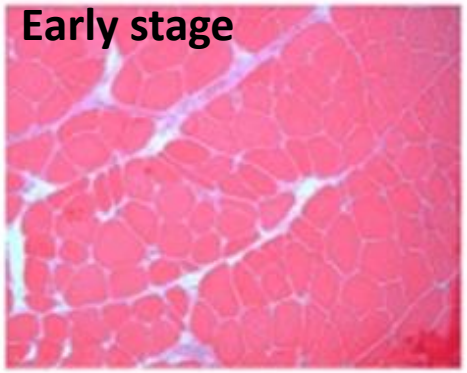
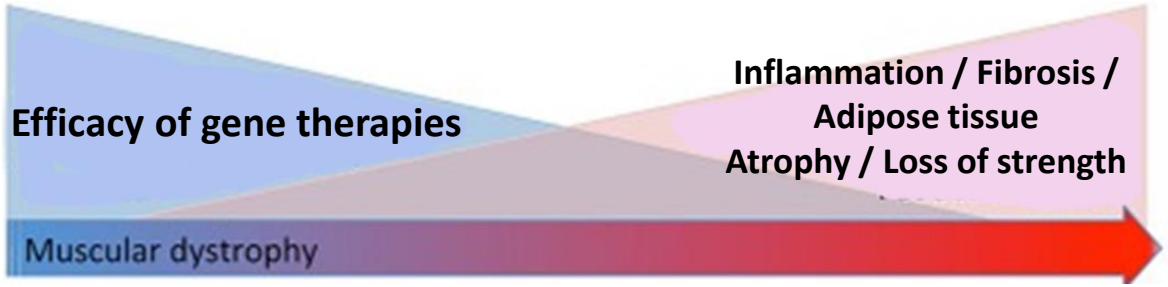
AAV-microDystrophin and AAV-GDF5: A combined treatment to optimize DMD gene therapy ?

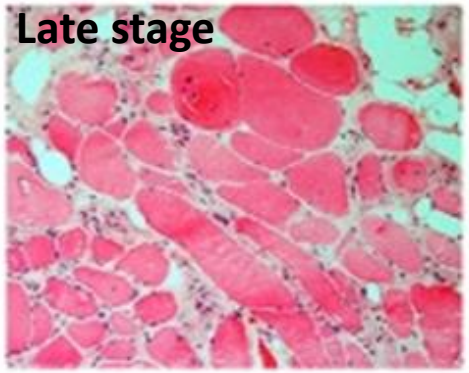
France Piétri-Rouxel

Duchenne Muscular Dystrophy - DMD

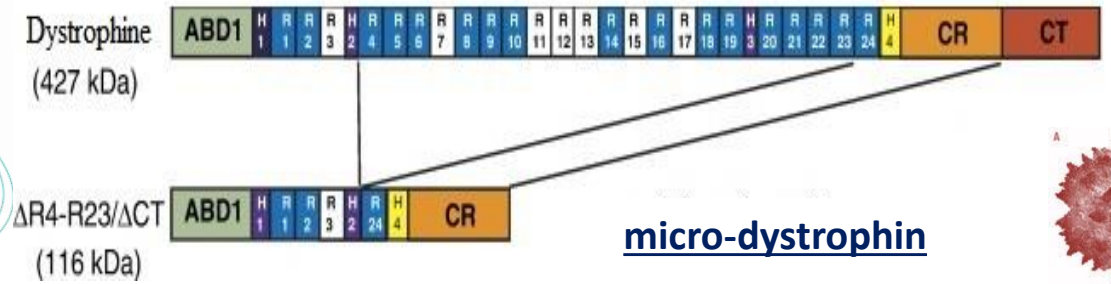
Lack of Dystrophin → **muscle fiber damage** → **necrosis/regeneration process** → **Degeneration**



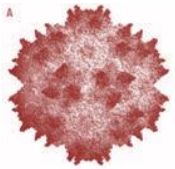




Adapted from Gordova G et al, Front Genet. 2018



micro-dystrophin



Limitation for AAV gene therapy : loss of viral genome

Need for a combined treatment to optimize dystrophin restoration

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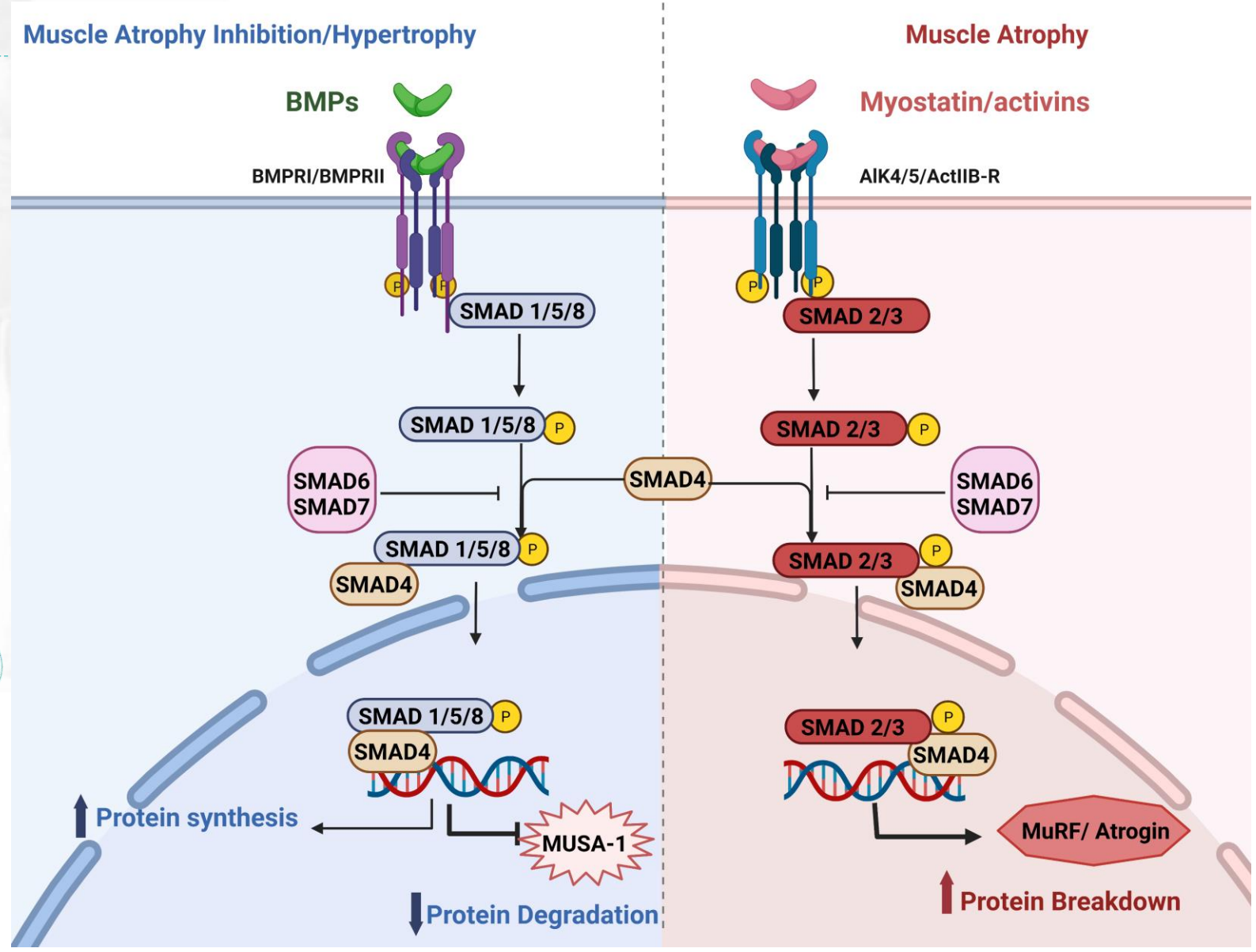
CURE THROUGH INNOVATION

Muscle mass homeostasis



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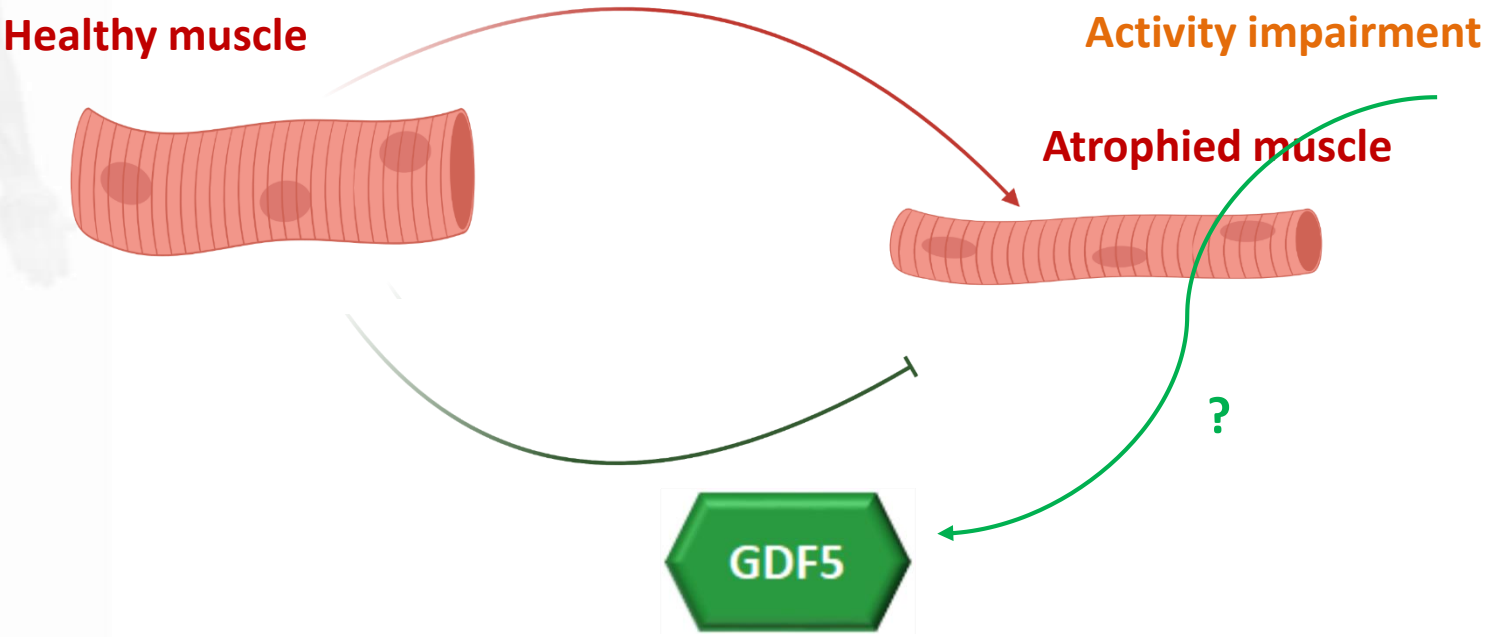
BMP14/GDF5: compensatory response to electrical activity impairment



