European Reference Network
for rare or low prevalence complex diseases

Network Neuromuscular Diseases (ERN EURO-NMD)

Present and future of gene therapy in Neuromuscular Diseases

Satellite Scientific Symposium endorsed by ERN EURO-NMD

February, 22nd 2024

Gene therapy and new avenues - Safety, limitations and new techniques

Engineering human skeletal muscle for advanced modelling of neuromuscular diseases and therapeutics

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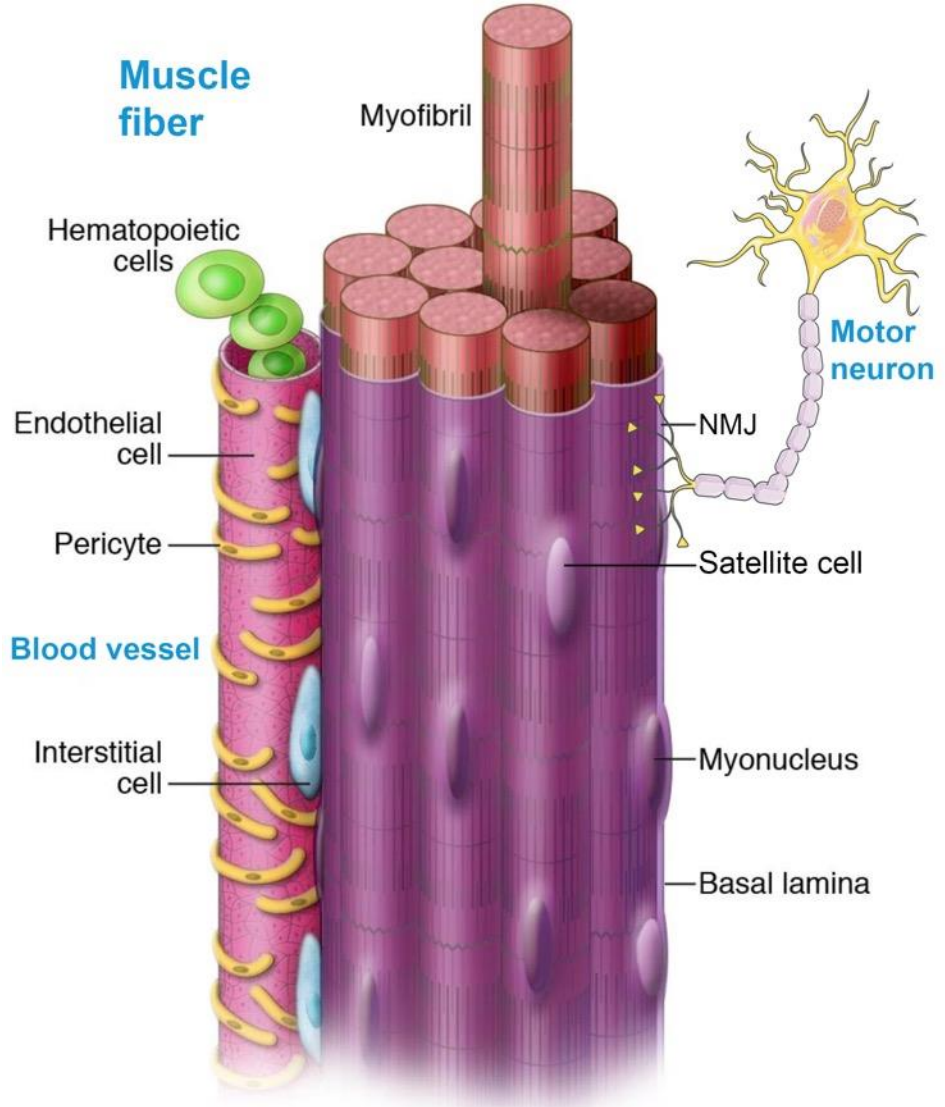
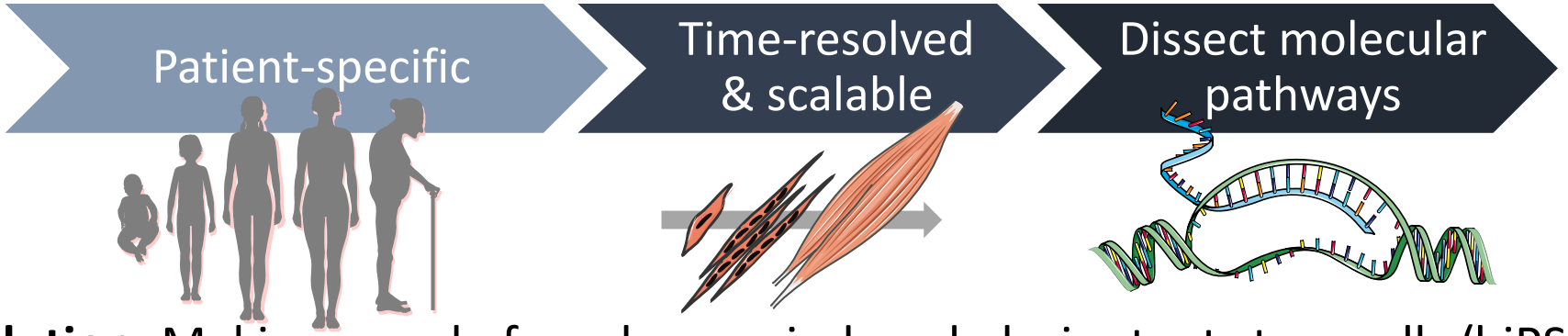
Skeletal Muscle

- Most abundant human tissue and main CNS output
- Complex structure and functions
- Myofibres: multinucleated syncytia
- Severe and incurable diseases, mutations impacting on different cellular/tissue compartments (e.g., nuclear envelope, sarcomeres, sarcolemma, ECM)
- ***Desperate need to understand disease mechanisms and develop therapies***

