

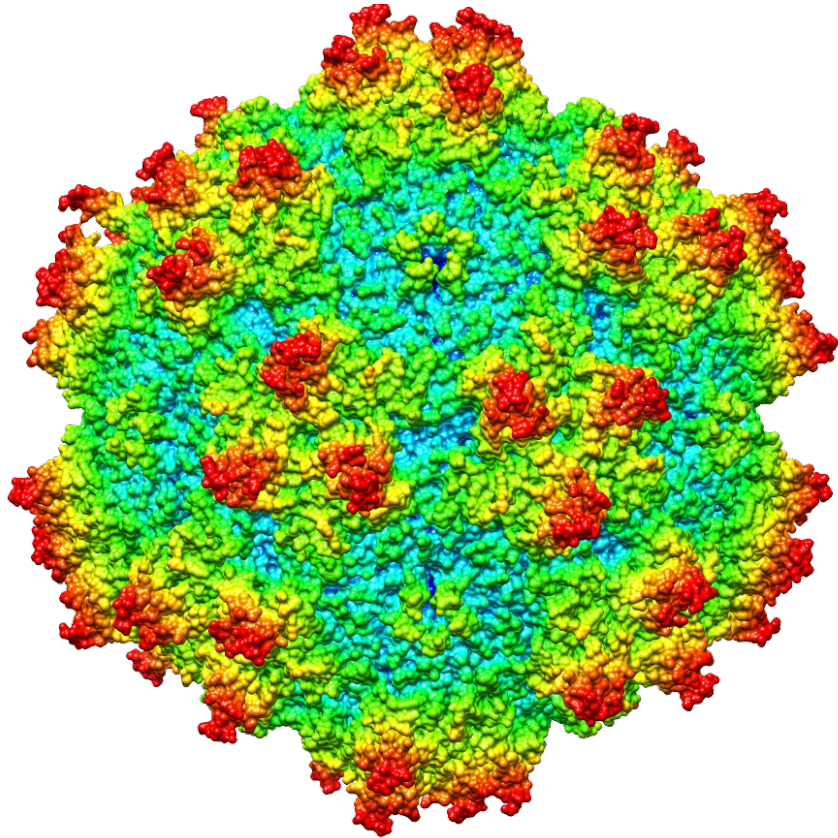
Discovery and Characterization of Novel Cross-Species BBB-Penetrant Capsids

Brett Hoffman, Tatiana Knox, Tyler Moyer, Ishan Shah, Shanan Emmanuel,
Mathieu Nonnenmacher

Disclosures

- Full-time employee at Voyager Therapeutics

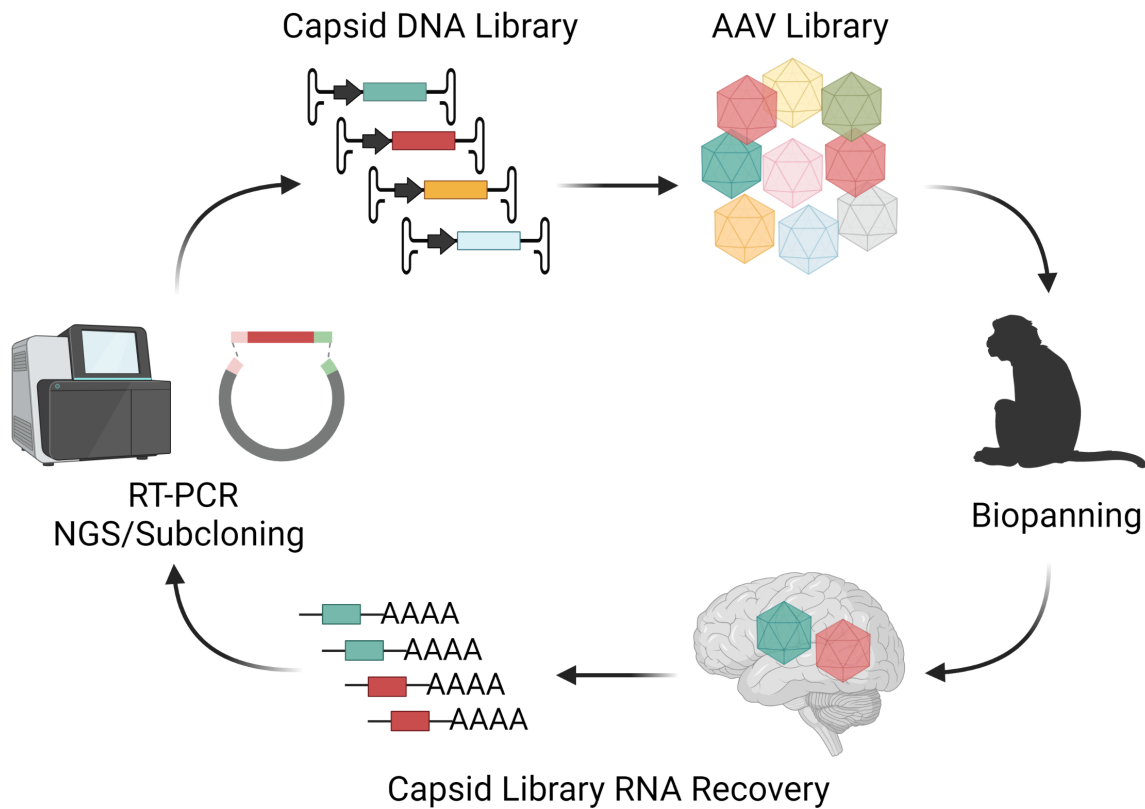
Delivery of Gene Therapies by Adeno-associated Virus (AAV)



- First AAV-mediated gene therapy approved in 2012
 - Glybera[®] (AAV1) - lipoprotein lipase deficiency
 - Luxturna[®] (AAV2) - Leber congenital amaurosis
 - Zolgensma[®] (AAV9) - spinal muscular atrophy
 - Hemgenix[®] (AAV5) - hemophilia B
- Entry of systemically delivered AAV to the CNS is largely impeded by the blood–brain barrier (BBB).
- AAV9 is the most efficient natural serotype at crossing the BBB and transducing the CNS
- Requires a high viral load to achieve limited CNS transduction

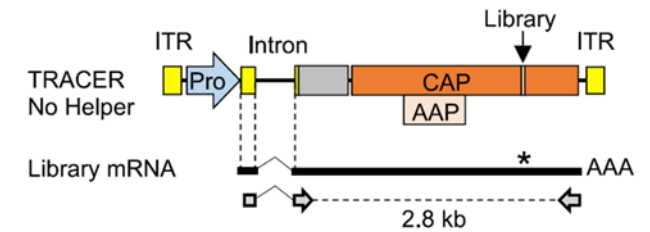
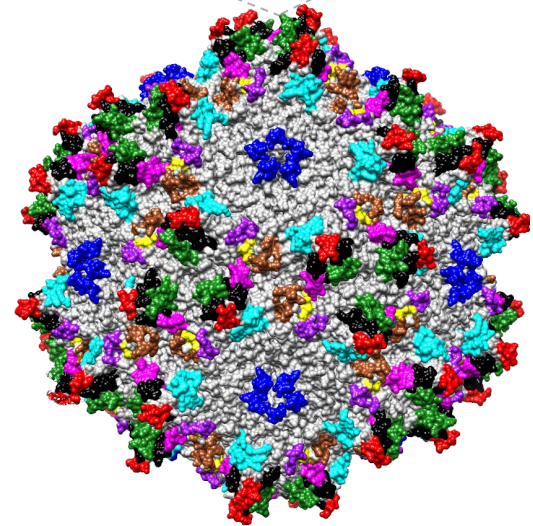
Directed Evolution of AAV for CNS Delivery - TRACER™

TRACER™ - Tropism Redirection of AAV by Cell-type-specific Expression of RNA



...GCCCAANNKNNKNNKNNKNNKNNKNNKNNKGCACAGGCG...
 ...A Q X X X X X X X A Q A

**VR-VIII
7-mer Insert**

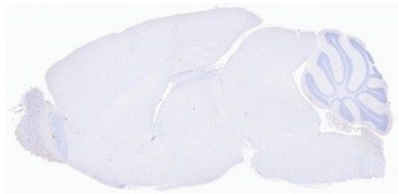


Directed Evolution of AAV for CNS Delivery - TRACER™

TRACER™ - Tropism Redirection of AAV by Cell-type-specific Expression of RNA



AAV9



9P31

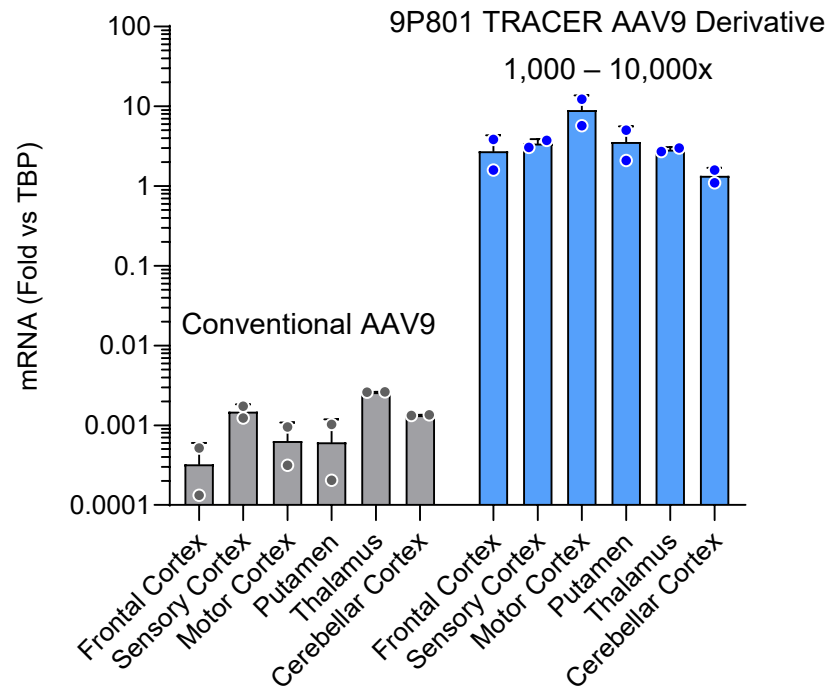


385-fold Increased EGFP Expression vs AAV9

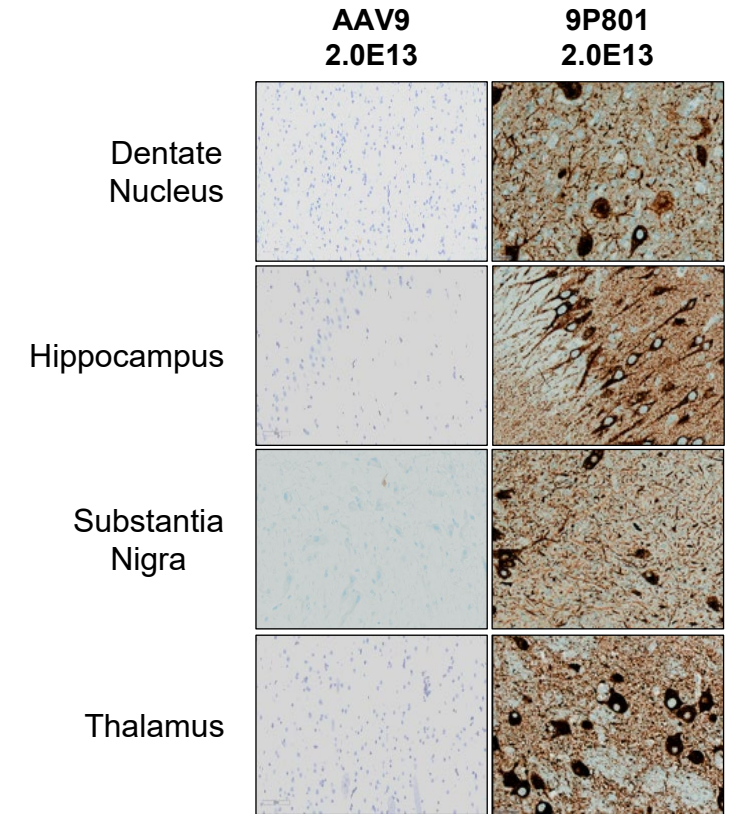
IV dosing, 4e11 VG, scAAV, anti-GFP staining



