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Highlights

- The diameter of C2C12 myotube is reduced by treatment with oncostatin M.
- Oncostatin M inhibits the myogenic pathway and activates the degradation pathway.
- The oncostatin M-induced effects on myotubes are dependent on the STAT3 signaling.
- The oncostatin M-induced STAT3 signaling potentially contributes to muscle atrophy.

Oncostatin M induces C2C12 myotube atrophy by modulating muscle differentiation and degradation

Yuya Miki [a], Tomoaki Morioka [a], Atsushi Shioi [b,c], Kenta Fujimoto [a], Takeshi Sakura [a], Hideki Uedono [a], Yoshinori Kakutani [a], Akinobu Ochi [a], Katsuhito Mori [d], Tetsuo Shoji [b,c], Masanori Emoto [a], Masaaki Inaba [a,c]

[a] Department of Metabolism, Endocrinology and Molecular Medicine, Osaka City University Graduate School of Medicine, 1-4-3, Asahi-machi, Abeno-ku, Osaka, 545-8585, Japan

[b] Department of Vascular Medicine, Osaka City University Graduate School of Medicine, 1-4-3, Asahi-machi, Abeno-ku, Osaka, 545-8585, Japan

[c] Vascular Science Center for Translational Research, Osaka City University Graduate School of Medicine, 1-4-3, Asahi-machi, Abeno-ku, Osaka, 545-8585, Japan

[d] Department of Nephrology, Osaka City University Graduate School of Medicine, 1-4-3, Asahi-machi, Abeno-ku, Osaka, 545-8585, Japan

Corresponding Author:

Tomoaki Morioka, MD, PhD.

m-tomo@med.osaka-cu.ac.jp

Tel. +81-6645-3806, Fax. +81-6-6645-3808

Abstract

Oncostatin M (OSM) is a cytokine of the interleukin-6 family and plays a role in various disorders such as cancer and inflammatory diseases, which are often accompanied by skeletal muscle atrophy, or sarcopenia. However, the role of OSM in the regulation of skeletal muscle mass remains to be identified. In this study, we investigated the effect of OSM on C2C12 myotube formation *in vitro*. C2C12 myoblasts were induced to differentiate into myotubes for 3 days and then treated with OSM for 24 or 48 hours. The diameter of differentiated C2C12 myotubes were reduced by 18.7% and 23.3% compared to control cells after treatment with OSM for 24 and 48 hours, respectively. The expression levels of MyoD and myogenin were decreased, while those of atrogin-1, CCAAT/enhancer binding protein δ, and OSM receptor were increased in C2C12 myotubes treated with OSM for 24 hours compared to control cells. Furthermore, the inhibitory effect of OSM on myotube formation was significantly attenuated by pretreatment with an inhibitor of signal transducer and activator of transcription (STAT) 3 or by knockdown of *Stat3*. Finally, the OSM-induced changes in the expression levels of MyoD, myogenin, and atrogin-1 were reversed by pretreatment with an inhibitor of STAT3 or by *Stat3* knockdown in C2C12 myotubes. In conclusion, OSM induces C2C12 myotube atrophy by inhibiting myogenic differentiation and activating muscle degradation in a STAT3-dependent manner.

Keywords: Oncostatin M; Myogenic differentiation; Muscle degradation; Sarcopenia; STAT3; C2C12 cell

Abbreviations: OSM, oncostatin M; MuRF, muscle RING-finger protein; CKD, chronic kidney disease; LIF, leukemia inhibitory factor; MHC, myosin heavy chain.

1. Introduction

Oncostatin M (OSM) is a member of the interleukin (IL)-6 family cytokine which is produced by macrophages, neutrophils and T lymphocytes [1,2]. OSM exerts various biological activities in inflammation, hematopoiesis, and development through the Janus kinase (JAK) - signal transducer and activator of transcription (STAT) pathway [1-4]. Accumulated evidence has indicated that OSM is involved in the disease process of several types of cancer, rheumatoid arthritis, heart failure, and pulmonary diseases [1]. Because those chronic diseases are characterized by skeletal muscle wasting, or sarcopenia [5], it is suggested that OSM plays a role in the process of sarcopenia.

The maintenance of skeletal muscle mass is determined by a balance between muscle protein synthesis and degradation [6]. Sarcopenia results from an excess of muscle protein degradation over synthesis and occurs during aging, disuse, or various chronic diseases [6,7]. Upon the excess of protein degradation, adult skeletal muscle has the ability to initiate a repair process for preventing loss of muscle mass, that is, muscle regeneration [8]. Muscle regeneration is a highly ordered multistep process which is composed of activation of myoblasts, or satellite cells, to proliferate, myogenic differentiation, fusion into multinucleated myotubes, and myotube maturation [9]. This process is dominantly regulated by the myogenic regulatory

factors including MyoD and myogenin, the expression level of which are established as markers of the myogenic differentiation pathway [8,9]. On the other hand, under muscle-wasting conditions, two major muscle-specific E3 ubiquitin ligases, atrogin-1 and muscle RING-finger protein-1 (MuRF-1), are transcriptionally upregulated in skeletal muscle, and are recognized as markers of the muscle degradation pathway [6,10]. Notably, recent studies have indicated that both myogenic differentiation pathway [11,12] and muscle degradation pathway [13] are involved in the formation of myotubes differentiated from C2C12 myoblasts, an *in vitro* model of skeletal muscle differentiation.

Accumulated evidence indicates a close link between inflammation and sarcopenia in cancer cachexia [14-17] and chronic kidney disease (CKD) [18-20]. Specifically, inflammatory signaling induced by the IL-6 family cytokines, including IL-6 [20-22] and leukemia inhibitory factor (LIF), [16,23] play a major role in regulating satellite cell function [22,24], myoblast differentiation [25,26], and muscle atrophy [16,17,20]. OSM was also indicated to play a role in preventing premature myoblast differentiation during the early phase of injury-induced muscle regeneration [27-29]. To date, no study has examined the direct role of OSM on sarcopenia in muscle-wasting conditions. In this study, we utilized C2C12 cells and examined the effect of OSM on the formation of differentiated C2C12 myotubes *in vitro*.

