Cochlin is required for maintenance of tendon structure

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Introduction: During adulthood, tendon homeostasis is maintained via ongoing turnover of extracellular matrix (ECM) components[1-3]. However, during aging, progressive changes in the tissue are observed, such as alterations in collagen fibril organization, density, and diameter[3-6], as well as decreases in cell density, and structural and functional mechanical properties.[3-6] Using a novel model of accelerated tendon ECM aging, via depletion of Scleraxis-lineage (Scx^{Lin}) cells, we demonstrated that Scx^{Lin} depletion impairs tendon ECM structure, composition, and material quality. Moreover, using integrated proteomic analysis, we demonstrated conserved downregulation of Cochlin (Coch), Aggrecan, Chondroadherin, and Keratocan following Scx^{Lin} cell depletion and natural aging, suggesting a potential requirement for these ECM components to maintain tendon homeostasis through the lifespan. Moreover, a substantial decline in *Coch*+ cells was observed in both aged and Scx^{Lin}-depleted tendons using single-cell RNA sequencing (scSeq) (Fig. 1). To begin to define the necessity of these ECM proteins in maintaining tendon structure-function, here we use Cochlin knockout ($Coch^{-/-}$) mice, and compare changes in tendon homeostasis and healing capacity to wildtype (WT) littermates. We hypothesize that $Coch^{-/-}$ will result in significant changes to ECM structure and function in both young and aged mice.

Methods: *Mice:* All studies were IACUC approved. $Coch^{-/-}$ mice (B6.129S1(Cg)-$Coch^{tm1.1Stw}$/YuanJ; KO) were purchased from Jackson labs, and WT littermates were used as controls. Six-month old male and female $Coch^{-/-}$ and WT mice were used for TEM analysis to determine structural changes in ECM organization. *Flexor tendon repair model:* 10-12 week old male and female, KO and WT mice underwent complete transection and repair of the FDL tendon in the right hind paw. *Functional assessment*: Metatarsophalangeal (MTP) flexion angle was measured by incremental loading of the FDL, and scar tissue formation was evaluated by calculation of gliding resistance. Reduced range of motion and increased gliding resistance indicates increased scar formation (n=8 per genotype). *Tensile testing:* Following functional assessment, tendons were dissected and underwent uniaxial displacement-controlled single load to failure (0.1% strain/second) tensile testing. Structural (stiffness, max load at failure) properties were determined from load/displacement curves and stress/strain curves, respectively. *Statistical analysis:* Changes in functional/biomechanical properties were analyzed by unpaired student's t-test with statistical significance set at $p \leq 0.05$

Results: *Cochlin is lost during natural aging concomitant with ECM disorganization:* Using scSeq analysis, we demonstrated a consistent loss of *Coch*+ cells during both natural aging, and in the context of accelerated ECM aging upon Scx^{Lin}-depletion in young animals (DTR) (Fig 1A). At 6 months of age, 60% of the total tenocyte population was $Coch^{+}$ (Fig 1B,C). In contrast, less than 10% of tenocytes were $Coch^{+}$ at 21 months of age (Fig 1B,C), indicating loss of this ECM molecule during natural aging, concomitant with disruptions in ECM organization[7]. *6-month old $Coch^{-/-}$ mice exhibit significant changes in ECM structure:* Compared to WT tendons, KO tendons had significantly higher collagen fibril density ($p = 0.01$) (Fig 2A-C), with smaller fibril diameter ($p < 0.0001$) (Fig 2D), whereas WT mice exhibited larger collagen fibril diameters with corresponding decreases in fibril density. Furthermore, $Coch^{-/-}$ alters collagen fibril distribution (WT median: 203, KO median: 161, $p < 0.0001$). Notably, the observed disruptions in structural properties are similar to those observed with natural aging and Scx^{Lin}-depletion.[6] *$Coch^{-/-}$ does not disrupt tendon healing:* Despite disruptions in ECM organization, $Coch^{-/-}$ does not functionally impair the healing process, as no changes in MTP flexion angle or gliding resistance were observed, relative to WT (Fig 3A-B). Moreover, no changes in max load at failure, or stiffness were observed between genotypes. Collectively, these data suggest that *Cochlin* is essential for homeostasis but not for healing.

