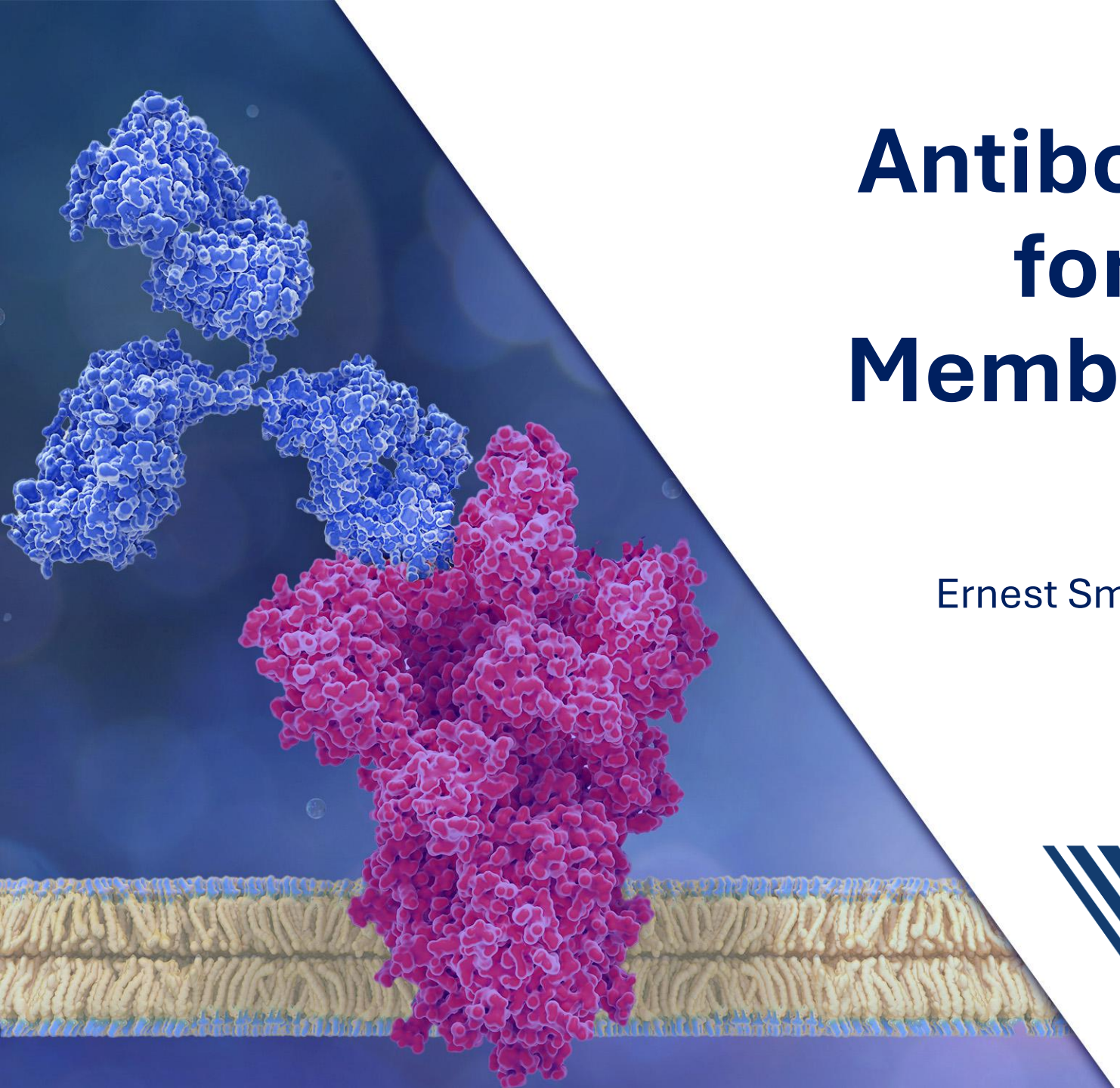


Antibody Discovery for Complex Membrane Antigens

Ernest Smith, Ph.D., General Manager

VACCINEX

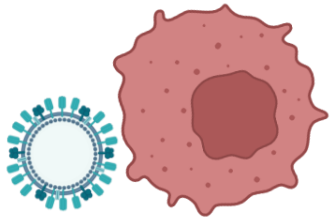
Unique Targets · Novel Mechanisms · New Medicines

Expression of Membrane Protein on Poxvirus for Antibody Discovery

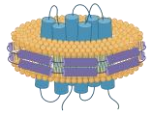
ActivMAb Technology

Antibody discovery for transmembrane proteins like GPCRs and Ion Channels is extremely challenging due to difficulty in obtaining high-quality antigen with native conformation

Alternative Approaches

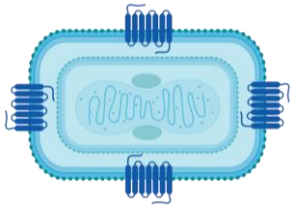


- Whole cells and VLPs: Complex antigen mixtures with low target expression, making them challenging for use as an antigen source for discovery
- Nanodiscs: Time consuming to generate and present both intra- and extra-cellular epitopes.



- Genetic immunization methods give variable results

ActivMAb



Vaccinex leverages its ActivMAb™ Technology to generate antigen for antibody discovery

- Antigen expression in the outer membrane of poxvirus
- Concentration of membrane protein
- Retains native conformation and orientation
- High purity

Created with Biorender.com

Seamless Incorporation of Target Antigens Into Poxvirus

ActivMAb Technology

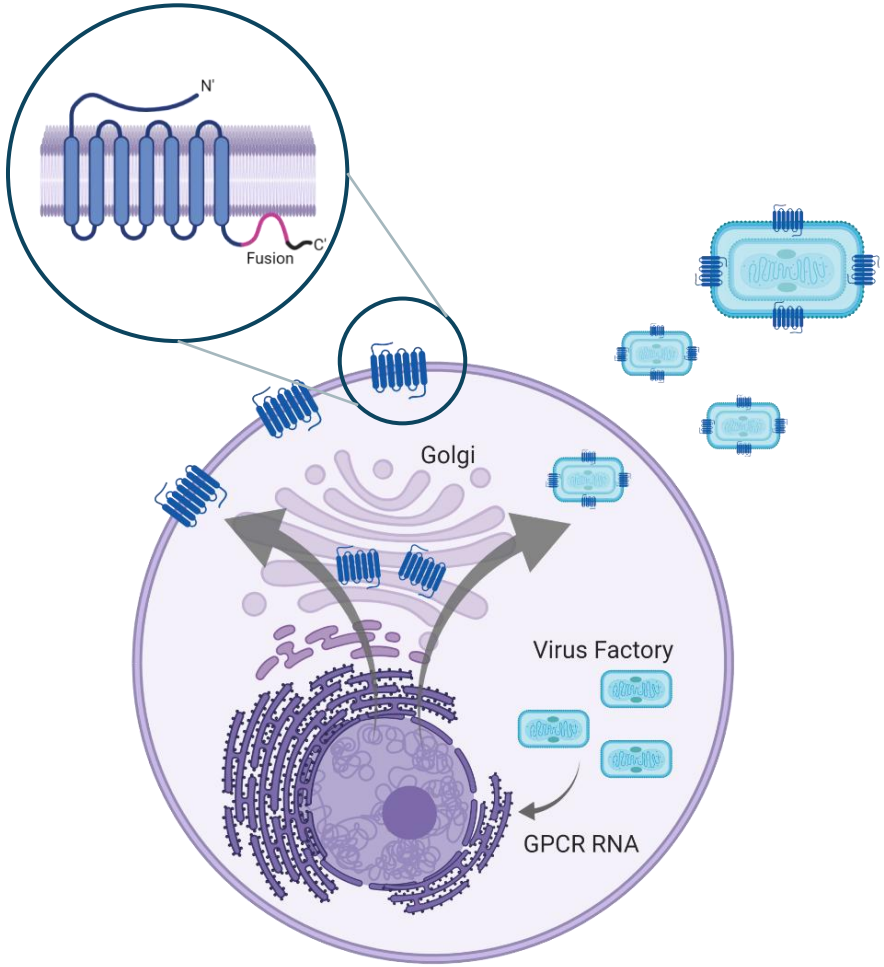
Vaccinex's fusion protein technology provides for efficient incorporation of multi-pass membrane proteins into the poxvirus.

