

HYBRID GENE THERAPY FOR AD-EDMD

Gene Therapy
Prof. Isabella Saggio
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AUTOSOMAL DOMINANT EMERY-DREIFUSS MUSCULAR DYSTROPHY

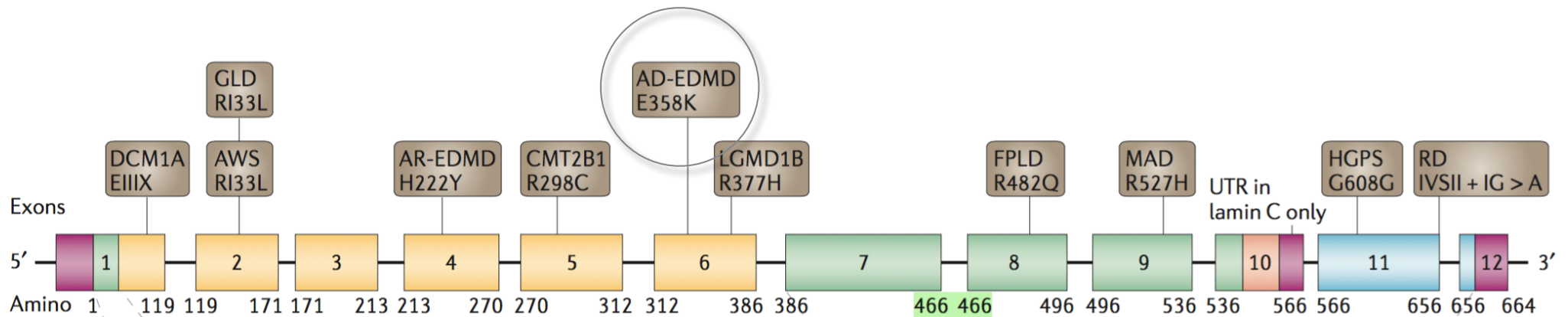


Fig.1 Adapted from B. Capell and F. Collins Nature Publishing Group, 2006

- EARLY CONTRACTURES ⁵
- MUSCLE WASTING
- **CARDIAC DEFECTS**

AUTOSOMAL DOMINANT EMERY-DREIFUSS MUSCULAR DYSTROPHY

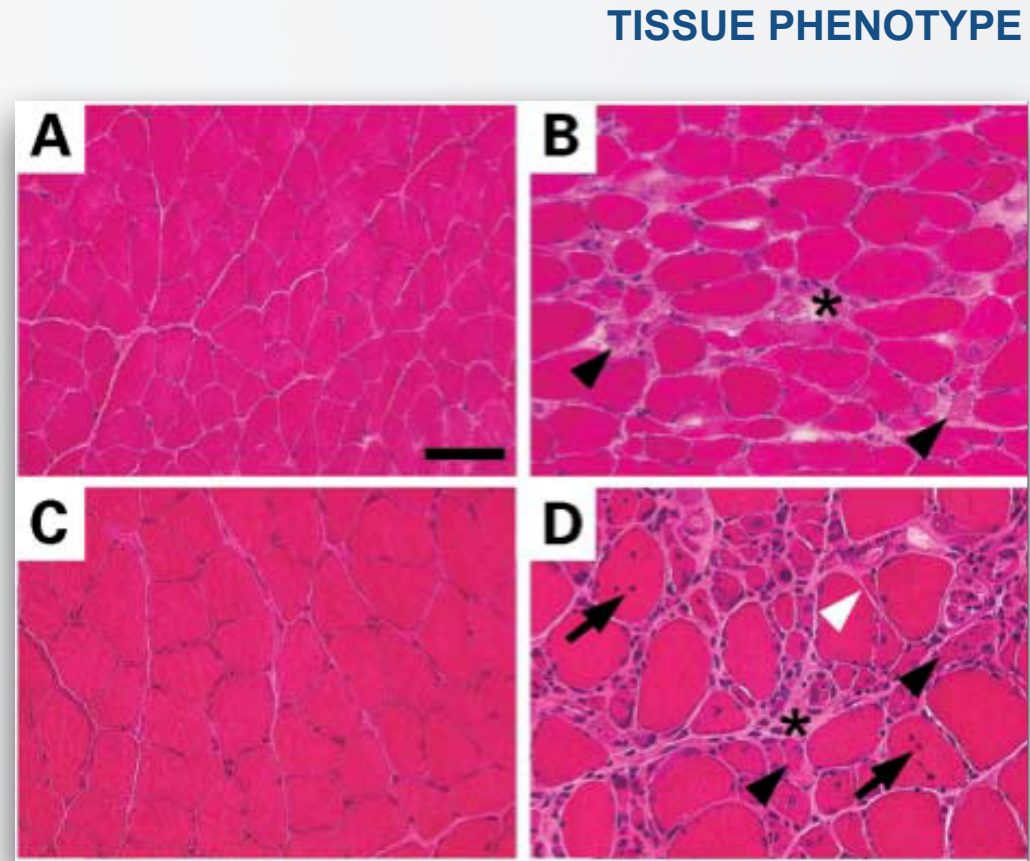
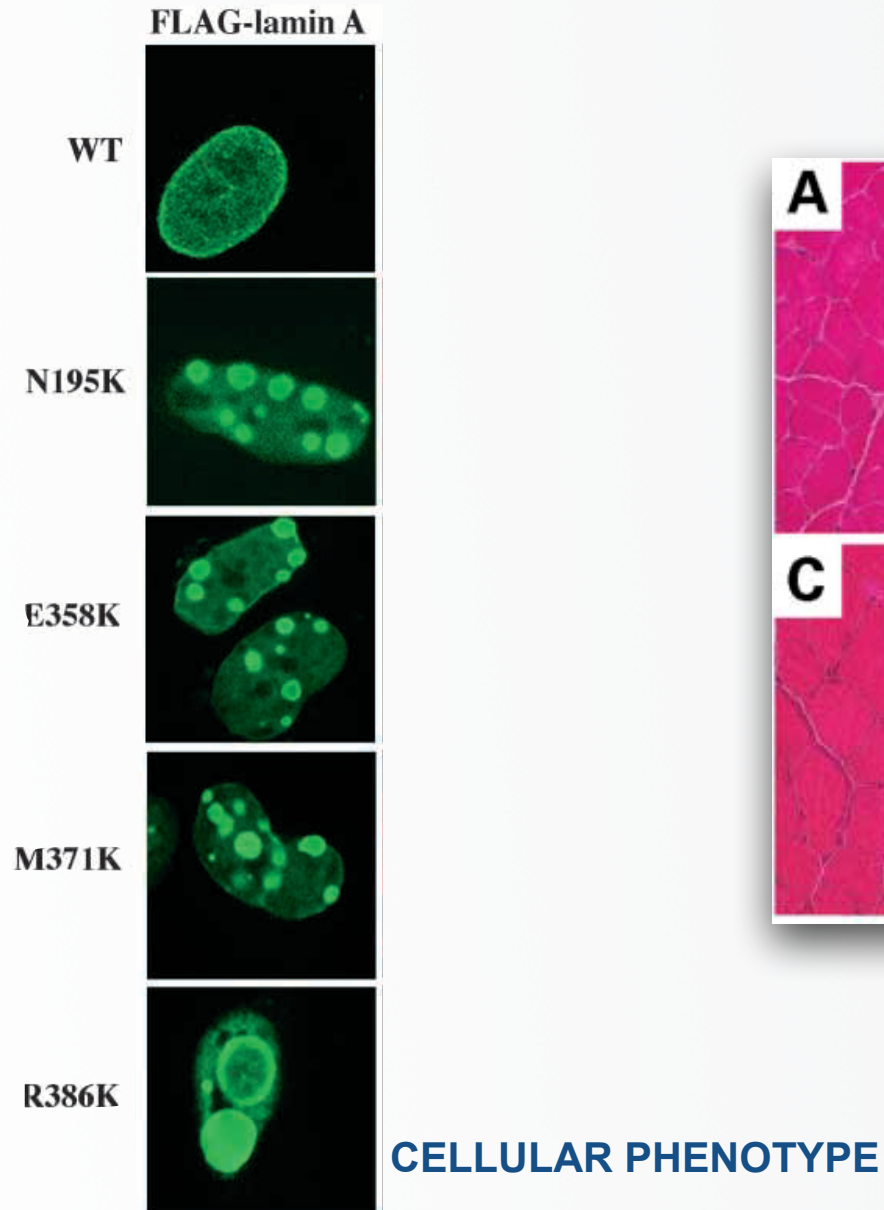