Compensatory response to limit muscle mass loss
Sartori et al, Nat Gen 2013

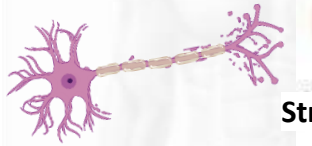
TGFβ family member: Growth Differentiation Factor 5

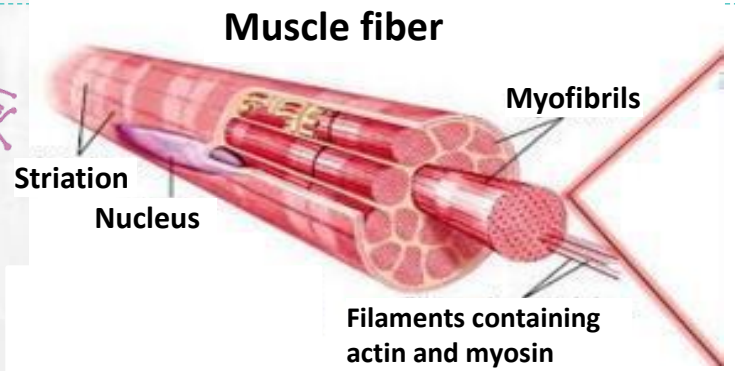
Circulating factor *(Storm et al. 1994)*

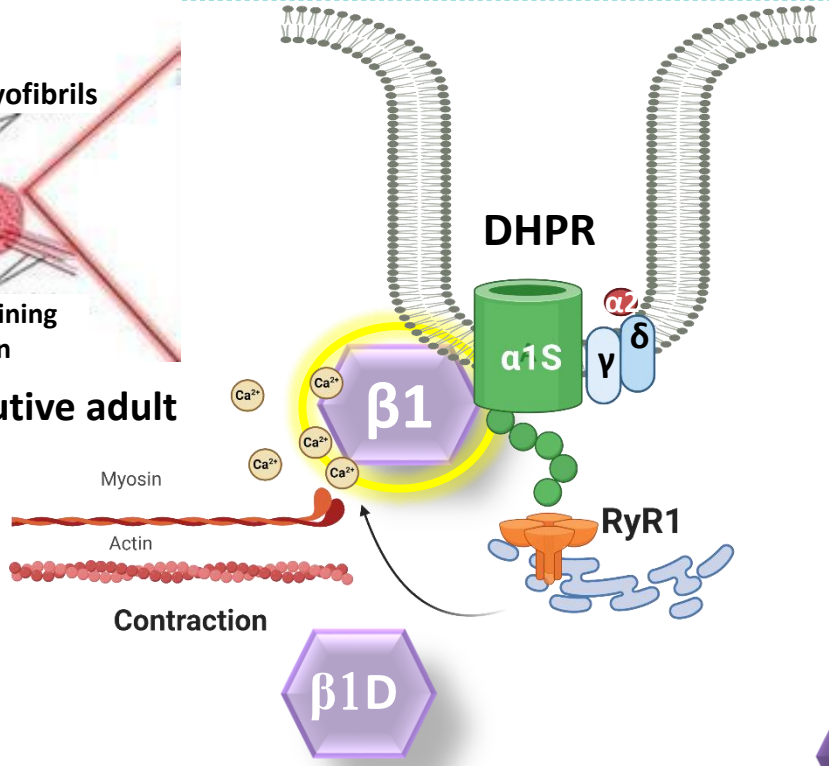
Needed for re-innervation *(MacPherson et al. 2005)*

Link between electrical activity impairment and compensatory response?

Nerve Damage





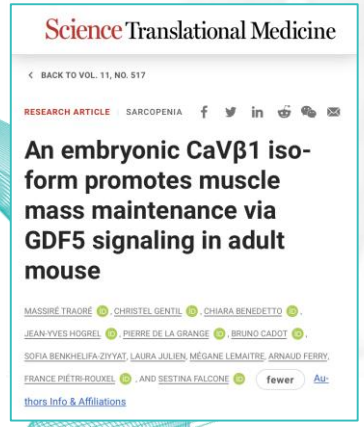


Constitutive adult

β1

-Modulates α1S fonctions

-Transcription factor function in MPC

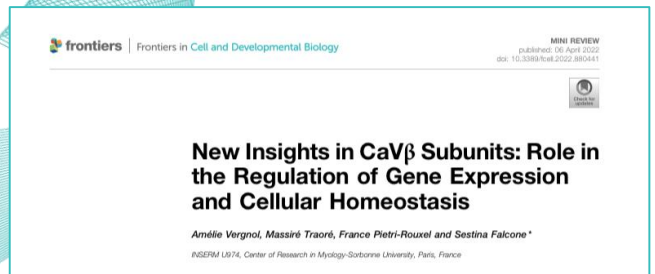

Science Translational Medicine

BACK TO VOL. 11, NO. 517

RESEARCH ARTICLE SARCOPENIA

An embryonic CaVβ1 isoform promotes muscle mass maintenance via GDF5 signaling in adult mouse

MASSIRÉ TRAORÉ, CHRISTEL GENTIL, CHIARA BENEDETTO, JEAN-YVES HOGREL, PIERRE DE LA GRANGE, BRUNO CADOT, SOFIA BENKHELIFA-ZIYYAT, LAURA JULIEN, MÉGANE LEMAITRE, ARNAUD FERRY, FRANCE PIÉTRI-ROUXEL, AND SESTINA FALCONE fewer Authors Info & Affiliations


frontiers | Frontiers in Cell and Developmental Biology

MINI REVIEW

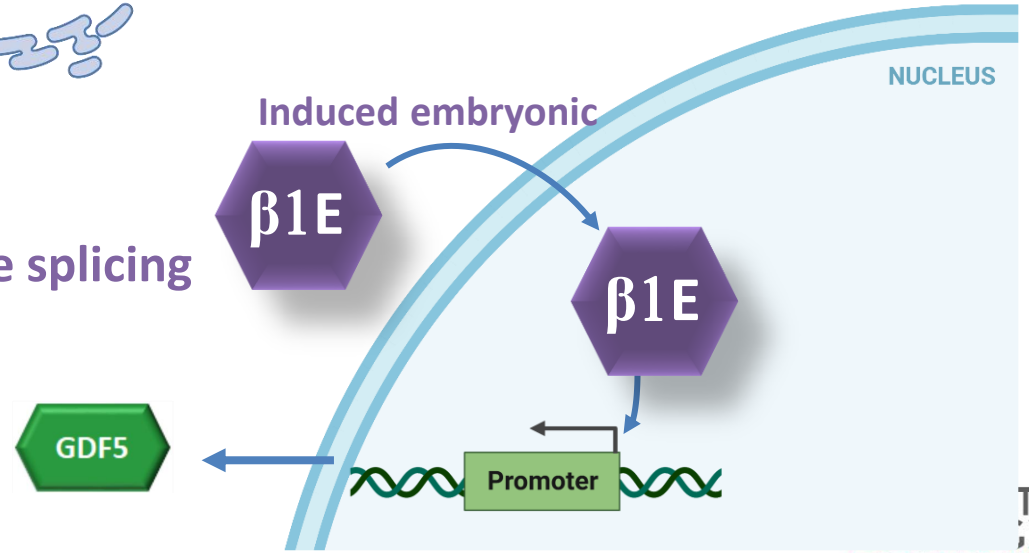
New Insights in CaVβ Subunits: Role in the Regulation of Gene Expression and Cellular Homeostasis