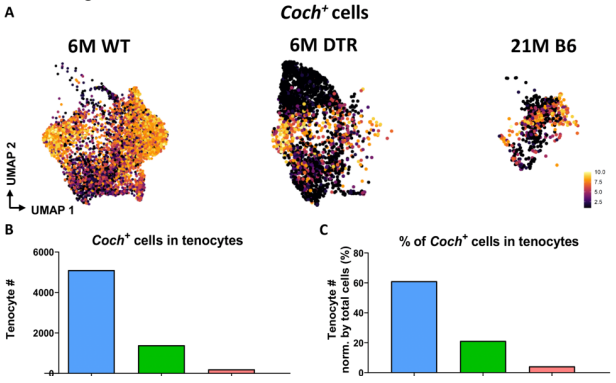

Figure 1. Significant decrease of Cochlin cells with scleraxis-lineage cell depletion and natural aging. **(A)** UMAP plots of $Coch^{+}$ cells in 6M WT, DTR, and 21M B6 groups. **(B)**Total tenocyte number of $Coch^{+}$ cells in each condition. **(C)** % of $Coch^{+}$ cells normalized to tenocyte number

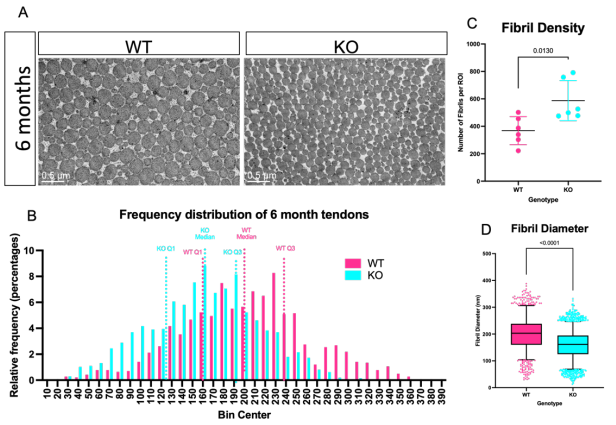

Figure 2. Assessment of collagen fibril diameter and distribution. **(A)** TEM axial images of the FDL tendon from KO and WT mice. **(B-D)** KO of *Cochlin* does not impair collagen fibril distribution in 3-month-old mice, **(E-F)** but results in altered fibril distribution at 6-months.

Discussion: The investigation of Cochlin's role in tendon aging provides further insight into the regulation of tendon structure during postnatal growth and natural aging. 6-month-old KO mice showed distinct structural properties relative to WT littermates. Specifically, KO mice displayed altered collagen fibril characteristics, which indicate disrupted ECM structure. Intriguingly, no substantial changes in healing capabilities or biomechanical properties were observed between KO and WT mice, suggesting that further ECM disruptions may be required to alter healing. Alternatively, these results may indicate that the healing deficits observed in aged mice may be due to intrinsic differences between young and aged cells. Further studies are being conducted to investigate the functional impact of Coch KO on tendon at more advanced ages.

Significance: This study demonstrates the requirement for Cochlin in maintaining tendon homeostasis during post-natal growth and natural aging, and thus identifies a novel intervention point to retain tendon structure-function through the lifespan.

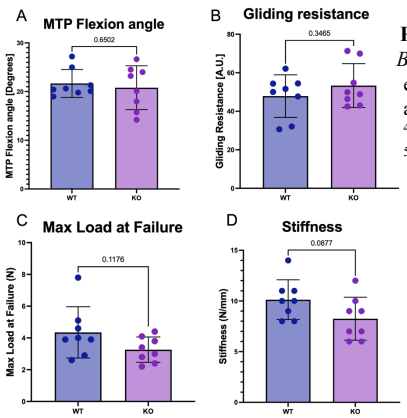

Figure 3. Assessment of functional mechanical outcomes during healing. Measurement of **(A)** Metatarsophalangeal (MTP) joint flexion angle, **(B)** Gliding resistance, **(C)** Maximum load at failure, and **(D)** Stiffness in 3-month-old KO and WT injured tendons 28 days post-op

References: [1]Samiric et al., *Eur. J. Biochem.* 2004; [2]Choi and Simpson et al., *eLife* 2020; [3]Heinemeier et al., *Sci. Transl. Med.* 2016; [4]Dunkman et al., *Matrix Biol* 2013; [5]Pardes et al., *J Biomech.* 2017; [6] Korcari et al., *eLife* 2023. [7]Best and Korcari et al., *eLife* 2021.