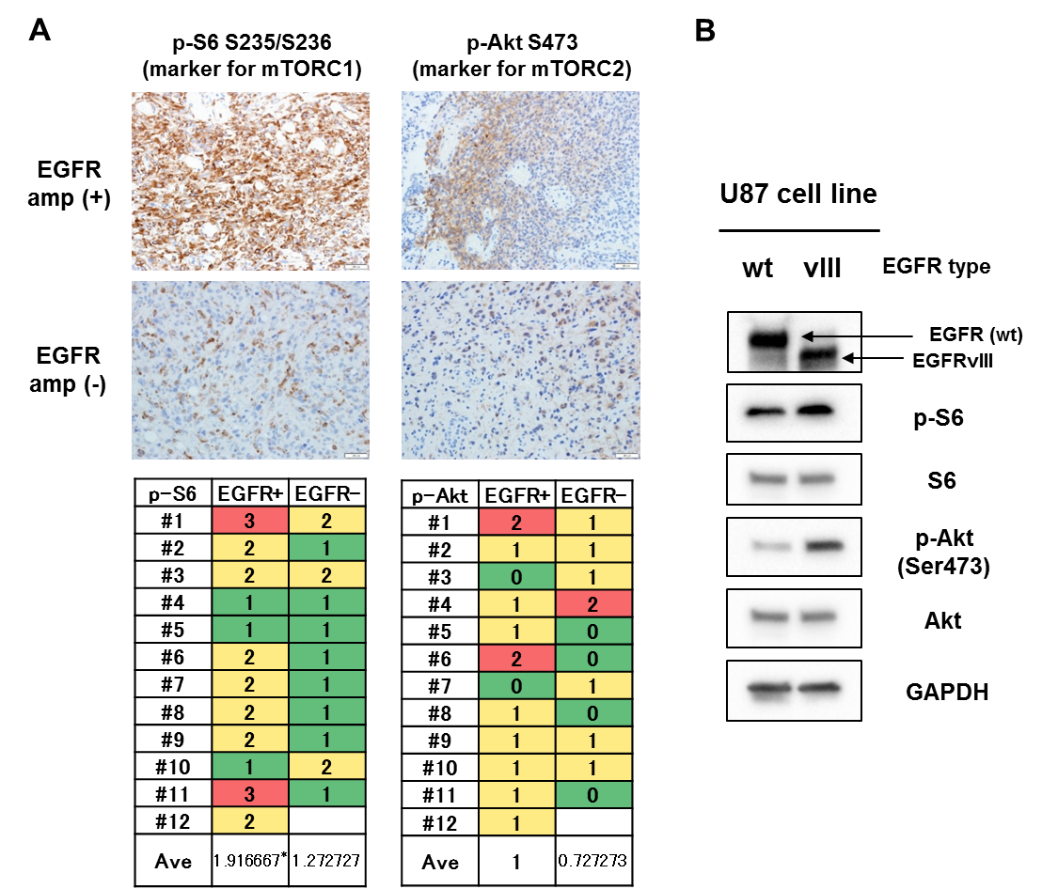
**Supplementary Data for**

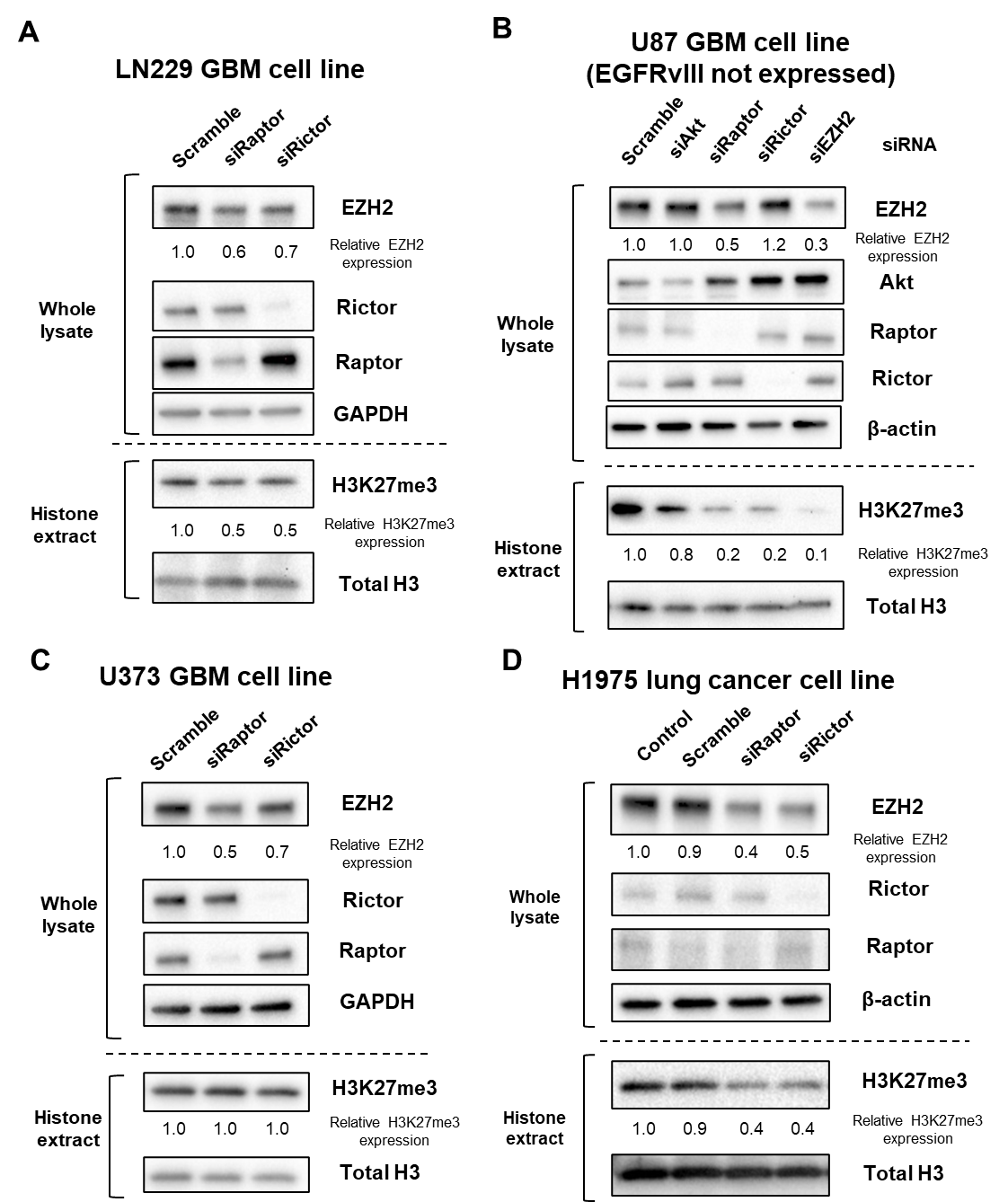
**Dual Regulation of Histone Methylation by mTOR Complexes Controls Glioblastoma Tumor Cell Growth via EZH2 and SAM**

Mio Harachi, Kenta Masui, Hiroaki Honda, Yoshihiro Muragaki, Takakazu Kawamata, Webster K. Cavenee, Paul S. Mischel and Noriyuki Shibata

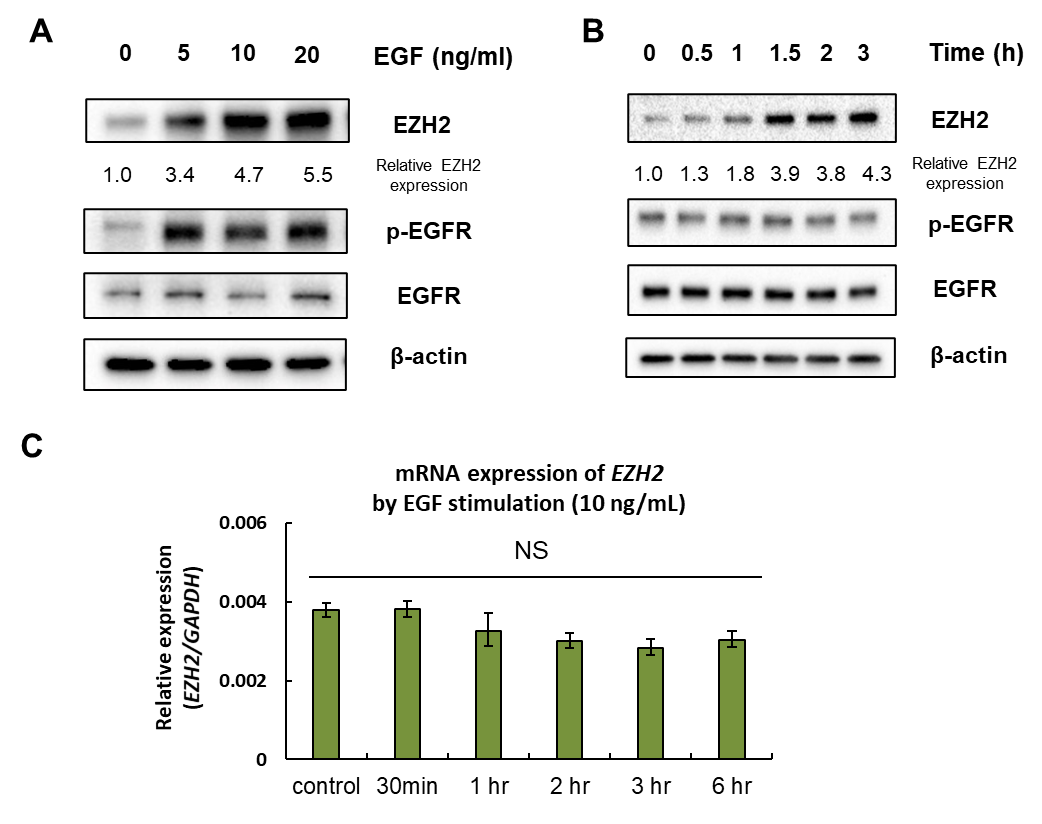
**・Supplementary Figures S1-S7**

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**Figure S1.** mTORC1 and mTORC2 are activated downstream of aberrant EGFR signaling in human GBM. **A**, Representative images for differential expression of p-S6 (mTORC1 activation marker) and p-Akt S473 (mTORC2 activation marker) in surgically resected