Amélie Vergnol, Massiré Traoré, France Pietri-Rouxel and Sestina Falcone*

INSERM U974, Center of Research in Myology-Sorbonne University, Paris, France

β1D

***Cacnb*1 (CaVβ1 mRNA) alternative splicing**
CaVβ1D/CaVβ1E



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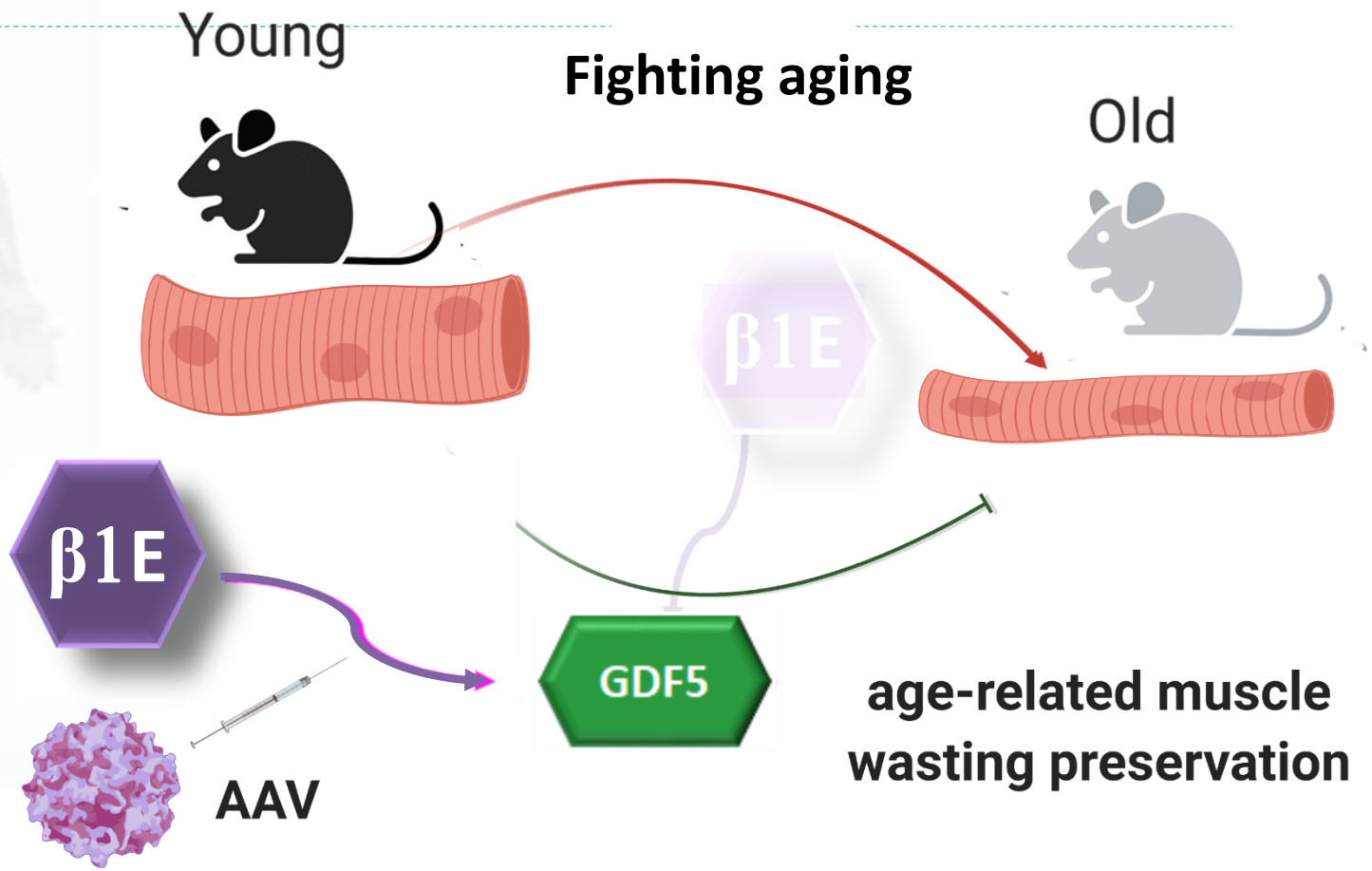
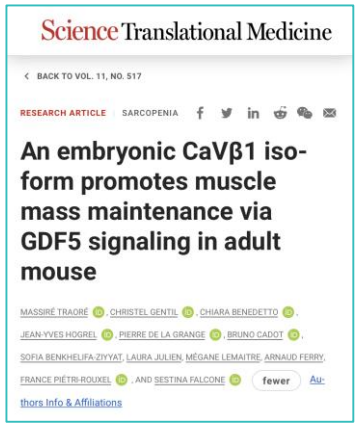
Counterbalancing age-related muscle atrophy through novel identified molecular pathways




Science Translational Medicine

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RESEARCH ARTICLE | SARCOPENIA

An embryonic CaVβ1 isoform promotes muscle mass maintenance via GDF5 signaling in adult mouse

MASSIRÉ TRAORÉ, CHRISTEL GENTIL, CHIARA BENEDETTO, JEAN-YVES HOGREL, PIERRE DE LA GRANGE, BRUNO CADOT, SOFIA BENKHELIFA-ZIYYAT, LAURA JULIEN, MÉGANE LEMAITRE, ARNAUD FERRY, FRANCE PIÉTRI-ROUXEL, AND SESTINA FALCONE fewer Authors Info & Affiliations

Poster #35. Therapeutic approach based on GDF5 to counteract age-related muscle wasting

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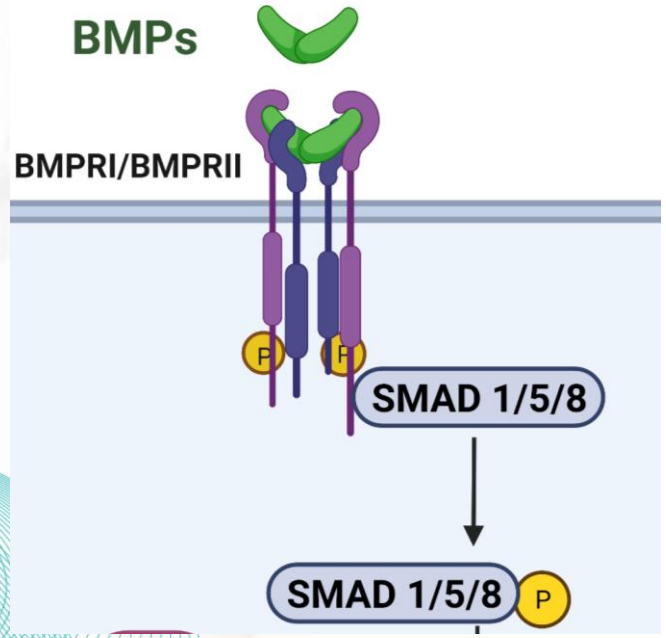
GDF5 in combined treatment to optimize gene therapy for DMD?

BMP signaling and skeletal muscle regeneration



Balances proliferation and differentiation of satellite cell descendants (Friedrichs et al., 2011).

Regulates myogenic differentiation in muscle satellite cells (Ono et al., 2011).

Has a key role in adult muscle regeneration (Clever et al., 2010).

Inhibits intramuscular adipogenesis (Huang et al., 2014).

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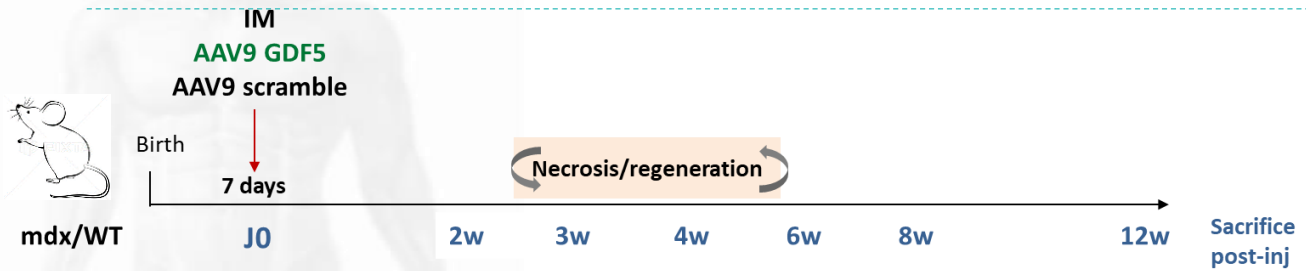
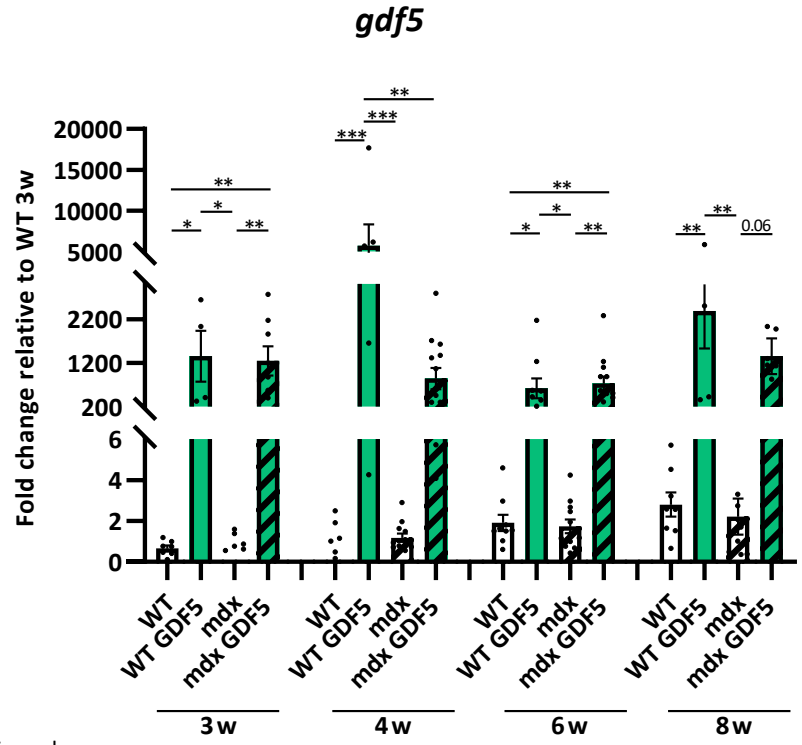
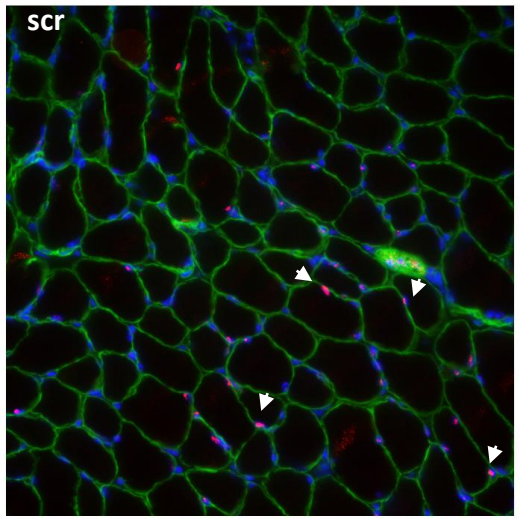
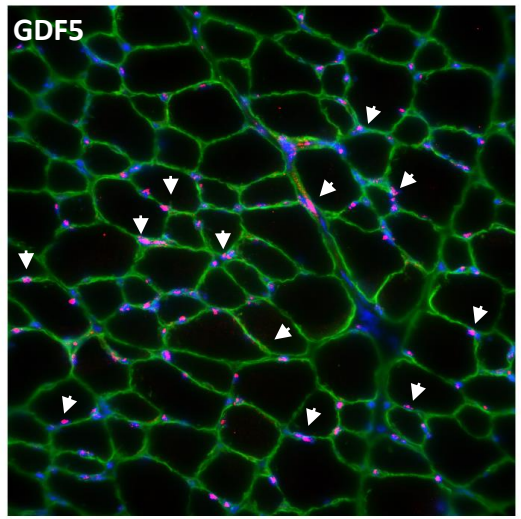
GDF5 overexpression in DMD mouse model (mdx)



