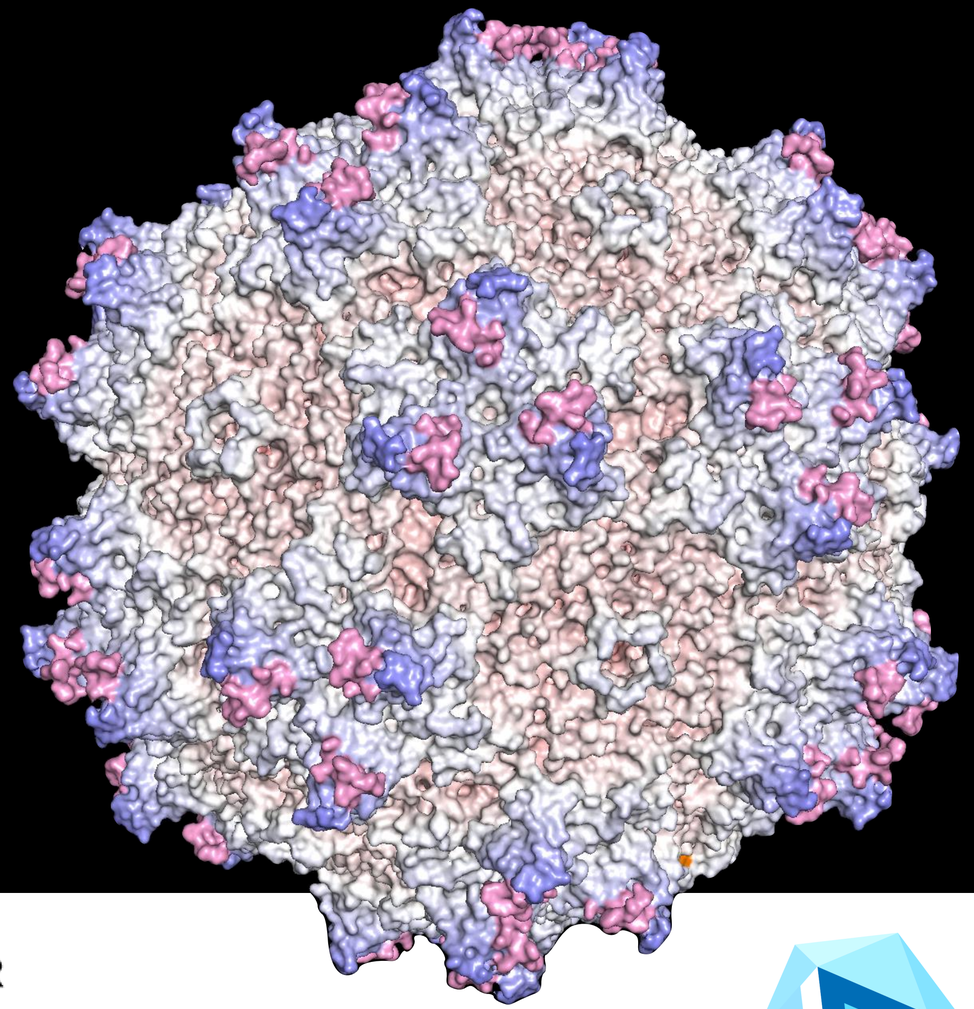
Delivering a prion protein lowering gene therapy across the human blood-brain barrier

Ken Chan
Group Leader of Vector Engineering
Deverman Lab
Stanley Center for Psychiatric Disease
Broad Institute of MIT and Harvard



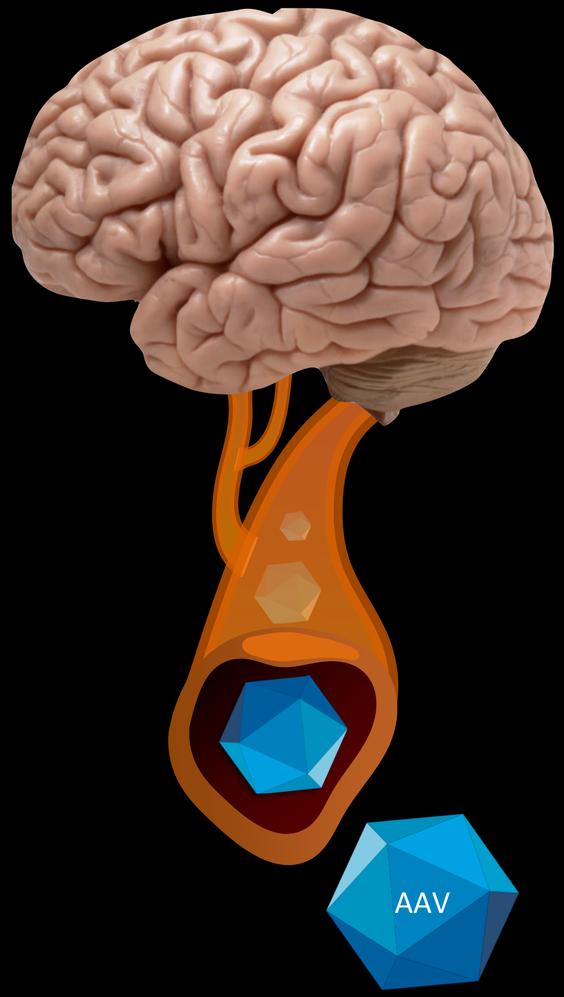
2024 Creutzfeldt-Jakob Disease Foundation Conference

Session: Rational drug design for prion diseases and how this informs other ADRDs

BROAD INSTITUTE

STANLEY CENTER FOR PSYCHIATRIC RESEARCH AT BROAD INSTITUTE

Engineering AAV vectors that enable efficient CNS-wide gene delivery in humans through the vasculature is challenging because of the blood brain-barrier



Advantages

- The vasculature provides access to all regions of the CNS
- Most uniform
- Non-invasive

Disadvantage

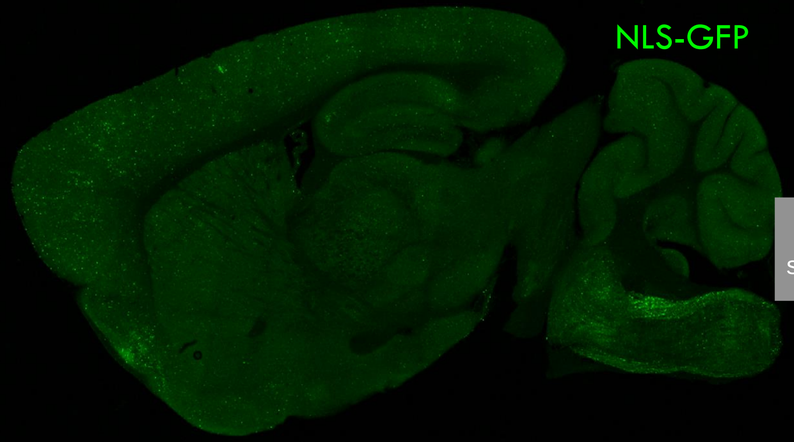
- Transduction efficiency is low across the CNS with naturally occurring AAVs
- Exposure to pre-existing neutralizing antibodies

BROAD INSTITUTE

Capsid engineering can dramatically enhance the targeting of AAVs to the CNS

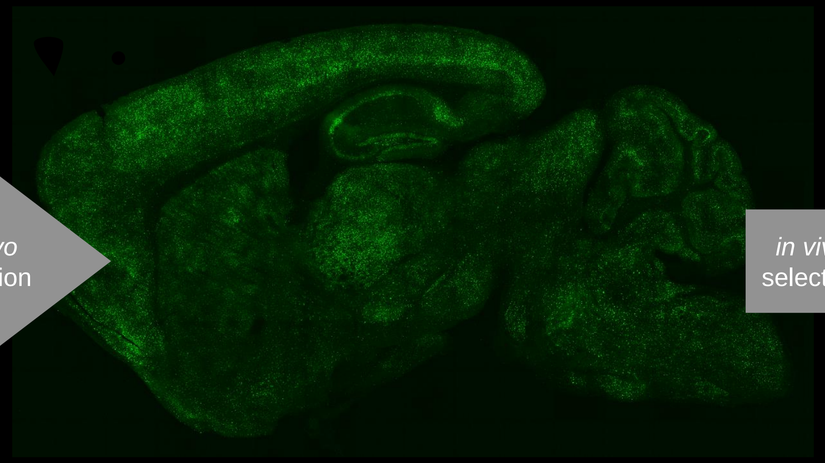
AAV9 (10^{12} vg)

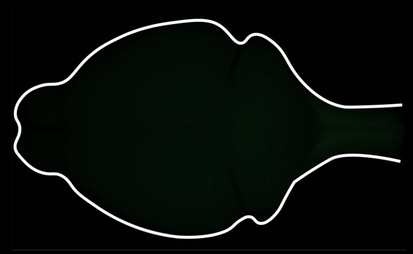
NLS-GFP

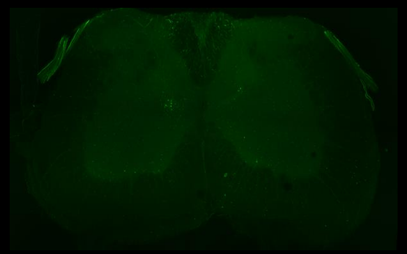


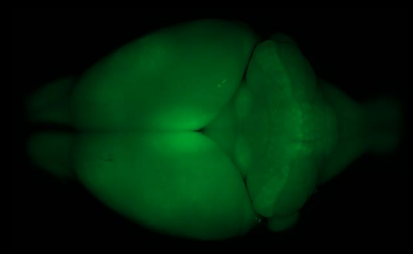
in vivo selection

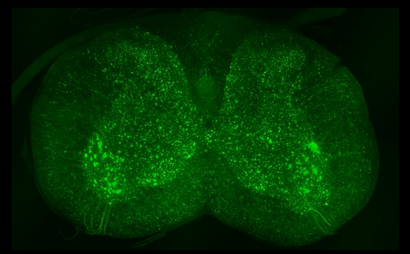
AAV-PHP.B (10^{11} vg) 10x lower dose







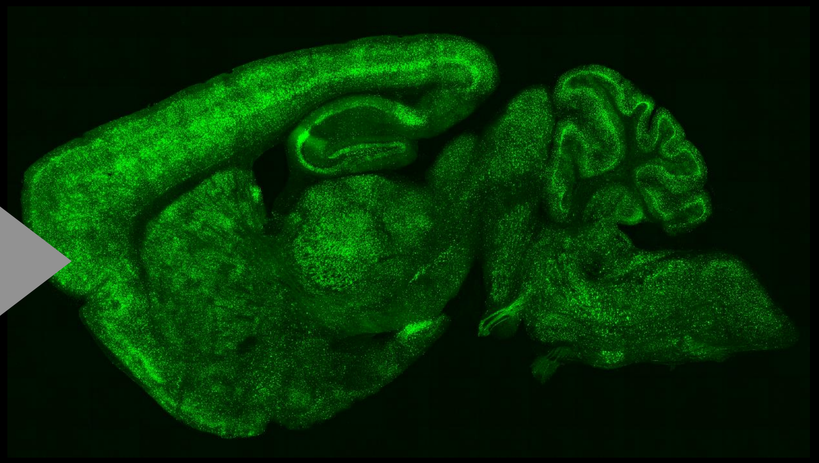


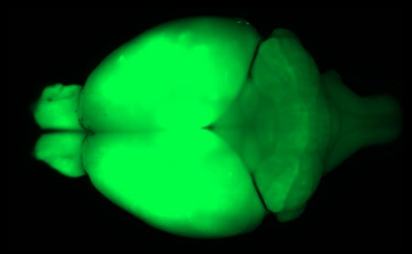


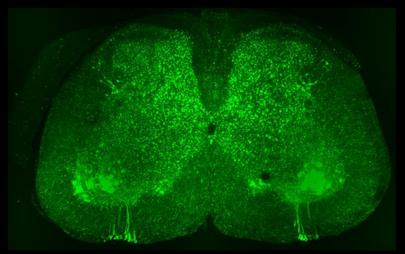
Deverman et al.
Nature Biotechnology 2016

