# Genome Editing for Duchenne Muscular Dystrophy

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Department of Orthopaedic Surgery
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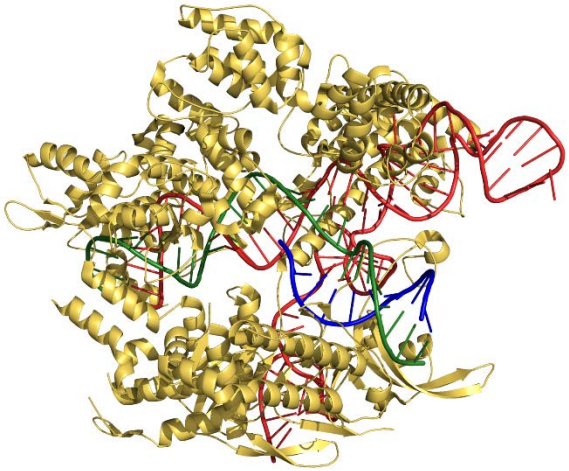
Duke University



November 28, 2018
2nd International Summit on Human Genome Editing
Hong Kong

# Disclosures

Locus Biosciences – stock, consultancy, royalties, sponsored research
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Sarepta Therapeutics – consultancy, sponsored research
Levo Therapeutics – sponsored research
Editas Medicine - royalties

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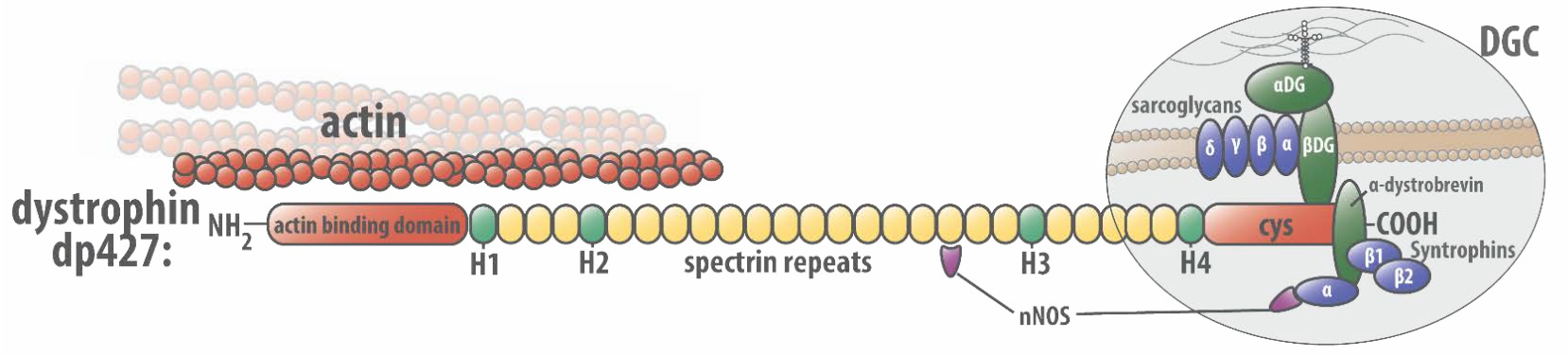
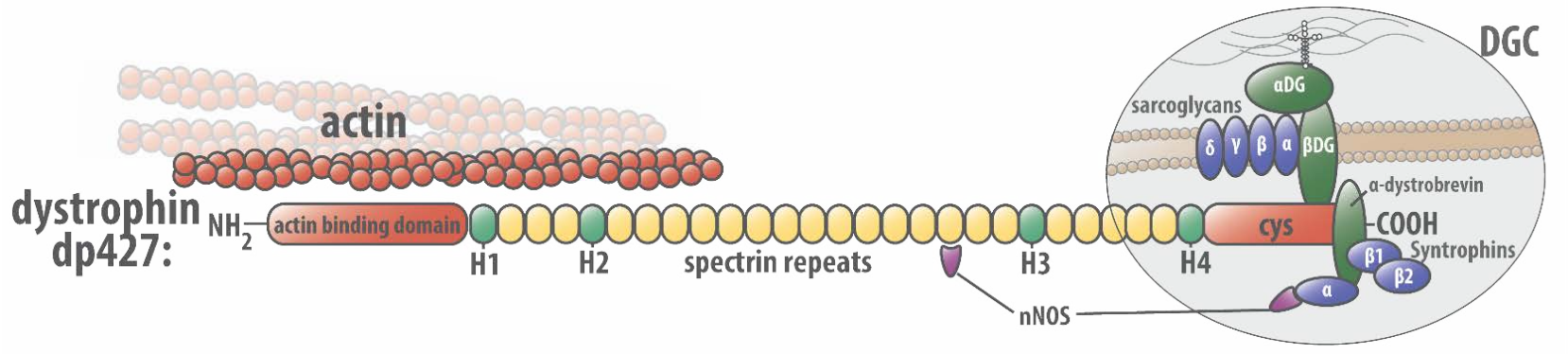
# Duchenne Muscular Dystrophy




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

# Duchenne Muscular Dystrophy

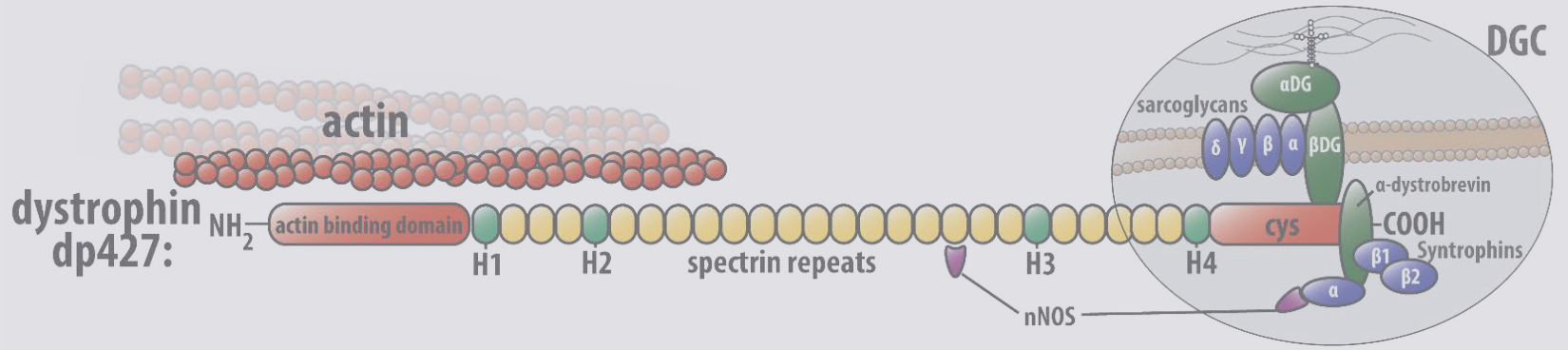



Why somatic cell gene editing for Duchenne?

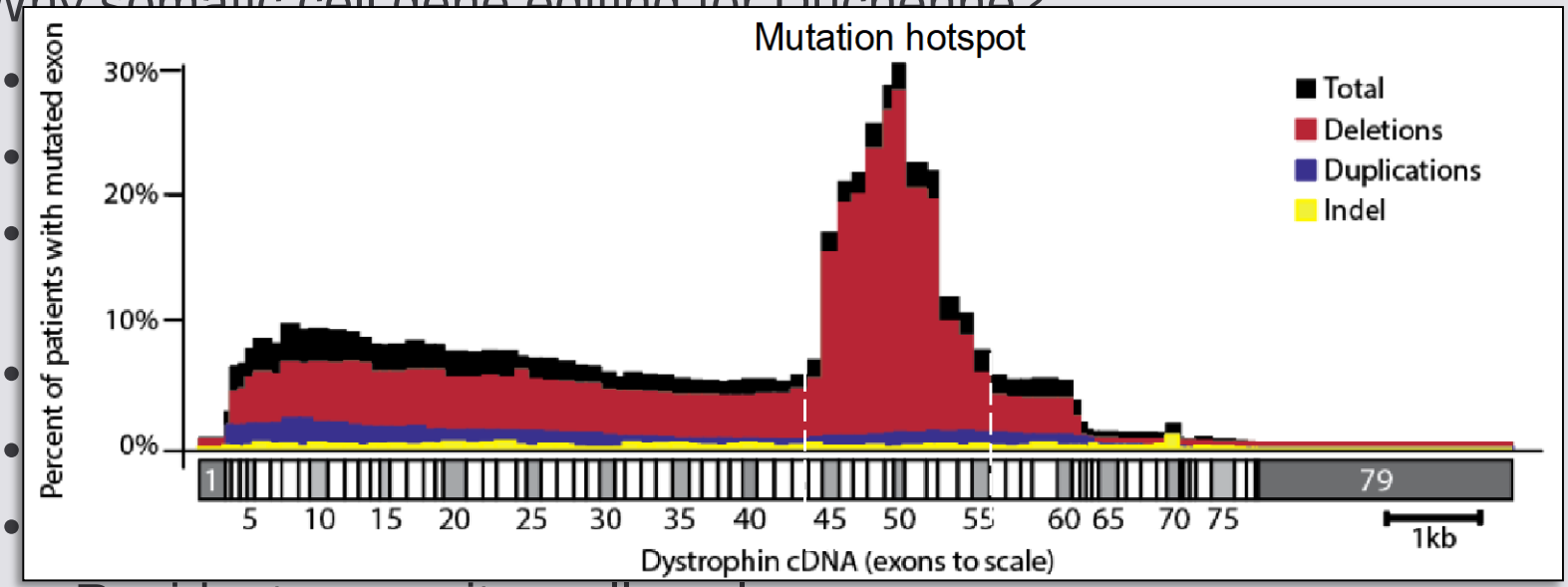
- Dire clinical need
- Limited therapeutic options
- **~30% of DMD cases are *spontaneous* mutations and thus germline editing not applicable**
- Cell delivery
- Gene delivery
- Full-length gene too large for delivery
- Resident progenitor cell pool
- Multinucleated target cells
- Correctable by NHEJ

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

# Duchenne Muscular Dystrophy




Why somatic cell gene editing for Duchenne?

