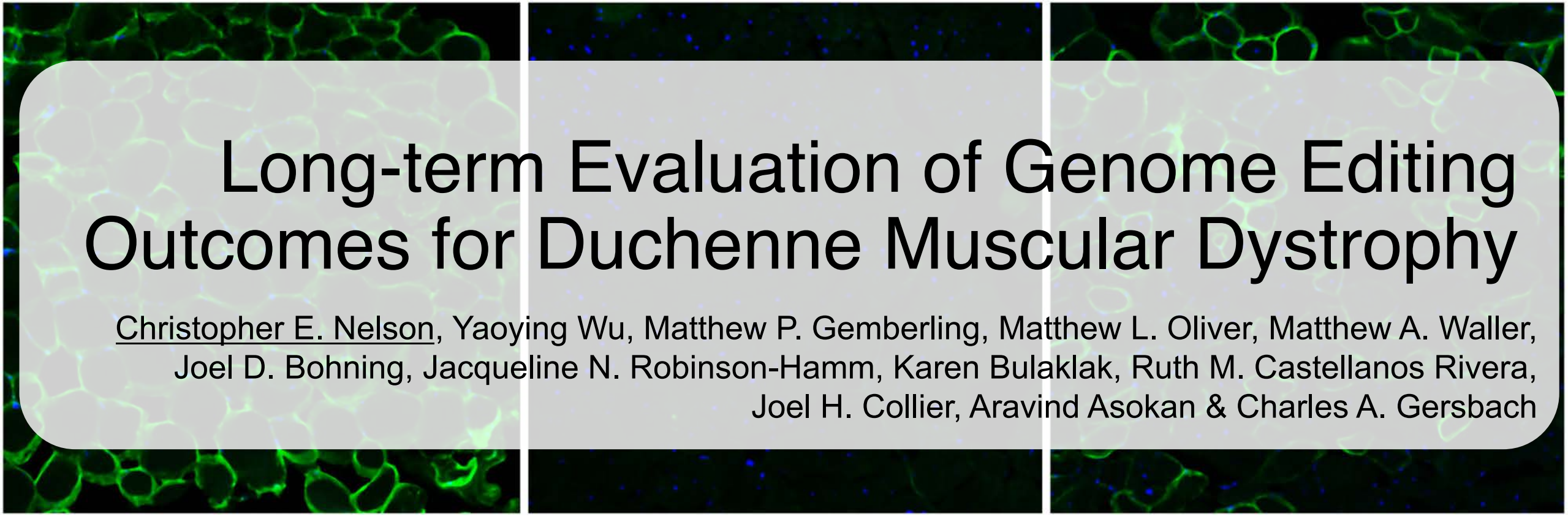
Duke UNIVERSITY



Long-term Evaluation of Genome Editing Outcomes for Duchenne Muscular Dystrophy

Christopher E. Nelson, Yaoying Wu, Matthew P. Gemberling, Matthew L. Oliver, Matthew A. Waller, Joel D. Bohning, Jacqueline N. Robinson-Hamm, Karen Bulaklak, Ruth M. Castellanos Rivera, Joel H. Collier, Aravind Asokan & Charles A. Gersbach

American Society for Gene and Cell Therapy 4/29/2019

Duchenne muscular dystrophy (DMD)

- **1:5000 live male births**
- **Debilitating and fatal**
  - Loss of ambulation in early teens
  - Loss of cardiac/pulmonary function in 20s
- **Limited treatments**
- **Molecular basis known**
  - Various mutations to dystrophin gene on X chromosome
- **Gene delivery problem**
  - 2.5 million base pairs gDNA
  - 14 kb mRNA product

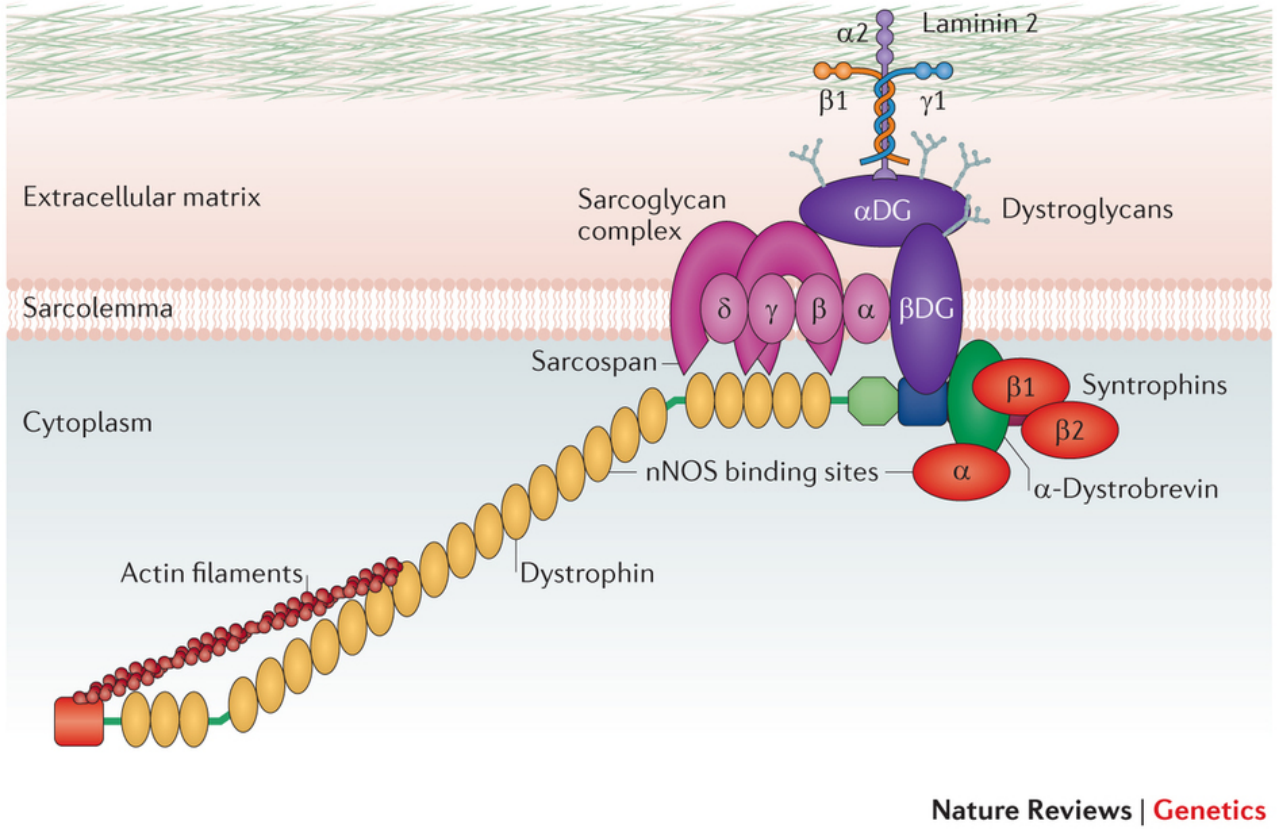
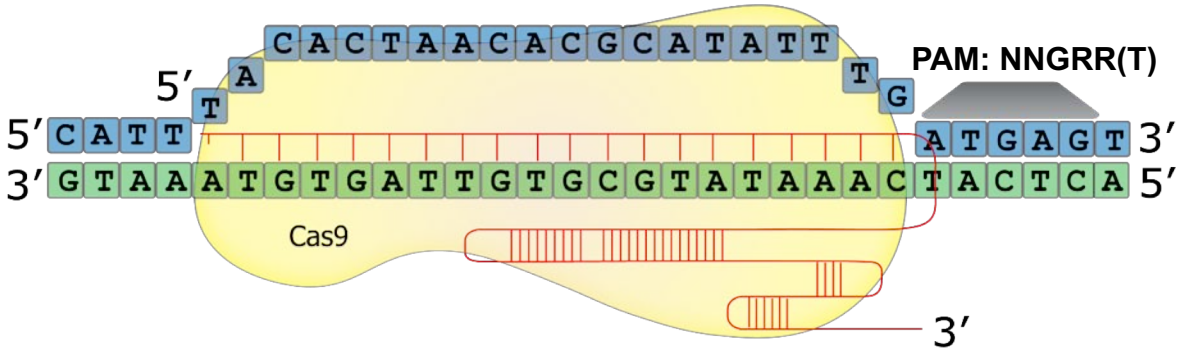


Image: Fairclough et al. *Nature Review Genetics.* 2013
Parent Project Muscular Dystrophy. 2013
Bushby et al. *Lancet Neurology.* 2010
Larkindale et al. *Muscle & Nerve.* 2014

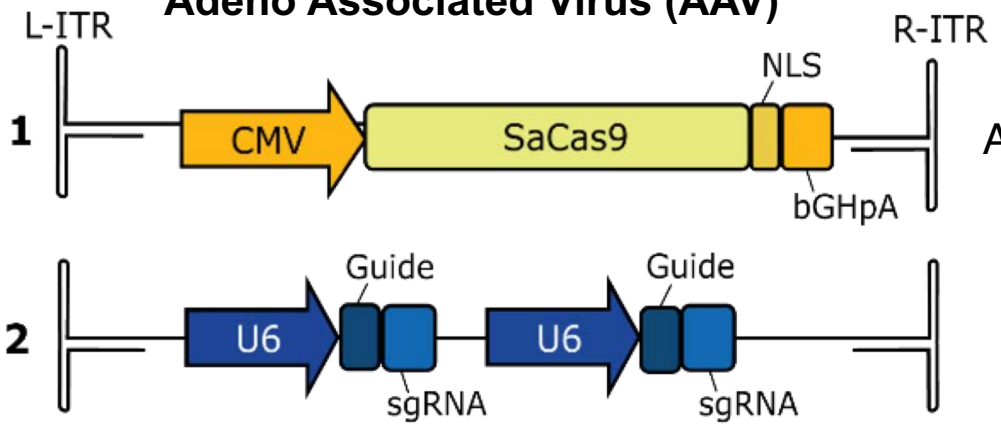
In vivo genome editing with Cas9

SaCas9

3.2 kb (vs. 4.2 kb)



