HHS Public Access

Author manuscript

Muscle Nerve. Author manuscript; available in PMC 2022 December 01.

Published in final edited form as:

Muscle Nerve. 2021 December ; 64(6): 765–769. doi:10.1002/mus.27412.

Krüppel-like factor 10 regulates the contractile properties of skeletal muscle fibers in mice

Malek Kammoun, PhD[1], **Philippe Pouletaut, PhD**[1], **Sandrine Morandat, PhD**[1], **Malayannan Subramaniam, PhD**[2], **John R. Hawse, PhD**[2], **Sabine F. Bensamoun, PhD**[1]

[1]Biomechanics and Bioengineering, Centre de recherche Royallieu, Université de technologie de Compiègne, Compiègne Cedex, France

[2]Department of Biochemistry and Molecular Biology, Mayo Clinic, Rochester, Minnesota, USA

Abstract

Introduction/Aims: *Klf10* is a member of the Krüppel-like family of transcription factors, which is implicated in mediating muscle structure (fiber size, organization of the sarcomere), muscle metabolic activity (respiratory chain), and passive force. The aim of this study was to further characterize the roles of Klf10 in the contractile properties of skeletal muscle fibers.

Methods: Fifty-two single fibers were extracted from female wild-type (WT) and *Klf10* knockout (KO) oxidative (soleus) and glycolytic (extensor digitorum longus [EDL]) skinned muscles. Each fiber was immersed successively in relaxing (R), washing (W), and activating (A) solutions. Calcium was included in the activating solution to induce a maximum contraction of the fiber. The maximum force (F_{max}) was measured and normalized to the cross-sectional area to obtain the maximum stress ($Stress_{max}$). After a steady state in contraction was reached, a quick stretch-release was performed; the force at the maximum stretch ($F_{stretch}$) was measured and the stiffness was assessed.

Results: Deletion of the *Klf10* gene induced changes in the contractile parameters (F_{max}, $Stress_{max}$, Stiffness), which were lower and higher for soleus and EDL fibers compared with littermates, respectively. These measurements also revealed changes in the proportion and resistance of attached cross-bridges.

Discussion: Klf10 plays a major role in the homeostasis of the contractile behavior of skeletal muscle fibers in a muscle fiber type–specific manner. These findings further implicate important roles for Klf10 in skeletal muscle function and shed new light on understanding the molecular processes regulating the contractility of skeletal muscle fibers.

Correspondence Sabine F. Bensamoun, Laboratoire Biomécanique et Bioingénierie, UMR CNRS 7338, Centre de recherche Royallieu, Université de Technologie de Compiègne, CS 60319, 60203 Compiègne Cedex, France. sabine.bensamoun@utc.fr.

CONFLICT OF INTEREST

The authors declare no potential conflicts of interest.

ETHICAL PUBLICATION STATEMENT

We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

Keywords

contractile properties; Klf10; skeletal muscle; stiffness active test; TEM

1 | INTRODUCTION

Krüppel-like transcription factor 10 (*Klf10*), also known as transforming growth factor-β (TGF-β)–inducible early gene-1 (*TIEG1*) is a member of the Krüppel-like family of transcription factors that regulates gene expression in multiple cell and tissue types.[1,2] Klf10 is implicated in multiple biological processes and diseases (i.e, osteoporosis,[3] cancer,[4,5] and hypertrophic cardiomyopathy).[6] Recently, *Klf10* has been implicated in Duchenne muscular dystrophy, suggesting a role for Klf10 in the regulation of fibrosis.[7]

Loss of *Klf10* expression results in multiple muscle phenotypes,[8,9] such as hyperplasia, hypertrophy, a decrease in succinate dehydrogenase, cytochrome c oxidase and menadione activities, ultrastructural muscle disorganization (smaller sarcomeres and absence of I bands), and changes in mitochondrial shape and respiration. All these phenotypes were only observed in female animals, with no significant defects or differences detected between male *Klf10* knockout (KO) and wild-type (WT) mice. Similar sex-specific differences have also been observed in the skeleton on *Klf10* KO mice where only female animals exhibited an osteopenic phenotype.[10] The basis for sex-specific differences in bone are attributed to defects in estrogen signaling in *Klf10* KO mice.[3]

