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ABSTRACT

Antibody 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. These libraries have repeatedly enabled the discovery of antibodies against multiple challenging or traditional targets. Screening of these libraries was performed using our liquid handling automation deck. TIGIT is reportedly a very challenging antibody immuno oncology target, since the discovery of human and cynomolgus TIGIT cross-reactive Abs has been often problematic.

We will hereby present TIGIT-CD155 ligand inhibition data and kinetic characteristics of selected human and cyno TIGIT binding antibodies derived from phage panning. Subsequently, we will demonstrate details of antigen specificity attributes, such as polyspecificity assessments.

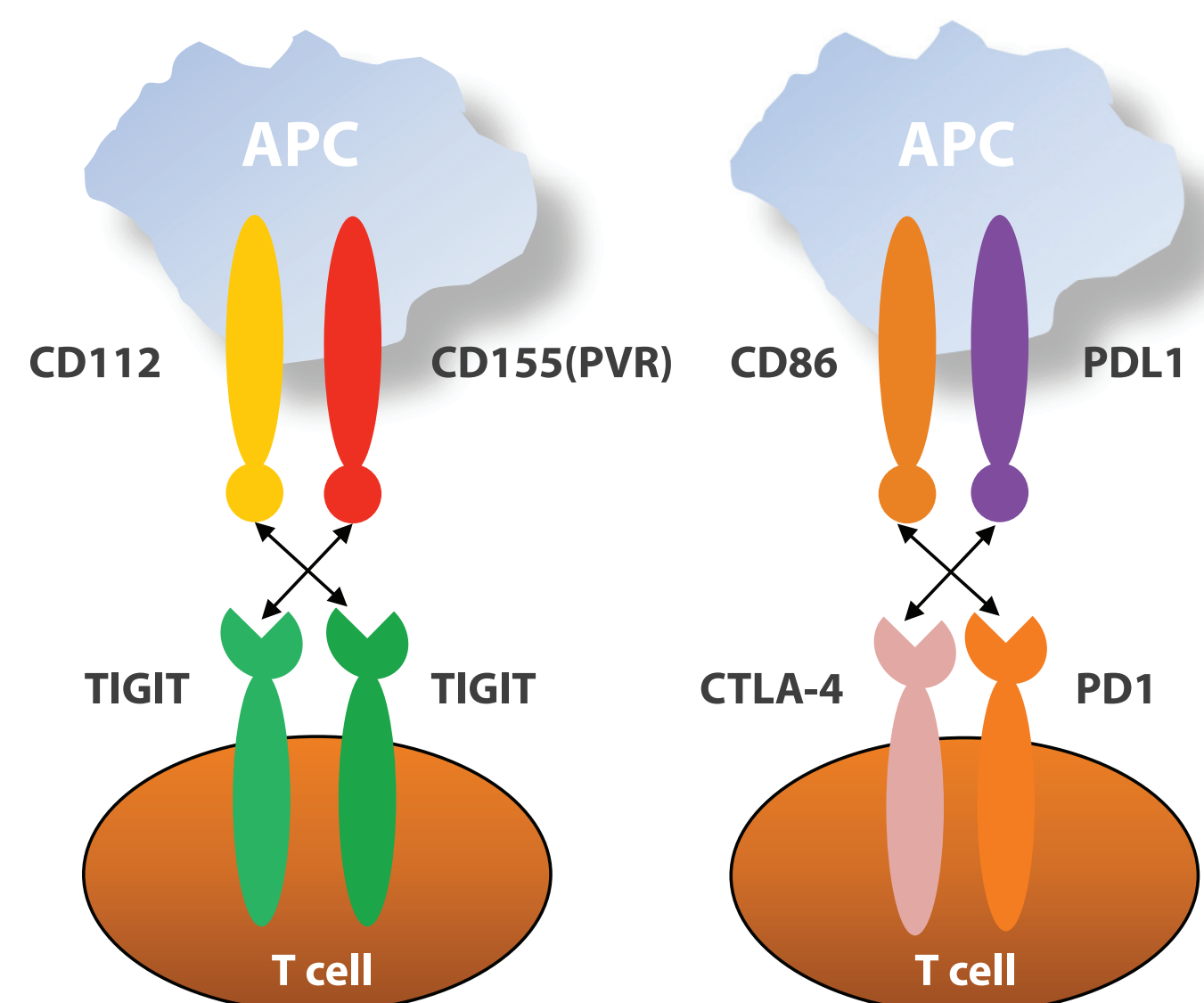
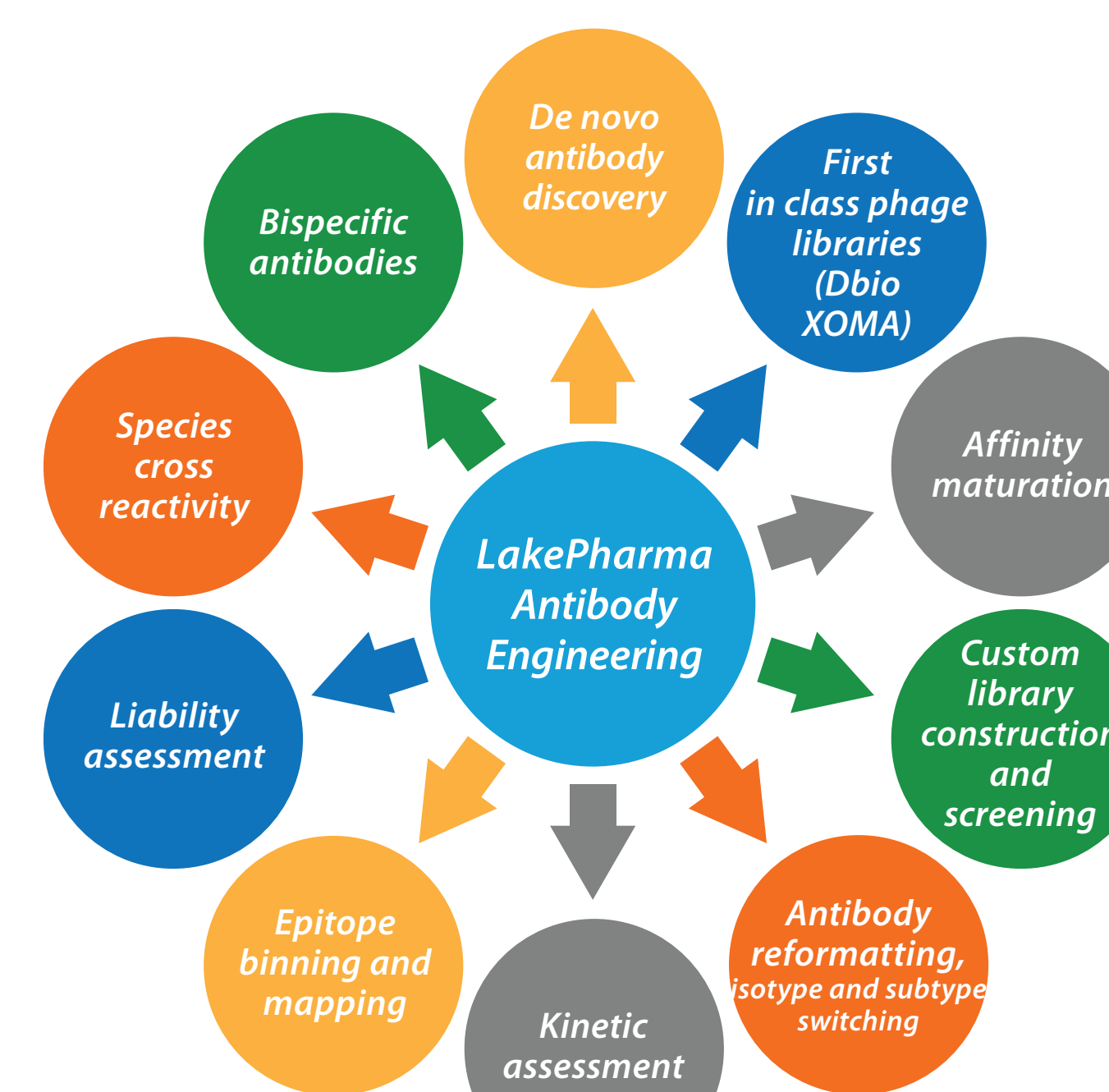


