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Supplemental Information

Piwi1 Regulates Glioma Stem Cell Maintenance and Glioblastoma Progression

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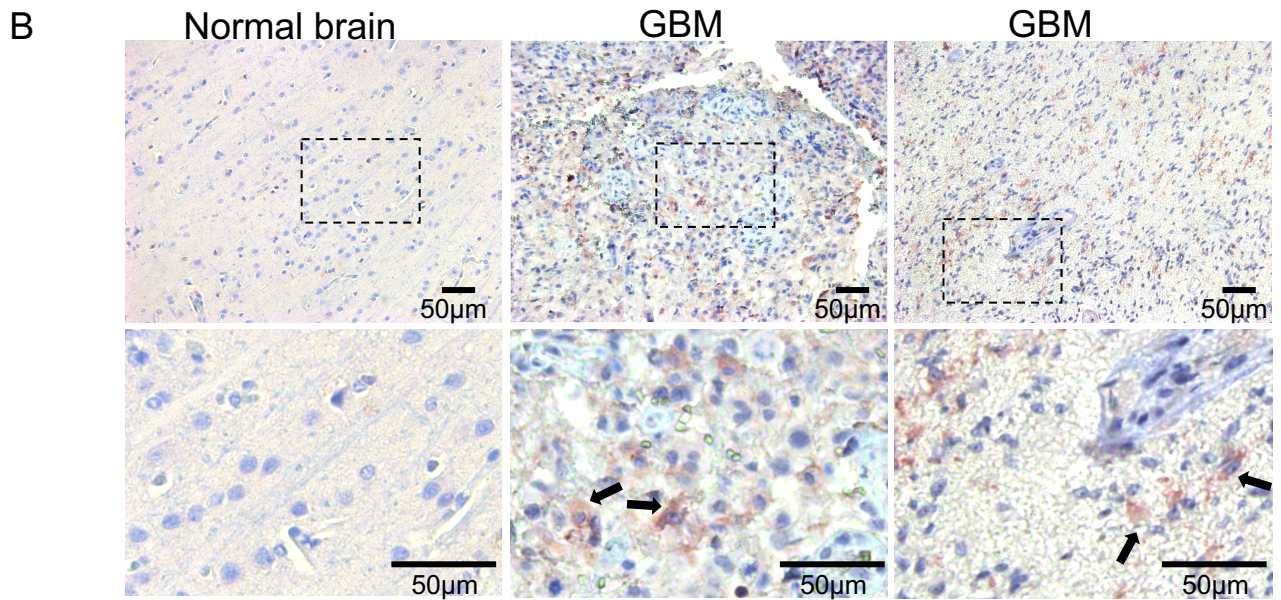
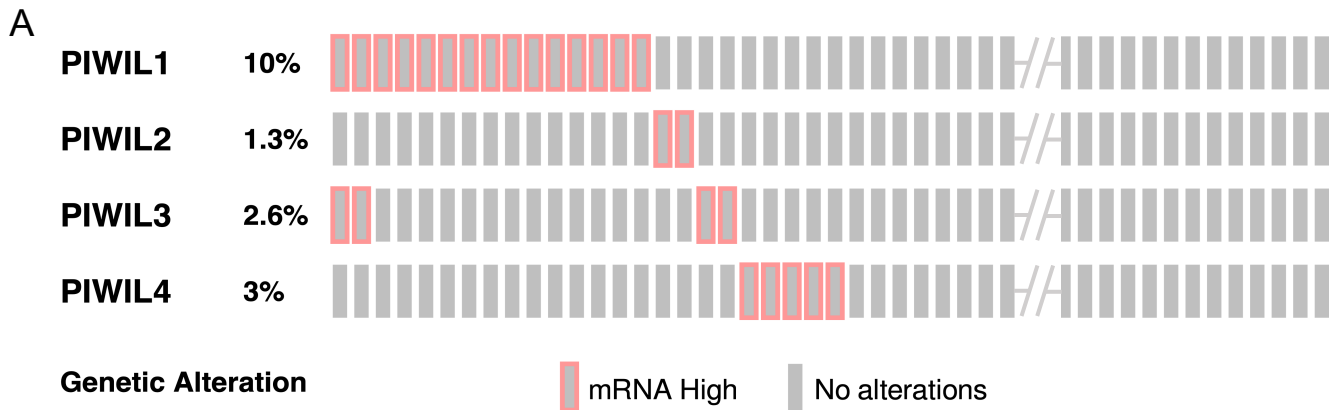


Figure S1. Piwil1 is overexpressed in glioblastoma. Related to Figure 1.

