Silencing Gain-of-Function KCNT1 Genetic Epilepsy with Divalent siRNA

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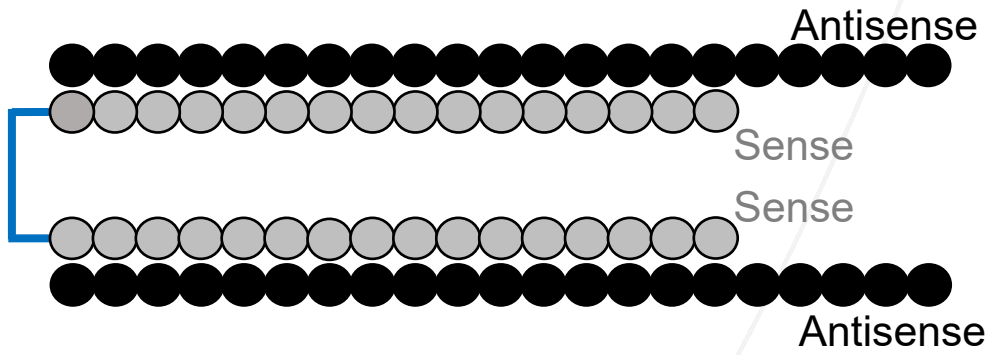
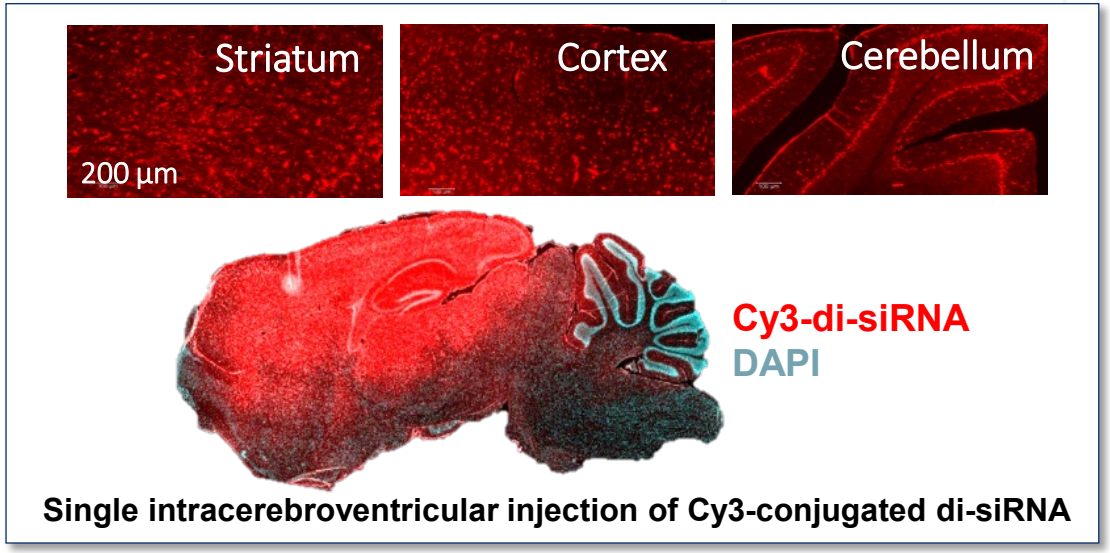
Divalent siRNA for RNA Silencing in the CNS

- Divalent structure builds on best-in-class **Distribution** of UMMS chemistry
  - **Excellent CNS distribution,** including to spinal cord and even to deep brain structures inaccessible to other oligo modalities including siRNA
- Increased **Potency**
- Enhanced **Durability**
- Improved **Tolerability**
- **No Conjugates, No LNP** needed for delivery compared to traditional siRNA





Single intracerebroventricular injection of Cy3-conjugated di-siRNA

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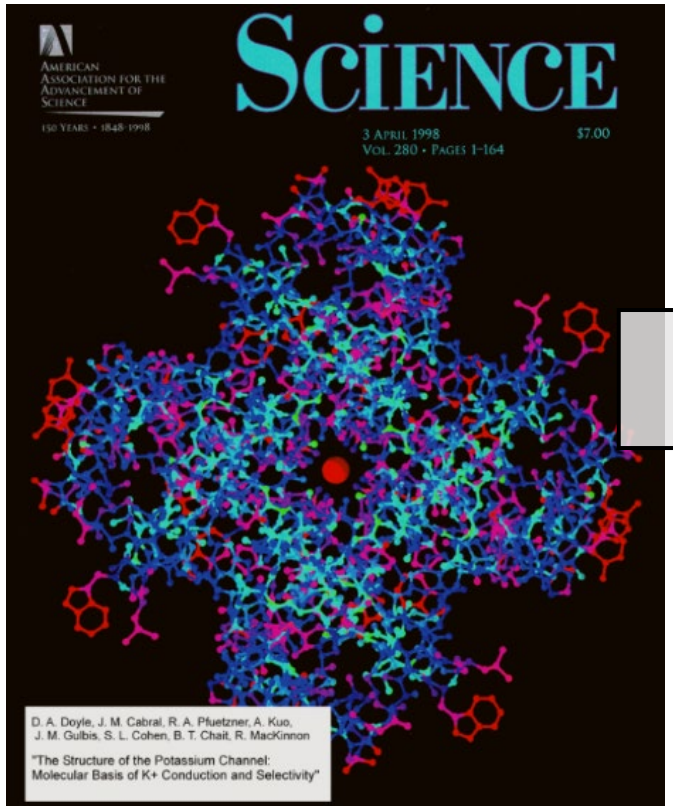
Di-siRNA is Well-Suited to Gain-of-Function Monogenic Neurological Diseases

KCNT1 encodes a potassium (K) ion channel in the brain activated by voltage and by intracellular Na+ ions