GBM cases with (n = 12) or without (n = 11) *EGFR* amplification. **B**, Immunoblot detection of mTOR activation markers (p-S6 for mTORC1, p-Akt for mTORC2) in U87 cells with wild type (wt) EGFR and constitutively active form of EGFR (EGFRvIII).



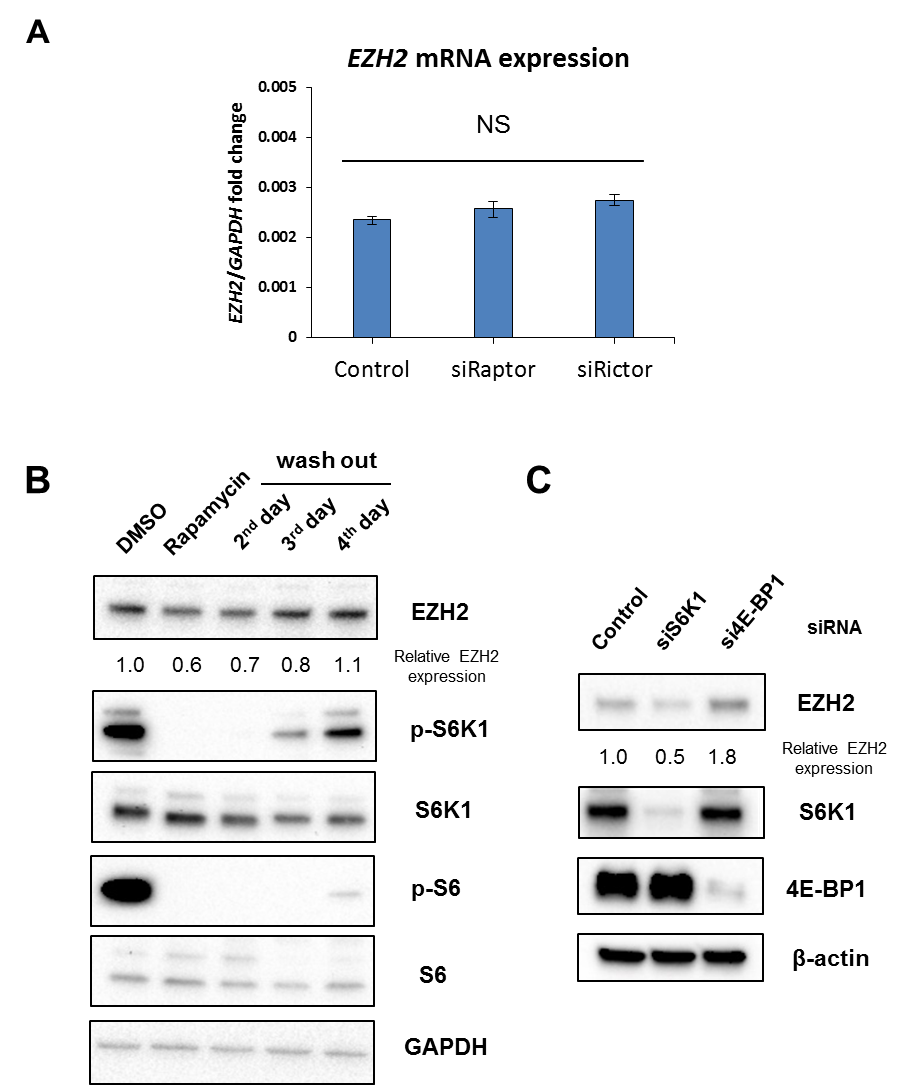
**Figure S2.** mTOR complexes regulate expression of EZH2 and H3K27me3 in GBM cell lines and other types of cancer cell lines. **A-D**, Knockdown of Raptor (mTORC1) or Rictor (mTORC2) reduces EZH2 and H3K27me3 expression at various levels in LN229, GBM cell line with EGFRvIII (tet-on EGFRvIII system) (**A**), U87 cell line without any known *EGFR* mutation (**B**), U373, GBM cell line with EGFRvIII (tet-off EGFRvIII system) (**C**), andH1975, lung cancer cell line with another *EGFR* mutation type (T790M) (**D**).

