



Chicken Heavy Chain only antibodies to SARS-CoV2

Phil Leighton

AET December 15, 2021

Ligand[®]

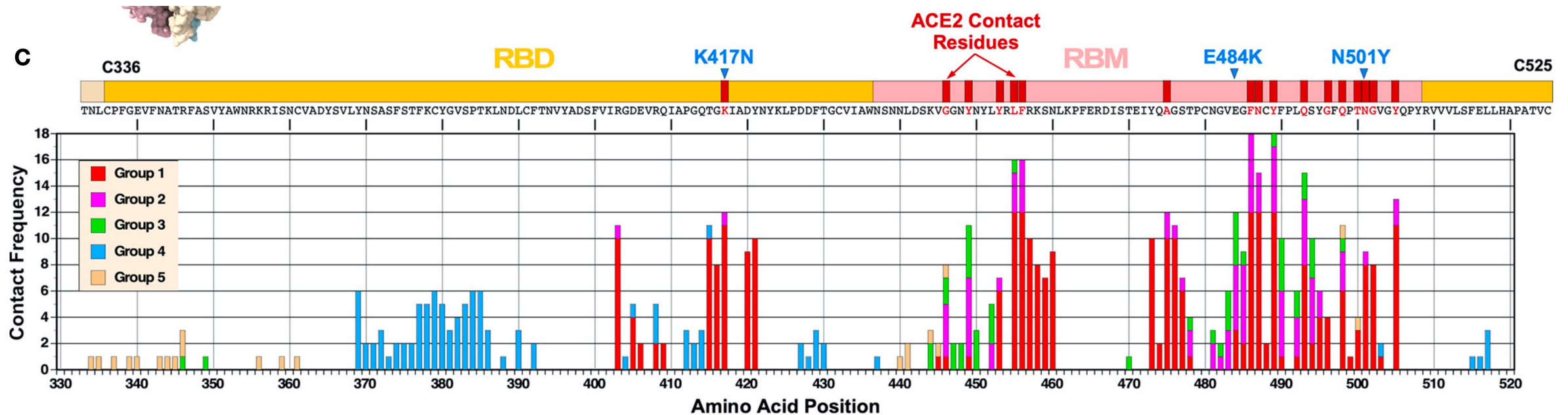
Antibody therapies for Covid-19

- mAb therapeutics authorized for emergency use:
 - casirivimab/imdevimab (Regeneron)
 - bamlanivimab/etesevimab (Lilly)
 - tixagevimab/cilgavimab (AstraZeneca)
-
- As of December 2021, 3968 antibodies and 485 nanobodies listed in CoV-AbDab database (Charlotte Deane, Univ. of Oxford)

Neutralizing antibodies and their modes of action

Indirect, such as by destabilizing prefusion conformation:

Direct competition with ACE2:



Niu L, Wittrock KN, Clabaugh GC, Srivastava V, Cho MW. A Structural Landscape of Neutralizing Antibodies Against SARS-CoV-2 Receptor Binding Domain. Front Immun 2021; 12:647934.

Advantages of single domain antibodies against SARS-CoV2

- Smaller size than antibodies, can access epitopes that may be inaccessible to full antibodies
- More stable than antibodies
- Can be made inhalable (Pittsburg Inhalable Nanobody-21, PiN-21)
- Thus can be used without injection, and applied more directly to the respiratory system

Single domain antibodies being developed to SARS-CoV-2

- Picobodies™ (cow ultralong CDR-H3), presented on Monday by Vaughn Smider and Diane Retallack
- Nanobodies – llama, alpaca
 - Koenig et al. (Dioscure, Bonn) 2021
 - Xu et al. (NIH) 2021
 - Huo et al. (UK) 2020
 - Xiang et al. (Pittsburgh) 2020 [PiN-21]
 - Li et al. (Pittsburgh) 2020
 - Twist
 - Schoof et al. (UCSF) 2020
 - Hanke et al. (Karolinska) 2020
 - Esparza et al. (NIH) 2020
 - Wrapp et al. (UT Austin) 2020
 - Custodio et al. (EMBL) 2020
- Shark VNAR
 - Gauhar et al. (Ossianix) 2021



Transgenic chickens expressing heavy chain-only antibodies: OmniDab



Normal antibodies in
chickens (IgY)
One IgH locus
One IgL locus



VHH transgene

Truncated light chain transgene



Chicken heavy-chain only antibodies

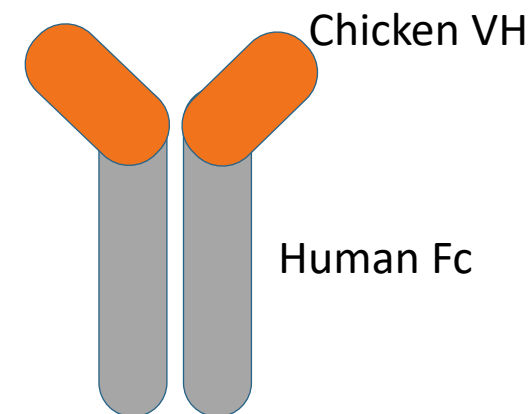


Normal
antibodies in
chickens
(IgY)



Truncated light chain (tLC)
Wild type heavy chain

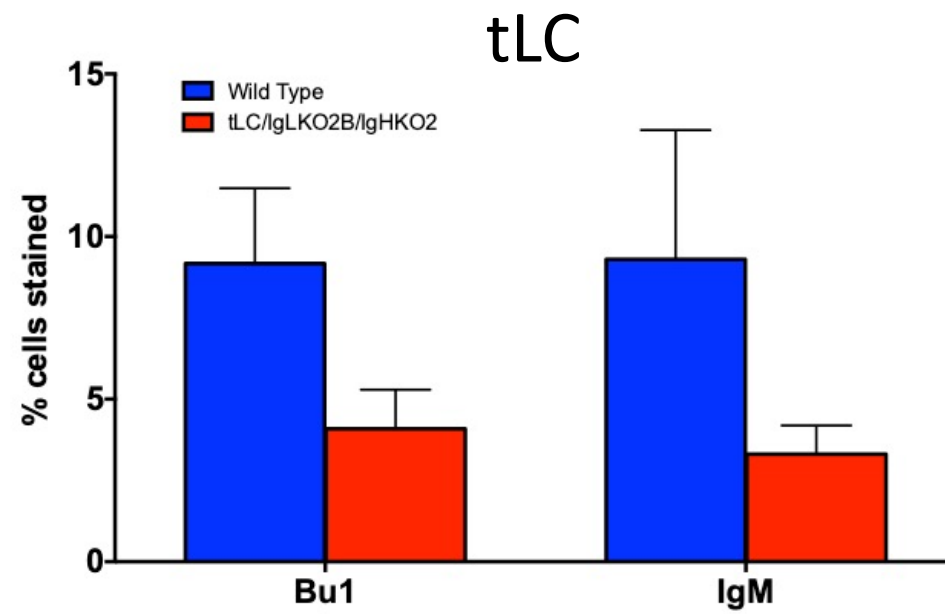
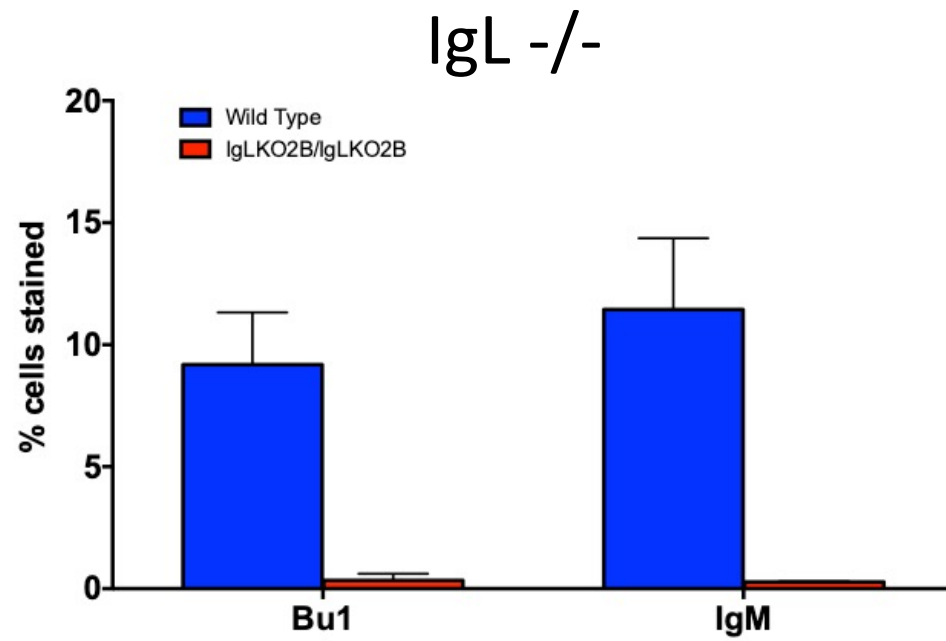
reformat



