



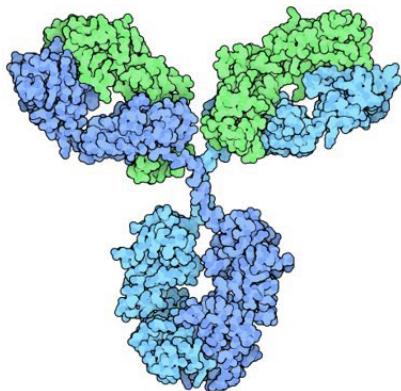
Phil Leighton

November 15, 2023

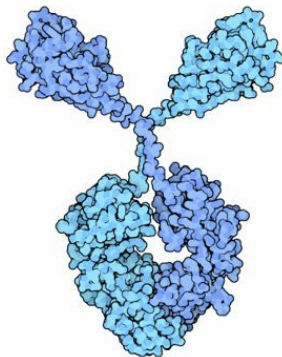
Single-Domain Antibodies (sdAbs)

ALSO KNOWN AS A VHH ANTIBODY OR NANOBODY®

Conventional Antibody
(IgG)



Heavy chain-only
(HcAb)



- Conventional IgG is comprised of 2 heavy chains and 2 light chains
 - Total MW ~150kD
 - Binding domain is VH x VL
- HcAb (found naturally in camelids) is comprised of 2 heavy chains, no LC
 - Total MW ~100kD
 - Binding domain is VH only
- VH domain of HcAb can be expressed independently as sdAb
 - Compact format of sdAb (~15kD) opens new opportunities

Clinical Landscape for Therapeutic sdAbs

ROBUST AND GROWING CLINICAL PIPELINE OF SDABS

Drug name	Target	Therapeutic Area	Phase
Cabliivi, Caplacizumab	von Willebrand Factor	Immune-mediated (rare blood) disorder	Approved (EU 2018, US 2019)
ENWEIDA, Envafohimab	PD-L1	Cancer	Approved (China 2021)
Nanozora, Ozoralizumab	TNFalpha	Immune-mediated disorder (rheumatoid arthritis)	Approved (Japan 2022)
CARVYKTI, Ciltacabtagene autoleucel	BCMA	Cancer	Approved (US 2022)
Erfonrilimab, KN046	PD-L1 x CTLA-4	Cancer	3
Gefurulimab, ALXN1720	C5 x albumin	Immune-mediated (rare neurological) disorder	3
LMN-201	C. difficile exotoxin TcdB	Infectious disease (C. difficile infection)	2/3 pending
JCT205, INBRX-109, Ozekibart	DR5	Cancer	2 (pivotal)
[68Ga]NOTA-Anti-HER2 VHH1	HER2	Cancer diagnostic (PET imaging)	2
V565	TNFalpha	Immune-mediated (Crohn's disease)	2
LMN-101	Flagellin FLAa	Infectious disease (Campylobacter infection)	2
ARP1, VHH batch 203027	rotavirus	Infectious disease (rotaviral diarrhoea)	2
Sonelokimab, M1095	IL17-A/F	Inflammation (psoriasis)	2
SAR442970	TNFalpha x OX40L	Inflammation/autoimmune	2
Vobarilizumab, ALX-0061	IL6R	Inflammation (rheumatoid arthritis)	Ph1/2
anti-PD1-MSLN-CAR-T Cells	PD1	Cancer	Ph1/2
ALX-0651	CXCR4 x albumin	Cancer	1
JS014	albumin	Cancer	1
CD19/20 bispecific CAR-T Cells	CD19 x CD20	Cancer	1
BCMA-Nb CAR-T Cells	BCMA	Cancer	1
[131I]-SGMIB Anti-HER2 VHH1	HER2	Cancer diagnostic (PET imaging)	1
ALX-0171	RSV x albumin	Infectious disease	1
M6495	ADAMTS-5	Inflammation (osteoarthritis)	1
MSB0010841	IL17-A/F	Inflammation (psoriasis)	1

