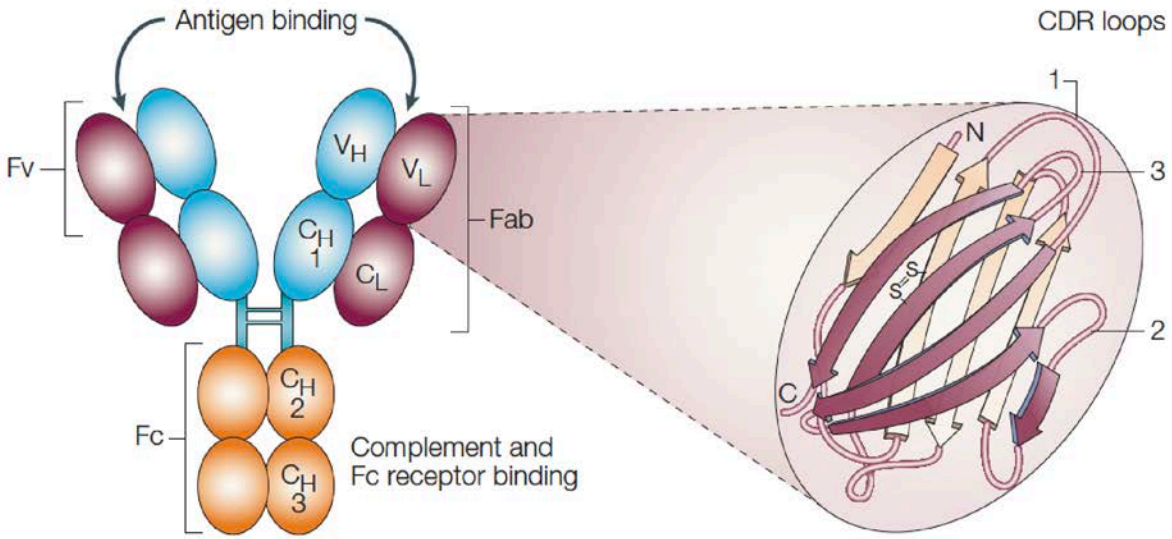
Assessment of human antibody repertoires to discover novel therapeutics

October 2nd 2018

Claudia Scheckel

Antibodies

Modular structure of immunoglobulin monomers

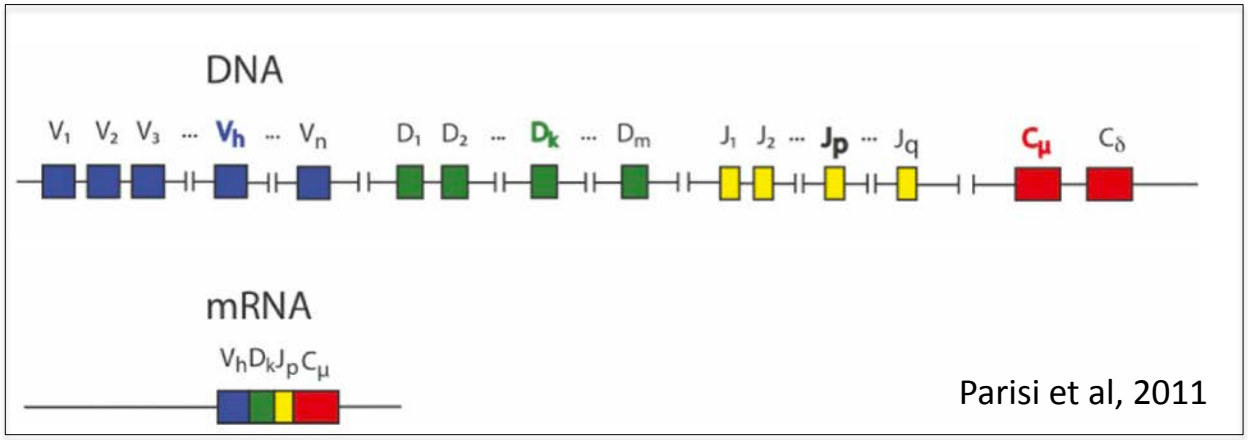


Modes of action to eliminate/neutralize disease targets:

- Blocking of molecules
- Targeting of cells
- Elimination of antigens

Beck et al, 2003

Antibody diversity



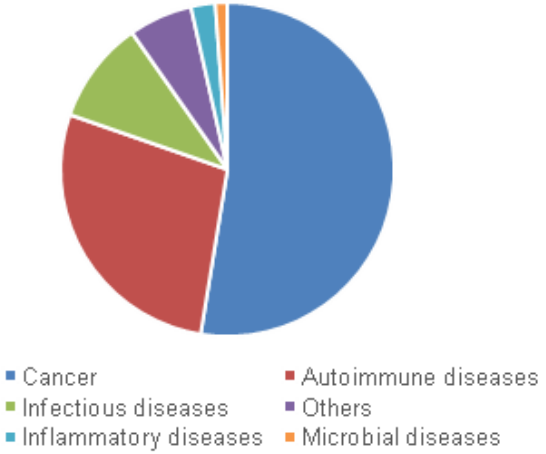
- Combinatorial joining of gene segments (287 different V_L's, 8262 different V_H's)
- Junctional diversification (2/3 non-functional)
- Combinatorial joining of V_L and V_H
- Somatic hypermutation

Monoclonal-Antibody based therapeutics

Advantages of antibodies:

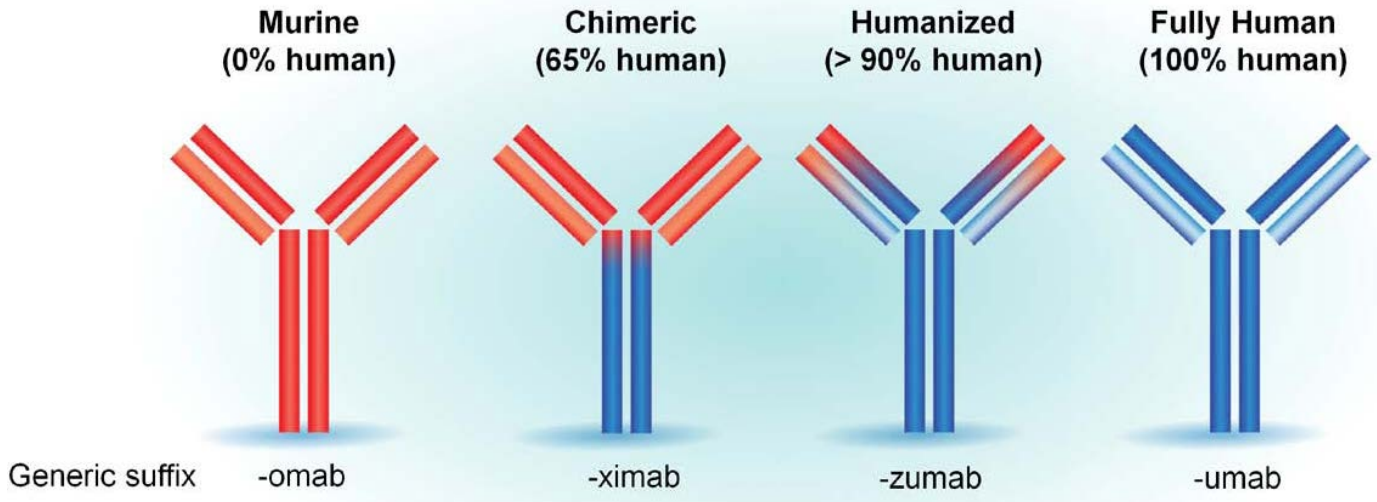
- Specific
- Non-toxic
- Function *in vivo*
- Long half-life

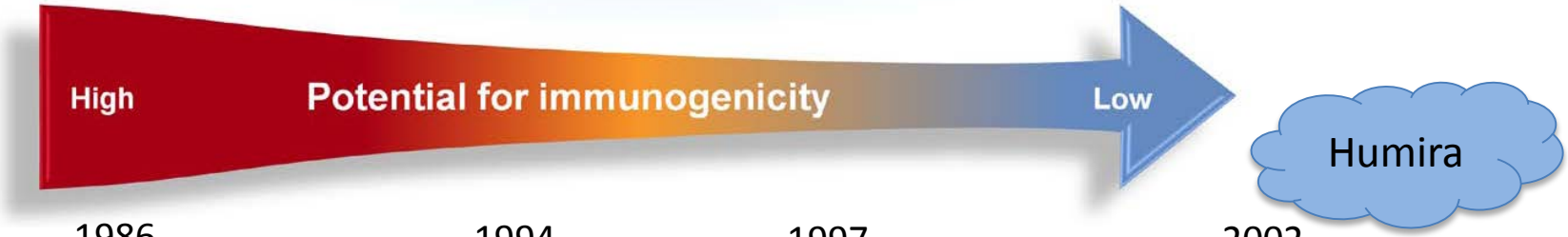
Monoclonal antibody market:
in 2017: 100 billion
in 2021: 150 billion
(~60% of biopharmaceutical sales)



Industry Report in 2016 (GrandViewResearch)

Monoclonal-Antibody based therapeutics





1986
Muromonab-CD3
allograft-rejection
→ HAMAs

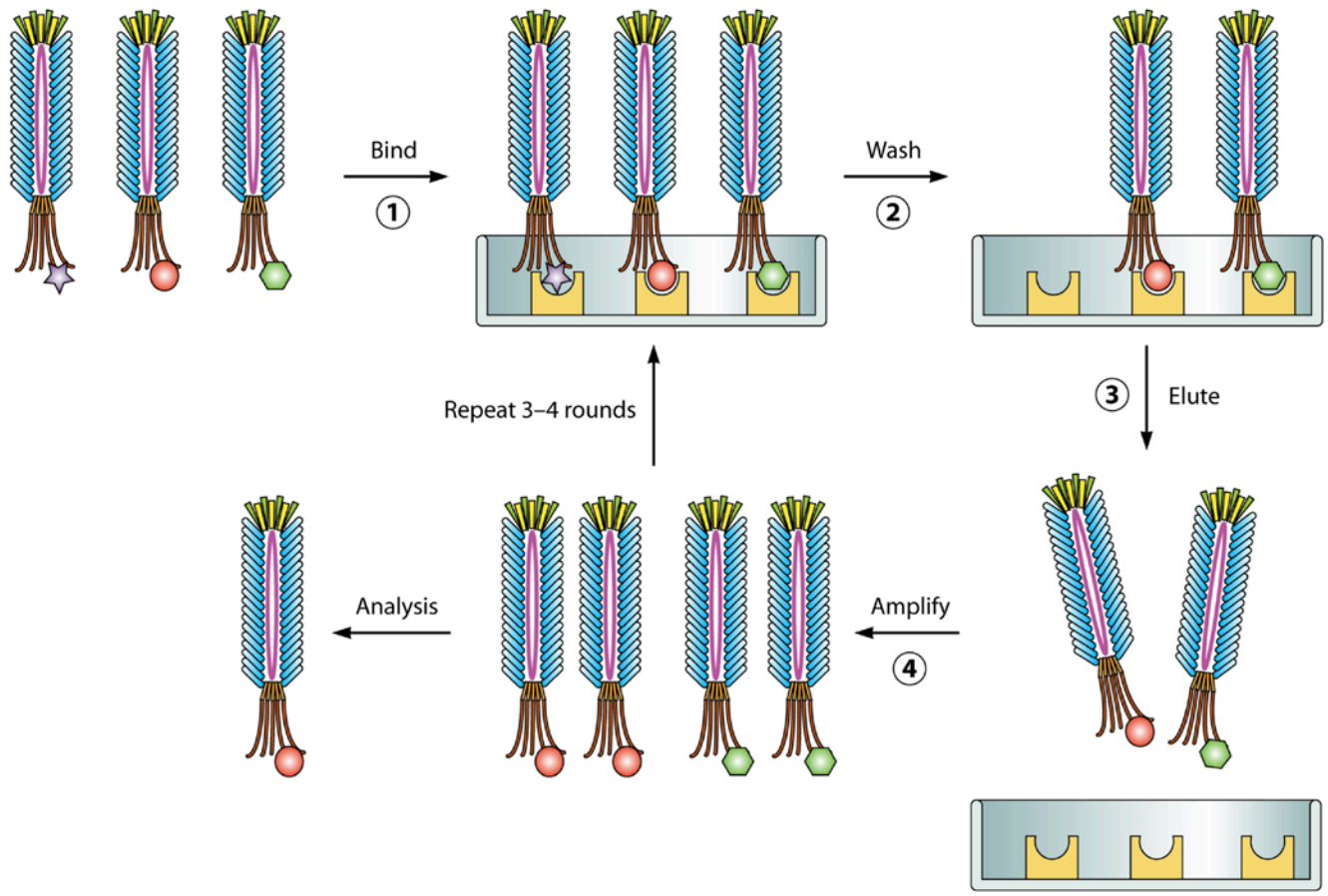
1994
Abciximab
blood clots
→ HACAs

1997
Daclizumab
transplant rejection

2002
Adalimumab
autoimmune diseases

Foltz et al, 2013

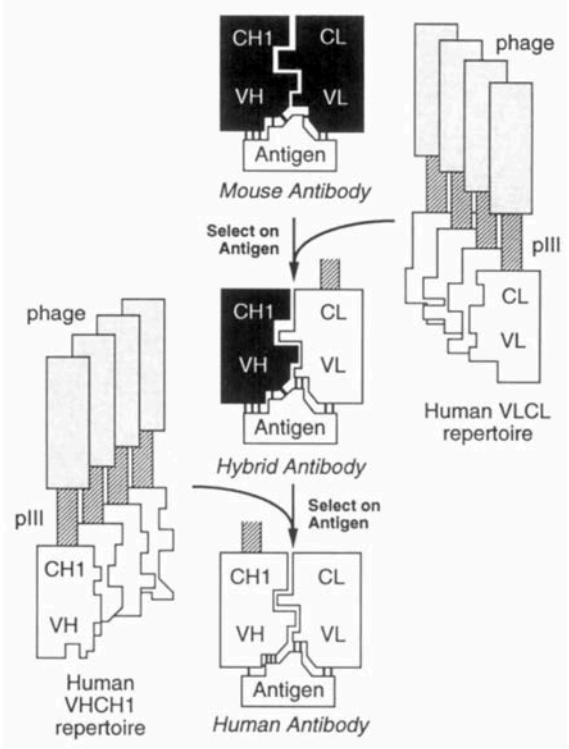
Phage Display



Huang et al, 2012

The first human antibody on market: Adalimumab (Humira)

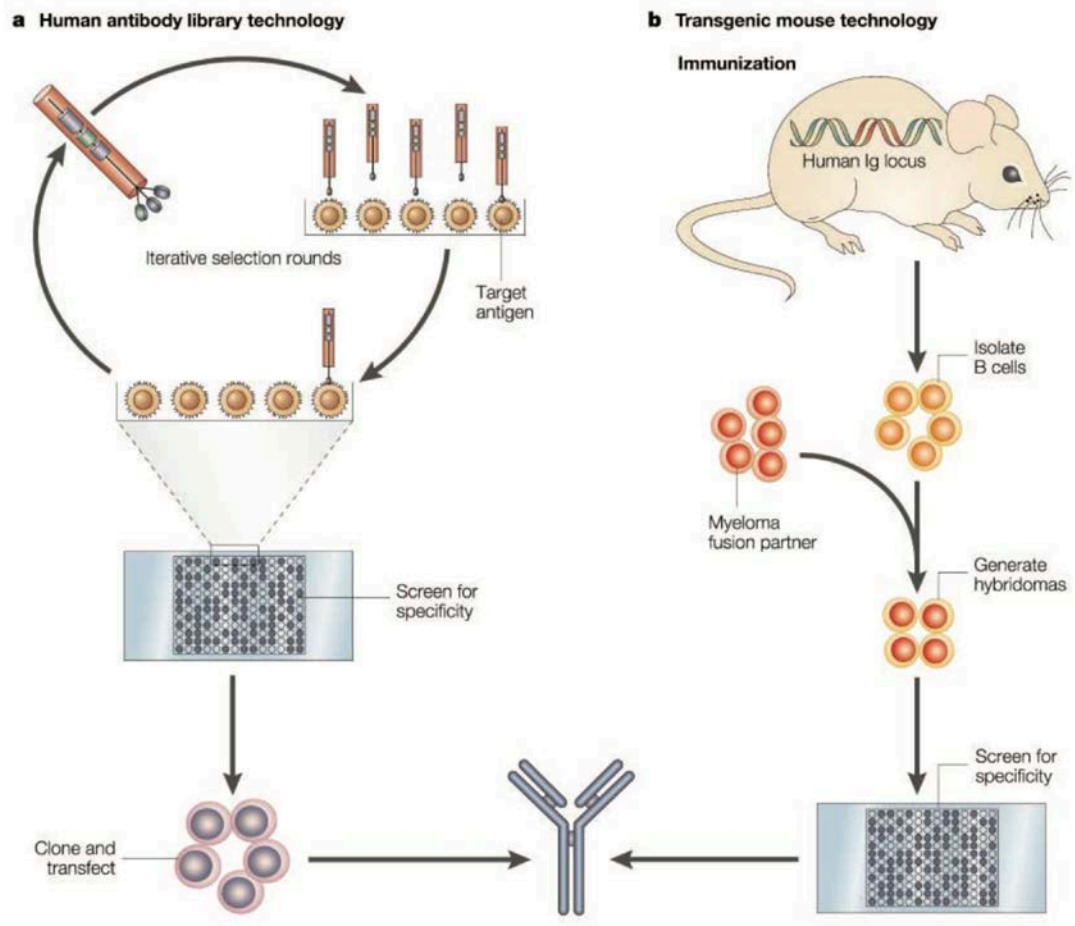
rodent MAB32 as template



Jespers et al, 1994

- Human TNF-a antibody
- D2E7 prevents arthritis in a murine RA model
- Phase I clinical trial in 1999
- FDA approval for RA treatment in 2002
- Since 2015 best selling drug (18 billion USD in 2017)