chVH-huFc

- Normal chicken heavy chain can express as VH alone
- VHH transgene in development

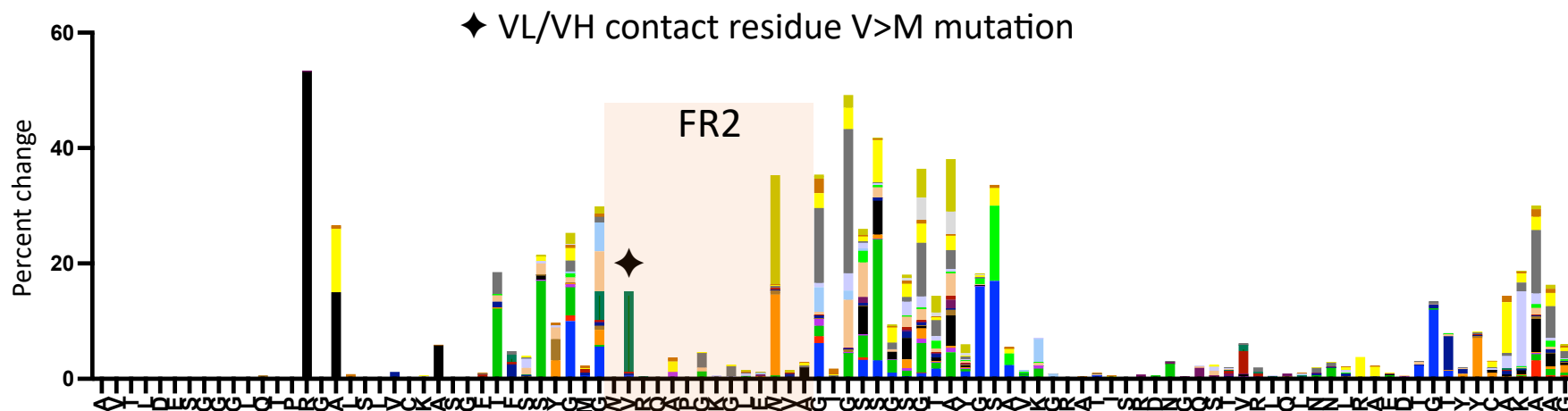
B cell development in tLC birds



NGS: VH diversity is similar in tLC and WT birds

Amino acid changes from germline at each position of VH. Splens from Progranulin-immunized birds

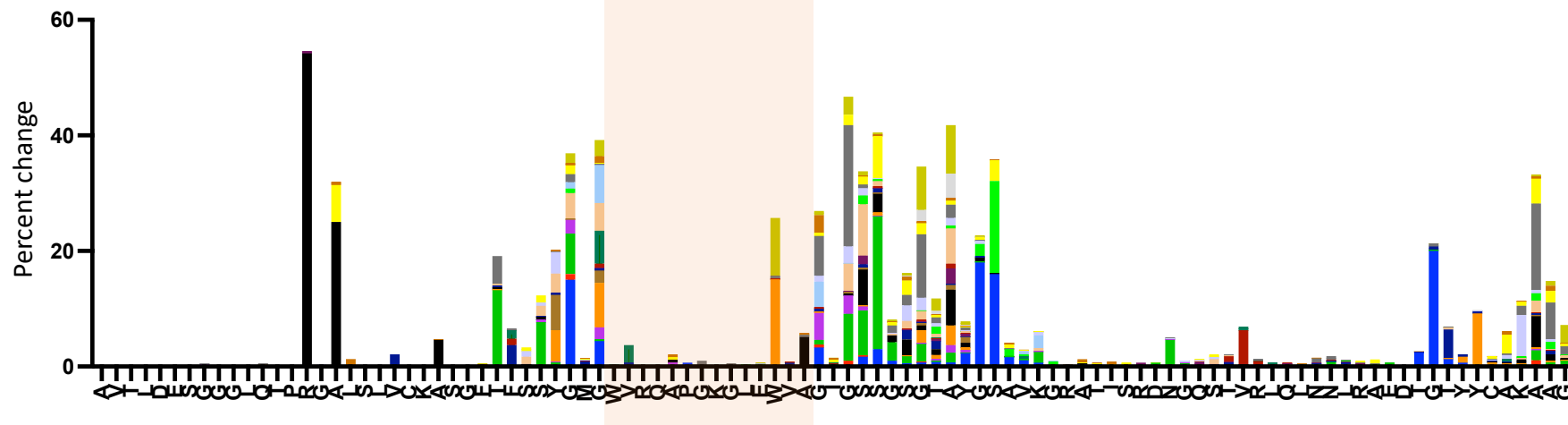
VH from tLC birds (3)



Mean mutation freq

15.1

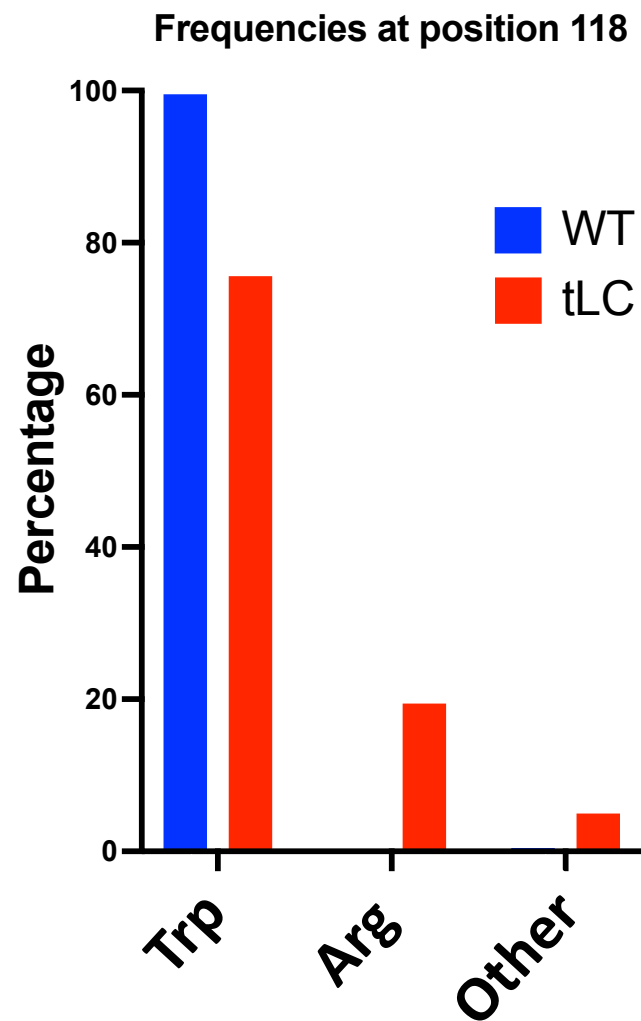
VH from normal birds (2)