· Proprietary fusion protein targets antigens to the membrane of the poxvirus. Untagged molecules do not incorporate efficiently.

· Specific membrane complexity – minimal contaminating cellular proteins.

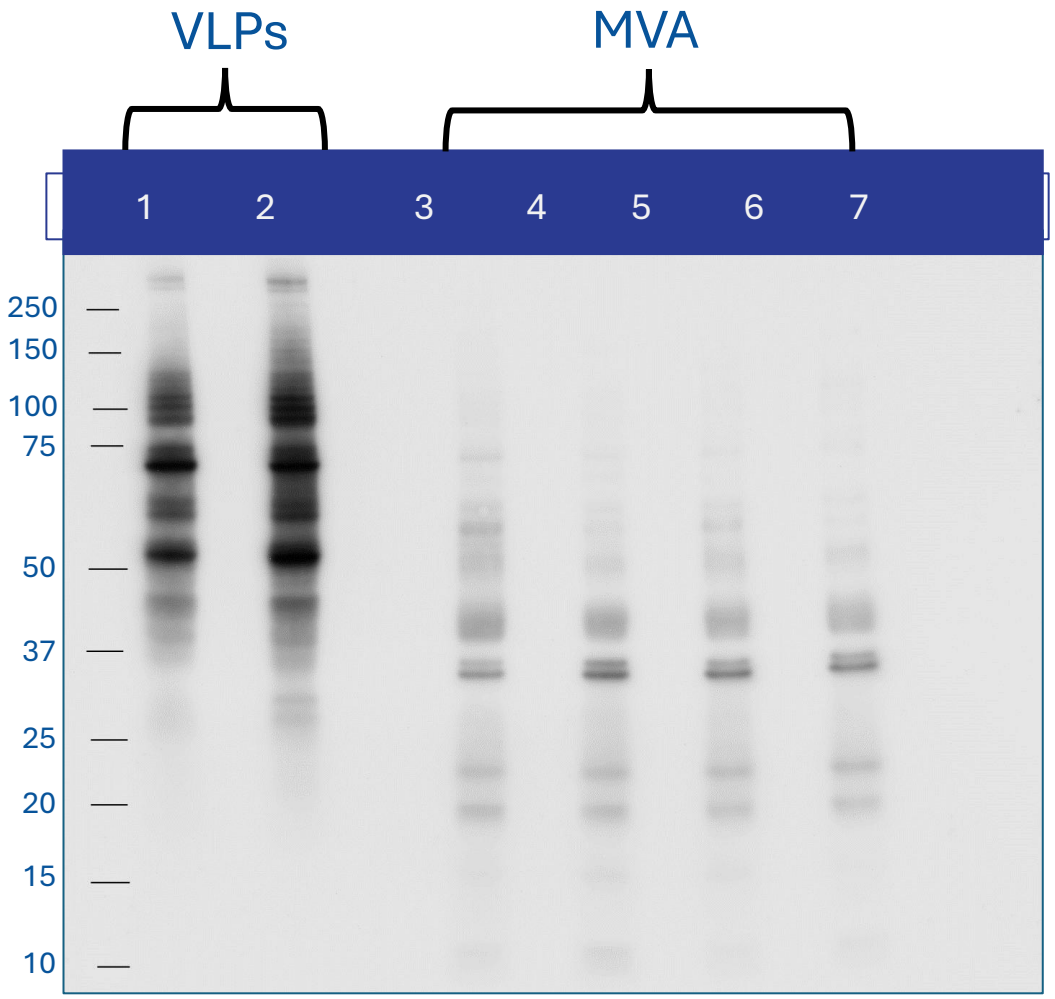
· Virions are harvested by centrifugation and used directly without detergents or refolding

· MVA (Modified Vaccinia Ankara) and FPV (Fowlpox) are BSL-1 DNA viruses with large cloning capacity – up to 25,000 bp inserts.



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Antigen Virions vs. VLPs



MVA has fewer surface exposed proteins than VLPs

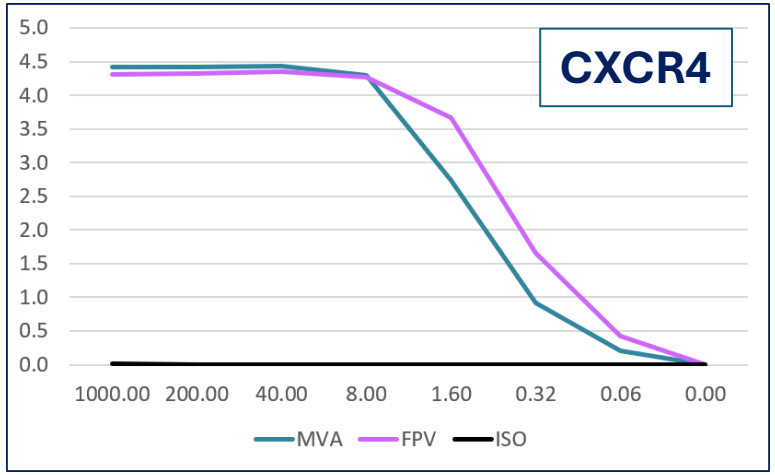
- MVA and VLPs were surface biotinylated and then denatured.
- Protein gel was loaded with equivalent protein/ well.
- Western was probed with Streptavidin-HRP to detect the surface exposed proteins.

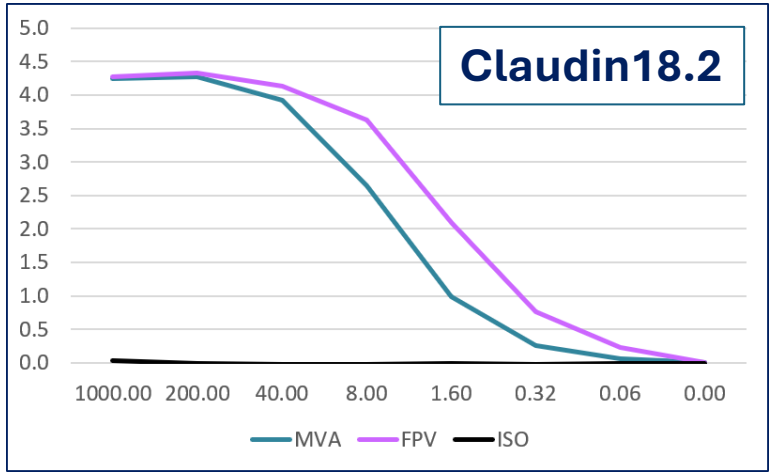
Lane	Sample
1	**GPR65** VLP's
2	**CXCR5**-VLP's
3	MVA-**GPR65**
4	MVA-**CXCR5**
5	MVA-**Sema4D**
6	MVA-**CXCR4**
7	MVA-**CXCR4 (not biotinylated)**