Generation/Identification of human antibodies



Affinity maturation *in vitro* vs *in vivo*

<u>Transgenic Mice</u>

Advantages:

- Diversity
- Expression
- Stability

Disadvantages:

- Antigenicity
- Difficult counter-screening (species cross-reactivity)

Beck et al, 2003

Exploiting the potential of human antibody repertoires

nature biotechnology

Functional interrogation and mining of natively paired human V_H:V_L antibody repertoires

B
Ji
Jo
L
V
K
W
M
Laboune[5], Julie E Ledgerwood[6], Barney S Graham[6], Mark Connors[8], Daniel C Douek[2], Nancy J Sullivan[2], Andrew D Ellington[9,10], John R Mascola[2] & George Georgiou[1,9–11]

COMMUNICATIONS BIOLOGY

ARTICLE

MedImmune

Parallelization/Miniaturization using microfluidics
High-throughput display methodology for screening
Identification of natively paired human antibodies

Herren Wu[1], William F. Dall'Acqua[1], Xiaodong Xiao[1,4] & Partha S. Chowdhury[1,5]

GigaGen

MABS
2017, VOL. 9, NO. 8, 1282–1296
https://doi.org/10.1080/19420862.2017.1371383

Taylor & Francis
Taylor & Francis Group

REPORT

OPEN ACCESS Check for updates

Rare, high-affinity anti-pathogen antibodies from human repertoires, discovered using microfluidics and molecular genomics

Adam S. Adler[a], Rena A. Mizrahi[a], Matthew J. Spindler[a], Matthew S. Adams[a], Michael A. Asensio[a], Robert C. Edgar[a], Jackson Leong[a], Renee Leong[a], Lucy Roalfe[b], Rebecca White[b], David Goldblatt[b], and David S. Johnson[a]

[a]GigaGen Inc., 407 Cabot Road, South San Francisco, CA, USA; [b]Immunobiology Section, Great Ormond Street Institute of Child Health, University College London, London, England, United Kingdom

COMMUNICATIONS BIOLOGY

ARTICLE

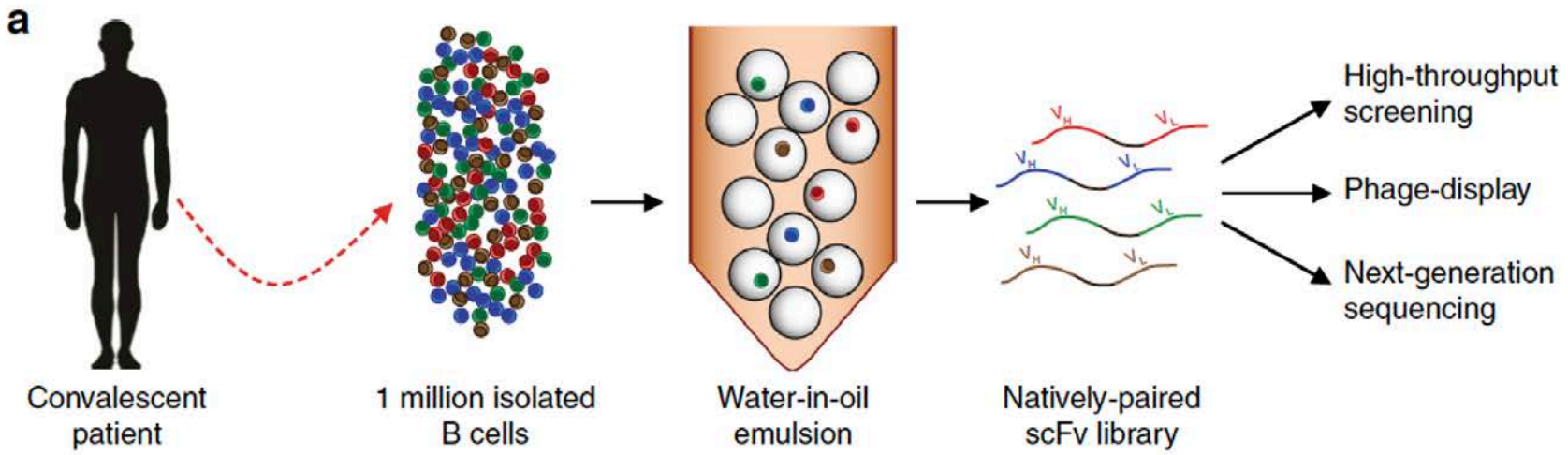
DOI: 10.1038/s42003-017-0006-2 OPEN

Recombinant human B cell repertoires enable screening for rare, specific, and natively paired antibodies

Saravanan Rajan[1], Michael R. Kierny[1], Andrew Mercer[1,3], Jincheng Wu[2], Andrey Tovchigrechko[2], Herren Wu[1], William F. Dall'Acqua[1], Xiaodong Xiao[1,4] & Partha S. Chowdhury[1,5]

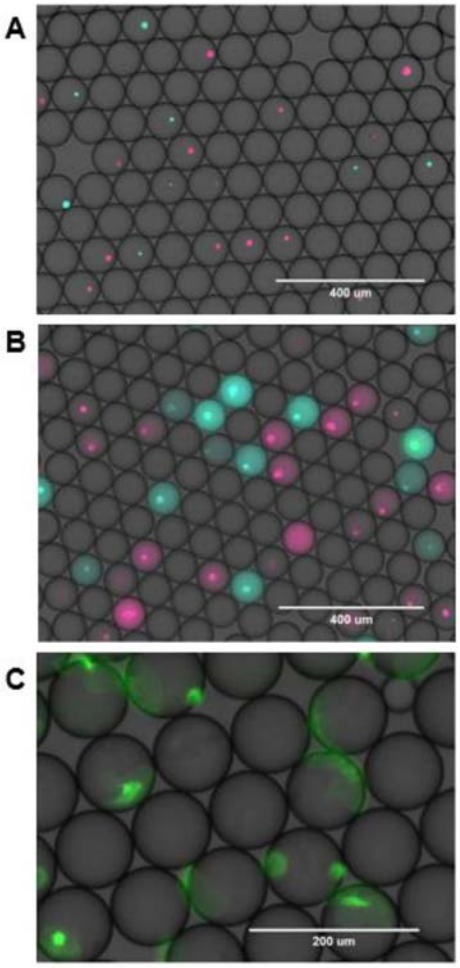
- Generation of natively paired scFv's from healthy donors using microfluidics (one-step emulsion)
- Cloning of scFv libraries for phage display
- Identification of cross-reactive antibodies against influenza hemagglutinin

Workflow


a
Convalescent patient
1 million isolated B cells
Water-in-oil emulsion
Natively-paired scFv library
High-throughput screening
Phage-display
Next-generation sequencing

Cell encapsulation and lysis

Encapsulation of cells in 10% of droplets
(Poisson statistics: 95% probability of single-cell encapsulation)



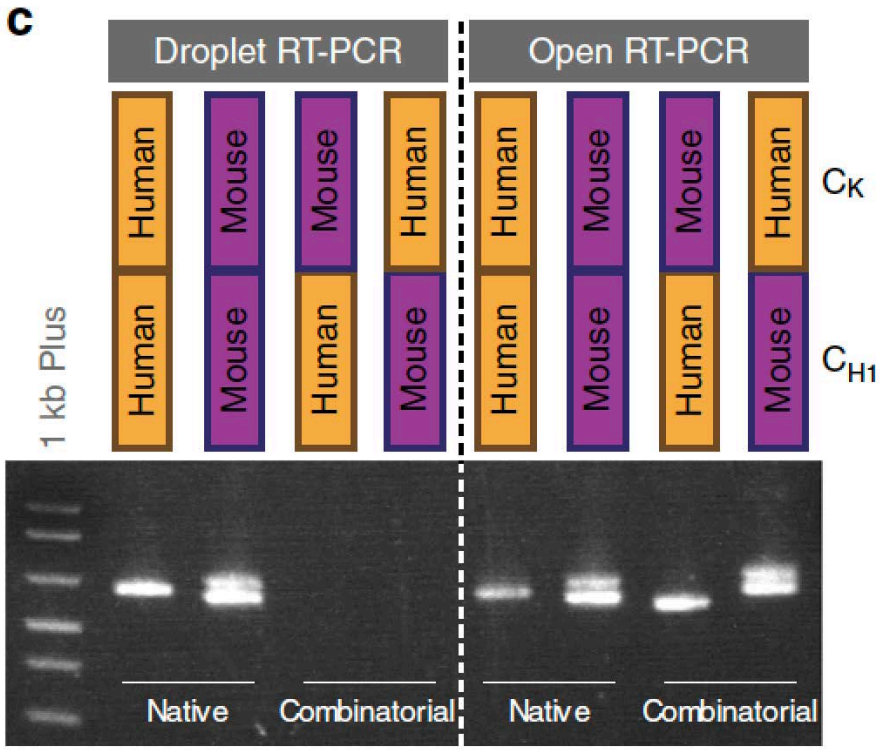
PBS (+ cytosolic dye)

RT-PCR buffer (+ cytosolic dye): release of dye

RT-PCR buffer (+ SYBR Green): release of nuclear dsDNA

Generation of natively paired scFv's

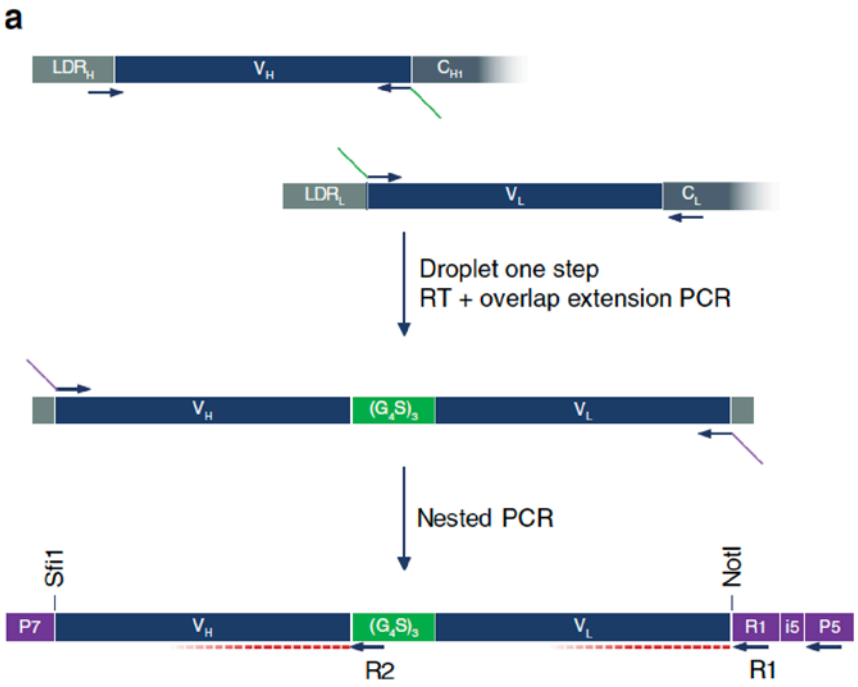
Encapsulation of human and mouse memory B cells



→ Amplification in droplets yields only correctly paired species

scFv library generation and sequencing

scFv library generation from memory B cells of healthy subjects and high-throughput sequencing

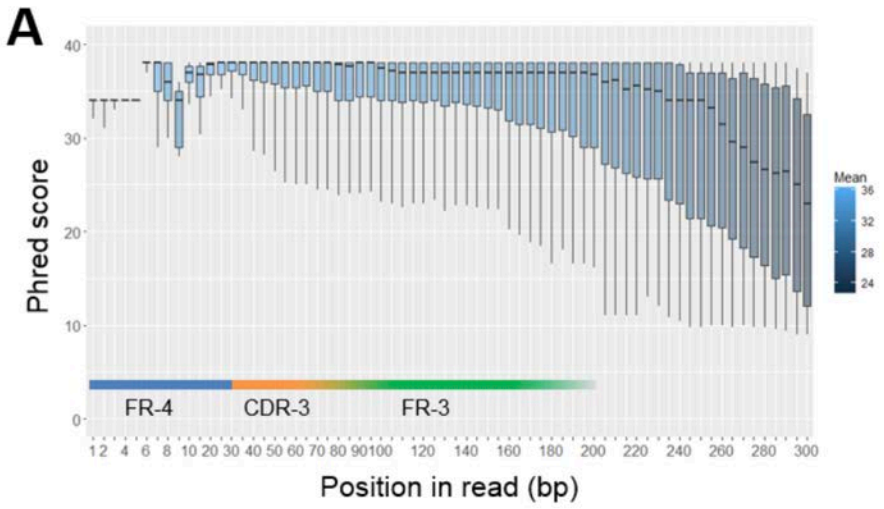


Addition of 1% IM-9 cells (expressing known V_H/V_L's)