14.3

$P < 0.0001$

NGS: position 118 is often mutated to Arg, as in camelids



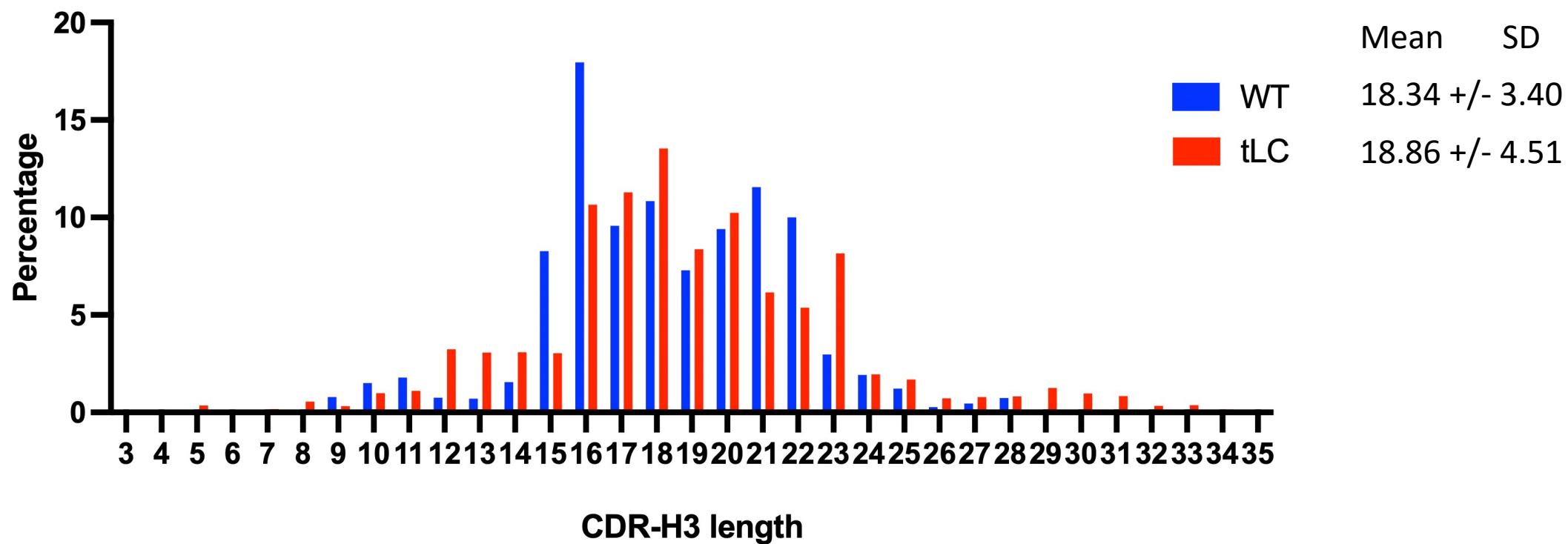
Framework 4

...SIDA**W**GHGTEVIVSS...

...SIDA**R**GHGTEVIVSS...

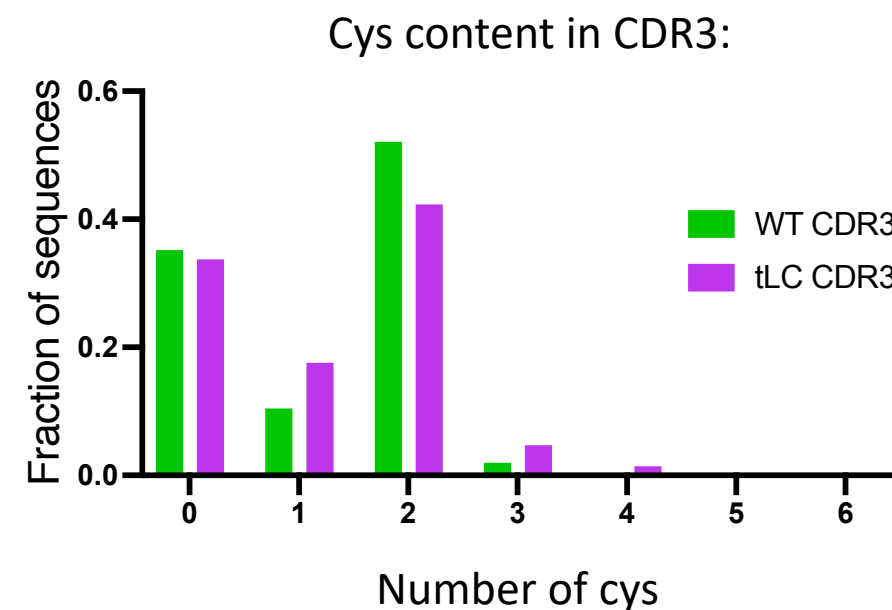
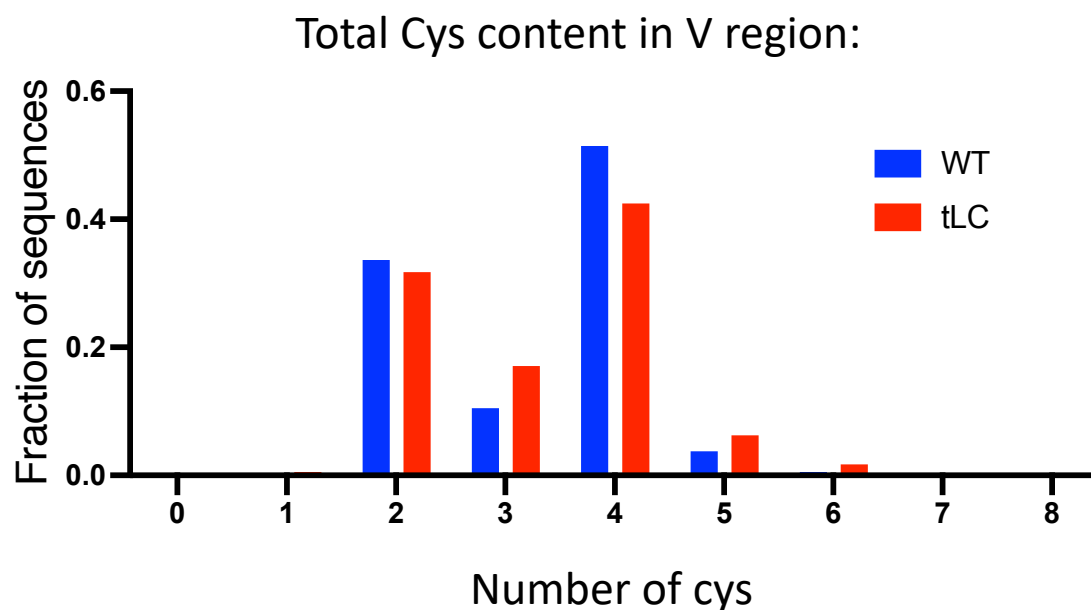
~10% Arg in camelid VHH

