Second generation micro-dystrophin
(2nd generation μDys AAV vector)

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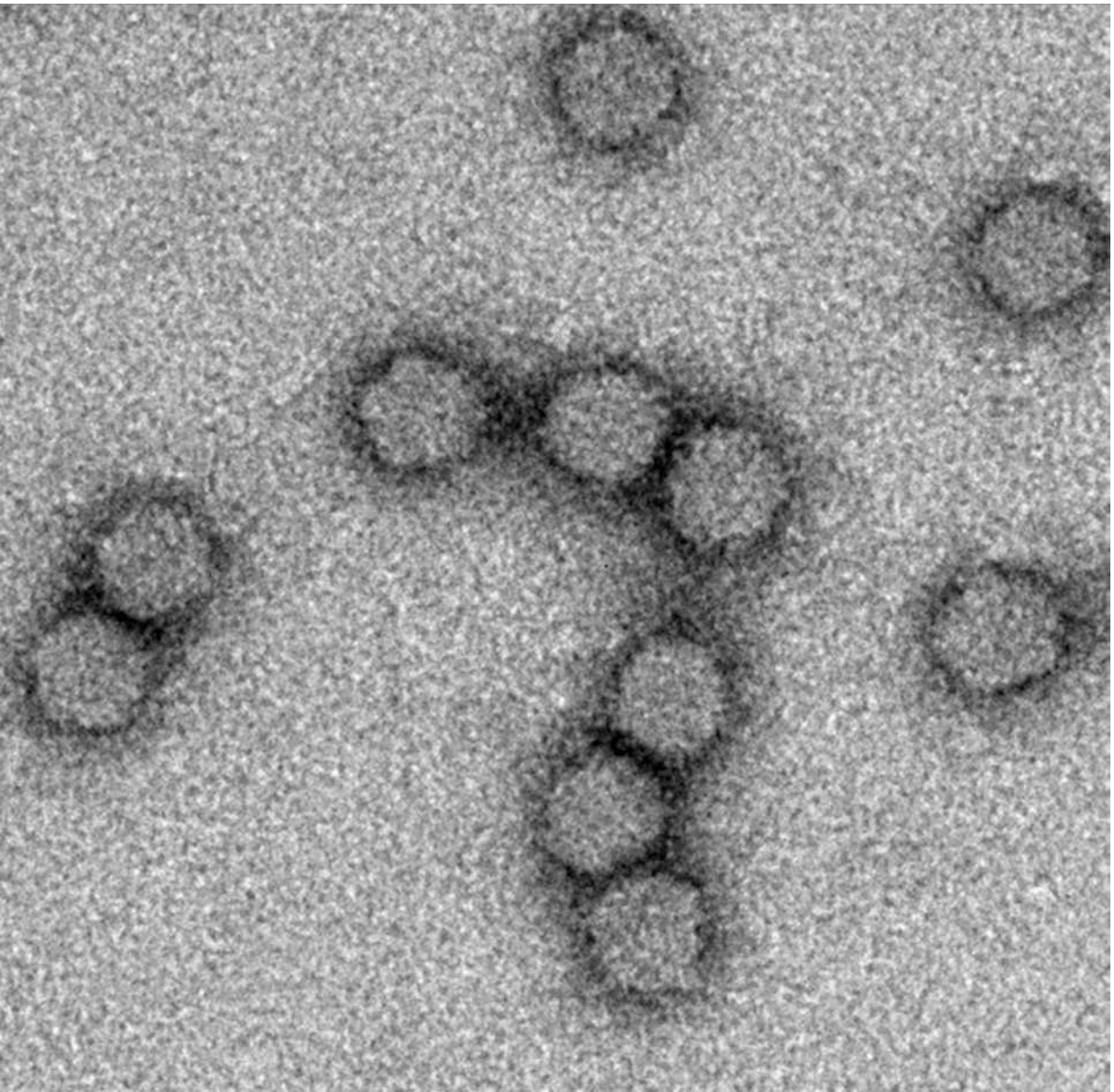
Orlando, FL, USA

Disclosure

Solid GT, LLC

- Member, Scientific Advisory Committee
- Equity holder
- Sponsored research contractor
- Patent licensed to Solid

Anatomy of Adeno-Associated Virus (AAV)



20-25 nm
4.5 kb genome
Smallest DNA virus

Anatomy of Adeno-Associated Virus (AAV)

Where is AAV?

20-25 nm

4.5 kb genome

Smallest DNA virus

Anatomy of Adeno-Associated Virus (AAV)

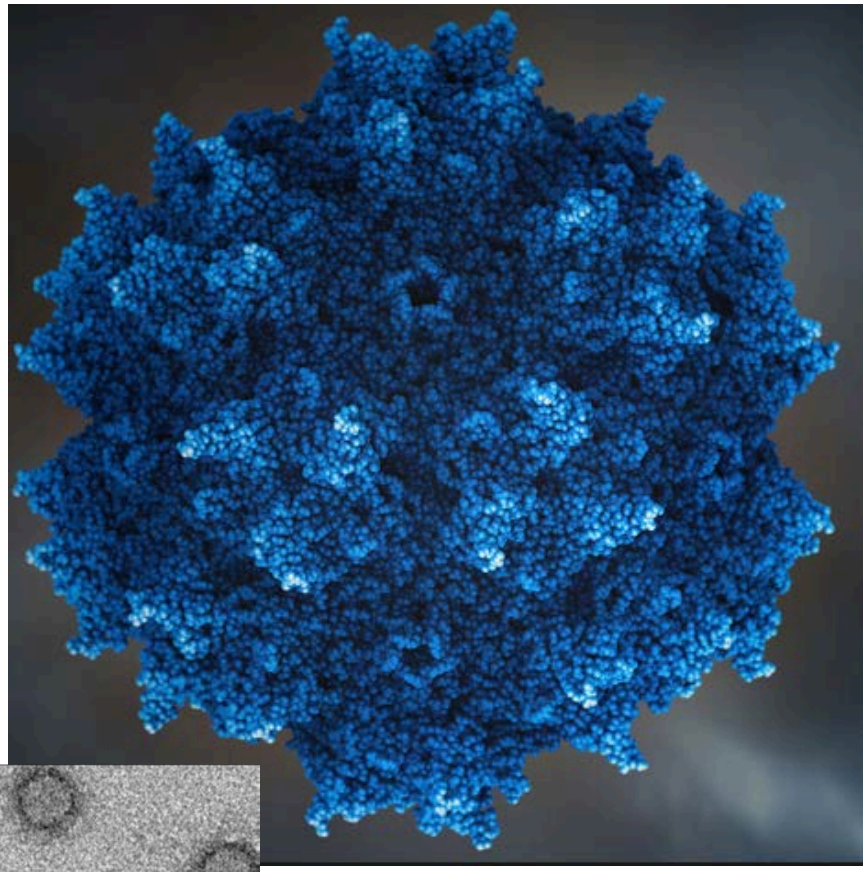
Where is AAV?

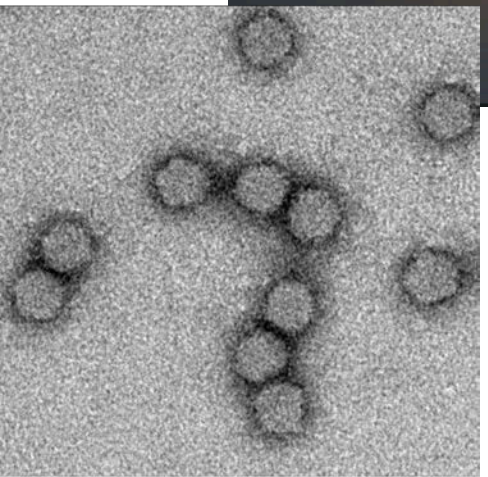
20-25 nm

4.5 kb genome

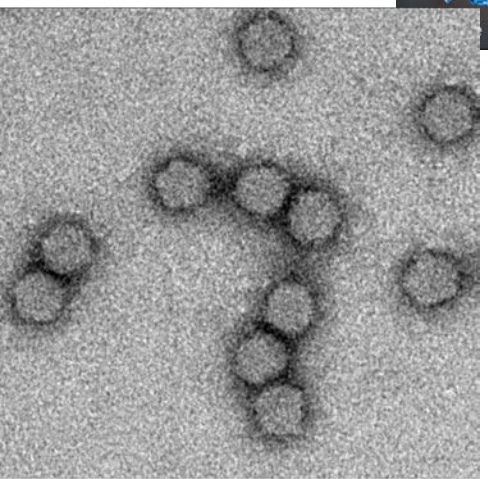
Smallest DNA virus

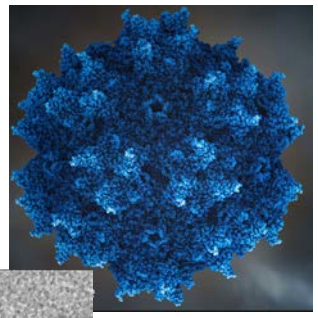
Anatomy of Adeno-Associated Virus (AAV)

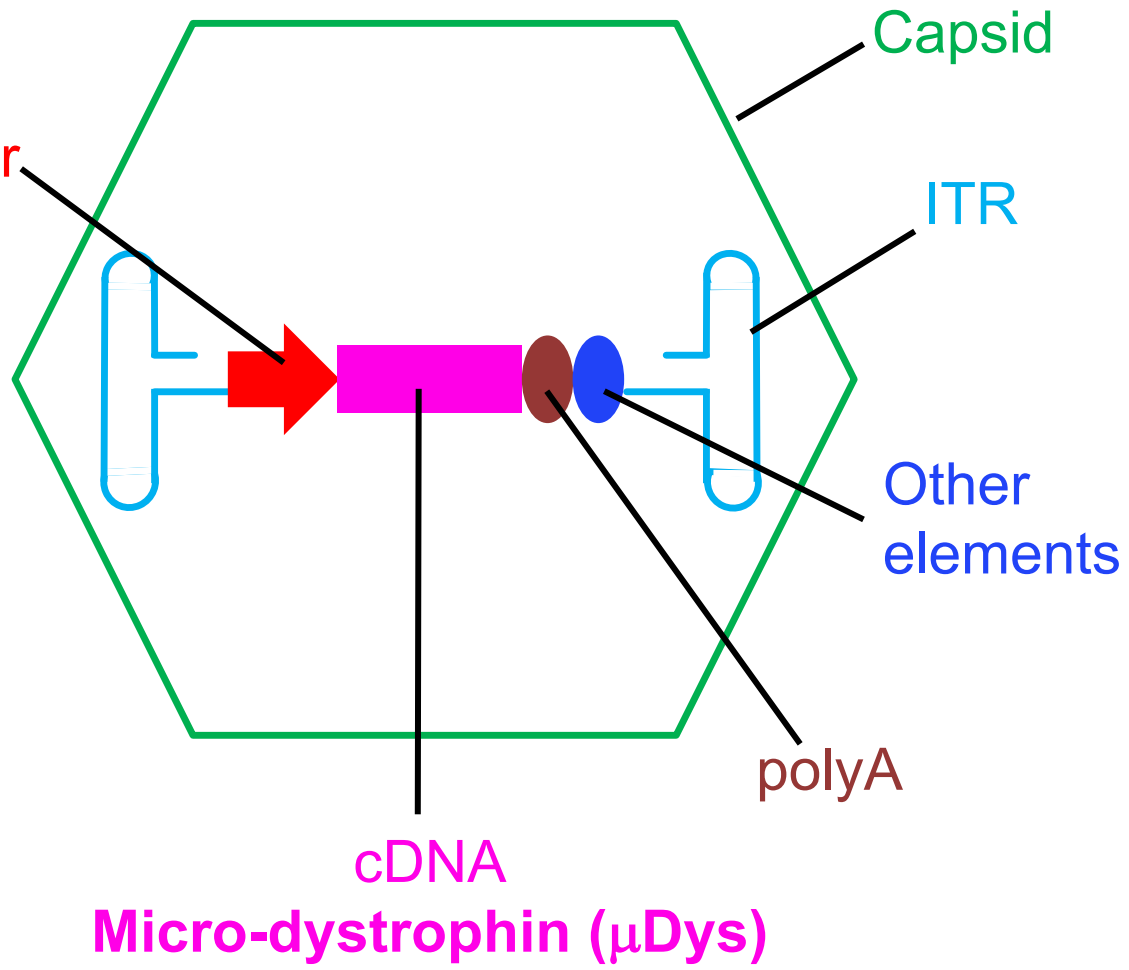




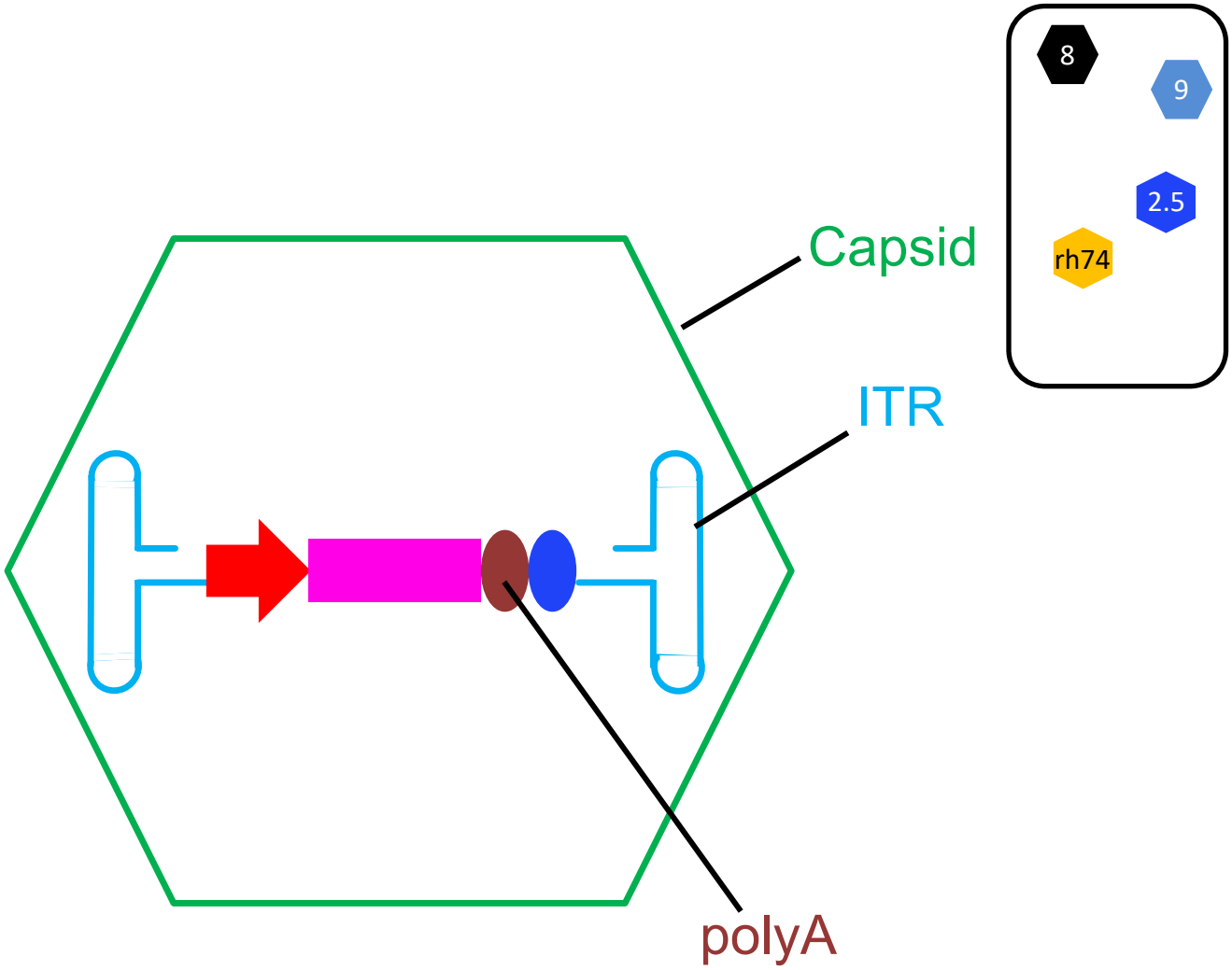
Anatomy of Adeno-Associated Virus (AAV)