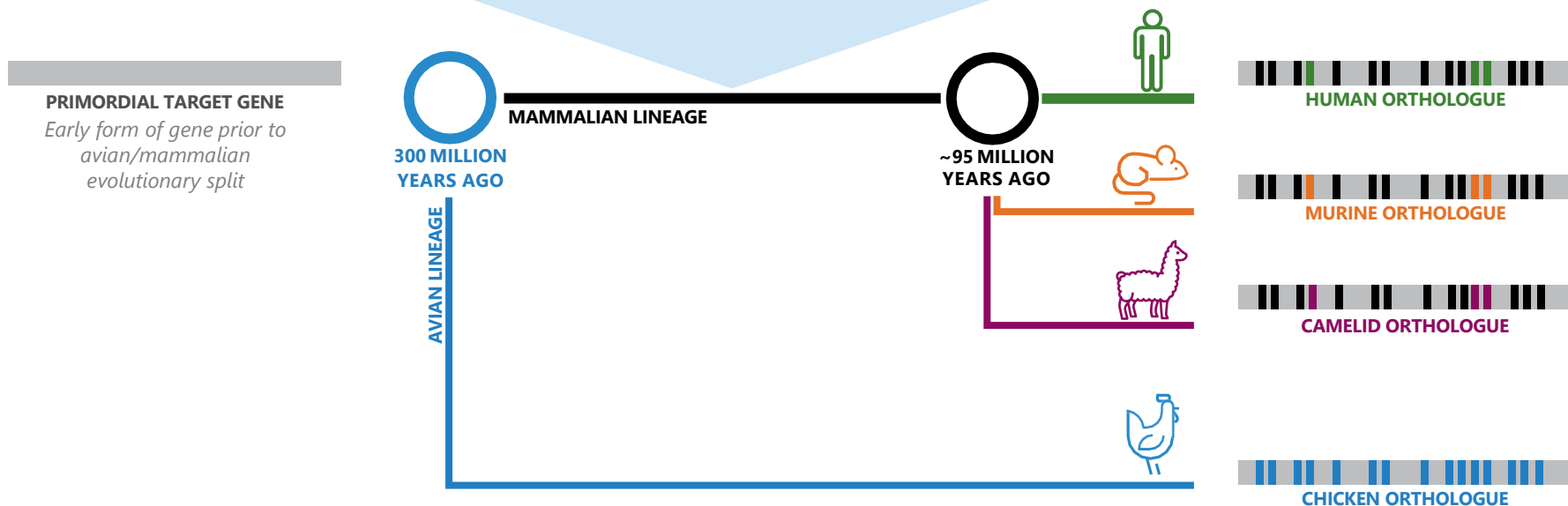
- **VHH-products comprise a growing segment of the Ab market**
- **4 approved VHH-based drugs**
- **20 VHH-based products in clinic**
- **Used to treat cancer, autoimmune & infectious diseases**
- **Various molecular formats**

Tandem VHH-VHH, VHH-Fc, VHH-albumin, bi/multi-specific, CAR-T, VHH cocktails...

Chicken Platforms: Powered by Evolution

OmniAb[®] OmniChicken[®] OmniClic[®]

**GREATER EVOLUTIONARY DISTANCE YIELDS
GREATER IMMUNOGENICITY AND MORE ANTIBODY DIVERSITY**



OmnidAb Platform: sdAb VH and tLC Transgenes

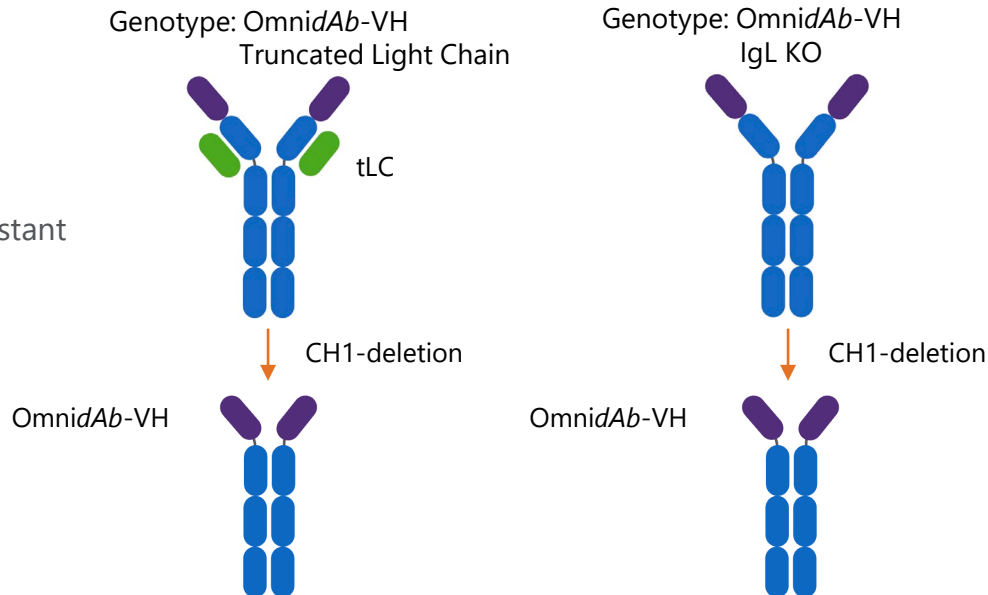
HUMAN VH3-23 WITH 10 STABILIZING MUTATIONS IN THE FRAMEWORKS

VH3-23*04



OmnidAb-VH

- Birds express VH but no VL
- Spliced to WT chicken heavy chain constant region (Fc)
- Spontaneous CH1 deletion