Average CDR-H3 length is the same in tLC and WT birds



NGS: frequency of non-canonical cysteine residues is similar in heavy chain only sequences from chicken

- *chicken CDR-H3 often contain a pair of cysteines*



>VHH from camelids often have CDR1-CDR3 paired cysteines

Antibody discovery for SARS-Cov2 spike binders



Cohort 1

Immunize with S1 protein (original strain)



Screen for binding to RBD,
S1, trimer
GEM single B cell screening

Genotype

tLC

Wild type IgH

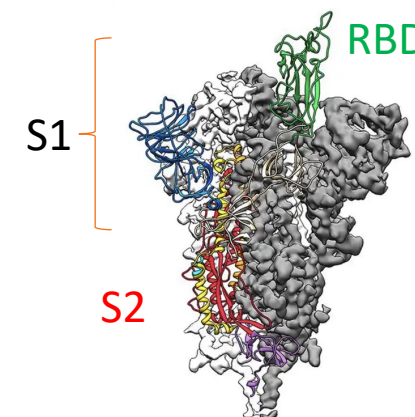
Cohort 2

Immunize with spike trimer. 2 boosts each:

Alpha

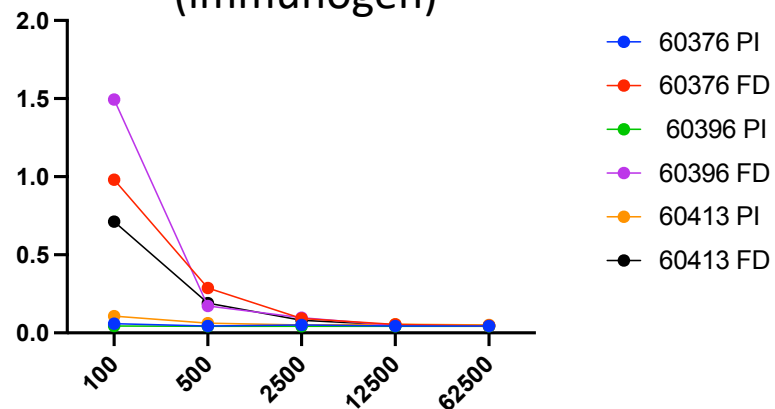
Beta

Delta

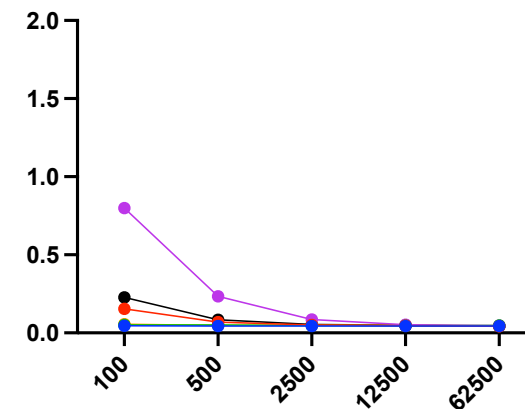


Serum titers – immune response

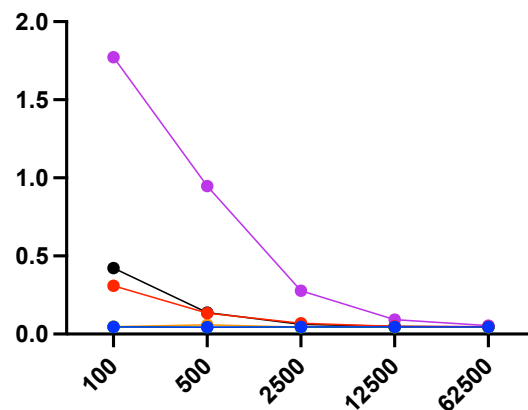
S1, D614G
(immunogen)



