The (elusive) perfect mouse model

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2016 MDF Annual Conference
MDF Drug Development Roundtable
September 15, 2016

Outline

What do we want to model?

What is the utility of a mouse model?

What are the ideal features?

What do we have?

What are the pros and cons?

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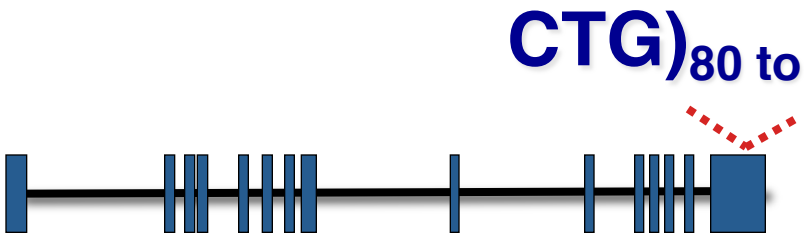
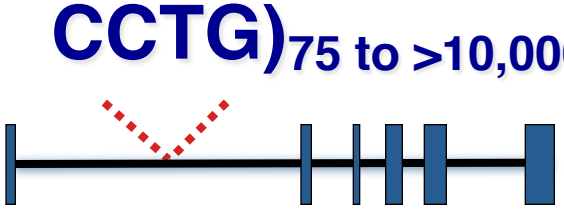
What are the pros and cons?

Two forms of Myotonic Dystrophy (DM)



DMPK (chrom. 19)

type 1 (DM1)

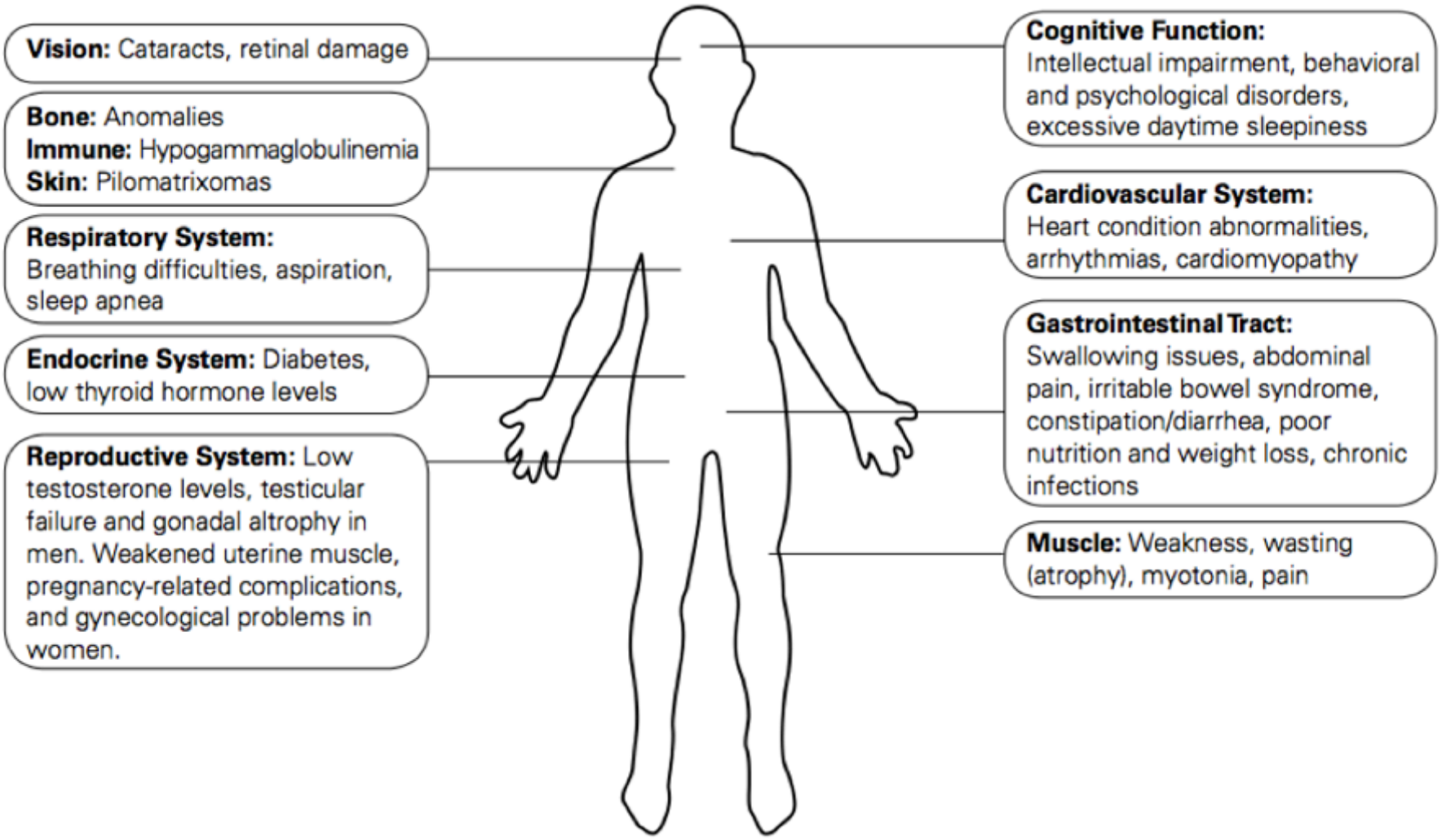


CNBP (chrom. 3)

type 2 (DM2)

- autosomal dominant
- most common form of adult onset muscular dystrophy
- second most common form of muscular dystrophy

Myotonic dystrophy is a multisystemic disease



Therapeutics need to access and address pathology in multiple tissues

From: Myotonic Dystrophy Foundation http://www.myotonic.org/

Clinical data informs development of mouse models

Patient-Reported Impact of Symptoms in Myotonic Dystrophy Type 2 (PRISM-2).

Chad Heatwole, Nicholas Johnson, Rita Bode, Jeanne Dekdebrun, Nuran Dilek, James E Hilbert, Elizabeth Luebbe, William Martens, Michael P McDermott, Christine Quinn, Nan Rothrock, Charles Thornton, Barbara G Vickrey, David Victorson, and Richard T Moxley

Neurology (2015)

DEVELOPMENTAL MEDICINE & CHILD NEUROLOGY ORIGINAL ARTICLE

Parent-reported multi-national study of the impact of congenital and childhood onset myotonic dystrophy

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1 Department of Neurology, The University of Utah, Salt Lake City, UT, USA. **2** Department of Pediatrics, The Queen Silvia Children's Hospital, Sahlgrenska Academy at the University of Gothenburg, Gothenburg, Sweden. **3** Department of Neurology, London Health Science Centre, London, ON, Canada. **4** Department of Orthopedics, The University of Utah, Salt Lake City, UT; **5** Division of Epidemiology, The University of Utah, Salt Lake City, UT; **6** Department of Neurology, The University of Rochester, Rochester, NY, USA.

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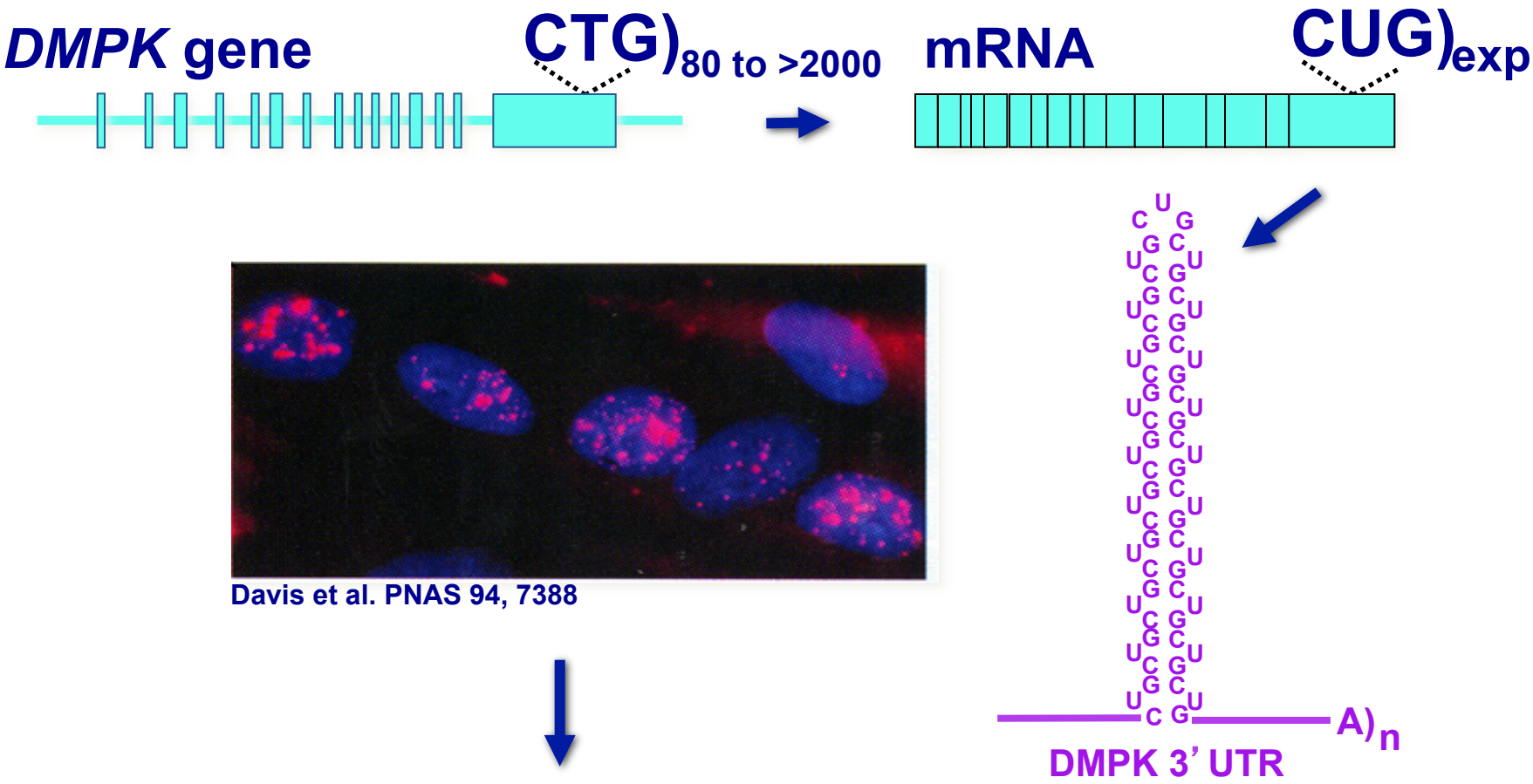
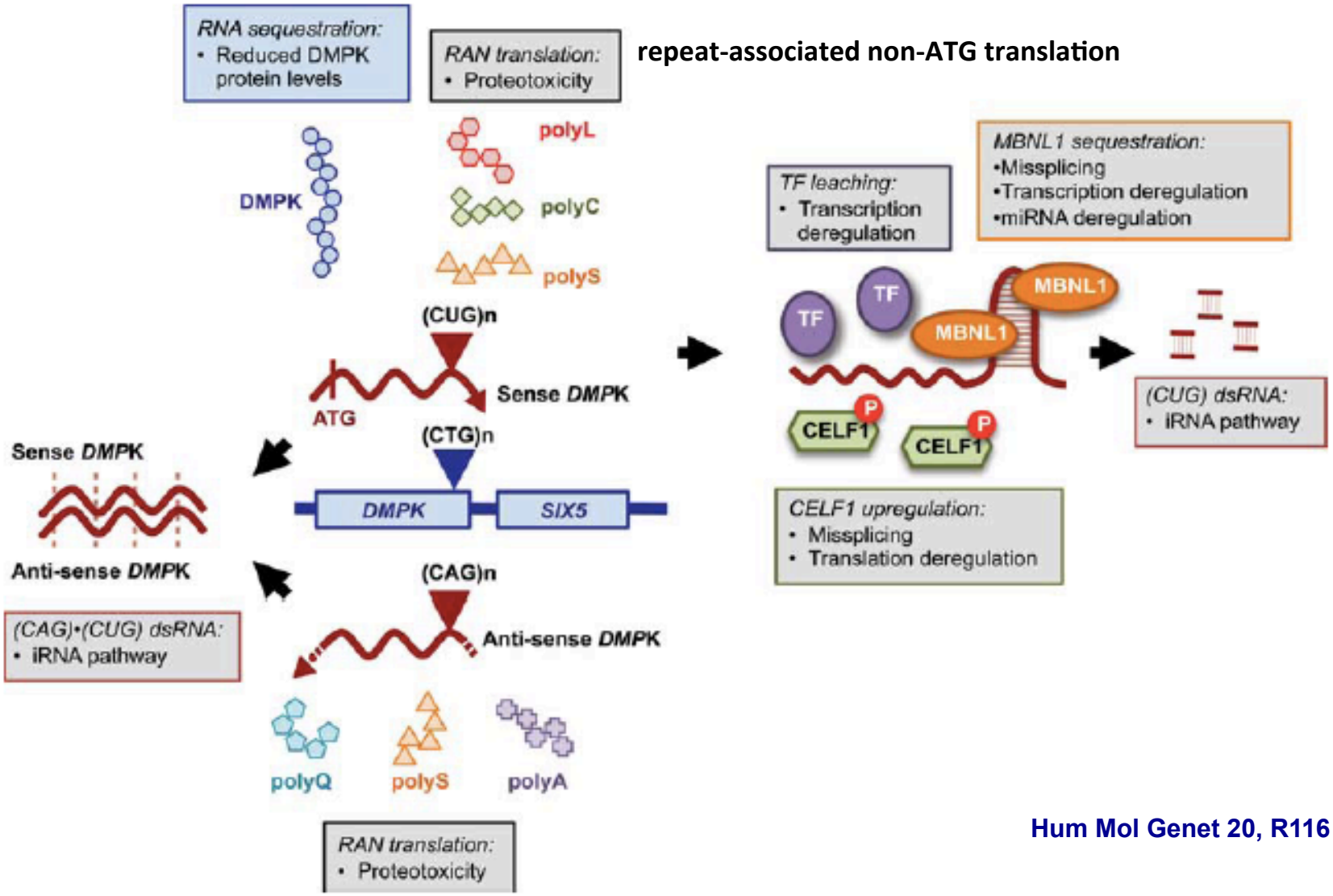
Johnson, N. E. *et al. Dev Med Child Neurol* **58,** 698–705 (2016).