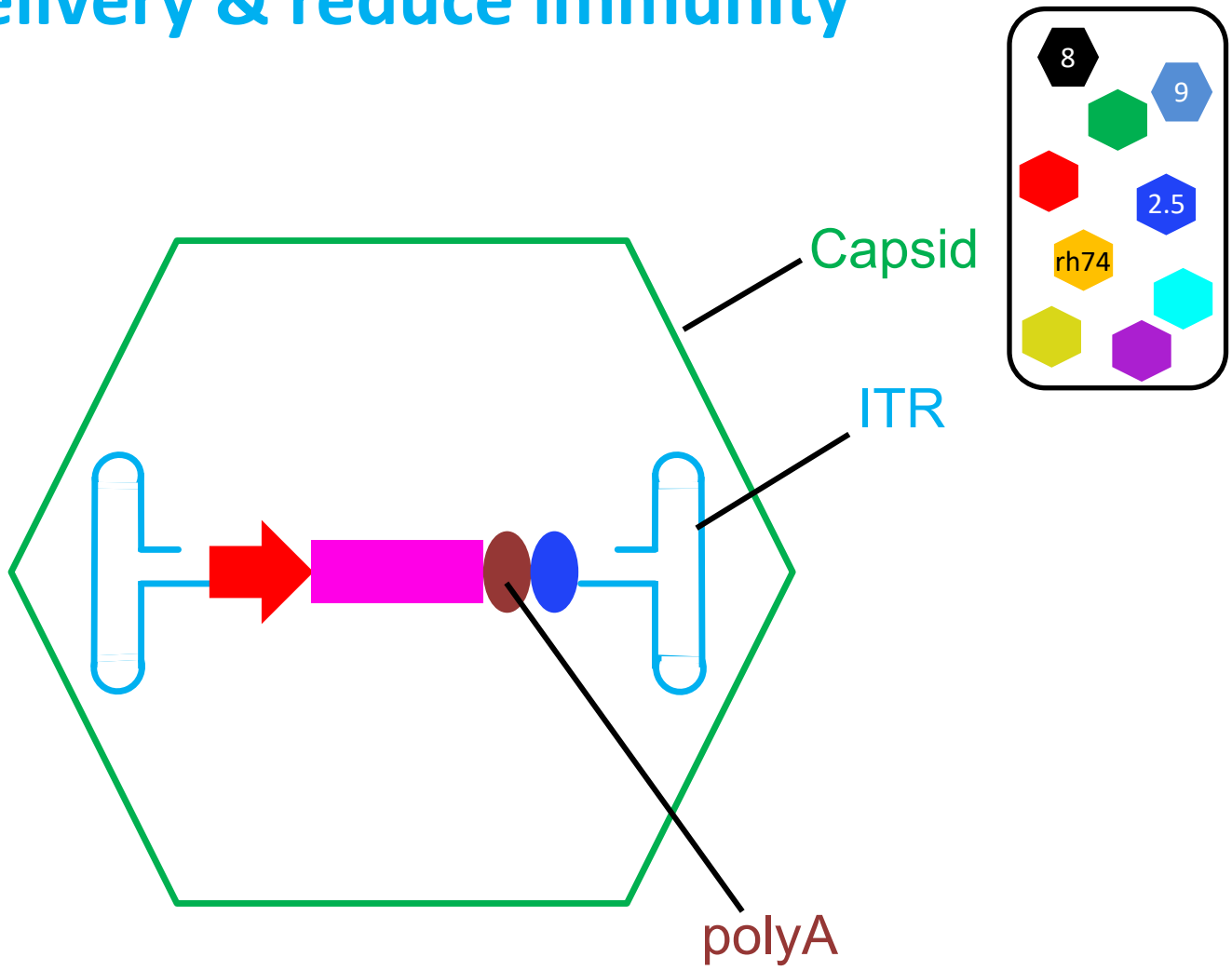


Strategies to improve AAV μDys vector



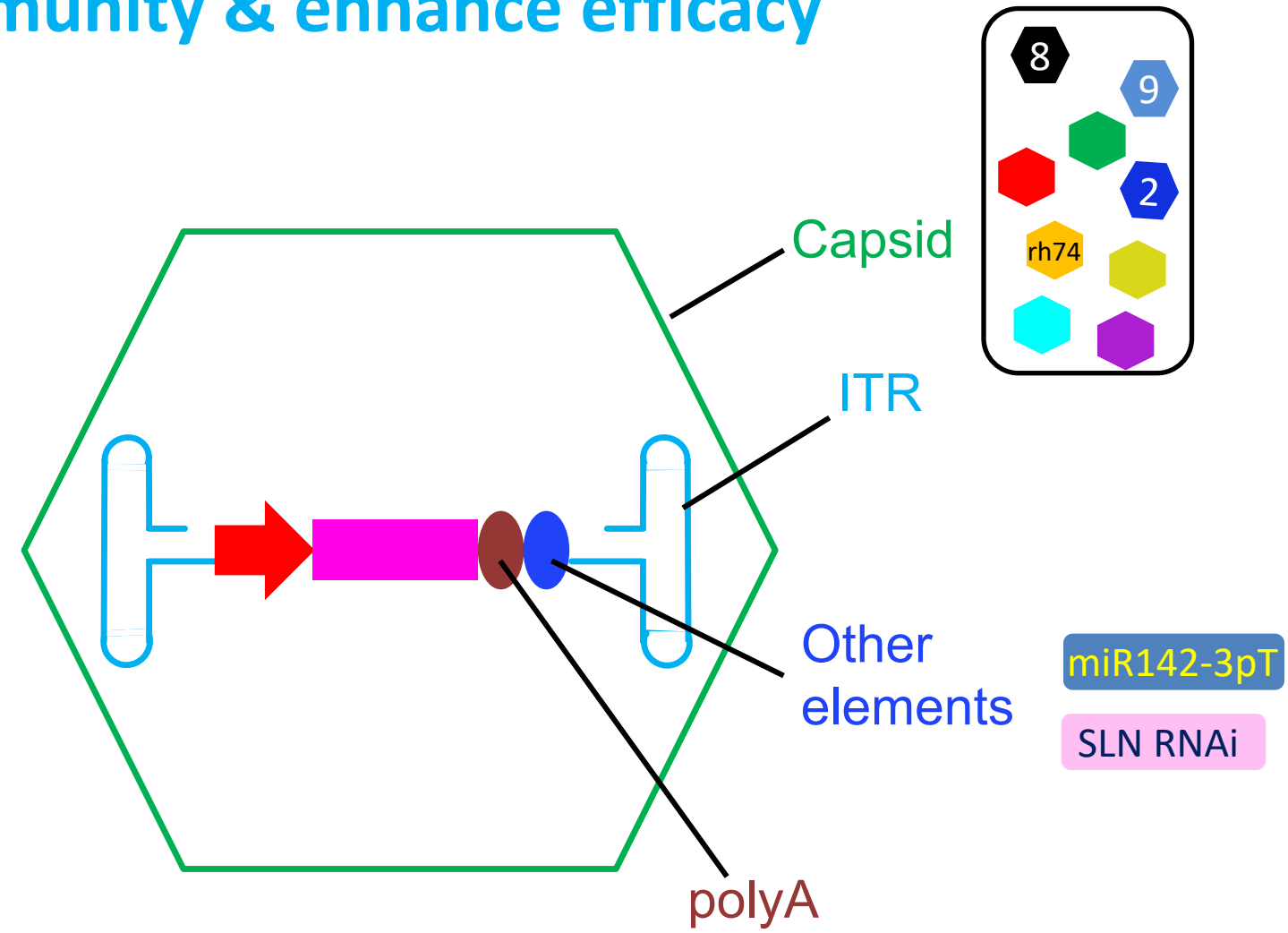
Strategies to improve AAV μDys vector

Improve delivery & reduce immunity



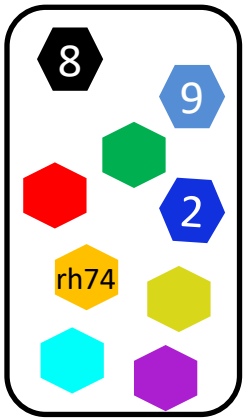
Strategies to improve AAV μDys vector

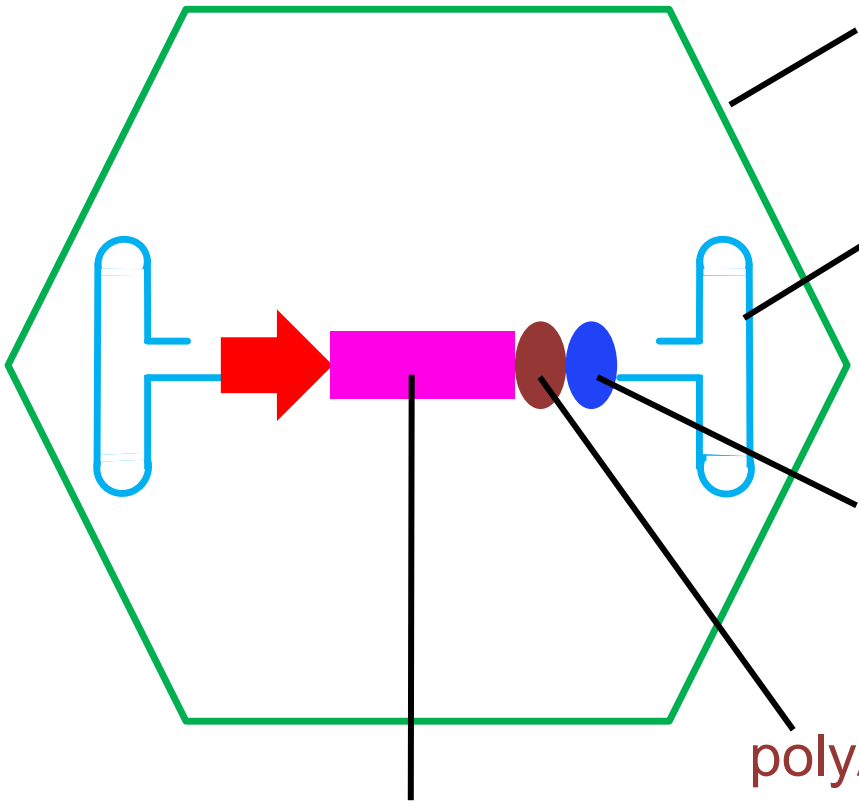
Reduce immunity & enhance efficacy



Strategies to improve AAV μDys vector

Increase expression & reduce immunity





miR142-3pT

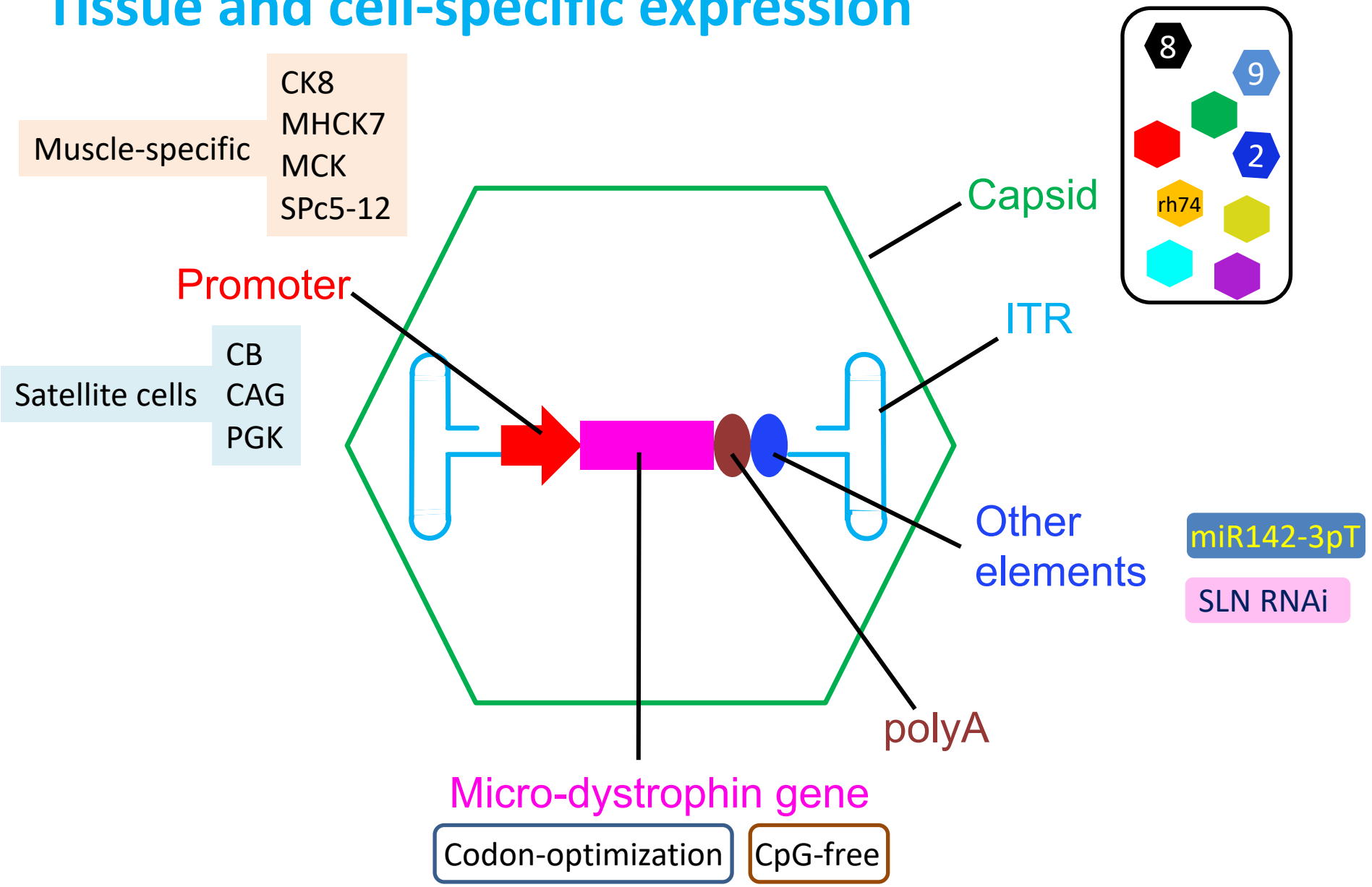
SLN RNAi

Codon-optimization

CpG-free

Strategies to improve AAV μDys vector

Tissue and cell-specific expression



AAV vector optimization

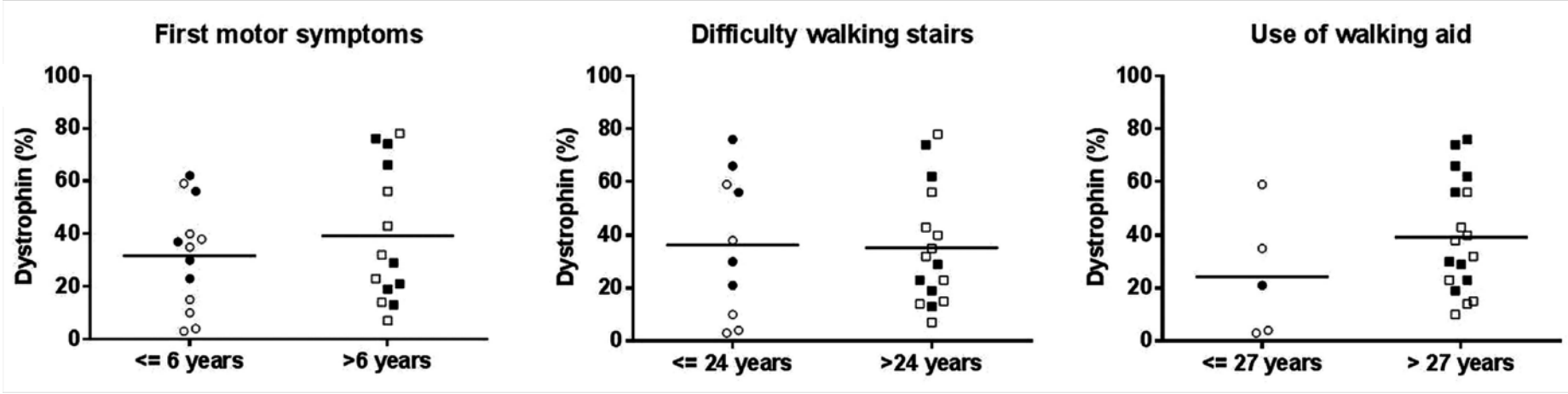
- enhances targeted delivery to muscle, heart and muscle stem cells.
- reduces immunogenicity of the capsid, micro-gene, and micro-dystrophin protein.
- increases micro-dystrophin expression level and duration.
- **But** does not improve micro-dystrophin quality.