Fig. 3 adapted from T. Arimura et al, Human Molecular Genetics, 2005

Fig. 2 adapted from C. Ostlund et al, Journal of Cell Science, 2001

WHY GENE THERAPY?

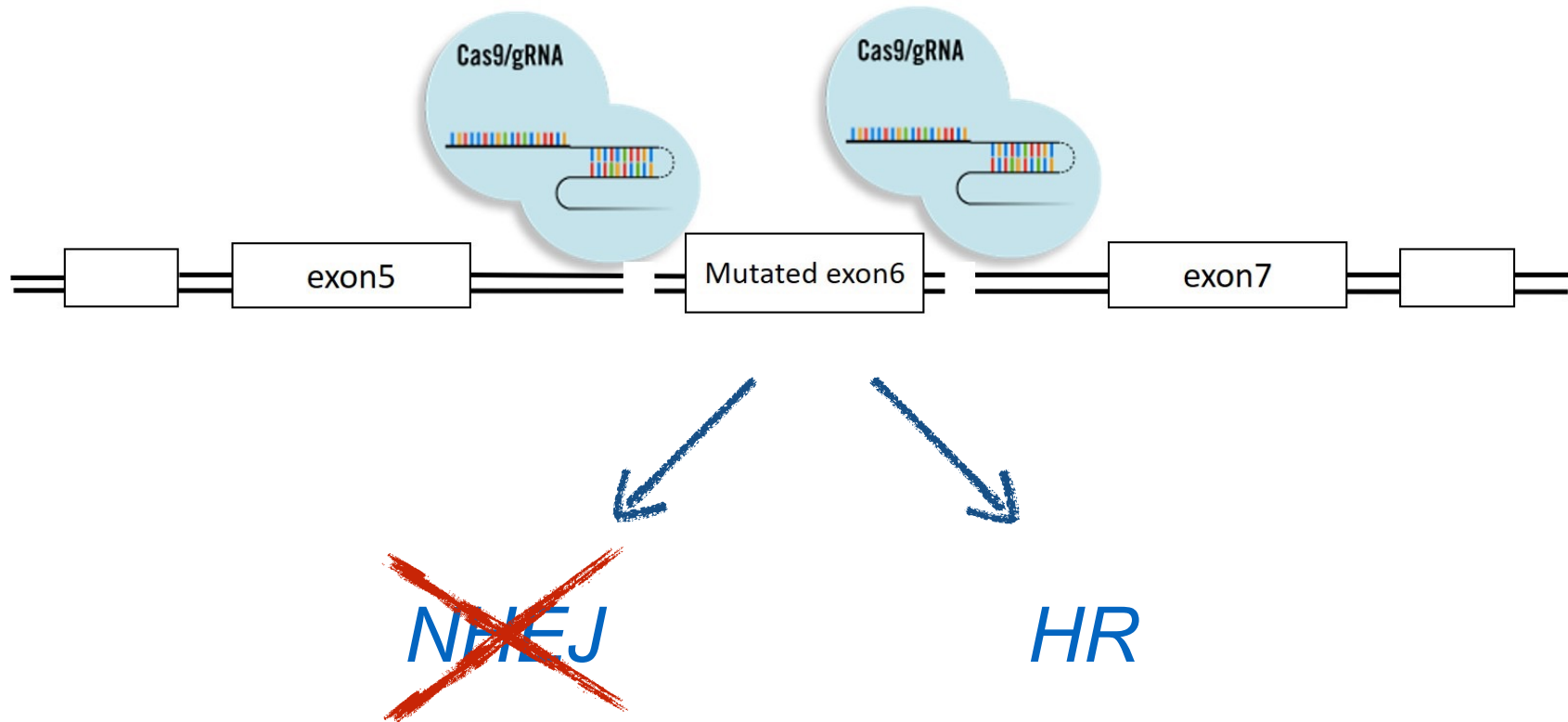
NO CURRENT TREATMENTS

RADICAL THERAPY



OUR STRATEGY

- AAV8 → CAS9 + 2 gRNAs
- AAV8 → ssDNA template
- LIPOSOME → SCR 7

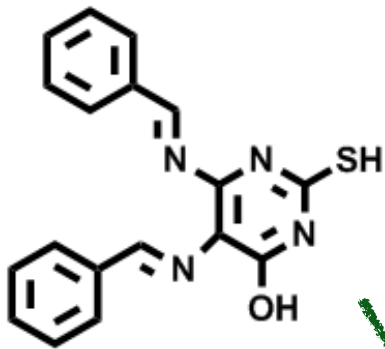


VECTORS STRUCTURE

1° AAV 8

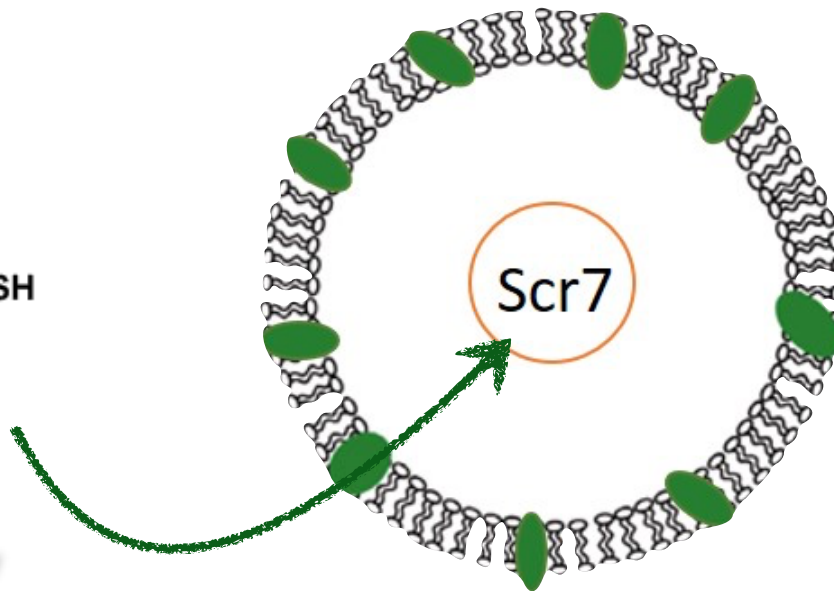


2° AAV 8



SCR7 protein = NHEJ inhibitor

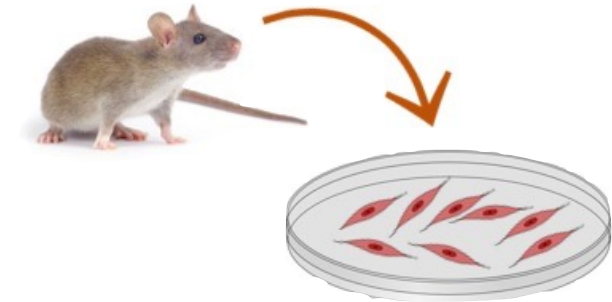