in vivo selection

AAV-PHP.eB (10^{11} vg) 10x lower dose





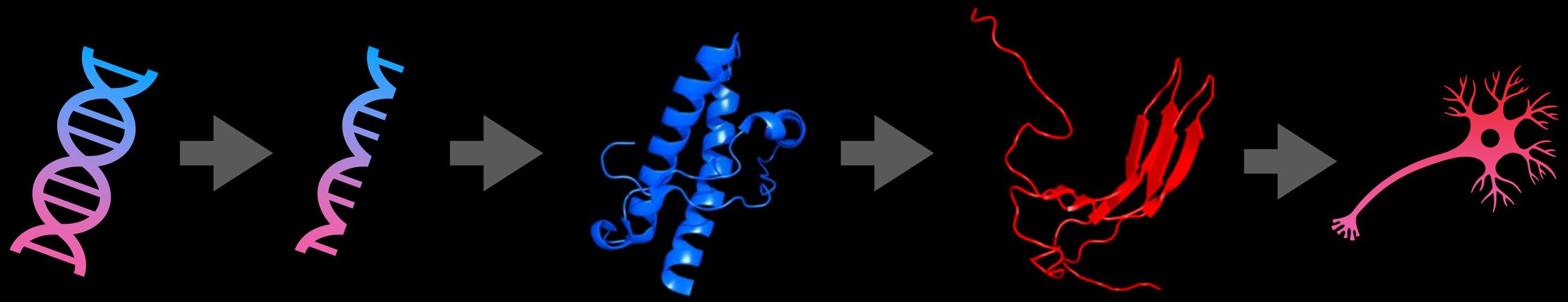


Chan et al. Nature Neurosci 2017

Cre based cell type specific in vivo selection

BROAD INSTITUTE

Pairing CNS-wide transducing capsids with genetic technologies as potential therapeutics for neurodegenerative disorders



DNA (PRNP)

RNA

Normal PrP

Misfolded PrP

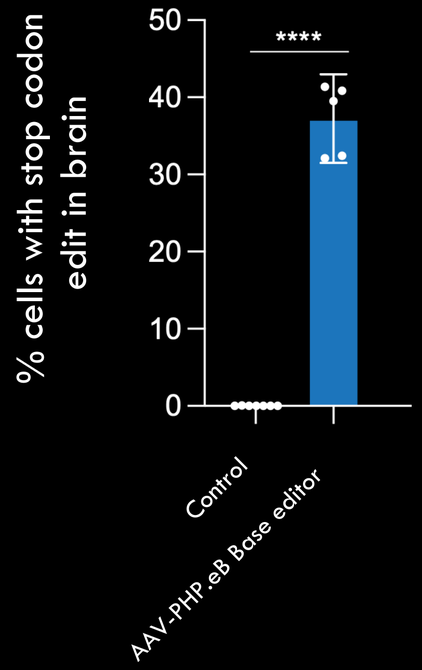
Cell death

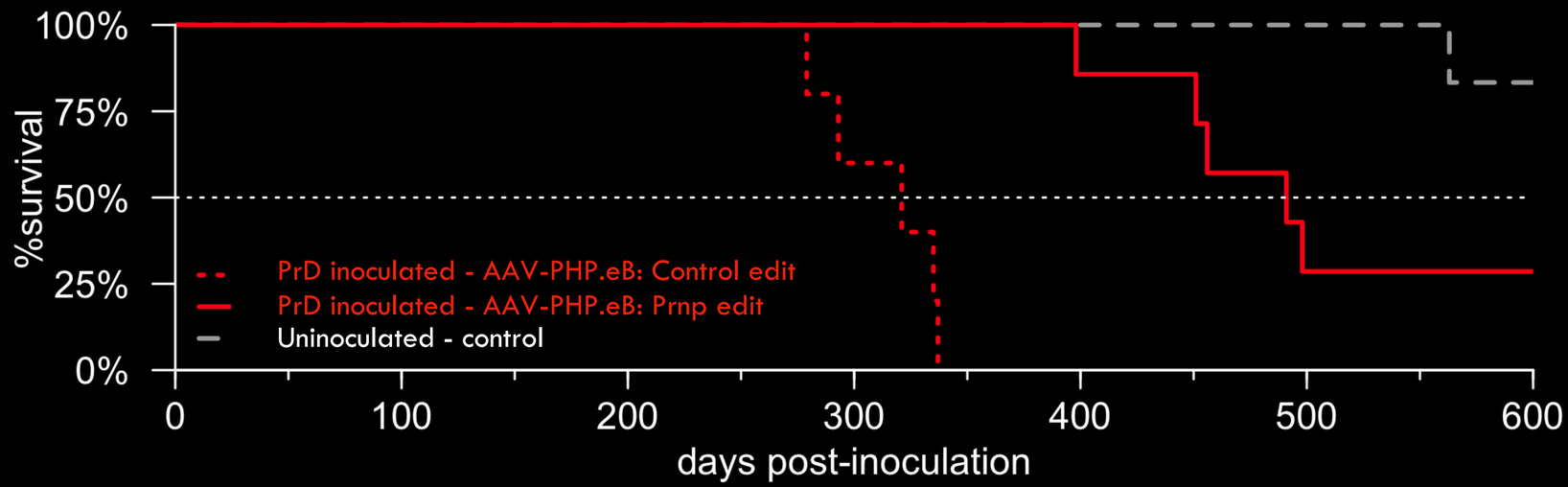
Lower PrP levels

- No established heath risk
- Prolong survival time

BROAD INSTITUTE

AAV-PHP.eB can be used to delivery a base editor to install early stop codons to Prnp to lower prion protein levels



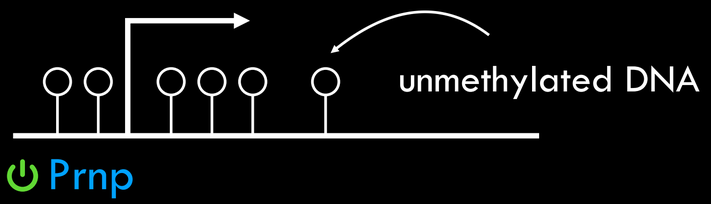


Meirui An, Eric Minikel, Sonia Vallabh, David Liu et al., unpublished

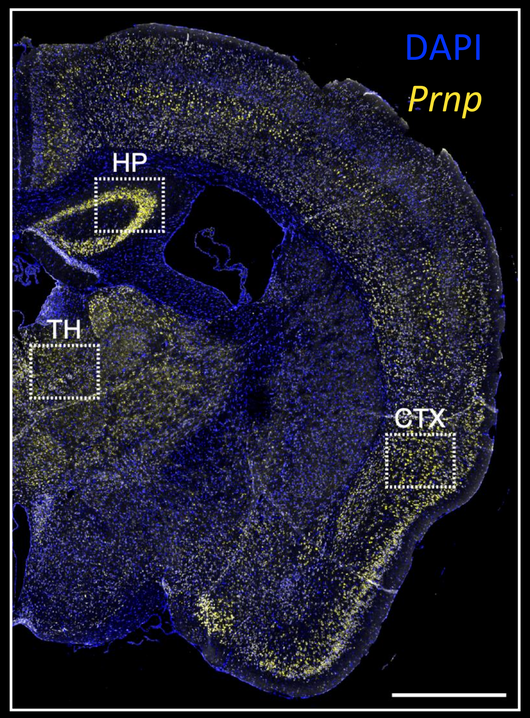
BROAD INSTITUTE

AAV-PHP.eB can be used to a delivery CHARM, an epigenetic silencer, to lower prion protein levels

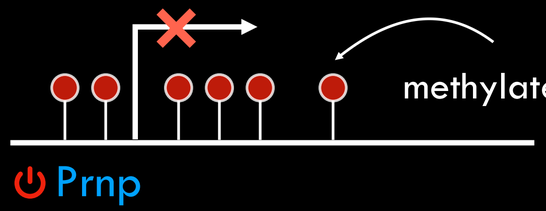
Control



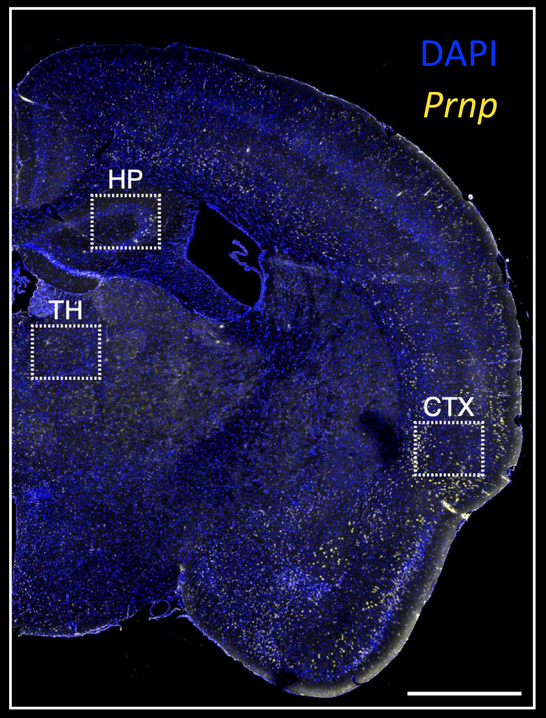
Prnp



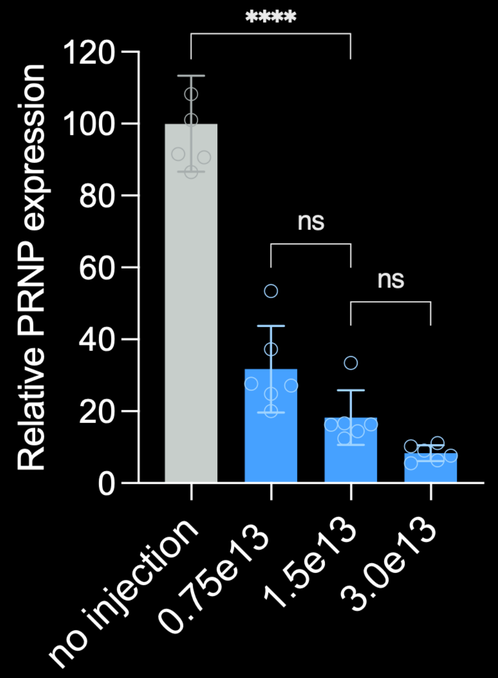
AAV-PHP.eB: CHARM



Prnp



AAV-PHP.eB: CHARM
Dose reponse (vg/kg)



Neumann & Bertozzi et al., Science (2024).
data modified for simplicity

BROAD INSTITUTE

Reprogrammable gene-based technologies paired with CNS-wide targeting capsids has great potential but…