2. Materials and methods

2.1. Cell culture and differentiation

C2C12 myoblast cells were obtained from the American Type Culture Collection and used within the first 15 passages. C2C12 cells were maintained at 37ºC in 5% CO_2 in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum containing 100 U/ml penicillin and 100 µg/ml streptomycin. After reaching confluence, myogenic differentiation of C2C12 cells was induced by DMEM supplemented with 2% horse serum for 3 days and then treated with the indicated concentrations of mouse recombinant OSM (R&D Systems, Minneapolis, MN) for 48 hours. To inhibit STAT3 activation, C2C12 myotubes were pretreated for one hour with a STAT3 inhibitor, C188-9 (10 µM; Merck KGaA, Darmstadt, Germany) before treatment with OSM. To analyze the diameter of differentiated C2C12 myotubes, 10 random culture fields were photographed for cells treated with OSM using the Olympus IX-70 inverted microscope equipped with a DP-10 digital camera (Olympus Co. Ltd., Tokyo, Japan). The mean value of myotube diameter was determined from the averaged diameter of myotubes measured in at least 200 myotubes per each well using National Institutes of Health ImageJ.

2.2. Knockdown of Stat3 by siRNA in differentiated C2C12 myotubes

Two days after induction of differentiation, a siRNA targeting *Stat3* was transfected into C2C12 myotubes according to the manufacturer's instructions. Briefly, C2C12 myotubes were treated with 50 nM of siRNA for *Stat3* (MSS209601, Invitrogen, San Diego, CA) in combination with RNAiMAX transfection reagent (Invitrogen) for 24 hours. Then, the myotubes were washed and treated with OSM for 24 hours.

2.3. Real-time quantitative PCR

Total RNA was extracted from C2C12 myotubes with Sepazol® RNA I super G reagent (Nacalai Tesque, Kyoto, Japan) according to the manufacturer's instruction. cDNAs were reverse transcribed from 1 μg of extracted total RNA using High Capacity cDNA Reverse Transcription Kit (Thermo Fisher Scientific K.K., Tokyo, Japan). Real-time quantitative PCR was performed using the Applied Biosystems 7500 Fast Real-Time PCR System (Applied Biosystems, Foster City, CA). Expression values were normalized by those of glyceraldehyde-3-phosphate dehydrogenase (GAPDH). TaqMan® probe and primer sets for MyoD (*Myod1*) (Mm00440387_m1), myogenin (*Myog*) (Mm00446194_m1), atrogin-1 *(Fbxo32)* (Mm00499523_m1), MuRF-1 (*Trim63*) (Mm01185221_m1),

CCAAT/enhancer-binding protein (C/EBP) δ (*Cebpd*) (Mm00786711_s1), and oncostatin M receptor (*Osmr*) (Mm01307326_m1) were purchased from Applied Biosystems.

2.4. Western blot analysis

Western blot analysis was performed as previously described [30,31]. Antibodies against phospho-STAT3, STAT3, GAPDH (Cell Signaling Technology, Danvers, MA), atrogin-1 (Abcam, Cambridge, MA), myogenin (Santa Cruz Biotechnology, Dallas, TX), and myosin heavy chain (MHC) (R&D systems, Minneapolis, MN) were used as primary antibodies. Horseradish peroxidase-conjugated donkey anti-rabbit IgG (GE Healthcare, Little Chalfont, UK), goat anti-mouse IgG, and mouse anti-goat IgG (Santa Cruz Biotechnology) antibodies were used as secondary antibodies. The signal was detected using FUSION Solo S (Vilber Lourmat, France) chemiluminescence imaging system.

2.5. Statistical analysis

Results were expressed as mean ± standard error of mean (SEM). The statistical analyses were performed using unpaired *t*-test, paired one-way ANOVA with post hoc Bonferroni test, or one-way ANOVA with post hoc Tukey-Kramer test, as appropriate. *P*-values less than 0.05 were considered statistically significant. All statistical analyses were performed using EZR (Saitama Medical Center, Jichi Medical University, Saitama, Japan), which is a

graphical user interface for R (The R Foundation for Statistical Computing, Vienna, Austria) [32].

3. Results

3.1. OSM suppresses C2C12 myotube formation.

To examine the morphological effect of OSM on C2C12 myotube formation, C2C12 myoblasts were treated with a differentiation medium supplemented with 2% horse serum for 3 days, and then differentiated myotubes were treated with OSM for up to 48 hours. Of note, the diameter of untreated control myotubes was increased after 24 and 48 hours from baseline (Fig. 1B). On the other hand, the diameter of myotubes was not increased but relatively reduced by 23.4% and 18.9% compared with untreated myotubes, after treatment with OSM for 24 and 48 hours, respectively (Figs. 1A, 1B). In addition, a dose-dependent effect of OSM on reducing the diameters was observed in C2C12 myotubes (Fig.1C).

Next, we assessed the OSM-induced alterations in the gene expression of key factors involved in the myogenic differentiation pathway and the muscle degradation pathway in C2C12 myotubes. The expression levels of MyoD and myogenin were significantly decreased, while those of atrogin-1 and C/EBPδ, but not MuRF-1, were significantly increased, by OSM in

C2C12 myotubes (Fig. 2A). The expression level of OSM receptor was significantly increased by OSM (Fig. 2A). The protein expression levels of myogenin and MHC were significantly decreased, while those of atrogin-1 were increased by OSM in C2C12 myotubes (Figs. 2B, 2C). These results indicate that OSM suppresses myotube formation by both inhibiting the myogenic differentiation pathway and promoting the muscle degradation pathway.

