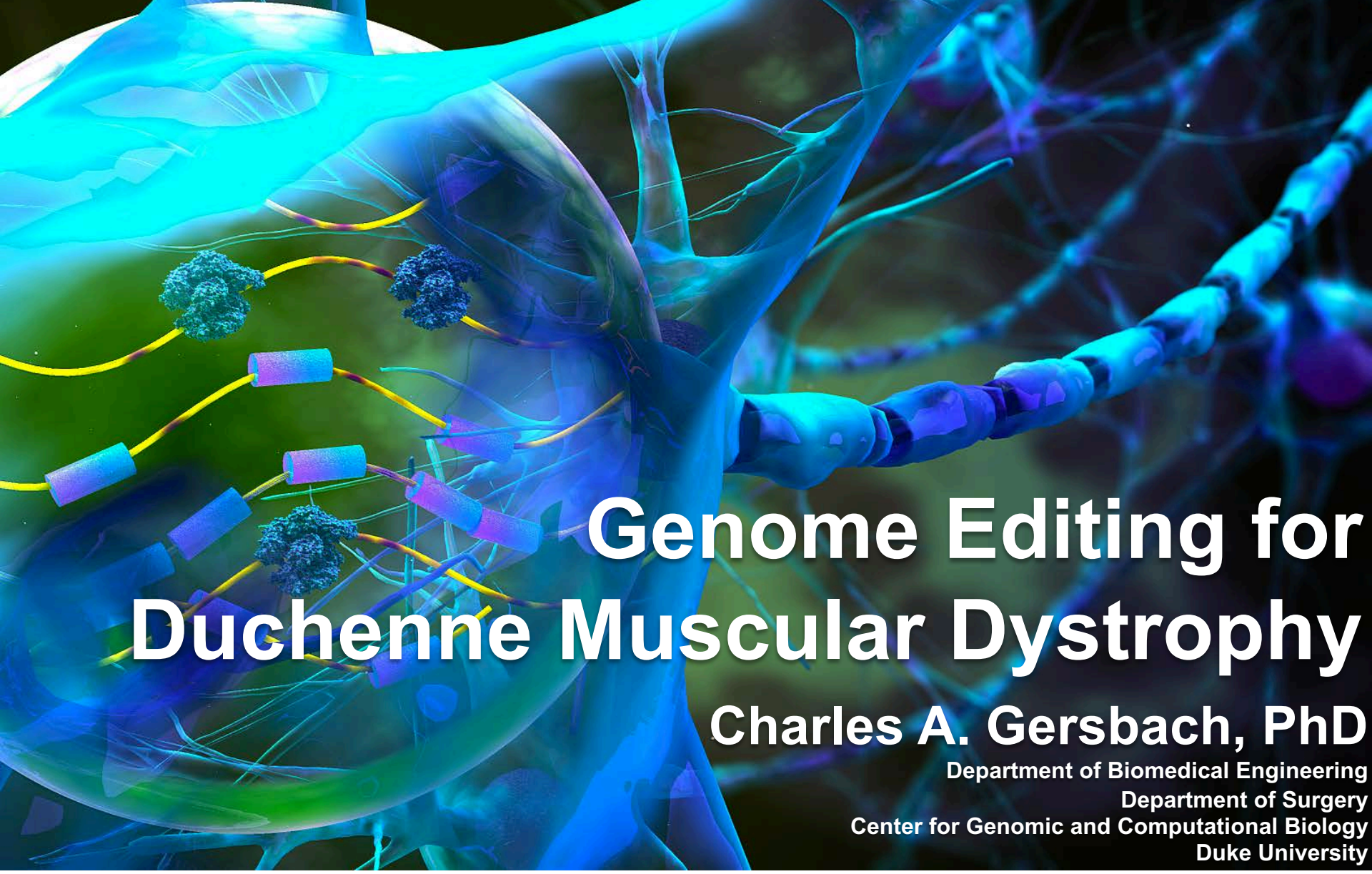


Genome Editing for Duchenne Muscular Dystrophy

Charles A. Gersbach, PhD

Department of Biomedical Engineering
Department of Surgery
Center for Genomic and Computational Biology
Duke University

Duke UNIVERSITY

June 28, 2019
Parent Project Muscular Dystrophy
Orlando, FL

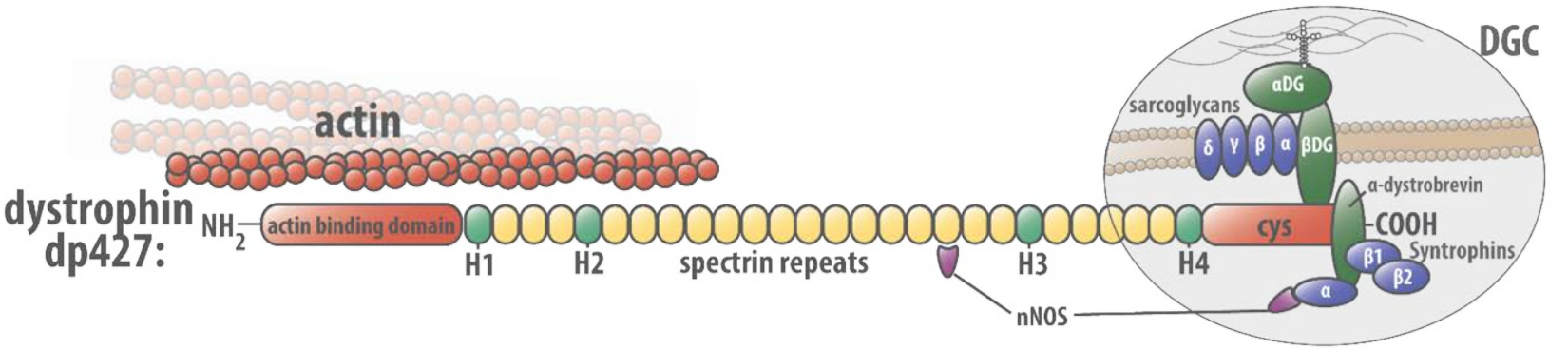
Disclosures

Sarepta Therapeutics – sponsored research, consultancy

These relationships are managed by Duke University.

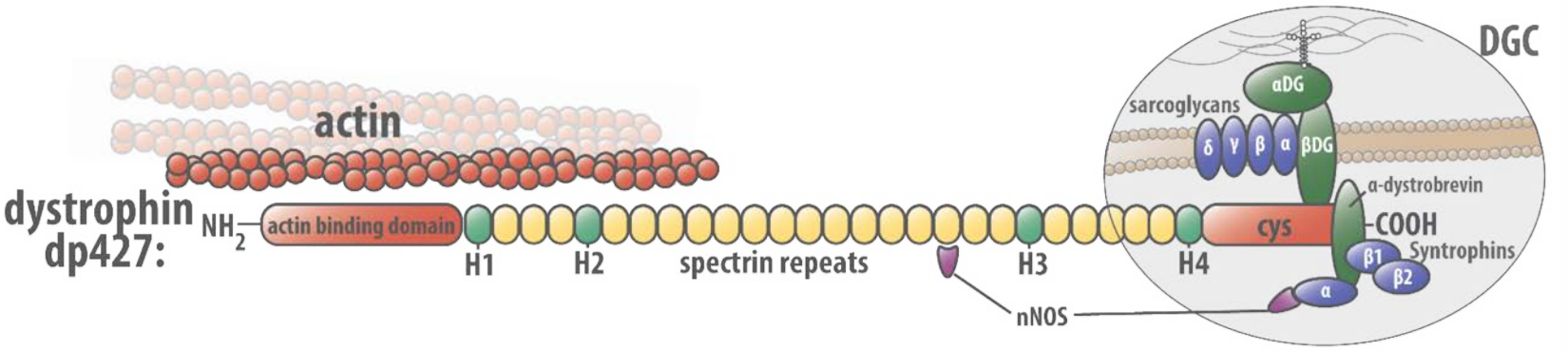
Gene Editing

Duchenne Muscular Dystrophy



Nelson and Gersbach, *Muscle Gene Therapy* (2018)

Duchenne Muscular Dystrophy



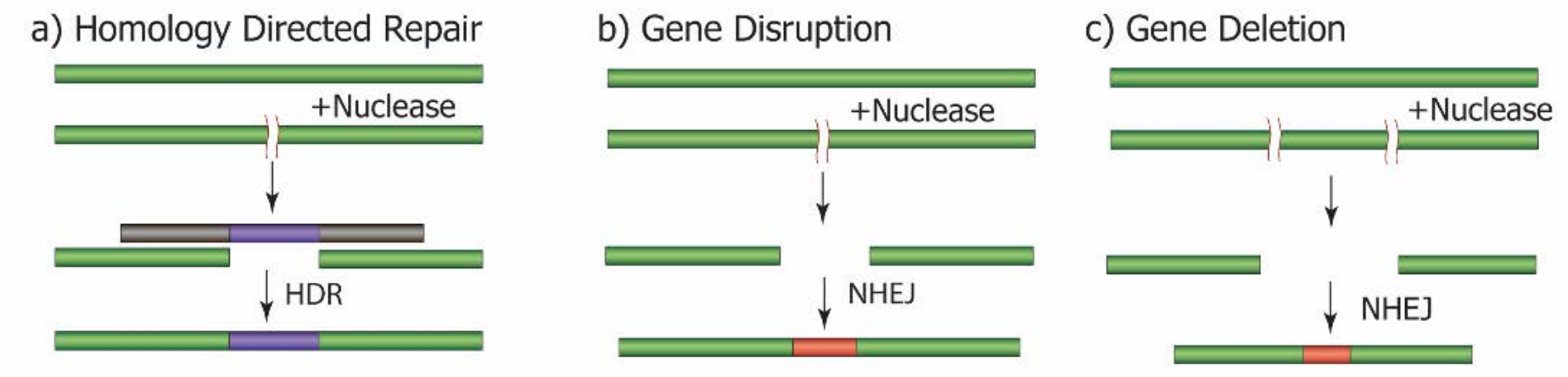
Why somatic cell gene editing for Duchenne?

- Clinical need
- Potential to create larger, perhaps full-length dystrophin protein
- Potential to edit genes in satellite cells
- Potential for long-term dystrophin restoration
- Multinucleated target cells means low level editing may be sufficient
- Correctable by exon excision
- Hotspot mutation regions can be addressed for large numbers of patients

Nelson and Gersbach, *Muscle Gene Therapy* (2018)

Genome Editing with Engineered Nucleases

Targeted breaks in DNA stimulate natural DNA repair pathways



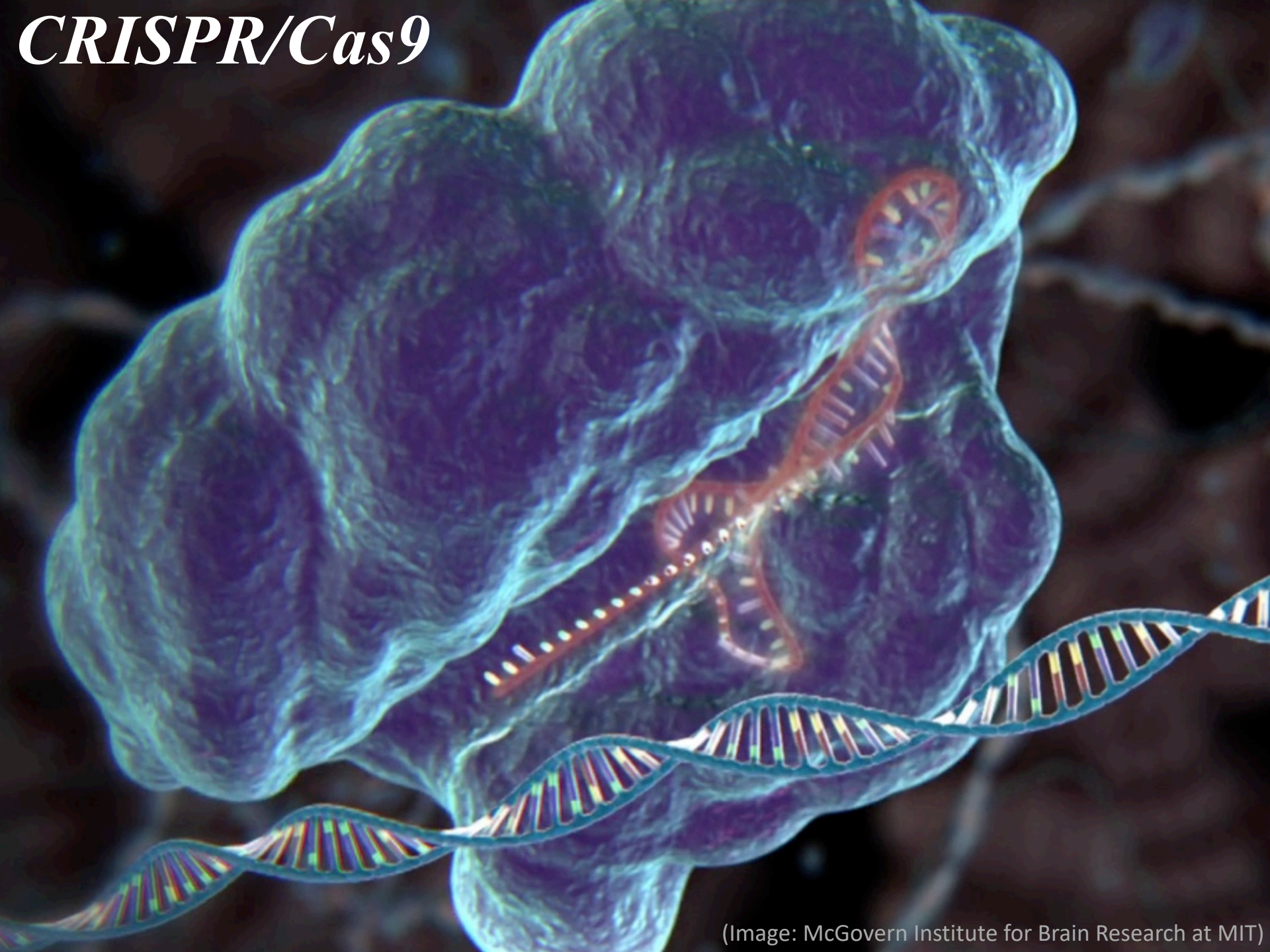
Targeted insertions

Splice site disruption
OR
re-framing

Exon excision

Nelson and Gersbach, *Annual Review Chem Biol Eng* (2016)