Versatile gene-based technologies

Base editing

Gene addition

Gene editing

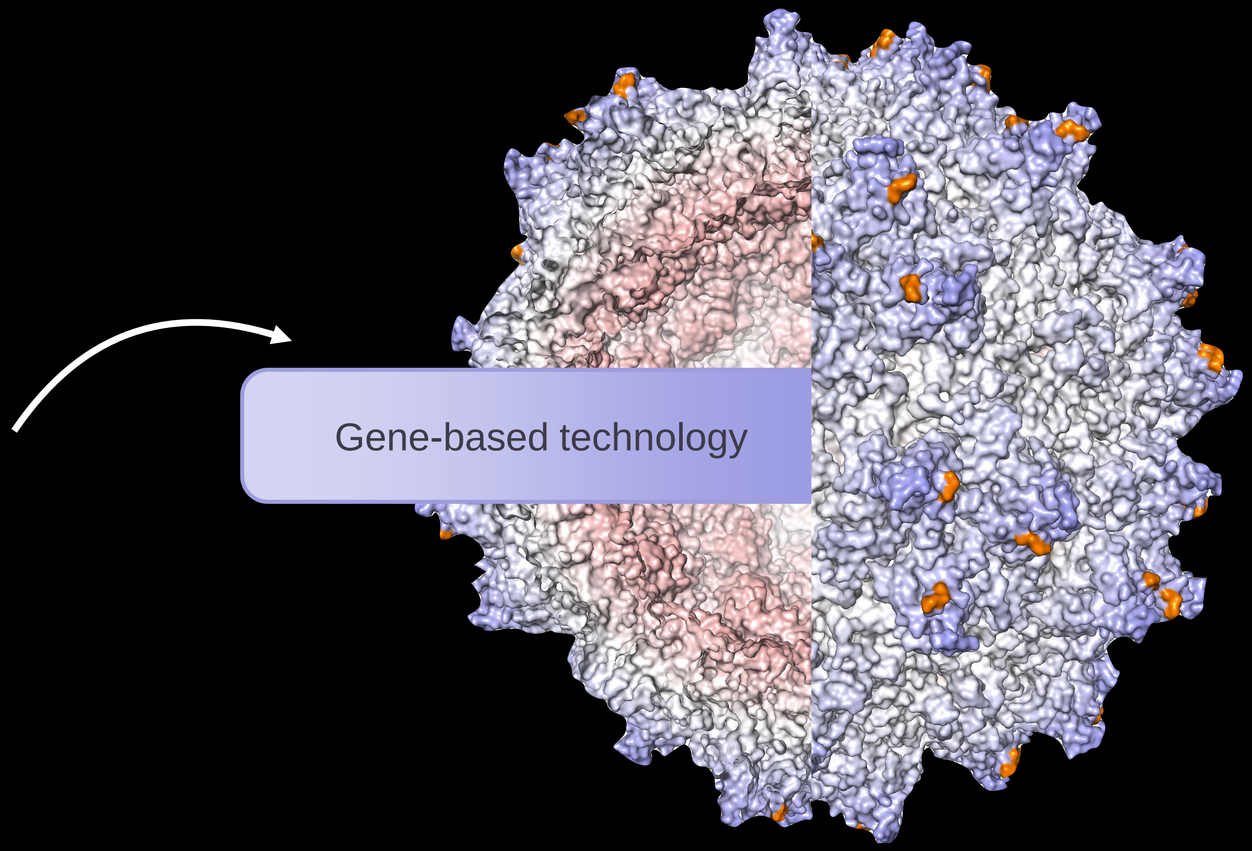
Prime editing

CRISPRoff

CHARM

miRNA KD

Trans-splicing



BROAD INSTITUTE

In vivo selections for CNS transducing AAVs have almost exclusively yielded capsids with species-specific enhancements

Mouse variants do not work in NHPs and vice versa

Papers describing improved mouse capsids

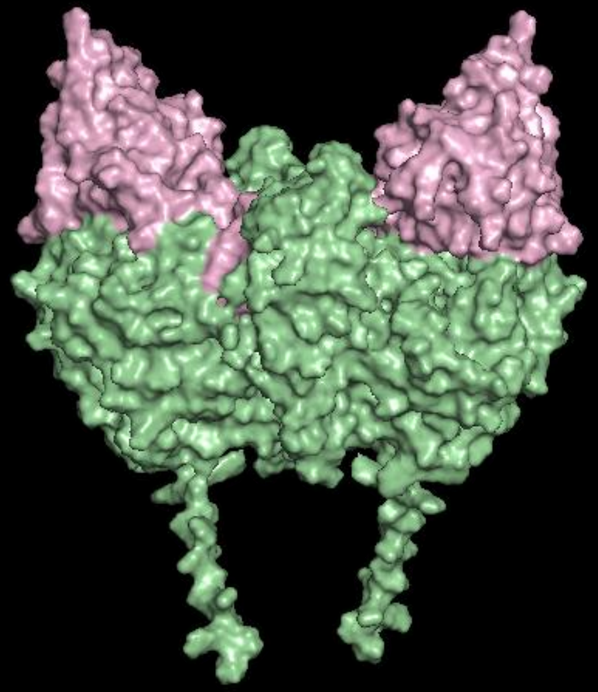
Deverman et al Nat Biotechnology 2016 (AAV-PHP.B)
Chan et al Nat Neurosci 2017 (AAV-PHP.eB)
Hanlon et al Mol Ther M&CD 2019
Kumar et al Nat Methods 2020
Nonnenmacher et al Mol Ther M&CD 2021
Huang et al bioRxiv 2022

Papers describing improved NHP capsids

Goertsen et al Nat Neurosci 2022
Chen et al Neuron 2022
Chuapoco et al bioRxiv 2022

BROAD INSTITUTE

We have reprogrammed AAV capsids to enter the CNS through interactions with the human Transferrin receptor



Human TfR1

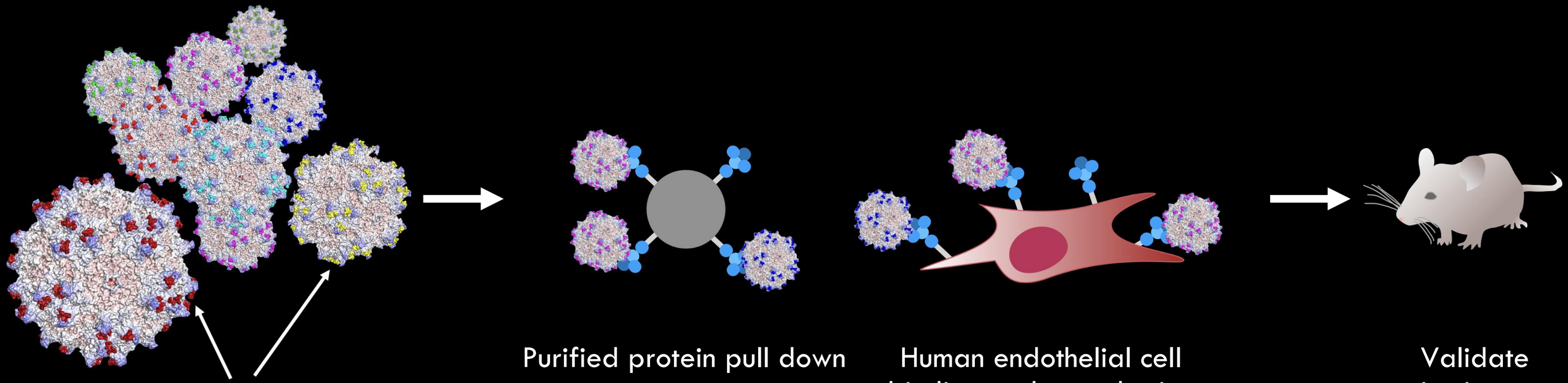
We chose TfR1 because

- ✓ It is highly expressed on human brain vasculature
- ✓ It is well characterized mediator of transcytosis across the blood brain-barrier
- ✓ Three TfR1 antibody-based therapeutics have demonstrated that TfR1 can be used to safely shuttle biologics into the CNS of humans

BROAD INSTITUTE

We screened highly diverse AAV libraries for variants that bind TfR1 using in vitro assays

Generate AAV capsid libraries with millions of variants



Randomized peptide insertion libraries

Purified protein pull down assays

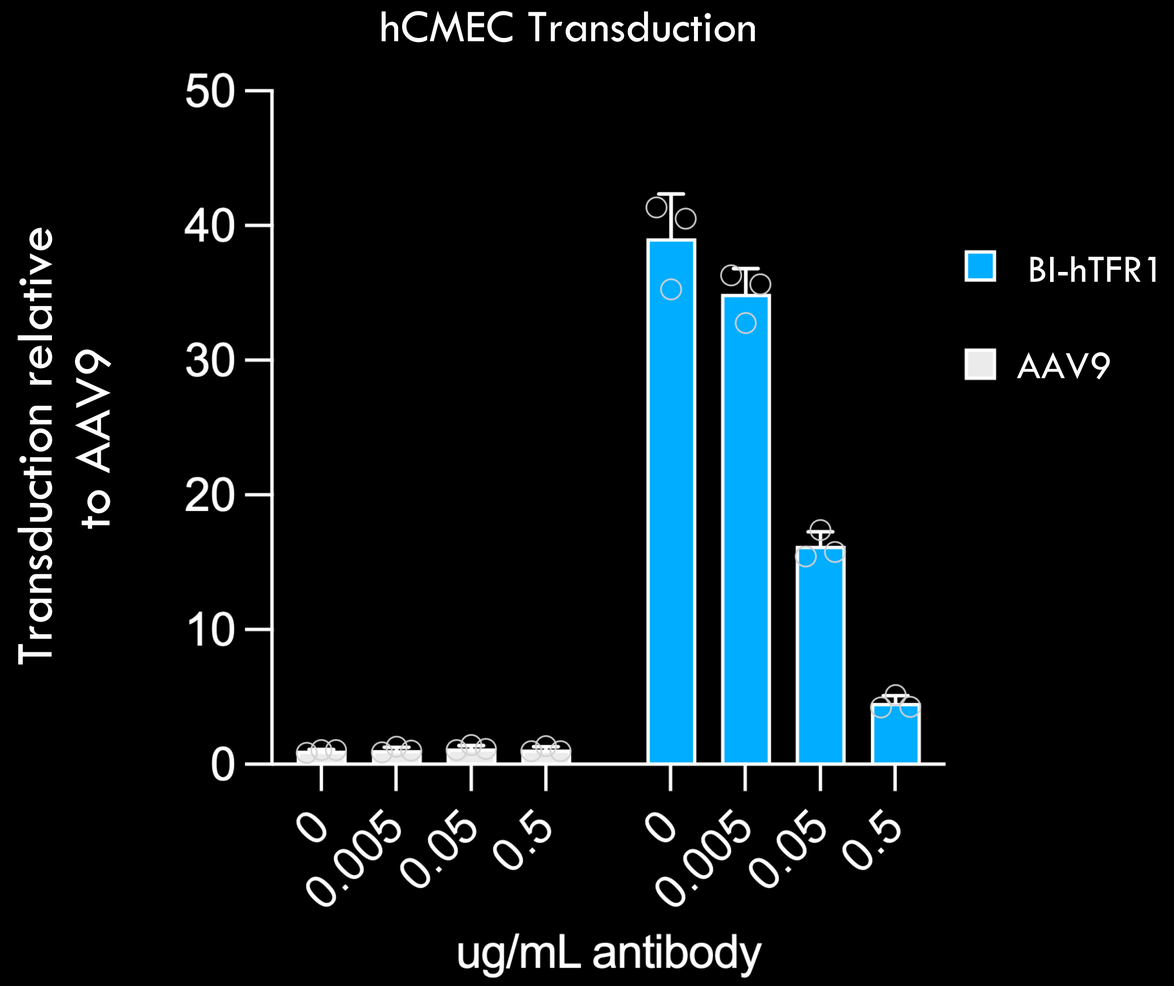
Human endothelial cell binding and transduction

