Piezo1 loss of function reduces Scx$^+$ cell migration and accelerates recovery of tendon stiffness after injury

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INTRODUCTION: Tendon is strong, stiff, yet flexible connective tissue which connects muscle to bone, enabling optimal skeletal movement. Tendon injuries heal slowly and often never recover full mechanical properties. Our lab recently discovered PIEZO1 to be a crucial shear stress sensor in tendons, positively correlating with mechanical properties during tendon homeostasis[1,2]. While we have established a role of PIEZO1 in tendon adaptation to exercise, we speculated that it may play a separate role in healing. For instance, studies in skin wound repair have shown that PIEZO1 is essential to cell migration[3]. On this basis, we hypothesized that tendon healing would be dysregulated by knocking out Piezo1 in Scx$^+$ cells, leading to altered healing.

METHODS: Animal experiments were approved by the Zurich Veterinary Office (license: ZH018/2022). We used *Piezo1$^{f/f}$* mice with a Scleraxis (Scx)-Cre driver to selectively knock out Piezo1 from Scx-containing tissues. We surgically induced a full-thickness, central third defect in the patellar tendon of the left leg of ScxCre;Piezo1$^{f/f}$ ("knockout") mice and floxed littermate controls (WT). The right leg underwent sham surgery for an intra-subject control. After 2 or 4 weeks of healing, patellar tendons were isolated and mechanically tested to failure. Tensile testing occurred in 1% RT PBS and followed the protocol: preload to 0.1 N, precondition 25x to 1% strain, 30 s rest, stretch to failure at 1.0% L_0/s. Data was analyzed with custom-altered MATLAB Stress-Strain code (R2019a). We isolated patellar tendon cells from Scx-GFP mice using collagenase and, at passage 3-5, transfected the cells with siRNA to knock down Piezo1 and used a non-selective siRNA as a scramble control. We performed a scratch assay by seeding the cells on a collagen-coated 12-well plate, serum starving with 1% serum for 24 hours, scratching the surface with a p200 pipette tip, and imaging with brightfield and confocal microscopes at 0, 4, 8, 12, 24, and 46 hours post-scratch. Images were analyzed with FIJI v.2.14[4]. All statistical analysis was performed in GraphPad Prism v.9.5.1.

RESULTS: After 2 weeks of healing, the stiffness of the injured patellar tendon of the ScxCre;Piezo1$^{f/f}$ mice had recovered to 89% of the sham stiffness on average (n=7 mice); those of the WT mice recovered to 72% of the sham stiffness on average (n=7). These data demonstrated a large Cohen's d effect size (0.98), indicating a practical significance between the two groups. The elastic modulus (E-mod) recovered to 67% and 40% of sham E-mod for ScxCre;Piezo1$^{f/f}$ and WT, respectively (p=0.02). After 4 weeks, the stiffness of the injured patellar tendon on average recovered to 89% and 82% of the ScxCre;Piezo1$^{f/f}$ sham and WT sham stiffnesses, respectively (n=3 WT & 4 ScxCre;Piezo1$^{f/f}$), indicating a moderate (0.5) Cohen's d effect size for practical significance. The E-mod of ScxCre;Piezo1$^{f/f}$ recovered to 76% and WT to 47% of sham E-mod (p=0.14). Results from the *in vitro* scratch assay (n=12 replicates from 4 mice) align with the mechanical data: after 4 and 12 hours, the knockdown cells exhibit significantly more migration into the defect (i.e., the width of the scratch has closed more) than the control cells (p=0.03 and 0.05, respectively). After 46 hours, the control cell migration has caught up to that of the knockdown cells. When examining the Scx$^+$ cells specifically at timepoints of faster migration, we found significantly fewer Scx$^+$ cells in the scratch of the knockdown cells after 12 h than in that of the control cells when normalized to the amount of Scx$^+$ cells in the scratch at 0 h (p<0.001).

