TOWARDS A UNIVERSAL INFLUENZA VIRUS VACCINE

Peter Palese

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ISIRV - Options IX for the Control of Influenza

Peter Palese, PhD
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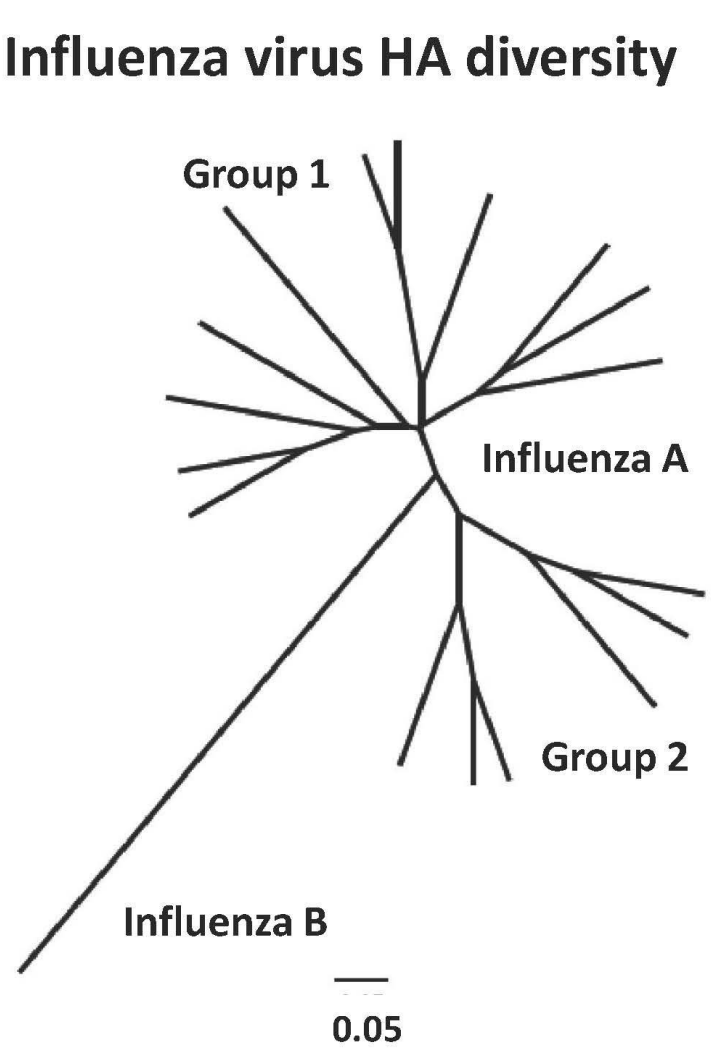
Department of Microbiology
Icahn School of Medicine,
New York

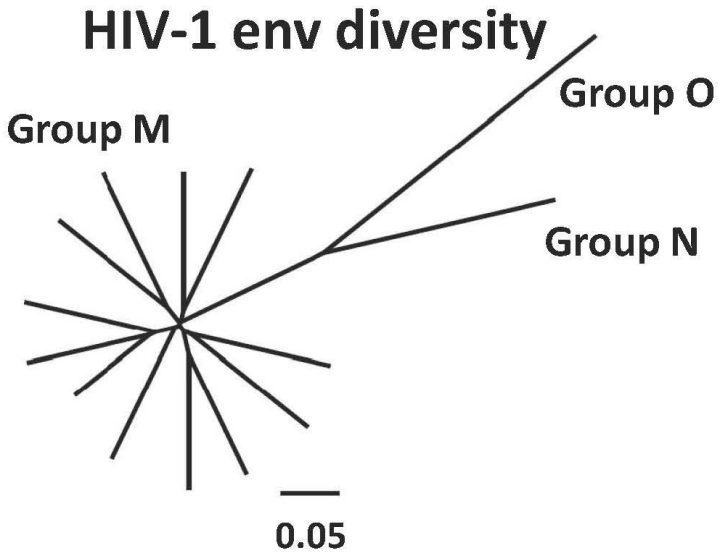
Mount Sinai has submitted patent applications for a universal influenza virus vaccine

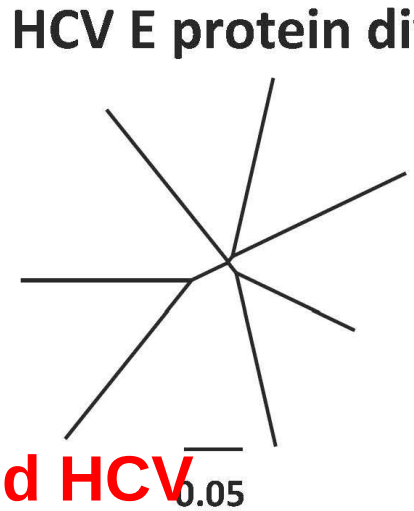
Work has been supported by the NIH, The Bill & Melinda Gates Foundation, GSK

My presentation does not include discussion of off-label or investigational use.

Surface glycoprotein diversity of different viruses



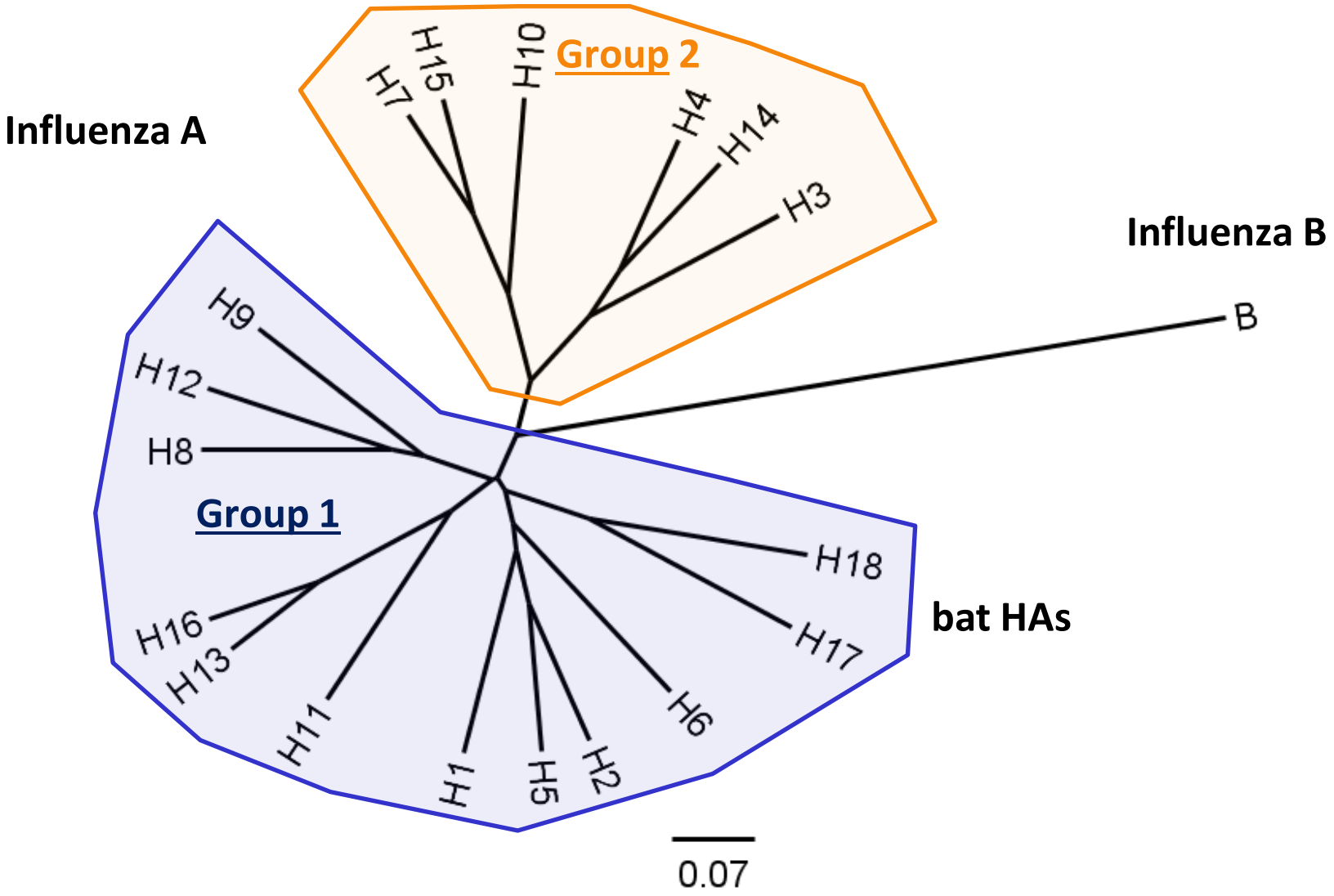




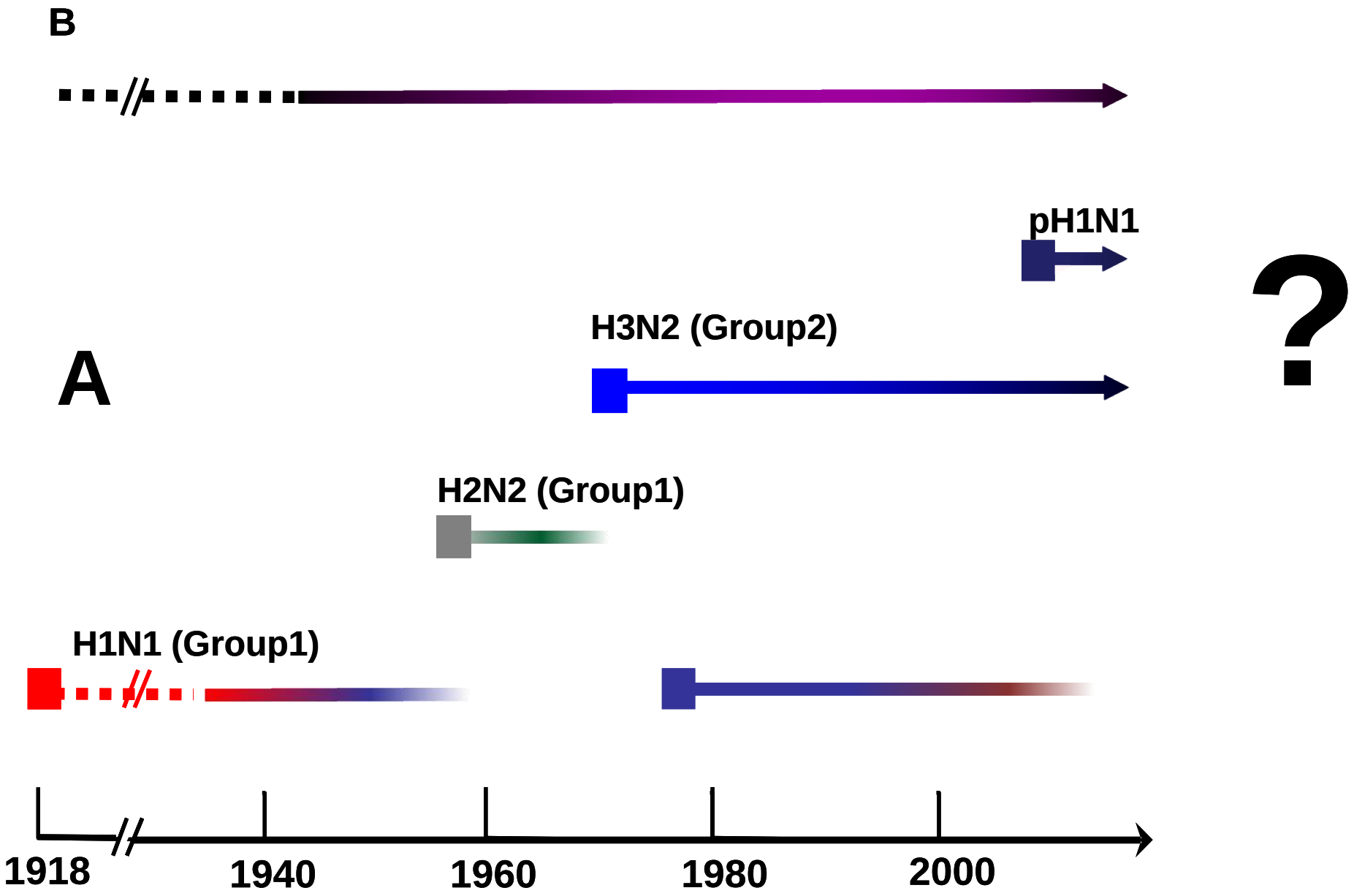
Similar variation for influenza, HIV and HCV

F. Krammer

EIGHTEEN SUBTYPES OF INFLUENZA A VIRUS HEMAGGLUTININS



Influenza viruses circulating in the human population



AVIAN INFLUENZA VIRUSES INFECTING HUMANS

H5N6	China	2016
H7N9	China	2015, 2014, 2013
H10N8	China	2013
H6N1	Taiwan	2013
H10N7	Australia,Egypt	2010,2004
H7N3	Mexico,UK,Canada,Italy	2012,2006,04,03
H7N2	UK,USA	2007,2003
H9N2	Hong Kong	1999
H5N1	Asia,Europe,Africa, Hong Kong	2015-2003 , 1997
H7N7	Netherlands,UK,USA,Austr.,USA	2003,96,80,77,59