LIPOSOME with SCR7



EXPERIMENTAL PLAN

IN VITRO (H222P mouse model)

- *Testing SCR 7 in muscles cells*
- *Verify the rescuing of cellular phenotype*



IN VIVO (H222P mouse model)

- *Verify liposomes targeting muscles cells*
- *Intravenous injection of the two AAV8 and liposome*



IN VITRO MOUSE

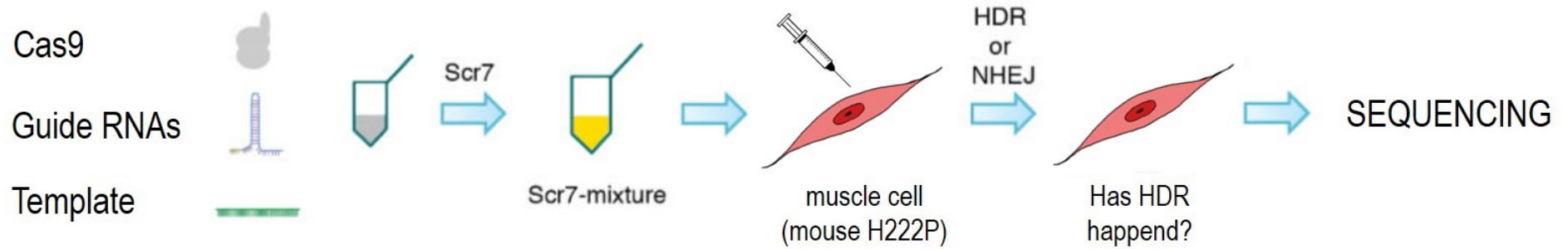
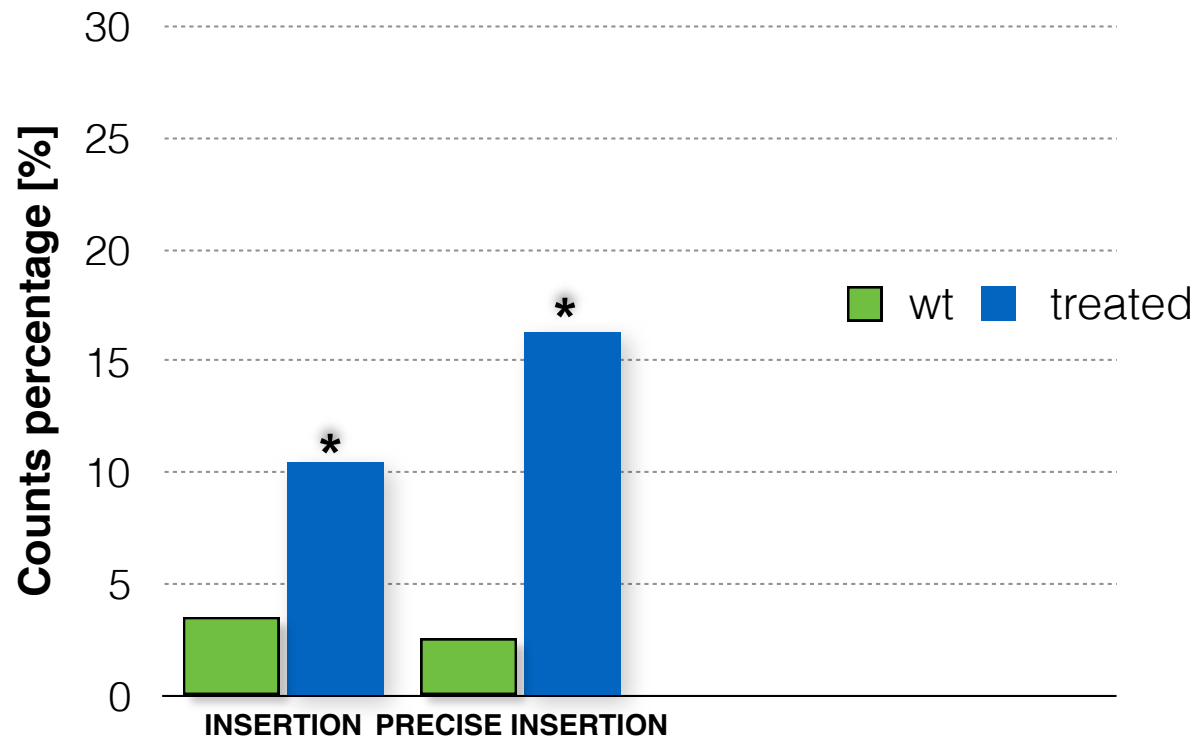


Fig. 4 Adapted from T. Maruyama et al, Nature biotechnology, 2015



IN VITRO MOUSE: is the wt cellular phenotype rescued?

