**Supplementary Information**

**Supplemental Materials and methods**

**Primers**

| **primers used for subcloning and plasmid construction** | |
|---|---|
| HOXC10-FL-up | GCCATGGACTACAAGGACGACGATGACAAGACATGCCCTCGCAATGTAAC |
| HOXC10-FL-dn | AGTGCTAGCAAGCTTCATATGTCAGGTGAAATTAAAATTGGAGGTC |
| HOXC10-F1-up | GCCATGGACTACAAGGACGACGATGACAAGGACTCCTGGGGCGACCCCAA |
| HOXC10-F1-dn | AGTGCTAGCAAGCTTCATATGTCAGGTGAAATTAAAATTGGAGGTC |
| HOXC10-F2-up | GCCATGGACTACAAGGACGACGATGACAAGTTCCCTGAGACCCCCAAGTC |
| HOXC10-F2-dn | AGTGCTAGCAAGCTTCATATGTCAGGTGAAATTAAAATTGGAGGTC |
| HOXC10-F3-up | GCCATGGACTACAAGGACGACGATGACAAGGCGAAAGAGGAGATAAAGGC |
| HOXC10-F3-dn | AGTGCTAGCAAGCTTCATATGTCAGGTGAAATTAAAATTGGAGGTC |
| HOXC10-F4-up | GCCATGGACTACAAGGACGACGATGACAAGGACTCCTGGGGCGACCCCAA |
| HOXC10-F4-dn | AGTGCTAGCAAGCTTCATATGACTCACTTTGCCCCCCAGC |
| **primers used for real-time RT-PCR** | |
| GAPDH-up | GGAGCGAGATCCCTCCAAAAT |
| GAPDH-dn | GGCTGTTGTCATACTTCTCATGG |
| HOXC10-up | CTATCCGTCCTACCTCTCGCA |
| HOXC10-dn | CCTGCCAACAGGTTGTTCC |
| VEGFA-up | AGGGCAGAATCATCACGAAGT |
| VEGFA-dn | AGGGTCTCGATTGGATGGCA |
| WDR5-up | TGCTGCAACTTCAATCCCCA |
| WDR5-dn | GTGTCCCAGATGCGACAGAG |
| PRMT5-up | TCACTCTGAGTATCCGTCCA |
| PRMT5-dn | ATTGCTGCATCGCCAGAAAC |

| primers used for VEGFA promoter reporter construction | |
|---|---|
| P1-luc-up | CGAGCTCTTACGCGTGCTAGCAAGATCTGGGTGGATAATCAGACTG |
| P1-luc-dn | ACTTAGATCGCAGATCTCGAGAGAAGTTGGACGAAAAGTTTCAGTG |
| P2-luc-up | CGAGCTCTTACGCGTGCTAGCCCCATTTCTATTCAGAAGATGAGCT |
| P2-luc-dn | ACTTAGATCGCAGATCTCGAGAGAAGTTGGACGAAAAGTTTCAGTG |
| P3-luc-up | CGAGCTCTTACGCGTGCTAGCCACTCCAGGATTCCAATAGATCTGT |
| P3-luc-dn | ACTTAGATCGCAGATCTCGAGAGAAGTTGGACGAAAAGTTTCAGTG |
| P4-luc-up | CGAGCTCTTACGCGTGCTAGCTCTACTTCCCCAAATCACTGTGG |
| P4-luc-dn | ACTTAGATCGCAGATCTCGAGAGAAGTTGGACGAAAAGTTTCAGTG |
| P5-luc-up | CGAGCTCTTACGCGTGCTAGCAAGATCTGGGTGGATAATCAGACTG |
| P5-luc-dn | ACTTAGATCGCAGATCTCGAGACAGATCTATTGGAATCCTGGAGTG |
| P6-luc-up | CGAGCTCTTACGCGTGCTAGCGATGGGTAATTTTCAGGCTGTGA |
| P6-luc-dn | ACTTAGATCGCAGATCTCGAGAGTGATTTGGGGAAGTAGAGCAA |
| P7-luc-up | TTTTTTTAAAACTGTATTGTTTCTCCCGCTAATTTATTTTTGCTTGCCATTCCC |
| P7-luc-dn | GGGAATGGCAAGCAAAAATAAATTAGCGGGAGAAACAATACAGTTTTAAAAAAA |
| **VEGFC promoter primer used for ChIP assays** | |
| Region 1-up | AATAGCCAGGTCAGAAACCAGCTAG |
| Region 1-dn | CTCCAACTCTCCACATCTTCCCTAA |
| Region 2-up | CCTGGGCTCTCTGTACATGAAGCAA |
| Region 2-dn | CTAAAGAGGGAATGGGCTTTGGAAA |
| Region 3-up | ATATTCATTGATCCGGGTTTTATCC |
| Region 3-dn | GTGATTTGGGGAAGTAGAGCAATCT |

**Immunohistochemistry (IHC)**

Immunohistochemistry assays were performed and quantified, as previously described[1].

IHC analysis was performed to study differential HOXC10 protein expression between

normal brain tissue and 94 paraffin-embedded and archived glioma tissue samples, using an antibody against HOXC10 (Abcam). The degree of immunostaining of formalin-fixed, paraffin-embedded sections was reviewed and scored separately by three independent pathologists who were blinded to the histopathological features and patient data of the samples. Scores were determined by combining the proportion of positively stained tumor cells and the intensity of staining. The scores given by the three independent pathologists were combined into a mean score for further comparative evaluation. Tumor cell proportions were scored as follows: 0: no positive tumor cells; 1: <10% positive tumor cells; 2: 10%-35% positive tumor cells; 3: 35%-75% positive tumor cells; 4: >75% positive tumor cells. Staining intensity was graded according to the following standard: 1: no staining; 2: weak staining (light yellow); 3: moderate staining (yellow brown); 4: strong staining (brown). The staining index (SI) was calculated as the product of the staining intensity score and the proportion of positive tumor cells. Using this method of assessment, we evaluated protein expression in malignant lesions by determining the SI, with possible scores of 0, 2, 3, 4, 6, 8, 9, 12, and 16. The median value, SI = 8, was chosen as the cutoff value; by this metric, samples with a SI ≥ 8 were defined as high expression and samples with a SI < 8 were defined as low expression.