Skeletal muscle must be characterized in passive[11] and active[12] conditions to achieve a comprehensive understanding of this tissue. Previous studies have only implemented passive stretch[13] and compressive[9] tests to reveal changes in elasticity of *Klf10* KO muscle fibers. Herein we assess the role of Klf10 in the contractile properties of muscle fibers from slow-twitch (soleus) and fast-twitch (extensor digitorum longus [EDL]) muscles.

2 | METHODS

2.1 | Animals

Klf10 KO mice were generated using a neomycin targeting vector to delete approximately 5.5 kb of the *Klf10* locus, including the entirety of exons 1 and 2.[14] *Klf10* KO animals utilized in this study were on a congenic C57BL/6 background. WT and *Klf10* KO animals were derived from heterozygous breeding. Female animals, 3 months of age, were utilized in these experiments. The protocol was approved by the French Ministry of Higher Education, Research and Innovation (DUO-4776) and the ethics animal care and use committee of the Picardie region.

2.2 | Transmission electron microscopy

Eight soleus and EDL muscles were harvested from 4 WT and 4 *Klf10* KO mice using a published protocol.[15] Transverse ultrathin (90 nm) sections were cut using a Leica UC7 ultramicrotome and were placed on copper grids. Five micrographs of each specimen were randomly captured across the muscle using a transmission electron microscope (TEM)

(JEOL 1400Plus; JEOL, Ltd, Tokyo, Japan) operating at 80 kV with a magnification of 42 000×. ImageJ 1.46/Java 8 software (National Institutes of Health, Bethesda, Maryland) was used with the plugin "Analyze Particles" to determine the myosin (myo) cross-sectional area (myoCSA).[16]

2.3 | Skinned muscle preparation

For this analysis, a second group of mice was utilized for TEM experiments. Soleus muscles were dissected from the right hindlimbs of 8 WT mice and 8 *Klf10* KO mice, and then permeabilized at 4°C using a relaxing solution with different percentages of glycerol (12.5%, 25%, and 50%) added.[13] Fiber bundles were stored in 50% glycerol relaxing solution at -20°C for 2 weeks.[17]

2.4 | Active mechanical tests

Single muscle fibers were isolated from each *Klf10* KO (N_{soleus} = 11, N_{EDL} = 16) and WT (N_{soleus} = 11, N_{EDL} = 14) skinned muscles. First, the fibers were placed in a small bath filled with the relaxing solution at 20°C. The extremities were connected to a force transducer (5 mN) and to a motor (Model 1400A; Aurora Scientific, Aurora, Ontario, Canada). The fiber length, diameter (D), and the distance between the successive sarcomeres were measured using a camera (UI-1220LE). The fibers were placed with an initial[13] sarcomere length of 2.5 μm and immersed in a bath containing a washing solution (W) free of calcium for 8 seconds. To activate the actin-myosin cross-bridges, fibers were then immersed in another bath containing an active solution of calcium (pCa = 4.5), resulting in muscle fiber contraction and the measurement of the maximum force (F_{max}).[18,19] This value was measured as an average of data during 1 second when the force had reached a steady-state plateau. The maximum stress ($Stress_{max}$) was measured by dividing the maximum force F_{max} by the cross-sectional area (CSA) of the fiber. After 31 seconds of immersion in the calcium solution, a quick stretch-release cycle was performed with an amplitude ($\varepsilon_{stretch}$) of 0.2% of the fiber length during 1 second, and an edge at a speed of 1 fiber length per second.[20] The force ($F_{stretch}$) at the peak of the stretch was determined and the stiffness was calculated as follows:

$$\text{Stiffness} = \frac{F_{stretch} - F_{max}}{\varepsilon_{stretch} \cdot CSA}$$

2.5 | Statistical analysis

SYSTAT version 11 (SYSTAT Software, Inc, San Jose, California) software was used and two-sample Mann-Whitney *U* tests were used to compare all parameters (myoCSA, D, stiffness, stress) as a function of genotype. $P < .05$ was considered significant.