Validate in vivo

Huang et al PLOS Bio 2023

BROAD INSTITUTE

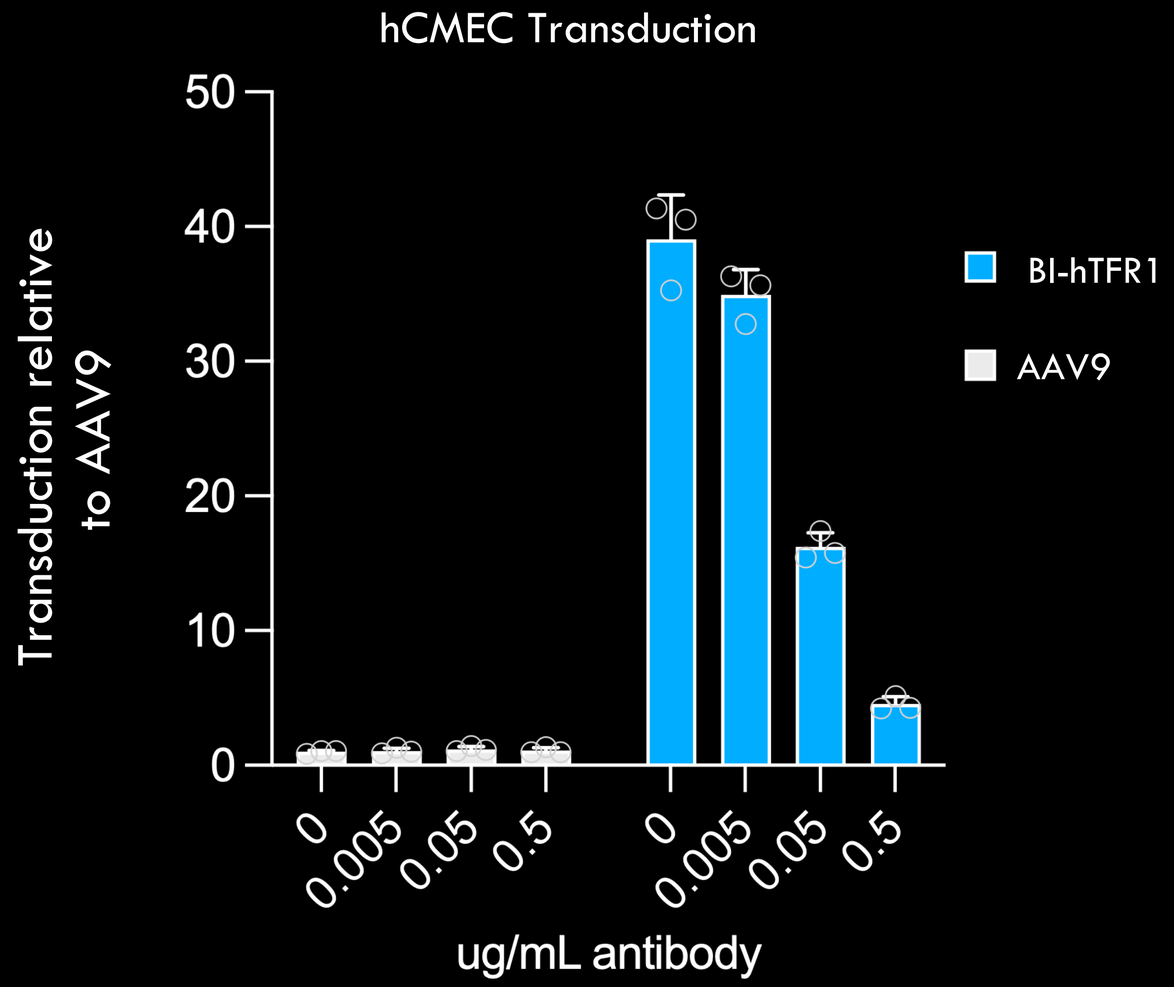
BI-hTFR1, a reprogrammed AAV that directly binds with Human Transferrin Receptor

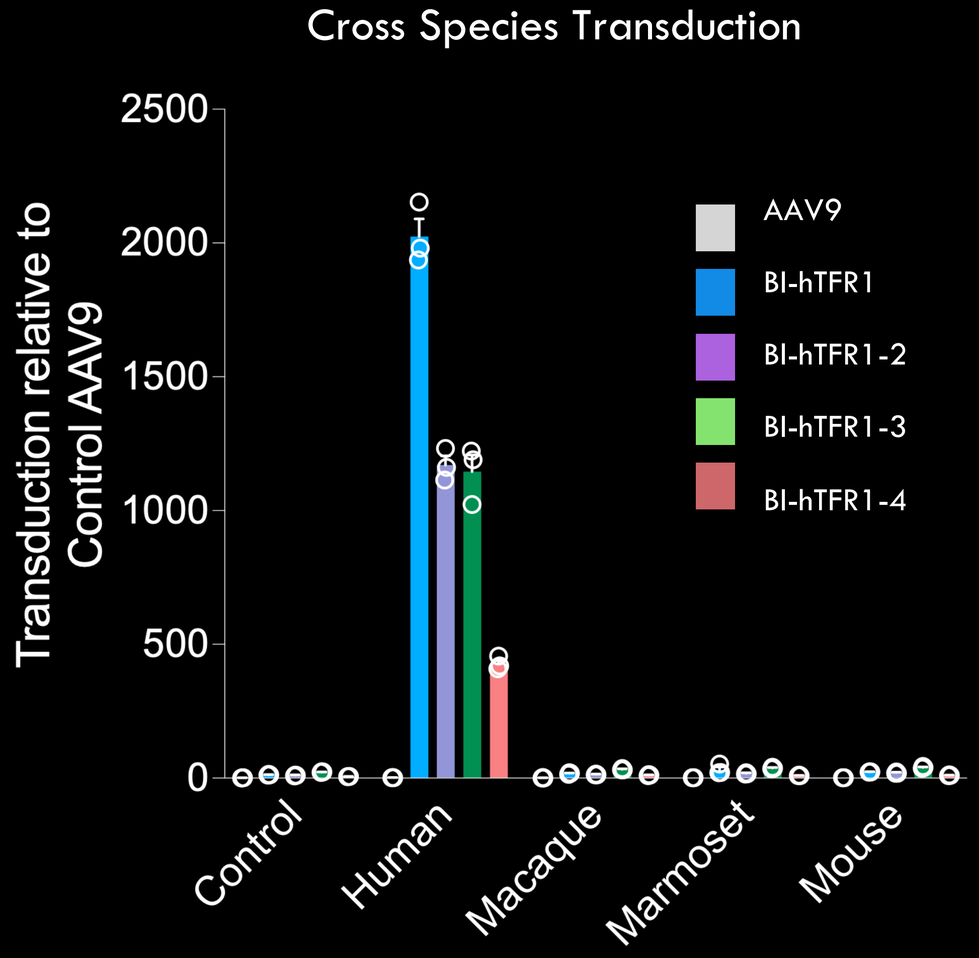


Huang and Chan et al, Science 2024

BROAD INSTITUTE

BI-hTFR1, a reprogrammed AAV that directly binds with Human Transferrin Receptor

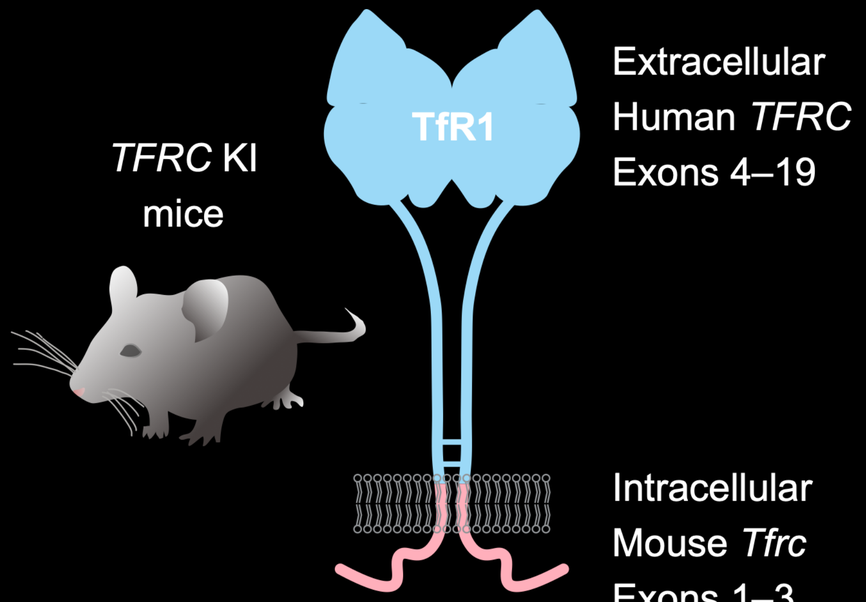




Huang and Chan et al, Science 2024

BROAD INSTITUTE

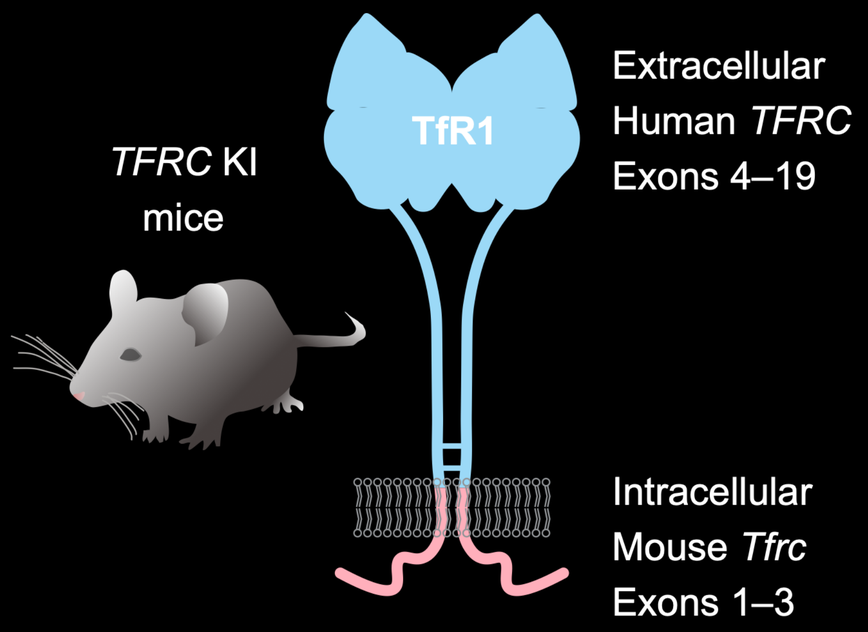
To evaluate BI-hTFR1 in vivo, we used human transferrin receptor knock in mice