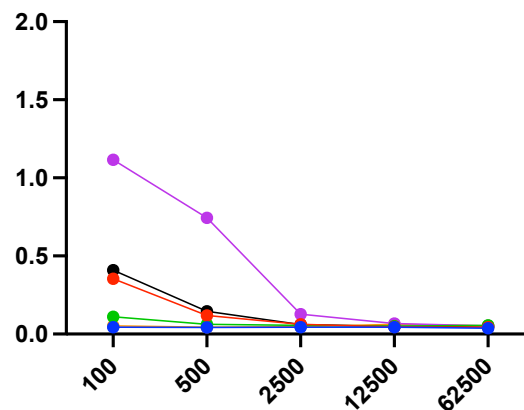
Cohort 1 RBD



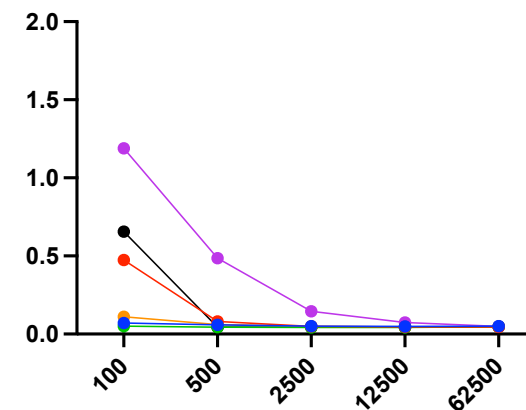
Cohort 1 Alpha



Cohort 1 Beta



Cohort 1 Delta

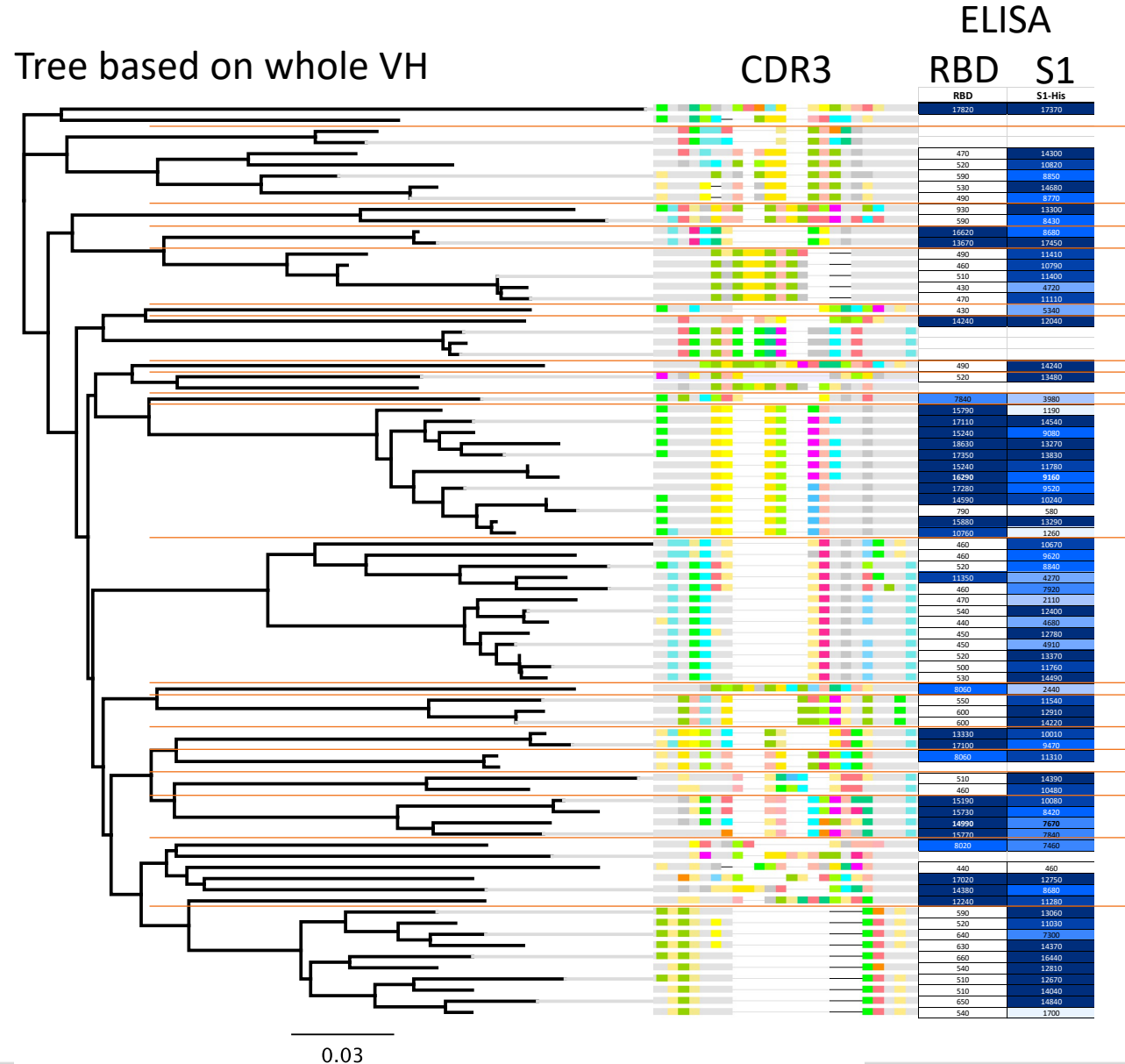


Antibody Discovery by GEM single B cell screening

Bird	Genotype	GEM plates	On beads	# unique	# confirmed
60376	TLC, chVH	1	S1	6	5
60396	TLC, chVH	1-4	RBD	19, 15, 26, 11	17, 14, 22, 8
		5-6	SA and UK trimer	6, 22	4, 10
60413	TLC, chVH	1	RBD	14	8
		2	SA or UK trimer	20	8

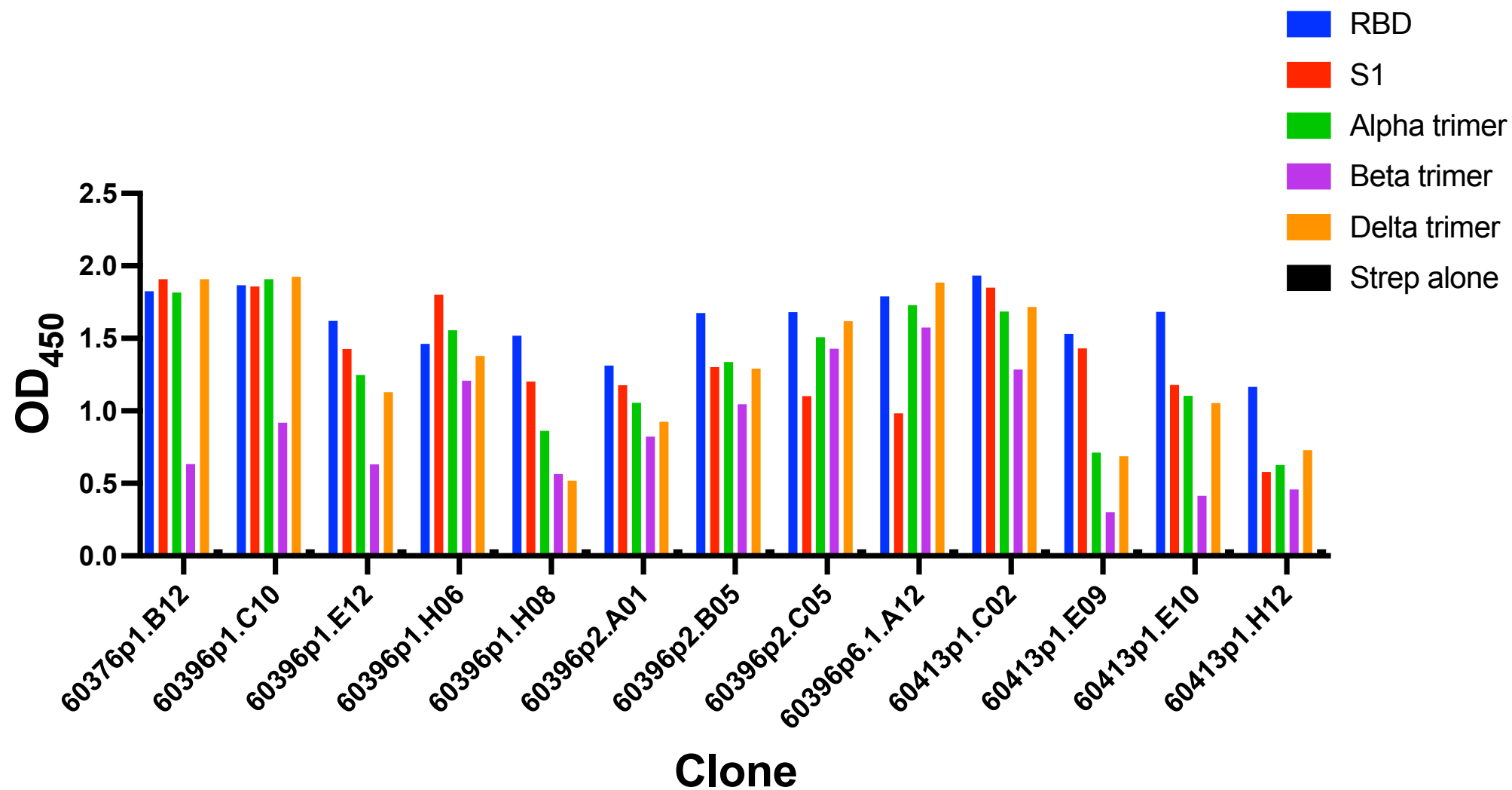
Amplify chicken VH from single cells and clone with human Fc

Many unique clones and clonotypes identified



- Diverse sequences and multiple clonotypes
- Antibodies show specificity
- About half the clones end up binding to RBD
- All bind to S1 (immunogen)

