

Tendinopathy, Tendon Healing & Tendon Regeneration

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Associate Professor

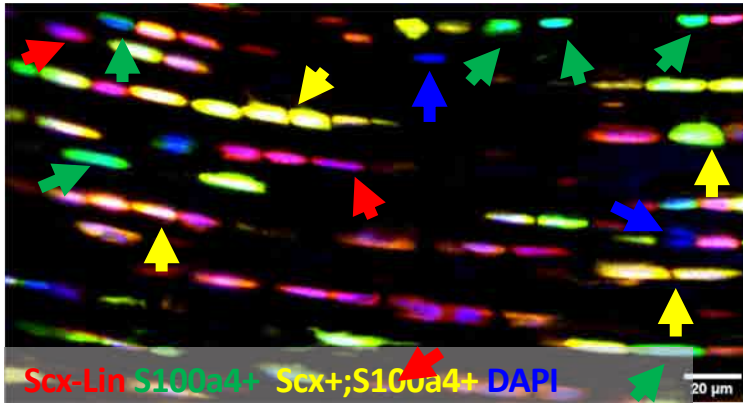
IND 464 Musculoskeletal Basic Science Course

October 21, 2019

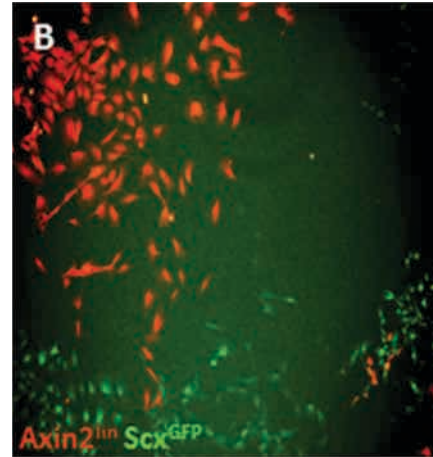
Outline

- Review of homeostasis
- Effects of Aging
- Disruptions in homeostasis
- Pathogenesis of tendinopathy/ tendinitis
- Mechanisms of regeneration and healing

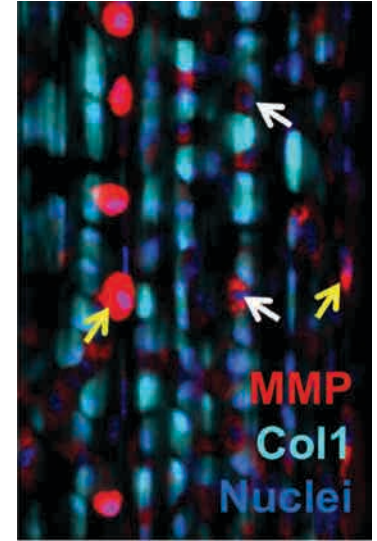
Tendon fibroblasts are not a uniform population



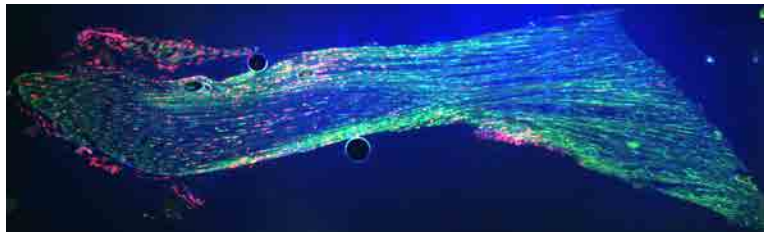
Best FASEB 2019



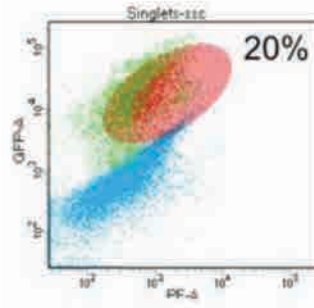
Walia *et al.* ORS 2019



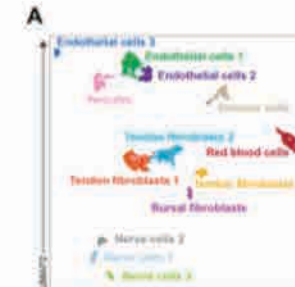
Bautista *et al.* ORS 2019



Loiselle Lab, unpublished data



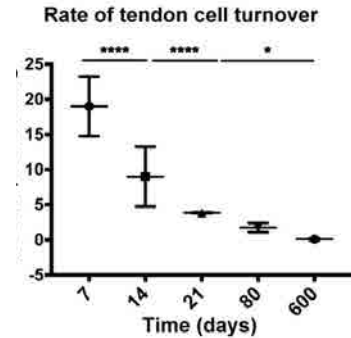
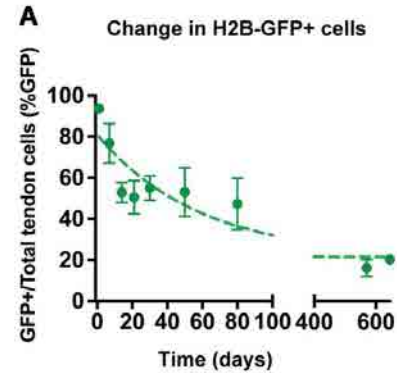
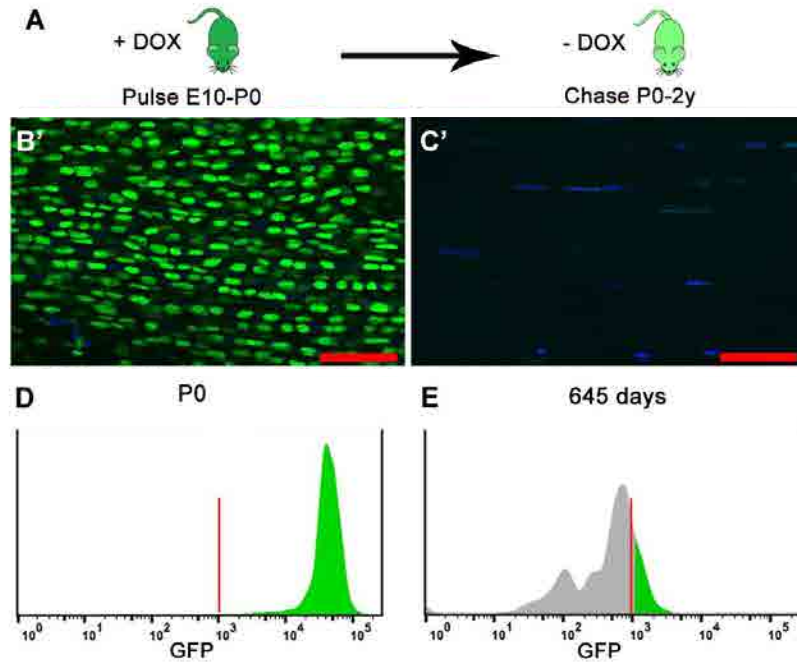
Grinstein *et al.* ORS 2019



Swanson *et al.*, BioRxiv. 2019

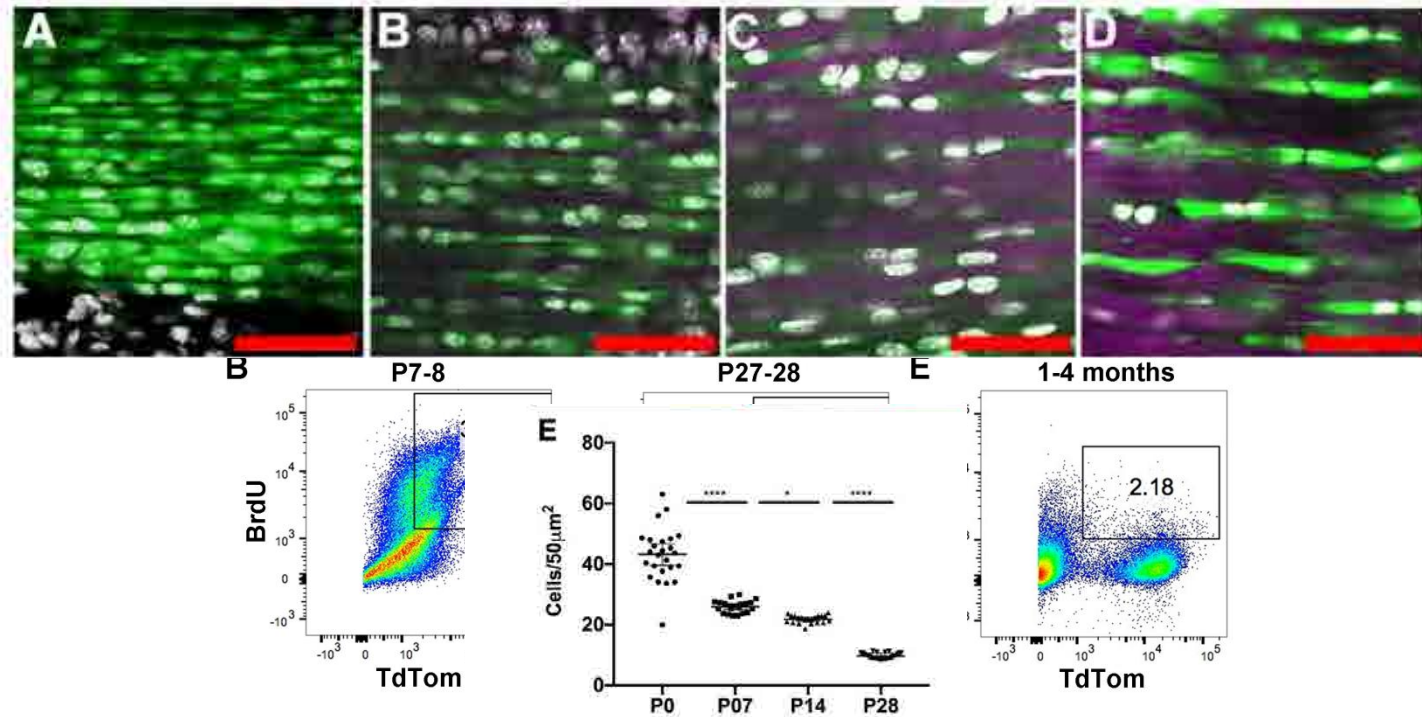
Tendon Cell Proliferation During Growth

Figure 1



Grinstein eLIFE 2019

Tendon Cell Proliferation During Growth



Grinstein eLIFE 2019

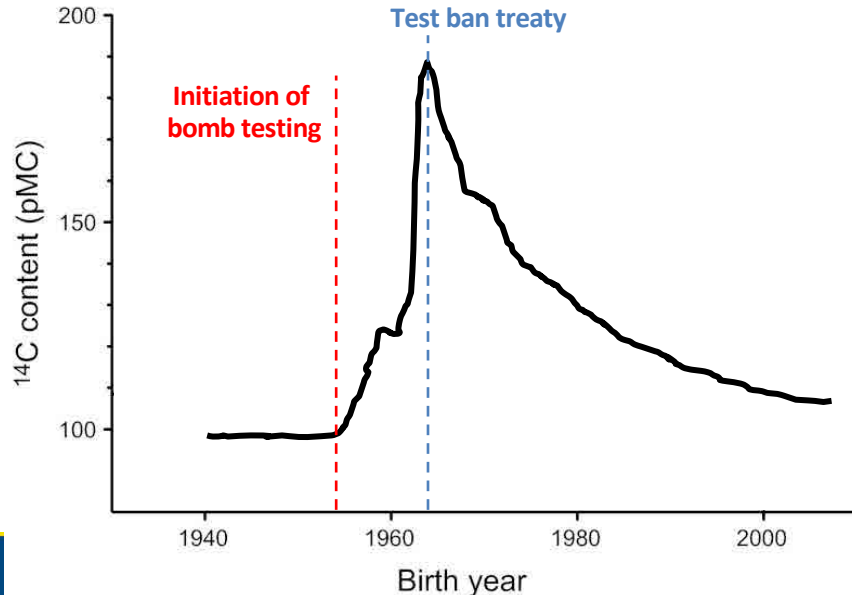
Tissue Renewal

Nuclear bomb testing between 1955-1963

Doubled atmospheric ^{14}C

Tendon and muscle biopsies from patients born ~1940-1980

Assessment of tissue turnover rate



National Library of Medicine



National Institutes of Health

The FASEB Journal

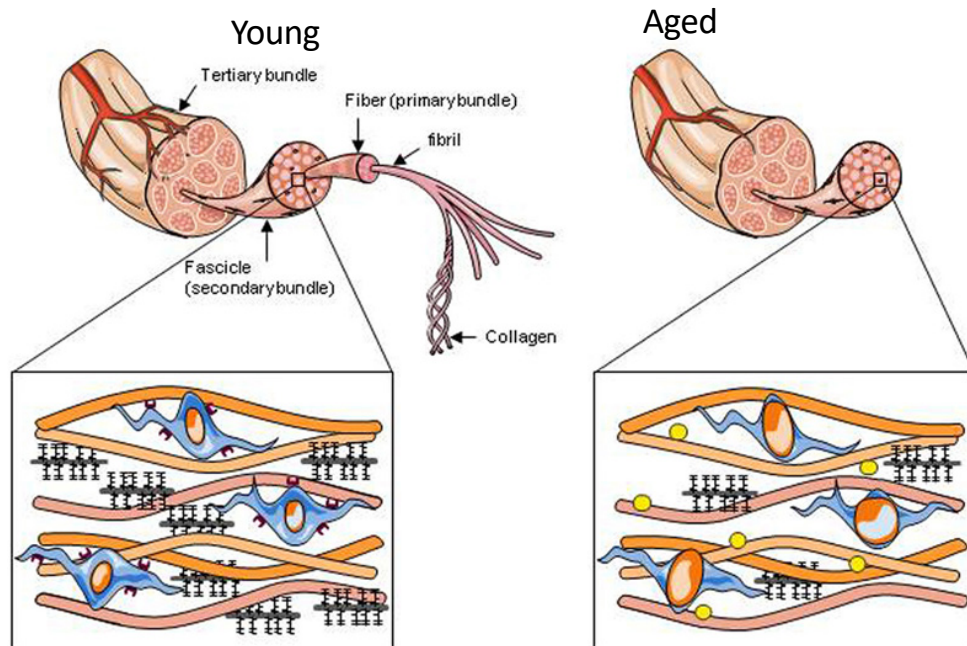
FASEB J. 27(5): 2074-2079

Lack of tissue renewal in human adult Achilles tendon is revealed by nuclear bomb ^{14}C

Katja Maria Heinemeier^{*†}, Peter Schjerling[†], Jan Heinemeier[‡], Stig Peter Magnusson^{*†}, Michael Kjaer^{*†}



Tendon Homeostasis

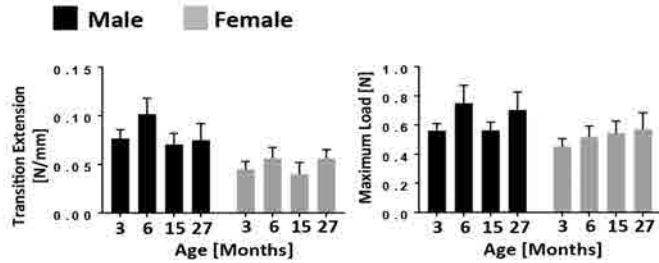


- Matrix composition and organization is not dramatically different
- Decreased proteoglycan content
- Changes in cell morphology

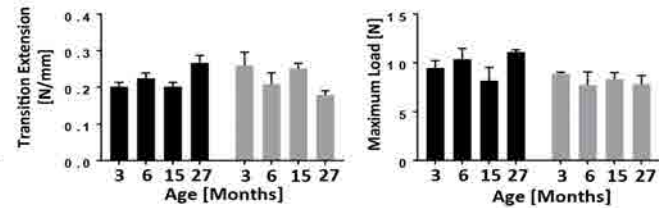
Functional Consequences of Aging?

Tendon is not mechanically sensitive to aging

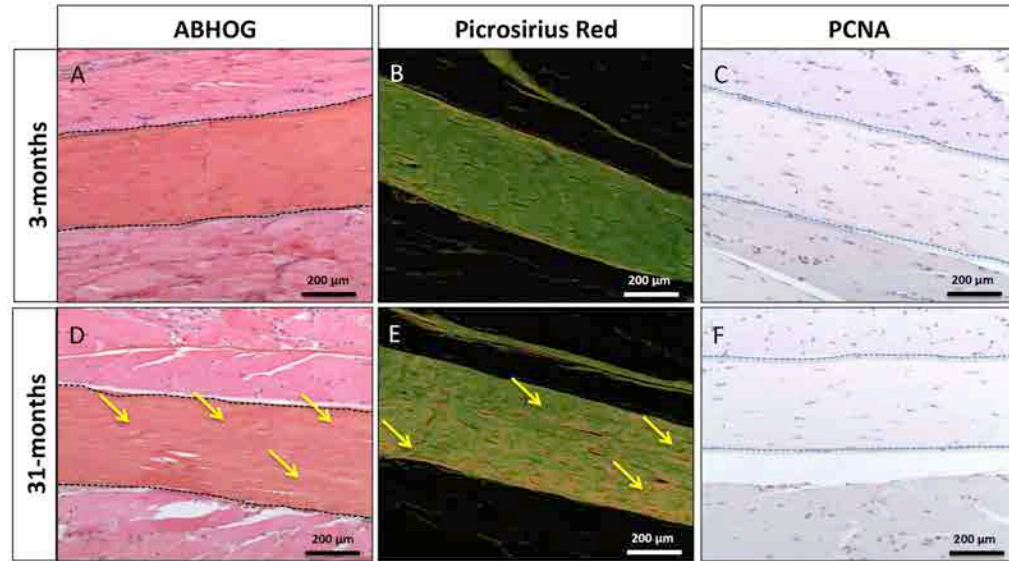
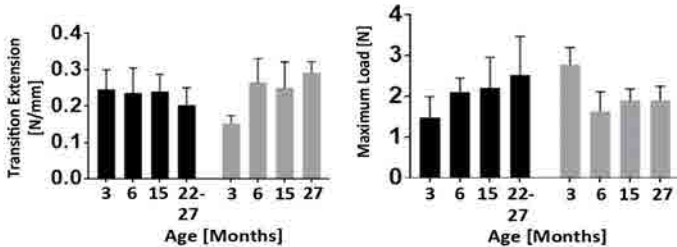
TT Fascicle



