Research Square

Preprints are preliminary reports that have not undergone peer review.
They should not be considered conclusive, used to inform clinical practice,
or referenced by the media as validated information.

EGR1-associated inflammatory and neurovascular mechanisms linking migraine with ischemic stroke risk

Wenzheng Rong
Zhengzhou University
Jing Xu
Zhengzhou University
Bo Li
Zhengzhou University
Xiaofeng Zhang
Zhengzhou University
Yapeng Li
Zhengzhou University
Bo Song
Zhengzhou University
Yuming Xu
xuyuming@zzu.edu.cn
Zhengzhou University

Research Article

Keywords: Ischemic stroke, Migraine, EGR1, Neuroinflammation

Posted Date: April 13th, 2026

DOI: https://doi.org/10.21203/rs.3.rs-9244440/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License.
Read Full License

Additional Declarations: No competing interests reported.

Version of Record: A version of this preprint was published at The Journal of Headache and Pain on July 11th, 2026. See the published version at https://doi.org/10.1186/s10194-026-02447-3.

Abstract

Background

Migraine is associated with an increased risk of ischemic stroke, but the molecular mechanisms linking these two disorders remain unclear.

Methods

We performed an integrated analysis of bulk RNA-seq data from ischemic stroke and single-cell RNA-seq data from a mouse migraine model. Differential expression, Gene Ontology enrichment, cell-cell communication, protein-protein interaction, disease association, and drug-gene interaction analyses were conducted to identify shared molecular signatures and pathways. A nitroglycerin-induced migraine mouse model was further used to validate neurovascular alterations in vivo.

Results

Integrated transcriptomic analysis identified shared upregulated genes between migraine and ischemic stroke, with IL1B and EGR1 emerging as key candidates. In ischemic stroke, enriched pathways were mainly related to immune and inflammatory responses, particularly immune response-regulating cell surface receptor signaling and interleukin-1-mediated signaling, with IL1B and EGR1 serving as central nodes. Single-cell analysis showed that EGR1 was the only significantly shared upregulated gene in migraine, with elevated expression in PEP neurons, NF neurons, vascular cells, and fibroblasts, while the interleukin-1 production pathway was activated in most of these cell types. Cell-cell communication analysis revealed enhanced interactions among neuronal, vascular, and fibroblast populations, especially through ANGPTL signaling. Hub network analysis identified EGR1, IL1B, TLR4, and ANGPTL2 as core molecules. In vivo, the migraine model showed increased neuronal activation, persistent mechanical hypersensitivity, and reduced ZO-1 expression in the trigeminocervical complex, indicating vascular tight junction impairment.

Conclusion

These findings suggest that EGR1-associated interleukin-1 inflammatory signaling and enhanced neurovascular-fibroblast communication may link migraine to increased ischemic stroke risk. This study highlights a potential neuroinflammatory and vascular mechanism underlying stroke susceptibility in migraine and suggests candidate therapeutic targets.

Introduction

Migraine is a complex brain disorder characterized by recurrent headaches accompanied by various neurological and vascular symptoms. Patients typically experience photophobia, phonophobia, nausea, vomiting, as well as cognitive and emotional disturbances. Although traditionally regarded as a "benign" functional disorder, long-term epidemiological evidence and clinical observations increasingly indicate

that there is a meaningful and persistent association between migraine and cerebrovascular events, especially in patients with migraine with aura [1][2, 3]. This association cannot be attributed to a single risk factor. Instead, it reflects a multi-level imbalance within the neurovascular unit [4]. Abnormal activation of the trigeminovascular system [5], impaired endothelial function, enhanced platelet reactivity, low but persistent immune activation, and accumulated oxidative stress are all considered to be the causes of this phenomenon. Understanding how these molecular and cellular dysfunctions interact with each other to link migraine to vascular fragility has become a key issue in the interdisciplinary field of neurology and vascular biology.

Chronic inflammation and endothelial dysfunction have been identified as the key mechanism link between migraine and increased risk of cerebrovascular events [6, 7]. Under normal circumstances, endothelial cells maintain the homeostasis of cerebral microcirculation by regulating the bioavailability of nitric oxide, vascular tension, the expression of adhesion molecules, and the balance between coagulation and fibrinolysis [8]. When inflammatory mediators and reactive oxygen species persistently increase chronically, endothelial cells will switch to an activated state, characterized by increased permeability, enhanced expression of adhesion molecules, and a pro-thrombotic tendency [7, 9]. Immune cells play a dual role in this process. Cytokines released by monocytes/macrophages, T lymphocytes, and natural killer cells can sensitize trigeminal nerve endings and promote vasogenic pain [10]. At the same time, immune-endothelial interactions mediated through ligand-receptor signaling pathways can alter the behavior of endothelial cells, promote microthrombosis, and promote uneven perfusion [11]. Previous transcriptomic analyses have reported that inflammatory and coagulation-related genes in the peripheral blood of migraine patients have been upregulated; however, these findings mostly come from overall-level data and lack cellular-level analysis or multi-layer validation across different independent datasets.

Most previous studies relied on a single data layer, usually based on data from a peripheral blood microarray cohort, which made it difficult to rule out the influence of cohort-specific factors and platform-related variability [12]. Secondly, evidence at the cellular resolution level is relatively limited [13], which leaves the question of which immune cell populations drive these interrelated pathways unanswered. Thirdly, endothelial dysfunction are rarely studied jointly within the same analytical framework, and there is a lack of systematic integration of population-level transcriptomics, single-cell analysis, pathway and cell communication analysis, as well as cross-dataset validation research [14]. Constructing a stepwise evidence chain from overall differential expression, cell type localization, cell-to-cell communication, and cross-dataset pathway convergence will help strengthen mechanism inference and better meet the clinical need for actionable and cell-specific therapeutic targets.

In the present study, we integrated bulk RNA-seq data from ischemic stroke with single-cell RNA-seq data from a mouse migraine model to identify shared genes, pathways, and cellular interaction networks. We further combined bioinformatic analyses with in vivo validation in a nitroglycerin-induced migraine model to test whether migraine is associated with vascular barrier disruption. Using this strategy, we

sought to define the inflammatory and neurovascular mechanisms linking migraine to ischemic stroke risk, with particular attention to EGR1- and IL1-related signaling.

Methods

Animals

Male C57BL/6J mice, aged 10 weeks, were acquired from the Experimental Animal Center of the First Affiliated Hospital of Zhengzhou University. The animals were housed in specific pathogen-free (SPF) conditions at a controlled temperature of 22 ± 2°C, with a 12-hour light/dark cycle and ad libitum access to standard chow and water. After a one-week acclimatization period, 12 mice were randomly assigned to experimental and control groups based on a sequence generated using R (version 4.0.2). The study protocol was reviewed and approved by the Animal Ethics Committee of the First Affiliated Hospital of Zhengzhou University (approval number ZZU-LAC2025042202), and all experimental procedures followed the ethical guidelines set forth by the International Association for the Study of Pain [15].

NTG-induced model preparation

A chronic migraine model induced by NTG was established using slightly modified protocols from previously validated methods [16, 17]. The NTG stock solution (5 mg/mL) was prepared in a vehicle composed of 30% ethanol, 30% propylene glycol, and 40% distilled water. Before administration, the stock solution was diluted with sterile 0.9% saline to achieve a final concentration of 1 mg/mL. Mice in the model group were administered intraperitoneal injections of NTG at a dose of 10 mg/kg, while control animals received an equivalent volume of saline. The injections were given every two days for a total of five sessions over a 9-day period to establish a stable migraine-like hypersensitivity phenotype.

Behavioral assessment

Mechanical nociceptive thresholds were assessed using von Frey filaments with calibrated bending forces ranging from 0.008 to 2 g, following the up-down method described previously [18]. Two areas were tested: the periorbital region and the hind paw. The filament was applied perpendicularly to the skin for approximately 3 seconds, starting with a 0.16 g filament. A positive response was characterized by an immediate head withdrawal in the periorbital region or a quick paw flick/withdrawal in the hind paw. If no response occurred, the next stronger filament was used, and if a response was elicited, a weaker filament was applied. To prevent sensitization, a 3-minute interval was maintained between consecutive stimulations.

Behavioral measurements were taken 2 hours before and 2 hours after each injection to monitor both immediate and delayed nociceptive responses. All assessments were conducted under double-blind

conditions, with experimenters and data analysts unaware of group assignments throughout data collection and analysis.