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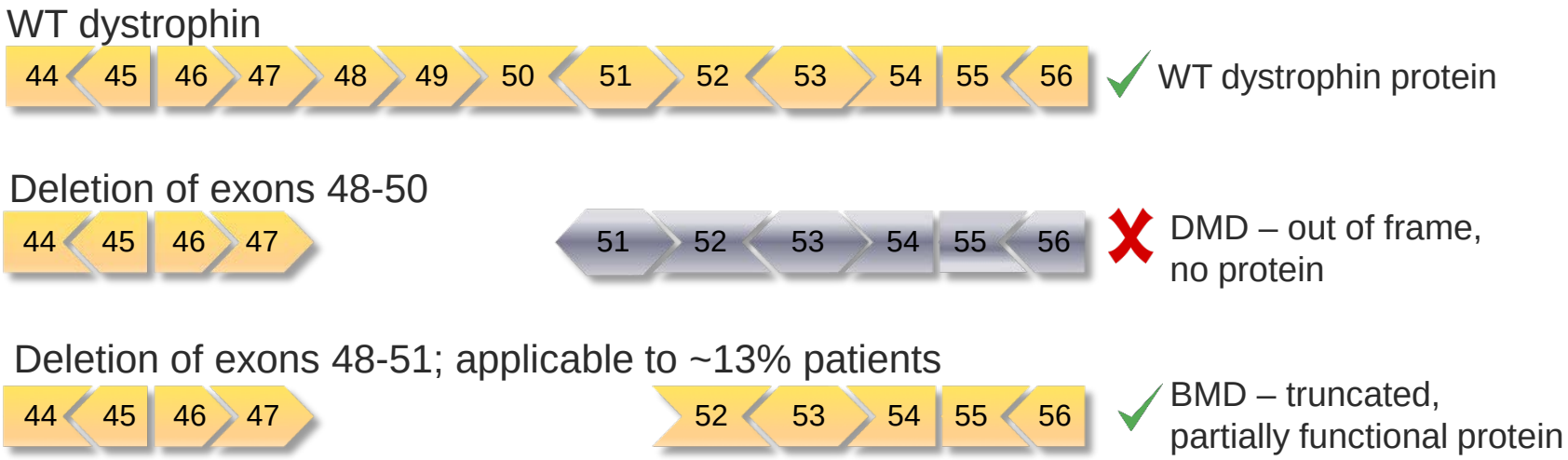
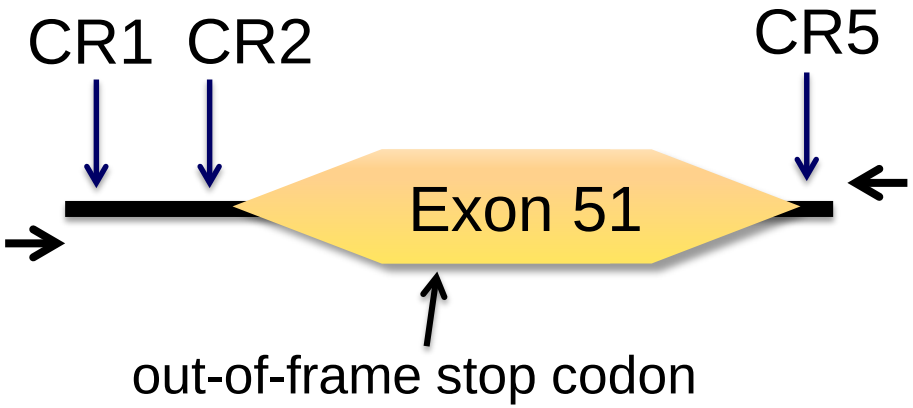
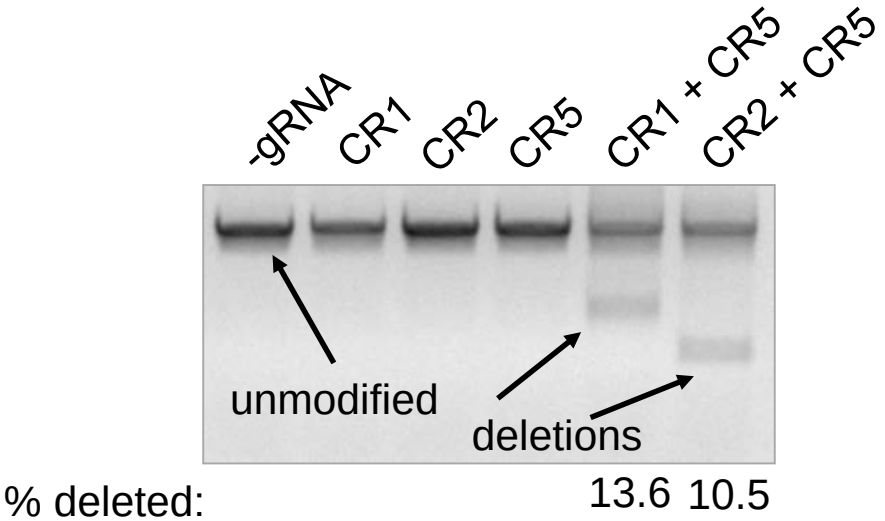


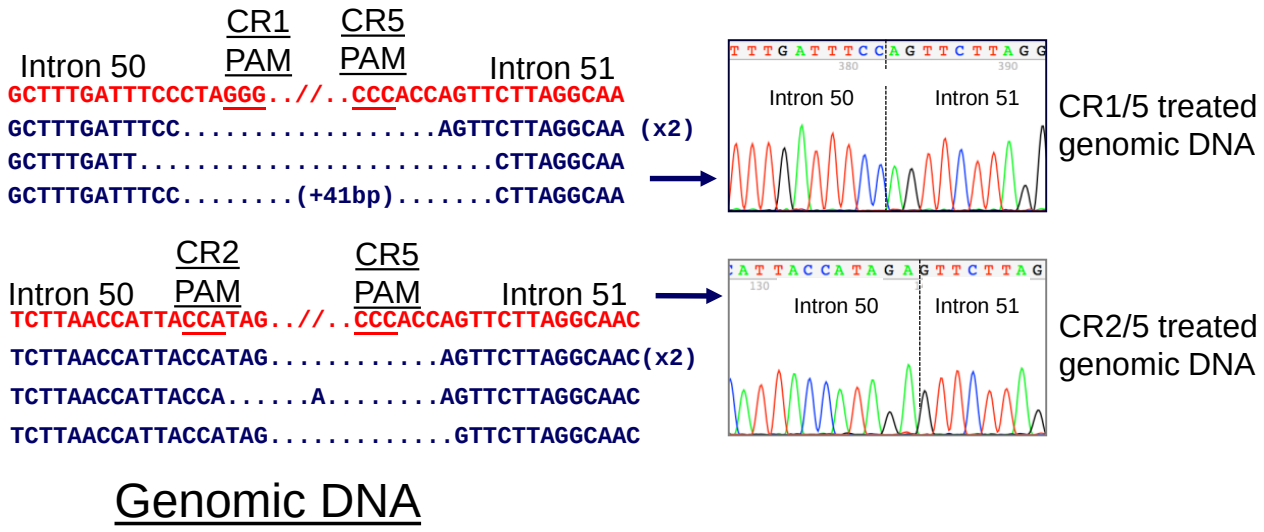

- Exon 51 skipping can correct 13% of DMD mutations

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

# Editing the Dystrophin Gene with CRISPR/Cas9

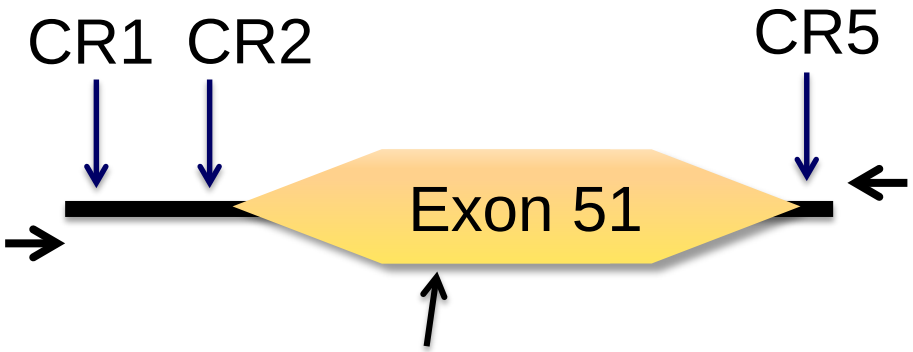


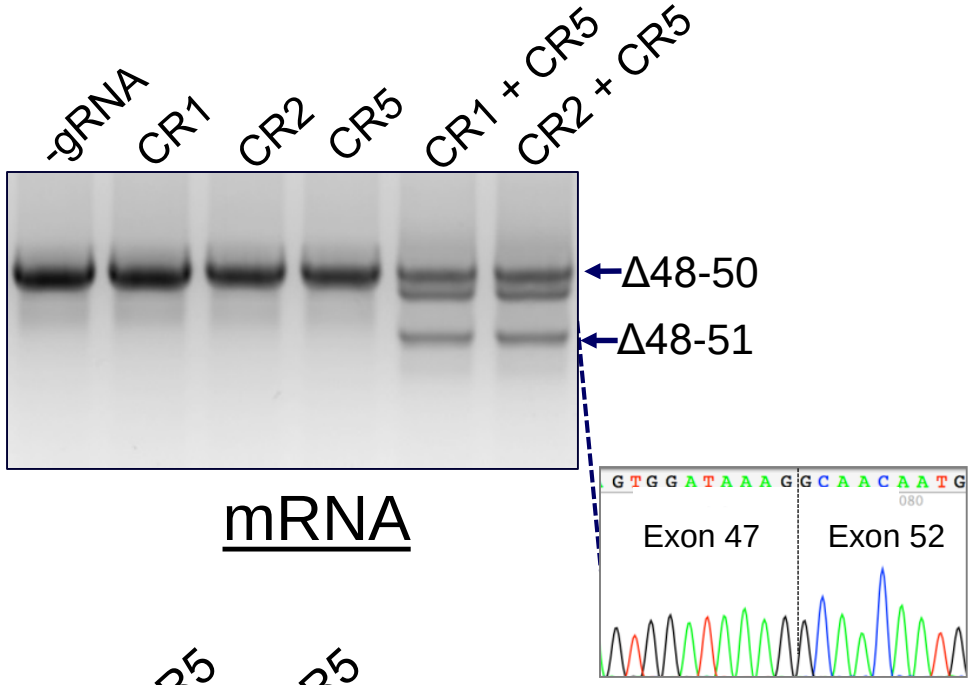




Ousterout et al. *Nature Communications* (2015)

# Editing the Dystrophin Gene with CRISPR/Cas9



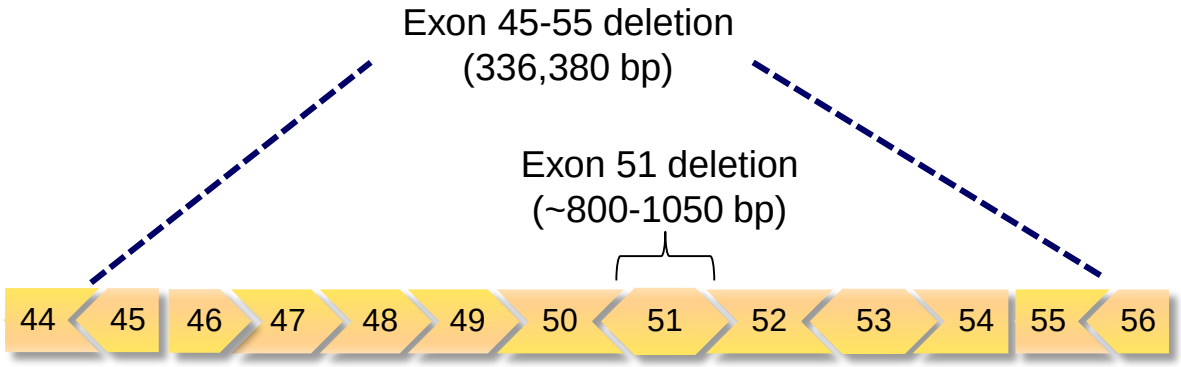

mRNA


Western blot

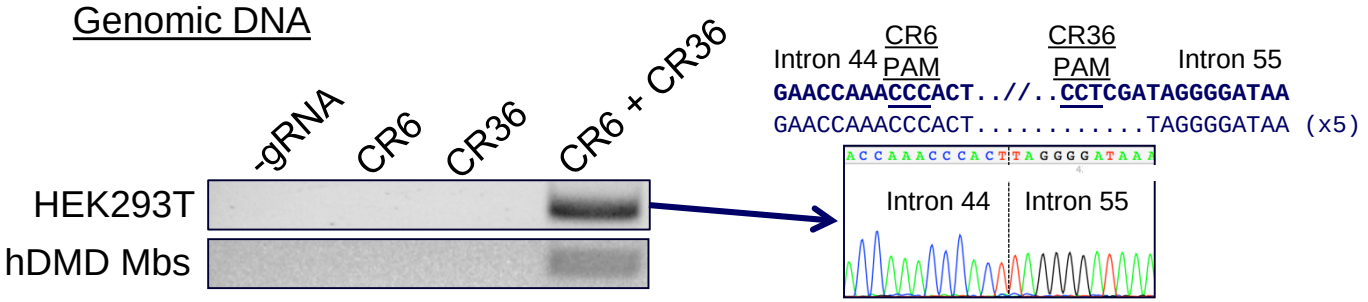
Deletion of exon 51 from the genome results in restored dystrophin expression

