

Microtubule-associated protein HAUS8 regulates glioma proliferation, invasion and migration through EMT mediated by RhoA/ ROCK1

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Abstract

Background

Glioma is a highly invasive brain tumor in which epithelial-to-mesenchymal transition (EMT) drives dissemination. The microtubule-associated protein HAUS8 is markedly over-expressed in high-grade gliomas, yet its role in invasion remains undefined.

Methods

We evaluated HAUS8 expression and prognosis using bioinformatic analyses of TCGA, GTEx, and CGGA datasets. Functional studies were performed in U251 and U87 glioma cell lines through siRNA-mediated knockdown and plasmid-based overexpression of HAUS8. Assays included cell proliferation (CCK-8), migration (wound healing), invasion (Transwell), cell cycle progression (flow cytometry), and EMT marker expression (Western blot, immunofluorescence). RhoA-GTP levels were measured by pull-down assays, and ROCK pathway dependence was verified using the inhibitor Y-27632.

Results

HAUS8 expression increased with glioma grade and correlated with poorer patient survival. Knockdown of HAUS8 inhibited glioma cell proliferation, migration, and invasion, whereas overexpression enhanced these phenotypes. Cell cycle analysis revealed that HAUS8 overexpression increased the proportion of cells in S and G2/M phases. RNA sequencing identified differential expression of genes related to cell cycle, migration, and GTPase activity upon HAUS8 knockdown. HAUS8 overexpression elevated RhoA-GTP levels, induced EMT marker shifts (increased N-cadherin, decreased E-cadherin), and promoted F-actin polymerization—effects reversed by Y-27632. Clinical datasets indicated that high HAUS8 expression correlates with distinct immune cell infiltration patterns.

Conclusion

HAUS8 acts as a key driver of glioma progression, promoting EMT and malignant phenotypes through the RhoA/ROCK1 signaling pathway. It represents an independent prognostic biomarker and a potential therapeutic target, offering a novel rationale for targeting glioma invasion and metastasis.

1 Introduction

Glioma is a malignant brain tumor. Despite advances in surgery, radiotherapy, and chemotherapy, treatment options remain limited and patient prognosis poor. High-grade gliomas are particularly aggressive, with a very low 5-year survival rate^[1–2]. Invasion of glioma cells into brain tissue underlies

key symptoms, and local tissue destruction contributes to recurrence and mortality^[3–4]. Epithelial-mesenchymal transition (EMT) has been increasingly recognized as a critical driver of glioma initiation, progression, and therapeutic resistance^[5]. Elucidating the molecular mechanisms governing glioma proliferation, invasion, and metastasis is therefore critical for understanding recurrence, developing new therapeutic strategies, and ultimately improving patient survival and quality of life.

Microtubules and microtubule-associated proteins (MAPs) are important targets in cancer therapy, involved in mitosis, cell signaling, and other fundamental processes, and have been a focus of tumor biology and drug research in recent years^[6–7]. Recent studies have identified multiple microtubule-associated genes that are significantly associated with glioma prognosis, highlighting the clinical relevance of this protein family in glioma biology^[8]. Disrupted microtubule stability can lead to mitotic defects, dysregulated signaling, impaired migration and invasion, and compromised vascular architecture^[9]. HAUS8 is a novel microtubule-associated protein and a subunit of the HAUS complex. HAUS8 is a novel MAP and a subunit of the eight-protein HAUS complex, which is essential for microtubule generation, centrosome integrity, and proper mitotic spindle assembly^{[10][11]}. Epithelial-mesenchymal transition (EMT) plays a pivotal role in glioma invasion, metastasis, and malignant progression^[12]. Through EMT, tumor cells acquire migratory and invasive properties and develop resistance to radiotherapy and chemotherapy^{[13][14]}. During glioma progression, an EMT-like process occurs, characterized by loss of epithelial traits and gain of mesenchymal features, ultimately facilitating tumor cell dissemination^[15]. Microtubule dynamic instability has been shown to promote cell depolarization and movement during EMT, thereby enhancing cancer invasion^[16]. Consequently, suppressing EMT is considered a crucial therapeutic strategy in glioma^[17]. The RhoA/ROCK signaling pathway is a key regulator of cytoskeletal reorganization, cell migration, adhesion, and EMT maintenance^[18]. Aberrant activation of this pathway sustains phosphorylation of myosin light chain and stress fiber formation, driving invasion and metastasis in multiple cancers^[19]. Previous studies confirm that ROCK inhibition significantly reduces migration and invasion of glioma cells^[20]. Given that RhoA/ROCK signaling cooperatively activates EMT and metastasis, we hypothesized that interfering with HAUS8 may inhibit this pathway, thereby attenuating EMT and metastatic potential in glioma.

This study integrates bioinformatic analysis with experimental validation to systematically investigate HAUS8 in glioma progression. We assess the correlation between HAUS8 expression and patient prognosis, examine its effects on proliferation, migration, invasion, and cell cycle in glioma cell lines, and determine whether HAUS8 promotes malignant phenotypes through the RhoA/ROCK1-EMT axis. Our findings aim to provide new insights into the molecular mechanisms of glioma invasion and identify potential therapeutic targets.

2 Materials and methods

2.1 Bioinformatics analysis

HAUS8 expression and its prognostic significance in glioma were analyzed using multiple publicly available datasets. The Chinese Glioma Genome Atlas (CGGA, <http://www.cgga.org.cn/>) provided two RNA-seq datasets (mRNAseq_325 and mRNAseq_693) for expression profiling, survival analysis, and immune infiltration assessment across glioma grades. The Cancer Genome Atlas (TCGA, <https://portal.gdc.cancer.gov/>) and Genotype-Tissue Expression (GTEx, <https://www.gtexportal.org/>) datasets were used to compare HAUS8 expression between low-grade glioma (LGG), glioblastoma (GBM), and normal brain tissues. Gene Expression Profiling Interactive Analysis (GEPIA, <http://gepia.cancer-pku.cn/>) was employed to generate overall survival curves based on HAUS8 expression levels. Univariate and multivariate Cox regression analyses were performed to evaluate the independent prognostic value of HAUS8, adjusting for WHO grade, IDH mutation status, 1p/19q co-deletion, age, and other clinical covariates. Time-dependent receiver operating characteristic (ROC) curves were constructed to assess the predictive accuracy of HAUS8 for 1-, 3-, and 5-year survival. The relationship between HAUS8 expression and tumor-infiltrating immune cells was explored using the CIBERSORT algorithm on both CGGA and TCGA datasets, and the TIMER database (<https://cistrome.shinyapps.io/timer/>) was used to evaluate correlations between HAUS8 expression and immune cell infiltration levels in LGG and GBM. Gene set enrichment analysis (GSEA) was performed to identify KEGG pathways and Gene Ontology (GO) terms associated with high HAUS8 expression.

