

## Abveris Antibody Discovery Case Study

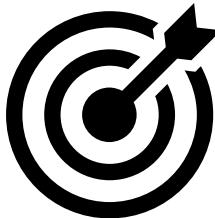
Accelerated Therapeutic Antibody Discovery  
Targeting a Cell Surface Receptor using a  
Beacon-Based Single B Cell Workflow

## Case study background



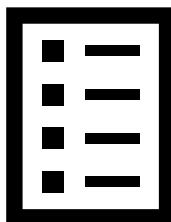
### Partner

- Well-established public biopharma
- Multiple successful campaigns completed in partnership with Abveris



### Target

- Cell surface receptor (FM1)
- Receptor family contains 9 highly conserved members (FM2-9)



### End Application

- Therapeutic antibody development for Partner's desired disease indication

### Goals

- 1 Specific on-cell binding to Family Member 1 (FM1) without off-target binding
- 2 Functional ligand blocking activity required
- 3 Sequence diversity desired
- 4 Tight timeline requirement

Lack of functional candidate antibodies out of previous efforts necessitated de novo discovery

## Previous Discovery Attempts Prior to Abveris



Hybridomas generated from wild-type mice were screened by ELISA then moved to flow validation

6 lead hybridomas were identified

- 1 FM1-specific binder, ligand blocker
- 2 non-specific binders, ligand blockers
- 3 FM1-specific binders, non-blocking

Additional Discovery Required

Primary Partner Goals

- Broader antibody diversity for discovery of rare candidates
- Function-forward, accelerated single B cell workflow for efficient lead identification

# OVERVIEW OF SINGLE B CELL WORKFLOW

Include parallel assay development

Workflow

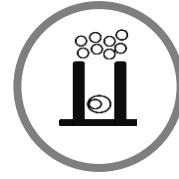
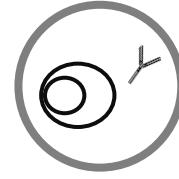
Immunization &  
assay development

Tissue harvest &  
import of plasma B cells

Beacon workflow  
(Specificity & Function)

Sequencing &  
Recombinant Expression

Functional Validation



- Immunization of two strains of DiversimAb Mice for diversity
- Accelerated immunization protocols (3 weeks) using FM1 recombinant protein
- Simultaneous antigen QC and Beacon assay development

- Titer analysis and harvest of lymph tissues
- Approximately  $10^6$  plasma B cell isolated per mouse
- Enrichment of plasma B cell using proprietary process

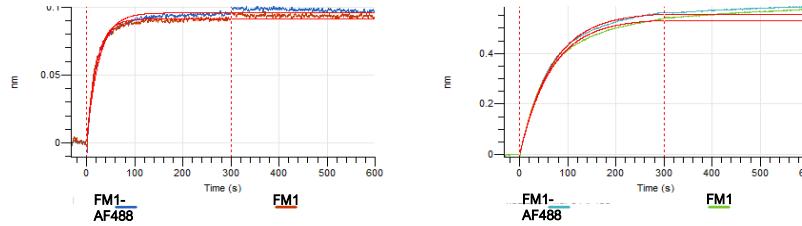
- 3 Sequential assays for specificity and functional screen
- Screened against cell lines expressing family members of highest homology and FM3 (FM2)
- Bead-based blocking assay

- Exported to lysis buffer in PCR plates
- cDNA synthesis
- Sequencing
- Small-scale expression in a HEK-based system and purified for validation

- Top clones were selected for functional validation
- Antibodies validation by flow cytometry and ELISA for specificity
- Antibodies validation of function by Octet BLI
- Binning of antibodies by Carterra SPR

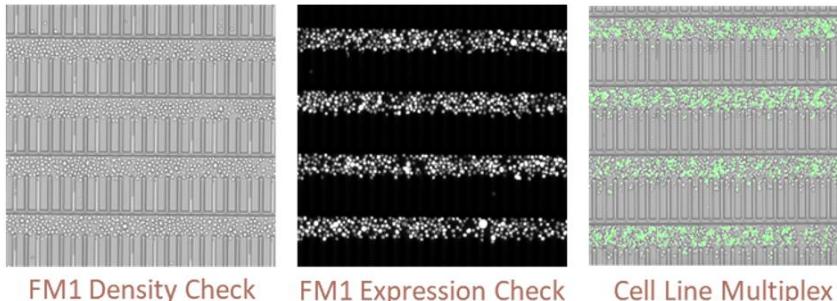
Labeled antigen QC both on and off Beacon enabled successful assay development

## Antigen QC



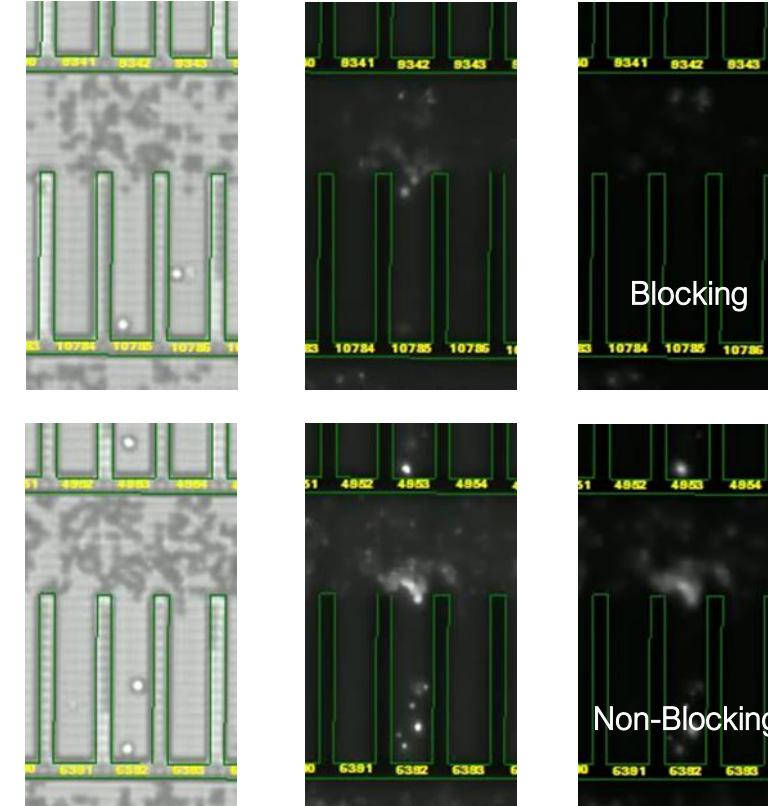
Octet QC using multiple control antibodies to labeled and unlabeled protein confirmed labeling did not impact protein integrity

## Cell Line Optimization



Import cell density and cell line multiplex conditions were optimized for assay

## FM1-AF488 + Ligand-AF647 in channel



Brightfield: Anti-Mouse IgG Capture Beads

FITC: FM1-AF488 Binding to Captured Control Antibody

CY5: Ligand-AF647 Binding to FM1- Control Antibody Complex

## Assay Development

Optimized on-Beacon ligand blocking assay using hybridomas expressing blocking and non-blocking control antibodies