3 | RESULTS

The muscle fiber active force (Figure 1) revealed lower and higher forces for *Klf10* KO soleus and *Klf10* KO EDL muscle, compared with WT littermates, respectively. TEM acquisitions revealed a reduction in the mean number of myosin filaments per region of interest (Figure 2A,B) within the *Klf10* KO muscles (N_{Soleus} = 90, N_{EDL} = 103) compared

with the WT muscles (N_{Soleus} = 117, N_{EDL} = 112). TEM analysis of the myosin area (Figure 2C,D) also revealed a significant increase ($P < .001$) in cross-sectional area (myoCSA) in *Klf10* KO soleus and EDL compared with WT littermates. No significant differences in mean ± SEM muscle fiber diameter (D) for the EDL (D_{WT} = 44 ± 6 μm, D_{Klf10_KO} = 44 ± 1 μm) or soleus (D_{WT} = 46 ± 3 μm, D_{Klf10_KO} = 39 ± 1 μm ; $P = .065$) a s afunctio no genotype were detected.

The maximum normalized active force ($Stress_{max}$) was significantly ($P = .001$) lower for the *Klf10* KO soleus fibers compared with the WT littermates (Figure 3A). Opposing results were observed for the EDL with *Klf10* KO soleus fibers, showing significantly higher ($P = .008$) values compared with the WT littermates (Figure 3A). For the WT genotype, no significant difference was noted for maximum stress between soleus and EDL fibers.

Results of stiffness measurements were similar to those for maximum stress, with significantly lower ($P = .008$) and higher ($P = .004$) values for the *Klf10* KO soleus fibers and *Klf10* KO EDL fibers, respectively, compared with the WT littermates (Figure 3B). No significant differences in stiffness were observed between WT soleus and WT EDL fibers.

4 | DISCUSSION

In this study we have elucidated the impact of *Klf10* expression on parameters related to muscle fiber contraction for oxidative and glycolytic muscles. In previous work we demonstrated that sarcomeric length is shorter in skeletal muscles of *Klf10* KO mice.[13] In spite of this phenotype, we conclude that the *Klf10* KO sarcomere is functioning normally based on the data reported here.

Thus, the decrease in the maximum stress for *Klf10* KO oxidative fibers compared with WT fibers may be indicative of a smaller proportion of attached cross-bridges or a weaker adhesion between the myosin head and the actin molecule during the isometric contraction.[21] This possibility is supported by the TEM results showing a decreased density of myosin in soleus *Klf10* KO fibers.

The weaker stiffness observed for the soleus *Klf10* KO compared with WT fibers is consistent with reduced contractile properties. Indeed, the stretch applied after the active steady state reflects the resistance of the attached cross-bridges,[12] which are weaker in soleus *Klf10* KO and associated with the reduced density of myosin.

In a previous study[8] we demonstrated that the *Klf10* KO soleus and EDL muscles exhibited hypertrophy, with an increase in the wet weight of *Klf10* KO soleus (7.8 ± 0.6 mg) compared with WT soleus (6.3 ± 0.7 mg), and *Klf10* KO EDL (8.3 ± 0.4 mg) compared with WT EDL (6.8 ± 0.4 mg). This may be one causative factor underlying the force measurement differences reported herein. Another potential explanation would be differences in myosin content or myosin isoforms, which need to be investigated in future studies. Although we have analyzed myofiber structures using various methods, it is difficult to compare the diameter of intrinsic structure (myosin) and the diameter of the entire muscle fiber. We previously demonstrated that the *Klf10* KO sarcomeres are shorter than WT sarcomeres with a shorter H band where the myosin is located. Therefore, it could be

assumed that the myosin may be compressed and this phenomenon may induce an increase of the CSA.