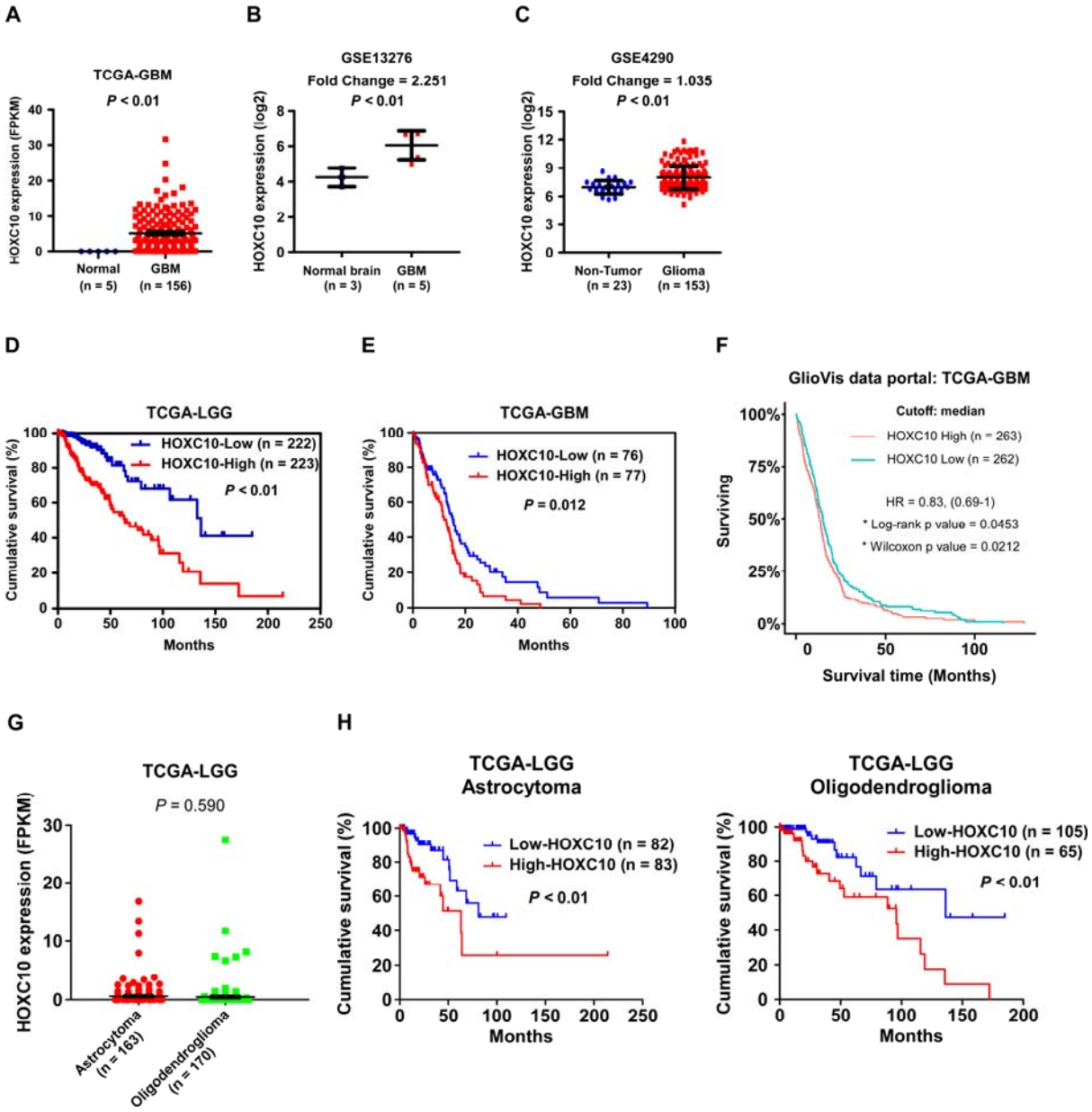
The method of Mean Optical Density (MOD) is defined as the mean of immunostaining intensities per positive pixels in specimens of each grade, and was performed as previously described. Briefly, the stained slides were evaluated at 200× magnification using the AxioVision Rel.4.6 computerized image analysis system, and ten random fields in each specimen were analyzed to determine the MOD of each specimen. In order to avoid possible bias in selecting fields that are more cellular or heavily stained, the representative staining fields of each tumor sample were analyzed and scored independently by three observers. The MOD data were statistically analyzed using t-test to compare the MOD differences between groups, and $P < 0.05$ was considered significant.

## Reference

1 Chen, X. *et al*. Acylglycerol kinase augments JAK2/STAT3 signaling in esophageal squamous cells. *J. Clin. Invest.* **123**, 2576-2589, doi:10.1172/JCI68143 (2013).