FIGURE 1: TIGIT has the unique ability to suppress immunity in a malignant microenvironment when the interaction with ligands CD155 (PVR) and CD122 is not properly regulated.

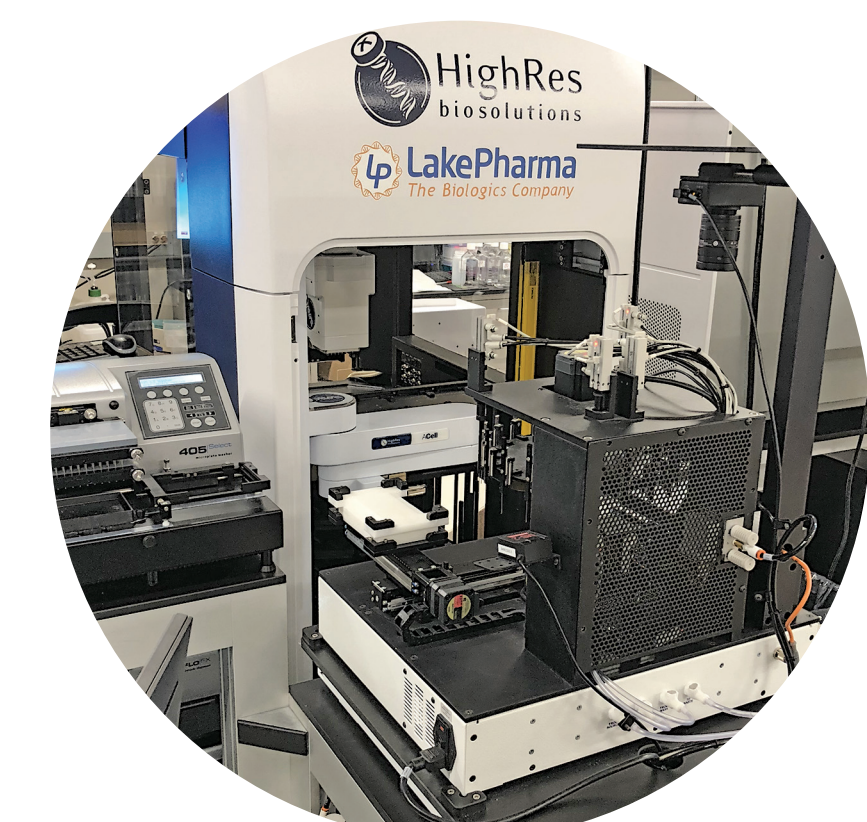
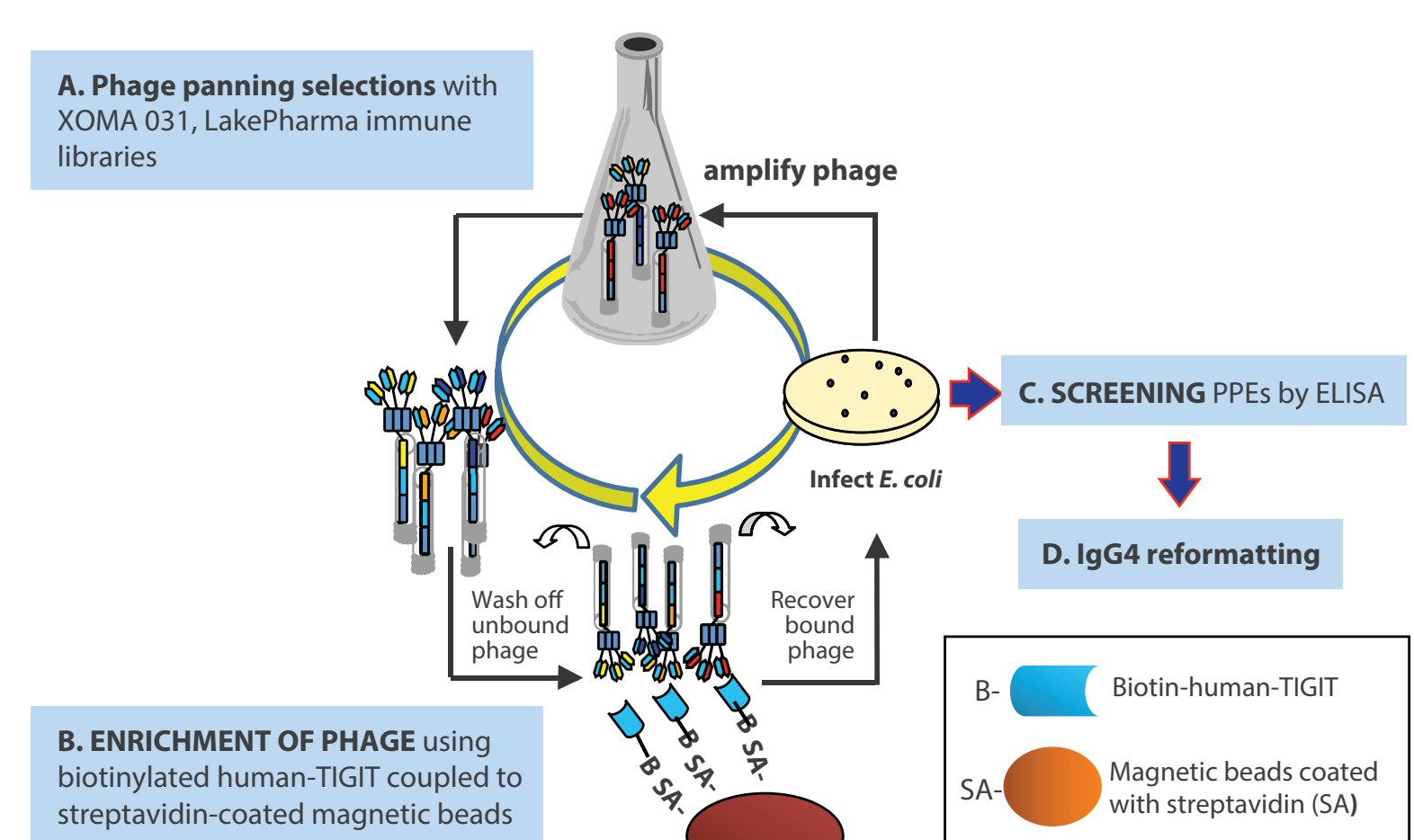
INTRODUCTION