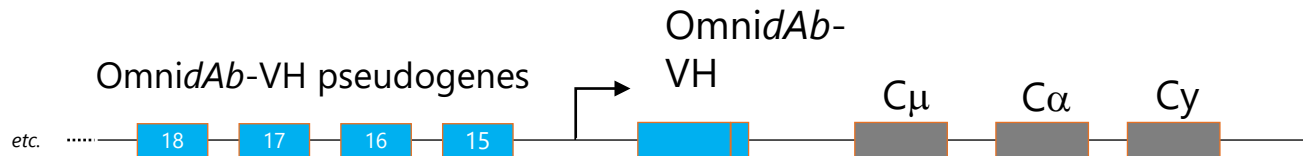
OmnidAb Pseudogene Array: Diversity Through Gene Conversion

STABILIZED FRAMEWORKS, CDR DIVERSITY

Stabilizing mutations maintained in FRs	CDR1*	CDR2*	CDR3**
15-D·YS-·SSY·RDR·GIAAQ-----DTH·
16-NAWKSKTD·TKD·TV·W-----YRAL·QH
17-NYYSKDLGYYS·DYG--GSRGM·V
18-WKQ-·D·SEKKPEG·RGAAA·PF-----DA·II
19-DHYTRNKANSYT·KDGIAA·HT-----RARGS·
20S-DDW-NS·IIRSKEDYDILT·YNLQYYYYGM·V
21YSIS·G·YYH--SKENNRHG·V·TFGGVLTSPH·
22-DD·TGT-·A·DTYKIS·DIVVPA-----AFH·
23-ENW-·NKGA·RN·LL-----PY·L
24-GY·PWY-·D·SNKKD·AY·EVRGV--ITNW·P
25-YSIS·NWRSKANSYA·KIP·LA·YFDTF--GRGA·Q
26GSTSS·GD·YYY-----IIKDYRANDRAT·GY--WDDA·II
27-GS·-G·YIH--KDPF·YSNY·F-----DY·A
28GSIS·-YNS-·D·SKNSDKWEL-----RDA·II

*From human VH3 germline genes

**From human VH3 somatic sequences

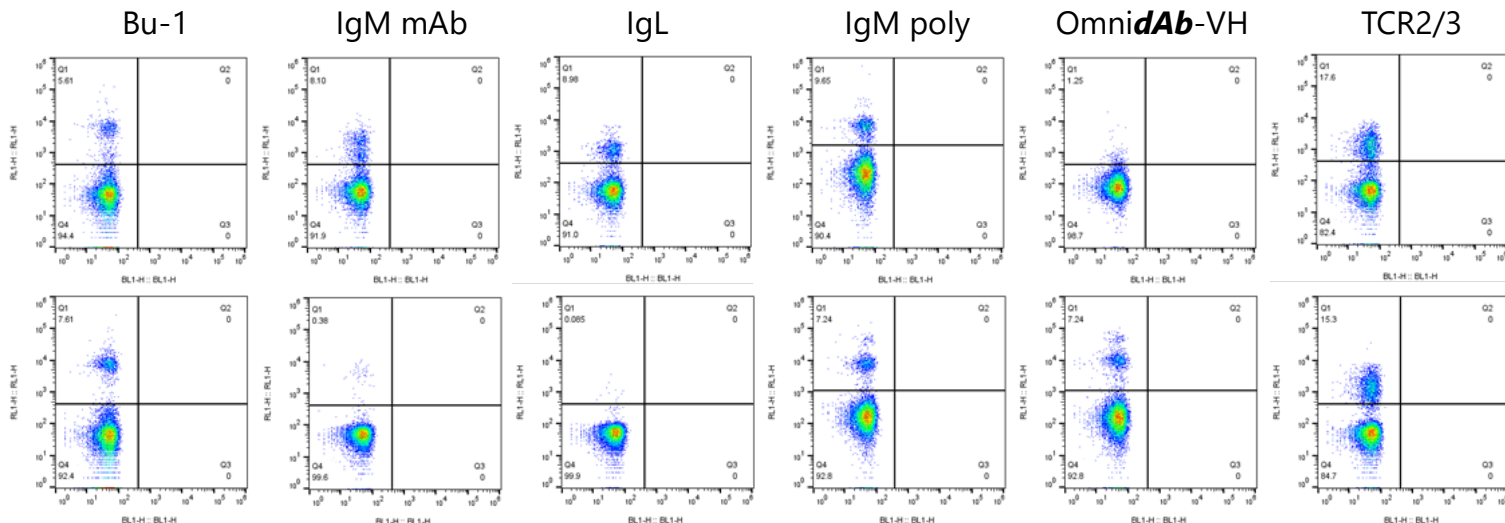
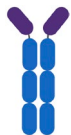


B Cell Development in *OmnidAb* Chickens

Wild type



OmnidAb



CH1-deletion:

- No IgL
- IgM epitope is in CH1

Robust B cell development in *OmnidAb* chickens

Immunizations

THREE COHORTS: NKp46, TIGIT+PGRN COCKTAIL, Kv1.3

	NKp46	TIGIT PGRN	Kv1.3
Immunogen	Protein	Protein	Nanodiscs DNA RNA
sdAb/IgL-/-	1 bird	1 bird	6 birds
sdAb/tLC	2 birds	3 birds	7 birds