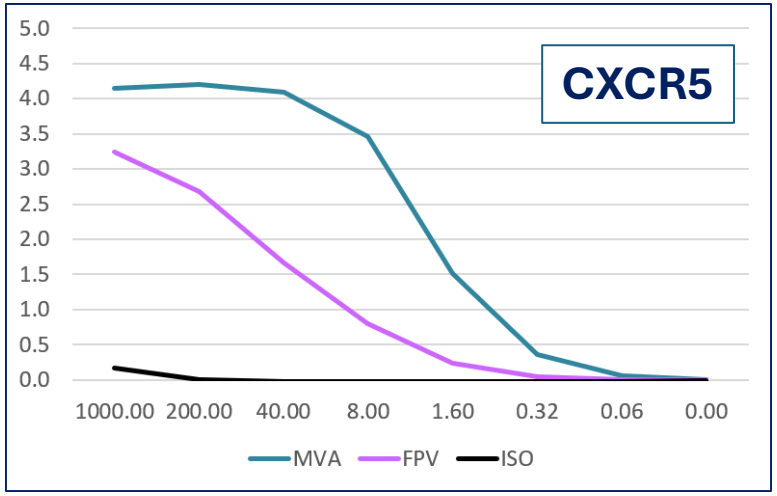
Antigens are Properly Expressed

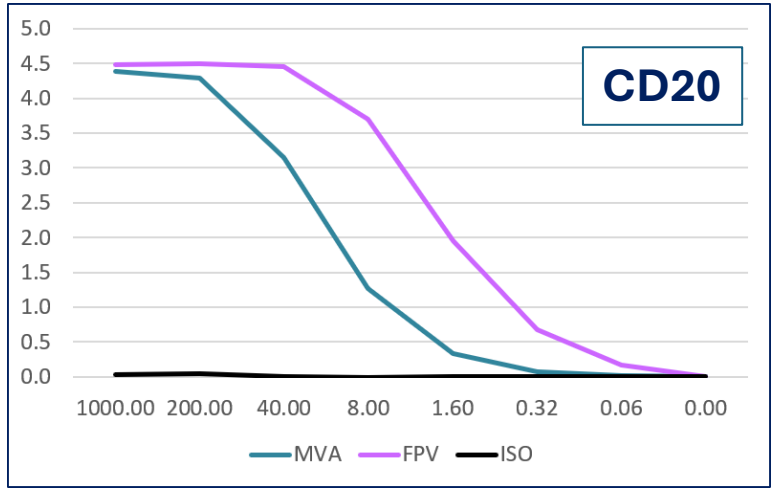
ActivMAb Technology

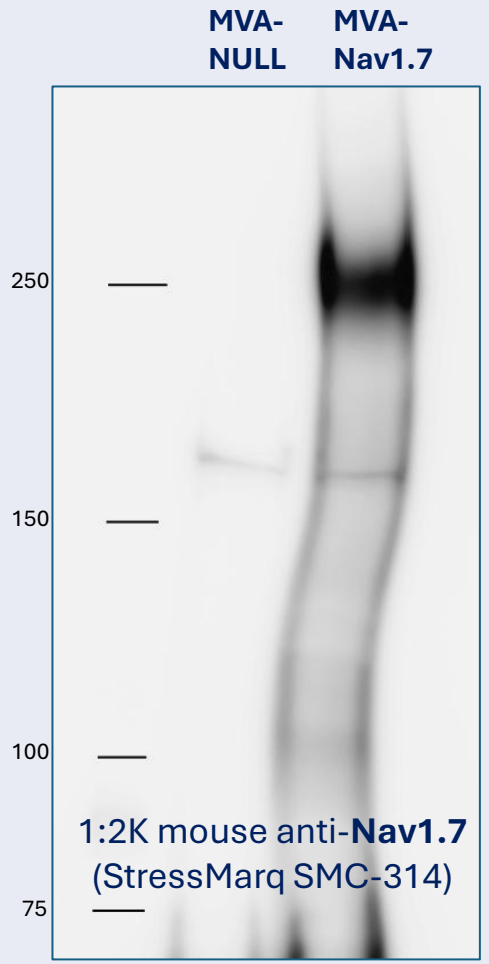
ELISA using commercially available, conformationally specific antibodies.











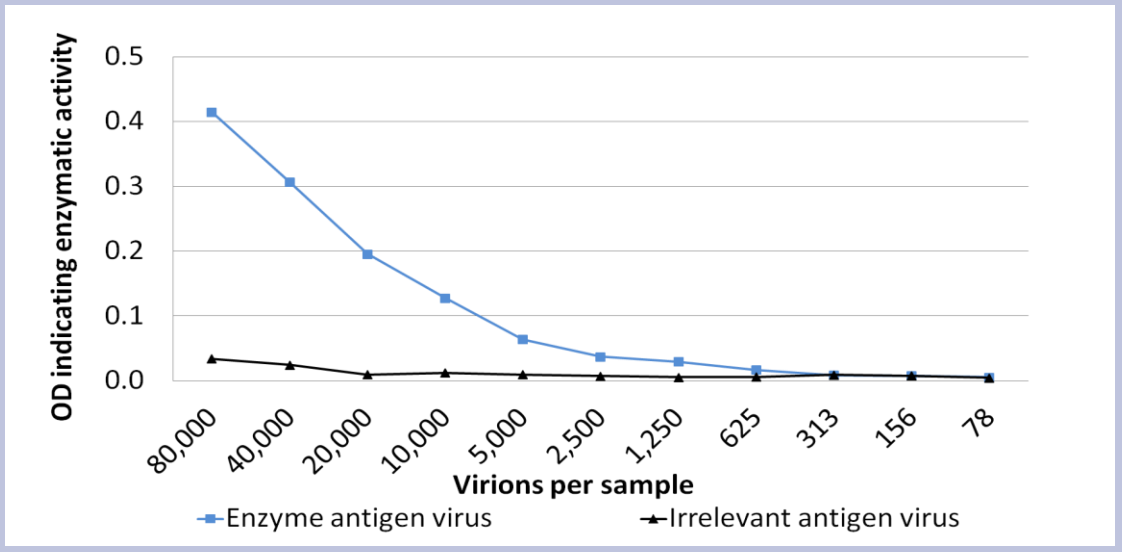
Western blot of MVA-Nav1.7 antigen virions (MW 253 kDa) vs MVA-NULL on a low% Tris-Acetate gel.

Antigens are Properly Folded

ActivMAb Technology

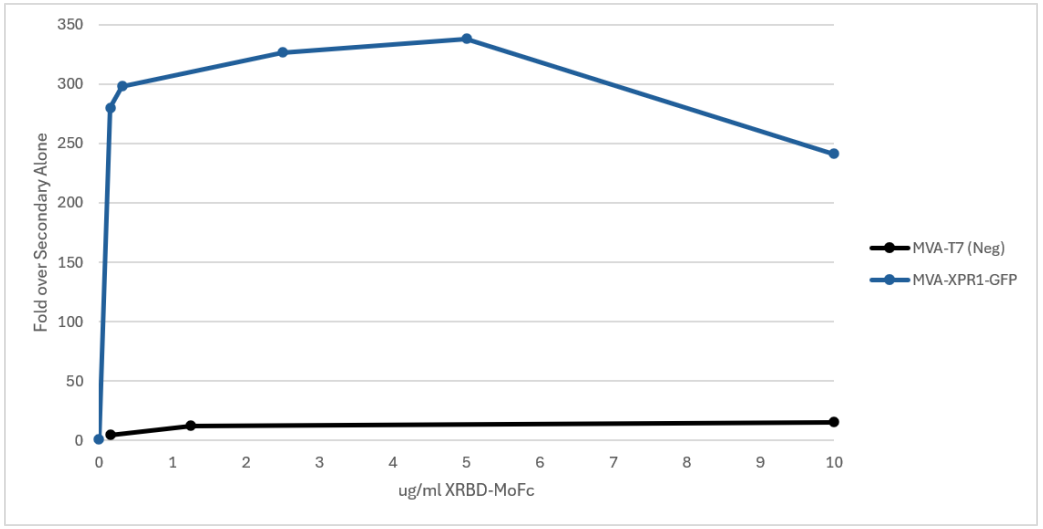
CD39 antigen virions demonstrated enzymatic activity

CD39 is an extracellular enzyme that converts ATP to AMP. Enzymatic activity was determined by the detection of free phosphate using Malachite Green.



