

Original Research

AIM2 Promotes Neuronal Apoptosis Through NF- κ B Activation in an OxyHb-Induced *In Vitro* Subarachnoid Hemorrhage

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Abstract

Background: Subarachnoid hemorrhage (SAH) induces early brain injury (EBI) characterized by neuronal apoptosis, inflammation, and neurological dysfunction, yet the underlying mechanisms remain incompletely understood. Although absent in melanoma 2 (AIM2) has been implicated in post-SAH pathology, its role in neuronal injury remains unclear. **Methods:** Transcriptomic analyses of Gene Expression Omnibus (GEO) datasets (GSE36791 and GSE73378) were performed to investigate the association between AIM2 and nuclear factor κ B (NF- κ B)-related signaling. An oxyhemoglobin (OxyHb)-induced *in vitro* SAH model, *Aim2* knockdown, and *Rela* co-silencing experiments were used to evaluate neuronal apoptosis, viability, and NF- κ B activation. *In vivo* validation was performed in SAH brain tissues. **Results:** Transcriptomic analyses showed a positive association between *AIM2* expression and NF- κ B signaling. In OxyHb-treated neurons, AIM2 expression and p65 phosphorylation were increased, accompanied by enhanced neuronal apoptosis and reduced viability. *Aim2* knockdown attenuated NF- κ B activation, reduced neuronal apoptosis, and improved cell viability, whereas co-silencing of *Aim2* and *Rela* exerted stronger neuroprotective effects. *Aim2* silencing showed limited effects on pyroptosis-related responses, and *in vivo* analyses further supported a positive association between AIM2 expression and NF- κ B activation after SAH. **Conclusions:** AIM2 contributes to OxyHb-induced neuronal apoptosis, at least in part, through NF- κ B signaling. These findings provide new insight into the molecular mechanisms underlying EBI after SAH.

Keywords: subarachnoid hemorrhage; nuclear factor κ B; AIM2 protein; apoptosis

1. Introduction

Subarachnoid hemorrhage (SAH) represents a life-threatening type of stroke caused by hemorrhage into the subarachnoid space, typically following rupture of a cerebral aneurysm. Despite advances in surgical and medical management, SAH continues to carry high rates of mortality and long-term neurological disability [1,2]. Early brain injury (EBI), which develops within the first 72 hours after SAH, has been recognized as a major determinant of poor outcome [3,4]. EBI involves a cascade of pathological events, including neuronal apoptosis, oxidative stress, blood–brain barrier (BBB) disruption, cerebral edema, and neuroinflammation [5,6]. Among these, inflammation and programmed cell death are pivotal contributors to secondary neuronal injury and are closely linked to activation of cytosolic pattern-recognition receptors such as inflammasomes [7,8].

Absent in melanoma 2 (AIM2), a critical cytosolic double-stranded DNA sensor and member of the AIM2-like receptor (ALR) family, forms an inflammasome complex with apoptosis-associated speck-like protein containing a CARD (ASC) and caspase-1 to induce the maturation of Interleukin (IL)-1 β and IL-18, leading to pyroptotic or apoptotic cell death [9,10]. Recent studies have demonstrated

that AIM2 is markedly upregulated in ischemic stroke and intracerebral hemorrhage, contributing to neuroinflammation and neuronal death. In experimental SAH models, AIM2 inflammasome activation has been linked to exacerbation of early brain injury, mostly via pyroptotic pathways [11,12,13]. However, the molecular mechanisms through which AIM2 regulates neuronal apoptosis (rather than pyroptosis) after SAH remain incompletely understood.

The nuclear factor κ B (NF- κ B) signaling pathway, a canonical mediator of inflammatory and apoptotic responses, is rapidly activated after SAH and contributes to neuronal injury and BBB dysfunction [14]. Although AIM2 has been shown to interact with NF- κ B signaling in other inflammatory contexts, whether AIM2 drives neuronal apoptosis through NF- κ B activation in the setting of SAH is unknown.

In this study, we aimed to elucidate the role of AIM2 in oxyhemoglobin (OxyHb)-induced neuronal injury and to define its relationship with NF- κ B signaling. First, transcriptomic analyses of GEO datasets (GSE36791 and GSE73378) were performed to examine the association between *AIM2* expression and NF- κ B-related pathways. Second, an *in vitro* OxyHb-induced SAH model was established to investigate the effects of AIM2 modulation on neu-



ronal apoptosis, viability, and pyroptosis-related responses. Finally, co-silencing of *Aim2* and *Rela* was conducted to determine whether AIM2-mediated neuronal injury is dependent on NF- κ B activation. In addition, *in vivo* analyses were performed to validate the association between AIM2 expression and NF- κ B activation in SAH brain tissues. This study provides new insight into the molecular mechanisms underlying early brain injury after SAH and highlights the AIM2-NF- κ B axis as a candidate pathway involved in neuronal injury.

2. Materials and Methods

2.1 Mice

At the experimental endpoint, mice were anesthetized with xylazine (10 mg/kg; Xyla-Ject, 20 mg/mL, NDC 57319-362-26, Phoenix Pharmaceutical, Inc., St. Joseph, MO, USA) and ketamine (100 mg/kg; Ketaject, 100 mg/mL, NDC 57319-542-02, Phoenix Pharmaceutical, Inc., St. Joseph, MO, USA) prior to euthanasia and tissue collection. Euthanasia was performed by carbon dioxide (CO₂) inhalation in accordance with institutional guidelines. Death was confirmed by the absence of respiration and heartbeat.

2.2 Cell Culture and Treatment

Primary cortical neurons were isolated from the cerebral cortex of foetal C57BL/6J mice (E16-18; Beijing Vital River Laboratory Animal Technology Co., Ltd., Beijing, China) using a modified protocol as previously described [13]. Briefly, foetal mice were decapitated and briefly immersed in 75% ethanol for sterilization. The brains were rapidly dissected in HBSS (Cat. No. 14175095, Gibco, Thermo Fisher Scientific, Waltham, MA, USA) under a stereomicroscope (SMZ745; Nikon, Tokyo, Japan). After careful removal of the leptomeninges and white matter, the cortical tissues were enzymatically digested in 0.125% trypsin (prepared from Trypsin-EDTA; Cat. No. 25200056, Gibco, Thermo Fisher Scientific) at 37 °C for 5 min in a water bath. The dissociated tissues were filtered through a 40 μ m cell strainer and centrifuged at 1500 r/min for 5 min. The resulting cell pellets were resuspended in Dulbecco's modified Eagle's medium (DMEM, Cat. No. 11965092, Gibco, Thermo Fisher Scientific) supplemented with foetal bovine serum (FBS; Cat. No. 10099141C, Gibco, Thermo Fisher Scientific) and penicillin-streptomycin (Cat. No. 15140122, Gibco, Thermo Fisher Scientific). Cells were then plated onto poly-D-lysine-coated culture plates (Cat. No. 354413, Corning, Corning, NY, USA). After 2 h, the medium was replaced with Neurobasal medium (Cat. No. 21103049, Gibco, Thermo Fisher Scientific) supplemented with GlutaMAX-I (Cat. No. 35050061, Gibco, Thermo Fisher Scientific), B27 (Cat. No. 17504044, Gibco, Thermo Fisher Scientific), and penicillin-streptomycin. Cells were maintained at 37 °C in a humidified incubator (Heracell 150i, Gibco, Thermo Fisher Scientific) con-

taining 5% CO₂ and 95% air, and half of the medium was refreshed every 2 days. The identity of primary cortical neurons was confirmed by microscopic examination based on their characteristic neuronal morphology, including typical neuronal cell bodies and extensive neurite networks. Mycoplasma contamination was assessed before experiments using a PCR-based Mycoplasma Detection Kit (Cat. No. 13100-01, SouthernBiotech, Birmingham, AL, USA) according to the manufacturer's instructions. No mycoplasma-specific amplification was detected in the tested neuronal cultures, whereas the internal control was successfully amplified, confirming that all tested samples were negative for mycoplasma contamination. Primary cortical neurons were cultured for 7 days *in vitro* prior to subsequent experiments. To establish the *in vitro* SAH model, neurons were treated with 10 μ M OxyHb purity $\geq$ 90%, Cat. No. 07109, Ruibio/Bomei Biotechnology Co., Ltd., Hefei, China.

