THE GOOD FOOD INSTITUTE

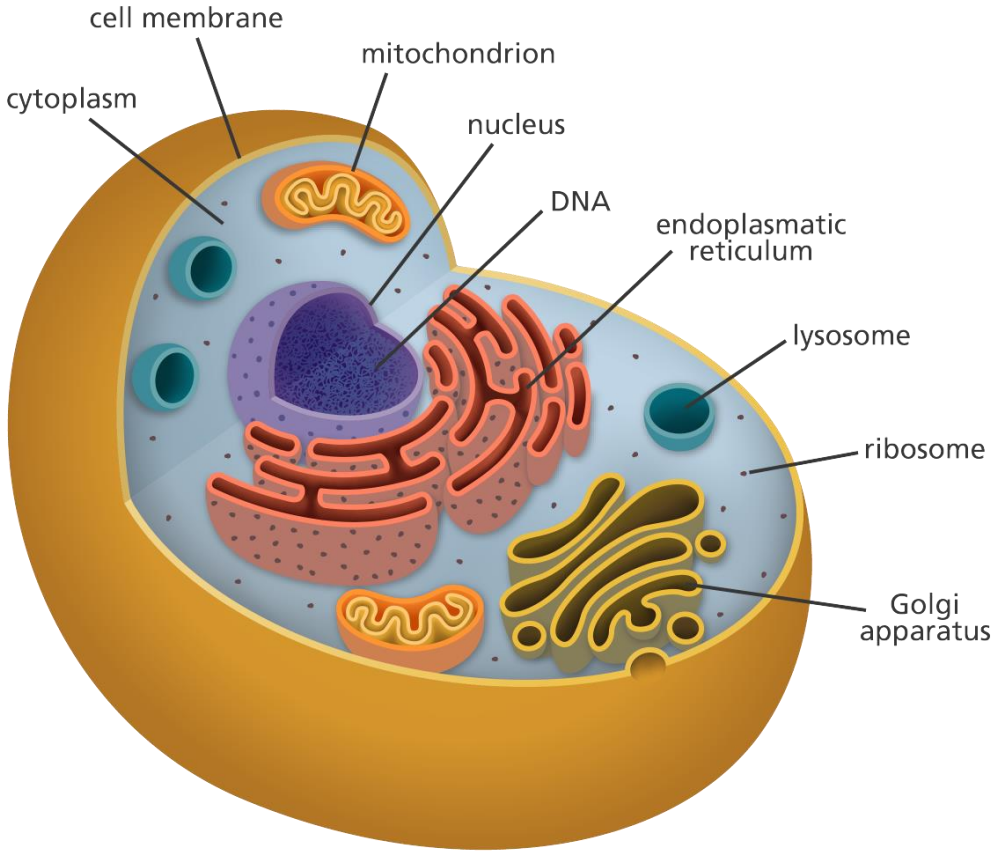
Cultivated meat: Cells and cell differentiation

Module content

- Cell overview
- Stem cells
- iPSCs
- Satellite cells
- Fibroblasts
- Adipocytes

Cells for biotechnology

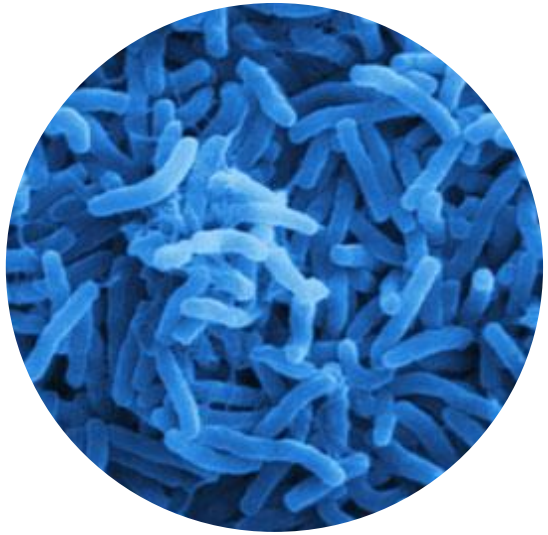
- Building block of life
- Multiply
- Functionality
- Thousands of different molecules

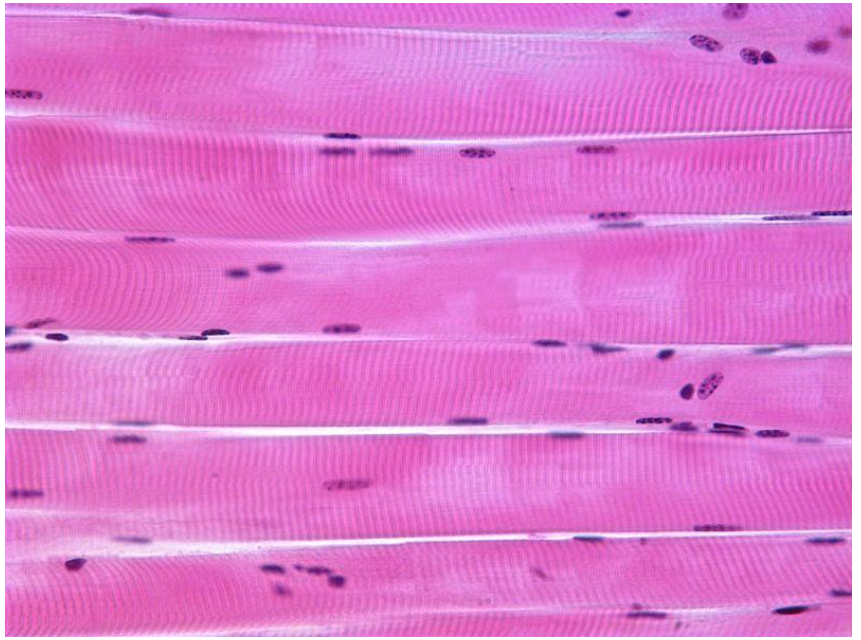


Cells of interest

- Single cell organisms
  - Adaptive
  - Focus on proliferation, nutrients
  - All-in-one

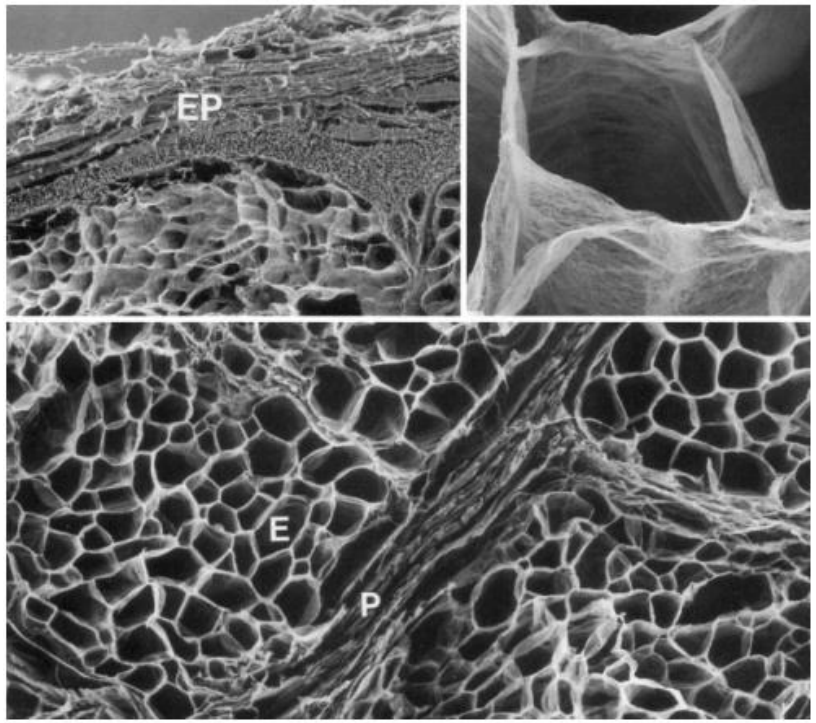
- Multicellular organisms
  - Sensitive
  - Proliferation regulation
  - Vascularization
  - Specific functionality
  - Adherent cells

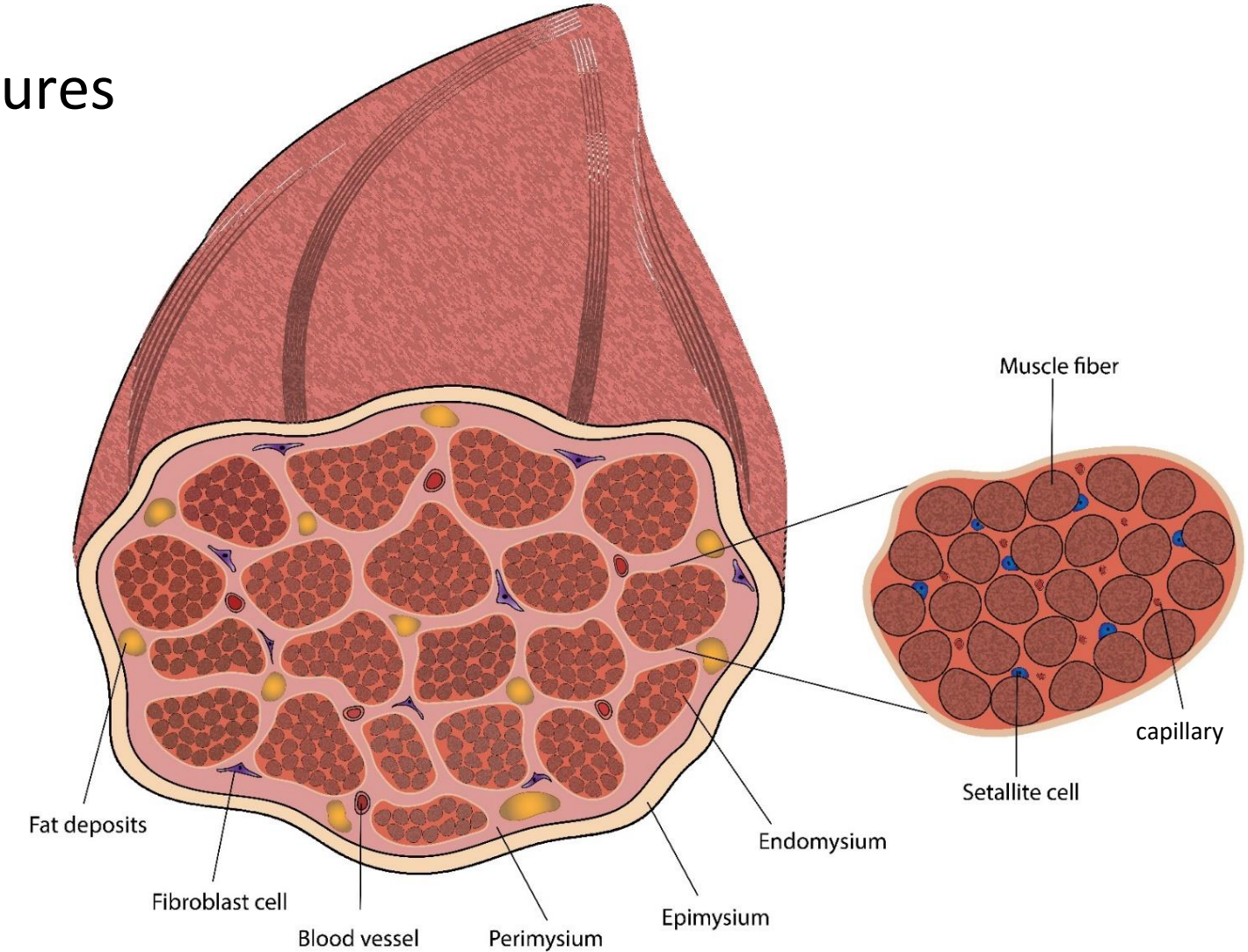




Tissues

- Hierarchical designed structures
- Extracellular matrix (ECM)

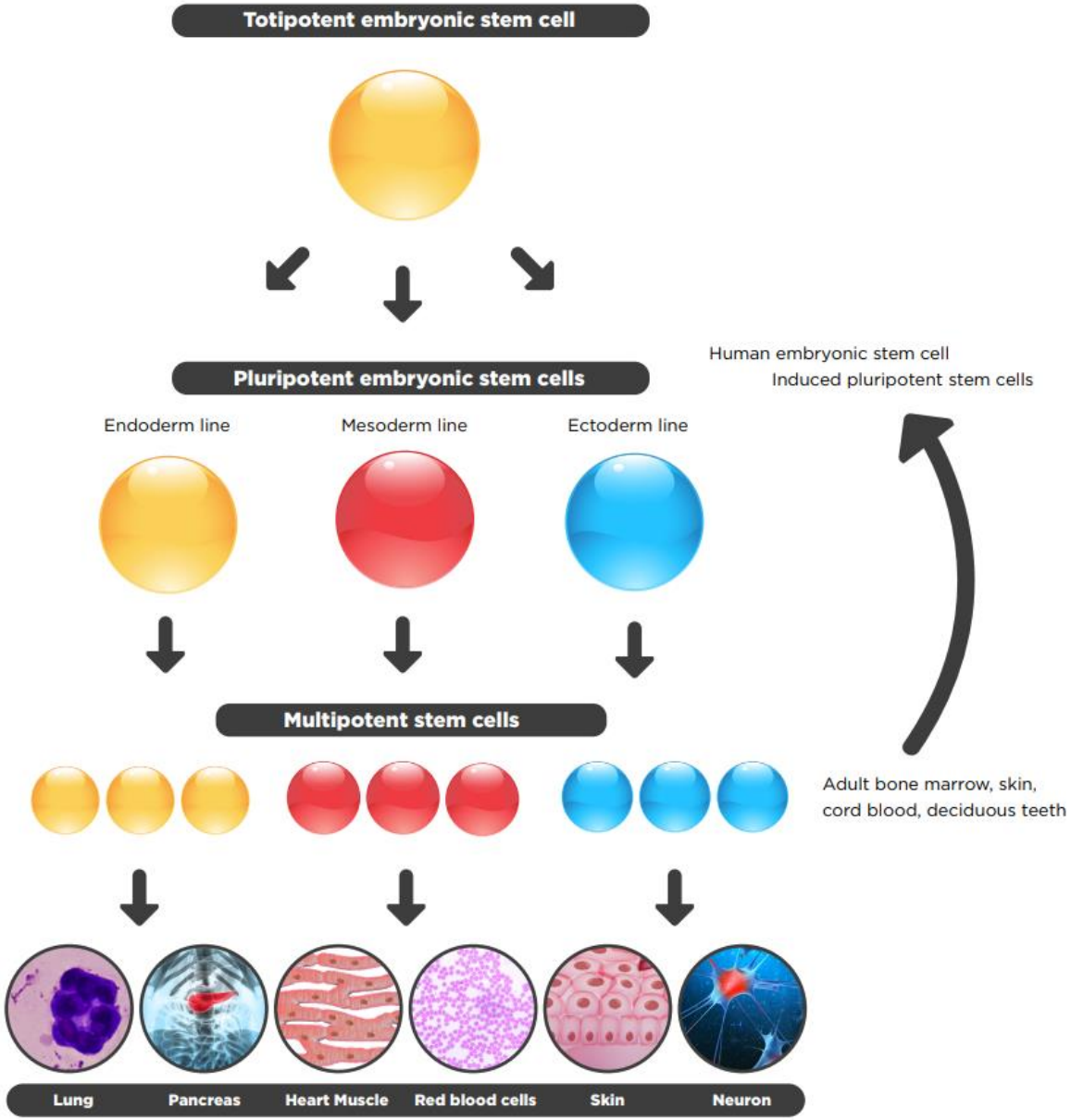




Stem cells

- A stem cell is a cell defined by its ability to **self-renew** and **differentiate**