Quantity and **quality** of dystrophin are both important for DMD gene therapy.

Once the dystrophin level reaches a threshold, **quality** becomes more important than quantity.

Dystrophin levels and clinical severity in Becker muscular dystrophy patients

J C van den Bergen,[1] B H Wokke,[1] A A Janson,[2] S G van Duinen,[3] M A Hulsker,[4] H B Ginjaar,[5] J C van Deutekom,[2] A Aartsma-Rus,[4] H E Kan,[6] J J Verschuuren[1]



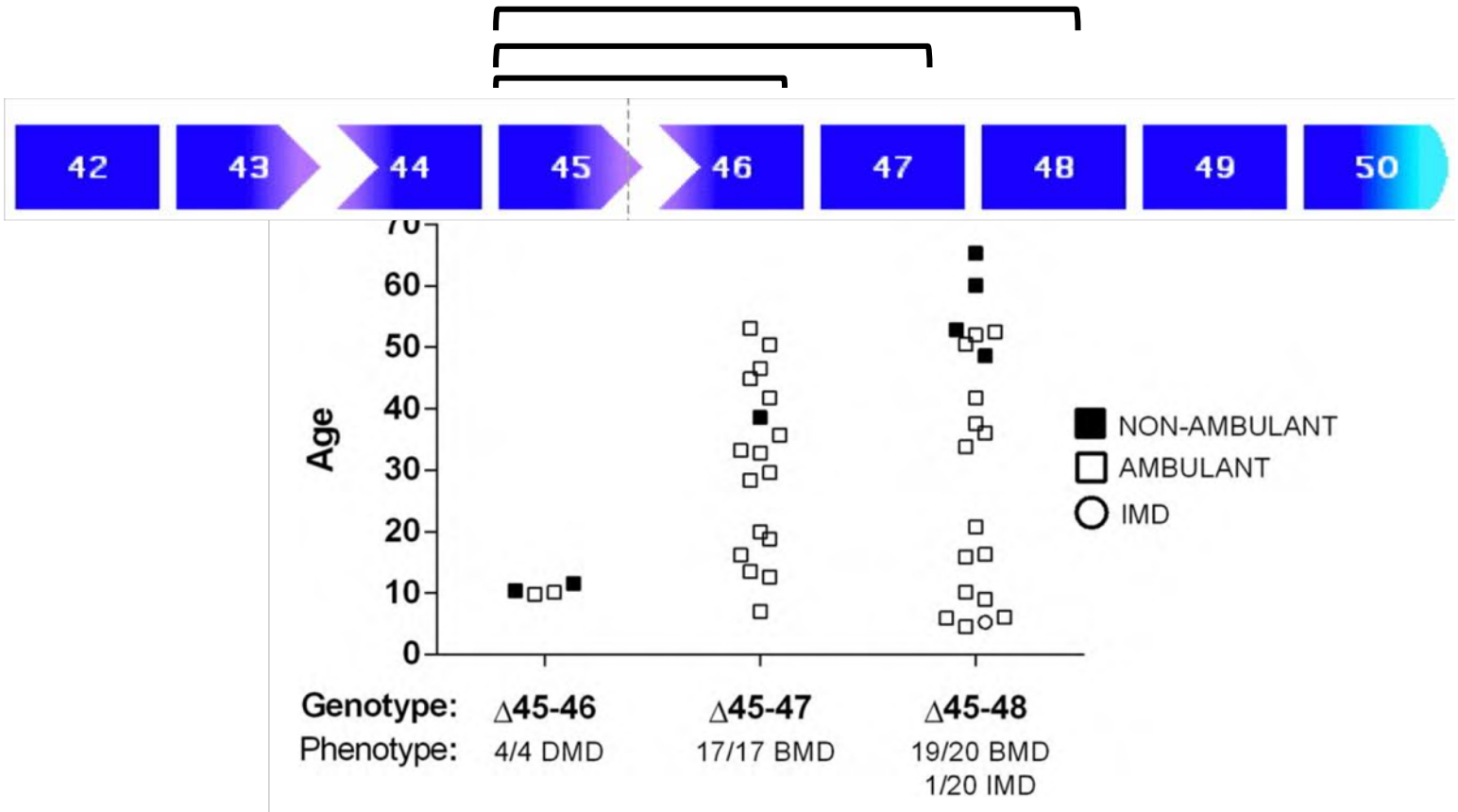
Conclusions: Our study shows that **dystrophin levels appear not to be a major determinant of disease severity in BMD**, **as long as it is above approximately 10%.** A significant relation between age and disease course was only found in the exon 45–47 deletion subgroup. This suggests that at higher dystrophin levels, **the disease course depends more on the mutation site than on the amount of the dystrophin protein produced.**

van den Bergen JC, *et al. J Neurol Neurosurg Psychiatry* 2013;

Clinical Phenotypes as Predictors of the Outcome of Skipping around *DMD* Exon 45

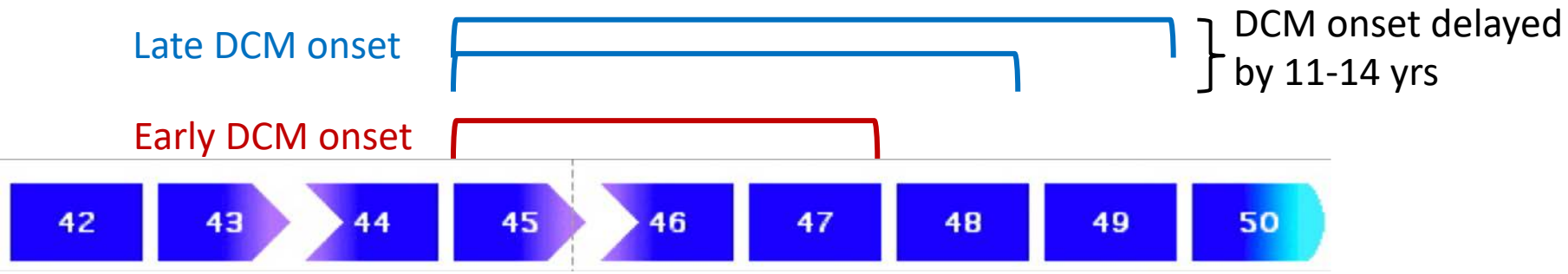
ANN NEUROL 2015;77:668–674

Andrew R. Findlay, M.D.,[1] Nicolas Wein, Ph.D.,[1] Yuuki Kaminoh, B.S.,[1]
Laura E. Taylor, B.S.,[1] Diane M. Dunn, B.S.,[2] Jerry R. Mendell, M.D.,[1,3,4]
Wendy M. King, P.T.,[4] Alan Pestronk, M.D.,[5] Julaine M. Florence, D.P.T., Ph.D.,[5]
Katherine D. Mathews, M.D.,[6] Richard S. Finkel, M.D.,[7]
Kathryn J. Swoboda, M.D.,[8] Michael T. Howard, Ph.D.,[2]
John W. Day, M.D., Ph.D.,[9] Craig McDonald, M.D.,[10] Aurélie Nicolas, Ph.D.,[11,12]
Elisabeth Le Rumeur, Ph.D.,[11,12] Robert B. Weiss, Ph.D.,[2] and
Kevin M. Flanigan, M.D.,[1,3,4] for the United Dystrophinopathy Project



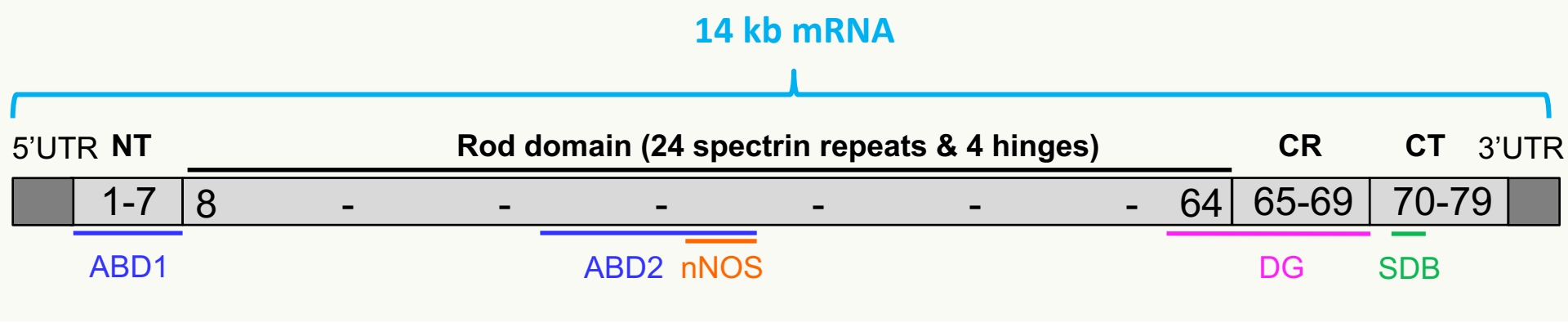
Becker muscular dystrophy severity is linked to the structure of dystrophin

Aurélie Nicolas[1,2], Céline Raguénès-Nicol[1,2], Rabah Ben Yaou[3,4,†], Sarah Ameziane-Le Hir[1,2], Angélique Chéron[1,2], Véronique Vié[1,5], Mireille Claustres[6,7,8], France Leturcq[3,9,†], Olivier Delalande[1,2], Jean-François Hubert[1,2], Sylvie Tuffery-Giraud[7,8,†], Emmanuel Giudice[1,2], Elisabeth Le Rumeur[1,2,*], and the French Network of Clinical Reference Centres for Neuromuscular Diseases (CORNEMUS)

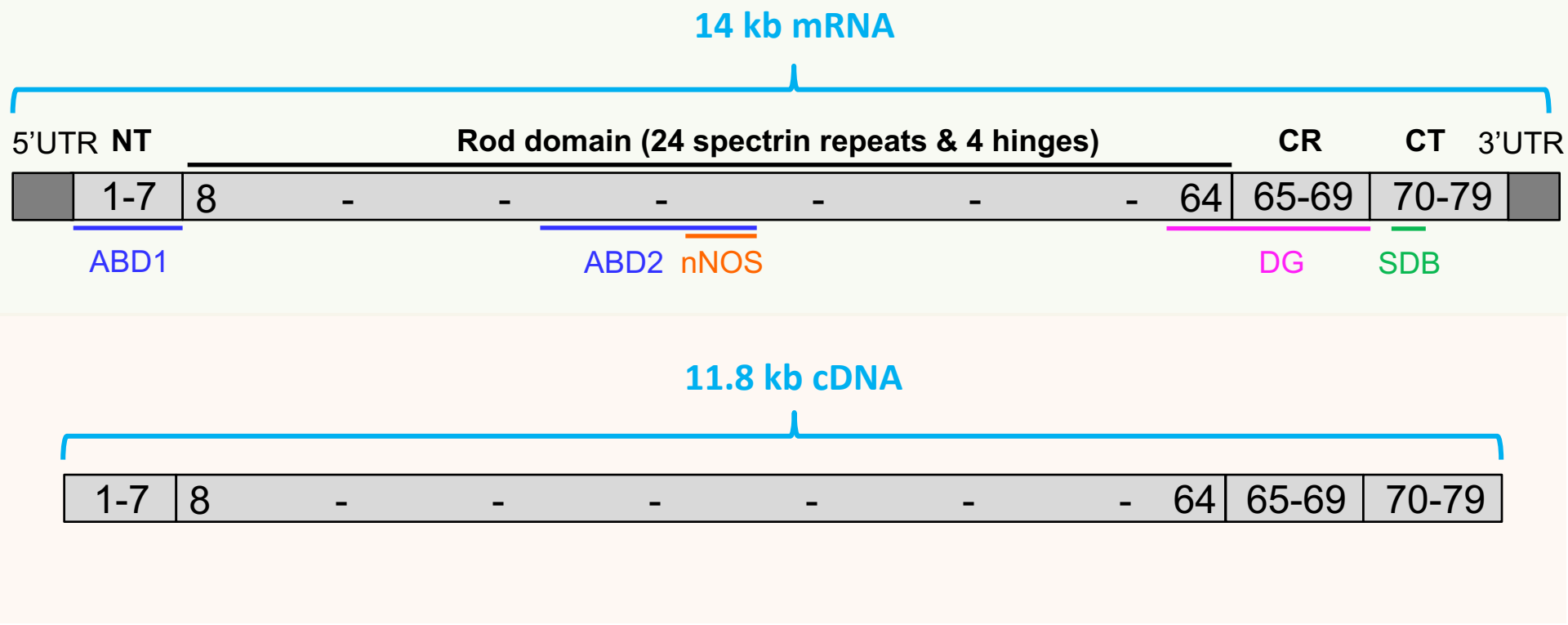


By retrospectively collecting data for a series of French BMD patients, we showed that the age of **dilated cardiomyopathy (DCM) onset was delayed by 11 and 14 years in Δ45–48 and Δ45–49 compared with Δ45–47 patients**, respectively. A clear trend toward earlier wheelchair dependency (minimum of 11 years) was also observed in Δ45–47 and Δ45–49 patients compared with Δ45–48 patients. Muscle dystrophin levels were moderately reduced in most patients without clear correlation with the deletion type. Disease progression in BMD patients appears to be **dependent on the deletion itself and associated with a specific structure of dystrophin at the deletion site**.

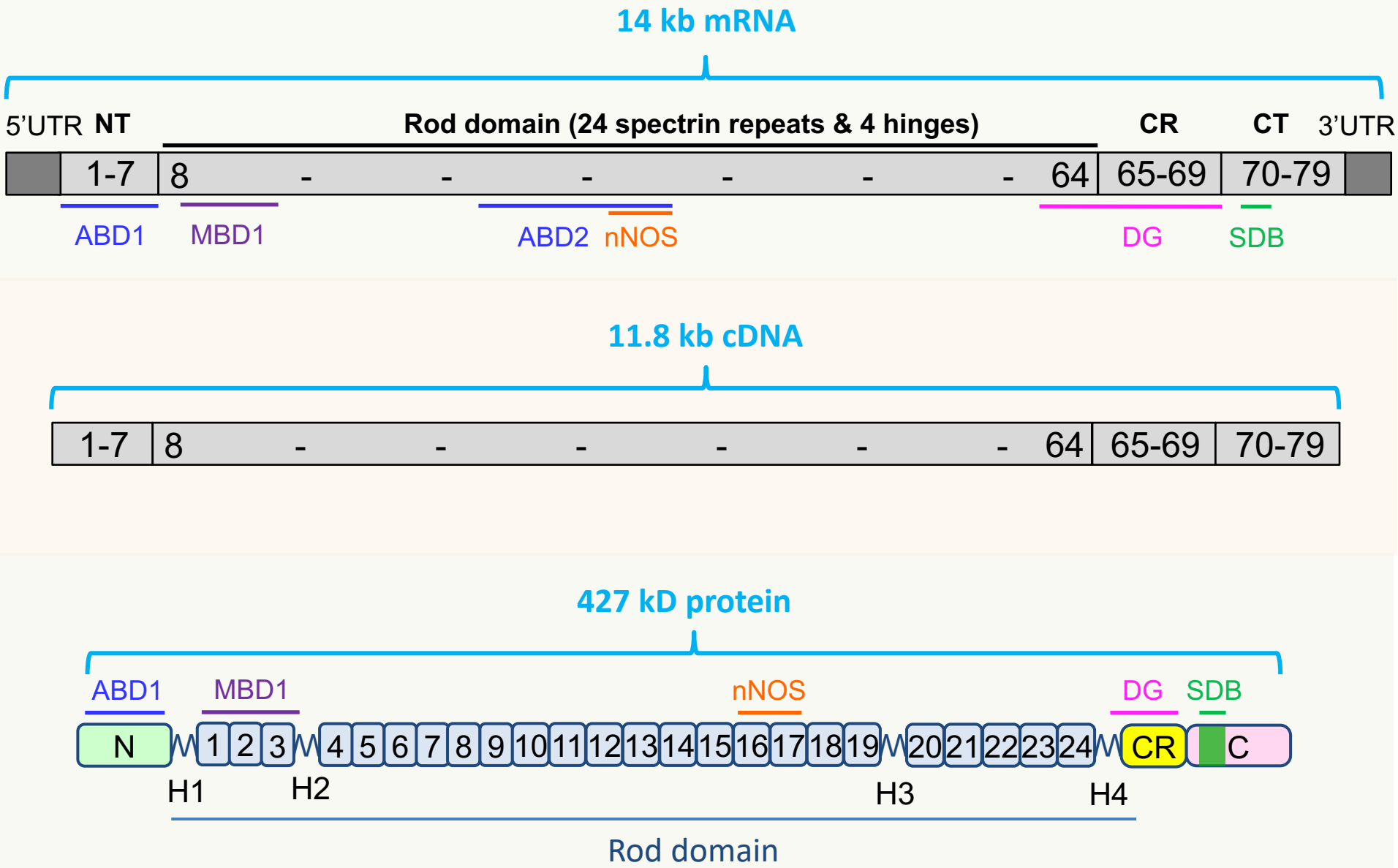
Full-length dystrophin



Full-length dystrophin



Full-length dystrophin



Full-length dystrophin vs micro-dystrophin

	Full-length dystrophin
mRNA size	14 kb
cDNA size	11.8 kb
Protein size	427 kD
Exons	76
Domains	4
Spectrin repeats	24
Hinges	4