9P801 CNS Transduction in Adult Macaques

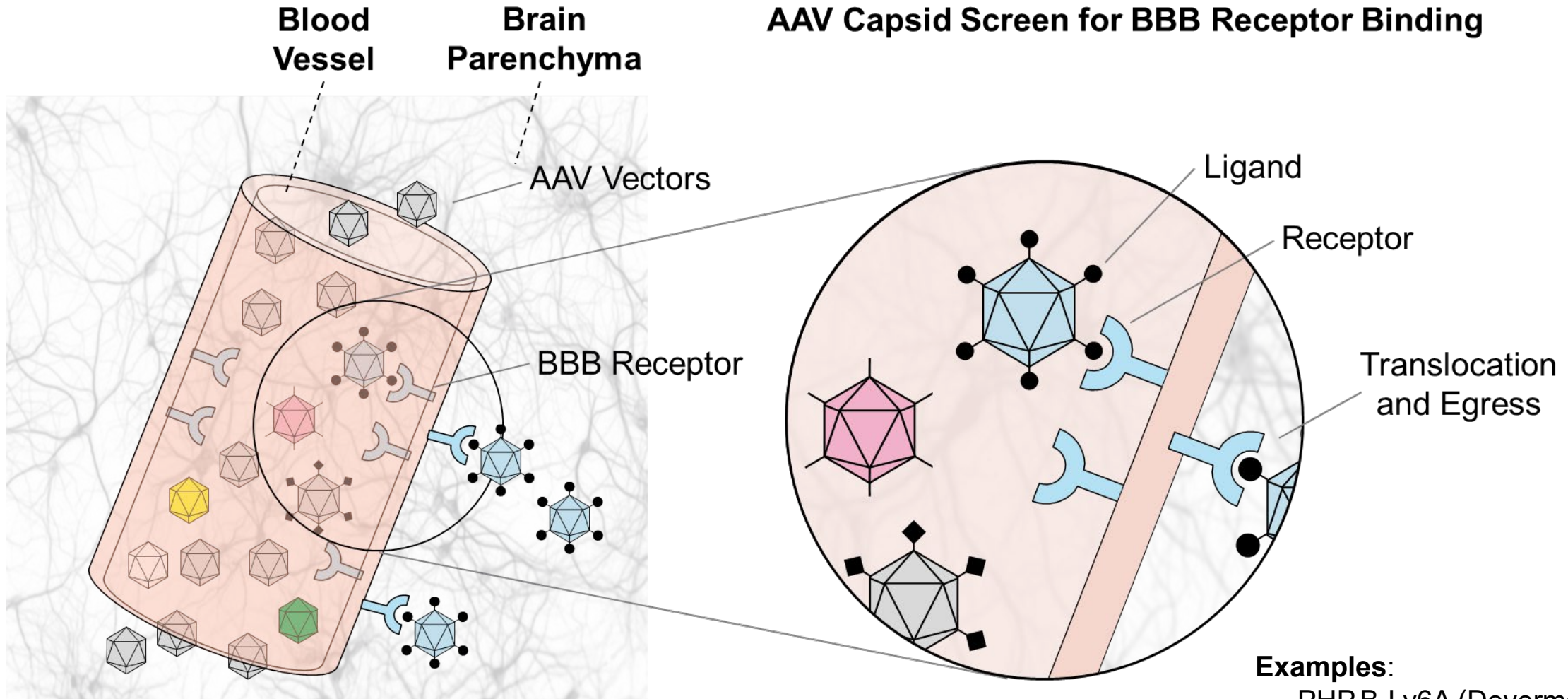


IV dosing, 2e13 VG/kg, ssAAV



IHC detection of transgene protein

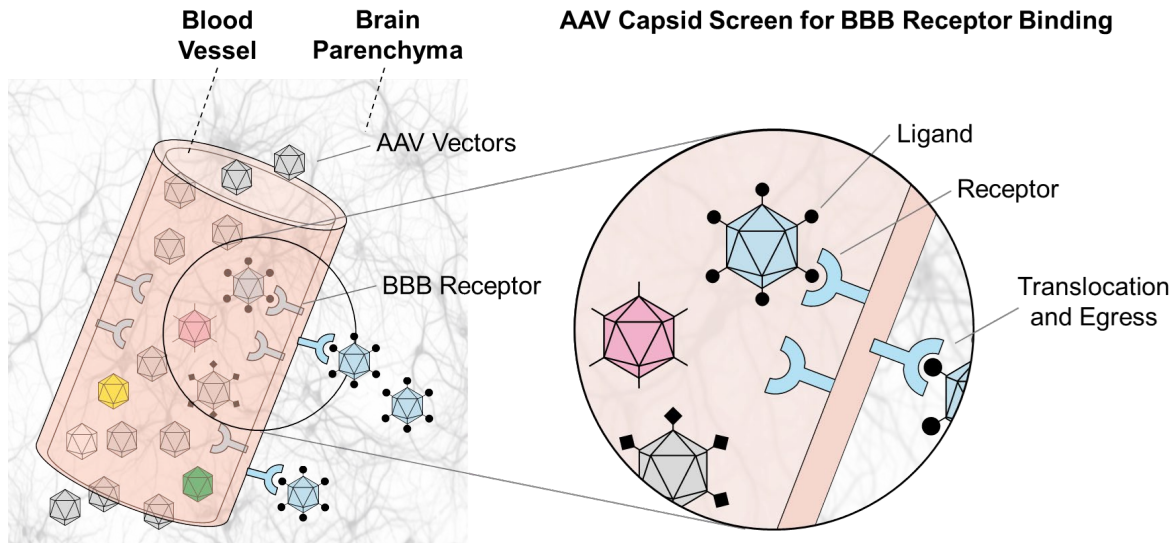
Receptor/Ligand Paradigm in BBB-penetrant Capsid Engineering



Examples:

- PHP.B-Ly6A (Deverman, Wilson)
- 9P39-Ly6C1 (Deverman)
- 9P31-CA4 (Gradinaru)

Importance of Novel Capsid Receptor Identification

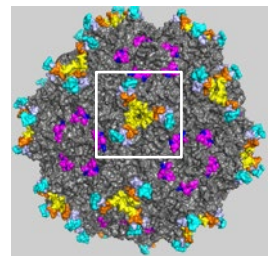


- Receptor identification will provide confidence in the transferability of novel capsids to humans
- Facilitate prediction of capsid behavior in humans based on receptor expression patterns
- A mechanistic understanding of how novel capsids outperform WT AAV9 in crossing the BBB
- Provide learnings which can be applied to future CNS targeting efforts

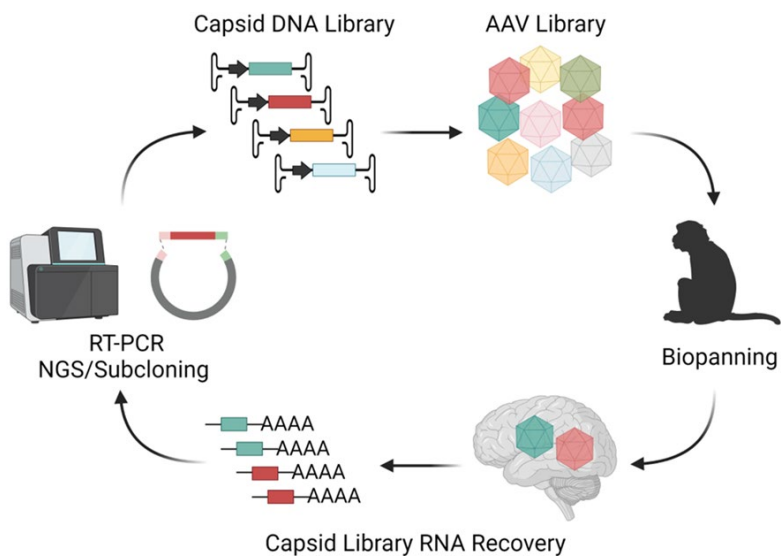
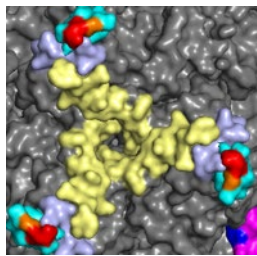
AAV9 VR-IV TRACER™ Screen

AAV Capsid

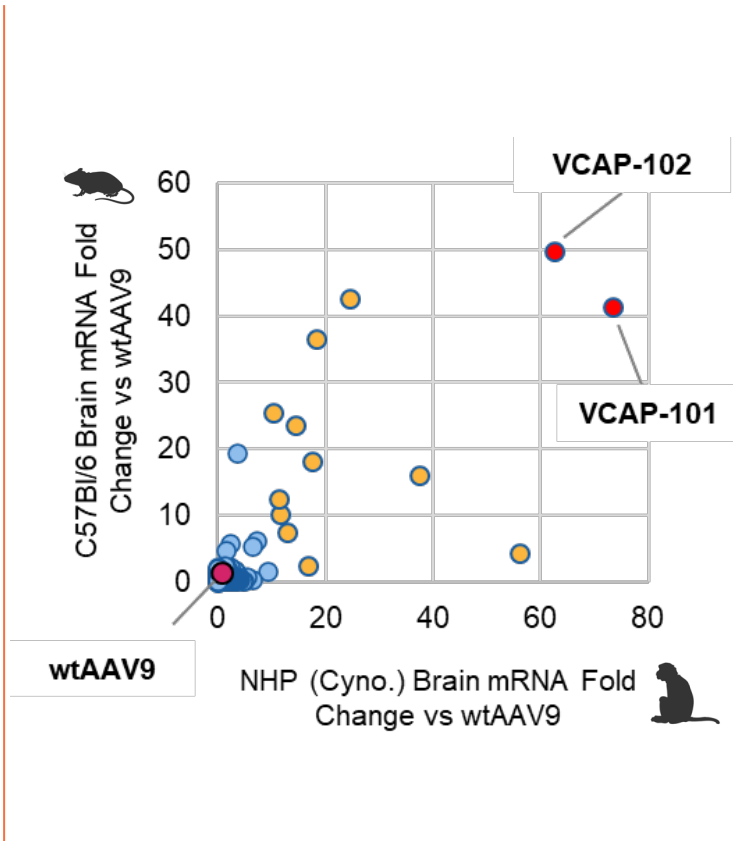
Library



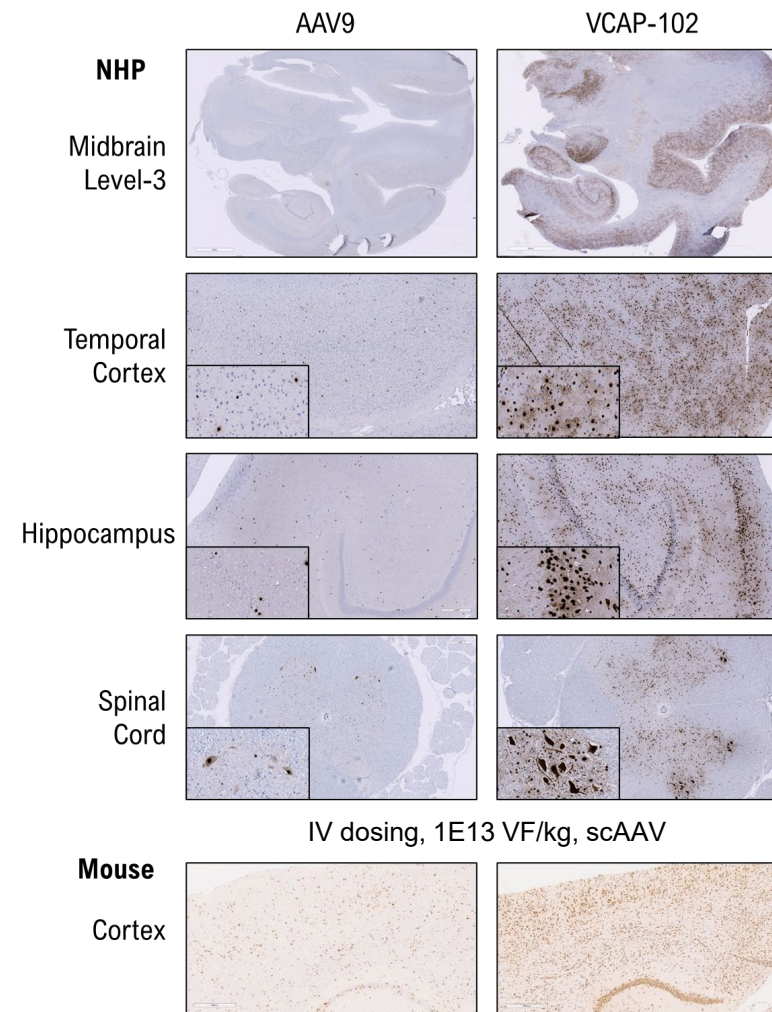
VR4 ins



NHP/Mouse Translation



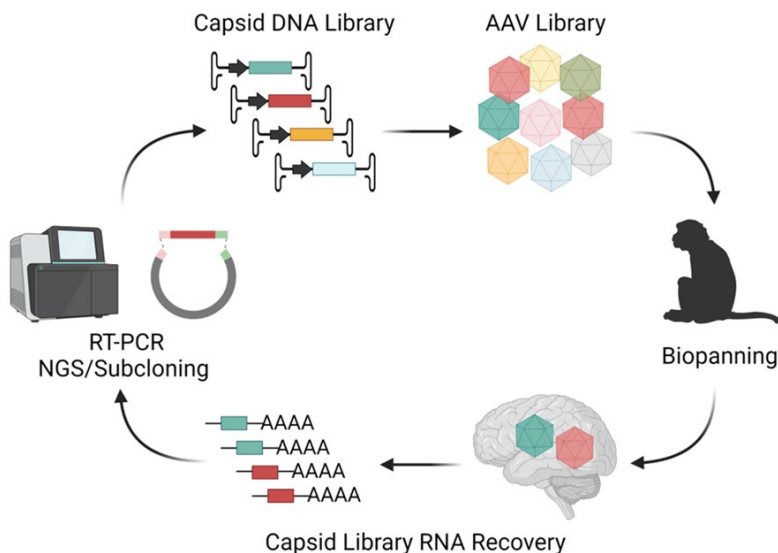
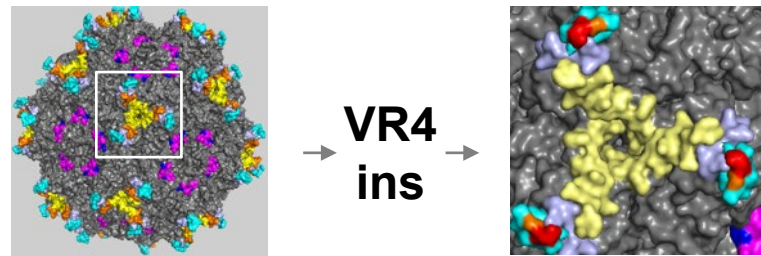
Brain Tropism



