Role of extracellular matrix in age-related declines of muscle regeneration

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Biochemistry of Aging

- The hallmarks of aging are widely known.
- However, how this takes place on a chemical level is only partially understood.
- We can use biochemistry and bioinformatics to develop a mechanistic understanding of aging.

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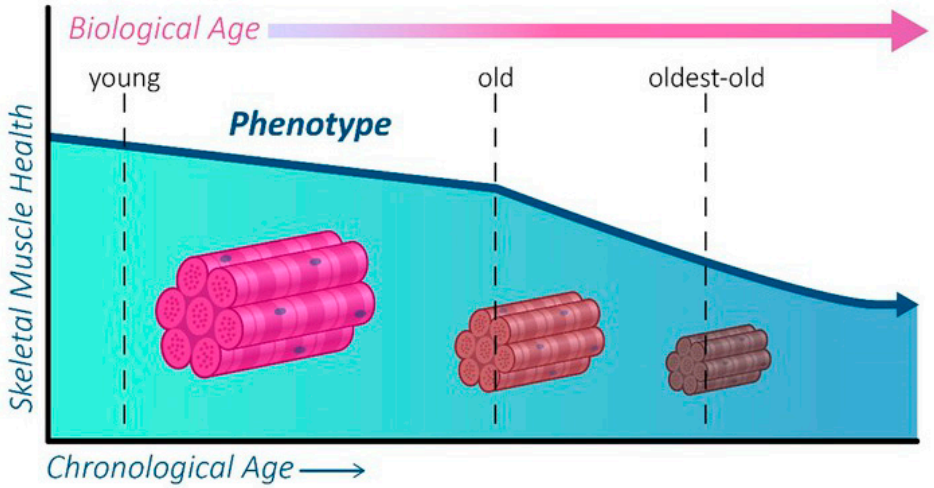
Aging effects on Skeletal Muscles Regeneration

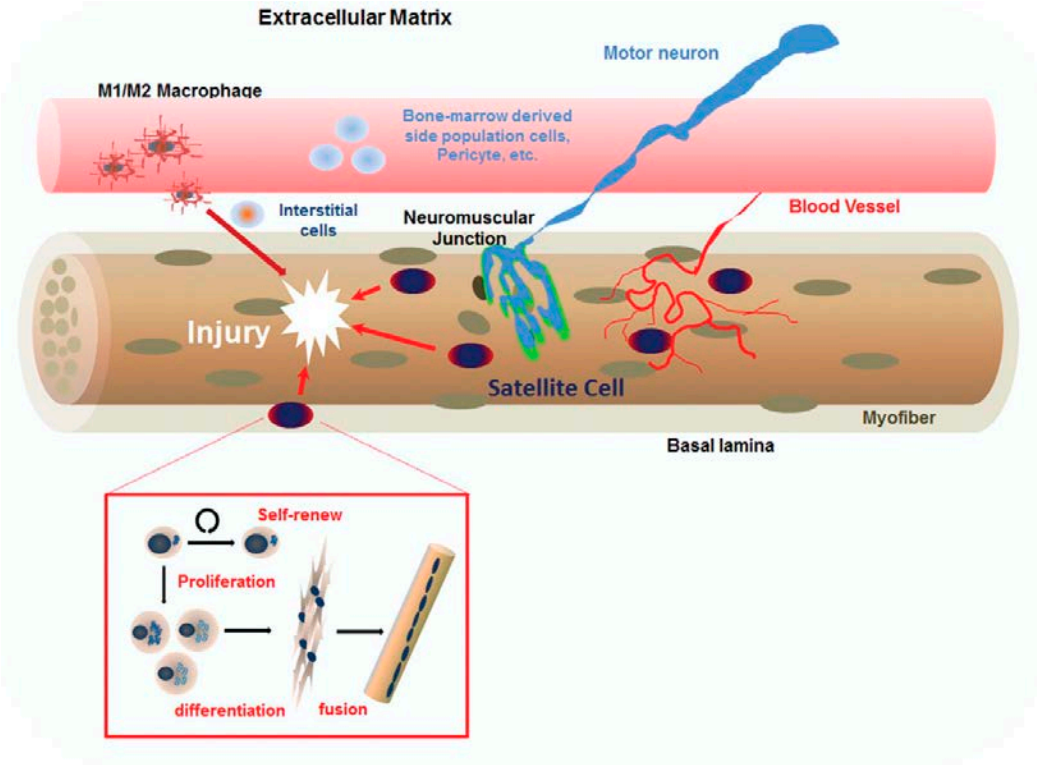
- Loss of mass and function known as sarcopenia.
- Impaired ability to regenerate after injury due to deficiency in the number and functionality of stem cells.
- Satellite cell function is controlled by both intrinsic and extrinsic regulatory cues, whose integration determines the success of muscle regenerative responses.