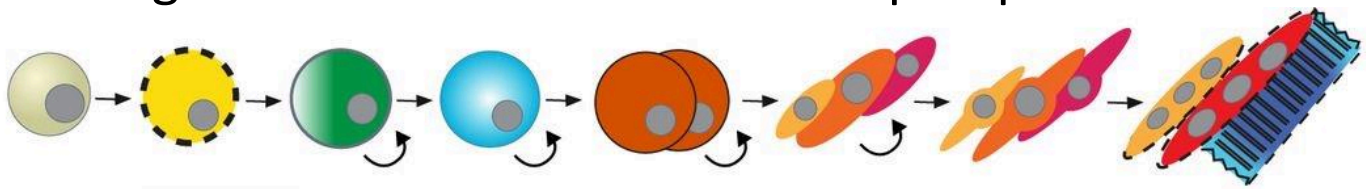


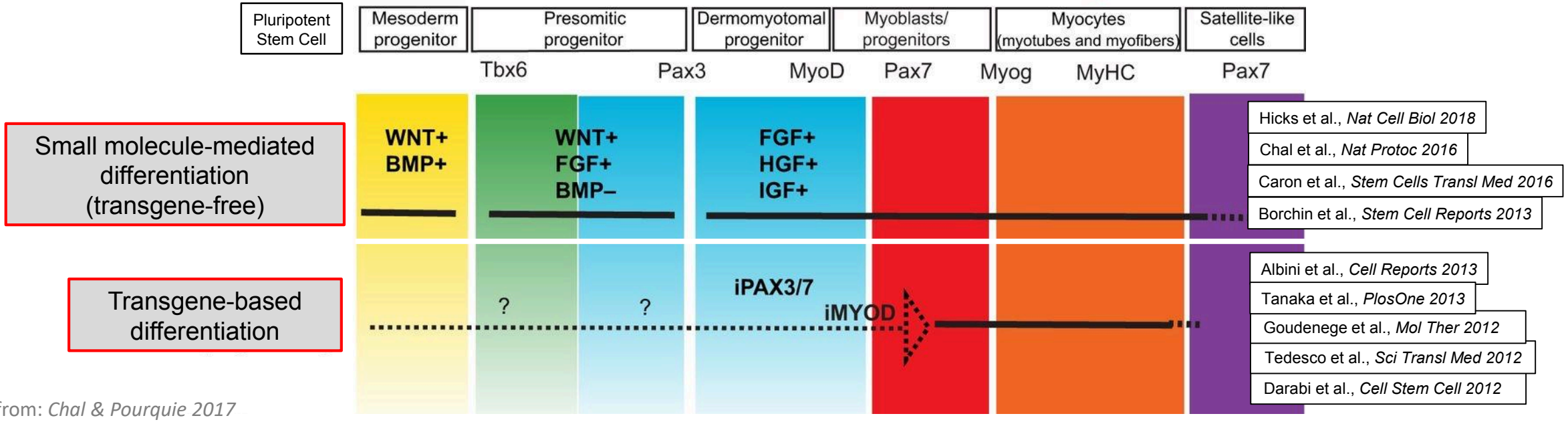
Adapted from *Tedesco et al., 2010*

Modelling human muscle diseases (in a dish)



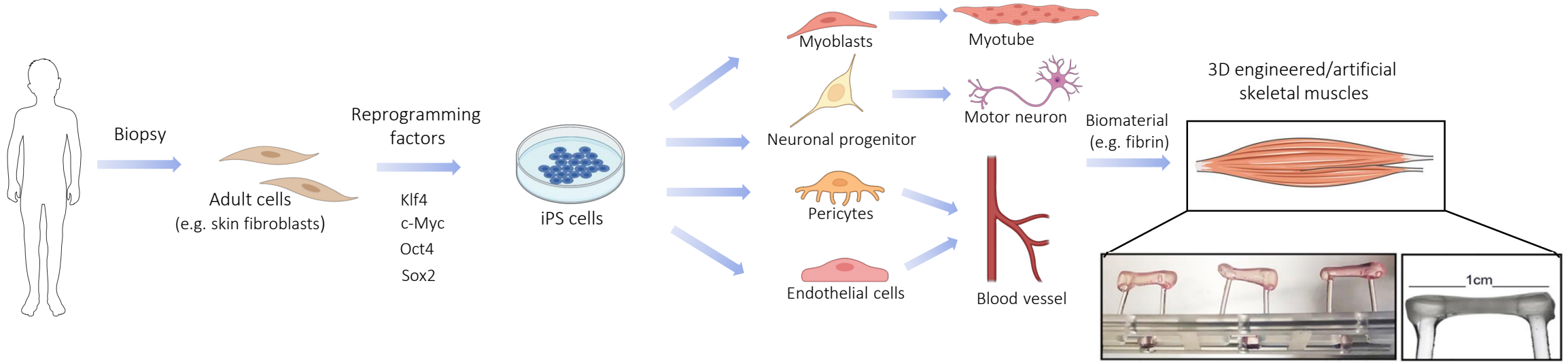
Solution: Making muscle from human induced pluripotent stem cells (hiPSCs)





Adapted from: *Chal & Pourquie 2017*

From 2D to 3D: hiPSC-derived Engineered Skeletal Muscles



Cell Reports *23*, 899–908, April 17, 2018

Cell Reports

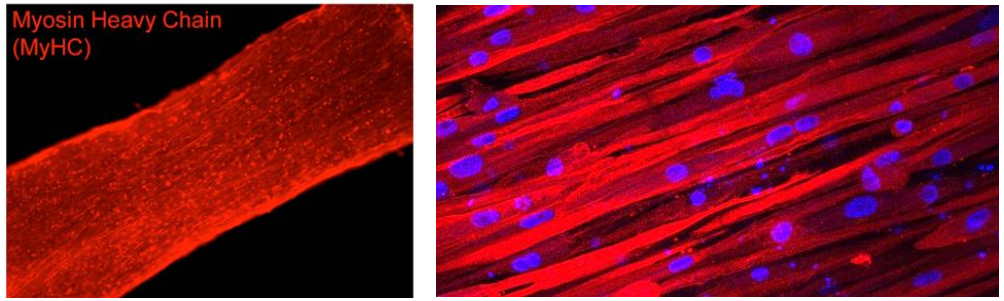
Resource

OPEN ACCESS

CellPress

Three-Dimensional Human iPSC-Derived Artificial Skeletal Muscles Model Muscular Dystrophies and Enable Multilineage Tissue Engineering

