

Supplementary figure and figure legends

Golgi phosphoprotein 3 promotes Wls recycling and Wnt secretion in glioma progression

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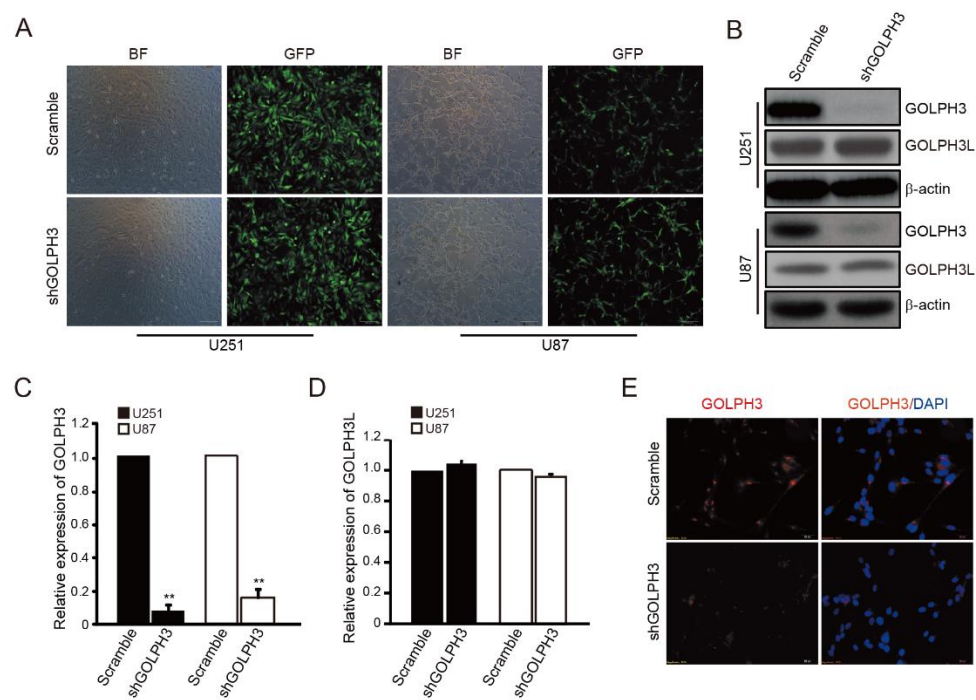
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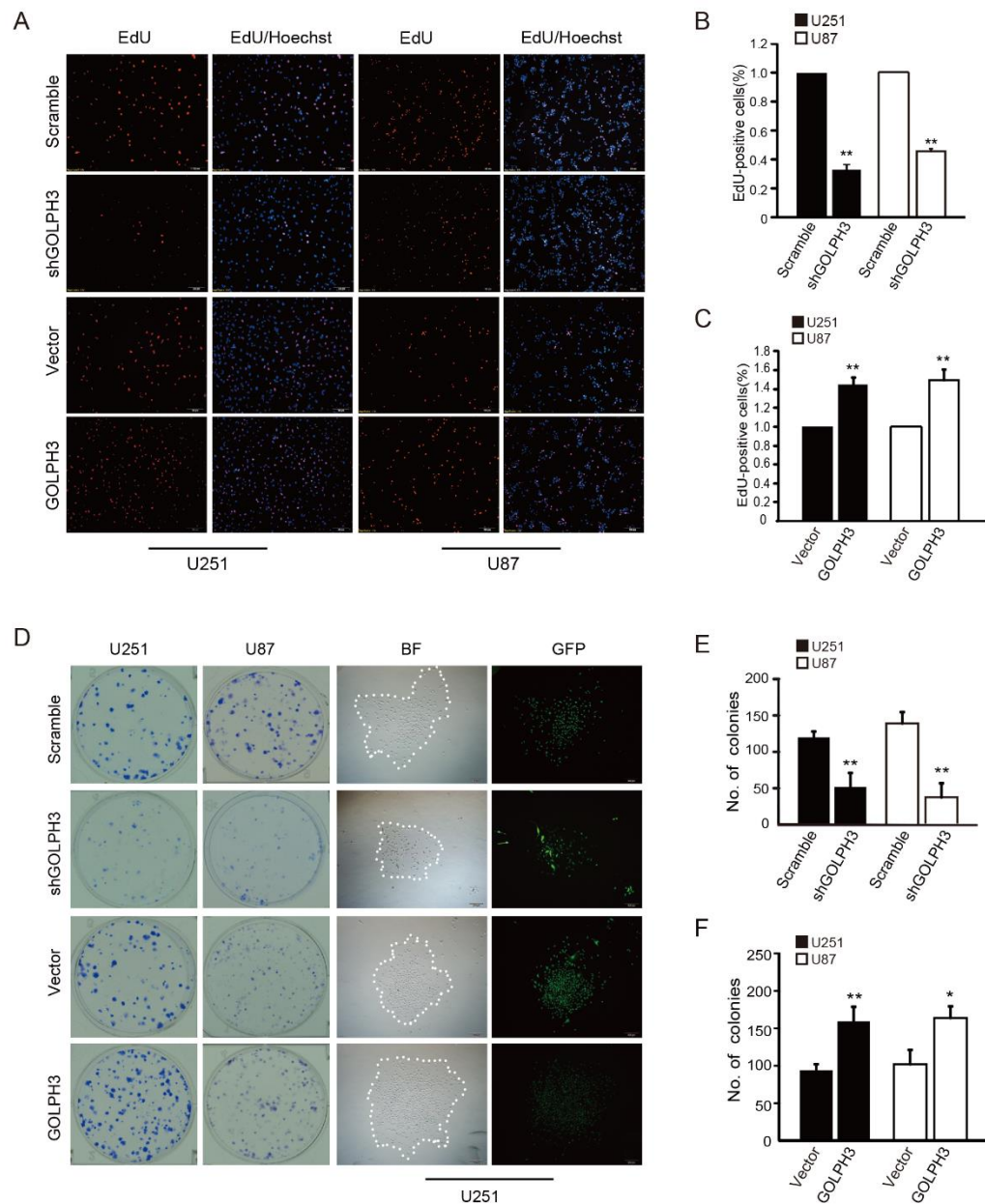
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sFig 1