2.2 Cell culture

Human glioblastoma glioma cell lines U251 (Cat # CL-0237) and U87 (Cat # CL-0238) of unknown origin (Procell, Wuhan, China) were cultured in DMEM (Keygen, #KGL1206-500) supplemented with 10% FBS (LONSERA, #S711-001S) and 1% penicillin/streptomycin (Beyotime, #C0223) at 37°C under 5% CO₂. Cell line authentication was assessed using short tandem repeat (STR) DNA profiling method every six months in our laboratory.

2.3 Cell transfection

Cells were seeded in 6-well plates and transfected at appropriate confluence. For plasmid transfection, 2 µg of HAUS8 plasmid (pcDNA4 vector, Shanghai Chengxin Biotechnology) was mixed with 200 µL jet buffer and 4 µL jet transfection reagent (Polyplus, #101000001). For siRNA transfection, 5–10 µL of 2.5 nmol HAUS8-siRNA was used. Complexes were incubated 15 min at room temperature before dropwise addition to cultures. Protein and RNA were harvested at 48 h and 24 h post-transfection, respectively. siRNA sequences synthesized by GenePharma (Shanghai, China) and are listed in Table 1.

Table 1
Haus8-si-RNA sequence

Name	Sequence (5' to 3')
Haus8-si-1059	sense: GCCGGUGGUAUUUCAACATT
	antisense: UGAUUGAAAUACCACCGGCTT
Haus8-si-90	sense: GUGCCAAGAAGAAGGAUAATT
	antisense: UUAUCCUUCUUCUUGGCACTT
Haus8-si-334	sense: GGAUCUCUCUGCUAUUAAUTT
	antisense: AUUAAUAGCAGAGAGAUCCCTT
NC	sense: UUCUCCGAACGUGUCACGUdTdT
	antisense: ACGUGACACGUUCGGAGAAAdTdT

2.4 RNA extraction and real-time fluorescence quantitative PCR (qPCR)

Total RNA was extracted using the TriQuick Reagent (Cat: R1100, Solarbio, Beijing, China). Reverse transcription was performed with 5× qRT SuperMix (RP1105, Solarbio). qPCR reactions contained 5 µL 2× ChamQ Universal SYBR qPCR Master Mix (QPK-201, Solarbio), 0.5 µL each of forward and reverse primers (10 µM), 1 µL cDNA, and 3 µL ddH₂O. Cycling conditions: 95°C for 30 s; 40 cycles of 95°C for 10 s and 60°C for 30 s; melt curve analysis. Relative gene expression was normalized to the internal control GAPDH and quantified using the 2^{-ΔΔCt} method. The sequences of the primers used in qPCR have been fully detailed in the supplementary file.

2.5 RhoA activity detection

Active RhoA was quantified using the G-LISA RhoA Activation Assay Kit (Cytoskeleton, #BK124, Denver, USA) according to the manufacturer's protocol. Absorbance was measured at 490 nm in triplicate.

2.6 CCK8 assay

Cells were seeded at 5,000 per well in 96-well plates 24 h after lentiviral infection. Proliferation was assessed using the CCK-8 kit (Beyotime, #C0037, Beijing, China).

2.7 Wound-healing assay

Confluent cells in 6-well plates were treated with 10 µg·mL⁻¹ Mitomycin C (MCE, #HY-13316, NJ, USA) for 2 h to inhibit proliferation. A scratch was made with a sterile pipette tip. After PBS washes, cells were cultured in serum-free medium. Images were taken at 0 and 24 h, and migration rate was calculated using ImageJ.

2.8 Transwell assay

For migration assays, 2×10^4 cells in serum-free DMEM (Gibco, Thermo Fisher Scientific, Waltham, MA, USA; cat# 11995-065) were seeded into the upper chamber of a Transwell insert (Corning Costar, Corning, NY, USA; cat# 3422, 8 μm pore size). Medium containing 20% fetal bovine serum (FBS; Gibco; cat# 10099-141) (600 μL) served as chemoattractant in the lower chamber. For invasion assays, chambers were pre-coated with Matrigel (Corning Matrigel Basement Membrane Matrix, Corning, NY, USA; cat# 354234) at 1:8 dilution and air-dried before use. After 24 h (migration) or 48 h (invasion), cells on the lower membrane were fixed with 4% paraformaldehyde (Sigma-Aldrich, St. Louis, MO, USA; cat# P6148), stained with 0.1% crystal violet (Sigma-Aldrich; cat# C3886), and counted in five random fields per well under a light microscope.

2.9 Flow cytometry for cell cycle

Cells were fixed in 70% ethanol (Sigma-Aldrich, St. Louis, MO, USA; cat# 459844) overnight at 4°C, stained with DNase-free RNase A (100 $\mu\text{g} \cdot \text{mL}^{-1}$; Sigma-Aldrich; cat# R4875) and propidium iodide (50 $\mu\text{g} \cdot \text{mL}^{-1}$; Sigma-Aldrich; cat# P4170) at 37°C for 30 min, and analyzed on a BD FACSCanto II flow cytometer (Becton, Dickinson and Company [BD Biosciences], San Jose, CA, USA). Cell cycle distribution was determined using ModFit LT software (Verity Software House, Topsham, ME, USA).

2.10 Confocal microscopy for F-actin

Cells on coverslips were fixed with 4% PFA (Sigma-Aldrich, St. Louis, MO, USA; cat# P6148), permeabilized with 0.1% Triton X-100 (Sigma-Aldrich; cat# T8787), blocked with 1% BSA (Sigma-Aldrich; cat# A7030), and stained with Alexa Fluor 488-phalloidin (1:200) (Invitrogen, Carlsbad, CA, USA; cat# A12379) followed by DAPI (Sigma-Aldrich; cat# D9542). Z-stack images were acquired on a Zeiss LSM980 confocal microscope (63 $\times$ oil) (Jena, Germany). Filopodia length was quantified using ImageJ (National Institutes of Health, Bethesda, MD, USA).

2.11 Western blot

Cells were lysed in RIPA buffer (Thermo Fisher Scientific, Waltham, MA, USA; cat# 89900); protein concentration was determined by BCA assay (Thermo Fisher Scientific; cat# 23225). Proteins (30 μg) were separated by 10% SDS-PAGE, transferred to PVDF membranes (Millipore, Billerica, MA, USA; cat# IPVH00010), blocked with 5% skim milk, and incubated overnight at 4°C with primary antibodies against RhoA (1:1500, Cell Signaling Technology [CST], Danvers, MA, USA; cat# 2117), phospho-MLC2 (1:1000, pMLC; CST; cat# 3674), E-cadherin (1:1500, CST; cat# 3195), total MLC2 (1:1500, CST; cat# 8505), ROCK1 (1:2000, CST; cat# 4035), GAPDH (1:3000, CST; cat# 5174), and N-cadherin (1:1500, CST; cat# 13116). HRP-conjugated secondary antibody (1:5000, CST; cat# 7074). HRP-conjugated secondary antibody (1:5000, CST; cat# 7074) was applied for 1 h at room temperature. Bands were visualized with ECL (Thermo Fisher Scientific; cat# 34580) and quantified using Image Lab (Bio-Rad Laboratories, Hercules, CA, USA). GAPDH was served as a loading control.

