

SOCIETY FOR MUSCLE BIOLOGY

FRONTIERS IN MYOGENESIS

INNOVATIONS IN MYOGENESIS, FROM MOLECULAR
MECHANISMS TO THERAPEUTIC INTERVENTIONS

OCTOBER

6-11, 2025

SUNRIVER, OREGON



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FRONTIERS IN MYOGENESIS

INNOVATIONS IN MYOGENESIS, FROM MOLECULAR
MECHANISMS TO THERAPEUTIC INTERVENTIONS

WELCOME

We are thrilled to welcome you to *Frontiers in Myogenesis: Innovations in Myogenesis, From Molecular Mechanisms to Therapeutic Interventions*, hosted by the Society for Muscle Biology and held October 6–11, 2025, at the Sunriver Resort in Oregon, USA.

The field of myogenesis continues to grow rapidly, with exciting advances in molecular mechanisms, regenerative strategies, and therapeutic applications. This year's conference reflects that breadth, highlighting innovative research from across the globe and bringing together a multidisciplinary community of investigators at all levels.

Over the course of the week, you can expect keynote lectures, thematic scientific sessions, and poster presentations that showcase cutting-edge science and clinical perspectives. The program was carefully developed by the Scientific Program Committee to foster collaboration and exchange of ideas, and we are confident you will find the discussions to be both rigorous and inspiring.

We are deeply grateful to our sponsors— **The National Institute of Arthritis and Musculoskeletal and Skin Diseases (NIAMS), Hevolution, The Alliance for Regenerative Rehabilitation Research and Training, AFMTelethon, Regeneron, The Company of Biologists, The Discovery Center for Musculoskeletal Recovery at Spaulding Research Institute – Harvard Medical School, Stem Cell Network, Helen M. Blau, Cure Duchenne, Satellos Biosciences, The Developmental Studies Hybridoma Bank and Stemcell Technologies**—for their generous support, which makes this meeting possible.

We thank you for joining us in Sunriver and for contributing your expertise and insight to this year's meeting. We hope you will not only find the scientific program rewarding, but also take the opportunity to enjoy the natural beauty of Sunriver, Oregon. We look forward to the conversations, collaborations, and new ideas that will emerge throughout the week!

Sincerely,

Frontiers in Myogenesis 2025 Organizers

Drs. Fabrisia Ambrosio, Colin Crist, & Yu Xin (Will) Wang

Society for Muscle Biology

SUNRIVER RESORT MAP



Meeting Rooms

SUNRIVER RESORT LODGE		RIVER LODGE	GREAT HALL CONFERENCE CENTER		HOMESTEAD
Abbot I	Vandevent	Links Gallery	<u>Great Hall</u>	Great Hall Courtyard	<u>Homestead I</u>
Abbot II	Upper Gallery	Cascade Gallery	Landmark I & II	<u>Heritage I & II</u>	<u>Homestead II</u>
Hearth Room	Fremont		Landmark Gallery	Heritage Gallery	Homestead III
Merchant Trader Lawn			Mt. Bachelor Lawn	Fireside Room	Homestead Lawn

Key

 **Foot Path**
 **Hot Tub**

 **Bike Path**
 **Unit Numbers**

 **Ice Machine**
 **Electric Charging Stations**

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MEETING PROGRAM

Monday, 6 October 2025

2:00 PM - 6:00 PM - Homestead Foyer

Registration

6:00 PM - 6:15 PM - Homestead 1

Welcome Address

6:15 PM - 7:15 PM - Homestead 1

Keynote 1

6:15 PM - 7:15 PM **Cell state conversion in development and disease** [Stephen Tapscott](#)¹. ¹Fred Hutchinson Cancer Center.

7:15 PM - 10:30 PM - Great Hall

Welcome Reception

Tuesday, 7 October 2025

7:30 AM - 9:00 AM - Homestead 2

Breakfast

9:00 AM - 10:30 AM - Homestead 1

Session 1: Muscle Formation and Development

Session Chairs: Gabrielle Kardon & Jessica Orton

9:00 AM - 9:30 AM **Control of muscle growth: the importance of working, resting and dining well** [Simon M. Hughes](#)¹.

¹King's College London and Medical Research Council.

9:30 AM - 10:00 AM **Oscillatory control of gene expression in myogenesis** [Carmen Birchmeier](#)¹. ¹Max Delbrueck Center Berlin.

10:00 AM - 10:30 AM **Muscle fusion and fission: can they go hand in hand?** [Elizabeth Chen](#)¹. ¹UT Southwestern Medical Center.

10:30 AM - 11:00 AM - Homestead Foyer

Coffee Break

11:00 AM - 12:00 PM - Homestead 1

Session 1: Muscle Formation and Development

11:00 AM - 11:30 AM **Protein-protein interactions regulating myogenesis** [D Cornelison](#)¹. ¹University of Missouri.

11:30 AM - 11:45 AM **Development of a split-toxin CRISPR screening platform to systematically identify upstream regulators of human myoblast fusion** [Pengpeng Bi](#)¹. ¹University of Georgia.

11:45 AM - 12:00 PM **Novel regulatory regions controlling expression of the muscle determination gene, MyoD** [Heather Jamieson](#)¹. ¹University of Connecticut.

12:00 PM - 2:00 PM - Homestead 2

Lunch and Meet the Speakers

1:00 PM - 2:00 PM - Heritage Room

Poster Setup

2:00 PM - 3:30 PM - Homestead 1

Session 2: Muscle Stem Cell Regulation

Session Chairs: David Goldhamer & Grace Jones

2:00 PM - 2:30 PM **PAX3-SIX2 muscle stem cell heterogeneity drives muscle-specific repair dynamics diversification**

[Frederic Relaix](#)¹. ¹Paris East Creteil University (UPEC).

2:30 PM - 3:00 PM **Non-canonical roles of the Keap1/Nrf2 axis in regulating quiescence and early activation in adult muscle**

stem cells [Zhenguo Wu](#)¹. ¹Hong Kong University of Science & Technology.

3:00 PM - 3:30 PM **CPT1A enables promoter-confined acetyl-CoA production to prevent muscle stem cell activation and**

myofiber hyperplasia [Thomas Braun](#)¹. ¹Max Planck Institute HLR.

3:30 PM - 4:00 PM - Homestead Foyer

Coffee Break

4:00 PM - 5:30 PM - Homestead 1

Session 2: Muscle Stem Cell Regulation

4:00 PM - 4:30 PM **Fibroblast Deactivation Across Species** [Fabio Rossi](#)¹. ¹University of British Columbia.

4:30 PM - 5:00 PM **“Stem cell quiescence: a microcosm of evolutionary tradeoffs”** [Thomas Rando](#)¹.

¹University of California, Los Angeles.

5:00 PM - 5:15 PM **Regulation of Muscle Stem Cell Dynamics and Quiescence by Netrin-Cytoskeleton Signaling** [Hsiao-Fan Lo](#)¹.

¹Icahn School of Mount Sinai.

5:15 PM - 5:30 PM **A myofiber-embedded hydrogel system reveals that constitutive FGFR1 activation restores integrin- β 1-**

mediated fibronectin sensing in aged satellite cells [Tze-Ling Chang](#)¹. ¹University of Colorado Boulder.

5:30 PM - 5:45 PM - Homestead 1

Poster Blitz Session 1

6:00 PM - 8:00 PM - Homestead 2

Dinner

8:00 PM - 10:00 PM - Heritage Room

Poster Session 1

7:30 AM - 9:00 AM - Homestead 2

Breakfast

9:00 AM - 10:30 AM - Homestead 1

Session 3: Muscle Stem Cell Dysfunction

Session Chairs: Clarissa Henry & Aline Yamashita

9:00 AM - 9:30 AM **Stem cell dynamics in Duchenne Muscular Dystrophy: insights from live imaging** [Brendan Evano](#)¹.

¹Pasteur Institute.

9:30 AM - 10:00 AM **New molecular signatures of muscle stem cells** [Foteini Mourkioti](#)¹. ¹University of Pennsylvania.

10:00 AM - 10:30 AM **Autophagy maintains the regenerative capacity of muscle stem cells** [Natasha Chang](#)¹. ¹McGill University.

10:30 AM - 11:00 AM - Homestead Foyer

Coffee Break

11:00 AM - 12:00 PM - Homestead 1

Session 3: Muscle Stem Cell Dysfunction

11:00 AM - 11:30 AM **Bioactive lipid-mediated regulation of stem cell fate in muscle regeneration** [Nicolas Dumont](#)¹.

¹Université de Montréal.

11:30 AM - 11:45 AM **Role of the TAK1 signaling pathway in FAP in tissue remodeling** [Marine Theret](#)¹. ¹UBC.

11:45 AM - 12:00 PM **Small molecule augmentation of Notch signaling rescues models of DMD and FSHD** [Duc Dong](#)¹.

¹Scintillon Research Institute.

12:00 PM - 2:00 PM - Homestead 2

Lunch and Meet the Speakers

1:00 PM - 2:00 PM - Heritage Room

Poster setup

2:00 PM - 3:00 PM - Homestead 1

Keynote 2

2:00 PM - 3:00 PM **Photoresponsive hydrogel matrices as muscle satellite cell microenvironments to study mechanotransduction during regeneration** [Kristi Anseth](#)¹. ¹University of Colorado Boulder.

3:00 PM - 3:30 PM - Homestead 1

Session 4: Pluripotent Stem Cell Derived Myogenic Cells

Session Chairs: Ning Liu & Shiv Sharma

3:00 PM - 3:30 PM **Making human skeletal muscle in vitro and in vivo** [Olivier Pourquie](#)¹. ¹Harvard Medical School.

3:30 PM - 4:00 PM - Homestead Foyer

Coffee Break

4:00 PM - 5:30 PM - Homestead 1

Session 4: Pluripotent Stem Cell Derived Myogenic Cells

4:00 PM - 4:30 PM **Human Myogenesis in Development and Disease** [April Pyle](#)¹. ¹UCLA Broad Stem Cell Center.

4:30 PM - 5:00 PM **Pluripotent stem cell-derived myogenic progenitors: from development to therapeutics** [Rita Perlingeiro](#)¹.
¹University of Minnesota.

5:00 PM - 5:15 PM **Modulation of the JAK2-STAT3 pathway promotes expansion and maturation of human iPSCs-derived myogenic progenitor cells** [Luca Caputo](#)¹. ¹Sanford Burnham Prebys.

5:15 PM - 5:30 PM **Creation of a stem cell-derived neuromuscular junction for disease modeling and drug discovery** [David Mack](#)¹. ¹ISCRM University of Washington Medicine.

5:30 PM - 5:45 PM - Homestead 1

Poster Blitz

6:00 PM - 8:00 PM - Homestead 2

Dinner

8:00 PM - 10:00 PM - Heritage Room

Poster Session 2

7:00 AM - 8:30 AM - Homestead 2
Breakfast

8:30 AM - 10:00 AM - Homestead 1
Session 5: Innovations in Myogenesis Session Chairs: Penney Gilbert & Tamas Nagy

8:30 AM - 9:00 AM **Probing biophysical regulation of myogenesis with dynamic biomaterials** [Christopher Madl](#)¹.
¹University of Pennsylvania.

9:00 AM - 9:30 AM **New Insights into Mechanisms of Muscle Disease** [Eric Olson](#)¹. ¹UT Southwestern Medical Center.

9:30 AM - 9:45 AM **Editing of Postmitotic Muscle and Satellite Cells for Continuous Correction of Dystrophin Expression in Duchenne Muscular Dystrophy** [Niclas Bengtsson](#)^{1, 2, 3, 4}. ¹Department of Neurology,
²Senator Paul D. Wellstone Muscular Dystrophy Specialized Research Center Seattle,
³Institute for Stem Cell and Regenerative Medicine, ⁴University of Washington.

9:45 AM - 10:00 AM **More Than a Bystander: Intramuscular Adipose Tissue Actively Impedes Muscle Regeneration**
[Daniel Kopinke](#)¹. ¹University of Florida.

10:00 AM - 10:30 AM -
Session 6: Emerging Technologies to Counteract Muscle Aging Session Chairs: Lorenzo Puri & Paolo Aguilari

10:00 AM - 10:30 AM **Post-transcriptional regulation of muscle stem cell aging** [Tom Cheung](#)¹.
¹The Hong Kong University of Science and Technology.

10:30 AM - 11:00 AM - Homestead Foyer
Coffee Break

11:00 AM - 12:15 PM - Homestead 1
Session 6: Emerging Technologies to Counteract Muscle Aging

11:00 AM - 11:30 AM **Enhancing muscle strength in aging by targeting the gerozyme 15-PGDH** [Helen Blau](#)¹. ¹Stanford University.

11:30 AM - 12:00 PM **Metabolic and epigenetic regulation of myogenesis in adulthood and aging** [Vittorio Sartorelli](#)¹.
¹NIAMS, National Institutes of Health.

12:00 PM - 12:15 PM **Aged Matrix Stiffness Disrupts Muscle Stem Cell Regeneration via RNA Surveillance** [Kandarp Dave](#)¹.
¹Department of Physical Medicine and Rehabilitation, Spaulding Rehabilitation, Harvard Medical School, Charlestown, MA.

12:30 PM - 2:00 PM - Homestead 2

Lunch and Meet the Speakers

2:00 PM - 6:00 PM -

Free Time: Networking and Informal Meetings

6:00 PM - 8:00 PM - Homestead 2

Dinner

8:00 PM - 10:00 PM -

Star Party

7:30 AM - 9:00 AM - Homestead 2

Breakfast

9:00 AM - 10:15 AM - Homestead 1

Session 7: Advances in Understanding and Treatment of Cachexia Session Chairs: Tom Cheung & Amanda Ignacz

9:00 AM - 9:30 AM **Role of SAA-1 in pancreatic cancer cachexia** [Alessandra Sacco](#)¹. ¹Sanford Burnham Prebys.

