

A targeted approach for gene therapy in *SLC6A1* related disorder

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<https://galibraneuroscience.com/>

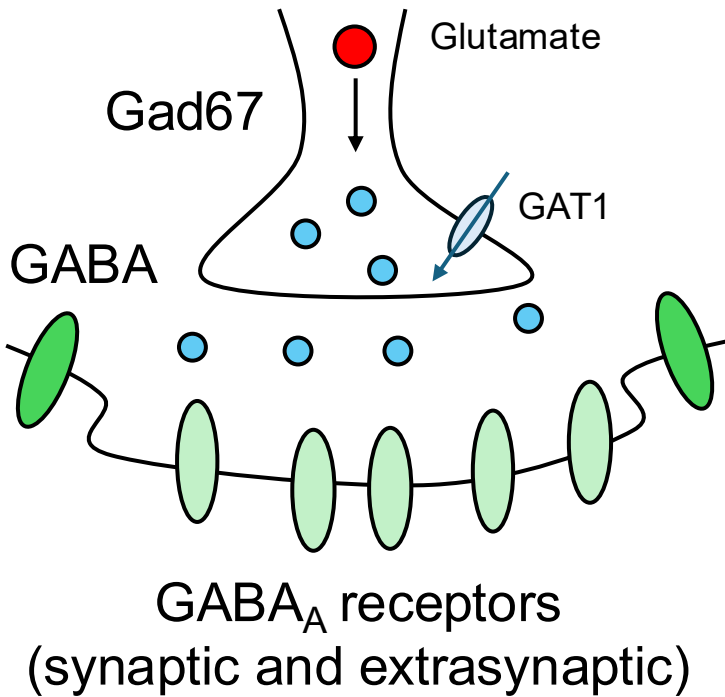
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Outline

- Introduce key challenges for gene therapy in *SLC6A1* disorders
- Review current status of *SLC6A1*-targeted gene therapy
- Discuss ongoing work and preliminary findings

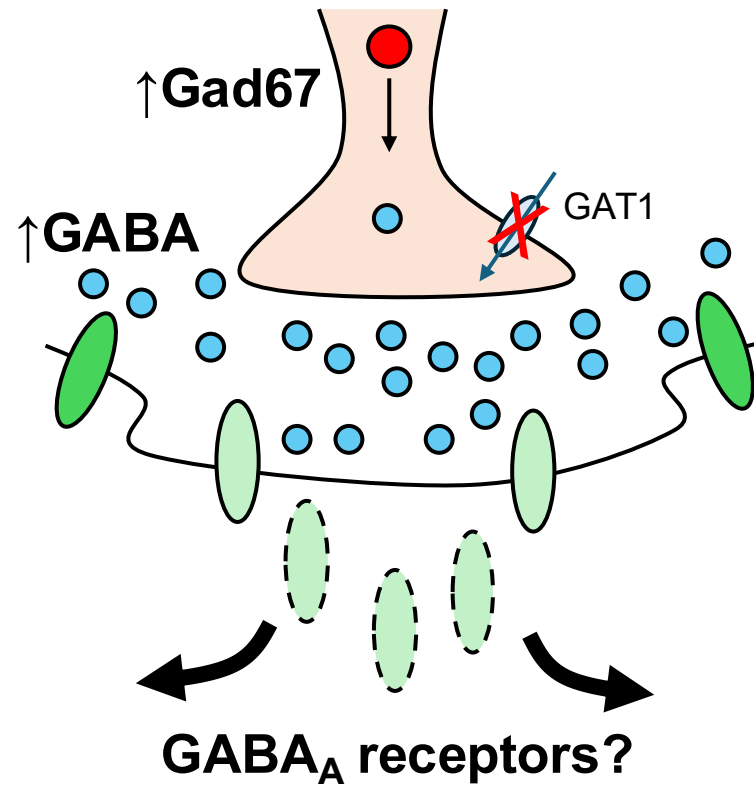
Challenge #1: compensatory GABA signaling in *SLC6A1*-related disorders

A Neurotypical



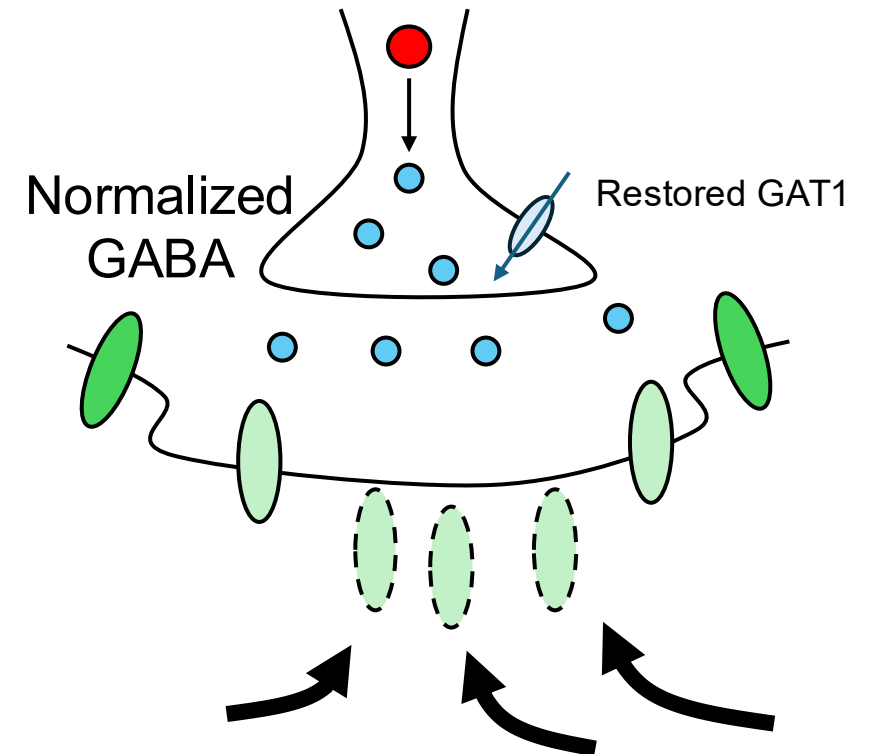
Balanced GABA and GABA_AR
Normal inhibitory tone

