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Broad biodistribution and expression of allele selective LETI-101 in critical brain regions for treatment of Huntington's disease following intrastriatal delivery in NHP.

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ASSOCIATE DIRECTOR
TRANSLATIONAL BIOLOGY

ASGCT ANNUAL MEETING

MAY 17TH, 2025

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Unlocking the potential of genomic medicines through proprietary editing and delivery capabilities

Proprietary Type II and V RNA-Guided Nucleases

- Compact (~800-1,100 aa) and efficient
- Facilitates delivery
- US Patent No. 11,162,114 (expiry June 2040)
- US Patent No. 11,859,181 (expiry May 2041)

Full-Spectrum Editing Modalities

- Knock-out
- Insertion/Repair
- A & C Base Editing
- Reverse Transcriptase Editing

Protein Discovery/ Engineering

- 20B+ proteins and counting
- Millions of candidates across diverse editing modalities
- Actively leverage AI during discovery and engineering

Broad Genome Access with PAM Diversity

- Protospacer Adjacent Motifs (PAMs) increase the number of specific sites where therapeutically meaningful edits can be made

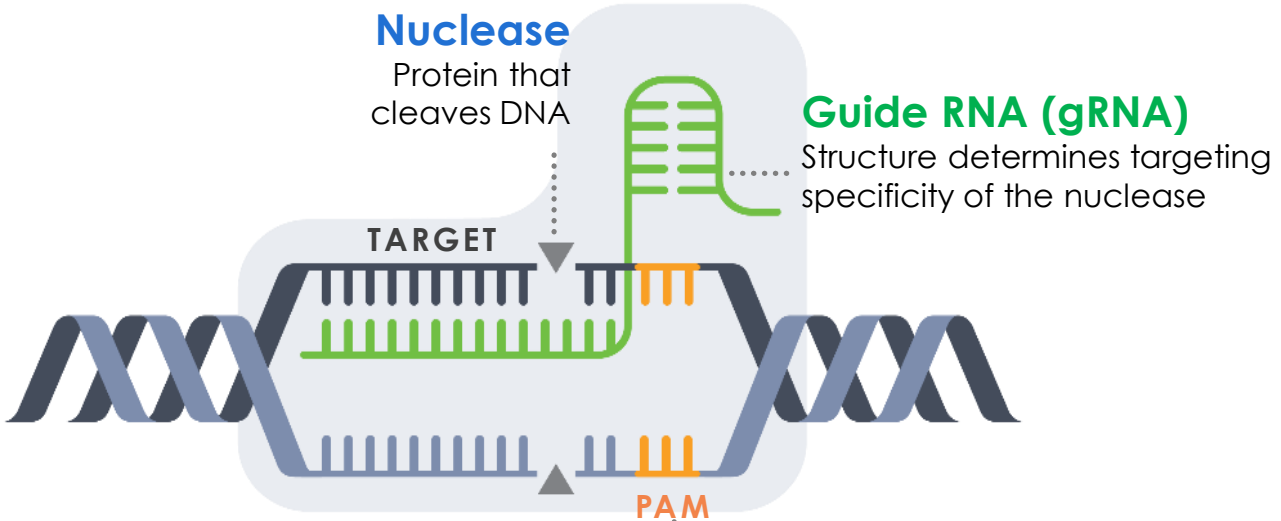
Flexible Delivery Platforms

- AAV
- Lipid nanoparticle (LNP)

In Vivo and *Ex Vivo* Therapeutics

- Multiplex base editing of up to 5 genes
- Simultaneous knock-in /knock-out in primary T-cells
- *In vivo* editing in the liver and CNS

Diverse arrays of novel RNA-guided nucleases



Life Edit nucleases
(**L**ife **E**dit **G**enes or **LEG**s)

- Smaller LEGs facilitate delivery
- Unique and diverse PAM recognition sequences
- Flexible targeting strategies

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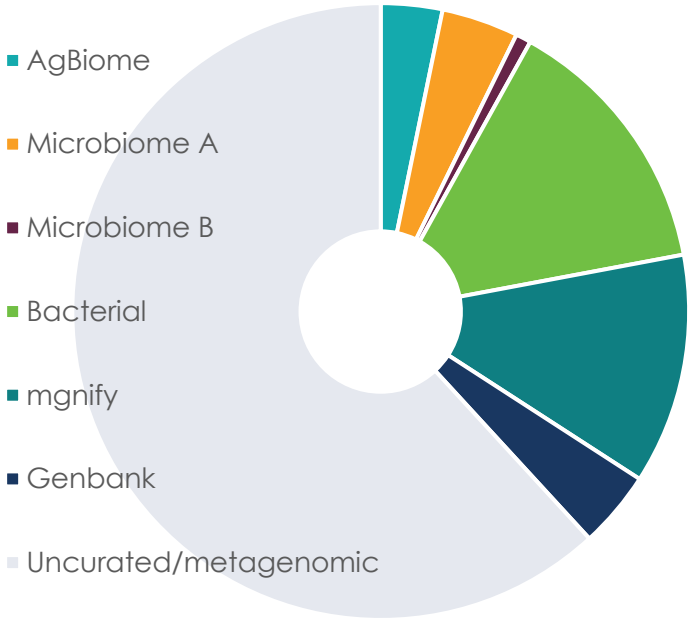
Life Edit has a proprietary library of evolutionarily distant nuclease with PAM sequence diversity



Over 2M CRISPR-Cas systems

All known Cas subtypes

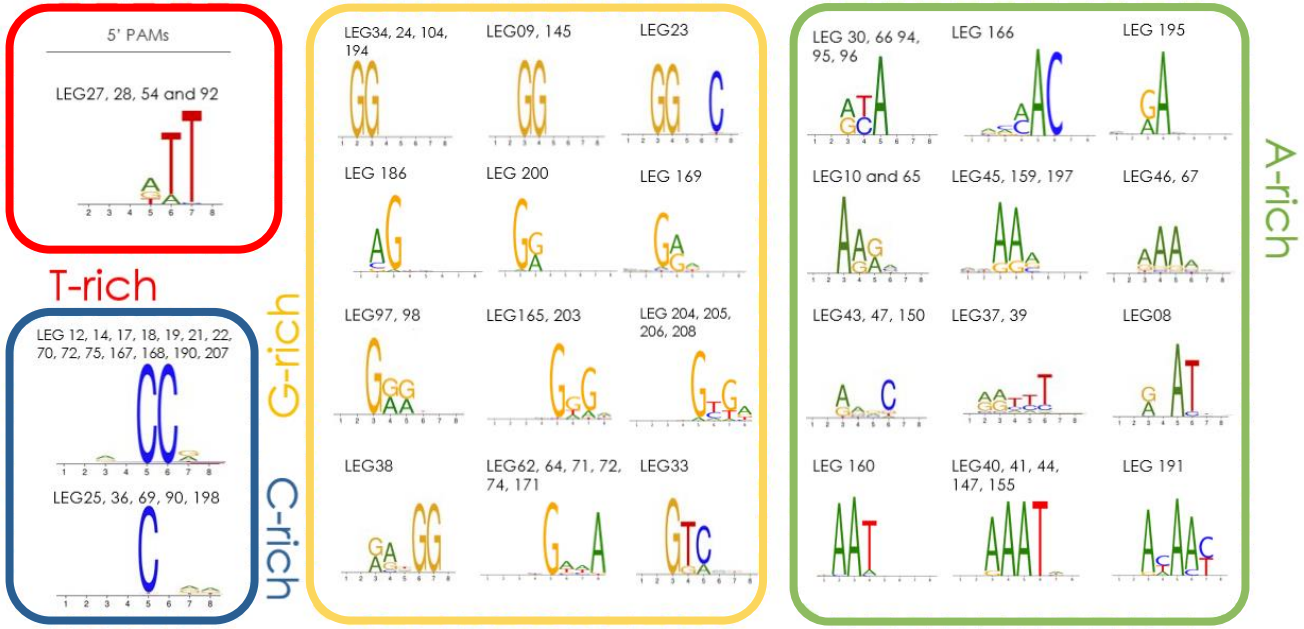
- Focus on Type II and Type V

Cataloged to capture annotations and *gene context*

10+ billion unique proteins (and counting)

