In vitro binding to human and NHP orthologs of candidate receptors identify novel systemically-delivered AAVs with enhanced CNS tropism

John Reece-Hoyes, Jackson Lee, Jie Tan, Estrella Lopez-Gordo, Caroline Curll, Fion Chan, Sri Siripurapu, Stephanie Malyszka, Kimberly Le, Allegra Fieldsend, Alex Needham, Haley Knight, Paul Freeman, Matt Bennett, Esther Aribilola, Ramin Kamran Sami, Shahrzad Parker, Jordan Shufro, Graham Lilley, Bryan Mastis, Roberto Calcedo, Matt Edwards, Sherry Cao, Charles F. Albright

P0144

Summary

- We have previously discovered BBB-penetrant AAV capsids that when delivered intravenously transduce >50% neurons in NHPs

- To identify additional capsids that lead to high CNS expression and broad CNS distribution, we screened AAV capsid libraries (7mer peptide insertions in AAV9 VR8) for binding to 10 putative human receptors that could facilitate BBB transcytosis, including Transferrin Receptor (TFRC), SLC3A2 (CD98), ALPL and CA4

- To address cross-species tropism early in the discovery pipeline we selected novel capsids that bound both human and NHP receptor orthologs

- This approach identified candidates with improved CNS tropism compared to AAV9

Receptor selection

Candidate receptors identified from scRNA-seq data from mouse (left) or human (right) brain endothelial cells
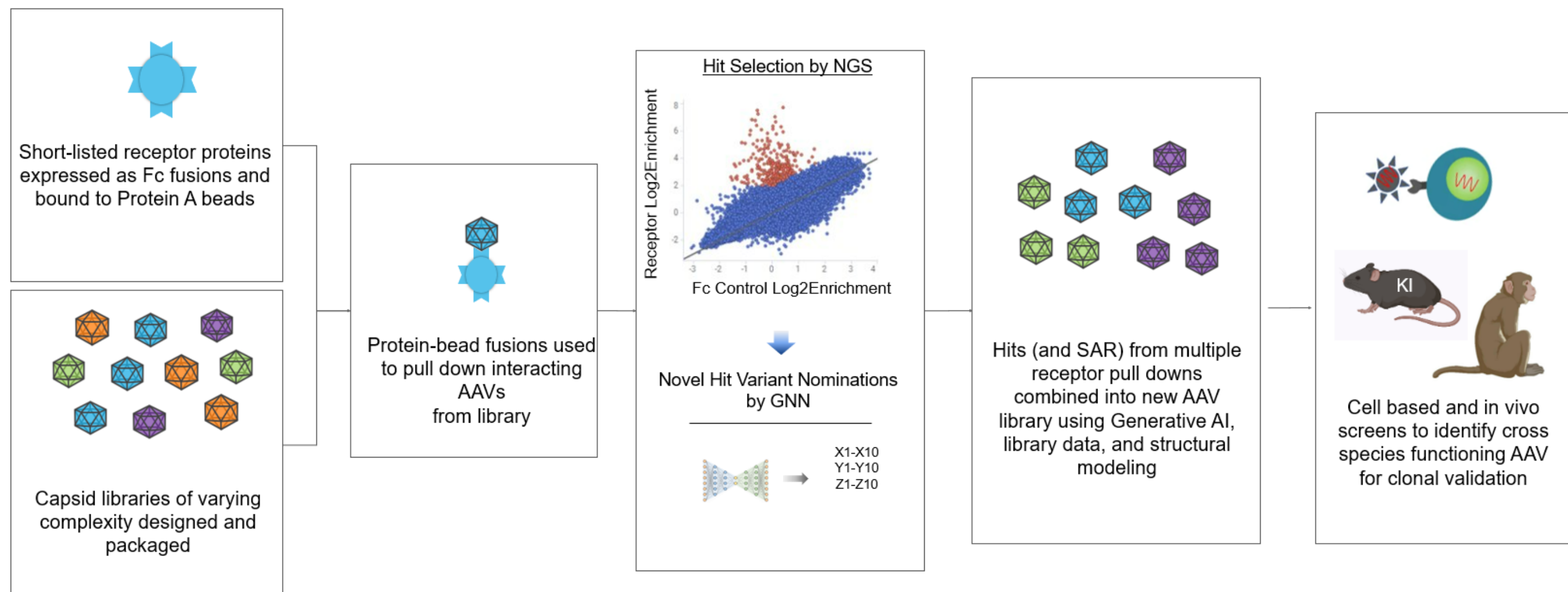