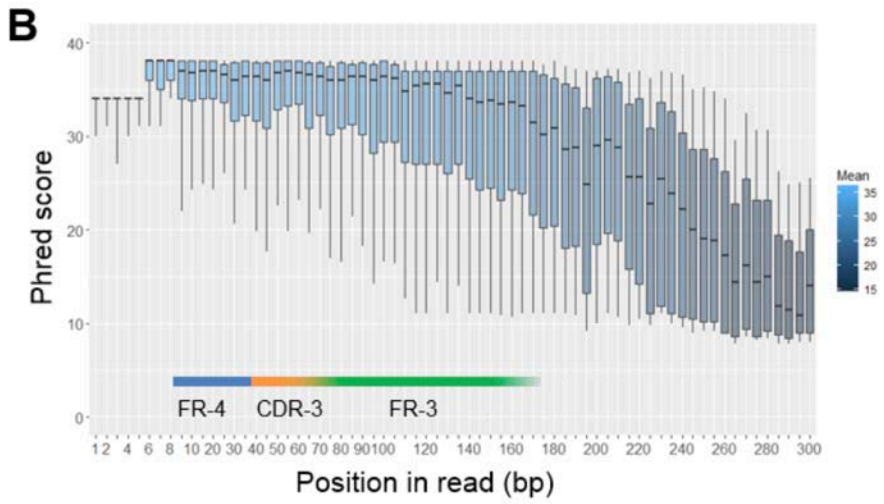
⟶ 96% accurate pairing

scFv sequence analysis

V_H sequences



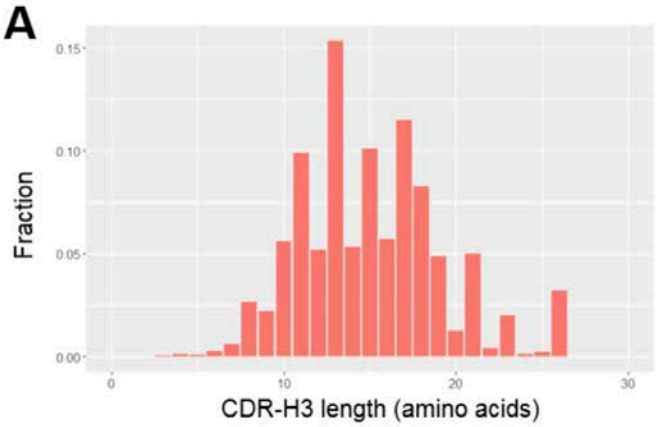
V_L sequences

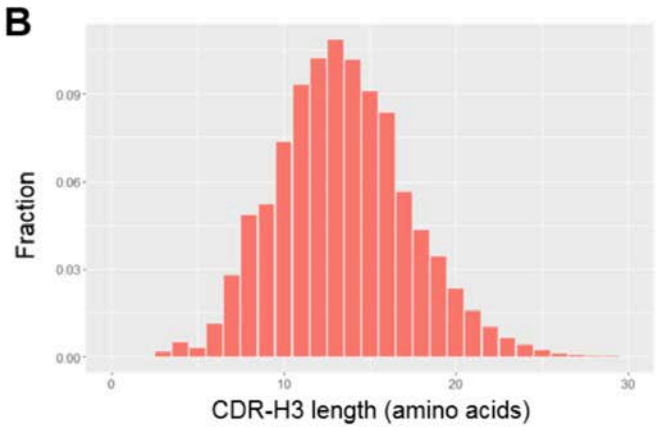


scFv sequence analysis - V_H

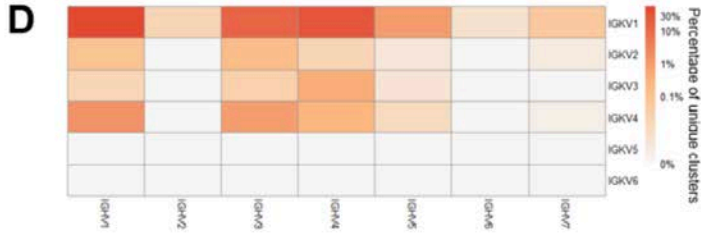
Droplets

Bulk

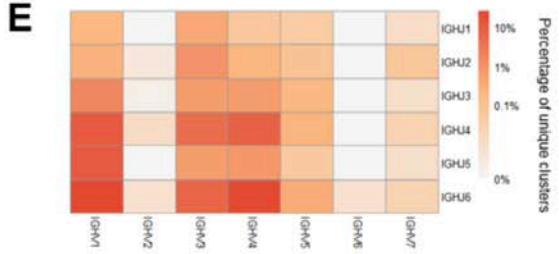




IGHV:IGLV



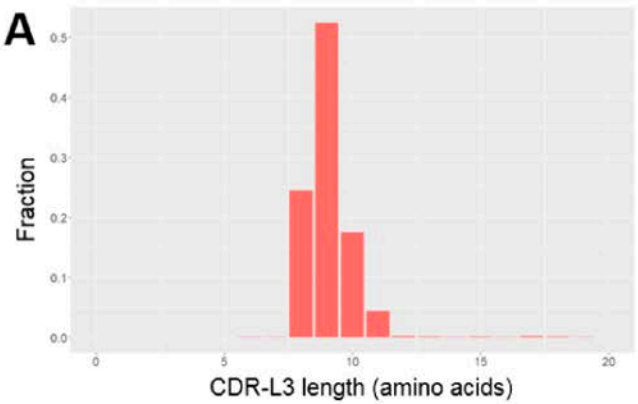
IGHV:IGKV

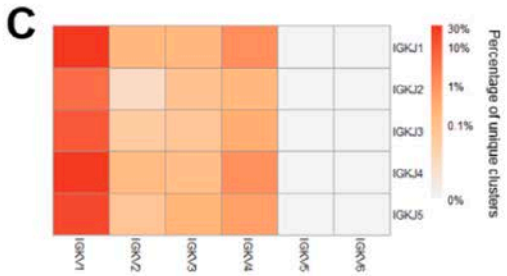


IGHV:IGHJ

scFv sequence analysis - V_L

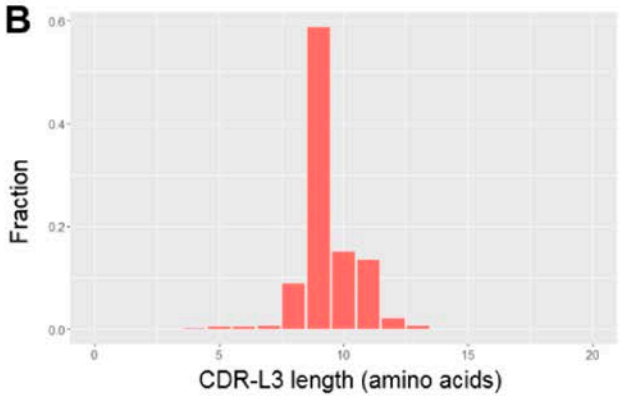
Droplets

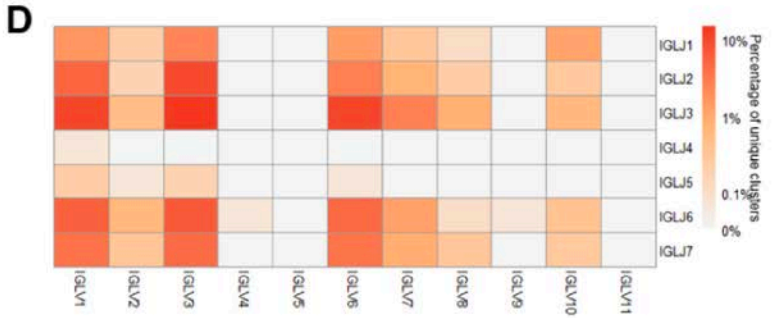




IGKV:IGKJ

Bulk

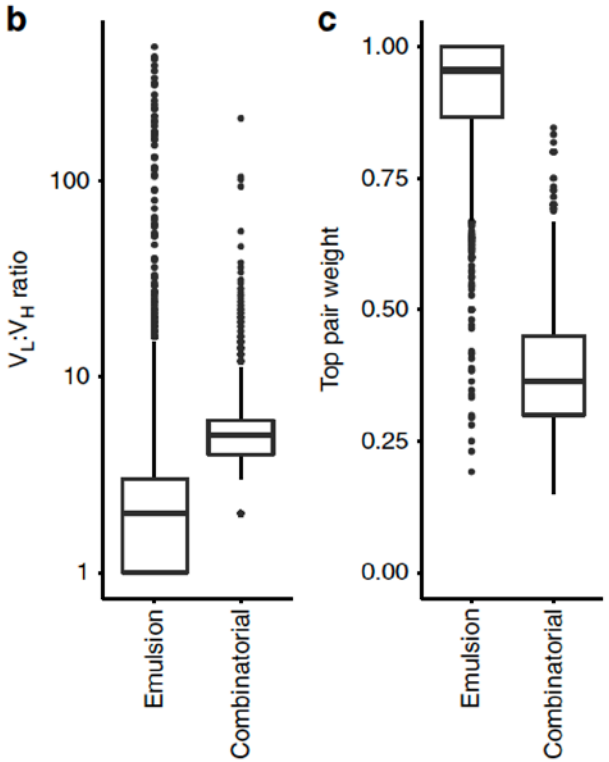




IGLV:IGLJ

scFv sequence analysis

- Droplets: 212k unique CDR-H3/L3 cluster
- Bulk: 2.5M unique CDR-H3/L3 cluster

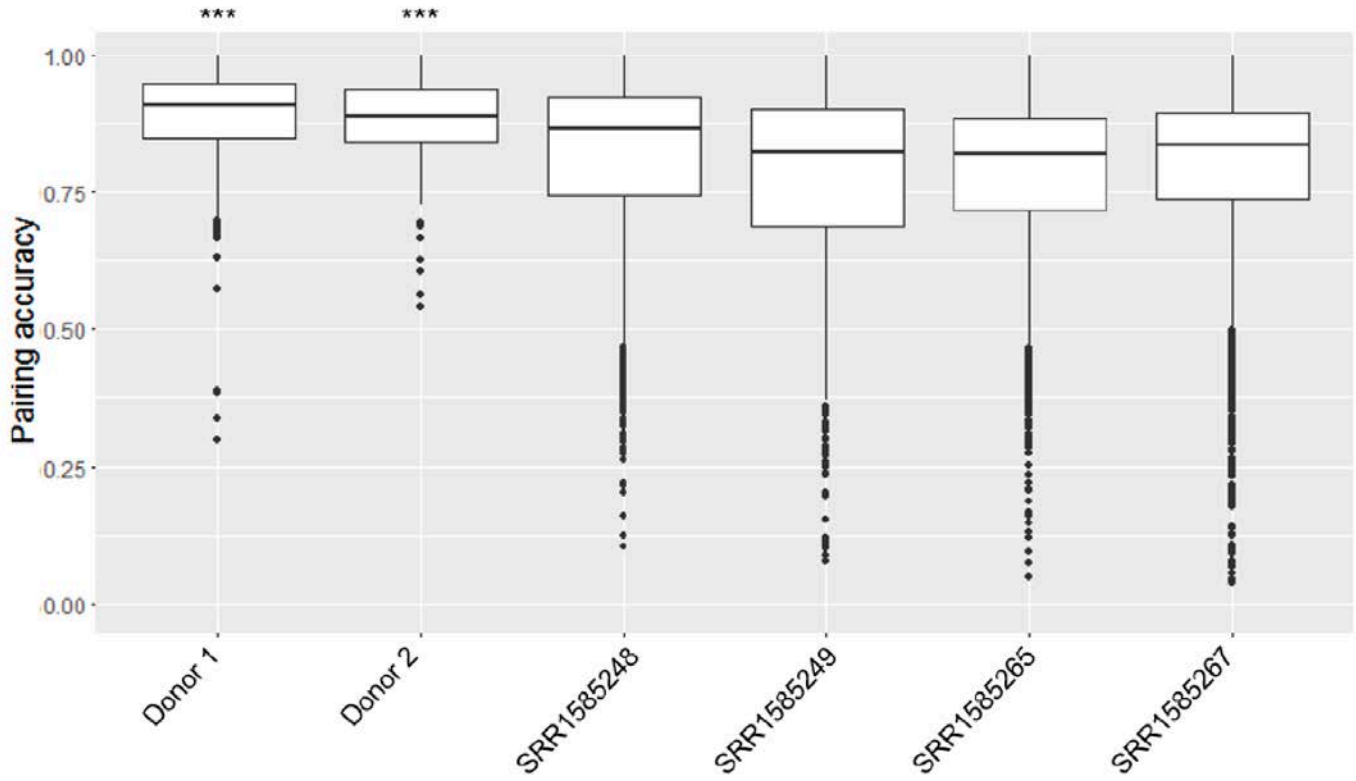


→ Droplets: 1 V_H pairs with 2 V_L's
top-pair ~96%

Bulk: 1 V_H pairs with 5-9 V_L's
top-pair ~25%

Amplification of natively paired scFv's

Top-pair analysis



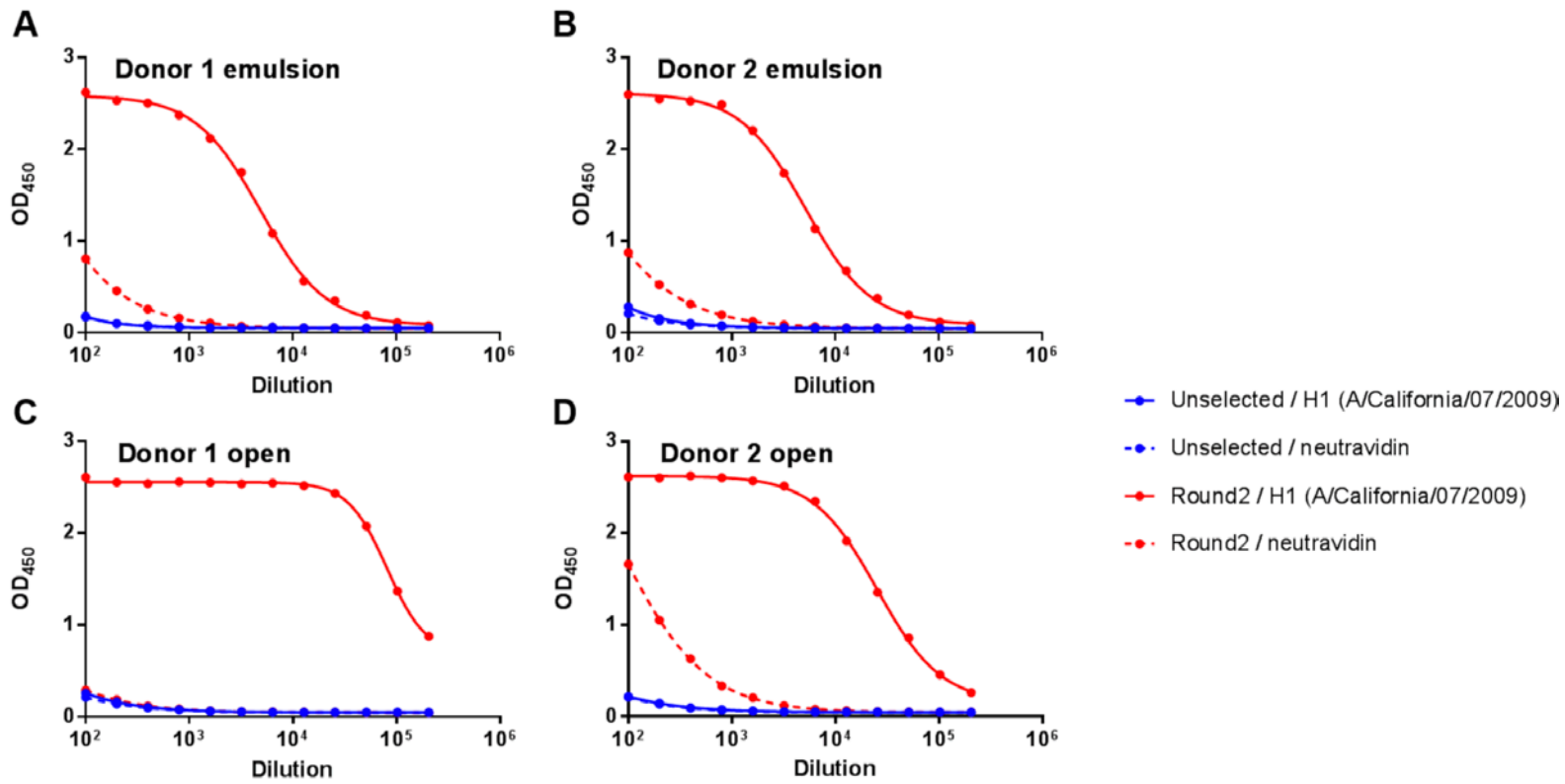
→ Better pairing accuracy than in previously published libraries

Identification of antigen-specific antibodies

Screening of Myc:scFv libraries via phage display:

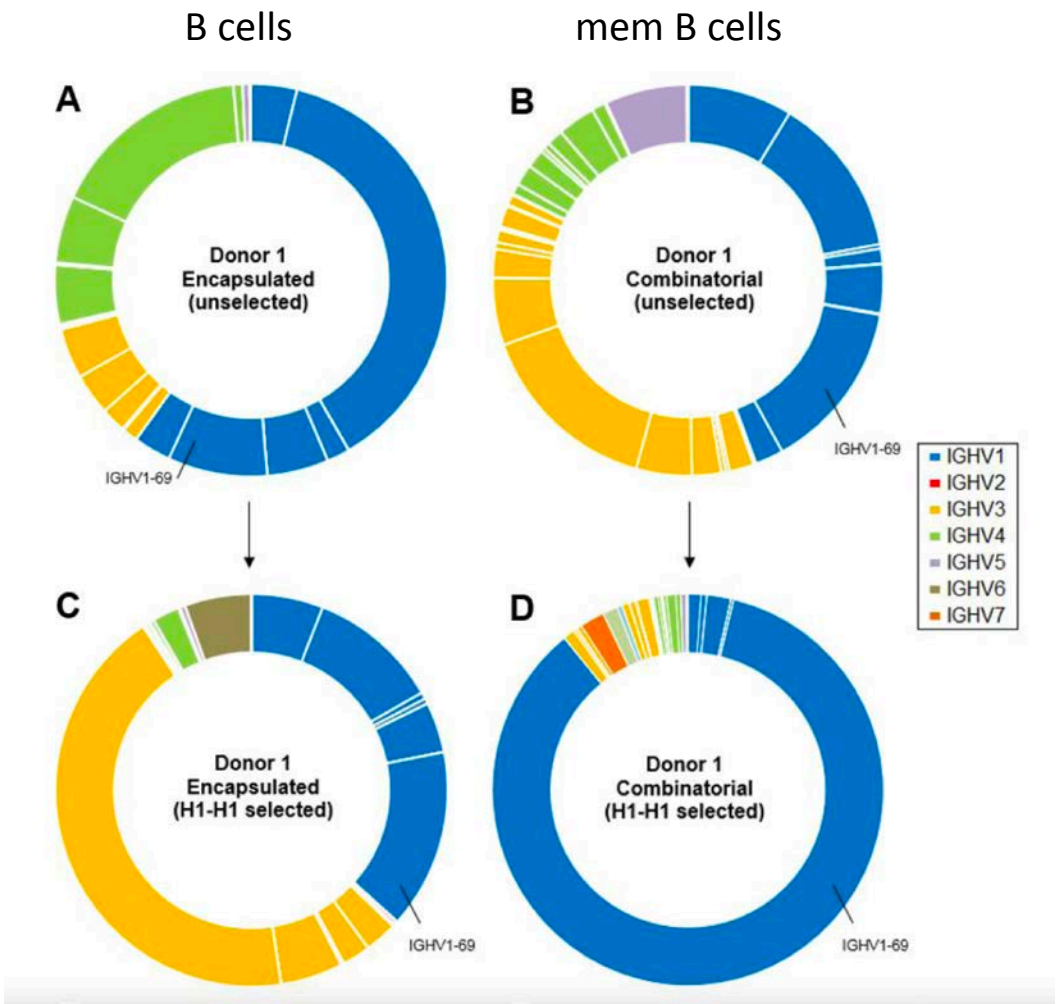
2x selection on influenza A hemagglutinin (H1N1) → polyclonal phage ELISA

B cells mem B cells



→ robust enrichment of binders (regardless of B cell source and pre-selection)

Identification of antigen-specific antibodies



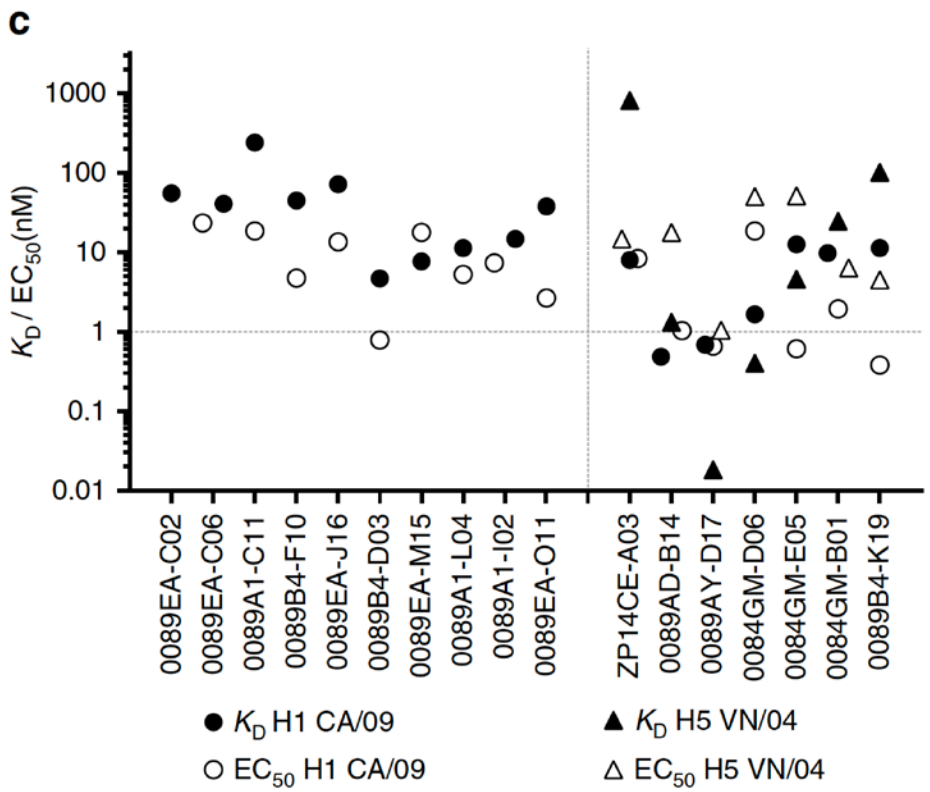
- strong bias for IgHV1-69 antibodies in bulk libraries
- IgHV1-69 can bind hemagglutinin through heavy-chain interactions alone

