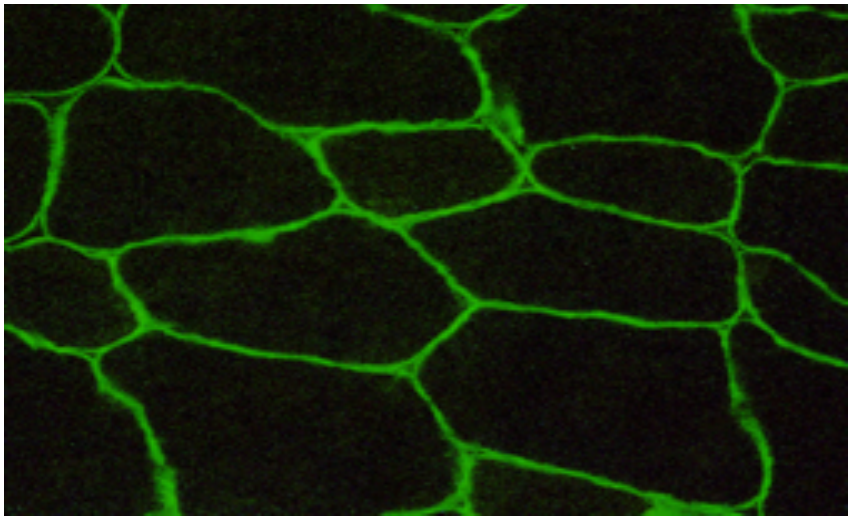
Mouse models of muscular dystrophies

- *mdx* : spontaneously occurring mutation of dystrophin-deficiency.
  - muscle pathology: degeneration and regeneration of muscle fibers
  - absence of DAPC from the muscle membrane
  - deficits in muscle force generation and elevated CK

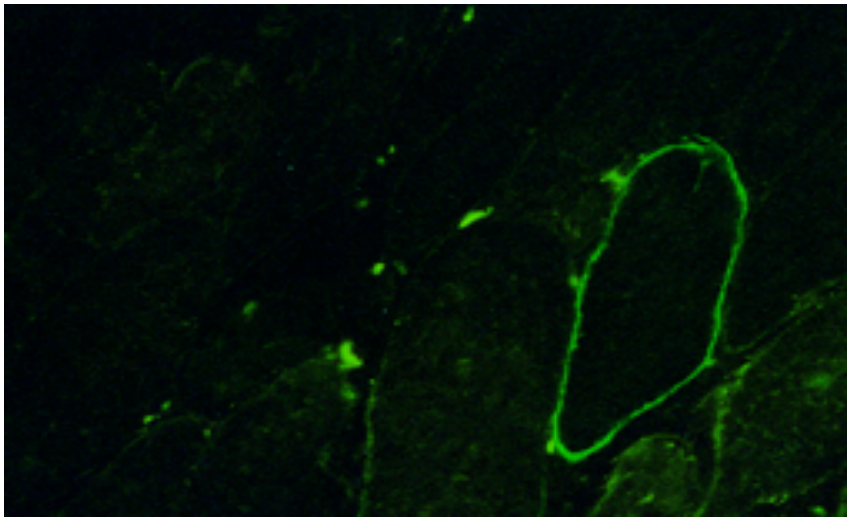
- Expression of truncated dystrophin transgenes in skeletal muscle of *mdx* mice have lead to the understanding of dystrophin protein domains and their functions, including the interaction of dystrophin with the DAPC.

Localization of dystrophin in skeletal muscle

normal

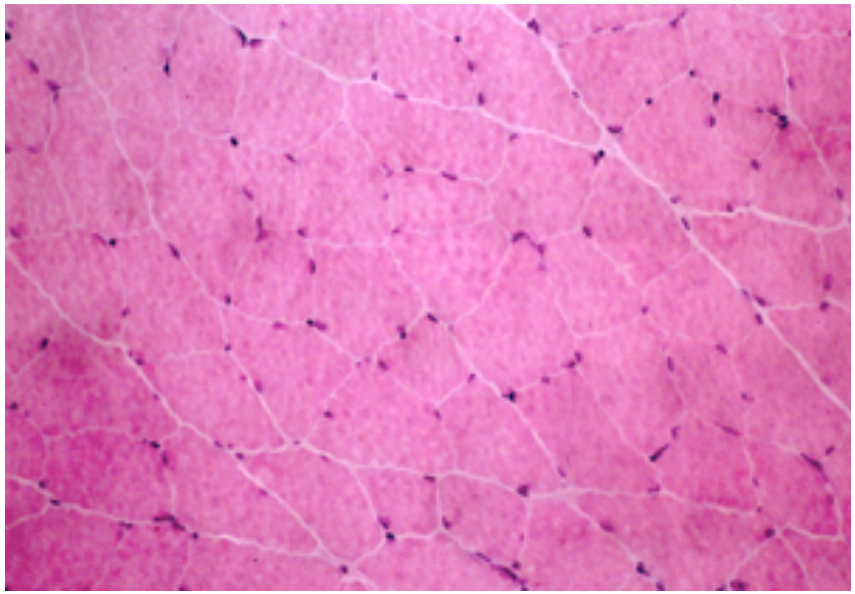


mdx

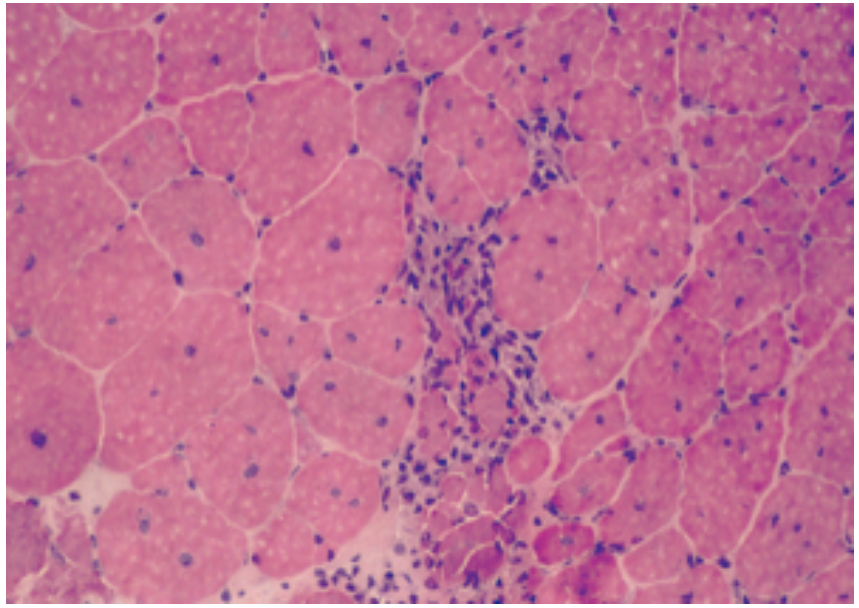


Morphology of dystrophic muscle

normal



mdx



Mating strategy to produce Tg/*mdx* mice

F0 male: **Tg/+ XY** X **+/+ $X^{mdx}X^{mdx}$**

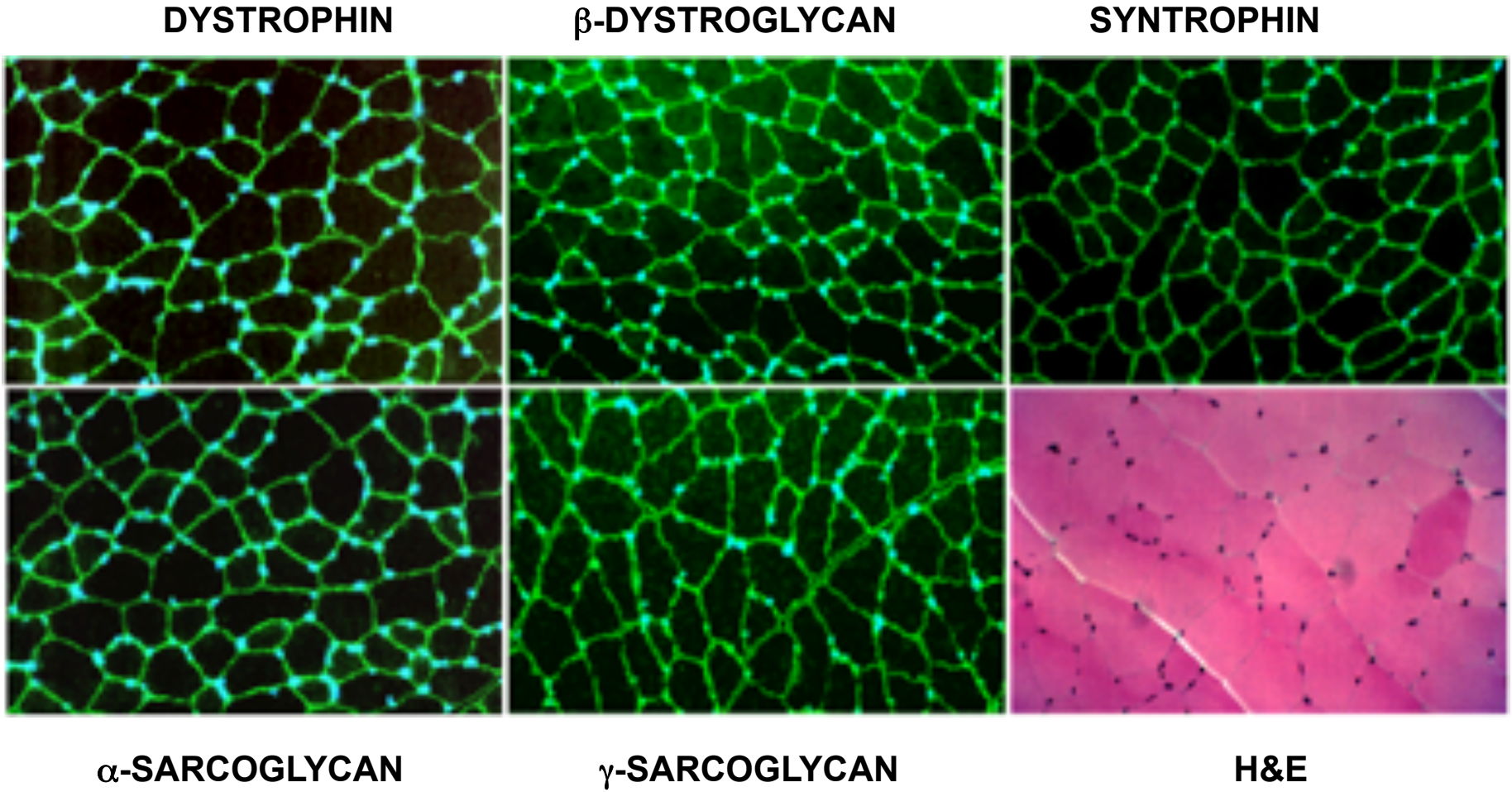
25%	25%	25%
Tg/+ XX^{mdx}	**+/+ XX^{mdx}**	**+/+ $X^{mdx}Y$**

