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Supplemental Information

Genome Editing Reveals Glioblastoma Addiction to MicroRNA-10b

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Supplementary Figures

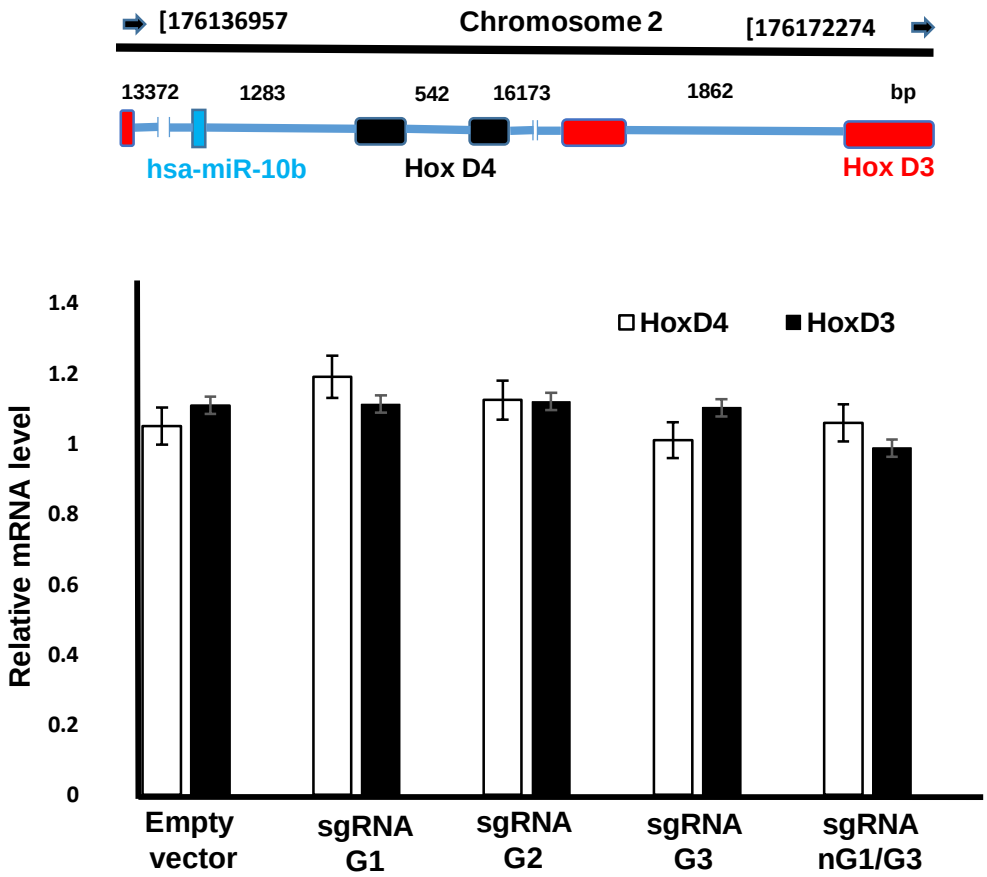

Figure S1: miR-10b editing with G1-G3 sgRNAs does not affect the expression of adjacent HOXD4 and HOXD3 genes. Schematic presentation of miR-10b located upstream of the *HOXD4* and embedded in the first intron separating two non-coding exons of HOXD3. Expression levels of HOXD3 and HOXD4 mRNAs were examined in LN229 glioma cells 48 hours after transfections with G1-G3 sgRNAs or double sgRNA guide nG1/G3.

