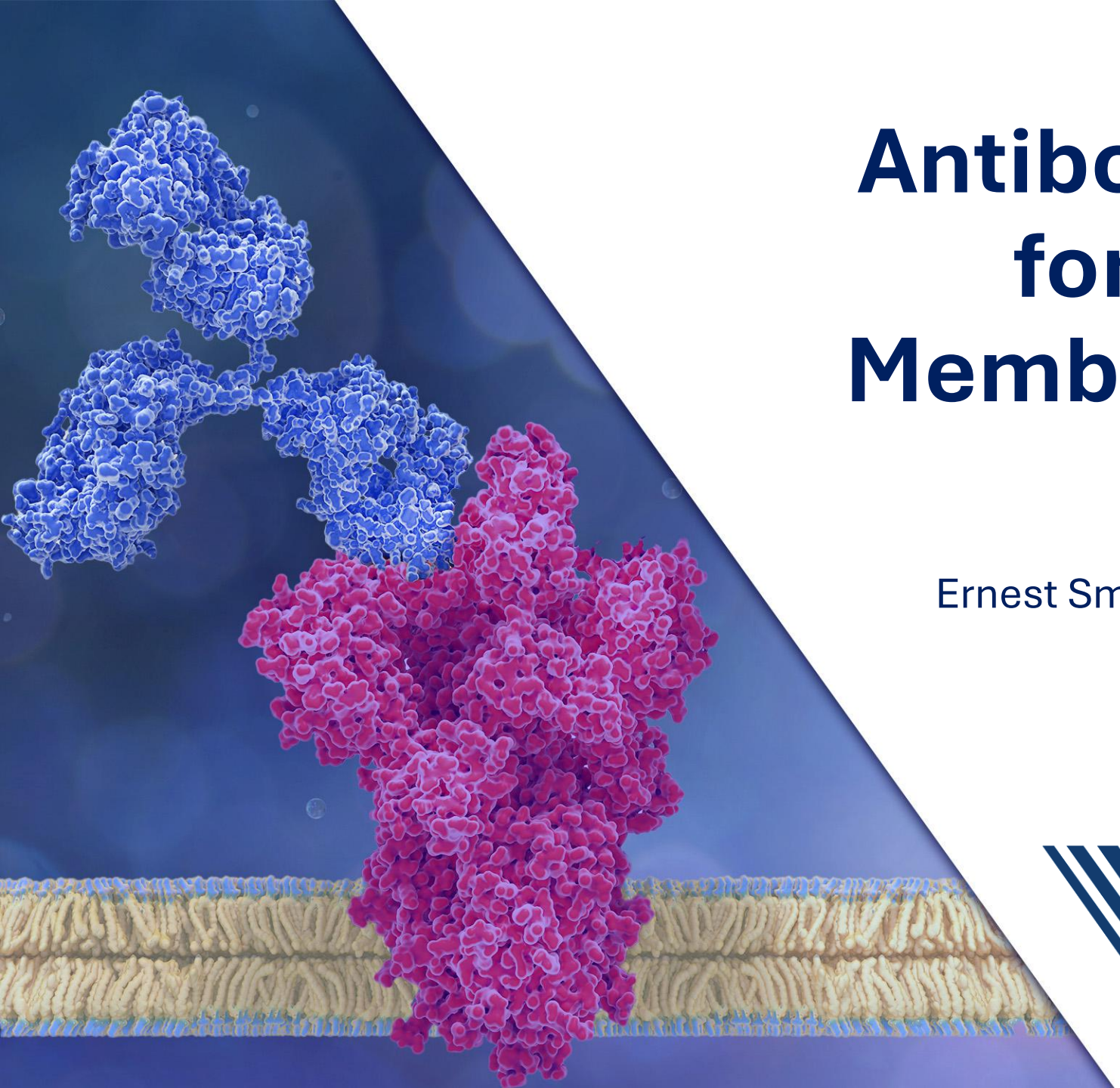


Antibody Discovery for Complex Membrane Antigens

Ernest Smith, Ph.D., General Manager

VACCINEX

Unique Targets · Novel Mechanisms · New Medicines

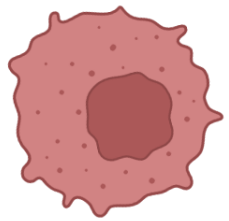
Expression of Challenging Membrane Proteins on Poxvirus

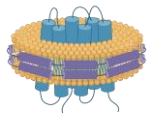
ActivMAb Technology

Alternative Solutions

- VLPs & whole cells: 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: variable results

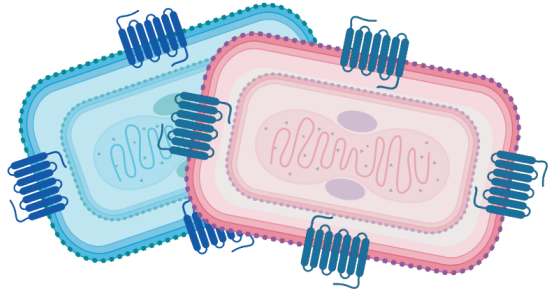






ActivMAb® Technology

- Antigen expression in the outer membrane retains native conformation, orientation and post translational modifications
- Concentration of membrane protein in virion
- High purity



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Seamless Incorporation of Target Antigens Into Poxvirus

ActivMAb Technology

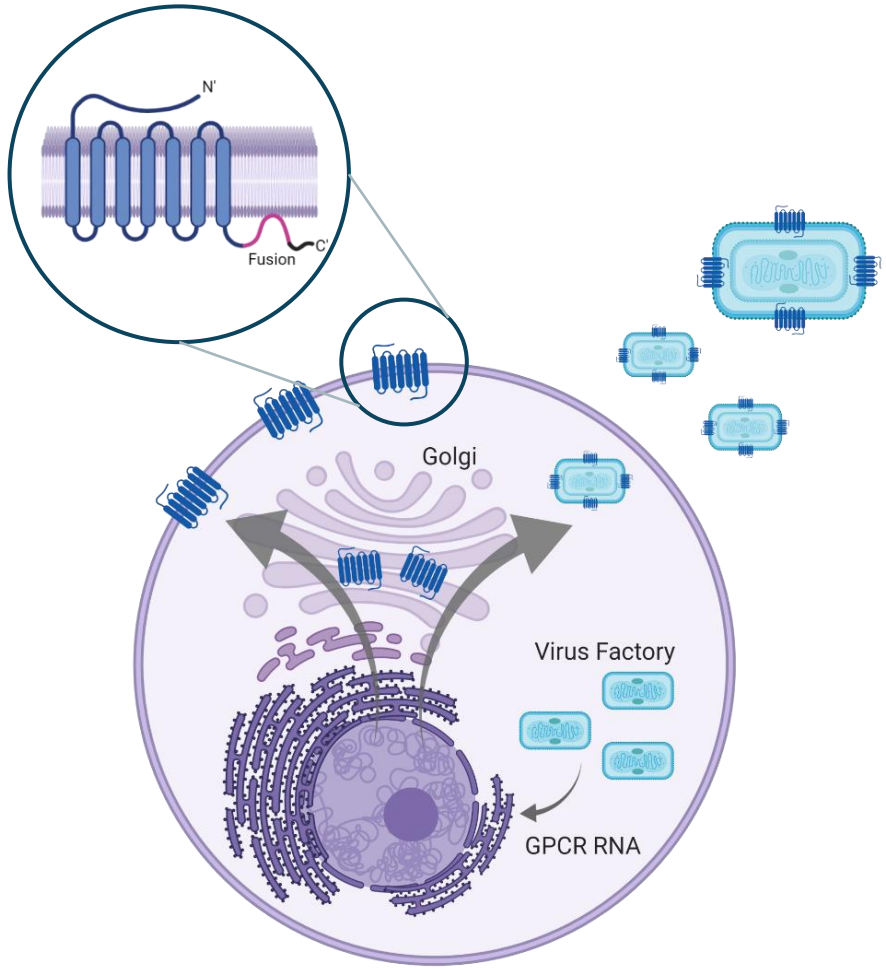
Vaccinex's ActivMAb® technology provides for efficient incorporation of challenging membrane proteins into the poxvirus