The increase in the contractile properties for *Klf10* KO EDL fibers compared with littermate controls could be due to the homeostasis of the muscle in an effort to maintain the contractile properties.

In conclusion, we have demonstrated that Klf10 plays a crucial role in the homeostasis of the contractile behavior of skeletal muscle fibers and functions in a muscle type–specific manner.

In future studies, eccentric contraction force could be measured with repetitive stimulations to provide evidence for a greater role of Klf10 in muscle physiological forces. It is of interest to determine whether differences in calcium sensing, extracellular matrix composition, and excitation-contraction coupling exist in *Klf10* KO muscles and whether such differences contribute to deficits in the force measurements described in this study.

ACKNOWLEDGMENTS

The authors thank the Mayo Microscopy and Cell Analysis Core for experimental and technical support.

Funding information

Idex Sorbonne University, Investments for the Future programs, Project SU-19–3-EMRG-12; National Institutes of Health, Grant No. R01 DE14036 (to J.R.H. and M.S.)

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Abbreviations:

A	activating solution
CSA	cross-sectional area
D	diameter
EDL	extensor digitorum longus
F_{max}	maximum force
$F_{stretch}$	stretch force
Klf	Krüppel-like factor
KO	knockout
Myo	myosin
pCa	-log(10) of the calcium concentration

R	relaxing solution
$Stress_{max}$	maximum stress
TEM	transmission electron microscopy
TIEG1	transforming max growth factor-β–inducible early gene-1
W	washing solution
WT	wild-type

REFERENCES

1. Subramaniam M, Harris SA, Oursler MJ, Rasmussen K, Riggs BL, Spelsberg TC. Identification of a novel TGF-beta-regulated gene encoding a putative zinc finger protein in human osteoblasts. Nucl Acids Res. 1995;23:4907–4912. [PubMed: 8532536]
2. Subramaniam M, Hawse JR, Rajamannan NM, Ingle JN, Spelsberg TC. Functional role of KLF10 in multiple disease processes. Biofactors. 2010;36:8–18. [PubMed: 20087894]
3. Hawse JR, Pitel KS, Cicek M, et al. TGFbeta inducible early gene-1 plays an important role in mediating estrogen signaling in the skeleton. J Bone Miner Res. 2014;29:1206–1216. [PubMed: 24190163]
4. Subramaniam M, Hefferan TE, Tau K, et al. Tissue, cell type, and breast cancer stage-specific expression of a TGF-beta inducible early transcription factor gene. J Cell Biochem. 1998;68:226–236. [PubMed: 9443078]
5. Nilsson E, Benrick A, Kokosar M, et al. Transcriptional and epigenetic changes influencing skeletal muscle metabolism in women with polycystic ovary syndrome. J Clin Endocrinol Metab. 2018;103:4465–4477. [PubMed: 30113663]
6. Bos JM, Subramaniam M, Hawse JR, et al. TGFbeta-inducible early gene-1 (TIEG1) mutations in hypertrophic cardiomyopathy. J Cell Biochem. 2012;113:1896–1903. [PubMed: 22234868]
7. DiMario JX. KLF10 gene expression modulates fibrosis in dystrophic skeletal muscle. Am J Pathol. 2018;188:1263–1275. [PubMed: 29458012]
8. Kammoun M, Meme S, Meme W, et al. Impact of TIEG1 on the structural properties of fast and slow twitch skeletal muscle. Muscle Nerve. 2017;55:410–416. [PubMed: 27421714]
9. Kammoun M, Ternifi R, Dupres V, et al. Development of a novel multiphysical approach for the characterization of mechanical properties of musculotendinous tissues. Sci Rep. 2019;9:7733. [PubMed: 31118478]
10. Hawse JR, Iwaniec UT, Bensamoun SF, et al. TIEG-Null mice display a severe osteopenic gender-specific phenotype characterized by normal cancellous bone microarchitecture. Bone. 2008;42:1025–1031. [PubMed: 18396127]
11. Bensamoun SF, Stevens L, Fleury MJ, Bellon G, Goubel F, Ho Ba Tho MC. Macroscopic-microscopic characterization of the passive mechanical properties in rat soleus muscle. J Biomech. 2006;39:568–578. [PubMed: 16389097]
12. Joumaa V, Fitzowich A, Herzog W. Energy cost of isometric force production after active shortening in skinned muscle fibres. J Exp Biol. 2017;220:1509–1515. [PubMed: 28232399]
13. Kammoun M, Pouletaut P, Canon F, et al. Impact of TIEG1 deletion on the passive mechanical properties of fast and slow twitch skeletal muscles in female mice. PLoS One. 2016;11:e0164566. [PubMed: 27736981]
14. Subramaniam M, Gorny G, Johnsen SA, et al. TIEG1 null mouse-derived osteoblasts are defective in mineralization and in support of osteoclast differentiation in vitro. Mol Cell Biol. 2005;25:1191–1199. [PubMed: 15657444]
15. Kammoun M, Piquereau J, Nadal-Desbarats L, et al. Novel role of Tieg1 in muscle metabolism and mitochondrial oxidative capacities. Acta Physiol (Oxf). 2020;228:e13394. [PubMed: 31560161]