INFLUENZA VIRUS VACCINES

INACTIVATED
LIFE ATTENUATED
RECOMBINANT

INFLUENZA VIRUS VACCINE STRAINS 2016-2017

A/California/7/2009 (H1N1)pdm09
A/Hong Kong/4801/2014 (H3N2)

B/Phuket/3073/2013
B/Brisbane/60/2008

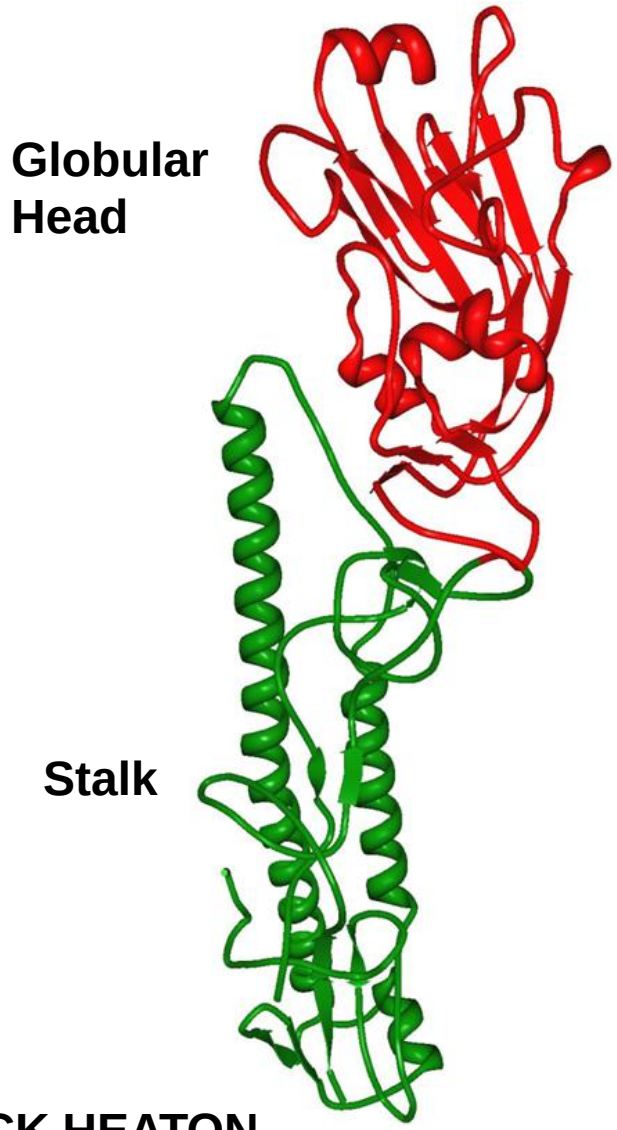
- **INFLUENZA VIRUS VACCINES ARE UNIQUE.**
- **THEY HAVE TO BE GIVEN ANNUALLY, BECAUSE NOVEL VACCINE FORMULATIONS HAVE TO BE PREPARED REFLECTING THE RAPID ANTIGENIC CHANGE OF THE VIRUS.**

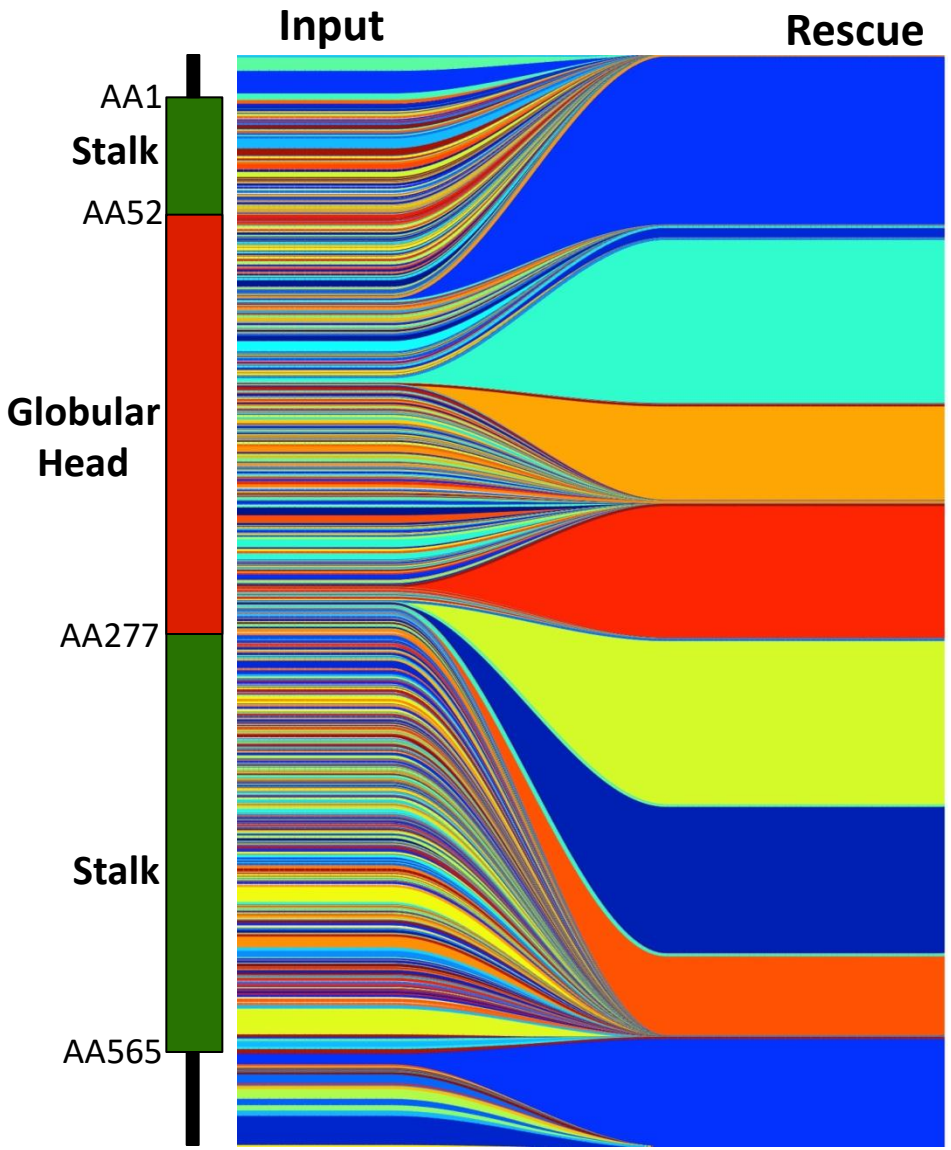
Antigenic diversity: analysis of the flexible influenza A virus and rigid measles virus glycoproteins

