SIRT2 overexpression alleviates remifentanil-induced postoperative hyperalgesia through microglia

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Research Article

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Abstract

Background: Sirtuin 2 (SIRT2), a member of the mammalian sirtuin family, plays an important role in the pathogenesis of various neurological diseases. However, whether SIRT2 is involved in the regulation of remifentanil-induced postoperative hyperalgesia remains unclear. This study aimed to investigate the potential role of SIRT2 in regulating remifentanil-induced postoperative hyperalgesia.

Methods: Remifentanil-induced postoperative hyperalgesia model was established in rats. SIRT2 was over-expressed by injecting recombinant adenoviruses. Nociceptive behaviors of mechanical allodynia and thermal hyperalgesia were measured with paw withdrawal mechanical threshold and paw withdrawal thermal latency, respectively.

Iba1 (marker of microglia activation) and SIRT2 levels were detected with Western blot. Iba1 was also measured with immunofluorescence.

Results: We found that SIRT2 was downregulated in the spinal cord in remifentanil-induced postoperative hyperalgesia rats. Intrathecal injection of a recombinant plasmid expressing SIRT2 markedly alleviated mechanical allodynia and thermal hyperalgesia in remifentanil-induced postoperative hyperalgesia rats. Iba1 were upregulated after surgical incision and remifentanil infusion, and the up-regulation was more obvious after the combination of surgical incision and remifentanil infusion. Moreover, our results showed that overexpression of SIRT2 inhibited the activation of the microglia in the spinal cord of remifentanil-induced postoperative hyperalgesia rats. Overexpression of SIRT2 significantly attenuated incision- and/or remifentanil induced pronociceptive effects and spinal microglia activation.

Conclusions: Overexpression of SIRT2 alleviates remifentanil-induced postoperative hyperalgesia possibly via inhibition of spinal microglia activation. Therefore, SIRT2 may serve as a potential therapeutic target for treatment of neuropathic pain.

Trial registration: Not applicable.

Background

Remifentanil is a novel super short acting μ-opiate receptor agonist. The analgesic effect of remifentanil is 1.5 ~ 3 times that of fentanyl. However, after discontinuation of remifentanil, the development of the nociceptive stimulation is also more rapid and prominent. The hyperalgesia induced by remifentanil is obviously stronger than that by long-acting opioids, which not only results in significant increase postoperative pain score and analgesic drugs requirements [1, 2], but also affects the patients' recovery after the surgery, thus limiting its wide application.

Currently, there are several main mechanisms for postoperative hyperalgesia caused by remifentanil. For example, neuroimmune mechanisms (activation of microglia) are involved in postoperative hyperalgesia. Microglia are immune cells of the central nervous system. When pathologic event or tissue damage

occurs, glia cells are activated to release pro-inflammatory regulatory factors, such as cytokines (TNF-α and IL-1β), chemokines (CCL2), reactive oxygen species (ROS), NO (nitric oxide), etc. [3]. The release of these factors will lead to lower threshold of response to pain signals and increased excitability of neurons. In the production and maintenance of pain, proinflammatory factors released by glial cells in the peripheral and central nervous system play an important role in the sensitization of pain. Experimental studies on animal models such as peripheral inflammation, spinal cord injury, and nerve injury [4] have suggested that spinal cord glial cells play an important role in central sensitization [5] and pathological pain [6]. The important role of microglia activation and release of regulatory factors in chronic pain models due to the introduction of nociceptive information has been confirmed [7, 8]. Recent studies have found that chemokines (CXCL1 and CXCR2), hydrogen peroxide, cytokines, oxidative stress and other inflammatory mediators play an important role in opioid-induced postoperative hyperalgesia [9–11]. Previous study of our group has confirmed that spinal microglia cells were highly activated in remifentanil-induced hyperalgesia, and the expressions of pro-inflammatory factors, including IL-6, TNF-α and p-NR2B, in cells were significantly increased [12], suggesting that spinal microglia cells may play an important role in remifentanil-induced hyperalgesia. However, our understanding of the possible mechanism by which spinal microglia contributes to remifentanil-induced hyperalgesia is still limited.

Recently, Sirtuin 2 (SIRT2) has attracted much attention due to its important role in microglia activation. SIRT2 is expressed in microglia cells and can inhibit the activation of microglia cells and the release of inflammatory cytokines [13]. It can also mediate the neuroimmune response of central glial cells [14]. *In vivo* and *in vitro* experiments have shown that inhibition or knockout of SIRT2 can significantly increase the response of glial cells to proinflammatory products, and up-regulate the transformation of ROS and reactive nitrogen free radicals [14, 13]. It has been reported that the activation of microglia induced by LPS (lipopolysaccharide) and TNF-α could lead to a significant reduction in SIRT2 phosphoric acid levels [13]. *In a vitro* experiment showed that N9 microglia transfected with wild-type SIRT2 could significantly reduce the expressions of NO induced by LPS and TNF-α, pro-inflammatory cytokine IL-6, and activation marker CD40, which were seemingly dependent on phosphorylation at S331 [13]. Both *in vivo* and *in vitro* experiments have confirmed that the SIRT2 inhibitor AK-7 could significantly increase the activation of microglia induced by traumatic brain injury and increase the expression and release of inflammatory factors [14–16]. Lys310 acetylation of p65 of NF-κB subunit is increased after SIRT2 inhibition or knockout in microglia, which induces NF-κB into the nucleus and mediates inflammatory factor transcription [14, 13]. The regulation of oxidative stress by SIRT2 may be achieved through its deacetylation of FoxO3a (Forkhead box protein O3), thereby increasing the transcription of FoxO3a-dependent MnSOD (manganese superoxide dismutase) [17], which is considered to be one of the most important endogenous antioxidant enzymes in the body and can catalyze the degradation of ROS. However, whether SIRT2 is involved in the regulation of remifentanil-induced postoperative hyperalgesia remains unclear.

Herein, we explored the involvement of SIRI2 in remifentanil-induced postoperative hyperalgesia. We established remifentanil-induced postoperative hyperalgesia model in rats. SIRT2 overexpression was

induced. The effect and mechanism of SIRT2 overexpression on remifentanil-induced postoperative hyperalgesia were analyzed and discussed.

Methods

Animals

Adult male Sprague-Dawley rats (n= 64), weighing 200 to 250 g, were supplied by the Laboratory Animal Center of Nanjing Drum Tower Hospital (Nanjing, China). All animals were housed in cages and maintained under a 12-h light/12-h dark cycle in a temperature controlled room (22±2°C) with food and water available ad libitum. All methods were carried out in accordance with relevant guidelines and regulations. All animal procedures were approved by the Experimental Animals Welfare and Ethics Committee of Nanjing Drum Tower Hospital and were performed in accordance with the guidelines for the use of laboratory animals. Efforts were made to minimize the number and suffering of animals.

