# SOCIETY FOR MUSCLE BIOLOGY

Presents the 14th meeting on:

# Skeletal Muscle Stem Cells and Regeneration

## Fairmont Empress Hotel
## Victoria, BC, Canada
## July 19 – 24th, 2026

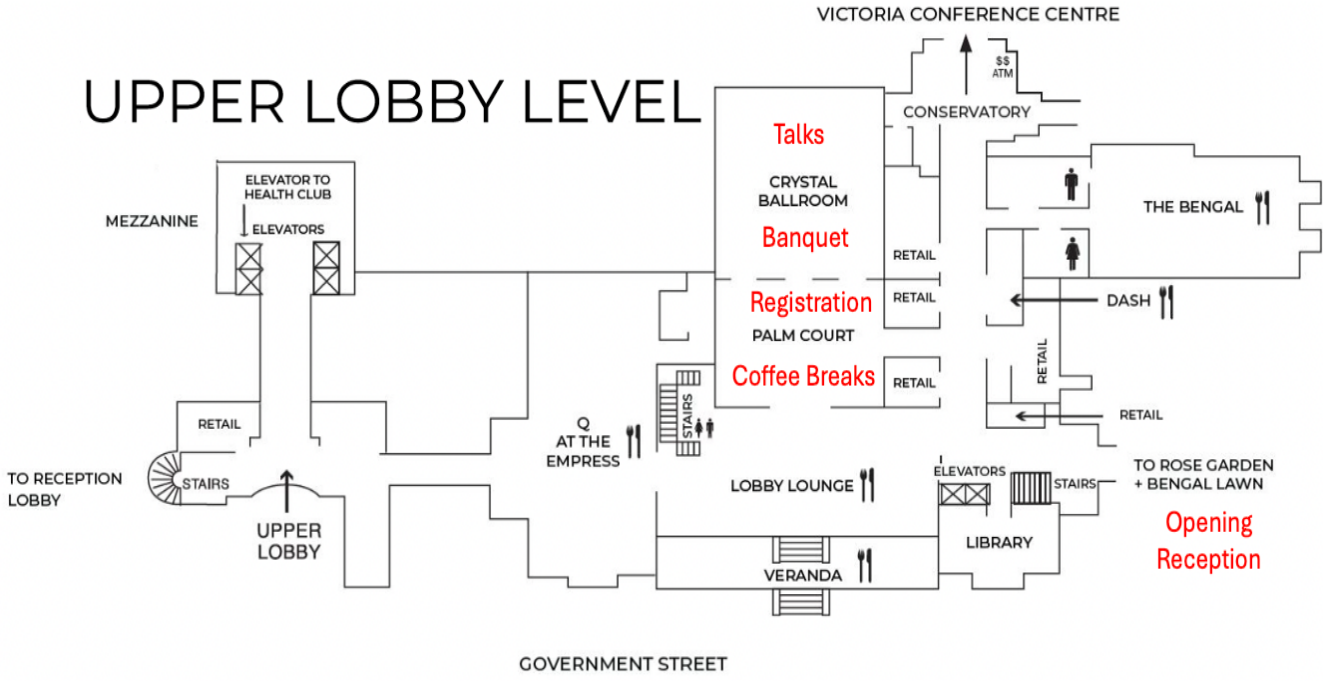
## Co-Organizers

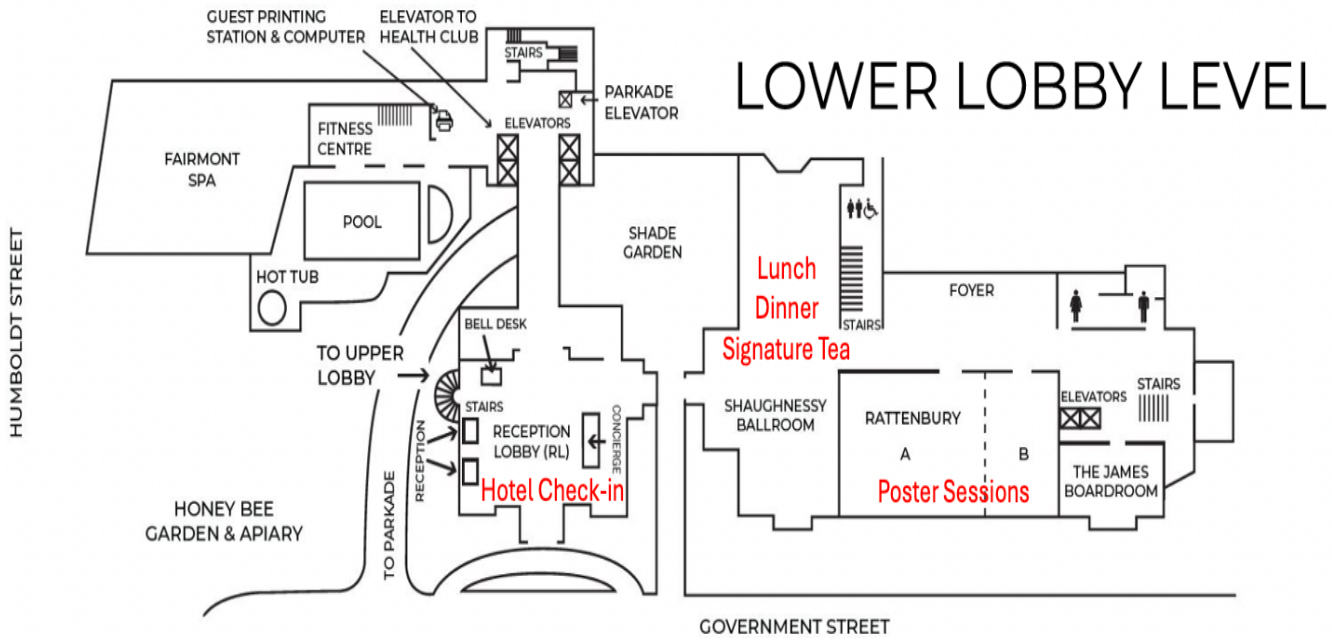
April Pyle
University of California, Los Angeles

Ben Cosgrove
Cornell University

Pete Zammit
King's College London

# MEETING VENUE FLOORPLAN



# MEETING PROGRAM

## SUNDAY, JULY 19

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| 3:00 – 6:00 pm | **Registration** (Palm Court) |
| 4:00 pm onwards | **Hotel Room Check-In** (Reception Lobby) |
| 7:30 – 7:45 pm | **Welcome & Introduction to Mauro Lecture** (Crystal Br)<br>April Pyle, Ben Cosgrove, Pete Zammit |
| 7:45 – 8:45 pm | **10th Alexander Mauro Lecture** (Crystal Ballroom)<br><br>**Shahragim Tajbakhsh** (Pasteur Institute, France)<br>*"Maintaining Orbit"* |
| 8:45 – 10:45 pm | **Opening Reception** (Rose Garden)<br>Refreshments and appetizers |

# MONDAY, JULY 20

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| **7:00 – 8:30 am** | **Breakfast** (Shaughnessy Ballroom)<br>(Fairmont Empress Hotel guests only) |
| **8:30 – 12:30 pm** | **Session 1: Muscle stem cell regulation and metabolism** (Crystal Ballroom)<br>Chairs: Colin Crist, Zhenguo Wu |
| 8:30 – 9:00 am | **Peter Currie** (Monash University, Australia)<br>*"Development, evolution and function of distinct muscle stem cell compartments within the vertebrate myotome"* |
| 9:00 – 9:30 am | **Rob Krauss** (Icahn School of Medicine at Mount Sinai, USA)<br>"*Regulation of muscle stem cell dynamics and quiescence"* |
| 9:30 – 10:00 am | **Tom Rando** (University of California, Los Angeles, USA)<br>"*Molecular regulation of stem cell quiescence"* |
| 10:00 – 10:15 am | **Ha My Ly** (University of Ottawa, Canada)<br>"*Dynamic peroxisomal remodeling supports metabolic transitions in regenerating muscle stem cells"* |
| **10:15 – 10:45 am** | **Coffee Break** (Palm Court) |
| 10:45 – 11:15 am | **Mireille Khacho** (University of Ottawa, Canada)<br>*"Mitochondrial dynamics direct stem cell fates through redox and metabolite signaling"* |
| 11:15 – 11:45 am | **Natasha Chang** (McGill University, Canada)<br>*"Autophagy maintains muscle stem cell quiescence"* |
| 11:45 – 12:00 pm | **Jared Talbot** (University of Maine, USA)<br>"*Retinoic acid signaling specifies anterior-posterior identity of muscle-forming cell streams in zebrafish"* |
| 12:00 – 12:15 pm | **Mark Danesh** (York University, Canada)<br>"*Obesity primes cellular senescence of female muscle stem cells by their epigenetic reprogramming during quiescence"* |

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| 12:15 – 12:30 pm | **Paola Pisterzi** (Leiden University Medical Center, NL)<br>"*Spatial transcriptomics and histopathologicalanalysis reveal selective loss of type 2a fibers in Duchenne muscular dystrophy"* |
| **12:30 – 2:00 pm** | **Lunch and Meet the Speakers** (Shaughnessy)<br>S. Tajbakhsh, T. Rando, P. Currie, M. Khacho, N. Chang |
| 1:30 – 2:00 pm | **All Posters to be Set-up (Rattenbury)** |
| **2:00 – 5:35 pm** | **Session 2: Muscle stem cell niche and environmental cues** (Crystal Ballroom)<br>Chairs: Rachelle Crosbie, Valentina Taglietti |
| 2:00 – 2:30 pm | **Thomas Braun** (Max Planck Institute for Heart and Lung Research, Germany)<br>*"Environmental control of muscle stem cells"* |
| 2:30 – 3:00 pm | **Clarissa Henry** (University of Maine, USA)<br>*"Impacts of muscle-matrix adhesion on muscle development, regeneration, and homeostasis"* |
| 3:00 – 3:30 pm | **Fabio Rossi** (University of British Columbia, Canada)<br>*"Exomuscular sources of FAPs in the early response to acute damage"* |
| 3:30 – 4.00 pm | **Isabella Scionti** (Institut NeuroMyoGène, France)<br>"*Interplay between lysine demethylases and the microenvironment during skeletal muscle regeneration"* |
| **4:00 – 4:30 pm** | **Coffee Break** (Palm Court) |
| 4:30 – 4:45 pm | **Daniel Kopinke** (University of Florida, USA)<br>"*Circadian regulation of FAP fate and function"* |
| 4:45 – 5:00 pm | **Joana Esteves de Lima** (East Paris University - France)<br>"*Epigenetic regulation of skeletal muscle FAPs identity"* |

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| 5:00 – 5:15 pm | **Mary-Jean Rowson** (Cornell University, USA)<br>"*High-resolution spatial total transcriptomic analysis of skeletal muscle regeneration and disease"* |
| 5:15 – 5.35 pm | **Poster Blitz 1** (Crystal Ballroom) |
| **6:00 – 7:30 pm** | **Dinner** (Shaughnessy Ballroom) |
| **7:30 – 8:15 pm** | **Career Stories: Academia and Industry** (Crystal)<br><br>Penney Gilbert (University of Toronto) (Moderator)<br>David Glass (Regeneron Pharmaceuticals)<br>Eva Chin (Solve FSHD)<br>Tom Cheung (Hong Kong Univ of Science and Technology)<br>Francesco Saverio Tedesco (University College London)<br>Will Wang (Regeneron Pharmaceuticals) |
| **8:15 – 10:15 pm** | **Poster Session 1** (Rattenbury) |

# TUESDAY, JULY 21

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| **7:00 – 8:30 am** | **Breakfast** (Shaughnessy Ballroom)<br>(Fairmont Empress Hotel guests only) |
| **8:30 – 10:30 am** | **Session 3: Genetic & epigenetic regulation** (Crystal Ballroom)<br>Chairs: Dada Pisconti, Yarui Diao |
| 8:30 – 9:00 am | **Jeffrey Dilworth** (University of Wisconsin-Madison, USA)<br>"*Epigenetic integration of niche signaling during regeneration*" |
| 9:00 – 9:30 am | **Chiara Mozzetta** (University of Rome, Italy)<br>"*Chromatin at the edge: Anchoring muscle homeostasis*" |
| 9:30 – 10:00 am | **Colin Crist** (McGill University, Canada)<br>"*CTCF insulates against inappropriate gene expression to safeguard migratory myogenic progenitor cells*" |
| 10:00 – 10:30 am | **Vittorio Sartorelli** (National Institutes of Health, USA)<br>*"Transcriptional and epigenetic control of muscle stem cell aging"* |
| **10:30 – 11:00 am** | **Coffee Break** (Palm Court) |
| **11:00 – 12:30 pm** | **Session 4: Aging and muscle stem cell dynamics** (Crystal Ballroom)<br>Chairs: Simon Hughes, Aaron Johnson |
| 11:00 – 11:30 am | **David Glass** (Regeneron, USA)<br>"*Age-related perturbations leading to sarcopenia*" |
| 11:30 – 12:00 pm | **Tom Cheung** (Hong Kong University of Science and Technology, China)<br>"*Post-transcriptional regulation of muscle stem cell function during aging*" |

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| 12:00 – 12:30 pm | **Bradley Olwin** (University of Colorado, USA)<br>*"Complex changes in muscle tissue during aging prevent rejuvenated stem cells from improving muscle function"* |
| **12:30 – 2:00 pm** | **Lunch and Meet the Speakers** (Shaughnessy)<br>T. Braun, C. Christ, D. Glass, V. Sartorelli, C. Mozzetta |
| **2:00 – 6:05 pm** | **Session 5: Muscle disorder mechanisms**<br>(Crystal Ballroom)<br>Chairs: Clarissa Henry, Jared Talbot |
| 2:00 – 2:30 pm | **Rachelle Crosbie** (University of California, LA, USA)<br>*"From membrane stability to regenerative competence in dystrophic muscle"* |
| 2:30 – 3:00 pm | **Bénédicte Chazaud** (Institut NeuroMyoGène, France)<br>*"The role of resident macrophages in skeletal muscle homeostasis"* |
| **3:00 – 4:15 pm** | **Signature Tea at the Empress**<br>(Shaughnessy Ballroom) |
| 4:15 – 4:45 pm | **Julia von Maltzahn** (Brandenburg Technische Universität Cottbus-Senftenberg, Germany)<br>*"Driving rhabdomyosarcoma into myogenic differentiation"* |
| 4:45 – 5:15 pm | **Alessandra Sacco** (Sanford Burnham Prebys, USA)<br>*"Targeting cachexia in pancreatic cancer"* |
| 5:15 – 5:30 pm | **Sonia Alonso-Martin** (IIS BIOGIPUZKOA, Spain)<br>"*From skeletal muscle regeneration and aging to ALS… and back again: revisiting ALS"* |
| 5:30 – 5:45 pm | **Jimmy Massenet** (Sanford Burnham Prebys MDI, USA)<br>"*Ctcf deficiency in myofibers induces pathological genome reprogramming toward development of idiopathic myopathy"* |
| 5:45 – 6:05 pm | **Poster Blitz 2** (Crystal Ballroom) |
| **6:05 – 8:00 pm** | **Dinner** (Shaughnessy Ballroom) |
| **8:00 – 10:00 pm** | **Poster Session 2** (Rattenbury) |

# WEDNESDAY, JULY 22

**7:00 – 8:30 am** — **Breakfast** (Shaughnessy Ballroom)
(Fairmont Empress Hotel guests only)

**8:30 –10:30 am** — **Session 6.1: Engineering and translating muscle stem cells** (Crystal Ballroom)
Chairs: Penney Gilbert, Hyojung Choo

8:30 – 9:00 am — **Olivier Pourquie** (Harvard Medical School, USA)
*"Reconstructing human skeletal muscle in vivo and in vitro"*

9:00 – 9:30 am — **Francesco Saverio Tedesco** (University College London, UK)
*"Advanced modelling of congenital muscle disorders to improve translation of neuromuscular (gene) therapies"*

9:30 – 10:00 am — **Stephanie Willerth** (University of Victoria, Canada)
"*3D bioprinting complex tissues*"

10:00 – 10:15 am — **Dieu Huong Hoang** (University of California, LA, USA)
"*Development of a universal cell therapy for skeletal muscle"*

10:15 – 10:30 am — **Charles Emerson** (UMass Chan Medical School, USA)
"*A role for regeneration in the initiation of FSHD muscle pathology as revealed by innate immune cell/FSHD patient muscle xenograft model"*

**10:30 – 11:00 am** — **Coffee Break** (Palm Court)

**11:00 – 11:15 pm** — **Conference Photo** (Location TBD)

**11:15 – 12:30 pm** **<u>Session 6.2</u>: Emerging Topics**
Chairs: Penney Gilbert, Hyojung Choo

11:15 – 11:30 pm **Aaron Johnson** (Washington University School of Medicine, USA)
"*Muscle injuries of different magnitudes activate distinct regenerative programs*"

11:30 – 11:45 pm **Francesca Boscolo Sesillo** (University of California San Diego, USA)
"*Pelvic floor muscle stem cells drive protective adaptations during pregnancy*"

11:45 – 12:00 pm **Yarui Diao** (Duke University Medical Center, USA)
"*Stem cells age when they lose their shape*"

12:00 – 12:15 pm **Krzysztof Jagla** (iGReD, Clermont Ferrand, France)
"*Loss of muscle identity and affected myonuclear differentiation lead to muscle fiber splitting*"

12:15 – 12:30 am **Poster Blitz 3** (Crystal Ballroom)

**12:30 pm – 8:00 pm** **Free Time and Activities**
See website for suggested activities

**8:00 – 10:00 pm** **Poster Session 3** (Rattenbury)

# THURSDAY, JULY 23

**7:00 – 8:30 am** **Breakfast** (Shaughnessy Ballroom)
(Fairmont Empress Hotel guests only)

**8:30 – 11:00 am** **Session 7: Building Muscle and Niches** (Crystal)
Chairs: Fabien Le Grand, Justin Fallon

8:30 – 9:00 am **Gabrielle Kardon** (University of Utah, USA)
"*Muscle regeneration after sterile and pathogenic injuries*"

9:00 – 9:30 am **Michael Hicks** (University of California, Irvine, USA)
*"Spatial transcriptomics and niche establishment by engrafted human muscle across time and disease"*

9:30 – 10:00 am **Doug Millay** (Cincinnati Children's Hospital Medical Center, USA)
*"Building muscle: Mechanisms of myoblast fusion and myonuclear function"*

10:00 – 10:30 am **D Cornelison** (University of Missouri, USA)
*"Protein-protein interactions regulating proliferation vs. differentiation in muscle satellite cells"*

**10:30 – 11:00 am** **Coffee Break** (Palm Court)

**11:00 – 12:30 pm** **Business and Conference Meeting** (Crystal)
*Society for Muscle Biology Discussion*
Bénédicte Chazaud, Tom Cheung, Jeff Dilworth, Penney Gilbert, David Glass, Clarissa Henry, Rob Krauss, Doug Millay, April Pyle, Alessandra Sacco

*2028 SMB Meeting Planning (Site, Scheduling, Integration)*
*Nominations and Election of Co-organizers with Ben Cosgrove*
*Conference Evaluation Forms*
*April Pyle, Ben Cosgrove, Pete Zammit*

**12:30 – 2:00 pm** **Lunch and Meet the Speakers** (Shaughnessy)
R. Crosbie, B. Chauzaud, D. Millay, F. S. Tedesco, S. Willerth G. Kardon

**2:00 – 6:00 pm** **Session 8: Diversity of Muscle Stem Cells and targeting for therapies** (Crystal Ballroom)
Chairs: Fred Relaix, Sonia Alonso-Martin

2:00 – 2:30 pm **Dada Pisconti** (SUNY Stony Brook, USA)
*"Multifaceted role of p53 in the establishment and maintenance of skeletal muscle identity"*

2:30 – 3:00 pm **Yusuke Ono** (University of Kumamoto, Japan)
"*Distinct functions of Notch signaling in muscle satellite cells and myofibers*"

3:00 – 3:15 pm **Paige Rudy** (University of Michigan, USA)
"*Regulation of Fibrosis by Human Antigen R in fibro-adipogenic progenitors after skeletal muscle injury*"

3:15 – 3:30 pm **Joshua Greig** (King's College London, UK)
"*Defective autophagy in facioscapulohumeral muscular dystrophy (FSHD) skeletal muscle provides a viable target for therapeutic intervention*"

3:30 – 3:45 pm **Duc Dong** (Scintillon Research Institute, USA)
"*Pharmacological restoration of muscle stem cell health and function in FSHD*"

**3:45 – 4:15 pm** **Coffee Break** (Palm Court)

4:15 – 4:30pm **Justin Fallon** (Brown University, USA)
"*MuSK-BMP signaling in adult muscle stem cells maintains quiescence and regulates myofiber size and muscle strength*"

4:30 – 5:00 pm **Philippos Mourikis** (Institut Necker Enfants Malades, France)
"*Multipotency of postnatal Pax7 cells and contribution to non-myogenic lineages*"

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| 5:00 – 5:30 pm | **Valentina Taglietti** (Paris Est-Creteil University, France "*Distinctive regenerative properties of extraocular muscle stem cells and their niche*" |
| 5:30 – 6:00 pm | **Michael Rudnicki** (Ottawa Hospital Research Institute, Canada) *"Developing innovative regenerative therapies for neuromuscular diseases"* |
| **6:00 – 7:00 pm** | **Free Time and Poster Removal** |
| **7:00 – 7:30 pm** | **Pre-Banquet Refreshments (Palm Court)** |
| **7:30 – 9:00 pm** | **Closing Banquet Dinner and Awards (Crystal)** |
| **9:00 – 11:30 pm** | **Dance Party and Karaoke (Crystal)** |

# FRIDAY JULY 24, 2026

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| **7:00 – 8:30 am** | **Quick Start Continental Breakfast**<br>(Shaughnessy Ballroom)<br>(Fairmont Empress Hotel guests only) |
| **To 11:00 am** | **Check-Out & Departure** |

# Talk Abstracts (in presentation order)

## Maintaining Orbit

Shahragim Tajbakhsh[1]

[1]Stem Cells & Development, Department of Developmental & Stem Cell Biology, Institut Pasteur, Paris, France

Sixty five years ago, Alexander Mauro described a small, wedge-shaped cell tucked between the plasma membrane and basal lamina of skeletal muscle fibers in frog. Bernard Katz noted comparable cells the same year, in passing. Both observations were anatomical, made by electron microscopy, and focused primarily on morphology. Neither paper anticipated that this quiescent, niche-dwelling cell would become one of the most studied adult stem cells in biology.

This lecture traces those initial discoveries and conceptual leaps that followed diverse trajectories, from descriptive anatomy to functional genetics, from a structural curiosity to a paradigm for stem cell self-renewal, heterogeneity, and niche dependency.

Work from different labs, including our own, addressed how satellite cells are regulated in different anatomical locations, how their behaviour is altered with age, and what the field has learned, or not, about mechanisms governing quiescence, activation, growth, regeneration, and then some... A single electron micrograph opened a field that has answered many questions, yet also left many stubbornly unresolved.

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# Development evolution and function of distinct muscle stem cell compartments within the vertebrate myotome.

Frank Tulenko[1], Avnika Ruparelia[1], Kevin Lu[1], Peter Currie[1]

[1]Australian Regenerative Medicine Institute

While the function of adult tissue-resident stem cells during regeneration and disease have received much attention, the mechanistic basis of stem cell driven organ growth remains relatively poorly defined. Consequently, our understanding of processes that drive organ development remains piecemeal. Understanding stem cell dynamics within the organ systems that deploy them to generate growth is critical for the success of ongoing efforts to generate complex functioning organs from organoid rudiments *in vitro*. Thus, we have sought to understand the stem cell processes that regulate and drive myotomal growth from the template of the embryonic myotome using zebrafish as our main model system. Here we compare and contrast modes of myotomal growth and muscle regeneration and their molecular regulation in distinct niches across the phylogeny, in attempt to generate a fuller understanding of the way muscle stem cells act within different vertebrate clades.

