The Role of the Mechanosensing Pathway Piezo 1 in Skeletal Muscle

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INTRODUCTION: Mechanotransducers convert the mechanical stimuli of the cellular microenvironment into biochemical signals that cells use to coordinate physiological processes. Piezo1, an ion channel, translates mechanical cues into electrical and chemical signals, influencing calcium influx and cell development. Our lab recently showed that muscle stem cells (MuSCs) have varying branch-like protrusions, which help them sense their environment and respond to injury. Our recent findings show that the Piezo1-regulated morphological characteristics of MuSCs protrusions can be directly linked to their activation status. Despite Piezo1's significance and diverse roles across various cell types, it's association with different aspects of muscle biology has only recently emerged. Here, we aim to explore the role of Piezo1 in myofiber physiology, using genetic mouse models to understand its impact on muscle function and regeneration.

METHODS: Muscle Injury: To observe difference in regeneration at various timepoints the Tibialis Anterior (TA) muscles of wildtype and Piezo1^{FiberKO} mice were injected with 10µg/ml of notexin at 3-mo-old. TAs were isolated at 2-, 3-, 5-, and 7- days post injury (DPI). Two-Photon Imaging: TA muscles were isolated and mounted on a custom glass chamber and submerged in 1X PBS. High-resolution serial optical sections were collected from the top of the muscle after the collagen deposition layer, using a Leica SP* Confocal/Multiphoton Microscope system equipped with a Chameleon Vision II Sapphire laser. A 910-nm laser was focused through a 20× HCX APO L Lens. Serial optical sections were collected in 1.5-µm steps for a total range of 100- to 200-µm depth, flattened, and normalized to the volume scanned. Force Generation *in vivo*: The hindlimb of 3-mo-old anesthetized mice immobilized and foot secured to the footplate of an Aurora 1300A. Plantarflexion was elicited by stainless steel EMG electrodes to deliver 0.1ms supramaximal pulses. The force frequency relationship (500msec trains 1-150Hz) followed by a fatigue protocol (200msec trains @80Hz delivered every 4 sec for 5 min) was performed. Statistical Analyses: Values presented as means ± SEM. *n* = number of mice/genotype. *N* = number of cells/mouse. Significance was determined using unpaired Student's *t* tests with Welch's correction or two-way analysis of variance (ANOVA) using GraphPad Prism 7 software.

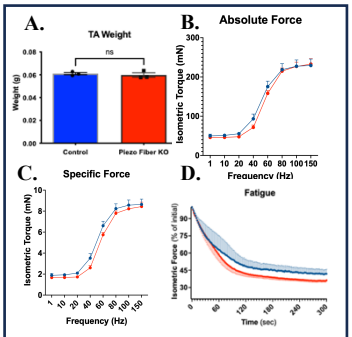

Fig1: A. TA weight, **B.** Functional analysis data measuring the Specific force out put **C.** Absolute force output **D.** and force output over time of the hindlimbs of untrained control (n= 4) and KOs (n=9).