sFig. 1 Generation of GOLPH3 down-regulation cells. (A) The infection efficiency of GFP tagged scramble and shGOLPH3 lentivirus in U251 and U87 cells. Scale bar, 100 μ m. (B) The silencing efficiency and specificity of shGOLPH3 were examined by western blotting. (C&D) Quantitative analysis. (E) shGOLPH3 knocking-down efficiency was detected by immunofluorescence in U87 cells. Scale bar, 20 μ m.

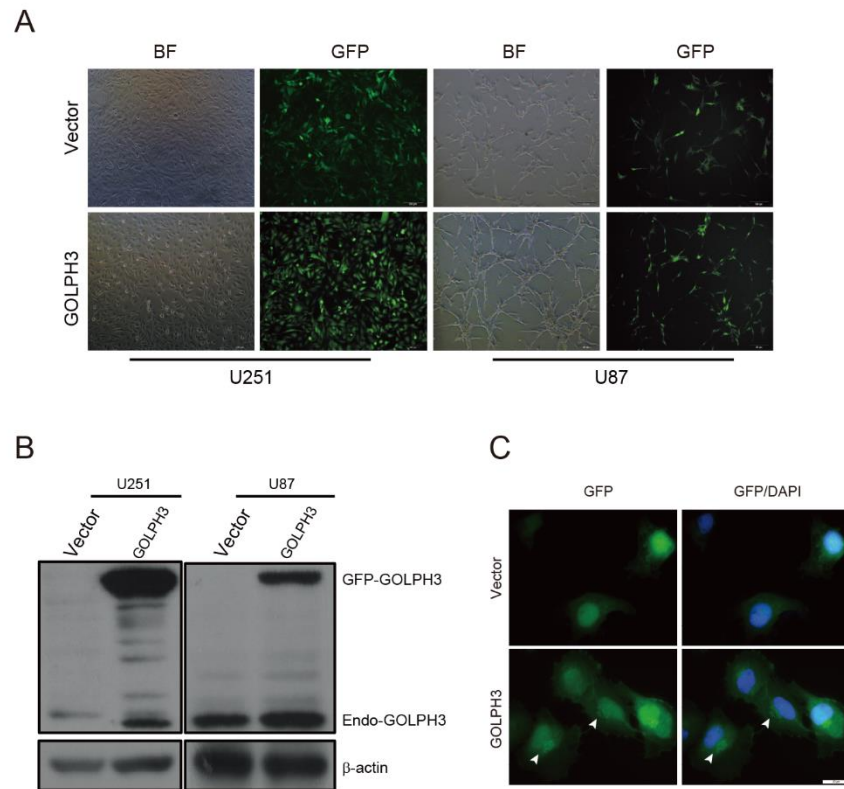
sFig 2



sFig.2 GOLPH3 regulates the proliferation of human glioma cells (A) Representative images of EdU incorporation assay after infecting U251 and U87 cells with indicated lentivirus. The cell proliferation was examined after plating for 48 h. Scale bar, 100 μ m. (B&C) Quantification of the EdU positive cells. (D) Representative images of U251 and U87 colonies infected with indicated lentivirus. Colonies stained with crystal violet after 14 days

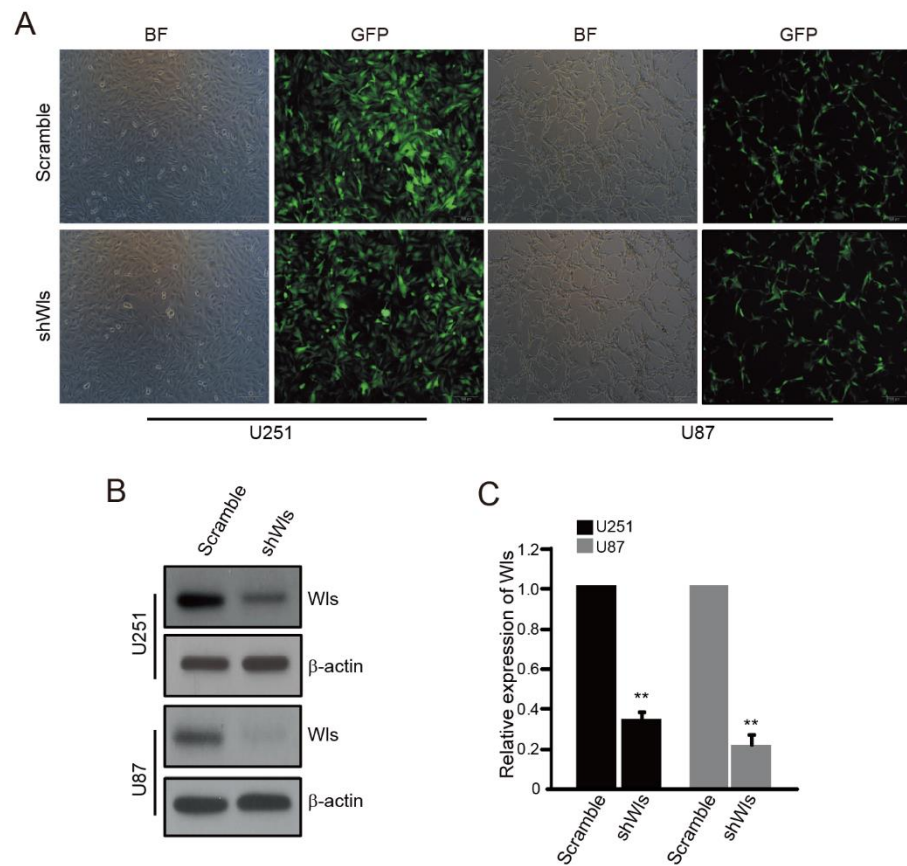
of seeding are shown. Scale bar, 100 μm . Right panel: Bright field (BF) and fluorescent (GFP) images of typical single colonies formed by U251 cells infected with the indicated GFP-tagged lentivirus. **(E&F)** Quantification of the colonies. * $P < 0.05$, ** $P < 0.01$.