(A) Oncoprint plot of mRNA expression of Piwil1 through Piwil4 using TCGA GBM dataset from cBioPortal. Each gray line represents a different tumor specimen. Samples showing increased expression of Piwi-like genes is highlighted in red.

(B) Immunohistochemical staining of Piwil1 in normal brain and human GBM. The dashed rectangles in the upper panels denotes the magnified views shown in the lower panels. Representative images are shown. Arrows denote cells expressing Piwil1.

Human GBM #16

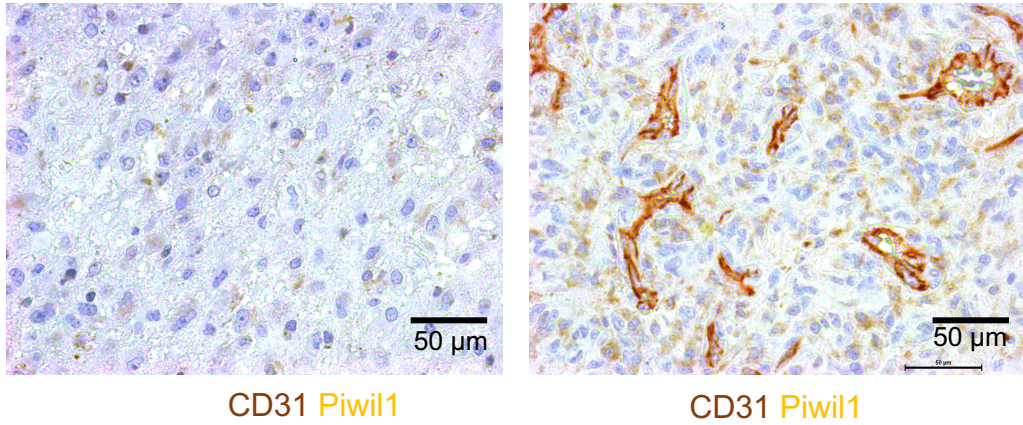
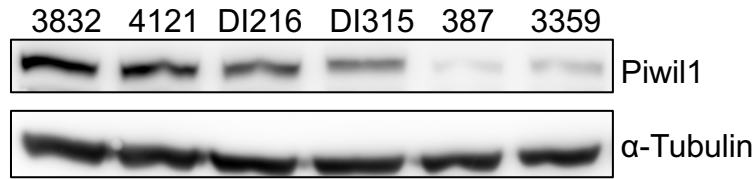


Figure S2. Piwil1 is localized in perivascular niche. Related to Figure 2.

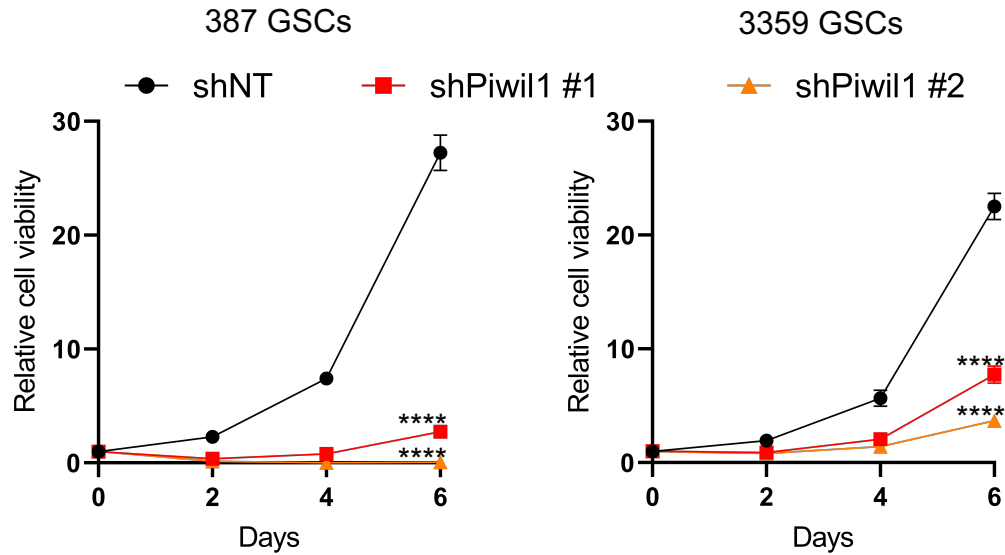
Immunohistochemical staining of Piwil1 (yellow) and CD31 (brown) in human GBM.