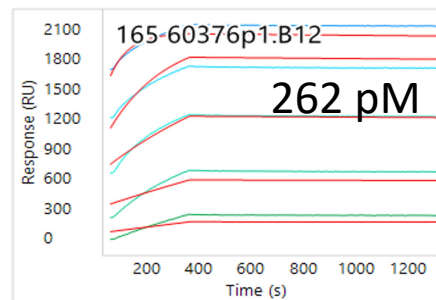
ELISAs on selected RBD binders



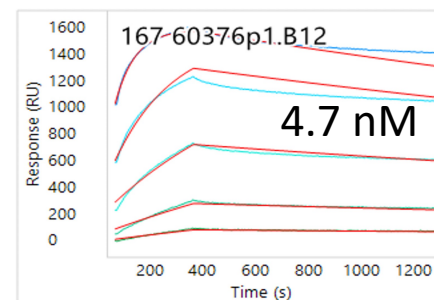
Kinetics - examples

- avidity effect of trimer
- Affinities to variants may differ

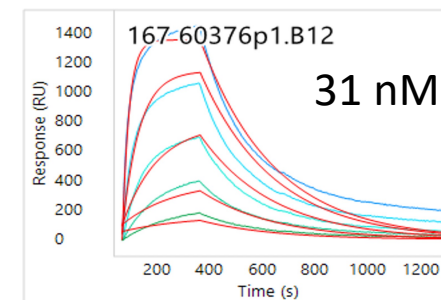
WT Trimer



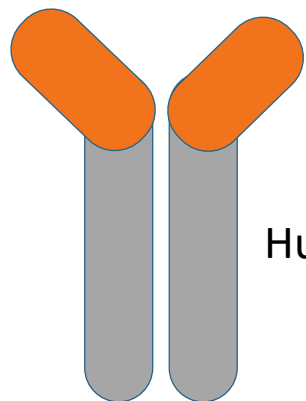
Beta (SA) Trimer



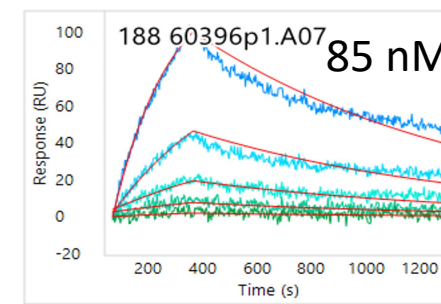
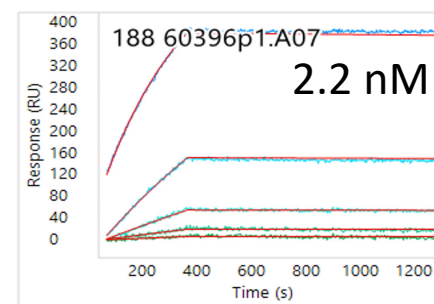
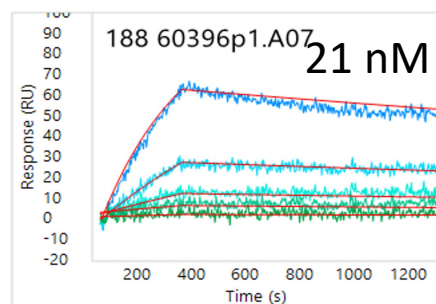
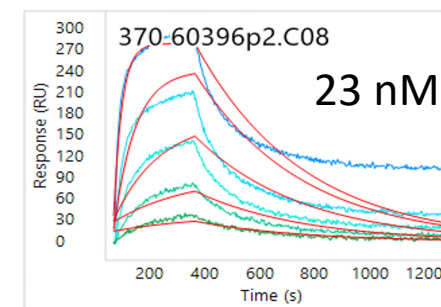
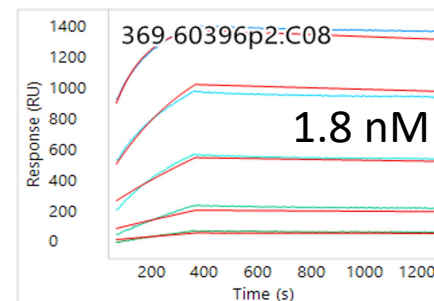
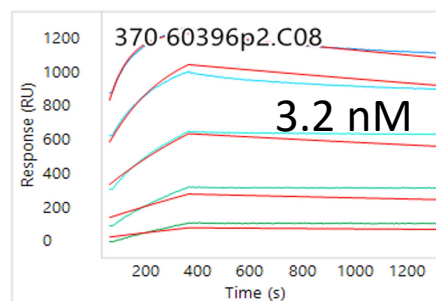
S1 (WT) monomer



Bivalent format:

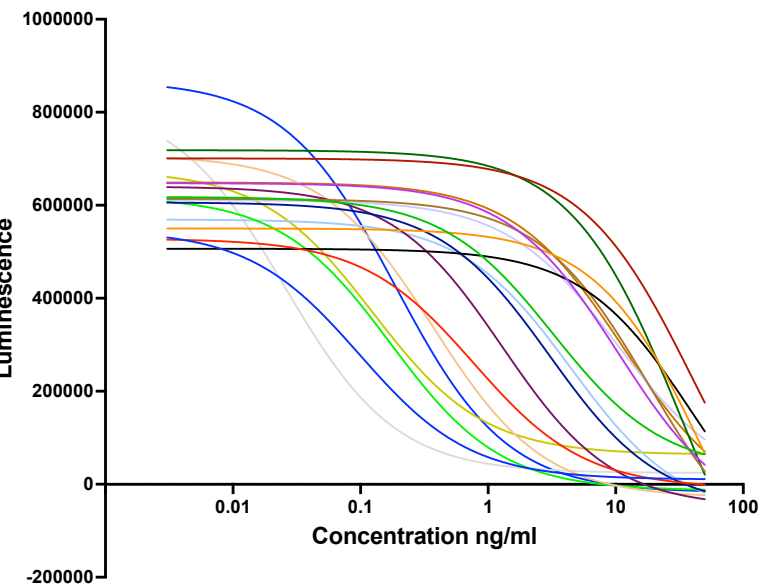


Human Fc



Pseudovirus neutralization

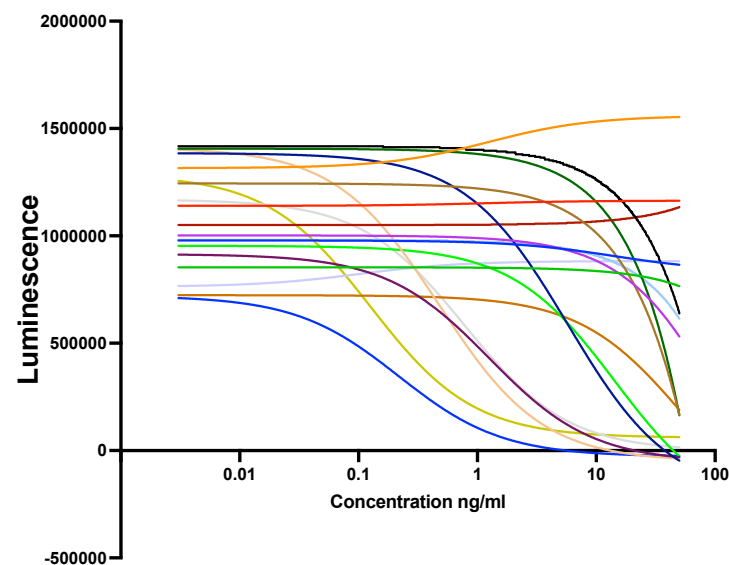
Alpha (B.1.1.7)



