# Stat3 Mediates the Function of mTORC1 in Fibrovascular Scar Formation During Postnatal Tendon Development

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## Background

Tendon injuries are challenging clinical problems due to slow, incomplete healing with fibrovascular scar formation, which reduces tendon function and causes chronic complications such as pain and tendon ruptures. The limited understanding of the regulatory mechanisms underlying fibrovascular scar formation hinders the development of effective treatment modalities for tendon diseases. Our recent study showed that constitutive activation of mTORC1 signaling during postnatal tendon development caused fibrovascular scar-like phenotypes in tendons, including disorganized ECM, high cellularity, and neovascularization [1]. However, the downstream mechanism mediating mTORC1 function in fibrovascular scar formation is not clear. Stat3 is a transcription factor and plays a crucial role in fibrosis and inflammation via the regulation of cell proliferation and ECM organization [2]. Interestingly, a previous study showed that Stat3 can be activated by mTORC1 signaling [3]. This study aims to determine Stat3 as a mediator of mTORC1 function in fibrovascular scar formation in tendons.

Referances: [1] Lim+, 2017 [2] Kasembeli+, 2018 [3] Saleiro+, 2015

## Method

All procedures were approved by UPenn's IACUC. To genetically determine Stat3 as a mediator of mTORC1 function in fibrovascular scar formation in tendons, we performed a genetic rescue experiment by generating three types of the tendon-specific deficient mouse: 1) *Scx-Cre; Tsc1*$^{fl/fl}$ (tendon-specific mTORC1 gain-of-function mouse model), 2) *Scx-Cre; Stat3*$^{fl/fl}$ (tendon-specific Stat3 knockout mouse model), and 3) *Scx-Cre; Tsc1*$^{fl/fl}$*; Stat3*$^{fl/fl}$ (tendon-specific Tsc1 and Stat3 double knockout mouse model for rescue experiment). Histological analyses were conducted on patellar and Achilles tendons at 1 month of age. RNA sequencing analysis was used to examine gene expression changes in Achilles tendons of wildtype and *Scx-Cre; Tsc1*$^{fl/fl}$ mice. Primary tenocytes were isolated from tail tendon to perform in vitro molecular studies using monolayer cell culture. A western blotting experiment was performed to examine the alteration in phosphorylated protein *in vitro*. An immunohistochemistry experiment was performed to examine the alteration in protein expression *in vivo*. Anti-CD31 antibody (Abcam, ab182981) was used to confirm neovascularization. Anti-F4/80 antibody (Abcam, ab111101) was used to check macrophage infiltration. Stattic (50µl/10g, Abcam, ab120952) was daily injected intraperitoneally from P21 to P27 daily in wildtype and *Scx-Cre; Tsc1*$^{fl/fl}$ mice. All quantitative data were analyzed using student's t-test.

## Results

### 1. mTORC1 activation increased pathogenic tendon markers.

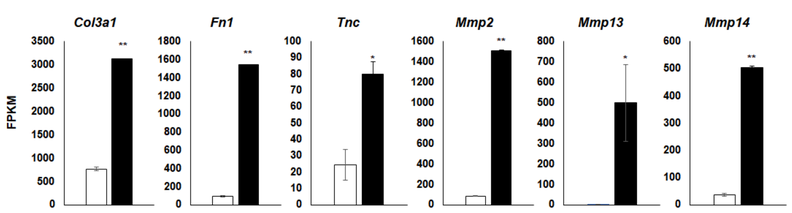


**Figure 1. Transcriptome analysis of tendon from mTORC1 gain-of-function (*Scx-Cre; Tsc1*$^{fl/fl}$) mouse.** FPKM (Fragment Per Kilobase of transcript per Million mapped reads) values of RNA-seq data from Achilles tendon of wildtype and *Scx-Cre;Tsc1*$^{fl/fl}$ mice in Co3a1, Fibronectin1 (Fn1), Tenascin C (Tnc), and Metalloproteinases (Mmps). (White bar indicates wildtype mice, Black bar indicates *Scx-Cre;Tsc1*$^{fl/fl}$ mice, * indicate P<0.05 and ** indicate P<0.001 between genotypes, n=3)

