Abveris Antibody Discovery Case Study

Accelerated, Function-Forward Therapeutic Antibody Discovery Targeting an Intractable Cell Surface Receptor

Concurrent use of Hyperimmune (DiversimAb) & Humanized Mice in a Beacon-Based Single B Cell Workflow

DISCOVERY OF FUNCTIONAL HUMAN ANTIBODIES AGAINST TARGET X

Case study background

Partner

- Well-established public biopharma
- Outsources all antibody discovery, requires highly capable partners

Target

- Type II TM cell surface receptor
- Difficult to target – immunogenicity challenges
- Failed a campaign at another CRO

End Application

- Therapeutic antibody development

Goals

1. Human/cyno cross-reactivity required
   Murine cross-reactivity desired, not required
2. Ligand blocking activity required
3. On-cell binding required
4. Fully human mAb preferred (Hu)
   Murine mAbs acceptable (ABV)
5. Binning profiling against benchmark (BM) antibody desired, not required

OVERVIEW OF SINGLE B CELL WORKFLOW

Project Goal: Hu/cy/mu cross-reactivity, ligand blocking, and on-cell binding

Workflow

① Immunization & Assay Development — 3 – 8 weeks

② Beacon Workflow (Specificity & Function) — 1 Day

③ Sequencing & Recombinant Expression — 6 weeks

④ Functional Validation and Characterization — 8 weeks

⑤ Developability Assessment — 1 week

- Immunization of humanized mice (Hu) & hyperimmune mice (ABV, DiversimAb)
- Custom DNA/cell/protein & protein-only immunization protocol for humanized mice
- Simultaneous antigen QC and Beacon assay development

- Titer analysis for mice selection
- Tissue harvest and enrichment of plasma B cells
- Sequential single-cell assays performed to identify:
  - On-cell binding
  - Ligand blocking activity
  - Species cross-reactivity
  - Benchmark binning profile (only for ABV)

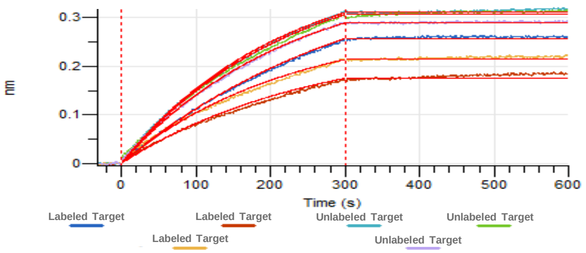
- Exported to lysis buffer in PCR plates and cDNA synthesis
- Sequencing for hit recovery
- Small-scale expression in a HEK-based system and purification for validation
- Repertoire sequencing of memory B cells by NGS to expand scope of hit selection (by partner)

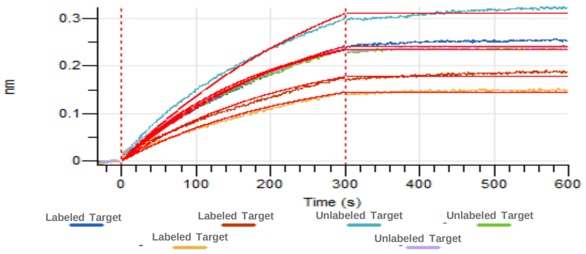
- Antibody validation by flow cytometry and ELISA for binding specificity confirmation
- Antibody validation by Octet BLI for ligand blocking confirmation
- Mid-scale expression for further characterization of selected antibodies
- K_D determination & binning of mAbs (and BM) by Carterra SPR

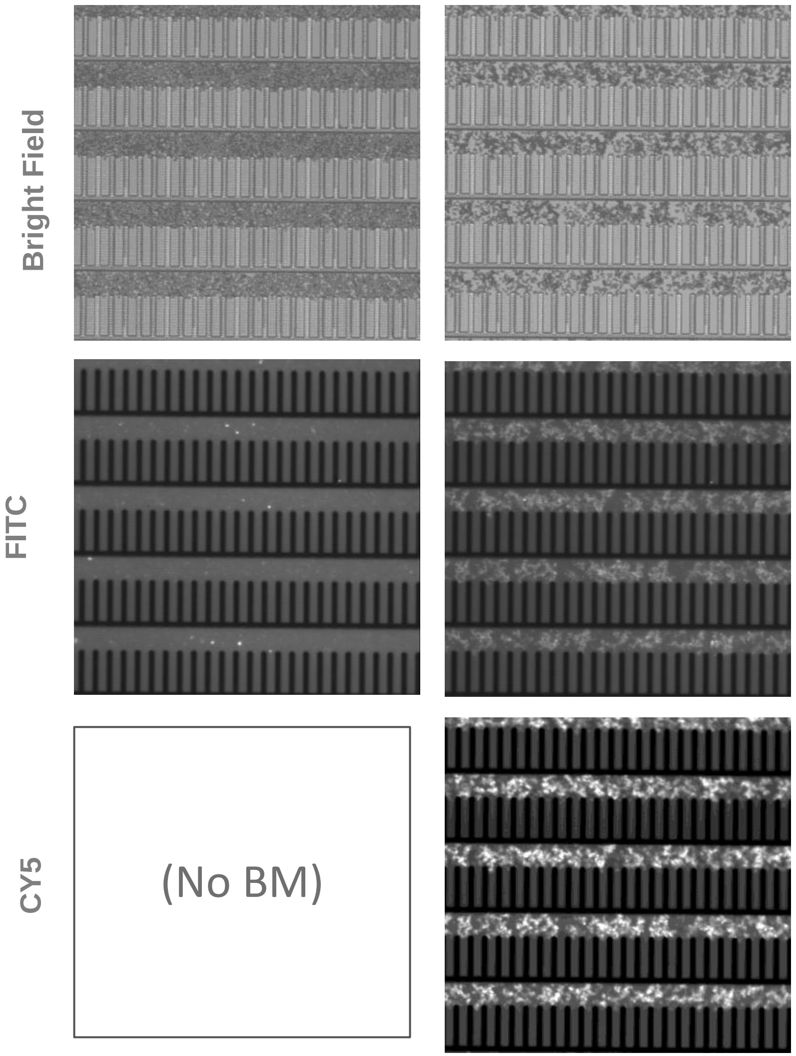
- Developability assessment using 3 bioanalytical assays
  - HIC (hydrophobicity)
  - CIC (cross-interaction)
  - CSI-BLI (self association)
- Developability profiles established for leads to provide further data for downstream candidate selection

ANTIGEN QC, ASSAY DEVELOPMENT, AND CELL LINE OPTIMIZATION

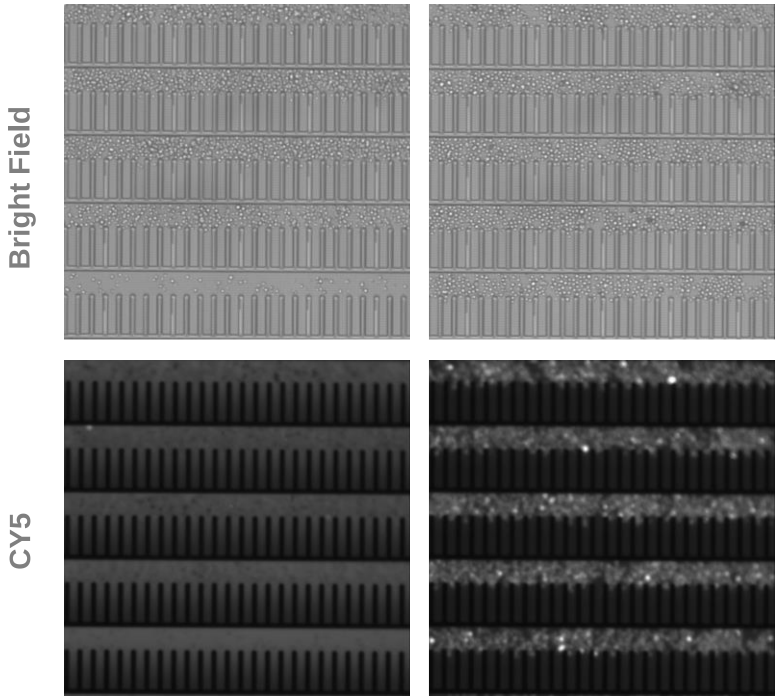
Labeled antigen QC both on and off Beacon enabled successful assay development


BM Binding Hu Target X


BM-AF647 Binding Hu Target X


Anti-BM Beads + Hu Target X-AF488 (no BM)

Anti-BM Beads + BM-AF647 + Hu Target X-AF488


Parental Cells + BM + Anti-BM-AF647

Target Cells + BM + Anti-BM-AF647

DIVERSE IMMUNIZATION STRATEGY AND TITER ANALYSIS

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Use of different antigens produced target-specific titer responses in both DiversimAb and humanized mice

Immunization

Summary of Immunization Methods

	Strain	Immunogen(s)
Cohort 1	**Hu**	**Protein-only**
Cohort 2	**ABV (DiversimAb)**	**Protein-only**
Cohort 3	**Hu**	**Cell/DNA/protein mixed**

- DiversimAb mice demonstrated hu/cy/mu triple cross-reactive titers while the humanized mice did not
- Abveris proprietary protein and mixed immunizations produced recombinant protein and on-cell titer response
- 1 Mouse was chosen from each cohort for tissue harvest and Beacon screening workflow

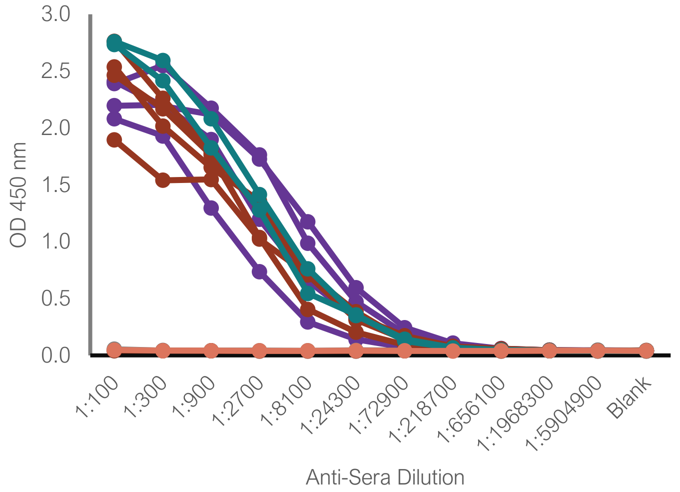
Figure 1A. Anti-hu Target X ELISA Titer

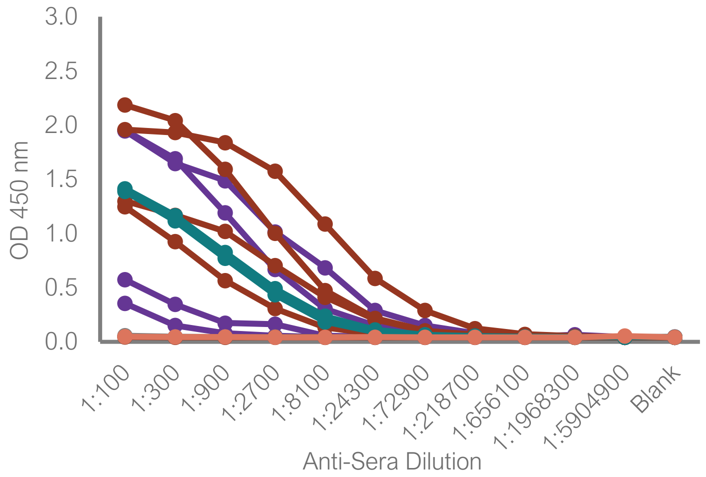
Figure 1B. Anti-cyno Target X ELISA Titer

