

Expanded View Figures

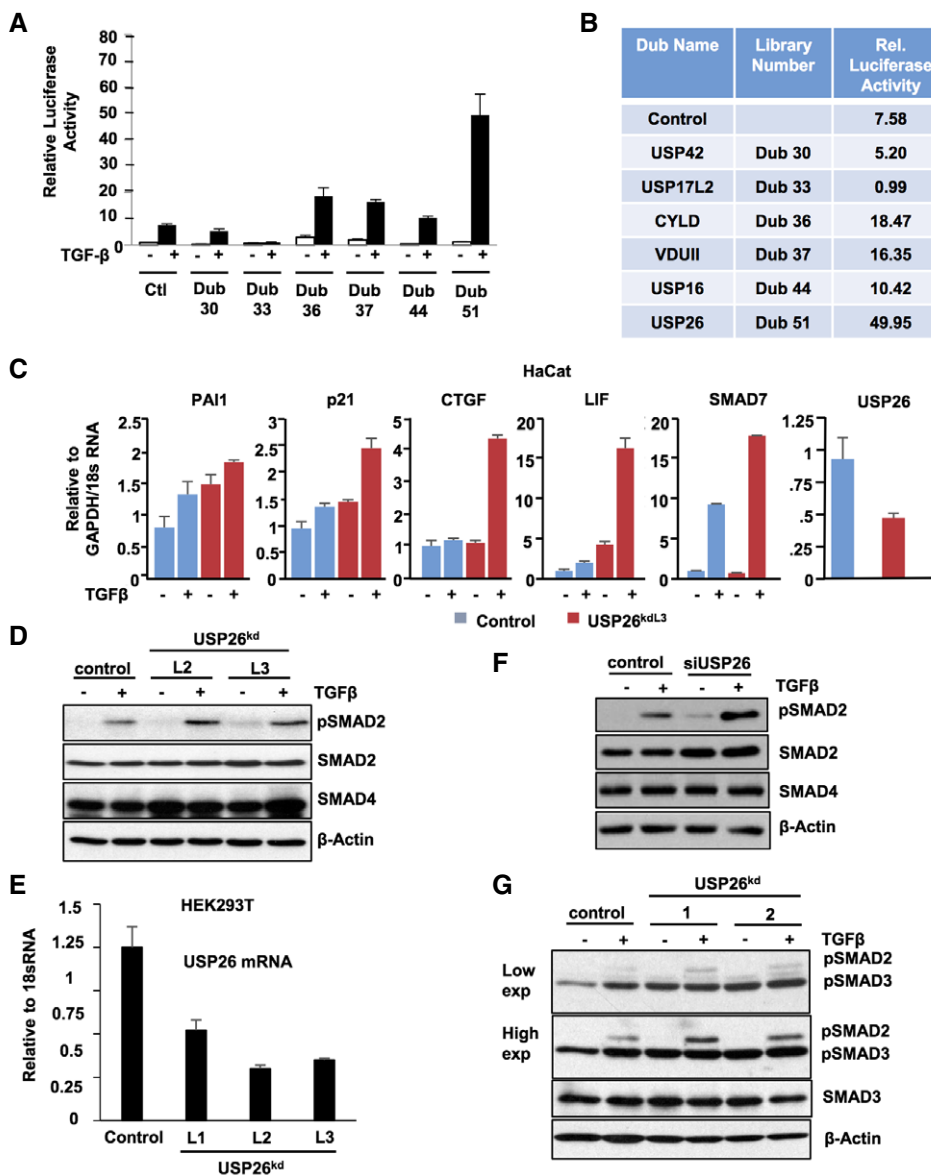


Figure EV1. USP26 enhances SMAD2 phosphorylation and TGF- β -mediated transcription.