9:30 AM - 10:00 AM **Human data to identify mechanisms of cancer-associated muscle wasting** [Erin Talbert](#)¹. ¹University of Iowa.

10:00 AM - 10:15 AM **Oncogenic alterations in Twist2 progenitors drive soft tissue sarcomas in vivo** [Alinne C. Costa](#)¹.

¹UT Southwestern Medical Center.

10:15 AM - 10:30 AM -

Group Photo

10:30 AM - 11:00 AM - Homestead Foyer

Coffee Break

11:00 AM - 12:00 PM - Homestead 1

Business Meeting

12:00 PM - 2:00 PM - Homestead 2

Lunch and Meet the Speakers

2:00 PM - 3:00 PM - Homestead 1

Session 8: Professional Development Workshop

3:00 PM - 3:30 PM - Homestead Foyer

Coffee Break

3:30 PM - 5:00 PM - Homestead 1

Session Chairs: Ling Liu & Beatrice Silvestri

Session 9: At the Frontiers of Myogenesis: Novel mechanisms, insights and therapeutic strategies.

3:30 PM - 4:00 PM **Multimodal spatial profiling of muscle regeneration dynamics in Duchenne muscular dystrophy**

Lorenzo Giordani¹.

¹Sorbonne Université, INSERM UMRS974, Association Institut de Myologie, Centre de Recherche en Myologie, 75013, Paris, France.

4:00 PM - 4:30 PM **Teratoma-derived myogenic progenitors for human muscle grafts in mice: applications to FSHD**

Michael Kyba¹. ¹University of Minnesota.

4:30 PM - 4:45 PM **Interrogating the role of fibro-adipogenic progenitors in muscle loss and impaired regeneration in the disease fibrodysplasia ossificans progressiva** Russell Hanson¹. ¹University of Connecticut.

4:45 PM - 5:00 PM **CTCF mediated long-term maintenance of skeletal muscle fiber homeostasis by control of chromatin structure** Jimmy Massenet¹.

¹Center for Cardiovascular and Muscular Diseases, Sanford Burnham Prebys Medical Discovery Institute, La Jolla, California 92037, USA.

5:00 PM - 5:30 PM - Homestead 1

Closing Remarks

6:00 PM - 8:00 PM - Great Hall

Banquet and Awards Dinner

Saturday, 11 October 2025

7:00 AM - 11:00 AM -

Checkout and Departure

Abstract Booklet

Short talks (O-1 to O-14 abstracts)

Posters (P-1 to P-46 abstracts)

O-1 - Development of a split-toxin CRISPR screening platform to systematically identify upstream regulators of human myoblast fusion

Pengpeng Bi¹

¹University of Georgia

Muscle defects are a common feature in human developmental disorders and often lead to severe functional impairment. These defects arise from intricate tissue crosstalk and rare genetic mutations, underscoring the need to systematically identify cell-autonomous mechanisms regulating human myogenesis. Despite the clinical significance, our understanding of human development remains limited, due in part to the absence of a scalable genetic approach to study this process. Here, we introduce a rationally designed high-throughput CRISPR screening platform that integrates human myoblast models, muscle-specific CRISPR knockout libraries, and a split-toxin strategy that acts as a functional readout for myoblast fusion—a fundamental step of human myogenesis. This screening strategy enables selection of CRISPR-induced fusion-defective myocytes in a quantifiable manner. Leveraging this platform, our initial genetic screen uncovered 236 new hits essential for human myoblast fusion. The majority of these hits converge into 23 protein complexes, most of which have not previously been functionally linked to myogenesis in any species. Notably, mutations in 41 of fusion screen hits cause human diseases presenting abnormal skeletal muscle morphology. Applying a new single-cell CRISPR & RNA-seq approach, we show that majority of these hits control human myoblast fusion as well as influence early-stage myogenic differentiation. Together, this work presents a new systematic approach to study human myogenesis and uncovers promising candidates governing human muscle differentiation and fusion. A broader application of this split-toxin based CRISPR screening platform would accelerate the study of cell-autonomous mechanisms of human muscle development and diseases at scale.

O-2 - Novel regulatory regions controlling expression of the muscle determination gene, *MyoD*

Heather Jamieson¹, Masakazu Yamamoto¹, Leighton Core¹, David Goldhamer¹

¹University of Connecticut

Two known enhancers regulate *MyoD* expression in the mouse: the core enhancer (CE) at -20 kb relative to the transcriptional start site (TSS), and the distal regulatory region (DRR) at -5 kb. Previous studies showed that neither the CE nor DRR is necessary for *MyoD* expression during development. To address enhancer redundancy, we developed two double knockout (dKO) mouse models in which both the CE and DRR were knocked out, with or without deletion of the 15 kb of genomic sequence between these enhancers. In both dKOs, the expression of *MyoD* represented an additive effect of the single KOs, characterized by delayed initiation of *MyoD*

expression in the forelimb buds and branchial arches, and decreased overall staining intensity. By E12.5, however, the mutants closely resemble their wild-type counterparts, demonstrating that the CE and DRR together are not necessary for *MyoD* expression and indicating the existence of additional *MyoD* regulatory elements. We used precision run-on sequencing (PRO-seq) on cultured myoblasts to predict novel enhancer regions by identifying genomic regions that exhibit common hallmarks of enhancer regions. PRO-seq data identified three candidate enhancer regions within 96 kb of the *MyoD* TSS. We developed transgenic lines to test the ability of these putative enhancer regions to drive *MyoD*-like reporter gene expression in embryos. Two of these regions recapitulated key aspects of *MyoD* expression during development, and genetic studies suggested that they may be targets of auto- and cross-regulatory mechanisms. These data indicate that at least four regulatory regions control murine embryonic *MyoD* expression.

O-3 - Regulation of Muscle Stem Cell Dynamics and Quiescence by Netrin-Cytoskeleton Signaling

Hsiao-Fan Lo¹, David Sachs¹, Robert Krauss¹

¹Icahn School of Mount Sinai

Muscle stem cells (MuSCs) are the source of skeletal muscle's regenerative properties. The hallmark property of MuSCs at homeostasis is quiescence. Quiescent MuSCs have long, elaborate, cellular protrusions, which retract upon activation after injury. These protrusions are heterogeneous, and cells with long protrusions display features of deeper quiescence than those with short or no protrusions. The morphology and cytoskeletal architecture of quiescent MuSC protrusions resemble that of pathfinding axons, suggesting that they are dynamic structures that act as sensors of the MuSC niche. We hypothesized that niche factors that signal directly to the MuSC cytoskeleton via the small GTPase, Rac1, stimulate protrusion outgrowth and thereby also promote MuSC quiescence. To identify such factors, we developed an *ex vivo* live imaging assay involving short-term culture of muscle bundles. Using this assay, we identified the axonal chemoattractant Netrin-1 as a stimulator of MuSC protrusion outgrowth. Netrin-1 is expressed by muscle-resident FAPs, tenocytes, and endothelial cells, and MuSCs express the Netrin-1 chemoattractant receptors, Dcc and Neo1. These receptors promote axon outgrowth by signaling via Rac1 to the actin-branching factor Arp2/3. Using genetic and small molecule inhibitor approaches, we find that MuSC protrusion outgrowth induced by Netrin-1 *ex vivo* is dependent on Dcc, Neo1, Rac1 and Arp2/3. Conditional genetic removal of *Dcc*, *Neo1*, and *Rac1* from MuSCs *in vivo* each resulted in shorter protrusions, loss of quiescence, and MuSC attrition. Netrin-mediated regulation of MuSC projection dynamics is therefore required for maintenance of quiescence, and guidance cues represent a new class of MuSC quiescence regulator.

O-4 - A myofiber-embedded hydrogel system reveals that constitutive FGFR1 activation restores integrin- β 1-mediated fibronectin sensing in aged satellite cells

Tze-Ling Chang¹, Alexandra Borelli¹, Thea Zlatkov¹, Kristi Anseth¹, Bradley Olwin¹

¹University of Colorado Boulder

Muscle satellite cells (SCs), essential for skeletal muscle maintenance and repair, decline in number and function with age, contributing to sarcopenia. Although intrinsic changes in SCs during aging are well-documented, how SCs from young and aged mice differentially respond to extracellular matrix (ECM) signals is understudied. We encapsulated myofibers in a synthetic viscoelastic hydrogel that mimics the native microenvironment and supports tunable densities of fibronectin-derived RGD ligands to assess SC responses. Encapsulated myofibers preserved SC polarity, SC-myofiber-matrix interactions, and SC stemness as well as preventing myofiber hypercontraction. Increasing RGD density activated SCs from young mice and promoted their proliferation, whereas SCs from older mice failed to respond. In SCs from young mice, polarized FGFR1 co-localized with syndecan-4 and integrin- β 1, key receptors for matrix sensing. In contrast, FGFR1 in SCs from aged mice neither polarized nor co-localized with syndecan-4 and integrin- β 1. Activating integrin- β 1 in SCs from young mice promoted asymmetric division, while ectopically activating FGFR1 and integrin- β 1 promoted symmetric expansion. Expressing constitutively active FGFR1 in SCs from aged mice restored FGFR1 polarization, enabled receptor co-localization, rescued self-renewal, and enhanced fibronectin responsiveness. Using our *in vitro* model, we demonstrated that FGFR1 activation combined with high RGD density amplified symmetric expansion and increased the number of MyoD+/Pax7- committed myoblasts. Thus, FGFR1 integrates RGD and FGF signals, identifying the ECM as a potential therapeutic target to enhance SC function and regeneration in aging muscle.

O-5 - Role of the TAK1 signaling pathway in FAP in tissue remodeling

Marine Theret¹, Fabio Rossi¹

¹UBC

Fibro-adipogenic progenitors (FAPs) have been described as key players of skeletal muscle homeostasis at resting state, during regeneration, and when repair occurs. We and others have shown that inflammatory cell-derived factors modulate the survival of FAPs as well as their ability to acquire a fibrogenic phenotype, at the same time limiting the activity of the myogenic progenitors driving. This is a reciprocal interaction as FAPs also provide beneficial support toward infiltrating cells, orchestrating the inflammatory response. Yet, little is known on which cellular pathway are required for FAP immunomodulatory function. Of interest, the TGF β -Activated Kinase 1 (TAK1) pathway is a key component of various inflammatory responses.

Deletion of TAK1 in adult FAPs strongly affected their behaviour by turning on a systemic type 2 inflammatory response. Despite this supposedly pro-regenerative environment, skeletal muscle regeneration (fusion, myofiber growth) was delayed. This was potentially due changes in FAP fate, as they pre-committed to the fibrogenic lineage and stopped proliferating, which dampened their pro-myogenic functions. While a better understanding of the relationship between FAP immuno-response and their fate is needed, our central hypothesis is that TAK1 in FAPs is a key-signaling node regulating both functions. Preliminary data suggests that the TAK1-NF-kB signaling governs the inflammatory response while the TAK1-SMAD pathway may regulate their lineage commitment. Here, we confirm that skeletal muscle homeostasis relies on a multiple-cell interactome that needs to be efficiently orchestrated. Beyond acute injury, the immunomodulatory functions of FAPs are of importance as they may participate in the development of muscle pathologies.

O-6 - Small molecule augmentation of Notch signaling rescues models of DMD and FSHD

Duc Dong¹, Shiv Kumar¹, Joseph Lancman¹, Sophie Hao¹

¹Scintillon Research Institute

Loss of Notch signaling in MuSCs impairs muscle regeneration and can cause muscular dystrophy. Conversely, increased Notch signaling rescues DMD dogs and DMD zebrafish, and more recently in DMD mice. Genetic induction of Notch signaling was shown to promote MuSC renewal, increase expression of the MuSC master regulator Pax7, and improve regeneration and exercise performance in DMD mice. Here, we investigate if a pharmacological augmentation of Notch signaling will have analogous outcomes to indicate therapeutic potential for DMD. Our studies reveal that *in vivo* treatment with a Notch agonist increases Notch activity in Pax7+ satellite cells and rescues locomotor behavior in DMD zebrafish. Importantly, this compound is orally available in adult mice, reaching limb and diaphragm muscles to increase Notch signaling targets, including *Hes1* and *Pax7*, as well as heart muscles to increase, *TRF2* and the *Dystrophin* compensatory gene, *Utrophin*, both previously shown to be individually sufficient to resolve DMD heart pathologies. Consistently, treatment of primary MuSCs with this Notch agonist increases key stem cell genes including *Pax7*, *HeyL*, *Col5a1*, *Trf2*, and *Sprouty1*, as well as Utrophin protein. Moreover, in line with published genetic approaches showing that increased Pax7 can prevent DUX4 myoblast cytotoxicity, treatment of mouse myoblasts with very high Dux4 expression, or FSHD patient myoblasts, with our Notch agonist robustly prevents apoptosis and enhances maturation into myotubes. Consistently, treated FSHD myoblasts show increased expression of Pax7 and anti-apoptosis genes. Together, these findings suggest therapeutic potential for a pharmacological Notch agonist in treating both DMD and FSHD.