Myotonic Dystrophy type 1 (DM1)



RNA gain-of-function

Pathogenic effects of CUG)exp RNA



Hum Mol Genet 20, R116

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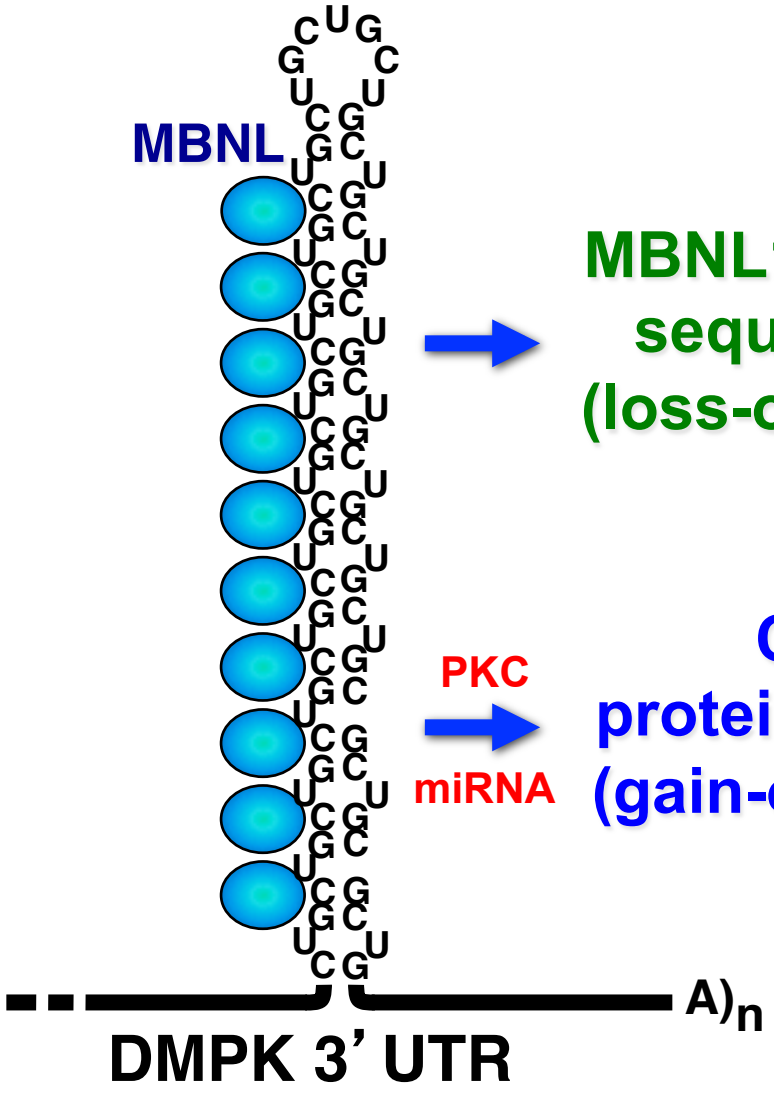


MBNL1 & MBNL2
sequestration
(loss-of-function)

PKC
miRNA

CELF1
protein induction
(gain-of-function)

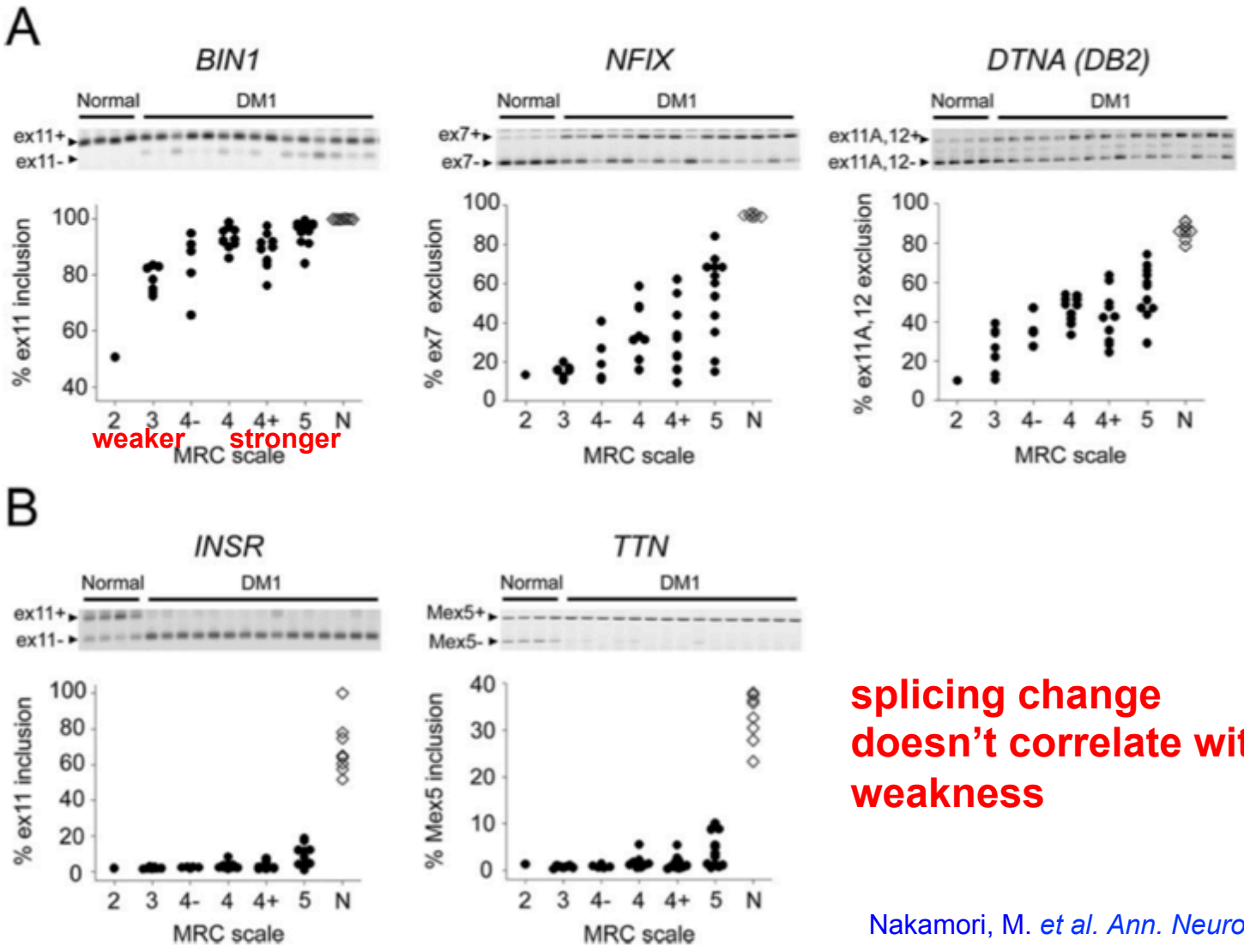
disrupted
developmental
splicing

misregulated
translation

mislocalized
mRNA

altered
mRNA stability

Extent of aberrant splicing for 20 events correlates with muscle weakness (TA dorsiflexion)



splicing change correlates with weakness

n = 45 DM1
n = 8 controls

splicing change doesn't correlate with weakness

Nakamori, M. *et al. Ann. Neurol.* **74,** 862 (2014).

What is the utility of a mouse model?

1. Reproduce pathogenic mechanisms for studies to identify additional therapeutic targets

2. Model for productive preclinical testing

What are ideal features?

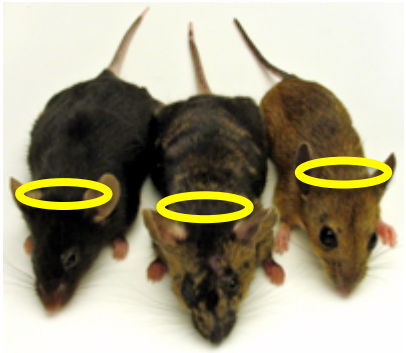
1. All affected tissues in one mouse model (CNS, heart, muscle, GI, etc.)
   - e.g., use DMPK to drive expression in correct tissues

2. Alternatively use clinical data to determine what promoters to use to express the CUGexp RNA
   - e.g., are GI symptoms due to autonomic nervous system or smooth muscle (or both?)

3. Straightforward mouse population maintenance and expansion

4. Goldilocks mouse: phenotype that is progressive, not too subtle and not too severe

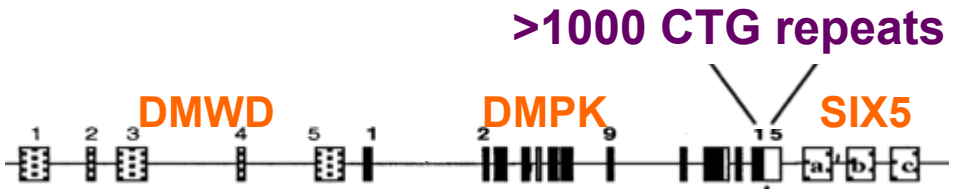
5. Model adult and congenital DM1



What do we have: published DM1 models

skeletal muscle specific (transgene)

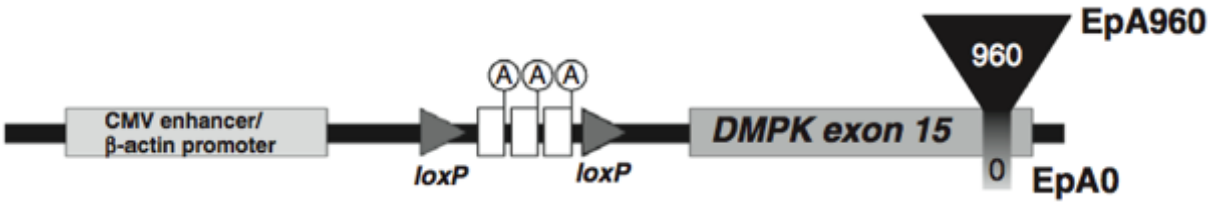
Mankodi, A. *et al*. 2000. *Science* 289, 1769–1773



multisystemic expression (transgene)

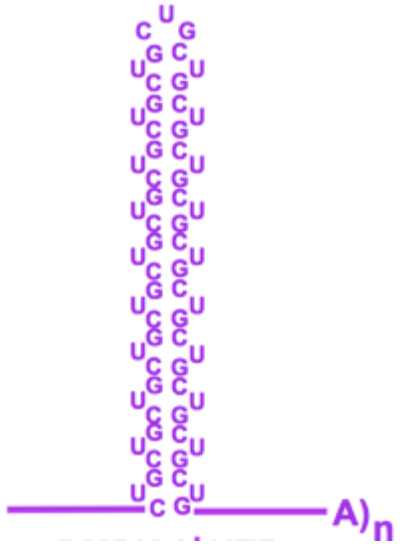
Seznec, H. *et al*. 2001. *HMG*. 10, 2717–2726

Huguet, A. *et al*. 2012 *PLoS Genet* 8, e1003043



Conditional, skeletal muscle or heart specific (transgene)

Orengo, J.P. *et al*. 2008. *PNAS*. 105, 2646–2651



Conditional, skeletal muscle and heart expression (transgene)

Mahadevan, M.S. *et al*. 2006. *Nat. Genet*. 38, 1066–1070

Gomes-Pereira et al *Trends Mol Med* 17, 506 (2011)

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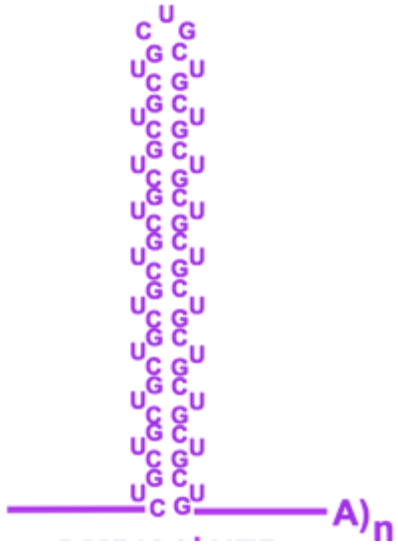

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HSA^{LR}

Mankodi, A. *et al*. 2000. *Science* 289, 1769–1773

1. Charles Thornton M.D., Univ. Rochester
2. 250 CTG repeats in the 3' UTR of the human skeletal alpha actin gene
3. expressed only in skeletal muscle
4. used as homozygote for stronger phenotype
5. >1000 fold higher expression than endogenous DMPK

6. molecular features
   - robust splicing abnormalities
   - CUGexp RNA foci with Mbnl co-localization
   - characteristic transcriptomic changes