Microbial database from multiple sources

Example PAM sequences



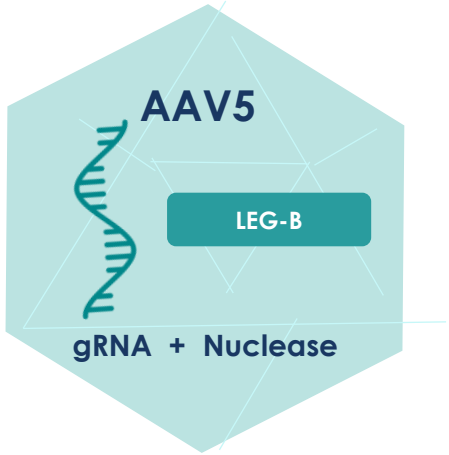
Collection of nucleases with diverse PAM sequences

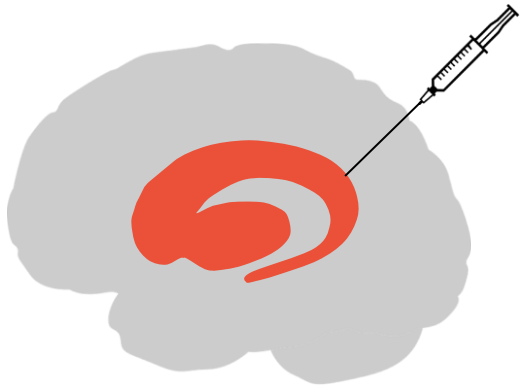
Founded on exclusive access to gene editing systems identified in a proprietary microbe collection for use in human therapeutics, and **expanded** by mining additional genomic data sources

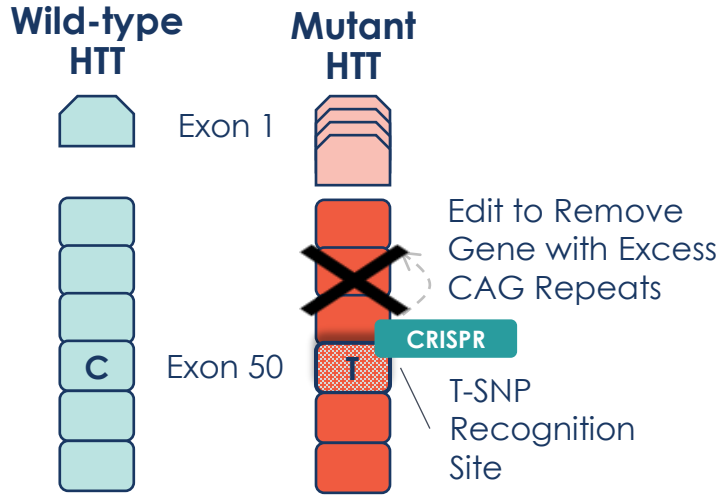
Collection enables the ability to **find additional enzymatic activities** to build future editing systems (e.g., proprietary base editors, transposases, others)

LETI-101: A precision editing approach as potential one-time treatment for huntington's disease

NOVEL CRISPR SYSTEM	TARGETED CNS DELIVERY	ALLELE-SELECTIVE EDITING







Proprietary, compact CRISPR system, packaged in AAV5 vector

One-time, bilateral intrastriatal administration

Potent and selective reduction in mutant while preserving wild-type; selective approach made possible by diverse genomic recognition sites

LETI-101 OFFERS POTENTIAL FOR A DURABLE, **ONE-TIME TREATMENT** WITH AN IMPROVED SAFETY PROFILE THROUGH SELECTIVE TARGETING

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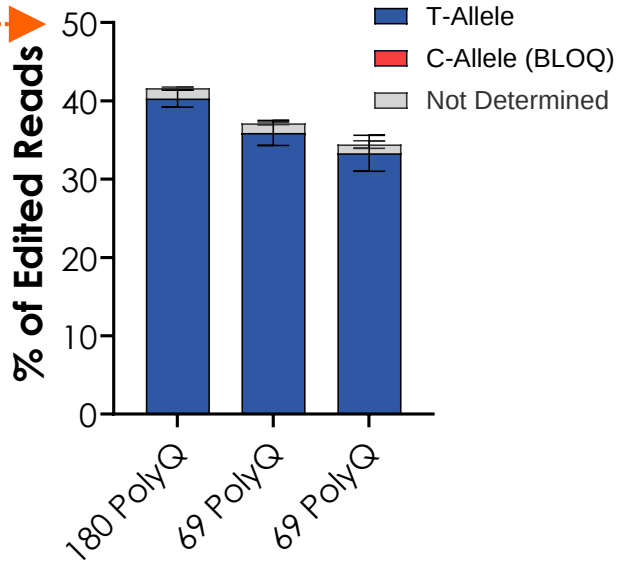
Potent allele-selective editing in cells derived from Huntington's disease patients

LETI-101 Targeted Editing of Mutant *HTT* Gene

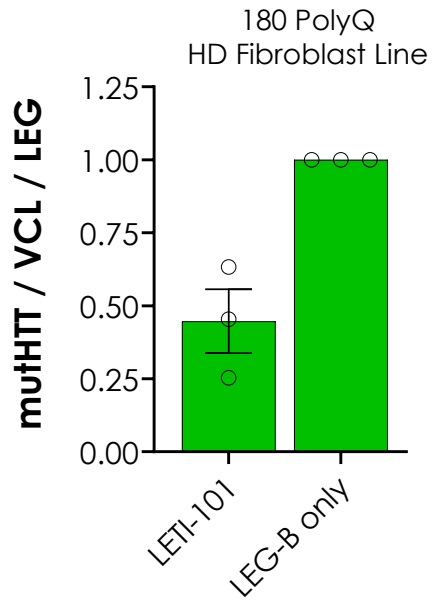
Maximum editing of 50% for T-allele



HD Fibroblast Cell Lines

Selective Reduction of Mutant HTT Protein only

mHTT Protein



- LETI-101 composition 'LEG-B-Guide1' **selectively edits the *mHTT* allele** only in the presence of the PAM-forming 'T' SNP rs362331
- LETI-101 composition 'LEG-B-Guide1' **selectively reduces mHTT protein**, but does not affect wtHTT protein levels in either patient-derived or healthy donor cell lines

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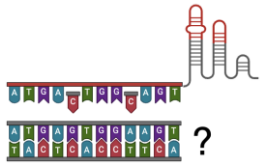
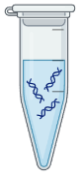
RNA transfection. Protein quantification by capillary electrophoresis. Error bars represent mean ± SE
Note: A small proportion of edited reads could not be categorized as coming from 'C' or 'T' allele due to the deletion covering the targeted SNP ("Not Determined")

