

Deficient Expression of PTPN2 Contributes to JAK/STAT3 Hyperactivation in Juvenile Dermatomyositis via Impaired Negative Regulation

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Abstract

Objective

Interleukin-6/janus kinase/signal transducer and activator of transcription 3 (IL-6/JAK/STAT3) signaling pathway was abnormally activated in patients with juvenile dermatomyositis (JDM). This study aimed to elucidate the negative regulation mechanisms of JAK/STAT3 signaling pathway in the pathogenesis of JDM.

Methods

Primary human skeletal muscle cells (HSMCs) were isolated from treatment naive JDM and controls. Transcription levels of negatively regulated genes of STAT3 were screened by quantitative real time polymerase chain reaction (qPCR). Based on the above results, protein tyrosine phosphatase non-receptor type 2 (*PTPN2*) was selected as the target gene for the following experiments. Western blot analysis of T cell protein tyrosine phosphatase (TC-PTP) was conducted in the biopsy samples of JDM and controls. Phosphorylation level of STAT3 in HSMCs knocked down of *PTPN2* (HSMCs *PTPN2*-) or over-expression of *PTPN2* (HSMCs *PTPN2*+) were tested.

Results

In the major negative regulatory genes of STAT3, suppressors of cytokine signaling (*SOCS*) 2 and *SOCS3* were increased. *SOCS4* and protein tyrosine phosphatase (*PTPN1*, *PTPN2*, *SHP1*, *SHP2*, *PIAS1*, *PIAS2*, and *PTPRD*) were decreased in JDM. *PTPN2* transcriptional levels exhibited the most pronounced and stable decline in primary skeletal muscle cells derived from JDM patients. TC-PTP was observed deficient expression in the muscle tissues of JDM. Over-expression of *PTPN2* in HSMCs leads to declined phosphorylation of STAT3, both in the cytoplasm and within the mitochondria. *PTPN2* deficiency indirectly upregulated *SLN* transcription in primary JDM myocytes.

Conclusion

PTPN2 plays an important role in the regulation of phosphorylation of STAT3 in HSMCs. Deficient expression of *PTPN2* may lead to over-activation of JAK/STAT3 signaling pathway, both in the cytoplasm and within the mitochondria, with dysregulation of the calcium-regulatory protein *SLN*.

Introduction

Juvenile dermatomyositis (JDM) is a rare autoimmune disease with an estimated annual incidence of 2.7 ~ 3.4/10,000 in children ^[1]. It is characterized by chronic non-suppurative inflammation in muscle and skin, manifested as proximal muscle weakness and typical rashes (such as Gottron's papules and

heliotrope rash)^[2]. Activation of type I interferon (IFN), interleukin 6 (IL-6) and tumor necrosis factor (TNF) and subsequent janus-kinase signal transducer and activators of transcription 3 (JAK/STAT3) pathway was considered to play a pathogenic role in the pathogenesis of dermatomyositis. JAK inhibitor (JAKi) is therefore proposed as a mechanism-based treatment for dermatomyositis. A series of clinical cohort studies displayed that nearly 2/3 of patients with JDM respond to JAKi^[3, 4]. Nevertheless, the detailed molecular mechanism for the JAK/STAT3 pathway, especially how JAK/STAT3 signaling was modulated in dermatomyositis was not fully understood.

STAT3 is transiently activated through phosphorylation, thereby transmitting transcriptional signals of cytokines and growth factors from the plasma membrane to the nucleus. Phosphorylation of STAT3 (pSTAT3) undergoes homo or hetero dimerization through reciprocal phosphotyrosine-SH2 interactions and then translocates into the nucleus where they bind to DNA response elements of targeted genes^[5]. The upper-stream activators of STAT3 including epidermal growth factor receptor (EGFR), amphiregulin, transforming growth factor- α (TGF- α), Src, and IL-6 which interacted with cell cycle progression (*Cyclin D1*, *Fos*, *c-Myc*, etc) and anti-apoptotic genes (*Survivin*, *Bcl-2*, *Bcl-XL* and others). STAT3 activation is also regulated by a negative feedback mechanism mediated by protein inhibitor of activated STAT (*PIAS*), suppressors of cytokine signaling (*SOCS*), protein tyrosine phosphatase (*PTPN1*, *PTPN2*, *SHP1*, *SHP2*, *PTPRD*, *PTPRT*, *dusp22*) and ubiquitinase^[6, 7].

Our previous study displayed that patient with JDM had over activation of IL-6/JAK/STAT3 signaling pathway with elevated serum IL-6 and increased phosphorylation of STAT3 in the muscle tissues. Blocking the IL-6 receptor resulted in decreased reaction oxygen species (ROS) generation in skeletal muscle cells, which is consistent with the results of clinical reports^[8]. Aberrant phosphorylation of STAT3 could be due to disruption of these negative regulation mechanisms or persistent upstream signaling activity. The in-depth study of the mechanism is beneficial to the stratified management of patients and more precise targeted therapy.

