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# Mechanical press stimulation alleviates MTrPs pain through modulation of the ERK/NF-κB/Piezo2 signaling axis

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# Abstract

This study investigated whether mechanical press stimulation alleviates myofascial trigger point (MTrPs) pain by suppressing the ERK/NF-κB/Piezo2 signaling axis and limiting abnormal activation of unmyelinated sensory nerve endings (USNEs). Adult male Sprague-Dawley rats were subjected to blunt impact combined with eccentric exercise to establish MTrPs. Bioinformatics analysis was first used to identify candidate pathways linking press stimulation to MTrPs. A force-response experiment then compared light (0.3 kg), moderate (0.5 kg), and heavy (0.7 kg) press stimulation with a Piezo2 inhibitor comparator. Pressure pain threshold (PPT), soft tissue tension (STT), histomorphology, inflammatory cytokines and pain mediators (IL-6, IL-1β, TNF-α, COX-2, SP, and BK), and Piezo2 co-expression in $CGRP^+$ and $IB4^+$ USNEs were assessed. Moderate press stimulation (MP) was subsequently selected for pathway validation by measuring p-ERK/ERK, p-p65/p65, and p-IκB/IκB. Finally, an ERK agonist and a Piezo2 inhibitor were introduced in sequential perturbation and rescue experiments to test pathway dependence. Bioinformatics enrichment implicated MAPK/ERK, NF-κB, inflammatory, and mechanosensitive signaling. All press intensities improved pain-related outcomes, but MP and heavy press produced greater reductions in inflammation and Piezo2 expression than light press; MP was selected because it achieved comparable efficacy with a lower theoretical risk of excessive tissue loading. MP significantly reduced ERK, p65, and IκB phosphorylation. ERK activation weakened the analgesic, anti-inflammatory, and Piezo2-suppressive effects of MP, whereas subsequent Piezo2 inhibition restored pain-related outcomes and reduced abnormal $CGRP^+/IB4^+$ sensory-ending activation. These findings indicate that MP alleviates MTrPs pain primarily by inhibiting ERK/NF-κB signaling, thereby reducing inflammatory amplification and Piezo2-dependent USNE hyperexcitability. The data support interruption of an ERK/NF-κB–inflammation–Piezo2 feed-forward circuit as a central mechanism of press stimulation-induced analgesia.


# Introduction

Myofascial trigger points (MTrPs) are a defining pathological feature of myofascial pain syndrome and are clinically recognized as hypersensitive nodules within taut skeletal-muscle bands that provoke local and referred pain [1, 2]. Repetitive mechanical overload, local ischemia, and impaired energy metabolism can destabilize the MTrPs microenvironment, promoting the release of IL-1β, IL-6, TNF-α, COX-2, substance P (SP), and bradykinin (BK) [3, 4]. These mediators lower peripheral nociceptor thresholds, sustain local inflammation, and contribute to persistent mechanical hypersensitivity[5].

The extracellular signal-regulated kinase (ERK)/nuclear factor-κB (NF-κB) pathway provides a plausible molecular bridge between the inflammatory microenvironment and nociceptor sensitization[6]. ERK phosphorylation can facilitate NF-κB activation through p65 and IκB signaling, thereby increasing the transcription and release of pro-inflammatory mediators [7]. In MTrPs, persistent activation of this pathway may therefore maintain both tissue inflammation and pain-related signaling. Conversely, an intervention that reduces ERK/NF-κB phosphorylation would be expected to weaken inflammatory amplification and decrease downstream sensory-terminal excitability[8].

MTrPs pain is also associated with abnormal activation of unmyelinated sensory nerve endings (USNEs), particularly peptidergic CGRP⁺ and non-peptidergic IB4⁺ C-fiber terminals[9-11]. Piezo2 is a mechanically gated ion channel expressed in peripheral sensory neurons and contributes to the conversion of tissue deformation into depolarizing currents [12, 13]. In an inflammatory environment, increased Piezo2 expression or sensitization can enhance mechanically evoked firing and amplify nociceptive input. Excessive Piezo2 activity in CGRP⁺ and IB4⁺ endings may therefore represent a downstream effector through which ERK/NF-κB-dependent inflammation is translated into mechanical pain [14].

Mechanical press stimulation is widely used to reduce MTrPs-associated muscle tension and pain and has been reported to influence local circulation, inflammatory mediator release, and nociceptive processing [15, 16]. However, its analgesic mechanism is often described only at the tissue or behavioral level[17]. The previous research of our research group found that press stimulation could directly inhibit the activation of ERK/NF-κB [8]. However, it is still unclear whether this inhibition is related to the decreased expression level of Piezo2 in different USNE groups, and whether the re-activation of ERK would negate the therapeutic effect[18]. Resolving this sequence is essential for moving from an empirical press intervention to a pathway-based explanation of analgesia.

Accordingly, we combined bioinformatics prediction, force-response testing, pathway-protein analysis, and pharmacological perturbation/rescue experiments in a rat MTrPs model. Light, moderate, and heavy press stimulation were first compared to identify an effective and tissue-tolerable parameter. The selected moderate press stimulation was then tested for its ability to suppress ERK/NF-κB phosphorylation. Finally, an ERK agonist was used to challenge the effect of moderate press stimulation, followed by Piezo2 inhibition to determine whether Piezo2 functions as a critical downstream mediator. We hypothesized that press stimulation relieves MTrPs pain through the sequential mechanism of ERK/NF-κB inhibition, reduced inflammatory signaling, downregulation of Piezo2 in CGRP⁺/IB4⁺ USNEs, and attenuation of abnormal nociceptive input.

# Results

### Screening of Press Stimulation and MTrPs-Related Targets and Bioinformatics Analysis Results

To investigate the mechanism underlying the therapeutic effect of pressure stimulation (Press) on MTrPs, we performed bioinformatics analysis to explore how Press alleviates MTrPs pathology. A total of 647 overlapping targets were identified between press stimulation-related and MTrPs-related datasets. The protein–protein interaction network contained 596 nodes and 9,265 edges, indicating extensive connectivity among the candidate targets. GO enrichment highlighted responses to mechanical stimuli, inflammatory processes, regulation of the ERK1/2 cascade, canonical NF-κB signaling, and cytokine production. KEGG analysis further enriched MAPK, NF-κB, and TNF signaling and inflammatory regulation of mechanosensitive channels. Core nodes included IL1B, IL6, TNF, MAPK1, and NFKB1. These results did not establish causality but supported the testable hypothesis that press stimulation may relieve MTrPs by suppressing inflammatory ERK/NF-κB signaling and downstream mechanosensitive nociceptive transmission (Figure 2).

### Effects of Press Stimulation with Different Forces on Inflammatory in MTrPs and Piezo2 Expression in USNEs

Guided by the bioinformatics results, we next determined whether press stimulation altered pain behavior, local inflammation, and Piezo2 expression in a force-dependent manner. Control, model, LP, MP, HP, and PI groups were established. LP, MP, and HP were delivered locally at 0.3, 0.5, and 0.7 kg, respectively, at 10 cycles/min for 7.5 min per session, once every other day for seven sessions[19]. The PI group received GsMTx-4 TFA (270 μg/kg, intraperitoneally) on the same schedule and served as a pathway-oriented comparator for Piezo channel inhibition[20] (Figure 3A).