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

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

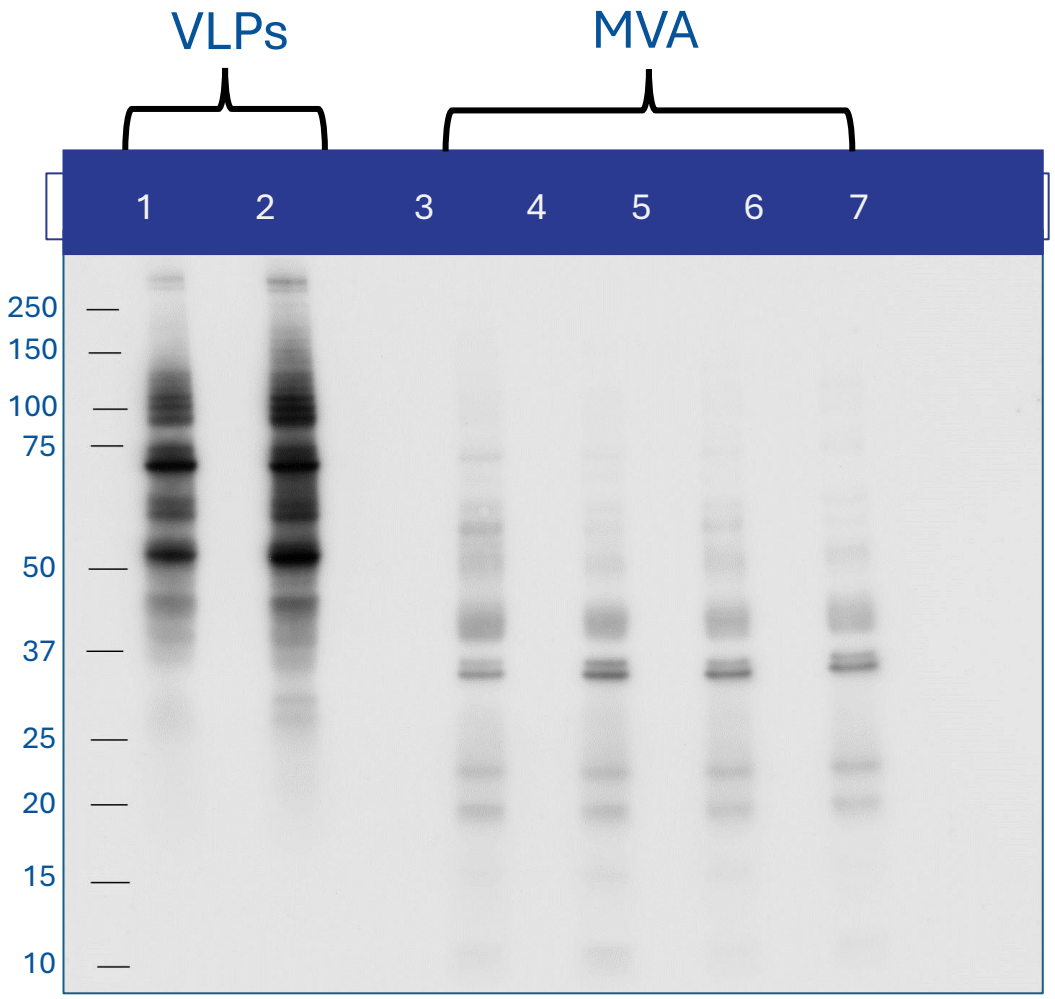
· Specific membrane complexity & minimal contaminating cellular proteins.

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



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Antigen Virions vs. VLPs



MVA has fewer surface exposed proteins than VLPs

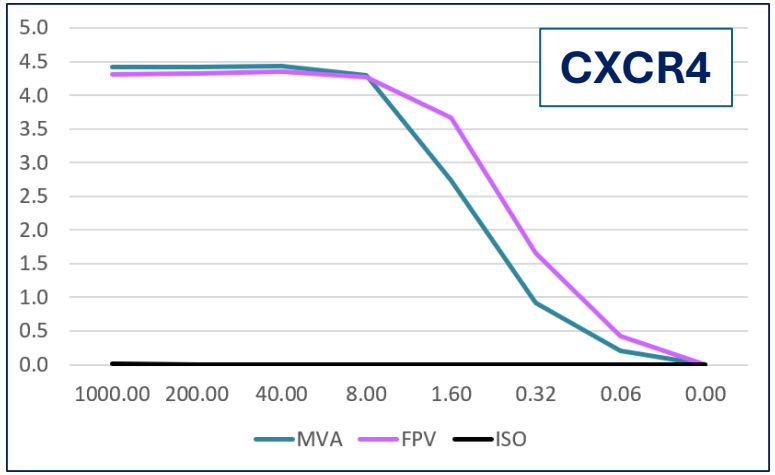
- MVA and VLPs were surface biotinylated and then denatured.
- Protein gel was loaded with equivalent protein/ well.
- Western was probed with Streptavidin-HRP to detect the surface exposed proteins.

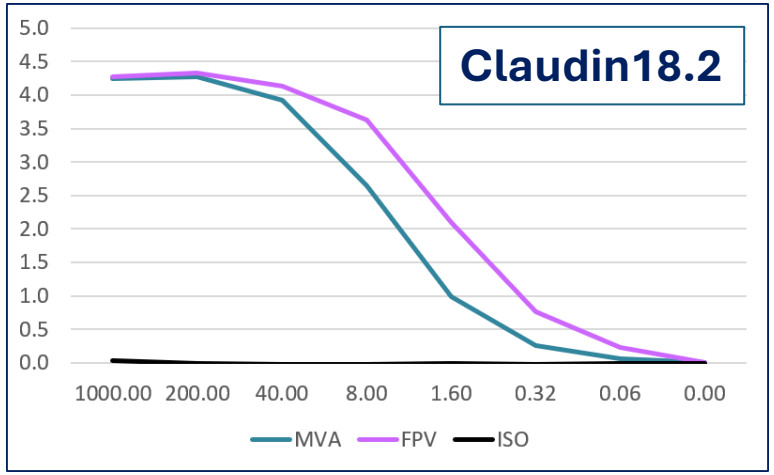
Lane	Sample
1	**GPR65** VLP's
2	**CXCR5**-VLP's
3	MVA-**GPR65**
4	MVA-**CXCR5**
5	MVA-**Sema4D**
6	MVA-**CXCR4**
7	MVA-**CXCR4 (not biotinylated)**