Tissue Perfusion and Cryosection Preparation

Following deep anesthesia, mice underwent transcardial perfusion with pre-chilled 0.9% saline, followed by 4% paraformaldehyde (PFA) in phosphate-buffered saline (PBS, pH 7.4). The brains were carefully extracted and post-fixed in 4% PFA at 4°C for 12–16 hours. The segment of the medulla containing the trigeminal nucleus caudalis (TNC) was isolated and cryoprotected sequentially in 20% and 30% sucrose solutions in PBS until the tissue sank. Once dehydrated, the samples were embedded and frozen for sectioning. Coronal cryosections (10 μm thick) were made using a cryostat microtome (Leica, Japan) at low temperature, and the sections were mounted onto adhesive microscope slides for subsequent immunofluorescence analysis.

Immunofluorescence Staining and Imaging

Cryosections were allowed to equilibrate to room temperature and were washed gently with phosphate-buffered saline (PBS). To minimize nonspecific antibody binding, the sections were preincubated with 5% bovine serum albumin (BSA) for 1 hour at room temperature. Following this, primary antibodies diluted 1:500 in PBS containing 1% BSA were applied and incubated overnight at 4°C. After thorough washing with PBS, fluorophore-conjugated secondary antibodies were added and incubated for 2 hours at room temperature in the dark. Nuclei were counterstained with 4',6-diamidino-2-phenylindole (DAPI) for 10 minutes.

Once staining was complete, the slides were mounted with an antifade reagent and examined using a confocal laser scanning microscope (Zeiss 980, Germany). Image acquisition and quantitative analysis of fluorescence intensity and positive signal area were performed using ImageJ software (version 2.3.0, NIH, USA), ensuring that the exposure parameters were consistent across all samples for reliable comparison.

Data Collection

Publicly available peripheral blood bulk and single-cell RNA sequencing datasets were utilized for this analysis. Batch bulk data related to cerebrovascular diseases were obtained from the GEO database (GSE22255 and GSE58294), both of which include clinical cases as well as matched healthy controls from peripheral blood samples. The differentially expressed genes in the peripheral blood RNA-seq of migraine patients were sourced from the study by Tiago Krug et al. [19]. For the mouse single-cell data, it was obtained from the research by Lite Yang et al., available in the NCBI GEO database under accession number GSE197289 [20]. All datasets were analyzed in compliance with public data access and privacy regulations.

Data preprocessing and differential expression analysis

Raw microarray probe signals were mapped to standard human gene symbols using the platform annotation file. For genes with multiple probes, the median probe value was taken to obtain a unique expression value. Each dataset underwent independent background correction, normalization, and quality control, with outlier samples removed prior to integration.

To remove technical batch effects, we applied an empirical Bayes-based batch correction method, ensuring expression distributions and clustering patterns were primarily driven by biological rather than technical variation. Differential expression analysis was conducted using the limma package's empirical Bayes moderated linear model, followed by multiple testing correction. Significant genes were defined as those with |Log2 fold change| > Log2(1.5) and adjusted P-value < 0.05.

Single-cell data analysis

The single-cell data were processed using Seurat, including quality control, normalization, dimension reduction and clustering [21]. Low-quality cells were removed, and the dataset was integrated through anchor-based alignment to correct batch differences. Cell maps were constructed through principal component analysis and nonlinear embedding. Cell subpopulations were annotated based on standard immune cell marker genes, and the annotations were confirmed and refined with the help of SingleR and a reference peripheral blood mononuclear cell dataset. Then, the expression patterns of pathways and key genes related to migraine identified from the batch analysis were examined in immune subpopulations to determine the cellular sources and activation characteristics of peripheral immune signals in migraine.

Functional Enrichment

Functional enrichment analysis of differentially expressed genes (DEGs) was performed using the clusterProfiler package (version 4.16.0) in R [22]. Gene Ontology (GO) enrichment analysis was conducted to categorize genes into biological process, molecular function, and cellular component terms (http://www.geneontology.org/) [23]. GO term overrepresentation was evaluated using the Wallenius non-central hypergeometric distribution, as implemented in the GOseq framework. To further investigate the enrichment of core gene sets in disease, the DisGeNET and GWAS Catalog entries were enriched using the Enrichr platform (https://maayanlab.cloud/Enrichr/) [24].

Cell communication analysis

CellChat is used to construct ligand-receptor interaction networks [25]. Based on the transcriptional expression levels of ligands and receptors, this model infers the signal strength and pathway activity between cell subpopulations, and determines the main signaling axes as well as the changes in the sending and receiving capabilities of specific cell populations. We focus on the immune communication

networks related to inflammation, vascular homeostasis, and iron metabolism, and combine these molecular-level findings with cell behavior and intercellular regulatory patterns.

Statistical analysis

All analyses were conducted in the R environment. The key packages used include limma (version 3.64.3), Seurat (version 5.4.0), SingleR (version 2.12.0), CellChat (version 2.1.2), and clusterProfiler (version 4.16.0), which were employed for data processing, differential expression analysis, functional enrichment, and communication modeling. The statistical tests were two-sided tests, and the P-values were corrected for multiple comparisons. Data visualization was carried out using basic plotting functions and ggplot2 (version 4.0.1). The analysis process underwent multiple independent reviews to ensure the reproducibility and stability of the results.

Results

Integrated transcriptomic analysis identifies shared upregulated genes between migraine and ischemic stroke

To investigate the molecular association between migraine and ischemic stroke, we performed an integrated analysis of bulk RNA-seq and single-cell RNA-seq datasets, followed by experimental validation in a migraine mouse model (Fig. 1A). Differential expression analysis of the ischemic stroke RNA-seq dataset revealed a distinct transcriptional profile, as illustrated by the volcano plot (Fig. 1B, Supplemental Table S2). Cross-condition comparison further identified a subset of commonly upregulated genes, including IL1B and EGR1, as shown in the Venn diagram (Fig. 1C, Supplemental Table S2). These findings suggest that migraine and ischemic stroke share overlapping molecular signatures and support the existence of common pathogenic pathways that may contribute to stroke susceptibility in migraine.

Immune-inflammatory pathways and stress-response genes are centrally involved in ischemic stroke

Next, we performed Gene Ontology enrichment analysis to characterize the biological functions of the differentially expressed genes identified in ischemic stroke. The most significantly enriched biological process terms were predominantly related to immune and inflammatory responses, including the immune response-regulating cell surface receptor signaling pathway, interleukin-1-mediated signaling pathway, and cellular response to mechanical stimulus (Fig. 2A). We further visualized the relationships between these pathways and representative genes, including CD177 and IL1B, in a circular plot (Fig. 2B). Network analysis showed that the immune response-regulating cell surface receptor signaling pathway and interleukin-1-mediated signaling pathway occupied central positions in the functional network, with IL1B and EGR1 identified as key hub genes (Fig. 2C). Together, these results support a central role for immune-inflammatory dysregulation and cellular stress responses in ischemic stroke.

Single-cell transcriptomics identifies EGR1 as a shared upregulated gene in migraine-relevant cell populations

Single-cell RNA-seq data from a mouse migraine model were analyzed to define the cellular context of shared molecular signals. UMAP visualization delineated the overall cellular composition and revealed disease-associated shifts across cell populations (Figs. 3A and 3B), while the dot plot identified characteristic marker genes for each cell type (Fig. 3C). Grouped differential expression analysis showed that EGR1 was the only shared gene significantly upregulated in the migraine condition relative to naive controls, with increased expression observed in PEP neurons, NF neurons, vascular cells, and fibroblasts (Fig. 3D). In parallel, pathway enrichment analysis demonstrated activation of the interleukin-1 production pathway in the majority of these cell populations. These observations suggest that EGR1-associated inflammatory activation is broadly distributed across multiple migraine-related cell types and may represent a shared molecular feature linking migraine and ischemic stroke.

Enhanced neurovascular and fibroblast communication characterizes the migraine-associated inflammatory microenvironment

To further explore how these cell populations interact, we examined intercellular communication in the migraine and naive groups. Compared with the naive group, the migraine group showed markedly altered communication patterns among NF neurons, PEP neurons, vascular cells, and fibroblasts, with an overall increase in interaction strength (Fig. 4A). Within this communication network, ANGPTL signaling was notably enhanced, particularly in interactions between NF neurons and immune fibroblasts as well as vascular cells (Fig. 4B). We also constructed a protein-protein interaction network based on the STRING database and identified EGR1, IL1B, TLR4, and ANGPTL2 as core hub molecules (Fig. 4C). These findings support the existence of an intensified neurovascular-fibroblast inflammatory network in migraine that may contribute to stroke-related susceptibility.

