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Fli-1 transcription factor regulates the expression of caspase-1 in lung pericytes

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Abstract

Our previous data demonstrated that Friend leukemia virus integration 1 (Fli-1), an ETS transcription factor, governs pericyte loss and vascular dysfunction in cecal ligation and puncture-induced murine sepsis by regulating essential pyroptosis markers including caspase-1. However, whether Fli-1 regulates caspase-1 expression levels *in vitro* and how Fli-1 regulates caspase-1 remain unknown. Our present work further demonstrated that overexpressed Fli-1 significantly increased caspase-1 and IL-18 expression levels in cultured mouse lung pericytes. Bacterial outer membrane vesicles (OMVs) have been found to induce cell pyroptosis through transferring LPS intracellularly. Using OMVs to induce an *in vitro* model of pyroptosis, we observed that OMVs significantly increased protein levels of Fli-1 in mouse lung pericytes. Furthermore, knockdown of Fli-1 by siRNA blocked OMVs-induced caspase-1, caspase-11 and IL-18 expression levels. As caspase-1 was predicted as a potential target of Fli-1, we cloned murine caspase-1 promoter into a

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Competing interests

The authors declare no competing financial interests.

luciferase construct. Our data demonstrate for the first time that Fli-1 regulates caspase-1 expression by directly binding to its promoter regions measured by chromatin immunoprecipitation (ChIP) assay and luciferase reporter system. In summary, our findings demonstrated a novel role and mechanism of Fli-1 in regulating caspase-1 expression in lung pericytes.

Keywords

Fli-1; pericytes; caspase-1

1. Introduction

Pericytes are embedded in the basement membrane of the microvasculature which wrap around the microvascular endothelial cells (EC) [1]. LPS-induced pericyte loss has been associated with microvascular dysfunction and mortality; however, this loss is not caused by apoptosis [2]. Our previous study demonstrated that cecal ligation and puncture (CLP)-induced lung pericyte loss was attributed to pyroptosis [3]. Pyroptosis, an inflammatory form of programmed cell death, is dependent on caspases 1 and 11 and is accompanied by the release of pro-inflammatory cytokines including IL-18 [4-7]. Pyroptosis can be induced by intracellular stimulation with LPS [8]. Recent studies have demonstrated that Gram-negative bacteria outer membrane vesicles (OMVs), contain abundant LPS and can induce pyroptosis of host cells [9]. In addition, OMVs induce inflammasome activation and pro-cytokine release in macrophages in a caspase-1 dependent manner [10]. As an inflammatory caspase and the most important pyroptosis marker, caspase-1 can be activated by inflammasomes, and processes pro-IL-18 into the active form and cleaves gasdermin D to trigger pyroptosis [11]. Circulating microvesicular caspase-1 activity was higher in septic patients and plays a critical role in sepsis-induced endothelial cell injury [12, 13]. Caspase-1-dependent pyroptosis of peripheral blood mononuclear cells predicts the development of sepsis in severe trauma patients [14]. Inflammasome NLRP3/caspase-1 pathway also mediated cognitive deficits in a CLP-induced murine septic model [15]. However, inhibition of caspase-1 by either antimicrobial cathelicidin peptide LL-37 or its inhibitor was associated with reduced inflammation, improved organ injury and increased survival in animal sepsis [6,16] Therefore, signaling pathways controlling caspase-1 expression may provide beneficial effects in sepsis.

Friend leukemia virus integration 1 (Fli-1), an ETS transcription factor, regulates a wide spectrum of biological processes including cancer development, fibrosis, vasculopathy and inflammation [17-22]. Fli-1 is expressed in endothelial cells, macrophages, B cells and T cells, and regulates expression of several important cytokines and chemokines including monocyte chemoattractant protein-1 (MCP-1), IL-6, granulocyte colony stimulating factor (G-CSF) and CCL5 by directly binding to these respective promoters [18, 19, 23-28]. Our previous study suggested that Fli-1 mediated lung pericyte loss and vascular function via regulating expression levels of essential pyroptosis markers including caspase-1 and IL-18 in CLP-induced septic mice [3]. However, the role and mechanism of Fli-1 in regulating caspase-1 expression in lung pericytes remains unknown. We hypothesized that Fli-1

transcription factor regulates caspase-1 gene expression in lung pericytes by directly binding and activating the caspase-1 promoter.

2. Materials and methods

2.1. OMVs isolation and characterization

OMVs were purified from *E. coli* K12 as previously described [9]. Briefly, the bacterial strain was grown in 600 ml of LB to an OD600 of 0.4-0.6 and centrifuged at 4000 g for 20 min at 4 °C. The bacteria-free supernatant was filtered through a 0.22 μm filter and OMVs were pelleted by ultracentrifugation at 400,000 g for 2 h at 4 °C. After removing the supernatant, OMVs were resuspended in 500 μl sterile PBS. The total protein concentration of the OMVs was measured by protein assay (Bio-Rad, Hercules, CA). The LPS concentration of OMVs was measured by Pierce LAL Chromogenic Endotoxin (Thermo Fisher Scientific, Rockford, IL). The size distribution and the total number of OMVs were analyzed by nanoparticle tracking analysis software (ZetaView 8.04.02).

2.2. Mouse lung pericyte isolation, culture and stimulation

Mouse lung pericytes were isolated as described previously [29, 30]. Briefly, single-cell preparations from whole lung digests were expanded, negatively selected by CD31, CD45 and CD326 magnetic beads (Miltenyi Biotec Inc., Auburn, CA), and positively selected by PDGFRβ magnetic beads (Miltenyi Biotec Inc., Auburn, CA). PDGFRβ positive lung pericytes were cultured in pericyte medium (ScienCell Research Laboratories, Carlsbad, CA) supplemented with pericyte growth supplement, 2% fetal bovine serum and 1% penicillin/streptomycin (ScienCell Research Laboratories, Carlsbad, CA). Pericytes were transfected with control plasmid or Fli-1 plasmid at different concentration (0.25-4 μg/ml) for 48 h. In another set of experiment, pericytes were transfected with Fli-1 siRNA or scrambled siRNA for 24 h and further stimulated with OMVs (25 μg/ml, containing 5.2 μg/ml LPS) for 16 h. Total RNA and protein were collected for further analysis. To determine intracellular LPS levels after OMVs treatment, the mouse pericytes were seeded into six-well plates and treated with 25 μg/ml OMVs (containing 5.2 μg/ml LPS) for 16 h. Then cells in each well were washed three times with PBS and lysed by 500 μl RIPA lysis buffer. The intracellular LPS in the lysate was measured by Pierce LAL Chromogenic Endotoxin (Thermo Fisher Scientific, Rockford, IL).

