RESEARCH ARTICLE
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Hsa_circRNA_092488 Exacerbates the Progression of Deep Vein Thrombosis Through the NLRP3/NFkB Signaling Pathway

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ABSTRACT
Objective: Deep vein thrombosis (DVT) is a type of vascular disorder and the incidence is around 0.1%. Endothelial progenitor cells (EPCs) are precursor cells of endothelial cells and contribute to vascular repair and regeneration. CircRNAs become a new research hotspot as they are involved in various biological processes including the progression of DVT.
Material and Methods: The expression of hsa_circRNA_092488 was evaluated in venous blood samples obtained from DVT patients (n=42) and healthy controls (n=42). Gain- and loss-of-function studies of hsa_circRNA_092488 were carried out. Expression of related RNA and protein was examined by qRT-PCR, western blotting and immunofluorescence assays. The proliferation, migration, cell cycle and apoptosis of transfected cells were measured by CCK-8, Transwell assay as well as Flow cytometry. The association of hsa_circRNA_092488 and NLRP3 in EPCs was unrevealed using RNA pull-down analysis. Furthermore, stabilities of NLRP3 mRNA were examined in transfected EPCs.
Results: In this study, up-regulation of hsa_circRNA_092488 was detected in DVT samples, which could suppress the proliferation and migration of EPCs, induce cell cycle arrest from S to G0/G1 phase and trigger cell apoptosis. Furthermore, NLRP3 was identified as the potential downstream molecule of hsa_circRNA_092488, and it could exert its regulatory functions through activating the NLRP3/NF-kB signaling. Overexpressed hsa_circRNA_092488 in the cell notably elevated the protein expression of caspase-1, IL-1b, P-NF-κB-p65/NF-κB-p65 and P-IκBa/IκBa; and vice versa, knockdown of hsa_circRNA_092488 significantly reduced the levels of these related proteins in EPCs.
Conclusion: The hsa_circRNA_092488/NLRP3/NF-kB signaling could be novel therapeutic candidate for the treatment of DVT.
KEYWORDS: Hsa_circRNA_092488; NLRP3; NFkB; DVT

INTRODUCTION

Deep vein thrombosis (DVT) is a multifactorial disorder, and there are numerous risk factors which can trigger DVT, such as pregnancy, immobility and thrombophilias [1]. And the risk factors for DVT could be sorted into fundamental elements which favor the formation of thrombus, including venous stasis, vascular injury and hypercoagulability [2]. However, the detailed mechanisms underlying the onset and progression of DVT are not fully understood. It has been reported that alterations in venous blood flow, endothelial activation, adhesion of platelets and leukocytes and activation of coagulation serve essential roles in the pathogenesis of DVT [1,2]. The diagnosis is based on a high degree of suspicion, including risk factor history, D-Dimer measurement, and ultrasound scans showing the presence of deep vein clots [1]. Other relevant tests include pulmonary artery CT scans and ventilation-perfusion scans when PE is suspected [1,2].
Endothelial progenitor cells (EPCs) are precursor cells of endothelial cells and contribute to vascular repair and

regeneration [3]. Under physiological or pathological stimulation, they can be mobilized from the bone marrow into the peripheral blood to participate in repairing damaged blood vessels [4]. Studies have shown that endothelial progenitor cells play important roles in cardiovascular and cerebrovascular diseases, peripheral vascular diseases, tumor angiogenesis, and wound healing [5].

CircRNAs are associated with vascular dysfunctions in several vascular disorders and could be used as disease biomarkers [6]. CircRNAs are a group of essential post-transcriptional regulators, and they are formed by head-to tail splicing of exons, which indicates the unrecognized regulatory potential of coding sequences [6[. For instance, circ_0020123 and hsa_circ_0001020 could affect EPC migration, invasion and tube formation during the development of DVT [7,8]. Knockdown of circ_0020123 enhanced the proliferation and angiogenesis of HUVEC cells in vitro, and it was considered as a putative biomarker for the onset of DVT in pregnant women [7[. In addition, up-regulation of hsa_circ_0001020 was found in both DVT patients and mouse model, and it suppressed the migration, invasion of EPC and tube formation through regulating the miR-29c-3p/MDM2 signaling pathway [8]. Furthermore, knockdown of hsa_circ_0001020 in DVT mice inhibited thrombosis and enhanced homing ability of EPCs into thrombi [8]. In previous study, up-regulation of hsa_circRNA_092488 was observed in DVT, and its involvement in inflammation has also been reported [9,10].

In our research, up-regulated hsa_circRNA_092488 was revealed in DVT patients, which inhibited EPCs proliferation and migration, caused shift of cell cycle from S to G0/G1 stage and induced cell apoptosis. In addition, hsa_circRNA_092488 exerted its regulatory functions via the NLRP3/NF-kB pathway.

MATERIALS AND METHODS

Characterization of circRNAs

For reverse transcription, oligo-dT and random primers were mixed with isolated RNA, then the levels of circular and linear RNAs were evaluated. Incubation of total RNA (~2 μg) and RNase R (3U/μg; Epicentre Technologies, Madison, USA) was carried out at 37°C for half an hour. After being treated with RNase R, the expression of RNA was measured using RT-qPCR.

Patient specimens and cell culture

The expression of hsa_circRNA_092488 was evaluated in venous blood samples obtained from DVT patients (n=42) and healthy controls (n=42) in xxx between June 2018 and May 2020. Venous blood samples were collected from the median elbow vein after 6 hours of fasting immediately after diagnosis. The median value of hsa_circRNA_092488 expression was used as the cutoff. Samples with values below the median were classified as “low”, while those above were classified as “high”. Demographic factors were assessed such as gender, age, history of smoking and obesity, and no significant differences on these factors were revealed between low- and high-has_circRNA_092488 group in patients. The clinical features of enrolled patients were summarized in Table I. This study included patients who were diagnosed with DVT using Color Doppler Ultrasound and lower extremity angiography. It excluded individuals with a history of diabetes mellitus, hypertension, other chronic diseases, recent immobilization or surgery, as well as those using anticoagulants or platelet inhibitors, undergoing hormone therapy, or having concurrent tumors. The experiments were performed in consistence with the Helsinki declaration and the protocols were reviewed by the Ethic Committee of xxx. Informed consents were obtained from all the participants.

Table I: Clinical features of enrolled patients.

