Differentiation of pluripotent stem cells to muscle fiber to model Duchenne muscular dystrophy

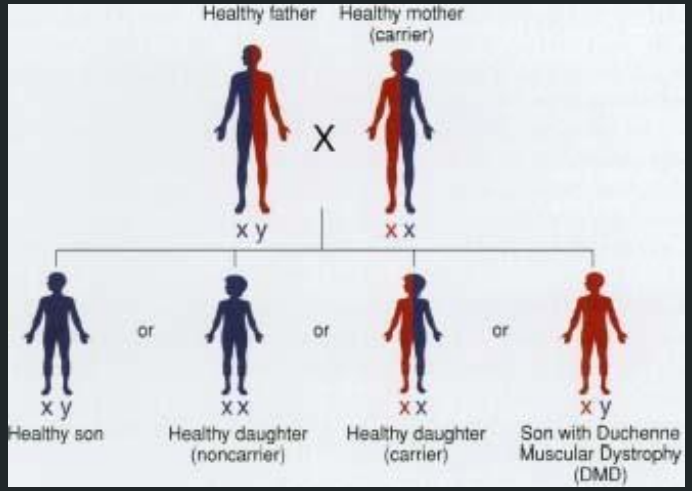
Jérome Chal, Masayuki Oginuma, Ziad Al Tanoury, Bénédicte Gobert, Olga Sumara, Aurore Hick, Fanny Bousson, Yasmine Zidouni, Caroline Mursch, Philippe Moncuquet, Olivier Tassy, Stéphane Vincent, Ayako Miyanari, Agata Bera, Jean-Marie Garnier, Getzabel Guevara, Marie Hestin, Leif Kennedy, Shinichiro Hayashi, Bernadette Drayton, Thomas Cherrier, Barbara Gayraud-Morel, Emanuela Gussoni, Frédéric Relaix, Shahragim Tajbakhsh & Olivier Pourquié

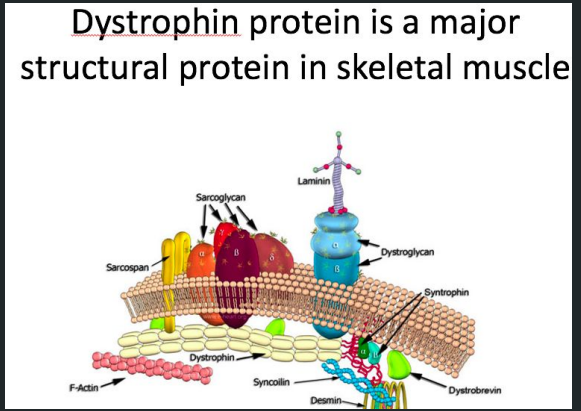
Katherine Duncan & Diana Lin

PHGY 488

Duchenne Muscular Dystrophy (DMD)

- Degenerative disease of the muscle
- X-linked recessive
- Dystrophin-deficient muscle cells
- Dystrophin: major structural protein in skeletal muscle
- Model: mdx mice