2.3. Real-time reverse transcription-polymerase chain reaction (RT-PCR)

Total RNA was extracted from cultured pericytes with RNeasy plus mini kit (Qiagen, Germantown, MD). cDNA was synthesized with High Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA). Quantitative real-time PCR was performed by CFX96 Real-Time PCR system (Bio-Rad, Hercules, CA, USA) using SYBR Green PCR Kit (Qiagen, Germantown, MD) in a final reaction volume of 25 μl with each primer (Qiagen, Germantown, MD). Data were analyzed with $2^{-\Delta\Delta Ct}$ value calculation using GAPDH for normalization.

2.4. Western blot analysis

Lung pericytes were lysed with ice-cold RIPA lysis buffer (Cell Signaling, Danvers, MA). Western blot was performed as described [31]. All lysed samples were kept on ice for 30 min, and centrifuged for 10 min at 4°C at 12,000 g. Cell lysates were subjected to 10% SDS-PAGE and transferred onto a polyvinylidene difluoride membrane. The membranes were blocked with 7% milk in TBST (20 mM Tris, 500 mM NaCl, and 0.1% Tween 20) for 1 h. After washing with TBST twice, membranes were incubated with primary antibody overnight at 4°C. Fli-1 primary antibody was provided by Dr. Xiankui Zhang (Medical University of South Carolina). Primary antibodies α-tubulin were from Cell Signaling. The membranes were washed twice with TBST and incubated with HRP conjugated secondary antibody in blocking buffer for 1 h. After washing three times with TBST, immunoreactive bands were visualized by incubation with ECL plus detection reagents (GE Healthcare, Waukesha, WI). The densitometry of bands was quantified with Image J2 software.

2.5. Chromatin immunoprecipitation (ChIP) assay

ChIP assay was performed using an anti-Fli-1 rabbit polyclonal antibody and normal IgG control (Cell Signaling, Danvers, MA) using EpiTect ChIP OneDay Kit (Qiagen, Germantown, MD) as described [17]. The primers used in the ChIP assay are available upon request. Briefly, mouse lung pericytes (10^6) were cross-linked with 1% formaldehyde at room temperature and lysed in IP lysis buffer (Qiagen, Germantown, MD). DNA was sheared by sonication and immunoprecipitation was performed by using Fli-1 specific antibody and normal IgG control. After immunoprecipitation, the DNA was purified and amplified by PCR according to the manufacturer's instructions (Qiagen, Germantown, MD).

2.6. Reporter and expression constructs

Mouse caspase-1 (GeneID: 12362) was predicted as a potential target of Fli-1 analyzed by the Genomatix online software. We found 14 potential binding sites of Fli-1 in the mouse caspase-1 promoter. The −2410 to +30 region of the mouse caspase-1 promoter, which covers all the potential Fli-1 binding sites, was cloned into the pGL3 basic vector upstream of the luciferase gene. The mouse Fli-1 gene cloned into the pcDNA3.0 expression vector has been described previously [28].

2.7. Luciferase reporter assays

To measure the luciferase activity for the transient transfection experiments, the luciferase assay systems (Promega, Madison, WI) was employed. Briefly, mouse lung pericytes were transfected with caspase-1/pGL3 luciferase reporter construct (1 μg) along with increased amounts of Fli-1/pcDNA3.0 plasmid (0.25, 0.5, 1, 2 and 4 μg) for 48 h. Luciferase activity was determined by a plate reader (Biotek, Winooski, VT). All experiments were normalized using the fold activation of luciferase activity compared to the control luciferase reporter construct.

2.8. Data analyses

Data are expressed as means ± standard error of the mean (SE). Statistical significance was determined by analysis of variance (ANOVA) with Fisher's probable least-squares difference

test or Student's *t*-test using GraphPad Prism software. A value of $p<0.05$ was considered statistically significant.

3. Results

3.1. Fli-1 regulates caspase-1 gene expression in mouse lung pericytes

Our previous study showed that Fli-1 regulates caspase-1 expression levels in lung pericytes of septic mice *in vivo* [3]. Here we sought to determine if Fli-1 regulates caspase-1 gene expression using an *in vitro* system. Isolated mouse lung pericytes were transfected with control or Fli-1 plasmid for 48 h. Fli-1 mRNA levels were significantly increased after transfection with Fli-1 plasmid ($p < 0.05$; Fig. 1A). Increased Fli-1 further significantly upregulates caspase-1 and IL-18 expression levels ($p < 0.05$; Fig. 1B-C).

3.2. Fli-1 regulates bacterial OMVs-induced caspase-1 expression in mouse lung pericytes

To further determine if Fli-1 regulates caspase-1 gene expression in activated lung pericytes *in vitro*, OMVs were isolated from *E.coli* K12 as previously described [9]. The isolated OMVs were characterized by nanoparticle tracking analysis (NTA) with Zetaview PMX 120 (Particle Metrix, Meerbusch, Germany). The size distribution and the total number of OMVs were analyzed by nanoparticle tracking analysis software (ZetaView 8.04.02). We isolated 6.5×10^{10} particles (containing 0.3 mg protein and 62.5 μg LPS) from 600 ml bacterial supernatant with a concentration of 1.3×10^{11} particles/ml. More than 90% of OMVs are within 70-130 nm range (Fig. 2A). Bacterial OMVs were reported to induce pyroptosis by transferring LPS intracellularly with increased caspase-1 [9]. We further determined intracellular LPS levels after OMV treatment, 25 μg/ml OMVs (containing 5.2 μg/ml LPS) were used to treat mouse pericytes for 16 h. Our results showed that 568 ± 32.4 ng/ml of intracellular LPS was detected in the 500 μl lysate from each well of pericytes. To investigate the role of Fli-1 in the regulation of caspase-1 expression under OMVs stimulation, we first determined the effect of OMVs on Fli-1 protein levels. Our data demonstrated that treatment with OMVs for 16 h significantly increased Fli-1 protein levels in mouse lung pericytes ($p < 0.05$; Fig. 2B). Lung pericytes were transfected with Fli-1 siRNA or scrambled siRNA and treated with bacterial OMVs (25 μg/ml) for 16 h. Fli-1 mRNA levels were significantly reduced by transfecting Fli-1 siRNA into pericytes (data not shown). Exposure of lung pericytes to OMVs significantly increased mRNA levels of pyroptosis markers including caspase-1, caspase-11 and IL-18, which were mitigated in cells transfected with Fli-1 siRNA ($p < 0.05$; Fig. 3A-C).