Jang, Y. C., Sinha, M., Cerletti, M., Dall'Osso, C., & Wagers, A. J. (2011). Skeletal muscle stem cells: Effects of aging and metabolism on muscle regenerative function. *Cold Spring Harbor Symposia on Quantitative Biology*, *76*, 101–111. https://doi.org/10.1101/sqb.2011.76.010652

Intrinsic and Extrinsic Regulation of Muscle stem cells (MuScs)

- The intrinsic factors include damage of key cellular macromolecules, such as proteins, lipids, and nucleic acids.
- The extrinsic factors affecting MuSCs describe MuSCs niche which includes:
  - The extracellular matrix (ECM)
  - The muscle fiber itself.
  - Motor neurons.
  - Inflammatory cytokine and cells.
  - Fibro/adipogenic precursor cells, and other nonhematopoietic cell types.



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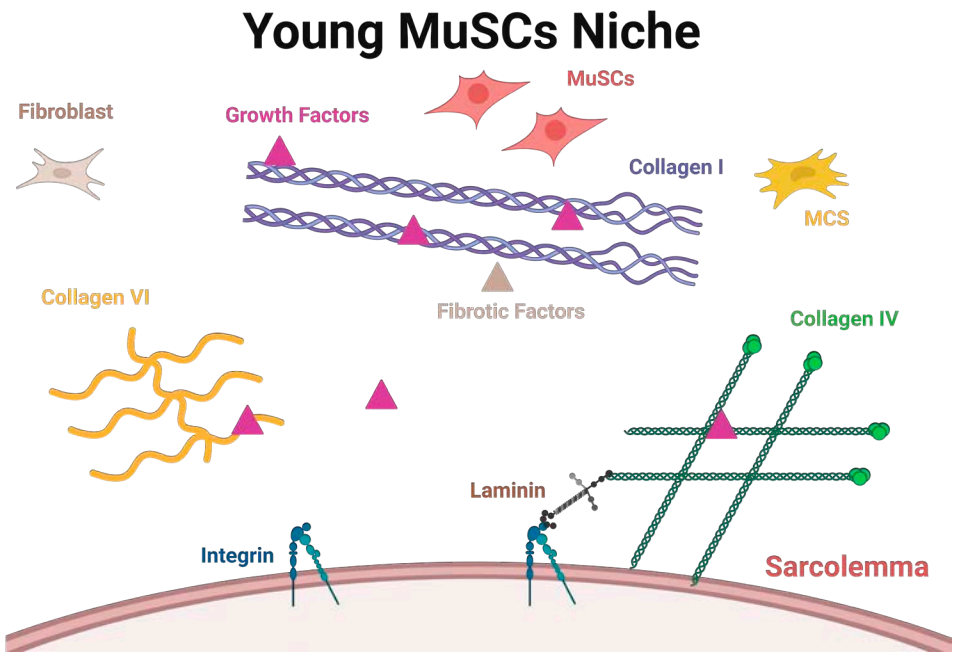
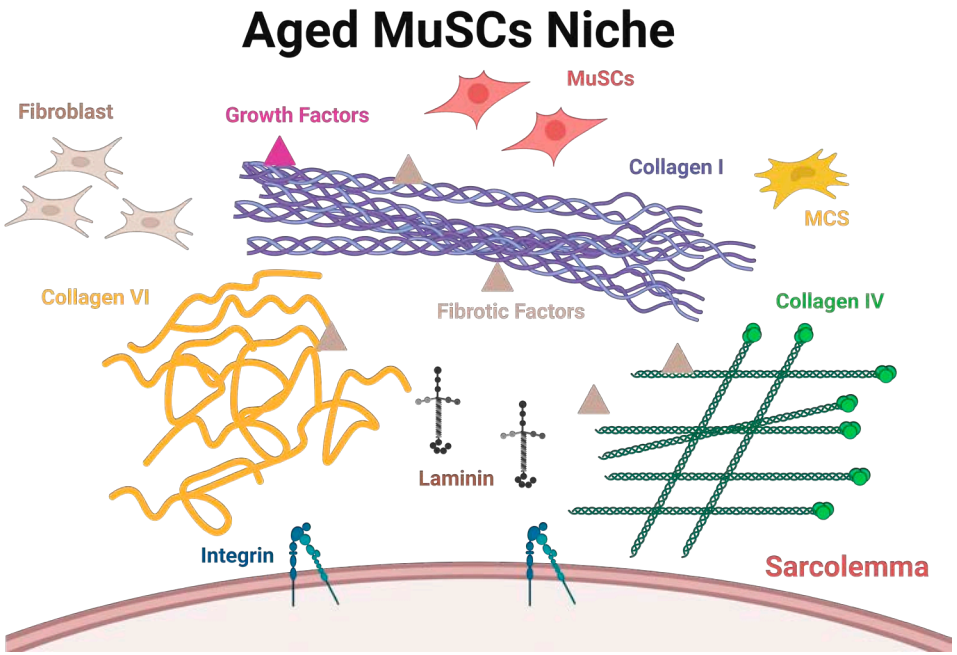
Aging Effects on Muscle Stem Cells (MuSCs) Niche