Disease enrichment and drug prediction analyses support the translational relevance of the hub gene network

Furthermore, we evaluated the potential clinical relevance of the identified hub genes through disease association, GWAS enrichment, and drug-gene interaction analyses. DisGeNET analysis showed that these genes were significantly enriched in disease categories related to inflammation, arteriosclerosis, and atherosclerosis (Fig. 5A, Supplemental Table S3). Consistently, GWAS Catalog enrichment associated the hub gene set with cytokine network levels, dysmenorrheic pain severity, and cardiovascular risk factors (Fig. 5B, Supplemental Table S4). Drug-gene interaction analysis using the DGIdb database further identified several candidate compounds targeting this network, among which SUNITINIB ranked among the top predicted agents (Fig. 5C). Taken together, these findings indicate that

the hub gene network is closely associated with inflammatory vascular pathology and may provide candidate targets for therapeutic intervention.

Migraine induces neuronal activation and vascular tight junction disruption in vivo

To validate the vascular effects of migraine in vivo, we established a nitroglycerin-induced mouse model. Immunofluorescence analysis of the trigeminocervical complex (TNC) demonstrated a significant increase in both the number of cFos-positive activated neurons and the proportion of $cFos^{+}/NeuN^{+}$ neurons in NTG-treated mice compared with vehicle controls (Figs. 6A and 6B). Behavioral assessment further confirmed successful model induction, as NTG-treated mice exhibited a sustained reduction in periorbital mechanical threshold after treatment (Figs. 6C and 6D). Importantly, expression of the vascular tight junction marker ZO-1 was markedly reduced in the TNC of the migraine model group (Figs. 6E and 6F). These results indicate that migraine induces both neuronal activation and disruption of vascular endothelial tight junction integrity, providing experimental evidence for a neurovascular mechanism that may underlie the increased risk of ischemic stroke in migraine.

Discussion

Our study demonstrates that EGR1-associated interleukin-1 inflammatory signaling and enhanced neurovascular–fibroblast communication constitute a shared molecular framework linking migraine to increased susceptibility to ischemic stroke. These findings suggest that convergent inflammatory and vascular mechanisms may underlie the epidemiological association between these two disorders.

The present results contribute to resolving the long-standing question of how migraine predisposes individuals to ischemic stroke by identifying shared transcriptional signatures and cellular mechanisms. First, our integrated transcriptomic analysis revealed that IL1B and EGR1 are commonly upregulated in both ischemic stroke and migraine, indicating a conserved inflammatory axis (Fig. 1B and 1C). Previous studies have established IL1B as a key mediator of neuroinflammation and ischemic injury, promoting leukocyte recruitment, blood–brain barrier disruption, and neuronal damage [26, 27]. Similarly, EGR1 has been implicated as an immediate early response gene that regulates stress-induced transcriptional programs in vascular and neuronal cells [28]. Our findings extend these observations by demonstrating that these molecules are not only involved in stroke pathology but are also activated in migraine, supporting a shared pathogenic pathway.

Second, our Gene Ontology enrichment analysis highlighted immune response-regulating receptor signaling and interleukin-1-mediated signaling as central pathways in ischemic stroke, with IL1B and EGR1 acting as hub genes (Fig. 2). These results are consistent with prior reports emphasizing the critical role of innate immune activation in stroke progression [29–31]. However, our study provides new insight by linking these pathways to migraine-related molecular changes, suggesting that repeated inflammatory activation during migraine attacks may prime the neurovascular unit for ischemic

vulnerability. This aligns with emerging theories proposing that chronic neuroinflammation contributes to vascular dysfunction and stroke risk [32–34].

Third, single-cell RNA sequencing revealed that EGR1 is the only significantly shared upregulated gene across migraine-relevant cell populations, including neurons, vascular cells, and fibroblasts. Importantly, the interleukin-1 production pathway was activated in most of these cell types, indicating a multicellular inflammatory response (Fig. 3D). Previous single-cell studies have shown cell-type-specific inflammatory signatures in neurological disorders [35], but our findings uniquely demonstrate a coordinated EGR1-driven inflammatory program spanning neuronal and vascular compartments in migraine. This broad cellular distribution suggests that EGR1 may function as a central regulator of neurovascular inflammation, bridging neuronal activation and vascular responses.

In addition, our cell–cell communication analysis identified enhanced interactions among neurons, vascular cells, and fibroblasts, particularly through ANGPTL signaling (Fig. 4). ANGPTL family proteins have been implicated in angiogenesis, vascular permeability, and inflammation [36–38]. The observed increase in ANGPTL-mediated signaling suggests that migraine induces a pro-inflammatory and pro-angiogenic microenvironment that may compromise vascular integrity. This is further supported by our protein–protein interaction network, which identified EGR1, IL1B, TLR4, and ANGPTL2 as core molecules. TLR4 signaling has been widely associated with neuroinflammation and stroke severity [39–42], reinforcing the relevance of our identified network.

Furthermore, in vivo validation using a nitroglycerin-induced migraine model demonstrated increased neuronal activation and reduced expression of the tight junction protein ZO-1 in the trigeminocervical complex (Fig. 6E and 6F). Disruption of tight junctions is a hallmark of blood–brain barrier dysfunction and has been strongly linked to ischemic injury [43–45]. Our findings provide direct experimental evidence that migraine can induce vascular barrier impairment, supporting the hypothesis that recurrent migraine attacks may weaken neurovascular integrity and increase stroke susceptibility.

Despite these advances, several limitations should be acknowledged. First, the integration of bulk and single-cell transcriptomic data from different species and experimental conditions may introduce bias, and cross-species differences could affect the generalizability of the findings. Second, although our analyses identified key hub genes and pathways, causal relationships between EGR1 activation and stroke risk remain to be established. Functional studies using genetic or pharmacological manipulation of EGR1 and related pathways are needed to confirm their mechanistic roles. Third, our in vivo validation focused primarily on vascular integrity and neuronal activation, without directly assessing ischemic outcomes, limiting our ability to fully link migraine-induced changes to stroke pathology.

Future studies should aim to validate these findings in human clinical samples and longitudinal cohorts to determine whether EGR1 and IL1B can serve as predictive biomarkers for stroke risk in migraine patients. In addition, targeting the identified inflammatory and neurovascular pathways may offer new therapeutic strategies. For example, pharmacological modulation of interleukin-1 signaling or ANGPTL-mediated communication could potentially reduce vascular vulnerability. Ultimately, a deeper

understanding of the interplay between neuronal activity, inflammation, and vascular function will be essential for developing effective interventions to mitigate stroke risk in individuals with migraine.

In conclusion, our integrative transcriptomic and single-cell analyses, together with in vivo validation, reveal a potential molecular and cellular link between migraine and ischemic stroke. The findings identify EGR1-associated interleukin-1 inflammatory signaling as a shared pathogenic feature and further suggest that enhanced communication among neurons, vascular cells, and fibroblasts contributes to the establishment of a pro-inflammatory neurovascular microenvironment. In addition, the observed reduction of ZO-1 in the migraine model supports the presence of vascular barrier impairment, providing experimental evidence that migraine-related neurovascular dysfunction may increase susceptibility to ischemic stroke. Collectively, these results highlight a plausible inflammatory-neurovascular axis connecting migraine to stroke risk and nominate EGR1, IL1B, TLR4, and ANGPTL2 as potential therapeutic targets for future mechanistic and translational studies.

Declarations

Data Availability

The data generated and analyzed in this study are available as Supplemental Materials. The code used for the analyses is available from the corresponding author upon reasonable request.