FDL



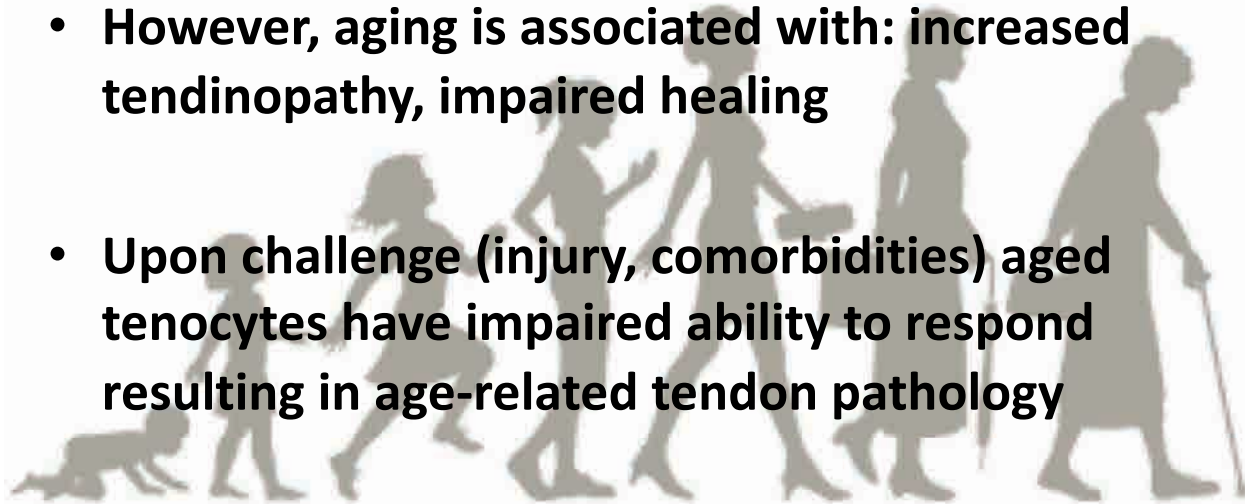
FCU



Ackerman et al., 2017. JOR

Tendon & Aging

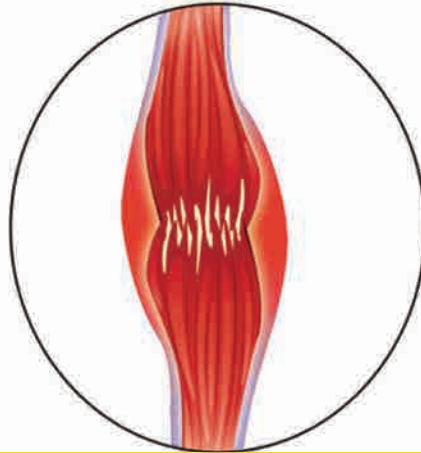
- **Tenocyte quiescence and low-frequency ECM turnover= decreased sensitivity to aging (vs. bone)**
- **However, aging is associated with: increased tendinopathy, impaired healing**
- **Upon challenge (injury, comorbidities) aged tenocytes have impaired ability to respond resulting in age-related tendon pathology**



Tendinopathy

Disease of the tendon

- Painful
- Exacerbated by activity
- Most common in the Achilles
- Tendonitis: acute inflammation and injury

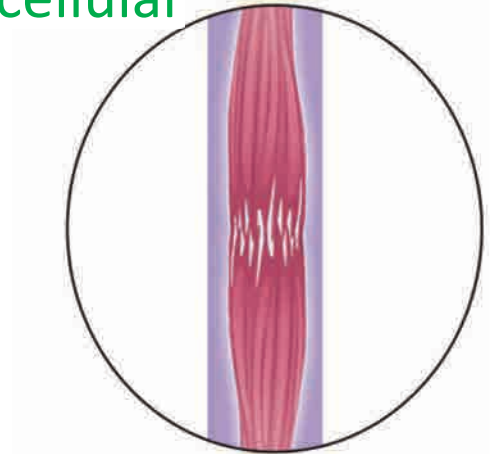


Scivet.com

Tendinopathy




Disease of the tendon

- Painful
- Exacerbated by activity
- Tendinosis: chronic with degenerative cellular changes, no inflammation
- More common than tendinitis
- Continuing for longer than 6 months



Scivet.com

Animal Models of Tendinopathy & Healing

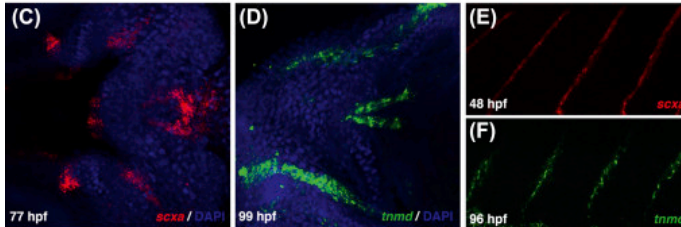
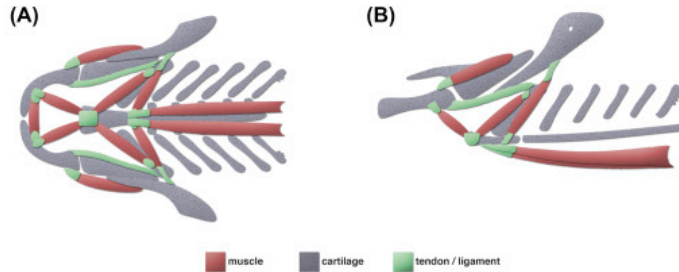
<u>Supraspinatus (rotator cuff)</u>	<u>Achilles Tendon</u>	<u>Patellar Tendon</u>	<u>Flexor Tendon</u>
Over 30 animals characterized ¹	Mouse Rat	Mouse Rat Rabbit	Mouse Canine Chicken Rat Rabbit Horse Pig
most common: Rat Mouse			
<u>Supraspinatus (rotator cuff)</u>	<u>Achilles Tendon</u>	<u>Patellar Tendon</u>	<u>Flexor Tendon</u>
Mechanical	Mechanical chemical	Fatigue loading	Diabetic tendinopathy
Full thickness, partial width Tendon to bone healing	Complete transection +/- repair Partial transection Biopsy punch	Biopsy punch	Complete transection + repair Partial transection Biopsy punch
		 Tendinopathy	 Healing

(1) Soslowky LJ, et al., J Shoulder Elbow Surg 1996;5:383–392

(2) Thomopoulos et al., JOR. 2015 (review)



Zebrafish!



JW Chen and JL Galloway. *Methods in Cell Biology*, Volume 138, 2017, 299–320

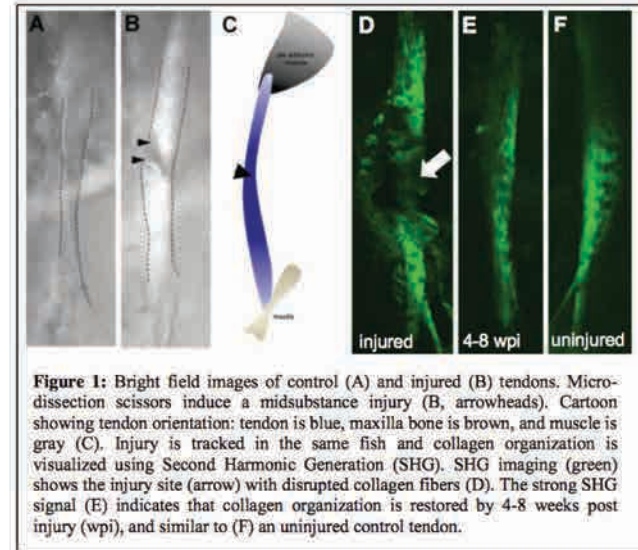
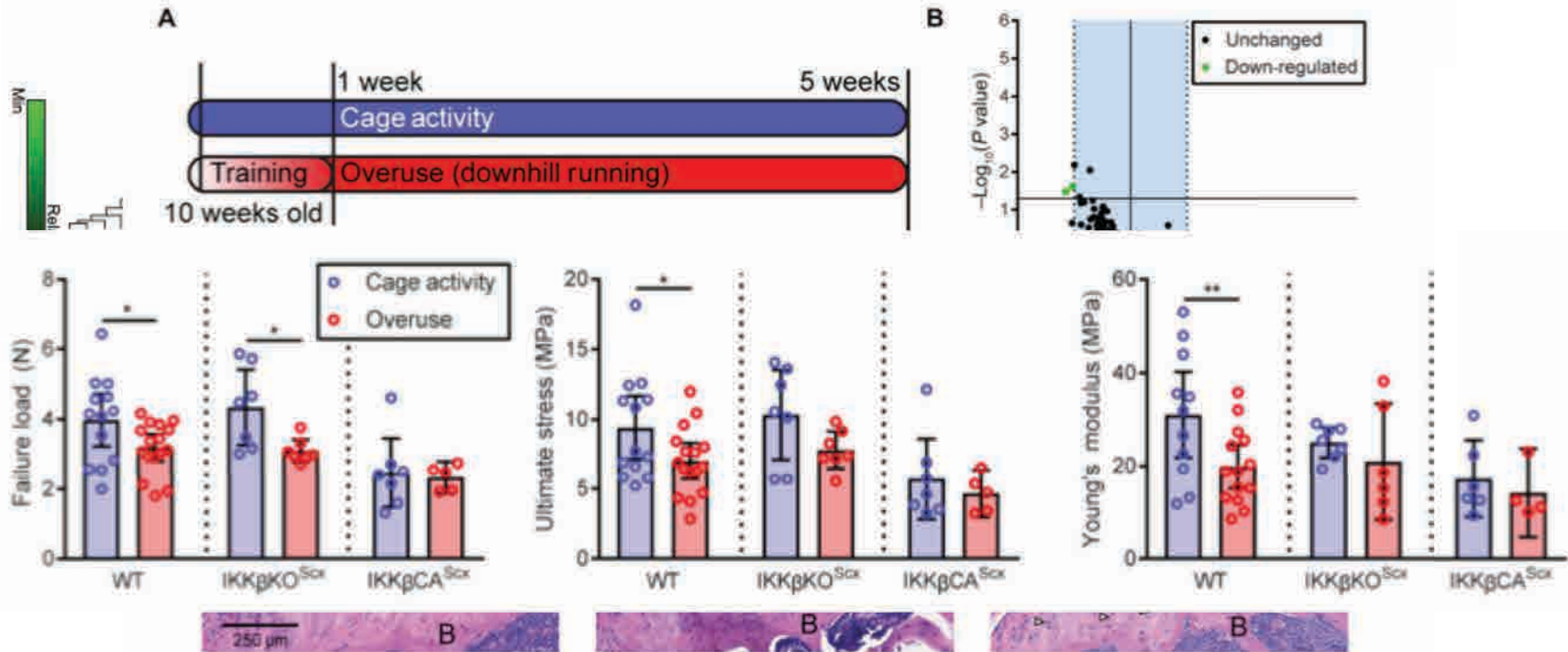


Figure 1: Bright field images of control (A) and injured (B) tendons. Microdissection scissors induce a midsubstance injury (B, arrowheads). Cartoon showing tendon orientation: tendon is blue, maxilla bone is brown, and muscle is gray (C). Injury is tracked in the same fish and collagen organization is visualized using Second Harmonic Generation (SHG). SHG imaging (green) shows the injury site (arrow) with disrupted collagen fibers (D). The strong SHG signal (E) indicates that collagen organization is restored by 4-8 weeks post injury (wpi), and similar to (F) an uninjured control tendon.

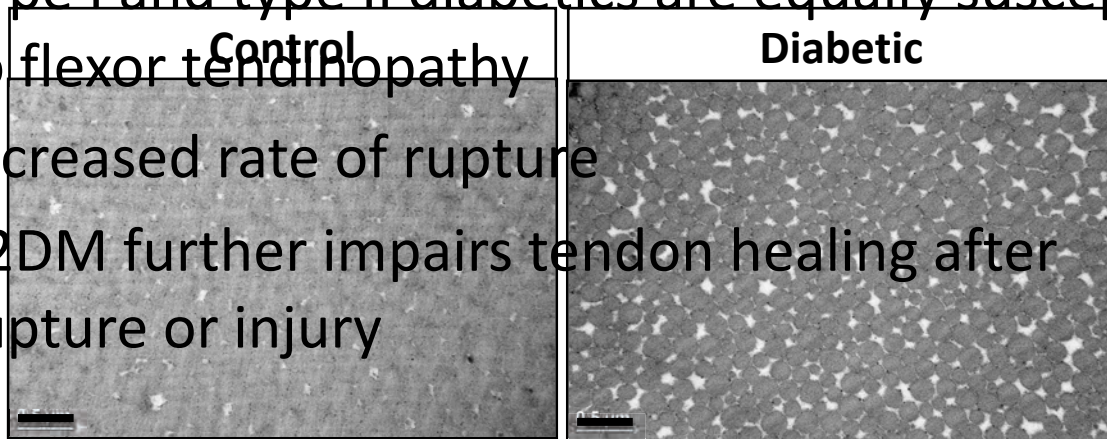
Inflammation is a potent mediator of tendinopathy



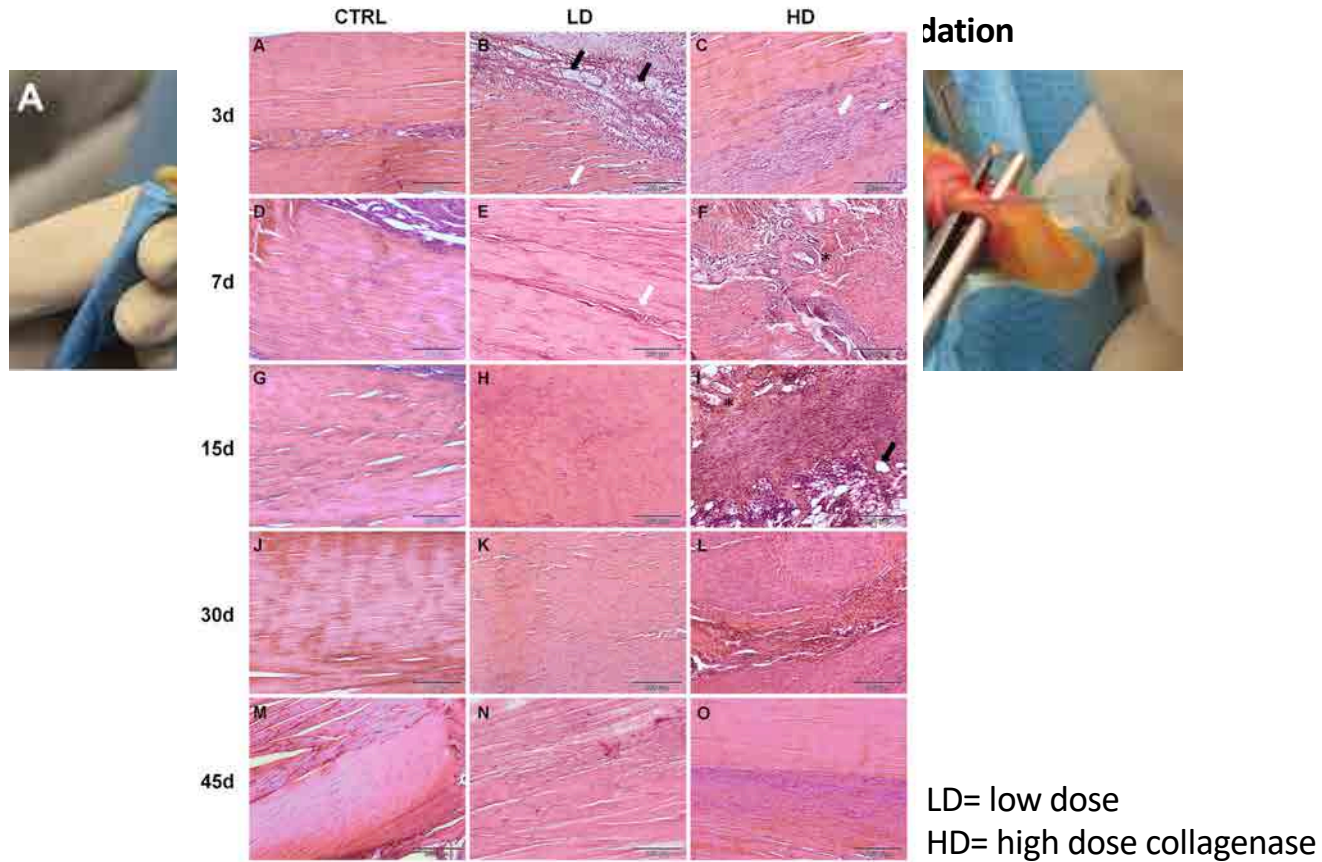
Abraham et al., SciTM 2019.

Disrupted Homeostasis: Diabetes

- Sensitivity varies by tendon*
- Flexors are most sensitive
- Pathological changes increase with disease duration
- Type I and type II diabetics are equally susceptible to flexor tendinopathy
- Increased rate of rupture
- T2DM further impairs tendon healing after rupture or injury



Collagenase Induced Tendinopathy



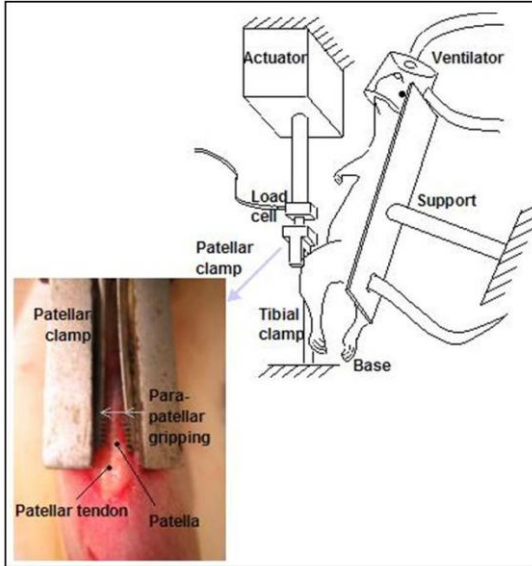
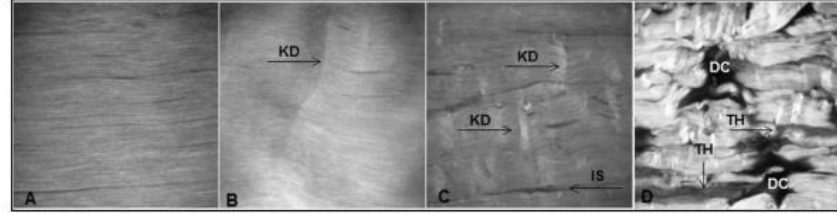
Orfei C et al. (2016) PLOS ONE 11(8): e0161590.