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# Regulation of muscle stem cell dynamics and quiescence

Robert Krauss[1]

[1]Icahn School of Medicine at Mount Sinai

The hallmark property of muscle stem cells (MuSCs) during homeostasis is quiescence. Quiescence is an actively maintained state, but homeostatic MuSCs are not generally viewed as morphologically dynamic. However, we and others have shown that quiescent MuSCs possess cellular protrusions that are heterogeneous, complex, and tipped with filopodia, characteristics of motile structures. We have proposed that MuSC protrusions extend and partially retract during quiescence as a means of niche sensation, and that this morphological equilibrium is governed by a balance of niche-regulated RhoA and Rac1 GTPase activity. MuSC-specific loss of one copy of either *RhoA* or *Rac1* leads to a break in quiescence in the absence of injury. Strikingly, *RhoA*;*Rac1* double heterozygotes display fewer activated MuSCs than either single heterozygote, revealing that the ratio of GTPase activity is more important than absolute activities, consistent with an equilibrium state. To identify regulatory pathways in this process, we developed of an ex vivo live imaging assay for MuSC protrusion dynamics. The axon guidance cue Netrin-1 promotes MuSC protrusion outgrowth ex vivo in a manner dependent on its receptors Dcc and Neogenin, Rac1, and the actin-branching factor Arp2/3; this pathway is also required for Netrin-dependent axonal growth cone motility. Adult MuSC-specific genetic removal of Netrin-1 receptors, Rac1, and Arp2/3 each result in failure to maintain homeostatic protrusion lengths, spontaneous quiescence exit, and MuSC attrition in uninjured mice. These findings reveal an unanticipated level of morphological dynamism by a quiescent stem cell at homeostasis and link this phenomenon to preservation of the quiescent state.

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# Molecular regulation of stem cell quiescence

Tom Rando[1]

[1]University of California, Los Angeles

Molecular regulation of stem cell quiescence

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# Dynamic peroxisomal remodeling supports metabolic transitions in regenerating muscle stem cells.

Ha My Ly[1], George Cairns[1], Nikita Larionov[2], Yan Burelle[1, 2, 3]

[1]Interdisciplinary School of Health Sciences, Faculty of Health Sciences, University of Ottawa, Ottawa, Canda, [2]Department of Biochemistry, Microbiology and Immunology, University of Ottawa, Ottawa, Canda, [3]Center for Neuromuscular Disease (CNMD), Faculty of Medicine, University of Ottawa, Canada

Peroxisomes are emerging as important regulators of cellular metabolism, yet their roles in muscle stem cell (MuSC) fate and regeneration remain unresolved. Here, we investigated how peroxisomal remodeling coordinates metabolic transitions during MuSC activation and skeletal muscle regeneration using inducible genetic models targeting distinct steps of peroxisome biogenesis. Multi-omic profiling and immunostaining analyses revealed quiescent MuSCs contain minimal peroxisomal content, which rapidly expands upon activation prior to lipid droplet accumulation and alongside mitochondrial remodeling. These changes were accompanied by increased interactions among peroxisomes, mitochondria and lipid droplets, suggesting that peroxisomes act as early organizers of metabolic adaptation during MuSC state transitions. To define peroxisomal functions, we generated MuSC-specific knockout models for Pex10 and Pex16, that regulate peroxisomal matrix protein import and membrane assembly, respectively. Pex10 deletion impaired early regenerative expansion following cardiotoxin injury, resulting in reduced MuSC proliferation and decreased $Pax7^{+}$ cell numbers while preserving differentiation capacity. In contrast, Pex16 ablation caused defective early differentiation and impaired long-term regeneration, characterized by reduced myofiber size and depletion of the MuSC pool. Both Pex10 and Pex16 deficiency reduced lipid droplet accumulation without altering mitochondrial abundance. However, Pex16-deficient myoblasts displayed reduced oxygen consumption rates with impaired mitochondrial respiratory complex activity, indicating that peroxisomal integrity supports mitochondrial function independently of mitochondrial content. Preliminary rescue experiments using lipid metabolite supplementation partially restored differentiation capacity, suggesting that altered peroxisome-derived lipid metabolism contributes to impaired myogenesis. Collectively, these findings identify distinct peroxisomal biogenesis pathways as critical regulators of MuSC fate and metabolic remodeling during skeletal muscle regeneration.

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# Mitochondrial dynamics direct stem cell fates through redox and metabolite signaling

Mireille Khacho[1]

[1]University of Ottawa

The regulation of adult stem cell quiescence is essential for stem cell maintenance, longevity and sustained tissue regeneration, however, the upstream regulatory mechanisms are not fully understood. Our studies have uncovered that changes in stem cell mitochondrial shape serve as an upstream signaling mechanism that communicates retrogradely with the nucleus to regulate the quiescent state of stem cells. We show that mitochondria in stem cells rapidly fragment in response to an environmental activation stimulus to drive the exit from deep quiescence. Manipulation of mitochondrial dynamics, by changing OPA1 levels, or redox states is sufficient to alter stem cells function and muscle regeneration. This occurs through activation of an intracellular reactive oxygen species (ROS) and glutathione (GSH) mediated signaling pathway that initiates a nuclear transcriptional program to suppress quiescence and self-renewal genes and promote myogenic gene expression. We provide evidence that impaired mitochondrial dynamics and GSH homeostasis may be an early contributor to age-related muscle stem cell dysfunction. Together, our data demonstrate that mitochondrial plasticity and redox balance are essential upstream regulators of stem cell function and longevity.

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# Autophagy maintains muscle stem cell quiescence

Natasha Chang[1]

[1]McGill University

Muscle stem cells, also known as satellite cells, are essential for muscle regeneration and for maintaining skeletal muscle homeostasis throughout life. Satellite cell fate decisions, including quiescence, activation, differentiation, and self-renewal, are tightly regulated by multiple molecular pathways. Increasing evidence suggests that cellular metabolism plays a key role in governing these fate transitions. Autophagy is a conserved cellular recycling pathway that removes and repurposes damaged proteins and organelles and has been implicated in the maintenance and differentiation of numerous stem cell populations. Consistent with its role in tissue homeostasis, defective autophagy has been linked to age-related muscle degeneration, neurodegenerative disorders, and myopathies such as Duchenne muscular dystrophy (DMD), the most common and severe form of muscular dystrophy.

Our recent work demonstrates that satellite cells in DMD exhibit reduced autophagy and impaired autophagy dynamics during regenerative myogenesis. Furthermore, pharmacological induction of autophagy enhances the differentiation capacity of DMD satellite cells. Using mice with satellite cell-specific conditional deletion of *Atg7*, we are investigating the role of autophagy in satellite cell fate, muscle homeostasis, and regeneration. Our data indicate that loss of autophagy impairs the ability of satellite cells to maintain quiescence, highlighting a critical role for autophagy in preserving the stem cell state. Understanding how autophagy contributes to satellite cell function provides insight into the impact of defective satellite cell autophagy in muscle disease and informs therapeutic strategies aimed at modulating autophagy to improve muscle regeneration.

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# Retinoic Acid Signaling Specifies Anterior-Posterior Identity of Muscle-Forming Cell Streams in Zebrafish

Alexandra Myles[1], Teresa Easterbrooks[1], Jared Talbot[1]

[1]University of Maine

The ‘migratory muscle precursor streams’ (MiMPS) are collective migrations of myogenic cells that originate in somites and build muscles elsewhere. Zebrafish have a simplified set of three MiMPS that emigrate from the first six somites; MiMPS-1 produces an anterior 'neck'-like muscle, MiMPS-2 builds pectoral fin muscles, and MiMPS-3 builds a posterior muscle in the chest. It remains unclear how these cell streams are patterned to migrate to different destinations. We find that a combinatorial code of retinoic acid (RA) pathway gene expression differs in each cell: MiMPS-1 expresses the RA-degrading gene *cyp26a1*; MiMPS-3 expresses RA synthesis genes, *aldh1a2* and *rdh10a*; the pectoral fin, populated by MiMPS-2, is a 'neutral territory' that lacks these RA pathway genes. Consistent with the hypothesis that RA synthesis patterns posterior MiMPS identity, RA inhibitors impede outgrowth of the 'chest' muscle, and RA activators shift it toward the midline. Consistent with the hypothesis that RA inhibition specifies anterior identity, RA activators move MiMPS-1 posteriorly, where it often fuses with MiMPS-3. The pathway appears to affect tissue identity (not just position), since preliminary experiments show that RA activation increases expression of posterior markers in MiMPS-1. The muscles in the pectoral fin are also affected by RA modulation, through a combination of direct effects on the MiMPS-2 and indirect effects via lateral plate mesoderm. Together, our findings strongly suggest that RA signaling levels impart different identities to MiMPS-1 through MiMPS-3, providing an initial explanation for how muscle-forming cell migrations are differentially patterned along the anterior-posterior axis.

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# Obesity primes cellular senescence of female muscle stem cells by their epigenetic reprogramming during quiescence

Mark Danesh[1], Jaryeon Lee[1], Lucas Campagna[1], Ethan Sooklal[2], Alexandra Pislaru[2], Quentin Sastourne-Arrey[1], Alexandre Blais[3], Tara Haas[1, 2], Anthony Scimè[1, 2]

[1]Molecular, Cellular, and Integrative Physiology, Faculty of Health, York University, [2]Department of Biology, York University, [3]Department of Biochemistry, Microbiology, and Immunology, University of Ottawa

Quiescent satellite cells (QSCs) integrate systemic and local cues, yet whether the metabolic environment imprints long-lasting programs on their function remains unclear. Fluorescence-activated cell-sorted QSCs from normal chow or high-fat (HF) fed male and female mice were profiled using ATAC-seq. HF diet reduced chromatin accessibility at promoters of core myogenic regulators such as MyoD and Myf5, indicating compromised myogenic competence. Consistent with this, myofiber cultures from HF mice demonstrated impaired SC activation, proliferation, self-renewal, and fusion, with sex-dependent effects. Strikingly, female HF QSCs exhibited chromatin signatures consistent with senescence priming, including increased accessibility linked to oxidative stress and inflammation. A pre-senescent phenotype in female HF SCs was confirmed, ex vivo and in vivo, by elevated levels of p16, γH2AX and senescence associated b-galactosidase activity. This coincided with pronounced deficits in muscle regenerative capacity in HF female mice after induced muscle injury. Unexpectedly, dietary normalization reversed HF-associated chromatin changes in males but not females. The female QSCs remained impaired in activation and proliferation, retained a HF-like epigenome, and continued to show heightened senescence sensitivity, indicating a sex-specific failure to erase metabolic memory. These findings identify dietary lipid excess as a driver of epigenetic reprogramming in QSCs that reshapes stem cell function in a sexually dimorphic manner. Our data link metabolic state to stem cell chromatin landscapes, showing how obesity perturbs muscle maintenance and repair.

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# Spatial Transcriptomics and Histopathological Analysis Reveal Selective Loss of Type 2a Fibers in Duchenne Muscular Dystrophy

Clara Martinez Mir[1,2], Paola Pisterzi[1,2], Isabel De Poorter[1,2], Jamilla Beek[1,2], Anna Alemany[1,2], Fanny Sage[1,2], Niels Geijsen[1,2]

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Duchenne muscular dystrophy (DMD) leads to progressive muscle wasting and alters fiber-type composition and metabolism. To investigate spatial variation across entire muscles and overcome the limitations of localized biopsies, we employed an integrated approach combining spatial transcriptomics, immunohistochemistry, and quantitative fiber-type profiling in dystrophic (hDMDdel52/mdx) and control mice. In healthy tibialis anterior (TA) muscle, oxidative fibers are typically enriched in the central region, while glycolytic fibers are more prevalent at the proximal and distal ends. In DMD, this organization is disrupted: central enrichment of oxidative fibers is diminished, accompanied by an increase in glycolytic fibers in these regions. Histological analysis confirmed reduced oxidative enzyme activity and a decreased abundance of MYH2-expressing fibers. A reduction in MYH2-positive fibers was also detected in multiple muscles, including the extensor digitorum longus and soleus. Spatial modeling revealed that pathological features are distributed heterogeneously, forming region-specific microenvironments defined by extracellular matrix remodeling, cytoskeletal changes, and immune cell infiltration. These findings indicate that DMD pathology is spatially heterogeneous, producing a mosaic of localized disease states within individual muscles. This multimodal, spatially resolved framework enhances understanding of muscle remodeling and informs the development of biomarkers and therapeutic strategies that address regional heterogeneity, potentially enabling more precise and targeted interventions for DMD.

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# Environmental control of muscle stem cells

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Skeletal muscle stem cells (MuSCs) are assumed to exist in a dormant, quiescent state but become highly active to repair muscle damage or support muscle growth following stress. Their activity is determined by an interplay between microenvironment signals and intrinsic genetic/metabolic controls. Extensive work has been done to decipher the impact of the muscle stem cell niche on the regulation of MuSCs. Moreover, various stimuli have been identified, such as exercise and resistance training that place mechanical stress on muscle tissue, directly activating satellite cells to proliferate, supply new nuclei, and drive muscle hypertrophy. Likewise, immune cells like macrophages flood the site after muscle injury to clear debris and release signaling proteins that suppress dormancy and trigger stem cell activation. Intrinsic genetic & epigenetic controls as well as metabolic reprogramming, processes associated with aging and disease also pay decisive functions in controlling MuSC activity.

We became interested whether environmental cues such as the composition of the intestinal microbiome also affect MuSC activity. We found that germ free mice devoid of intestinal microbes show a pronounced increase of MuSCs, mostly located outside the basal lamina. We uncovered that the intestinal microbiome affects the concentration of metabolites in the serum, which has a direct effect on the activation of MuSCs. The underlying molecular mechanisms will be presented.

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# Impacts of muscle-matrix adhesion on muscle development, regeneration, and homeostasis

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Musculoskeletal function requires integration of skeletal muscle with two different junctions: neuromuscular junctions (NMJs) and myotendinous junctions (MTJs). Disruption of either of these junctions can lead to incurable neuromuscular degenerative diseases that shorten lifespan and deleteriously impact quality of life. Despite their importance, the mechanisms underlying coordinated development and homeostasis of NMJs, muscle, and MTJs are not entirely understood. NMJs and MTJs connect to muscle through cell-extracellular matrix (ECM) adhesion complexes such as the Dystrophin Glycoprotein Complex. Dystroglycan (DG) is a critical transmembrane receptor in the Dystrophin Glycoprotein Complex that requires glycosylation to bind to the ECM. Dystroglycanopathies are clinically heterogeneous disorders caused by disrupted glycosylation of α-Dystroglycan. Dystroglycanopathies display a wide range of phenotypic diversity with mutations in the same gene resulting in severe congenital onset muscular dystrophy with eye/brain involvement, milder adult-onset limb girdle muscular dystrophies, congenital myasthenic syndrome, epilepsy, and all of the above can occur with or without cardiac involvement. A major gap in our understanding of basic muscle biology is how do mutations in the same gene cause variable phenotypes across individuals? Published zebrafish models of dystroglycanopathies capture the two ends of the phenotypic spectrum: either severe dystrophy and lethal early in development or viable with only subtle adult phenotypes. We generated a novel zebrafish dystroglycanopathy model that captures the middle of the phenotypic spectrum and provides new insight into how NMJ, muscle, and MTJ integrity are coordinated during development and progressively lost in disease.

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# Exomuscular sources of FAPs in the early response to acute damage

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Exomuscular sources of FAPs in the early response to acute damage.

Upon acute damage, fibro/adipogenic progenitors rapidly increase in numbers within the tissue. During the first 36 hours following damage, this increase takes place in the absence of proliferation, suggesting that FAPs migrate into the tissue from the outside. We have identified the fascia as a reservoir of a specific subset of FAPs with progenitor characteristics. We will present a characterization of these cells and their role during the response to damage. In addition we will present our recent efforts towards he beating the TGFb pathway by engineering hiPSC-derived myogenic progenitors to improve engraftment and reduce local fibrosis upon transplantation.

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# Interplay between Lysine demethylases and the Microenvironment during skeletal muscle regeneration

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Communication between muscle stem cells (MuSCs) and their microenvironment is essential for maintaining skeletal muscle homeostasis and supporting efficient regeneration. During aging, the muscle microenvironment undergoes profound changes, including alterations in inflammatory signals, growth factors, and regenerative pathways, which can negatively impact MuSC function and regenerative capacity. Among the signaling pathways involved, Wnt/β-catenin signaling has emerged as a key regulator of MuSC fate and muscle regeneration in both physiological and aging contexts. Lysine-specific demethylase 1 (LSD1), an epigenetic regulator involved in transcriptional control and stem cell dynamics, has been implicated in the modulation of MuSC responses to Wnt/β-catenin signaling cues. This project aims to investigate the crosstalk between lysine demethylases and the aging muscle microenvironment, focusing on how epigenetic regulation shapes MuSC function and regenerative potential. Understanding these interactions may provide new insights into the molecular mechanisms underlying age-associated regenerative decline.

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# Circadian regulation of FAP fate and function

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FAPs are critical regulators of muscle regeneration because they can either support myogenesis or adopt maladaptive fates that drive intramuscular adipose tissue (IMAT) formation. A central question in our laboratory is what controls this switch. In peripheral artery disease, impaired limb recovery is strongly associated with pathological muscle remodeling, yet the upstream mechanisms that redirect FAPs away from regenerative support towards adipocyte differentiation remain poorly defined. Our current work identifies the circadian clock as a cell-intrinsic regulator of this process. Our preliminary data show that ischemic human and murine muscle display misregulated clock gene expression, including within FAPs. To test whether the FAP clock directly governs FAP fate, we generated a mouse model that deletes the core clock gene Bmal1 specifically in FAPs. Strikingly, disruption of Bmal1 suppresses adipogenic differentiation, limits intramuscular adipose tissue formation, and improves muscle recovery after both adipogenic injury and hindlimb ischemia. These findings identify the FAP clock as a key determinant of pathological versus regenerative remodeling. Importantly, the effect is not limited to reduced fat formation. FAP-specific clock disruption also enhances myofiber regeneration, indicating that circadian regulation of FAPs shapes the broader niche that supports muscle repair. We therefore hypothesize that ischemia induces maladaptive circadian reprogramming in FAPs, shifting them away from pro-regenerative paracrine functions and toward an adipogenic lineage that interferes with myogenesis and functional recovery. Overall, our findings suggest that circadian programs within stromal progenitors may represent a fundamental mechanism by which injured tissues coordinate the choice between regenerative repair and maladaptive remodeling.

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# Epigenetic regulation of skeletal muscle FAPs identity

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Chromatin architecture impacts gene expression and defines cell lineage specification. The role of the histone variant H3.3 and its chaperone HIRA, in the gene regulation of somatic cell lineage acquisition remains under investigated. We recently showed that satellite cells lacking HIRA lose myogenic cell identity and fail to repair the muscle. Since HIRA is a ubiquitous protein, we aimed to assess whether the HIRA-H3.3 deposition axis is required to drive the cell fate choices of other lineages. By combining tissue-specific and inducible mouse lines, we addressed the consequences of HIRA loss in fibroadipogenic progenitors (FAPs) in skeletal muscle tissue. While only transcriptomic changes are observed in FAPs lacking HIRA in homeostasis, we found that upon muscle injury, FAP numbers are reduced, leading to an impaired regeneration. Additionally, HIRA-depleted FAPs display increased fibrogenic potential, *i.e.*, increased extracellular matrix deposition resulting in fibrosis *in vivo* and increased fibroblast versus *adipocyte* differentiation *in vitro*. CellChat analysis on scRNA-seq datasets unveiled the WNT signalling pathway activity to be increased in FAPs lacking HIRA. Consistently, we validated *in vitro* and *in vivo*, that inhibition of WNT pathway in *Hira* cKO FAPs restores FAP cell fate choice. In addition to intrinsic defects, HIRA depleted FAPs display a disruptive crosstalk with neighbouring cells, with satellite cell and macrophage numbers being impaired. Taken together, we conclude that HIRA-H3.3 deposition axis sustains FAPs lineage progression during muscle regeneration and safeguards lineage-specific gene expression and cell identity.

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# High-resolution spatial total transcriptomic analysis of skeletal muscle regeneration and disease

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Skeletal muscle development, regeneration, and disease progression are all critically dependent on signaling and transcriptional regulatory circuits coordinated between multiple cell types. While signaling communication between cell types has been well documented, the roles of non-coding RNAs (ncRNAs) as transcriptional regulators remains less examined due in part to a paucity of assay technologies that map their abundance with spatial resolution. Here, we established a new spatial transcriptomics method called STRS-HD to capture both coding and ncRNAs at high spatial resolution and applied it to a mouse model of injury-associated skeletal muscle regeneration. Quantification and spatial mapping of ncRNAs revealed that ncRNAs broadly exhibit spatial correlations within and outside zones of injury, marking ncRNAs as potential regulators of myogenesis. Cell-type inference illustrated that the initiation of injury is marked by fibro-adipogenic progenitor proliferation and immune cell infiltration, both with accumulation in the injury zone. With cell types inferred and mapped, we analyzed cellular communication changes and the influence of ncRNAs on these pathways. On-going work extends STRS-HD to study facioscapulohumeral muscular dystrophy (FSHD) in human skeletal muscle biopsies. This aims to understand how sporadic *DUX4* expression leads to myotoxic effects and FSHD pathogenesis by leveraging STRS-HD data to characterize the differences in ncRNA composition, biological pathways, and ncRNA regulation of *DUX4*-affected myonuclei with spatial resolution. Together, these findings will be the first to exhaustively map ncRNAs in mouse and human skeletal muscle with cellular-scale definition, contributing to a greater understanding of how ncRNAs impact myogenic cell fate and disease.

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# From MyoD Binding to Enhancer Activation: Mechanisms of Enhancer Commissioning During Myogenesis

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The transcription factor MyoD is widely recognized as the master regulator of skeletal muscle differentiation, yet the mechanisms that convert MyoD occupancy into productive enhancer activation remain incompletely understood. While MyoD binds thousands of genomic sites during myogenesis, only a subset acquire the chromatin features and transcriptional activity characteristic of functional enhancers, indicating that additional regulatory mechanisms govern enhancer commissioning.

Our work has identified the H3K4 methyltransferase KMT2D as a critical epigenetic regulator required for activation of the myogenic enhancer landscape. Building upon these findings, we have investigated how MyoD coordinates the recruitment of enhancer regulatory complexes to establish active chromatin. We find that MyoD associates with components of the Mediator complex and that Mediator occupancy is closely linked with enhancer activation and transcription of myogenic target genes. Functional disruption of Mediator impairs activation of the myogenic transcriptional program, supporting a model in which Mediator acts together with KMT2D to establish productive enhancer function downstream of MyoD binding.

These studies support a hierarchical model in which lineage-determining transcription factors recruit multiple classes of epigenetic and transcriptional co-regulators to transform transcription factor occupancy into active enhancers. This work provides new mechanistic insight into how enhancer activation is orchestrated during skeletal muscle differentiation and regeneration and offers a broader framework for understanding lineage-specific gene regulation.

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# Chromatin at the edge: Anchoring muscle homeostasis

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Tissue homeostasis requires stromal cell populations to preserve lineage identity while maintaining accurate cell division over time. In skeletal muscle, fibro-adipogenic progenitors (FAPs) are essential niche cells that support regeneration and tissue integrity, yet the nuclear mechanisms that ensure their functional stability across cell divisions remain unclear. Here, we show that Prdm16 serves as a critical tether for H3K9me2-marked peripheral chromatin in FAPs, a function required to maintain nuclear lamina integrity and mitotic fidelity. Loss of Prdm16 disrupts heterochromatin anchoring and nuclear lamina organization, leading to chromosome mis-segregation, micronuclei formation, and the accumulation of DNA damage during mitosis. Crucially, this defect renders FAP nuclei mechanically unstable and prone to envelope rupture specifically under conditions of injury-induced proliferation, linking chromatin anchoring to nuclear structural resilience during tissue repair. These combined defects ultimately compromise FAP identity and function, driving fibro-adipogenic degeneration and impaired muscle regeneration. Restoration of functional FAPs within the muscle rescues the tissue phenotype, establishing a direct link between progenitor nuclear stability and tissue maintenance. Together, these findings uncover a novel Prdm16-dependent chromatin-tethering mechanism governing nuclear resilience in stromal progenitors, redefining how nuclear architecture coordinates niche stability and skeletal muscle homeostasis.

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# CTCF insulates against inappropriate gene expression to safeguard migratory myogenic progenitor cells

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During embryogenesis, multipotent progenitors present in the dermomyotome compartment of the somite generate multiple lineages including skeletal muscle, dermis and endothelial cells of the vasculature. The transcriptional programs governing these fate choices, coordinated by paired box transcription factor PAX3, are well established. How genome organization contributes to lineage specification remains poorly understood. CTCF, a key architectural protein that maintains chromatin domain boundaries to insulate gene expression, also cooperates with myogenic determination factor MYOD in muscle differentiation. However, the functional requirement for CTCF during cell fate specification *in vivo* has not been defined. Here we show that genetic inactivation of *Ctcf* in *Pax3*-expressing multipotent progenitors disrupts the specification of migratory myogenic progenitors while closely related lineages, such as non-migratory myogenic progenitors and vascular endothelial cells, are spared. Our findings demonstrate a cell-autonomous requirement for CTCF's insulator function in safeguarding migratory myogenic progenitors from inappropriate enhancer-promoter interactions and aberrant gene expression. This model is illustrated by aberrant activation of *Ikaros* in the somitic mesoderm, through neighbouring enhancers normally associated with somite expression, and resulting in impaired myogenic differentiation. Our findings define CTCF as a context-dependent regulator of cell fate specification, ensuring the emergence of the migratory myogenic progenitors of the *Pax3* lineage.