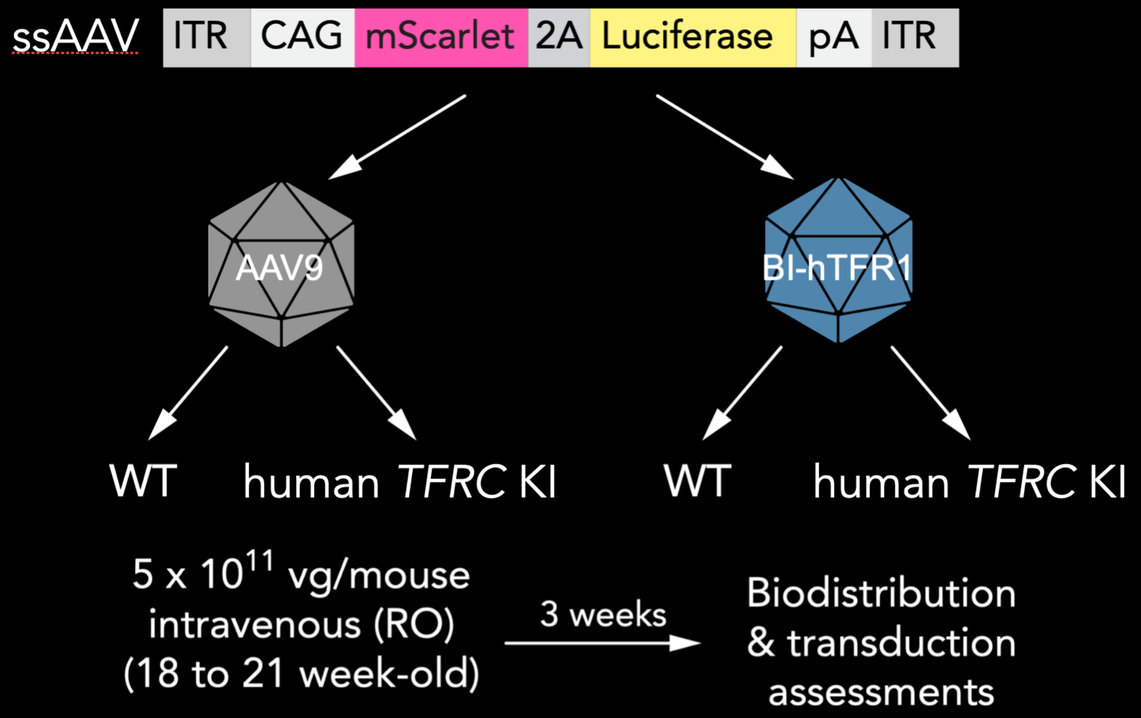


Huang and Chan et al, Science 2024

BROAD INSTITUTE

To evaluate BI-hTFR1 in vivo, we used human transferrin receptor knock in mice

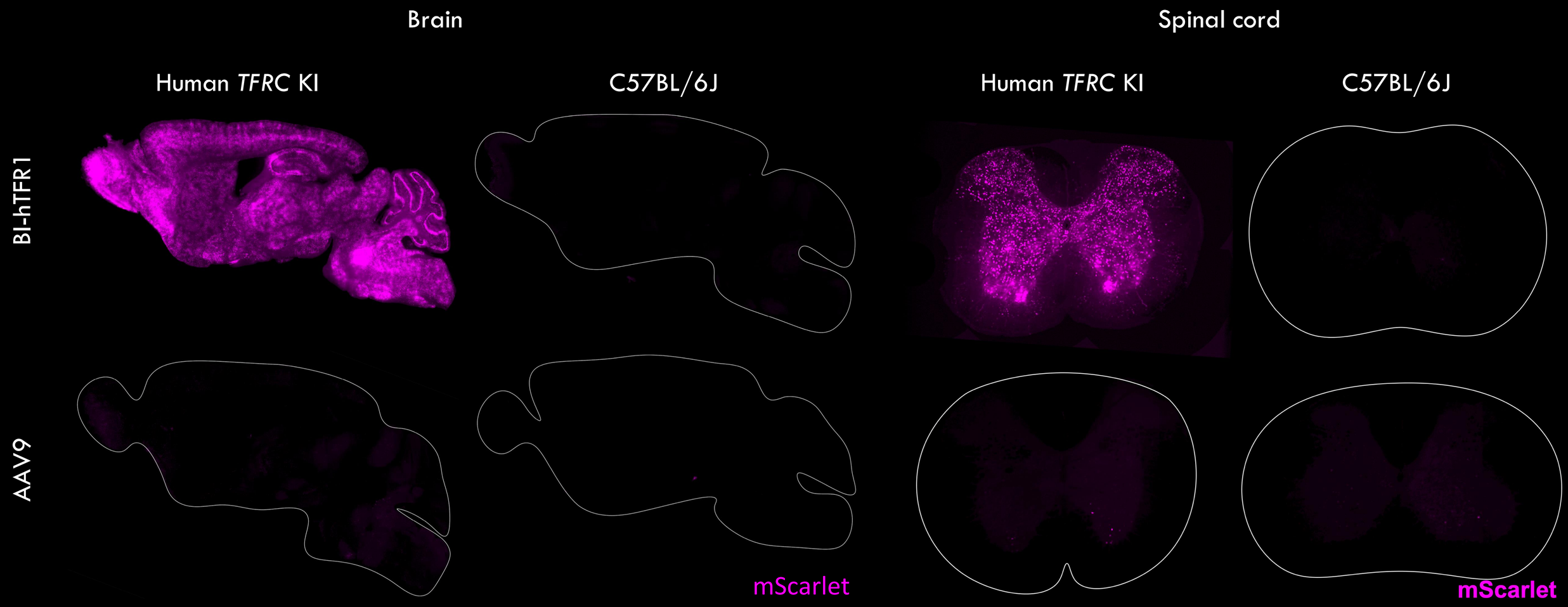




Huang and Chan et al, Science 2024

BROAD INSTITUTE

BI-hTFR1 has a strongly enhanced CNS tropism that requires human transferrin receptor expression

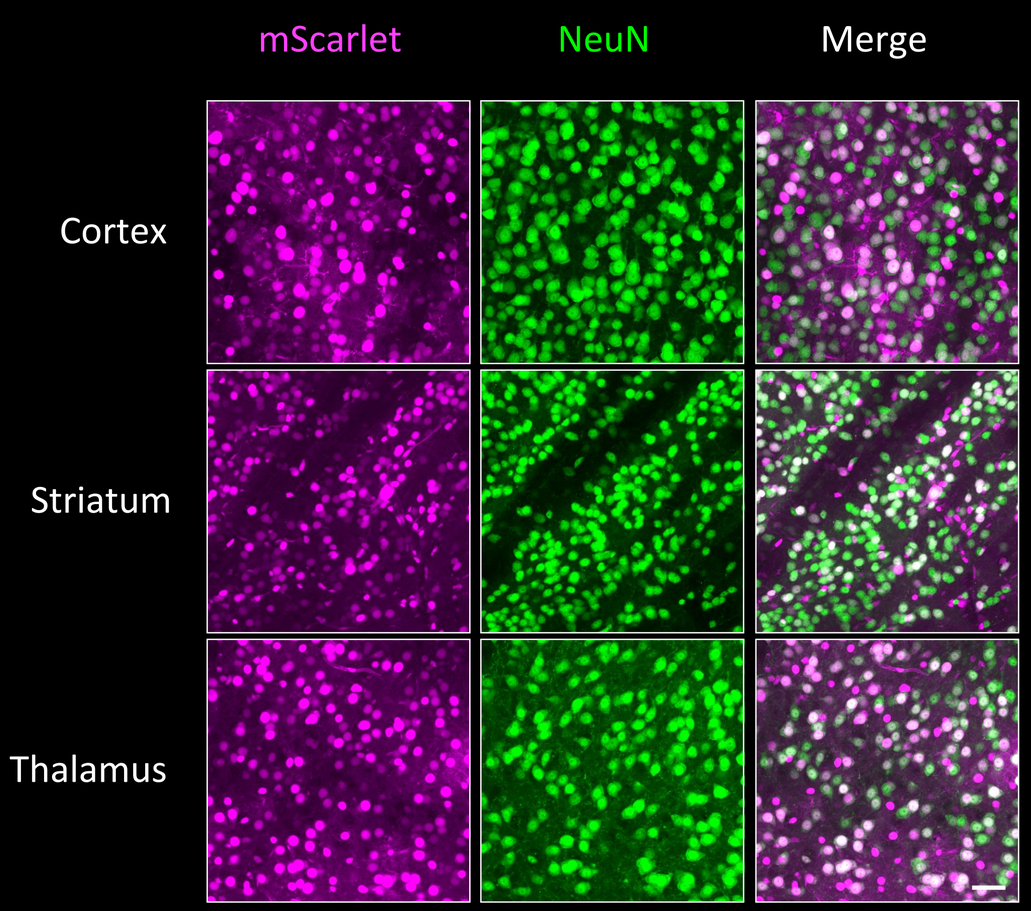


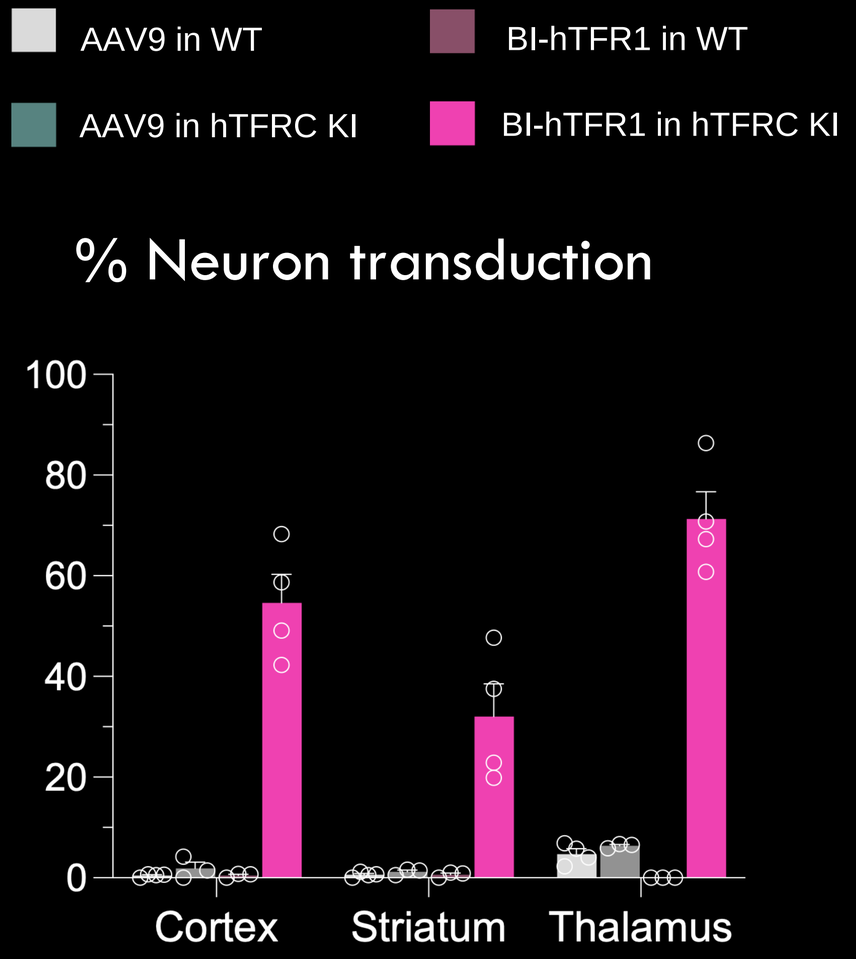
$5x10^{11}$ vg ssAAV:CAG-NLS-mScarlet-2A-luc-pA, 21 days post injection

Huang and Chan et al, Science 2024

BROAD INSTITUTE

BI-hTFR1 efficiently transduces neurons and glia throughout the brain when delivered to Human TFRC KI Mice