MVA-XPR1 infected cells show XRBD binding by flow cytometry

XRBD-moFc was titrated and incubated with QT35 cells infected overnight with MVA-XPR1-GFP or MVA-T7. Binding was detected with anti-Mo-Fc-APC by flow cytometry.

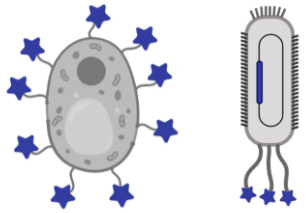


High Versatility Enables Antibody & Protein Engineering Solutions

ActivMAb Technology

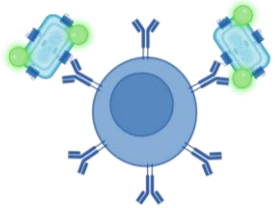
- Proteins retain native conformation, membrane orientation and post translational modifications
- Virions expressing multi-pass membrane proteins in their outer membrane and are released into the culture medium for simple purification and direct use
- Much cleaner than VLPs / easier than nanodiscs

Antigen virions can be used for antibody discovery using established methods for purified protein



Phage and yeast display

- No non-specific binding of phage or yeast
- Works with bead or plate-based screening



Can be labelled for sorting

- Compatible with ex-vivo B cell sorting



Immunization

- BSL-1 does not replicate in mice or rats
- Potent immunogens – no adjuvant needed



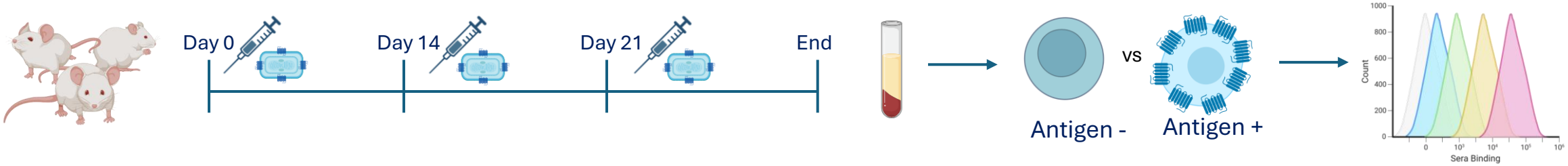
Two antigenically distinct strains

- Eliminate anti-viral background by using two strains: MVA & FPV

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Antigen Virions Can Be Used for Mouse Immunization

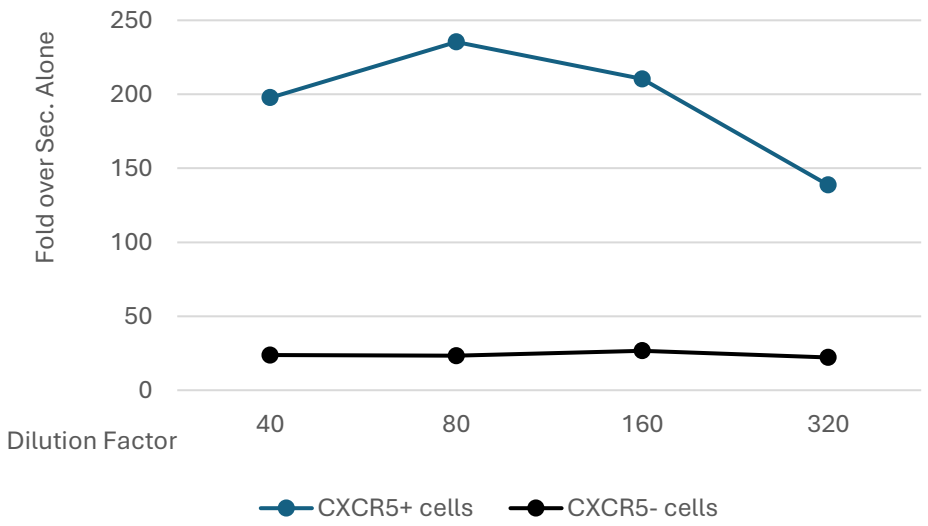
ActivMAb Technology



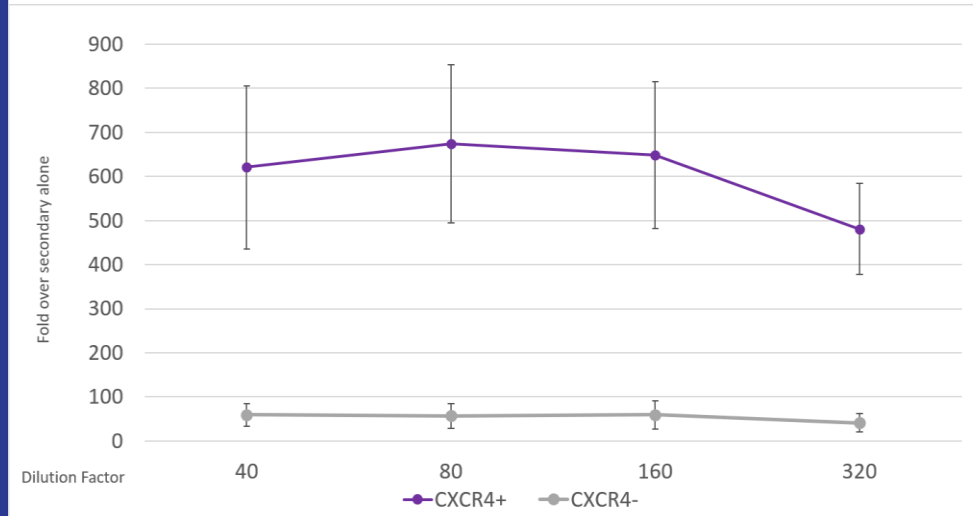
· BSL-1 Antigen virions are well tolerated and do not require adjuvant.

· Sera is analyzed by flow cytometry to confirm native reactivity.