B *SLC6A1* disorders



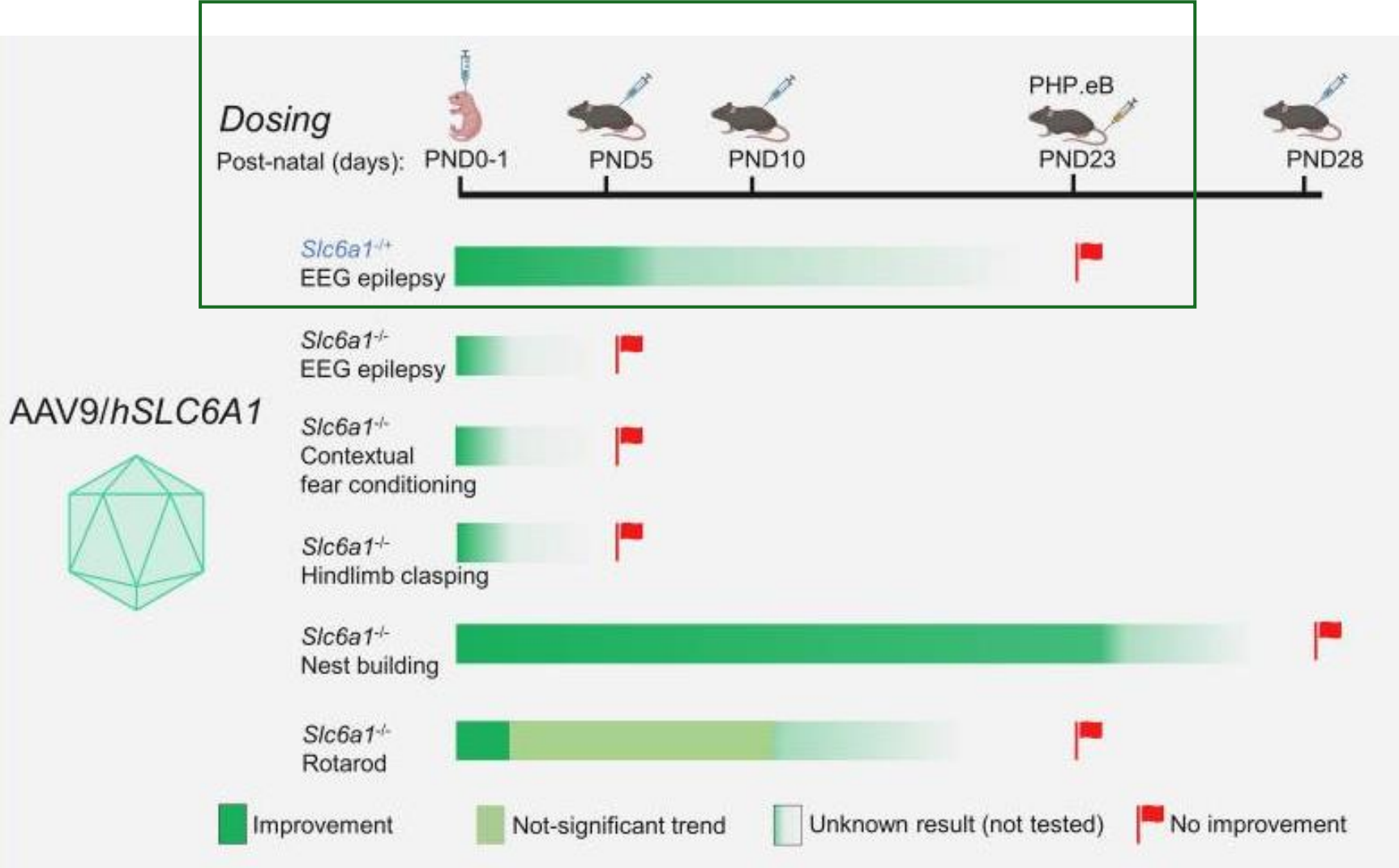
↑Tonic inhibition
Absence seizures

C *SLC6A1* disorders+ gene therapy



Tonic inhibition renormalized?
GABA/Gad67 restored?

Challenge #2: therapeutic window of effective *SLC6A1*-targeted gene therapy might be limited

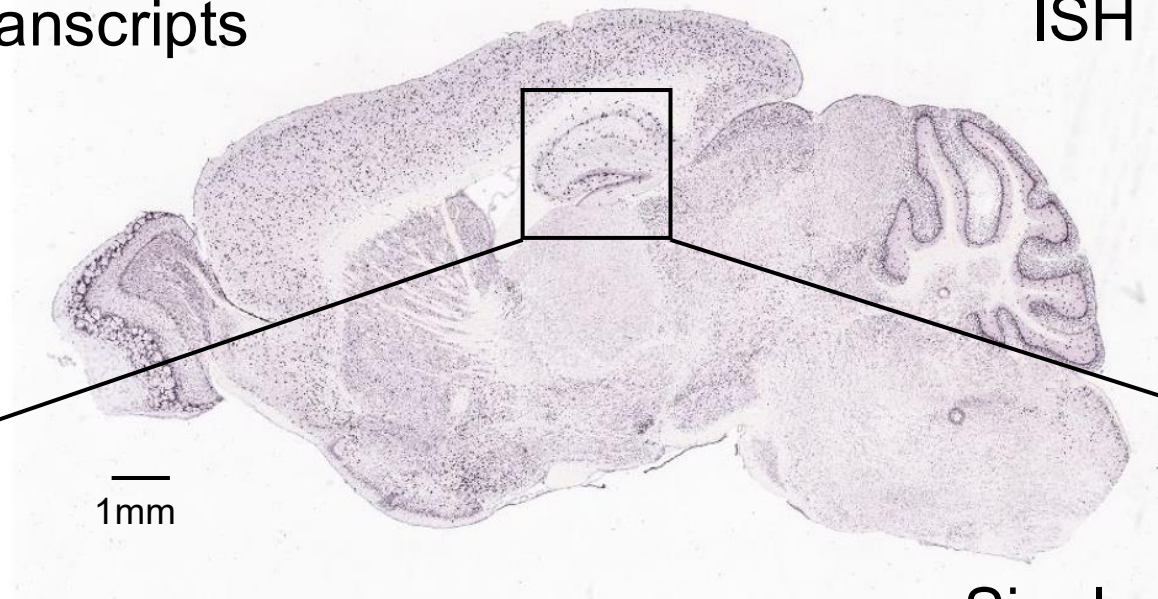


Challenge #3: *Slc6a1* expression is cell-restricted

A

Slc6a1 transcripts

ISH data



Allen Brain Institute

Linnarsson Lab
Karolinska Institute

B

Molecules/cell

150
100
50
0

Interneurons

Pyramidal neurons

Others (astrocytes, oligodendrocytes)