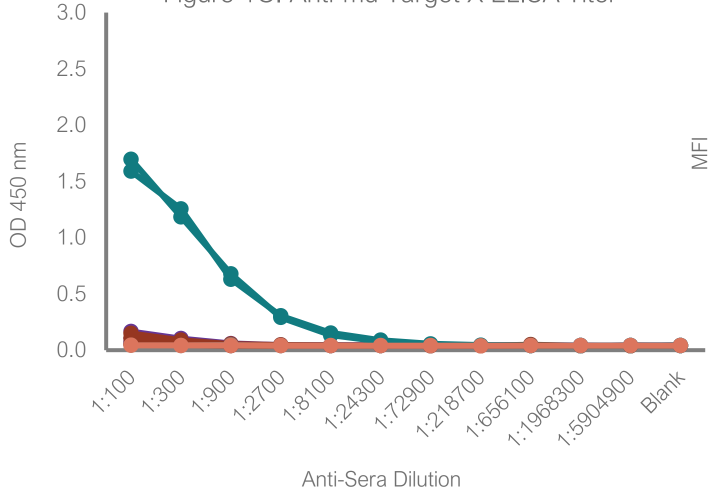
Figure 1C. Anti-mu Target X ELISA Titer

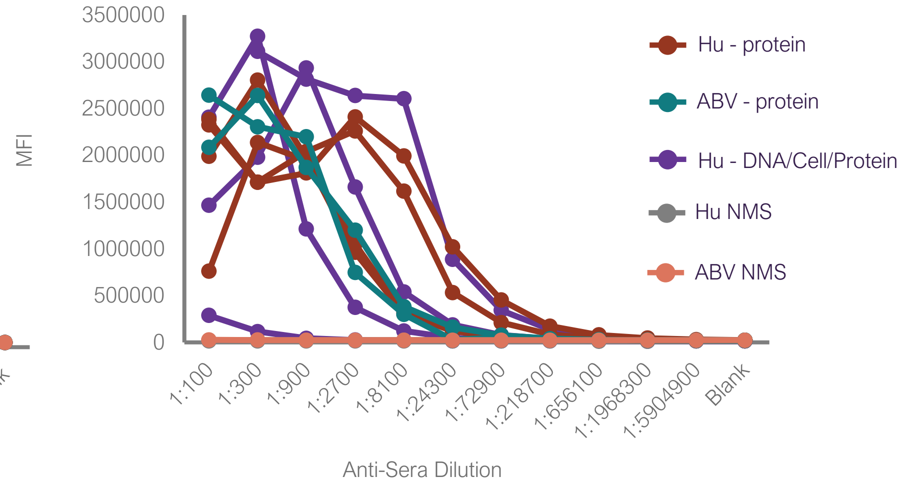
Figure 1D. Anti-hu Target X On-Cell Flow Titer

UP TO FOUR SEQUENTIAL ASSAYS WERE INCLUDED IN THE SCREENING WORKFLOW

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High resolution screening of entire plasma cell population in one single day

Beacon Screen

Goals (required):

- ☐ Human/cyno cross-reactivity
- ☐ Ligand blocking
- ☐ On-cell binding

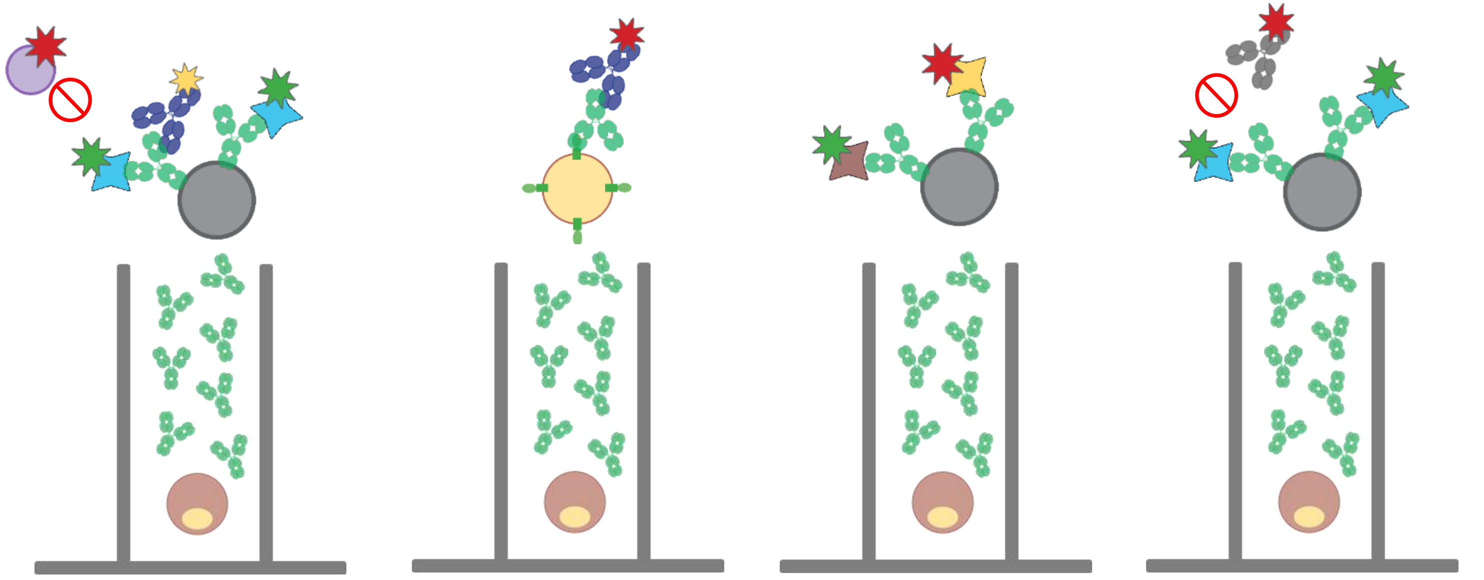
Goals (desired):

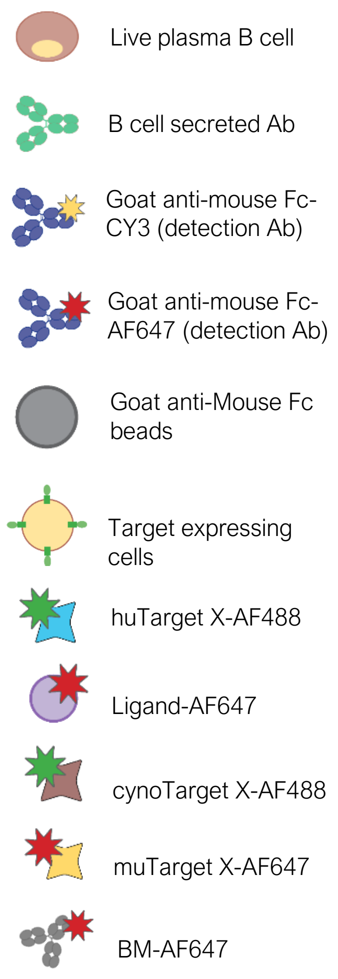
- ☐ Binning profiling using benchmark antibody ("BM")
- ☐ Murine cross-reactivity
- ☐ *Strong preference for human Abs from humanized mice*

Humanized Mice Screening Workflow – 3 Sequential Assays (2 Runs)

DiversimAb Mice Screening Workflow – 4 Sequential Assays (1 Run)

Assay 1 Ligand Blocking and IgG Secretion Check	Assay 2 On-Cell Binding Check	Assay 3 Species Cross-Reactivity	Assay 4 Benchmark Ab Pairing Assay
Goat anti-Mouse Fc beads + hu Target X-AF488 + Ligand-AF647 + Goat anti-Mouse Fc CY3	Target cells + Goat anti-Mouse Fc AF647	Goat anti-mouse Fc beads + cyno Target X-AF488 + mu Target X-AF647	Goat anti-Mouse Fc beads + hu Target X-AF488 + BM-AF647





Legend

- Live plasma B cell
- B cell secreted Ab
- Goat anti-mouse Fc-CY3 (detection Ab)
- Goat anti-mouse Fc-AF647 (detection Ab)
- Goat anti-Mouse Fc beads
- Target expressing cells
- huTarget X-AF488
- Ligand-AF647
- cynoTarget X-AF488
- muTarget X-AF647
- BM-AF647

BEACON SCREENING RESULTS

Project Goal: Hu/cy/mu cross-reactivity, ligand blocking, and on-cell binding

Beacon Screen

Assay 1 Ligand Blocking and IgG Secretion Check

Goat anti-Mouse Fc beads +
Goat anti-Mouse Fc CY3
hu Target X-AF488 +
Ligand-AF647

Assay 2 On-Cell Binding Check

Target cells + Goat anti-Mouse Fc AF647

Assay 3 Species Cross-Reactivity

Goat anti-mouse Fc beads +
cyno Target X-AF488 +
mu Target-AF647

Assay 4 Benchmark Ab Pairing Assay

Goat anti-Mouse Fc beads +
hu Target X-AF488 +
BM-AF647

Results:

- ☐ 2 Runs were performed to screen plasma B cells from two Hu mice, respectively
- ☐ 1 Run was performed to screen single B cells from one ABV
- ☐ Identification of hits from both Hu and ABV mice with the required binding and blocking profiles
- ☐ Clones were thus exported for single cell sequencing and expression

		Run #1	**Run #2**	**Run #3**
	Mouse	**Co 1-2**	**Co 2-2**	**Co 3-3**
	Strain	**Hu**	**ABV**	**Hu**
	Immunogen	**Protein**	**Protein**	**Mixed**
Priority 1 Hit Count	hu/cy/mu target cross reactive binding + target cell binding + ligand blocking	**0**	**1**	**0**
Priority 2 Hit Count	hu/cy target cross reactive binding + target cell binding + ligand blocking	**3**	**35**	**8**
Priority 3 Hit Count	hu-only target binding + target cell binding + ligand blocking	**0**	**3**	**1**
Priority 4 Hit Count	All other ligand blockers	**24**	**44**	**15**
Priority 5 Hit Count	Other potentially interesting hits	**19**	**109**	**48**
Total Hit Exported		**46**	**192***	**72**