Materials and methods

Human skeletal muscle cells (HSMCs) isolation and culture

Skeletal muscle samples were obtained from three treatment naive JDM donors (MDA5⁺, NXP2⁺ and TIF1- γ ⁺ one for each). Primary HSMCs was isolated and expanded by tissue explants in sterile culture medium. Samples were sectioned into 1 mm³ columnar tissue blocks and spaced at 0.5 cm intervals in a culture flask (NEST, Jiangsu, China). The tissue culture medium was composed of high-glucose DMEM (GIBCO, USA), 20% fetal bovine serum (ExCell Bio, Jiangsu, China), and 2% Penicillin-Streptomycin Solution (beyotime, Shanghai, China). Primary myocytes from passages 1–3 were used for experiments. HSMCs were cryopreserved in a 90% growth medium and 10% DMSO mixture for long-term storage.

Lentiviral transduction and stable cell line establishment (*PTPN2* knock-down & *PTPN2* over-expression)

Lentivirus production was outsourced to OBiO Technology (Shanghai). Lentivirus infection was performed according to a previously reported protocol^[9]. For knockdown experiments, normal human muscle cells were transduced with either shControl (GL401NC) or shPTPN2 (Sense: 5'-CCUAACACAUGCUGCCAUTT-3'; Antisense: 5'-AAUGGCAGCAUGUGUUAGGTT-3'). The full-length cDNA of the human *PTPN2* gene was inserted into a lentiviral vector (OBiO Technology, Shanghai, China). To establish stable HSMC cell lines, cells were transduced with either shPTPN2 or the negative control (shNC) for knockdown, or with OE-PTPN2 or the negative control (OE-NC) for over-expression, in the presence of 10 µg/ml polybrene for 6 hours. The medium was then refreshed, and two days later, cells were split and selected with 5 µg/ml puromycin. *PTPN2* knockdown and over-expression were confirmed by qPCR and western blotting.

Quantitative reverse transcription polymerase chain reaction (qPCR)

Total RNA was extracted by using TRIzol reagent (Invitrogen, Carlsbad, CA, USA), from muscle tissues (JDM and normal controls), HSMCs isolated from JDM and control muscle cell line. RNA was reverse transcribed into cDNA using the PrimeScript RT Master Mix (Takara, Shiga, Japan). Quantitative PCR was performed on the CFX Opus 96 Real-Time PCR System (Bio-Rad, USA) using SYBR Green reagents (Monad Biotech, Jiangsu, China). Transcription levels of negative regulatory genes of STAT3, including suppressors of cytokine signaling (*SOCS* 2, *SOCS3*, *SOCS4* and protein tyrosine phosphatase (*PTPN1*, *PTPN2*, *SHP1*, *SHP2*, *PIAS1*, *PIAS2*, and *PTPRD*), and *SLN* were detected by qPCR (the corresponding primers are listed in Table 1). Transcription of *PTPN2* in different groups: HSMCs wild type (WT), HSMCs *PTPN2* knock-down negative control (shNC), HSMCs *PTPN2* knock-down (shPTPN2), HSMCs *PTPN2* over-expression negative control (oe-NC) and HSMCs *PTPN2* over-expression (oe-PTPN2) as well as STAT3 were tested by qPCR.

Table 1
Primer sequences of STAT3 negative regulatory genes

Gene	Primer sequences	Gene	Primer sequences
<i>CIS</i>	F: TCCTTTCTCCTTCCATCCCG R: TGCTCCACAGCCAGCAAAG	<i>SHP1</i>	F: GGAAGTGAGAGCAGATCCCTA A R: TTAGAAGCTACC TTCCCTGGC T
<i>SOCS1</i>	F: CTCGTCCTCGTCTTCGTCCT R: GAAGGTGCGGAAGTGAGTGT	<i>SHP2</i>	F: GGATTACAGGCATA GCCAC R:GACCACTAAACTTCTTAAATGAGC
<i>SOCS2</i>	F: GTTGCCGGAGGAACAGTC R: AACAGTCATACTTCCCCAGTACC	<i>PTPN1</i>	F: GATGGACAAGAGGAAAGACCC R: AGCTCCTTC CAC TGA TCC TG
<i>SOCS3</i>	F: ACTGAGCCGACCTCTCTCCT R: GGCAGCTGGGTCACTTTCTC	<i>PTPN2</i>	F: TGCTGTGCTGCGTGAGTT R: CACCATTGAGCGAAGTCC
<i>SOCS4</i>	F: GGCGGCAGGCGCTCGGACAG R: TCTGGGCACTTTCTGGACGG	<i>PRPRT</i>	F: CTCCTCTCTGTGGTGATGCTGA R: ATGTCTGCGGAGACGAACTC
<i>SOCS5</i>	F: GACGGCTTAGTATCGAAGAA R: GCTTATACAATGGGTTGACC	<i>PTPRD</i>	F: AATTGCATATGACTCATTTCTCTT R: GAAACCATGAGCTCCCTTCATAGGG
<i>SOCS6</i>	F: TACGGAGGAGCTGAAGAAGC R: AGAACCATCTGGCACATTTGC	<i>PIAS1</i>	F: AGCCTAAGGGAAAGCCATAGC R: ATGTCTGGTATGATGCCAAAGATG
<i>SOCS7</i>	F: TTCGATCACAGGGTATCACC R: ACTGACAGCGATCCTCAAAC	<i>PIAS2</i>	F:CACGAACTCTTGAAGGACTTTCTG R: AAGTGGAAGGCAACGAGTGG
<i>ISG15</i>	F: GAGGCA GCGAACTCATCTTT R: AGCATCTTCACCGTCAGGTC	<i>SLN</i>	F: ATGGTCCTGGGATTGACTGAG R: GTGCCCTCGGATGGAGAATG