Single-cell RNAseq data

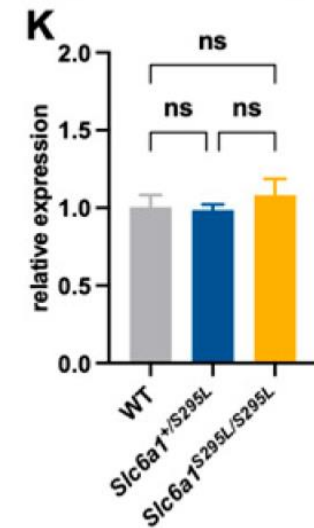
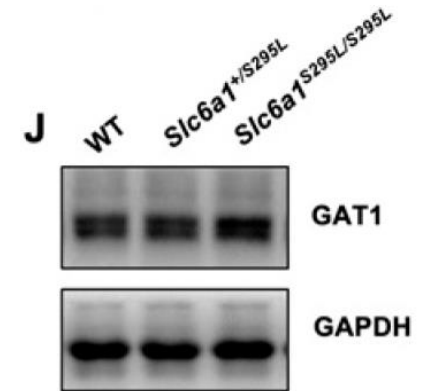
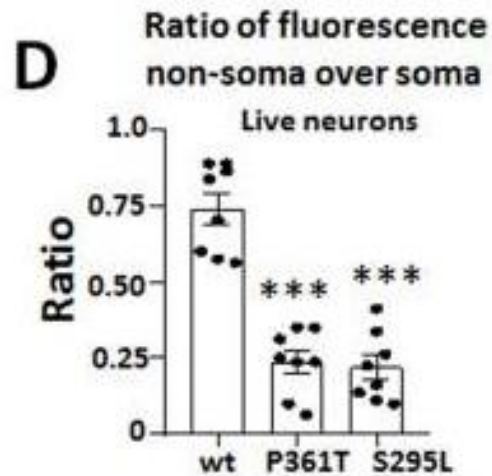
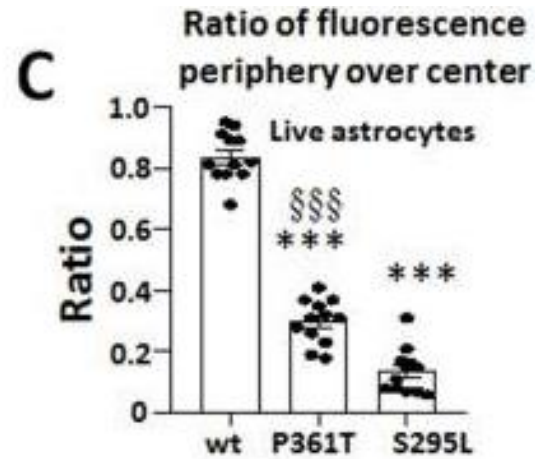
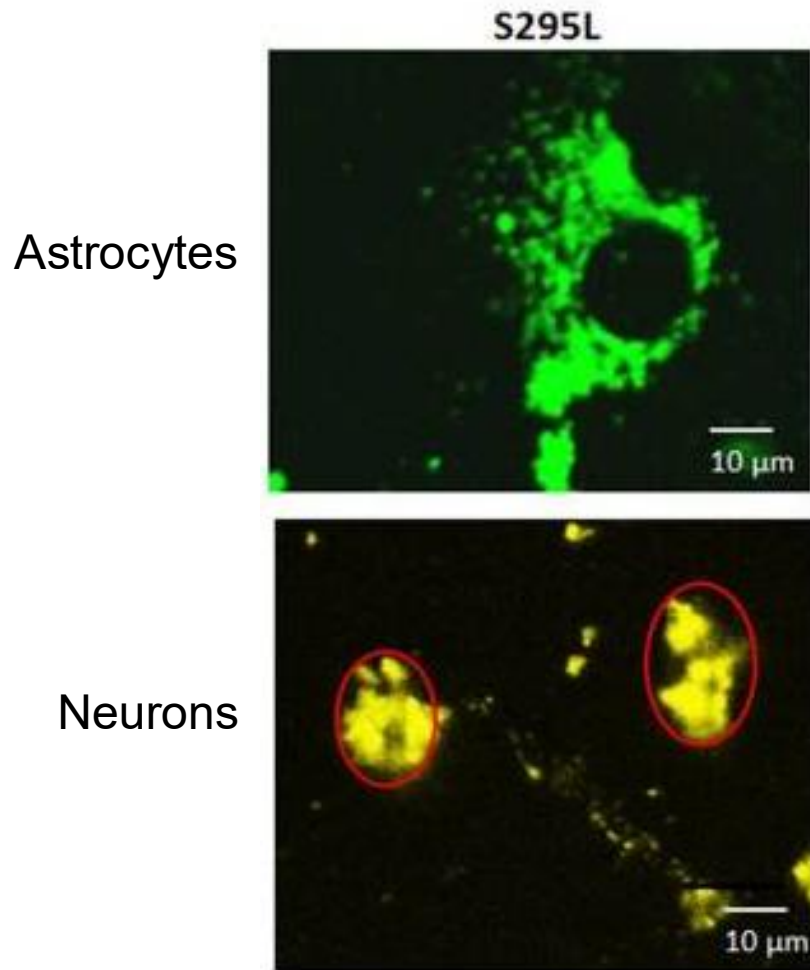
1mm