** Maximum number of hits exported is 192 per run*

BEACON SCREENING RESULTS IN NUMBERS

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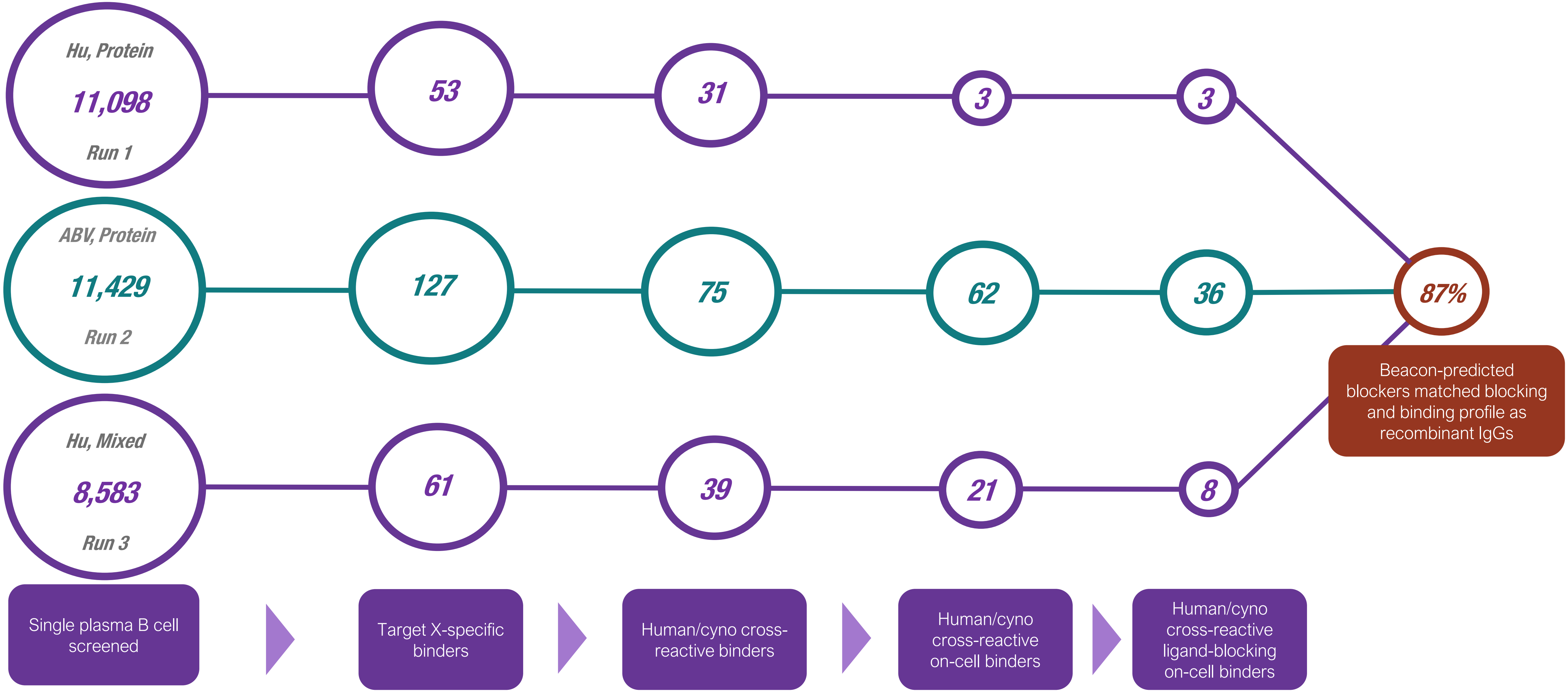
Number of clones screened and profile of hits from the single B cell workflow



	Single plasma B cell screened	Target X-specific binders	Human/cyno cross-reactive binders	Human/cyno cross-reactive on-cell binders	Human/cyno cross-reactive ligand-blocking on-cell binders
Hu, Protein Run 1	11,098	53	31	3	3
ABV, Protein Run 2	11,429	127	75	62	36
Hu, Mixed Run 3	8,583	61	39	21	8

87%

Beacon-predicted blockers matched blocking and binding profile as recombinant IgGs

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VALIDATION RESULTS: SINGLE-POINT FACS, ELISA FOR SPECIFICITY

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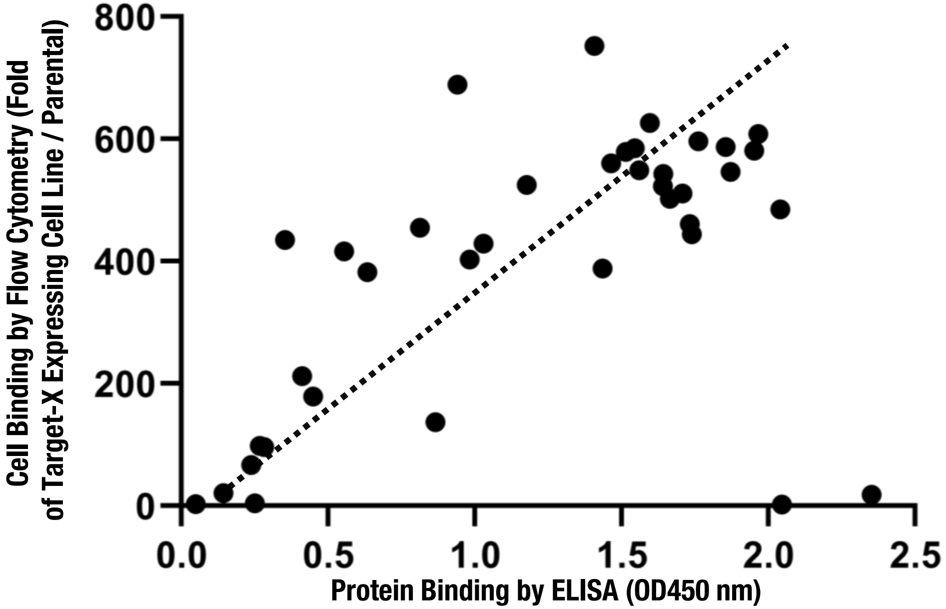
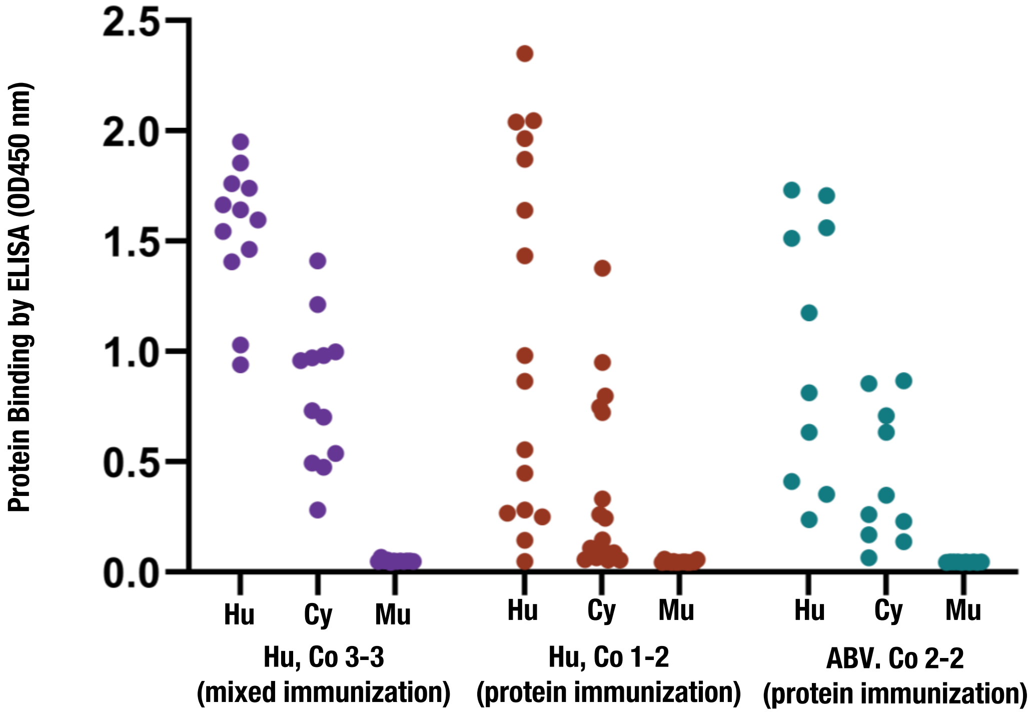
Candidates recapitulated Beacon binding profiles as recombinantly expressed antibodies

Figure 2A. Recombinant Antibody Binding Validation Flow Cytometry vs. ELISA



- 49 clones exported from Beacon were expressed and validated off-Beacon for binding to hu Target X (all 3 mice)
- 45 of 49 (92%) of sequences expressed as recombinant IgG
- 84% of recombinantly expressed clones exhibited on-cell binding to hu Target X expressing cells

Figure 2B. Recombinant Antibody Cross-Reactivity by ELISA



- 84% of hu/cy Target X cross-reactive binders maintained expected species binding specificity off-Beacon

TWIST BIOSCIENCE

VALIDATION RESULTS: RECOMBINANT HUMAN VS. CYNO PROTEIN BINDING

Off-Beacon validation of IgG off-Beacon by Octet BLI revealed a wide range of affinity and confirmed hu/cy cross-reactivity

Validation Results:

- Expressed mAbs were validated by Octet BLI to reveal a wide range of affinities
- Affinities of mAbs to recombinant human and cyno Target X proteins were analyzed via single-point kinetics assessment
- A positive correlation between affinities for human and cyno Targets X was observed, which is consistent with the degree of homology between the two orthologs