CRISPR/Cas9



(Image: McGovern Institute for Brain Research at MIT)

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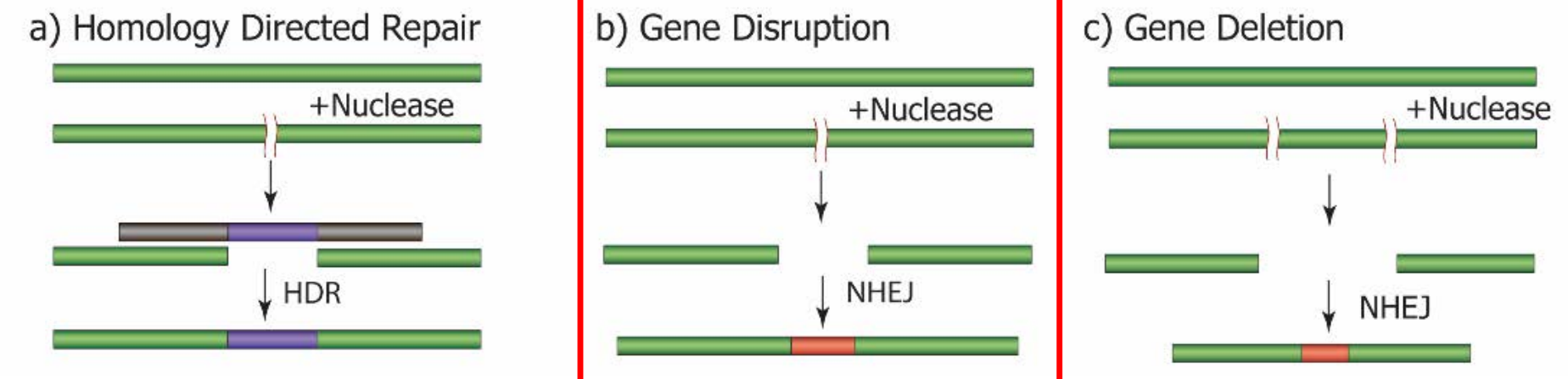

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Nelson and Gersbach, *Annual Review Chem Biol Eng* (2016)

Single-Cut Reframing for DMD

WT dystrophin

✓ WT dystrophin protein

Deletion of exons 48-50

✗ DMD – out of frame, no protein

Deletion of exons 48-51; applicable to ~13% patients

✓ BMD – truncated, partially functional protein

Dave Ousterout

Ousterout et al. *Molecular Therapy* (2013), *Molecular Therapy* (2014), *Nature Communications* (2015)

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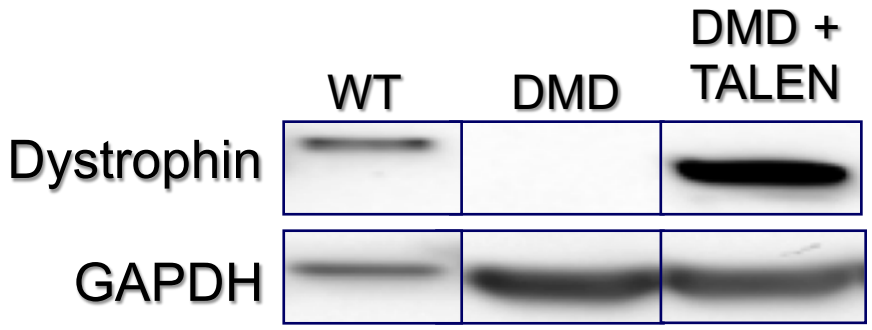
Reading Frame Correction by Targeted Genome Editing Restores Dystrophin Expression in Cells From Duchenne Muscular Dystrophy Patients

David G Ousterout[1], Pablo Perez-Pinera[1], Pratiksha I Thakore[1], Ami M Kabadi[1], Matthew T Brown[1], Xiaoxia Qin[2], Olivier Fedrigo[2], Vincent Mouly[3], Jacques P Tremblay[4] and Charles A Gersbach[1,2,5]



WT	CTCAGACTGTTACTCTGGTGACACAA	shift	(frame)
32	CTCAGACTGTT----TGGTGACACAA	-4	(+2)
40	CTCAGACTGTTACTCTG-TGACACAA	-1	(+2)
67	CTCAGACTG---------TGACACAA	-9	(+3)
96	CTCAGACTGTTACTC-GGTGACACAA	-1	(+2)
106	CTCAGACTGTTACT-----GACACAA	-5	(+1)
127	CTCAGACTGTTACTCTG---ACACAA	-3	(+3)
141	CTCAGACTGTTACT---GTGACACAA	-3	(+3)
145	CTCAGACTGTTACTCTG---ACACAA	-3	(+3)

Ousterout et al. *Molecular Therapy* (2013), *Molecular Therapy* (2014), *Nature Communications* (2015)

Single-Cut Reframing for DMD

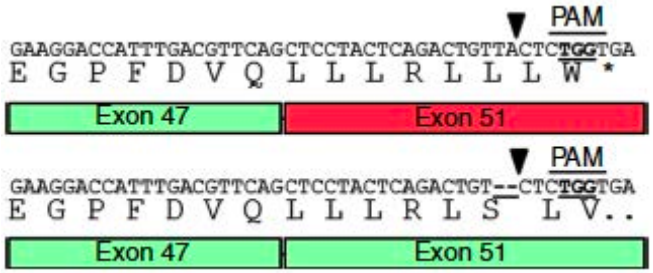
ARTICLE

Received 16 Nov 2014 | Accepted 8 Jan 2015 | Published 18 Feb 2015

DOI: 10.1038/ncomms7244

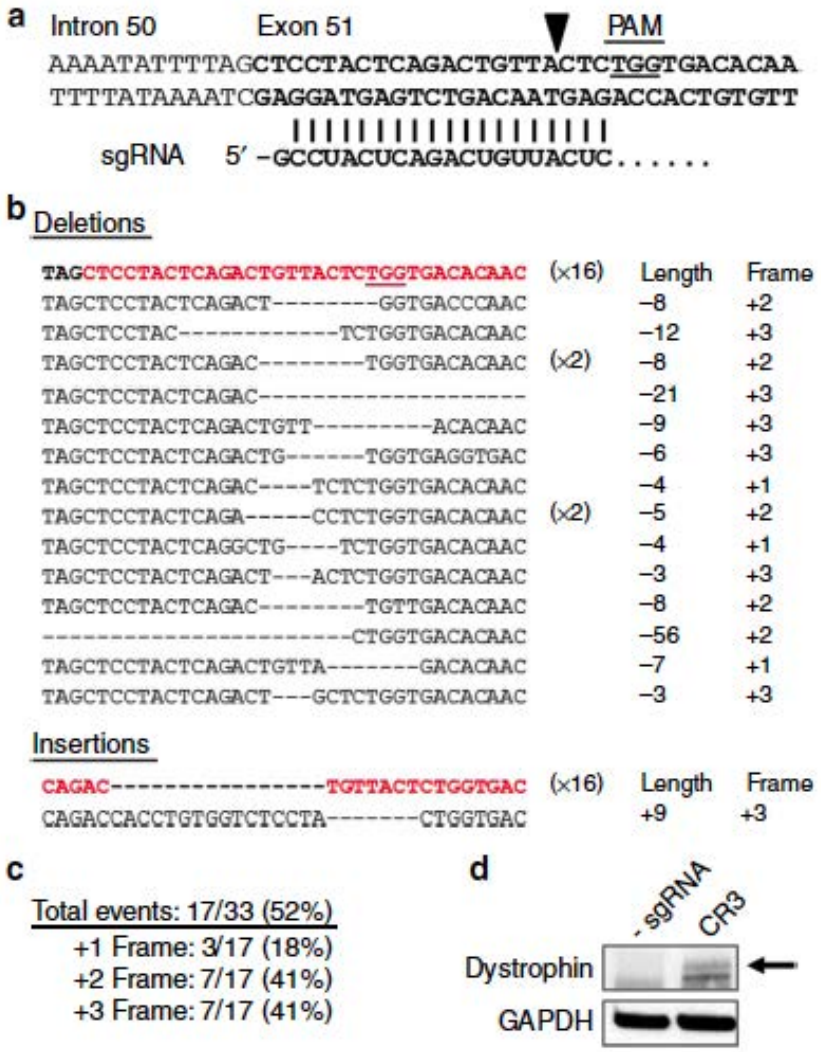
Multiplex CRISPR/Cas9-based genome editing for correction of dystrophin mutations that cause Duchenne muscular dystrophy

David G. Ousterout[1], Ami M. Kabadi[1], Pratiksha I. Thakore[1], William H. Majoros[2], Timothy E. Reddy[3,4] & Charles A. Gersbach[1,3,5]



Out-of-frame (Δ48–50)

Frame restored by small targeted deletion



Ousterout et al. *Molecular Therapy* (2013), *Molecular Therapy* (2014), *Nature Communications* (2015)

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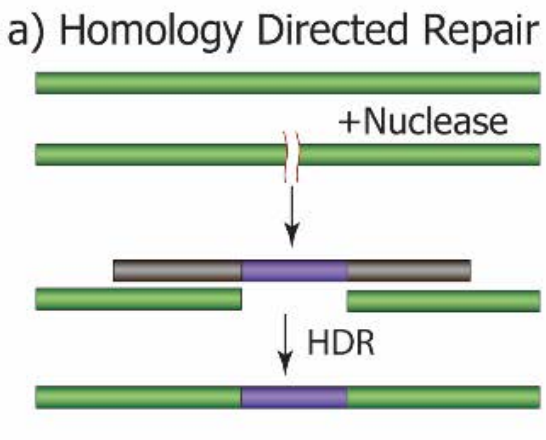
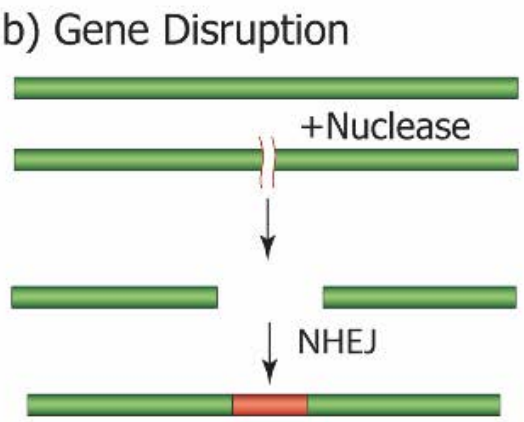
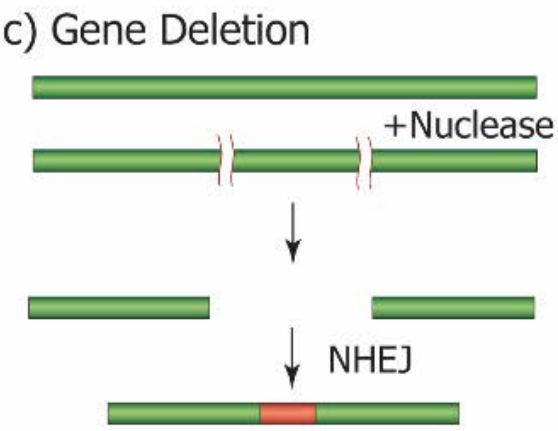


Targeted insertions



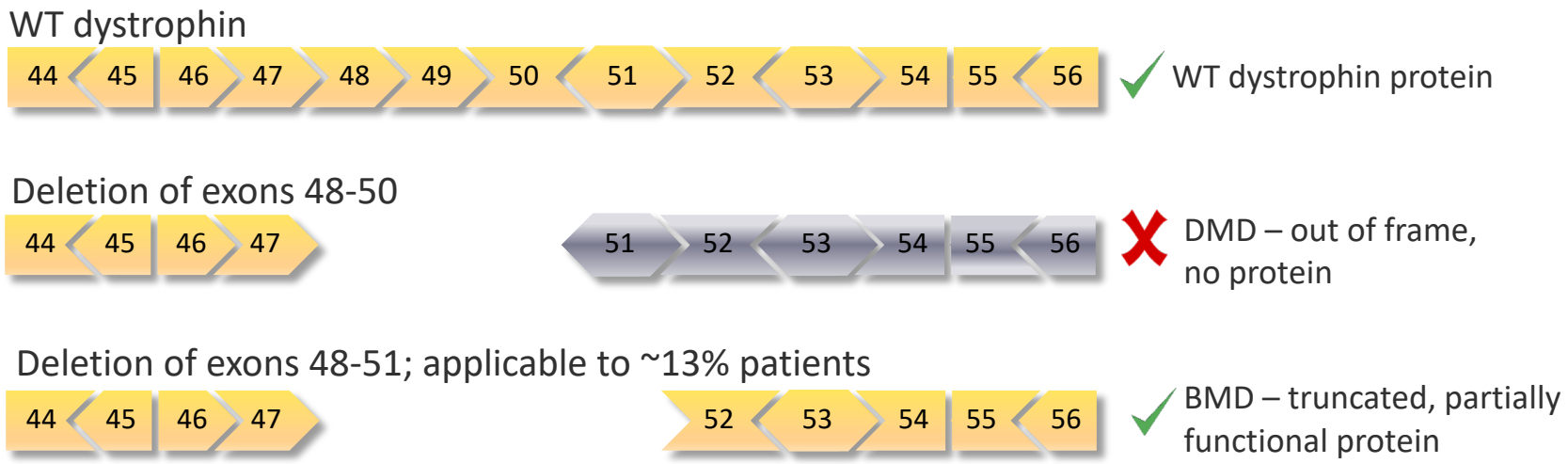
Splice site disruption
OR
re-framing



Exon excision

Nelson and Gersbach, *Annual Review Chem Biol Eng* (2016)