Identification of cross-reactive antigen-specific antibodies

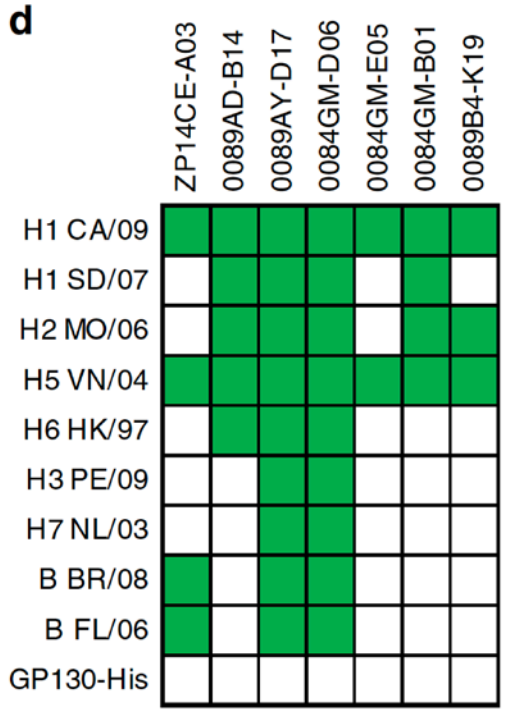
Screening of 5632 clones for H5N1 binding

- 320 clones bind H5N1
  - 17 unique antibodies

ELISA (some with pM affinities):



Biolayer inferometry:



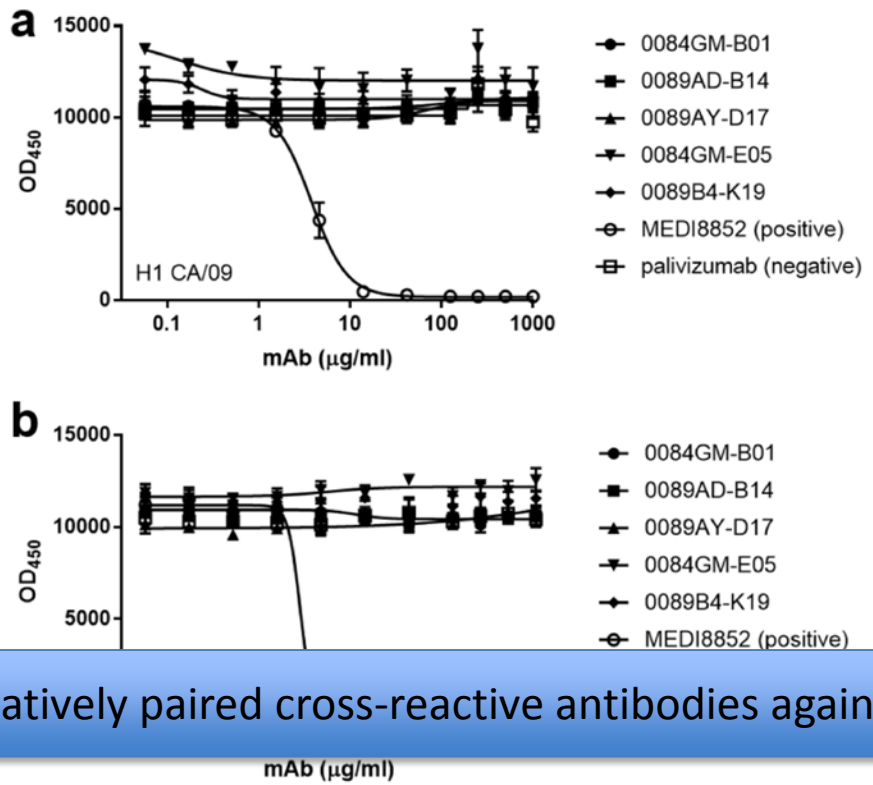
neg. ctrl

Characterization of cross-reactive antibodies

- No competition with previously identified cross-reactive antibodies
- Expression/purification of 7 antibodies as IgG1's
  - 5 antibodies retained binding
  - 0 antibodies exhibited neutralizing activity

seasonal H1N1

non-seasonal H5N1



Identification of natively paired cross-reactive antibodies against influenza hemagglutinin

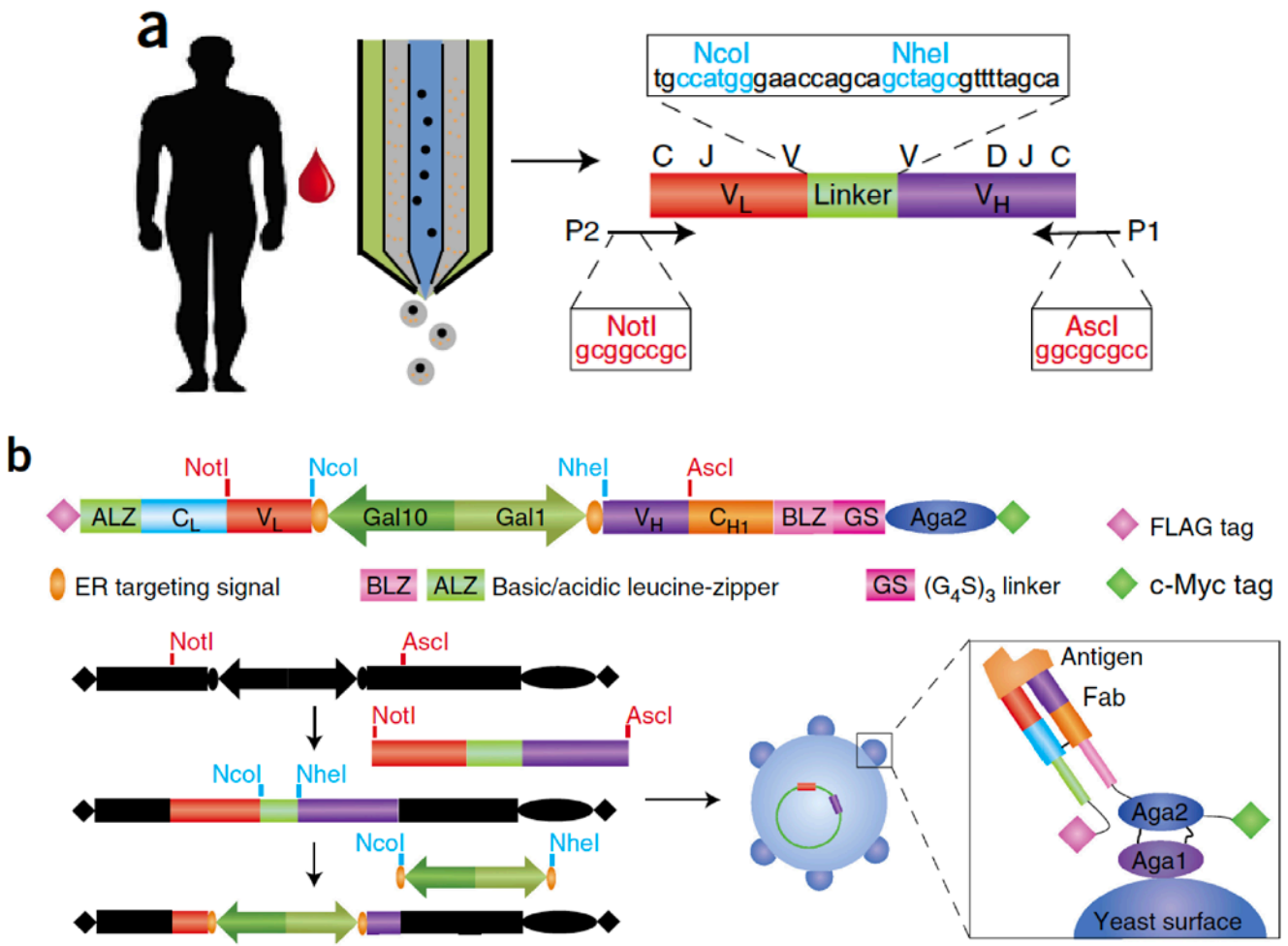
nature biotechnology

Functional interrogation and mining of natively paired human V_H:V_L antibody repertoires

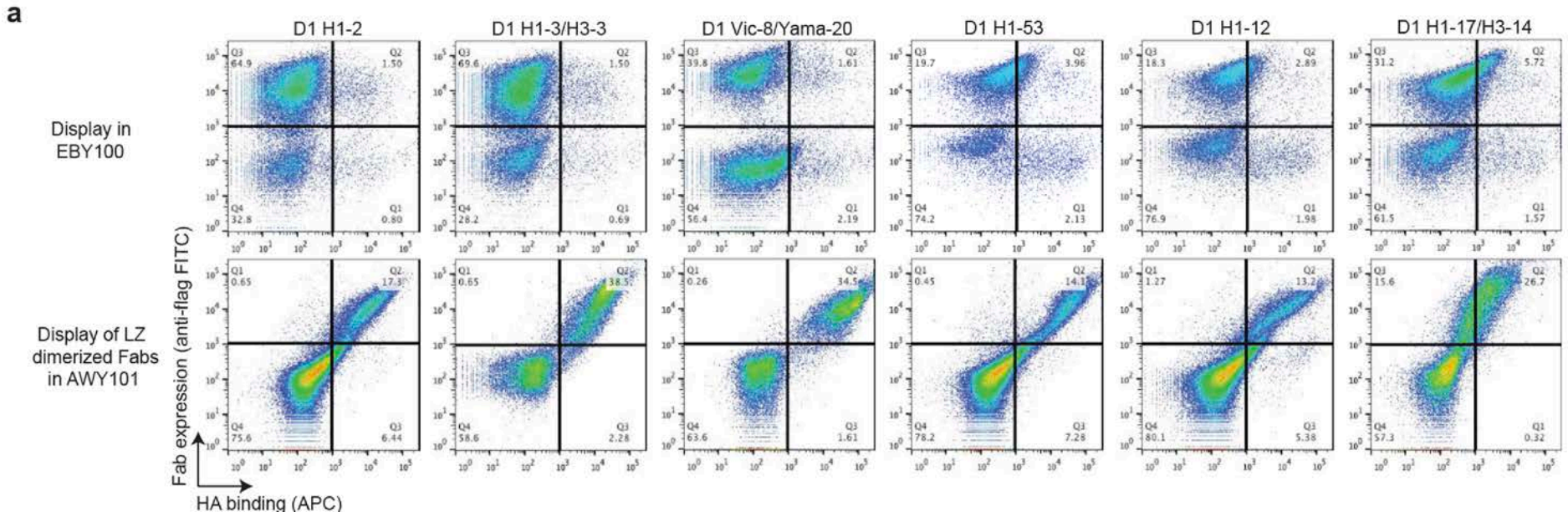
Bo Wang[1,12], Brandon J DeKosky[2–4,12], Morgan R Timm[2], Jiwon Lee[1], Erica Normandin[2], John Misasi[2], Rui Kong[2], Jonathan R McDaniel[1], George Delidakis[1], Kendra E Leigh[2], Thomas Niezold[2], Chang W Choi[2], Elise G Viox[2], Ahmed Fahad[4], Alberto Cagigi[2], Aurélie Ploquin[2], Kwanyee Leung[2], Eun Sung Yang[2], Wing-Pui Kong[2], William N Voss[1], Aaron G Schmidt[5], M Anthony Moody[6,7], David R Ambrozak[2], Amy R Henry[2], Farida Laboune[2], Julie E Ledgerwood[2], Barney S Graham[2], Mark Connors[8], Daniel C Douek[2], Nancy J Sullivan[2], Andrew D Ellington[9,10], John R Mascola[2] & George Georgiou[1,9–11]

- Generation of natively paired V_H/V_L amplicons from vaccinated/infected donors using microfluidics (two-step emulsion)
- Cloning of Fab libraries for yeast display
- Identification of antibodies against Ebola/Influenza/HIV

Workflow


a
NcoI
NheI
tgccatgggaaccagcagctagcgttttagca
C J V
V D J C
V_L
Linker
V_H
P2
P1
NotI
gcggccgc
AscI
ggcgcgcc
b
NotI
NcoI
NheI
AscI
ALZ
C_L
V_L
Gal10
Gal1
V_H
C_{H1}
BLZ
GS
Aga2
FLAG tag
ER targeting signal
BLZ ALZ Basic/acidic leucine-zipper
GS $(G_4S)_3$ linker
c-Myc tag
Antigen
Fab
Aga2
Aga1
Yeast surface

Optimization of Fab expression



c

Antibody ID	Displayed Fab bound antigen?		
	EBY100	AWY101 (with PDI)	LZ dimerized Fabs expressed in AYW101
D1 H1-2	No	Yes	Yes
D1 H1-3/H3-3	No	No	Yes
D1 Vic-1/Yama-1	Yes	Yes	Yes
D1 Vic-9/Yama-5	Yes	Yes	Yes
D1 Vic-8 Yama-20	No	No	Yes
D1 H1-53	No	Yes	Yes
D1 H1-12	No	Yes	Yes
D1 Vic-20/Yama-18	Yes	Yes	Yes
D1 H1-9/H3-7	Yes	Yes	Yes
D1 H1/H3/Vic	Yes	Yes	Yes
D1 H1-17/H3-14	No	No	Yes
D1 H1-34	Yes	Yes	Yes
D1 H3-9	Yes	Yes	Yes