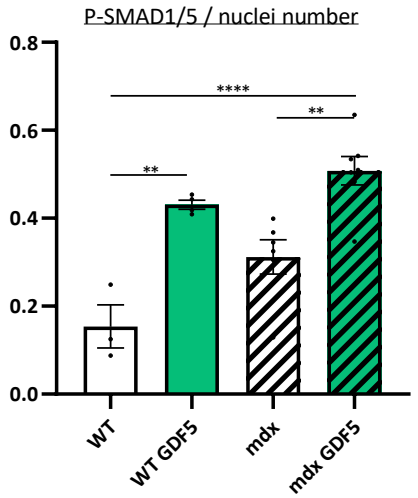


mdx P-SMAD1/5 / laminin / DAPI





2w post-inj



Activation of GDF5 pathway

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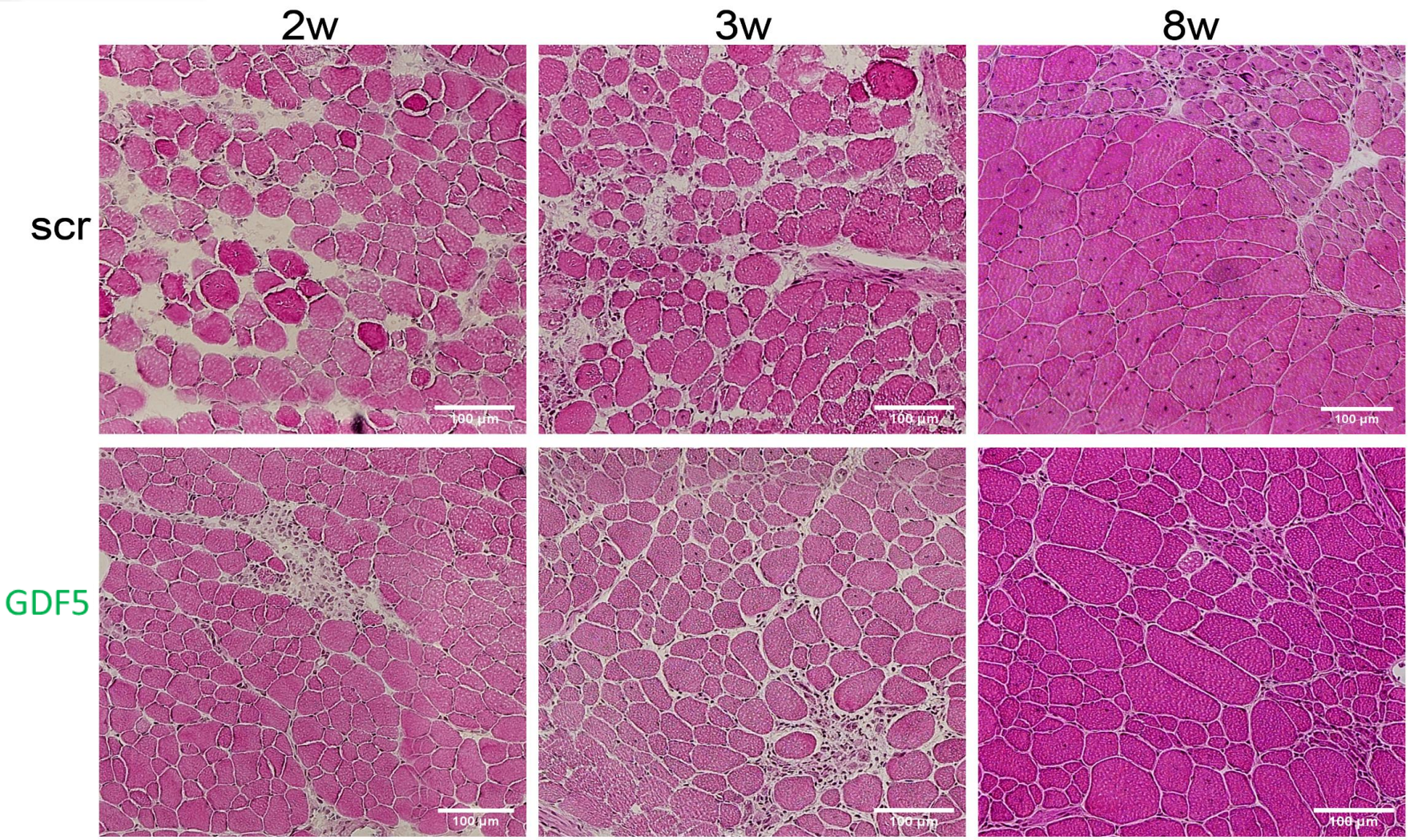
Effect of GDF5 overexpression on DMD muscle



Improvement of DMD muscle histology

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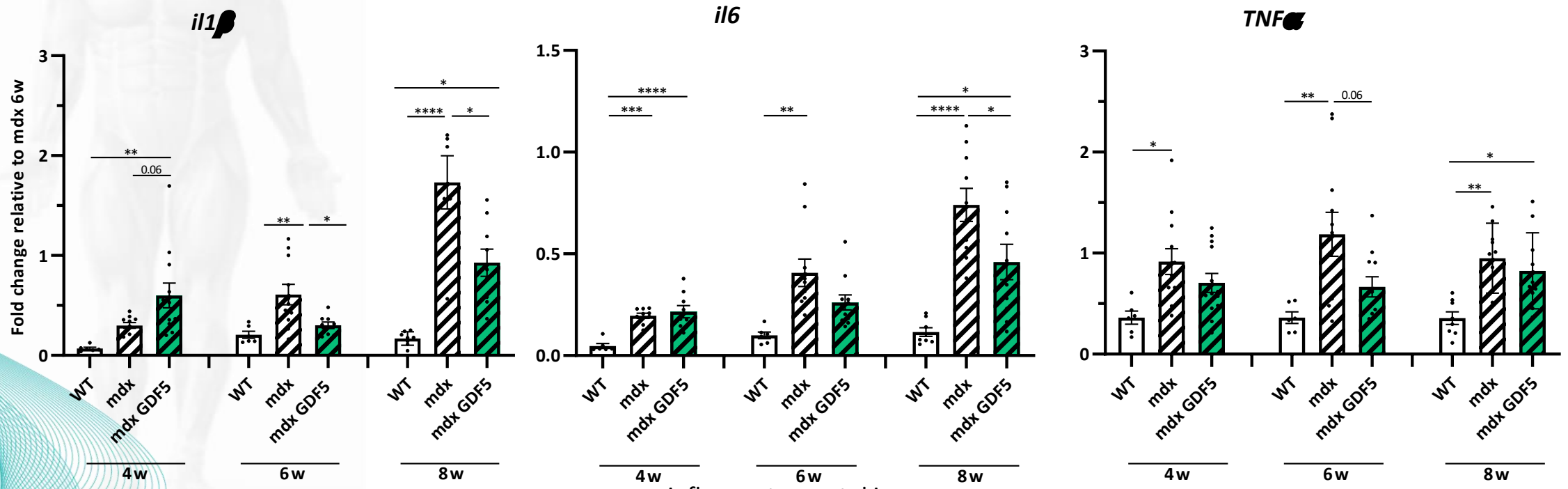
GDF5 overexpression and inflammatory marker expression