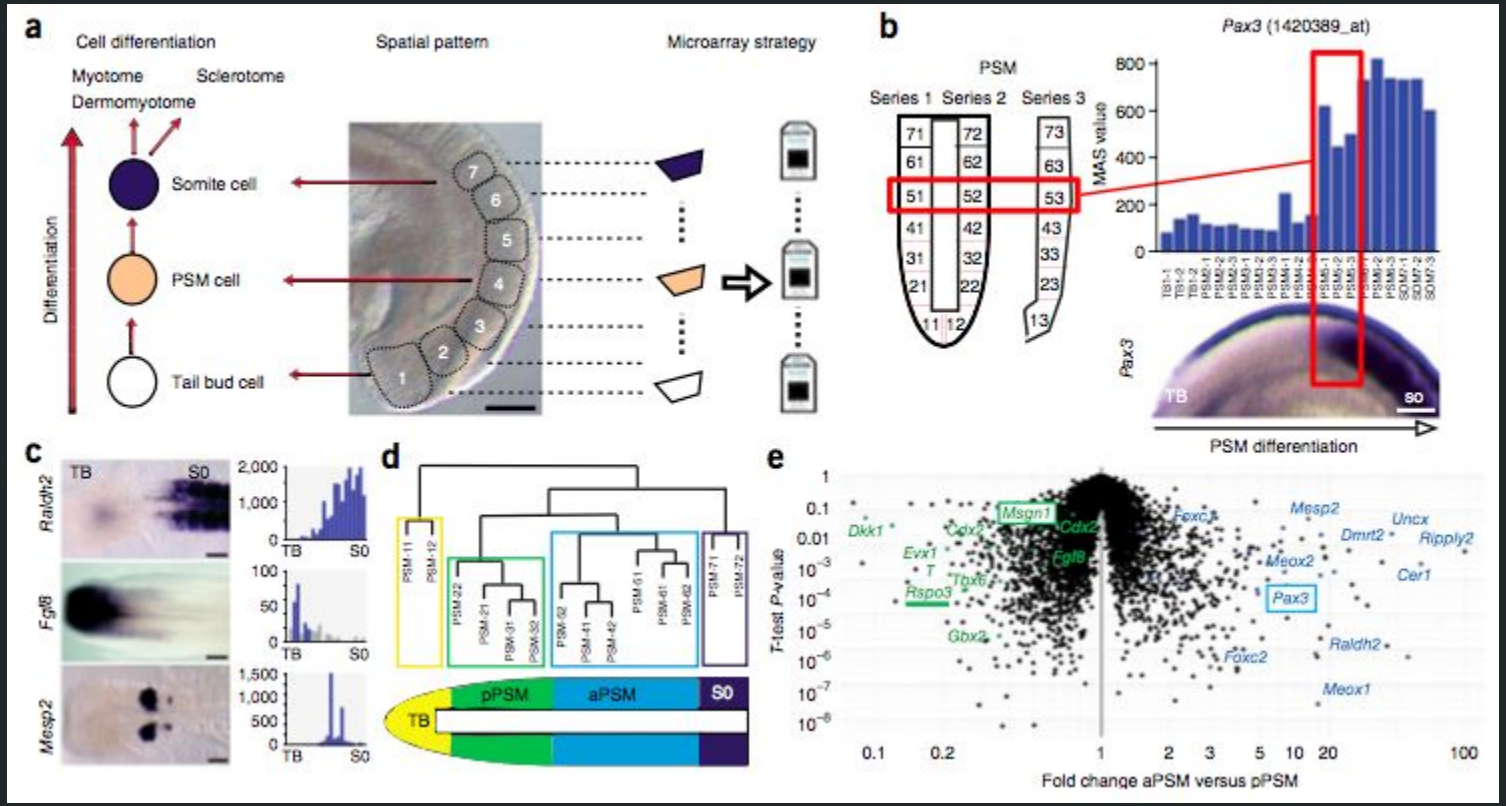


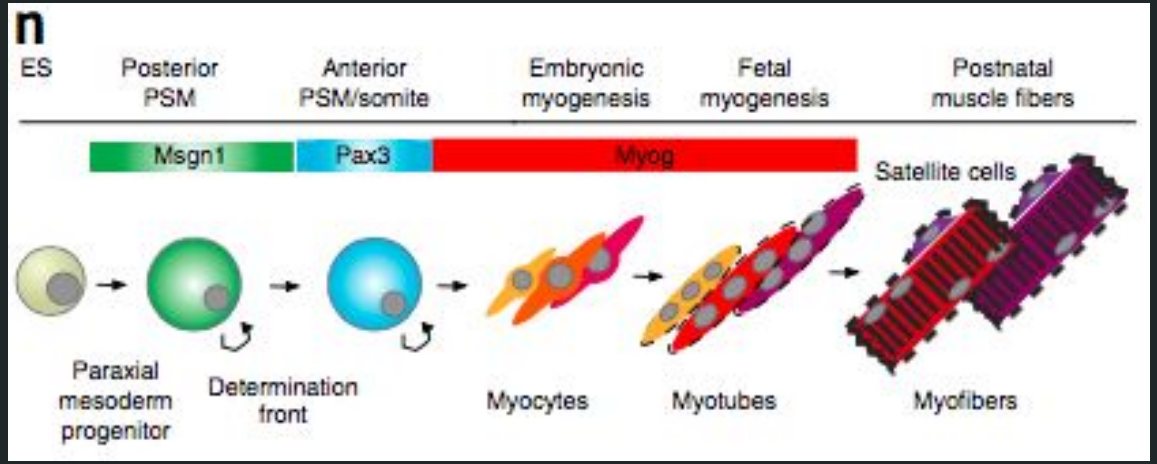


Goal of the study

- Develop a protocol for ES cells to differentiate into Pax7+ cells in order to generate muscle fibers in dystrophin-deficient cells, with the goal of restoring dystrophin in conditions such as Duchenne muscular dystrophy
  ➜ Generation of striated, contractile fibers from mouse and human pluripotent cells

Figure 1: Anterior-posterior maturation gradient




n
ES
Posterior PSM
Anterior PSM/somite
Embryonic myogenesis
Fetal myogenesis
Postnatal muscle fibers
Msgn1
Pax3
Myog
Satellite cells
Paraxial mesoderm progenitor
Determination front
Myocytes
Myotubes
Myofibers

