Figure S1. Expressing IL13-PE or ENb-PE in toxin-resistant cell lines. (A) Schematic demonstration of establishing toxin-resistant cell lines. (B) Viability of either wild-type or toxin-resistant 293T cells (293 oligo) treated with 0-1,000 ng/ml diphtheria toxin. (C) Fluorescence images of toxin resistant 293 oligo cells expressing IL13-PE, ENb-PE or TRAIL. (D) Western blot analysis of targeted toxin and TRAIL secretion into conditioned medium collected from toxin resistant 293 oligo cells. (E) Dot blot analysis of IL13-PE and ENb-PE in conditioned medium from 293 oligo cells (band intensities were determined by ImageJ analysis and used for quantification). TRAIL, tumor necrosis factor-related apoptosis-inducing ligand.

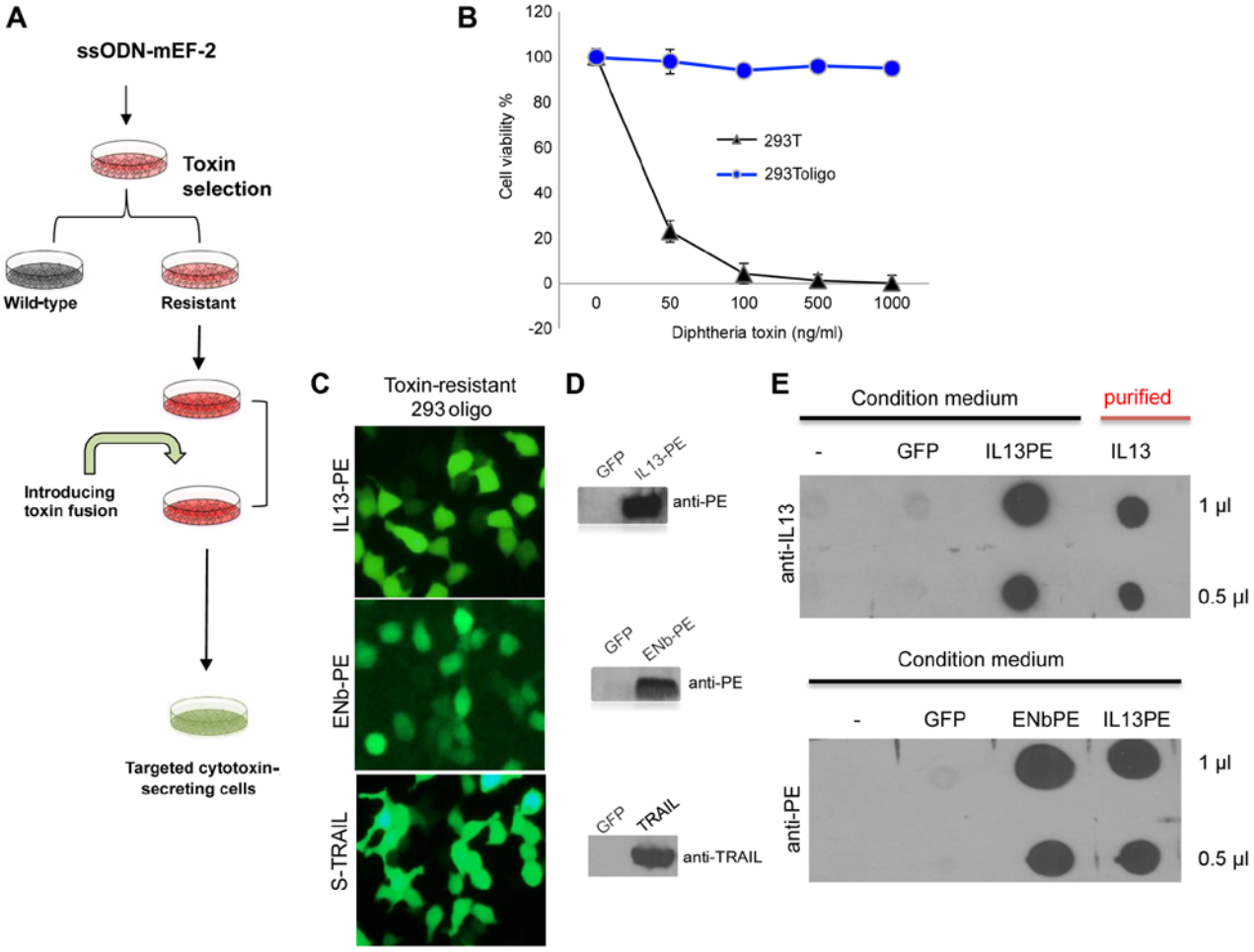

Figure S2. Targeted toxins inhibit protein synthesis and reduce cell proliferation. (A) Tracking protein synthesis in destabilized luciferase (dsluc)-expressing LN229-dsluc and U251-dsluc GBM cells following 24 h of treatment with 25 ng/ml ENb-PE or IL13-PE. (B and C) Fluorescent images of Ki67 in LN229-dsluc, or U251-dsluc cells treated with either 25 ng/ml ENb-PE or IL13-PE, respectively (red, Ki-67; blue, DAPI and pink, merged). (D-G) Plots showing the quantification of Ki67 immunostaining in targeted toxin-treated GBM cells. Data represent the means ± SEM. Significance was determined by an unpaired Student's t-test when comparing 2 groups. For multiple comparisons, one-way ANOVA and a post hoc test (Bonferroni) was applied ($^{*}P<0.05$ and $^{**}P<0.001$ compared to control). TRAIL, tumor necrosis factor-related apoptosis-inducing ligand; GBM, glioblastoma.