- In-licensing of XOMA's 031 human Fab naïve phage library
 - Routinely selected pM affinity antibodies
 - Fully human, very large (>10¹¹), multiple open reading frames
 - Leads in multiple clinical programs
- LakePharma's 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, KD assessments using Octet or SPR



RESULTS

FIGURE 2: Phage Panning and Screening Workflow

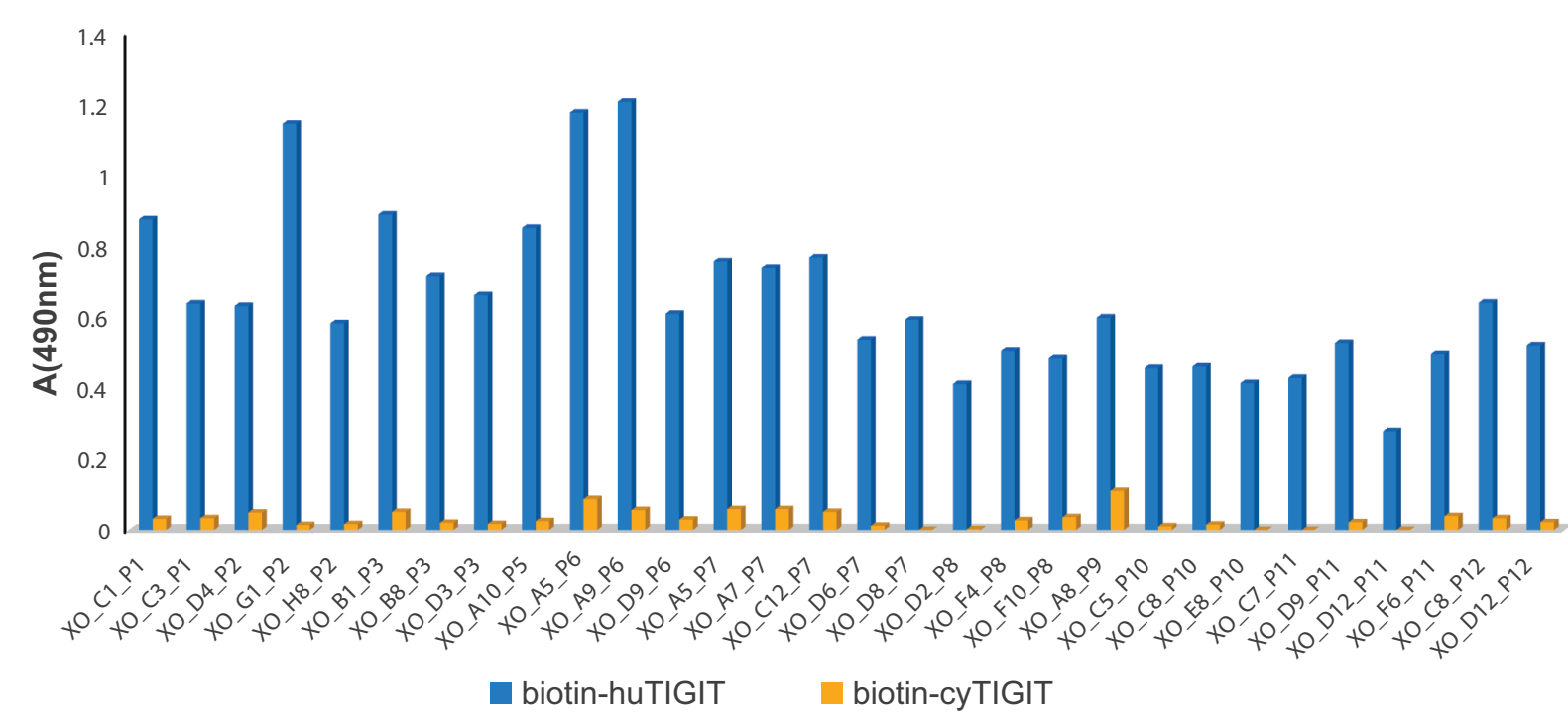


HighRes
biosolutions

- 4 rounds of selections with XOMA library:
 - Subsequent 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 library, following human and cyno-TIGIT mouse immunizations:
 - 3 rounds of selections with Biotin-huTIGIT on Streptavidin-coated ELISA plate wells
- 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

FIGURE 3: ELISA binding of TIGIT-specific Fab clones in bacterial PPEs following panning with the XOMA Fab phage library