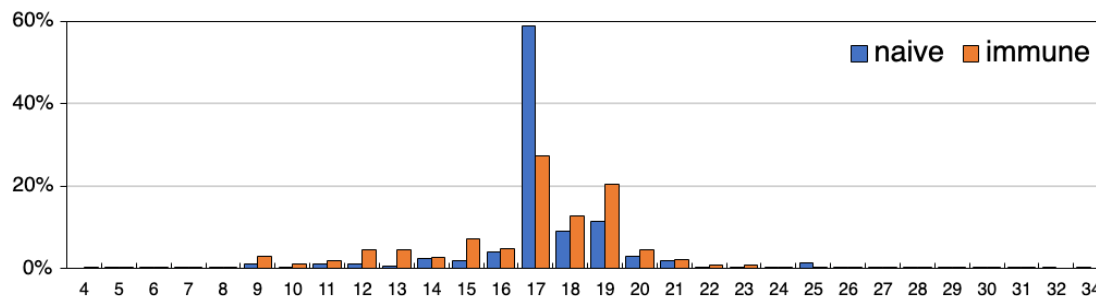
2-3 boosts (4-5 injections)

(in process)

NGS: Diversity increases upon immunization

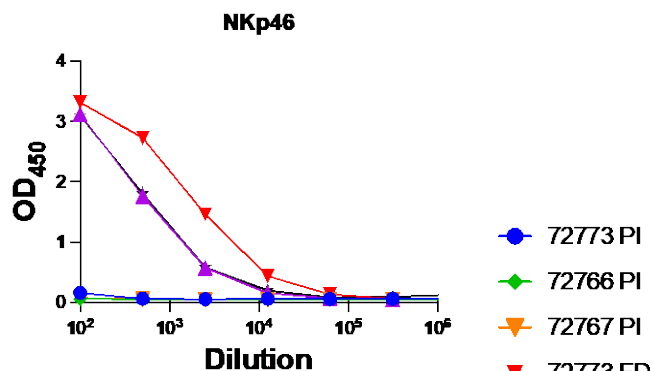
	Bird ID:	72766	72767	72773	72776	72806	72809	72847
Naïve PBMC	# uniq seq:	19323	25764	14061	6565	9948	8855	17094
	# lineage:	176	50	163	247	315	500	45
Immunized splenocyte	# uniq seq:	35240	23160	35890	30810	17693	29436	17551
	# lineage:	1270	2099	3490	5190	3252	3266	604

CDR-H3 length:

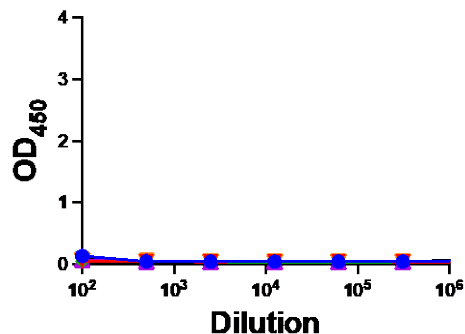


OmniAb Birds Raise Robust, Specific Immune Responses

NKp46-immunized

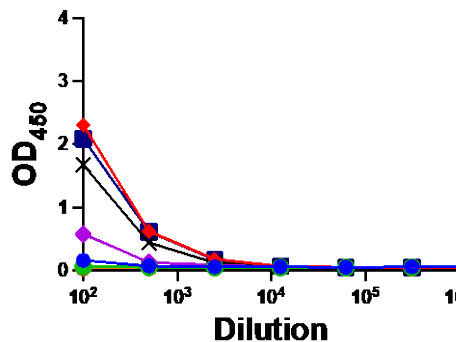


TIGIT-His control

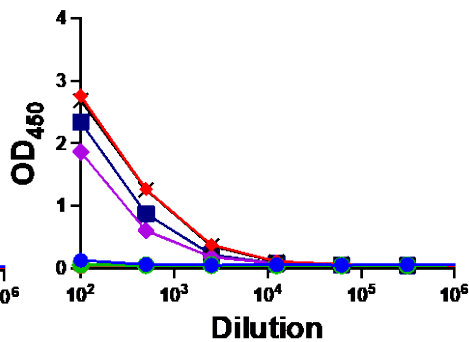


TIGIT/PGRN-immunized

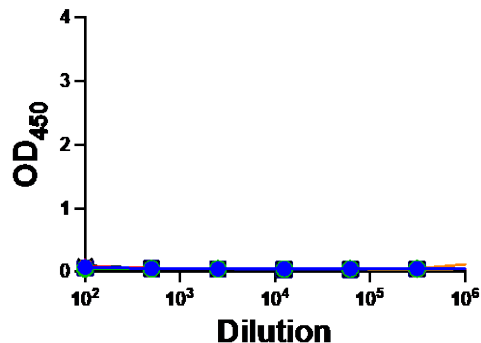
TIGIT



PGRN



NKp46-His control



- 72776 PI
- 72806 PI
- 72809 PI
- 72847 PI
- 72776 FD
- 72806 FD
- 72809 FD
- 72847 FD

OmnidAb sdAbs are Antigen-Specific

96w plate of sdAb-huFc supernatants (bird 72776), binding either to TIGIT or Progranulin:

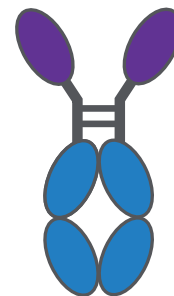
Yield: 0.2 – 1 mg/ml

TIGIT

Coat: TIGIT-Bio at 2 ug/mL												
Detect: anti-HuFc-HRP at 1:5000												
	1	2	3	4	5	6	7	8	9	10	11	12
A	0.113	0.051	1.757	0.049	0.055	0.052	0.048	0.051	0.052	0.046	0.049	0.05
B	0.07	0.091	0.048	0.053	0.045	0.047	0.083	0.046	0.047	0.046	0.047	0.062
C	0.058	0.049	0.051	0.056	0.049	0.047	0.044	0.052	0.053	0.046	0.046	0.053
D	0.104	0.051	1.982	0.051	0.047	0.049	0.058	0.049	0.045	0.054	0.046	0.047
E	0.063	0.05	0.056	0.047	0.053	0.058	0.053	0.049	0.049	0.051	0.073	0.051
F	0.058	0.051	0.064	0.049	0.046	0.041	0.048	0.052	0.047	0.137	0.068	0.05
G	0.059	0.048	0.056	0.056	0.055	0.049	0.071	0.077	0.045	0.049	0.049	0.05
H	0.082	0.071	0.057	0.061	0.08	0.055	0.067	0.058	0.061	0.06	0.061	1.703
Coat: PGRN-his at 2ug/ml												
Detect: anti-HuFc-HRP at 1:5000												
	1	2	3	4	5	6	7	8	9	10	11	12
A	0.14	0.051	0.048	1.75	0.057	1.732	0.05	0.056	0.048	0.079	0.053	0.175
B	0.063	0.082	0.046	0.06	0.135	0.049	0.052	0.049	0.048	0.049	0.046	0.056
C	0.066	0.047	0.048	0.052	0.059	0.061	0.047	0.047	0.048	0.602	0.059	1.556
D	1.62	0.048	0.048	0.047	0.101	0.048	0.046	0.047	0.05	1.418	1.543	0.052
E	1.749	0.053	0.053	1.122	0.046	0.888	0.046	0.049	0.046	1.689	0.06	0.055
F	0.071	0.048	1.682	0.047	0.046	1.78	0.048	0.063	1.528	0.065	0.051	0.054
G	0.052	0.048	1.723	0.047	1.69	0.047	0.048	0.06	0.24	1.842	1.013	1.689
H	0.061	0.063	0.051	0.881	0.067	0.155	1.68	1.613	1.752	0.057	0.392	0.083

Progranulin

sdAb-huFc



The way the GEMs were screened we could get either TIGIT or PGRN binders, serving as controls for each other in the ELISA

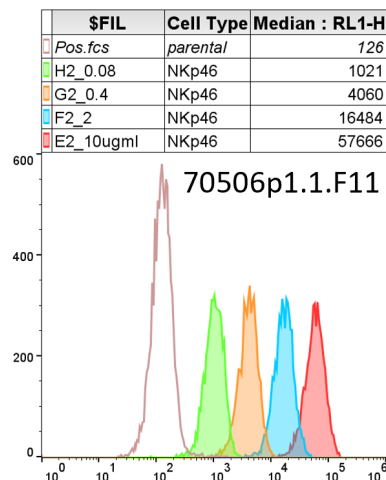
Specific Cell Binding to NKp46-Expressing Cells

Representative OmnidAb clone 70506p1.1.F11 in flow cytometry:

Most clones show specific binding:

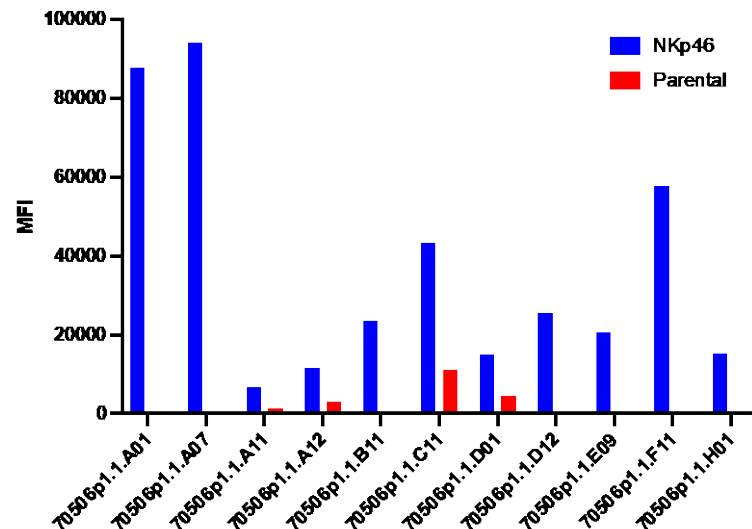
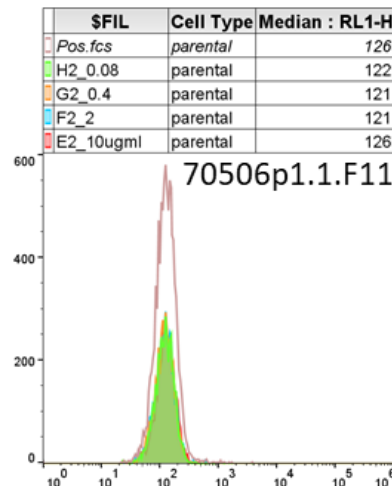
NKp46 expressing cells