Author information

Authors and Affiliations

Department of Neurology, The First Affiliated Hospital of Zhengzhou University, Zhengzhou University, Zhengzhou, Henan, PR China

Wenzheng Rong, Jing Xu, Bo Li, Xiaofeng Zhang, Yapeng Li, Bo Song, Yuming Xu

Tianjian Laboratory of Advanced Biomedical Sciences, School of life sciences, Zhengzhou University, Zhengzhou, Henan, China

Wenzheng Rong, Yapeng Li, Bo Song, Yuming Xu

NHC Key Laboratory of Prevention and Treatment of Cerebrovascular Diseases; Henan Key Laboratory of Cerebrovascular Diseases

Wenzheng Rong, Bo Li, Xiaofeng Zhang, Yapeng Li, Bo Song, Yuming Xu

Department of Endocrinology, The First Affiliated Hospital of Zhengzhou University

Jing Xu

Author Contributions

WZR and YMX designed the study and established the animal models. They also performed the behavioral experiments and confirmed the hypersensitivity phenotypes. JX and BL were responsible for tissue dissociation, library construction, and sequencing quality assessment. XFZ and YPL conducted the bioinformatic analyses, processed the multi-omics data, and interpreted the biological significance of the results. WZR and BS prepared the initial draft of the manuscript and contributed substantial mechanistic interpretation. All authors participated in revising the manuscript and approved the final version for publication.

Corresponding authors

Correspondence to Yuming Xu.

Funding

The National Natural Science Foundation of China [Grant U1904207], National Key R&D Program of China [Grant 2017YFA0105003], Non-profit Central Research Institute Fund of Chinese Academy of Medical Sciences [Grant 2020-PT310-01], and Innovative and Scientific and Technological Talents Training Project of Henan Province [Grant YXKC2021062] all provided funding for this work.

Conflict of Interest

The authors declare that they have no competing interests.

Ethics declarations

Ethics approval and consent to participate

All experimental procedures involving animals were approved by the Animal Ethics Committee of the First Affiliated Hospital of Zhengzhou University. Animal suffering was minimized as far as possible by using minimally invasive behavioral testing, optimized pain management strategies, and humane euthanasia in compliance with institutional ethical guidelines.

Informed Consent Statement

Not applicable.

References

1. Al-Hassany L, MaassenVanDenBrink A, Kurth T (2024) Cardiovascular Risk Scores and Migraine Status. JAMA Netw Open 7(10):e2440577
2. Nathan N, Ngo A, Khoromi S (2024) Migraine and Stroke: A Scoping Review. J Clin Med 13(18)
3. Ravi V, Osouli Meinagh S, Bavarsad Shahripour R (2024) Reviewing migraine-associated pathophysiology and its impact on elevated stroke risk. Front Neurol 15:1435208

4. Silvestro M, Esposito F, De Rosa AP, Orologio I, Trojsi F, Tartaglione L, García-Polo P, Tedeschi G, Tessitore A, Cirillo M et al (2024) Reduced neurovascular coupling of the visual network in migraine patients with aura as revealed with arterial spin labeling MRI: is there a demand-supply mismatch behind the scenes? J Headache Pain 25(1):180
5. Frimpong-Manson K, Ortiz YT, McMahon LR, Wilkerson JL (2024) Advances in understanding migraine pathophysiology: a bench to bedside review of research insights and therapeutics. Front Mol Neurosci 17:1355281
6. Tana C, Onan D, Messina R, Waliszewska-Prosół M, Garcia-Azorin D, Leal-Vega L, Coco-Martin MB, Ornello R, Raffaelli B, Souza MNP et al (2025) From Headache to Heart Health: Investigating the Migraine-Cardiovascular Disease Connection. Neurol Ther 14(4):1229–1268
7. Paolucci M, Altamura C, Vernieri F (2021) The Role of Endothelial Dysfunction in the Pathophysiology and Cerebrovascular Effects of Migraine: A Narrative Review. J Clin Neurol 17(2):164–175
8. Sacco S, Ripa P, Grassi D, Pistoia F, Ornello R, Carolei A, Kurth T (2013) Peripheral vascular dysfunction in migraine: a review. J Headache Pain 14(1):80
9. Caminero AB (2012) Sánchez Del Río González M: [Migraine as a cerebrovascular risk factor]. Neurologia 27(2):103–111
10. Yamanaka G, Hayashi K, Morishita N, Takeshita M, Ishii C, Suzuki S, Ishimine R, Kasuga A, Nakazawa H, Takamatsu T et al (2023) Experimental and Clinical Investigation of Cytokines in Migraine: A Narrative Review. Int J Mol Sci 24(9)
11. Ha WS, Chu MK (2024) Altered immunity in migraine: a comprehensive scoping review. J Headache Pain 25(1):95
12. Zhou H, Peng Y, Huo X, Li B, Liu H, Wang J, Zhang G (2025) Integrating Bulk and Single-Cell Transcriptomic Data to Identify Ferroptosis-Associated Inflammatory Gene in Alzheimer's Disease. J Inflamm Res 18:2105–2122
13. Acarsoy C, Ruiter R, Bos D, Ikram MK (2023) No association between blood-based markers of immune system and migraine status: a population-based cohort study. BMC Neurol 23(1):445
14. Zhang J, Zhao H (2023) eQTL studies: from bulk tissues to single cells. J Genet Genomics 50(12):925–933
15. Zimmermann M (1983) Ethical guidelines for investigations of experimental pain in conscious animals. Pain 16(2):109–110
16. Pradhan AA, Smith ML, McGuire B, Tarash I, Evans CJ, Charles A (2014) Characterization of a novel model of chronic migraine. Pain 155(2):269–274
17. Guo G, Zhang L, Liu X, Deng Y, Wu P, Zhao R, Wang W (2025) Fibroblast reprogramming in the dura mater of NTG-induced migraine-related chronic hypersensitivity model drives monocyte infiltration via Angptl1-dependent stromal signaling. J Headache Pain 26(1):130
18. Chaplan SR, Bach FW, Pogrel JW, Chung JM, Yaksh TL (1994) Quantitative assessment of tactile allodynia in the rat paw. J Neurosci Methods 53(1):55–63

19. Krug T, Gabriel JP, Taipa R, Fonseca BV, Domingues-Montanari S, Fernandez-Cadenas I, Manso H, Gouveia LO, Sobral J, Albergaria I et al (2012) TTC7B emerges as a novel risk factor for ischemic stroke through the convergence of several genome-wide approaches. J Cereb Blood Flow Metab 32(6):1061–1072
20. Yang L, Xu M, Bhuiyan SA, Li J, Zhao J, Cohrs RJ, Susterich JT, Signorelli S, Green U, Stone JR et al (2022) Human and mouse trigeminal ganglia cell atlas implicates multiple cell types in migraine. Neuron 110(11):1806–1821e1808
21. Hao Y, Stuart T, Kowalski MH, Choudhary S, Hoffman P, Hartman A, Srivastava A, Molla G, Madad S, Fernandez-Granda C et al (2024) Dictionary learning for integrative, multimodal and scalable single-cell analysis. Nat Biotechnol 42(2):293–304
22. Wu T, Hu E, Xu S, Chen M, Guo P, Dai Z, Feng T, Zhou L, Tang W, Zhan L et al (2021) clusterProfiler 4.0: A universal enrichment tool for interpreting omics data. Innov (Camb) 2(3):100141
23. Ashburner M, Ball CA, Blake JA, Botstein D, Butler H, Cherry JM, Davis AP, Dolinski K, Dwight SS, Eppig JT et al (2000) Gene ontology: tool for the unification of biology. The Gene Ontology Consortium. Nat Genet 25(1):25–29
24. Chen EY, Tan CM, Kou Y, Duan Q, Wang Z, Meirelles GV, Clark NR (2013) Ma'ayan A: Enrichr: interactive and collaborative HTML5 gene list enrichment analysis tool. BMC Bioinformatics 14:128
25. Jin S, Guerrero-Juarez CF, Zhang L, Chang I, Ramos R, Kuan CH, Myung P, Plikus MV, Nie Q (2021) Inference and analysis of cell-cell communication using CellChat. Nat Commun 12(1):1088
26. Iadecola C, Anrather J (2011) The immunology of stroke: from mechanisms to translation. Nat Med 17(7):796–808
27. Dinarello CA (2018) Overview of the IL-1 family in innate inflammation and acquired immunity. Immunol Rev 281(1):8–27
28. Pagel JI, Deindl E (2011) Early growth response 1–a transcription factor in the crossfire of signal transduction cascades. Indian J Biochem Biophys 48(4):226–235
29. Chamorro Á, Meisel A, Planas AM, Urra X, van de Beek D, Veltkamp R (2012) The immunology of acute stroke. Nat Rev Neurol 8(7):401–410
30. Cheng W, Zhao Q, Li C, Xu Y (2022) Neuroinflammation and brain-peripheral interaction in ischemic stroke: A narrative review. Front Immunol 13:1080737
31. Thapa K, Shivam K, Khan H, Kaur A, Dua K, Singh S, Singh TG (2023) Emerging Targets for Modulation of Immune Response and Inflammation in Stroke. Neurochem Res 48(6):1663–1690
32. Moskowitz MA, Macfarlane R (1993) Neurovascular and molecular mechanisms in migraine headaches. Cerebrovasc Brain Metab Rev 5(3):159–177
33. Kleeberg A, Luft T, Golkowski D, Purrucker JC (2025) Endothelial dysfunction in acute ischemic stroke: a review. J Neurol 272(2):143
34. Luo L, Qiao S (2026) Neuroinflammation and blood-brain barrier dysfunction in cerebral small vessel disease: mechanisms, biomarkers, and therapeutic implications. Eur J Med Res 31(1):307

