Supplementary Materials

The EGFR-TMEM167A-p53 Axis Defines the Aggressiveness of Gliomas

Berta Segura-Collar, Ricardo Gargini, Elena Tovar-Ambel, Esther Hernández-SanMiguel, Carolina Epifano, Ignacio Pérez de Castro, Aurelio Hernández-Lain, Sergio Casas-Tintó and Pilar Sánchez-Gómez

Figure S1. TP53 opposes EGFR in glioblastoma. (A) Kaplan–Meier overall survival curves of patients from the TCGA LGG + GBM cohort. Patients were separated based on the TP53 status. (B,C) Frequency analysis of somatic mutations (B) and copy number amplification (C) in glioblastomas grouped according to TP53 status. * Statistically significant $p < 0.0001$.

Figure S2. Expression and implication of DYRK1A in the pathology of gliomas. (A) Expression of DYRK1A in gliomas, grouped according to the histological type. (B) Kaplan–Meier overall survival
curves of patients from the TCGA LGG + GBM cohort. Patients were separated based on high and low DYRK1A expression values. ** $p \leq 0.01$; **** $p \leq 0.0001$.

Figure S3. Vesicular transport genes associated to EGFR in gliomas. (A) Levels of EGFR mRNA measured by RNAseq in gliomas (LGG + GBM TCGA cohort), comparing wild-type and mutant TP53 tumors. (B, C) Vesicular transport signature in gliomas of the TCGA (LGG + GB cohort). Tumors were classified based on high or low EGFR expression. **** $p \leq 0.0001$.
Figure S4. TMEM167A downregulation in subcutaneous tumor tissue. (A,B) qRT-PCR analysis of TMEM167A in our own cohort of glioma samples. Patients (A) and PDXs (B) were stratified based on TP53 status: p53 wt and p53 mut. HPRT was used for normalization. (C-F) TMEM167A levels were determined by qRT-PCR in U87 (A), U373 (B), GBM2 (C), and GBM3 (D) tumor tissue (control, shTMEM167Aa, or shTMEM167Ab). HPRT was used for normalization. * p ≤ 0.05.

Figure S5. Downregulation of TMEM167A in intracranial tumors. (A,B) TMEM167A expression was determined by qRT-PCR in U87 (A) and U373 (B) tumor tissue (control or shTMEM167Aa). ** p ≤ 0.01.
Figure S6. Downregulation of TMEM167A in cultured GBM cells. (A, B) TMEM167A levels were determined by qRT-PCR in U87 (A) and U373 (B) cells (control or shTMEM167Aa). HPRT was used for normalization. (C) WB analysis of p53 in U87 and U87 p53 mut (R273H) cells. GAPDH was used as a loading control. (D) TMEM167A levels were determined by qRT-PCR in U87 p53 mut (R273H) cells (control or shTMEM167Aa). ** p ≤ 0.01.

Figure S7. Analysis of autophagy-related proteins after TMEM167A downregulation. (A) Growth-factor-starved U87 and U373 cells (Control or shTMEM167Aa) were stimulated with 100 ng/mL of EGF for the indicated times; p62 and LC3B were tested by WB; GAPDH was used as a loading control. (B) WB analysis of p62 and Cathepsin B (CTSB) in tumors from Figure 5A. GAPDH was used as a loading control.
Figure S8. Full scan of the different WBs.
Table S1. Human samples (n.d. = not diagnosed; wt = wild type; and mut = mutated).

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