MicroRNA-29 regulates infiltration of glioblastoma stem like cells in orthotopic mouse model

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Purpose: Emerging evidence suggests that glioblastoma-stem like cells (GSCs) substantially influence and communicate with multiple aspects of the tumor microenvironment, including the infiltration. However, the identity of migrating cells and the molecular mechanisms remain unclear. To elucidate the molecular mechanism involved in the infiltration of GSCs, 448T GSC were injected into nude mice brain and sampled infiltrating tumor mass and main mass (infiltrate vs. demarcate) separately. And we compared the expression profiles between two groups using Homo sapiens transcriptome analysis. Finally, 88 miRNAs were upregulated and 44 miRNAs were downregulated in the infiltrated tissue. Among them, we choose miR-29 family, very highly upregulated in infiltrated tumor tissue, and carried out experiment to know how to miR-29 orchestrate GSC infiltration.

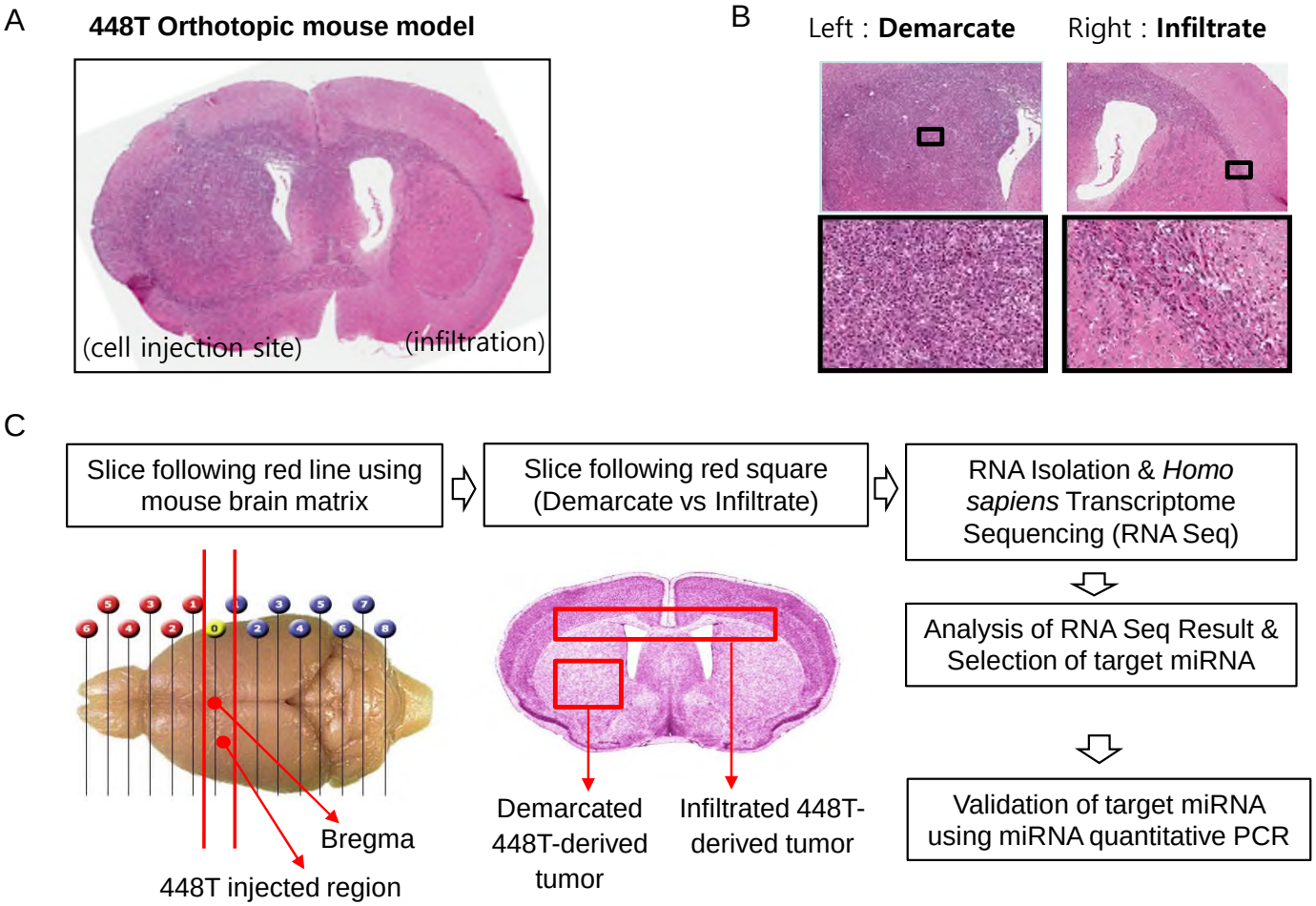

Figure 1. Confirmation of demarcated and infiltrating tumor in 448T glioblastoma stem-like cells (GSCs) orthotopic mouse model. (A,B) H&E staining to confirm the infiltration of 448T GSCs derived tumor in orthotopic model. (C) Flow chart from slice of mouse brain matrix to profiling of genes and miRNAs expression using *Homo sapiens* transcriptome sequencing.