Establishment of remifentanil-induced postoperative hyperalgesia model

After being anesthetized with sevoflurane (Jiangsu Hengrui Pharmaceutical Co., Ltd., China) delivered via a nose mask (induction, 3.5%; maintenance, 3.0%), rats (n= 32) were subcutaneously administered with remifentanil (Yichang Humanwell Pharmaceutical Co., Ltd., China) (0.04 mg/kg, 0.4 ml) at a rate of 0.8 ml/h over a period of 30 min using an infusion pump. This treatment was to induce pronociceptive effects as described in our previous study [12]. Control rats were subcutaneously administered the same volume of NS (0.8 ml/h, 30 min) (n= 32).

The surgical incision to induce postoperative pain was performed as previously described by Brennan et al. [18]. Briefly, after sterilization of the right hind paw with 10% povidoneiodine, a 1-cm longitudinal incision was made through the skin and fascia, starting at 0.5 cm from the edge of the heel and extending toward the toes. The underlying flexor muscle was elevated, incised longitudinally, and retracted, leaving the muscle origin and insertion intact. After suture of the lesion, the wound site was covered with erythromycin ointment. The surgical incision was started approximately 5 min after the infusion of remifentanil or NS. The control group received sham surgery consisting of anesthesia with sevoflurane, sterilization of the hind paw, and erythromycin ointment on the plantar surface, without plantar incision.

Implantation of intrathecal catheter

Intrathecal catheter implantation was conducted according to a method described previously [19]. Briefly, rats were anesthetized, and the polyethylene catheter was inserted in the cisterna magna through an incision advanced 7.0 cm caudally to the lumbar enlargement. Proper location of the intrathecal

implantation was confirmed by bilateral hind limb paralysis with injection of 2% lidocaine (Sigma, St. Louis, MO, USA). Afterwards, the catheter was fixed and the incision was sutured.

Administration of recombinant lentiviruses

Recombinant lentiviruses-SIRT2 were purchased from Applied Biological Materials Inc. (GENE, Shanghai, China). The recombinant adenoviruses were amplified using 293T cells and then purified using double CsCl purification. For gene transfer, 1×10^8 pfu recombinant adenoviruses were injected with a microinjection syringe linked with the intrathecal catheter.

Sample collection

At 1 month after administration of recombinant lentiviruses, rats were euthanized. The L4–L5 lumbar spinal cords were removed and the dorsal root ganglia (DRG) from L4–L5 lumbar spinal cords were dissected for detection.

Animal grouping

According to different treatments, rats were divided into eight groups: Group Sham+NS (received sham surgery and NS infusion); Group Inci+NS received (surgical incision and NS infusion); Group Sham+Remi (received sham surgery and remifentanil infusion); Group Inci+Remi (received surgical incision and remifentanil infusion); Group Sham+LV-control (received sham surgery and LV-control); Group Sham+LV-SIRT2 (received sham surgery and LV-SIRT2); Group Inci+Remi+LV-control (received surgical incision and remifentanil infusion treatment after LV-control); Group Inci+Remi+LV-SIRT2 (received surgical incision and remifentanil infusion treatment after LV-SIRT2).

For behavioral tests, 32 rats were randomly divided into Sham+NS, Inci+NS, Sham+Remi, and Inci+Remi groups (n=8 each group) and were tested at 1 d prior to the surgical procedure (baseline value) and at 2 h, 6 h, 24 h, 48 h after the surgical procedure; For Western blot analysis, 32 rats were randomly divided into Sham+NS, Inci+NS, Sham+Remi and Inci+Remi groups (n=8 each group), and specimens were collected at 2 d after the surgical procedure. For immunofluorescence, 16 rats were randomly divided into Sham+LV-control, Sham+LV-SIRT2, Inci+Remi+LV-control, and Inci+Remi+LV-SIRT2 groups (n=4 each group), and specimens were collected at 2 d after the surgical procedure. The observers were blinded to the experimental groups in the experiments.

Nociceptive behavior tests

To assess mechanical allodynia, the paw withdrawal mechanical threshold (PWMT) was measured using a set of von Frey filaments. The animals were placed in plastic boxes (20×20×15 cm) with a wire mesh

bottom (1×1 cm) and allowed to acclimatize for 30 min. Each von Frey filament was applied vertically to the plantar surface adjacent to the wound of right hind paw for 6 to 8 s with sufficient force. Positive responses were defined as paw flinching or brisk withdrawal. There was a 5-min interval between withdrawal responses. The PWMT was determined by sequentially increasing and decreasing the stimulus strength as described previously [20]. Each rat was tested five times per stimulus strength, and three or more positive responses of the lowest strength were considered as PWMT. The measurement of PWMT was conducted three times at each time point.

To evaluate thermal hyperalgesia, paw withdrawal thermal latency (PWTL) was measured using a biological research apparatus (Ugo Basile 37370, Comerio, Italy). Rats were placed into glass-floored testing cages and allowed to acclimate for approximately 15 min. Before starting the experiment, the infrared heat intensity of the apparatus was adjusted to give an average PWTL of approximately 10 s, and the cutoff latency was set at 15 s to avoid tissue damage. The infrared source was positioned directly beneath the area adjacent to the wound of the right hind paw. PWTL was defined as the time from onset of the infrared heat stimulus to withdrawal of the paw from the heat source. We repeated the stimulation three times with intervals of approximately 5 min, recorded the latencies, and calculated the mean value.

Western blot analysis

Spinal cord segments (right dorsal part of L4–L5) were homogenized in lysis buffer. The homogenate was centrifuged at 13,000 r/min for 10 min at 4°C, and the supernatant was collected. The protein concentration was determined by the BCA Protein Assay Kit. Samples (50 mg) were separated on sodium dodecyl sulfate polyacrylamide gel electrophoresis and transferred onto a nitrocellulose membrane. The membranes were blocked with 5% skimmed milk for 1 h at room temperature and incubated overnight at 4°C with the following primary antibodies: Iba1 (1:600, ab15690, Abcam), and SIRT2 (1:2000, ab211033, Abcam). After washing, the membrane was incubated with the goat anti-mouse secondary antibody (1:5000, ab97040, Abcam) or goat anti-rabbit secondary antibody (1:5000, ab7090, Abcam) conjugated with horseradish peroxidase for 1 h at room temperature. Next, the immune complexes were detected using the electrochemiluminescence system (Millipore Immobilon). β-actin was used as a loading control for total protein. Image-Pro Plus (version 6.0, Media Cybernetics, USA) was used to analyze the density of specific bands.

