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Discovery and Characterization of Potent TIGIT-specific Antibodies Derived from Very Diverse Phage Display Libraries

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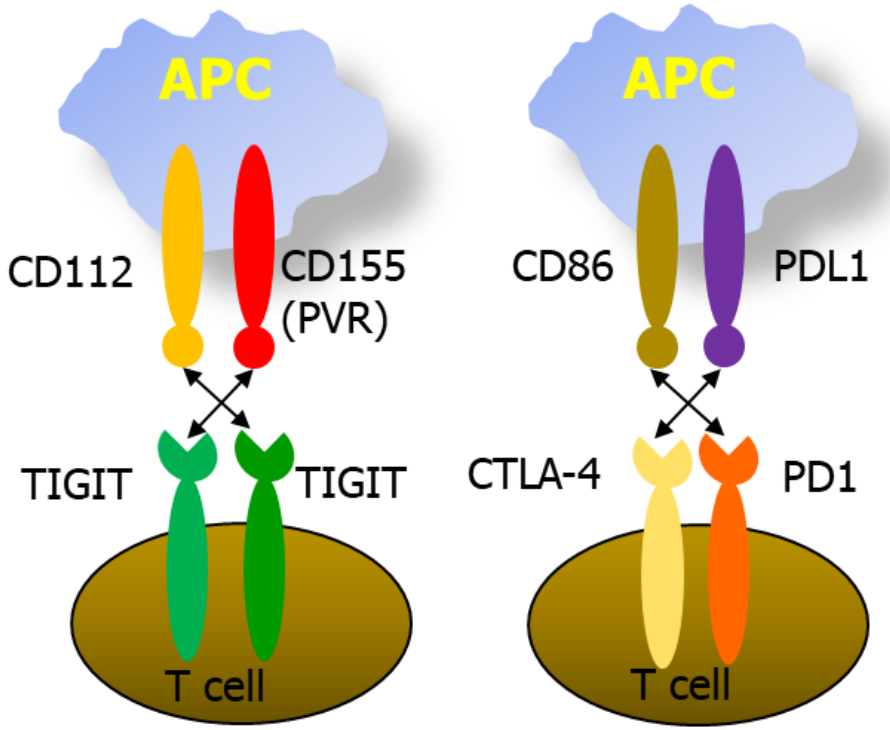
ABSTRACT

- Ab candidates targeting TIGIT (T cell immunoreceptor with Ig and ITIM domains) were discovered via phage panning selections using LakePharma's licensed state-of-the-art Fab naïve (XOMA) and in-house antigen-specific immune libraries
- TIGIT is a challenging antibody immuno oncology target
  - discovery of human and cynomolgus TIGIT cross-reactive Abs has been often problematic
- We will present TIGIT-CD155 ligand inhibition data and kinetics of selected human and cyno TIGIT binding antibodies derived from phage panning



TIGIT suppresses immunity in a malignant microenvironment when the interaction with ligands CD155 (PVR) and CD112 is not properly regulated.

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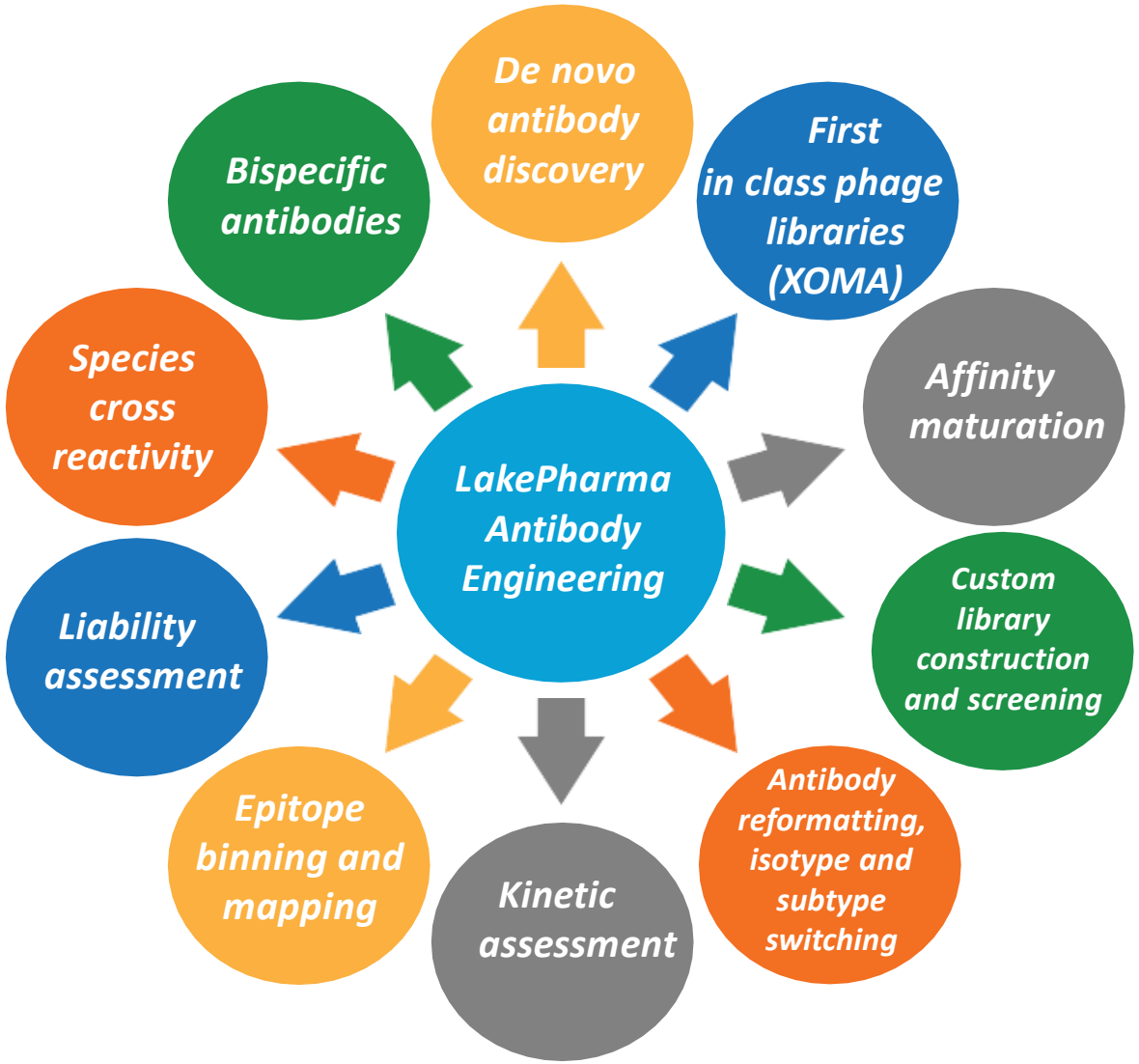
INTRODUCTION

- In-licensing of XOMA's 031 human Fab and 040 human scFv naïve phage libraries
  - Routinely selected pM affinity antibodies
  - Fully human, very large (>10^{11}), multiple ORFs
  - Leads in multiple clinical programs
- In-house diverse immune custom phage libraries
- High throughput reformatting into different isotypes
- High throughput 96-well transfections and high-yield purifications from proprietary CHO cell lines
- IgG specificity, K_D assessments (BLI, Carterra)