O-7 - Modulation of the JAK2-STAT3 pathway promotes expansion and maturation of human iPSCs-derived myogenic progenitor cells

Luca Caputo¹, Cedomir Stamenkovic¹, Alessandra Cecchini¹, Matt Tierney², Monica Nicolau¹, Gabriele Guarnaccia¹, Jesus R Barajas¹, Maria Sofia Falzarano³, Rhonda Bassel-Duby⁴, Alessandra Ferlini³, Eric N Olson⁴, Pier Lorenzo Puri¹, Alessandra Sacco¹

¹Sanford Burnham Prebys, ²The Rockefeller University, ³University of Ferrara, ⁴University of Texas Southwestern Medical Center

Generation of *in vitro* human induced pluripotent cells (hiPSCs)-derived skeletal muscle progenitor cells (SMPCs) holds great promise for regenerative medicine for skeletal muscle wasting diseases, as for example Duchenne Muscular Dystrophy (DMD). While multiple approaches have been described to obtain SMPCs *in vitro*, hiPSCs-derived SMPC generated using transgene-free protocols are usually obtained in a low amount and resemble a more embryonal/fetal stage of differentiation. Here we demonstrate that modulation of the JAK2/STAT3 signaling pathway in myogenic cultures with AG490 generates a population of SMPCs able to engraft *in vivo* and contribute to skeletal muscle regeneration. Furthermore, using a single purification marker, CD54, we are able to efficiently purify a population of SMPCs that are able to engraft in recipient mice and contribute to skeletal muscle regeneration. Furthermore, we show that upon modulation of JAK/STAT signaling pathway, in controlled *in vitro* conditions, CD54+ SMPCs present a transcriptional signature that more closely resemble the one of late fetal MuSCs (Stage 4 of the developmental trajectory). In line with this finding, we observed that SMPCs from AG490-treated cultures showed higher engraftment ability based on the detection of hDYS+ myofibers. Importantly, we could observe hDYS+ myofibers even when unpurified AG490 myogenic population was transplanted in recipient animals. These findings reveal that modulation of the JAK2/STAT3 signaling pathway is a potential therapeutic avenue to generate SMPCs *in vitro* addressing two major bottlenecks in the field of cell-therapy, increasing the yield of *in vitro* generated hSMPCs and promoting their maturation towards late fetal/adult human MuSCs.

O-8 - Creation of a stem cell-derived neuromuscular junction for disease modeling and drug discovery

Jacob Fleming¹, Molly McCloskey², changho chun², Juyoung Seong², Nicholas Geisse¹, Alec Smith², David Mack²

¹Curi Bio, Inc., ²ISCRM University of Washington Medicine

The neuromuscular junction (NMJ) has been widely studied using animal models, but species differences limit their utility for disease modeling and drug discovery. Challenges with scarcity, scalability, throughput, and ethics further undermine the suitability of animal models for preclinical validation studies. Engineered tissue models have emerged as alternatives for studying NMJ functionality in response to genetic and/or pharmacological challenges. However, these models face challenges associated with their poorly scalable creation, sourcing suitable cells, and the extraction of reliable, quantifiable metrics. We present a turnkey iPSC-based model of the NMJ employing channelrhodopsin-2 expression within the motor neuron (MN) population driving muscle contraction in response to blue light. MNs co-cultured with engineered skeletal muscle tissues produced twitch forces of $34.7 \pm 22.7 \mu\text{N}$ in response to blue light, with a response fidelity > 92%. Histological analysis revealed characteristic punctate acetylcholine receptor staining co-localized with the presynaptic marker synaptic vesicle protein-2. Dose-response studies using botulinum neurotoxin showed loss of function in a dose- and time-dependent manner ($\text{EC}_{50} = 0.11 \pm 0.015 \mu\text{g}$). Variability of the EC_{50} values between 2 different iPSC differentiations of both cell types and 2 users was less than 2%. Further testing with the acute neurotoxins acetylcholine mustard and d-tubocurarine validated the biological relevance of the postsynaptic machinery of the model. This model marks a meaningful progression of 3D engineered models of the NMJ, providing engineered tissues at a throughput suitable for potency and screening applications with abundant iPSC cell sources and standardized hardware-software ecosystem allowing technology transfer across laboratories.

O-9 - Editing of Postmitotic Muscle and Satellite Cells for Continuous Correction of Dystrophin Expression in Duchenne Muscular Dystrophy

Niclas Bengtsson^{1, 2, 3, 4}, Stephen Hauschka^{2, 3, 4, 5}, Nuoying Ma^{1, 2, 3, 4}, Quynh Nguyen^{1, 2, 3, 4, 5}, Jeffrey Chamberlain^{1, 2, 3, 4, 5, 6}

¹Department of Neurology, ²Senator Paul D. Wellstone Muscular Dystrophy Specialized Research Center Seattle, ³Institute for Stem Cell and Regenerative Medicine, ⁴University of Washington, ⁵Department of Biochemistry, ⁶Department of Medicine

Our longtime research focuses on correcting dystrophin expression and pathophysiology in Duchenne muscular dystrophy (DMD) via adeno-associated virus (AAV)-mediated gene therapy. An integral part of this effort involves use of muscle-specific gene regulatory cassettes (RCs) to

express therapeutic proteins delivered to muscles body-wide using AAV vectors. RCs developed by our group have been used to restore dystrophin expression and function in cardiac and post-mitotic skeletal muscles via gene replacement, and by editing the endogenous dystrophin gene. However, the lifelong workloads of skeletal muscle may cause therapeutic efficacy to decrease over time due to muscle remodeling in response to growth, exercise, or trauma. Thus, therapeutic longevity could benefit greatly from effective and safe editing of satellite cells (SCs) to ensure a lifelong supply of skeletal muscle myonuclei that express functional dystrophins.

We have designed promising AAV-size-compatible RCs with preferential activities in SCs (SCRCs). These SCRCs are comprised of highly conserved regulatory regions from the human *PAX7* and *MYOD1* genes and exhibit a range of activities in primary SC-derived myoblasts *in vitro* and in FACS-isolated SCs from wildtype versus dystrophic mice. *In vivo*, top SCRC candidates display varied myogenic transcriptional patterns, ranging from preferential activity in SCs to more widespread activity in SCs and postmitotic striated muscle. Importantly, they show promise for systemic targeting of SCs in dystrophic mouse muscles.

Ultimately, efficient gene correction in SCs constitutes an important element for addressing current limitations of skeletal muscle gene therapies aiming to treat DMD and potentially many other genetic muscle diseases.

O-10 - More Than a Bystander: Intramuscular Adipose Tissue Actively Impedes Muscle Regeneration

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The differentiation of fibro/adipogenic progenitors (FAPs) into adipocytes drives intramuscular adipose tissue (IMAT) accumulation in skeletal muscle pathology. While correlated with functional decline, whether IMAT is a passive biomarker or an active pathogenic agent has remained unclear. We investigated the functional impact of IMAT by genetically modulating the FAP-to-adipocyte fate switch. First, we created a “mFATBLOCK” mouse model where FAP-specific deletion of *Pparγ* prevents their differentiation into adipocytes. Following an adipogenic injury, blocking IMAT formation in these mice led to more efficient muscle regeneration and significantly improved functional recovery. Mechanistically, IMAT acts primarily as a physical barrier, preventing myofiber formation and restricting their growth, rather than operating through altered paracrine signaling. We also developed a “mFATGAIN” model, where FAP-specific overexpression of *Pparγ* increased adipocyte differentiation. Applying both models to hindlimb ischemia (HLI), a model for peripheral artery disease (PAD), our preliminary results show that increasing IMAT worsened muscle strength; while preventing it improved functional outcomes. Together, these studies establish IMAT as an active contributor to muscle dysfunction. Our results highlight a novel, detrimental structural role for adipocytes in the regenerative muscle niche, where their physical presence—distinct from their endocrine function—can override or obstruct the intercellular communication necessary for effective repair. This demonstrates that

IMAT physically impedes repair and that targeting the FAP-to-adipocyte fate switch represents a promising therapeutic avenue to enhance muscle function.

O-11 - Aged Matrix Stiffness Disrupts Muscle Stem Cell Regeneration via RNA Surveillance

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Skeletal muscle regeneration relies on muscle stem cells (**MuSCs**) progression along a myogenic continuum, a process that falters with aging. One key culprit is age-related extracellular matrix (**ECM**) stiffening, driven by excessive collagen crosslinking, which disrupts MuSC fate. However, the mechanisms by which ECM stiffening governs MuSC fate remain elusive. Using matrices engineered to mimic the stiffness of young versus aged muscle, we found that aged MuSCs exhibited hypersensitivity to ECM biophysical cues. In contrast, young MuSCs were resilient, regardless of substrate stiffness. Modulation of aged muscle stiffness by reducing collagen crosslinking promoted regeneration, as evidenced by increased force recovery, larger regenerating fibers, and more Pax7+ MuSCs, compared to untreated aged muscle. Single-cell RNA-sequencing of young and aged MuSCs seeded onto soft or stiff substrates revealed no significant differences in static gene counts. However, dynamic modeling of transcriptomic vector fields using RNA velocity revealed altered RNA surveillance with aging, with nonsense-mediated decay implicated as a central pathway underlying aged MuSC hypersensitivity to matrix stiffness cues. Computational modeling showed that heightened activity of the RNA helicase, UPF1, diverts aged MuSC trajectories toward precocious terminal differentiation, while *in silico* inhibition of UPF1 restored MuSC trajectories towards an undifferentiated cell state (i.e., quiescence and cycling). Experimental validation of these computational findings using siRNA-mediated UPF1 knockdown in aged MuSCs on stiff substrates increased cellular Pax7 levels. These findings demonstrate that aged MuSCs lose resilience to biophysical cues through dysregulated post-transcriptional dynamics, suggesting that targeting ECM mechanics and RNA surveillance may restore regenerative capacity.

O-12 - Oncogenic alterations in Twist2 progenitors drive soft tissue sarcomas in vivo

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Soft-tissue sarcomas are rare, aggressive tumors with high recurrence. Although originating from mesenchymal stem cells, soft-tissue sarcomas are diverse, each with distinct characteristics and cells of origin. Rhabdomyosarcoma and undifferentiated pleomorphic sarcoma (UPS) are the most common types in children and adults, respectively. Rhabdomyosarcoma features myogenic cells that fail to differentiate, while UPS consists of undifferentiated mesenchymal cells. Identifying the cell of origin and mechanisms of recurrence can help to uncover therapeutic targets to overcome tumor resistance. We previously discovered a distinct Twist2⁺ progenitor population within skeletal muscle. Here, we investigated whether the Twist2⁺ progenitor could serve as cell of origin of soft-tissue sarcomas and contribute to tumor heterogeneity and recurrence.

We generated two genetically engineered mouse models driven by Twist2-CreERT2, one with p53 deletion and KRAS overactivation (TPKT), and the other with p53 deletion in the mdx background to provide a regenerative microenvironment (TPMT). Our results showed that TPKT mice gave rise to UPS tumors, while TPMT mice generated rhabdomyosarcomas in the Twist2 lineage. We also established Twist2⁺ tumor allografts that recapitulated their original differentiation status. Single-cell RNA-sequencing analysis of the Twist2-driven tumors revealed distinct subpopulations of tumor cells in TPKT and TPMT and the predominance of an immunosuppressive tumor microenvironment. We also identified tumor cell hierarchies that resembled rhabdomyosarcoma patient tumors in the TPMT mouse model. These results highlight an unrecognized role of Twist2⁺ progenitors as a cell of origin for soft tissue sarcomas, providing insights into their heterogeneity, which has implications for patient tumor recurrence.

O-13 - Interrogating the role of fibro-adipogenic progenitors in muscle loss and impaired regeneration in the disease fibrodysplasia ossificans progressiva

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In the rare disease fibrodysplasia ossificans progressiva (FOP), progressive skeletal muscle loss is a debilitating and poorly understood feature accompanying heterotopic ossification (HO). Using mouse models expressing the disease-causing *Acvr1*^{R206H} mutation in fibro-adipogenic progenitors (FAPs), this study reveals that slight injury to the fascia, without affecting the underlying muscle, triggers both HO formation and directional muscle degeneration. Despite the absence of direct muscle damage, FOP mice exhibited a striking loss of muscle fibers directly beneath the fascial lesion, accompanied by cellular infiltrates but lacking signs of muscle regeneration. This degeneration was anatomically confined, temporally progressive, and not associated with adipogenic or fibrotic tissue replacement, distinguishing it from classical

dystrophic or impaired-regeneration models. Histological analysis showed that areas of fiber loss coincided with the accumulation of PDGFR α + FAPs, implicating these cells in driving muscle destruction. In exacerbated models achieved via genetic loss of wild-type *Acvr1* or treatment with the ACVR1 agonist JAB0505, muscle degeneration was markedly more extensive, often obliterating large muscle regions adjacent to growing HO lesions, which followed fascial planes. A lengthened fascial incision spanning the length of the TA produced similar results as genetic and pharmacological interventions. Together with previous work, these data suggest that fascial injury is both necessary and sufficient for formation of HO in FOP and suggest that fascial-resident *Acvr1*^{R206H} FAPs are key mediators of progressive muscle loss. The fascial injury model provides the opportunity to dissect cellular interactions that underlie muscle degeneration and failed regeneration in the context of FOP.

O-14 - CTCF mediated long-term maintenance of skeletal muscle fiber homeostasis by control of chromatin structure

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The regulation of gene expression is tightly controlled at the genomic level through the intricate interplay of promoters and regulatory sequences. However, maintenance of gene expression by chromatin conformation in postmitotic skeletal muscle fibers remains poorly understood. A key player in this process is the CTCF protein, which enables the formation of DNA loop structures, crucial for establishing topologically associated domains (TADs). These TADs are imperative for orchestrating long-range control of cell-specific gene expression, thereby governing cellular identity.

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. Using a mouse model with constitutive deletion of CTCF in myofibers (HSA-cre; CTCF-fl), we observed that the absence of CTCF in myofiber nuclei (myonuclei) leads to progressive atrophy of skeletal muscle myofibers, associated with

inflammation. Employing a multi-omic strategy, we analyzed gene expression using RNA-seq, chromatin accessibility using ATAC-seq, and chromatin conformation using Promoter capture. Our findings indicate that the loss of CTCF protein in myonuclei results in disrupted patterns of chromatin accessibility at promoters of myogenic genes, which dysregulates their expression, impacting muscle homeostasis.

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.