Genome Editing for Duchenne Muscular Dystrophy



Ousterout et al. *Molecular Therapy* (2013), *Molecular Therapy* (2014), *Nature Communications* (2015)

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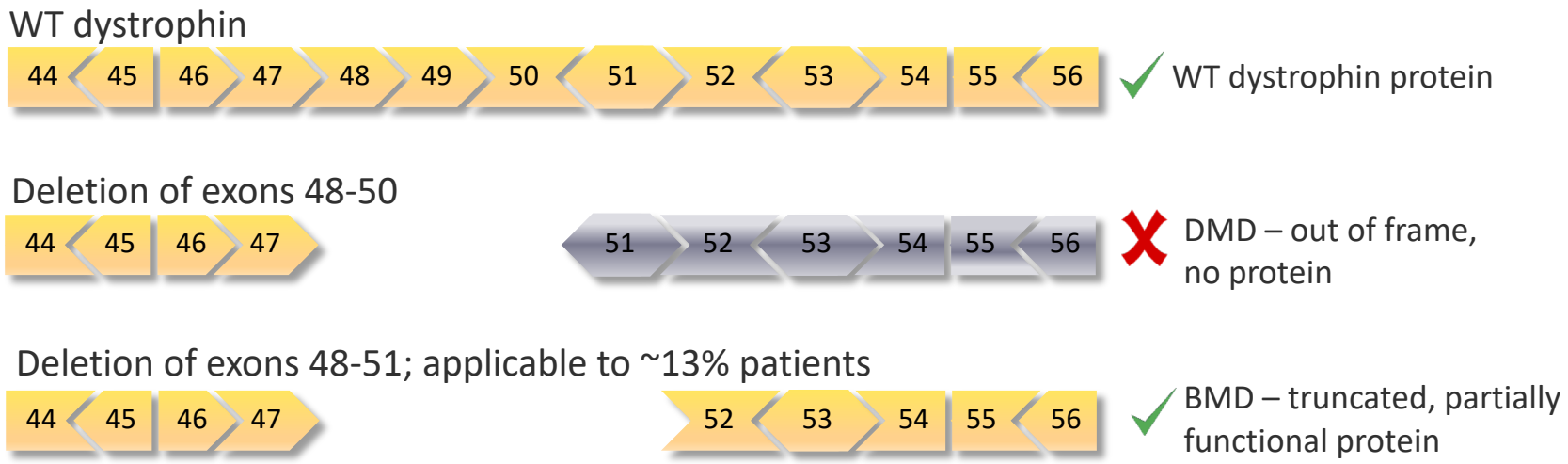


Why exon deletion?

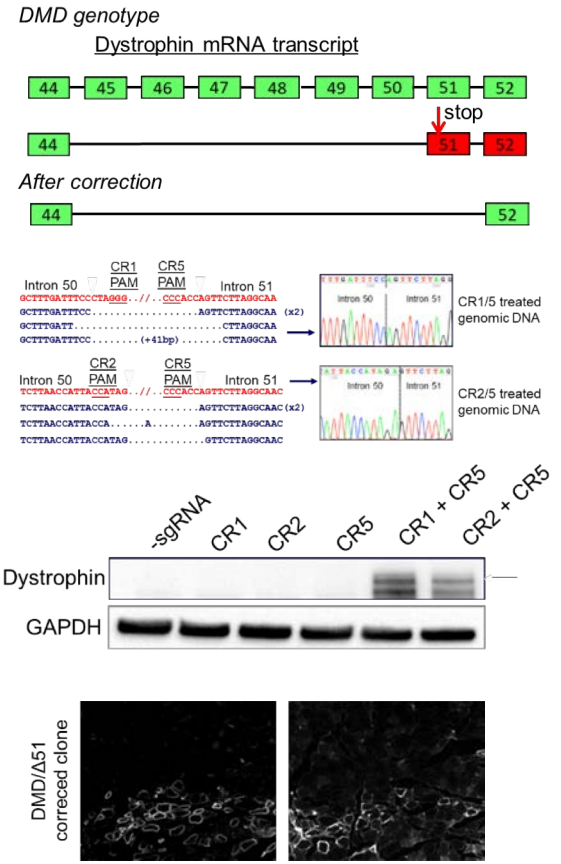
- NHEJ > HDR
- Predictable and reproducible protein product
- Large intronic regions allow for gRNA optimization
- Multi-exon skipping can increase applicability

Dave Ousterout

Ousterout et al. *Molecular Therapy* (2013), *Molecular Therapy* (2014), *Nature Communications* (2015)

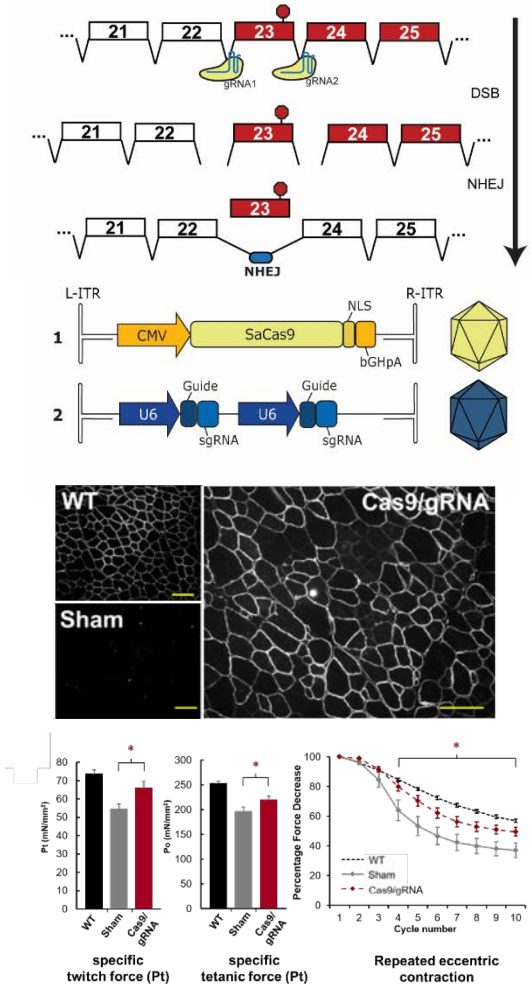
Genome Editing for Duchenne Muscular Dystrophy

DMD patient cells



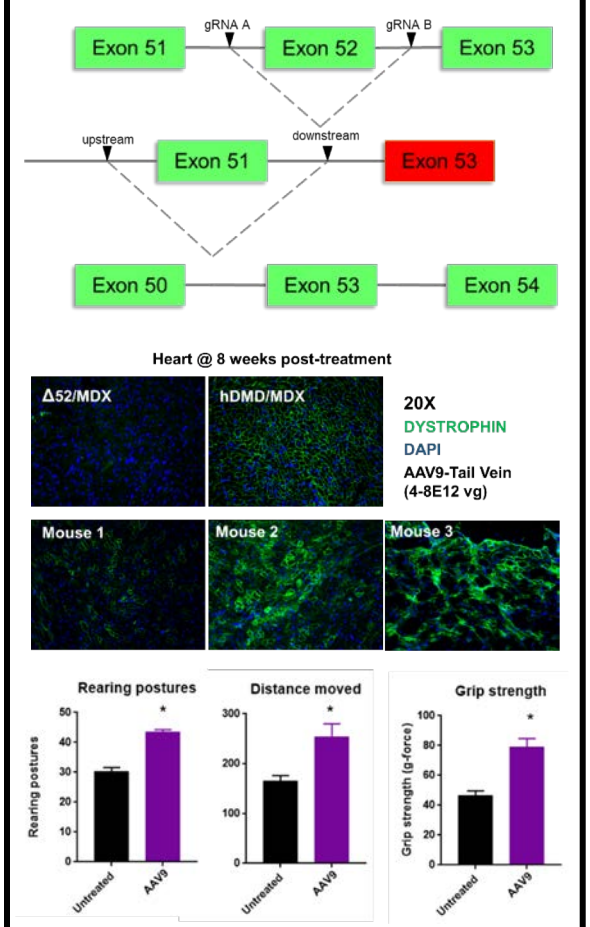
Ousterout et al., *Nature Communications* (2015), *Molecular Therapy* (2013, 2015)

mdx mouse



Nelson et al., *Science* (2016)

hDMD mouse



Robinson-Hamm et al., *unpublished*

Genome Editing for Duchenne Muscular Dystrophy

Realizing the promise of gene editing:

- Immunogenicity
- Durability
- Satellite Cell Targeting
- Restoration of a Full-Length Dystrophin

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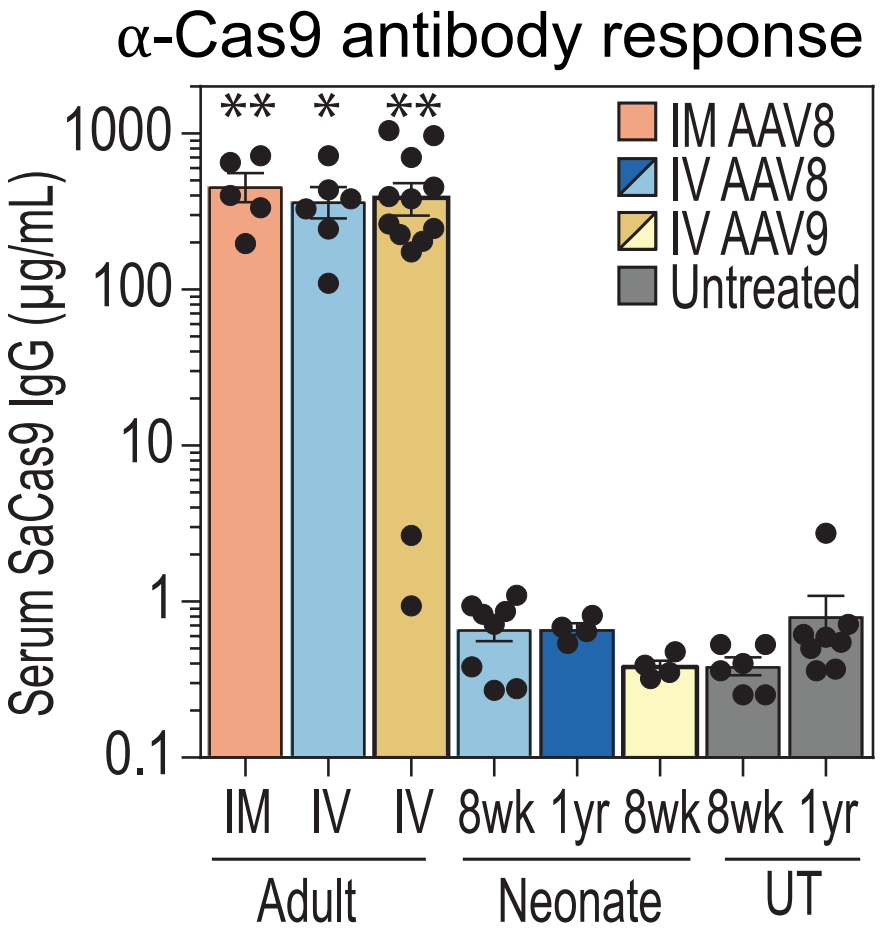
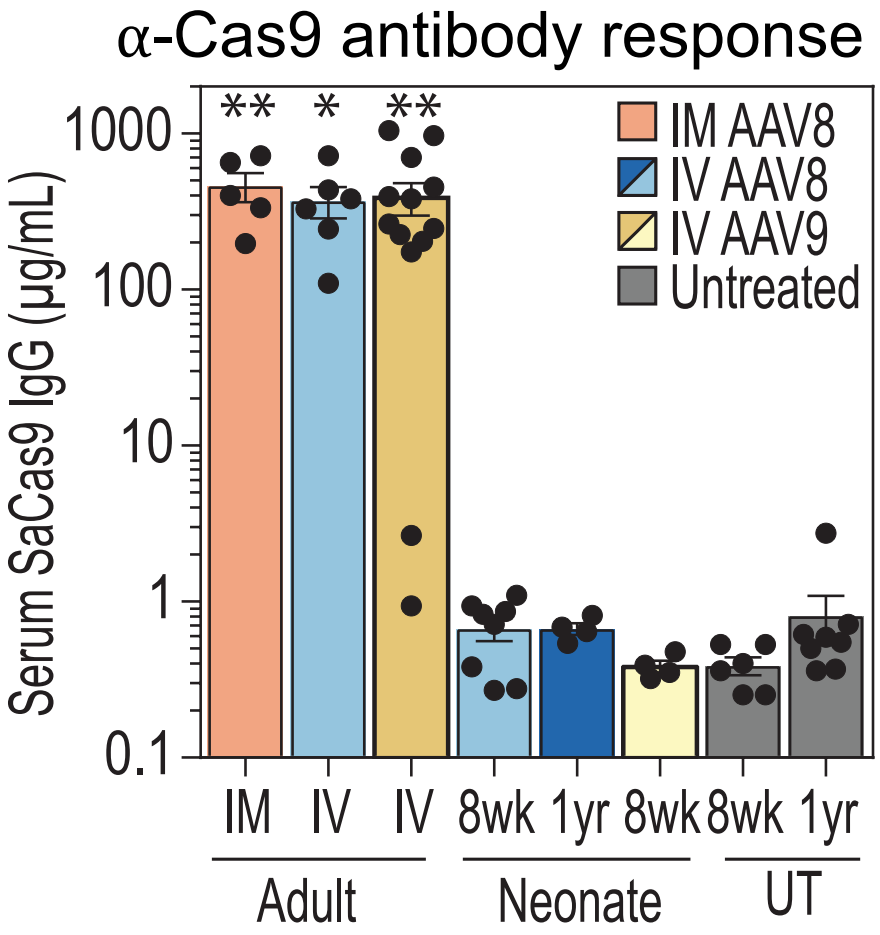
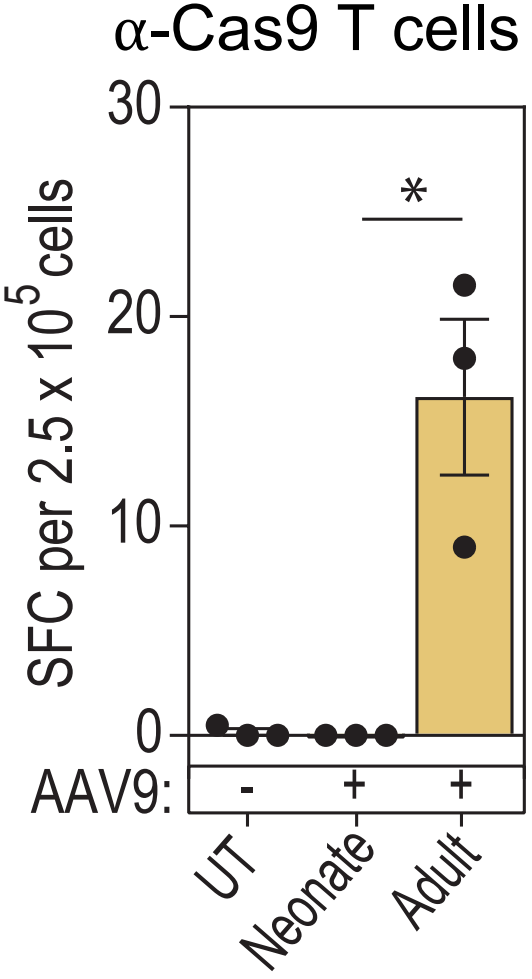
Host Immune Response to CRISPR/Cas9