Sara Martina Maffioletti,[1,8,9] Shilpita Sarcar,[1,8] Alexander B.H. Henderson,[1] Ingra Mannhardt,[2,3] Luca Pinton,[1,4] Louise Anne Moyle,[1] Heather Steele-Stallard,[1,4] Ornella Cappellari,[5] Kim E. Wells,[5] Giulia Ferrari,[1] Jamie S. Mitchell,[6,7] Giulia E. Tyzack,[6,7] Vassilios N. Kotiadis,[1] Moustafa Khedr,[1] Martina Ragazzi,[1,10] Weixin Wang,[1,11] Michael R. Duchen,[1] Rickie Patani,[6,7] Peter S. Zammit,[4] Dominic J. Wells,[5] Thomas Eschenhagen,[2,3] and Francesco Saverio Tedesco[1,12,*]



nature protocols

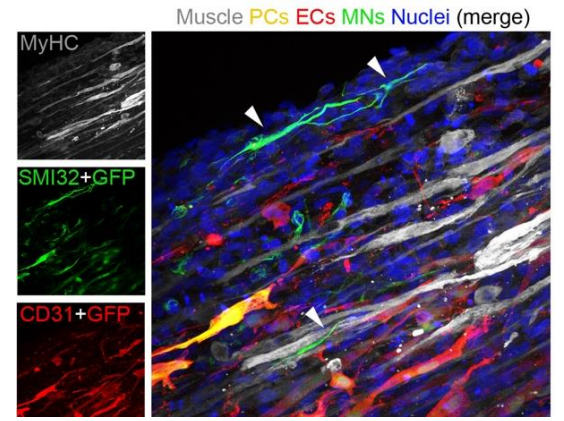
PROTOCOL

https://doi.org/10.1038/s41596-022-00790-8

Check for updates

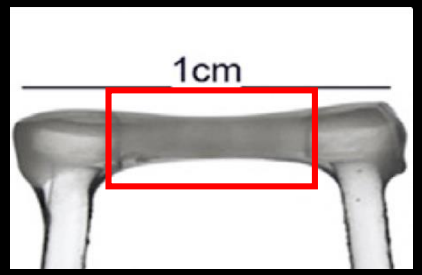
3D human induced pluripotent stem cell–derived bioengineered skeletal muscles for tissue, disease and therapy modeling

Luca Pinton[1,2,3,9], Moustafa Khedr[1,2,9], Valentina M. Lionello[1,2], Shilpita Sarcar[1], Sara M. Maffioletti[1,8], Sumitava Dastidar[1,2], Elisa Negroni[1,4], SungWoo Choi[1,2], Noreen Khokhar[1,2,3], Anne Bigot[4], John R. Counsell[5,6], Andreia Sofia Bernardo[2,7], Peter S. Zammit[3] and Francesco Saverio Tedesco[1,2,6]✉

