# Collagen V Knockdown During Phases of Tendon Healing Differentially Impacts Gene Expression

**Ryan J. Leiphart**
**Brittany L Taylor**
**Stephanie N Weiss**
**Nathaniel A Dyment**
**Louis J Soslowsky**

McKay Orthopedic Research Laboratory
University of Pennsylvania,
Philadelphia, PA

## Disclosures

None

## Introduction

Classic Ehlers-Danlos Syndrome (cEDS) is a connective tissue disorder often caused by mutations in *COL5A1*, which encodes the primary collagen V alpha chain[1]. Surprisingly, acute *Col5a1* knockdown at the time of murine tendon injury mitigated the quasi-static mechanical deficits of healing tendons[2]. This effect on mechanical parameters was reversed and diminished with *Col5a1* knockdown during the late inflammatory and early remodeling phases of tendon healing, respectively[3]. While this demonstrates that the timing of *Col5a1* knockdown during tendon healing differentially impacts tendon mechanics, the genetic mechanisms underlying this regulation remain unknown. Therefore, the objective of this study was to define the effect of acute *Col5a1* knockdown throughout the phases of tendon healing on injured tendon gene expression.

Based on prior mechanical findings, we hypothesized that *Col5a1* knockdown at the time of tendon injury would lead to diminished inflammatory expression and an earlier increase in matrix remodeling gene expression. We hypothesized that this effect would be reversed with *Col5a1* knockdown in the late inflammatory phase, leading to increased inflammatory expression at later healing timepoints. We also hypothesized that *Col5a1* knockdown in the early remodeling phase would cause negligible changes in gene expression.

## Methods

### *Animals*

Male wild-type (WT) (n = 20), bitransgenic *Col5a1*$^{flox/+}$ (n = 34) and *Col5a1*$^{flox/flox}$ (n = 34) mice with a tamoxifen (TM)-inducible *ROSA-CreER*$^{T2}$ were used (IACUC approved). At 120 days old, mice received bilateral, full-thickness partial width patellar tendon injuries[4]. Mice received two consecutive daily doses of TM (2 mg/40 g body weight) for Cre-mediated excision of the *Col5a1* gene, resulting in I-*Col5a1*$^{+/-}$ and I-*Col5a1*$^{-/-}$ mice. The first TM injections were administered on the day of injury (WT, TM0), 5 days post-injury (TM5), or 21 days post-injury (TM21) for *Col5a1* knockdown during the different phases of tendon healing. Mice were sacrificed at 1 week (WT, TM0), 3 weeks (WT, TM0, TM5), or 6 weeks (WT, TM0, TM5, TM21) post-injury. Healthy WT control mice received TM doses (3 days of 4mg/40g body weight) at 120 days old and were sacrificed 30 days later. At sacrifice, right patellar tendons were dissected and immediately flash frozen at −80°C.

### *Gene Expression*

For RNA extraction, patellar tendons were thawed in RNA*later* ICE. Tendons were homogenized with plastic pestles in TRIzol and vortexed. RNA was extracted (Direct-zol RNA Microprep, Zymo), and cDNA was reverse transcribed (High Capacity cDNA RT, Thermo). cDNA was pre-amplified for 15 cycles with Taqman assays for 96 target genes and was loaded into a Fluidigm 96.96 Dynamic Array. The 96 target genes included categories of collagens, non-collagenous matrix, matrix remodeling, cell-ECM proteins, cell markers, inflammatory markers, and housekeepers (*Abl1* and *Rps17*). ΔCt was calculated by subtracting the gene Ct from average housekeeping Ct.

### *Statistics*

One-way ANOVAs with Tukey post-hoc tests were used to compare ΔCt values across genotypes within knockdown induction timepoint and healing timepoint. Significance was set at $p \pm 0.05$, and trends were set at $p \pm 0.1$.

## Results

I-*Col5a1*$^{-/-}$ tendons had decreased *Col5a1* expression compared to WT tendons at each healing timepoint (TM0: 2.2-fold decrease, TM5: 2.4-fold decrease, TM21: 1.4-fold decrease). I-*Col5a1*$^{+/-}$ tendons had decreased *Col5a1* expression compared to WT tendons at 3 weeks post-injury (1.3-fold decrease, TM0 trend).