Nelson et al., *Nature Medicine* (2019)

Host Immune Response to CRISPR/Cas9





- α-Cas9 response following treatment of adults but not neonates
- Used ubiquitous promoter – tissue-restricted promoters may help

Nelson et al., *Nature Medicine* (2019)

Genome Editing for Duchenne Muscular Dystrophy

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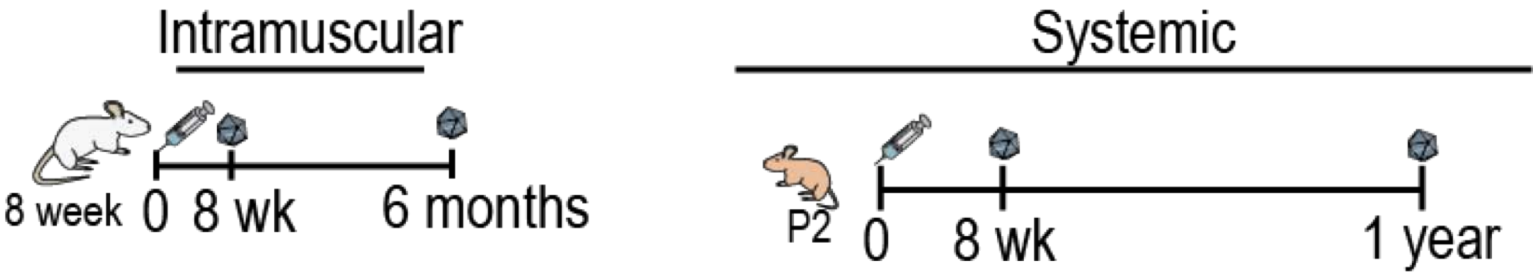
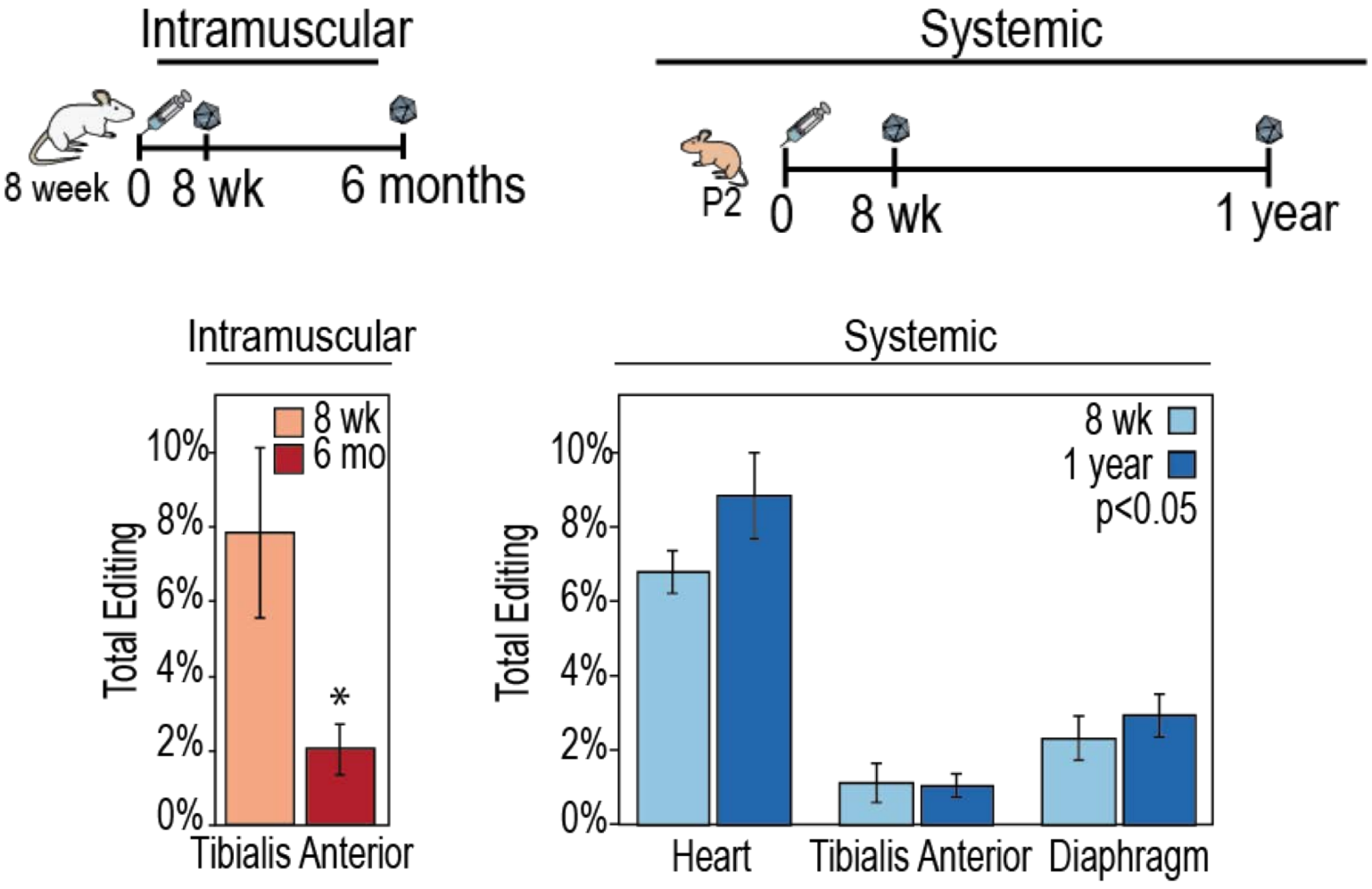
Persistence of Editing in Skeletal and Cardiac Muscle



Nelson et al., *Nature Medicine* (2019)

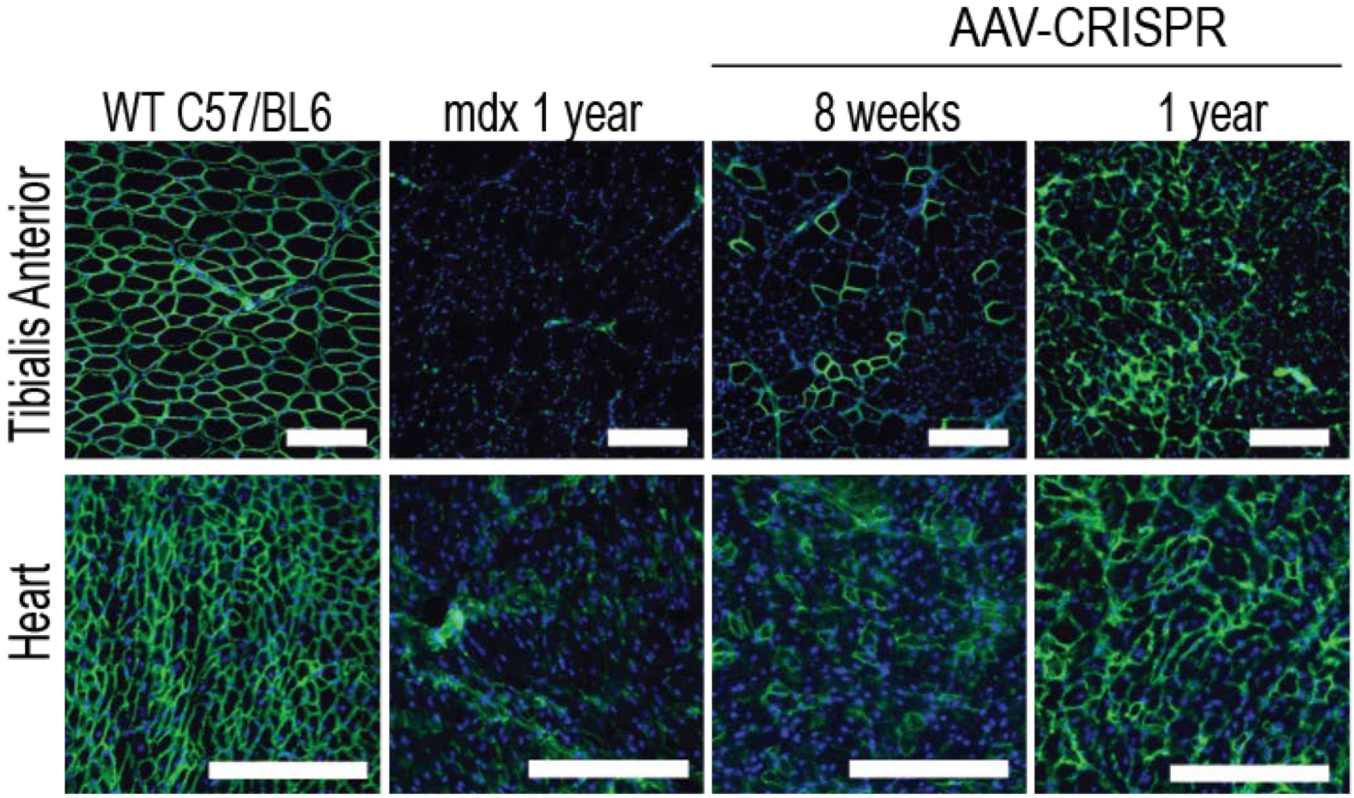
Persistence of Editing in Skeletal and Cardiac Muscle



Significant increase in *DNA* editing frequency from 8 weeks to 1 year

Nelson et al., *Nature Medicine* (2019)

Persistence of Editing in Skeletal and Cardiac Muscle



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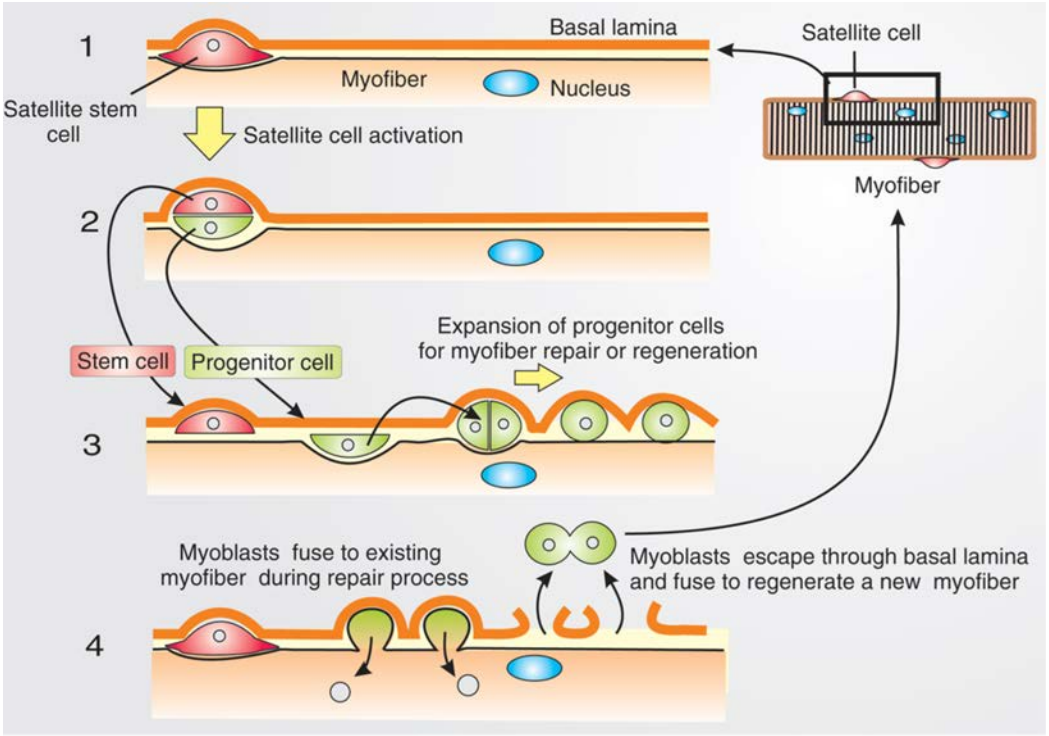
Satellite Cells are the Stem Cells of Skeletal Muscle