2.12 RNA sequencing

Total RNA from 5×10^6 U87 cells was extracted and subjected to high-throughput sequencing on the Illumina HiSeq platform (Novogene). The initial data processing were performed in collaboration with the laboratory of Prof. Lufeng Zheng at China Pharmaceutical University, who also deposited the raw and processed data to the Gene Expression Omnibus (GEO) database on behalf of our research group. Reads were aligned to GRCh38.p10 with HISAT2, and expression was quantified with RSEM. Differential expression analysis used DESeq2; GO enrichment was performed with GOATools. Data are available in GEO (<https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE237818>.) under accession GSE237818.

2.13 Statistical analysis

The statistical analysis was carried out using GraphPad Prism 9.0 software (GraphPad Software, Inc., La Jolla, CA, USA). Results are presented as the mean $\pm$ standard deviation (S.D.) and verified by the Kolmogorov–Smirnov test to ensure they were normally distributed. Declared group size (n) refers to the number of independent values rather than technical replicates, and statistical analyses were undertaken only where group size was at least $n = 3$. The group size is the number of independent values, and statistical analysis was done using these independent values, and there were no outliers in the data analysis. Differences between group means were assessed by unpaired Student's t test (two groups) or (more than two groups) by one-way ANOVA followed with Bonferroni's multiple comparisons test. One-way ANOVA was conducted only if F in ANOVA achieved $P < 0.05$, and there was no significant variance inhomogeneity.

3 Results

3.1 HAUS8 expression correlates with poor prognosis in glioma

HAUS8 expression was significantly upregulated in low-grade glioma (LGG) and glioblastoma (GBM) compared to normal brain tissue (Fig. 1A). High HAUS8 expression correlated with shorter overall survival (Fig. 1B). Univariate Cox analysis indicated HAUS8 expression was significantly associated with outcome (Fig. 1C). Multivariate analysis confirmed HAUS8 as an independent prognostic factor after adjusting for WHO grade, IDH mutation, and 1p/19q co-deletion (Fig. 1D). ROC curves showed good predictive accuracy for 1-, 3-, and 5-year survival (Fig. 1E). HAUS8 expression increased with WHO grade (Fig. 1F) and was elevated in IDH wild-type and 1p/19q non-codeleted subtypes (Fig. 1G). These findings indicate that high HAUS8 expression aligns with established malignant drivers of glioma, collectively contributing to tumor aggressiveness and progression.

3.2 HAUS8 promotes glioma cell proliferation, migration, and invasion

To investigate the biological function of HAUS8 in glioma, we established HAUS8 overexpression and siRNA-mediated knockdown models in U87 and U251 glioma cell lines. We first verified the efficiency of

genetic manipulation by quantitative real-time PCR. Compared with the negative control (NC), the relative RNA levels of HAUS8 were significantly reduced in si-Haus8-1, si-Haus8-2, and si-Haus8-3 groups, with si-Haus8-3 showing the most pronounced knockdown effect. In contrast, the OE-Haus8 group exhibited a substantial increase in HAUS8 expression compared with the Vector control (Fig. 2A). These results confirm the successful establishment of HAUS8 knockdown and overexpression models. The CCK-8 assay demonstrated that HAUS8 overexpression significantly promoted glioma cell proliferation, while its knockdown markedly inhibited cell growth (Fig. 2B). Wound healing assays further revealed that HAUS8 overexpression enhanced cell migration, whereas HAUS8 silencing substantially impaired this capacity (Fig. 2C, D). Similarly, Transwell assays confirmed that HAUS8 overexpression increased both migration and invasion of glioma cells, while knockdown produced the opposite effects (Fig. 2E). Given the close relationship between cell cycle progression and proliferative capacity as well as invasiveness, we further analyzed the effect of HAUS8 on cell cycle distribution using flow cytometry. In U87 cells, compared with the vector control group, HAUS8 overexpression significantly increased the proportion of cells in G2/M phase and decreased the population in G0/G1 phase. Conversely, HAUS8 knockdown led to a notable accumulation of cells in G0/G1 phase and a reduction in G2/M phase (Fig. 2F). A similar trend was observed in U251 cells: HAUS8 overexpression reduced the G1 phase proportion to 28.30% and increased the S phase fraction to 62.00%, whereas HAUS8 silencing decreased the S phase population (Fig. 2G). These results suggest that HAUS8 may enhance the proliferative capacity of glioma cells by driving cell cycle progression, particularly facilitating G1/S transition and G2/M phase progression. Collectively, these findings demonstrate that HAUS8 not only promotes glioma cell proliferation by regulating the cell cycle but also enhances their migratory and invasive capabilities, thereby revealing the multifaceted role of HAUS8 in glioma malignant progression.

3.3 Transcriptomic analysis identifies HAUS8-regulated pathways

RNA sequencing of HAUS8-knockdown U251 cells showed altered expression of genes involved in migration, cytoskeleton, and GTPase signaling (Fig. 3A, B). GSEA indicated HAUS8 suppression negatively regulated cell migration, mesenchymal–epithelial transition, and Ca^{2+} signaling (Fig. 3C–G). Enrichment analyses highlighted Rho GTPase signaling and actin cytoskeleton reorganization (Fig. 3H–J).

3.4 HAUS8 regulates the expression of EMT markers in glioma cells

To elucidate the mechanism by which HAUS8 promotes glioma progression, we first examined its effect on RhoA pathway activity. In U87 and U251 glioma cells, RhoA activity assays showed that HAUS8 knockdown significantly inhibited RhoA activation, whereas HAUS8 overexpression markedly enhanced it, compared with their respective controls (si-NC or Vector) (Fig. 4A). Given the established role of the RhoA pathway in regulating epithelial–mesenchymal transition (EMT), we next assessed the expression