Decrease of pro-inflammatory markers at 6and 8 weeks post- treatment

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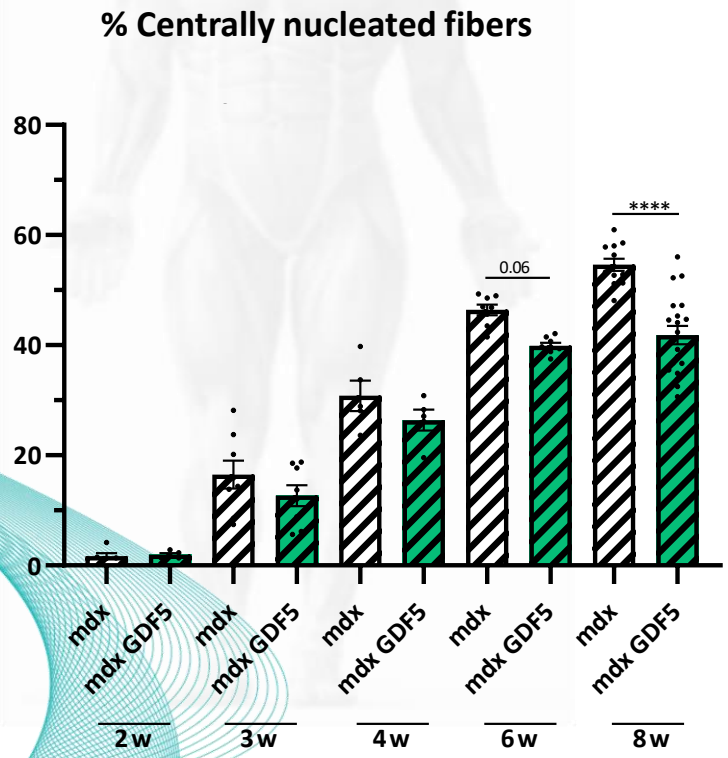
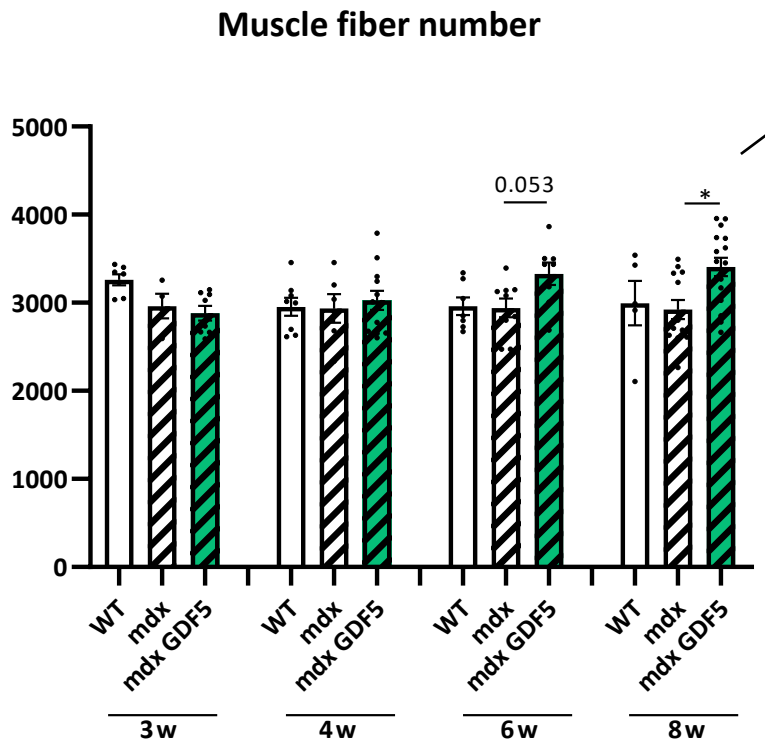
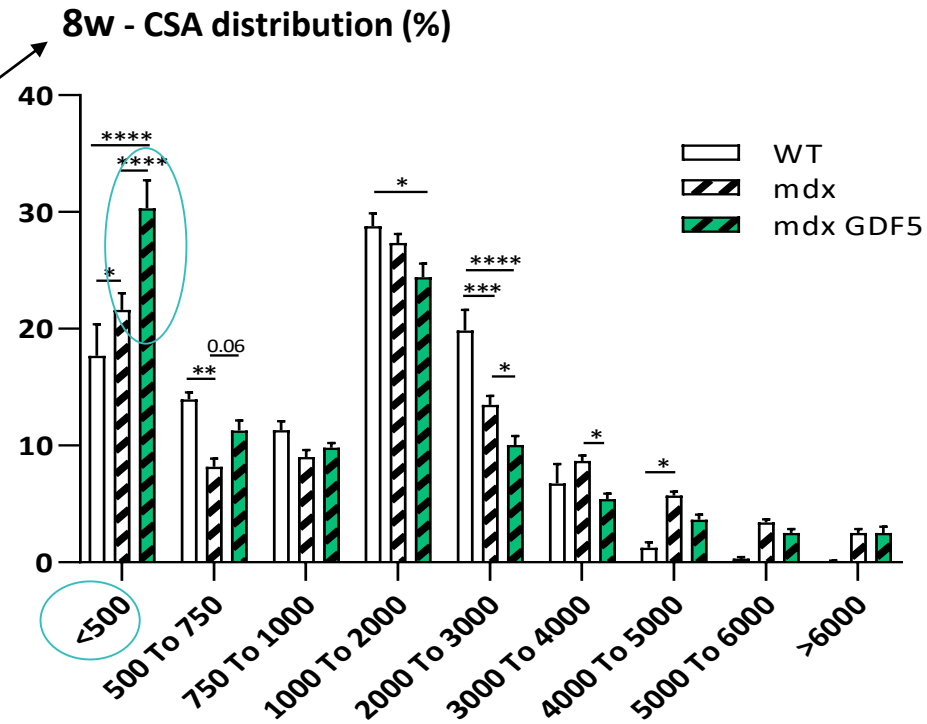
GDF5 overexpression and differentiation / regeneration process







Decrease of centrally nucleated fibers
->modulation of regeneration

->hyperplasia

->Increase of small fiber number

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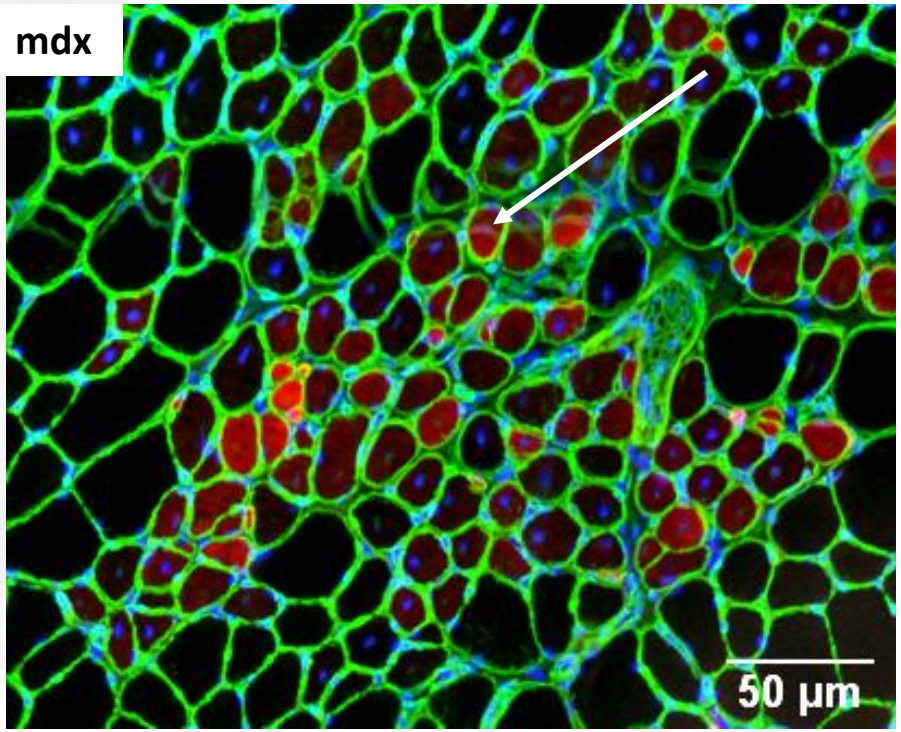
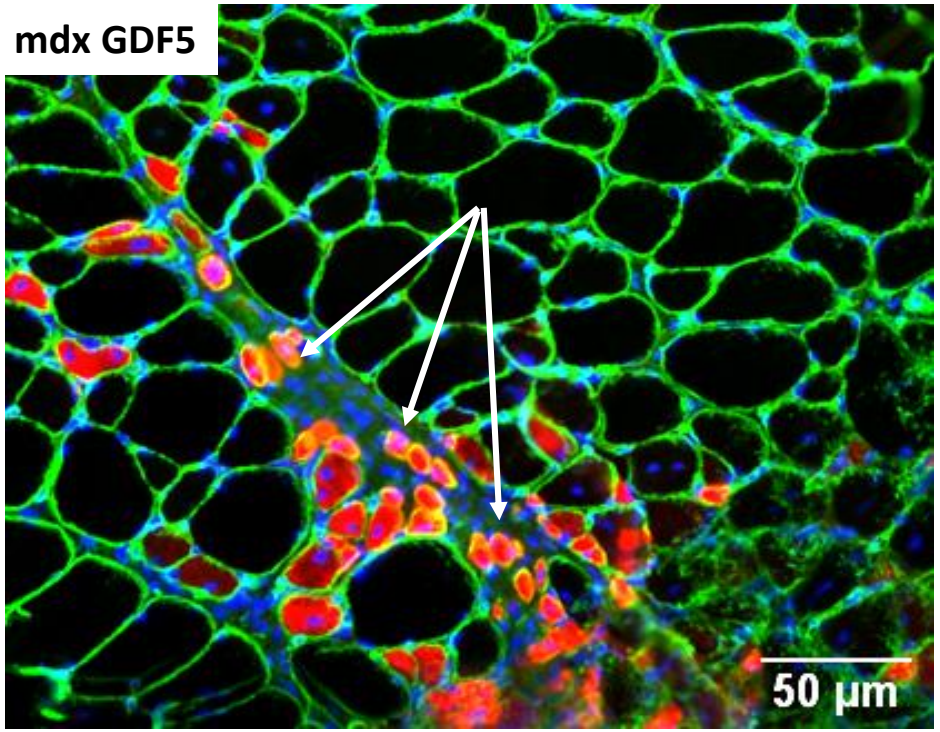
GDF5 and hyperplasia

eMHC/laminin / DAPI





3w post-inj

Presence of new fibers in interstitial space

GDF5 could regulate myogenic commitment of resident muscle cells

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GDF5 overexpression effects in mdx muscle

- ✓ Improvement of DMD muscle histology
- ✓ Modulation of inflammatory markers
- ✓ Increased of small fiber number
  - ->hyperplasia
  - ->appearance of new fibers in interstitial space

Could GDF5 promote long term and more microDystrophin + fibers ?