- A Graph representing relative luciferase values obtained from DUB screen. 293T cells were transfected with CAGA-Luc and indicated DUB pools. Forty-eight hours later, cells were treated with TGF- β for 16 h and a luciferase assay was performed. Data are mean \pm SD of triplicate samples.
- B Table indicates relative luciferase value of each gene tested in (A).
- C HaCat cells stably transduced with a hairpin targeting USP26 or vector control were stimulated with TGF- β for 3 h. *PAI1*, *CDKN1A* (p21), *CTGF*, *LIF*, *SMAD7*, and *USP26* mRNA levels relative to *GAPDH* or 18S are shown as evaluated by quantitative real-time PCR. Data are mean \pm SD of triplicate samples.
- D 293T cells were stably infected with two independent hairpins (L2 and L3) targeting USP26 and treated with TGF- β overnight. Whole-cell extracts were probed with the indicated antibodies.
- E 293T cells were stably infected with three independent hairpins (L1, L2, and L3) targeting USP26. *USP26* mRNA levels relative to 18S are shown as evaluated by quantitative real-time PCR. Data are mean \pm SD of triplicate samples.
- F 293T cells transfected with siRNA targeting USP26 or control vector were treated with TGF- β overnight. Whole-cell extracts were probed with the indicated antibodies.
- G 293T cells expressing knockdown vectors targeting USP26 or control vector were treated with TGF- β overnight. Whole-cell extracts were probed with the indicated antibodies.

Source data are available online for this figure.

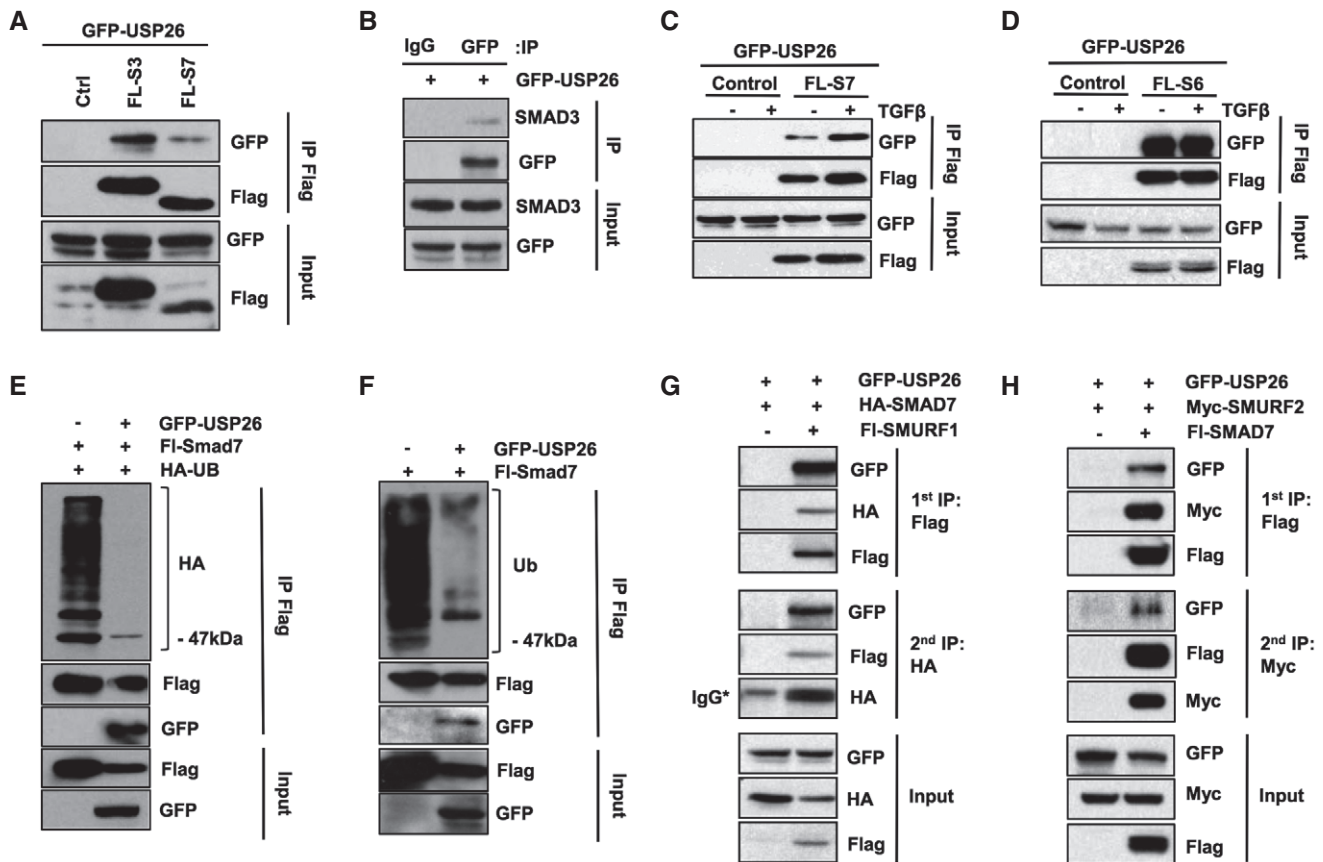


Figure EV2. USP26 binds to and deubiquitinates SMAD7.