Figure 3A. Hu Target X Affinity

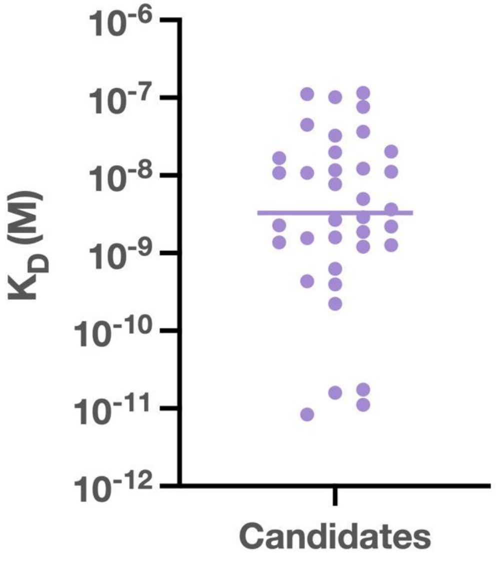
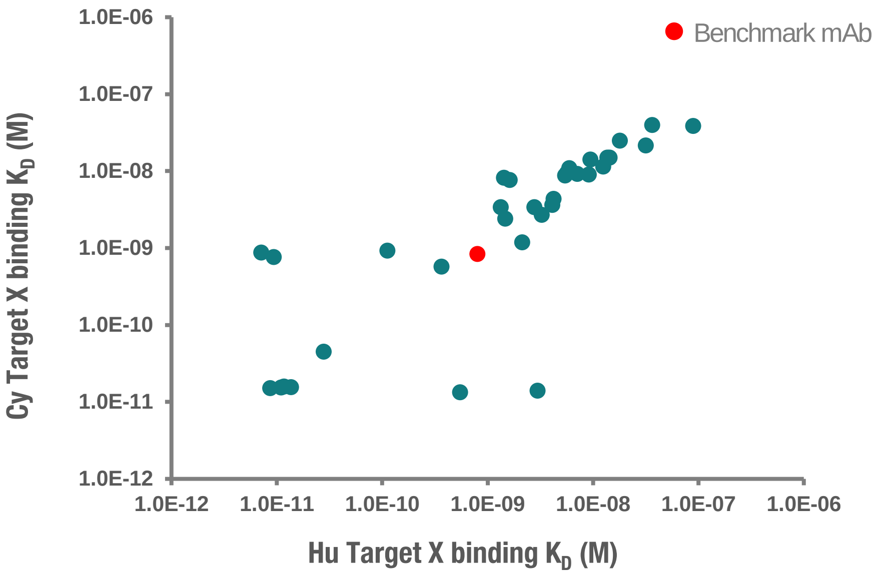


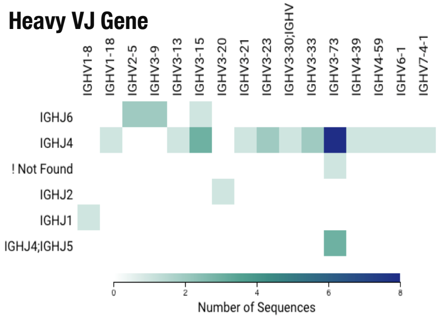
Figure 3B. Cy Target X vs. Hu Target X Affinity



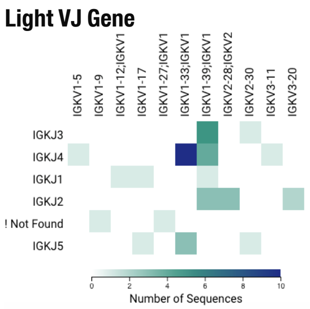
VALIDATION RESULTS: SEQUENCING OF B CELLS FROM BEACON

89% of paired HC/LC sequences were unique across all three workflows

Sequence Diversity of Run #1 (Humanized Mouse Protein Immunization)

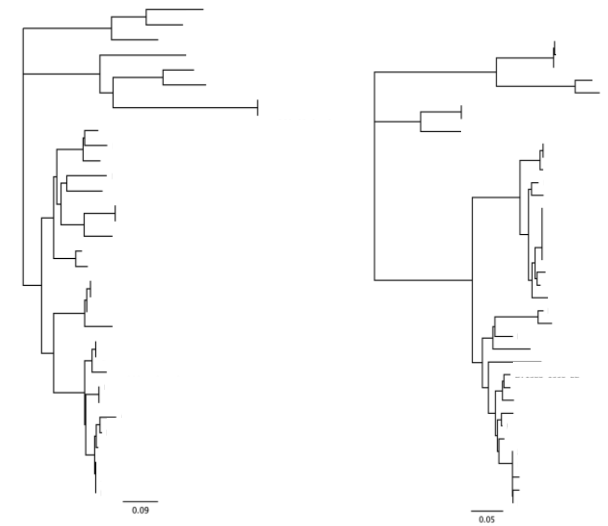


Heavy VJ Gene

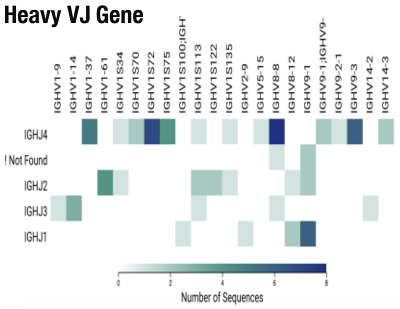


Light VJ Gene

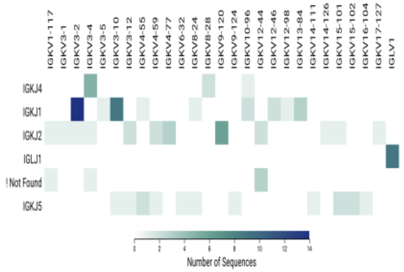
Phylogenic Diagram of Hu mAbs (protein immunization)



Sequence Diversity of Run #2 (ABV Mouse Protein Immunization)

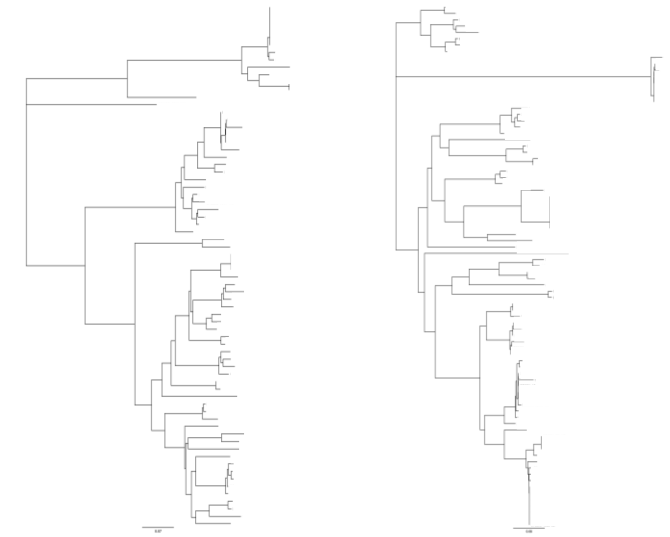


Heavy VJ Gene

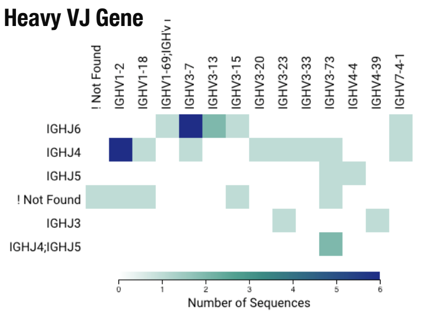


Light VJ Gene

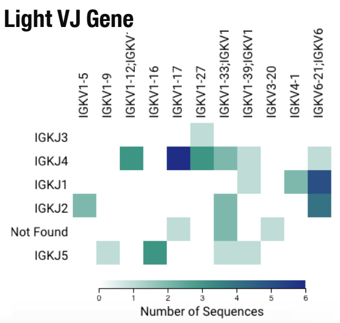
Phylogenic Diagram of ABV mAbs (protein immunization)



Sequence Diversity of Run #3 (Humanized Mouse Mix Immunization)

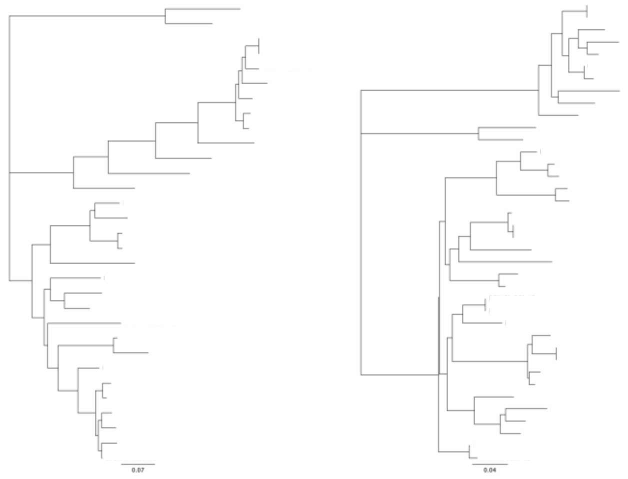


Heavy VJ Gene



Light VJ Gene

Phylogenic Diagram of Hu mAbs (mix immunization)