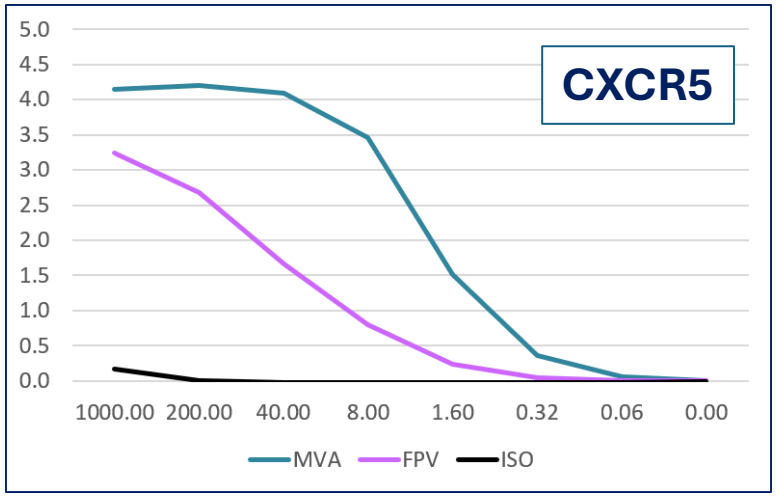
Antigens are Properly Expressed

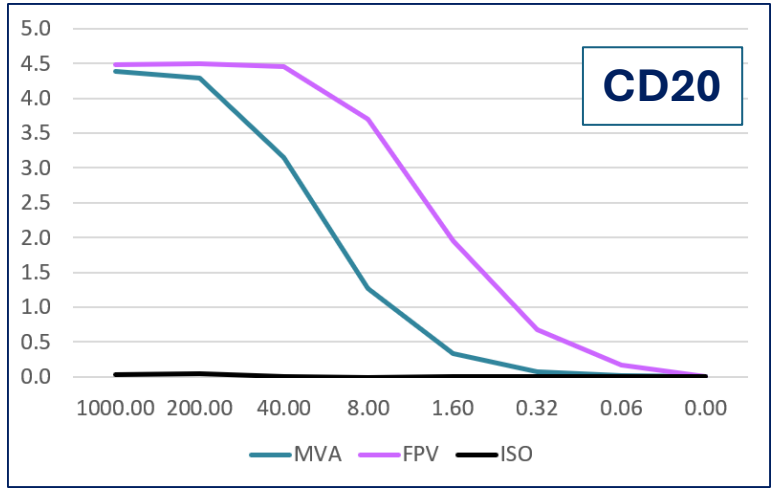
ActivMAb Technology

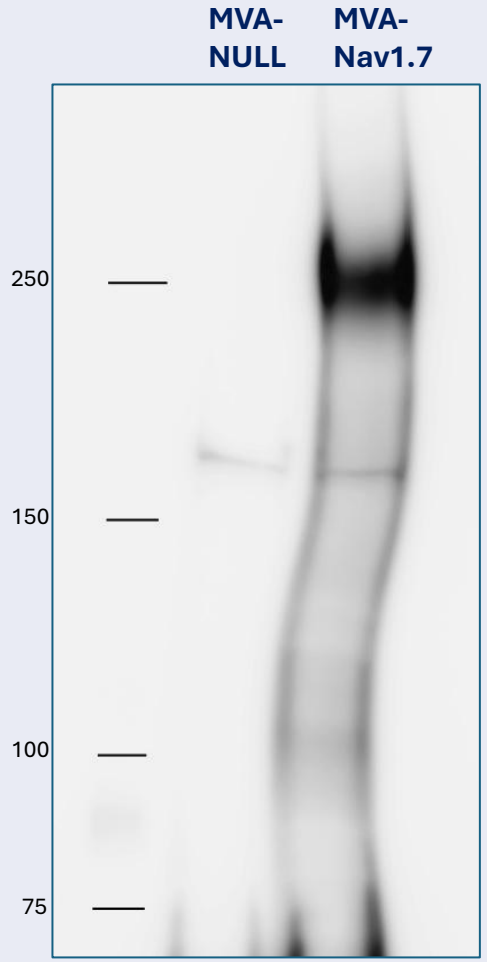
ELISA using commercially available, conformationally specific antibodies.











1:2K mouse anti-**Nav1.7**
(StressMarq SMC-314)

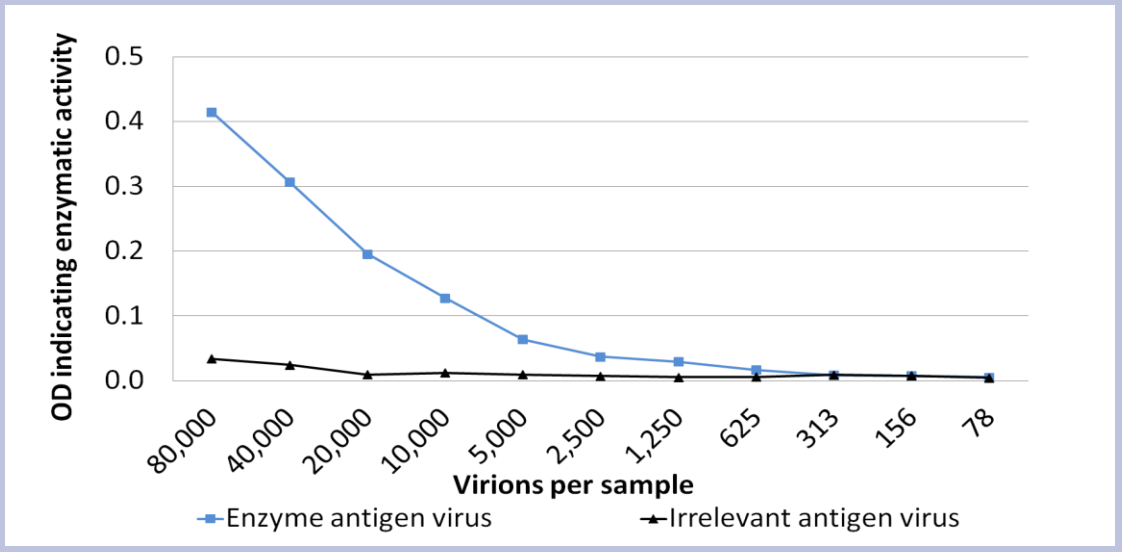
Western blot of MVA-Nav1.7 antigen virions (MW 253 kDa) vs MVA-NULL on a low% Tris-Acetate gel.