3.3. Fli-1 binds to the caspase-1 promoter in mouse lung pericytes

To determine if Fli-1 directly regulates caspase-1 expression levels, we used MatInspector Software (Genomatix) analysis to predict potential Fli-1 binding sites on the murine caspase-1 promoter. We identified 14 putative Fli-1 binding sites on the caspase-1 promoter and 9 pair primers were designed to cover these sites (Fig. 4A). After immunoprecipitation of the cultured lung pericytes with a Fli-1 specific antibody and normal IgG control, ChIP1, ChIP2, ChIP7 and ChIP9 were significantly enriched for Fli-1 specific antibodies (8.1 ± 0.6

fold for ChIP1, 4.8 ± 1.3 fold for ChIP2, 9.3 ± 1.7 fold for ChIP7 and 13.5 ± 2.3 fold for ChIP9, respectively, $p < 0.05$) compared to the IgG negative control (Fig. 4B-C).

3.4. Fli-1 drives transcription from the caspase-1 promoter

To further confirm that Fli-1 regulates the expression of caspase-1, transient transfection assays were performed. The Fli-1 transcription factor was transfected into mouse lung pericytes along with the mouse caspase-1 promoter/pGL3 reporter construct. We determined the Fli-1 protein expression levels after transfection by immunoblot. As shown in Fig. 5A, Fli-1 protein expression corresponds with increasing amounts of the Fli-1 plasmid transfected into the cells. Furthermore, the Fli-1 transcription factor strongly induced activation from the caspase-1 promoter in a statistically significant manner when compared to the activation of the reporter construct (Fig. 5B). The results demonstrate that Fli-1 drives transcription from the caspase-1 promoter in a concentration-dependent manner, with as little as 500 ng of Fli-1 needed to significantly activate transcription from the caspase-1 promoter.

4. Discussion

Our previous data showed that Fli-1 regulates both mRNA and protein levels of caspase-1 *in vivo*. This study demonstrates several novel and important discoveries. First, Fli-1 regulates caspase-1 and IL-18 expression in cultured lung pericytes with or without stimulation *in vitro*. Secondly, we provide the first molecular evidence that the Fli-1 transcription factor directly drives transcription from the murine caspase-1 promoter. Collectively, these findings reveal a novel mechanism for Fli-1 in the regulation of the inflammatory response and pyroptosis. Thus, Fli-1 may be a novel target for treating pyroptosis/inflammation-related diseases including sepsis

Recent studies highlight a critical role of pyroptosis in sepsis. Sepsis-associated pyroptosis occurred widely in the various type of cells including peripheral blood mononuclear cells, neurons, macrophages, pericytes, endothelial cells and liver cells in patients and animal models [3, 6, 12, 14, 15, 32]. During sepsis, pyroptosis is required for defense against invasive pathogenic bacteria and microbial infections; however, when overactivated it can result in the inflammatory response, multi-organ dysfunction and septic shock [33-35]. In addition, circulating microvesicular caspase-1 activity and IL-18 levels were significantly higher in septic patients [12, 35]. However, inhibition of pyroptosis has been proven beneficial in reducing the inflammatory response, organ injury and mortality in animal models [6, 15, 16, 32]. Consistently, our previous study demonstrated that inhibition of lung pericyte pyroptosis was associated with reduced lung vascular leak and decreased mortality in CLP-induced murine sepsis; this beneficial effect was mediated by knockout of Fli-1 in pericytes [3]. Our present data further showed that Fli-1 upregulated caspase-1 and IL-18 gene expression by directly binding to the caspase-1 promoter. Pericyte Fli-1 levels were elevated in CLP-induced septic mice [3]. Thus, the beneficial effects of Fli-1 pericyte knockout in sepsis may be partly attributed to attenuated caspase-1 expression. Similar, inhibition of caspase-1 was also found to improve sepsis outcomes in animal models [6, 15,

16]. Therefore, Fli-1 is a potential novel target for inhibition of pyroptosis via controlling caspase-1 expression.

Besides pyroptosis, the uncontrolled inflammatory response is another hallmark of sepsis. LPS-induced overwhelming production of inflammatory mediators during sepsis was thought to be mediated by activation of the Toll-like receptor (TLR) 4 signaling pathway [34, 36]. However, recent studies suggest that TLR4-independent recognition of intracellular LPS leads to non-canonical activation of the inflammasome and caspase-1 which, coupled with pyroptosis, triggers further inflammatory response [34, 37, 38]. Our previous data demonstrated that extracellular LPS increased Fli-1 expression levels in cultured lung pericytes; while inhibition of Fli-1 expression by transfecting Fli-1-specific siRNA blocked extracellular LPS-induced production of sepsis-related pro-inflammatory cytokines including G-CSF and IL-6 [3]. The present study further showed that intracellular LPS, the major components of OMVs, significantly upregulated Fli-1 protein levels in lung pericytes; while knockdown of Fli-1 expression attenuated OMVs-induced caspase-1 and IL-18 expression levels. Thus, inhibition of Fli-1 can block both extracellular and intracellular LPS-mediated inflammation in lung pericytes. Taken together, these findings provide new insights into the mechanisms by which Fli-1 exacerbates the inflammatory response: 1) Fli-1 transcription factor directly regulates the transcription of several important cytokines and chemokines including MCP-1, IL-6, G-CSF, chemokine C-X-C motif ligand 2 (CXCL2) and CCL5 through direct binding to their respective promoters [17-19, 23, 28]; 2) Fli-1 mediates extracellular LPS-induced inflammatory response; 3) Fli-1 mediates intracellular LPS-induced inflammatory caspase-1 and IL-18 expression. Therefore, blockade of Fli-1 may exert multiple beneficial effects to inhibit inflammation during sepsis.

There are several limitations to our study. We demonstrated that both extracellular and intracellular LPS stimulate Fli-1 expression levels; however, the specific mechanism of how LPS induces Fli-1 expression needs further investigation. Although we found that Fli-1 transcription factor directly regulates caspase-1 gene expression by binding to its promoter, activated caspase-1 is the key factor for producing active IL-18 and inducing pyroptosis. Whether Fli-1 has the impact on activated caspase-1 remains unknown and could be an interesting topic for future study.

In summary, the Fli-1 transcription factor drives transcription from the murine caspase-1 promoter in a dose-dependent manner and affects the regulation of the pyroptosis and inflammation. Further, our previous study demonstrated that inhibition of Fli-1 reduces inflammatory response, attenuates vascular leak and increases survival in cultured lung pericytes and in CLP-induced sepsis [3]. Thus, we provide evidence that signaling pathways reducing Fli-1 expression may represent a novel potential way for inhibiting pyroptosis and inflammation during sepsis.