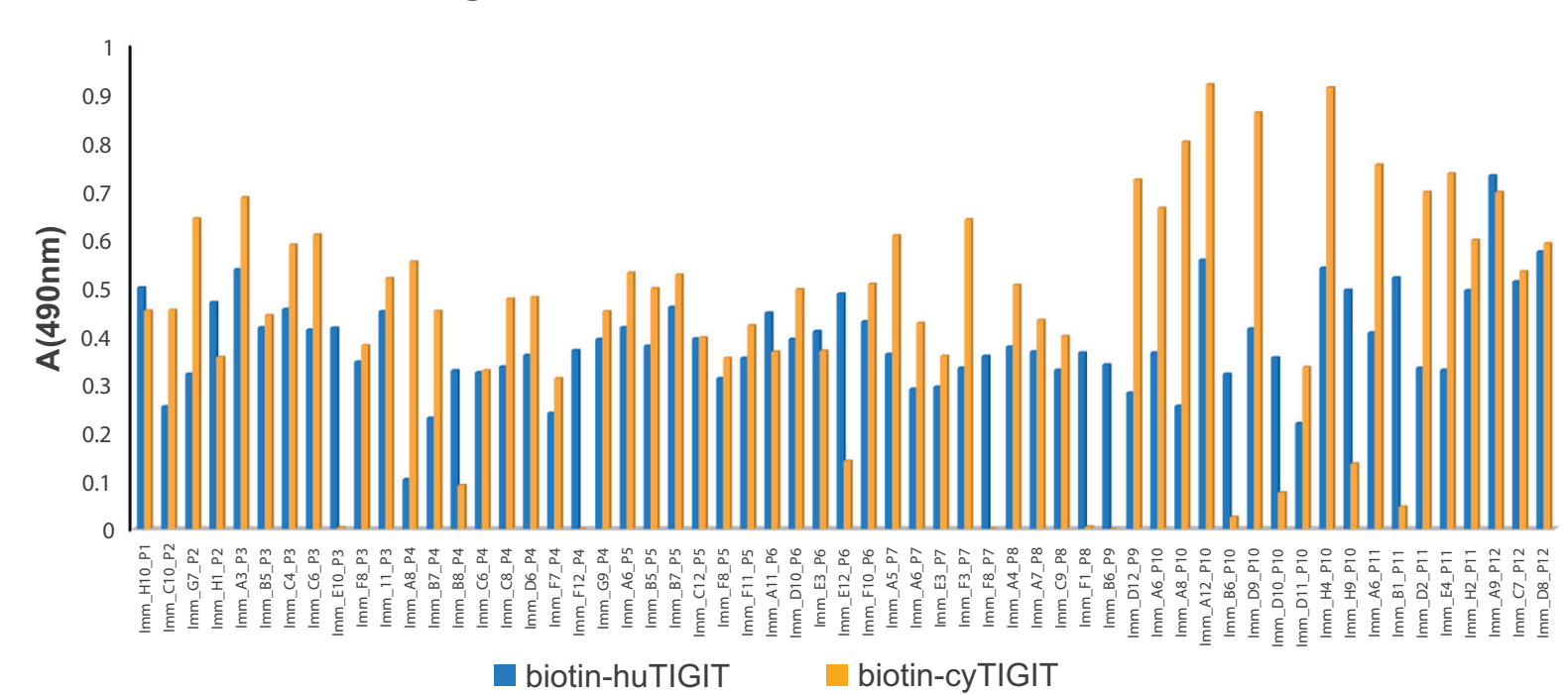
- 1116 individual clones of bacterial Fab PPEs screened by ELISA against cynomolgus and human TIGIT, using the HighRes deck
- No cynomolgus TIGIT binders from XOMA Fab library selections



Sequencing analysis allowed the selection of 30 unique human TIGIT Fab binders (Fig.3) which were reformatted into human IgG4 for further kinetic and functional characterizations

FIGURE 4: ELISA binding of TIGIT-specific scFv clones in bacterial PPEs following panning with a custom in-house immune phage library

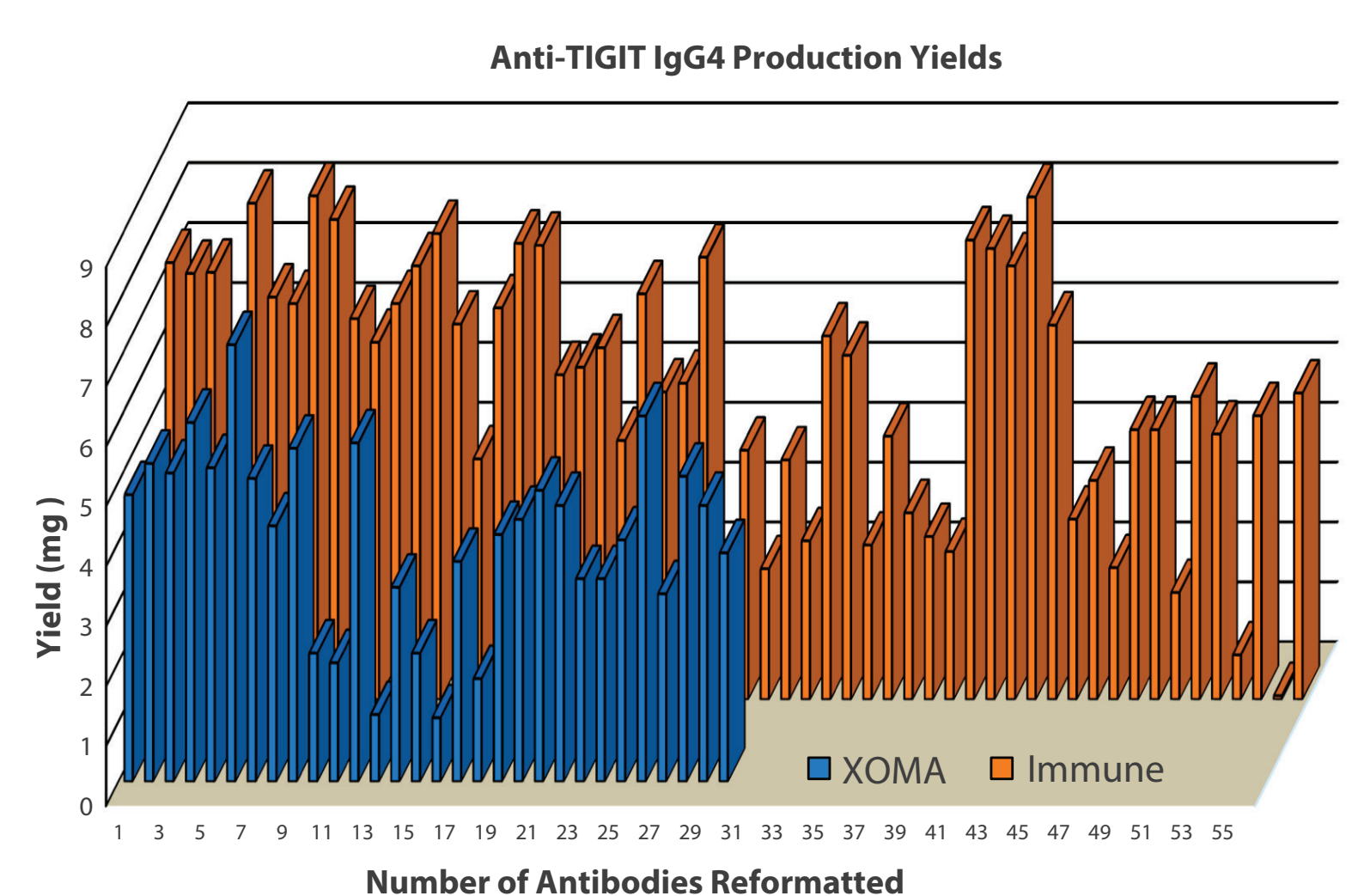
- Following co-immunization of mice with human and cynomolgus TIGIT-Fc using LakePharma's optimized hybridoma protocols, a diverse scFv phage library was generated with cDNA template from murine spleen mRNA
- 1116 individual clones of bacterial scFv PPEs screened by ELISA against cynomolgus and human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