25%

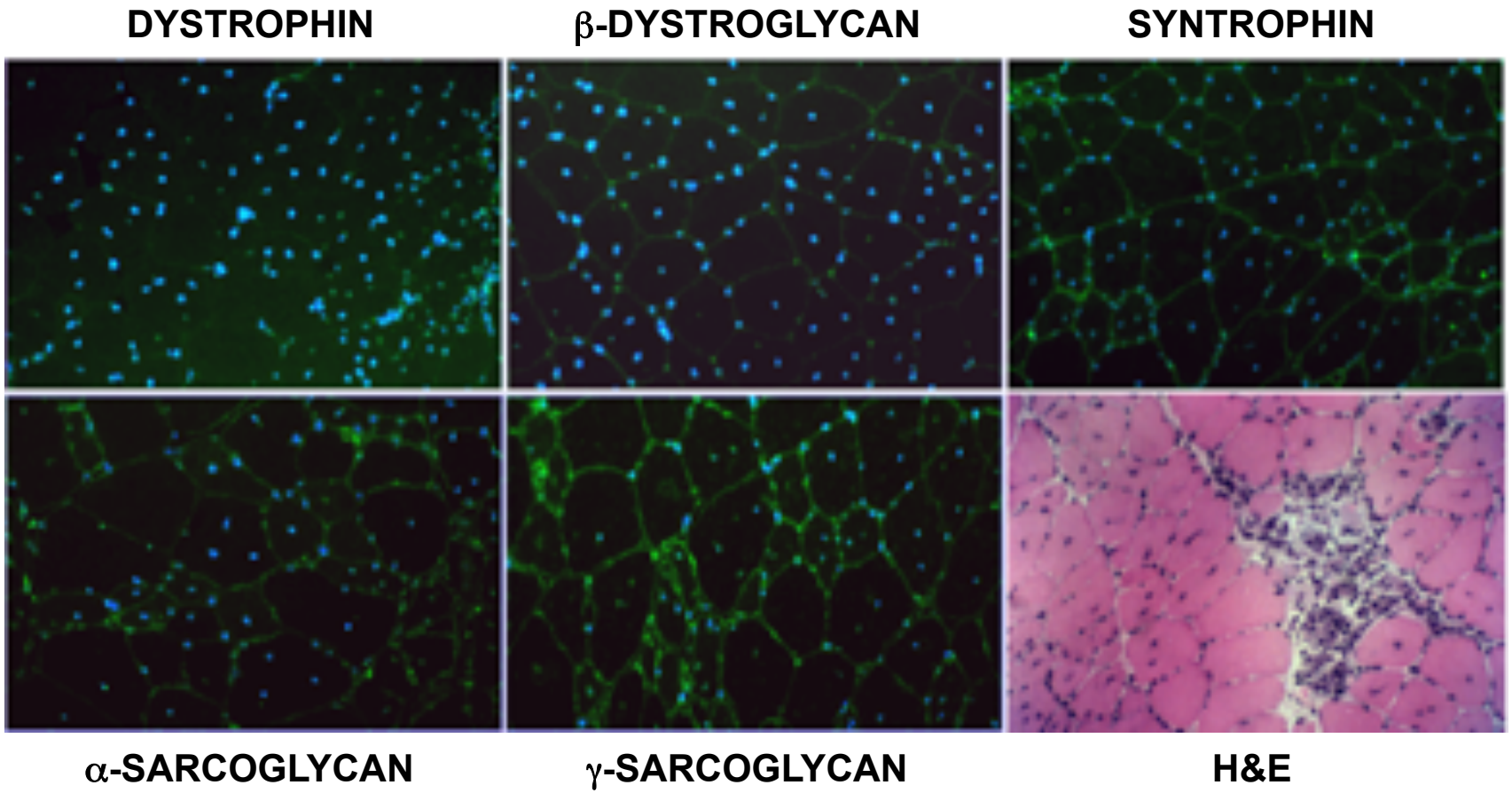
Tg/+ $X^{mdx}Y$ X **+/+ $X^{mdx}X^{mdx}$**

50% Tg/mdx	50% mdx
Tg/+ $X^{mdx}X^{mdx}$	**+/+ $X^{mdx}X^{mdx}$**
Tg/+ $X^{mdx}Y$	**+/+ $X^{mdx}Y$**

DAPC localization in C57 wild-type muscle



DAPC is greatly reduced at the membrane of *mdx* skeletal muscle



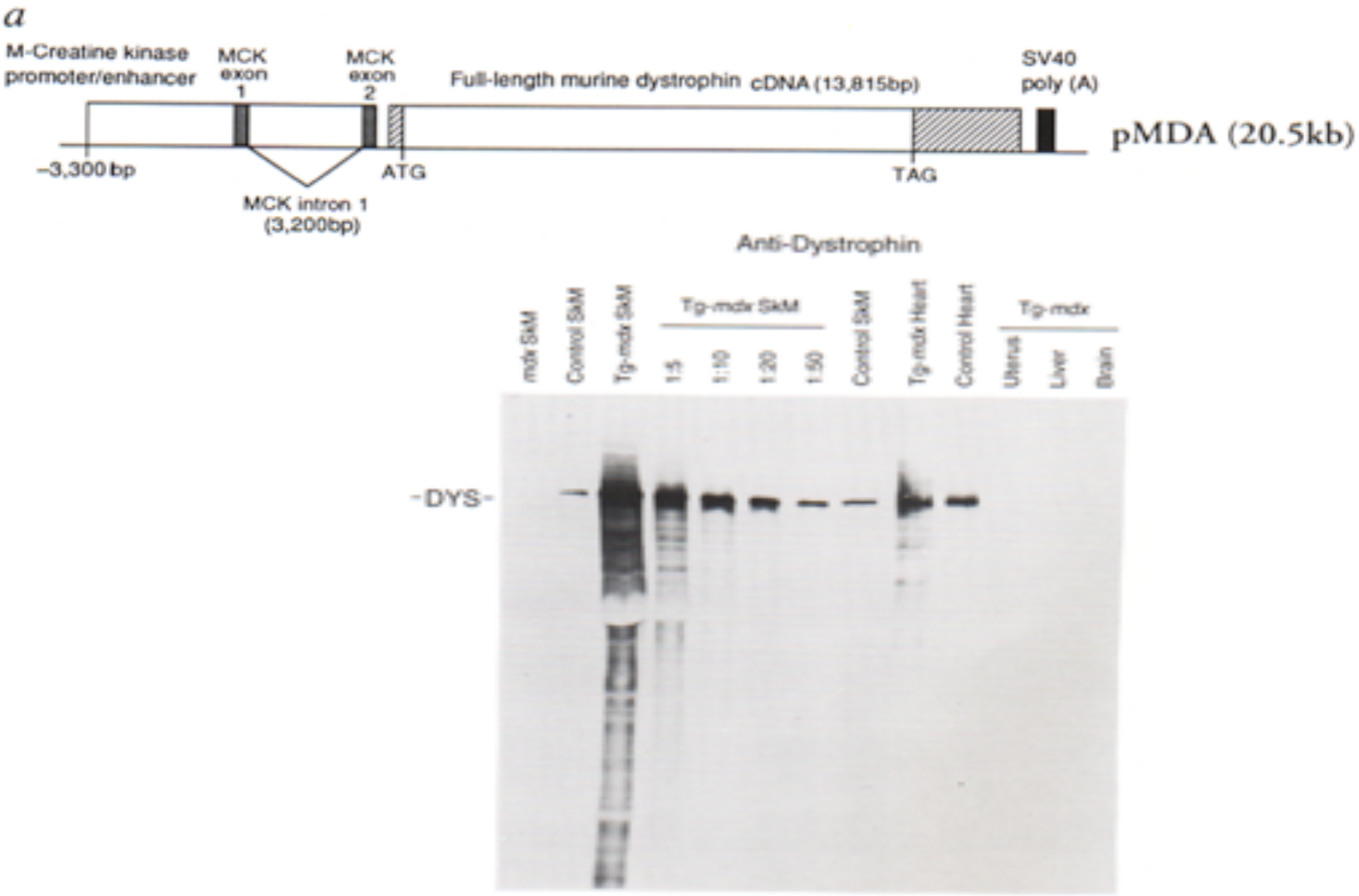
MDA/*mdx*

- expression of a full-length dystrophin cDNA transgene in *mdx* skeletal muscle
  - muscle pathology prevented
  - DAPC restored to membrane
  - normal CK levels and force generation

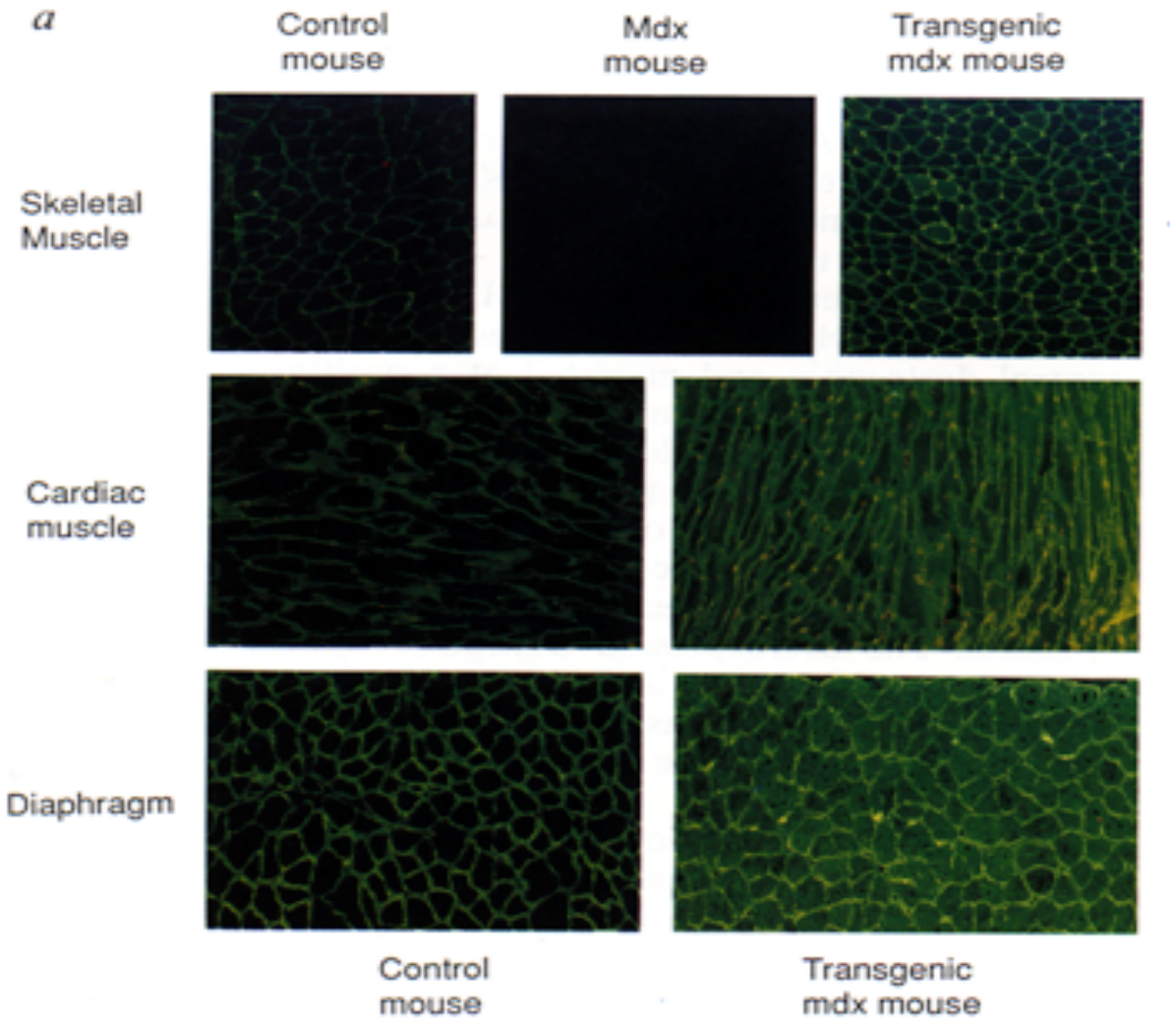
This experiment showed that expression of a **14kb cDNA** (from a 2.5 Mb gene) **in skeletal muscle only** was sufficient to prevent all signs of muscular dystrophy in the *mdx* mouse.

Nature (1993) 364:725

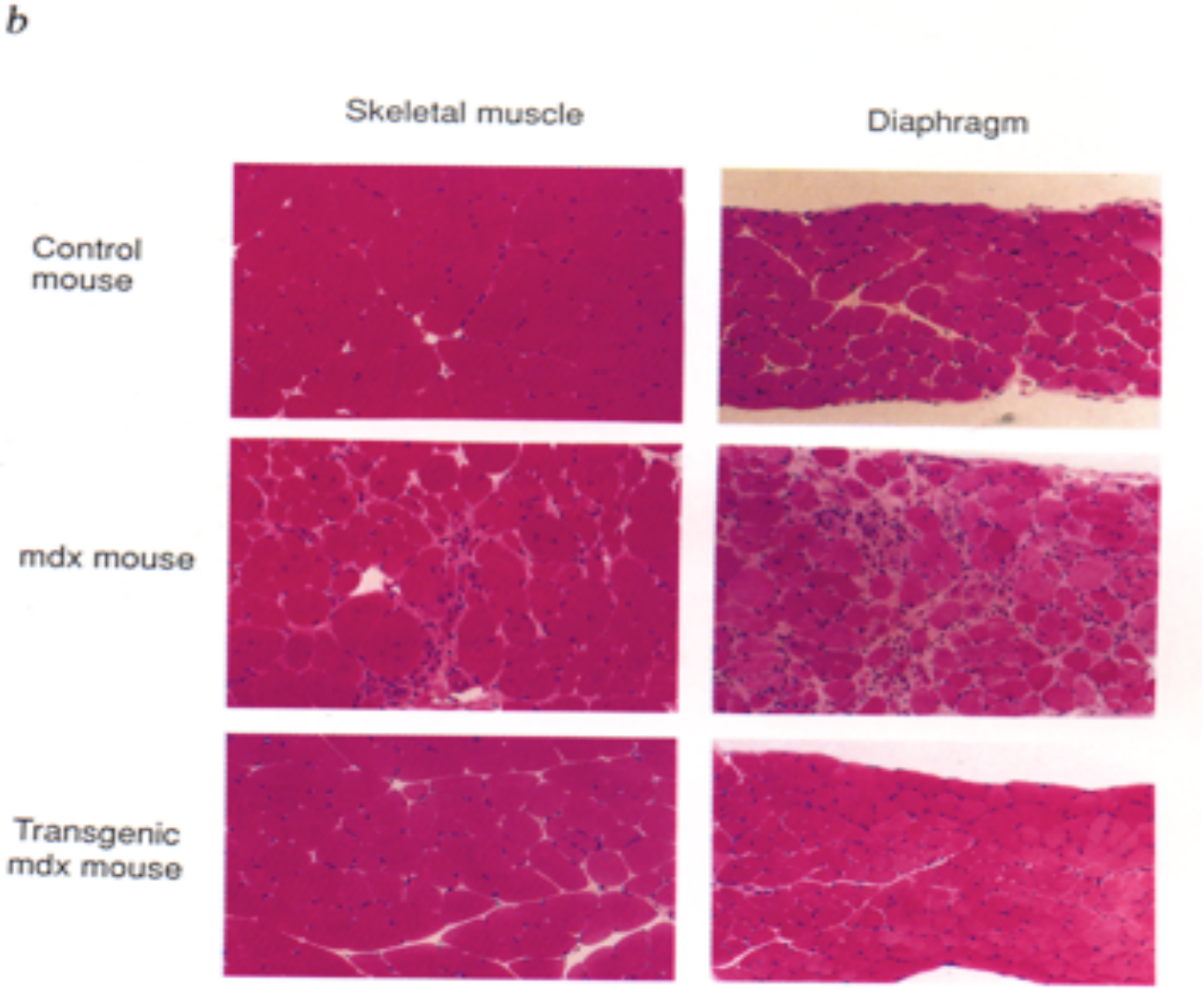
Full-length dystrophin transgene and expression