Doyle et al, 1998

Pathological KCNT1 mutations drive severe, treatment-refractory epilepsy

- Cortical seizures
- Epilepsy of Infancy with Migrating Focal Seizures
- Sleep-Related Hypermotor Epilepsy

kcnt1epilepsy.org

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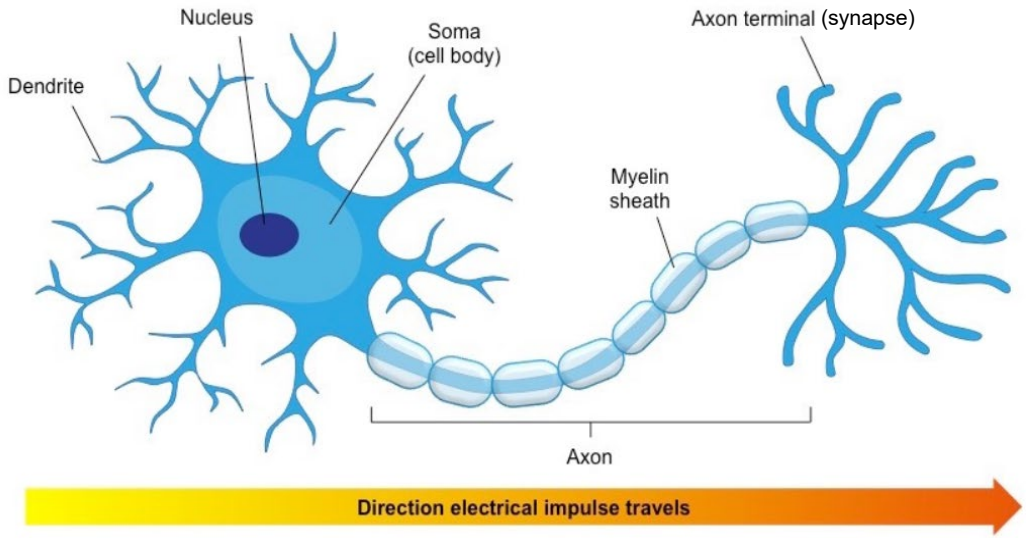
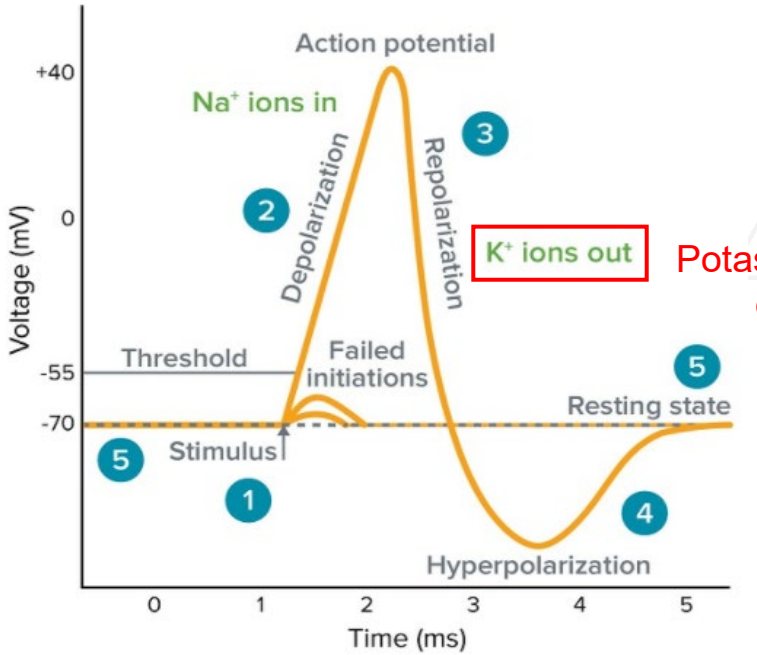
Seizures Are Caused by Aberrant Excitability Within Neuronal Networks

Input signal accumulation from afferent neurons → Current through **ion channels** (action potential) → Action potential travels down axon → Neurotransmitter release at synapse → Signal passed onto efferent neuron





Potassium Channels e.g. KCNT1

Potassium ion channels govern action potential generation and neuronal spiking

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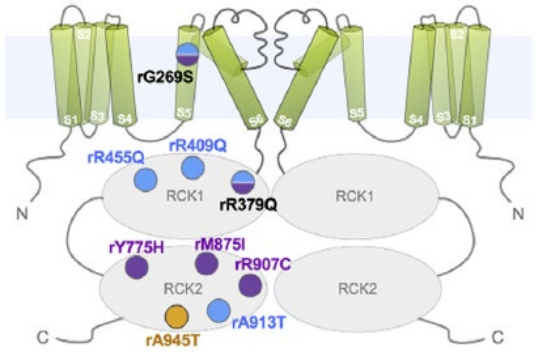
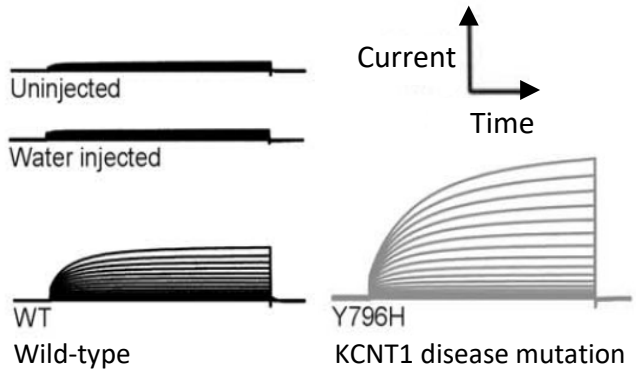
KCNT1 Gain-of-Function Variants Drive Epilepsy

Pathological mutations in KCNT1 increase the potassium ion current



Full channel is a tetramer



Milligan et al, 2014

Reduction of KCNT1 will treat root cause of KCNT1 epilepsy

- Robust efficacy expected from less than total knockdown
  - Mutations increase existing K+ current
  - Knockdown of wildtype as well as mutant allele reduces K+ current
- Safety window for knockdown
  - Global knockout mice & ASO-treated mice have subtle if any behavioral defects

Bausch et al., 2015; Quraishi et al., 2020; Burbano et al., 2022

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No Adverse Effects Seen in Humans With One KCNT1 Loss-of-Function Allele

- Mining of UK Biobank supports safety of knockdown in humans
  - **1,463** plasma protein levels & **483** hospital diagnostic codes (phecodes) with more than 1,000 cases were searched for association with **190 KCNT1 pLOF carriers** across 52 variants

- **No significant associations for KCNT1 pLoF found across plasma proteins or phecodes** corresponding to loss of one allele