Sequencing analysis allowed the selection of 56 unique TIGIT scFv binders (Fig.4) that were subsequently reformatted into a chimeric IgG4 isotype for further kinetic and functional characterizations

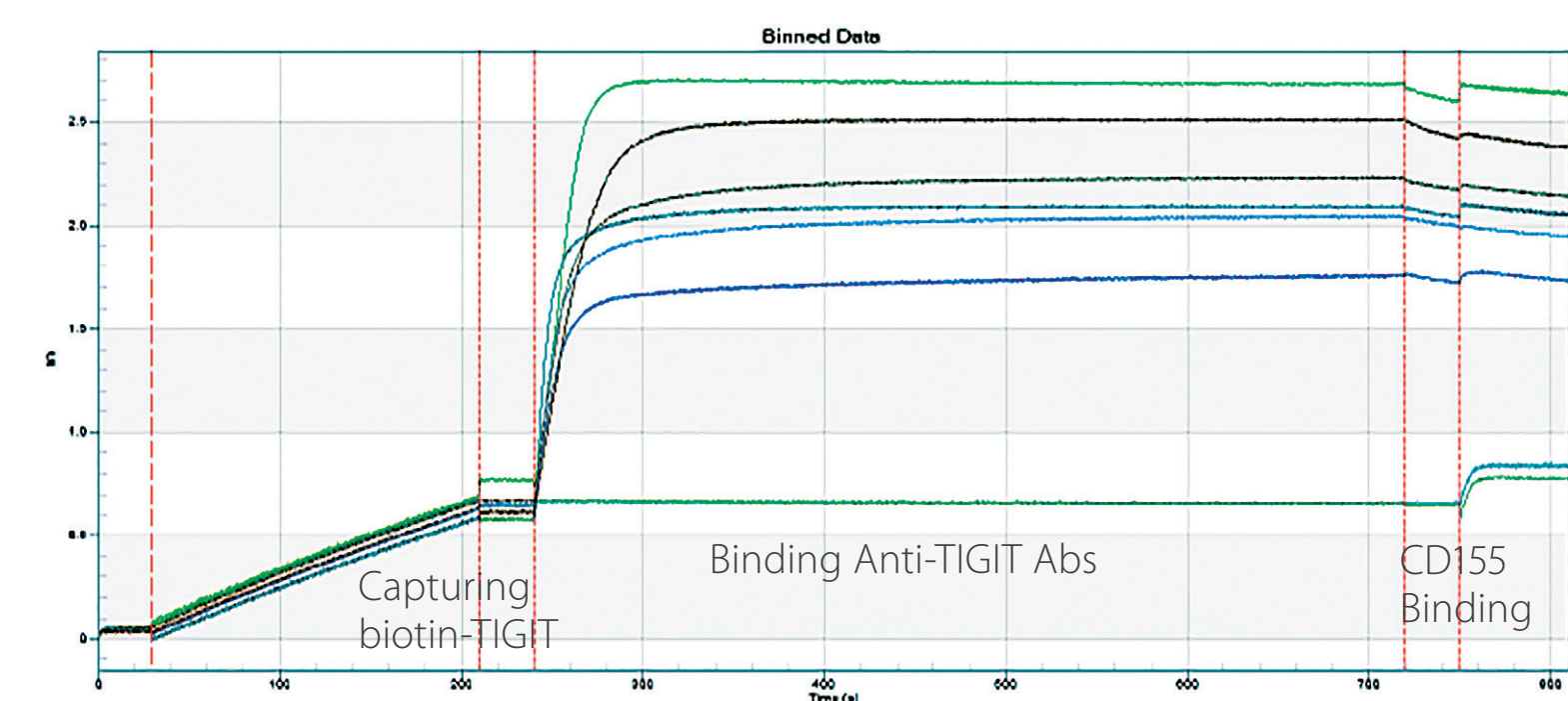
FIGURE 5: HT IgG4 Transient Production using LakePharma's TunaCHO™ cells

- 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



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

FIGURE 6: Octet analysis (sensogram example shown) demonstrates CD155 (PVR) blocking of TIGIT-binding antibodies



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

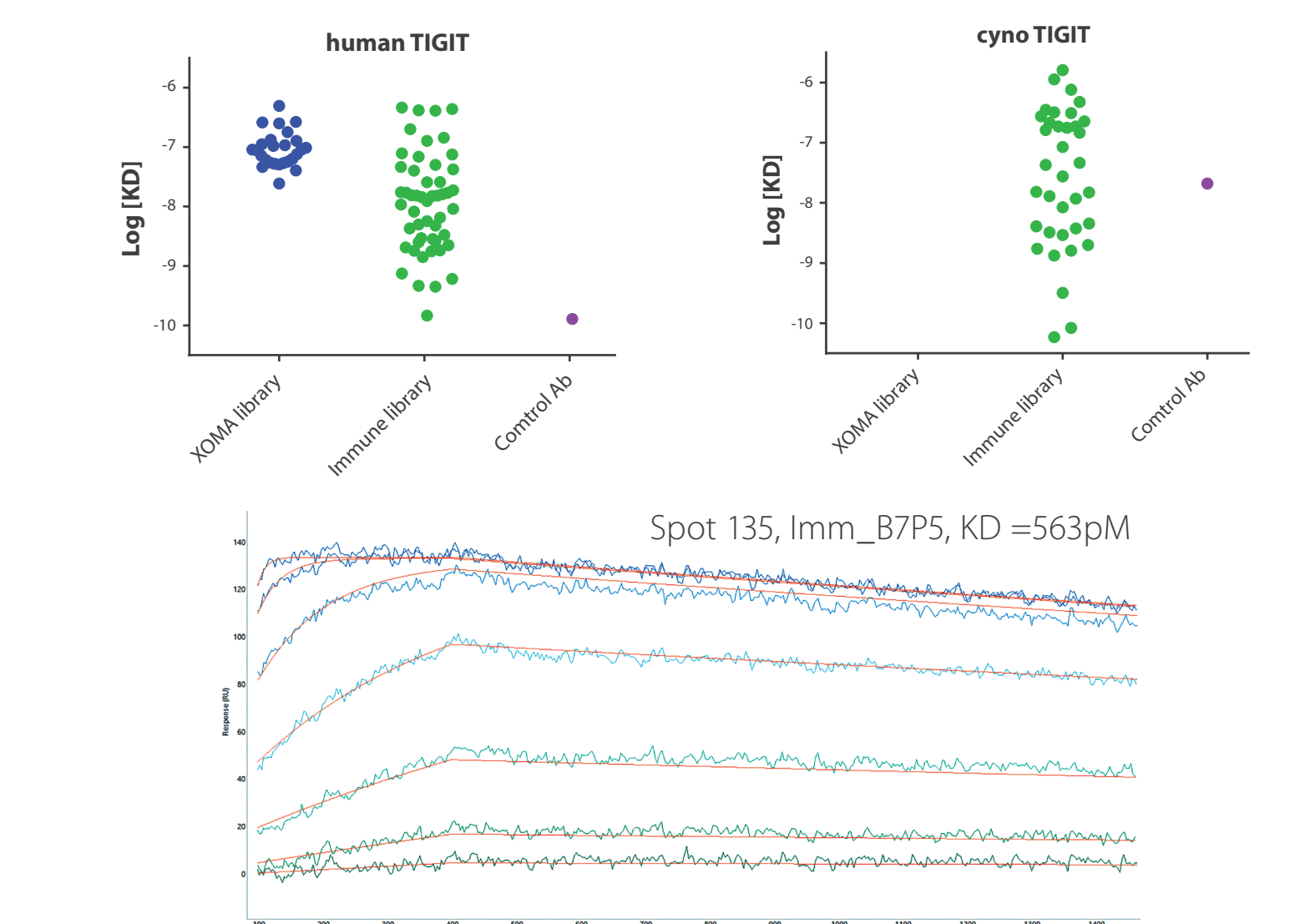
XOMA blocking IgG4		Immune blocking IgG4	
XO_A5_P6	XO_C5_P10	Imm_A12P10	Imm_D9P10
XO_A5_P7	XO_C8_P12	Imm_A3P3	Imm_E10P3
XO_A7_P7	XO_D3_P3	Imm_A6P10	Imm_E3P5
XO_A8_P9	XO_D4_P2	Imm_A6P5	Imm_E3P7
XO_A9_P6	XO_D9_P11	Imm_A7P8	Imm_E4P11
XO_B1_P3	XO_D9_P6	Imm_B1P11	Imm_E9P12
XO_B8_P3	XO_F10_P8	Imm_B5P3	Imm_F10P6
XO_C1_P1	XO_F6_P11	Imm_B6P10	Imm_F12P4
XO_C12_P7	XO_G1_P2	Imm_B7P5	Imm_F3P7
XO_C3_P1		Imm_B8P4	Imm_F8P7
		Imm_C4P3	Imm_H11P3
		Imm_C6P3	Imm_H4P10
		Imm_D10P10	Imm_H9P10
		Imm_D2P11	
		Imm_D6P4	
		Imm_D8P12	