Fab surface expression in yeast

+ protein disulfide isomerase

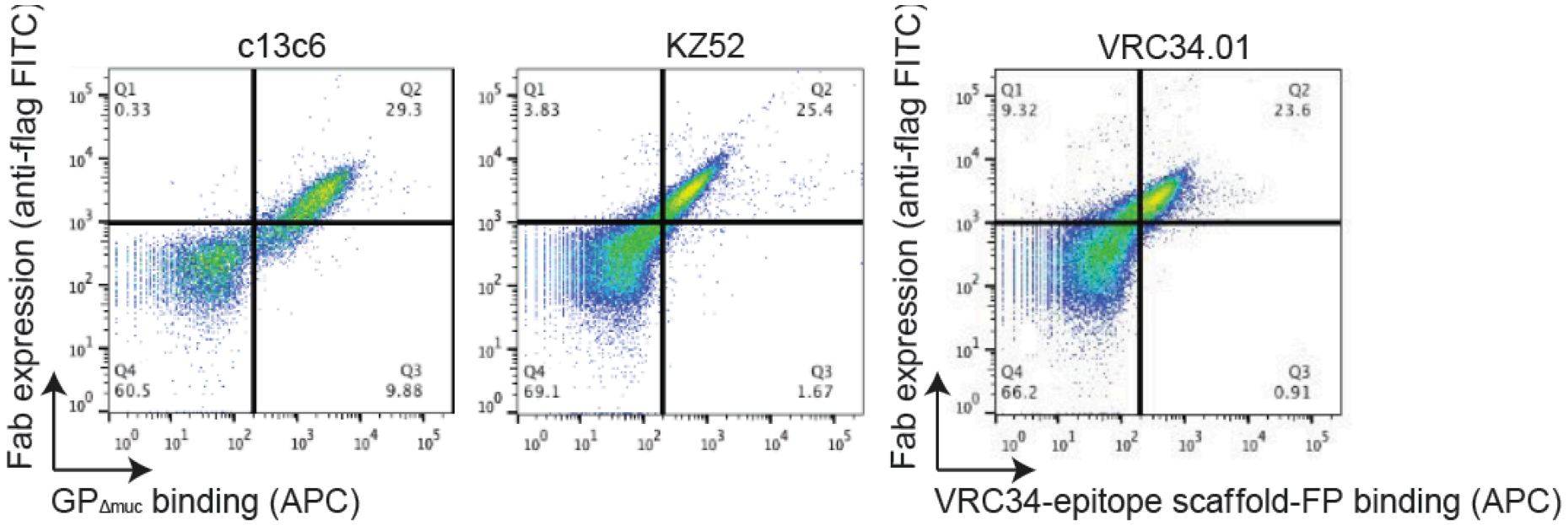
+ dimerization

13 (13) human antibodies show antigen binding

Assessment of known antibodies

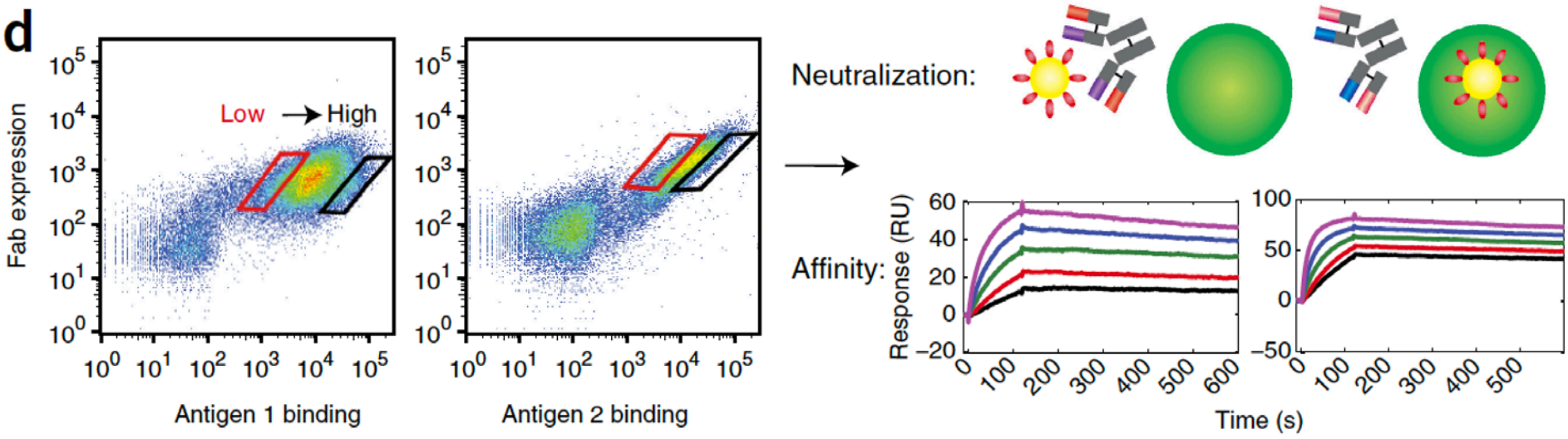
Anti-Ebola

Anti-HIV



Identification of high-affinity binders

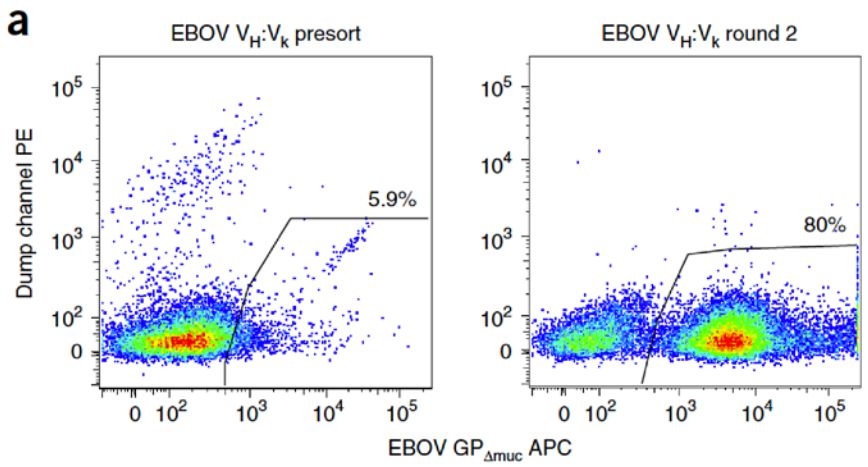
Sequential rounds of FACS



Identification of anti-Ebola antibodies

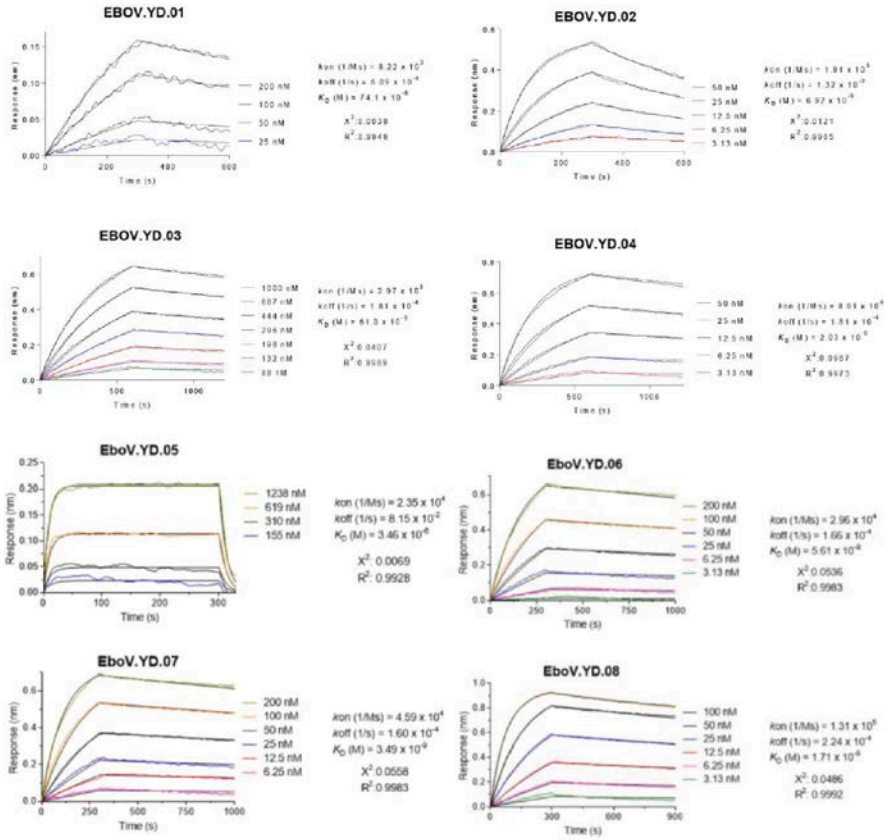
Donor analysis 6 days after EBOV immunization:

- 5002 plasmablasts
- 1189 unique CDR3H:CDR3L clusters
- 6% of Fabs in pre-sort bind antigen
- 7 antibody lineages (>100x enriched)
- IgG1 expression of eight antibodies



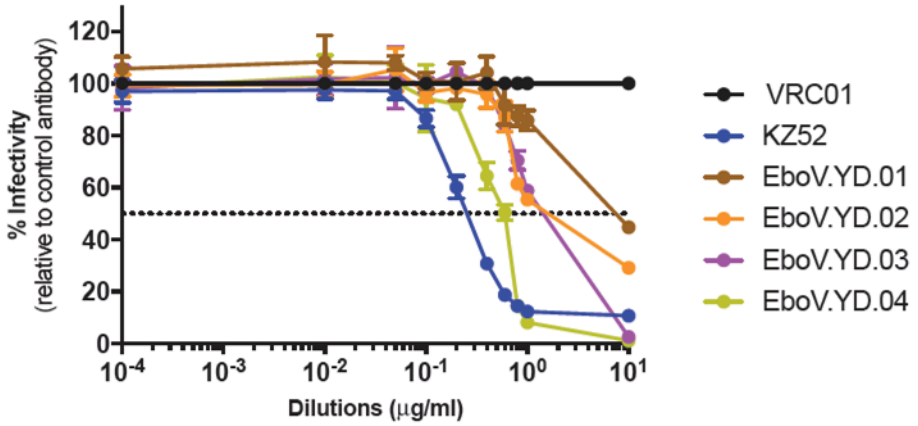
Biolayer interferometry

7 (8) antibodies bind with single-digit nM affinity



Identification of anti-Ebola antibodies

all of four antibodies neutralized infection



one antibody competes with KZ52
(generated during natural infection)

b

EBOV antibody	HV gene	% EBOV Neut. (10 μg/mL)	Fab affinity (nM)
EBOV.YD.01	3–21	55.1 ± 1.1	74.1 ± 8.9
EBOV.YD.02	1–46	70.8 ± 1.7	6.92 ± 0.11
EBOV.YD.03	4–4	97.3 ± 0.3	61.0 ± 1.3
EBOV.YD.04	3–15	98.8 ± 0.1	2.03 ± 0.07

c

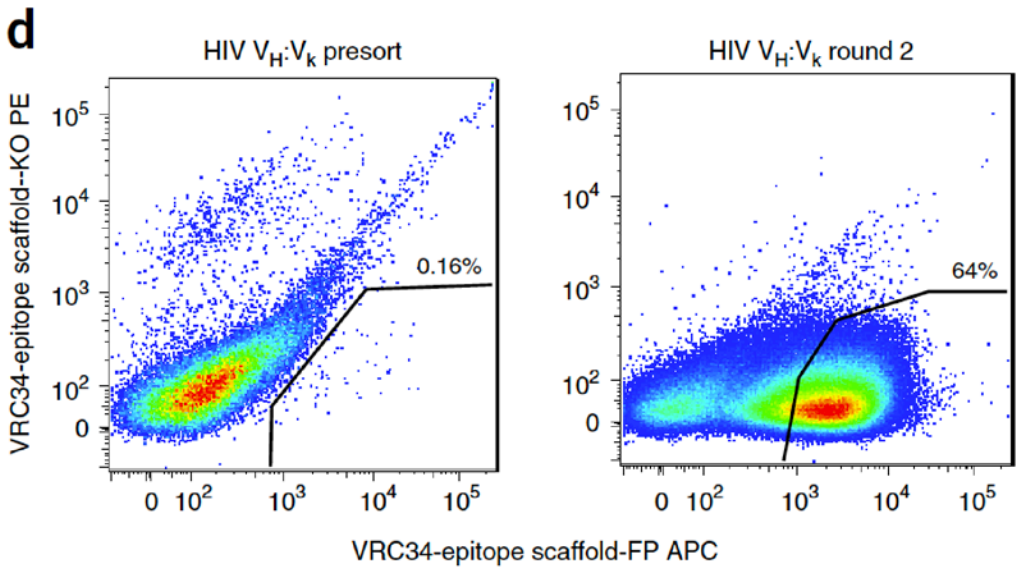
Competitor \ Analyte	KZ52	EBOV YD.01	EBOV YD.02	EBOV YD.03	EBOV YD.04
KZ52	92.8%	22.9%	9.7%	83.6%	10.4%
EBOV YD.01	26.7%	82.5%	11.6%	28.1%	27.4%
EBOV YD.02	30.6%	26.1%	97.6%	56.4%	15.5%
EBOV YD.03	12.8%	10.8%	4.5%	79.6%	6.3%
EBOV YD.04	32.4%	49.6%	13.2%	22.0%	94.1%

Identification of anti-HIV antibodies

HIV-infected patient harboring a broadly neutralizing anti-FP antibody N123-VRC34

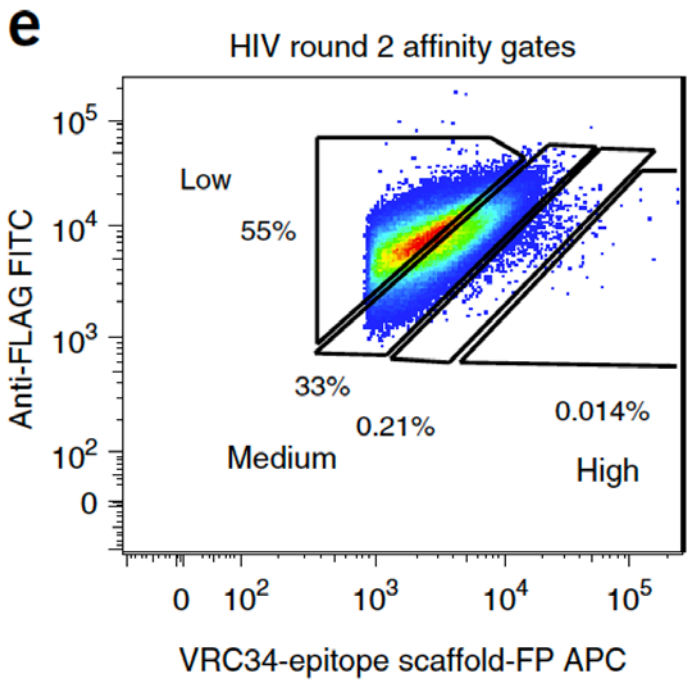
- Rare antibody (0.003% of B cells)
- Mutations in FR1

→ requires lineage-specific primers

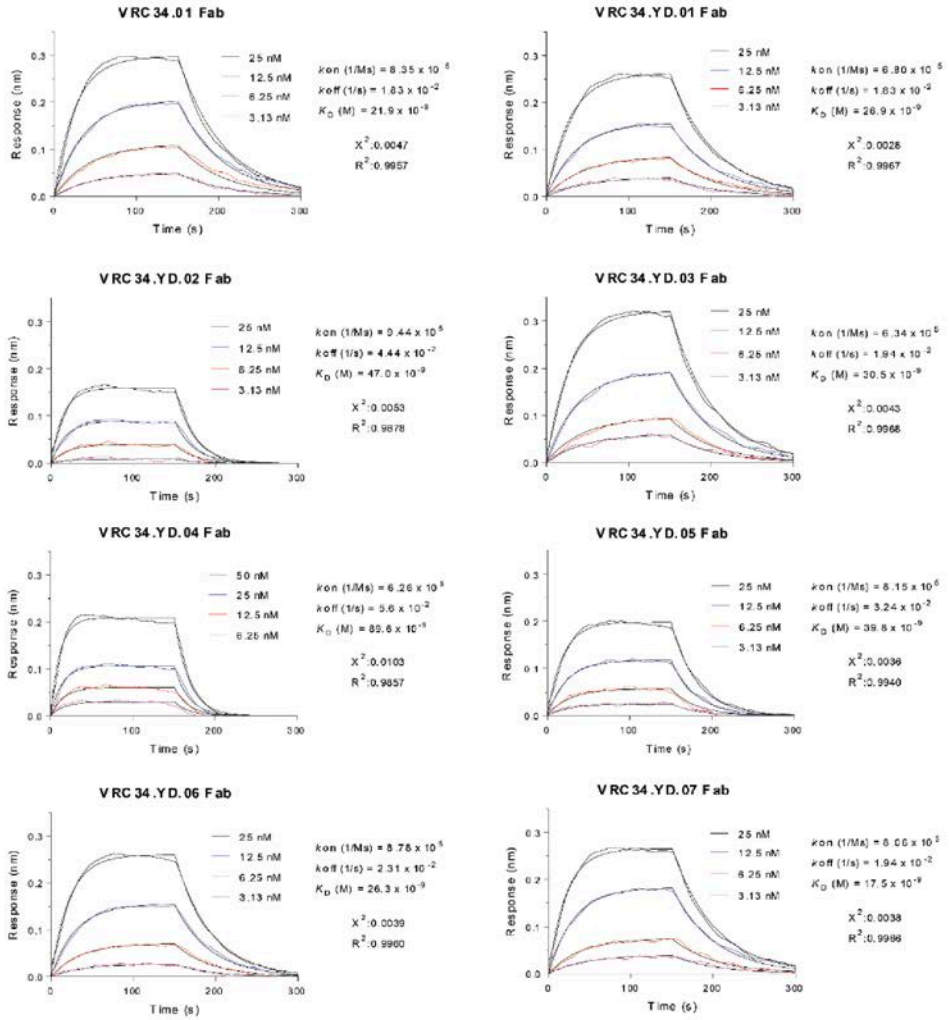


Identification of anti-HIV antibodies

Validation of low/middle/high affinity antibodies via biolayer interferometry



KD values correlated with FACS signal



Identification of anti-HIV antibodies

7 unique lineages (three non-synonymous aa substitutions within one codon suggest site-specific selection)

Virus	Clade	VRC34 YD.01	VRC34 YD.02	VRC34 YD.03	VRC34 YD.04	VRC34 YD.05	VRC34 YD.06	VRC34 YD.07	VRC34 01	VRC34 02*	VRC34 03*	VRC34 04*	VRC34 06*
TRO.11	B	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50
DU156.12	C	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	1.0	0.40
BG505.W6M.C2	A	0.22	0.38	0.17	0.27	0.22	0.17	0.21	0.14	0.10	0.71	0.11	>50
Q168.a2	AD	0.19	0.65	0.28	0.36	0.37	0.29	0.30	0.18	0.10	0.30	0.25	>50
Q23.17	A	0.13	0.29	0.08	0.13	0.08	0.05	0.11	0.11	0.062	0.24	0.28	>50
CAAN.A2	B	2.4	>50	>50	4.5	>50	>50	>50	2.1	0.44	8.5	>50	>50
ZM55.28a	C	0.28	1.0	0.25	0.33	0.22	0.15	0.44	0.21	0.47	19.0	>50	>50
KER2018.11	A	0.28	1.5	0.38	0.32	0.38	0.25	0.47	0.21	0.094	0.56	0.55	>50
BaL.01	B	0.90	2.9	1.3	1.5	2.0	0.72	1.1	0.71	0.14	0.31	0.071	>50
JRCSF.JB	B	>50	>50	>50	>50	>50	>50	>50	>50	0.56	>50	>50	>50
JRFL.JB	B	0.28	0.56	0.28	0.42	0.38	0.30	0.29	0.20	0.12	0.31	>50	>50
TRJO.58	B	6.7	47.3	8.2	19.3	35.3	9.8	8.0	4.8	5.5	>50	>50	>50
RW020.2	A	2.4	>50	3.3	0.44	1.8	0.59	4.5	1.1	0.78	>50	2.0	>50
YU2.DG	B	2.9	>50	>50	12.3	>50	>50	>50	2.0	2.8	>50	>50	>50
PVO.04	B	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50
THRO.18	B	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50
ZM106.9	C	5.1	>50	>50	>50	>50	>50	>50	6.4	>50	>50	>50	>50
Q769.h5	A	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50
ZA012.29	C	1.9	3.1	2.8	2.7	3.6	2.4	2.0	1.6	1.0	>50	>50	>50
DU422.01	C	0.30	2.6	0.72	0.44	4.8	0.72	0.62	0.29	2.2	>50	>50	>50
6101.1	B	1.9	6.6	2.1	2.1	3.0	2.1	1.8	0.91	0.60	1.5	>50	>50
BG1168.01	B	1.2	7.2	1.7	1.9	5.7	2.9	1.5	1.2	0.47	>50	>50	>50