Δ69-70, Δ144, N501Y, A570D, D614G, P681H, T716I, S982A, D1118H

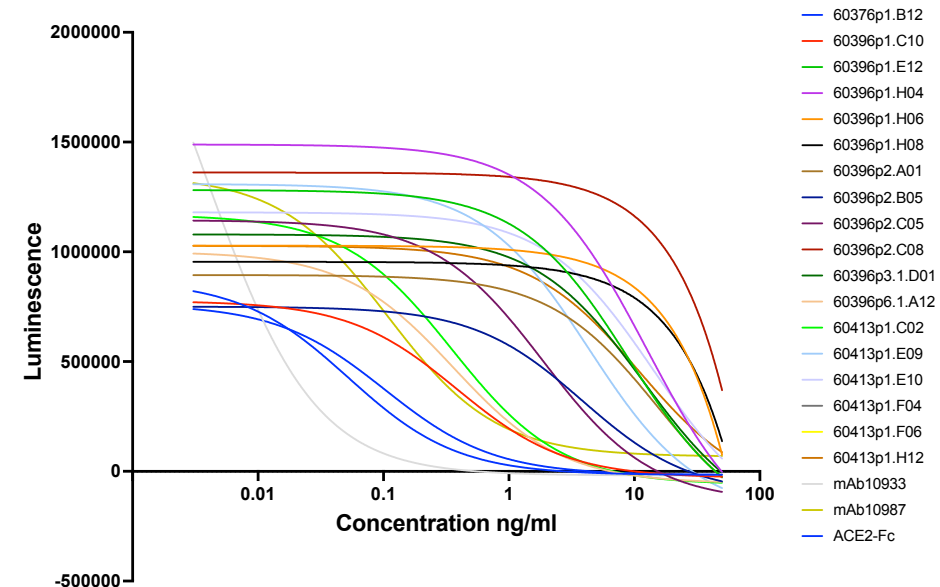
RBD: 336-525

Beta (B.1.351)



L18F, D80A, D215G, R246I, K417N, **E484K**, N501Y, D614G, A701V, Δ242-244

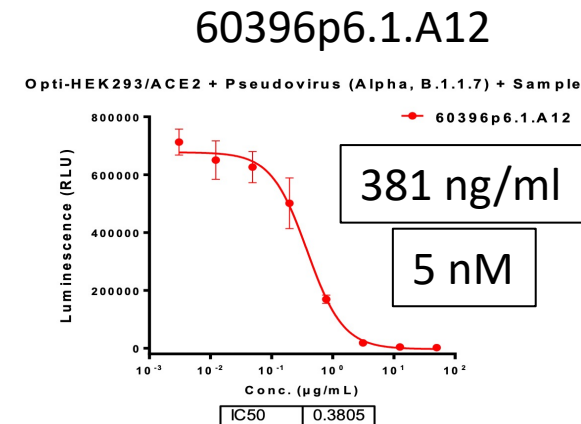
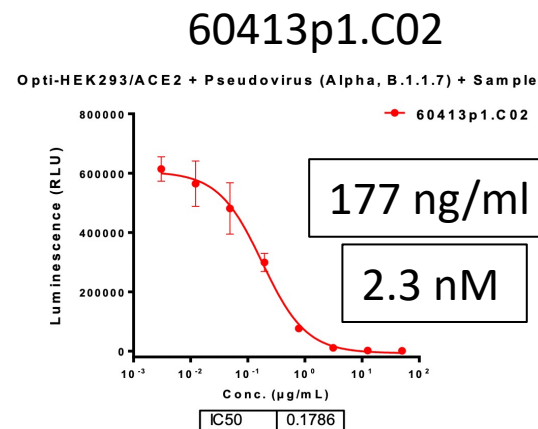
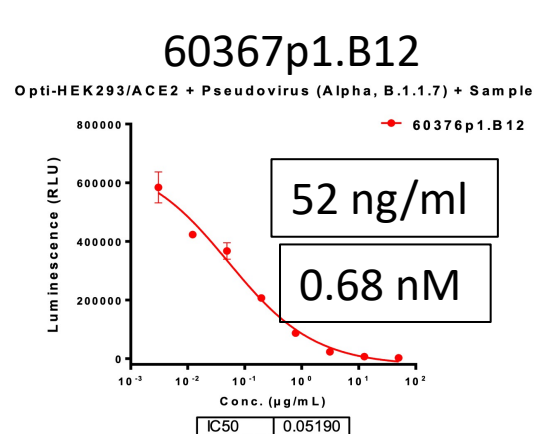
Delta (B.1.617.2)



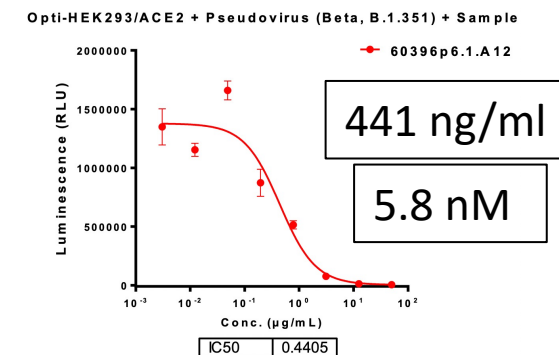
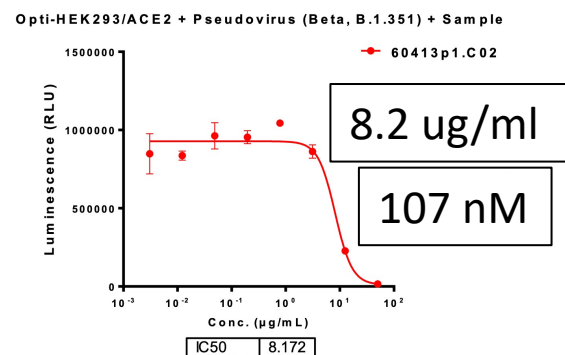
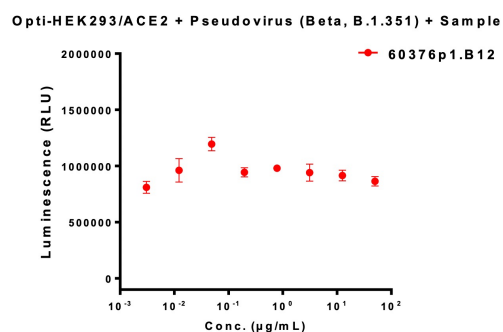
T19R, Δ156-158, L452R, T478K, D614G, P681R, D950N

Pseudovirus neutralization on 3 best clones

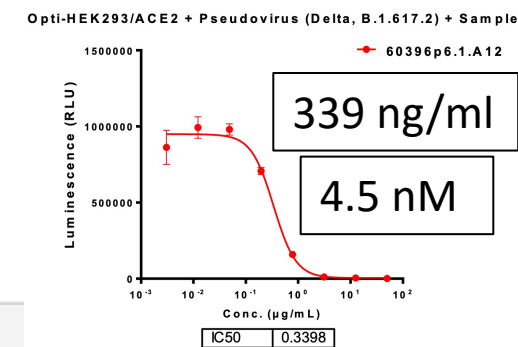
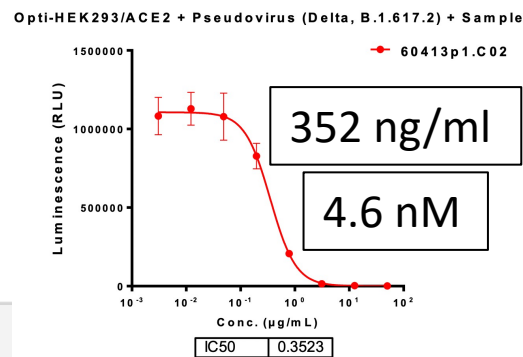
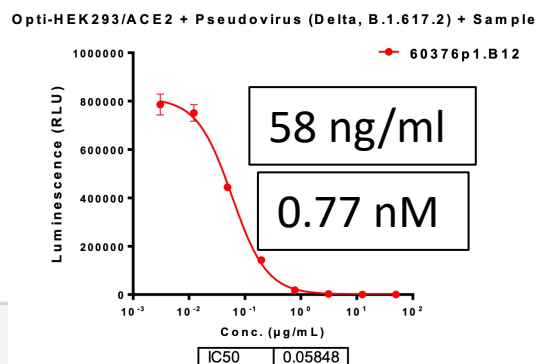
Alpha (B.1.1.7)