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# The Catalytic Activity of Ezh2 is Required for Mesodermal Lineage Development

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The Polycomb Repressive Complex 2 (PRC2) catalyzes repressive di- and trimethylation of histone H3 lysine 27 (H3K27me2/3) at regulatory regions of developmental genes through its catalytic subunit Ezh2. Much of our understanding of Ezh2 function derives from genetic deletion models; however, interpreting the resulting phenotypes is complicated by the structural interdependence of the PRC2 core subunits. Ezh2 catalytic activity requires assembly with the core components Suz12 and Eed, and complex stability depends on the integrity of each subunit. This mutual interdependence suggests that deletion of *Ezh2* may not only abolish H3K27 methyltransferase activity but also broadly compromise PRC2 integrity- confounding the attribution of observed phenotypes specifically to loss of catalytic function *per se*. This distinction is particularly relevant given emerging evidence that histone-modifying enzymes, including the methyltransferases MLL3/MLL4, the demethylase JMJD2, and Ezh2 itself can exert catalysis-independent functions.

To disentangle the structural and catalytic contributions of PRC2, we generated a knock-in mouse harboring a point mutation in the *Ezh2* SET domain that selectively abolishes H3K27 methyltransferase activity while preserving PRC2 assembly and stability. Given the established roles of Ezh2 in MuSCs and brown adipocytes, and the common developmental origin of these two cell types from Myf5-expressing progenitors, we crossed this knock-in line with *Myf5*-Cre mice to generate compound mutants bearing one conditional nullallele and one catalytically inactive *Ezh2* allele. This genetic strategy enabled direct assessment of the requirement for Ezh2 catalytic activity — independently of PRC2 structural integrity — in the development of skeletal muscle, and brown adipose tissue.

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# Age-related perturbations leading to sarcopenia

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Understanding how gene expression and specific pathways shape tissue aging, muscle mass, and metabolic health is central to developing interventions against age- and obesity-related decline. We present two complementary studies. First, to identify genes and pathways consistently regulated across the rodent lifespan, we generated a high-N, age-related gene-expression atlas spanning 28 tissues in male and female C57BL/6J mice and 32 tissues in male Sprague Dawley rats (>5,000 samples) across multiple ages. We classified genes and pathways by trajectory—changing early, at mid-age, late, or linearly across lifespan. Linear changes dominated many, though not all, tissues, and certain tissues were relatively spared. We examined shared and divergent features of aging across tissues, sexes, and species, yielding a resource for understanding the timing and tissue-, sex-, and species-specificity of age-related change. Second, because myostatin negatively regulates muscle size and is a therapeutic target for preserving muscle, including during GLP1R-agonist-induced weight loss, we quantified the effects of reduced myostatin function in humans. A meta-analysis of ~1.1 million individuals showed that carriers of rare function-disrupting MSTN variants had decreased adiposity and increased lean mass, grip strength, and creatinine. Deep-learning segmentation of Dixon whole-body MRI (UK Biobank; 62,308 individuals) confirmed enlargement across muscle groups, exceeding 10% in heterozygous loss-of-function-like carriers. Structural modeling suggested that stabilizing inactive myostatin conformations mimics partial loss-of-function. Together, these studies illuminate the molecular architecture of tissue aging and the therapeutic potential of myostatin blockade for muscle and metabolic health.

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# Spectral Autofluorescence Resolves Muscle Stem Cell States During Muscle Injury and Aging

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Skeletal muscle stem cells (MuSCs) undergo metabolic reprogramming during muscle regeneration. These metabolic changes can be reflected in corresponding alterations of cellular autofluorescence (AF). Here, we exploit spectral AF, the intrinsic fluorescence of endogenous biomolecules, as a non-invasive, label-free readout of MuSC state. We demonstrate that distinct spectral AF signatures are associated with the dynamic state transitions that occur during muscle regeneration, enabling discrimination of functionally distinct cell states without external labeling. Furthermore, computational modeling of AF profiles generates a continuous atlas of MuSC state transitions, revealing structured trajectories that mirror canonical regenerative progression. Notably, specific AF signals correlate with cellular senescence, positioning spectral AF as a candidate biomarker of aging. Collectively, our findings establish spectral AF profile as a robust readout of MuSC heterogeneity and state dynamics, with potential applications in measuring real-time cellular metabolic flux at a label-free manner.

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# The Muscle Tissue Environment Limits Rejuvenated Muscle Stem Cell Capacity in Aged Mice

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Frailty arising from loss of muscle function and mass is a significant health concern impacting quality of life and dramatically increasing health care costs as our population ages. Ameliorating frailty derived from reduced muscle function is thus a critical research priority to improve health span. Cell intrinsic defects in muscle stem cells occur as skeletal muscle ages, reducing the capacity of muscle stem cells to maintain and repair skeletal muscle and are accompanied by cell nonautonomous changes. Ectopically expressing an inducible FGF Receptor 1 restores muscle stem cells to their youthful numbers, restores self-renewal and a more youthful transcriptome. Although rejuvenating stem cells in aged tissues or organs has potential to improve muscle aging phenotypes, we found that the extracellular environment in aged mice abrogates rejuvenated muscle stem cell potential. Muscle stem cells from young mice were unable to grow on extracellular matrix derived from aged mice that contains elevated collagen protein levels, establishing a critical role for the environment in contributing to muscle phenotypes in aging. Combining an inducible FGF Receptor 1 to rescue muscle stem cell intrinsic aging defects with a drug to reduce fibrosis rescued muscle mass loss in aged mice. We conclude that aging affects tissues, and particularly skeletal muscle tissue, via complex multifactorial processes requiring multifaceted interventions to improve aging phenotypes

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# From membrane stability to regenerative competence in dystrophic muscle

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Duchenne muscular dystrophy (DMD) results from loss of dystrophin and disruption of the dystrophin-glycoprotein complex, yet therapies that restore membrane integrity often produce incomplete functional recovery. Understanding this disconnect requires identifying biological programs that remain coordinated across multiple layers of regulation.

To address this, we developed a computational framework to quantify RNA-protein concordance and applied it across multiple tissues and disease states, including mild and severe DMD, spinal muscular atrophy, and aging muscle. Across these datasets, only a subset of genes exhibited concordant regulation, whereas many were discordant, highlighting the complexity of post-transcriptional regulation. Comparison of mild and severe DMD revealed limited conservation of coordinated biological programs, supporting the concept that severe disease represents a distinct molecular state warranting independent investigation.

We therefore examined sarcospan (SSPN)-mediated rescue in the severe D2-mdx model. SSPN restored membrane stability, improved muscle pathology, and reduced fibrosis. However, despite recovery of membrane adhesion complexes, extracellular matrix coupling remained incomplete because α-dystroglycan glycosylation was not restored. Integrating bulk RNA sequencing with direct measurements of RNA synthesis and degradation revealed selective destabilization of transcripts required for matriglycan biosynthesis, including persistent instability of the early glycosyltransferase POMGNT1. Similar alterations were observed in AAV-microDys treated D2-mdx mice and in human DMD myoblasts.

Together, these findings demonstrate that membrane stabilization alone is insufficient to restore regenerative competence in severe DMD. More broadly, RNA-protein concordance provides a framework for identifying disease-state-specific biology and reveals selective RNA instability as a potential therapeutic barrier limiting recovery of extracellular matrix coupling.

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# The role of resident macrophages in skeletal muscle homeostasis

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Resident macrophages are known to regulate homeostasis in various organs, but their roles in skeletal muscle remain poorly defined. In skeletal muscle, they are localized within the connective tissues. We aimed to identify reliable markers of muscle-resident macrophages at steady-state and during skeletal muscle regeneration.

We used the CX3CR1-GFP mouse to track blood monocyte recruitment. We evaluated, by immunofluorescence, the expression of canonical residency markers (CD169, MHCII, Lyve1, and Tim4) in muscle macrophages. Post-lesional muscle regeneration was studied using a physiological injury model, combined with clodronate-mediated monocyte depletion to isolate the contribution of resident macrophages.

Using histology, we found that, at steady state, macrophages predominantly reside in the epimysium surrounding the muscle. Only 10-15% of resident macrophages expressed CX3CR1-GFP. CD169 was expressed by all resident macrophages, while Lyve-1 and Tim4 were expressed by 75 and 50 %, respectively. Following injury, recruited macrophages rapidly expressed CD169 and MHCII, indicating a rapid acquisition of residency markers. Under monocyte depletion, resident macrophages expanded, with 60% more macrophages in severely versus mildly injured muscle

We showed that blood monocytes contribute to resident macrophage population in skeletal muscle both at steady state and during regeneration. Current work investigates their inflammatory profile and function to define their contributions to homeostasis and repair.

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# Driving rhabdomyosarcoma into myogenic differentiation

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Rhabdomyosarcoma is the most common pediatric soft tissue tumor comprising two major subtypes, the alveolar subtype subtype being positive for PAX3/7-FOXO1 and the embryonal being negative for PAX3/7-FOXO1. The current research project focuses on induction of myogenic differentiation especially in embryonal rhabdomyosarcoma. Therefore, we generated reporter cell lines for different human rhabdomyosarcoma cell lines with which we can follow the induction of myogenic differentiation. Next, we performed a screen for factors which induce myogenic differentiation. We identified E2F3 and KIF14 to be upregulated in embryonal rhabdomyosarcoma cells. Of note, reduction of either E2F3 or KIF14 induced myogenic differentiation suggesting that they are therapeutic targets for treatment of embryonal rhabdomyosarcoma. Currently, we are deciphering the signaling pathways through which aberrant KIF14 expression in embryonal rhabdomyosarcoma is causing the impaired myogenic differentiation in embryonal rhabdomyosarcoma.

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# Targeting Cachexia in Pancreatic Cancer

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Cancer cachexia is a devastating systemic syndrome that drives morbidity, limits therapeutic response, and contributes substantially to mortality in pancreatic ductal adenocarcinoma (PDAC) patients, yet the tumor-derived factors that initiate tissue dysfunction remain poorly defined. Here, we identify serum amyloid A1 (SAA1) as a key tumor-secreted mediator that links PDAC progression to cachexia-associated muscle dysfunction. Across independent patient cohorts, PDAC tumors exhibited marked SAA1 expression, and elevated SAA1 levels were associated with sarcopenia and systemic inflammation. In a syngeneic orthotopic PDAC mouse model, rising circulating SAA1 preceded overt cachexia and coincided with early muscle dysfunction and depletion of muscle stem cells (MuSC). Mechanistically, tumor-derived SAA1 activated Toll-like receptor 4 (TLR4) signaling in skeletal muscle, driving both myofiber atrophy and MuSC depletion through inflammatory and metabolic reprogramming. Single-cell and spatial transcriptomic analyses further uncovered extensive immune and stromal remodeling downstream of SAA1/TLR4 signaling, establishing this pathway as a regulator of cachexia-associated muscle dysfunction. Therapeutically, pharmacologic inhibition of TLR4 attenuated muscle wasting and restored muscle performance and MuSC abundance in cachectic PDAC-bearing mice. Together, these findings define a previously unrecognized tumor-to-host signaling axis in which PDAC-derived SAA1 drives muscle dysfunction and cachexia, revealing the SAA1/TLR4 axis as a therapeutic vulnerability in PDAC cachexia.

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# From skeletal muscle regeneration and aging to ALS… and back again: revisiting ALS

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Skeletal muscle regeneration is a highly coordinated process essential for preserving tissue integrity after injury or stress. This process is primarily mediated by satellite cells (SCs), quiescent muscle stem cells that activate, proliferate, and differentiate into myoblasts to repair damaged myofibres. However, aging profoundly impairs SC function altering commitment from stem-like to primed states, ultimately contributing to sarcopenia (muscle mass/strength loss). Similarly, amyotrophic lateral sclerosis (ALS), traditionally considered a motor neuron disease, also presents intrinsic muscle pathologies that resemble accelerated muscular aging.

We have demonstrated that ALS muscle exhibits impaired myoblast fusion, defective trophic signalling, and disrupted glycolytic metabolism, all of which exacerbate muscle wasting. ALS-derived myotubes display abnormal morphology and reduced differentiation capacity, while neuromuscular junctions (NMJs), the synapsis allowing for muscle contraction, undergo early fragmentation, preceding denervation. These findings support the concept that skeletal muscle is not merely a passive target in ALS, but an active contributor to disease progression. In this context, we identified kranocytes as novel players in NMJ maintenance and injury response, acting as stress sensors whose degeneration parallels disease progression.

In addition, we identified TDP-43 aggregates in skeletal muscle from ALS patients while nuclear expression remained preserved. Given the role of TDP-43 in muscle differentiation and regeneration, we investigated its deletion in SCs, revealing a marked inability to regenerate after cardiotoxin injury and the development of a premature stem cell aging phenotype. Together, these findings reframe ALS as a neuromuscular disease and highlight new therapeutic opportunities to restore muscle regeneration and delay disease progression.

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# Ctcf deficiency in myofibers induces pathological genome reprogramming toward development of idiopathic myopathy

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Maintenance of cell identity in perennial, postmitotic tissues, such as myofibers, and preservation of their transcriptional competence to properly respond to homeostatic perturbations through adult life, require long-term integrity of the 3D genome organization. A key player in this process is the CTCF protein, an genomic architectural protein. Idiopathic autoimmune myositis (IAM) is characterized by the inflammation of muscles of unknown etiology, accompanied by muscle atrophy. Exome sequencing of patients has revealed recurrent mutations in CTCF gene. Using a mouse model with constitutive deletion of CTCF in myofibers (*Ctcf*$^{mKO}$), we observed that the mice were born with no evident phenotype, but they developed a progressive myofiber atrophy, with features reminiscent of myopathies. *Ctcf*$^{mKO}$ mice myofibers exhibited dynamic alterations in chromatin accessibility and interactions between gene promoters and distal regulatory elements, resulting in patterns of dysregulated gene expression. Specifically, myofibers exhibited downregulation of muscle contractile, structural, metabolic and NMJ-related genes while presenting increased expression of catabolic genes and an ectopic activation of inflammatory genes. These alterations lead to atrophic myofibers accumulation that exhibited histological features, transcriptional and proteomic signatures resembling those observed in patients affected by idiopathic myopathies. These findings highlight the role of CTCF and the maintenance of 3D chromatin conformation for the regulation of gene expression in post-mitotic myofibers. It is particularly pertinent considering the established associations between CTCF dysregulation and various muscle diseases, including IAM, sarcopenia, and cachexia. This observation emphasizes the potential for therapeutic interventions targeting this novel common aspect of muscle disease.

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# Reconstructing human skeletal muscle in vivo and in vitro

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Skeletal muscles and vertebrae derive from precursors located in the embryonic segments called somites. These structures form periodically from a posterior tissue called Presomitic Mesoderm (PSM). The rhythmic formation of somites involves a molecular oscillator called segmentation clock which drives pulses of Notch, Wnt and FGF signaling in the PSM. Virtually nothing is known on human somitogenesis as it proceeds between 3- and 5-weeks post conception when embryos are extremely difficult to access. We have developed protocols to differentiate human pluripotent stem cells (ES/iPS) *in vitro* into PSM. Single cell RNA-sequencing comparison of these human cells differentiating in vitro with mouse embryo PSM reveals that they faithfully recapitulate the PSM differentiation sequence in vitro. Using our in vitro system as a proxy for human somitogenesis, we were able to demonstrate that human iPS reporter cells harboring a HES7 fluorescent reporter differentiated to PSM exhibit 5-hour oscillations, thus identifying the human segmentation clock. We have also succeeded in generating PSM organoids that can sequentially form somites exhibiting a normal antero-posterior pattern in vitro. By mimicking key signaling events leading to muscle formation in the embryo, we developed directed differentiation protocols which recapitulate the developmental sequence of myogenesis. We then used these cells to generate new in vitro models of Duchenne Muscular Dystrophy and to pioneer the production of human satellite cells for cell therapy strategies for muscular dystrophies. Our work provides a framework to study early stages of human myogenesis which are poorly accessible in the embryo.

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# Advanced modelling of congenital muscle disorders to improve translation of neuromuscular (gene) therapies

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LMNA-related congenital muscular dystrophy (L-CMD) is one of the most severe muscle disorders, caused by pathogenic variants in *LMNA*, which encodes key components of the nuclear lamina that regulate nuclear architecture, chromatin organisation and gene expression. Progress towards effective therapies has been limited by the lack of human-relevant models that faithfully recapitulate disease pathology. To address this challenge, we developed a suite of humanised skeletal muscle models ranging from patient-derived induced pluripotent stem cell (iPSC)-based systems to complex multicellular 3D engineered muscle tissues.

Using transgene-free myogenic differentiation of L-CMD patient iPSCs, we demonstrated robust recapitulation of disease-associated phenotypes across multiple *LMNA* variants, including dysmorphic nuclei, nuclear envelope protein mislocalisation and transcriptomic alterations, despite largely preserved developmental myogenesis. These models enabled therapeutic evaluation of gene-editing approaches, revealing that precise correction of an *LMNA* mutation fully restored nuclear morphology and normalised a pro-inflammatory transcriptional signature, whereas exon-removal strategies produced stable Lamin A/C isoforms but failed to substantially rescue disease phenotypes.

Building on these findings, we expanded this approach to model and study several other congenital myopathies and muscular dystrophies with defects affecting distinct muscle tissue compartments. Finally, we demonstrate the application of this technology for the development and preclinical evaluation of clinically relevant next-generation genetic therapies, including assessment of viral vector specificity and efficacy, establishing a versatile human platform for precision medicine in neuromuscular diseases.

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# Bioprinting complex human tissues

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3D bioprinting can create living human tissues on demand based on specifications contained in a digital file. One of the benefits of using this additive manufacturing technique is the ability to generate complex constructs containing a variety of cells that mimic structures found in the human body. Such highly customized, physiologically relevant 3D human tissue models can screen potential drug candidates as an alternative to expensive pre-clinical animal testing as well as hold the potential to regenerate diseased and damaged tissues. Over the past few years, the Willerth lab has been a leader in the 3D bioprinting space. The Willerth lab has developed a novel fibrin-based bioink called TissuePrint for bioprinting human tissues that has been commercialized through her award-winning spin-off company - Axolotl Biosciences. We have developed several tissue models using TissuePrint, including constructs derived from human induced pluripotent stem cells (hiPSCs), which can become any cell type found in the body. More recent work has focused on developing our “smart” bioinks that contain drug releasing microspheres produced using microfluidics as a way to improve the reproducibility of the bioprinting process. Using our novel, patent-pending BrainPrint bioink, we have generated tissues with chemical and electrical properties similar to that of the brain and spinal cord.

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# Development of a universal cell therapy for skeletal muscle

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Several groups have developed advanced protocols for efficiently differentiating human pluripotent stem cells (hPSCs) into potent skeletal muscle progenitor cells (SMPCs). hPSC-SMPCs are an attractive source of cells for muscle replacement therapy, with the potential capacity to generate new myofibers and replenish the muscle stem cell niche *in vivo*. However, translating hPSCs-SMPCs from bench to bedside remains a major challenge especially regarding overcoming immune rejection.

One groundbreaking technology cloaks the cells from the immune system, bypassing the need for immunosuppression. The immune cloaking property is achieved through the overexpression of 8 genes in hPSCs, which allows evasion from innate and adaptative immune cells. This overexpression prevents the immune system from recognizing the cell as foreign and generate induced allogenic cell tolerance (iACT) stealth hPSCs.

Taking advantage of the new iACT stealth technology, we have investigated the therapeutic potential of iACT-SMPCs. Using different directed differentiation protocols, we showed that the overexpression of immunomodulatory genes in iACT hPSC does not prevent their differentiation into SMPCs. *In vitro* and *in vivo* myogenesis confirmed ability of iACT-SMPCs to fuse into myotubes and myofibers. *In vitro* coculture with human primary NK cells demonstrated robust cytotoxicity toward WT cells, whereas iACT-SMPCs showed resistance to NK cell-mediated killing. Finally, engraftment in humanized mouse muscles showed immune-shielding capacity of iACT-SMPCs *in vivo*, while maintaining myogenic potency. Overall, our results provide a proof-of-concept for the use of an immune-shielding SMPCs as a universal donor for cell therapy, a strategy that could transform the landscape of future muscle therapies.

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# A role for regeneration in the initiation of FSHD muscle pathology as revealed by innate immune cell/FSHD patient muscle xenograft model.

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We have investigated the role of innate immunity in FSHD muscle pathology using a novel human innate immune-FSHD muscle xenograft mouse model. An immune-compromised host NSG-SGM3 mouse strain has been engineered to selectively expand human innate immune cell lineages and allows engraftment of umbilical cord blood (UCB)-derived hematopoietic stem cells (HSCs) and patient-derived FSHD (or unaffected patient control) muscle stem cells into the mouse tibialis anterior muscle. FSHD muscle xenografts preferentially accumulated human macrophages and B cells and expressed activators of both the classical and alternative complement pathways and C3 complement, as also observed in FSHD patient muscle biopsies. Significantly, FSHD muscle xenografts were deficient in the expression of embryonic isoforms of Myosin Heavy Chain and Light Chain 2 as well as DUX4, the toxic FSHD disease gene, but expressed adult Myosin Heavy Chain isoforms. These findings support a hypothesis that the expression of toxic DUX4 by regenerating muscle fibers expressing embryonic genes activates C3 complement and macrophage expansion, leading to destruction of DUX4-expressing regenerating muscle fibers by macrophage engulfment. Experiments are in progress to further investigate the roles of C3 complement, DUX4 and regeneration in FSHD muscle pathology using the human innate immune-FSHD muscle xenograft mouse experimental model and FSHD patient muscle biopsies.

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# Large scale muscle injury activates a systemic repair program: a new model for therapeutic development

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Although skeletal muscle is highly regenerative, volumetric muscle loss (VML) caused by orthopedic trauma frequently results in permanent functional deficits, disability, and limb loss. Current clinical treatments are limited and do not adequately restore muscle tissue or strength. We hypothesized that highly regenerative organisms, such as zebrafish, activate distinct regenerative programs in response to different magnitudes of muscle injury. To test this, we developed a transgenic zebrafish model that induces systemic skeletal muscle injury throughout the organism. Remarkably, zebrafish are capable of fully repairing this large-scale muscle damage. Transcriptomic analyses revealed that small- and large-scale injuries activate distinct regenerative programs. Mechanistically, Heparin-binding epidermal growth factor (Hb-EGF) and Macrophage inhibitory factor (MIF) are induced in the epidermis following systemic muscle injury and are required for large-scale muscle regeneration, suggesting that distant tissues coordinate repair beyond the local muscle stem cell niche. To translate these findings toward therapy, we developed an implantable biomatrix that provides sustained Hb-EGF release over one week. Ongoing studies are evaluating whether Hb-EGF delivery improves regeneration and functional recovery following VML in mice. Together, these studies identify systemic regenerative signaling mechanisms in vertebrate muscle repair and establish a foundation for developing innovative therapies to treat large-scale muscle injuries.

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# Pelvic floor muscle stem cells drive protective adaptations during pregnancy

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Pelvic floor muscle (PFM) trauma from parturition-associated strains predisposes women to pelvic floor dysfunction. However, not all women who deliver vaginally sustain PFM birth injury. The differential extent of pregnancy-induced protective PFM adaptations, sarcomerogenesis and myofiber elongation, identified in preclinical model, could be responsible for clinically observed differences. Muscle stem cells (MuSCs) drive sarcomerogenesis in limb muscles, but their role in pregnancy-induced adaptations of PFMs is unknown, constituting the current study's objective.

Pubocaudalis (PCa, pelvic) and tibialis anterior (TA, limb) muscles from nonpregnant and pregnant Sprague-Dawley rats and Pax7-CreERT2-tdTomato mice were used for analyses.