Nicholas Heaton, PhD
Ben Fulton

Palese Lab
Icahn School of Medicine at Mount Sinai

INSERTION MUTATIONS ARE TOLERATED IN THE HEAD OF THE HEMAGGLUTININ

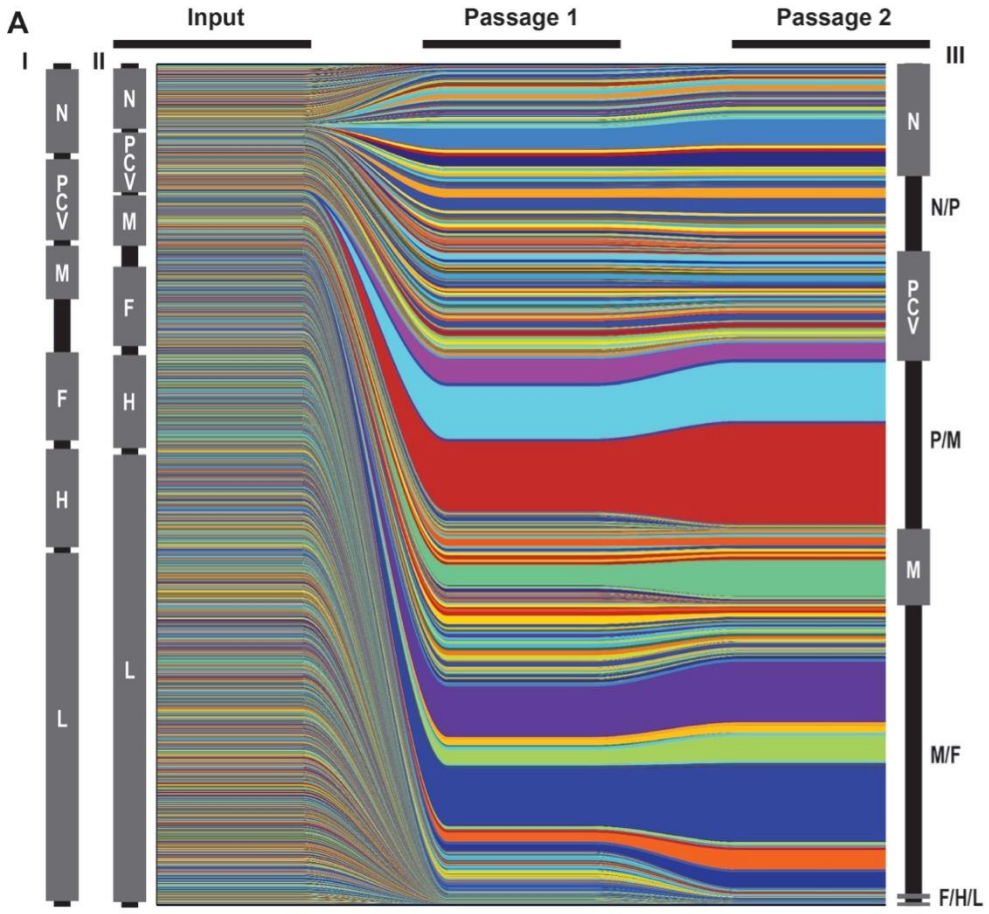




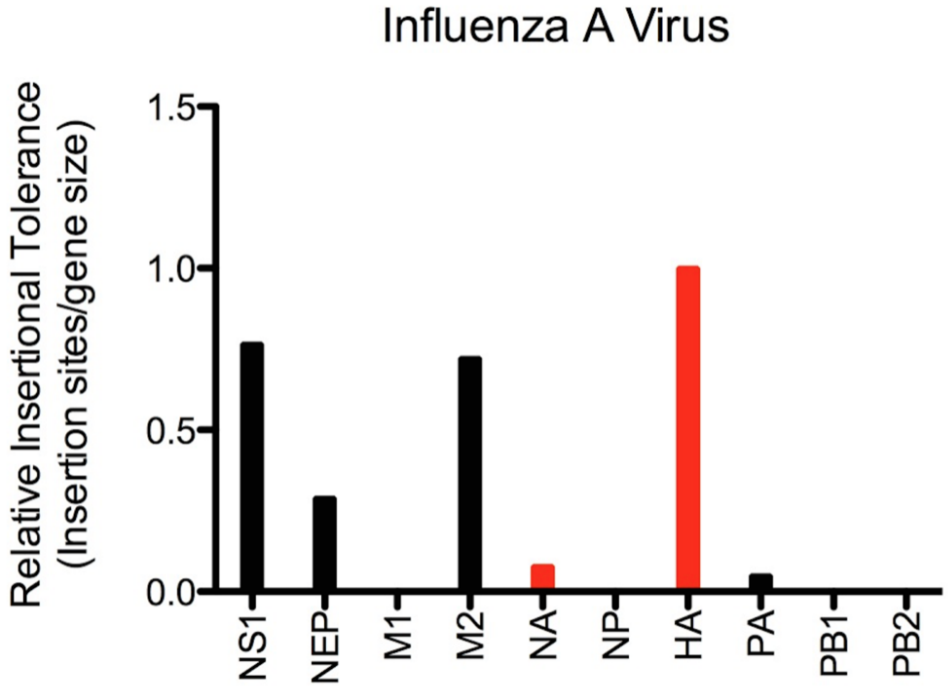
NICK HEATON

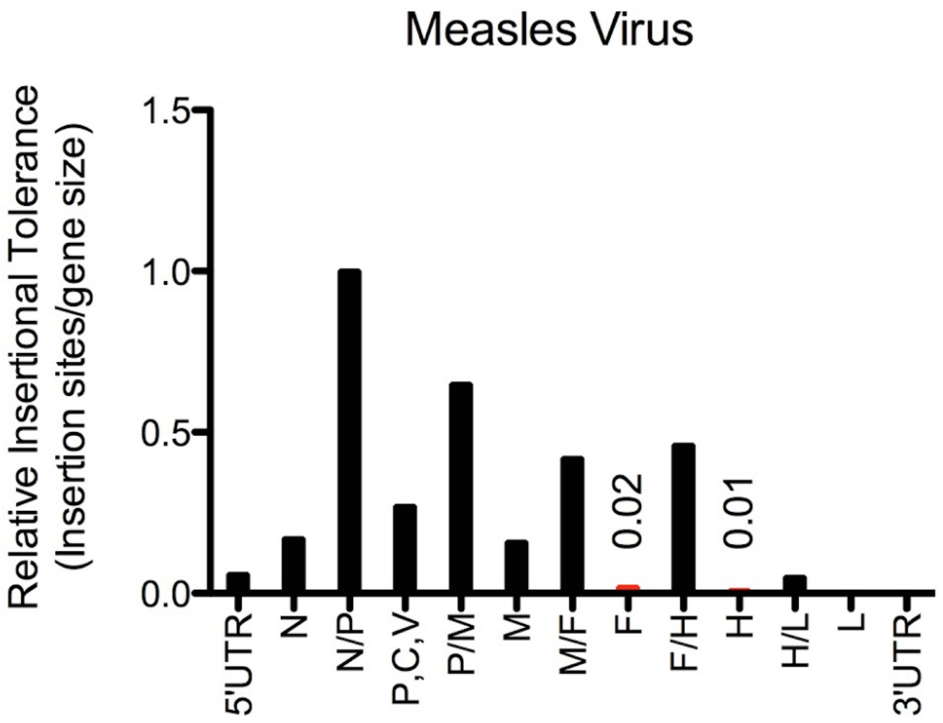
Rescue in Eggs

The measles virus glycoproteins (and the polymerase) are resistant to insertions



TOLERANCE OF THE INFLUENZA A VIRUS AND OF MEASLES VIRUS GENOMES

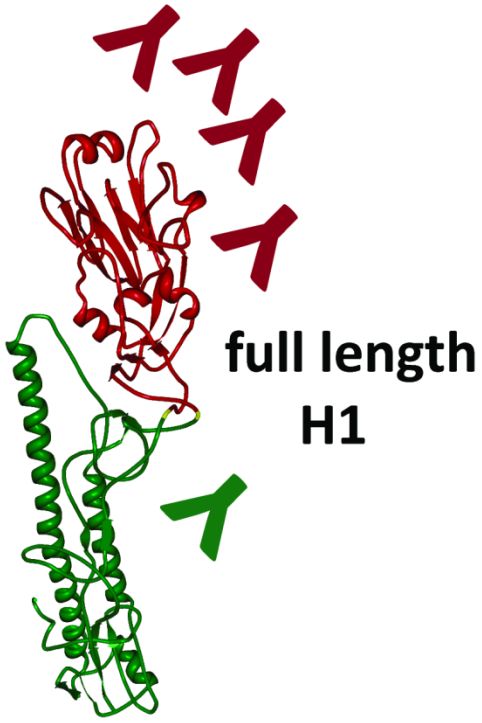




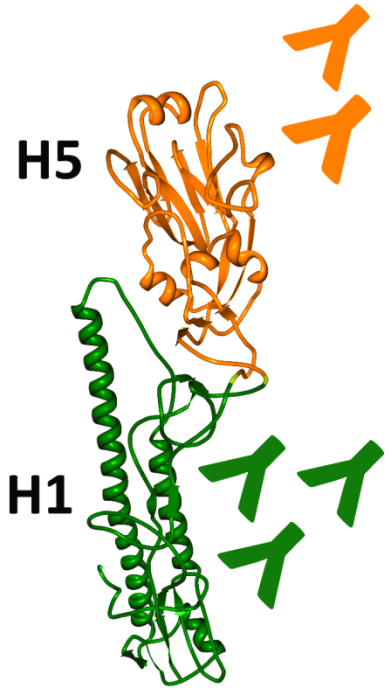
HOW CAN WE DO BETTER?

UNIVERSAL INFLUENZA VIRUS VACCINES

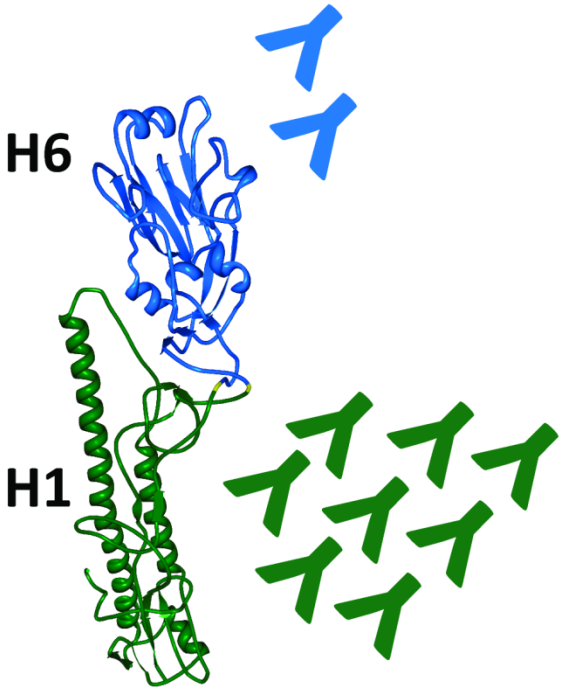
Vision for a human universal influenza virus vaccine



pre-existing immunity
against e.g. H1



boost with cH5/1 construct



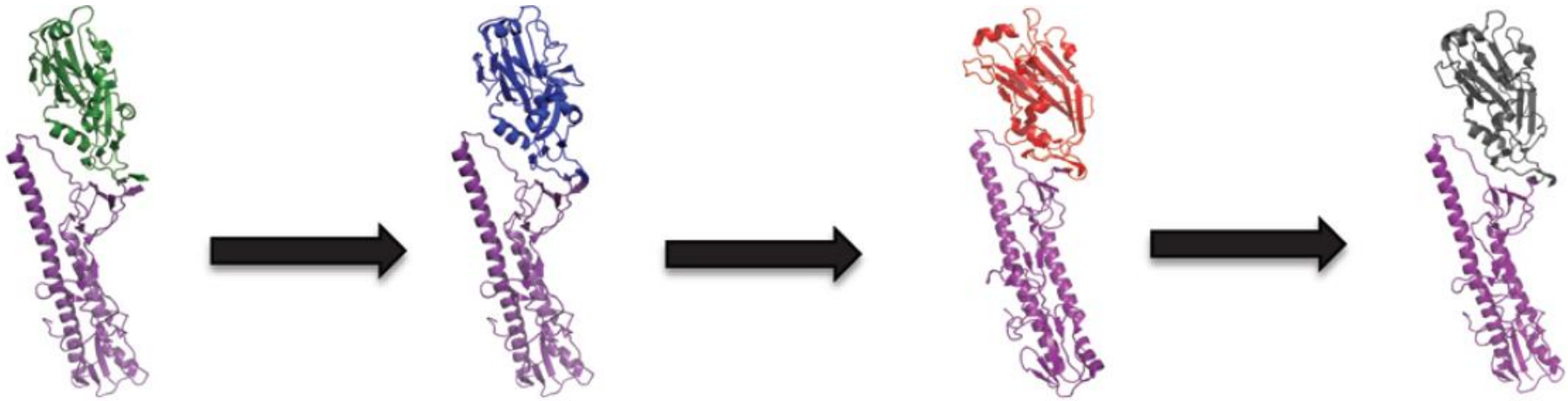
boost with cH6/1 construct

FLORIAN KRAMMER ADOLFO GARCÍA-SASTRE PETER PALESE

APPROACHES

- **ADJUVANTS**
- **MVA-VECTORED**
- **M2e-BASED**
- **EPITOPES/PEPTIDES**
- **NEURAMINIDASE**
- **COBRA (computationally optimized broadly reactive antigens)**
- **STALK ONLY, HEADLESS HEMAGGLUTININ**
- **CHIMERIC HEMAGGLUTININ**

Induction of protective levels of stalk-reactive antibodies using chimeric HA constructs in mice



cH9/1 DNA

PRIME

cH6/1 protein

BOOST

cH5/1 protein

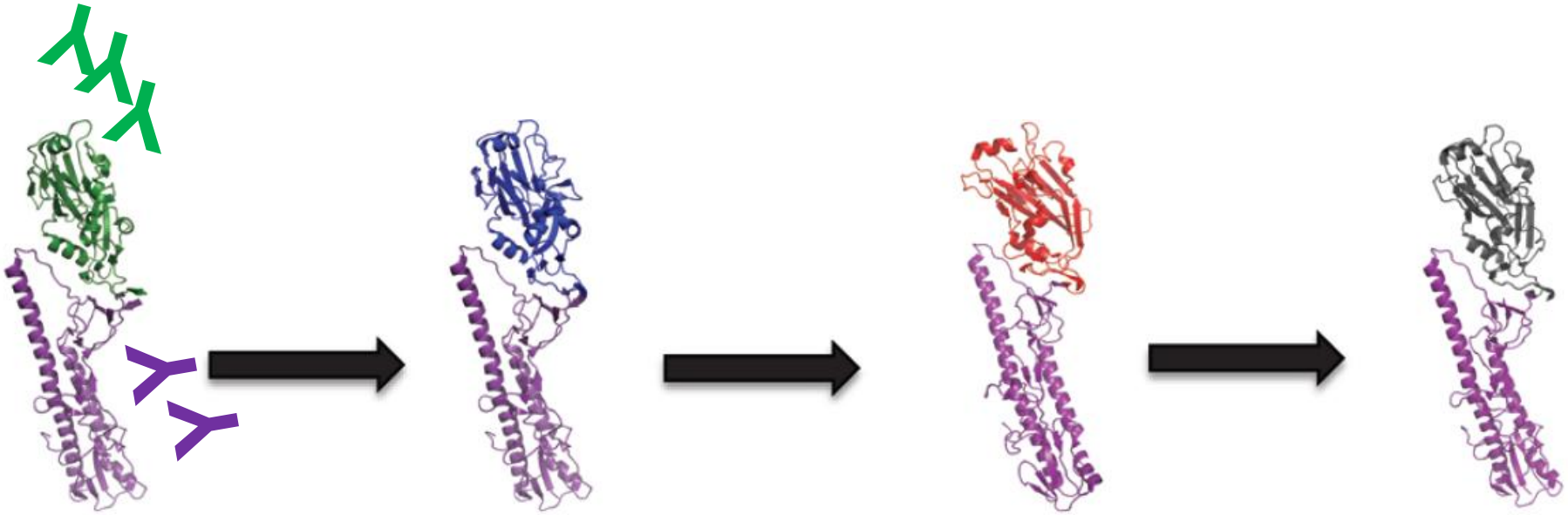
BOOST

PR8 H1N1(1934)
FM1 H1N1(1947)
pH1N1(2009)
H5N1
H6N1

CHALLENGE

Control groups:
cH9/1 DNA + BSA + BSA
matched vaccine (pos. contr.)

Induction of protective levels of stalk-reactive antibodies using chimeric HA constructs in mice



cH9/1 DNA

cH6/1 protein

cH5/1 protein

PR8 H1N1
FM1 H1N1
pH1N1
H5N1
H6N1
challenge

Control groups:
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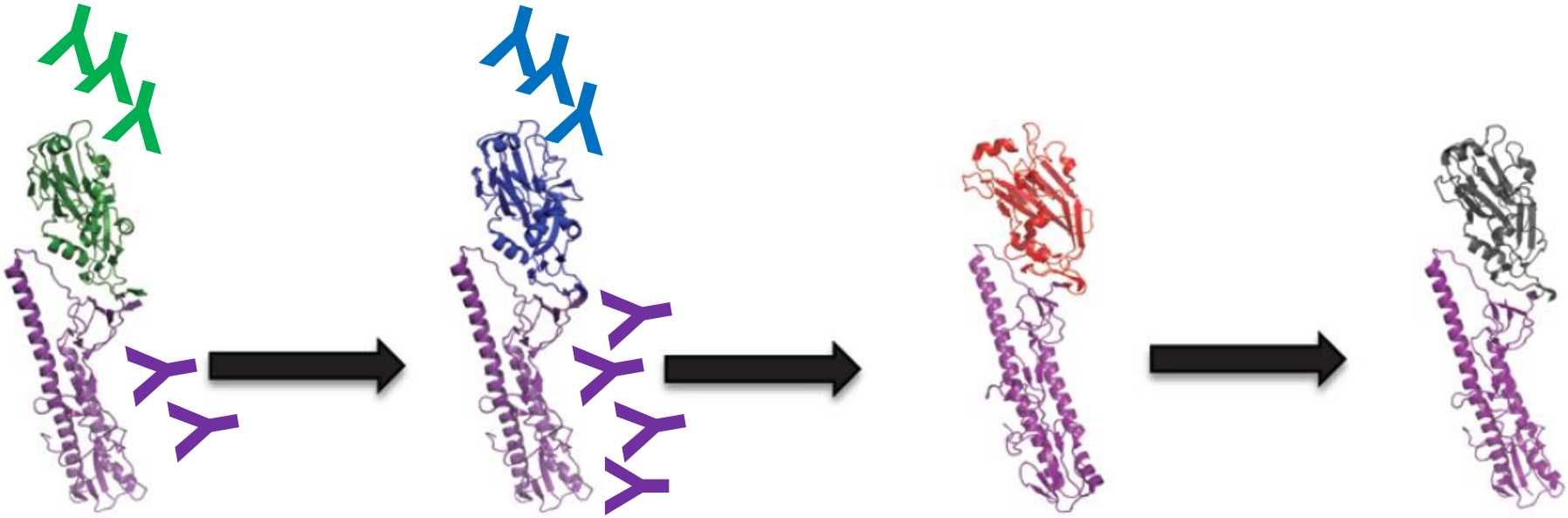
matched vaccine (pos.