DOI:10.3389/fimmu.2021.730825

DOI:10.1126/science.add7046
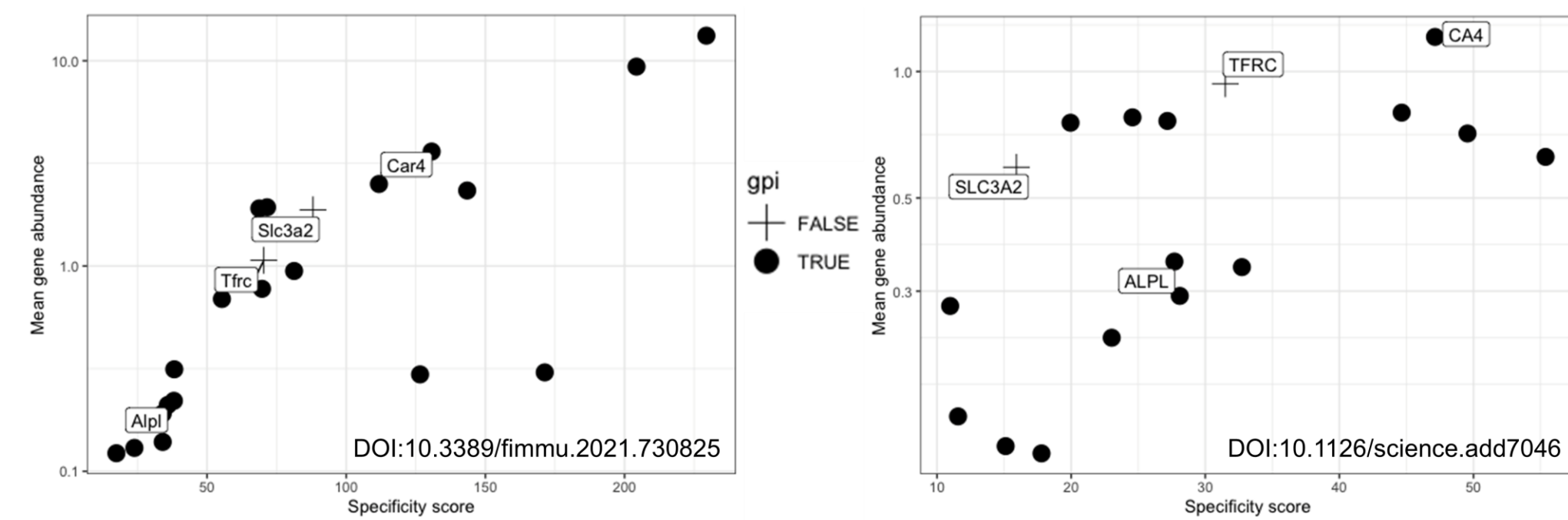
Defining characteristics of receptors of interest

Gene	GPI Anchored	Brain Endothelial Abundance	Brain %CV (v. FLT1)
1		1.61E-03	-28.71
2		1.54E-03	-33.11
3		1.16E-03	-15.07
TFRC		1.10E-03	0.27
5	+	1.00E-03	2.40
CA4	+	7.48E-04	-2.39
SLC3A2		5.28E-04	-18.42
8	+	4.73E-04	-14.57
9	+	4.27E-04	27.26
ALPL	+	3.54E-04	-6.19

- Single cell RNA-seq of brain tissues was used to identify highly expressed (y-axis) and brain endothelial cell specific (x-axis) receptors in brain endothelial cells of mouse or vascular cells of human. Other features such as %CV across GTEx brain data were normalized to an endothelial specific gene (FLT1)