of EMT markers and key components of the RhoA/ROCK cascade by Western blotting. In both U87 and U251 cells, HAUS8 knockdown downregulated mesenchymal markers (Vimentin, N-cadherin) and upregulated the epithelial marker E-cadherin, while reducing the levels of RhoA, ROCK, and its downstream effector p-MLC. Conversely, HAUS8 overexpression promoted the expression of mesenchymal markers and RhoA pathway proteins and suppressed E-cadherin expression. These data indicate that HAUS8 drives EMT in glioma cells via activation of the RhoA/ROCK pathway. Densitometric analysis revealed that HAUS8 knockdown significantly reduced the p-MLC/total MLC ratio in both U87 (si-NC: 0.833 ± 0.074 vs. si-Haus8: 0.370 ± 0.020 , $p < 0.001$) and U251 cells (si-NC: 0.610 ± 0.069 vs. si-Haus8: 0.387 ± 0.021 , $p < 0.001$). Conversely, HAUS8 overexpression markedly increased the p-MLC/total MLC ratio in U87 cells (Vector: 1.027 ± 0.025 vs. HAUS8 OE: 1.400 ± 0.050 , $p < 0.001$) and U251 cells (Vector: 0.640 ± 0.020 vs. HAUS8 OE: 0.923 ± 0.025 , $p < 0.001$) (Fig. 4B). Because RhoA/ROCK signaling critically regulates cytoskeletal dynamics, we further evaluated the effect of HAUS8 on F-actin organization by immunofluorescence staining (Fig. 4C). In U251 cells, HAUS8 knockdown reduced F-actin fluorescence intensity compared with the NC group, whereas HAUS8 overexpression increased it relative to the Vector control. Similar changes were observed in U87 cells. These findings align with the alterations in pathway protein expression and demonstrate that HAUS8 promotes F-actin polymerization and cytoskeletal remodeling through RhoA/ROCK activation.

3.5 HAUS8 Promotes EMT in Glioma Cells through Activation of the RhoA/ROCK1 Pathway

We next used the ROCK-specific inhibitor Y-27632 to functionally interrogate pathway dependence. CCK-8 assays showed that HAUS8 overexpression increased cell viability over 72 h, and this proliferative advantage was attenuated by Y-27632 co-treatment (Fig. 5A). Similarly, wound-healing and Transwell invasion assays demonstrated that HAUS8-driven migration and invasion were markedly suppressed by Y-27632 (Fig. 5B, C). Flow cytometry further revealed that HAUS8 overexpression reduced apoptosis, an effect reversed by Y-27632 (Fig. 5D). Mechanistically, Western blotting indicated that Haus8 regulated epithelial-mesenchymal transition (EMT) and the RhoA/ROCK pathway: Haus8 overexpression upregulated mesenchymal markers (Vimentin, N-Cadherin) and downregulated the epithelial marker (E-Cadherin), while also increasing the expression of RhoA, ROCK, and phosphorylated MLC2 (pMLC). Notably, Y-27632 treatment partially reversed these changes, including restoring E-Cadherin levels, reducing mesenchymal markers, and downregulating RhoA/ROCK/pMLC in Haus8-overexpressing cells. Quantification of the p-MLC/total MLC ratio further confirmed these observations. In U87 cells, HAUS8 overexpression significantly increased the p-MLC/total MLC ratio compared with the Vector control (1.161 ± 0.109 vs. 0.830 ± 0.066 , $p < 0.001$), and this effect was significantly attenuated by Y-27632 co-treatment (0.545 ± 0.161 , $p < 0.01$). Similarly, in U251 cells, HAUS8 overexpression elevated the p-MLC/total MLC ratio (0.384 ± 0.025 vs. 0.293 ± 0.085 for Vector, $p < 0.01$), which was reversed by Y-27632 treatment (0.219 ± 0.021 , $p < 0.001$) (Fig. 5E).

Together, these results demonstrate that HAUS8 promotes glioma cell proliferation, migration, invasion, and EMT while suppressing apoptosis—effects that are largely mediated through the RhoA/ROCK1 signaling axis, as evidenced by their reversal upon ROCK inhibition. This stepwise validation, from initial phenotypic and transcriptomic profiling to targeted pharmacological inhibition, establishes HAUS8 as an upstream activator of the RhoA/ROCK1-EMT pathway in glioma.

4 Discussion

These findings fill a critical gap in understanding how microtubule-associated proteins actively regulate signaling pathways to drive glioma invasion and metastasis. This study is the first to systematically elucidate the molecular mechanism by which HAUS8 promotes epithelial-mesenchymal transition (EMT) and invasion in glioma via the RhoA/ROCK1 signaling pathway. We found that HAUS8 is significantly overexpressed in glioma tissues, and its expression level is closely associated with adverse prognostic features such as WHO grade, IDH wild-type status, and non-co-deletion of 1p/19q, establishing its role as an independent prognostic biomarker. Functional experiments demonstrated that overexpression of HAUS8 markedly enhances the proliferation, invasion, and migration capabilities of glioma cells. Furthermore, we observed that these phenotypic changes are closely linked to EMT. Mechanistic investigations revealed that HAUS8 drives malignant progression in glioma by activating the RhoA/ROCK1 pathway, thereby regulating the expression of EMT markers and inducing cytoskeletal reorganization.

HAUS8 (HAUS augmin-like complex subunit 8), also known as HICE1, is a core component of the eight-subunit augmin complex that promotes microtubule generation and maintains spindle stability during mitosis^{[21][22]}. Although initially thought to function only in cell division, accumulating evidence indicates that microtubule-associated proteins like HAUS8 also regulate tumor cell migration, polarity, and cytoskeletal dynamics—key processes underlying EMT and invasion^[23–25]. Like other MAPs such as stathmin and KIF3C—which promote EMT and glioma invasion by modulating microtubule dynamics and activating signaling pathways like PI3K/AKT—HAUS8 also exerts its pro-invasive effects through RhoA/ROCK1-mediated cytoskeletal remodeling and EMT induction^[26–29]. Compared with the main MAPs such as EB1^{[30][31]} that regulate the dynamics of the positive end of microtubules, HAUS8 may be more directly involved in the establishment of cell polarity by influencing centrosome function, which is a key step in the EMT process. However, unlike stathmin, which primarily enhances cancer cell motility through microtubule destabilization, HAUS8, as a core component of the augmin complex, may maintain the fidelity of microtubule generation, thereby providing a structural basis for sustained activation of the RhoA/ROCK1 signaling pathway. Furthermore, in contrast to certain MAPs that mainly affect cell cycle progression (such as some kinesin family members), the pro-proliferative effect of HAUS8 is independent of cell cycle alterations, indicating that its mechanism of action focuses more on directly regulating cell motility and phenotypic plasticity. This characteristic may confer unique advantages in targeted therapy. It is noteworthy that, compared to the mechanism by which miR-940 suppresses EMT via targeting ZEB2 in another study^[32], HAUS8 represents a novel pathway that positively regulates EMT

through the microtubule–RhoA signaling axis, thereby enriching our understanding of how the microtubule system participates in glioma invasion.