Significant increase in MuSC proliferation ($Pax7^+/EdU^+$) and differentiation ($myogenin^+$) was noted in PCa of pregnant compared to nonpregnant rats and mice, indicating MuSC activation and myogenic lineage progression during pregnancy. Consistently, myonuclei isolated from PCa of pregnant animals showed increased H19 and Igf2 expression and increased amount of $EdU^+$ nuclei within myofibers were present in PCa tissue slices in pregnancy, suggesting MuSC fusion. Pax7-CreERT2-tdTomato mice were employed to validate this finding. The number of $td\text{-}tomato^+$ myofibers, derived from $td\text{-}Tomato^+$ MuSCs, was significantly increased in PCa of pregnant compared to nonpregnant mice. None of these differences were present in TA in either species. Using scRNA sequencing analysis, validated through western blots, we identified plasminogen pathway, through phospho-ERK activation, to be significantly upregulated in PCa, but not in TA, of pregnant compared to nonpregnant animals.

During pregnancy, activation of the plasminogen system induces MuSC activation and fusion with the existing myofibers, enabling protective adaptations observed in PFMs.

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# Stem cells age when they lose their shape

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Aging is associated with progressive morphological decay and loss of biological order across tissues and organisms, yet at the cellular level, how these changes contribute to stem cell functional decline is not fully understood. Here, using muscle stem cells (MuSCs) as a model, we found that the transmembrane protein Betaglycan is markedly reduced during aging. Furthermore, we show that Betaglycan preserves protrusion architecture and deep quiescence of MuSCs. Importantly, inducible-knockout of Betaglycan in young MuSCs recapitulates key features of aging, including premature activation, impaired regeneration, protrusion retraction and a rounded, less structured morphological state. Mechanistically, Betaglycan recruits β-arrestin2, enabling cell-membrane localization of dephosphorylated Cofilin, which severs actin filaments and promotes protrusion. In aged MuSCs, the deregulated Betaglycan-β-arrestin2-Cofilin axis leads to protrusion retraction and loss of deep quiescence. Together, our findings identify Betaglycan as a key regulator of MuSC morphology and function, suggesting loss of morphological complexity as a feature of stem cell aging.

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# Loss of muscle identity and affected myonuclear differentiation lead to muscle fiber splitting

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Muscle fiber identity programs underlie the diversity of body wall muscles in the Drosophila embryos. We previously identified homeodomain transcription factor Lateral Muscle Scarcer (Lms) that sets the identity of lateral transverse muscles (LTs) and reported that its loss could lead to LT split phenotype. Negative myoblast fusion regulator Gelsolin (Gel) that acts downstream of Lms prevents LT muscles from splitting. Here, we follow LT muscle fiber splitting in vivo and observe that it is initiated during early phase of multinucleated myotube formation with a subset of myonuclei that express repressors of myogenic differentiation Twist and Him. Inhibition of Twist (and Him) is a prerequisite of myogenic differentiation and formation of functional muscle fibers. Consistent with this notion maintenance of Twi in developing LT myotubes interfere with differentiation and, as we show here, promotes their splitting. This indicates that affected myogenic differentiation of a subset of myonuclei within a developing myotube could be at the origin of split phenotype.

Split muscle fibers are hallmarks of aging muscle and muscular diseases including Duchenne (DMD) and Steinert (DM1) muscular dystrophies. We previously reported LT muscle splitting in Drosophila DM1 models. Here we show that it occurs, like in *lms* mutants, during early multinucleated LT myotube formation and involves affected myonuclear differentiation.

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# Acute and chronic damage of skeletal muscle after alphavirus infection

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Ross River (RRV) and Chikungunya (CHIKV) viruses are emerging pathogens that target the musculoskeletal system and a major health concern as they cause explosive outbreaks in humans. RRV and CHIKV are mosquito-borne enveloped RNA viruses that cause severe arthritis and myopathy that typically resolves within weeks of infection, although a significant fraction of RRV and CHIKV patients develop debilitating chronic disease. While the etiology of virally-induced arthritis has been intensely studied, the mechanisms underlying myopathy are poorly understood. Using mouse models, we show that these viruses abrogate weight gain and impair overall maximal workload capacity during treadmill running. The viruses readily infect skeletal myofibers and muscle stem cells. Interestingly, there is great heterogeneity in the effects of viral infection on different skeletal muscles, myofibers, and stem cells. Surprisingly some myofibers and stem cells survive infection, but infected myofibers and stem-cell derived regenerated myofibers are consistently smaller. We also find that a continued elevated immune response is a key difference between muscle recovering after viral infection versus after sterile injury.

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# Spatial transcriptomics and niche establishment by engrafted human muscle across time and disease

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Skeletal muscle cell therapies are limited by poor long-term engraftment, particularly in diseased muscle, where retention of a PAX7+ stem cell pool depends on the formation of a supportive niche. Here, we used spatial transcriptomics to map niche development across engraftment time in healthy and dystrophic in vivo micro-environments, and after secondary injury. We found engrafted myogenic cells underwent rapid early transcriptional remodeling and substantial donor-cell loss, whereas regenerating myofibers acquired niche-associated programs that stabilized surviving PAX7+ cells. In dystrophic muscle, niche maturation was impaired, donor-cell states were altered, and regenerative adaptation after secondary injury was blunted.

Through spatial transcriptomics, MEGF10 emerged as an early niche-associated regulator that was transiently expressed during healthy regeneration but persisted in dystrophic muscle. Using CRISPR, we engineered a tetracycline-inducible system in hPSCs to temporally control MEGF10 gene expression and found its induction in vivo early after transplantation more than doubled PAX7+ cell retention and donor-derived myofiber output. In contrast, MEGF10 loss of function, which is associated with the early-onset myopathic disease, EMARDD, failed to maintain engrafted PAX7+ cells. Spatial profiling and functional studies with Tet-On engraftments identified a MEGF10-responsive regenerative myofiber programs linked to early niche support.

These findings demonstrate that the transplanted myogenic niche changes dynamically over time, such that receptor programs that are beneficial during early regeneration may become ineffective or maladaptive if sustained at later stages. We identified an early therapeutic window to boost transplantation via targeted manipulation of niche development that in turn may improve regenerative outcomes in muscular dystrophy.

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# Building muscle: Mechanisms of myoblast fusion and myonuclear function

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Skeletal myofibers acquire nuclei through fusion with activated myogenic progenitors, and this multinucleation is established as a key determinant of muscle size and function. However, the molecular circuitry linking myonuclei to growth, and why myofibers accrue additional nuclei, has remained unclear. We found that functional growth is still possible after restriction of nuclear content in myofibers, and that this was associated with elevated levels of RNA Polymerase II (Polr2a) and increased mRNA content. Using a genetic mouse model in which endogenous Polr2a is upregulated specifically in myofibers, we demonstrated that increased transcriptional output is sufficient to drive functional myofiber growth, and notably, that Polr2a overexpression curtails accretion of additional nuclei. These findings reveal transcriptional output per nucleus as a previously underappreciated contributor of muscle growth. Building on this, we asked whether myonuclear number itself is actively regulated over time, since the magnitude of fusion-mediated nuclear addition and whether nuclei are also lost from this syncytial cell is highly debated. Using a lineage tracing strategy to quantify myonuclear accretion at nuclear-level resolution in fast and slow muscles across the mouse lifespan, we found that despite persistent accrual of new myonuclei, total nuclear content does not increase with age indicating myonuclear loss, which is most evident in slow, oxidative soleus myofibers. Together, these studies show that myofiber nuclear content is dynamically calibrated through both accrual and loss, and that transcriptional output from existing nuclei is a key, targetable lever for muscle growth, with implications for combating muscle wasting conditions.

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# Protein-protein interactions regulating proliferation vs. differentiation in muscle satellite cells

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Syndecan-4 has no intrinsic signaling activity of its own, but is necessary for coordination and transmission of signals from multiple integrins, receptor tyrosine kinases, and G protein-coupled receptors. In the context of satellite cells, syndecan-4 appears to be critical for activation, proliferation, migration, and differentiation, but how it promotes these disparate activities is not fully understood. By leveraging domain mutants re-expressed in primary satellite cells lacking syndecan-4 we are working to understand what interactions of syndecan-4 with which other proteins are necessary in each of these activities.

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# Multifaceted role of p53 in the establishment and maintenance of skeletal muscle identity

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The tumor suppressor p53, also known as “the guardian of the genome” is a master regulator of cell proliferation, and its dysregulation in cancer is well documented. However, p53 has also evolved differentiation regulation functions that are independent of its role in cell proliferation and genome integrity, and which are beginning to be appreciated. We have previously shown that tight regulation of p53 protein levels is associated with cell fate determination during the cell cycle. Here I will present and discuss our recent data highlighting the importance of p53 in postnatal muscle regeneration. Using muscle stem cell (MuSC)-specific p53 knockout mice (*Pax7*$^{CreER}$*;Trp53*$^{flox}$) we have identified a role for p53 in regulating MuSC biology at multiple levels, including preservation of genome integrity, prevention of uncontrolled proliferation and especially promotion of differentiation as well as maintenance of a healthy differentiated state. The latter is strikingly associated with regulation of mitochondrial function and of the balance between glucose and lipid metabolism. Our data show that the ultimate result of losing p53 in adult MuSCs is not cancer, but defective regenerated myofibers, which show impaired mitochondrial function and structural decay.

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# Distinct functions of Notch signaling in muscle satellite cells and myofibers

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The Notch signaling pathway is evolutionarily conserved and regulates stem cell function through cell-cell interactions during tissue development and regeneration. We previously reported that muscle satellite cells (MuSCs) express Notch1 and Notch2, which coordinately maintain the quiescent and undifferentiated states of MuSCs and are essential for muscle regeneration in adult mice. Moreover, we demonstrated that Notch2 is expressed in fully differentiated myofibers and acts as a key mediator of mechanical unloading- or hyperglycemia-induced muscle atrophy through a γ-secretase-dependent, but RBP-J-independent, pathway activated by vascular endothelial cell (EC)-derived soluble Dll4 (sDll4). Here, we show that sDll4 is upregulated and released from ECs in a soluble full-length form in aged muscle. sDll4 promotes age-related sarcopenia through activation of Notch2. Notably, sDll4 selectively binds the extracellular domain of Notch2, but not those of Notch1 or Notch3, and collagen I functions as a scaffold that facilitates the interaction between sDll4 and Notch2. While immobilized Dll4 activates Notch signaling in both MuSCs and myofibers, sDll4 has no effect on MuSCs. Consistently, prolonged treatment with a Dll4-blocking antibody improved muscle mass and strength in aged mice without affecting MuSC numbers. Furthermore, analyses of human and mouse cohorts demonstrated that renal dysfunction increases circulating sDll4 levels, leading to muscle atrophy and implicating a kidney-vascular-muscle axis in the pathogenesis of sarcopenia. Collectively, these findings indicate that endothelium-derived sDll4 is a key driver of muscle aging, revealing systemic and local microenvironmental mechanisms underlying muscle wasting diseases.

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# Regulation of Fibrosis by Human Antigen R in Fibro-Adipogenic Progenitors after Skeletal Muscle Injury

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Skeletal muscle is a unique tissue composed of a constellation of cell types that coordinates all voluntary movements and adapts to modify its structure and function. Pathological fibrosis is the primary barrier to functional recovery after irrecoverable volumetric muscle loss (VML), yet few anti-fibrotic therapies exist for skeletal muscle. One of the primary cell types responsible for fibrotic scarring after VML is fibro-adipogenic progenitors (FAPs), interstitial mesenchymal cells that can expand and differentiate into fibroblasts and myofibroblasts following trauma. The post-transcriptional regulation of FAPs remains virtually unexplored, particularly regarding RNA-binding proteins (RBPs) that control mRNA splicing, localization, stability, and translation. We have profiled an RBP known as human antigen R (HuR), a ubiquitously expressed post-transcriptional regulator that primarily stabilizes and promotes the translation of target mRNAs. We have shown that as FAPs become fibrogenic and increase extracellular matrix (ECM) deposition and pro-fibrotic gene expression, HuR exhibits hallmark nuclear-cytoplasmic translocation. Under this mechanism, integrated RNA-sequencing, Ribo-sequencing, and ARTR-sequencing for profiling RBP-RNA interactions revealed increased HuR binding to ECM-associated and pro-fibrotic transcripts crucial to FAP fibrotic differentiation. Results suggest that HuR-mRNA binding in FAPs promotes differentiation towards myofibroblasts, a pattern we've identified as disruptible with in vitro treatment by HuR small-molecule inhibitor KH-3. Here, we present HuR as a driver of FAP-mediated fibrosis, and demonstrate its inhibition attenuates fibrotic FAP differentiation. Defining how HuR shapes transcript structure, diversity, and lifecycle to influence FAP fate establishes a new therapeutic paradigm potentially applicable to a wide range of fibrotic conditions.

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# Defective autophagy in facioscapulohumeral muscular dystrophy (FSHD) skeletal muscle provides a viable target for therapeutic intervention

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Autophagy is a key cellular pathway for the turnover of larger cellular components and organelles. The perturbation of autophagy is implicated in many pathological conditions. Facioscapulohumeral muscular dystrophy (FSHD) is characterised by the slowly progressive weakness and wasting of specific skeletal muscle groups. Ectopic expression of the transcription factor DUX4 underlies FSHD pathology, in part, by affecting many metabolic and homeostatic cellular pathways in muscle.

We have examined autophagy in FSHD and found that myotubes fail to respond to environmental stimuli that normally stimulate autophagy, increasing apoptosis. Autophagy can still be activated in FSHD myotubes however, since pre-treating FSHD myotubes with an activator of the ULK1 complex, that initiates autophagy, protects FSHD myotubes from apoptosis. Moreover, prolonged low-level activation of autophagy also enhances myogenesis and myogenic differentiation. Finally, we show that the autophagy and metabolic regulating compound Metformin, the first-line anti-diabetic drug, can enhance muscle function *in vivo* in a mouse model of FSHD. In summary, defective autophagy contributes to FSHD pathology and this pathway is a viable therapeutic target.

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# Pharmacological restoration of muscle stem cell health and function in FSHD

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Therapeutics in development for FSHD primarily target the causal *DUX4* gene in myofibers. However, DUX4 is rarely detected in FSHD muscle, rendering it a temporally elusive therapeutic target. Further, as a pioneer transcription factor, brief expression of DUX4 in muscle stem cells (MuSCs) may be sufficient to trigger lasting epigenetic changes that can impair their capacity to differentiate into healthy myofibers. Therefore, restoration of muscle regenerative capacity in FSHD will likely require a resolution of the damage caused by DUX4 in muscle progenitors. We have identified a first-in-class small molecule that can restore myogenic potential and decrease apoptosis in differentiating FSHD patient myoblasts, as well as inhibit DUX4 target genes. Specifically, this compound resolves the PAS/DAS ratio of *DUX4* transcripts, reduces oxidative stress, and promotes healthy differentiation. In mouse myoblasts with DUX4 induced, our compound increases expression myogenic genes, improves healthy myofiber maturation, and reduces cell death, outperforming the Phase3 FSHD drug, Losmapimod. This compound is orally available, increasing Pax7 and decreasing *Atrogin1* in limb and diaphragm muscles in old mice. Independent testing revealed increased muscle force in a mouse FSHD model after 28 days of treatment. Together, these studies implicate our small molecule as a potential therapeutic for resolving DUX4 damage and restoring myogenic potential in MuSC to enhance muscle regeneration in FSHD and likely other muscular dystrophies.

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# MuSK-BMP signaling in adult muscle stem cells maintains quiescence and regulates myofiber size and muscle strength

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The signaling pathways underpinning the balance of satellite cell (SC) quiescence and activation are incompletely understood. MuSK is a BMP co-receptor that binds BMPs via its Ig3 domain. snRNAseq revealed that MuSK transcripts are expressed in ~30% of quiescent SCs and that this subpopulation is in a state of shallow quiescence. MuSK protein is localized to SCs on isolated myofibers. To test the role of the MuSK-BMP pathway in SC quiescence we examined muscle growth in both body-wide and SC-conditional mutants lacking the MuSK Ig3 domain (ΔIg3-MuSK), which reduces BMP signaling. SC and myonuclei numbers as well as myofiber size were comparable to WT at 3 months of age. However, by 5 months myofiber size, myonuclear number and grip strength were increased - indicating that SCs had activated and productively fused into myofibers over this interval. Myonuclear domain size was maintained. Transcriptomic analysis showed that SCs from uninjured ΔIg3-MuSK mice exhibit signatures of activation. Damage experiments showed that ΔIg3-MuSK SCs maintain full stem cell function and that regeneration is accelerated. Conditional ΔIg3-MuSK expression in SCs in 3-month-old animals was sufficient to break quiescence and increase myofiber size and grip strength. Splice-modifying ASOs that create ΔIg3-MuSK activate quiescent SCs in WT mice in vivo. We conclude that the MuSK-BMP pathway regulates SC quiescence and myofiber size in a cell autonomous, age-dependent manner. Targeting MuSK-BMP signaling in muscle stem cells thus emerges as a therapeutic strategy for promoting muscle growth and function in settings such as disease, muscle atrophy, and aging.

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# Beyond myogenesis: unexpected multipotency of postnatal Pax7 cells uncovers an additional layer of muscle assembly..

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The core function of mesodermal Pax7 cells is to generate myofibers and muscle stem cells. During embryogenesis, however, somitic Pax7 cells can also give rise to minor non-muscle populations, including dermis and adipocytes. We asked whether these cells retain such multipotency during early postnatal growth of limb muscles. Using lineage tracing, we uncovered unexpected plasticity at early postnatal days, leading to the generation of multiple non-myogenic lineages, including a previously unrecognized Pax7-derived subpopulation of fibro-adipogenic progenitors that we termed Pax7$^{FAPs}$. The mechanisms governing this fate switch, as well as the long-term fate of Pax7$^{FAPs}$ during adult muscle homeostasis and regeneration, will be discussed. Together, our findings reveal an unsuspected layer of multipotency in postnatal Pax7+ cells, broadening our understanding of cellular contributions during postnatal muscle development and regeneration.

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# Distinctive regenerative properties of extraocular muscle stem cells and their niche

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Extra-ocular muscles (EOMs) possess remarkable regenerative capacity and remain selectively spared in Duchenne muscular dystrophy (DMD), yet the cellular and molecular mechanisms underlying these unique properties are largely unknown. Our work aims to uncover the biological basis of EOM resilience by dissecting the interplay between the muscle stem cell niche and its surrounding environment. We demonstrate that EOM FAPs differ from their limb counterparts in both their transcriptional identity and functional properties. Using scRNAseq, we identified CCL11 as a novel FAP-derived signal that promotes muscle stem cell (MuSC) proliferation via CCR5 signaling, maintaining a robust regenerative stem cell pool specifically in EOMs. Beyond cellular crosstalk, we further show that the extracellular matrix of EOMs is compositionally distinct from limb muscle, with differences that directly influence MuSC behaviour, adding an additional layer to the EOM-specific regenerative niche. Finally, to investigate EOM regeneration in vivo, we developed the first EOM injury model enabling precise tracking of regenerative kinetics. EOMs regenerate significantly more efficiently than tibialis anterior muscles, exhibiting rapid clearance of necrotic fibers and a markedly higher proportion of newly regenerated fibers. Strikingly, DMD EOMs still outperform dystrophic limb muscles in regenerative capacity, though they display a decline compared to wild-type, suggesting partial susceptibility to dystrophic pathology. Together, this work establishes FAP-MuSC crosstalk as a key regulator of EOM regeneration, identifies CCL11 as a novel pro-regenerative factor, and provides new insights into the exceptional resilience of EOMs.

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# Developing Regenerative Therapeutics for Neuromuscular Disease

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Loss of dystrophin causes Duchenne Muscular Dystrophy (DMD), a neuromuscular disease characterized by muscle fragility, wasting, and muscle stem cell (MuSC) impairment. Dystrophin is expressed in activated MuSC to establish apical-basal polarity. In the absence of dystrophin, MuSC undergo reduced asymmetric division resulting in stem cell hyperplasia and insufficient numbers of progenitor cells to repair ongoing muscle damage. Thus, this deficiency contributes to the progressive muscle loss in DMD. Using an *in situ* MuSC screening platform, we identified Adapter Associated Kinase 1 (AAK1) as a potent modulator of asymmetric division. Inhibition of AAK1 promotes rescue of asymmetric MuSC divisions by stimulating increased levels of membrane localized NUMB, resulting in the robust production of progenitors *in vitro* and *in vivo*. Examination of *mdx* mouse embryos lacking dystrophin reveals no impairment of the primary myogenic program. By contrast, histological and single cell RNA-sequencing analysis during secondary myogenesis uncovers an increase in the proportion of fetal MuSCs that exhibit reduced NUMB and PARD3 polarization, and a marked reduction in myogenic progenitors and myocytes, leading to fewer smaller-caliber myofibers. Deletion of AAK1 rescued polarization of NUMB and myogenic progenitor generation in *mdx* fetal muscle. Satellos has developed a small molecule inhibitor of AAK1. SAT-3247 is efficacious in both mouse and dog pre-clinical models of DMD and has recently completed a phase 1a/b clinical trial in healthy human volunteers and adult Duchenne patients. An open label clinical trial for older DMD patients and a placebo controlled paediatric trial have been initiated.

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# Poster Abstracts (in alphabetical order)

## Selective mTORC1 Signaling in Skeletal Muscle

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Aging is characterized by a decline in physiological function throughout the body, including loss of skeletal muscle mass. The size of a tissue reflects its ability to build or degrade cellular mass, via activation of anabolism or catabolism, respectively. These processes are regulated by the mTORC1 (mechanistic Target of Rapamycin Complex 1) pathway, which pairs activation of anabolic processes with suppression of lysosome-dependent catabolism. In the muscle, under- or overactivation of mTORC1 activity can lead to dysfunction, suggesting that a complex interplay of anabolism and catabolism is needed for tissue homeostasis. How this balance is achieved, however, remains unclear. As a consequence, drugs targeting mTORC1 in the muscle aging context exert inconsistent benefits, underscoring the lack of therapies for muscle loss.

We and others have shown that mTORC1 is not simply ‘on’ or ‘off’, but can be selectively linked to distinct downstream outputs. Notably, phosphorylation of mTORC1 substrates that promote anabolism by enhancing protein synthesis can be uncoupled from phosphorylation of substrates that regulate catabolism through lysosome biogenesis control. To address whether selective activation of mTORC1 occurs in vivo, we will use whole-cell and lysosome isolation coupled with molecular assays to assess how mTORC1 activity is regulated across muscles and their cell types, and how this regulation changes with age. Ultimately, by elucidating how metabolism downstream of mTORC1 is regulated, we aim to identify new targets for preserving muscle health.

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# Functional Effects of C19MC in Human Skeletal Muscle Cells

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Previous work from our laboratory showed that expression of microRNAs from the paternally expressed chromosome-19-microRNA-cluster (C19MC) is associated with fat-free-mass-index in COPD patients, an association also reported in lung cancer, where cachectic patients exhibit reduced C19MC-miRNA expression. C19MC is the largest known human miRNA cluster and includes miR-518e and miR-519a, which are exclusively expressed from the paternally inherited chromosome. However, the mechanisms by which C19MC-miRNAs influence skeletal muscle phenotype remain unclear.

To identify pathways associated with miR-518e and miR-519a, we correlated their expression with skeletal muscle transcriptomes from patients undergoing aorticsurgery and from a COPD cohort. In both populations, higher miRNA expression was positively associated with oxidative phosphorylation-related genes and negatively associated with stress- and cell-cycle-related pathways, including p53/G2M-checkpoint and E2F-target-gene-sets (NES=-1.8,-1.9 and -2.1, FDR q-val<0.002, 0.001, and 0.0001 respectively). These associations are consistent with reported roles of C19MC miRNAs but do not establish causality.

To directly assess function, miR-518e was overexpressed in LHCN human myoblasts followed by RNA sequencing. miR-518e significantly altered the expression of 5,103 genes (2,531 upregulated and 2,572 downregulated; adj.$p$<0.05). Among the downregulated genes, 923 contained predicted miR-518e seed-sequence complementarity, including multiple components of the TGF-β-signaling-network. miR-518e overexpression also induced ECM gene expression and upregulated myogenic differentiation markers, including *MYOD*,*MYOGENIN*, and *MYH7* ($log_2$FC=0.6, $log_2$FC=3.2 adj.p<0.001, $log_2$FC=2, adj.p<0.04 respectively). qPCR validation and differentiation experiments confirmed enhanced myogenic differentiation with a bias toward slow-twitch muscle program.

Collectively, these data suggest that miR-518e promotes myogenic differentiation and extracellular matrix remodeling, supporting a role for C19MC-miRNAs in skeletal muscle maintenance.

