



HAL
open science

EGR1 Regulates Transcription Downstream of Mechanical Signals during Tendon Formation and Healing

Ludovic Gaut, Nicolas Robert, Anthony Delalande, Marie-Ange Bonnin, Chantal Pichon, Delphine Duprez

► **To cite this version:**

Ludovic Gaut, Nicolas Robert, Anthony Delalande, Marie-Ange Bonnin, Chantal Pichon, et al.. EGR1 Regulates Transcription Downstream of Mechanical Signals during Tendon Formation and Healing. PLoS ONE, 2016, 11 (11), pp.e0166237. <10.1371/journal.pone.0166237.t001>. <hal-01394467>

HAL Id: hal-01394467

<https://hal.sorbonne-universite.fr/hal-01394467v1>

Submitted on 9 Nov 2016

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons CC BY 4.0 - Attribution - International License

RESEARCH ARTICLE

EGR1 Regulates Transcription Downstream of Mechanical Signals during Tendon Formation and Healing

Ludovic Gaut¹✉, Nicolas Robert¹✉, Antony Delalande², Marie-Ange Bonnin¹, Chantal Pichon², Delphine Duprez^{1*}

1 Sorbonne Universités, UPMC Univ Paris 06, CNRS UMR7622, Inserm U1156, IBPS-Developmental Biology Laboratory, F-75005 Paris, France, **2** CNRS UPR4301-CBM, 45071 rue Charles Sadron, Orléans CEDEX2, France

✉ These authors contributed equally to this work.

* delphine.duprez@upmc.fr



OPEN ACCESS

Citation: Gaut L, Robert N, Delalande A, Bonnin M-A, Pichon C, Duprez D (2016) EGR1 Regulates Transcription Downstream of Mechanical Signals during Tendon Formation and Healing. PLoS ONE 11(11): e0166237. doi:10.1371/journal.pone.0166237

Editor: Chunfeng Zhao, Mayo Clinic Minnesota, UNITED STATES

Received: May 3, 2016

Accepted: October 25, 2016

Published: November 7, 2016

Copyright: © 2016 Gaut et al. This is an open access article distributed under the terms of the [Creative Commons Attribution License](https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Data Availability Statement: All relevant data are within the paper.

Funding: This work was supported by the Fondation pour la Recherche Médicale (FRM DEQ20140329500). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing Interests: The authors have declared that no competing interests exist.

Abstract

Background

Tendon is a mechanical tissue that transmits forces generated by muscle to bone in order to allow body motion. The molecular pathways that sense mechanical forces during tendon formation, homeostasis and repair are not known. EGR1 is a mechanosensitive transcription factor involved in tendon formation, homeostasis and repair. We hypothesized that EGR1 senses mechanical signals to promote tendon gene expression.

Methodology/Principal findings

Using *in vitro* and *in vivo* models, we show that the expression of *Egr1* and tendon genes is downregulated in 3D-engineered tendons made of mesenchymal stem cells when tension is released as well as in tendon homeostasis and healing when mechanical signals are reduced. We further demonstrate that EGR1 overexpression prevents tendon gene downregulation in 3D-engineered tendons when tension is released. Lastly, ultrasound and microbubbles mediated EGR1 overexpression prevents the downregulation of tendon gene expression during tendon healing in reduced load conditions.

Conclusion/Significance

These results show that *Egr1* expression is sensitive to mechanical signals in tendon cells. Moreover, EGR1 overexpression prevents the downregulation of tendon gene expression in the absence of mechanical signals in 3D-engineered tendons and tendon healing. These results show that EGR1 induces a transcriptional response downstream of mechanical signals in tendon cells and open new avenues to use EGR1 to promote tendon healing in reduced load conditions.

Introduction

Tendon is a crucial component of the musculo-skeletal system, which transmits forces generated by skeletal muscle to bone to allow body motion. Mechanical signals are known to be involved in tendon development, homeostasis and repair [1–4]. However, the mechanotransduction pathways involved in tendon cell differentiation in normal or pathological situations are not fully understood.

The functional component of tendon is type I collagen, which displays a tendon-specific spatial organization that confers tendon mechanical properties [5]. Type I collagen is composed of a triple helix of collagen chains ($\alpha 1(2)$, $\alpha 2(1)$) coded by two different genes, *Col1a1* and *Col1a2*. Unfortunately, neither of the *Col1a* genes is specific to tendons. They are also expressed in many other connective tissues, making it difficult to assess tenogenesis using *Col1a* gene expression. The bHLH transcription factor Scleraxis (*Scx*) is specifically expressed in embryonic, fetal and postnatal tendons [6–8]. From the 4th postnatal month, *Scx* expression is restricted to the epitenon, but is reactivated in the tendon core by treadmill exercise [8]. Moreover, *Scx* expression is upregulated in tendons upon injury in animal models [9, 10]. The type II transmembrane glycoprotein tenomodulin (*Tnmd*) is also considered as a relevant marker of tendon cell differentiation in mouse, rat and human [11–13]. During development, *Scx* is required and sufficient for *Tnmd* expression in limb tendons [14, 15]. *Tnmd*^{-/-} mice show defective postnatal tendons [16] and reduced self-renewal of tendon stem cells in adult [17].

In addition to *Scx*, the Zinc finger transcription factor, Early Growth Response-1 (EGR1) has been identified as being involved in pre- and postnatal tendon formation [10, 18]. EGR1 is not specific to tendons, since it is expressed in many other tissues; however, EGR1 has the remarkable ability to be sufficient for promoting tendon gene expression, including *Scx* and *Col1a1*, during development [18], in mouse mesenchymal stem cells [10] and in rabbit tendon stem cells [19]. The DNA-binding protein EGR1 is known to be a mechanosensitive gene in various cellular systems. Mechanical stretch increases *Egr1* transcription in endothelial cells [20], vascular smooth muscle cells [21] or skeletal muscle cells [22]. Dynamic compression also activates *Egr1* transcription in 3-dimensional (3D) cultures of mouse primary chondrocytes [23]. EGR1 has been shown to be a mediator of mechanical input that contributes to vascular remodeling of vein grafts [24].

Since EGR1 is known to be a mechanosensitive gene and involved in tendon development, homeostasis and repair, we hypothesized that EGR1 transduces mechanical signals into transcriptional regulation to promote tendon cell differentiation during tendon formation, homeostasis and repair. We used *in vitro* 3-dimensional culture system and *in vivo* models to test this hypothesis.

Materials and Methods

Animals

The *Egr1*^{LacZ/+} mice bred in a C57BL/6j background carry an insertion of a LacZ-neo cassette that inactivates the *Egr1* gene [25] and allow the visualization of *Egr1* expression with LacZ activity in a heterozygous context [10]. C57BLj wild-type mice were purchased from Janvier (France). All animal experiments were conducted in accordance with the guidelines of the french national ethic comity for animal experimentation N°05. The animal experiments shown in this study have been approved by the french national ethic committee for animal experimentation N°05 and are registered under the number 01789.02.

Engineered tendons made of mesenchymal stem cells

Mouse mesenchymal stem cells, C3H10T1/2 [26] were used to establish fibrin-based 3D constructs. Tendon-like structures from mouse C3H10T1/2 cells or C3H10T1/2-EGR1 cells [10]

were performed as previously described [27]. For each construct, 400 μ l of cell suspension (7.5×10^5 cells) were mixed with 20 mg/ml fibrinogen (Sigma, St Louis, MO, USA) and 200 U/ml thrombin (Sigma, St Louis, MO, USA). The fibrin gels containing cells were seeded in already prepared SYLGARD-covered wells (Dow Chemical, Midland, MI, USA), in which two 8 mm-sutures (Ethicon, Somerville, NJ, USA) were pinned 10 mm apart. Culture medium containing 200 μ M of L-ascorbic acid 2-phosphate was added to the wells and gels were scored every day for a proper contraction into a linear construct. After 7 days, the C3H10T1/2 and C3H10T1/2-EGR1 cells formed continuous tendon-like constructs between the 2 anchors. Tension release was obtained by cutting one end of the construct as previously described in [28]. Gene expression was analyzed 24 hours after tension release. Each tendon construct made of C3H10T1/2 or C3H10T1/2-EGR1 cells under tension or after tension release was considered as a biological sample. We analyzed 12 constructs made of C3H10T1/2 cells, 7 constructs made of C3H10T1/2-EGR1 cells, 7 de-tensioned-constructs made of C3H10T1/2 cells and 5 de-tensioned-constructs made of C3H10T1/2-EGR1 cells. The mRNA levels of each construct were analyzed by q-RT-PCR.

Botox injection in muscles and Achilles tendon injury in adult mice

For the analysis of mechanical signal involvement in tendon homeostasis, 6 UI/kg of Botox preparation (Allergan) [29] and physiological saline solution were injected into the Gastrocnemius muscles of right and left legs, respectively, of *Egr1*^{LacZ/+} (N = 6) and wild-type (N = 12) adult mice (two- to four-month-old). Botox injection reduces muscle contraction and movements and consequently mechanical signals to tendons [30]. Experimental animals did not display any obvious difficulty in moving, but limped on the Botox-injected legs. The Botox and physiological saline manipulated *Egr1*^{LacZ/+} animals were kept for one week (N = 6) and analyzed for LacZ staining. Tendons were fixed for 20 minutes in 4% paraformaldehyde and incubated in X-gal staining solution for 4 hours at 37°C. The Botox and physiological saline manipulated wild-type animals were kept for one (N = 8) or two (N = 11) weeks after injection (3 independent experiments) and then analyzed for gene expression.