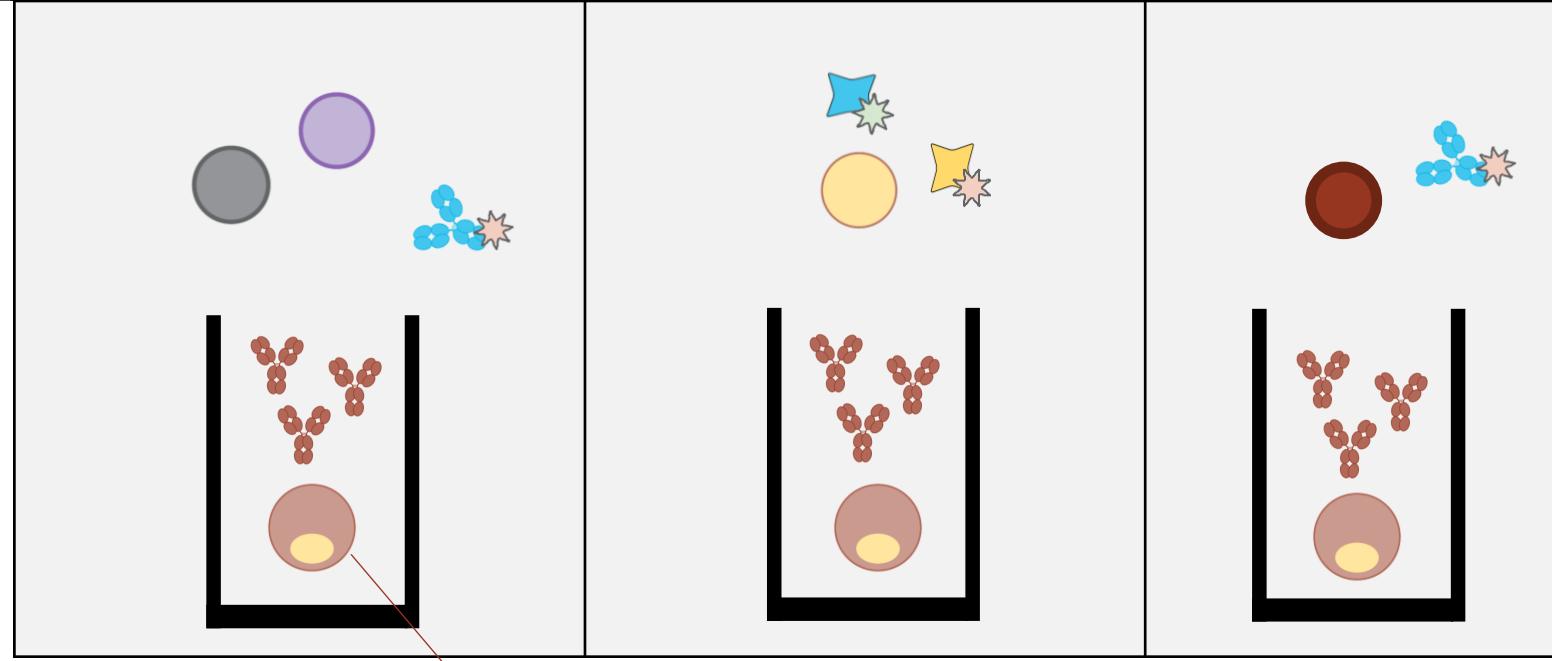
# THREE SEQUENTIAL ASSAYS WERE EMPLOYED IN THE SCREENING WORKFLOW

## Assay format overview

	Assay 1 Off-Target Binding		Assay 2 Blocking Assay		Assay 3 FM1 Specificity
Screening Reagent	FM2 cell line	FM3 cell line	FM1 protein (+)	Ligand (-)	FM1 cell line
Detection Channel	Cy5	Cy5	FITC	Cy5	Cy5
FM1 Specific, blocking		-	+	-	+
FM1 Specific, non-blocking		-	+	+	+

### Legend

	Secondary antibody-AF647		FM1-expressing cell line
	B cell-secreted antibody		FM2-expressing cell line
	FM1 recombinant protein-AF488		FM3-expressing cell line
	Ligand-AF647		Anti-mouse IgG capture beads

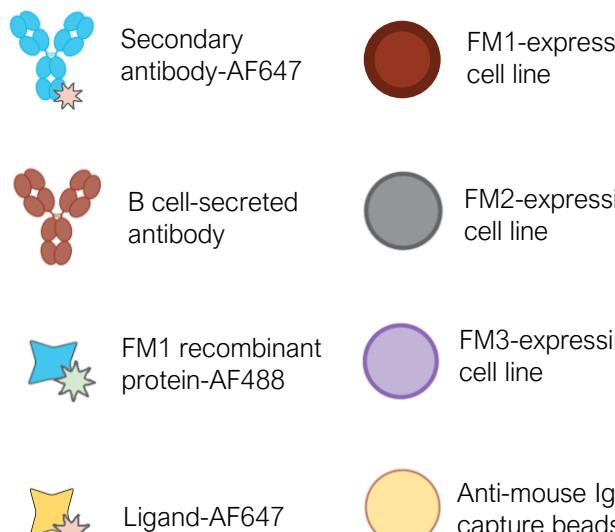


# REPRESENTATIVE ASSAY RESULTS – SPECIFIC BLOCKING HITS

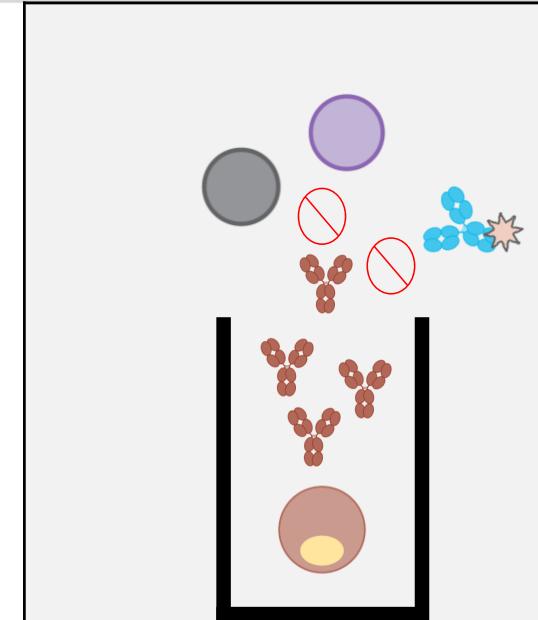
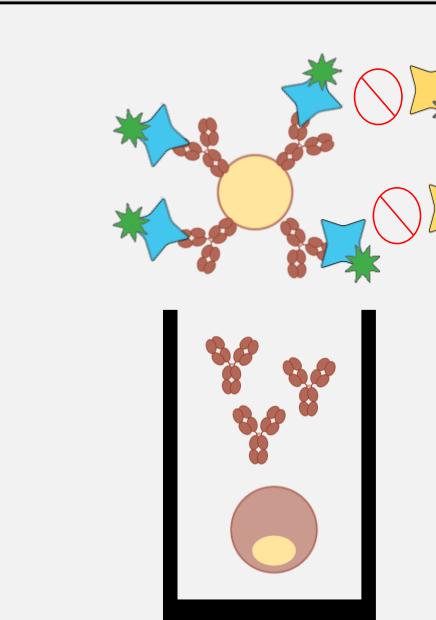
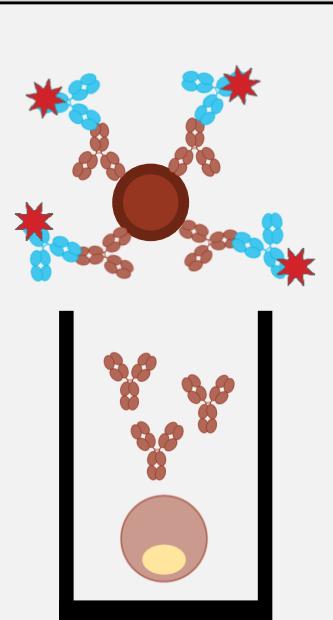
## Assay results