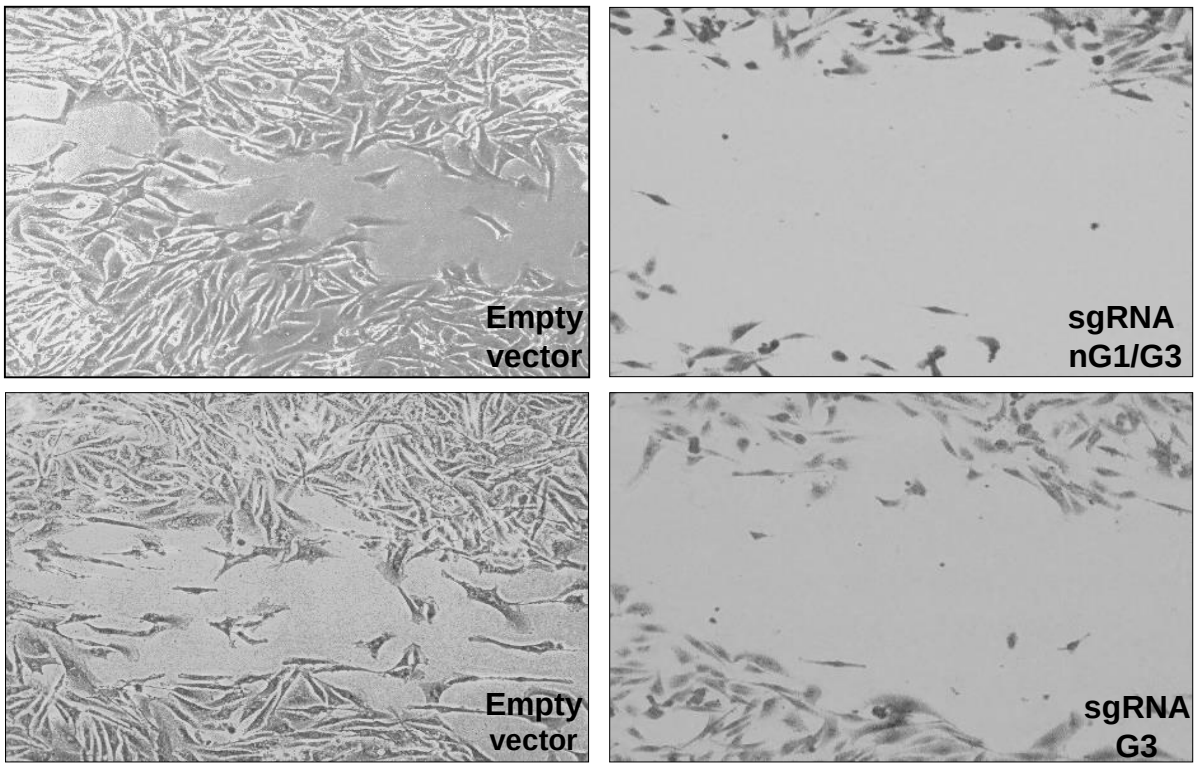

Figure S2. CRISPR-Cas9/G3 mediated editing of miR-10b reduces migration of MDA-MB-231 cells as indicated by the scratch motility assay. The cell viability was not affected.