35. Habib N, Li Y, Heidenreich M, Swiech L, Avraham-Davidi I, Trombetta JJ, Hession C, Zhang F, Regev A (2016) Div-Seq: Single-nucleus RNA-Seq reveals dynamics of rare adult newborn neurons. Science 353(6302):925–928
36. Hato T, Tabata M, Oike Y (2008) The role of angiopoietin-like proteins in angiogenesis and metabolism. Trends Cardiovasc Med 18(1):6–14
37. Huang D, Sun G, Hao X, He X, Zheng Z, Chen C, Yu Z, Xie L, Ma S, Liu L et al (2021) ANGPTL2-containing small extracellular vesicles from vascular endothelial cells accelerate leukemia progression. J Clin Invest 131(1)
38. Takano M, Hirose N, Sumi C, Yanoshita M, Nishiyama S, Onishi A, Asakawa Y, Tanimoto K (2021) ANGPTL2 Promotes Inflammation via Integrin α5β1 in Chondrocytes. Cartilage 13(2suppl):885s–897s
39. Mao L, Wu DH, Hu GH, Fan JH (2023) TLR4 Enhances Cerebral Ischemia/Reperfusion Injury via Regulating NLRP3 Inflammasome and Autophagy. *Mediators Inflamm* 2023:9335166
40. Nalamolu KR, Challa SR, Fornal CA, Grudzien NA, Jorgenson LC, Choudry MM, Smith NJ, Palmer CJ, Pinson DM, Klopfenstein JD et al (2021) Attenuation of the Induction of TLRs 2 and 4 Mitigates Inflammation and Promotes Neurological Recovery After Focal Cerebral Ischemia. Transl Stroke Res 12(5):923–936
41. Oo TT (2024) Ischemic stroke and diabetes: a TLR4-mediated neuroinflammatory perspective. J Mol Med (Berl) 102(6):709–717
42. Huang X, Chen S, Zhang W, Li J, Yang S, Zhou L, Zhou H, Xu K (2026) Targeting the intestinal TLR4-GABA(A) axis to promote stroke recovery. J Neuroinflammation 23(1):61
43. Chen H, Tang X, Li J, Hu B, Yang W, Zhan M, Ma T, Xu S (2022) IL-17 crosses the blood-brain barrier to trigger neuroinflammation: a novel mechanism in nitroglycerin-induced chronic migraine. J Headache Pain 23(1):1
44. Duan Y, Deng Y, Tang F, Li J (2024) Lifibrate attenuates blood-brain barrier damage following ischemic stroke via the MLCK/p-MLC/ZO-1 axis. Aging 16(7):6135–6146
45. Xue S, Zhou X, Yang ZH, Si XK, Sun X (2023) Stroke-induced damage on the blood-brain barrier. Front Neurol 14:1248970

Figures

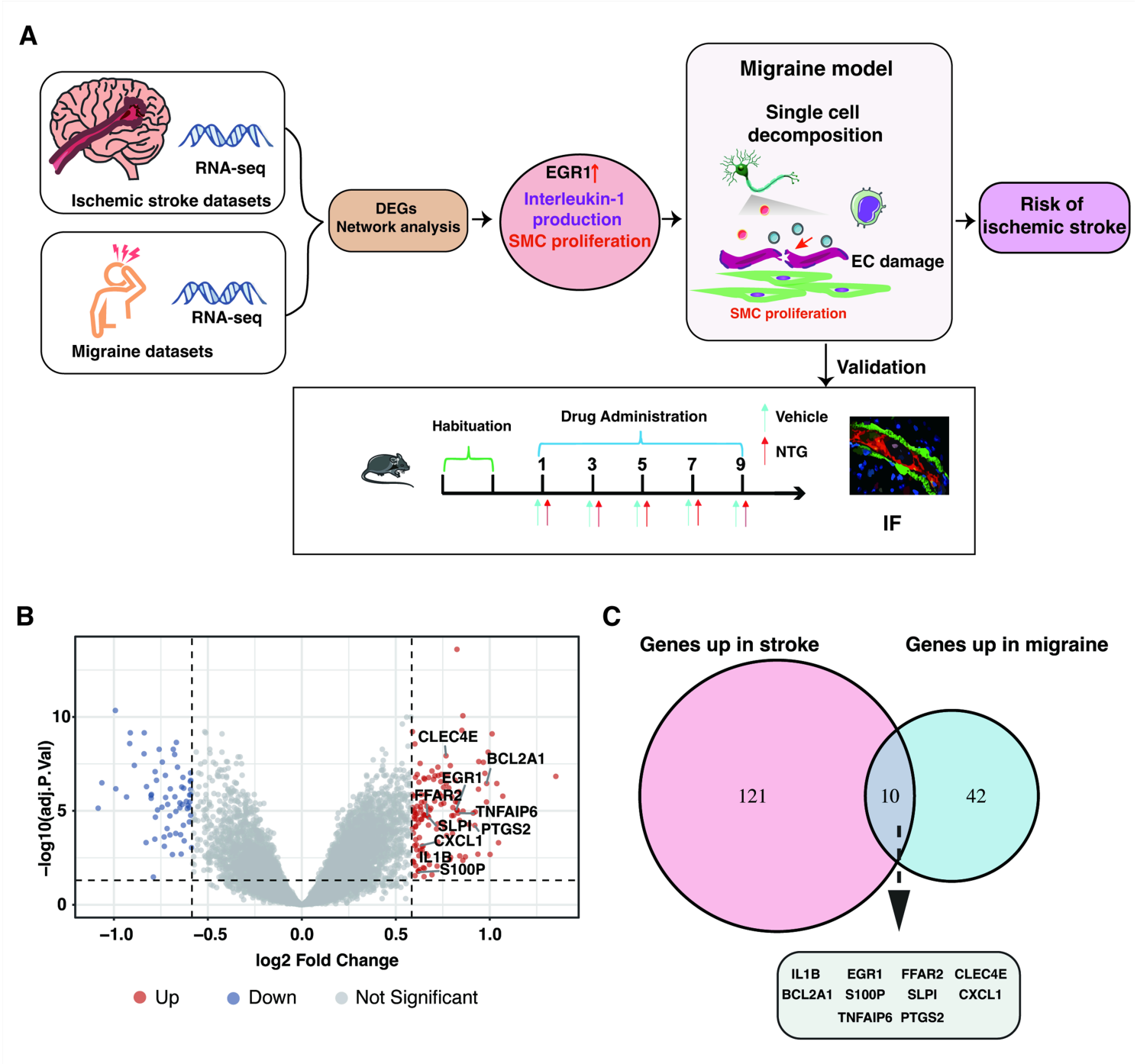
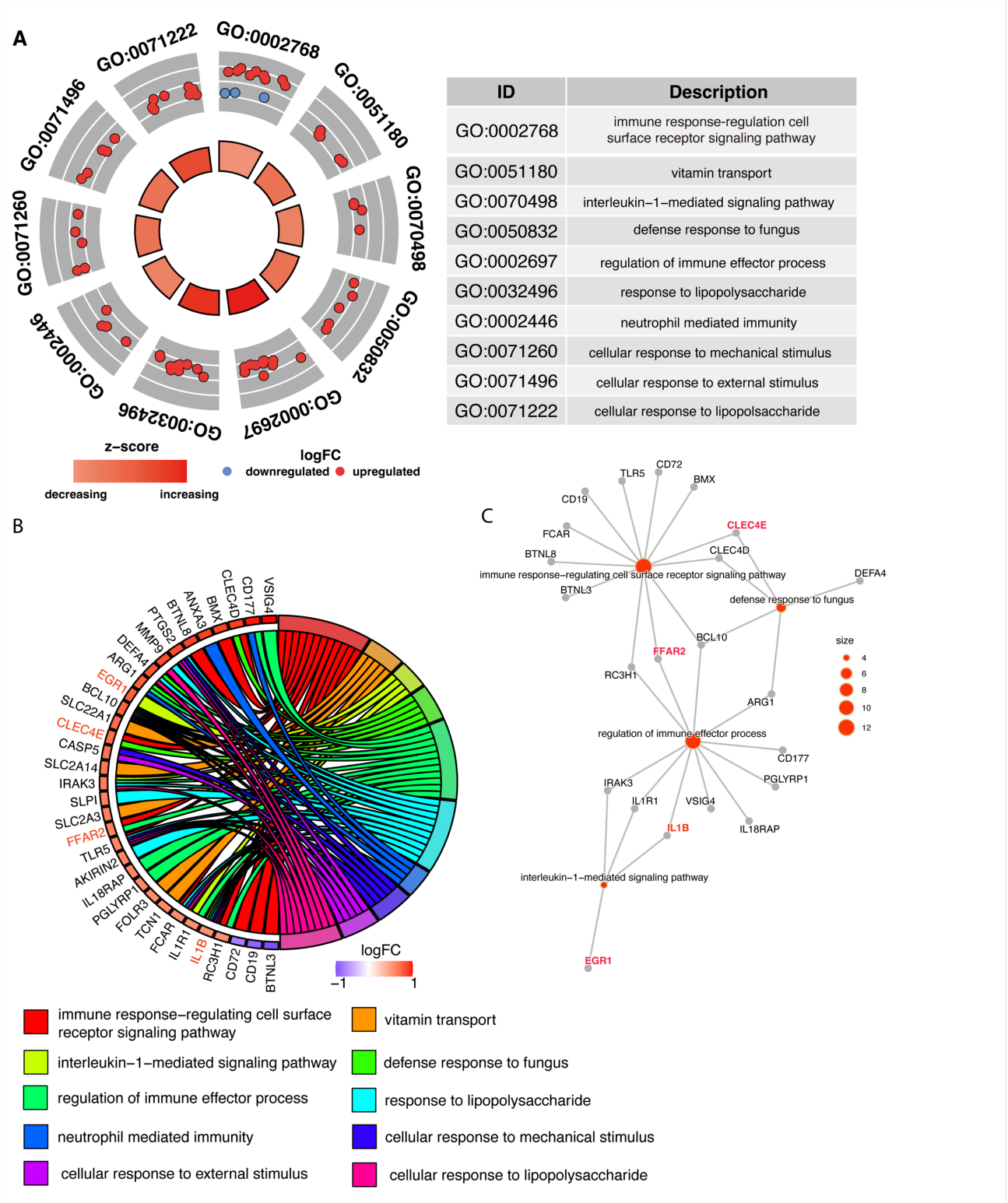