2.3 SAH Model

For *in vivo* experiments, the SAH model was established as previously described [13]. Male C57BL/6J mice were anesthetized with 2% isoflurane (R510-22, RWD Life Science Co., Ltd., Shenzhen, China) in 100% O₂ and maintained under 1% isoflurane during the procedure. SAH was induced by stereotaxic injection of arterial blood into the prechiasmatic cistern. Briefly, a needle was inserted 4.5 mm anterior to the bregma along the midline at a 40° angle in the sagittal plane. Meanwhile, a donor mouse was euthanized, and arterial blood was collected via left ventricular cardiac puncture. A total of 60 μ L of non-heparinized arterial blood was slowly injected into the prechiasmatic cistern over 10 s using a 27-gauge needle (Cat. No. 305109, BD, Franklin Lakes, NJ, USA). The needle was left in place for 5 min to minimize reflux and prevent cerebrospinal fluid leakage. The burr hole was then sealed immediately with bone wax. Sham-operated mice underwent the same surgical procedure but received an equivalent volume of physiological saline instead of arterial blood.

2.4 ELISA

The concentrations of IL-1 β and IL-18 in neuronal culture supernatants were measured using commercially available mouse ELISA kits for IL-1 β (Cat. No. E-EL-M0037, Elabscience Biotechnology Co., Ltd., Wuhan, Hubei, China) and IL-18 (Cat. No. E-EL-M0730, Elabscience Biotechnology Co., Ltd.), respectively. Following the indicated treatments, culture supernatants were collected and centrifuged to remove cellular debris. ELISA assays were performed according to the manufacturer's instructions. Absorbance was measured at 450 nm using a microplate reader (ELx800; BioTek Instruments, Inc., Winooski, VT, USA), and cytokine concentrations were calculated from the corresponding standard curves.

2.5 Nissl Staining

Brain tissues were fixed in 4% paraformaldehyde, dehydrated, embedded in paraffin, and cut into 5- μ m-thick coronal sections. Sections containing the peri-hemorrhagic cortical region were deparaffinized, rehydrated, and subjected to Nissl staining using a cresyl violet-based Nissl Stain Kit (Cat. No. G1430, Beijing Solarbio Science & Technology Co., Ltd., Beijing, China) according to the manufacturer's instructions. After staining, sections were dehydrated, cleared, and mounted. Images were acquired under a light microscope, and neuronal density in the peri-hemorrhagic cortical region was quantified using ImageJ software (version 1.53t, National Institutes of Health, Bethesda, MD, USA). Neurons with intact cell bodies and clearly visible Nissl substance were considered viable neurons.

2.6 Transfection

To achieve stable knockdown of target genes, short hairpin RNAs (shRNAs) targeting *AIM2* or p65 (*RELA*) were designed and synthesized by GenePharma (Shanghai, China). The shRNA sequences were cloned into a lentiviral vector under the control of the U6 promoter. Primary mouse cortical neurons were seeded in 6-well plates (Cat. No. 3516, Corning) and transfected with sh-*AIM2*, sh-p65, or a non-targeting negative control shRNA using Lipofectamine 2000 (Cat. No. 11668019, Invitrogen, Thermo Fisher Scientific, Waltham, MA, USA) according to the manufacturer's instructions. After 48 h of transfection, total protein and RNA were extracted to evaluate knockdown efficiency by Western blotting and quantitative reverse transcription polymerase chain reaction (qRT-PCR), respectively. The final concentration of shRNA was 50 nM. The sequences used for gene silencing are listed in Table 1.

Table 1. shRNA sequences targeting *Aim2* and *Rela*.

sh- <i>Aim2</i>	Sense: 5'-GGAAGAAAGTTCTGGAAGA-3'
	Antisense: 5'-TCTTCCAGAACTTCTTCC-3'
sh- <i>Rela</i>	Sense: 5'-GGACTTCTATGAGGCTGGA-3'
	Antisense: 5'-TCCAGCCTCATAGAAGTCC-3'
shRNA, short hairpin RNA; Aim2, absent in melanoma 2.	

2.7 Western Blotting

Total protein was extracted from cultured cells and mouse tissues and separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). The proteins were subsequently transferred onto polyvinylidene difluoride (PVDF) membranes (0.45 μ m; Cat. No. 88518, Thermo Scientific, Waltham, MA, USA). After blocking, membranes were incubated with the indicated primary antibodies, followed by incubation with horseradish peroxidase (HRP)-conjugated anti-rabbit IgG secondary antibody

(1:5000; Cat. No. ab6721, Abcam, Cambridge, UK). The primary antibodies used in this study included AIM2 (1:1000; Cat. No. ab93015, Abcam), NF- κ B p65 (1:1000; Cat. No. ab76311, Abcam), phospho-NF- κ B p65 (1:1000; Cat. No. ab28856, Abcam), GAPDH (1:1000; Cat. No. 5174, Cell Signaling Technology, Danvers, MA, USA), Bax (1:1000; Cat. No. 14796, Cell Signaling Technology), Bcl2 (1:2000; Cat. No. ab182858, Abcam), N-GSDMD (1:1000; Cat. No. ab215203, Abcam), cleaved Caspase-1 (1:1000; Cat. No. 89332, Cell Signaling Technology, Danvers, MA, USA) and cleaved caspase-3 (1:500; Cat. No. ab32042, Abcam). Protein bands were detected using enhanced chemiluminescence (ECL; Cat. No. 32106, Thermo Scientific) reagents and quantified with ImageJ software (version 1.53t, National Institutes of Health).

2.8 TUNEL Staining

Neuronal apoptosis was assessed using a terminal deoxynucleotidyl transferase dUTP nick-end labeling (TUNEL) assay. Following the indicated treatments, primary mouse cortical neurons were fixed with 4% paraformaldehyde (Cat. No. P0099, Beyotime, Shanghai, China) in PBS for 20 min and permeabilized with 0.2% Triton X-100 (Cat. No. T8787, Sigma-Aldrich, St. Louis, MO, USA) for 10 min. Cells were subsequently incubated with the TUNEL reaction mixture at 37 °C for 1 h according to the manufacturer's instructions. After washing three times with PBS, nuclei were counterstained with 4',6-diamidino-2-phenylindole (DAPI; Cat. No. C1002, Beyotime) for 5 min in the dark. TUNEL-positive cells were then visualized and imaged using a fluorescence microscope (C2 Si; Nikon, Tokyo, Japan).

2.9 Cell Viability Assay

Cell viability was evaluated using the Cell Counting Kit-8 (CCK-8; Cat. No. C0038, Beyotime) in accordance with the manufacturer's instructions. Primary mouse cortical neurons were seeded into 96-well plates at a density of 6×10^3 cells per well and maintained at 37 °C in a humidified incubator containing 5% CO₂. Following the indicated treatments, 10 μ L of CCK-8 solution was added to each well containing 90 μ L of fresh culture medium and incubated for 1.5 h. Absorbance was measured at 450 nm using a microplate reader (ELx800; BioTek Instruments, Inc., Winooski, VT, USA). Each group included three technical replicates, and all experiments were independently repeated three times. Cell viability was normalized to the control group and expressed as a fold change.

2.10 Transcriptomic Correlation Analysis

Transcriptomic data were obtained from the publicly available GEO datasets GSE36791 and GSE73378, which include gene expression profiles from peripheral blood mononuclear cells of patients with subarachnoid hemorrhage (SAH) and healthy controls. Correlation analysis

between *AIM2* expression and NF- κ B signaling pathway activity was performed using R software (version 4.3.2; R Foundation for Statistical Computing, Vienna, Austria). Pearson correlation coefficients (r) and corresponding p -values were calculated using the `cor.test()` function in the `stats` package. Correlation plots and visualization of enrichment scores were generated using the `ggplot2` (version 3.5.1) and `ggpubr` (version 0.6.0) packages.