*Reported in Kong *et al.*, Science 2017

Potency (μg/mL) 0.010-0.10 | 0.10-1.0 | 1.0-10 | 10-50 | >50

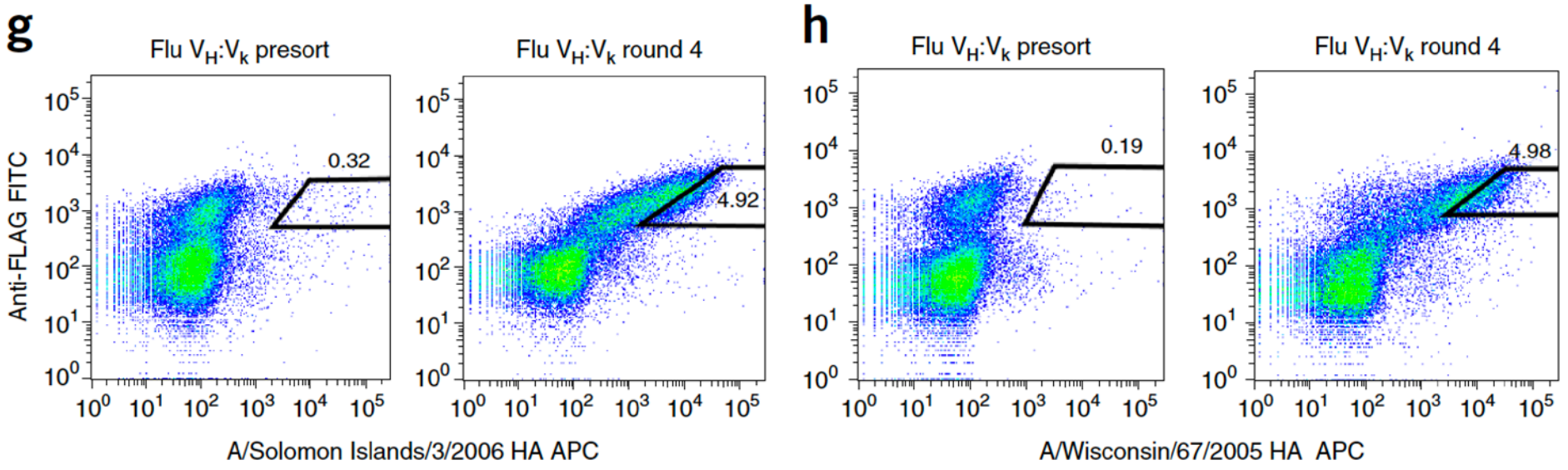
f

HIV antibody clone	HIV-1 neutralization breadth	Fab affinity (nM)	From affinity gates
VRC34.YD.01	16/22 (73%)	26.9 ± 1.4	–
VRC34.YD.02	12/22 (55%)	47.0 ± 9.2	–
VRC34.YD.03	13/22 (59%)	30.5 ± 1.7	–
VRC34.YD.04	15/22 (68%)	89.6 ± 20.1	Low
VRC34.YD.05	13/22 (59%)	39.8 ± 4.2	Medium
VRC34.YD.06	13/22 (59%)	26.3 ± 1.7	Medium
VRC34.YD.07	13/22 (59%)	17.5 ± 0.8	High

→ Identification of novel broadly neutralizing antibodies

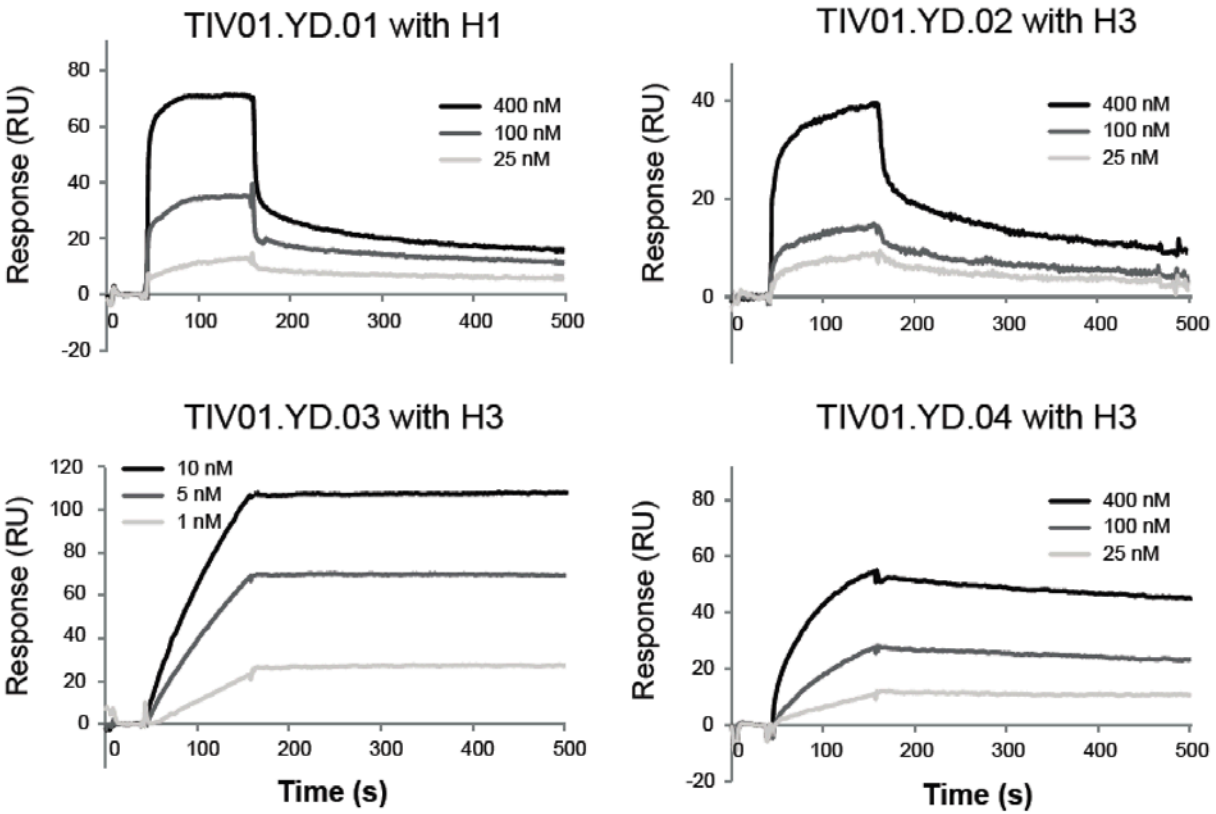
Identification of anti-influenza antibodies

Libraries from 12 million B cells 270 days post influenza vaccination
(0.01% B cells recognizing influenza hemagglutinin)



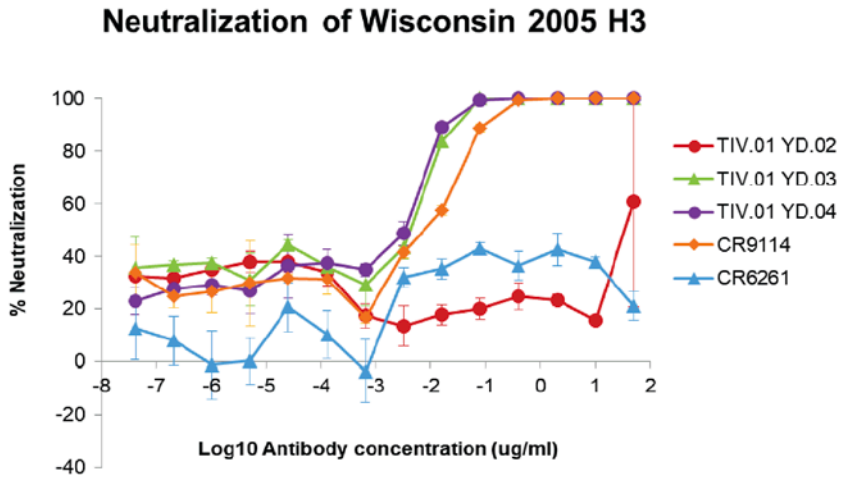
Identification of anti-influenza antibodies

4 antibody lineages with K_D = 0.35-39.9nM when expressed as IgG1's



Identification of anti-influenza antibodies

2 (3) antibodies neutralized influenza at pM concentrations



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Influenza antibody	IgG affinity (nM)		Neut. IC_{50} (pM)	
	H1	H3	H1	H3
TIV01.YD.01	11.1 ± 0.4	n.b.	n.n.	n.n.
TIV01.YD.02	n.b.	39.9 ± 6.0	n.n.	>333
TIV01.YD.03	n.b.	0.35 ± 0.25	n.n.	15.9
TIV01.YD.04	n.b.	4.5 ± 1.6	n.n.	10.5

Identification of natively paired

- HIV-1 broadly neutralizing antibodies
- high affinity neutralizing antibodies against Ebola virus glycoprotein and influenza hemagglutinin

Assessment of human antibody repertoires

MABS
2017, VOL. 9, NO. 8, 1282–1296
https://doi.org/10.1080/19420862.2017.1371383

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REPORT

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Rare, high-affinity anti-pathogen antibodies from human repertoires, discovered using microfluidics and molecular genomics

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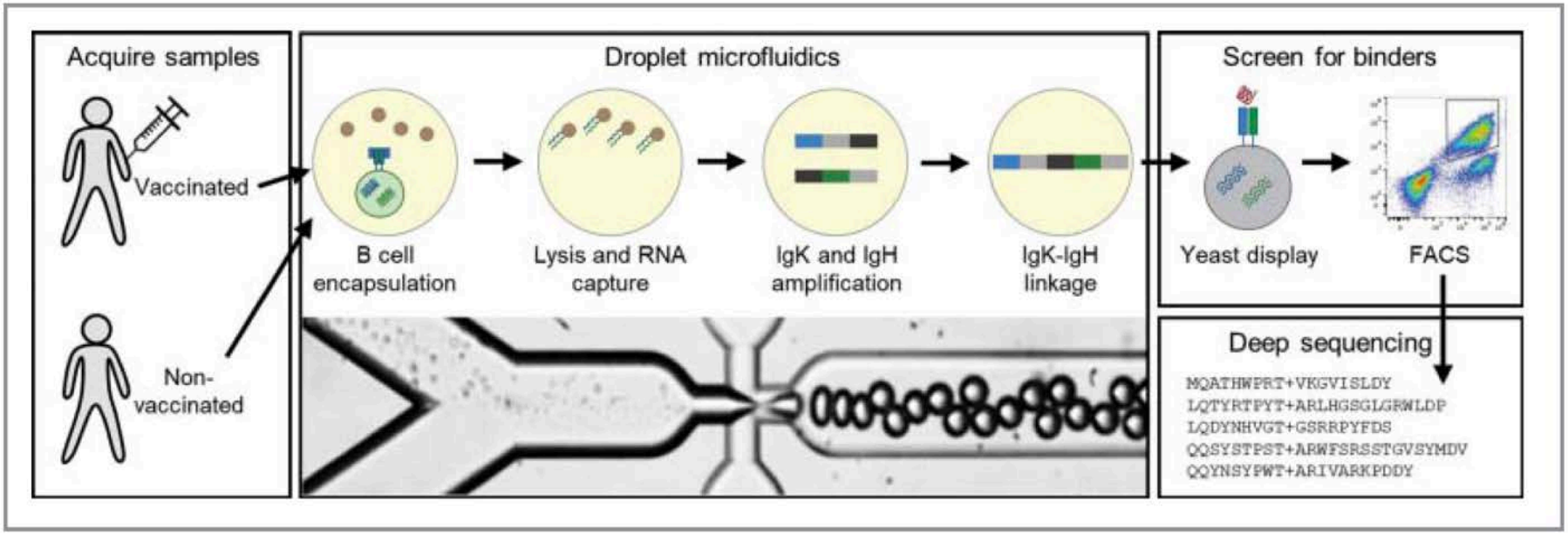
[a]GigaGen Inc., 407 Cabot Road, South San Francisco, CA, USA; [b]Immunobiology Section, Great Ormond Street Institute of Child Health, University College London, London, England, United Kingdom

- Generation of natively paired scFv's from healthy vaccinated/non-vaccinated donors using microfluidics (two-step emulsion)
- Cloning of scFv libraries for yeast display
- Identification of high-affinity antibodies against Influenza A virus and pneumococcus bacteria

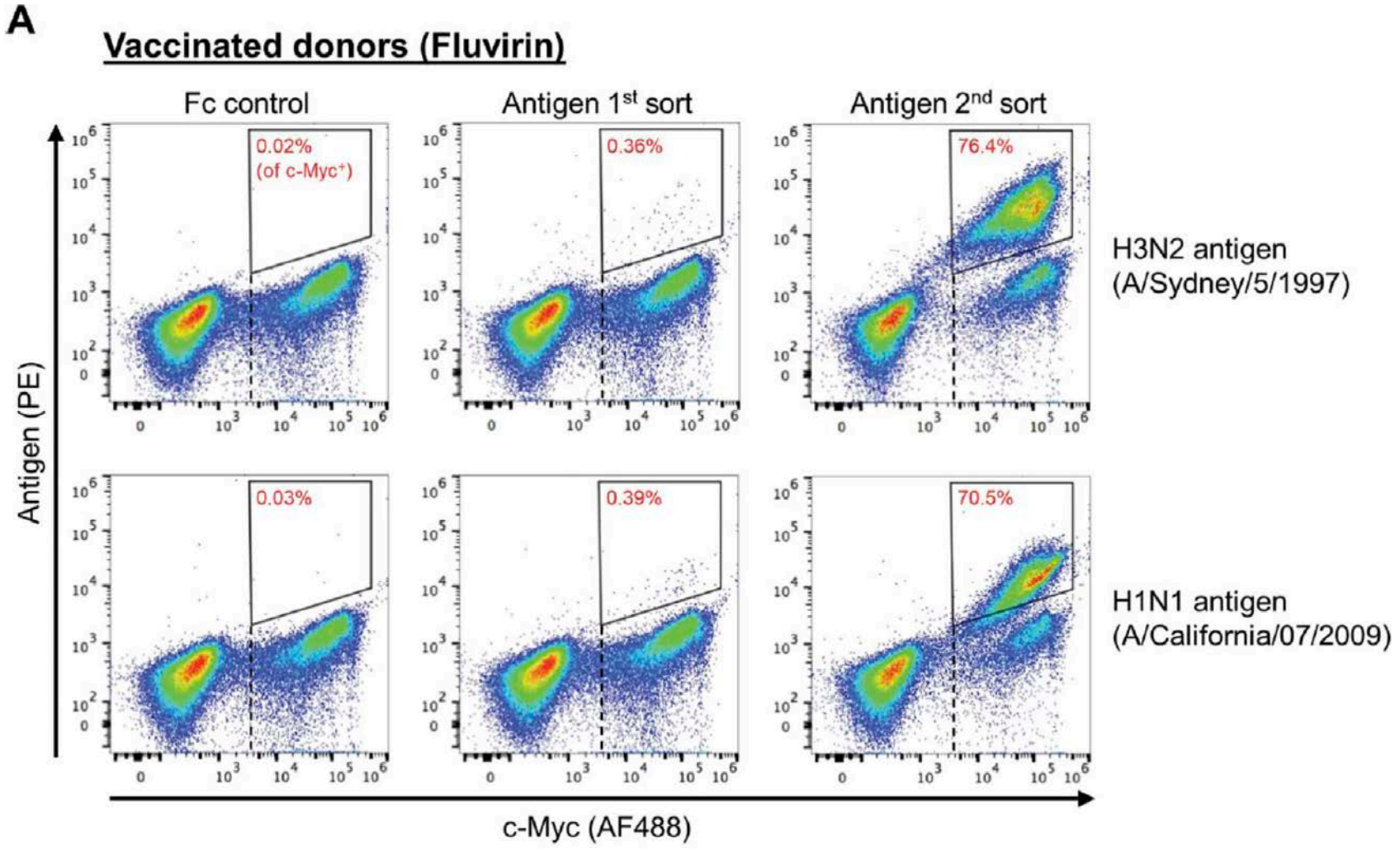
Workflow Overview



scFv libraries subjected to FACS for influenza A virus

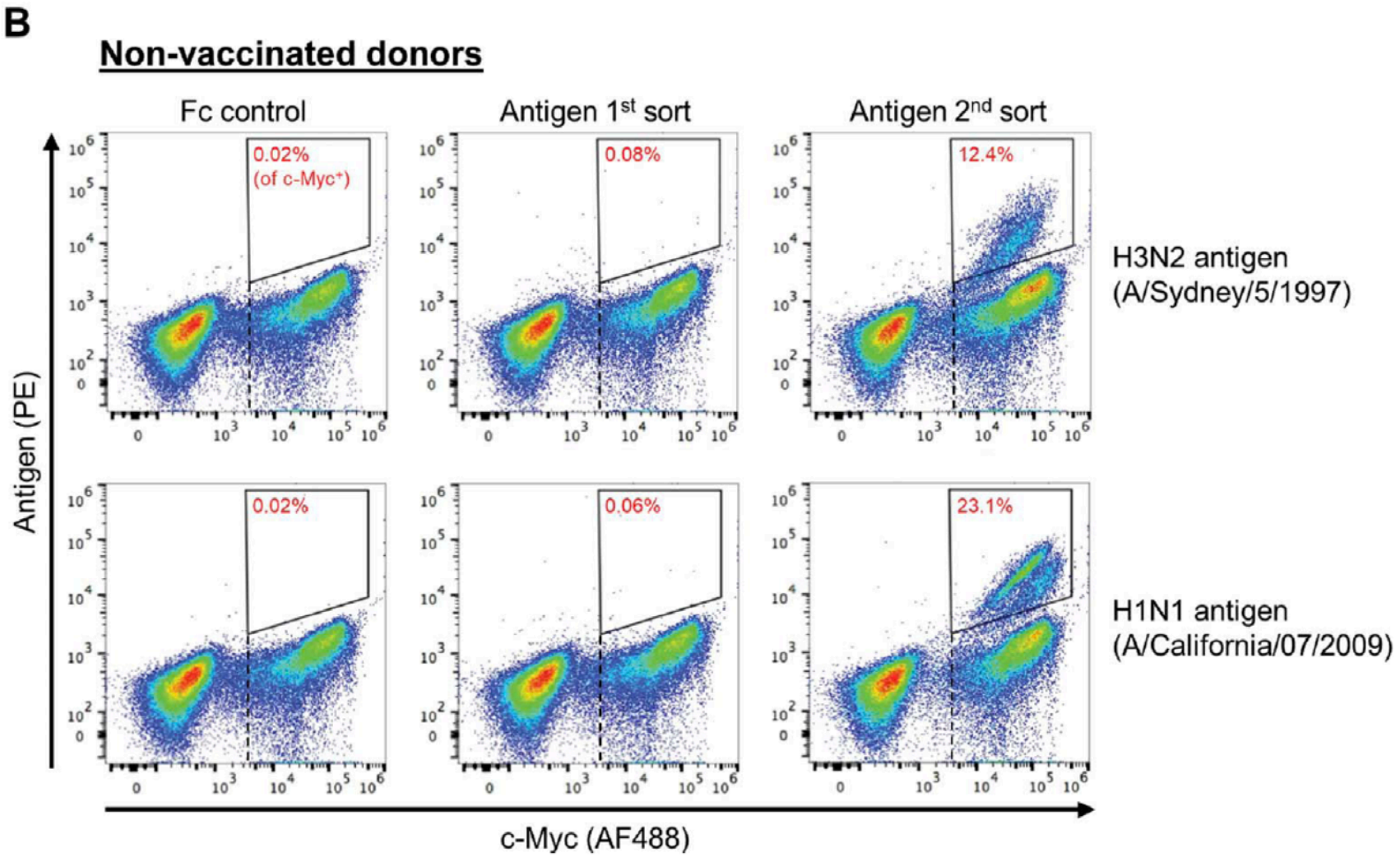
Donors vaccinated for Influenza A (Fluvirin, pool of 3 patients)