- A 293T cells were transfected as indicated with GFP-USP26 and Flag-tagged SMAD3 and SMAD7. After 48 h, cells were lysed and immunoprecipitated with anti-Flag affinity resin. Whole-cell extracts were probed with the indicated antibodies.
- B 293T cells were transfected with GFP-USP26, and cells were lysed and immunoprecipitated with anti-GFP affinity resin. Whole-cell extracts were probed for SMAD3.
- C 293T cells were transfected as indicated with GFP-USP26 and Flag-tagged SMAD7. After 48 h, cells were treated with TGF- β for 1 h and lysed. Flag-tagged proteins were immunoprecipitated with anti-Flag affinity resin and whole-cell extracts were probed with the indicated antibodies.
- D 293T cells were transfected as indicated with GFP-USP26 and Flag-tagged SMAD6. After 48 h, cells were treated with TGF- β and lysed. Flag-tagged proteins were immunoprecipitated with anti-Flag affinity resin and whole-cell extracts were probed with the indicated antibodies.
- E 293T cells transfected with FL-SMAD7, GFP-USP26, control vector, and HA-tagged ubiquitin. Following immunoprecipitation of SMAD7, lysates were resolved by SDS-PAGE and probed with the indicated antibodies.
- F Endogenous ubiquitination assay. 293T cells transfected with FL-SMAD7, GFP-USP26, or control vector. Following immunoprecipitation of SMAD7, lysates were resolved by SDS-PAGE and probed with the indicated antibodies.
- G 293T cells were transfected as indicated with GFP-USP26, Flag-tagged SMAD7, and Myc-tagged SMURF2. After 48 h, cells were lysed and immunoprecipitated with anti-Flag affinity resin overnight, eluted with anti-Flag peptide, and re-immunoprecipitated with anti-Myc affinity resin. Whole-cell extracts were probed with the indicated antibodies.
- H 293T cells were transfected as indicated with GFP-USP26, HA-tagged SMAD7, and Myc-tagged SMURF1. After 48 h, cells were lysed and immunoprecipitated with anti-Flag affinity resin overnight, cleaved with an anti-Flag peptide, and re-immunoprecipitated with anti-Myc affinity resin. Whole-cell extracts were probed with the indicated antibodies.

Source data are available online for this figure.