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References

1. Armulik A, Abramsson A and Betsholtz C (2005) Endothelial/pericyte interactions. Circulation research 97:512–23. doi: 10.1161/01.RES.0000182903.16652.d7 [PubMed: 16166562]
2. Zeng H, He X, Tuo QH, Liao DF, Zhang GQ and Chen JX (2016) LPS causes pericyte loss and microvascular dysfunction via disruption of Sirt3/angiopoietins/Tie-2 and HIF-2alpha/Notch3 pathways. Scientific reports 6:20931. doi: 10.1038/srep20931 [PubMed: 26868537]
3. Li P, Zhou Y, Goodwin AJ, Cook JA, Halushka PV, Zhang XK, Wilson CL, Schnapp LM, Zingarelli B and Fan H (2018) Fli-1 Governs Pericyte Dysfunction in a Murine Model of Sepsis. J Infect Dis 218:1995–2005. doi: 10.1093/infdis/jiy451 [PubMed: 30053030]
4. Aachoui Y, Sagulenko V, Miao EA and Stacey KJ (2013) Inflammasome-mediated pyroptotic and apoptotic cell death, and defense against infection. Current opinion in microbiology 16:319–26. doi: 10.1016/j.mib.2013.04.004 [PubMed: 23707339]
5. Cookson BT and Brennan MA (2001) Pro-inflammatory programmed cell death. Trends in microbiology 9:113–4. [PubMed: 11303500]
6. Hu Z, Murakami T, Suzuki K, Tamura H, Reich J, Kuwahara-Arai K, Iba T and Nagaoka I (2016) Antimicrobial cathelicidin peptide LL-37 inhibits the pyroptosis of macrophages and improves the survival of polybacterial septic mice. International immunology 28:245–53. doi: 10.1093/intimm/dxv113 [PubMed: 26746575]
7. Aglietti RA and Dueber EC (2017) Recent Insights into the Molecular Mechanisms Underlying Pyroptosis and Gasdermin Family Functions. Trends in immunology 38:261–271. doi: 10.1016/j.it.2017.01.003 [PubMed: 28196749]
8. Pfalzgraff A, Heinbockel L, Su Q, Brandenburg K and Weindl G (2017) Synthetic anti-endotoxin peptides inhibit cytoplasmic LPS-mediated responses. Biochemical pharmacology 140:64–72. doi: 10.1016/j.bcp.2017.05.015 [PubMed: 28539262]
9. Vanaja SK, Russo AJ, Behl B, Banerjee I, Yankova M, Deshmukh SD and Rathinam VAK (2016) Bacterial Outer Membrane Vesicles Mediate Cytosolic Localization of LPS and Caspase-11 Activation. Cell 165:1106–1119. doi: 10.1016/j.cell.2016.04.015 [PubMed: 27156449]
10. Cecil JD, O'Brien-Simpson NM, Lenzo JC, Holden JA, Singleton W, Perez-Gonzalez A, Mansell A and Reynolds EC (2017) Outer Membrane Vesicles Prime and Activate Macrophage Inflammasomes and Cytokine Secretion In Vitro and In Vivo. Front Immunol 8:1017. doi: 10.3389/fimmu.2017.01017 [PubMed: 28890719]
11. Sun Q and Scott MJ (2016) Caspase-1 as a multifunctional inflammatory mediator: noncytokine maturation roles. J Leukoc Biol 100:961–967. doi: 10.1189/jlb.3MR0516-224R [PubMed: 27450556]
12. Exline MC, Justiniano S, Hollyfield JL, Berhe F, Besecker BY, Das S, Wewers MD and Sarkar A (2014) Microvesicular caspase-1 mediates lymphocyte apoptosis in sepsis. PLoS One 9:e90968. doi: 10.1371/journal.pone.0090968 [PubMed: 24643116]
13. Mitra S, Exline M, Habyarimana F, Gavrilin MA, Baker PJ, Masters SL, Wewers MD and Sarkar A (2018) Microparticulate Caspase 1 Regulates Gasdermin D and Pulmonary Vascular Endothelial Cell Injury. Am J Respir Cell Mol Biol 59:56–64. doi: 10.1165/rcmb.2017-0393OC [PubMed: 29365280]
14. Wang YC, Liu QX, Liu T, Xu XE, Gao W, Bai XJ and Li ZF (2018) Caspase-1-dependent pyroptosis of peripheral blood mononuclear cells predicts the development of sepsis in severe trauma patients: A prospective observational study. Medicine (Baltimore) 97:e9859. doi: 10.1097/MD.0000000000009859 [PubMed: 29465571]
15. Fu Q, Wu J, Zhou XY, Ji MH, Mao QH, Li Q, Zong MM, Zhou ZQ and Yang JJ (2018) NLRP3/Caspase-1 Pathway-Induced Pyroptosis Mediated Cognitive Deficits in a Mouse Model of Sepsis-Associated Encephalopathy. Inflammation. doi: 10.1007/s10753-018-0894-4
16. Chen YL, Xu G, Liang X, Wei J, Luo J, Chen GN, Yan XD, Wen XP, Zhong M and Lv X (2016) Inhibition of hepatic cells pyroptosis attenuates CLP-induced acute liver injury. American journal of translational research 8:5685–5695. [PubMed: 28078039]

17. Lou N, Lennard Richard ML, Yu J, Kindy M and Zhang XK (2017) The Fli-1 transcription factor is a critical regulator for controlling the expression of chemokine C-X-C motif ligand 2 (CXCL2). Molecular immunology 81:59–66. doi: 10.1016/j.molimm.2016.11.007 [PubMed: 27889620]

18. Sato S, Lennard Richard M, Brandon D, Jones Buie JN, Oates JC, Gilkeson GS and Zhang XK (2014) A critical role of the transcription factor fli-1 in murine lupus development by regulation of interleukin-6 expression. Arthritis & rheumatology 66:3436–44. doi: 10.1002/art.38818 [PubMed: 25155007]

19. Suzuki E, Karam E, Williams S, Watson DK, Gilkeson G and Zhang XK (2012) Fli-1 transcription factor affects glomerulonephritis development by regulating expression of monocyte chemoattractant protein-1 in endothelial cells in the kidney. Clinical immunology 145:201–8. doi: 10.1016/j.clim.2012.09.006 [PubMed: 23108091]

20. Akamata K, Asano Y, Yamashita T, Noda S, Taniguchi T, Takahashi T, Ichimura Y, Toyama T, Trojanowska M and Sato S (2015) Endothelin receptor blockade ameliorates vascular fragility in endothelial cell-specific Fli-1-knockout mice by increasing Fli-1 DNA binding ability. Arthritis & rheumatology 67:1335–44. doi: 10.1002/art.39062 [PubMed: 25707716]