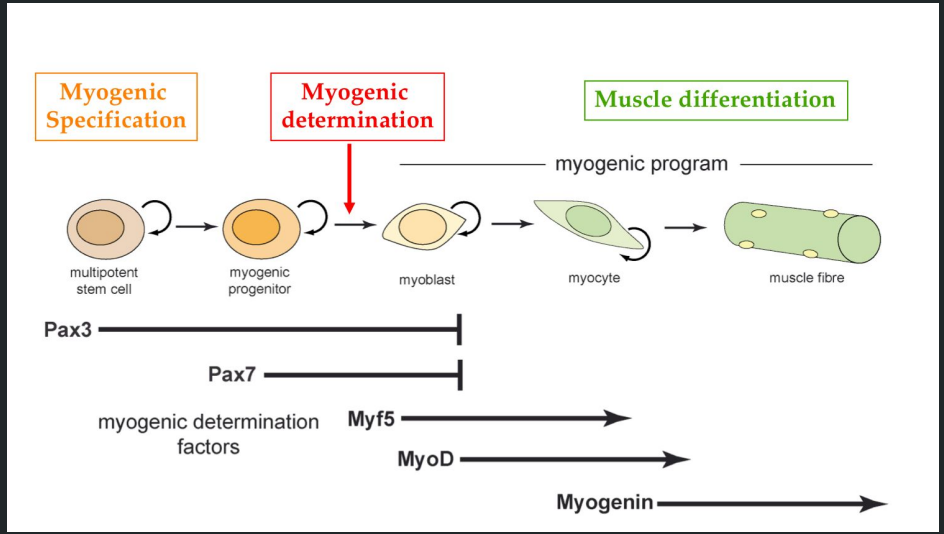
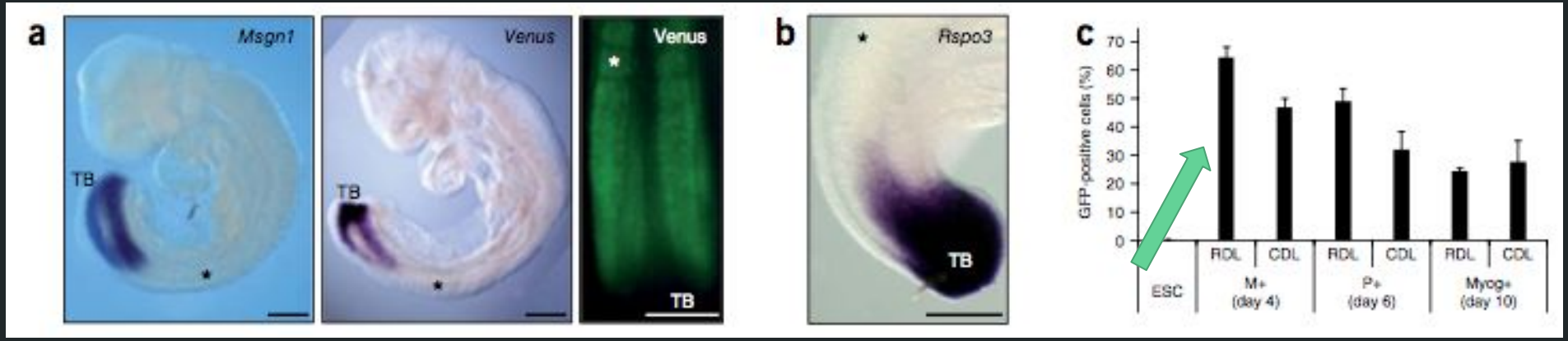

Myogenic Specification
Myogenic determination
Muscle differentiation
myogenic program
multipotent stem cell
myogenic progenitor
myoblast
myocyte
muscle fibre
Pax3
Pax7
myogenic determination factors
Myf5
MyoD
Myogenin

Figure 2: Wnt activation and BMP inhibition lead to a PSM fate



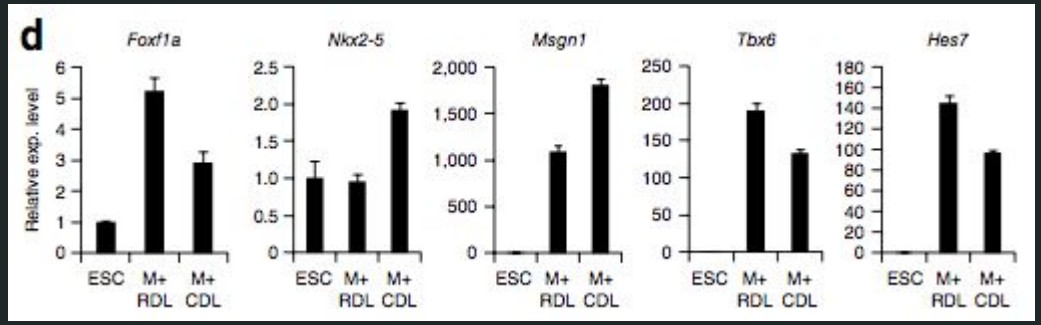
Rspo3: Wnt activator
LDN193189: BMP inhibitor

M+ → Msgn1-repV+ cells
P+ → Pax3-GFP+ cells
Myog-repV+ cells

CDL: contains Chir, control (activates Wnt pathway)
RDL: contains Rspo3

PSM → Presomitic Mesoderm
Pax3 → Early and essential myogenic inducer expressed in the anterior PSM and in myogenic precursors

