Engineered AAV capsids that target a novel human brain endothelial receptor achieve robust transduction in non-human primate central nervous system after intravenous dosing

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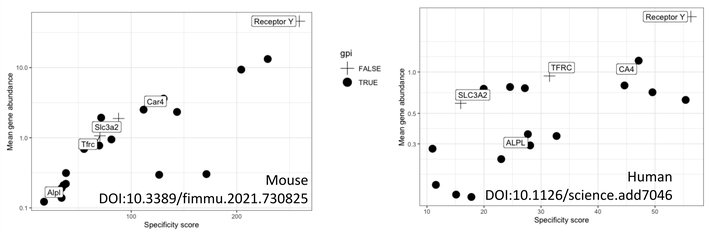
Estrella Lopez-Gordo, Jackson Lee, Jie Tan, Esther Aribilola, Amany Awad, Amanda Berry, Kyle Chamberlain, Fion Chan, Caroline Curll, Hanna Czeladko, Anthony DeMarco, Spencer Fielder, Paul Freeman, Emily Grandell, Charles Gualtieri, Tyler Ironside, Jennifer Joubert, Haley Knight, Stephanie Malyszka, Bryan Mastis, Alex Needham, Shahrzad Parker, Cynthia Pryce, Nick Robinson, Ramin Kamran Sami, Lauren Sargent, Elizabeth Scott, Jordan Shufro, Roberto Calcedo, Matt Edwards, Graham Lilley, Giridhar Murlidharan, John Reece-Hoyes, Sherry Cao, Charles Albright

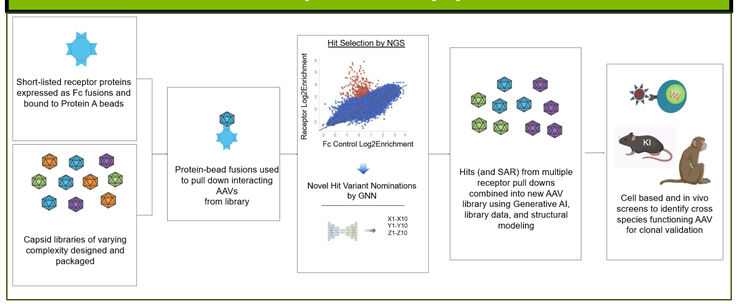
Summary

- The therapeutic utility of engineered capsids for intravenous delivery to the CNS has been complicated by a lack of cross-species tropism
- To address this risk, we identified capsids that bind both the human and non-human primate (NHP) orthologs of a novel protein (referred to as Receptor-Y) that is highly expressed in brain endothelium of the mouse, NHP, and human
- We first screened a library of AAV9 capsids with random 7mer peptide insertions for binding the extracellular domain of human or NHP Receptor-Y protein immobilized on beads
- A library of ~13K Gen1 binding hits was screened in NHPs using intravenous dosing and revealed that a minority of binding hits were detectable in the CNS, and at relatively low levels
- A Gen2 library of ~50K capsids was generated using SAR of the Gen1 in vivo active sequence motif families and then used for a second round of screening in NHPs
- While several Gen2 capsids achieved ~250-fold higher mRNA expression than AAV9 across NHP cortical and deep-brain regions, a third round of optimization is being pursued
- A Gen3 library of ~50K capsids was generated that modified the flanking residues of the top several Gen2 hits and then used for a third round of screening in NHPs with results due soon

Receptor-Y is highly expressed in brain endothelial cells



In vitro pulldown pipeline



Binding screens reveal families of variants with cross-species activity

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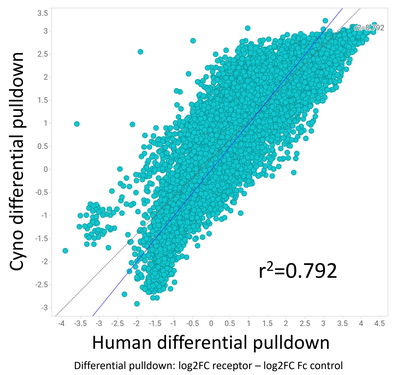
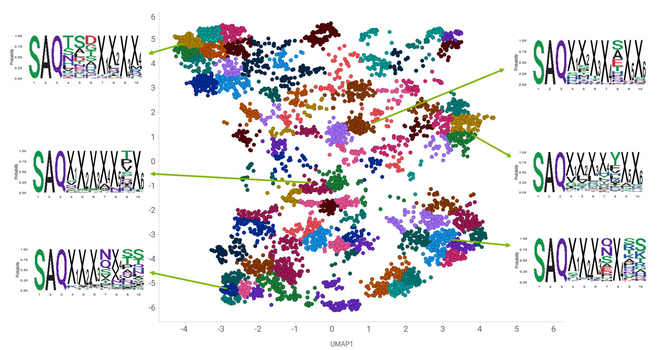
SAQ - XXXXXXX - AQA

NNK library of millions of 7-mer inserts in AAV9 VR-VIII region



High overlap in binding between human and cyno orthologs



Conserved binding motifs revealed by hit clustering

Iterative NHP library screens to improve CNS transduction

Gen1 NHP library design

~13K binding hits

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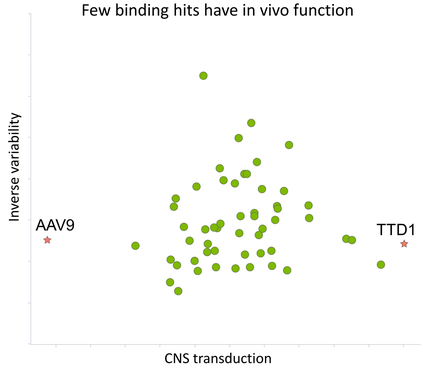
Gen1 NHP library data

Few binding hits have in vivo function



Gen2 NHP library design

~50K including Gen1 hits and expansion within leading conserved motifs

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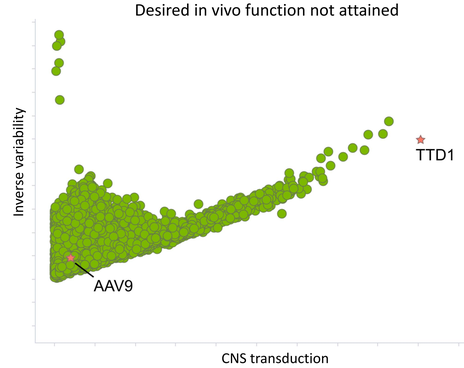
Gen2 NHP library data

Desired in vivo function not attained



Gen3 NHP library design

~50K including top leads from Gen2 with flanking residues mutated

Gen3 NHP library data pending

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