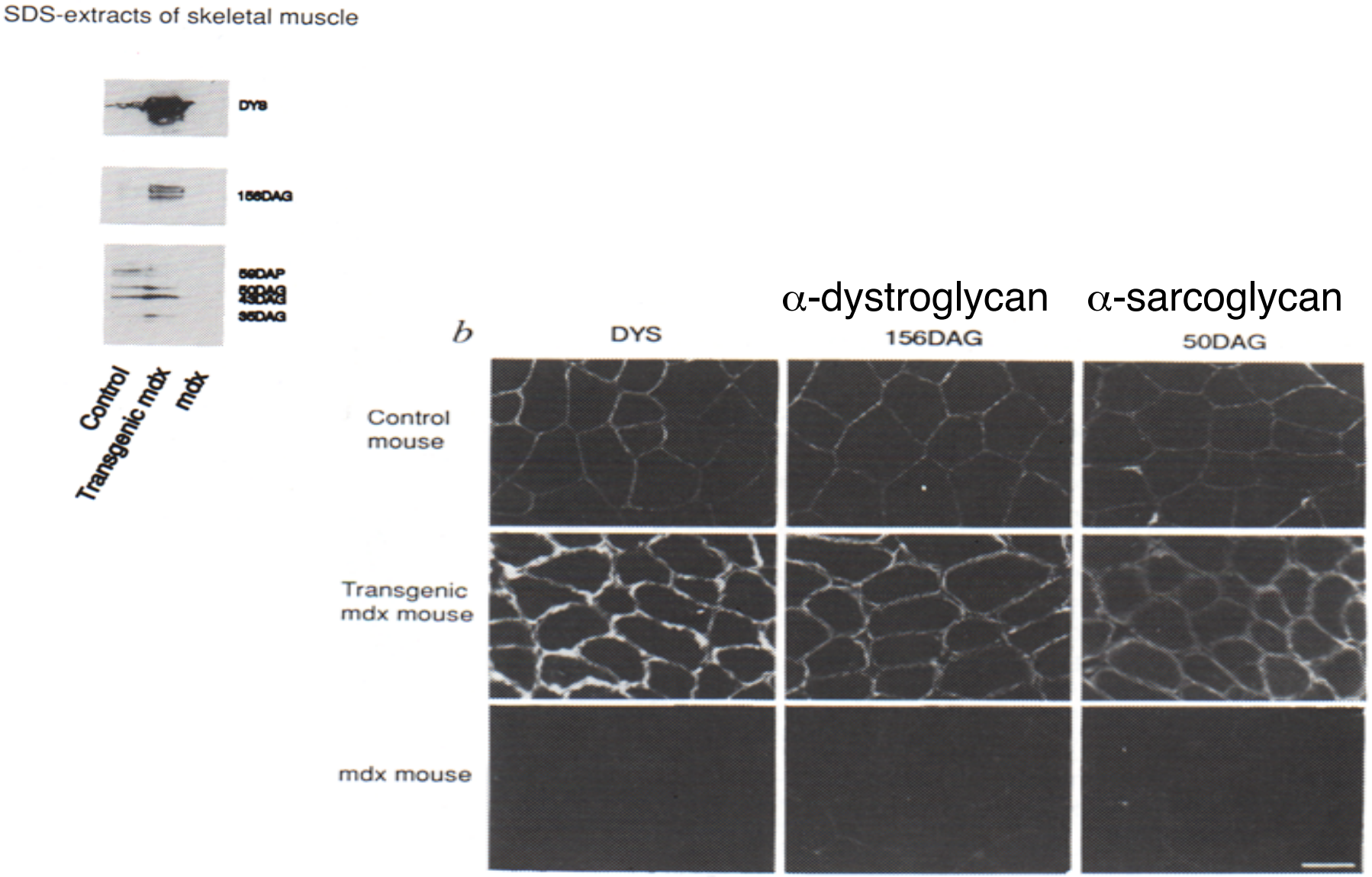
Localization of transgenic dystrophin in striated muscles



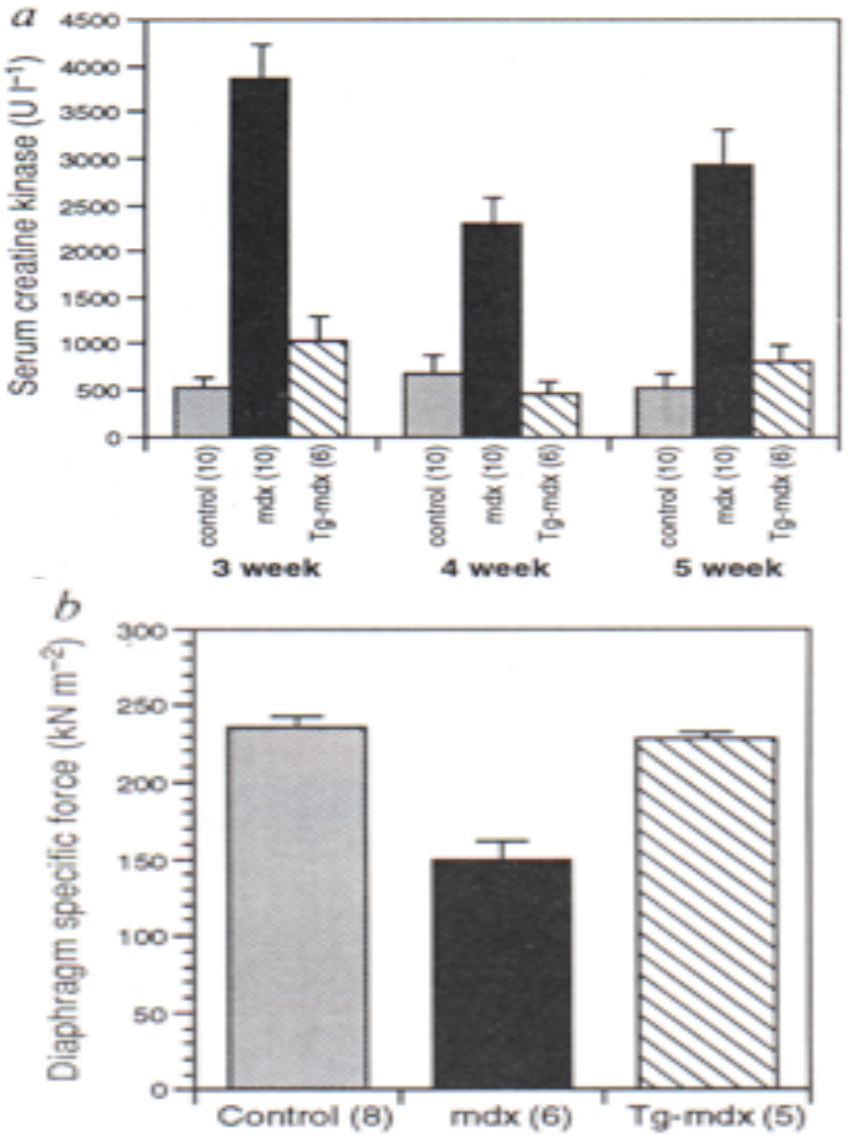
Prevention of *mdx* skeletal muscle pathology