Antigens are Properly Folded

ActivMAb Technology

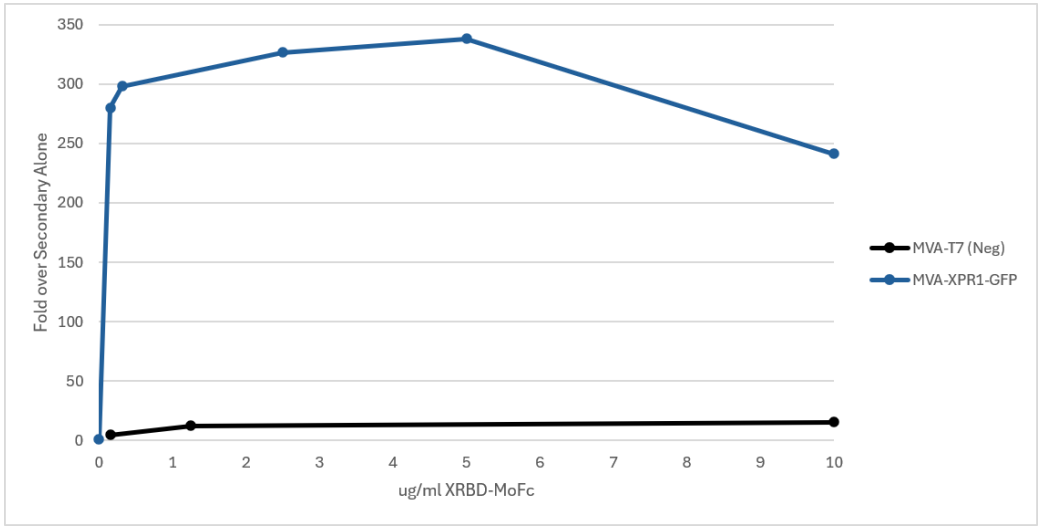
CD39 antigen virions demonstrated enzymatic activity

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

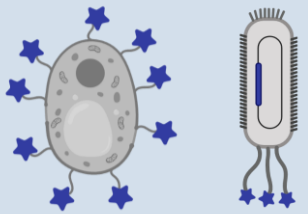
XRBD-moFc was titrated and incubated with QT35 cells infected overnight with MVA-XPR1-GFP or MVA-T7. Binding was detected with anti-Mo-Fc-APC by flow cytometry.



High Versatility of Antigen Virions

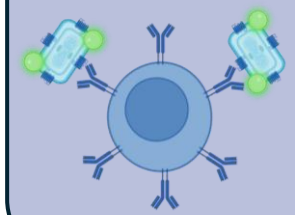
ActivMAb Technology

Antigen virions can be used for your established methods just like purified protein



Phage and yeast display

- No non-specific binding
- Compatible with bead or plate-based screening



Fluorescent sorting

Compatible with ex-vivo B cell sorting



Immunization

- BSL-1 does not replicate in mice/rats
- Potent immunogens, no adjuvant needed



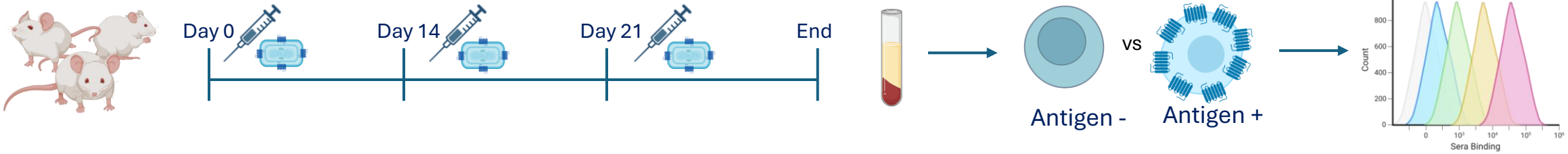
Two antigenically distinct strains

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

Created with Biorender.com

Antigen Virions Can Be Used for Mouse Immunization

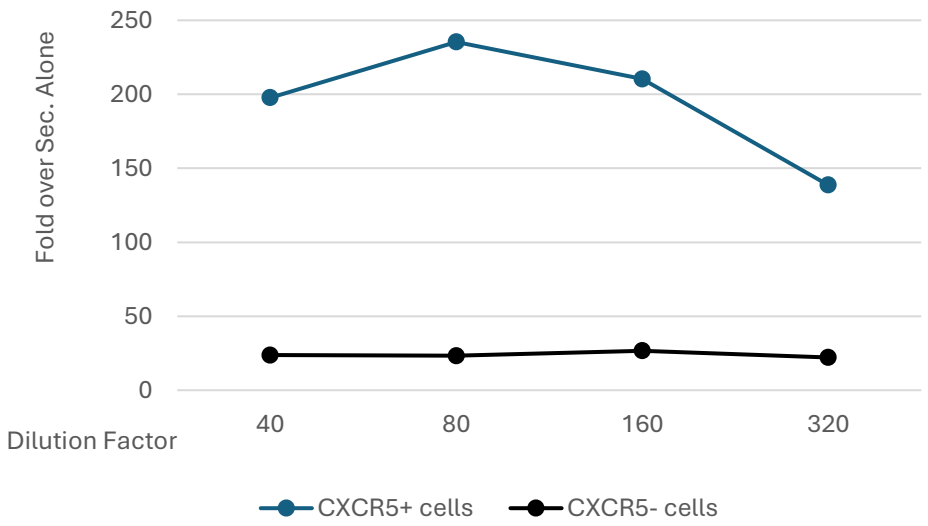
ActivMAb Technology



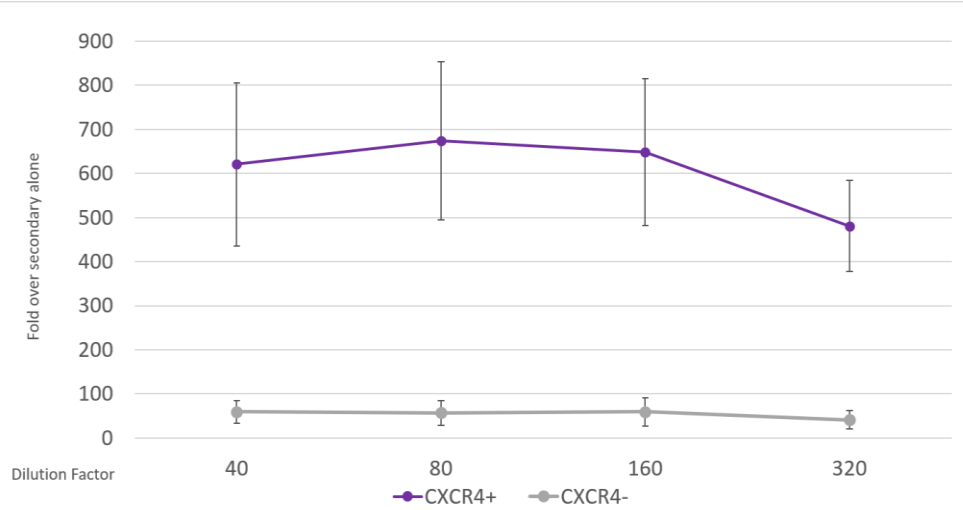
· BSL-1 antigen virions are well tolerated and do not require adjuvant.