➤ staining in dilution series



Parental cells

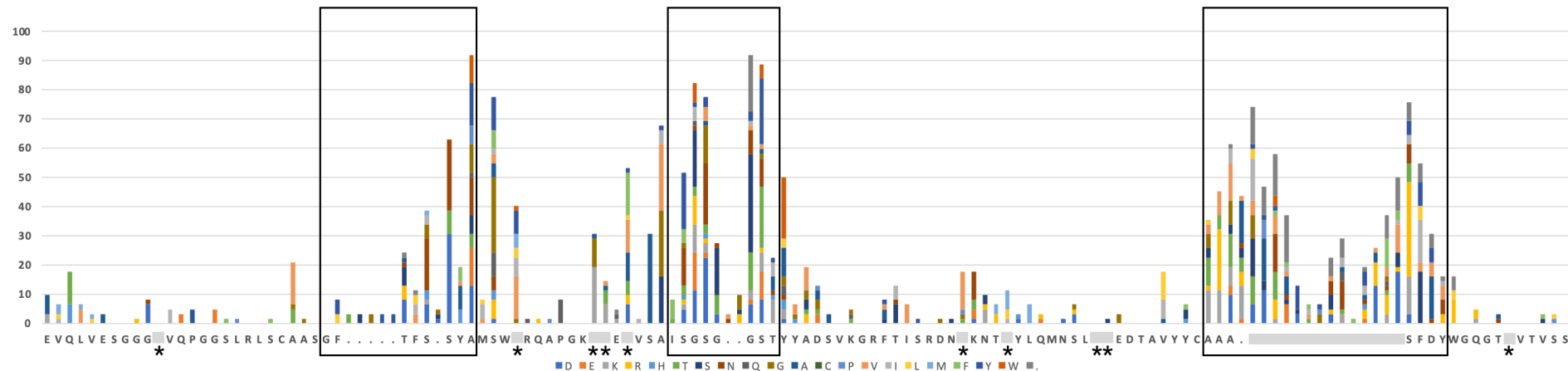
➤ no staining



Mutational Levels in Cloned sdAbs

DATA FROM PGRN, TIGIT AND NKP46 CLONES. N = 62

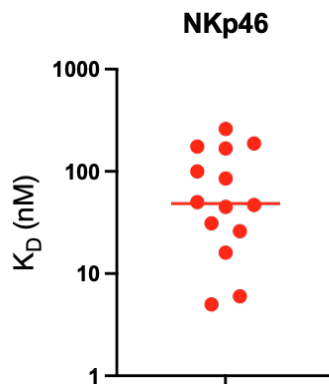
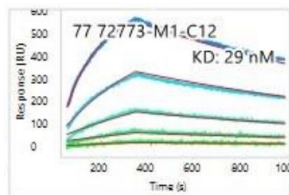
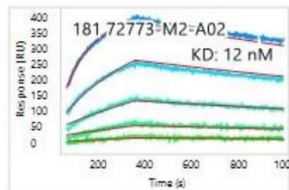
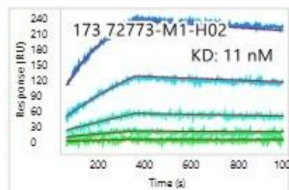
FR2



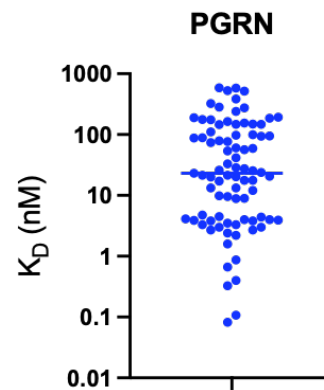
* Stabilizing mutations

More change of FR2 stabilizing mutations than others, in particular V37 and W47

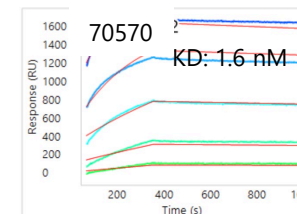
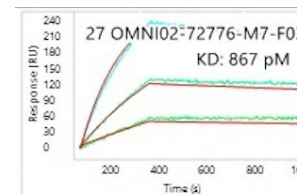
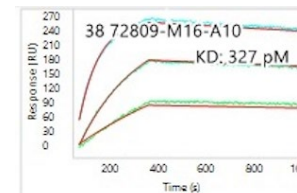
OmnidAb sdAbs Have High Affinity