2.11 Statistical Analysis

All statistical analyses were performed using GraphPad Prism 8 software (GraphPad Software, San Diego, CA, USA). Comparisons between two groups were conducted using an unpaired Student's t -test. Differences among multiple groups were analyzed by one-way analysis of variance (ANOVA) followed by Tukey's post hoc test for multiple comparisons. Data are presented as mean $\pm$ standard error of the mean (SEM). A value of $p < 0.05$ was considered statistically significant, whereas $p < 0.01$ indicated a highly significant difference.

3. Results

3.1 *AIM2* Expression Positively Correlates With NF- κ B Signaling Activity in Datasets Simulating Subarachnoid Hemorrhage

To explore the potential involvement of *AIM2* in the inflammatory response following SAH, we first analyzed its relationship with the NF- κ B signaling pathway, a key mediator of inflammation and apoptosis. Using the GSE36791 dataset, which models inflammatory transcriptional profiles in SAH-related blood samples, gene set enrichment analysis (GSEA) revealed that high *AIM2* expression was associated with increased enrichment of multiple NF- κ B-related pathways, including canonical, non-canonical, and tumor necrosis factor- α (TNF- α)-mediated signaling cascades (Fig. 1A). Consistently, *AIM2* expression exhibited significant positive correlations with several gene sets related to NF- κ B signaling (Fig. 1B), suggesting a strong linkage between *AIM2* activation and NF- κ B pathway activity.

To validate these findings, similar analyses were conducted using the GSE73378 dataset. The results showed comparable enrichment patterns, where *AIM2* expression was again positively associated with canonical and TNF- α -dependent NF- κ B signaling (**Supplementary Figs. 1,2,3**). Correlation analysis further confirmed that *AIM2* expression levels were significantly correlated with the activity scores of NF- κ B signaling components, supporting the robustness of this association across independent datasets. Together, these data indicate that *AIM2* expression is tightly linked to NF- κ B pathway activation, implicating *AIM2* as a potential upstream regulator of inflammatory signaling in OxyHb-induced SAH models.

3.2 *OxyHb* Exposure Upregulates *AIM2* Expression and Promotes Neuronal Apoptosis in the *In Vitro* SAH Model

To further validate the potential involvement of *AIM2* in OxyHb-induced neuronal injury, we established an *in vitro* subarachnoid hemorrhage (SAH) model using increasing concentrations of oxyhemoglobin (OxyHb). Cell viability analysis using the CCK-8 assay showed a dose-dependent decline in neuronal survival following OxyHb exposure, with a significant reduction observed at concentrations of 10 μ M and 20 μ M (Fig. 2A). Western blotting analysis revealed a gradual elevation of *AIM2* protein expression with increasing OxyHb concentration (Fig. 2B), indicating that *AIM2* is activated in response to OxyHb-induced stress.

To investigate whether *AIM2* upregulation contributes to neuronal apoptosis, we examined the expression of apoptosis-related proteins. As shown in Fig. 2C, OxyHb stimulation led to decreased expression of the anti-apoptotic protein Bcl2, while the pro-apoptotic protein Bax and cleaved caspase-3 were markedly increased. Quantitative analysis confirmed that the Bcl2/Bax ratio was significantly reduced, whereas cleaved caspase-3 levels were elevated in a concentration-dependent manner. Together, these findings demonstrate that OxyHb exposure induces *AIM2* expression and promotes neuronal apoptosis, suggesting that *AIM2* may play a critical role in OxyHb-induced neuronal injury in the SAH model.

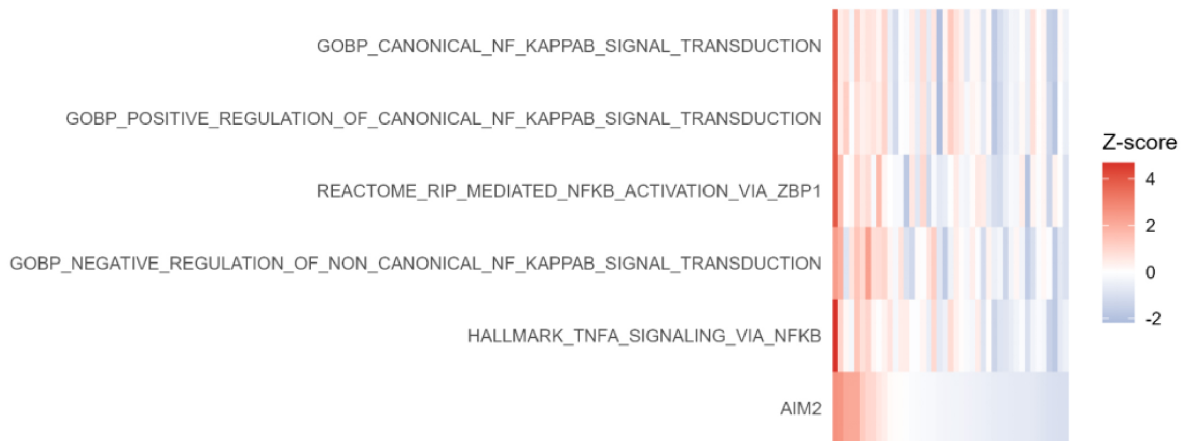
3.3 Knockdown of *Aim2* Alleviates OxyHb-Induced Neuronal Apoptosis and Inhibits NF- κ B Activation in the *In Vitro* SAH Model

To elucidate the functional role of *AIM2* in OxyHb-induced neuronal injury, *AIM2* expression was silenced using a specific shRNA construct in the *in vitro* SAH model. Western blotting analysis confirmed that *Aim2* knockdown markedly reduced the phosphorylation level of p65, indicating suppression of NF- κ B pathway activation (Fig. 3A). Consistently, *AIM2* silencing significantly decreased the expression of pro-apoptotic markers Bax and cleaved caspase-3, while restoring the Bcl2/Bax ratio (Fig. 3B), suggesting that *AIM2* promotes neuronal apoptosis through NF- κ B signaling activation.

To further determine whether *AIM2* is involved in pyroptosis, key pyroptosis-related markers were examined. OxyHb stimulation slightly increased the expression of cleaved caspase-1 and N-GSDMD, whereas *Aim2* knockdown did not significantly reverse these changes (Fig. 3C), indicating that *AIM2* has a limited effect on pyroptosis under these conditions.

Functionally, enzyme-linked immunosorbent assay (ELISA) analysis showed that OxyHb markedly increased IL-1 β and IL-18 levels, while *Aim2* knockdown showed no significant reduction in these inflammatory cytokines (Fig. 4A,B). In contrast, CCK-8 assays demonstrated that *Aim2* silencing significantly improved neuronal viability follow-