Compared with the model group, all three press intensities and PI increased PPT and $D_{0.2}$ values, indicating reduced mechanical hypersensitivity and improved soft-tissue compliance (Figure 3B–C). MP and HP produced greater improvements than LP, whereas MP, HP, and PI did not differ significantly in the principal behavioral outcomes. H&E staining showed that LP partially reduced inflammatory infiltration, while MP and HP more clearly restored myofiber arrangement and reduced round, deeply stained fibers; MP showed robust histological improvement without the higher mechanical load applied in HP (Figure 3D). ELISA demonstrated that LP, MP, HP, and PI reduced IL-1β, IL-6, TNF-α, COX2, SP, and BK. MP and HP generally produced larger reductions than LP, and IL-1β was lower after MP than after HP (Figure 3E–J). Immunofluorescence further showed that all press intensities decreased Piezo2 co-expression in both $CGRP^+$ and $IB4^+$ USNEs, with MP and HP producing greater suppression than LP and effects broadly comparable to PI (Figure 3K–N).

Together, these findings establish a coherent force-response pattern: press stimulation reduces pain, tissue inflammation, and Piezo2-associated sensory-ending activation, and the effect plateaus between moderate and heavy force. Because MP achieved efficacy comparable to HP while avoiding unnecessary additional loading, MP was selected as the optimal condition for testing the upstream ERK/NF-κB mechanism.

### Moderate Press Stimulation Suppresses ERK/NF-κB Activation in MTrPs

Figure 3 demonstrated that MP simultaneously improved pain-related behavior, reduced inflammatory mediators, and downregulated Piezo2 in sensory endings. These coordinated effects suggested involvement of an upstream inflammatory signaling pathway, but the force-response experiment alone could not determine whether ERK/NF-κB activity was directly altered by MP. We therefore examined phosphorylation of ERK, p65, and IκB in control, model, and MP groups. The model group showed significantly increased p-ERK/ERK, p-p65/p65, and p-IκB/IκB ratios relative to controls, confirming activation of ERK/NF-κB signaling in MTrPs. MP significantly reduced all three phosphorylation ratios compared with the model group (Figure 4A–D). Thus, the transition from the phenotypic effects in Figure 3 to the signaling results in Figure 4 indicates that MP does not merely suppress downstream inflammatory markers and Piezo2 expression; it also inhibits the upstream ERK/NF-κB pathway that can coordinate these changes.

### ERK Activation Counteracts MP Analgesia, Whereas Piezo2 Inhibition Rescues the Phenotype

To determine whether MP stimulation suppresses abnormal USNEs activation by targeting Piezo2 channels via the ERK/NF-κB pathway, we established control, model, MP, MP+ERK agonist (EA), and MP+EA+Piezo2 inhibitor (PI) groups. MP (0.5 kg, 10 times/min, 7.5 min/session, every other day for 7 sessions) was applied

locally. EA (LM22B-10, 50 mg/kg, i.p.) and PI (GsMTx-4 TFA, 270 μg/kg, i.p.) were administered as described. Rats in the MP+EA group were intraperitoneally injected with LM22B-10 (MedChemExpress, HY-104047, China) at 50 mg/kg, followed by MP stimulation 1 h later, intervened once every other day for a total of 7 interventions [21]. Rats in the MP+EA+PI group were intraperitoneally injected with LM22B-10 at 50 mg/kg, followed by intraperitoneal injection of GsMTx-4 TFA at 270 μg/kg 1 h later,and received moderate pressure intervention 30 min later, intervened once everyother day for a total of 7 interventions [21, 22] (Fig 5A).

MP stimulation alone markedly improved pain behaviors, as reflected by increased PPT and $D_{0\text{-}2}$ compared to the model group. Co-administration of MPand EA (MP+EA) reversed this effect, reducing PPT and $D_{0\text{-}2}$, while subsequent addition of PI (MP+EA+PI) restored these parameters to levels comparable to MP alone (Fig 5B–C). HE staining revealed that MP treatment ameliorated myocyte disarray, reduced the number of round, deeply stained cells, and decreased inflammatory infiltration. EA counteracted these improvements, and PI reversed the EA-induced aggravation (Fig 5D). WB analysis showed that MP suppressed the ERK/NF-κB pathway, as evidenced by reduced p-ERK, p-p65 and p-IκB levels. EA increased these phosphorylation levels, and PI reversed this effect (Fig 5E–H). ELISA results demonstrated that MP reduced the levels of pro-inflammatory cytokines (TNF-α, IL-1β, IL-6) and pain mediators (SP, BK, COX2). EA elevated these mediators, and PI significantly lowered them compared to the MP+EA group (Fig 5I–J). IF revealed that MP downregulated Piezo2 expression in both $CGRP^+$ and $IB4^+$ USNEs. EA upregulated Piezo2 co-localization, and PI reversed this upregulation (Fig 5K–N).

Taken together, these results indicate that MP stimulation targets Piezo2 channels in USNEs by inhibiting the ERK/NF-κB pathway, thereby suppressing abnormal USNEs activation and alleviating pain in MTrPs rats.

## Discussion

This study establishes a stepwise mechanistic explanation for press stimulation-induced analgesia in MTrPs. Bioinformatics first identified ERK/NF-κB, inflammatory, and mechanosensitive signaling as candidate components. Force-response experiments then showed that press stimulation simultaneously improved pain behavior, reduced local inflammatory mediators, and decreased Piezo2 expression in $CGRP^+$ and $IB4^+$ USNEs, with MP providing the most favorable balance between efficacy and mechanical load. Direct protein analysis demonstrated that MP suppressed ERK, p65, and IκB phosphorylation. Finally, ERK activation weakened the effects of MP, whereas Piezo2 inhibition rescued the ERK-activated phenotype. The convergent results support a central logic: MP inhibits ERK/NF-κB signaling, reduces inflammatory amplification and Piezo2 expression, limits abnormal USNE activation, and thereby alleviates MTrPs pain.

The enrichment of MAPK/ERK, NF-κB, TNF, cytokine-production, and mechanical-stimulus pathways provided an unbiased rationale for examining ERK/NF-κB and Piezo2 experimentally. ERK/NF-κB signaling is a major regulator of inflammatory transcription [23, 24], whereas Piezo2 converts mechanical deformation into sensory-neuron excitation [14, 25]. Their convergence is biologically plausible in MTrPs, where tissue loading, ischemia, inflammatory mediator accumulation, and nociceptor sensitization coexist. Nevertheless, database enrichment alone cannot determine pathway direction. For this reason, the subsequent

experiments were deliberately arranged to move from dose-response association to direct pathway measurement and finally to agonist challenge and downstream rescue.

All tested press intensities improved PPT, STT, histological injury, inflammatory mediator levels, and Piezo2 expression, demonstrating that the analgesic response was accompanied by coordinated tissue, biochemical, and neural-terminal changes. MP and HP were consistently more effective than LP, indicating that insufficient mechanical input may not adequately modify the pathological microenvironment [26]. However, HP did not provide a clear additional benefit over MP, and MP produced favorable histomorphological recovery with lower applied force[27]. This plateau supports MP as a rational parameter for mechanistic testing and is consistent with evidence that appropriately dosed deep pressure can modulate pain sensitivity, whereas excessive loading may increase discomfort or tissue stress [28]. Importantly, the similarity between MP and the Piezo inhibitor comparator suggested that suppression of mechanosensitive sensory signaling contributed substantially to the behavioral effect[29].