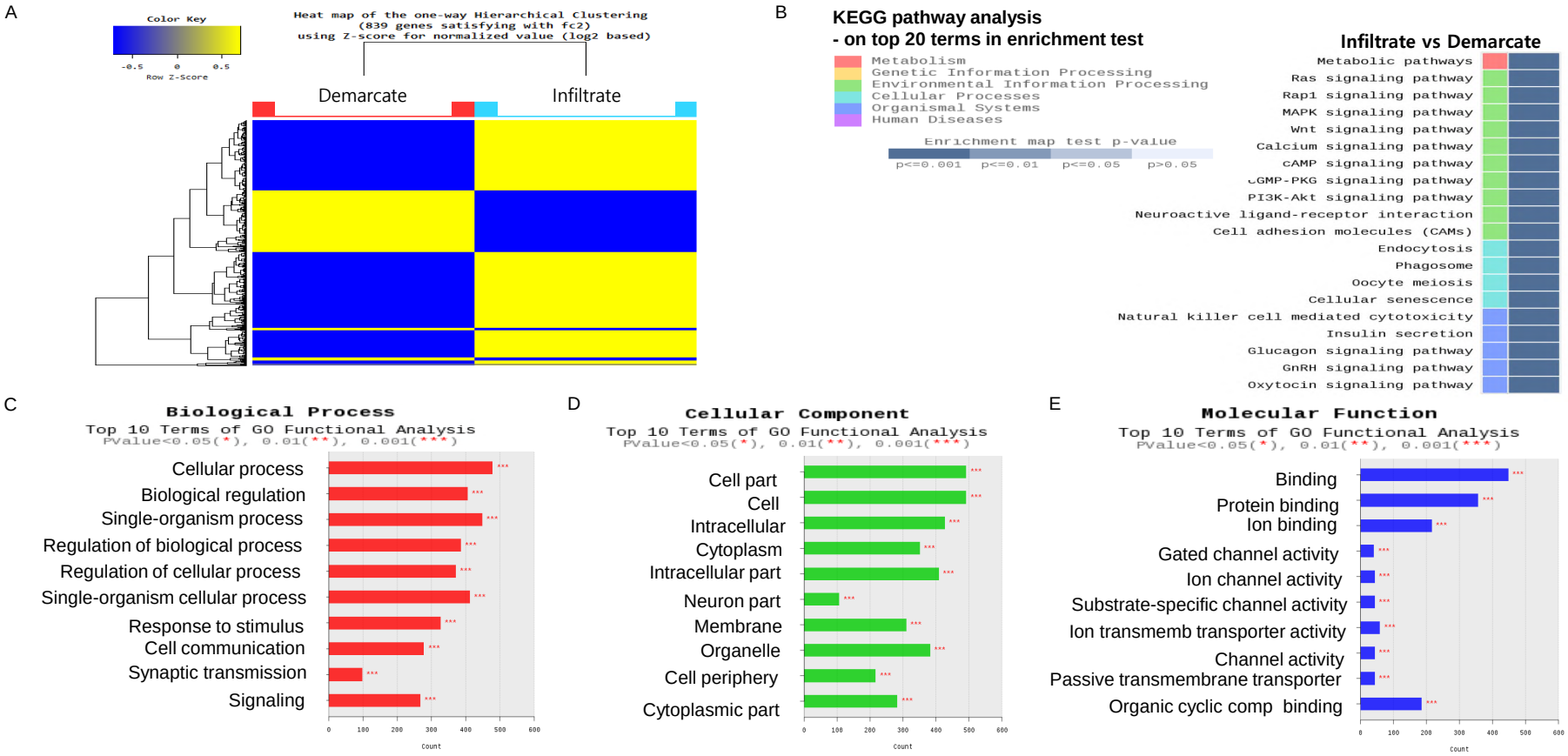

Figure 2. Diverse expression patterns of infiltrated and demarcated tumor tissue of 448T GSC mouse model. (A) Hierarchical clustering map of gene expression levels in infiltrated tumor tissue vs demarcated tissue. (B) The graph shows the top 20 terms in KEGG pathway analysis. (C–E) The graph shows the top 10 terms in Gene Ontology (GO) functional analysis (C, biological process; D, cellular component; E, molecular function).

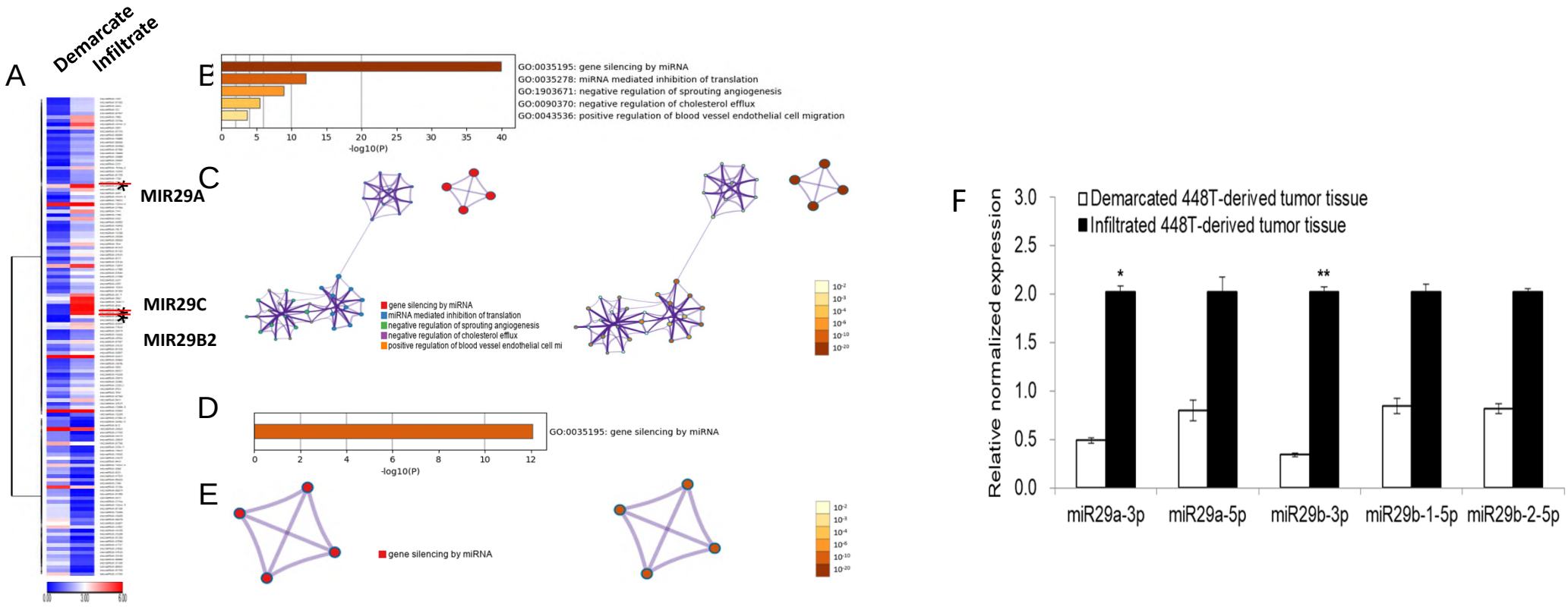

Figure 3. Expression patterns of infiltrated and demarcated tumor tissue of 448T GSC mouse model. (A) Hierarchical clustering map of miRNA expression levels in infiltrated tumor tissue compared with those in demarcated tissue. (B,C) The graph shows the top 5 functional terms (C) and protein-protein interaction clustering map (D) in upregulated miRNA expression. (D,E) The graph shows the top 5 functional terms (D) and PPI clustering map (E) in the downregulated miRNA expression. (F) We could confirmed the enhanced expression of miR29 family (miR29a-3p, miR29a-5p, miR29b-3p, miR29b-1-5p, miR29b-2-5p) in infiltrated 448T tumor tissue using quantitative PCR.