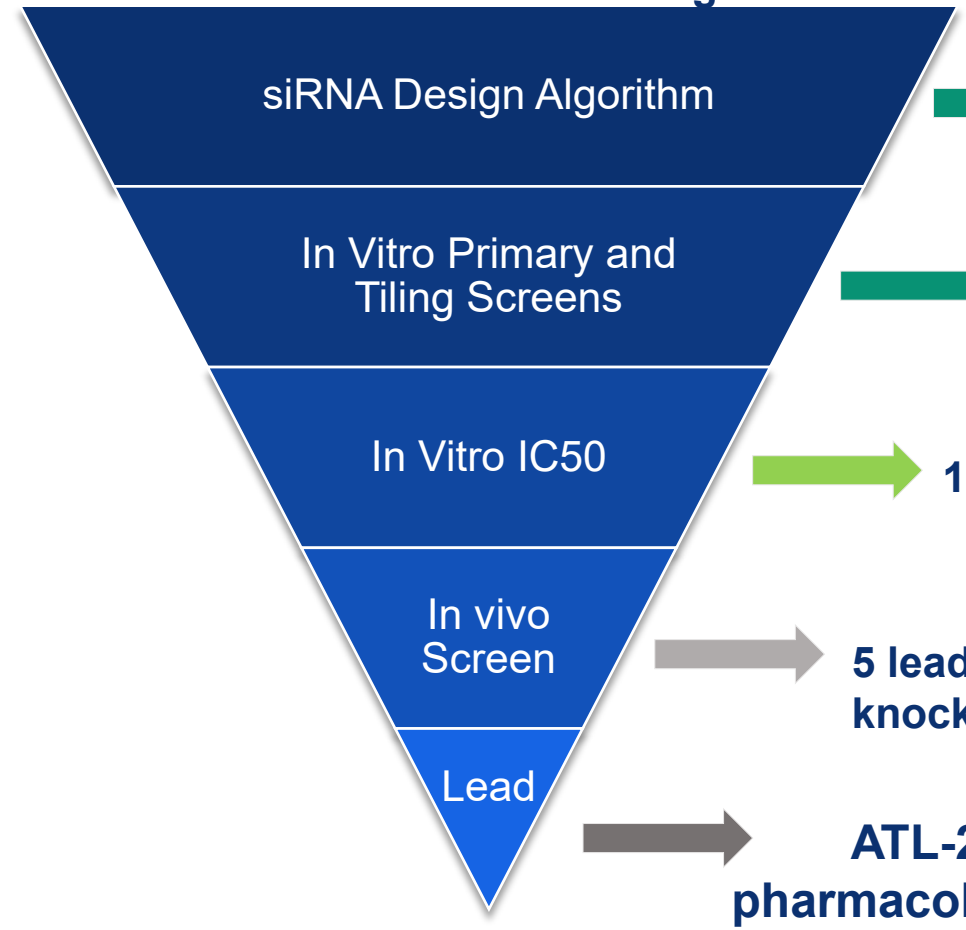
- High impact variants
- High confidence loss-of-function (LOFTEE)
- 24 single carrier variants
- 8 carriers in Olink population

QC metrics:

Limited to European ancestry
Minor allele frequency < 1%
Hardy-Weinberg equilibrium p-value > $1e^{-15}$
Missing rate < 10%

siRNA Screening at Atalanta to Identify ATL-201, a di-siRNA That Knocks Down Human KCNT1 and Mouse Kcnt1 Transcripts

All 7102 Possible siRNAs That Target Human KCNT1



436 siRNAs prioritized based on algorithm score

44 siRNAs prioritized based on in vitro knockdown

11 siRNAs prioritized based on IC50

5 lead candidate siRNAs prioritized based on in vivo knockdown

ATL-201 identified based on superior pharmacology, tolerability, and efficacy profiles

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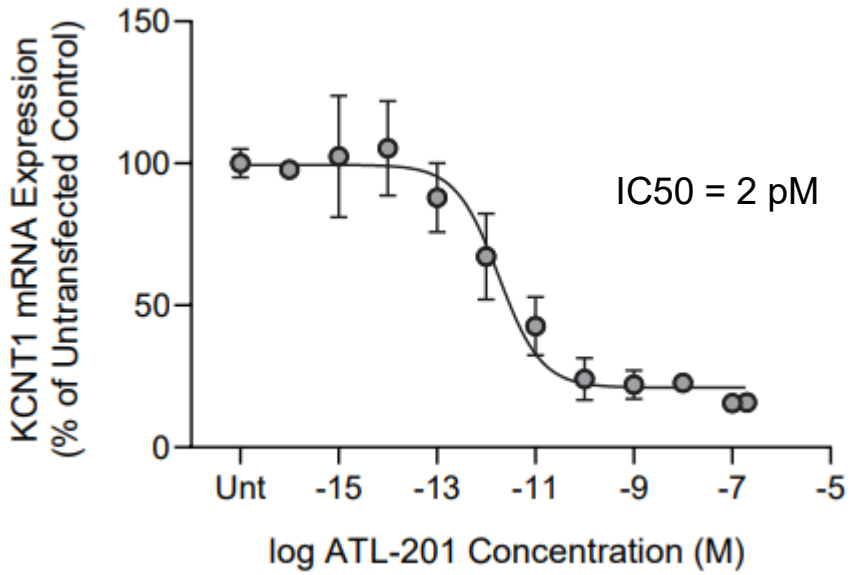
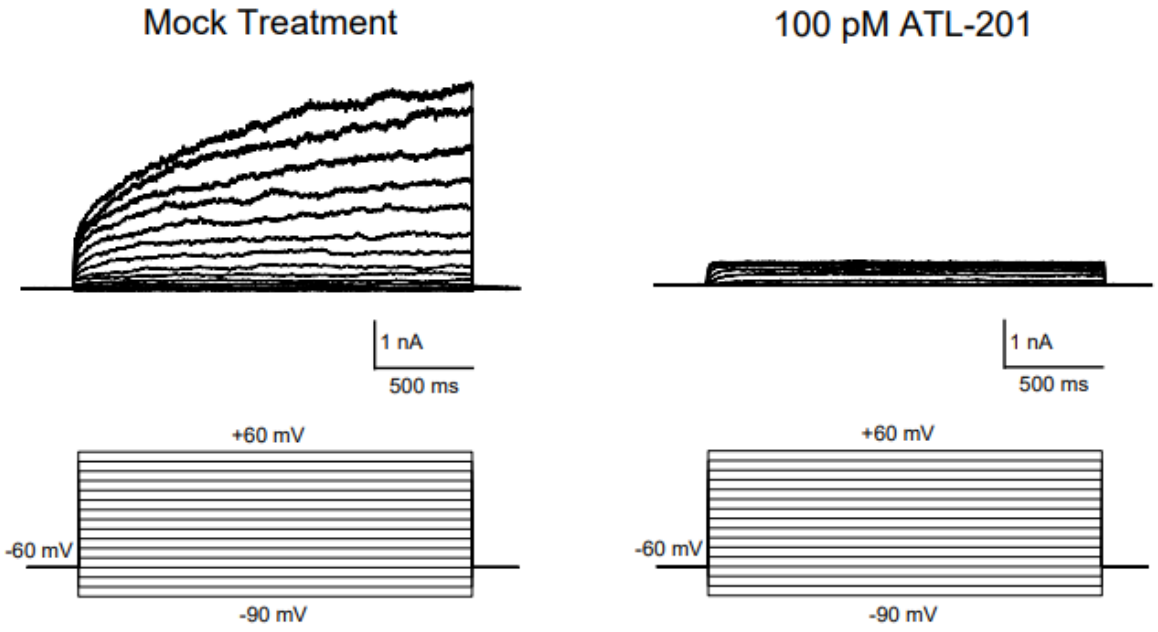
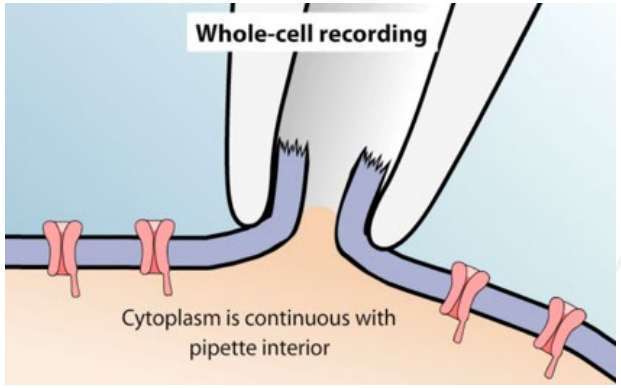
ATL-201 Knockdown of Human KCNT1 Transcript in Heterologous Expression