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Legend

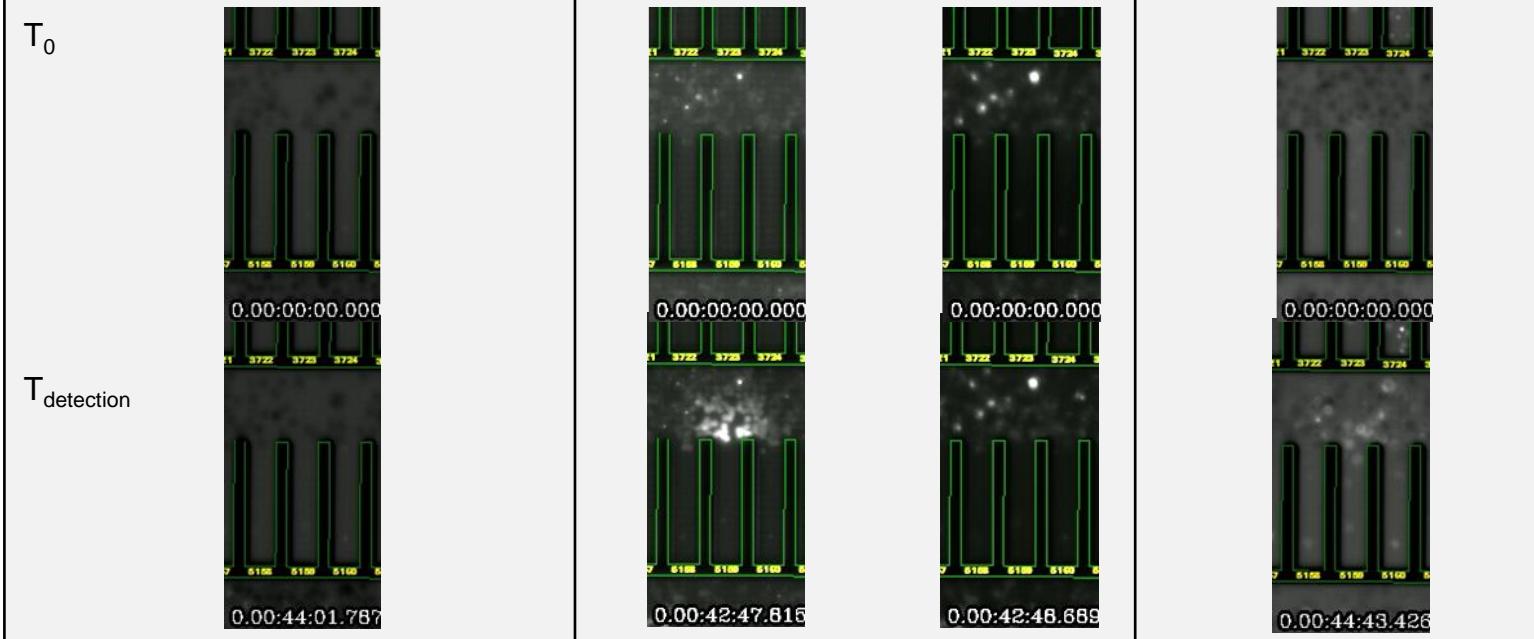


- Secondary antibody-AF647
- B cell-secreted antibody
- FM1 recombinant protein-AF488
- Ligand-AF647
- FM1-expressing cell line
- FM2-expressing cell line
- FM3-expressing cell line
- Anti-mouse IgG capture beads

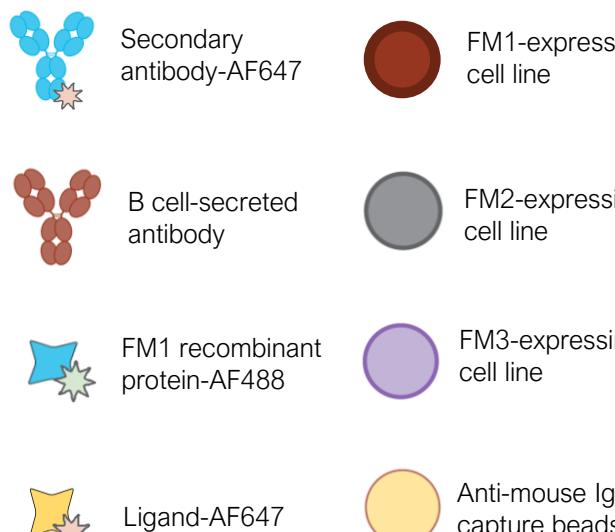
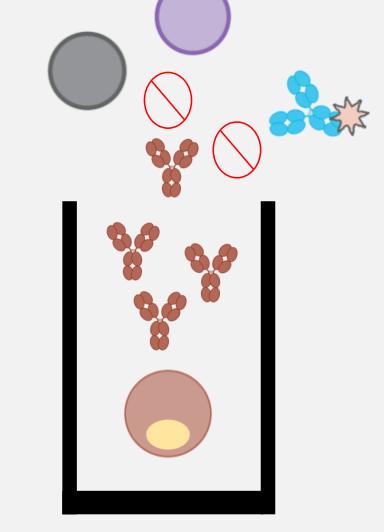
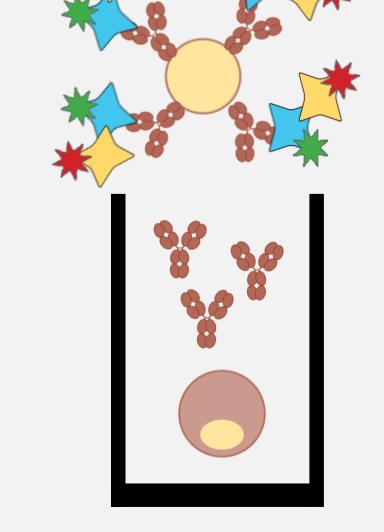
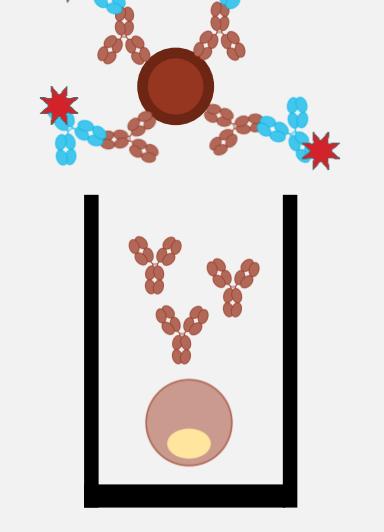
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FM1 Specific, non-blocking		-	+	+	+
<u>Legend</u> <ul style="list-style-type: none"> <li>Secondary antibody-AF647</li> <li>B cell-secreted antibody</li> <li>FM1 recombinant protein-AF488</li> <li>Ligand-AF647</li> <li>FM1-expressing cell line</li> <li>FM2-expressing cell line</li> <li>FM3-expressing cell line</li> <li>Anti-mouse IgG capture beads</li> </ul>					
 <p><math>T_0</math></p> <p><math>T_{\text{detection}}</math></p>					