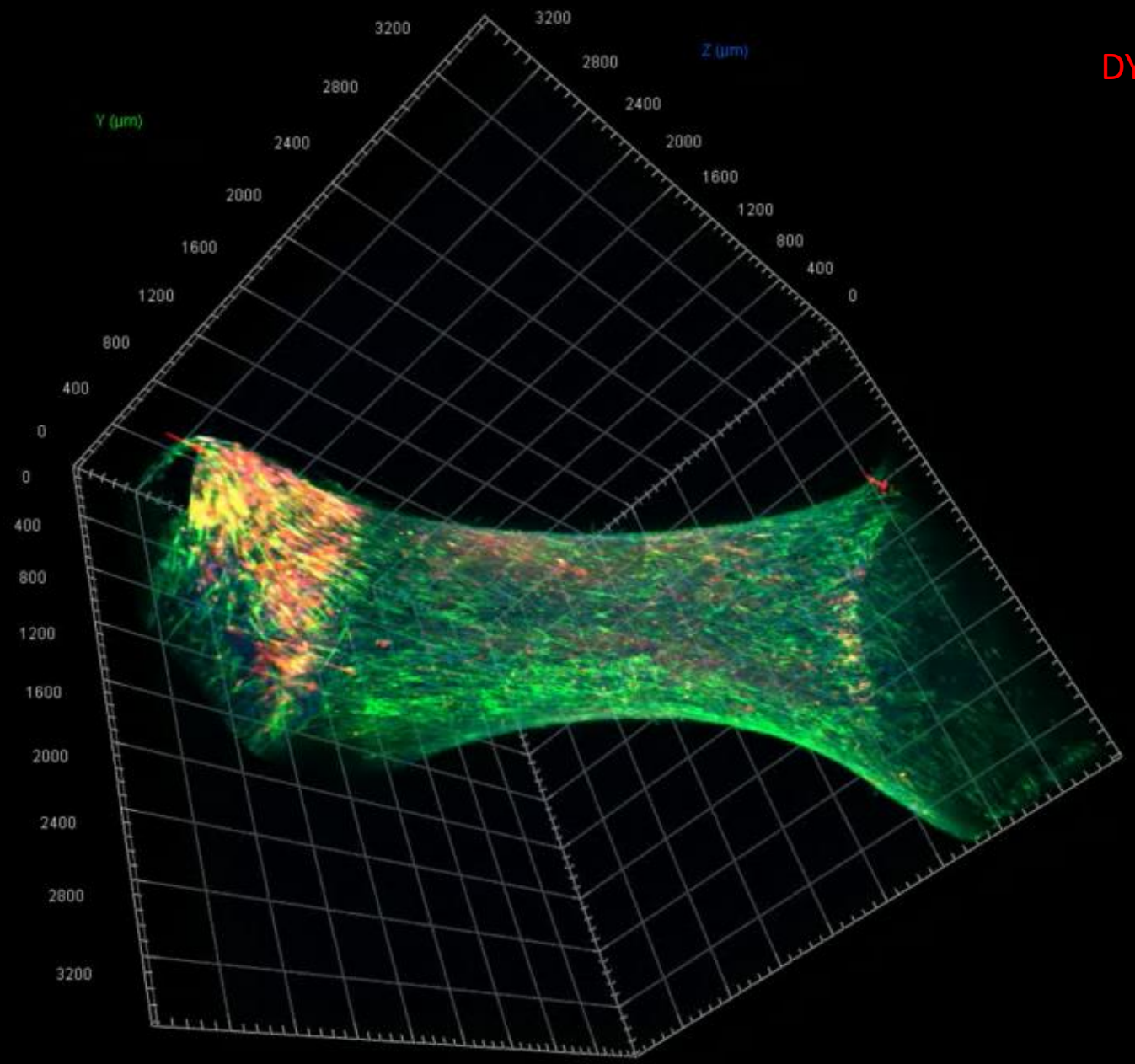


- 3D muscles, EMTs, myobundles, artificial muscles, muscle organoids…
- Academia: Gilbert, Bursac, Zimmerman, Mack, Eschenhagen, Pijnappel…
- Industry: CuriBio, Optics11, Dinabios, Myriamed, Bi/ond…

Light sheet microscopy
7 days differentiation

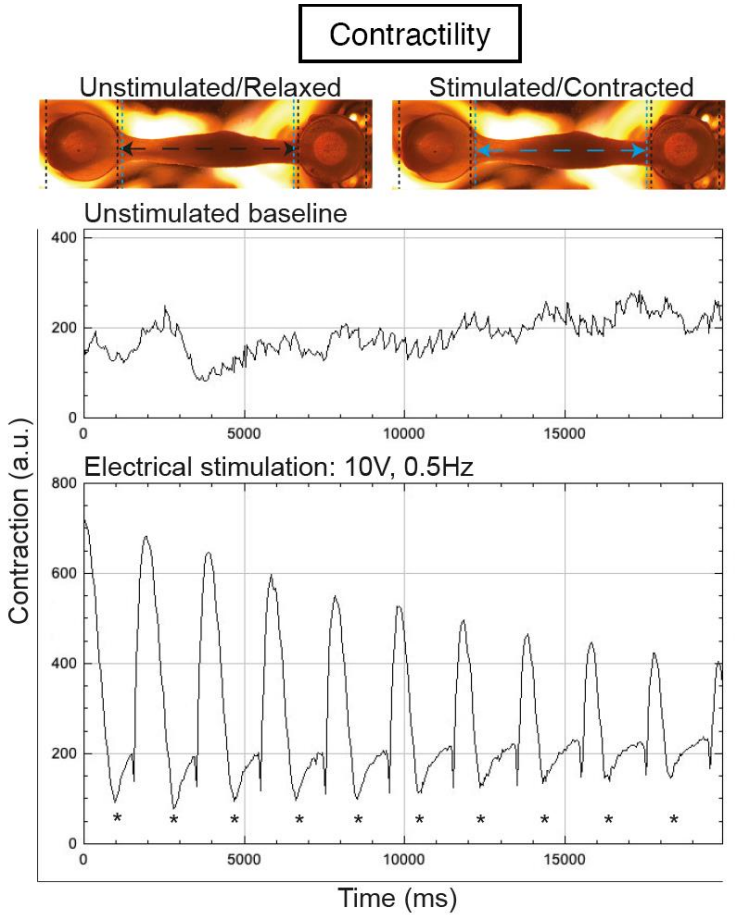


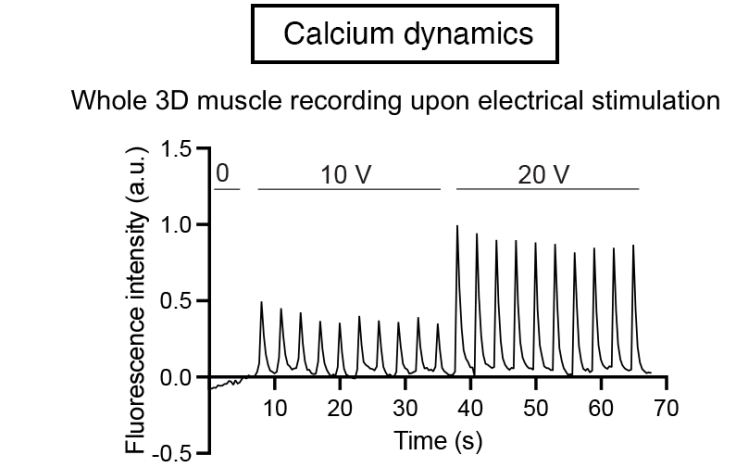
TITIN (sarcomeres)
DYSTROPHIN (sarcolemma)
Hoechst (nuclei)