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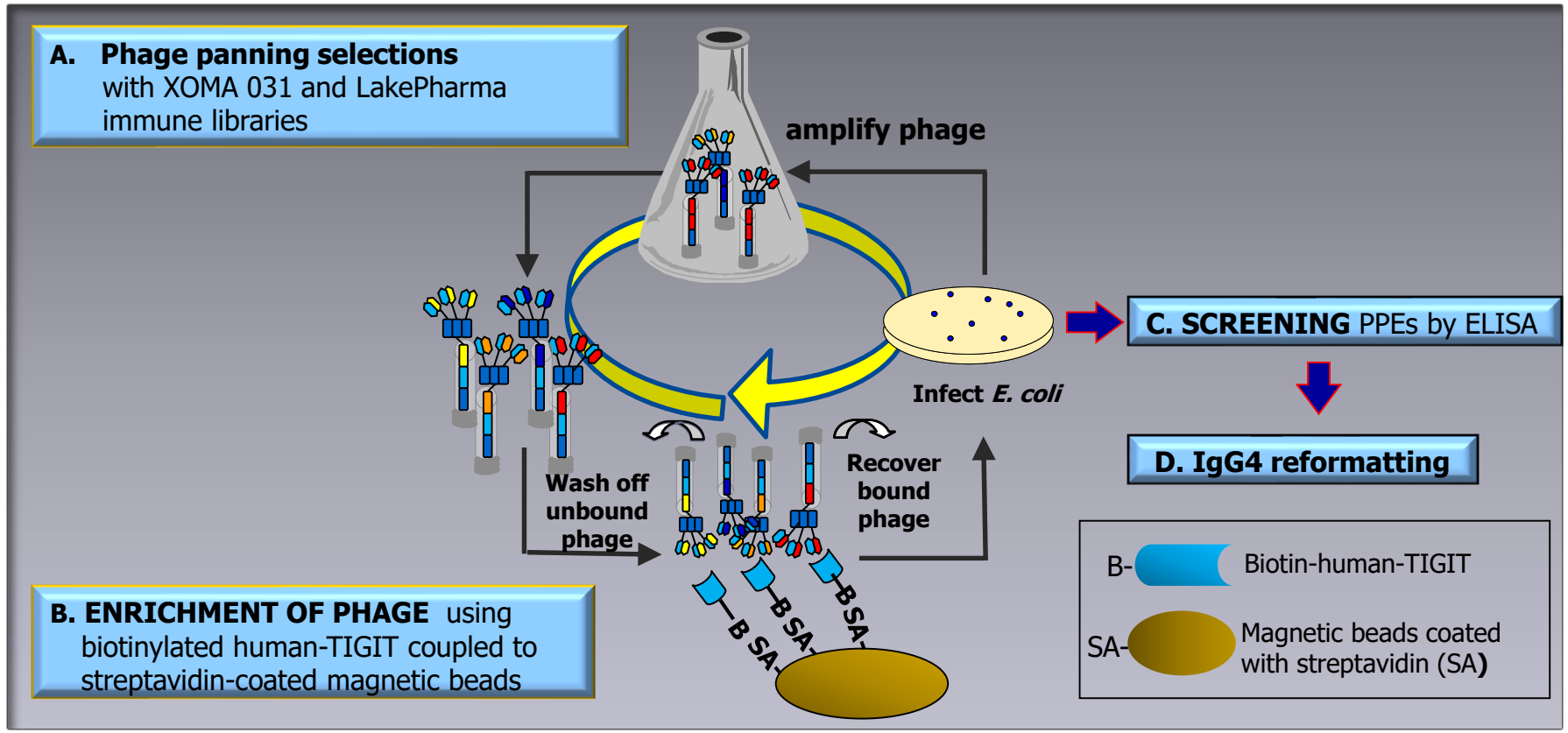
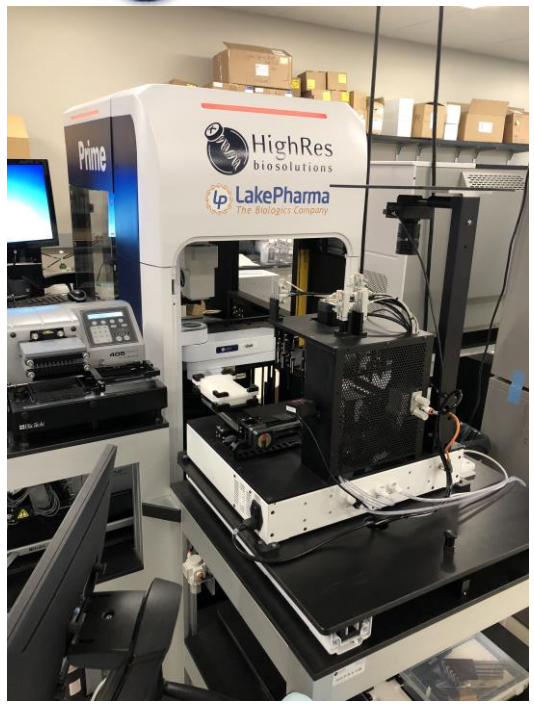
Phage Panning and Screening Workflow

- **4 rounds of selections with XOMA Fab library:**
  - Rounds of selections performed with decreasing biotin-huTIGIT coupled to Streptavidin-coated magnetic beads
  - Stringency of selections increased with number and duration of washes in successive selection rounds
  - Bacterial periplasmic extracts (PPEs) screened for human and cyno-TIGIT binding by ELISA (HighRes automation deck)
- **3 rounds of selections with LakePharma immune scFv library, following human and cyno-TIGIT mouse immunizations:**
  - 3 rounds of selections with Biotin-huTIGIT on Streptavidin-coated ELISA plate wells





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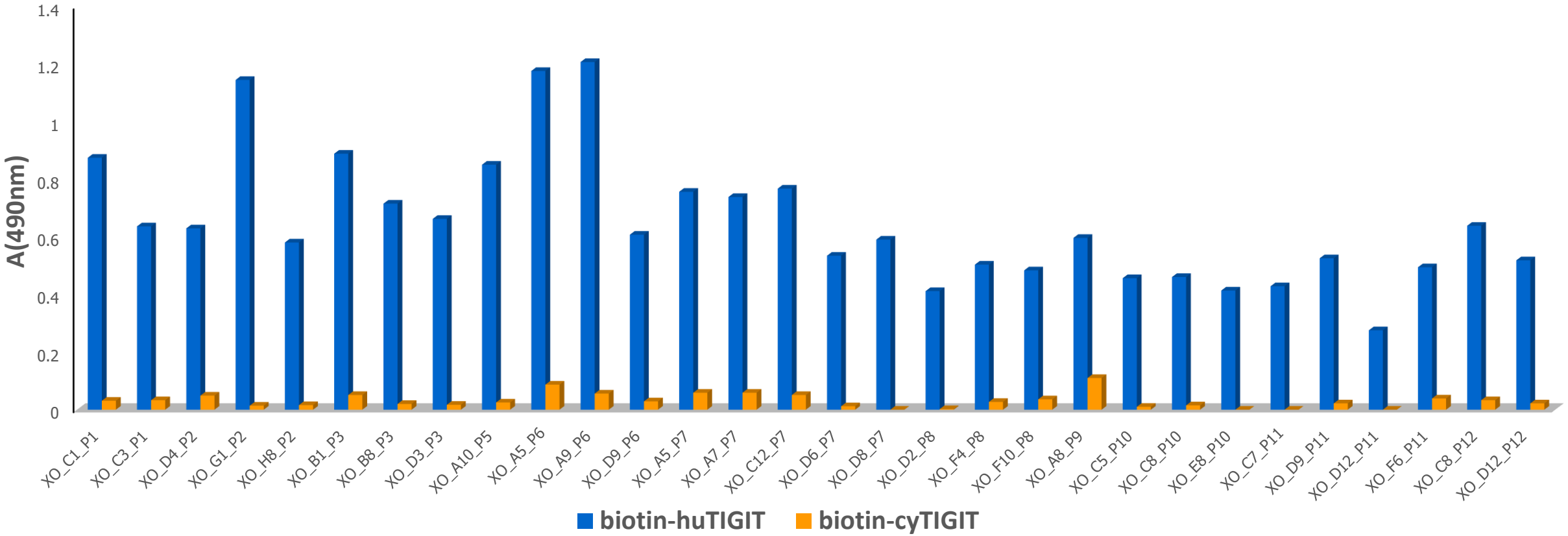
Sequences of TIGIT-binding Abs from naïve and immune library selection campaigns

- **XOMA Fab naïve Library: Sequenced 1116 huTIGIT binders from 12 x 96-well plates of clones**
  - 61 sequence-unique binders identified
  - 30 unique human TIGIT binders, including all VH-unique sequences, were selected for human IgG4 reformatting and characterization
  - No clones were able to recognize cynomolgus TIGIT
- **LakePharma scFv Immune Library: Sequenced 1116 huTIGIT binders from 12 x 96-well plates of clones**
  - 67 sequence-unique binders identified
  - 56 sequence-unique human-TIGIT binders, including all VH-unique clones, selected for human IgG4 reformatting

ELISA binding of TIGIT-specific Fab clones in PPEs following panning with XOMA phage library

- 1116 individual clones of bacterial Fab PPEs screened by ELISA against cynomolgus and human TIGIT, using the HighRes deck
- No cynomolgus TIGIT binders from XOMA Fab library selections