Full-length dystrophin vs micro-dystrophin

	Full-length dystrophin	Micro-dystrophin
mRNA size	14 kb	
cDNA size	11.8 kb	≤ 4 kb (**~1/3 of full**)
Protein size	427 kD	≤ 160 kD (**~1/3 of full**)
Exons	76	
Domains	4	≤ 4
Spectrin repeats	24	≤ 6
Hinges	4	≤ 3

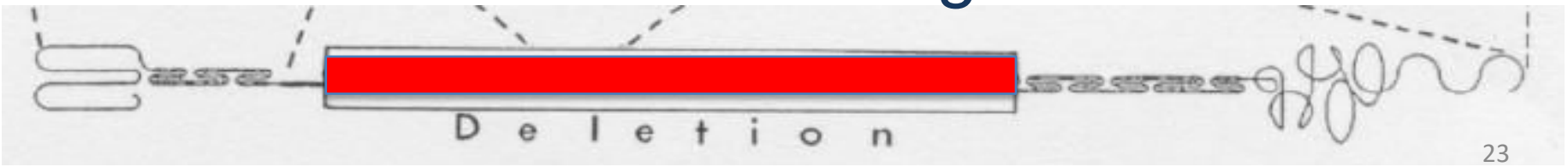
Mini-dystrophin, the era before micro-dystrophin

NATURE · VOL 343 · 11 JANUARY 1990

Very mild muscular dystrophy associated with the deletion of 46% of dystrophin

S. B. England*, L. V. B. Nicholson†, M. A. Johnson†, S. M. Forrest*, D. R. Love*, E. E. Zubrzycka-Gaarn‡, D. E. Bulman‡, J. B. Harris§ & K. E. Davies*||

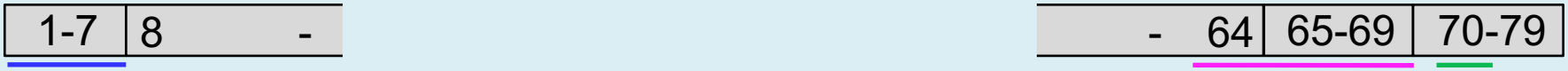
6.2 kb Δ17-48 minigene



Proband
25-yr-old
Body builder

61-yr-old relative
Walk with a stick

∆17-48 mini-gene (6.2 kb, naturally existing minigene, Davies lab, 1990)



Can protect muscle but **cannot** fit into AAV

Generation 0 μDys (≤ 2 repeats)
Can fit into AAV but cannot protect muscle

Δ17-48 mini-gene (6.2 kb, naturally existing minigene, Davies lab, 1990)

Can protect muscle but **cannot** fit into AAV

Δ10-53 micro-gene (~4 kb, naturally existing μDys gene, Angelini lab, 1996)

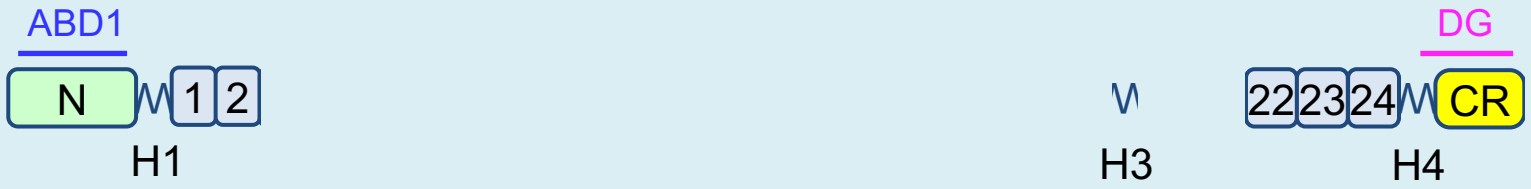
Can fit into AAV but **cannot** protect muscle

ΔDysM3 (3.7 kb, 1st synthetic μDys gene, Takeda lab, 1997)

Can fit into AAV but **cannot** protect muscle

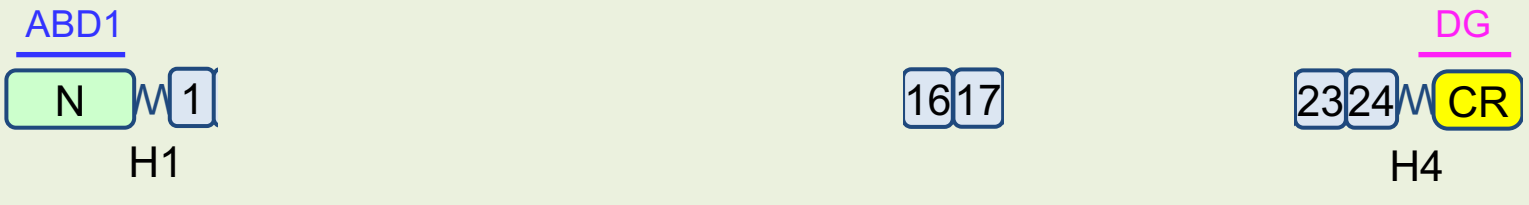
Generation 1 μDys (4 or 5 repeats)
Can fit into AAV and can protect muscle

Pfizer (Xiao lab, 2000)

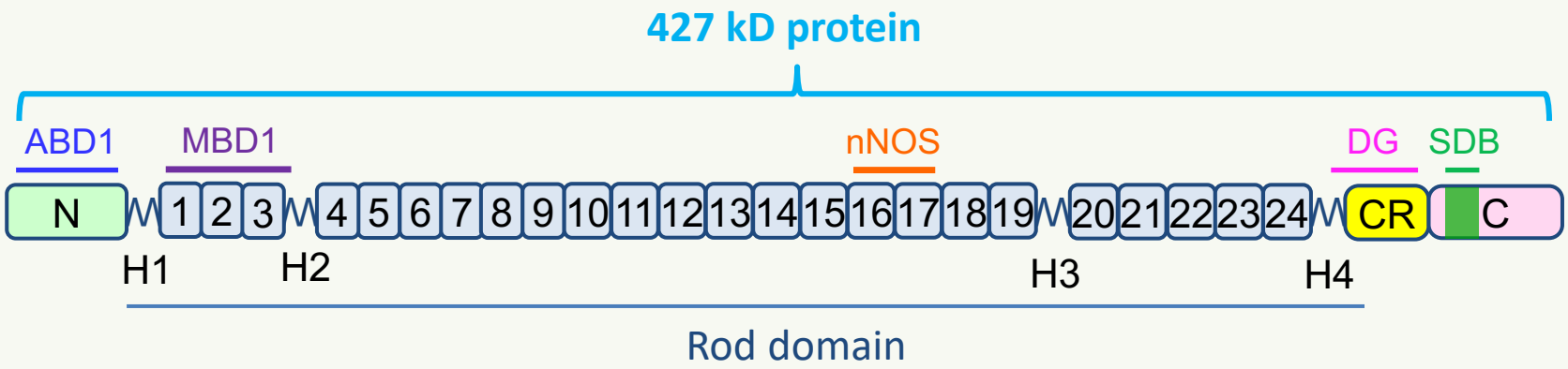


Sarepta (Chamberlain lab, 2002)

Solid (Duan lab, 2017)

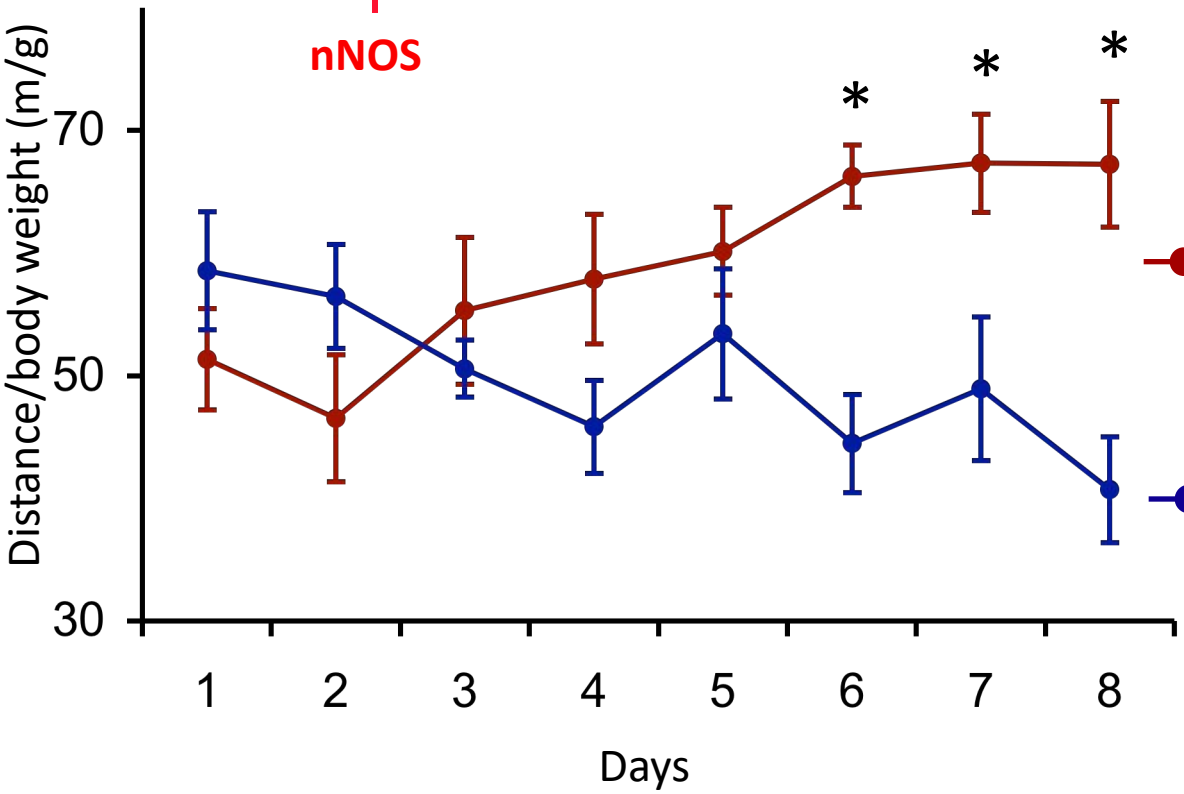


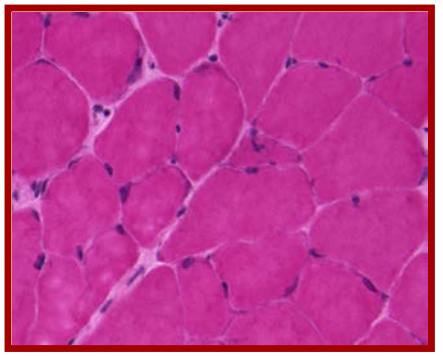
Full-length dystrophin



Dose nNOS Binding Make a Difference?

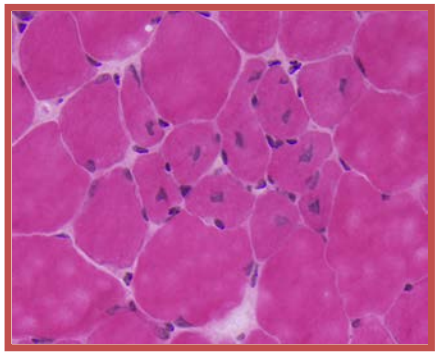
nNOS





with nNOS binding (N=7)

w/o nNOS binding (N=7)



Lai et al Journal of Clinical Investigation 119:624, 2009
Lai et al PNAS 2013

Human Molecular Genetics, 2012, Vol. 21, No. 15 **3449–3460**
doi:10.1093/hmg/dds176
Advance Access published on May 15, 2012

Variable phenotype of del45-55 Becker patients correlated with nNOSμ mislocalization and RYR1 hypernitrosylation

Christel Gentil[1], France Leturcq[2,3], Rabah Ben Yaou[2,4], Jean-Claude Kaplan[3], Pascal Laforet[5], Isabelle Pénisson-Besnier[6], Caroline Espil-Taris[7], Thomas Voit[1], Luis Garcia[1] and France Piétri-Rouxel[1,*]

Cases	Exons deleted in dystrophin gene	Combined severity class	nNOSμ localization Sarcolemmal
2	45–55	Severe +	No
4	45–55	Severe +	No
5	45–55	Severe ++	No
6	45–55	Severe ++	No
9	45–55	Severe ++	No
10	45–55	Severe +	No

Without nNOS → Severe disease

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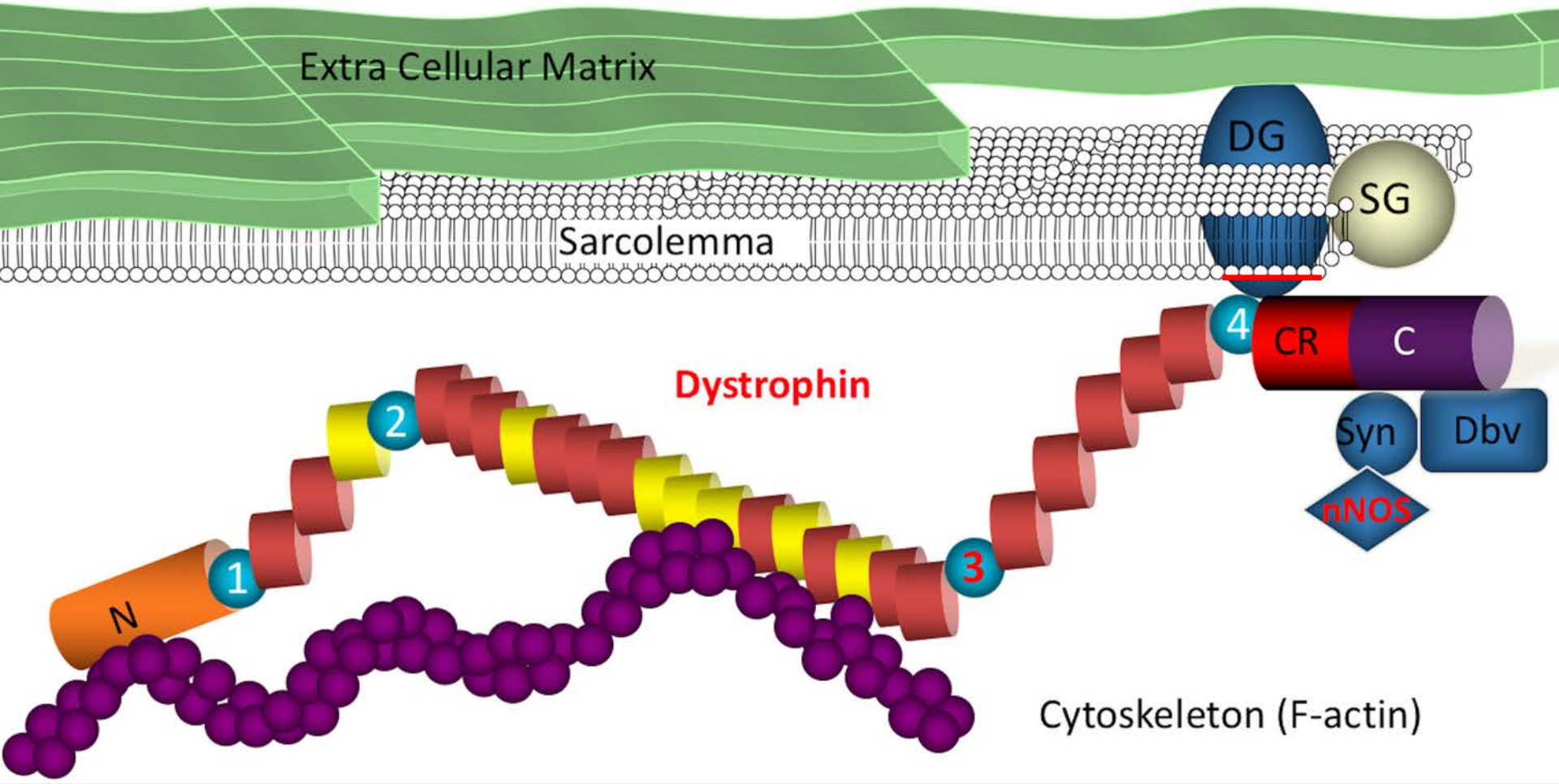
Cases	Exons deleted in dystrophin gene	Combined severity class	nNOSμ localization Sarcolemmal
1	45–55	Moderate	Yes
3	45–55	Moderate	Yes
7	45–55	Moderate	Yes
8	45–55	Moderate	Yes
11	45–55	Mild	Yes
12	45–55	Moderate	Yes
2	45–55	Severe +	No
4	45–55	Severe +	No
5	45–55	Severe ++	No
6	45–55	Severe ++	No
9	45–55	Severe ++	No
10	45–55	Severe +	No