21. Theisen ER, Pishas KI, Saund RS and Lessnick SL (2016) Therapeutic opportunities in Ewing sarcoma: EWS-FLI inhibition via LSD1 targeting. Oncotarget 7:17616–30. doi: 10.18632/oncotarget.7124 [PubMed: 26848860]

22. Takahashi T, Asano Y, Sugawara K, Yamashita T, Nakamura K, Saigusa R, Ichimura Y, Toyama T, Taniguchi T, Akamata K, Noda S, Yoshizaki A, Tsuruta D, Trojanowska M and Sato S (2017) Epithelial Fli1 deficiency drives systemic autoimmunity and fibrosis: Possible roles in scleroderma. The Journal of experimental medicine 214:1129–1151. doi: 10.1084/jem.20160247 [PubMed: 28232470]

23. Lennard Richard ML, Brandon D, Lou N, Sato S, Caldwell T, Nowling TK, Gilkeson G and Zhang XK (2016) Acetylation impacts Fli-1-driven regulation of granulocyte colony stimulating factor. European journal of immunology 46:2322–2332. doi: 10.1002/eji.201646315 [PubMed: 27431361]

24. Gao P, Yuan M, Ma X, Jiang W, Zhu L, Wen M, Xu J, Liu Q and An H (2016) Transcription factor Fli-1 positively regulates lipopolysaccharide-induced interleukin-27 production in macrophages. Molecular immunology 71:184–91. doi: 10.1016/j.molimm.2016.02.006 [PubMed: 26900987]

25. Zhang XK and Watson DK (2005) The FLI-1 transcription factor is a short-lived phosphoprotein in T cells. Journal of biochemistry 137:297–302. doi: 10.1093/jb/mvi032 [PubMed: 15809330]

26. Zhang XK, Moussa O, LaRue A, Bradshaw S, Molano I, Spyropoulos DD, Gilkeson GS and Watson DK (2008) The transcription factor Fli-1 modulates marginal zone and follicular B cell development in mice. Journal of immunology 181:1644–54.

27. Richard EM, Thiyagarajan T, Bunni MA, Basher F, Roddy PO, Siskind LJ, Nietert PJ and Nowling TK (2013) Reducing FLI1 levels in the MRL/lpr lupus mouse model impacts T cell function by modulating glycosphingolipid metabolism. PloS one 8:e75175. doi: 10.1371/journal.pone.0075175 [PubMed: 24040398]

28. Lennard Richard ML, Sato S, Suzuki E, Williams S, Nowling TK and Zhang XK (2014) The Fli-1 transcription factor regulates the expression of CCL5/RANTES. J Immunol 193:2661–8. doi: 10.4049/jimmunol.1302779 [PubMed: 25098295]

29. Hung C, Linn G, Chow YH, Kobayashi A, Mittelsteadt K, Altemeier WA, Gharib SA, Schnapp LM and Duffield JS (2013) Role of lung pericytes and resident fibroblasts in the pathogenesis of pulmonary fibrosis. American journal of respiratory and critical care medicine 188:820–30. doi: 10.1164/rccm.201212-2297OC [PubMed: 23924232]

30. Hung CF, Mittelsteadt KL, Brauer R, McKinney BL, Hallstrand TS, Parks WC, Chen P, Schnapp LM, Liles WC, Duffield JS and Altemeier WA (2017) Lung pericyte-like cells are functional interstitial immune sentinel cells. American journal of physiology. Lung cellular and molecular physiology 312:L556–L567. doi: 10.1152/ajplung.00349.2016 [PubMed: 28188224]

31. Li P, Cook JA, Gilkeson GS, Luttrell LM, Wang L, Borg KT, Halushka PV and Fan H (2011) Increased expression of beta-arrestin 1 and 2 in murine models of rheumatoid arthritis: isoform specific regulation of inflammation. Molecular immunology 49:64–74. doi: 10.1016/j.molimm.2011.07.021 [PubMed: 21855149]

32. Cheng KT, Xiong S, Ye Z, Hong Z, Di A, Tsang KM, Gao X, An S, Mittal M, Vogel SM, Miao EA, Rehman J and Malik AB (2017) Caspase-11-mediated endothelial pyroptosis underlies endotoxemia-induced lung injury. J Clin Invest 127:4124–4135. doi: 10.1172/JCI94495 [PubMed: 28990935]

33. Gao YL, Zhai JH and Chai YF (2018) Recent Advances in the Molecular Mechanisms Underlying Pyroptosis in Sepsis. Mediators Inflamm 2018:5823823. doi: 10.1155/2018/5823823 [PubMed: 29706799]

34. Pfalzgraff A, Heinbockel L, Su Q, Brandenburg K and Weindl G (2017) Synthetic anti-endotoxin peptides inhibit cytoplasmic LPS-mediated responses. Biochem Pharmacol 140:64–72. doi: 10.1016/j.bcp.2017.05.015 [PubMed: 28539262]

35. Esquerdo KF, Sharma NK, Brunialti MKC, Baggio-Zappia GL, Assuncao M, Azevedo LCP, Bafi AT and Salomao R (2017) Inflammasome gene profile is modulated in septic patients, with a greater magnitude in non-survivors. Clin Exp Immunol 189:232–240. doi: 10.1111/cei.12971 [PubMed: 28369745]

36. Palsson-McDermott EM and O'Neill LA (2004) Signal transduction by the lipopolysaccharide receptor, Toll-like receptor-4. Immunology 113:153–62. doi: 10.1111/j.1365-2567.2004.01976.x [PubMed: 15379975]

37. Shi J, Zhao Y, Wang Y, Gao W, Ding J, Li P, Hu L and Shao F (2014) Inflammatory caspases are innate immune receptors for intracellular LPS. Nature 514:187–92. doi: 10.1038/nature13683 [PubMed: 25119034]

38. Schmid-Burgk JL, Gaidt MM, Schmidt T, Ebert TS, Bartok E and Hornung V (2015) Caspase-4 mediates non-canonical activation of the NLRP3 inflammasome in human myeloid cells. Eur J Immunol 45:2911–7. doi: 10.1002/eji.201545523 [PubMed: 26174085]

Highlights

- Fli-1 induces caspase-1 and IL-18 expression in cultured lung pericytes.
- Knockdown of Fli-1 inhibits intracellular LPS-induced caspase-1 and IL-18 expression.
- Fli-1 transcription factor drives transcription of the murine caspase-1 promoter.

