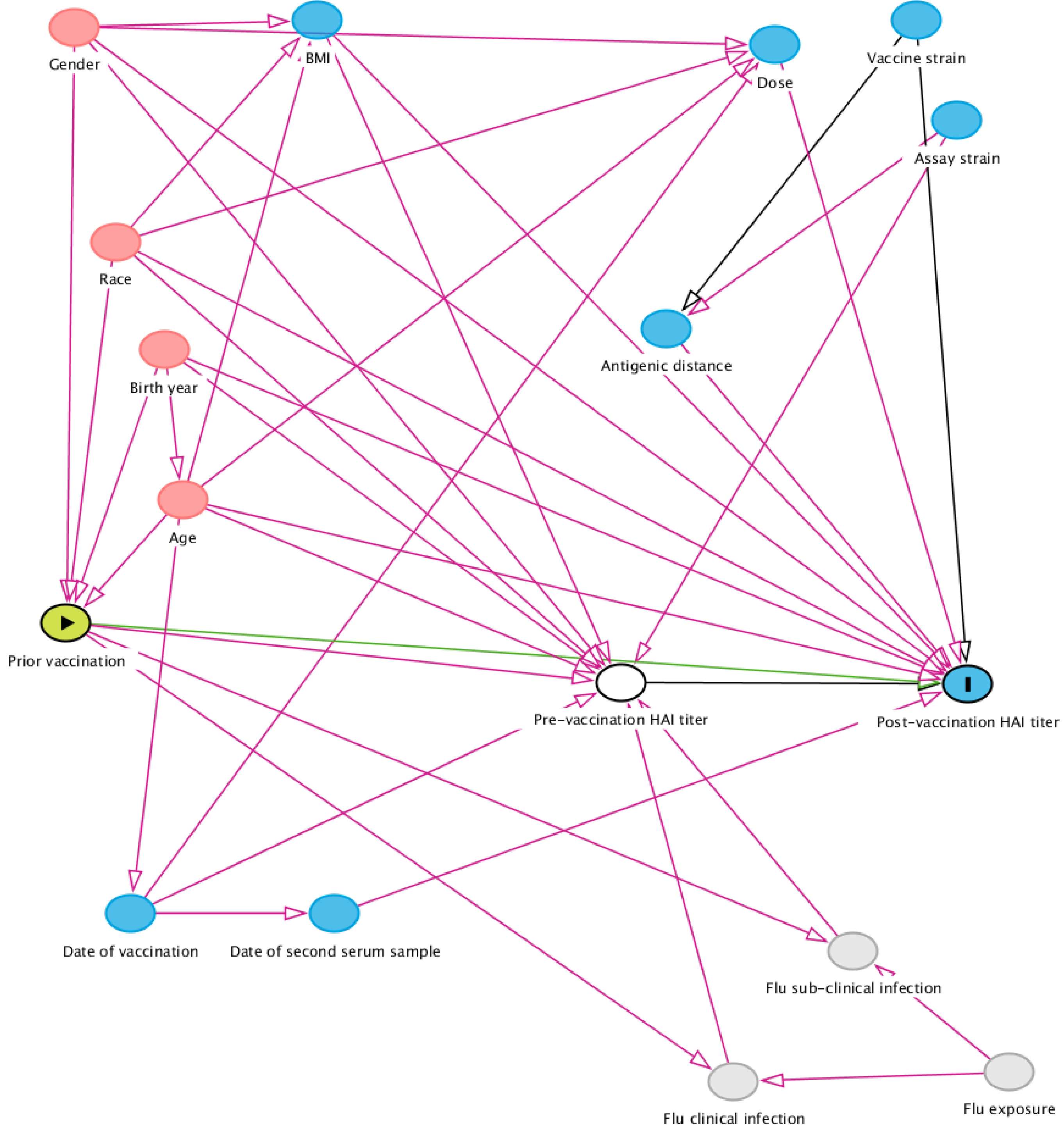
$\delta=1$, $\alpha=0$ (Basic, ACM); $\delta=1$, $\alpha>0$ (FIM); $\delta=0$, $\alpha=0$ (EMM)