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

FIGURE 7: KD distribution of IgG4-reformatted TIGIT-binding antibodies by Carterra



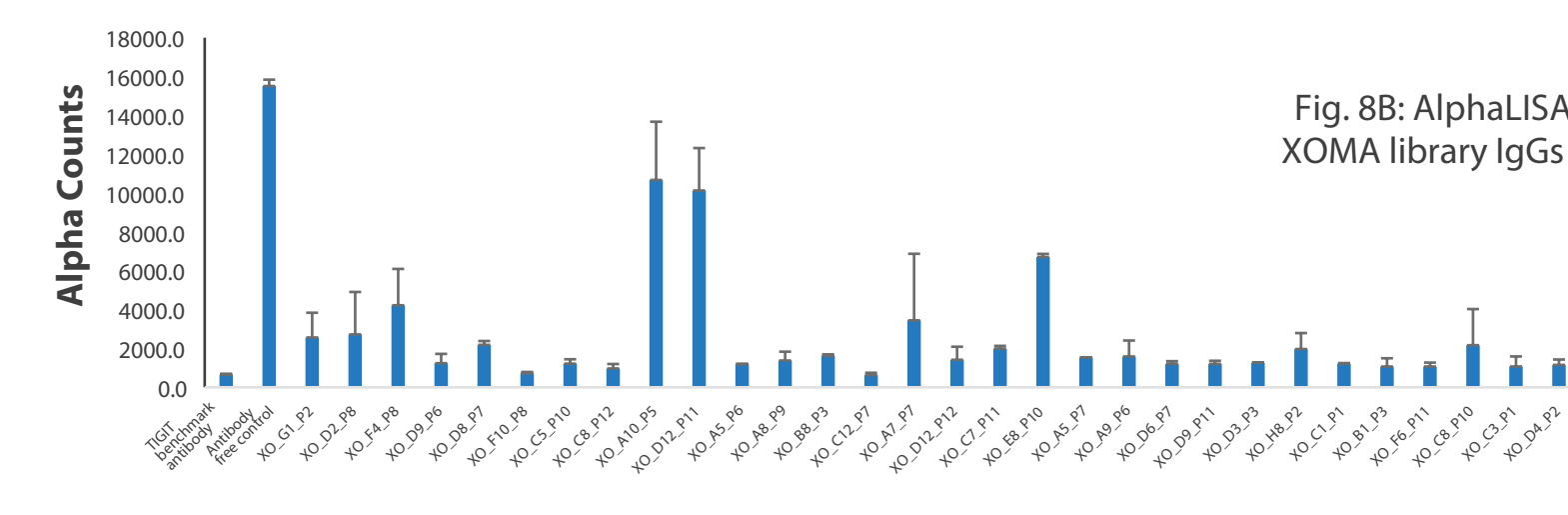
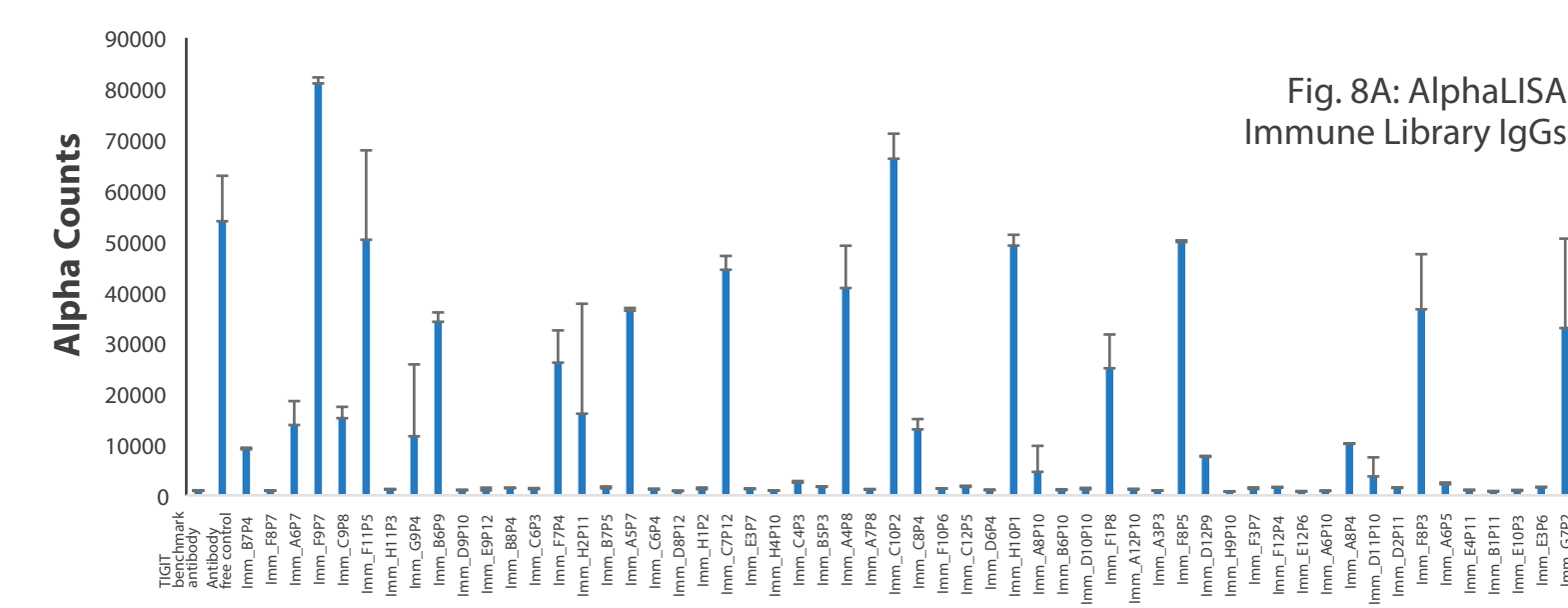
- Binding assays performed using Array SPR
- Purified human and chimeric antibodies (4.5 µg/ml) were loaded onto Anti-Human IgG Fc coated CMD50M chip
- Cyno and Human TIGIT were injected as monovalent analytes as a 3-fold dilution series (0.4-300nM) over IgGs captured onto discrete spots to create a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