For the analysis of mechanical signal involvement in tendon healing, Achilles tendon injury was performed on left legs of wild-type animals, as previously described in [10]. Briefly, adult mice were anesthetized by isoflurane inhalation. A 0.5-mm longitudinal full-thickness lesion parallel to the axis of the tendon was performed using a scalpel. In this type of injury, the tendon tension was maintained. After injury, the skin was sutured using 2–0 Mersilk, and the animals were kept for two weeks. The Botox or physiological saline was injected in the Gastrocnemius muscles of the manipulated left legs. The mouse group, which had undergone Achilles tendon injury and Botox injection, constitute the experimental group (N = 11) that was compared to the control group with Achilles tendon injury and physiological saline injection in muscles (N = 10). Experimental animals did not display any obvious difficulty in moving. However, the animals in the group that had Achilles tendon injury and Botox injection were limping compared to those in the group with Achilles tendon injury and physiological saline injection. Achilles tendons, in the above experimental conditions, were processed for RT-q-PCR analyzes.

Ultrasound and microbubbles-mediated EGR1 gene delivery in Achilles tendons followed by tendon injury and Botox injection into muscles

Ultrasound and microbubbles-mediated gene delivery is a technique also known as sonoporation, allowing for a high and sustained gene expression in mouse Achilles tendons [31]. 10 μ g of EGR1 encoding plasmid DNA [18, 32] and 3.75 μ l of MicroMarkerTM microbubbles

(Bracco) in a total volume of 10 µl were injected into the Achilles tendon. Injection was immediately followed by a 1-MHz ultrasound stimulation of 200 kPa (negative peak) during 10 minutes. Ultrasound was generated from a 0.5" diameter, IBMF-014 transducer with a frequency of 1 MHz (Sofranel, Sartrouville, France). A signal consisting of 40 cycles with a frequency of 1.0 MHz and a pulse repetition frequency of 10 kHz, a duty cycle of 40%, was generated by a 33220A arbitrary function generator (Agilent technologies, Les Ulis, France). The signal was amplified by a RF power amplifier (ADECE, Artannes, France) and used as the input for the transducer. The transducer was calibrated in a Perspex container using an HGL-200 PVDF bullet type hydrophone (Onda, Sunnyvale, CA). EGR1 sonoporation experiments were performed one day before tendon injury and Botox treatment because intratendinous injection requires the integrity of tendon sheath. Mice were euthanatized 15 days after tendon injury and Botox injection and tendons were harvested in 500 µl of RNAlater solution (ThermoScientific) for RNA isolation and RT-q-PCR analyses. 10 mice displaying EGR1 overexpression, tendon injury and Botox injection and 6 mice displaying tendon injury and Botox injection were analyzed.

RNA isolation, reverse transcription and RT-q-PCR

Total RNAs were extracted from fibrin gel constructs made of C3H10T1/2 (N = 11) or C3H10T1/2-EGR1 cells (N = 7), tension-released fibrin gel constructs made of C3H10T1/2 cells (N = 7) or C3H10T1/2-EGR1 cells (N = 5). Total RNAs were extracted from adult mouse tendons under various experimental designs (following physiological saline or Botox injection in normal or injured conditions, after ultrasound-forced EGR1 expression followed by injury and Botox injection) according to the Qiagen RNeasy Minikit (QIAGEN, Germany). RNA (300 ng to 1 µg) was reverse transcribed using the High Capacity Retrotranscription Kit (Applied Biosystems). RT-q-PCR was performed using SYBR Green PCR Master Mix (Applied Biosystems). Relative mRNA levels were calculated using the $2^{-\Delta\Delta Ct}$ method [33]. In all our experimental designs, the Cts of two housekeeping genes *Gapdh* and *Hprt* did not show any variations in control versus experimental conditions. The ΔCts were obtained from Cts normalized with *Gapdh* levels in each sample. RNA samples originating from 5 to 11 biological samples originating from 3 independent experiments were analyzed in duplicate. Primers used for RT-q-PCR are listed in Table 1.

Table 1. Primers used for quantitative RT-PCR.

Col1a1	Fwd 5' CCAGCGAAGAACTCATACAGC
	Rev 5' GGACACCCCTTCTACGTTGT
Col1a2	Fwd 5' CCAGCGAAGAACTCATACAGC
	Rev 5' GGACACCCCTTCTACGTTGT
Egr1	Fwd 5'-CAGCGCTTCAATCCTCAAG
	Rev 5'-GCGATGTCAGAAAAGGACTCTGT
Gapdh	Fwd 5' TTGTGGAAGGGCTCATGACC
	Rev 5' TCTTCTGGGTGGCAGTGATG
Hprt	Fwd 5'AGGGCATATCCAACAACAACTT
	Rev 5'GTTAAGCAGTACAGCCCCAAA
Scx	Fwd 5' CCTTCTGCCTCAGCAACCAG
	Rev 5' GGTCCAAAGTGGGGCTCTCCGTGACT
Tgfb2	Fwd 5' GAATAAAAGCGAAGAGCTCGAGG
	Rev 5' GAGGTGCCATCAATACCTGCA
Tnmd	Fwd 5' AACACTTCTGGCCCGAGGTAT
	Rev 5' AAGTGTGCTCCATGTCATAGGTTTT

doi:10.1371/journal.pone.0166237.t001

Statistical analysis

Sample sizes were based on historical experience of effect sizes for these types of experiments. Error bars in graphics represent the standard deviations or the standard errors of the mean, depending of the size of the samples. Statistics were calculated using non-parametric tests with the GraphPad Prism V6 software. The Mann-Whitney test was used for unpaired samples and the Wilcoxon test for paired samples.

Results

EGR1 overexpression prevents the decrease of tendon gene expression in 3D tendon constructs after tension release

Fibrin-based 3D cell cultures recapitulate tendon formation based on tenogenic marker expression and tendon-like collagen fibrillogenesis [27, 34–37]. This *in vitro* engineered tendon system involves tension [34, 35]. Forced expression of EGR1 in fibrin-based 3D C3H10T1/2 cell cultures increases *Scx*, *Col1a1* and *Col1a2* expression levels compared to control fibrin-based 3D C3H10T1/2 cell cultures [10]. We now show that the expression of the tendon differentiation marker *Tnmd* was also increased in the presence of EGR1 in 3D constructs (Fig 1A). The *Tgfb2* mRNA levels were also increased in EGR1-producing 3D constructs compared to 3D constructs (Fig 1A). The tension release of the 3D constructs induces the appearance of immature collagen fibrils with no preferred orientation in engineered chick tendons [27] and loss of *TNMD* expression in engineered human tendons [28]. The tension was released by cutting one edge of the engineered mouse tendons made of C3H10T1/2 cells (Fig 1B). Tension release led to a decrease in the expression of *Egr1* and tendon genes including *Scx*, *Tnmd*, *Col1a1* and *Col1a2* (Fig 1B). The expression of *Tgfb2* was also decreased in tension-released engineered tendons (Fig 1B). Tension release in *Egr1*-producing 3D-constructs did not trigger any significant changes in tendon gene expression compared to *Egr1*-producing 3D constructs under tension (Fig 1C). This shows that EGR1 overexpression is able to activate tendon gene expression independently of tension in engineered tendons. We conclude that *Egr1* expression is sensitive to tension in engineered mouse tendons and EGR1 forced expression prevents the downregulation of tendon gene expression in the absence of mechanical input.

The expression of tendon genes is downregulated in adult tendons when movements are reduced

In order to assess the importance of mechanical signals for tendon gene expression in homeostasis, we developed a reduced load model in adult mice based on botulinum toxin A (Botox) injection into the Gastrocnemius muscle of hindlimbs (Fig 2A). One week after injection of Botox or physiological saline solution in muscles of *Egr1^{LacZ/+}* mice, LacZ expression (reflecting *Egr1* expression) appeared to be decreased in Achilles tendons (Fig 2B and 2C). Consistently, the *Egr1* mRNA levels were decreased in Achilles tendons of Botox-injected legs compared to saline solution-injected legs, one and two weeks after injection in wild-type mice (Fig 2D). *Scx* expression was also decreased in tendons of Botox-injected legs compared to control legs, one and two weeks after Botox injection (Fig 2D), consistent with the decrease in *Scx* expression one week after Botox injection in *Scx*-GFP mice [30]. The tendon-associated collagen gene *Col1a2* also displayed decreased expression levels one and two weeks after Botox injection (Fig 2D). The expression of the terminal differentiation tendon marker, *Tnmd* did not significantly change (Fig 2D). Interestingly, *Tgfb2* expression was also decreased in tendons, two weeks after Botox injection (Fig 2D). This showed that the expression of *Egr1*, *Tgfb2* and tendon genes (*Scx*