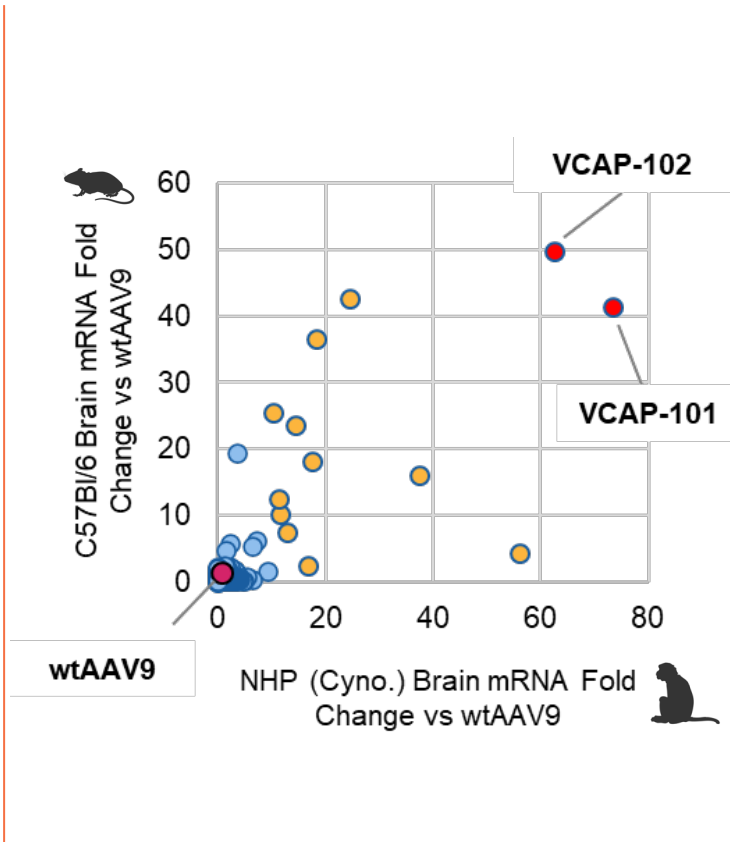
AAV9 VR-IV TRACER™ Screen

AAV Capsid

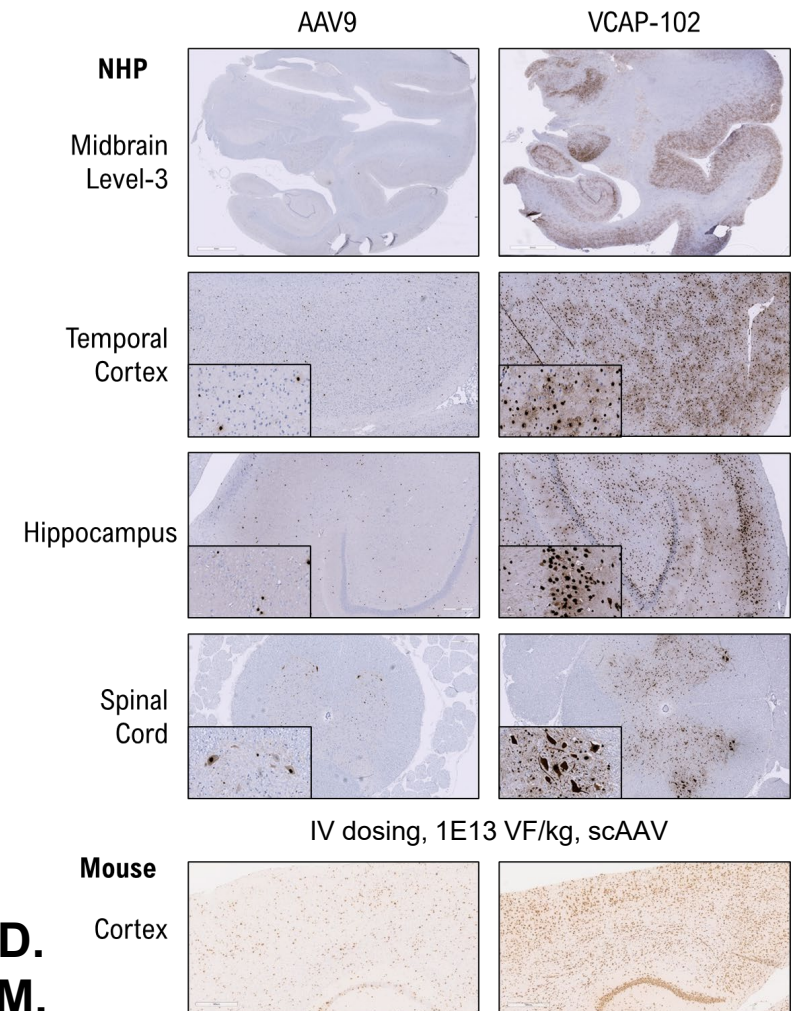
Library



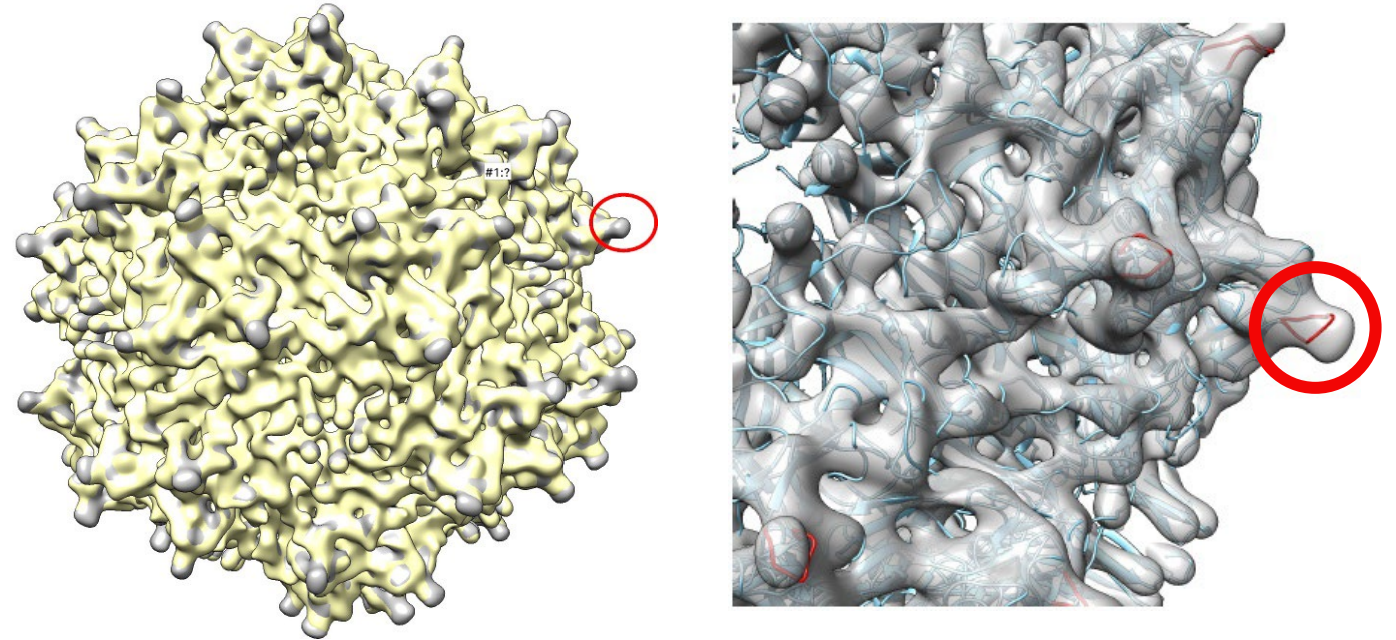
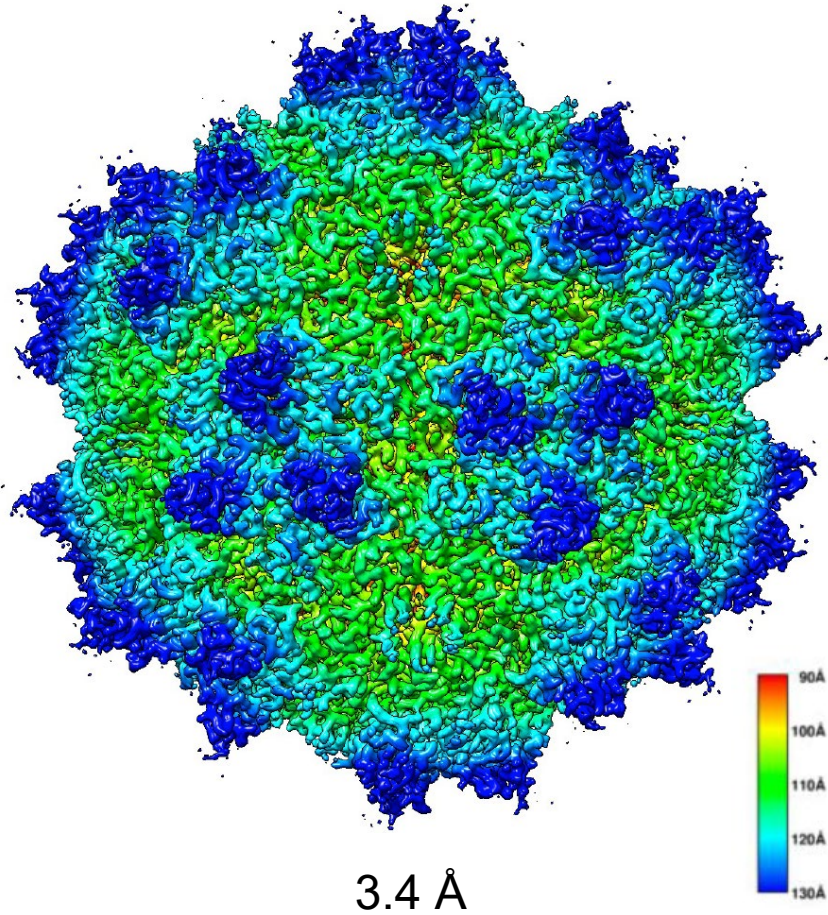
NHP/Mouse Translation



Brain Tropism



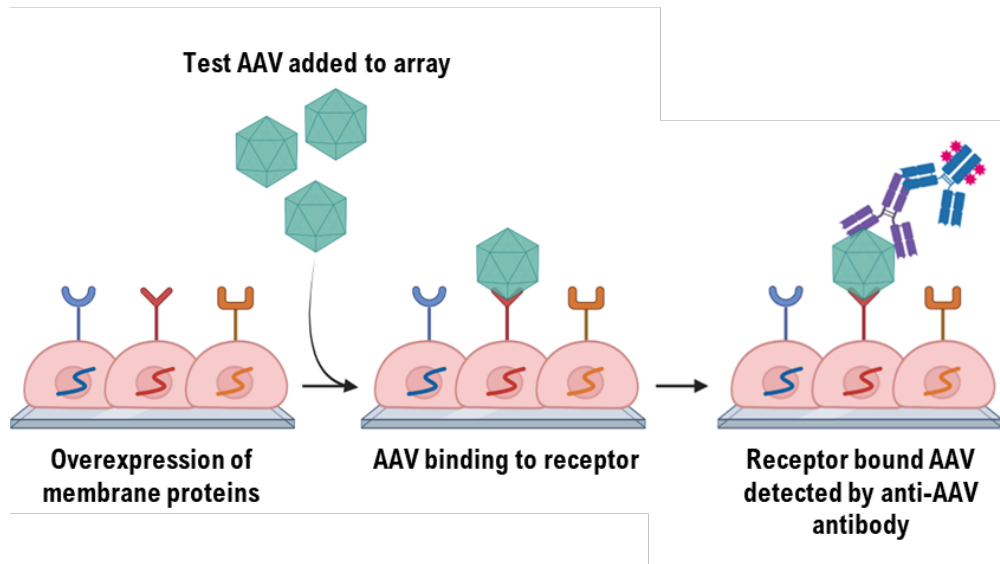
Cryo-EM structure of VCAP-102



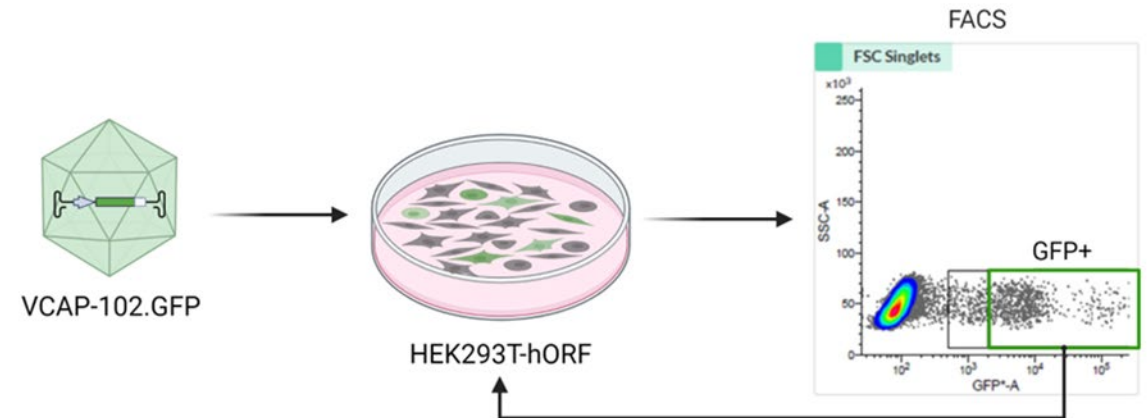
- Overlay of VCAP-102 map (grey) and AAV9 map (yellow)
- A difference within a surface loop is highlighted
- This region contains the VCAP-102 peptide insert
- This region is disordered at higher resolution indicating flexibility

Receptor Identification – Orthogonal Approaches

Receptor Binding Array



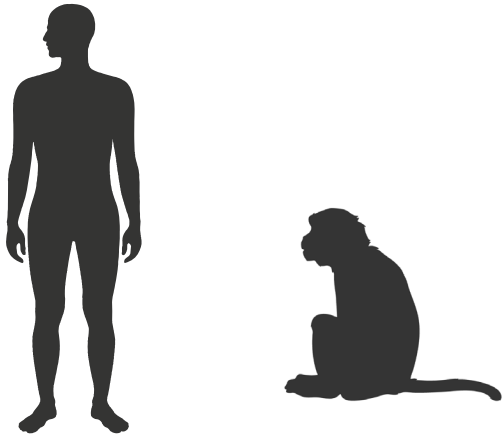
Human ORFeome Transduction Screen



- Both approaches converged on a surface membrane protein expressed on the blood brain barrier

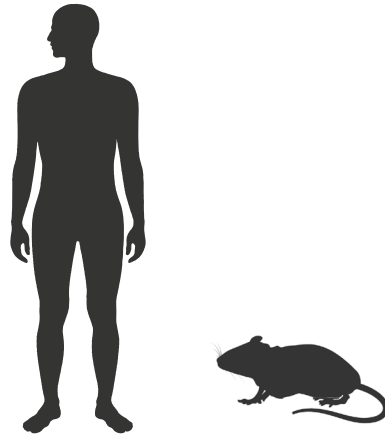
Receptor X is Highly Conserved - Protein Sequence Alignment

Cell Binding Array Identified an Interaction Between “Receptor X” and VCAP-102



Human / Cyno. Macaque

Identity	>95%
Similarity	>95%



Human / Mouse

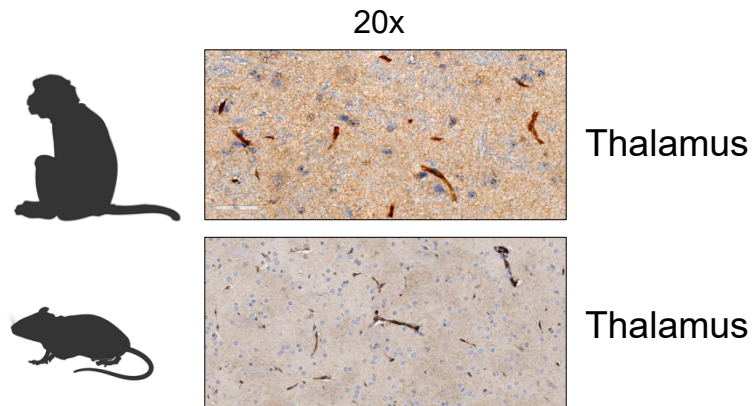
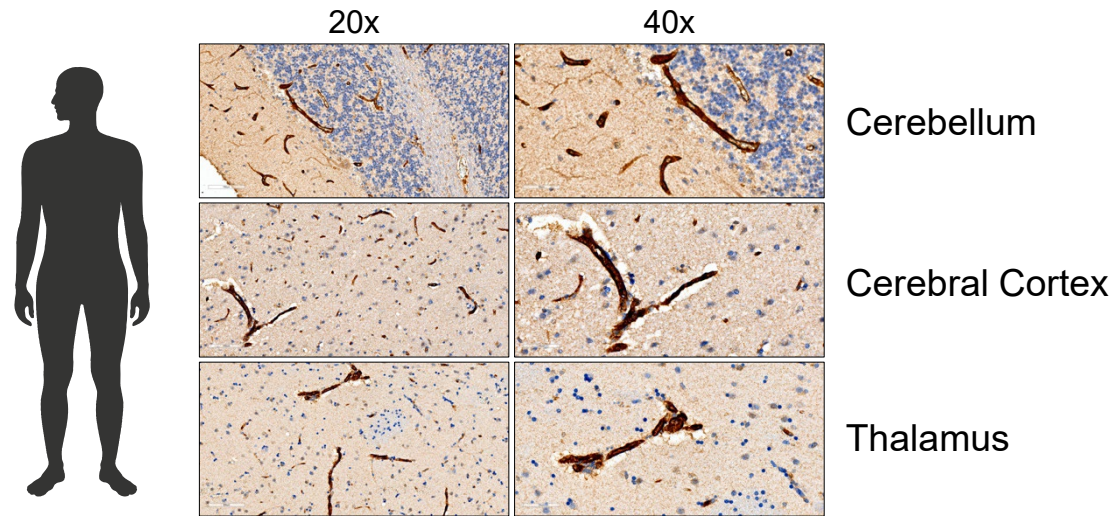
Identity	~90%
Similarity	>90%