Off-target analysis reveals exquisite specificity of LETI-101

Off-Target Identification Strategy





Bioinformatic Prediction

Predicts off-targets based on homology to guide

Population Variation Analysis

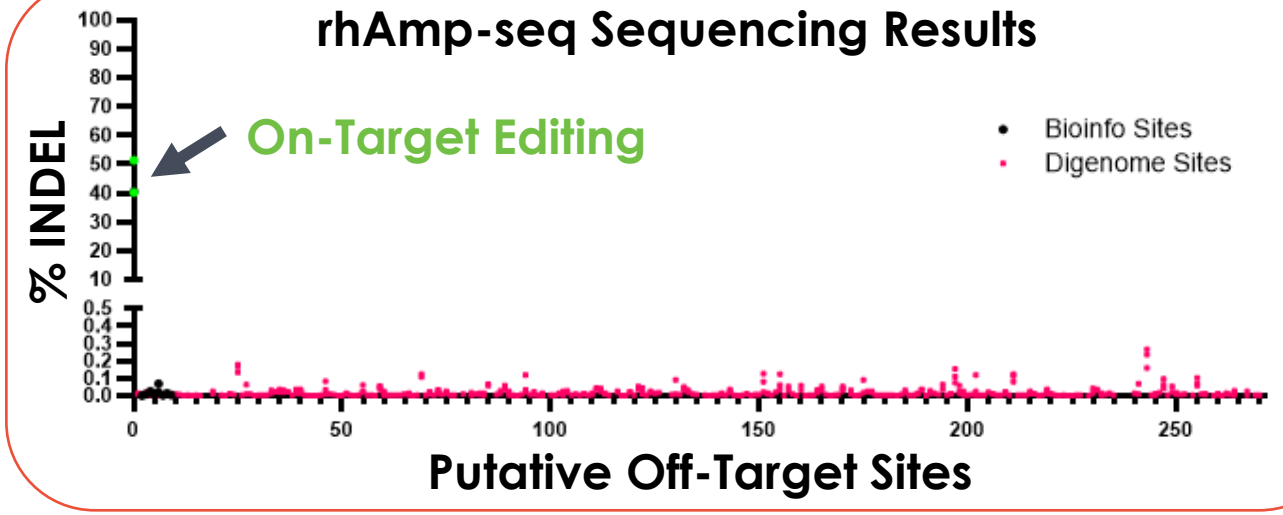
Identifies SNPs that could pose off-target risks

Digenome-Seq

Unbiased biochemical method to identify putative off-targets

~300 putative off-target sites identified and profiled via amplicon sequencing using genomic material from HD patients edited with mRNA/RNA delivery

Off-Target Results Compiled Across 3 HD Fibroblast lines



Indels

No off-target editing observed at sequenced sites & no off-target liabilities identified

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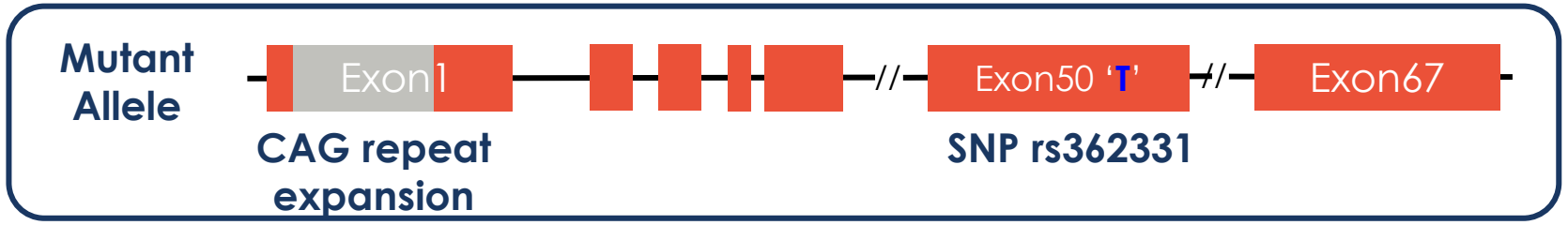
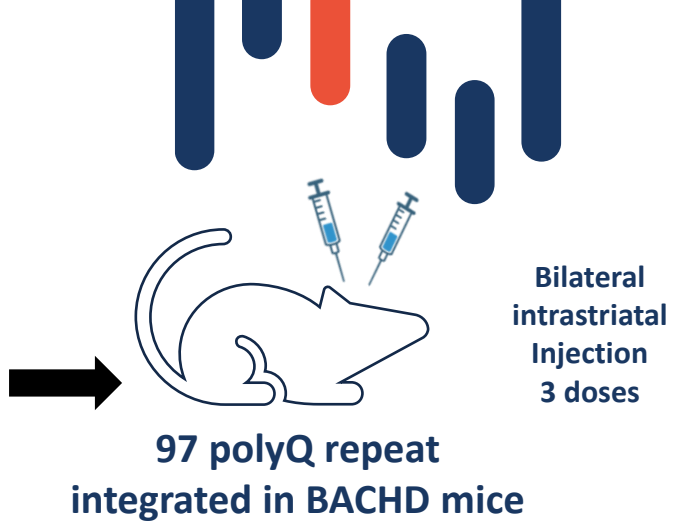
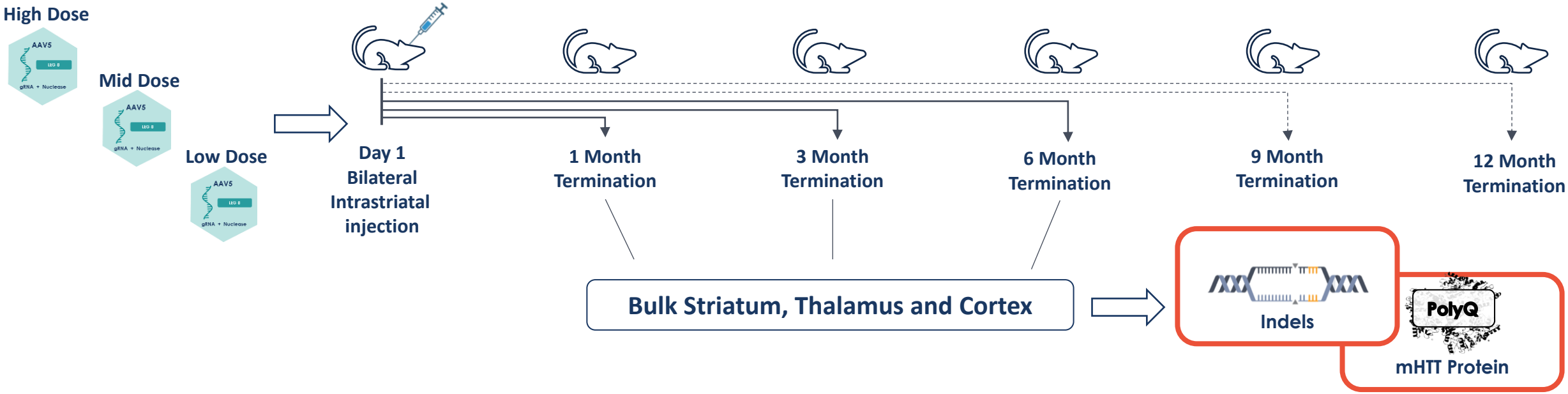
LETI-101 Dose-dependent activity in striatum of BACHD transgenic rodent model







Intrastriatal delivery of LETI-101 resulted in:

- Dose-dependent AAV vector copy, guide RNA expression, & LEG expression
- Dose-dependent, on-target editing of mHTT allele and up to **80% reduction of mHTT protein**

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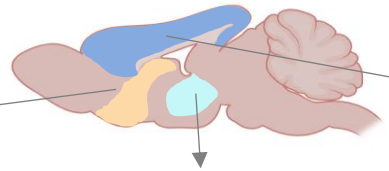
LETI-101 Activity increases with dose and time in BACHD transgenic rodent model