Pinton, Khedr et al., 2023

Excitation-contraction coupling





V. Lionello;
Pinton, Khedr et al., 2023

Modelling muscle disorders using iPSC-derived myogenic cells

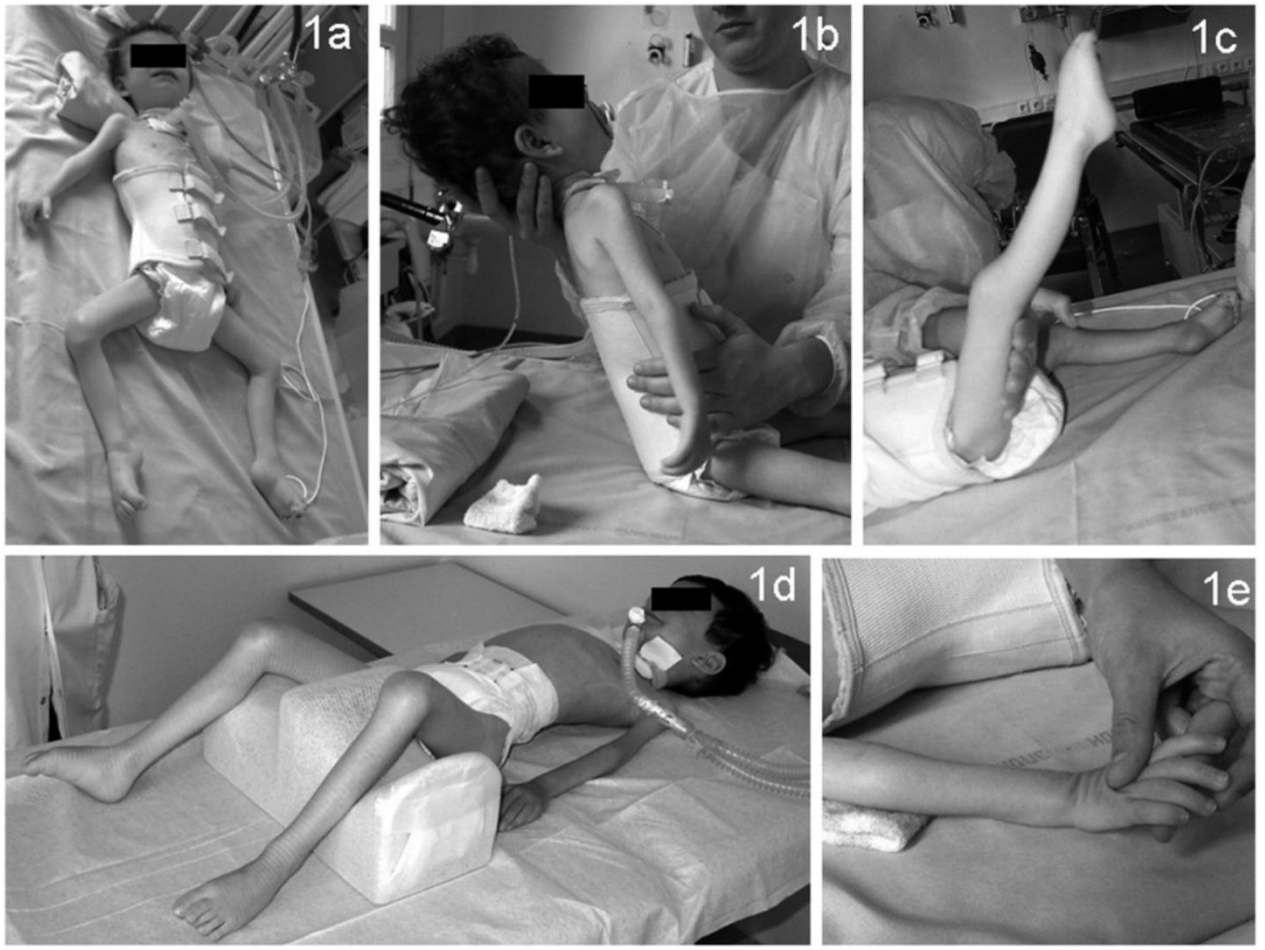
Striated muscle laminopathies

- Subgroup of laminopathies (AD)
- Dysfunctional nuclear envelope:
  - Mechanical / Signalling / Epigenetic

- *LMNA*-related muscular dystrophies:
  - Emery-Dreifuss muscular dystrophy
  - LGMD1B
  - Congenital muscular dystrophy (L-CMD)

Why?

- Study pathophysiology (genotype-phenotype)
- Insights on myonuclear dynamics
- Developing therapies



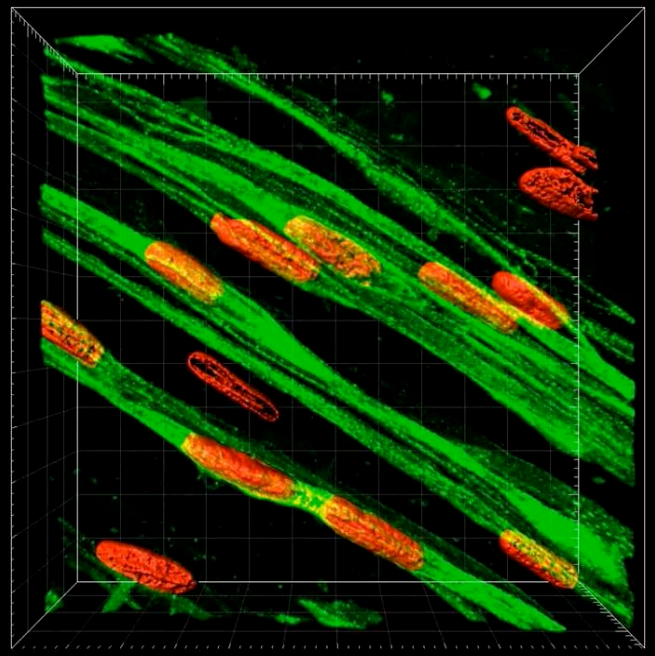
Worman 2012
Quijano-Roy et al., 2008

With P. Zammit, King's College London (UK)
Cells: CureCMD & Cellular Dynamics (USA)