A

GSC lines



B



C

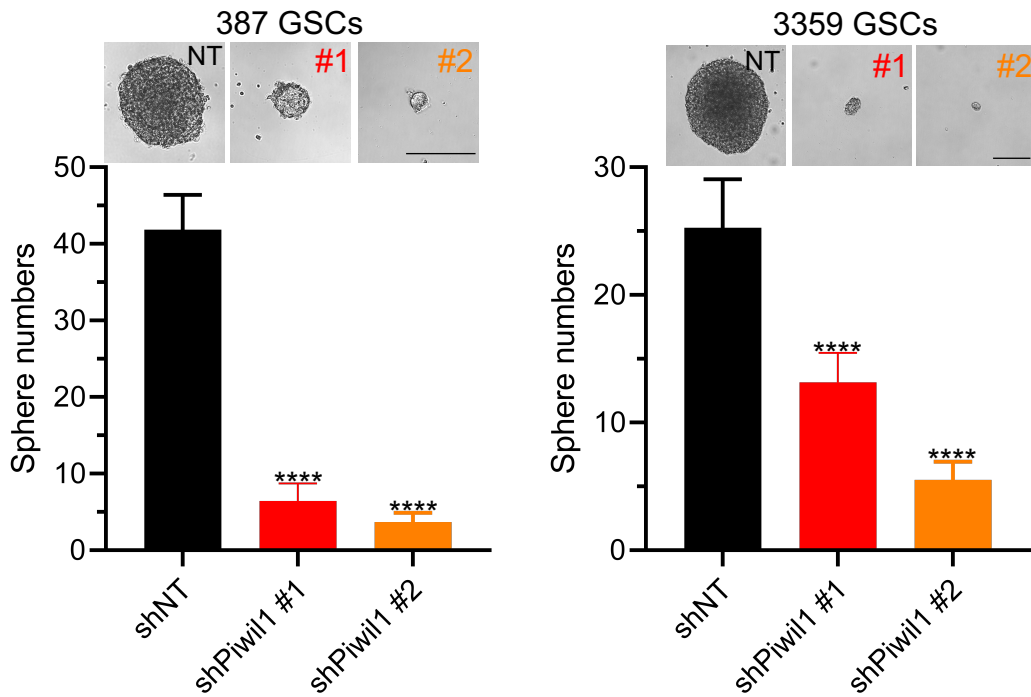


Figure S3. Piwil1 is required for GSC maintenance and survival. Related to Figure 3.

(A) Immunoblot of Piwil1 in 6 GSC lines.

(B) Cell viability of 387 and 3359 GSCs after Piwil1 knockdown (n=4 replicates for each group).

(C) Top panels: Representative images of tumorspheres in control shNT and shPiwil1 #1 and #2 GSCs. Scale bar: 100 μ m. Bottom panels: Quantification of tumor sphere numbers after Piwil1 knockdown (n=12 replicates for each group).