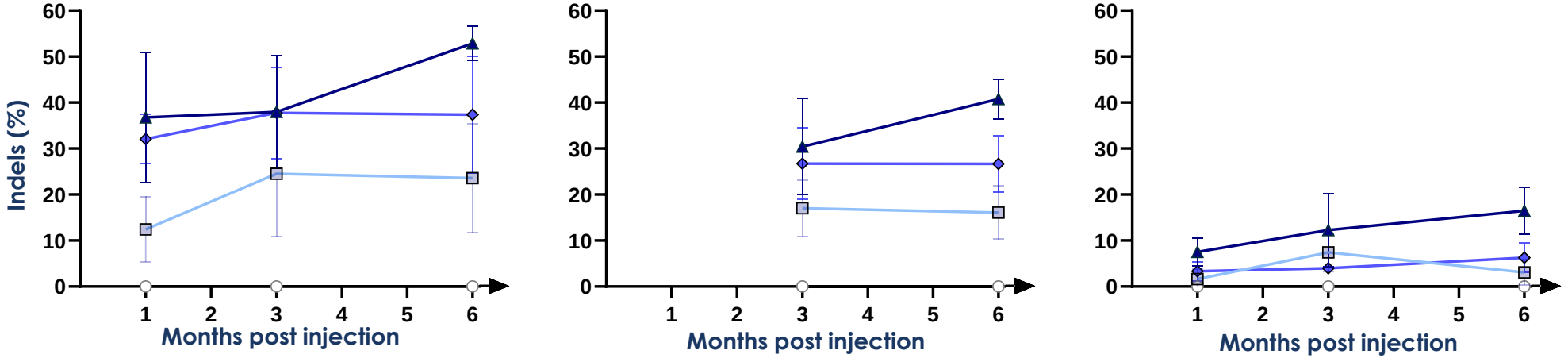
Striatum

Thalamus

Cortex

Indels

mHTT Protein



Potentially clinically relevant threshold of 40% reduction

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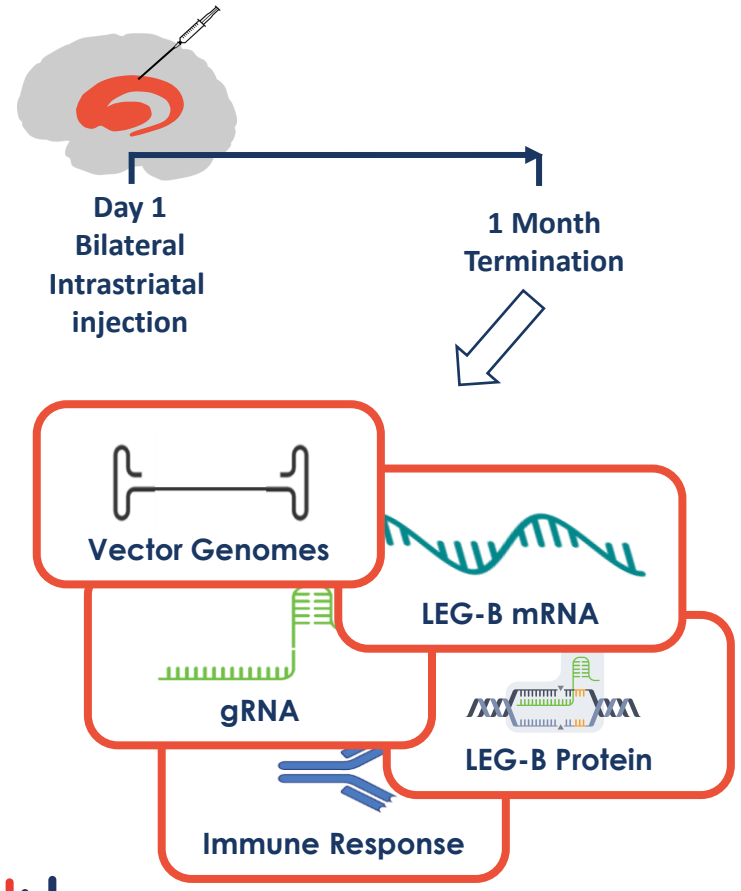
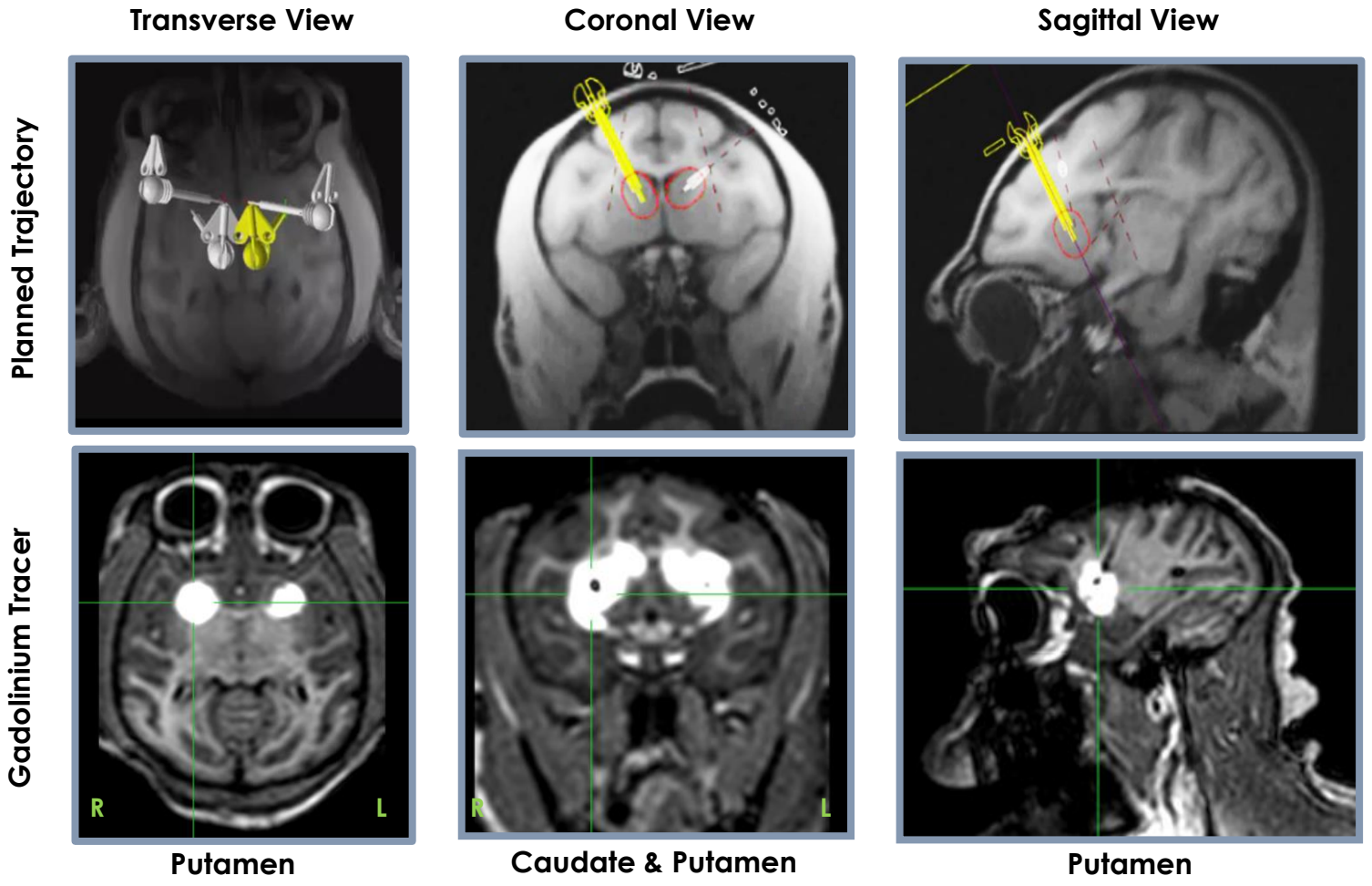
Bilateral intrastriatal delivery of AAV5-LEG-B-gRNA in NHPs with MRI guided CED resulted in high level biodistribution and expression in brain regions critical for HD pathology.