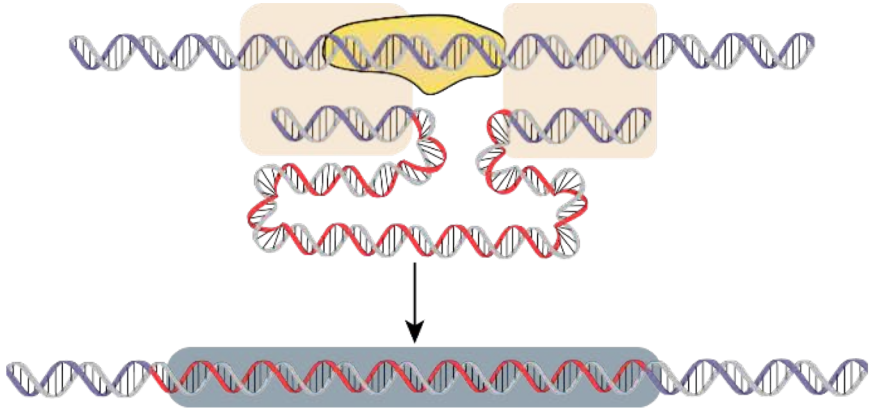
Adeno Associated Virus (AAV)



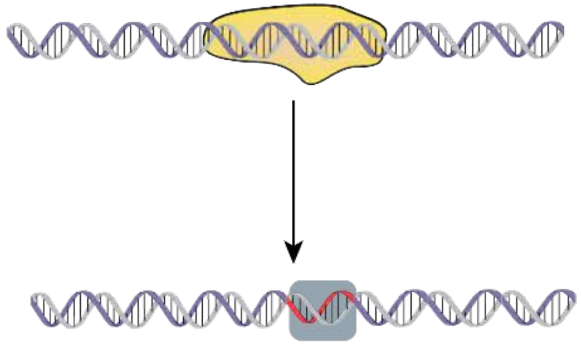
Available Space = 4.7 kb
Replication deficient
Non-pathogenic
>100 clinical trials

Nishimasu H et al. *Cell.* 2015 (PDB 5AXW)
Nelson CE, Gersbach CA. *ARCBE.* 2016

Gene editing approaches for DMD

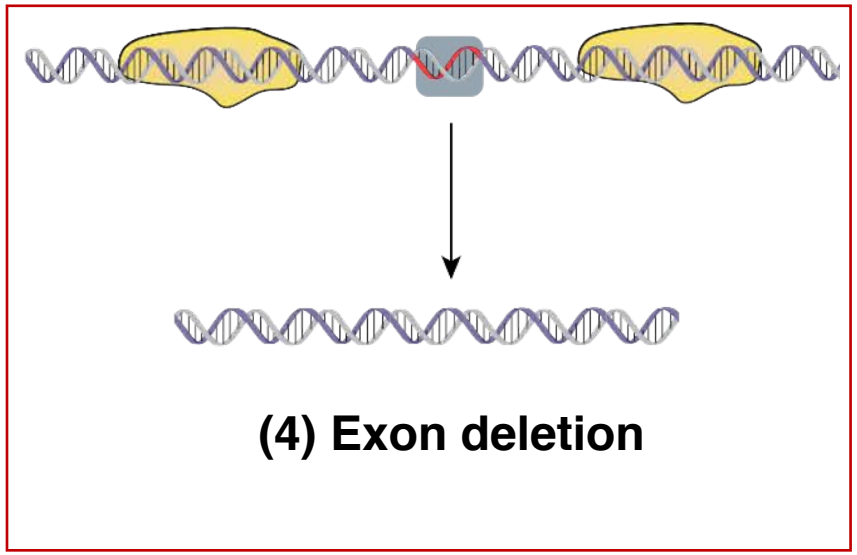


(1) HDR exon replacement
(2) NHEJ targeted integration



(2) NHEJ - frame shifting

(3) Splice-site targeting



(4) Exon deletion

Why exon deletion?

- Doesn't require HDR
- Large introns provide flexibility in protospacer design
- Single gene-editing strategy may apply to as much as 40-63% of DMD patients

Figures adapted from:
Nelson et al. *ARCBE.* 2016

DMD mutation spectrum

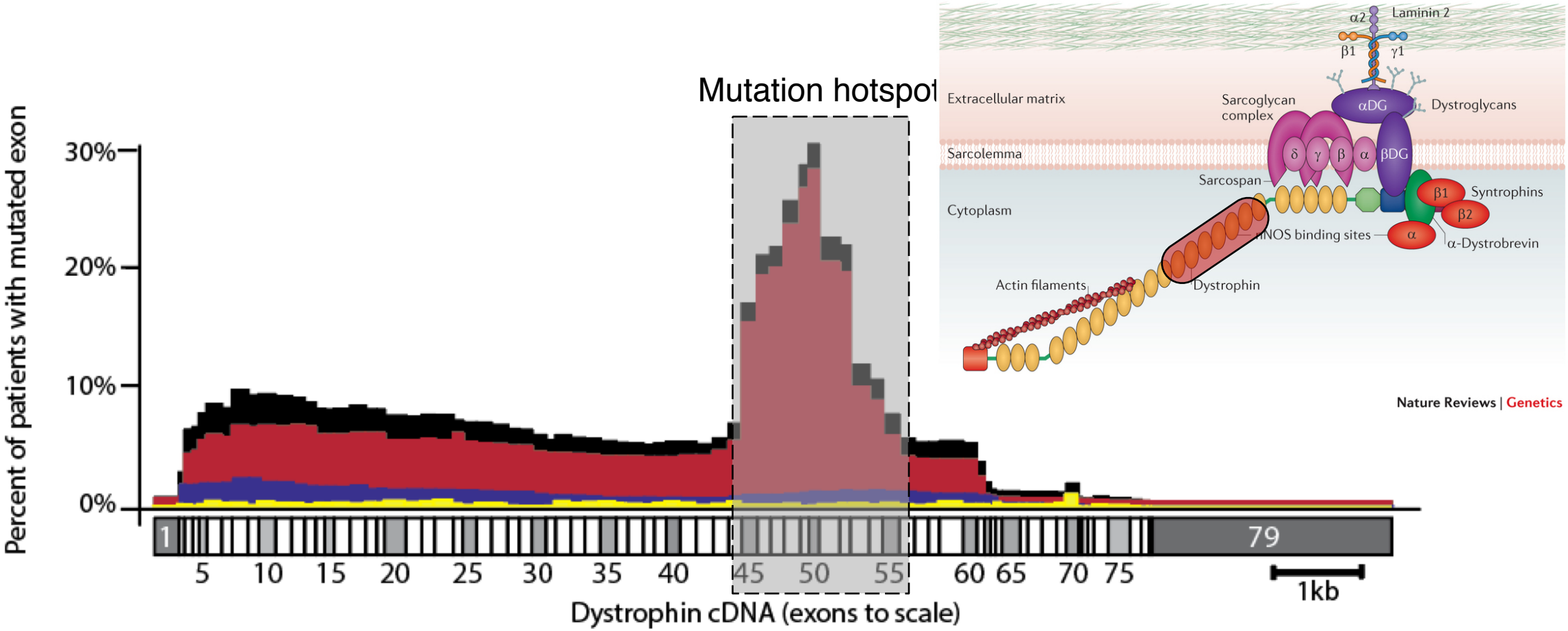
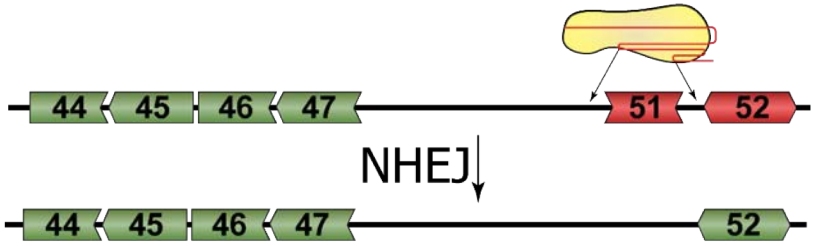


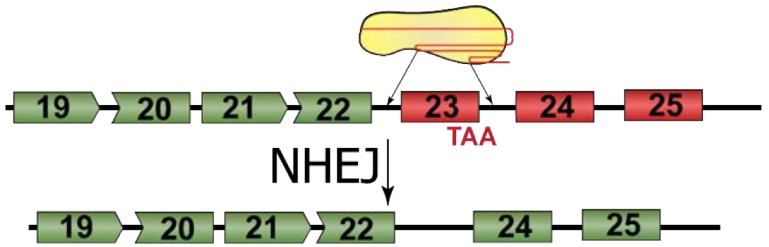
Figure adapted from:
Nelson CE and Gersbach CA. *Muscle Gene Therapy.* In press.
Data derived from the UMD database