Parameter	Number of patients	Low hsa_circRNA_092488 expression (n=21)	High hsa_circRNA_092488 expression (n=21)	p
Age (years)				
≤40	22	12	10	0.54
>40	20	9	11	
Gender				
Female	21	11	10	0.76
Male	21	10	11	
Smoking				
Yes	19	8	11	0.35
No	23	13	10	
BMI (kg/m^2)		24.62±6.17	25.24±6.35	0.75

To isolate mononuclear cells (MNCs), circulating blood (~100 ml) of patients with DVT and healthy donors was obtained using BD vacutainer EDTA tube, and specimens were kept in dark. Upon collection, the specimens were processed as follows. Briefly, isolation of MNCs was performed at 5003g for half an hour using a density gradient centrifuge (Biocoll; Biochrom, Berlin, Germany), and subsequently, the samples were rinsed by PBS

for three times. Then, isolated cells were innoculated on a cell culture dish which were already pre-coated using commercial fibronectin (Sigma, Deisendorf, Germany), and the cells were cultured using EGM growth media for endothelial cell (GE Healthcare Life Science), which contains human epidermal growth factor (10 ng/ml), bovine brain extract (12 mg/ml), hydrocortisone (1 mg/ml), human insulin-like growth factor-1 (50 ng/ml) and pen/strep ((all from Gibco, Grand Island, NY, USA). In addition, heparin (10U/ml) was added to prevent the platelet coagulation, and cells were incubated at 37°C with the supply of 5% CO_2. Colony of EPC was visible ~two weeks after.

Transfection

Annealing of shRNA segments was carried out and then the plasmids were cloned into pU6-Luc-Puro lentivirus vector (Genepharm Co. Ltd.). In order to produce the cell model with overexpression of hsa_circRNA_092488, WT (oe- hsa_circRNA_092488) and MUT (oe-NC) fragments were amplified using PCR, then the segments were inserted into PLCDH-cir expression vector (Invitrogen; Waltham, MA, USA). To verify the transfection efficiencies, down- or up-regulation of hsa_circRNA_092488 was confirmed using RT-qPCR. Briefly, cells were seeded onto a 24-well plate, and 1ug of plasmids were used per well for each transfection.

qRT-PCR

The extraction of total RNA was performed using Trizol reagent (Sobao Biotechnology, Shanghai, China). Subsequently, reverse transcription was carried out using PrimeScript™ RT Reagent kit (Invitrogen, Shanghai, China), and SYBR Green PCR reagent was used for qPCR on an Applied Biosystems (7500; Applied Biosystems, USA). Paired primers were produced by Xinbei Biotechnology (Shanghai, China): hsa_circRNA_092488 Forward 5'- GCAGGTTGTTCATCGGGCATTC -3', Reverse 5'-GGCTTACAGCACGGAAGTGTTTC-3'; Ki-67 5'- AGCGGCTCTCTTTAACACAGT-3', 5'-TGACCCCCAAAGGATACACG-3'; NLRP3 5'-GGACTGAAGCACCTGTTGTGCA-3', 5'-TCCTGAGTCTCCCAAGGCATTC-3'; GAPDH 5'- GTCTCCTCTGACTTCAACAGCG -3', 5'-ACCACCCTGTTGCTGTAGCCAA-3'.; U6, 5'-CTCGCTTCGGCAGCACATA-3' and 5'-AACGATTCACGAATTTGCGT-3'. Endogenous U6 or GAPDH were used as controls for normalization of the expression of miRNA or mRNA. For mRNA expression analysis, untreated RNA samples were used. For circRNA expression analysis, RNA samples treated with RNase R were used to remove linear RNA.

Western blotting

Concentrations of extracted protein were determined using a BCA kit (Dingguo Biotechnology, Guangzhou, China). Separation of proteins (~40 μg) were carried out on SDS-PAGE. Subsequently, the samples were transferred onto a PVDF membrane (Thermo Fisher Scientific). Then the membranes were blocked with 5% skimmed milk at room temperature for one hour and then incubated using primary antibodies against NLRP3 (1:1000; cat no. ab263899; Abcam), caspase-1 (1:500; cat no. ab207802; Abcam), IL-1b (1:500; cat no. ab283818; Abcam), p-NFkB-p65 (1:500; cat no. 3033; Cell Signaling Technology), NFkB-p65 (1:500; cat no. 3034; Cell Signaling Technology), p-IKBa (1:1000; cat no. 2859; Cell Signaling Technology), IKBa (1:1000; cat no. 4818; Cell Signaling Technology) and GAPDH (1:2000; cat no. 4967; Cell Signaling Technology) in a cold room overnight. Next day, incubation was performed using anti-mouse IgG (1:2000; cat no. ab6728; Abcam) or anti-rabbit IgG (1:2000; cat no. ab6721; Abcam) for one hour.

CCK-8 assay

Cell proliferation at various transfection conditions was measured using a CCK-8 kit. Transfected cells were inoculated using 96 wells plates. Then, 10μL of CCK-8 mixture (Dojindo, Kumamoto, Japan) was added on cells. Cell proliferation was measured at different time points (Day 1, 2, 3 and 4). Then, further incubation of cells was carried out for additional two hours, and the absorbance (wl=450nm) was recorded by microplate reader (Bio-Rad Laboratories, Inc., Hercules, CA, USA).

Transwell experiment

Transwell kit (ChenGong Biotechnology, Shanghai, China) was employed to evaluate cell migration. Cell suspension (~$5x10^5$ cells) was added in the upper chamber (pore size= 8μm; BD Biosciences, New Jersey, USA) which was pre-coated using Matrigel® (Sigma-Aldrich, MO, USA). Then, 500 μl of culture medium supplemented with 10% FBS was loaded on the lower chamber. Following 48 hours, non-migratory cells were discarded by cotton bud. Fixation of cells remained in lower chamber was carried out using ice-cold methanol for 15 mins. Following this, cells were stained using 0.5% crystal violet solution.

Cell cycle analysis and measurement of cell apoptosis

Cells with a density at $5x10^5$ cells/well were seeded on a 6 wells plate. Subsequently, cells were spun down at low-speed (1000rpm) at 4˚C for 5 mins. The pellets were then washed three times and well suspended using

PBS, subsequently, fixation of cells was performed using 70% ice-cold ethanol and samples were then stored at 4˚C for 48n hours. Then, lysis of cells was conducted before flow cytometry, and cells were centrifugated and well suspended using PI staining solution with 50 µl/ml PI and 250 µl/ml RNase A (Sigma-Aldrich). Distribution of cell cycle was evaluated using flow cytometry (BD Biosciences) and the data were analysed using Flowjo (7.6; Flowjo LLC). For cell apoptosis, cell suspension was incubated at 4˚C in dark for half an hour and staining using 5 µl annexin V-FITC was performed (JingMei Biotech, Beijing, China).