The potential mechanism of the HAUS8–RhoA/ROCK1–EMT signaling axis warrants further investigation. Based on our experimental results, we propose the following mechanistic hypothesis: First, as a key component of the augmin complex, HAUS8 maintains centrosome integrity and microtubule generation fidelity, thereby providing a structural basis for RhoA activation^{[33][34]}. The stability of the microtubule network may influence the membrane localization or activity of RhoGEF (guanine nucleotide exchange factor), thereby regulating the GTP/GDP cycle of RhoA^{[35][36]}. Second, HAUS8 may indirectly modulate the spatiotemporal activation pattern of RhoA by influencing centrosomal function. As the microtubule-organizing center, the centrosome plays an essential role in establishing cell polarity—a critical step in the EMT process. Our data show that HAUS8 overexpression significantly increases RhoA-GTP levels and enhances the expression of ROCK1 and its downstream effector p-MLC, suggesting that HAUS8 may serve as a scaffold for the effective assembly of the RhoA/ROCK1 signaling module by maintaining proper microtubule architecture. We observed that HAUS8 overexpression promotes F-actin polymerization and stress fiber formation, consistent with the classical functions of the RhoA/ROCK1 pathway. ROCK1 promotes actomyosin contractility through a dual mechanism: on the one hand, it directly phosphorylates myosin light chain (MLC), and on the other hand, it phosphorylates and inhibits myosin light chain phosphatase (MLCP), thereby enhancing myosin ATPase activity^{[37][38]}. Simultaneously, ROCK1 phosphorylates LIM kinase, which in turn inhibits cofilin-mediated actin filament depolymerization, stabilizing invasive pseudopodia at the cell front. These cytoskeletal remodeling events ultimately drive alterations in the expression of key EMT markers, including upregulation of N-cadherin and vimentin, and downregulation of E-cadherin. Notably, our mechanistic experiments provide strong evidence: treatment with the ROCK-specific inhibitor Y-27632 completely reversed the HAUS8 overexpression-induced enhancements in EMT phenotype, proliferation, and invasion, further confirming the functional necessity of this signaling axis. The proposed HAUS8–RhoA/ROCK1–EMT pathway represents a novel microtubule-dependent signaling route, unique in that it promotes tumor cell invasive behavior by maintaining microtubule network stability rather than modulating dynamics. This finding not only expands our understanding of the functional diversity of microtubule-associated proteins but also provides new insights for developing targeted therapeutic strategies.

In summary, this study systematically elucidates for the first time the expression profile, clinical significance, and molecular mechanism by which HAUS8 promotes glioma invasion through the RhoA/ROCK1-mediated EMT pathway. Our findings not only expand the understanding of microtubule-associated protein functions in tumor biology but also identify a potential novel therapeutic target for glioma. Although the functional role of this signaling axis has been confirmed at the cellular level, further validation in animal models is required to verify its effects *in vivo*, and a more detailed molecular mechanism underlying HAUS8-mediated regulation of RhoA activity remains to be elucidated. Future studies will focus on investigating the specific roles of HAUS8 across different molecular subtypes of

glioma and evaluating the therapeutic potential of targeting this pathway, thereby providing a theoretical foundation for the development of new treatment strategies for glioma.

Declarations

Competing Interests:

The authors declare no conflict of interest.

Consent to publish:

not applicable.

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Author Contribution

H.H. and X.T. contributed equally to this work. J.J. and Y.X. conceived and designed the study. H.H. and X.T. performed the experiments, collected and curated the data, and wrote the original draft. J.Z., J.L., and K.W. developed the methodology and conducted the formal analysis. X.W. and K.W. provided resources and performed software analysis. J.L. and X.W. handled data visualization. J.J. and Y.X. acquired funding, supervised the project, and administered the study. J.J., Y.X., and K.W. reviewed and edited the manuscript. H.H. and J.J. have accessed and verified all the raw data underlying this study. All authors read and approved the final version of the manuscript.

Data Availability

The RNA sequencing data generated in this study have been deposited in the Gene Expression Omnibus (GEO) database under accession number GSE237818 (<https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE237818>). The clinical and expression data from TCGA [Glioblastoma Multiforme project TCGA-GBM (<https://portal.gdc.cancer.gov/projects/TCGA-GBM>) and Brain Lower Grade Glioma project TCGA-LGG (<https://portal.gdc.cancer.gov/projects/TCGA-LGG>), GTEx (<https://www.gtexportal.org/>), and CGGA (<http://www.cgga.org.cn/>, datasets mRNAseq_325 and mRNAseq_693) are publicly available and were accessed in accordance with the respective data use policies. All other data supporting the findings of

this study, including western blot, qPCR, cell proliferation, migration, invasion, flow cytometry, and immunofluorescence data, are available from the corresponding author upon reasonable request. Materials (plasmids, siRNAs, cell lines) used in this study are available from the corresponding author subject to material transfer agreements where applicable.