## Supplementary Figures and Figure Legends

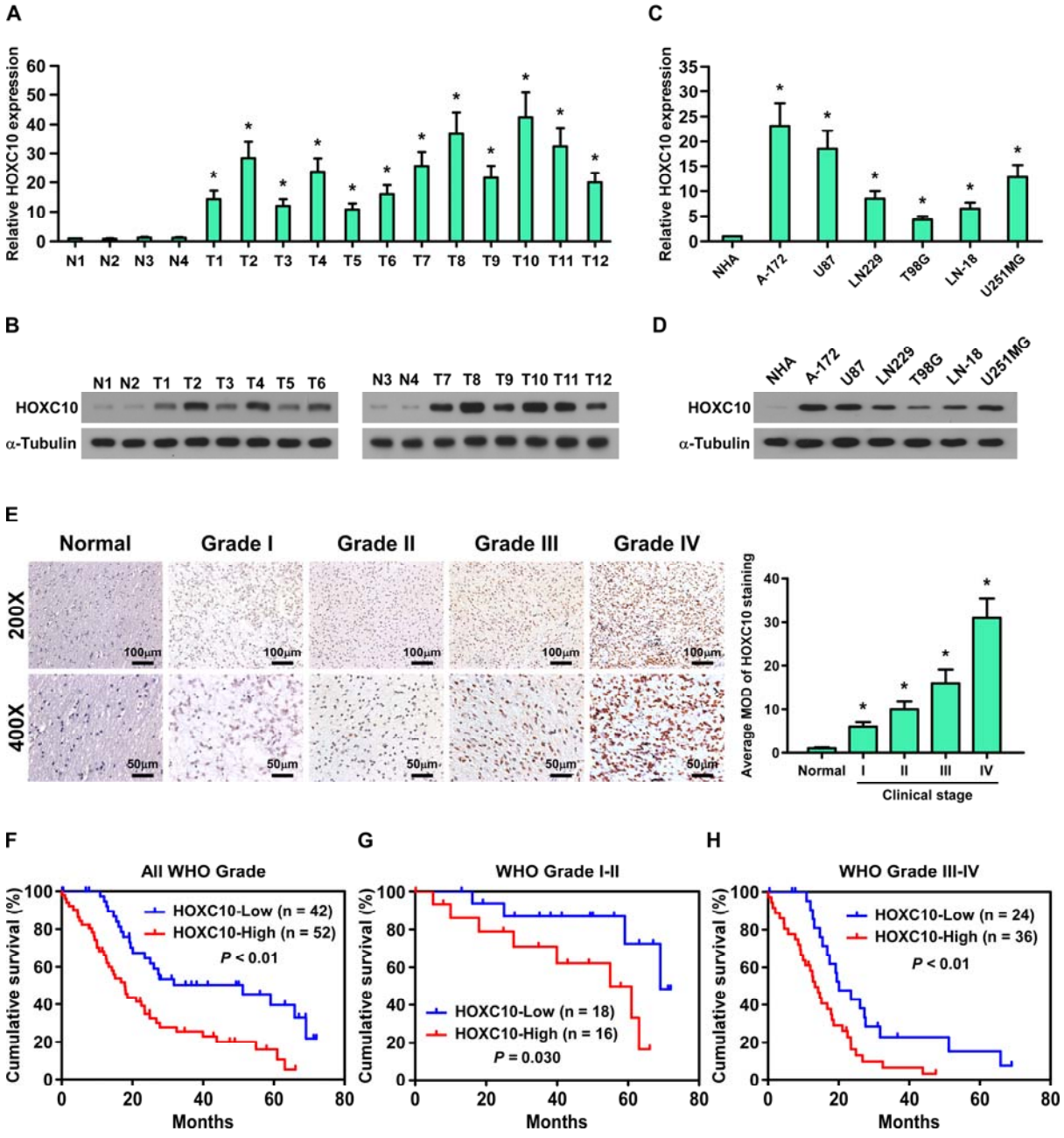
### Supplementary Figure S1




**Supplementary Figure S1. HOXC10 is predicted to be overexpressed in gliomas and elevated HOXC10 predicts poor outcome of patients with glioma**. (**A**) Expression analysis of *HOXC10* mRNA in GBM (n = 156) and normal brain tissues (n = 5; $P < 0.01$; TCGA). (**B**) Expression analysis of *HOXC10* mRNA in GBM tissues (n = 5) and normal brain tissues (n = 3; $P < 0.01$; GSE13276). (**C**) Expression analysis of *HOXC10* mRNA in glioma tissues (n = 153) and non-tumor tissues (n = 23; $P < 0.01$; GSE4290). Error bars, SD. (**D**) Kaplan–Meier

analysis of overall survival in patients with LGG with low and high *HOXC10* expression levels (TCGA-LGG; n = 445; $P < 0.01$). (**E**) Kaplan–Meier analysis comparing overall survival in patients with GBM with low and high *HOXC10* expression levels (TCGA-GBM; n = 153; $P = 0.012$). (**F**) Kaplan–Meier analysis comparing survival in patients with GBM with low and high *HOXC10* expression levels (HG-UG133A dataset; n = 525; $P = 0.0453$; TCGA). (**G**) TCGA analysis of expression of HOXC10 in astrocytomas and oligodendrogliomas. (**H**) Kaplan–Meier analysis comparing survival in patients with astrocytomas (left) and oligodendrogliomas (right) with low and high *HOXC10* expression levels.