Sequencing analysis allowed the selection of 30 unique human TIGIT Fab binders which were reformatted into human IgG4 for further kinetic and functional characterizations

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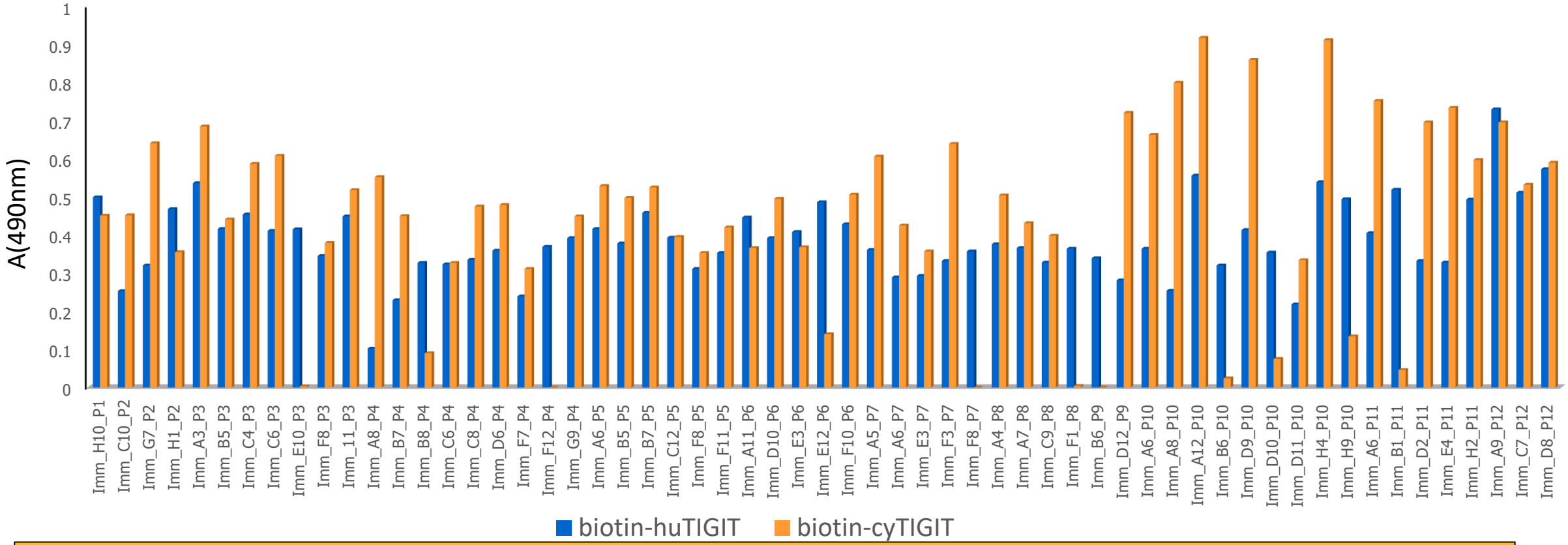
ELISA binding of TIGIT-specific scFv clones in bacterial PPEs following panning with in-house immune phage library

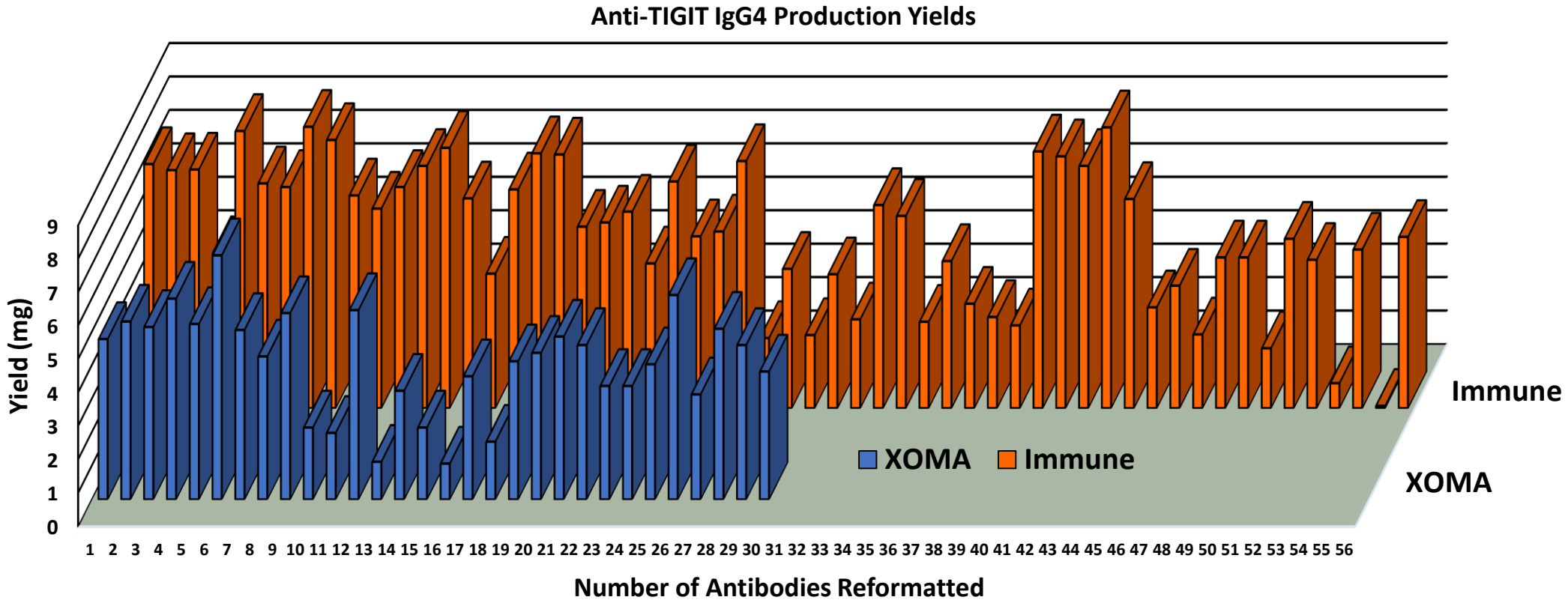
- Following co-immunization of mice with human and cynomolgus TIGIT-Fc using LakePharma's optimized hybridoma protocols, a diverse scFv phage library was generated with cDNA template from murine spleen mRNA
- 1116 individual clones of bacterial scFv PPEs screened by ELISA against cynomolgus and human TIGIT protein, using LakePharma's HighRes automation deck



Sequencing analysis allowed the selection of 56 unique TIGIT scFv binders that were subsequently reformatted into a chimeric IgG4 isotype for further kinetic and functional characterizations

High Throughput IgG4 Transient Production using LakePharma's TunaCHO™ cell line

- TunaCHO™ is LakePharma's proprietary cell line (same parental cell line as CHO-GSN)
- Consistent anti-TIGIT IgG4 production in 10ml scale transient transfections
- More cost-effective than other premium CHO transient cell line production systems



Superb expression of 86 IgG4-reformatted anti-TIGIT Abs from XOMA and immune phage display libraries (average yield ~4.6mg from 10ml transfections)