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

CXCR5 sera



CXCR4 sera



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In vivo Antibody Discovery

ActivMAb Technology

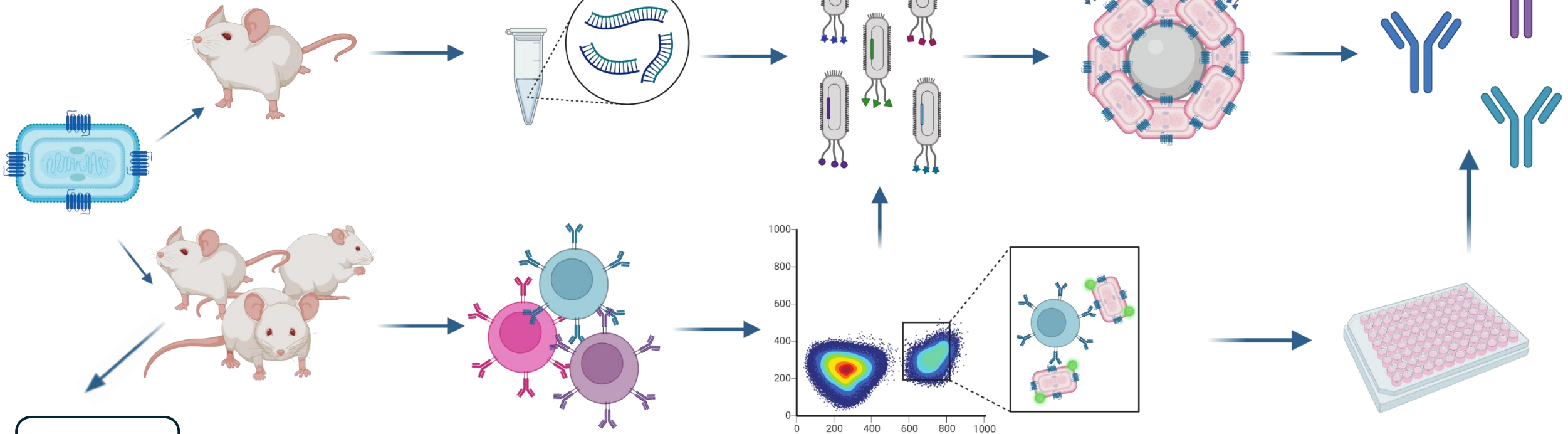
Mouse immunization with antigen virus

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

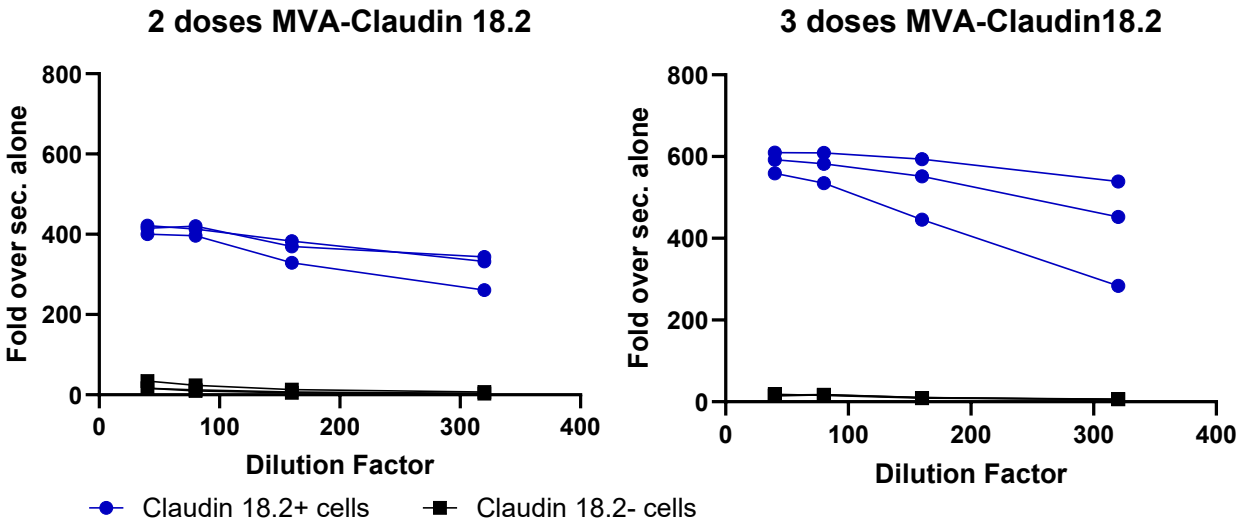
FACS for memory B cells bound to fluorescent labelled Antigen Virus

Single Cell Analysis

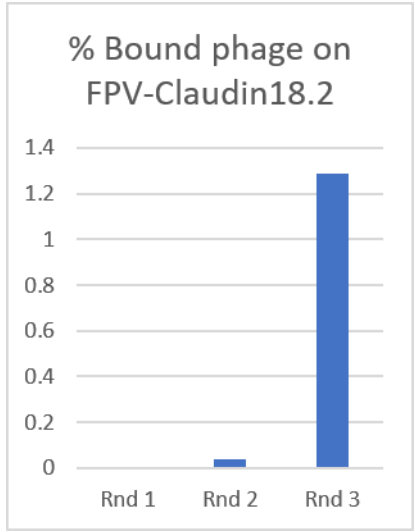
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Case Study: Claudin18.2
Immunized Library Antibody Discovery