Mechanically Induced Tendinopathy

- Fatigue loaded under anaesthesia
- Uphill treadmill running
- Downhill treadmill running

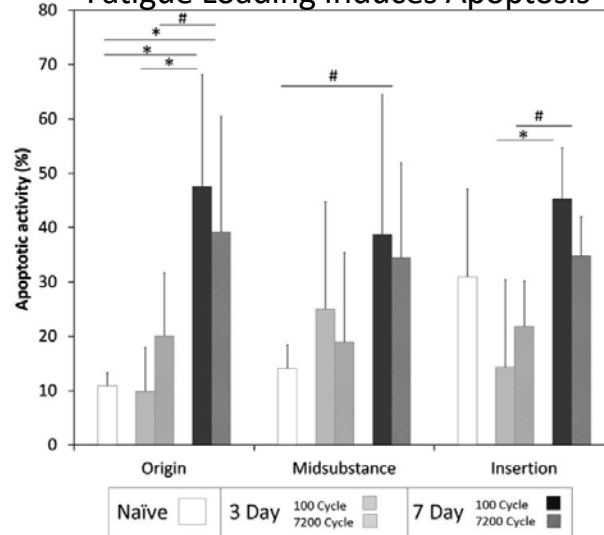
Induces matrix and cellular changes

Unloaded Low level loading Moderate loading High level loading



Neviasser A. 2012. J Shoulder Elbow Surg.

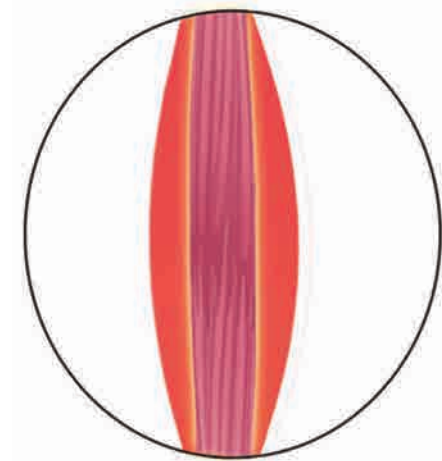
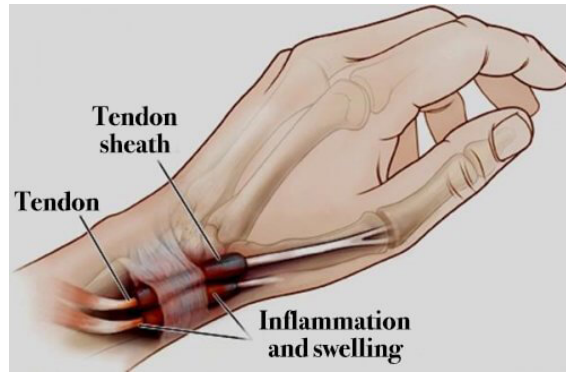
Fatigue Loading Induces Apoptosis



N Andarawis-Puri et al., 2014. JOR

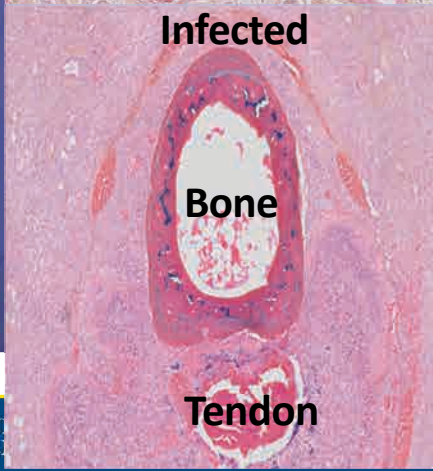
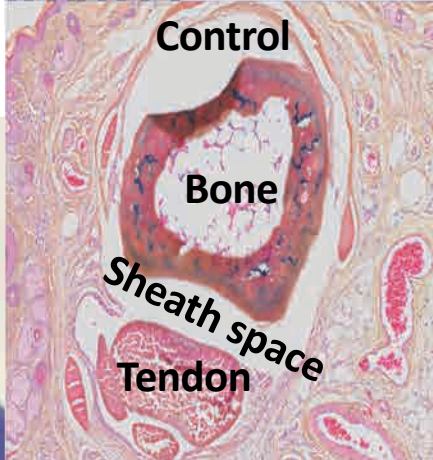
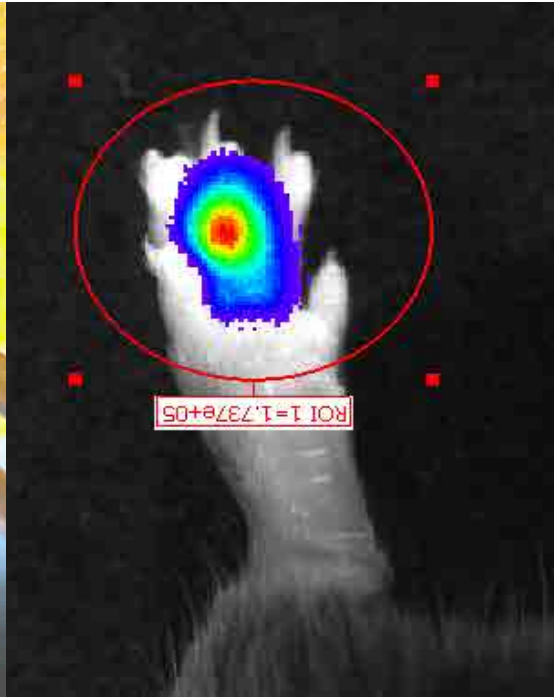
Tenosynovitis

- Inflammation of the sheath
- Causes: inflammatory diseases, infection, injury
- Most commonly in hand/ wrist



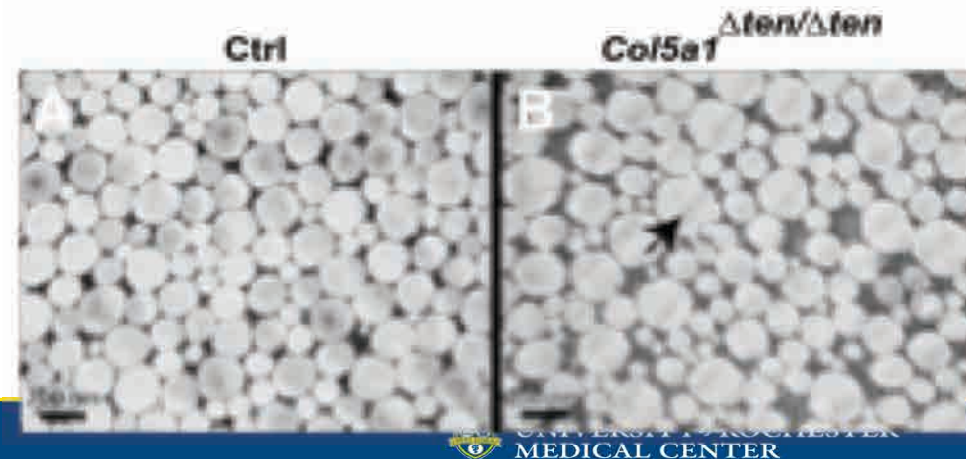
Scivet.com

Developing a mouse model of pyogenic tenosynovitis



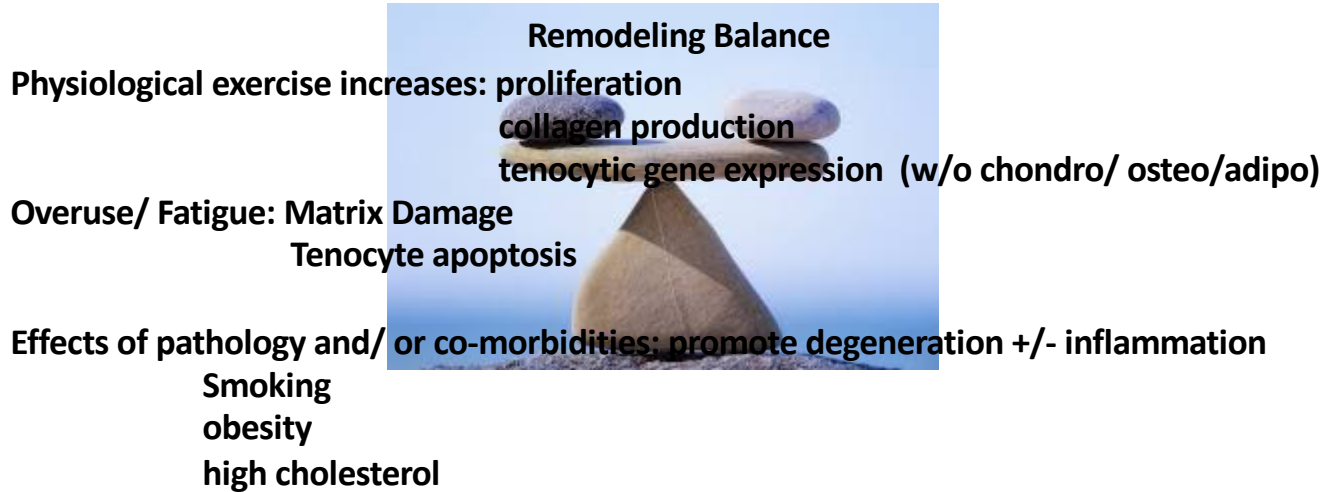
Genetic models of tendinopathy: Ehlers-Danlos Syndrome

- Joint hypermobility and frequent dislocations
- Mutation in ColV
- Tendon-specific ColV KO- EDS
 - Abnormal gait, joint laxity, altered collagen structure
- ColV^{+/-} altered tendon structure, impaired tendon healing



Sun et al., AJP. 2015, Johnston et al., JOR. 2017

Summary of Tendinopathy

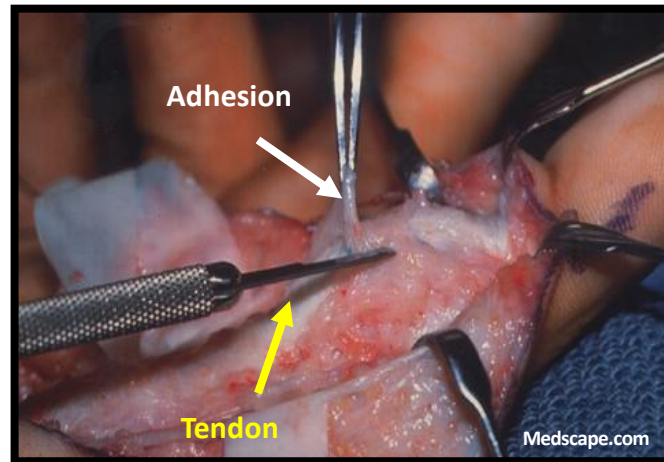
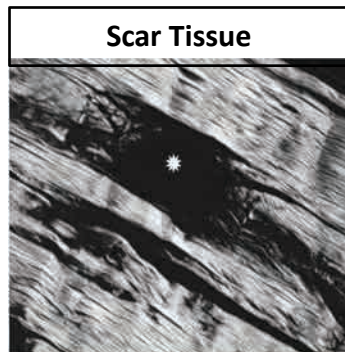
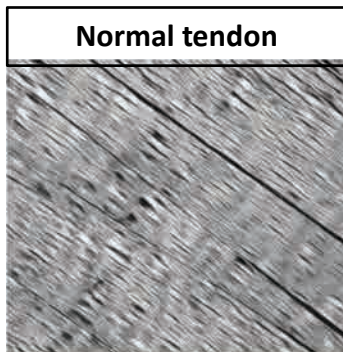


Future Directions

- Continue to identify co-morbidities that predispose or accelerate tendinopathy
- Most clinical data are from late stage pathology
- Beginning to use genetic animals models to better understand tendinopathy

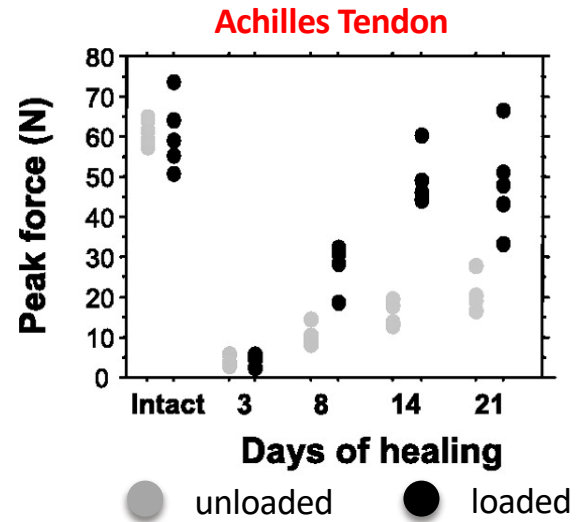
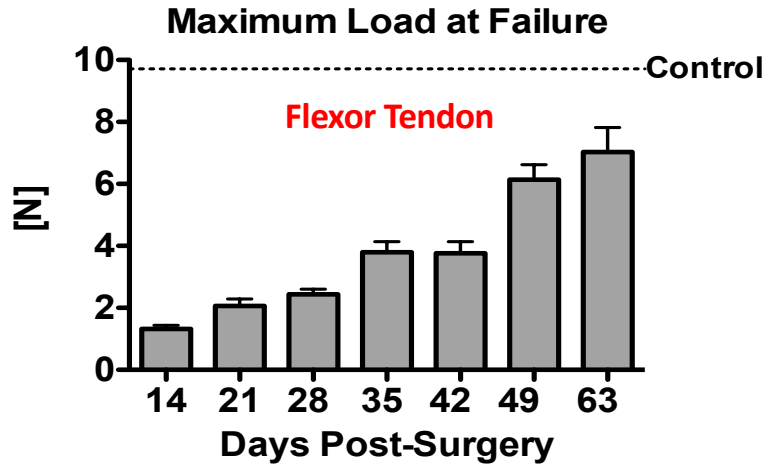
Tendon Healing

- ~300,000 tendon repair procedures per year
- Over \$20 Billion in associated health care costs
- Healing is complicated by scar formation



de Jong 2014; Pennisi 2002; Beredjiklian 2003; Defranco 2004

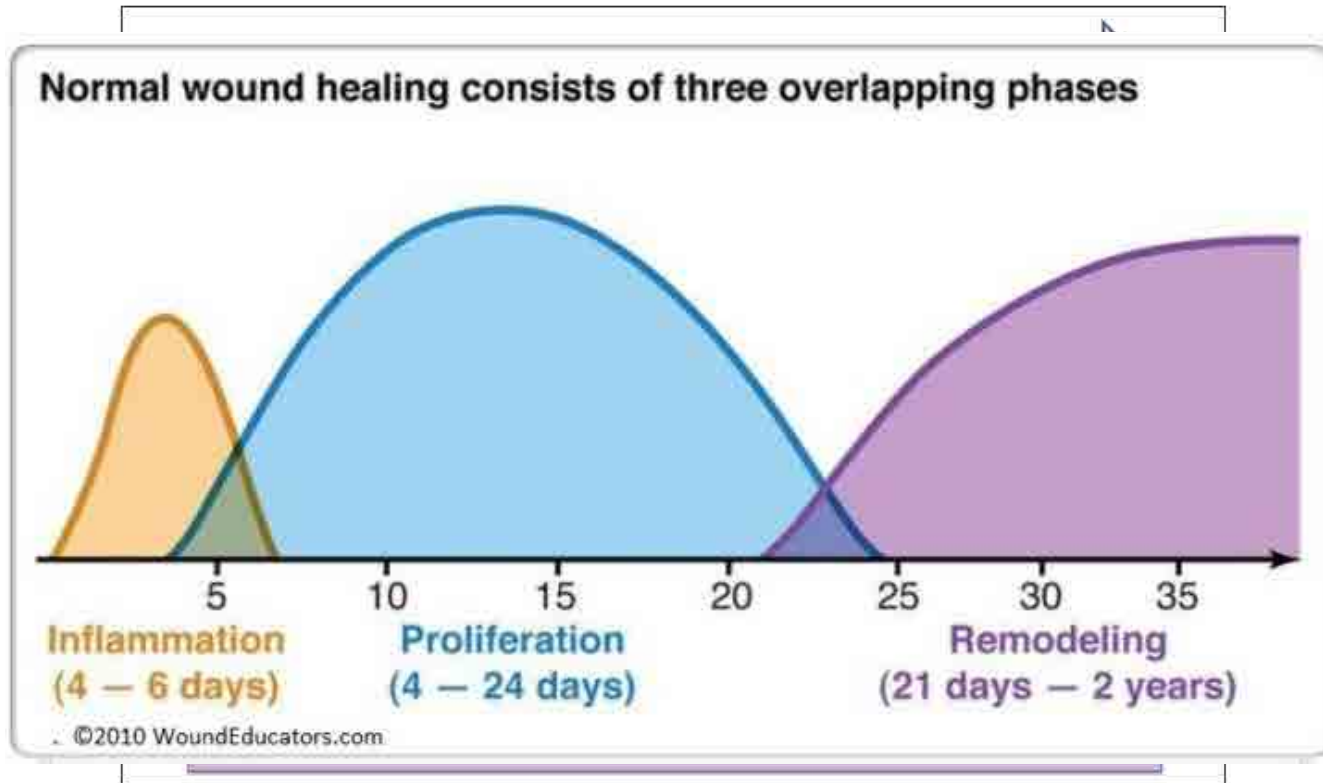
Acquisition of Mechanical Properties



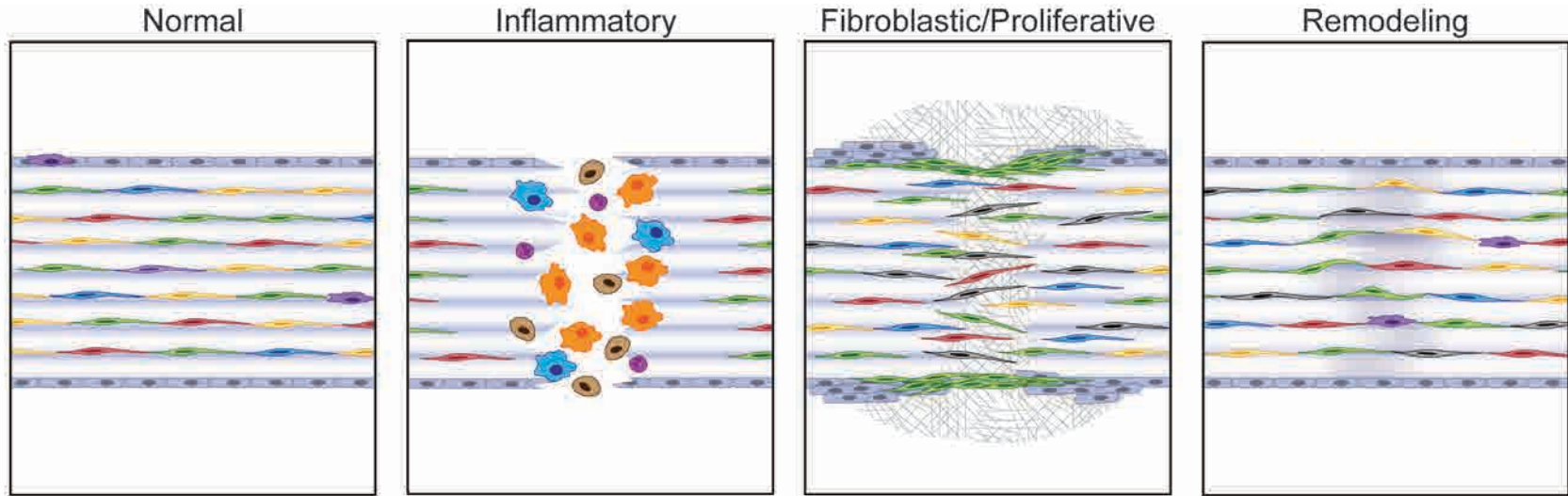
Loiselle et al., 2009. JOR

P Eliasson et al., 2009. J Appl Phys.