RNA pulldown assay
The probes of NLRP3 and negative control which were labelled by biotin were generated by GenePharma (Shanghai, China). The labeling of cell lysates was carried out by Dynabeads M-280 Streptavidin. Then, beads conjugated with immobilized NLRP3 were treated using ethylenediaminetetraacetic acid (10mM).

Assessment of mRNA stabilities
To prevent the additional synthesis of RNA, treatment with actinomycin D (5µg/mL; MedChemExpress, USA) was applied on the cells. Subsequently, treatment with actinomycin D of the cells was carried out at different time points. Then extraction of RNA was performed, and samples were subsequently used for RT-qPCR. Remaining levels of NLRP3 RNAs at different time points were normalized to the level at start of the treatment.

Immunofluorescence analysis
The fixation of cells was conducted with pre-chilled acetone (Sigma-Aldrich) for 20 mins. Subsequently, fixed cells were washed using PBS and further incubated in blocking buffer for half an hour. Then incubation with primary anti-Ki67 antibody (1:500; cat. no. ab15580; Abcam) was carried out at 4˚C overnight. The following day, samples were then washed for three times, and incubation of cells with secondary antibody conjugated with Alexa-Fluor 568 (1:1000, Molecular Probes, Eugene) was carried out for 60 mins. Subsequently, nuclei were stained with DAPI solution. Finally, stained cells were washed for three times and mounted to slides using Mowiol reagent (10% Mowiol D488; Calbiochem, Nottingham, U.K).

Statistics analysis
Data generated by the experiments were shown as mean ± standard error of mean. Data were interpreted by SPSS (26; Chicago, USA). Statistical significance was evaluated with Student's t-test or one-way analysis of variance. Post-hoc Tukey test was conducted after ANOVA. In Pearson's correlation test, the correlation of relative gene expression was examined.

RESULTS
CircRNAs characterization in EPCs
For the characterization of circRNAs in EPCs, the levels of linear RNAs were measured following the addition of oligo-dT primer, whereas the levels of circRNAs were not observed (Fig. 1A). Furthermore, linear RNAs were sensitive to digestion induced by RNase R but not circRNAs (Fig. 1B).

Up-regulation of hsa_circRNA_092488 was revealed in DVT specimens
The levels of hsa_circRNA_092488 were remarkably elevated in patients with DVT than matched healthy controls (Figure 1C). In order to explore the roles of hsa_circRNA_092488 up-regulation in the progression of DVT, function experiments were carried out. Cell models with hsa_circRNA_092488 over-expression and knock-down were generated, respectively (Figure 1D and E).

Hsa_circRNA_092488 was involved in the biological behavior changes of EPCs
The results of CCK-8 assay revealed that over-expression of hsa_circRNA_092488 significantly inhibited the proliferation of EPCs (Figure 2A), while knockdown of hsa_circRNA_092488 remarkably promoted the proliferative activity of cells (Figure 2B). In addition, the levels of Ki-67 were decreased in EPCs with hsa_circRNA_092488 over-expression (Figure. 2C and D), while Ki-67 expression was enhanced in cells treated with sh-hsa_circRNA_092488 (Figure. 2E and F).
Furthermore, the migration of EPCs was suppressed by the over-expressed hsa_circRNA_092488 (Figure 3A and B), while cell migration was elevated after the transfection with sh-hsa_circRNA_092488 (Figure 3C and D). Moreover, the results of cell cycle distribution suggested the shift of EPCs from S to G0/G1 phase following the treatment with oe-hsa_circRNA_092488 (Fig. 3E and F). Vice versa, the proportion of cells at G0/G1 phase was reduced, whereas cell percentage at S phase was elevated after the knockdown of hsa_circRNA_092488 (Fig. 3G and H).
Moreover, flow cytometry revealed that hsa_circRNA_092488 over-expression triggered cell apoptosis in EPCs (Fig. 4A and B). Vice versa, cell apoptotic rate was decreased after the transfection of sh-hsa_circRNA_092488 (Fig. 4C and D).

NLRP3 was the novel target of hsa_circRNA_092488

The association of hsa_circRNA_092488 and NLRP3 in EPCs was unrevealed using RNA pull-down analysis (Figure 5A). Furthermore, stabilities of NLRP3 mRNA in EPCs was enhanced after the transfection with oe-hsa_circRNA_092488 (Figure 5B). Vice versa, the mRNA stability was decreased in EPCs with hsa_circRNA_092488 knockdown (Figure 5C). In addition, NLRP3 expression was enhanced in DVT samples (Figure 5D). Moreover, the levels of hsa_circRNA_092488 and NLRP3 were positively correlated in DVT specimens (Figure 5E; $R=0.602$, $p<0.05$). Western blotting also suggested that NLRP3 protein levels were increased in EPCs treated with oe-hsa_circRNA_092488 and reduced in cells transfected with sh-hsa_circRNA_092488, respectively (Figure 5F and G).

Hsa_circRNA_092488 could function via the NLRP3/NF-kB pathway

Furthermore, the protein expression of NLRP3/NF-κB-associated molecules were examined (Figure 6). The results of western blotting suggested that overexpressed hsa_circRNA_092488 in the cell notably elevated the protein expression of caspase-1, IL-1b, P-NF-κB-p65/NF-κB-p65 and P-IκBa/IκBa; and vice versa, knockdown of hsa_circRNA_092488 significantly reduced the levels of these related proteins in EPCs.

DISSCUSSION

In previous studies, circRNAs bind to miRNA to regulate the expression of target genes and they become new research hotspot as they are involved in various biological processes [6] Furthermore, circ_0020123 and hsa_circ_0001020 modulated EPC migration and tube formation and regulate the progression of DVT [7,8]. Up-regulated hsa_circRNA_092488 was detected in DVT, and its involvement in inflammation was reported [9,10]. In our study, the biological functions of hsa_circRNA_092488 in DVT were explored and the underlying mechanisms were evaluated.

In our experiments, up-regulated hsa_circRNA_092488 was confirmed in DVT patients, which inhibited EPCs proliferation and migration, triggered the shift of cell cycle from S to G0/G1 phase and induced cell apoptosis. Similarly, up-regulation of hsa_circ_0001020 accelerated the development of DVT via sponging miR-29c-3p to promote the expression of MDM2 [8]. In addition, long non-coding RNA Crnde promoted DVT by sequestering miR-181a-5p away from thrombogenic Pcyox1l [11]. In their findings, both Crnde and Pcyox levels were enhanced in the blood from DVT mice, and miR-181a-5p was the novel target of Crnde. Furthermore, knockdown of Crnde or restoration of miR-181a-5p both inhibited inflammatory injury, therefore suppressing the formation of thrombus in mice [11].