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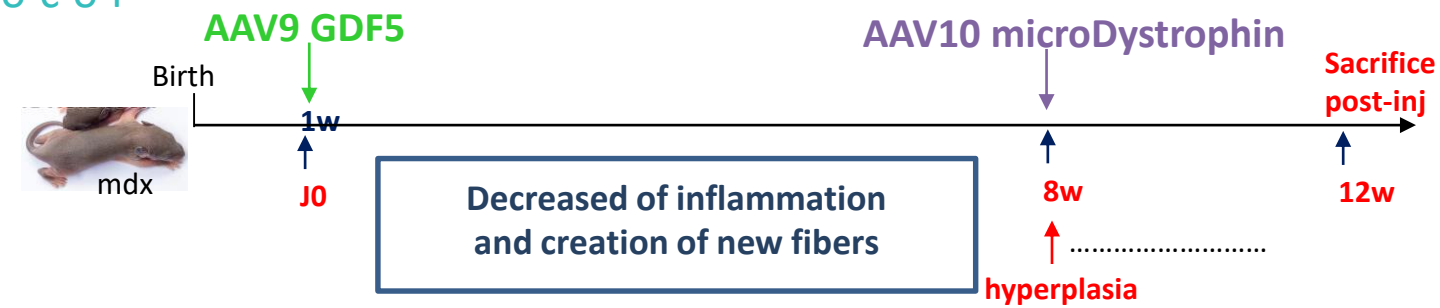
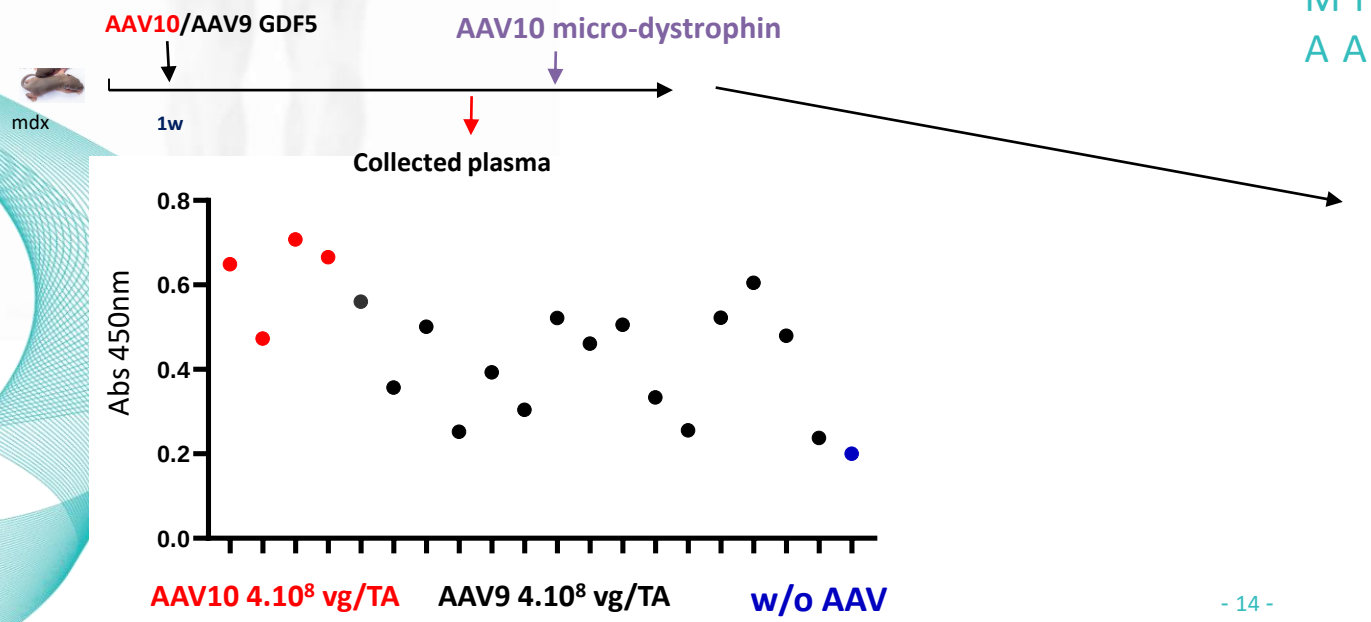
A combined treatment to optimize DMD gene therapy
AAV-GDF5 and AAV-microDystrophin

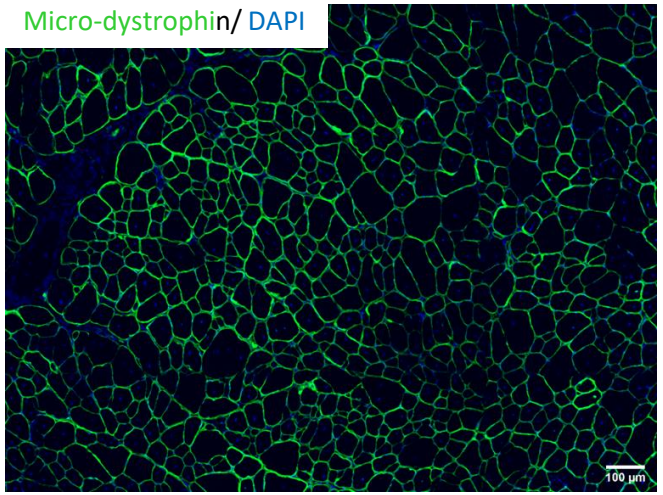
An innovative protocol



AAV10-Ab serology



MicroDystrophin expression
AAV9-GDF5 then AAV10-microDystrophin



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Perspectives

Evaluation of combined treatment benefits on

-> number of microDystrophin positive fibers

-> muscular function parameters

Characterization of muscle precursors responding to GDF5

-> in vitro study of myogenic commitment

-> spatial transcriptomic single cell (Collab L. Giordani)

Combined treatment to optimize DMD gene therapy

AAV-GDF5 and AAV-microDystrophin

-> optimization of the treatment in adult mice

-> in more severe DMD models

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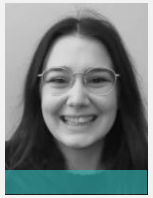
AAV-microDystrophin and AAV-GDF5:
A combined treatment to optimize DMD gene therapy ?

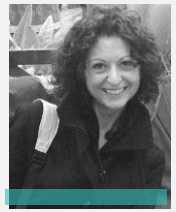
C. Gentil, PhD

L. Saillard



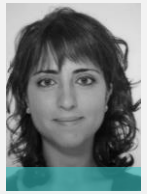
C. Parry

A. Vergnol



S. Falcone, PhD

M. Traoré, PhD



C Noviello, PhD

A. Forand, PhD

A. Bourguiba

Collaborators

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Piera Smeriglio, PhD

Bruno Cadot, PhD

Facilities

Force measurement

Mégane Lemaitre

Sorbonne Université, Inserm, phénotypage du petit animal

Imaging-Morphometric analysis

Zoehir Guesmia

Sorbonne Université, Inserm, Centre de Recherche en Myologie, Institut de Myologie

AAV production

Sofia Benkhelifa Zyyat

Pierre Meunier

Sorbonne Université, Inserm, Centre de Recherche en Myologie, Institut de Myologie

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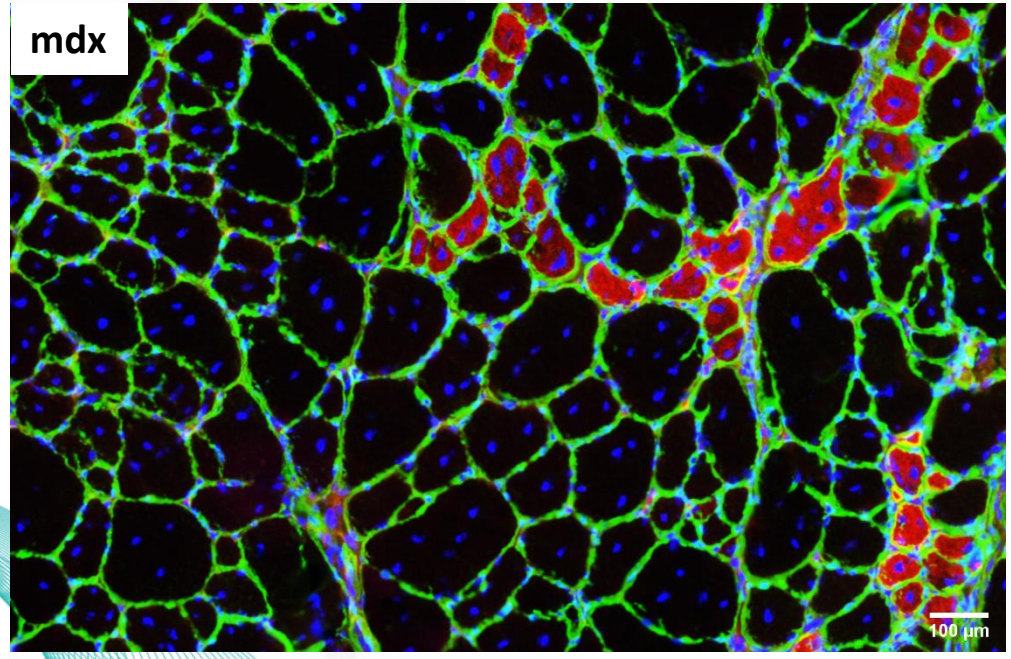
AAV9-GDF5 → **5 weeks** → CTX 6µm → **1 week** → sacrifice