3.2. OSM inhibits C2C12 myotube formation through the STAT3 signaling.

To clarify the signaling pathway through which OSM inhibits myotube formation, we inhibited the STAT3 pathway, a major downstream of OSM receptor signaling [1], by utilizing a STAT3 inhibitor, C188-9, before treating C2C12 myotubes with OSM. Phosphorylation of STAT3 induced by OSM was attenuated by pretreatment with C188-9 in C2C12 myotubes (Fig. 3A). In a morphological analysis, pretreatment with C188-9 completely reversed the OSM-induced reduction in the diameter of C2C12 myotubes (Fig. 3B). Pretreatment with C188-9 also reversed the OSM-induced changes in the mRNA expression of MyoD, myogenin, and atrogin-1 in C2C12 myotubes (Fig. 3C). The OSM-induced decrease in myogenin and increase in atrogin-1 were also reversed by C188-9 at protein level (Figs. 3D, 3E).

Finally, we utilized siRNA for *Stat3* (si*Stat3*) and evaluated the effect of *Stat3* knockdown on the OSM-induced changes in C2C12 myotube formation. Twenty-four hours

after transfection of si*Stat3*, protein expression of STAT3 was reduced by approximately 40% (Fig. 4A). Treatment with OSM reduced the diameter of C2C12 myotubes, and the effect was significantly reversed in myotubes treated with si*Stat3* (Fig. 4B). Moreover, treatment with OSM decreased MyoD and myogenin gene expression, and increased atrogin-1 gene expression, and those effects were completely or partially reversed in myotubes treated with si*Stat3* (Fig. 4C). The effects of *Stat3* knockdown on the OSM-induced changes in myogenin and atrogin-1 levels were also reversed at protein level (Fig. 4D, 4E). These results indicate that reduced STAT3 signaling by a chemical inhibitor or gene knockdown approach reverses the inhibitory effect of OSM on C2C12 myotube formation.

4. Discussion

In the present study, we demonstrated that OSM induced C2C12 myotube atrophy by inhibiting the myogenic differentiation pathway and activating the muscle degradation pathway. Furthermore, the effect of OSM on the regulation of myotube formation was dependent on the STAT3 signaling. Because no study has examined the role of OSM in the regulation of skeletal muscle mass, this is the first study to demonstrate an inhibitory effect of OSM on the growth of differentiated myotubes *in vitro*.

This study clearly demonstrated that OSM inhibited the myogenic differentiation pathway as evidenced by reduced expression of MyoD and myogenin in C2C12 myotubes. It is currently accepted that MyoD is required for determination of myoblasts, and then, myoblasts can proliferate and further differentiate into myocytes and mature into myofibers under the action of myogenin [9]. In addition, recent *in vitro* studies indicate that myogenic differentiation regulates terminally differentiated myotube formation, namely, late myogenic differentiation [11,12]. Particularly, siRNA-mediated down-regulation of myogenin caused a reduction of terminally differentiated C2C12 myotubes [12]. In our study, an increase in the diameter of C2C12 myotubes was observed even after the 3 days differentiation period, which was completely suppressed by OSM (Fig. 1B). Collectively, our data suggest that there exists late myogenic differentiation in mature C2C12 myotubes and that OSM inhibits the process.

Regarding the effect of OSM on skeletal muscle, prior studies showed inhibitory effects of OSM on myoblast proliferation [27,29] and early-phase differentiation [28] in primary mouse satellite cells [29] and/or C2C12 myoblasts [27,28]. Unlike those studies [27-29], our study focused on the effect of OSM on the formation of mature myotubes and demonstrated the reduction of myotube diameter and the expression of MyoD, myogenin, and MHC in C2C12 myotubes treated with OSM which was administered after the 3 days differentiation period.

Considering together with prior studies, our data suggest that OSM acts not only on myoblasts, but also on mature myotubes to inhibit late myogenic differentiation *in vitro*.

Our data also demonstrated that OSM activated the muscle degradation pathway as evidenced by upregulated expression of atrogin-1 and C/EBPδ with reduced one of MHC in C2C12 myotubes. It is currently accepted that atrogin-1 and MuRF-1 are muscle-specific ubiquitin E3-ligases, which target MHC for proteasomal degradation, are activated in skeletal muscle under catabolic conditions [10]. In addition, recent studies indicate a major role for C/EBPδ in mediating the STAT3-induced activation of ubiquitin-proteasome system in skeletal muscle [17,20]. A number of previous studies evaluated the regulation of muscle atrophy by using differentiated C2C12 myotubes [11,12,16,20,33,34]. Among those studies, IL-6 [20] and LIF [16] were shown to play key roles in C2C12 myotube atrophy through activating the STAT3 signaling. In a study by Zhang et al. [20], IL-6 and phospho-STAT3 were elevated in skeletal muscle of patients with CKD, and IL-6 activated STAT3 and increased the expression of C/EBPδ and myostatin in C2C12 myotubes. In a study by Seto, et al. [16], conditioned media from C26 cancer cells induced atrophy of C2C12 myotubes in a STAT3-dependent manner, and LIF, but not IL-6 or OSM, was responsible for the atrophic effect caused by C26-conditioned media. As with those prior studies [16,20], our study demonstrated that OSM activated STAT3 signaling and upregulated the expression of atrogin-1and C/EBPδ in C2C12 myotubes. Because

OSM and LIF are among the IL-6 family cytokines, it is speculated that OSM mediates an inflammatory signal and promotes the degradation pathway to induce C2C12 myotube atrophy. Nevertheless, OSM failed to upregulate the expression of MuRF-1 in our study, suggesting a differential regulation of atrogin-1 and MuRF-1, as shown in several previous studies [6,10], in C2C12 myotubes treated with OSM.