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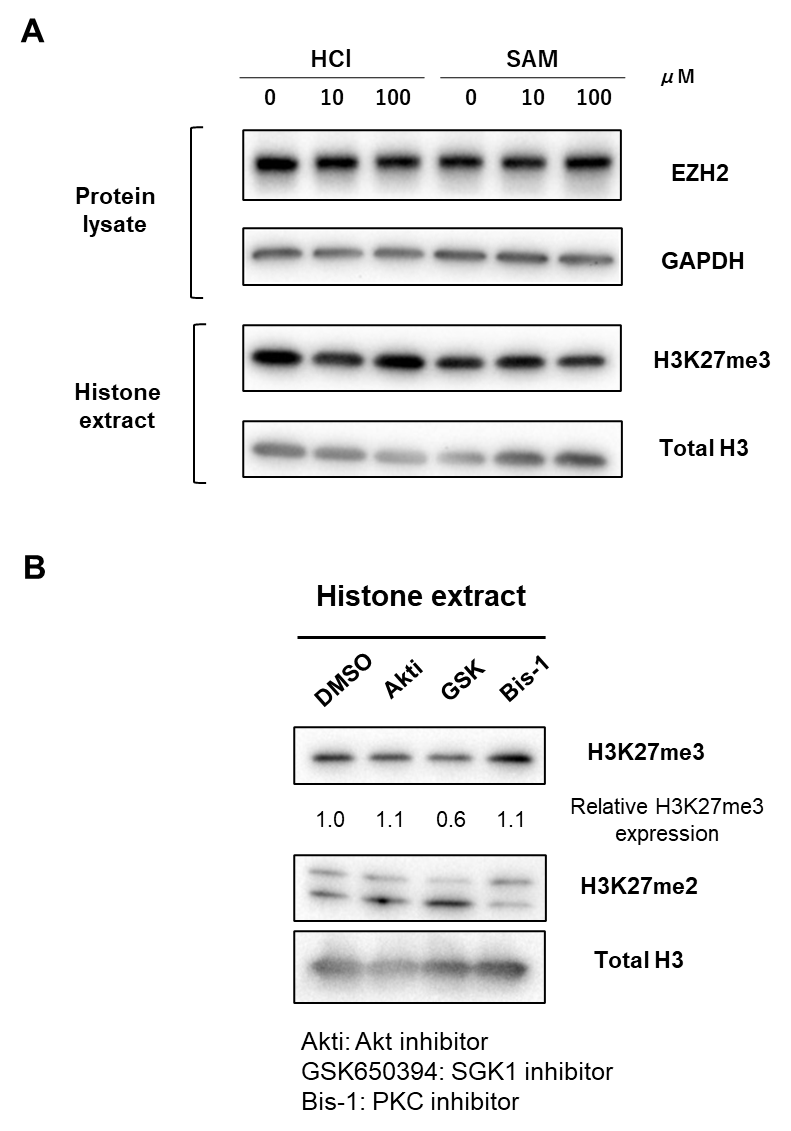
**Figure S3.** EGFR activation translationally increases the level of EZH2. **A**, Immunoblot detection of dose-dependent increase of EZH2 in U87-wild type EGFR with EGF stimulation (indicated dose) for 48 hours. **B**, Immunoblot detection of time-dependent increase of EZH2 in U87-wild type EGFR with EGF stimulation (EGF 10 ng/ml). **C**, Quantification of mRNA expression of *EZH2* in U87-EGFRvIII cells with EGF stimulation in an indicated time frame. NS, not significant.



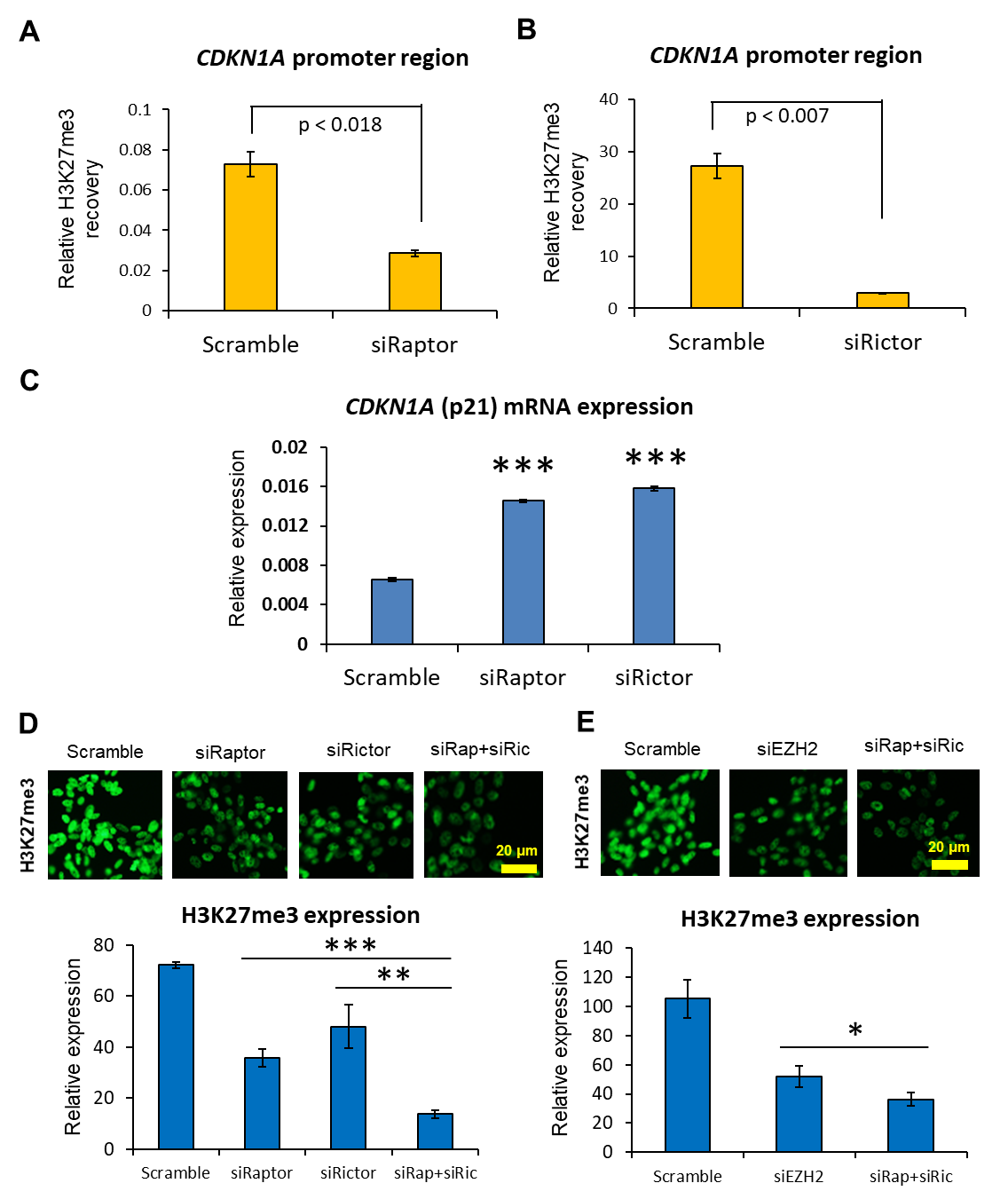