Cyno. Macaque / Mouse

Identity	>90%
Similarity	>95%

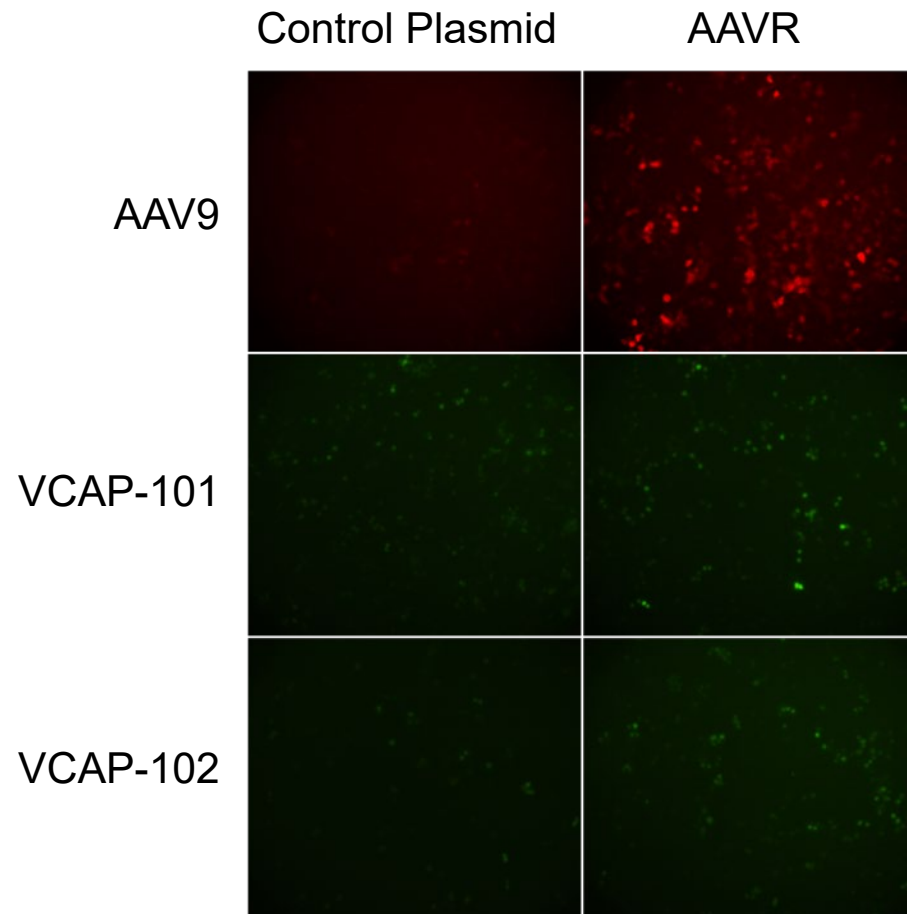
Receptor X is Highly Conserved and Expressed on Brain Endothelial Cells



Receptor X
mRNA
ISH Staining

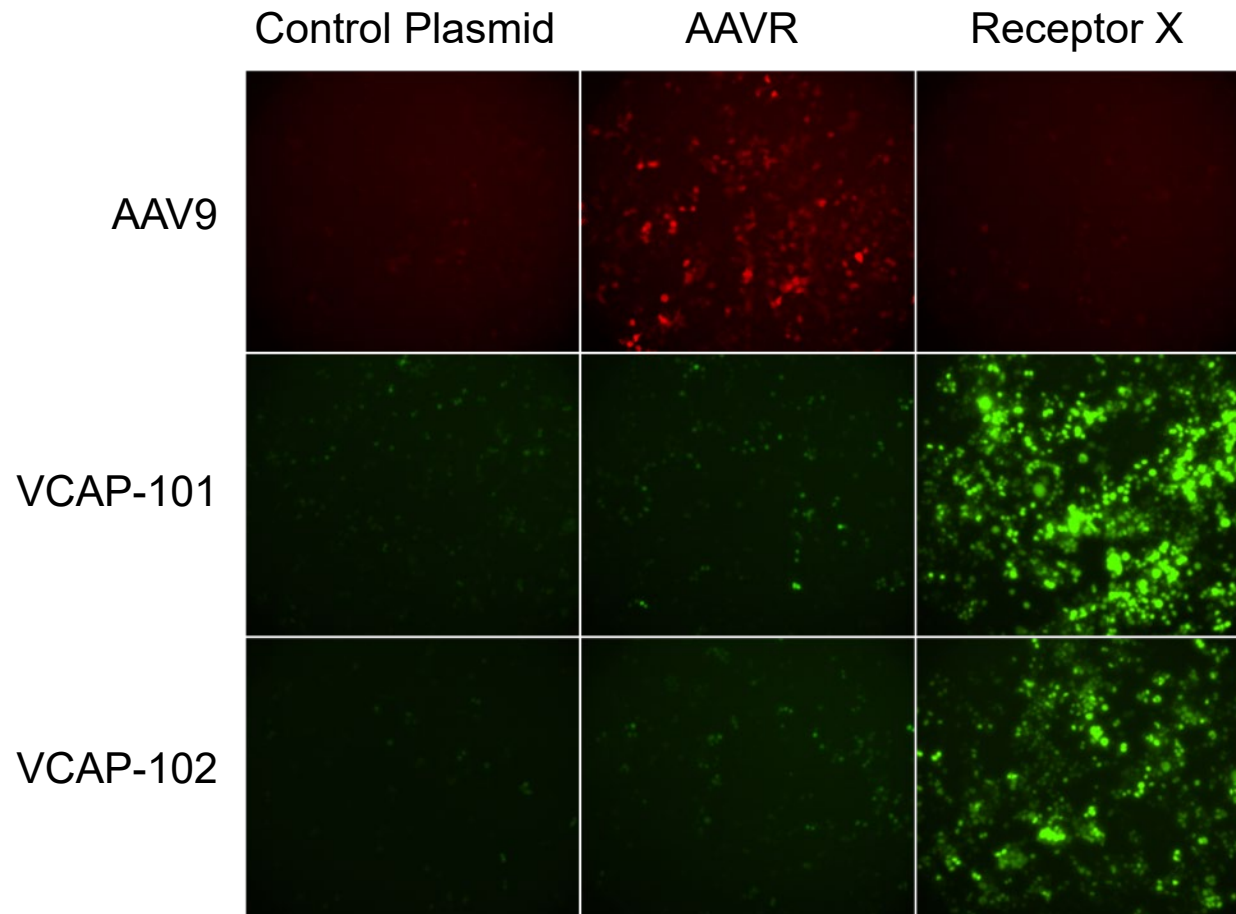
VCAP-101/102 Transduction Assay – Human Receptor X

Effect of Receptor X Expression is Specific to VCAP-101/102 Capsid Family

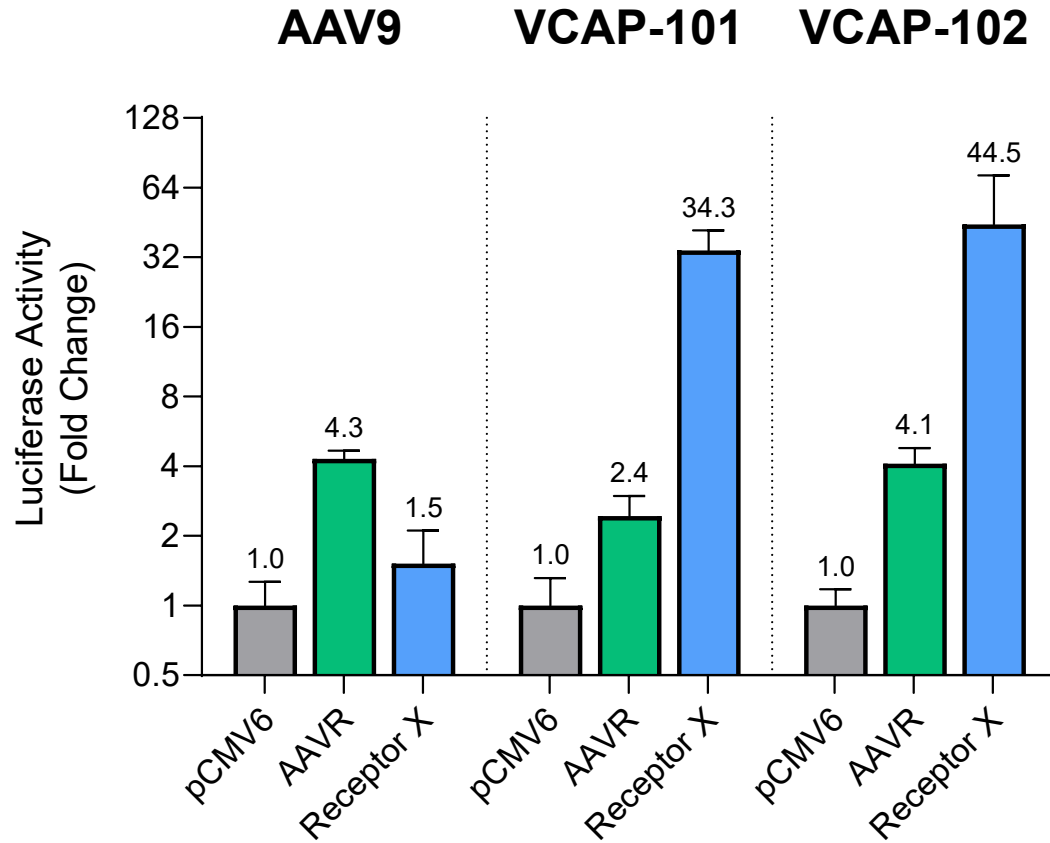


VCAP-101/102 Transduction Assay – Human Receptor X

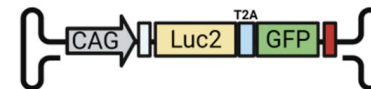
Effect of Receptor X Expression is Specific to VCAP-101/102 Capsid Family



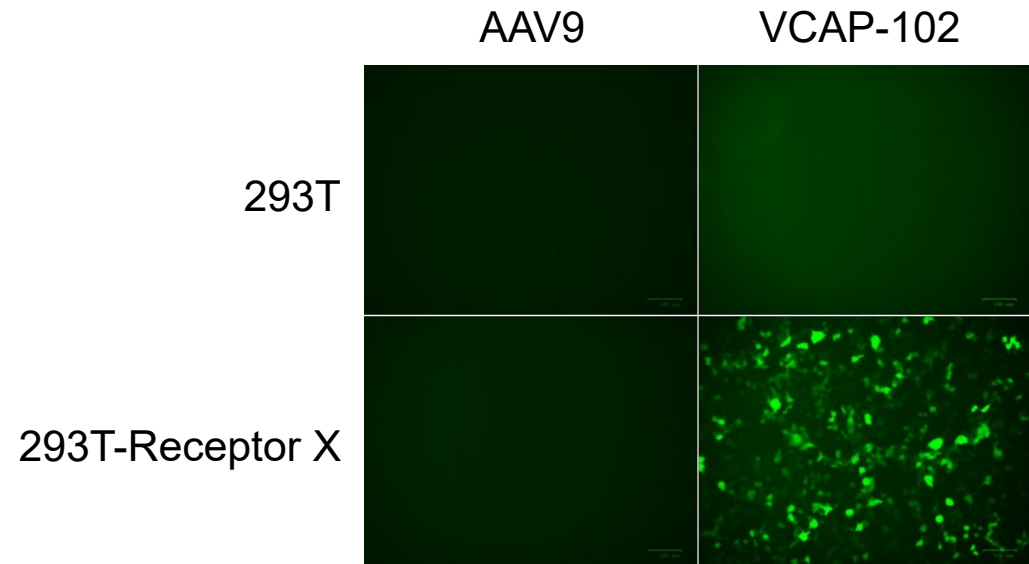
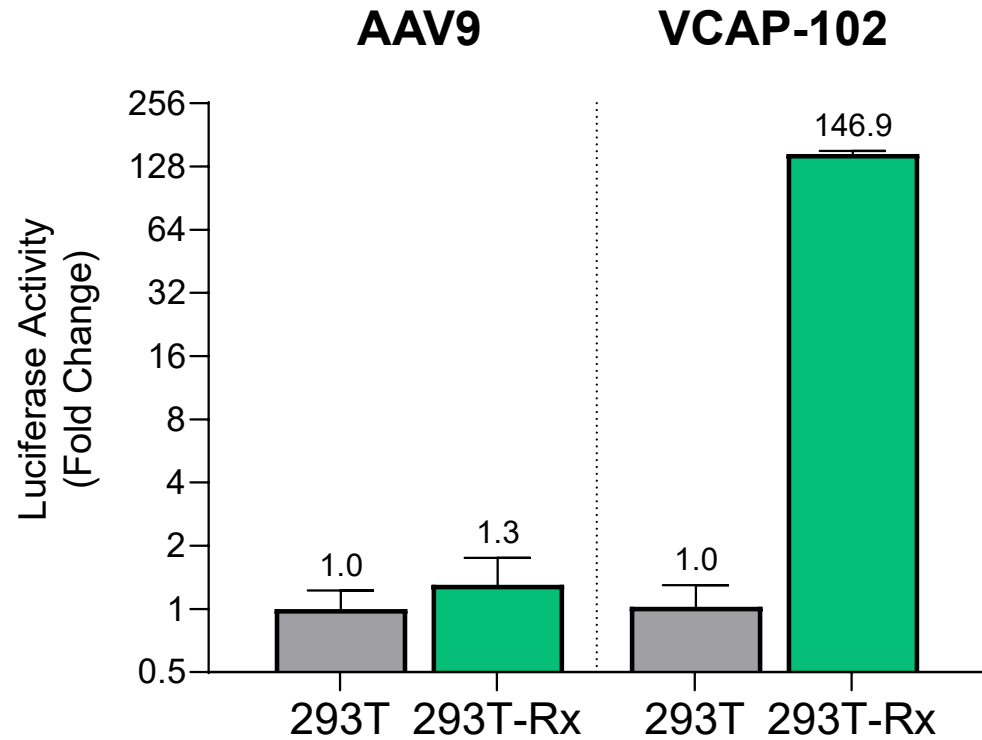
VCAP-101/102 Transduction Assay – Human Receptor X