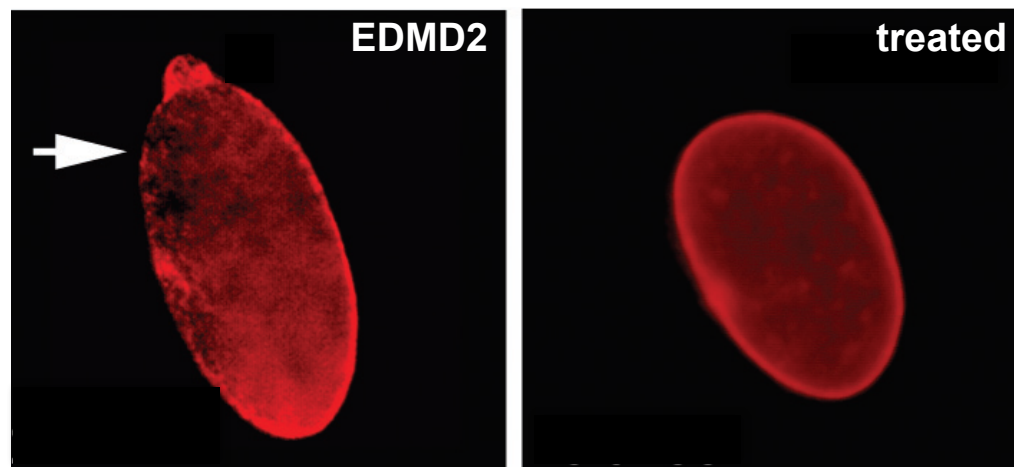


Fig.5 Adapted from D. Camozzi et al., Nucleus, 2014

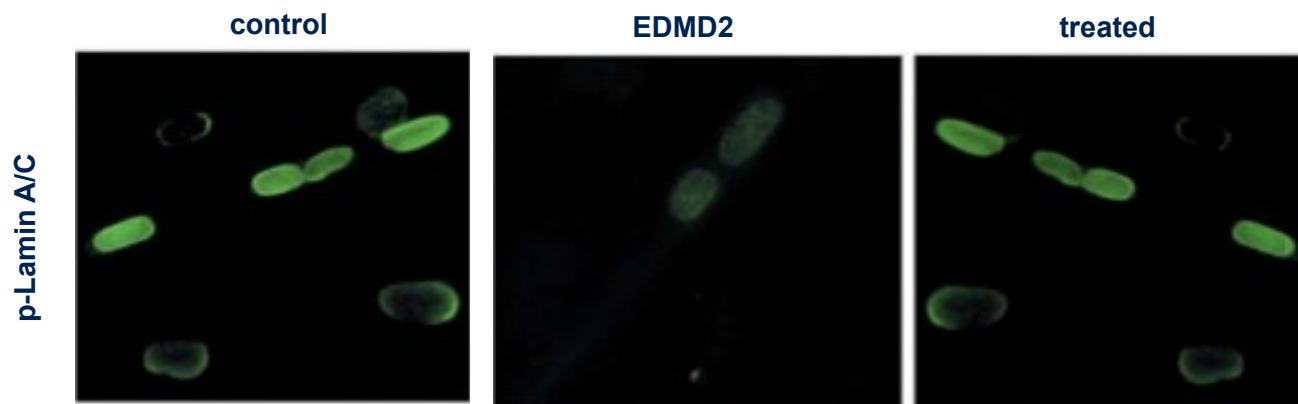
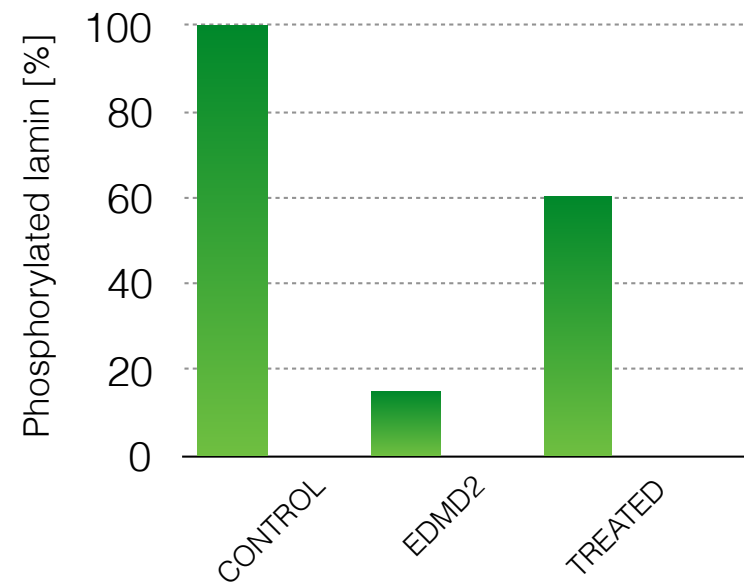


Fig. 6 Adapted from V. Cenni et al, J Med Genet, 2005



IN VITRO MOUSE: is the wt cellular phenotype rescued?