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# The role of non-coding RNAs in the molecular regulation of muscle stem cells

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Skeletal muscle maintenance and regeneration depends on satellite cells (SCs). Our lab has shown that quiescent SCs comprise muscle stem (8-10%) and progenitor cells. Quiescence maintenance is central for ensuring the long-term stemness of muscle stem cells (MuSCs). However, whether quiescence maintenance regulates stemness and if both phenomena underlie shared molecular mechanisms is unknown. Non-coding RNAs (ncRNAs), such as microRNAs (miRNAs) and long non-coding RNAs (lncRNAs), are regulatory RNAs that act via epigenetic (post-transcriptional and chromatin) regulation, with reports on dynamic signatures in SCs during early muscle regeneration. Thus, we hypothesize that ncRNAs play a central role in MuSC stemness maintenance via epigenetic regulation of MuSC quiescence. We identified and isolated MuSCs via cell-surface markers extracted from single-cell RNAseq (scRNAseq) of quiescent/activated SCs with differing commitment. Using a novel bulk RNAseq approach that enables the capture of coding and non-coding RNAs, we molecularly characterized the complete - functional and regulatory - transcriptomics of MuSCs. The former supported our functional characterization of MuSCs (deeper quiescence, lower metabolism, reduced translation), whereas the latter led to the identification of MuSC-specific miRNAs and lncRNAs. We were also able to complement these findings at the protein-level through low-input proteomics. Moreover, we constructed a post-transcriptional lncRNA-miRNA-mRNA regulatory axis using *in silico* RNA-RNA interaction tools. We experimentally validated candidate ncRNAs' overexpression and are unraveling their functional role in MuSC stemness/quiescence maintenance via knockdown experiments in cultured myofibers and freshly-sorted MuSCs. Our study supports regenerative muscle therapies by presenting a multi-targetable epigenetic regulatory axis capable of MuSC mobilization.

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# Anisotropic Rod-Shaped Microgels with Tunable Surface Topography and Electrical Conductivity for Multi-Scale Alignment and Maturation of Skeletal Muscle Cells in 3D Bioprinted Constructs

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Accurate modeling of structural anisotropy is critical for engineering functional skeletal muscle tissues, where aligned myofibers and extracellular matrix organization drive force generation and regeneration. Here, we present a scalable platform of rod-shaped microgels fabricated via flow-focusing droplet microfluidics, featuring tunable surface grooves that guide cellular alignment. These anisotropic microgels serve as modular building blocks and microcarriers, significantly enhancing myogenic differentiation, sarcomere organization, and alignment of mouse C2C12 myoblasts and human induced pluripotent stem cell-derived myogenic progenitors compared to isotropic controls. Electrically conductive variants of the rod microgels further modulate cellular organization and contraction under electrical stimulation. The microgels are compacted into a shear-thinning bioink for extrusion-based 3D bioprinting, during which the rods align along the print path due to shear forces. This process establishes hierarchical alignment across subcellular (sarcomeric), cellular, and macroscopic (construct) scales, resulting in markedly increased expression of myogenic differentiation markers (e.g., myosin heavy chain) and improved tissue-like architecture. This bottom-up approach using tailorable anisotropic microgels offers a versatile, modular strategy to recapitulate the aligned architecture essential for skeletal muscle function and regeneration. The platform holds strong potential for applications in muscle stem cell research, disease modeling, and regenerative therapies. We anticipate these engineered constructs will advance our understanding of mechanobiological cues in muscle stem cell behavior and support the development of functional in vitro muscle models.

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# Investigating developmental and regenerative myogenesis in FSHD using human iPSC-derived models

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Facioscapulohumeral muscular dystrophy (FSHD) is the third most common muscular dystrophy and currently incurable. It causes progressive skeletal muscle weakness and wasting, usually with onset in the second to third decade of life. In FSHD, epigenetic derepression causes aberrant expression of the transcription factor DUX4 in skeletal muscle. Sporadic bursts of *DUX4* trigger persistent downstream effects including mitochondrial dysfunction, and activation of anti-myogenic and pro-apoptotic pathways. To further investigate metabolic defects, I am characterizing mitochondrial respiratory function using respirometry in FSHD patient versus isogenic control iPSC-derived myoblasts and myotubes. Preliminary data shows iPSC FSHD myotubes have altered mitochondrial function and reduced ATP production, as well as higher apoptosis, compared to isogenic controls.

Critically, DUX4 and PAX7, a master regulator of muscle stem cells, share similar homeodomains and exhibit mutual transcriptional inhibition. Thus, we hypothesize that *DUX4* expression impairs PAX7 (and the closely related PAX3) function during muscle development and myogenic specification. To investigate the expression dynamics and consequences of co-expression of *DUX4* and *PAX7,* I have engineered the isogenic iPSCs via CRISPR/Cas9-mediated knock-in and lentiviral transduction to report for DUX4 and PAX7 proteins. Using live imaging and transcriptomic analysis, I am investigating the consequences of DUX4 bursts on the expression and transcriptional function of PAX7 in 2D cultures and 3D engineered muscle tissues. Additionally, I am studying early development in FSHD by modelling somitogenesis *in vitro*.

This work will highlight interactions between DUX4 and PAX3/PAX7, potentially yielding novel FSHD biomarkers and therapeutic targets, as well as understanding myodevelopment in FSHD.

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# Investigation of Pulsed Frequency Ultrasound Stimulation of the Spleen as an Immunomodulatory Therapy for Volumetric Muscle Loss

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Volumetric muscle loss (VML) leads to irrecoverable, chronic functional deficits. Following VML, a dysregulated shift toward chronic inflammation inhibits myogenesis and nerve reinnervation. Pulsed Frequency Ultrasound (PFUS) is a non-invasive technology that delivers localized acoustic energy that can modulate the splenic anti-cholinergic pathway (CAP). Male Sprague-Dawley rats (~70-76 days) received either a full thickness VML defect of the tibialis anterior muscle or a sham surgery. Animals were stratified by injury and treatment, with PFUS treatment beginning 24 hours post-surgery. Systemic inflammatory response was quantified via plasma TNF levels. Inflammation, myogenesis and neurogenesis were measured via local protein and histologic analyses. Systemic TNF expression exhibited an interaction between injury and time; circulating TNF was reduced in VML animals at 3 days *($p<0.0001$)*, with no difference between groups by 14 days. PFUS had no effect on systemic TNF. Locally, TNF expression increased over time. At 3 days, VML increased local TNF, but PFUS had no effect. At 14 days, PFUS reduced local TNF in the sham group (*$p=0.0009$*) but increased it in VML animals (*$p=0.0133$*). Local NGF expression was lower at both timepoints in VML animals relative to sham ($p<0.0001$), unaltered by PFUS. Systemic TNF reduction over time is consistent with resolving inflammation. In sum, PFUS did not exhibit the anticipated modulatory effect on systemic or local TNF following injury; nor did it impact NGF expression. While additional neuromuscular outcomes are forthcoming, this cross-sectional analysis suggests that spleen-based PFUS activation of CAP may not be suitable as a primary intervention for VML.

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# FGFR1 drives muscle stem cell self-renewal through the adaptor protein FRS2

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The loss of skeletal muscle function and mass during aging is a major health burden that severely impacts quality of life. Muscle stem cells (MuSCs), which maintain and repair skeletal muscle, lose their regenerative capacity and ability to self-renew during aging due to compromised signaling from FGF receptor 1 (FGFR1). Restoring FGFR1 activity in MuSCs of aged mice re-establishes receptor polarity, rescues asymmetric division, and rescues self-renewal. How FGFR1 polarizes is not understood, and understanding the mechanisms involved may provide therapeutic targets for improving MuSC function during aging. FGFR1 signals via ligand-induced recruitment of intracellular substrates, conformationally changing the pre-dimerized receptor to trans-autophosphorylate eight intracellular tyrosines. Two tyrosines are required for catalytic activity and the remaining six recruit intracellular signal transducers. Mutating FGFR1 intracellular tyrosines to phenylalanine, I show that FGFR1 catalytic activity is essential to polarize the receptor but that the remaining six tyrosines are dispensable. Since the adaptor protein FGF receptor substrate 2 (FRS2) is constitutively associated with FGFR1 and phosphorylated upon activating FGFR1, I knocked down FRS2. Attenuating FRS2 abolishes FGFR1 polarization, indicating that FRS2-dependent signaling is required. Surprisingly, activating an FGFR1 construct lacking the extracellular domain polarizes the receptor and promotes self-renewal. Therefore, FRS2 likely integrates additional extracellular signals to polarize FGFR1. Since Syndecan-4 and ß1-integrin colocalize and polarize with FGFR1 during asymmetric division, these co-receptors are potential FRS2 signaling targets. Elucidating signaling pathways polarizing FGFR1 during MuSC self-renewal will clarify how MuSC numbers are regulated and identify pathways that fail to maintain MuSCs during aging.

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# Mechanisms of polarity dysregulation in dystrophin-deficient satellite cells

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Duchenne muscular dystrophy (DMD) is muscle wasting disease caused by a mutation in DMD gene encoding the dystrophin protein. This protein is highly expressed in skeletal muscle and in activated muscle stem cells (MuSCs). Dystrophin is required to establish apicobasal polarity, necessary to asymmetric division and efficient regeneration. In addition, asymmetric division depends on the formation of the PAR-complex composed of Pard3, Pard6 and aPKC in apical pole of MuSCs. We have previously demonstrated that the absence of dystrophin leads to reduced Pard3 polarization on the apical cortex, resulting in a reduction of apicobasal polarity and asymmetric division. Finally, we found the lack of dystrophin induced downregulation of Mark2, the kinase recruited to the basal pole by direct binding to dystrophin. However, the specific mechanisms involved remains unclear. We purpose to study the polarity establishment and the mechanisms involved on polarity dysregulation in dystrophin-deficient MuSCs. First, we showed polarity establishment is sequential in time between the basal and apical sides of MuSCs. Moreover, despite Pard3 expression being increased in dystrophin-deficient MuSCs, the lack of dystrophin reduces apicobasal polarity and alters the formation of PAR-complex. siMark2-treated-myofibers showed Pard3 upregulation while displaying reduced polarization, analogous of the deficits observed in *mdx* MuSCs, supporting the involvement of Mark2. Finally, the Mark2 downregulation observed in mdx MuSCs appears to be induced by proteasome degradation and hyper-phosphorylation (inactivated form of Mark2 protein). Together, these experiments provide important insight into the dysregulation of polarity caused by dystrophin-Mark2 deficiency in MuSCs in Duchenne muscular dystrophy.

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# Fascia-derived DPP4+ fibroadipogenic progenitors orchestrate the earliest stromal response to skeletal muscle injury

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Skeletal muscle regeneration depends on precisely coordinated interactions between immune cells and stromal progenitors that rebuild the damaged tissue. Fibro-adipogenic progenitors (FAPs) within muscle are known to expand early after injury and support myogenesis, yet the origin of the first waves of FAPs remains unclear. Here, we reveal the fascia, the connective tissue surrounding muscle, as an immediate external reservoir of progenitor cells that initiates the stromal response to injury. Fascia FAPs are transcriptionally distinct, exhibiting enriched expression of immune-regulated and migration-associated genes. In vivo models demonstrate their directed movement toward macrophage-infiltrated zones of injury. Importantly, fascia FAPs are also characterized by the expression of surface marker DPP4, which enabled identification of an equivalent population in skeletal muscle. Skeletal muscle DPP4+ FAPs exhibit elevated colony forming capacity and display a higher rate of proliferation. Lineage tracing in DPP4CreERT2:ROSA26tdTomato mice further revealed DPP4+ FAPs contributing to adipogenesis and osteogenesis upon *in vivo* stimulation. We also observed unidirectional flux of DPP4+ to DPP4- FAPs following injury, indicating that DPP4+ FAPs are the precursors of DPP4- FAPs. We observed a proportional reduction of DPP4+ FAPs in a mouse model of Duchenne muscular dystrophy, suggesting diminished trophic function of FAPs in chronic myopathy. Mechanistically, pharmacological inhibition of DPP4 does not impaired muscle regeneration, implying the enzymatic activity is dispensable in the regeneration process. Collectively, these findings identify fascia-derived DPP4+ FAPs as early responders that prime the damaged muscle environment for regeneration.

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# Alterations in Muscle Transcriptome and Motor Function with DUX4-Associated Pathology in a Tamoxifen-Induced FSHD Mouse Model

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Facioscapulohumeral muscular dystrophy (FSHD) is caused by aberrant expression of the DUX4 transcription factor, resulting in impaired myogenesis, inflammation, and progressive muscle weakness. The tamoxifen-inducible FLExDUX4 mouse model enables controlled DUX4 expression in skeletal muscle, providing a valuable platform to study acute disease mechanisms.

**Methods:** Male FLExDUX4 mice received tamoxifen or vehicle control. Hindlimb skeletal muscles, including tibialis anterior (TA) and gastrocnemius, were collected for bulk RNA-seq (DESeq2 analysis, adjusted p-value < 0.05) and histological evaluation. In vivo muscle contractility (specific force/torque) was assessed in the TA.

**Results:** RNA-seq identified a large number of altered genes with tamoxifen induction. The upregulated genes showed strong enrichment for the canonical DUX4 transcriptional program, robust induction of direct targets, and numerous Gm-family transcripts, as well as keratins, proteases, stress-response, and inflammatory pathways. Downregulated genes included mature muscle structural components, energy metabolism, and signaling regulators. These molecular changes were accompanied by significant reductions in in vivo muscle force production in tamoxifen-induced mice compared to vehicle controls. Histological analyses of TA and gastrocnemius muscles revealed moderate to marked myopathic changes, including increased myocyte cell death, mononuclear inflammatory infiltrates, myofiber degeneration/regeneration with centralized nuclei and fiber size variation, in tamoxifen (+DUX4) vs. vehicle control conditions.

**Conclusion:** Tamoxifen-induced DUX4 expression in the FLExDUX4 model rapidly recapitulates key molecular, functional, and histopathological features of FSHD. The pronounced DUX4 signature, combined with contractile deficits and myopathic histology in multiple hindlimb muscles, establishes this as a robust preclinical platform for mechanistic studies and evaluation of DUX4-targeted and other myogenic therapies.

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# Age-related PABPN1 Decline in Muscle Stem Cells Drives Dysregulation of Alternative Polyadenylation and Impairment of Muscle Regeneration

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Skeletal muscle regeneration depends on tightly regulated mechanisms within satellite cells (SCs) that are progressively disrupted with age. Alternative polyadenylation (APA) is a key post-transcriptional regulatory mechanism that alters the 3'UTR length of target transcripts by selectively using different polyadenylation sites, thereby affecting mRNA stability, translational efficiency, and microRNA-mediated repression. Despite its critical role in post-transcriptional regulation, whether APA is disrupted in aged SCs and which regulators are responsible remain largely unexplored, leaving a significant gap in our understanding of age-related muscle decline.

To characterize age-associated APA changes, we performed transcriptomic analyses on quiescent (QSC), freshly isolated (fiSC), and activated SCs (ASC) isolated from young and old mice. Aged QSCs exhibit widespread APA dysregulation, including increased transcript heterogeneity and global 3'UTR shortening, preferentially affecting genes involved in proteostasis, cell cycle, and longevity pathways. These changes persisted across all cell states, establishing APA dysregulation as a cell-intrinsic feature of aged SCs independent of quiescence. Proteomic screening identified PABPN1, an APA regulator that promotes distal polyadenylation site selection, as one of the most significantly downregulated factors in aged SCs, potentially contributing to global 3'UTR shortening. Furthermore, conditional PABPN1 *in vivo* knockout recapitulated SC aging, leading to premature differentiation, SC pool depletion, and impaired muscle repair.

These findings identify PABPN1 loss in aged SCs as a driver of APA dysregulation and regenerative failure. Ongoing studies investigating the therapeutic potential of restoring PABPN1 expression in aged SCs may inform novel strategies to combat skeletal muscle aging and muscle diseases such as oculopharyngeal muscular dystrophy.

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# NLRP3 inhibition improves myopathy in the limb girdle muscular dystrophy model

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Innate immunity is activated in several types of muscular dystrophies associated with a fragile sarcolemmal membrane or impaired membrane repair, such as Duchenne Muscular Dystrophy (DMD) and Limb-Girdle Muscular Dystrophy (LGMD) R1-R6 and R12. Among innate immune pathways, the **NLR family pyrin domain-containing 3 (NLRP3) inflammasome** is activated by danger-associated molecular patterns (**DAMPs**), such as extracellular ATP and HMGB1, which are released from damaged cells. Initially, the inflammasome is activated in immune cells and releases pro-inflammatory cytokines, such as IL-1β, via non-selective membrane pores, inducing **pyroptosis**, a programmed cytolytic cell death. However, inflammasomes are not exclusive to immune cells; they also operate within skeletal muscle, where their activation induces muscle degeneration via pyroptosis. We investigated how the inflammasome is activated in skeletal muscle, specifically in dystrophic muscle. We utilized known DAMPs, such as extracellular ATP, HMGB1, and intracellular calcium, to induce inflammasome activation in muscle fibers and myotubes expressing the inflammasome reporter. Furthermore, we investigated the contribution of NLRP3 activation using an in vivo LGMD R2 mouse model (dysferlin knock-out mice, 8-month-old, n=8) and in vitro LGMD R2 patient muscle cells (n=3) treated with pharmacological NLRP3 inhibition. We confirmed increased NLRP3-inflammasome activity in both in vivo and in vitro models compared with healthy controls. Our findings reveal that NLRP3 inhibition treatment delayed motor function decline, reduced muscle damage in BlaJ mice, and decreased cell death in LGMD R2 muscle cells.

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# Modelling MuSC Niche Repopulation Using Ordinary Differential Equations

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Muscle stem cell (MuSC) quiescence, characterized by arrest in $G_0$ phase of cell cycle, is critical to long-term tissue maintenance and regeneration following injury or stress. Failure to return activated MuSCs to quiescence can deplete the stem cell pool, contributing to impaired regeneration in aging and muscle disease. Because in vivo extraction procedures rapidly activate MuSCs through stress responses, experimentally studying true quiescence remains challenging. The Inactivation and Dormancy LEveraged in vitro (mini-IDLE) platform addresses this limitation by providing a three-dimensional MuSC niche composed of murine myotubes that promotes re-entry of implanted cells into the $G_0$ phase, bringing them back into quiescence in vitro, while reliably restoring the MuSC pool to a baseline size. In this work, ordinary differential equations (ODEs) are used to model the MuSC population dynamics and transitions between quiescent (QSCs), activated (ASCs), and proliferating stem cells (PSCs), as well as post-mitotic myocytes and multinucleated myofibers. Model parameters are estimated and fitted to mini-IDLE data using gradient-based optimization across multiple initializations to ensure robustness and assess parameter identifiability. An ODE model of only canonical transitions between MuSC states cannot explain the key property of a homeostatic steady-state, and alternative ODE models suggest that a type of cell-cell interaction with QSCs is likely necessary to achieve a homeostatic steady-state (i.e. baseline size). This ODE framework provides a tool for interpreting MuSC behaviour within mini-IDLE, enabling efficient simulation of experimental predictions for various hypotheses about population-level mechanisms and interactions. These findings contribute to a deeper understanding of muscle regeneration.

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# Beyond the Defect: Evidence Against the Exhaustion of Myogenic Potential in VML Injuries

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Volumetric muscle loss (VML) causes permanent disability, often attributed to an exhaustion of regenerative capacity that necessitates cell-based therapies. To test this regenerative capacity, we compared tibialis anterior (TA) healing in Lewis rats across four groups: 1) VML (6-mm biopsy), 2) $BaCl_2$ (50% necrosis), 3) VML$BaCl_2$, and 4) $BaCl_2$ administered 28 days post-VML. All injuries initially caused an ~84% strength loss. By day 56, the $BaCl_2$-only group fully recovered, while VML-injured groups plateaued at a 30% deficit. Despite the added injury, the VML + $BaCl_2$ group achieved strength recovery equivalent to VML-only. Histologically, VML caused fiber loss in the defect region and smaller fibers in both the defect and proximal residual muscle, whereas $BaCl_2$ alone maintained normal fiber metrics. While fibrosis was elevated in VML defect regions, it did not increase in the proximal residual muscle compared to $BaCl_2$ controls. The animals that received $BaCl_2$ 28 days after VML had equivalent outcomes to the VML + $BaCl_2$ group mentioned above with the exception of higher muscle fiber counts. Bulk RNAseq revealed few differentially expressed genes (DEGs) between injury groups, with all groups showing similar DEG profiles compared to healthy muscle. This data demonstrates that while muscle fails to regrow within the VML defect itself, the residual muscle retains a significant capacity for regeneration, challenging the paradigm that VML fundamentally exhausts the tissue's innate regenerative potential.

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# scRNA-seq enables assessment of myogenic progenitor identity, purity and differentiation capacity for 2D and 3D studies

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Congenital myopathies (CM) are a heterogeneous group of muscle disorders that cause muscle weakness, typically present from birth. Increasingly, patient cell-derived 3D tissues are being used to model disease, providing platforms to more accurately predict clinical outcomes of candidate drugs. A significant field-wide barrier is the variability that exists in skeletal muscle differentiation potential between stem cell lines that are used to generate these models.

Here, we differentiated four control and five CM patient iPSC lines to myogenic progenitors (MPCs) using the STEMdiff™ Myogenic Progenitor kit and assessed their quality and performance in 2D and 3D culture. Quality control assessments included: a) growth curves, b) immunostaining, c) scRNA-seq, d) bulk RNA-seq, e) fusion index. We found scRNA-seq to be particularly informative to understand why certain MPC batches have poor myotube fusion potential or fuse more rapidly, and to benchmark suitable control-patient combinations that are likely to yield biologically robust comparisons.

MPCs were subsequently used to generate 3D muscle tissues and contractile force measured for up to three weeks using the Mantarray™ platform. We observed a correlation between 3D tissue maturation rate, 2D fusion rate and MPC population composition. We are currently investigating how contractile performance of 3D tissues correlates with tissue maturation using quantitative proteomics. Overall, this work helps guide establishment of new models which reduce biases that stem from variation in iPSC differentiation behaviour.

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# Using bioengineered skeletal muscle to model bed rest in a dish

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Age-related loss of skeletal muscle mass, strength, and function is referred to as sarcopenia. Skeletal muscle is a mechanically sensitive tissue that requires constant tension to maintain its structure and function. An acute period of bed rest in older people can result in a substantial decline in muscle mass.

To elucidate the mechanisms of muscle wasting that result from acute periods of bed red, we have used bioengineered skeletal muscle tissue to develop a model of mechanical unloading. Our platform utilises a central seeding reservoir, containing two elastomeric poles around which the tissue self-organise and generate passive tension. By freeing one end of the tissue from the pole we can induce in an immediate loss of tension. Loaded controls, 4 days unloaded, and 7 day unloaded tissue samples were analysed by proteomics. We identified reductions in protein translation markers, sarcomere organization, and fatty acid biosynthesis. Proteins associated with extracellular matrix organization, collagen organization, and actin filament organization were upregulated.

Characterization has demonstrated that following 4 days of mechanical unloading, we can observe an atrophy phenotype including a 25% reduction in fibre size ($p<0.05$, ~18μm). Morphological changes including loss of typical tissue formation, fibre uniformity and spacing were observed. MuRF1 and Atrogin-1 were upregulated on day 4 ($p<0.05$) indicating upregulation of protein degradation. Mitochondrial membrane integrity was quantified using JC-1 accumulation assays, where a 29% ($p<0.05$) reduction in membrane integrity was observed. These findings indicate that there is dysfunction occurring in multiple systems that can contribute to sarcopenia.

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# Loss of periostin results in increased number of donor-derived myofibers in adult and neonatal satellite cell transplants

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Conditional expression of PAX3 (iPAX3) in differentiating pluripotent stem cells (PSCs) allows for the generation of skeletal muscle progenitors that can replace damaged myofibers and repopulate the pool of tissue-resident stem cells, satellite cells, to ensure long-term regenerative capacity. Our previous work identifies factors like Notch1/3 that regulate the *in vitro*-to-*in vivo* maturation switch that occurs in iPax3-derived myogenic progenitors to facilitate regeneration. The matricellular protein periostin (Postn) was also found to be highly expressed in the iPAX3 *in vivo* setting. Thus, is periostin also important in muscle progenitor maturation? Knowing that Postn is secreted into the environment, we also sought to separate Postn from donor cell and host tissue to pinpoint exactly which source was most impactful on donor cell outcome. Here, we take a multipronged approach using comparative analyses with i) adult and ii) neonatal satellite cells in the presence/absence of Postn, and iii) loss-of-function studies using donor (iPax3) and host (murine muscle) knockouts. We show that, surprisingly, there is a trend toward *increased* engraftment in the absence of Postn expression in the wildtype (NSG), dystrophic (NSG*mdx*$^{4cv}$) and Postn-null (NSG-PostnKO) muscle settings. We also note that though the number of donor-derived myofibers increases in knockout conditions, the average myofiber size is reduced, suggesting a possible defect in full maturation without Postn. While more studies are still needed to understand the full impact of Postn on bona fide and PSC-derived satellite cells, we are one step closer to defining the molecular mechanisms that control developmental regeneration and maturation.