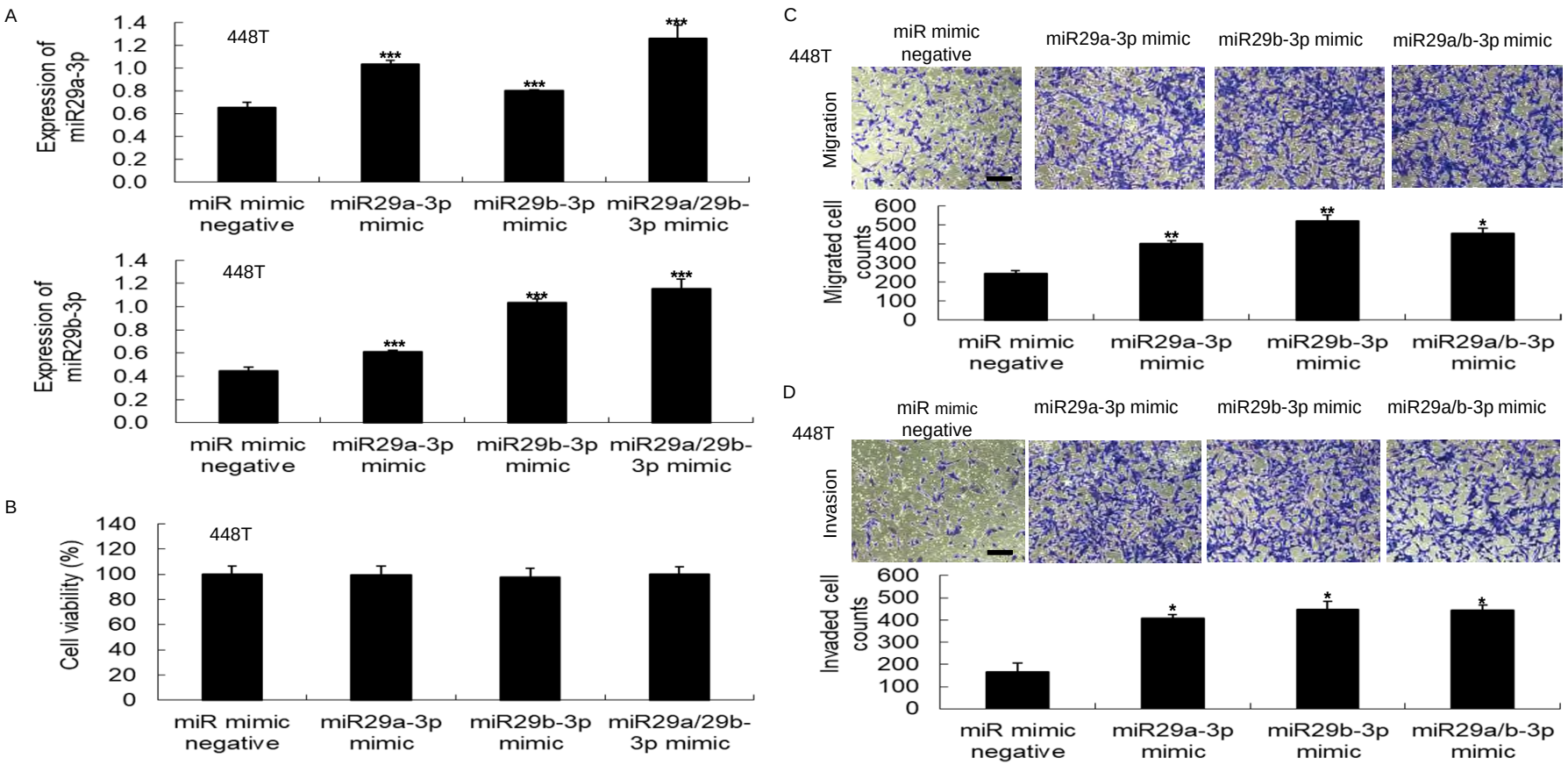

Figure 5. Effect of miR29a-3p/miR29b-3p mimic treatment in 448T. (A) Relative normalized expression of miR29a-3p/miR29b-3p mimic using qPCR in 448T. (B) The cell viability was not significantly increased by miR29a-3p/miR29b-3p mimic treatment in 448T. (C,D) Transwell assays showed that the treatment of miR29a-3p/miR29b-3p mimic enhanced migration and invasion activity of the 448T cells.