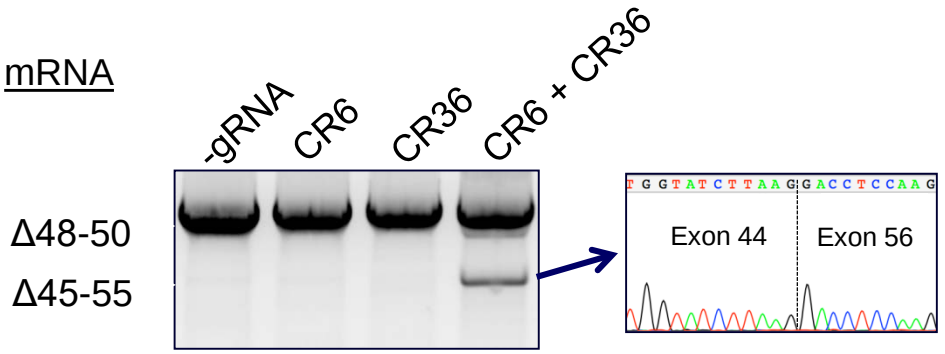
Ousterout et al. *Nature Communications* (2015)

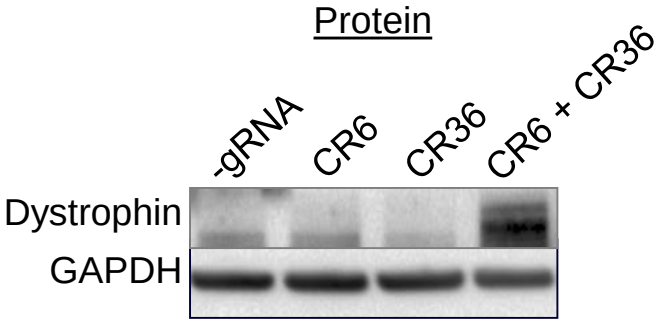
# Editing the Dystrophin Gene with CRISPR/Cas9




- Exon 51 skipping can correct 13% of DMD mutations
- Skipping 45-55 can correct 40-62% of DMD mutations (Aartsma-Rus et al., *Hum Mutat* 2009)
- Multi-exon skipping in preclinical development (Aoki et al., *PNAS* 2012)

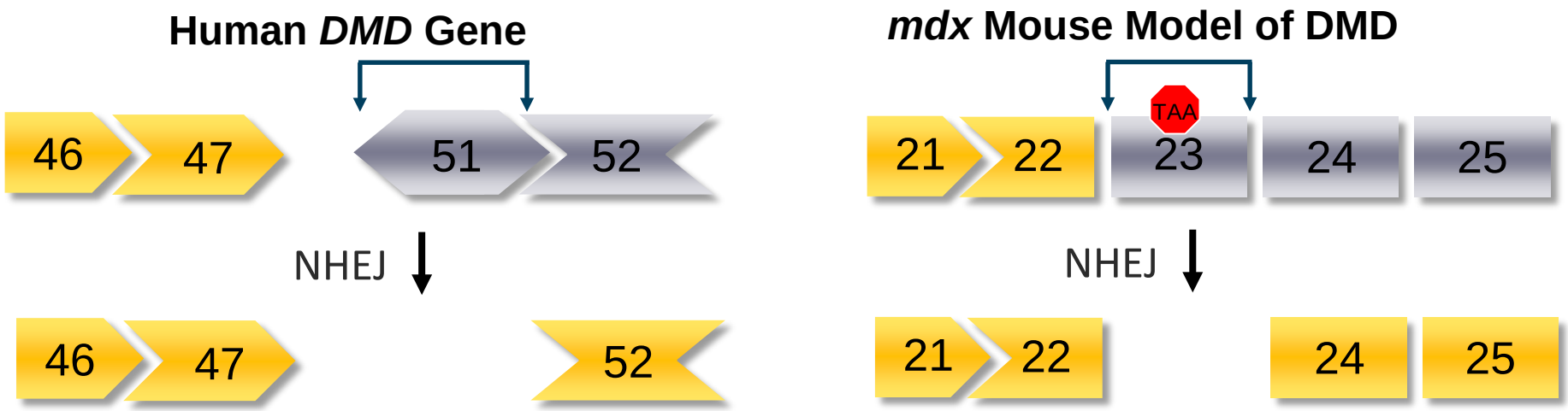



Ousterout et al. *Nature Communications* (2015)

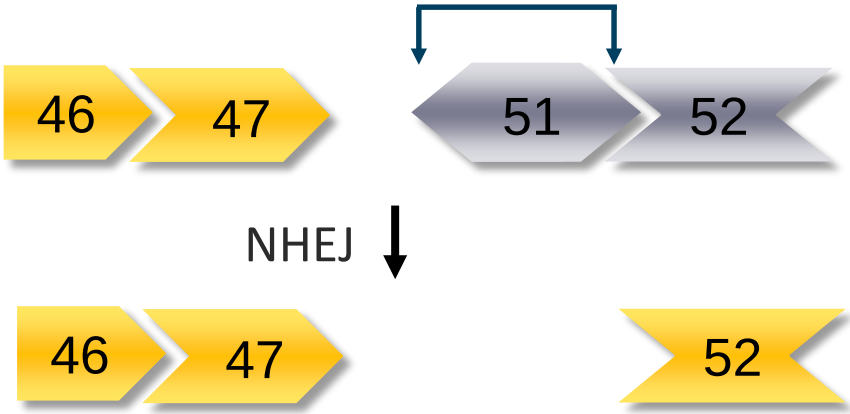
# Genome Editing to Treat the *mdx* Mouse Model of DMD



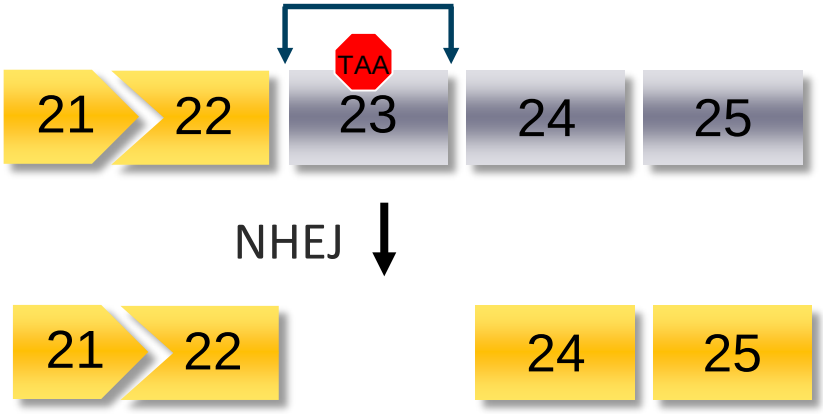

Nelson et al. *Science* (2016)

# Genome Editing to Treat the *mdx* Mouse Model of DMD

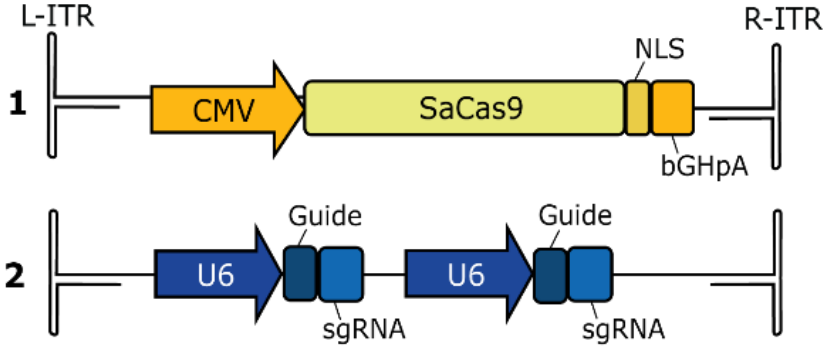
## Human *DMD* Gene



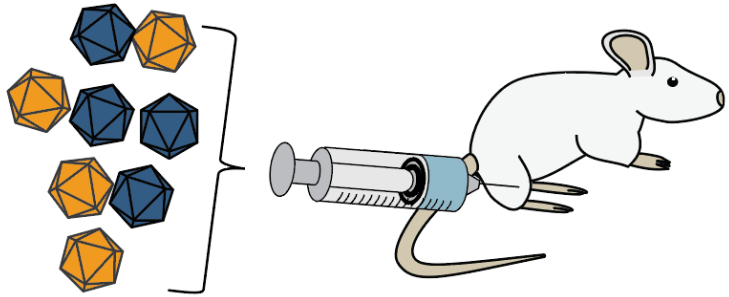

## *mdx* Mouse Model of DMD




## AAV Viral Vector Design



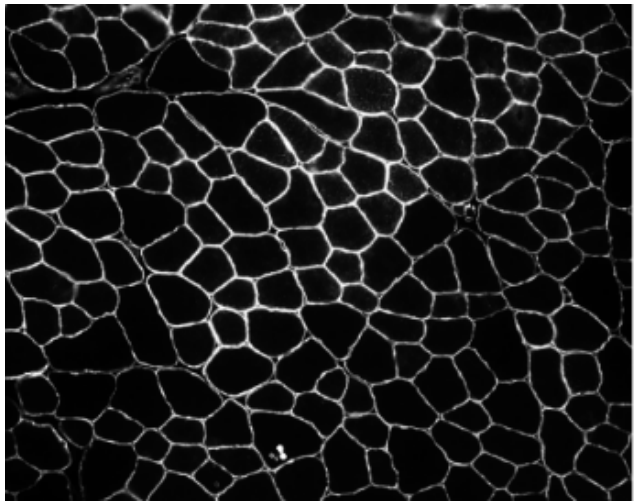

## Local Injections Into Tibialis Anterior



Nelson et al. *Science* (2016)

# Restoration of Dystrophin Expression by Genome Editing

wild type

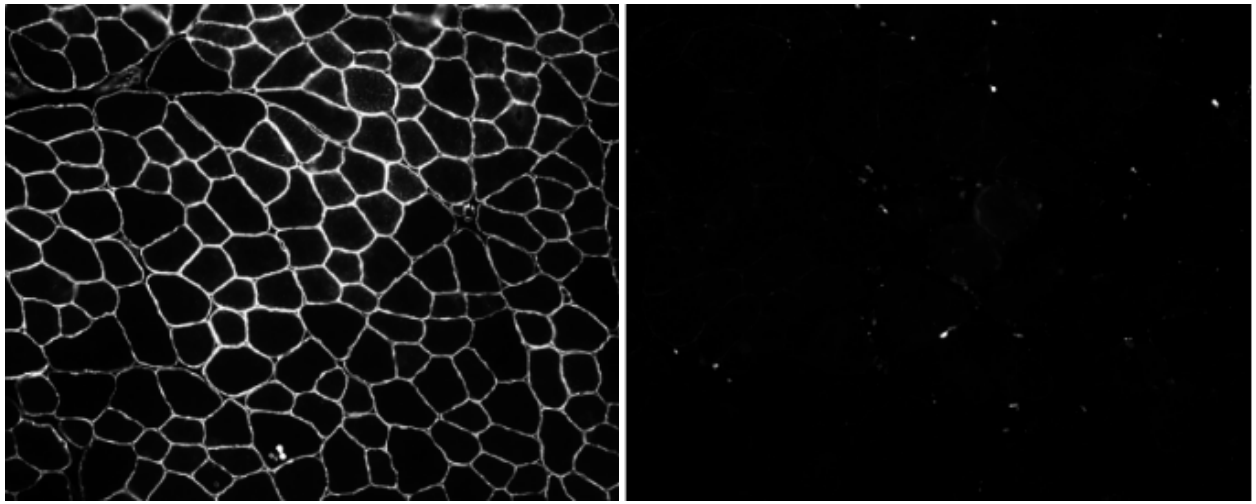


Nelson et al., *Science* (2016)

# Restoration of Dystrophin Expression by Genome Editing

wild type

*mdx*



Nelson et al., *Science* (2016)

# Restoration of Dystrophin Expression by Genome Editing

wild type

*mdx*

*mdx* + AAV-CRISPR

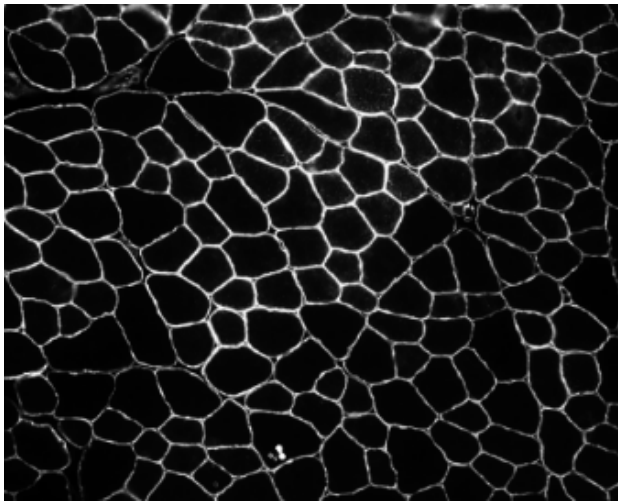
Nelson et al., *Science* (2016)