Immunofluorescence analysis

The L4–L5 spinal cord segments were removed, post-fixed in 4% paraformaldehyde, and then incubated with 30% sucrose. Transverse spinal sections (25 mm) were blocked with 10% goat serum in 0.3% Triton for 1 h at room temperature, and incubated overnight at 4°C with primary antibodies (Iba1, 1:200, Abcam). After washing in phosphate-buffered saline, sections were incubated with secondary antibody (Alexa Fluor 488 for Iba1, 1:1500, ThermoFisher) for 1 h at room temperature. The stained sections were

mounted on glass slides, air-dried, covered with coverslips, and examined under a Leica multiphoton confocal microscope (Leica Microsystems, Wetzlar, Germany).

Statistical analysis

Statistical analysis was performed using SPSS 15.0 software. Data were expressed as mean±SD. Two-way analysis of variance with repeated measures followed by post hoc Bonferroni multiple comparisons were used to analyze behavioral data. One-way analysis of variance followed by post hoc Bonferroni multiple comparisons were used to analyze Western blot data. $P<0.05$ was set as the level of statistical significance.

Results

Effects of remifentanil infusion on mechanical allodynia and thermal hyperalgesia

In order to evaluate whether remifentanil can induce hyperalgesia, we tested the pain behavior changes (PWMT and PWTL) of rats in Sham+NS group, Sham+Remi group, Inci+NS group, and Inci+Remi group. As shown in Figure 1, there were no significant differences in the baseline PWMT and PWTL among all four groups ($P>0.05$). Sham+NS group did not induce significant changes in nociceptive thresholds compared with baseline ($P>0.05$). In Inci+NS group, surgical incision induced mechanical allodynia and thermal hyperalgesia at 2 h after the surgical procedure and lasted for 2 days, respectively ($P<0.05$). In Sham+Remi group, remifentanil infusion also induced mechanical allodynia and thermal hyperalgesia, which was observed as early as 2 h after the surgical procedure and lasted for 2 days ($P<0.05$). Notably, in Inci+Remi group, remifentanil infusion exaggerated incision-induced mechanical allodynia and thermal hyperalgesia ($P<0.05$) when compared with group Inci+NS. Thus, our data demonstrates that remifentanil could induce hyperalgesia.

Incision- and/or remifentanil-induced upregulation of glial markers in the spinal cord

To analyze whether remifentanil induced hyperalgesia is related to the activation of microglia, we used Western blot and immunofluorescence to detect the changes in Iba1 expression of microglia at the spinal cord level. Western blot analyses showed that both surgical incision and remifentanil infusion alone increased the levels of Iba1, when compared with sham+NS group ($P<0.05$) (Figure 2). Furthermore, remifentanil infusion aggravated the upregulation of Iba1 induced by surgical incision in Inci+Remi group ($P<0.05$). Similarly, immunofluorescence results showed that compared with Sham+NS group, the expression of Iba1 moderately increased in Inci+NS group and Sham+Remi group and dramatically

increased in Inci+Remi group (Figure 3). These results indicate that remifentanil may cause excessive activation of microglia at the spinal cord level of normal rats and rats with surgical incision.

Incision- and/or remifentanil-induced down regulation of SIRT2 in the spinal cord

To further evaluate whether excessive activation of microglia is related to SIRT2, Western blot analyses were used to quantify the expression of SIRT2 in the spinal cord at 2 d after the surgical procedure. Both surgical incision and remifentanil infusion decreased the levels of SIRT2 protein, when compared with sham+NS group ($P<0.05$) (Figure 4). Furthermore, SIRT2 level was further reduced in Inci+Remi group ($P<0.05$). Then, immunofluorescence was conducted to examine the expression and location of SIRT2 in the spinal cord at 2 d after the surgical procedure. Similar results were obtained. Compared with Sham+NS group, the expression of SIRT2 moderately decreased in Inci+NS group and Sham+Remi group, but it was dramatically decreased in Inci+Remi group (Figure 4). These results showed that remifentanil reduced SIRT2 protein levels at the spinal cord level of normal rats and rats with surgical incision, indicating that remifentanil induced hyperalgesia may be related to the decreased expression of SIRT2.

The co-expression of SIRT2 and Iba1 in the spinal cord

Immunofluorescence was used to examine the co-expression of SIRT2 and Iba1 in the spinal cord at 2 d after the surgical procedure. The results demonstrated that SIRT2 were co-expression with Iba1 in the spinal card at 2d in Inci+Remi group after the surgical procedure (Figure 5). This phenomenon suggests that SIRT2 may play an important role in the activation of microglia cells.

Overexpression of SIRT2 attenuates mechanical allodynia and thermal hyperalgesia in model Rats

To investigate the biological function of SIRT2 in regulating remifentanil-induced postoperative hyperalgesia, we overexpressed SIRT2 in remifentanil-induced postoperative hyperalgesia rats by intrathecal injection of recombinant lentivirus expressing SIRT2 (LV-SIRT2). We then examined the effect of SIRT2 overexpression on remifentanil-induced postoperative hyperalgesia by assessment of thermal hyperalgesia and mechanical allodynia. The results showed that overexpression of SIRT2 significantly alleviated thermal hyperalgesia and mechanical allodynia of rats (Figure 6), implying that overexpression of SIRT2 inhibits remifentanil-induced postoperative hyperalgesia.

Overexpression of SIRT2 down-regulates the expression of Iba1

To investigate the mechanism of SIRT2 in regulating remifentanil-induced postoperative hyperalgesia, we detected the effect of SIRT2 overexpression on Iba1, which is a glia marker. We found that overexpression of SIRT2 significantly suppressed the expression of Iba1 protein in the spinal of remifentanil-induced postoperative hyperalgesia rats (Figure 7). The results showed that SIRT2 overexpression suppressed Iba1 expression, suggesting that SIRT2 may inhibit microglia activation in remifentanil-induced postoperative hyperalgesia.

SIRT2 overexpression inhibits the activation of microglia

Immunofluorescence was further conducted to verity the role of SIRT2 on microglia activation. As shown in Figure 8, there was enhanced expression of Iba1 in the dorsal horn of L4–L5 spinal cord induced by Inci+Remi+LV-control at 2 d after the surgical procedure. Compared with Inci+Remi+LV-control group, the expression of Iba1 moderately decreased in Inci+Remi+Lv-SIRT2 group. This result further confirmed that SIRT2 could inhibit microglia activation in remifentanil-induced postoperative hyperalgesia.

Discussion

With the increasing use of remifentanil as an analgesic in clinical anesthesia, lines of evidence have demonstrated that patients receiving remifentanil to control pain during operation may paradoxically become more sensitive to postoperative pain [21, 22]. We selected the dosage of remifentanil (0.04 mg/kg) based on our previous studies [12, 23], which showed that this dosage could enhance postoperative hyperalgesia in rats. The results in this study showed that treatments including sevoflurane anesthesia, sham surgery, and NS infusion had no significant influence on pain behavior, which is consistent with our previous studies [12, 23] and other studies [24, 25]. Both surgical incision and remifentanil infusion induced significant mechanical allodynia and thermal hyperalgesia. However, when remifentanil was infused as an analgesic during surgical incision as a part of general anesthesia, it significantly enhanced incisional pain and prolonged mechanical allodynia and thermal hyperalgesia.