- Aging causes increased extracellular matrix (ECM) stiffness which hinder skeletal muscle contractibility and affect the regeneration of the neighboring muscle stem cells (MuSCs) post injury.

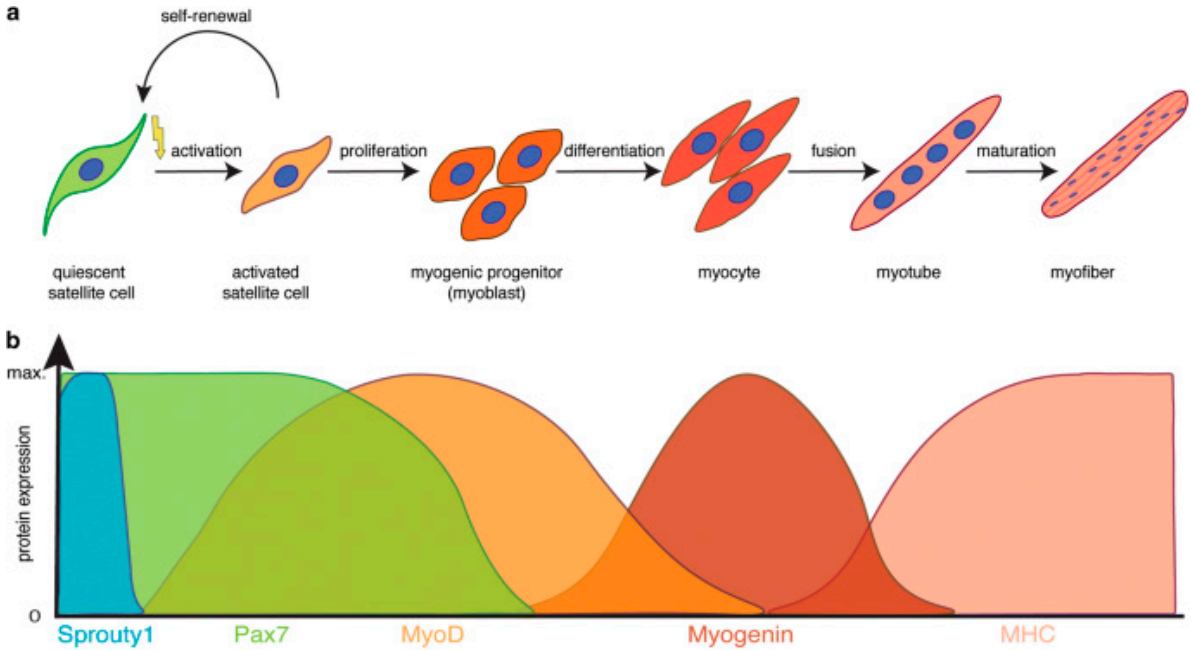
- These effects are reversible if the MuSCs were transferred to a soft environment, but the molecular mechanisms of this remains poorly understood.