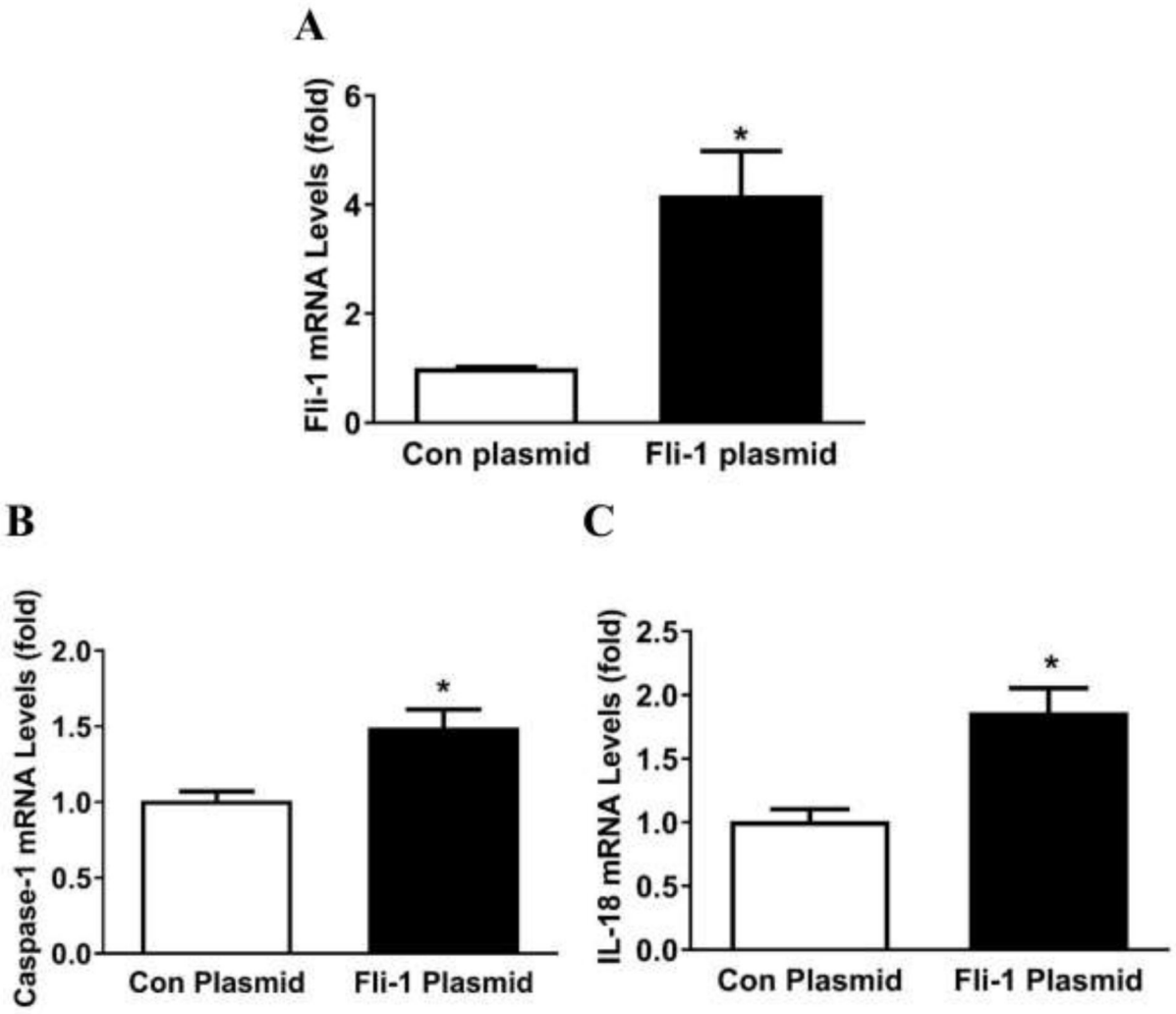

Figure 1.

Fli-1 overexpression increases caspase-1 in mouse lung pericytes. Lung pericytes were isolated from normal C57/BL6 mice and transfected with control or Fli-1 plasmid for 48 h. The mRNA levels of (A) Fli-1, (B) caspase-1 and (C) IL-18 were determined by Real-time PCR. *$p < 0.05$ compared to control plasmid group. Con: control.

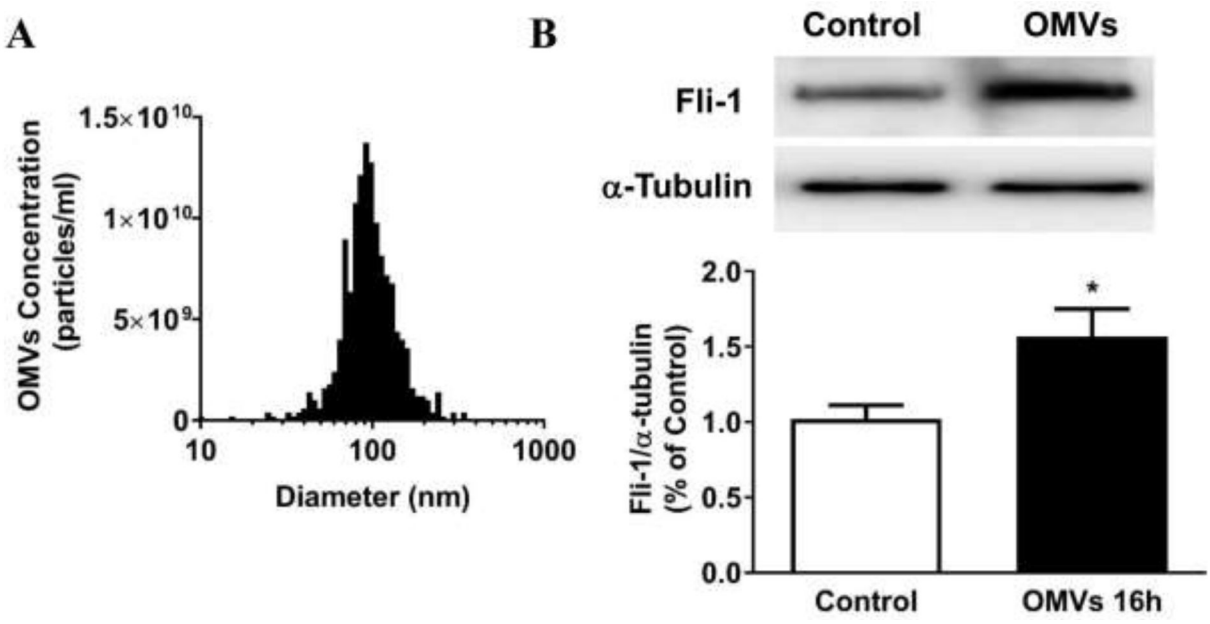

Figure 2.