Western blot

The protein extracted from JDM (patient with positive NXP2, patient with positive MDA5 and patient with negative specific myositis antibody) and control muscle tissues were degenerated, electrophoresed, and transferred to the polyvinylidene fluoride membrane (Bioss, Beijing, China). The membrane was blocked using 5% non-fat milk and incubated with primary antibodies-rabbit PTPN2 (1:1000, A21812, ABclonal), antibodies-rabbit stat3 (1:1000, ab68153, abcam) and p-stat3 (1:1000, ab32143, abcam) and mouse anti- β -actin (Bioss, Beijing, China) overnight at 4°C. Membranes were washed and incubated with the horseradish peroxidase-conjugated secondary antibodies (Bioss, Beijing, China) and detected with an ECL reagent (Bioss, Beijing, China) using the chemiluminescence system (GBOX-CHEMI-XRQ, Syngene,

USA). The grayscale of the detected bands was assessed by ImageJ software (National Institutes of Health).

Detection of phosphorylated STAT3 within mitochondria

In the western blot assay, protein lysates were extracted from primary skeletal muscle cells derived from JDM patients, including wild-type cells, *PTPN2* over-expression negative control (oe-NC), and *PTPN2* over-expression (oe-*PTPN2*) groups. Cells were seeded into T75 culture flasks, and mitochondria were isolated by homogenization and centrifugation using a mitochondrial isolation kit (C3601, Beyotime, China) according to the manufacturer's instructions. Mitochondrial proteins (10 µg) from each sample were subjected to SDS-PAGE and transferred onto PVDF membranes. The membranes were incubated with primary antibodies against STAT3 (rabbit anti-STAT3, 1:1000, ab68153, Abcam) and phosphorylated STAT3 (rabbit anti-P-STAT3, 1:1000, ab32143, Abcam). Anti-TOM20 antibody (ET1609-25, HuBio, China) was used as a loading control for mitochondrial proteins. This experiment aimed to assess the effect of *PTPN2* over-expression on STAT3 expression as well as phosphorylation levels in mitochondria.

Enzyme-linked immunosorbent assay (ELISA) for SLN quantification

SMC and primary HSMCs were treated with or without recombinant *PTPN2* protein (100 µg/mL, 31592, proteintech, China). After treatment, cells were harvested and lysed to obtain total cellular protein. Intracellular SLN protein levels were quantified using a commercial human SLN ELISA kit (ED-100104, LunChangShuoBiotech, China) following the manufacturer's instructions. Optical density values were measured on a microplate reader, and SLN protein concentrations were calculated based on the standard curve. All samples were assayed in duplicate.

Statistical analysis

All statistical analyses were performed using graphpad prism 10. Continuous data were expressed as mean (standard deviation) and were tested by Student's t test. Non-normal continuous data were expressed as median (interquartile ranges) and were tested by Wilcoxon test. P-values were adjusted by using the "Benjamini–Hochberg" method and a two-tailed t-test ($p < 0.05$) was used to test each term for significance.

Results

Transcription and expression of *PTPN2* was significantly decreased in the muscle tissue or myocytes of JDM

Negative regulatory genes of STAT3, including *SOCS2*, *SOCS3*, *SOCS4*, *PTPN1*, *PTPN2*, *SHP1*, *SHP2*, *PIAS1*, *PIAS2*, and *PTPRD* were detected from the muscle tissues of JDM and controls. Repeated experiments confirmed *PTPN2* was stable deficiency transcription in the muscle tissues of patients with

JDM (Fig. 1). Transcription of *SOCS2*, *SOCS3*, *PIAS1*, and *SHP1* were also significantly different in patients with JDM and normal control tissues. However, due to the differences in the results of muscle cell lines isolated from JDM, only *PTPN2* was selected as target gene in the subsequent experiments. Expression of *PTPN2* was detected by western blot from three patients with JDM (one patient with positive NXP2, one patient with positive MDA5 and one patient with negative specific myositis antibody). All the three patients had decreased expression of *PTPN2* protein in the muscle tissues, comparing with normal controls (Fig. 3A).

Relative mRNA expression levels were quantified by qRT-PCR and normalized to β -ACTIN. Data are presented as mean $\pm$ SEM. Statistical significance was determined by unpaired two-tailed Student's t-test: * $P < 0.05$, $P < 0.01$, $P < 0.001$, *** $P < 0.0001$; ns, not significant.

(a) *SOCS1*, (b) *SOCS2*, (c) *SOCS3*, (d) *SOCS4*, (e) *SOCS6*, (f) *SOCS7*, (g) *SHP1*, (h) *SHP2*, (i) *PTPN1*, (j) *PTPN2*, (k) *PIAS1*, (l) *PIAS2*, (m) *PRPRT*, (n) *PTPRD*, (o) *ISG15*.