7. phenotypic features
   - centralized nuclei
   - myotonia
   - age-dependent myopathy (centralized nuclei, fiber hypertrophy, ringed fibers, size variability)

HSA^{LR}

Mankodi, A. *et al*. 2000. *Science* 289, 1769–1773

Cons

1. limited to skeletal muscle expression
2. does not contain DMPK sequence
3. expression of CUGexp RNA very high compared to DM1 muscle
4. weak muscle wasting phenotype despite robust histopathology

DMSXL

>1000 CTG repeats

Seznec, H. *et al*. 2001. *HMG*. 10, 2717–2726

1. Genvieve Gourdon, Inserm Paris, France
2. transgene containing 45 kb human genomic segment, >1000 CTG repeats
3. used as homozygote for stronger phenotype
4. expression:
   - heart (0.3x endogenous DMPK)
   - muscle (0.1x endogenous DMPK)
   - brain (3x endogenous DMPK)

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   - weak splicing abnormalities muscle, heart, brain; lessen with aging in muscle and heart
   - RNA foci in muscle, heart, brain (neurons and glia)
   - Celf1 increased in brain

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6. phenotypic features
   - general
     - 60% mortality of HOM from HET matings before weaning
     - 50% size first month and 60-80% of wild type size at 2 months

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     - grip strength reduced but not significant when standardized to muscle weight
     - weak and variable myotonia

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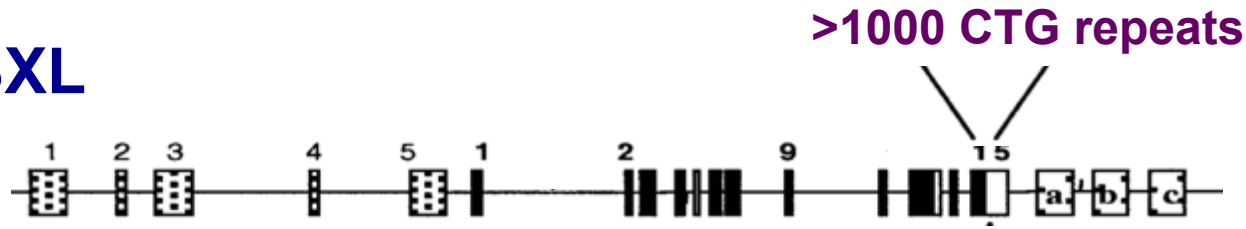
     - weak and variable myotonia
   - heart:
     - normal ECG at baseline, enhanced sensitivity to sodium channel blocker flecainide in 8-month-old DMSXL mice
     - developed mild abnormal echo parameters by 8 months of age
     - abnormal gating properties of the sodium current in isolated cardiomyocytes
   - brain:
     - behavioral differences (anxiety)
     - spatial memory reduced

DMSXL



Seznec, H. *et al*. 2001. *HMG*. 10, 2717–2726

Cons

1. animals born small and “sick”; phenotypic features in heart and muscle are weak. Therefore difficult to assay for rescue of phenotype beyond assays for molecular rescue
2. definitely “sick” but concerns about whether all phenotypes represent DM1
3. transgene inserted into a protein coding gene for which model is homozygous knock out
4. potential somatic instability

DM5 and DM200

Conditional, skeletal muscle or heart specific (transgene)

Mahadevan, M.S. *et al.* 2006. *Nat. Genet.* 38, 1066–1070

1. Mani Mahadevan, Univ. Virginia
2. CTG5 used in most papers; CTG200 poorly expressed - used only in one recent paper as back up
3. heart and muscle phenotypes described
4. reversible pathology

Cons

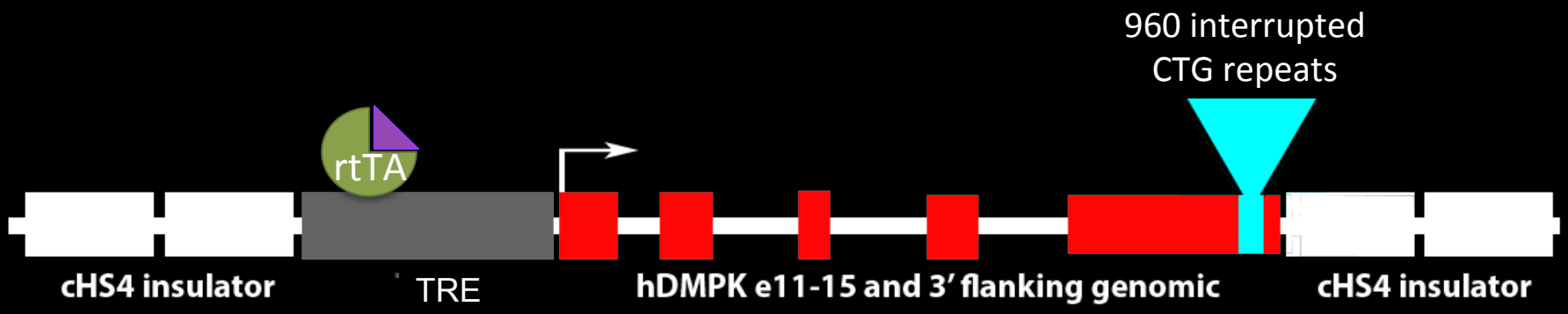
1. CTG5 pathogenic without expansion; potentially other aspects of transgene are pathogenic
2. CTG200 only used in one publication as back up
3. Extremely high level of expression

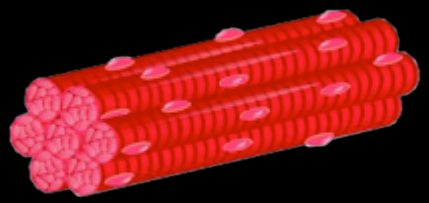
Additional mouse models

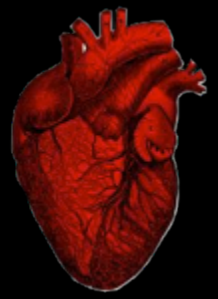
Additional mouse models

MDF and the Wyck Foundation have entered into a one-year partnership with Dr. Cat Lutz and Jackson Laboratory (Bar Harbor, ME) to develop a new mouse model of myotonic dystrophy type 1 (DM1).

Tet-inducible expression of DMPK-CUG$_{960}$ RNA in heart or skeletal muscle







Ginny Morris, Ph.D.

RNA foci
Mild alternative splicing changes
Histopathology
Significant muscle wasting by 10 weeks

RNA foci
Strong alternative splicing changes
altered ECG
altered echocardiography

Induce at postnatal day 1 (through nursing doe) or adult (6-10 weeks old)

Tissue	Model	HSA^{LR}	DMSXL	CTG5	TRE-H muscle	TRE-H heart
muscle	Myotonia	✔	✔	✔	✔	
muscle	Histopathopathology	✔	✔	✔	✔	
muscle	Wasting	+14 mo	✔	✔*	✔	
muscle	Weakness (grip)	✔	✔#	✔	✔	
muscle	RNA foci	✔	✔	no	✔	
muscle	MBNL colocalization	✔	✔	no	✔	
muscle	Celf1 upregulation	inconsistent	?	✔**	✔@	
muscle	Mis-splicing	✔	mild, resolves	✔	✔	
heart	Histopathopathology		?	✔		✔
heart	Abnormal ECG		stimulated	✔		✔
heart	Abnormal echo		8 mo	✔		✔
heart	RNA foci		✔	no		✔
heart	MBNL colocalization		✔	no		✔
heart	Celf1 upregulation		mild; 1 of 4	no		?
heart	Mis-splicing		mild, resolves	✔		✔
brain	RNA foci		✔	?		
brain	MBNL colocalization		?	?		
brain	Celf1 upregulation		✔	?		
brain	Mis-splicing		✔	?		
brain	Functional abnormal.		✔	?		

* severe degeneration

** potentially secondary to severe degeneration

@ only by immunofluoresence; not deterected by western.

grip strength reduced but not significant when standardized to muscle weight

Should we consider other mammalian models?

Acknowledgements

The Cooper Lab

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Muge Kuyumcu-Martinez, Ph.D.
Johanna Lee, Ph.D.
Donnie Bundman

Early onset myotonic dystrophy

DEVELOPMENTAL MEDICINE & CHILD NEUROLOGY ORIGINAL ARTICLE

Parent-reported multi-national study of the impact of congenital and childhood onset myotonic dystrophy

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Patient-scored symptoms in adult onset DM1

	Total population
Total no. of respondents	278
Symptomatic themes	
1: Problems with hands or arms	
Prevalence, %	93.5
Relative impact on lives (SD)	2.27 (1.22)
2: Fatigue	
Prevalence, %	90.8
Relative impact on lives (SD)	2.49 (1.22)
3: Myotonia	
Prevalence, %	90.3
Relative impact on lives (SD)	2.09 (1.30)
4: Impaired sleep or daytime sleepiness	
Prevalence, %	87.9
Relative impact on lives (SD)	2.25 (1.31)
5: Problems with physical health[c]	
Prevalence, %	86.9
Relative impact on lives (SD)	1.97 (1.21)
6: Limitations with mobility or walking	
Prevalence, %	85
Relative impact on lives (SD)	2.42 (1.21)
7: Inability to do activities	
Prevalence, %	84.6
Relative impact on lives (SD)	2.35 (1.20)
8: Gastrointestinal issues	
Prevalence, %	83.4
Relative impact on lives (SD)	1.86 (1.31)
9: Pain	
Prevalence, %	74
Relative impact on lives (SD)	1.82 (1.35)
10: Problems with vision, hearing, or smell	
Prevalence, %	71.2
Relative impact on lives (SD)	1.57 (1.18)

Neurology **79,** 348–357 (2012)

I have not used these mice so this is from the literature but those who have worked with them can speak up

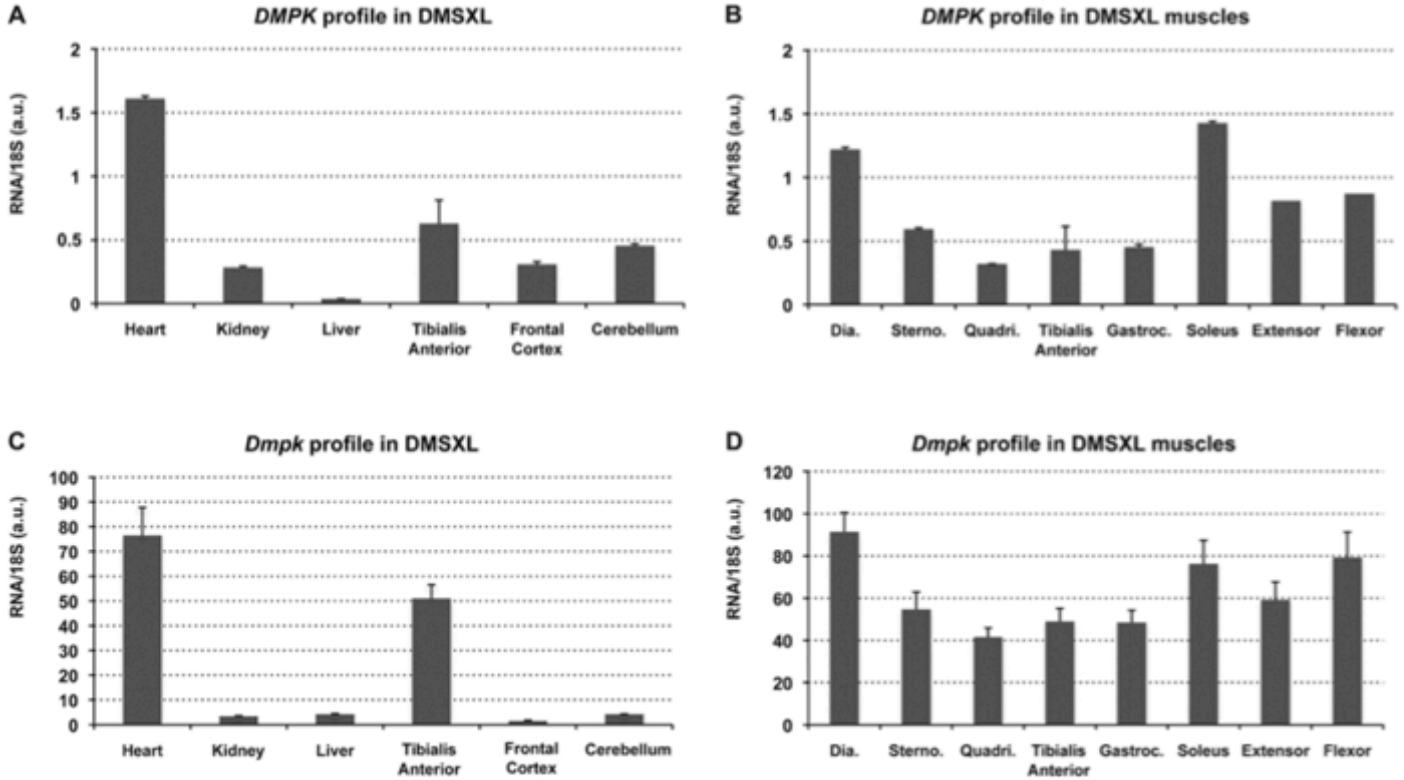
45 kb genomic segment
low expression in muscle
WHAT IS EXPRESSION IN HEART
HIGH EXPRESSION IN FRONTAL CORTEX
transgene is inserted into an endogenous gene; the impact needs to be evaluated
HOM 50% size “during” first month and 60-80% at 2 months
60% mortality of HOM from HET matings before weaning then only 5%
lower fasting levels of IGFBP3 and insulin
muscle fibers 31% reduction in area in TA, no difference fiber number
weak splicing changes in heart and muscle lessen over time
myotonia weak, variable and only with needle insertion
grip strength reduced but not sig with normalized to body weight
brain – foci through out in neurons and glia region specific differences
brain – mild splicing changes but revert to fetal, elevated Celf1 and 2 and increased PO4 of Celf1
brain – increased anxiety open field test but only first minute
brain – increased anxiety indicated by better buried marbles
brain – spatial memory Morris water maze – reduced
brain - electrophysiological profiling of DMSXL hippocampus -no major deficits in basal transmission
brain - the repeat expansion may affect (directly or indirectly) a limited number of synaptic targets

1. Gudde, A. E. E. G., González-Barriga, A., van den Broek, W. J. A. A., Wieringa, B. & Wansink, D. G. A low absolute number of expanded transcripts is involved in myotonic dystrophy type 1 manifestation in muscle. *Hum. Mol. Genet.* **25,** 1648–1662 (2016).