With nNOS → **Mild to moderate disease**

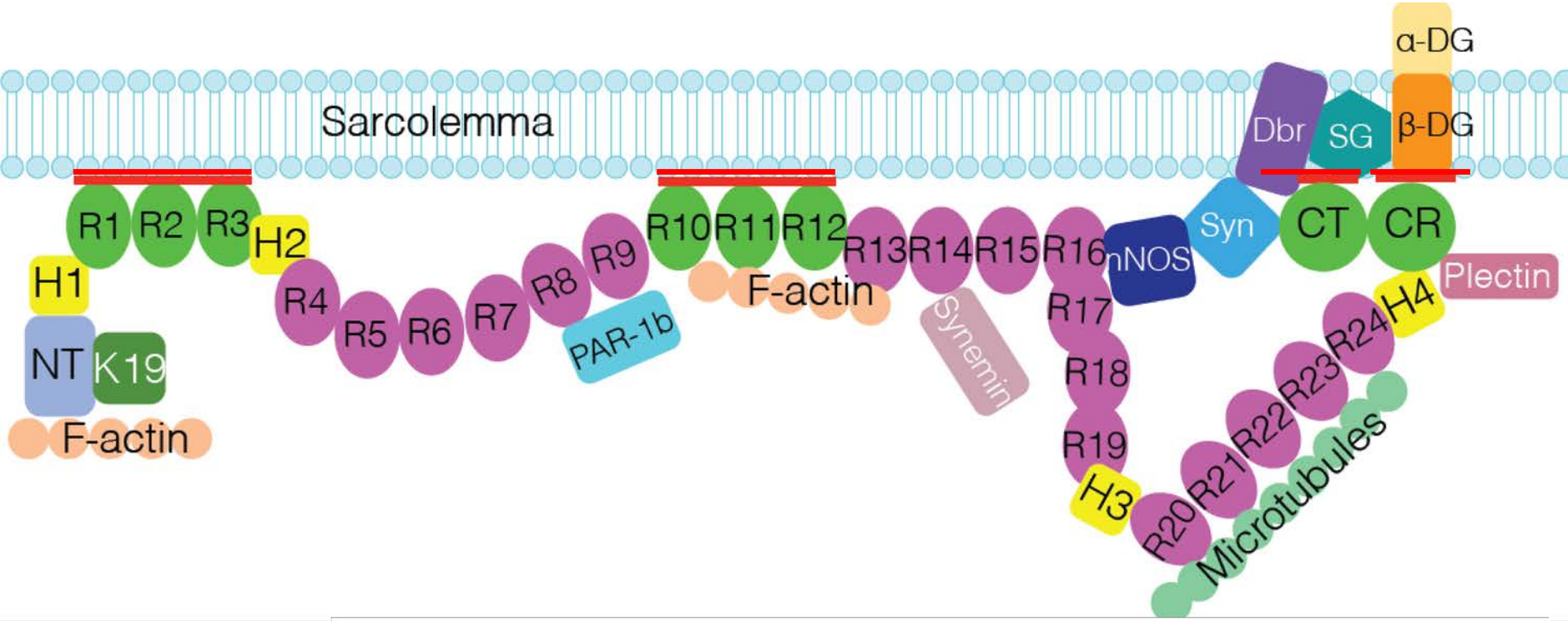
Without nNOS → **Severe disease**

Dystrophin contains one membrane binding domain



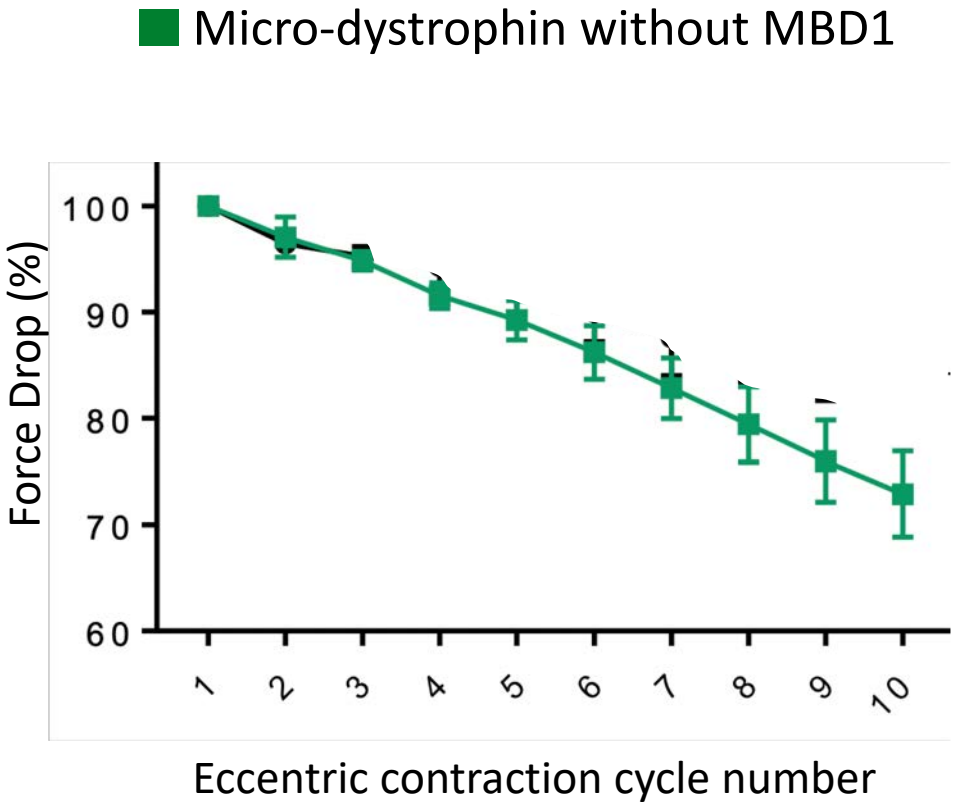
Dystrophin contains multiple independent membrane-binding domains

Junling Zhao[1], Kasun Kodippili[1], Yongping Yue[1], Chady H. Hakim[1,2], Lakmini Wasala[1], Xiufang Pan[1], Keqing Zhang[1], Nora N. Yang[2], Dongsheng Duan[1,3,4,5,*] and Yi Lai[1,*]



Human Molecular Genetics, 2016, Vol. 25, No. 17 3647–3653

Inclusion of MBD1 better prevented eccentric contraction-induced force drop

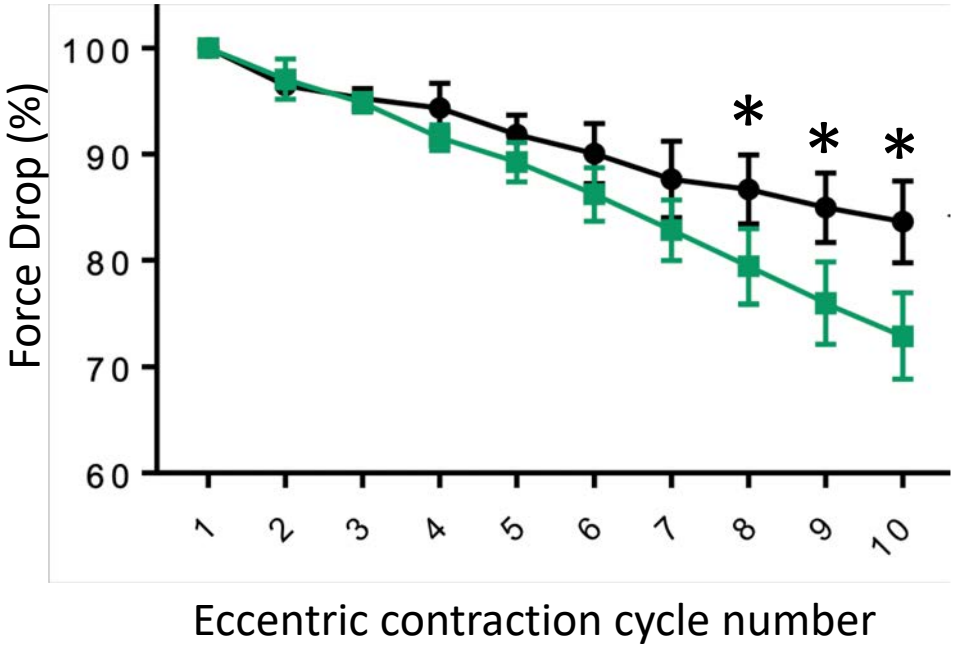


EDL (limb muscle)

Inclusion of MBD1 better prevented eccentric contraction-induced force drop

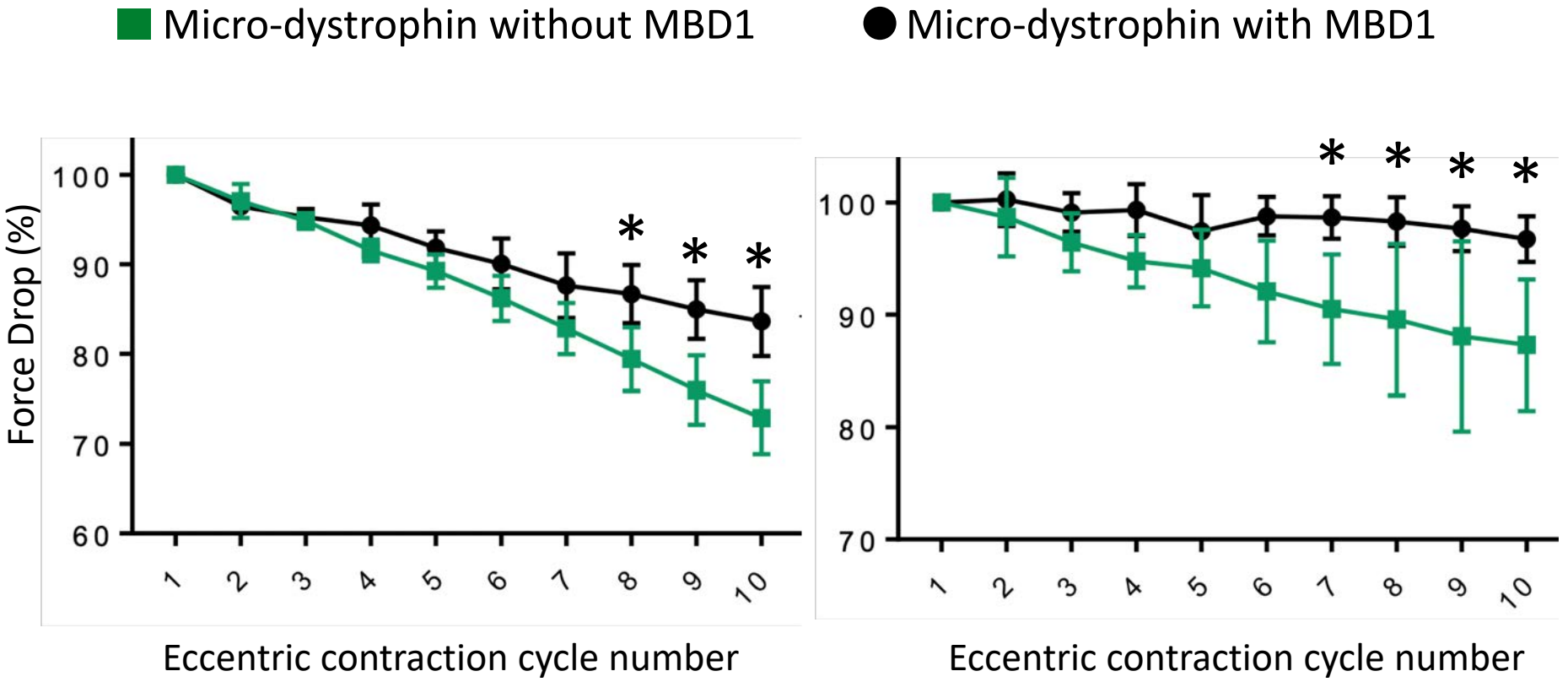
Micro-dystrophin without MBD1

Micro-dystrophin with MBD1



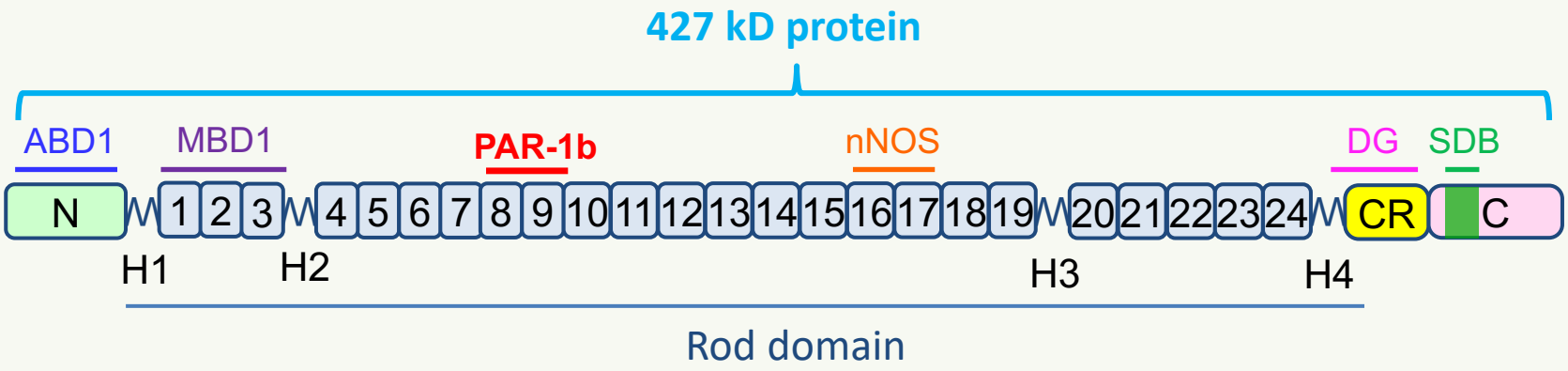
EDL (limb muscle)

Inclusion of MBD1 better prevented eccentric contraction-induced force drop



EDL (limb muscle)