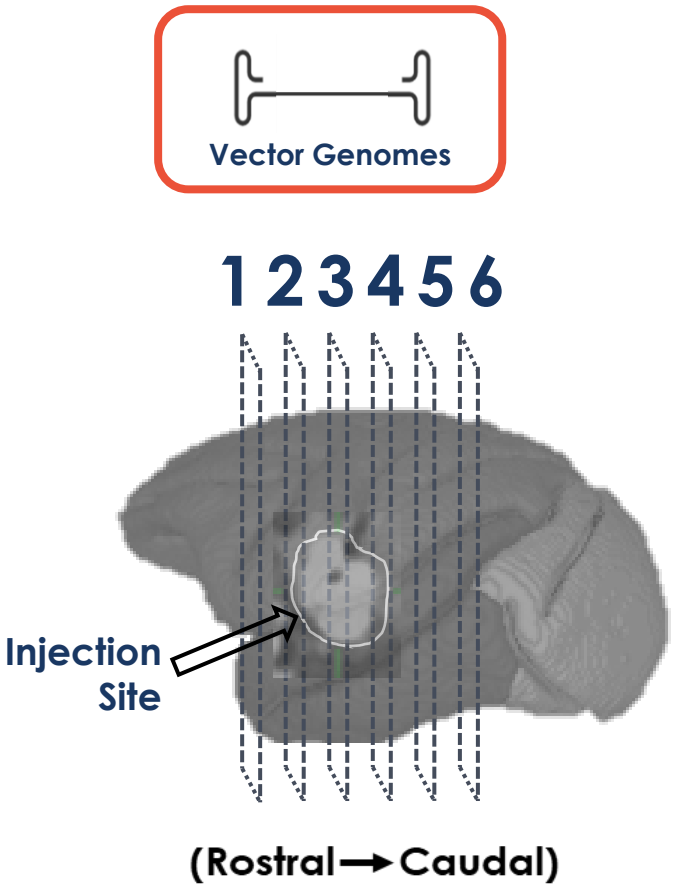
TARGETED CNS DELIVERY

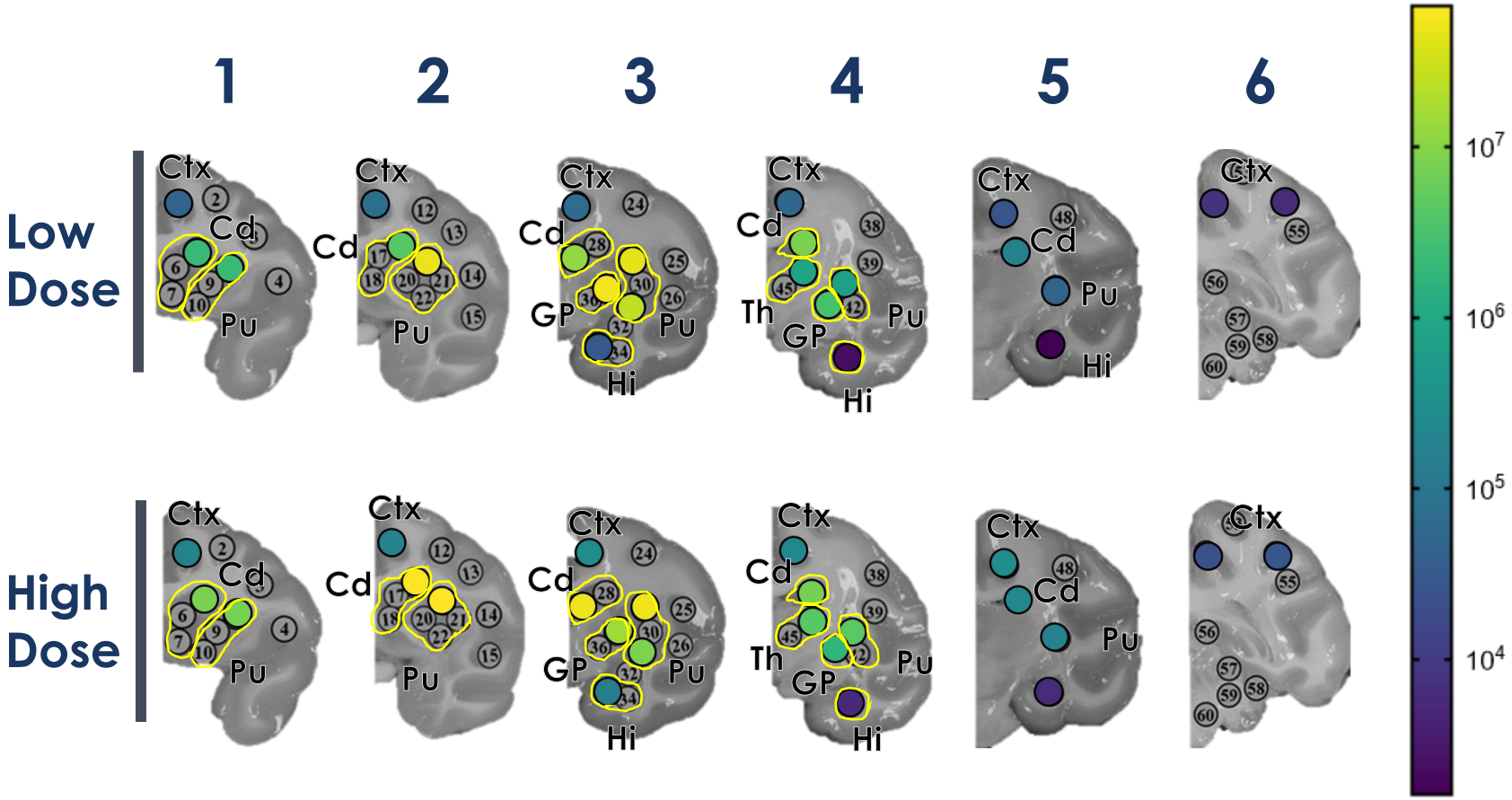




CED: convection enhanced delivery, infusion conducted with neuroinfuse™ (Renishaw, UK) device.

One-month biodistribution in NHP: Vector genomes observed in striatum and across brain regions





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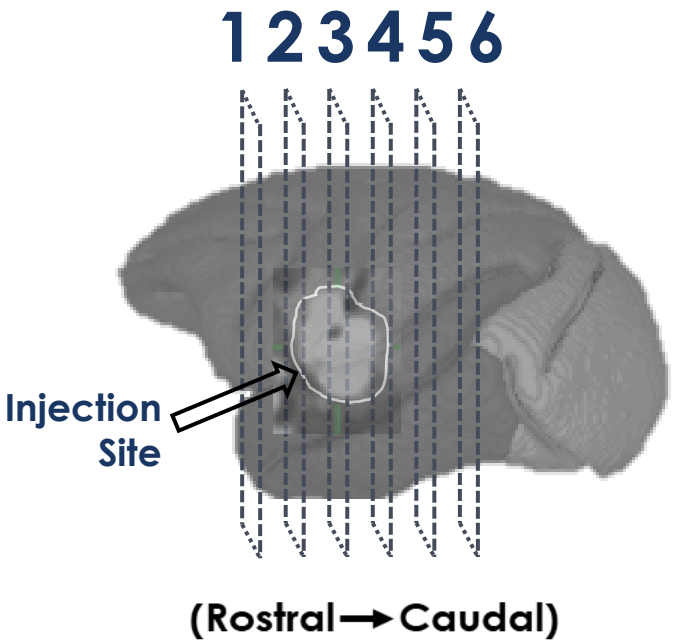
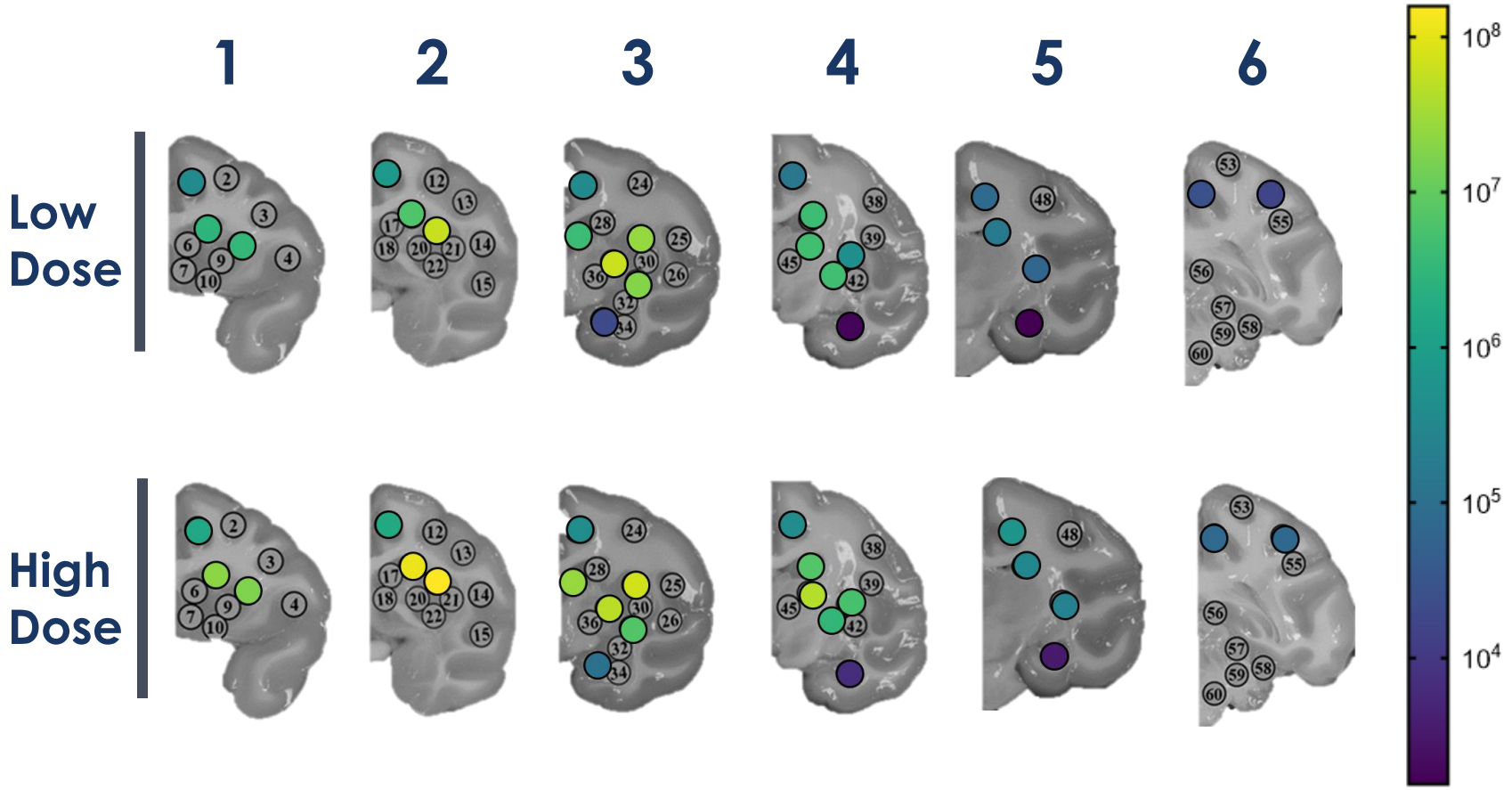
Each circle represents an average across NHPs from low dose (n=2) or high dose (n=3)