Figure 2: Wnt activation and BMP inhibition lead to a PSM fate

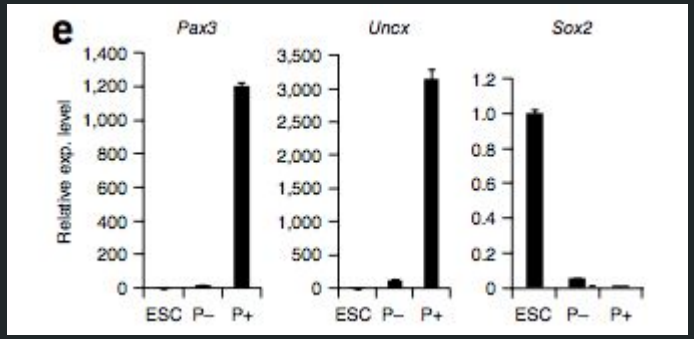


Lateral plate marker

Cardiac marker

Paraxial mesoderm markers

Pax3-GFP ES reporter line



Paraxial mesoderm markers

Neural marker

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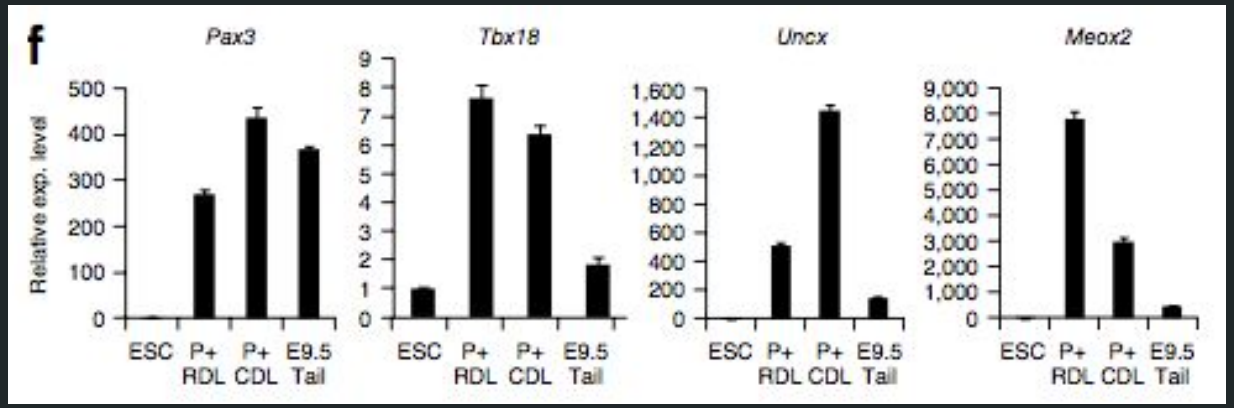
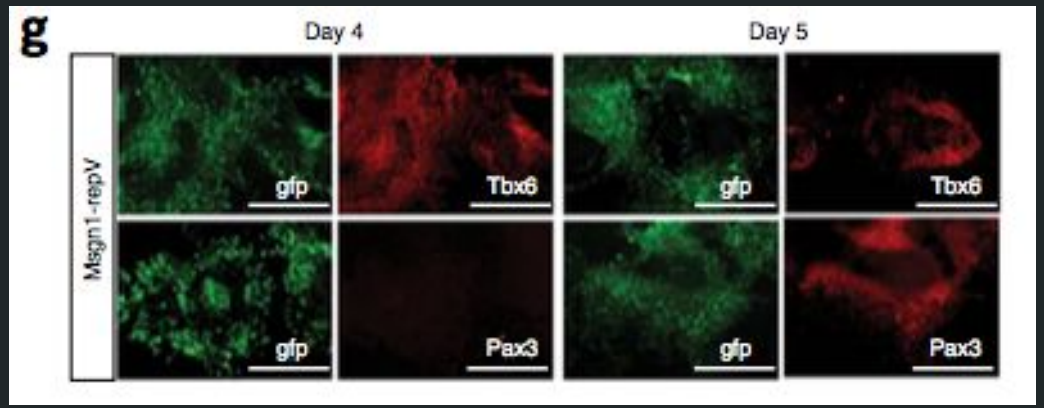