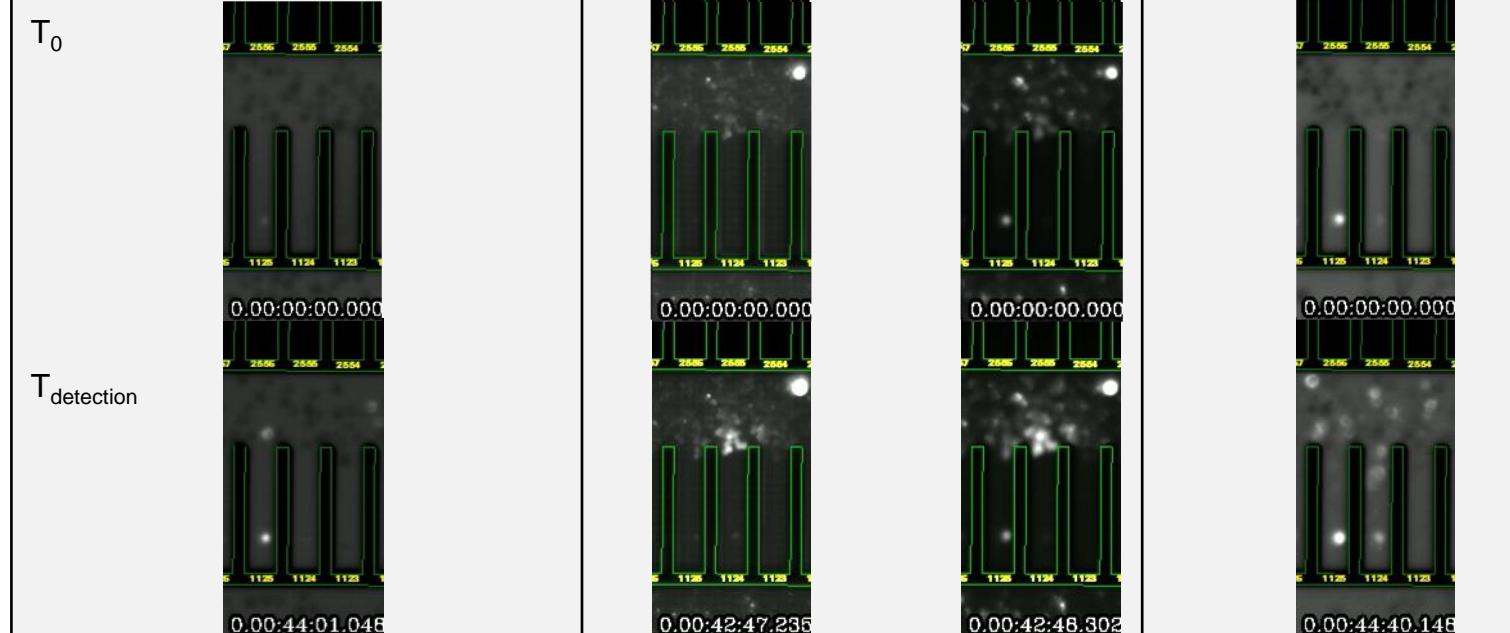
# REPRESENTATIVE ASSAY RESULTS – SPECIFIC NON-BLOCKING HITS

## Assay results

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FM1 Specific, blocking		-	+	-	+
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<u>Legend</u> 					
					
					
					

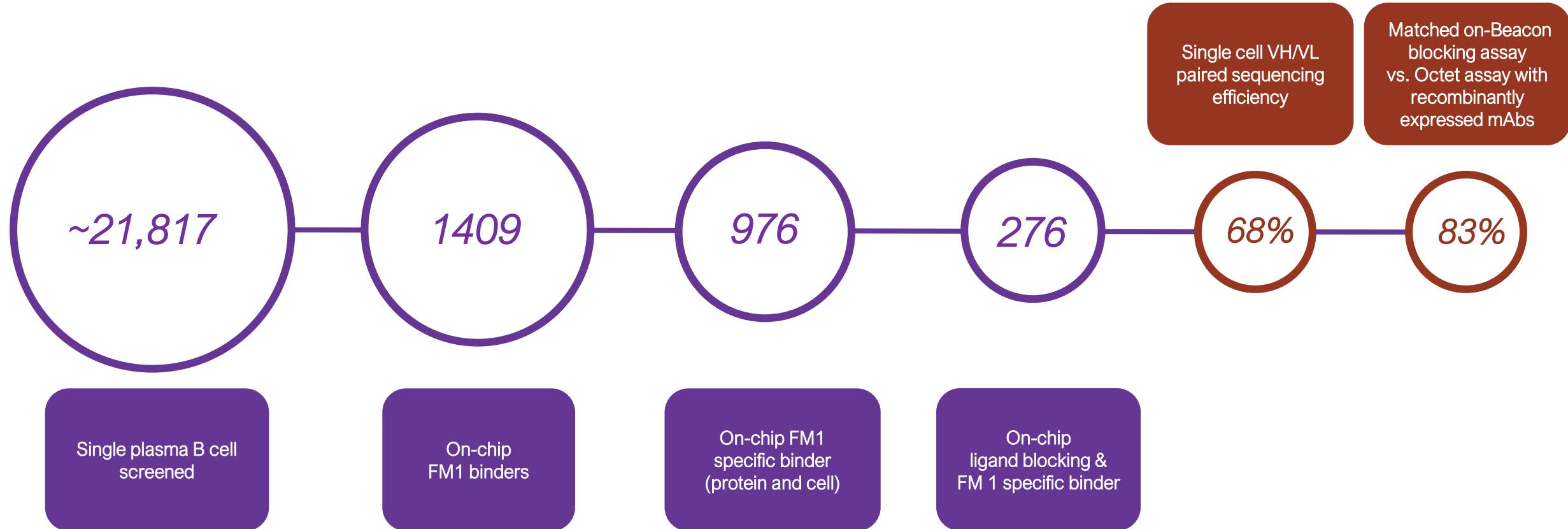
# REPRESENTATIVE ASSAY RESULTS – SPECIFIC NON-BLOCKING HITS

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# SCREENING RESULTS IN NUMBERS

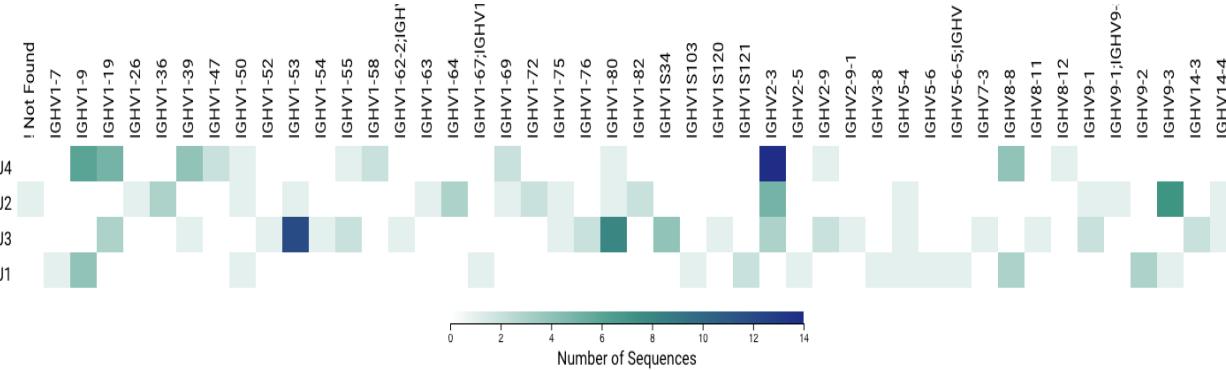
Number of clones screened and hits in each stage of the single B cell workflow