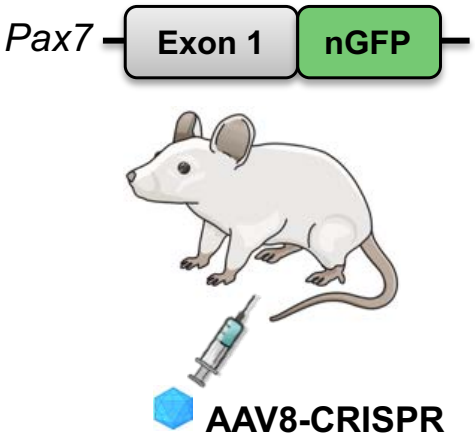


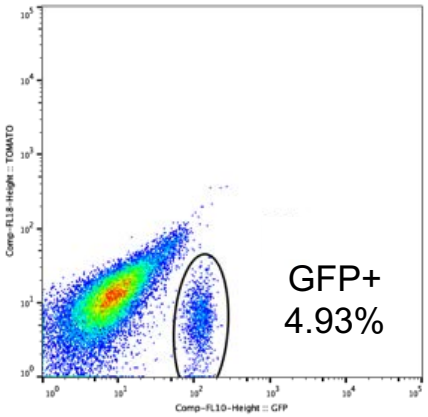
Does AAV transduce satellite cells *in vivo*?

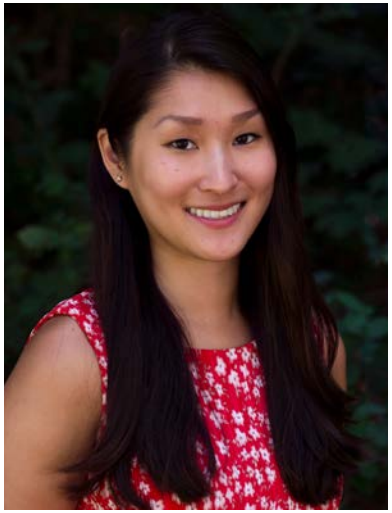
Does CRISPR edit satellite cells *in vivo*?

Does satellite cell editing facilitate long-term dystrophin restoration?

AAV-CRISPR Gene Editing of Satellite Cells



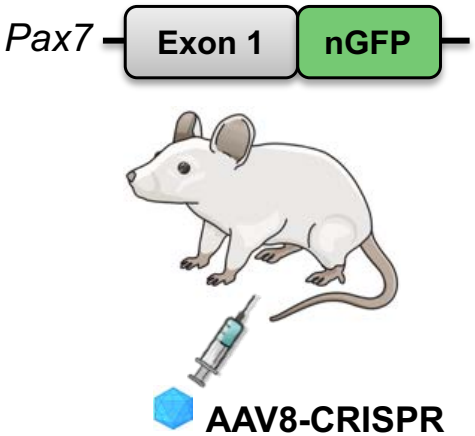


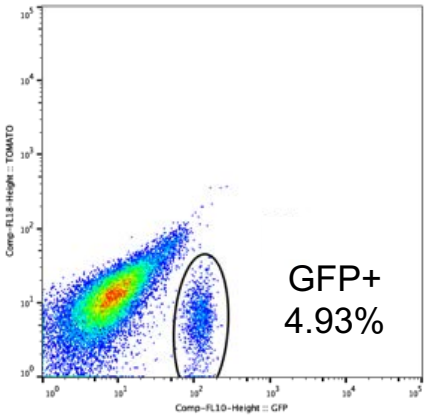


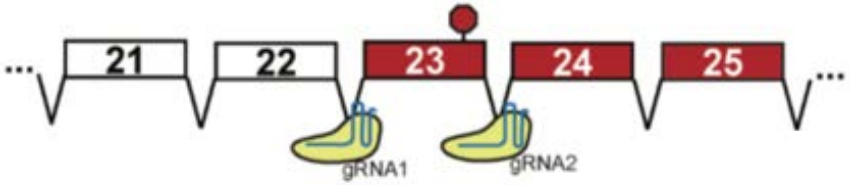
Jennifer Kwon

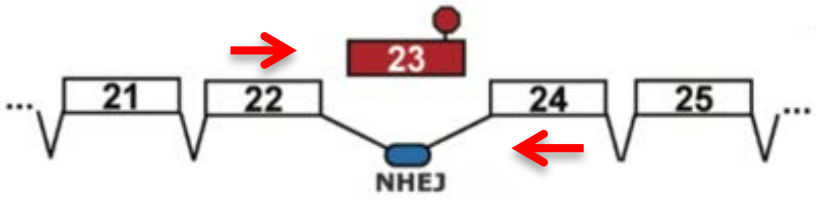
Kwon et al., *unpublished*

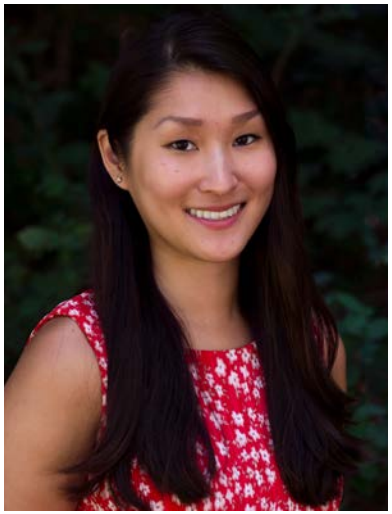
AAV-CRISPR Gene Editing of Satellite Cells











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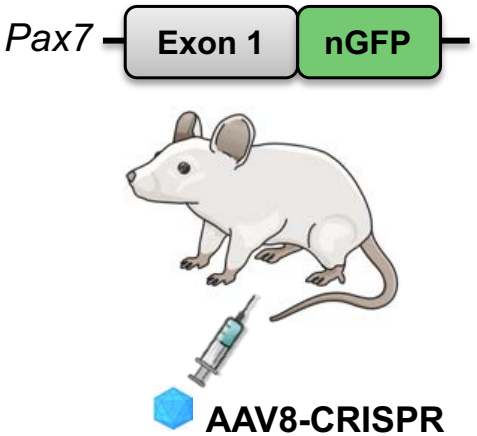
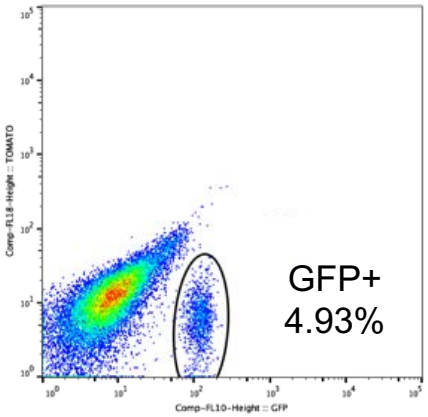
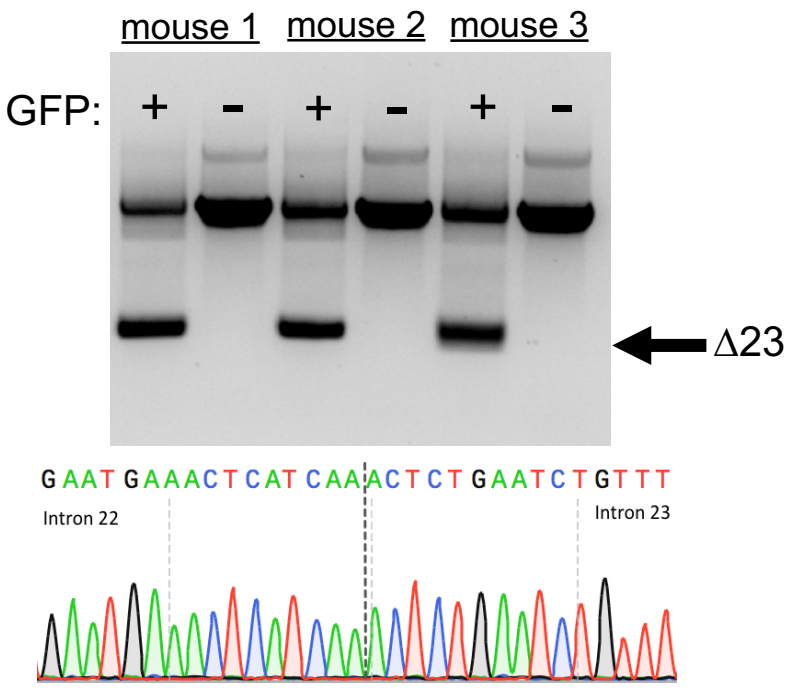
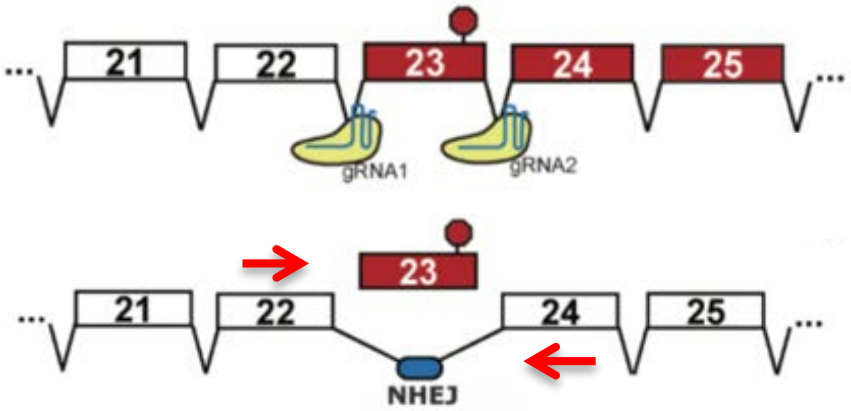