CXCR5 sera



CXCR4 sera



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In vivo Antibody Discovery

ActivMAb Technology

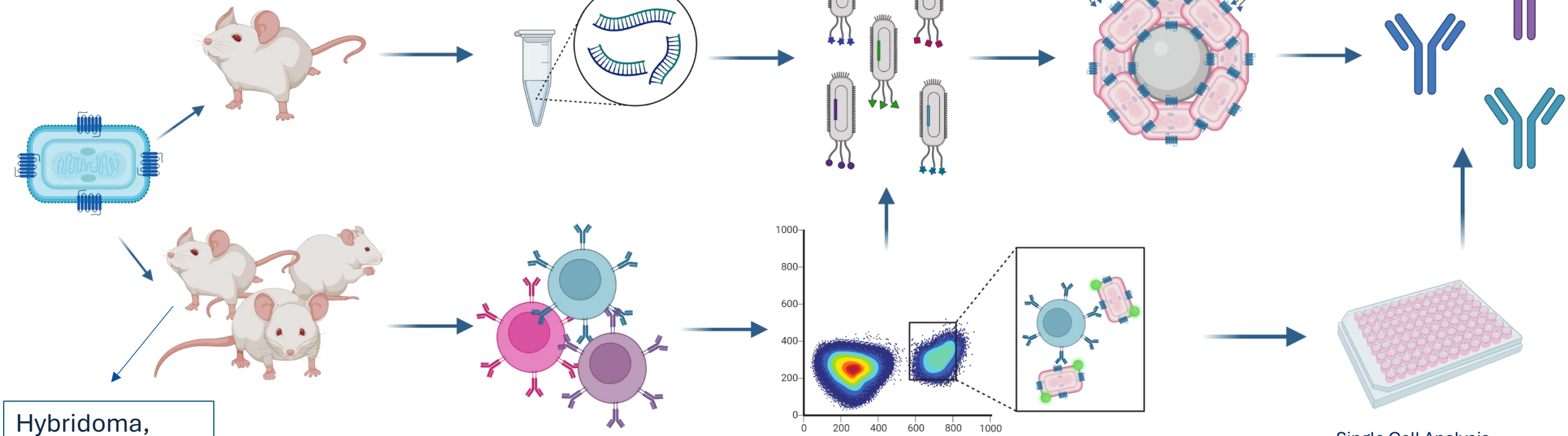
Mouse immunization with Antigen virus

mRNA extraction from Whole spleen

Phage library generation

Phage panning antigen virus Coupled to magnetic beads

Specific anti-antigen antibodies



Hybridoma, Beacon, etc

B Cells from spleens & Bone marrow

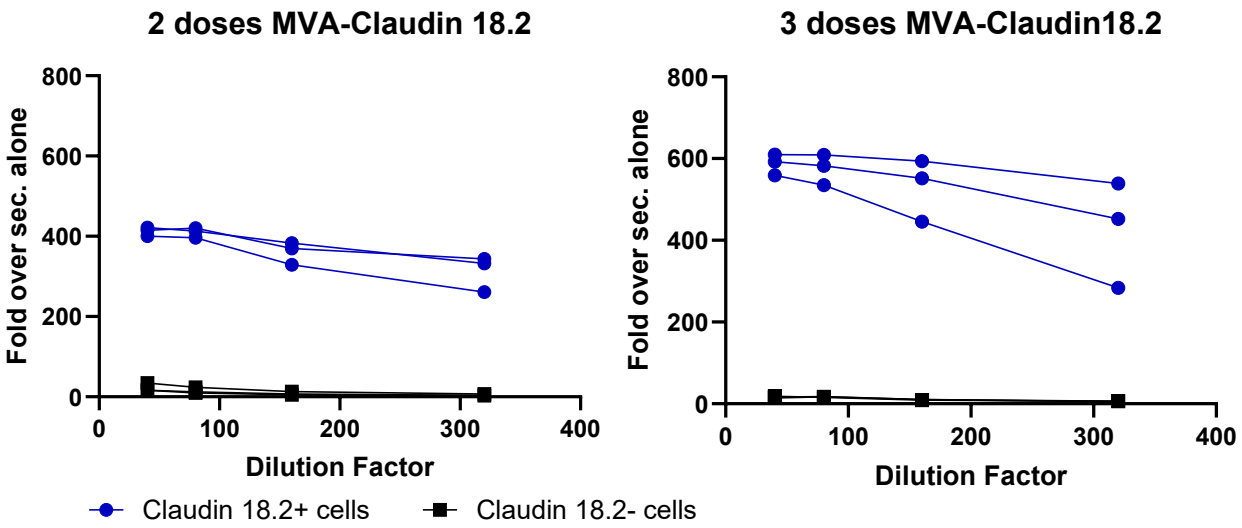
FAC Sorting for Memory B cells
[B220+, IgM-, IgG+]
Bound to Fluorescent labelled Antigen Virus

Single Cell Analysis

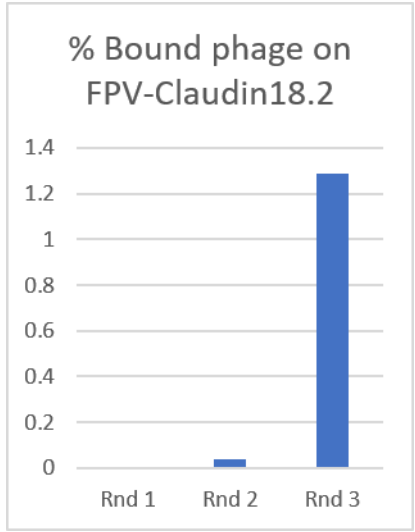
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Case Study: Claudin18.2 Immunized Library Antibody Discovery

ActivMAb Technology

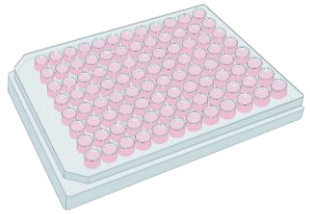


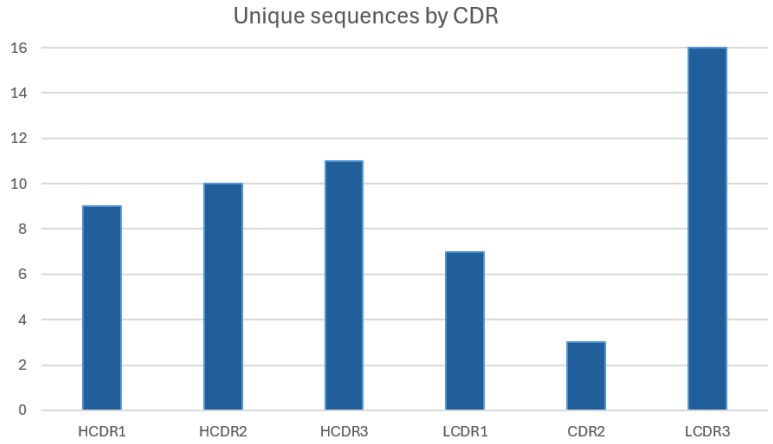
Immune Phage library generation



Cloning Transfection

Testing of 94 clones by flow cytometry





53 unique clones were discovered.
After additional specificity and binding testing,
The top 5 were chosen for humanization,
purification and functional testing.