RESULTS: To address the *in vivo* impact of Piezo1 in skeletal myofibers, mice with a myofiber-specific inducible Cre driver (human alpha skeletal actin-MerCreMer or αHSA-MerCreMer) with the Piezo1$^{Fl/Fl}$ mice to generate Piezo1 fiber-specific knockouts (Piezo1$^{\textbf{Fiber-KO}}$). To genetically label the Piezo1-deficient fibers, these mice also have the Ai9 floxed allele to track and image myofibers (tdTomato+). To induce Cre expression Controls (αHSA-MerCreMer;Ai9$^{Fl/Fl}$) and Piezo1$^{\textbf{Fiber-KO}}$ mice were subjected to 7 daily intraperitoneal (i.p.) injections of Tamoxifen (Tam), starting at 6 weeks of age **(Fig. 2A),** resulting in >98% Cre recombination efficiency and a significant downregulation of Piezo1 expression **(Fig. 2B).** To investigate if muscle strength is affected in Piezo1$^{Fiber-KO}$ mice in steady state conditions over time, we performed *in vivo* force measurements. In preliminary studies, while we found no impact either on muscle/body weights (**Fig. 1A**) or on specific and absolute force **(Fig. 1B,C)**, we found that 4-mo-old Piezo1$^{\textbf{Fiber-KO}}$ mice appear to fatigue faster and to a greater extent than control aged-matched mice (**Fig. 1D**), suggesting that there is not enough refilling of their Ca^{2+} stores to sustain repetitive contractions. These *in vivo* force generation data show that Piezo1 is essential to maintaining long lasting force production and that Piezo1 maintains muscle strength in adult muscles. To understand how mechanical cues in the fiber affect MuSC function and fate decisions, morphological properties of MuSCs (EGFP+), we crossed Pax7EGFP mice, previously generated in our lab [2], with the Piezo1$^{\textbf{Fiber-KO}}$ mice to generate double Pax7EGFP/Piezo1$^{\textbf{Fiber-KO}}$ mice, which labels MuSCs in green and fibers with lack of Piezo1 in red (Fig. 2A). As these mice permit non-invasive evaluation of MuSCs in their endogenous microenvironment, we have the unique advantage of undertaking longitudinal studies of Piezo1-dependent changes in the regenerative properties of MuSCs over time. We found few MuSCs infiltrating the site of injury 3 DPI in Piezo1$^{\textbf{Fiber-KO}}$ compared to controls, suggesting that there is some form of regenerative delay (Fig. 2B, C). While more analysis is required to further evaluate the mechanistic basis of these regeneration defects, our images suggest that Piezo1-dependent alterations in fiber mechanoproperties impact MuSC function. Altogether, these data suggest that Piezo1 controls the mechanoproperties of both MuSCs and skeletal muscle fibers which is essential for proper muscle regeneration.

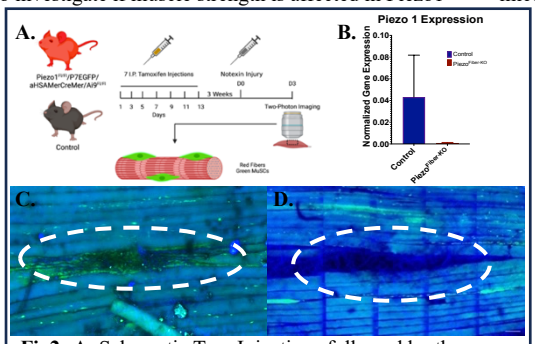

Fig2: A. Schematic Tam Injections followed by the injury of the TA and subsequent two-photon imaging. **B.** qPCR confirming Piezo 1 KO **C.** Two photon images of control and **C.** KO 3 DPI. The green image highlights Pax7EGFP positive MuSCs and the blue shows muscle fibers within the SGH.

DISCUSSION: Our data suggest that Piezo$^{Fiber-KO}$ adult mice have no deficits in the ability to produce force yet have a greater rate and extent of fatigue. Our current work is focused on treadmill running capacity, coupled with analysis of fiber type composition changes, to evaluate Piezo1's role in fatigue. presumably due to their inability to properly sense their microenvironment and alter their metabolic state appropriately. In addition, MuSCs are largely responsible for sensing the environment and responding to injury. As communication between MuSCs and their muscle environment changes with over time, my findings suggest that Piezo 1 specific alterations in the myofiber contributes to dysregulation of MuSCs upon injury. Next, I will explore the cellular events that influence these regeneration delays by conducting stainings and quantifications of MuSCs activation markers, such as Pax7 and MyoD. These experiments will further determine whether Piezo1-deletion in the myofibers affect the myogenic properties of MuSCs upon injury. Such mechanistic understanding of communication between stem cells and their microenvironment has pre-clinical implications for regenerative medicine. Knowing how mechanical signals deliver agents with respect to repair, paves the way for enhancing mechano-transduction information to delay or restore defects for the replacement of the muscular tissue.

SIGNIFICANCE: Overall, our work delineates the exact mechanism of action as well as the potential functional contribution of Piezo1 in mechanosensing regulation of both myofibers and MuSCs in adult muscles. Knowing how mechanical signals deliver agents with respect to repair, paves the way to delay or restore defects for the replacement of the muscular tissue upon chronic injuries, disease and/or aging.

REFERENCES: [1] Ma et al., Piezo1 regulates the regenerative capacity of skeletal muscles via orchestration of stem cell morphological states. *Sci. Adv.* (2022). [2] Tichy, E.D., *et al.* A robust Pax7EGFP mouse that enables the visualization of dynamic behaviors of muscle stem cells. *Skeletal Muscle* (2018).

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