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# Pax3 protein induction enhances muscle stem cell activation in mdx5cv mice

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Duchenne Muscular Dystrophy (DMD) is a severe genetic disorder characterised by muscle degeneration. The prevailing pathogenesis model holds that the loss of dystrophin protein causes myofiber breakdown, which leads to activation, proliferation, and differentiation of muscle stem cells (MuSCs) and new myofiber formation. However, lacking dystrophin, these new myofibers soon degenerate, resulting in cycles of degeneration and regeneration until the MuSC pool is exhausted and regeneration stops. The mechanisms underlying MuSC exhaustion remain poorly understood. We observed a delayed activation phenotype in MuSCs from gastrocnemius muscles from *mdx*$^{5cv}$ mice compared to wild type controls, as measured by EdU incorporation. Analysis of myogenic regulatory factors revealed reduced expression of Pax3 protein, a key regulator of MuSC activation. Conditional deletion of Pax3 further reduced MuSC activation, while pharmacological induction of Pax3 protein expression with antisense morpholino oligonucleotides could partially restore MuSC activation. Prolonged treatment with these Pax3-inducing morpholinos resulted in increased grip strength and ambulation.Our data suggest that MuSCs from *mdx*$^{5cv}$ mice have an activation defect that might be targeted as a potential therapeutic strategy for improving MuSC function in DMD.

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# Fibroblast growth factor 2 scales the muscle stem cell pool in the mini-IDLE biomimetic culture assay

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Muscle stem cells (MuSCs) sustain skeletal muscle regeneration by balancing proliferation with a return to quiescence, yet how this balance is choreographed by extrinsic cues remains poorly understood. Fibroblast growth factor 2 (FGF2) is a potent MuSC mitogen, but chronic exposure depletes the Pax7+ pool, suggesting that the timing of FGF2 exposure dictates regenerative outcome. Here, we leverage mini-IDLE, a 3D biomimetic assay that supports MuSC re-entry into dormancy, to dissect how FGF2-FGFR signalling shapes MuSC pool scaling. Exogenous FGF2 expanded the Pax7+ population in a dose- and time-dependent manner, but only a single pulse during peak proliferation simultaneously expanded the Pax7+ pool and increased the proportion of quiescent-like Pax7+CalcR+ MuSCs, whereas prolonged exposure did not alter Pax7+CalcR+ proportions. Re-analysis of regeneration scRNA-seq data revealed selective enrichment of FGFR1 and FGFR4 in proliferating and self-renewing MuSC states, and pharmacologic inhibition confirmed that both receptors are required for FGF2-driven scaling of the Pax7+ population. We tested whether these principles translate in vivo by delivering a single intramuscular FGF2 bolus during peak proliferation following injury. Pooled across the 1 µg and 5 µg doses tested, FGF2-treated muscles showed a significant increase in Pax7+ density relative to saline controls, although an increase in Pax7+CalcR+ proportions was not yet observed at 14 days post-injury. Aged Pax7+ MuSCs were refractory to FGF2 stimulation, exposing an age-dependent ceiling on this axis. Together, these findings identify temporal precision as a key determinant of productive FGF2-FGFR signalling, providing a tunable framework for engineering MuSC fate in regenerative therapies.

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# Fine-Tuning Agrin, Fixing NMJs: A Heparan Sulfate Strategy for DMD

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The neuromuscular junction (NMJ) is a specialized tripartite synapse in which motor neurons, muscle fibers, and terminal Schwann cells coordinate to convert neuronal signals into muscle contraction. This process depends on the agrin-LRP4-MuSK signaling complex, which organizes acetylcholine receptor (AChR) clusters and sustains effective neuromuscular transmission. In Duchenne muscular dystrophy (DMD), disruption of this pathway may contribute to NMJ fragmentation and aggravate muscle degeneration associated with the loss of functional dystrophin proteins.

To address NMJ abnormalities, restore optimal neurotransmition, and limit muscle degeneration in DMD, we adopted a glycobiology-based strategy. At synapses, heparan sulfate proteoglycans (HSPGs) consist of a core protein attached to heparan sulfate (HS) chains. HS-modifying enzymes such as polymerases, epimerases, sulfotransferases, and sulfatases determine HS structural diversity by controlling polysaccharide conformation, making HSPGs key interaction platforms that regulate selective protein binding and signaling via HSPG.

We hypothesized that specific HS modifications could modulate agrin-LRP4 interaction and thereby influence AChR aggregation. Transcriptomic and glycomic analyses of the rodent NMJ revealed synaptic enrichment of two HS-specific enzymatic modifiers and their product. In C2C12 myotubes as in rat primary 3D pillars, overexpression of these enzymes, as well as treatment with synthetic HSPG mimetics carrying their product, both enhanced agrin-induced AChR aggregation. ELISA assays and SPR suggested that these mimetics act by strengthening agrin-LRP4 interaction. In rat models of DMD, reduced levels of both the HS modifiers and their product coincided with NMJ fragmentation. By *in vivo* gain-of-function strategy we restored HS levels in DMD rats and strikingly improved NMJ morphology.

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# PRDM16 supports Fibro-Adipogenic Progenitors regenerative competence by coupling heterochromatin architecture to Mitotic Fidelity

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Fibro-adipogenic progenitors (FAPs) are muscle-resident mesenchymal multipotent cells essential for skeletal muscle homeostasis and regeneration. Upon injury, FAPs rapidly expand to support muscle stem cell function and tissue repair. However, how their proliferative competence is maintained during regeneration remains poorly understood. Here, we identified Prdm16 as a critical regulator of FAPs' mitotic fidelity. In FAPs, Prdm16 localizes to the NL where it anchors H3K9-methylated chromatin. Loss of Prdm16 disrupts peripheral heterochromatin tethering and triggers severe nuclear lamina defects, such as invaginations, blebbing and envelope ruptures. Notably, these abnormalities become particularly evident upon injury-induced proliferation, suggesting that Prdm16 is required to preserve nuclear integrity under proliferative stress. Consistent with this model, Prdm16-deficient FAPs display profound mitotic defects, including impaired mitotic progression, cytokinesis failure, micronuclei formation and accumulation of tetraploid/aneuploid cells. *In vivo*, these defects hamper injury-induced expansion of FAPs, culminating with impaired muscle regeneration. Mechanistically, we further show that loss of Prdm16 alters H3K9me2 genomic distribution, reducing its occupancy over gene-associated regions while increasing it at both peri-centromeric and centromeric regions. Together, these data identify Prdm16 as a previously unrecognized factor important to maintain FAPs' mitotic fidelity by preserving the correct spatial patterning of heterochromatin.

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# Sarcospan promotes rapid sarcolemma repair through membrane microdomain organization

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Skeletal muscle fibers experience frequent membrane disruptions during contraction, relying on rapid cell-intrinsic repair mechanisms to maintain sarcolemmal integrity. Membrane repair defects cause muscular dystrophies, highlighting the importance of coordinated repair for muscle homeostasis. Tetraspanins, four-pass membrane scaffolding proteins, directly regulate membrane repair, suggesting that sarcospan (SSPN), a tetraspanin-like component of the muscle-stabilizing dystrophin-glycoprotein complex, may contribute to sarcolemmal resealing. SSPN forms lateral-intermembrane homo-oligomers preferentially in less-reducing environments, such as the oxidizing extracellular milieu entering injured fibers following membrane disruption. Using laser injury of *ex vivo* myofibers, we found that SSPN rapidly accumulates at sites of membrane injury, where it is required to mitigate damage, as evidenced by increased $Ca^{2+}$ influx in SSPN-deficient myofibers. Conversely, SSPN overexpression conferred protection from acute $BaCl_2$-induced injury *in vivo*, reducing membrane permeability relative to wildtype muscle. Laser injury experiments further demonstrated that membrane lipid protrusion at injury sites positively correlated with SSPN expression, suggesting that SSPN facilitates membrane budding and/or lipid accumulation during repair. By selectively visualizing membrane-embedded SSPN, we observed rapid lateral migration of SSPN to repair sites, consistent with the formation of injury-associated microdomains. To define SSPN-dependent repair pathways, we performed multi-omic analyses and found that SSPN expression altered the localization and abundance of membrane repair machinery. Additionally, SSPN was found to interact with membrane repair and $Ca^{2+}$ signaling proteins. Because SSPN was recruited sites of injury within seconds of membrane disruption and influenced membrane trafficking and repair complex organization, we conclude that SSPN plays an orchestrative role in coordinating sarcolemma repair.

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# Human Primary Myotubes Display Diminished Muscle Protein Synthesis After Doxorubicin Treatment

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Loss of skeletal muscle is an inherent feature of ageing. Stable isotope tracers show that aged muscle responds poorly to anabolic stimuli such as protein feeding, termed anabolic resistance. Its mechanisms remain unclear, partly because manipulatable human in vitro models are lacking. Doxorubicin (DOX) induces senescence in human myoblasts; here we asked whether DOX impairs muscle protein synthesis (MPS) in mature myotubes.

Human primary CD56+ve myoblasts (18-35 years, n=9) were differentiated into myotubes for 96 hours, then exposed to 0.2 μM DOX in 15% FBS growth medium, or growth medium alone, for 24 hours. All were refed with growth medium for 72 hours, then analysed for fusion index and senescence markers. Parallel wells received 4% D2O for 72 hours to measure MPS alongside anabolic signalling.

Compared with controls, DOX-treated myotubes showed 20.6±4.4% lower D2O incorporation over 72 h, demonstrating impaired MPS ($p<0.01$), alongside a trend for reduced phosphorylation of the mTOR pathway proteins eEF2, p70 and 4EBP1. DOX did not affect fusion index or myotube area, and Senescence-Associated β-Galactosidase, p16, p21 and senescence-associated secretory gene expression did not differ between groups.

Using direct D2O incorporation, we show DOX impairs chronic MPS in human primary myotubes. Notably, the absence of increased senescence markers suggests DOX blunts MPS through a distinct mechanism, with accumulated cellular damage a possible contributor. This model provides a tractable system to probe anabolic resistance and to screen therapeutics for restoring MPS.

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# TDP-43 ribonucleoprotein aggregates build sarcomeres

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Ribonucleoprotein aggregates, a hallmark of neurodegenerative and progressive neuromuscular diseases, often appear in the skeletal muscle cytoplasm and are considered cytotoxic. Surprisingly, ribonucleoprotein aggregates transiently appear in the cytoplasm of regenerating muscle. The components of these cytoplasmic aggregates, or myo-granules, include the RNA-binding protein TDP-43, VCP (Vasolin-Containing Protein), and mRNAs encoding sarcomeric structural proteins that are bound to TDP-43. When muscle is building, myo-granules transiently appear in the cytoplasm of regenerating myofibers, peaking at 5 days post-injury (dpi), and clear once the muscle rebuilds at 10 days post-injury. When TDP-43 is ablated before muscle injury, sarcomeres fail to assemble, the muscle cannot rebuild, and cytoplasmic ribonucleoprotein aggregates persist. When muscle cells commit to terminally differentiate, TDP-43 multimerizes, appearing in the cytoplasm as an aggregate, and participates in building sarcomeres. Myo-granules require TDP-43 to bind and sequester mRNAs encoding sarcomere structural proteins, likely to traffic and deliver these mRNAs to build sarcomeres. By incorporating common disease mutations or deleting the nuclear localization signal encoded on TDP-43, I have assessed the effects on the myo-granule requirement for TDP-43-dependent sarcomerogenesis and the ramifications of myo-granule dysregulation. Understanding the involvement of TDP-43 in myo-granule assembly, trafficking, and clearance will aid in understanding why myo-granules clear in healthy muscle but persist in diseased muscle, contributing to skeletal muscle pathologies.

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# Advancing human models of muscle regeneration through stem cell enrichment

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Human muscle stem cells are essential for muscle regeneration and repair in the context of injury and disease. A major obstacle in developing human muscle models is the isolation of pure human muscle stem cell populations from biopsies, due to limited access to human tissues and frequent contamination by other cell types. Although a variety of isolation methods have been described, no comparative framework exists to establish a gold-standard approach for achieving high efficiency, purity, and viability of human muscle stem cells. We evaluated isolation protocols to enrich human muscle stem cells, with the goal of advancing human models to study muscle regeneration. We compared three isolation methods from a vastus lateralis biopsy of a healthy donor: (i) explant culture of primary muscle tissue, (ii) selective enrichment of stem cells using ice-cold PBS treatment, and (iii) magnetic-activated cell sorting. The resulting populations were evaluated for their proliferative and myogenic capacity. Compared with the routine explant isolation technique, the pre-plating/ice-cold PBS and CD56-positive methods yielded purer cultures of stem cells, as evidenced by a higher proportion of Desmin-positive cells, enhanced fusion index, and altered mitochondrial profiles. Our findings suggest that although cells derived from the routine explant procedure can differentiate into myotubes, their purity remains limited. The removal of contaminating cells such as fibroblasts resulted in a higher proportion of Desmin-positive cells that formed thicker and larger myotubes. This highlights the importance of optimised isolation strategies for improving culture purity and generating reliable human muscle stem cell models.

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# Murine arylamine n-acetyltransferase 2 as a modulator of skeletal muscle responses in MND: Implications for disease progression and myogenesis.

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Skeletal muscle dysfunction occurs early in MND and likely contributes to disease onset and progression. Improved understanding of factors that impact muscle in response to disease will help facilitate the development of therapeutics that can preserve muscle function in MND. Here, we investigate the role of murine n-acetyltransferase 2 (mNat2), an enzyme involved in mitochondrial function, as a modulator of muscle responses to disease. We assessed the *in vivo* expression and activity of mNat2 in skeletal muscles of SOD1$^{G93A}$ MND mice, and subsequent germline deletion of mNat2 on disease progression. Additionally, we investigated the role of mNat2 on the differentiation of murine C2C12 myoblasts. mNat2 expression and activity increased early and remained elevated throughout disease in skeletal muscles of SOD1$^{G93A}$ mice. Germline deletion of mNat2 worsened disease progression, evidenced by an earlier and steeper decline in disease phenotype. *In vitro*, mNat2 activity and expression increased during the differentiation of C2C12 muscle cells. Knockdown of mNat2 reduced C2C12 differentiation and myotube formation, with bulk sequencing revealing dysregulation of pathways related to muscle regeneration and mitochondrial function. Findings suggest that an early and sustained increase in mNat2 activity in muscle may support muscle responses following the onset of the disease. *In vitro*, mNat2 was shown to contribute to myogenesis, with its knockdown leading to perturbations in pathways related to muscle regeneration and mitochondrial function. These results provide novel evidence that mNat2 may influence muscle responses in MND; however, a direct role in muscle physiology specific to the disease remains unknown.

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# Building Duchenne in a dish: Engineered human Muscle Tissues (EMTs) for disease modelling and drug testing

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Duchenne Muscular Dystrophy (DMD) is a severe and progressive X-linked neuromuscular disorder that is caused by mutations in the dystrophin gene, leading to the absence of a functional protein. The lack of dystrophin results in myofibre degeneration, disruption of muscle integrity leading to loss of ambulation, and ultimately death due to cardiac or respiratory failure.

There are no available treatments for DMD able to slow muscle degeneration. Recent strategies have led to dystrophin replacement therapies; however, low efficacy and safety concerns related to both viral vectors used and transgene expression still remain a big challenge. Therefore, no animal model fully recapitulates the complexity of DMD. Moreover, different attempts at modelling this disease with human-derived cells have reproduced specific features of DMD. Organoids are self-organizing, renewing 3D cellular structures that resemble whole organs in composition and function.

By the Mantarray platform (CuriBio), we are developing a 3D Engineered Muscle Tissue (EMT), starting from patient-derived myoblasts and primary fibroblasts. EMTs allow tissue to self-assemble into a polarized 3D structure, between two pillars acting as tendons, capable of contraction. External electrodes can stimulate muscle bundle contraction, record electrophysiological signatures and monitoring force and rate of contraction. This allows for characterizing muscle function before and after potential pharmacological treatments, along with molecular and bioenergetic characterizations.

Our results demonstrate that DMD-deriving EMTs recapitulate some typical aspects of DMD in terms of functionality as well as structural defects such as disorganized myotubes distribution, delayed maturation of the muscle fibers and reduced expression of maturation markers.

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# Single-nucleus RNA sequencing reveals metabolic reprogramming of diaphragm muscle stem cells during mechanical ventilation

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Up to 40% of intensive care patients require mechanical ventilation (MV). While lifesaving, prolonged MV can cause infections, lung injury, and diaphragm damage. Ventilator-induced diaphragmatic dysfunction (VIDD) is characterized by muscle fiber atrophy, contractile impairment, and oxidative damage. While VIDD is well characterized at the tissue level, the impact of prolonged MV at the cellular level remains poorly understood.

In this study, we 1) investigate the impact of prolonged MV on diaphragm muscle stem cells (satellite cells) and (2) evaluate the therapeutic potential of extracellular vesicles (EVs) derived from human mesenchymal stem cells (MSCs) during prolonged MV.

Adult Sprague-Dawley rats underwent MV for five days and received either intravenous delivered MSC-derived EVs on day 1 of the MV, or no treatment. Diaphragms were collected and analyzed by single-nucleus RNA sequencing, revealing 13 distinct cell populations. Prolonged MV altered myonuclear composition, reduced satellite cell abundance, and induced the presence of differentiating myocytes. Interestingly, we identified a population of *Alb*$^{high}$ and *Ephb4*$^{high}$ nuclei unique to MV. Subclustering of satellite cells revealed increased immune signalling and a lack of myogenic differentiation during prolonged MV. Moreover, gene set enrichment analysis revealed metabolic pathway alterations in prolonged MV satellite cells that were partially restored following EV treatment.

This study provides the first snRNA-seq atlas of the rat diaphragm in healthy and prolonged MV conditions. By defining prolonged MV-induced changes across diaphragm cell populations, we aim to identify molecular pathways contributing to VIDD and develop therapeutic strategies to prevent diaphragm dysfunction.

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# Niche mechanical integrity is a critical regulator of MuSC self-renewal in vivo

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Mechanical signals are well-known instructors of muscle stem cell (MuSC) fate, but a paucity of mechanically selective *in vivo* models has limited our understanding of biophysical cue integration on MuSC behaviors in health and disease. Addressing this, we introduce a novel murine injury model that combines the well-characterized tibialis anterior (TA) $BaCl_2$ chemical injury with a ~25% muscle length retraction resulting from distal tendon transection (tenotomy). Independently controlling tenotomy timing allows interrogation of mechanical inputs on distinct MuSC functions during regeneration. In concurrent injury (BT) muscles feature deficient myofiber regeneration with significantly depleted $Pax7^+$populations at 28 days post injection (dpi) when compared to $BaCl_2$ injury alone (BS), indicating impaired regeneration due to MuSC loss. To investigate the role of niche mechanics on MuSC self-renewal, we applied tenotomy at 7 dpi (BTd) when MuSC self-renewal activity is greatest. $Pax7^+$ depletion is more severe in BTd muscles at 14 and 28 dpi, suggesting intact niche mechanics are critical for MuSC maintenance. We observe differential MuSC loss between interior and peripheral muscle regions, which correlate to distorted myofiber geometries and increased strain surrounding the tendon insertion. eMyHC expression observed in BTd vs. BT at 14 dpi indicates MuSCs are lost to terminal differentiation, perhaps due to ejection from the niche. In support of a mechanical over inflammatory basis for MuSC loss, we find $CD45^+$ cell distributions do not correlate to regions of MuSC depletion. Collectively, these findings establish a tractable *in vivo* system to causally interrogate how mechanical inputs drive MuSC fate decisions.

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# iPSC-derived myogenic progenitors deliver in situ therapeutics

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Fibrosis is a hallmark of chronic disease and a key driver of pathophysiology in disease progression and long-term morbidity. While the role of TGF-β in fibrosis is well-established, attempts to attenuate its signaling have been met with limited clinical success due to its global role in homeostatic regulation of the immune system and tissue resident cells. However, recent work suggests that isoform-specific targeting holds promise to alleviate fibrosis while limiting off-target effects.

Both traumatic and chronic skeletal muscle injuries result in degenerative muscle loss underpinned by severe fibrosis. This fibrosis is driven by loss of tissue architecture, persistent TGFβ signaling, and failure of resident muscle stem cells to regenerate damaged tissue. Effective intervention must therefore address the physical, cellular, and biochemical dysregulation driving these myopathies. Our therapeutic strategy rests on three pillars: 1) generation of an off-the-shelf supply of iPSC-derived myogenic progenitors (iMPs) capable of rebuilding muscle mass *in vivo*, 2) isoform-specific targeting of pro-fibrotic TGF-β signaling at the site of injury, and 3) a biocompatible material capable of restoring tissue architecture.

In pursuit of these goals, we optimized a protocol generating iMPs with robust engraftment capacity that produce de novo human skeletal muscle fibers and retain functional, long-term, Pax7 expression *in vivo*. We further developed an isoform-specific therapeutic and a novel local delivery method that attenuates fibrosis while minimizing systemic effects. Finally, we engineered a composite biomaterial that mimics skeletal muscle and supports host-tissue integration. Together, these aims function synergistically to address the pathophysiology of degenerative myopathies.

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# IQGAP2 and IQGAP3 act as Regulators of Human Myoblast Differentiation

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Aging of skeletal muscle is characterized by the loss of skeletal muscle mass and impaired functionality including impaired regeneration. During aging muscle stem cell functionality and their ability to differentiate is affected. Therefore, we aimed to identify new regulators of myogenic differentiation focusing on genes which show a differential expression in muscle stem cells of young and old mice. Thereby we identified IQGAPs as interesting candidates. IQGAPs are a class of scaffold molecules which regulate multiple cellular processes and pathways including differentiation making them ideal candidates.

Knockdown of IQGAP2 or IQGAP3 resulted in an increased myotube diameter and fusion index. IQGAP proteins are known signaling transducers of canonical and non-canonical Wnt signaling, known regulators of myogenic differentiation and myotube growth. While induction of myotube growth by knockdown of IQGAP2 or IQGAP3 was independent of canonical Wnt signaling, knockdown of IQGAP3 and Wnt7a seem to act synergistically resulting in myotubes with even more enhanced diameters. Interestingly, knockdown of IQGAP3 rescued the reduced formation of myotubes caused by inhibition of mTOR by rapamycin, while knockdown of IQGAP2 had no beneficial effect here. Knockdown of IQGAP3 caused an increase in S6 phosphorylation while knockdown of IQGAP2 did not affect S6 phosphorylation. We are currently deciphering the signaling cascade controlled by IQGAP2 and IQGAP3 in myogenic differentiation.

These current data identify IQGAP2 and IQGAP3 as negative regulators of terminal myogenic differentiation in human myoblasts. The data support a model in which IQGAP3 constrains a rapamycin-sensitive myogenic differentiation and supports a Wnt7a-responsive myogenic program.

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# Pro-fibrotic Activation of Fibro-Adipogenic Progenitors Impairs Myogenesis Through Extracellular Matrix Synthesis and Soluble Factors

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Fibro-adipogenic progenitors (FAPs) are mesenchymal stromal cells that play an important role in maintaining and stimulating regeneration in skeletal muscle. However, profibrotic activation of FAPs stimulates excessive secretion of extracellular matrix (ECM) and can result in pathological fibrosis. Duchenne muscular dystrophy is a X-linked progressive lethal disorder affecting 1:5000 male births where patients experience pathological fibrosis because of progressive muscle loss and injury. While FAPs play a pivotal role in skeletal muscle maintenance and pathogenic fibrosis, how soluble factors and the ECM from profibrotic FAPs affect muscle satellite cell (MuSC) differentiation is largely unknown. We hypothesize that FAPs can impair myogenic differentiation of MuSCs through paracrine signaling and variable ECM architecture attributed to profibrotic signaling.