Ctrl = healthy control; JDM = juvenile dermatomyositis.

***PTPN2* negatively regulates the phosphorylation of STAT3 in HSMCs**

PTPN2 knock-down (sh*PTPN2*) and *PTPN2* over-expression (oe*PTPN2*) human muscle cell lines were constructed using lentiviral transduction method. The established cell lines were verified by qPCR. Expression of total STAT3 was not significantly affected by knock-down of *PTPN2*, but was largely affected by over expression of *PTPN2*, resulting in decreased expression of STAT3 (Fig. 2A). The negative regulatory effect of *PTPN2* was manifested during phosphorylation of STAT3 in the HSMCs. Since the wild type muscle cells isolated from JDM (JDM-WT) had deficient expressed *PTPN2*, we constructed over-expression *PTPN2* muscle cell lines of JDM (JDM-oe-*PTPN2*) through lentiviral vector. Phosphorylation level of STAT3 was compared among JDM-WT, JDM (oe-NC) and JDM (oe-*PTPN2*). Western blot analysis displayed that restoring *PTPN2* function can lead to a decreased level of phosphorylation of STAT3 in the mitochondria (Fig. 2B).

1. qRT-PCR quantification of *PTPN2* and STAT3 mRNA in WT, sh(NC), sh(*PTPN2*), oe(NC), and oe(*PTPN2*) cells; transcript levels normalized to α -ACTIN.
 2. Immunoblots for p-STAT3, total STAT3, *PTPN2*, and α -ACTIN loading control. Densitometric analysis quantifies the p-STAT3/total STAT3 ratio, total STAT3, and p-STAT3 protein expression normalized to α -ACTIN. STAT3 expression as well as phosphorylation levels in mitochondria (TOM-20).
- Data are presented as mean $\pm$ SEM. ns = non-significant; * $P < 0.05$, ** $P < 0.01$, *** $P < 0.0001$.

***PTPN2* downregulation is associated with elevated sarcolipin (SLN) expression in JDM**

Accumulating evidence positions the IL-6/JAK/STAT3 axis as an upstream regulator of SLN transcription in inflammatory skeletal muscle. SLN is a critical mediator of the muscle's adaptive response to stress, including inflammation, oxidative damage, and metabolic overload. Our previous study revealed that IL-6 selectively potentiated *SLN* transcription in inflammatory myotubes in vitro, leaving basal expression in standard myogenic cultures unaffected^[8].

In this study, *SLN* mRNA expression was found markedly increased in the JDM group compared with the control group (Fig. 3B, left; $P < 0.0001$). Intriguingly, *PTPN2* overexpression did not significantly alter *SLN* mRNA expression, whereas *PTPN2* knockdown significantly increased *SLN* mRNA levels (Fig. 3B, right; $P < 0.0001$), suggesting a potential negative regulatory role of *PTPN2* in *SLN* transcription. At the protein level, SLN concentrations were significantly higher in the JDM group than in the SMC group (Fig. 3C, $P < 0.0001$). Recombinant PTPN2 supplementation was associated with a modest increase in SLN levels in SMCs ($P < 0.01$), whereas it significantly reduced SLN levels in primary JDM myocytes ($P < 0.0001$), although SLN remained higher than that in SMCs. Collectively, these findings indicate that decreased PTPN2 expression is associated with elevated SLN expression in JDM, and restoration of PTPN2 may partially attenuate SLN up-regulation in the JDM setting.

1. (A) Western blot detection of PTPN2 protein in control skeletal muscle cells (SMC), NXP2-positive JDM myocytes, MDA5-positive JDM myocytes, and negative control samples. α -Actin serves as the internal loading control. Quantitative densitometry analysis shows significantly lower relative PTPN2 protein expression in JDM myocytes compared with healthy SMCs.
2. (B) Quantification of *SLN* mRNA transcript levels normalized to β -ACTIN. Left panel: *SLN* mRNA is markedly upregulated in JDM muscle cells relative to control SMC. Right panel: *SLN* mRNA expression in wild-type (WT), empty vector overexpression control (OE[NC]), PTPN2-overexpressing (OE[PTPN2]), empty vector knockdown control (sh[NC]), and PTPN2-knockdown (sh[PTPN2]) myocytes. No significant differences are observed between WT, OE(NC), OE(PTPN2), and sh(NC) groups; *SLN* transcription is drastically increased upon PTPN2 silencing.
3. (C) ELISA quantification of secreted SLN protein concentrations (ng/mL) in culture supernatants from healthy SMC, PTPN2-overexpressing SMC (SMC-PTPN2), JDM myocytes, and PTPN2-overexpressing JDM myocytes (JDM-PTPN2). Loss of endogenous PTPN2 in JDM cells increases extracellular SLN release, while exogenous PTPN2 supplementation reverses this phenotype.

All quantitative data are presented as mean $\pm$ standard error of the mean (SEM). ns = not significant; ** $P < 0.01$, **** $P < 0.0001$.