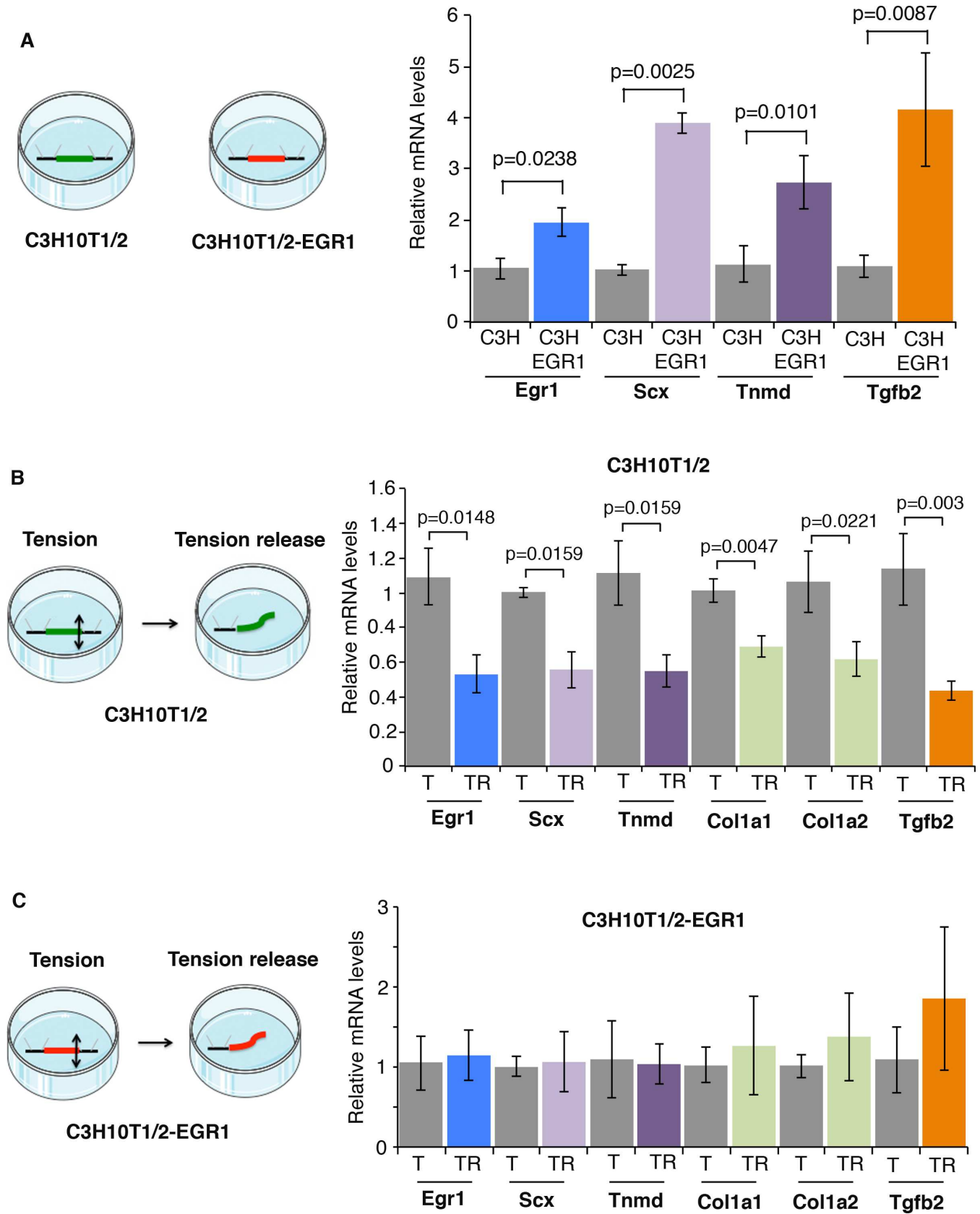


Fig 1. EGR1 overexpression prevents the downregulation of tendon-associated gene expression in tension released engineered tendons. (A) Two week-old fibrin gel constructs made of mouse C3H10T1/2 cells or C3H10T1/2-EGR1 cells were analyzed for tendon gene expression by RT-q-PCR analyses. The mRNA levels of C3H10T1/2 constructs were normalized to 1. Errors bars represent standard errors of the mean of 5 C3H10T1/2 constructs and 7 C3H10T1/2-EGR1 constructs. The *p* values were calculated using the Mann-Whitney test. The mRNA levels of *Egr1*, *Scx*, *Tnmd* and *Tgfb2* genes were increased in C3H10T1/2-EGR1 constructs compared to those of C3H10T1/2 constructs. (B) Tension was released in C3H10T1/2 constructs by sectioning one end of the construct. Transcript levels were analyzed by RT-q-PCR analyses in tension-released C3H10T1/2 constructs and compared to C3H10T1/2 constructs. The mRNA levels of C3H10T1/2 constructs were normalized to 1. Errors bars represent standard errors of the mean of 7 C3H10T1/2 constructs and 7 tension-released C3H10T1/2 constructs. The *p* values were calculated using the Mann-Whitney test. We observed a decrease in the transcript levels of *Egr1*, *Scx*, *Tnmd*, *Col1a1*, *Col1a2* and *Tgfb2* genes in tension-released C3H10T1/2 constructs (TR) compared to tensioned C3H10T1/2 constructs (T). (C) The mRNA levels of tendon genes were analyzed in tension-released C3H10T1/2-EGR1 constructs and compared with tensioned C3H10T1/2-EGR1 constructs by RT-q-PCR analyses. The mRNA levels of C3H10T1/2-EGR1 constructs were normalized to 1. Errors bars represent standard errors of the mean of 7 C3H10T1/2-EGR1 constructs and 5 tension-released C3H10T1/2-EGR1 constructs. The *p* values were calculated using the Mann-Whitney test. There was no significant change in tendon gene expression in tension-released C3H10T1/2-EGR1 constructs (TR) compared to tensioned C3H10T1/2-EGR1 constructs (T). T, Tension, TR, Tension release.

doi:10.1371/journal.pone.0166237.g001

and *Col1a2*) is sensitive to mechanical input in adult tendons. We conclude that mechanical signals are required for tendon gene expression in homeostasis.

Reduced mechanical signals decrease the transcriptional response during tendon healing after injury

In order to analyze the effect of mechanical signals during healing, we used an Achilles tendon injury model in adult wild-type mice as previously described [10]. In this tendon injury model, a longitudinal incision was performed along the axis of the Achilles tendon, in which the tension was maintained. One week after tendon injury, there is a dramatic increase in tendon gene expression, including *Scx*, *Tnmd*, *Col1a1* and *Col1a2* [10]. *Egr1* expression is also increased by fold-3.6 in tendons, after injury and *Egr1* is required for the normal tendon transcriptional response during the healing process [10]. In order to determine whether mechanical signals would influence the transcriptional response during tendon healing, we injected Botox or physiological saline solution in muscles in this mouse model of tendon injury (Fig 3A). We observed a significant decrease in the expression of *Egr1*, *Scx*, *Tnmd*, *Col1a1*, *Col1a2* and *Tgfb2* genes after Botox injection compared to physiological saline solution injections, in injury conditions (Fig 3B). The decrease of mRNA levels of tendon genes ranged from 40% to 60% (Fig 3). This shows that a diminution of mechanical signals modifies the transcriptional response during the tendon healing process following injury. We conclude that mechanical signals are required for the full transcriptional response during tendon healing.

EGR1 forced expression in tendons prevents the diminution of tendon gene response in reduced load conditions after injury

The expression of the mechanosensitive gene *Egr1* was decreased in reduced load conditions in tendon homeostasis (Fig 2) and healing (Fig 3). To assess whether EGR1 acts downstream of mechanical signals during tendon healing, we performed *in vivo* EGR1 rescue experiments in reduced load conditions and after tendon injury. We took advantage of an ultrasound-based gene delivery method, which has been found to be efficient in tendons [31]. Plasmid DNA encoding for the *Egr1* gene was delivered to the tendons with optimized parameters (1 MHz, 200 kPa, 10 min, 40% duty cycle and 10 kHz pulse repetition frequency). The following day, Botox was injected in muscle followed by tendon injury (Fig 4A). Two weeks after Botox injection in muscle and tendon injury, we compared the expression of tendon genes in tendons overexpressing EGR1 or not during tendon healing and in reduced load conditions. In this experiment, *Egr1* was increased by fold-2.6. EGR1 forced expression in tendons increased the expression of tendon-associated genes including *Scx*, *Tnmd*, *Col1a1*, *Col1a2* and *Tgfb2*, when