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

FIGURE 8: AlphaLISA ligand CD155 blockade assay for immune (A), XOMA (B) library reformatted clones

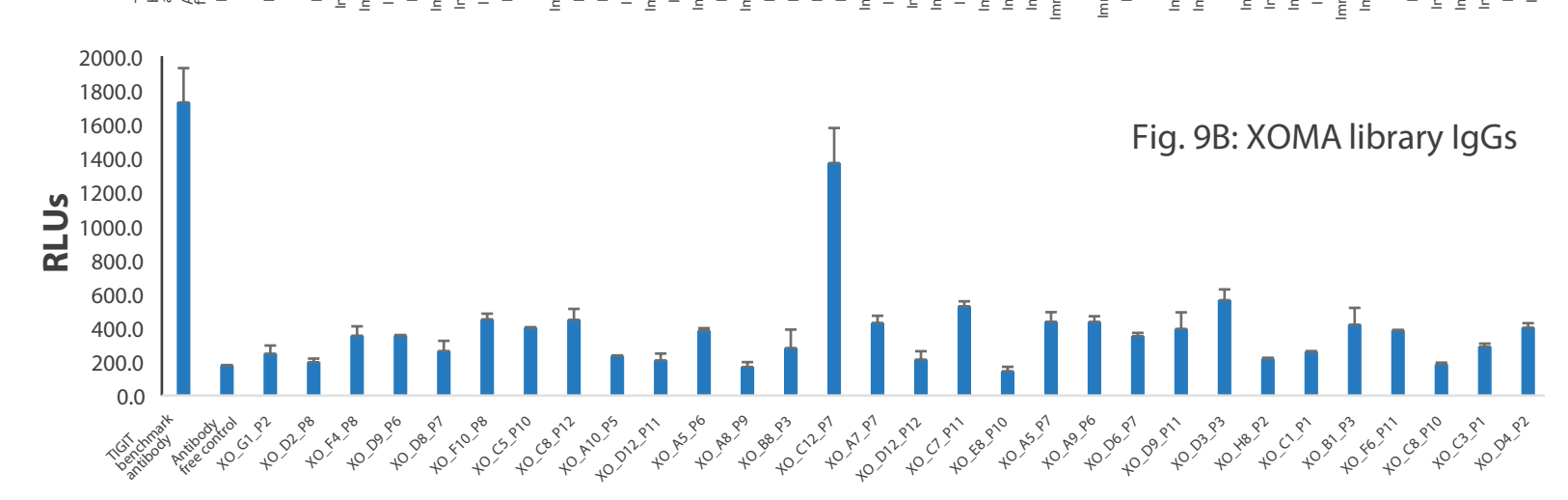
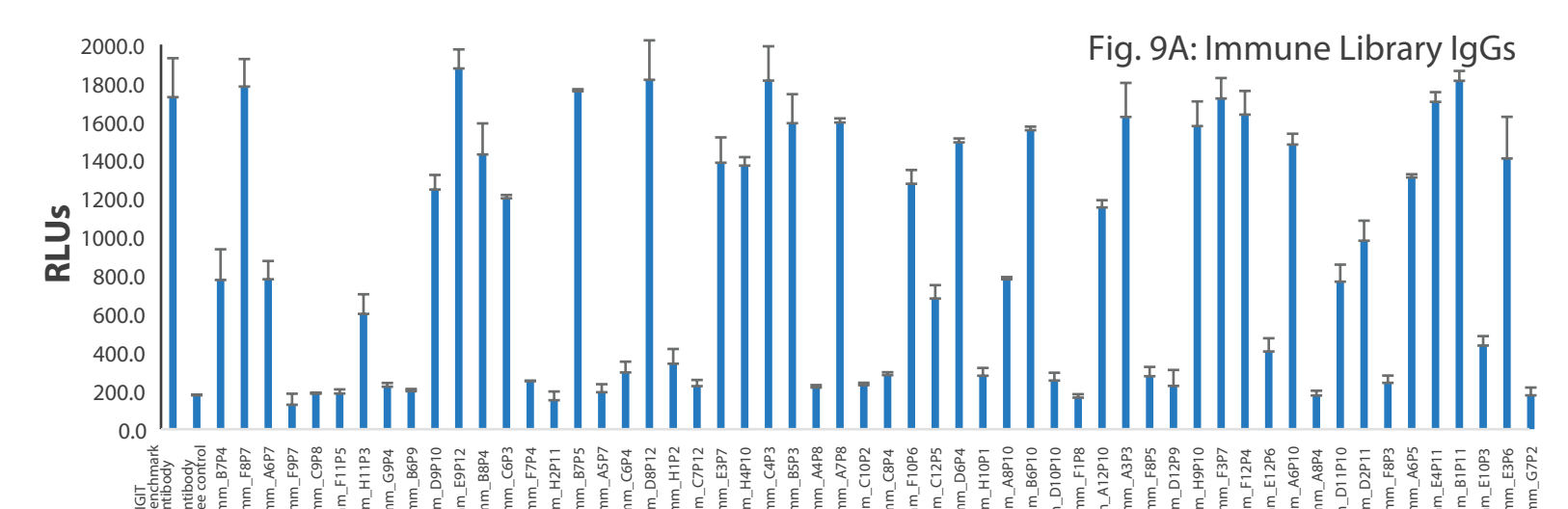
- TIGIT:CD155 Homogeneous Assay Kit in AlphaLISA format (BPS Bioscience) using biotin-huTIGIT and human CD155 ligand
- Anti-TIGIT IgGs (40-160 µg/ml) incubated with human TIGIT before adding CD155
- Acceptor and donor beads added before monitoring Alpha Counts
- Lower Alpha Count values indicate CD155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