In recent years, a growing body of evidence has found that SIRT2 plays a role in many neurological disorders. For example, Zhang et al. reported that overexpression of SIRT2 could attenuate neuropathic pain and neuroinflammation via NF-κB [26]. Wang et al. reported that inhibition of SIRT2 reduced striatal dopamine consumption and improved antioxidant capacity and behavioral abnormalities in rats with Parkinson's disease [27]. Loss of SIRT2 restored microtubule stability and promoted elimination of toxic Aβ oligomers from Alzheimer's disease [28]. In Huntington's disease, SIRT2 inhibition lowered steroid levels and reduced the toxicity of the mutated Huntington protein [29]. SIRT2 inhibition can induce antidepressant-like action, and overexpression of SIRT2 can inhibit depressive behaviors [30, 31]. Xie et al. reported that down-regulation of SIRT2 protected the mouse brain against ischemic stroke [32]. Krey et al. reported that SIRT2 knockout preserved neural function after experimental stroke in mice [33]. In contrast, Yuan et al. reported that SIRT2 inhibition aggravated blood brain barrier destruction and traumatic brain injury in mouse models [14]. However, whether SIRT2 is involved in the regulation of

opioid-induced postoperative hyperalgesia such as remifentanil is unclear. In this study, our results showed that SIRT2 was down-regulated in DRG of remifentanil-induced postoperatively hyperalgesic rats. The overexpression of SIRT2 inhibited mechanical hypersensitivity and thermal hyperalgesia in model rats. These findings suggest that SIRT2 overexpression, induced by microglial activation, plays an important role in the pathogenesis of remifentanil induced postoperative hyperalgesia.

It is now well established that glial cells, in addition to neurons, are involved in the initiation and maintenance of various pain conditions[34]. Evidence has suggested that glial cells can be activated after painful stimuli and injuries, which then release various glial mediators including proinflammatory cytokines to modulate neuronal activity and synaptic strength [35–40]. Opioid treatment can also activate glial cells to induce opioid induced hyperalgesia in previous studies [41, 42]. Although studies on the mechanisms underlying opioid induced hyperalgesia have mainly focused on neuronal cells, accumulating evidence has demonstrated that activation of glial cells is closely implicated in the development and maintenance of opioid induced hyperalgesia [42, 43]. Our previous study showed that glial cells were activated and proinflammatory cytokines were increased during the process of remifentanil-induced postoperative hyperalgesia [12]. The present study also demonstrated that there were activated glial cells in spinal cord in remifentanil induced hyperalgesia rats.

Conclusion

In conclusion, our results showed that SIRT2 was expressed in microglia and that remifentanil-induced pain reduced SIRT2 levels in the spine. Importantly, we showed that overexpression of SIRT2 alleviated remifentanil induced hyperalgesia and inhibited microglia activation in spinal cord in remifentanil induced hyperalgesia rats. Immunofluorescence results showed that SIRT2 co-localized with microglia. The results demonstrated that SIRT2 may play an important role in remifentanil induced hyperalgesia via inhibiting activated glia cells. Our findings not only confirmed that remifentanil had a certain effect on postoperative hyperalgesia, but also demonstrated that the overexpression of SIRT2 in microglia had a significant inhibitory effect on postoperative hyperalgesia induced by remifentanil. Further investigations are needed to further support the rational use of SIRT2 as a therapeutic target.

Abbreviations

SIRT2	Sirtuin 2
CCL2	chemokines
ROS	reactive oxygen species
NO	nitric oxide

Declarations

Ethics approval and consent to participate:

All animal procedures were approved by the Experimental Animals Welfare and Ethics Committee of Nanjing Drum Tower Hospital and were performed in accordance with the guidelines for the use of laboratory animals.

Consent for publication:

Not applicable.

Availability of data and materials:

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests:

The authors declare that they have no competing interests.

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Authors' contributions:

Conceived and designed the experiments: Zhengliang Ma, Jinhua Bo; Performed the experiments: Wei Zhu, Xinmei Wang, Jinhua Bo, Ming Jiang, Ying Liang, Zhengliang Ma; Analyzed the data: Wei Zhu, Xinmei Wang; Writing–original draft: Wei Zhu, Xinmei Wang; Writing–review & editing: Ming Jiang, Zhengliang Ma, Jinhua Bo. All authors have read and reviewed the manuscript.