P+ cells positive for anterior PSM/somitic markers Pax3, Tbx18, Uncx, and Meox2



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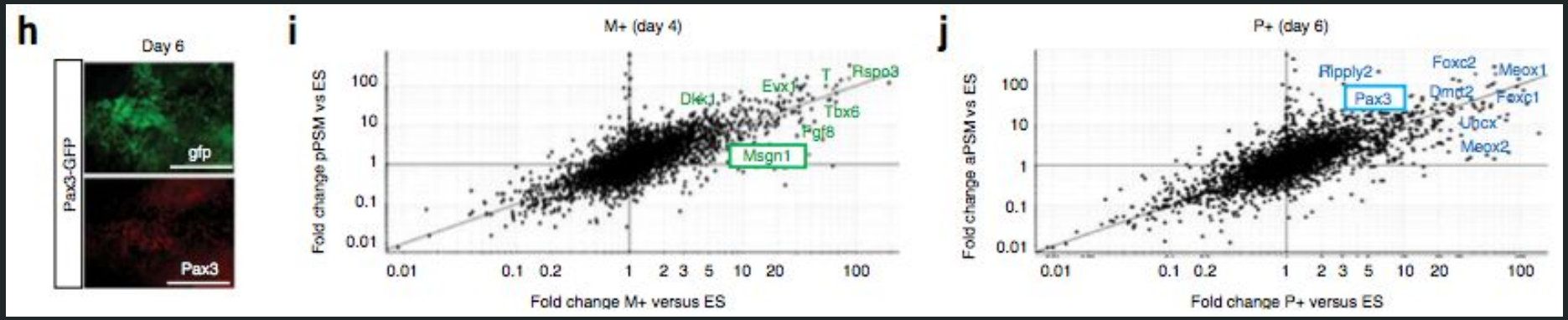
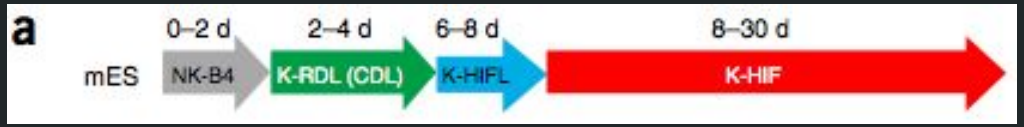


Green: genes specific for pPSM

Blue: genes specific for aPSM

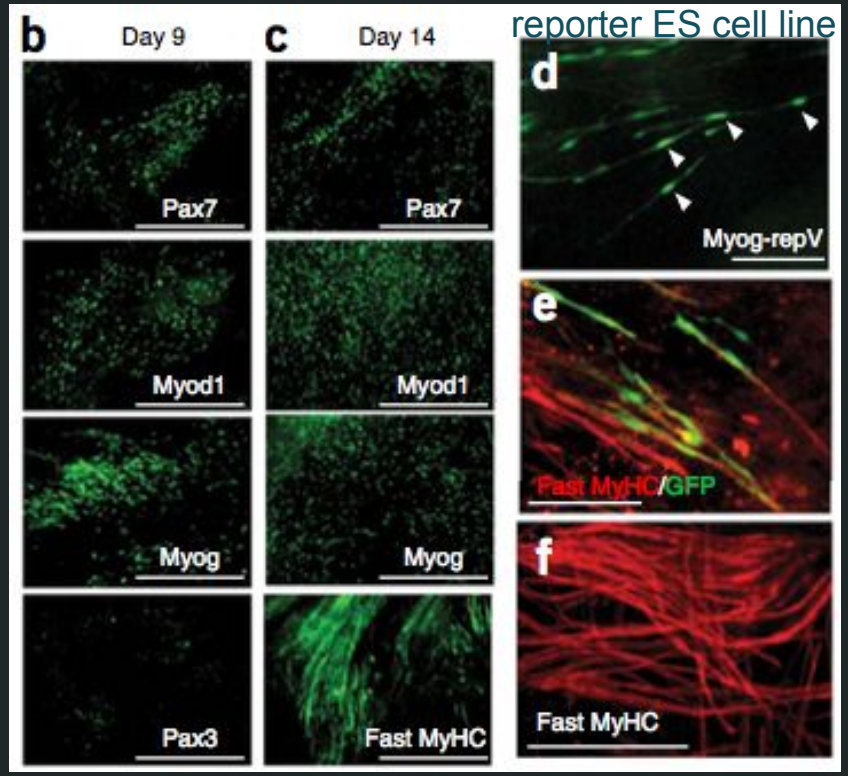
Figure 3: Differentiation of multinucleated striated muscle fibers and associated Pax7+ cells from mouse ES cells *in vitro*



Myod: myoblasts

Myog: myocytes

(Primary Myogenesis)
Muscle fibers expressing slow myosin heavy chain (MyHC) form Pax3+ precursors
(Secondary/Fetal Myogenesis)
Pax7+ myogenic precursors derived from Pax3+ cells form larger multinucleated fibers with mature (fast) MyHC