Additionally, NLRP3 was explored as the putative target of hsa_circRNA_092488. NOD-like receptor protein 3 (NLRP3) a protein encoded by the NLRP3 gene located on the long arm of chromosome 1 [12]. NLRP3 is abundantly detected in macrophages and as a component of inflammasome. Research has indicated that various inflammatory diseases are associated with the NLRP3 inflammasome [12]. NLRP3 inflammasome could be activated by multiple stimuli, such as mitochondrial dysfunction and the elevated levels of ROS [15]. Dysregulated activation of NLRP3 inflammasome has been implicated in numerous disorders including diabetes, athrosclerosis and Alzheimer's disease. Small molecule inhibitors targeting the NLRP3 inflammasome have shown certain therapeutic potential, but their clinical feasibility still requires further exploration. Once activated, NLRP3, along with the adaptor protein ASC and the effector protein caspase-1, initiates the assembly of inflammasomes, leading to the activation of caspase-1 and subsequent cleavage of IL-1β and IL-18. Aberrant activation of the NLRP3 inflammasome is associated with the pathogenesis of various inflammatory diseases [13]. Upon activation, NLRP3 inflammasome could promote the activation of NF-κB and production of cytokines in inflammatory diseases [14,15]. In the previous study, knockdown of NLRP3 inhibited the activation of NF-kB and secretion of cytokines in both microbially induced and sterile inflammation. NLRP3 not only trigger the activation of caspase-1 post-translationally, but it also promote the expression of cytokines in the innate immune system [15]. In consistence with these findings, our results suggested that hsa_circRNA_092488 could exert its regulatory functions through activating the NLRP3/NF-kB signaling, as overexpression of hsa_circRNA_092488 enhanced the protein levels of caspase-1, IL-1b, P-NF-κB-p65/NF-κB-p65 and P-IκBa/IκBa; and vice versa.

Similarly, previous study also revealed that the NF-κB signaling could modulate the pro-inflammatory and coagulation response in DVT [16]. They revealed that miRNAs or drugs for the interference with the NF-kB signaling could be a promising therapeutic approach to improve thrombosis, but the appropriate dose and possible side effects should also be considered and require further investigation [16]. In another study, the essential regulatory role of NLRP3/HIF-1a signaling within a complex network of coagulation and inflammation during the progression of thrombosis have been revealed [17]. Furthermore, another study also indicated that NLRP3 inflammasome was able to promote the production of IL-1b, and it was elevated in the activation of platelet and the formation of thrombus [18]. Therefore, targeted therapies against NLRP3 or IL-1b could also be beneficial for the treatment of inflammation-related thrombosis. However, there were some

limitations in current study. For instance, to confirm the existing findings, the expression of apoptosis-related molecules could be examined in transfected cells. Furthermore, more in vivo experiments should be carried out in future work to confirm the findings in this study.
In summary, up-regulation of hsa_circRNA_092488 in DVT was able to suppress the proliferation and migration of EPCs, induce cell cycle arrest from S to G0/G1 phase and trigger cell apoptosis. Moreover, hsa_circRNA_092488 could function via activating the NLRP3/NF-kB signaling, as overexpressed hsa_circRNA_092488 elevated the protein levels of caspase-1, IL-1b, P-NF-κB-p65/NF-κB-p65 and P-IκBa/IκBa; and vice versa. Therefore, the hsa_circRNA_092488/ NLRP3/NF-kB pathway could be associated with the progression of DVT, and this novel signaling may be considered as putative therapeutic candidate for the treatment of DVT such as targeting circRNAs, drugs for the interference with the NF-kB signaling or targeted therapies against NLRP3 or IL-1b; however, the accurate dose and potential side effects should also be considered and require further investigation.

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Authors' contributions
Both authors initiated the study and performed the experiments and analyzed data. They both wrote the paper.
Availability of data and materials
The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.
Ethics approval
The protocol was reviewed by the Ethics Committee of the First Affiliated Hospital of Jinzhou Medical University,
Consent to participate
Not applicable.
Competing interests
The authors have no competing interests.

References
1. Navarrete S, Solar C, Tapia R, Pereira J, Fuentes E, Palomo I. Pathophysiology of deep vein thrombosis. Clin Exp Med. 2023;23(3):645-654.
2. Stone J, Hangge P, Albadawi H, Wallace A, Shamoun F, Knuttien M, Naidu S, Oklu R. Deep vein thrombosis: pathogenesis, diagnosis, and medical management. Cardiovasc Diagn Ther. 2017;7 (Suppli 3):S276-S284.
3. Hristov M, Erl W, Weber P. Endothelial progenitor cells: mobilization, differentiation, and homing. Arterioscler Thromb Vasc Biol. 2003:23(7):1185-1189.
4. Yan F, Liu X, Ding H, Zhang W. Paracrine mechanismas of endothelial progenitor cells in vascular repair. Acta Histochem. 2022;124(1):151822.
5. Ribatti D, Nico B, Crivellato E, Vacca A. Endothelial progenitor cells in health and disease. Histol Histopathol. 2005;20(4):1351-1358.
6. Ding S, Zhu Y, Liang Y, Huang H, Xu Y, Zhong C. Circular RNAs are a large class of animal RNAs with regulatory potency. Nature. 2013;495: 333-338.
7. Cui M, Wang L, Xu P, Fu L, Hu R. Circ_0020123, a new circular RNA biomarker for deep vein thrombosis in pregnant women. 2023;69(9).
8. Lou Z, Ma H, Li X, Zhang F, Du K, Wang B. Hsa_circ_0001020 accelerates the lower extremity deep vein thrombosis via sponging miR-29c-3p to promote MDM2 expression. Thromb Res. 2022;211:38-48.
9. Lou Z, Li X, Li C, Li X, Du K, Zhang F, Wang B. Microarray profile of circular RNAs identifies hsa_circ_000455 as a new circular RNA biomarker for deep vein thrombosis. Vascular. 2022;30(3):577-589.
10. Wang X, Li L. Cell type-specific potential pathogenic genes and functional pathways in Alzheimer's Disease. BMC Neurology. 2021;21:381.
11. He X, Liu Y, Li Y, Wu K. Long non-coding RNA crnde promotes deep vein thrombosis by sequestering miR-181a-5p away from thrombogenic Pcyox1l. Thromb J. 2023;21(1):44.
12. Kelly N, Jeltema D, Duan Y, He Y. The NLRP3 inflammasome: An overview of mechanisms of activation and regulation. Int J Mol Sci. 2019;20(13):3328.
13. Xu J, Nunez G. The NLRP3 inflammasome: activation and regulation. Nature Reviews Immunology. 2023;48(4):331-344.
14. Chen W, Wang J, Hua Z, Zhang Y. Du Huo Ji Sheng Tang relieves knee osteoarthritis via suppressing NLRP3/NF- κB inflammatory signals in rats. European Journal of Inflammation. 2020;18:1-11.