Our data additionally demonstrated that the OSM-induced effects on C2C12 myotubes were reversed by a STAT3 inhibitor or siRNA-mediated *Stat3* knockdown, indicating that OSM utilizes the STAT3 signaling to regulate the formation of C2C12 myotubes. OSM is a multifunctional cytokine and recruits a broad array of cell signaling pathways, such as JAK/STAT, MAP kinase and PI3-kinase, on the basis of cellular expression of the OSM receptor β subunit [1,2]. Although no study has examined the OSM-induced intracellular signaling in mature myotubes, previous studies indicate a dominant role for STAT3 as a downstream of OSM receptor signaling to inhibit proliferation [27] or differentiation [28] of myoblasts *in vitro*. In addition, the JAK/STAT pathway plays a major role in mediating the effects of IL-6 family cytokines on satellite cell function [22,24], myoblast differentiation [25,26], and myotube atrophy [16,17,20]. Particularly, activation of the STAT3 signaling upregulated the expression of myostatin, atrogin-1, MuRF-1, and C/EBPδ, leading to proteolysis in C2C12 myotubes [17]. Taken together, our study is in line with previous studies

indicating OSM-induced STAT3 activation and STAT3 activation leading to sarcopenia, and is the first to demonstrate a direct role of the OSM-induced STAT3 pathway in promoting atrophy of mature myotubes *in vitro*.

This study has potential implications in the pathogenesis of sarcopenia associated with chronic wasting diseases. It is evidently shown that activation of the IL-6 family of cytokines and the downstream STAT3 pathway impairs myoblast function [22,24] and promotes muscle degradation in age-related and muscle-wasting conditions [14,16,17,20]. A prior *in vivo* study showed that prolonged expression of OSM in muscle suppressed myoblast differentiation and delayed muscle regeneration after injury [28]. Our study indicates that OSM inhibits myogenic differentiation and promotes degradation to cause myotube atrophy *in vitro*. Considering that OSM is upregulated in muscle-wasting diseases including cancer [1,35,36], it is tempting to speculate that the continuously upregulated OSM may contribute to sarcopenia by modulating myogenic differentiation and muscle degradation in several kinds of chronic diseases. However, because this was an *in vitro* study, this working hypothesis needs to be clarified by *in vivo* studies in future.

In conclusion, this study demonstrated that OSM induces C2C12 myotube atrophy by inhibiting the myogenic pathway and activating the muscle degradation pathway. Our data also demonstrated that the effect of OSM in the regulation of myotube formation is dependent on the

STAT3 pathway. Our data provide *in vitro* evidence for the OSM-induced STAT3 signaling as a potential mechanism contributing to skeletal muscle atrophy.

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Figure legends

Fig. 1. OSM inhibits C2C12 myotube formation. (A) C2C12 myoblasts were induced to differentiate into myotubes for 3 days and then treated with vehicle control (upper panels) or 20 ng/mL OSM (lower panels) for 24 (left) or 48 (right) hours. The cells were stained with hematoxylin and eosin. Scale bars, 100 μm. **(B)** The diameter was measured for C2C12 myotubes treated with vehicle or OSM for 24 or 48 hours (mean ± SEM, n = 3). #, $p < 0.01$ vs. baseline by a paired one-way ANOVA with post hoc Bonferroni test; *, $p < 0.05$; **, $p < 0.01$ vs. vehicle control cells by unpaired *t*-test. **(C)** The diameter was measured for C2C12 myotubes treated with increasing doses of OSM for 24 hours (mean ± SEM, n = 3). *, $p < 0.05$; **, $p < 0.01$ vs. vehicle control cells by one-way ANOVA with post hoc Tukey-Kramer test.

Fig. 2. OSM modulates the expression of key factors regulating muscle mass in C2C12 myotubes. (A) Differentiated C2C12 myotubes were treated with (black bars) or without (white bars) OSM for 24 hours and mRNA expression for MyoD, myogenin, atrogin-1, MuRF-1, C/EBPδ, or OSM receptor was analyzed by real-time quantitative PCR. mRNA level normalized by GAPDH was expressed as relative to controls (mean ± SEM, n = 3). **(B)** C2C12 myotubes were treated with or without OSM for 12 or 24 hours and protein expression was analyzed by Western blotting for myogenin, MHC, atrogin-1, or GAPDH. **(C)** Protein expression level for

myogenin, MHC, or atrogin-1, was quantified by densitometry, normalized by GAPDH and expressed as relative to control cells (mean ± SEM, n = 3). *, $p < 0.05$; **, $p < 0.01$ vs. control by unpaired *t*-test.

Fig. 3. Inhibition of STAT3 signaling reverses the inhibitory effect of OSM on C2C12 myotube formation. (A) Differentiated C2C12 myotubes were preincubated with or without a STAT3 inhibitor, C188-9, for 1 hour, and then treated with or without 20 ng/mL OSM for the time indicated. Protein expression level was analyzed by Western blotting for phospho-STAT3, STAT3, or GAPDH. Representative results from three independent experiments are shown. **(B)** The diameter was measured in C2C12 myotubes treated with or without 20 ng/mL OSM for 24 or 48 hours after preincubation with or without C188-9 (mean ± SEM, n = 3). #, $p < 0.05$ vs. control without OSM/C188-9 by one-way ANOVA with post hoc Tukey-Kramer test. **(C)** C2C12 myotubes were treated with or without 20 ng/mL OSM for 24 hours after preincubation with or without C188-9. mRNA expression level for MyoD, myogenin, or atrogin-1 was analyzed by real-time quantitative PCR. mRNA level normalized by GAPDH was expressed as relative to controls without OSM (mean ± SEM, n = 3). **(D)** Protein expression was analyzed by Western blotting for myogenin, atrogin-1, or GAPDH in C2C12 myotubes treated with or without OSM after pretreatment with or without C188-9. Representative results from three

independent experiments are shown. **(E)** Protein expression level was quantified by densitometry, normalized by GAPDH and expressed as relative to controls without OSM (mean ± SEM, n = 3). *, $p < 0.05$ vs. control without OSM/C188-9 by unpaired *t*-test.