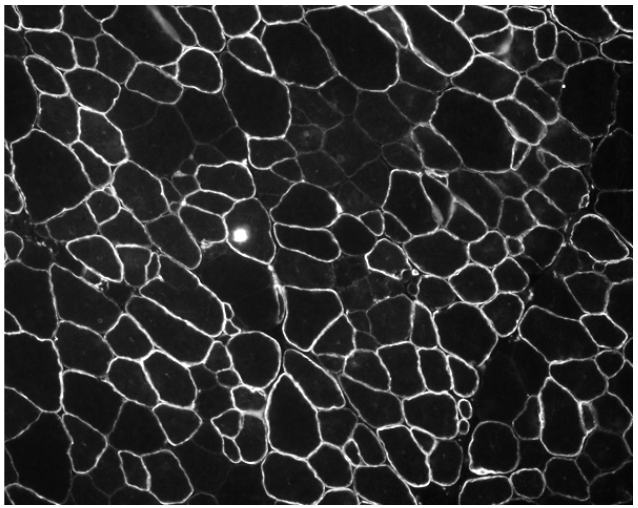
# Restoration of Dystrophin Expression by Genome Editing

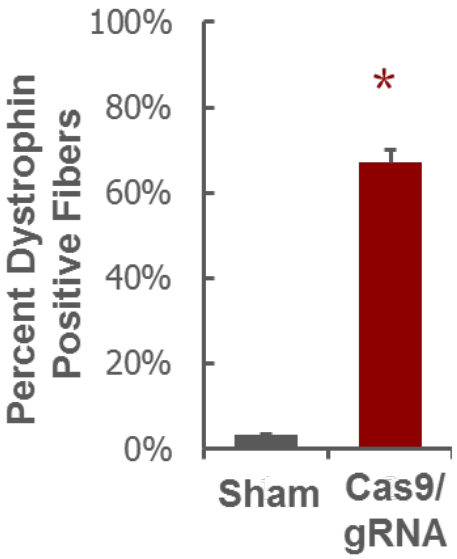
wild type

*mdx*

*mdx* + AAV-CRISPR



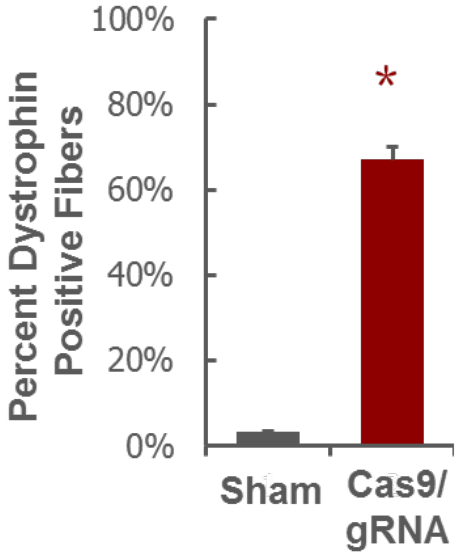


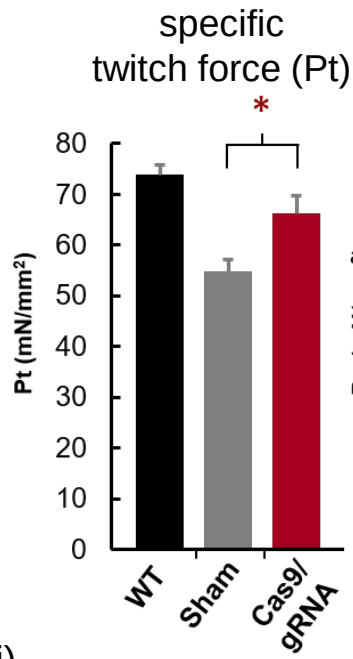


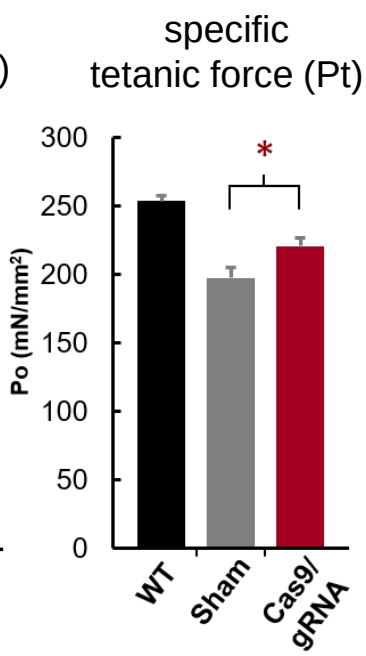

Nelson et al., *Science* (2016)

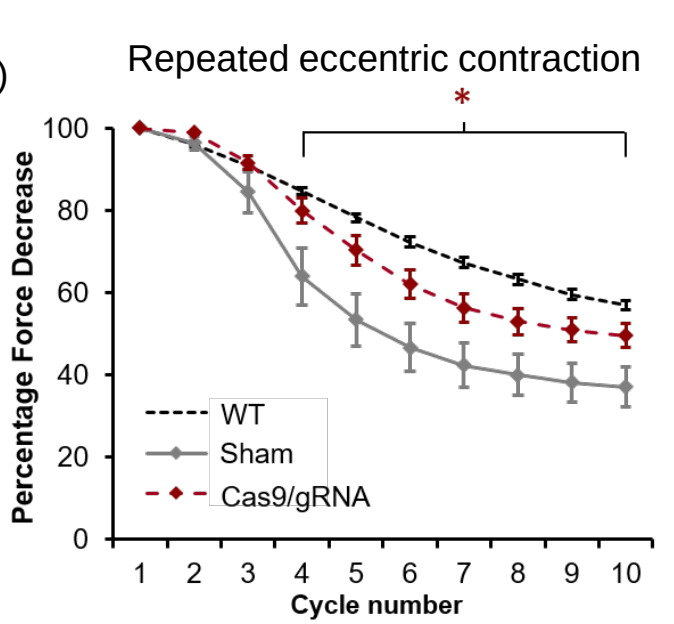
# Restoration of Dystrophin Expression by Genome Editing

wild type | *mdx* | *mdx* + AAV-CRISPR




w/ Dongsheng Duan (U Missouri)

Nelson et al., *Science* (2016)

# Genome Editing for Muscular Dystrophies

## In vivo genome editing improves muscle function in a mouse model of Duchenne muscular dystrophy

Christopher E. Nelson,[1,2] Chady H. Hakim,[3] David G. Ousterout,[1,2] Pratiksha I. Thakore,[1,2] Eirik A. Moreb,[1,2] Ruth M. Castellanos Rivera,[4] Sarina Madhavan,[1,2] Xiufang Pan,[3] F. Ann Ran,[5,6] Winston X. Yan,[5,7,8] Aravind Asokan,[4] Feng Zhang,[5,9,10,11] Dongsheng Duan,[3,12] Charles A. Gersbach[1,2,13]*

## In vivo gene editing in dystrophic mouse muscle and muscle stem cells

Mohammadsharif Tabebordbar,[1,2]* Kexian Zhu,[1,3]* Jason K. W. Cheng,[1] Wei Leong Chew,[2,4] Jeffrey J. Widrick,[5] Winston X. Yan,[6,7] Claire Maesner,[1] Elizabeth Y. Wu,[1]† Ru Xiao,[8] F. Ann Ran,[6,7] Le Cong,[6,7] Feng Zhang,[6,7] Luk H. Vandenberghe,[8] George M. Church,[4] Amy J. Wagers[1]‡

## Postnatal genome editing partially restores dystrophin expression in a mouse model of muscular dystrophy

Chengzu Long,[1,2,3]* Leonela Amoasii,[1,2,3]* Alex A. Mireault,[1,2,3] John R. McAnally,[1,2,3] Hui Li,[1,2,3] Efrain Sanchez-Ortiz,[1,2,3] Samadrita Bhattacharyya,[1,2,3] John M. Shelton,[4] Rhonda Bassel-Duby,[1,2,3] Eric N. Olson[1,2,3]†

## CRISPR-mediated Genome Editing Restores Dystrophin Expression and Function in *mdx* Mice

Li Xu[1], Ki Ho Park[1], Lixia Zhao[1], Jing Xu[1], Mona El Refaey[1], Yandi Gao[1], Hua Zhu[1], Jianjie Ma[1] and Renzhi Han[1]

## Muscle-specific CRISPR/Cas9 dystrophin gene editing ameliorates pathophysiology in a mouse model for Duchenne muscular dystrophy

Niclas E. Bengtsson[1,2], John K. Hall[1,2], Guy L. Odom[1,2], Michael P. Phelps[3], Colin R. Andrus[4,5], R. David Hawkins[4,5], Stephen D. Hauschka[2,6], Joel R. Chamberlain[2,4] & Jeffrey S. Chamberlain[1,2,4,6]

## Selection-free gene repair after adenoviral vector transduction of designer nucleases: rescue of dystrophin synthesis in DMD muscle cell populations

Ignazio Maggio[1], Luca Stefanucci[1,2], Josephine M. Janssen[1], Jin Liu[1], Xiaoyu Chen[1], Vincent Mouly[3] and Manuel A.F.V. Gonçalves[1,*]

## Genetic Modulation of RNA Splicing with a CRISPR-Guided Cytidine Deaminase

Juanjuan Yuan,[1,5] Yunqing Ma,[1,5] Tao Huang,[1] Yanhao Chen,[2] Yuanzheng Peng,[1] Bing Li,[4] Jia Li,[1] Yuchen Zhang,[1] Bing Song,[3] Xiaofang Sun,[3] Qiurong Ding,[2] Yan Song,[4,*] and Xing Chang[1,6,*]

## CRISPR-Induced Deletion with SaCas9 Restores Dystrophin Expression in Dystrophic Models *In Vitro* and *In Vivo*

Benjamin L. Duchêne,[1,2] Khadija Cherif,[1] Jean-Paul Iyombe-Engembe,[1,2] Antoine Guyon,[1,2] Joel Rousseau,[1] Dominique L. Ouellet,[1] Xavier Barbeau,[3] Patrick Lague,[3] and Jacques P. Tremblay[1,2]

## Functional Rescue of Dystrophin Deficiency in Mice Caused by Frameshift Mutations Using *Campylobacter jejuni* Cas9

Taeyoung Koo,[1,2] Ngoc B. Lu-Nguyen,[3] Alberto Malerba,[3] Eunji Kim,[1] Daesik Kim,[4] Ornella Cappellari,[5] Hee-Yeon Cho,[1] George Dickson,[3] Linda Popplewell,[3] and Jin-Soo Kim[1,2,4]

## In Vivo Genome Editing Restores Dystrophin Expression and Cardiac Function in Dystrophic Mice

Mona El Refaey, Li Xu, Yandi Gao, Benjamin D. Canan, T.M. Ayodele Adesanya, Sarah C. Warner, Keiko Akagi, David E. Symer, Peter J. Mohler, Jianjie Ma, Paul M.L. Janssen, Renzhi Han

## Correction of a splicing defect in a mouse model of congenital muscular dystrophy type 1A using a homology-directed-repair-independent mechanism

Dwi U Kemaladewi[1], Eleonora Maino[1,2], Elzbieta Hyatt[1], Huayun Hou[1,2], Maylynn Ding[1], Kara M Place[1], Xinyi Zhu[1], Prabhpreet Bassi[1,2], Zahra Baghestani[1], Amit G Deshwar[3], Daniele Merico[1,3,4], Hui Y Xiong[3], Brendan J Frey[3,5], Michael D Wilson[1,2,6], Evgueni A Ivakine[1] & Ronald D Cohn[1,2,7]