15. Kinoshita T, Imanura R, Kushiyama H, Suda T. NLRP3 mediates NF-kB activation and cytokine induction in microbially induced and sterile inflammation. PLOSONE. 2015;10(3):e0119179.
16. Wang Z, Fang C, Yao M, Wu D, Chen M, Guo T, Mo J. Research progress of NF-κB signaling pathway and thrombosis. Front Immunol. 2023;14:1257988.
17. Gupta N, Sahu A, Prabhakar A, Chatterjee T, Tyagi T, Kumari B, Khan N, Nair V, Bajaj N, Sharma M, Ashraf M. Activation of NLRP3 inflammasome complex potentiates venous thrombosis in response to hypoxia. Biological Sciences. 2016;114(18):4763-4768.
18. Qiao J, Wu X, Luo Q, Wei G, Xu M, Wu Y, Liu Y, Li X, Zi J, Ju W, Fu L, Chen C, Wu Q, Zhu S, Qi K, Li D, Li Z, Andrews R, Zeng L, Gardiner E, Xu K. NLRP3 regulates platelet integrin αIIbβ3 outside-in signaling, hemostasis and arterial thrombosis. Haematologica. 2018;103(9):1568-1576.

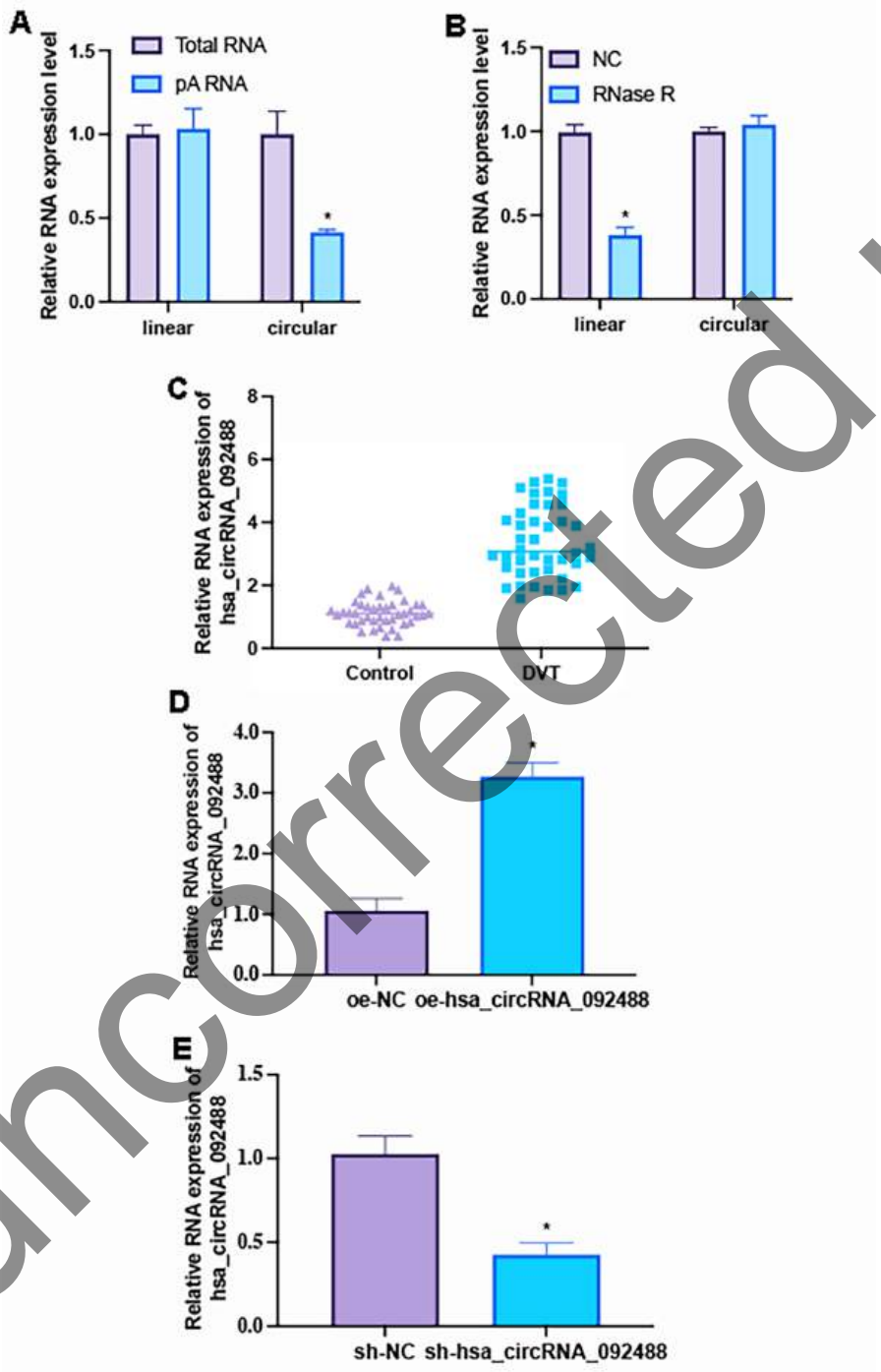

FIGURE 1. Characterization of circRNAs in EPCs and up-regulated hsa_circRNA_092488 in DVT. (A) Levels of linear RNAs were observed after adding of oligo-dT primer, while there was no expression of circRNAs. (B)

The results revealed resistance in circRNAs to digestion induced by RNase R but not in linear RNAs. (C) Hsa_circRNA_092488 was up-regulated in DVT patients than matched healthy donors. (D and E) Cells with hsa_circRNA_092488 over-expression and knock-down were produced using lentiviral vectors .* p<0.05.