Figure 1

Identification of shared molecular signatures between migraine and ischemic stroke through transcriptomic analysis

(A) Overview of the experimental workflow. Bulk RNA-seq and single-cell RNA-seq datasets were analyzed to explore the molecular association between ischemic stroke and migraine. Differentially expressed genes (DEGs) were identified and subjected to network analysis, highlighting key pathways such as EGR1 regulation, interleukin-1 production, and smooth muscle cell (SMC) proliferation. A migraine mouse model was utilized for experimental validation, where drug administration (NTG) was

performed across multiple time points (1, 3, 5, 7, and 9 days) following habituation, with immunofluorescence (IF) imaging for validation of endothelial cell (EC) damage. (B) Volcano plot of the ischemic stroke RNA-seq dataset. The plot illustrates the log2 fold changes (x-axis) and -log10 adjusted P-values (y-axis) for differentially expressed genes. Upregulated genes are highlighted in red, downregulated genes in blue, and non-significant genes in gray. (C) Venn diagram showing the overlap of genes upregulated in both stroke and migraine conditions.



ID	Description
GO:0002768	immune response-regulation cell surface receptor signaling pathway
GO:0051180	vitamin transport
GO:0070498	interleukin−1−mediated signaling pathway
GO:0050832	defense response to fungus
GO:0002697	regulation of immune effector process
GO:0032496	response to lipopolysaccharide
GO:0002446	neutrophil mediated immunity
GO:0071260	cellular response to mechanical stimulus
GO:0071496	cellular response to external stimulus
GO:0071222	cellular response to lipopolsaccharide

Figure 2

Key immune-inflammatory pathways and stress-response genes in ischemic stroke highlighted by GO enrichment and network analysis

(A) Gene Ontology (GO) enrichment analysis of differentially expressed genes in ischemic stroke. The most significantly enriched biological processes are shown, primarily associated with immune and inflammatory responses. Terms such as immune response-regulating cell surface receptor signaling pathway, interleukin-1-mediated signaling pathway, and cellular response to mechanical stimulus are highlighted. Red indicates upregulated genes, while blue indicates downregulated genes. (B) Circular plot visualizing the relationships between enriched biological processes and representative genes in ischemic stroke. (C) Network analysis of immune-inflammatory pathways in ischemic stroke. Gene size corresponds to the degree of connectivity, with larger nodes representing more central genes in the network.

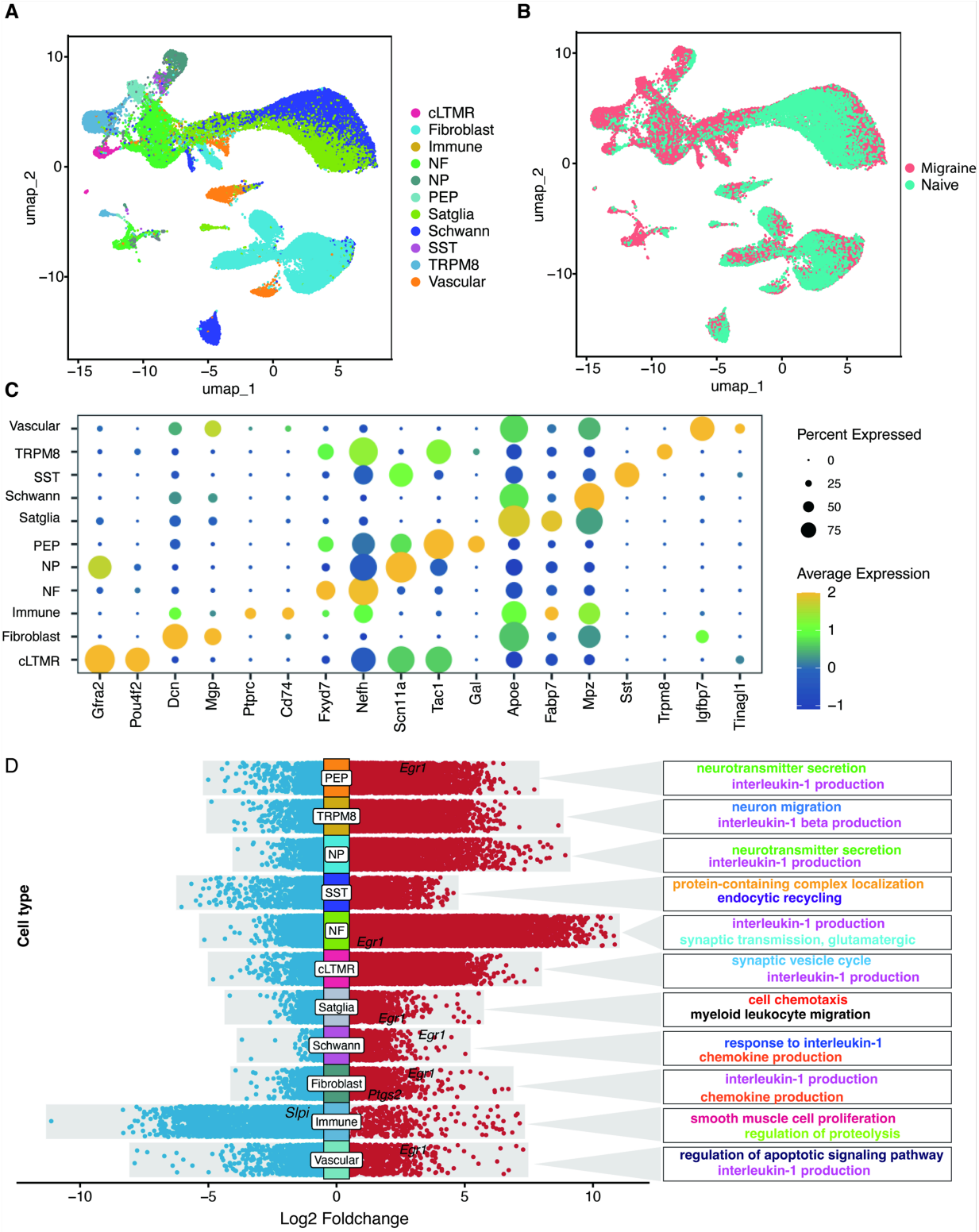


Figure 3

Single-cell transcriptomic profiling reveals EGR1 as a key upregulated gene in migraine-related cell populations