The mdx mouse is a model of DMD for *in vivo* genome editing

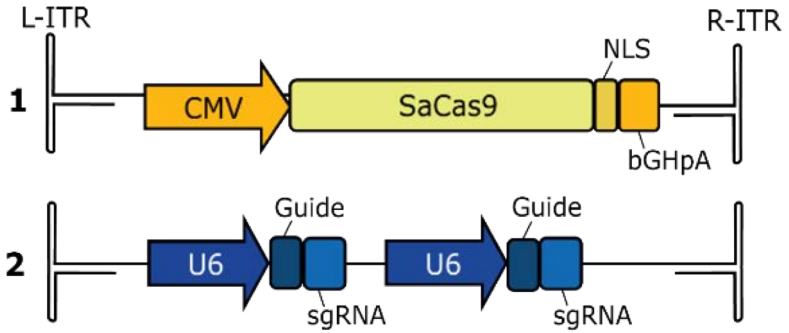
Human *DMD* gene



The mdx mouse model of DMD



Viral Vector Design



Local injections into tibialis anterior

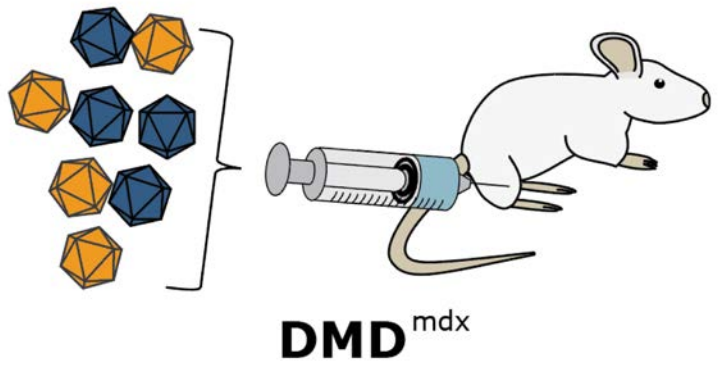
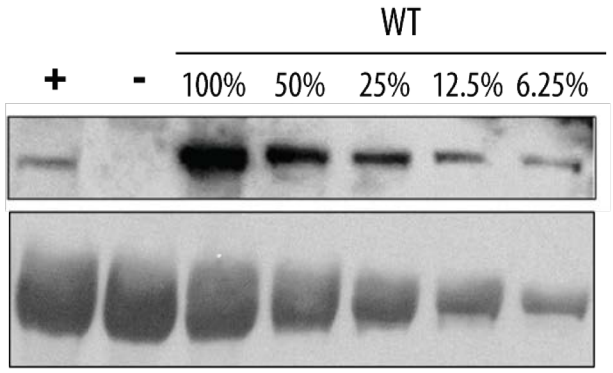
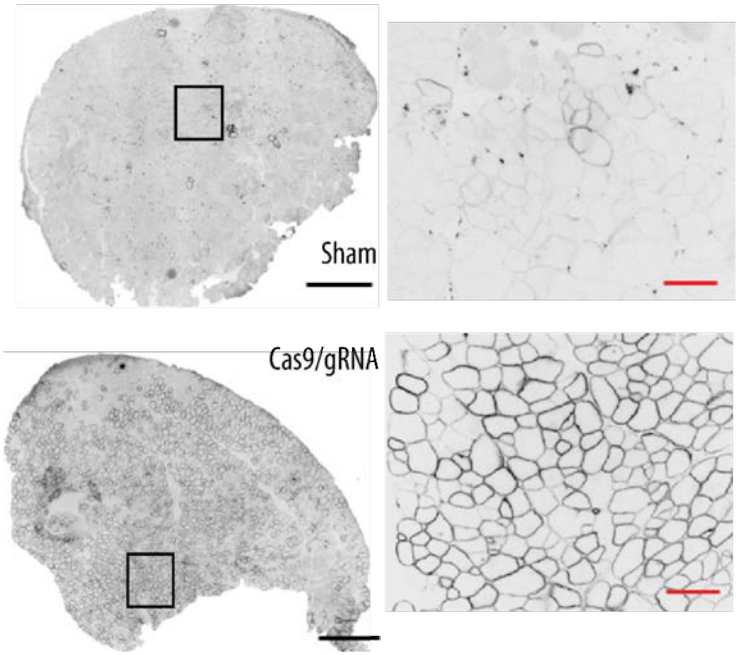


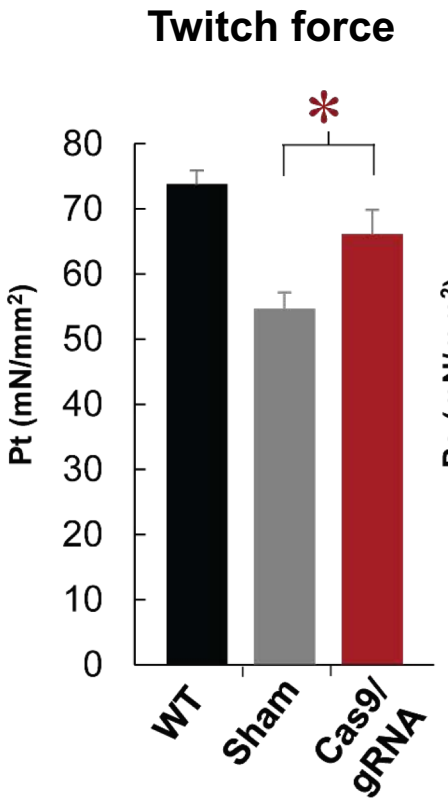
Figure adapted from:
Nelson CE et al. *Nature Reviews Neurology*. 2017

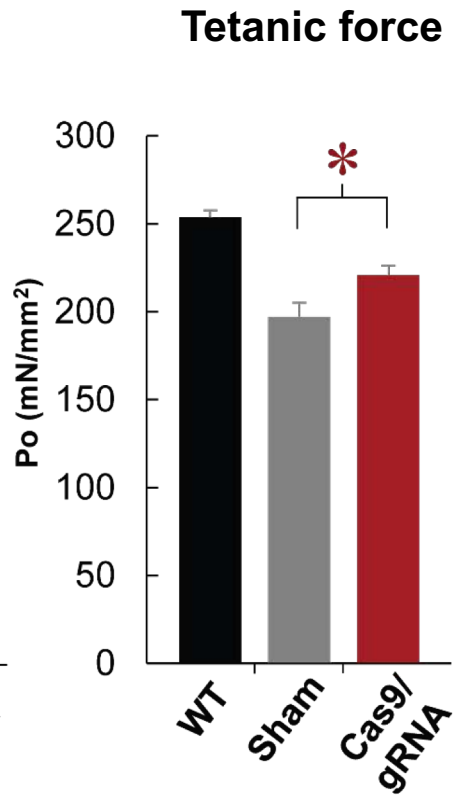
Dystrophin restoration improves muscle function in the mdx mouse

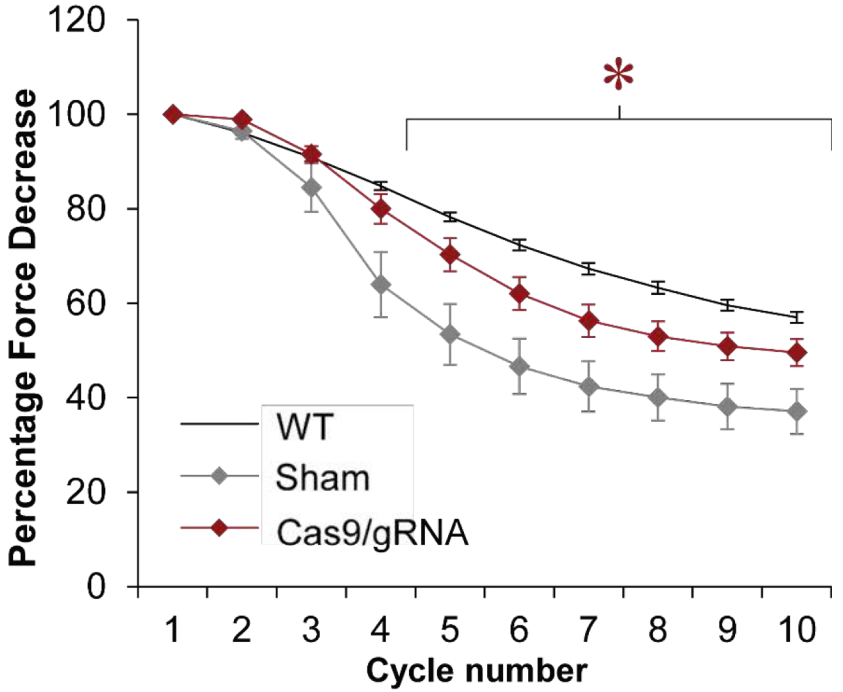




***In situ* muscle function assay of tibialis anterior[1]**

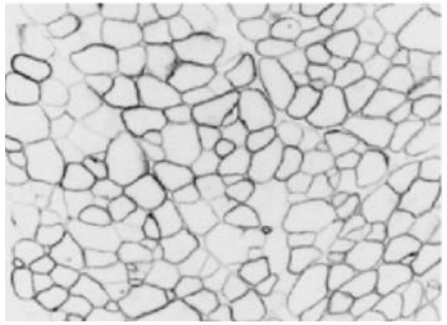






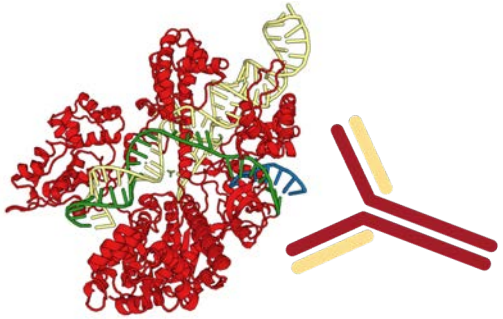
Nelson CE et al. *Science.* 2016
1. Hakim, Wasala, Duan. *Jove.* 2013 (method)

Remaining questions

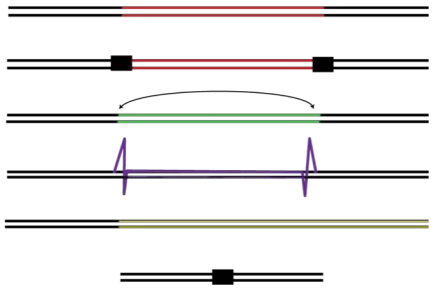


→ ?

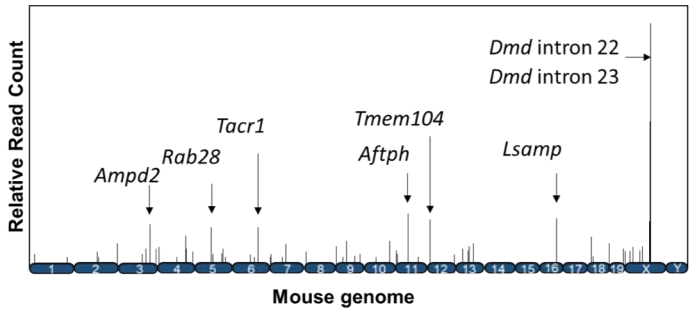
Long-term evaluation of genome editing efficiency



Host response to AAV-CRISPR



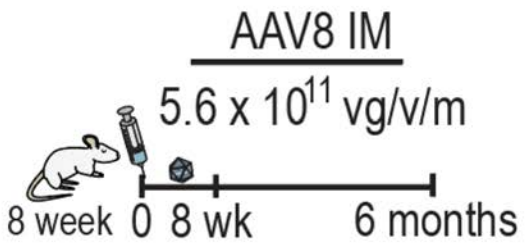
Detect and quantify alternate genome modifications