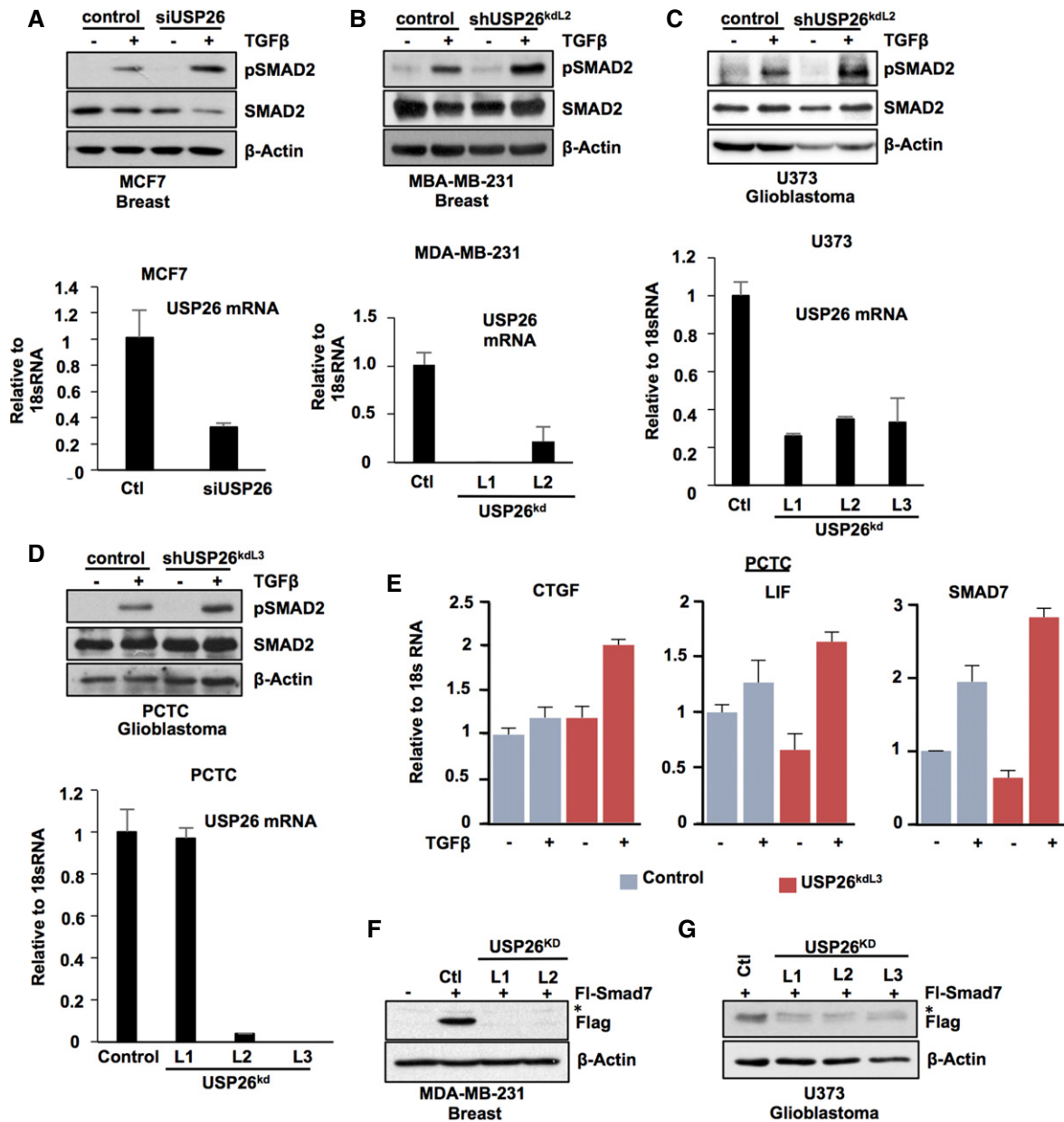


Figure EV3. Regulation of the TGF- β by USP26 in breast cancer and GBM cell lines.

- A** MCF7 cells transfected with siRNA targeting USP26 or control vector were treated with TGF- β overnight. Whole-cell extracts were probed with the indicated antibodies (top panel). Corresponding *USP26* mRNA levels relative to 18S are shown as evaluated by quantitative real-time PCR (bottom panel). Data are mean \pm SD of triplicate samples.
- B–D** MDA-MB-231 (**B**), U373 (**C**), and PCTC (**D**) cells stably expressing lentiviral knockdown vectors targeting USP26 or control vector were treated with TGF- β overnight. Whole-cell extracts were probed with the indicated antibodies (top panel). Corresponding *USP26* mRNA levels relative to 18S are shown as evaluated by quantitative real-time PCR (bottom panel). Data are mean \pm SD of triplicate samples.
- E** PCTC cells stably transduced with a hairpin targeting USP26 or vector control were stimulated with TGF- β for 3 h. *CTGF*, *LIF*, and *SMAD7* mRNA levels relative to 18S are shown as evaluated by quantitative real-time PCR. Data are mean \pm SD of triplicate samples.
- F** MDA-MB-231 cells stably expressing lentiviral knockdown vectors targeting USP26 or control vector were treated with transfected with FL-SMAD7 (1 μ g). Whole-cell extracts were probed with the indicated antibodies. * denotes background band.
- G** U373 cells stably expressing lentiviral knockdown vectors targeting USP26 or control vector were treated with transfected with FL-SMAD7 (1 μ g). Whole-cell extracts were probed with the indicated antibodies. * denotes background band.

Source data are available online for this figure.