P-1 - How to divide a multinucleated cell - lessons from myofiber splitting of *Drosophila* indirect flight muscle

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While most cell-cell fusion events generate multinucleated cells, most cell division events begin with a mononucleated parent cell that divides into mononucleated daughter cells. It has been a longstanding question as to how a multinucleated cell divides into two daughter cells. Here, we use the splitting of *Drosophila* indirect flight muscle (IFM) as a model to address this question. During metamorphosis, each multinucleated IFM divides into two multinucleated myofibers. Our genetic and live imaging analyses revealed several unexpected cellular events leading to IFM division. First, muscle precursor cells (MPCs) infiltrate the mechanically soft, multinucleated myofiber to form a cell-in-myofiber structure. The infiltration process is facilitated by DE-cadherin-mediated adhesion between MPCs and the myofiber. The infiltrated MPCs then upregulate Notch expression and rapidly proliferate within the myofiber leading to increase the number of cells aligned along the midline of the myofiber. These mononucleated muscle cells exert mechanical forces that push existing myonuclei to opposite edges. Gradually, the plasma membrane of the myofiber is pulled apart around the midline by the mechanical forces, leading to the splitting of the myofiber into two multinucleated daughter fibers. During the splitting process, the muscle cells within the myofiber continue to differentiate and fuse with the inner myofiber membrane, thus adding nuclei to the myofiber and contribute to its growth. Taken together, our study has revealed, for the first time, a novel mechanism of how a multinucleated cell is divided into two daughter cells and highlighted critical functions of cell infiltration, proliferation, and mechanical forces in this process.

P-2 - Single nuclei multiome reference atlas of skeletal muscle through human pediatric development

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Insights from single-cell RNA sequencing have advanced our understanding of skeletal muscle biology, however these data are limited by poor capture of large syncytial myonuclei. Additionally, existing datasets primarily profile adult or diseased tissues. Human muscle formation and maturation is comparatively understudied by such methods yet is critical for understanding a variety of pediatric muscle diseases. We generated a single nucleus multiomic

dataset of healthy pediatric skeletal muscle, using joint profiling of transcriptomes and chromatin accessibility.

We collected 28 pediatric muscle samples from both biopsy and autopsy sources and performed 10X's Epi Multiome ATAC + Gene Expression assay. After ambient RNA correction, doublet detection and filtering of low-quality cells, we obtained a dataset of over 130,000 nuclei, across a broad range of cell types. Integrated multimodal analysis revealed robust representation of myogenic populations and highlighted changes in cell type composition with development stage. Compared with existing single-cell datasets, our approach yielded improved myonuclear capture, enabling detailed characterization of fiber type specific transcriptional programs. Paired ATAC data enabled regulatory inference by linking peaks to gene expression to uncover cell-type specific chromatin accessibility landscapes and regulatory mechanisms underlying myogenesis.

This work provides a valuable reference atlas of human pediatric skeletal muscle development. By linking gene expression to underlying chromatin regulation, our dataset offers new insights into normal muscle development and serves as a foundation for studying pediatric muscle disorders, regeneration and aging.

P-3 - CHAMP1 is an essential regulator for human myoblast fusion and muscle development

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Human skeletal muscle is composed of myofibers, each formed by the fusion of thousands of individual myoblasts. This process requires tightly regulated expression of muscle-specific fusogens, which confer both efficiency and muscle specificity to cell-cell fusion. Currently, the genetic regulation of this process remains poorly understood. Here, we identify CHAMP1, a chromosome alignment factor, as a surprising upstream activator of human myoblast fusion. Genetic deletion of CHAMP1 in human myoblasts led to profound fusion defects in vitro and in vivo following transplantation. Leveraging genomics approach and protein interaction assays, we uncovered a noncanonical role for CHAMP1 as a transcriptional cofactor for MyoD, directly activating the expression of the key muscle fusogen Myomaker. CHAMP1 mutations in human patients cause developmental delay, hypotonia, and muscle weakness. Importantly, CHAMP1 patient-derived cells exhibit pronounced fusion defects that can be fully rescued by restoring Myomaker expression. Structure and function analysis identified C2H2-type zinc finger motifs on CHAMP1 protein that are both necessary and sufficient for interaction with MyoD, activation of Myomaker transcription and human myoblast fusion. These findings highlight the muscle-autonomous role of CHAMP1 in muscle development and disease, and point to therapeutic avenues for treating muscle development defect caused by CHAMP1 mutations.

P-4 - Removal of *dpm3* in zebrafish leads to impaired neuromuscular development and failure to thrive

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Dystroglycanopathies lead to progressive neuromuscular degeneration and are frequently lethal. Healthy muscle requires the development of neuromuscular junctions (NMJs), which contain cell-matrix adhesion complexes replete with glycosylated proteins, for neuromuscular crosstalk to occur. In the context of dystroglycanopathies, which disrupt glycosylation of Dystroglycan, it is still unknown whether muscle tissue or neural tissue degenerates first, driving disease progression. An important question that remains unanswered is which tissue or tissues that express genes associated with these diseases should be targeted to mitigate disease progression. The ability to address this question has been hindered by the lack of viable vertebrate models to facilitate the discovery of tissue-specific impacts of disrupted glycosylation on cell-matrix adhesion throughout embryonic development and disease progression. To address this, we have generated a zebrafish model through CRISPR/Cas9 gene editing of a known dystroglycanopathy gene, *dpm3*, which assists in initial steps of multiple glycosylation events. Our model exhibits early NMJ defects resulting in failure to thrive and impaired growth. Through tissue specific gene expression studies, we have determined that one aspect of NMJ development is dependent on expression of *dpm3* in muscle while other aspects are not, suggesting that *dpm3* is necessary in multiple tissues including muscle for healthy NMJ formation. Ultimately, we aim to elucidate which tissues require *dpm3* expression to understand the basic biology underlying abnormal neuromuscular development in dystroglycanopathies, serving as a basis for identifying future therapeutic targets and significantly impacting the field of neuromuscular development and homeostasis.

P-5 - Human FKRP mutant satellite cells display defective muscle regeneration

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Mutations in the *fukutin-related protein* (FKRP) gene cause a spectrum of muscular dystrophies, including limb-girdle muscular dystrophy (LGMD2I/LGMDR9), Walker-Warburg Syndrome (WWS), and muscle-eye-brain disease (MEB). These mutations impair the glycosylation of α -

dystroglycan, leading to compromised membrane integrity and progressive muscle degeneration. While satellite cells are critical for muscle regeneration, their role and molecular profile in the context of FKRP mutations remain poorly understood. To investigate FKRP's role in human muscle regeneration, we utilized human induced pluripotent stem cell (iPSC)-derived myogenic progenitors, which can repopulate the satellite cell compartment and support long-term regeneration. We generated an iPSC line harboring a WWS-associated FKRP mutation alongside its isogenic control. Upon transplantation into irradiated and cardiotoxin-injured tibialis anterior muscles of NSG mice, WWS mutant myogenic progenitors exhibited reduced myofiber engraftment compared to controls, with no significant difference in satellite cell frequency. Secondary transplantation using equal numbers of FACS-purified human satellite cells again showed impaired regeneration in the WWS group. These findings suggest that FKRP mutations compromise satellite cell regenerative function rather than abundance. To explore underlying mechanisms, we performed transcriptional profiling of donor-derived satellite cells. WWS mutants showed significant upregulation of monosaccharide metabolism processes and downregulation of Golgi complex organization, intracellular transport, and glycoprotein biosynthesis pathways. Collectively, our study provides the first in vivo evidence that FKRP mutations impair satellite cell function and identifies disrupted molecular pathways potentially contributing to WWS pathology. These insights enhance our understanding of disease mechanisms and may inform future therapeutic strategies for FKRP-related muscular dystrophies.

P-6 - Regulating Stem Cell Fate: From Biophysical Cues to DNA Methylation

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Skeletal muscle stem cells, also called Satellite cells (SCs), reside in a mechanically dynamic niche, where they interpret biophysical cues to maintain quiescence. We show that substrate stiffness and RhoA-dependent signaling converge to regulate SC fate through epigenetic mechanisms. SCs cultured on soft matrices or depleted of RhoA adopt altered morphology, lose cytoskeletal integrity, and prematurely transition from quiescence to activation. RhoA loss reshapes the DNA methylation landscape, alters exon usage and isoform expression, and reduces expression of the de novo DNA methyltransferase Dnmt3a at both transcriptional and translational levels. Mechanistically, RhoA maintains Dnmt3a expression by inhibiting a kinase that phosphorylates and inactivates SP1, a transcription factor required for Dnmt3a transcription. Direct loss of Dnmt3a in quiescent SCs similarly drives activation, establishing Dnmt3a as a key epigenetic effector of mechanical signaling. Together, these findings uncover a novel mechanotransduction to epigenetic axis in which RhoA sustains DNA methylation programs to preserve SC quiescence.

P-7 - A cell-autonomous function of DLL1 in satellite cells

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Notch signaling is essential for embryonic and postnatal myogenesis, but the role of various ligands and receptors are poorly understood. Using conditional knockout and over expression approaches, we identify a cell-autonomous role of DLL1 intracellular domain in muscle satellite cell maintenance and regenerative function.

P-8 - Expression of a disease-causing BMP receptor gain-of-function allele in muscle stem cells impairs skeletal muscle regeneration

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Fibrodysplasia ossificans progressiva (FOP) is a rare and debilitating disease caused by dominant mutations in the gene that encodes the bone morphogenetic protein (BMP) receptor, ACVR1. The most prevalent mutation in FOP patients results in an arginine-to-histidine amino acid substitution, which is a gain-of-function mutation that renders the receptor responsive to activin A. Consequently, this neo-function results in overactive BMP signaling in cells that express ACVR1(R206H). We and others have demonstrated that fibro-adipogenic progenitor cell-specific or global expression of ACVR1(R206H) leads to heterotopic ossification and impairs skeletal muscle regeneration. Here, we directly target satellite cells for ACVR1(R206H) expression to test whether dysregulated BMP signaling affects muscle regeneration cell-autonomously. We show that skeletal muscle regeneration is delayed at early post-injury stages, but regenerated muscle resembled controls by day 14 post-injury. Regeneration deficits are exacerbated by genetically increasing the level of ACVR1(R206H) signaling, and this impaired regeneration is accompanied by fat and fibrotic accumulations. Lineage tracing showed that these non-myogenic cell accumulations are not of satellite cell origin. Additionally, exacerbated ACVR1(R206H) signaling can result in the accumulation of satellite cell-derived heterotopic bone under some conditions. We also show that cultured ACVR1(R206H)-expressing satellite cells exhibit impaired differentiation, particularly in the presence of exogenously added activin A. Collectively, these data demonstrate that dysregulated BMP signaling affects satellite cell function cell-autonomously and that expression of ACVR1(R206H) in both fibro-adipogenic progenitors and satellite cells contributes to FOP pathogenesis.

P-9 - Branched actin polymerization drives invasive protrusion formation to promote myoblast fusion during skeletal muscle regeneration

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Skeletal muscle regeneration is a multistep process involving the activation, proliferation, differentiation, and fusion of muscle stem cells, known as satellite cells. The fusion of satellite cell-derived mononucleated muscle cells (SCMs) is indispensable for the generation of multinucleated, contractile myofibers during muscle repair. However, the molecular and cellular mechanisms underlying SCM fusion during muscle regeneration remain poorly understood. In this study, we uncovered an essential role for branched actin polymerization in SCM fusion. Using conditional knockouts of the Arp2/3 complex and its actin nucleation-promoting factors, N-WASP and WAVE, we demonstrated that branched actin polymerization is required for the SCM fusion, but not for satellite cell proliferation, differentiation, and migration. We showed that the N-WASP and WAVE complexes have partially redundant functions in regulating SCM fusion. Furthermore, we revealed that branched actin polymerization is essential for generating invasive protrusions at the fusogenic synapses in SCMs. Taken together, our study has identified new components of the myoblast fusion machinery in skeletal muscle regeneration and demonstrated a critical role for branched actin-propelled invasive protrusions in this process.

P-10 - The Role of Notch Oscillatory Synchronization in the Stem Cell Self-Renewal Decision

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How tissues achieve the correct ratio of cell types during regeneration is an important open question. We study this in the context of muscle regeneration where, in response to injury, muscle stem cells (MuSCs) activate, proliferate, differentiate, and then fuse to form new multinucleated myofibers. Intriguingly, a small fraction of the activated cells self-renew into stem cells, ready to repair future injuries. The fraction of cells that self-renew is carefully controlled at the population level to balance restoration of tissue function and future regenerative capacity. Decades of work has shown that proliferating cells use the Notch pathway to decide whether to become Notch-high (self-renew) or Notch-low (differentiate) and through lateral inhibition adjacent cells choose opposite fates. However, recent work has shown that the key Notch signaling factor Hes1 rapidly oscillates in proliferating stem cells (every 3 hrs) and that it directly represses the myogenic program. The synchronization of these rapid oscillations may be key to how cells are able to robustly compute the appropriate cell ratios. We are using timelapse microscopy and live-cell biosensors to visualize Hes1 oscillations during regeneration and link

them with cell fate decisions. Additionally, we are using optogenetics to specifically manipulate the temporal properties of the oscillations to understand their causative role in MuSC decision-making. Ultimately, we are interested in how these oscillations are affected by the aging process and how they contribute to the aging-related decline in stem cell self-renewal.