sFig 3



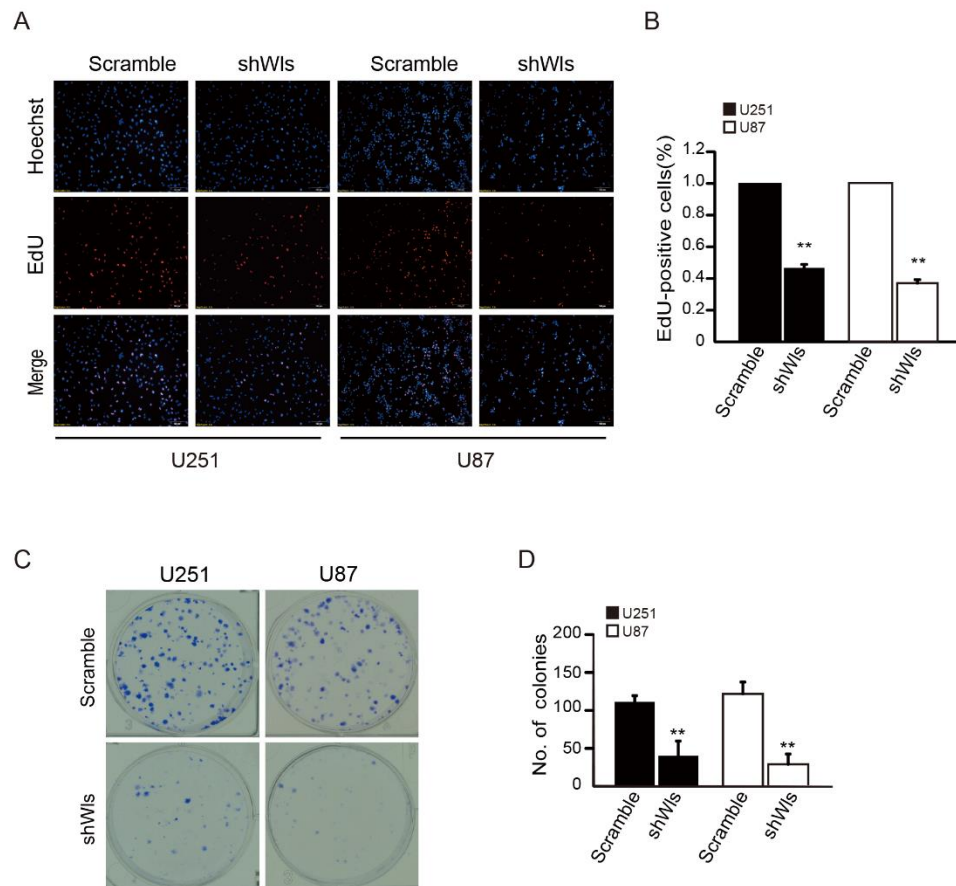
sFig. 3 Generation of GOLPH3 over-expression cells. (A&B) The infection efficiency of vector and GOLPH3 lentivirus in U251 and U87 cells. Scale bar, 100 μ m. **(C)** The location of GFP and GFP-GOLPH3 in U251 cells was shown by immunofluorescence. White arrowheads indicated the GFP-GOLPH3. DAPI indicated the cell nucleus. Scale bar, 10 μ m. BF: bright field. The lentivirus was GFP tagged.