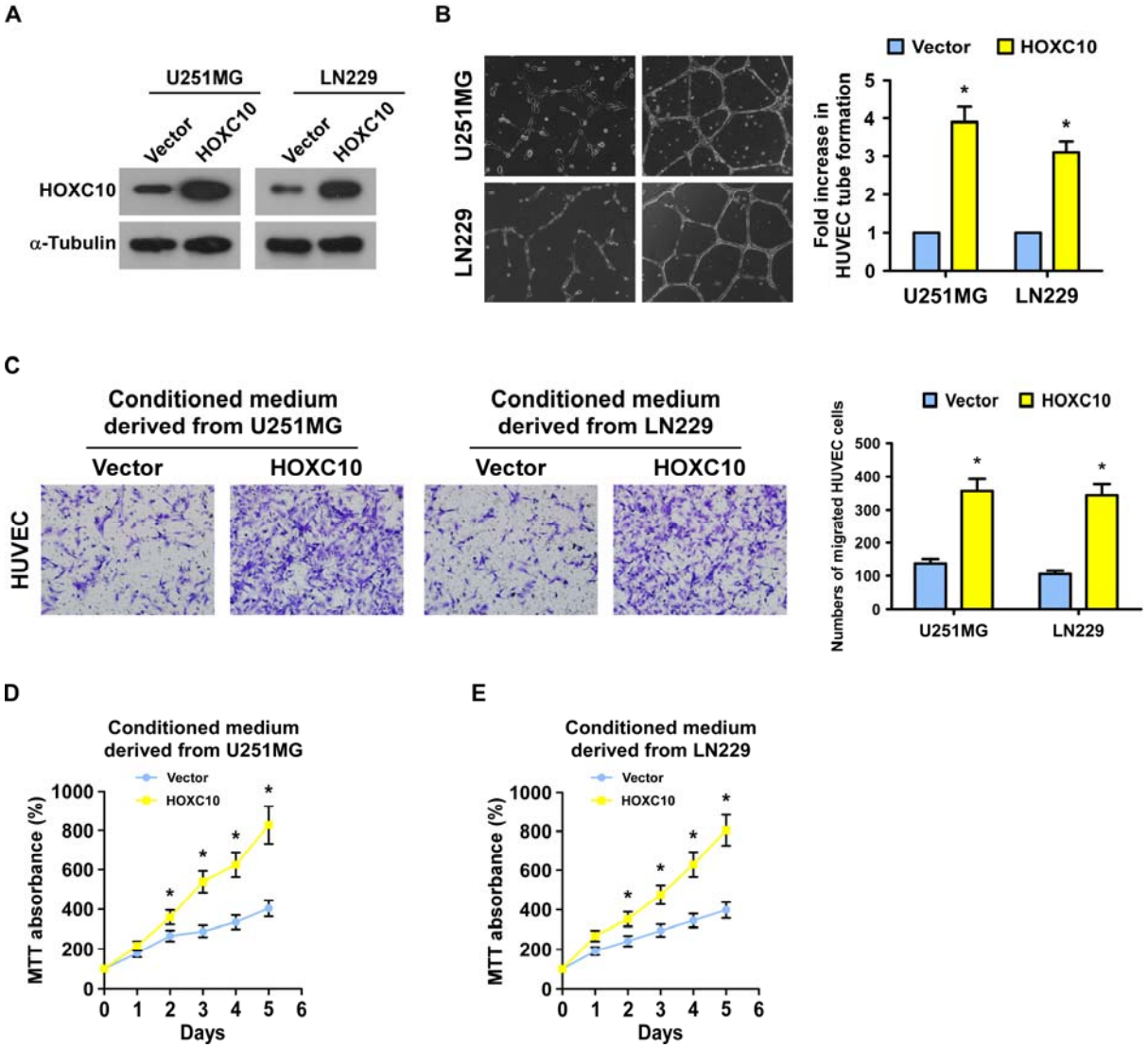
**Supplementary Figure S2**




**Supplementary Figure S2. HOXC10 is overexpressed in gliomas and elevated HOXC10 expression correlates with poor outcome of gliomas.** (**A**) Real-time PCR analysis of *HOXC10* expression in normal brain tissues (N) and human glioma tissues (T). The assay was replicated independently three times. (**B**) Western blotting of HOXC10 expression in four normal brain tissues (N) and 12 human glioma tissues (T). α-Tubulin was used as a loading control. The assay was replicated independently three times. (**C**) Real-time PCR analysis of *HOXC10* expression in NHA and six glioma cell lines. Transcript levels were

normalized to *GAPDH* expression. Error bars, SD. * $P < 0.05$. (**D**) Western blotting of HOXC10 expression in NHA and six glioma cell lines. (**E**) IHC staining indicating that HOXC10 levels were upregulated in human gliomas (WHO Grade I-IV) compared with that in normal brain tissues (left panel); statistical quantification of the average mean optical density (MOD) of HOXC10 staining between normal brain tissues and glioma specimens of different WHO grades (right panel). MOD is defined as the mean of immunostaining intensities per positive pixels in the specimens. Error bars, SD. (**F**) Kaplan–Meier analysis of overall survival comparing all WHO grade patients with low and high HOXC10 expression levels (n = 94; $P < 0.01$). (**G**) Kaplan–Meier analysis of overall survival comparing WHO grade I-II patients with low and high HOXC10 expression levels (n = 34; $P = 0.030$). (**H**) Kaplan–Meier analysis of overall survival comparing WHO grade III-IV patients with low and high HOXC10 expression levels (n = 60; $P < 0.01$).