P-11 - A Non-Canonical Intracellular Function of ADAMTS1 in Regulating Muscle Stem Cell Quiescence

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Muscle stem cells (MuSCs) typically reside in a quiescent state, unless activated by external stimuli like muscle injury. Maintenance of this quiescent state is required for the long-term preservation of the functional MuSC pool and for ensuring proper muscle regeneration; however, the metabolic regulation of quiescence is largely unknown. Here, we found that expression of ADAMTS1, an extracellular matrix metalloproteinase, is high in quiescent MuSCs and diminishes upon activation. We generated an inducible MuSC-specific ADAMTS1 knockout mouse model and found that the absence of ADAMTS1 leads to upregulation of genes related to cell division and proliferation in MuSCs and results in spontaneous MuSC activation. Through immunoprecipitation-mass spectrometry (IP-MS) and AlphaFold protein-protein interaction predictions, we identified phosphofructokinase muscle isoform (PFKM), a key glycolytic enzyme, as a top interacting partner of ADAMTS1 in MuSCs. Our findings show that ADAMTS1 regulates PFKM tetramer formation and phosphofructokinase (PFK) activity, suggesting that ADAMTS1 may influence MuSC quiescence by regulating metabolic processes through its interaction with PFKM. We identify a significant intracellular role of ADAMTS1 beyond its canonical roles in ECM remodeling and extracellular functions. Together, our studies establish ADAMTS1 as a regulator of MuSC quiescence through metabolic control.

P-12 - Roles of Rho family GTPase signaling in muscle stem cell quiescence and activation

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Muscle stem cells (MuSCs) are quiescent during homeostasis, but upon injury activate, proliferate, and differentiate to repair damaged myofibers. Quiescent MuSCs possess cellular projections that retract upon MuSC activation. The quiescence-to-activation (Q-A) transition is driven by a Rac1-to-RhoA GTPase activity switch; Rac1 promotes outgrowth of cytoplasmic projections, while RhoA drives projection retraction and downstream activation events. *Rac1* is required for quiescence in a gene dosage-sensitive manner. High RhoA activity is required for activation, but *RhoA* is also required for quiescence in a gene dosage-sensitive manner. To

address this dichotomy, MuSC-specific *RhoA* conditional knockout (cKO) mice were assessed by a combination of *in vivo* and *ex vivo* analyses. Removal of one or both copies of *RhoA* led to MuSC activation, but a higher percentage of *RhoA* heterozygous MuSCs were activated than *RhoA* homozygotes. These results indicate that both alleles of *RhoA* are needed for maintenance of quiescence *and* for complete activation. Rac1 and RhoA signal in a mutually antagonist way. That both *Rac1* and *RhoA* heterozygous cKO MuSCs are activated, raised the possibility that heterozygosity of either causes an imbalance in Rac-Rho signaling that is sensed by MuSCs as a quiescence-breaking perturbation of the system. We report that fewer MuSC-specific *Rac1*;*RhoA* double-heterozygous MuSCs are activated than single *Rac1* or *RhoA* heterozygotes, indicating that rebalancing *Rac1* and *RhoA* recreates a more quiescent equilibrium. This equilibrium likely underlies dynamic cytoskeletal regulation that is responsive to niche signals during homeostasis, while also promoting rapid responses to injury that are essential for regeneration.

P-13 - Modulation of Muscle Stem Cell Dynamics through Fibro/Adipogenic Progenitors-mediated Paracrine Signaling during Muscle Repair

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Skeletal muscle regeneration relies on coordinated interactions between muscle stem cells (MuSCs) and their supporting niche cells to restore tissue function after injury. Among the key niche populations, fibro/adipogenic progenitors (FAPs) emerge as critical regulators of MuSC dynamics through secreted paracrine factors. Following muscle injury, FAPs rapidly proliferate and create a supportive microenvironment that subsequently promotes MuSC expansion. As regeneration progresses, MuSC numbers peak while FAPs undergo programmed cell death, suggesting a temporally regulated communication network. To investigate this crosstalk, we demonstrated that conditioned medium from FAPs isolated from injured muscle significantly enhanced MuSC proliferation both *in vitro* and *ex vivo*, confirming that FAPs control MuSC proliferation through paracrine signaling during early regeneration phases. Integrated proteomics analysis of FAP conditioned medium and transcriptomic profiling of treated MuSCs identified a spectrum of proteins with potential roles in promoting MuSC proliferation. These

findings reveal multiple candidate mediators of FAP-MuSC communication and highlight promising molecular targets for strategies aimed at enhancing skeletal muscle repair. Elucidating the specific mechanisms of these proteins may open new avenues for therapeutic interventions in muscle injuries, age-related muscle loss, and degenerative myopathies.

P-14 - Understanding Sex-Based Divergence in Muscle Stem Cell Function and Aging

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Women are known to be more susceptible to sarcopenia and frailty with age. Recent longitudinal studies show that, across ethnic groups, women experience an accelerated loss of lean mass during the menopausal transition. They are also at higher risk for musculoskeletal aging-related diseases, including osteoporosis and autoimmune conditions such as rheumatoid arthritis. Understanding the mechanisms underlying muscle loss in women is therefore critical to improving health outcomes during aging. Sexual dimorphism in skeletal muscle regeneration has been suggested since the 1990s. However, most studies in females have relied on hormone-manipulated animal models—such as ovariectomy or estrogen receptor knockout—to examine estrogen's role in muscle regeneration and muscle stem cell (MuSC) function. Few studies have directly compared MuSC activation between males and females. To address this gap, we applied a multiomics approach to profile sex-specific differences in skeletal muscle and MuSCs from young and old male and female mice. Preliminary analyses revealed sex-specific responses to exercise in aged muscle metabolism. Transcriptomic profiling of MuSCs further showed that old female mice exhibit heightened inflammatory signatures and altered proportions of inflammation-associated MuSC subpopulations compared to males. This work lays the foundation for uncovering the epigenetic and metabolic mechanisms regulating MuSC quiescence and activation across sexes, with the ultimate goal of understanding and addressing sex differences in muscle aging.

P-15 - Myogenin directs muscle stem cell fate during muscle regeneration

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Muscle stem cells (MuSCs) replenish their population through self-renewal during regeneration. However, the molecular mechanisms that regulate MuSC self-renewal remains incompletely understood. Here, we identified that a population of progenitors co-expressing *Pax7* and myogenin (*Myog*) that emerges during early muscle regeneration. Single cell RNA sequencing and genetic lineage tracing revealed that, unexpectedly, *Myog*⁺ progenitors can give rise to self-

renewed MuSCs after injury. Conditional deletion of *Myog* increased MuSC self-renewal capacity without impairing differentiation. Using Cut&Tag and single nuclei RNA sequencing, we further identified the G protein-coupled receptor ADGRE5(CD97) as the downstream target of *Myog*. Blocking CD97 both in vitro and in vivo attenuated the elevated MuSC self-renewal associated with *Myog* deficiency. Together, our study reveals a noncanonical role of myogenin in cell fate decisions and regulating the MuSC pool.

P-16 - FGF Receptor 1 Drives Muscle Stem Cell Self-Renewal via Asymmetric Division

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Skeletal muscle, vital for movement, breathing, and reproduction, relies on a population of rare, typically quiescent muscle stem cells (MuSCs) to maintain and regenerate muscle tissue. Residing between the basal lamina and myofiber membrane, MuSCs activate upon injury or stress, exiting G0 to divide symmetrically or asymmetrically. Daughter cells differentiate to repair muscle or self-renew to sustain the stem cell pool. Impaired self-renewal depletes MuSCs, contributing to muscle loss in aging and neuromuscular diseases including Duchenne Muscular Dystrophy and sarcopenia. Fibroblast Growth Factor Receptor 1 (FGFR1) drives MuSC self-renewal via asymmetric division. FGFR1 polarizes upon ligand binding, asymmetrically activating p38 α / β MAPK to inhibit MyoD mRNA degradation, driving myogenic commitment in one daughter cell, while the other daughter regains quiescence. Impaired FGFR1 signaling abolishes protein polarization, precluding MuSC self-renewal, reducing the MuSC pool, and compromising muscle repair. Ectopically activating FGFR1 *in vivo* in MuSCs from aged mice restores polarization, restores MuSC numbers, and rejuvenates MuSCs to a transcriptionally youthful state. Thus, restoring the ability of FGFR1 to polarize appears sufficient to rescue MuSC self-renewal in aged mice, and the signal to polarize and self-renew emanates from the intracellular kinase domain. However, the mechanisms driving MuSC self-renewal via asymmetric division are incompletely understood. Using a bioengineered tissue platform that recapitulates the MuSC niche and supports self-renewal, the intracellular mechanisms required to polarize FGFR1 and the downstream signaling pathways driving self-renewal will be identified. Understanding the mechanisms driving self-renewal may reveal therapeutic targets to stimulate MuSC production for combating pathological and age-related muscle degeneration.

P-17 - Cell composition and gene-expression dynamics of skeletal muscle regeneration after contraction-induced injury

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Efficient skeletal muscle regeneration is essential for preserving muscle mass, strength, and function, with direct implications for physical performance and quality of life. Impaired regeneration contributes to the disruption of skeletal muscle homeostasis associated with aging and disease, yet the underlying mechanisms remain unclear and effective treatments are limited. Single-nucleus RNA sequencing (snRNAs-seq) has enabled cell-type-specific analysis of muscle regeneration, but most studies rely on myotoxic models where the injury extent surpasses typical muscle contraction-induced damage. In this study, we applied snRNA-seq to human and mouse skeletal muscle after eccentric contraction-induced injury. We generated transcriptomic profiles from 85,009 human and 102,630 mouse nuclei, identifying 13 and 17 distinct cell types, respectively. These included myogenic populations and a range of supportive cell types. Trajectory analysis revealed a continuous transition from quiescent muscle stem cells through committed progenitors to mature myofibers. Known and novel regulators of myogenesis, including heat shock genes, were identified. Cell-cell communication analysis identified fibro-adipogenic progenitors as primary signal senders, particularly toward muscle stem cells, with strongest interactions observed at day 2 post injury. Endothelial and smooth muscle/pericyte cells emerged as major signal receivers. Cross-species comparisons of differentially expressed genes and temporal correlation analyses highlighted conserved transcriptional responses in muscle stem cells. Together, this work provides a comprehensive, cross-species reference of the cellular and molecular events underpinning skeletal muscle regeneration using a physiologically relevant injury model.

P-18 - Long-chain Ceramide Impairs Insulin Signaling in Ovine Muscle Stem Cells Through PP2A Activation

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Ceramide accumulation has been implicated in insulin resistance across species, Its role in ruminant skeletal muscle remains unclear. In this study, we investigated the effects of extracellular long-chain ceramide on insulin signaling in ruminant muscle stem cells (MuSCs). MuSCs were isolated from tissue digests using a nonFACS based method that uses their weak adherence properties to gelatin-coated plates and distinct adhesion kinetics compared to other cell populations. Following ceramide treatment, insulin stimulated glucose uptake was significantly diminished. Western blot analysis revealed reduced phosphorylation of AKT. To

determine whether this effect involved phosphatase activity, we treated cells with okadaic acid (OKA), a potent PP2A inhibitor. OKA restored AKT phosphorylation in ceramide treated cells, indicating that ceramide impairs insulin signaling through PP2A mediated AKT dephosphorylation. These findings provide insights into the cellular mechanisms by which ceramide induces insulin resistance in ruminant muscle, and demonstrate a practical and effective strategy for MuSC isolation without the need for specialized sorting equipment or antibodies.

P-19 - Role of Nesprin2G-Dependent Mechanotransduction in Maintenance of Heterochromatin Landscape and Muscle Stem Cell Function.

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Muscle stem cells are primarily responsible for muscle regeneration. These cells rely on their microenvironment for signals, including changes in mechanical stimuli like muscle stretching, which impact their cellular state and function. The nucleus, through the nuclear membrane complex, translates these external forces into genetic modifications. Mutations of proteins within this complex, such as nesprins, are linked to muscular dystrophies. So, using a C-terminal truncated Nesprin-2 Giant (*SYNE2*^{cpfl8-/-}) mutant, we study how the disruption of continuous propagation of mechanical stimuli to the nucleus affects muscle stem cells. We observed that this led to a shift in the cellular state towards a more activated state and an altered heterochromatin state. In this study, I am investigating how these changes affect stem cell function by inducing muscle injury and assessing its regenerative potential. This study hopes to investigate the interplay between mechanotransduction-regulated heterochromatin changes and stem cell function in associated disorders and aging.

P-20 - Innervation Induces a Distinct Force-Frequency Phenotype in 3D Human Neuromuscular Junction Tissues

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Neuromuscular junction (NMJ) dysfunction underlies many devastating neuromuscular diseases, highlighting the need for clinically relevant human NMJ models. While embedding motor neurons within 3D skeletal muscle (SkM) improves biochemical and contractile properties in primary cell-derived models, comparable benefits in induced pluripotent stem cell (iPSC) systems remain unexplored. Here, we present an iPSC-derived 3D NMJ model showing an advanced contractile phenotype absent from SkM-only tissues.

3D NMJs and SkM-only control tissues were generated from iPSC-derived myoblasts and motor neurons on the Mantarray platform in two independent experiments (n=3/group/experiment). Contractility was assessed every 2-3 days from Day 17 through Day 31 using electrically stimulated twitch and force-vs-frequency (FVF) protocols.

While twitch and tetanus forces were comparable between groups, NMJ tissues exhibited a distinct FVF plateau above 50 Hz, a phenotype absent from SkM-only controls. By Day 31, NMJs showed a 72% reduction in Freq50 (the frequency required to elicit 50% of maximal tetanic force) and a 42% decrease in tetanus-to-twitch ratio. Both differences were statistically significant (t-test; p<.001).

This study demonstrates a robust, previously unreported divergence in the FVF phenotype between SkM-only tissues and NMJs. We hypothesize that this differential FVF response may be due to a shift in myosin isoform and ion channel expression, and our ongoing work will characterize the transcriptomic profiles of SkM-only and NMJ tissues through RNA-seq analysis. This work advances *in vitro* models of the NMJ for high-throughput therapeutic screening, potency testing, and neuromuscular disease modeling.