Fig. 4. Knockdown of *Stat3* reverses the inhibitory effect of OSM on C2C12 myotube formation. (A) Differentiated C2C12 cells were transfected with scramble control or si*Stat3* for 24 hours and STAT3 protein expression was analyzed by Western blotting. **(B)** The diameter was measured in C2C12 myotubes treated with or without OSM for 24 hours after transfection with scramble control or si*Stat3* (mean ± SEM, n = 3). #, $p < 0.05$ vs. scramble control without OSM by one-way ANOVA with post hoc Tukey-Kramer test. **(C)** C2C12 myotubes were treated with or without OSM for 24 hours after transfection with scramble control or si*Stat3*. mRNA expression level for MyoD, myogenin, or atrogin-1 was analyzed by real-time quantitative PCR. mRNA level normalized by GAPDH was expressed as relative to controls without OSM (mean ± SEM, n = 3). **(D)** Protein expression was analyzed by Western blotting for myogenin, atrogin-1, or GAPDH in C2C12 myotubes treated with or without OSM after transfection with scramble control or si*Stat3*. Representative results from three independent experiments are shown. **(E)** Protein expression level was quantified by densitometry, normalized by GAPDH

and expressed as relative to controls without OSM (mean ± SEM, n = 3). *, $p < 0.05$ vs. scramble control without OSM by unpaired *t*-test.

Figure

Fig. 1

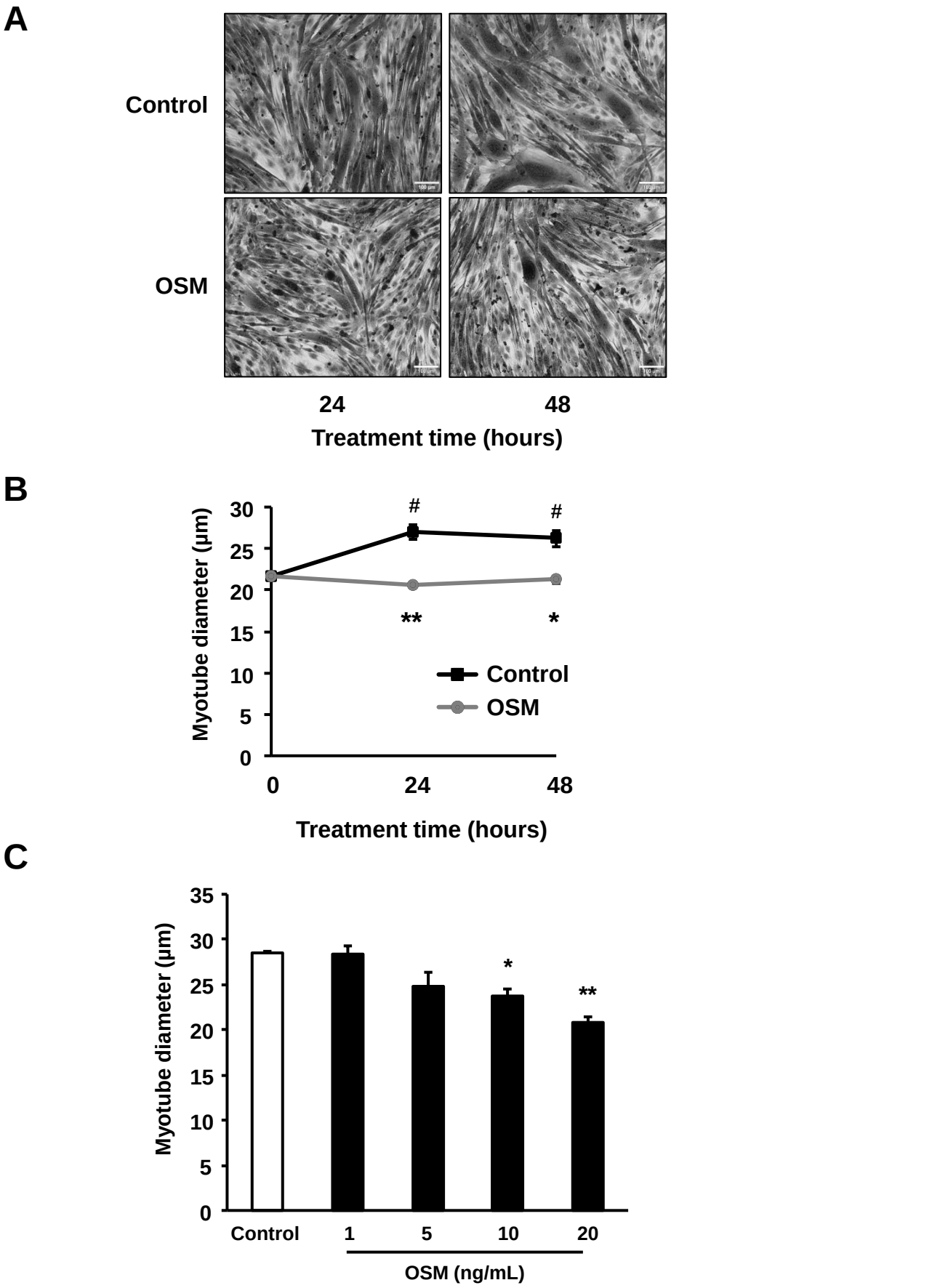

A
Control
OSM
24
48
Treatment time (hours)
B
Myotube diameter (μm)
30
25
20
15
10
5
0
#
#
**
*
Control
OSM
0
24
48
Treatment time (hours)
C
Myotube diameter (μm)
35
30
25
20
15
10
5
0
*
**
Control
1
5
10
20
OSM (ng/mL)