- Cell potency is defined by a cell's ability to differentiate into other cell types
  - **toti**potent - all cells
  - **pluri**potent - all cell lineages (endoderm, ectoderm, mesoderm)
  - **multi**potent - many cell types
  - **oligo**potent - some cell types
  - **uni**potent - one cell type



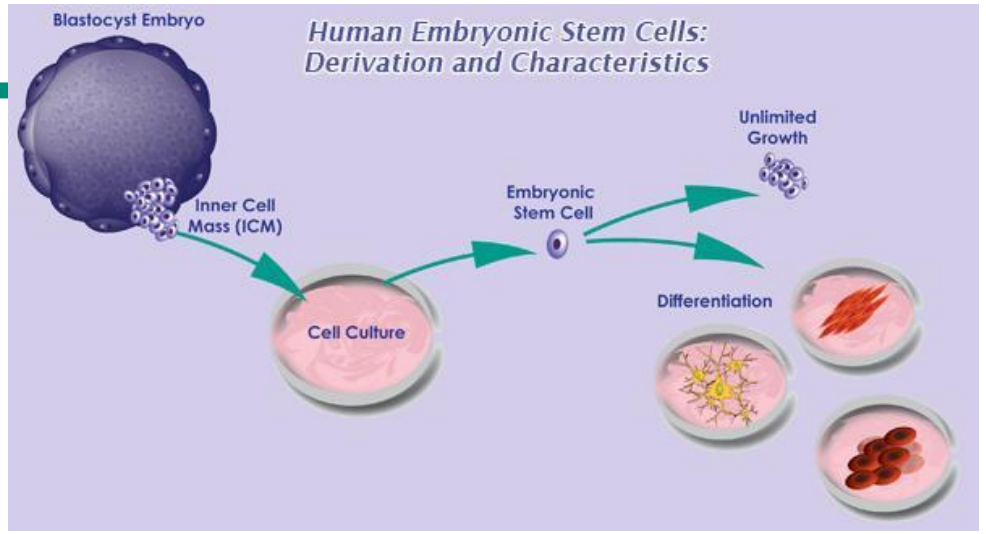
Pluripotency and cell reprogramming

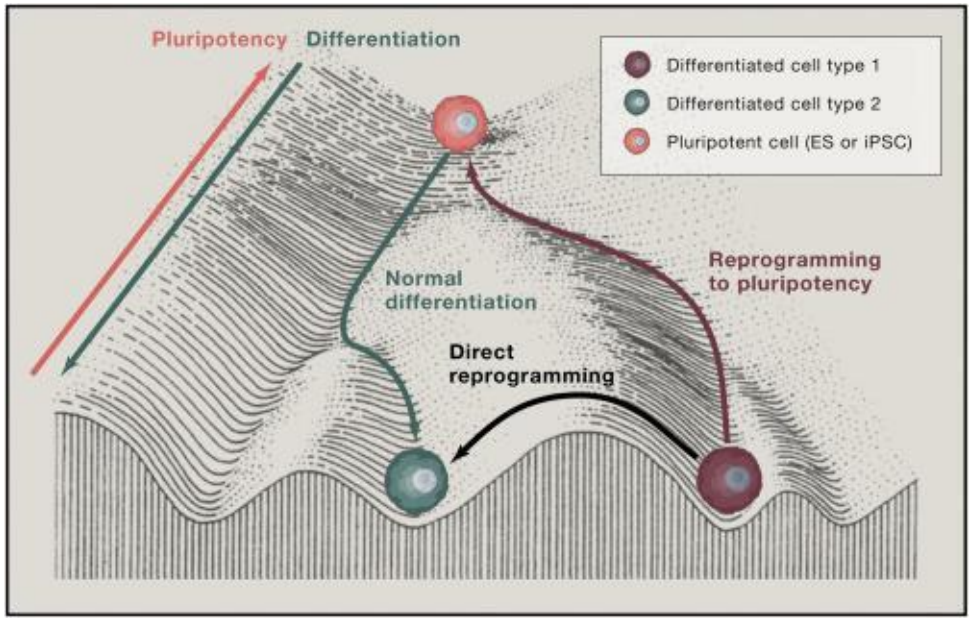
- Embryonic stem cells are obtained from the inner cell mass of an embryo during the blastocyst stage
  - Challenging to do with delicate materials and variable growth conditions

- In 2012, Shinya Yamanaka won the Nobel Prize for induced pluripotency, a form of cell reprogramming

- Cell reprogramming enables the direct conversion of one cell type into any other cell type based on the expression of a defined set of important genes of the final cell type, typically a set of transcription factors

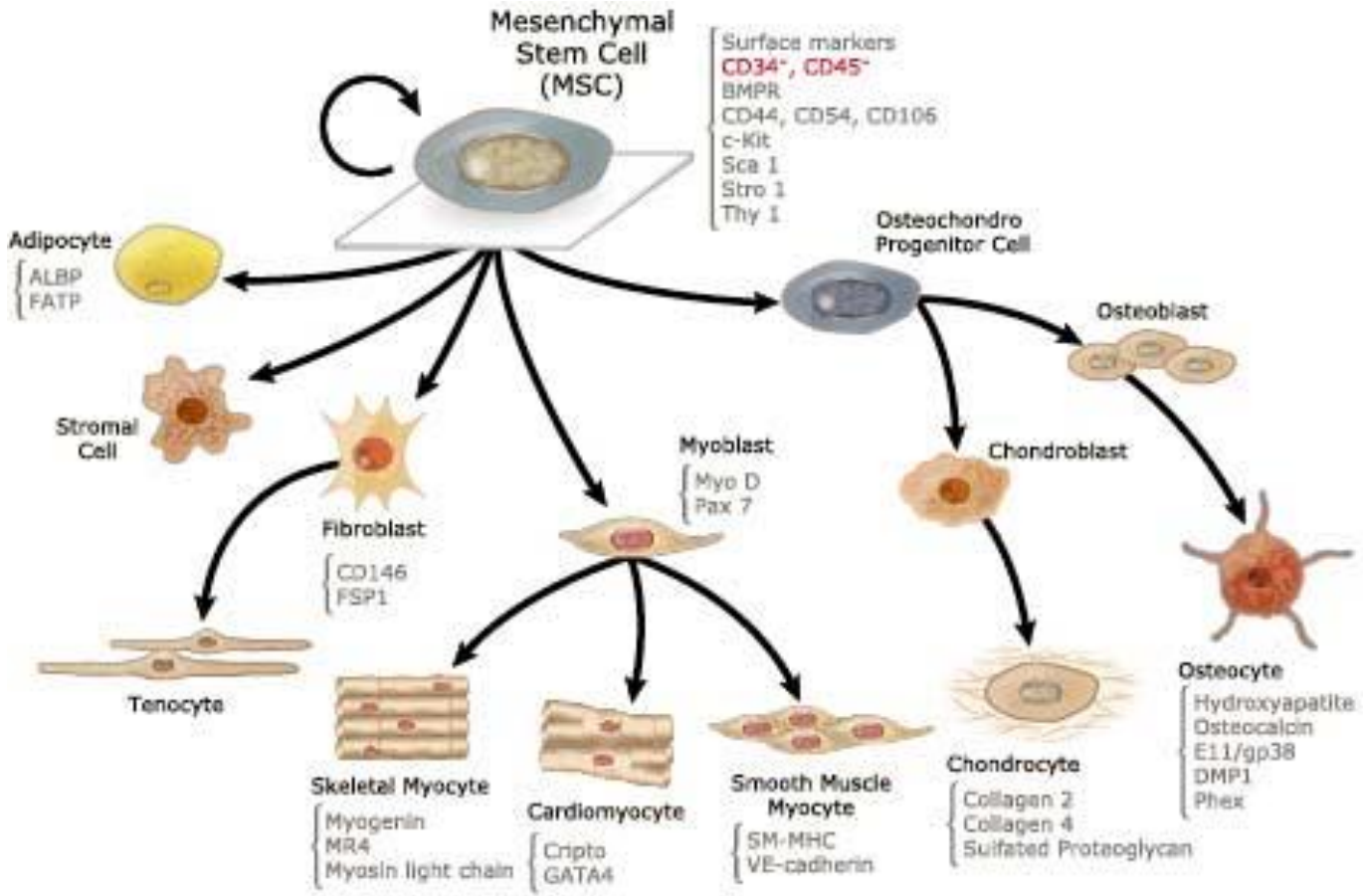
- Induced pluripotent stem cells (iPSCs) are functionally identical to embryonic stem cells and significantly easier to obtain





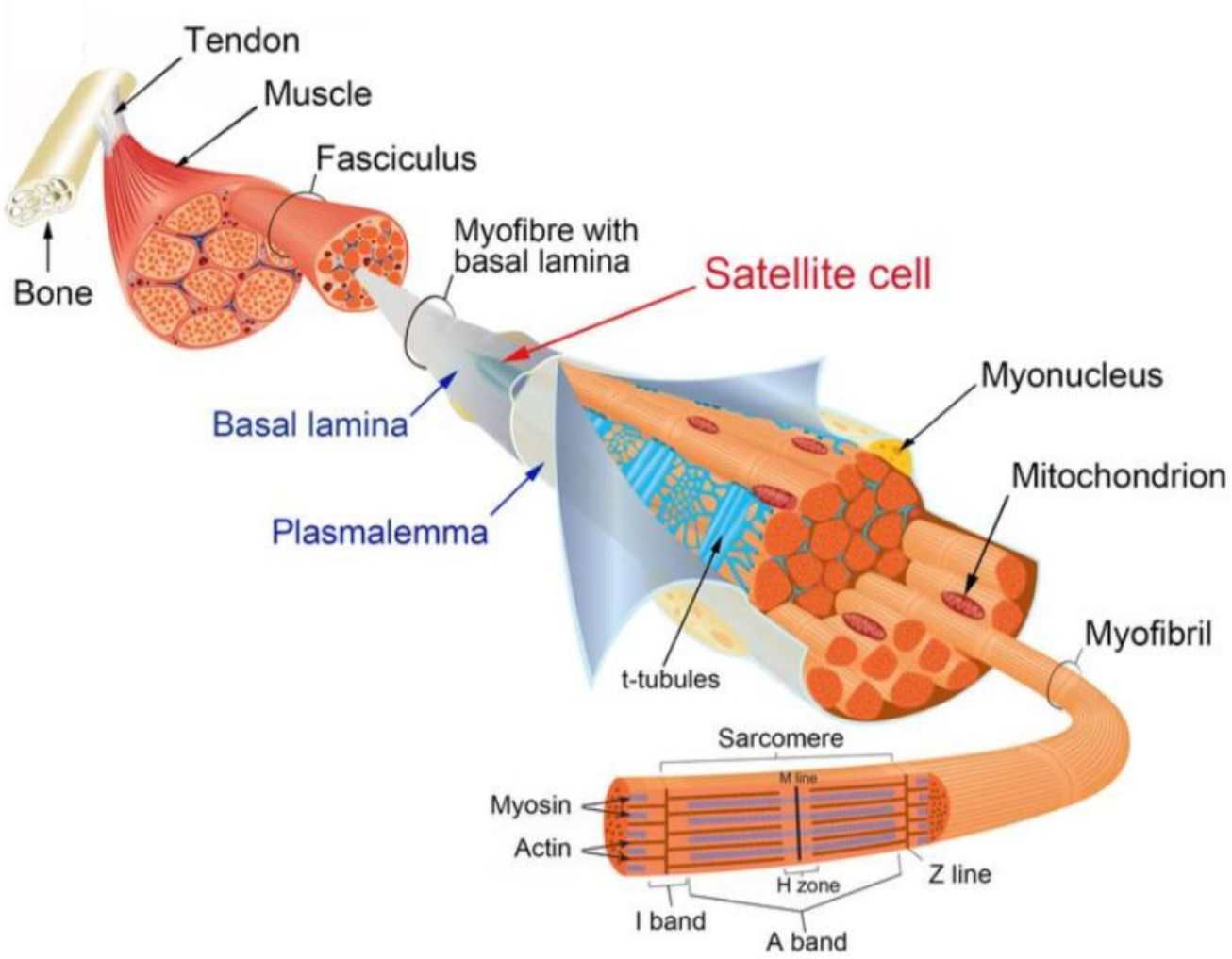
Mesenchymal stem cells

- Mesenchymal stem cells (MSCs) are multipotent stem cells that differentiate into a variety of cell types
- Can be obtained from bone marrow, adipose tissue, umbilical cord, dental pulp, etc
- How to specifically define these stem cells is an active scientific debate