sFig 4



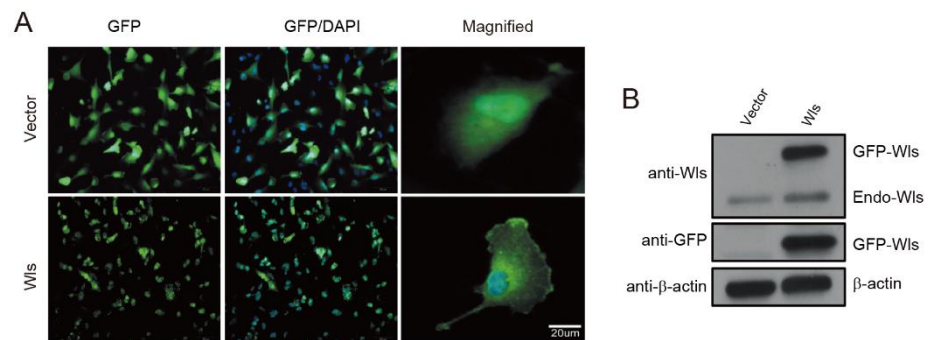
sFig. 4 Generation of Wls down-regulation cells. (A) The infection efficiency of GFP tagged scramble and shWls lentivirus in U251 and U87 cells. Scale bar, 100 μ m. **(B)** The silencing efficiency of shWls was examined by western blotting. **(C)** Quantitative analysis of **(B)**. ** $P < 0.01$.

sFig 5



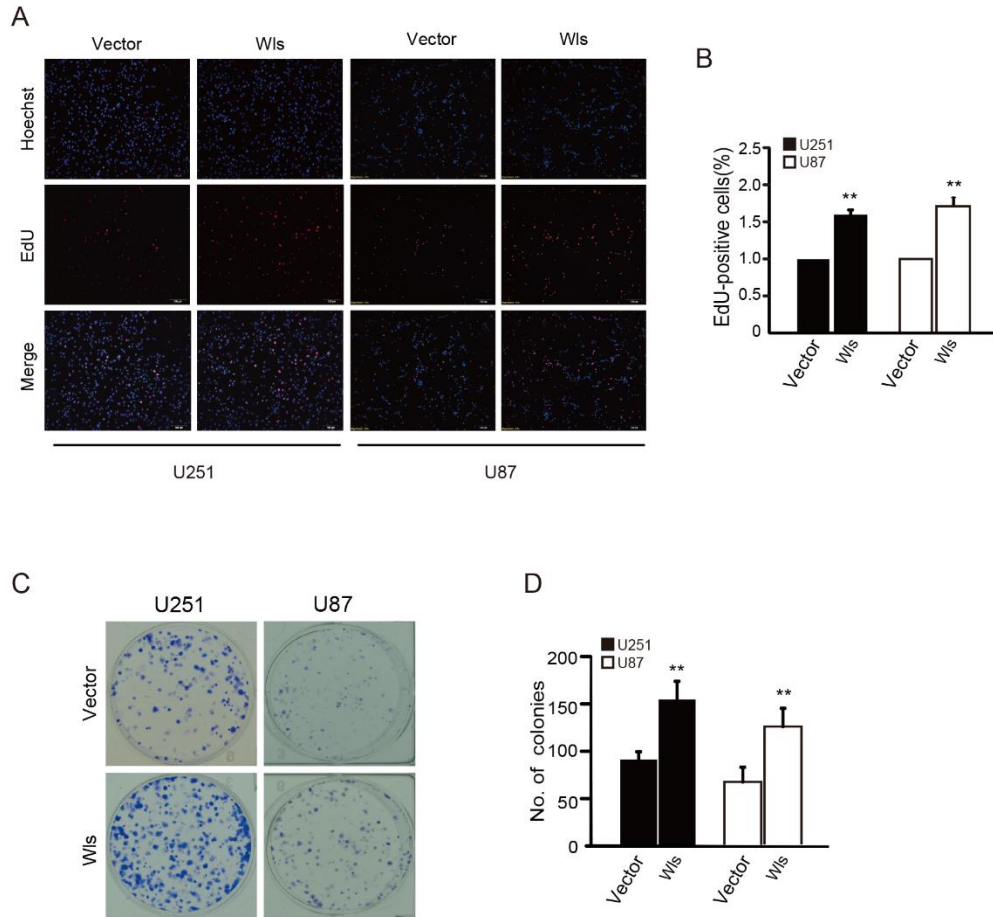
sFig.5 Wls down-regulation inhibits the proliferation of human glioma cells. (A) Representative images of EdU incorporation assay after down-regulation of Wls with indicated lentivirus. Scale bar, 100 μ m. **(B)** Quantification of the EdU positive cells. **(C)** Representative images of colonies infected with indicated lentivirus. Scale bar, 100 μ m. **(D)** Quantification of the colonies. * P <0.05, ** P <0.01.