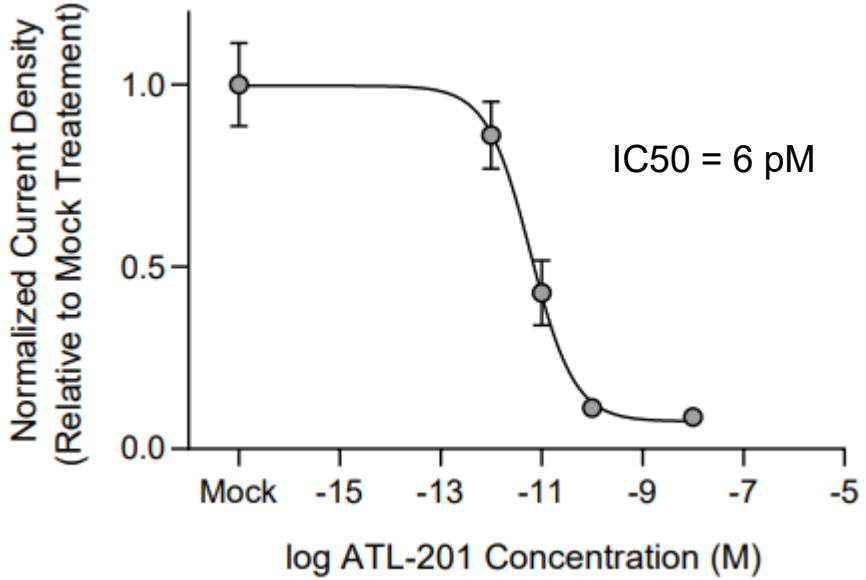


293 cells stable pool-tf hKCNT1
RT-PCR counts normalized to ATP5B with ΔΔ method
72 hour incubation, active tf with 0.1% RNA Max
Normalized to % of control
Triplicate technical replicates

ATL-201 Knockdown of Human KCNT1 Functional Protein in Heterologous Expression: Electrophysiology







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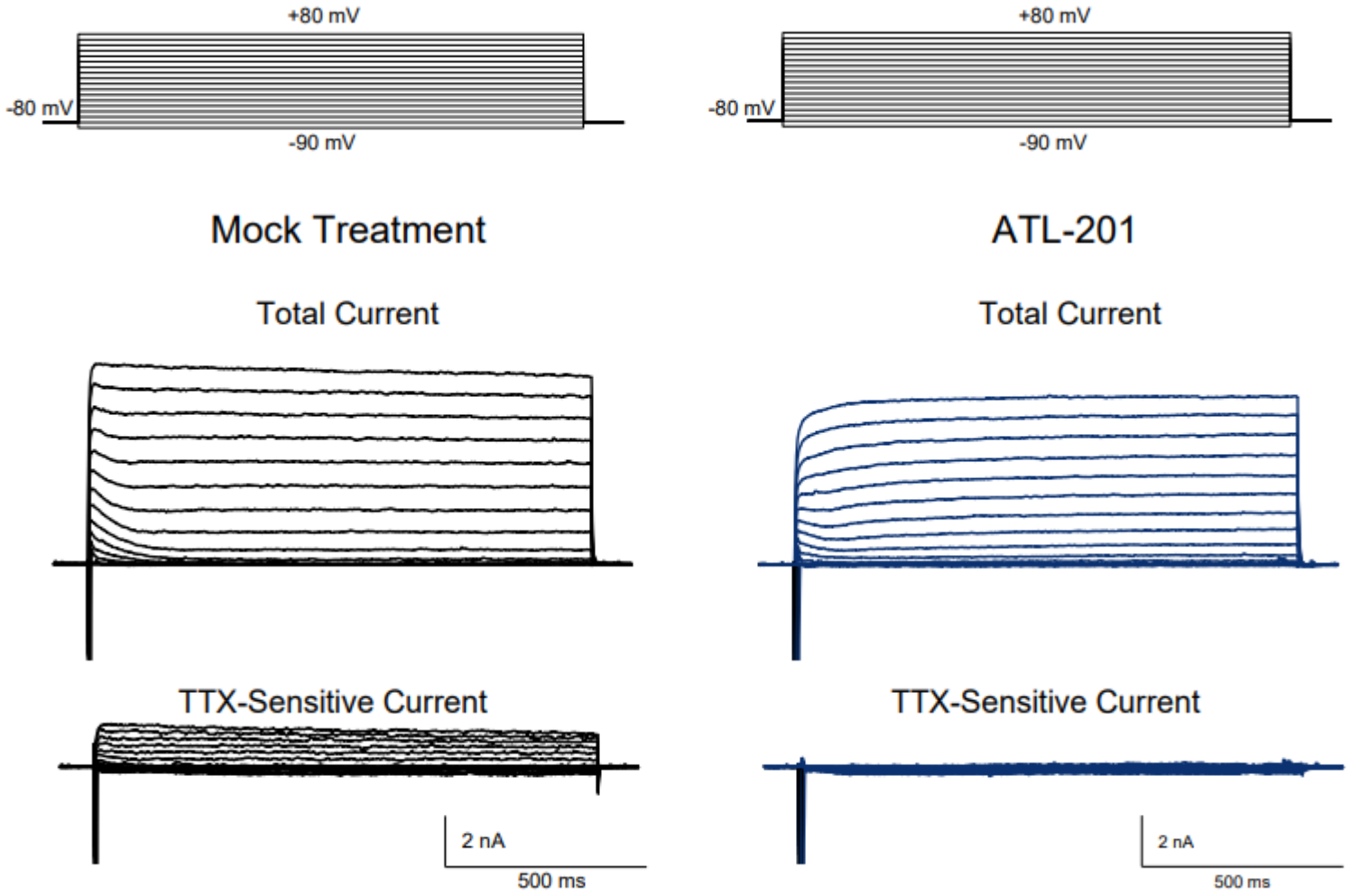
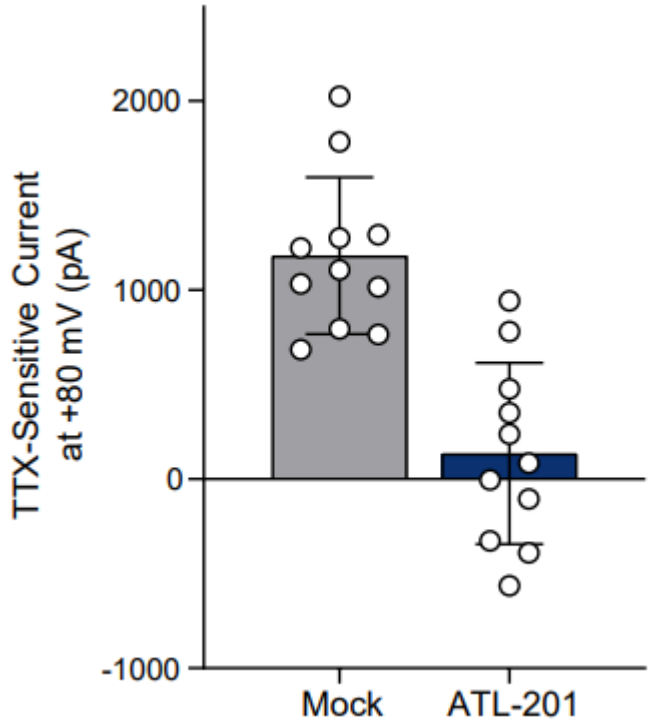
ATL-201 Inhibits Native TTX-Sensitive K+ Current from Mouse Cortical Neurons