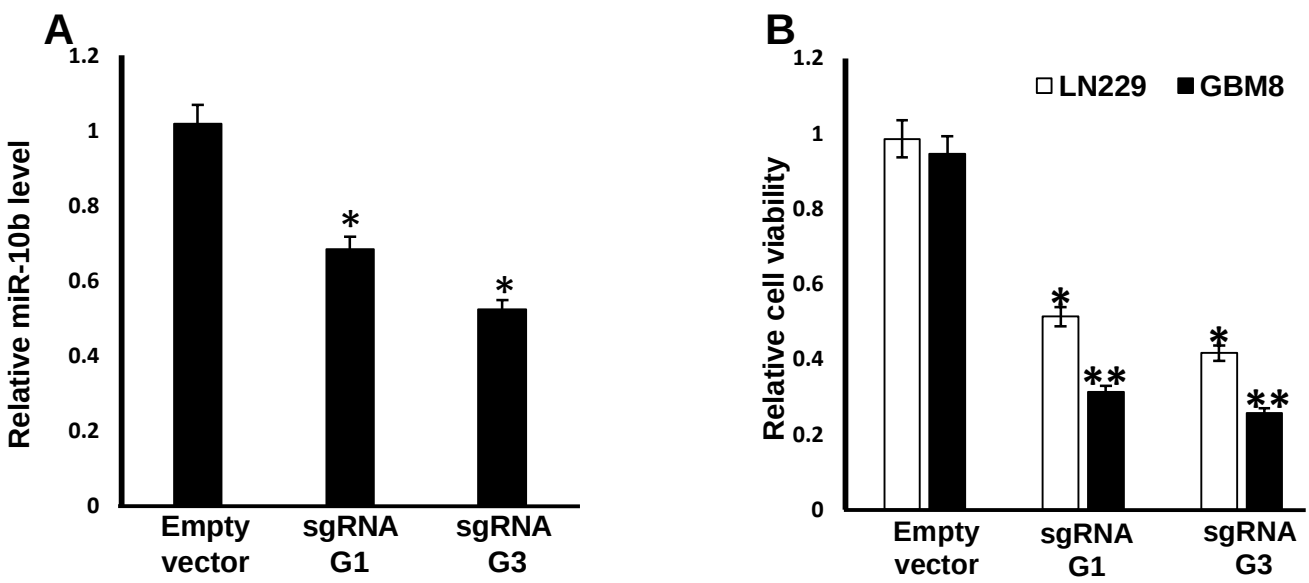

Figure S3. **Lentivirus-mediated miR-10b CRISPR-Cas9 editing reduces (A) miR-10b levels and (B) glioma cell viability** as monitored by qRT-PCR and WST1 assays, respectively.

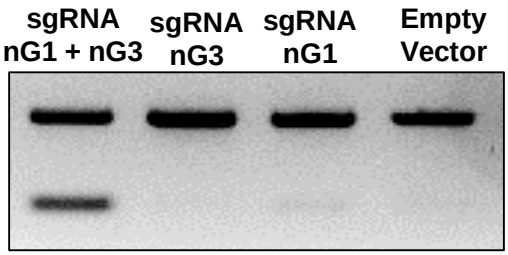

Figure S4. Functional validation of lentivirus nCas9 in LN229 cells demonstrates efficient editing guided by a pair of sgRNAs targeting both strands (sgRNA nG1/G3), but not individual G1 or G3 sgRNAs.