## Gene editing restores dystrophin expression in a canine model of Duchenne muscular dystrophy

Leonela Amoasii[1,2], John C. W. Hildyard[3], Hui Li[1], Efrain Sanchez-Ortiz[1], Alex Mireault[1], Daniel Caballero[1], Rachel Harron[3], Thaleia-Rengina Stathopoulou[4], Claire Massey[3], John M. Shelton[5], Rhonda Bassel-Duby[1], Richard J. Piercy[3], Eric N. Olson[1]*

# Genome Editing for Duchenne Muscular Dystrophy

Enthusiasm predicated on:

1. Potential to translate to human disease
   - How to model human mutations?

2. Long-term stability of editing
   - Most study outcomes at 2-8 weeks post-treatment

3. Which cells are edited and do they persist?
   - Most studies assessing bulk tissues

4. Tolerable immune response
   - Most studies ignore immune response

5. Destiny and safety of delivery systems
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# Humanized Mouse Model of DMD


hDMD/*mdx* mouse (t'Hoen et al., *JBC* 2008)

Germline CRISPR/Cas9 editing (**Δ**52)


Robinson-Hamm et al., unpublished

# Humanized Mouse Model of DMD


hDMD/*mdx* mouse (t'Hoen et al., *JBC* 2008)

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AAV-CRISPR editing (**Δ**51)


Robinson-Hamm et al., unpublished

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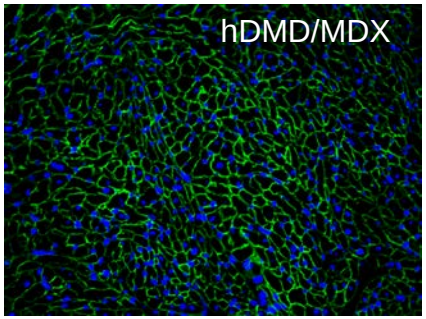
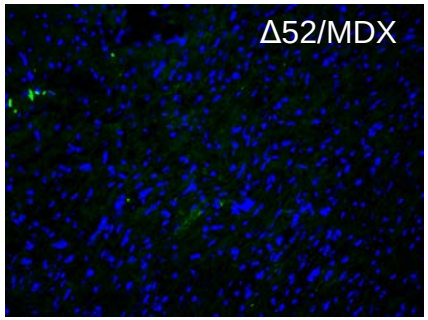
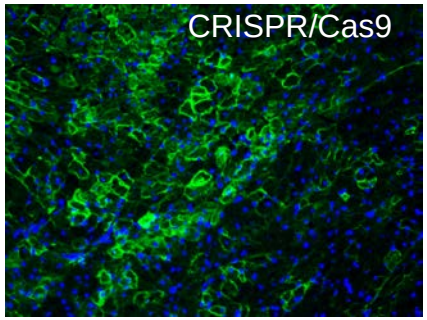
hDMD/*mdx* mouse (t'Hoen et al., *JBC* 2008)

Germline CRISPR/Cas9 editing (**Δ**52)


AAV-CRISPR editing (**Δ**51)

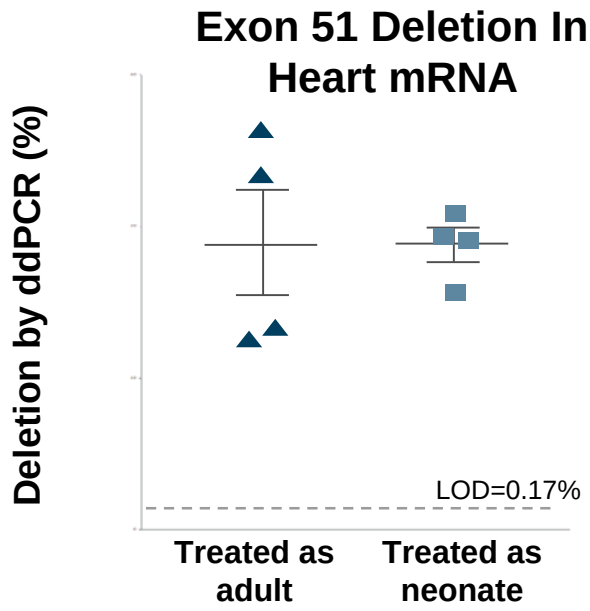




**DYSTROPHIN**
**NUCLEI**

Systemic AAV9
(4-8 x $10^{12}$ vg/mouse)




Robinson-Hamm et al., unpublished

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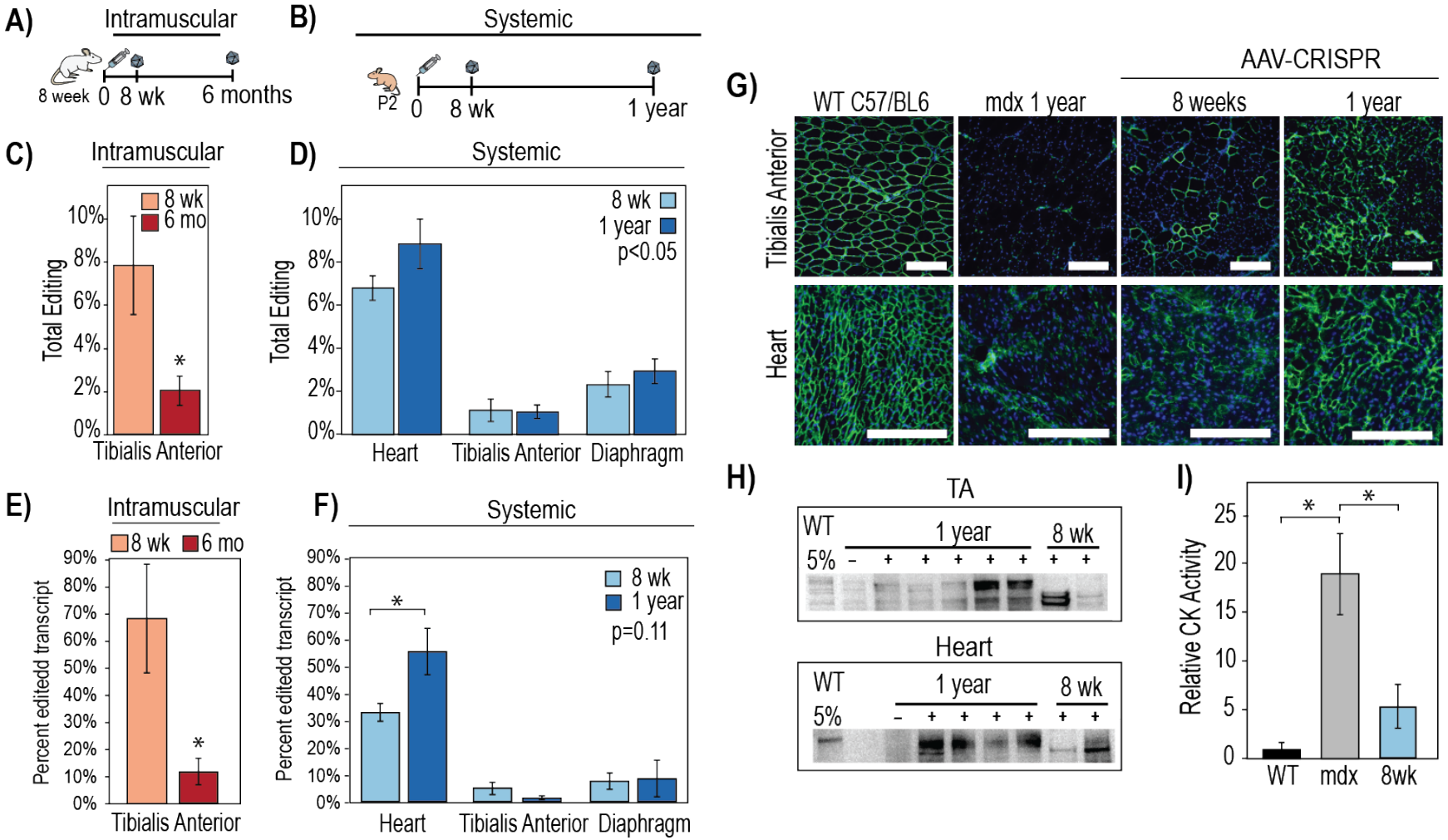
# Persistence of Editing in Skeletal and Cardiac Muscle




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

Nelson et al., *in review*

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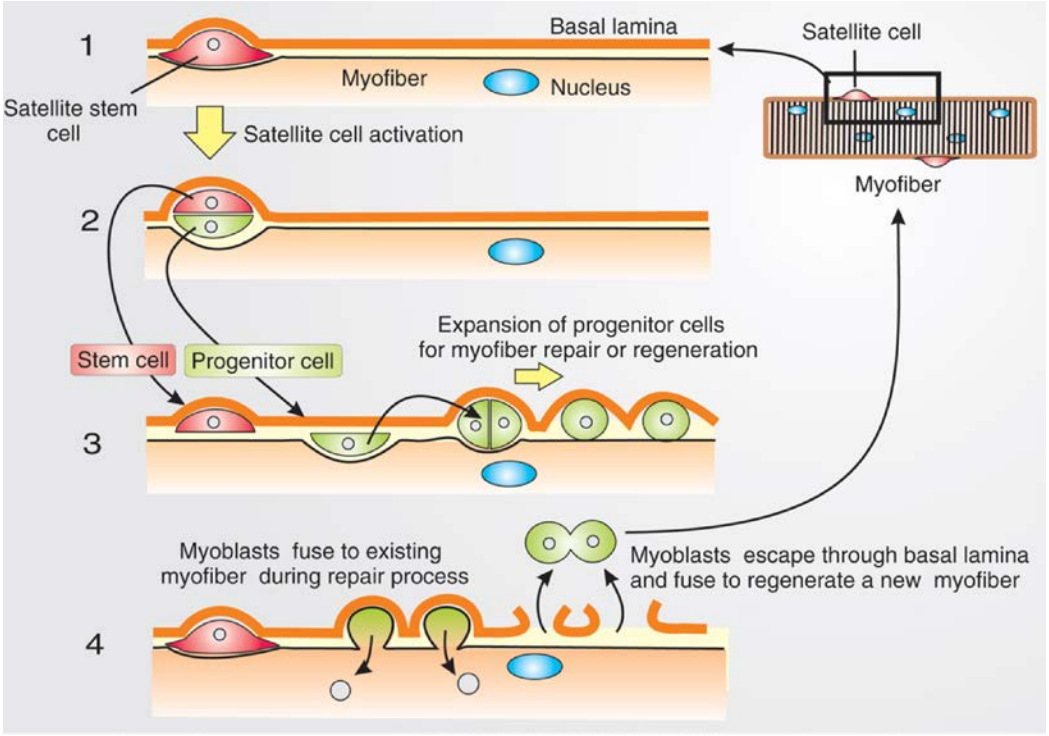
# Satellite Cells are the Stem Cells of Skeletal Muscle

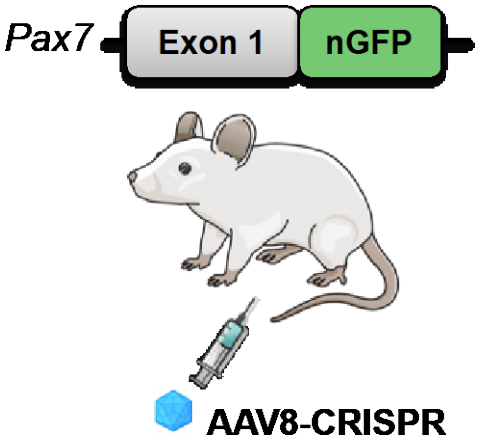


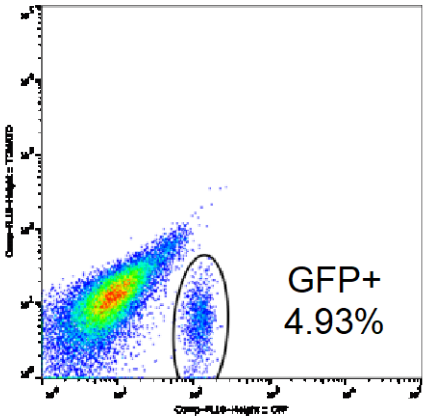

Does AAV transduce satellite cells *in vivo*?