ActivMAb Technology

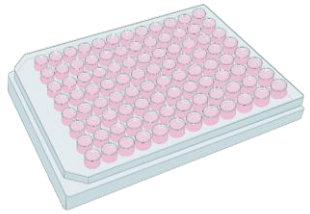


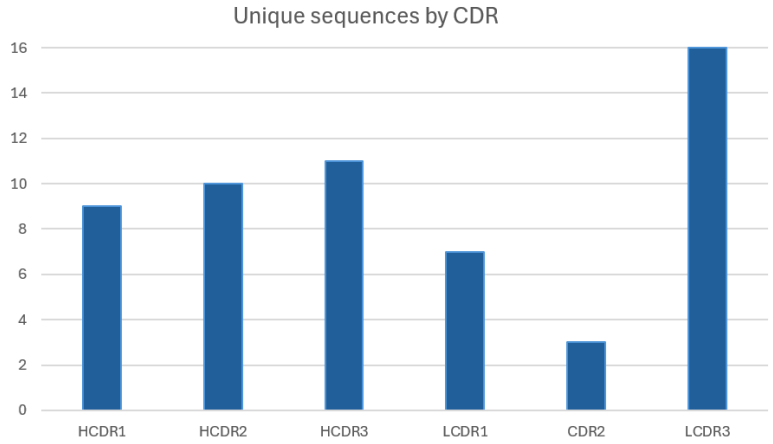
Immune phage library generation



Cloning Transfection

Testing of 94 clones by flow cytometry





53 unique clones

Top 5 humanized for further testing

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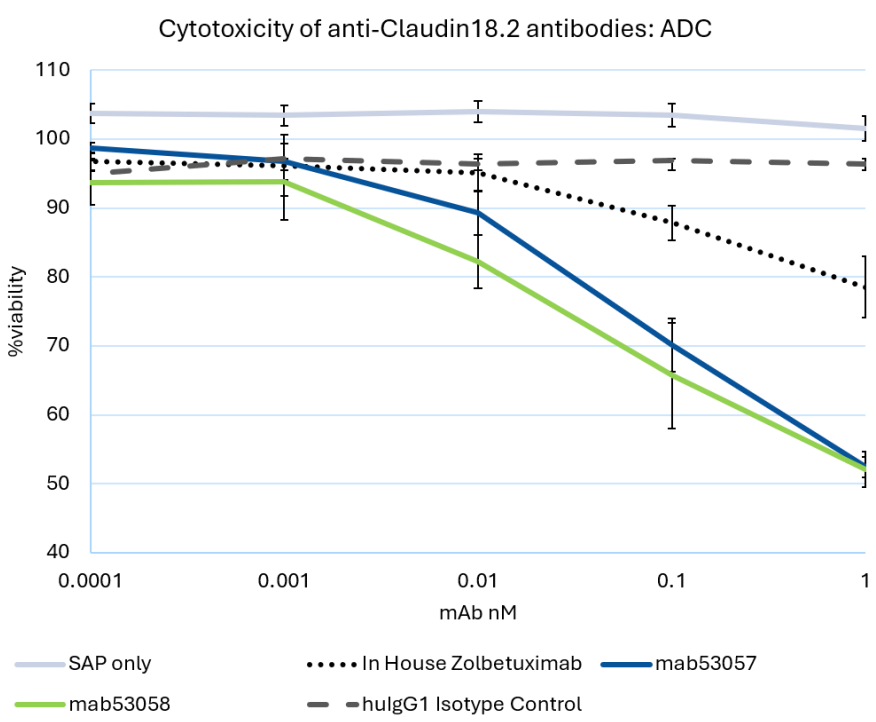
Case Study: Claudin18.2
Functional Testing

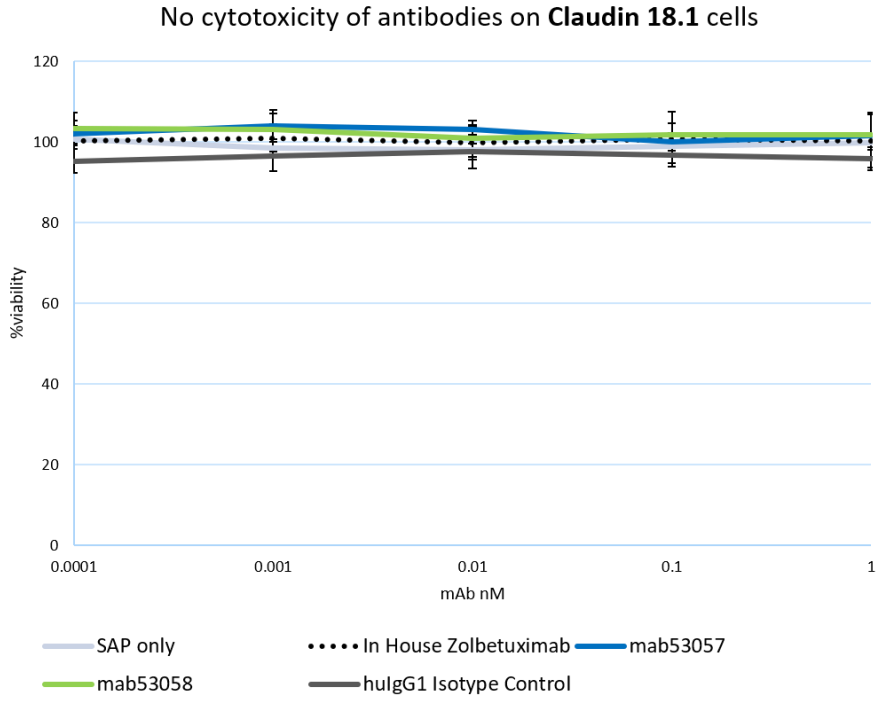
ActivMAb Technology

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

Affinity was determined by a flow cytometry based Scatchard Assay

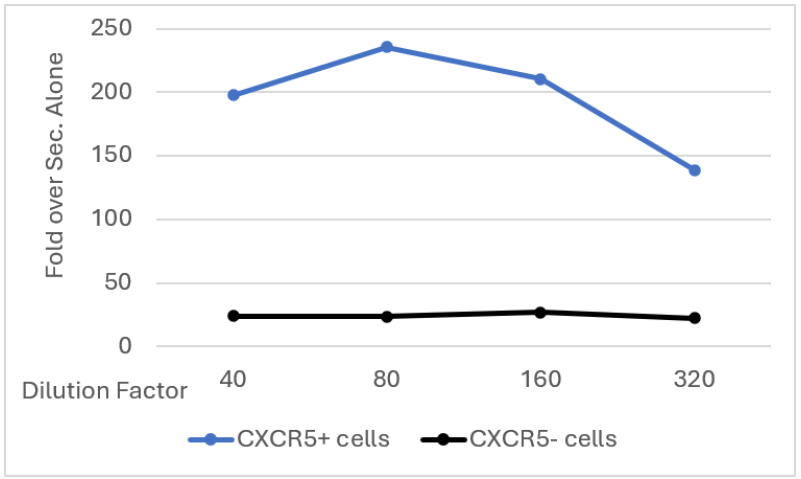
Claudin 18.2 – Antibodies show specific ADC activity