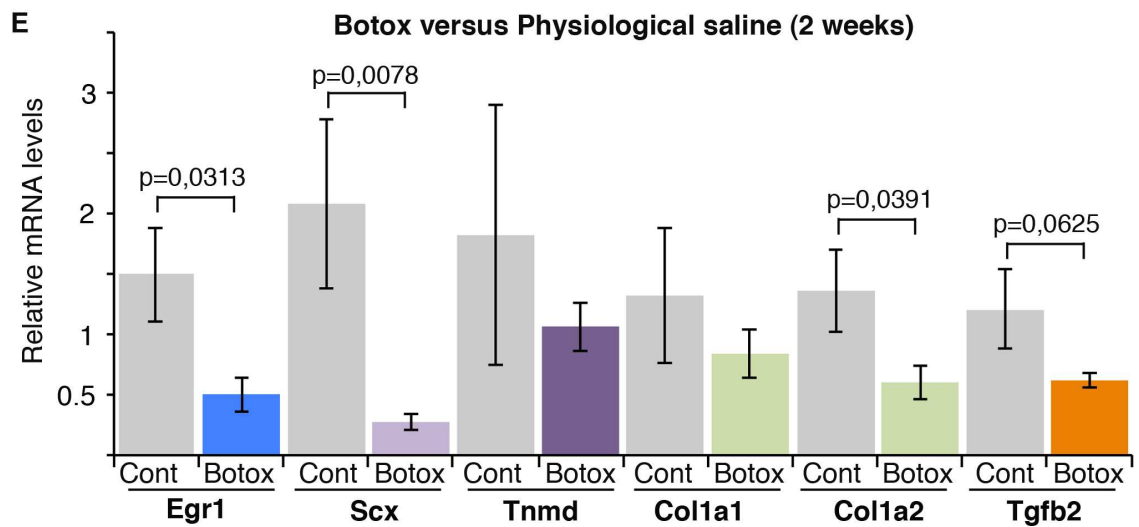
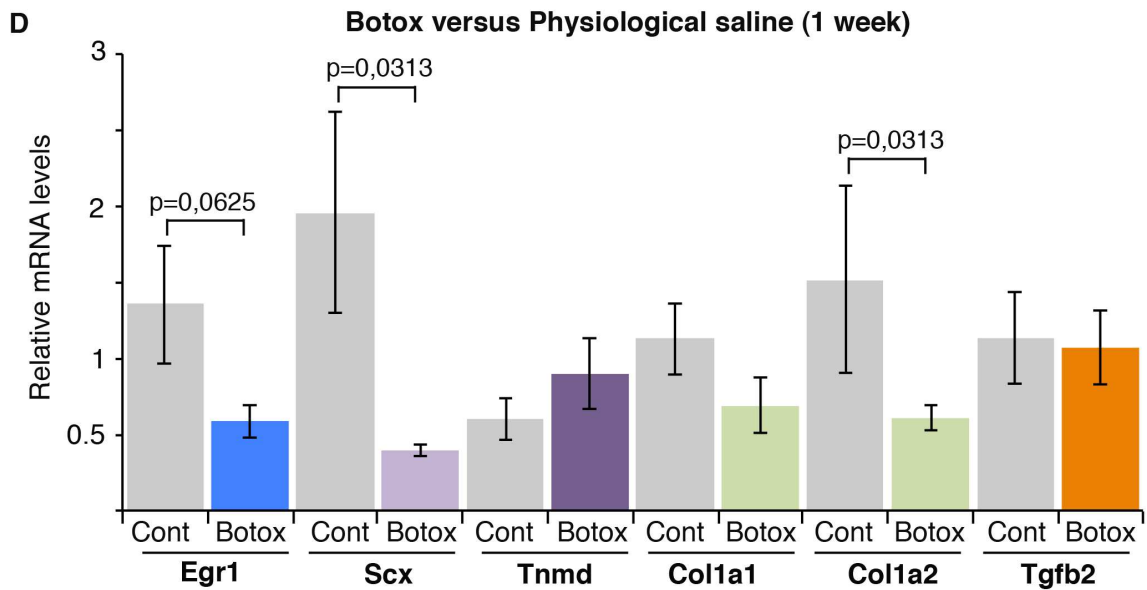
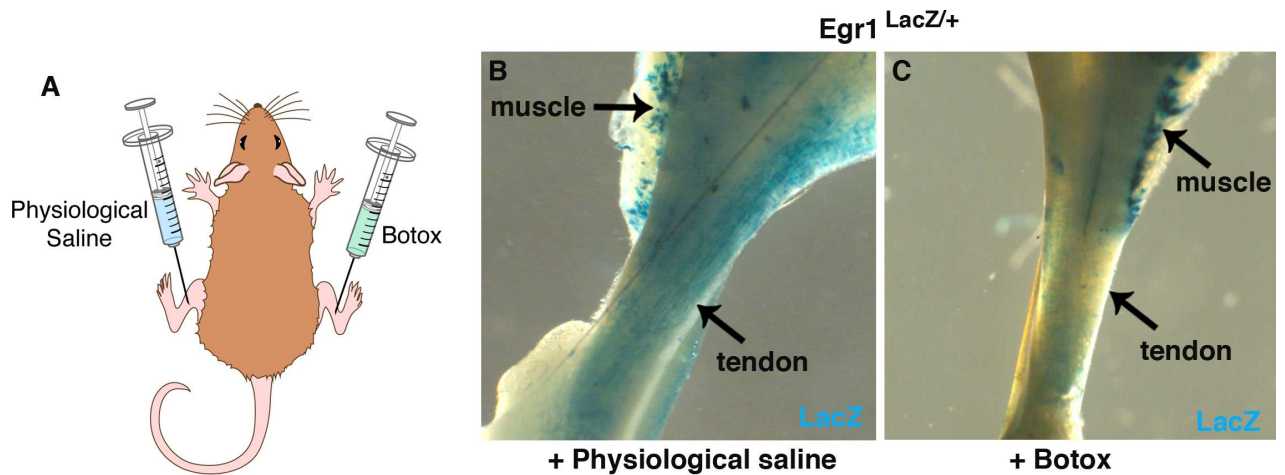


Fig 2. Reduced mechanical input induces a diminution of *Egr1* and *Scx* expression in adult tendons. (A) Botox or physiological saline injections in Gastrocnemius muscles of adult mice. (B,C) LacZ staining (reflecting *Egr1* expression) in tendons, 1 week following physiological saline or Botox injection in *Egr1*^{LacZ/+} adult mice. (D) RT-q-PCR analyses of tendons, one or two weeks after Botox or physiological saline injections in muscles in adult mice. The mRNA levels of tendons following Botox injection were compared to those of tendons with physiological saline injection. Errors bars represent the standard deviations of 5 (one week) and 7 (two weeks) biological samples. The mRNA levels of *Egr1*, *Scx*, *Col1a2*, *Tgfb2* genes were decreased one or two weeks after Botox injection compared to physiological saline injection. *Tnmd* mRNA levels were not significantly decreased after Botox injection. The *p* values were calculated using the Wilcoxon test.

doi:10.1371/journal.pone.0166237.g002

compared to control (Fig 4B). The levels of induction were similar to that of *Egr1* overexpression (Fig 4A). This shows that EGR1 is sufficient to prevent the diminution of tendon gene expression that is observed during healing in reduced load conditions.

Discussion

We show that reduced load conditions consistently lead to a decrease in expression of the transcription factor *Egr1* and tendon genes in tendon homeostasis, tendon healing and *in vitro* engineered tendons. We also demonstrate that EGR1 forced expression prevents the decrease of tendon gene expression in reduced load conditions during tendon healing and in engineered tendons.

Egr1 is a mechanosensitive gene, which has been shown to act downstream of mechanical signals in the vascular system [20, 21, 24]. Consistently, *Egr1* expression is systematically downregulated in reduced load conditions in the *in vivo* and *in vitro* models of tendon biology. The decrease of *Egr1* expression at the transcription level in reduced load conditions is consistent with the upregulation of *Egr1* expression in overload conditions in adult tendons [38, 39]. *Egr1* expression is induced as early as 15 minutes after a loading episode in rat Achilles tendons [40]. This indicates that *Egr1* expression reflects a rapid transcriptional response following loading changes in tendons. In addition to being sensitive to mechanical signals, *Egr1* appears to be sufficient to drive the tendon genetic program in the absence of mechanical signals. The presence of exogenous EGR1 abolishes the downregulation of *Scx*, *Col1a1* and *Tnmd* in engineered tendons subjected to tension release and EGR1 also prevents the decrease of *Scx*, *Col1a1* and *Tnmd* expression in Achilles tendons in reduced load conditions during tendon healing following injury. This shows that the Zinc-finger transcription factor *Egr1* senses mechanical signals in tendons and modulates tendon gene expression upon loading changes. Although EGR1 has the ability to induce the expression of tendon genes in normal [10] or reduced load (Figs 1 and 4) conditions, it remains unclear whether EGR1 directly regulates the transcription of tendon genes. Previous promoter and Chromatin Immuno-Precipitation (ChIP) analyses indicate that EGR1 trans-activates the tendon promoter of the mouse *Col1a1* gene [18, 32]. Moreover, EGR1 directly regulates *Tgfb2* transcription in adult mouse tendons [10]. *Tgfb2* expression is downregulated in reduced load conditions in tendon homeostasis or healing in adult mice (Figs 2 and 3) and in engineered tendons made of mouse mesenchymal stem cells (Fig 1). EGR1 rescue experiments in conditions of reduced mechanical signals induced *Tgfb2* expression in engineered tendons and during the tendon healing process (Figs 1 and 4). These results suggest that *Egr1* acts upstream of *Tgfb2* to activate tendon gene expression upon loading. It should be noted that TGF- β 1 supplementation was not sufficient to prevent the decrease of tendon gene expression in de-tensioned 3D engineered tendons derived from human tendon cells [28]. It is not clear whether TGF- β ligand is not sufficient to activate tendon gene expression in reduced load conditions or TGF- β 1 activity differs from TGF- β 2 activity. Antagonist effects between TGF- β 1 and TGF- β 2 ligands have been reported on *COL1A1* transcription in rat tendon fibroblasts [41]. TGF- β supplementation activates *Scx* expression in various cellular