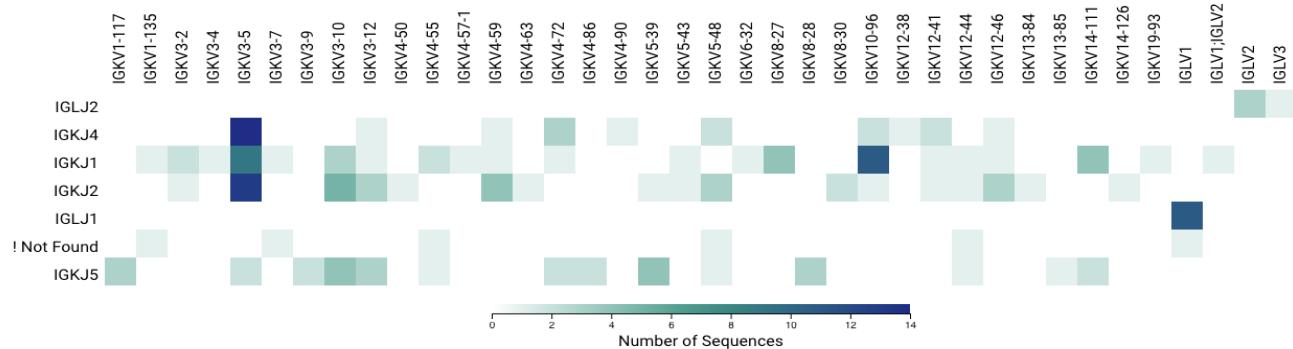
## VALIDATION RESULTS: SEQUENCING OF B CELLS FROM BEACON

78% of paired HC/LC sequences were unique

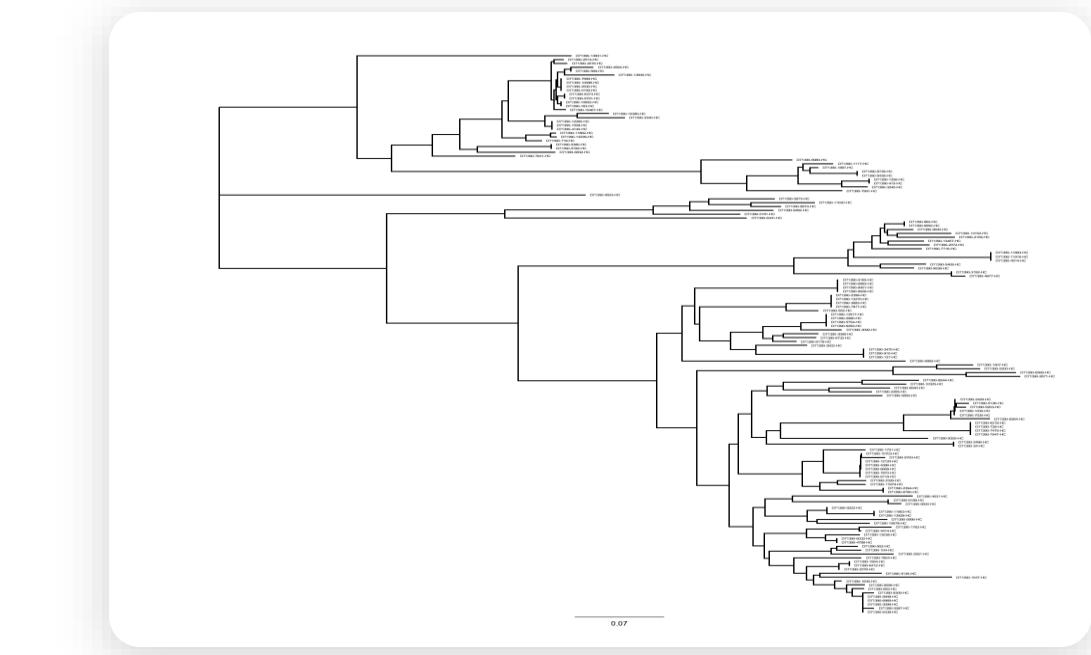
## Heavy VJ Gene



## Light VJ Gene



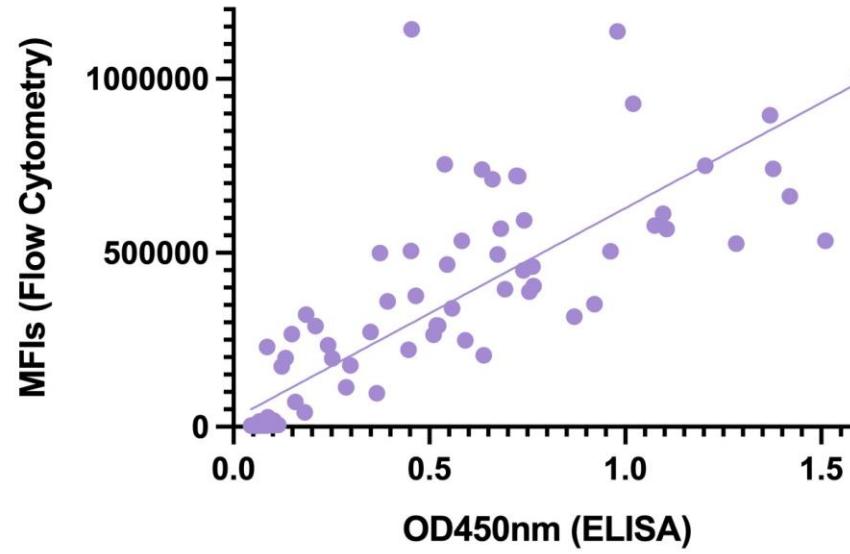
## Phylogenetic Diagram



- 129 paired HC/LC sequences recovered from 191 B cells
- 100 out of 129 unique sequences identified
- Broad V-gene usage across both HC and LC
- Diverse clonal lineages identified