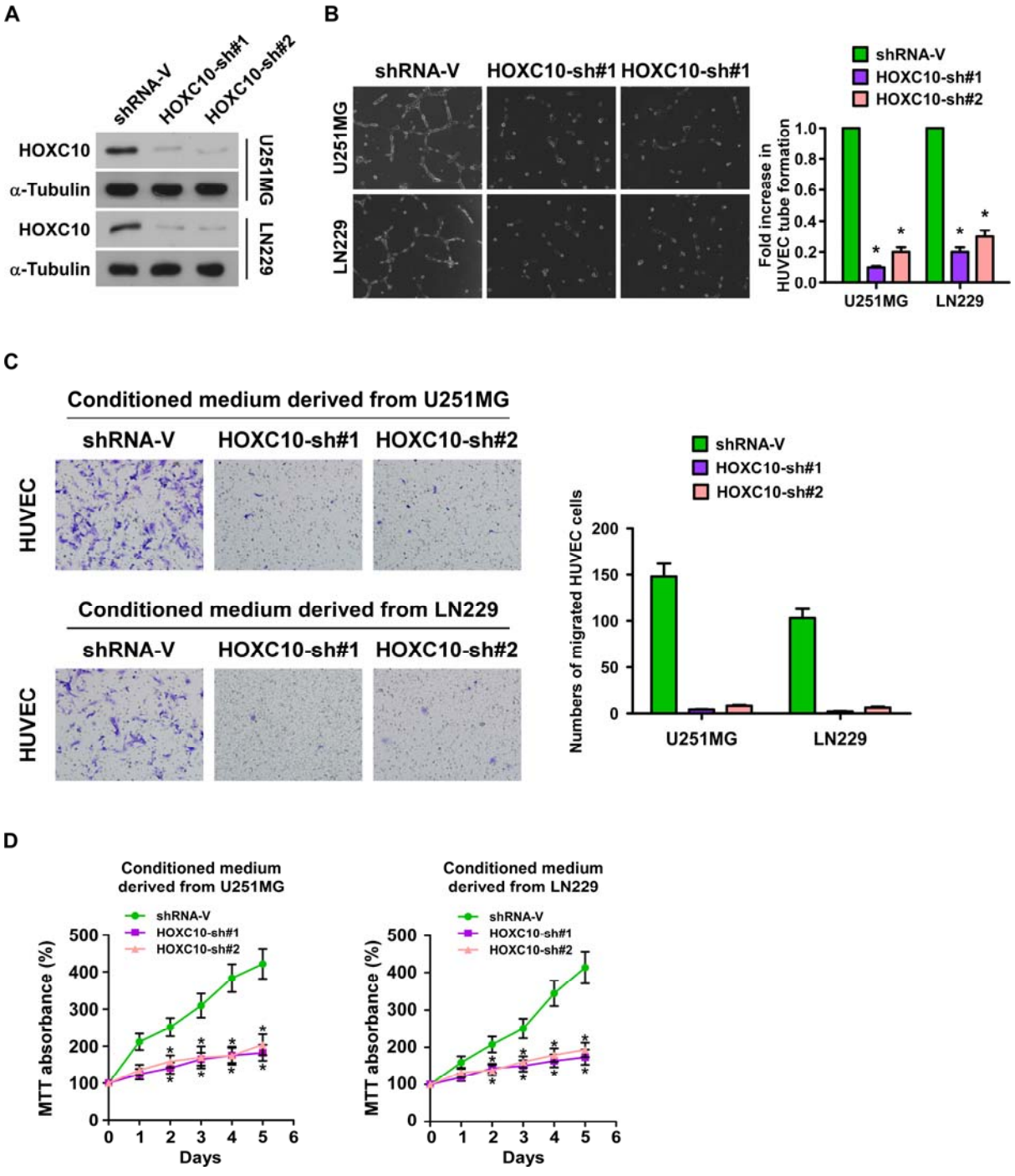
**Supplementary Figure S3**




**Supplementary Figure S3. Ectopic expression of HOXC10 enhances the pro-angiogenic activity of glioma cells *in vitro*.** (**A**) Western blotting of HOXC10 protein levels in the vector control or *HOXC10*-transduced glioma cells. (**B**) Representative images (left) and quantification (right) of HUVEC formed tube-like structures on Matrigel-coated plates with CM derived from the vector control or *HOXC10* transduced glioma cells. (**C**) Representative images (left) and quantification (right) of migrated HUVECs treated with the indicated conditioned medium analyzed using a Transwell migration assay. (**D**-**E**) HUVEC viability was determined using the MTT proliferation assay. HUVECs were treated with CM from the

indicated cells for the specified number of days. All the assays shown in the figure were independently replicated three times. Error bars, SD. * $P < 0.05$.

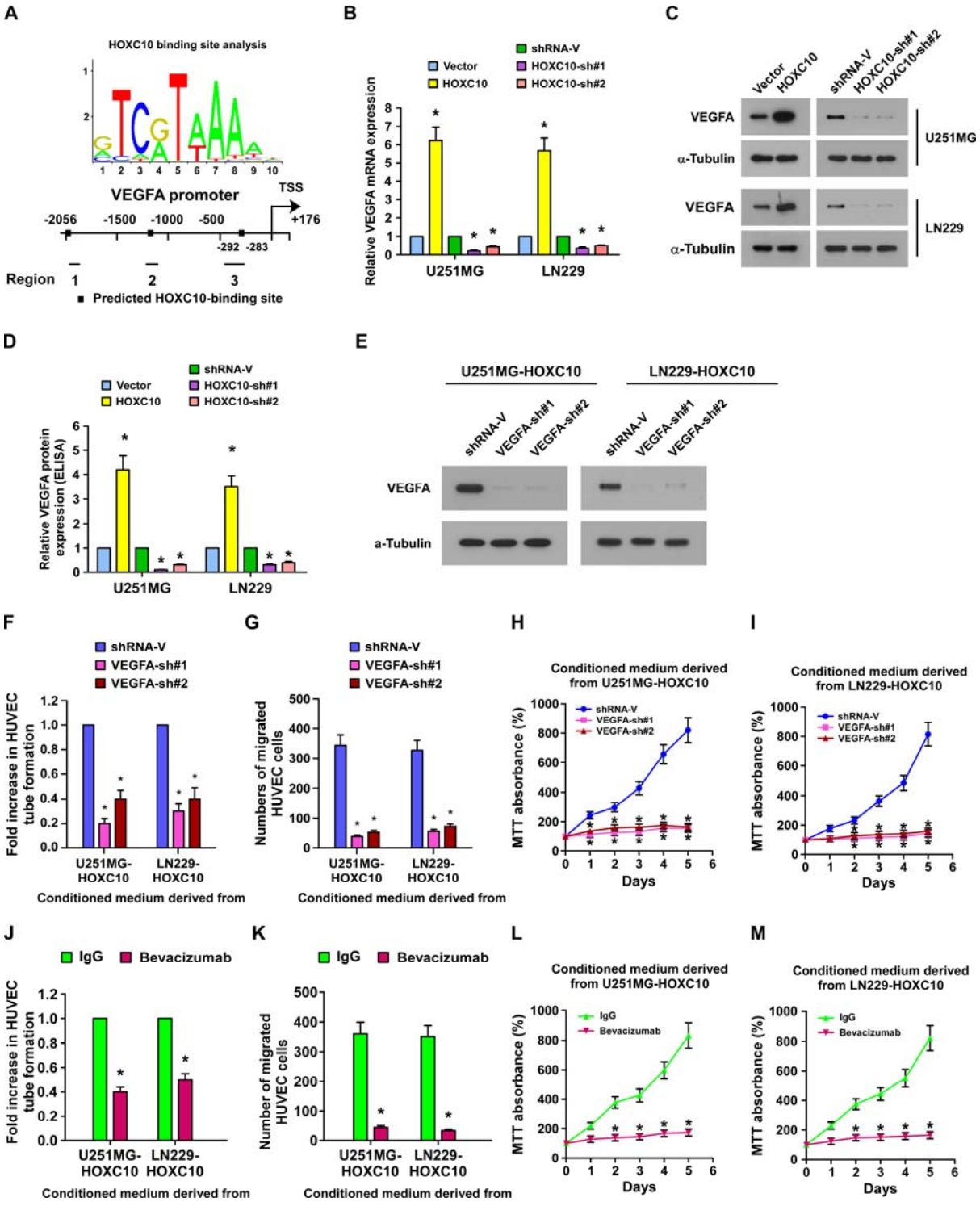
**Supplementary Figure S4**




**Supplementary Figure S4. Knockdown of *HOXC10* reduces the pro-angiogenic activity of glioma cells *in vitro*.** (**A**) Western blotting of HOXC10 levels in the shRNA-vector control or *HOXC10*-silenced glioma cells; α-Tubulin was used as a loading control. (**B**) Representative images (left) and quantification (right) of HUVEC formed tube-like structures on Matrigel-coated plates with CM derived from the shRNA control vector cells or