2. hemizygous DMSXL RNA is 10 fold LOWER than endogenous DMPK in muscle tissue which is 1-20 copies per cell
3. homozygous HSALR RNA 1000 fold higher than endogenous DMPK in muscle tissue which is 1-20 copies per cell
4. DMPK mRNA in human skeletal muscle is same in DM1 and normal and equal expanded and non expanded alleles; muscle tissue which is 1-20 copies per cell and each foci in cell culture is one to a few RNA molecules

1. Huguet, A. *et al.* Molecular, Physiological, and Motor Performance Defects in DMSXL Mice Carrying >1,000 CTG Repeats from the Human DM1 Locus. *PLoS Genet* **8,** e1003043 (2012).

2. antisense transcripts, mild splicing defects, muscle affected and motor performance
3. DMSXL mRNA 1/3 endog in muscle and 3x in frontal cortex



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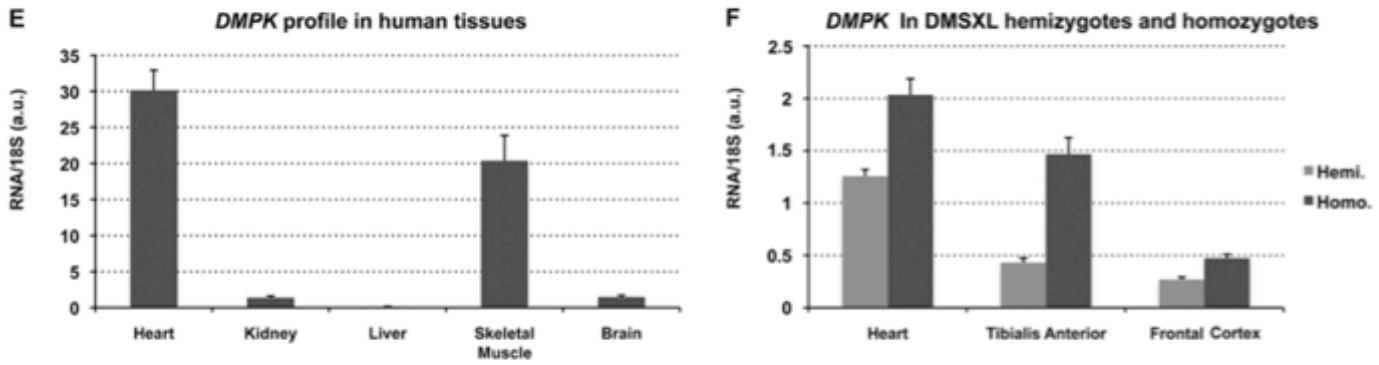
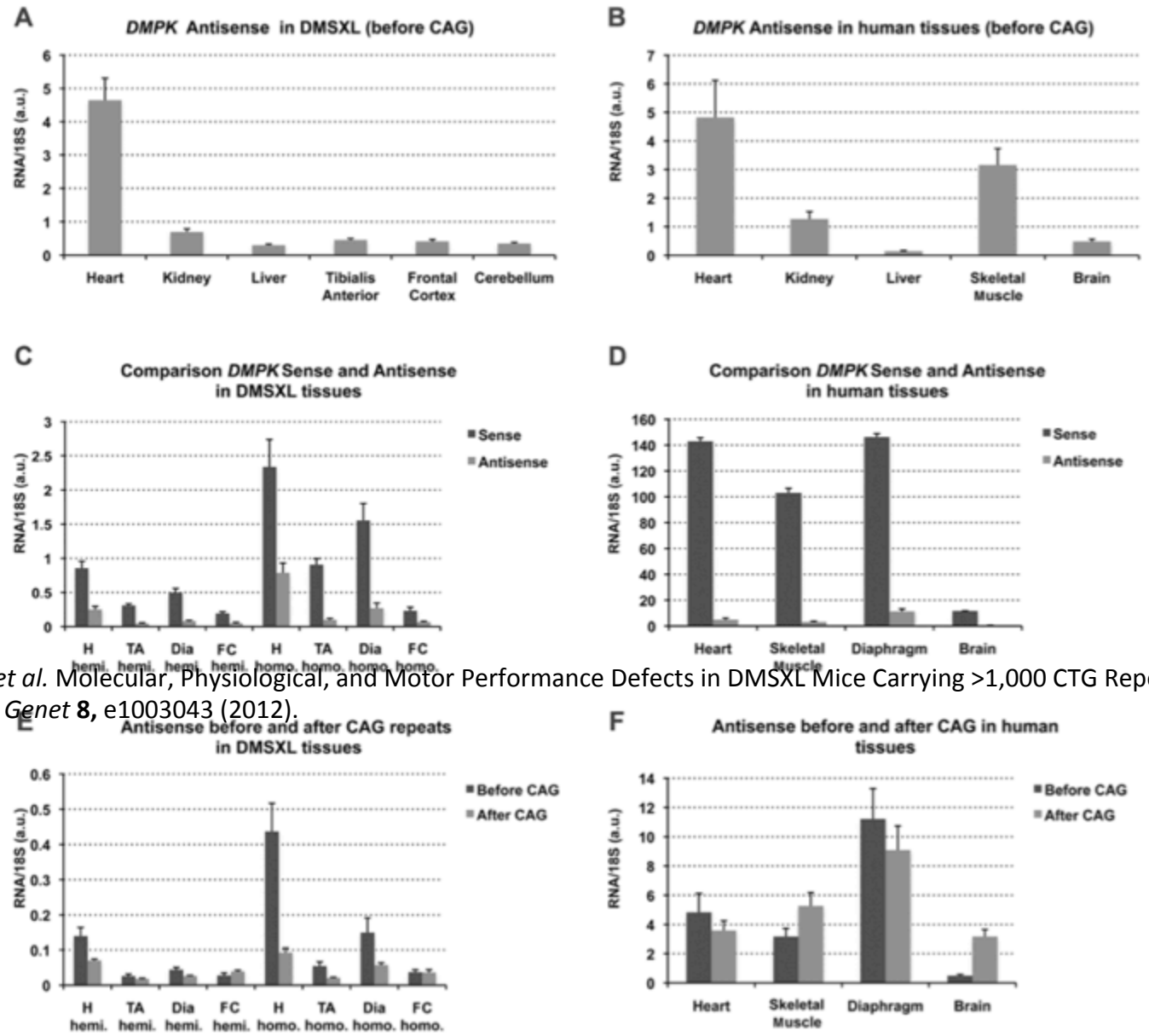


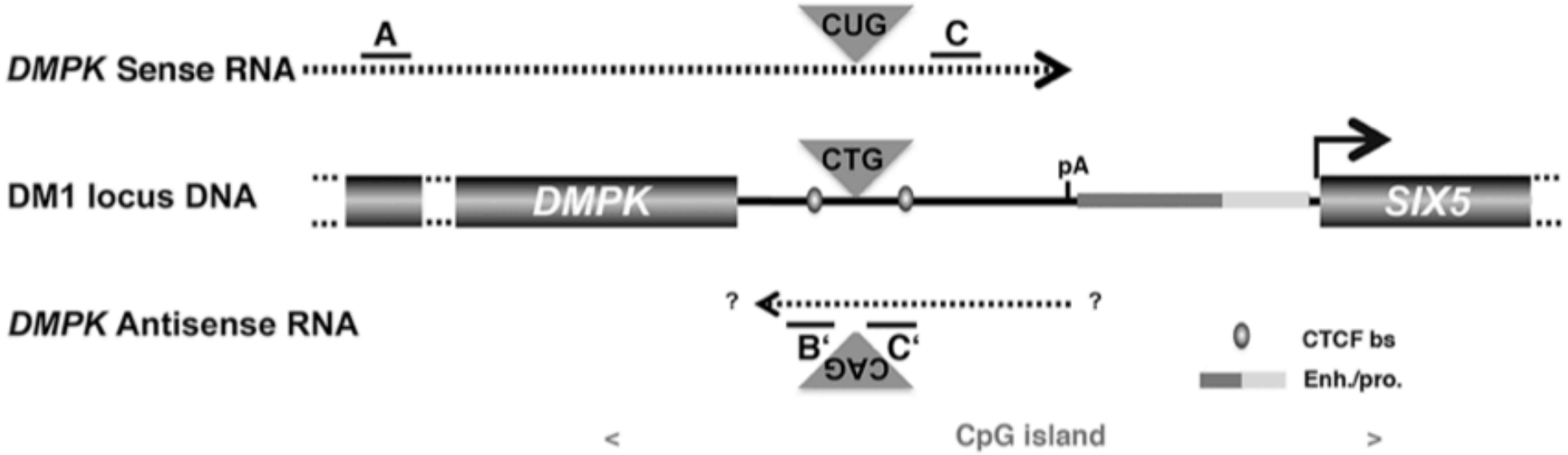
Figure 2. *DMPK* expression profiles. Expression of the human *DMPK* transgene was studied in various hemizygous DMSXL tissues (A) and muscles (B), in parallel with the endogenous *Dmpk* mouse gene (C and D) (n = 3). (E) Expression of *DMPK* in human tissues. Dia., Diaphragm; Sterno., Sternomastoid; Quadri, Quadriceps; TA, Tibialis Anterior; Gastroc. Gastrocnemius. (a.u.): arbitrary units. (F) Expression of *DMPK* in hemizygous (Hemi.) and homozygous (Homo.) DMSXL tissues. Data are presented as means ± standard deviation.
doi:10.1371/journal.pgen.1003043.g002



Huguet, A. *et al.* Molecular, Physiological, and Motor Performance Defects in DMSXL Mice Carrying >1,000 CTG Repeats from the Human DM1 Locus. *PLoS Genet* **8,** e1003043 (2012).

Figure 3. Expression of sense and antisense *DMPK* transcripts. (A–B) *DMPK* antisense expression profile in 4-month-old DMSXL homozygotes (n = 3) and human control adult tissues (commercial panel) using amplicon B′ located upstream the CAG repeat. (C–D) Comparison of *DMPK* sense and antisense transcript levels in 4-month-old DMSXL homozygotes (n = 3) and human control tissues. (E–F) Comparison of antisense transcript levels measured in 5′ (before) and in 3′ (after) of the CAG repeat using amplicons B′ and C′ in DMSXL and control human tissues. H, heart; TA, tibialis anterior; Dia, diaphragm; FC, Frontal Cortex; Hemi., Hemizygous; Homo. Homozygous. Data are presented as means ± standard deviation in arbitrary units (a.u.).

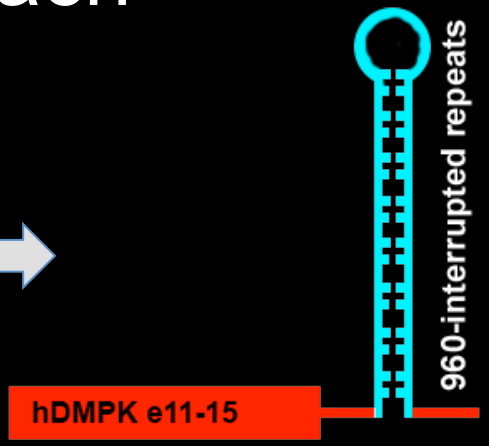
doi:10.1371/journal.pgen.1003043.g003

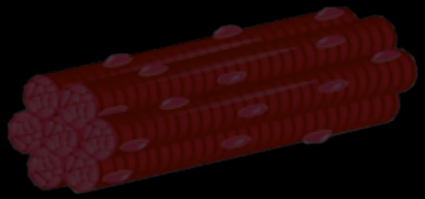


Huguet, A. *et al.* Molecular, Physiological, and Motor Performance Defects in DMSXL Mice Carrying >1,000 CTG Repeats from the Human DM1 Locus. *PLoS Genet* **8,** e1003043 (2012).