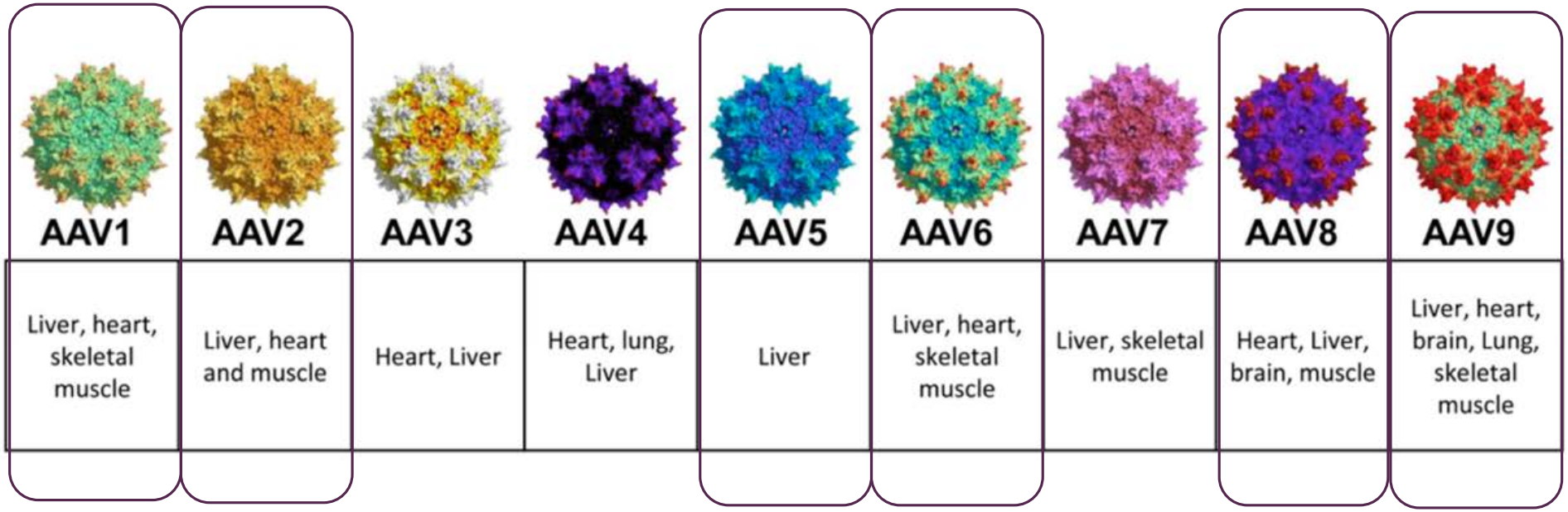






Kwon et al., *unpublished*

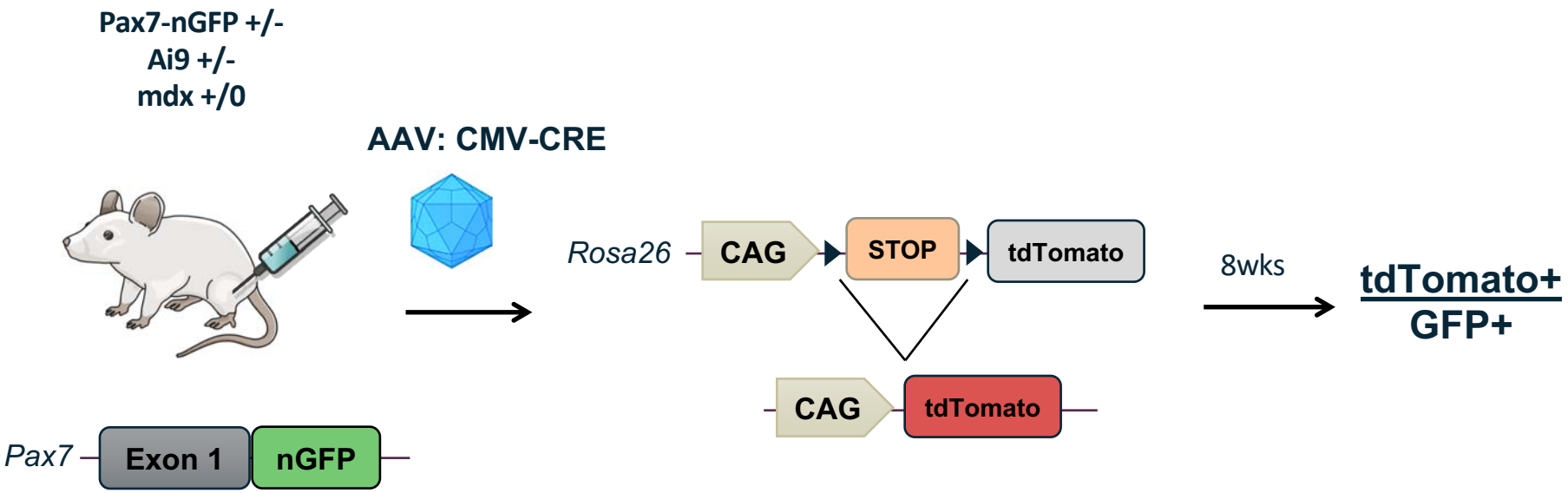
Diverse AAV Serotypes with Various Tissue Tropisms



AAV1	AAV2	AAV3	AAV4	AAV5	AAV6	AAV7	AAV8	AAV9
Liver, heart, skeletal muscle	Liver, heart and muscle	Heart, Liver	Heart, lung, Liver	Liver	Liver, heart, skeletal muscle	Liver, skeletal muscle	Heart, Liver, brain, muscle	Liver, heart, brain, Lung, skeletal muscle

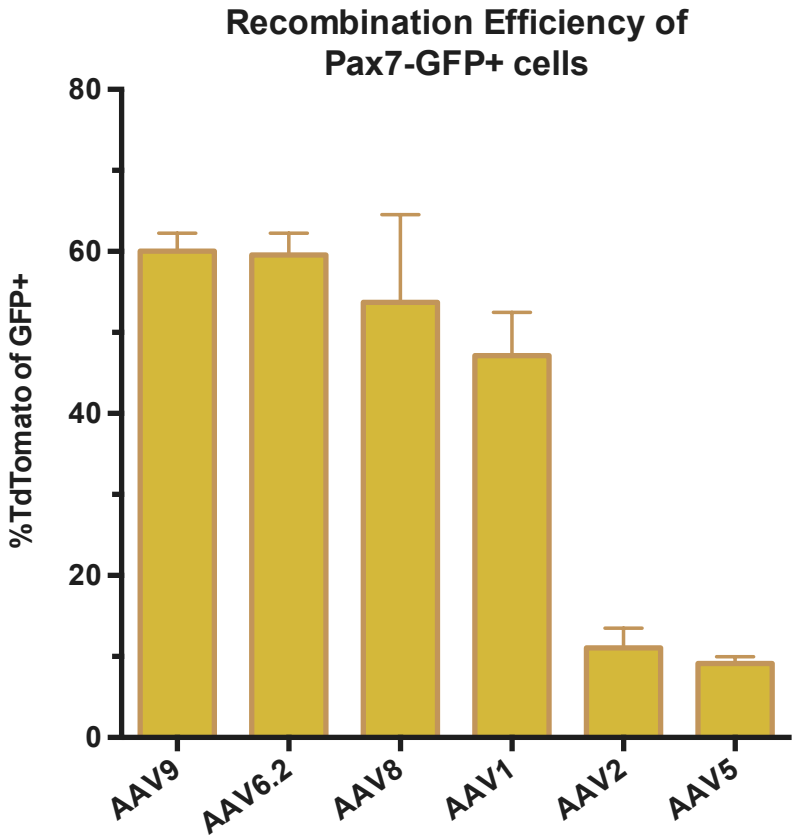
Vance et al., 2015

Systematic Assessment of AAV Transduction of Satellite Cells



Kwon et al., *unpublished*

Efficient Genetic Labelling of Satellite Cells by Multiple AAV Serotypes

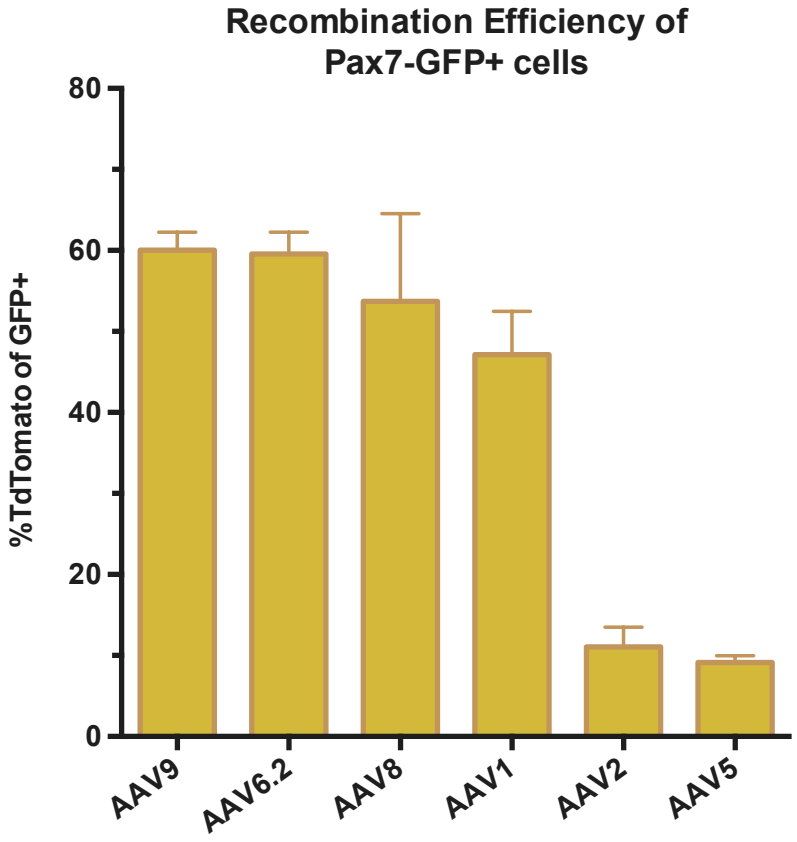


Local AAV Delivery
(4.72E+11 vg/TA muscle)

n=5, mean + SEM; 8 weeks post-injections

Kwon et al., *unpublished*

Efficient Genetic Labelling of Satellite Cells by Multiple AAV Serotypes



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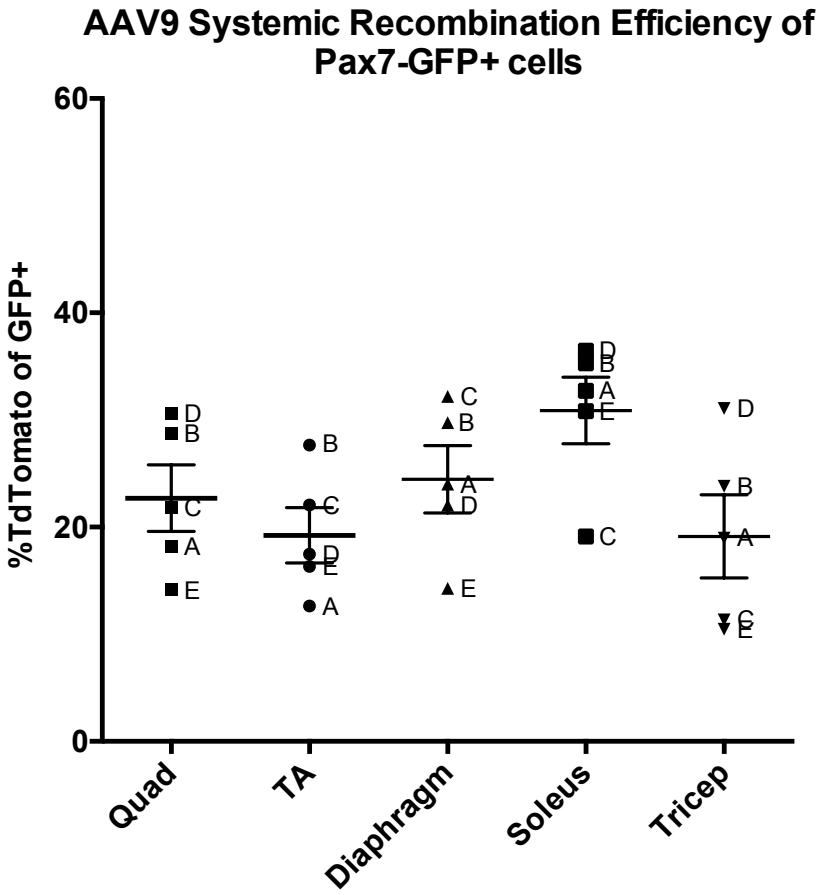
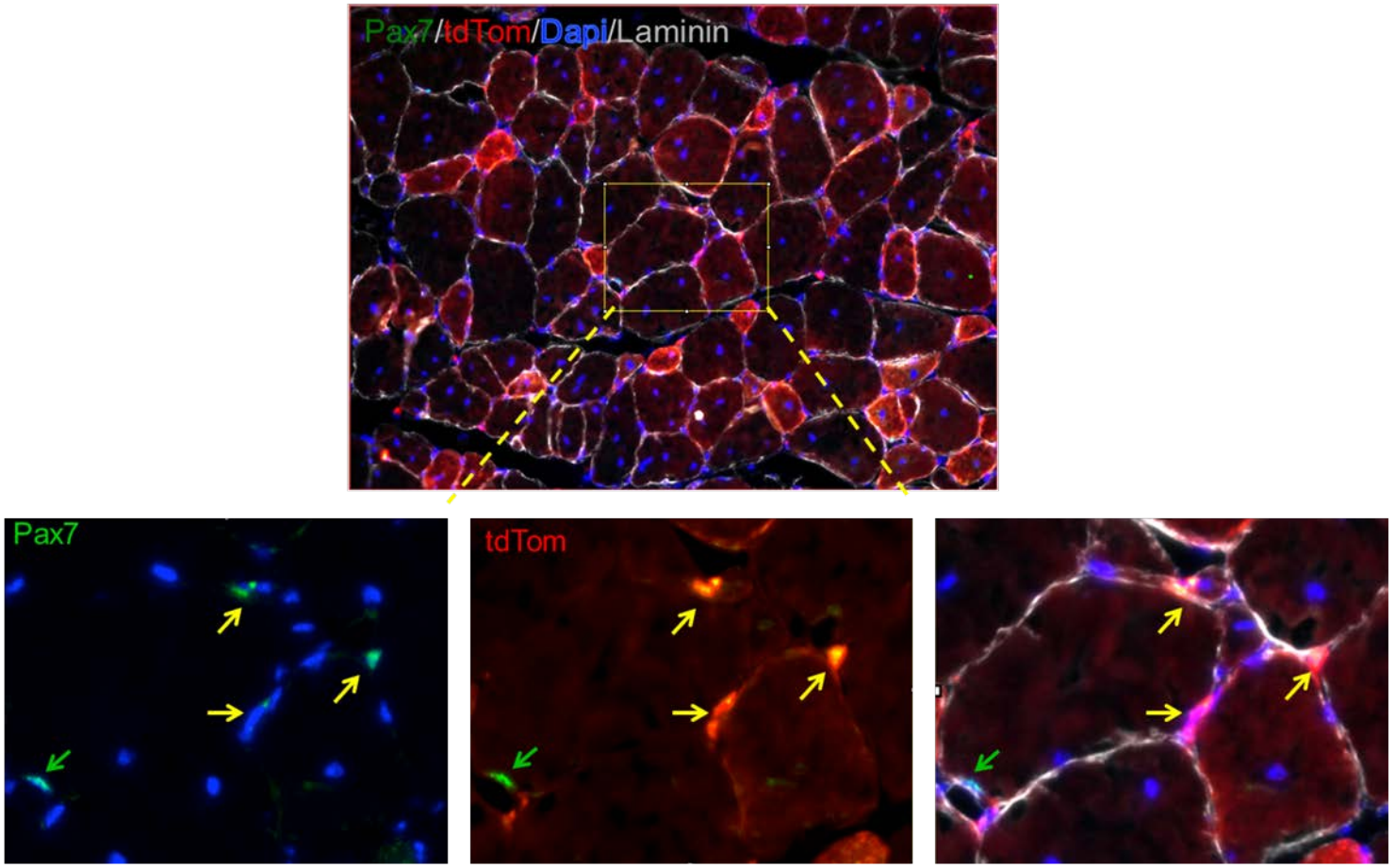