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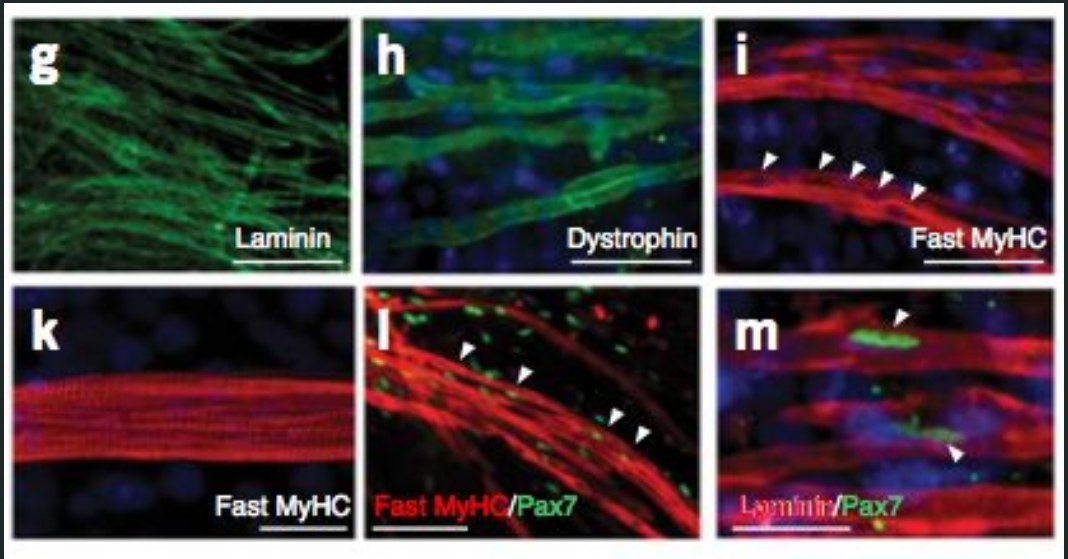
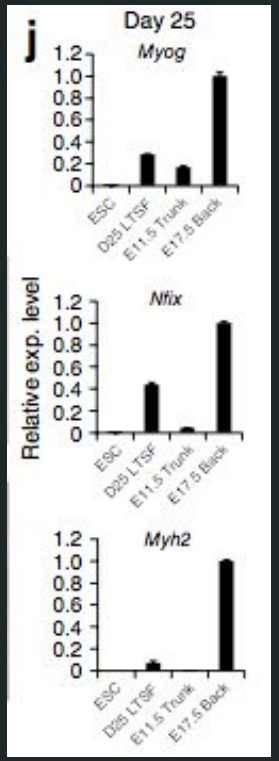


k: highly organized striation

l, m: Pax7 cells show satellite-like activity

Differentiated fibers exhibited spontaneous in vitro contractions, indicating that the sarcomeric organization of the fibers was functional



Fetal muscle fiber marker

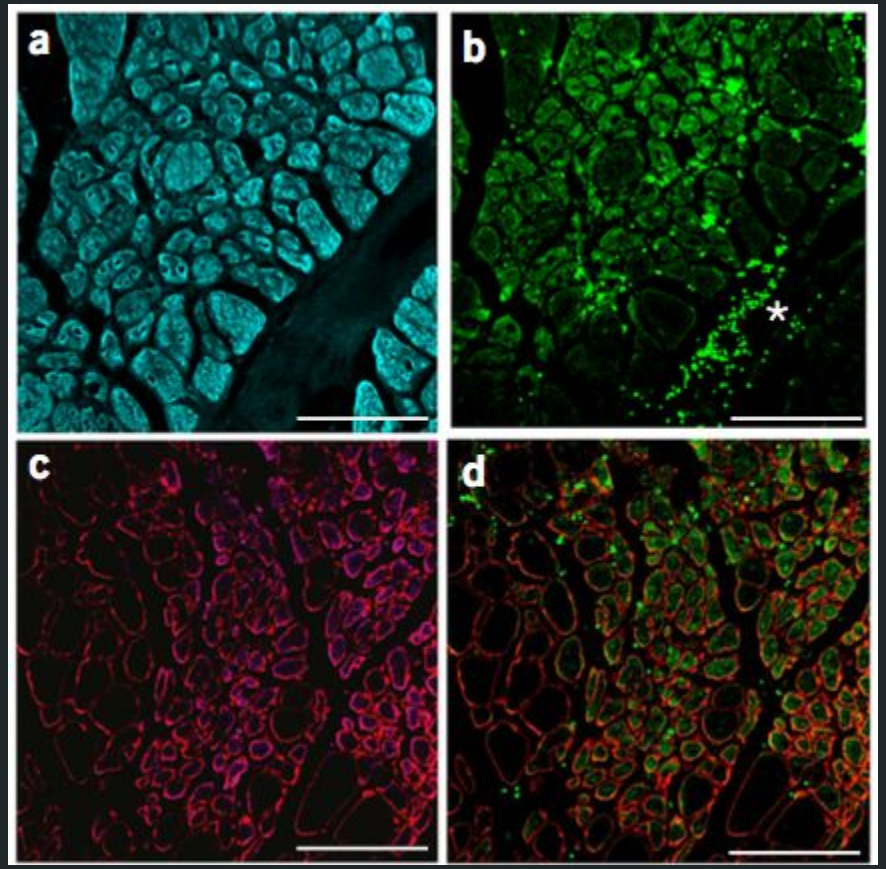
Myosin fast heavy chain

E11.5 trunk muscles: contain primary myofibers

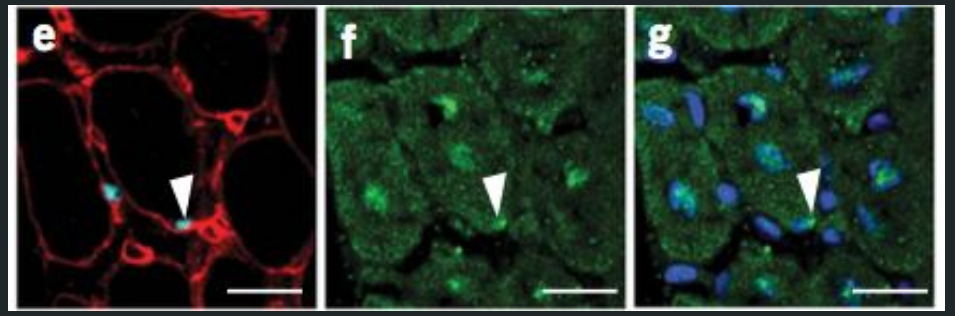
E17.5 back muscles: ongoing secondary myogenesis

