Figure S1. miR-324-5p inhibits glioma cell proliferation and promotes apoptosis. (A) miR-125a, miR-495-3p and miR-504 expression in glioma tissues. Data were obtained from The Cancer Genome Atlas. (B) NEAT1 expression was monitored using RT-qPCR in U251 and LN229 cells following transfection with miR-324-5p inhibitor or mimics. (C) RT-qPCR of miR-324-5p expression in U251 and LN229 cells transfected with miR-324-5p mimics or a control sequence. (D) Colony formation (magnification, x40) and (E) EdU (magnification x200) assays were used to determine U251 and LN229 cell proliferation following transfection with miR-324-5p mimics or negative control. Flow cytometry of the (F) cell cycle phase distribution and (G) apoptotic rate of U251 and LN229 cells transfected as described for (C). (H) Western blot analysis of the expression levels of CDK4, cyclin D1, Bcl-2 and Bax in cells transfected as described for (C) GAPDH was used as the loading control. The experiments were performed in triplicate and data are presented as the mean ± SD. *P<0.05, **P<0.01 and ****P<0.0001 vs. NBT, Anti-Ctrl, miR-NC or as indicated. Anti-Ctrl, control sequence; EdU, ethynyldeoxyuridine; GBM, glioblastoma; miR, microRNA; NBT, normal brain tissue; NC, negative control; NEAT1, nuclear paraspeckle assembly transcript 1; NS, not significant; RT-qPCR, reverse transcription-quantitative PCR.