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Figures

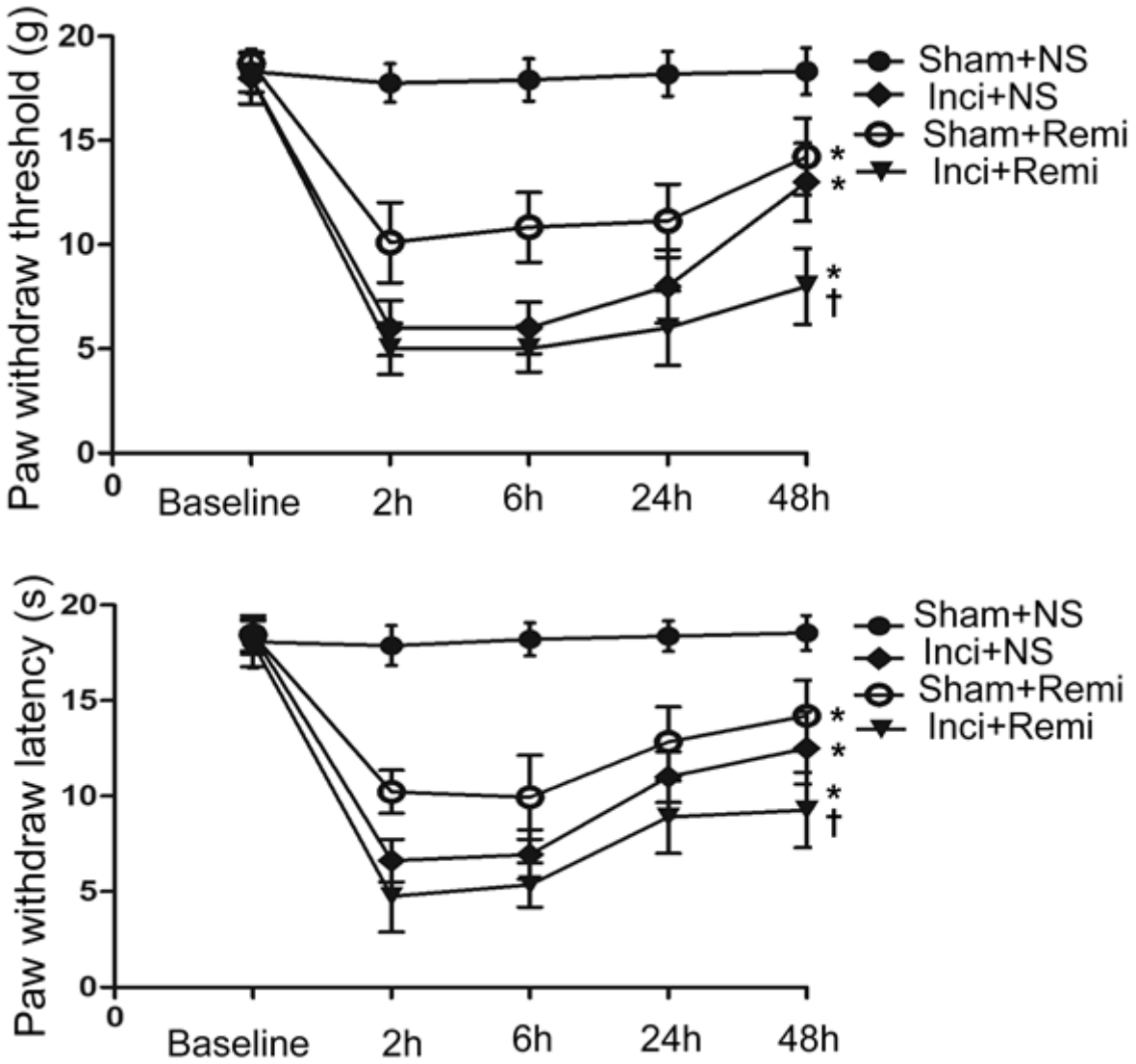

Figure 1

Analysis of incision- and/or remifentanil-induced mechanical allodynia and thermal hyperalgesia (n=6). PWMT and PWTL was measured at 1 d before (baseline value) and 2 h, 6 h, 24 h, 48 h after the surgical procedure. Data were expressed as mean±SD. *P<0.05, compared with Sham+NS; †P<0.05, compared with Inci+NS group. Inci: incision; Remi: remifentanil; NS: normal saline; Sham: sham surgery.

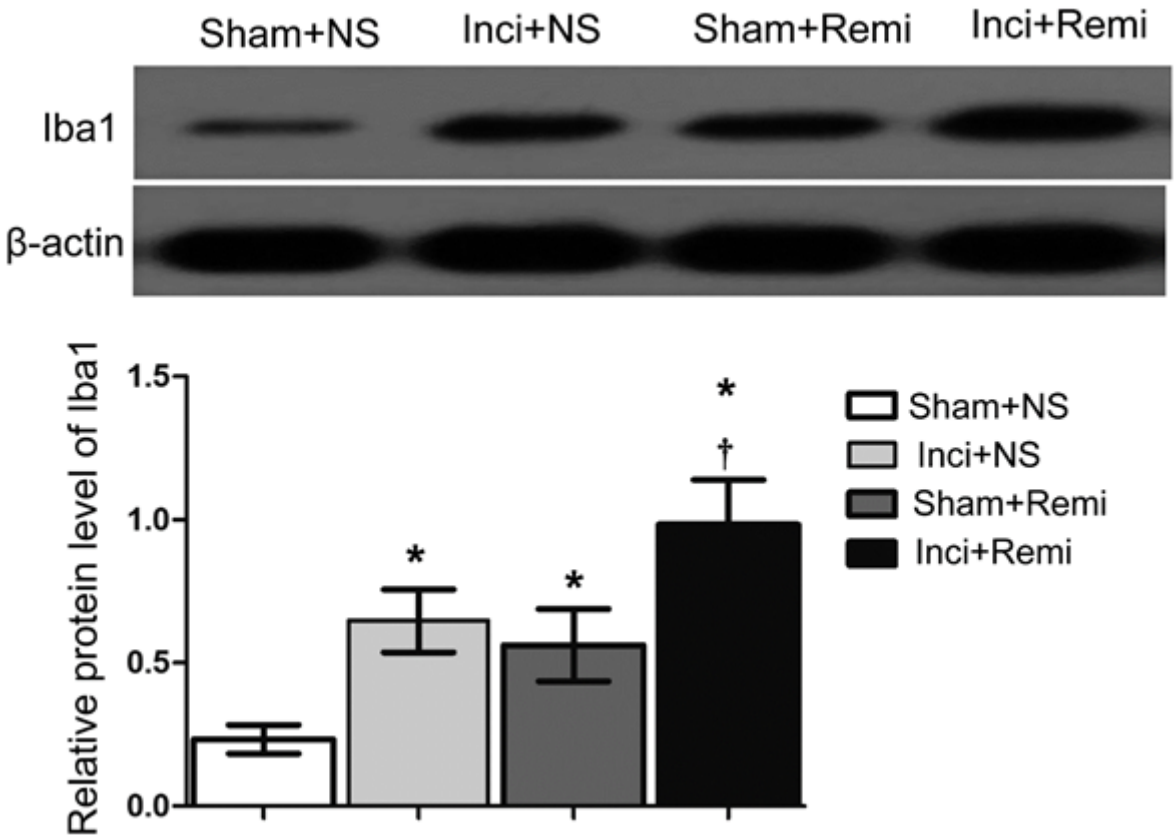

Figure 2

Analysis of Iba1 protein expression. The expression of Iba1 protein in the dorsal horn of L4–L5 spinal cord after induction by surgical incision, remifentanil infusion, or their combination at 2 d after the surgical procedure was detected by Western blot (n=4). β-actin was used as the internal control. Data were expressed as mean±SD. *P<0.05 compared with Sham+NS group; †P<0.01 compared with Inci+Remi group. Inci: incision; Remi: remifentanil; NS: normal saline; Sham: sham surgery.