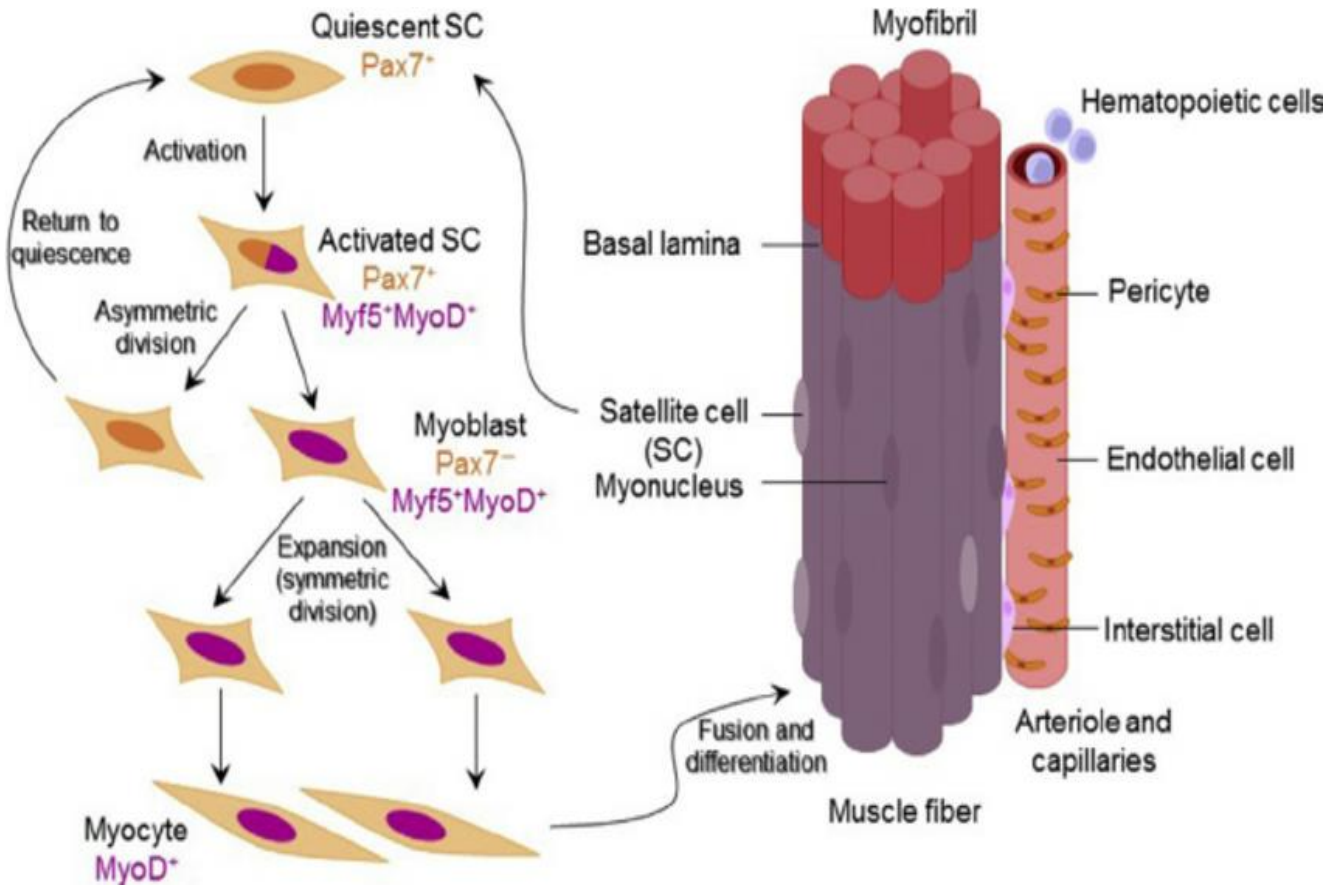
Satellite cell & myoblasts

- Satellite cells are the resident stem cell population in skeletal muscle. They lie quiescent under the “basal lamina” until activated upon injury or stress.
- Activated satellite cells are called myoblasts
- Obtained by a muscle biopsy
- Generally considered to be unipotent in that they give rise to skeletal myocytes



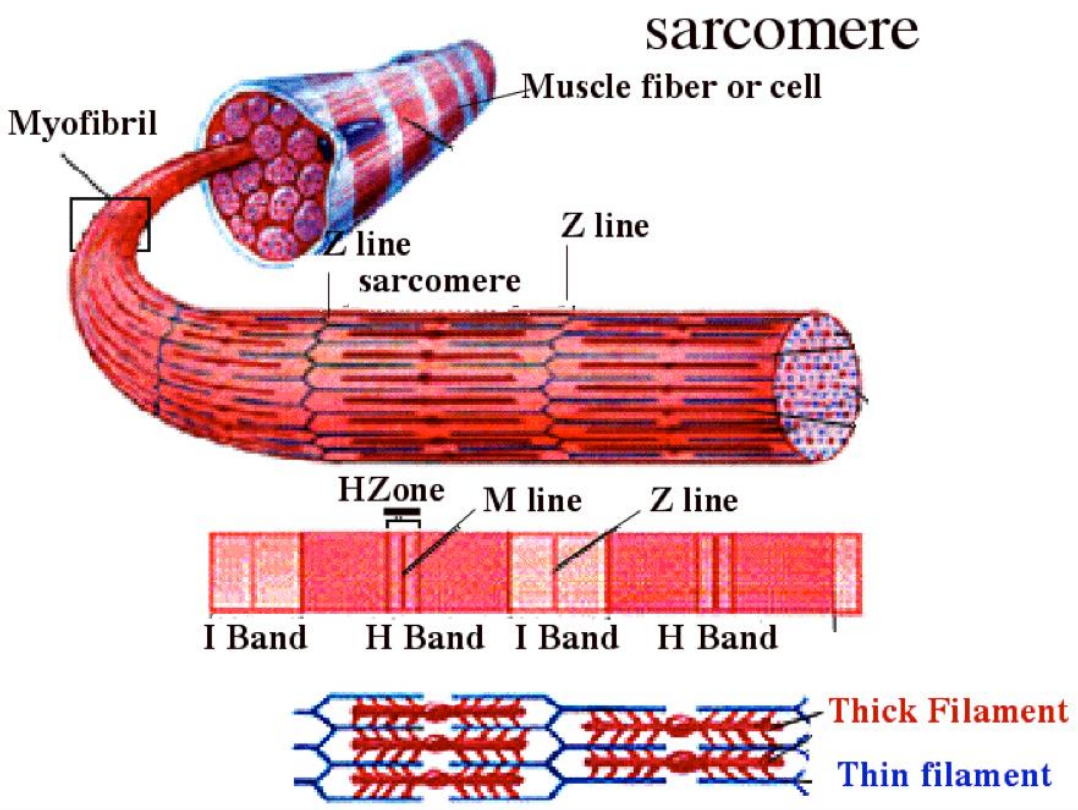
Myogenesis

- During injury, activated myoblasts differentiate into myocytes. Myocytes eventually fuse together, differentiating into multinucleated myotubes, which make up myofibrils.

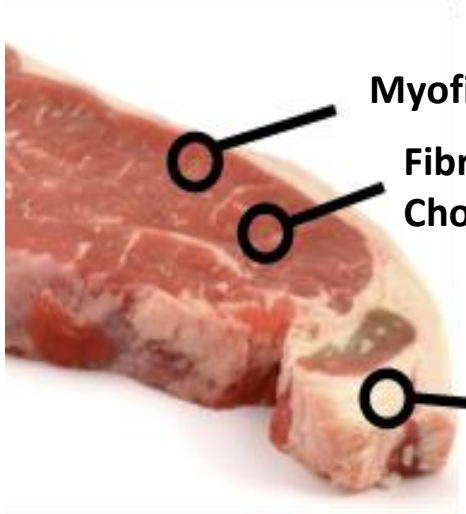


Myogenesis

- Mature myofibrils form a complete muscle fiber.
- The structural unit of the myofibril is called the **sarcomere**. The sarcomere is organized by specific proteins that permit contraction through sliding.
- The two most important proteins are **actin** and **myosin**
- Actin makes up the thin filaments of the I band, whereas myosin makes up thick filaments of the A band. Additional proteins serve as anchor points and allow the actin-myosin unit to contract.



Cell types used in cultivated meat production

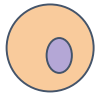


Cell Type	Cell Types Created	Difficulty to Obtain	Proliferative Capacity	Species-relevant Work
Embryonic Stem Cell (ESC) Induced Pluripotent Stem Cell (iPSC)	All	+++ (ESC) ++ (iPSC)	Immortal	++ (bovine, porcine, ovine, avian)
Mesenchymal Stem Cell (MSC)	Bone, Muscle, Fat	+	Limited (10-30 doublings)	++ (bovine, porcine, ovine, avian, piscine)
Myosatellite Cell	Muscle Fat, Bone (?)	+	Limited (10-30 doublings)	+ (bovine, porcine, ovine, avian, piscine)