Similarities between wound and tendon healing



Similarities between wound and tendon healing



- | | | | | | |
|--|--------------------------|--|-----------------------------|--|---------------------------|
| | S100a4+ tendon cell | | Tendon stem/progenitor cell | | M2 macrophage |
| | Scx+ tendon cell | | αSMA+ cell | | M1 macrophage |
| | Scx+ S100a4+ tendon cell | | Epitenon cell | | Neutrophil |
| | Scx+αSMA+ tendon cell | | Macrophage | | Bone marrow-derived cells |

Nichols, Best, Loisel. 2019

Inflammation

Well-regulated inflammation is beneficial

- activates healing cascade
- recruitment/activation of cells

Excessive/ Chronic inflammation is pathological

- degenerative matrix changes
- fibrotic healing

Benefits of Anti-inflammatory therapy is controversial

Timing may be key!

Generally effective at preventing excess scar formation

Early inhibition decreases mechanics

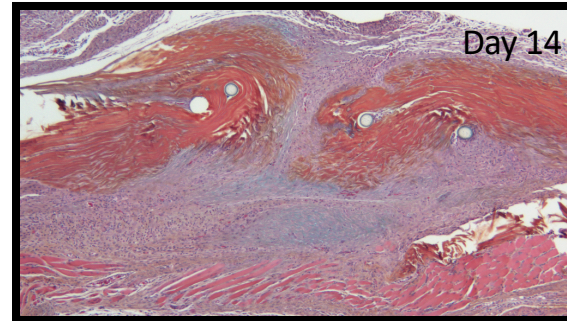
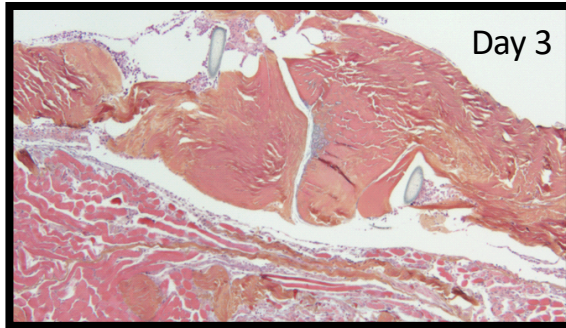
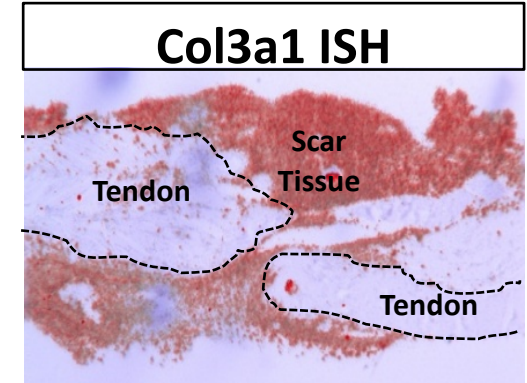
Delayed healing maintains mechanics

Cell-type specific considerations

Virchenko et al., 2004. Am J Sports Med, Geary et al. 2015. PlosOne

Proliferative/ Granulation Phase

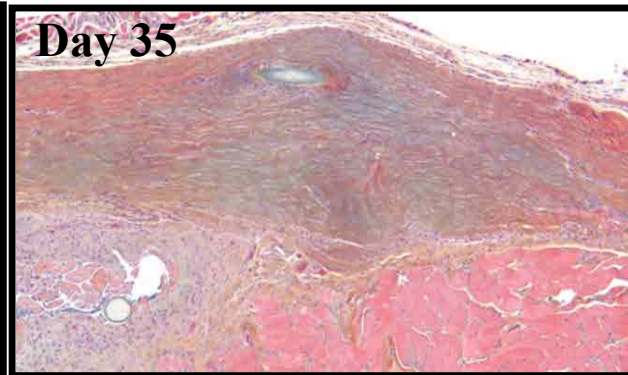
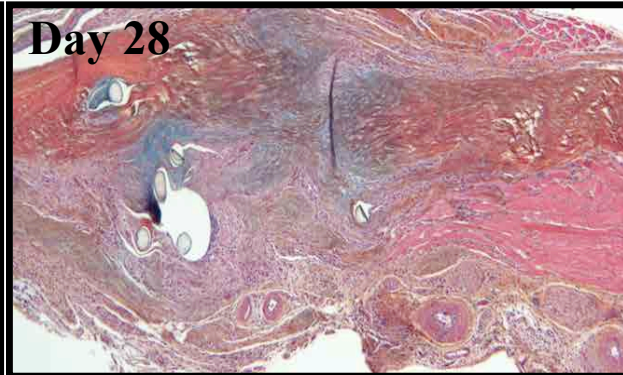
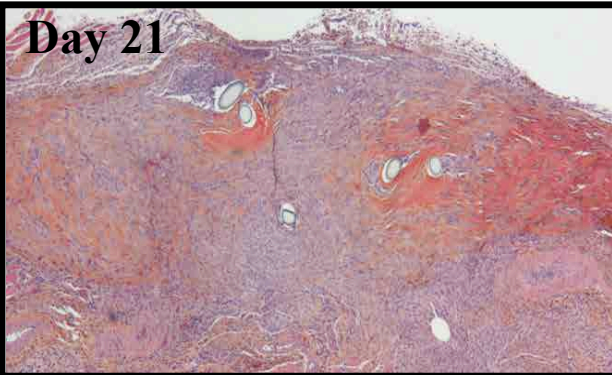
- Lasts a few weeks
- Begins ~day 7 in mouse model
- Proliferation of ‘fibroblasts’
- Bridging on injury site
- Production of ECM components (Col1/ Col3)
- Rapid deposition of disorganized ECM



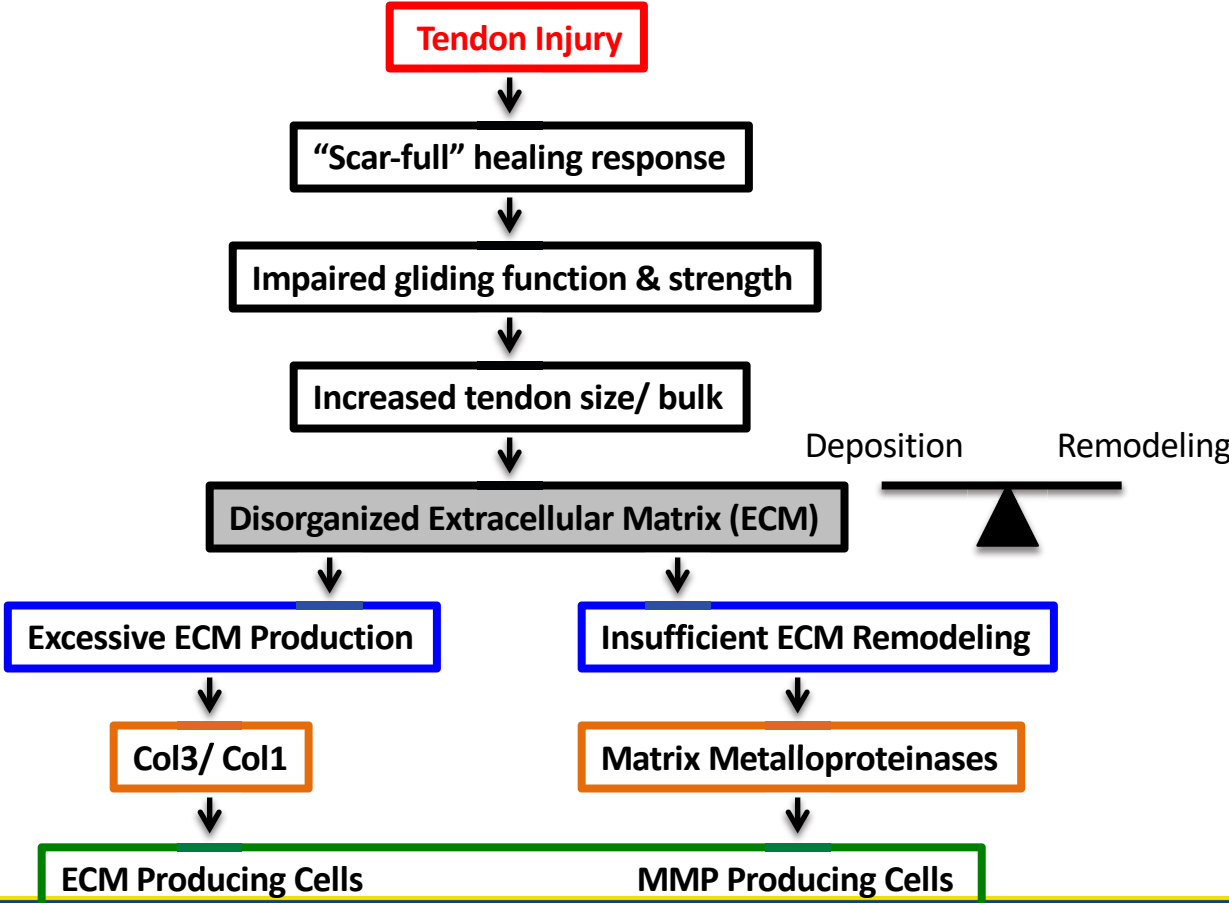
Thomopoulos et al., 2009. JOR, Thomopoulos et al., 2010. JBJS, Katznel et al. 2010. JOR, Awad Lab, Loiselle Lab

Remodeling Phase

- Lasts many months
- Begins ~day 21 in mouse model
- Reorientation of ECM
- Mmp-mediated remodeling



Successful Repair: A Delicate balance of deposition and remodeling



Double-edged sword of tendon healing

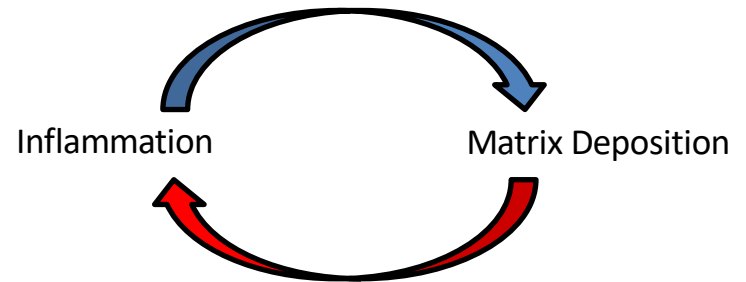
Matrix deposition



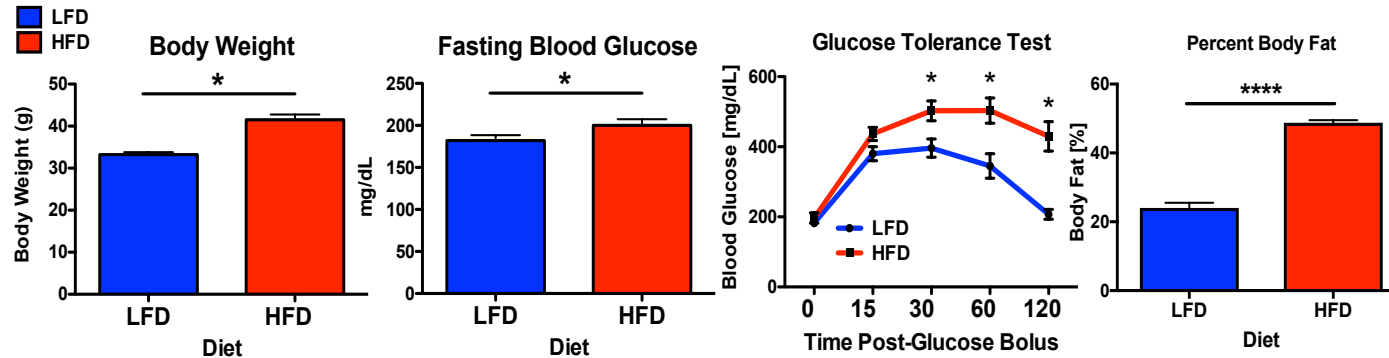
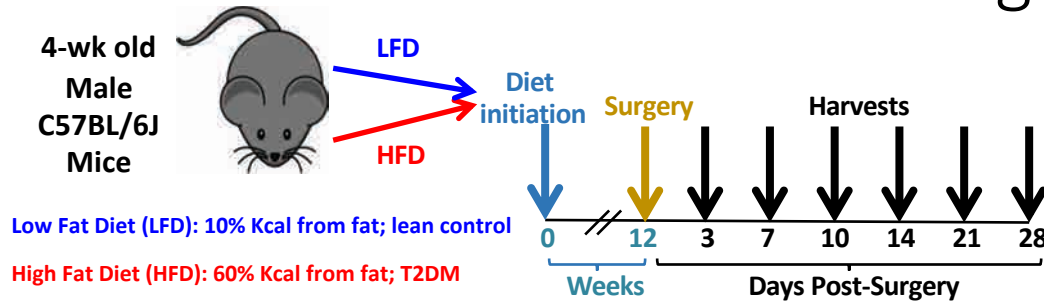
Mechanical properties

Fibrosis

- Thickening and/or scarring of connective tissue
- Typically in response to injury
- In response to injury fibrosis= scar tissue
- Excess matrix deposition
- Disorganized matrix
- Exuberant healing response



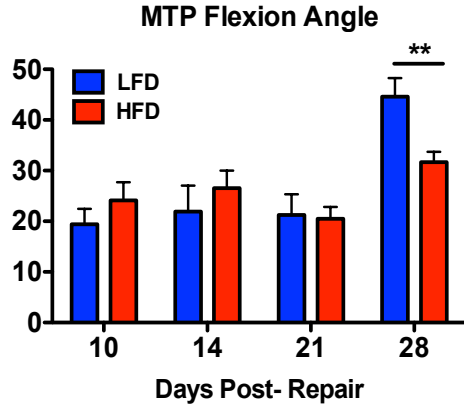
Impact of Diet induced Obesity and Type II Diabetes on Tendon Healing



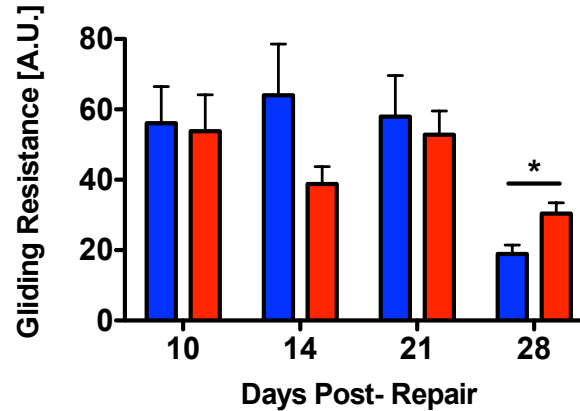
Ackerman⁺ PlosOne. 2017.

T2DM Impairs Tendon Healing

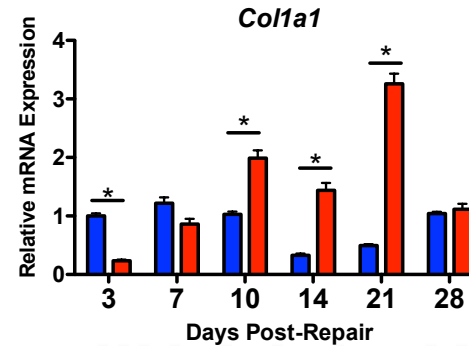
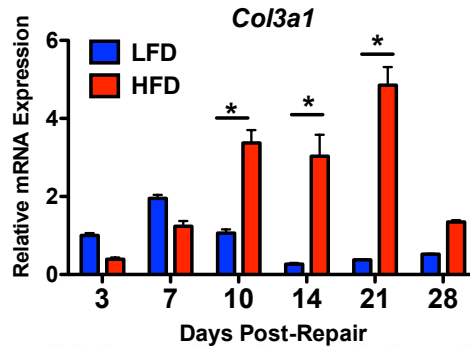
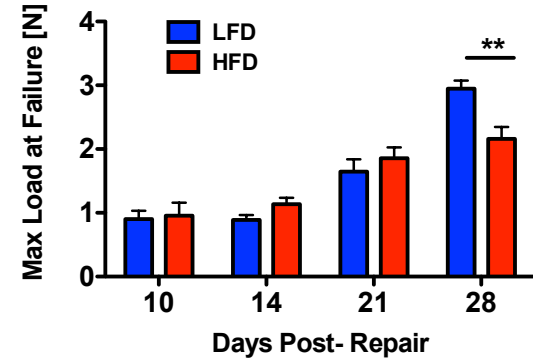
MTP Flexion Angle [Degrees]



Gliding Resistance

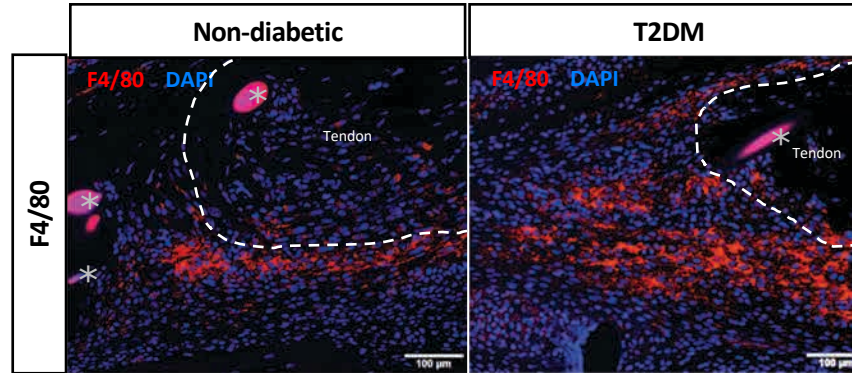
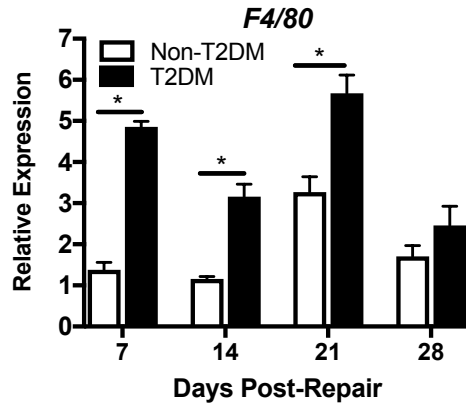


Max Load at Failure



Ackerman⁺ PlosOne. 2017.