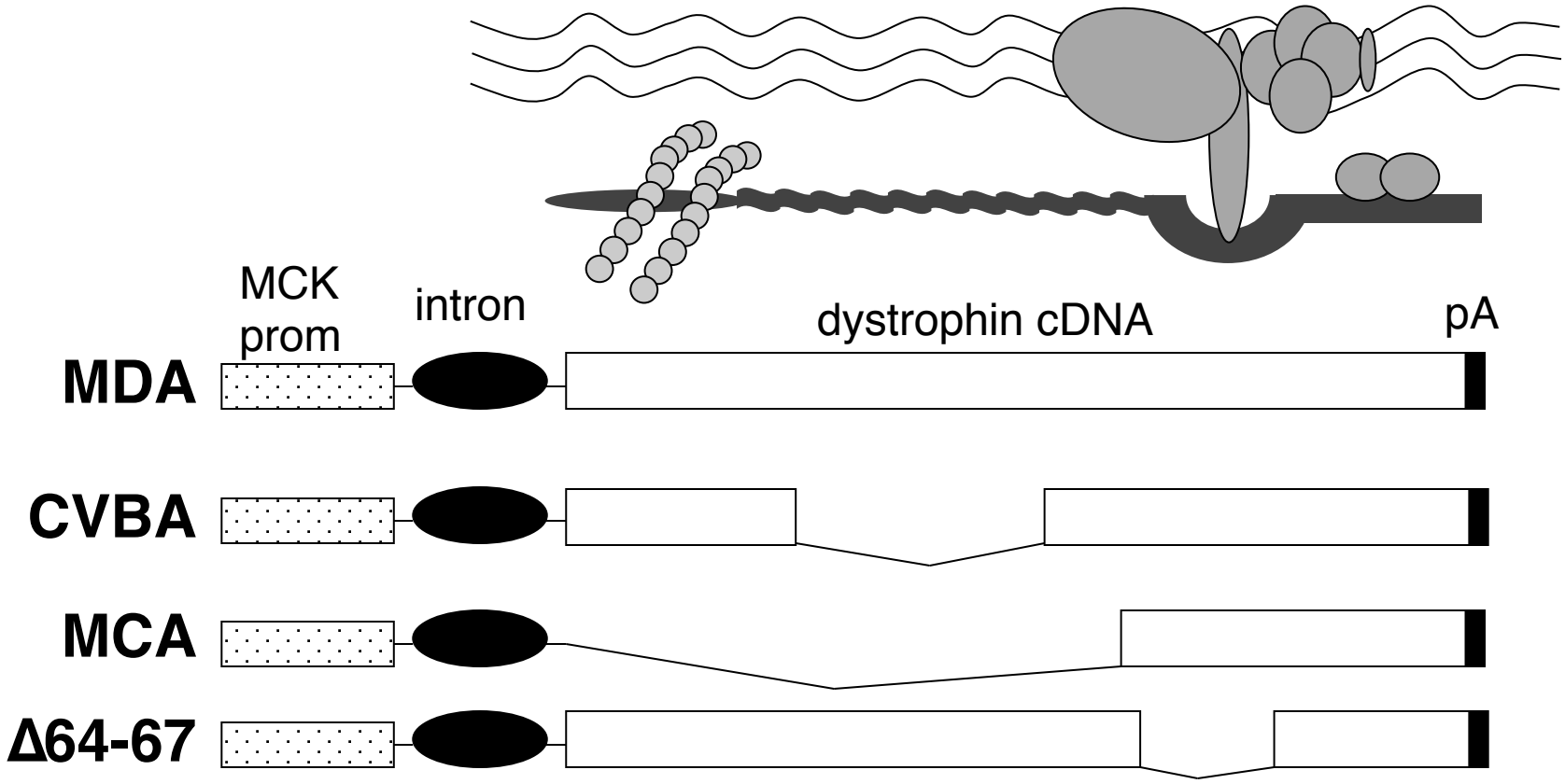
Relocalization of the DAPC



Prevention of abnormal CK levels and reduction in normalized force



Dystrophin transgenes



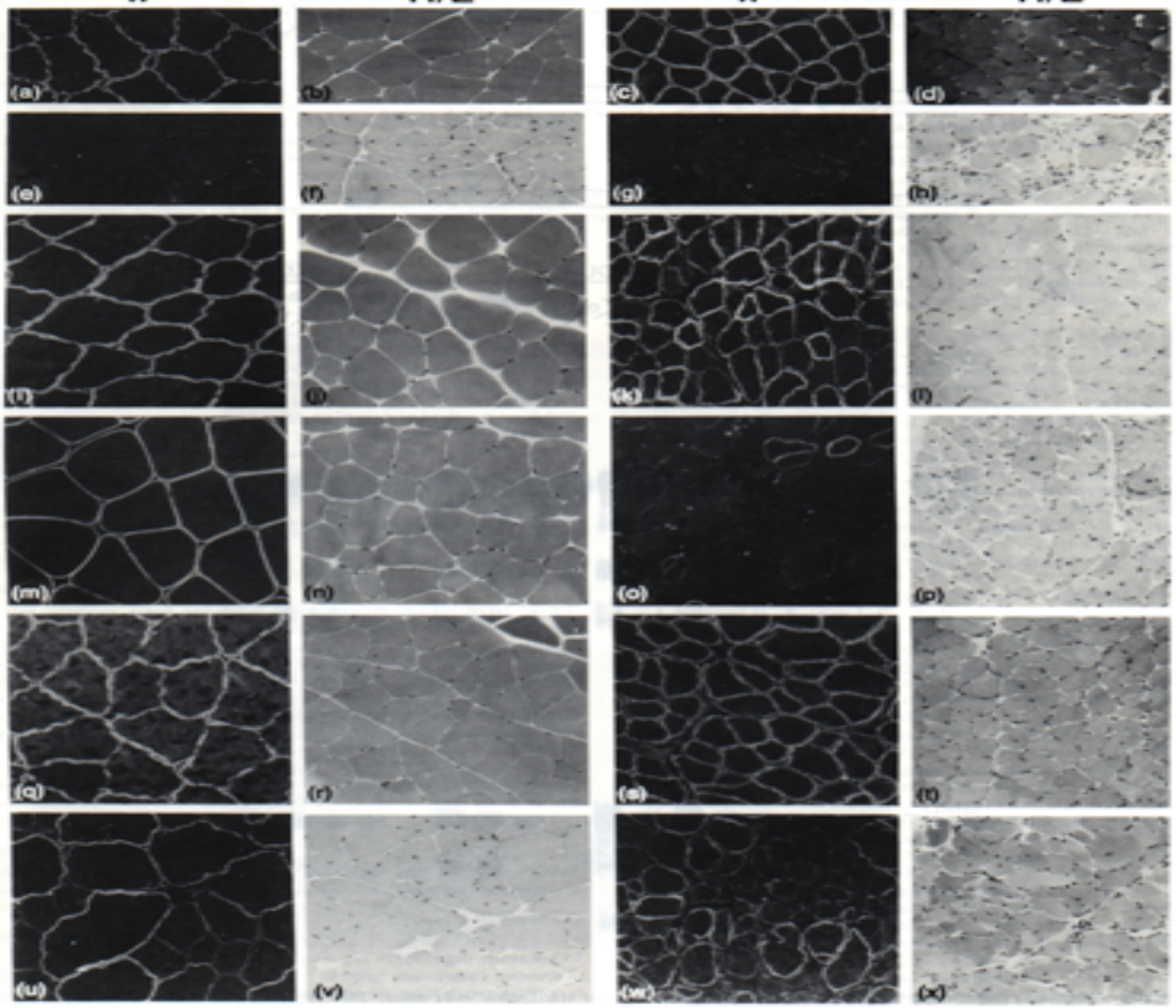
CVBA/*mdx*

- expression of a dystrophin transgene deleted for exons 17-48 of the rod-domain in *mdx* skeletal muscle. (Based on patient with mild phenotype.)
  - muscle pathology ameliorated
  - DAPC restored to membrane
  - almost normal muscle force generation
  - insight into uniform vs. variable expression

Hum Mol Genet (1995) 4:1251

Full-length and Δ17-48 transgenic lines and phenotypes

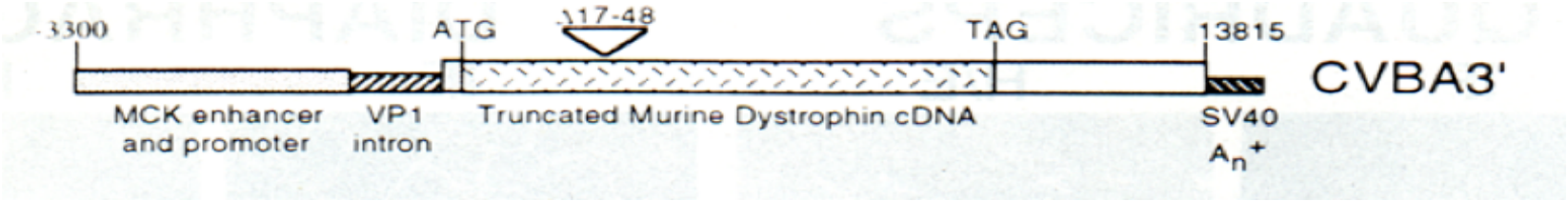
	Quadriceps			Diaphragm		
	Level of expression	Central nuclei	M-pyruvate kinase (U/L)	Level of expression	Central nuclei	Specific force (kN/m^2)
C57Bl/10*mdx*	–	88.7%	12008 ± 4228(12)	–	54.8%	117 ± 9(3)
C57Bl/10	1X(U)	0.67%	664 ± 202(10)	1X(U)	0.19%	222 ± 9(3)
8440CVAA	>5X(U)	1.9%	684 ± 114(7)	2X(U)	0.34%	249 ± 21(5)
862CAA	2X(U)	1.0%	633 ± 50(7)	0.5X(U)	0.52%	211 ± 21(5)
852CAA	0.7X(U)	1.9%	643 ± 134(7)	0.2X(SV)	1.2%	205 ± 20(5)
847CAA	1X(U)	5.8%	845 ± 153(7)	0.3X(V)	18.9%	206 ± 20(5)
8487CAVA	0.15X(V)	52.1%	7509 ± 2827(7)	<0.05X(V)	45.1%	148 ± 14(5)
C57Bl/10*mdx*	–	88.7%	11427 ± 1626(6)	–	54.8%	126 ± 19(5)
C57Bl/10	1X(U)	0.67%	503 ± 152(6)	1X(U)	0.19%	237 ± 23(5)
11808CVHBA	>10X(U)	3.0%	N/D	0.9X(U)	6.0%	212 ± 10(4)
11922CVHBA	>10X(V)	9.1%	731 ± 146(9)	0.2X(U)	8.3%	192 ± 11(4)
11929CVHBA	0.7X(SV)	39.7%	2281 ± 1030(7)	<0.05X(V)	46.2%	N/D
11956CVBA3′	>10X(U)	7.1%	N/D	0.9X(U)	0.14%	N/D
12042CVBA3′	>10X(V)	34.0%	1692 ± 362(8)	0.65X(SV)	8.9%	196 ± 17(4)
12142CVBA	5X(U)	7.0%	813 ± 164(4)	0.7X(V)	12.8%	194 ± 28(4)
12157CVBA	0.4X(U)	10.2%	1885 ± 417(2)	0.2X(V)	37.8%	123 ± 20(4)
12210CVBA	5X(U)	2.8%	N/D	0.1X(V)	40.8%	N/D

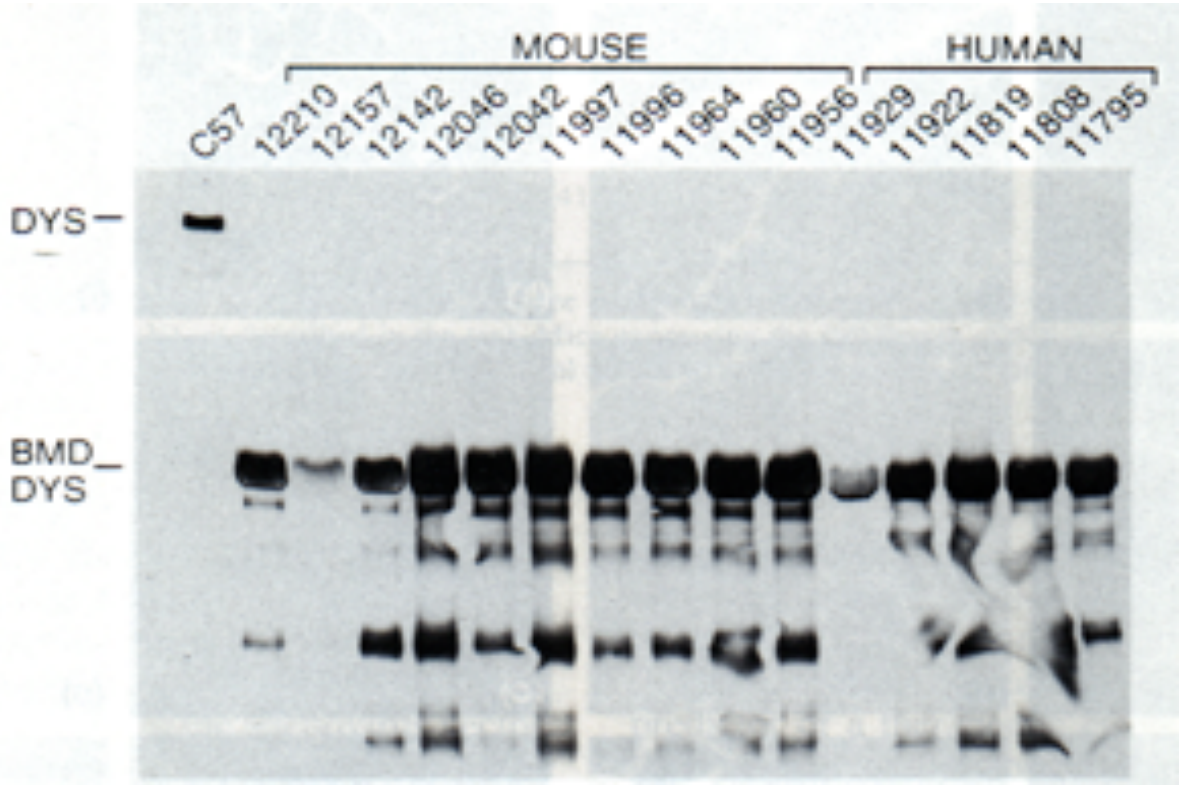

QUADRICEPS
DIAPHRAGM
IF
H/E
IF
H/E
(a)
(b)
(c)
(d)
(e)
(f)
(g)
(h)
(i)
(j)
(k)
(l)
(m)
(n)
(o)
(p)
(q)
(r)
(s)
(t)
(u)
(v)
(w)
(x)

CVBA/*mdx*

- This experiment showed that expression of a 5.5 kb (instead of a full-length 14 kb) dystrophin cDNA was sufficient to greatly ameliorate the phenotypic signs of muscular dystrophy in the *mdx* mouse. The majority of the rod domain of dystrophin is not critical for protein function.