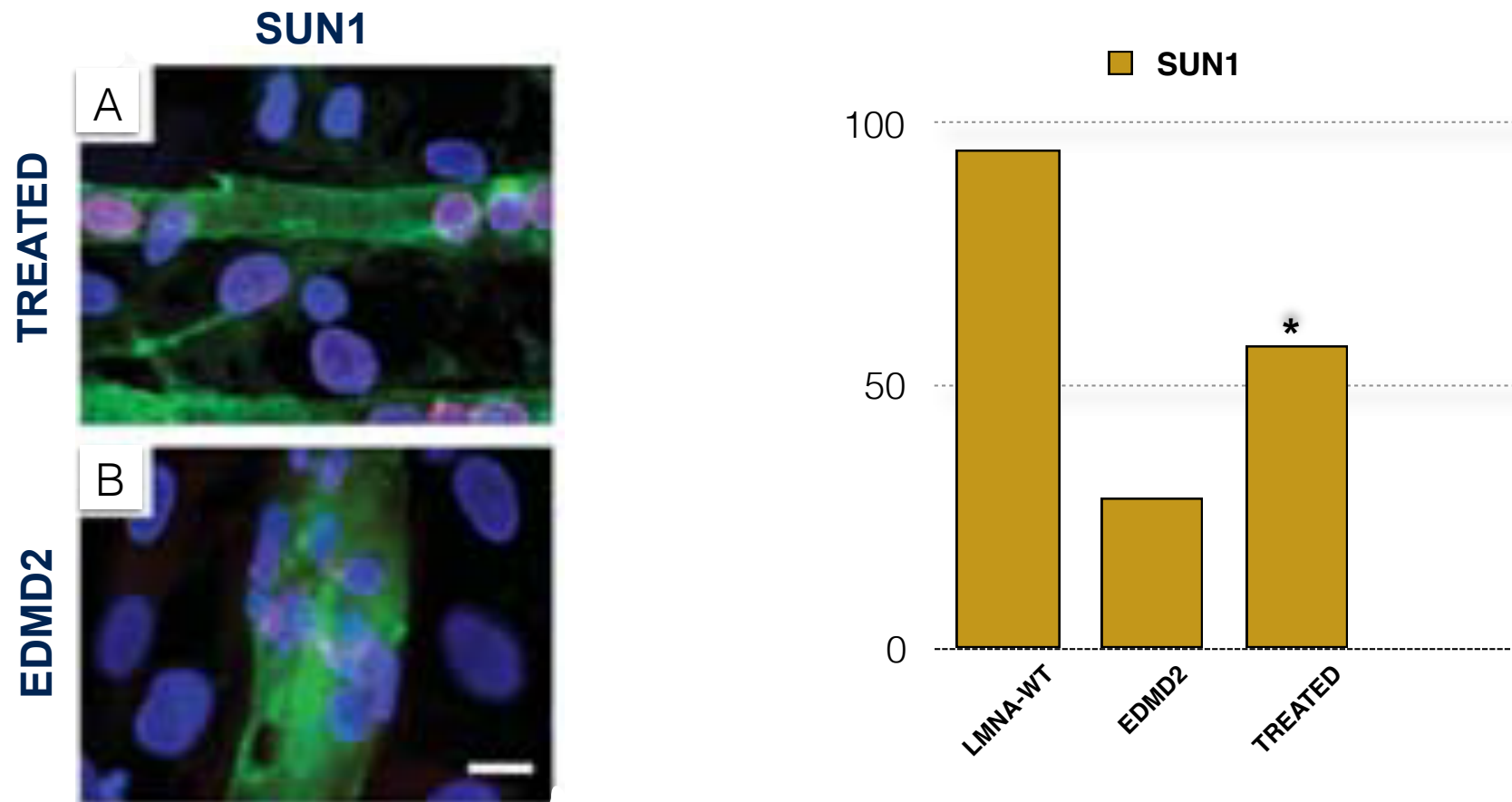


Fig. 7 Adapted from E. Mattioli et. Nature publication group, 2011

IN VIVO MOUSE: do our liposomes target muscle cells?

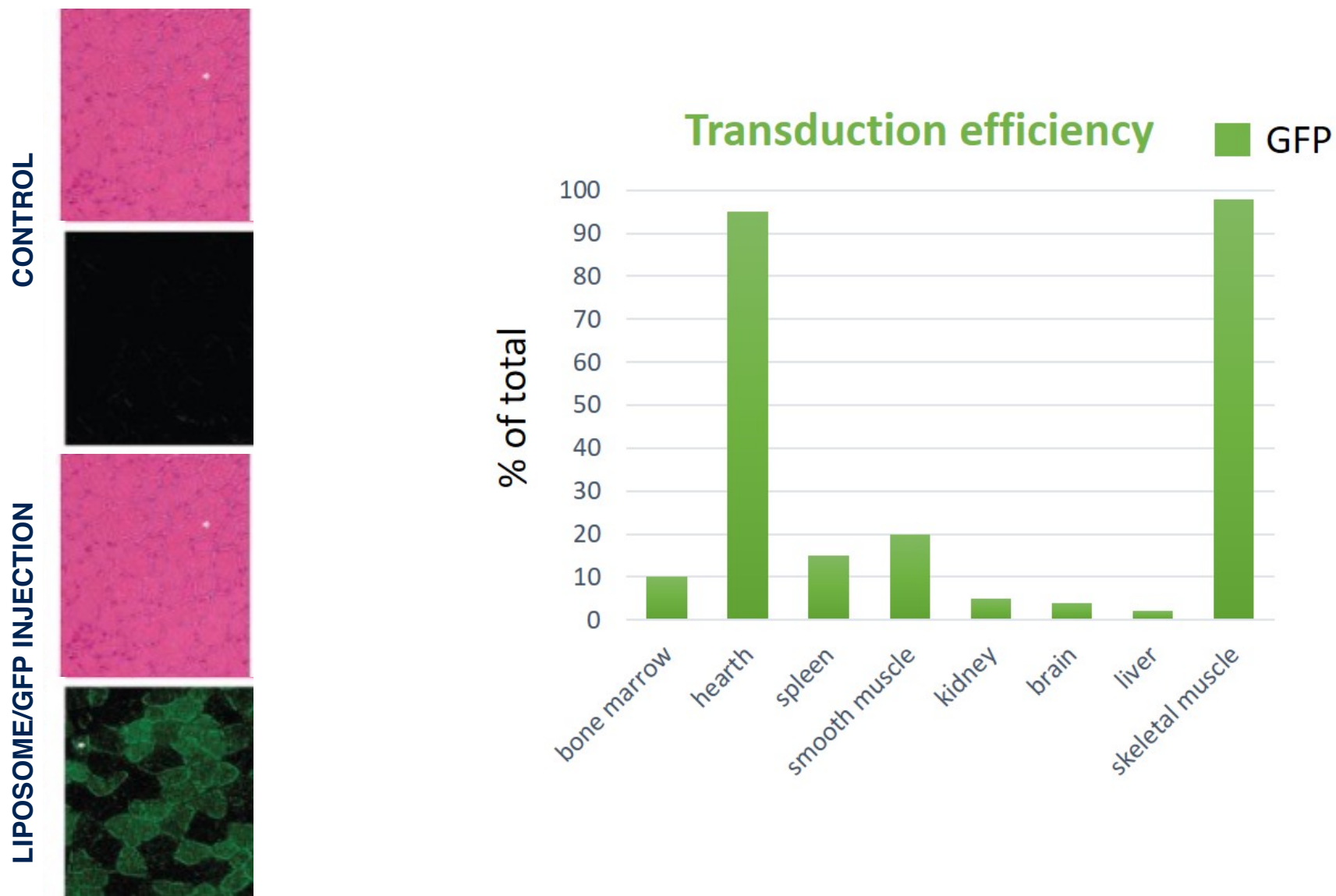


Fig.9 Adapted from C. Winbanks et al, Plos One, 2012

IN VIVO MOUSE: is the wt cellular phenotype rescued?