Figure 4 demonstrated that MP also reduced p-ERK/ERK, p-p65/p65, and p-IκB/IκB, placing inhibition of the ERK/NF-κB pathway upstream of the observed anti-inflammatory and sensory-terminal changes[30]. ERK/NF-κB activation can promote IL-1β, IL-6, TNF-α, and COX2 expression and amplify SP- and BK-related nociceptive signaling [31, 32]. These mediators can enhance mechanosensitive-channel expression or function, while Piezo2-dependent $Ca^{2+}$ entry can further promote neuronal excitability and inflammatory signaling [33]. MP may therefore interrupt a self-reinforcing circuit rather than acting on a single endpoint[34].

The ERK agonist experiment provided a stronger test of causality than comparisons between untreated and MP groups alone. Reactivating ERK during MP treatment restored ERK/NF-κB phosphorylation, increased inflammatory and pain mediators, upregulated Piezo2 in $CGRP^+$ and $IB4^+$ endings, worsened histological injury, and reduced the behavioral benefit of MP[23, 35]. Thus, intact suppression of ERK signaling is necessary for the full analgesic effect of MP. The subsequent rescue experiment was particularly informative: Piezo2 inhibition improved pain-related outcomes and sensory-ending activation despite ERK agonism. This places Piezo2 downstream of ERK/NF-κB in the pathway that converts inflammatory signaling into abnormal mechanical nociception[33].

The observation that Piezo2 inhibition also reduced inflammatory and phosphorylation readouts suggests reciprocal amplification. A parsimonious model is that MP initially reduces pathological mechanical and inflammatory signaling at the MTrPs site, suppressing ERK/NF-κB activation and decreasing Piezo2 availability in USNEs[29]. Reduced Piezo2-dependent depolarization then lowers nociceptive input and neurogenic inflammatory reinforcement, which further stabilizes ERK/NF-κB inhibition[36]. Accordingly, the ERK/NF-κB/Piezo2 axis should be interpreted as a hierarchically organized but bidirectionally reinforced circuit. The present data support MP as an intervention that interrupts this circuit at the upstream inflammatory-signaling level and propagates the effect to peripheral sensory terminals[34].

A major strength of this study is the alignment of multiple evidence layers with a single mechanistic question. Bioinformatics generated the hypothesis; force-response testing selected MP; behavioral, histological, ELISA, and immunofluorescence outcomes defined the phenotype; Western blotting established

pathway inhibition; and ERK activation followed by Piezo2 rescue tested pathway dependence. This progression strengthens the interpretation that MP-mediated pain relief is associated with inhibition of the ERK/NF-κB/Piezo2 axis rather than with a nonspecific change in a single biomarker.

Several limitations should be acknowledged. First, all experiments were performed in rats, and the effective force cannot be directly translated to human treatment without accounting for tissue depth, contact area, and individual tolerance. Second, GsMTx-4 TFA is not completely selective for Piezo2 and may also inhibit Piezo1; sensory-neuron-specific Piezo2 knockdown or conditional knockout would provide stronger evidence[37]. Third, $CGRP^+$ and $IB4^+$ endings were evaluated by co-localization rather than cell-type-specific electrophysiology or calcium imaging[33]. Fourth, systemic agonist and inhibitor administration lacks spatial and cellular resolution[38]. Fifth, the study demonstrates pharmacological pathway dependence but does not directly show NF-κB binding to the PIEZO2 promoter or define how press stimulation is sensed upstream of ERK. These questions should be addressed using genetic manipulation, transcriptional assays, in vivo neural recordings, and clinical validation.

# Conclusion

Mechanical press stimulation alleviates MTrPs pain through a force-dependent mechanism that converges on the ERK/NF-κB/Piezo2 signaling axis. MP provided an effective and mechanically conservative condition, increased PPT and STT, reduced tissue inflammation, suppressed ERK, p65, and IκB phosphorylation, and downregulated Piezo2 in $CGRP^+$ and $IB4^+$ USNEs. ERK activation counteracted these effects, whereas Piezo2 inhibition rescued the pain phenotype, identifying Piezo2-dependent sensory-ending hyperactivation as a critical downstream component of ERK/NF-κB-mediated pain amplification. The findings support a model in which MP interrupts an ERK/NF-κB–inflammation–Piezo2 feed-forward circuit and thereby reduces peripheral nociceptive input. This mechanism provides a biological rationale for optimizing press stimulation in MTrPs and identifies the ERK/NF-κB/Piezo2 network as a candidate target for future translational studies.

# Materials and Methods

### Source of Experimental Animals

All 6–8-week-old healthy adult male Sprague-Dawley (SD) rats, weighing 160–200 g, were provided by Hunan Aokelaisi Laboratory Co., Ltd. (certification number: 430727231101163252). Rats were purchased from the Animal Center of Hunan University of Chinese Medicine, with a license number of SYXK (Xiang) 2019-0009. Rats were housed 3 per cage in the Experimental Animal Center of Hunan University of Chinese Medicine under controlled conditions (temperature: 24–26°C, humidity: 50–70%, 12 h/12 h light/dark cycle) with free access to food and water. After 7 days of environmental adaptation, experiments were initiated.All experimental procedures involving animals were conducted in accordance with the ARRIVE guidelines and relevant institutional and national regulations for the care and use of laboratory animals. This study was approved by the Experimental Animal Ethics Committee of Hunan University of Chinese Medicine (ethical approval number: LL2023020803).

### Establishment and Evaluation of MTrPs Model

The model was established by modified blunt impact combined with eccentric exercise, as previously described by Huang Qiangmin's team[39]. Rats underwent adaptive training on a treadmill at a 0° incline and 16 m/min speed for 15 minutes, once every 2 days for a total of 3 sessions. Subsequently, an 8-week modeling protocol was implemented. Blunt impact was administered on the first day of each week, followed by treadmill eccentric exercise on the second day, with normal housing and no additional interventions for the remaining 5 days. Rats were anesthetized via inhalation of 4% isoflurane, fixed to the base of the impact device, and the medial thigh of the left hindlimb was exposed and marked. A blunt weighted rod (mass 1200 g, kinetic energy 2.352 J) was dropped from a height of 20 cm onto the marked site. After recovery from anesthesia, rats were returned to their cages. On the day following impact, rats performed eccentric exercise on a treadmill set at a -16° incline, 16 m/min speed, for 90 minutes, with mechanical and auditory stimuli used to encourage running. A 4-week recovery period followed, during which all rats received normal housing and regular observation. Successful model establishment was confirmed by post-recovery palpation of distinct nodules or cord-like taut bands at the impact site, accompanied by significantly lower PPT and STT values compared to control rats.

**Experimental Design, Grouping, and Interventions**

The experimental workflow was organized to test the proposed analgesic pathway in a stepwise manner. After bioinformatics screening, a force-response experiment compared control, model, light press (LP), moderate press (MP), heavy press (HP), and Piezo2 inhibitor (PI) groups to determine whether press stimulation reduced pain, inflammation, and Piezo2 expression in a force-dependent manner. Because MP and HP produced comparable effects and MP imposed a lower mechanical load, MP was selected for mechanistic validation. ERK/NF-κB activation was then compared among control, model, and MP groups. Finally, control, model, MP, MP plus ERK agonist (MP+EA), and MP plus ERK agonist plus Piezo2 inhibitor (MP+EA+PI) groups were used to test whether ERK activation counteracted MP-induced analgesia and whether Piezo2 inhibition rescued the phenotype. The detailed intervention protocols are summarized in Table 1.