Does CRISPR edit satellite cells *in vivo*?

Does satellite cell editing facilitate long-term dystrophin restoration?

# AAV-CRISPR Gene Editing of Satellite Cells




Kwon et al., *unpublished*

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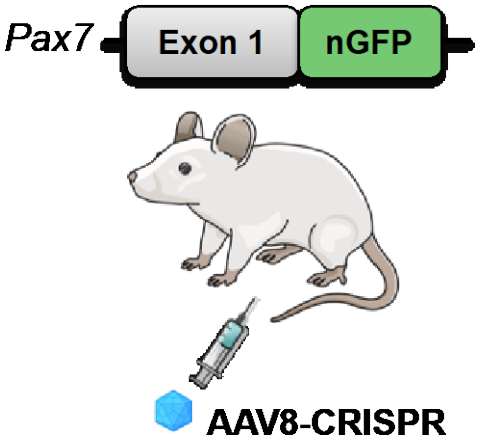


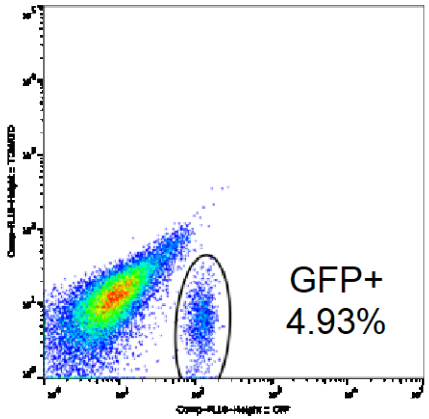


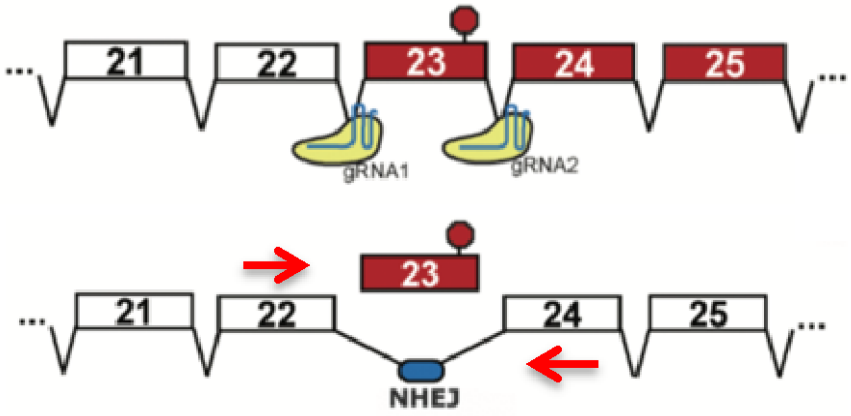


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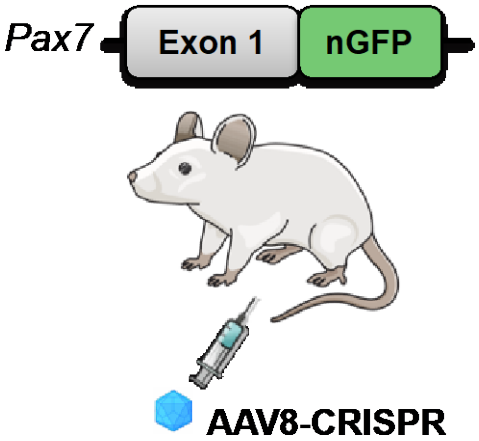
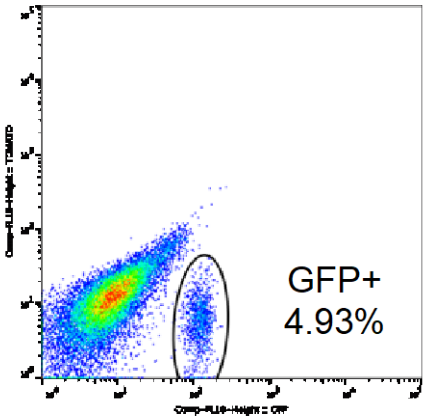
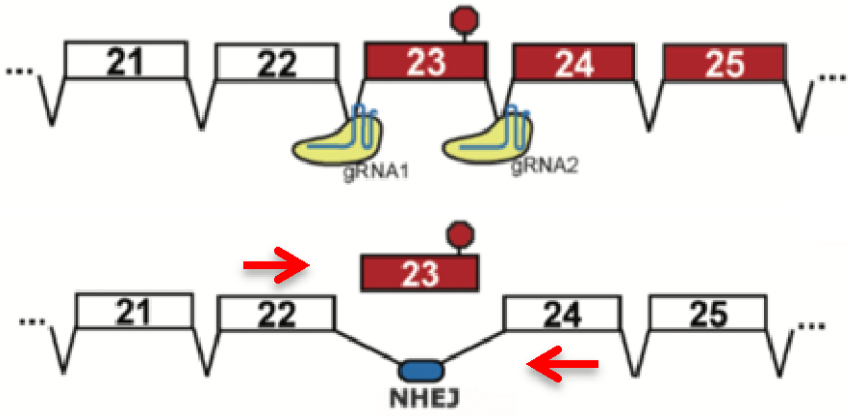
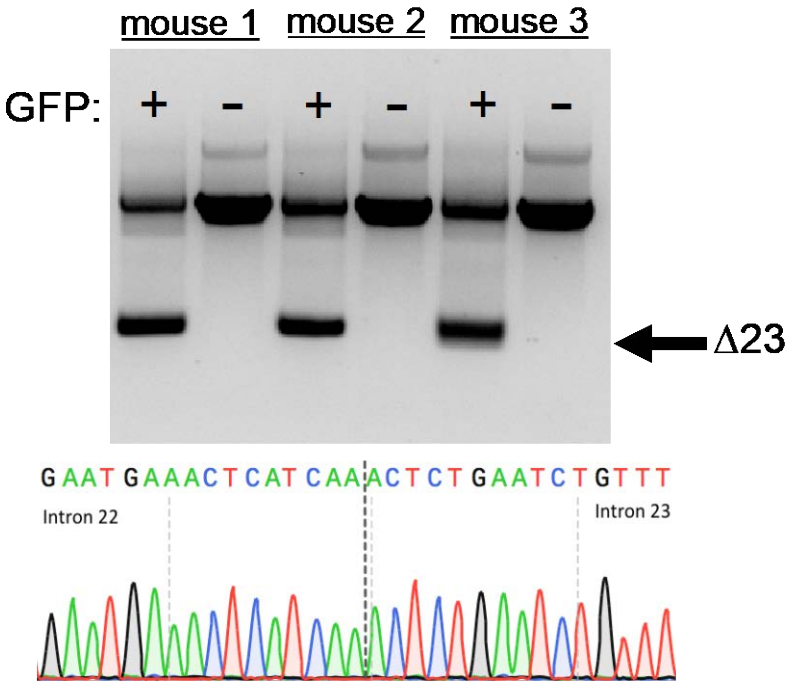



Kwon et al., *unpublished*

# Genome Editing for Duchenne Muscular Dystrophy

Enthusiasm predicated on:

1. Potential to translate to human disease
   - How to model human mutations?

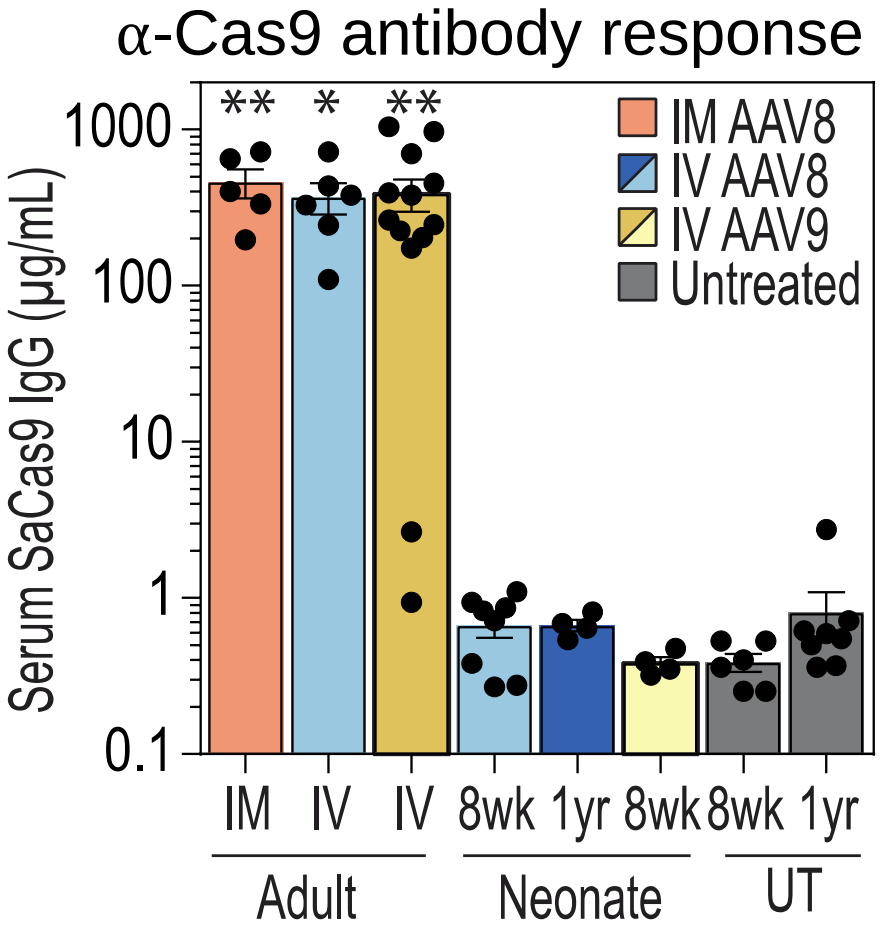
2. Long-term stability of editing
   - Most study outcomes at 2-8 weeks post-treatment

3. Which cells are edited and do they persist?
   - Most studies assessing bulk tissues

4. Tolerable immune response
   - Most studies ignore immune response

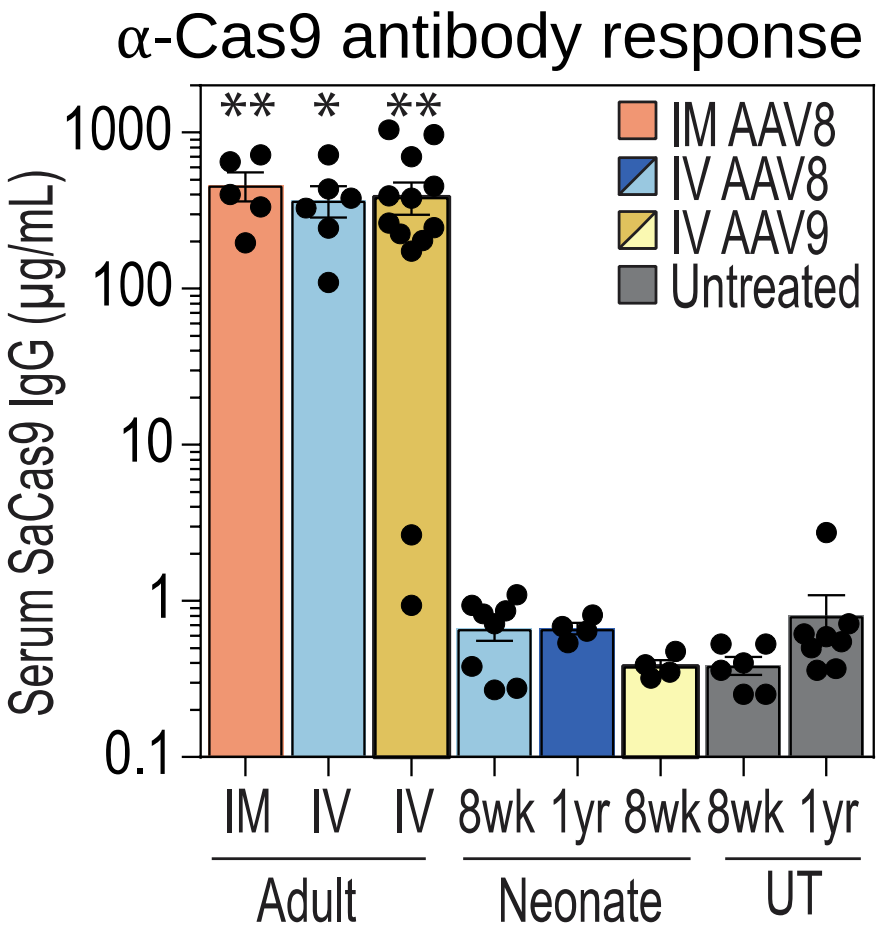
5. Destiny and safety of delivery systems
   - Most studies do not assess vector stability