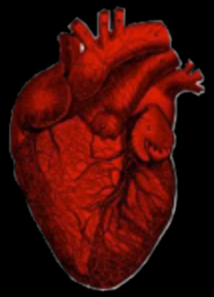
Experimental Approach

dox diet
(2 or 6 g/kg dox chow)





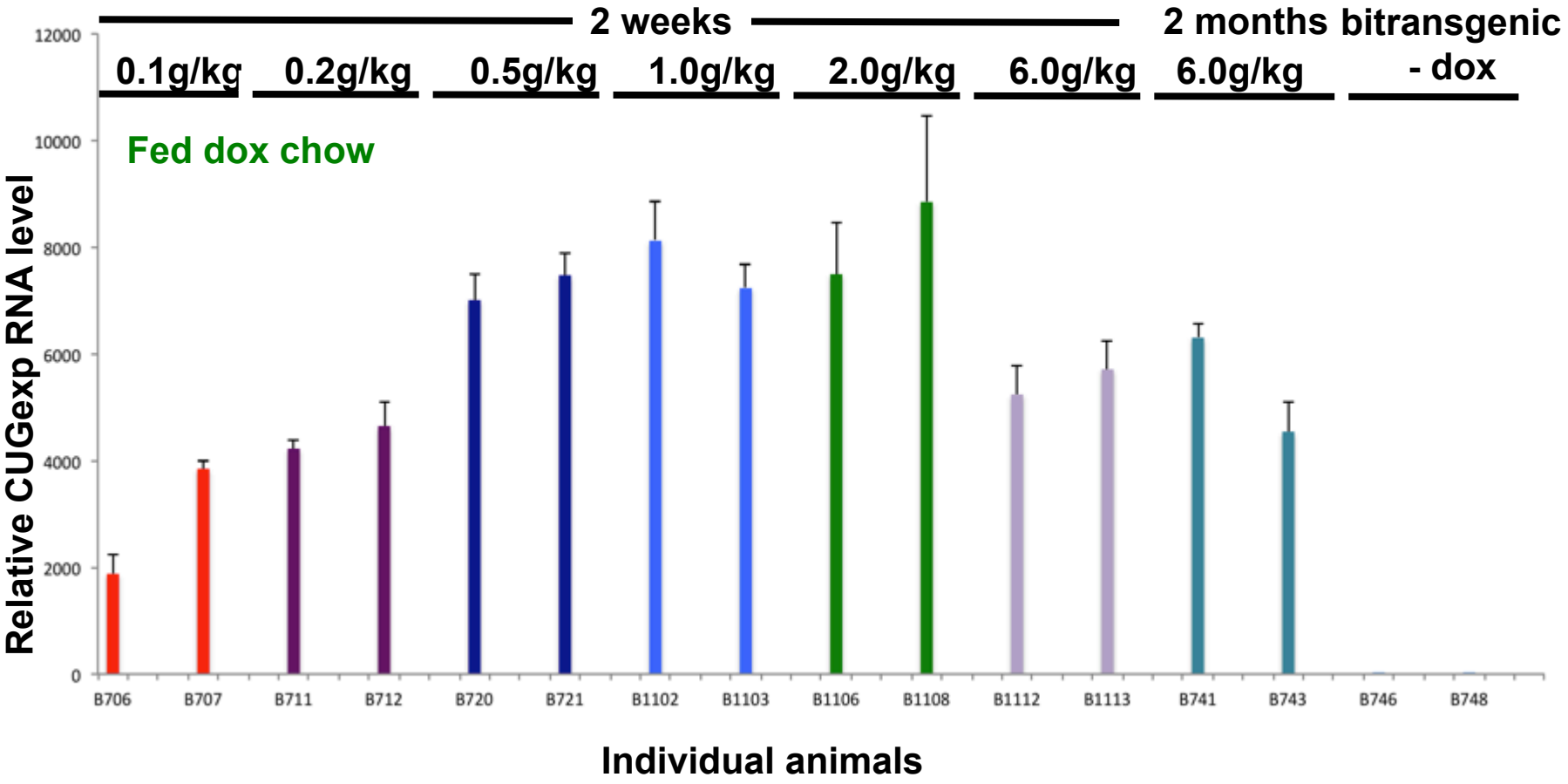
RNA foci
Alternative splicing changes
Histology
Muscle wasting



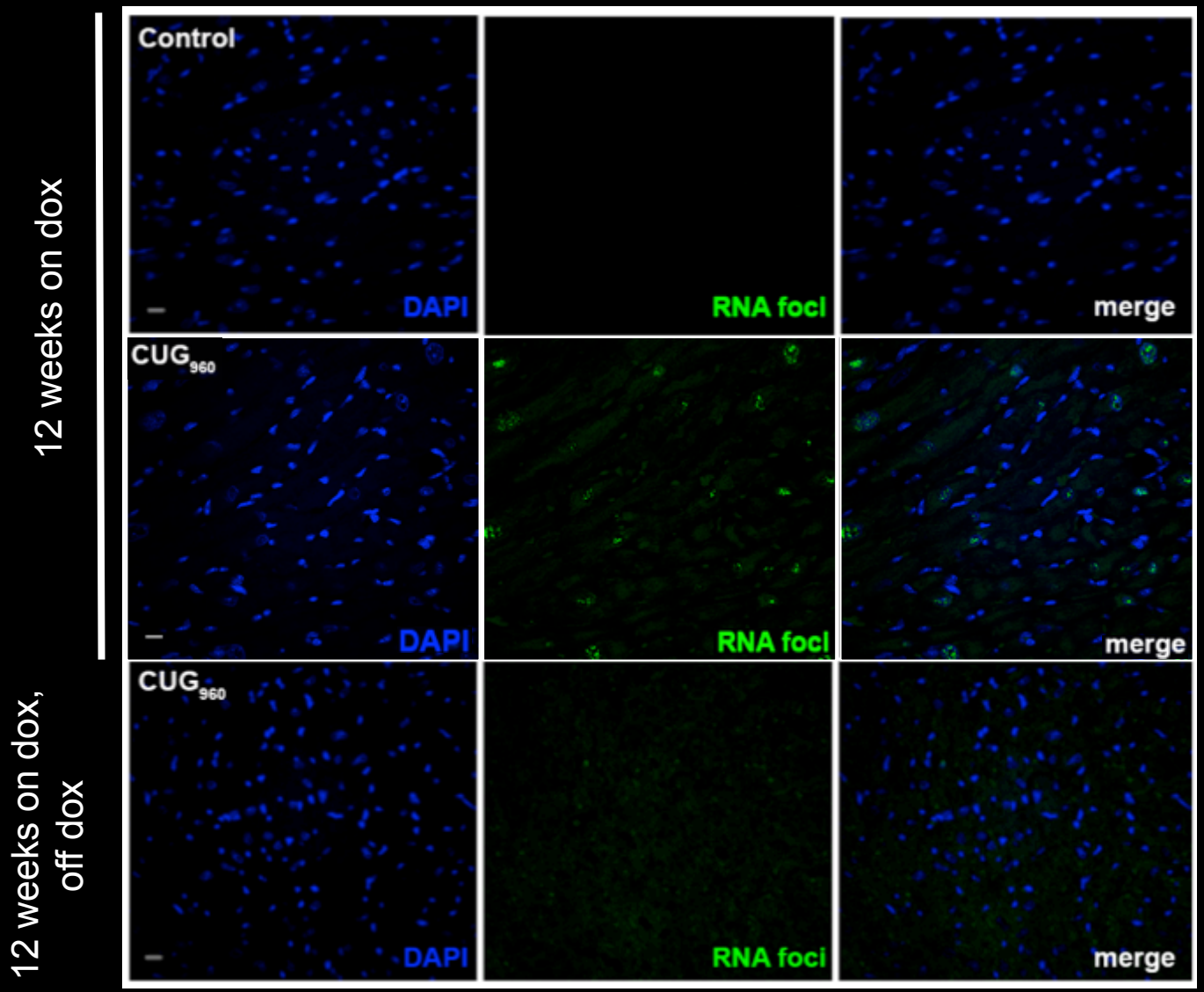
RNA foci
Alternative splicing changes
Heart size
Cardiac Function

Induce at postnatal day 1 (through nursing doe) or adult (6-10 weeks old)

Dose-response CUGexp RNA expression in DM1 heart model

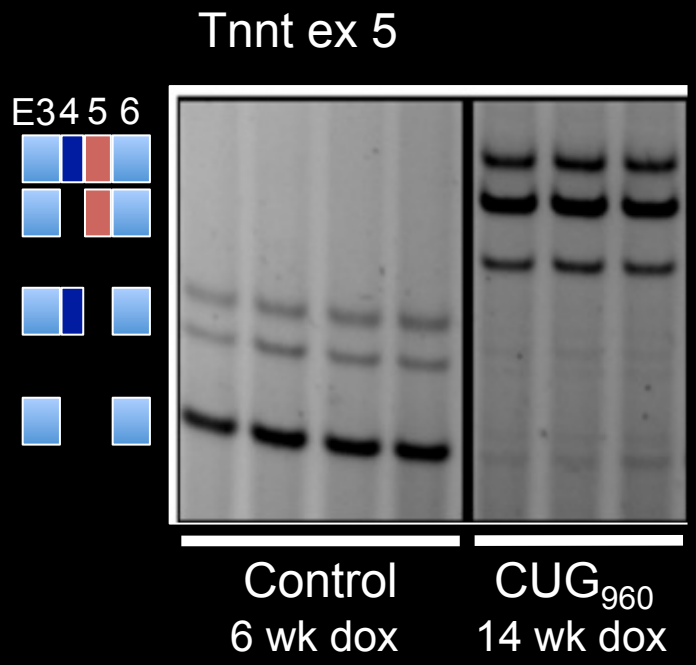


RNA foci are observed in CUG_{960} hearts and lost upon doxycycline removal

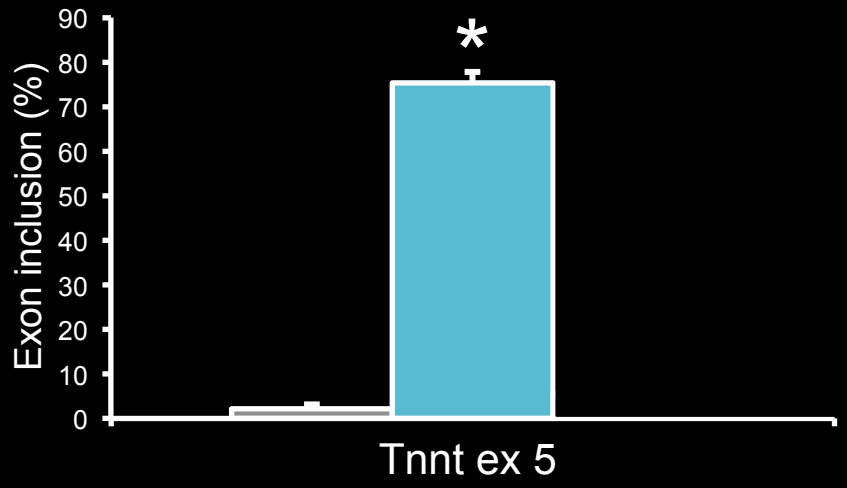


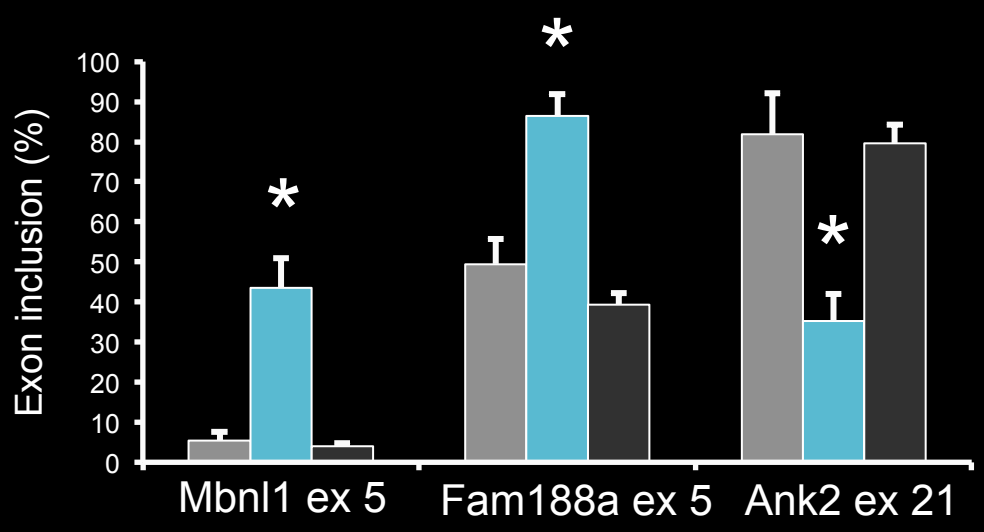
6 g/kg dox

DM1 splicing events are misregulated and reversible in CUG_{960} heart



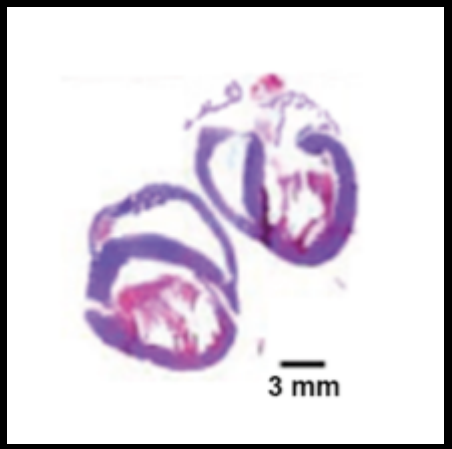
Control 6 wk dox, n = 4
CUG_{960} 14 wk dox, n = 3
CUG_{960} 14 wk dox/6 wk off, n = 4
*p < 0.05
6 g/kg dox induced PN1