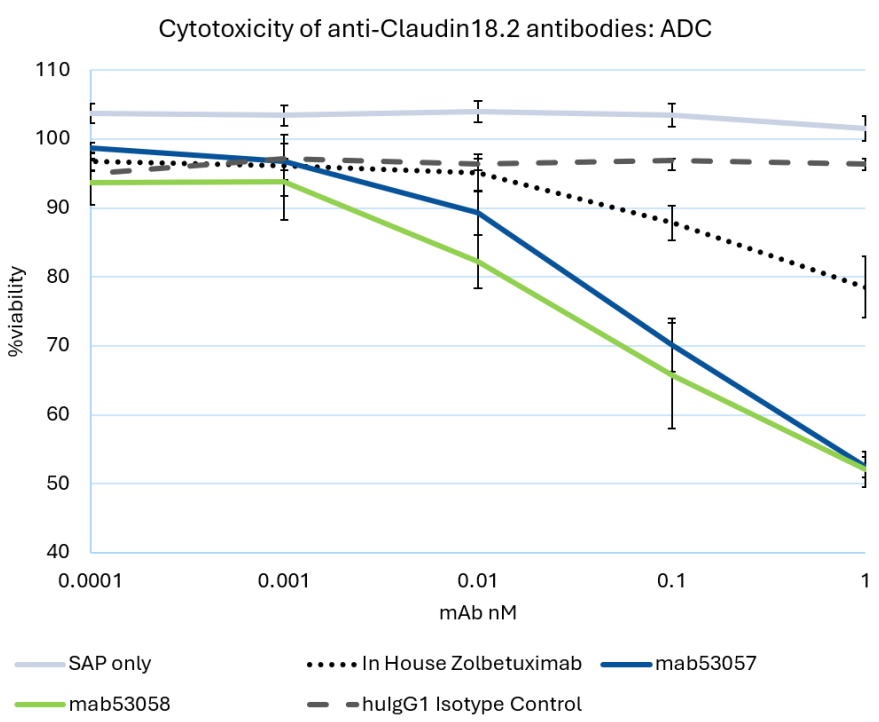
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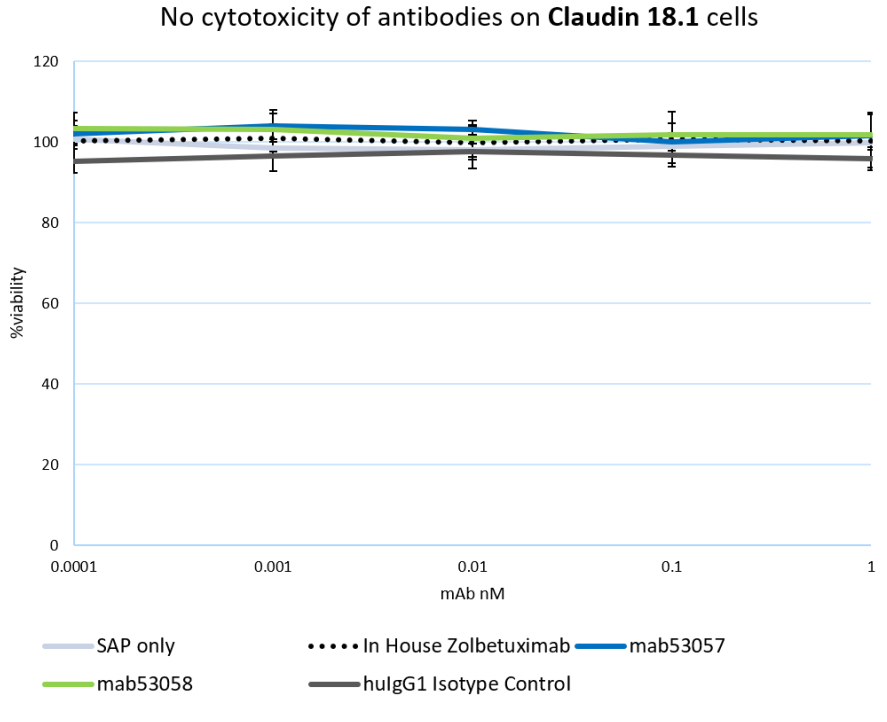
Claudin18.2 Antibody Functional Testing

Affinity was determined by a flow cytometry based Scatchard Assay.

MAb	Affinity (nM)
In house Zolbetuximab	0.95
mAb53054	0.75
mAb53055	1.11
mAb53056	0.98
mAb53057	**0.73**
mAb53058	**0.49**