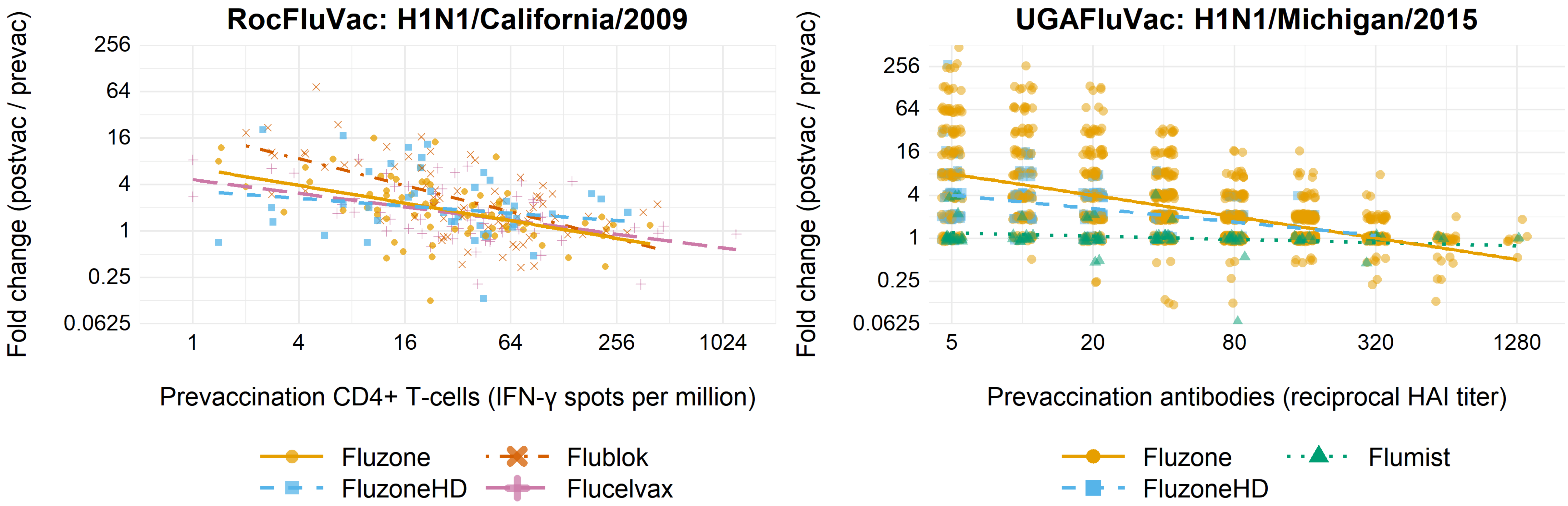
A. Four states of antigen HA in the two-epitope model



Our goal: can we do something here to add an amount of antigenic difference that controls the rate at which states occur, rather than parametrizing in terms of steric hindrance?

Zarnitsyna et al, PLOS Path 2016


Gender
BMI
Dose
Vaccine strain
Assay strain
Race
Antigenic distance
Birth year
Age
Prior vaccination
Pre-vaccination HAI titer
Post-vaccination HAI titer
Date of vaccination
Date of second serum sample
Flu sub-clinical infection
Flu clinical infection
Flu exposure



RocFluVac: H1N1/California/2009
Fold change (postvac / prevac)
256
64
16
4
1
0.25
0.0625
1
4
16
64
256
1024
Prevaccination CD4+ T-cells (IFN-γ spots per million)
Fluzone
FluzoneHD
Flublok
Flucelvax
UGAFluVac: H1N1/Michigan/2015
Fold change (postvac / prevac)
256
64
16
4
1
0.25
0.0625
5
20
80
320
1280
Prevaccination antibodies (reciprocal HAI titer)
Fluzone
FluzoneHD
Flumist