One-month biodistribution in NHP: gRNA highly expressed in striatum and across brain regions





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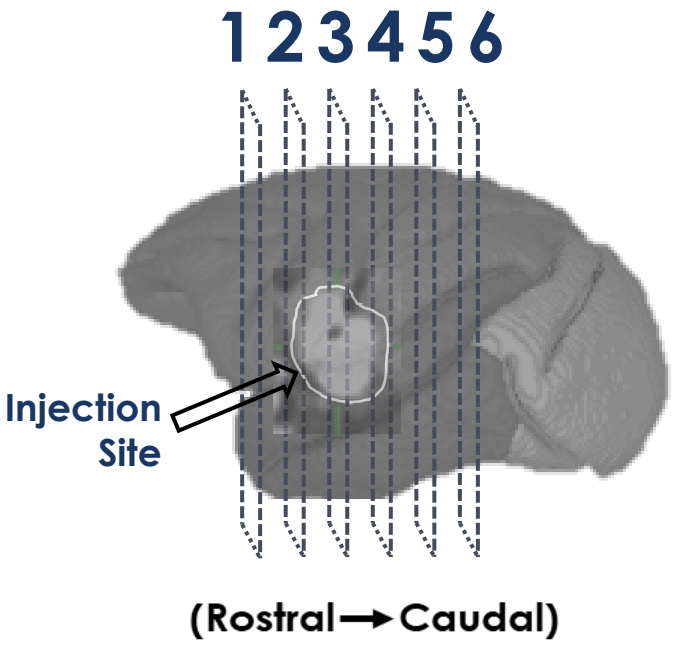
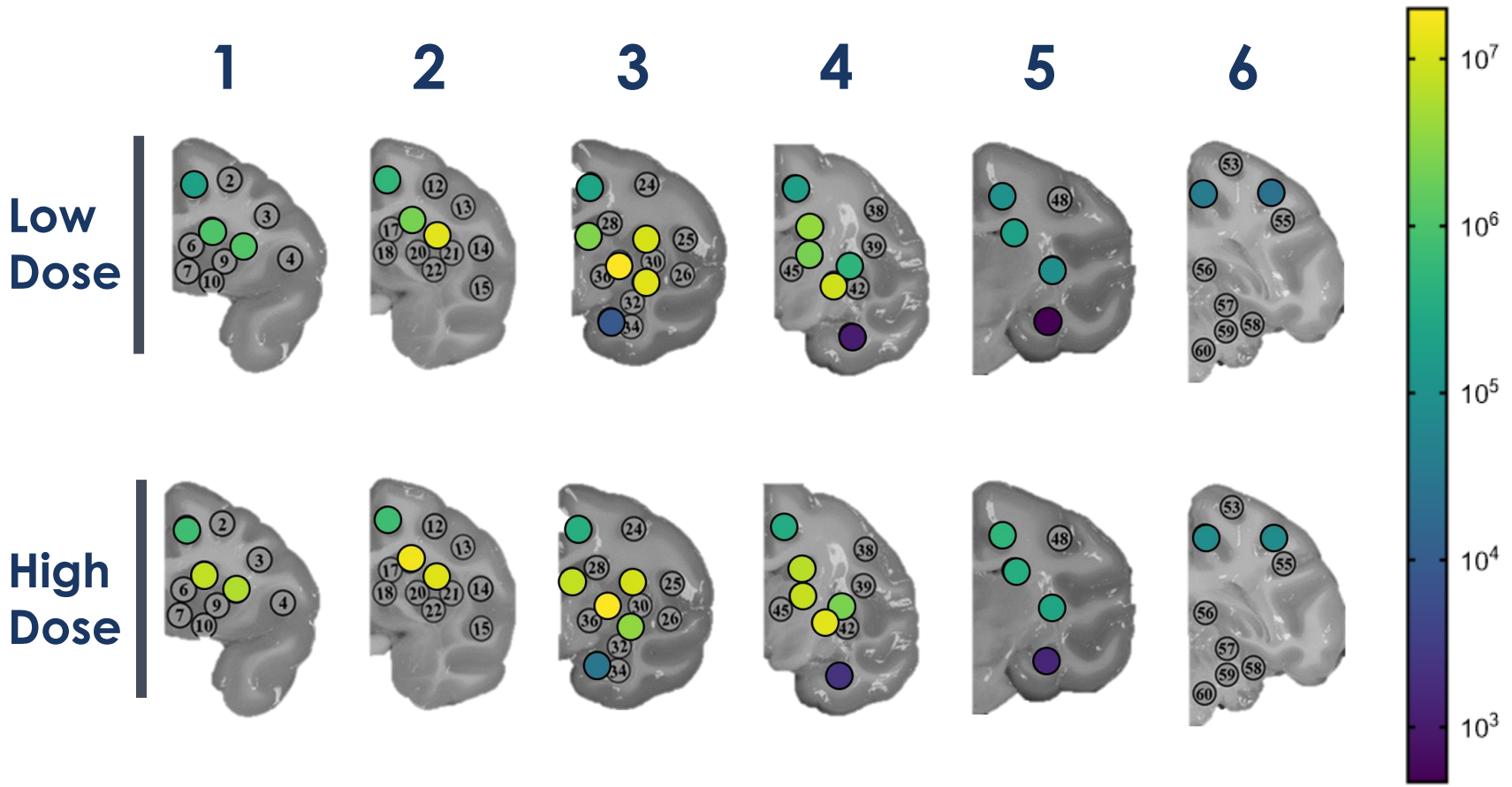
Each circle represents an average across NHPs from low dose (n=2) or high dose (n=3)