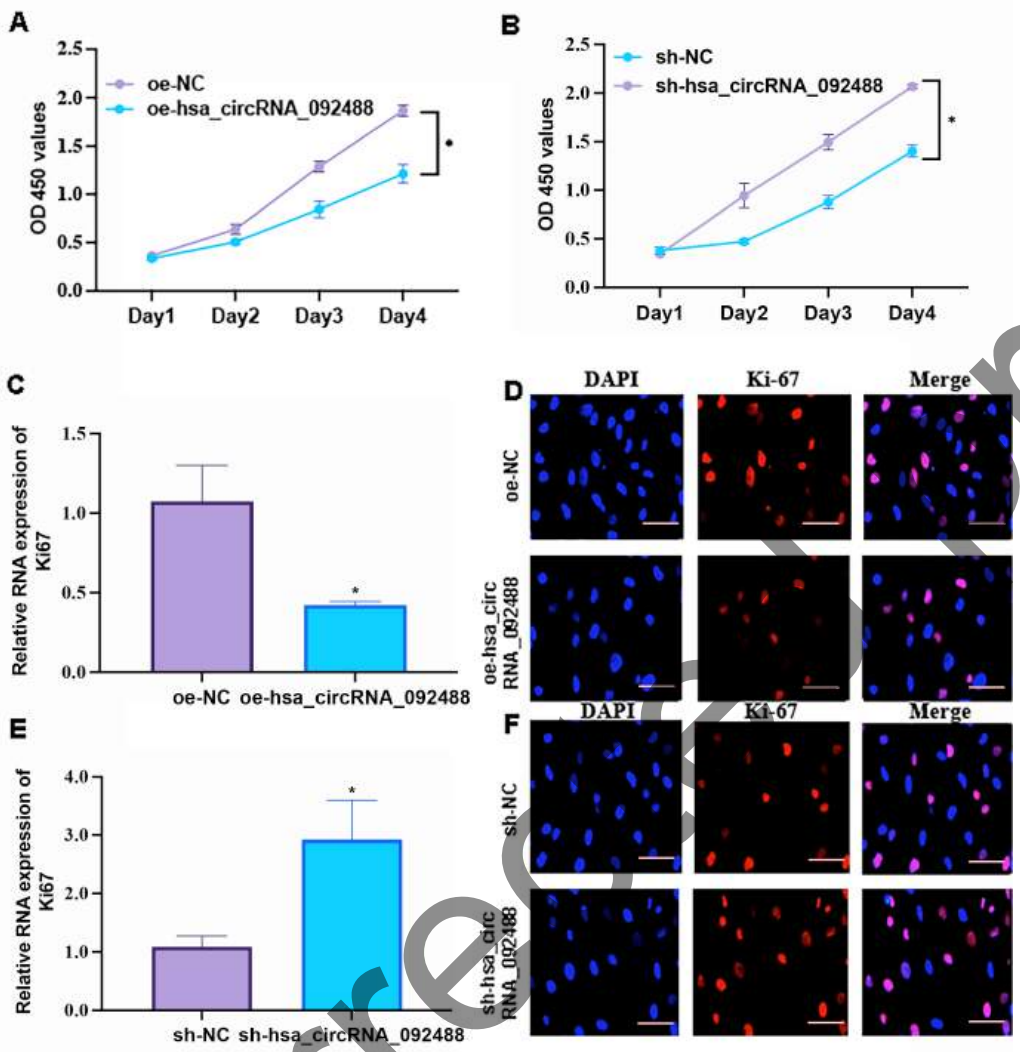

FIGURE 2. Hsa_circRNA_092488 participated in the regulation of biological behaviours of EPCs. (A) Overexpression of hsa_circRNA_092488 notably down-regulated the proliferative activity of EPCs. (B) Vice versa, knockdown of hsa_circRNA_092488 enhanced cell proliferation. (C-F) The subcellular staining of Ki-67 were also evaluated in EPCs following the treatment with oe-hsa_circRNA_092488 and sh-hsa_circRNA_092488. * p<0.05. Magnification: 40x; scale bar: 50 μm.