Fibroblast

FUTURE MEAT

ESC



CUBIQ FOODS
Clean Innovations

iPSC

Hs Higher Steaks

Meatable

MSC

NEW AGE MEATS

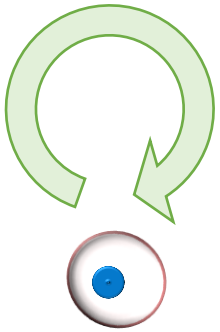
Satellite cell

mosa meat

BlueNalu

Myogenesis induction

bFGF, LIF, PDGF
TGF-ß, HGF, IGF-1

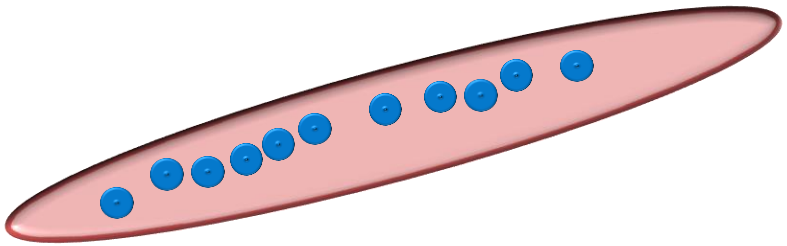


BSC

Contact inhibition
Starvation media
IGF-I, IGF-II
myogenic miRNA

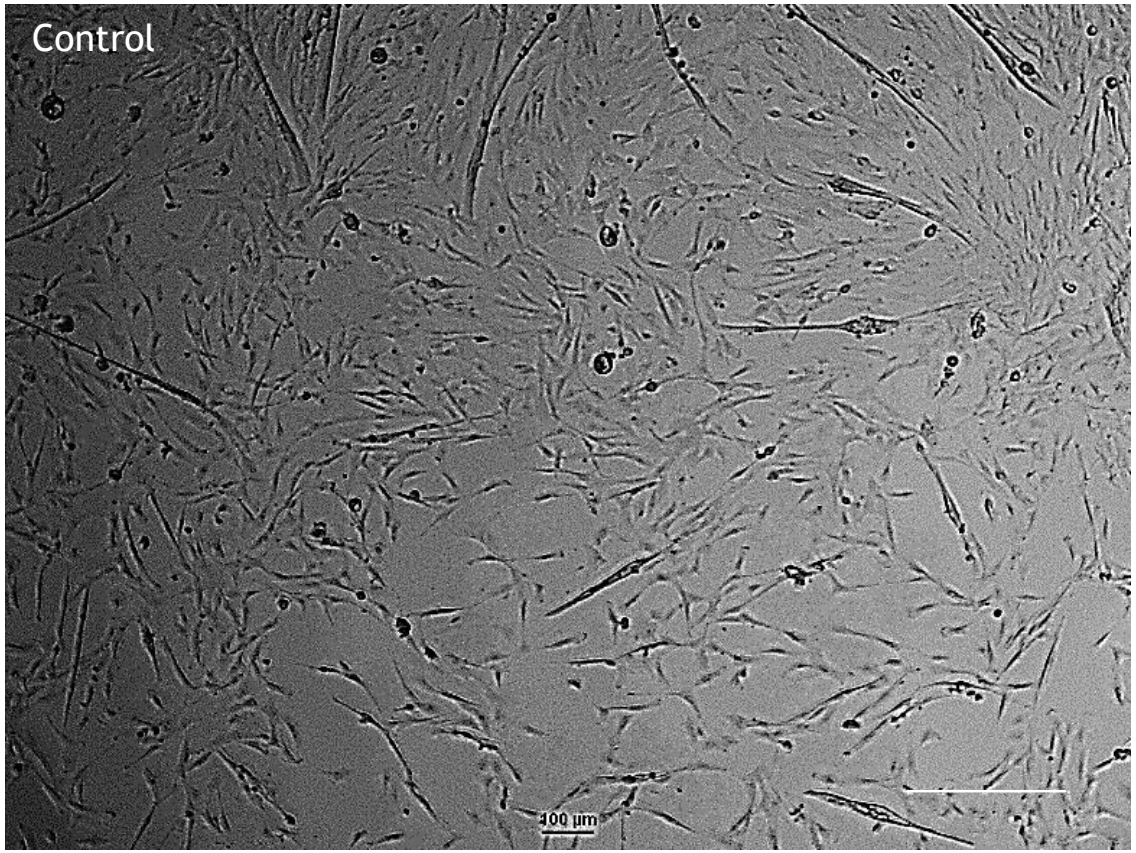
Myogenesis

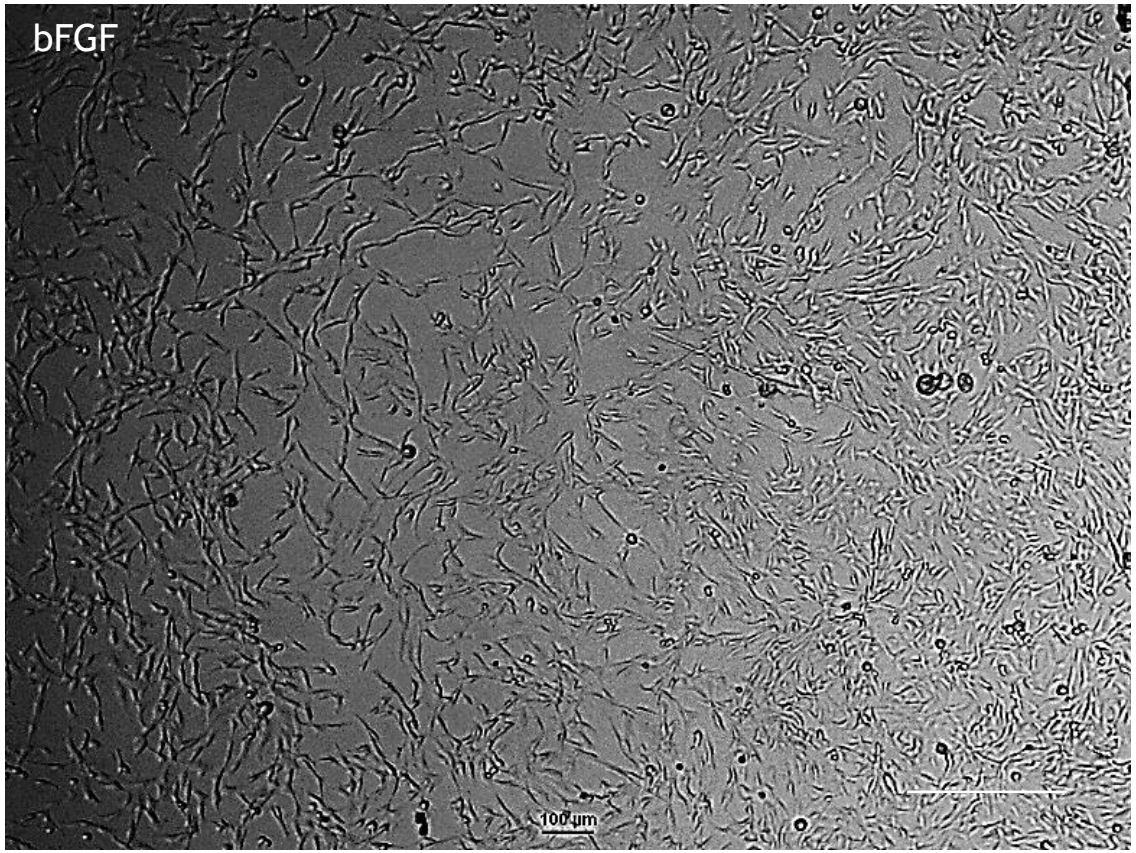
bFGF, LIF, HGF



Myotube

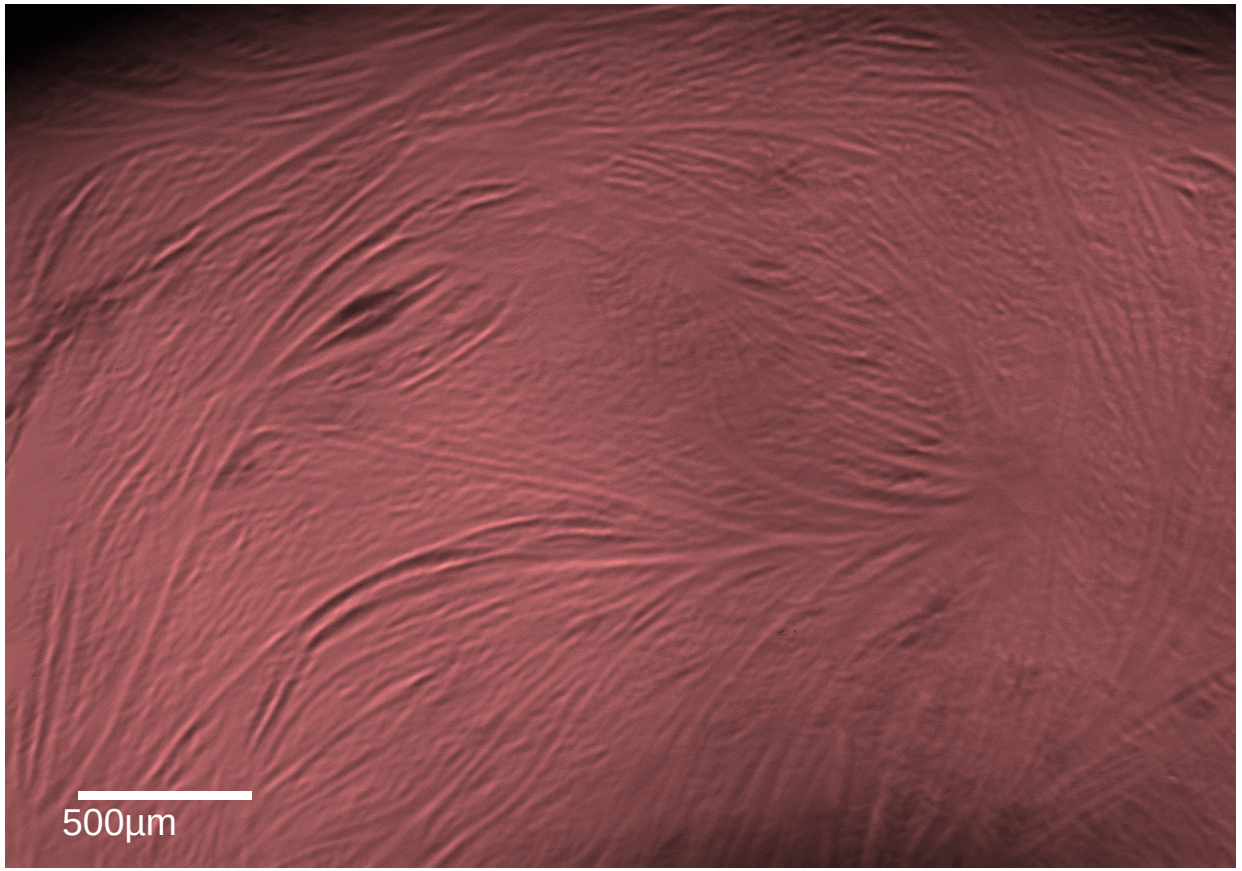
bFGF prevent myogenesis

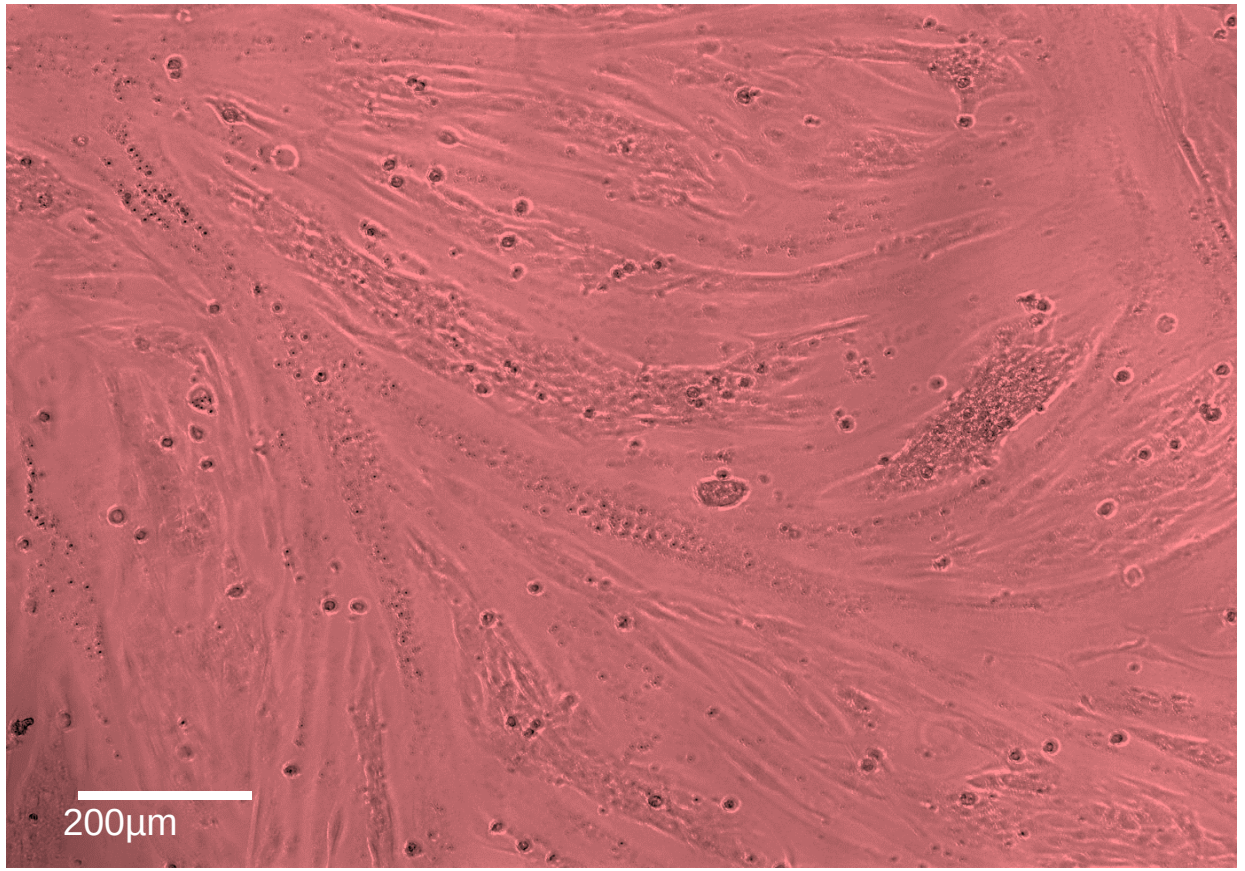




