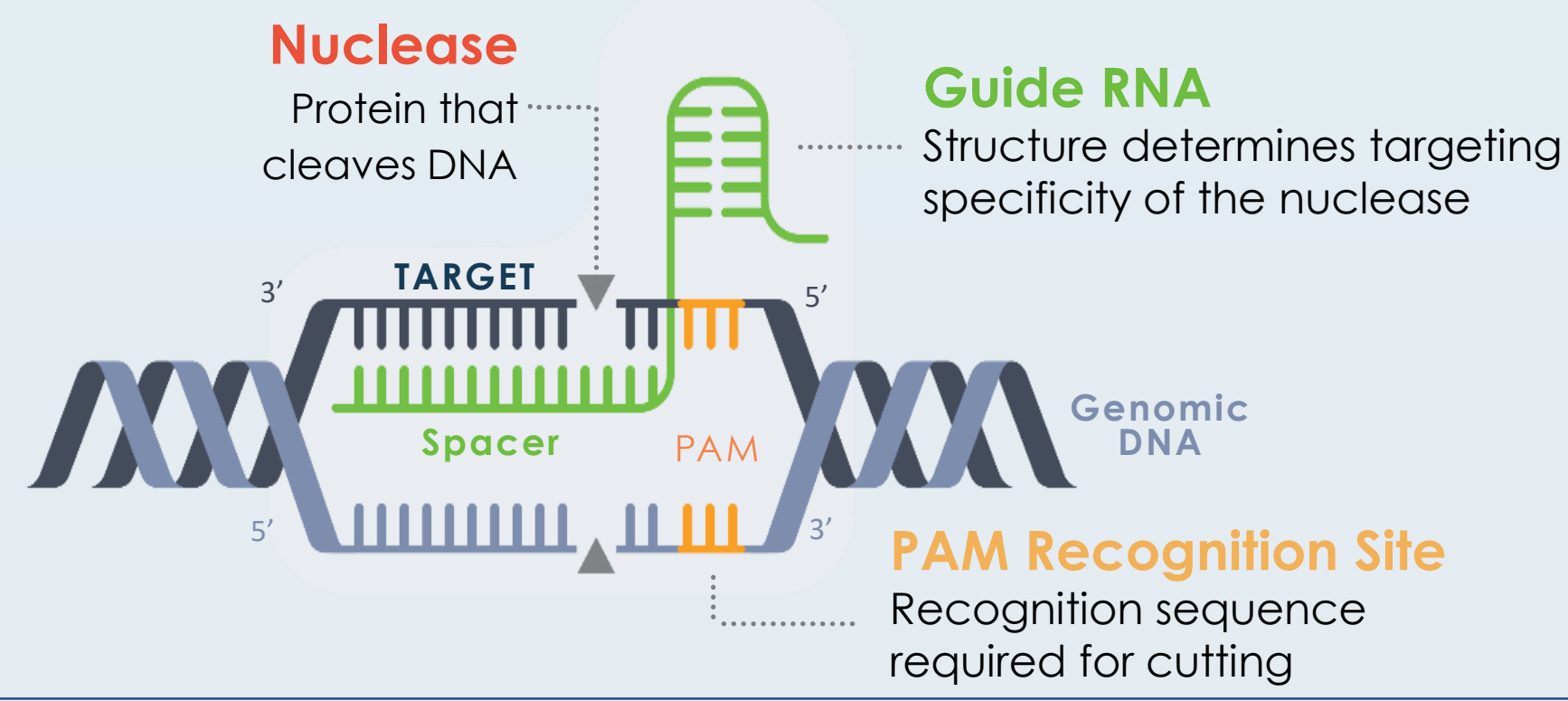


## Life Edit Therapeutics

**OUR PLATFORM** Life Edit's genome editing platform offers a large and diverse collection of novel RNA guided nucleases (LEGs), base editors, & reverse transcriptase editors that provide flexible editing strategies and unprecedented access to genomic loci of interest. Our platform is derived from both an ever-growing proprietary collection of microbes and extensive mining of metagenomic data.