Discussion

The cellular infiltrates consisting of CD4⁺ T cells, B cells, macrophages, plasmacytoid dendritic cells, and T helper (Th) 17 cells, were observed in the muscle of patients with JDM and adult dermatomyositis (DM). Increased levels of IL-6, TNF- α , IL-17F, IL-23 and IFN in blood and affected muscle were also one of the inflammatory features of JDM^[10]. Microarray datasets analysis displayed that IL6/JAK/STAT3

signaling was one of the important pathways that correlated with disease pathogenesis^[11]. Our previous studies confirmed that overactivation of JAK/STAT3 pathway may play an important role in the occurrence and development of myositis in JDM^[8]. Clinical reports showed that Tocilizumab (IL-6 receptor inhibitor) can rapidly relieve muscle inflammation in NXP2-positive DM and some patients with polymyositis^[12, 13]. In-depth research on regulatory mechanisms of JAK/STAT3 signaling pathway is helpful for stratified treatment of JDM.

Phosphorylated STAT3 plays an important role in the modulation of electron transport chain in mitochondria, which may correlate with the oxidative damage in muscle tissues^[14]. Oxidative stress assay confirmed that knockdown of *PTPN2* led to increased sensitivity of skeletal muscle cells to extracellular cytokines, which is consistent with our previous study. Dephosphorylation of the tyrosine residue is one of the major mechanisms that negatively regulates STAT3 activation. In our study, we found that transcriptional level of negative regulator (*PTPN2*) was extremely low in the muscle tissues as well as the primary skeletal muscle cells of patients with JDM comparing with normal control. Expression of *PTPN2* in the muscle tissues of JDM were also lower than controls, which inferred that decreased negative regulation may contribute to the over-activation of STAT3 pathway in JDM. To verify this hypothesis, we isolated skeletal muscle cells from JDM and constructed *PTPN2* overexpression cell model (HSMCs *PTPN2*⁺). JDM wild type skeletal muscle cells (JDM WT) were served as *PTPN2* deficient expression cell model. The results showed that *PTPN2* overexpression significantly reduced pSTAT3 both in cytoplasm and mitochondria, which convinced that *PTPN2* plays a critical negative regulatory role in the STAT3 signaling pathway in JDM.

Research on the function of STAT3 in the mitochondria of skeletal muscle cells in JDM was largely unknown. The amount of STAT3 in the mitochondria would increase in response to stimuli such as ischemia^[15]. Recent study displayed that accumulation of calcium phosphate salts preferentially localized to mitochondria in a time and dose dependent way in patients with JDM complicated with calcinosis. Proinflammatory factors such as IFN- α can amplify mitochondrial calcification in ROS dependent manner^[16]. *PTPN2* deficiency led to excessive STAT3 phosphorylation and its aberrant translocation into mitochondria of human keratinocytes^[17]. This mitochondrial STAT3 accumulation exacerbated oxidative stress, impaired mitochondrial respiration, and amplified calcium phosphate deposition—effects partially reversed by STAT3 knockdown. Based on previous studies and our current findings, we propose that *PTPN2* is a key suppressor of mitochondrial STAT3-driven pathology in JDM, offering a potential therapeutic target for mitigating muscle damage and calcinosis.

SLN is a small regulatory peptide that inhibits the sarco/endoplasmic reticulum Ca²⁺-ATPase (SERCA) in skeletal muscle. By promoting SERCA uncoupling, SLN enhances ATP consumption and heat production while optimizing Ca²⁺ refilling into the sarcoplasmic reticulum. These properties enable SLN to regulate muscle relaxation kinetics, sustain contractile performance during repetitive activity, and protect against fatigue. Given its central role in calcium handling and stress adaptation, aberrant SLN expression has been implicated in various neuromuscular disorders characterized by impaired Ca²⁺ homeostasis^[18, 19].

In inflammatory myopathies, heightened *SLN* expression likely reflects a maladaptive attempt to restore impaired Ca^{2+} reuptake. However, excessive *SLN* can hyper-inhibit SERCA, leading to delayed muscle relaxation, cytosolic Ca^{2+} overload, and activation of proteolytic pathways that drive muscle atrophy and weakness^[20]. In JDM myotubes, phosphorylated STAT3 is elevated and IL-6 selectively induces *SLN* mRNA in diseased but not control cells, implying that STAT3 activation is required for the inflammatory context-specific upregulation of *SLN*, which positions the IL-6/JAK/STAT3 axis as an upstream regulator of *SLN* transcription in inflammatory skeletal muscle.

Our findings imply that *PTPN2* may serve as a promising biomarker for the precision treatment of JDM. Notably, we observed a significant downregulation of *PTPN2* expression specifically in patients harboring anti-MDA5 or anti-NXP2 autoantibodies, as well as in those lacking detectable myositis-specific autoantibodies (MsAs). However, the generalizability of these observations is limited by the relatively small cohort size and the incomplete coverage of all MsA subtypes. Future studies with larger, multi-center cohorts are warranted to validate *PTPN2*'s utility across diverse serological profiles.

Declarations

Ethics approval and consent to participate

This study was approved by institutional research ethics committee of Zhejiang University School of Medicine (2021-IRBAL-303). Informed consents were obtained from guardians as well as children's age over 8 years.