C

Slc6a1
Gad1
Olig1
Gfap



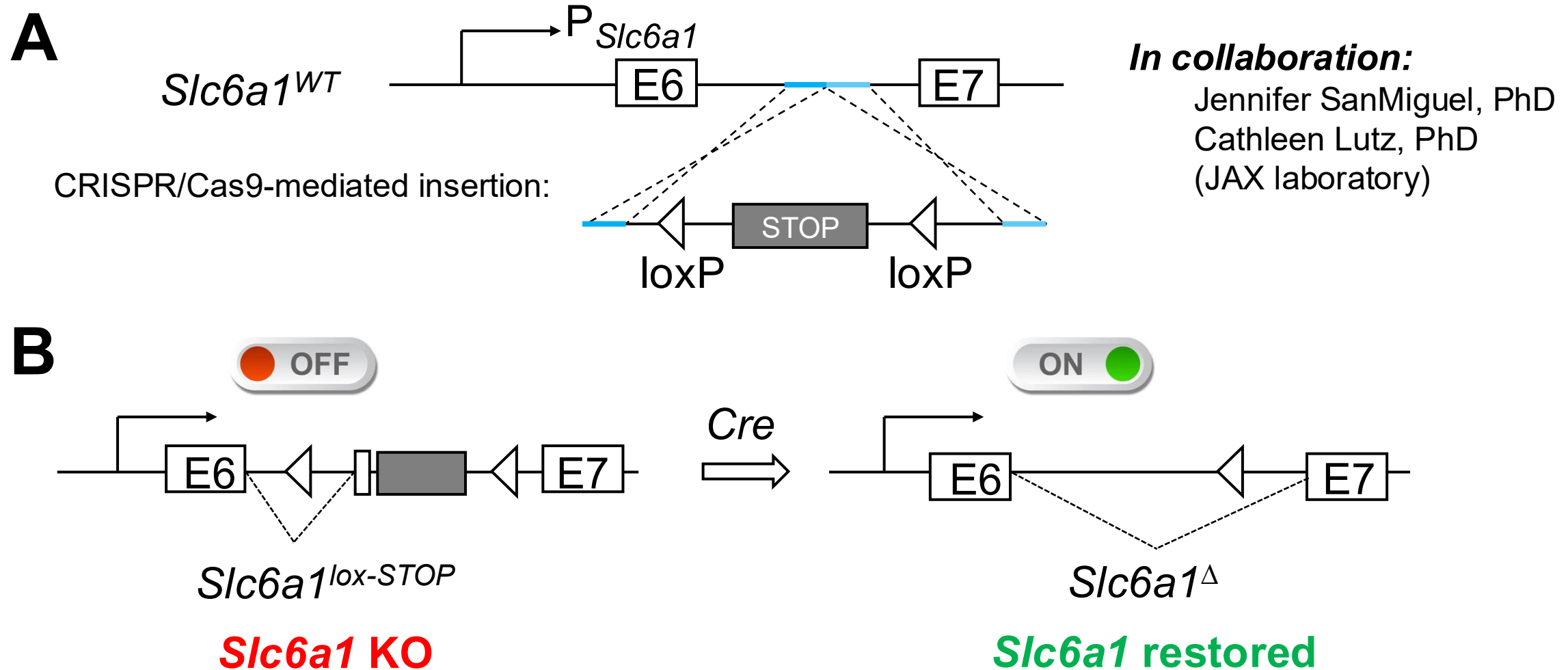
Challenge #4: *SLC6A1* variants might interfere intracellular trafficking of functional proteins



Key considerations for *SLC6A1*-targeted gene therapy

1. Mapping a therapeutic window
 - Systematic age testing in a Cre-lox mouse system
2. Brain-wide delivery
 - Use of brain-penetrating AAV capsid
3. Transgene expression restricted to relevant cell targets
 - Incorporation of native promoter regulatory elements
4. Intracellular trafficking interfered by mutant proteins
 - Testing in animal model harboring patient mutations

Construction of an inducible *Slc6a1* mouse

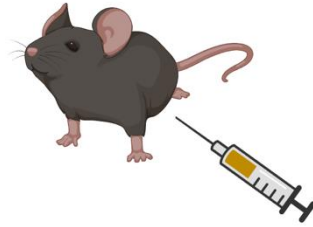


Cre-dependent *Slc6a1* restoration currently ongoing at JAX laboratory

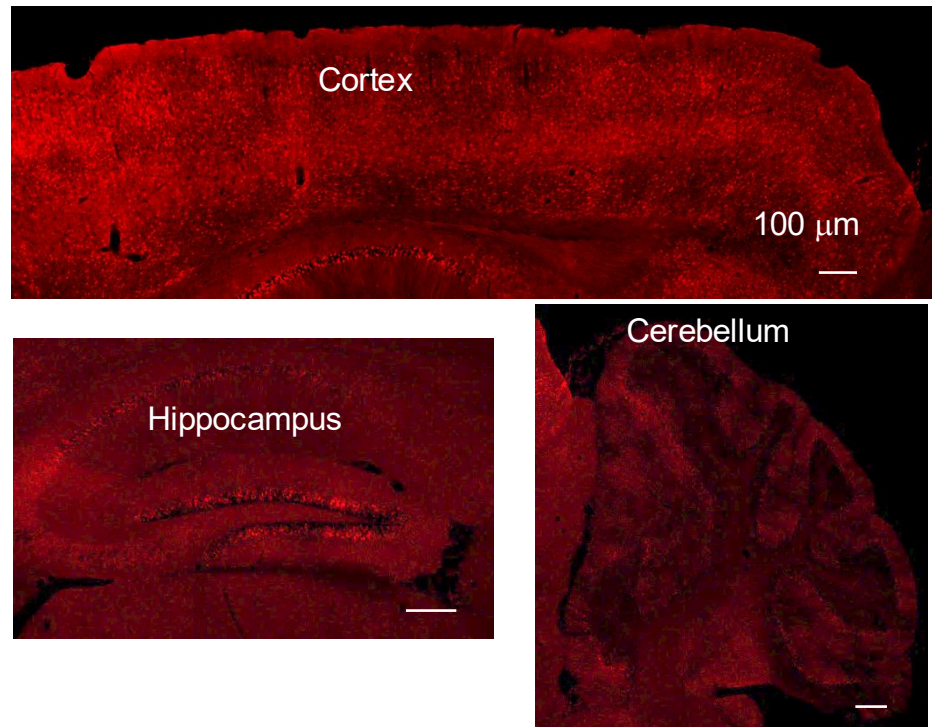
Brain-wide targeting via AAV-PHP.eB injection