**Table 1. Experimental groups and intervention protocols**

| Group | Abbreviation | Intervention | Agent / Parameter | Route | Regimen |
|---|---|---|---|---|---|
| Control | Control | No intervention (normal feeding) | — | — | — |
| Model | Model | MTrPs modeling | — | — | — |
| Light press | LP | Mechanical press stimulation | 0.3 kg, 10 times/min, 7.5 min/session | Local pressing on MTrPs | Once every other day, 7 sessions |
| Moderate press | MP | Mechanical press stimulation | 0.5 kg, 10 times/min, 7.5 min/session | Local pressing on MTrPs | Once every other day, 7 sessions |
| Heavy press | HP | Mechanical press stimulation | 0.7 kg, 10 times/min, 7.5 min/session | Local pressing on MTrPs | Once every other day, 7 sessions |
| Piezo2 inhibitor | PI | GsMTx-4 TFA(MedChemExpress, HY-P1410A, China) | 270 μg/kg | Intraperitoneal injection | Once every other day, 7 administrations |
| Moderate press + ERK agonist | MP+EA | LM22B-10(MedChemExpress, HY-104047, China) i.p., then MP stimulation 1 h later | 50 mg/kg + 0.5 kg press | Intraperitoneal + local pressing | Once every other day, 7 sessions |
| Moderate press + ERK agonist + Piezo2 inhibitor | MP+EA+PI | LM22B-10 i.p., then GsMTx-4 TFA 1 h later, then MP 30 min later | 50 mg/kg + 270 μg/kg + 0.5 kg press | Intraperitoneal + local pressing | Once every other day, 7 sessions |

**Tissue Collection and Euthanasia**

At the end of the experimental procedures, rats were euthanized by exposure to an overdose of isoflurane (>5%) until respiratory arrest occurred. Death was confirmed before tissue collection.

**Outcome Measures**

**PPT Measurement of MTrPs**

A mechanical pain threshold meter was used for assessment. One researcher restrained the rat in a supine position with hindlimbs extended to ensure calmness. Another researcher identified and marked muscle taut bands or contraction nodules at the modeled site via palpation. The marked area was pressed slowly using the mechanical pain threshold meter, with the control group receiving pressure at the corresponding site. The threshold value was recorded when the rat exhibited sudden leg withdrawal or vocalization. Each rat was

tested 3 times at 10-minute intervals, and the mean value was taken as the PPT. All PPT measurements were performed on six independent rats per group.

**STT Measurement of MTrPs**

A soft tissue tension meter was employed for evaluation. The detector probe was aligned perpendicular to the target area, applying uniform pressure for 3 seconds followed by gradual release over 3 seconds. The displacement corresponding to 0.2 kg force (D0.2kg) from the tension-displacement curve was selected as the primary indicators to reflect muscle soft tissue tension. Each rat was tested 3 times at 10-minute intervals, and the mean value was recorded as the D0.2 for soft tissue tension. All STT measurements were performed on six independent rats per group.

**Histopathological Assessment by HE Staining**

MTrPs samples were fixed in 4% paraformaldehyde. Sections were dewaxed in xylene I and II for 10 minutes each, followed by gradient hydration in absolute ethanol I, absolute ethanol II, 95% ethanol, 80% ethanol, and 70% ethanol, with 2 distilled water rinses. Sections were stained in hematoxylin solution for 10 minutes at room temperature, rinsed under running tap water for 2 minutes, differentiated in 1% hydrochloric acid-ethanol for 10 seconds, immediately blued in tap water for 2 minutes, and rinsed in distilled water for 1 minute. Subsequent eosin staining was performed using 0.5%-1% eosin solution for 5 minutes at room temperature. Water-soluble eosin was rinsed with distilled water to remove excess stain, while alcohol-soluble eosin was quickly differentiated with 95% ethanol. Sections were dehydrated in 95% ethanol, absolute ethanol I, and absolute ethanol II, cleared in xylene I and II for 1 minute each, and mounted with neutral balsam. Finally, images were captured using a microscopic imaging system. HE staining was performed on three independent rats per group (n=3), with three non-overlapping fields per section analyzed.

**ELISA Quantification of Inflammatory and Pain Mediators**

The levels of IL-6, TNF-α, IL-1β, COX2, SP and BK in MTrPs were measured using IL-6 kit (Shanghai Jianglai Biotechnology Co., Ltd., cat. no.: JL20896, China), IL-1β kit (Shanghai Jianglai Biotechnology Co., Ltd., cat. no.: JL20884, China), TNF-α kit (Shanghai Jianglai Biotechnology Co., Ltd., cat. no.: JL13202, China), COX2 kit (Shanghai Jianglai Biotechnology Co., Ltd., cat. no.: JL21044, China), SP kit (Shanghai Jianglai Biotechnology Co., Ltd., cat. no.: JL12596, China), and BK kit (Shanghai Jianglai Biotechnology Co., Ltd., cat. no.: JL21045, China). Each ELISA assay was performed in triplicate on samples from three independent rats per group.

**Immunofluorescence Analysis of Piezo2 in USNEs**

Paraffin-embedded MTrPs sections were dewaxed in xylene and hydrated with gradient ethanol, followed by antigen retrieval in sodium citrate buffer (pH 6.0) under high temperature and pressure. Non-specific binding was blocked with PBS containing 5% bovine serum albumin (BSA) for 1 hour at room temperature. Sections were then incubated overnight at 4°C with primary antibody mixtures: Anti-CGRP antibody (abcam, ab283568, 1:100) to label peptidergic nerve endings, IB4 lectin (Sigma-Aldrich, L2140, 1:100) to label non-peptidergic nerve endings, and PIEZO2 antibody (Novus Biologicals, NBP1-78624, 1:200) to detect mechanosensitive channel expression. The next day, sections were incubated with Alexa Fluor 488-

conjugated goat anti-rabbit IgG (1:500) and Cy3-conjugated streptavidin (1:1000) as secondary reagents, followed by DAPI counterstaining for nuclei. Multichannel images were acquired using a confocal microscope (Zeiss LSM 880), and the co-localization ratios of CGRP with Piezo2 and IB4 with Piezo2 were quantified using ImageJ software. Immunofluorescence was performed on three independent rats per group (n=3), with three random fields per section analyzed.

### Western Blot Analysis of ERK/NF-κB Signaling in MTrPs

Western blotting was performed to determine whether MP altered activation of the ERK/NF-κB pathway. Total protein was extracted from MTrPs tissue, separated by SDS-PAGE, transferred to membranes, and blocked with 5% non-fat milk. Membranes were incubated with antibodies against ERK1/2 (Abcam, ab184699, 1:10000), p-ERK (Abcam, ab229912, 1:1000), NF-κB p65 (Huaan Bio, ET1603-12, 1:1000), p-NF-κB p65 (Bioss, bs-3485R, 1:200), IκBα (Huaan Bio, ET1603-6, 1:5000), p-IκBα (Huaan Bio, HA722770, 1:5000), and GAPDH (Huaan Bio, EM1101, 1:10000). After secondary-antibody incubation and chemiluminescent detection, band intensities were quantified using Image-Pro Plus 6.0. Pathway activation was expressed as the phosphorylation ratios p-ERK/ERK, p-p65/p65, and p-IκB/IκB; GAPDH was used to verify protein loading. The MP pathway-validation experiment included five independent rats per group, whereas the combined pharmacological experiment was analyzed using the sample size indicated in the corresponding figure legend.