Beta (B.1.351)



Delta (B.1.617.2)



Pseudovirus neutralization

- Several clones are strong neutralizers against Delta variant
- Some clones neutralize all three variants assayed

IC50, nM	60376p1. B12	60396p1. C10	60396p1. E12	60396p1. H04	60396p1. H06	60396p1. H08	60396p2. A01	60396p2. B05	60396p2. C05	60396p2. C08	60396p3.1. D01	60396p6.1. A12	60413p1. C02	60413p1. E09	60413p1. E10
Alpha (B.1.1.7)	0.68	9.78	50.51	88.74	167.63	191.32	186.71	35.91	14.78	161.84	193.82	5.01	2.35	50.82	103.17
Beta (B.1.351)	NA	NA	NA	171.32	NA	NA	182.76	61.70	15.29	NA	NA	5.80	107.53	NA	NA
Delta (B.1.617.2)	0.77	5.23	64.92	110.47	261.05	193.68	120.37	39.76	17.97	200.00	106.53	4.47	4.64	49.51	110.14

Controls:

IC50, nM	mAb10933	mAb10987	ACE2-Fc
Alpha (B.1.1.7)	0.07	0.00	4.90
Beta (B.1.351)	4.90	0.62	1.83
Delta (B.1.617.2)	0.06	0.28	1.01

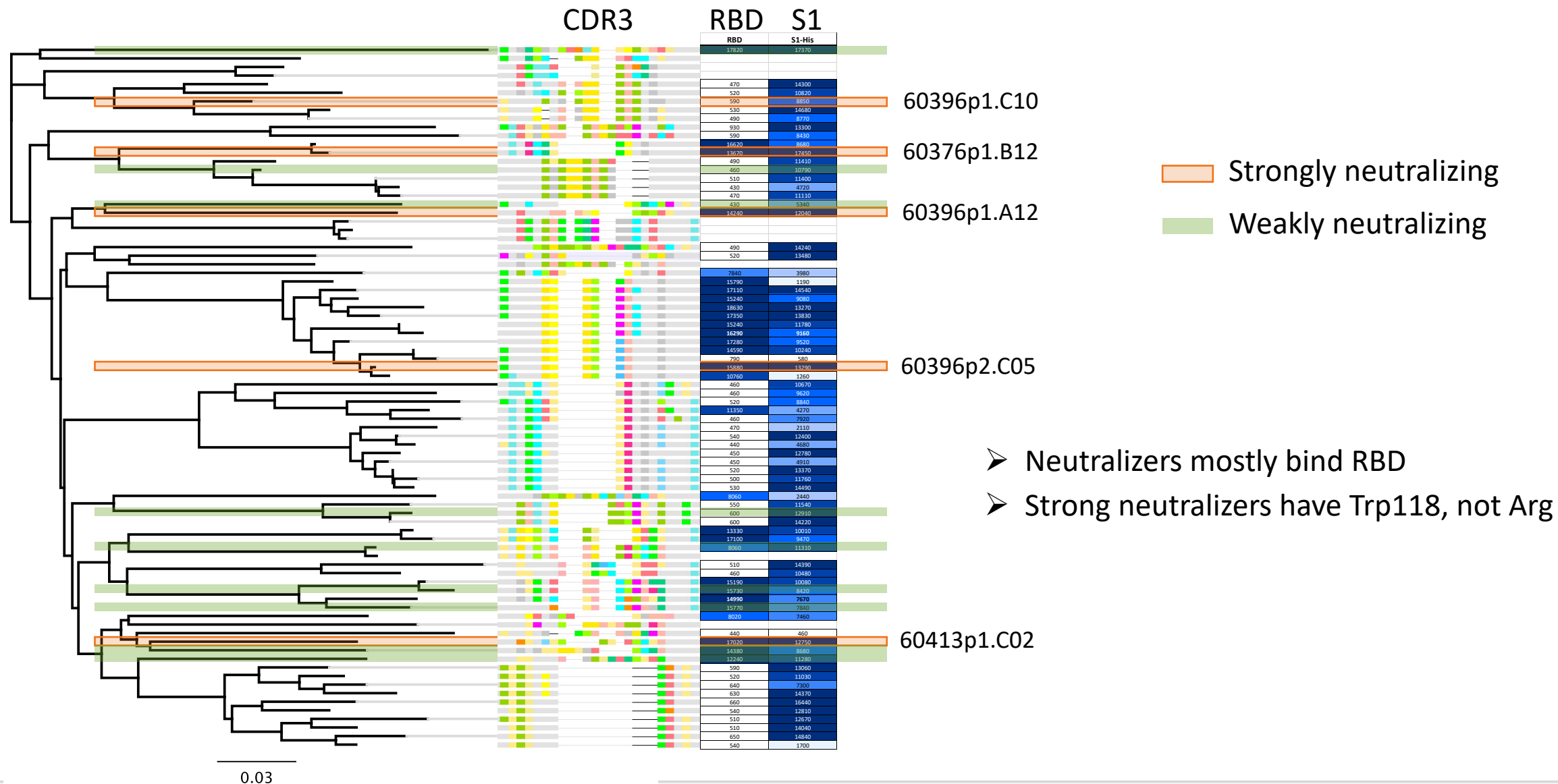
Pseudovirus neutralization: Beta

IC50, nM	60376p1. B12	60396p1. C10	60396p1. E12	60396p1. H04	60396p1. H06	60396p1. H08	60396p2. A01	60396p2. B05	60396p2. C05	60396p2. C08	60396p3.1. D01	60396p6.1. A12	60413p1. C02	60413p1. E09	60413p1. E10
Beta (B.1.351)	NA	NA	NA	171.32	NA	NA	182.76	61.70	15.29	NA	NA	5.80	107.53	NA	NA

KD Beta (nM)	4.69	1.52	2.56	2.78	4.35	13.49	68.99	135.15	N.D.	1.77	N.D.	N.D.	967.48	49.48	5.33
RBD binding	13670	590	15730	17200	600	15770	460	17110	15880	430	17820	14240	17020	8060	12240

➤ Neutralization does not correlate with binding affinity or RBD-binding

Neutralizing activity across a broad range of sequences



Summary

- Transgenic chickens have been made that express heavy chain-only antibodies of high affinity and potency
- Retains advantage of chicken host for difficult or highly conserved mammalian targets
- Neutralizing single domain antibodies have been produced to SARS-CoV2 spike protein

Next steps:

- Humanization
- Structural studies – stability, aggregation propensity
- Epitope identification
- Multimerization to increase avidity

Acknowledgements – members of the OmniAb[®] Team

- Antibody discovery and characterization:
 - Katie Ching
 - Kevin Reynolds
 - Sam Zeng
 - Abheepsa Gupta
 - Christine Vuong
- Transgenic animal generation:
 - Marie-Cecile van de Lavoie
 - Shelley Izquierdo
 - Renee Kopulos
 - Darlene Pedersen
 - Robyn Cotter
 - Ellen Collarini