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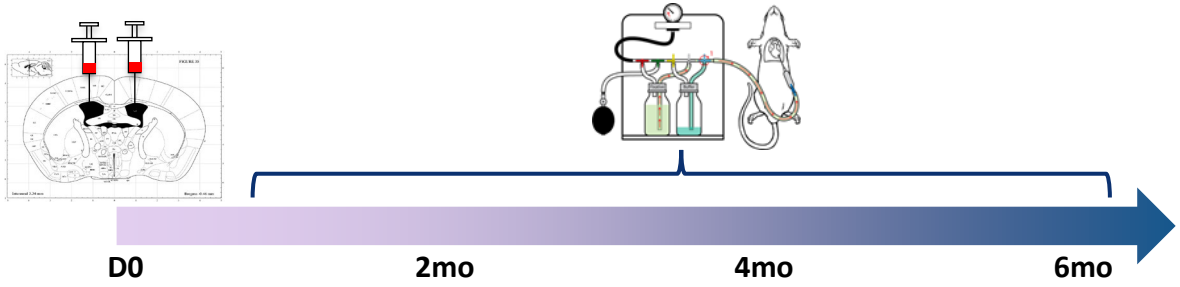
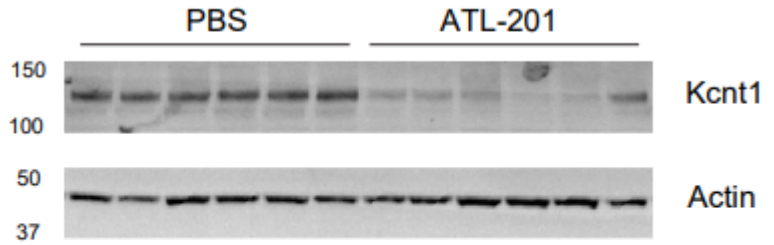
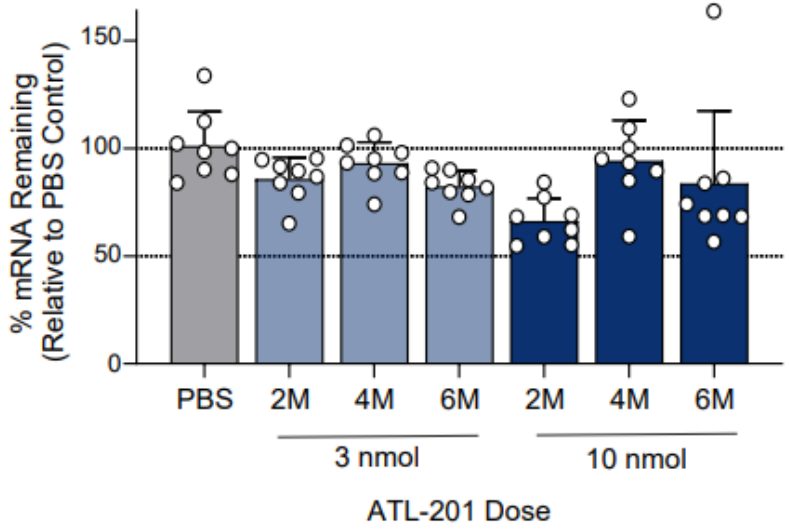
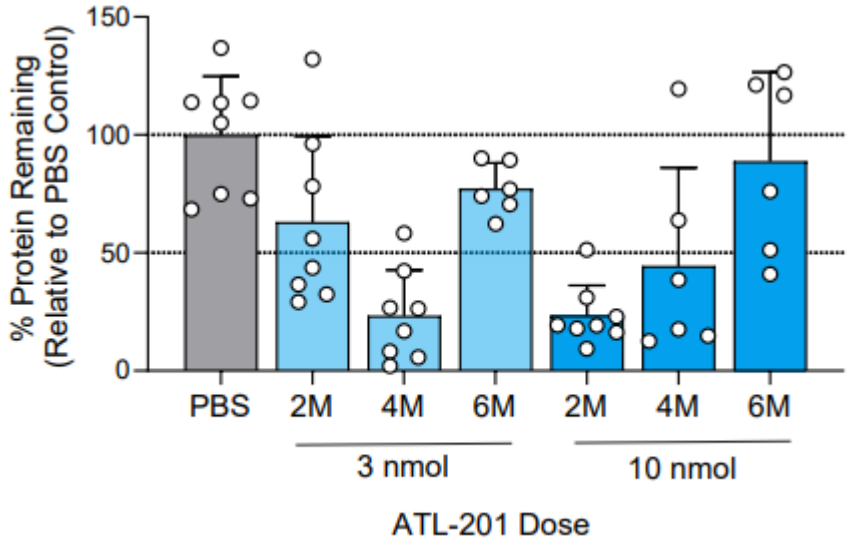
Single ICV Dose of ATL-201 Knocks Down Native Kcnt1 Transcript and Kcnt1 Protein in Wildtype Mice For 4 to 6 Months