bFGF - basic fibroblast growth factor

Myogenesis quantification

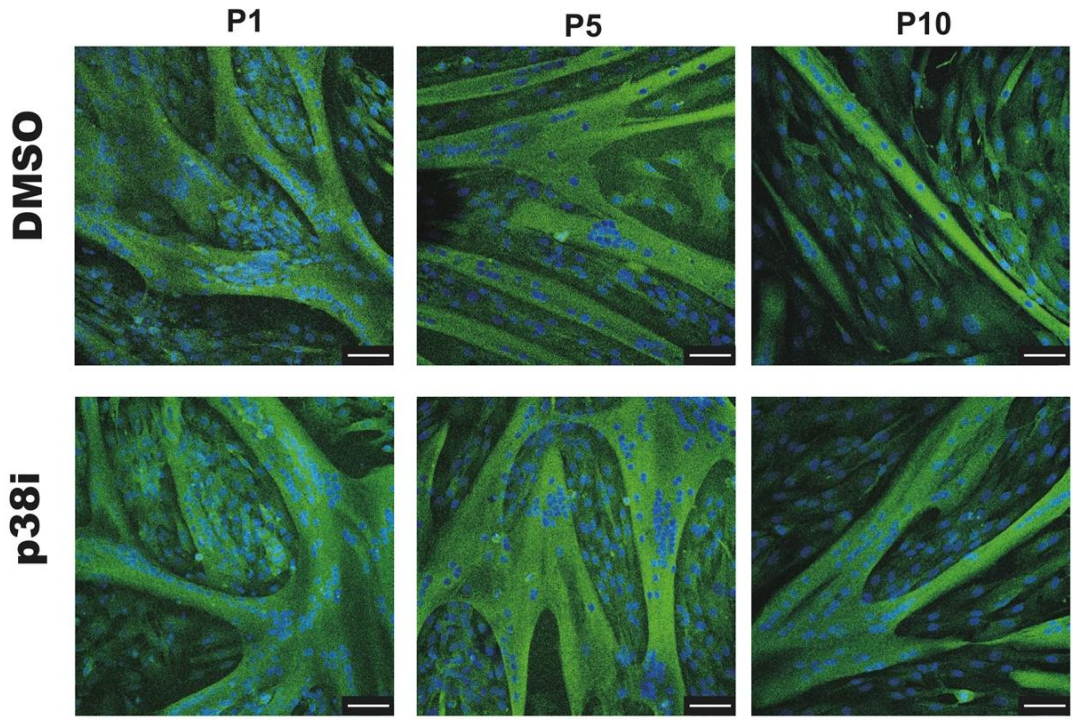


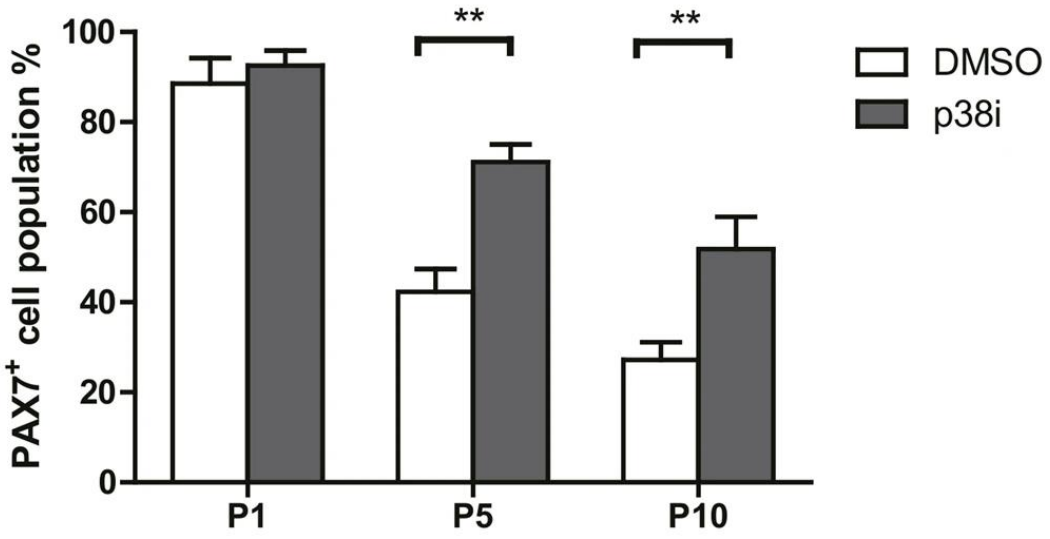


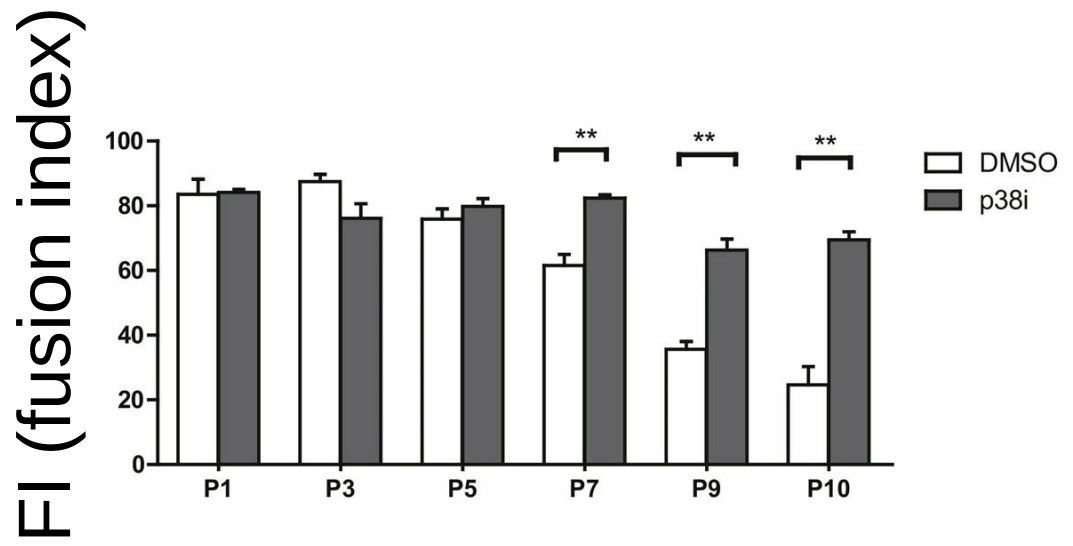
Bovine satellite cells (BSC)

- First CM burger

P38 inhibitor maintain BSC stemness

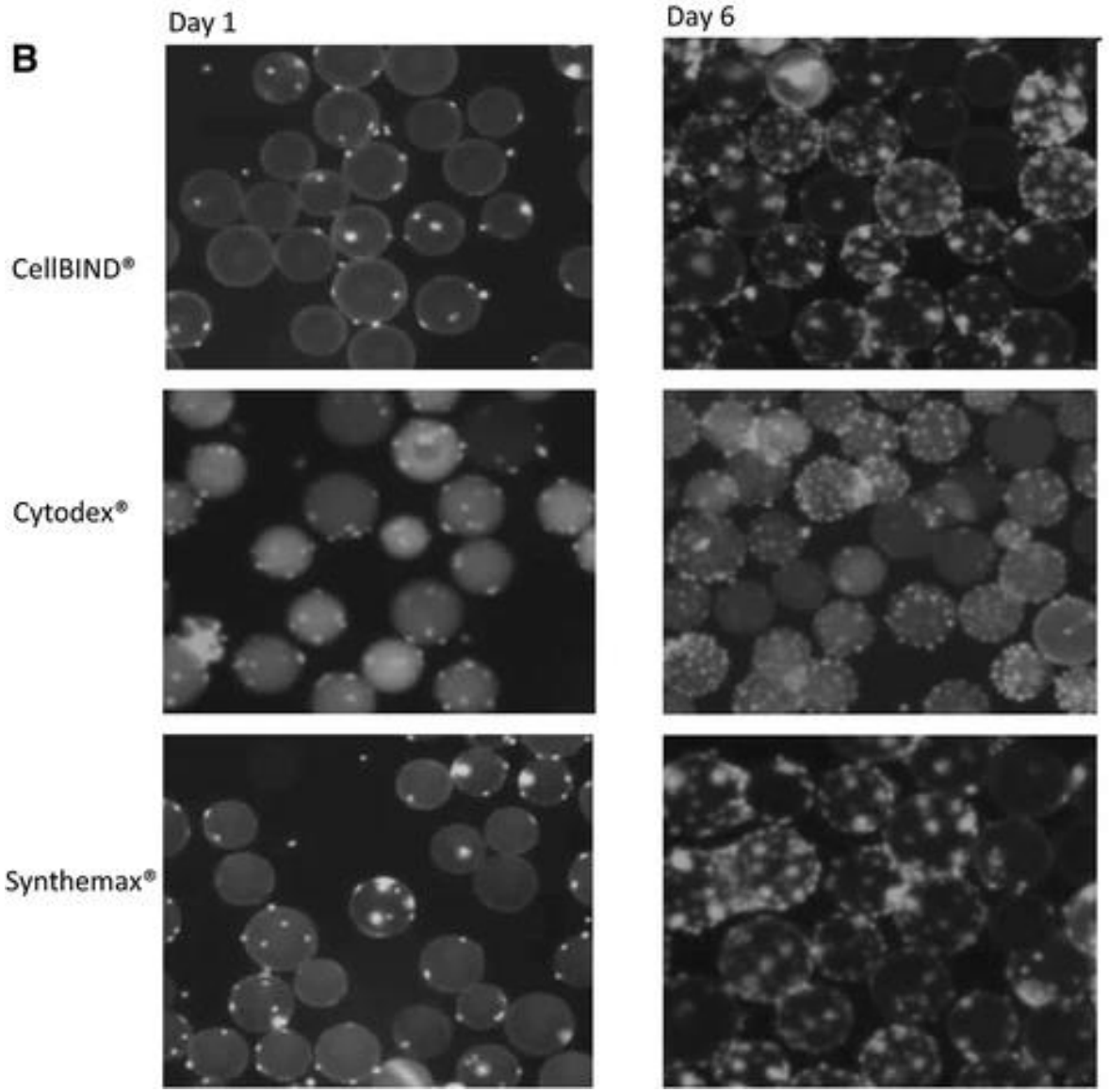






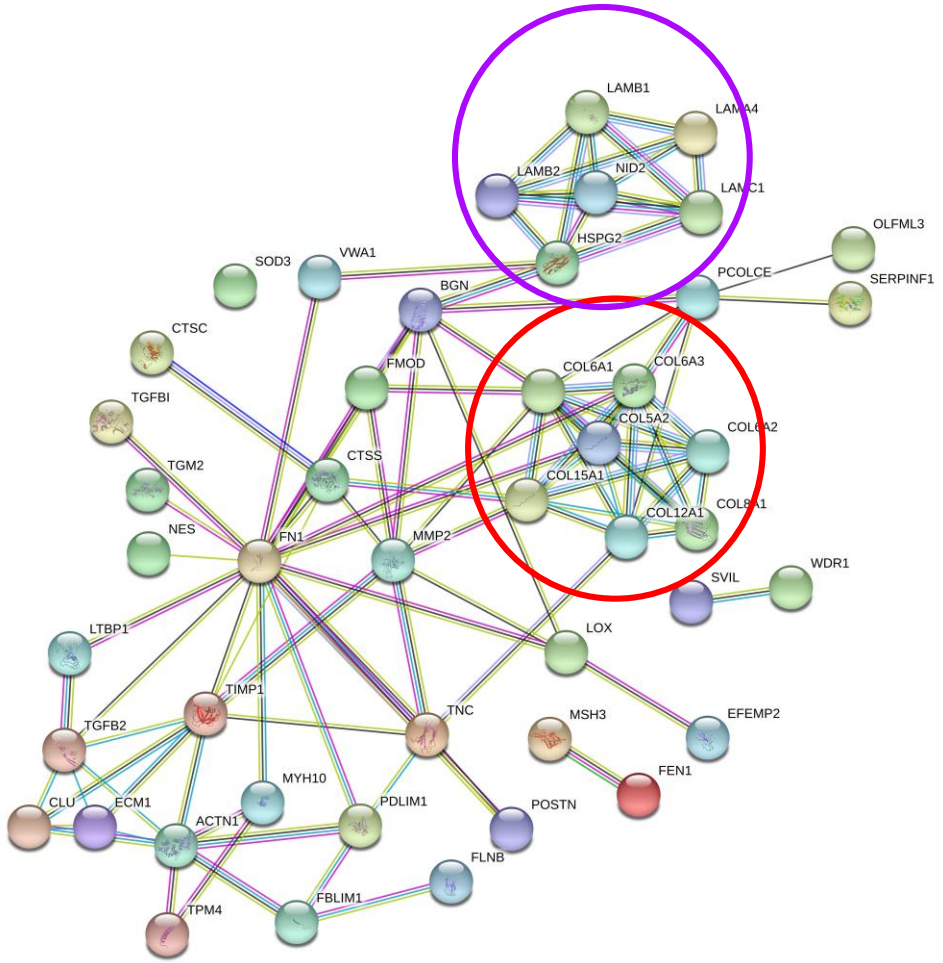
Ding et al (2018)

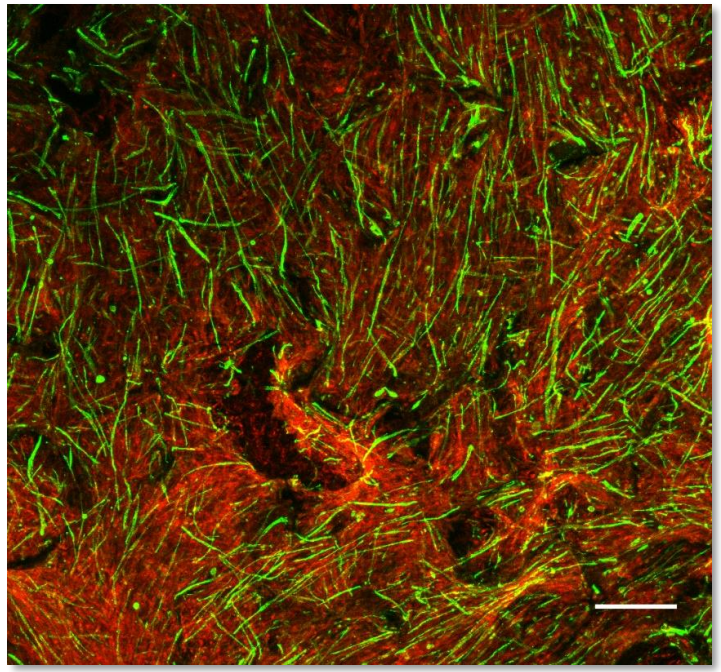
BSC on micro-carriers



Verbruggen et al (2018)

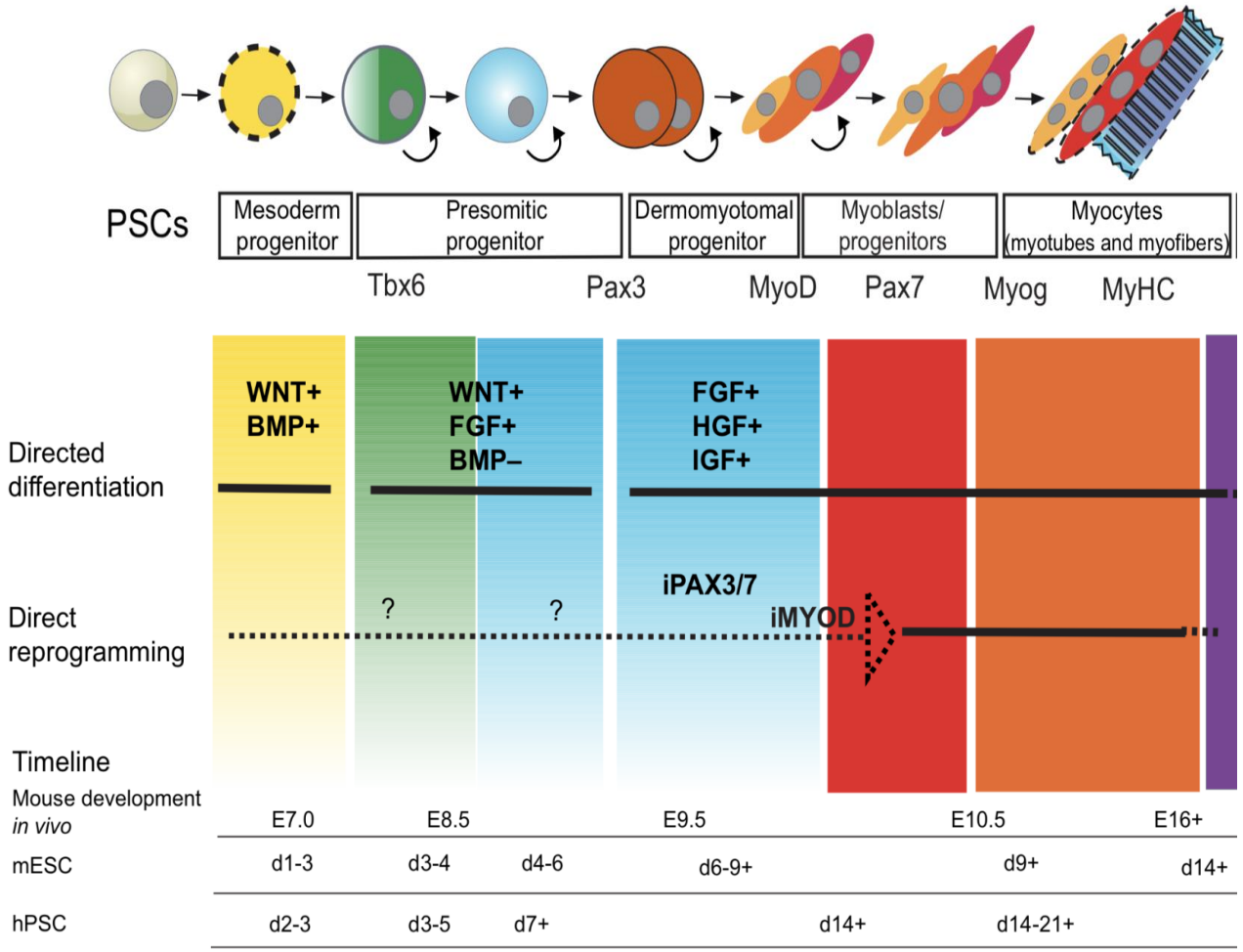
BSC co-culture on TVP scaffolds





Ben-Arye et al (2020)