Case Study: CXCR5
Immunized Library Antibody Discovery

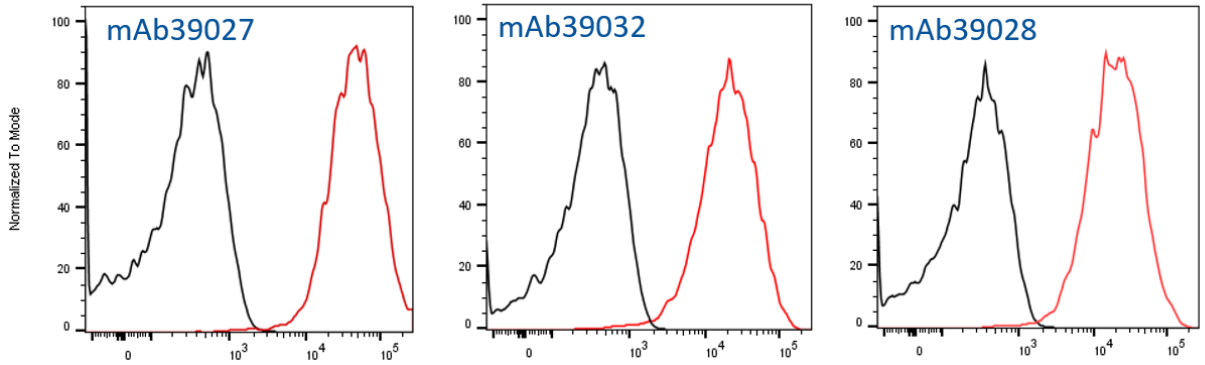
ActivMAb Technology

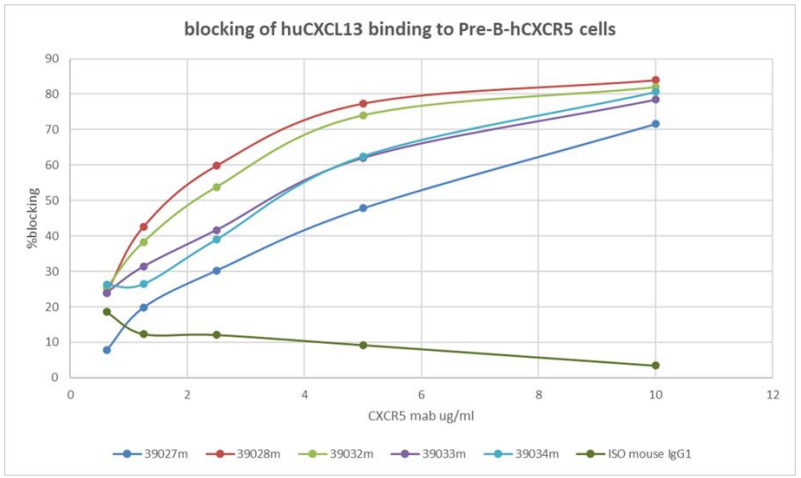


Phage library generation
&
panning

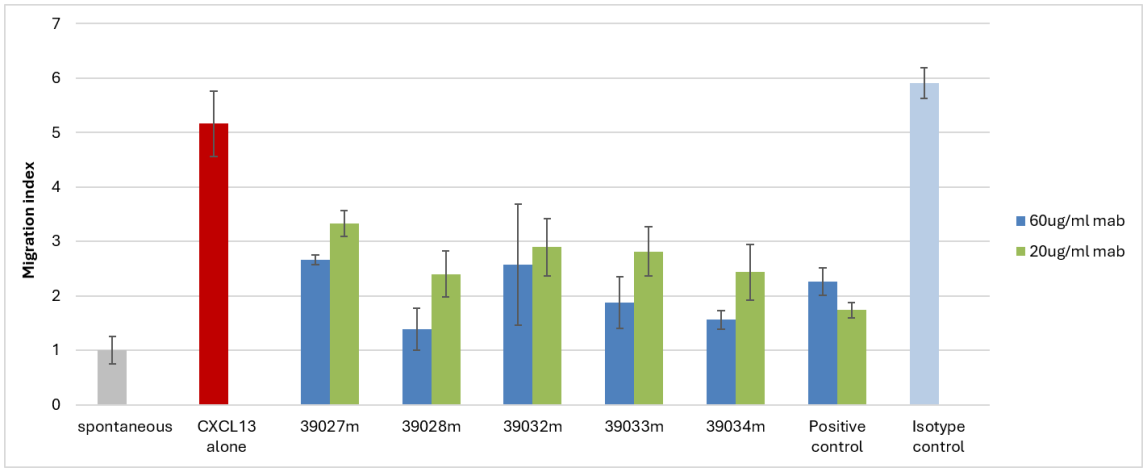
>150 unique mabs

18 unique H-CDR3s





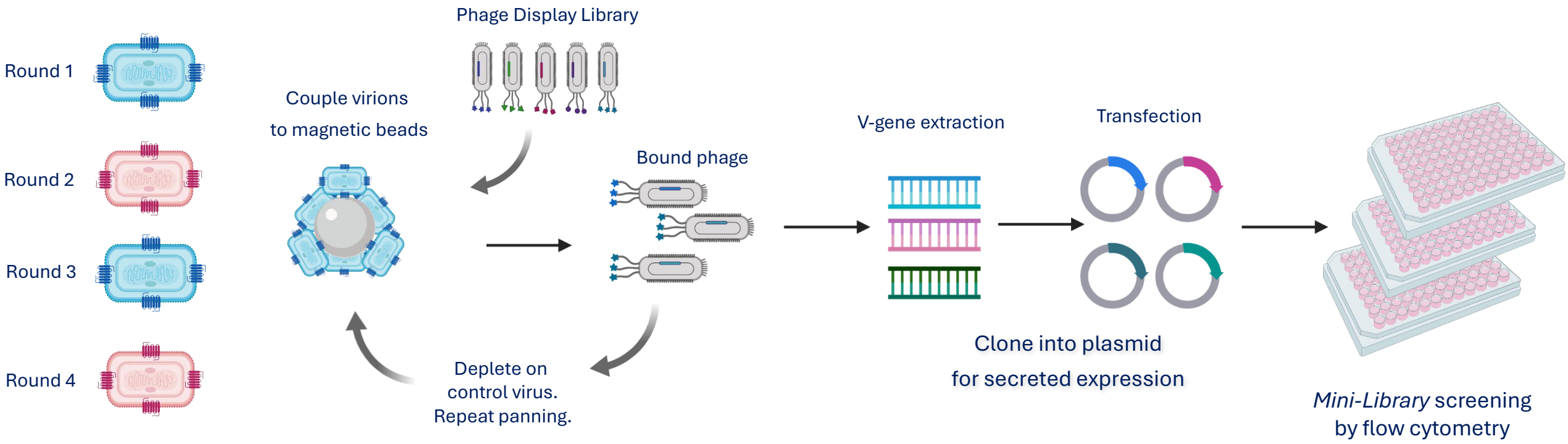
MAb	nM Affinity
39027m	0.04
39028m	0.56
39032m	0.53
39033m	0.34
39034m	0.35



In vitro Antibody Discovery

ActivMAb Technology

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



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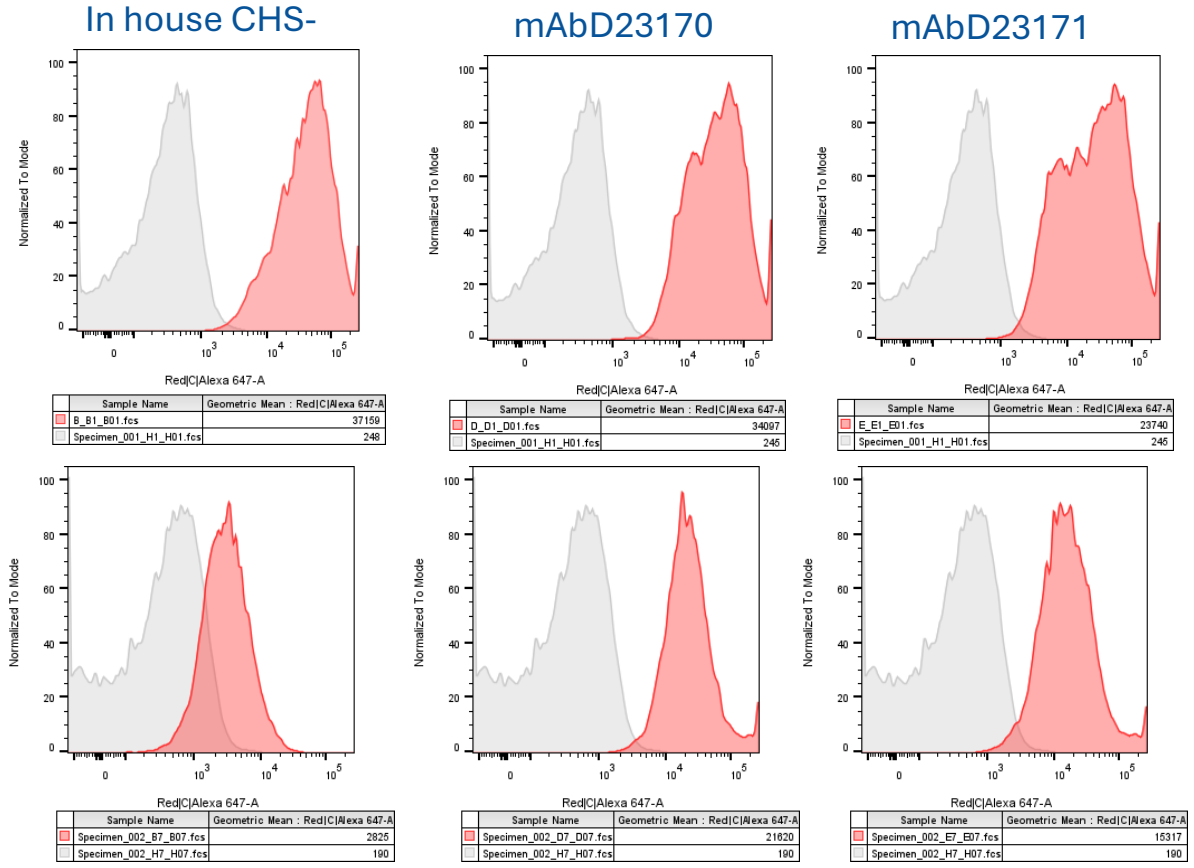
Case Study: CCR8 *In vitro* Antibody Discovery