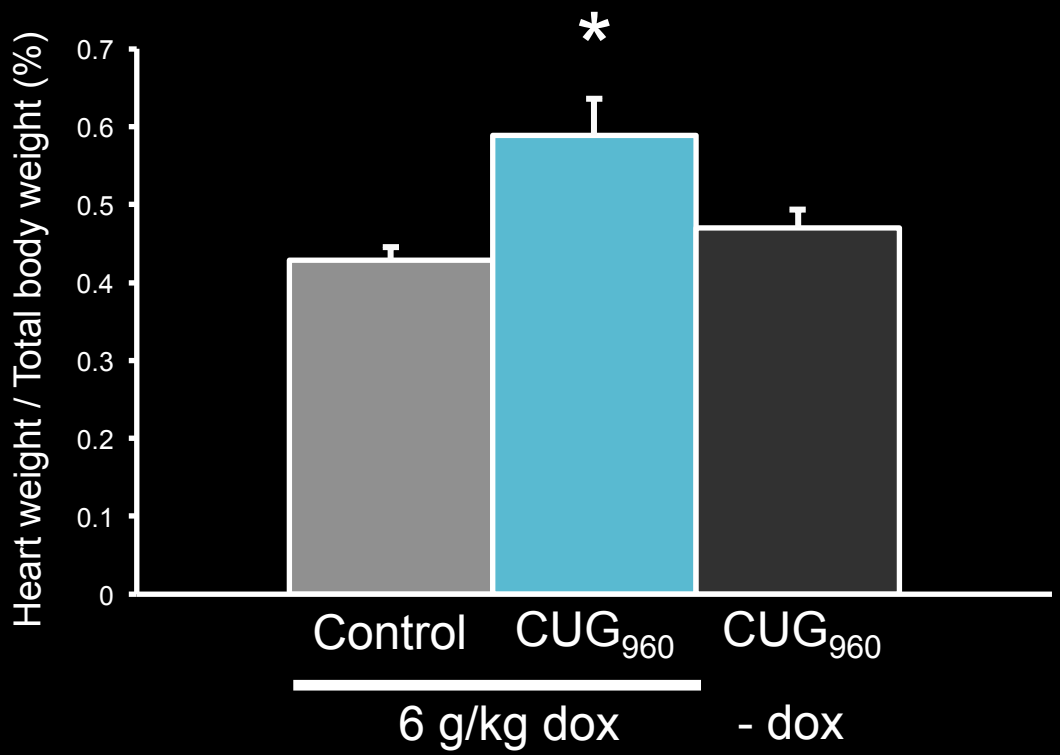


CUG_{960} hearts are enlarged compared with controls

CUG_{960}

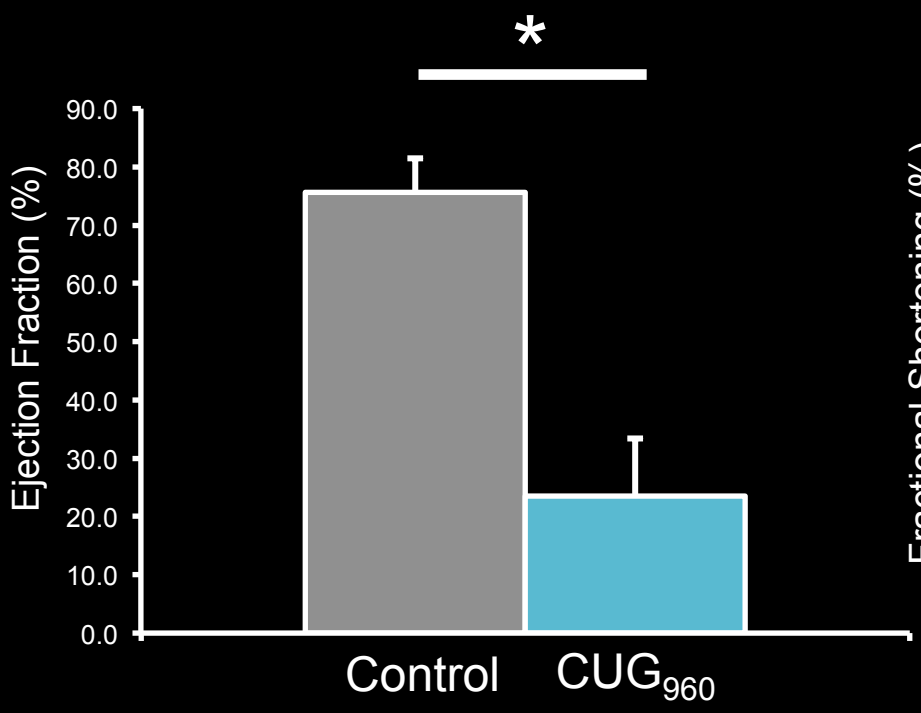


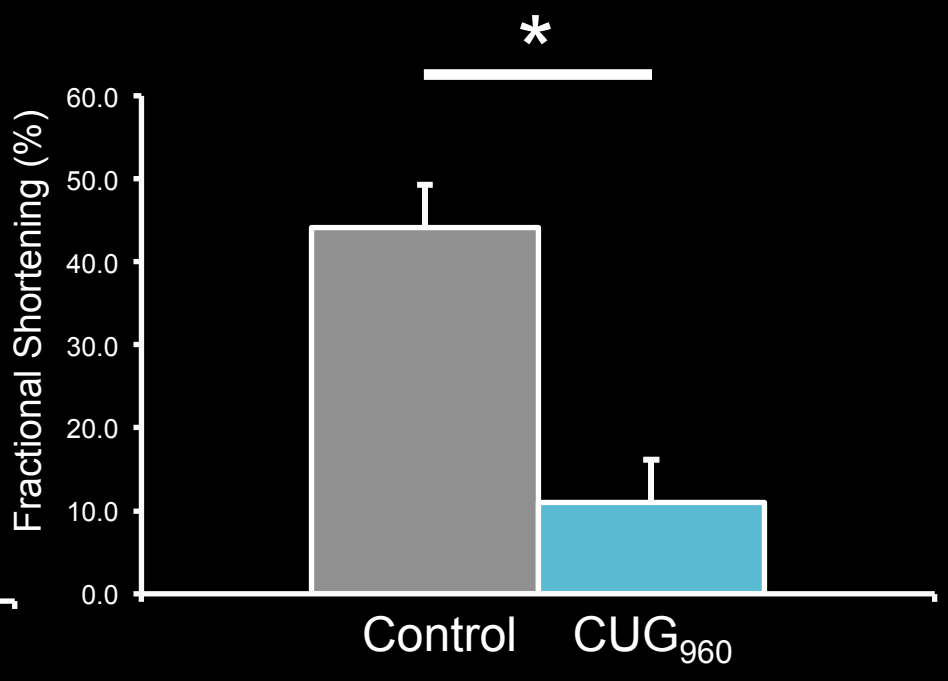
Control



* $p < 0.05$
Control n = 6
CUG_{960} +dox n = 10
CUG_{960} - dox n = 4
6 g/kg dox induced PN1

CUG_{960} mice show abnormal cardiac function



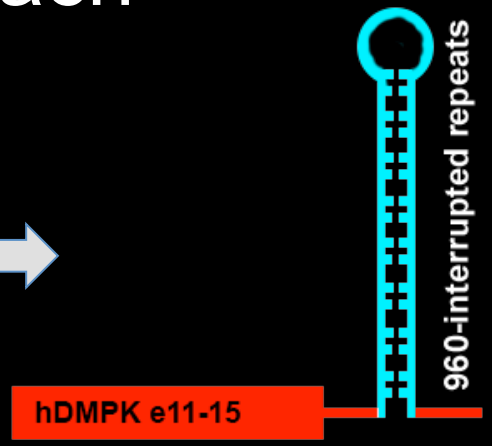


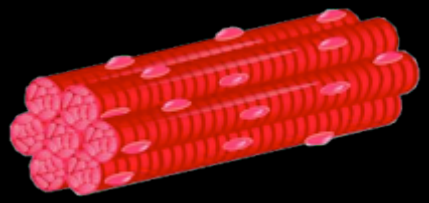
- ECG abnormalities
- 17 of 19 CUG_{960} mice abnormal…
- …but 8 of 16 control mice also abnormal
- addressing issues with background

Control n = 16
CUG_{960} n = 19
*p < 0.05
6 g/kg dox induced PN1

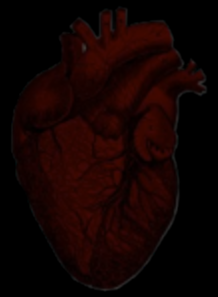
Experimental Approach

dox diet
(2 or 6 g/kg dox chow)





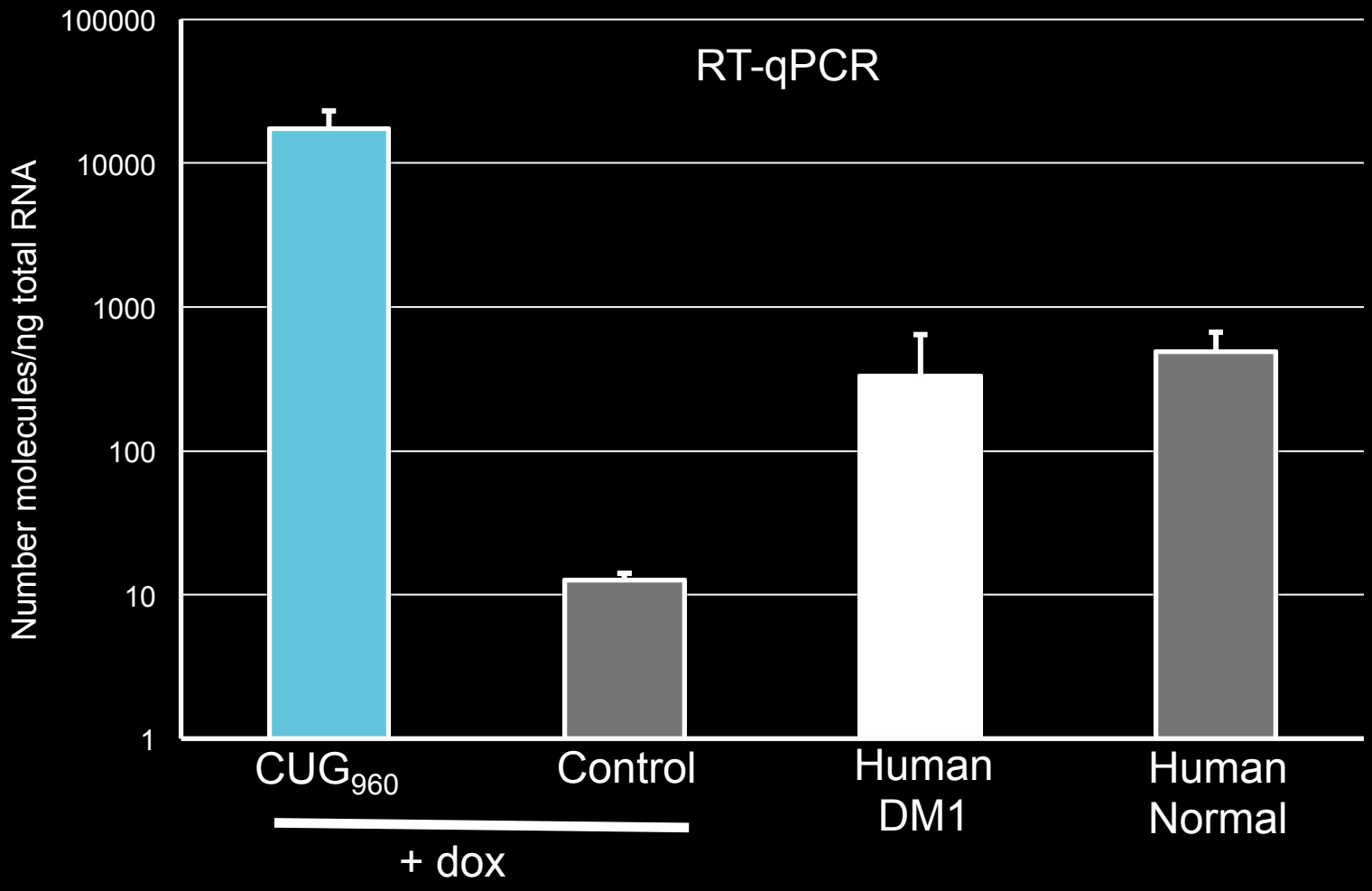
RNA foci
Alternative splicing changes
Histology
Muscle wasting