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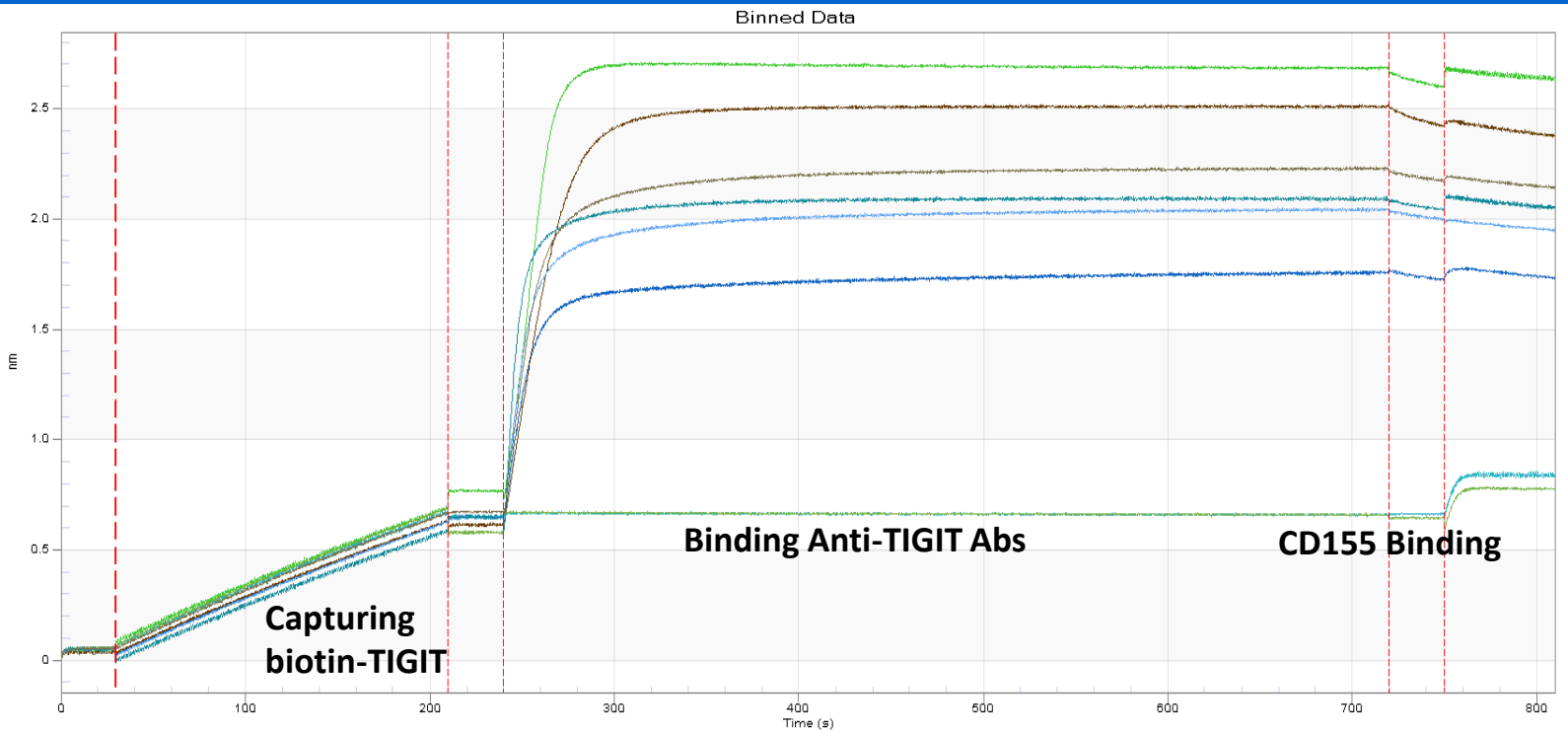
Kinetic analysis (sensogram example shown) demonstrates CD155 (PVR) blocking of TIGIT-binding antibodies



XOMA blocking IgG4	
XO_A5_P6	XO_C5_P10
XO_A5_P7	XO_C8_P12
XO_A7_P7	XO_D3_P3
XO_A8_P9	XO_D4_P2
XO_A9_P6	XO_D9_P11
XO_B1_P3	XO_D9_P6
XO_B8_P3	XO_F10_P8
XO_C1_P1	XO_F6_P11
XO_C12_P7	XO_G1_P2
XO_C3_P1	

Immune blocking IgG4	
Imm_A12P10	Imm_D9P10
Imm_A3P3	Imm_E10P3
Imm_A6P10	Imm_E3P6
Imm_A6P5	Imm_E3P7
Imm_A7P8	Imm_E4P11
Imm_B1P11	Imm_E9P12
Imm_B5P3	Imm_F10P6
Imm_B6P10	Imm_F12P4
Imm_B7P5	Imm_F3P7
Imm_B8P4	Imm_F8P7
Imm_C4P3	Imm_H11P3
Imm_C6P3	Imm_H4P10
Imm_D10P10	Imm_H9P10
Imm_D2P11	
Imm_D6P4	
Imm_D8P12	

- Streptavidin Octet biosensor loaded with Biotin-human TIGIT (3 min)
- Human TIGIT-specific IgG4 Abs added at saturating concentration (8 min)
- Human CD155 ligand allowed to bind (1 min)

CD155-blocking antibodies were discovered following XOMA and in-house immune phage display library selections

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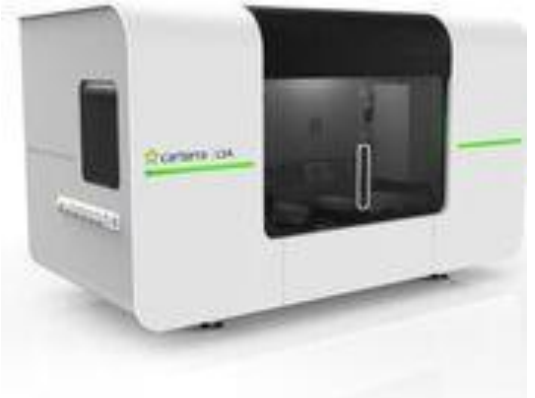
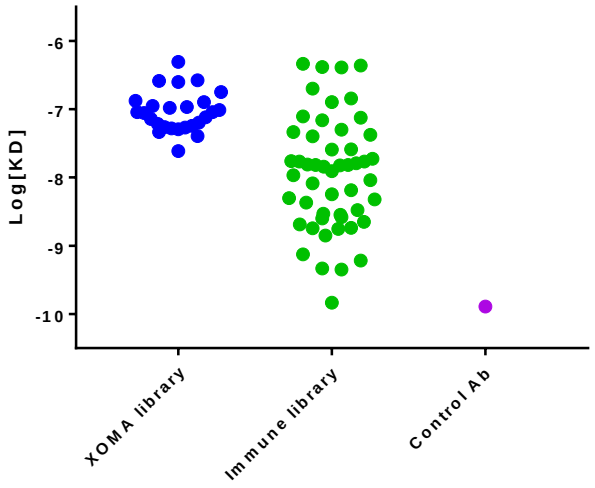
K_D distribution of IgG4-reformatted TIGIT-binding antibodies by Carterra LSA

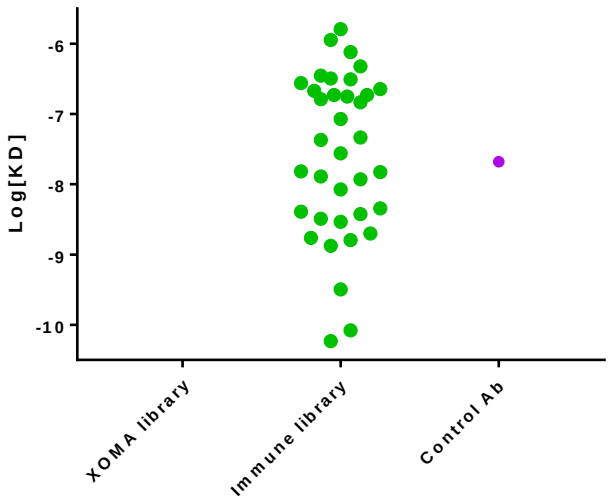
- Binding assays performed using array SPR
- Purified human and chimeric antibodies (4.5 μg/mL) were loaded onto Anti-Human IgG Fc coated CMD50M chip
- Cyno and Human TIGIT were injected as monovalent analytes as a 3-fold dilution series (0.4-300nM) over IgGs captured onto discrete spots to create a 192-spotted array via anti-human IgG Fc-coated CMD50M chip
- No cynomolgus TIGIT IgGs from XOMA library

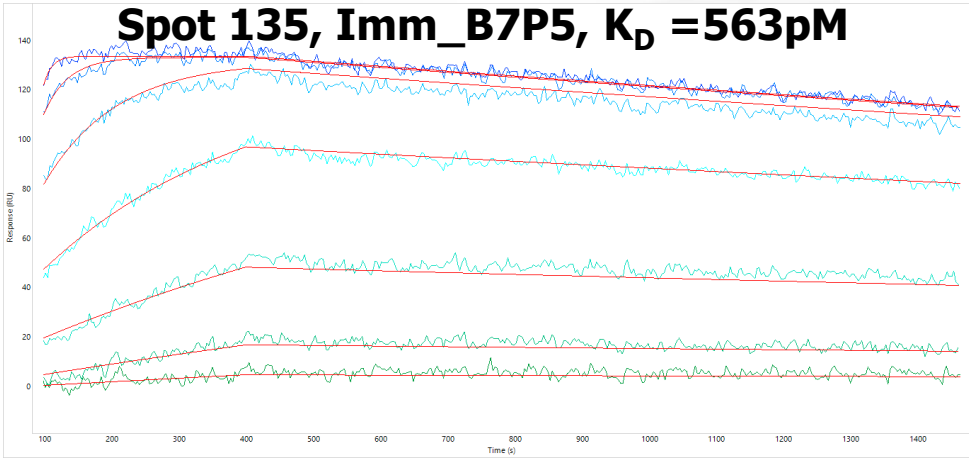


Log[KD] distribution for two libraries against huTIGIT-His



Log[KD] distribution for two libraries against cyTIGIT-His





TIGIT-binding reformatted antibodies derived from LakePharma's immune scFv libraries exhibited superior K_D values