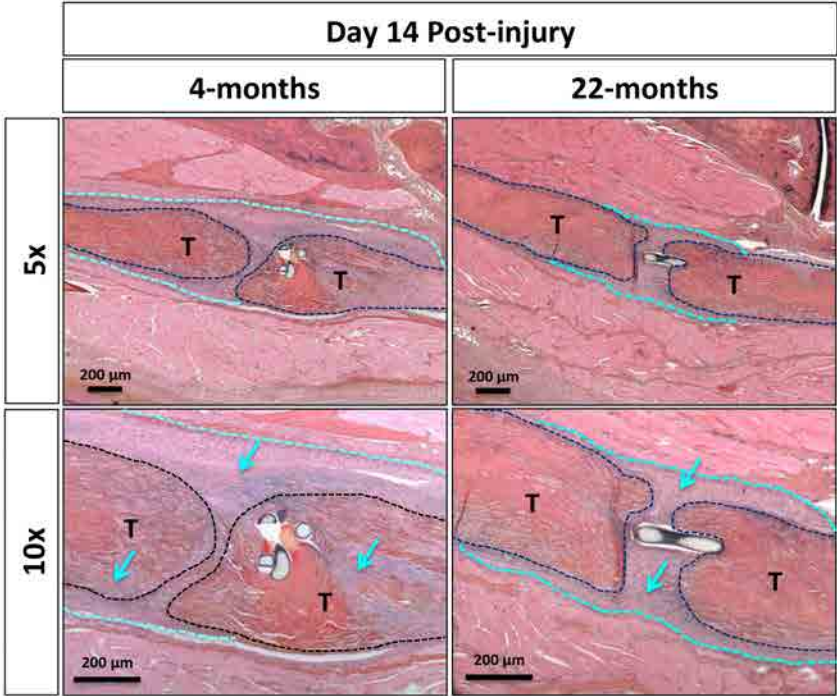
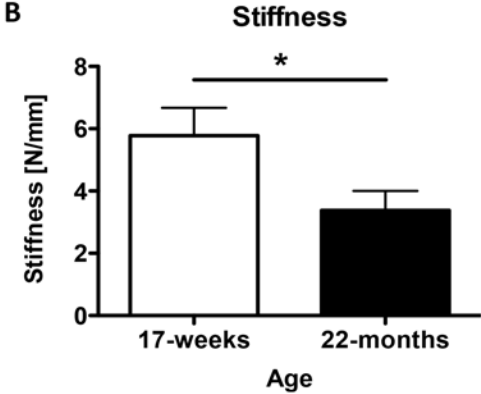
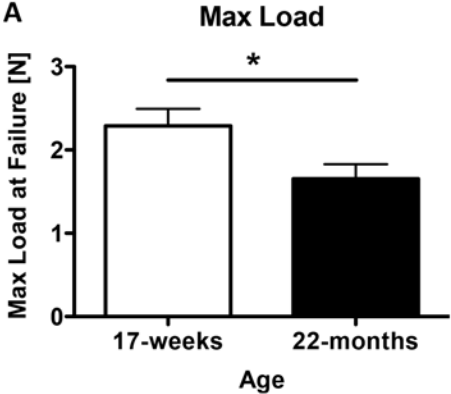
T2DM Prolongs and Alters Inflammation



Ackerman⁺ PlosOne. 2017.

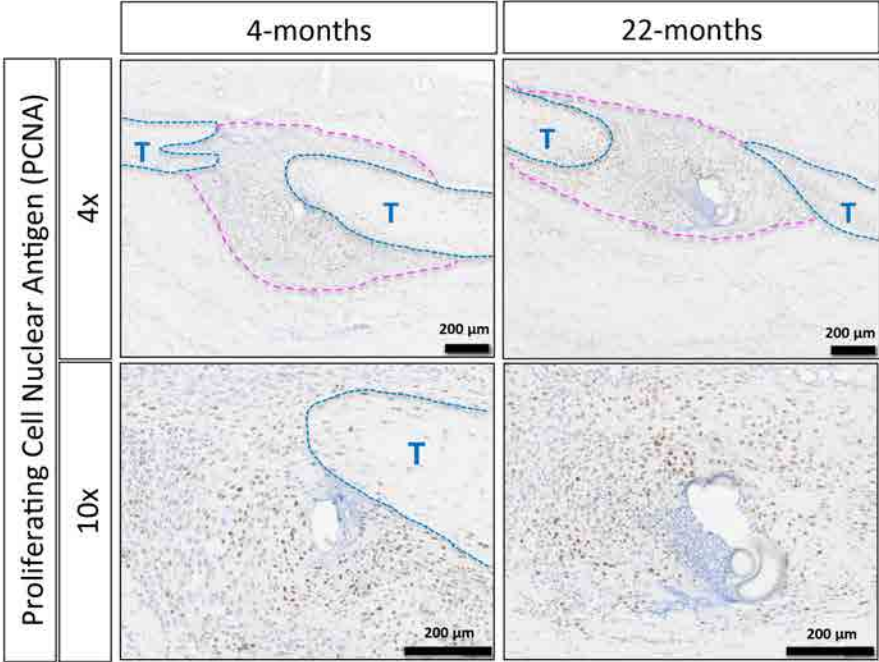
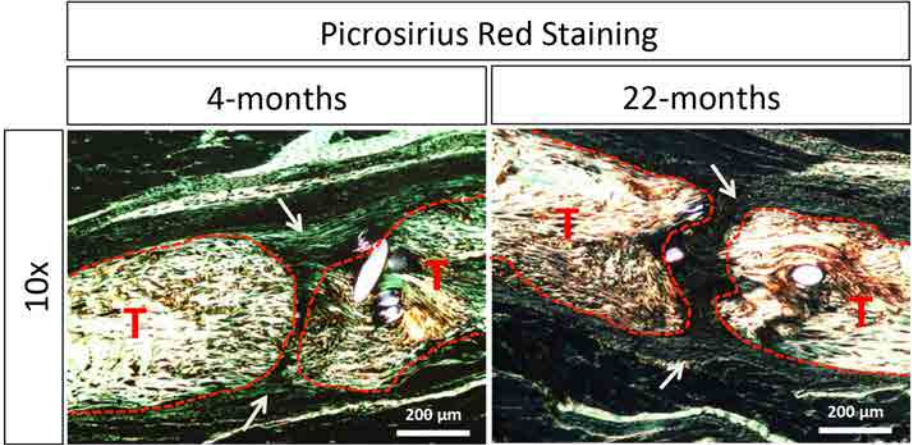
Increased macrophage content
Prolonged macrophage presence
Increased, prolonged pro-inflammatory M1 macs
Increased, early anti-inflammatory* M2 macs

Aging decreases fibrotic tendon healing (too much)



Ackerman et al., JOR. 2017.

Aging decreases fibrotic tendon healing (too much)

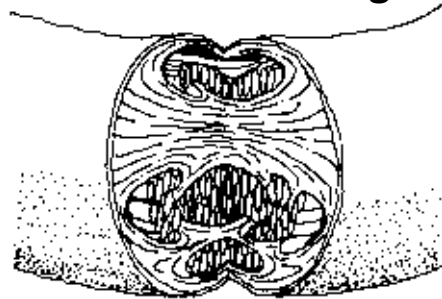


No change in proliferative capacity
 Decreased matrix production on a per cell basis
 Is the cellular environment the same?

Origin of Cells During Tendon Healing: Intrinsic & Extrinsic

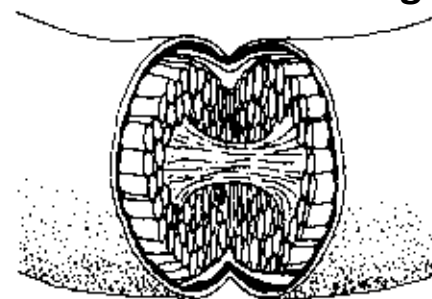
The cellular components of tendon healing are not well characterized

Extrinsic Healing

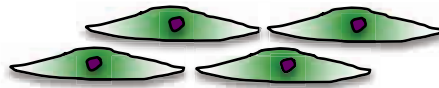


Beredjikian 2003

Intrinsic Healing

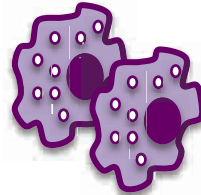


Resident Tenocytes

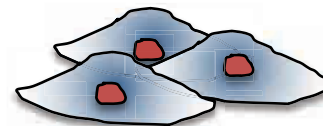


Scx-lineage

Macrophages/ Inflammatory cells



Bone marrow/ circulating cells

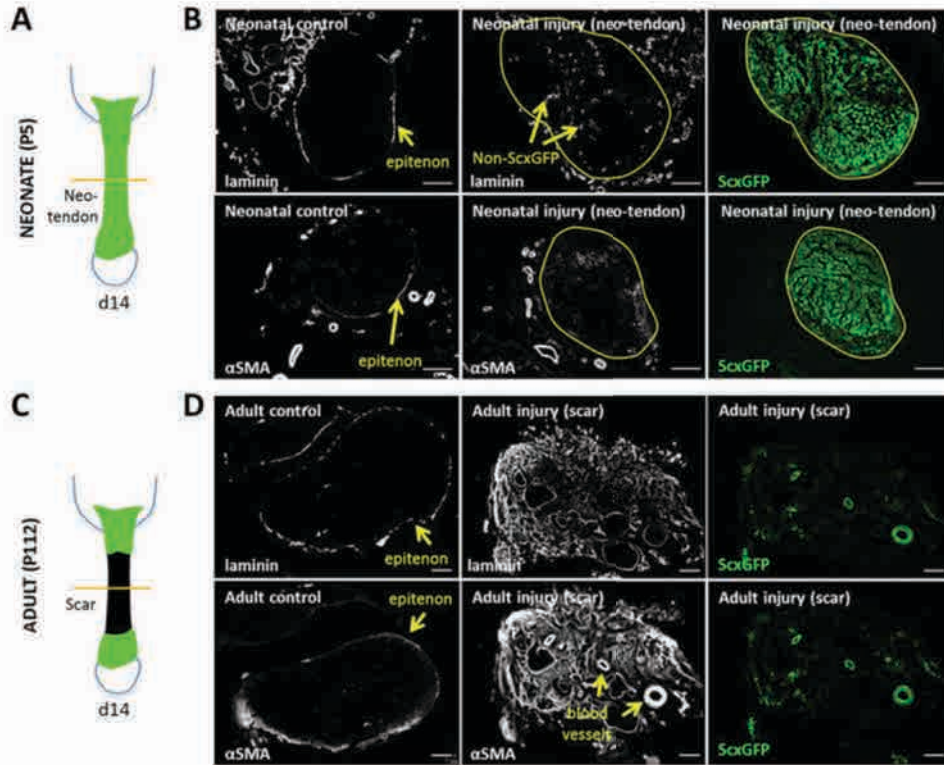


Undefined Milieu

- Myofibroblasts (α -SMA)
- Sheath (Prg4)
- Basement membrane (laminin)
- ?

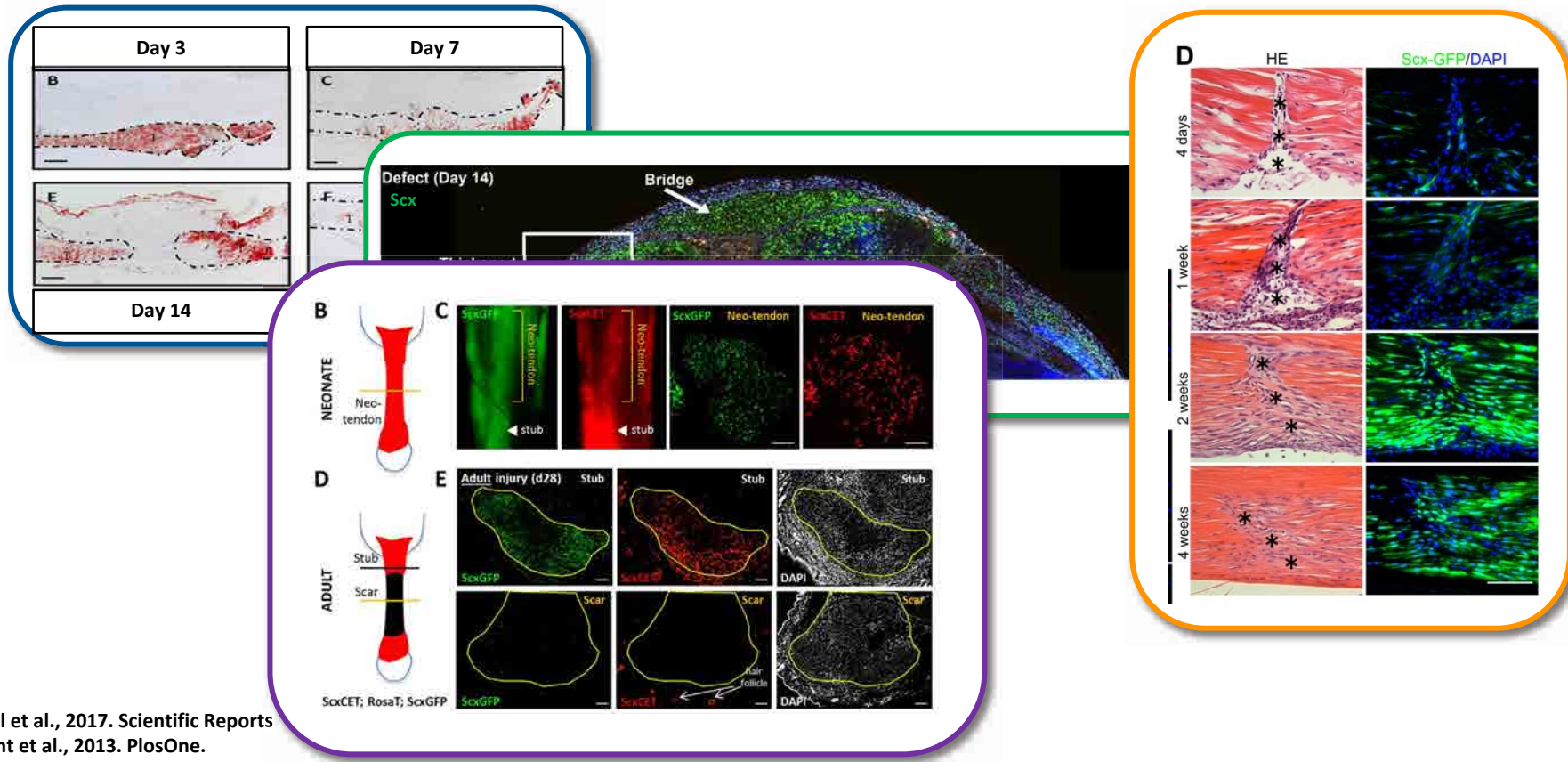
Cellular Basis of Healing: Intrinsic Cells

Tenocytes: Scx remains best marker with genetic tools



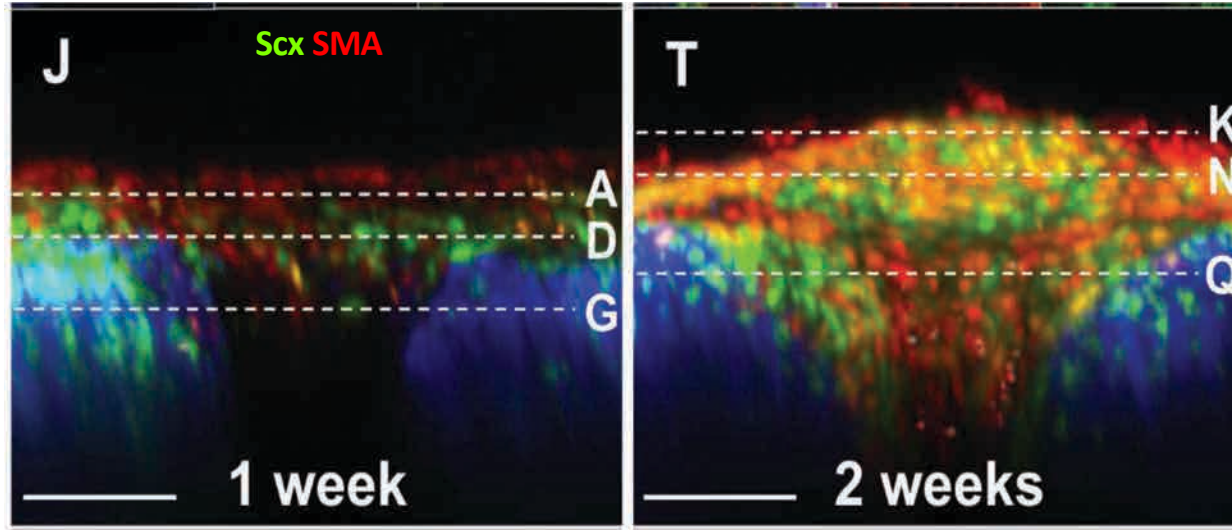
Howell et al., 2017. SciRep

Contribution of Scx-cells to healing is controversial and context dependent



Howell et al., 2017. Scientific Reports
 Dymont et al., 2013. PlosOne.
 Loiselle et al., 2009. JOR
 Sakabe et al., 2018. JBC.