*HOXC10*-silenced glioma cells. (**C**) Representative images (left) and quantification (right) of migrated HUVECs treated with the indicated CM analyzed using a transwell migration assay. (**D**) HUVEC cell viability determined using the MTT proliferation assay. HUVECs were treated with the indicated CM from the indicated cells for the indicated number of days. All the assays shown in the figure were independently replicated three times. Error bars, SD. * $P < 0.05$.

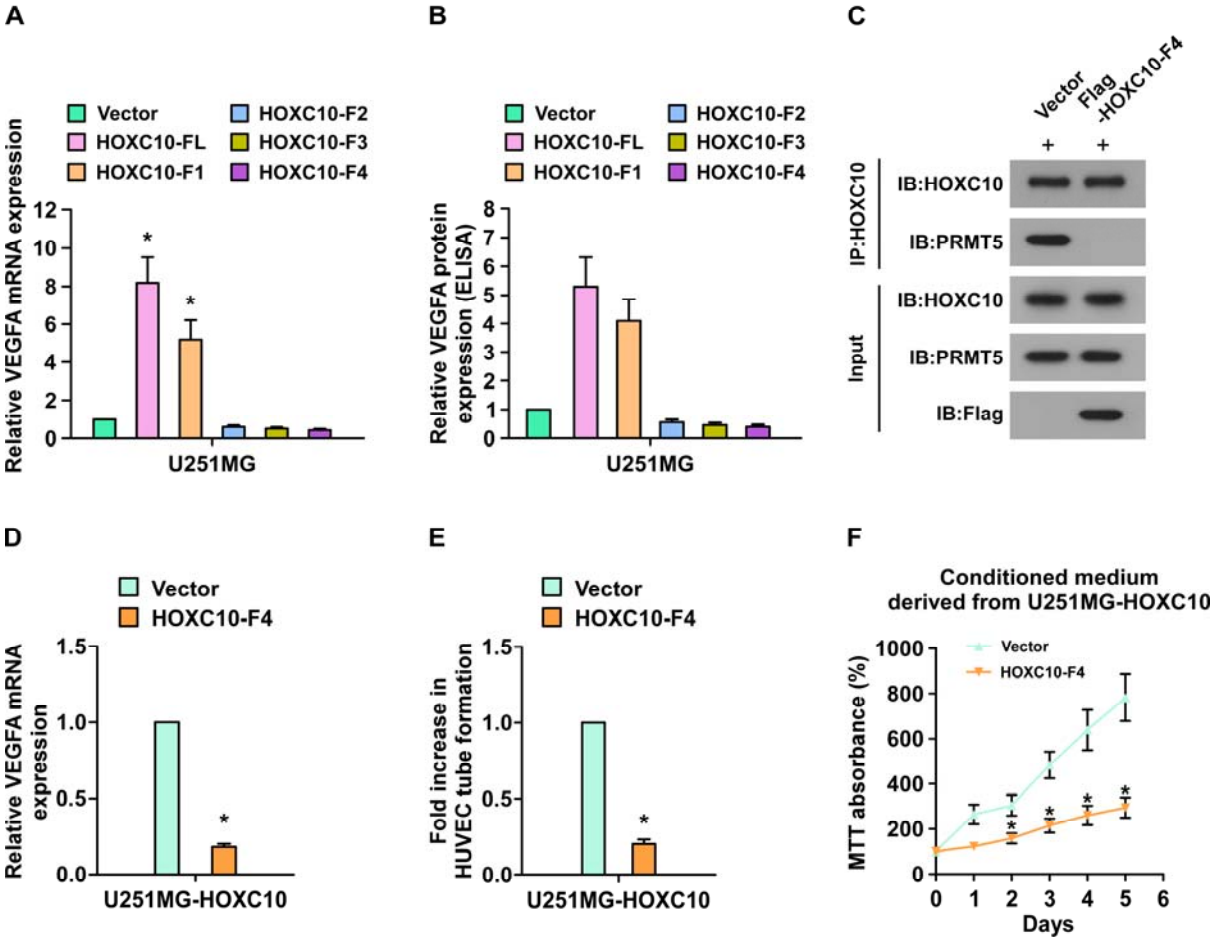
**Supplementary Figure S5**




**Supplementary Figure S5. HOXC10 modulates angiogenesis via regulating *VEGFA* expression.** (**A**) Three potential HOXC10 binding sites were predicted in the promoter of *VEGFA* using the JASPAR database. TSS: Transcription start site. (**B**) Real-time PCR analysis of *VEGFA* mRNA expression in the vector control, *HOXC10*-transduced,