### Bioinformatics Analysis of Press Stimulation- and MTrPs-Related Targets

#### Target Retrieval and Intersection Analysis

Press stimulation-related targets were retrieved from GeneCards and OMIM using the terms “physical stimulation,” “massage,” and “manipulation.” MTrPs-related targets were obtained from GeneCards, OMIM, and DisGeNET. After duplicate removal, targets meeting the predefined database thresholds were intersected and visualized using the Weishengxin platform. This in silico analysis was used to generate a pathway hypothesis for subsequent experimental testing and was not interpreted as causal evidence by itself.

#### Protein–Protein Interaction Network Construction

Intersecting database targets and literature-supported candidates were imported into STRING with the species restricted to Homo sapiens and the confidence score set to 0.400. Network data were visualized in Cytoscape v3.10.1. Degree centrality and related topological parameters were used to identify highly connected targets and to prioritize signaling modules potentially linking press stimulation to inflammation and mechanotransduction.

#### GO and KEGG Enrichment Analyses

Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analyses were performed using DAVID. The KEGG database was used for pathway annotation and enrichment analysis[40, 41]. Biological Process, Molecular Function, and Cellular Component terms, together with enriched signaling pathways, were ranked and visualized using Weishengxin. Particular attention was paid to mechanical-

stimulus responses, inflammatory signaling, MAPK/ERK and NF-κB pathways, and mechanosensitive ion-channel regulation because these categories directly informed the subsequent press-stimulation experiments.

### Statistical Analysis

All data were analyzed using SPSS 26.0. Quantitative data are presented as mean ± standard deviation ($\bar{x}\pm s$). For normally distributed and homoscedastic data, paired t-tests were used for intragroup comparisons before and after treatment, and one-way analysis of variance (ANOVA) was used for intergroup comparisons. Otherwise, the rank sum test was applied. $P < 0.05$ was considered statistically significant.

## Declarations

### Availability of data and materials

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

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### Contributions

Experiment design: LYT, WL. Experiment development: LYT, XYL. Project management: JSL, WL. Analysis of data: LYT, LYT, YQZ, KSL, FL. Paper review: XF, JC. Paper writing: LYT, XYL, WL. Experimental support: JSL, WL, LYT. All authors approved the final version of the manuscript.

### Ethics approval and consent to participate

This study was approved by Hunan University of Traditional Chinese Medicine Animal Welfare and Ethics Committee (Approval No. LL2023020803).

**Informed consent**

Not applicable.

**Competing interests**

All authors declare no competing of interest.

**Clinical trial number**

Not applicable.

# References


1. Kobylarz, M. D. et al. Shear-Wave Elastography Supports That Muscle Stiffness Should Not Be Considered a Criterion to Determine the Presence of Myofascial Trigger Points in the Infraspinatus Muscle. *Am. J. Phys. Med. Rehabil*. **104** (10), 890–895 (2025).
2. Ziembicki, T. Nerve entry points - The anatomy beneath trigger points. *J. Bodyw. Mov. Ther.* **35**, 121–123 (2023).
3. Lam, C. et al. Myofascial pain - A major player in musculoskeletal pain. *Best Pract. Res. Clin. Rheumatol.* **38** (1), 101944 (2024).
4. Eloqayli, H. Subcutaneous accessory pain system (SAPS): A novel pain pathway for myofascial trigger points. *Med. Hypotheses*. **111**, 55–57 (2018).
5. Mazza, D. F., Boutin, R. D. & Chaudhari, A. J. Assessment of Myofascial Trigger Points via Imaging: A Systematic Review. *Am. J. Phys. Med. Rehabil*. **100** (10), 1003–1014 (2021).
6. Yu, C., Li, Z., Nie, C., Chang, L. & Jiang, T. Targeting Src homology phosphatase 2 ameliorates mouse diabetic nephropathy by attenuating ERK/NF-κB pathway-mediated renal inflammation. *Cell. Commun. Signal.* **21** (1), 362 (2023).
7. Guo, J. & Yang, Y. Parecoxib sodium alleviates ischemia reperfusion-induced pulmonary injury via inhibiting ERK/NF-κB and further activating the HIF–1α pathway. *Immun. Inflamm. Dis.* **10** (9), e684 (2022).
8. Liu, D. et al. Modulation effects of pressing manipulation on local inflammatory responses and ERK/NF-κB pathway in trigger point model rats. *J. Acupunct. Tuina Sci.* **22** (5), 371–380 (2024). (in Chinese).
9. Matsumoto, K. et al. Immunohistochemical characterization of transient receptor potential vanilloid types 2 and 1 in a trinitrobenzene sulfonic acid-induced rat colitis model with visceral hypersensitivity. *Cell. Tissue Res.* **391** (2), 287–303 (2023).
10. Oikawa, I. et al. A descending inhibitory mechanism of nociception mediated by an evolutionarily conserved neuropeptide system in Drosophila. *Elife* 12. (2023).

11. Lu, H. E. et al. Proof-of-concept: Differentiating upper trapezius muscle with myofascial trigger point using deep learning model on a small prospective sEMG dataset. *J. Electromyogr. Kinesiol.* **88**, 103132 (2026).
12. Tang, F., Jiang, C., Chen, J., Wang, L. & Zhao, F. Global hotspots and trends in Myofascial Pain Syndrome research from 1956 to 2022: A bibliometric analysis. *Med. (Baltim).* **102** (12), e33347 (2023).
13. Sánchez-Carranza, O. et al. Piezo2 voltage-block regulates mechanical pain sensitivity. *Brain* **147** (10), 3487–3500 (2024).
14. Sonkodi, B. PIEZO2 Proton Affinity and Availability May Also Regulate Mechanical Pain Sensitivity, Drive Central Sensitization and Neurodegeneration. *Int. J. Mol. Sci.* **26**(3). (2025).
15. Dinçer, Ş., Devran, S., Savaş, E. Ç., Pasin, Ö. & Bayraktar, B. Efficacy of capacitive-resistive therapy on the treatment of myofascial pain - A randomized control trial. *J. Bodyw. Mov. Ther.* **38**, 86–91 (2024).
16. Yang, Z. et al. Characteristics of Pain Changes in Rats with Nerve Injury within 24 Hours after One-Time Tuina Intervention. J Vis Exp. (203). (2024).
17. Nahomi Kuroda, M. et al. Effect of ischemic compressions versus extracorporeal shockwave therapy on myofascial trigger points: A protocol of a randomized controlled trial. *PLoS One*. **18** (3), e0283337 (2023).
18. Kartaloglu, I. F. & Kus, A. A. Evaluation of Radial Extracorporeal Shock Wave Therapy on Treatment-Resistant Trigger Points Using Sonographic Shear Wave Elastography. *J. Coll. Physicians Surg. Pak*. **33** (10), 1159–1164 (2023).
19. Jiang, Q. et al. Effects of different strength of pressing massage on myofascial trigger points with chronic pain in rats. **28**(27): 4360–4366. (2024). (in Chinese).
20. Park, S. P. et al. A tarantula spider toxin, GsMTx4, reduces mechanical and neuropathic pain. *Pain* **137** (1), 208–217 (2008).
21. Jia, X. et al. Infiltrated Macrophages Aggravate TMJOA Chronic Pain via Piezo2 in IB4+-TG Neurons. *Oral Dis.* **31** (6), 1841–1853 (2025).
22. Schaefer, I. et al. PKA mediates modality-specific modulation of the mechanically gated ion channel PIEZO2. *J. Biol. Chem.* **299** (6), 104782 (2023).
23. Lin, L. L. et al. GPR34 Knockdown Relieves Cognitive Deficits and Suppresses Neuroinflammation in Alzheimer's Disease via the ERK/NF-κB Signal. *Neuroscience* **528**, 129–139 (2023).
24. Zhang, R. et al. Protective Effect of an Isoflavone, Tectorigenin, Against Oxidative Stress-induced Cell Death via Catalase Activation. *J. Cancer Prev.* **21** (4), 257–263 (2016).
25. Villarino, N. W. et al. Labeling PIEZO2 activity in the peripheral nervous system. *Neuron* **111** (16), 2488–2501e8 (2023).
26. Zhang, M., Zhu, Y., Jin, F., Liu, Y. & Yin, L. The peripheral PI3K/AKT/MLC signalling pathway alleviates myofascial pain in rats by inhibiting abnormal contraction at myofascial trigger points. *Mol. Pain*. **21**, 17448069251376205 (2025).
27. Strickland, M., Luera, M. J., Parra, M. E. & Riggs, C. The protective model of myofascial trigger points: a testable systems-level framework based on strain-repair mismatch. *Front. Physiol.* **17**, 1818589 (2026).