Expression of Δ17-48 transgene



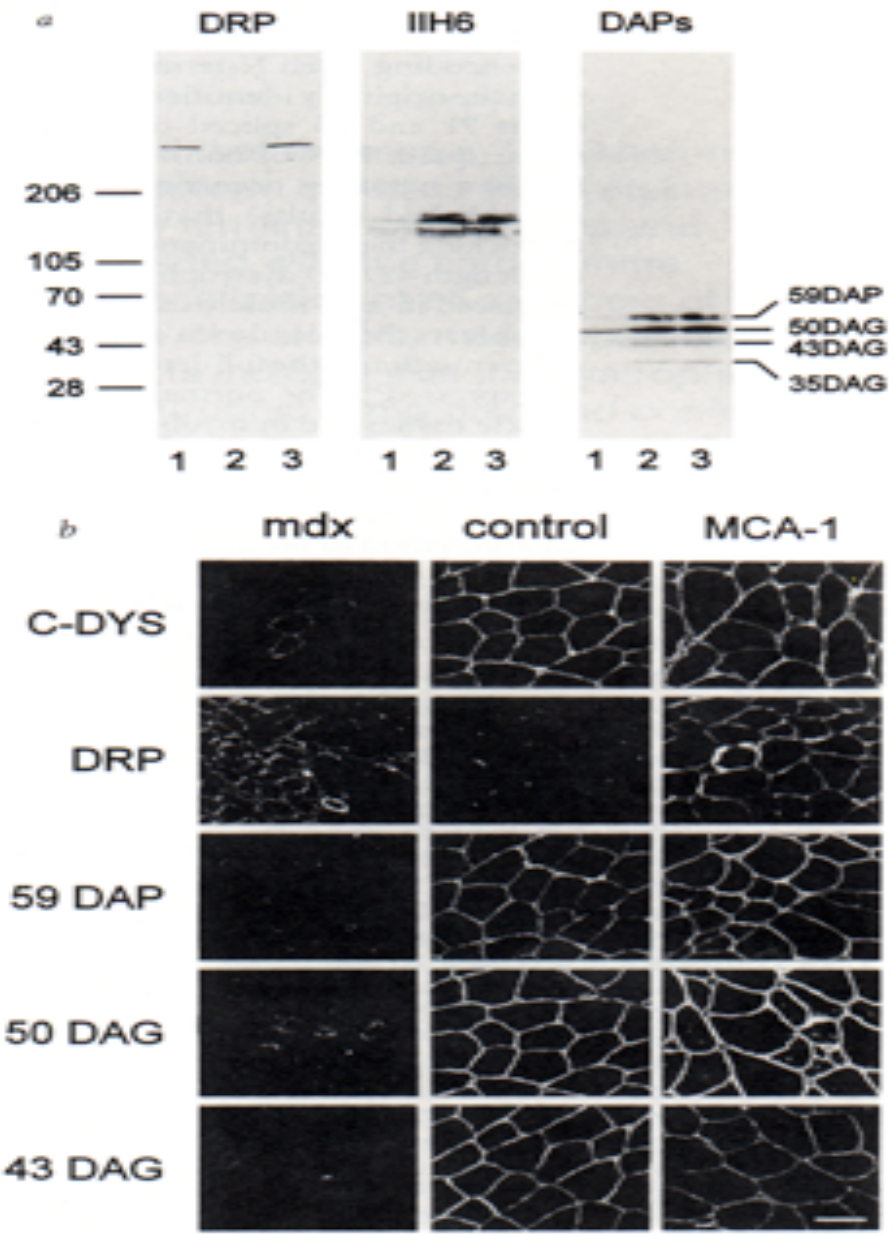


MCA/*mdx*

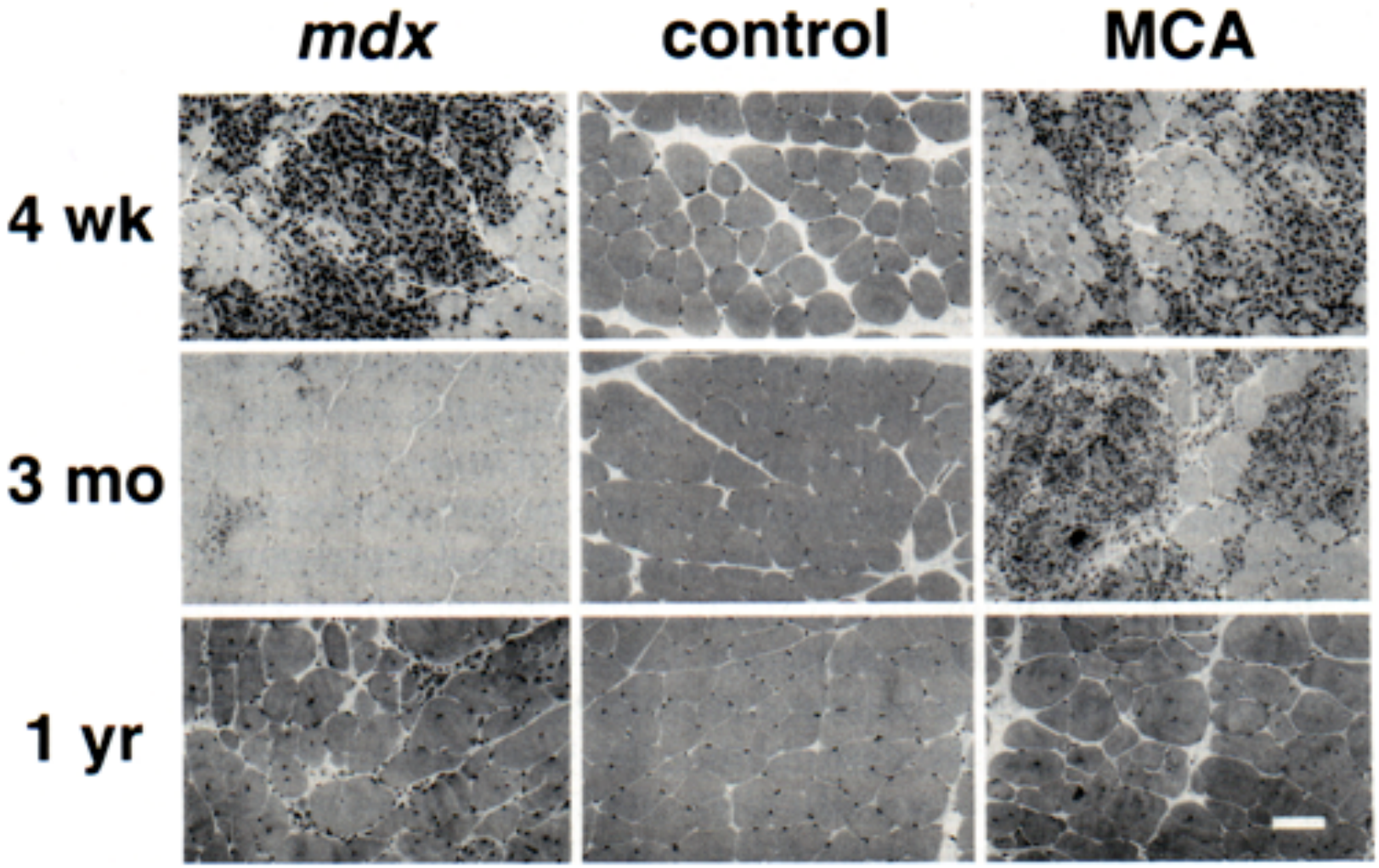
- expression of a C-terminal dystrophin transgene (exons 63-79) encoding the entire DAPC binding region in *mdx* skeletal muscle
  - muscle pathology not improved
  - DAPC restored to muscle membrane
  - deficits in muscle force generation

Nat Genet (1994) 8:333

Association of MCA dystrophin with the DAPC



MCA transgene is unable to prevent muscular dystrophy



MCA/*mdx*

This experiment showed that the restoration of the DAPC to the muscle membrane is not sufficient to prevent the phenotypic signs of muscular dystrophy

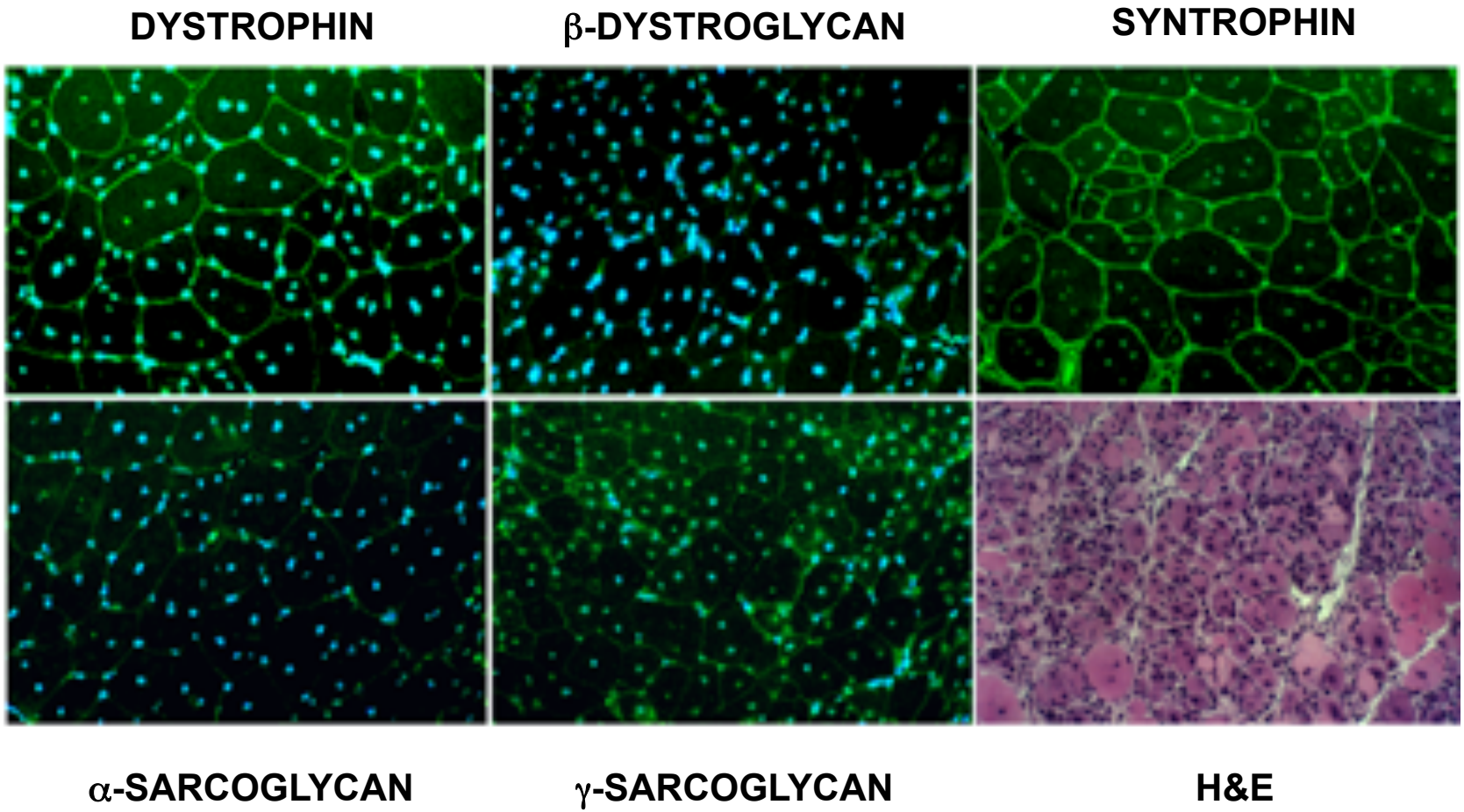
Nat Genet (1994) 8:333

Δ64-67/*mdx*

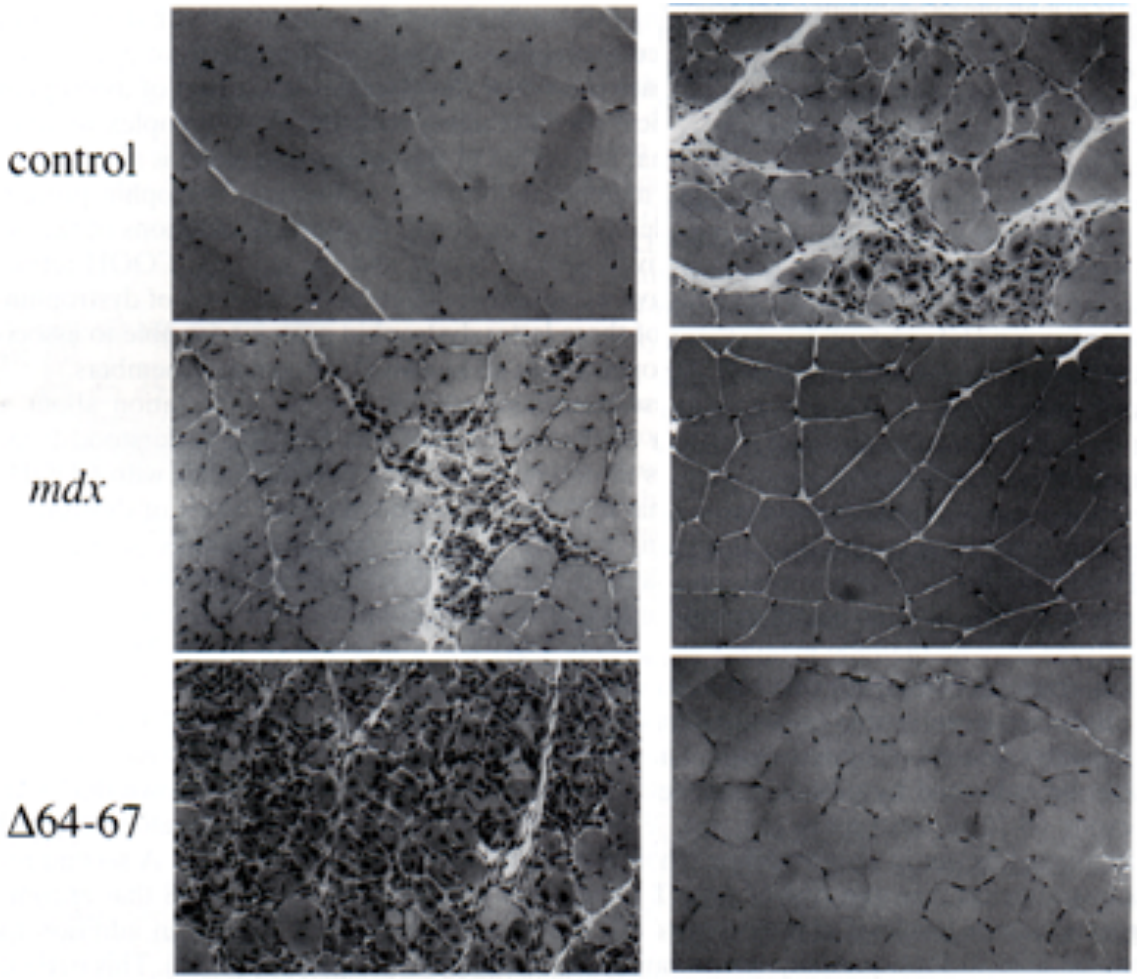
- expression of a dystrophin transgene deleted for the β-dystroglycan binding site in *mdx* skeletal muscle
  - muscle pathology is not improved
  - DAPC (dystroglycans and sarcoglycans) are not restored to muscle membrane
  - deficits in muscle force generation

