Novel AAV capsids that bind human transferrin receptor (TFRC) demonstrate widespread and preferential CNS tropism in hTFRC-KI mice after low dose systemic dosing

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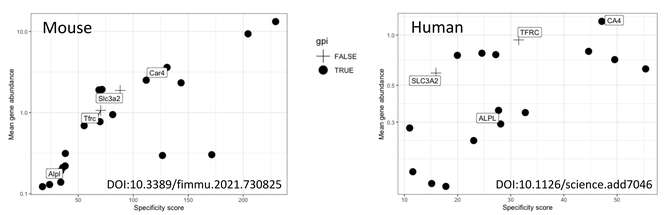
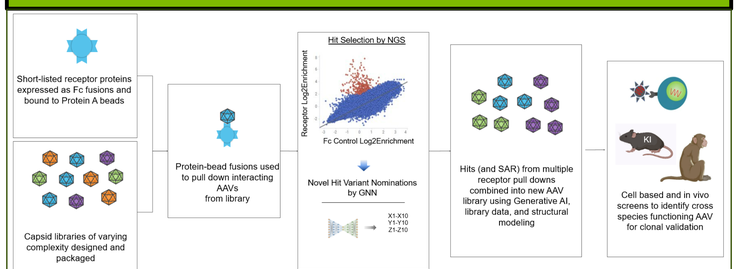
Summary

- Human TFRC can bind and enable AAVs and antibodies to cross the BBB
- We screened a library of AAV9 capsids with random 7mer peptide VR-VIII insertions for binding the human and cyno TFRC extracellular domain immobilized on beads
- A deep learning generative AI framework was applied to the binding data to predict additional TFRC hit capsids
- A library of observed binding hits and AI-predicted hits was dosed intravenously to transgenic mice harboring human TFRC receptor on the BBB (hTFRC-KI)
- Interestingly, the best performing capsids were all AI-predicted
- Three lead capsids were individually packaged with reporter cargo for dosing hTFRC-KI mice at 2.5e13, 2.5e12 and 2.5e11 vg/kg
- All three capsids outperformed both AAV9 (140- to 270-fold higher CNS mRNA expression) and BI19 (10-fold higher CNS mRNA expression), with one capsid (ATC-0260) also demonstrating 10-fold lower expression in the liver
- Importantly these novel capsids all demonstrated 50% neuronal transduction at the clinically low dose of 2.5e12 vg/kg
- We show that these novel capsids bind the apical domain of human TFRC via R208
- In sum, we developed a high throughput screening platform to identify capsids that can bind human TFRC protein in vitro and penetrate the BBB via TFRC in vivo

TFRC is highly expressed in brain endothelial cells



In vitro pulldown pipeline



Library screens reveal variants with high in vivo activity in hTFRC-KI mice

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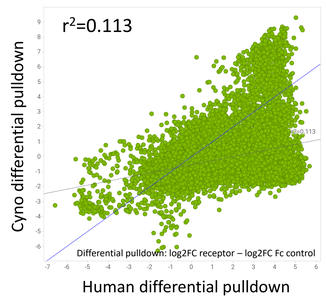
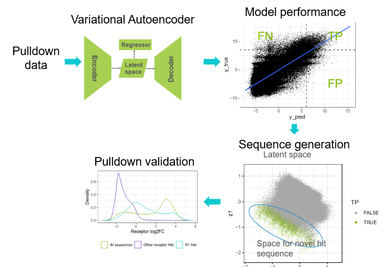
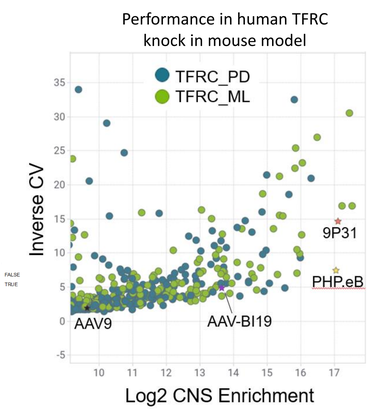
NNK library of 7-mer inserts into AAV9 VR-VIII region