28. Zhai, T., Jiang, F., Chen, Y., Wang, J. & Feng, W. Advancing musculoskeletal diagnosis and therapy: a comprehensive review of trigger point theory and muscle pain patterns. *Front. Med. (Lausanne)*. **11**, 1433070 (2024).
29. Fernández-Trillo, J., Villaro-Capellán, M., de la Peña, E., Viana, F. & Gomis, A. Piezo2 mediates mechanical sensitivity and injury-induced hypersensitivity in MrgprD-expressing nociceptors. *Pain* **167** (6), 1404–1416 (2026).
30. Huang, Z. et al. Interleukin–19 enhances eosinophil infiltration through upregulation of epithelium-derived RANTES expression via the ERK/NF-κB signalling pathway in patients with eosinophilic CRSwNP. *Inflamm. Res.* **73** (4), 499–513 (2024).
31. Cai, J. et al. PCSK9 promotes T helper 1 and T helper 17 cell differentiation by activating the nuclear factor-κB pathway in ankylosing spondylitis. *Immun. Inflamm. Dis.* **11** (5), e870 (2023).
32. Li, M. et al. Tumor necrosis factor α upregulates the bile acid efflux transporter OATP3A1 via multiple signaling pathways in cholestasis. *J. Biol. Chem.* **298** (2), 101543 (2022).
33. Lv, L. et al. Pressure-mediated mechanosensitive Piezo2 channel is activated in neurogenic bladder and promote bladder fibrosis in children. *J. Mol. Med. (Berl)* **104**(1). (2026).
34. Lin, C. M. et al. Adenine suppresses inflammatory response in vascular smooth muscle cells via modulating AMPK/p53/NF-κB cascade. *Eur. J. Pharmacol.* **1007**, 178239 (2025).
35. Xia, A. et al. The neuroprotection of hyperbaric oxygen therapy against traumatic brain injury via NF-κB/MAPKs-CXCL1 signaling pathways. *Exp. Brain Res.* **240** (1), 207–220 (2022).
36. Xu, H. et al. Piezo2 Mediates a Vicious Cycle of Mechanical Homeostasis Imbalance-Inflammation in Sensory Nerves and the Cartilage Endplate. *Adv. Sci. (Weinh)*. **13** (38), e07299 (2026).
37. Lee, P. R. et al. Piezo1 mediates mechanical signals in TRPV1-positive nociceptors in mice. *Acta Physiol. (Oxf)*. **240** (11), e14236 (2024).
38. Yang, W. et al. 4-DMB, a diclofenac derivative, alleviates DSS-induced ulcerative colitis in mice by binding TNFR1 to inhibit inflammation. *Biochem. Pharmacol.* **252**, 118207 (2026).
39. Huang, Q. M., Ye, G., Zhao, Z. Y., Lv, J. J. & Tang, L. Myoelectrical activity and muscle morphology in a rat model of myofascial trigger points induced by blunt trauma to the vastus medialis. *Acupunct. Med.* **31** (1), 65–73 (2013).
40. Kanehisa, M. & Goto, S. KEGG: kyoto encyclopedia of genes and genomes. *Nucleic Acids Res.* **28** (1), 27–30 (2000).
41. Kanehisa, M., Sato, Y., Kawashima, M., Furumichi, M. & Tanabe, M. KEGG as a reference resource for gene and protein annotation. *Nucleic Acids Res.* **44** (D1), D457–D462 (2016).

# Figures

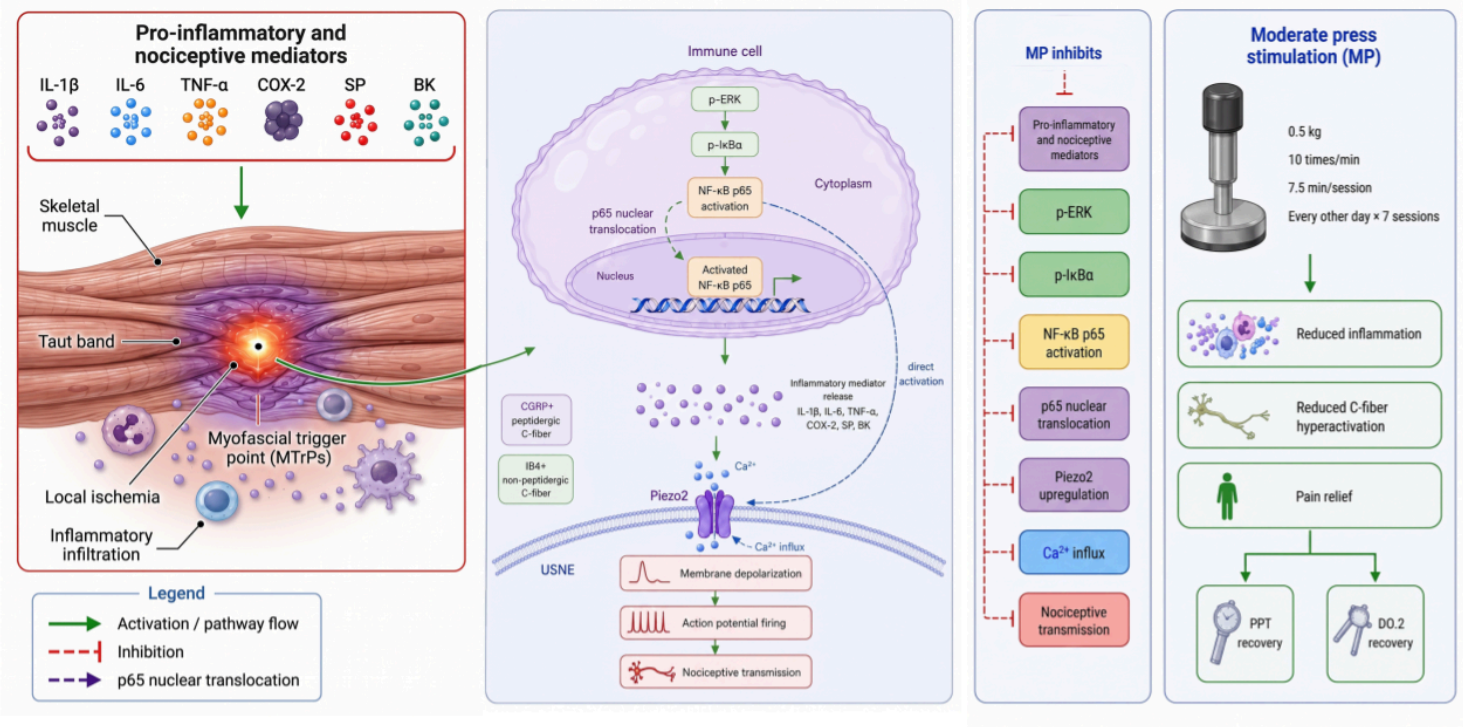



**Figure 1**

**Graphic abstract**

Mechanism diagram of press stimulation inhibiting the ERK/NF-κB/Piezo2 pathway to target abnormal activation of USNEs in MTrPs.