A



B

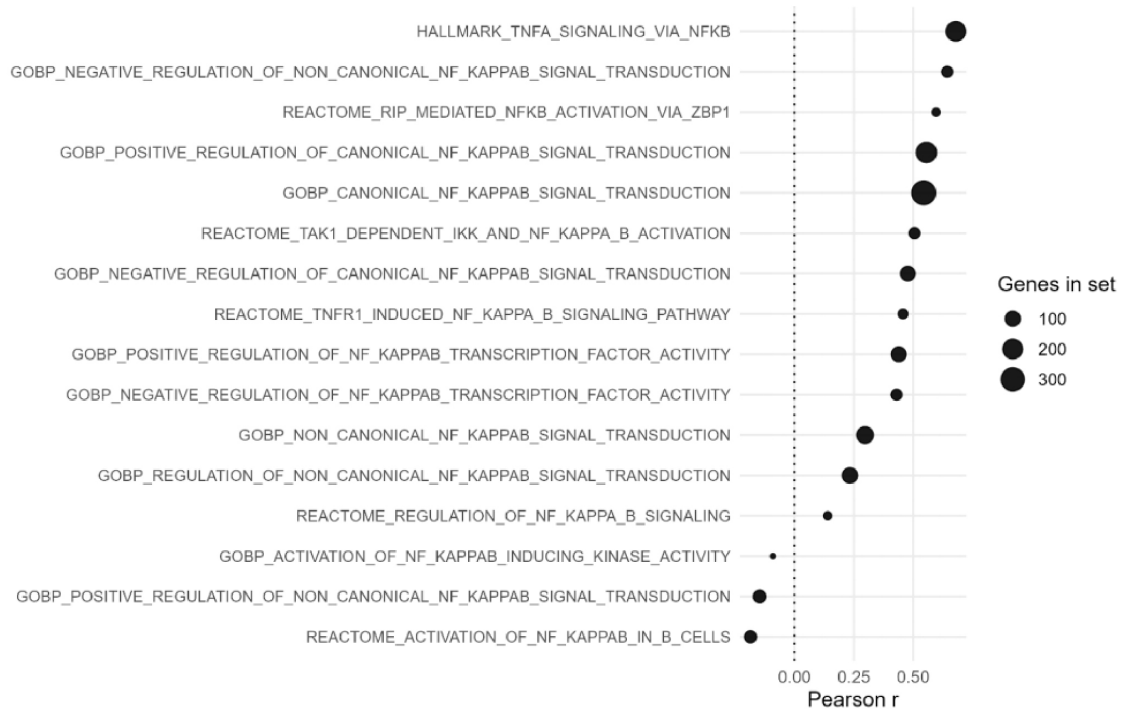


Fig. 1. Correlation between *AIM2* expression and nuclear factor κ B (NF- κ B) signaling pathway activity in the GSE36791 dataset. (A) Heatmap showing the enrichment scores (Z-score transformed) of representative NF- κ B-related gene sets, including canonical, non-canonical, and TNF- α -mediated signaling pathways, in relation to *AIM2* expression; (B) Bubble plot depicting the Pearson correlation between *AIM2* expression and NF- κ B-related signaling pathways. The size of each bubble indicates the number of genes included in each gene set. Data are derived from the GSE36791 dataset.

ing OxyHb exposure (Fig. 4C). Furthermore, TUNEL staining revealed that *Aim2* knockdown markedly reduced the proportion of apoptotic cells (Fig. 4D).

Collectively, these findings suggest that *AIM2* primarily promotes neuronal apoptosis rather than pyroptosis in OxyHb-induced injury, at least in part through activation of the NF- κ B signaling pathway.

3.4 *AIM2 Promotes OxyHb-Induced Neuronal Apoptosis Through NF- κ B Signaling, Which Is Further Suppressed by Rel α Knockdown*

To confirm that *AIM2*-mediated neuronal injury occurs via activation of the NF- κ B pathway, we performed co-silencing experiments targeting *Aim2* and *Rela* in OxyHb-treated neurons. Western blotting analysis revealed that

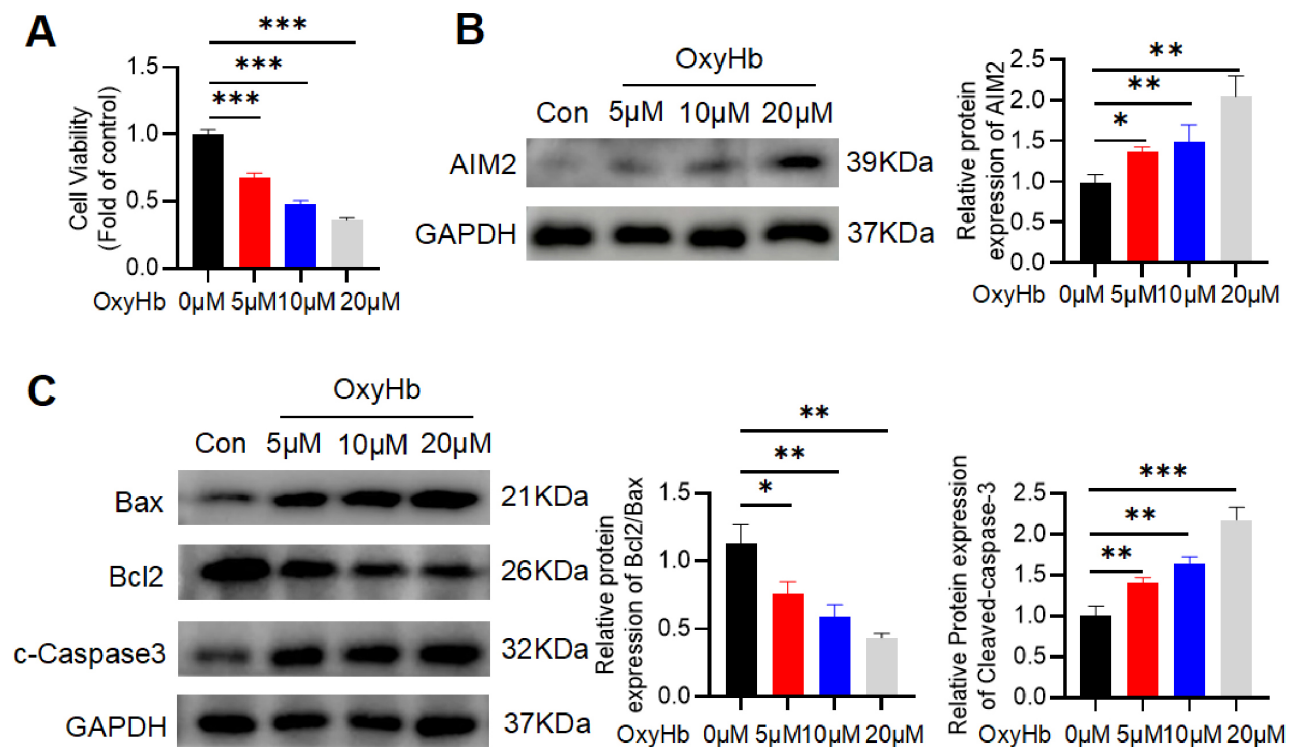


Fig. 2. AIM2 expression is upregulated by OxyHb stimulation and promotes neuronal apoptosis. (A) Cell Counting Kit-8 (CCK-8) assay showing reduced cell viability in response to increasing concentrations of oxyhemoglobin (OxyHb); (B) Western blotting and quantification demonstrating that AIM2 protein expression increases in a dose-dependent manner following OxyHb exposure; (C) Western blotting analysis of apoptosis-related proteins (Bax, Bcl2, and cleaved caspase-3) and corresponding quantification, showing enhanced pro-apoptotic signaling with higher OxyHb concentrations. Data are expressed as mean $\pm$ standard error of the mean (SEM); Data are derived from three independent biological replicates ($n = 3$). Statistical differences among multiple groups were analyzed by one-way ANOVA followed by Tukey's post hoc test.; * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

AIM2 knockdown significantly decreased p65 phosphorylation, whereas co-silencing of *Rela* further inhibited p-p65 expression without altering AIM2 levels (Fig. 5A). This indicates that AIM2 functions upstream of NF- κ B signaling.

As shown in Fig. 5B, *Aim2* knockdown restored the Bcl2/Bax ratio and reduced cleaved caspase-3 expression, while additional suppression of p65 produced a more pronounced reduction in pro-apoptotic signaling. Consistently, cell viability assays demonstrated that simultaneous inhibition of AIM2 and p65 markedly improved neuronal survival compared with *Aim2* knockdown alone (Fig. 5D).

Moreover, TUNEL staining confirmed that co-silencing *Aim2* and *Rela* further reduced neuronal apoptosis relative to *Aim2* silencing alone (Fig. 5C). Collectively, these results demonstrate that AIM2 aggravates OxyHb-induced neuronal apoptosis primarily through activation of the NF- κ B signaling pathway, and inhibition of NF- κ B effectively counteracts AIM2-mediated neurotoxicity.