leukapheresis (and ELISA on serum) at Day 10



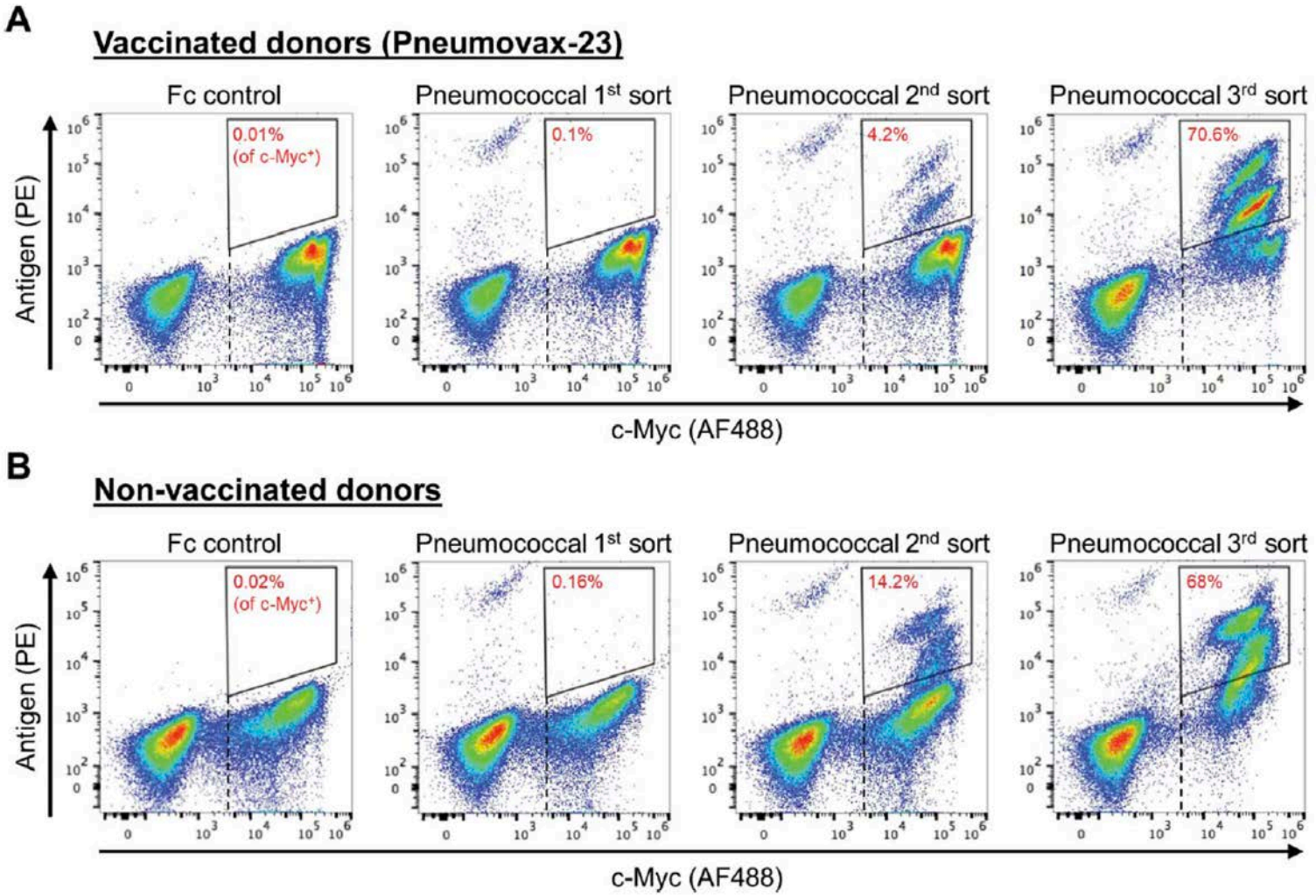
scFv libraries subjected to FACS for influenza A virus

Non-vaccinated healthy donors (pool of 52 patients)

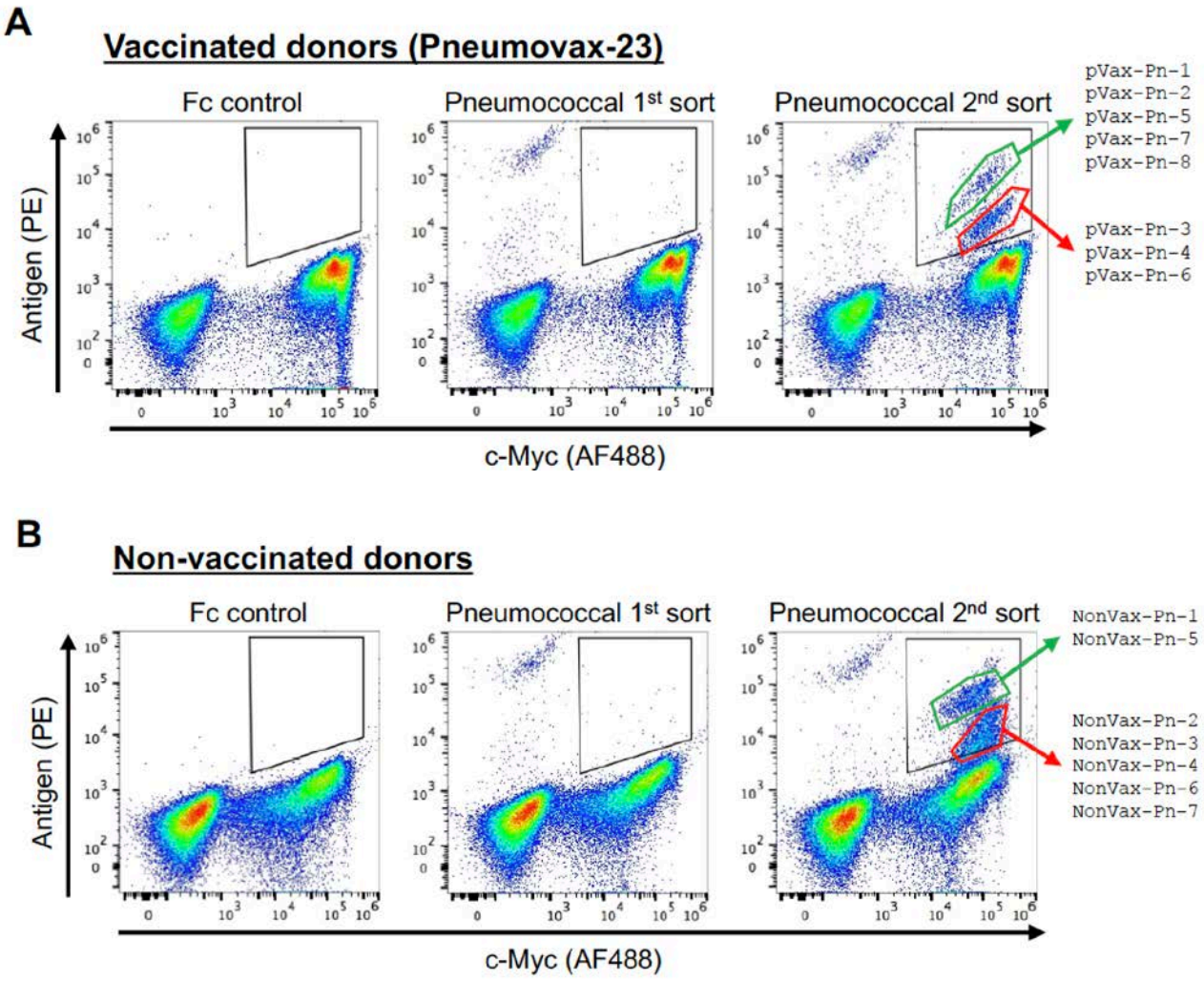


scFv libraries subjected to FACS for pneumococcus polysaccharides

Donors vaccinated for pneumococcus (Pneumovax-23, pool of 3 patients)
leukapheresis (and ELISA on serum) at Day 8



scFv libraries subjected to FACS for pneumococcus polysaccharides



- Distinct scFv sequences binding to different pneumococcal epitopes
- Increased signal due to binding multiple epitopes or differential polysaccharide labeling

Identification of antibodies against Influenza A/pneumococcus

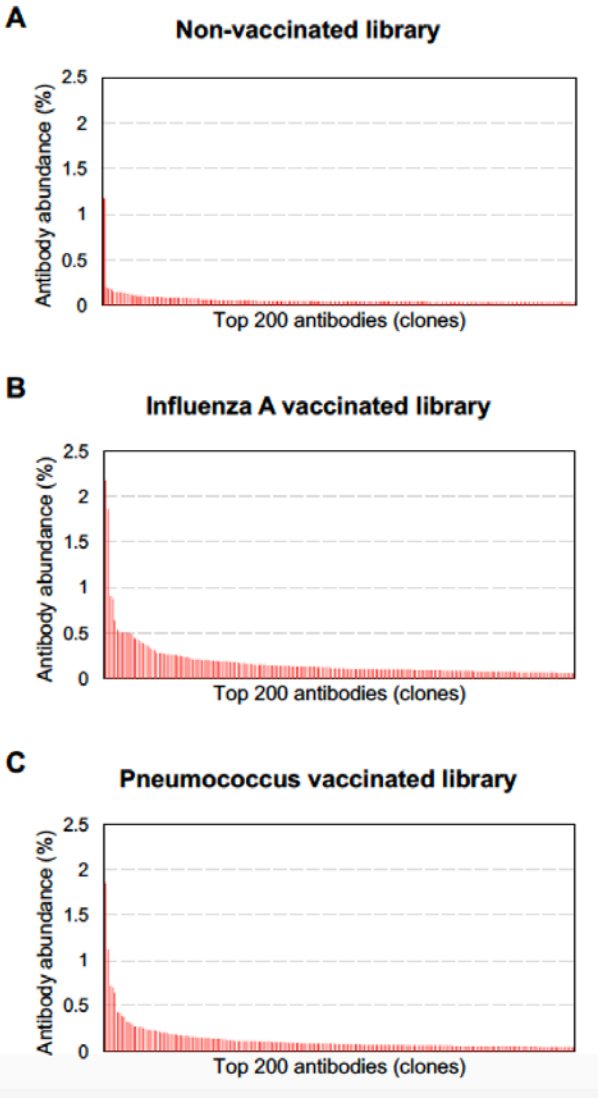
Pre-sort libraries

- 10 000 – 17 500 clones/library
  (clones: <2aa difference)
- Divergence:
  92.5% for IgHV
  95% for IgHK
- Vaccinated libraries have more abundant sequences
- Pneumococcal vaccinated library:
  higher R/S ratio for IgH
  lower R/S ratio for IgK
  (measure of somatic hypermutations)

46% IgG1
30% IgG2

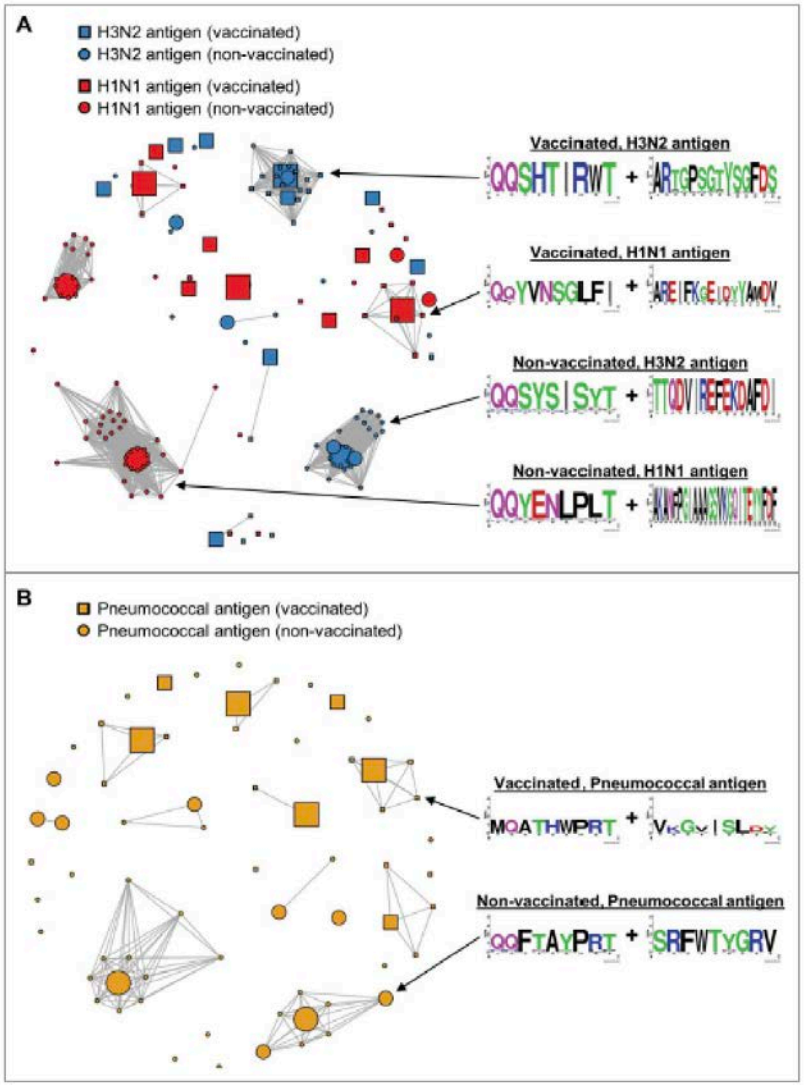
57% IgG1

81% IgG2

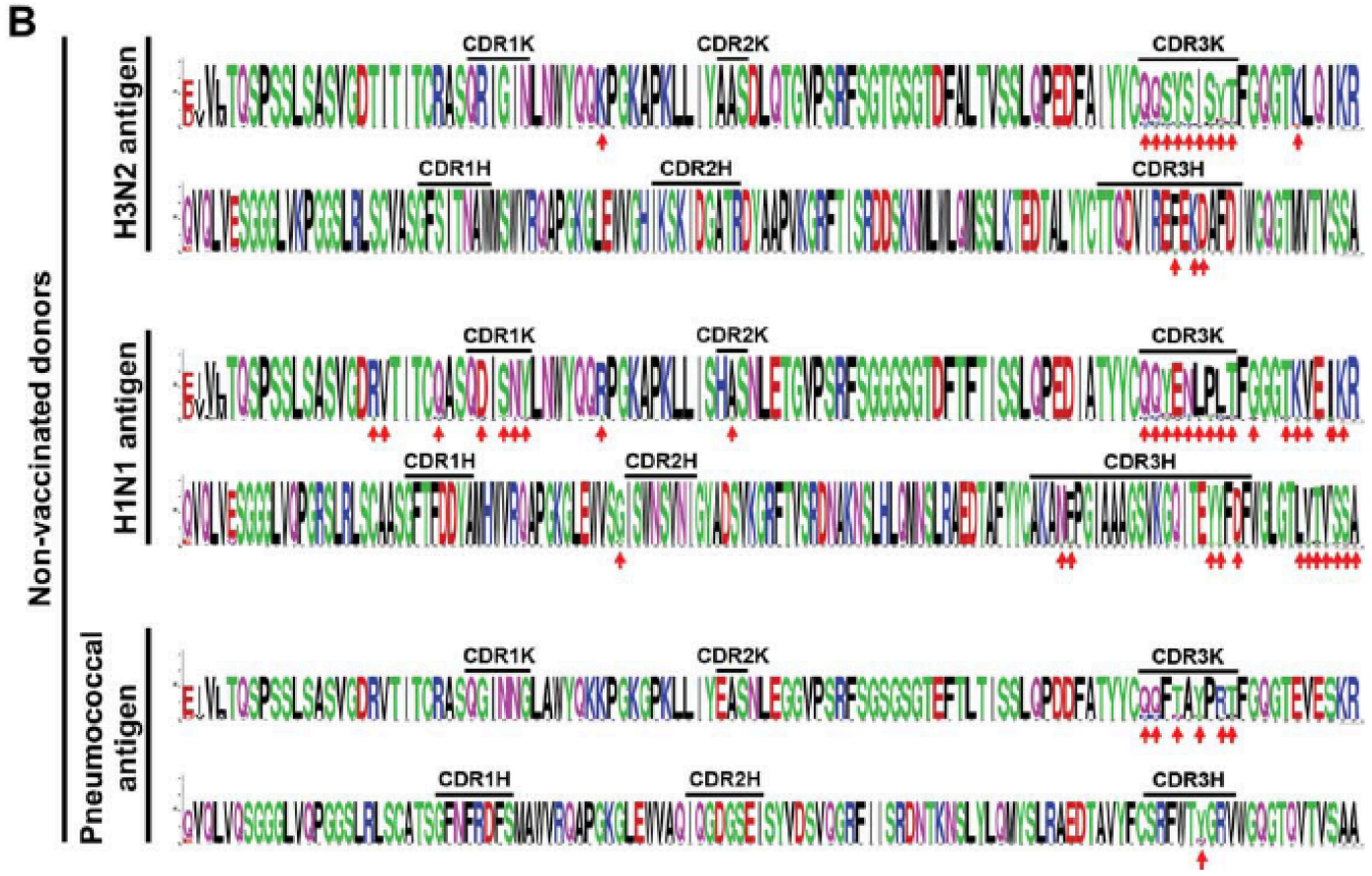


Identification of antibodies against influenza A/pneumococcus

- 247 antibodies are present at >0.1% frequency in post-sort (31-59/library)
- Identification of antibodies that were not present in pre-sort
- Identification of clonal lineages (arise from affinity maturation and selection in vivo: <9aa differences) clusters contain scFv's from either of the libraries