Consent for publication

The paper was approved for publication by all authors listed in this manuscript.

Competing interests

The authors declare no conflict of interests.

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Author Contribution

Qi Zheng: Study design and manuscript writing. Zhaoling Wang: Lab work and statistical analysis. Lixia Zou, Rongjun Zheng & Yan Zhang: Clinical data collection; reviewed the manuscript. Meiping Lu:

Supervisor; review and modification of the article.

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Availability of data and material

All data generated in this study are included in this published article and its supplementary information files.

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Figures

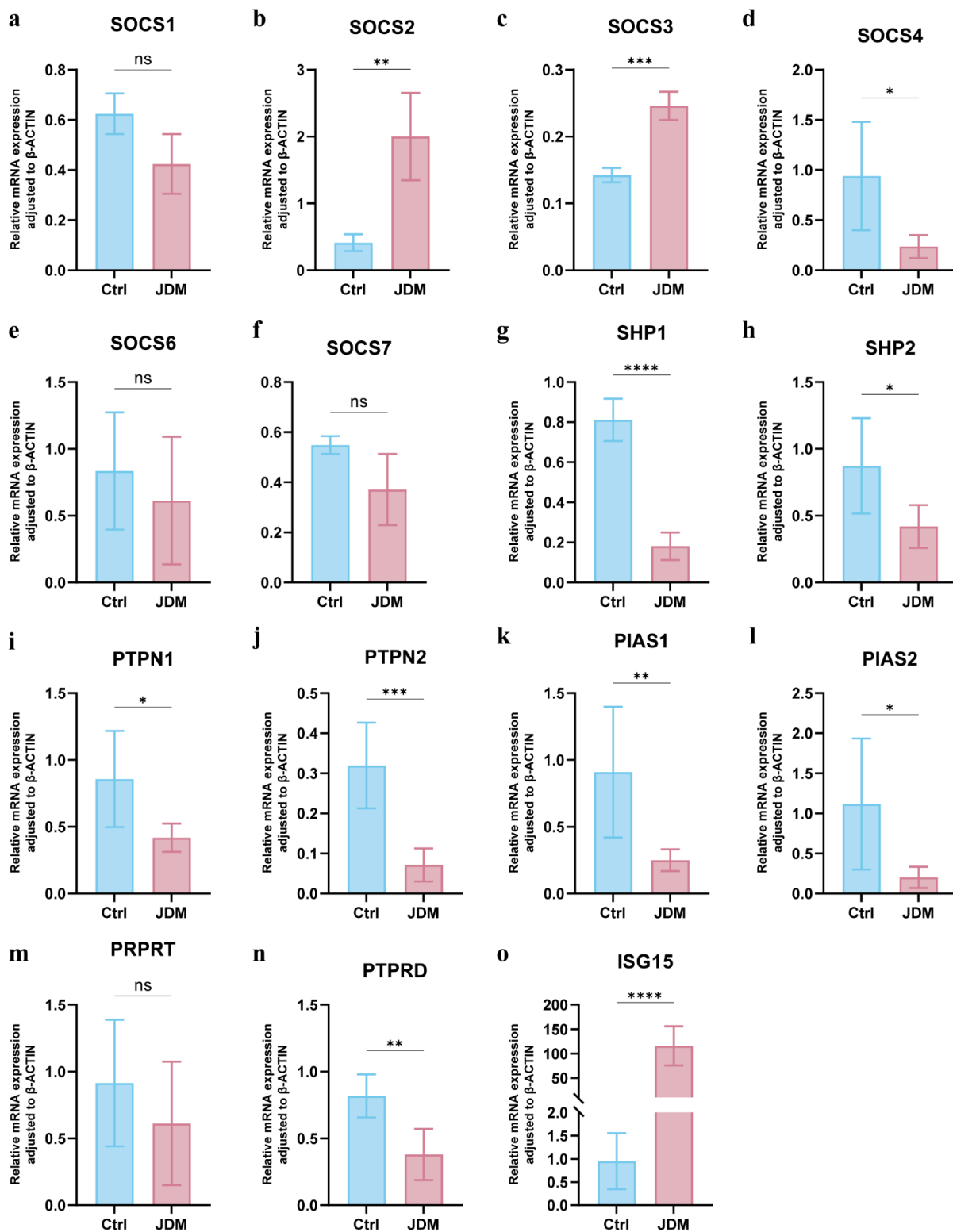


Figure 1

Transcription levels of negatively regulated genes of STAT3 screened by qPCR

Relative mRNA expression levels were quantified by qRT-PCR and normalized to β -ACTIN. Data are presented as mean $\pm$ SEM. Statistical significance was determined by unpaired two-tailed Student's t-test: * $P < 0.05$, $P < 0.01$, $P < 0.001$, *** $P < 0.0001$; ns, not significant.

(a) SOCS1, (b) SOCS2, (c) SOCS3, (d) SOCS4, (e) SOCS6, (f) SOCS7, (g) SHP1, (h) SHP2, (i) PTPN1, (j) PTPN2, (k) PIAS1, (l) PIAS2, (m) PRPRT, (n) PTPRD, (o) ISG15.