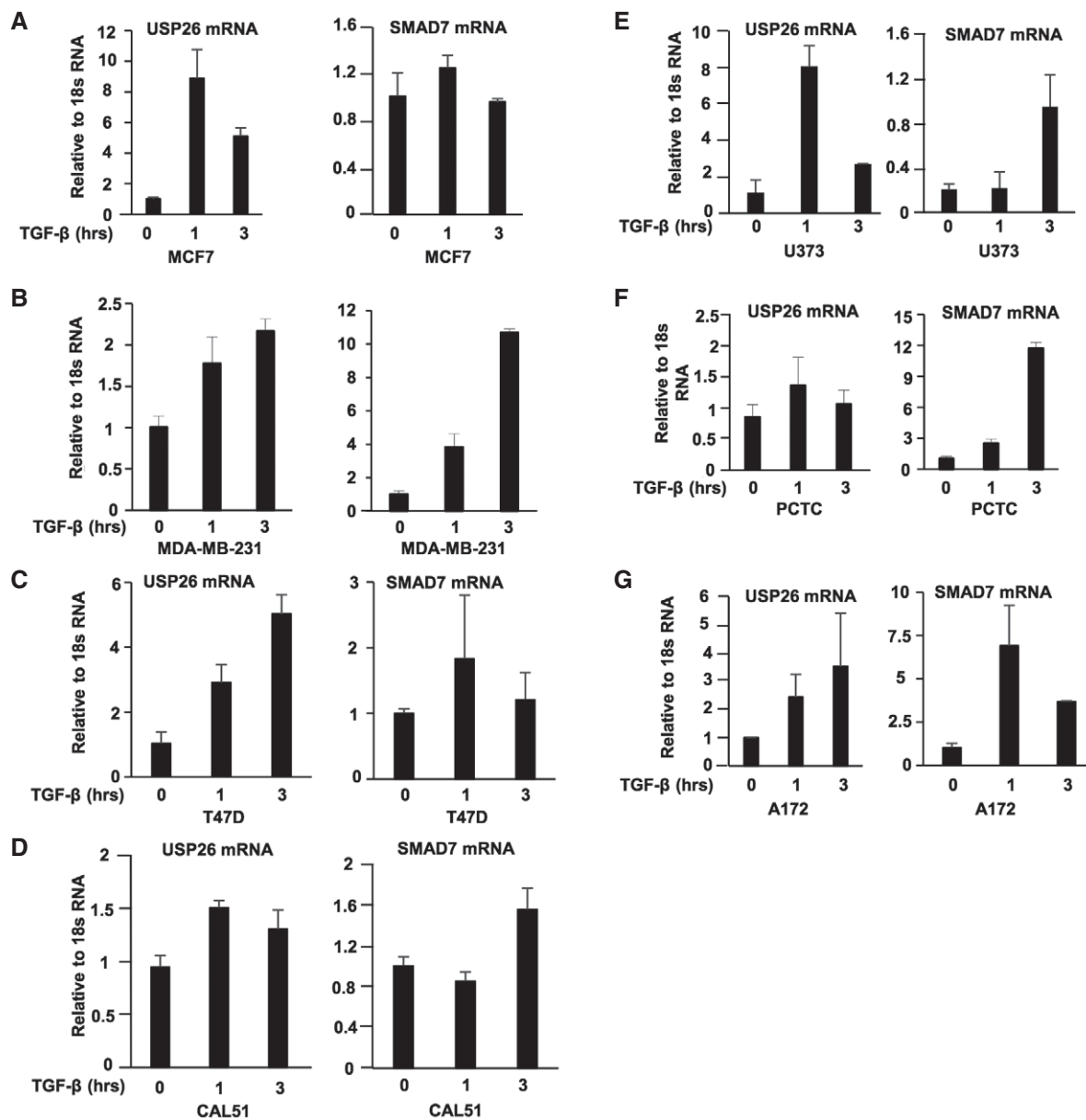


Figure EV4. Expression of SMAD7 and USP26 following TGF- β induction in breast cancer and GBM cell lines.

A–D The breast cancer cell lines MCF7 (A), MDA-MB-231 (B), T47D (C), and CAL51 (D) were stimulated with TGF- β for 1 and 3 h. USP26 mRNA (left panel) and SMAD7 (right panel) levels relative to 18S are shown as evaluated by quantitative real-time PCR. Data are mean \pm SD of triplicate samples.