Systemic AAV9 Delivery
(2E+12 vg/mouse)

n=5, mean + SEM; 8 weeks post-injections

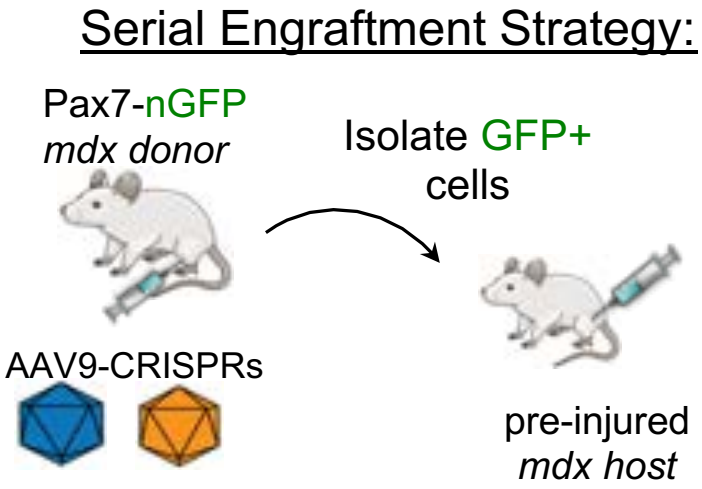
Kwon et al., *unpublished*

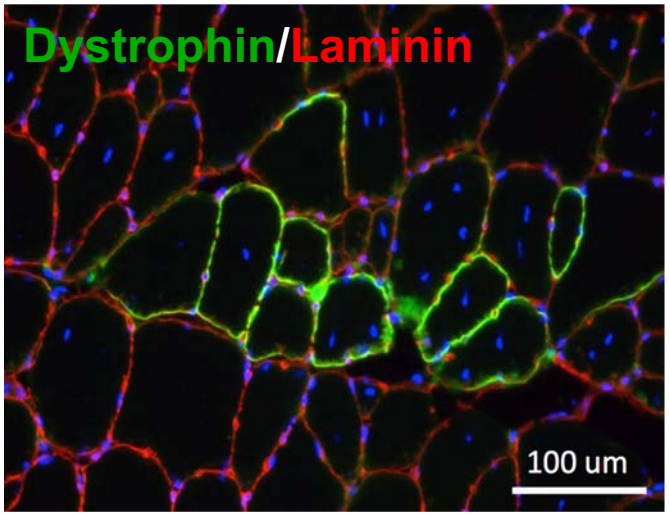
Efficient Genetic Labelling of Satellite Cells by Multiple AAV Serotypes

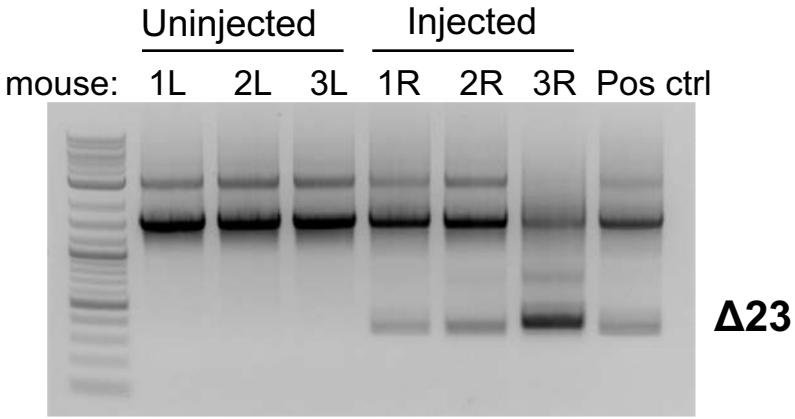


Kwon et al., *unpublished*

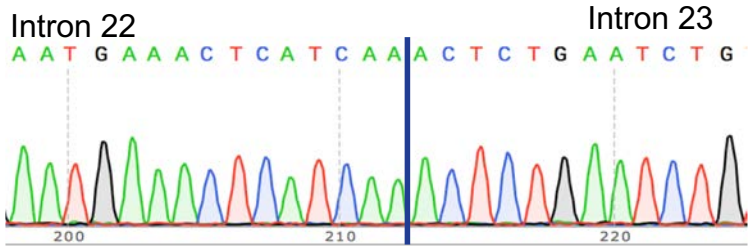
Regenerative Potential of CRISPR-Edited Satellite Cells







Deletion PCR at *Dmd* locus



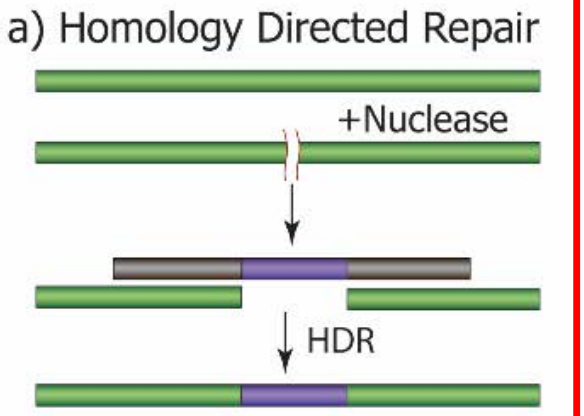
Kwon et al., *unpublished*

Genome Editing for Duchenne Muscular Dystrophy

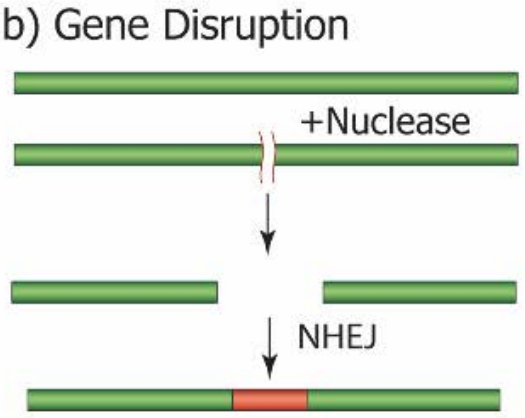
Realizing the promise of gene editing:

- Immunogenicity
- Durability
- Satellite Cell Targeting
- Restoration of a Full-Length Dystrophin

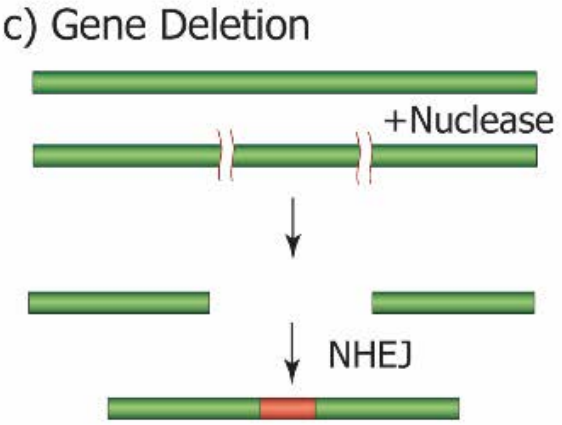
Genome Editing with Engineered Nucleases



Targeted insertions



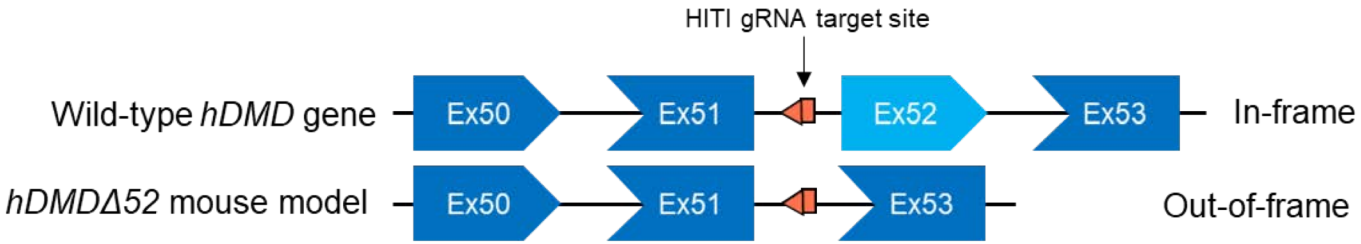
Splice site disruption
OR
re-framing



Exon excision

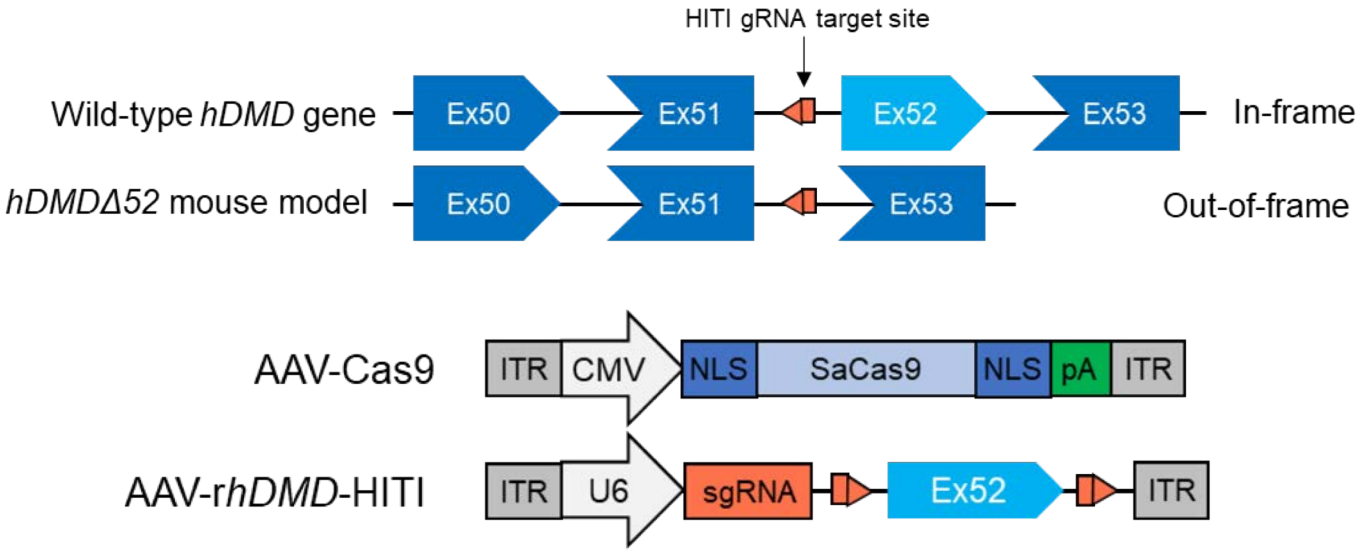
Nelson and Gersbach, *Annual Review Chem Biol Eng* (2016)