### Life Edit Genes (LEGs)



Life Edit nucleases (Life Edit Genes or LEGs) have unique PAM recognition sequences enabling flexible target strategies for diverse genomic targets, including many disease-linked genes.

Life Edit Lead Nucleases				
Nuclease	Base Pairs	Amino Acids	MW (kDa)	PAM
LEG-A	3213	1071	126	NNNCC
LEG-B	3450	1150	133	NNRYA
LEG-C	3156	1052	124	NNGRR
LEG-D	3390	1130	130	NNGG
SpCas9	4104	1368	158	NGG

## Therapeutic Strategy

<b>SNP Based Allele-Selective Editing</b>	The PAM site generated by HTT Exon50 rs362331 SNP allows selective targeting of HTT alleles with Life Edit nucleases based on the presence of 'C' (LEG-A) or 'T' (LEG-B) nucleotide
<b>Target Product Profile</b>	AAV5-delivered Life Edit nuclease (LEG-B) and guide RNA (SGN2) targeting Huntington's Exon50 rs362331 'T' allele, to be expressed in at least 50% of striatal neurons, resulting in ≥ 40% knockdown of mutHTT
<b>Mode of Action</b>	Allele-specific DSBs activate NHEJ repair pathway, form INDELS that cause frameshift leading to mRNA containing premature-stop codons which are degraded by non-sense mediated decay
<b>Compact Nucleases Enable AAV Delivery</b>	<p>LEG-B (3.4 kb) ~4.7 kb</p> <p>SpCas9 (4.1 kb) ~5.4 kb</p>

## Transgenic Murine Models and Striatal Injections

**Qualification of Intrastratial Injections**

Mouse Brain Dorsal View  
Automated Stereotaxic Delivery  
2µl/site

AAV5-eGFP  
DARPP32 (MSNs)

**BACHD Alleles**

Human HTT: 20 kbp flanking, Exon1 (97 CAG/CAA), Exon50-T, Exon67, 50 kbp flanking