LONGER IMMUNIZATION REGIMENS RESULT IN MORE SOMATIC HYPERMUTATION

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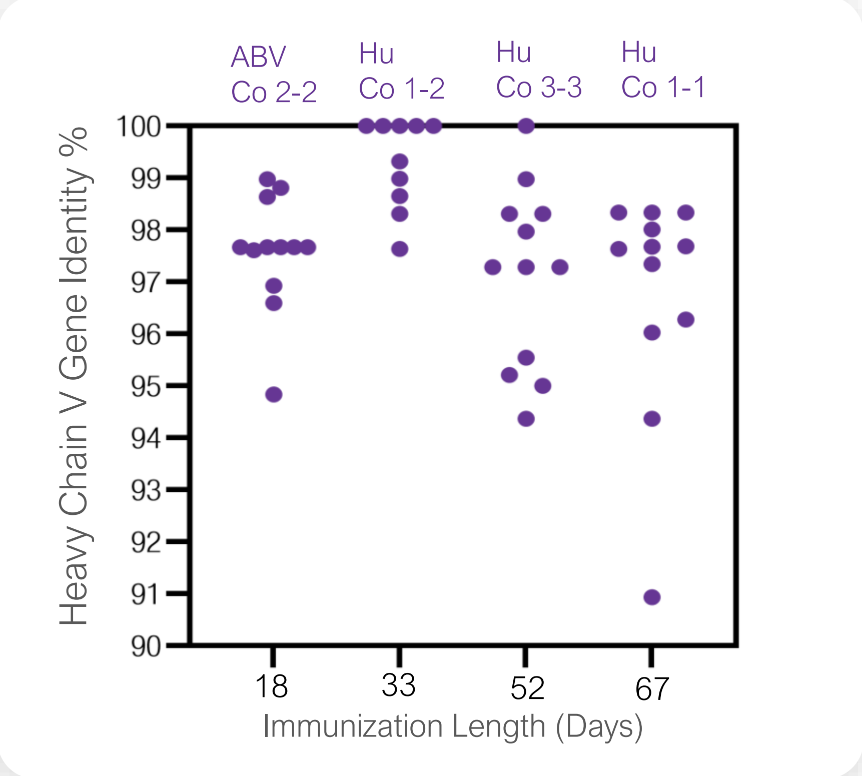
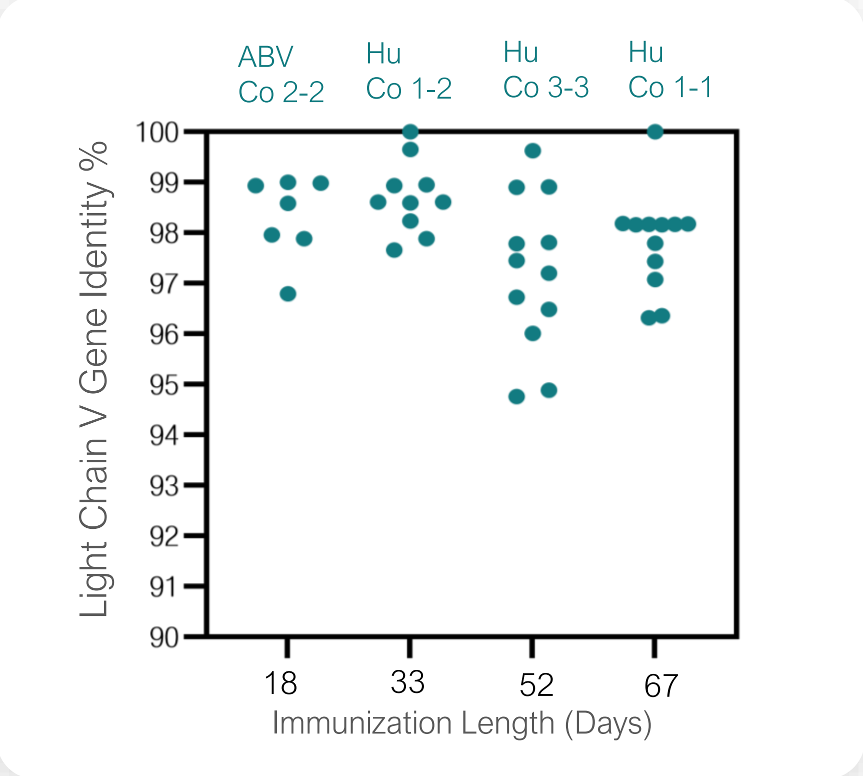
ABV mouse achieved a similar degree of somatic hypermutation to Hu mice despite divergent immunization strategies

Mouse #	Strain	Cohort	Immunization Regimen	Days
Co 2-2	ABV	2	Accelerated, cyno/hu Target X + ABV1	18
Co 1-2	Hu	1	Extended, cyno/hu Target X + ABV1	33
Co 3-3	Hu	3	Custom, cells/DNA/protein + SAS/Ribi	52
Co 1-1*	Hu	1	Extended, cyno/hu Target X + ABV1	67



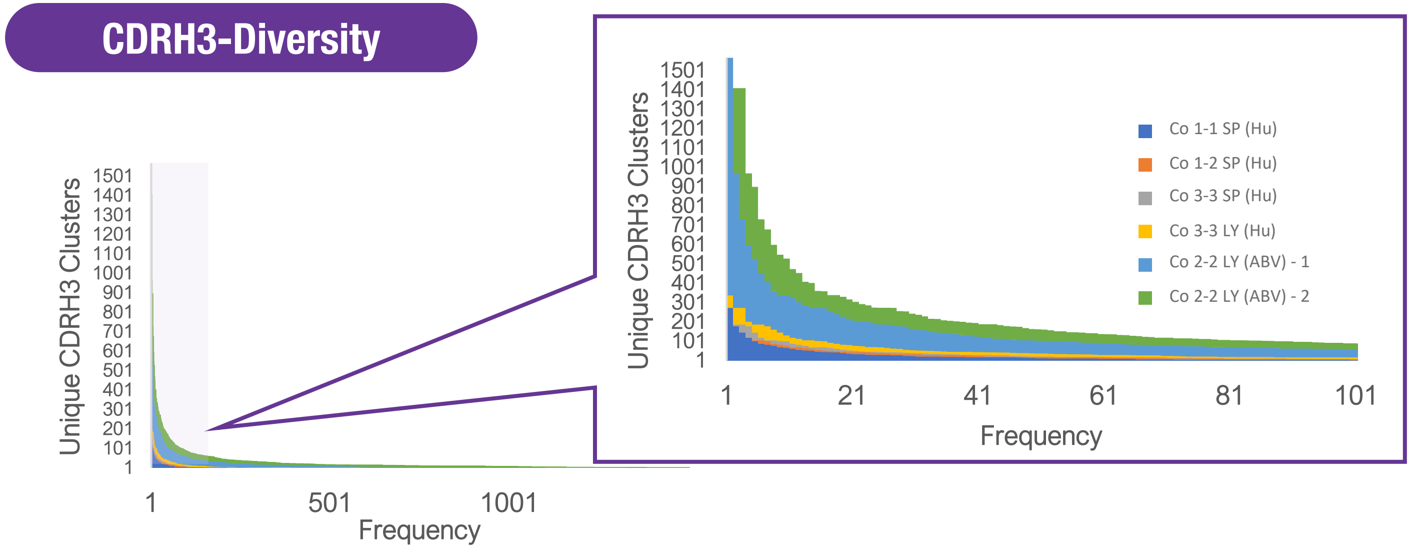


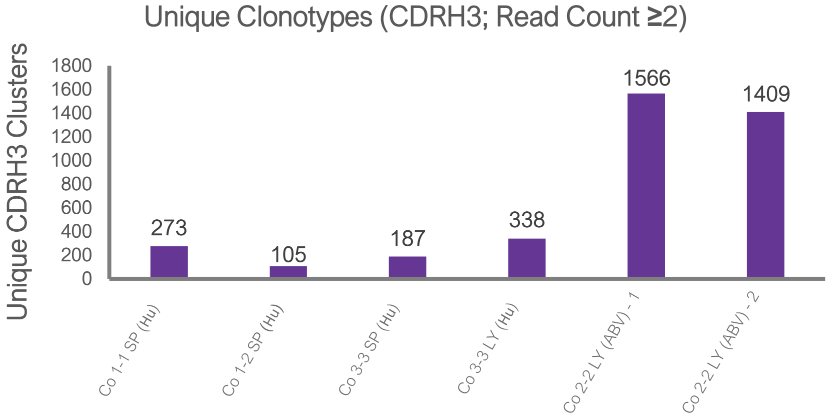
** A simplified Beacon screen was performed with an additional mouse from Cohort 1 to identify huTarget X ligand blockers*

NGS REPERTOIRE SEQUENCING – HIT EXPANSION WORKFLOW

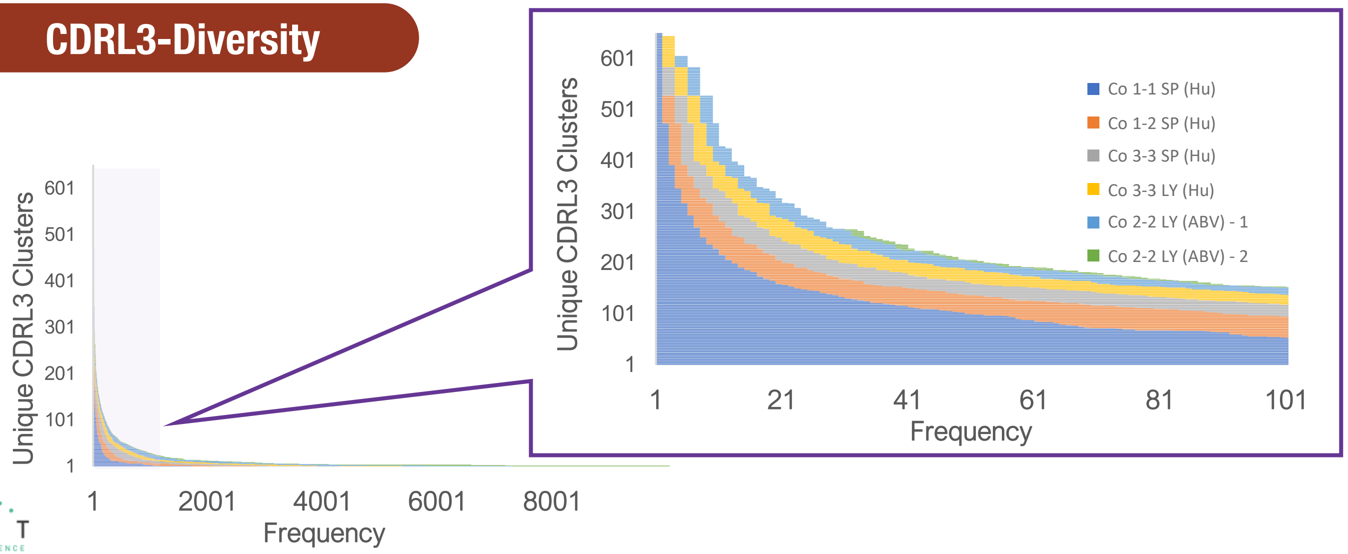
NGS repertoire clonotype analysis of various mice led to opportunities to identify additional hits from repertoire sequences