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# Zebrafish larvae compensate for deficiencies in fast-twitch muscle development via slow-twitch fiber growth and utilization

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Muscle becomes stronger by expanding sarcomeres from the cell's edge to its center. Here we investigate how Mylpf (Myosin Light Chain Phosphorylatable Fast) abundance impacts sarcomere growth. The two zebrafish Mylpf genes (*mylpfa* and *mylpfb*) are exclusively expressed in fast-twitch muscle, with *mylpfa* expressed at least twice as abundantly as *mylpfb,* and matching ratios on the protein level. Mutations in both genes cause full mRNA and protein loss, as well as loss of sarcomere formation. The degree of sarcomere reduction in fast-twitch muscle is predicted by Mylpf protein dosage using a high-slope Hill equation, with severe sarcomere loss in *mylpfa*$^{-/-}$ mutant larvae, and no defect in the *mylpfb*$^{-/-}$ mutant even at adult stages. The *mylpfa*$^{-/-}$ defects can be rescued by Mylpfa-GFP*,* Mylpfb-GFP*,* and by human MYLPF-GFP with equivalent efficiency, but not by a MYLPF protein variant that causes Distal Arthrogryposis, suggesting conservation and disease relevance. The fast-twitch sarcomere defect may be driven by thick filament localization, which is impaired in *mylpfa*$^{-/-}$ and obliterated in *mylpfa*$^{-/-}$*;mylpfb*$^{-/-}$. Despite the severe fast-twitch phenotype, *mylpfa*$^{-/-}$ mutants outdistances their wild-type siblings by 6 dpf, owing to increased slow-twitch size and activity. Preliminary analysis shows that motoneurons become enlarged in the slow-twitch region of the *mylpfa*$^{-/-}$ mutant by 2 dpf which precedes the slow-twitch muscle hypertrophy, suggesting that neural compensation may drive the effects on muscle. Our findings show that impaired sarcomere formation in fast-twitch muscle is partially offset by compensatory growth and function of slow-twitch muscle fibers.

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# FAPs lineage progression regulation by the histone chaperone HIRA

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Chromatin architecture is fundamental to the regulation of gene expression and cell lineage determination. However, the specific contribution of the histone variant H3.3 and its chaperone HIRA to transcriptional regulation during somatic cell lineage progression, remains largely unexplored. Using tissue-specific and inducible mouse models, we assessed the consequences of HIRA deletion in Fibro-Adipogenic Progenitors (FAPs), a key muscle-resident stem cell population, in homeostasis and injured skeletal muscled.

HIRA deficient FAPs show an intrinsic defect in proliferation and increased apoptosis highlighted by an altered cell trajectory during muscle regeneration. HIRA cKO FAPs are also primed towards a fibrotic fate both in vivo and in vitro, as seen by higher intramuscular fibrosis throughout regeneration and strong fibrotic differentiation in culture. scRNA-sequencing in homeostasis and at different regeneration timepoints validated these phenotypes and revealed a metabolic defect in the cKO. Seahorse stress testing confirmed global mitochondrial dysfunction.

Mechanistically, we identified WNT signaling as the mediator of the increased fibrotic potential, and its inhibition completely restores FAP fibrotic differentiation in HIRA cKO. ATAC-sequencing confirmed WNT-mediated fibrosis and revealed a global shift in chromatin accessibility at key fibrotic loci. HIRA cKO FAPs further adopt a pro-inflammatory phenotype, altering their crosstalk with satellite and immune cells as confirmed by co-culture experiments. Ongoing CUT&Tag for H3.3, HIRA and key PTMs will establish the mechanistic link between HIRA-mediated H3.3 deposition and these defects.

Collectively, these results identify HIRA-mediated H3.3 deposition as a critical epigenetic safeguard of FAP identity and function, with implications for understanding fibrosis in skeletal muscle.

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# Altered functions and fates of satellite cells lacking MyoD and Myf5

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*MyoD* and *Myf5* are master regulators of embryonic myogenesis and are upregulated by satellite cells as an early response to muscle injury. We previously showed that inactivation of both *MyoD* and *Myf5* (dKO) in satellite cells results in loss of differentiation capacity and injured muscle fails to regenerate. dKO satellite cells remain in the injured tissue and continue to express PAX7, but the extent to which satellite cell programming is disrupted is unknown. We are using precision run-on sequencing (PRO-seq), bulk RNA-seq, and scRNA-seq to understand how loss of *MyoD* and *Myf5* affects satellite cell programming and other functions. As expected, PRO-seq of cultured satellite cells revealed decreased polymerase density over myogenic genes, and gene ontology (GO) showed a decrease in myogenic biological processes. Interestingly, GO analysis revealed an upregulation of regeneration and tissue remodeling, despite the inability of dKO cells to drive regeneration in vivo or to differentiate in culture. Previous lineage tracing studies showed that some dKO satellite cells adopt an adipocyte fate after muscle injury. However, adipogenesis was not evident in culture, suggesting that adoption of an adipogenic fate may be regulated by both intrinsic changes in cell programming and the abnormal, non-regenerating, muscle environment. scRNA-seq of non-regenerating muscle will reveal the alternative fates dKO satellite cells assume and will suggest cell-non-autonomous changes in the muscle environment that affect satellite cell fate choices and functions. Finally, comparisons to control muscle and published datasets of impaired regeneration will reveal core molecular features of regenerating and non-regenerating muscle tissue.

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# Sex-Dependent Responses to Moringa oleifera During Skeletal Muscle Regeneration After Injury

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***Background / Research Objectives:***

Moringa oleifera (MO) is a tropical plant rich in natural antioxidants, minerals, phytochemicals, and protein. Although widely used for its nutritional and medicinal properties, its effects on skeletal muscle regeneration remain unknown. Because MO contains bioactive compounds that could influence tissue repair, this study aimed to evaluate its impact on muscle regeneration in male and female mice.

***Materials and Methods:*** Male and female mice aged 8-10 weeks were divided into two treatment groups. One group received daily intraperitoneal injections of MO leaf powder diluted in saline, while the control group received saline alone. Muscle injury was induced by injecting cardiotoxin into the tibialis anterior (TA) muscles of both legs on the first day of treatment. Mice were weighed daily for 21 days. TA muscles were collected on days 3, 7, 15, and 21 post-injury, weighed, frozen, and processed for histological evaluation using hematoxylin and eosin staining.

***Results:*** Our results indicate that MO significantly decreases tibialis anterior mass in female mice on days 15 and 21. There was no significant difference in body weight. Normalizing TA weight by animal weight revealed a significant difference between the Moringa and saline groups on days 15 and 21 in females, though no such difference was observed in males.

In ***conclusion***, Moringa does not change muscle damage resolution but reveals robust sex-specific differences in regeneration. These findings highlight the importance of including both sexes in studies of botanical interventions.

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# Functional screening of sarcomere repair factors identifies the role of Usp28

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Daily muscle activity imposes recurrent damage on sarcomeres, the fundamental contractile units of myofibers. The timely repair of this damage is essential for maintaining muscle integrity, yet the underlying mechanisms remain poorly understood, with only a handful of factors identified to date. Here, we establish an *in vitro* platform in which controlled electric pulse stimulation (EPS) induces sarcomere damage followed by robust repair. Using this system, we performed a focused siRNA screen and identified a diverse set of genes required for sarcomere repair, including factors involved in basal sarcomere organization, transcriptional regulation, protein chaperoning, and the ubiquitination system. We further investigated USP28, the only deubiquitinase recovered in the screen. Muscle-specific deletion of Usp28 in mice caused sarcomere disorganization and muscle weakness in vivo. Mechanistically, Usp28 stabilized multiple sarcomeric and repair factors by counteracting their degradation. Together, our findings establish a scalable platform for dissecting sarcomere repair mechanisms, identify Usp28 as a new regulator of this process, and provide a framework for understanding how myofibers maintain contractile integrity under mechanical stress.

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# Environmental and metabolic exposures accelerate skeletal muscle dysfunction during aging

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Aging is associated with a progressive loss of skeletal muscle mass and function, contributing to frailty and reduced independence in older adults. While intrinsic aging processes drive this decline, the impact of accumulating environmental toxicants and microbiome-derived metabolites remains poorly understood.

This study investigates Cadmium (Cd), a widespread toxic metal known to induce severe oxidative stress and systemic organ toxicity, and δ-Valerobetaine (VB), a gut microbiota metabolite that impairs fatty acid oxidation and promotes lipid accumulation. Both exposures increase significantly with age in humans, potentially driving metabolic dysfunction.

To evaluate their combined impact on muscle health, aged mice were exposed to Cd and VB. The exposure significantly exacerbated skeletal muscle damage and age-related chronic inflammation, characterized by increased centrally nucleated fibers and macrophage infiltration. Furthermore, circulating markers of muscle injury were elevated, as reflected by increased serum creatine kinase levels. Functional assessments revealed impaired physical performance, demonstrated by prolonged beam traversal times. Additionally, Cd and VB exposure impaired the regenerative capacity of aged skeletal muscle, suggesting reduced ability of aged muscle to recover from injury.

In conclusion, these findings indicate that the age-related accumulation of environmental toxicants and microbiome-derived metabolites can exacerbate muscle injury, chronic inflammation, and functional decline. These results suggest that Cd-induced systemic toxicity and VB-mediated metabolic dysregulation are critical contributors to skeletal muscle dysfunction during aging, significantly increasing susceptibility to muscle damage and limiting recovery capacity in older individuals.

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# Trained to repair: repeated muscle injury imprints a ‘healthy regenerative memory’

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In healthy skeletal muscle, eccentric exercise confers protection against subsequent bouts of damage; the repeated bout effect. In chronic neuromuscular diseases such as Duchenne muscular dystrophy, repeated cycles of satellite cell-driven degeneration and regeneration instead drive progressive pathological remodelling. Whether repeated complete myofibre regeneration in otherwise healthy muscle drives cumulative functional degradation or encodes a beneficial adaptive state remains unresolved.

We tested whether repeated injury and regeneration produce a mechanically resilient rather than progressively compromised phenotype. Adult male C57BL/10 mice underwent one (1X) or three (3X) rounds of notexin-induced myotoxic injury alongside undamaged controls and dystrophic *mdx* mice, assessed using *ex vivo* contractile physiology, histology, RNA sequencing, and proteomics.

Repeated injury produced persistent structural remodelling including reduced fibre cross-sectional area, increased mass, branching, and fibre-type redistribution towards slower, more damage-resistant phenotypes. Despite this, 3X muscles exhibited preserved isometric force, marked resistance to eccentric damage, and superior post-eccentric recovery, exceeding singly injured and *mdx* muscles. Multi-omics profiling revealed coordinated remodelling of contractile identity, ECM composition, and oxidative metabolism, alongside widespread post-transcriptional regulation in immune and calcium handling pathways, indicating that the repeat-injury proteome cannot be inferred from transcript abundance alone.

These findings define a healthy regenerative memory: a stably remodelled, mechanically resilient state induced by repeated satellite cell-mediated regeneration. This state is molecularly and functionally distinct from dystrophic remodelling, with relevance to muscle stem cell biology, exercise physiology, and neuromuscular disease.

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# Functionally Informed High Throughput Screening of Gene Therapy Capsid Transduction Efficiency Using Human 3D Engineered Muscle Tissues

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Gene therapy is a promising treatment strategy for neuromuscular disorders, yet the rapid expansion of engineered viral capsid libraries has outpaced our ability to efficiently screen candidates in systems that predict human outcomes. Evaluation of capsid transduction efficiency in skeletal muscle remains largely dependent on animal models, which may not capture human-specific biology, or 2D myoblast cultures lacking the structural organization and maturity to evaluate tissue response over time.

To bridge this gap, we established a human iPSC-derived 3D engineered muscle tissue (EMT) platform for longitudinal high-throughput screening of capsid transduction efficiency in a 96-well format. Ten candidate AAV capsids encoding an mCherry reporter, provided by Dyno Therapeutics (Watertown, MA; Dyno did not design, conduct, or analyze the experiments), were applied to EMTs on Day 11 for a 24-hour transduction across a range of MOIs ($10^2$-$10^5$; n=4 per group), alongside untreated and vehicle controls. Transduction efficiency was quantified via fluorescence imaging of intact EMTs, and functional readouts including contractility and calcium dynamics were measured to contextualize transgene expression within muscle performance.

All capsids achieved detectable transgene expression at higher MOIs, demonstrating robust, dose-responsive expression across multiple conditions. Notably, the most efficiently expressed reporters induced dose-dependent functional impairment at elevated MOIs, including reduction in contraction force to ~25% of untreated controls and near-complete loss of calcium signaling.

These results highlight the limitations of evaluating transgene expression alone. By integrating functional measurements with transduction readouts, this 3D EMT platform provides a scalable, human-relevant filter for prioritizing capsid candidates prior to clinical translation.

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# Lentiviral Optogenetic Engineering of Human 3D Neuromuscular Junctions for Disease Modeling Applications

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Neuromuscular junction (NMJ) dysfunction is a hallmark of several neuromuscular diseases and neuropathies. While human iPSC-derived 3D NMJ models offer a physiologically relevant alternative to animal studies, broader adoption is limited by challenges in rapidly introducing disease-relevant genetic modifications. Here, we demonstrate lentiviral delivery of a light-sensitive ion channel to generate an optogenetically controlled human 3D NMJ within a commercially available platform, expanding its utility for disease modeling.

Human iPSC-derived myoblasts were co-cultured with motor neuron neurospheres on flexible posts to generate 3D NMJ tissues. Motor neurons were transduced with a blue-light-responsive channelrhodopsin (ChR2) lentiviral construct at MOIs of 1, 5, or 10 prior to tissue assembly. NMJs generated using inducible CRISPR-edited ChR2+ motor neurons served as a control. Transduction efficiency was assessed via fluorescent reporters, and functional outcomes were quantified by measuring blue-light-evoked contractile responses normalized to electrical contractions to determine functional percent innervation.

Lentiviral transduction produced robust, dose-dependent reporter expression in motor neurons within 3D NMJ tissues with no evidence of toxicity. Optogenetic stimulation reliably elicited innervation-driven contractions, with the highest MOIs yielding ~110% increased blue-light-evoked twitch force and percent innervation relative to the lowest MOI. Optogenetic responses in lentivirally transduced NMJs exceeded those in NMJs with edited motor neurons, highlighting the efficiency of this approach.

This work establishes a human iPSC-derived 3D NMJ platform supporting optogenetic, innervation-driven contractility through lentiviral genetic manipulation, providing a scalable tool for studying NMJ dysfunction and accelerating therapeutic development by enabling the rapid integration of diverse motor neuron sources.

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# Machine learning-directed exercise protocol development for 3D neuromuscular junction tissues

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Models of exercise and atrophy in tissue engineered muscles are critical for therapeutic discovery in neuromuscular indications and for advancing fundamental understanding of skeletal muscle physiology. However, interactions between culture conditions and donor variability make it difficult to identify optimal exercise protocols, and conditions often fail to translate across systems and laboratories. We present a human-in-the-loop optimization workflow combining scalable functional readouts with well-by-well experimental assignment to efficiently identify electrical stimulation parameters that enhance function in a human 3D neuromuscular junction (NMJ) tissue model.

Using a commercial platform enabling independently programmable stimulation across a 24-well plate, we sampled four stimulation variables: frequency (1-40 Hz), block intensity (1-10s), bouts per day (1-24), and bout intensity (60-360 repeats). Sampling across all parameter combinations revealed twitch forces ranging from no improvement to 6.5-fold above unstimulated controls, underscoring the sensitivity of 3D NMJs to stimulation parameters. A Gaussian process regression model mapped the four-dimensional space and guided subsequent protocol selection, supporting rapid identification of effective exercise protocols. Notably, optimized protocols improved relaxation kinetics following optogenetic NMJ activation, suggestive of enhanced acetylcholinesterase localization and isoform distribution.

This workflow supports more efficient, reproducible, and physiologically relevant skeletal muscle research. Running all conditions within a single plate eliminates inter-experiment variability while reducing time, cost, and reagent use. This approach removes the requirement for prior cell line characterization and could be applied to use cases ranging from disuse atrophy to peak exercise adaptation, enabling interrogation of functional adaptive mechanisms and context-dependent therapeutic efficacy.

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# Muscle stem cell commitment is orchestrated by Retinoblastoma-like protein 1 associated mitochondrial fragmentation

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Maintenance of appropriate muscle stem cell (MuSC) fate decisions is crucial for limiting regenerative decline in various myopathies and muscular complications. We find that the nuclear co-transcriptional repressor, Retinoblastoma-like protein 1 (Rbl1), can direct MuSC commitment to differentiation by its activity in the mitochondria. Using ex-vivo myofiber culture, we found MuSC self-renewal significantly increased when Rbl1 was either absent or predominately sequestered in the cytosol. However, Rbl1 mitochondrial localization significantly promoted more commitment of MuSCs. This was confirmed by transfection of Rbl1 specifically targeted to the mitochondria of MuSCs on myofibers that exclusively caused commitment. We found that the function of Rbl1 to induce commitment is linked to the mitochondria organization, which has a known role in mediating fate decisions. In this case, localization of Rbl1 in the mitochondria of MuSCs is associated with significantly smaller mitochondria lengths and volumes, as well as increased sphericity that are hallmarks of commitment. Taken together, this data underscores Rbl1's association with mitochondrial remodelling in shifting MuSC fates. Understanding the mechanisms affecting Rbl1 activity and subcellular localization may potentially provide avenues in rescuing muscle regenerative deficits.

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# Reconstruction of hPSC Myogenic Lineage Transitions through Multi-omics Single Cell Optimal Transport Identifies CREB5 and JAK Signaling as Regulators of Developmental Myogenesis

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Transient cell states that precede and support human myogenic lineage commitment, and the intrinsic and extrinsic signals that control them, remain poorly defined *in vitro.* Here, we combined longitudinal single-nucleus profiling with multi-omics single-cell optimal transport (Moscot), and a SIX1:H2B-GFP hPSC reporter for lineage tracing to resolve previously uncaptured transient intermediates and sequential waves of human myogenesis across differentiation and *in vivo* maturation. Moscot identified dynamic transcriptomic drivers of myogenic subsets that establish an initial wave of myotubes and PAX7+ skeletal muscle progenitor cells (SMPCs), which then give rise to a secondary wave of myotubes, mirroring embryonic transitions *in vivo* including LEF1 as the top regulator of PAX3 commitment and CREB5 as the top regulator of the PAX3-to-PAX7 state in human myogenesis. We next investigated complementary small molecule screen and lentiviral overexpression strategies to enhance skeletal muscle differentiation from hPSCs. From this screen, the JAK inhibitor Pyridone-6 emerged as a potent inducer of early myogenesis and may act in parallel to LEF1-associated programs to enhance myogenic specification. Using lentiviral-mediated overexpression, we demonstrated the effects CREB5 on improving $PAX7^{+}$ SMPC specification, progenitor expansion, and downstream myotube differentiation. Myogenic induction with SIX1:H2B-GFP hPSCs further identified a critical time window for autonomous myogenic potential and demonstrated how CREB5 mediates survival and proliferation during PAX7 initiation. Together, this work improves the efficiency and reproducibility of hPSC-derived skeletal muscle differentiation and identifies molecular regulators that can be leveraged for regenerative muscle therapies.

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# Screening of Korean Plant Extracts on Hedgehog Signaling Activation and Evaluation of Muscle Atrophy Improvement by Torreya nucifera and Ginkgetin

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Many studies have demonstrated that Hedgehog (Hh) signaling plays an important role in the regulation of skeletal muscle homeostasis. Therefore, natural compounds capable of activating Hh signaling may exert the potential activities ameliorating the muscle atrophy. Here, we screened Korean native plant extracts (n = 752) to determine their effect on Gli1-dependent transcriptional activities in Shh-Light II cells. Using luciferase reporter assay, 21 extracts were found to significantly enhance Gli1 transcriptional activity, among which the ethanol extract of *Torreya nucifera* (L.) Siebold & Zucc. (TNE) was selected for further study. We first analyzed TNE with LC-MS/MS and identified 19 compounds, and ginkgetin was one of the potential ingredients responsible for Hh signaling activation. We next confirmed the effect of TNE and ginkgetin on improvement of dexamethasone (DEX)-induced muscle atrophy in C2C12 cells. TNE at 5 and 10 μg/mL and ginkgetin at 1 and 5 μM enhanced myotube formation dose dependently. TNE and ginkgetin also suppressed the protein expression of MuRf1 and MAFbx which are well-known E3 ubiquitin ligases responsible for the protein degradation during muscle atriphy, indicating that TNE and ginkgetin reversed the protein degradation induced by DEX in C2C12 cell. Taken together, TNE exerting the strong Hh signaling activation and its functional component ginkgetin were confirmed to show the improvement of DEX-induced muscle atrophy by enhancing protein synthesis, suggesting that it may be developed as a potent agent for functional foods.

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# Development of Satellite Cell-Targeted Gene Regulatory Elements for AAV-based Gene Therapy Approaches

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Tissue- and cell-type-selective gene regulatory elements (REs) capable of driving targeted gene expression within the packaging constraints of gene therapy vectors hold promise for advancing the treatment of Duchenne muscular dystrophy (DMD) and other skeletal muscle diseases. Robust transgene expression in satellite cells (SCs) is particularly desirable, as these cells support early muscle growth and sustain myofiber replacement throughout life. Our work has demonstrated correction of the *Dmd* mutation via AAV-mediated base editing in myofibers and SCs; however, correction efficiency in SCs remains limited by the moderate activity of the EFS promoter used for adenine base editor (ABE) expression. To address this limitation, the present study aimed to develop novel REs capable of driving robust gene expression in SCs following systemic AAV delivery. Multiple ATAC-seq datasets were analyzed to generate genome-wide chromatin accessibility maps in sorted SCs and identify candidate cis-REs 200 bp or shorter in length. This effort nominated 14 candidate short elements (“mini-REs"), which were functionally evaluated via plasmid transfection of primary mouse SCs *in vitro*. Several of the tested mini-REs successfully drove EGFP and ABE expression and enabled ABE-mediated correction of the *mdx*$^{4cv}$ nonsense mutation. Cell specificity was further validated through *in vivo* assessment. Collectively, these data indicate that selected candidate mini-REs demonstrate comparable potency to, and greater SC-expression specificity than, widely used AAV-compatible promoters, including EFS (250 bp) and MHCK7 (771 bp), which should prove helpful for achieving potent and selective gene delivery to SCs for permanent rescue of inherited muscle diseases.

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# Identifying mechanistic differences between regenerative and degenerative infarcted hearts

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The heart is one of the least regenerative organs, with both *Mus musculus* (*Mus*) and humans exhibiting fibrosis-driven adverse remodeling following myocardial infarction (MI). Previous studies in *Acomys* MI models have revealed reduced scar formation and minimal pathological remodeling compared to *Mus*. Here, we investigate the cellular and molecular differences underlying these divergent repair responses in *Acomys* and *Mus* identify signaling pathways that promote functional repair over fibrosis. Single-nucleus RNA sequencing (snRNA-seq) of post-MI hearts revealed distinct fibroblast dynamics between species. *Mus* hearts showed persistent fibroblast activation, whereas *Acomys* fibroblasts underwent activation followed by resolution. Temporal analysis identified day 5 post-MI as a critical diverging point in *Acomys* fibroblast response, characterized by increased apoptosis, and a transient dip in profibrotic gene expression, suggesting attenuation of the fibroblast activation trajectory. Through regulatory network analysis, we identified RAR related orphan receptor alpha (RORa) as a novel and critical transcription factor potentially driving fibroblast resolution in Acomys. Pharmacologic modulation of RORα, using SR1078 (agonist) and SR3335 (inverse agonist), demonstrated that RORα activity is necessary for fibroblast resolution in *Acomys* and sufficient to promote resolution in *Mus*. In *Mus*, RORα activation post-MI produced marked improvements in cardiac function and restored fibroblast state. These effects were recapitulated in the pressure overload injury model (angiotensin II) in *Mus*, thus providing strong proof-of-concept for therapeutic modulation. Building on these findings, we next aim to elucidate the mechanisms by which RORα drives fibroblast resolution following cardiac injury.

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# Balancing efficacy and cardiac risk in AAV-mediated micro-dystrophin therapy for Duchenne muscular dystrophy using SIRT1 activation as a combinatorial strategy

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Duchenne muscular dystrophy (DMD) is an X-linked recessive disorder caused by mutations affecting the dystrophin gene, leading to progressive deterioration of muscle structure and function. Despite significant advances in disease management, no curative treatment is currently available, and existing therapies mainly target symptom control.