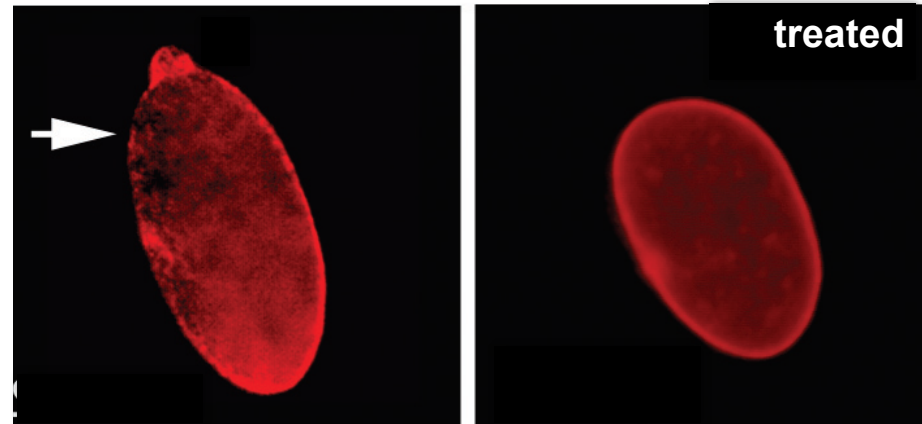


Fig. 10 Adapted from D. Camozzi et al., *Nucleus*, 2014

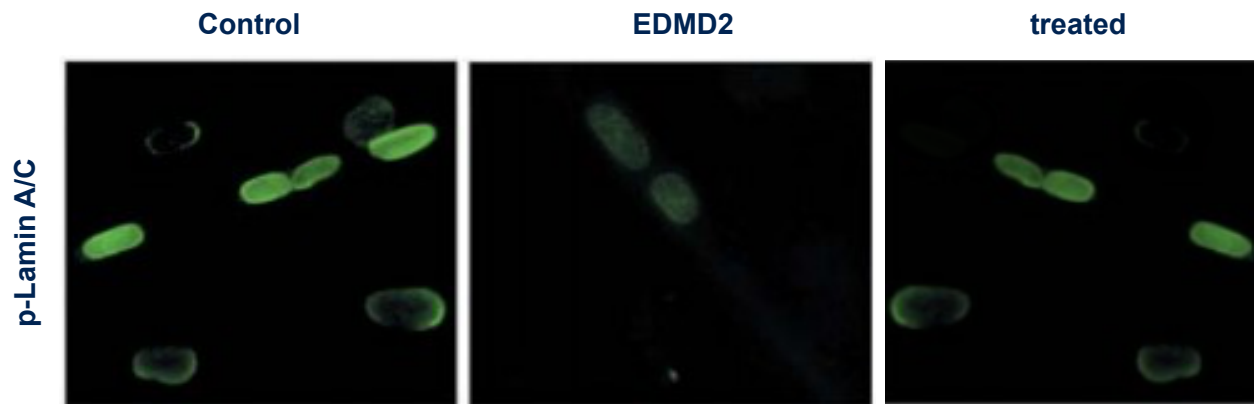
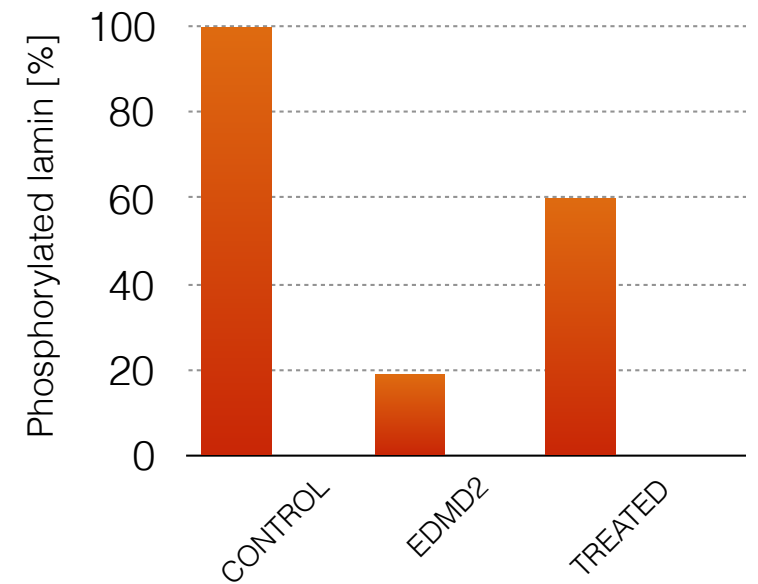


Fig. 11 Adapted from V. Cenni et al, *J Med Genet*, 2005



IN VIVO MOUSE: is the wt cellular phenotype rescued?