Contribution of Scleraxis Cells to Healing, Regeneration and Scar Formation is Unclear

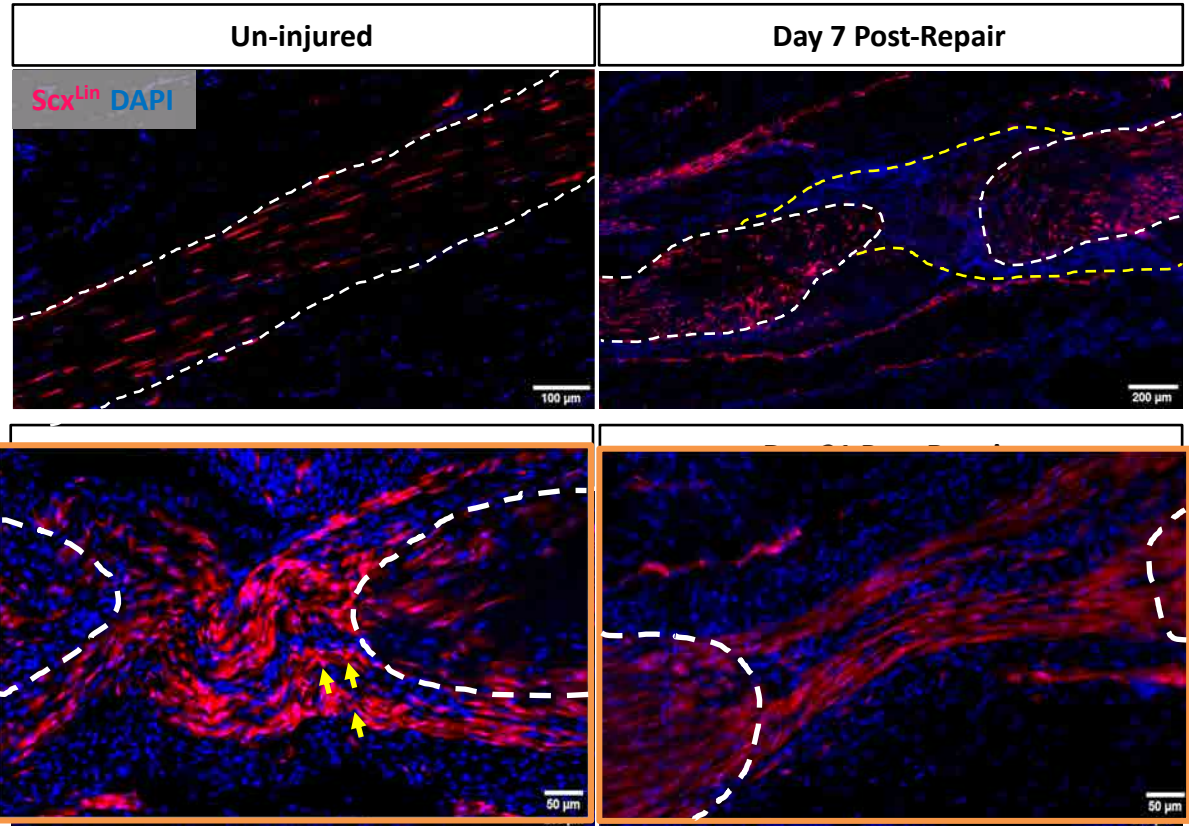
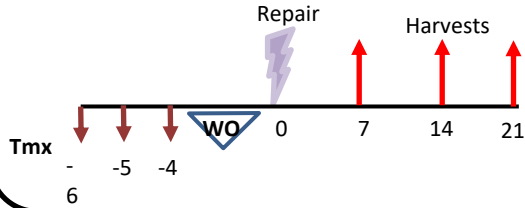


Function is likely to be context dependent

Dyment et al., 2014. PlosOne

Scx^{Lin} Cells Contribute to Bridging Population by Day 14

A- Scx^{Lin} - Tmx Washout

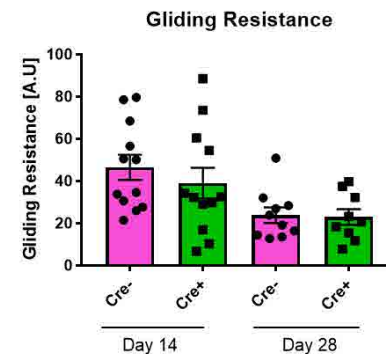
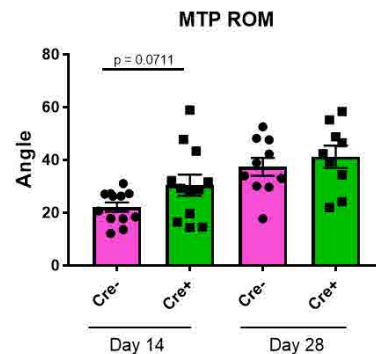
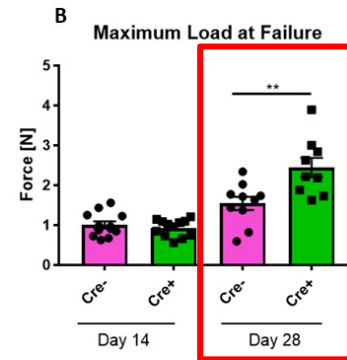
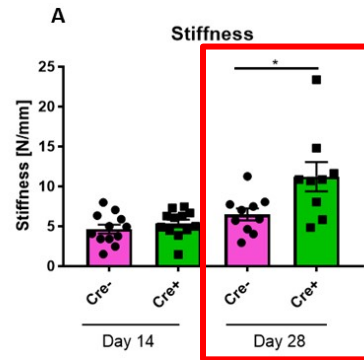
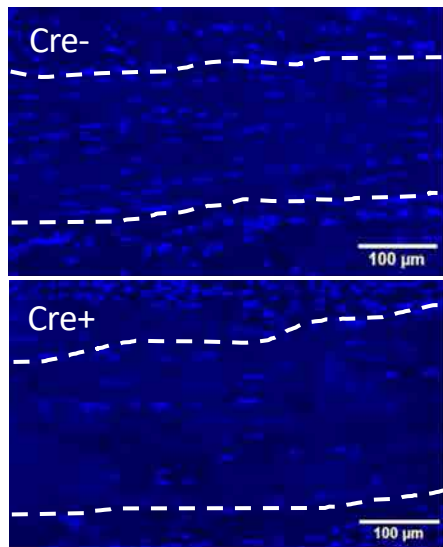
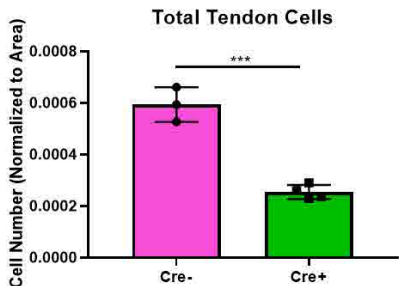


Best & Loiselle FASEB 2019

Scx+ Cell Death Improved Healing Tendon Strength



Scx-Cre; ROSA-DTR^{F/+}

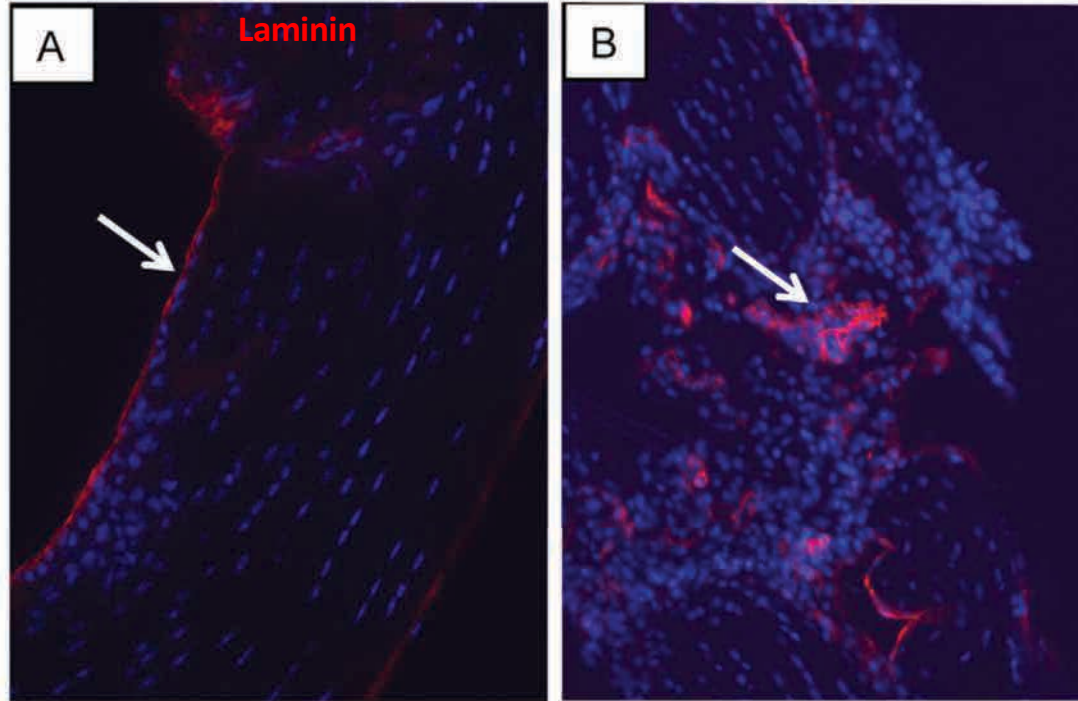


➤ **Loss of Scx cells is beneficial to late healing**

Best, Unpublished Data

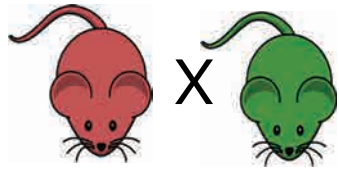
Cellular Basis of Healing: Intrinsic Cells

Contribution of basement membrane (laminin⁺) cells to healing and adhesion formation

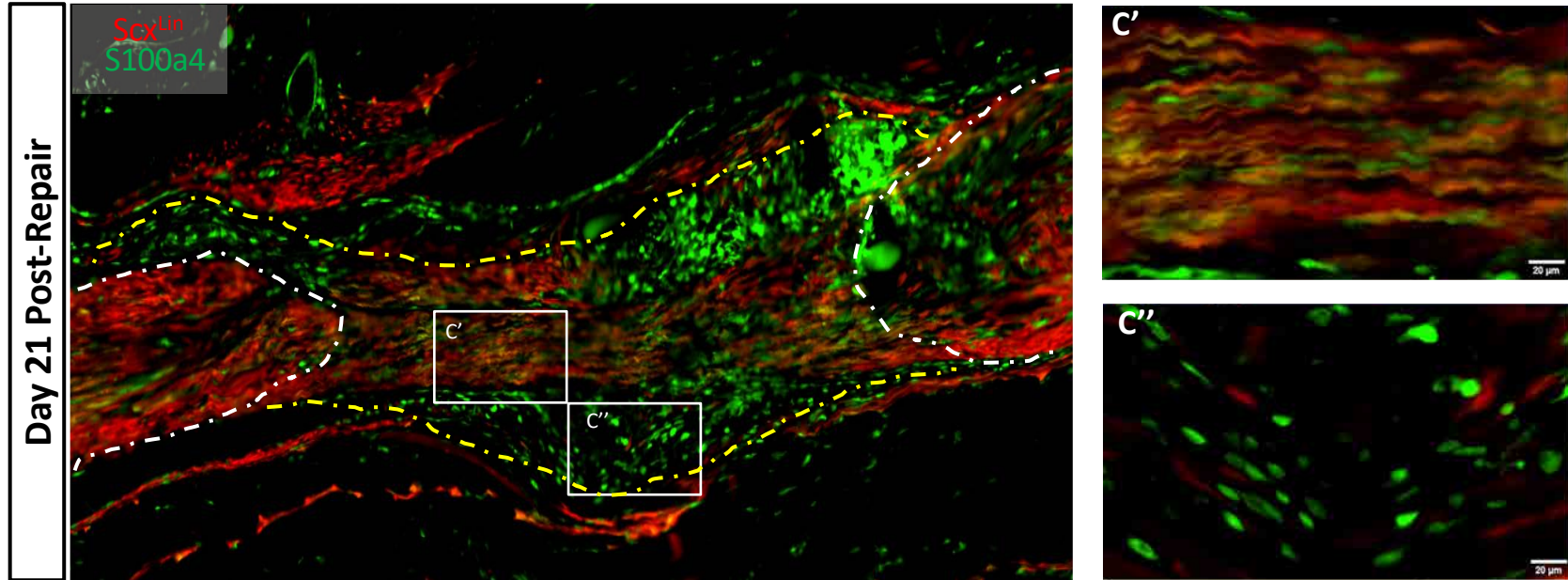
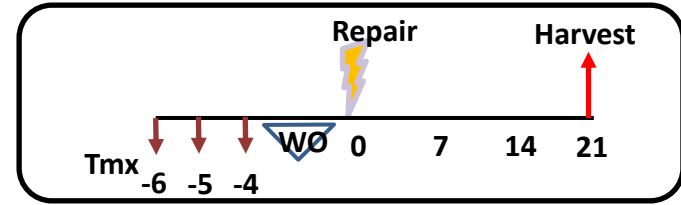


Taylor et al., 2011. PlosOne

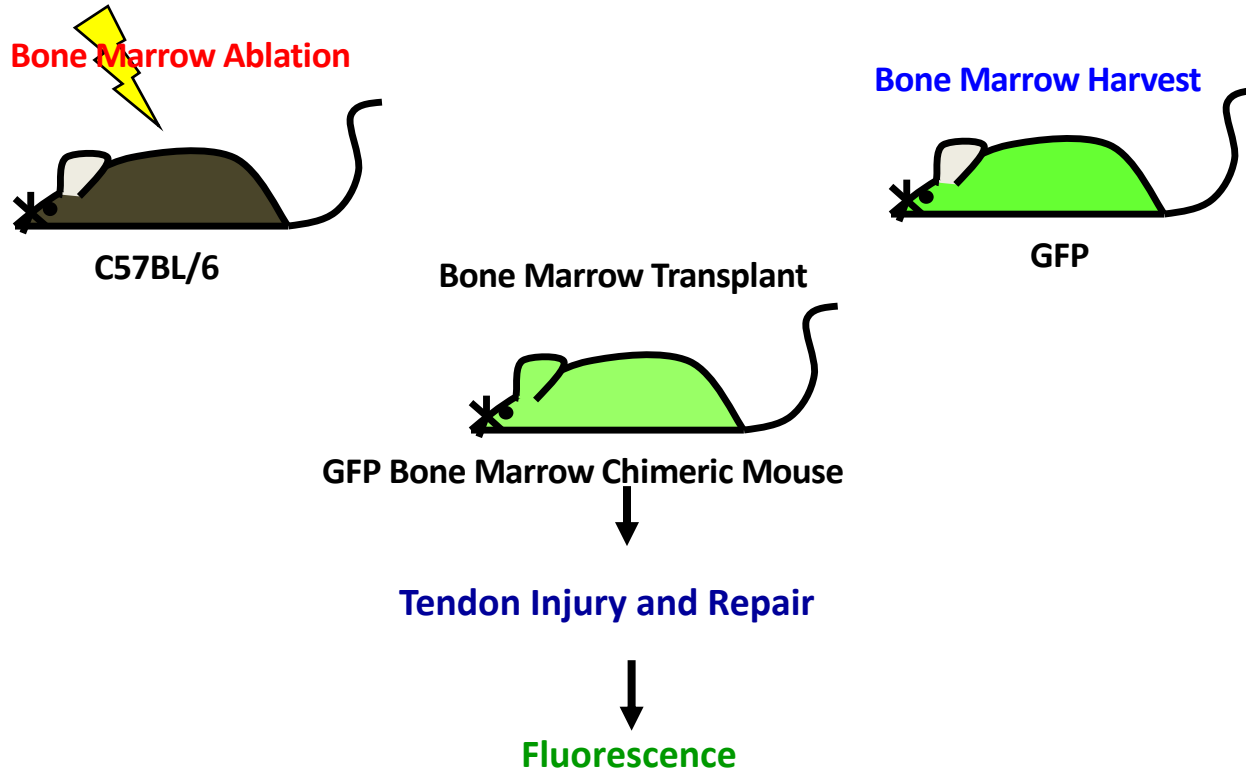
S100a4 and Scx cells have distinct spatial expression profiles during healing



Scx-Cre^{ERT2}; Rosa-Ai9; S100a4-GFP^{promoter}

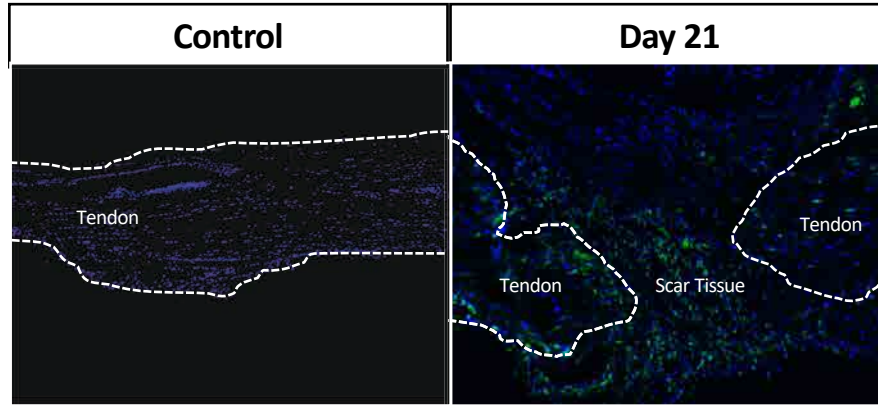


Cellular Basis of Healing: Extrinsic Cells

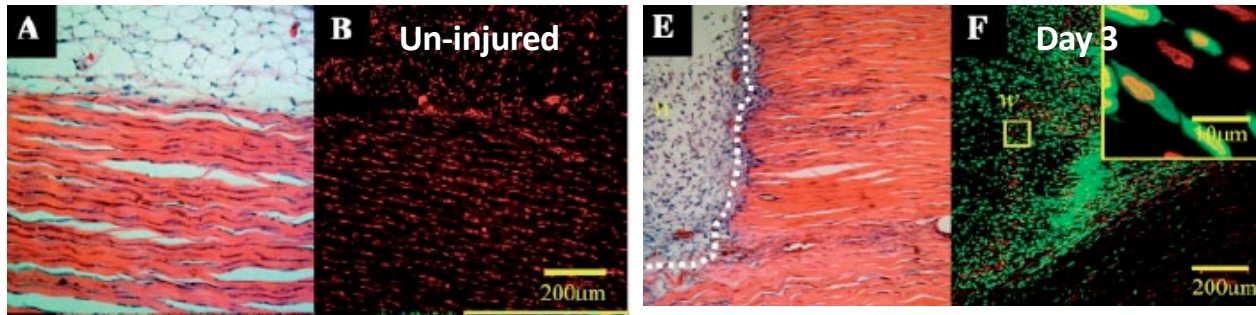


Cellular Basis of Healing: Extrinsic Cells

Bone Marrow Derived Cells Migrate Specifically to the Repair Site



- Specific Sub-populations remain unknown
- Function not ~clear
- BM-Mmp9 sufficient for adhesion formation



Loiselle et al., 2012. PlosOne, Kajikawa, Y. et al. Journal of Cell Physiology. 2007

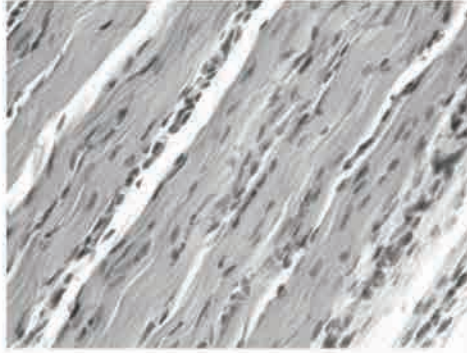
Future Work

- Delineate intrinsic vs. extrinsic contributions of cell types
 - Macrophages
 - S100a4
- Define functions of intrinsic & extrinsic populations
- Understand how homeostatic populations change in response to injury

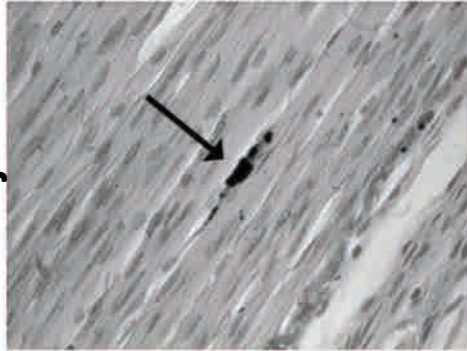
Regenerative Healing: Embryonic

Fetal Sheep

Un-injured



Injured

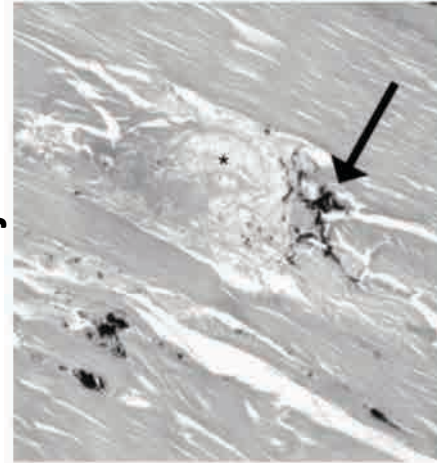


Mature Sheep

Un-injured



Injured



Beredjikian PK et al., 2003. Annals of Biomedical Engineering

Regenerative Healing: Embryonic

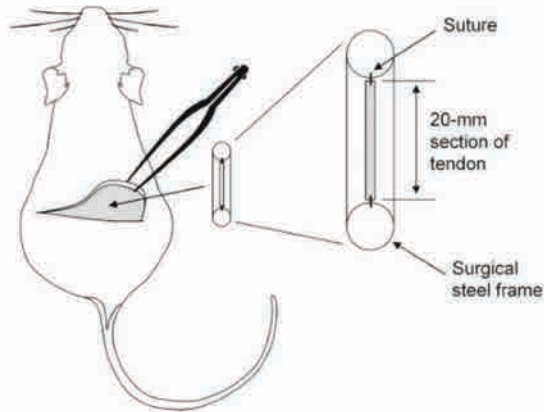


Figure 1. Schematic of subcutaneous transplantation procedure. Each mouse received one graft of either adult or fetal tendon.