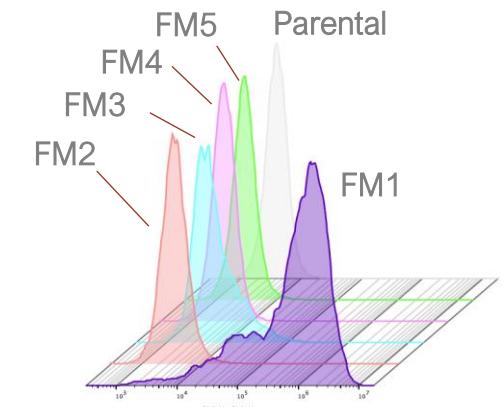
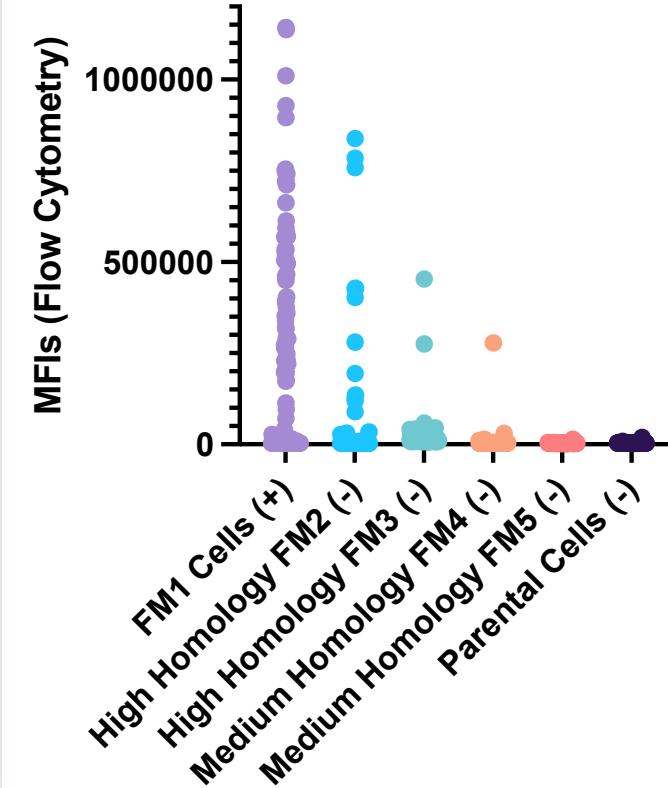
Candidates recapitulated Beacon binding profiles as recombinantly expressed antibodies

## Recombinant Expression Binding



- 96 clones from Beacon were expressed and validated off-Beacon for binding to FM1
- 92% of sequences produced appreciable IgG quantities
- Strong correlation between FM1 recombinant protein binding and FM1 on-cell binding

## Flow Cytometry Binding

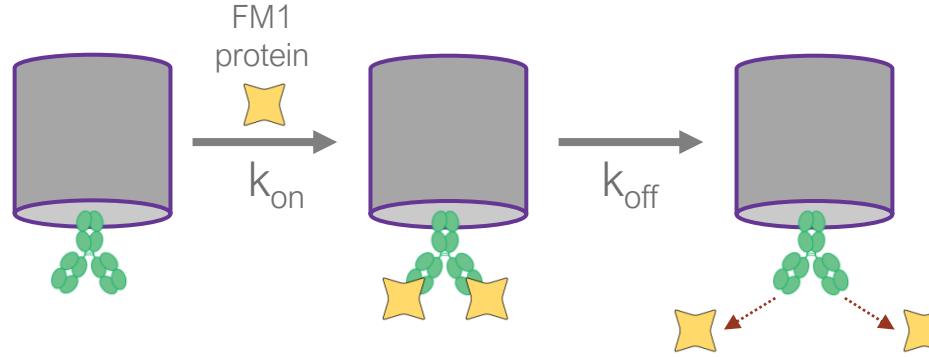


- 70% of clones exhibited binding to FM1 cells
- 61% of FM1 binders maintained stringent specificity off-Beacon

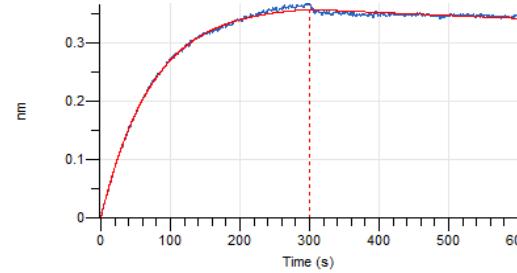
# VALIDATION RESULTS: OCTET BLI – AFFINITY ANALYSIS

A diverse range of affinities was obtained with clones in nanomolar and picomolar range