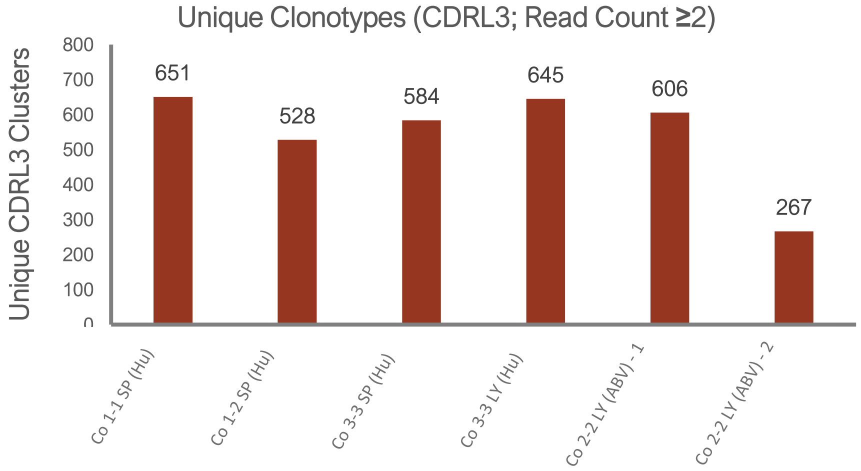
CDRH3-Diversity





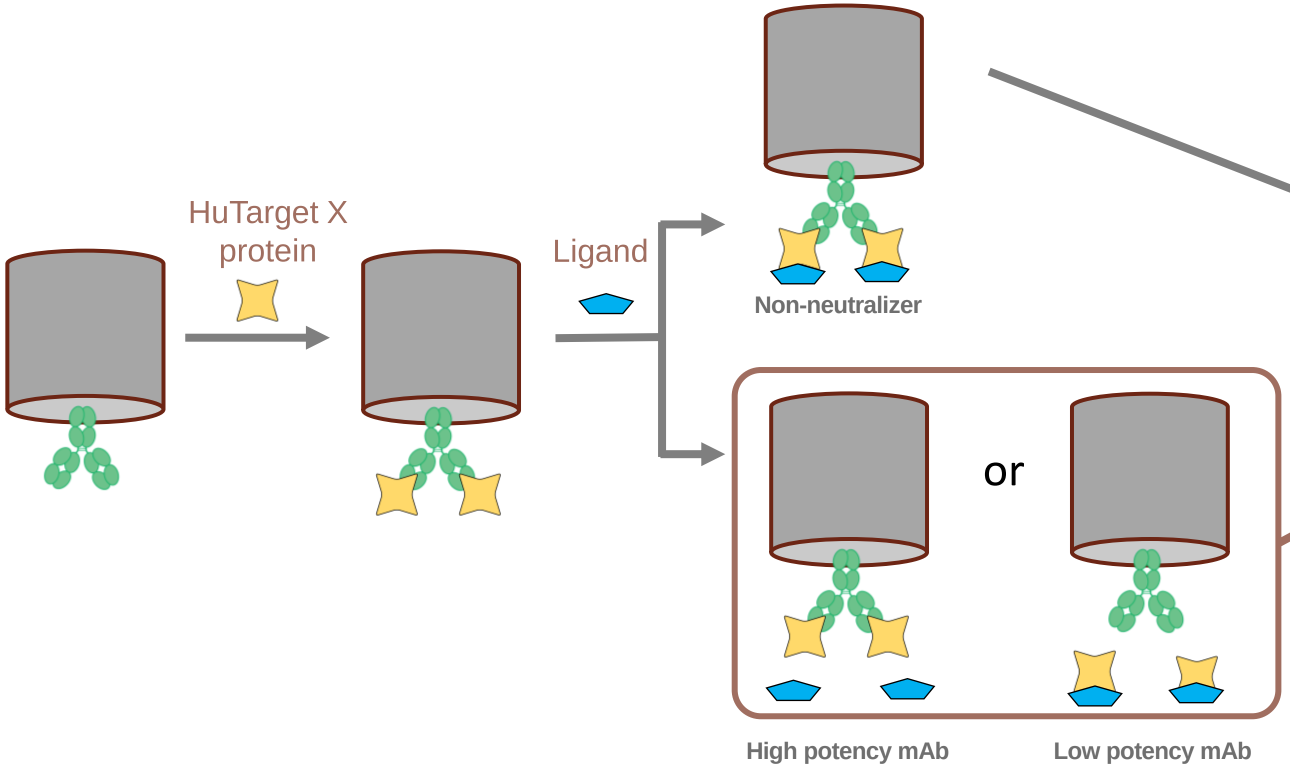
CDRL3-Diversity

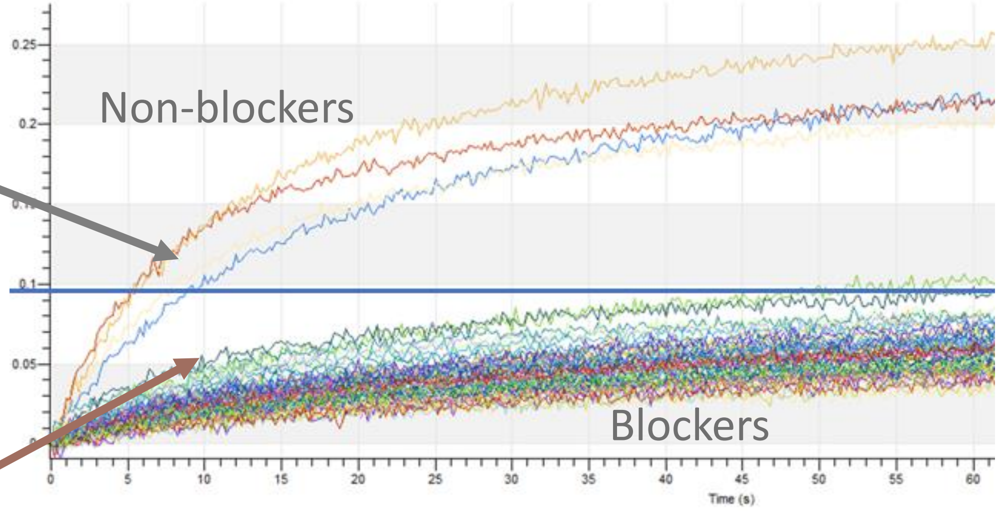




VALIDATION RESULTS: OCTET BLI – SEQUENTIAL FORMAT FOR BLOCKING ANALYSIS

Sequential format allows for definitive non-blocking clone determination

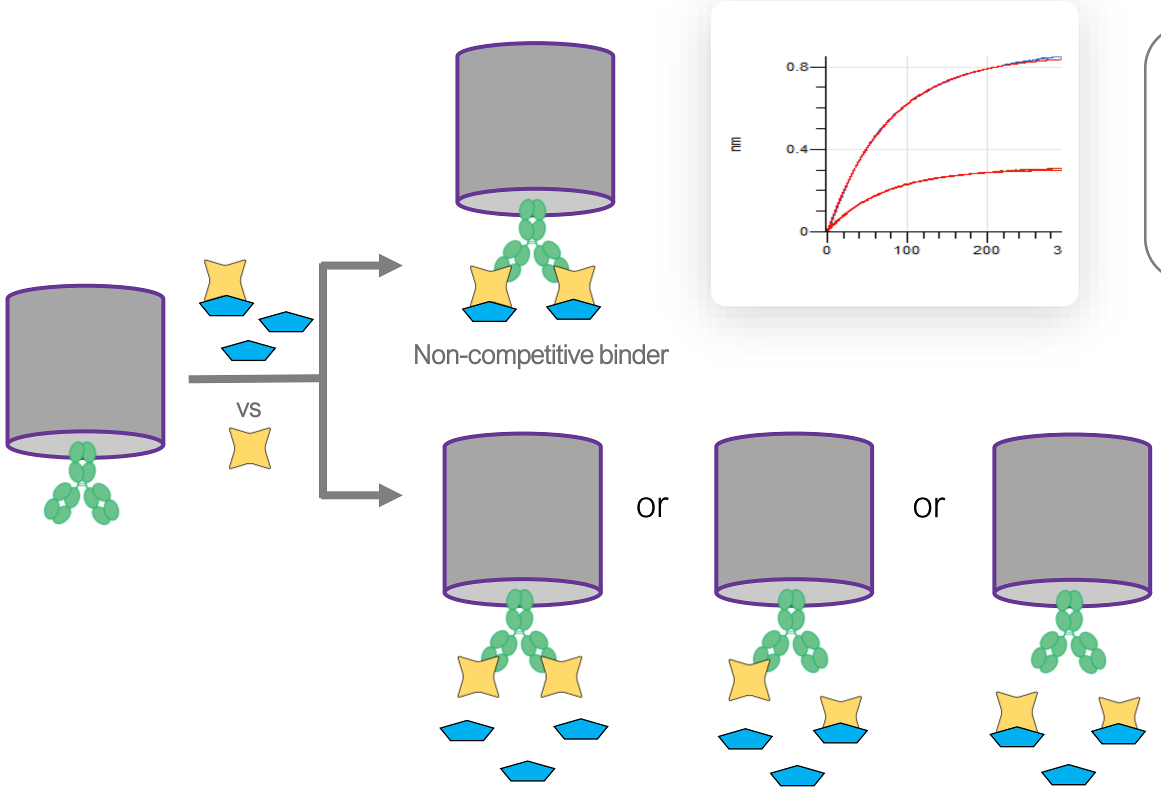




- Of the clones that exhibited blocking on-Beacon, 87% maintained blocking activity as recombinantly expressed IgGs

VALIDATION RESULTS: OCTET BLI – PRE-MIXED FORMAT FOR BLOCKING ANALYSIS

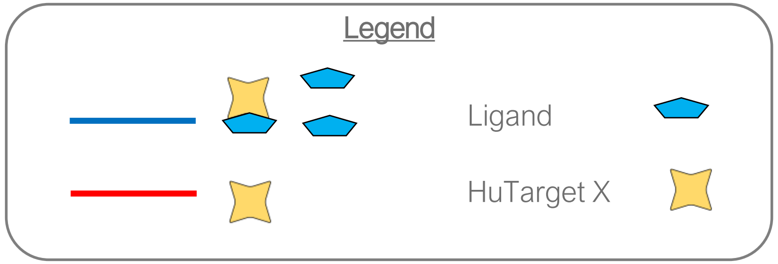
Pre-mixed format allows differentiation in potency among blocking clones