- Candidates were enriched for known transcytosis receptors (eg transferrin receptor TFRC) and GPI-anchored proteins
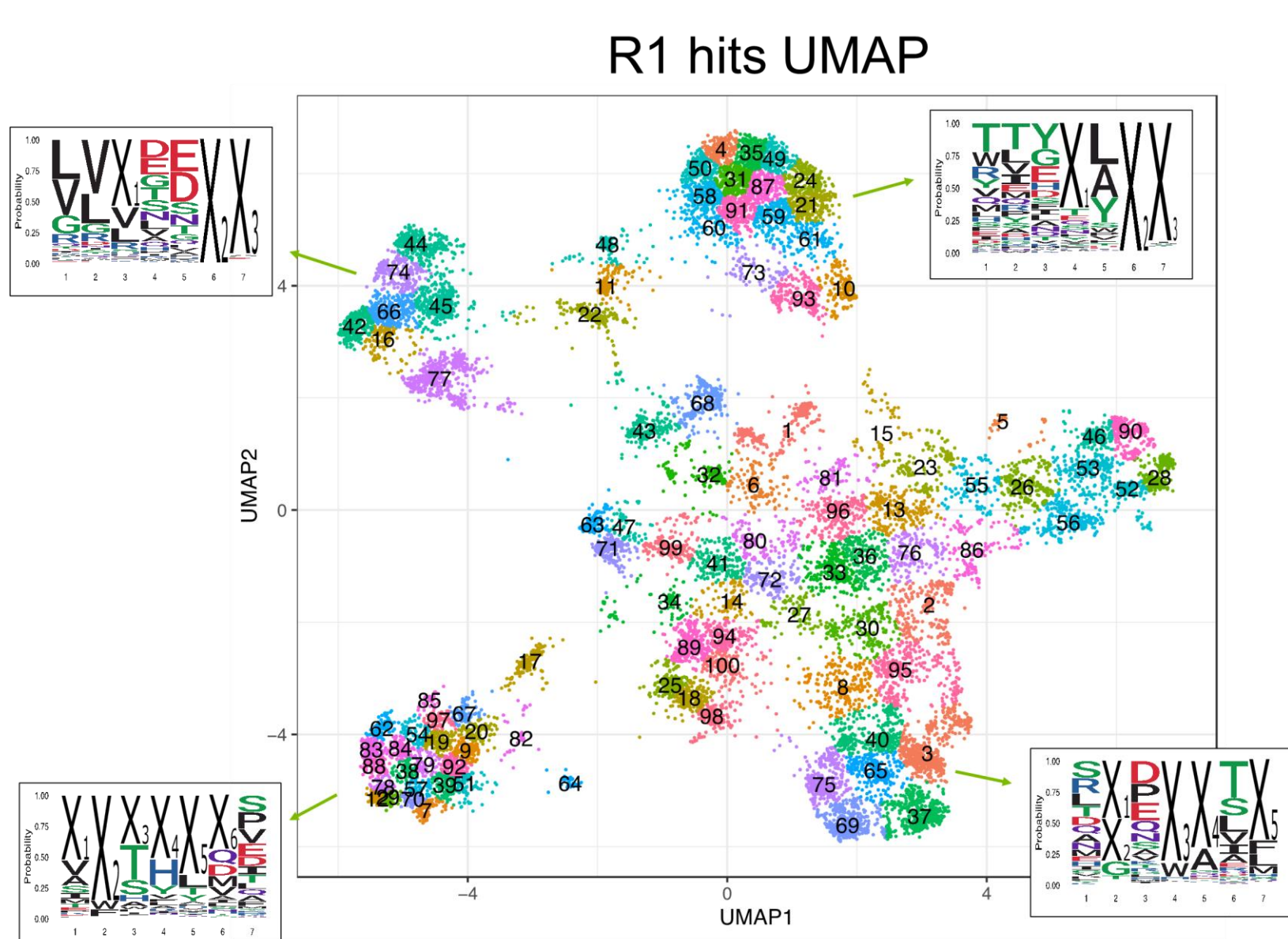
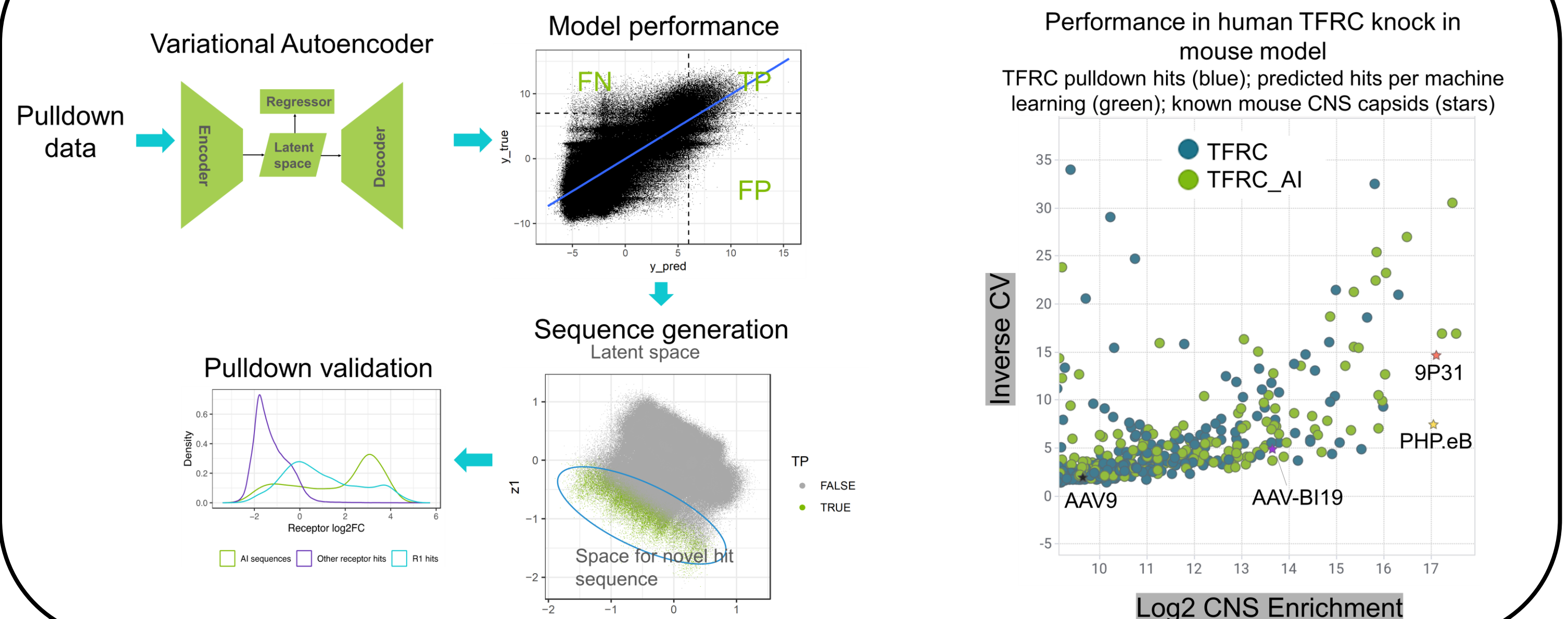## Method to isolate capsids using in vitro binding assay