Induction of protective levels of stalk-reactive antibodies using chimeric HA constructs in mice



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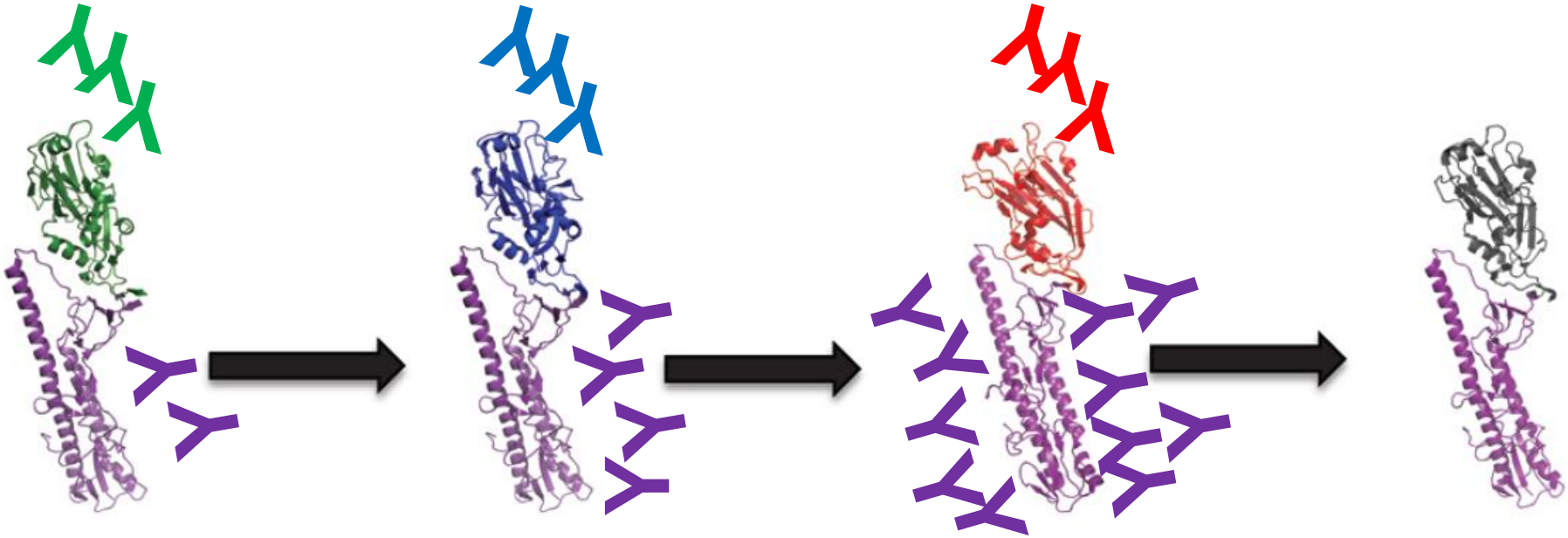

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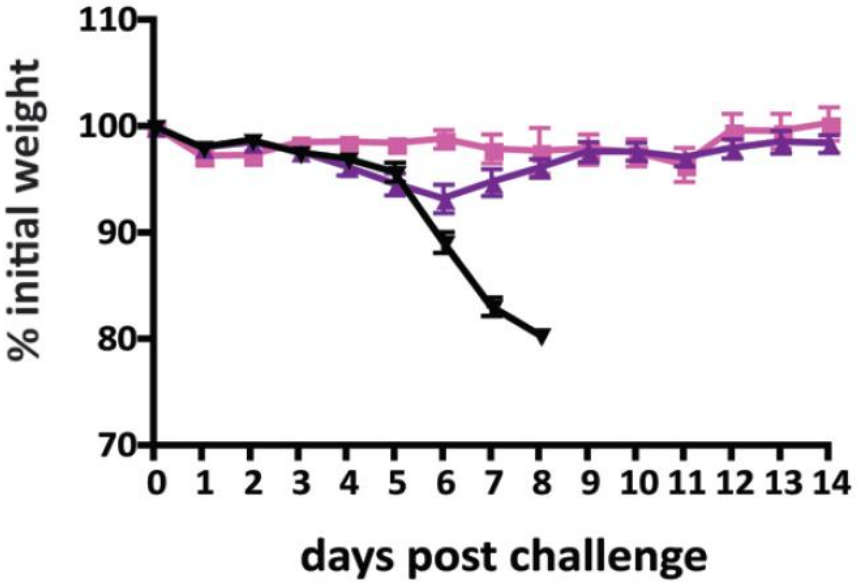
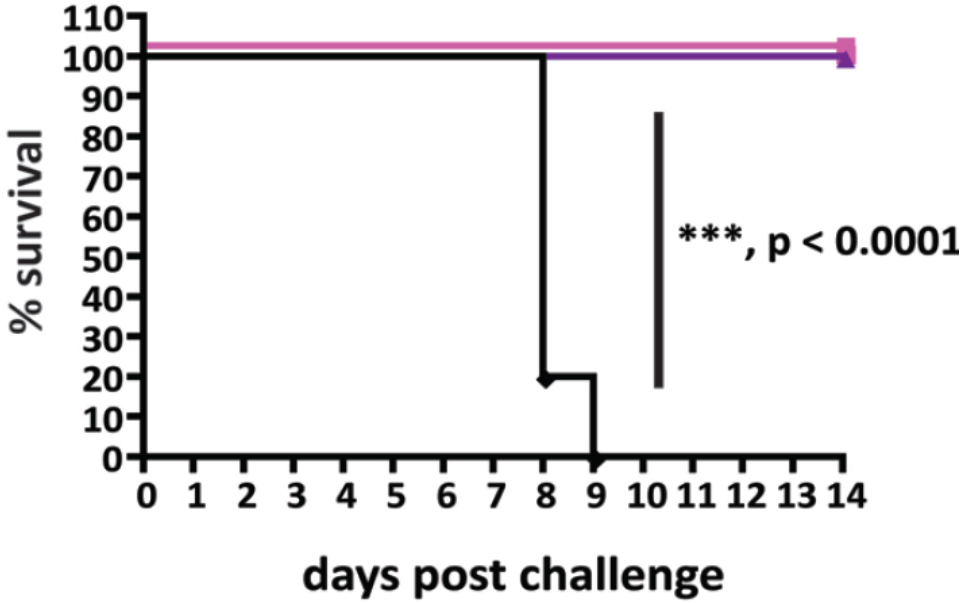
pH1N1(2009)
H5N1
H6N1

CHALLENGE

Control groups:
cH9/1 DNA + BSA + BSA
matched vaccine (pos. contr.)

Vaccination with cHA constructs protects from pH1N1 (A/Netherlands/602/09) challenge



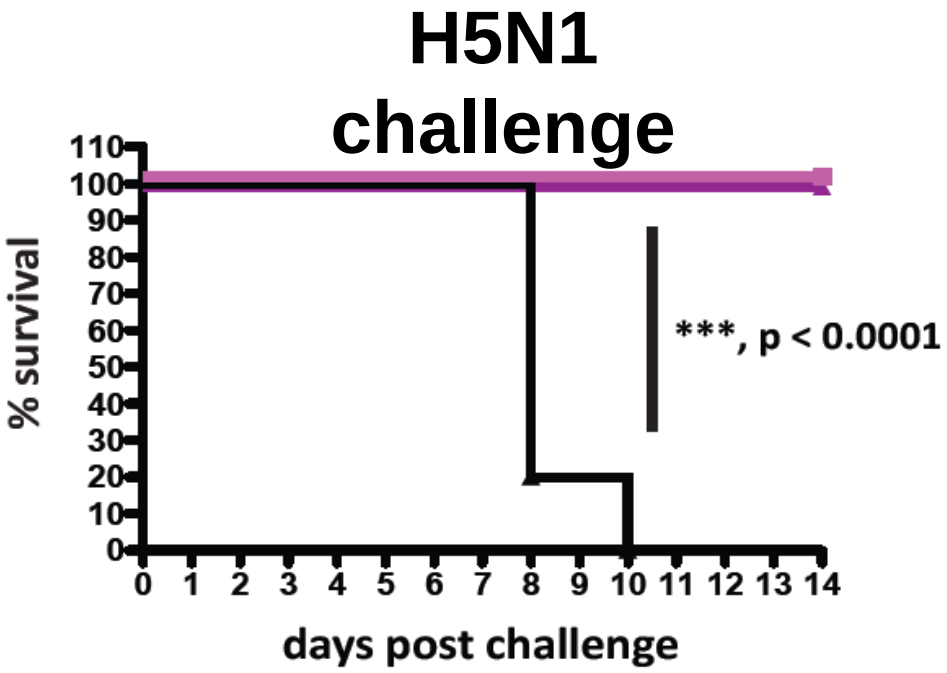


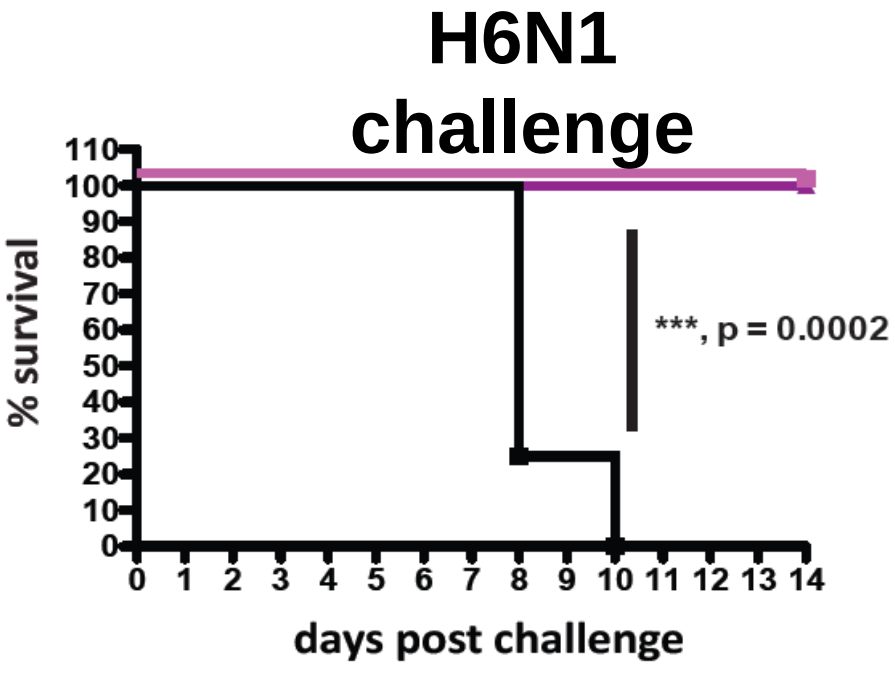
- positive control (matched inactivated)
- cH9/1 DNA + cH6/1 protein + cH5/1 protein
- cH9/1 DNA + BSA +BSA

Similar results for A/PR/8/34 H1N1 and A/FM/1/47 challenges

Krammer et al. JVI, 87, 6542,2013

cHA constructs protect mice from heterosubtypic challenge





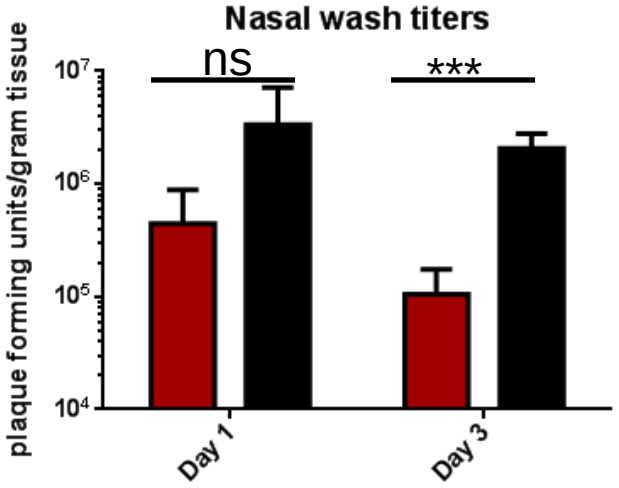
positive control (matched inactivated)

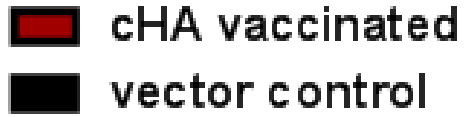
cH9/1 DNA + H1 protein/cH6/1 protein + cH5/1 protein/H1 protein

cH9/1 DNA + BSA +BSA

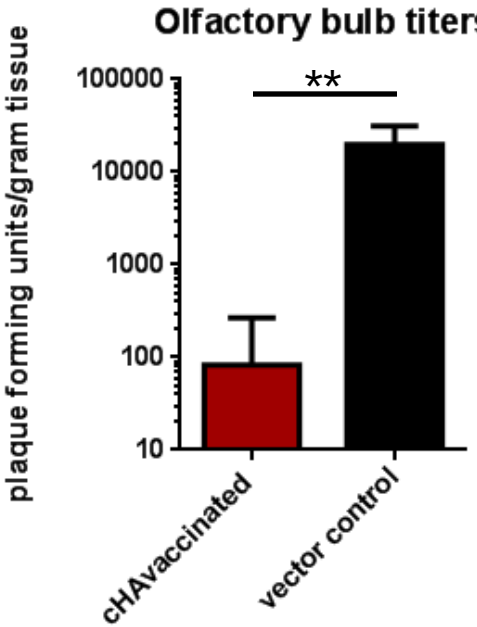
cH5/1 (H5 challenge) or cH6/1 (H6 challenge) protein was replaced by full length H1 protein to exclude head-based protection