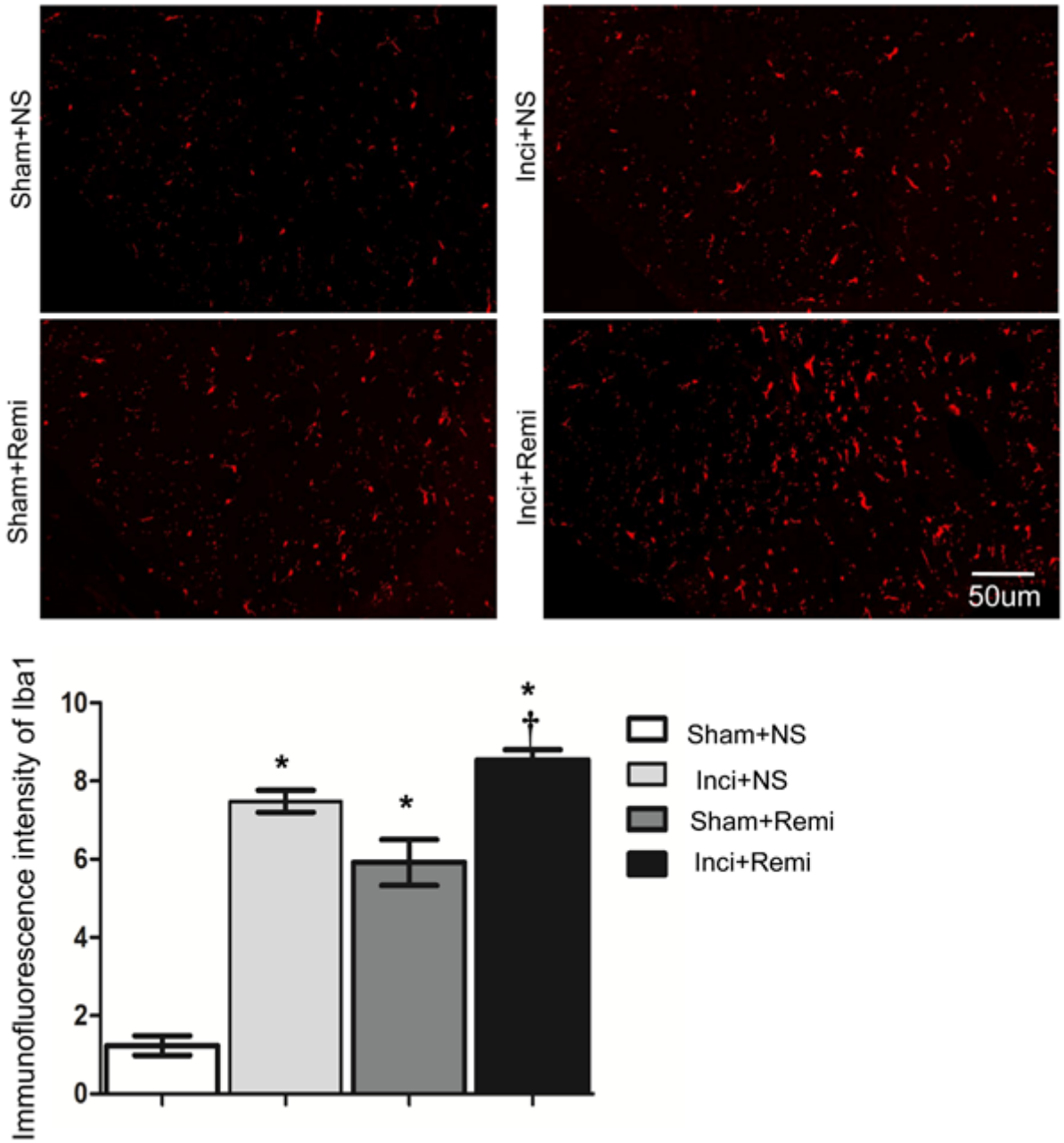

Figure 3

Analysis of Iba1 protein expression and localization. Immunofluorescence was performed to detect the expression and localization of Iba1 in the dorsal horn of L4–L5 spinal cord after induction by surgical incision, remifentanil infusion, or their combination at 2 d after the surgical procedure (n=4). Scale bar: 50 μm. *P<0.05, compared with Sham+NS; †P<0.05, compared with Inci+NS group. Inci: incision; Remi: remifentanil; NS: normal saline; Sham: sham surgery.

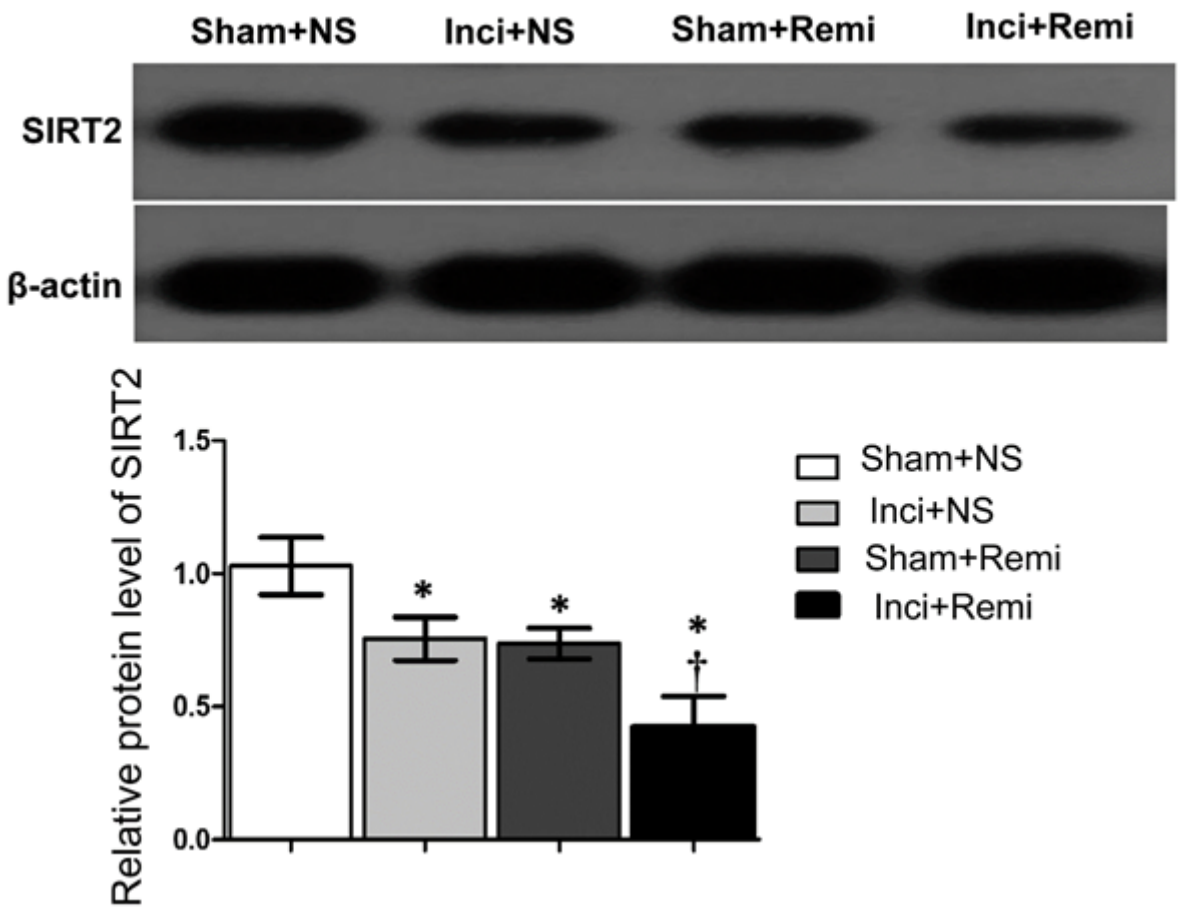

Figure 4

Analysis of SIRT2 protein expression. Western blot was conducted to measure SIRT2 expression in the dorsal horn of L4–L5 spinal cord after treatment with surgical incision, remifentanil infusion, or their combination at 2 d after the surgical procedure (n=4). *P<0.05, compared with Sham+NS; †P<0.05, compared with Inci+NS group. Inci: incision; Remi: remifentanil; NS: normal saline; Sham: sham surgery.