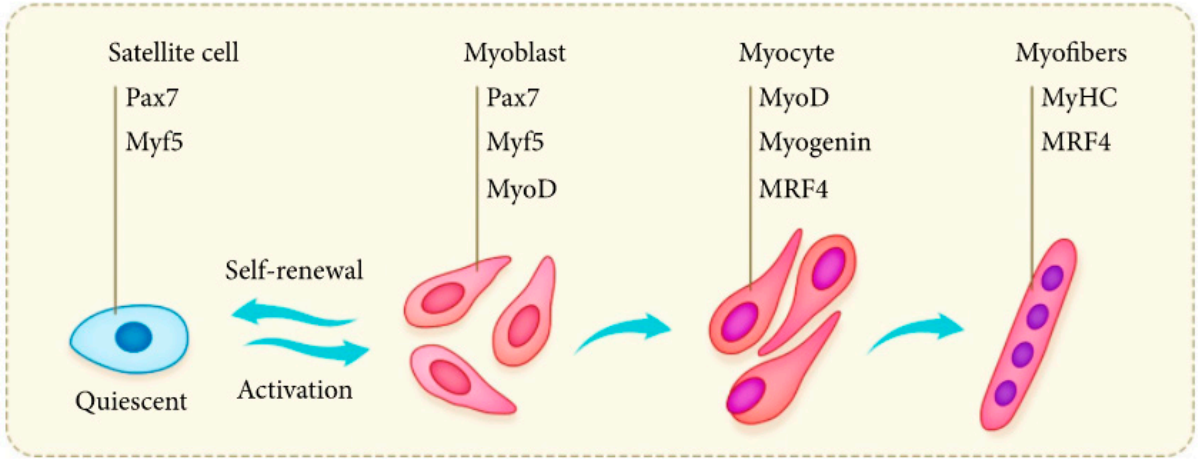




Differentiation of Muscle Stem Cells in Healthy Young Mice

- Stages of differentiation is dictated by time-dependent gene expression patterns.
- What happens to aged MuSCs in stiff environments?





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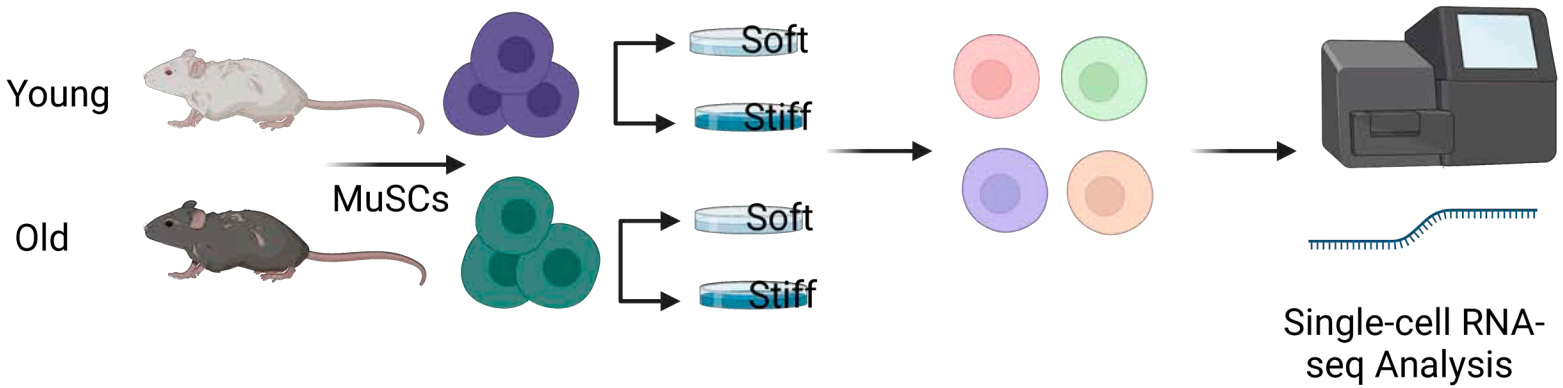
Experimental Design for Harvesting MuSCs