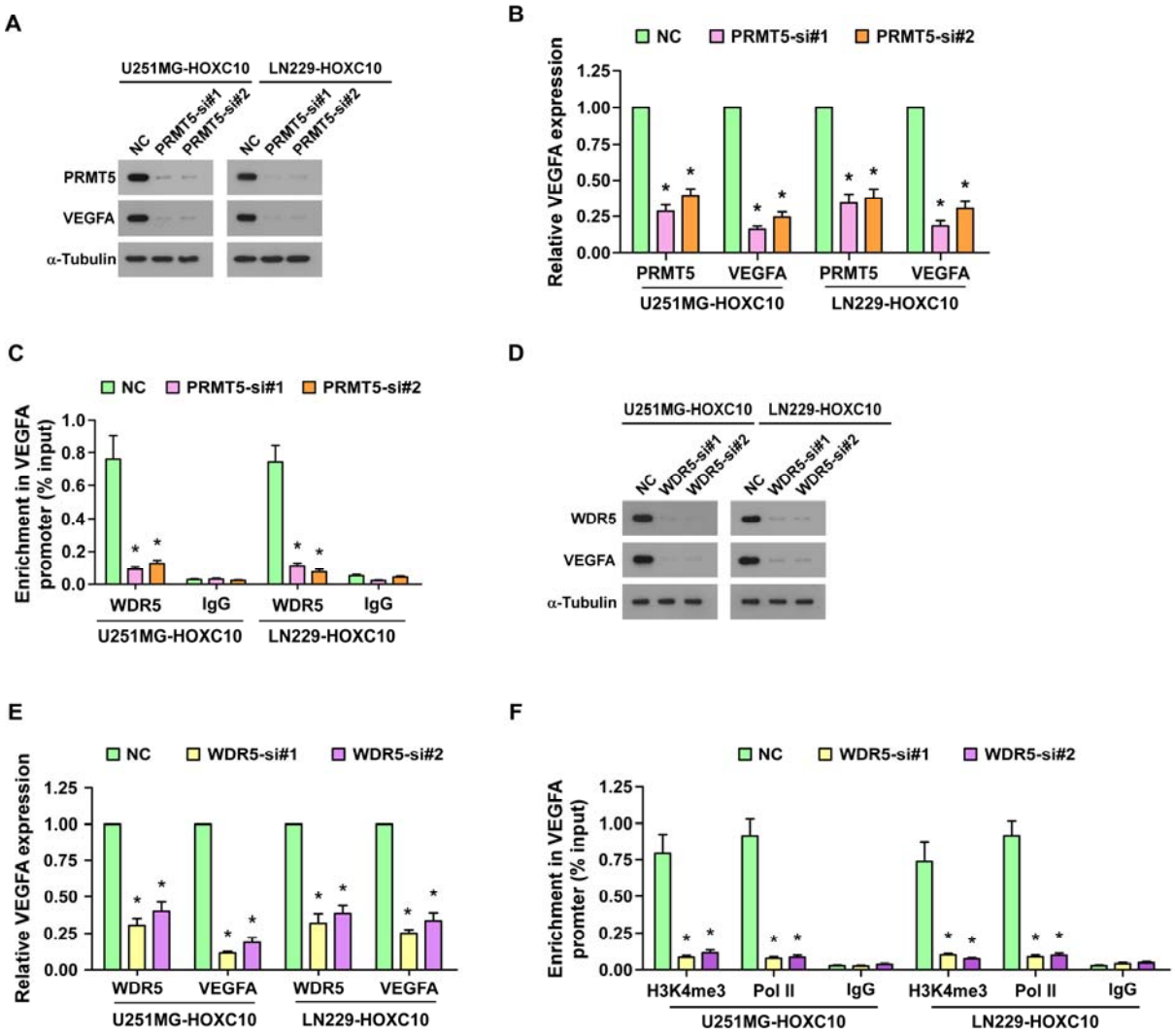
shRNA-vector control or *HOXC10*-silenced glioma cells. Expression levels were normalized to that of *GAPDH*. (**C**) Western blotting analysis showing VEGFA protein levels in the indicated cells. (**D**) ELISA of VEGFA protein levels in the supernatants of the indicated cells. (**E**) Western blotting analysis showing the VEGFA level in control-shRNA-transduced, *HOXC10*-overexpressing glioma cells or *VEGFA*-shRNA-transduced, *HOXC10*-overepxressing glioma cells. (**F**) Quantification of HUVEC formed tube-like structures on Matrigel-coated plates with CM derived from the indicated glioma cells cultured for 24 h. (**G**) Quantification of migrated HUVECs treated with CM derived from the indicated glioma cells cultured for 24 h, analyzed using a transwell migration assay. (**H-I**) HUVEC cell viability determined using the MTT proliferation assay. HUVECs were treated with the indicated CM from the indicated cells for the indicated number of days. (**J**) Quantification of HUVEC formed tube-like structures on Matrigel-coated plates with the indicated CM added with IgG or 150 μg/ml Bevacizumab. (**K**) Quantification of migrated HUVECs treated with the indicated CM added with IgG or 150 μg/ml Bevacizumab. The results were demonstrated using a transwell migration assay. (**L-M**) HUVEC cell viability determined using the MTT proliferation assay. HUVECs were treated with the indicated CM added with IgG or 150 μg/ml Bevacizumab for the indicated number of days. All the assays show in the figure were independently replicated three times. Error bars, SD. * $P < 0.05$.

**Supplementary Figure S6**




**Supplementary Figure S6. HOXC10 regulates *VEGFA* expression through interaction with PRMT5.** **(A-B)** Relative mRNA expression (A) and protein level (B) of VEGFA in the cells transfected with indicated HOXC10 fragments, analyzed via Real-time PCR analysis and ELISA assay. **(C)** Co-IP assay showing that HOXC10-F4 inhibited the interaction of HOXC10 and PRMT5. **(D)** Relative mRNA expressionof VEGFA in the U251-HOXC10 cells transfected with HOXC10-F4 peptide analyzed via Real-time PCR analysis. (**E**) Quantification of HUVEC formed tube-like structures on Matrigel-coated plates with CM derived from the indicated glioma cells cultured for 24 h. (**F**) MTT proliferation assay analysis of HUVEC cell viability treated with CM harvested form indicated cells. Error bars, SD. * $P < 0.05$.