16. O'Brien J, Hayder H, Peng C. Automated quantification and analysis of cell counting procedures using ImageJ plugins. J Vis Exp. 2016;17:54719.

17. Kammoun M, Pouletaut P, Nguyen TN, Subramaniam M, Hawse JR, Bensamoun SF. The effect of freezing time on muscle fiber mechanical properties. Annu Int Conf IEEE Eng Med Biol Soc. 2019;5356–5359. [PubMed: 31947066]

18. Joumaa V, Herzog W. Calcium sensitivity of residual force enhancement in rabbit skinned fibers. Am J Physiol Cell Physiol. 2014;307: C395–C401. [PubMed: 24965591]

19. Herzog W, Schachar R, Leonard TR. Characterization of the passive component of force enhancement following active stretching of skeletal muscle. J Exp Biol. 2003;206:3635–3643. [PubMed: 12966055]

20. Joumaa V, Macintosh BR, Herzog W. New insights into force depression in skeletal muscle. J Exp Biol. 2012;215:2135–2140. [PubMed: 22623202]

21. Huxley AF. Muscle structure and theories of contraction. Prog Biophys Biophys Chem. 1957;7:255–318. [PubMed: 13485191]

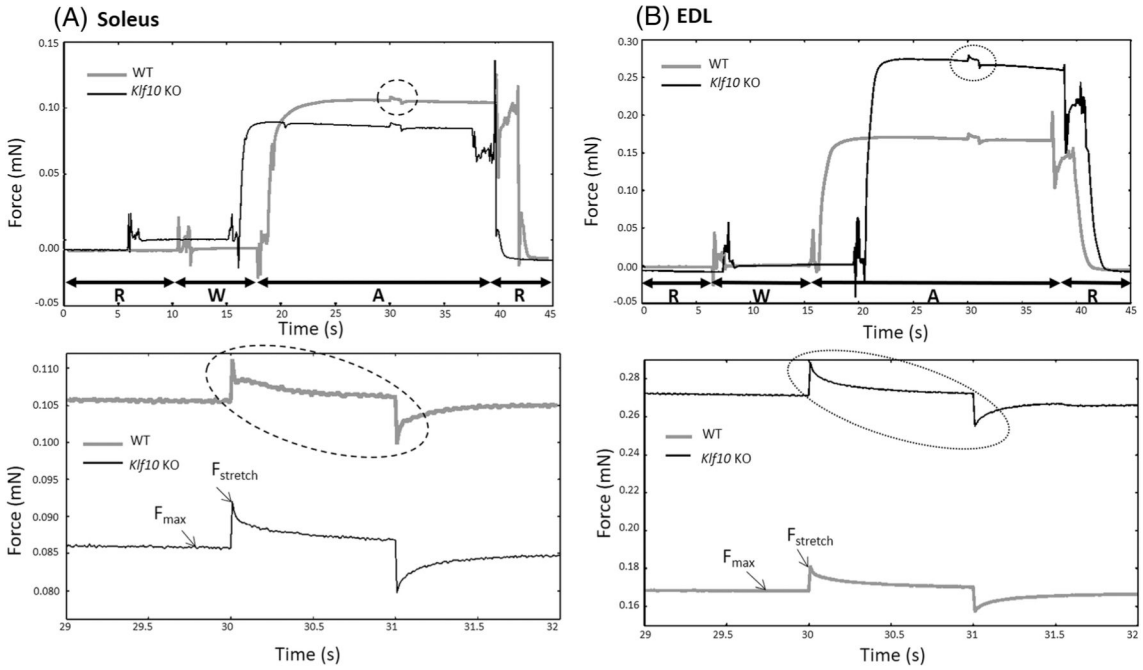

FIGURE 1.

Force behavior of the WT and *Klf10* KO muscle fiber from soleus (A) and EDL (B) in the relaxing (R), washing (W), and activating (A) solutions. The dotted circle indicates the stretch-release test, which is enlarged in the figure below for soleus and EDL, respectively. The stretch-release test was performed at 30 seconds for both WT and *Klf10* KO fiber. Abbreviations: EDL, extensor digitorum longus; F_{max}, maximum force; $F_{stretch}$, stretch force; WT, wild-type

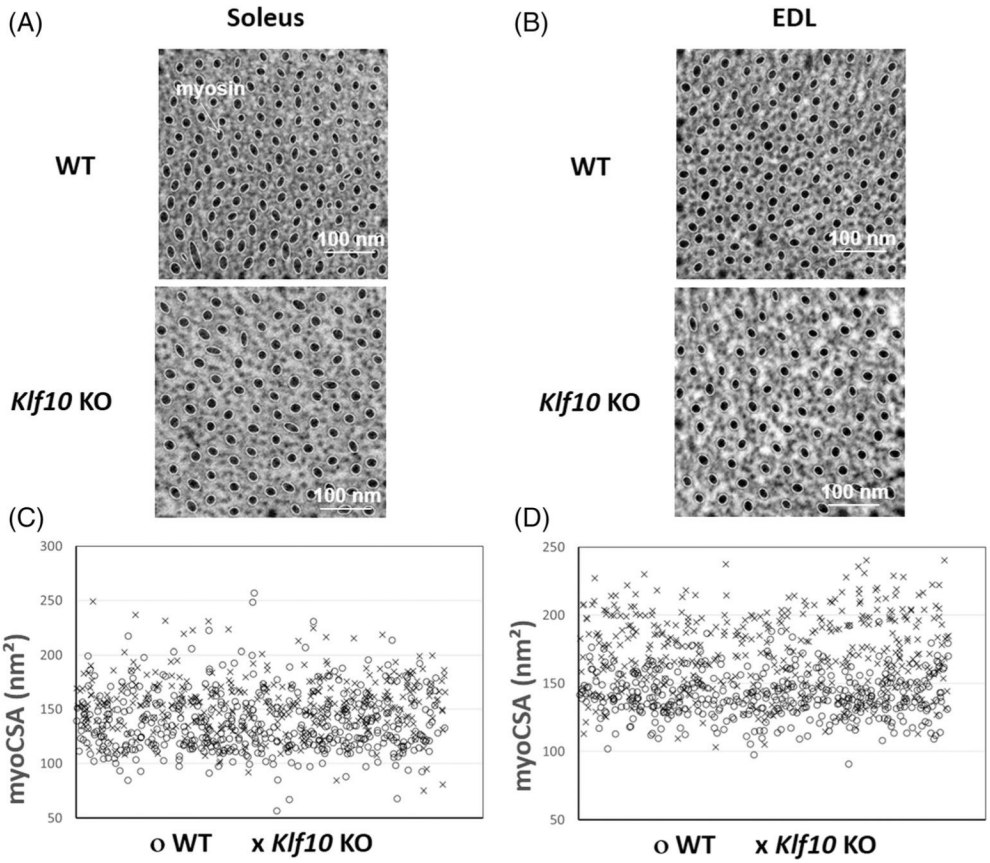

FIGURE 2.