TM0: I-*Col5a1*$^{+/-}$ (trend) and I-*Col5a1*$^{-/-}$ tendons had increased *Postn* expression compared to WT tendons at 3 weeks post-injury, which persisted at 6 weeks post-injury

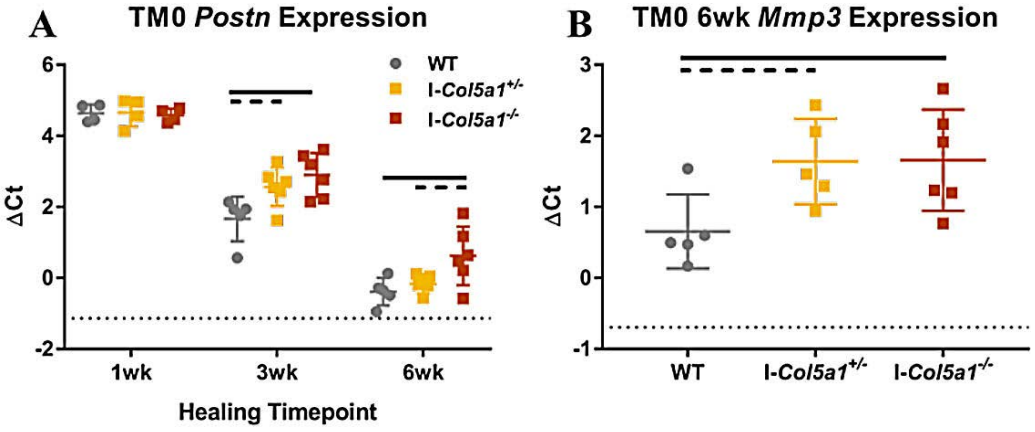


**Figure 1. TM0 Gene Expression. (A)** I-*Col5a1*$^{-/-}$ tendons had increased Postn expression at 3 weeks post-injury that persisted at 6 weeks post-injury. **(B)** TM0 *Mmp 3* expression was increased in collagen V-deficient tendons at 6 weeks post-injury. Dotted lines indicate WT uninjured expression. Solid lines denote $p \leq 0.05$, while dashed lines denote $p \leq 0.1$.

for I-*Col5a1*$^{-/-}$ tendons (Figure 1A). At 6 weeks post-injury, I-*Col5a1*$^{+/-}$ (trend) and I-*Col5a1*$^{-/-}$ tendons had increased *Mmp3* expression compared to WT tendons (Figure 1B).

TM5: At 6 weeks post-injury, I-*Col5a1*$^{+/-}$ and I-*Col5a1*$^{-/-}$ tendons had increased expression of *Aspn*, *Lum*, *Igf1*, *Mmp3*, and *Thbs4* compared to WT tendons (Figure 2). At this timepoint, I-*Col5a1*$^{+/-}$(trend) and I-*Col5a1*$^{-/-}$ tendons also had increased expression of *Bgn*, *Lox*, and *Mmp2* compared to WT tendons.

TM21: I-*Col5a1*$^{+/-}$ and I-*Col5a1*$^{-/-}$ tendons had increased expression of *Thbs4* compared to WT tendons at 6 weeks post-injury (data not shown). I-*Col5a1*$^{-/-}$ tendons had increased *Mmp3* expression compared to WT tendons at 6 weeks post-injury.

## Discussion

Results indicate that collagen V temporally regulates healing tendon gene expression. Effective *Col5a1* knockdown was demonstrated in the *Col5a1*$^{flox/flox}$ model at all healing timepoints. When *Col5a1* was knocked down at the time of injury (TM0), *Postn* and *Mmp3* expression was increased. In addition to increased matrix remodeling expression (*Lox, Mmp2, Mmp3,* and *Thbs4*), *Col5a1* knockdown during the late inflammatory phase of tendon healing (TM5) led to increased small leucine rich proteoglycan expression (SLRPs, here *Aspn, Bgn,* and *Lum*). When *Col5a1* was knocked down during the early remodeling phase of tendon healing (TM21), less robust increases in matrix remodeling expression were observed. The observed expression changes support the mechanical changes seen with *Col5a1* knockdown during different tendon healing phases[2,3]. While *Col5a1* knockdown at the time of injury mitigated mechanical deficits of healing tendons, *Col5a1* knockdown during the late inflammatory phase worsened the mechanical deficits seen with wild-type healing. Compared to TM0 tendons, TM5 tendons exhibited more robust changes in gene expression, including increased SLRP expression. Limitations of this study are the global nature of the *Col5a1* knockdown, which could lead to off-