Fig. 2

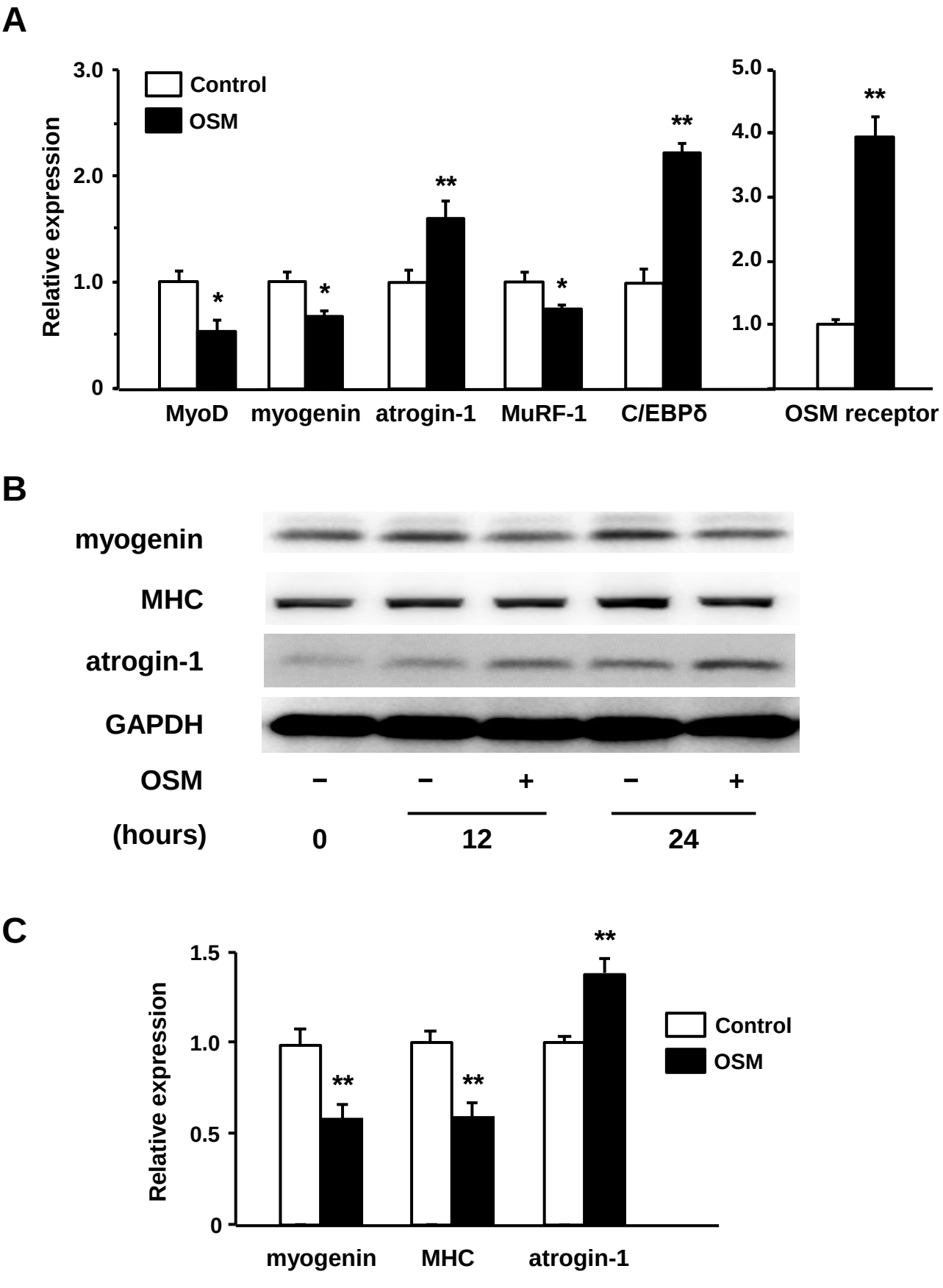