A

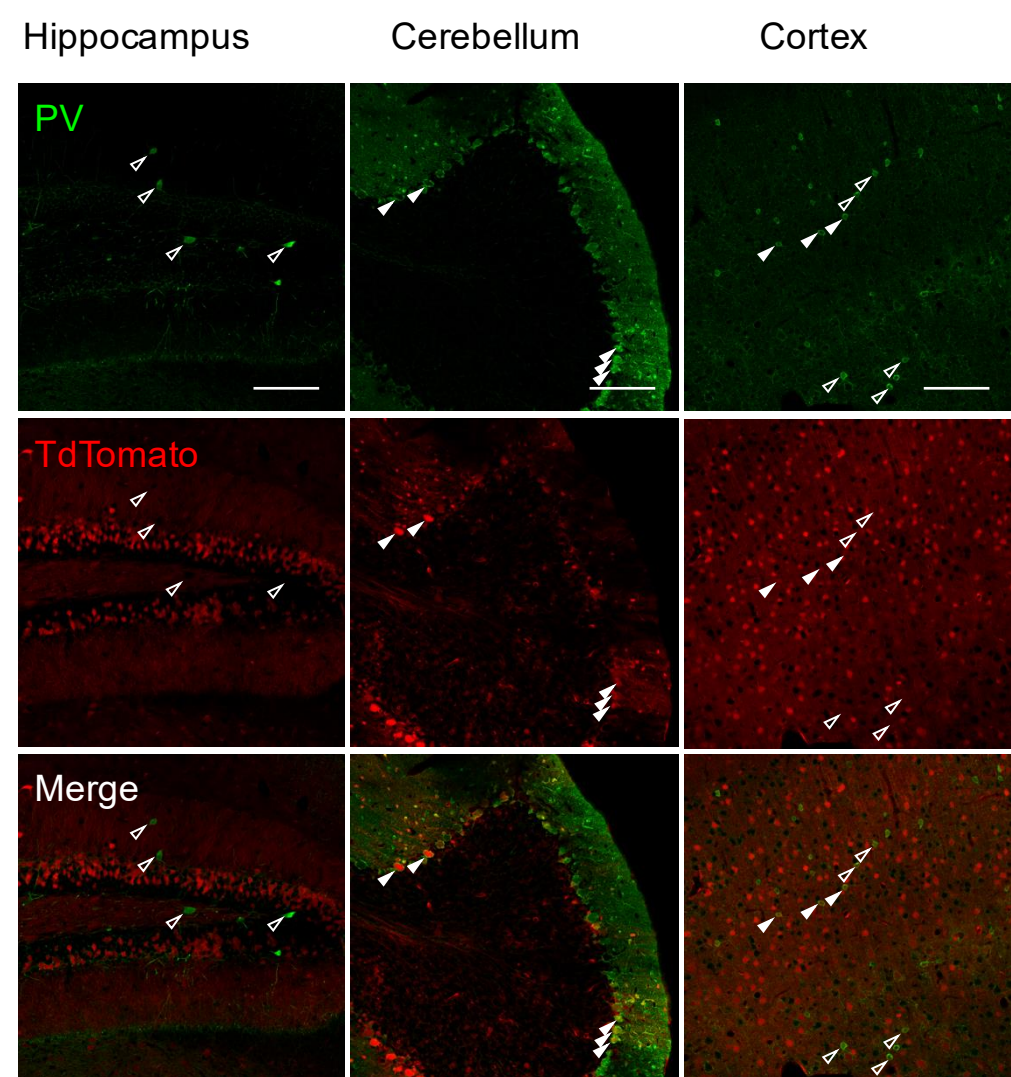
1×10^{14} vg/kg AAV-Cre
Td-TOMATO



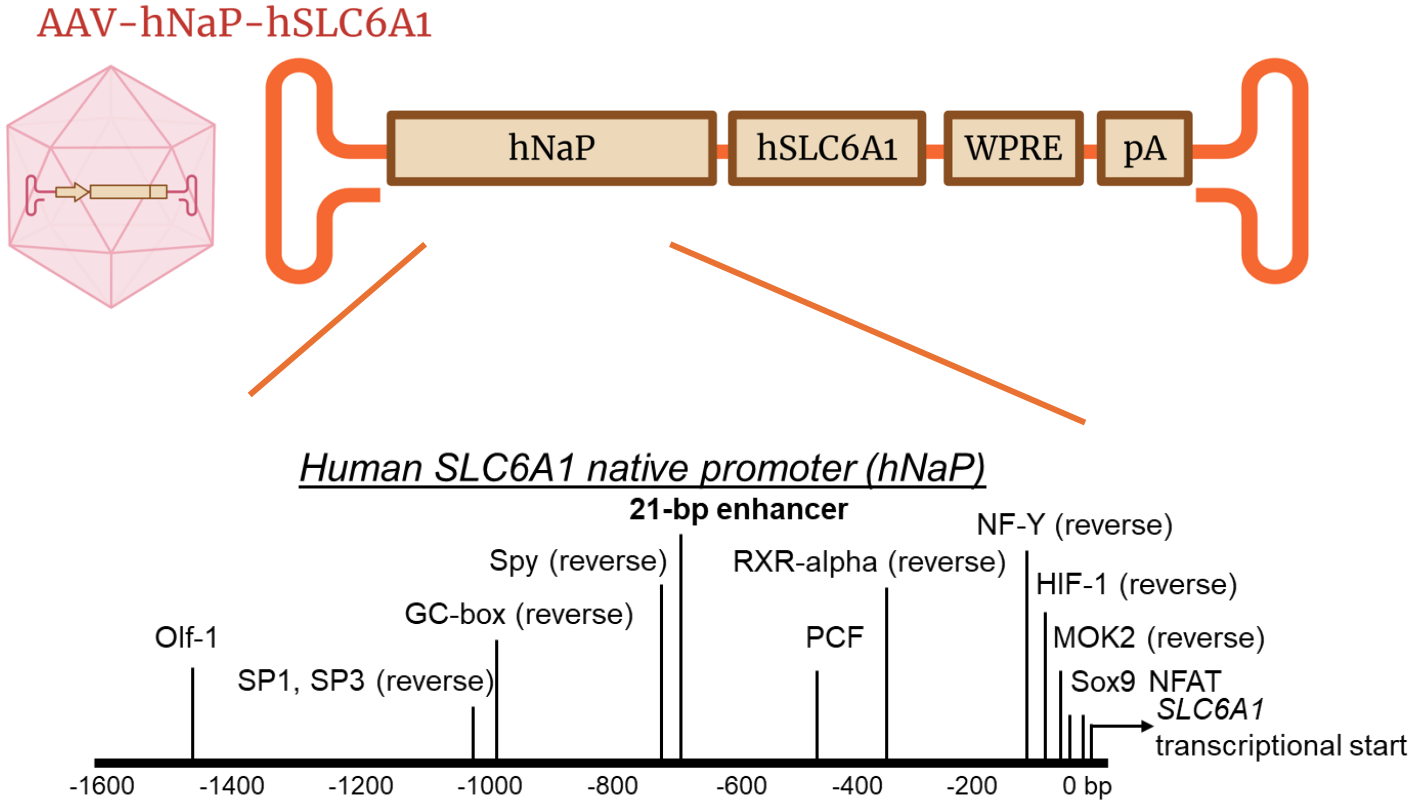
B



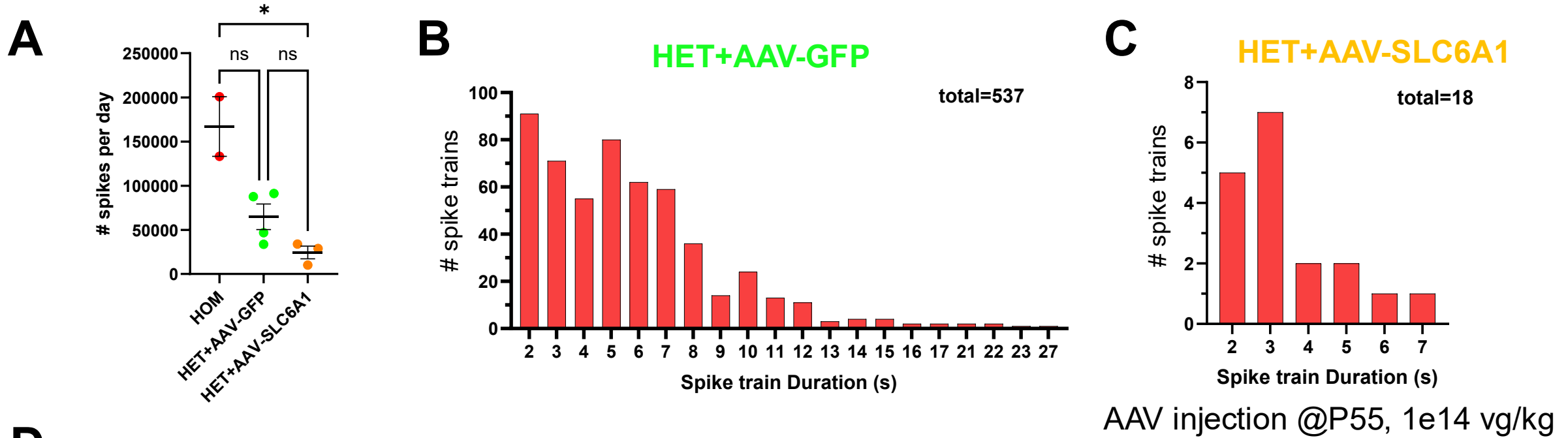
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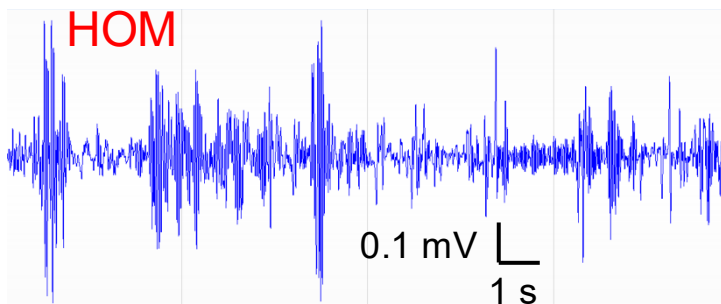
Molecular design and cloning of AAV-hNaP-hSLC6A1