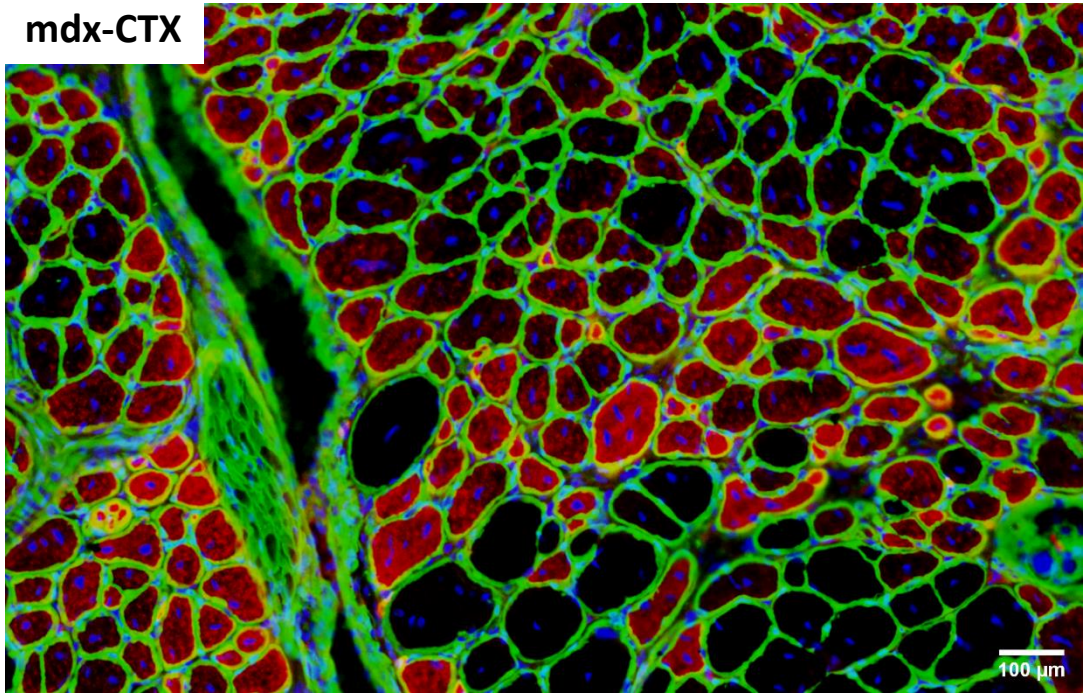
mdx adult
2months

eMHC / Laminin / DAPI

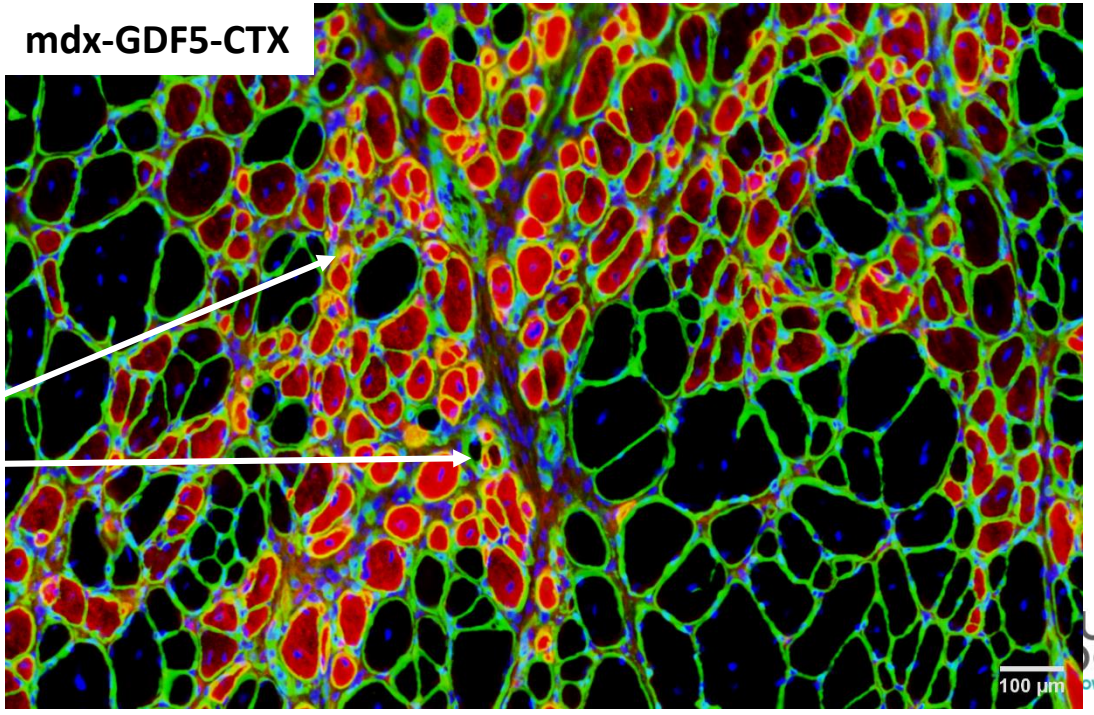
mdx



mdx-CTX



mdx-GDF5-CTX



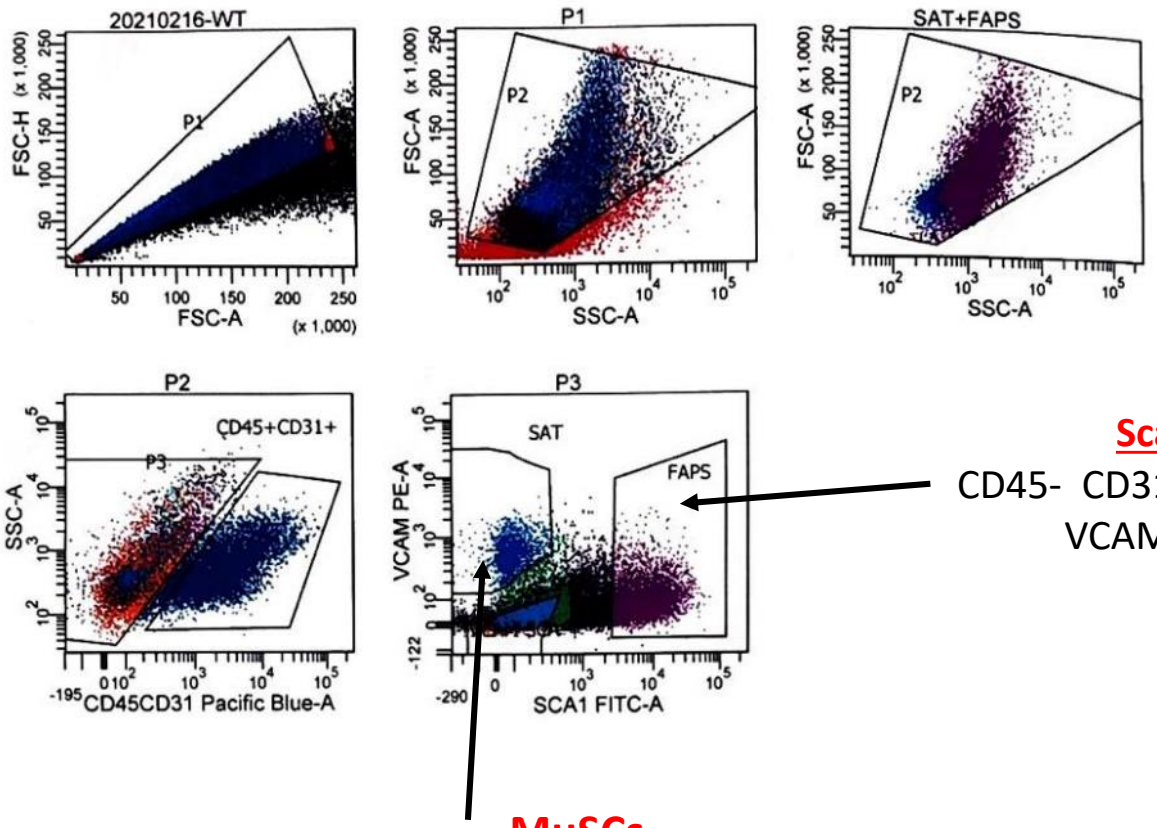
Small new fibers

In vitro experiment

Isolation of sca1+ cells

Blk6 vs mdx

3 week old mice



Sca1+ cells

CD45- CD31- **α7 integrin -/+**
VCAM1- **Sca1+**

MuSCs

CD45- CD31- Sca1- **α7 integrin+ VCAM1+**

Culture
Sca1+ cells

+/- GDF5

3 days of proliferation

3 days of myogenic differentiation

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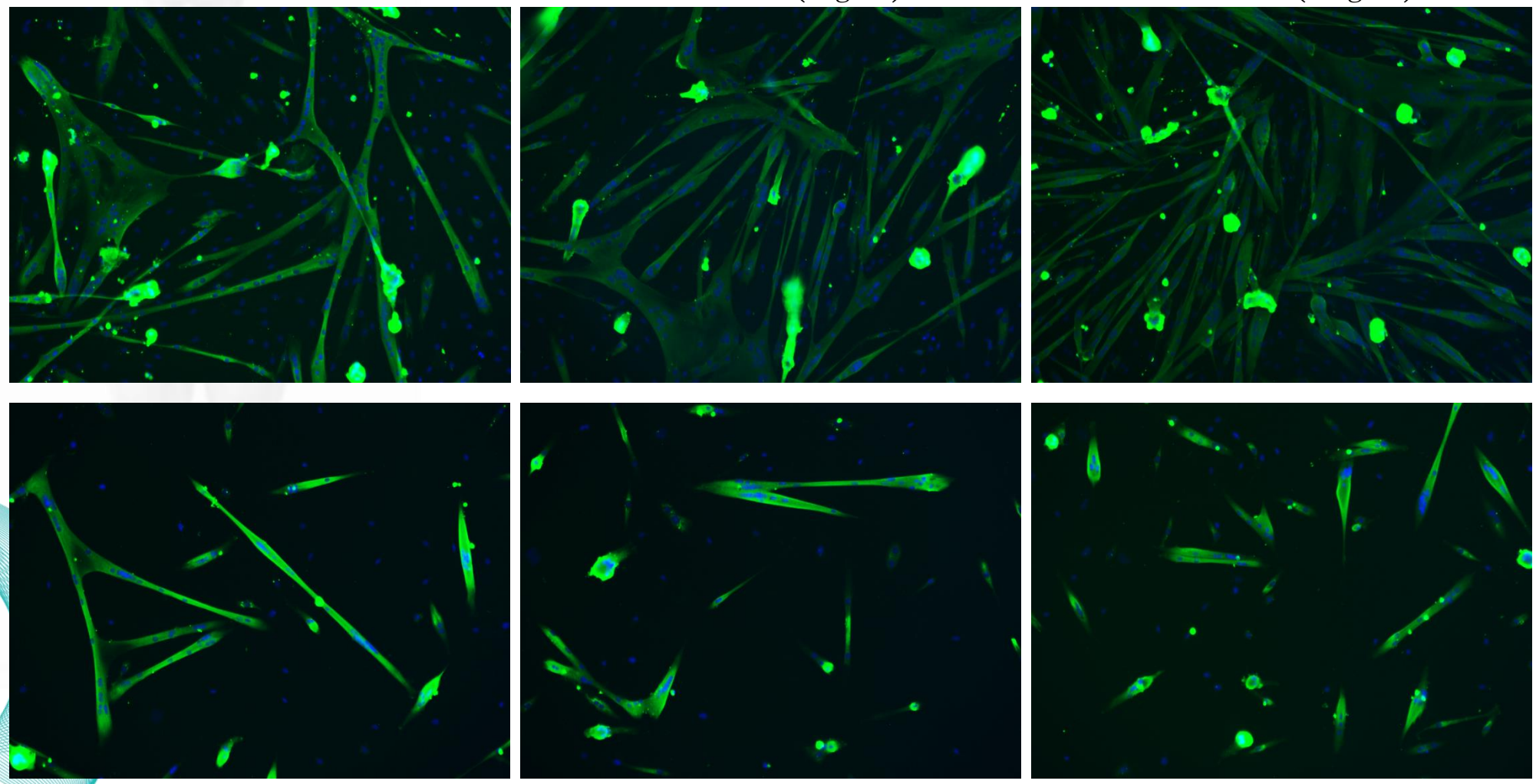
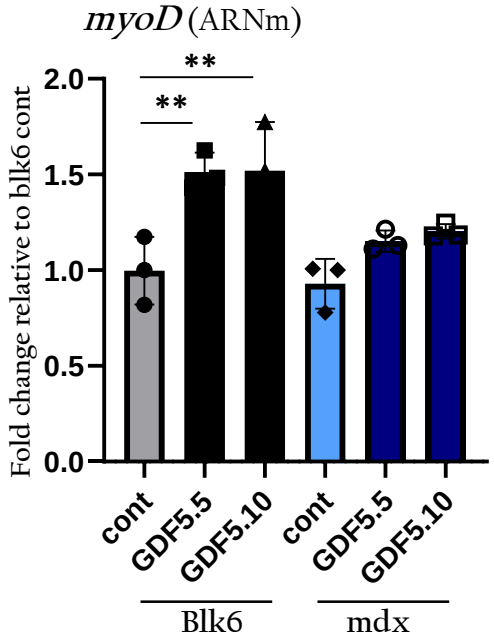
CD31- Sca1- **a7 integrin+ VCAM1+)**