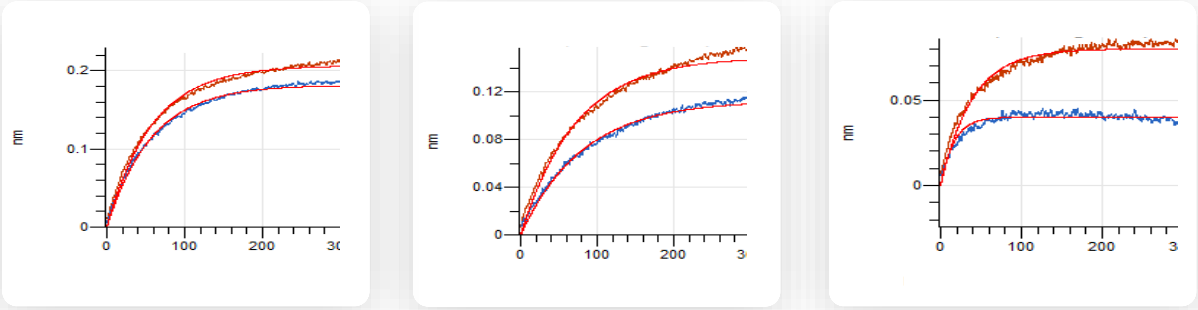
High potency mAb

Mid potency mAb

Low potency mAb



Antibodies with different potencies were identified



High potency mAb

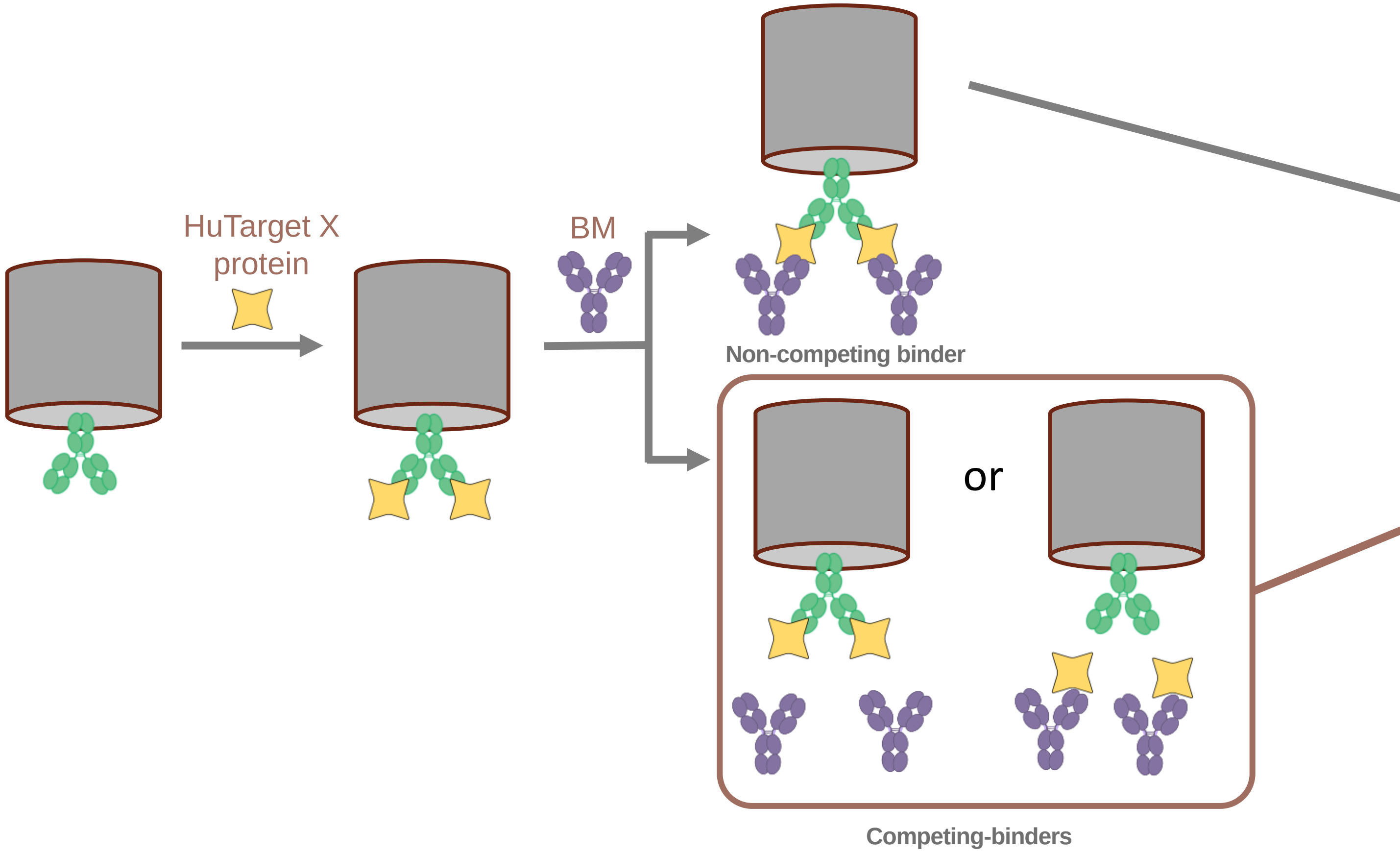
Mid potency mAb

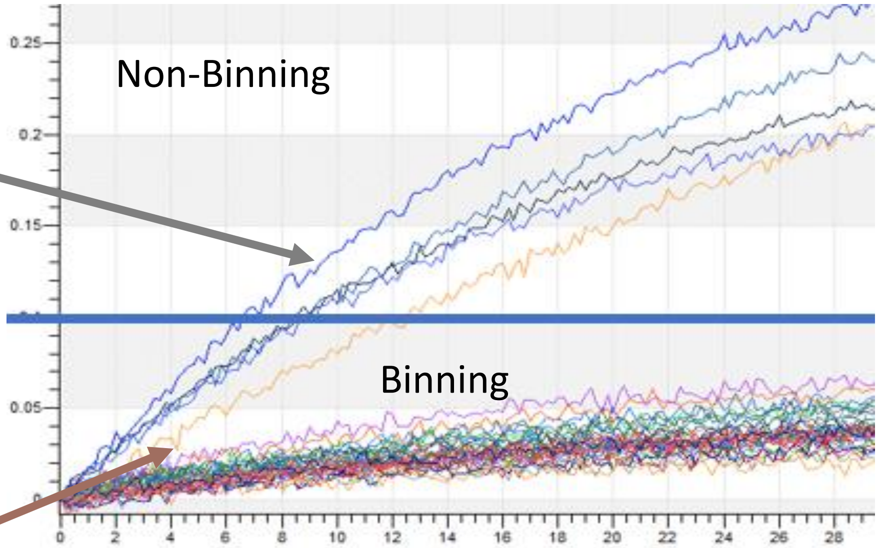
Low potency mAb

- Ligand blockers were further differentiated by potency to identify both high and low potency blockers

VALIDATION RESULTS: OCTET BLI – SEQUENTIAL FORMAT FOR BM BINNING ANALYSIS

Sequential format allows for benchmark binning profiling, indicating 88% of clones





- 88% of clones bin/compete with the benchmark antibody for Target binding

FUNCTIONAL VALIDATION: CARTERRA SPR FOR AFFINITY ASSESSMENT AND BINNING

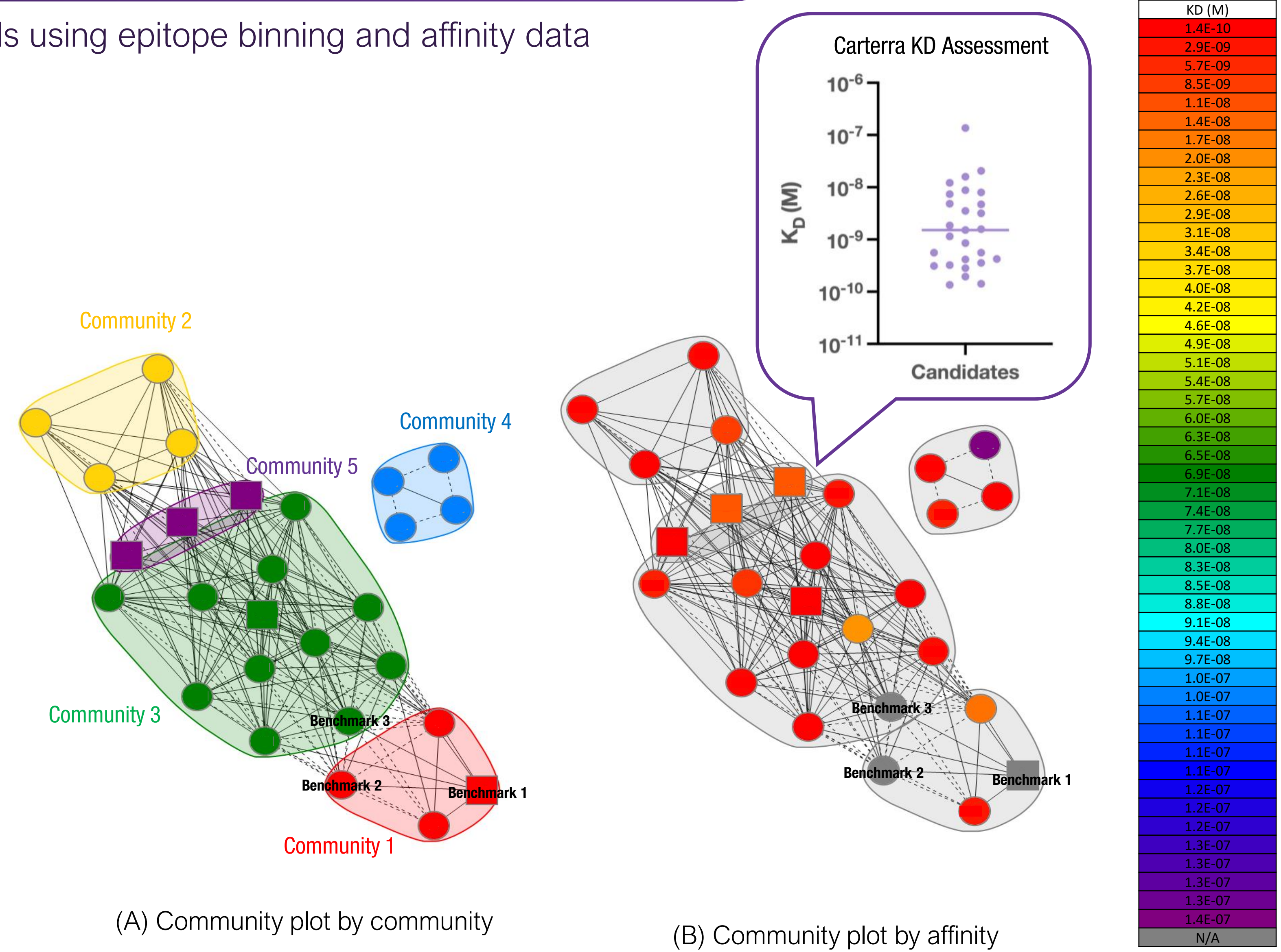
Deep analysis allows for the final selection of leads using epitope binning and affinity data

Further Functional Validation:

- 24 clones were selected for mid-scale production
- Purified antibodies were analyzed for epitope binning and affinity assessment alongside 3 benchmark antibodies (including Beacon BM antibody) by Carterra SPR
- This panel contained 6, 8, and 10 antibodies from Cohort 1 (Hu/protein), Cohort 2 (ABV/protein), and Cohort 3 (Hu/mixed), respectively

Conclusions:

- A core epitope was identified that included many ligand neutralizing clones (Community 3 and 5)
- Two directly adjacent epitopes also included neutralizing clones (Communities 1 and 2)
- A distal epitope included non-neutralizing clones (Community 4)
- Kinetics revealed that most clones possessed affinities in the single-digit nanomolar and subnanomolar range