**Figure S4.** Aberrant EGFR signaling renders GBM cells sensitive to EZH2 inhibitors. **A-C**, Cell proliferation (**A**, **B**) and Western blotting (**C**) assays revealed that *EGFR*-mutated GBM cells (U87-EGFRvIII) were more sensitive to EZH2 inhibitors (DZNep and GSK126) over *EGFR*-wildtype GBM cells (U87). Note that the effect of DZNep on *EGFR*-mutated GBM cells was more potent than that of GSK126 in terms of cell proliferation.



**Figure S5.** mTORC1 regulates the expression of EZH2 translationally. **A**, Quantification of *EZH2* mRNA expression in U87-EGFRvIII cells transfected with siRNA against Raptor (mTORC1) or Rictor (mTORC2). The mRNA expression was normalized against *GAPDH*. NS, not significant. **B**, Immunoblot detection of EZH2 protein change in U87-EGFRvIII with an addition and subsequent wash-out treatment of Rapamycin (10 nM). **C**, Immunoblot detection of EZH2 in U87-EGFRvIII cells with siRNAs against the effector molecules downstream of mTORC1 including S6K1 and 4E-BP1.

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**Figure S6.** mTORC2 regulates the production of S-adenosylmethionine (SAM). **A**, Immunoblot detection of H3K27me3 in U87 cells with treatment of exogenous SAM or vehicle (HCl solution) in an indicated concentration for 24 hours. **B**, Immunoblot detection of H3K27me3 in U87-EGFRvIII cells with inhibitors against mTORC2 downstream substrates: Akti-1/2 (Akt inhibitor, 2.5 µM), GSK650394 (SGK1 inhibitor, 2.0 µM) or Bis-1 (PKC-alpha inhibitor, 10 µM) for 48 hours.



**Figure S7.** mTOR-dependent histone methylation epigenetically regulates tumor suppressor gene *CDKN1A* (p21) in GBM cells. **A, B**, ChIP-PCR analysis on H3K27me3 recovery in the promoter region of tumor suppressor *CDKN1A* in U87-EGFRvIII cells with siRNA against Raptor (**A**) or Rictor (**B**). Raptor knockdown experiment was normalized by input DNA, and Rictor knockdown one by negative control samples without any IP antibodies. **C**, mRNA expression of *CDKN1A* (p21) in U87-EGFRvIII cells with indicated siRNAs. mRNA expression was normalized against *GAPDH*. **D, E**, Immunocytochemisty revealed that double knockdown of Raptor (mTORC1) and Rictor (mTORC2) was more potent in decreasing H3K27me3 than knockdown of each gene (Raptor or Rictor) (**D**) or EZH2 (**E**).