I (MF20)



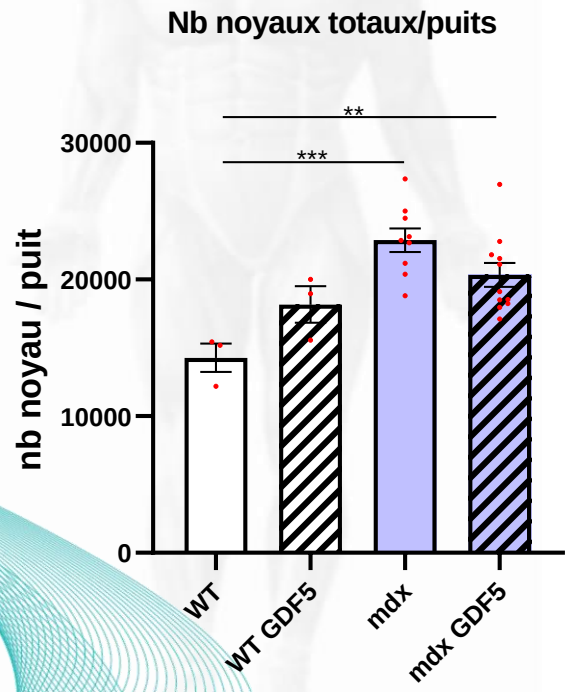


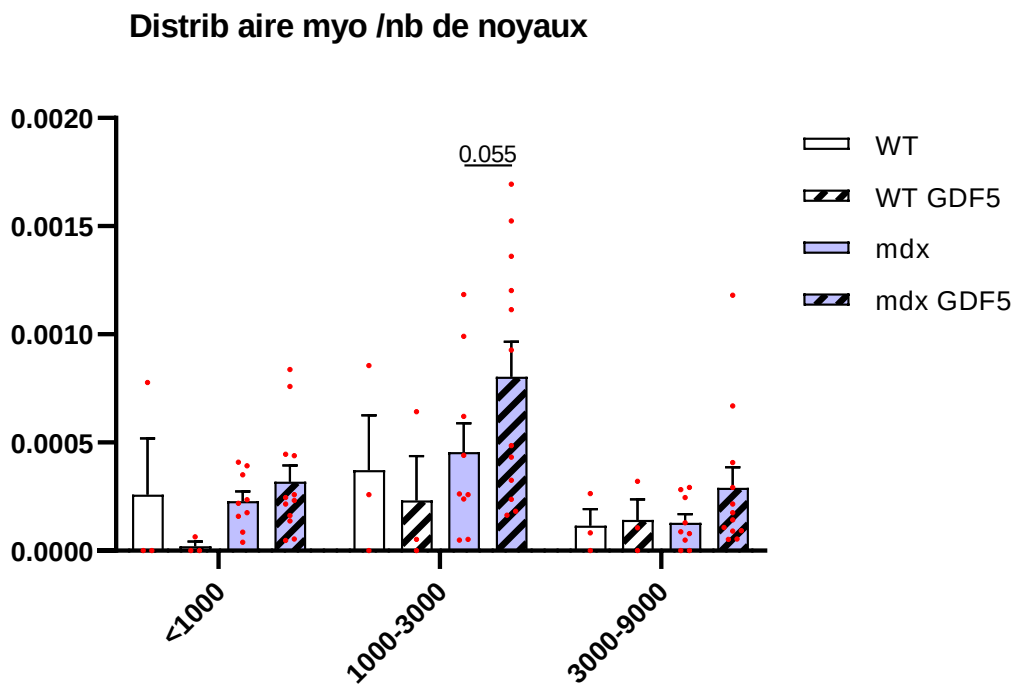
Blk6 : GDF5 promotes MuScs différenciation

Blk6 mdx

In vitro experiment

Isolation of sca1+ cells





More cells in mdx conditions

No difference between mdx +/- GDF5

Higher proportion of large myotubes in mdx GDF5

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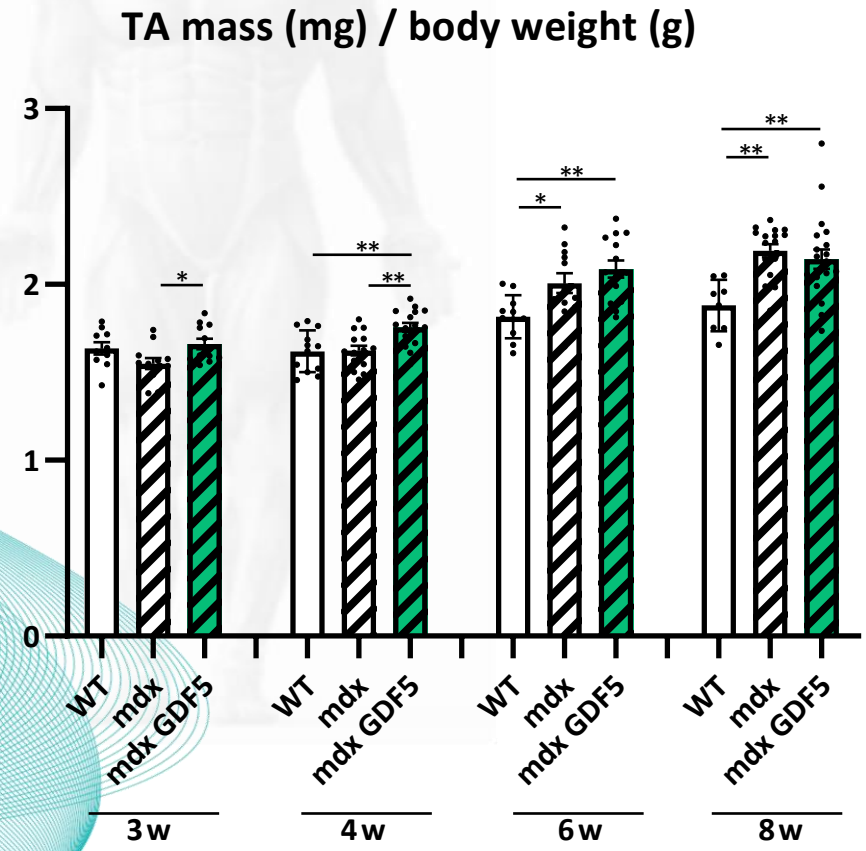
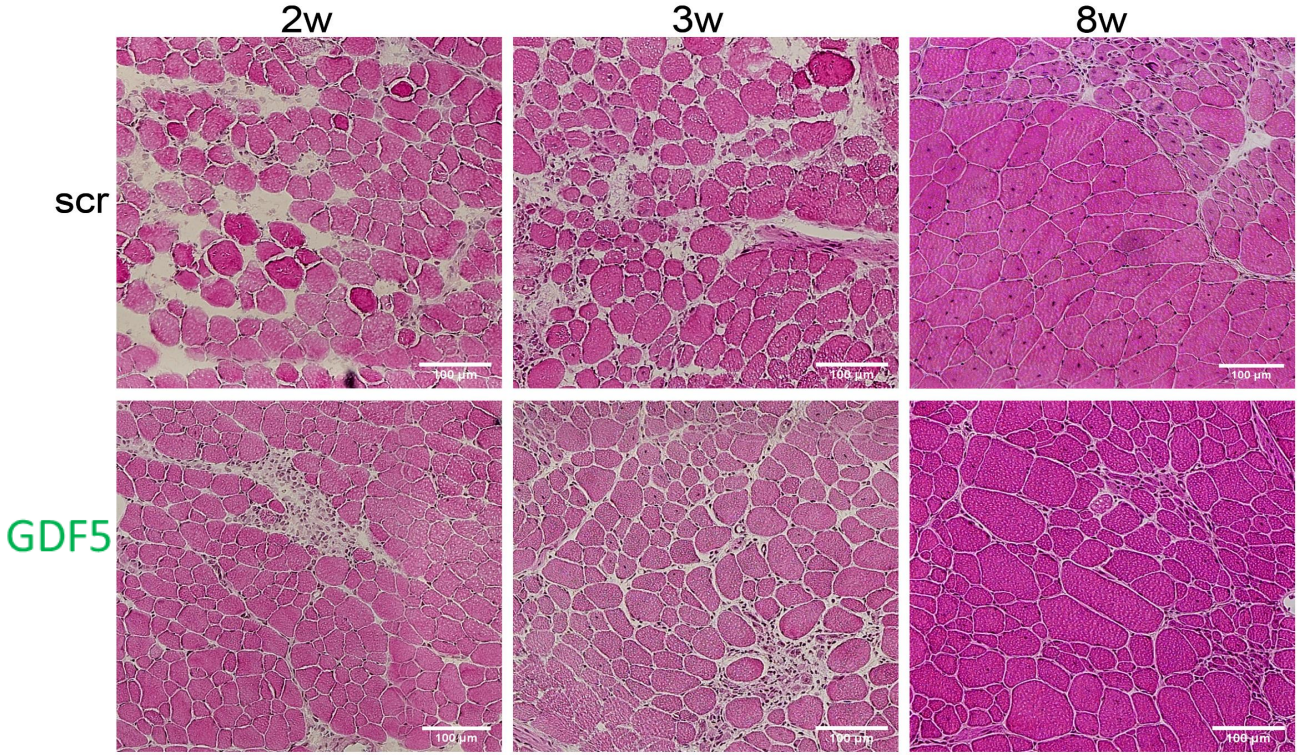
Effects of GDF5 overexpression on DMD muscle pathophysiology



GDF5 OE increases TA mass up to 4 weeks



GDF5 OE improves histological features

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Cardiotoxin

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YYYY/MM/DD

Title Firstname LASTNAME

Function, Entity

Tel/Email

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