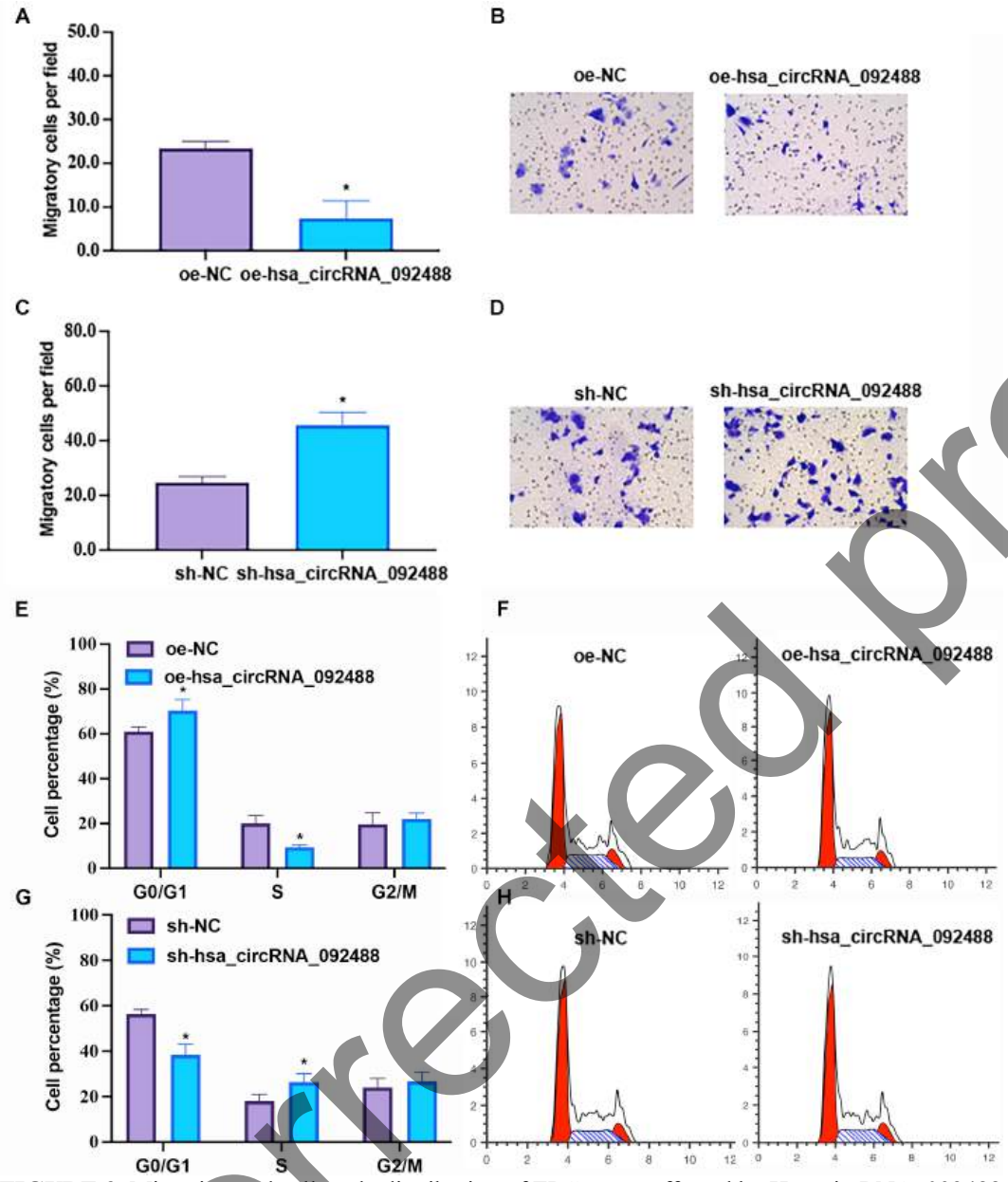

FIGURE 3. Migration and cell cycle distribution of EPCs were affected by Hsa_circRNA_092488. (A-D) Migrative ability of EPCs were evaluated following the treatment with oe-hsa_circRNA_092488 and sh-hsa_circRNA_092488. (E-H) Cell cycle distribution of EPCs were also measured after the transfection with oe-hsa_circRNA_092488 and sh-hsa_circRNA_092488.* $p<0.05$.

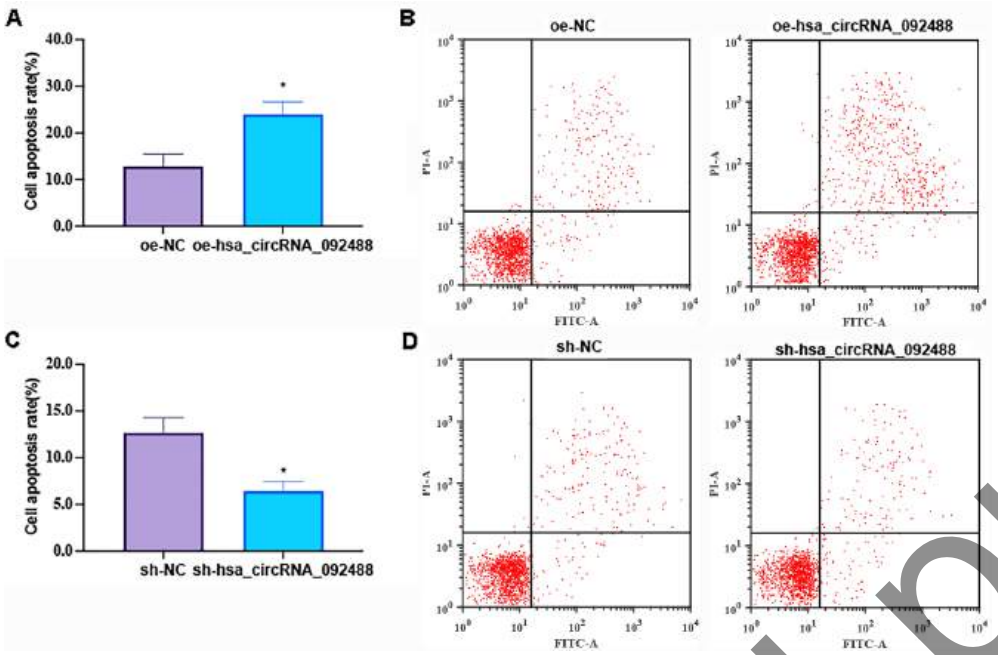

FIGURE 4. Cell apoptosis was triggered by up-regulated hsa_circRNA_092488. (A and B) Cell apoptotic rates were elevated in EPCs transfected with oe-hsa_circRNA_092488. (C and D) Cell apoptosis was inhibited by sh-hsa_circRNA_092488. * $p<0.05$.