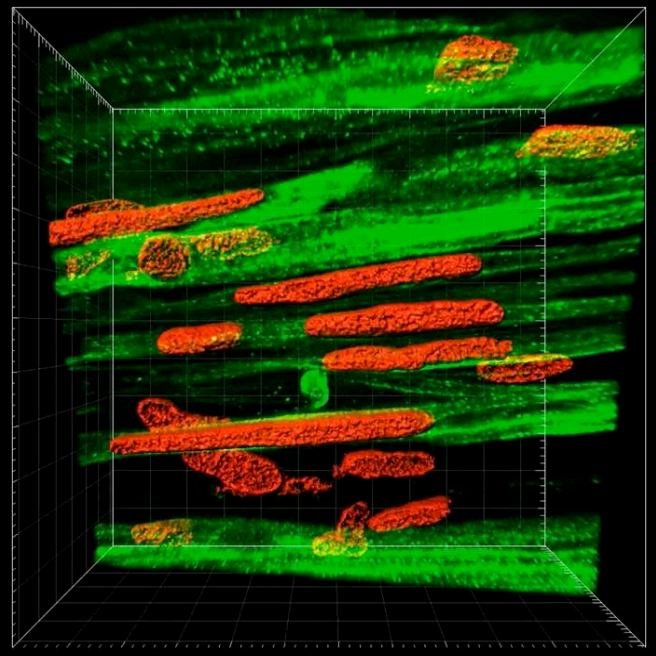
Modelling nuclear abnormalities of *LMNA* (LAMIN A/C)-related congenital muscular dystrophy (L-CMD) in human engineered muscles

LAMIN A/C
TITIN

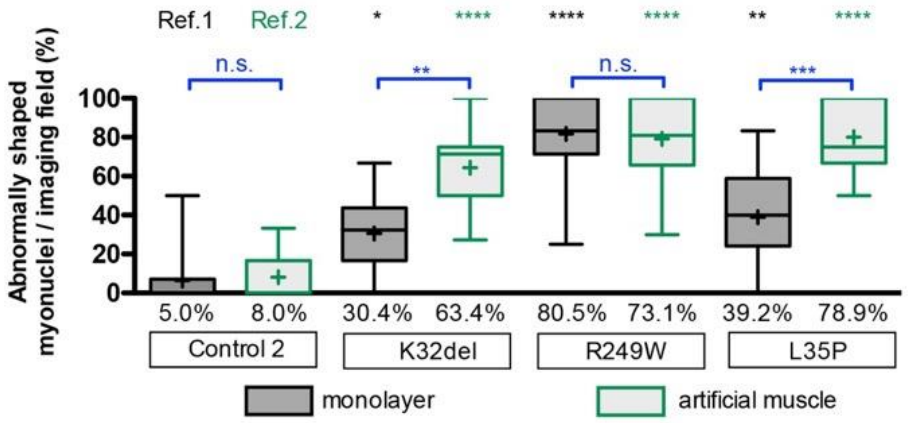
Healthy donor

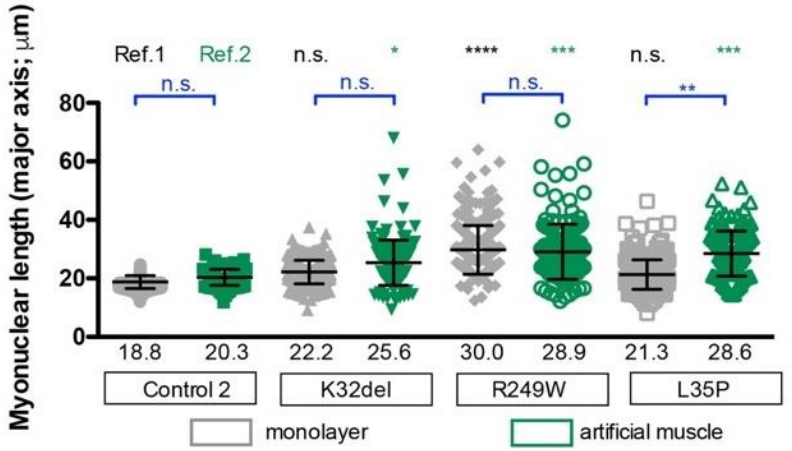


***LMNA*-mutant**



Maffioletti et al., 2018
Steele-Stallard et al., 2018
Pinton et al., 2023
Moore et al., unpublished

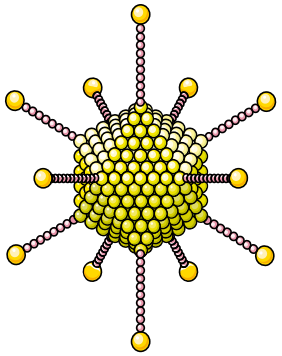




- ✓Nuclear abnormalities correlate with disease severity (mutation specific)
- ✓Nuclear elongation: disease-associated objective readout for therapy development
- ✓Independently validated (*Rose et al., Biomaterials, 2023*)

From disease to (advanced) therapy modelling

Advanced Therapy Medicinal Products (ATMPs)