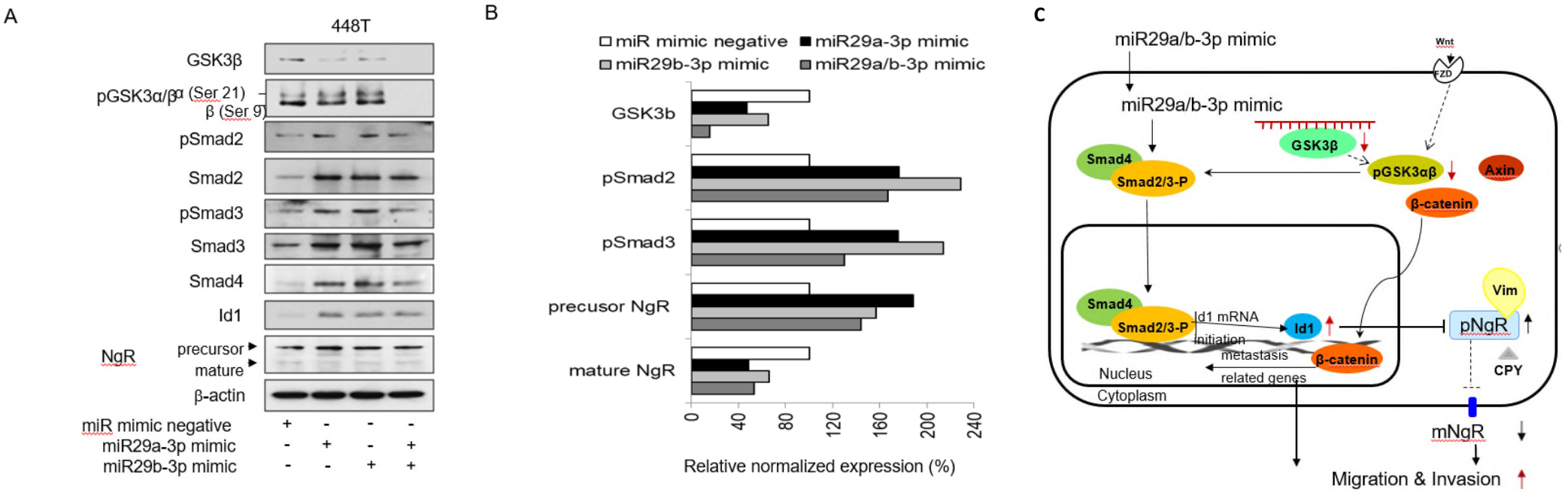

Figure 6. Treatment of miR29a-3p and miR29b-3p mimic increases migration and invasion activity by suppressing NgR maturation through phosphorylation of Smad 2/3 in glioblastoma cells. (A) Western blot analysis shows the expression levels of GSK3β, Smad 2/3/4, Id1, and Nogo receptor in miR29a-3p mimic-treated 448T cells. (B) Expression levels of all proteins were normalized to that of β-actin. After treatment with miR29a-3p mimic upregulated the expression of NgR1 precursor in 448T, resulting in the suppression of NgR maturation. (C) Based on these results, we suggest that the upregulation of miR29a/b-3p expression induces migration and invasive activity in 448T GSC through the involvement of the NgR1 maturation-related pathway.

Conclusion

- Expression of miR29a-3p, miR29a-5p, miR29b-3p, miR29b-1-5p, and miR29b-2-5p were upregulated in the infiltrated 448T-derived tumor tissues compared to demarcated mass and confirmed again using quantitative PCR.
- We treated 448T GSC with a mimic of miR-29 isotypes and determined that miR-29 isotype mimics enhanced GSC migration and invasion activity.
- Previously, we found out that the maturation of Nogo receptor (NgR1) regulates myelin associated glioma cell migration and invasion. In this study, we observed that miR-29 family as a candidate of regulator in infiltration of GSCs involved pathway of NgR1 maturation.
- Our results demonstrate that the miR-29 regulates the infiltration of GSCs, suggesting that inhibition of miR-29 expression in vivo may be a new potential therapeutic target for the suppression of GSC infiltration.