## Results

### 2. Stat3 is activated in mTORC1 gain-of-function mouse model.

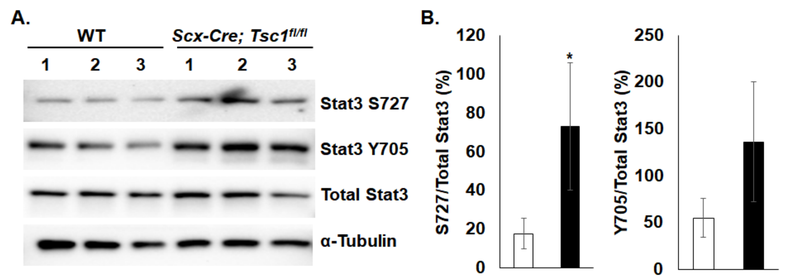


**Figure 2. Stat3 is activated in primary tendon cells from mTORC1 gain-of-function mouse.** Western blots to assess Stat3 signaling, phosphorylation of S727 is mTOR dependent and phosphorylation of Y705 is mTOR independent, activation in *Scx-Cre;Tsc1*$^{fl/fl}$ cells (A). Quantification of the western blot bands (B). (White bar indicates wildtype cells, Black bar indicates *Scx-Cre;Tsc1*$^{fl/fl}$ Cells, * indicate P<0.05 between genotypes, n=3)

### 3. The abnormal growth caused by mTORC1 activation was not rescued by the deletion of Stat3.

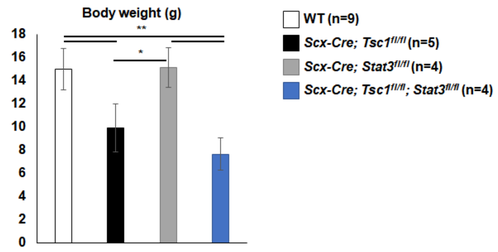


**Figure 3. Body wight of wildtype, *Scx-Cre; Tsc1*$^{fl/fl}$, *Scx-Cre; Stat*$^{fl/fl}$, and *Scx-Cre; Tsc1*$^{fl/fl}$*; Stat3*$^{fl/fl}$ mouse at 1 month old.** (* indicate *P*<0.05, and ** indicate *P*<0.01 in between genotypes)

### 4. Stat3 deletion partially rescued the fibrovascular scar-like phenotypes in Achilles and patellar tendon.

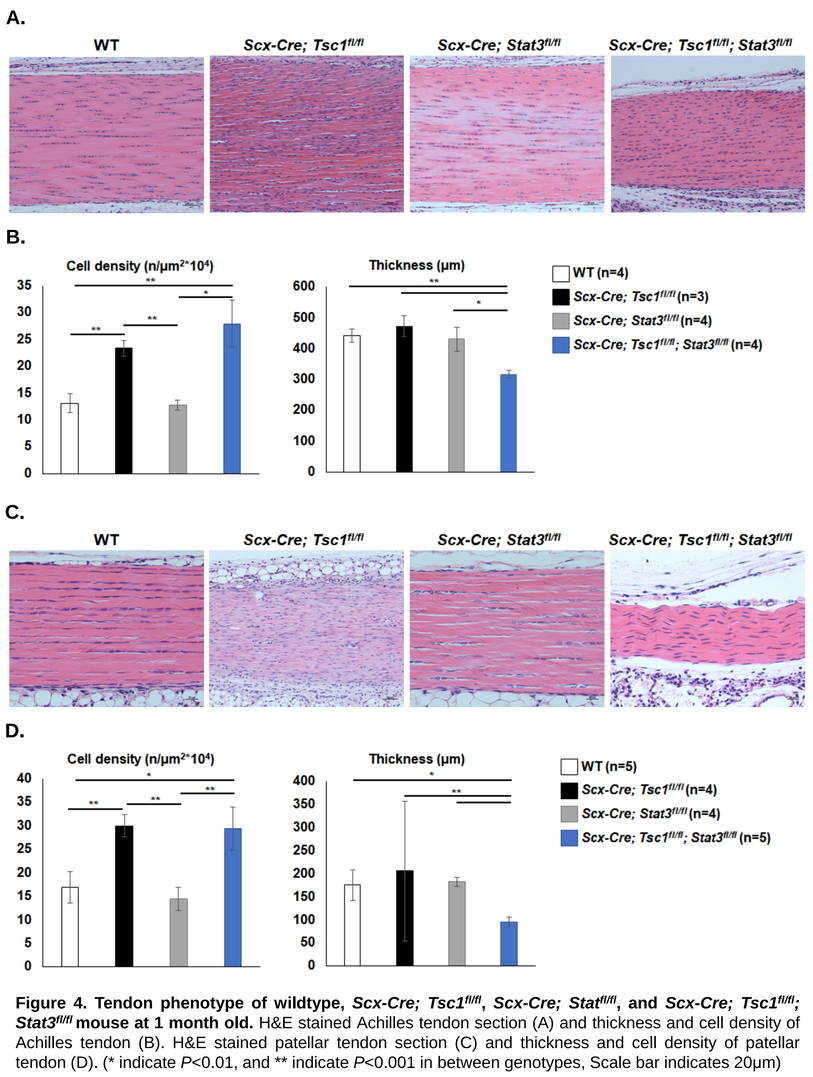


**Figure 4. Tendon phenotype of wildtype, *Scx-Cre; Tsc1*$^{fl/fl}$, *Scx-Cre; Stat*$^{fl/fl}$, and *Scx-Cre; Tsc1*$^{fl/fl}$*; Stat3*$^{fl/fl}$ mouse at 1 month old.** H&E stained Achilles tendon section (A) and thickness and cell density of Achilles tendon (B). H&E stained patellar tendon section (C) and thickness and cell density of patellar tendon (D). (* indicate *P*<0.01, and ** indicate *P*<0.001 in between genotypes, Scale bar indicates 20µm)