Ctrl = healthy control; JDM = juvenile dermatomyositis.

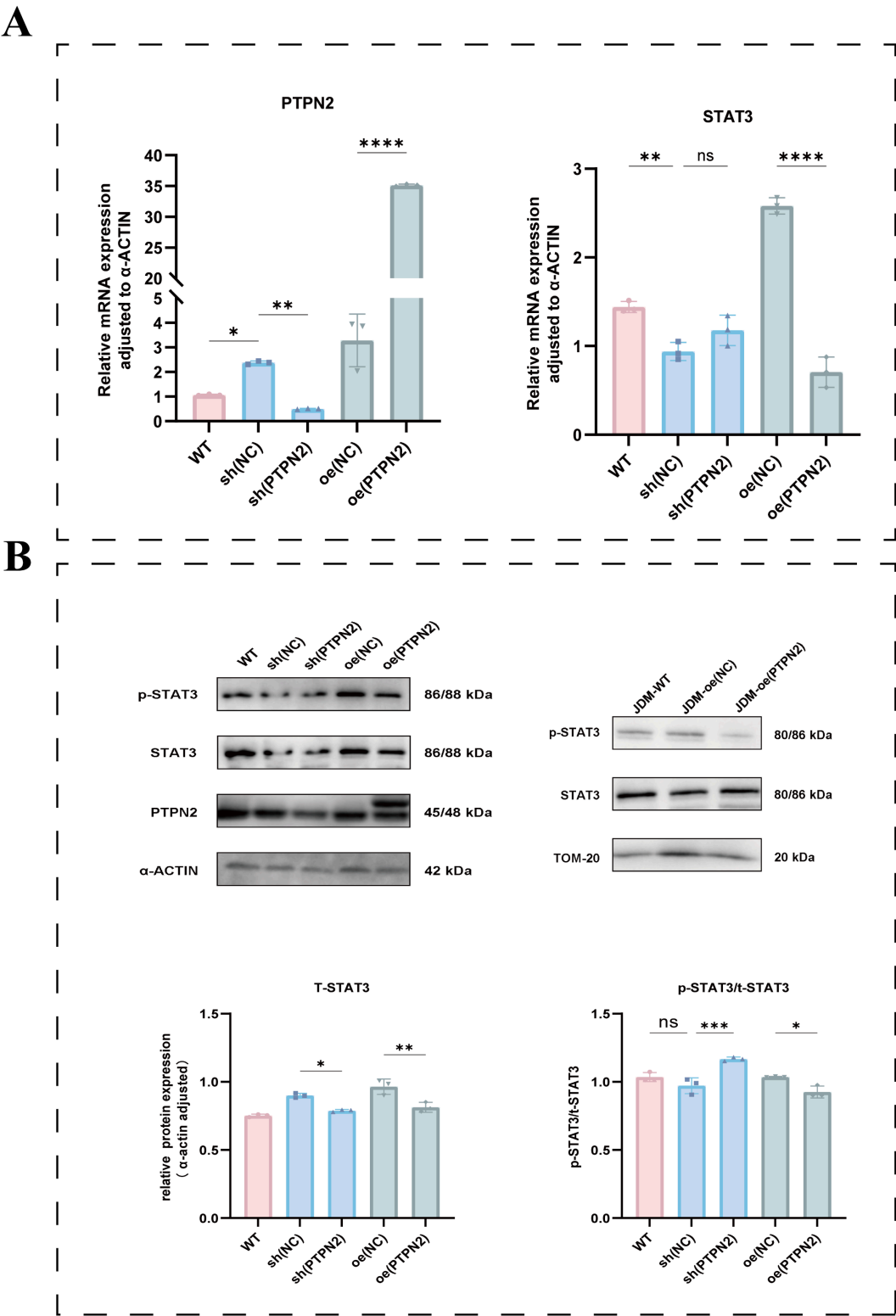


Figure 2

PTPN2negatively regulates STAT3 transcription and activation.

(A) qRT-PCR quantification of *PTPN2* and *STAT3* mRNA in WT, sh(NC), sh(*PTPN2*), oe(NC), and oe(*PTPN2*) cells; transcript levels normalized to α -ACTIN.

(B) Immunoblots for p-STAT3, total STAT3, PTPN2, and α -ACTIN loading control. Densitometric analysis quantifies the p-STAT3/total STAT3 ratio, total STAT3, and p-STAT3 protein expression normalized to α -ACTIN. STAT3 expression as well as phosphorylation levels in mitochondria (TOM-20).

Data are presented as mean $\pm$ SEM. ns = non-significant; * $P < 0.05$, ** $P < 0.01$, **** $P < 0.0001$.