sFig 6



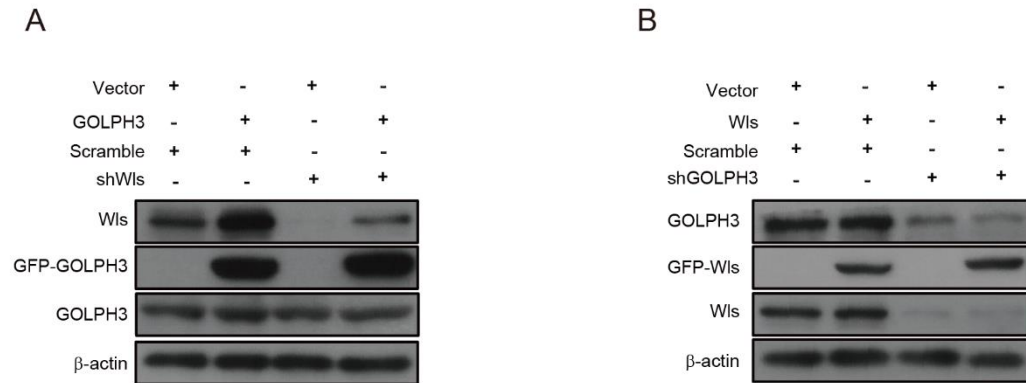
sFig.6 Over-expression of Wls in glioma cells. **(A)** The transfection efficiency of GFP-Wls was shown in U251 cells. Scale bar, 50μm. The Magnified image showed the specific subcellular localization of GFP-Wls. Scale bar, 20μm. **(B)** The over-expression efficiency of GFP-Wls was examined by western blotting.

sFig 7



sFig.7 Over-expression of Wls promotes the proliferation of human glioma cells. (A) Representative images of EdU incorporation assay after over-expression of Wls with indicated lentivirus. Scale bar, 100 μ m. **(B)** Quantification of the EdU positive cells. **(C)** Representative images of colonies infected with indicated lentivirus. Scale bar, 100 μ m. **(D)** Quantification of the colonies. * $P < 0.05$, ** $P < 0.01$.

sFig 8

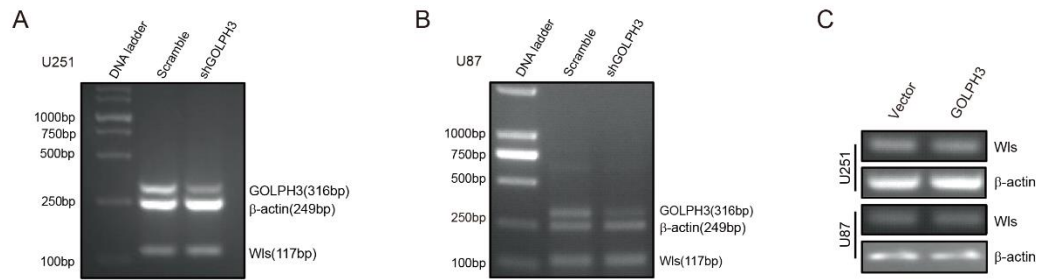


sFig.8 Co-infection of shWls and GOLPH3 or Wls and shGOLPH3 in U251 cells. (A)

Representative blots showed the co-infection of GOLPH3 and shWls lentivirus in U251 cells.

(B) Representative blots showed the transfection of GFP-Wls in shGOLPH3 glioma cells.

sFig 9



sFig.9 GOLPH3 does not affect the Wls mRNA level. (A&B) GOLPH3 knocking down did not affect the mRNA level of Wls in U251 and U87 cells. **(C)** GOLPH3 over-expression did not affect the mRNA level of Wls in U251 and U87 cells.