RNA foci
Alternative splicing changes
Heart size
Cardiac Function

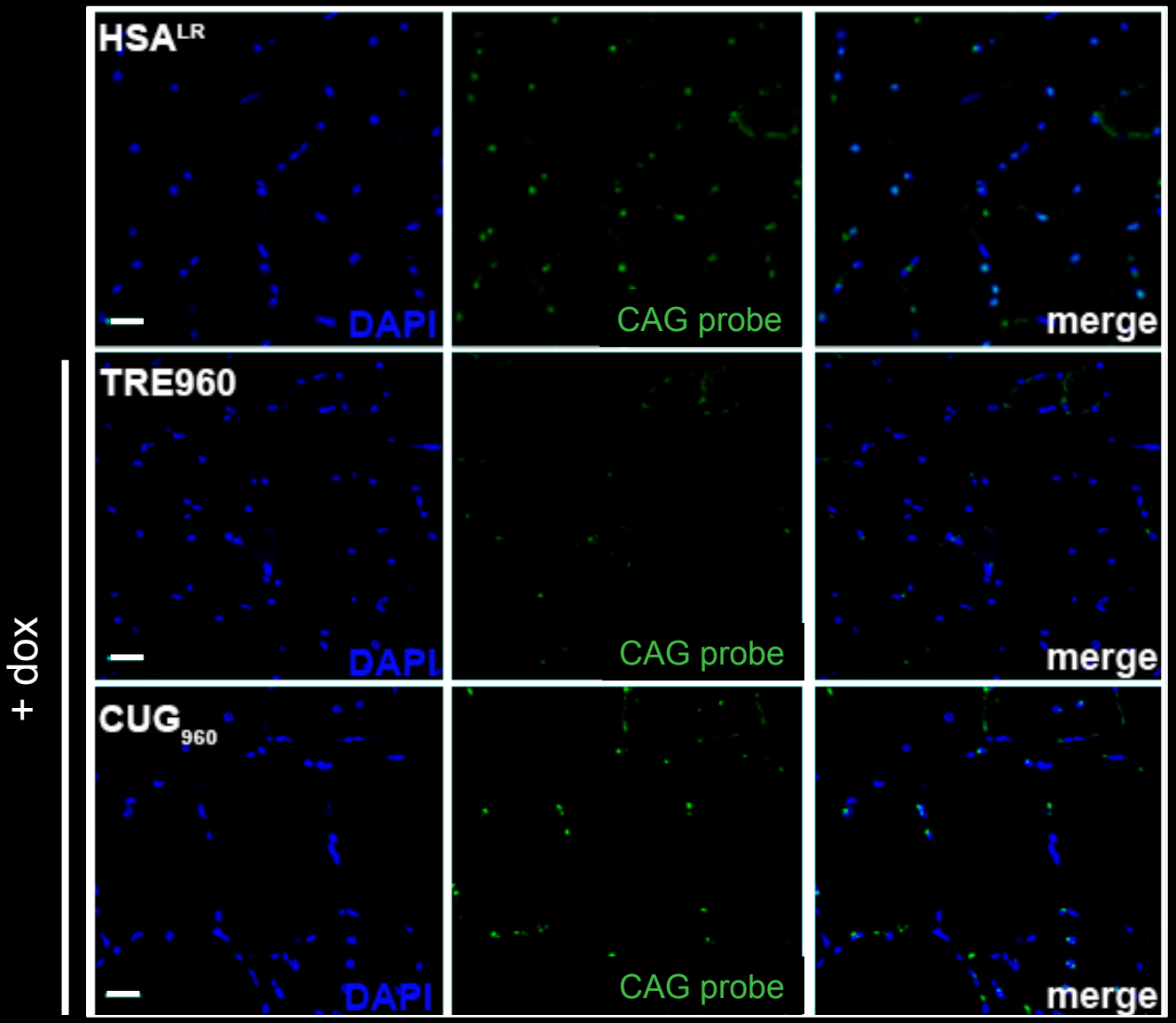
Induce at postnatal day 1 (through nursing doe) or adult (6-10 weeks old)

Expression of CUG_{960} transgene is >30x greater than DMPK expression in human skeletal muscle

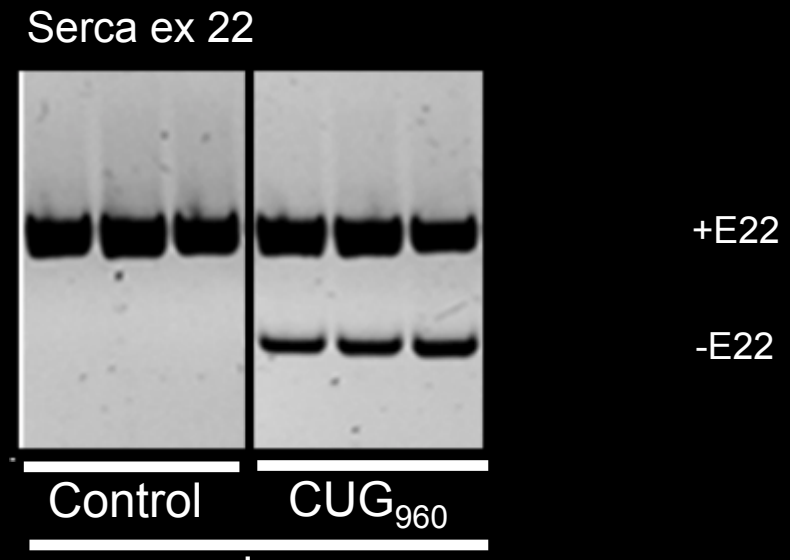


CUG_{960} +dox n = 4
Control + dox n = 2
Human DM1 n = 4
Human Normal n = 3

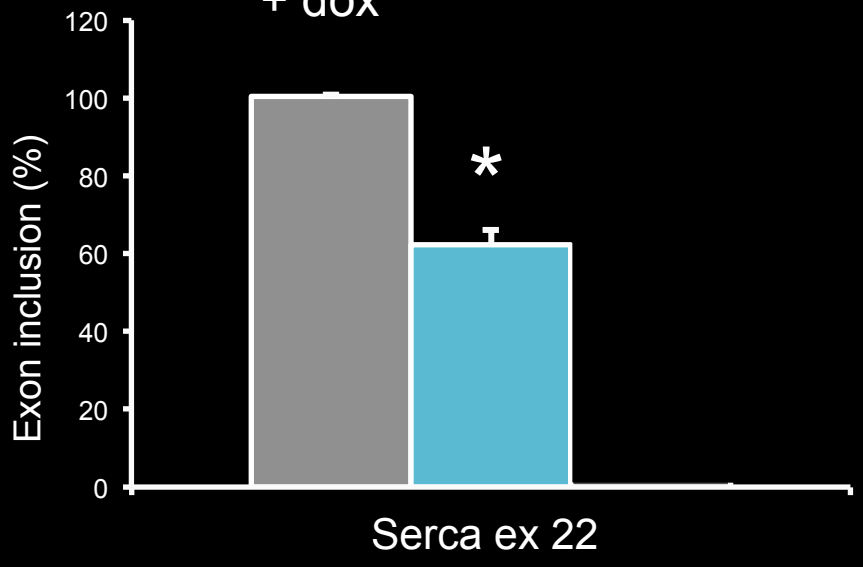
CUG_{960} skeletal muscles contain RNA foci

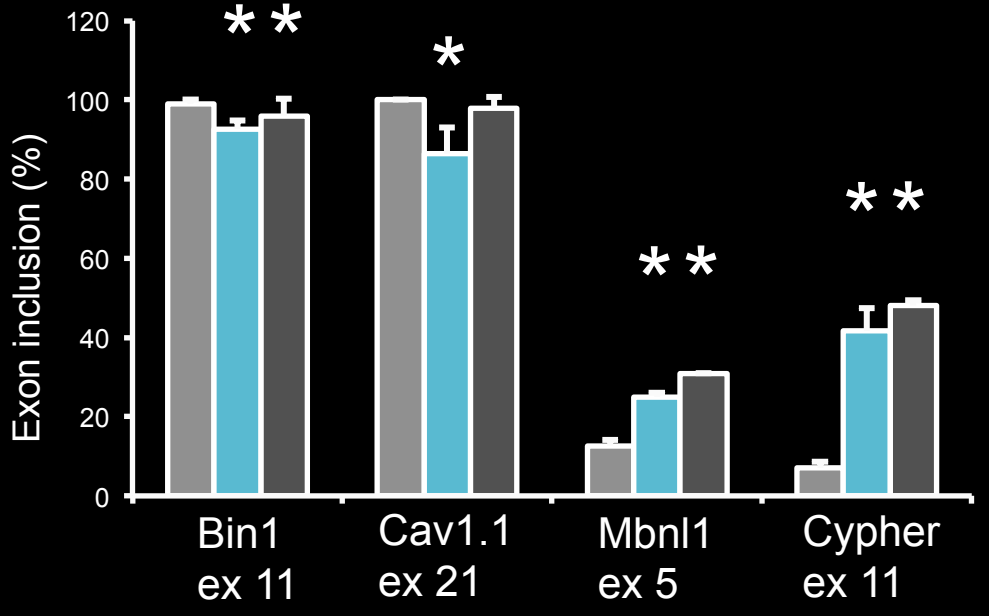


Splicing events misregulated in DM1 are misregulated in CUG_{960} muscle

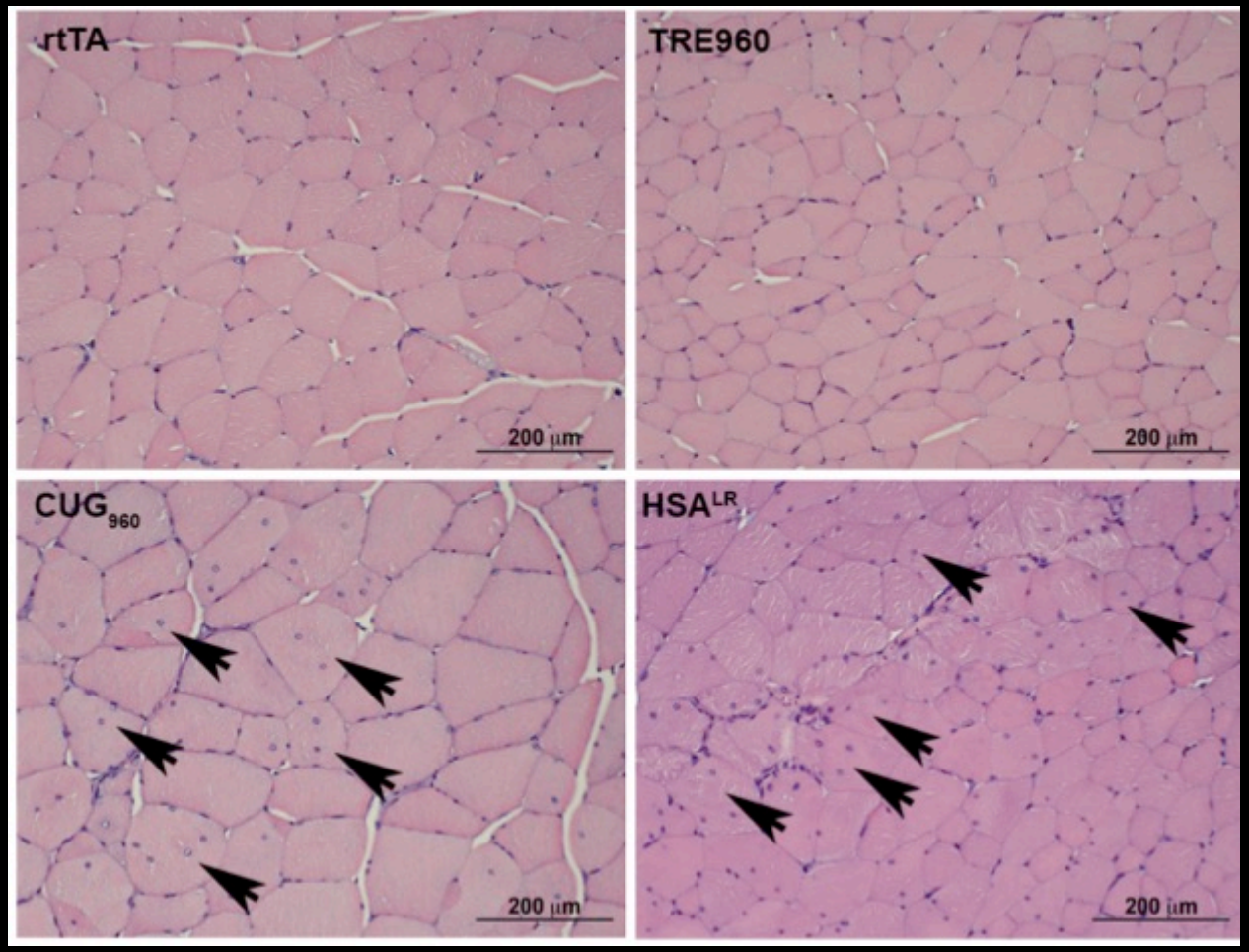


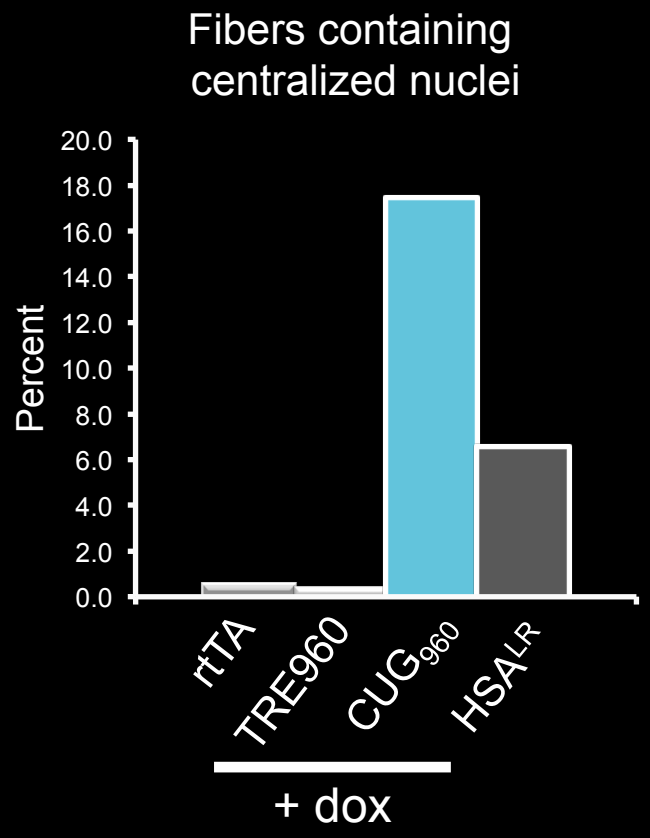
Control + dox, n = 3
CUG_{960} + dox, n = 3
HSA^{LR}, n = 2
*p < 0.05