One-month biodistribution in NHP: LEG-B mRNA highly expressed in striatum and across brain regions





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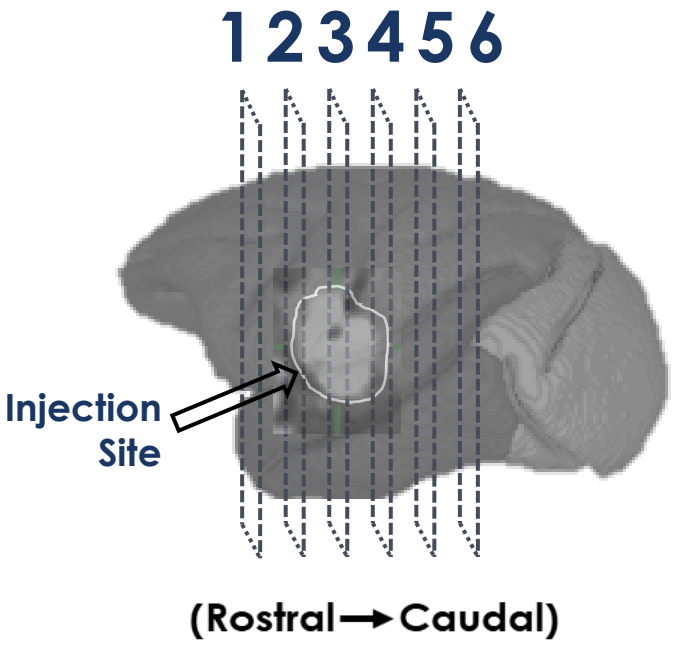
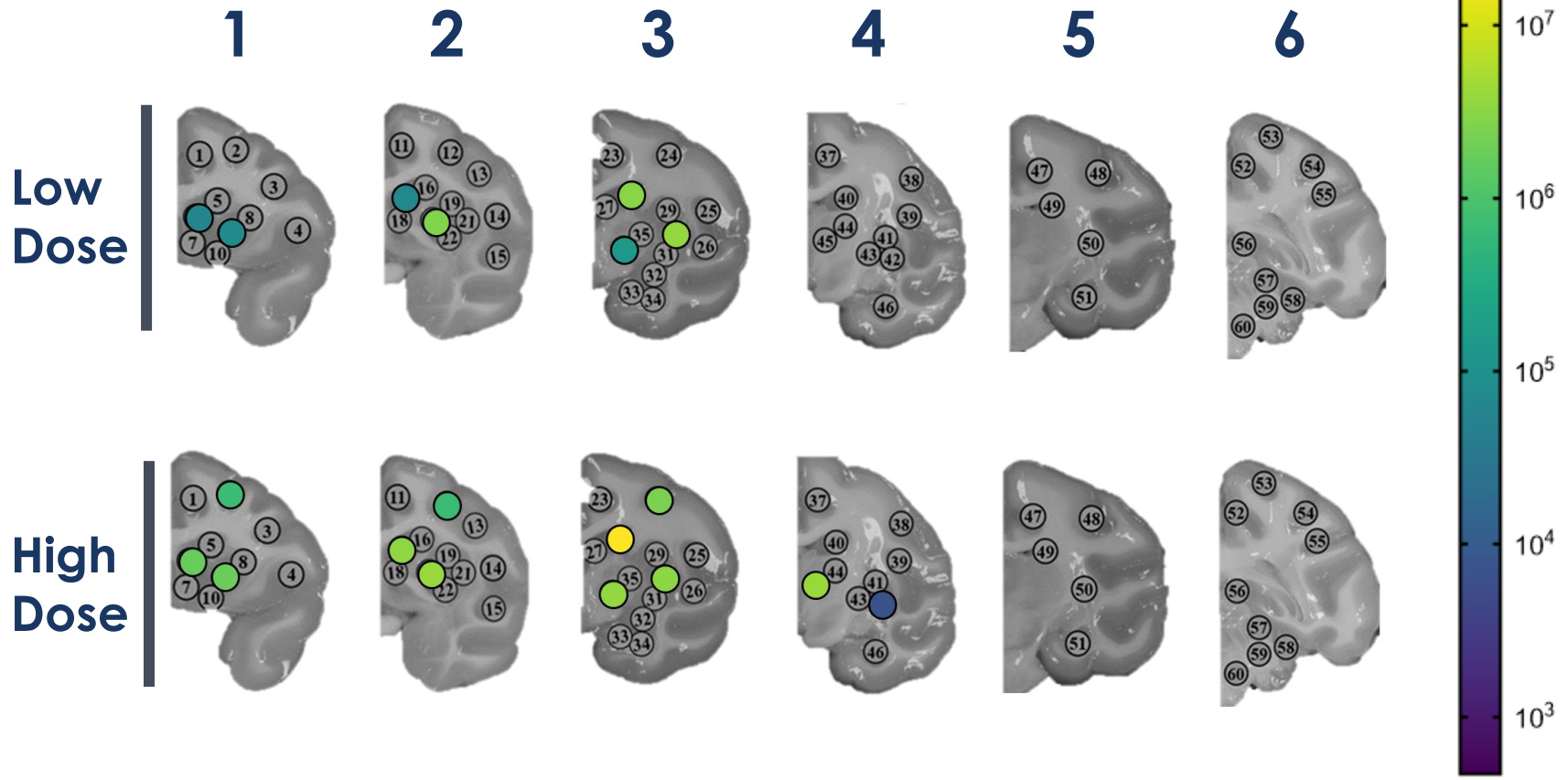
Each circle represents an average across NHPs from low dose (n=2) or high dose (n=3)

One-month biodistribution in NHP: LEG-B protein highly expressed in striatum and across brain regions





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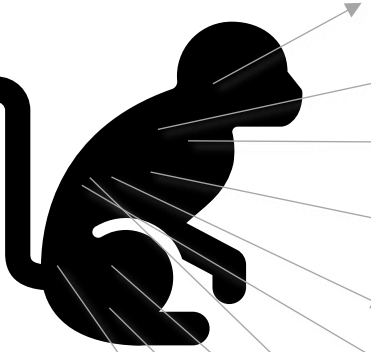
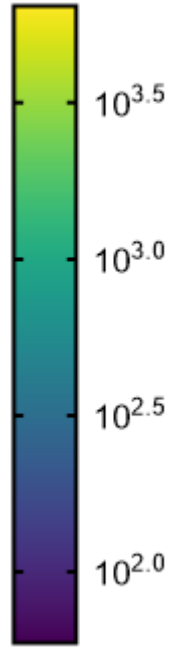
Each circle represents an average across NHPs from low dose (n=2) or high dose (n=3)

Minimal systemic biodistribution of vector with none detected in gonads



	Low Dose			High Dose		
	Vector Genomes	LEG-B mRNA	gRNA	Vector Genomes	LEG-B mRNA	gRNA
Lymph Node	○	○	○	●	●	●
Heart	○	○	N/A	○	○	N/A
Lung	○	○	N/A	●	○	N/A
Liver	●	●	●	●	●	●
Kidney	●	○	○	●	●	○
Spleen	●	●	○	●	●	●
Adrenal Gland	○	○	N/A	●	○	●
Gonads	○	○	N/A	○	○	N/A
Muscle	○	○	N/A	○	○	N/A
Sciatic Nerve	○	○	N/A	○	○	N/A



Copies/µg DNA or RNA

NHP in Low dose: male n=1 and female n=1. High dose : male n=1 and female n=2.
Variable tissue sample n based on availability