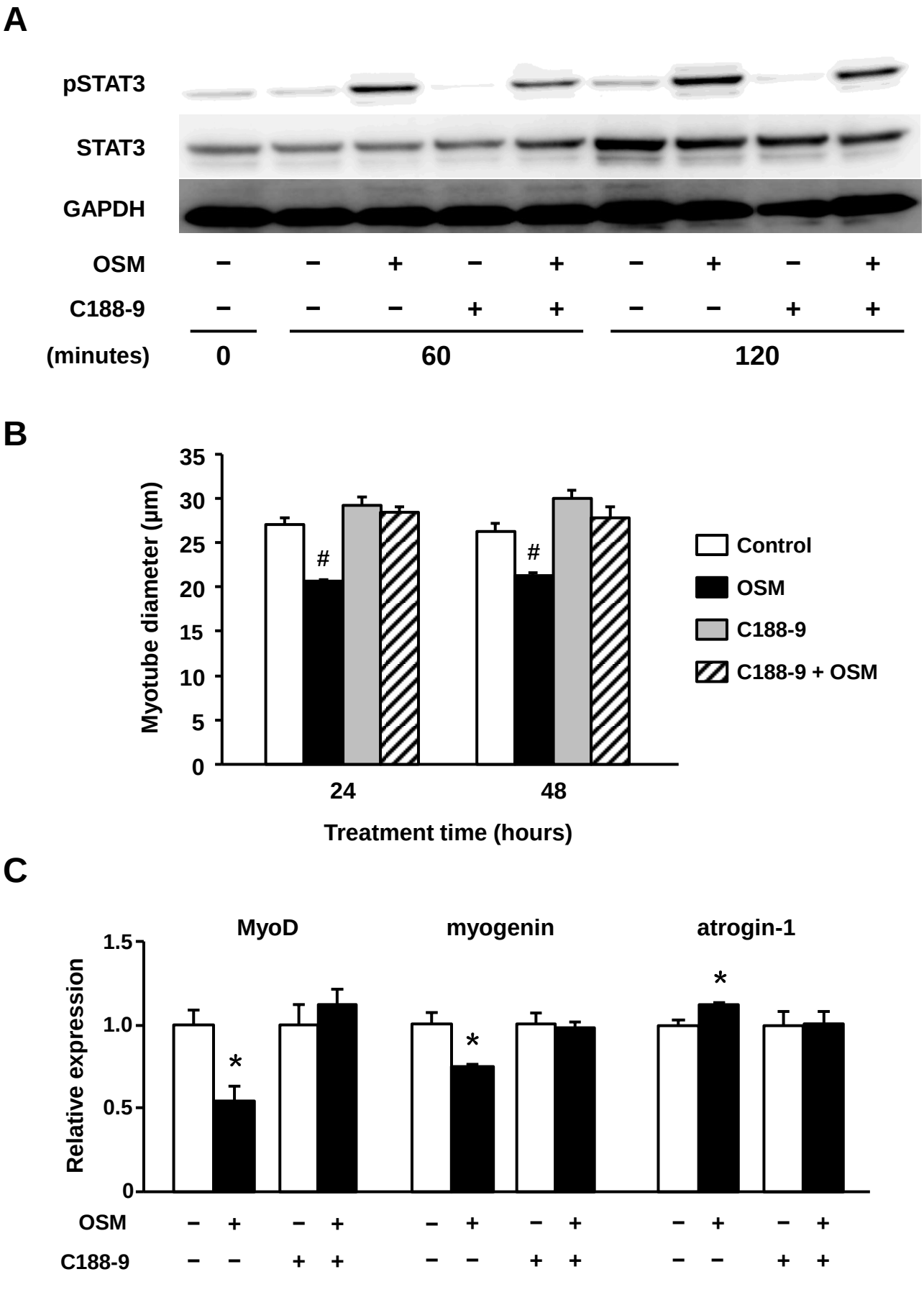
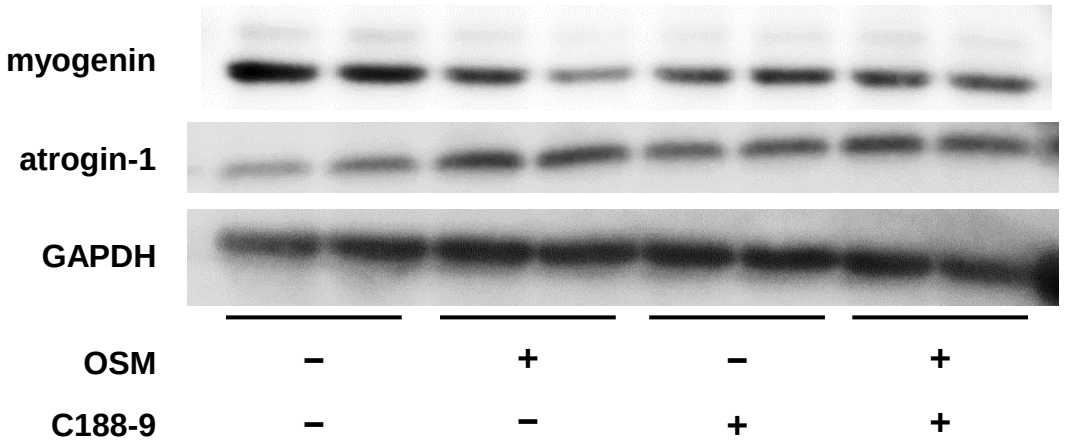