Figure 4: Pax7+ cells grafted in dystrophin-deficient environment

Adult mdx muscle 1 month post-engraftment



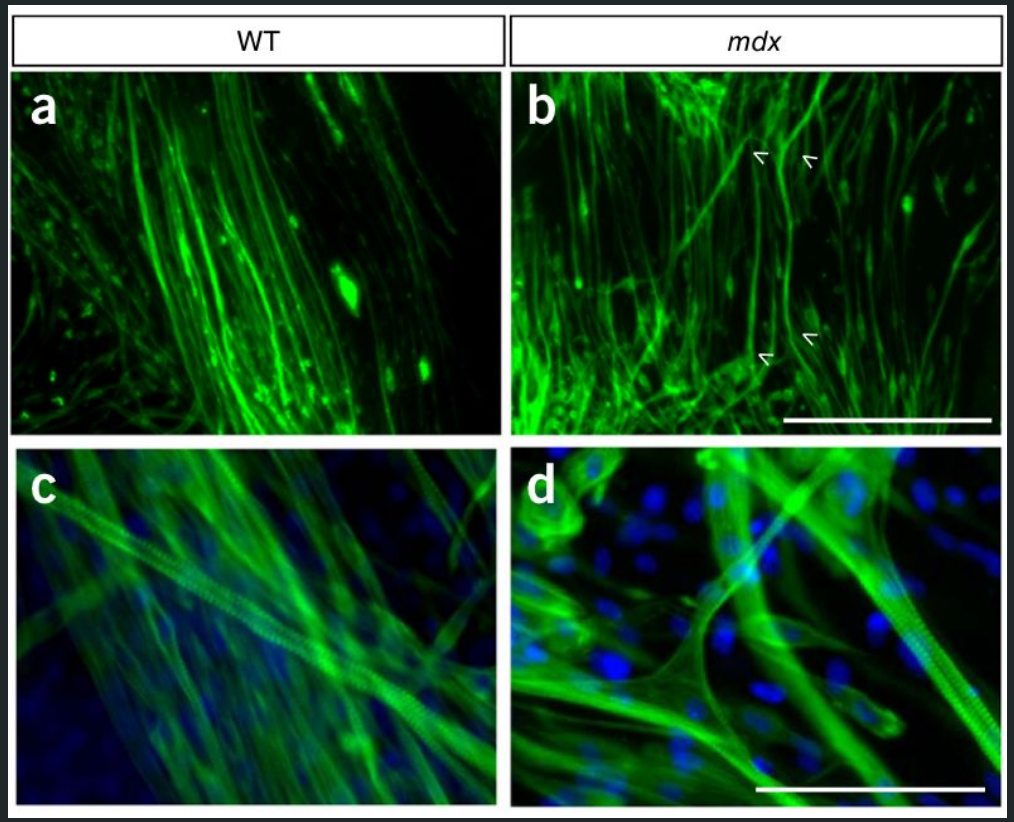
Rag1−/− Dmd$^{mdx\text{-}5Cv}$ (mdx) mice → immunodeficient mice lacking dystrophin