P-21 - Uncovering the Role of Motor Neuron Rewiring in Cancer-induced Cachectic Muscle Wasting

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Cancer-induced cachexia (CIC) is a systemic muscle-wasting syndrome associated with poor prognosis, reduced treatment tolerance and shortened survival. Despite its prevalence in cancer patients, effective therapies remain elusive. While previous studies have focused on muscle catabolic factors, recent evidence suggests that motor neuron pathology and neuromuscular

junction (NMJ) loss are central to CIC. However, the timing and cellular context of cachectic denervation are poorly understood.

To address this gap, I will use an engineered reversible-C26 colorectal cancer model, developed by our collaborators at CSHL, that enables the controlled study of cachexia onset and recovery, providing an unprecedented opportunity to dissect the dynamics of neuromuscular disruption and insights into the repair process.

I will apply spatial proteomics (CODEX) to profile cellular composition, muscle atrophy heterogeneity, fiber type transitions, and immune infiltration at single-cell resolution; while harness cutting-edge 3D light-sheet fluorescence microscopy (LSFM) to generate unbiased, whole-muscle reconstructions of motor axons and NMJs across the time course of cachexia and recovery. These integrated approaches will reveal how motor and sensory nerves in muscle are remodeled by cachectic de- and re-innervation, uncovering cellular and structural mechanisms that drive cachectic muscle wasting and recovery. Insights gained could lead to cellular targets to preserve or restore neuromuscular connectivity. By integrating innovative models with advanced imaging, this project will provide a foundation for new understanding of the pathophysiology of CIC.

P-22 - Characterizing 3D Muscle Tissues to Accelerate Novel Target Discovery and Advance Disease Models

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Three-dimensional engineered muscle tissues (EMTs) bridge the gap between traditional monolayer cultures and native tissues by better recapitulating the in vivo microenvironment. This study evaluates the molecular fidelity of iPSC-derived EMTs generated on the Curi Bio Mantarray platform to validate their utility for target discovery and disease modeling. We constructed EMTs using control and Duchenne Muscular Dystrophy (DMD) iPSC-myoblasts co-cultured with human dermal fibroblasts. Functional assessment revealed significant reductions in contractile force and velocity metrics in DMD EMTs compared to controls. Proteomic analysis demonstrated decreased expression of oxidative metabolism proteins in DMD EMTs, while proteins involved in calcium regulation, extracellular matrix organization, and glycolysis were upregulated. Comparative analysis with a published human DMD muscle tissue proteome (Capitanio, D. et al., *Journal of cachexia, sarcopenia and muscle*, 11(2), 547-563, 2020) revealed strong overlap of dysregulated pathways despite some differences in specific protein regulation patterns. These findings confirm that DMD EMTs effectively recapitulate both the contractile deficits and key proteomic alterations observed in primary DMD muscle tissue. Our comprehensive functional and molecular characterization validates EMTs as physiologically relevant models for investigating muscular dystrophy pathophysiology and supports their application in therapeutic target identification and preclinical drug development.

P-23 - Small Molecule Inhibitor of PAX3::FOXO1 to Reverse Rhabdomyosarcoma Treatment Resistance

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Rhabdomyosarcoma (RMS) is the most common soft tissue sarcoma of childhood and often also occurs in adolescent and young adults. Clinically, survival amongst metastatic RMS patients has remained dismal yet unimproved for decades. RMS harboring the driver PAX3::FOXO1 fusion oncoprotein is generally not survivable when metastatic. PAX3::FOXO1 facilitates G₂/M checkpoint adaptation and hence treatment resistance, causing a 45% drop in 10-year survival. PAX3::FOXO1 inhibition induces significant chemotherapy sensitivity *in vitro* and *in vivo*. This finding resulted in a completed phase I clinical trial concept of an indirect PAX3::FOXO1 inhibition strategy using histone deacetylase inhibitor entinostat (NCT02780804) and led to a newly developing cooperative group Phase 2 clinical trial for entinostat in combination with chemotherapy in relapsed/refractory rhabdomyosarcoma. While we are hopeful this approach will impact patient outcomes, indirect targeting is not sufficient. From our preliminary studies, PAX3::FOXO1+ RMS may be “curable” with a very efficient PAX3::FOXO1 targeted drug. A series of PAX3::FOXO1 inhibitors have been identified with specificity and structure-activity relationships. We identified five molecules with ≥50% inhibition of PAX3 paired domain (PD) or homeodomain (HD) transcriptional reporters. The best hit has a PD and HD reporter inhibition EC₅₀'s at 1.8 and 5.3 μM respectively. By contrast, an EC₅₀ of > 10 μM in a FOXO1 reporter assay. We thus provide proof-of-concept that transcription factors can be druggable. With Hit-to-Lead development of an investigation small molecule compound, we seek to reverse the trend that no curative-intent clinical trial for relapsed and refractory rhabdomyosarcoma has been open for enrollment since July 2013.

P-24 - A Translational Pipeline for DMD Therapeutics: Engineered Muscle Tissue Screening of Novel Epigenetic Molecules and Gene Therapies

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Although Duchenne Muscular Dystrophy (DMD) is a progressive and degenerative disease, there is evidence for embryonic and fetal stage defects during myogenesis, including transcriptional and epigenetic perturbations, suggesting early intervention potential. Two promising treatment approaches have emerged: epigenetic small molecules, which target transcriptional dysregulation, and gene therapy for dystrophin replacement. While epigenetic

compounds like Duvyzat (Givinostat) show promise in animal models, many have failed in clinical trials. Separately, gene therapies such as Elevidys face distinct challenges, particularly regarding efficacy and safety due to dystrophin-specific T-cell immunity.

Our dystrophin-null hiPSC derived EMTs demonstrated characteristic DMD pathology, including force deficits and dysregulated contractile kinetics. Using these models, we validated the therapeutic efficacy of histone deacetylase inhibitors Trichostatin-A, Givinostat, and our novel compound SR-4370 to reverse these phenotypes. They all displayed unique therapeutic action; however, they showed limited functional restoration in EMTs in contrast to zebrafish models. Additionally, we developed deimmunized micro-dystrophin vectors using in silico prediction tools to minimize immune responses while maintaining functionality. These vectors achieved efficient lentiviral transduction in hiPSC-derived myoblasts with consistent expression across regulatory cassettes. Preliminary data in DMD EMTs show persistent expression and functional restoration. By combining EMT modeling, high-throughput screening, and computational protein design, we've established a pipeline for optimizing DMD therapeutics. This integrated approach of epigenetic modulation and deimmunized vectors addresses both functional recovery and immune tolerance, potentially expanding treatment accessibility to previously excluded patient populations. Our most promising candidates are advancing to animal studies, representing a significant step toward more effective and safer therapies.

P-25 - Contractile human skeletal muscle organoids with an in vivo-like satellite cell niche for modeling sarcopenia

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Sarcopenia is a progressive disorder of aging, marked by the gradual loss of muscle mass, strength, and regenerative capacity. Age-related chronic inflammation impairs muscle homeostasis, accelerating disease progression. Sarcopenia leads to frailty, reduced mobility, and higher mortality in older adults. It is now recognized as a global public health concern requiring effective therapies and human-relevant models. Existing animal and 2D human cell-based models are insufficient to replicate the physiological complexity of human skeletal

muscle, limiting their relevance for sarcopenia research. Therefore, to overcome these limitations, we established a human skeletal muscle organoid (hSkMO) platform based on 3D self-organizing culture conditions. Upon long-term culture, the hSkMOs underwent progressive maturation, resulting in the formation of a satellite cell niche and increased myofiber thickness. In addition, we observed the formation of ChAT⁺ motor neurons and αBTX⁺ neuromuscular junctions, which exhibited spontaneous contractile activity, indicating functional integration. Single-nucleus RNA sequencing further revealed the cellular composition and transcriptional landscape of the hSkMOs, confirming the presence of key myogenic and neural cell populations. To model sarcopenia, we exposed hSkMOs to chronic TNF-α, a pro-inflammatory cytokine elevated during aging. This treatment led to satellite cell exhaustion, followed by muscle atrophy and significant downregulation of androgen receptor (AR) expression. To assess therapeutic potential, we co-treated the organoids with testosterone during TNF-α exposure. Testosterone maintained AR expression in satellite cells and mitigated myofiber atrophy. Collectively, we demonstrated the utility of hSkMOs as a robust platform for evaluating therapeutic interventions, providing valuable insights into the pathophysiology of human sarcopenia.

P-26 - Bioengineered muscle constructs to evaluate the effect of progesterone on muscle function and regeneration

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Aging leads to declines in skeletal muscle strength and regenerative capability, changes that progress more rapidly in females than in males. In women, these declines are accelerated during the menopausal transition, when estrogen and progesterone levels drop sharply. While estrogen loss is a well-recognized factor of muscle quality decline, the impact of progesterone loss on muscle health remains largely unexplored. Understanding the role of progesterone could provide new insights into female muscle aging and reveal new therapeutic opportunities to improve aged muscle health. Therefore, in this study, we investigate the effects of progesterone on muscle strength and regeneration using a bioengineered *in vitro* muscle construct system.

We first generated 3D muscle constructs by culturing female mouse muscle progenitor cells in Matrigel/fibrin hydrogels through our established approach. To evaluate the effects of progesterone on muscle function, constructs were cultured either in hormone-free (HF) media or HF media supplemented with progesterone. Our data showed that muscle constructs cultured in HF media displayed reduced contractile forces, whereas progesterone treatment prevented the force decline. To examine regenerative capacity, constructs were injured with cardiotoxin and cultured with or without progesterone. Injured muscle constructs treated with progesterone exhibited significantly higher force recovery than those cultured in HF media. These results were further confirmed *in vivo* using an aged, ovariectomized mouse model of menopause.

Overall, our findings highlight the potential of the bioengineered muscle constructs for studying the influences of progesterone on muscle health in aging females.

P-27 - An Automated MATLAB Pipeline Accelerates Unbiased Quantification of Zebrafish Skeletal Muscle and Shows that Inactivity Exacerbates Muscle Degeneration in the Zebrafish Model of Duchenne Muscular Dystrophy

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Skeletal muscle is critical for health. The quality and quantity of skeletal muscle mass predicts longevity and organismal resilience. The zebrafish system has a number of advantages for the study of skeletal muscle. Some of these advantages are the ability to conduct large scale drug screens in a vertebrate disease model, time-lapse analyses of organogenesis, and longitudinal studies of individual larvae through time. The most common approach for quantifying zebrafish skeletal muscle structure in the above contexts is to use birefringence imaging, which allows visualization of organized structures such as skeletal muscle. One rate-limiting aspect of birefringence analysis that can also introduce human error and bias is the manual annotation and quantification of birefringence images. Thus, we developed a MATLAB-based pipeline to automate the zebrafish annotation and calculate the mean gray value. The method combines image analysis tools and image thresholding techniques to eliminate background noises and accurately identify zebrafish 20-fold more quickly than manual annotation. We also used this pipeline and longitudinal analysis to show that early inactivity exacerbates the progression of muscle degeneration in the zebrafish model of Duchenne Muscular Dystrophy

P-28 - Specific non-myogenic mesenchymal cell populations contribute to the muscle pathology of massive rotator cuff tears and reveal novel therapeutic treatment options

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The rotator cuff is a group of four muscles in the shoulder, which aid in movement and rotation of the upper arm. Rotator cuff tears (RCTs) within tendons of these muscles are common musculoskeletal injuries, often resulting in intramuscular fat, fibrosis, and muscle atrophy. Fatty infiltration specifically correlates with high rates of retear following repair. The cellular sources and molecular cues that cause these pathologies are unknown and therefore non-surgical

cell/drug therapies for RCTs do not exist. Thus, we first sought to determine the cellular source(s) and molecular underpinnings of fatty atrophy and fibrosis associated with massive RCTs. Using a murine model of massive RCTs combined with lineage tracing, we demonstrate that muscle resident *Pdgfra*⁺ non-myogenic mesenchymal cells (NMMCs) are responsible for the fatty and fibrotic RCT pathologies. Utilizing sorted *Pdgfra*⁺ cells from rotator cuff muscles and “deep” single cell RNA-sequencing, we identified a specific *Dpp4*⁺ cell population associated with RCT-induced fibrosis, while *Gfra1*⁺ nerve-associated NMMCs are drivers of the RCT-induced intramuscular fat pathology. Finally, we demonstrate that RCT-induced fatty infiltration occurs at least partially via the loss of GDNF-GFRA1-RET signaling, since local treatment of murine RCTs with a small molecule RET agonist alters development of the RCT-induced intramuscular fat.

P-29 - Acute and Chronic Muscle Damage After Alphavirus Infection

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Ross River (RRV) and Chikungunya (CHIKV) viruses are emerging pathogens that target the musculoskeletal system. These mosquito-borne enveloped RNA viruses are a major and growing health concern as they undergo explosive outbreaks in humans. Furthermore, they have expanded their range due to climate change, geographic expansion of mosquitos, and virus evolution. Acute RRV and CHIKV infection causes severe arthralgia, arthritis, myalgia and myopathy that typically resolves within weeks of infection. However, a significant fraction of RRV and CHIKV patients develop debilitating chronic disease that includes arthralgia, arthritis, myalgia, and myopathy. While the etiology of virally-induced arthralgia and arthritis has been intensely studied, the mechanisms underlying RRV and CHIKV-induced myalgia and myopathy are poorly understood. Using new mouse models and viruses engineered to contain fluorescent reporters or Cre, we demonstrate that both viruses profoundly affect skeletal muscle. Myofibers are readily infected, but the fate of infected myofibers is variable; some myofibers die after infection while others survive. Importantly, myofibers that survive infection for months post infection are consistently smaller and likely functionally impaired across all muscles examined. This may contribute to the chronic muscle weakness found in patients. In addition, we found that compared with sterile injury, the dynamics and properties of muscle stem cells are altered after viral pathogenic injury.