A
Relative expression
Control
OSM
3.0
2.0
1.0
0
5.0
4.0
3.0
2.0
1.0
*
**
MyoD
myogenin
atrogin-1
MuRF-1
C/EBPδ
OSM receptor
B
myogenin
MHC
atrogin-1
GAPDH
OSM
– – + – +
(hours)
0
12
24
C
Relative expression
1.5
1.0
0.5
0
**
Control
OSM
myogenin
MHC
atrogin-1

Fig. 3


A
pSTAT3
STAT3
GAPDH
OSM
C188-9
(minutes)
0
60
120
B
Myotube diameter (μm)
Treatment time (hours)
24
48
Control
OSM
C188-9
C188-9 + OSM
C
MyoD
myogenin
atrogin-1
Relative expression
OSM
C188-9

D


myogenin
atrogin-1
GAPDH
OSM
C188-9
−
+
−
+
−
−
+
+

E

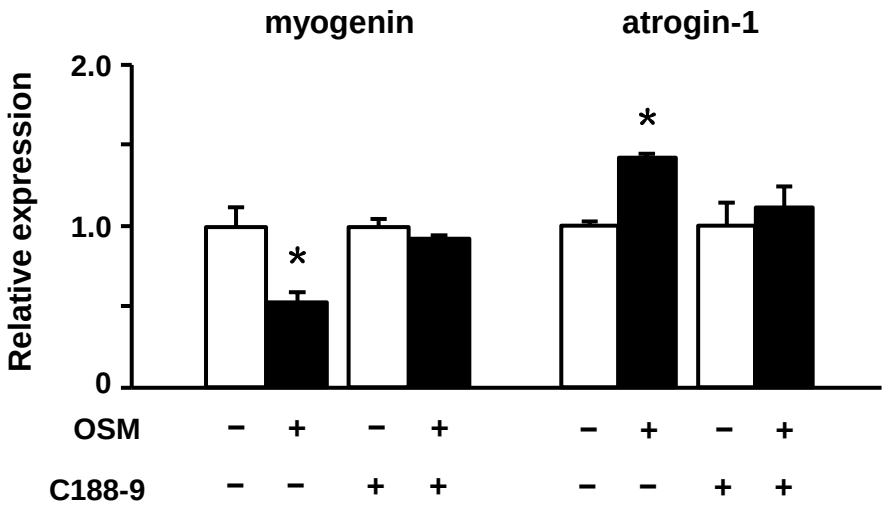
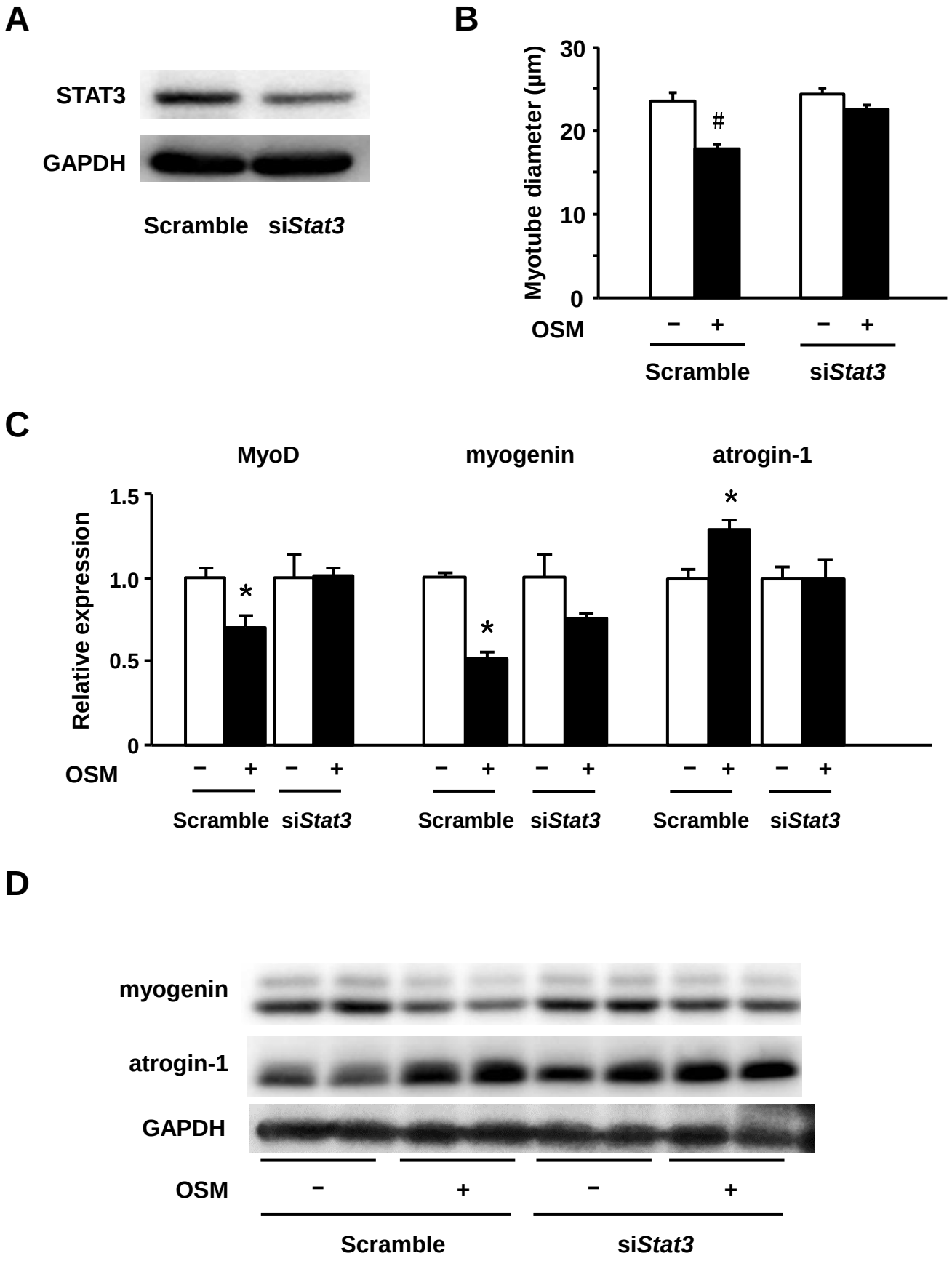
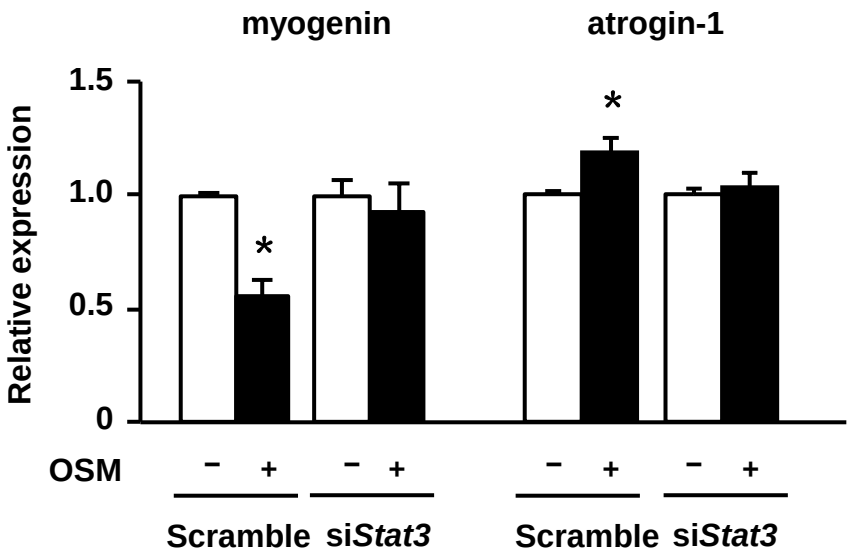

myogenin
atrogin-1
Relative expression
2.0
1.0
0
*
*
OSM
C188-9
− + − + − + − +
− − + + − − + +

Fig. 4


A
STAT3
GAPDH
Scramble
siStat3
B
Myotube diameter (μm)
30
20
10
0
#
OSM
−
+
−
+
Scramble
siStat3
C
MyoD
myogenin
atrogin-1
Relative expression
1.5
1.0
0.5
0
*
*
*
OSM
−
+
−
+
−
+
−
+
−
+
−
+
Scramble
siStat3
Scramble
siStat3
Scramble
siStat3
D
myogenin
atrogin-1
GAPDH
OSM
−
+
−
+
Scramble
siStat3

E


myogenin
atrogin-1
Relative expression
1.5
1.0
0.5
0
*
*
OSM
−
+
−
+
−
+
−
+
Scramble
siStat3
Scramble
siStat3