Full Length Dystrophin Restoration by Targeted Integration



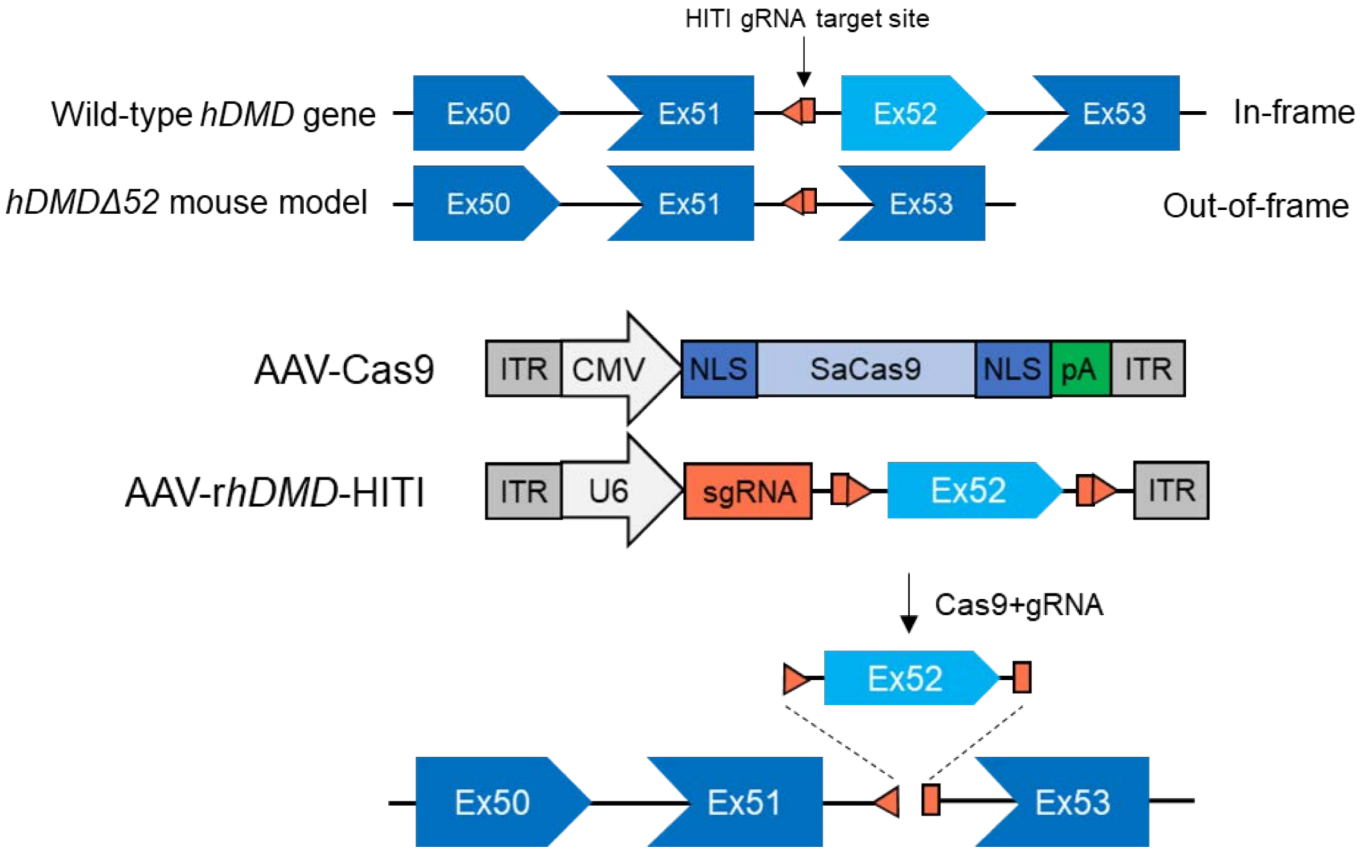
Adrian Pickar Oliver, PhD

Full Length Dystrophin Restoration by Targeted Integration



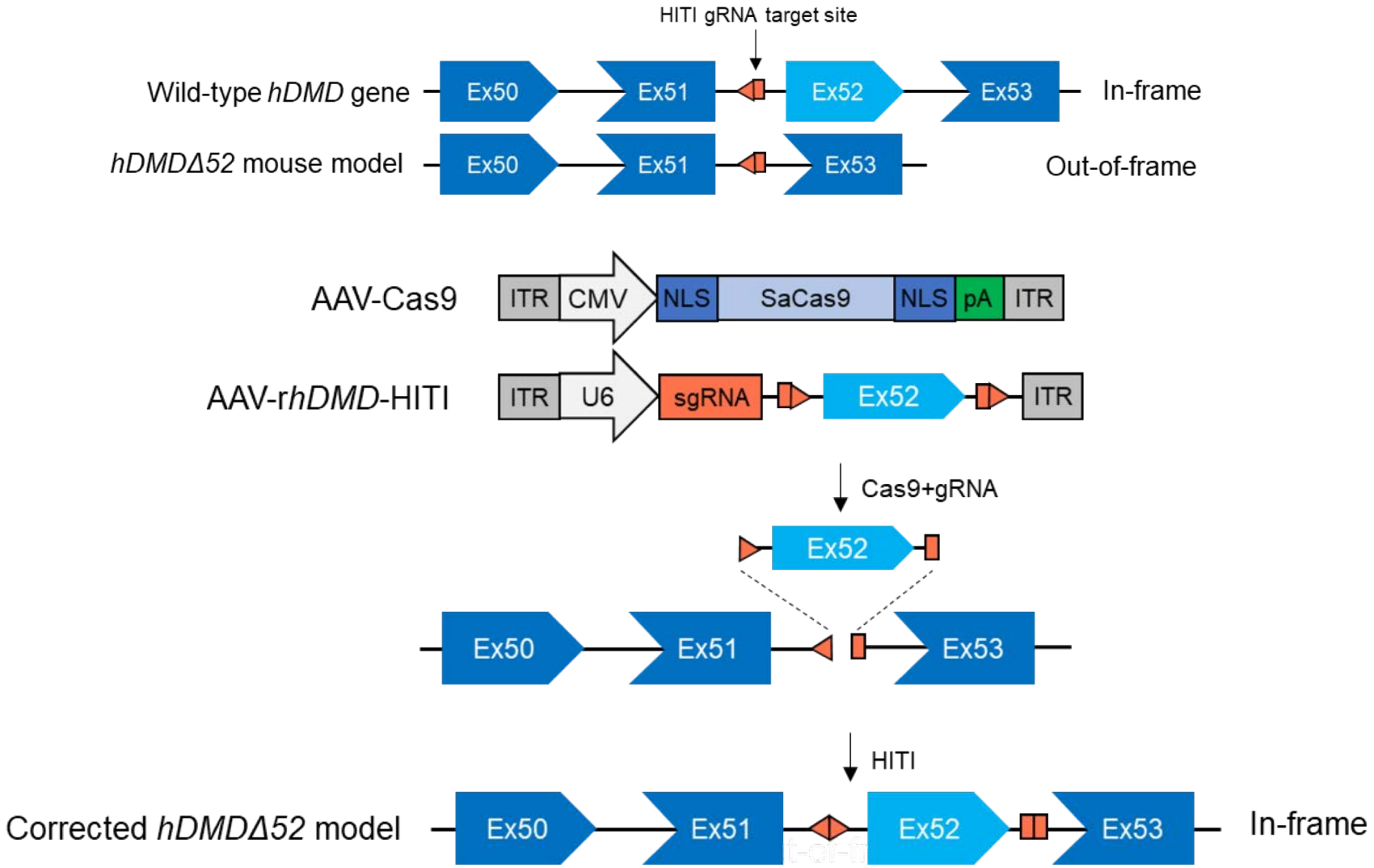
Adrian Pickar Oliver, PhD

Full Length Dystrophin Restoration by Targeted Integration



Pickar-Oliver et al., *unpublished*

Full Length Dystrophin Restoration by Targeted Integration



Pickar-Oliver et al., *unpublished*

Full Length Dystrophin Restoration by Targeted Integration

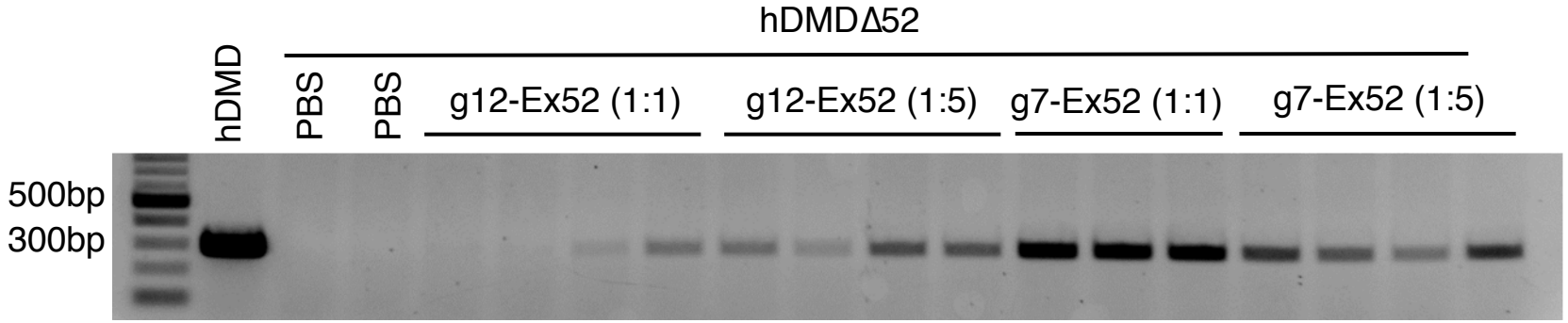
Expected size:
300bp

Pickar-Oliver et al., *unpublished*

Full Length Dystrophin Restoration by Targeted Integration

Expected size:
300bp

cDNA:

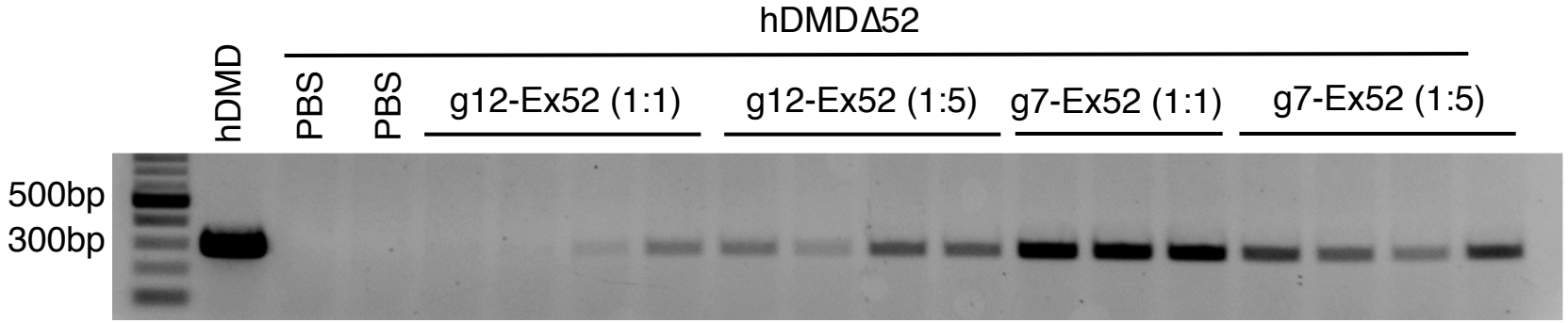


Pickar-Oliver et al., *unpublished*

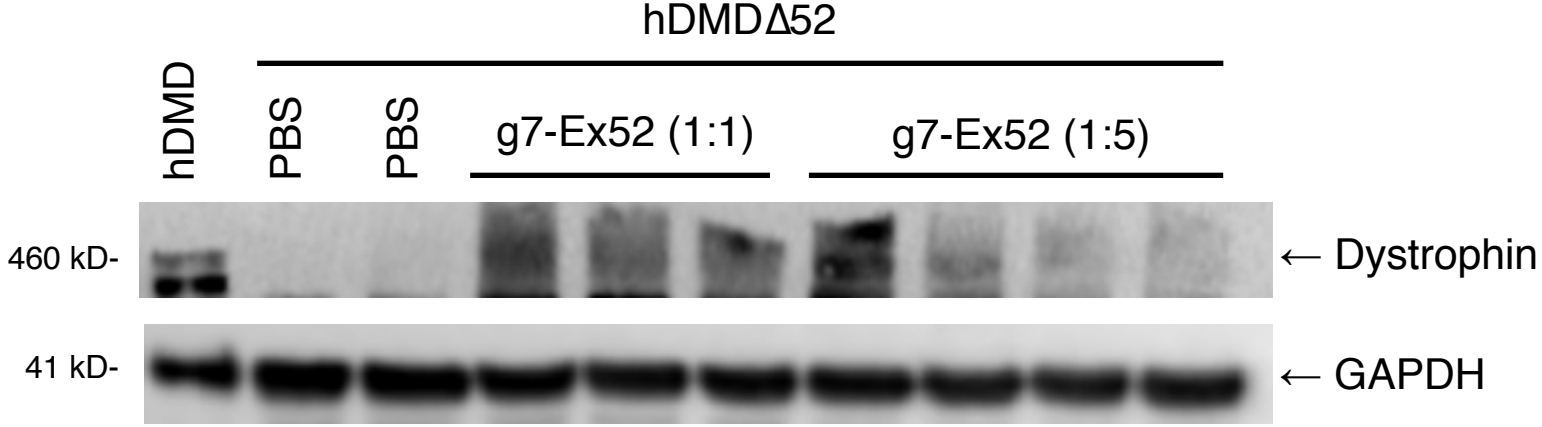
Full Length Dystrophin Restoration by Targeted Integration

Expected size:
300bp

cDNA:



Protein:



Pickar-Oliver et al., *unpublished*

Summary

- Genome editing for DMD typically focuses on *removing gene segments* to restore functional, truncated dystrophin
- Single gene editing strategy can treat *~50% of DMD patients*
- Robust *anti-Cas9 host immune response* that resolves without intervention
- *In vivo* CRISPR-based genome editing restores *long-term dystrophin expression* in many studies with *no reported adverse effects*
- *Unintended genomic outcomes*, including AAV integration, into on-target and off-target sites
- *Full-length dystrophin restoration* is possible but requires patient-specific approaches
- **Additional research required to understand implications of *immune response*, long-term presence of *delivery vectors*, and *alternative genome modifications***

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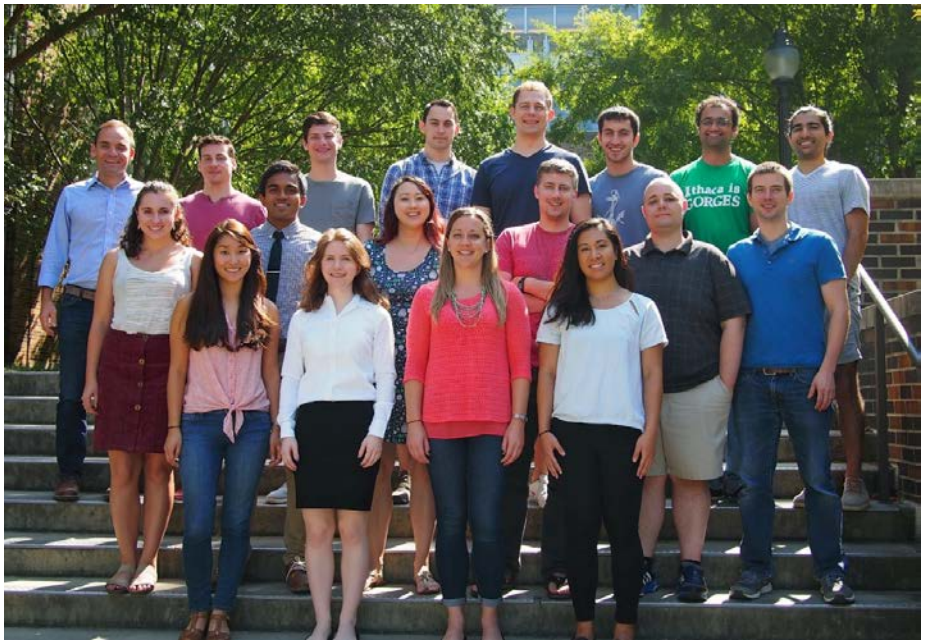

SAREPTA THERAPEUTICS

NATIONAL INSTITUTES OF HEALTH

CGE Somatic Cell Genome Editing

CDMRP

MDA

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