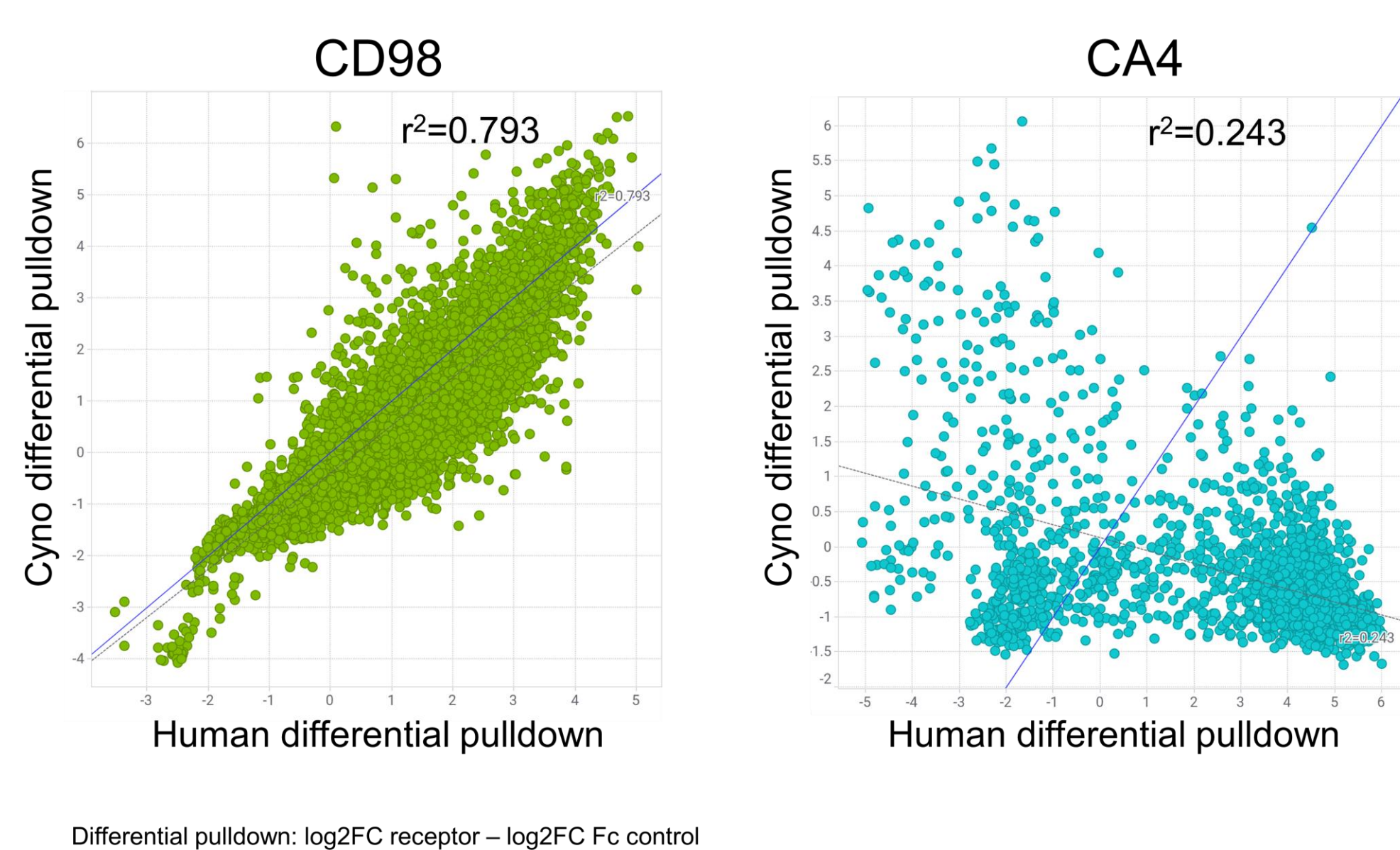## Binding hits are reproducible and unique to a given receptor