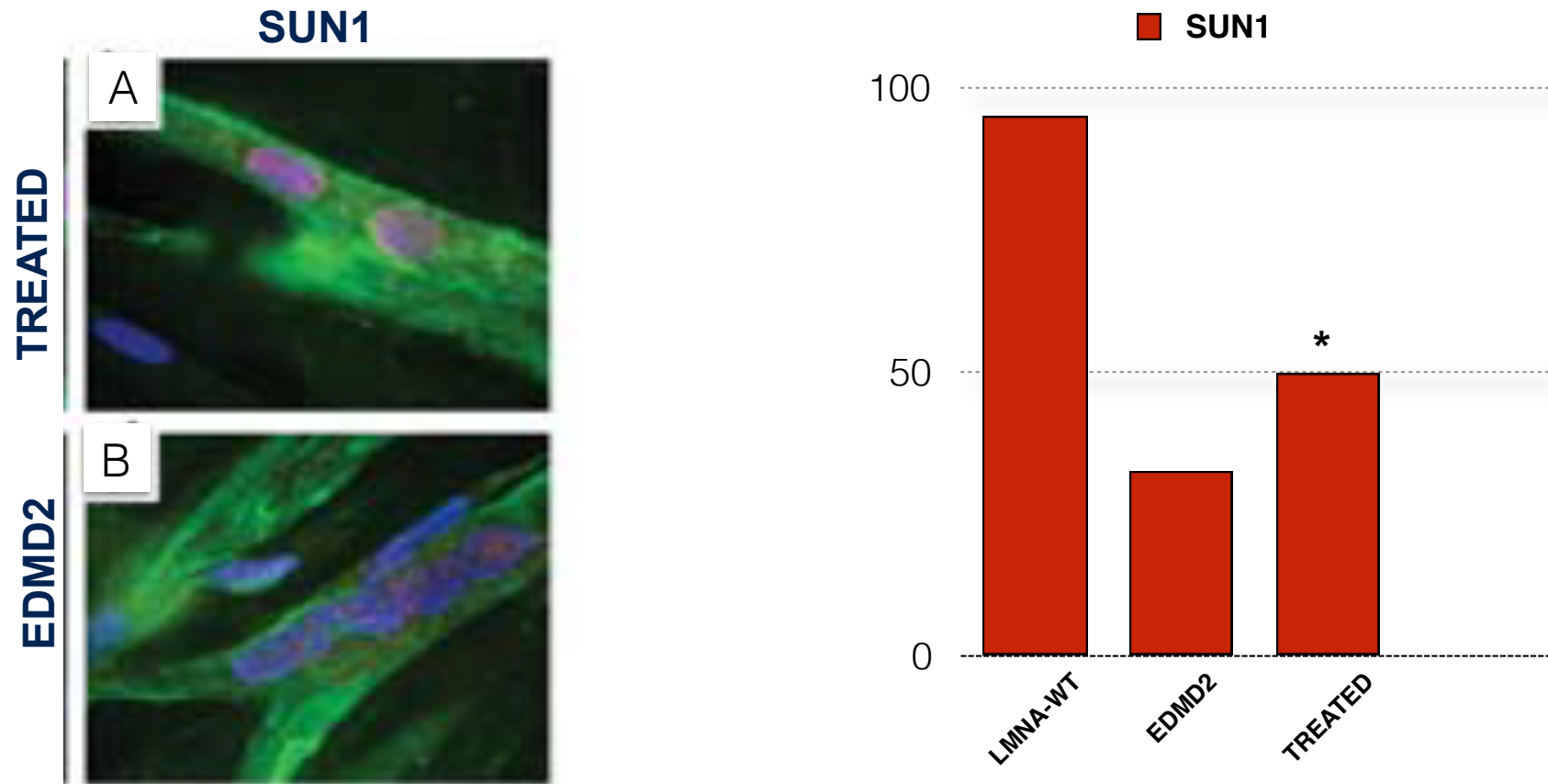


Fig. 12 Adapted from E. Mattioli et al. Nature publication group, 2011

IN VIVO MOUSE: is the wt tissue phenotype rescued?

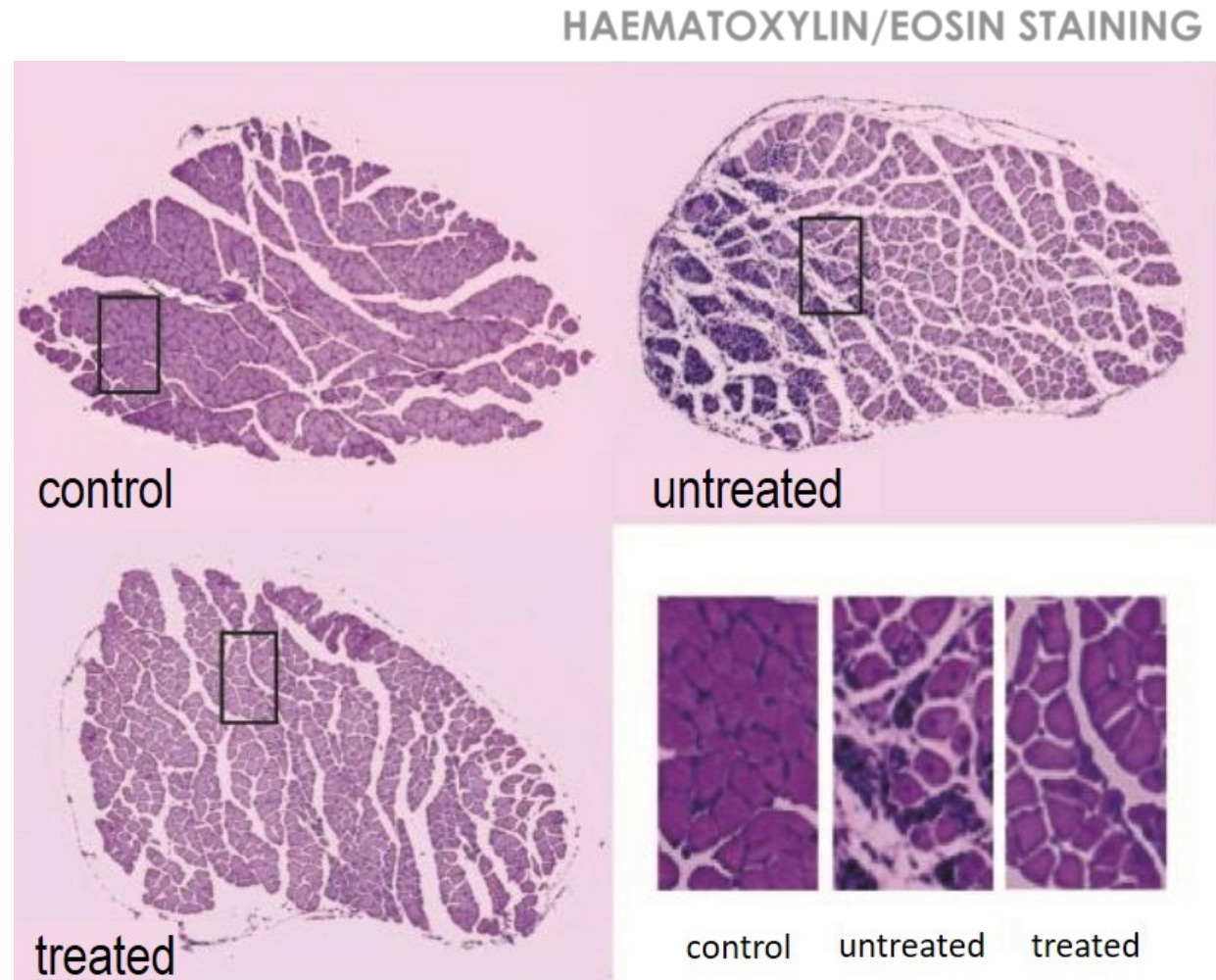
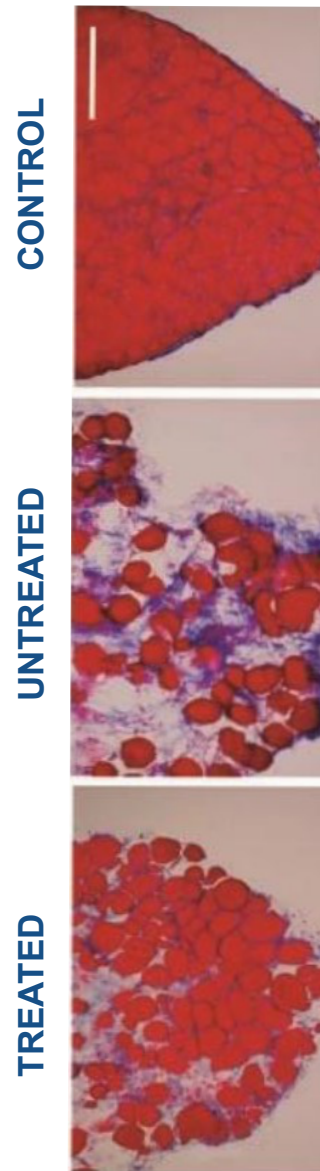
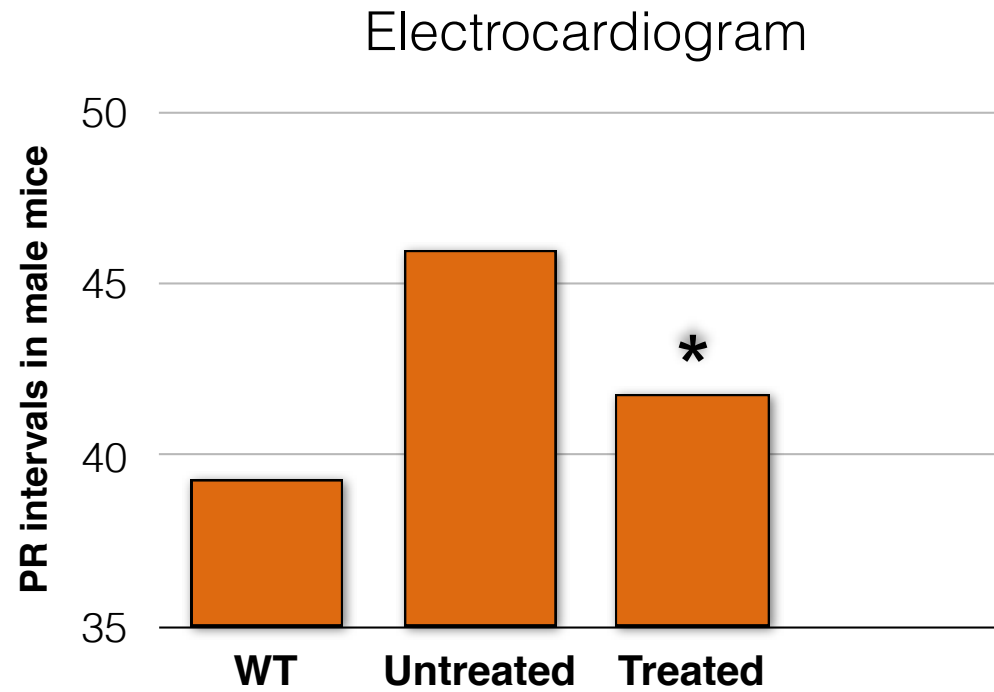