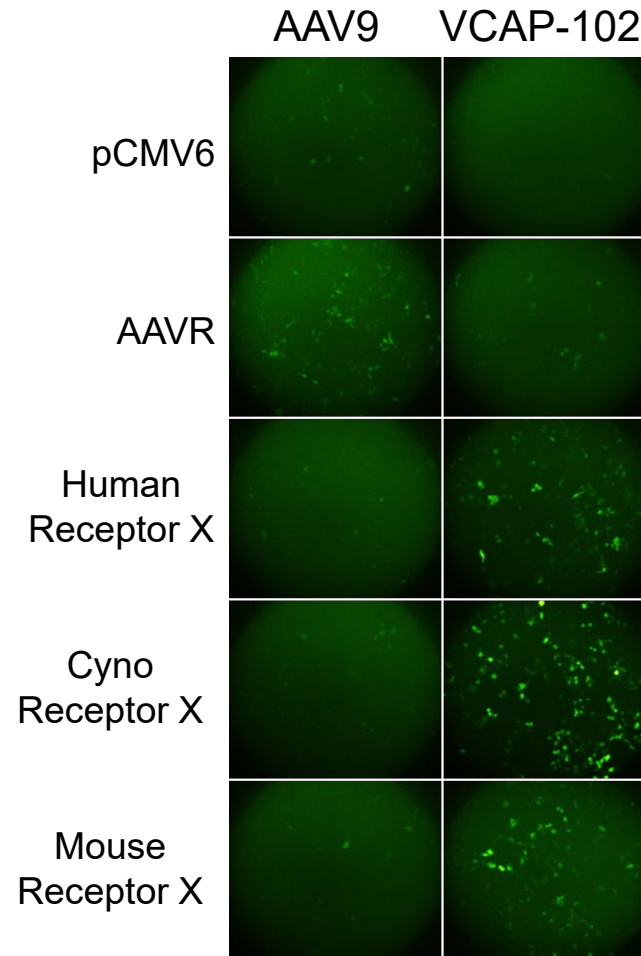
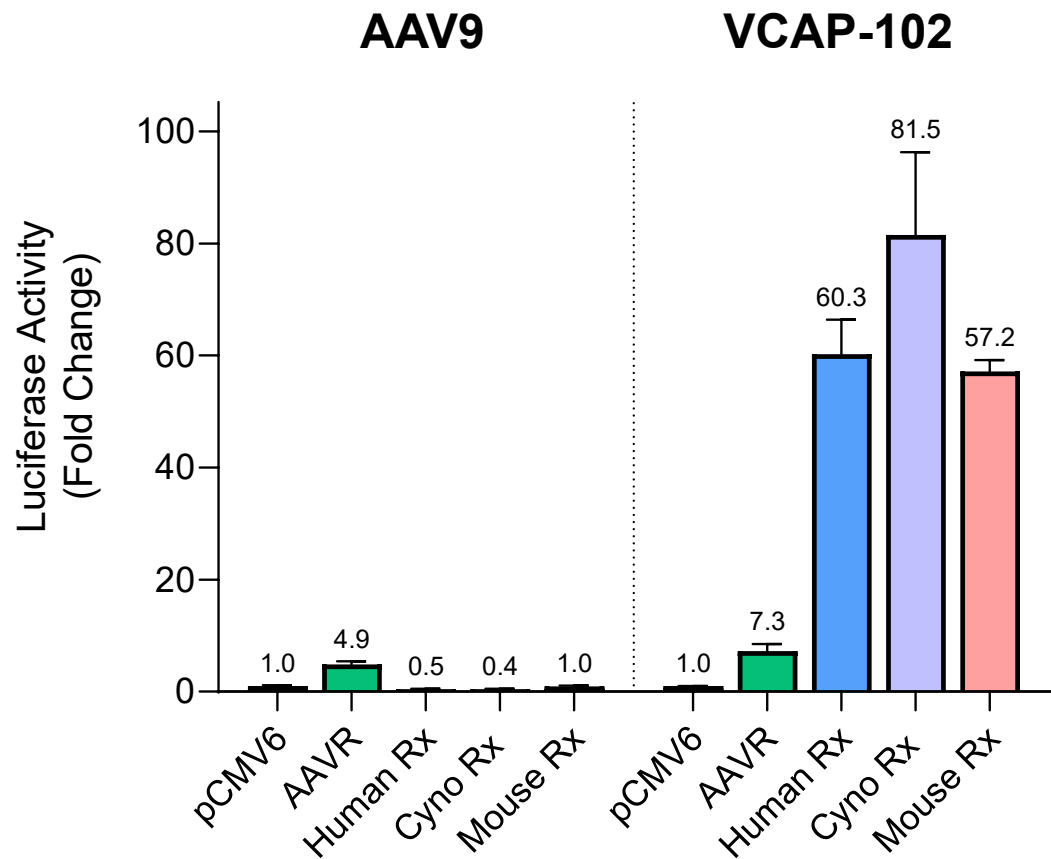
- Receptor X leads to a 35 and 45-fold increase in transduction by VCAP-101 and VCAP-102, respectively
- This effect is specific to this novel capsid family as Wt AAV9 transduction is unaffected by receptor X expression



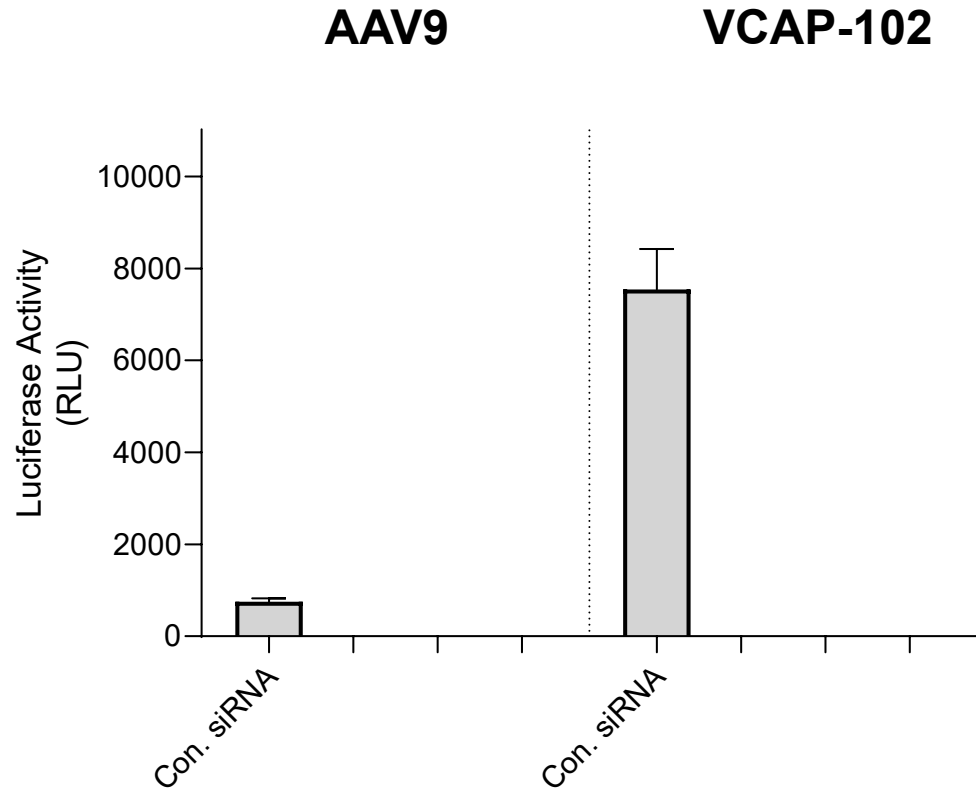
Transduction Assay – Human Receptor X Stable Cell Line



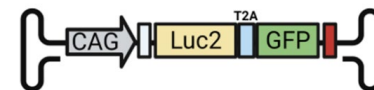
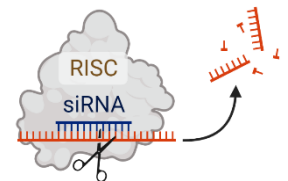
Transduction Assay – NHP and Mouse Receptor X are Functionally Equivalent



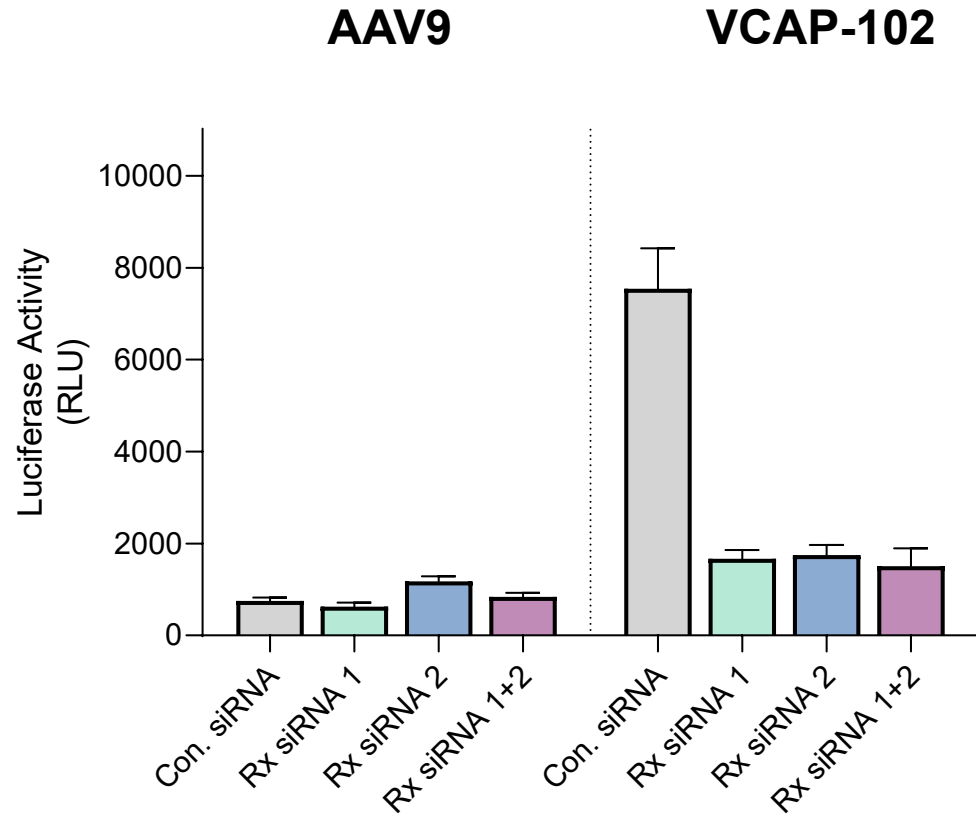
Knockdown of Endogenous Receptor X Expression Inhibits VCAP-102 Transduction



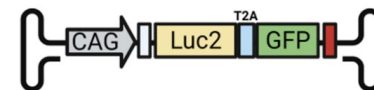
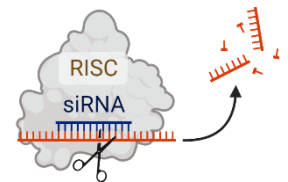
- VCAP-102 transduction of an alternative cell line is up to 10-fold higher than wt AAV9
- ~5-fold elevated levels of Receptor X expression compared to HEK293T cell line
- What effect on VCAP-102 transduction does siRNA mediated knockdown of Receptor X expression have?



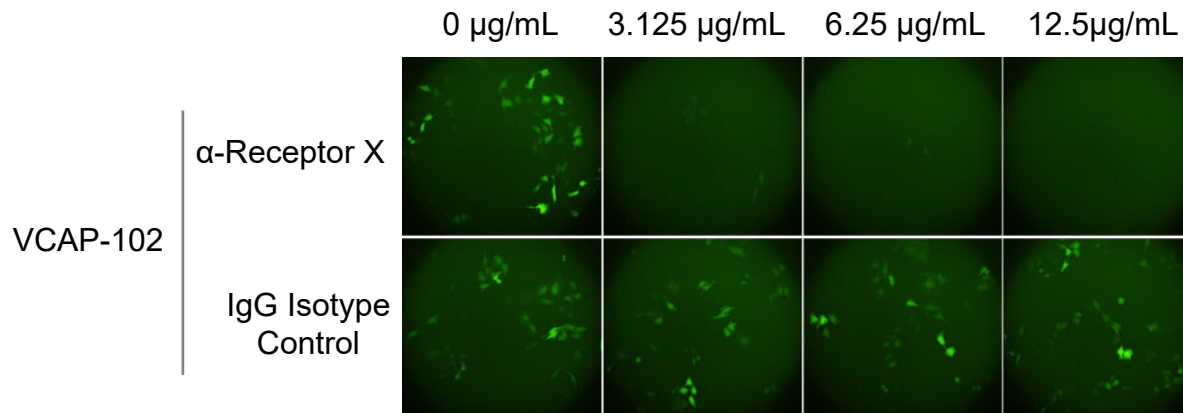
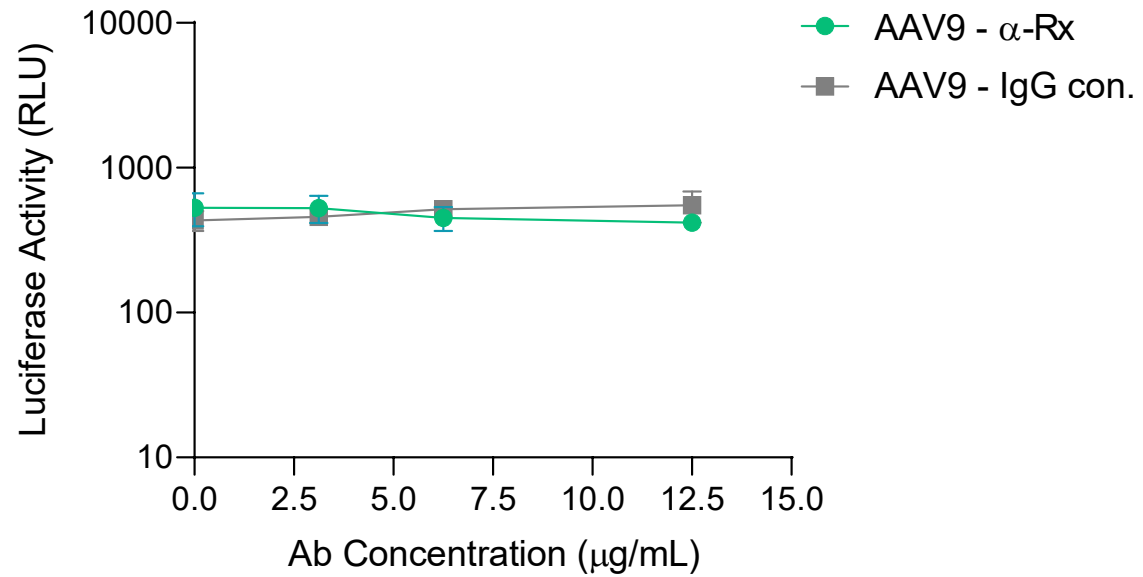
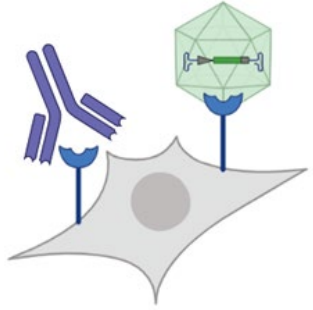
Knockdown of Endogenous Receptor X Expression Inhibits VCAP-102 Transduction