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Figures

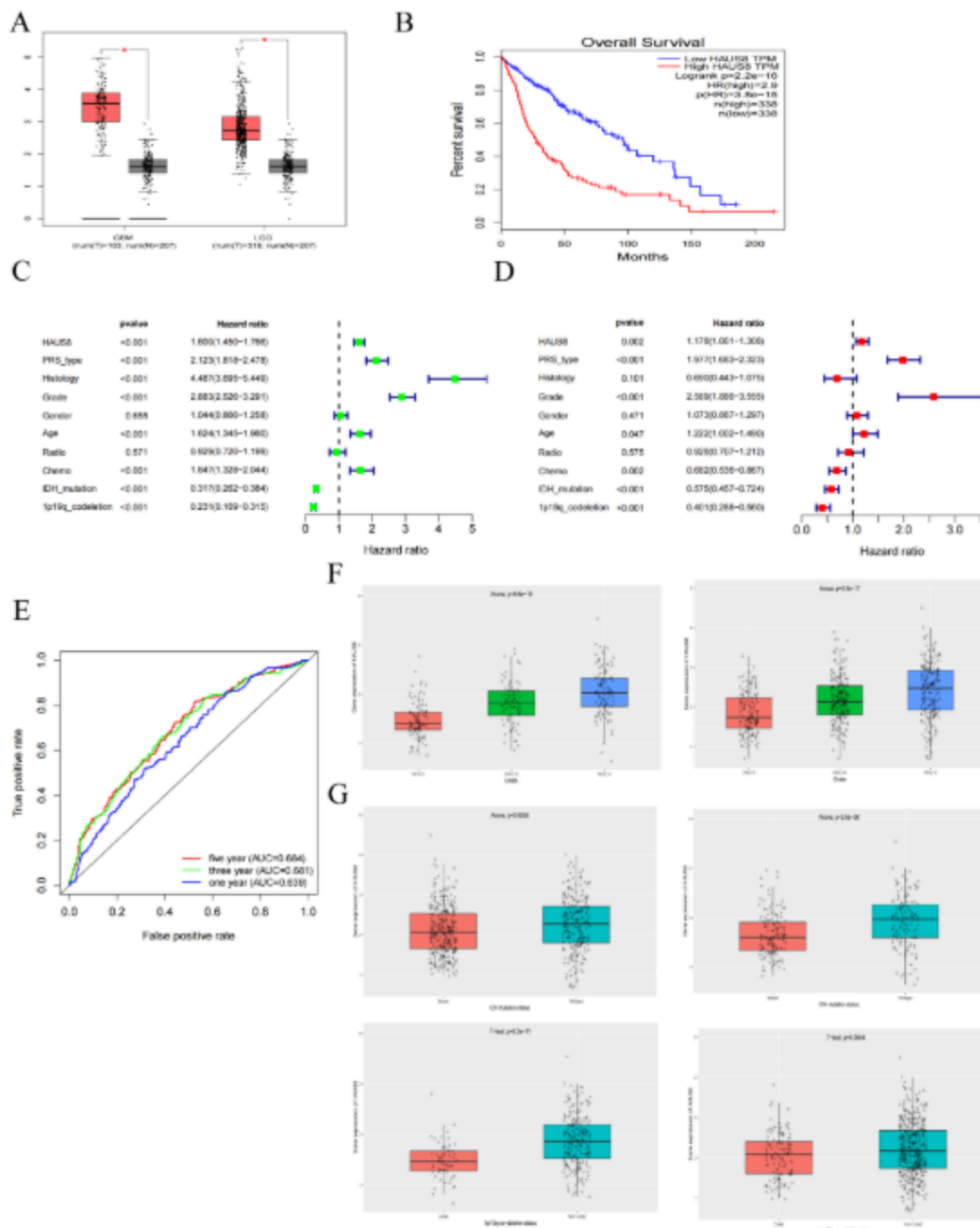


Figure 1

The correlation between HAUS8 expression and the prognosis of glioma. (A) Significant differences of HAUS8 expression in LGG and GBM. (B) Survival curve of differential HAUS8 expression were analyzed by GEPIA. (C, D) Univariate and Multivariate Cox analysis of HAUS8 expression and other clinical pathological factors. (E) The time-dependent receiver operating characteristic (ROC) curves for 1-, 3- and 5-years survival rate. (F) Differential expression of HAUS8 among different WHO grades (Dataset ID: mRNAseq_325) (Dataset ID: mRNAseq_693). (G) The KM survival curve of HAUS8 expression for LGG and GBM patients. (Dataset ID: mRNAseq_325) (Dataset ID: mRNAseq_693)

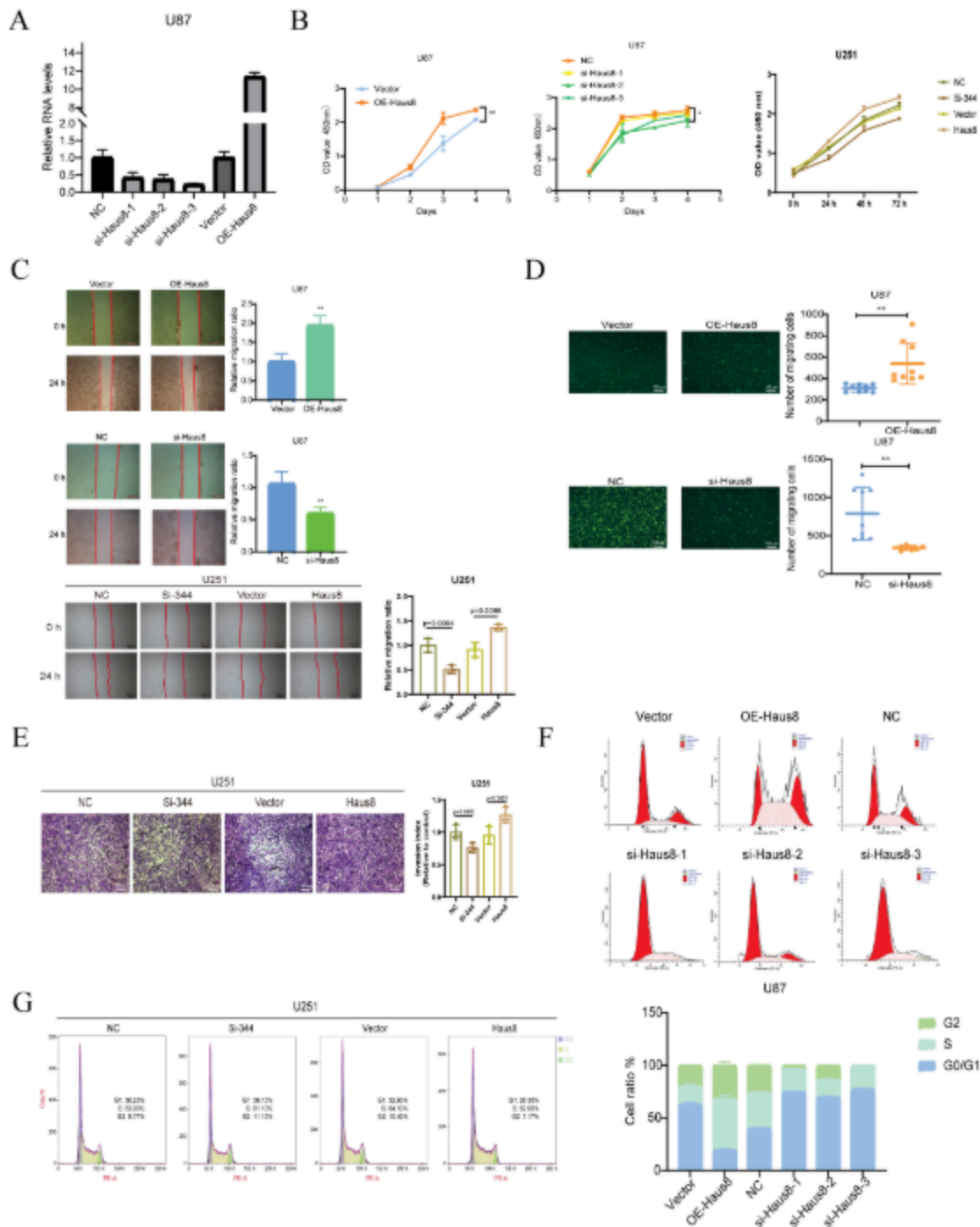


Figure 2

(A) RT-PCR was used to detect the knockdown (si-Haus8-1, si-Haus8-2, si-Haus8-3) and overexpression (OE-Haus8) efficiency of the Haus8 gene in U87 cells. NC is the negative control, and Vector is the vector control. (B) CCK8. (C, D) Wound healing assays. (E, F) Transwell invasion assays. (G) Flow cytometry analysis. Data are presented as mean $\pm$ SD ($n = 3$). Statistical comparisons among multiple groups were performed using one-way ANOVA followed by Bonferroni's multiple comparisons test.