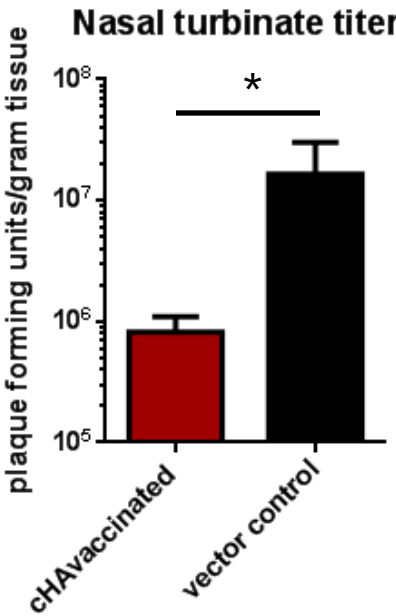
cHA constructs protect ferrets from pH1N1 challenge

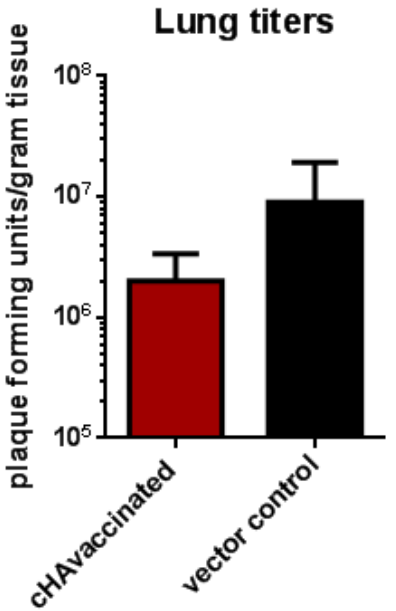




Krammer et al., JVI Jan. 8, 2014

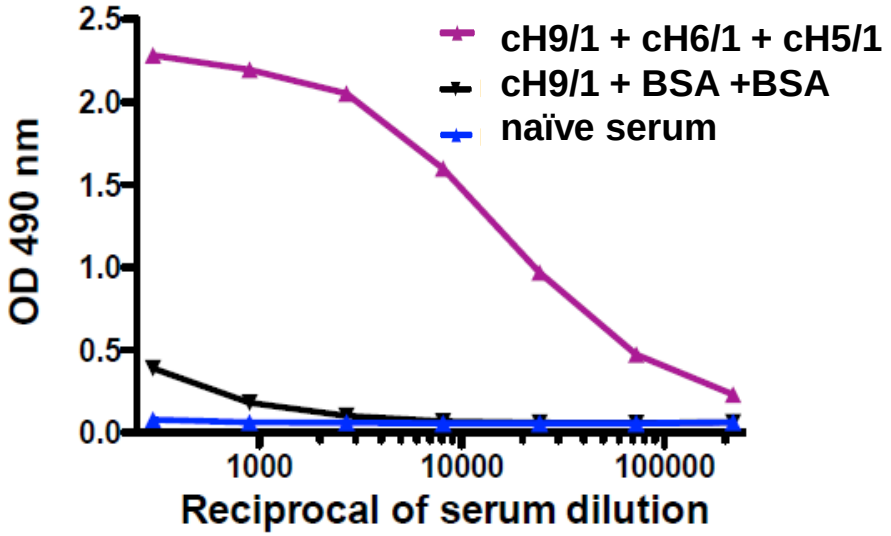




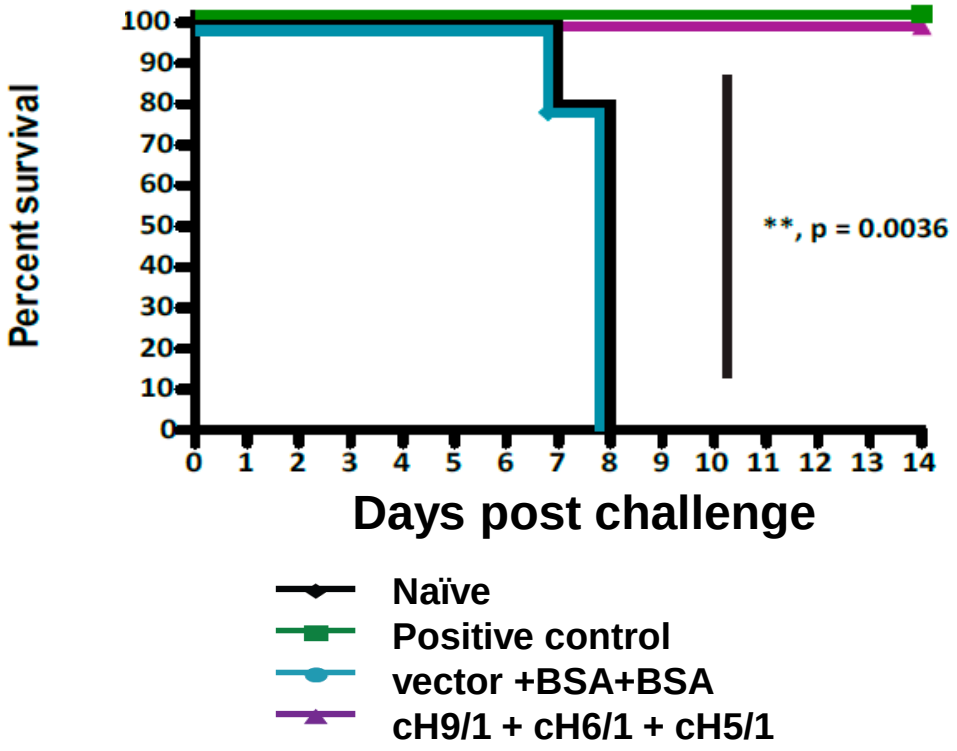


Protection is antibody mediated

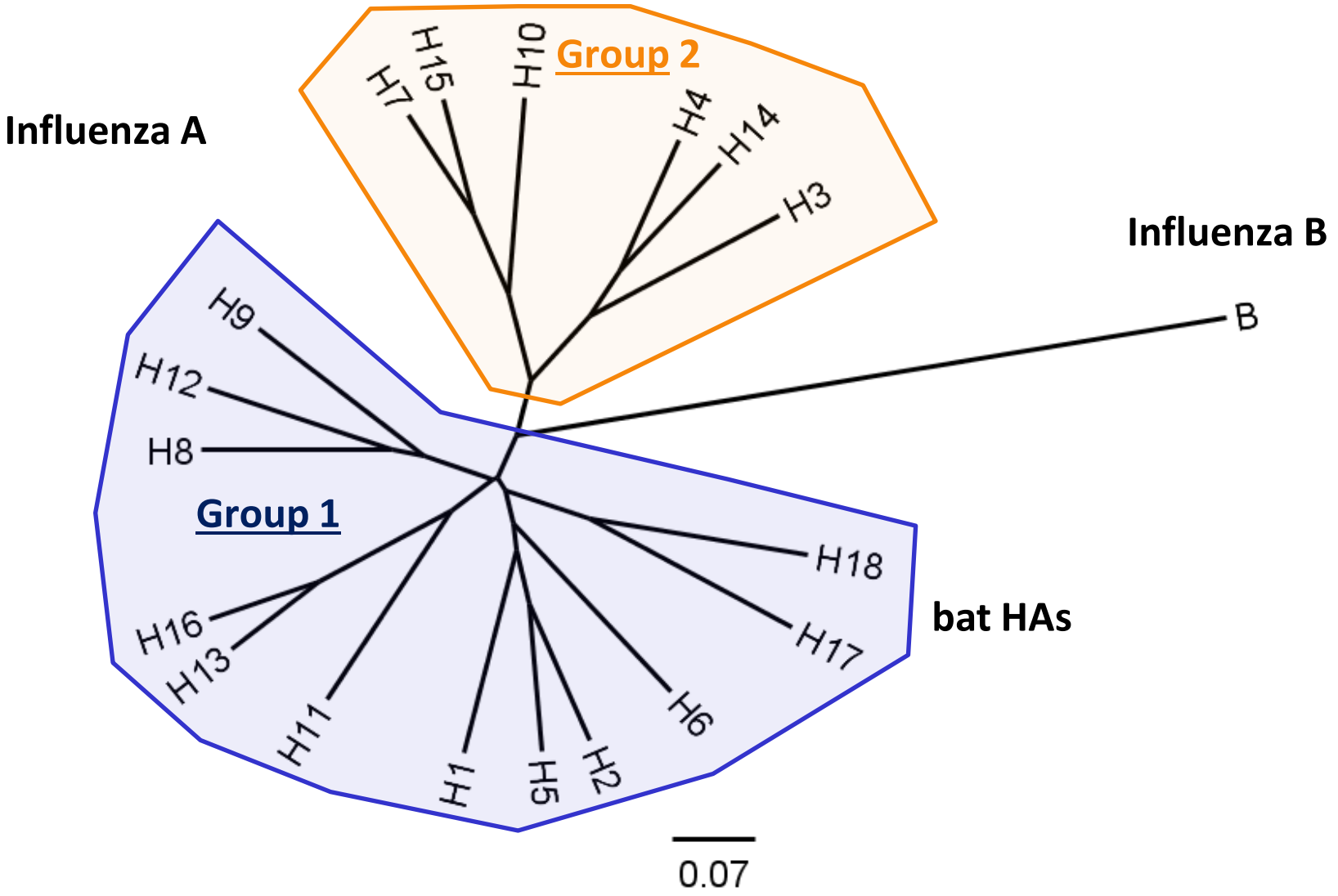
ELISA reactivity to Cal09 (pH1N1) protein



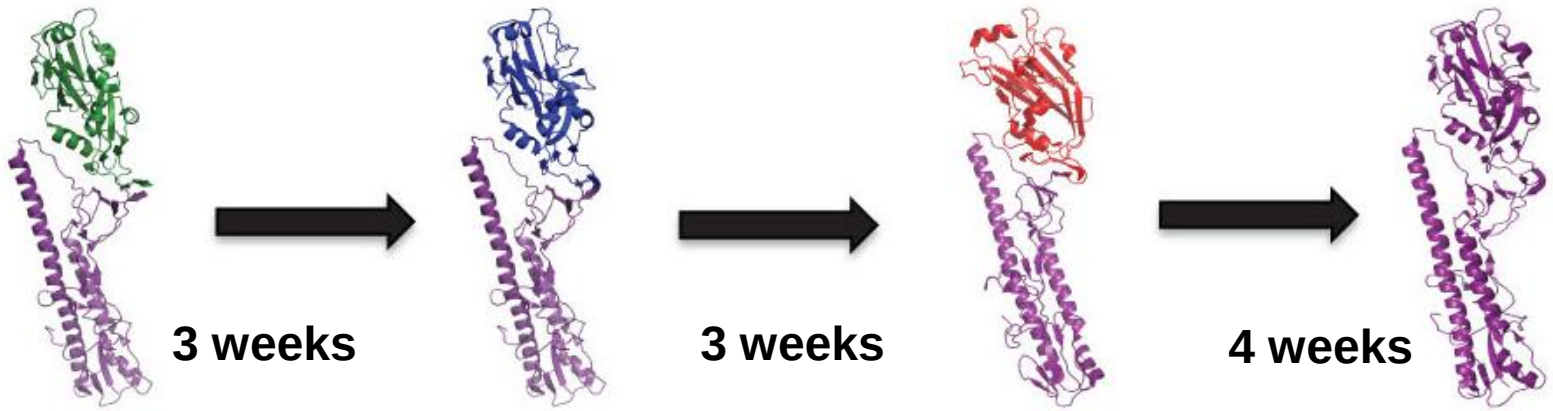
Passive transfer of serum protects from viral challenge