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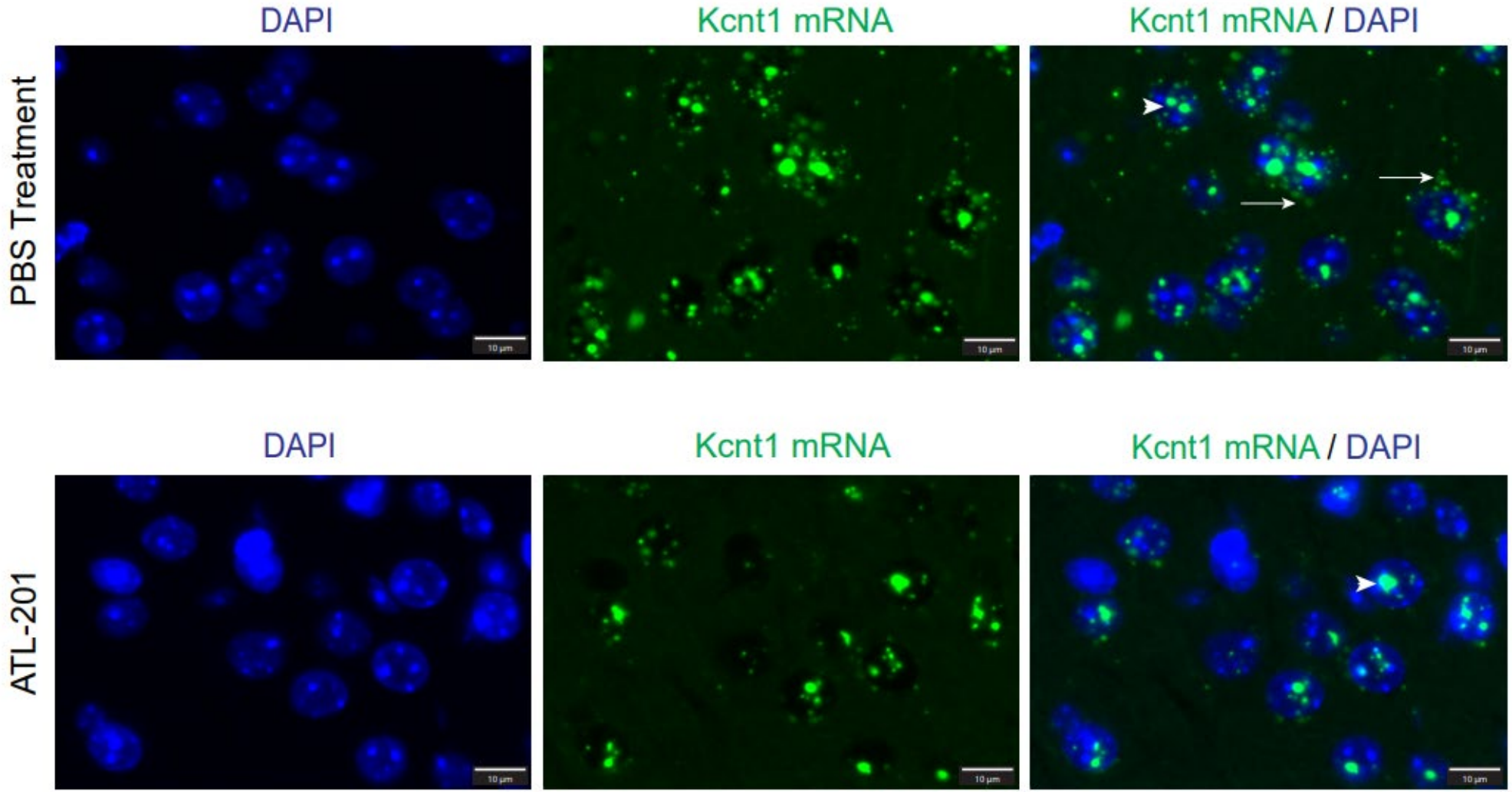
In Vivo, ATL-201 Knocks Down Cytoplasmic Transcript Driving Protein Expression Strongly, Nuclear Transcript Weakly



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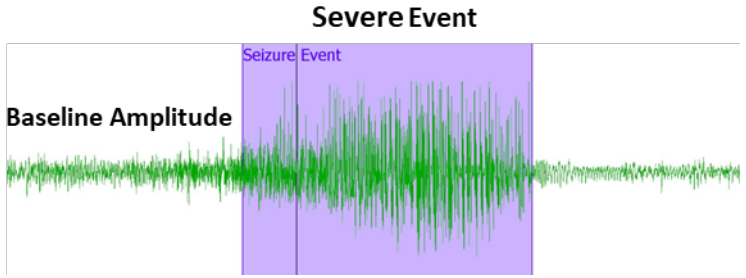
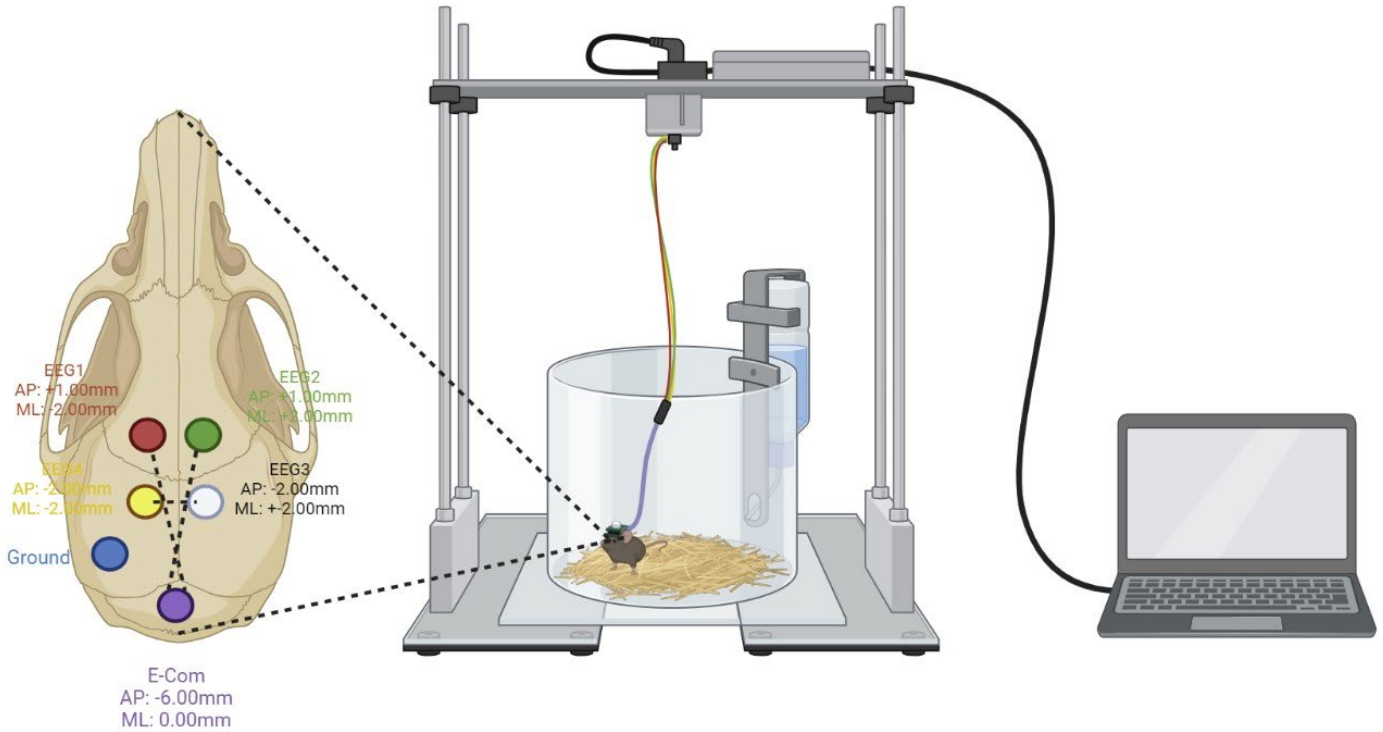
Efficacy Testing: Seizures Assayed In Freely-Behaving Kcnt1^{Y777H} Homozygous Mice With Cortical EEG Plus Video Recording