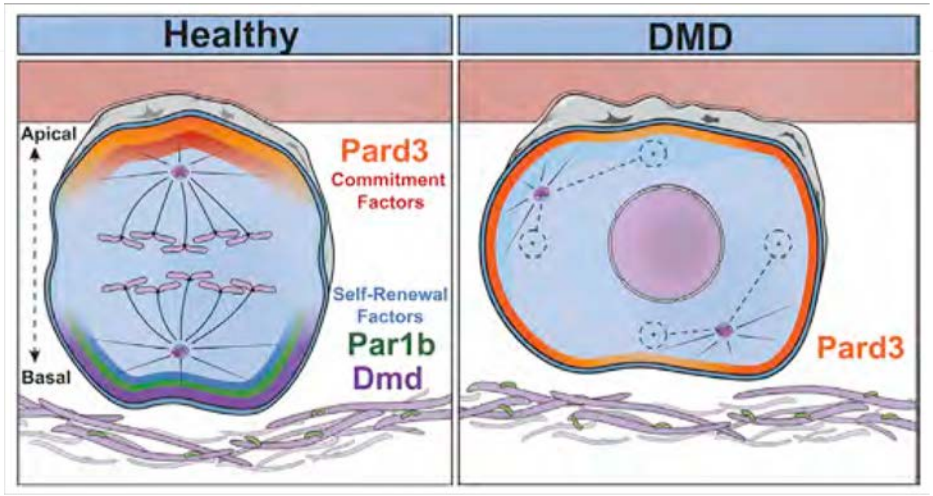
Diaphragm



nature medicine

Dystrophin expression in muscle stem cells regulates their polarity and asymmetric division

Nicolas A Dumont[1,2,4], Yu Xin Wang[1,2,4], Julia von Maltzahn[1–3], Alessandra Pasut[1,2], C Florian Bentzinger[1–3], Caroline E Brun[1,2] & Michael A Rudnicki[1,2]



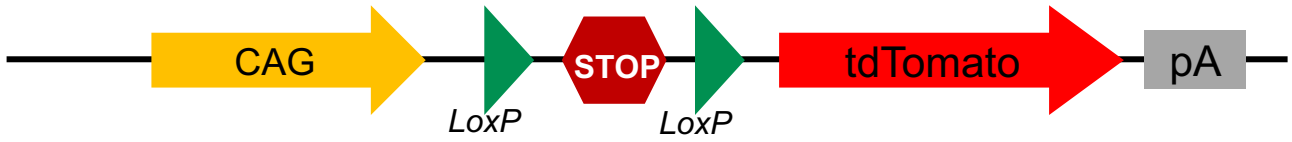
Orienting Muscle Stem Cells for Regeneration in Homeostasis, Aging, and Disease.

Feige P, Brun CE, Ritso M, **Rudnicki MA**.

Cell Stem Cell. 2018 Nov 1;23(5):653-664. doi: 10.1016/j.stem.2018.10.006.

AAV9 transduced Pax7+ muscle stem cells in Ai14 mice

Ai14 mice
(no fluorescence)



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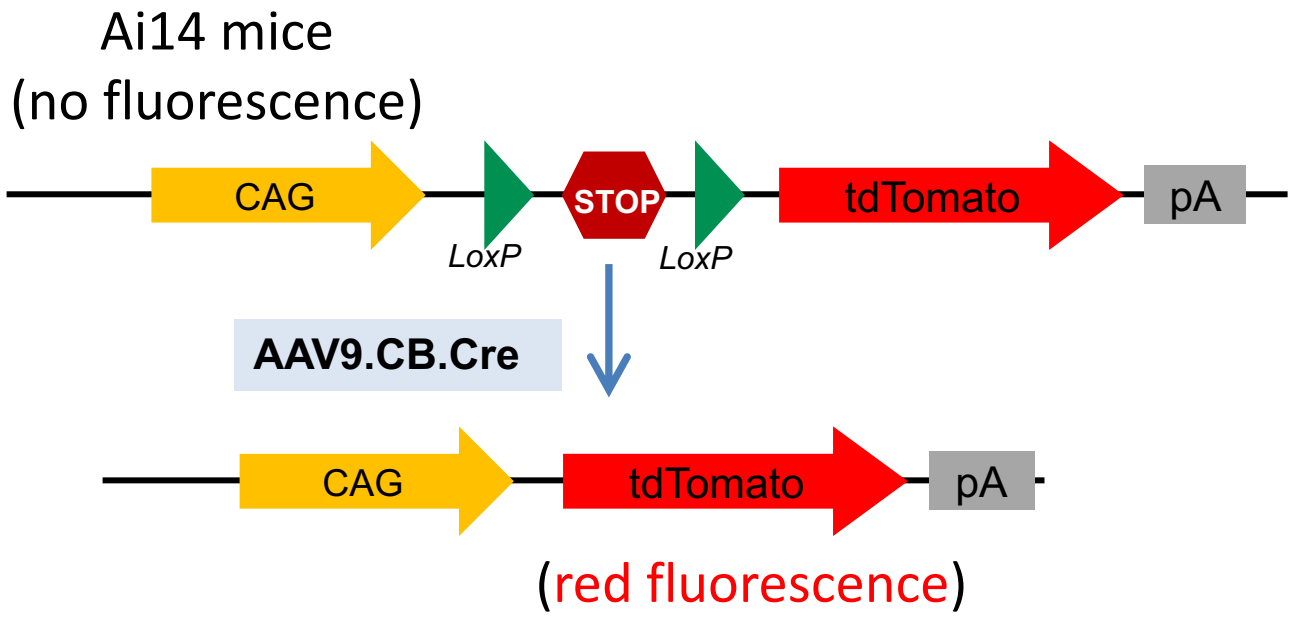
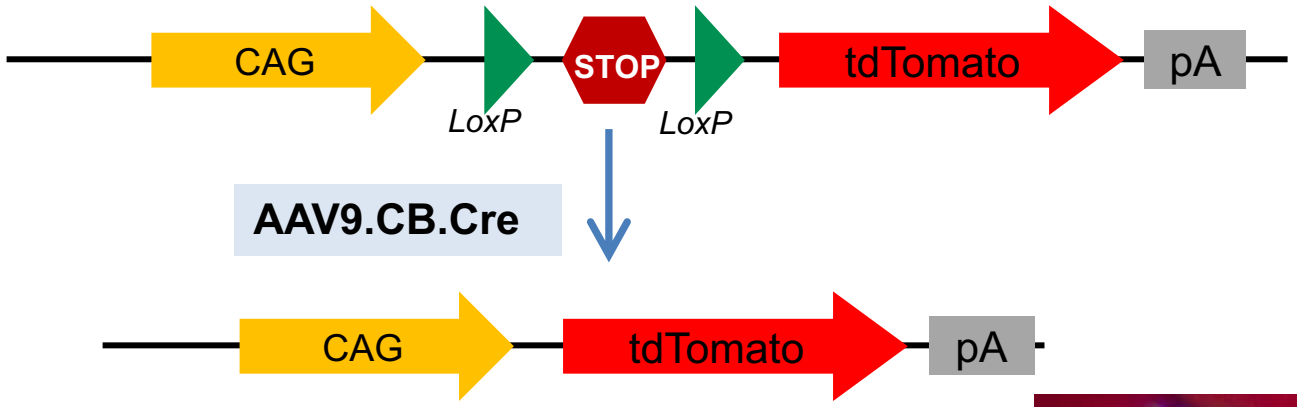
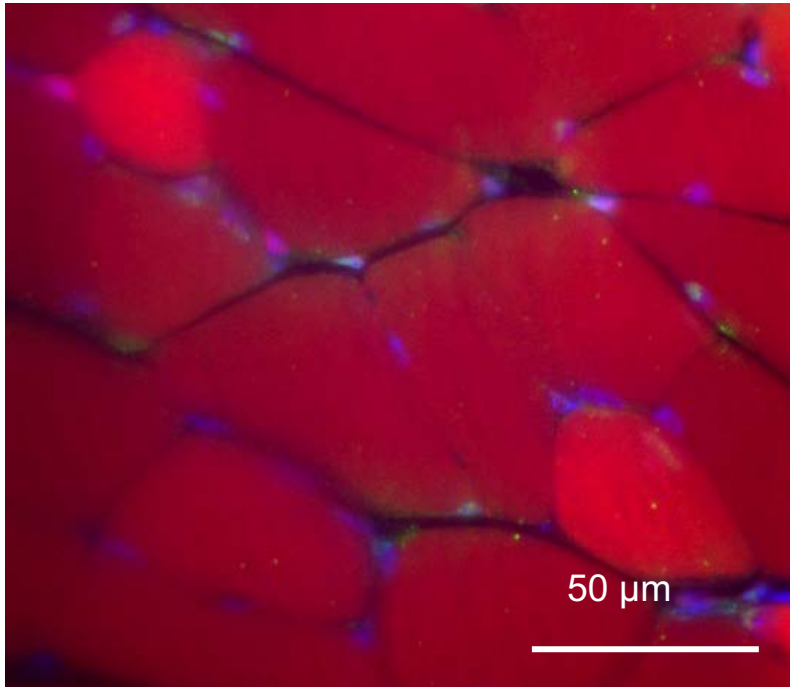


AAV9 transduced Pax7+ muscle stem cells in Ai14 mice

Ai14 mice
(no fluorescence)

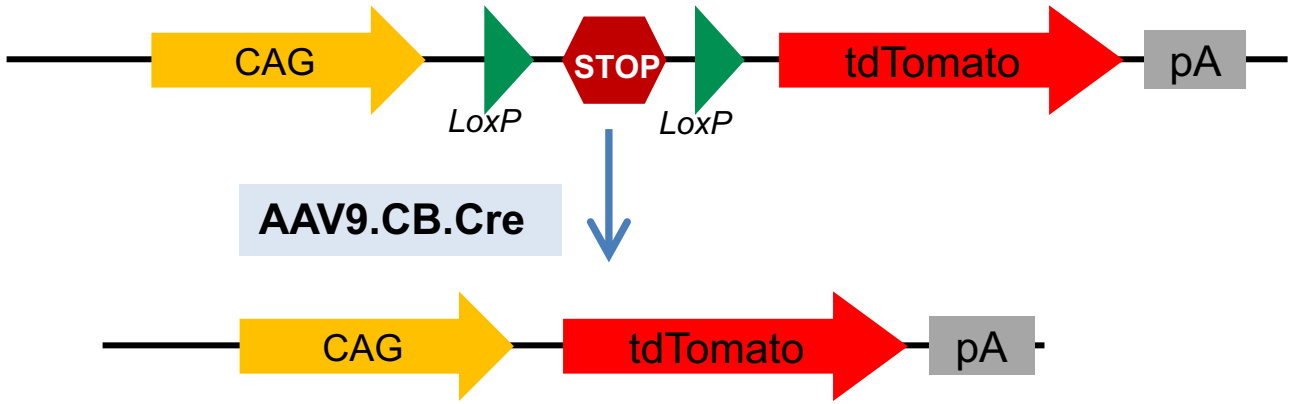


(red fluorescence)

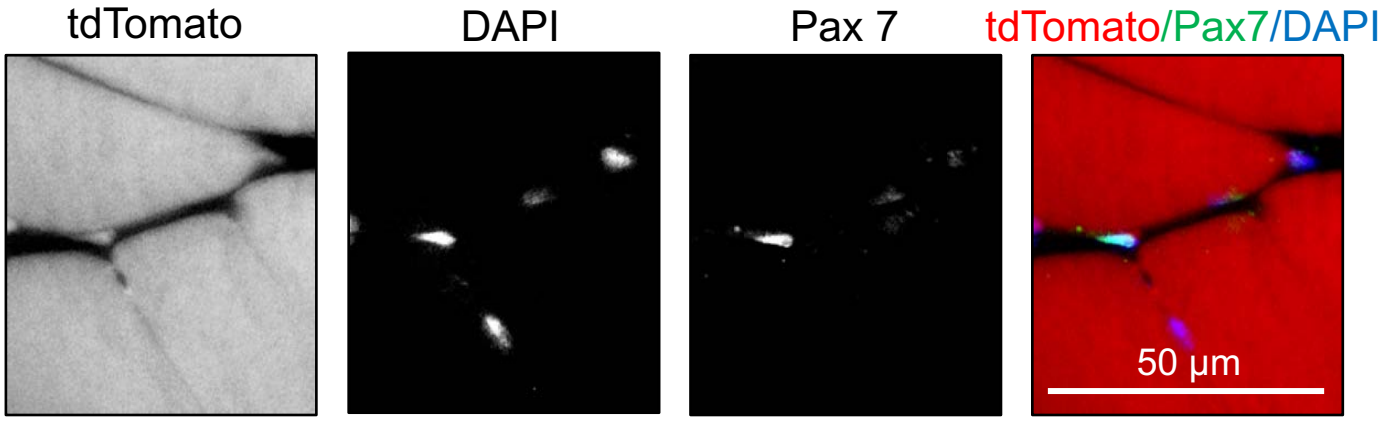


AAV9 transduced Pax7+ muscle stem cells in Ai14 mice

Ai14 mice
(no fluorescence)

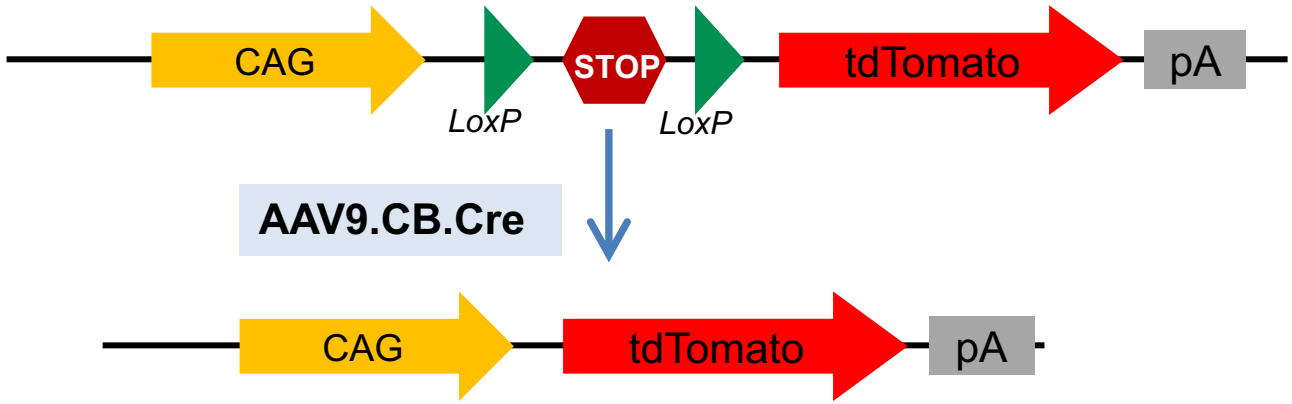


(red fluorescence)



AAV9 transduced Pax7+ muscle stem cells in Ai14 mice

Ai14 mice
(no fluorescence)