Targeting group 2 HA viruses



Protection against group 2 HA expressing viruses in the mouse model



cH4/3 DNA

PRIME

cH5/3 protein

BOOST

cH7/3 protein

BOOST

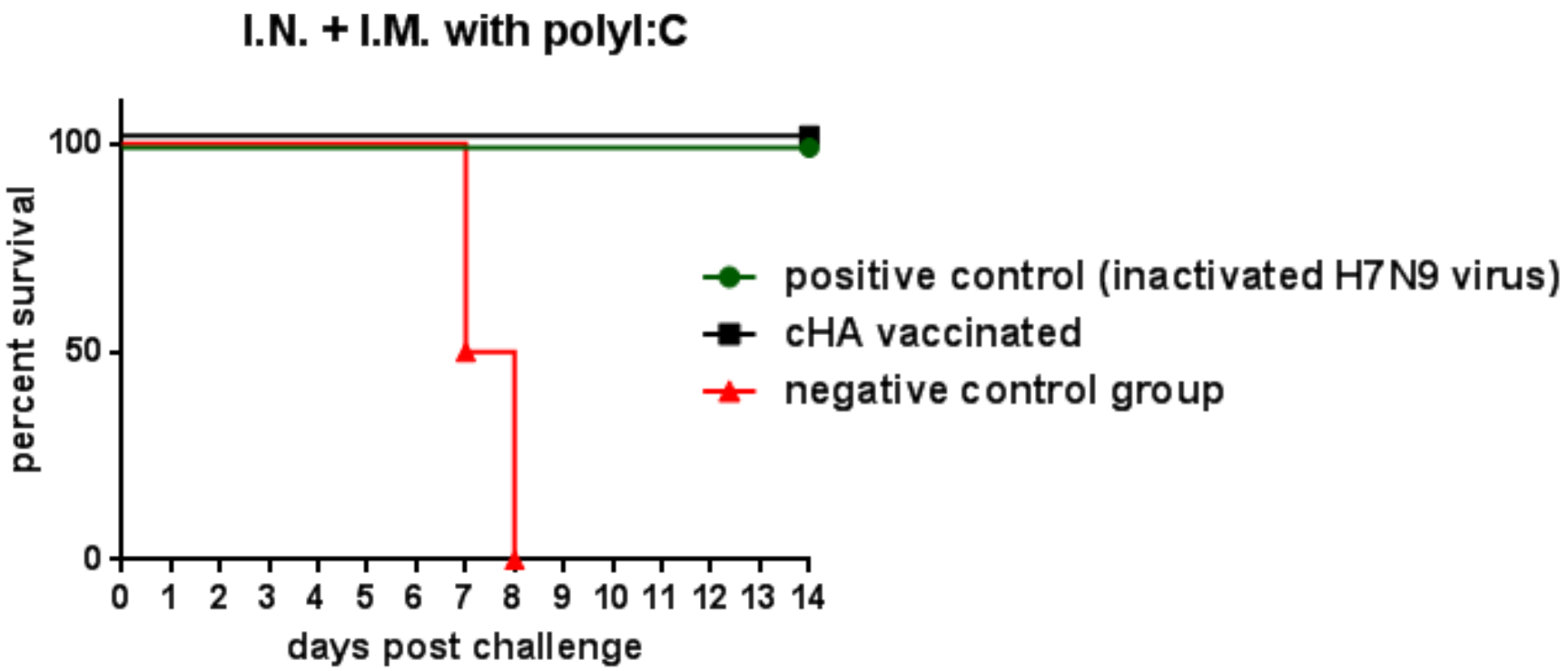
Phil/82 (H3N2)
X/31 (H3N2) 1968
Rhea (H7N1)

CHALLENGE

<u>Control groups:</u>
cH4/3 DNA + BSA + BSA
naïve (neg. contr.)
matched vaccine (pos. contr.)

Margine et al., JVI, 87,10435, 2013

Group 2 cHA vaccine protects against challenge with novel H7N9*virus



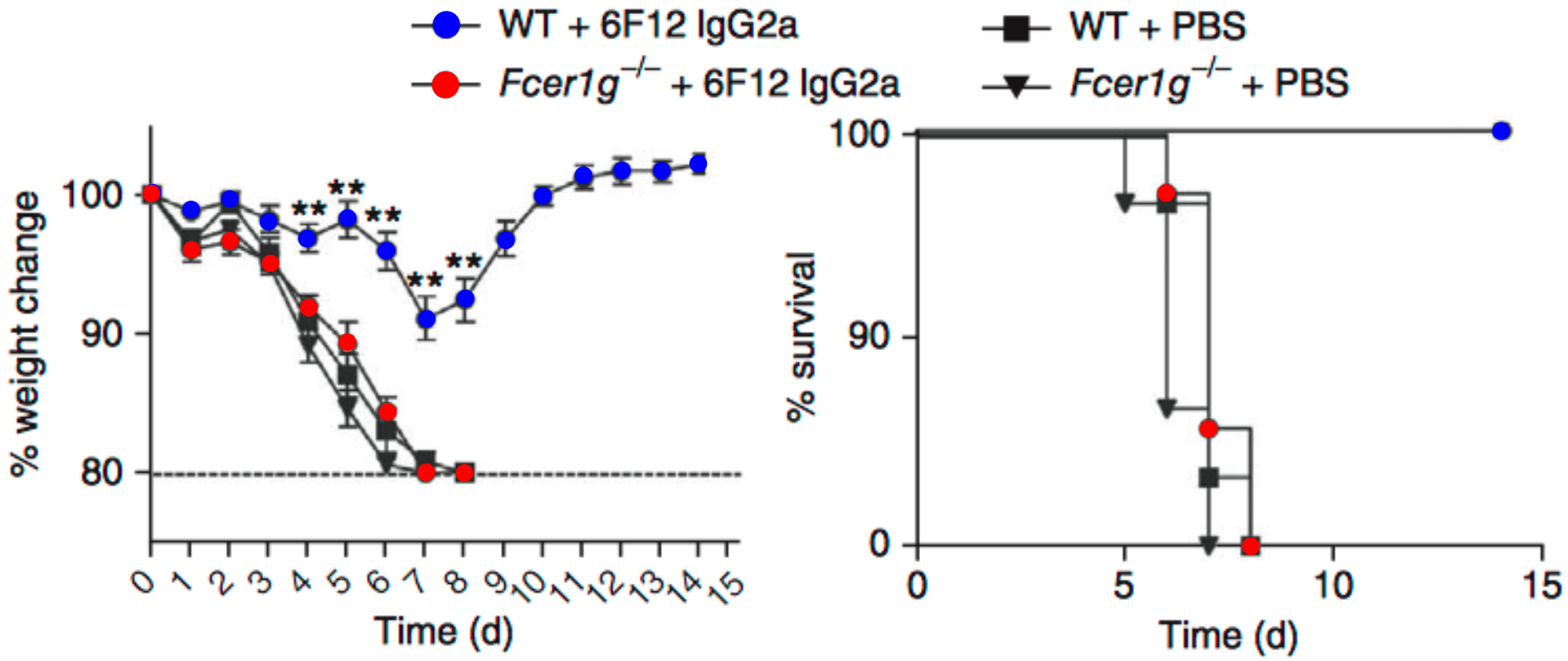
*cH7/3 protein was replaced by full length H3 protein for the H7N1 challenge group

Krammer et al. JVI,88, 2340, 2014

WHAT IS THE MECHANISM BY WHICH THESE BROADLY PROTECTIVE STALK-SPECIFIC ANTIBODIES MEDIATE THEIR ANTIVIRAL ACTIVITY?

nature medicine

Broadly neutralizing hemagglutinin stalk–specific antibodies require FcγR interactions for protection against influenza virus *in vivo*



DiLillo *et al. Nature Medicine* 2014

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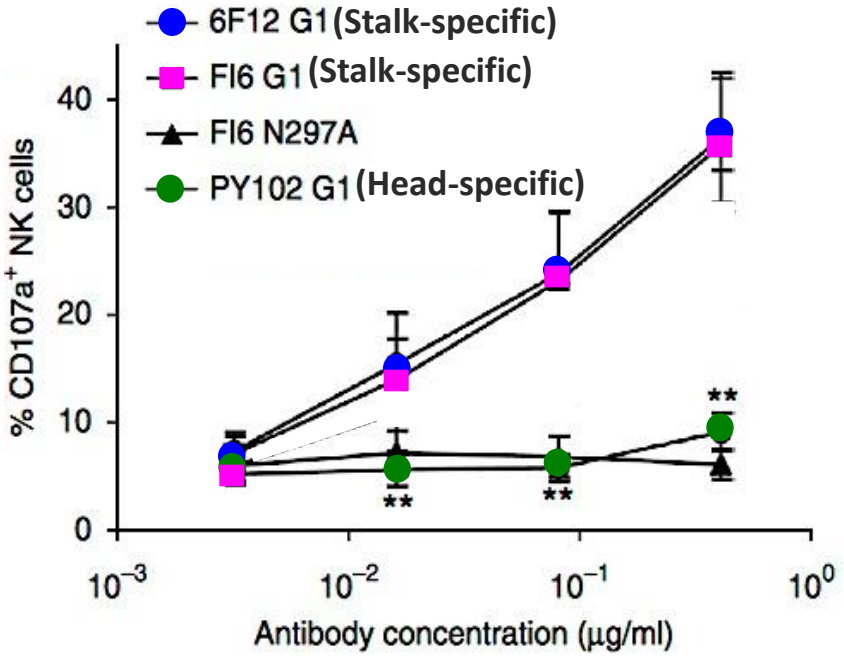
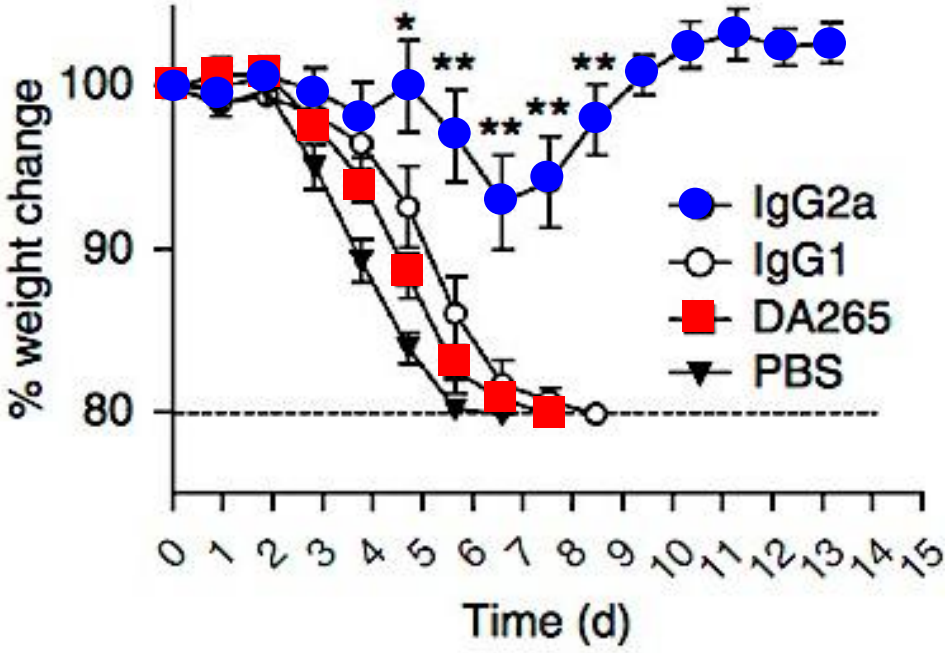
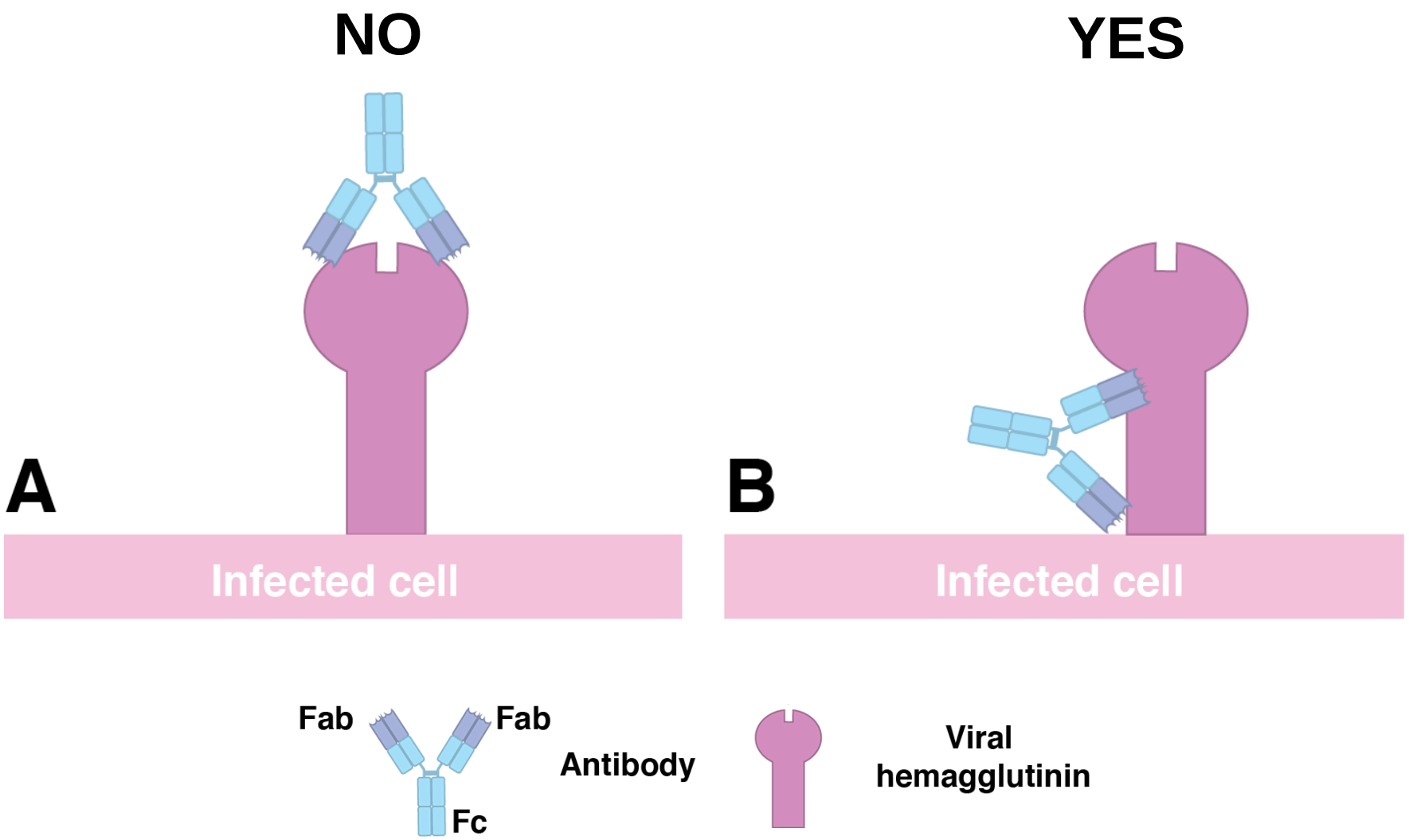




Antibody-dependent Cell-mediated Cytotoxicity (ADCC) can be induced by stalk-specific, but not head-specific antibodies.