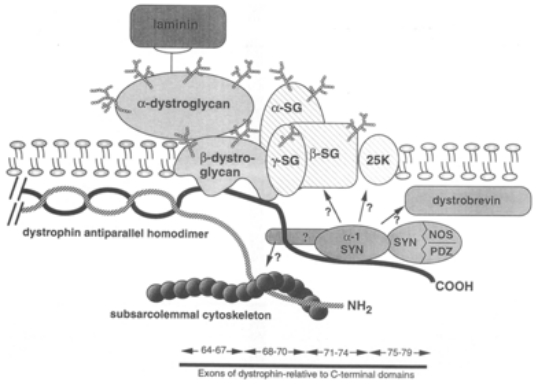
J Cell Biol. (1996) 134:93

Dystroglycans and sarcoglycans are not restored to the membrane in Δ64-67/*mdx* skeletal muscle



Restoration of DAPC to muscle membrane is necessary to prevent muscular dystrophy



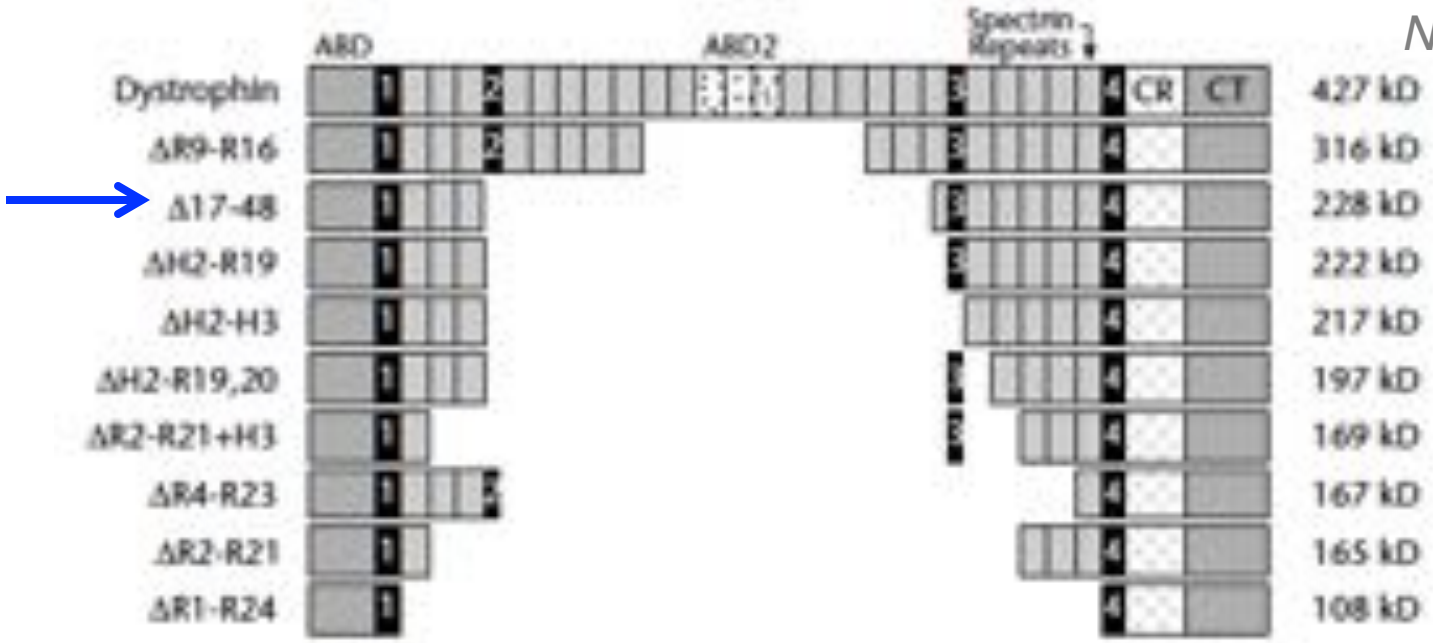


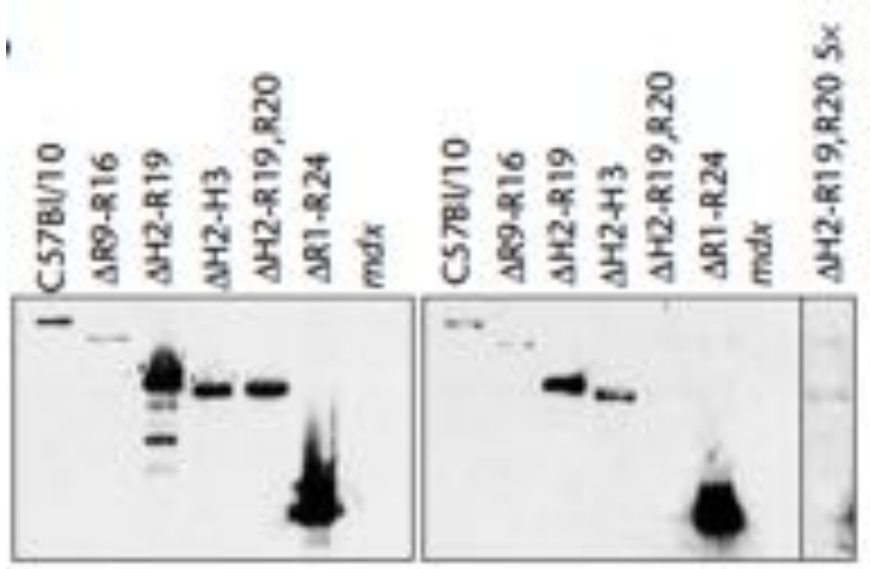
Δ64-67/*mdx*

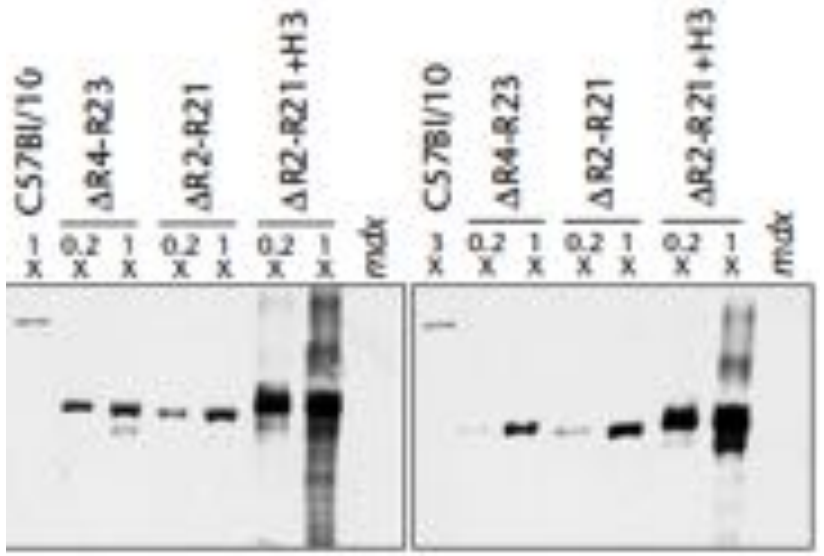
This experiment together with MCA/*mdx* shows that restoration of the DAPC to the muscle membrane is necessary, but not sufficient to prevent the phenotypic signs of muscular dystrophy in the *mdx* mouse. These experiments together show that both the amino and carboxy terminal domains are required for normal dystrophin function.