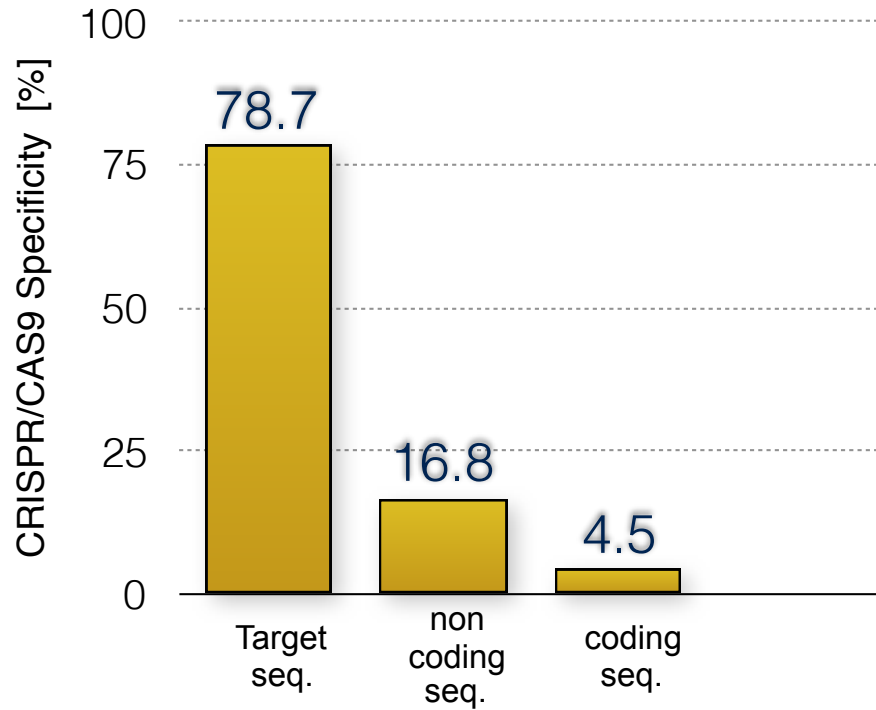
Fig. 13 and 14 Adapted from V. Cenni et al, J Med Genet, 2005

IN VIVO MOUSE: functional assays



	WT	UNTREATED	TREATED
ROTAROD TEST	100%	42%	98%

PITFALLS and FUTURE PERSPECTIVES



- **ALLELE SPECIFICITY**
- **OFF TARGET**

Timeline



COSTS

approximately **40000,00€** for year
for at least **3 years**
(without salary of researchers)

Mice lines:

c57/bl6 mice(wt) (20€x3)= 60€
H222P (300€x15)= 4500€
Stabulation = 3000€

SCR7, NHEJ inhibitor (2mg) = 130€
AVV 8 (300€x40)= 12000€
qRT.PCR kit = 500€
Immunohistochemistry kit = 2000€
Western blot kit = 2000€
Immunofluorescence kit = 1500€
Antibodies = 600€
Liposome kit (90€x18) = 1620€
Multiplex CRISPR/Cas9 Assembly System Kit = 350€
sgRNA Kit = 295€
Struments used in lab = 8000€

(eppendorf, termomixer, vials, flasks, tubes, buffers,
growth medium, chemical agents employed in our reserch,
enzymes employed, rotarod, ecg)



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