DiLillo *et al. Nature Medicine* 2014

Antibody-Dependent Cell-Mediated Cytotoxicity (ADCC)

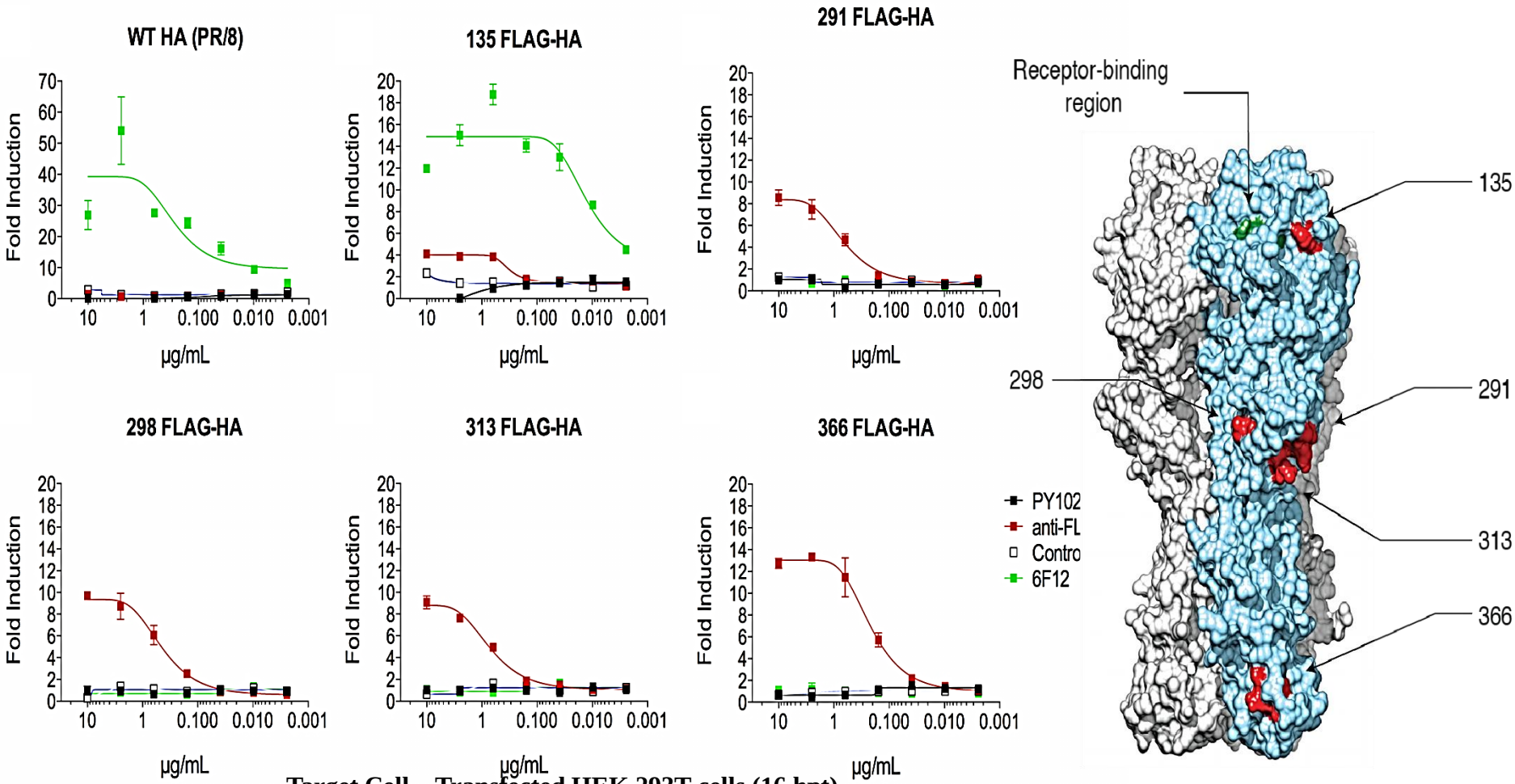


Can we elucidate the role epitope location plays in the induction of ADCC by broadly cross-reactive hemagglutinin antibodies?

Yes, by introducing FLAG epitopes into different locations in the viral hemagglutinin

Paul Leon, Wenqian He, Caitlin Mullarkey, Mark Bailey, Matt Miller, Florian Krammer, Gene Tan

A stalk-based FLAG epitope can induce FcγR-mediated effector function



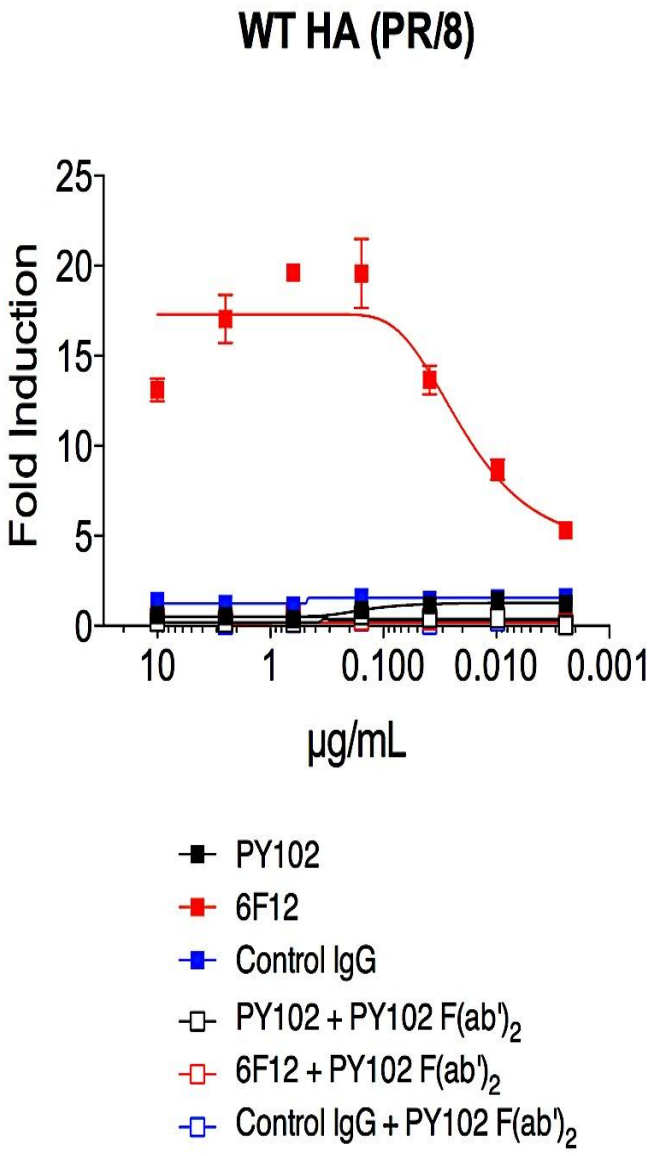
Target Cell – Transfected HEK 293T cells (16 hpt)
Effector Cells – Jurkat cells expressing murine FcγRIV
Fold Induction = $(RLU_{Induced}-RLU_{Background})/(RLU_{No\ mAb}-RLU_{Background})$

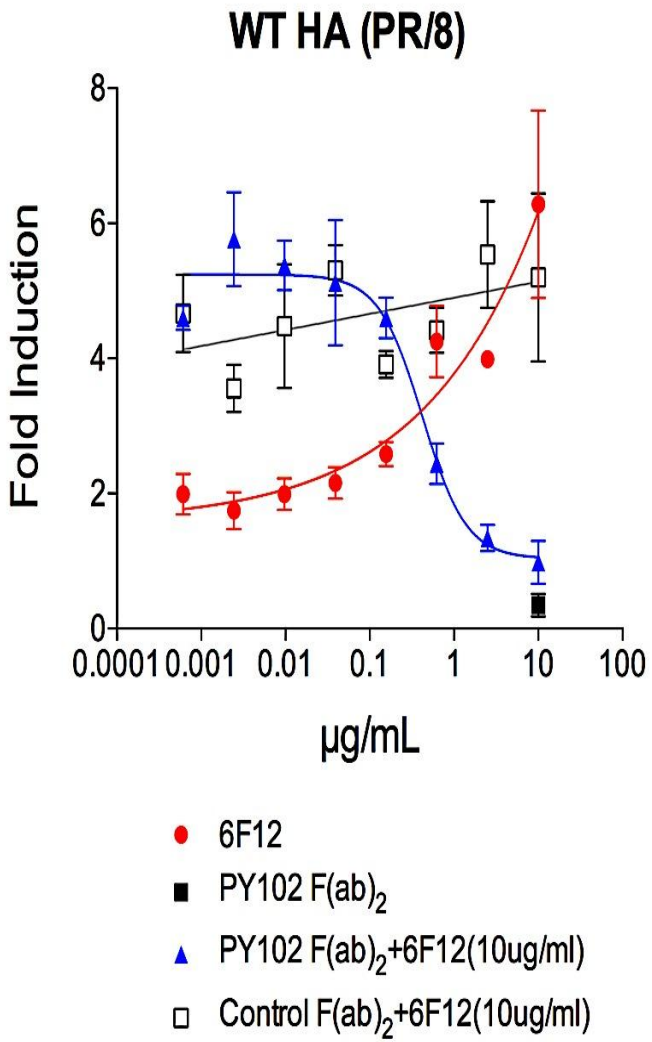
Why do antibodies targeting the hemagglutinin head domain lack the ability to optimally induce ADCC activity?

Disruption of sialic acid engagement

- **Blocking with Head-specific $F(ab)_2$**
- **Blocking with 6' Sialyllactose**
- **Mutating Y108F in Receptor Binding Site**

Head-specific F(ab)$_2$ prevents ADCC induction of stalk-specific 6F12 mAb



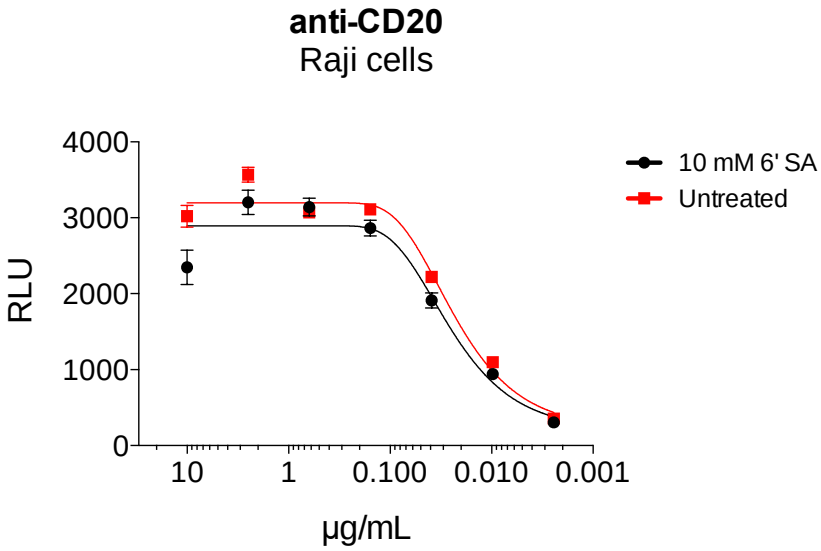


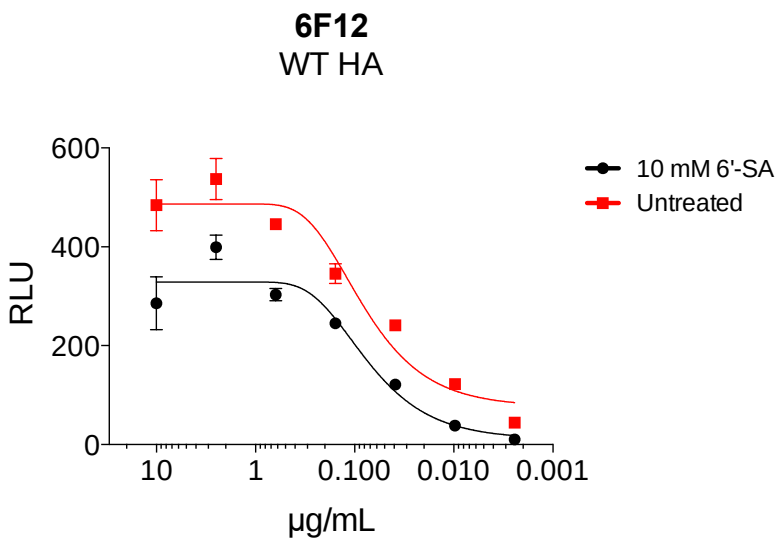
PY102 (Head-specific)
6F12 (Stalk-specific)