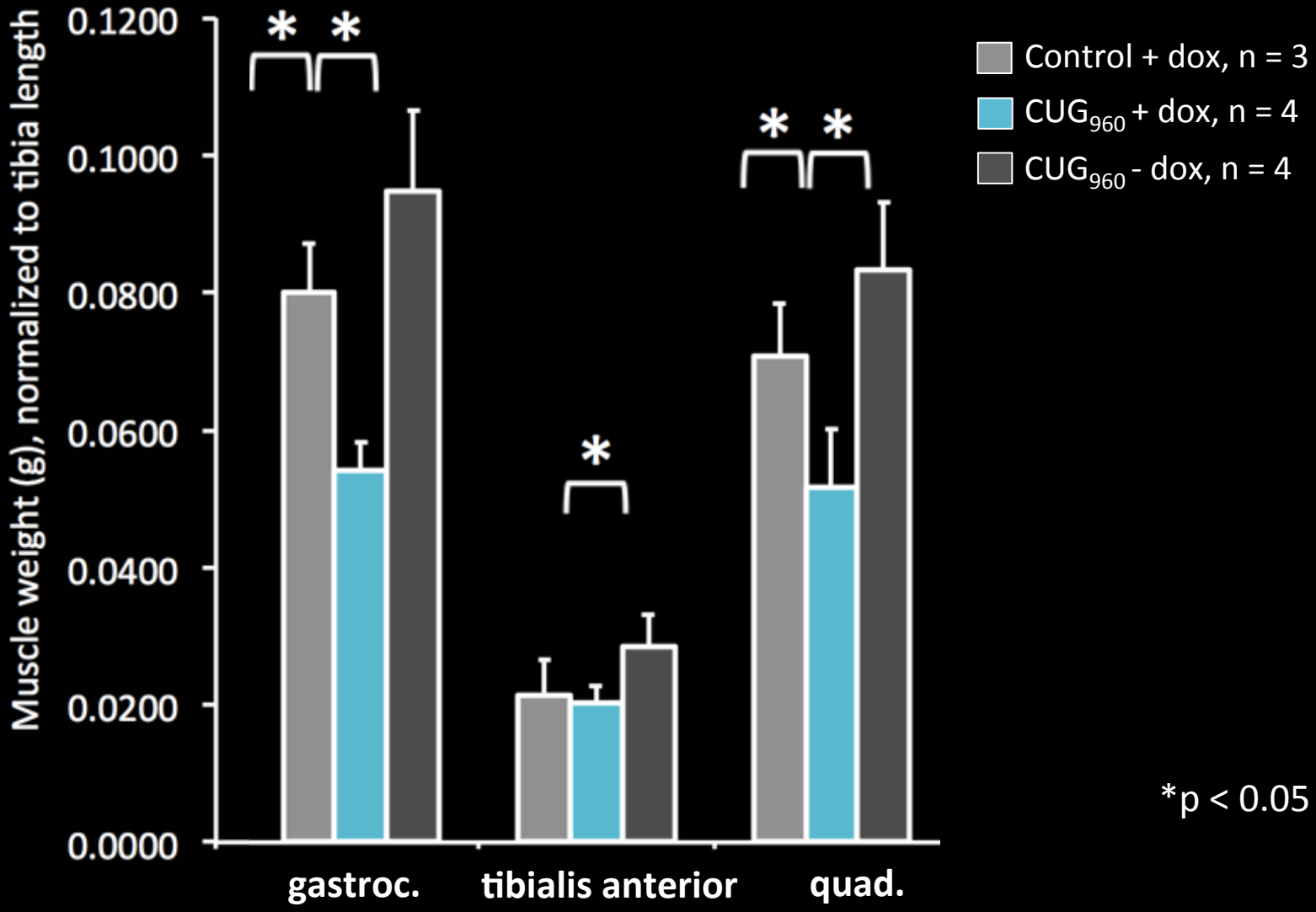


Severity of skeletal muscle histopathology is increased in CUG_{960} mice at four months





Muscle wasting is observed in CUG_{960} mice 12 weeks after induction at PN1



Summary of the skeletal muscle model

- CUG_{960} transgene is expressed at levels 30-50 x human tissue
- Splicing effects are mild while wasting is robust

Plans

- Determine whether turning off CUG_{960} RNA reverses muscle wasting
- Use transcriptome and signaling assays to identify changes relevant to mechanism of muscle wasting
- Use rescue as the assay to test mechanisms of muscle wasting
    - replace Mbnl1 and Mbnl2
    - deplete Celf1

Comparison of expanded repeat mouse models

Model	HSA^{LR}	DMSXL	CTG5
Myotonia	✔	✔	✔
Myopathy	✔	✔	✔
Wasting	Old mice only		
Weakness		✔	✔
Motor function			✔
RNA foci accumulation	✔	✔	
MBNL sequestration	✔		
Increased Celf1 levels	Inconsistent reports		✔
Mis-splicing events	✔	✔	✔

THERE IS MORE INFO ON BRAIN THAT CAN ADD TO THE CHART

Ginny Morriss, Ph.D.

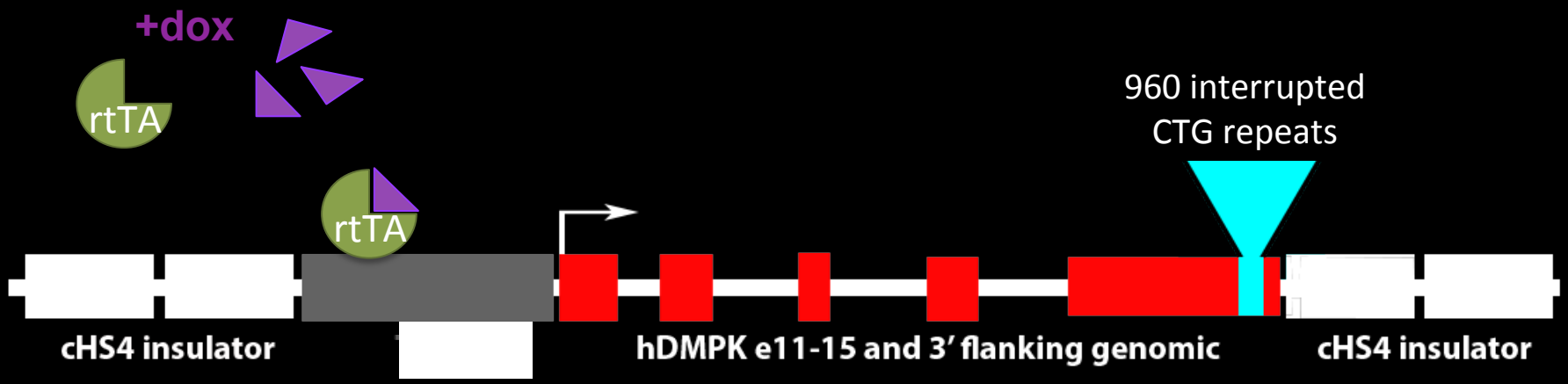
Comparison of expanded repeat models

Model	HSA^{LR}	DMSXL	EpA960	CTG5
Myotonia	✔	✔	✔	✔
Myopathy	✔	✔	✔	✔
Wasting	Old mice only		✔	
Weakness		✔		✔
Motor function			✔	✔
RNA foci accumulation	✔	✔	✔	
MBNL sequestration	✔		✔	
Increased Celf1 levels	Inconsistent reports		✔	✔
Mis-splicing events	✔	✔	✔	✔

Comparison of expanded repeat models

Model	HSA^{LR}	DMSXL	CTG5	TRE-H/MDAF
Myotonia	✔	✔	✔	✔
Myopathy	✔	✔	✔	✔
Wasting	old mice			
Weakness		✔	✔	✔
Motor function			✔	✔
RNA foci	✔	✔		✔
MBNL sequest	✔			✔
Celf1 upreg	inconsistent		✔	✔
Mis-splicing	✔	mild	✔	✔

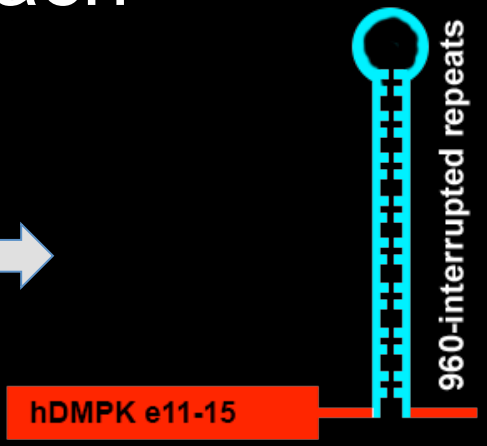
Tet-inducible expression of DMPK-CUG$_{960}$ RNA in heart or skeletal muscle

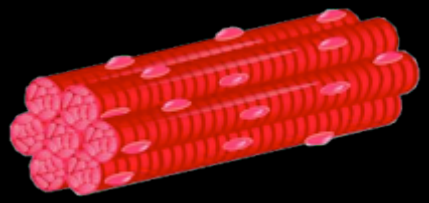


Ginny Morris, Ph.D.

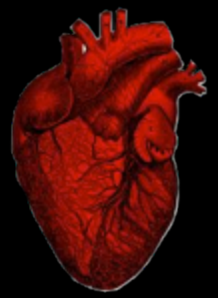
Experimental Approach

dox diet
(2 g/kg dox chow)





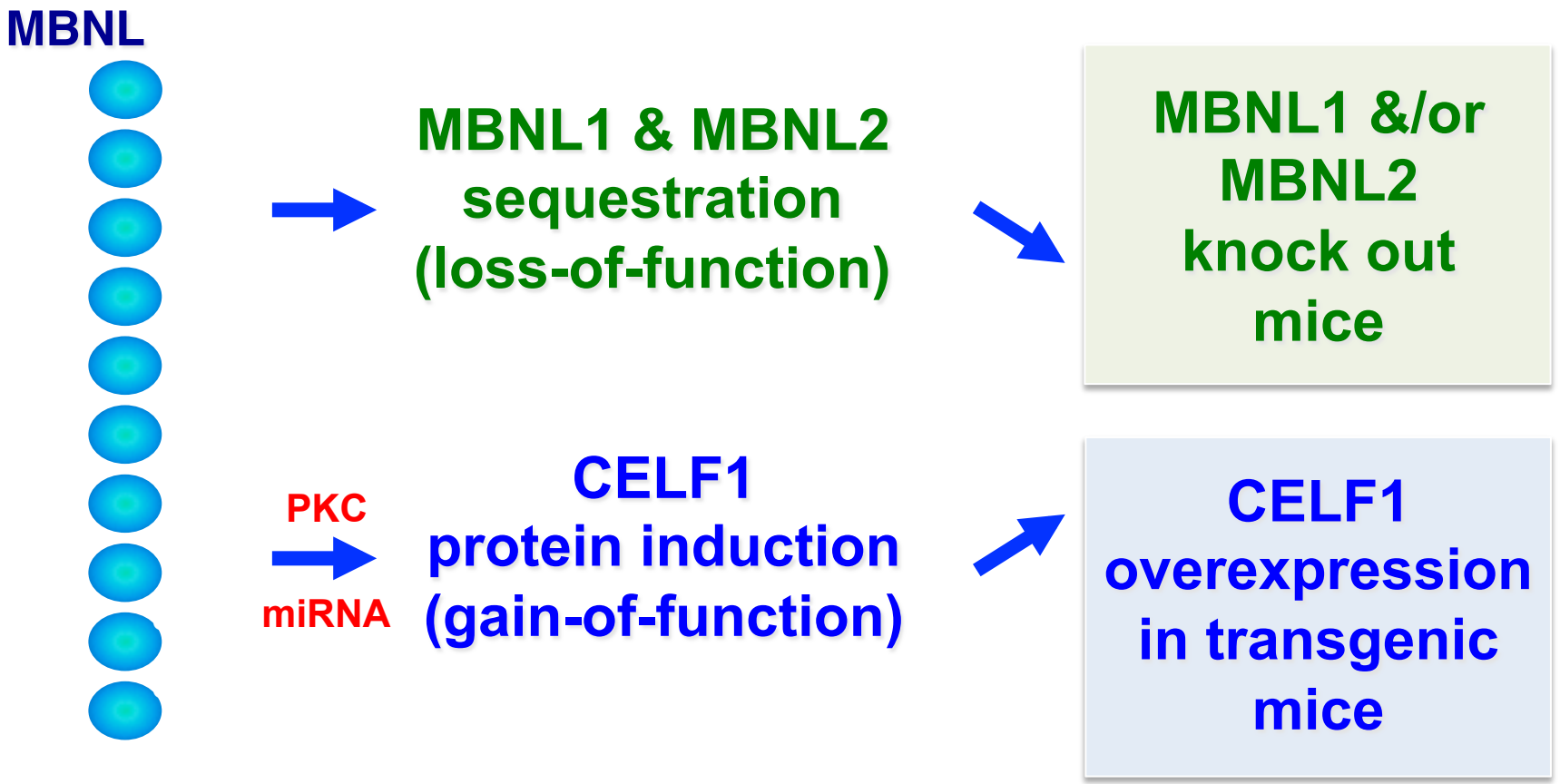
RNA foci
Mild alternative splicing changes
Histopathology
Muscle wasting



RNA foci
Strongalternative splicing changes
altered ECG
altered echocardiography

Induce at postnatal day 1 (through nursing doe) or adult (6-10 weeks old)

Modeling MBNL and CELF effects in mice



Modeling DM1 in mice

the RNA is the primary cause