Directed Differentiation of Pluripotent Stem Cells



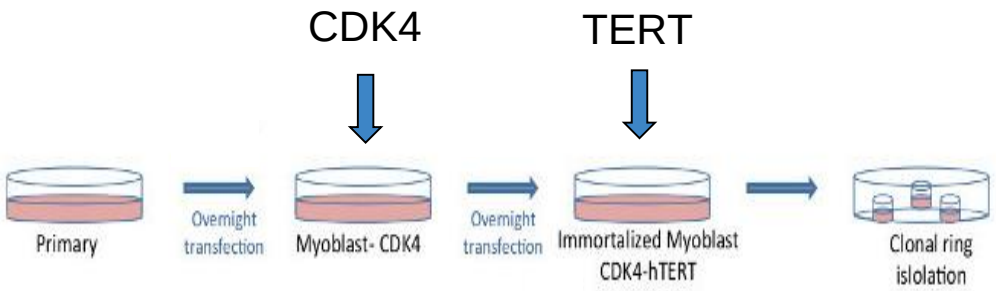
Chal & Pourquie, *Development*, 2017

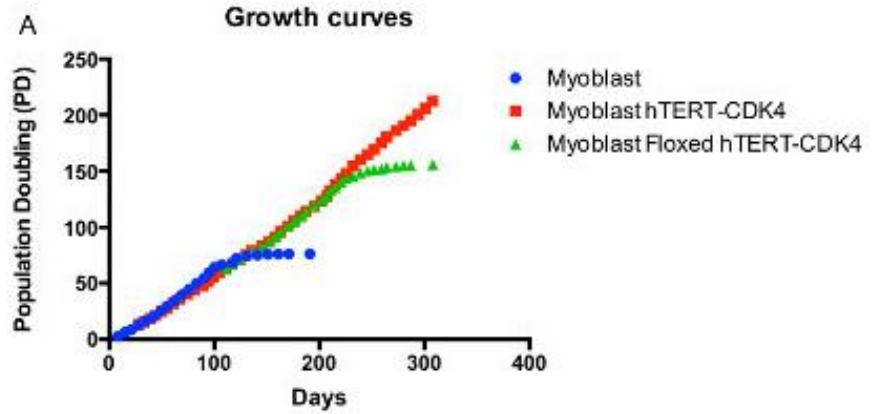
- Timing varies between species

Transgenic Methods for Myogenic Improvements

Immortalization

- Spontaneous immortalization





Robin *et al. JOVE*, 2015

Transgenic Methods for Myogenic Improvements

Immortalization

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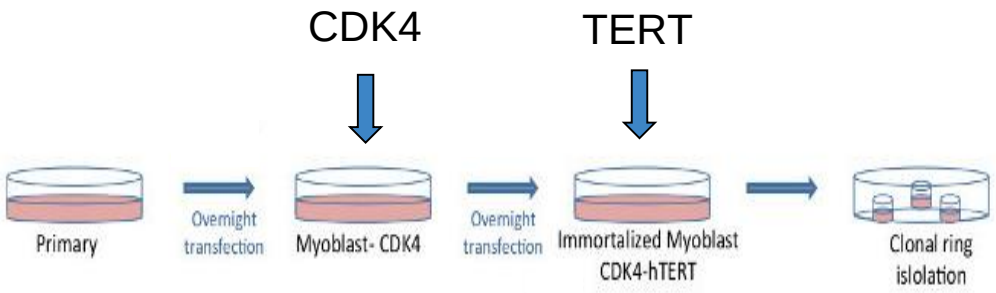
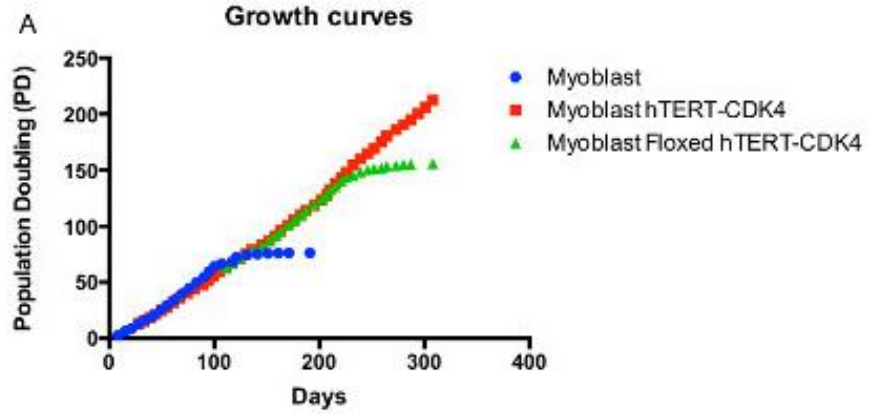
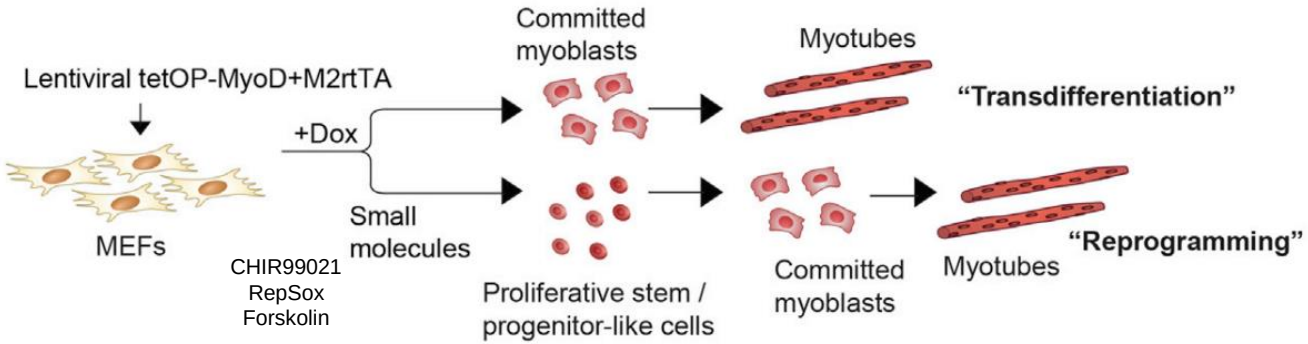


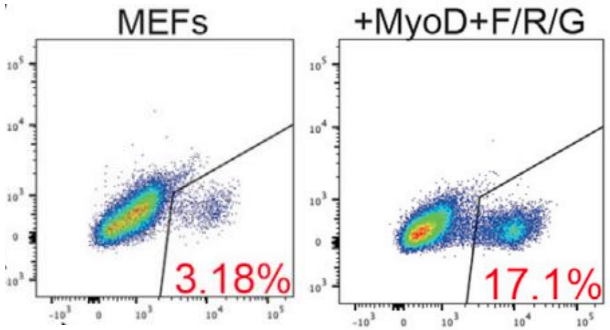


Robin *et al. JOVE*, 2015

Direct Reprogramming



Bar-Nur *et al. Stem Cell Reports*, 2018



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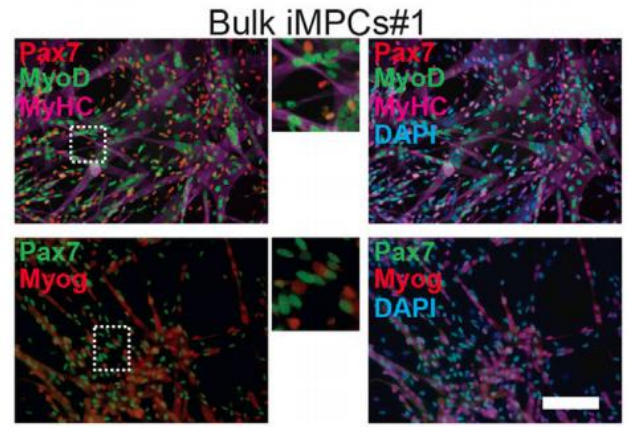
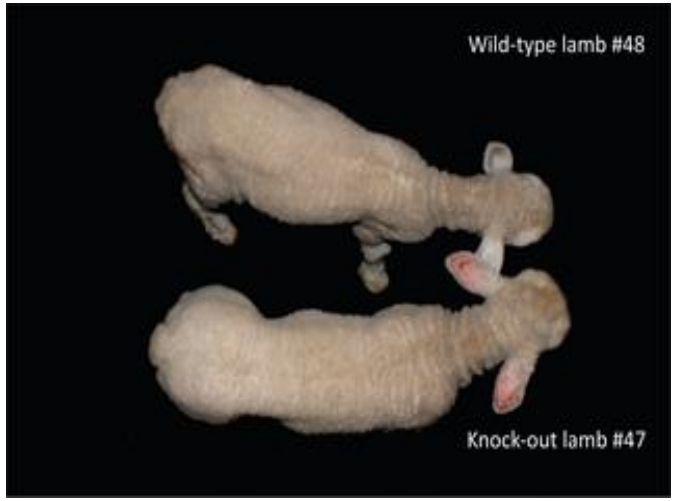
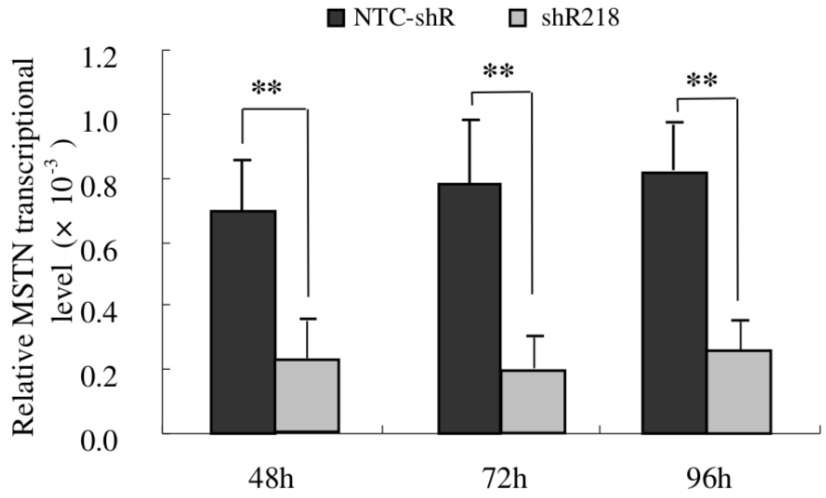
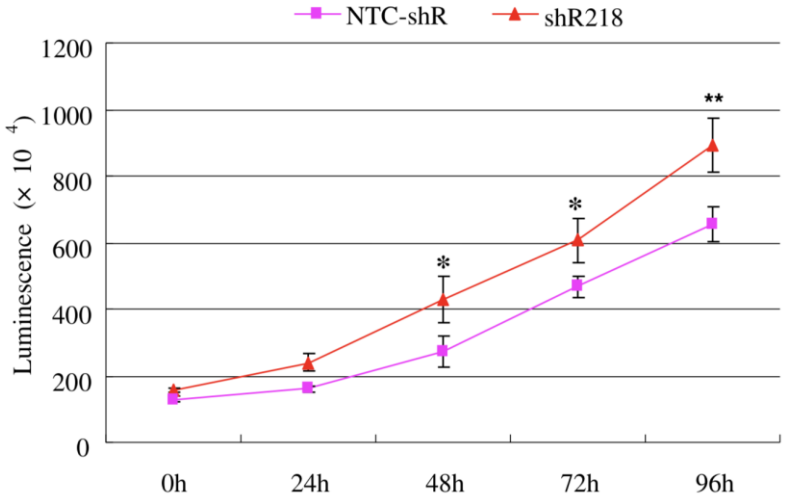


- Clonal populations propagated for >6 months, >20 passages

Transgenic Methods for Myogenic Improvements





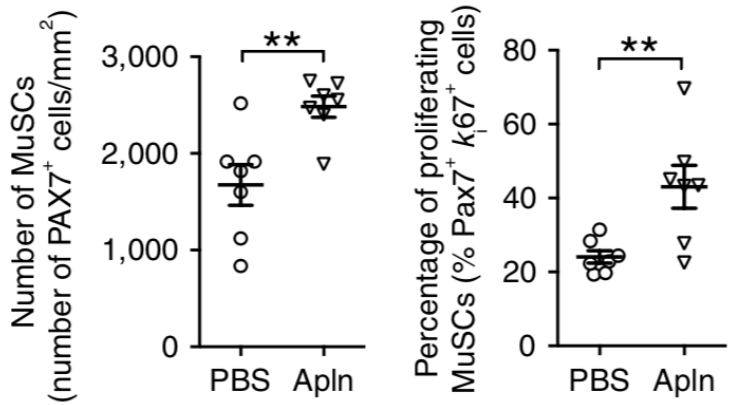


28% increase in proliferation rates

Liu *et al. In Vit Cell Dev Bio - Animal*, 2013

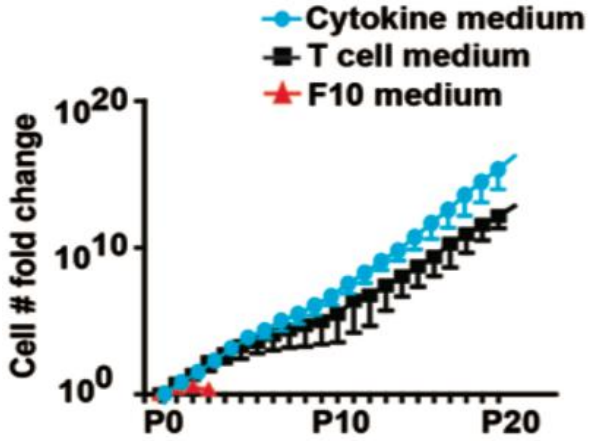
Transgene-Free Methods for Myogenic Improvements