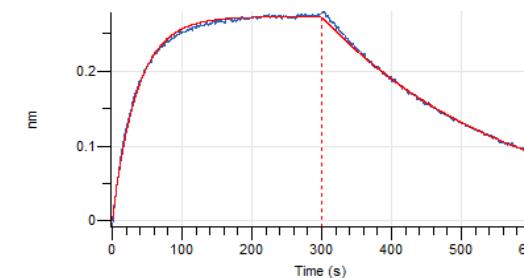
$K_D$  measurement



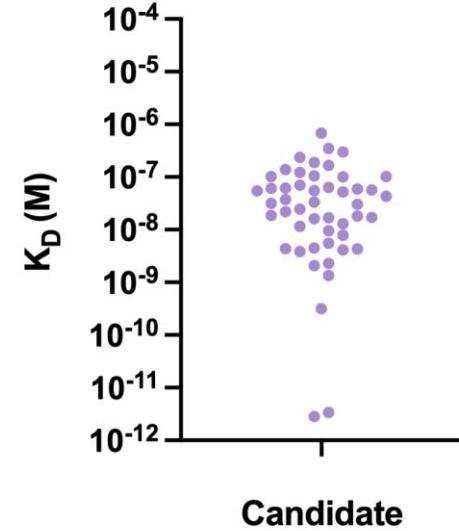
Example clone 1



Example clone 2

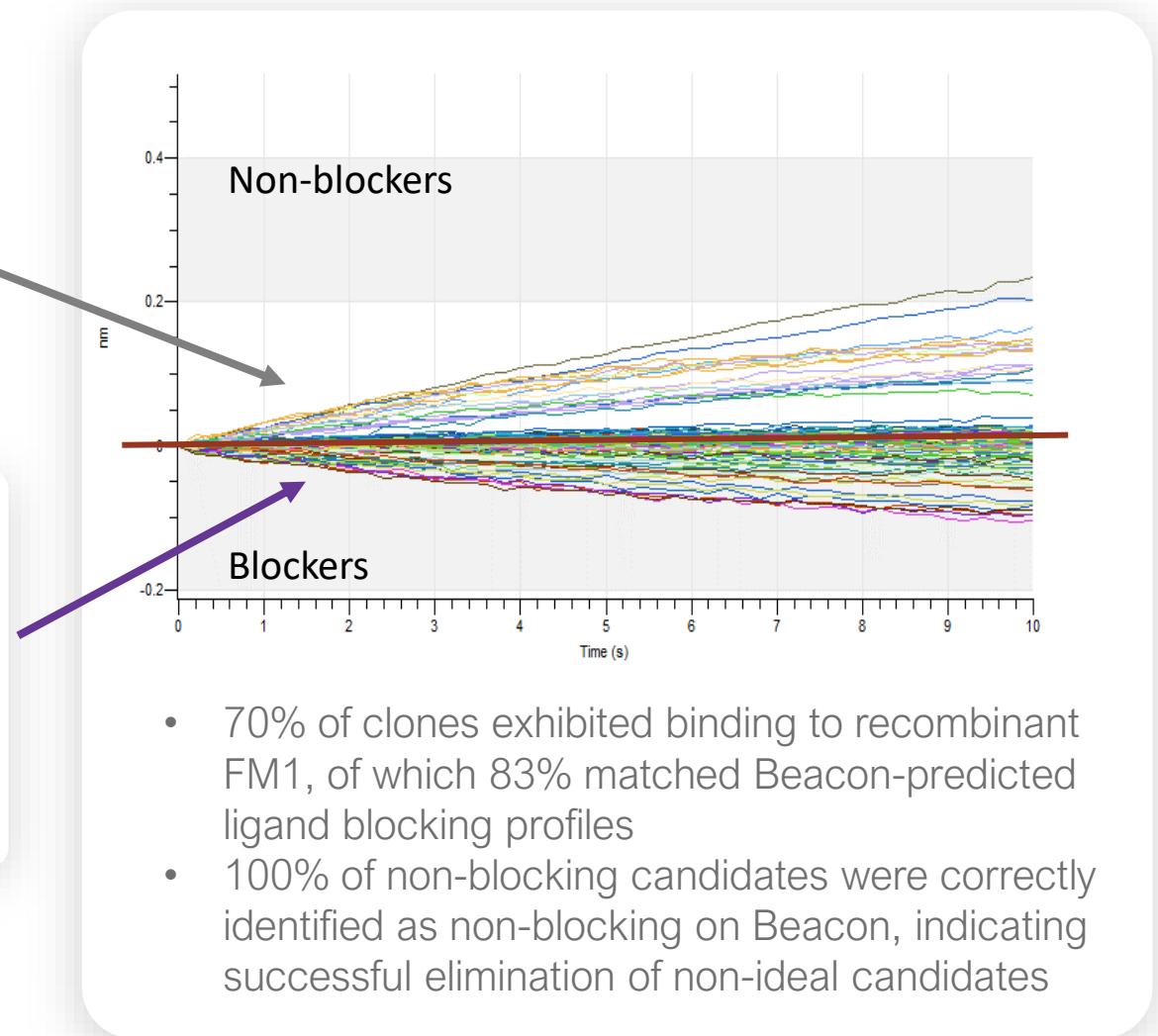
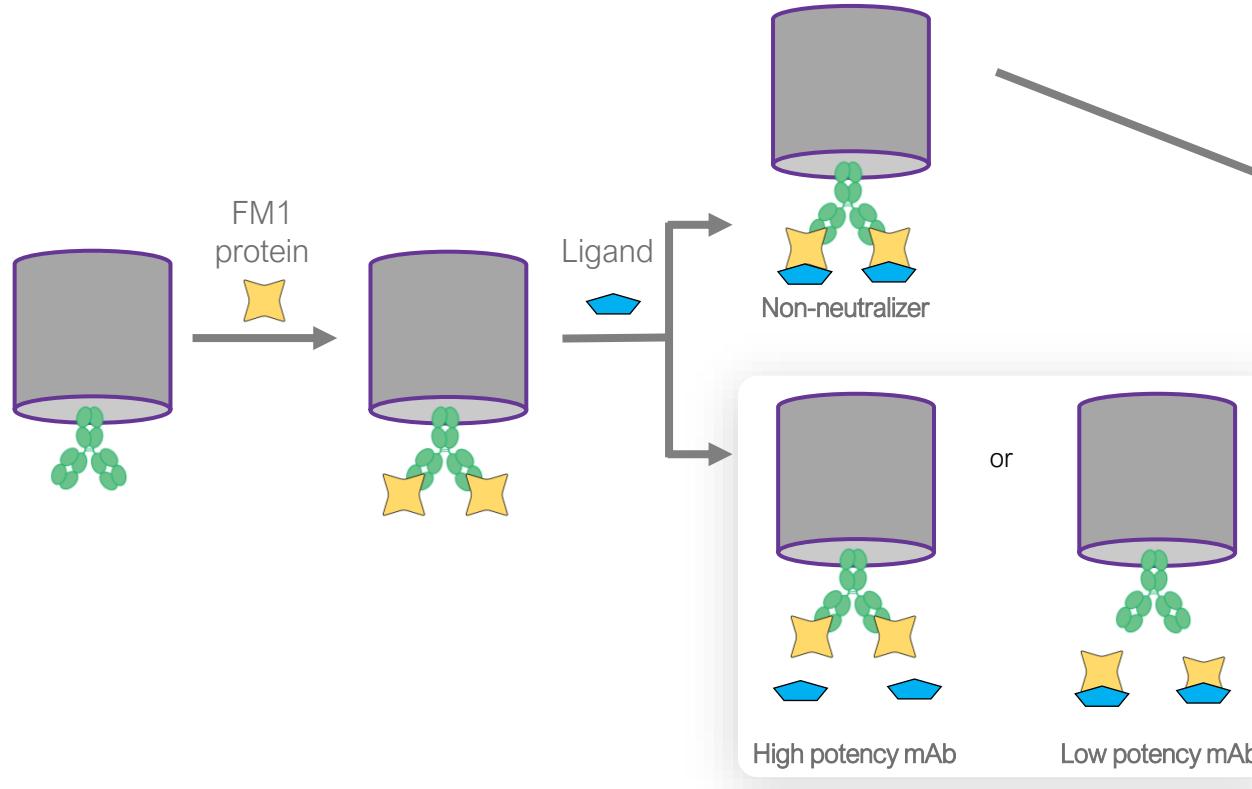


**Single-Point  $K_D$  Analysis**



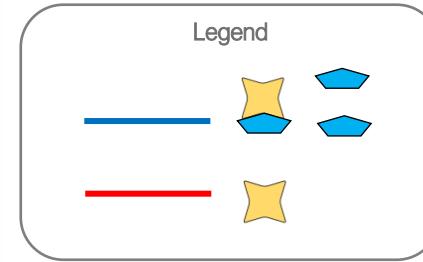
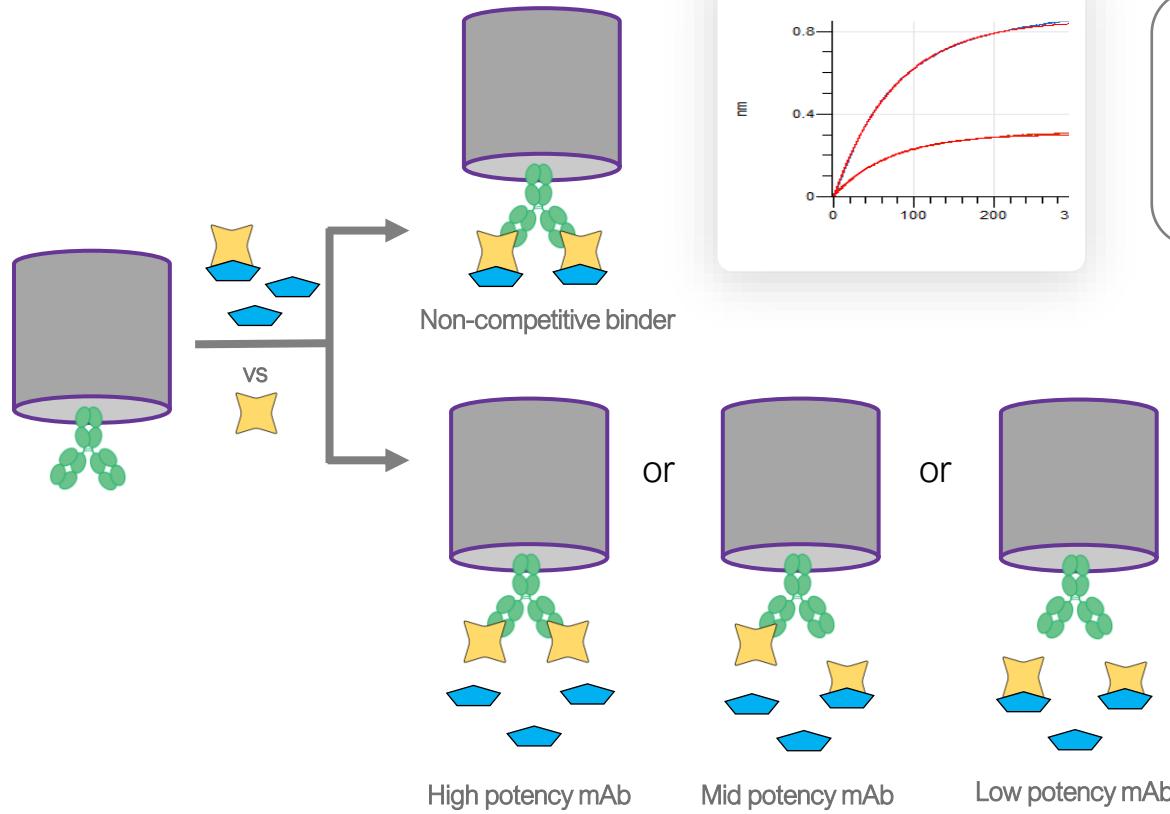
# VALIDATION RESULTS: OCTET BLI – SEQUENTIAL FORMAT FOR BLOCKING ANALYSIS