(A) UMAP plot of single-cell RNA-seq data from a mouse migraine model. Each cell is colored according to its identified cell type, including cLTMR, Fibroblast, Immune, NF, PEP, Satglia, Schwann, SST, TRPM8,

and Vascular. This plot highlights the overall cellular composition and shifts in cell populations associated with the migraine condition. (B) UMAP visualization showing the comparison between migraine and naive control conditions. Cells are colored by group (migraine vs. naive), revealing disease-associated shifts across cell populations. (C) Dot plot of marker genes for each cell type in the migraine model. The plot shows the percent of cells expressing each gene (dot size) and the average expression level (color intensity) across cell types, with distinct markers highlighted for each population. (D) Differential expression analysis comparing the migraine condition to naive controls. The plot shows log2 fold change (x-axis) for each gene across different cell types (y-axis). Pathway enrichment analysis reveals activation of the interleukin-1 production pathway in these cell populations, suggesting a broad inflammatory activation linked to EGR1.

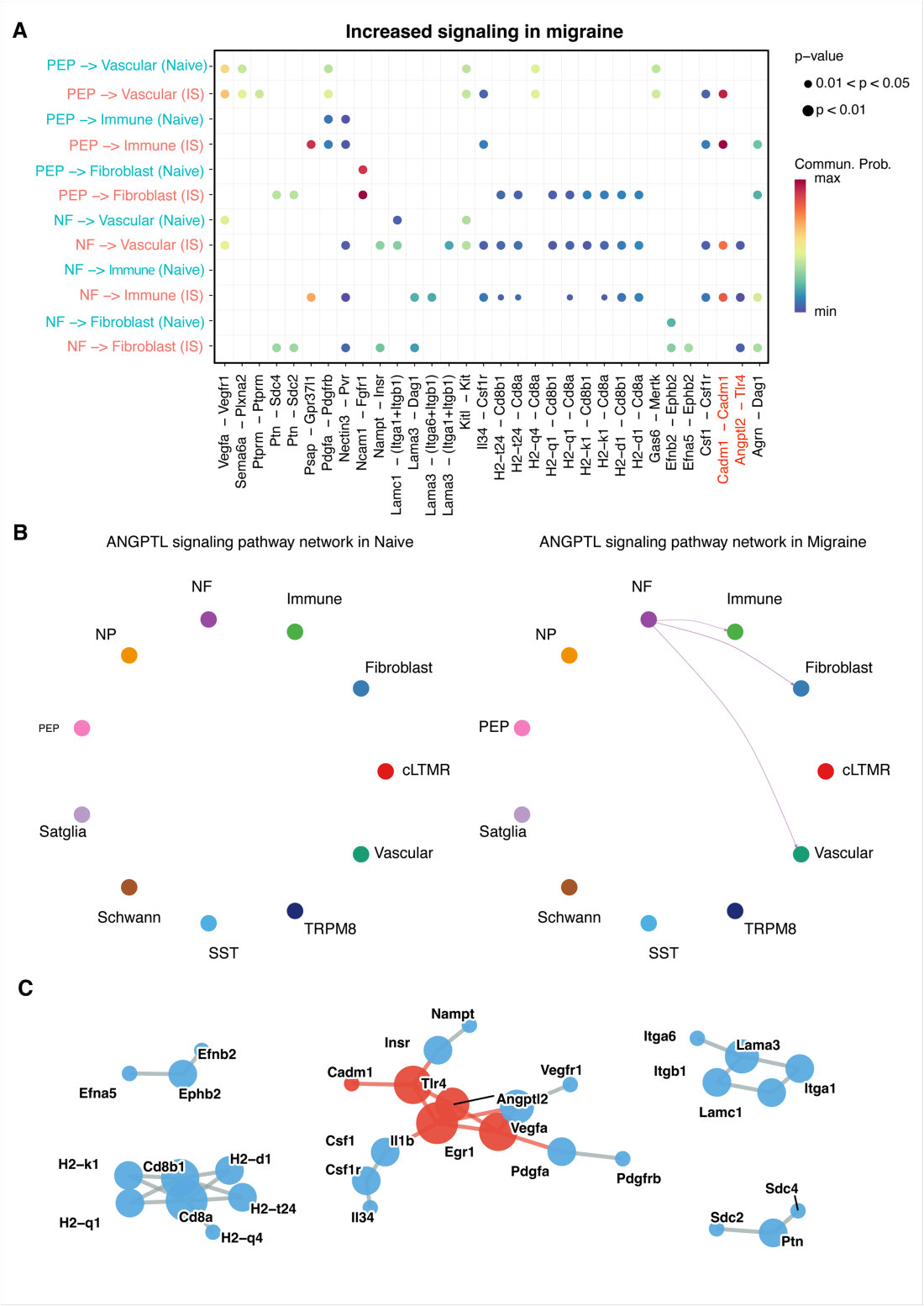


Figure 4

Altered neurovascular and fibroblast communication networks in migraine highlight enhanced inflammatory pathways

(A) Dot plot of intercellular communication signaling pathways in the migraine and naive groups. The plot shows increased interaction strength in the migraine group compared to the naive group, with

significant alterations observed in communication among NF neurons, PEP neurons, vascular cells, and fibroblasts. The size of the dots represents the p-value, with darker shades indicating stronger significance. (B) ANGPTL signaling pathway network in both naive and migraine groups. The network diagram illustrates enhanced ANGPTL signaling in the migraine group, particularly between NF neurons and immune fibroblasts, as well as vascular cells. The lines indicate the communication strength between cell types, with thicker lines representing stronger interactions. (C) Protein-protein interaction network constructed from the STRING database. Core hub molecules identified in the migraine-associated network include EGR1, IL1B, TLR4, and ANGPTL2. Red nodes represent upregulated molecules, while blue nodes represent downregulated molecules. The network highlights key inflammatory molecules central to neurovascular and fibroblast interactions in migraine.