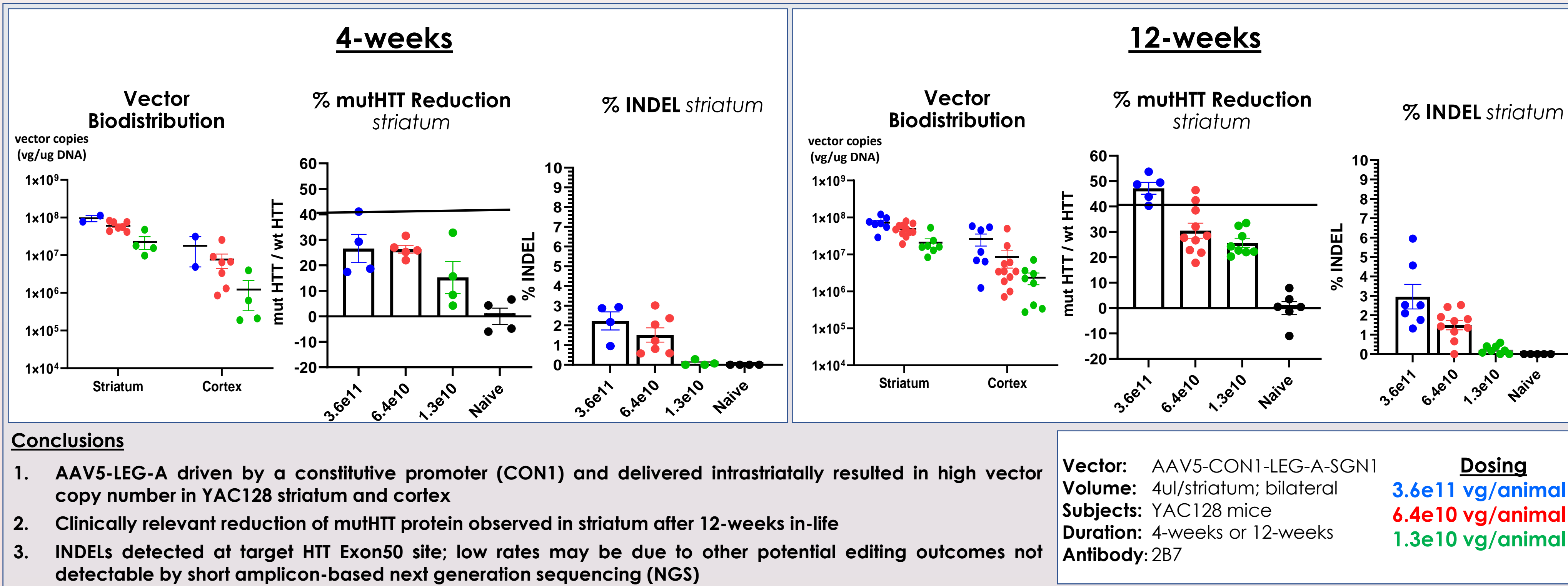
Mouse Hdh: Exon1 (7 CAG), Exon1

Transgenic Murine Models			
Model	rs362331 SNP	CAG/CAA Repeats	Transgene Copy #
BACHD	T	97	5x
YAC128	C	92 & 118	3x

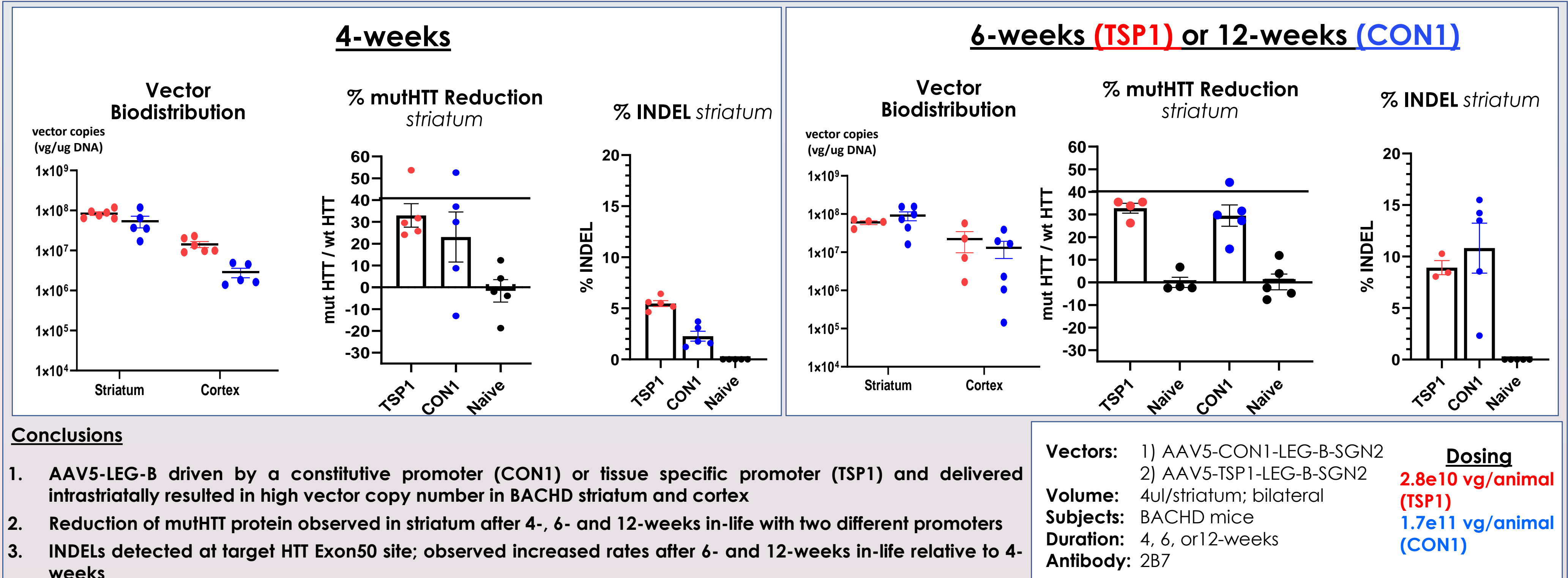
BACHD & YAC128 lines are humanized transgenic mice containing a full-length human mutHTT allele in addition to the normal mouse Hdh alleles. The mutHTT transgene contains a mixed CAG/CAA repeat expansion and, importantly, either the Exon50 rs362331 'T' (BACHD) or 'C' SNP (YAC128).