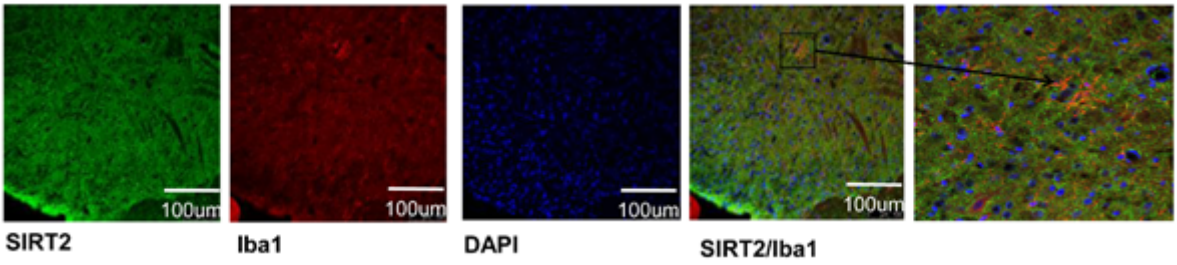

Figure 5

Analysis of SIRT2 and Iba1 co-localization. The expression of SIRT2 and Iba1 in the dorsal horn of L4–L5 spinal cord induced by surgical incision and remifentanil at 2 d after the surgical procedure was as detected by immunofluorescence (n=4).

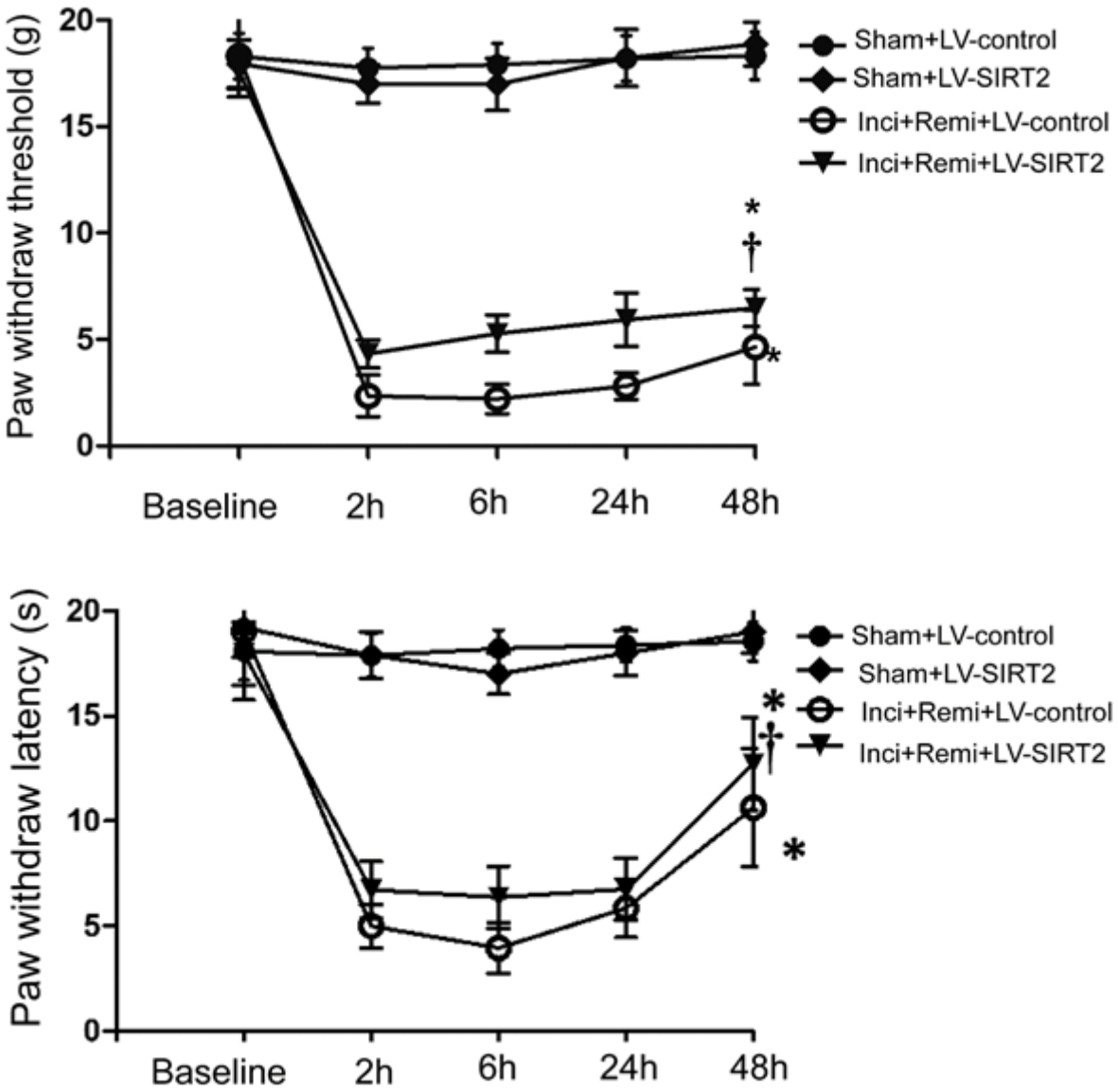

Figure 6

Overexpression of SIRT2 alleviates mechanical allodynia and thermal hyperalgesia in model rats. Mechanical allodynia was determined by measuring the paw withdrawal threshold in response to von Frey hair stimulation. Thermal hyperalgesia was determined by measuring paw withdrawal latencies in response to radiant heat stimulation. N = 6. Data were expressed as mean±SD. *P<0.05 compared with Sham+LV-control group; †P<0.01 compared with Inci+Remi+LV-control group. Inci: incision; Remi: remifentanil; Sham: sham surgery.