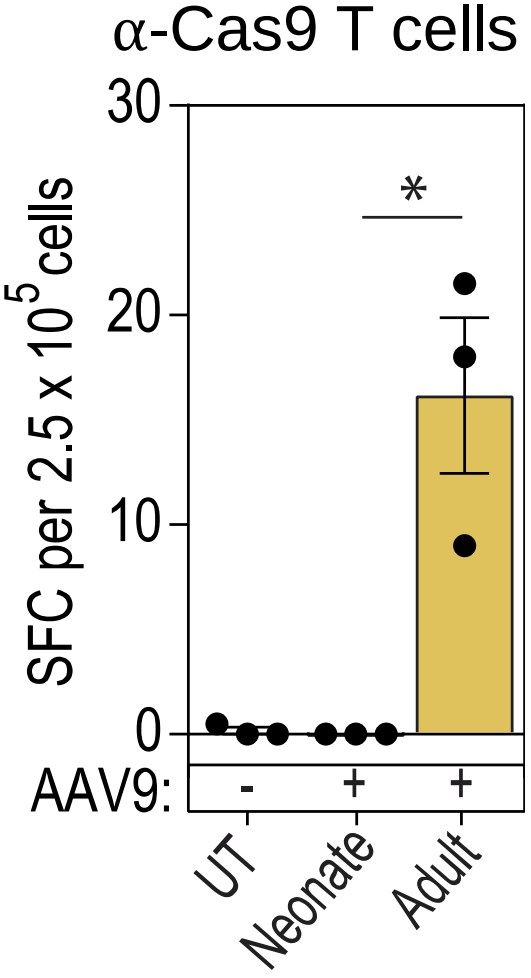
# Host Immune Response to CRISPR/Cas9




Nelson et al., *unpublished*

# 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., *unpublished*

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# Unbiased Detection of Genome Editing Events

Repair of double-strand breaks induced by CRISPR–Cas9 leads to large deletions and complex rearrangements

Michael Kosicki, Kärt Tomberg & Allan Bradley

NATURE BIOTECHNOLOGY

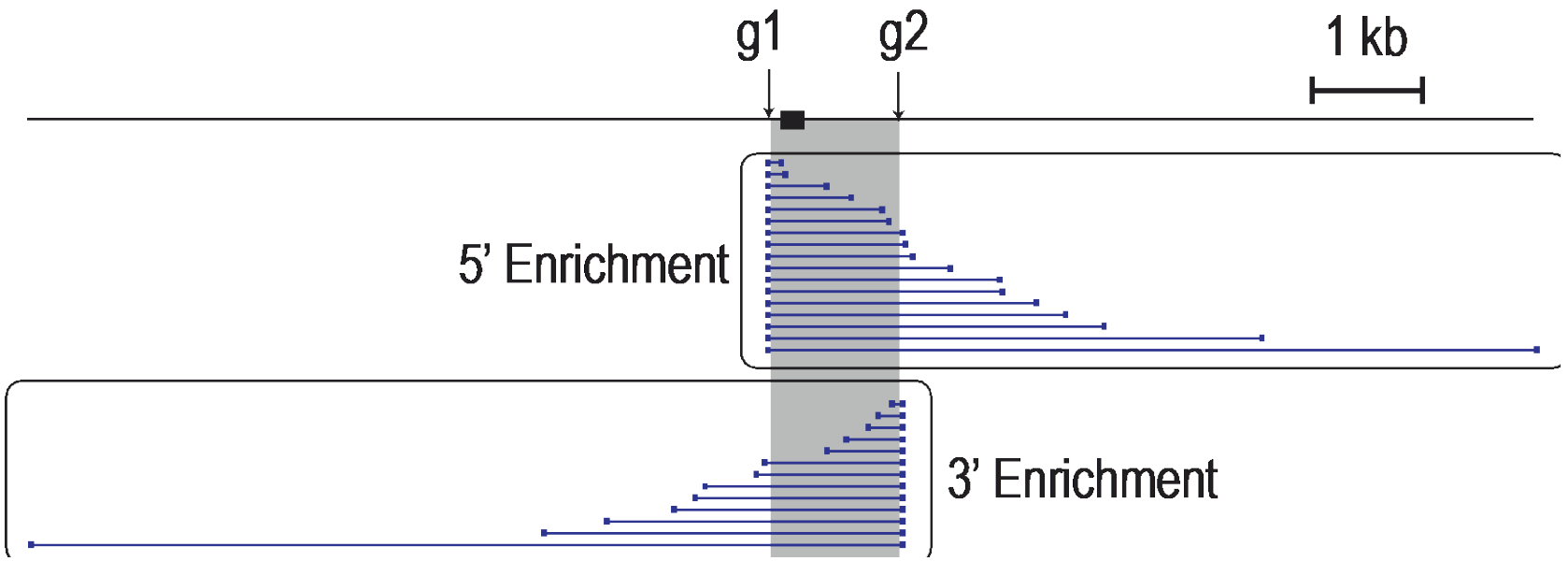
Received 27 Aug 2016 | Accepted 31 Mar 2017 | Published 31 May 2017

DOI: 10.1038/ncomms15464 OPEN

CRISPR/Cas9 targeting events cause complex deletions and insertions at 17 sites in the mouse genome

Ha Youn Shin[1,2,*,**], Chaochen Wang[1,*], Hye Kyung Lee[1,3,*], Kyung Hyun Yoo[1,4], Xianke Zeng[1], Tyler Kuhns[1], Chul Min Yang[1], Teresa Mohr[1], Chengyu Liu[5] & Lothar Hennighausen[1,**]

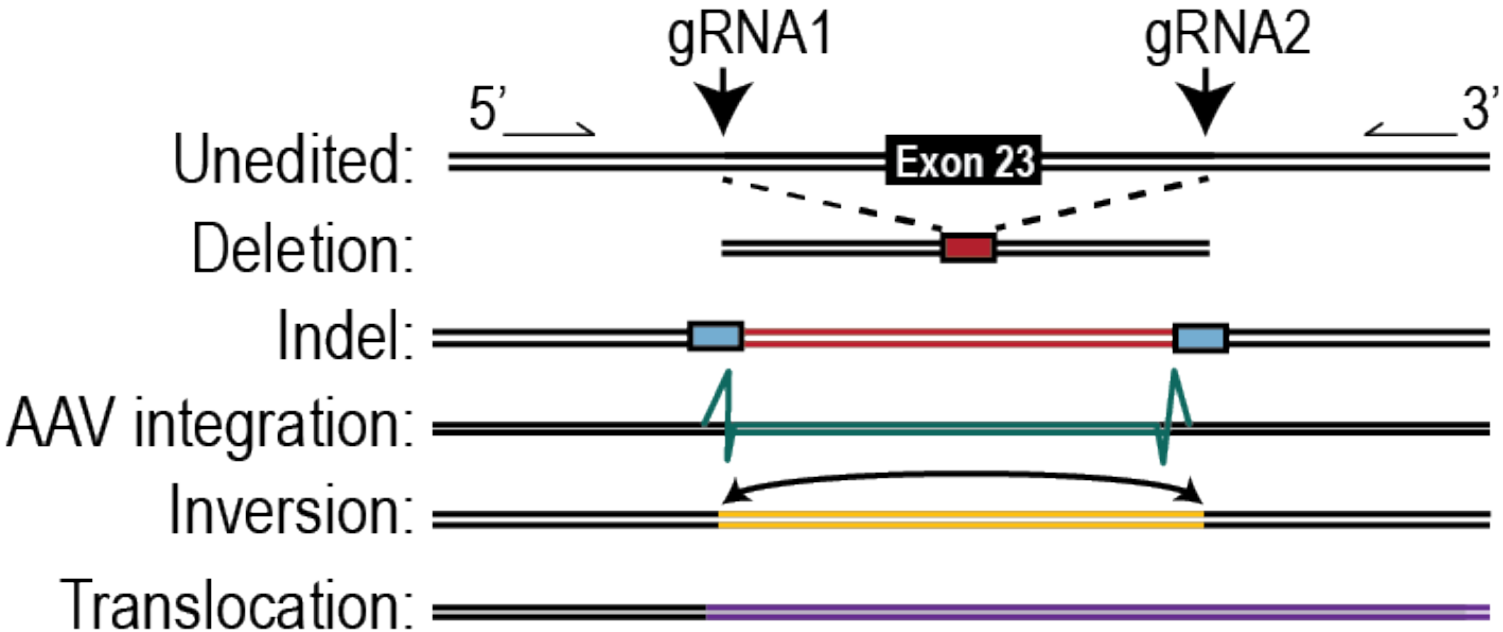
# Unbiased Detection of Genome Editing Events




Large deletions (multi-kilobase) in DMD locus
in the liver of AAV9-CRISPR treated mice