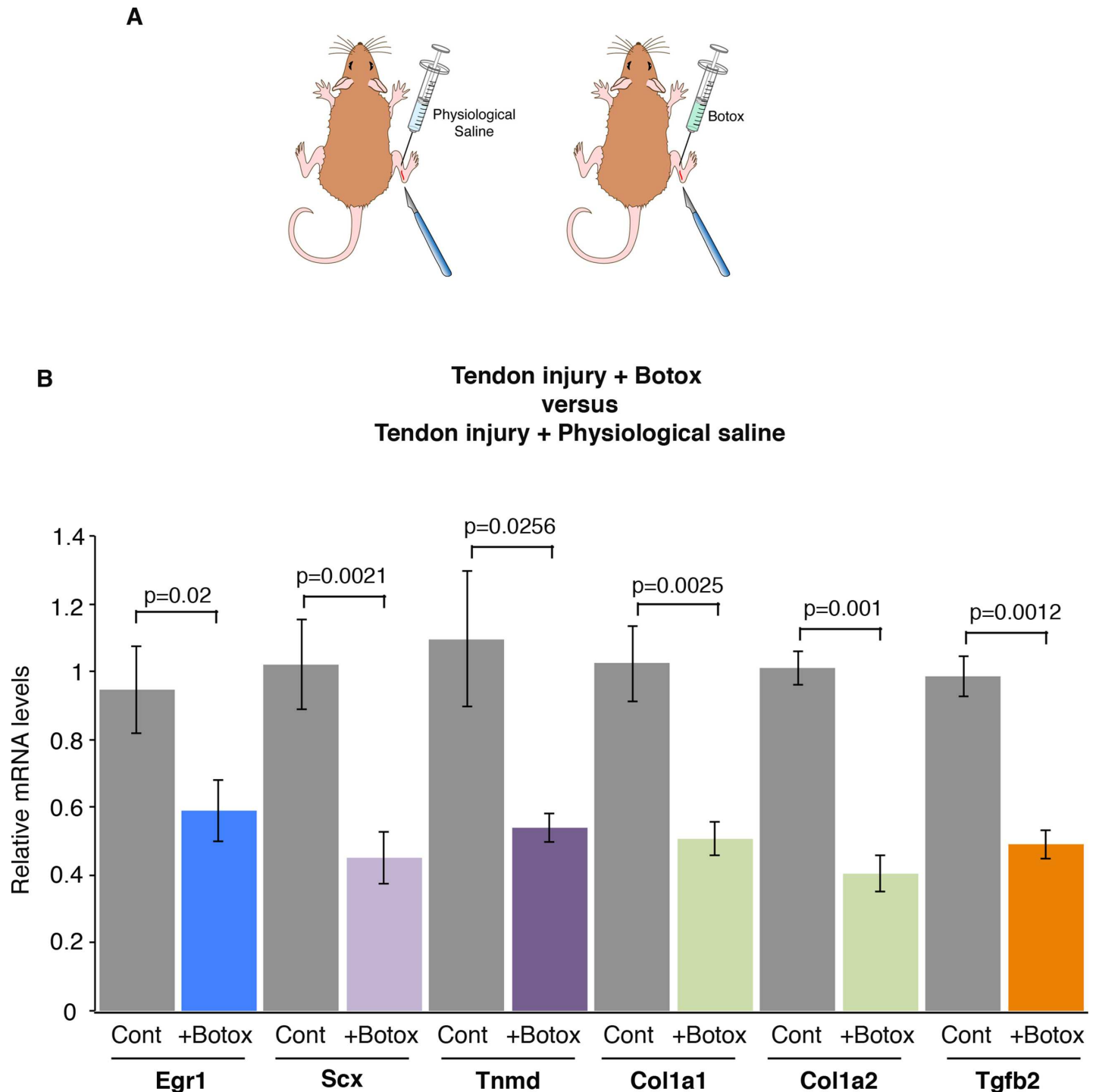


Fig 3. Mechanical signals are required for normal tendon gene response following tendon injury. (A) Botox or physiological saline injections in Gastrocnemius muscles and tendon injury in adult mice. (B) RT-q-PCR analyses of tendons, 2 weeks after tendon injury and Botox injection in muscles. The mRNA levels of tendons following injury and physiological saline injection in muscles were normalized to 1. The errors bars represent the standard error of the means of 10 and 11 biological samples of injured tendons after physiological saline or Botox injections, respectively. The *p* values were calculated using the Mann-Whitney test. The mRNA levels of the *Egr1*, *Scx*, *Tnmd*, *Col1a1*, *Col1a2* and *Tgfb2* genes were all significantly decreased in reduced movement conditions compared to controls during the healing process, following tendon injury.

doi:10.1371/journal.pone.0166237.g003

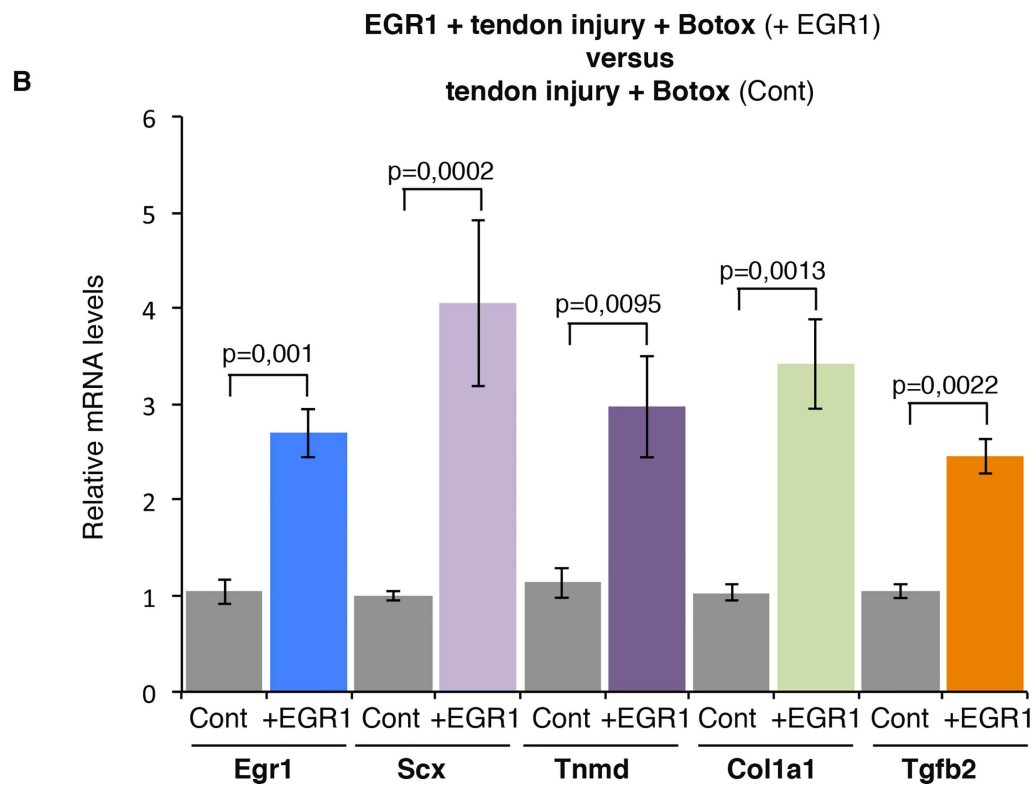
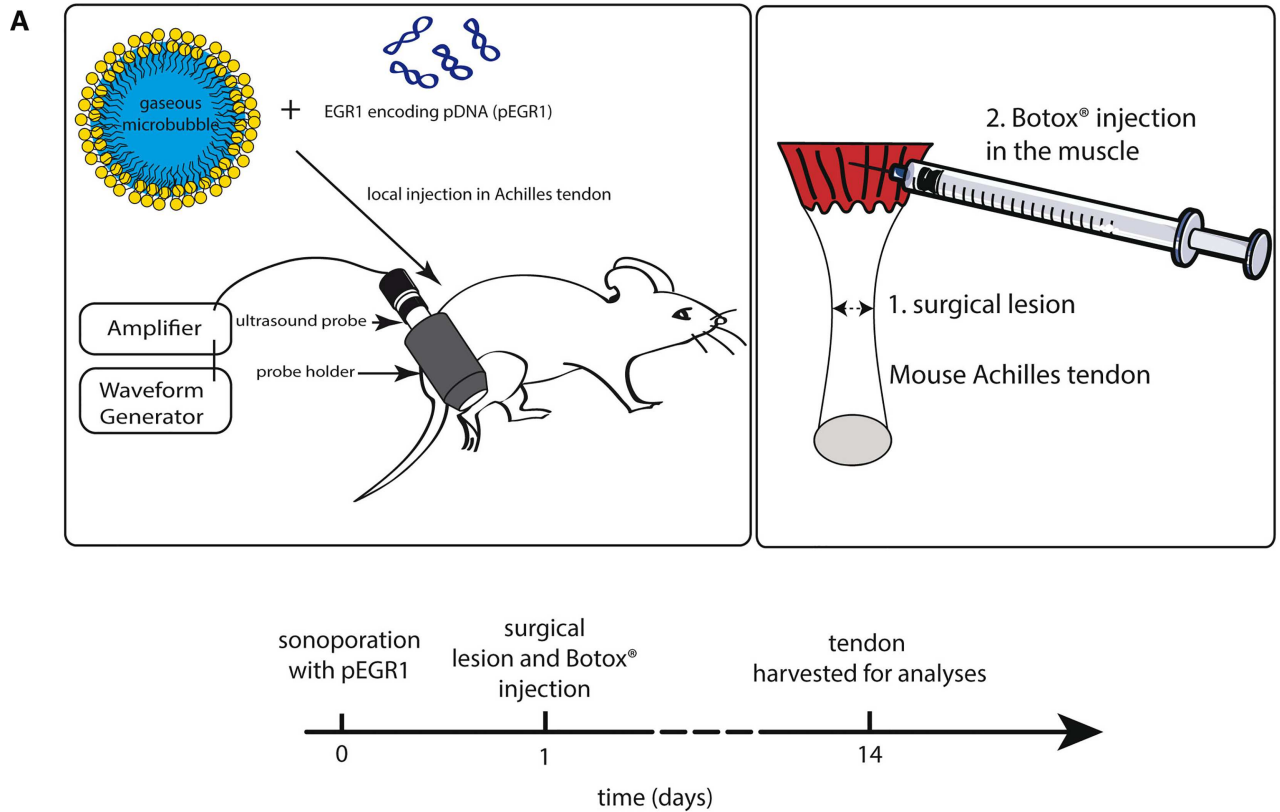


Fig 4. EGR1 forced expression in tendons prevents the diminution of tendon gene expression during tendon healing in reduced mechanical load. (A) Description of the experimental design for sonoporation. 10 µg of EGR1 encoding plasmid and 3.75 µl of MicroMarker microbubbles were injected in the Achilles tendon sheath. Tendons were then stimulated by ultrasound at 1 MHz during 10 minutes at 200 kPa, 40% duty cycle and 10 kHz pulse repeating frequency. The day after EGR1 sonoporation, a surgical lesion of the Achilles tendon was performed followed by a Botox or physiological saline solution injection in the muscle. Two weeks after treatment, tendons were harvested for analyses by RT-q-PCR. (B) RT-q-PCR analysis of tendon gene expression in EGR1-sonoporated tendons versus control-tendons, following tendon injury in immobilization conditions. The mRNA levels of control tendons following injury and Botox injection in muscles were normalized to 1. The error bars represent standard errors of the mean of 6 biological samples of injured tendons of Botox-injected legs in the absence of EGR1 and 10 biological samples of injured tendons of Botox-injected legs in presence of ectopic EGR1. The *p* values were calculated using the Mann-Whitney test. The mRNA levels of *Egr1*, *Scx*, *Tnmd*, *Col1a2* and *Tgfb2* were increased in Egr1-sonoporated tendons compared to control tendons, following tendon injury and Botox injection in muscles.