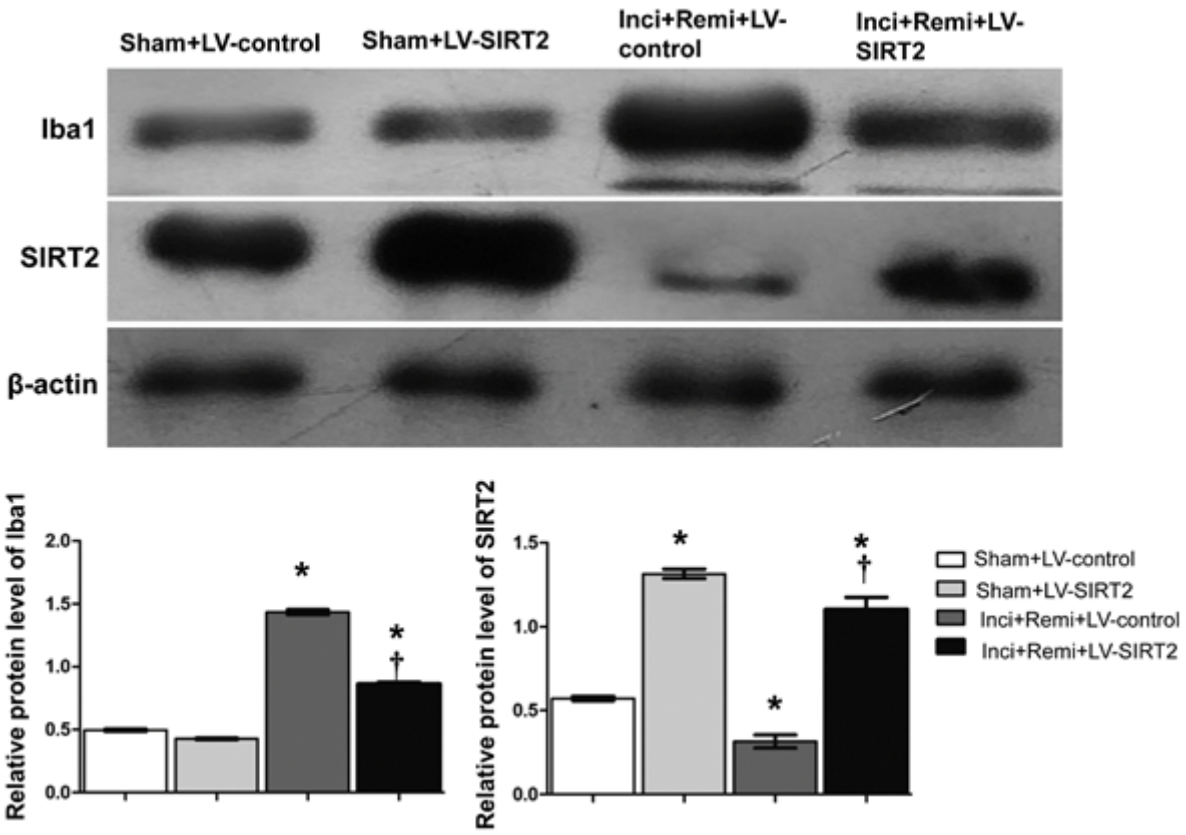

Figure 7

Overexpression of SIRT2 decreases the Iba1 expression. The DRG dissected from the L4–L5 lumbar spinal cords at postoperative day 2 was subjected to Western blot analysis of SIRT2 and Iba1 protein expression. Data were expressed as mean±SD. *P<0.05 compared with Sham+LV-control group; †P<0.01 compared with Inci+Remi+LV-control group. Inci: incision; Remi: remifentanil; Sham: sham surgery.

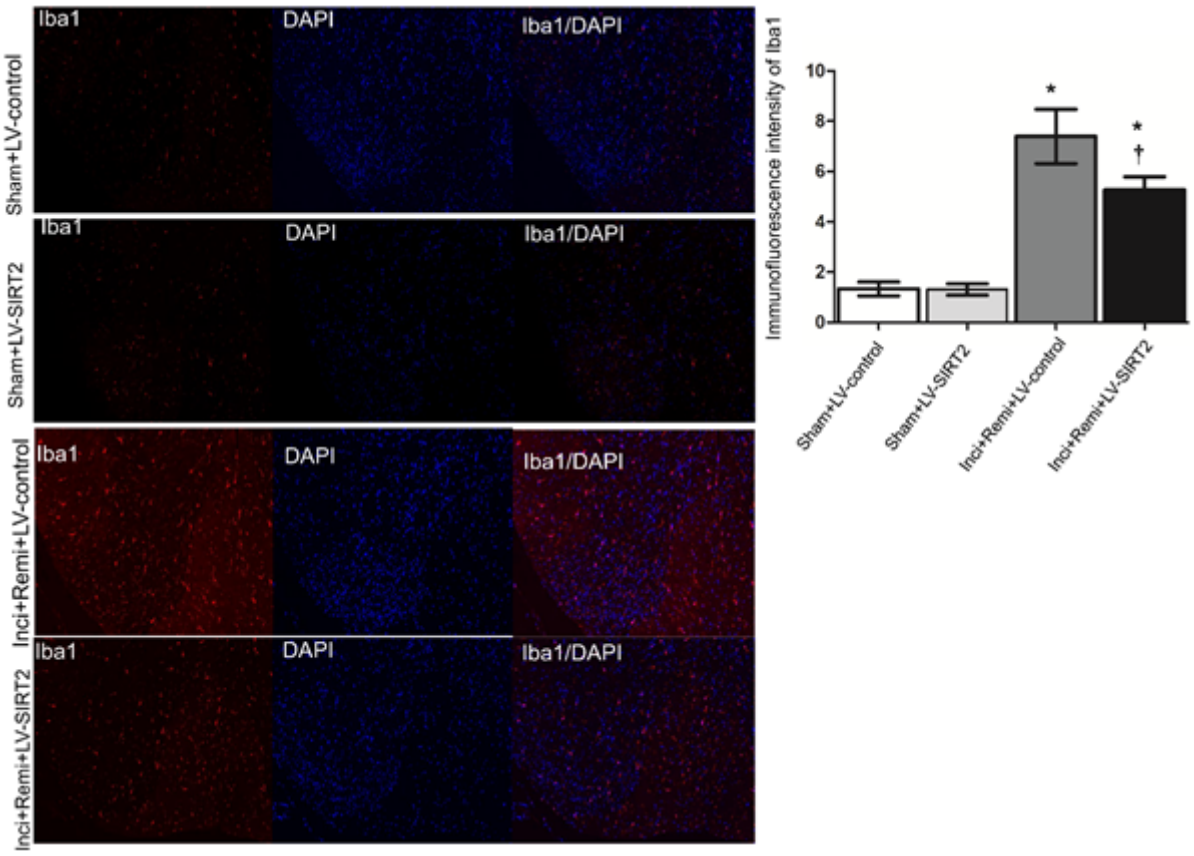

Figure 8

Analysis of Iba1 expression after overexpression of SIRT2. The expression of Iba1 in the dorsal horn of L4–L5 spinal cord induced by sham, Inci+Remi+LV-control and Inci+Remi+LV-SIRT2 at 2 d after the surgical procedure was detected by immunofluorescence (n=4). Data were expressed as mean±SD. *P<0.05 compared with Sham+LV-control group; †P<0.01 compared with Inci+Remi+LV-control group. Inci: incision; Remi: remifentanil; Sham: sham surgery.

Supplementary Files

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