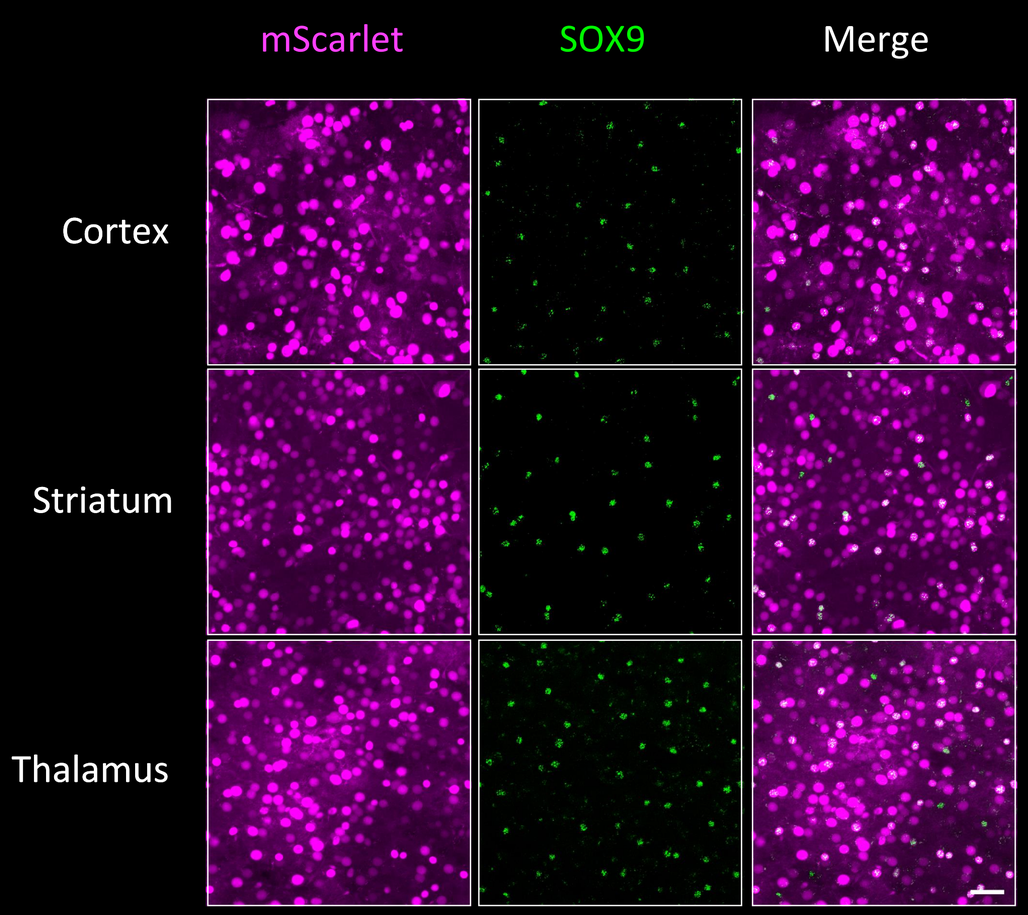


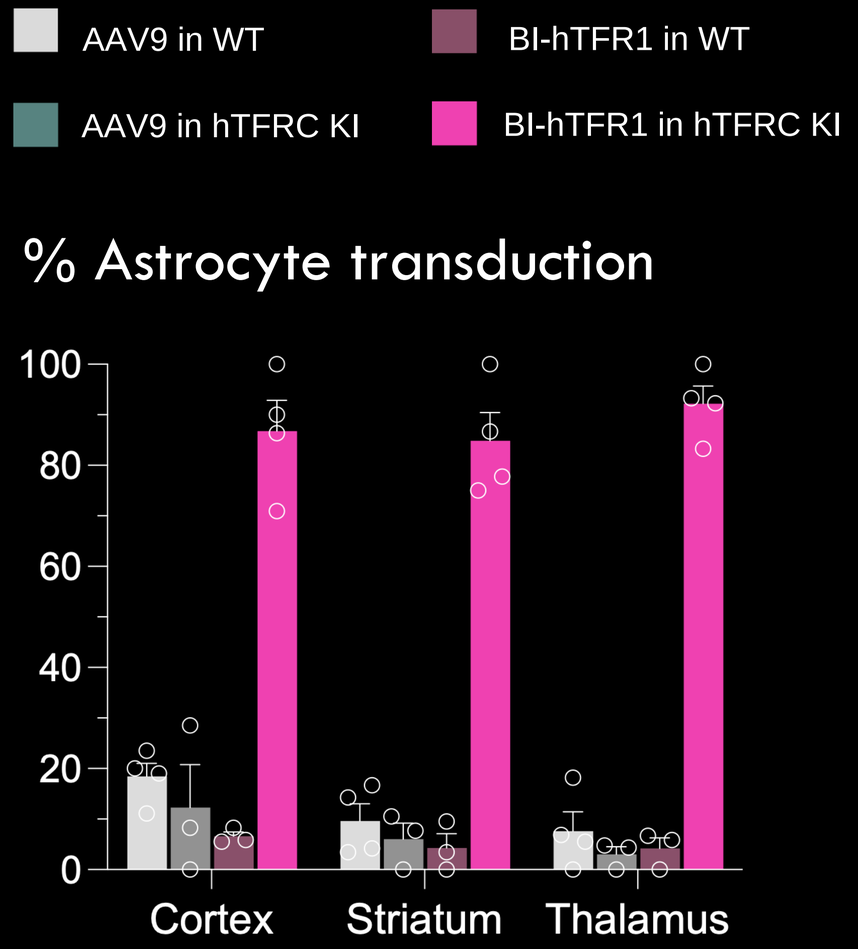


Huang and Chan et al, Science 2024

BROAD INSTITUTE

BI-hTFR1 efficiently transduces neurons and glia throughout the brain when delivered to Human TFRC KI Mice

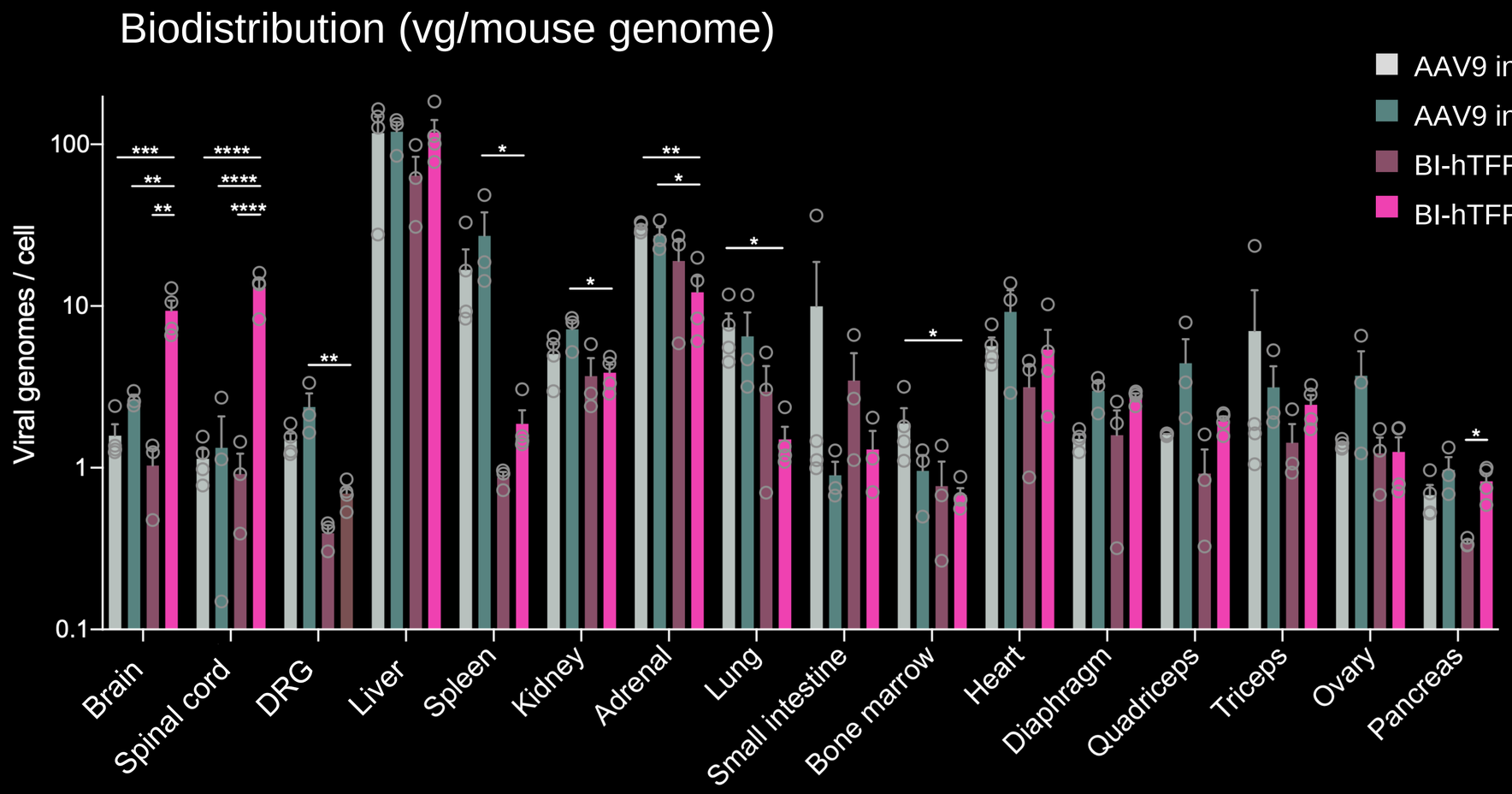




Huang and Chan et al, Science 2024

BROAD INSTITUTE

BI-hTFR1 has a CNS selective increase in biodistribution in transferrin receptor knock in mice



$5x10^{11}$ vg, 21 days post injection ssAAV:CAG-NLS-mScarlet-2A-luc

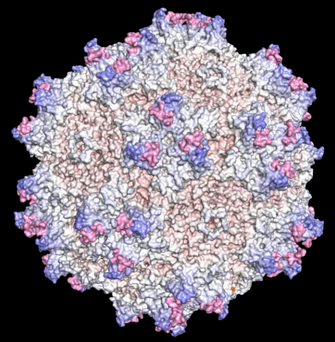
Huang and Chan et al, Science 2024

BROAD INSTITUTE

Reprogramming AAVs to target Human Transferrin Receptor with multiple optimized functions

in vitro TfR1 screen

- Cell based receptor expression
- Pull down with purified proteins



BI-hTFR1

in vivo and in vitro functional screen

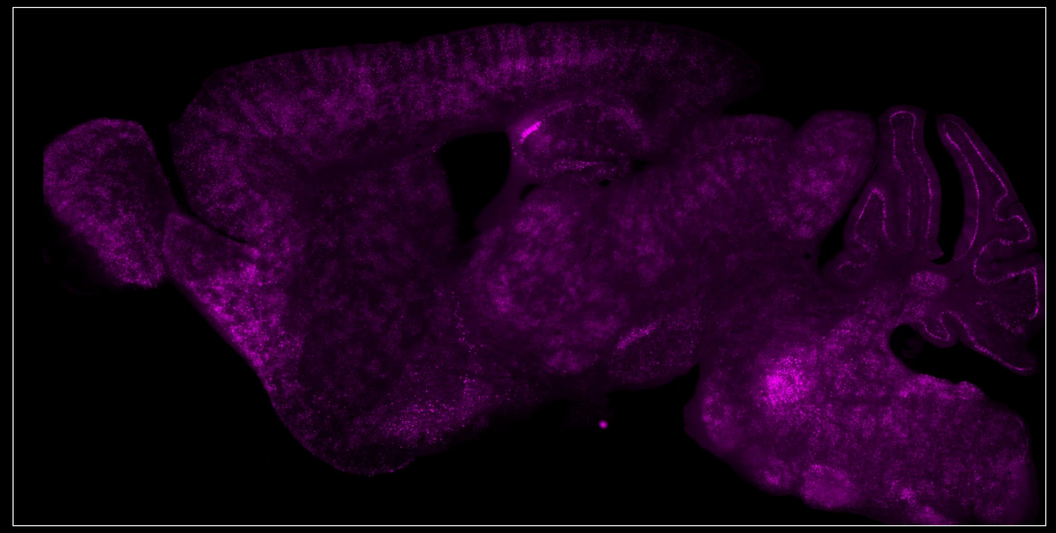
- Enhanced CNS transduction
- Liver detargetting
- Production fitness