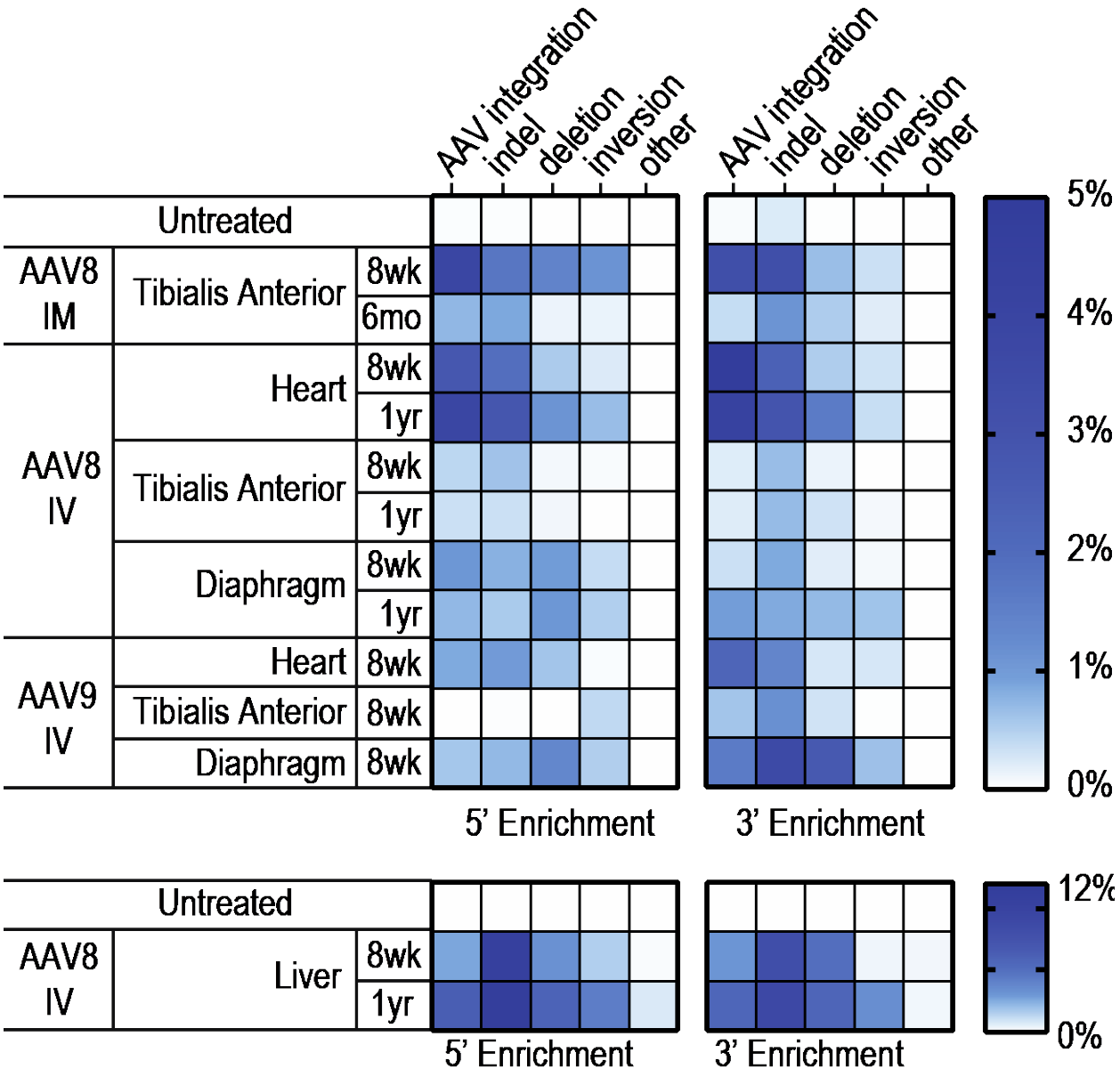
Nelson et al., *unpublished*

# Unbiased Detection of Genome Editing Events




Nelson et al., *unpublished*

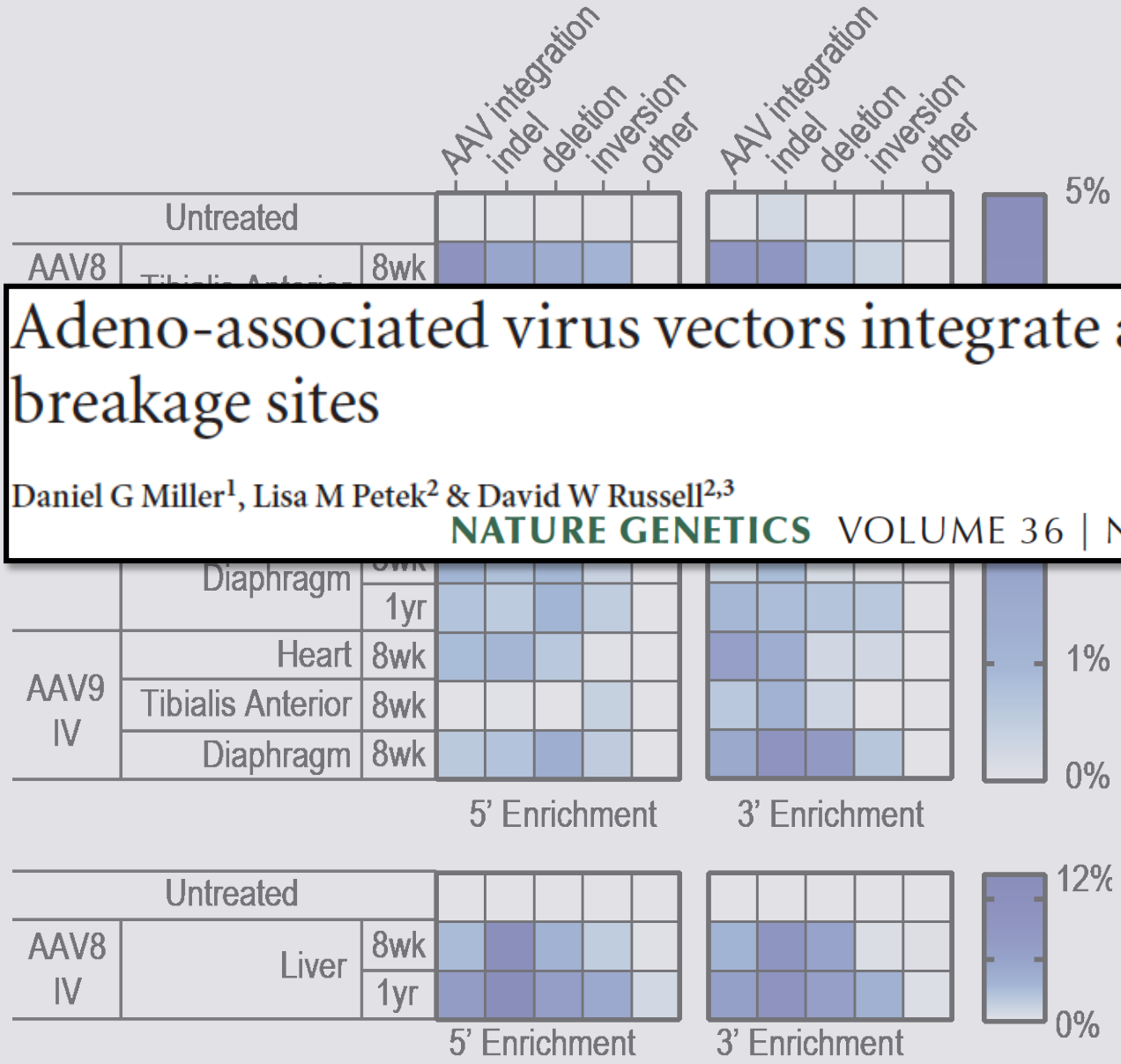
# Unbiased Detection of Genome Editing Events




Unexpectedly high levels of AAV integrations at target site

Nelson et al., *unpublished*

# Unbiased Detection of Genome Editing Events




Adeno-associated virus vectors integrate at chromosome breakage sites

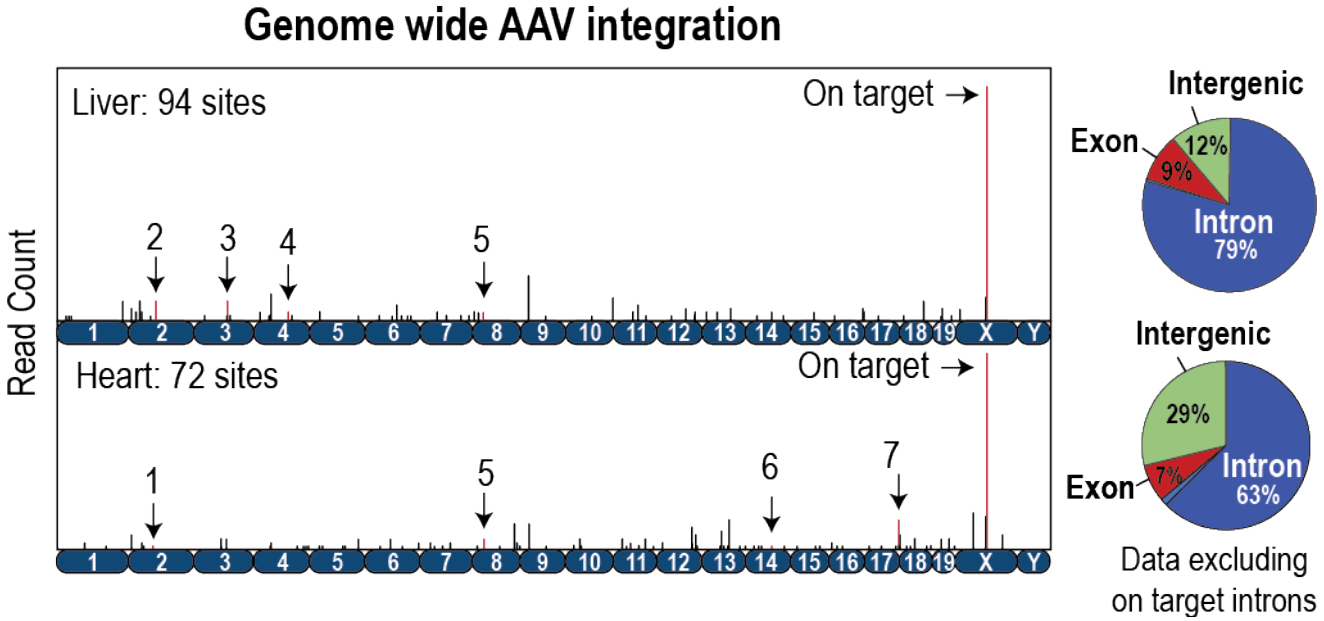
Daniel G Miller[1], Lisa M Petek[2] & David W Russell[2,3]

NATURE GENETICS VOLUME 36 | NUMBER 7 | JULY 2004

gh
levels of AAV
integrations at
target site

Nelson et al., *unpublished*

# Detection of AAV Integration at Off-Target Sites




| | | | |
|---|---|---|---|
| mm10<br>1. Chr2:65945524<br>Csrnp3 | PAM<br>CATTGCATCCATGT-CTGACTNNGRRT<br>\|\| \|\| \|\| \|\|\|\|\| \| \|\|\|\|\|\|\|\|\|\|<br>CAgTGgATaCATGTGCaGACTCTGAAT | gRNA1<br>22/26 | mm10<br>4. Chr4:96078165<br>Intergenic | CATTGCATCCA-TGTCTGACTNNGRRT<br>\| \|\|\|\|\| \| \|\|\|\|\|\|\|\|\|\|<br>tgatctatccaatagctgactgtgagc | gRNA1<br>18/26 |
| 2. Chr2:76763712<br>Ttn | TACACTAACACGCATATTTGNNGRRT<br>\|\| \|\|\| \| \| \|\|\|\|\|\|\|\|\|\|<br>tatgataaaaggtctatttgtggaaa | gRNA2<br>18/26 | 5. Chr8:27903718<br>Intergenic | CATTGCATCCATGTCTGACTNNGRRT<br>\|\|\|\| \|\| \|\|\|\|\|\|\|\|\|\|\|\|<br>CATT--AT-CATGTCTGACTTTGtta | gRNA1<br>20/26 |
| 3. Chr3:90484616<br>Ilf2 | CATTG----CATCCATGTCTGACTNNGRRT<br>\|\|\|\| \|\| \|\|\|\| \|\|\|\|\|\|\|\|\|\|<br>gattgaactcaggtttgtcagacttagggt | gRNA1<br>20/26 | 6. Chr14:38227210<br>Intergenic | TACACTAACACGCATATTTGNNGRRT<br>\|\|\|\|\| \|\|\| \|\|\|\|\|\|\|\|\|\|\|\|<br>TACACacACAtGCATATTTGGTGGAT | gRNA2<br>23/26 |
| 7. Chr17:88459505<br>Foxn2 | CATT-G-CATCCATGTCTGACTNNGRRT<br>\|\|\| \| \| \|\| \|\|\|\|\|\|\|\|\|\|<br>gtatttgtttttcctg-ctgactcagagt | gRNA1<br>19/26 | | | |

AAV integration at CRISPR off-target sites
not identified by traditional methods

Nelson et al., *unpublished*

# Summary

- Genome editing for DMD typically focuses on *removing gene segments* to restore functional, truncated dystrophin
- *In vivo* CRISPR-based genome editing restores *long-term dystrophin expression* in many studies with *no reported adverse effects*
- Potential for *editing adult stem cells* of skeletal muscle
- Robust *anti-Cas9 host immune response* that resolves without intervention
- *Unintended genomic outcomes*, including AAV integration, into on-target and off-target sites
- **Additional research required to understand implications of *immune response*, long-term presence of *delivery vectors*, and *alternative genome modifications***

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Kam Leong (Duke/Columbia)
**Dongsheng Duan (U Missouri)**
Yong-hui Jiang (Duke)
Xiling Sheng (Duke)

**Funding:** Paul G. Allen Frontiers Group, Open Philanthropy, NIH, NSF, **Muscular Dystrophy Association**, **Department of Defense**, Thorek Memorial Foundation, **The Hartwell Foundation**, **March of Dimes Foundation**, American Heart Association, Nancy Taylor Foundation, **Duke-Coulter Partnership, Duke Clinical and Translational Science Award, Sarepta Therapeutics**, Locus Biosciences, Element Genomics, Levo Therapeutics