Late AAV-hNaP-SLC6A1 treatment suppresses spiking in *Slc6a1*^{STOP/+} mice

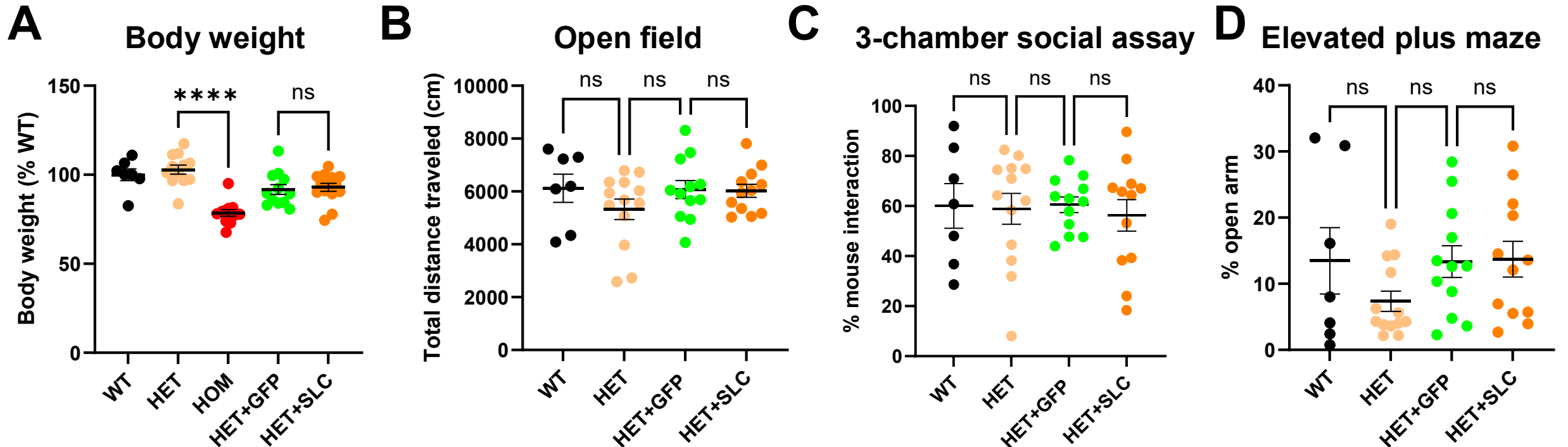
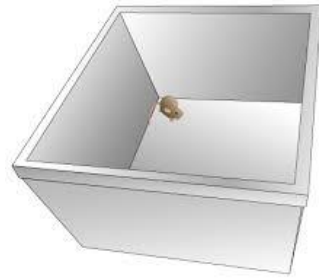


D Sample EEG traces

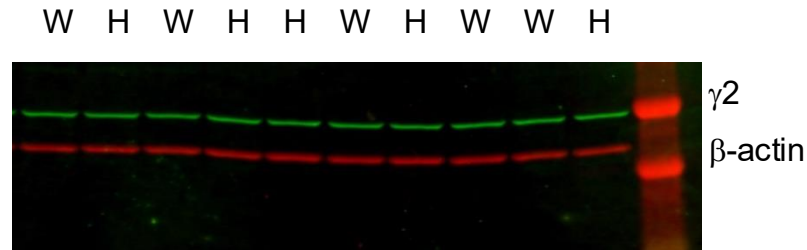
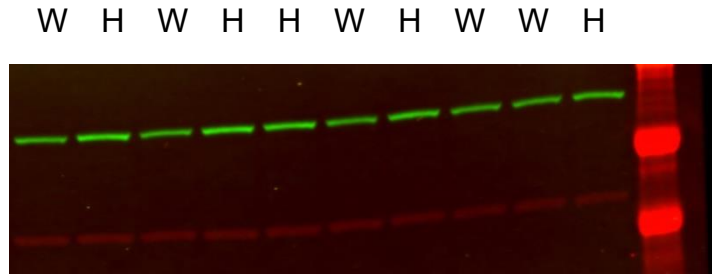


Unaffected body weight gain and behaviors upon AAV-hNaP-hSLC6A1 treatment

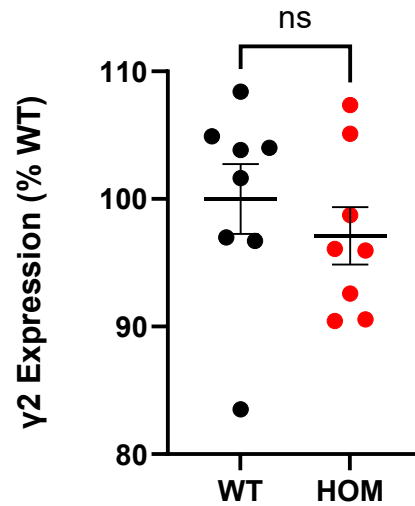
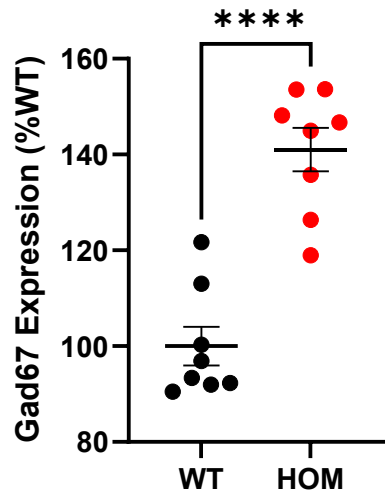
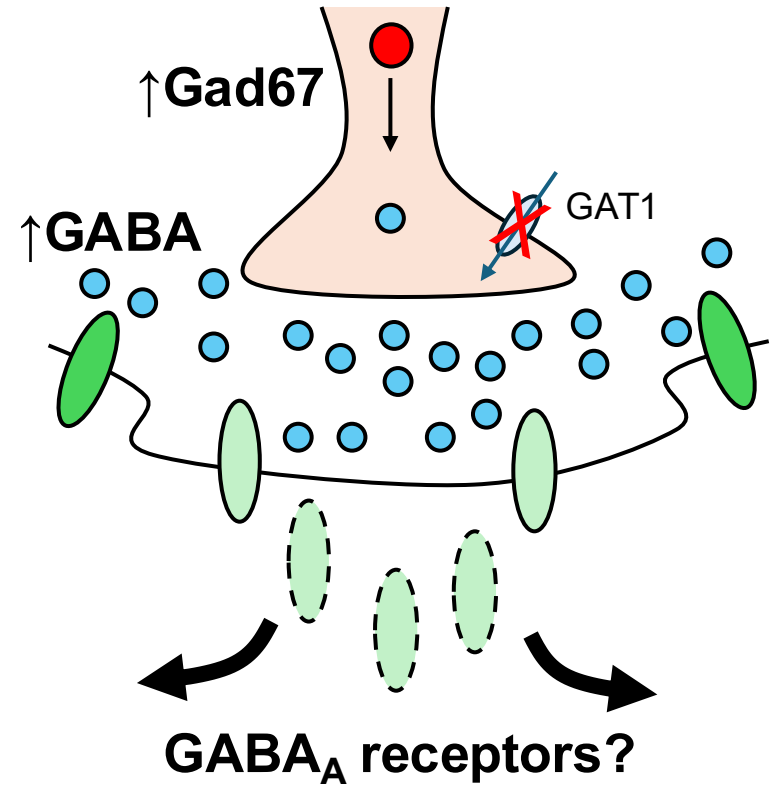
AAV injected @P55 or P25
1e14 vg/kg