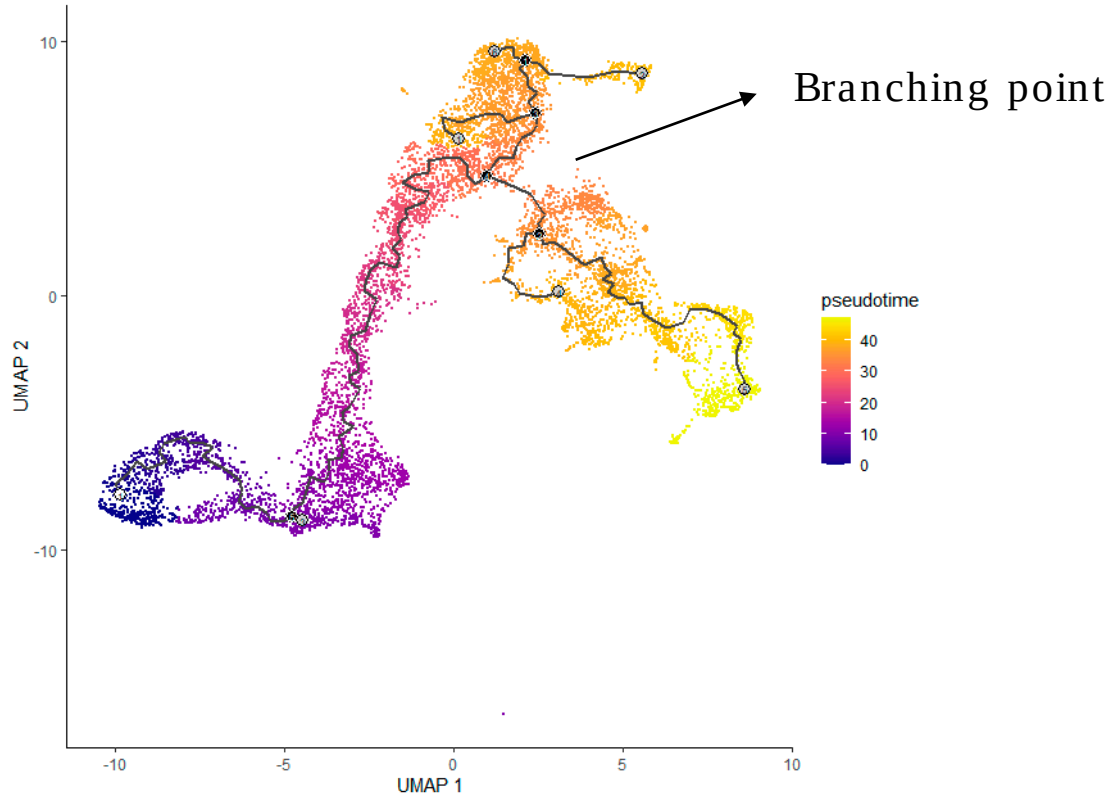
MuSCs derived from both aged and young mice were planted on fabricated silicone-based organic polymers (PDMS) substrates to mimic the stiffness of young (elastic modulus (E): 12 kPa)) or aged muscle (E: 29 kPa).

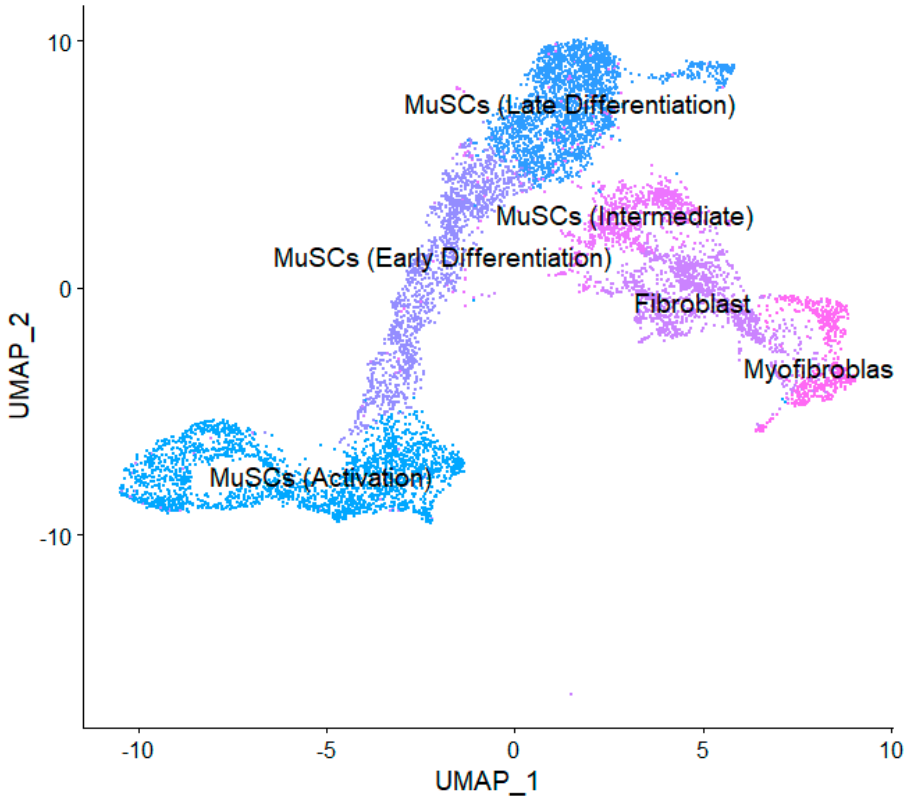
Followingly, cells were recovered for scRNA-seq.