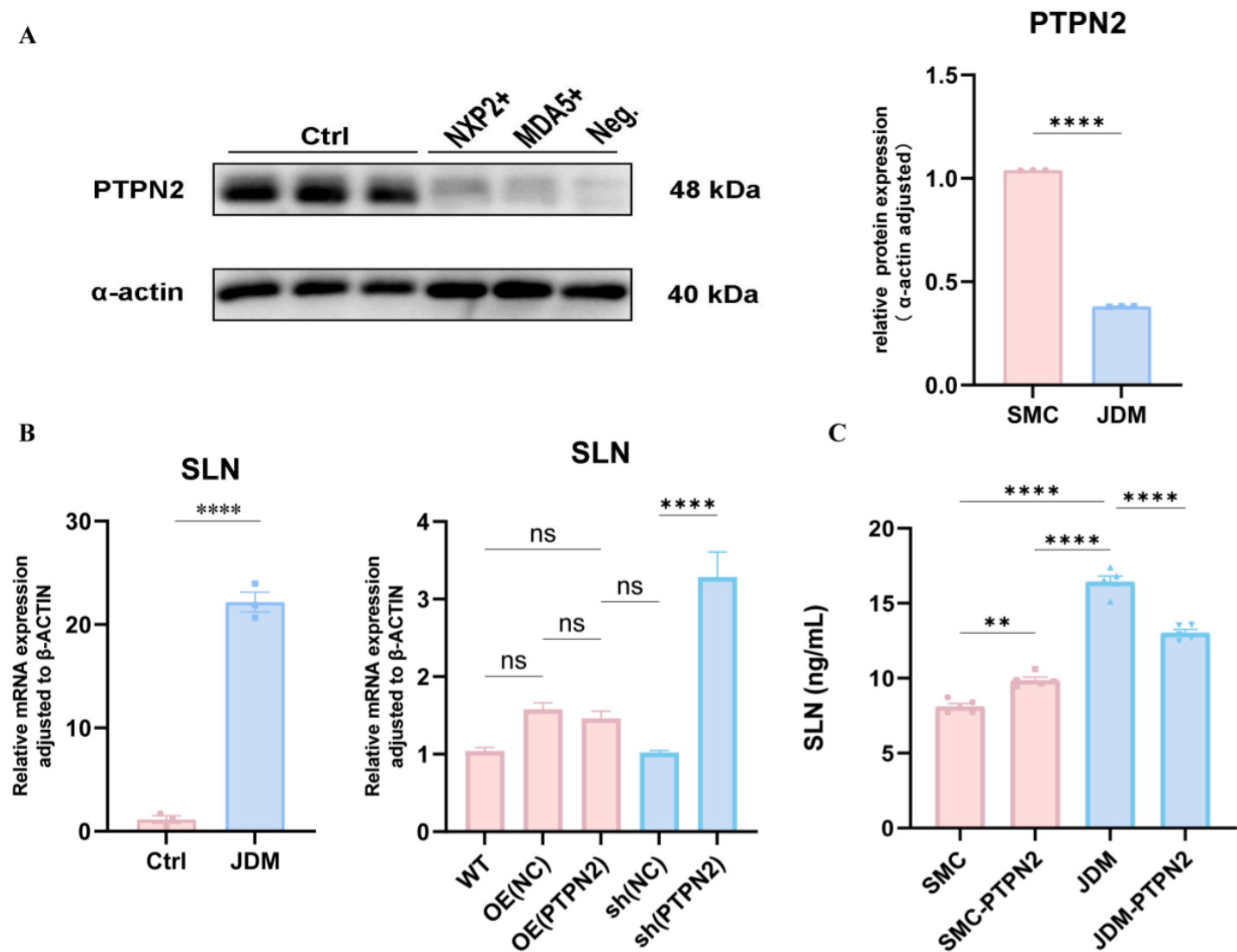


Figure 3

PTPN2 protein expression is reduced in JDM skeletal muscle compared to controls.

(A) Western blot detection of PTPN2 protein in control skeletal muscle cells (SMC), NXP2-positive JDM myocytes, MDA5-positive JDM myocytes, and negative control samples. α -Actin serves as the internal loading control. Quantitative densitometry analysis shows significantly lower relative PTPN2 protein expression in JDM myocytes compared with healthy SMCs.

(B) Quantification of *SLN* mRNA transcript levels normalized to β -ACTIN. Left panel: *SLN* mRNA is markedly upregulated in JDM muscle cells relative to control SMC. Right panel: *SLN* mRNA expression in wild-type (WT), empty vector overexpression control (OE[NC]), PTPN2-overexpressing (OE[PTPN2]), empty vector knockdown control (sh[NC]), and PTPN2-knockdown (sh[PTPN2]) myocytes. No significant differences are observed between WT, OE(NC), OE(PTPN2), and sh(NC) groups; *SLN* transcription is drastically increased upon PTPN2 silencing.

(C) ELISA quantification of secreted *SLN* protein concentrations (ng/mL) in culture supernatants from healthy SMC, PTPN2-overexpressing SMC (SMC-PTPN2), JDM myocytes, and PTPN2-overexpressing JDM myocytes (JDM-PTPN2). Loss of endogenous PTPN2 in JDM cells increases extracellular *SLN* release, while exogenous PTPN2 supplementation reverses this phenotype.

All quantitative data are presented as mean $\pm$ standard error of the mean (SEM). ns = not significant; ** $P < 0.01$, *** $P < 0.0001$.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [CTRLJDMPTPN21.zip](#)
- [pSTAT3STAT3.zip](#)
- [SHOEptpn2STAT3.zip](#)