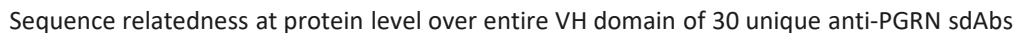


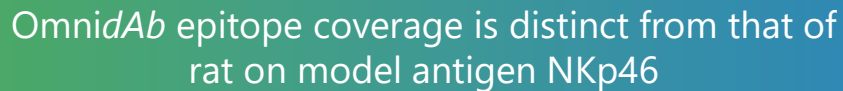
Number of clones	14
Minimum	5 nM
Maximum	261 nM
Median	48 nM



Number tested (some dups)	83
Minimum	0.08 nM
Maximum	500 nM
Median	23 nM

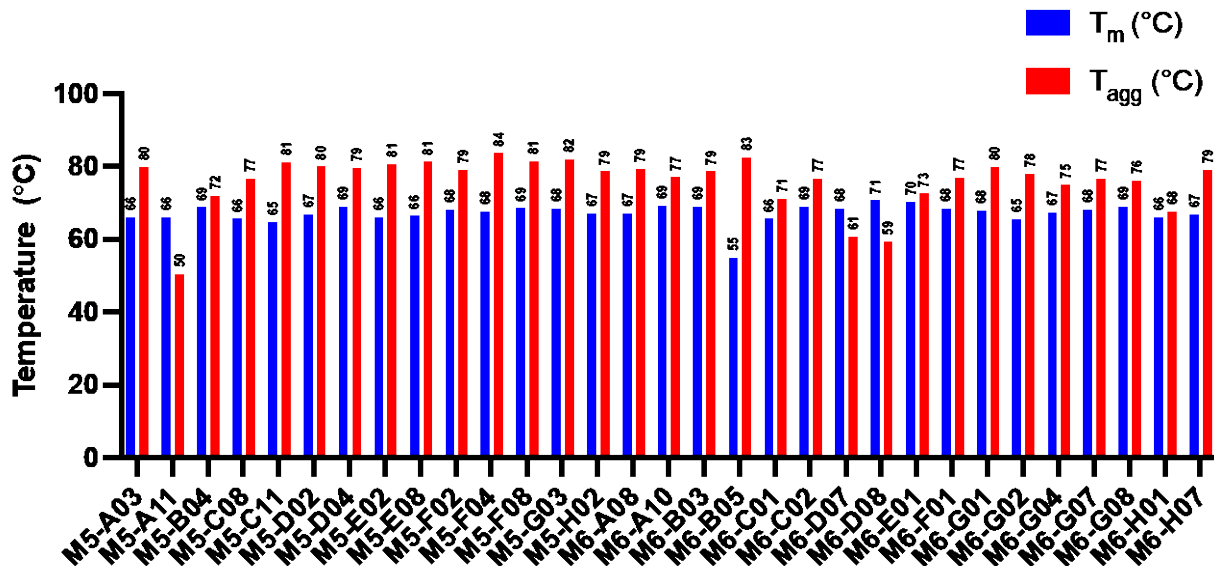






Developability Assessment

PHYSICAL PROPERTY CHARACTERIZATION OF NKP46 DAB PANEL

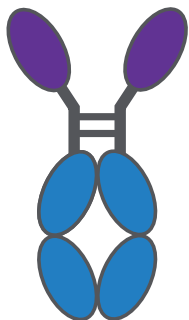


$T_m > 65^\circ\text{C}$
 $T_{agg} > T_m$

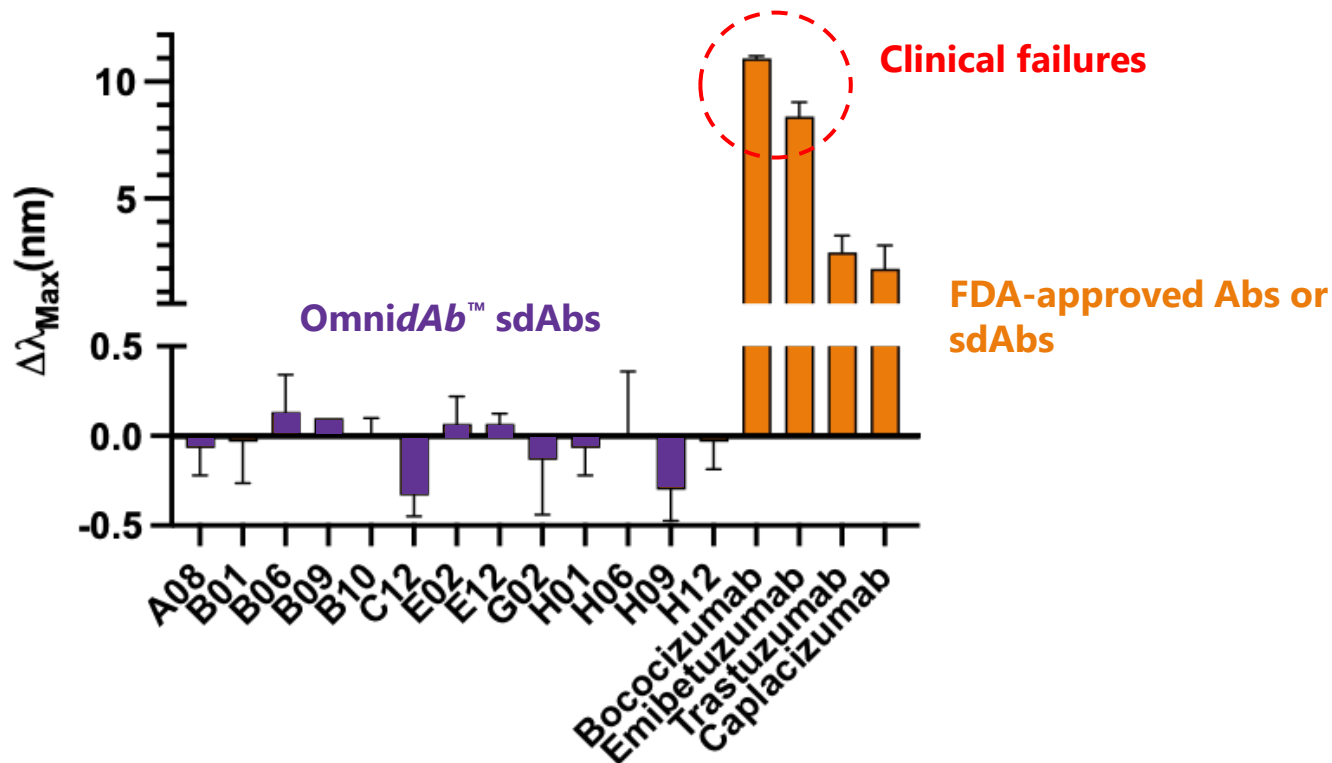
OmniAb clones meet "clinical grade" developability criteria

OmnidAb sdAbs Show No Self-Association

AC-SINS CHARACTERIZATION OF PGRN DAB PANEL



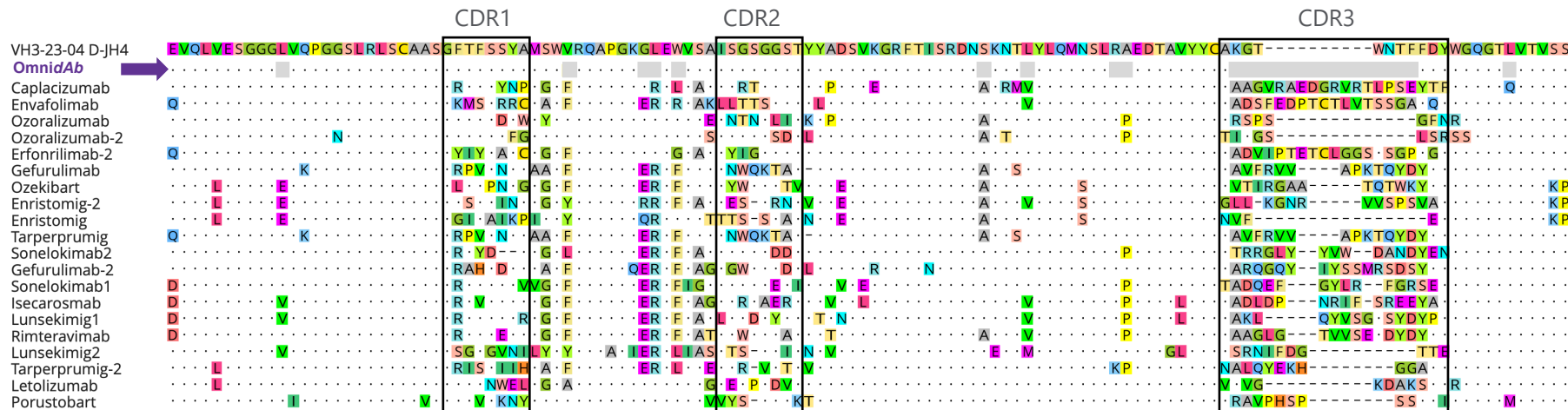
Format: sdAb-huFc



Sequence of Non-Germline Framework Positions

POTENTIAL FOR IMMUNOGENICITY

OmniAb vs.
other clinical-stage molecules:



OmniAb is designed to capture ideal attributes of clinical molecules,
bypassing need for extensive *in vitro* engineering

Summary

OmnidAb birds:

- Express an optimized single domain humanized framework
 - No non-canonical cysteines or PTM liabilities
- Produce robust titers upon immunization
- Develop diverse repertoires of sdAb VH sequences
- Target distinct epitopes
- Produce high-affinity, antigen-specific sdAbs with good developability metrics and high expression levels in mammalian cells