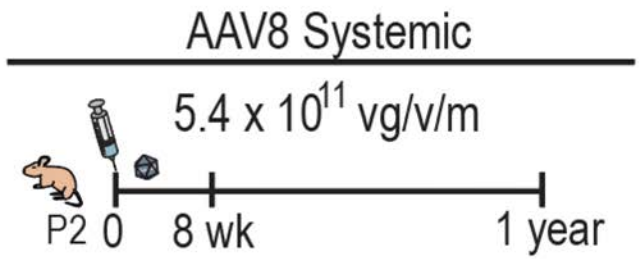


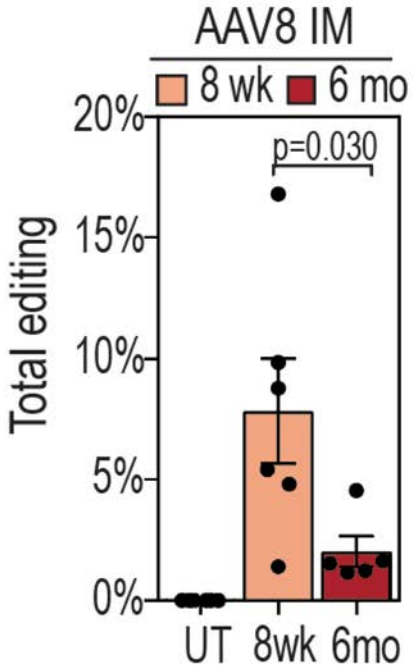
Genome-wide off targets in vivo

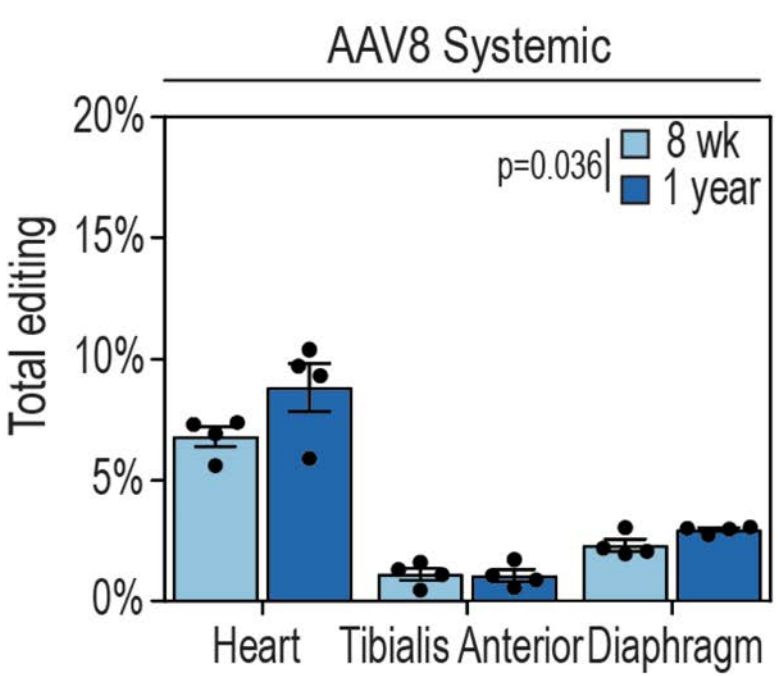
Characterizing efficiency and safety

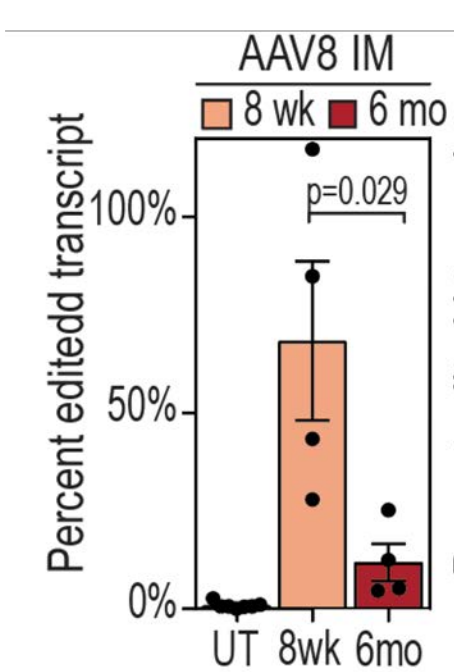
Genome editing is sustained for one year in systemically administered neonates