Sequential format allows definitive non-blocking clone determination

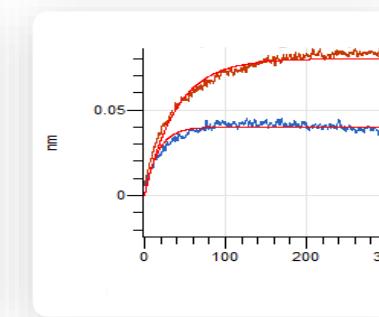
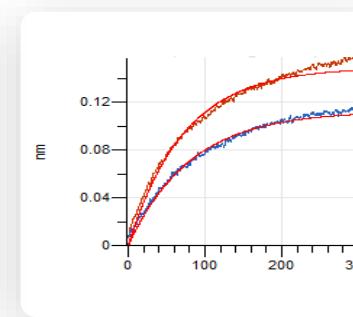
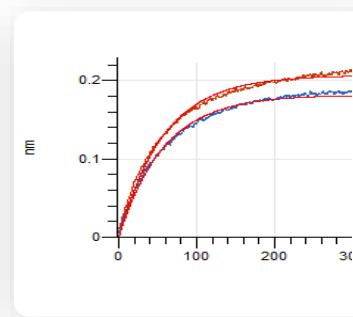


# VALIDATION RESULTS: OCTET BLI – PRE-MIXED FORMAT FOR BLOCKING ANALYSIS

Pre-mixed format allows differentiation in potency among blocking clones



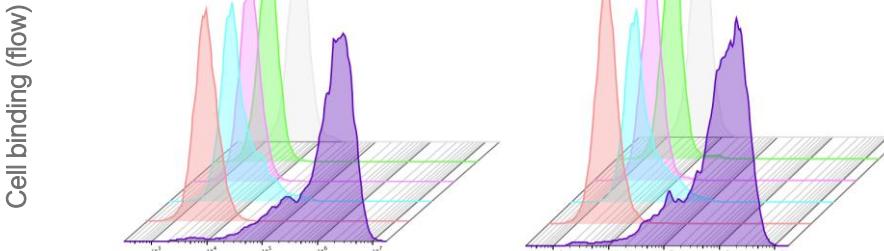
Antibodies with different potencies were identified



# LEAD IDENTIFICATION FOR THERAPEUTIC CANDIDATE NOMINATION

Representative lead candidates from the workflow

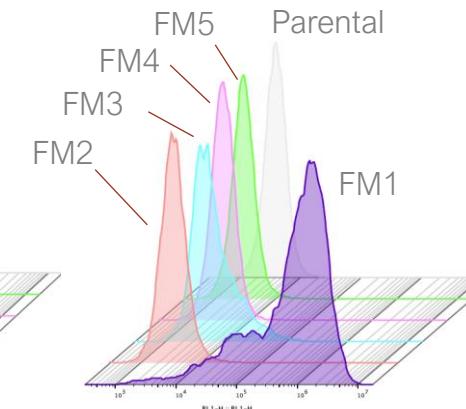
Clone 1



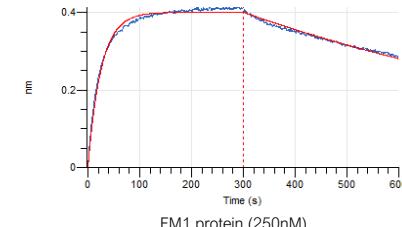
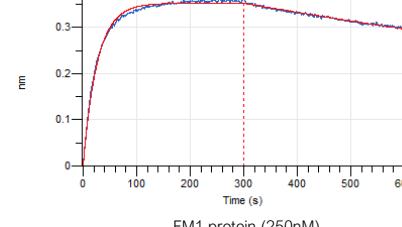
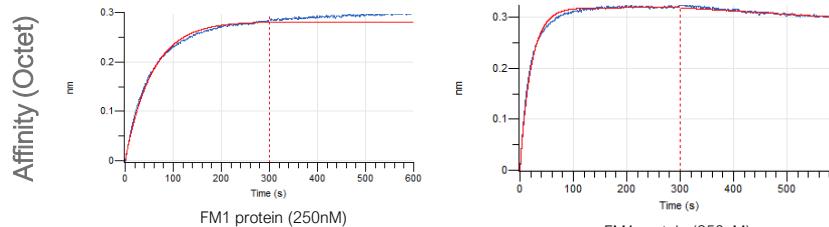
Clone 2

Clone 3

Clone 4

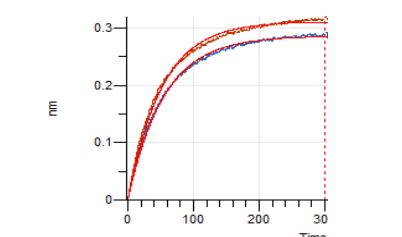
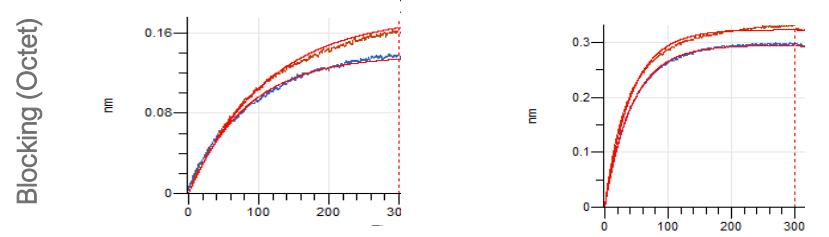


Successful  
Additional Discovery



## Primary Partner Goals

- ✓ Broader antibody diversity for discovery of rare candidates
- ✓ Function-forward, accelerated single B cell workflow for efficient lead identification



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

1 Specific on-cell binding to Family Member 1 (FM1) without off-target binding

2 Functional ligand blocking activity required

3 Sequence diversity desired

4 Tight timeline requirement

- 62 FM 1-specific binders identified and validated off-Beacon by flow cytometry
- 51 ligand blocking antibodies identified and validation off-Beacon by Octet
- Expression recovery of approximately 90% from unique sequences

- 100 unique sequences or 80% unique sequence recovery
- Diverse affinities down to nanomolar range

- Delivery of validated, purified antibodies in 2.5 months



# PREMIER ANTIBODY DISCOVERY PARTNER

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Contact: Garren Hilow  
[ghilow@abverisantibody.com](mailto:ghilow@abverisantibody.com)