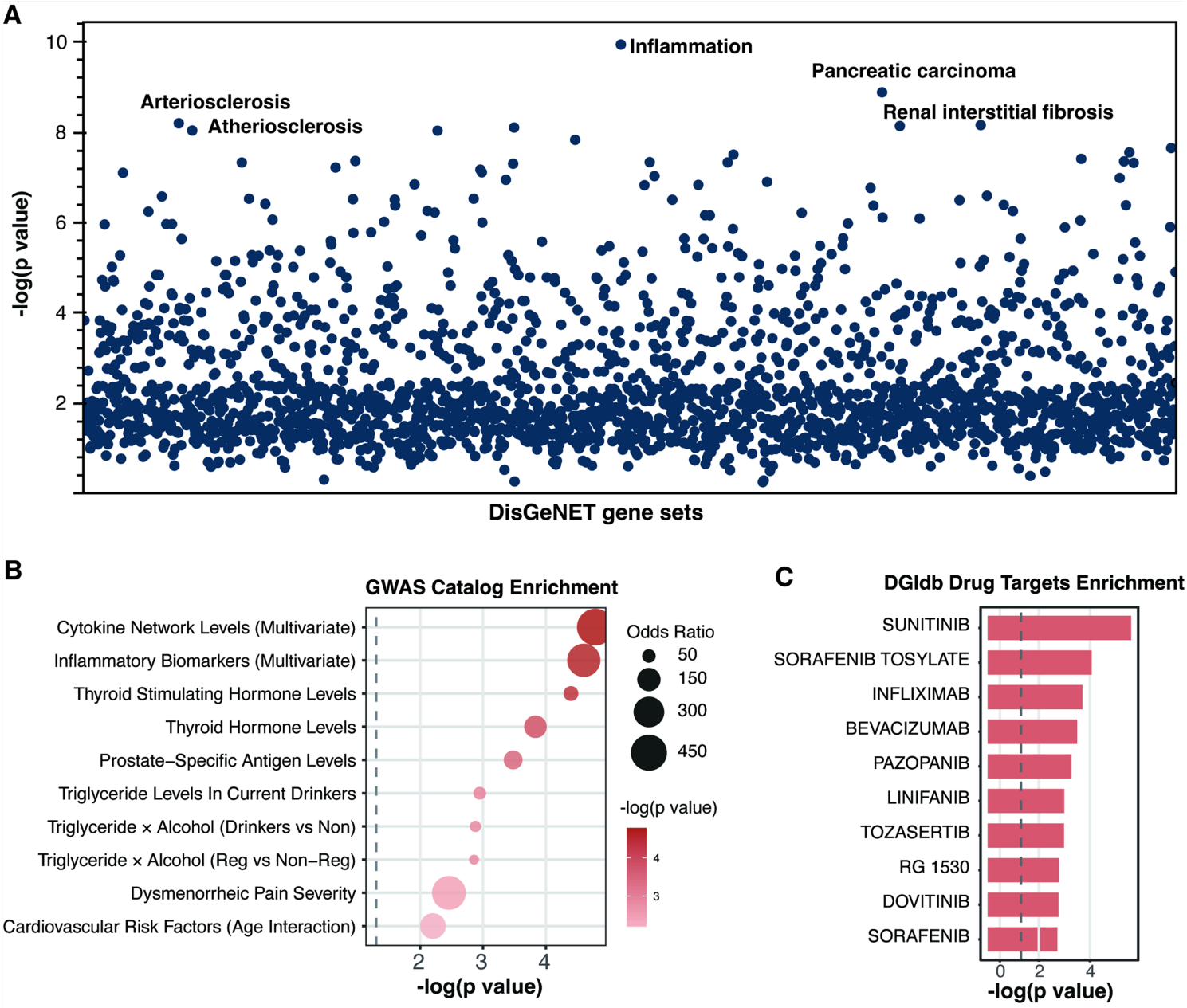


Figure 5

Translational relevance of the hub gene network supported by disease enrichment and drug atrget predictions

(A) DisGeNET disease enrichment analysis of the identified hub genes. The plot shows the association of the hub gene set with various disease categories. Significant enrichments are observed in inflammation, arteriosclerosis, and atherosclerosis, with the most prominent disease categories indicated. The -log(p value) is plotted on the y-axis. (B) GWAS Catalog enrichment analysis of the hub gene set. The plot displays associations with various traits, including cytokine network levels, dysmenorrheic pain severity, and cardiovascular risk factors. The size of the circles corresponds to the odds ratio, and the color intensity represents the significance level (-log(p value)). (C) Drug-gene interaction analysis using the DGIdb database. The bar plot shows the -log(p value) for candidate compounds targeting the hub gene network. SUNITINIB, ranked among the top predicted agents, is identified as a potential therapeutic candidate for targeting the gene network associated with inflammation and vascular pathology.

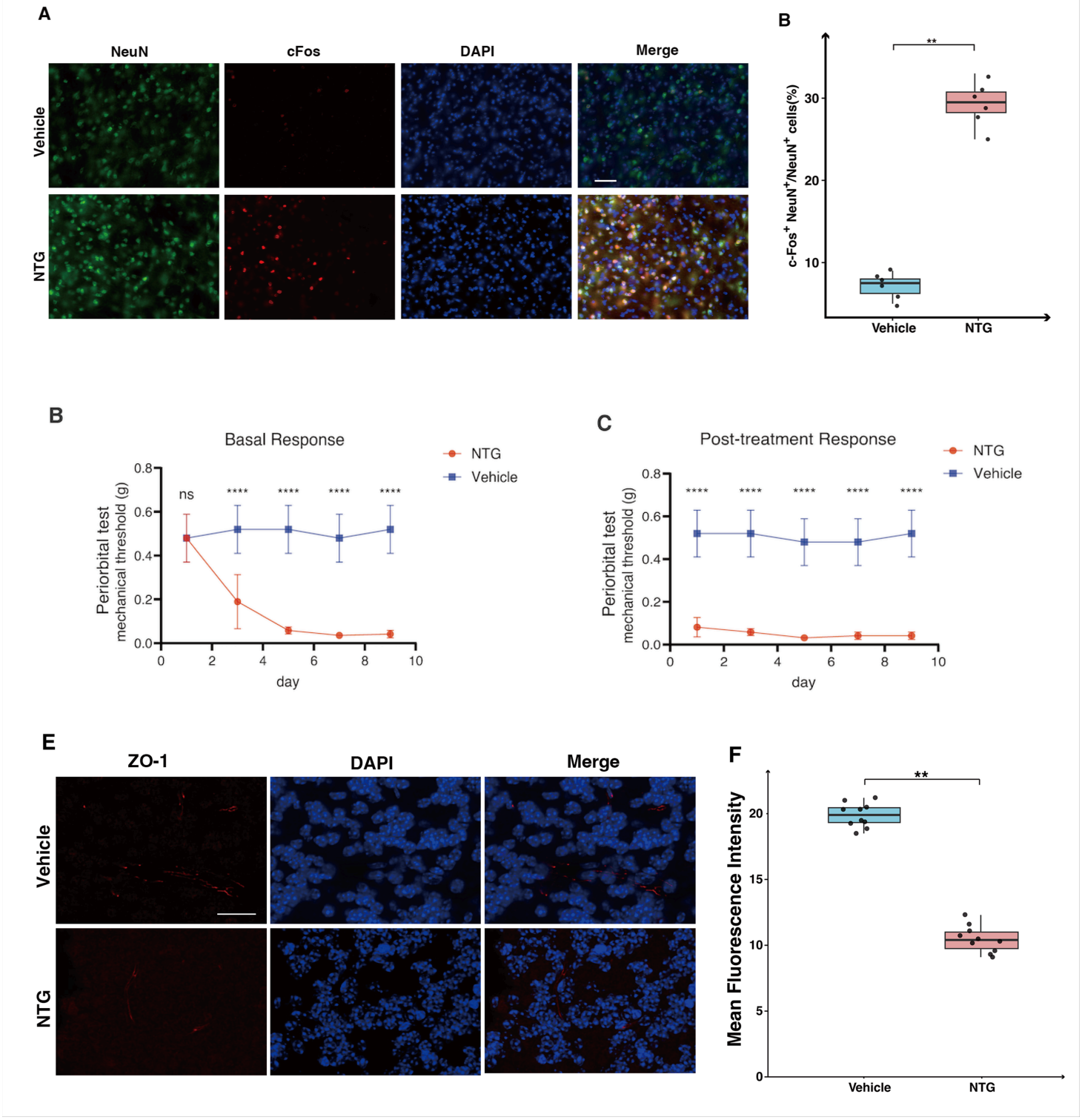


Figure 6

Neuronal activation and vascular tight junction impairment induced by migraine in vivo

(A) Immunofluorescence images of the trigeminocervical complex (TNC) showing cFos (red) and NeuN (green) staining in vehicle and NTG-treated mice. The DAPI stain (blue) is used to visualize cell nuclei. The merge panel shows the colocalization of cFos and NeuN in activated neurons. Scale bar = 50 μm. (B) Quantification of the proportion of cFos+/NeuN+ double-positive neurons in the TNC. NTG-treated

mice show a significant increase in the number of activated neurons compared to vehicle controls (**p < 0.01). (C) Basal mechanical threshold response in the periorbital test. NTG-treated mice exhibit a significant reduction in the mechanical threshold over the course of the study compared to vehicle-treated mice (****p < 0.001). (D) Post-treatment response in the periorbital test. NTG-treated mice show a sustained reduction in mechanical threshold, confirming the development of hypersensitivity after treatment (****p < 0.001). (E) Immunofluorescence images showing the expression of ZO-1 (red), a vascular tight junction marker, in the TNC of vehicle and NTG-treated mice. DAPI (blue) highlights cell nuclei. Scale bar = 50 μm. (F) Quantification of the mean fluorescence intensity of ZO-1 expression. NTG-treated mice display significantly reduced ZO-1 expression compared to vehicle controls (**p < 0.01).

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementalMaterial.docx