****p < 0.0001. Data are represented as mean \pm SD.

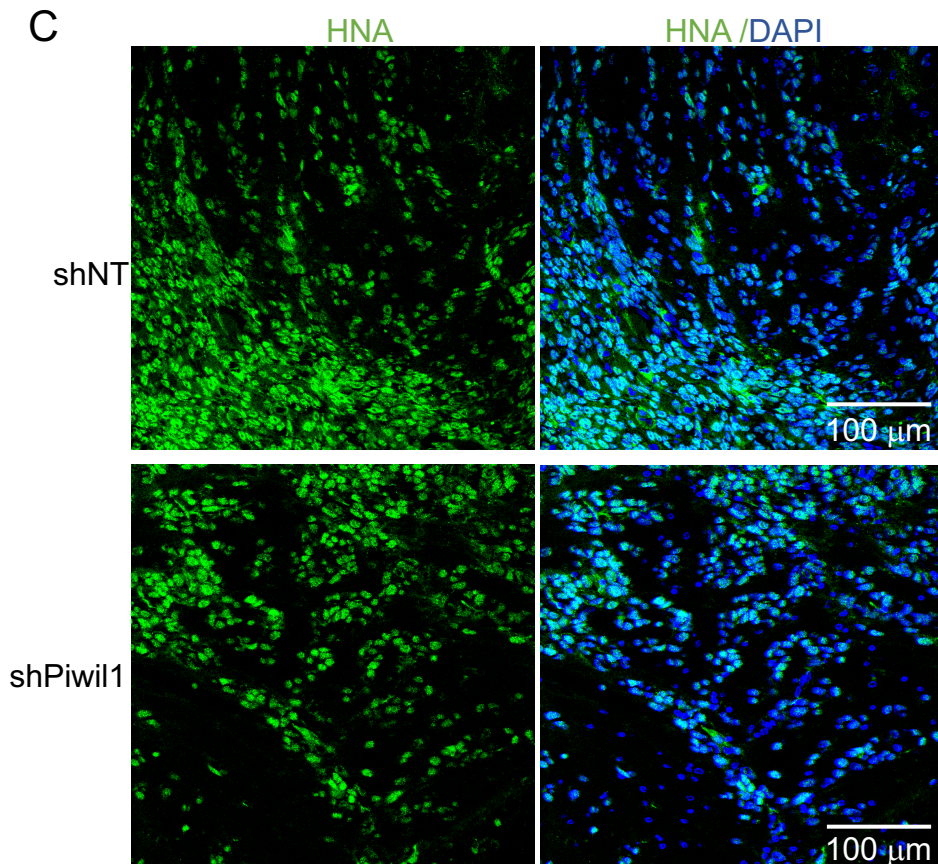
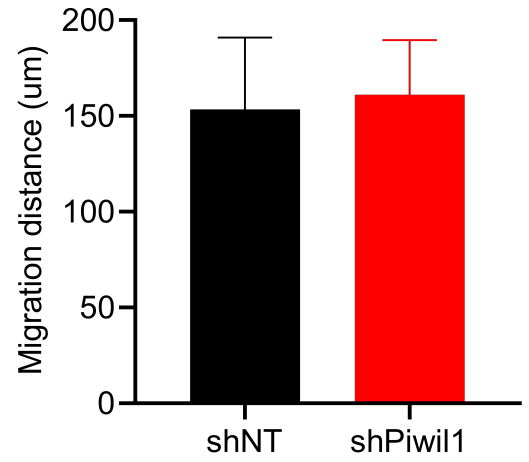
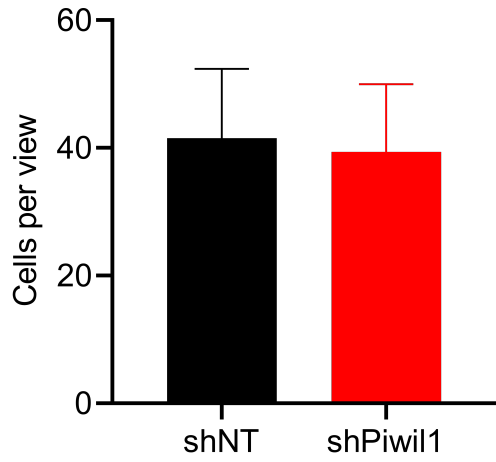
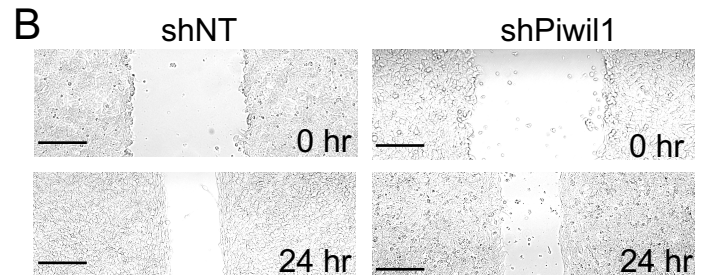
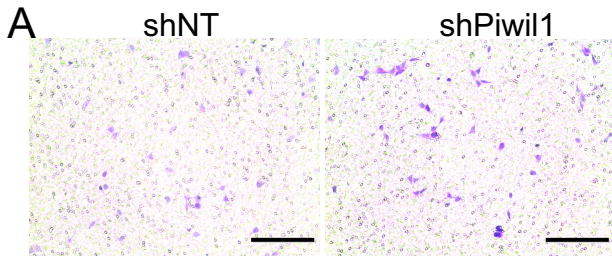