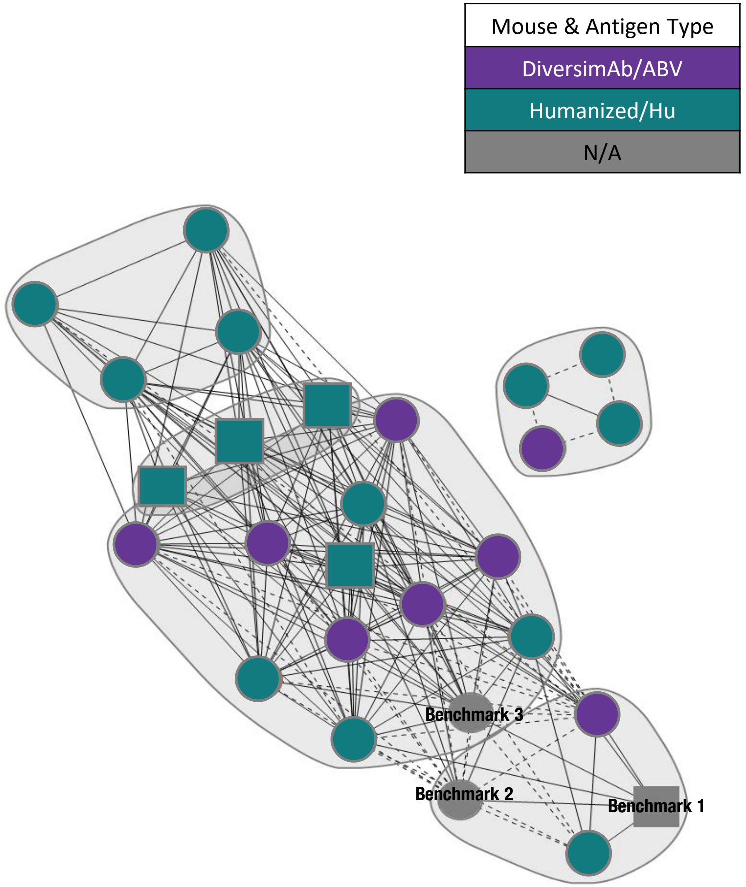


(A) Community plot by community

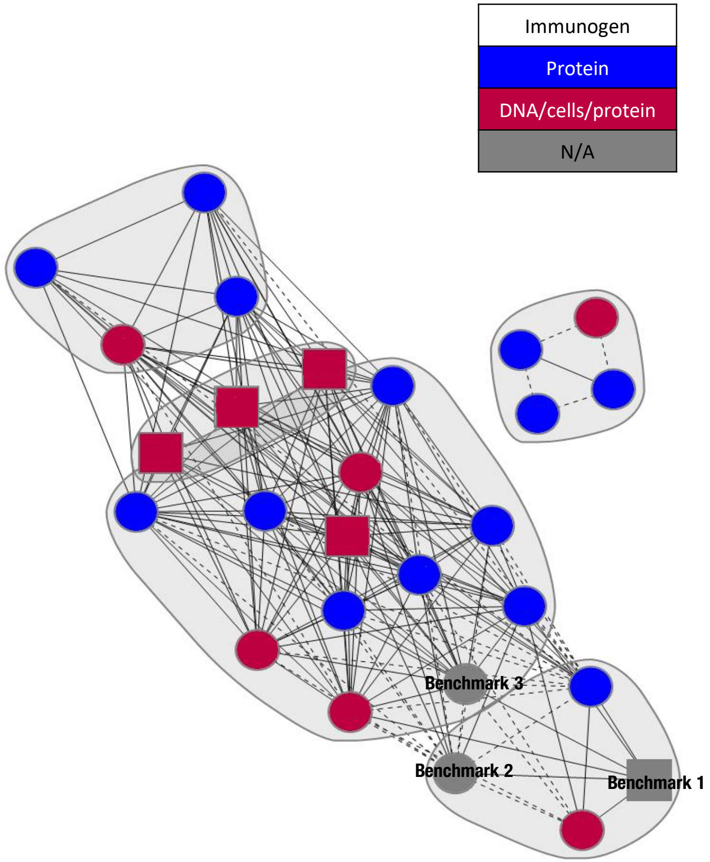
(B) Community plot by affinity

FUNCTIONAL VALIDATION: CARTERRA SPR FOR AFFINITY ASSESSMENT AND BINNING

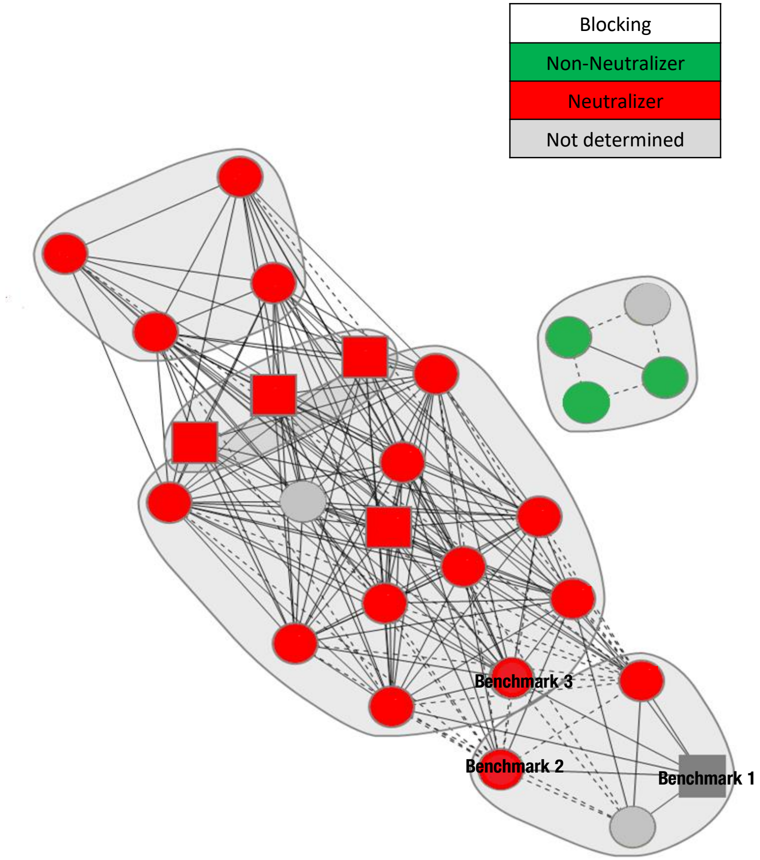
Deep analysis allows for the final selection of leads using epitope binning and neutralization data



(C) Community plot by mouse



(D) Community plot by immunogen



(E) Community plot by blocking activity

Conclusion:

- Diversity in immunization strategy and mouse strain results in broader epitopic coverage

Bioanalytical analysis of lead candidates revealed promising developability profiles

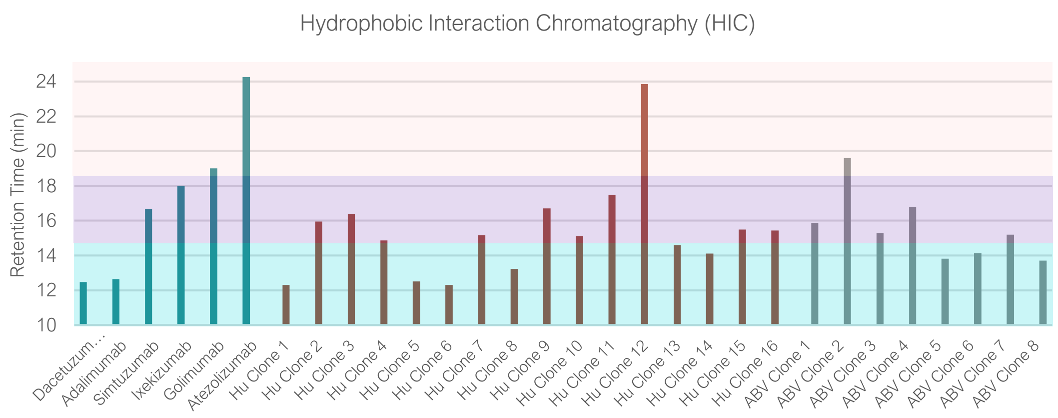
Developability analysis of leads helped guide candidate selection and inform potential downstream engineering

Developability Assessment:

- A set of reference standards was established using 6 approved therapeutic mAbs of varying biophysical profiles
- Overall developability score is represented by the aggregate score from 3 bioanalytical assays:
  - CSI-BLI for self-association
  - CIC for propensity to aggregate
  - HIC for hydrophobicity

Conclusions:

- Developability analysis of the 24 leads provided further information to guide lead candidate selection by the Partner
- mAbs from DiversimAb showed comparable aggregate scores to that of Humanized mice



Representative Developability Assessment Plot

		Developability Scores Summary			
Sample ID	Immunization	CSI-BLI Score	CIC Score	HIC Score	Aggregate Score*
Dacetuzumab	N/A	1	1	1	3
Adalimumab		1	1	1	3
Simtuzumab		2	2	2	6
Ixekizumab		3	2	2	7
Golimumab		3	3	3	9
Atezolizumab		2	3	3	8
Humanized Clone 1	Protein	2	2	1	5
Humanized Clone 2		1	2	2	5
Humanized Clone 3		1	3	2	6
Humanized Clone 4		1	3	2	6
Humanized Clone 5		1	3	1	5
Humanized Clone 6		1	1	1	3
Humanized Clone 7	Cells/DNA/Protein	1	2	2	5
Humanized Clone 8		3	2	1	6
Humanized Clone 9		3	2	2	7
Humanized Clone 10		2	2	2	6
Humanized Clone 11		1	1	2	4
Humanized Clone 12		3	3	3	9
Humanized Clone 13		2	1	1	4
Humanized Clone 14		3	2	1	6
Humanized Clone 15		3	2	2	7
Humanized Clone 16		3	2	2	7
DiversimAb (ABV) Clone 1	Protein	1	1	2	4
DiversimAb (ABV) Clone 2		1	3	3	7
DiversimAb (ABV) Clone 3		1	1	2	4
DiversimAb (ABV) Clone 4		1	1	2	4
DiversimAb (ABV) Clone 5		3	2	1	6
DiversimAb (ABV) Clone 6		2	2	1	5
DiversimAb (ABV) Clone 7		1	1	2	4
DiversimAb (ABV) Clone 8		2	2	1	5

* Aggregate Score, Poor (8-9); Aggregate Score Caution (6-7); Aggregate Score Good (3-5)

CASE STUDY SUMMARY

Successful delivery of rare hits with desired specificity and function in a tight timeline

1. Human/cyno cross-reactive binding required
2. Functional ligand blocking activity required
3. On-cell binding required
4. Delivery of fully human sequences preferred
5. Binning profiles against multiple benchmarks evaluated

- **45** unique sequences successfully recombinantly expressed for validation
- **87%** of Beacon-predicted ligand blockers maintained ligand blocking activity as expressed IgGs
- Expression recovery of approximately **92%** from unique sequences

- **96 (of 108)** unique sequences or **89%** unique sequence recovery
- Diverse affinities down to subnanomolar range

- Delivery of a diverse panel of ligand blocking human/cyno cross-reactive on-cell binders
- Unique ligand blocking epitopes identified

- Successfully established the binning profiles of a panel of antibodies against multiple benchmarks (both binning and non-binning)