Lineage Branching Observed in Trajectory Analysis

- The Monocle3 R package was used for trajectory analysis.
- Cell subpopulations are organized according to pseudo-time to infer developmental transitions.

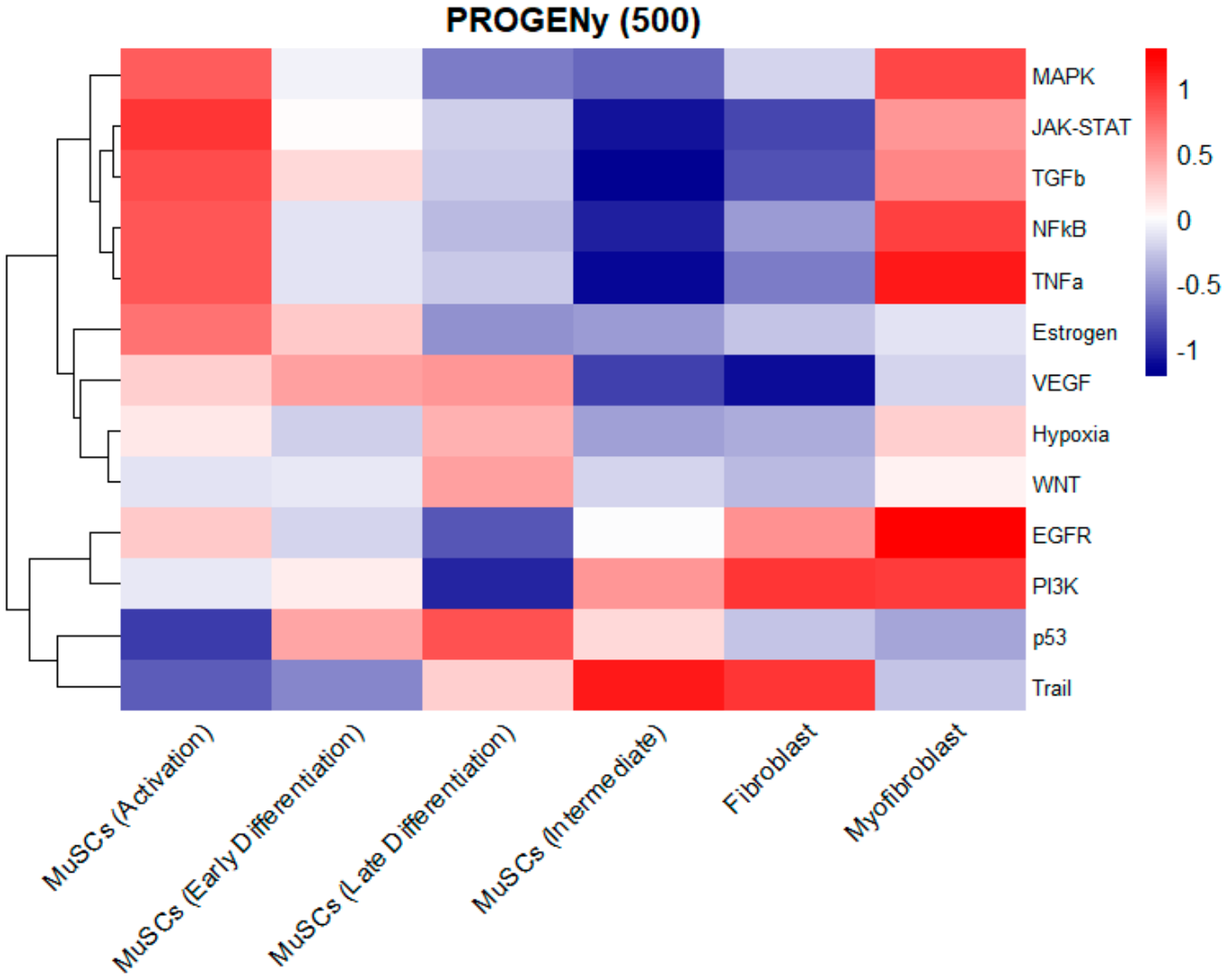




TRAIL Activation Trend in the Fibrogenic population

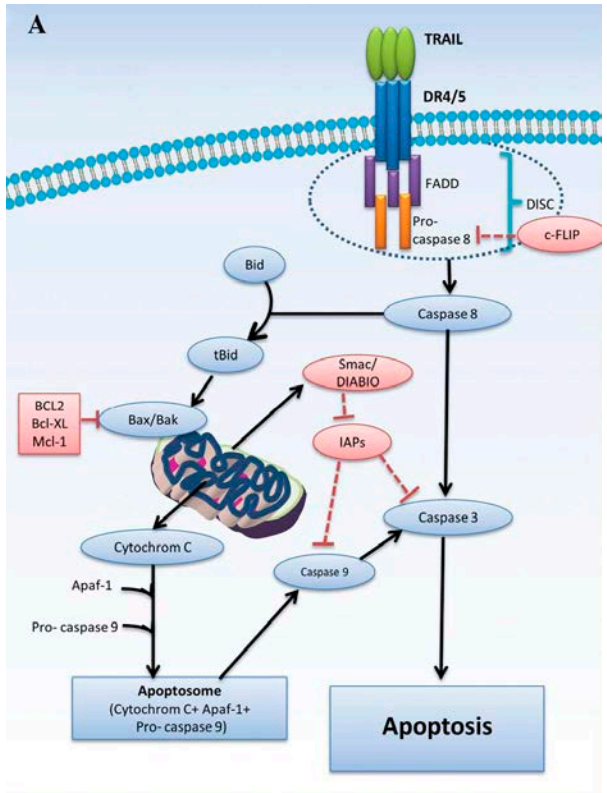
Three pathways were activated in the fibroblastic population:

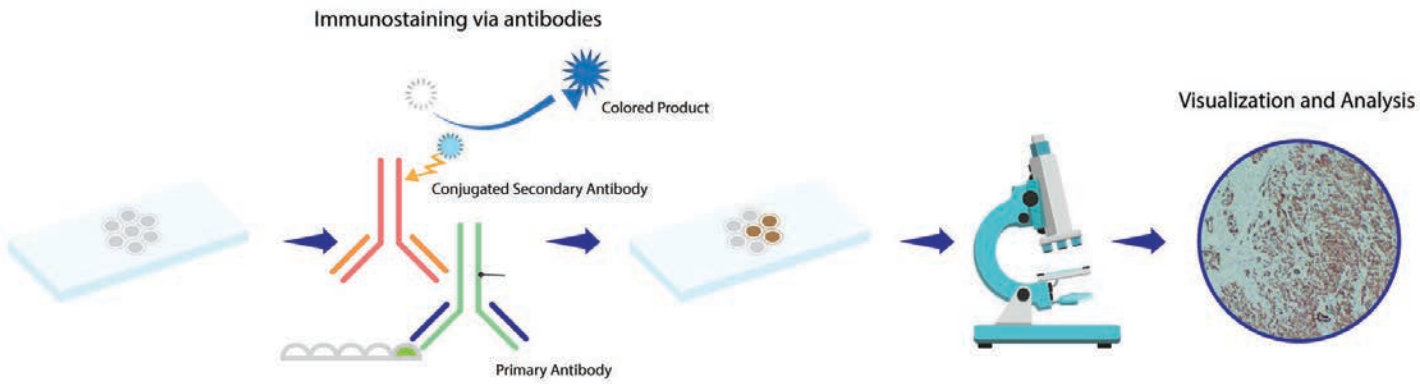
1. TRAIL was following a trend along the trajectory as it was highly activated in fibroblasts followed by the MuSCs (Intermediate), and slightly activated in the MuSCs (late differentiation).
2. the Phosphoinositide 3-kinases (PI3K) pathway that inhibits apoptosis by activating Akt.
3. Epidermal Growth Factor Receptor (EGFR) that mediates apoptosis through Stat3.



In-vivo Validation with TRAIL Immunostaining

- Caspase-3, a TRAIL downstream protein associated with cell apoptosis was stained and quantified.