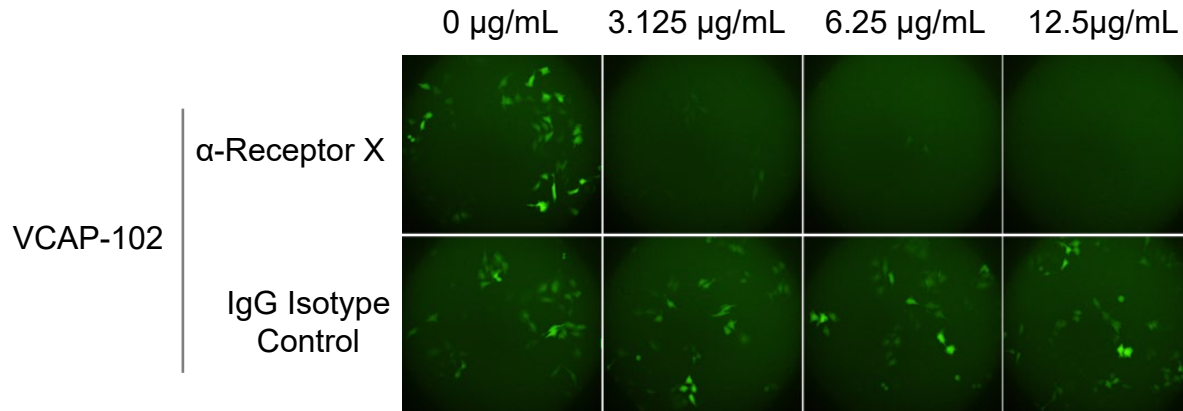
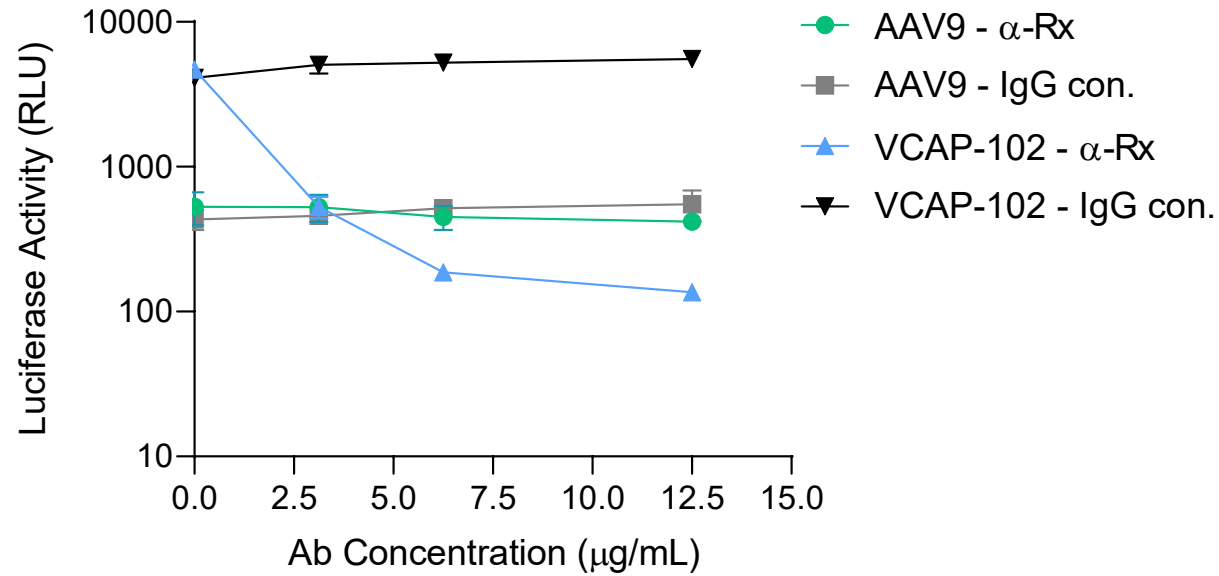
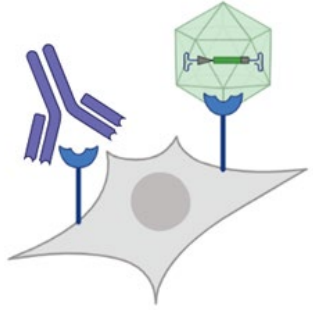
- AAV transduction at 48hr post-siRNA transfection followed by luciferase assay 24hr post-transduction
- siRNA mediated knockdown of Receptor X led to a ~80% reduction in VCAP-102 transduction
- No effect on transduction of AAV9



α -Receptor X Antibody Inhibits VCAP-102 Transduction

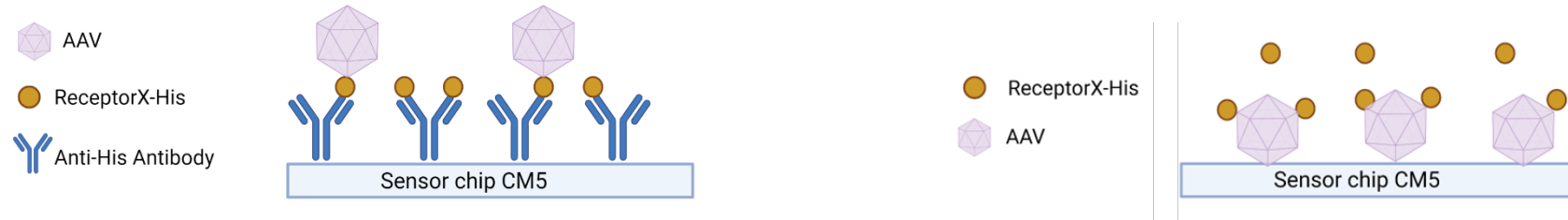


α -Receptor X Antibody Inhibits VCAP-102 Transduction



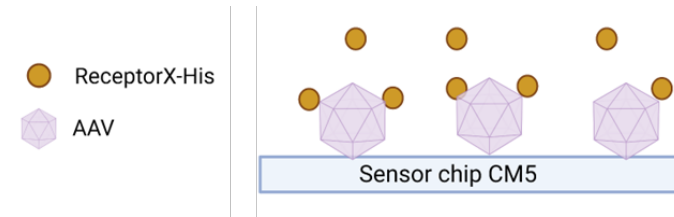
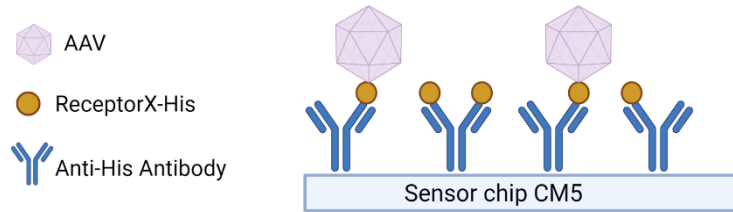
VCAP-102 Directly and Specifically Binds to Receptor X

- SPR measures molecular binding events in real-time and calculates binding affinity (KD)

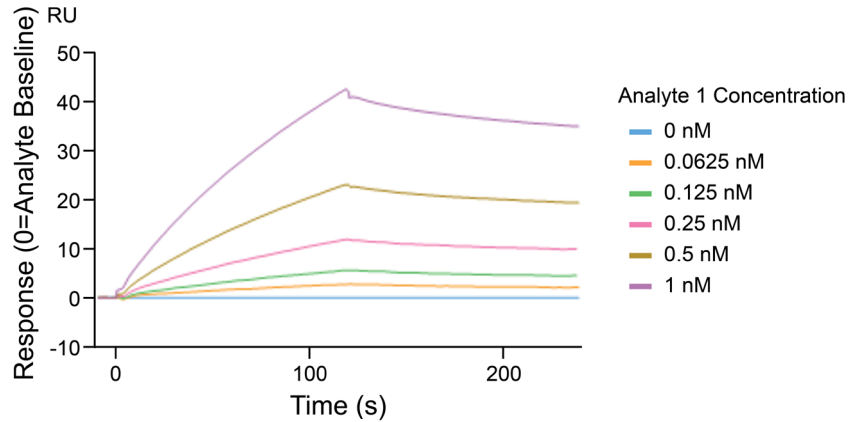


VCAP-102 Directly and Specifically Binds to Receptor X

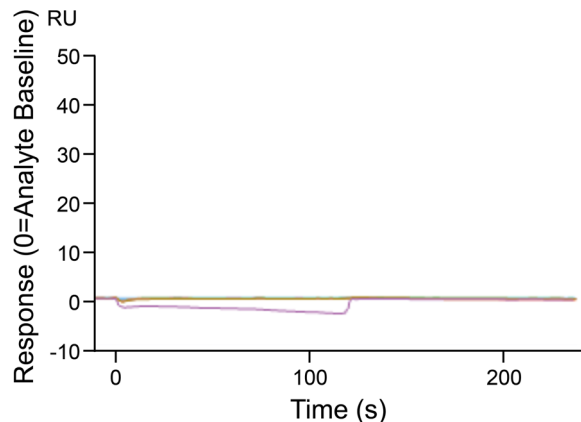
- SPR measures molecular binding events in real-time and calculates binding affinity (KD)



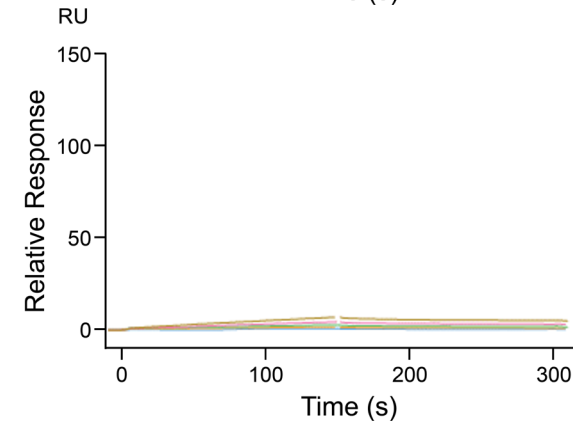
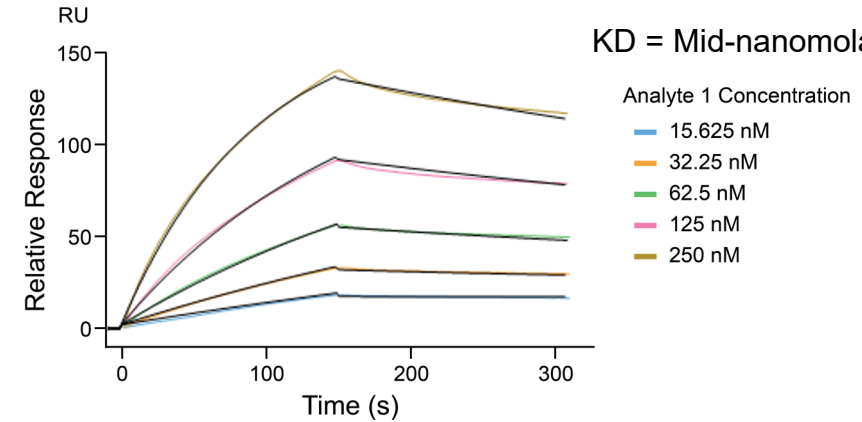
VCAP-102



AAV9

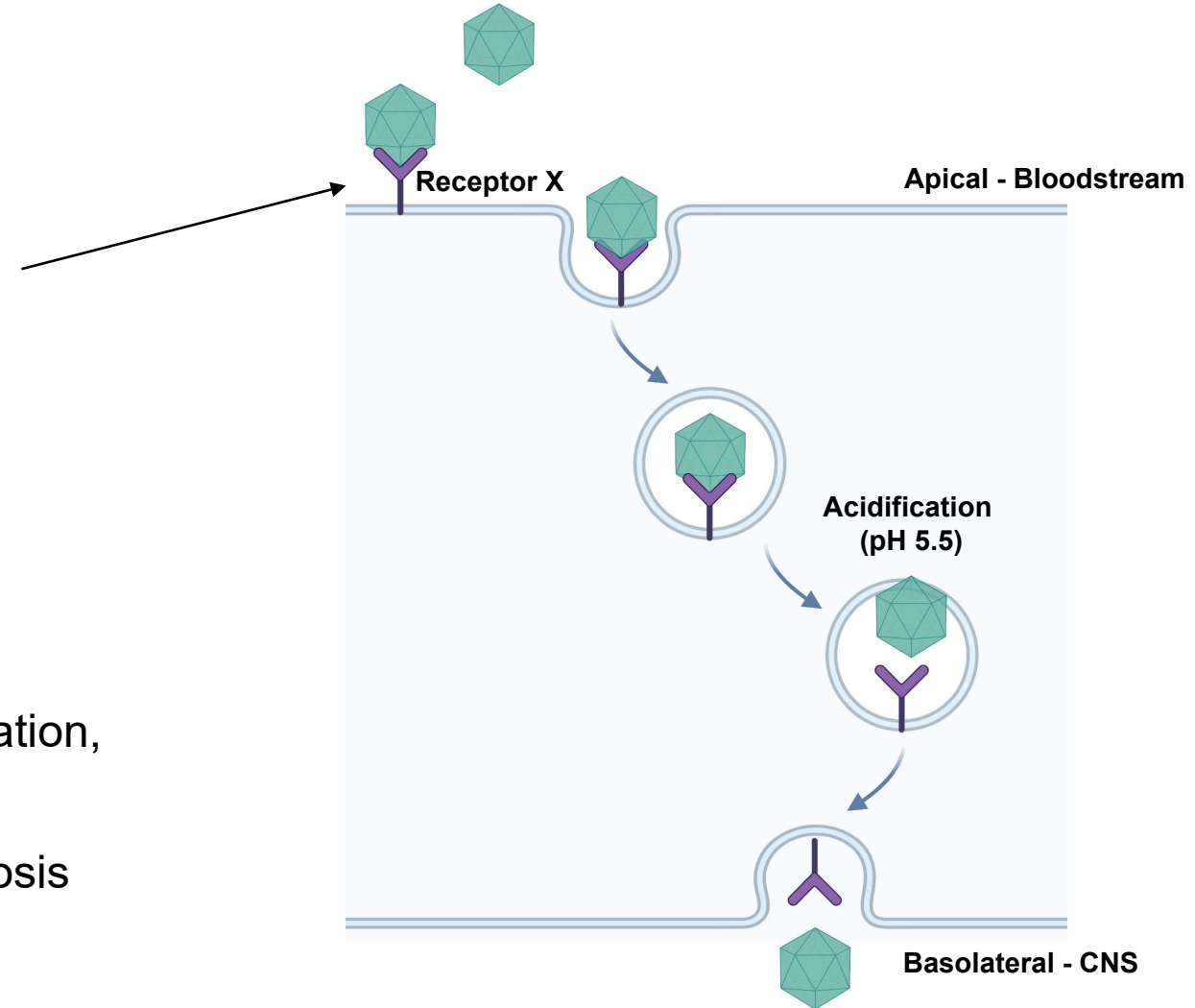
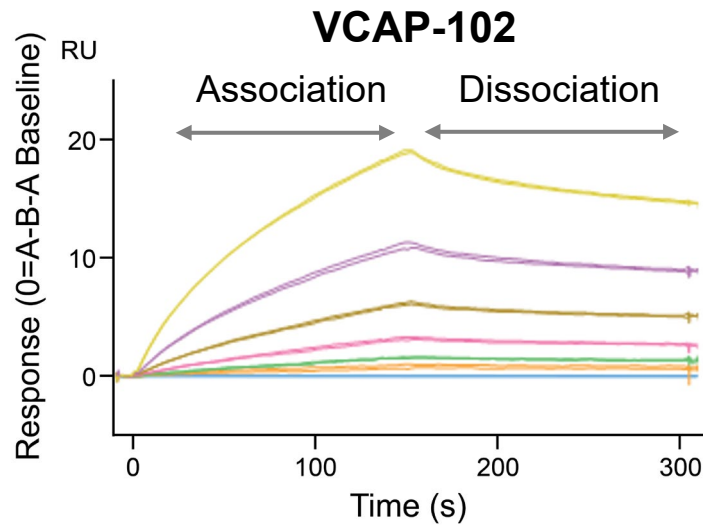


KD = Mid-nanomolar (10-100 nM)