Strong correlation between assay replicates

Different receptors show different pulldown profiles

Receptor pulldown hits are unique

Log2FC: difference in proportion of total reads a variant represents in pulldown data compared to input virus expressed in log2 format
Control log2FC = log2FC for pulldowns using Fc domain alone
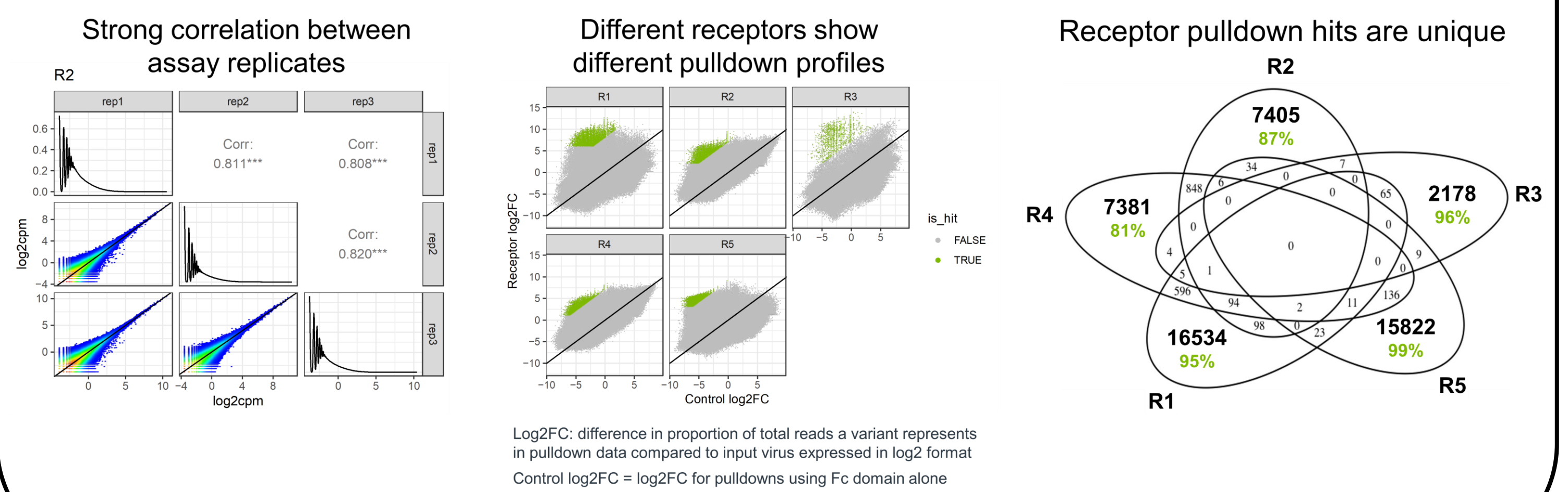
Binding hits form distinctive clusters with motifs

R1 hits UMAP

Machine learning successfully identifies further functional hits

Variational Autoencoder

Model performance

Performance in human TFRC knock in mouse model
TFRC pulldown hits (blue); predicted hits per machine learning (green); known mouse CNS capsids (stars)

Sequence generation

Pulldown validation

Overlap between human and cyno orthologs is variable

CD98

r²=0.793

CA4

r²=0.243

Differential pulldown: log2FC receptor – log2FC Fc control

Candidates identified with function better than AAV9

HEK-OE

KI mice

Relative expression data for five variants that bind TFRC, extracted from library studies in stable HEK TFRC overexpression cells (left) and CNS of humanized knock-in TFRC mice (Biocytogen, right)

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