doi:10.1371/journal.pone.0166237.g004

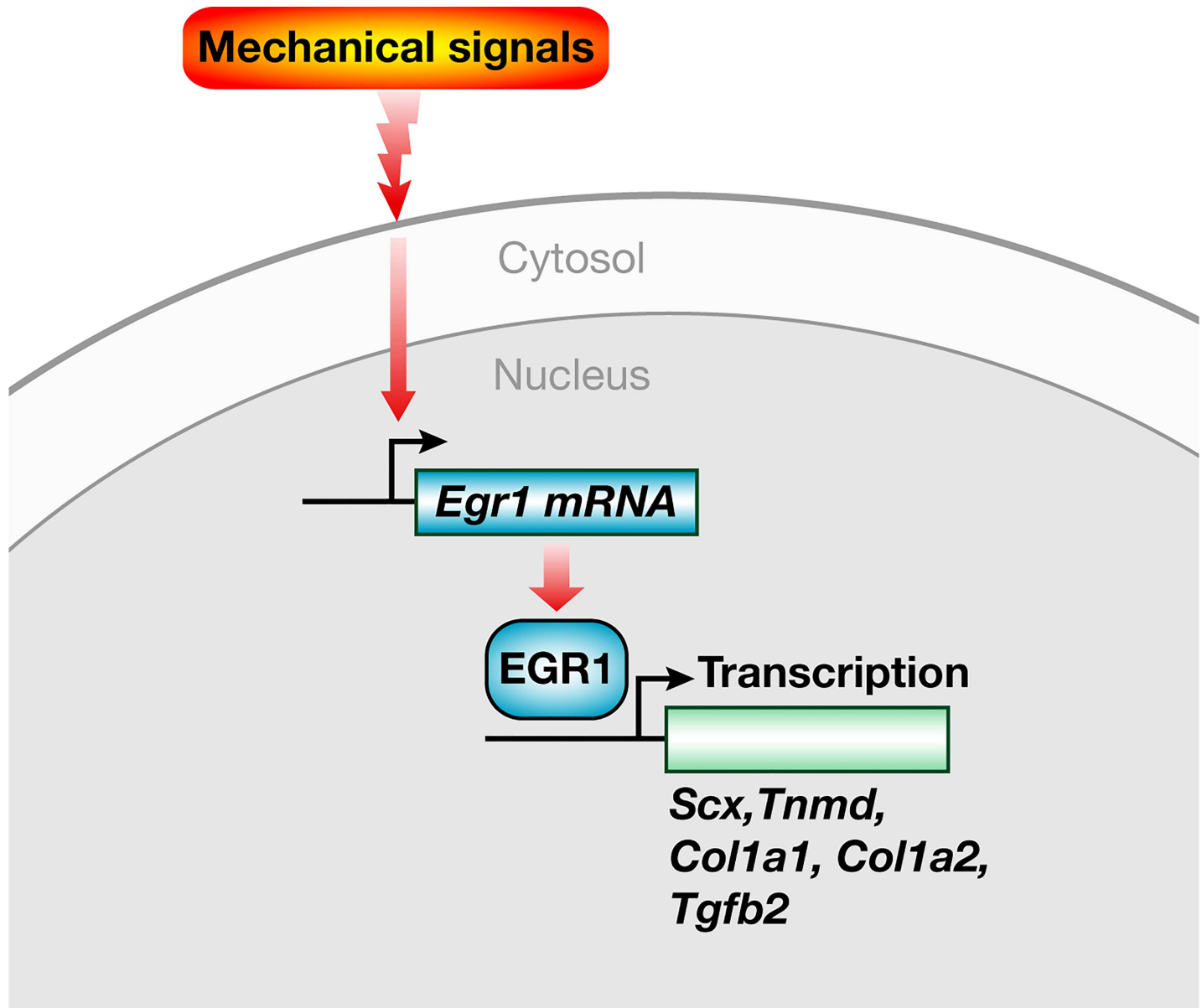


Fig 5. Schematic representation of EGR1 regulation and function downstream of mechanical signals. *Egr1* expression is regulated by mechanical signals in tendon cells. EGR1 positively regulates the transcription of tendon genes including *Scx*, *Tnmd*, *Col1a1* and *Col1a2*. The transcription of *Tgfb2* is also regulated by EGR1.

doi:10.1371/journal.pone.0166237.g005

models and loss of TGF- β leads to a decrease of *Scx* expression in developmental tendons [10, 42–44]. We failed to identify any EGR1 binding sites in mouse *Scx* promoter region and all our ChIP attempts to identify direct EGR1 recruitment to *Scx* promoter region were unsuccessful. Given the EGR1 direct binding to *Tgfb2* promoter [10], it is tempting to suggest that TGF- β 2 mediates the *Scx* induction by EGR1 in tendons. Consistently, SCX and SMAD3 (an intracellular component of the TGF- β pathway) physically interact in 10T1/2 cells [45] and SCX is required for Smad3-mediated gene transcription in cardiac fibroblasts [46]. Whether EGR1 activates *Tnmd* via TGF- β 2 is less clear, since TGF- β 2 has been shown to downregulate *Tnmd* expression in mouse stem cells cultured in 2D [10, 44, 47] and do not have any positive effect on *TNMD* expression in human 3D tissue cultures [28]. In contrast, SCX gain- and loss-of-function experiments lead to upregulation and downregulation of *Tnmd*, respectively, during development [14, 15]. This suggests that SCX could activate *Tnmd* expression during tendon differentiation.

In summary, we show that the expression of the zinc-finger transcription factor EGR1 is sensitive to mechanical signals and that EGR1 senses mechanical signals at the transcription level. Moreover, EGR1 is sufficient to promote tendon gene expression in the absence of mechanical forces during tendon formation and healing (Fig 5). This feature opens the possibility of exploiting EGR1 forced expression to accelerate tendon healing in reduced load conditions.

Acknowledgments

We thank laboratory members for comments on the manuscript and Sophie Gournet for illustrations.

Author Contributions

Conceptualization: DD CP.

Formal analysis: LG.

Funding acquisition: DD.

Investigation: LG NR AD MAB.

Methodology: LG NR AD MAB.

Writing – original draft: DD.

Writing – review & editing: DD LG AD CP.

References

1. Shwartz Y, Blitz E, Zelzer E. One load to rule them all: mechanical control of the musculoskeletal system in development and aging. *Differentiation; research in biological diversity*. 2013; 86(3):104–11. doi: [10.1016/j.diff.2013.07.003](https://doi.org/10.1016/j.diff.2013.07.003) PMID: [23953954](https://pubmed.ncbi.nlm.nih.gov/23953954/).
2. Wang JH, Guo Q, Li B. Tendon biomechanics and mechanobiology—a minireview of basic concepts and recent advancements. *Journal of hand therapy: official journal of the American Society of Hand Therapists*. 2012; 25(2):133–40; quiz 41. doi: [10.1016/j.jht.2011.07.004](https://doi.org/10.1016/j.jht.2011.07.004) PMID: [21925835](https://pubmed.ncbi.nlm.nih.gov/21925835/); PubMed Central PMCID: [PMC3244520](https://pubmed.ncbi.nlm.nih.gov/PMC3244520/).
3. Heinemeier KM, Kjaer M. In vivo investigation of tendon responses to mechanical loading. *Journal of musculoskeletal & neuronal interactions*. 2011; 11(2):115–23. PMID: [21625048](https://pubmed.ncbi.nlm.nih.gov/21625048/).
4. Lavagnino M, Wall ME, Little D, Banes AJ, Guilak F, Arnoczky SP. Tendon mechanobiology: Current knowledge and future research opportunities. *Journal of orthopaedic research: official publication of the Orthopaedic Research Society*. 2015; 33(6):813–22. doi: [10.1002/jor.22871](https://doi.org/10.1002/jor.22871) PMID: [25763779](https://pubmed.ncbi.nlm.nih.gov/25763779/); PubMed Central PMCID: [PMC4524513](https://pubmed.ncbi.nlm.nih.gov/PMC4524513/).