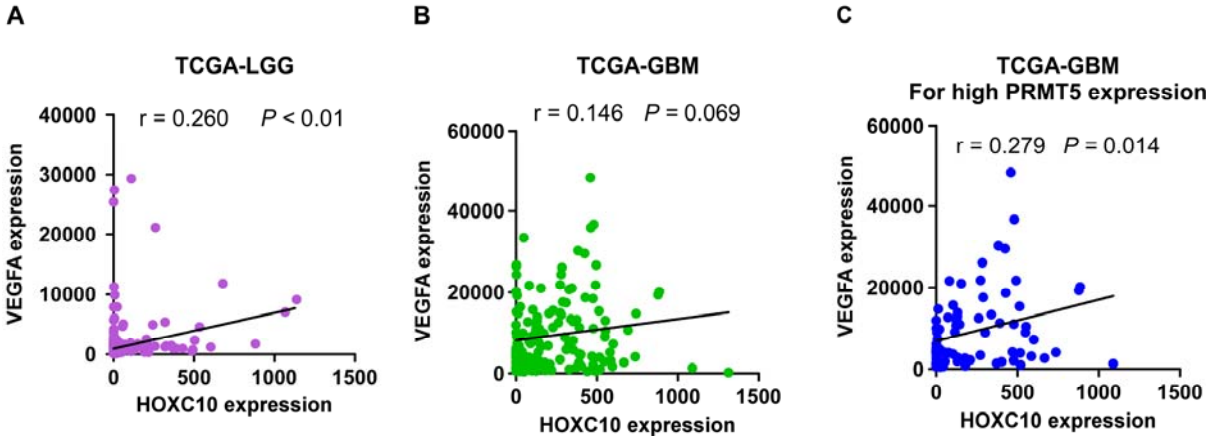
Supplementary Figure S7




**Supplementary Figure S7. Knockdown of *PRMT5* or *WDR5* efficiently reduced *VEGFA* expression. (A)** Western blotting analysis showed the PRMT5 and VEGFA levels in control-shRNA-transduced, or *PRMT5*-shRNA-transduced glioma cells overexpressing *HOXC10*. (**B**) Real-time PCR analysis showing *PRMT5* and *VEGFA* expression in control-shRNA-transduced, or *PRMT5*-shRNA-transduced glioma cells overexpressing *HOXC10*. (**C**) ChIP-qPCR enrichments of WDR5 or IgG on *VEGFA* promoter region 3 in the indicated cells. **(D)** Western blotting analysis showing the WDR5 and VEGFA levels in control-shRNA-transduced, or *WDR5*-shRNA-transduced glioma cells overexpressing *HOXC10*. (**E**) Real-time PCR analysis showing *WDR5* and *VEGFA* expression in control-shRNA-transduced, or *WDR5*-shRNA-transduced glioma cells overexpressing

*HOXC10*. (**F**) ChIP-qPCR enrichments of H3K4me3, Polymerase II or IgG on *VEGFA* promoter region 3 in the indicated cells. All the assays shown in the figure were independently replicated three times. Error bars, SD. * $P < 0.05$.

**Supplementary Figure S8**




**Supplementary Figure S8. HOXC10 levels were correlated with *VEGFA* expression in gliomas. (A)** TCGA-LGG analysis showing a significant correlation of HOXC10 and VEGFA expression in low-grade gliomas (n = 516; r = 0.260; $P < 0.01$). (**B-C**) TCGA-GBM analysis showing no correlation between HOXC10 and VEGFA in GBM group (n = 156; r = 0.146; $P = 0.069$) but a significant correlation of HOXC10 and VEGFA expression in PRMT5 higher-GBM subgroup (n = 78; r = 0.279; $P = 0.014$).

## Supplementary Tables

**Table S1**. Clinicopathological characteristics of studied patients and expression of HOXC10 in gliomas

| Factor | No. | (%) |
|---|---|---|
| **Gender** | | |
| Male | 50 | 53.2 |
| Female | 44 | 46.8 |
| **Age (years)** | | |
| ≤45 | 46 | 48.9 |
| >45 | 48 | 51.1 |
| **Glioma histopathology (WHO grading)** | | |
| Grade I | 17 | 18.1 |
| Grade II | 17 | 18.1 |
| Grade III | 35 | 37.2 |
| Grade IV | 25 | 26.6 |
| **Feature** | | |
| IDH wild-type | 44 | 46.8 |
| IDH mut and 1p/19q non-codel | 48 | 51.1 |
| IDH mut and 1p/19q codel | 2 | 2.1 |
| **Patient survival** | | |
| Alive | 31 | 41.5 |
| Deceased | 63 | 58.5 |
| **Expression of HOXC10** | | |
| Low expression | 42 | 44.7 |
| High expression | 52 | 55.3 |

**Table S2**. Correlation between the clinicopathological features and expression of HOXC10

| Patient characteristics | | HOXC10 expression | | *P*-value |
|---|---|---|---|---|
| | | Low or none | High | |
| **Sex** | Male | 22 | 28 | 1.000 |
| | Female | 20 | 24 | |
| **Age (years)** | ≤45 | 26 | 20 | 0.037 |
| | >45 | 16 | 32 | |
| **Glioma histology (WHO grading)** | I | 13 | 4 | 0.009 |
| | II | 5 | 12 | |
| | III | 17 | 18 | |
| | IV | 7 | 18 | |
| **IDH and 1p/19q codel subtype** | IDH wt | 21 | 23 | 0.207 |
| | IDH mut and non-codel | 19 | 29 | |
| | IDH mut and codel | 2 | 0 | |
| **Survival** | Alive | 20 | 11 | 0.008 |
| | Deceased | 22 | 41 | |

**Table S3**. Univariate and multivariate analysis of different prognostic parameters in glioma patients by Cox-regression analysis

| | **Univariate analysis** | | **Multivariate analysis** | |
|---|---|---|---|---|
| | ***P*** | **Hazard ratio (95% CI)** | ***P*** | **Hazard ratio-RR (95% CI)** |
| **Age (years)** | | | | |
| ≤45 | 0.016 | 1.854 (1.121-3.067) | 0.305 | 1.305 (0.785-2.170) |
| >45 | | | | |
| **Glioma histology (WHO grade)** | | | | |
| I | <0.001 | 2.537 (1.863-3.455) | <0.001 | 2.564 (1.854-3.545) |
| II | | | | |
| III | | | | |
| IV | | | | |
| **HOXC10 expression** | 0.001 | 2.485 (1.458-4.236) | 0.004 | 2.217 (1.283-3.834) |
| Low | | | | |
| High | | | | |