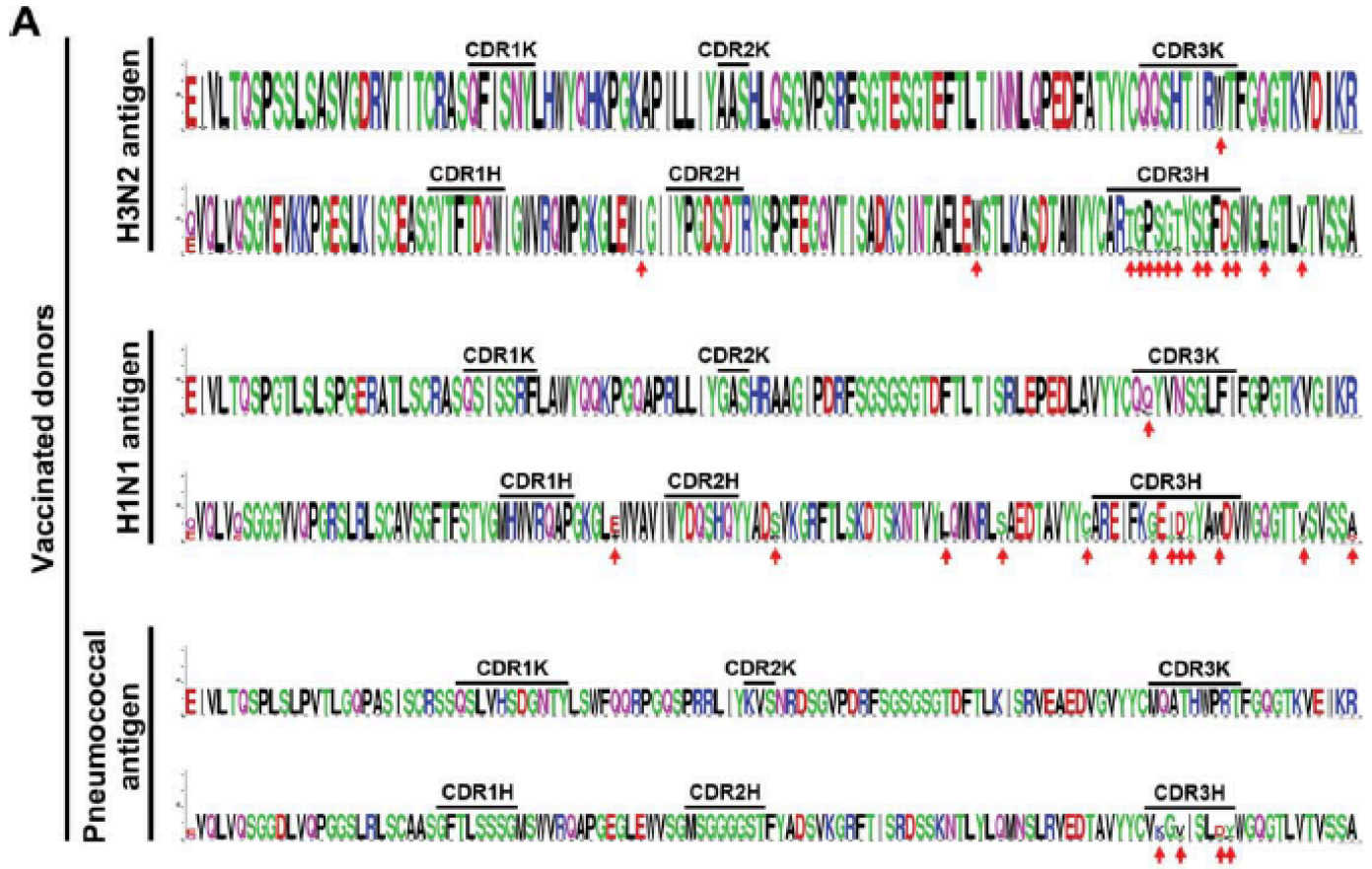


Analysis of scFv clonal lineages



→ Sequence variation in CDR3H and CDR3K

Analysis of scFv clonal lineages



→ Sequence variation mostly in CDR3H

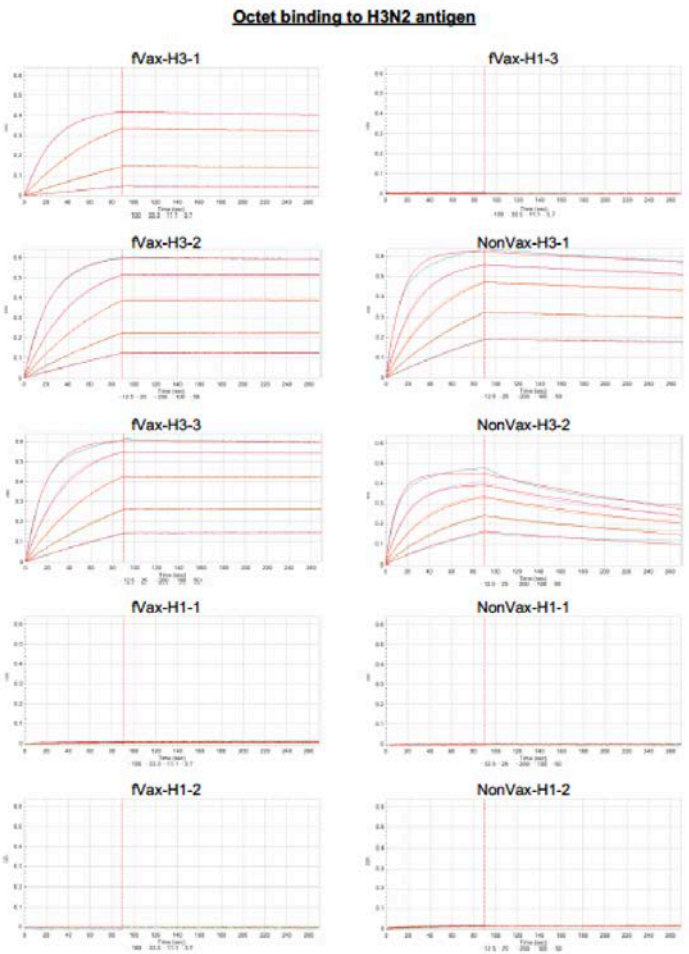
Higher R/S ratio in Pneumovax library (increased frequency of highly affinity mature antibodies)

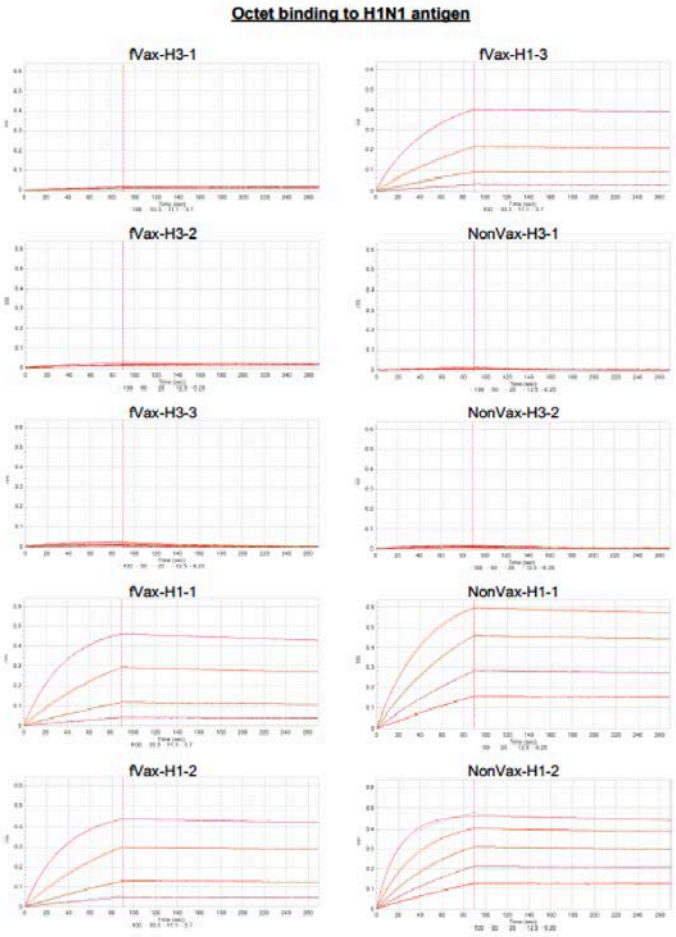
Similar divergence pre/post-sort

Generation of 10 anti-flu antigen IgG1 antibodies

AB expression in CHO and purification with protein A chromatography

→ Bio-layer inferometry: K_D = 73pM - 8.8nM





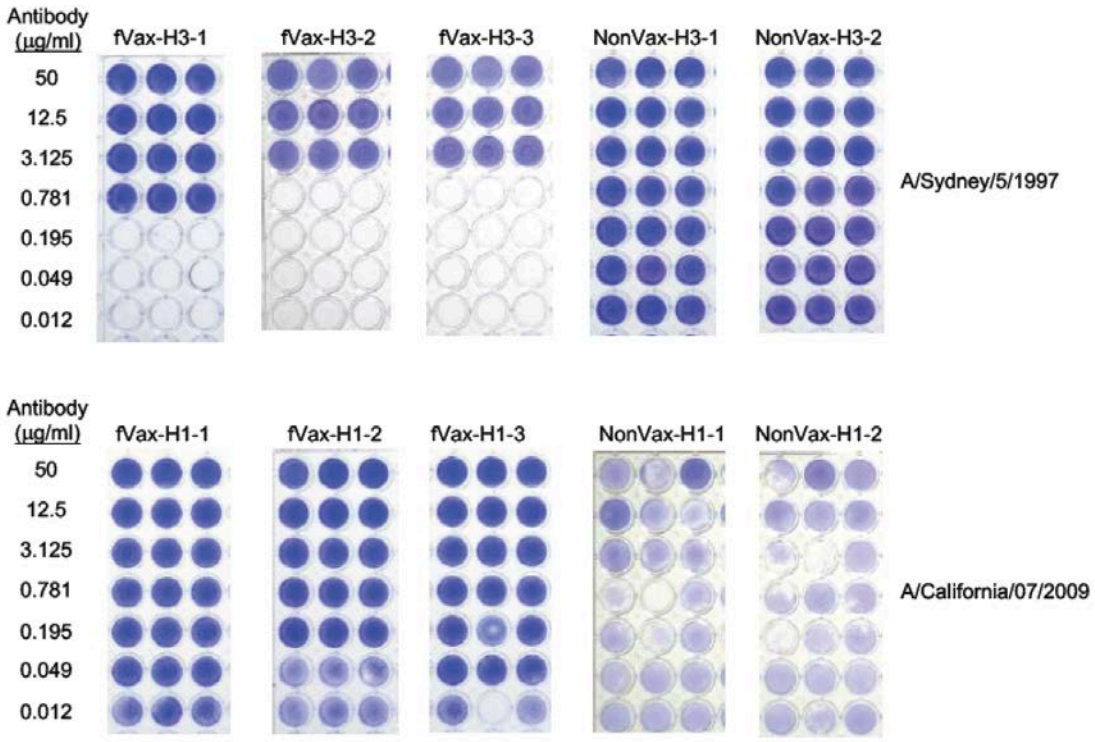
Generation of 10 anti-flu antigen IgG1 antibodies

AB expression in CHO and purification with protein A chromatography

→ Neutralization Assay: 10 (10) anti-flu antigen antibodies

Incubation of antibodies and influenza viral strains

Monitoring of cytophathic effect upon addition to MDCK cells (alive cells stain blue)



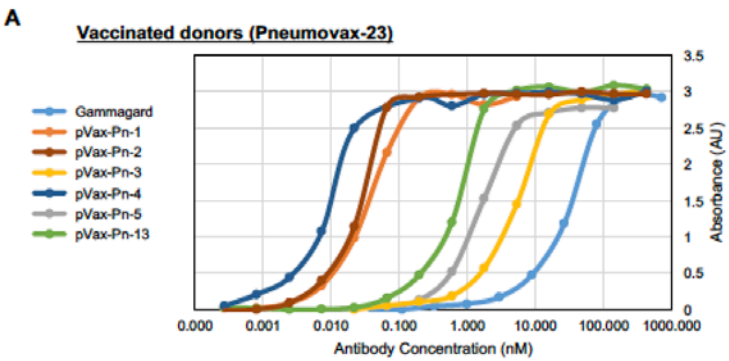
Generation of 9 pneumococcal antigen-binding IgG1 antibodies

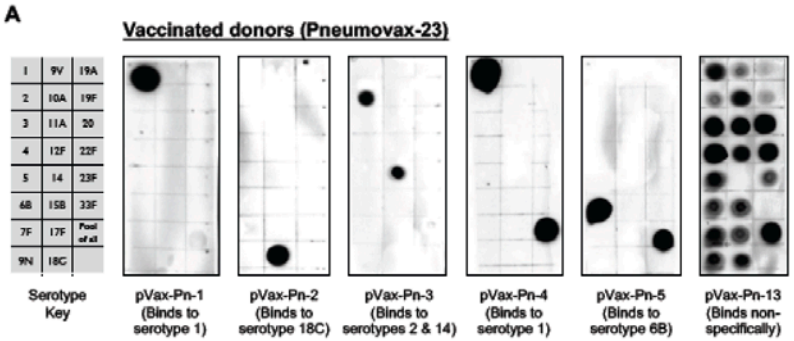
AB expression in CHO and purification with protein A chromatography

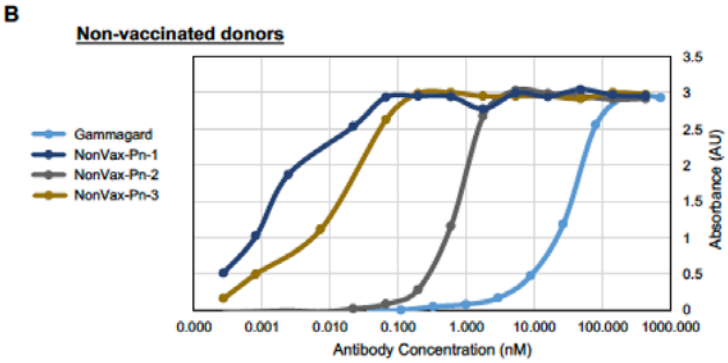
→ ELISA: EC50 = 0.001 - 5.3nM

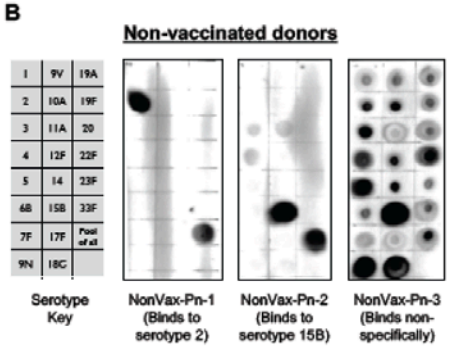
7 antibodies recognize one or two polysaccharides (serotype-specific dot blot)

2 antibodies recognize all polysaccharides (serotype-specific dot blot)









Generation of 9 pneumococcal antigen-binding IgG1 antibodies

AB expression in CHO and purification with protein A chromatography

→ Opsonophagocytic killing assay

6 (7) specific antibodies can prevent ingestion/phagocytosis/killing of bacteria expressing corresponding polysaccharide

1 (2) non-specific antibodies can prevent ingestion/phagocytosis/killing of bacteria expressing 2 (out of 8 tested) polysaccharides

- Generation of natively paired scFv libraries
- Identification of 247 antibodies (some being as rare as 1 in 100 000) and 76 clonal clusters
- Generation of 17 antibodies, all of which bind the respective antigen and 15 of which are active and therefore good candidates for passive immunization

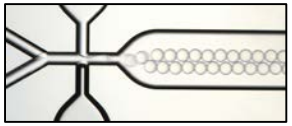
Exploiting the potential of human antibody repertoires

Affinity maturation through B cell selection

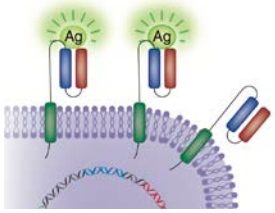
- Diversity
- Expression
- Stability
- Specificity

High-throughput approaches

- Microfluidics: miniaturization and parallelization
  Preservation of native V_H/V_L pairing



- Display methods (phage/yeast)
  Linkage of antibody sequence and function



Thank you !