5. Nourissat G, Berenbaum F, Duprez D. Tendon injury: from biology to tendon repair. *Nature reviews Rheumatology*. 2015; 11(4):223–33. doi: [10.1038/nrrheum.2015.26](https://doi.org/10.1038/nrrheum.2015.26) PMID: [25734975](https://pubmed.ncbi.nlm.nih.gov/25734975/).
6. Schweitzer R, Chyung JH, Murtaugh LC, Brent AE, Rosen V, Olson EN, et al. Analysis of the tendon cell fate using Scleraxis, a specific marker for tendons and ligaments. *Development*. 2001; 128(19):3855–66. Epub 2001/10/05. PMID: [11585810](https://pubmed.ncbi.nlm.nih.gov/11585810/).
7. Pryce BA, Brent AE, Murchison ND, Tabin CJ, Schweitzer R. Generation of transgenic tendon reporters, ScxGFP and ScxAP, using regulatory elements of the scleraxis gene. *Developmental dynamics: an official publication of the American Association of Anatomists*. 2007; 236(6):1677–82. doi: [10.1002/dvdy.21179](https://doi.org/10.1002/dvdy.21179) PMID: [17497702](https://pubmed.ncbi.nlm.nih.gov/17497702/).
8. Mendias CL, Gumucio JP, Bakhurin KI, Lynch EB, Brooks SV. Physiological loading of tendons induces scleraxis expression in epitenon fibroblasts. *Journal of orthopaedic research: official publication of the Orthopaedic Research Society*. 2012; 30(4):606–12. doi: [10.1002/jor.21550](https://doi.org/10.1002/jor.21550) PMID: [21913219](https://pubmed.ncbi.nlm.nih.gov/21913219/); PubMed Central PMCID: [PMC3245815](https://pubmed.ncbi.nlm.nih.gov/PMC3245815/).
9. Scott A, Sampaio A, Abraham T, Duronio C, Underhill TM. Scleraxis expression is coordinately regulated in a murine model of patellar tendon injury. *Journal of orthopaedic research: official publication of the Orthopaedic Research Society*. 2011; 29(2):289–96. doi: [10.1002/jor.21220](https://doi.org/10.1002/jor.21220) PMID: [20740671](https://pubmed.ncbi.nlm.nih.gov/20740671/); PubMed Central PMCID: [PMC3951487](https://pubmed.ncbi.nlm.nih.gov/PMC3951487/).
10. Guerquin MJ, Charvet B, Nourissat G, Havis E, Ronsin O, Bonnin MA, et al. Transcription factor EGR1 directs tendon differentiation and promotes tendon repair. *The Journal of clinical investigation*. 2013; 123(8):3564–76. doi: [10.1172/JCI67521](https://doi.org/10.1172/JCI67521) PMID: [23863709](https://pubmed.ncbi.nlm.nih.gov/23863709/).
11. Jelinsky SA, Archambault J, Li L, Seeherman H. Tendon-selective genes identified from rat and human musculoskeletal tissues. *Journal of orthopaedic research: official publication of the Orthopaedic Research Society*. 2010; 28(3):289–97. doi: [10.1002/jor.20999](https://doi.org/10.1002/jor.20999) PMID: [19780194](https://pubmed.ncbi.nlm.nih.gov/19780194/).
12. Sugimoto Y, Takimoto A, Akiyama H, Kist R, Scherer G, Nakamura T, et al. Scx+/Sox9+ progenitors contribute to the establishment of the junction between cartilage and tendon/ligament. *Development*. 2013; 140(11):2280–8. doi: [10.1242/dev.096354](https://doi.org/10.1242/dev.096354) PMID: [23615282](https://pubmed.ncbi.nlm.nih.gov/23615282/).
13. Havis E, Bonnin MA, Olivera-Martinez I, Nazaret N, Ruggiu M, Weibel J, et al. Transcriptomic analysis of mouse limb tendon cells during development. *Development*. 2014; 141(19):3683–96. doi: [10.1242/dev.108654](https://doi.org/10.1242/dev.108654) PMID: [25249460](https://pubmed.ncbi.nlm.nih.gov/25249460/).
14. Shukunami C, Takimoto A, Oro M, Hiraki Y. Scleraxis positively regulates the expression of tenomodulin, a differentiation marker of tenocytes. *Developmental biology*. 2006; 298(1):234–47. doi: [10.1016/j.ydbio.2006.06.036](https://doi.org/10.1016/j.ydbio.2006.06.036) PMID: [16876153](https://pubmed.ncbi.nlm.nih.gov/16876153/).
15. Murchison ND, Price BA, Conner DA, Keene DR, Olson EN, Tabin CJ, et al. Regulation of tendon differentiation by scleraxis distinguishes force-transmitting tendons from muscle-anchoring tendons. *Development*. 2007; 134(14):2697–708. Epub 2007/06/15. dev.001933 [pii] doi: [10.1242/dev.001933](https://doi.org/10.1242/dev.001933) PMID: [17567668](https://pubmed.ncbi.nlm.nih.gov/17567668/).
16. Docheva D, Hunziker EB, Fassler R, Brandau O. Tenomodulin is necessary for tenocyte proliferation and tendon maturation. *Molecular and cellular biology*. 2005; 25(2):699–705. doi: [10.1128/MCB.25.2.699-705.2005](https://doi.org/10.1128/MCB.25.2.699-705.2005) PMID: [15632070](https://pubmed.ncbi.nlm.nih.gov/15632070/); PubMed Central PMCID: [PMC543433](https://pubmed.ncbi.nlm.nih.gov/PMC543433/).
17. Alberton P, Dex S, Popov C, Shukunami C, Schieker M, Docheva D. Loss of tenomodulin results in reduced self-renewal and augmented senescence of tendon stem/progenitor cells. *Stem cells and development*. 2015; 24(5):597–609. doi: [10.1089/scd.2014.0314](https://doi.org/10.1089/scd.2014.0314) PMID: [25351164](https://pubmed.ncbi.nlm.nih.gov/25351164/); PubMed Central PMCID: [PMC4333258](https://pubmed.ncbi.nlm.nih.gov/PMC4333258/).
18. Lejard V, Blais F, Guerquin MJ, Bonnet A, Bonnin MA, Havis E, et al. EGR1 and EGR2 Involvement in Vertebrate Tendon Differentiation. *J Biol Chem*. 2011; 286(7):5855–67. Epub 2010/12/22. M110.153106 [pii] doi: [10.1074/jbc.M110.153106](https://doi.org/10.1074/jbc.M110.153106) PMID: [21173153](https://pubmed.ncbi.nlm.nih.gov/21173153/); PubMed Central PMCID: [PMC3037698](https://pubmed.ncbi.nlm.nih.gov/PMC3037698/).
19. Tao X, Liu J, Chen L, Zhou Y, Tang K. EGR1 induces tenogenic differentiation of tendon stem cells and promotes rabbit rotator cuff repair. *Cellular physiology and biochemistry: international journal of experimental cellular physiology, biochemistry, and pharmacology*. 2015; 35(2):699–709. doi: [10.1159/000369730](https://doi.org/10.1159/000369730) PMID: [25592085](https://pubmed.ncbi.nlm.nih.gov/25592085/).
20. Stula M, Orzechowski HD, Gschwend S, Vetter R, von Harsdorf R, Dietz R, et al. Influence of sustained mechanical stress on Egr-1 mRNA expression in cultured human endothelial cells. *Molecular and cellular biochemistry*. 2000; 210(1–2):101–8. PMID: [10976763](https://pubmed.ncbi.nlm.nih.gov/10976763/).
21. Morawietz H, Ma YH, Vives F, Wilson E, Sukhatme VP, Holtz J, et al. Rapid induction and translocation of Egr-1 in response to mechanical strain in vascular smooth muscle cells. *Circulation research*. 1999; 84(6):678–87. PMID: [10189355](https://pubmed.ncbi.nlm.nih.gov/10189355/).
22. Pardo PS, Mohamed JS, Lopez MA, Boriak AM. Induction of Sirt1 by mechanical stretch of skeletal muscle through the early response factor EGR1 triggers an antioxidative response. *J Biol Chem*. 2011;