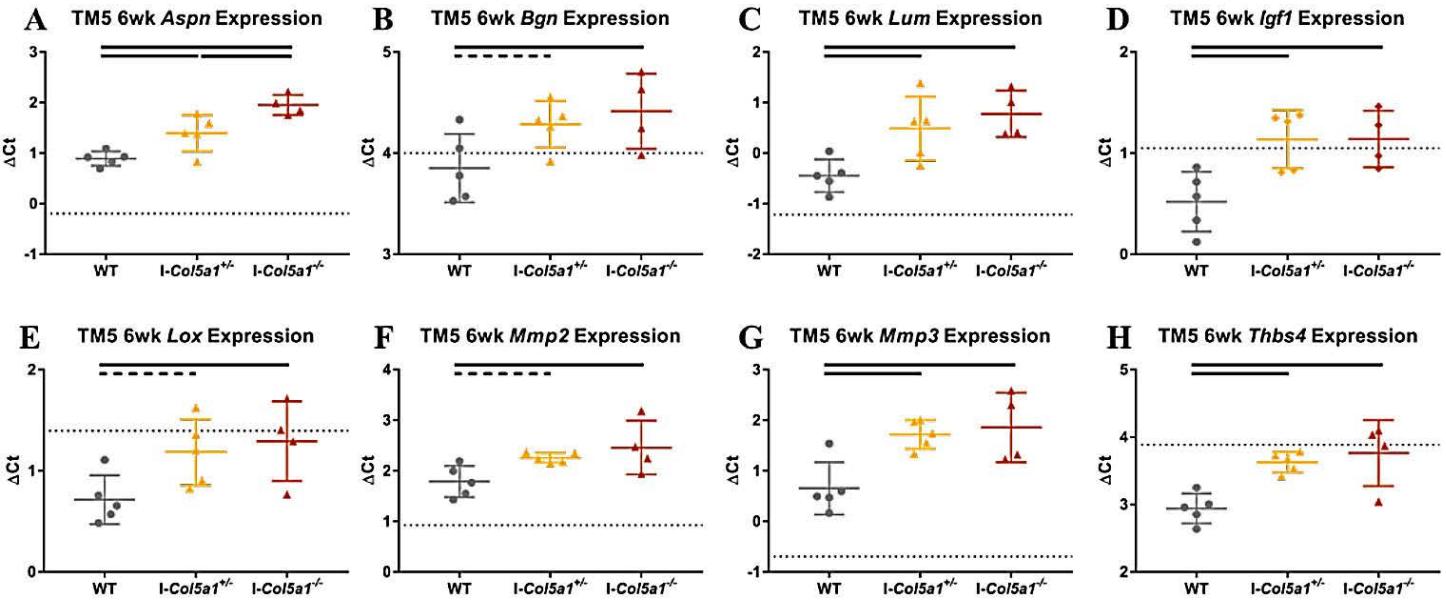


**Figure 2. TM5 Gene Expression.** At 6 weeks post-injury, *Col5a1* knockdown during the late inflammatory phase of tendon healing lead to increased expression of small leucine-rich proteoglycans **(A-C)**, *Igf1* **(D)**, and matrix remodeling genes **(E-H)**. Dotted lines indicate WT uninjured expression. Solid lines denote $p \leq 0.05$, while dashed lines denote $p \leq 0.1$.

target effects on neighboring tissues, particularly if longer knockdown periods were studied, and the study of gene expression without the addition of protein quantitation. Future work will analyze healing tendon matrix content to define how observed expression changes translate to functional matrix changes.

## Significance

This work elucidates the temporally dynamic role of collagen V on regulating healing tendon gene expression. These results inform which phases of tendon healing are most sensitive to collagen V presence.

## Acknowledgements

This work was supported by the NIH (R01AR065995, P30AR069619) and the NSF GRFP.

## References

**1. Malfait F, *et al.*** *Genet Med.* 2010.
**2. Leiphart RJ, *et al.*** *ORS Annual Meeting.* 2020.
**3. Taylor BL, *et al.*** *ORS Annual Meeting.* 2020.
**4. Beason DP, *et al.*** *J Biomech.* 2012.