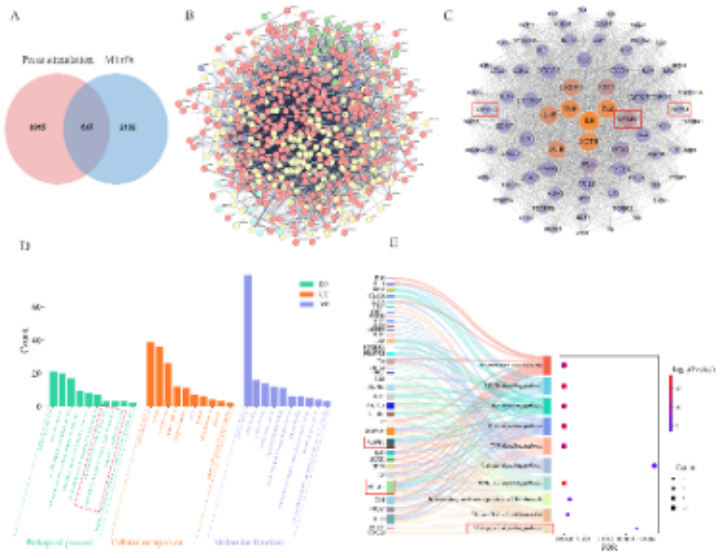
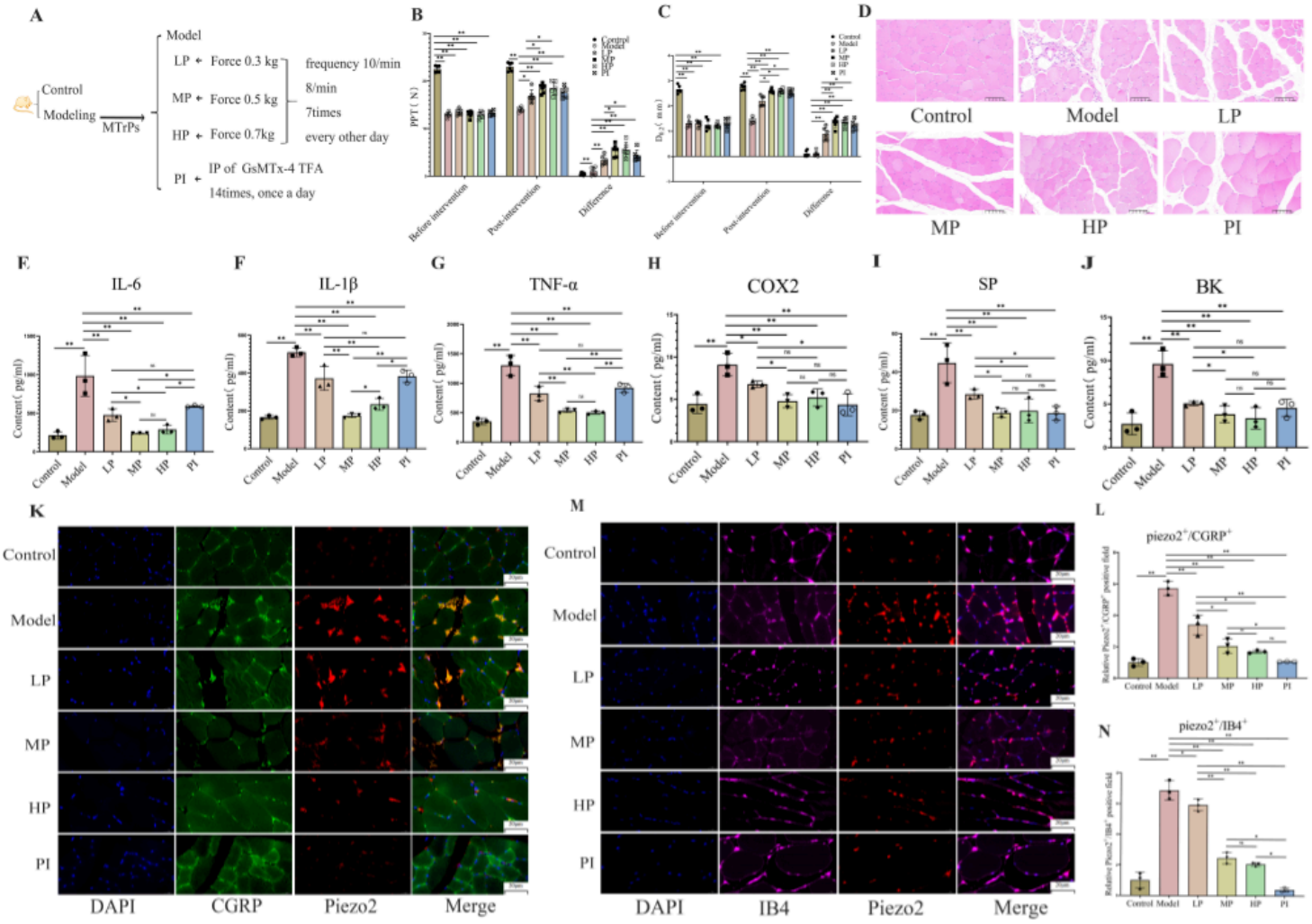


**Figure 2**

Bioinformatics analysis of press stimulation- and MTrPs-related targets. A, Venn diagram showing 647 intersecting targets. B, Protein–protein interaction network containing 596 nodes and 9,265 edges (confidence score, 0.400). C, Topological analysis of 77 highly connected genes; node size and color indicate

relative network scores. D, GO enrichment analysis, including Biological Process, Cellular Component, and Molecular Function categories. E, KEGG enrichment analysis of key targets and pathways.




## Figure 3

Effects of Press Stimulation with Different Forces on Inflammatory in MTrPs and Piezo2 Expression in USNEs. A Experimental scheme. B Comparison of PPT before and after intervention in rats of each group, Difference=Post intervention-Before intervention, n=6. C Comparison of STT before and after intervention in rats of each group, Difference=Post intervention-Before intervention, n=6. D Representative micrographs of HE staining of MTrPs, × 200/50 μm. E ELISA results for IL-6, n=3. F ELISA results for IL-1β, n=3. G ELISA results for TNF-α, n=3. H ELISA results for COX2, n=3. I ELISA results for SP, n=3. J ELISA results for BK, n=3. K-L Co-expression results of Piezo2+/CGRP+, n=3. M-N Co-expression results of Piezo2+/IB4+, n=3.