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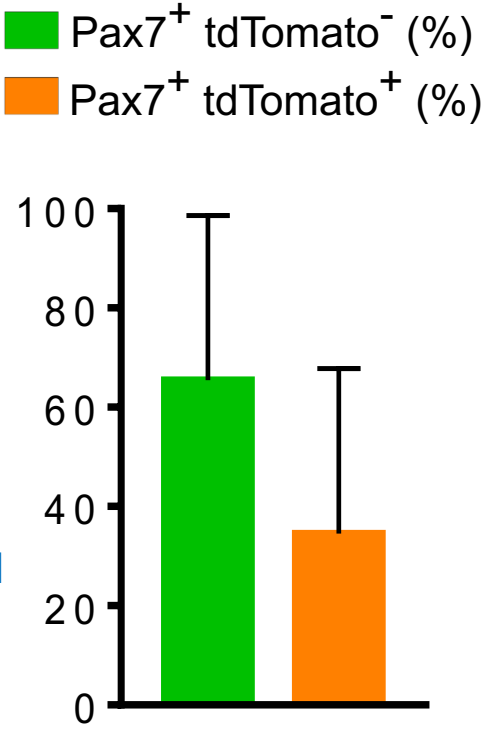
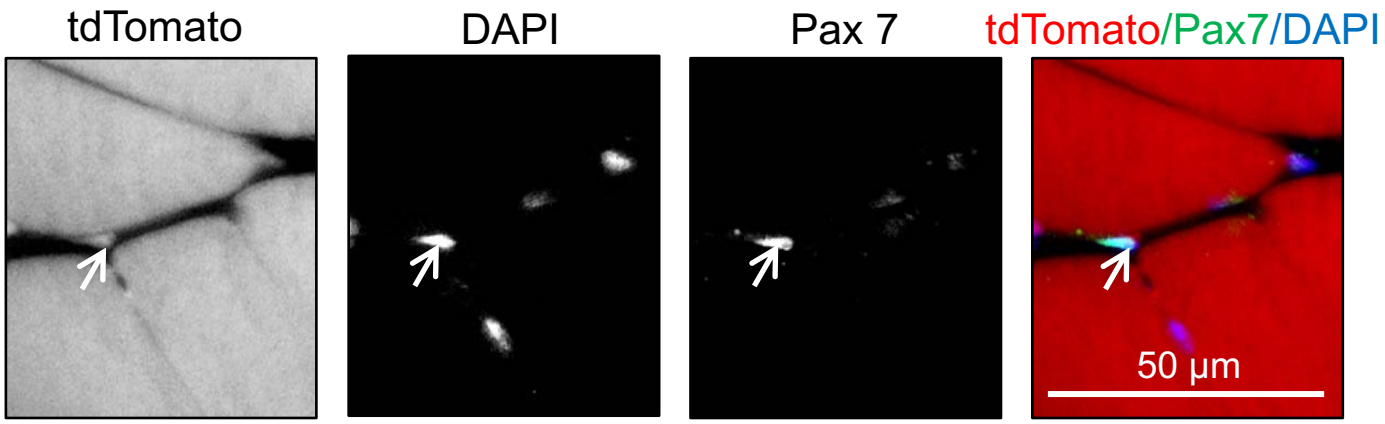
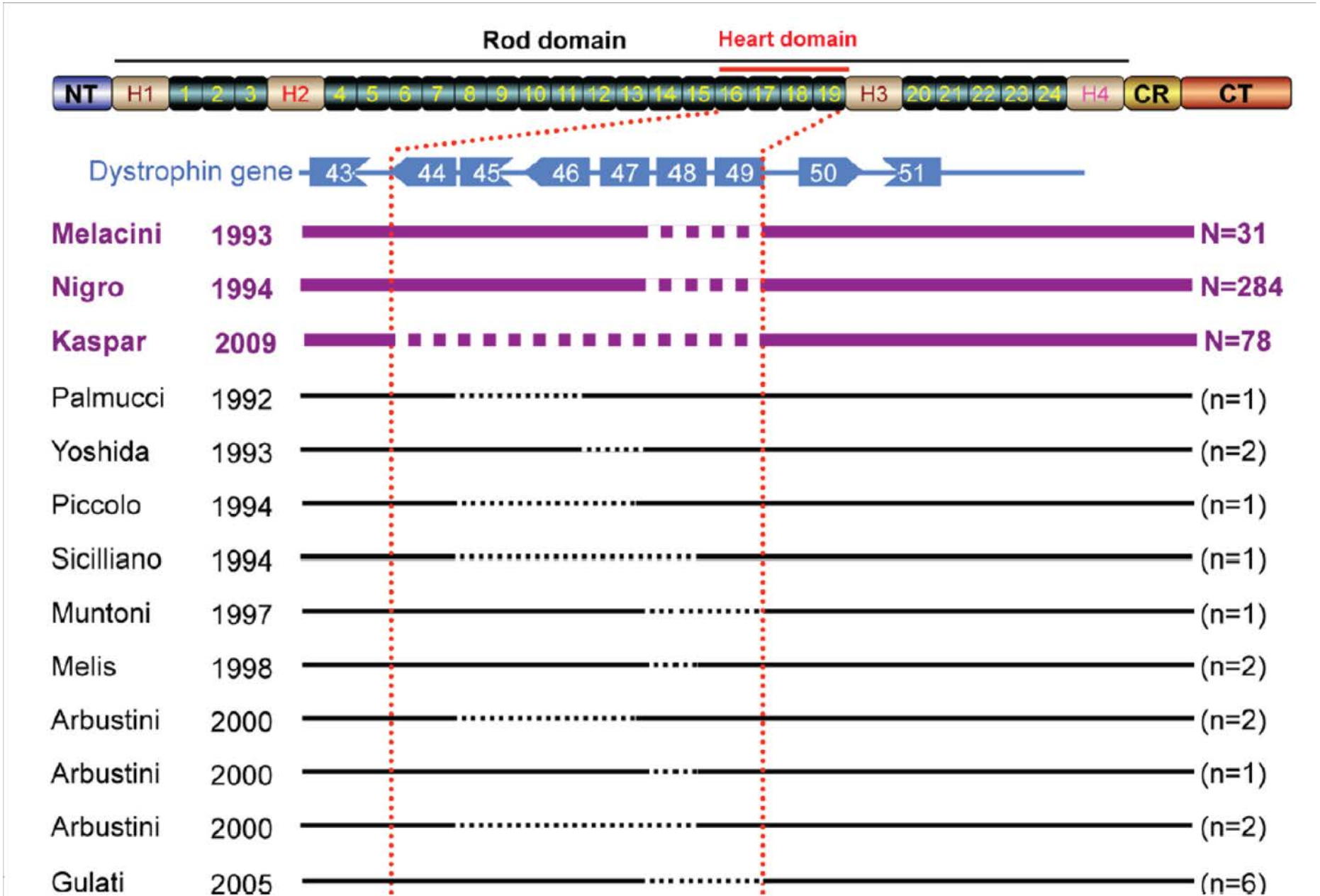




Dystrophin in the heart is different from that in skeletal muscle

	Skeletal muscle	Heart
actin	subsarcolemma filamentous γ-actinl	subsarcolemma filamentous γ-actinl α-actin in thin filament
nNOS	localize at membrane	localize in SR and mitochondria
excessive cytosolic nNOS	nitrosative sress, force reduction, agrevate disease	overexpression alleviate cardiomyopathy
sytrophin	α1, β1	α1, β1, β2
dystrobrevin	α1, α2	α1, α2, α3
Cardiac-specific DGC		cavin-1, ahnak-1, cypher and αB-crystallin

Partial or complete loss of R16-19 is associated with more severe cardiac disease



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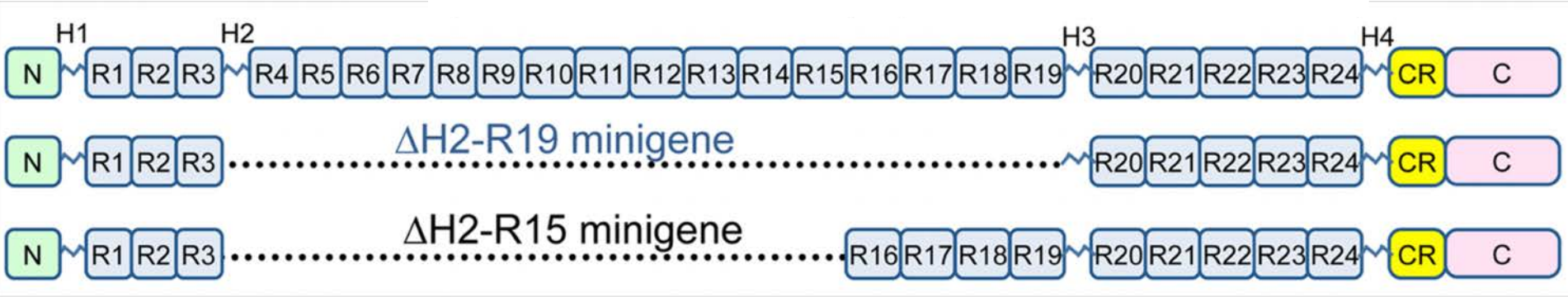
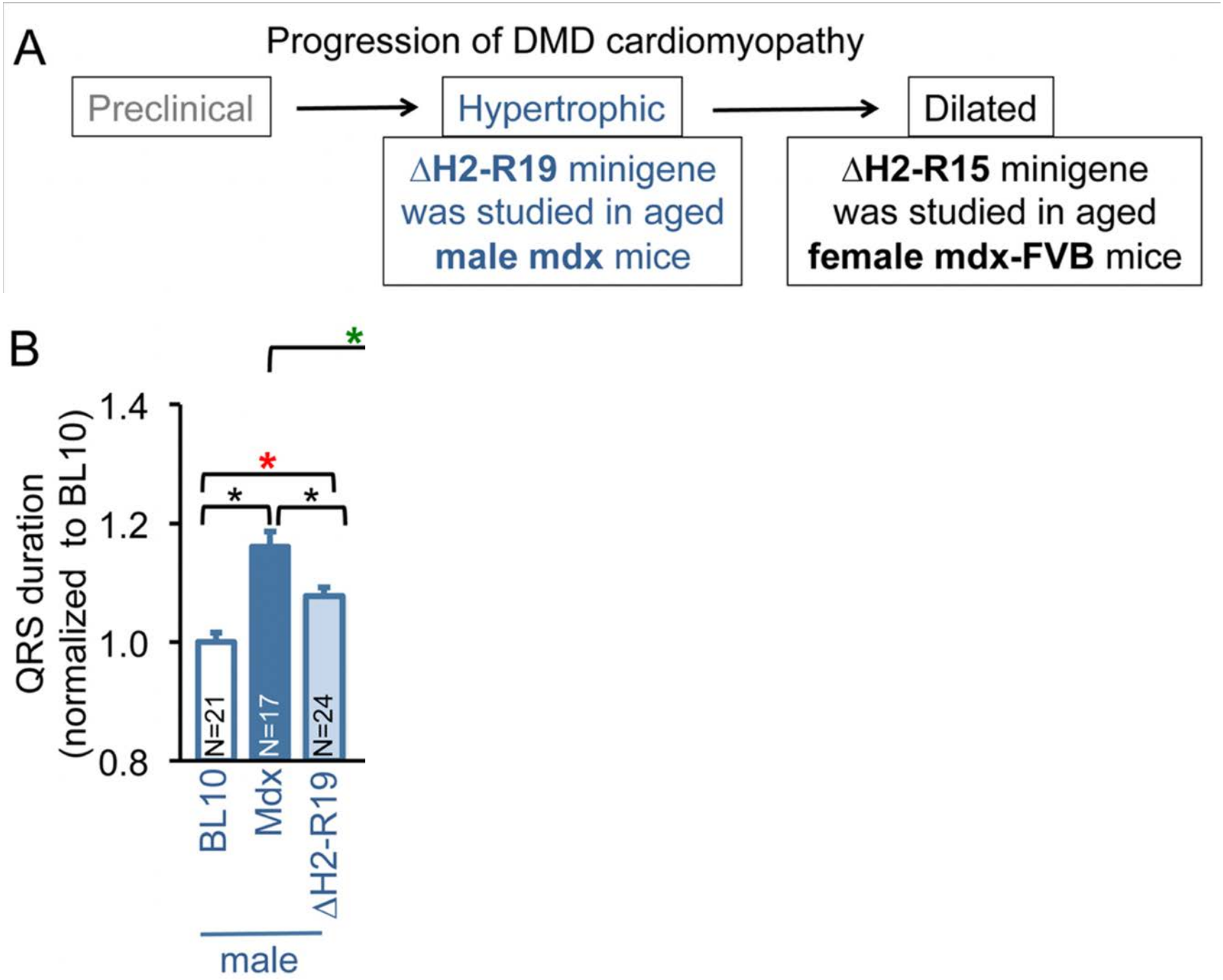
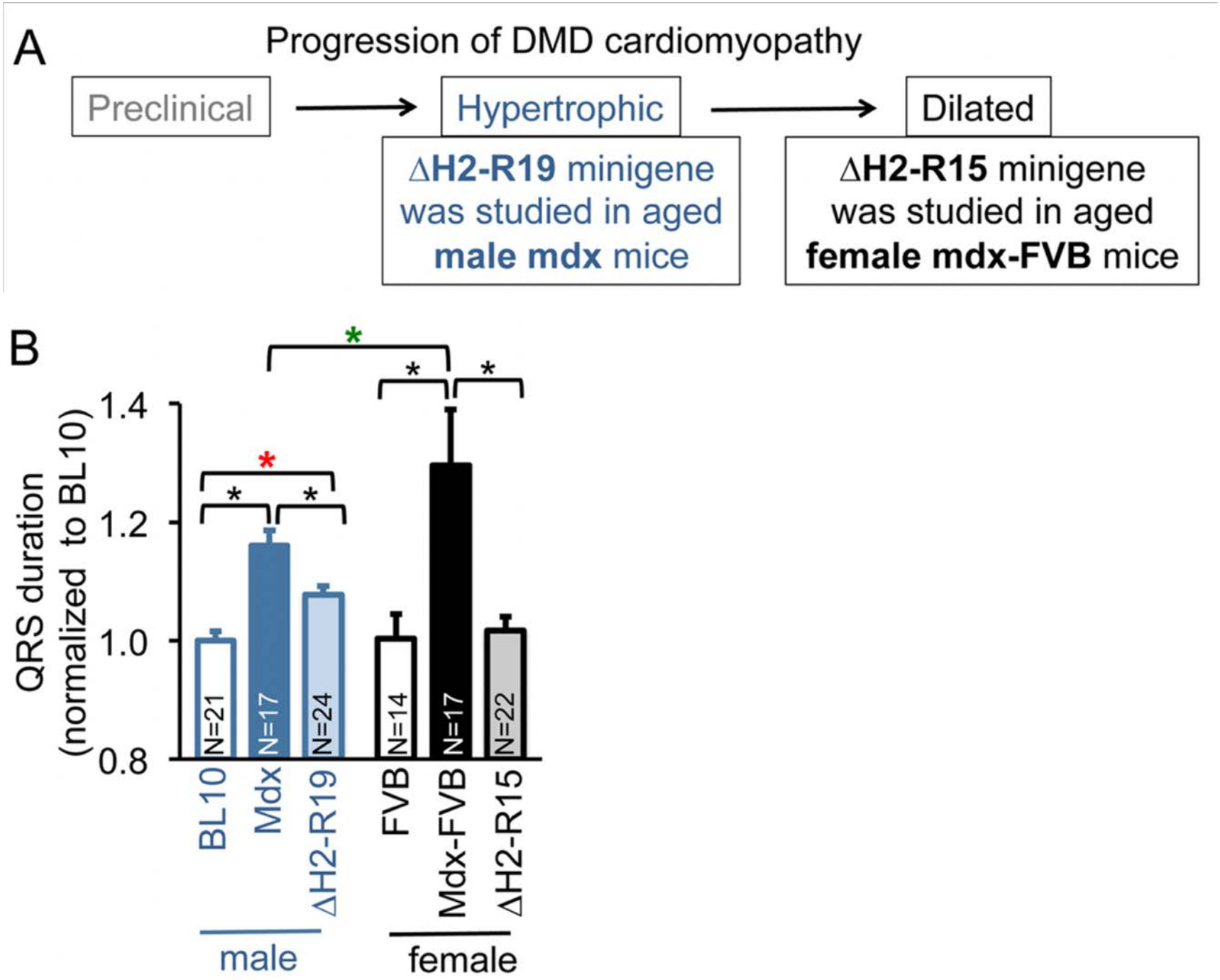
Cardiac-Specific Expression of ΔH2-R15 Mini-Dystrophin Normalized All Electrocardiogram Abnormalities and the End-Diastolic Volume in a 23-Month-Old Mouse Model of Duchenne Dilated Cardiomyopathy

Nalinda B. Wasala,[1] Jin-Hong Shin,[1,†] Yi Lai,[1] Yongping Yue,[1] Federica Montanaro,[2] and Dongsheng Duan[1,3–5,*]