Claudin 18.2 – Antibodies show specific ADC activity

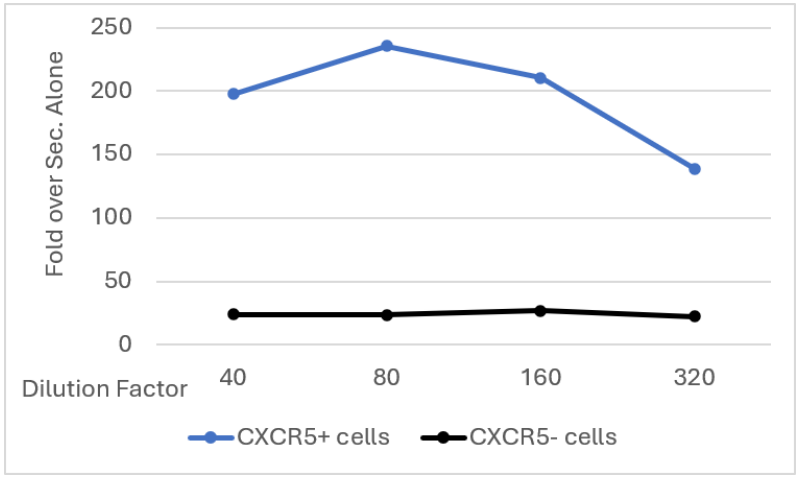




Case Study: CXCR5 Immunized Library Antibody Discovery

ActivMAb Technology

CXCR5 Immunization Titer

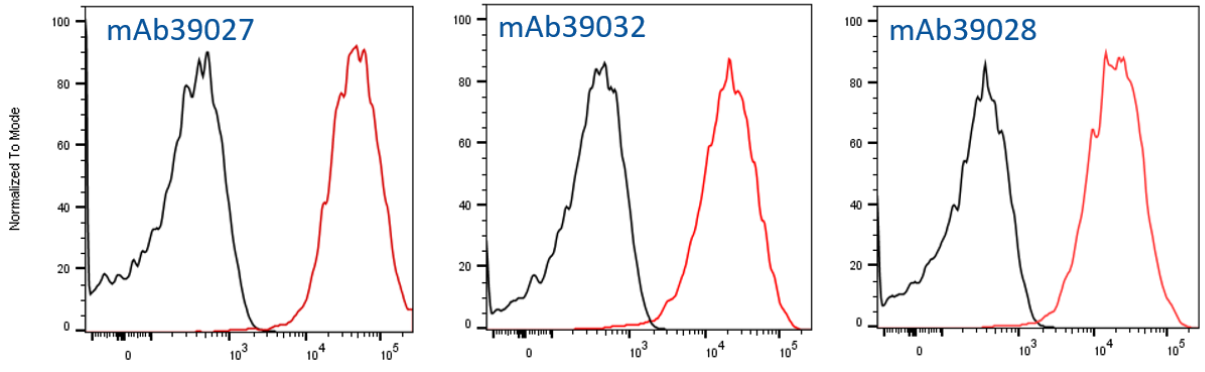


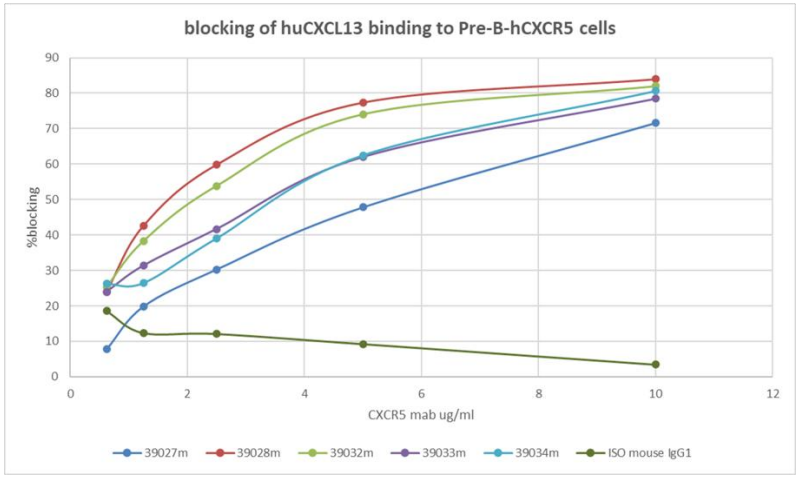
Phage library generation

Phage Panning

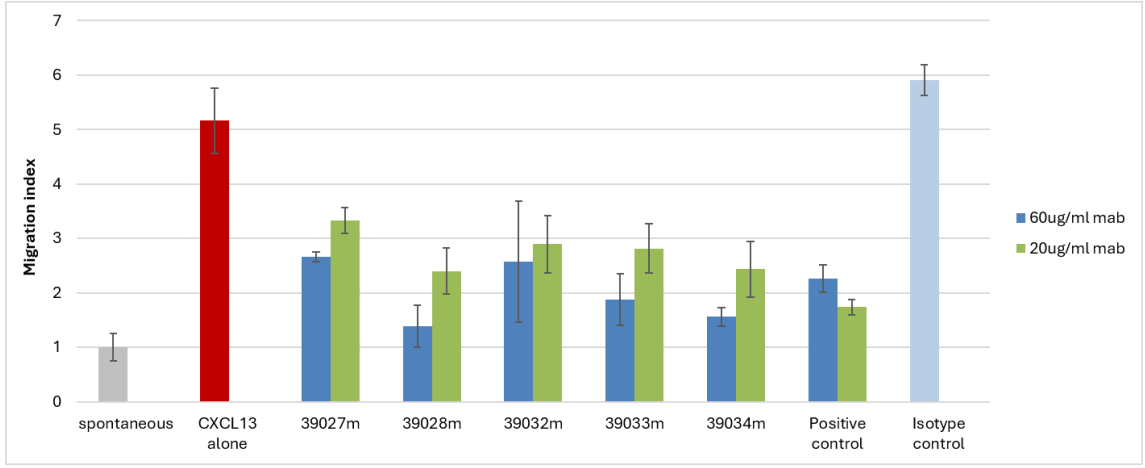
>150 unique mabs

18 unique H-CDR3s





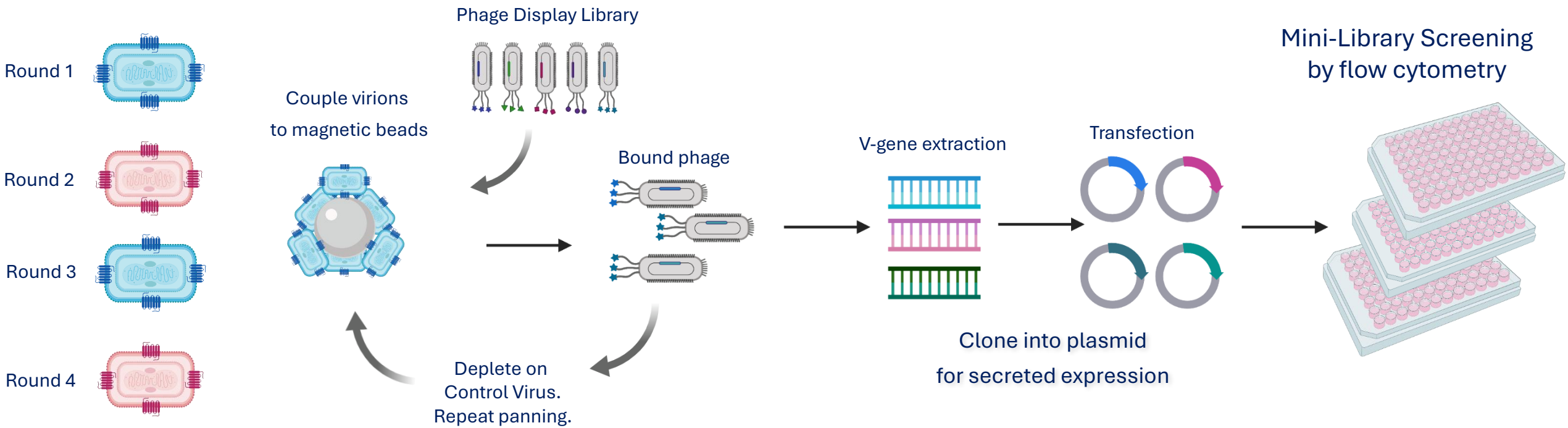
mAb	nM Affinity
39027m	0.04
39028m	0.56
39032m	0.53
39033m	0.34
39034m	0.35



In vitro Phage Panning

ActivMAb Technology

Antigen virions can be coupled to magnetic beads or ELISA plates for panning with various *in vitro* display technologies.



Antigen Virions:

Alternating rounds between MVA and FPV can improve specificity

mAbs, *15*(1). https://doi.org/10.1080/19420862.2023.2249947

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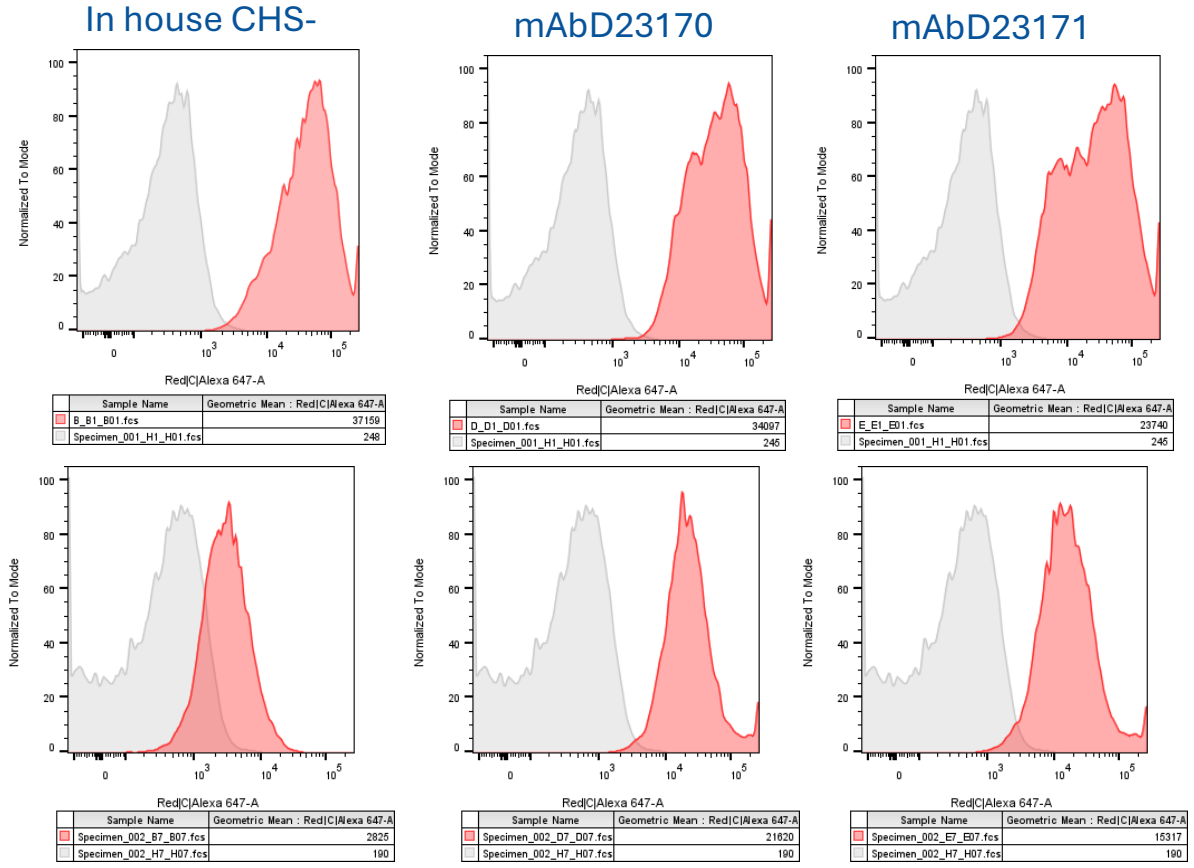
Case Study: CCR8 *In vitro* Antibody Discovery