3.5 AIM2 Expression Is Positively Associated With NF- κ B Activation in SAH

To validate the *in vitro* findings *in vivo*, brain tissues were collected from the peri-hemorrhagic cortical region of SAH mice and the corresponding cortical region of Sham controls. Nissl staining revealed a significant reduction in neuronal cell density in the cortex of SAH mice compared with the Sham group (Fig. 6A), indicating substantial neuronal injury following SAH. Consistently, Western blotting analysis demonstrated that AIM2 expression was markedly increased in the cortical tissue of SAH mice, accompanied by elevated phosphorylation of p65, suggesting activation of the NF- κ B pathway (Fig. 6B). Furthermore, correlation analysis demonstrated a significant positive association between AIM2 expression and NF- κ B activation (p-p65/p65) in SAH tissues (Fig. 6C), further supporting that AIM2 regulates NF- κ B signaling *in vivo*.

4. Discussion

In this study, we demonstrated that AIM2 is markedly upregulated after SAH and is associated with activation of the NF- κ B signaling pathway. Through transcriptomic

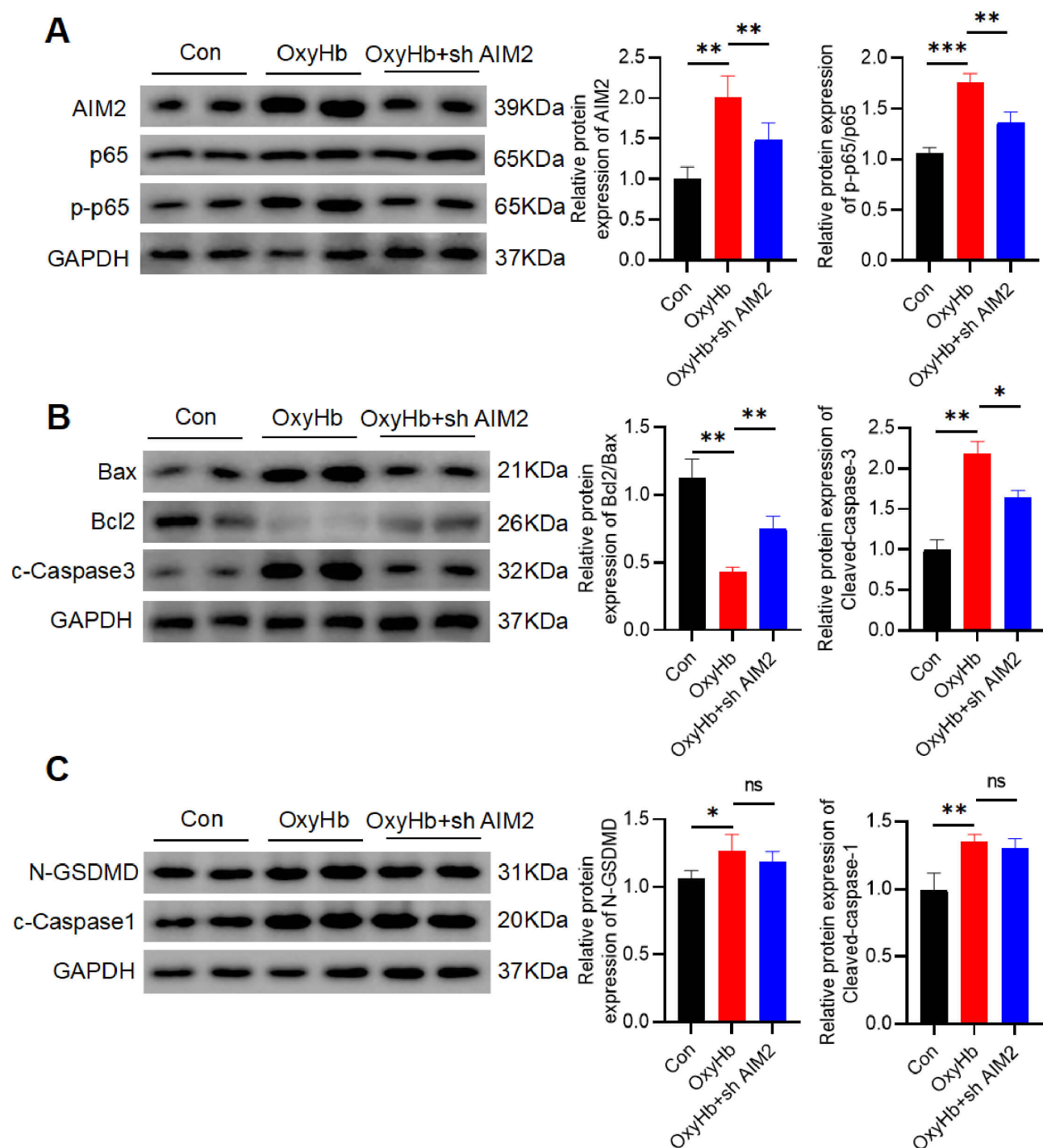


Fig. 3. *Aim2* knockdown suppresses NF- κ B activation and apoptosis and is associated with pyroptosis-related changes in OxyHb-treated neurons. (A) Western blotting and quantification showing that *Aim2* silencing reduces p65 phosphorylation (p-p65/p65) in OxyHb-treated cells, indicating inhibition of NF- κ B activation; (B) Western blot analysis of apoptosis-related proteins (Bax, Bcl2, and cleaved caspase-3) demonstrating that *Aim2* knockdown reverses OxyHb-induced pro-apoptotic signaling; (C) Western blotting analysis of pyroptosis-related proteins (N-GSDMD and cleaved caspase-1). Data are expressed as mean $\pm$ SEM; Data are derived from three independent biological replicates ($n = 3$). Statistical differences among multiple groups were analyzed by one-way ANOVA followed by Tukey's post hoc test; ns, not significant; * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