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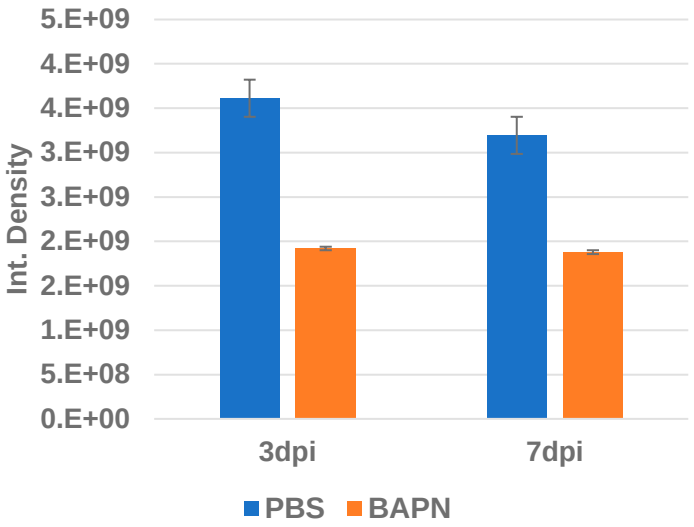
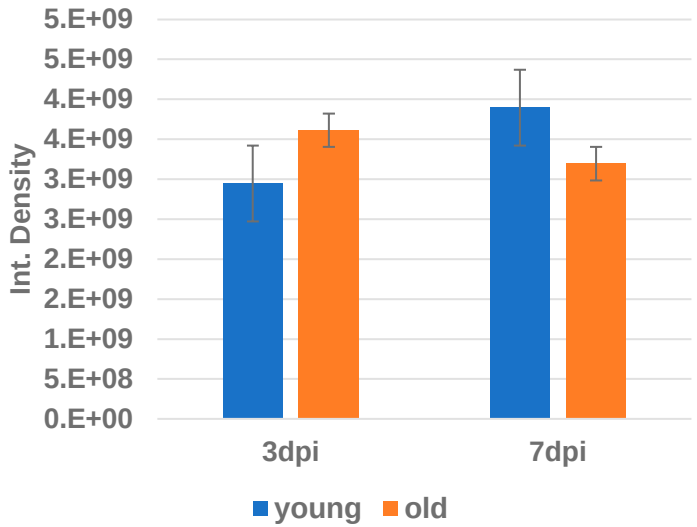
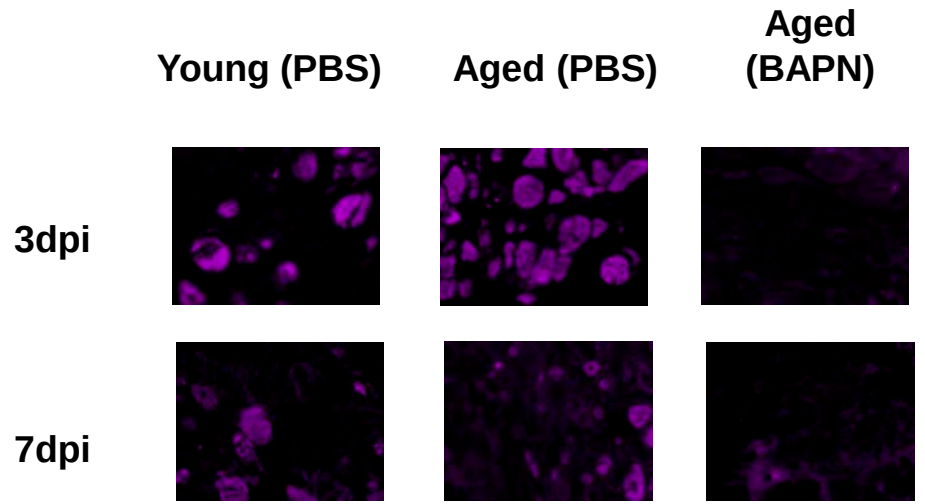
In-vivo Validation with TRAIL Immunostaining

- Whole tissues were harvested from young and aged animals.
- β-aminopropionitrile (BAPN), a collagen anti-crosslinking agent used to reverse stiffness, was applies to aged tissues mimicking the soft tissues environment and then those tissues were also stained for caspase-3.
- Caspase-3 was found to be downregulated in aged animals treated with BAPN, suggesting a connection between aging, fibrogenic conversion, matrix stiffness, and apoptosis







Conclusions and Acknowledgments

Single-cell RNA sequencing data were analyzed from muscle-derived stem cells

A novel mechanism for fibrogenic conversion was identified that involved the inflammatory mediator TRAIL

This mechanism was validated using immunohistochemical staining of muscle tissue sections

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