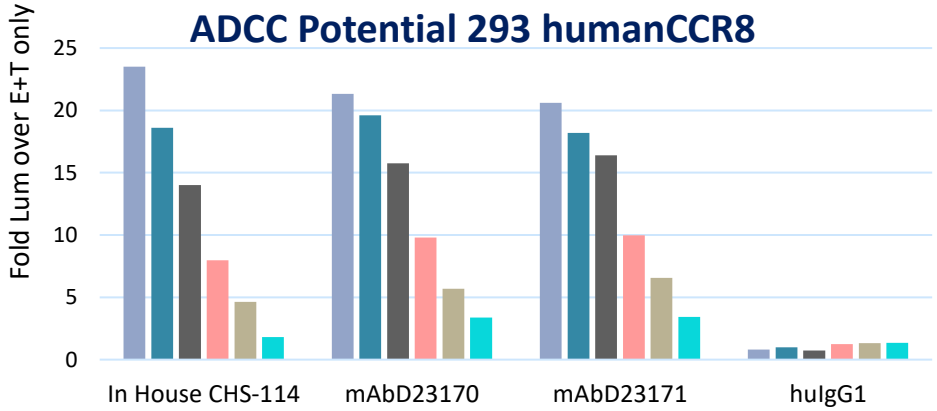
ActivMAb Technology

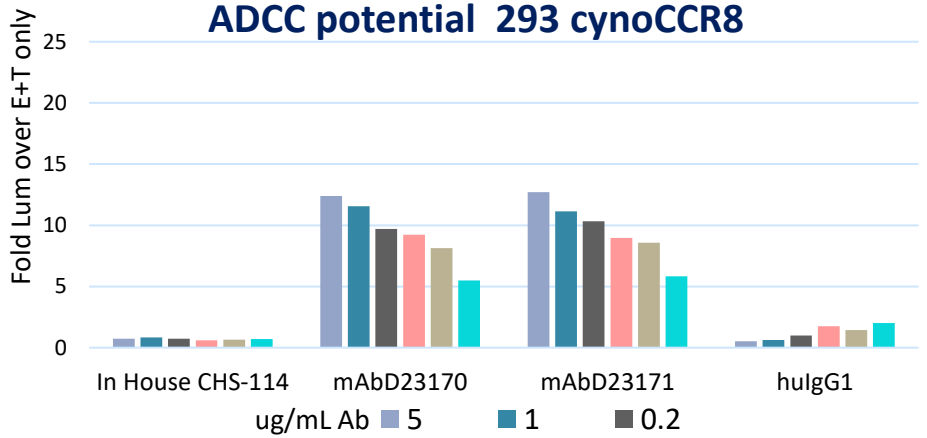
Utilizing CCR8 antigen virions, 4 rounds of phage panning were done using a naïve human Fab library. Individual antibodies were screened for binding to native CCR8 expressing cells. Top binders were purified and tested for ADCC potential using strep-ZAP.

293-HumanCCR8 cells

293-CynoCCR8 cells





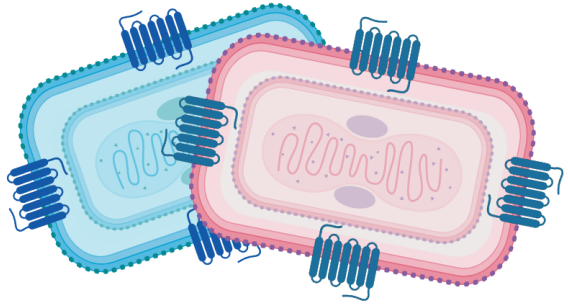


ActivMAb® Antibody Discovery

Vaccinex ActivMAb® Technology provides an ideal reagent to discover antibodies specific for complex membrane proteins

Any Membrane Protein

- Up to 24-pass transmembrane proteins
- Heterodimers
- Co-expression with co-factors
- Multiple isoforms



Better Antibody Discovery

- Native conformation without detergents
- Cleaner than VLPs and easier than nanodiscs
- Suitable for phage or yeast display
- Potent immunogens with low background

Over 50 deals signed in the last 2 years, including 8 top 20 Pharma

Created with Biorender.com

Discovery Options

Science EXCHANGE APPROVED VENDOR

REGISTERED scientist.com SUPPLIER

Option 1: Catalog of Virions



CCR2
CCR5*
CCR7*
CCR8*
CXCR4*
CXCR5*



FZD4
FZD7
GLP1R
GPR65
GPR75
GPRC5D



CD20*
CLDN 6
CLDN 9
CLDN 18.2*
XPR1



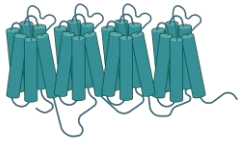
BCMA*
HER2*
Cov2-RBD



P2X2*
P2X7*



Kv1.3



Nav1.7
Nav1.8

Fee for Service; 2-3 weeks
*** Antibodies available for license**

Option 2: Custom Virion Generation



Vaccinex generates antigen expressing virions with client supplied sequence.

- MVA, FPV or both
- Biotinylation available

Fee for Service; 2-3 months

Option 3: Antibody Services

Complete antibody discovery pipeline available!

Services available:

- Custom antigen virus
- Mouse immunization
- Phage panning
- Purification & Affinity testing
- Stable Cell Lines
- Optimization
- Functional Assays
- Mouse Models

Our team has over 20 years of antibody discovery expertise. Let us do the work for you!

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ActivMAb® Discovery Solutions

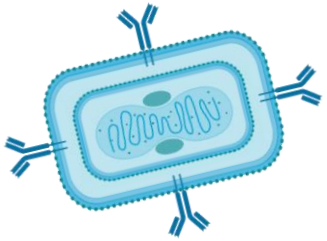
Poxvirus based antibody and protein engineering solutions

ActivMAb Technology



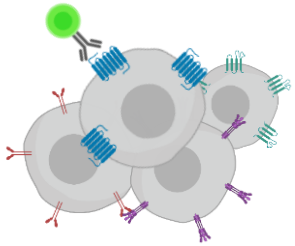
Antigen Virus

Membrane Protein
Expression Platform



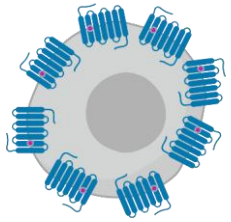
Antibody Library

Mammalian Human IgG
Display Library



Target Identification

FACS sorting of
human cDNA library



Protein Optimization

FACS sorting of mutant libraries
for higher expression isoforms

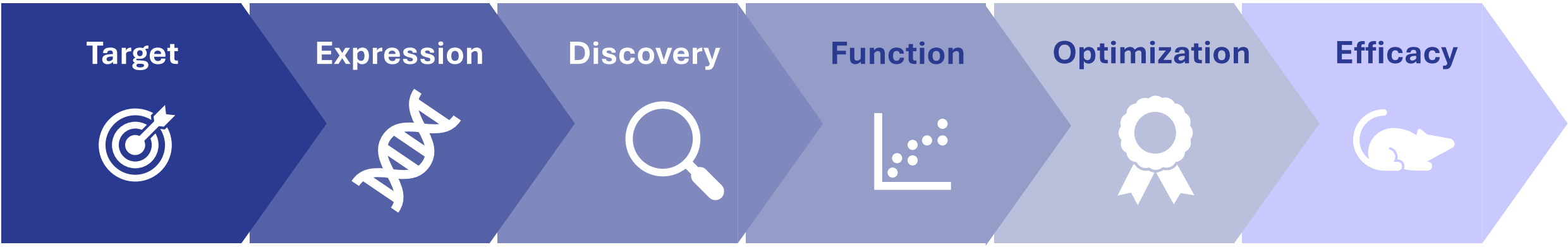
Learn more at Vaccinex.com/Activmab

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Fully Integrated Discovery Capabilities

Providing a suite of technologies to facilitate drug discovery

ActivMAb Technology



Target	Expression	Discovery	Function	Optimization	Efficacy
·Target ID	· Antigen Virus	· Immunization	· Cell assays	· Affinity	· Animal Models
· Mammalian Protein Optimization	· Protein & Antibody Production	· Phage Display	· Receptor blocking	· Humanization	· PK/PD
	· Stable Cell Lines	· Mammalian Display	·Phagocytosis	· Liability	
		· Cell Sorting	· Viability		

Thank You!

ActivMAb Technology



Ernest Smith
General Manager
esmith@vaccinex.com

Jeremy Rudman
Business & Corporate Development
jrudman@vaccinex.com

Elaine Gersz
Business Development, Associate
egersz@vaccinex.com

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