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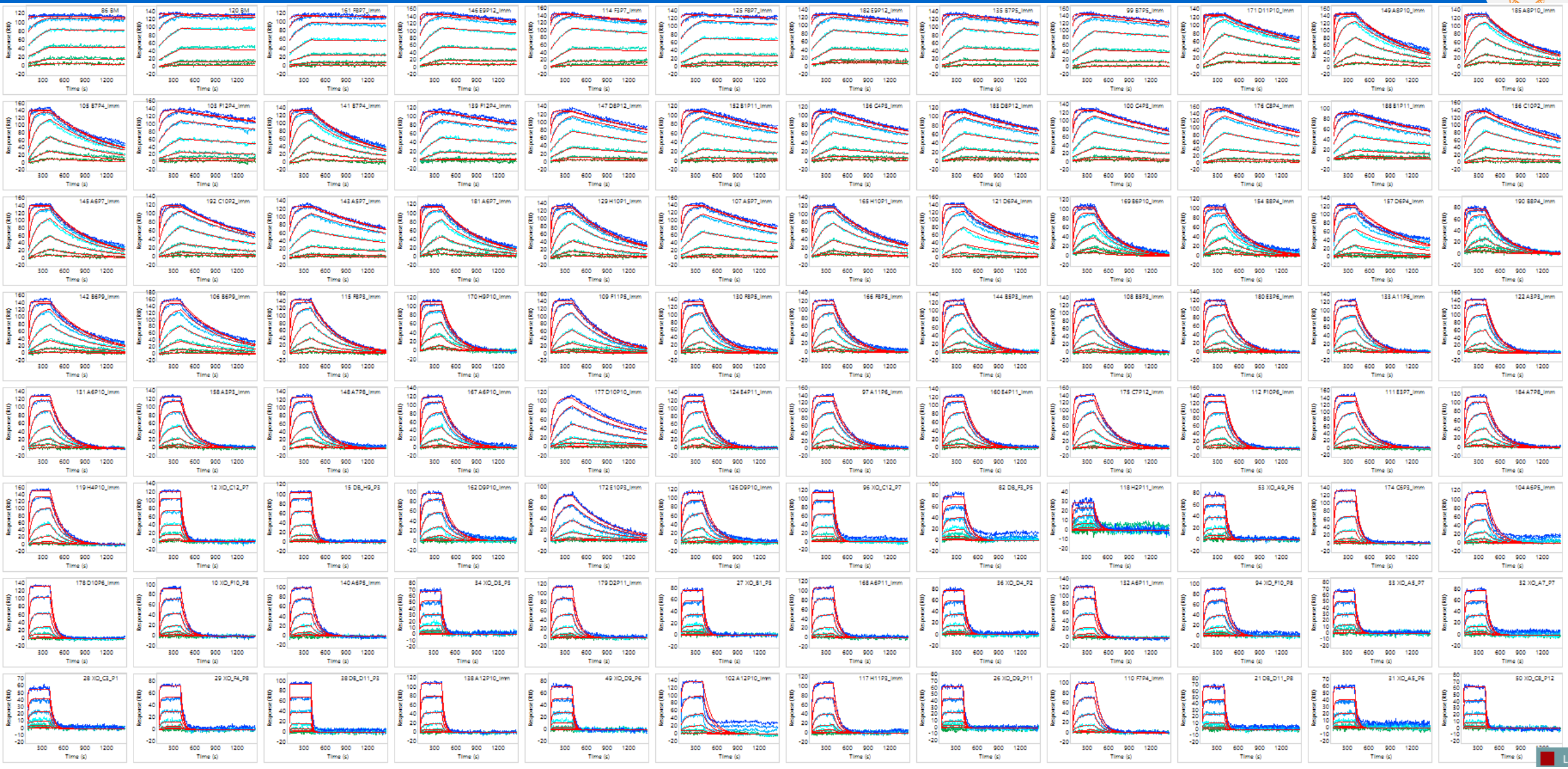
Carterra LSA: Human TIGIT IgG binding (affinity ranked from high to low)



HUMAN AND CYNO TIGIT (0.4-300NM, 3-FOLD DILUTION SERIES) BINDING TO 192-ARRAY OF HIGGS CAPTURED VIA ANTI-HIGG FC COATED CMD50M CHIP