P-30 - Exploration of IGF-2R blocking as potential therapeutic target for sarcopenia - effects on human skeletal muscle cell proliferation and differentiation

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Insulin-like-growth factors (IGF-1 and IGF-2) play a critical role in human neonatal and adult development. IGF-1/2 bind to IGF1-R (insulin-like growth factor 1 receptor) to initiate signaling critical for cell proliferation, differentiation and hypertrophy in skeletal muscles.

Transcriptomic profiling suggests a significant decline of IGF-1 and IGF-2 during ageing in humans and which could contribute to the development of sarcopenia. Of note, circulating levels of IGF-2 seem higher compared to circulating IGF-1 levels. However, IGF-2 levels are negatively regulated by the IGF-2R which internalizes IGF-2 upon binding and therefore reduces its bioavailability. At the tissue level the expression of IGF-2R is highest in skeletal muscle, suggesting a regulatory role in skeletal muscle biology. A recent publication showed that blocking of IGF-2R had beneficial effects on skeletal muscle in mdx mice¹. However, the underlying mechanism, whether this translates to human, and whether this could play a role in sarcopenia remains unknown.

Herein, we compared the signaling and the effects of IGF-1 and IGF-2 in human skeletal myoblasts and myotubes from young and aged donors. We then evaluated the effect of an IGF-2R blocking antibody on human myoblast proliferation and differentiation and examined if IGF-2 signaling in human myocytes can be modulated as a potential target for sarcopenia.

1. Bella, P. *et al.* Blockade of IGF2R improves muscle regeneration and ameliorates Duchenne muscular dystrophy. *EMBO Mol. Med.* **12**, EMMM201911019 (2020).

P-31 - A 3D Human Engineered Muscle Tissue Platform for Longitudinal Muscle Atrophy, Differential Insulin Sensitivity, and Metabolism

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Insulin resistance in skeletal muscle impairs systemic glucose regulation and contributes to metabolic disorders including obesity and type 2 diabetes. Coupled with emerging evidence of GLP-1-based treatment-associated muscle loss, these clinical challenges underscore the critical need for physiologically relevant models to study human muscle metabolism and atrophy. To address this, we developed functional models of skeletal muscle atrophy, insulin sensitivity, and glucose metabolism using primary human skeletal myoblasts cast into contractile engineered

muscle tissues (EMTs) with commercially available instruments, consumables, and media products. To induce atrophy, EMTs were treated with high-dose dexamethasone or activin A, resulting in significant reductions in tissue size and contractile force. To generate insulin sensitive EMTs, tissues were cultured in either standard or insulin-sensitizing media, followed by glucose starvation and insulin stimulation. EMTs maintained in insulin-sensitizing media showed elevated basal GLUT4 expression and enhanced AKT phosphorylation in response to insulin, indicating restored insulin responsiveness. To assess downstream metabolic function, EMTs were subjected to glucose starvation, insulin stimulation, and/or electrical stimulation-induced exercise. Exercise reduced glycogen and increased lactate content, while glucose starvation alone elevated lactate without altered glycogen. Glucose reintroduction with insulin significantly increased both glycogen and lactate content, indicating reactivation of glycolytic metabolism. In summary, this study establishes EMTs as a powerful, physiologically relevant platform for investigating human skeletal muscle metabolism with functional and biochemical markers. This experimental framework supports metabolic drug discovery by enabling simultaneous assessment of therapeutic efficacy and the potential risk of treatment-induced sarcopenia.

P-32 - Targeting C1q signaling in fibro-adipogenic progenitors prevents regenerative fibrosis of aged muscle

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Skeletal muscle fibrosis, as occurs with age, in response to injury, or in the setting of degenerative diseases, results in impairments of muscle regeneration and function. Fibro-adipogenic progenitors (FAPs) play an important role in promoting myogenic regeneration under normal conditions, while in pathological conditions such as severe or recurrent muscle injury, FAPs can aberrantly differentiate into fibrogenic cells, resulting in excessive deposition of extracellular matrix and fibrosis.

We explored the molecular regulation of FAP differentiation along the fibrogenic lineage in mice to gain insights into the mechanisms of fibrosis in aged muscle in response to injury.

We found that aging is associated with an increased expression of the complement Component 1q (C1q) in muscle-resident macrophages and elevated expression of the complement proteins C1r and C1s in FAPs, and that elevated C1q causes excess fibrosis following injury to skeletal muscle.

Exposure of proliferating FAPs to C1q results in the activation of the Wnt signaling pathway, elevated expression of collagen genes, and FAP fibrogenic differentiation, leading to increased

tissue fibrosis. Either pharmacological inhibition of the complement pathway or genetic ablation of C1s in FAPs in aged mice reduces fibrogenic differentiation of FAPs by suppressing Wnt signaling. This reduction in FAP differentiation attenuates the fibrotic response to injury in aged animals as well as in the mdx mouse model of muscular dystrophy.

Our studies suggest that non-canonical C1 signaling may represent a target for therapeutics to reduce or prevent fibrosis that arises from acute or recurrent injury to aged muscle.

P-33 - Spatial mapping of immune system drivers of NMJ degeneration in aging

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Aging is associated with progressive skeletal muscle degeneration, resulting in reduced strength, mobility, and functional capacity. Chronic low-grade inflammation, or "inflammaging," plays a central role in this muscle decline. Understanding the link between immune system aging and neuromuscular dysfunction is critical for developing targeted therapeutic strategies to improve health outcomes in the elderly. Our lab has identified antibody-mediated neuromuscular junction (NMJ) degeneration in aged mice, pointing to a potential role of autoimmune dysregulation in age-related muscle pathology. To investigate this, we apply single-cell spatial multi-omic approaches to characterize immune alterations with age. Using CODEX imaging, consisting of 53-antibody panel, we map cellular neighborhoods and interactions in the bone marrow (BM), revealing spatial and functional changes in T and B cells that may contribute to chronic inflammation and NMJ damage. Through single-cell analysis, we specifically investigate molecular mechanisms of B cell dysfunction in driving muscle pathology. We further assess the role of B cells through selective depletion experiments in aged mice, which reduced molecular signatures of aging and improved muscle function. Together, these studies highlight the contribution of immune aging to neuromuscular degeneration and paves the way for innovative immunotherapeutic strategies to preserve neuromuscular health in the elderly.

P-34 - Synergistically Enhancing Aged Muscle Regeneration via Mitochondrial Transplantation and Magnetic Field Stimulation

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Aging disrupts mitochondrial function, impairing skeletal muscle regeneration and leading to incomplete repair that diminishes muscle quality. Mitochondrial transplantation therapy (MTT) has emerged as a promising strategy to restore mitochondrial function in both preclinical and clinical settings. In this study, we aimed to develop an MTT-based approach to enhance aged muscle regeneration using a bioengineered three-dimensional muscle aging model. This model recapitulates age-related impairment in muscle regeneration while enabling high-throughput evaluation of therapeutic interventions. Our results showed that MTT improved functional recovery in aged muscle after injury. However, this improvement was limited, as recovery of aged muscle remained inferior to that of injured young muscle.

Magnetic field stimulation (MFS), a non-invasive strategy with rehabilitation potential, has been shown to enhance mitochondrial function by promoting mitophagy, stimulating mitochondrial biogenesis, and improving respiration. To further boost regeneration in aged muscle, we applied MFS to injured muscle constructs following MTT. Our findings revealed that combining MTT with MFS produced a synergistic effect, significantly improving functional recovery compared to either treatment alone. The findings were validated *in vivo*. Mechanistic analyses indicated that MFS augmented the effects of MTT by enhancing mitochondrial uptake and regulating mitophagy.

In summary, we present an innovative paradigm to rejuvenate aged skeletal muscle by integrating regenerative therapy (MTT) with a rehabilitation modality (MFS).

P-35 - Fibro-Adipogenic Progenitors maintain muscle mass in homeostatic muscle through prevention of immune cell invasion

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Fibro-Adipogenic progenitors (FAPs) are essential for maintaining muscle mass. When FAPs are depleted, rapid muscle atrophy ensues. Previous studies have implicated the loss of neuromuscular junction (NMJ) integrity as a potential cause of atrophy in the absence of FAPs. However, additional mechanisms may also contribute to muscle mass loss. To address those questions, we generated an inducible FAP knockout (FAP-KO) model by crossing *Pdgfra*^{CRE-ERT2}

and *Rosa26*^{DTA} mice. Following tamoxifen (TAM) administration, we confirmed ablation of FAPs and atrophy that mirrored previous findings. Isolated muscle functional testing of the extensor digitorum longus (EDL) and soleus (SOL) muscles revealed proportional force and mass deficits, leading to a preservation of specific force when muscles were stimulated directly. NMJ integrity was evaluated in both muscles using whole-mount immunofluorescence and confocal microscopy. NMJ structure remained intact, with no signs of denervation, muscle fiber fragmentation, or Schwann cell pathology, suggesting NMJ dysfunction was not responsible for the observed atrophy. Instead, we observed robust immune activation in FAP-KO muscles, including increased neutrophil and macrophage infiltration and a 10- to 50-fold upregulation of pro-inflammatory cytokines, chemokines, and interleukins. The inflammatory response in muscles of FAP KO mice was accompanied by a ~10-fold increase in atrogene expression. These data suggest that FAPs serve as gate keepers to immune cell invasion, and that the absence of FAPs enables an immune response which later drives atrophy. Together, these findings reveal a previously unrecognized protective mechanism by FAPs for preservation of skeletal muscle mass under homeostatic conditions.

P-36 - Defining the interplay of developmental and tumorigenic mechanisms in fusion-driven rhabdomyosarcoma

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Rhabdomyosarcoma (RMS) is the most common pediatric soft tissue sarcoma and has molecular characteristics of incomplete skeletal muscle differentiation. The infantile subtype of RMS is primarily driven by gene fusions, the most common being VGLL2::NCOA2. Vestigial like family member 2 (VGLL2) is associated with TEAD1-dependent skeletal muscle gene regulation and nuclear receptor coactivator 2 (NCOA2) is a common 3' fusion partner in multiple cancers. We hypothesize that the fusion between these two genes inappropriately leverages myogenic developmental programs for tumorigenesis. To understand VGLL2::NCOA2 mechanisms of transformation, we created complementary VGLL2::NCOA2-driven C2C12 mouse allograft and transgenic zebrafish models, which develop tumors that histologically and transcriptionally recapitulate the human disease. Shared cross-species transcriptional profiles revealed

suppression of muscle differentiation-specific genes and overexpression of the small GTPase ARF6. Knockout of *Arf6* in our mouse myoblast C2C12-VGLL2::NCOA2 cell culture model suppresses colony formation and restores the capacity to terminally differentiate. To define ARF6 and VGLL2::NCOA2 cooperation in vivo, we generated a zebrafish CRISPR/Cas9 *arf6b* knockout and injected VGLL2::NCOA2 into wildtype and *arf6b* knockout fish. The *arf6* knockout VGLL2::NCOA2 tumors had a longer latency to tumor formation and transcriptionally had an upregulation of skeletal muscle differentiation pathways. These results highlight the cooperation of ARF6 with VGLL2::NCOA2 to impede skeletal muscle differentiation pathways. We are now implementing a C2C12 mouse myoblast *Arf6* knockdown approach to understand temporal requirements of ARF6 for muscle differentiation and VGLL2::NCOA2 oncogenesis. Our results will provide insight into how ARF6 supports rhabdomyosarcoma tumorigenesis and ARF6's potential as a therapeutic target.

P-37 - FSH transforms fibro-adipogenic progenitors and promotes muscle non-contractile tissue accumulation in a murine menopause model

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Aging in female individuals leads to a more rapid decline in skeletal muscle function compared to males. This sex-specific aging trajectory is poorly understood, partly because muscle aging research to date has primarily used male animals. Additionally, conventional female aging animal models do not faithfully recapitulate human menopausal phenotypes, thus failing to capture a critical aspect of human female aging. Here, we used a chemically induced menopause model to characterize the impact of menopause on muscle structure and function in aged female mice. Menopause induced significant muscle weakness compared to age- and sex-matched control counterparts, though myofiber size and cross-sectional area were unchanged. Instead, we observed myosteatosis and fibrosis in the muscles of menopausal mice, changes in muscle quality that were concomitant with accumulation of fibro-adipogenic progenitor cells (FAPs). Exposing FAPs to menopausal serum *in vitro* increased adipogenesis, serving as further evidence that menopause-induced changes in circulating factors mediate downstream effects on FAPs. Upon interrogating direct hormone effects *in vitro*, we discovered that elevated follicle-stimulating hormone (FSH) levels, as occurs post-menopause, induced FAP adipogenic transformation and a secretory profile favoring extracellular matrix degradation and fat deposition. Next, we sought to evaluate the physiological relevance of our *in vitro*

findings using an *in vivo* menopause model. Administering FSH neutralizing antibody in a menopausal mouse model significantly decreased the number of PDGFR α + FAPs and enhanced muscle strength compared to non-treated menopausal counterparts. Collectively, these data suggest that FSH drives FAP adipogenesis, non-contractile tissue accumulation, and muscle weakness in aged post-menopausal female mice.

P-38 - SRSF8 Mediates Aberrant RNA Splicing and Neoantigen Production in DUX4-Induced Facioscapulohumeral Muscular Dystrophy

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Facioscapulohumeral muscular dystrophy (FSHD) is characterized by inappropriate activation of the early embryonic transcription factor DUX4 in skeletal muscle, where it is typically epigenetically silenced. Aberrant DUX4 expression in myoblasts causes widespread disruption of RNA splicing and RNA quality control, leading to the accumulation of transcripts with retained introns. These faulty transcripts produce toxic truncated proteins and immunogenic neoepitopes, both implicated in FSHD pathogenesis. We hypothesize that DUX4-induced splicing errors, alongside compromised RNA surveillance, further amplify the generation of these deleterious proteins and neoepitopes, thereby exacerbating disease development.