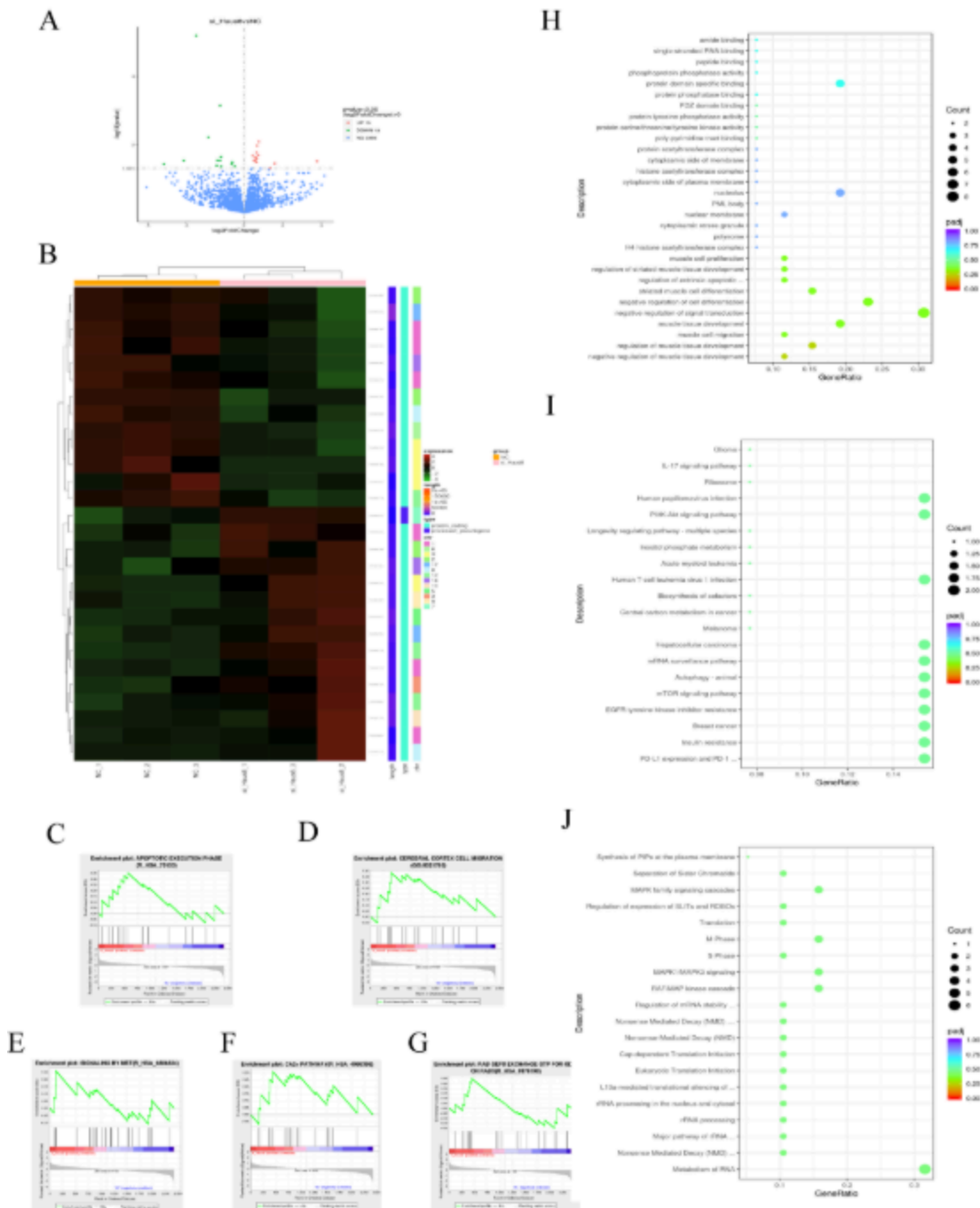


Figure 3

Analysis of transcriptome characteristics after HAUS8 knockdown. (A) Volcano map of differentially expressed genes (red: up-regulated, blue: down-regulated). (B) Based on the information of gene expression changes, draw a heat map and display the clustering tree. (C) GSEA showing apoptotic execution phase. (D) Cerebral cortex cell migration. (E) Signaling by MET. (F) Calcium signaling pathway. (G) RAB GEFs exchange GTP for GDP on RABS. (H) Gene Ontology (GO) enrichment analysis. (I) KEGG pathway analysis. (J) Reactome pathway analysis.