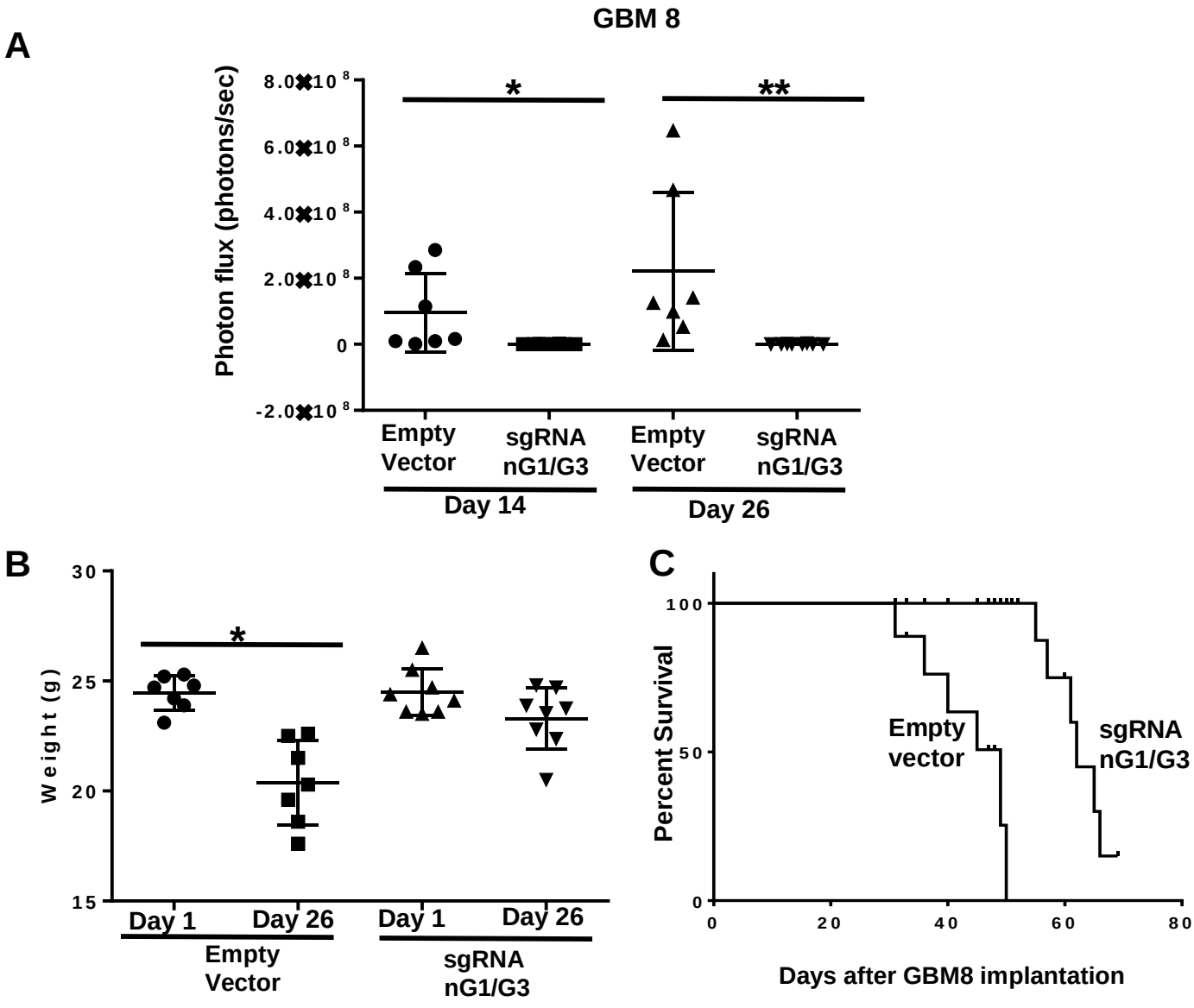

Figure S5. **Intratumoral injections of lentiviral miR-10b editing nCas9 "nickase" vectors (3x10^5 TU) strongly impair the growth of established orthotopic GBM8**. A. Tumor growth was monitored by luciferase imaging *in vivo*. There were 7-8 mice per group at the treatment initiation, and each dot represents an animal. The insert illustrates tumor imaging in representative animals. *P < 0.05, **P < 0.005 by Student's *t*-test**.** B. miR-10b gene editing helps maintain the body weight in mice bearing intracranial tumors. n= 7-8 animals per group. *P < 0.005. C. Survival curves. miR-10b editing significantly extends animal survival, analyzed by Kaplan-Meier plot. *N* = 8 mice per group. *P* = 0.0001 by log-rank (Mantel-Cox) test.

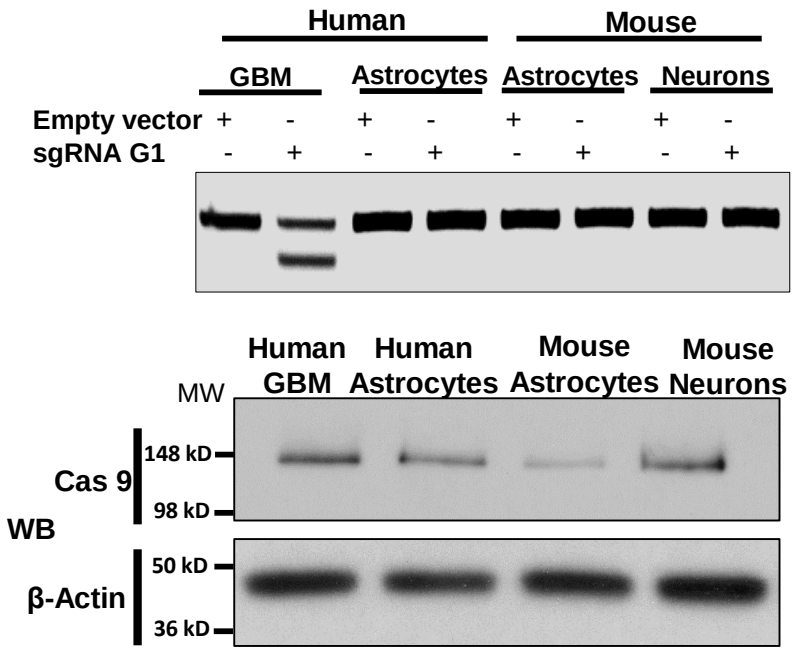

Figure S6. Transduction of normal mouse and human primary neuroglial cultures with lentiviral miR-10b editing CRISPR/Cas9 vectors at $3x10^5$ TU does not result in miR-10b gene editing. Western blot analysis (lower panel) demonstrates the corresponding Cas9 expression at 48h post-transduction.