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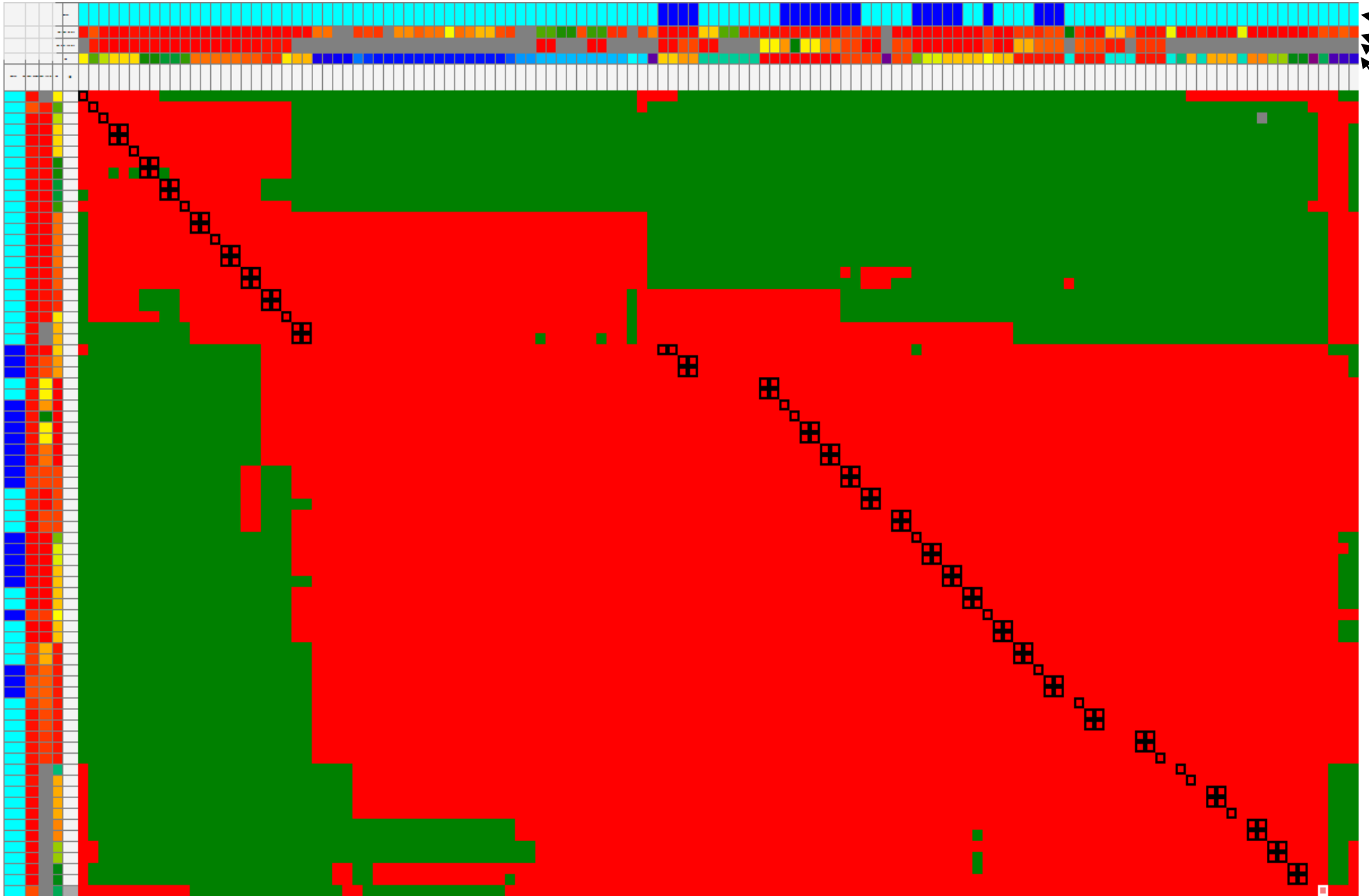
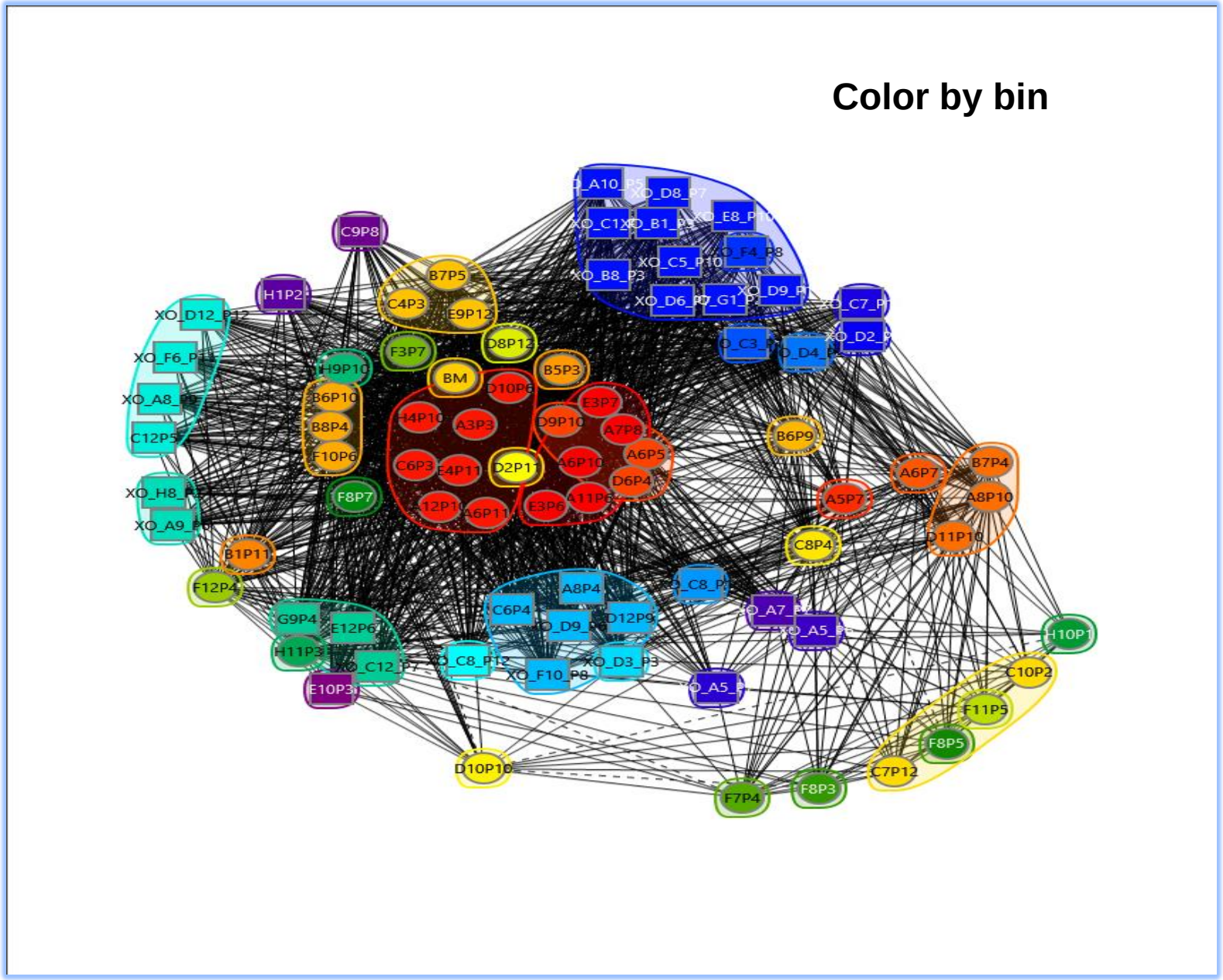
TIGIT binders: Heat Map indicating IgG interactions



Epitope Binning of TIGIT-specific IgGs: Network Plots



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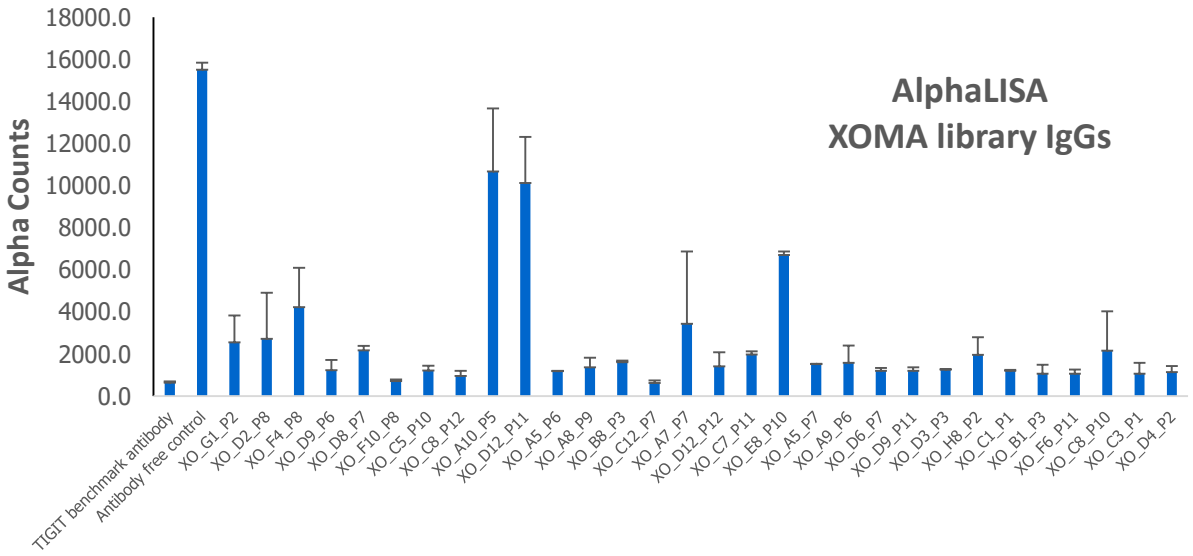
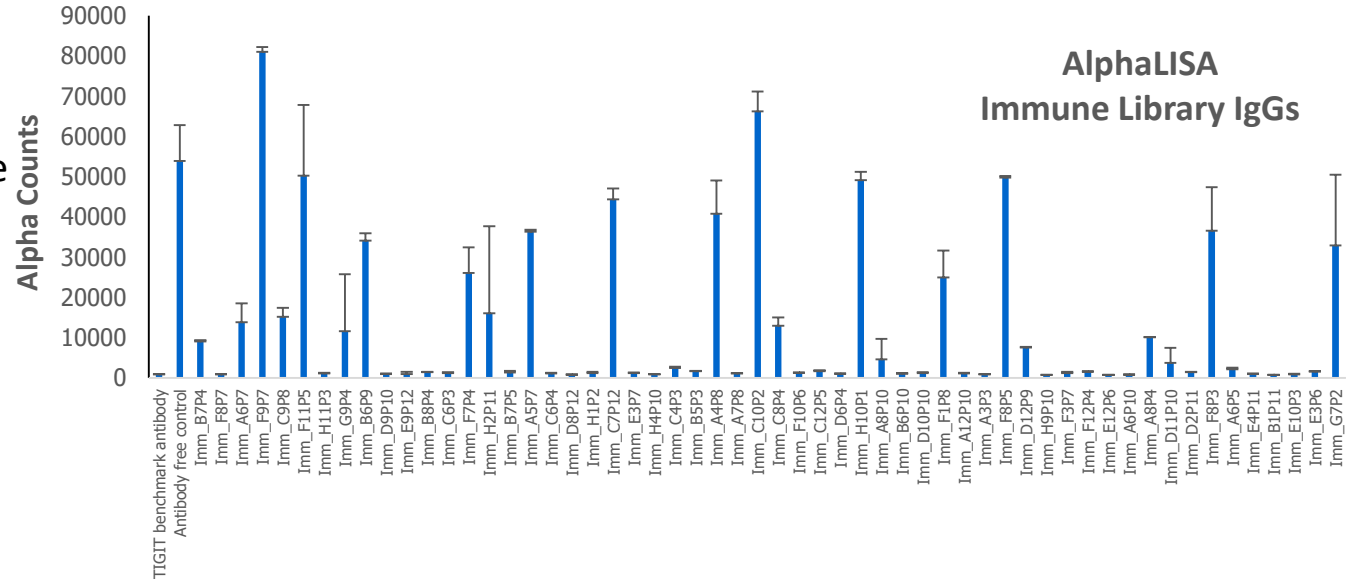
AlphaLISA ligand CD155 blockade assay for Immune and XOMA library reformatted clones