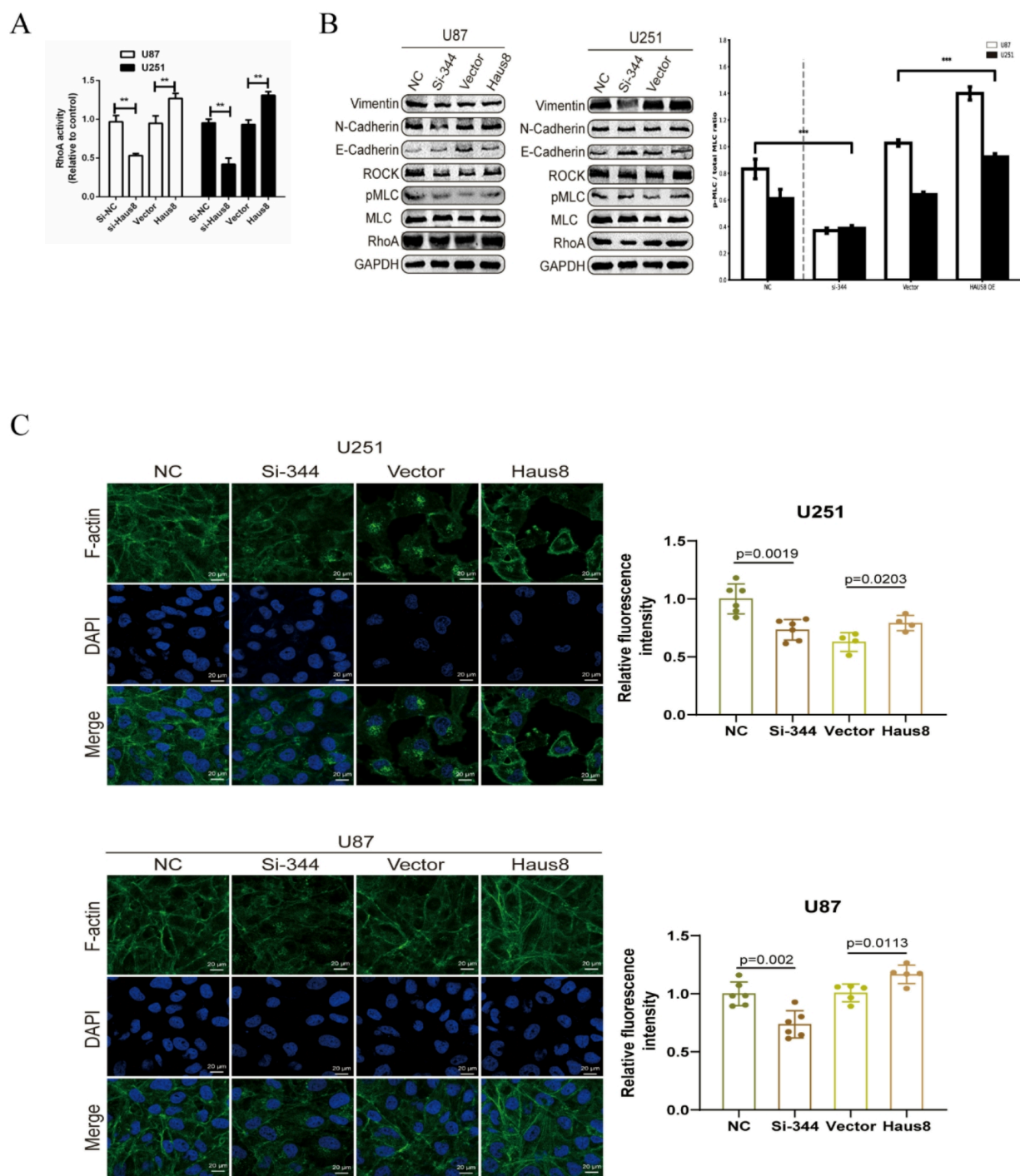


Figure 4

(A) Roha activity detection. (B) Western blot analysis: To clarify the regulatory effect of overexpression and interference of HAUS8 on the RhoA pathway. p-MLC/total MLC ratio quantification following HAUS8 knockdown or overexpression. Cells were transfected as indicated. Data are presented as mean $\pm$ SD ($n=3$). Statistical comparisons among multiple groups were performed using one-way ANOVA followed by Bonferroni's multiple comparisons test. *** $p < 0.001$; (C) Immunofluorescence detection of F-actin.

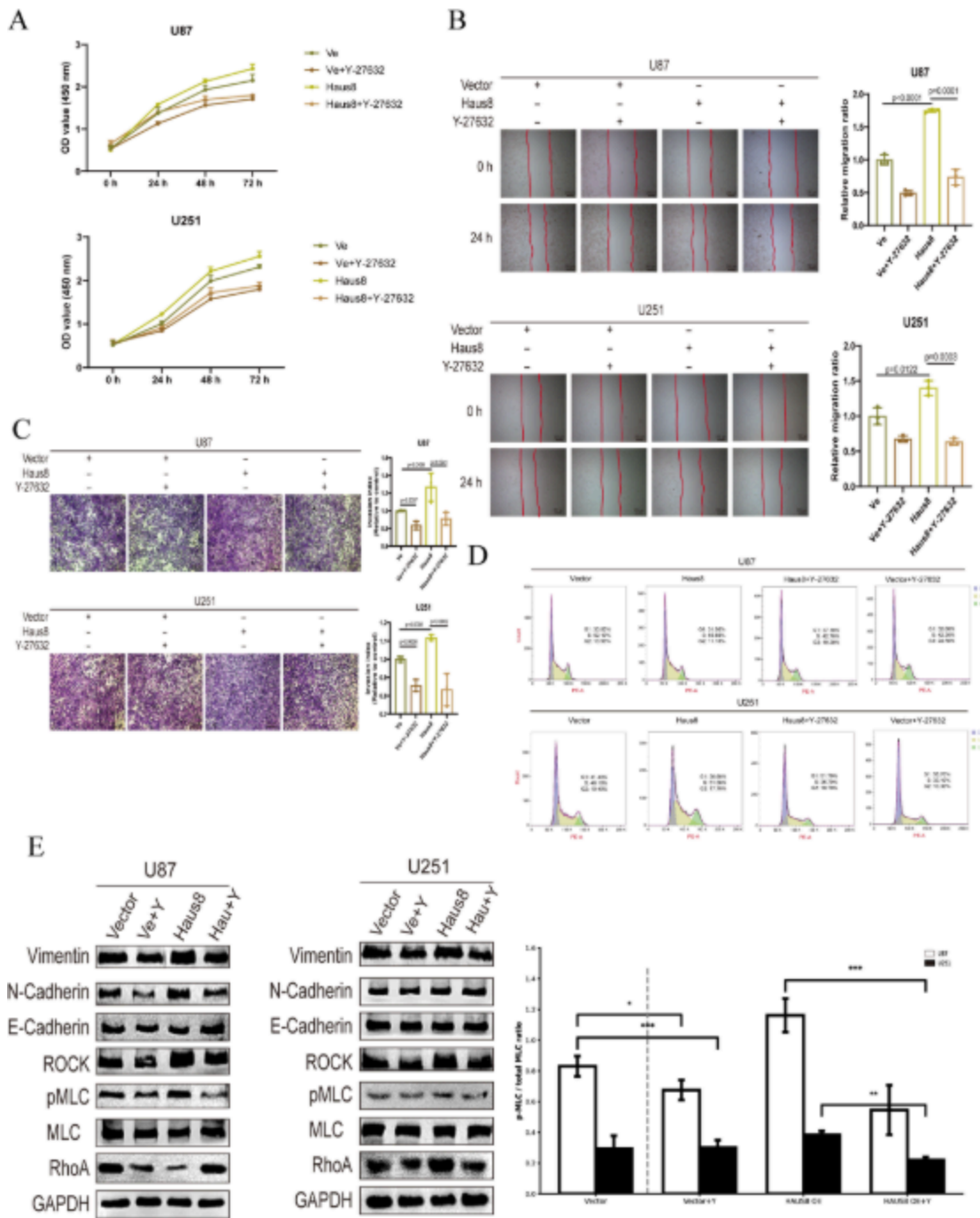


Figure 5

HAUS8 promotes EMT in glioma cells through the RhoA/ROCK1 pathway. (A) CCK8 assay. (B) Wound healing assays. (C) Transwell invasion assays. (D) Flow cytometry analysis. (E) Western blot analysis: To determine whether the role of HAUS8 in promoting epithelial-mesenchymal transition (EMT) is dependent on the RhoA/ROCK1 pathway. p-MLC/total MLC ratio quantification for HAUS8 overexpression with or without Y-27632 treatment. Cells were treated as indicated. Data are presented as mean $\pm$ SD (n=3). Statistical comparisons among multiple groups were performed using one-way ANOVA followed by Bonferroni's multiple comparisons test. *p < 0.05, **p < 0.01, ***p < 0.001; ns, not significant.

Supplementary Files

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