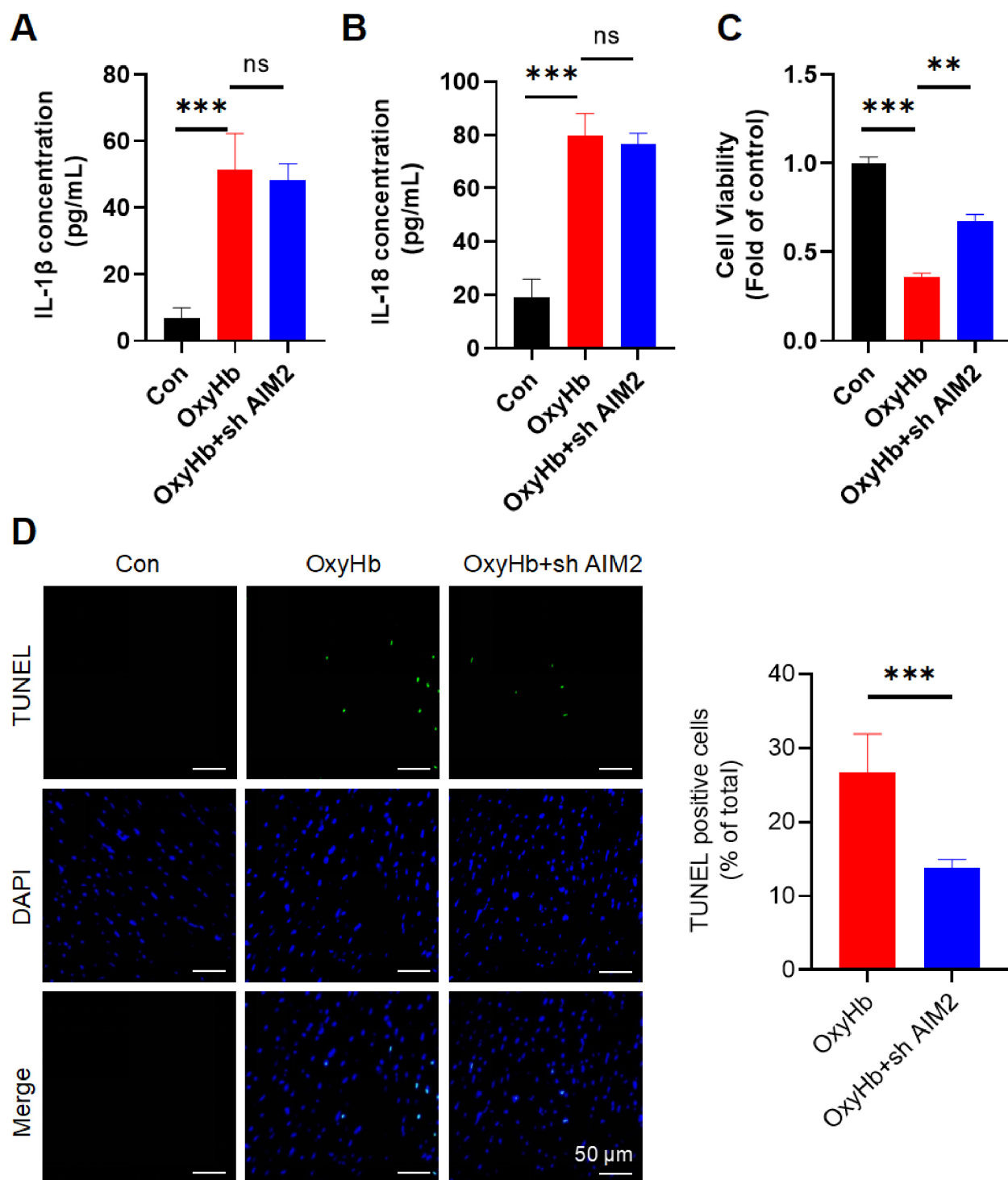


Fig. 4. *Aim2* knockdown reduces OxyHb-induced neuronal apoptosis and improves cell viability. (A,B) Enzyme-linked immunosorbent assay (ELISA) analysis of IL-1 β and IL-18 levels in control and OxyHb-treated neurons with or without *Aim2* knockdown; (C) Cell viability was assessed using CCK-8 assay; (D) Terminal deoxynucleotidyl transferase dUTP nick-end labeling (TUNEL) staining images and quantification demonstrating that *Aim2* knockdown reduces OxyHb-induced neuronal apoptosis, Scale bar = 50 μ m. Data are expressed as mean $\pm$ SEM; Data are derived from three independent biological replicates (n = 3). Statistical differences among multiple groups were analyzed by one-way ANOVA followed by Tukey's post hoc test; ns, not significant; ** $p < 0.01$, *** $p < 0.001$.

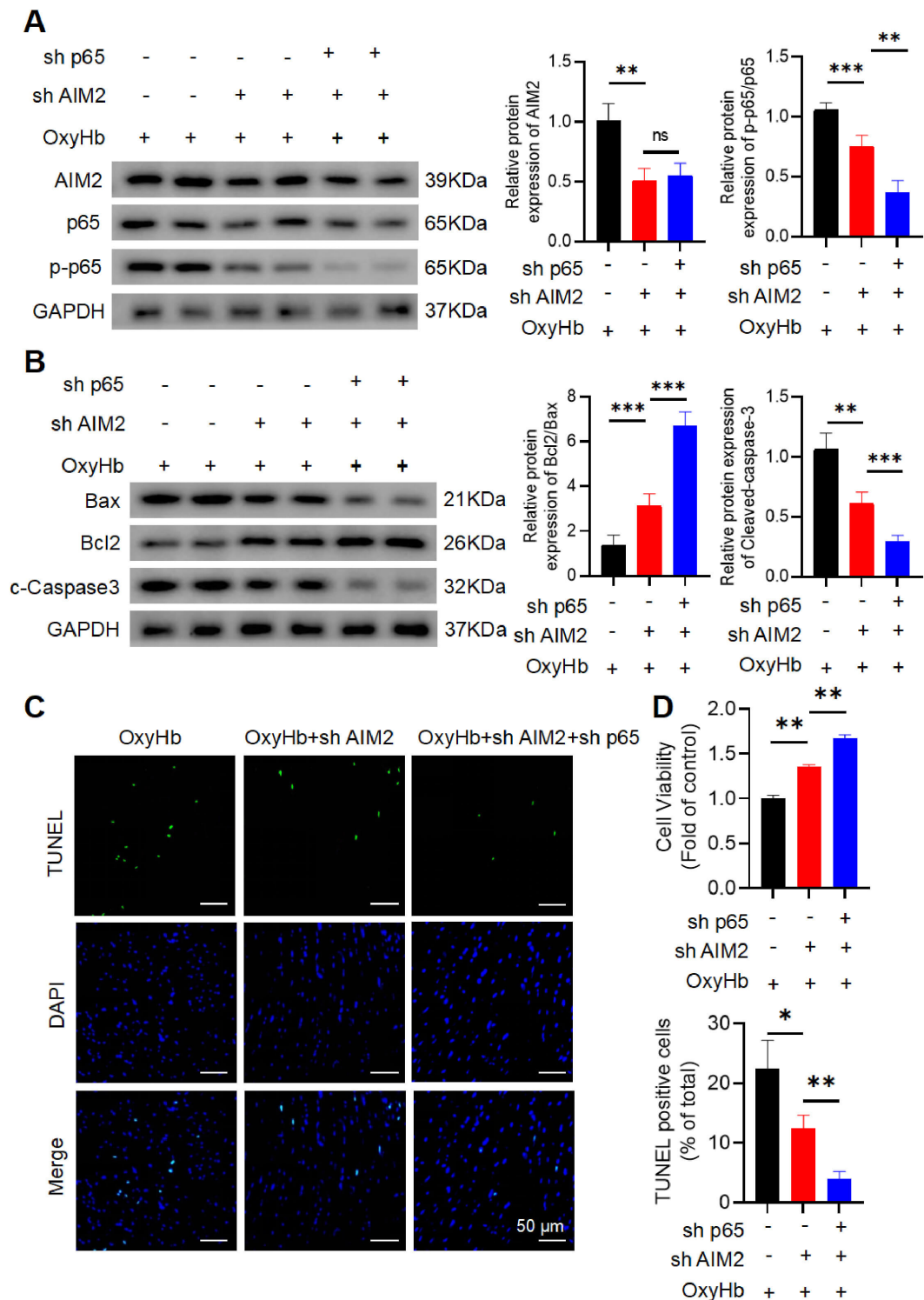


Fig. 5. AIM2 promotes OxyHb-induced neuronal apoptosis through NF- κ B signaling. (A) Western blotting and quantification showing that *Aim2* knockdown reduces phosphorylation of p65, while co-silencing of *Aim2* and *Rela* further reduces NF- κ B activation compared with *Aim2* knockdown alone in OxyHb-treated cells; (B) Western blotting analysis of apoptosis-related proteins (Bax, Bcl2, and cleaved caspase-3) indicating that inhibition of p65 mitigates AIM2-mediated pro-apoptotic effects under OxyHb stimulation; (C) TUNEL staining and quantification demonstrating that co-silencing *Aim2* and *Rela* further reduces OxyHb-induced apoptosis. Green fluorescence represents TUNEL-positive cells, and nuclei are stained with DAPI (blue). Scale bar = 50 μ m; (D) CCK-8 assay showing that combined *Aim2* and *Rela* silencing enhances cell viability compared with *Aim2* knockdown alone. Data are expressed as mean $\pm$ SEM; Data are derived from three independent biological replicates (n = 3). Statistical differences among multiple groups were analyzed by one-way ANOVA followed by Tukey's post hoc test; ns, not significant; * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