Gad67, but not GABA_AR γ 2, is up-regulated in *Slc6a1*^{STOP/STOP} cortex



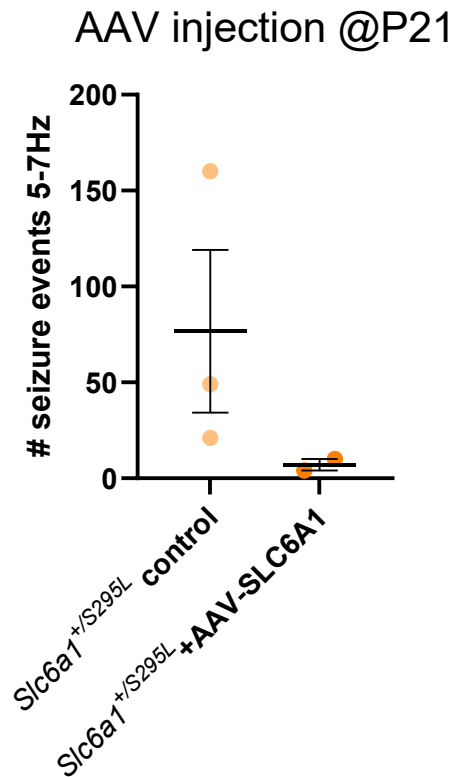
SLC6A1 disorders



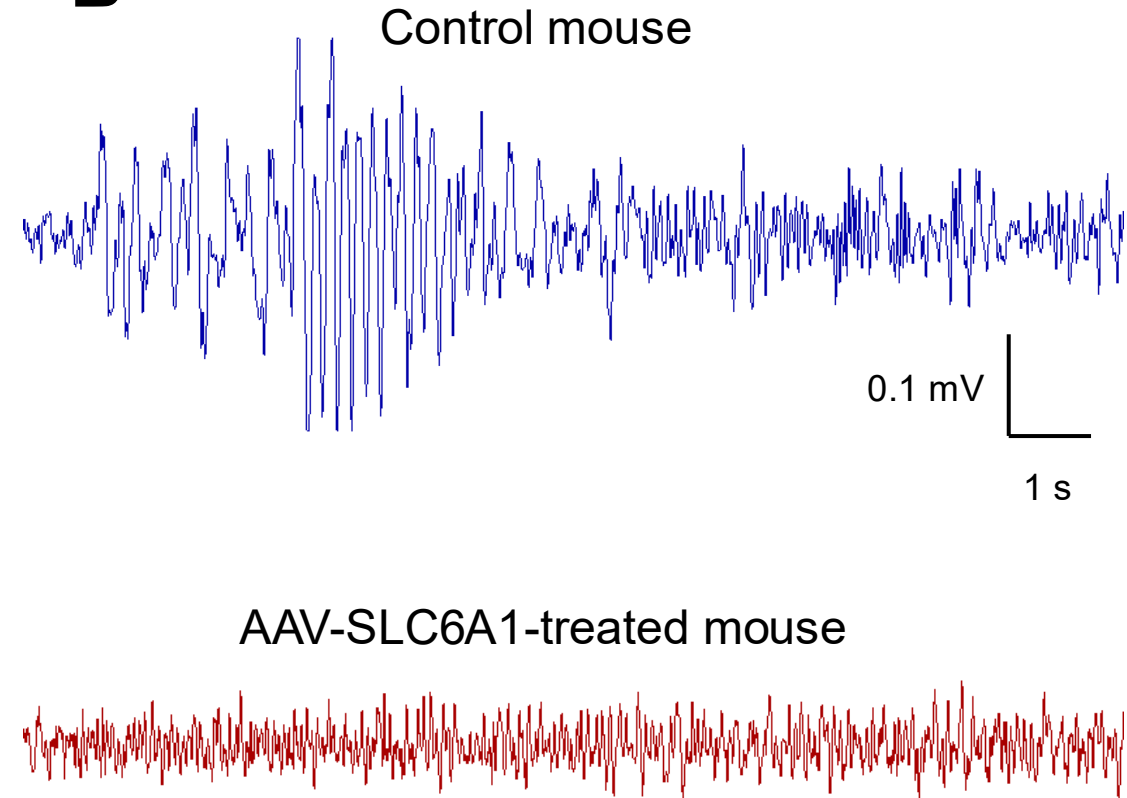
Reversible by gene therapy?

Spike suppression in *SLC6A1*^{+/-295L}

A



B



Summary and future directions

- A novel AAV construct encompassing a **native *SLC6A1* promoter (hNaP)** might be effective beyond the previously described treatment window (**up to P55, early adulthood**)
- AAV-mediated *SLC6A1* gene therapy development requires further **fine-tuning** of time and dose of administration, as well as testing in **disease-relevant** animal and human cell models

Acknowledgments



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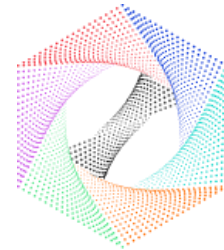
Broad Institute

Ben Deverman



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Amber Freed/SLC6A1 connect
CURE epilepsy
NIH/NINDS
BCH Translational
Neuroscience Center



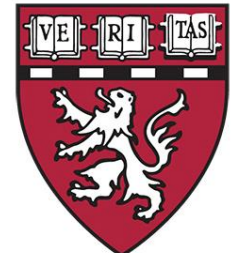
Apertura
GENE THERAPY



ROSAMUND
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TRANSLATIONAL
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University Hospitals | Cleveland, Ohio



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