A
Progression of DMD cardiomyopathy
Preclinical
Hypertrophic
Dilated
ΔH2-R19 minigene was studied in aged male mdx mice
ΔH2-R15 minigene was studied in aged female mdx-FVB mice
B
QRS duration (normalized to BL10)
1.4
1.2
1.0
0.8
N=21
N=17
N=24
BL10
Mdx
ΔH2-R19
male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N=17
N=22
BL10
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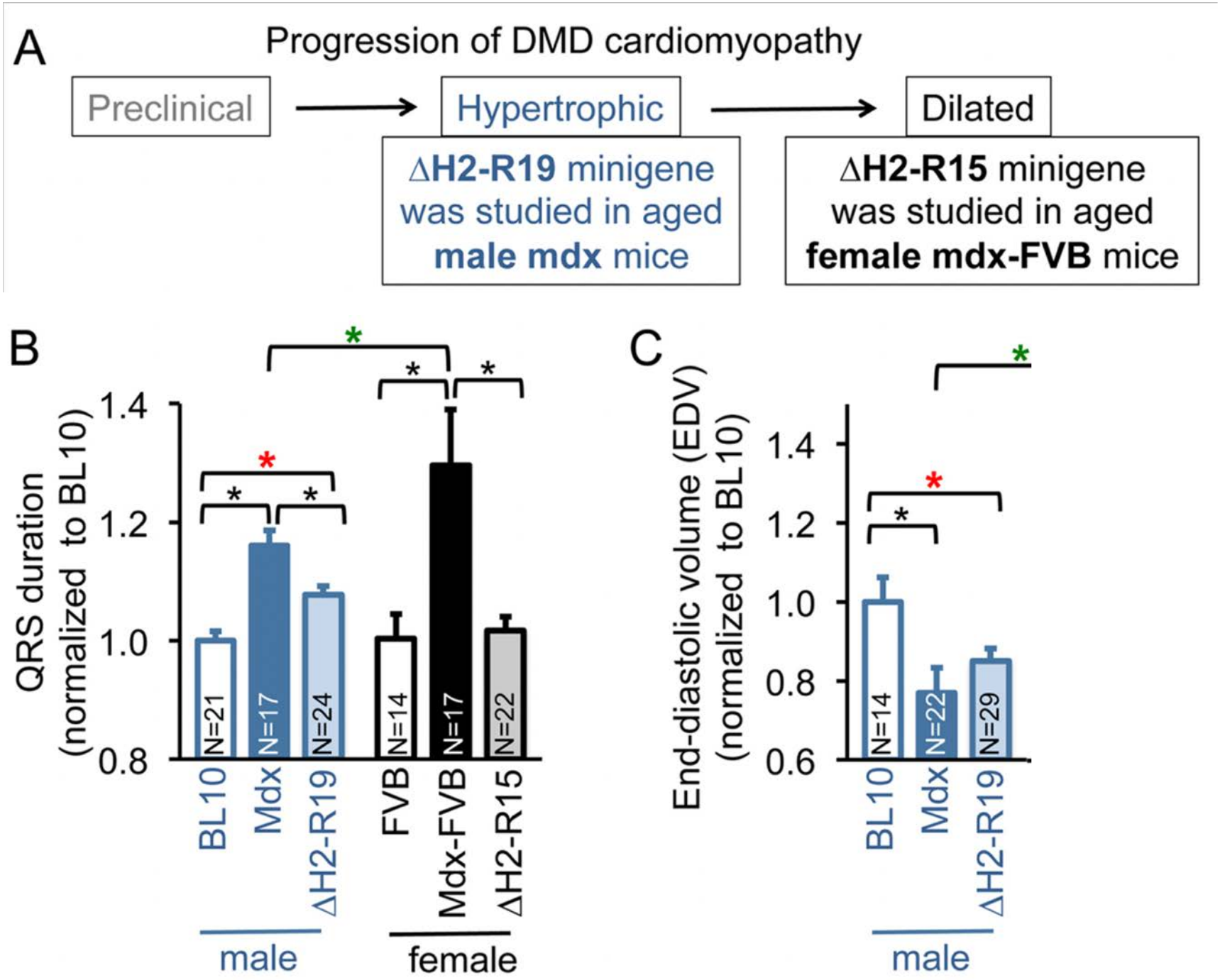
ΔH2-R19
FVB
Mdx-FVB
ΔH2-R15
male
female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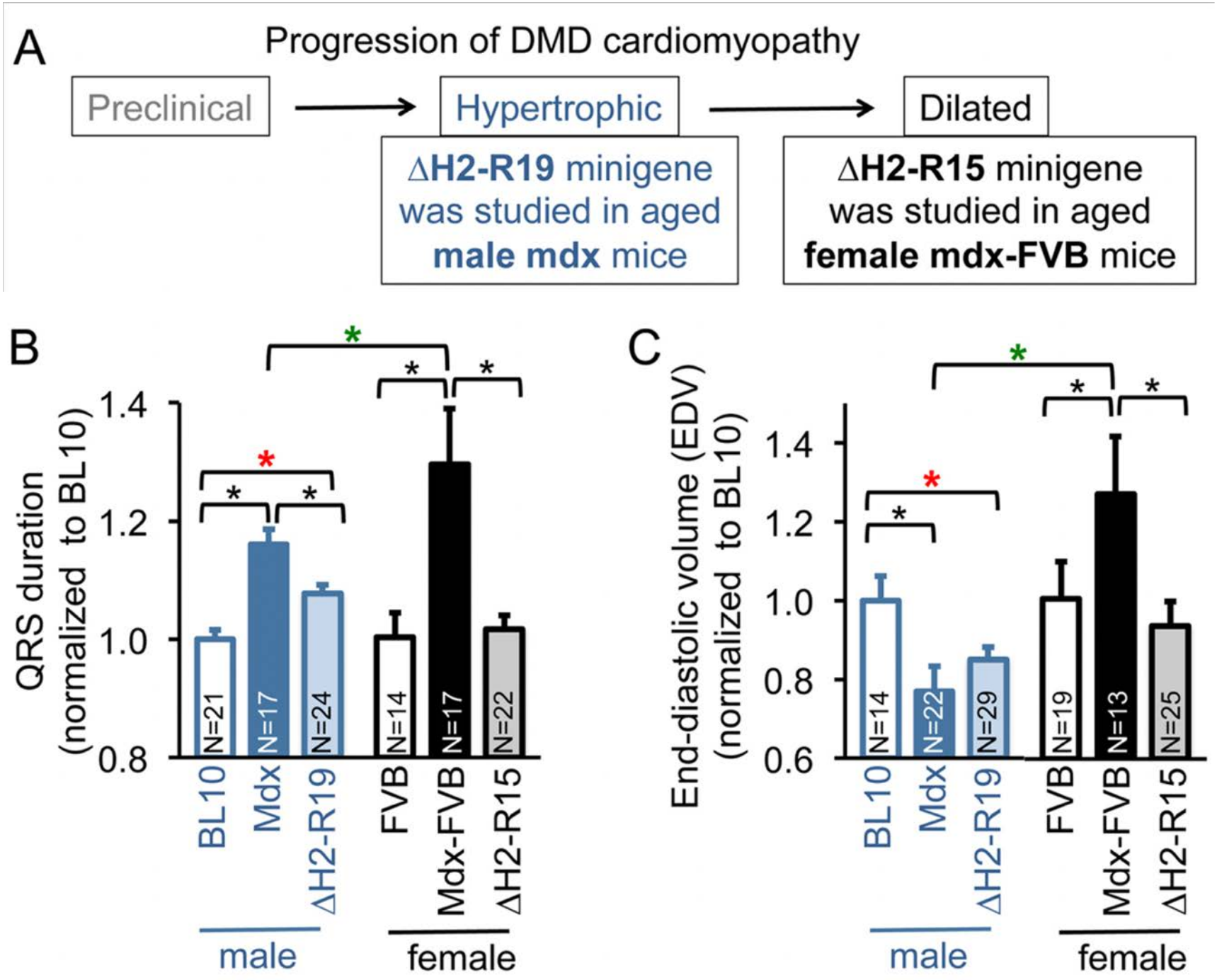
N=14
N=17
N=22
BL10
Mdx
ΔH2-R19
FVB
Mdx-FVB
ΔH2-R15
male
female
C
End-diastolic volume (EDV) (normalized to BL10)
1.4
1.2
1.0
0.8
0.6
N=14
N=22
N=29
BL10
Mdx
ΔH2-R19
male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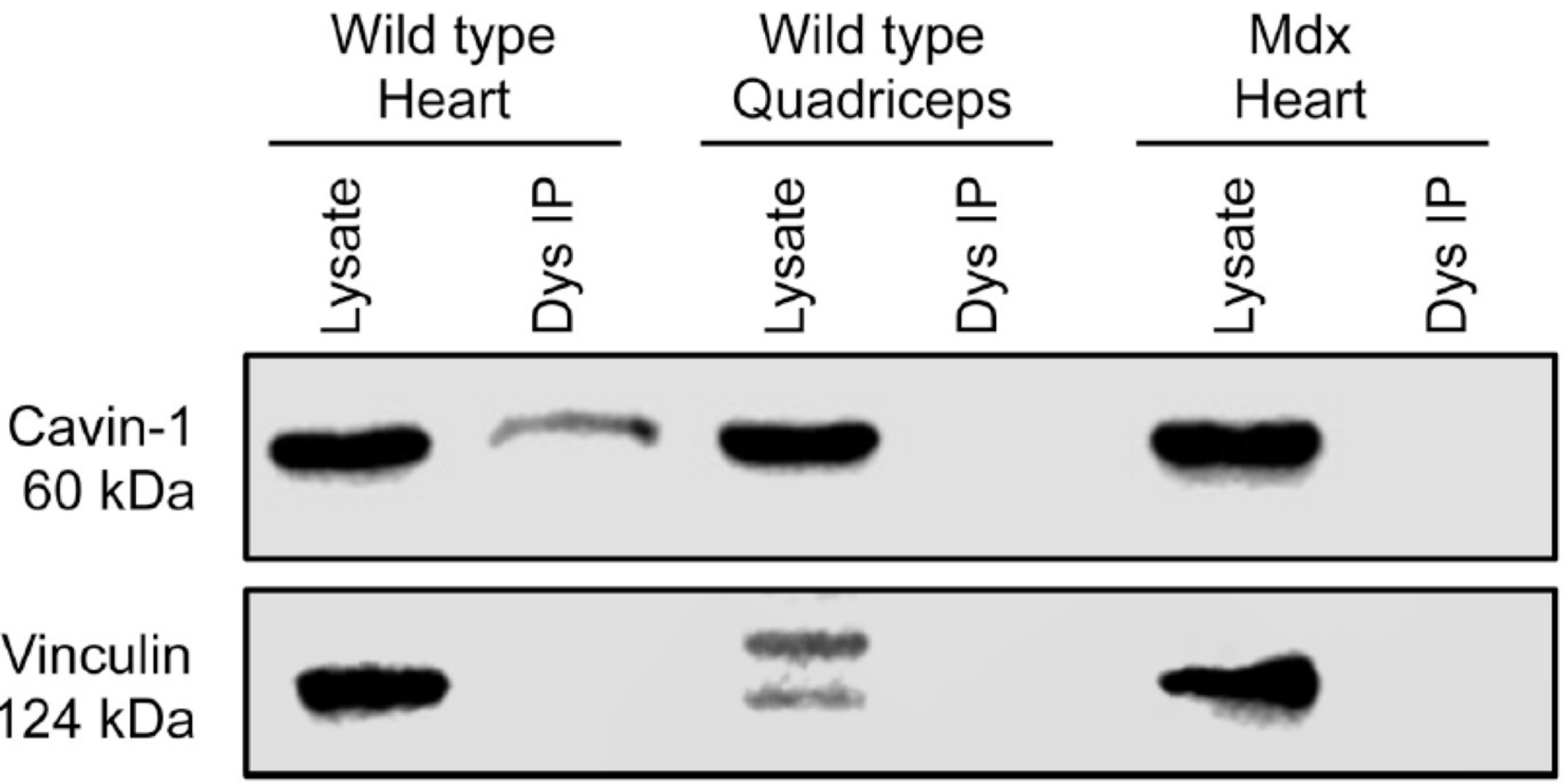
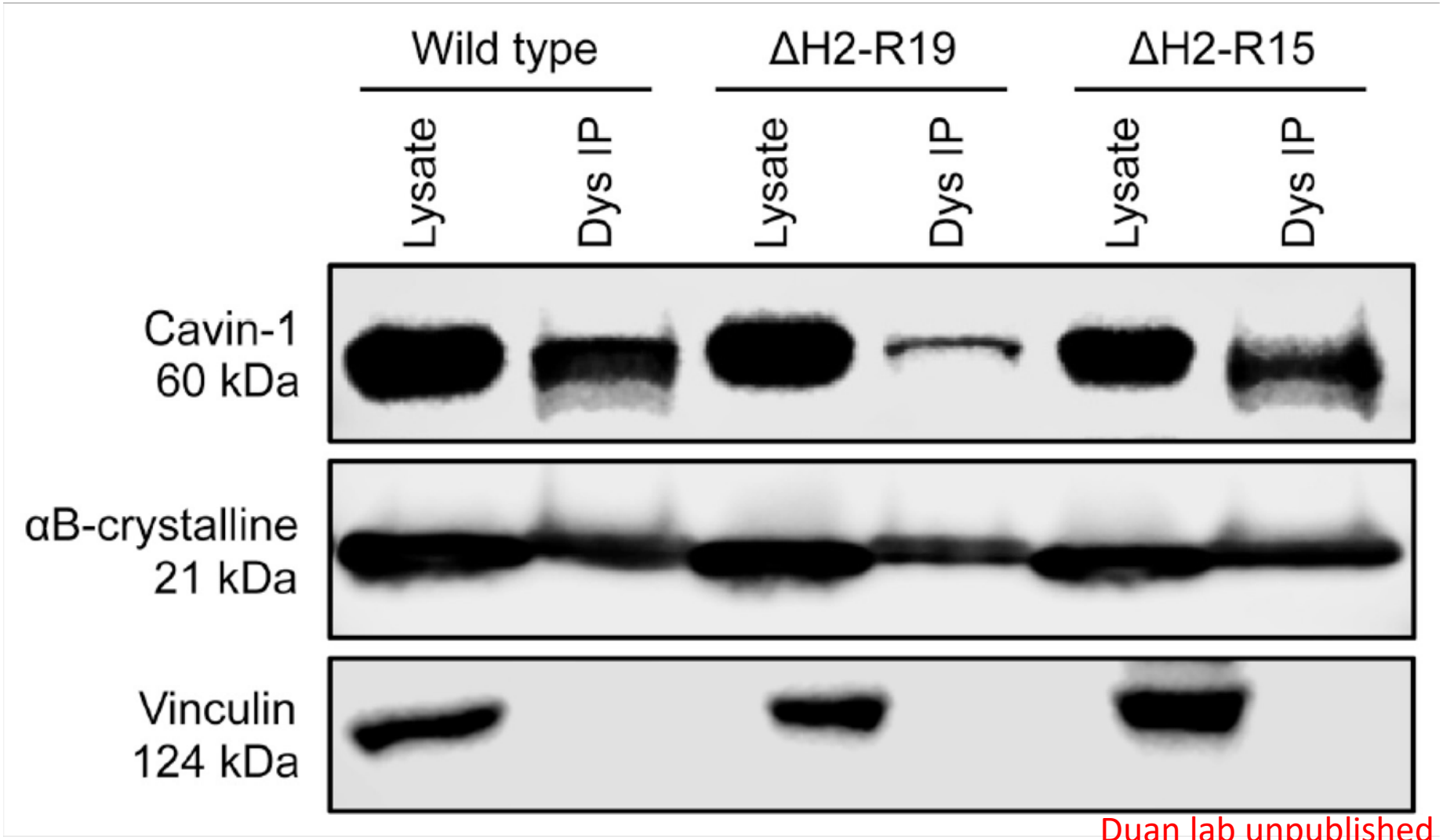
BL10
Mdx
ΔH2-R19
FVB
Mdx-FVB
ΔH2-R15
male
female

Cavin-1 interacts with dystrophin in the heart but not skeletal muscle



Duan lab unpublished

High affinity binding to cavin-1 may underlie enhanced heart protection by ΔH2-R15



Duan lab unpublished

Summary

- Current micro-dystrophin is suboptimal.
- Inclusion of nNOS-binding domain improves muscle perfusion and exercise capacity.
- Inclusion of additional membrane-binding domain leds better protection against eccentric contraction injury.
- Inclusion of heart protection domain may better preserve cardiac function.
- Ability to deliver dystrophin to muscle stem cells may improve muscle regeneration.

Acknowledgements

NIH

PPMD

Jesse’s Journey

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