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

FIGURE 9: Cell-Based Immunoblockade Assay (Promega) for immune (A), XOMA (B) library reformatted clones

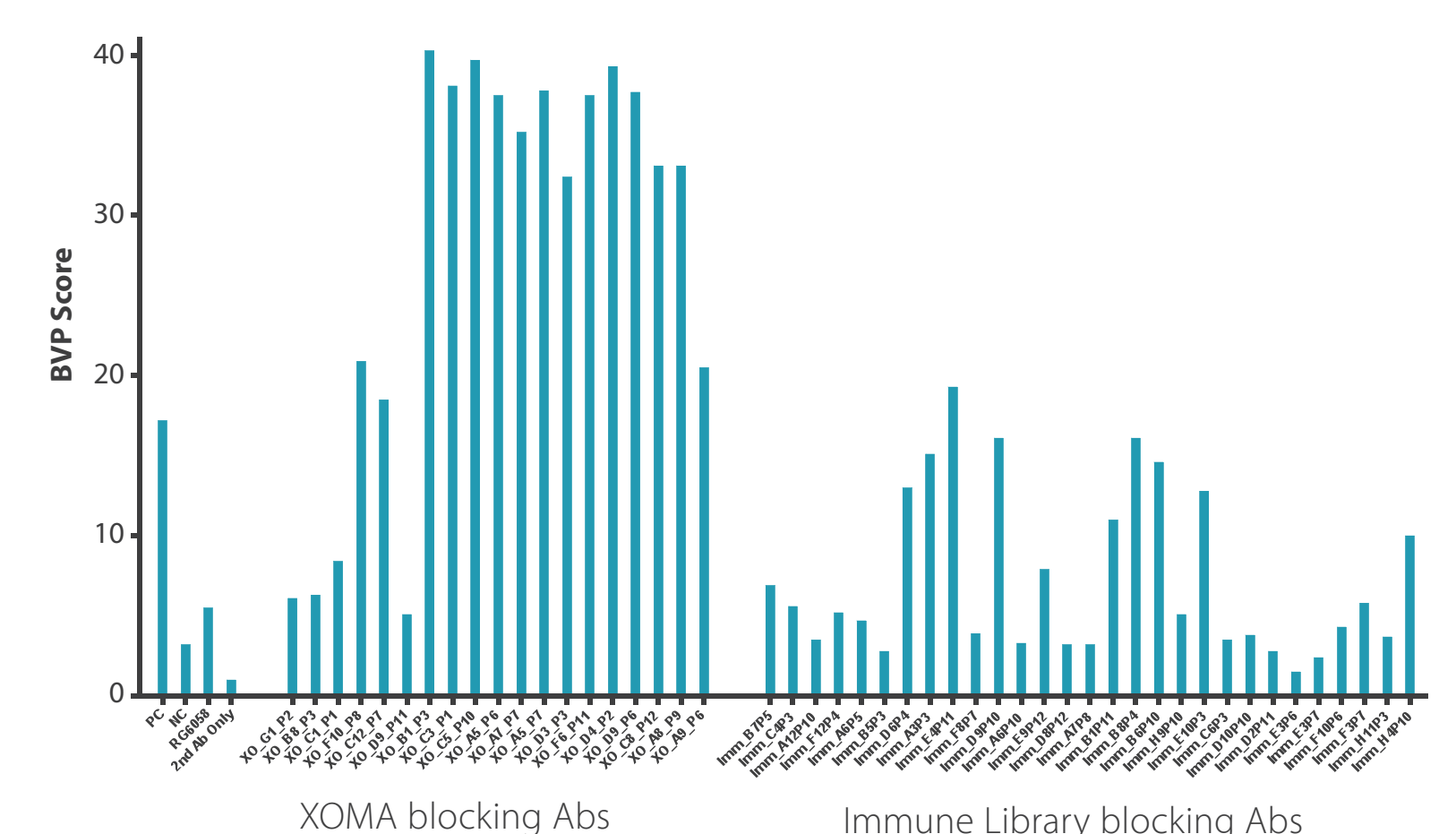
- CD226-induced luminescence is inhibited when Jurkat effector T cells expressing human TIGIT with luciferase reporter are co-cultured with CHO-K1 cells expressing CD155
- Anti-TIGIT IgGs (33-132 µg/ml) block interaction of TIGIT/CD155, resulting in CD226-activated luminescence (Higher RLU indicate CD155 blockade)



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

FIGURE 10: 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), negative control (NC), 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 determined by normalizing absorbance with 2nd Ab only
 - BVP score = OD₄₅₀ average of antibody (150 µg/mL) / OD₄₅₀ average of 2nd Ab only



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

TABLE 2: Lead human TIGIT-blocking reformatted antibodies from phage library selections (3 best candidates highlighted in green)

Blocking huTIGIT Abs (Octet)	Yield in CHOCHO™ (mg)	KD (human TIGIT) (nM)	KD (cyno TIGIT) (nM)	Blocking huTIGIT Abs AlphaLISA (<3000 counts)	AlphaLISA (IC50)	IO assay (>500 RLU)	IO assay (IC50)	Polyspecificity (BVP score < 5)
Imm_A9P5	6.61	46	177	✓	5.48E-09	✓	3.85E-07	✓
Imm_A6P10	5.87	15.3	351	✓	3.93E-09	✓	1.48E-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	✓	2.98E-09	✓	1.72E-08	✓
Imm_C4P3	7.13	4.2	4	✓	2.75E-09	✓	5.01E-08	✓
Imm_C6P3	7.67	42	311	✓	3.60E-09	✓	4.88E-08	✓
Imm_D2P11	6.25	50	186	✓	4.36E-09	✓	1.53E-01	✓
Imm_D9P10	5.19	16	41	✓	5.07E-09	✓	3.94E-09	✓
Imm_E3P5	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-07	✓
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-specific antibodies following selections with the XOMA 031 naïve Fab and LakePharma scFv immune libraries
- There was a high sequence diversity of TIGIT binders derived from the XOMA and LakePharma phage libraries
- Surprisingly, 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

References:

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