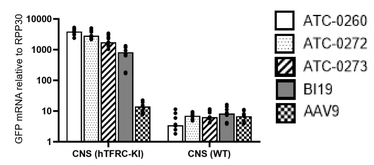
Few pulldown hits bind both human and cyno TFRC orthologs



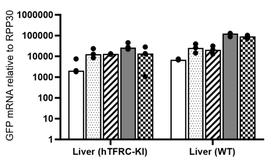


Machine Learning identified novel functional leads

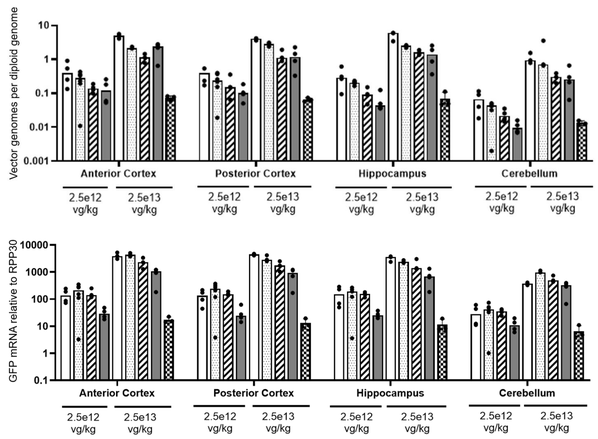
Clonal leads outperform AAV9 and BI19 in hTFRC-KI mice & transduce 50% neurons at low systemic dose

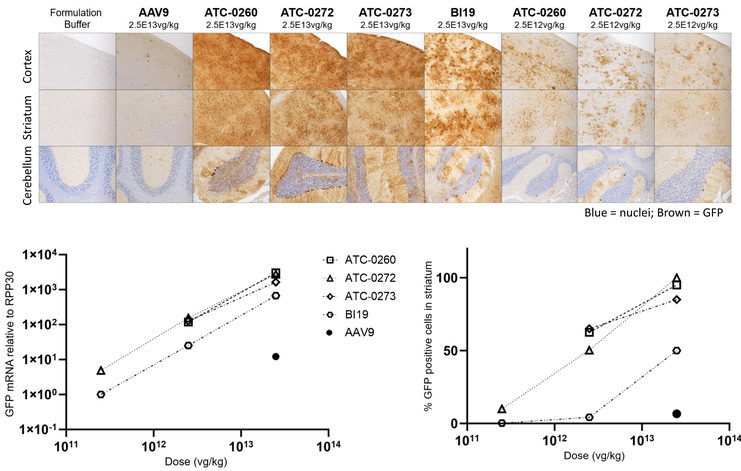


Presence of hTFRC necessary for CNS (but not liver) transduction

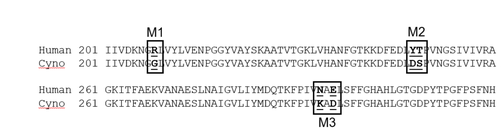


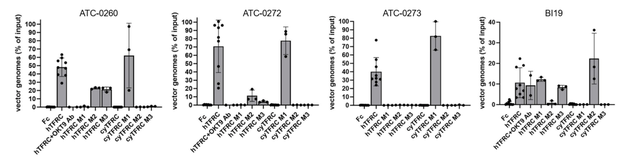
ATC-0260 low in liver





Clonal leads only bind human TFRC (via R208)





- Novel capsids all bind hTFRC, not cyno
- Blocking the apical domain of hTFRC using OKT9 antibody prevented capsid binding
- Mutating hTFRC R208 to G prevented novel capsid binding
- Mutating cynoTFRC G208 to R enabled novel capsid binding
- BI19 binding is via Y247/T248

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