Embryonic and adult tendon implanted in to the back to determine if embryonic tendons could heal scar-lessly in an adult environment

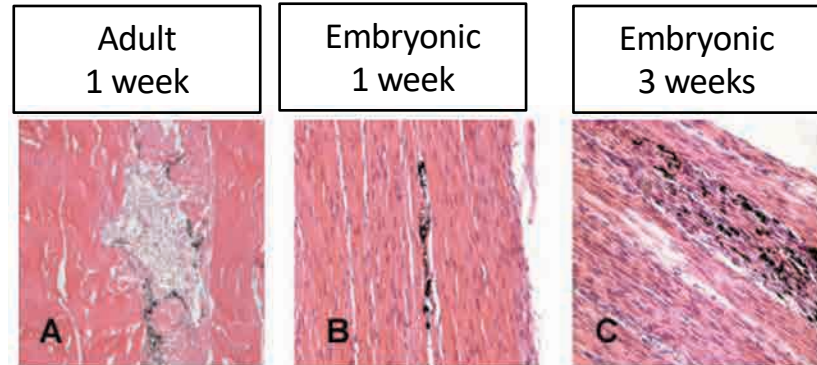
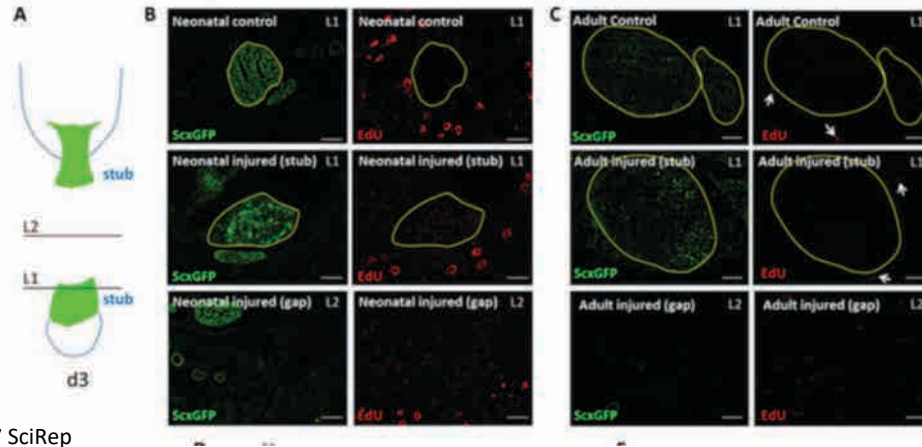
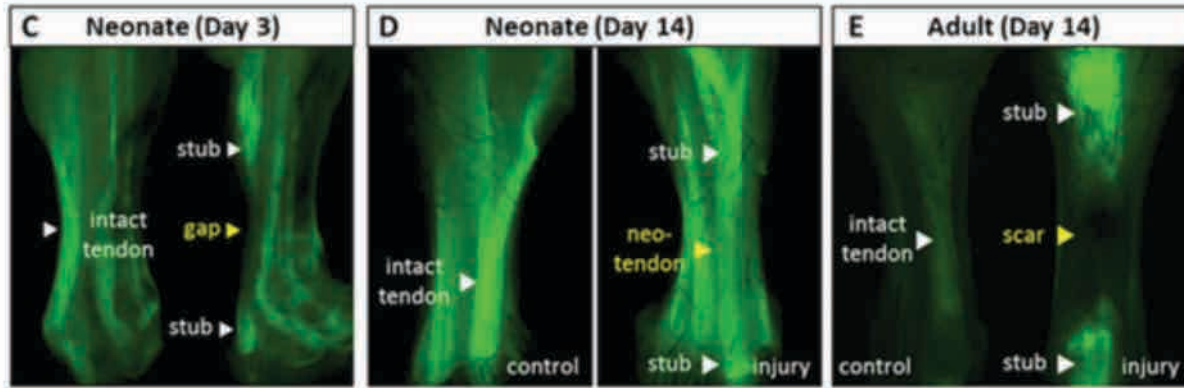


Figure 2. H&E sections of wounded tendons (black indicates wound). (A) 1-week adult, original magnification $\times 50$; (B) 1-week fetal, original magnification $\times 200$; (C) 3-week fetal, original magnification $\times 200$. Note the substantial inflammatory response in the adult, but not the fetal, specimens.

Beredjikian PK et al., 2006. JOR

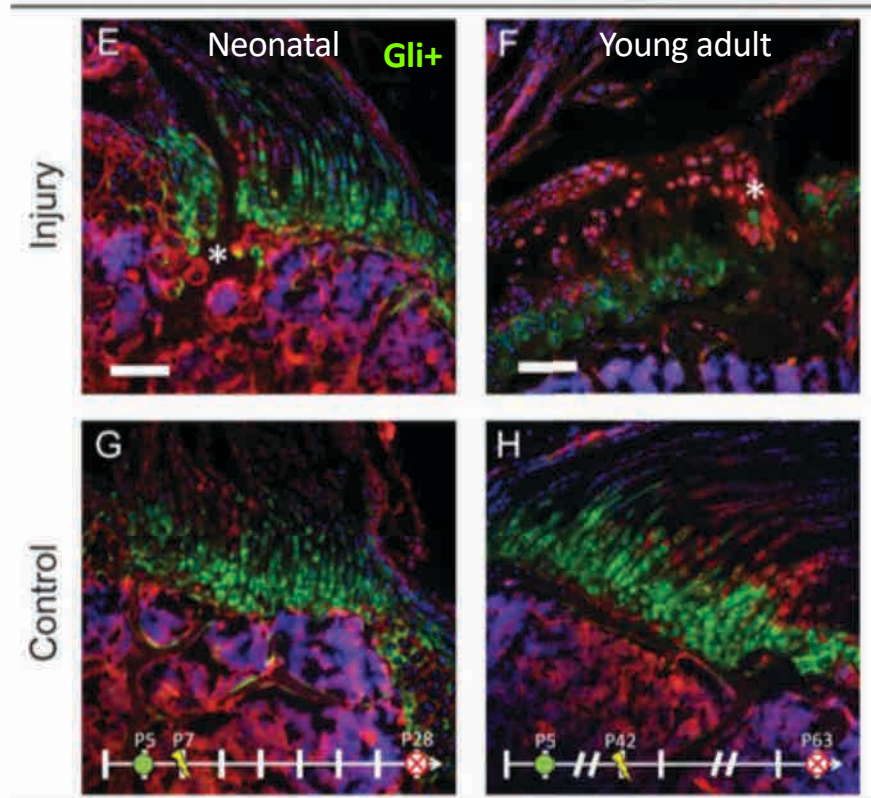
Regenerative Healing: Neonatal



Howell et al., 2017 SciRep

Regenerative Healing: Neonatal

Tracing Gli1-lineage cells after enthesis injury



Schwartz AG et al., 2017, Development

Where do we go from here? (2017)

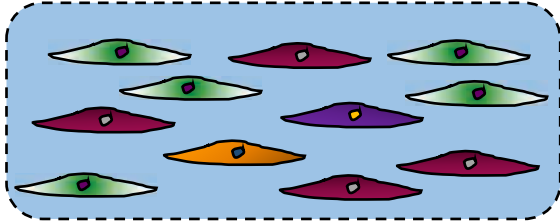
- ✓ Critical mass of labs studying cell lineage/fate/function
- ✓ Big data and single-cell RNAseq
- Development of tendon specific tools
- Identification of new 'tendon-specific' markers



WELCOME
TO THE
FUTURE

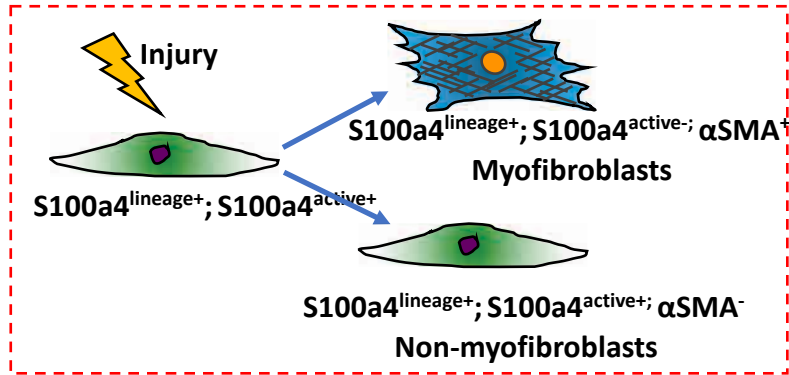
Where do we go now?

Tendon Cell Heterogeneity

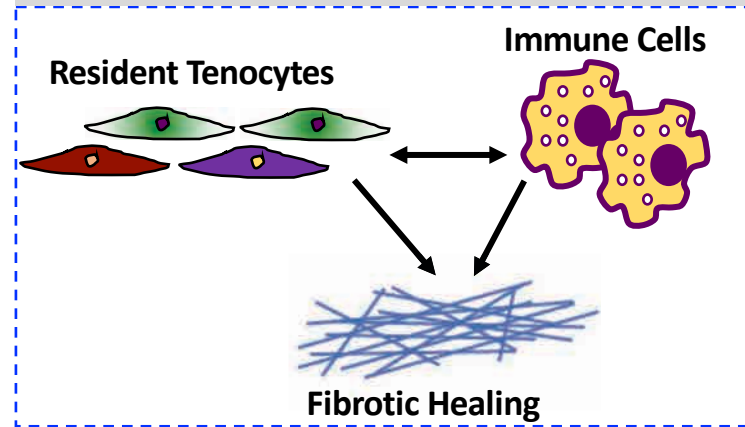


- Tendon Cell Heterogeneity
- Delineate functions of subpopulations
- **Need to define cell origin, lineage and fate**
- **Understanding of cell-cell, cell-matrix interaction → phenotype**

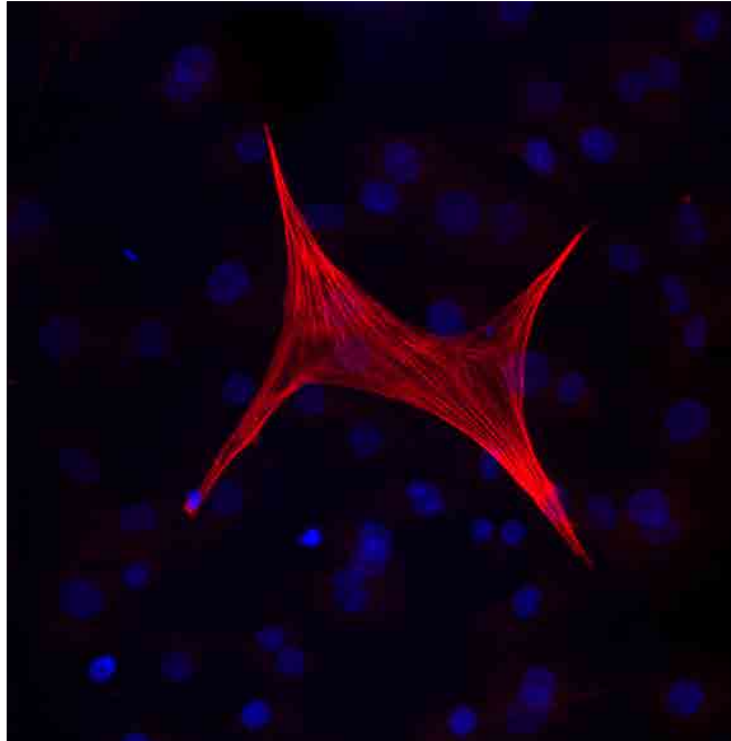
Defining Cell Lineage and Terminal Fate



Understanding Cell-Cell Interactions

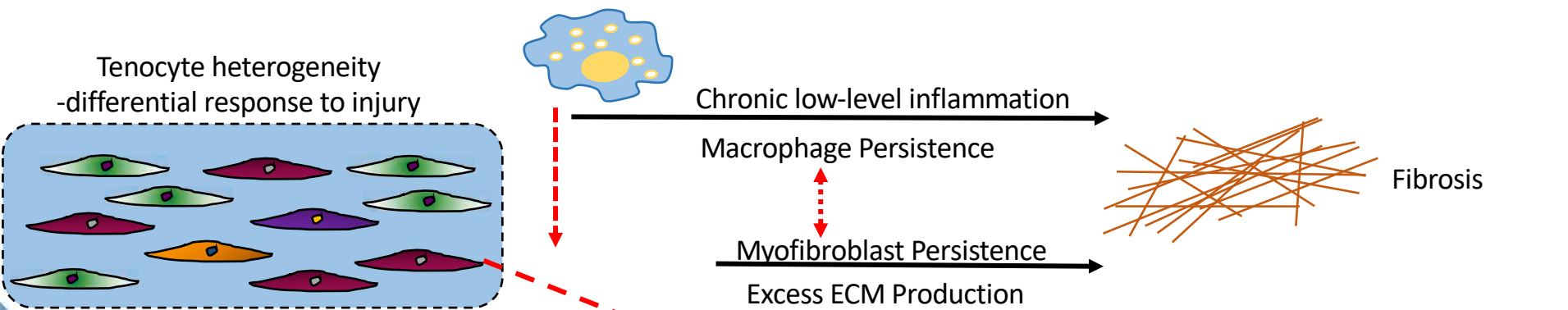
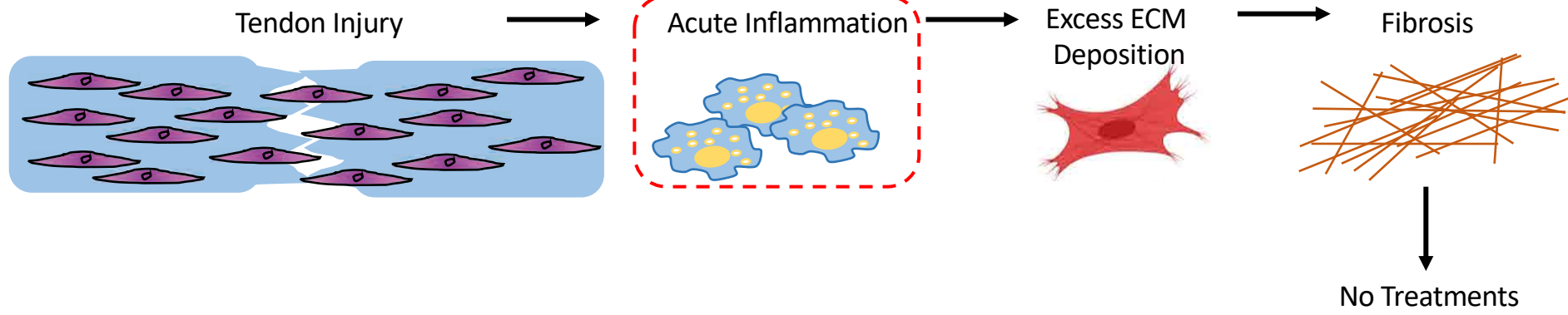


Tenocyte transition to myofibroblasts?