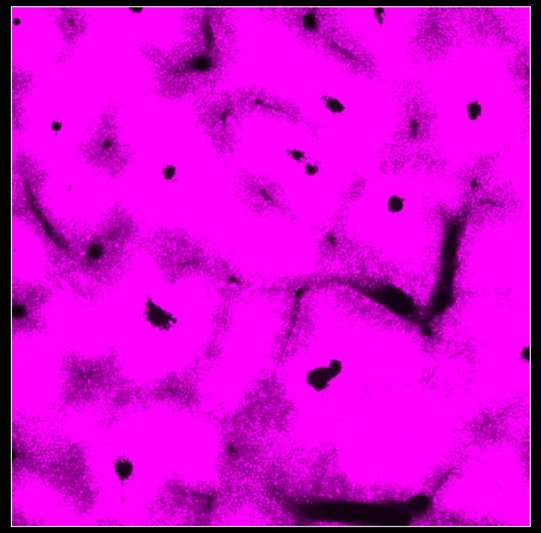
2nd generation variants

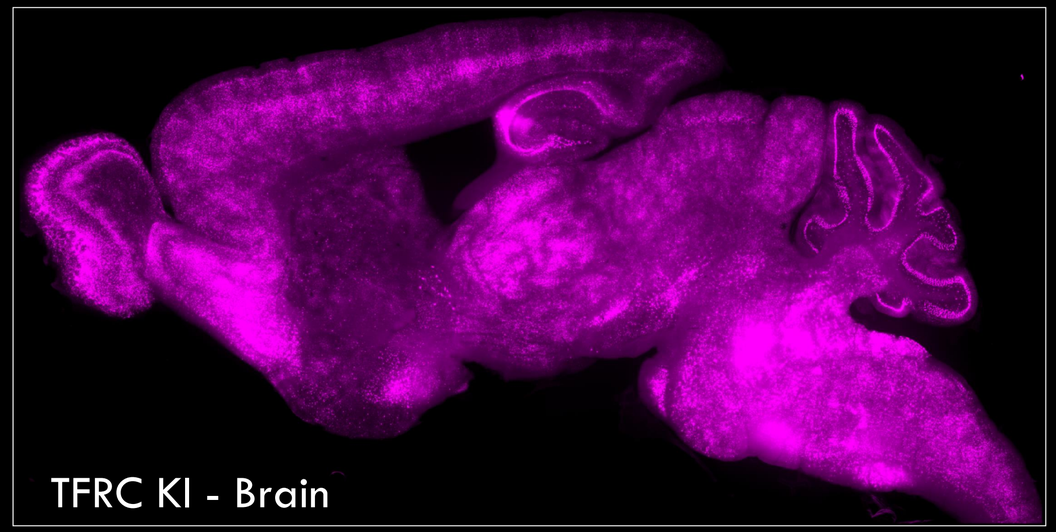
Huang and Chan et al, bioRxiv, 2023
Huang et al., PLOS Biology, 2023

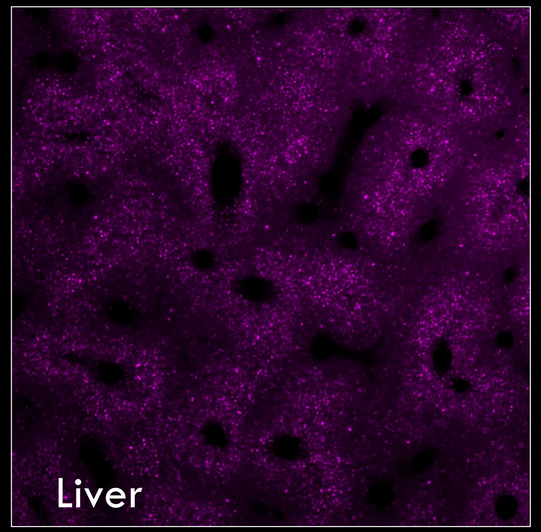
BROAD INSTITUTE

Preliminary data suggests that we have identified a 2nd generation BI-hTFR1 capsid with improved CNS tropisms and liver detargeting









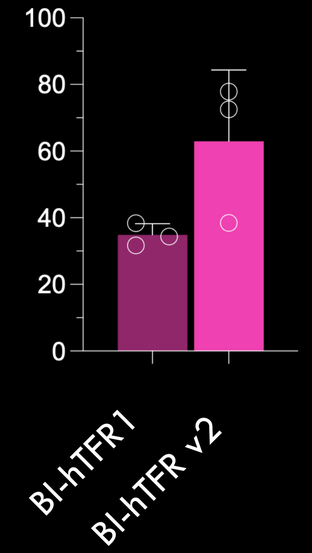
BI-hTFR 1

BI-hTFR v2

TFRC KI - Brain

Liver

% Cortical neuron transduction



ssAAV:CAG-NLS-mScarlet-2A-luc
$5x10^{10}$ vg, 21 days post injection

~50x lower dose (vg/kg) than that used for Zolgensma

Deverman lab Unpublished

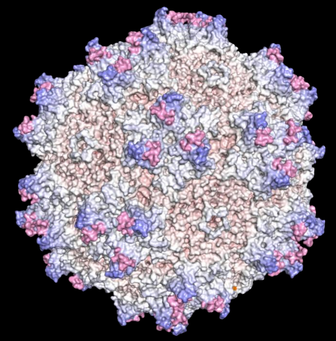
BROAD INSTITUTE

Reprogramming AAVs to target Human Transferrin Receptor with improved pre-existing nAB evasion

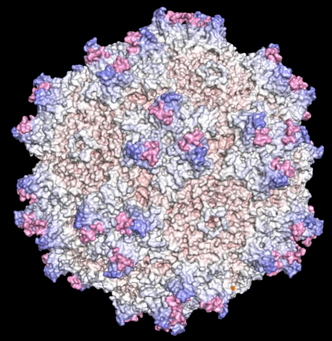
in vitro TfR1 screen

in vivo and in vitro functional screen

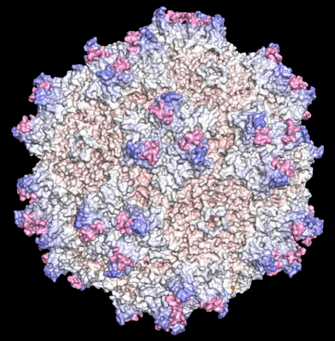
in vivo and in vitro functional screen for nAB evasion



BI-hTFR1



BI-hTFR v2

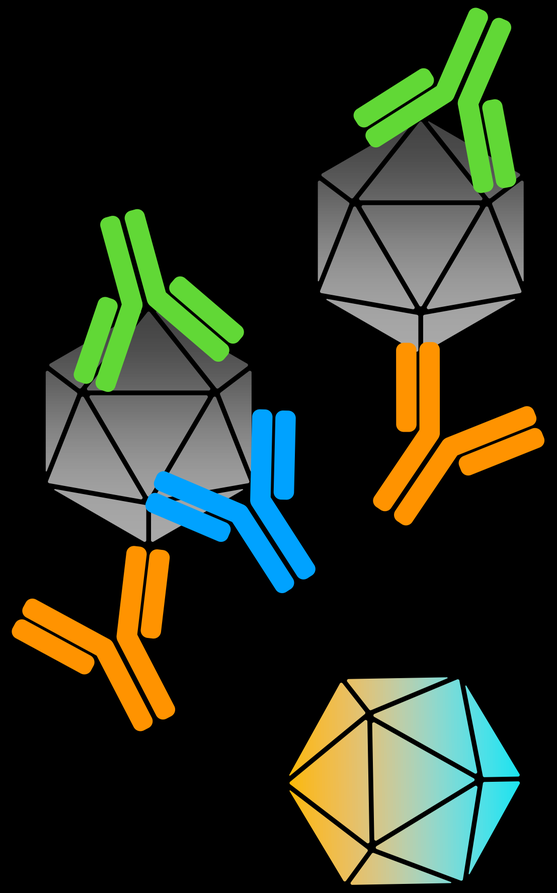


BI-hTFR v2 AE

- Better evasion of pre-existing nABs
- Similar CNS transduction to v2
- Similar production fitness

Huang and Chan et al, bioRxiv, 2023
Huang et al., PLOS Biology, 2023

Pre-existing neutralizing antibodies present in a substantial fraction of patients restrict access to gene therapies



Pre-existing antibodies

- AAV capsid antibodies are present in a large fraction of patients due to natural AAV infections
- Neutralizing antibodies block AAV function
- Anti-capsid antibodies increases the risk for complement activation which has been associated with adverse events in clinical trails.

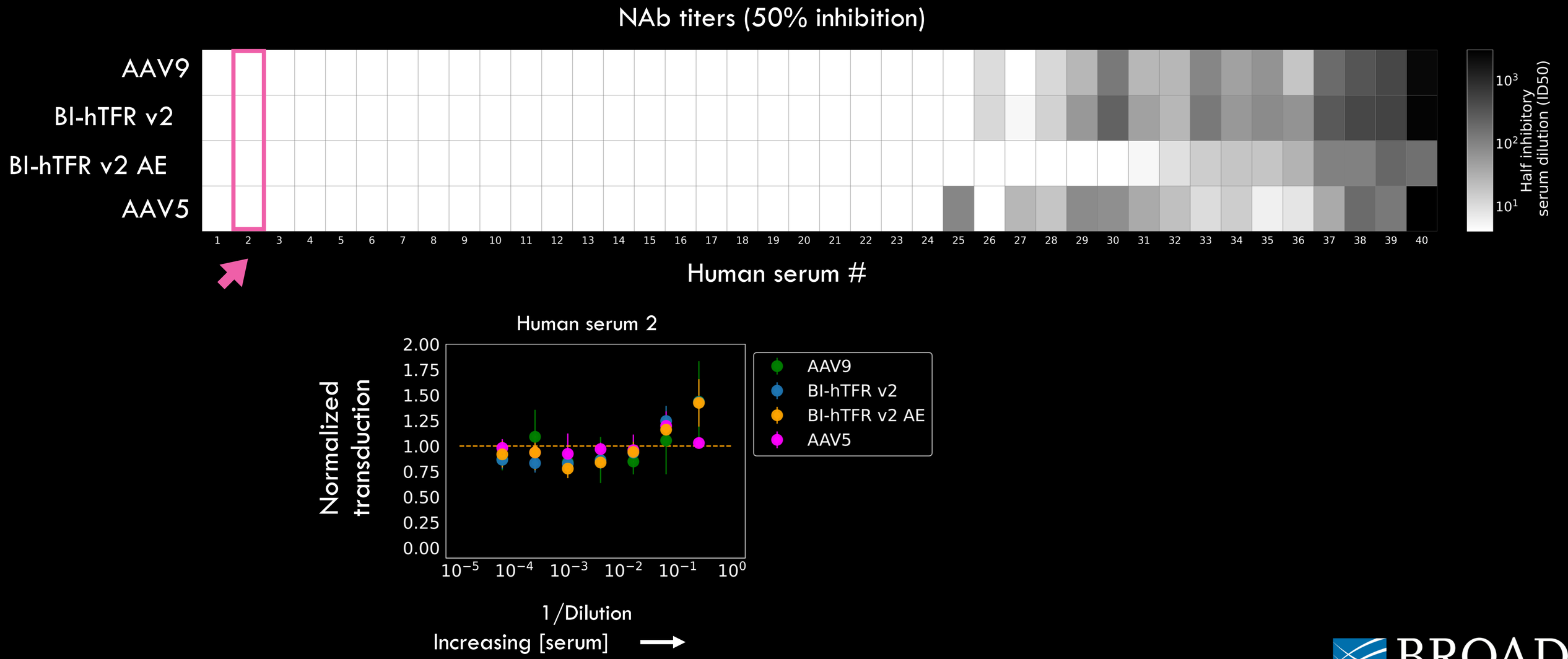
BROAD INSTITUTE

BI-hTFR v2 AE evades pre-existing neutralizing Ab in a subset of human serum samples with antibodies to AAV9 and AAV5