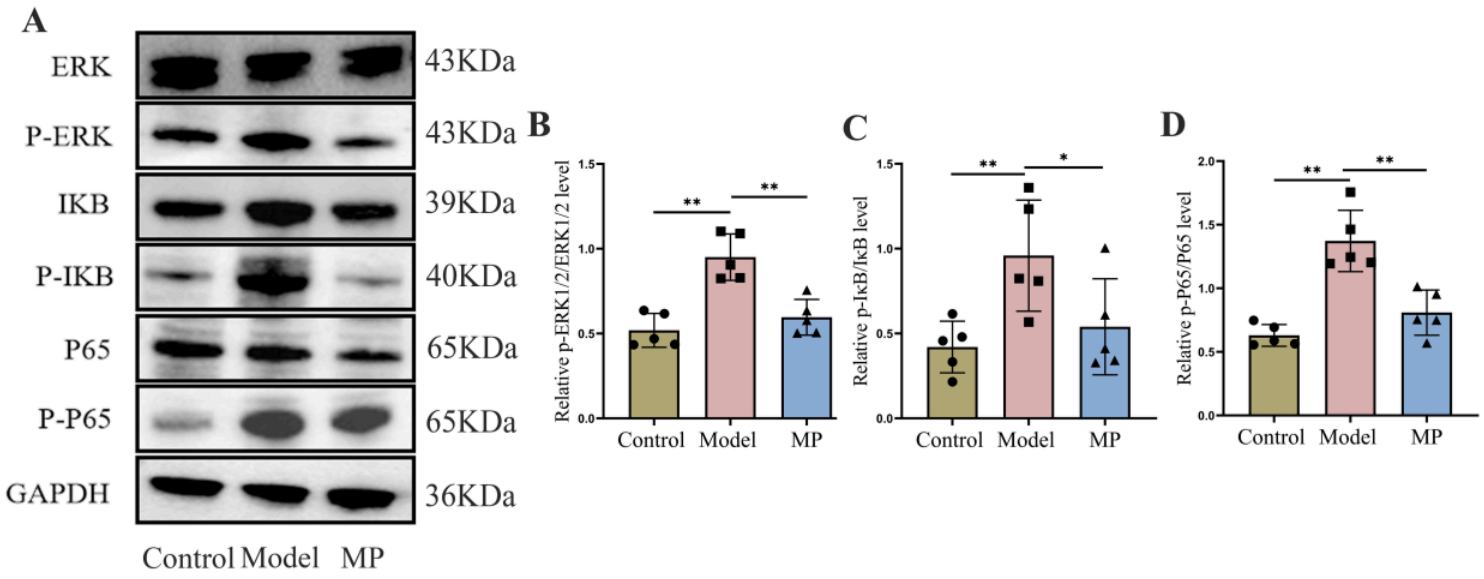
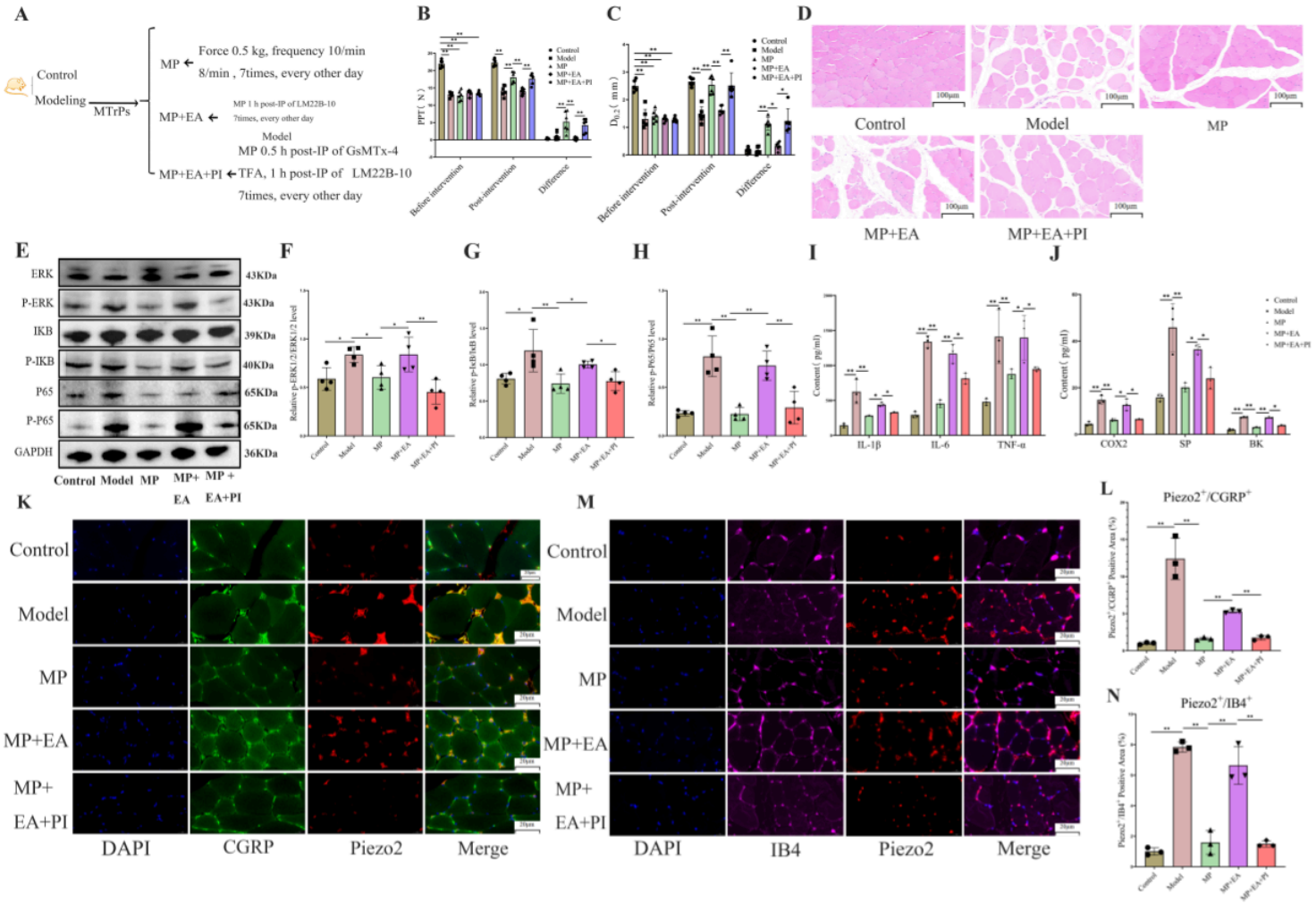



**Figure 4**

Moderate press stimulation suppresses ERK/NF-κB activation in MTrPs. A, Representative immunoblots of ERK, p-ERK, IκB, p-IκB, p65, p-p65, and GAPDH in control, model, and MP groups. B–D, Densitometric quantification of p-ERK/ERK, p-IκB/IκB, and p-p65/p65, respectively; n=5.All Western blots shown in this figure were obtained from full-length original membranes without cropping or splicing.




## Figure 5

ERK activation counteracts MP-induced analgesia, whereas Piezo2 inhibition rescues the phenotype. A, Experimental scheme. B, Change in PPT before and after intervention, n=6. C, Change in STT before and after intervention, n=6. D, Representative H&E-stained MTrPs sections, ×200; scale bar, 50 μm. E, Representative immunoblots of ERK/NF-κB pathway proteins. All Western blots shown in this figure were obtained from full-length original membranes without cropping or splicing. F–H, Quantification of ERK, p65, and IκB phosphorylation ratios, n=4. I, ELISA results for IL-1β, IL-6, and TNF-α, n=3. J, ELISA results for COX2, SP, and BK, n=3. K–L, Piezo2⁺/CGRP⁺ co-expression, n=3. M–N, Piezo2⁺/IB4⁺ co-expression, n=3.