Disruption of sialic acid engagement

- **Blocking with Head-specific $F(ab)_2$**
- **Blocking with 6’-sialyllactose**
- **Mutating Y108F in Receptor Binding Site**

10 mM of 6’-sialyllactose decreases ADCC induction of stalk-specific antibodies

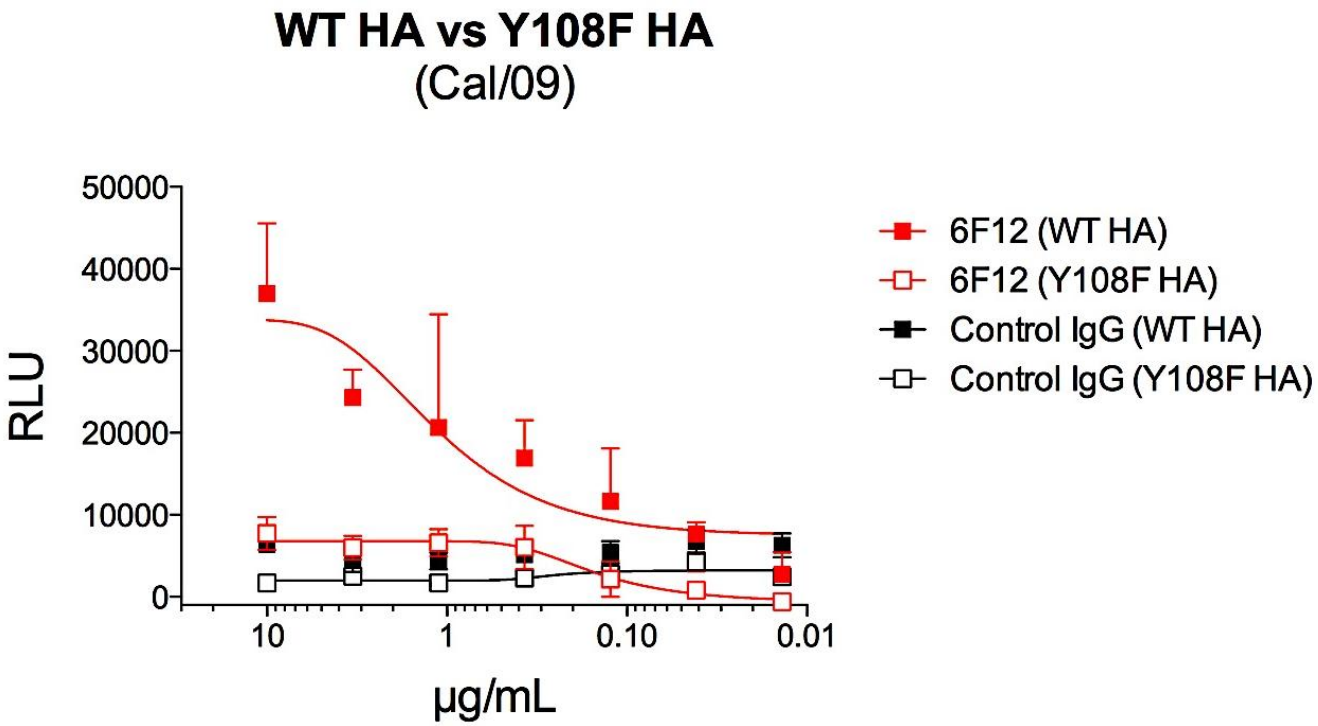




Disruption of sialic acid engagement

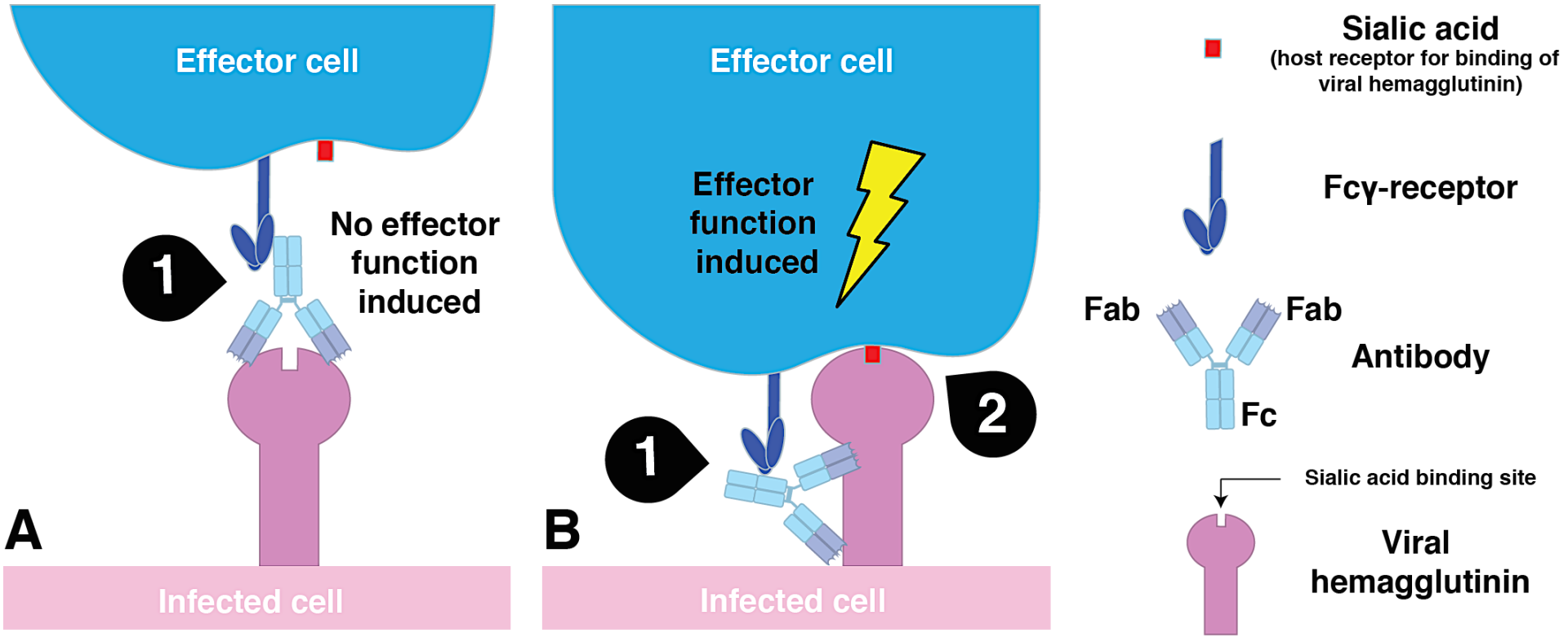
- **Blocking with Head-specific $F(ab)_2$**
- **Blocking with 6' Sialyllactose**
- **Mutating Y108F in Receptor Binding Site**

Y108F mutation lowers RLU values when compared to WT Cal09



Y108F plasmid was generated and provided by Madhu

Two-contacts model for optimal induction of ADCC by influenza virus-specific mAbs




1405
ON TEST
Property of the US Government
cH5/1N1 GMOs contained inside
Contract Nbr.: HHSO100201200011I
Order Nbr.: HHSO10033005T
Strain: A/Vietnam/1203/2004-
A/California/04/2009-(cH5/1N1)-PR8-IDCDC-RG37
Seedlot: VI-1593
DOM: 29/08/2013
Storage Conditions: 2-8°C
Bottle 1 of 1
Monovalent Pooled Harvest
bioCSL
MPH
Date of MPH Processing: 29/08/14
Product: IVV (Split Virion)
Lot No: 0901149Z000
Strain: A/Vietnam
Seed Lot: VI-1593
Operators: Proc.Stage: MPH
Bottle Tare: g
Expiry: 29/08/15
WET FLOOR


Property of the US Government
cH5/1N1 GMOs contained inside
Contract Nbr.: HHSO100201200011I
Order Nbr.: HHSO10033005T
Strain: A/Vietnam/1203/2004-
A/California/04/2009-(cH5/1N1)-PR8-IDCDC-RG37
Seedlot: VI-1593
DOM: 29/08/2013
Bottle 1 of 1
Storage Conditions: 2-8°C
Monovalent Pooled Harvest

Property of the US Government

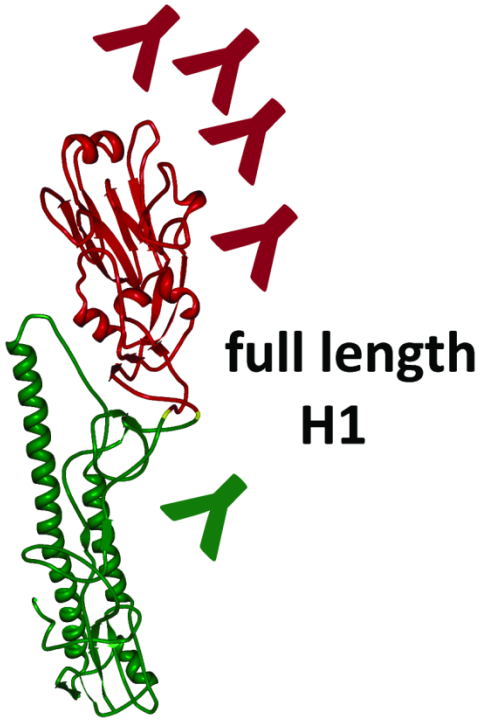
cH8/1N1 GMOs contained inside

Contract Nbr.: HHSO100201200011I
Order Nbr.: HHSO10033005T
Strain: A/mallard/Sweden/24/2002-California/04/2009-(cH8/1N1)-PR8-IDCDC-RG38A
Seedlot: VI-1592
DOM: 28/08/2013
Storage Conditions: 2-8°C
Bottle 1 of 1
Monovalent Pooled Harvest

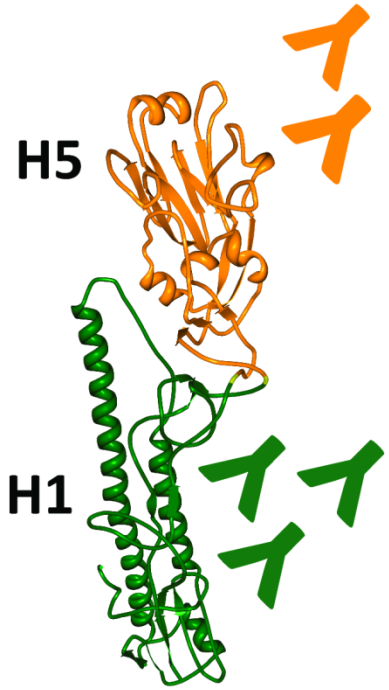
SUMMARY

Towards a universal influenza virus vaccine by reducing the immunodominance of the hemagglutinin head and thereby increasing the immunogenicity of the hemagglutinin stalk and of the neuraminidase

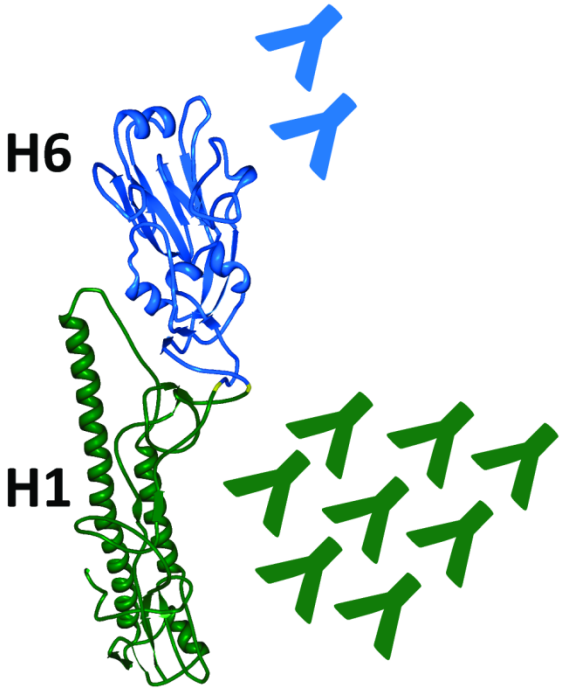
Vision for a human universal influenza virus vaccine



pre-existing immunity
against e.g. H1



boost with cH5/1 construct



boost with cH6/1 construct

Trivalent vaccine with group 1, group 2 and influenza B stalk component necessary

FLORIAN KRAMMER ADOLFO GARCÍA-SASTRE PETER PALESE

SUMMARY (cont.)

MECHANISM OF ADCC INDUCTION
(TWO-CONTACTS MODEL)

- **The location of a FLAG-Tag epitope plays a critical role in determining the level of Antibody-Dependent Cell-Mediated Cytotoxicity (ADCC) induction**

- **The ability of the hemagglutinin to bind to effector cells via its sialic acid receptor is required for optimal ADCC induction**

- **By blocking/mutating the sialic acid receptor binding site with $F(ab)_2$, 6'-sialyllactose or a Y108F mutation, ADCC induction can be lowered substantially**

ACKNOWLEDGEMENTS

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MEGAN ERMLER

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