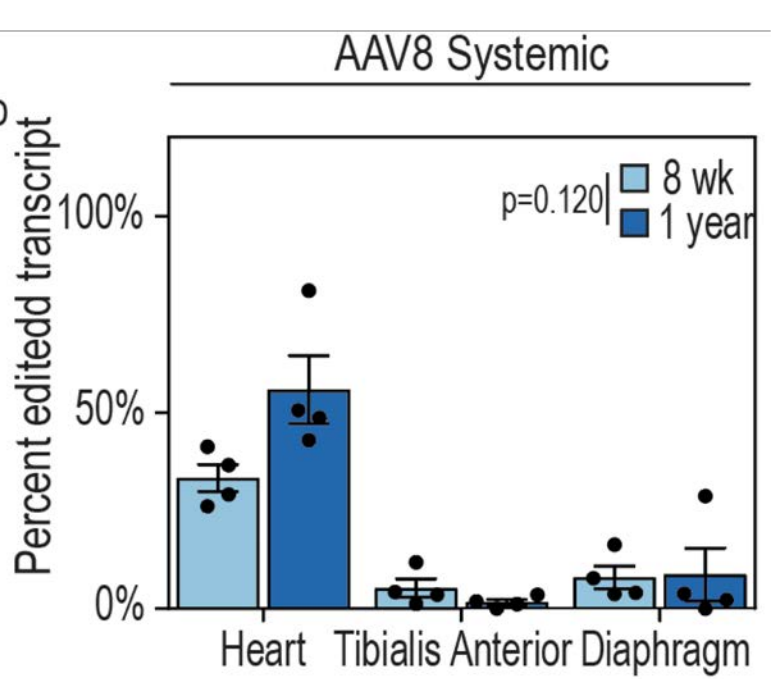




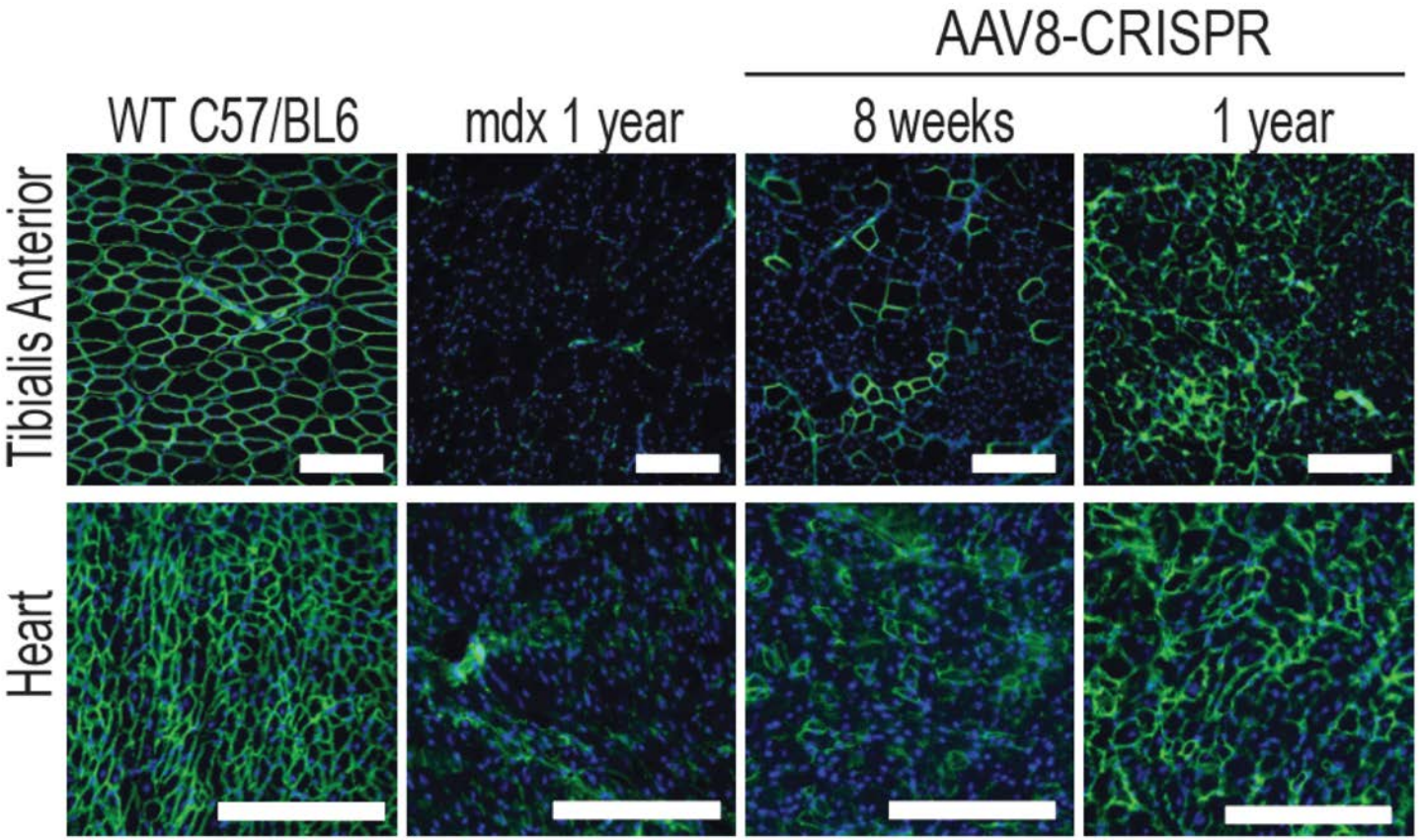


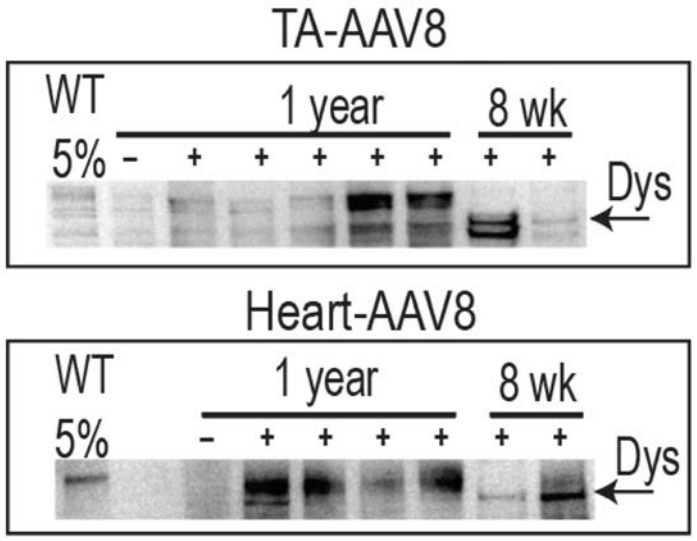


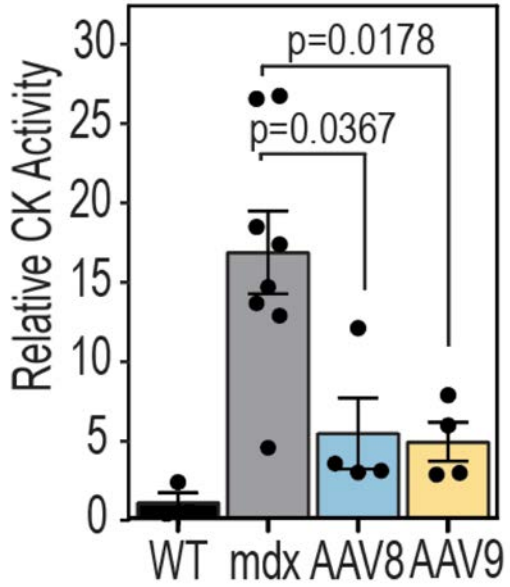




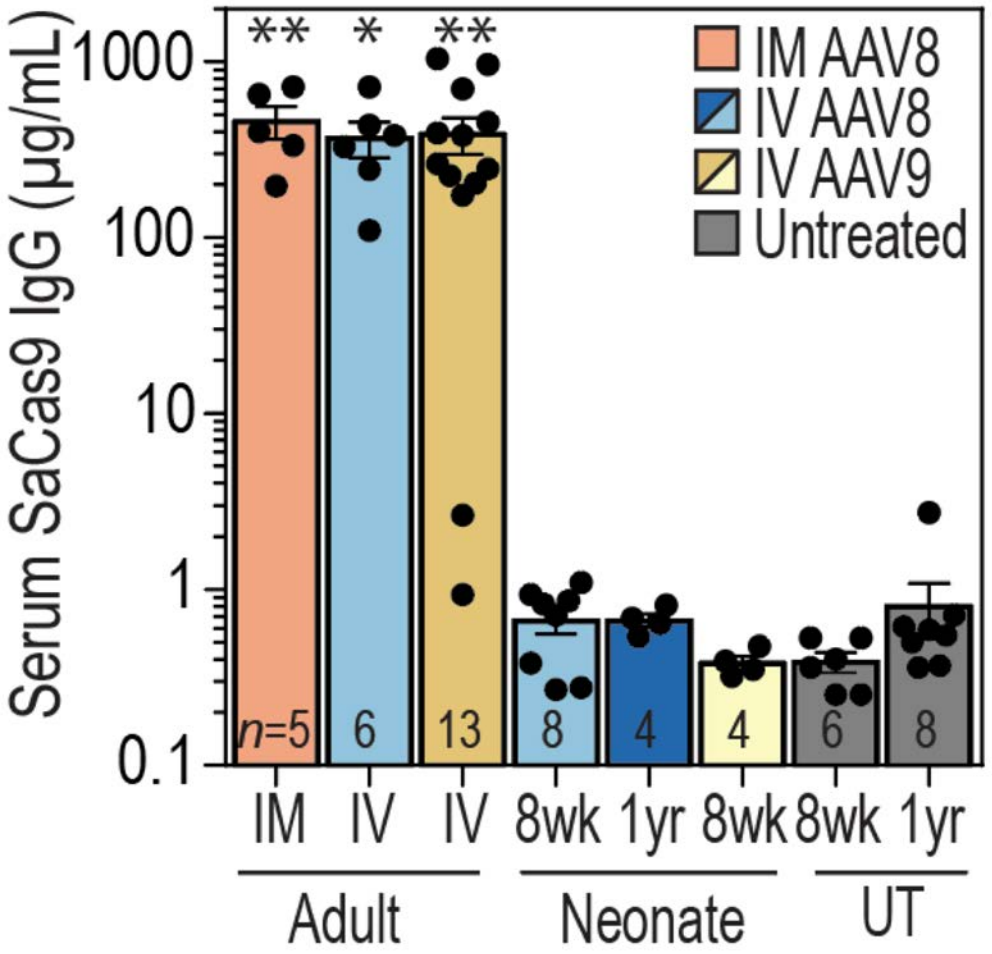
Dystrophin is restored and sustained for one year in systemically administered neonates

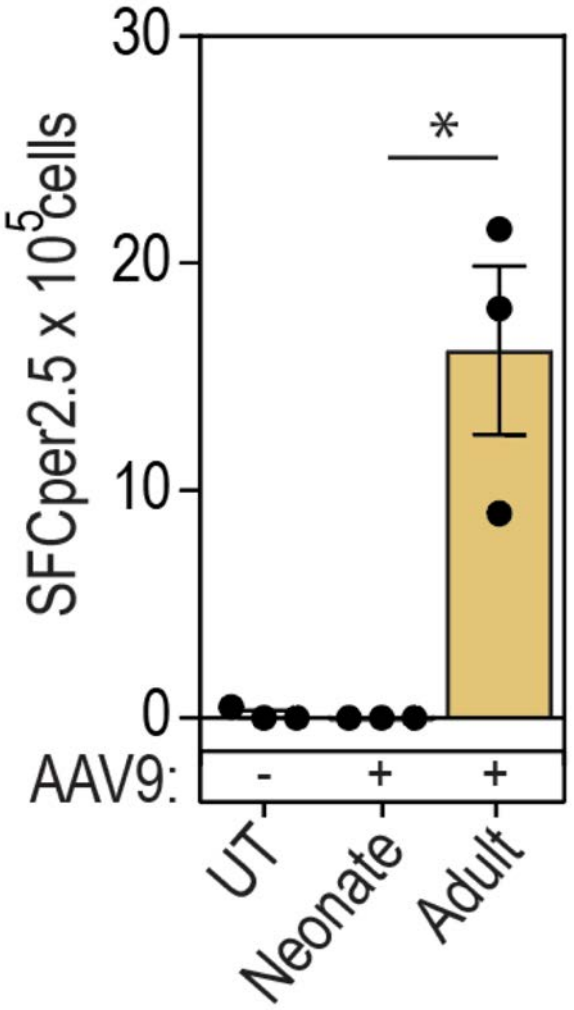




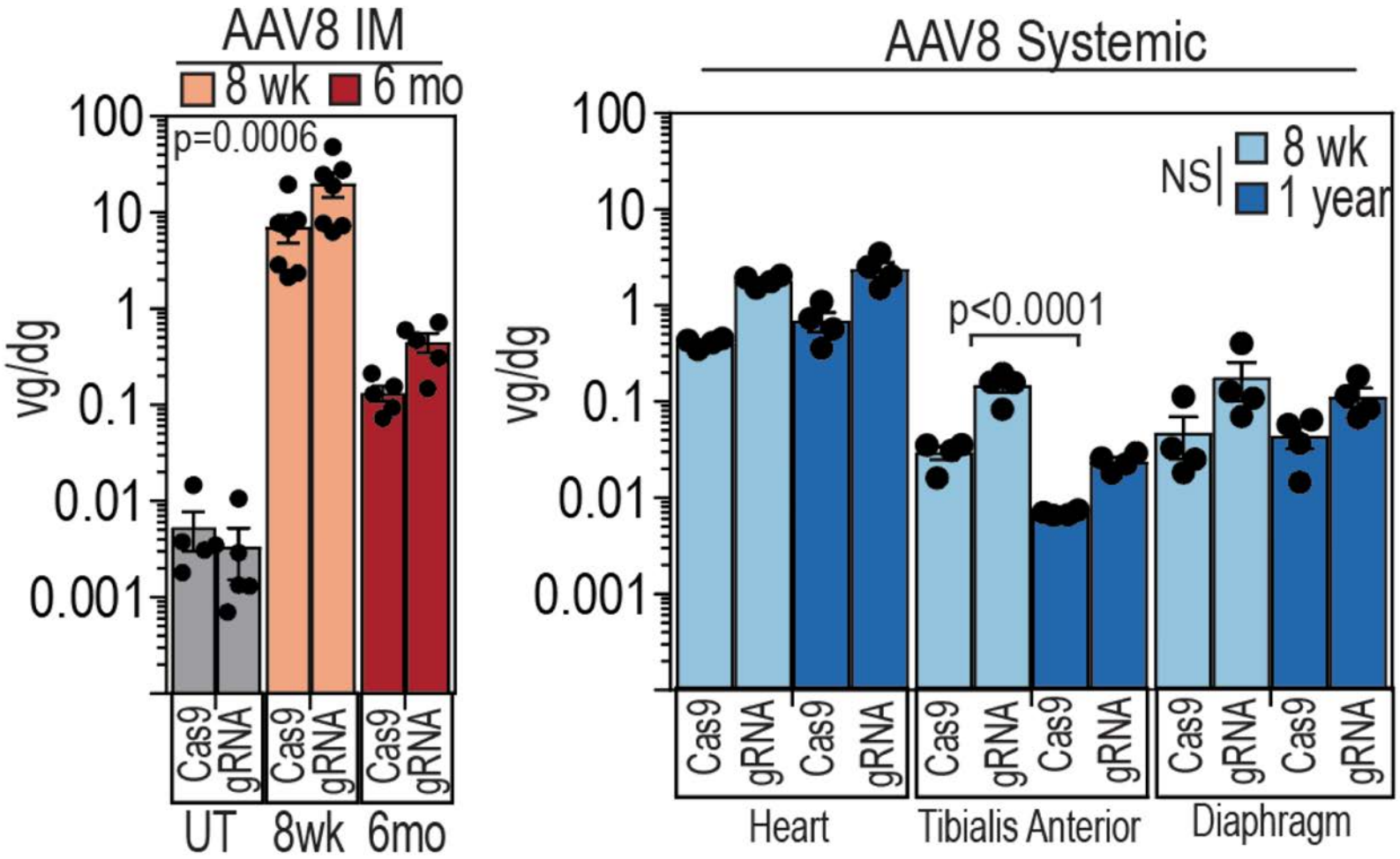


Humoral and cellular immune response detected in mice administered as adults but not neonates