A fluorescent eGFP reporter driven by the CAG promoter was packaged into an AAV5 vector and bilateral injections were performed via automated stereotaxic delivery. Brain sections subsequently underwent IHC using DARPP32 primary antibody conjugated to a fluorescent secondary for visualization using fluorescent microscope.

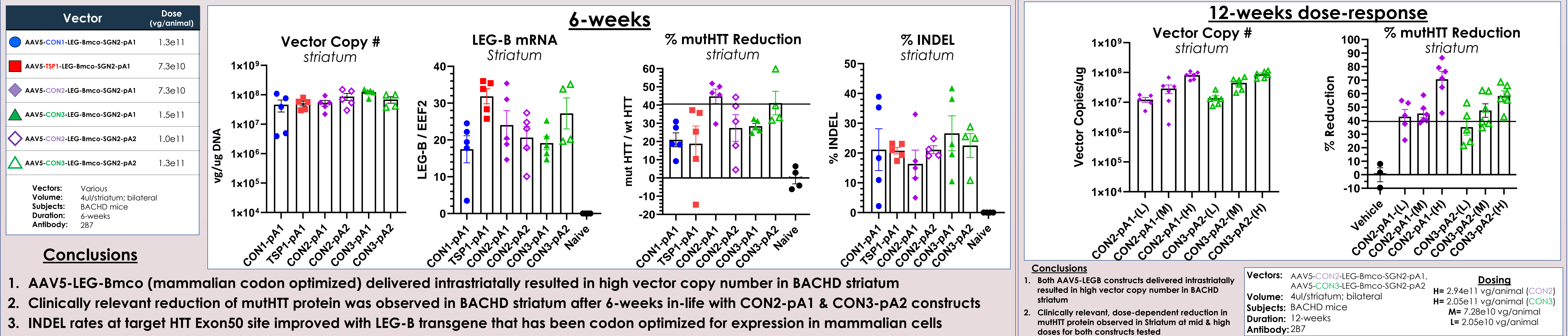
## Selective Editing of rs362331 'C' SNP with LEG-A



## Selective Editing of rs362331 'T' SNP with LEG-B



## Optimization of Selective Editing of rs362331 'T' SNP with LEG-B



## Summary

- Life Edit nucleases are compact, facilitating all-in-one delivery with a single AAV vector, and characterized by diverse PAM recognition sequences, enabling flexible targeting of genomic loci including many disease-linked genes
- Life Edit nucleases LEG-A and LEG-B enable allele specific targeting of mutant HTT based on the PAM generated by HTT Exon50 SNP rs362331
- Life Edit gene editing systems packaged into AAV5 efficiently transduce CNS tissue *in vivo* with high vector copy numbers detected up to 12-weeks post injection (furthest time point tested)
- AAV5-delivered Life Edit gene editing systems targeting mutant HTT allele resulted in clinically relevant reduction of mutant HTT protein in the striatum of YAC128 and BACHD transgenic mice which contain a full-length human mutant HTT gene
- Optimization of vectors using different combinations of promoters, polyA signals, and codon optimized transgenes resulted in improved editing rates and reduction of mutant HTT protein
- Life Edit is an ElevateBio company, enabling access to ElevateBio's proven expertise in gene therapy development including vector engineering, process development, and GMP manufacturing