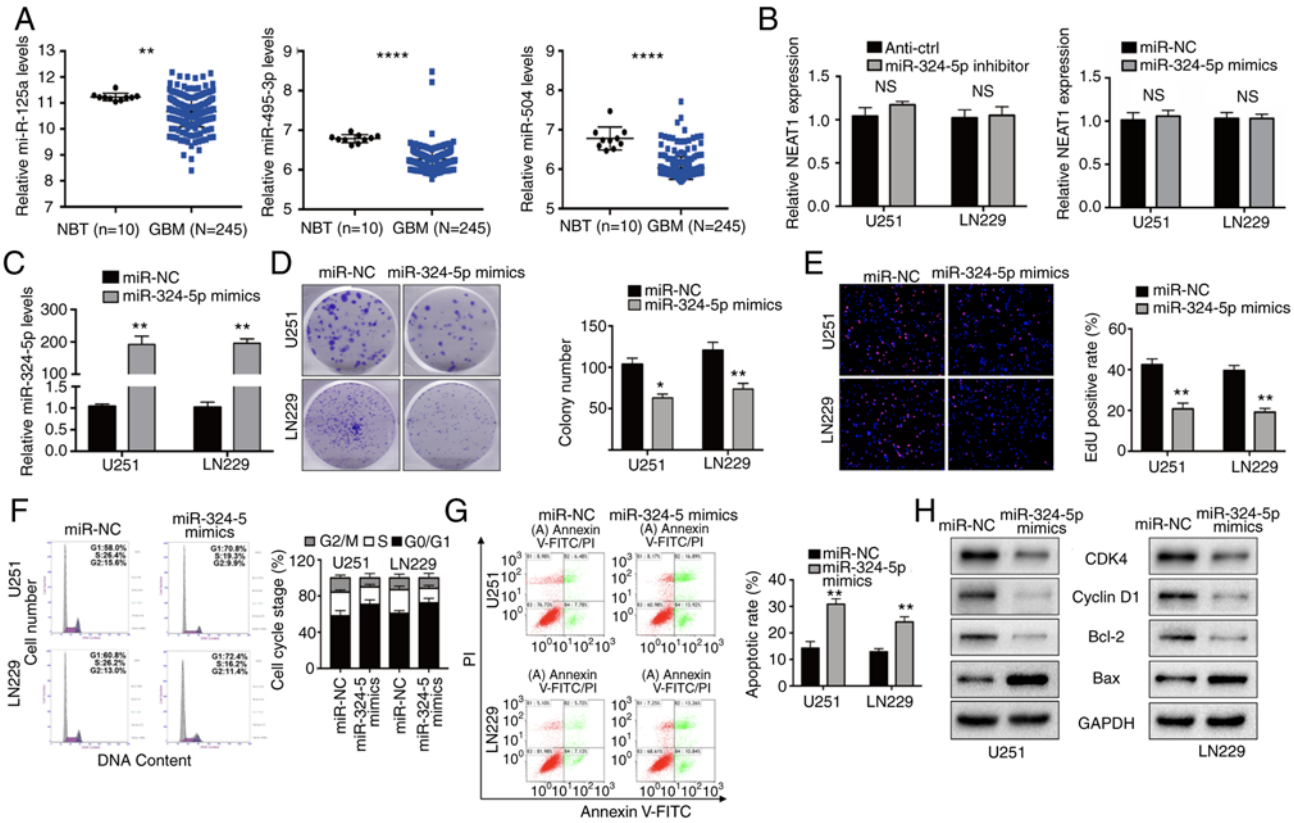

Figure S2. KCTD20 expression is upregulated in glioma tissues. (A) A TCGA dataset was initially used to analyze the mRNA expression levels of five potential miR-324-5p target genes: NIPBL, ELAVL1, KLF3, MMP19 and ZFX. (B) KCTD20 mRNA expression was evaluated in datasets from TCGA and CGGA, and the GSE16011 dataset. (C) Immunohistochemical staining of KCTD20 in sections of glioma tissues and adjacent normal brain tissues. Scale bar, 100 μm. (D) Sections of xenograft glioma tumors excised from mice injected with U251 cells stably expressing shNC or shNEAT1. Scale bar, 100 μm. The experiments were performed in triplicate and data are presented as the mean ± SD. ***P<0.001 and ****P<0.0001. CGGA, Chinese Glioma Genome Atlas; ELAVL1, ELAV like RNA binding protein 1; KCTD20, potassium channel tetramerization protein domain containing 20; KLF3, Kruppel like factor 3; miR, microRNA; NEAT1, nuclear paraspeckle assembly transcript 1; NIPBL, NIPBL cohesin loading factor; shNC, control shRNA; shNEAT1, NEAT1-specific shRNA; shRNA, short hairpin RNA; TCGA, The Cancer Genome Atlas; ZFX, zinc finger protein X-linked.

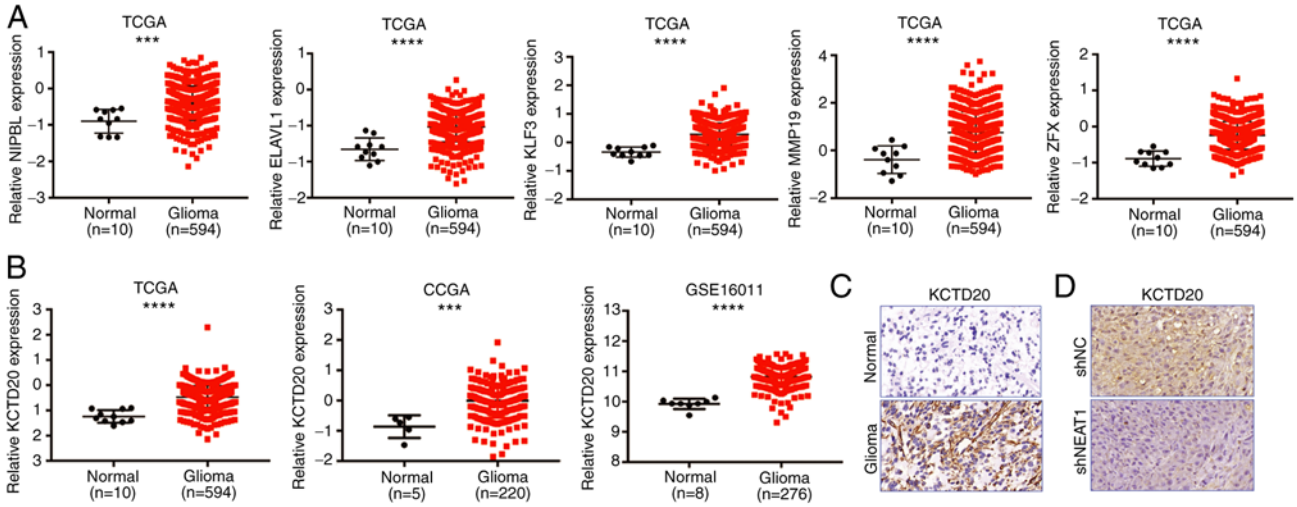

Figure S3. KCTD20 is involved in the tumor-suppressive effects of miR-324-5p. Flow cytometry analysis of the (A) cell cycle distribution and (B) apoptotic rate of U251 and LN229 cells transfected with siKCTD20 or a scrambled sequence. (C) Western blotting of the indicated cell cycle- and apoptosis-related proteins in U251 and LN229 cells transfected as described for (A and B) GAPDH served as the loading control. (D) Representative *in vivo* images of mice injected intracranially with U251 cells transfected with luciferase and either shNC or shKCTD20 (n=6 for each group). Mice were injected with D-luciferin before bioluminescence imaging. Overall survival was determined using Kaplan-Meier survival curves. (E) Representative H&E-stained sections of tumors (magnification, x100) excised from mice injected with U251 cells stably expressing shNC or shKCTD20. Arrows indicate the location of the tumor. (F) Western blot analysis of KCTD20 protein expression in shNC and shKCTD20 orthotopic tumor models. (G) Reverse transcription-quantitative PCR analysis of KCTD20 expression in U251 and LN229 cells transfected with a scrambled sequence, siKCTD20 or siKCTD20 plus miR-324-5p inhibitor. (H) EdU (magnification x200) staining of U251 and LN229 cells transfected as described for (G). (I) Spearman's correlation analysis of the association between miR-324-5p and KCTD20 expression in glioma tissues. The experiments were performed in triplicate and data are presented as the mean ± SD. *P<0.05 and **P<0.01 vs. scrambled, shNC or as indicated. EdU, ethynyldeoxyuridine; KCTD20, potassium channel tetramerization protein domain containing 20; miR, microRNA; shKCTD20, KCTD20-targeting shRNA; shNC, control shRNA; shRNA, short hairpin RNA; siKCTD20, KCTD20-targeting small interfering RNA; T, tumor.