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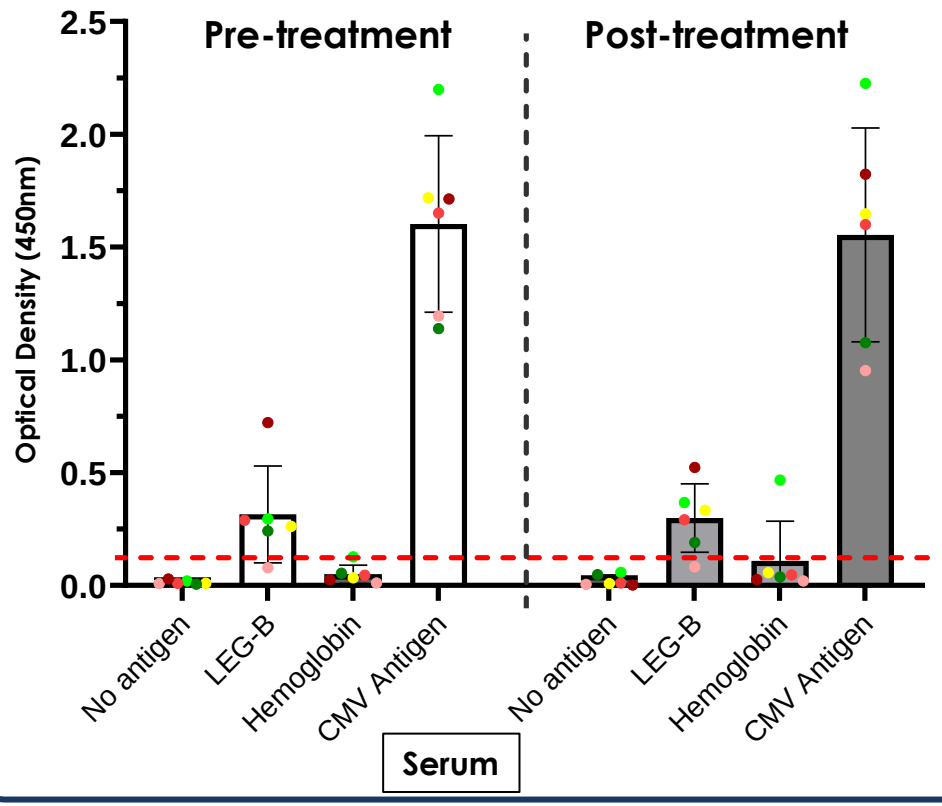
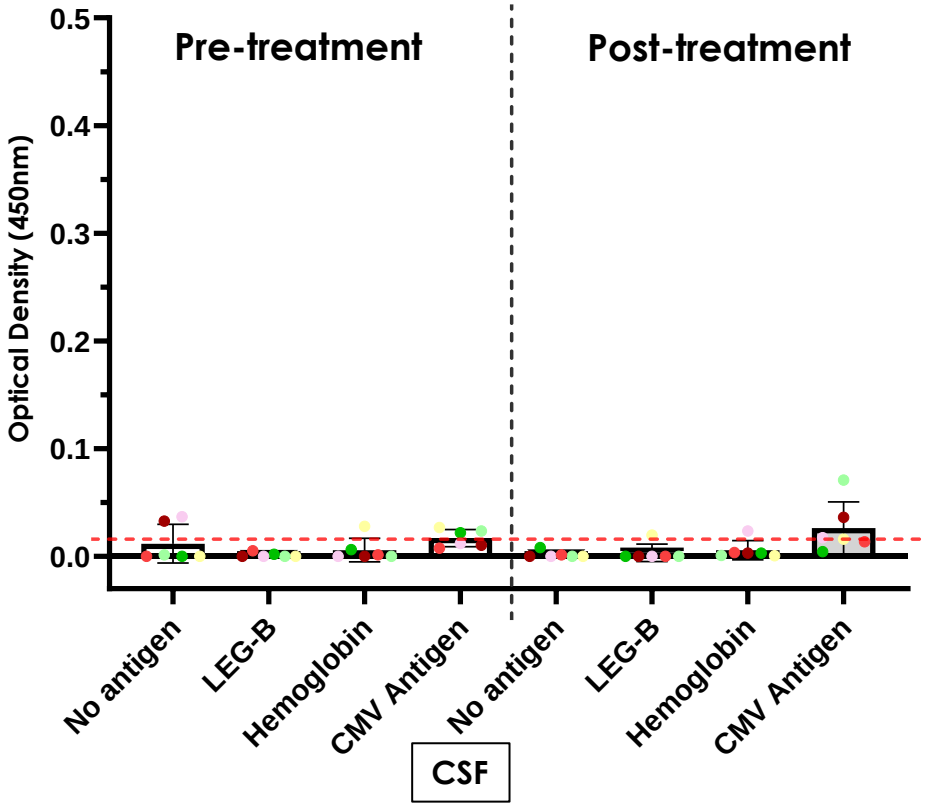
No change in immune response to the LEG-B nuclease observed and no detectable vector exposure in the gonads

Immune Response

Total Antibodies to LEG-B



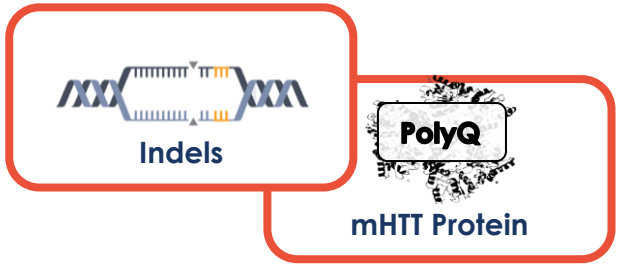


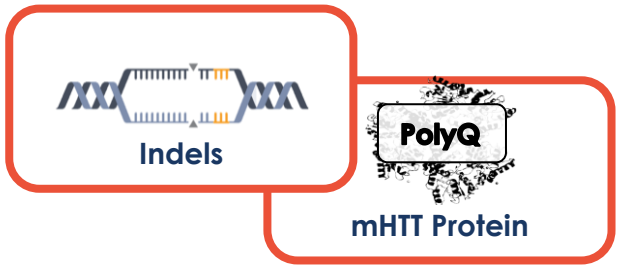
Vehicle
Low Dose
High Dose

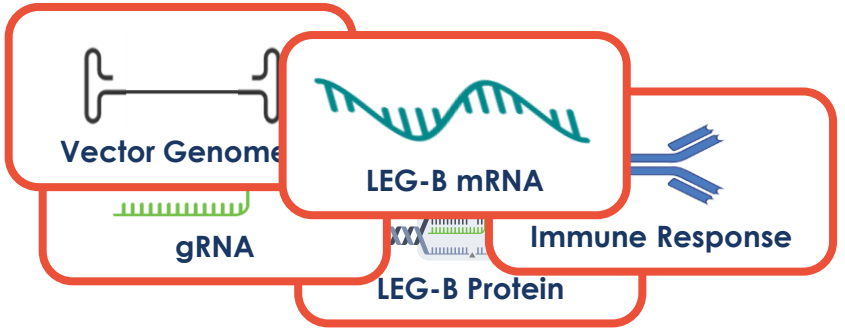
CSF: cerebral spinal fluid; gRNA: guide RNA. CED: Convection enhanced delivery; NHP: nonhuman primate. Results from one month tolerability and biodistribution study of AAV5-LEG-B-gRNA following bilateral intrastriatal administration in adult cynomolgus monkeys.

LETI-101: A precision editing approach as potential one-time treatment for Huntington's Disease

PROOF OF CONCEPT	PHARMACOLOGY IN RODENTS	BIODISTRIBUTION & TOLERABILITY IN NHP







LETI-101 (LEG-B-SGN) delivered by RNA in **patient-derived cells** resulted in allele selective editing of *mHTT* gene and reduction of mutant HTT protein while wildtype HTT protein levels were unaffected

LETI-101 delivered intrastriatally in **BACHD transgenic mice** resulted in dose-dependent vector disposition, transgene expression, and clinically relevant reduction of mHTT protein in striatum

LETI-101 delivered intrastriatally in **NHP** (cynomolgus macaque) resulted in dose-dependent vector biodistribution and transgene expression across brain regions that are critically vulnerable in HD. A **NOAEL of 1.13 x 10^{13} vg** (the highest dose evaluated) was obtained in the one-month tolerability and biodistribution study

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