Modular flexibility in spectrin-like repeats

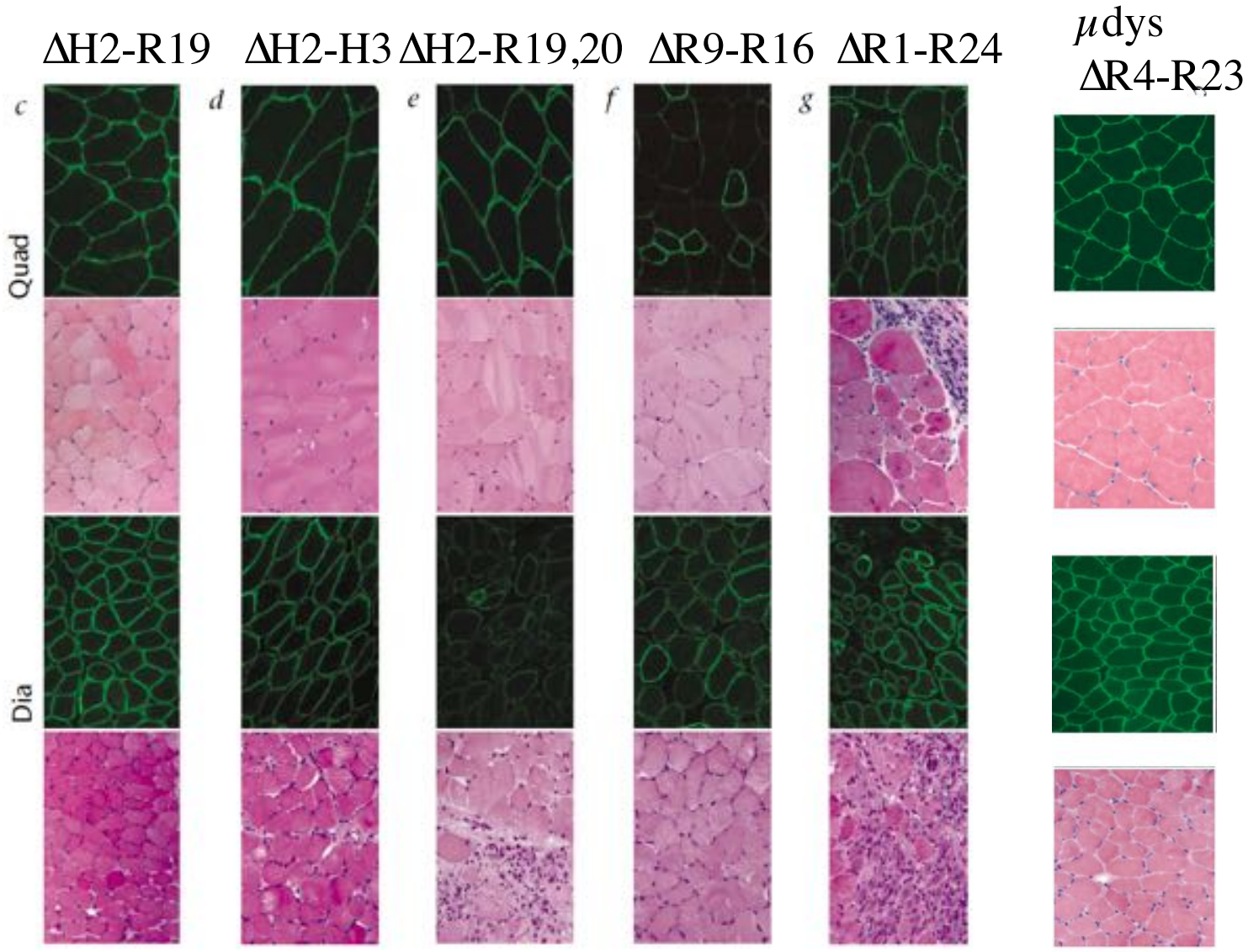
Nat Med (2002) 8:253







Different levels and uniformity of transgene expression in different muscles

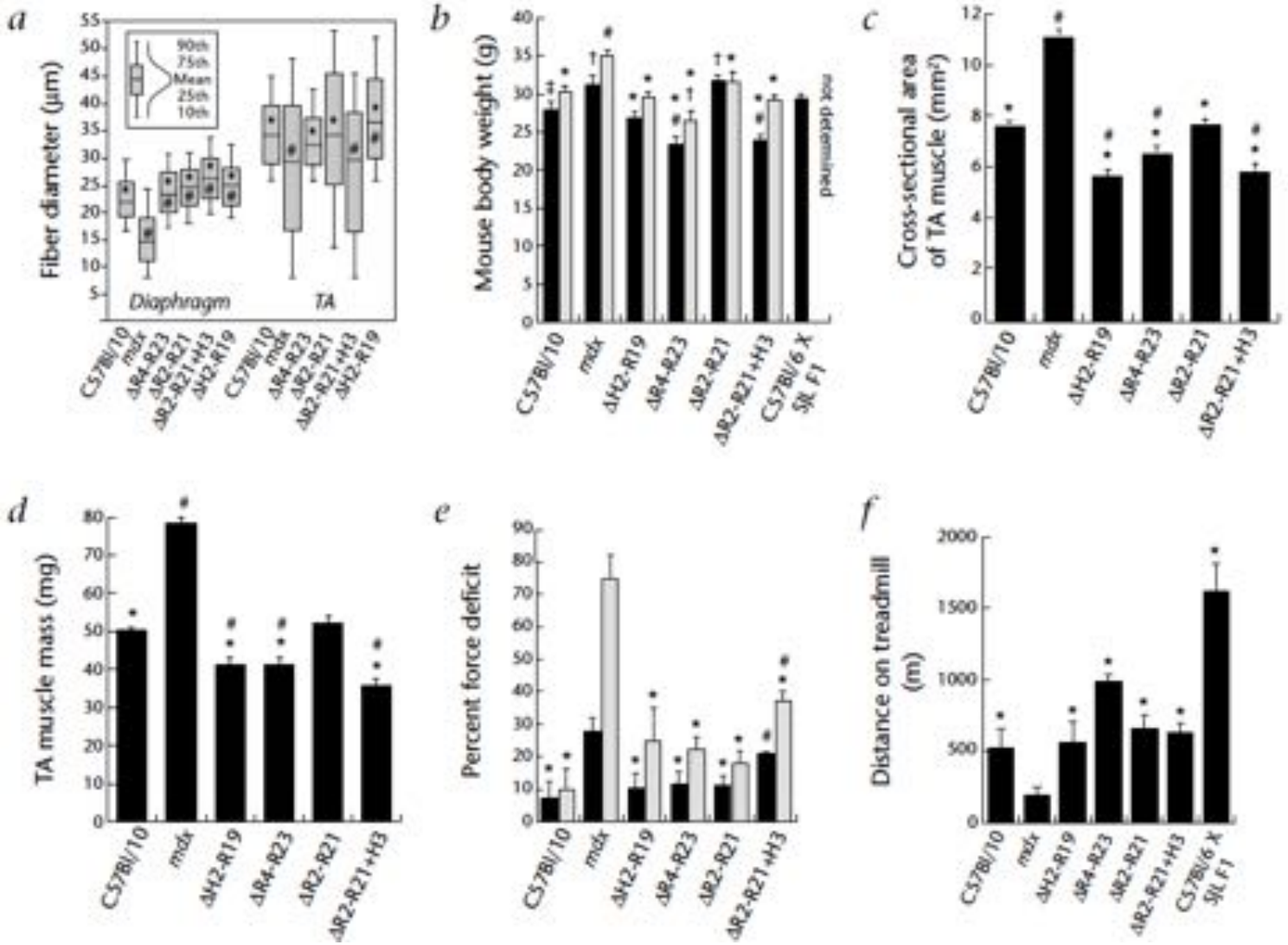


Some repeats are necessary for function, but not all repeats are required.

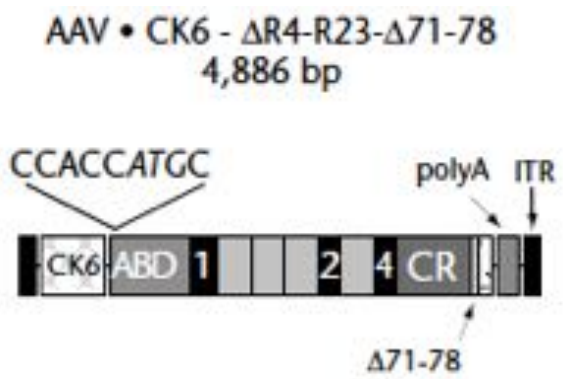
Table 1 Mini- and micro- dystrophin transgenic mouse % CN and specific force

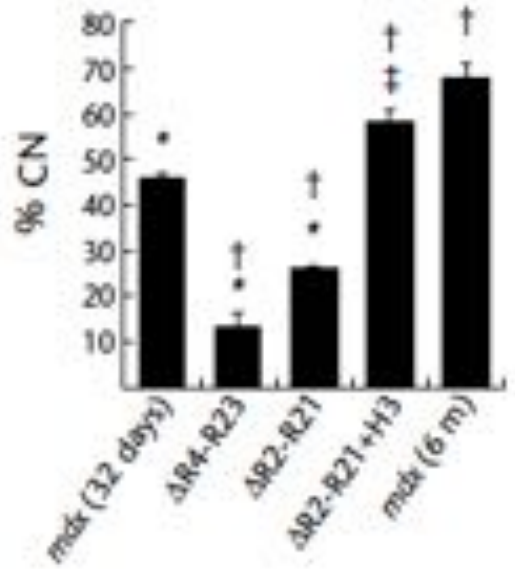
	Percentage of fibers with centrally located nuclei		Specific force (kN/m^2)		
	Diaphragm	Quadriceps	Diaphragm	EDL	TA
C57Bl/10 (3 mos)	<1	<1	200 ± 8[a]	222 ± 8[a]	235 ± 13[a]
C57Bl/10 (6 mos)	<1	<1	215 ± 17[a]	ND	ND
mdx (3 mos)	55	71	119 ± 5[b]	181 ± 6[b]	177 ± 8[b]
mdx (6 mos)	51	64	76 ± 7[b]	ND	ND
ΔH2-R19 (3 mos)	1	<1	207 ± 11[a]	231 ± 8[a]	252 ± 23[a]
ΔH2-H3 (3 mos)	5	4	150 ± 14[a,c]	194 ± 10[d]	ND
ΔH2-R19,R20 (3 mos)	25	27	104 ± 14[b]	206 ± 14	ND
ΔR9-R16 (het)	5	34	145 ± 18[b]	ND	ND
ΔR9-R16 (homo)	1	9	ND	ND	ND
ΔR1-R24 (3 mos)	65	74	ND	ND	ND
ΔR4-R23 (3 mos)	<1	<1	ND	ND	159 ± 12[b]
ΔR4-R23 (6 mos)	<1	<1	148 ± 23[a,b]	ND	ND
ΔR2-R21 (3mos)	<1	12	ND	ND	201 ± 6
ΔR2-R21 (6 mos)	<1	15	161 ± 8[a,b]	ND	ND
ΔR2-R21+H3 (3 mos)	<1	27	ND	ND	183 ± 17[b]
ΔR2-R21+H3 (6 mos)	<1	52	162 ± 11[a,b]	ND	ND

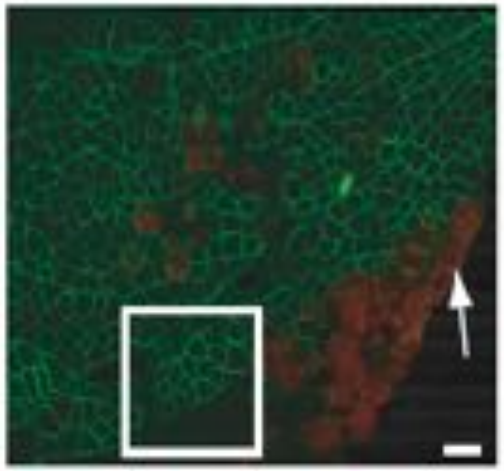
The strength of comprehensive functional and histological analyses

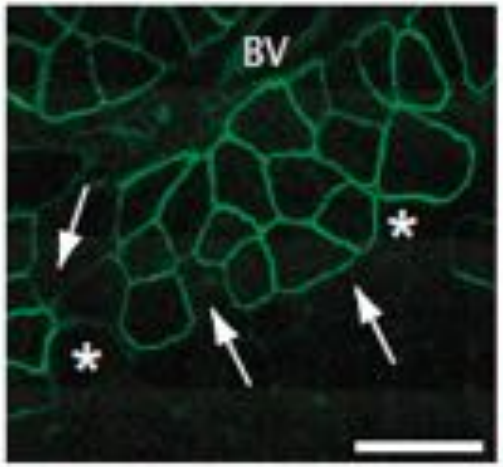


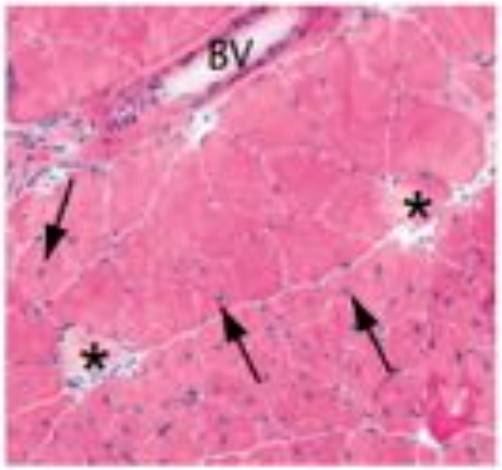
Truncated dystrophins can halt or reverse, in addition to prevent, the dystrophic phenotype