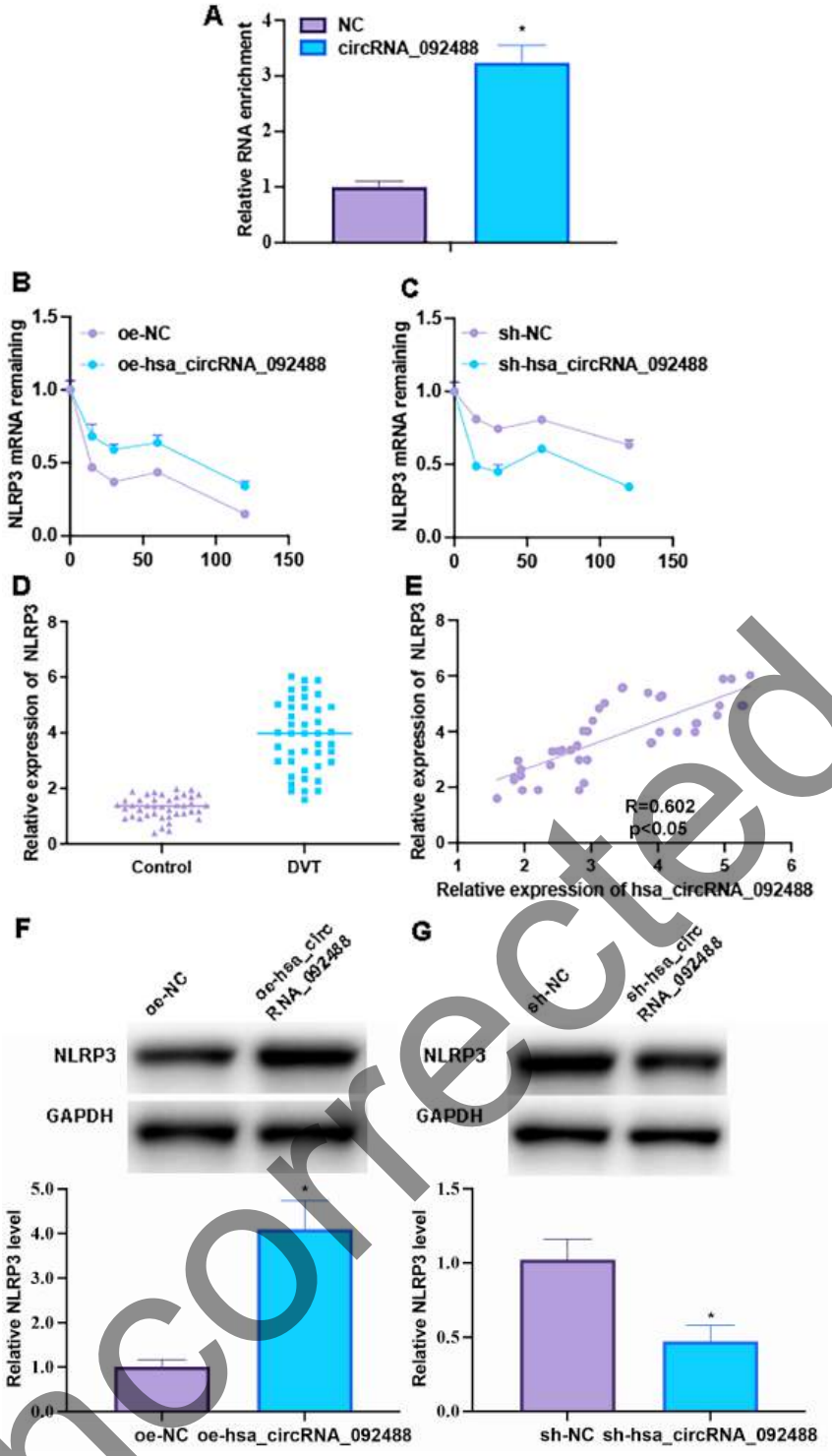

FIGURE 5. NLRP3 was the putative downstream molecule of hsa_circRNA_092488. (A) The interaction of hsa_circRNA_092488 and NLRP3 was revealed in RIP assay. (B) Stabilities of NLRP3 mRNA was elevated in EPCs transfected with oe-hsa_circRNA_092488. (C) Vice versa, NLRP3 mRNA stability was reduced by sh-hsa_circRNA_092488. (D) NLRP3 expression was enhanced in DVT specimens. (E) The levels of hsa_circRNA_092488 and NLRP3 were positively correlated in DVT. (F and G) NLRP3 protein levels were increased in EPCs treated with oe-hsa_circRNA_092488 and vice versa. * $p<0.05$.

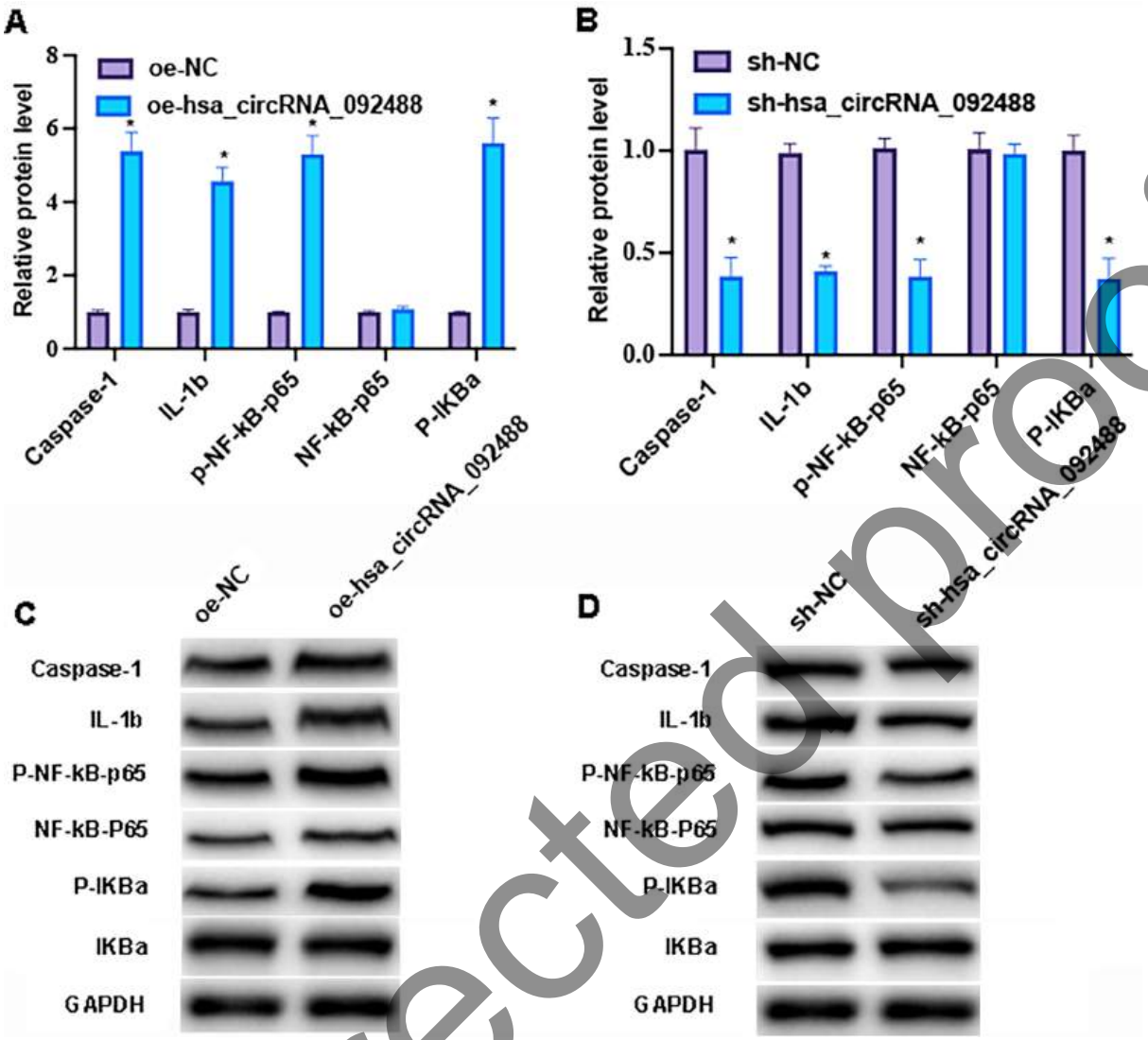

FIGURE 6. The levels of NLRP3/NF-κB-associated proteins were affected by hsa_circRNA_092488. Over-expression of hsa_circRNA_092488 in EPCs remarkably increased the protein levels of caspase-1, IL-1b, P-NF-κB-p65/NF-κB-p65 and P-IκBa/IκBa; and vice versa. * $p<0.05$.