Receptor X – VCAP-102 pH Dependent Dissociation

Dissociation at pH 7.4

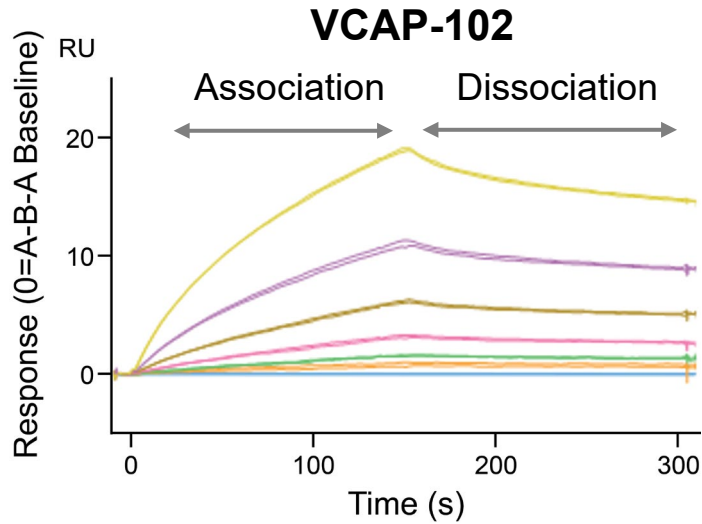


Lessons from transferrin receptor:

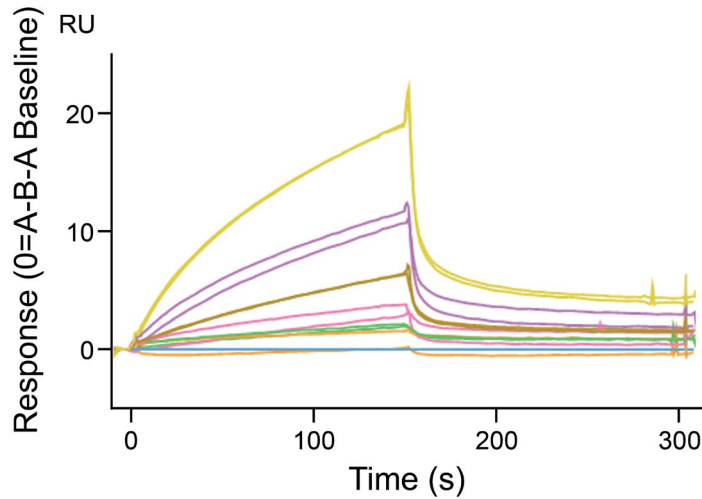
- Correlation between affinity and lysosomal degradation, high affinity results in poor transcytosis
- Lower affinity at pH 5.5 promotes efficient transcytosis

Receptor X – VCAP-102 pH Dependent Dissociation

Dissociation at pH 7.4

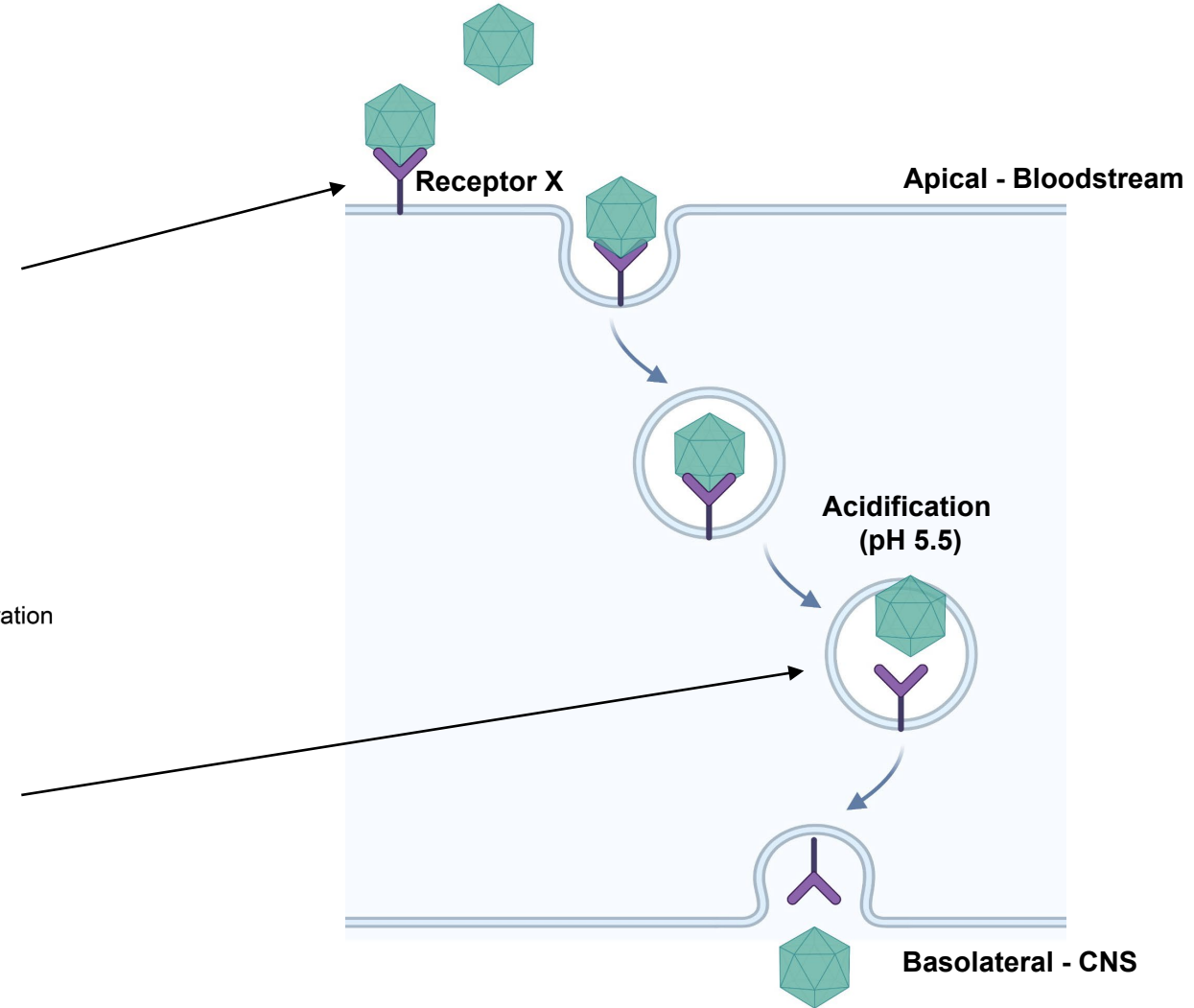


Dissociation at pH 5.5

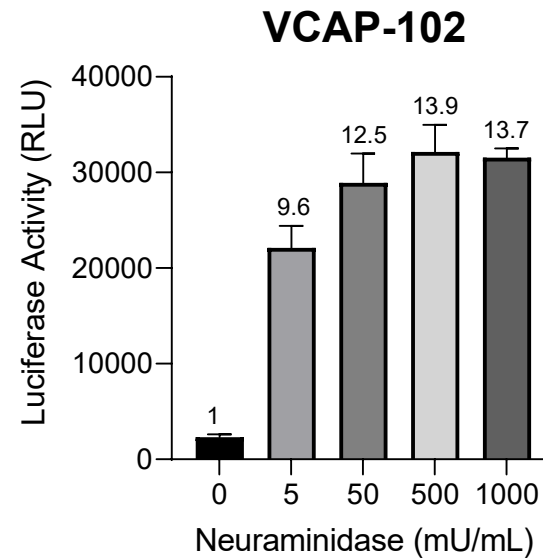
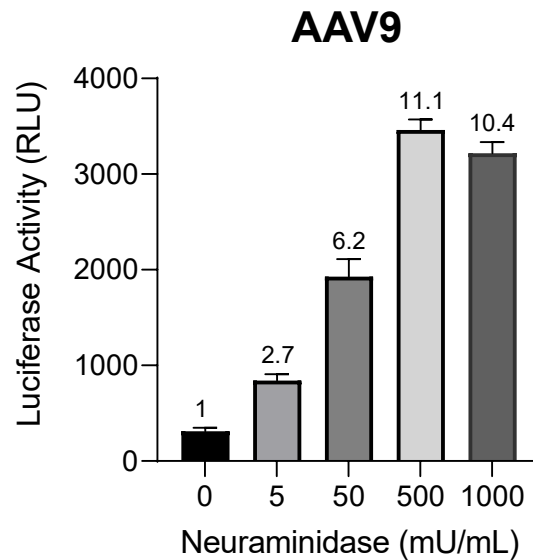
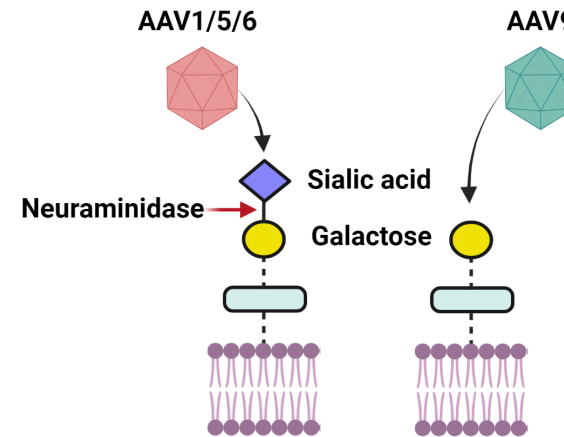
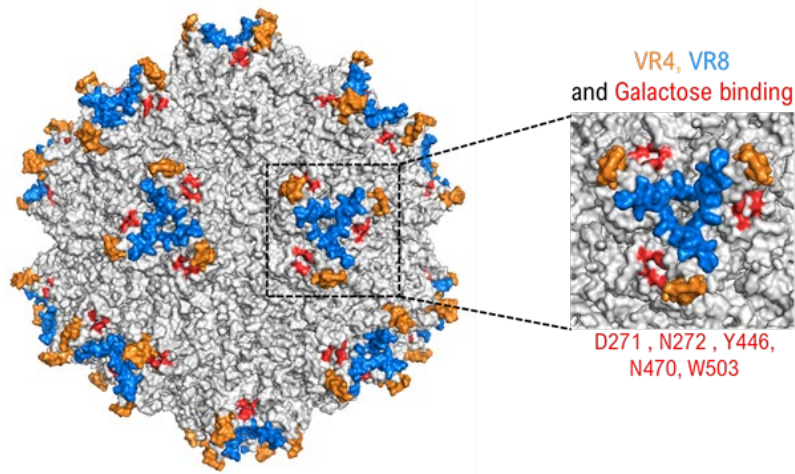


A-B-A 1 Concentration

- 0 nM
- 7.8 nM
- 15.6 nM
- 31.25 nM
- 62.5 nM
- 125 nM
- 250 nM

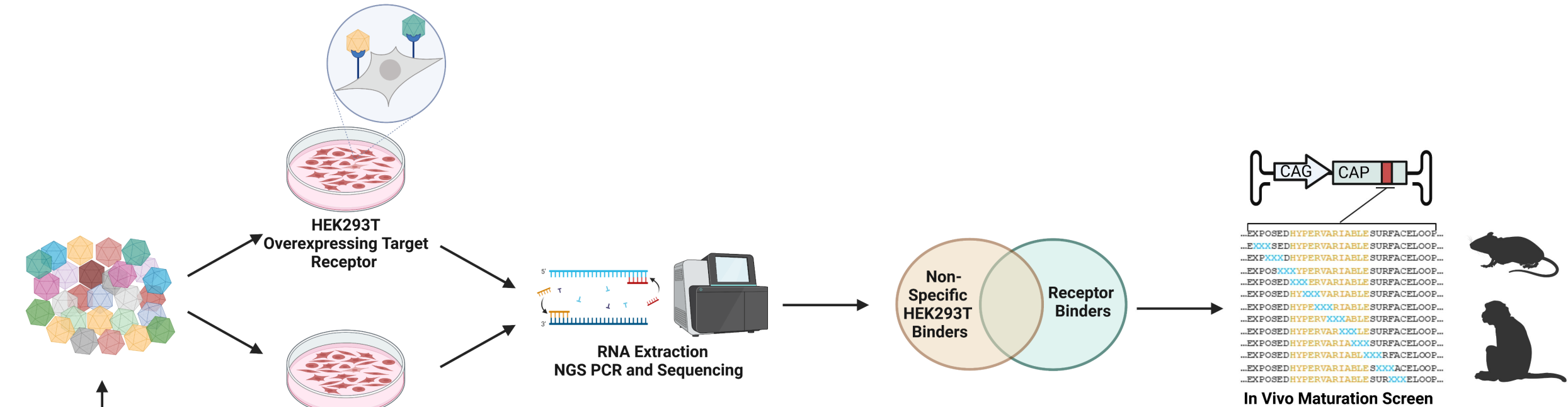


VCAP-102 Retains Galactose Binding – RX Binding is a Gain of Function



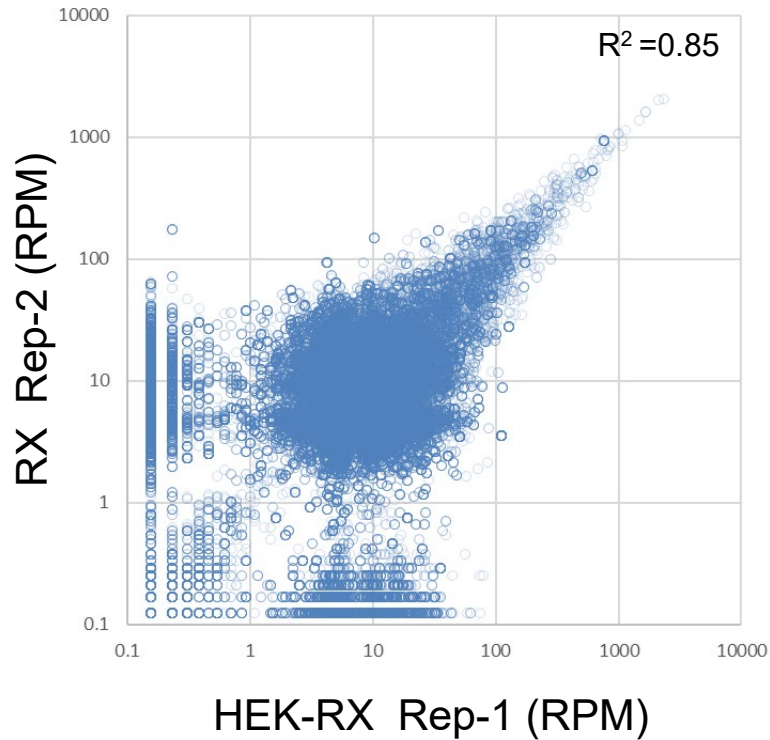
Receptor-Guided Capsid Evolution

- Can we utilize Receptor X to drive evolution of novel serotypes and alternative VR insert locations?