Kcnt1-Y777H is the mouse ortholog to human pathogenic mutation KCNT1-Y796H



ATL-201 or placebo dosed ICV and electrodes implanted in a single surgery at day zero



Experimental Settings (Sirenia Acquisition):

- Experiment Duration: 24 or 72 hrs
- Filter 40 Hz, sampled 400Hz, video 30 Hz
- Search threshold 2x baseline amplitude
- Window: 5 sec, step size 1 sec
- Manual observation and categorization of video from each event using modified RACINE scoring

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Efficacy Testing: Nesting Behavior Scored From 1 (None) to 5 (Full Nest)

Deacon, 2012; Burbano et al., 2022

Nest Building Protocol:

- Nestlets given to animals in a clean, single-house cage for 24 hr.
- The next day nests scored 1-5
- Mice were tested 2 months after ATL-201 dosing

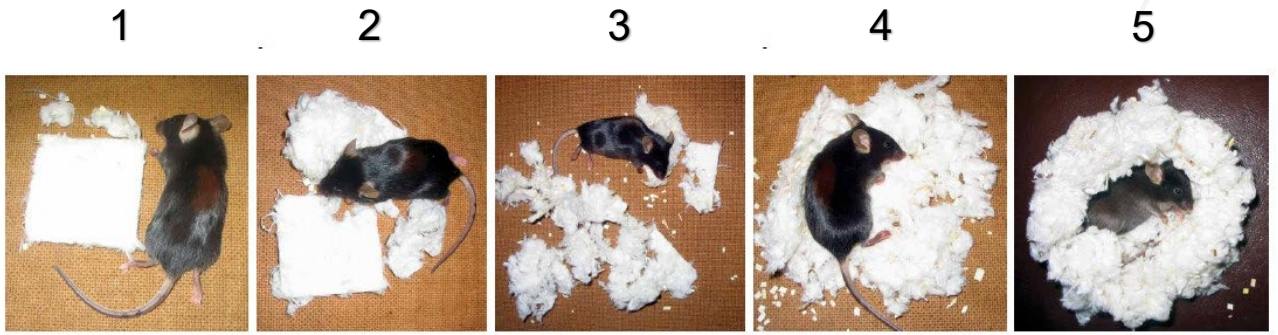
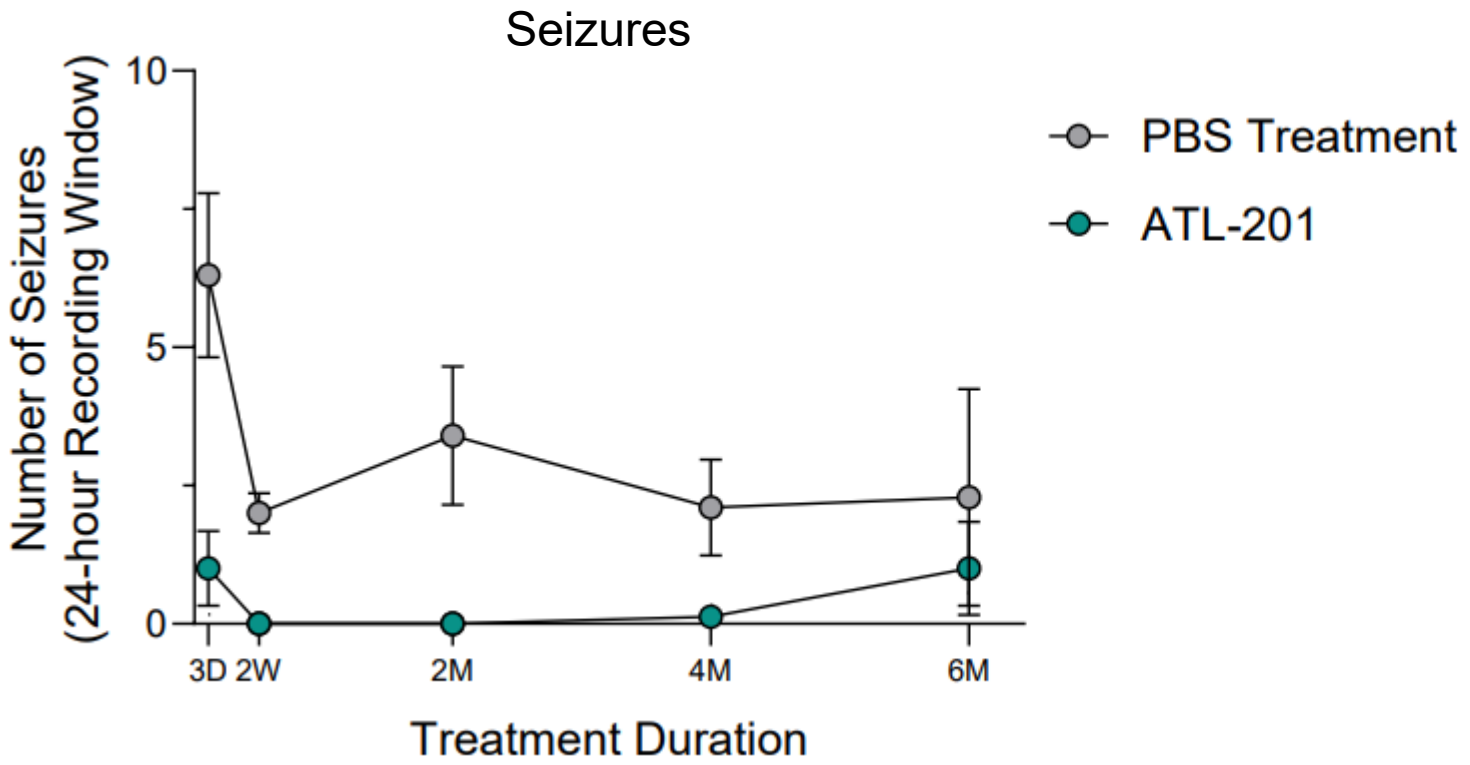
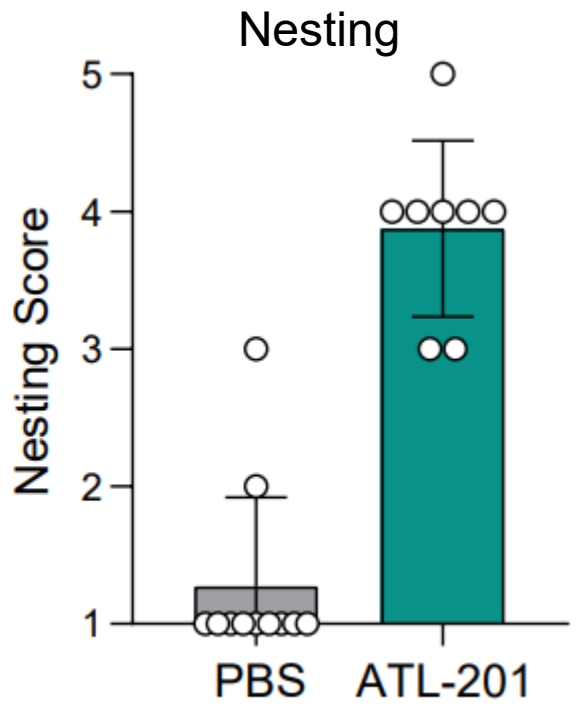