- TIGIT:CD155 Homogeneous Assay Kit in AlphaLISA format (BPS Bioscience) using biotin-huTIGIT and huCD155 ligand
- Anti-TIGIT IgGs (40-160 µg/ml) incubated with human TIGIT before adding CD155
- Acceptor and donor beads added before monitoring Alpha Counts
- Lower Alpha Count values indicate CD155 blockade





AlphaLISA CD155 blocking assay revealed multiple ligand-blocking antibodies from XOMA and LakePharma immune library selections (IC50s of top blockers not shown)

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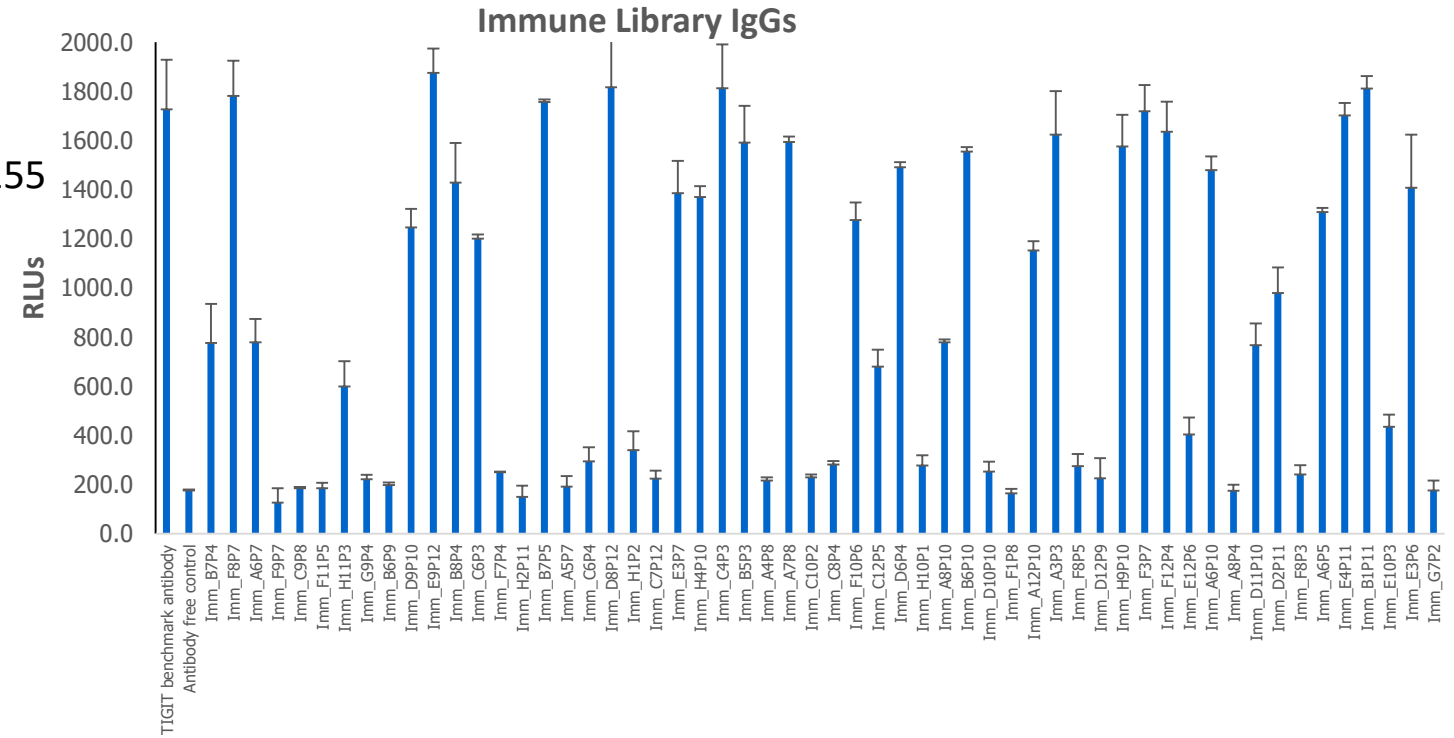
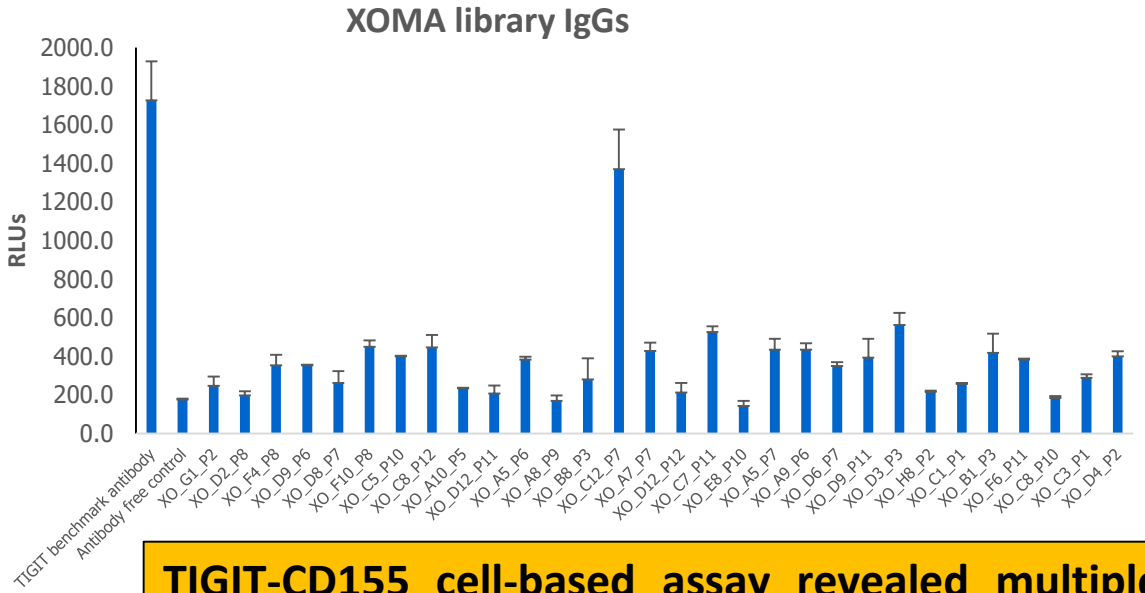
Cell-Based Immunoblockade Assay (Promega) for Immune and XOMA library reformatted clones

- CD226-induced luminescence is inhibited when Jurkat effector T cells expressing human TIGIT with luciferase reporter are co-cultured with CHOK1 cells expressing CD155
- Anti-TIGIT IgGs (33-132 μg/ml) block interaction of TIGIT/CD155, resulting in CD226-activated luminescence (Higher RLUs indicate CD155 blockade)





TIGIT-CD155 cell-based assay revealed multiple human TIGIT-specific functional antibodies, the vast majority originating from LakaPharma's immune phage display library (IC50s of top blockers not shown)