## Results

### 5. Stat3 deletion fully rescued neovascularization and macrophage infiltration.

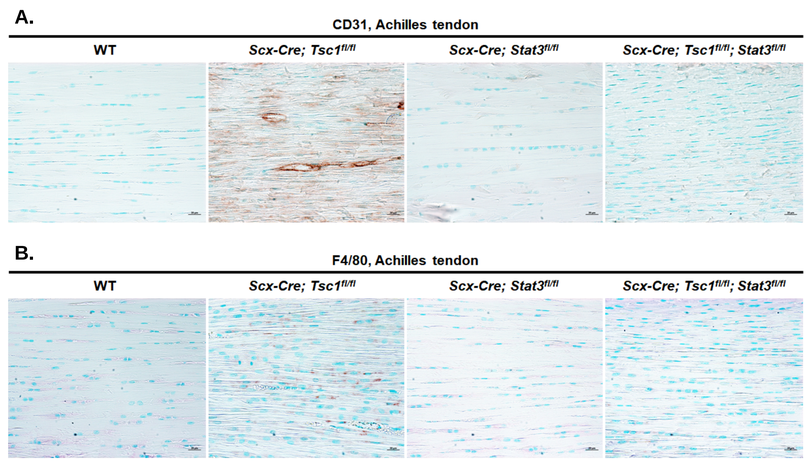


**Figure 5. Immunohistochemistry of wildtype, *Scx-Cre; Tsc1*$^{fl/fl}$, *Scx-Cre; Stat*$^{fl/fl}$, and *Scx-Cre; Tsc1*$^{fl/fl}$*; Stat3*$^{fl/fl}$ mouse at 1 month old.** Anti-CD31 antibody was used to confirm neovascularization (A). Anti-F4/80 antibody was used to check macrophage infiltration (B). (Brown color indicates positive cells, Scale bar indicates 20µm)

### 6. Pharmacological inhibition of Stat3 partially rescued fibrovascular scar-like phenotypes caused by mTORC1 activation.

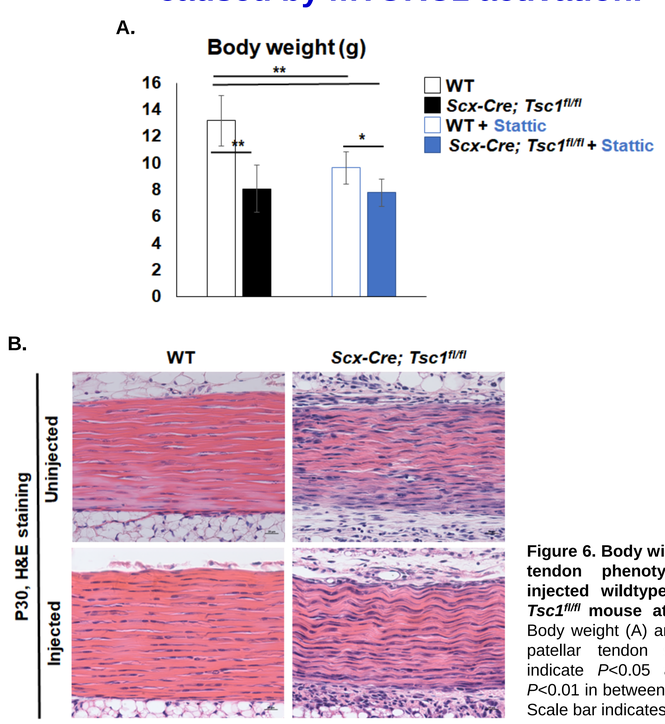


**Figure 6. Body wight and patellar tendon phenotype of stattic injected wildtype and *Scx-Cre; Tsc1*$^{fl/fl}$ mouse at 1 month old.** Body weight (A) and H&E stained patellar tendon section (B). (* indicate *P*<0.05 and ** indicate *P*<0.01 in between genotypes, n=3, Scale bar indicates 20µm)

## Summary

- mTORC1 can be a major biological mechanism regulating fibrovascular scar formation in pathogenic tendon condition
- Stat3 deletion in mTORC1 activated mouse
  - partially rescued tendon morphology
  - enhanced collagen organization
  - fully rescued neovascularization
  - fully rescued inflammatory cell infiltration

## Funding

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