- 286(4):2559–66. doi: [10.1074/jbc.M110.149153](https://doi.org/10.1074/jbc.M110.149153) PMID: [20971845](https://pubmed.ncbi.nlm.nih.gov/20971845/); PubMed Central PMCID: [PMC3024751](https://pubmed.ncbi.nlm.nih.gov/PMC3024751/).
23. Bougault C, Aubert-Foucher E, Paumier A, Perrier-Groult E, Huot L, Hot D, et al. Dynamic compression of chondrocyte-agarose constructs reveals new candidate mechanosensitive genes. *PLoS one*. 2012; 7(5):e36964. doi: [10.1371/journal.pone.0036964](https://doi.org/10.1371/journal.pone.0036964) PMID: [22615857](https://pubmed.ncbi.nlm.nih.gov/22615857/); PubMed Central PMCID: [PMC3355169](https://pubmed.ncbi.nlm.nih.gov/PMC3355169/).
 24. Wu X, Cheng J, Li P, Yang M, Qiu S, Liu P, et al. Mechano-sensitive transcriptional factor Egr-1 regulates insulin-like growth factor-1 receptor expression and contributes to neointima formation in vein grafts. *Arteriosclerosis, thrombosis, and vascular biology*. 2010; 30(3):471–6. doi: [10.1161/ATVBAHA.109.184259](https://doi.org/10.1161/ATVBAHA.109.184259) PMID: [19965784](https://pubmed.ncbi.nlm.nih.gov/19965784/).
 25. Topilko P, Schneider-Maunoury S, Levi G, Trembleau A, Gourdji D, Driancourt MA, et al. Multiple pituitary and ovarian defects in Krox-24 (NGFI-A, Egr-1)-targeted mice. *Molecular endocrinology*. 1998; 12(1):107–22. doi: [10.1210/mend.12.1.0049](https://doi.org/10.1210/mend.12.1.0049) PMID: [9440815](https://pubmed.ncbi.nlm.nih.gov/9440815/).
 26. Reznikoff CA, Brankow DW, Heidelberger C. Establishment and characterization of a cloned line of C3H mouse embryo cells sensitive to postconfluence inhibition of division. *Cancer research*. 1973; 33(12):3231–8. PMID: [4357355](https://pubmed.ncbi.nlm.nih.gov/4357355/).
 27. Kapacee Z, Richardson SH, Lu Y, Starborg T, Holmes DF, Baar K, et al. Tension is required for fibroblastic formation. *Matrix biology: journal of the International Society for Matrix Biology*. 2008; 27(4):371–5. doi: [10.1016/j.matbio.2007.11.006](https://doi.org/10.1016/j.matbio.2007.11.006) PMID: [18262777](https://pubmed.ncbi.nlm.nih.gov/18262777/).
 28. Bayer ML, Schjerling P, Herchenhan A, Zeltz C, Heinemeier KM, Christensen L, et al. Release of tensile strain on engineered human tendon tissue disturbs cell adhesions, changes matrix architecture, and induces an inflammatory phenotype. *PLoS one*. 2014; 9(1):e86078. doi: [10.1371/journal.pone.0086078](https://doi.org/10.1371/journal.pone.0086078) PMID: [24465881](https://pubmed.ncbi.nlm.nih.gov/24465881/); PubMed Central PMCID: [PMC3897642](https://pubmed.ncbi.nlm.nih.gov/PMC3897642/).
 29. Aoki KR. A comparison of the safety margins of botulinum neurotoxin serotypes A, B, and F in mice. *Toxicon: official journal of the International Society on Toxinology*. 2001; 39(12):1815–20. PMID: [11600142](https://pubmed.ncbi.nlm.nih.gov/11600142/).
 30. Maeda T, Sakabe T, Sunaga A, Sakai K, Rivera AL, Keene DR, et al. Conversion of mechanical force into TGF-beta-mediated biochemical signals. *Curr Biol*. 2011; 21(11):933–41. Epub 2011/05/24. S0960-9822(11)00423-4 [pii] doi: [10.1016/j.cub.2011.04.007](https://doi.org/10.1016/j.cub.2011.04.007) PMID: [21600772](https://pubmed.ncbi.nlm.nih.gov/21600772/); PubMed Central PMCID: [PMC3118584](https://pubmed.ncbi.nlm.nih.gov/PMC3118584/).
 31. Delalande A, Bouakaz A, Renault G, Tabareau F, Kotopoulos S, Midoux P, et al. Ultrasound and microbubble-assisted gene delivery in Achilles tendons: long lasting gene expression and restoration of fibromodulin KO phenotype. *Journal of controlled release: official journal of the Controlled Release Society*. 2011; 156(2):223–30. doi: [10.1016/j.jconrel.2011.08.020](https://doi.org/10.1016/j.jconrel.2011.08.020) PMID: [21888933](https://pubmed.ncbi.nlm.nih.gov/21888933/).
 32. Lejard V, Brideau G, Blais F, Salingcamboriboon R, Wagner G, Roehrl MH, et al. Scleraxis and NFATc regulate the expression of the pro-alpha1(I) collagen gene in tendon fibroblasts. *J Biol Chem*. 2007; 282(24):17665–75. doi: [10.1074/jbc.M610113200](https://doi.org/10.1074/jbc.M610113200) PMID: [17430895](https://pubmed.ncbi.nlm.nih.gov/17430895/).
 33. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2⁻(Delta Delta C(T)) Method. *Methods*. 2001; 25(4):402–8. doi: [10.1006/meth.2001.1262](https://doi.org/10.1006/meth.2001.1262) PMID: [11846609](https://pubmed.ncbi.nlm.nih.gov/11846609/).
 34. Kapacee Z, Yeung CY, Lu Y, Crabtree D, Holmes DF, Kadler KE. Synthesis of embryonic tendon-like tissue by human marrow stromal/mesenchymal stem cells requires a three-dimensional environment and transforming growth factor beta3. *Matrix biology: journal of the International Society for Matrix Biology*. 2010; 29(8):668–77. doi: [10.1016/j.matbio.2010.08.005](https://doi.org/10.1016/j.matbio.2010.08.005) PMID: [20736064](https://pubmed.ncbi.nlm.nih.gov/20736064/); PubMed Central PMCID: [PMC3611595](https://pubmed.ncbi.nlm.nih.gov/PMC3611595/).
 35. Bayer ML, Yeung CY, Kadler KE, Qvortrup K, Baar K, Svensson RB, et al. The initiation of embryonic-like collagen fibrillogenesis by adult human tendon fibroblasts when cultured under tension. *Biomaterials*. 2010; 31(18):4889–97. doi: [10.1016/j.biomaterials.2010.02.062](https://doi.org/10.1016/j.biomaterials.2010.02.062) PMID: [20356622](https://pubmed.ncbi.nlm.nih.gov/20356622/); PubMed Central PMCID: [PMC3485556](https://pubmed.ncbi.nlm.nih.gov/PMC3485556/).
 36. Breidenbach AP, Dyment NA, Lu Y, Rao M, Shearn JT, Rowe DW, et al. Fibrin gels exhibit improved biological, structural, and mechanical properties compared with collagen gels in cell-based tendon tissue-engineered constructs. *Tissue engineering Part A*. 2015; 21(3–4):438–50. doi: [10.1089/ten.TEA.2013.0768](https://doi.org/10.1089/ten.TEA.2013.0768) PMID: [25266738](https://pubmed.ncbi.nlm.nih.gov/25266738/); PubMed Central PMCID: [PMC4333253](https://pubmed.ncbi.nlm.nih.gov/PMC4333253/).
 37. Yeung CY, Zeef LA, Lallyett C, Lu Y, Canty-Laird EG, Kadler KE. Chick tendon fibroblast transcriptome and shape depend on whether the cell has made its own collagen matrix. *Scientific reports*. 2015; 5:13555. doi: [10.1038/srep13555](https://doi.org/10.1038/srep13555) PMID: [26337655](https://pubmed.ncbi.nlm.nih.gov/26337655/); PubMed Central PMCID: [PMC4559659](https://pubmed.ncbi.nlm.nih.gov/PMC4559659/).
 38. Eliasson P, Andersson T, Hammerman M, Aspenberg P. Primary gene response to mechanical loading in healing rat Achilles tendons. *Journal of applied physiology*. 2013; 114(11):1519–26. doi: [10.1152/jappphysiol.01500.2012](https://doi.org/10.1152/jappphysiol.01500.2012) PMID: [23519232](https://pubmed.ncbi.nlm.nih.gov/23519232/).

39. Hammerman M, Aspenberg P, Eliasson P. Microtrauma stimulates rat Achilles tendon healing via an early gene expression pattern similar to mechanical loading. *Journal of applied physiology*. 2014; 116(1):54–60. doi: [10.1152/jappphysiol.00741.2013](https://doi.org/10.1152/jappphysiol.00741.2013) PMID: [24177691](https://pubmed.ncbi.nlm.nih.gov/24177691/).
40. Eliasson P, Andersson T, Aspenberg P. Rat Achilles tendon healing: mechanical loading and gene expression. *Journal of applied physiology*. 2009; 107(2):399–407. doi: [10.1152/jappphysiol.91563.2008](https://doi.org/10.1152/jappphysiol.91563.2008) PMID: [19541731](https://pubmed.ncbi.nlm.nih.gov/19541731/).
41. Chan KM, Fu SC, Wong YP, Hui WC, Cheuk YC, Wong MW. Expression of transforming growth factor beta isoforms and their roles in tendon healing. *Wound repair and regeneration: official publication of the Wound Healing Society [and] the European Tissue Repair Society*. 2008; 16(3):399–407. doi: [10.1111/j.1524-475X.2008.00379.x](https://doi.org/10.1111/j.1524-475X.2008.00379.x) PMID: [18471258](https://pubmed.ncbi.nlm.nih.gov/18471258/).
42. Pryce BA, Watson SS, Murchison ND, Staverosky JA, Dunker N, Schweitzer R. Recruitment and maintenance of tendon progenitors by TGFbeta signaling are essential for tendon formation. *Development*. 2009; 136(8):1351–61. Epub 2009/03/24. 136/8/1351 [pii] doi: [10.1242/dev.027342](https://doi.org/10.1242/dev.027342) PMID: [19304887](https://pubmed.ncbi.nlm.nih.gov/19304887/); PubMed Central PMCID: [PMC2687466](https://pubmed.ncbi.nlm.nih.gov/PMC2687466/).
43. Brown JP, Finley VG, Kuo CK. Embryonic mechanical and soluble cues regulate tendon progenitor cell gene expression as a function of developmental stage and anatomical origin. *Journal of biomechanics*. 2014; 47(1):214–22. doi: [10.1016/j.jbiomech.2013.09.018](https://doi.org/10.1016/j.jbiomech.2013.09.018) PMID: [24231248](https://pubmed.ncbi.nlm.nih.gov/24231248/); PubMed Central PMCID: [PMC4157071](https://pubmed.ncbi.nlm.nih.gov/PMC4157071/).
44. Brown JP, Galassi TV, Stoppato M, Schiele NR, Kuo CK. Comparative analysis of mesenchymal stem cell and embryonic tendon progenitor cell response to embryonic tendon biochemical and mechanical factors. *Stem cell research & therapy*. 2015; 6:89. doi: [10.1186/s13287-015-0043-z](https://doi.org/10.1186/s13287-015-0043-z) PMID: [25956970](https://pubmed.ncbi.nlm.nih.gov/25956970/); PubMed Central PMCID: [PMC4425922](https://pubmed.ncbi.nlm.nih.gov/PMC4425922/).
45. Berthet E, Chen C, Butcher K, Schneider RA, Alliston T, Amirtharajah M. Smad3 binds Scleraxis and Mohawk and regulates tendon matrix organization. *Journal of orthopaedic research: official publication of the Orthopaedic Research Society*. 2013; 31(9):1475–83. doi: [10.1002/jor.22382](https://doi.org/10.1002/jor.22382) PMID: [23653374](https://pubmed.ncbi.nlm.nih.gov/23653374/); PubMed Central PMCID: [PMC3960924](https://pubmed.ncbi.nlm.nih.gov/PMC3960924/).
46. Bagchi RA, Roche P, Aroutiounova N, Espira L, Abrenica B, Schweitzer R, et al. The transcription factor scleraxis is a critical regulator of cardiac fibroblast phenotype. *BMC biology*. 2016; 14(1):21. doi: [10.1186/s12915-016-0243-8](https://doi.org/10.1186/s12915-016-0243-8) PMID: [26988708](https://pubmed.ncbi.nlm.nih.gov/26988708/); PubMed Central PMCID: [PMC4794909](https://pubmed.ncbi.nlm.nih.gov/PMC4794909/).
47. Liu H, Zhang C, Zhu S, Lu P, Zhu T, Gong X, et al. Mohawk promotes the tenogenesis of mesenchymal stem cells through activation of the TGFbeta signaling pathway. *Stem cells*. 2015; 33(2):443–55. doi: [10.1002/stem.1866](https://doi.org/10.1002/stem.1866) PMID: [25332192](https://pubmed.ncbi.nlm.nih.gov/25332192/).