Gene therapy/editing

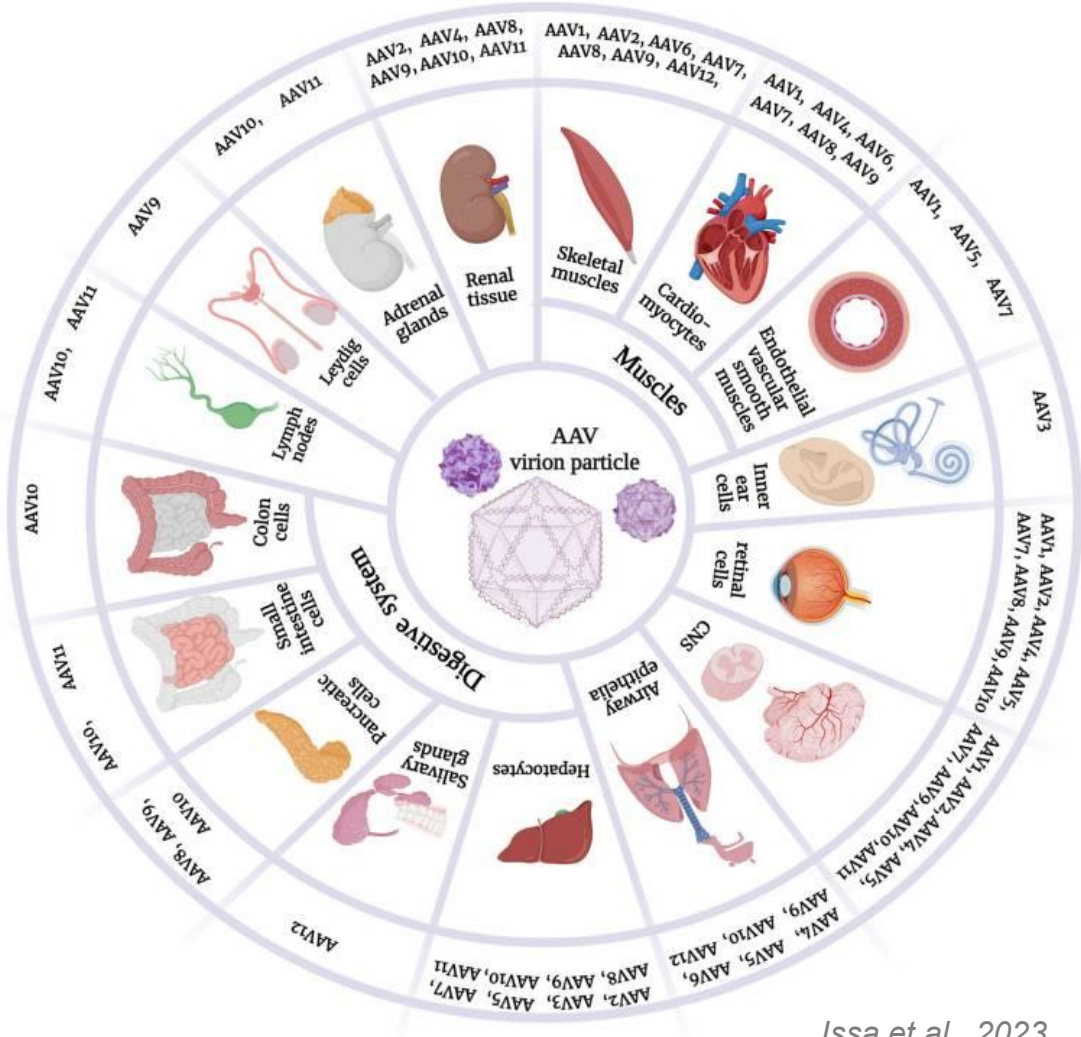
Cell therapy

Choi & Tedesco, in preparation

Choi, Ferrari et al., EMBO Mol Med 2022

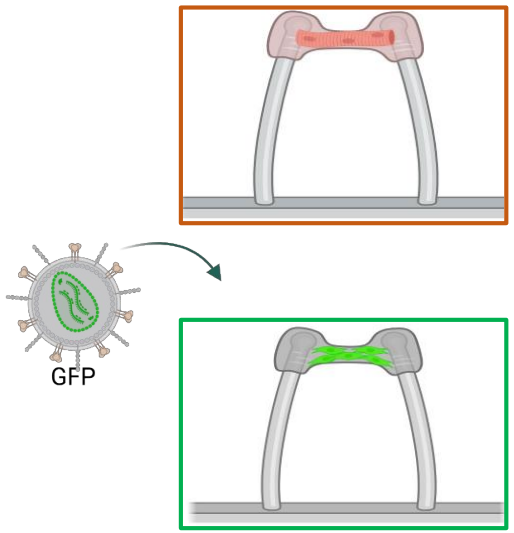
Tissue engineering/replacement

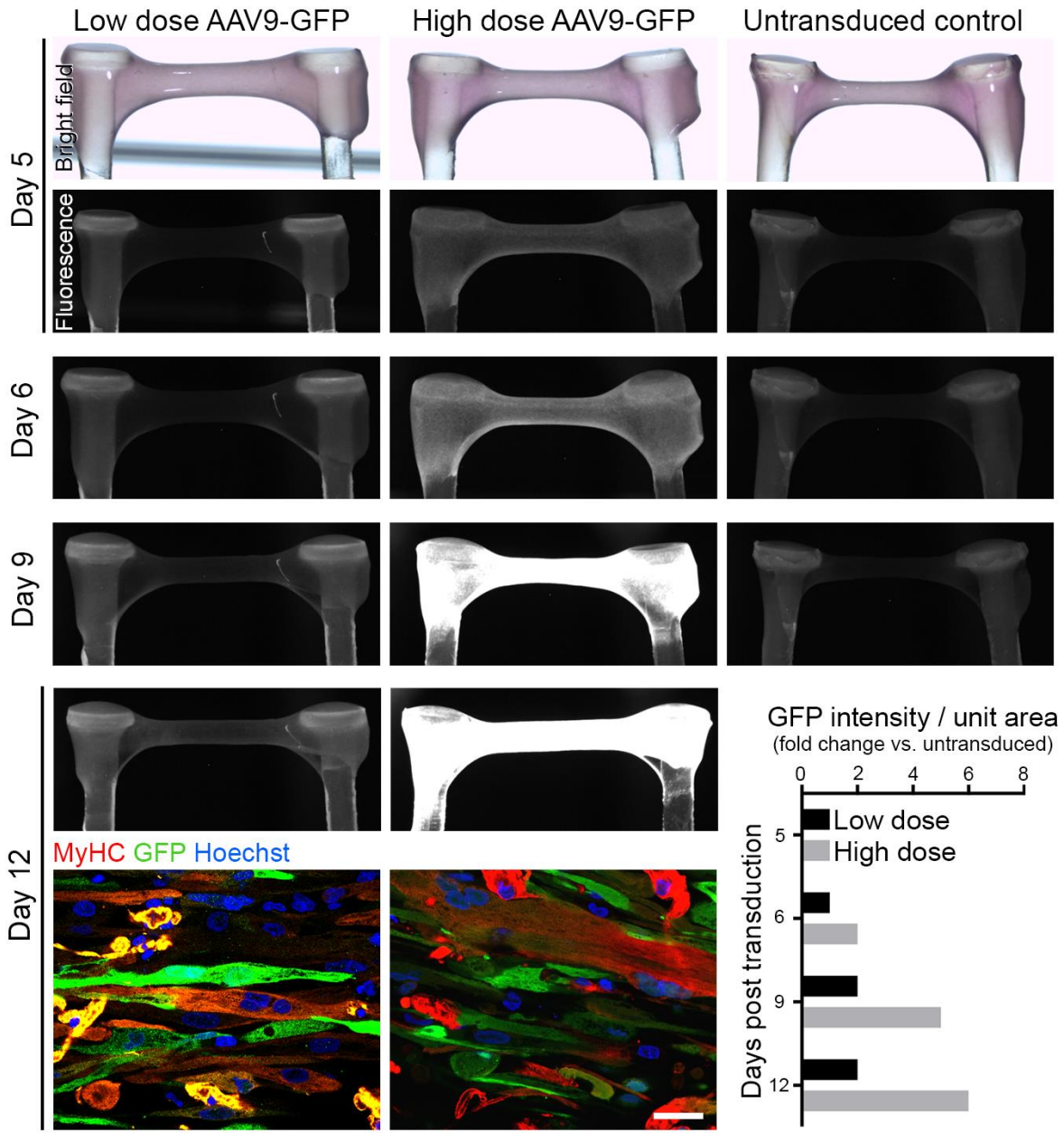
Maffioletti, Sarcar et al., Cell Reports 2018