Simon Pacouret, et al. unpublished

BROAD INSTITUTE

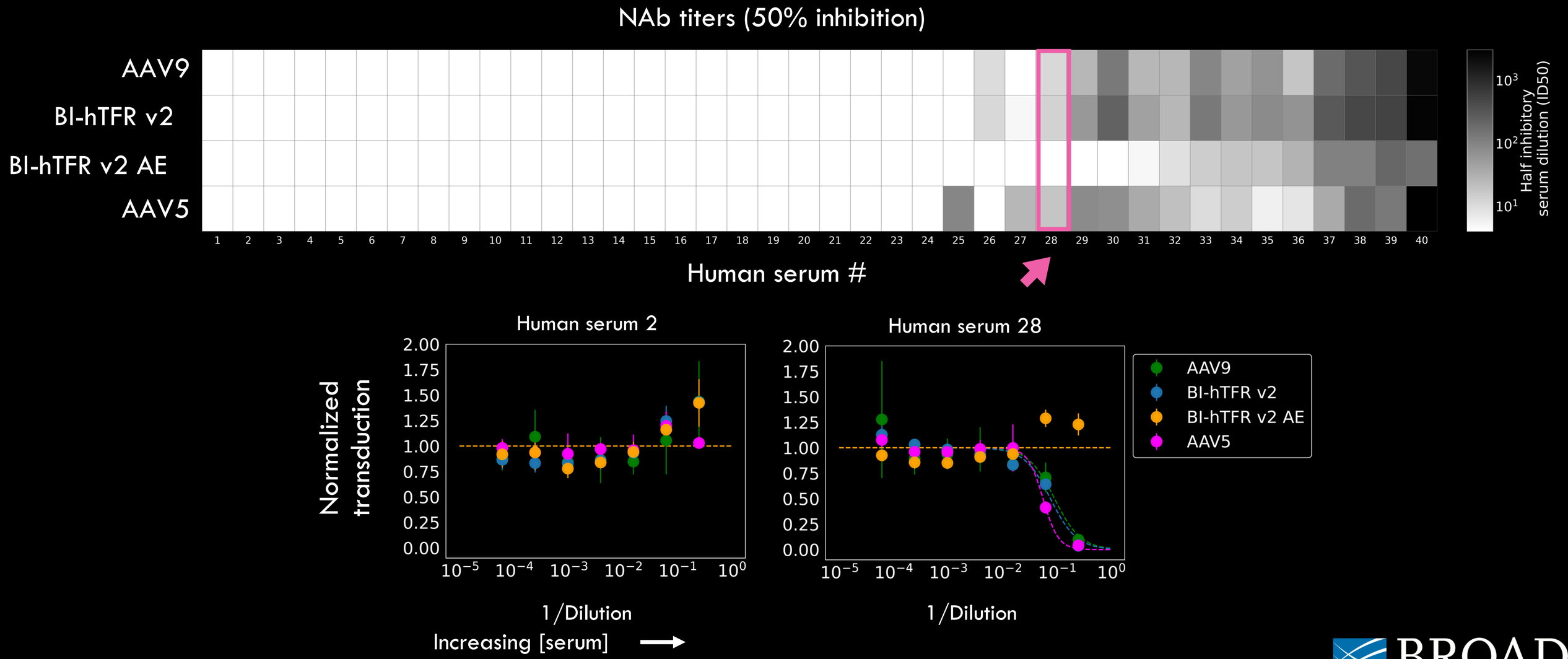
BI-hTFR v2 AE evades pre-existing neutralizing Ab in a subset of human serum samples with antibodies to AAV9 and AAV5



Simon Pacouret, et al. unpublished

BROAD INSTITUTE

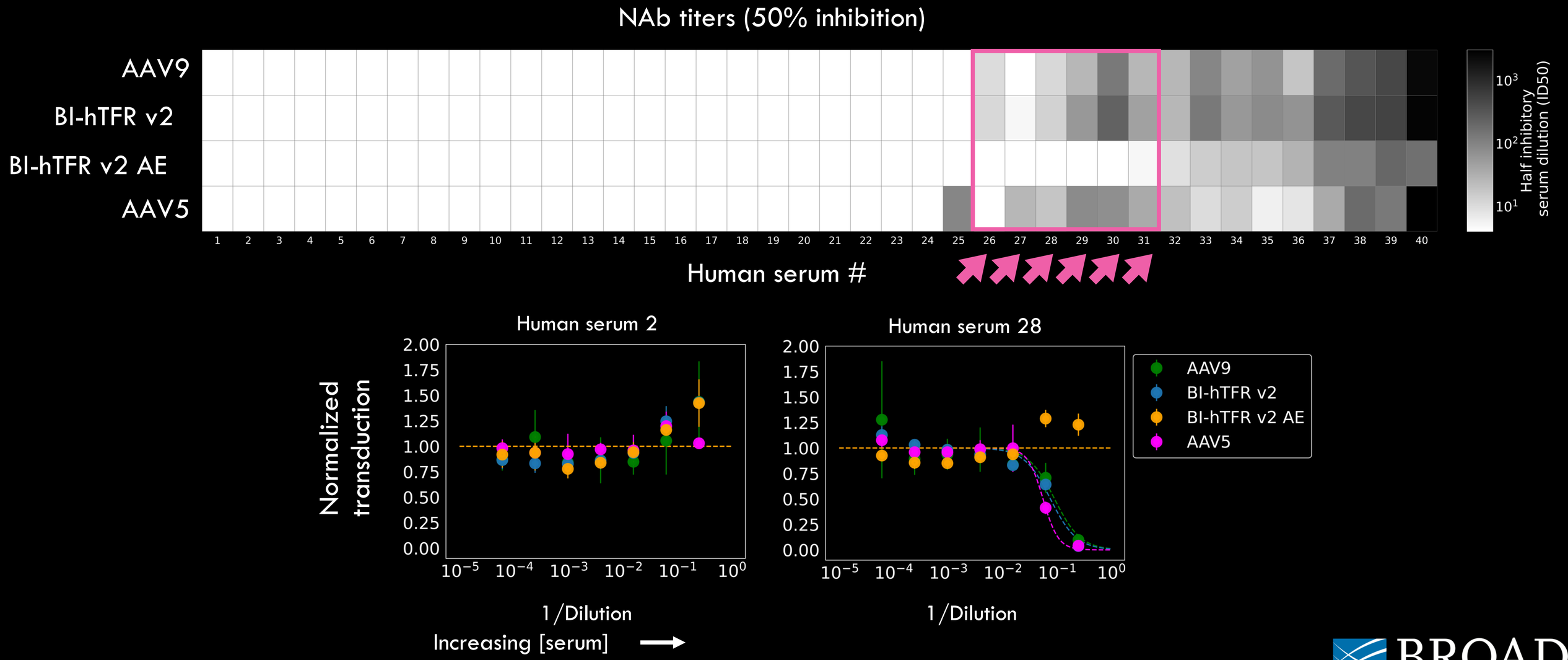
BI-hTFR v2 AE evades pre-existing neutralizing Ab in a subset of human serum samples with antibodies to AAV9 and AAV5



Simon Pacouret, et al. unpublished

BROAD INSTITUTE

BI-hTFR v2 AE evades pre-existing neutralizing Ab in a subset of human serum samples with antibodies to AAV9 and AAV5



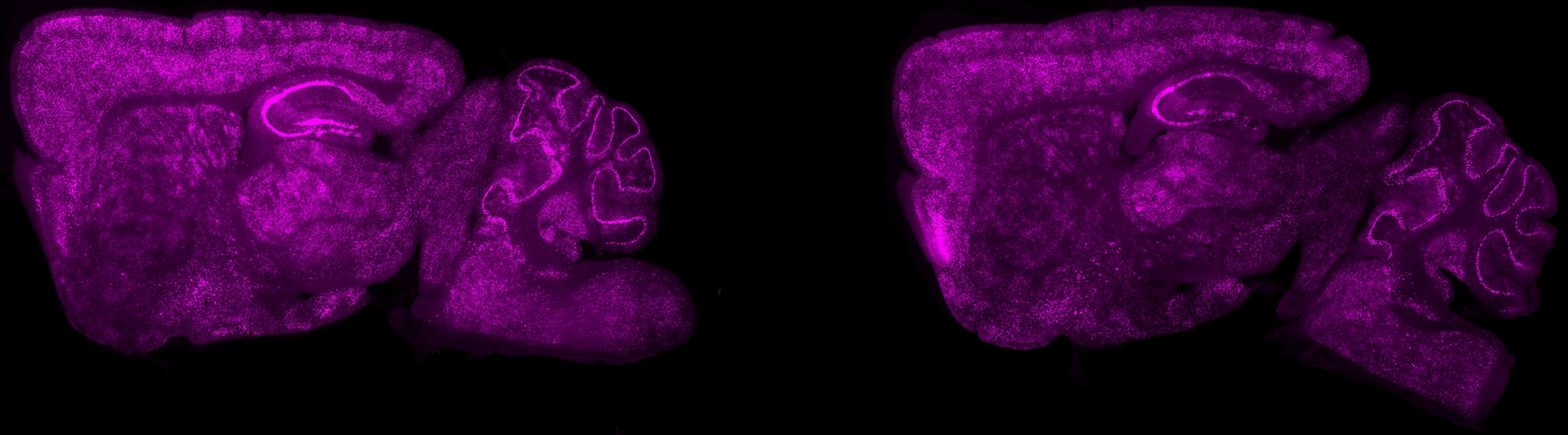
Simon Pacouret, et al. unpublished

BROAD INSTITUTE

BI-hTFR v2 AE maintains enhanced CNS transduction

BI-hTFR v2 (2nd gen capsid)

BI-hTFR v2 AE (better at nAB evasion)



ssAAV:CAG-NLS-mScarlet-2A-luc-WPRE
$1x10^{11}$ vg, 21 days post injection at 3 weeks

Simon Pacouret, et al. unpublished

BROAD INSTITUTE

We believe we are on track to achieve our ideal engineered AAV capsid that could be used in humans

Summary of capsid

Property	Status
Route of administration	IV with known mechanism-of-action
Neuron transduction efficiency	>40-80+% across all brain regions in our 2nd generation capsids
Target dose	~2E12 vg/kg
Organ detargeting	Potentially: 2nd gen capsids are detargeted from the liver by at least 10x
NAb evasion	Our top NAb evading capsid was free of neutralization in 31 of 40 (78%) of human sera
Manufacturability	Within 2-fold of AAV9

BROAD INSTITUTE

Conclusion / overall vision

- Reprogrammed an AAV to bind human transferrin receptor that provides efficient CNS-wide transduction and evaluated BBB transcytosis in humanized mice
- Working closely with Sonia Vallabh and Eric Minikel's lab who are leading efforts to pair these AAVs with promising genetic technologies to lower PrP levels forward for a clinical trial
    - Committed to sharing learnings and evaluation broadly to the community to apply towards other severe neurodegenerative diseases

BROAD INSTITUTE

Acknowledgements

Vector Engineering lab
Ben Deverman
Qin (Helen) Huang
Simon Pacouret
Jason Wu
Qingxia Zheng
Nuria Botticello-Romero
Jean Lin
Will Donahoe
Alexander Svanbergsson
Allan Mills
Alina Chan
Shan Lou
Casey Keyes
Andy Barry
Fatma-Elzahraa Eid

Albert Chen
Jencilin Johnston
Pam Brauer
Jenna Hurley
John Harvey
Jarrett Rios
Catherine Pirtle
Saša Jereb
Lia D'Alessandro
Binhui Zhao
Hikari Sorensen
Gabrielle Clouse
Thomas Beddow
Isabelle Tobey

Vallabh-Minikel lab
Sonia Vallabh
Eric Vallabh Minikel
Alissa Coffey
Fiona Search
Nikita Kamath

Weissman lab
Jonathan Weissman
Edwin Neumann
Tessa Bertozzi
Elaine Wu

Liu lab
David Liu
Meirui An
Jessie Davis
Jonathan Levy

CGE Somatic Cell Genome Editing

Apertura GENE THERAPY

STANLEY CENTER FOR PSYCHIATRIC RESEARCH AT BROAD INSTITUTE

BROAD INSTITUTE