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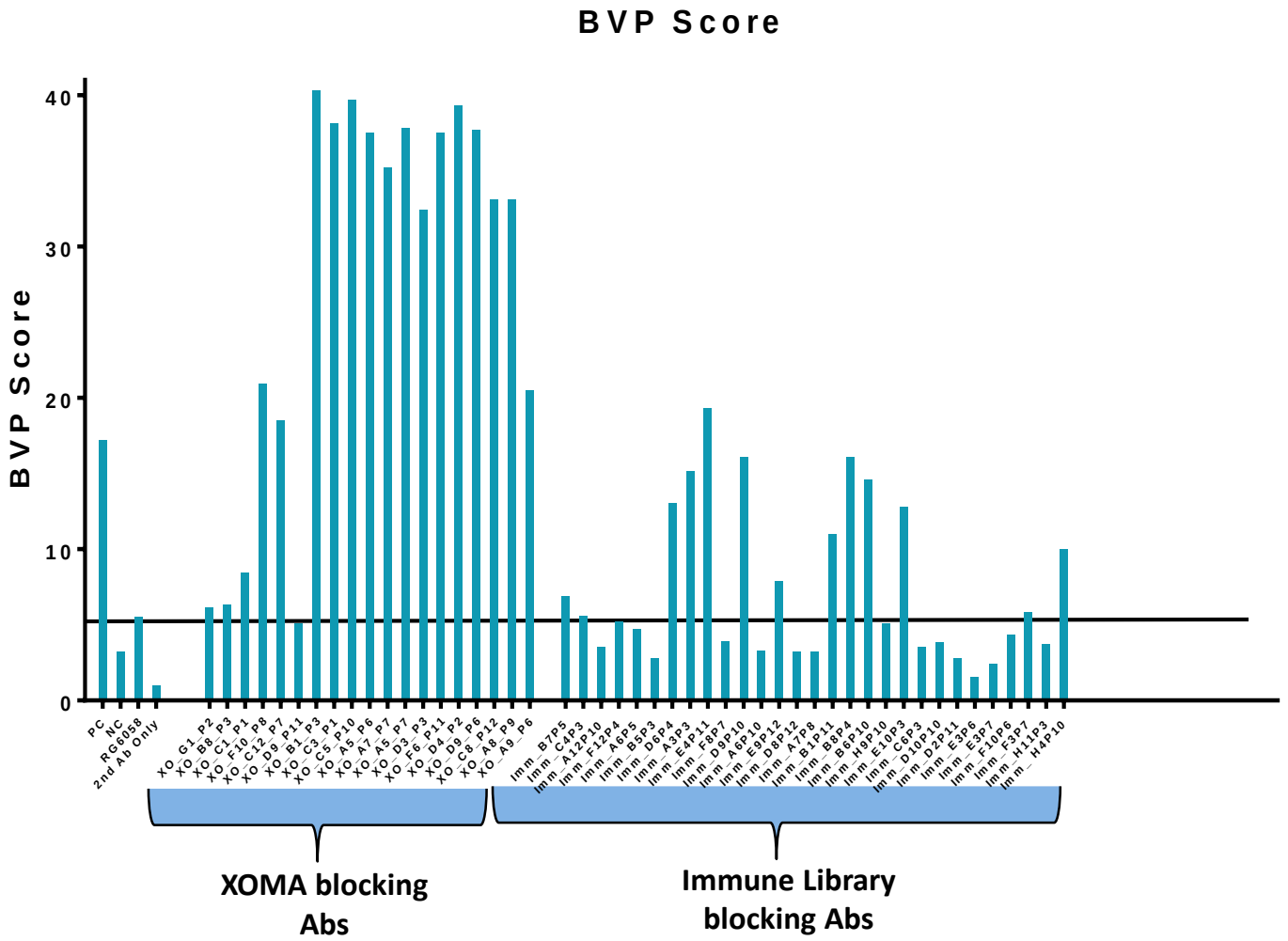
Polyspecificity ELISA of selected TIGIT-blocking antibodies

Polyspecificity ELISA experimental design[1]:

- Coating with Baculovirus particle (BVP)
- Loading anti-TIGIT Abs (150, 50, 16.7 μg/ml)
- Detection with HRP-conjugated anti-human Fc (2nd Ab)
- Positive control (PC) and negative control (NC) Abs
- RG6058 benchmark anti-TIGIT IgG4
- Background BVP wells coated only with 2nd Ab
- Cut-off: 5x coated background signal

BVP Score Calculation [1]:

- BVP score is determined by normalizing absorbance on control wells with 2nd Ab only
- BVP score = OD_{450} average of antibody (150 μg/mL) /OD_{450} average of 2nd Ab only



Polyspecificity ELISA assessment reveals multiple clones with low BVP score derived from the LakePharma immune library

[1] Jain *et al*. Biophysical properties of the clinical-stage antibody landscape. *PNAS*, 2017, 114(5), 944-949.

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Lead human TIGIT-blocking reformatted antibodies from phage library selections (3 best candidates highlighted in green color)

	Blocking huTIGIT Abs (Octet)	Yield in CHOTuna™ (mg)	K_D (human TIGIT) (nM)	K_D (cyno TIGIT) (nM)	Blocking huTIGIT Abs AlphaLISA (<3000 counts)	AlphaLISA (IC50)	IO assay (>500 RLUs)	IO Assay (IC50)	Polyspecificity (BVP score < 5)
Immune Library clones	Imm_A6P5	6.61	46	177	✓	5.46E-09	✓	3.85E-07	✓
	Imm_A6P10	5.87	15.3	351	✓	3.93E-09	✓	1.46E-07	✓
	Imm_A7P8	5.27	15.4	759	✓	2.94E-09	✓	3.02E-08	✓
	Imm_A12P10	8.28	69	276	✓	6.11E-09	✓	4.48E-08	✓
	Imm_B5P3	5.96	12.3	226	✓	2.99E-09	✓	1.72E-08	✓
	Imm_C4P3	7.13	2.2	4	✓	2.75E-09	✓	5.01E-09	✓
	Imm_C6P3	7.67	42	311	✓	3.60E-09	✓	4.88E-08	✓
	Imm_D2P11	6.25	50	186	✓	4.36E-09	✓	1.52E-01	✓
	Imm_D8P12	5.13	1.8	4	✓	6.07E-09	✓	3.94E-09	✓
	Imm_E3P6	3.01	14.4	476	✓	6.45E-09	✓	1.20E-08	✓
	Imm_E3P7	4.5	17.1	1609	✓	3.07E-09	✓	5.70E-07	✓
	Imm_F3P7	5.06	0.46	0.1	✓	3.61E-09	✓	3.90E-09	✓
	Benchmark		0.13	21	✓	4.12E-09	✓	6.12E-09	✓

Multiple TIGIT-blocking antibodies (12) from our immune library phage display selections were able to meet most ligand blocking, affinity, productivity and polyspecificity criteria

CONCLUSIONS

- We were able to discover multiple TIGIT antibodies following selections with the XOMA naïve Fab and LakePharma scFv immune libraries
- There was high sequence diversity of TIGIT binders from the XOMA and LakePharma libraries
- A high number of cynomolgus and human TIGIT cross-reactive binders was discovered following panning with the LakePharma immune libraries
- Co-immunization of mice with human and cynomolgus TIGIT resulted in higher affinity CD155 blocking antibodies, as shown by both AlphaLISA and cell-based immuno oncology assays
- Most TIGIT Abs originating from the LakePharma immune library were unable to recognize non-specifically baculovirus particles (low BVP score), possibly predicting fewer developability issues
- Presumably, affinity maturation of lower affinity TIGIT Abs derived from the XOMA library selections could lead towards the discovery of a diverse repertoire of potent human TIGIT-blocking antibodies

Numerous Carterra LSA platform advantages

- **Great customer and technical support**
- **High throughput screening**
  - Affinities, dissociation and association constants, epitope binning
- **Epitope binning can be performed with multiple (384 x 384) antibodies (ligands vs analytes)**
- **Sensitive kinetic characterization could allow preliminary selection prior to IgG reformatting**
  - Pros: Full kinetics using low concentrations of antibody fragments present in crude PPEs
- **Analysis software is very detailed and user-friendly**
  - (e.g. heat maps, sensograms, iso-affinity plots, binning networks)
- **Already multiple requests for Carterra LSA work from LakePharma clients**
  - as part of ongoing long-term antibody discovery projects
  - as stand alone requests for K_D or epitope binning analysis projects

Acknowledgements

- Daniel Bedinger and Yasmina Abdiche (Carterra)
- Antibody Center scientists (LakePharma)
  - Antibody Engineering
  - Discovery Immonology
  - Cell biology
  - Bacterial expression
  - Development center