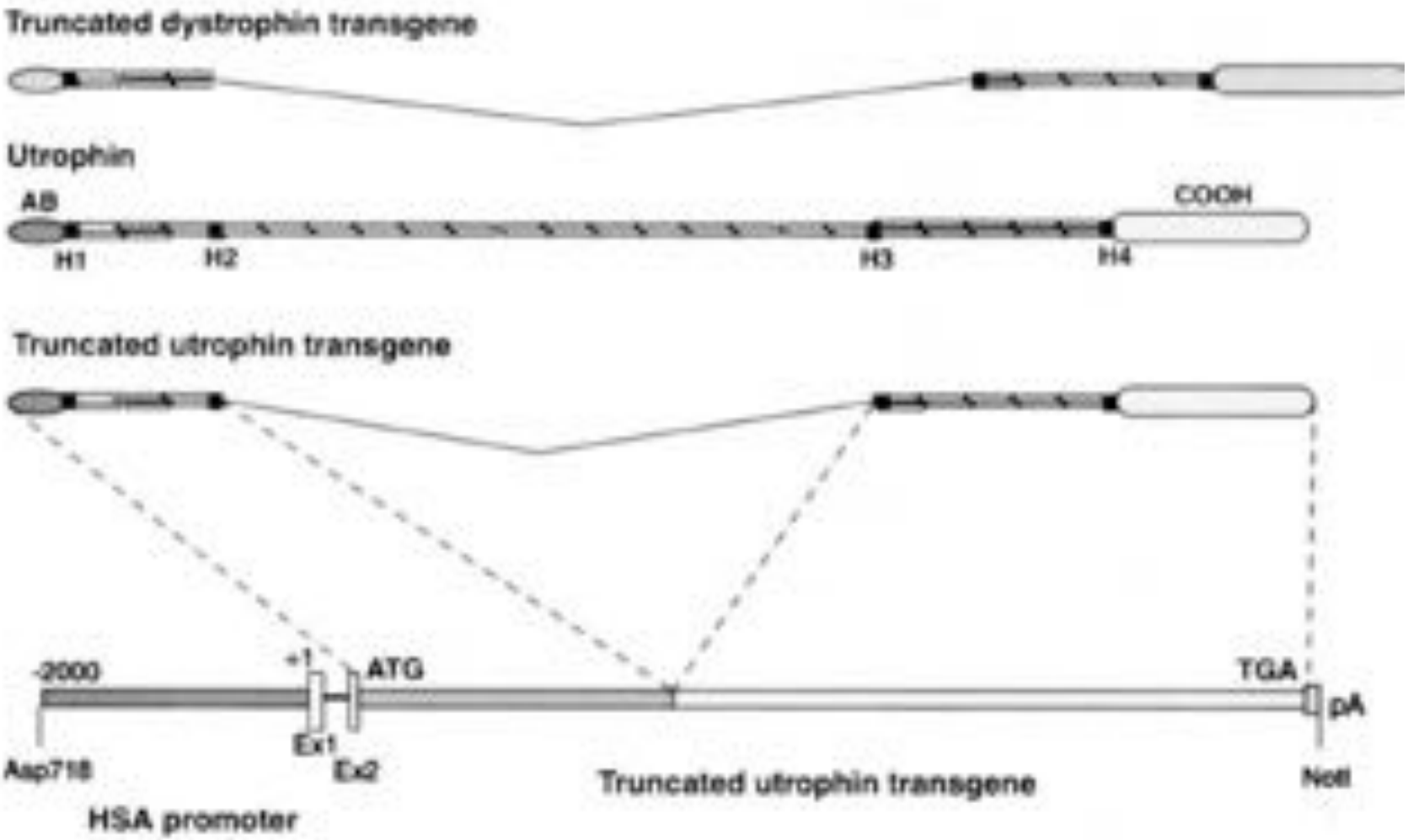






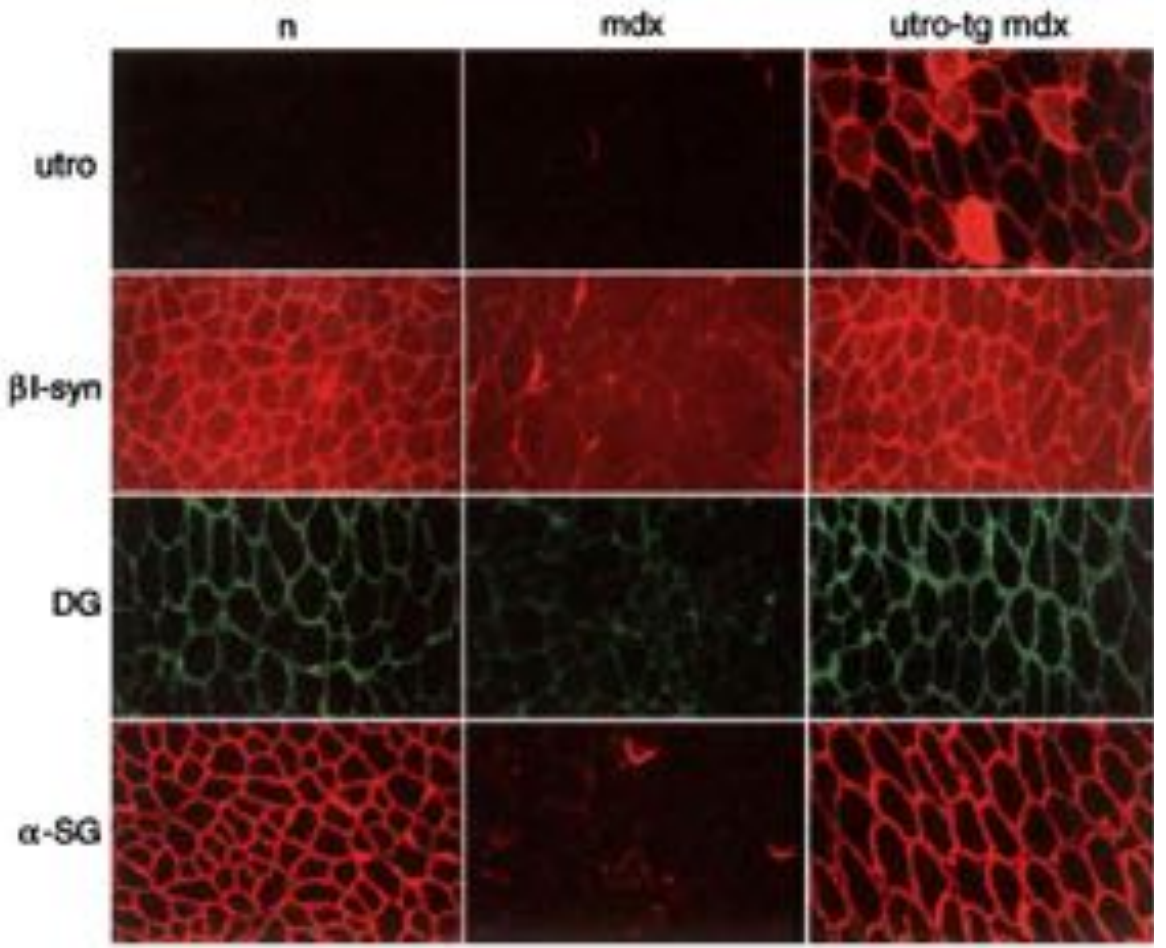


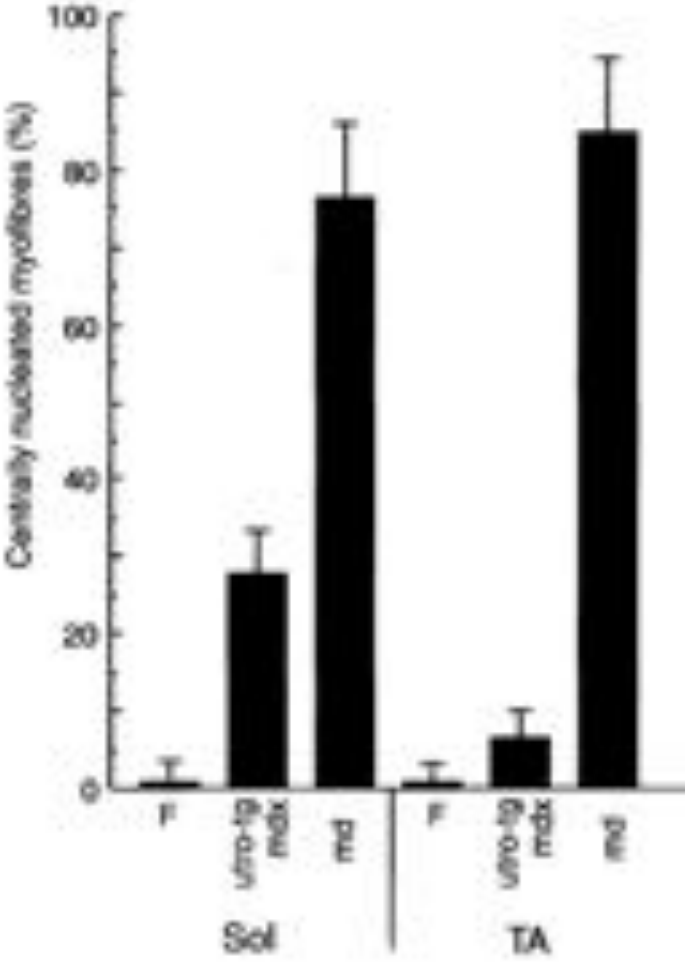
Utrophin/*mdx* transgenic



Nature (1996) 384:349

Mini-utrophin restores DGC and histology (and function)

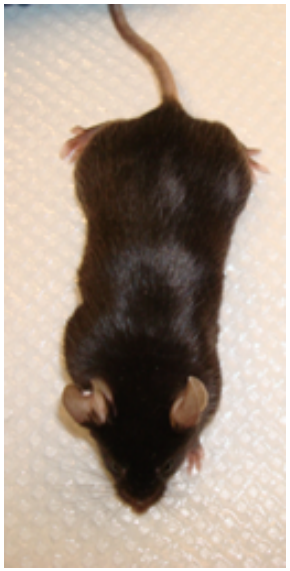




Mouse models

mdx
Dystrophin-deficient

- normal mouse lifespan (2 yrs)
- mild skeletal muscle fibrosis
- mild cardiomyopathy

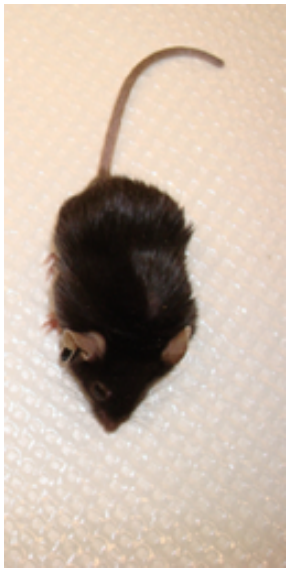


Het
Dystrophin-deficient; missing 1 copy of utrophin

- normal mouse lifespan (2 yrs)
- severe skeletal muscle fibrosis
- Cardiomyopathy progression more similar to DMD patients

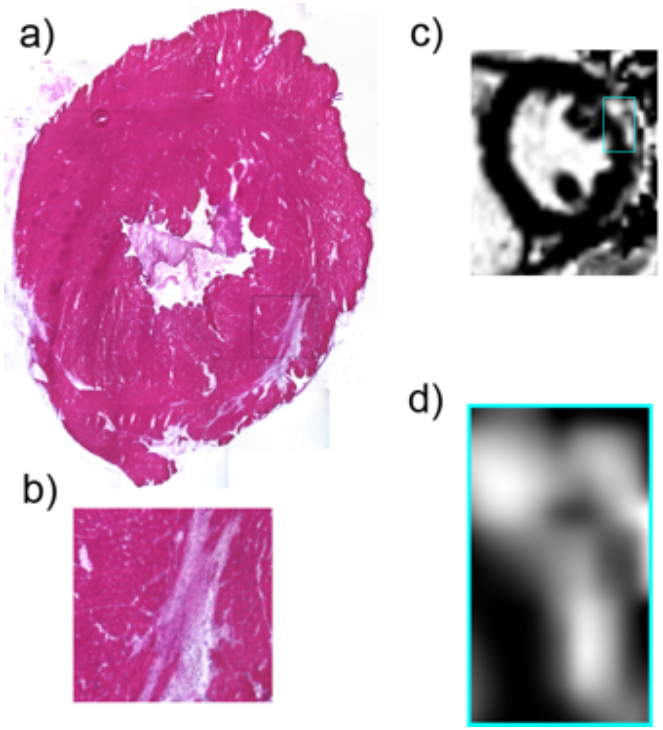
dko
Dystrophin/utrophin-deficient

- Dies 10-12 weeks-of-age
- mild skeletal muscle fibrosis
- severe cardiomyopathy

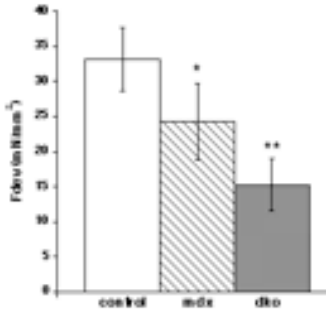


Cell (1997) 90:717; J Neurol Sci. Jan 15;264(1-2):106-11.

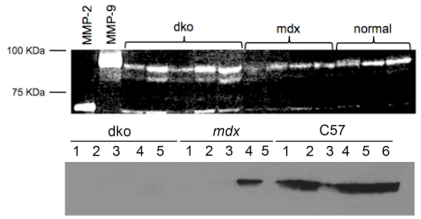
Dko mice and DMD patients show similar patterns of myocardial injury prior to whole heart dysfunction (reduced EF)



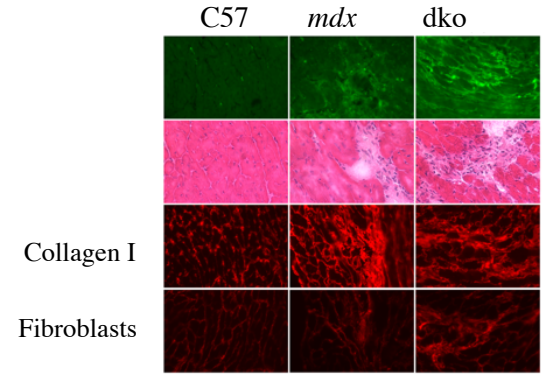
- Cardiac contractile dysfunction



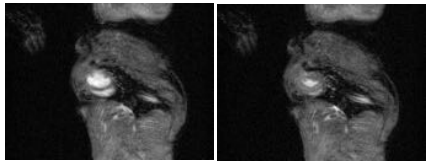
- Unregulated MMP remodeling



- Collagen scarring



- Normal Ejection Fraction



Some other *mdx* double mutant models

- Cmah/*mdx*
- Telomerase/*mdx*

GRMD = Golden Retriever Muscular Dystrophy

- **The homologue of the Duchenne locus is defective in X-linked muscular dystrophy of dogs.** (Nature, 1988 Jul 14;334(6178):154-6)
- **Eccentric contraction injury in dystrophic canine muscle.** (Arch Phys Med Rehabil. 2002 Nov;83(11):1572-8)
- **Chronic administration of membrane sealant prevents severe cardiac injury and ventricular dilatation in dystrophic dogs.** (J Clin Invest. 2010 Apr;120(4):1140-50.)
- **Age-matched comparison reveals early electrocardiography and echocardiography changes in dystrophin-deficient dogs.** (Neuromuscul Disord. 2011 Jul; 21(7):453-61.)
- **Microdystrophin ameliorates muscular dystrophy in the canine model of duchenne muscular dystrophy.** (Mol Ther. 2013 Apr;21(4):750-7)