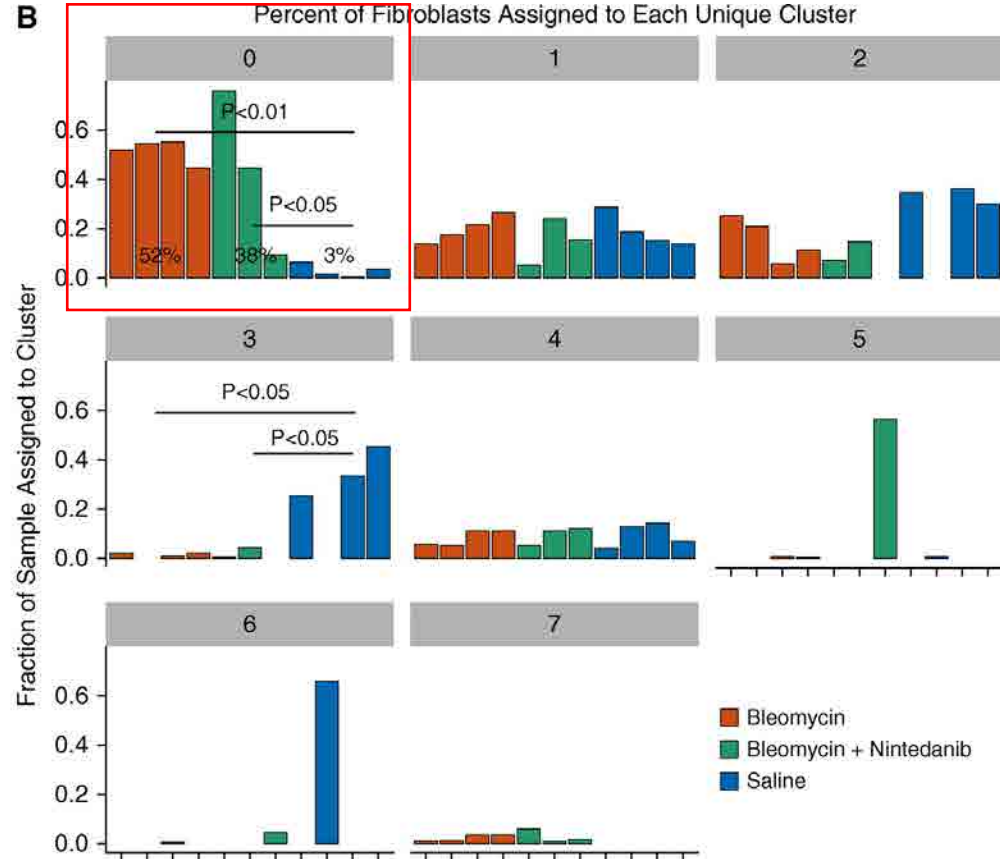
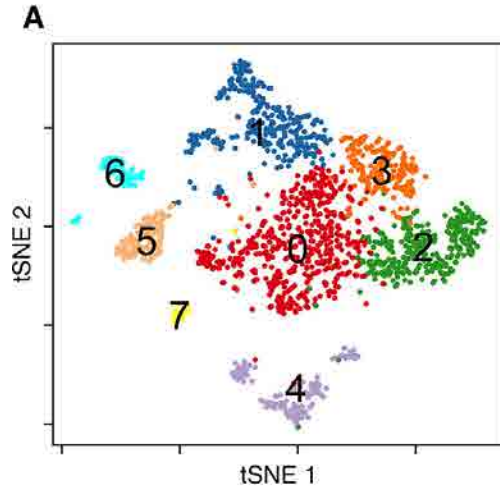


- **“Specialized” / Activated Fibroblasts**
- **Involved in matrix deposition**
- **Restoration of tissue integrity**

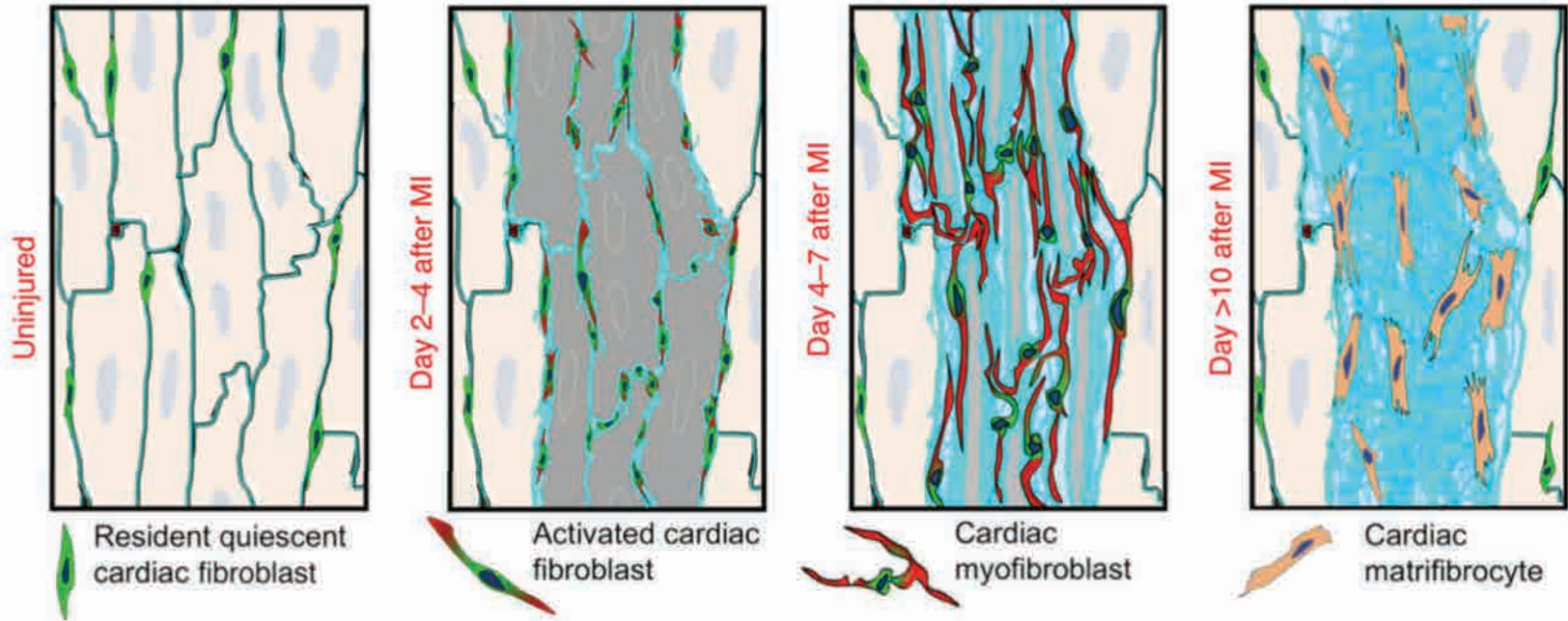




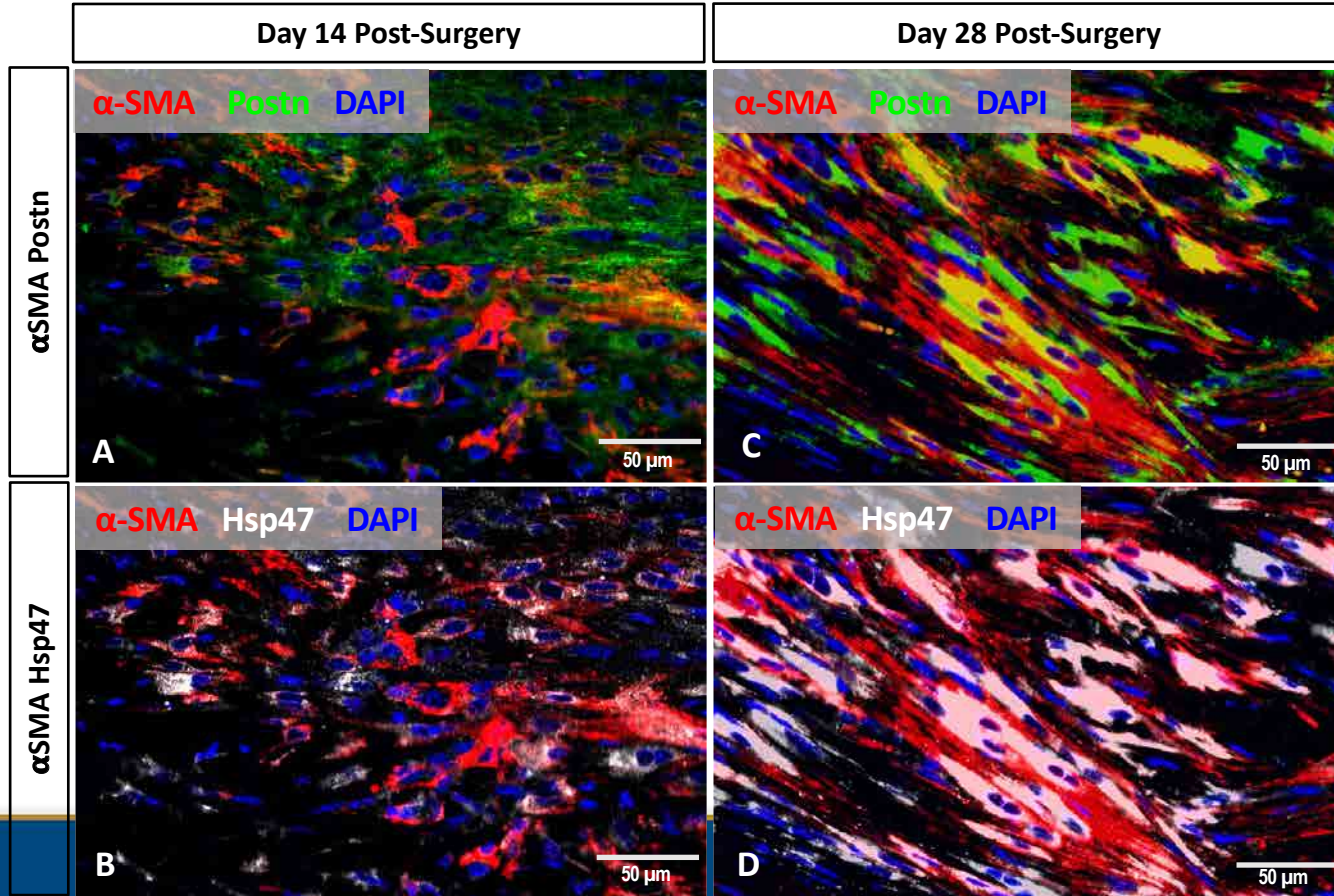
Fibroblast heterogeneity at baseline with differential contributions to myofibroblast fate



Phenotypic and functional shifts over time

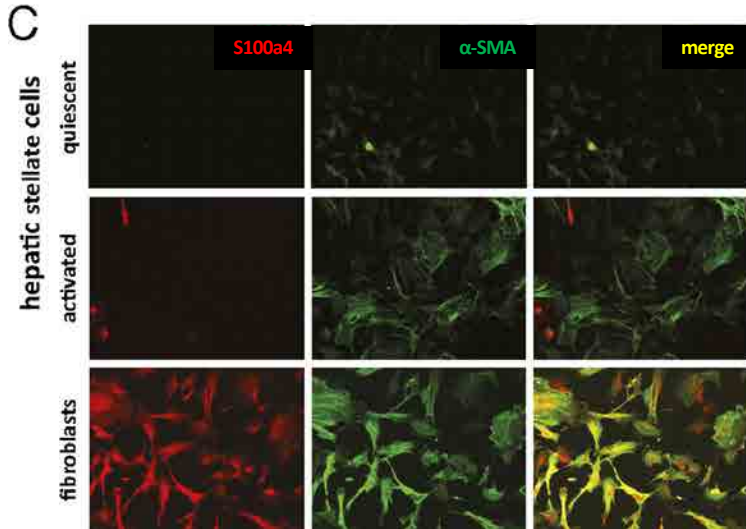


Phenotypic and functional shifts over time



The controversial relationship between S100a4 and α -SMA

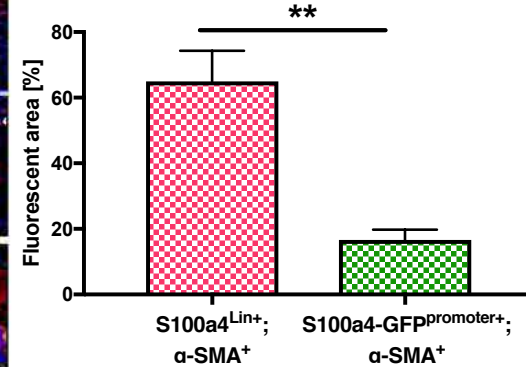
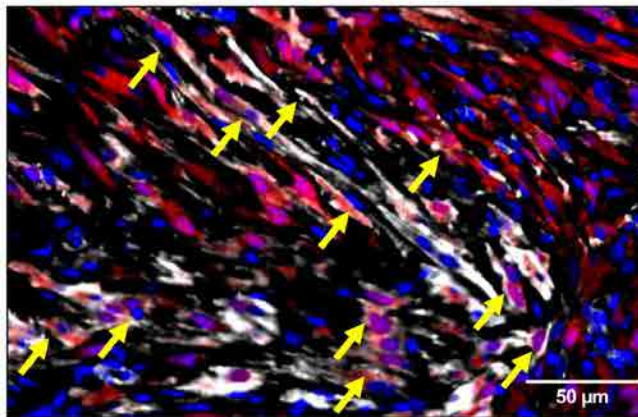
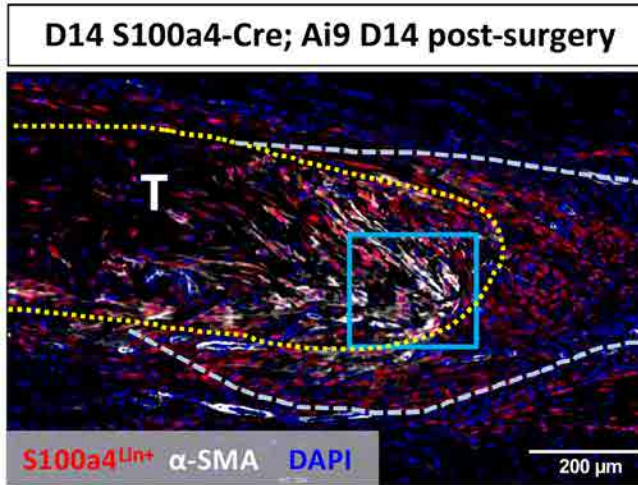
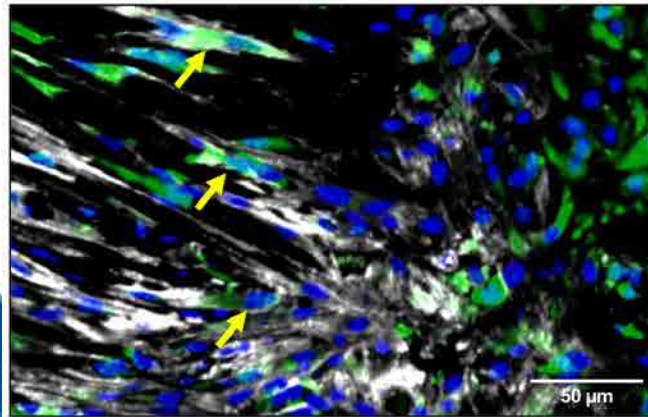
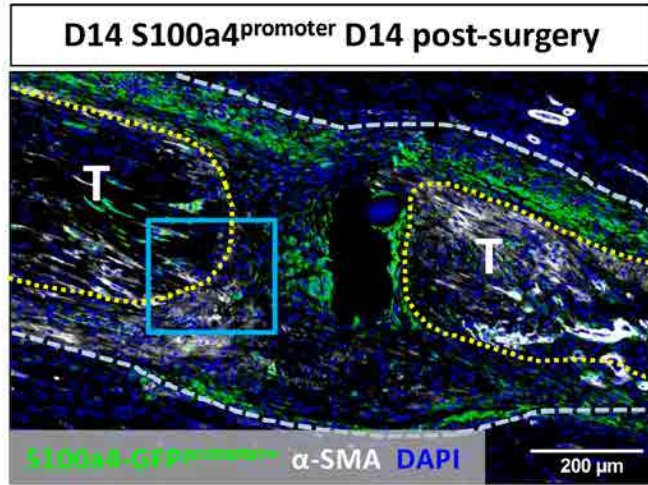
Defining the myofibroblast pre-cursors during tendon healing



- Nearly all dermal fibroblasts are S100a4⁺/ α -SMA⁺
- No co-localization seen in quiescent or activated hepatic stellate cells

Österreicher et al. PNAS 108:308-313. (2011)

S100a4⁺ cells are not myofibroblasts in the healing tendon But, S100a4-lineage cells become α -SMA⁺ myofibroblasts



Ackerman et al., eLife. 2019



UR
MEDICINE

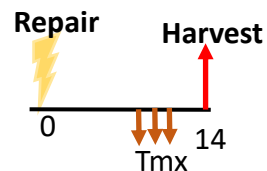
Orthopaedics
& Rehabilitation

Dynamic Contribution of ScxLin and Scx Expression to Myofibroblast Fate

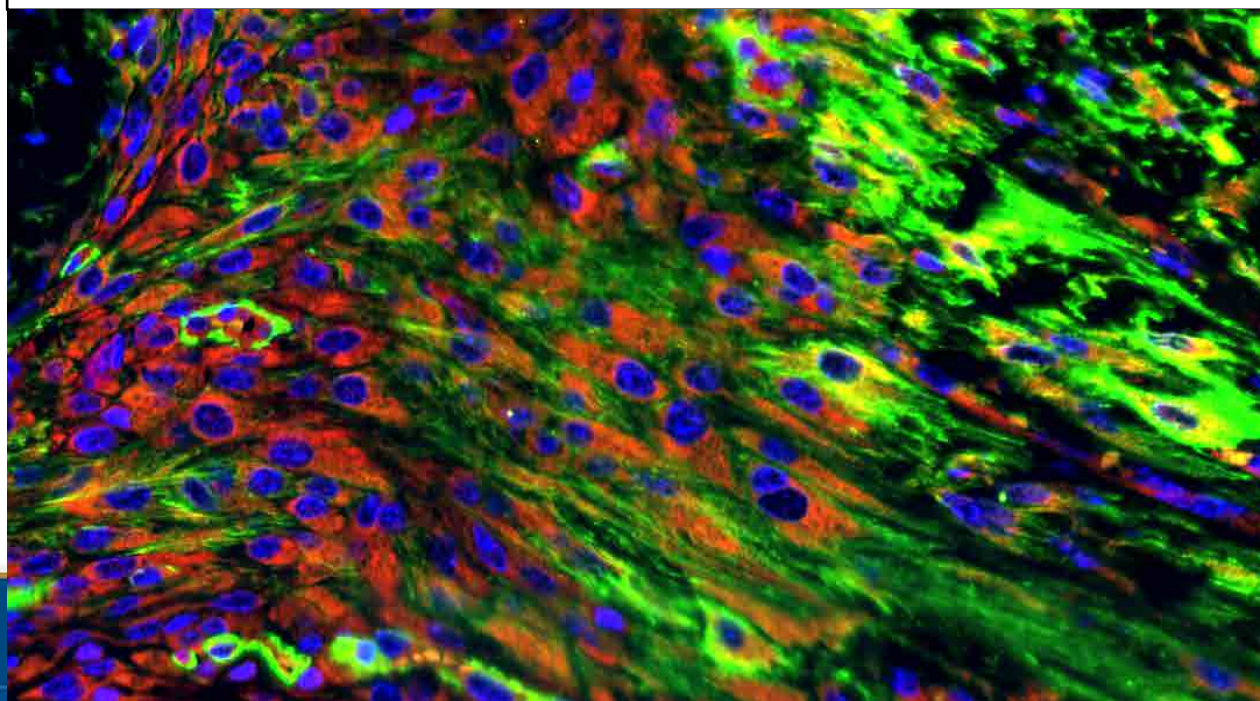


Scleraxis^{Ai910-12} Mice

Labels tendon cells that express Scx 10-12 days post-surgery



Scx⁺ at D10-12 post-op ⇒ myofibs



RFP (Scx^{Ai9})
αSMA
DAPI