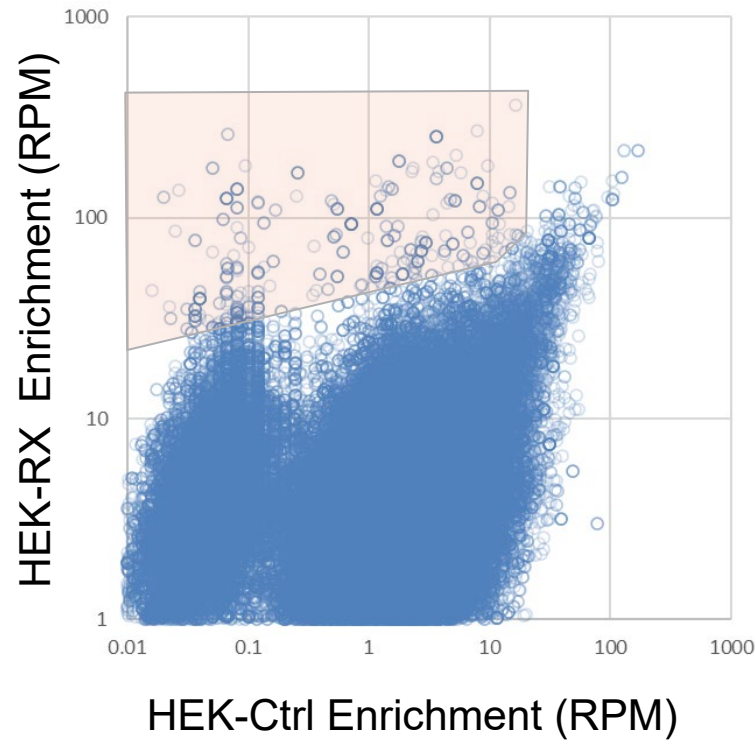


AAV9.VR-IV P0 Library – *In vitro* Evolution – HEK vs HEK-Receptor X

Correlation Between Receptor X Replicates

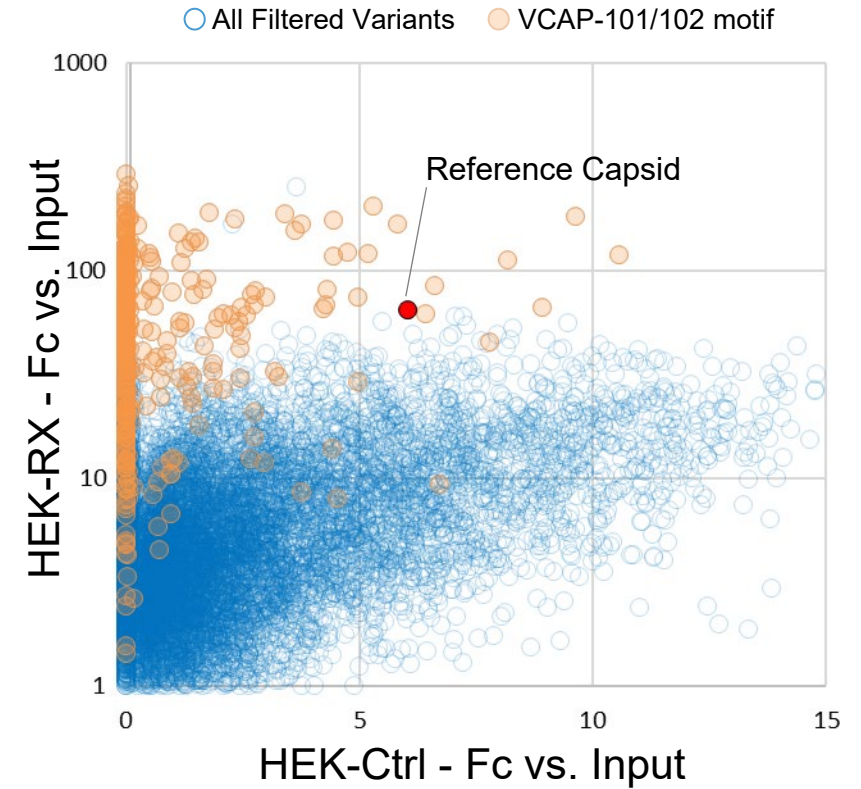


Receptor X Specific vs. Non-Specific Enrichment



Variant enrichment = Avg RPM between replicates normalized to input library RPM

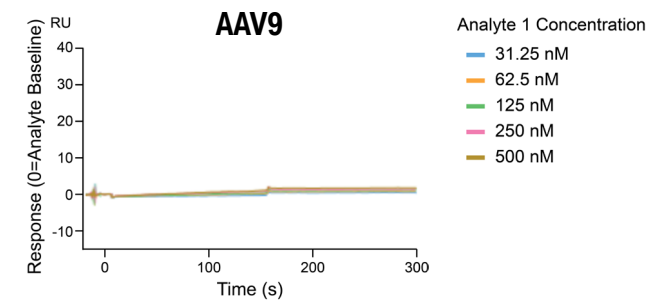
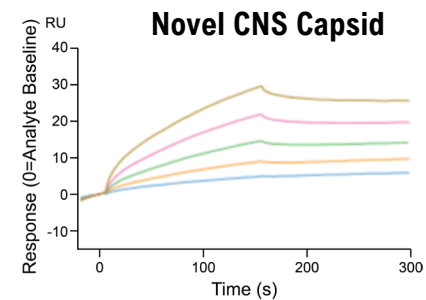
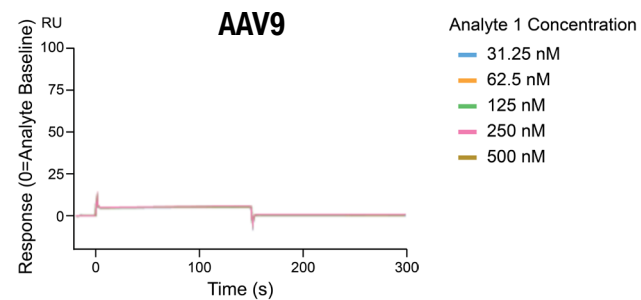
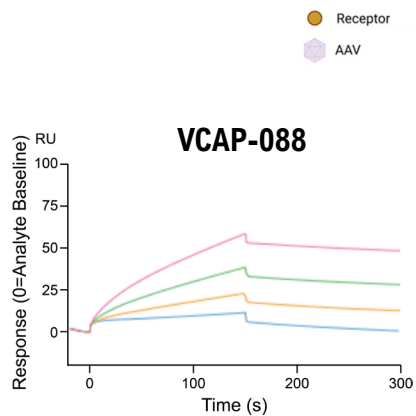
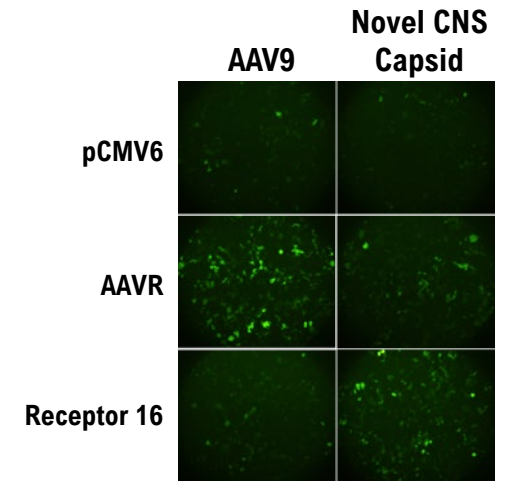
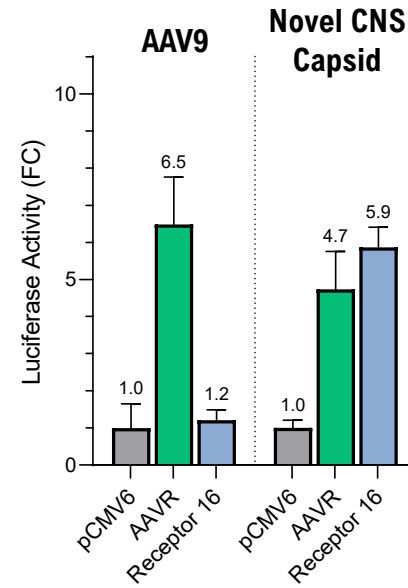
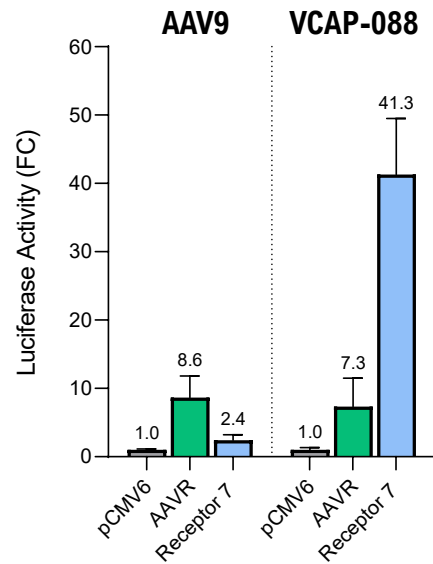
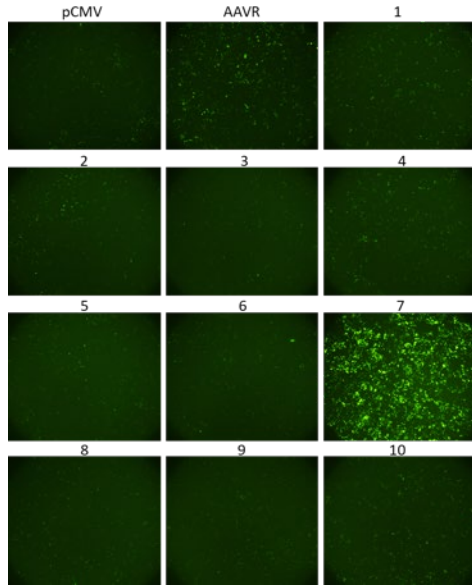
Fold Change vs. Input



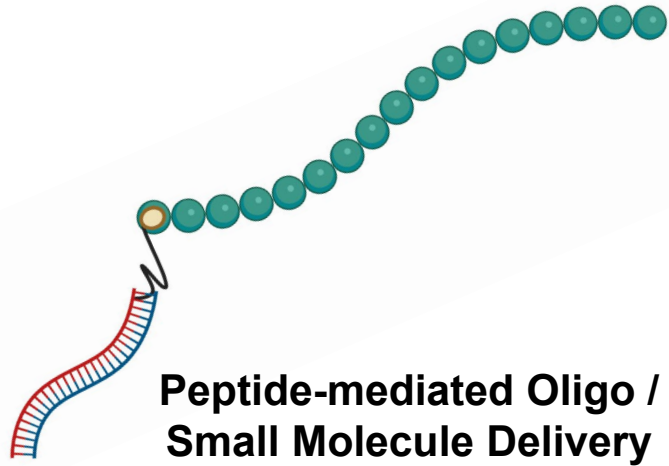
Filtering criteria - WT FC < 15, RX CV < 1

Preliminary Identification of Two Additional Human Receptors for Novel Capsids

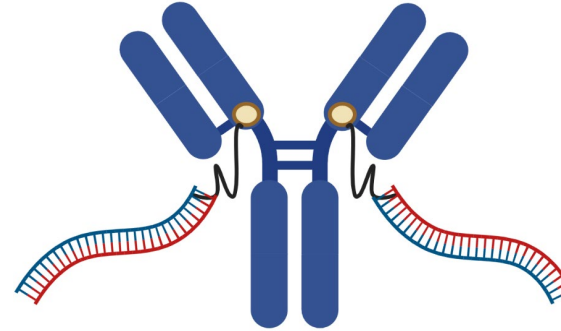
VCAP-088 Human Receptor Candidate Screen



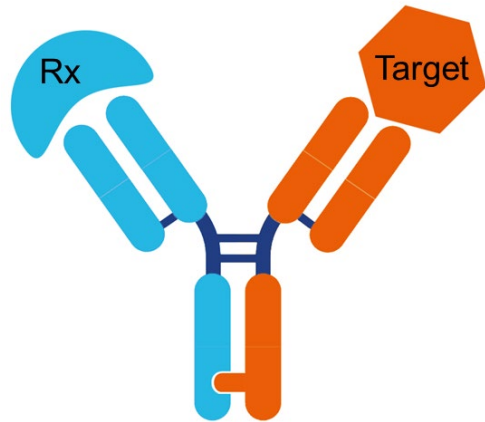
Receptor X – Non-viral CNS Delivery



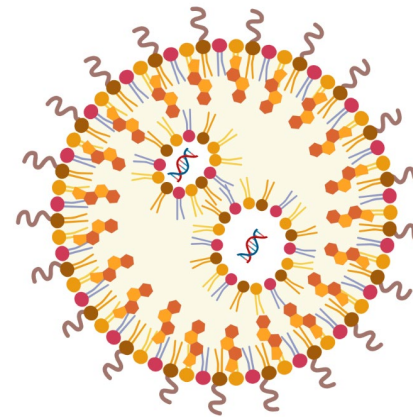
**Peptide-mediated Oligo /
Small Molecule Delivery**



**Antibody-mediated Oligo /
Small Molecule Delivery**



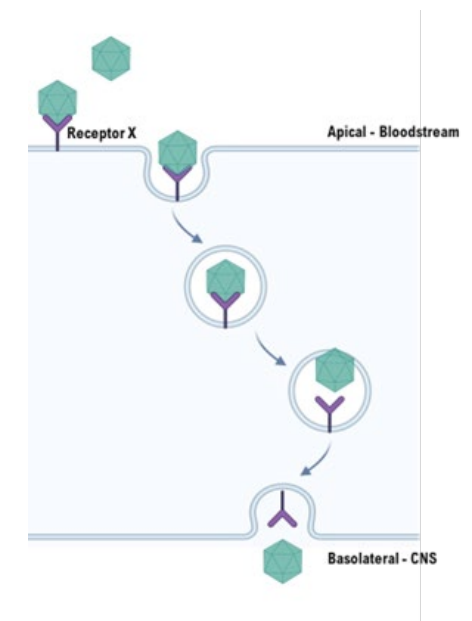
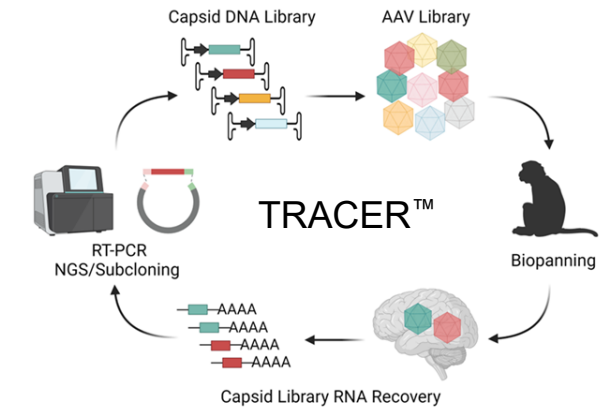
Bivalent Antibody



**Receptor X Targeted
Lipid Nanoparticle**

Conclusions

- The TRACER™ platform has resulted in the generation of multiple capsid families with enhanced CNS tropism
- We have identified Receptor X as the likely cell surface receptor for our novel cross-species BBB-penetrant capsid VCAP-102
 - Supports transferability of BBB-penetrant phenotype to humans
- *In vitro* evolution is being used to develop new AAV serotypes and new insert locations that bind Receptor X
- We continue efforts to identify receptors for our additional panel of novel capsids and have preliminary data supporting the identification of two additional receptors
- Investigating use of identified receptors for non-viral CNS delivery





**Thank you!
Questions?**

If you would like more information,
please contact:

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