DISCUSSION: Although the ScxCre;Piezo1$^{f/f}$ mice exhibited a functionally relevant increase in mechanical recovery of stiffness and strength after 2 weeks over WT, our 4-week data suggest that this does not result from fibrotic healing. Cross-sectional area (CSA) of the injured patellar tendons was 160% of sham for WT and 144% of sham for ScxCre;Piezo1$^{f/f}$ after 2 weeks of healing. This suggests that assembly of new collagen matrix may be more ordered in the ScxCre;Piezo1$^{f/f}$ tissue. After 4 weeks of healing, the CSA's reduced to 111% of sham for WT and 119% of sham for ScxCre;Piezo1$^{f/f}$. These data indicate that there is both healing and remodeling taking place in the tendons of both groups over time. Speculating that reduced migration of Scx$^+$ cells could potentially explain the improved healing in the ScxCre;Piezo1$^{f/f}$, we performed confocal imaging of the Scx$^+$ cell migration in a scratch assay. Indeed, there were fewer Scx$^+$ cells migrating into the scratch of the Piezo1 knockdown tenocytes than into that of the control tenocytes. This is consistent with previous work[5] describing accelerated tendon healing in a model with depletion of Scx$^+$ cells, giving credence to our current results. While the sample size for the 4-week *in vivo* data is currently small, our data suggest that a lack of the PIEZO1 channel in Scx$^+$ cells may slow the migration of Scx$^+$ cells to a tendon injury, which in turn could result in the observed acceleration in mechanical recovery.

SIGNIFICANCE: Our experiments suggest a novel role for PIEZO1 in tendon healing response that could potentially be exploited for therapeutic purposes.

REFERENCES: [1]https://doi.org/10.1038/s41551-021-00716-x [2]https://doi.org/10.1152/japplphysiol.00573.2022 [3]https://doi.org/10.7554/eLife.65415 [4]doi:10.1093/bioinformatics/btp184 [5]doi:10.7554/eLife.62203

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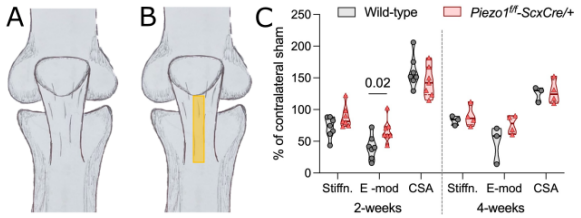

Fig.1. Surgically induced middle third patellar tendon injury in ScxCre;Piezo1$^{f/f}$ mouse recovers mechanical properties faster than WT. Illustration of sham tendon (A) and defect (B) made in tendon. (C) Violin plots showing the distribution of stiffness (Stiffn.), E-mod, and CSA after 2- and 4-weeks in both ScxCre;Piezo1$^{f/f}$ and WT mice. Each point represents the value of one mouse defect tendon normalized to the corresponding contralateral sham. Statistical analysis performed as unpaired two-sided t-test.

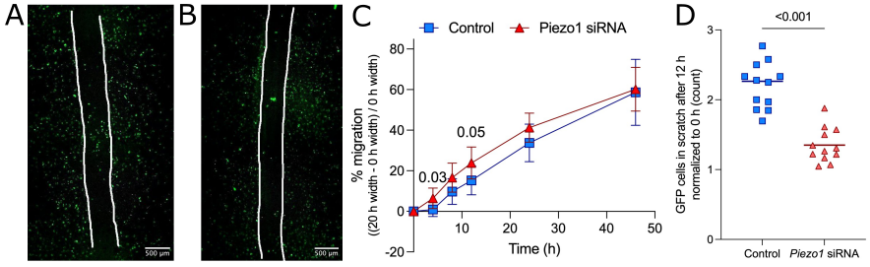

Fig. 2. Results from scratch assay. Representative confocal images of scramble control (A) and knockdown (B) 12 hours post-scratch. (C) Width of defect over time as percent migration. Data is avg ± SD of n=12 from 4 mice. Statistical analyses performed as unpaired two-sided t-tests with correction for multiple comparisons. (D) Number of Scx-GFP cells in the scratch after 12 h normalized to the number at 0 h. Each point is one of three replicates from n=4 mice. Statistical analyses performed as unpaired two-sided t-test.