Bacterial outer membrane vehicles (OMVs) increased Fli-1 protein levels in lung pericytes. (A) OMVs were purified from *E. coli* K12. The number of OMVs particles vs. particle size was generated by nanoparticle tracking analysis with ZetaView. Lung pericytes were stimulated with OMVs (25 μg/ml) for 16 h. (B) The effect of OMVs on Fli-1 protein levels were determined by western blot. *$p < 0.05$ compared to control group.

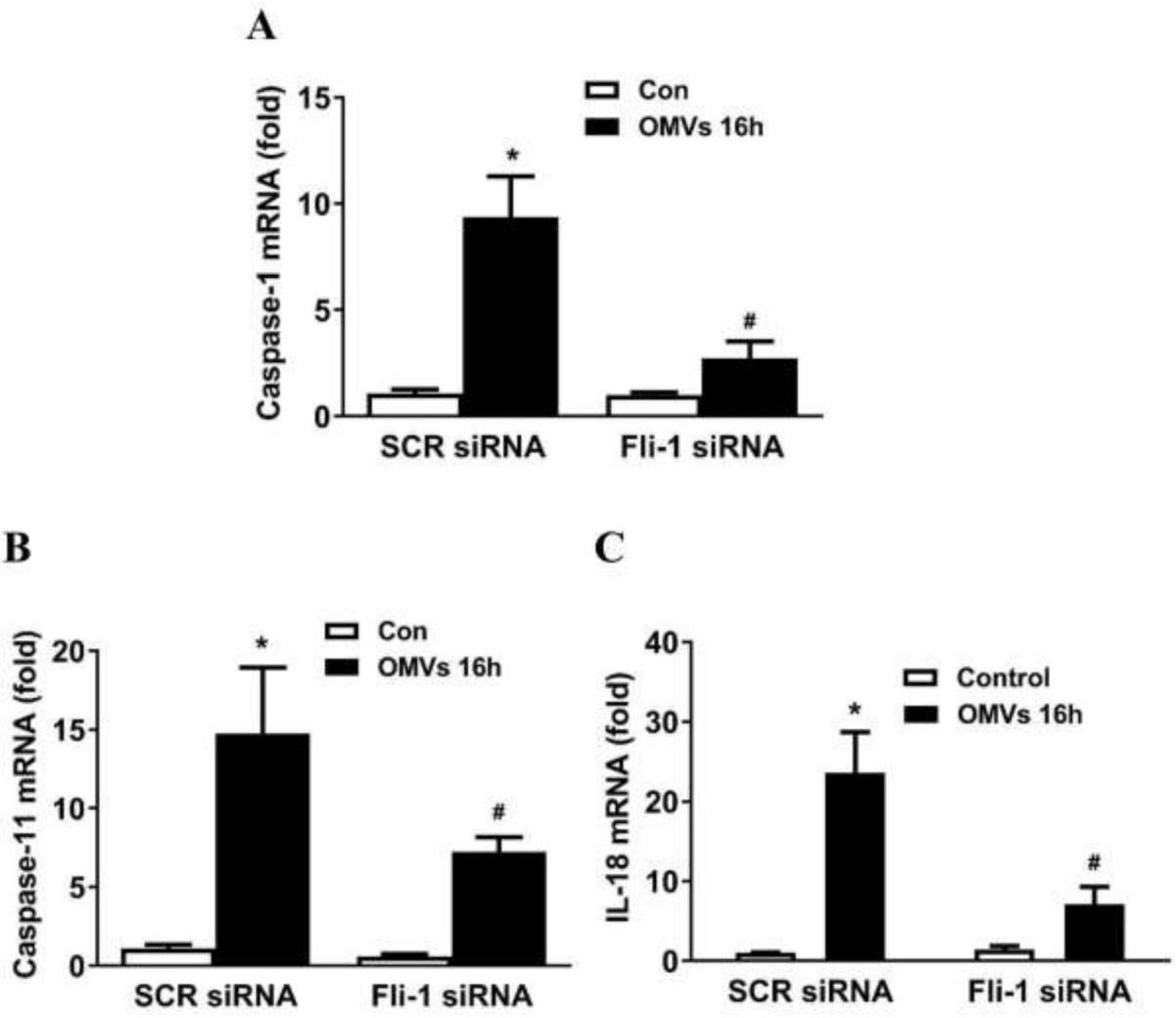

Figure 3.

Fli-1 regulates OMVs-induced caspase-1 expression in lung pericytes. Lung pericytes were transfected with Fli-1 specific siRNA or scrambled siRNA and further stimulated by OMVs (25 μg/ml, *E.coli* K12) for 16 h. The mRNA levels of (A) caspase-1, (B) caspase-11 and (C) IL-8 in lung pericytes were measured. N=3 experiments. Data are expressed as means ± SE. *$p < 0.05$ compared to scrambled siRNA control group; $^{\#}p < 0.05$ compared to scrambled siRNA LPS group. SCR: scramble.