Figure S4. Piwil1 is not required for GSC migration and invasion. Related to Figure 3.

(A) Transwell migration assay of 4121 GSCs after Piwil1 knockdown. Upper panels: Representative images of migratory cells stained with crystal violet. Bottom panels: Quantification of migratory cells (n=5 high-powered fields for each group). Scale bar: 100 μ m.

(B) Wound healing assay of 4121 GSCs after Piwil1 knockdown. Upper panels: Representative images of wound at 0 hr and 24 hr. Bottom panels: Quantification of migration distances (n=13 high-powered fields for each group). Scale bar: 200 μ m.

(C) Immunofluorescence staining of human nuclear antigen (HNA) in shNT and shPiwil1 mice. Representative data from 4121 GSC-derived xenografts are shown. Nuclei were counterstained with DAPI (blue).

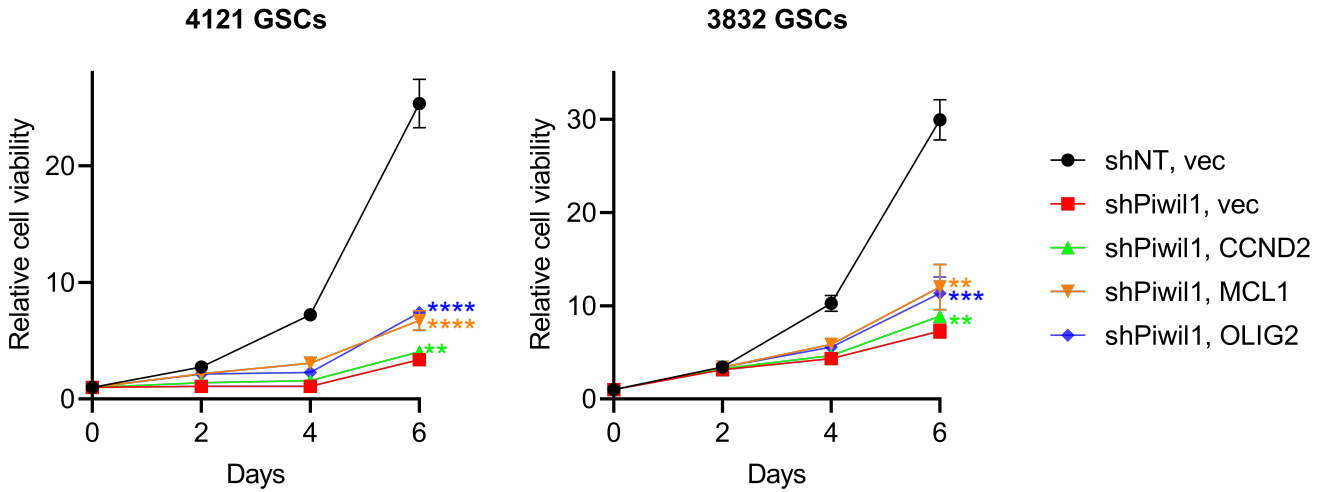


Figure S5. MCL1 and OLIG2 partially rescue Piwil1 knockdown phenotypes. Related to Figure 5.

Cell viability of 4121 and 3832 GSCs after Piwil1 knockdown and expression of vector control, CCND2, MCL1, and OLIG2 (n=4 replicates for each group).

p < 0.01; *p < 0.001, ****p < 0.0001. Data are represented as mean \pm SD.