Notably, DUX4 robustly induces the splicing factor SRSF8, with ChIP-seq confirming direct DUX4 binding at the SRSF8 promoter. Preliminary data show that partial knockdown of SRSF8 diminishes many DUX4-induced aberrant transcripts. Ongoing studies utilizing CRISPR-mediated knockout and inducible overexpression systems aim to define the precise role of SRSF8 in DUX4-driven splicing dysregulation—specifically testing whether SRSF8 loss can suppress, and its overexpression can recapitulate, mis-splicing and neoantigen production in the absence of DUX4.

Additionally, in-progress CLIP-seq analyses in FLAG-tagged SRSF8 lines will identify direct RNA-binding targets of SRSF8 under both basal and DUX4-activated conditions. Integration of these datasets with RNA-seq and BrU-seq will enable delineation of authentic SRSF8-dependent splicing events.

Together, these results support a model in which SRSF8 functions as a key mediator of RNA splicing dysregulation and neoantigen formation downstream of DUX4 in FSHD, and highlight SRSF8 as a promising target for restoring RNA homeostasis in this disease.

P-39 - Suppressing PDGFR β Signaling Enhances Myocyte Fusion to Promote Skeletal Muscle Regeneration

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Efficient myocyte fusion is fundamental for skeletal muscle development and regeneration, yet the molecular checkpoints that control this process remain elusive. Here, we reveal platelet-derived growth factor receptor beta (PDGFR β) signaling as a critical negative regulator of myocyte fusion and repair. Using genetic and pharmacological approaches, we show that *Pdgfr β* deletion accelerates myonuclear accretion and myofiber regeneration, while PDGFR β activation disrupts cell spreading and fusion. Mechanistically, PDGFR β cooperates with TGF β signaling and activates STAT1 to restrain fusion. Strikingly, PDGFR β inhibition in both mouse and human models robustly enhances myotube formation, positioning PDGFR β as a promising therapeutic target to drive rapid muscle repair and regeneration.

P-40 - Exploring a Neuromuscular Deficit in a Mouse Model of UCMD

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Ullrich Congenital Muscular Dystrophy (UCMD) is a genetic disorder characterized by distal joint hyperlaxity, proximal joint contractures, and muscle weakness that leads to significant morbidity and mortality. UCMD is caused by mutations in any of the genes (COL6A1, COL6A2, and COL6A3) that generate collagen VI, an extracellular matrix (ECM) protein that interacts with myriad other proteins in the ECM and at the cell surface. Despite recent work in mouse models of UCMD, there remains no curative therapy, necessitating further research into the mechanisms underlying UCMD. Here we confirmed pathological findings, such as muscle weakness and histological hallmarks of UCMD, in a mouse model with a genetic deletion of *Col6a2* (*Col6a2*^{-/-}). We then performed RNA sequencing on the quadriceps muscle of 4-week-old *Col6a2*^{-/-} vs WT mice and found dysregulated genes related to muscle atrophy and the neuromuscular junction (NMJ). To assess cell-type specific transcriptional differences, we performed single-nucleus RNA sequencing on quadriceps muscle from 4-week-old *Col6a2*^{-/-} vs WT mice, which revealed a unique “synaptically altered” myonuclear population based on the dysregulation of NMJ-related genes. To address this apparent neuromuscular phenotype, we injected *Col6a2*^{-/-} and WT pups with a MyoAAV vector overexpressing Dok7, a protein that potentiates the signal to form and stabilize NMJs and which has been shown to partially rescue other models containing neuromuscular defects. Our preliminary data suggests that Dok7 does not rescue the pathological features of UCMD and, in fact, has negative consequences on WT muscle function, suggesting the need to explore alternative therapeutic opportunities.

P-41 - DR1/DRAP1 safeguards the myogenic identity in human skeletal muscle by repressing IRF1-STAT1 mediated autonomous interferon signaling

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Immune regulation of tissue development has traditionally been viewed as cross-talk between immune cells and developing parenchymal cells, as described in the brain, pancreas, and lung. Whether progenitor cells within developing tissues themselves harbor immune-like programs that act autonomously has remained unclear. Using single-cell RNA-seq of human fetal myogenesis, we observed a progressive decline of a low grade of interferon signaling as myogenic precursors differentiate, suggesting that an intrinsic interferon program regulates myogenic fate. Through CRISPR-based screening, we identified the transcriptional repressor DR1-DRAP1 complex as essential for human myogenesis. Loss of DR1 or DRAP1 in human myoblasts completely abolished muscle differentiation and fusion, accompanied by the activation of the JAK-STAT pathway and robust upregulation of interferon- β together with a broad array of immune markers. Mechanistically, DR1-DRAP1 complex binds cis-regulatory elements on IRF1 and STAT1 to repress their expression. Deletion of either IRF1 or STAT1 in DR1- or DRAP1-deficient myoblasts partially restored muscle differentiation and fusion. Together, these findings demonstrate that myogenic precursor cells possess an autonomous interferon program that must be repressed by DR1-DRAP1 to enable normal myogenic differentiation. This work uncovers a previously unrecognized, cell-intrinsic barrier to muscle differentiation and highlights a new dimension of immune regulation in muscle development.

P-42 - The effect of fibrosis on the engraftment of myogenic progenitors in a mouse model of LGMDR9.

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One of the hallmarks of muscular dystrophy is the replacement of myofibers with fibrotic tissue, which we hypothesize may reduce the regenerative capacity of transplanted cells. We screened the levels of fibrosis in different muscles of early stage and moderate/advanced stage fukutin-related protein (FKRP)-mutant mice, a model of LGMDR9, characterized by defects in α -dystroglycan (α -DG) functional glycosylation. Using picrosirius red staining, we confirmed that the diaphragm is highly fibrotic (23%) whereas tibialis anterior muscles show minimal levels (2%) in 6-week-old mice. The gluteus (Glu) muscles also display significant levels of fibrosis

(8%) early on, with increased accumulation at later stages (15% and 22-25% at 6 and 9 months of age, respectively). To determine whether fibrosis interferes with engraftment, we injected 6-week and 6-9-month-old (mo) FKRP mice with RFP-labeled Pax3⁺ myogenic progenitors into the Glu muscles. At early stage, robust engraftment was detected by immunofluorescent staining with antibodies to IHH6 (α -DG functional glycosylation) and RFP (donor-tracking) in both cardiotoxin (CTX)-injured (185 ± 26 donor-derived myofibers) and uninjured (256 ± 41) groups. At the later stages, uninjured muscles showed a significant reduction in the number of donor-derived myofibers (114 ± 18 at 6-mo and 96 ± 22 at 9-mo cohorts), whereas engraftment in the CTX-injured counterparts was less impeded by increasing fibrosis (144 ± 29 at 6-mo and 132 ± 38 at 9-mo). When we examined engraftment with respect to levels of fibrosis, we found an inverse correlation only in the uninjured cohort, suggesting that CTX-injury disrupts the pathological architecture and signaling milieu, thus diminishing the impact of established fibrosis on cell engraftment.

P-43 - Investigating the Role of the SPP1/NF- κ B Axis in Muscle Recovery in CTS Patients

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Introduction: Carpal Tunnel Syndrome (CTS), a neuropathic condition resulting from median nerve compression, often leads to muscle atrophy, weakness, and fibrosis. Successful muscle recovery after CTS surgery requires efficient regeneration, reduced fibrosis, and inflammation resolution. This study delves into the role of pro-inflammatory cytokines, specifically examining how the SPP1/NF- κ B pathway impacts muscle cell proliferation, migration, and differentiation to aid in regeneration and repair.

Methods: Muscle myoblast cells were treated with recombinant cytokines (Osteopontin, TNF- α , IL-1 β , and IFN- γ) for 48 hours in culture medium containing 1% FBS. Following this, cells were cultured in DMEM with 2% horse serum for five days to induce myotube formation, and then subjected to an additional 48-hour treatment with the same pro-inflammatory cytokines. Protein and gene expression were subsequently analyzed, and morphological analysis was done using a Keyence microscope.

Results: Osteopontin (OPN) treatment enhances myoblast proliferation and migration. Furthermore, this treatment promotes myogenic differentiation, as evidenced by elevated Myogenin gene and protein expression levels in differentiated myotubes. Mechanistic studies demonstrated that recombinant osteopontin (OPN) enhanced proliferation and migration via PKC ζ activation and promoted multinucleated myotube formation through Akt pathway activation. Pharmacological inhibition of PKC ζ or Akt significantly reduced these effects.

Conclusion: These results highlight that osteopontin (OPN)-mediated PKC ζ /Akt signaling is a potential therapeutic target to preserve muscle integrity and improve recovery after CTS

surgery. Ongoing studies aim to elucidate the molecular mechanisms involved, potentially leading to new interventions for CTS- associated muscle degeneration.

P-44 - Role of Glyoxalase 1 (GLO1) in Human Myoblast Differentiation and Its Potential Impact on Obesity-Associated Muscle Defects

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Obesity is associated with impaired skeletal muscle regeneration and function, but the molecular mechanisms underlying these defects remain incompletely understood. Analyses of Glyoxalase 1 (GLO1) mRNA and protein from muscle biopsies revealed a consistent reduction in GLO1 expression in obese versus lean individuals. GLO1 is a critical enzyme in cellular detoxification pathways, but its role in myogenesis is unclear. To investigate the functional role of GLO1 in myogenesis, we generated GLO1 knockout (KO) human immortal myoblasts via CRISPR/Cas9 gene editing. Myogenic differentiation was induced by growth factor withdrawal using 2% horse serum. Differentiation and fusion were assessed morphologically and by quantifying mRNA expression of key myogenic (e.g., CD56, MYOD, MYOG), contractile (e.g., MYH1, 2, and 7, TNNT1, TNNT3), and fusogenic (e.g., MYOMAKER) markers using RT-droplet digital PCR. GLO1-KO myoblasts exhibited a failure to differentiate under standard conditions that was rescued with reintroduction of GLO1. Gene expression analyses revealed significant increases in CD56 (2.15-fold), contractile genes, and fusogenic gene expression, that were rescued upon reintroduction of GLO1, indicating broad disruption of the myogenic program upon GLO1 loss. We also observed that GAPDH was downregulated by GLO1 knockout and rescued by GLO1 reintroduction. These findings suggest GLO1 is an integral part of human myoblast differentiation and that GLO1 deficiency recapitulates molecular defects observed in muscle from obese and diabetic patients. Our data support a mechanistic link between GLO1 reduction and impaired muscle regeneration in obesity, highlighting GLO1 as a potential therapeutic target.

P-45 - Evaluating Drug-Responsive Gene Editing Constructs for In Vivo Gene Therapy of Duchenne Muscular Dystrophy

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Duchenne Muscular Dystrophy (DMD) is a devastating muscle degenerative disease caused by mutations in the dystrophin gene. One function of dystrophin is to stabilize and protect muscle cells from injury during contraction. Lack of functional dystrophin causes recurring muscle damage resulting in muscle necrosis and replacement of fibers with fibrotic tissue and fat. Experimental *in vivo* gene editing therapies aim to restore functional dystrophin protein expression through AAV-mediated CRISPR/Cas9 gene editing to correct causative mutations in the dystrophin gene. While these approaches have shown great promise, significant immunological concerns arise with the continuous expression of bacterial editing components, like Cas9. This project aims to address these complications by developing a system of drug-responsive and tunable gene editing that can toggle expression of editing components on and off. Here we present data from ongoing cell culture studies where we screened two classes of RNA elements capable of regulating expression of genes integrated in AAV expression constructs: riboswitches and pA regulators. Our results show that pA regulators clearly outperform riboswitches. Further *in vitro* tests helped identify a top pA regulator candidate, exhibiting a favorable dose response curve to the drug Tetracycline (Tet) at clinically relevant doses. Current studies are focused on adapting top pA regulators for temporal regulation of AAV-mediated dystrophin gene editing activity in the *mdx* mouse model of DMD. Overall, this tunable editing system is showing great promise for reducing risks associated with *in vivo* AAV-mediated gene therapy for DMD, presenting a safer and more efficacious approach to gene therapy.

P-46 - M-cadherin regulates fetal myogenesis and the muscle stem cell quiescence-to-activation transition

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Cadherin-based adhesion of skeletal muscle stem cells (MuSCs) to the myofiber is critical for quiescence, niche retention, and maintenance of the stem cell pool. While M-cadherin (*CDH15*) is one of the most abundant cadherins expressed in MuSCs, previous data indicates that M-cadherin is dispensable for pre- and post-natal myogenesis, as well as adult regeneration in response to injury. Here, we show that while loss of M-cadherin does not cause gross defects in development or regeneration, it does play a key role in these processes; M-cadherin knockout mice (*CDH15*^{KO}) display clear alterations in fetal development and regenerative processes.

During fetal development, loss of M-cadherin results in the loss of adherens junctions associated with reduced secondary myofiber formation, ultimately leading to reduced myofiber number and muscle mass that persists into adulthood. This is supported by human genetics data, where predicted loss-of-function (pLoF) mutations in the *CDH15* gene are associated with

reduced lean body mass. Adult CDH15^{KO} mice maintain a normal MuSC pool; however, their MuSCs are significantly larger compared to wildtype MuSCs and display a more activated transcriptional profile in the uninjured state. Consistent with CDH15^{KO} MuSCs appearing more primed for activation, they exit quiescence more rapidly *ex vivo*, and CDH15^{KO} mice have an accelerated regenerative response to acute cardiotoxin injury *in vivo*. Overall, these data demonstrate previously unrecognized roles of M-cadherin in controlling fetal myofiber formation and regulating the quiescence-to-activation transition in MuSCs.