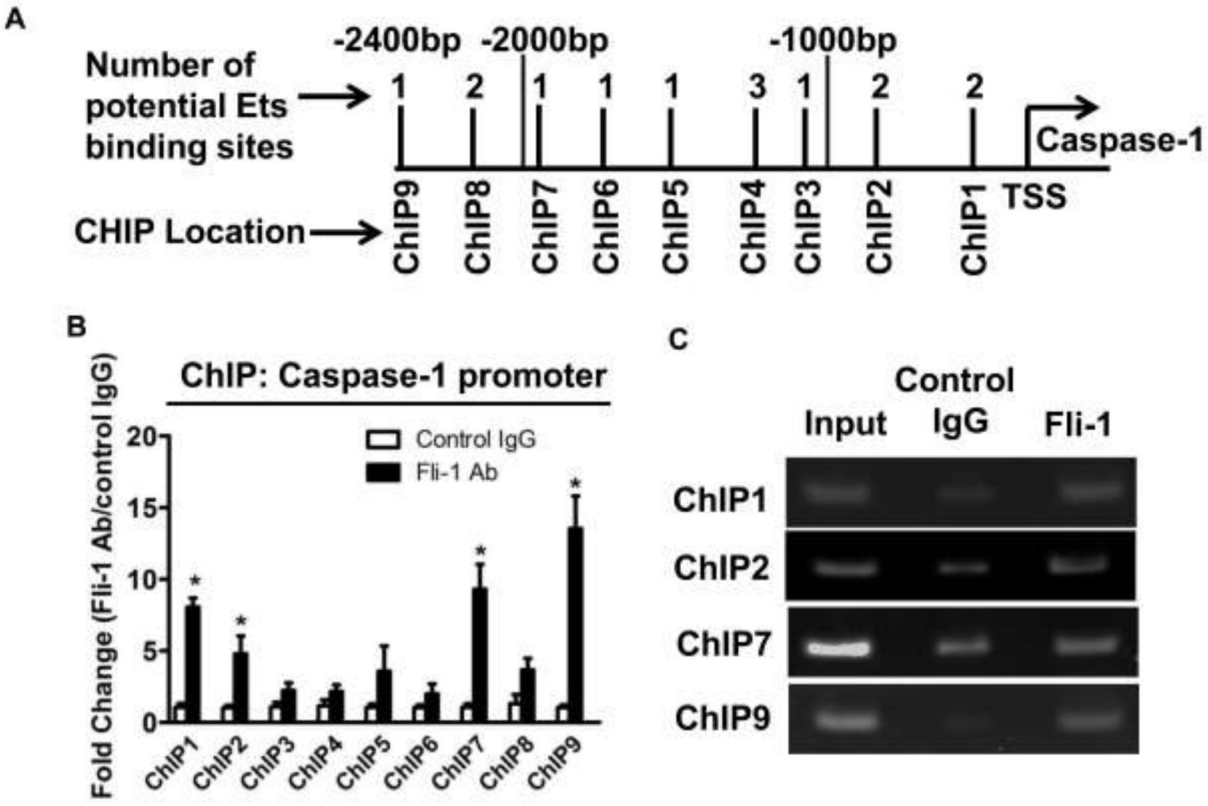

Figure 4.

Fli-1 regulates caspase-1 expression in lung pericytes by binding to its promoter. The potential Fli-1 binding sites on murine caspase-1 promoter region were determined by ChIP assay. A schematic diagram (A) showing the location of the fourteen putative Ets binding sites and nine primers designed for ChIP. ChIP analysis (B) of Fli-1 binding to the Caspase-1 promoter was performed. Representative agarose gel results (C) for ChIP1, 2, 7 and 9 were showed. N=3 independent experiments. *$p < 0.05$ compared to control IgG group.

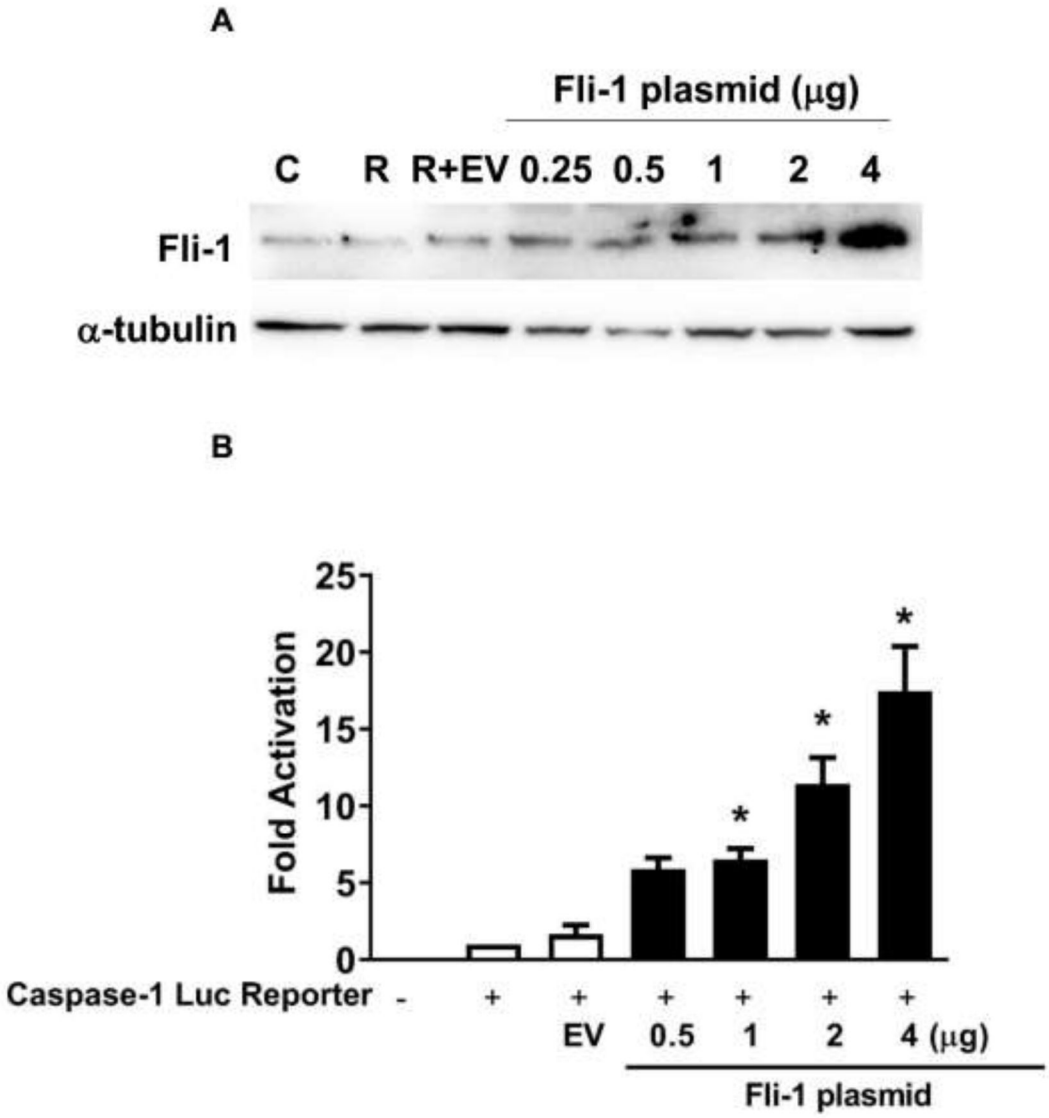

Figure 5.

Fli-1 drives transcription from the caspase-1 promoter. (A) Fli-1 protein concentrations after transfection into the pericytes. (B) Graph illustrating that Fli-1 drives transcription from the caspase-1 promoter in mouse lung pericytes. Transfections were carried out using increasing amounts of Fli-1 plasmid (0.5 μg, 1 μg, 2 μg and 4 μg) and mouse caspase-1 promoter/pGL3 reporter construct (1 μg). A luciferase assay was performed to determine activation from the caspase-1 promoter. Data presented are shown as fold activation over the activation of the empty reporter construct. N=3 independent experiments. *$p < 0.05$ compared to the activation of the reporter construct. C: control; R: reporter; R+EV: reporter + Empty vector.