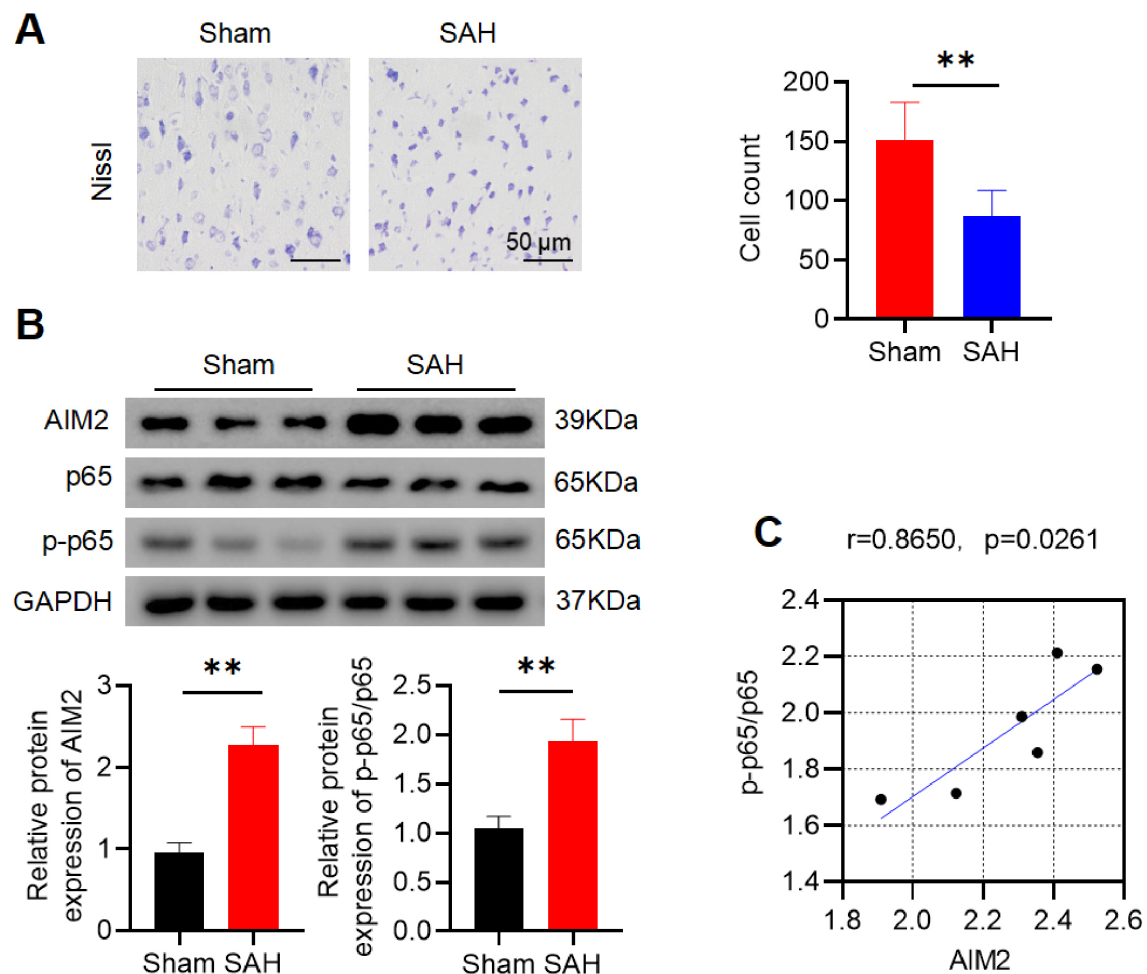


Fig. 6. AIM2 is upregulated in subarachnoid hemorrhage (SAH) brain tissue and correlates with NF- κ B activation. (A) Representative Nissl staining images and quantification of neuronal cell count in Sham and SAH groups, Scale bar = 50 μ m; (B) Western blotting analysis and quantification of AIM2, p65, and p-p65 in brain tissues from Sham and SAH mice; (C) Correlation analysis between AIM2 expression and NF- κ B activation (p-p65/p65) in SAH brain tissues. Data are expressed as mean $\pm$ SEM; $n = 6$ mice per group. Differences between the Sham and SAH groups were analyzed using an unpaired two-tailed Student's t -test; ** $p < 0.01$.

analyses, OxyHb-induced neuronal injury models, and *Aim2* knockdown experiments, we found that suppression of AIM2 significantly reduced p65 phosphorylation, restored the Bax/Bcl2 balance, alleviated neuronal apoptosis, and improved cell viability. Co-silencing of *Aim2* and *Rela* further enhanced neuroprotective effects, supporting the involvement of NF- κ B signaling in AIM2-mediated neuronal injury. In contrast, *Aim2* knockdown exerted limited effects on pyroptosis-related responses. Moreover, *in vivo* analyses confirmed a positive association between AIM2 expression and NF- κ B activation in SAH brain tissues. Collectively, these findings suggest that AIM2 contributes to early brain injury after SAH, at least in part, through NF- κ B signaling.

SAH remains a devastating form of hemorrhagic stroke with very high mortality and disability rates. Even with substantial advances in microsurgical clipping, en-

dovascular coiling, and neurocritical care, there is still no established pharmacological therapy to prevent secondary neuronal injury after SAH [15,16]. Early brain injury (EBI) developing within the first 72 hours post-SAH is now recognized as a critical determinant of patient outcome, with neuronal apoptosis and blood-brain barrier disruption being pivotal contributors to this phase [17]. Standard clinical care, such as nimodipine administration and blood pressure optimization, primarily targets vasospasm and maintains cerebral perfusion, but does not effectively address the molecular cascades of neuronal death [18]. Therefore, investigating novel molecular regulators of EBI is imperative for improving neurological outcomes.

AIM2, a cytosolic double-stranded DNA sensor belonging to the AIM2-like receptor family, exerts its canonical function by assembling inflammasome complexes and activating caspase-1-dependent pyroptosis. Recent studies

have suggested that AIM2 also participates in ischemic and hemorrhagic brain injury, indicating that its role may extend beyond classical immune activation [11,13,19]. For instance, Yuan et al. [13] demonstrated that inhibition of AIM2 inflammasome activation alleviated gasdermin D-mediated pyroptosis and improved neurological outcomes after SAH. Similarly, Zhang et al. [19] and Zheng et al. [11] reported that AIM2 contributes to early brain injury after SAH through neuronal inflammasome activation, whereas pharmacological or genetic inhibition of AIM2 exerts neuroprotective effects. Nevertheless, whether AIM2 also contributes to neuronal apoptosis beyond its canonical inflammasome-related function remains unclear. In the present study, *Aim2* knockdown reduced apoptotic markers, restored the Bcl2/Bax balance, and improved neuronal viability under OxyHb stimulation. In contrast, *Aim2* silencing exerted only limited effects on pyroptosis-related responses, suggesting that AIM2 predominantly contributes to neuronal injury through apoptotic signaling under the present experimental conditions.

NF- κ B is a key transcription factor involved in the regulation of inflammation, oxidative stress, and apoptosis. Upon activation, phosphorylated p65 translocates into the nucleus and promotes the transcription of pro-apoptotic and pro-inflammatory genes [20]. Previous studies have demonstrated that NF- κ B activation occurs in neurons and glial cells following SAH and contributes to neuronal death and neurological dysfunction [21,22]. In the present study, transcriptomic correlation analysis revealed a strong positive association between *AIM2* expression and NF- κ B signaling activity, suggesting a functional association between these pathways. *In vitro*, *Aim2* knockdown markedly reduced p65 phosphorylation and pro-apoptotic protein expression, whereas co-silencing *Aim2* and *Rela* further enhanced neuroprotective effects compared with AIM2 inhibition alone. Moreover, *in vivo* analyses demonstrated a positive association between AIM2 expression and NF- κ B activation in SAH brain tissues. Together, these findings support the involvement of AIM2-associated NF- κ B signaling in neuronal apoptosis following SAH.

Although this study provides evidence that AIM2 contributes to neuronal apoptosis through NF- κ B signaling after SAH, several limitations should be acknowledged. First, although *in vivo* validation was performed, the mechanistic experiments were mainly based on an OxyHb-induced neuronal injury model, which cannot fully recapitulate the complex pathological environment after SAH. Second, while *Aim2* knockdown and *Rela* co-silencing supported the involvement of NF- κ B signaling, the precise molecular mechanisms linking AIM2 to NF- κ B activation require further investigation. Finally, the role of AIM2 in inflammasome-associated responses after SAH and the temporal dynamics of AIM2/NF- κ B activation warrant further exploration in future studies.

5. Conclusions

In summary, this study demonstrates that AIM2 is up-regulated after SAH and contributes to neuronal apoptosis, at least in part, through NF- κ B signaling. AIM2 suppression attenuated neuronal apoptosis, improved cell viability, and was associated with reduced NF- κ B activation *in vitro*, while *in vivo* analyses further supported a positive association between AIM2 expression and NF- κ B activation after SAH. These findings provide new insight into the molecular mechanisms underlying early brain injury after SAH and suggest that the AIM2-NF- κ B axis may represent a promising pathway for future therapeutic investigation.