Gene replacement approaches have emerged as promising therapeutic options, with adeno-associated virus (AAV)-delivered micro-dystrophin (μDys) showing beneficial effects in experimental models and clinical trials through partial restoration of dystrophin expression. Nevertheless, treatment outcomes remain constrained by the dystrophic muscle milieu, which is characterized by persistent inflammation, membrane instability, and immune reactions directed against both the viral capsid and the therapeutic transgene. Collectively, these factors may limit the durability of μDys expression, attenuate therapeutic efficacy, and increase the risk of adverse events.

In this study, we examined how different levels of μDys expression influence skeletal and cardiac muscle outcomes and tested whether pharmacological activation of Sirtuin 1 (SIRT1) could potentiate the effects of gene therapy. Building on our previous work showing that the clinically advanced SIRT1 activator SRT2104 promotes muscle performance, regenerative capacity, and mitochondrial function, we evaluated its ability to improve μDys-mediated rescue.

Our data reveal the presence of a minimum μDys expression level required to obtain meaningful benefits in skeletal muscle. Conversely, excessive μDys expression was associated with the emergence of detrimental cardiac effects. These findings underscore the need to carefully calibrate μDys expression levels in order to maximize therapeutic efficacy in skeletal muscle while minimizing the risk of worsening cardiac involvement.

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# Hoxa11 interstitial cells preferentially contribute to fast twitch myofibers during homeostasis and mechanical overload

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Hox genes are responsible for producing transcription factors that are crucial for the developmental patterning of the skeleton across the animal kingdom. Proper development and muscle patterning of the zeugopod (radius/ ulna and tibia/ fibula) requires *HoxPG11* function. Loss-of-function of the paralogous genes *Hoxa11/Hoxd11* has been shown to cause stunted elongation of the skeleton and abnormal muscle attachments in the zeugopod during development. We have previously published a Hoxa11 lineage reporter (*Hoxa11*$^{CreERT2/+}$*;ROSA26*$^{LSL\text{-}tdTomato/+}$; Hox11iTom) that is expressed postnatally in muscular interstitial cells. Nuclear contribution from these cells to skeletal myofibers occurs rapidly during postnatal stages through one year post-induction. Recent findings show Hox11iTom interstitial cells contribute preferentially to fast twitch IIb and IIx myofibers, particularly in superficial muscle groups. However, the mechanism behind this preferential nuclear contribution remains unknown. To examine whether muscle use impacts Hox11iTom contribution to myofibers, we performed myotenectomy of the lateral and medial gastrocnemius, inducing overload in the soleus and plantaris. Following the surgery, Hox11iTom interstitial cells showed preferential nuclear contribution to fast twitch hypertrophied fibers. While the nuclear contribution rate remained similar to the homeostasis phenotype, we did observe a proliferation of Hox11iTom cells within the interstitium. Direct comparison of the plantaris and soleus after surgery illustrate a substantially different muscular niche between fast and slow-twitch dominant muscles. Our results demonstrate the complexity of the skeletal muscle niche and describe an additional cell type that may be influential in the longevity of fast-twitch myofibers.

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# Spatially Mapping Non-Coding RNA Regulatory Modules in Skeletal Muscle Regeneration

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keletal muscle (SkM) regeneration is a dynamic multicellular process coordinated by myogenic and non-myogenic cell populations within the regenerative niche. Proper SkM regeneration relies on the activation, proliferation, and differentiation of muscle stem cells, a process tightly controlled by intrinsic gene regulatory networks and extrinsic cellular dynamics within the SkM niche. Non-coding RNAs (ncRNAs), including microRNAs and long non-coding RNAs, are increasingly recognized as important regulators of myogenesis. However, their expression and regulatory roles during regeneration remain poorly understood due to limitations in transcriptome-wide spatial profiling approaches. We seek to understand whether ncRNA gene regulatory interactions are spatially orchestrated in myogenesis. To address this, we developed Spatial Total RNA-Sequencing High Definition (STRS-HD), a novel approach that enables unbiased capture of coding and non-coding RNAs through *in situ* polyadenylation on commercial spatial transcriptomics platforms. Using STRS-HD, we generated a high-resolution spatial atlas of mouse SkM regeneration across multiple timepoints following notexin injury. Spatial gene correlation analysis using Smoothie identified distinct spatially coordinated transcriptional modules associated with regenerative myogenesis, immune and fibro-adipogenic progenitor (FAP) cells, myofiber subtypes, and ncRNA-enriched regulatory programs. These modules revealed spatially heterogeneous injury-associated niches and cellular subtype-specific transcriptional organization during regeneration. Together, these findings demonstrate that STRS-HD enables comprehensive spatial profiling of the total transcriptome and provides a powerful framework for identifying ncRNA-associated regulatory programs during SkM regeneration. This work establishes a foundation for investigating spatial gene regulation in SkM regeneration and disease.

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# Reduction of SBNO2 levels enhances myogenic differentiation in cancer cachexia

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Cancer-related cachexia is a multifactorial syndrome characterized by progressive skeletal muscle wasting and systemic inflammation, leading to reduced quality of life and increased mortality in cancer patients. Importantly, cachexia cannot be fully reversed by nutritional supplementation and remains a major limitation in anti-cancer therapy, as affected patients often exhibit increased treatment toxicity and poorer clinical outcomes. Impaired myogenic differentiation further contributes to impaired regeneration of skeletal muscle in cachectic patients. Tumor-derived pro-inflammatory cytokines, including IL-6, activate catabolic signaling pathways such as the JAK-STAT signaling pathway and contribute to impaired myogenic differentiation.

Previous studies identified SBNO2 as a potential regulator of inflammatory signaling downstream of IL-6. Therefore, we investigated the expression of SBNO2 in myogenic cells under cachectic conditions in mice and human myoblasts. While expression of SBNO2 remains low during myogenic differentiation under physiological conditions, its expression is upregulated under cachectic conditions, suggesting a role in tumor-associated suppression of myogenesis. To analyze the role of SBNO2 in myogenic differentiation under cachectic conditions, we differentiated primary human skeletal muscle cells in the presence of conditioned medium from C26 cells as a model for cancer cachexia. Of note, knockdown of SBNO2 promoted myogenic differentiation under cachectic conditions. Furthermore, we can demonstrate that miR-124-3p regulates SBNO2 expression, under physiological and cachectic conditions. Interestingly, miR-124-3p affected expression of SBNO2 and STAT3-associated signaling responses in addition to enhancing myogenic differentiation.

Taken together, these results suggest that SBNO2 contributes to the impaired myogenic differentiation observed under cachectic conditions, presumably through modulation of JAK/STAT-associated signaling.

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# Multi-lineage specification from hPSCs enhances skeletal muscle progenitor maturity in vitro and regeneration in vivo

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Human pluripotent stem cell (hPSC)-derived skeletal muscle progenitor cells (SMPCs) represent a promising cell source for muscle regenerative therapies. However, current differentiation strategies often generate immature myogenic populations that lack key features of developmental muscle progenitors. During embryonic and fetal development, muscle stem and progenitor cells emerge within a complex cellular environment containing vascular, neural, and mesenchymal populations that contribute to muscle formation and growth.

Here, we developed a multicellular hPSC-derived skeletal muscle model designed to recapitulate aspects of the developing human muscle microenvironment. Directed differentiation generated cultures containing skeletal muscle fibers, PAX7+ SMPCs, endothelial cells, neuronal populations, and mesenchymal support cells. Single-nucleus RNA sequencing identified distinct myogenic, neural, endothelial, and stromal populations, demonstrating the successful generation of a diverse multicellular muscle tissue.

To further characterize the myogenic compartment, we performed single-cell RNA sequencing of purified SMPCs. Transcriptomic analyses revealed a population expressing canonical muscle stem and progenitor markers, including PAX7, and exhibiting molecular features consistent with fetal-like muscle progenitors. Following transplantation into a volumetric muscle loss model, human SMPCs successfully engrafted within host muscle and occupied the satellite cell niche. Quantitative analyses demonstrated the presence of donor-derived PAX7+ cells associated with regenerated muscle tissue.

Together, these findings establish a human multicellular skeletal muscle model that recapitulates key cellular components of developing skeletal muscle and generates fetal-like SMPCs with regenerative potential. This platform provides a valuable system for studying human muscle development and advancing stem cell-based therapies for muscle disease and injury.

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# CD26 Marks a Subpopulation of Multipotent Fibro/Adipogenic Progenitors That Declines with Aging

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Fibro/adipogenic progenitors (FAPs) are critical for muscle homeostasis and repair but paradoxically contribute to age-associated fibrosis and fatty infiltration. *We hypothesized that aging shifts the balance of homeostatic to pathological FAPs through a loss of cellular potency*. We performed CytoTRACE2 on publicly available mouse and human single-cell RNA sequencing datasets to stratify FAPs by cellular plasticity. We identified CD26+/PDGFRα+ FAPs as the most developmentally potent population of FAP subpopulations as well as in all of postnatal muscle. We developed a sorting strategy using the Cell Surface Protein Atlas to isolate and quantify age-related shifts in multipotent and lineage restricted subpopulations, which revealed an age-associated decline in CD26+/PDGFRα+ FAPs with an enrichment of CD26-/PDGFRα+ FAPs. We next assessed multipotency experimentally by isolating and culturing CD26+/PDGFRα+ and CD26-/PDGFRα+ FAPs from adult mice in ectodermal, endodermal, and mesodermal differentiation conditions. Consistent with our in-silico findings, CD26+/PDGFRα+ FAPs demonstrated ectodermal (β3-Tubulin+, GFAP+, Nestin+) and mesodermal competence (Perilipin+, Acta2+). However, CD26-/PDGFRα+ FAPs were restricted to mesenchymal fates only. *In vivo* lineage tracing using CD26-tdTomato mice supported our modeling studies by demonstrating skeletal muscle tdT+/β3-Tubulin+ and tdT+/Perilipin+ cell progeny. Finally, to determine whether age-related potency loss is cell-intrinsic, young CD26+/PDGFRα+ FAPs were cultured in serum isolated from young and aged mice. CD26+/PDGFRα+ FAPs readily underwent adipogenesis in aged serum while young serum maintained the progenitor state. Altogether, these findings identify multipotent CD26+/PDGFRα+ FAPs that are diminished with aging and suggest preservation of cell potency as a therapeutic strategy to combat fibrofatty infiltration with age.

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# Deciphering the role of HIF1a in skeletal muscle FAPs following acute muscle injury

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Loss of muscular mass and function due to traumatic injury impact millions of adults, frequently resulting in pathological fibrosis and fatty infiltration. Central to these processes is the dysregulation of a muscle resident mesenchymal population, the fibro-adipogenic progenitors (FAPs), which can maladaptively differentiate into fibrotic myofibroblasts or adipocytes. While FAPs have emerged as critical regulators of muscle homeostasis and repair, their complex behavior is still not fully understood. A largely unexplored modulator of FAP behavior is hypoxia signaling and the Hypoxia-Inducible Factor 1-Alpha (HIF-1a), a critical transcription factor and a key regulator of cellular adaptation (proliferation, survival, extracellular matrix deposition). Here we investigated the role of HIF-1a in modulating FAP behavior following Volumetric Muscle Loss (VML). We found that VML muscle experiences acute, transient hypoxia while FAPs robustly upregulated *HIF1a* expression. Crucially, HIF-1a stabilization in FAPs cultured under hypoxic conditions (1% $O_2$) restricted FAP proliferation, inducing a glycolytic shift and suppressing fibrotic gene expression. Next, the use of FAP-specific HIF-1a knockout mice (PDGFra-CreER$^{T2}$; Hif1a$^{fl/fl}$) will help us elucidate how HIF-1a controls FAP functions and their response to muscle injury. Overall, this study will help us understand the role of a critical transcriptional factor in regulating FAPs and their fibrotic response to muscle injury.

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# iPSC-derived myogenic progenitors: resolving lineage heterogeneity and maturation-dependent ERBB3 expression

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Induced pluripotent stem cell (iPSC)-derived skeletal muscle progenitors (iPS-SkM) provide a scalable platform for modelling skeletal muscle diseases. However, current differentiation approaches generate mixed myogenic and non-myogenic populations, complicating disease modelling by obscuring or confounding disease-associated phenotypes. This study aimed to characterise and benchmark iPS-SkM generated using STEMdiff™ Myogenic Progenitor Kit, with specific interest in expression of enrichment markers ERBB3 and NGFR.

Seven iPS-SkM lines were generated from five donors to capture intra- and inter-donor variation across differentiations. To evaluate myogenicity, PAX7 expression and fusion index were assessed by immunofluorescence and qPCR. An eleven-colour spectral flow cytometry panel was developed to evaluate co-expression of common myogenic surface markers reported in the literature. Cellular heterogeneity and identity were assessed in five lines by single-cell RNA sequencing, including one line that failed to form myotubes.

By scRNA-seq, iPS-SkM cultures comprised of 40.0 ± 3.6% (mean ± SD; n = 4) myogenic, 29.8 ± 14.8% early mesenchymal progenitors, and 32.0 ± 14.7% stromal, fibroblast and neural crest-like populations. Previously reported surface markers (ITGB1, MCAM, CD82, NCAM and MME) lacked myogenic specificity within the iPS-SkM pool. ERBB3 was absent and NGFR was lowly expressed under standard culture conditions. However, we show that secondary maturation in reduced serum medium markedly increase ERBB3 and NGFR expression, enabling isolation by FACS.

Altogether, we present an integrated toolkit to generate comparable iPS-SkM and enable more reliable targeted myogenic enrichment. These findings allow robust comparisons between iPS-SkM lines and facilitate improved downstream differentiation and *in-vivo* engraftment workflows.

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# Engineering Hypoimmunogenic Adult Skeletal Muscle Cells as Universal Donors for Allogeneic Regenerative Therapies

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The clinical translation of cell-based therapies is limited by immune rejection and by the high cost of manufacturing patient-specific medicinal products. Hypoimmunogenic “universal donor” cells represent a scalable alternative for allogeneic transplantation, enabling immune evasion and true off-the-shelf availability. Here, we report the generation of engineered adult human skeletal muscle-derived primary cells with reduced immunogenicity. These cells were genetically edited to eliminate HLA class I and II expression, thereby minimizing recognition by host T cells. Functional evaluation in mixed lymphocyte reactions confirmed a marked reduction in allogeneic immune activation. While HLA deficiency prevents direct TCR-mediated recognition, it renders cells susceptible to “missing-self” recognition by Natural Killer (NK) cells and does not fully address pro-inflammatory clearance mediated by the myeloid compartment. To overcome these innate, residual barriers, we developed an immunomodulatory strategy based on a lentiviral platform expressing selected immune-regulatory factors, combined with the incorporation of a suicide gene as a safety device. Co-culture experiments with NK cells demonstrated that the expression of immunomodulatory factors on engineered cells confers effective protection against NK cell-mediated cytotoxicity. *In vivo* studies in humanized mouse models demonstrate that these hypoimmunogenic cells effectively evade immune surveillance, achieve stable engraftment, and contribute to muscle regeneration. Altogether, this strategy provides a robust framework for the development of safe, universal, and cost-effective allogeneic cell therapies, addressing critical barriers in regenerative medicine.

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# Ex vivo gene therapy for Duchenne Muscular Dystrophy via cell-mediated exon skipping combined with a Ca2+ inhibitor COOH agrin peptide.

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Duchenne Muscular Dystrophy (DMD) is the most common genetic disorder affecting skeletal muscle. DMD results from out-of-frame mutations in the *DMD* gene, causing loss of dystrophin, a key component of the dystrophin-glycoprotein complex linking the cytoskeleton to the basal lamina. In contrast, in-frame mutations produce shorter, partially functional dystrophin associated with milder phenotypes. Dystrophin deficiency alters among others, also $Ca^{2+}$ channel activity, promoting $Ca^{2+}$ influx, myofiber damage, and progressive respiratory and cardiac dysfunction leading to premature death. We developed a combined cell and gene therapy strategy for DMD using immortalized human DMD myogenic cells transduced with a lentiviral vector (LV) expressing U7snRNA, a modified small RNA inducing exon 51 skipping and restoring expression of a shorter, in-frame dystrophin. The same cells were additionally transduced with a second LV expressing a COOH agrin peptide, capable to restore dystrophin-glycoprotein complex organization and block $Ca^{2+}$ influx in DMD cells. U7snRNA transduction restored dystrophin expression to levels comparable to primary cells from a healthy donor. Notably, co-transduction with both LVs further increased dystrophin expression at both mRNA and protein levels compared with U7snRNA alone. Biochemical analysis of muscle biopsies from DMD mice 30 days after intramuscular transplantation of transduced cells confirmed these findings. This combined approach may simultaneously restore functional dystrophin and correct $Ca^{2+}$ dysregulation, offering a potential therapeutic strategy for DMD and other muscular dystrophies.

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# Human satellite cells exhibit high basal p16 expression that increases in overweight females following injury

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The external environment affects the behavior and regenerative potential of adult muscle stem cells (SCs) in ways that are not completely understood. An important and highly modifiable factor is diet, which might lead to excessive caloric intake and weight gain. Our team recently discovered that exposure to a high-fat diet induces an irreversible senescent phenotype specifically in female mouse SCs. This is characterized by high levels of senescent markers p16, γH2AX, and senescence-associated β-galactosidase expression, as well as an impaired muscle regenerative capacity. To determine whether similar effects occur in humans, skeletal muscle biopsies from lean and overweight male and female individuals were analyzed by immunofluorescence for senescence-associated markers. Unexpectedly, we found widespread expression of p16 and γH2AX, independent of sex or adiposity status, which undermines their suitability as indicators of senescence for human SCs. Moreover, following exercise-induced muscle injury, p16 expression was significantly elevated in SCs only from overweight females compared to all other groups, whereas γH2AX levels remained unchanged. These findings challenge the prevailing notion of p16 as a definitive senescence marker in humans, raising the possibility that its expression may reflect a unique state of reversible cell cycle arrest for SCs. In addition, the muscle damage response through exercise found only in overweight females highlights the importance of stratifying male and female cohorts in human SC studies.

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# G9a/GLP inhibition induces an immunomodulatory program in DPP4+ FAPs to ameliorate regenerative capacity in advanced muscular dystrophy

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Duchenne Muscular Dystrophy (DMD) causes progressive muscle waste due to dystrophin deficiency. Despite the potential of gene therapies, their use is hindered by technical hurdles and mutation-specific requirements. Epigenetic drugs like the HDAC inhibitor Givinostat offer a viable alternative, yet their effectiveness is limited to early-stage patients due to emerging resistance in advanced disease. In this study, we demonstrate that inhibitors of H3K9 methyltransferases G9a and GLP (G9a/GLPi) overcome these stage-dependent limitations. In aged (>1-year-old) *mdx* mice, G9a/GLPi increase myofibers’ size and reduce fibro-adipogenic accumulation. Mechanistically, snATAC-seq analysis of aged mdx skeletal muscle suggests that G9a/GLPi promote regeneration by remodeling the chromatin landscape across the muscle niche. Specifically, our preliminary data identify DPP4+ stem FAPs as a central signaling hub of such beneficial effects, as G9/GLPi markedly increase their interactions across nearly all cellular compartments. This epigenetic reprogramming appears to induce a specialized immunomodulatory program within this FAP subpopulation, potentially coordinating the expansion of Self-Renewing Resident Macrophages (SRRMs). In line with these findings, treated tissues exhibit a notable reduction in degenerating fibers, suggesting that the activation of this stem-FAP/SRRM axis facilitates the efficient clearance of necrotic cells. Collectively, these results support a model where G9a/GLP inhibition restores the regenerative capacity of advanced dystrophic muscle by resolving chronic tissue degeneration through niche-wide reprogramming.

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# From modified to muscularized: Characterization of tRNA isopentenyltransferase 1 in skeletal muscle homeostasis and repair.

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tRNA isopentenyltransferase 1 (TRIT1) is a nuclear encoded protein that modifies select mitochondrial and cytoplasmic tRNA at Adenosine 37 in the anticodon loop, thereby stabilizing codon:anticodon base pairing and improving translation efficiency. In mice, deletion of Trit1 results in embryonic lethality whereas deficiency in humans leads to mitochondrial dysfunction and a rare disorder associated with neurodevelopmental delays called combined oxidative phosphorylation deficiency 35 (COXPD35). We previously identified that the metabolite byproducts of TRIT1-dependent tRNA modifications, isopentenyl adenosine 37 (aka i6A) and 2-methylthio-isopentenyl adenosine (aka ms-i6A), are affected by aging and injury in mouse skeletal muscle tissues. This suggests an uncharacterized role for TRIT1 in skeletal myogenesis and homeostasis. The objectives of this study were to: 1) characterize TRIT1 expression patterns and sub-cellular localization during skeletal muscle myogenesis using C2C12 cells and C57BL/6 mice and 2) to monitor post-natal development of a muscle-specific *Trit1* knockout mouse model. We found that *Trit1* expression increases during C2C12 myogenesis and that it primarily localizes to the cytosol. TRIT1 is abundant within the tibialis anterior muscle of 3-month-old C57BL/6 male mice, however levels did not change in response to cardiotoxin injury. Lastly, *ACTA1*-Cre was used to delete *Trit1* from striated muscles. No overt changes in physiology were observed, though in females, the terminal soleus weight was significantly increased, corresponding with a progressive reduction in grip strength with aging. Additional analyses are ongoing. Together these preliminary studies identify a foundation for further investigation into the importance of TRIT1 in skeletal muscle homeostasis and COXPD35.

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# Modelling X-linked myotubular myopathy in advanced human in vitro models for next generation genetic therapies

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X-linked myotubular myopathy (XLMTM) is a rare congenital muscle disorder characterised by muscle weakness, hypotonia and respiratory insufficiency. Histopathological hallmarks include smaller rounded myofibres and central localisation of nuclei and organelles. It is caused by mutations in *MTM1*, a lipid phosphatase involved in membrane trafficking, organelle organisation and phosphoinositide signalling. Despite substantial preclinical and clinical efforts, there is currently no cure, and the mechanisms underlying disease pathology remain incompletely understood.

Here, we report a platform to investigate XLMTM disease mechanisms and evaluate *MTM1* gene therapy strategies. Immortalised skeletal myoblasts derived from patient biopsies or human induced pluripotent stem cells (iPSC), were differentiated in both 2D monolayer cultures and 3D engineered muscle tissues, alongside healthy controls. These models recapitulate key features of XLMTM muscle pathology, including organelle mislocalisation, reduced myofibre size and mitochondrial abnormalities, with altered mitochondrial morphology and function in 2D cultures. In addition, small molecule-based differentiation yielded Pax7-positive myogenic precursors, indicating that the platform can capture early myogenic cell populations relevant to XLMTM disease modelling.

These phenotypic readouts were then used as treatment outcomes to evaluate novel therapy approaches. Using adeno-associated viral vectors (AAV), we show increased levels of MTM1 expression and investigated downstream effects through morphological, biochemical and functional readouts, demonstrating that myofibre size reduction can be ameliorated through AAV mediated gene replacement therapy. Together, our platform captures disease-relevant phenotypes across morphology, cellular organisation, and mitochondrial function in a fully human context, providing a scalable system to investigate disease mechanisms and assess treatment efficacy for XLMTM.

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# Understanding the regenerative capacity and repair mechanisms of the Myotendinous Junction

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The myotendinous junction (MTJ) is the biomechanical epicenter of muscle-tendon force transmission and the most vulnerable site of strain injury in athletes, yet it remains one of the least understood interfaces in musculoskeletal biology. Incomplete recovery, fibrotic scarring, and high reinjury rates define the current clinical reality.

We are pioneering a novel approach to decode MTJ injury and regeneration. By combining synergist ablation-induced mechanical overload with eccentric exercise, we have developed the first strain injury model that disrupts MTJ integrity without tendon excision, faithfully recapitulating sports injuries seen in the clinic. Critically, damage is spatially restricted to the myofiber tips with localized mononuclear cell infiltration, $eMHC^+$ centrally nucleated fibers and $Pax7^+$ MuSC activation, pinpointing the MTJ as the selective epicenter of injury. Longitudinal section immunofluorescence reveals disrupted Col22a1 and Dystrophin staining patterns at the fiber tips, confirming structural remodeling of the MTJ extracellular matrix. Using murine and human tenocytes isolated by FACS and validated by SCX and MKX expression, we are mapping the cellular landscape of the MTJ niche to decode the key molecular and cellular actors driving repair. Central to our investigation is understanding how MuSC and tenocytes cooperate to re-establish fiber branching, interdigitation, structural continuity and whether this can occur without fibrotic scar formation. Building on these findings, we aim to develop an implantable 3D engineered muscle-tendon construct designed to rescue MTJ integrity, rejuvenate repair, and offer a clinically translatable solution for musculoskeletal injury in athletes.

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