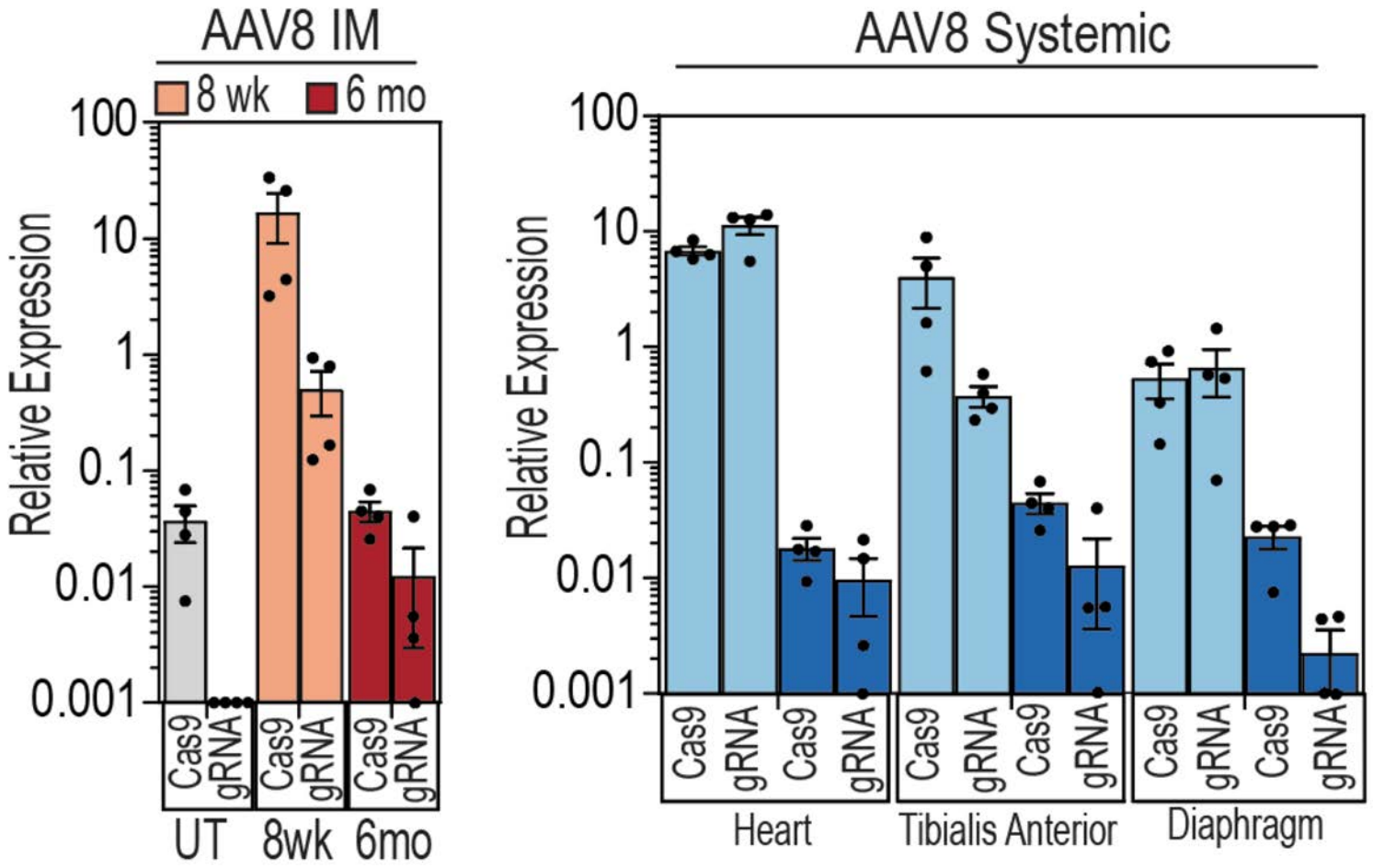




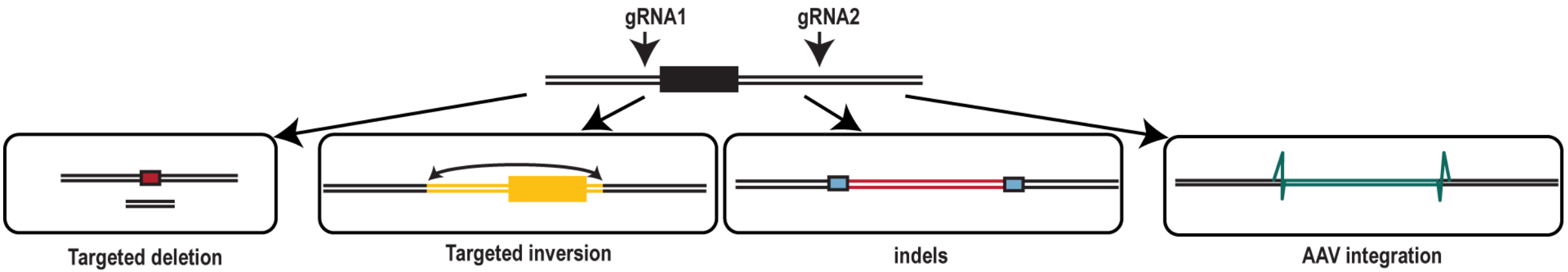
Viral vector is lost in skeletal muscle but not cardiac muscle or diaphragm