Issa et al., 2023

From disease to advanced therapy modelling: **gene therapy vectors**





Pinton & Khedr et al.,
Nat Protocol 2023

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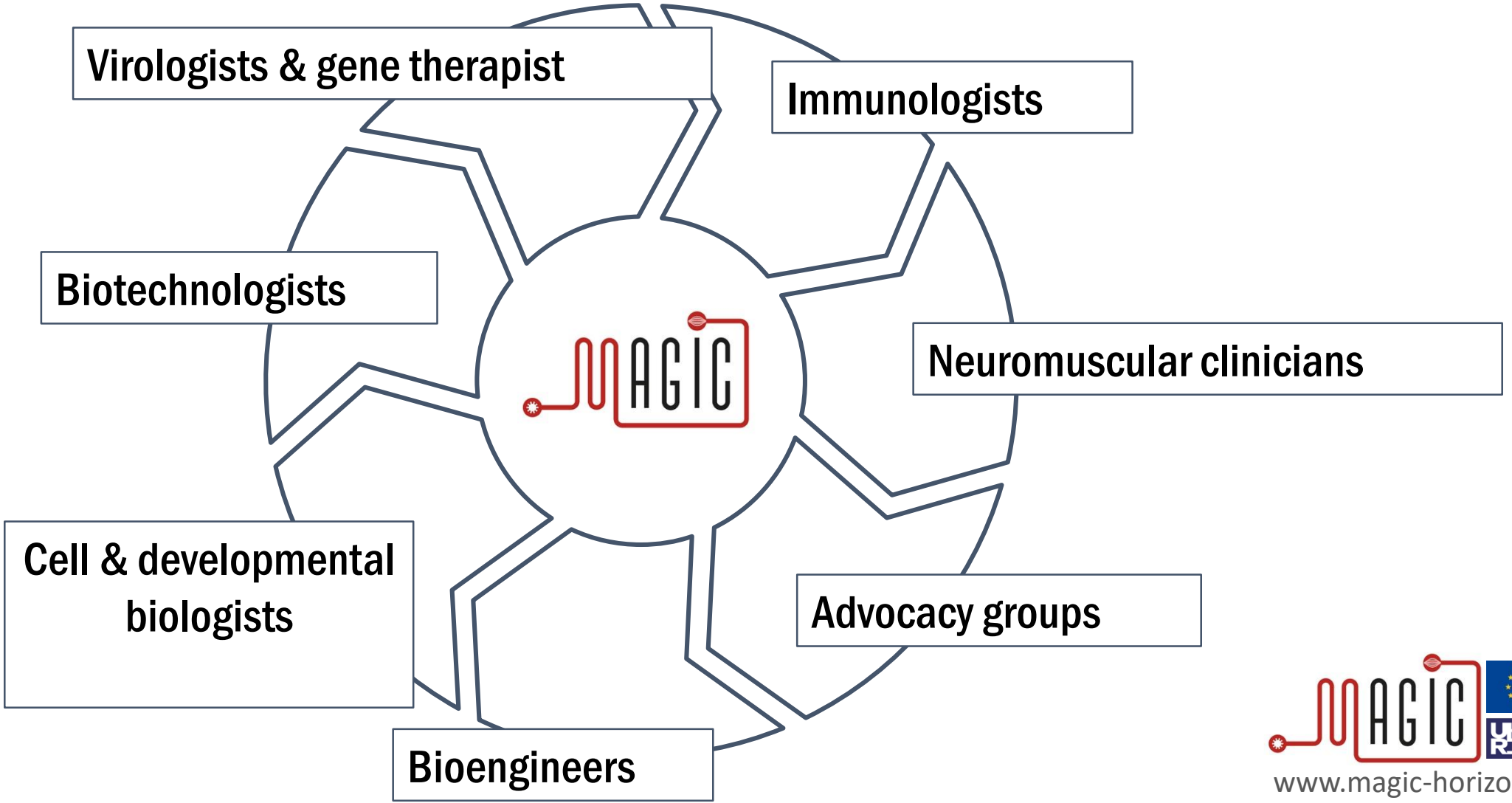
Inserm · UPEC Université Paris-Est Créteil Val de Marne Connaissance - Action · Cincinnati Children's · Maynooth University National University of Ireland Maynooth · REITHERA · VIVE biotech · cure CMD a cure is among us · DUCHENNE DATA FOUNDATION

The MAGIC Team



www.magic-horizon.eu

“All models are wrong…

…but some are useful”

George E. P. Box

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UKRI Biotechnology and Biological Sciences Research Council

Medical Research Council

Duchenne Parent Project

MAGIC

cure CMD a cure is among us

AFMTÉLÉTHON CURE THROUGH INNOVATION

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