Region of interest (1500 × 1500 pixels, pixel size = 0.22 nm) from transmission electron microscopy of transversal section from soleus (A) and EDL (B) muscles for both genotypes (WT, *Klf10* KO). The scatterplots depict that the individual data points between WT and *Klf10* KO mice segregate, more obviously for EDL than soleus. Cross-sectional area of myosin (myoCSA) was measured in the region of interest in four soleus (C) and four EDL (D) muscles for both genotypes (WT, *Klf10* KO). Myosin area is significantly different between WT and *Klf10* KO fibers for both soleus and EDL muscles *(P* < .001): in soleus muscle ($myoCSA_{WT}$ = 136.8 ± 1.4 nm^2, n = 466; $myoCSA_{Klf10\_KO}$ = 155.3 ± 1.3 nm^2, n = 360) and in EDL muscles ($myoCSA_{WT}$ = 141.4 ± 0.8 nm^2, n = 448; $myoCSA_{Klf10\_KO}$ = 174.0 ± 1.4 nm^2, n = 412). All data is presented as mean ± standard error of mean and "N" is the number of myosin filaments used in the analysis. Abbreviations: KO, knockout; myoCSA, myosin cross-sectional area

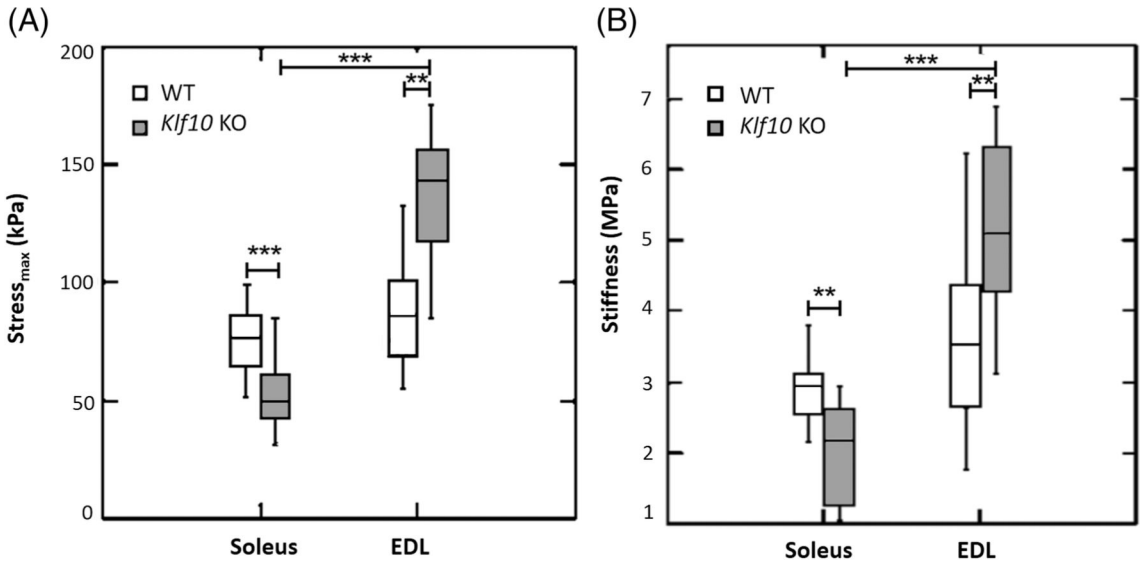

FIGURE 3.

Boxplots of $Stress_{max}$ values (A) and Stiffness (B) for soleus and extensor digitorum longus muscles as a function of genotype. $^{**}P < .01$, $^{***}P < .001$