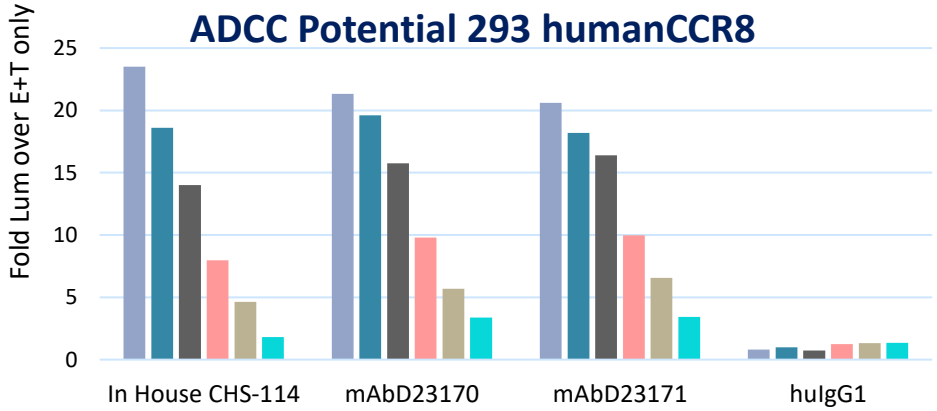
ActivMAb Technology

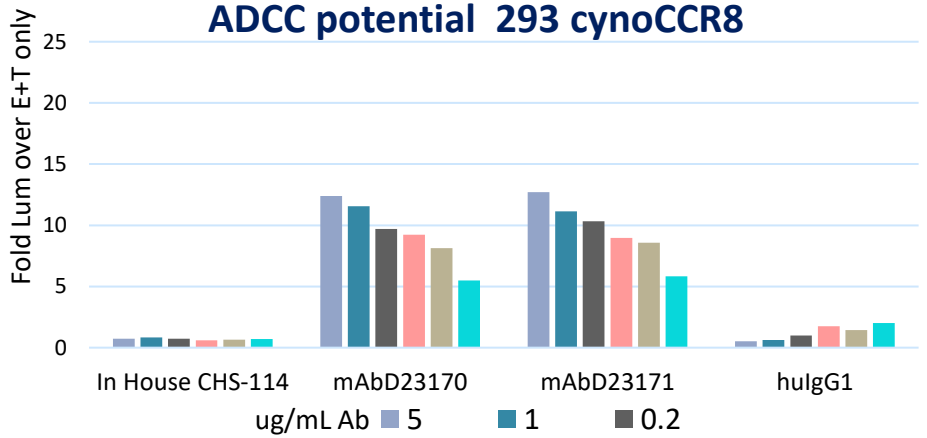
Utilizing CCR8 antigen virions, 4 rounds of phage panning were done using a naïve human Fab library. Individual antibodies were screened for binding to native CCR8 expressing cells. Top binders were purified and tested for ADCC potential using strep-ZAP.

293-HumanCCR8 cells

293-CynoCCR8 cells



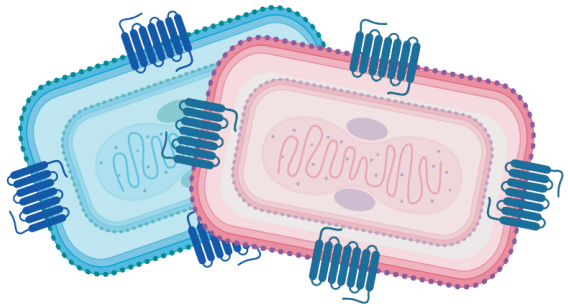




ActivMAb® Technology

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

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

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Discovery Options

Science EXCHANGE APPROVED VENDOR

REGISTERED scientist.com SUPPLIER

Option 1: Catalog 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

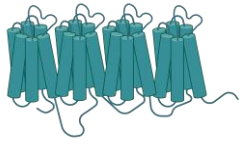




P2X2*
P2X7*



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

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

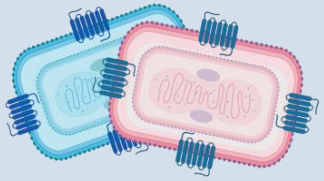
Our team has over 20 years of antibody discovery expertise. Let us do the work for you!

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ActivMAb® Discovery Solutions

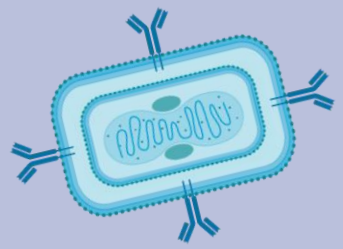
Poxvirus based antibody and protein engineering solutions

ActivMAb Technology



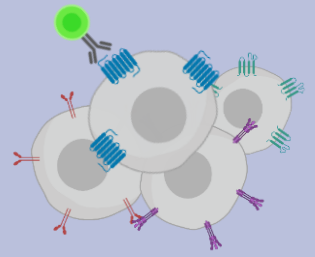
Antigen Virus

Membrane protein expression platform



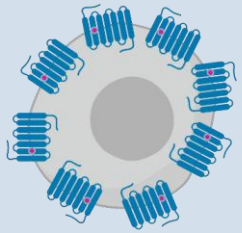
Antibody Library

Mammalian human IgG display library



Target Identification

FACS sorting of human cDNA library



Protein Optimization

FACS sorting of mutant libraries for higher expression isoforms

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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



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Science EXCHANGE APPROVED VENDOR

REGISTERED scientist.com SUPPLIER