Availability of Data and Materials

The data generated and analyzed during the current study are available from the corresponding author on reasonable request. The publicly available datasets analyzed in this study are available in the Gene Expression Omnibus (GEO) database under accession numbers GSE36791 and GSE73378.

Author Contributions

HLJ contributed to data collection and literature review, and wrote the first draft of the manuscript. TM, YC, ZJ and TY contributed to data collection, and performed statistical analysis. HHJ contributed to conception and design of the study, funding acquisition, and revision of the manuscript. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

All animal procedures were performed in accordance with the ethical standards approved by the Animal Research Ethics Committee of Wenzhou Medical University (Approval No. wyd-2025-0495) and complied with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and ARRIVE 2.0 guidelines.

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Conflicts of Interest

The authors declare no conflicts of interest.

Supplementary Material

Supplementary material associated with this article can be found, in the online version, at <https://doi.org/10.31083/JIN50383>.

References

- [1] Macdonald RL, Schweizer TA. Spontaneous subarachnoid haemorrhage. *Lancet*. 2017; 389: 655–666. [https://doi.org/10.1016/S0140-6736\(16\)30668-7](https://doi.org/10.1016/S0140-6736(16)30668-7)
- [2] Lawton MT, Vates GE. Subarachnoid Hemorrhage. *The New England Journal of Medicine*. 2017; 377: 257–266. <https://doi.org/10.1056/NEJMc1605827>
- [3] Cahill J, Zhang JH. Subarachnoid hemorrhage: is it time for a new direction? *Stroke*. 2009; 40: S86–S87. <https://doi.org/10.1161/STROKEAHA.108.533315>
- [4] Sehba FA, Hou J, Pluta RM, Zhang JH. The importance of early brain injury after subarachnoid hemorrhage. *Progress in Neurobiology*. 2012; 97: 14–37. <https://doi.org/10.1016/j.pneurobio.2012.02.003>
- [5] Yang Y, Chen S, Zhang JM. The Updated Role of Oxidative Stress in Subarachnoid Hemorrhage. *Current Drug Delivery*. 2017; 14: 832–842. <https://doi.org/10.2174/1567201813666161025115531>
- [6] Chen S, Feng H, Sherchan P, Klebe D, Zhao G, Sun X, et al. Controversies and evolving new mechanisms in subarachnoid hemorrhage. *Progress in Neurobiology*. 2014; 115: 64–91. <https://doi.org/10.1016/j.pneurobio.2013.09.002>
- [7] Amarante-Mendes GP, Adjemian S, Branco LM, Zanetti LC, Weinlich R, Bortoluci KR. Pattern Recognition Receptors and the Host Cell Death Molecular Machinery. *Frontiers in Immunology*. 2018; 9: 2379. <https://doi.org/10.3389/fimmu.2018.02379>
- [8] Voet S, Srinivasan S, Lamkanfi M, van Loo G. Inflammasomes in neuroinflammatory and neurodegenerative diseases. *EMBO Molecular Medicine*. 2019; 11: e10248. <https://doi.org/10.15252/emmm.201810248>
- [9] Rathinam VAK, Fitzgerald KA. Inflammasome Complexes: Emerging Mechanisms and Effector Functions. *Cell*. 2016; 165: 792–800. <https://doi.org/10.1016/j.cell.2016.03.046>
- [10] Kumari P, Russo AJ, Shivcharan S, Rathinam VA, et al. AIM2 in health and disease: Inflammasome and beyond. *Immunological Reviews*. 2020; 297: 83–95. <https://doi.org/10.1111/imr.12903>
- [11] Zheng Y, Tang W, Zeng H, Peng Y, Yu X, Yan F, et al. Probenecid-Blocked Pannexin-1 Channel Protects Against Early Brain Injury via Inhibiting Neuronal AIM2 Inflammasome Activation After Subarachnoid Hemorrhage. *Frontiers in Neurology*. 2022; 13: 854671. <https://doi.org/10.3389/fneur.2022.854671>
- [12] Denes A, Coutts G, Lénárt N, Cruickshank SM, Pelegrin P, Skinner J, et al. AIM2 and NLR4 inflammasomes contribute with ASC to acute brain injury independently of NLRP3. *Proceedings of the National Academy of Sciences of the United States of America*. 2015; 112: 4050–4055. <https://doi.org/10.1073/pnas.1419090112>
- [13] Yuan B, Zhou XM, You ZQ, Xu WD, Fan JM, Chen SJ, et al. Inhibition of AIM2 inflammasome activation alleviates GSDMD-induced pyroptosis in early brain injury after subarachnoid haemorrhage. *Cell Death & Disease*. 2020; 11: 76. <https://doi.org/10.1038/s41419-020-2248-z>
- [14] Xu P, Tao C, Zhu Y, Wang G, Kong L, Li W, et al. TAK1 mediates neuronal pyroptosis in early brain injury after subarachnoid hemorrhage. *Journal of Neuroinflammation*. 2021; 18: 188. <https://doi.org/10.1186/s12974-021-02226-8>
- [15] Rabinstein AA, Lanzino G, Wijdicks EF. Multidisciplinary management and emerging therapeutic strategies in aneurysmal subarachnoid haemorrhage. *The Lancet. Neurology*. 2010; 9: 504–519. [https://doi.org/10.1016/S1474-4422\(10\)70087-9](https://doi.org/10.1016/S1474-4422(10)70087-9)
- [16] Thilak S, Brown P, Whitehouse T, Gautam N, Lawrence E, Ahmed Z, et al. Diagnosis and management of subarachnoid haemorrhage. *Nature Communications*. 2024; 15: 1850. <https://doi.org/10.1038/s41467-024-46015-2>
- [17] Fujii M, Yan J, Rolland WB, Soejima Y, Caner B, Zhang JH. Early brain injury, an evolving frontier in subarachnoid hemorrhage research. *Translational Stroke Research*. 2013; 4: 432–446. <https://doi.org/10.1007/s12975-013-0257-2>
- [18] Sanicola HW, Stewart CE, Luther P, Yabut K, Guthikonda B, Jordan JD, et al. Pathophysiology, Management, and Therapeutics in Subarachnoid Hemorrhage and Delayed Cerebral Ischemia: An Overview. *Pathophysiology*. 2023; 30: 420–442. <https://doi.org/10.3390/pathophysiology30030032>
- [19] Zhang X, Xu C, Liu ZY, Zhang DY, Wang BH, Wang J, et al. The Inflammasome: A Promising Potential Therapeutic Target for Early Brain Injury Following Subarachnoid Hemorrhage. *Frontiers in Bioscience (Landmark Edition)*. 2025; 30: 33454. <https://doi.org/10.31083/FBL33454>
- [20] Geeviman K, Babu D, Prakash Babu P. Pantoprazole Induces Mitochondrial Apoptosis and Attenuates NF-κB Signaling in Glioma Cells. *Cellular and Molecular Neurobiology*. 2018; 38: 1491–1504. <https://doi.org/10.1007/s10571-018-0623-4>
- [21] Tian Y, Liu B, Li Y, Zhang Y, Shao J, Wu P, et al. Activation of RARα Receptor Attenuates Neuroinflammation After SAH via Promoting M1-to-M2 Phenotypic Polarization of Microglia and Regulating Mafk/Msr1/PI3K-Akt/NF-κB Pathway. *Frontiers in Immunology*. 2022; 13: 839796. <https://doi.org/10.3389/fimmu.2022.839796>
- [22] Shao J, Meng Y, Yuan K, Wu Q, Zhu S, Li Y, et al. RU.521 mitigates subarachnoid hemorrhage-induced brain injury via regulating microglial polarization and neuroinflammation mediated by the cGAS/STING/NF-κB pathway. *Cell Communication and Signaling*. 2023; 21: 264. <https://doi.org/10.1186/s12964-023-01274-2>