E–G The glioblastoma cancer cell lines U373 (E), PCTC (F), and A172 (G) were stimulated with TGF- β for 1 and 3 h. USP26 mRNA (left panel) and SMAD7 (right panel) levels relative to 18S are shown as evaluated by quantitative real-time PCR. Data are mean \pm SD of triplicate samples.

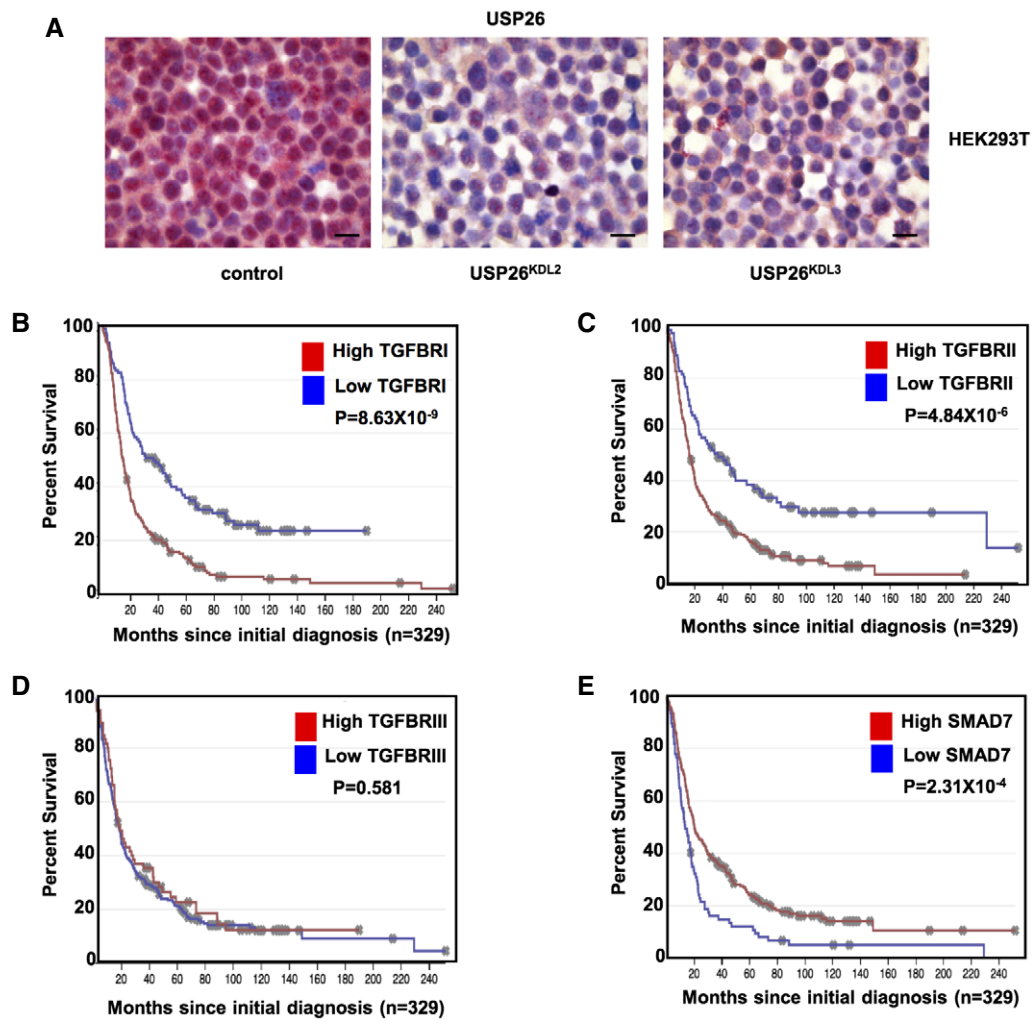


Figure EV5. Correlation of TGF- β pathway components with overall survival in GBM.

- A Validation of the USP26 antibody for immunohistochemistry. 293T cells stably infected with knockdown vectors targeting USP26 were analyzed by immunohistochemistry for USP26 expression. Representative images are shown. To prepare sections, cell pellets were fixed in formalin and embedded in paraffin. Red staining indicates positive immunoreactivity. Scale bars: 50 μ m.
- B–E Kaplan–Meier curves of glioblastoma patients ($n = 329$) with TGFBR1 (B), TGFBR2 (C), TGFBR3 (D), and SMAD7 (E) (REMBRANDT). P -value was obtained by log-rank test.