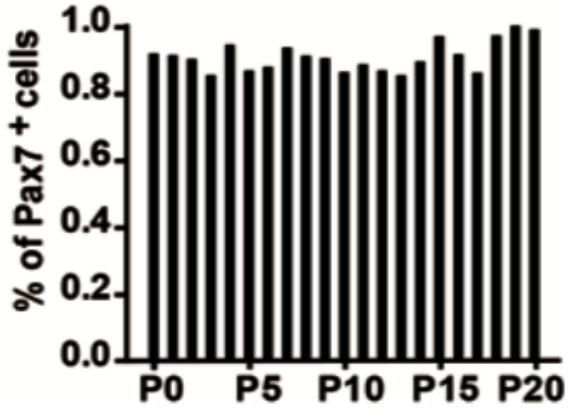
- Addition of the exerkine Apelin increases muscle stem cell proliferation



Vinel *et al. Nature Medicine*, 2018

- Addition of cytokines IL-1α, IL-13, TNF-α, and IFN-γ permit serial passaging of muscle stem cells

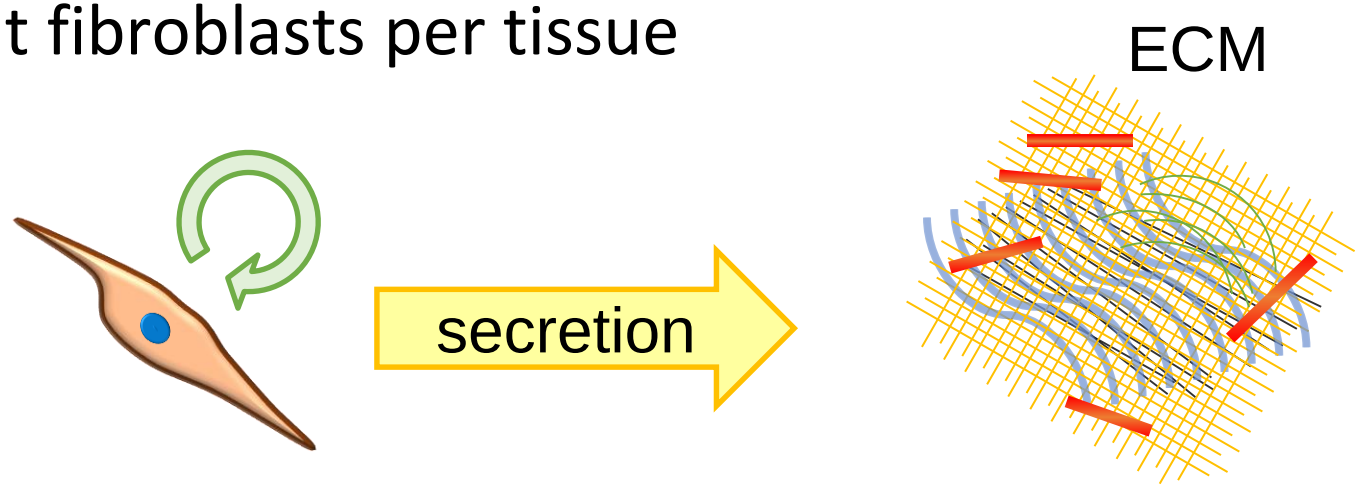




Fu *et al. Cell Research*, 2015

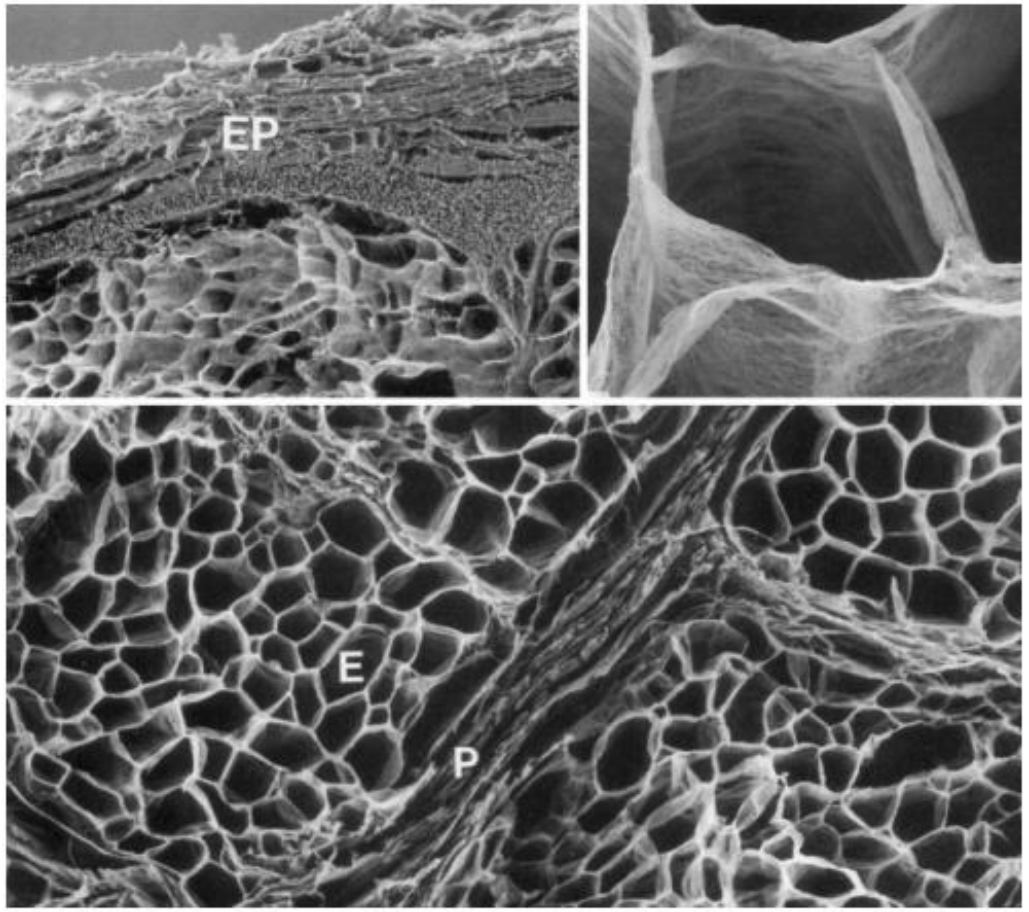
Fibroblasts

- Produce ECM (Extracellular Matrix)
- Not a single cell type
- Robust
  - Not sensitive to culture conditions
  - Short cell cycle
  - Simple isolation
- Different fibroblasts per tissue



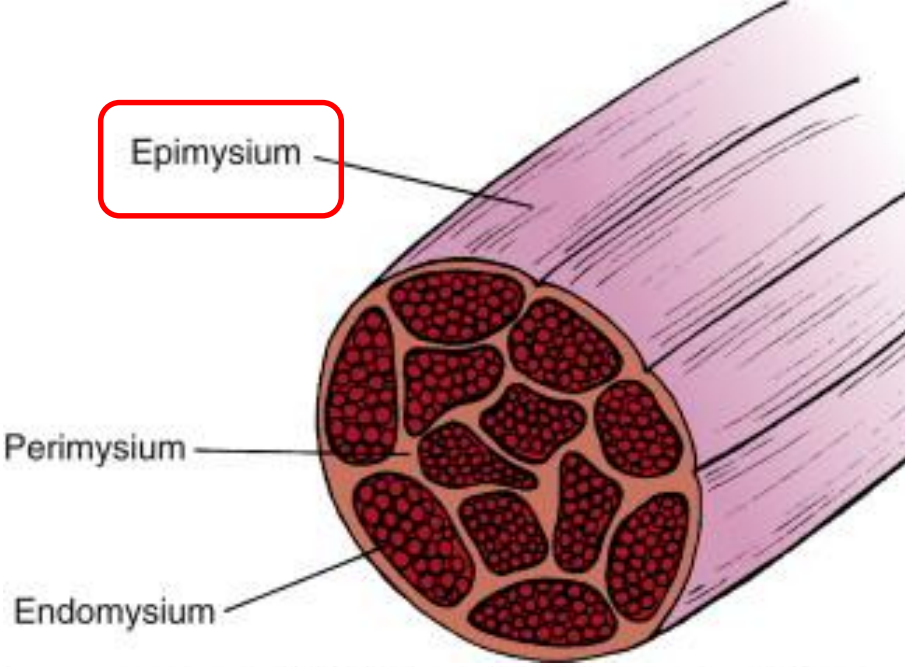
ECM (extracellular matrix)

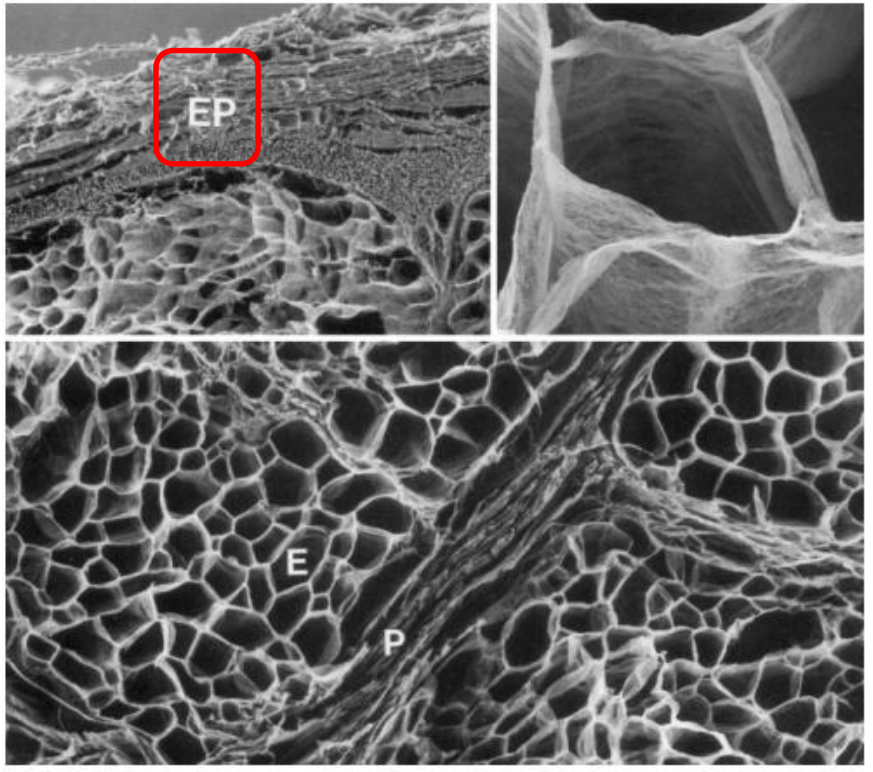
- Acellular part of meat
  - tissue elasticity
  - nutrients
  - cell adherence
  - 3D environment
  - Cell regulations
  - Changes during tissue development



Skeletal muscle ECM

- Epimysium: envelops the entire muscle
- Perimysium: honeycomb structure, compartmentalize fascicles (bundles of muscle fibers)
- Endomysium: fills the gaps between the muscle fibers
- Fibroblast should recapitulate the endomysium. Perhaps also develop the epimysium.





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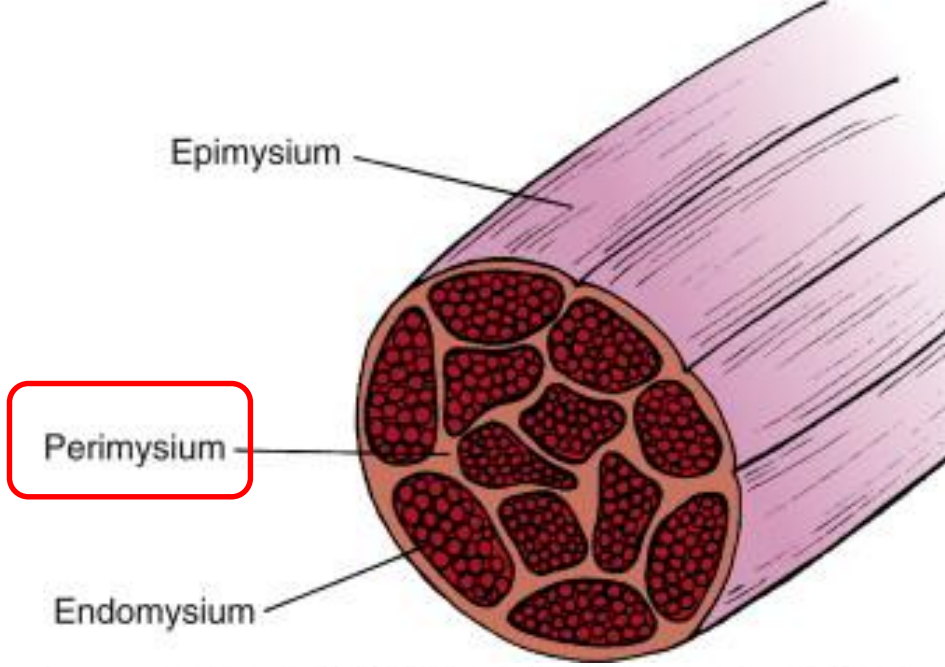