Figure 1 | Assigning scores to nests. (a–e) These nests are assigned scores of 1–5, respectively. An anesthetized mouse has been used here because of the difficulties in obtaining a satisfactory image with a freely moving mouse.

A Single Well-Tolerated 10 Nanomole Dose of ATL-201 Suppressed Seizures for 4 to 6 Months and Restored Nest-Building





ATL-201 or placebo dosed ICV and electrodes implanted in a single surgery at day zero

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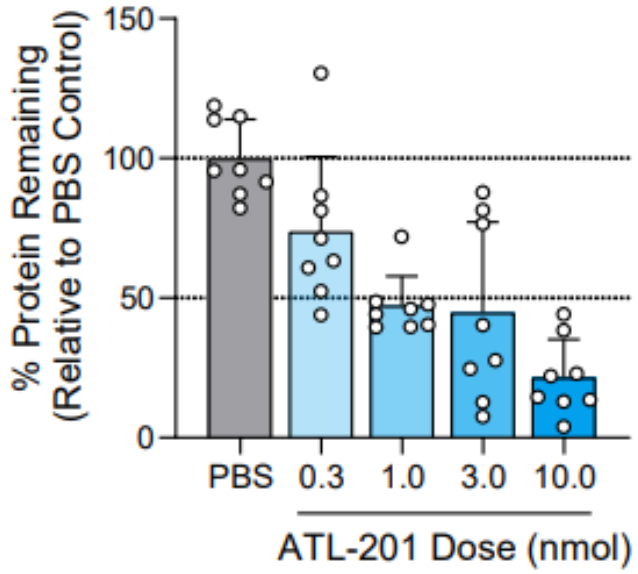
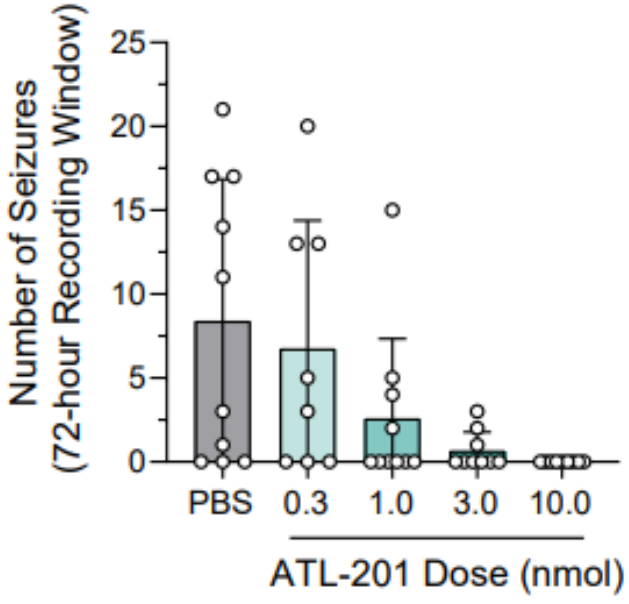
Dose-Efficacy Study Shows Dose-Dependent Kcnt1 Protein Knockdown and Seizure Reduction

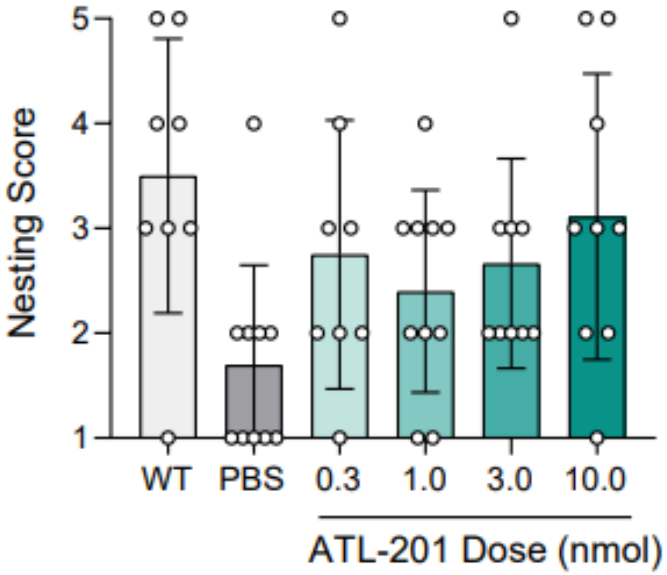
Kcnt1 Protein



Seizures



Nesting



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Summary

- ATL-201 is a di-siRNA that inhibits the sodium-activated potassium ion channel KCNT1
- KCNT1 variants drive a severe genetic developmental epileptic encephalopathy
  - ATL-201 targets an area of KCNT1 containing no annotated pathogenic variants
  - The area targeted is identical between human KCNT1 and mouse Kcnt1
- ATL-201 delivers dose-dependent knockdown of human and mouse transcript and protein
  - More knockdown of protein than transcript in mouse likely reflects nuclear transcript
- ATL-201 produced dose-dependent reduction of spontaneous seizures in Kcnt1^{Y777H} mice, a model of KCNT1 epilepsy, and restored nest-building behavior
- ATL-201 is being developed as a potential therapy of KCNT1 epilepsy

Acknowledgments – Atalanta KCNT1 Team

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