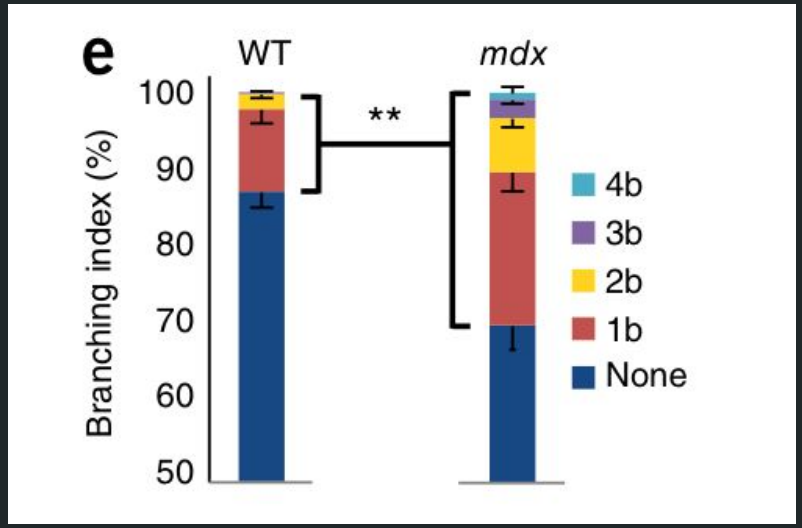


Pax7+ satellite cell–like cells located under the basal lamina of GFP+ fibers

Pax7-GFP construct shown in green

Figure 5: mdx ES cells show an abnormal branching phenotype

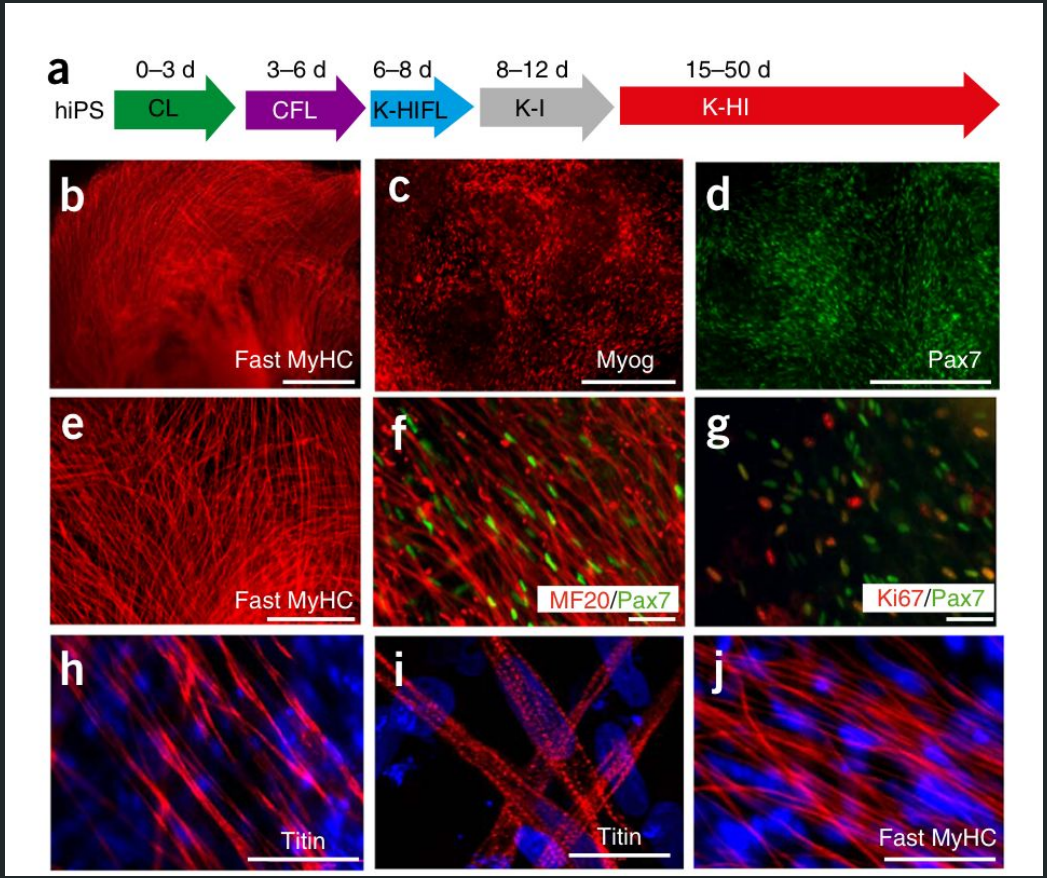




More branching in mdx fibers in vitro vs. WT

Figure 6: Myogenic differentiation of human iPS cells

MF20: antibody for myosin heavy chain

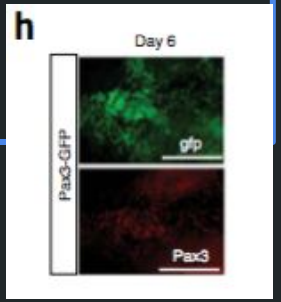


Discussion

- *In vitro* differentiation of mouse and human pluripotent cells into striated, linear, millimeter-long muscle fibers
- Strong BMP inhibition critical to prevent BMP4 expression → lateral plate formation
- Provides a tractable *in vitro* model to study the cell biology of DMD

Caveats - study

- *In vitro* cultures were only <u>similar</u> to *in vivo*: PSM-like, satellite cell-like
  - Cultures could behave differently in *in vivo* environment
- Does not address the issue of engraftment failure in human trials
- A lot of unnecessary data to get a simple point across
- When making comparisons, often data not shown

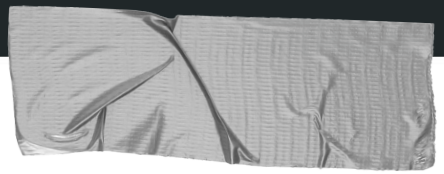


Caveats - paper

- Did not go into enough detail about related studies (more context needed)
- Difficult to understand figures
- Mechanisms were not thoroughly explained (required further research to understand)
- Authors assumed we had more background knowledge than we actually had

Future endeavours

- Figure out how to successfully engraft cells into humans
- Clinical trials: attempt to rescue DMD in humans by injecting human iPSC derived Pax7+ cells into human patients with DMD



Questions?