Cas9 and gRNA expression at later time points

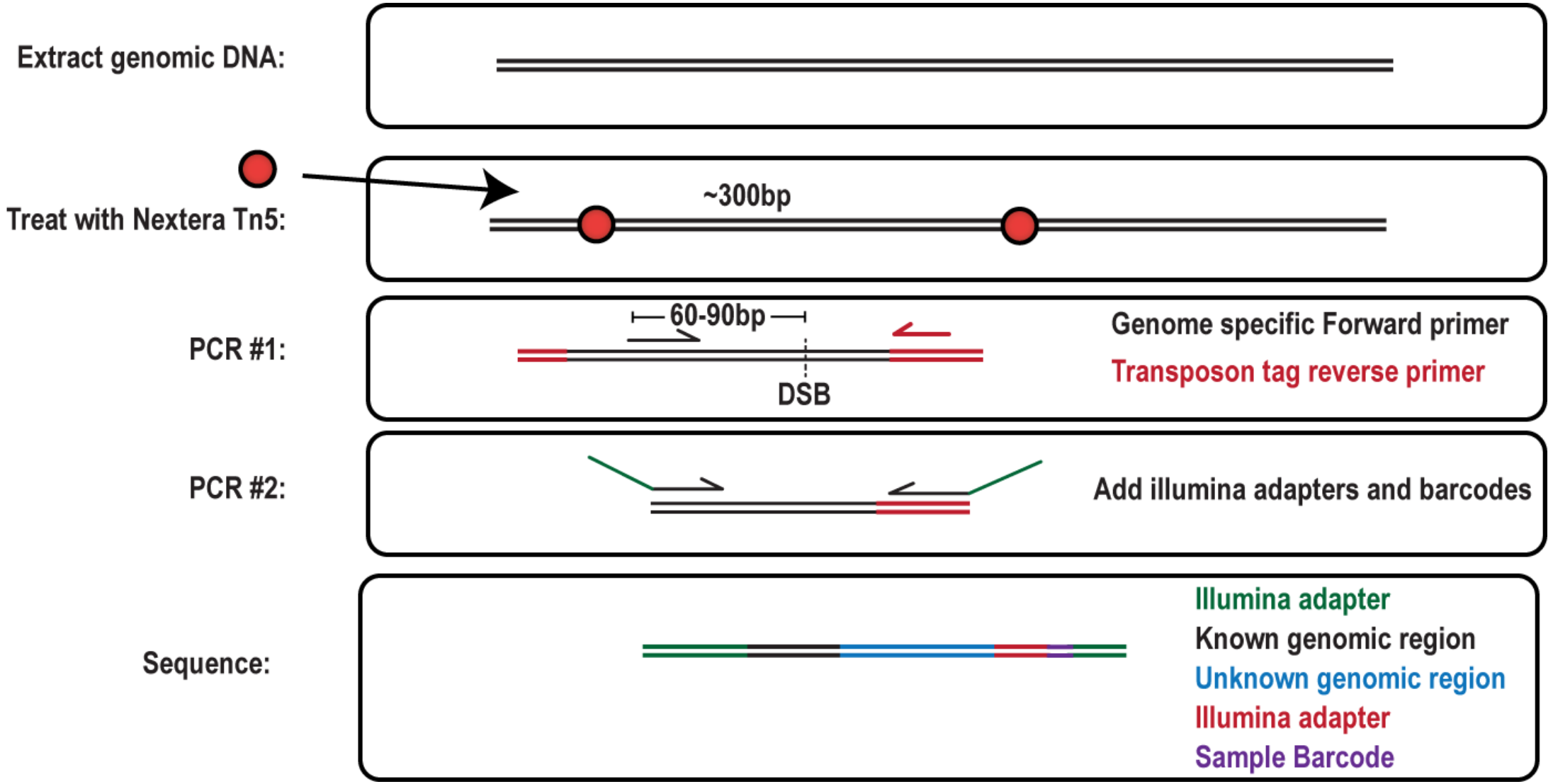


Heterogenous genome modifications induced by multiplex genome editing

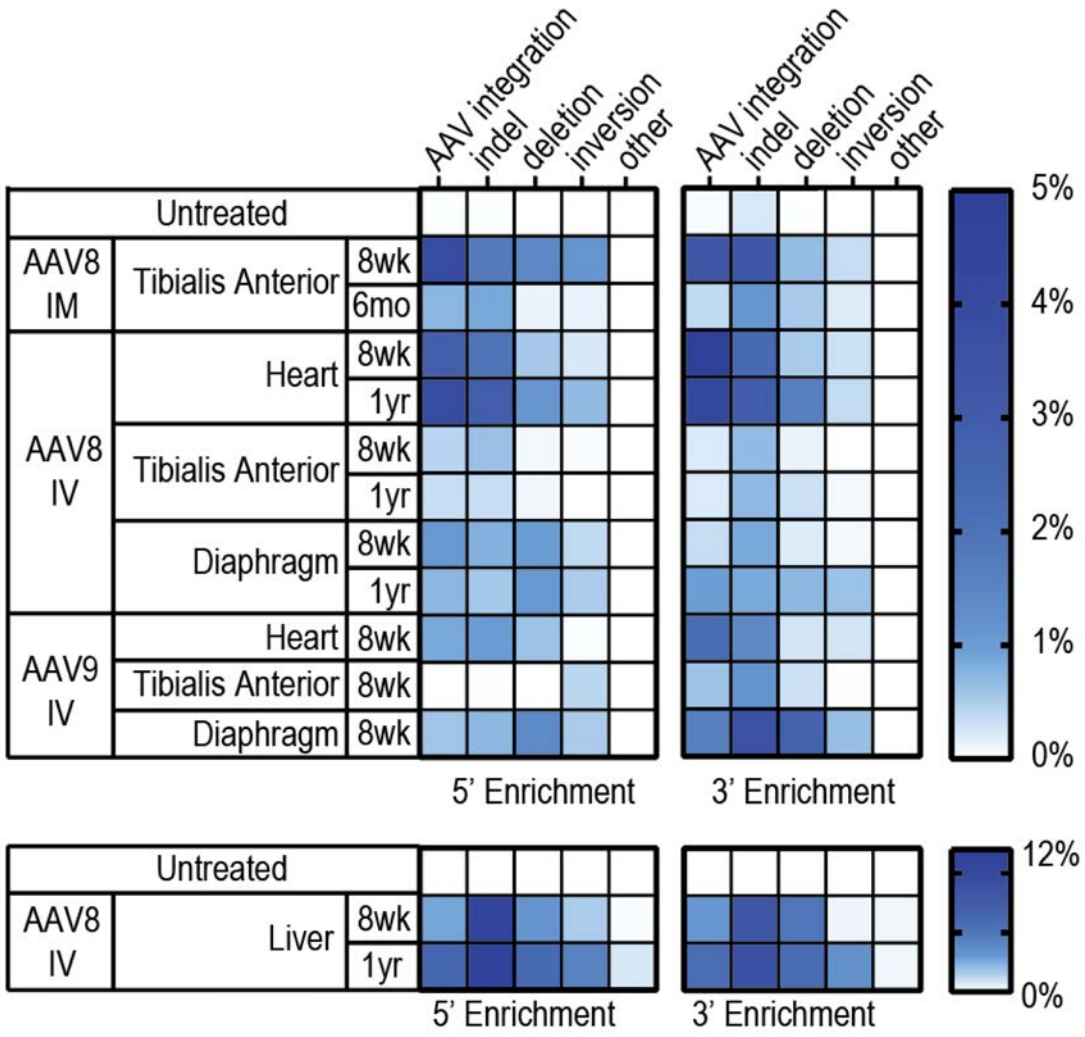


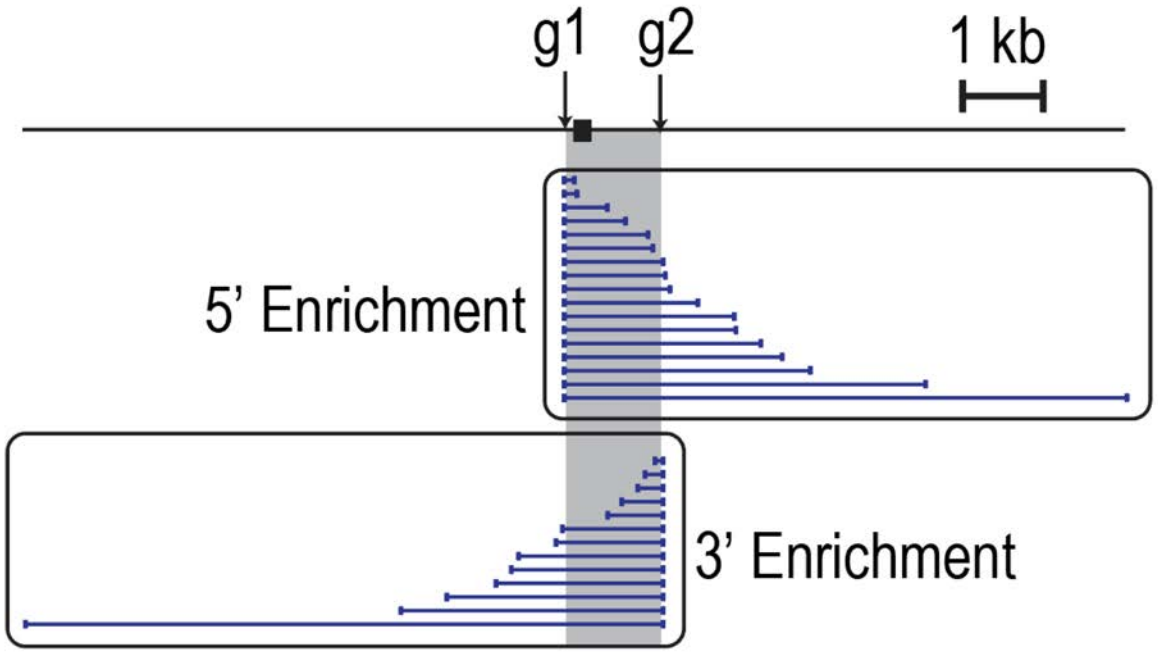
Quantitative, unbiased, sensitive to alternate events

Custom sequencing method to detect heterogenous modifications



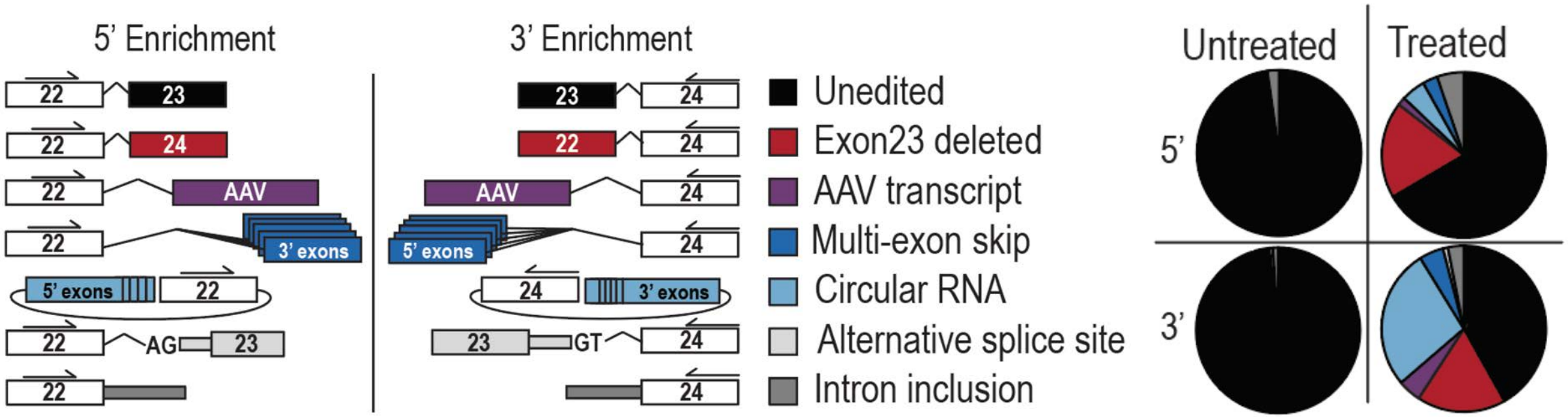
Deep sequencing shows sustained genome editing and heterogenous genome editing events



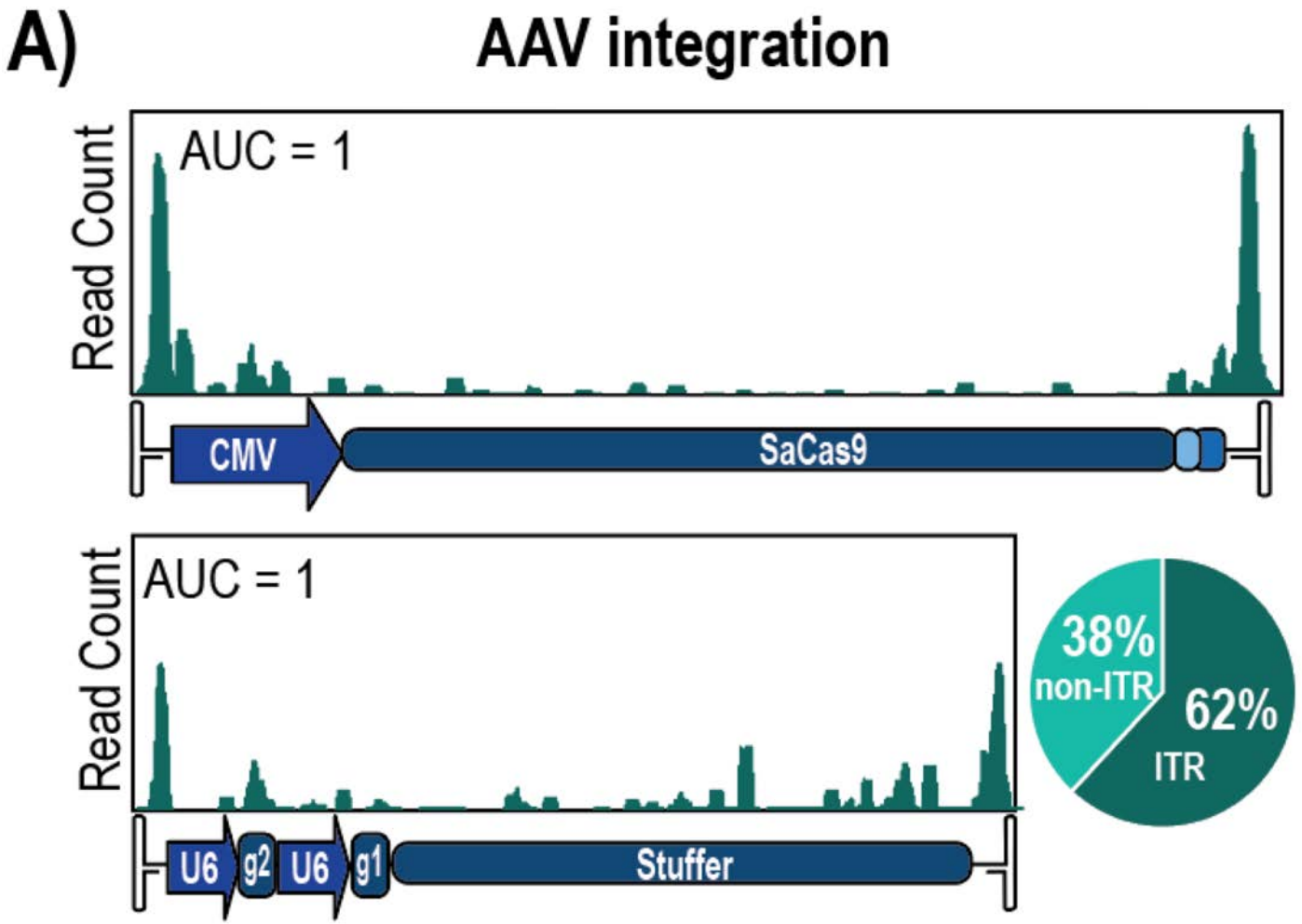


***Only detected in liver**

Heterogenous transcript modifications were detected in mice treated with AAV-CRISPR