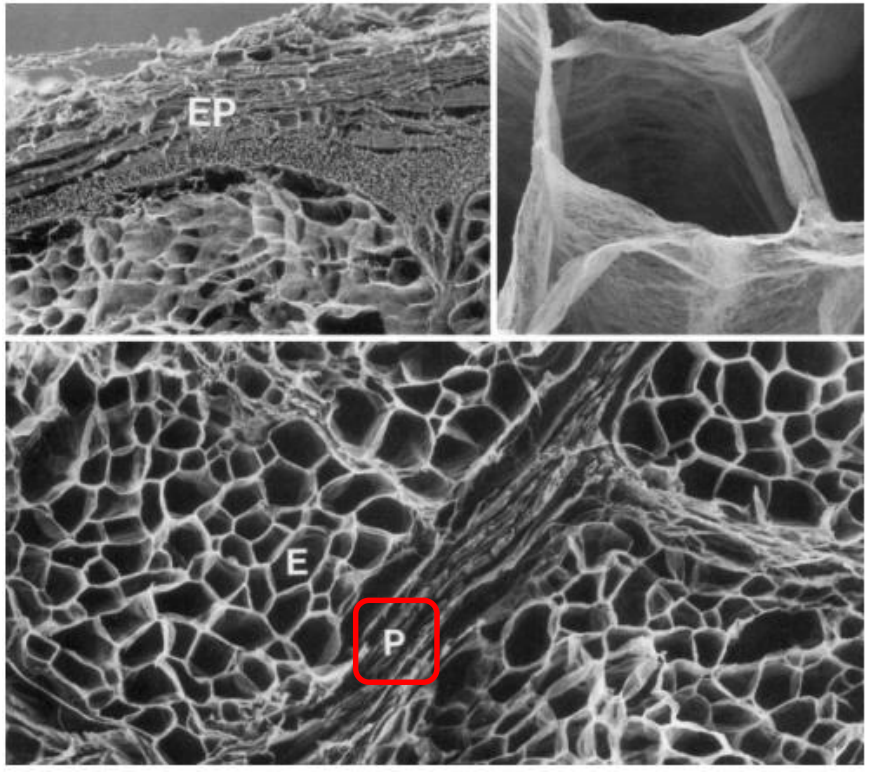

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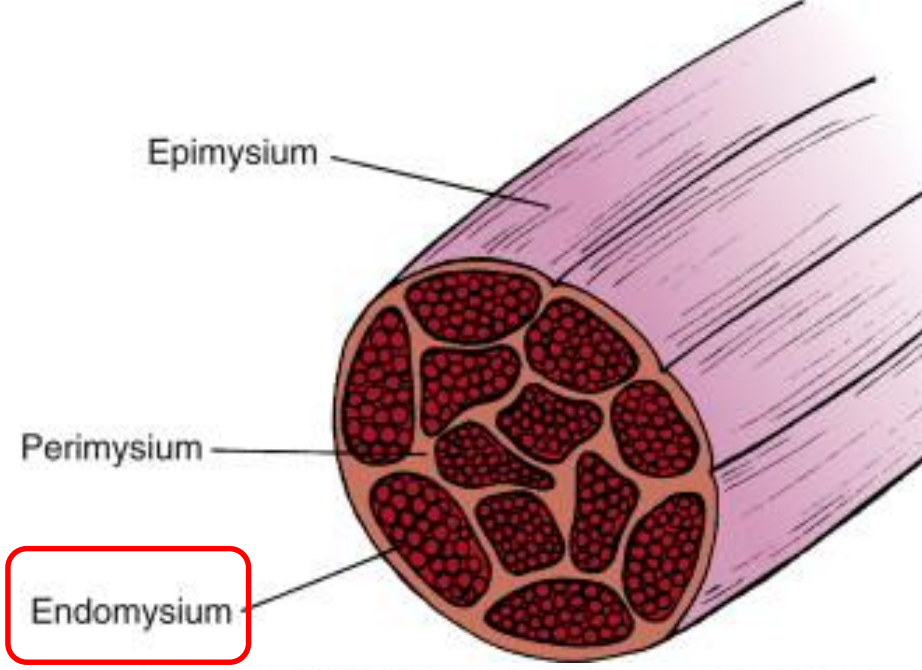
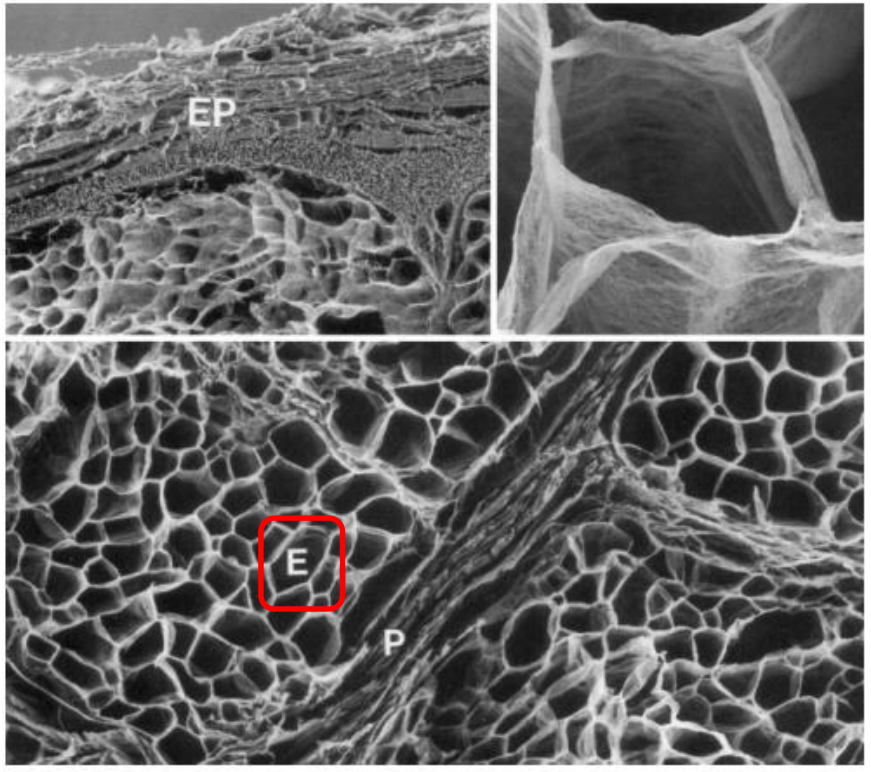




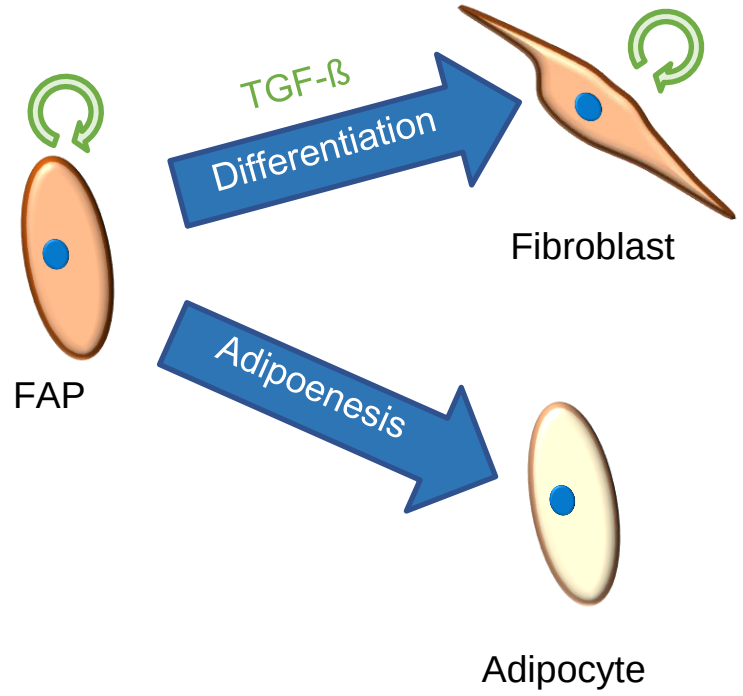
Fibroblasts

- Dermal fibroblast
- Skeletal muscle fibroblasts
- Embryonic fibroblasts

- Other supporting cell types
  - Smooth muscle cells
  - Mesenchymal stem cells
  - Dental pulp stem cells

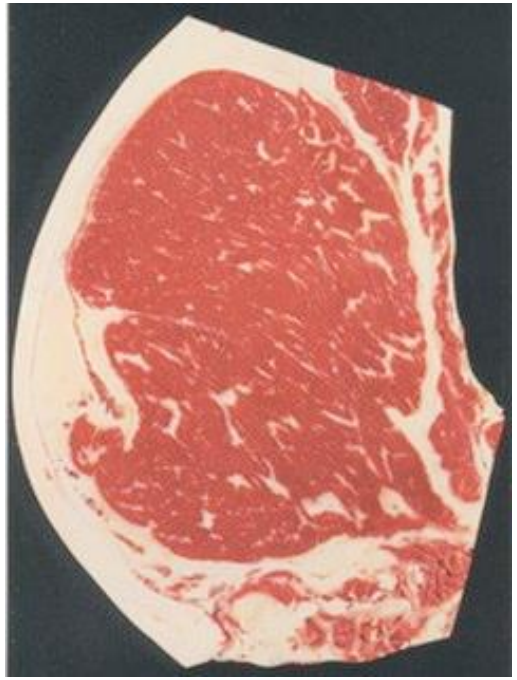
Fibroblasts sources

- **Cell isolation**
- Fibro-adipogenic precursor (FAP) cells



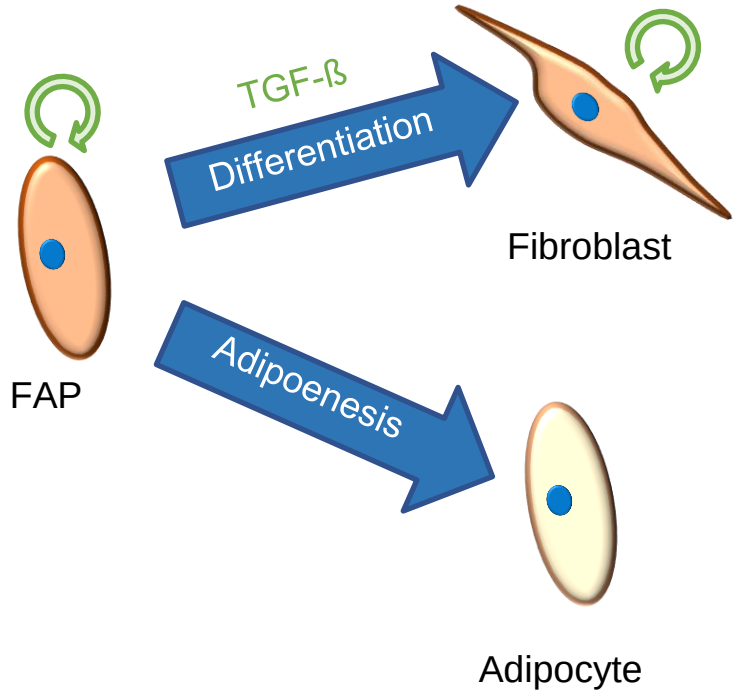
Intramuscular fat (IMF)

- Accounts for 80% of the muscle fat
- Also termed marbling
- meat quality, juiciness, flavor, tenderness and nutritional value



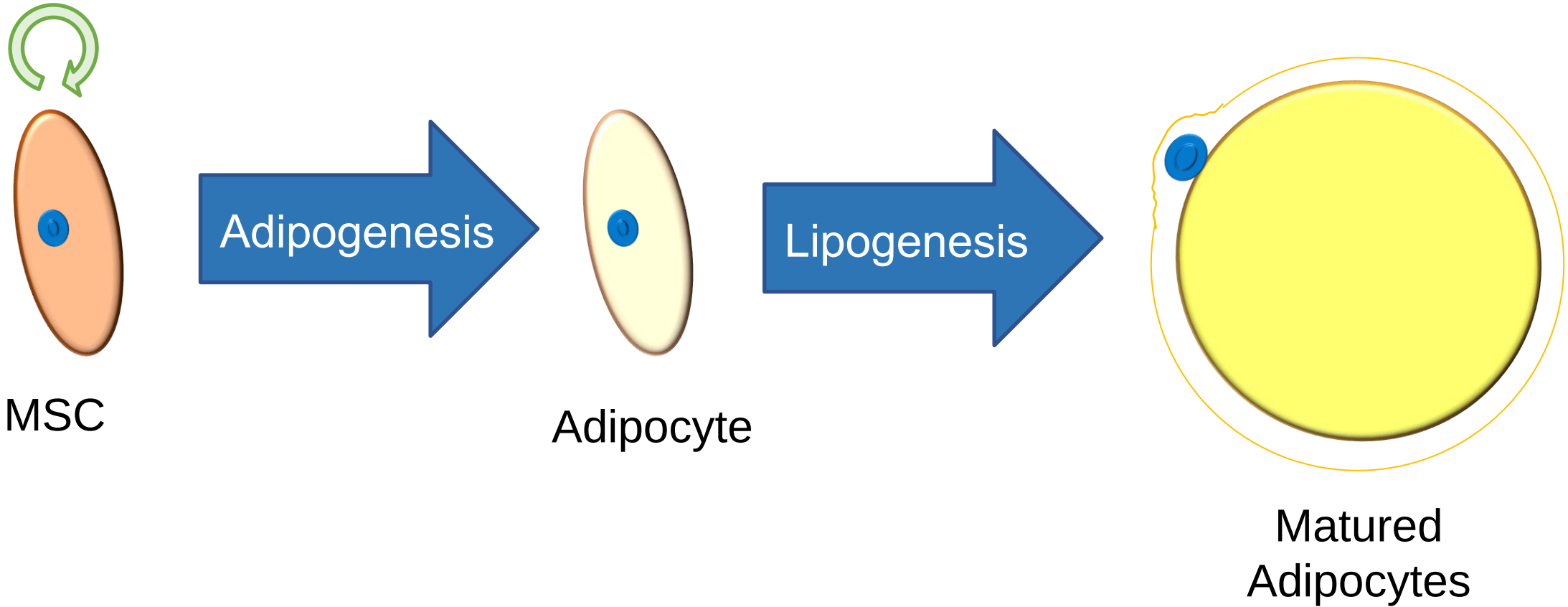
Adipocyte sources

- Adipocytes do not proliferate
- MSC differentiation (e.g. FAP cells)
- Can also be differentiated from Satellite Cells and fibroblasts



Adipogenic differentiation

- Adipogenesis – differentiation into adipocytes
- Lipogenesis – accumulation of triglycerides.



Lipogenesis

- Triglyceride synthesis in adipocytes