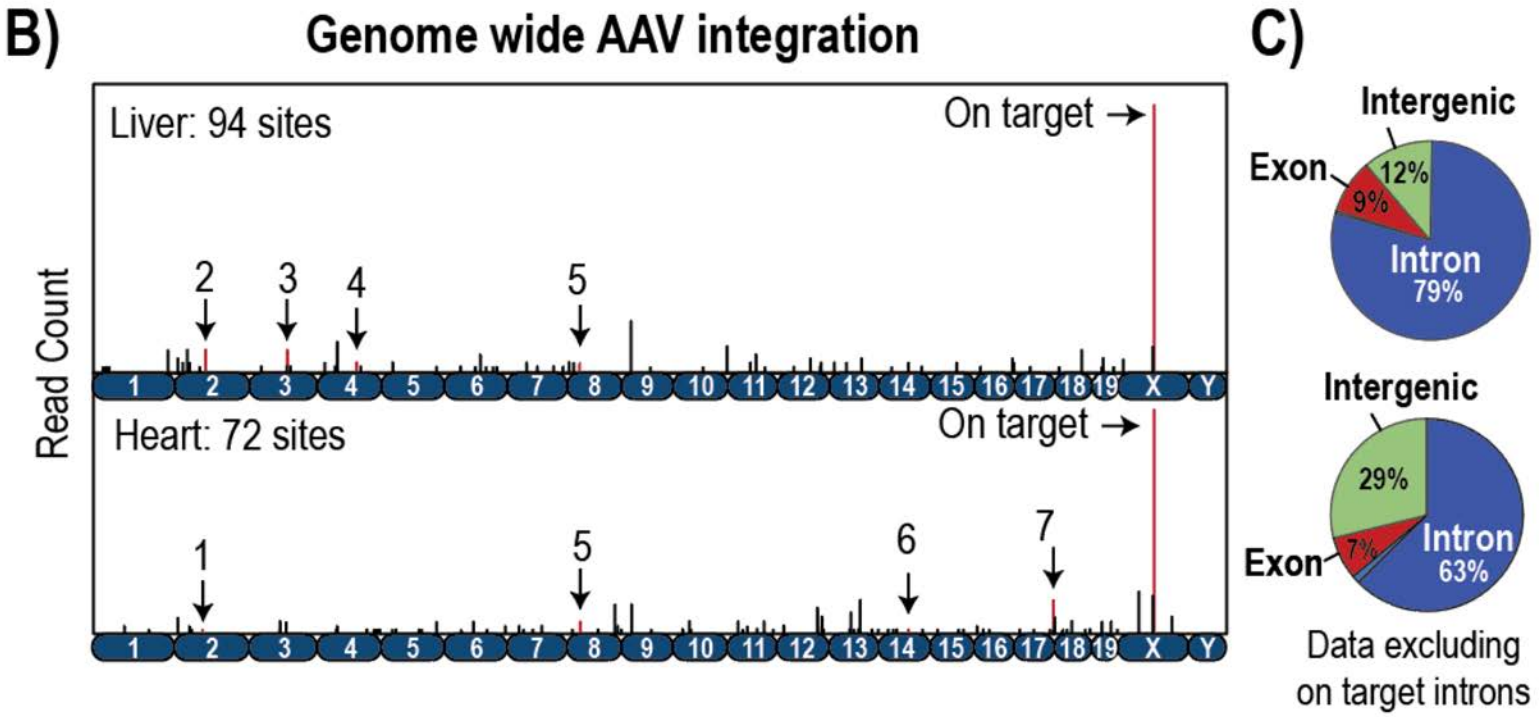


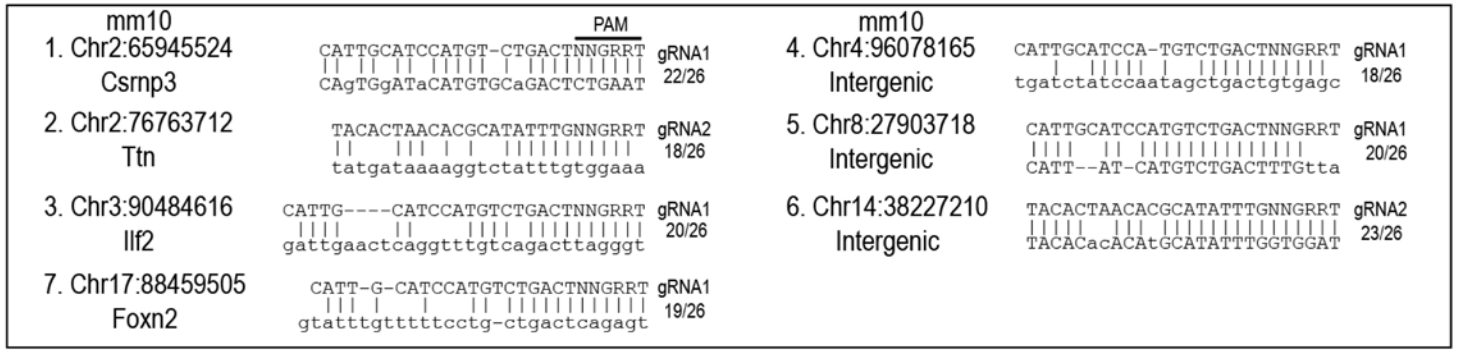
AAV integrations into CRISPR target site similar to canonical integration*



*Miller, D.G., Petek, L.M., and Russell, D.W. (2004). Adeno-associated virus vectors integrate at chromosome breakage sites. Nat Genet 36, 767-773.

AAV integrates genome wide nearby putative CRISPR off-target sites





Key points

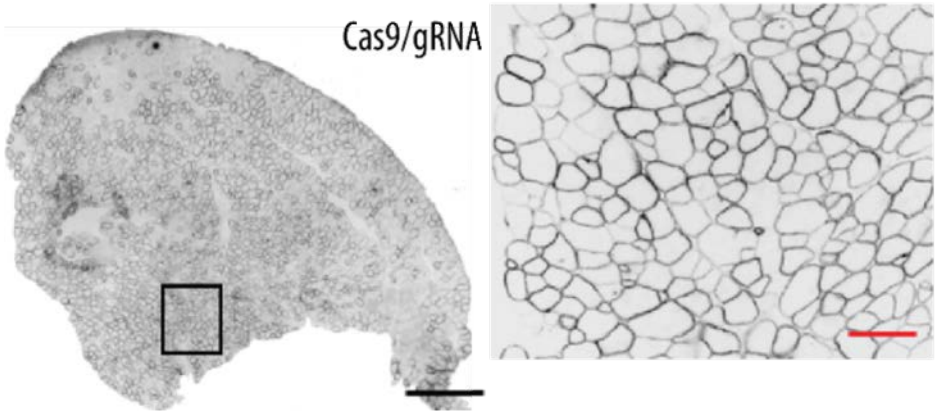
Method could be used to map genome wide off targets.

Also detects WT AAV integration.

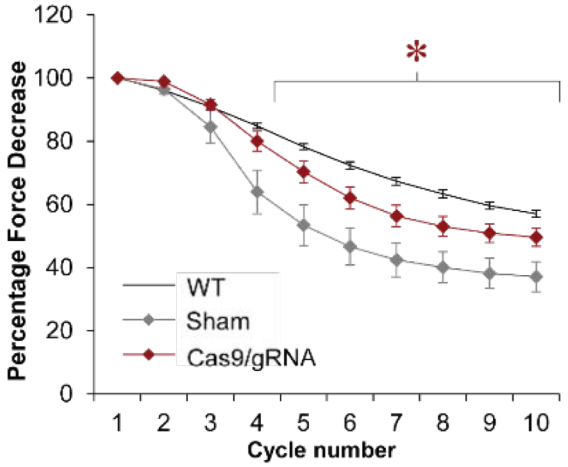
Follow up with deep sequencing

Conclusions

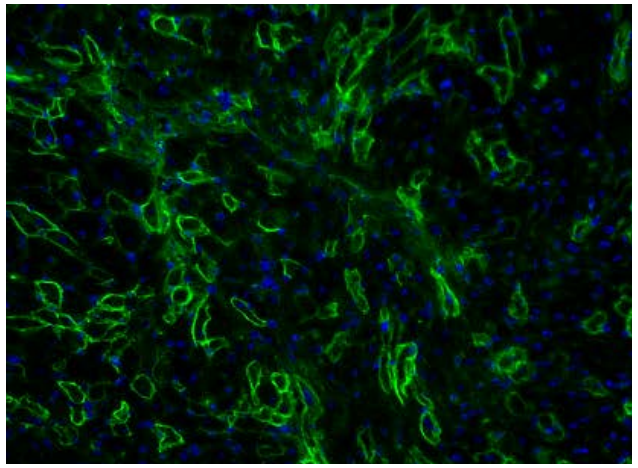
Improvements in muscle biochemistry



Improvements in muscle function



Sustained systemic gene editing



Cardiac tissue

Ongoing efforts:

1. Optimize delivery and efficiency
2. Characterizing cellular immunity
3. Genome-wide off-target effects

Acknowledgments

Collaborating labs

Asokan Lab (UNC) | Duan Lab (Missouri) | Zhang Lab (MIT)

Funding Support

SAREPTA THERAPEUTICS

Congressionally Directed Medical Research Programs CDMRP Department of Defense

DMDRP MD140071

NATIONAL INSTITUTES OF HEALTH

R01AR069085

Postdoctoral Support

NIH National Institute of Biomedical Imaging and Bioengineering

K99/R00: EB023979

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CRS Controlled Release Society