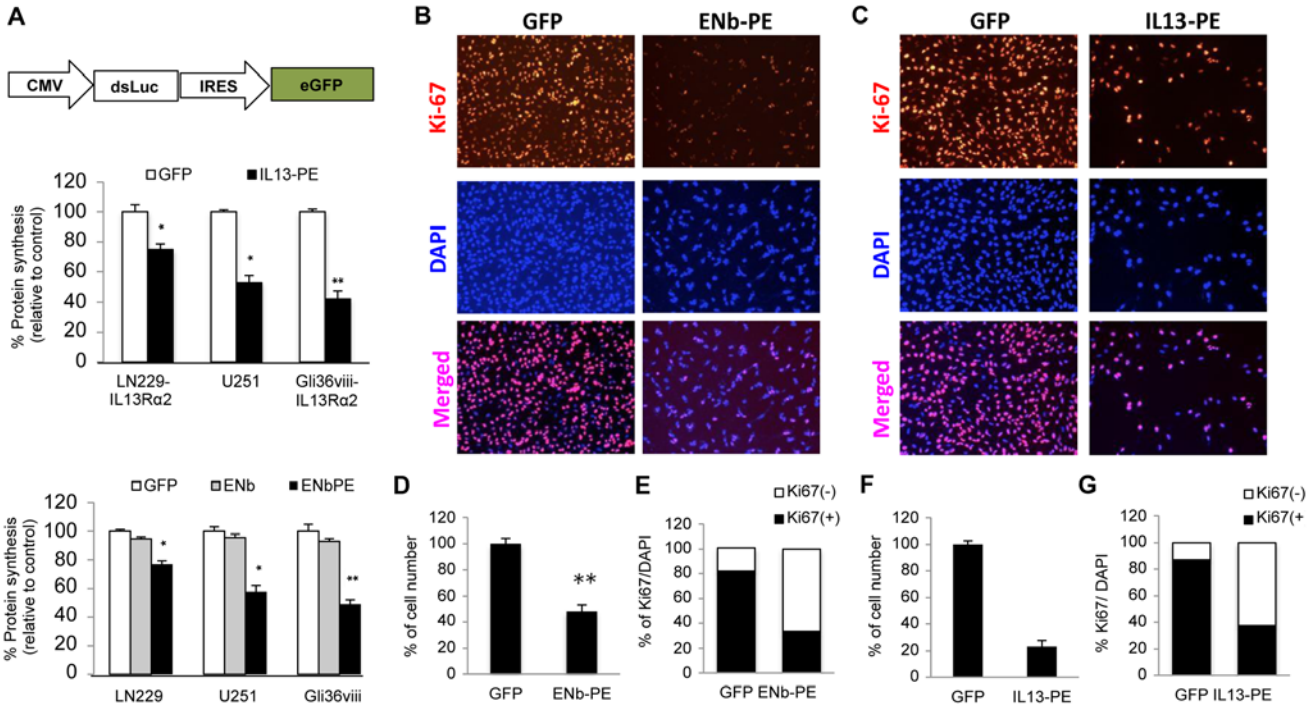

Figure S3. Response to TRAIL treatments in both established and patient-derived GBM cells. Viability of both established and patient-derived GBM cells following treatment with 0-500 ng/ml S-TRAIL Data represent the means ± SEM. Significance was determined by an unpaired Student's t-test (*P<0.05 vs. control). TRAIL, tumor necrosis factor-related apoptosis-inducing ligand; GBM, glioblastoma.

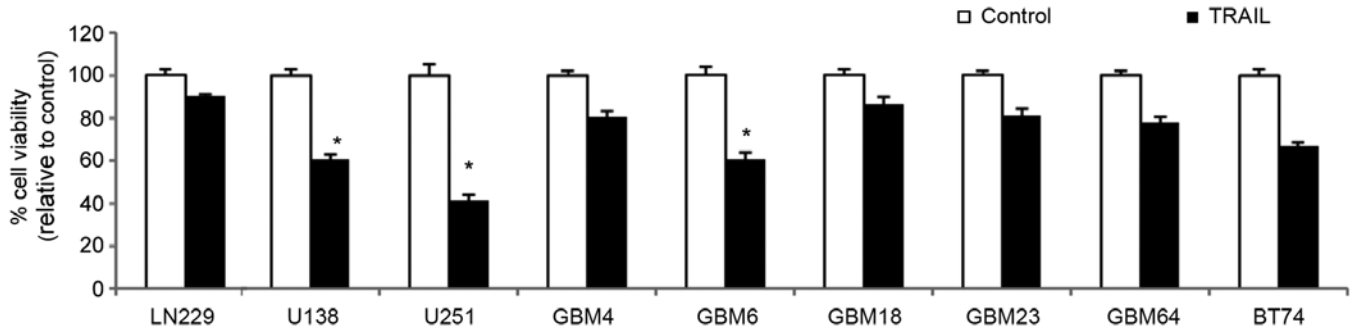

Figure S4. Co-treatment with IL13-PE and TRAIL enhances the death of TRAIL-sensitive U251 cells. (A) Plot showing photon emission assayed at various time points following treatment with 100 ng/ml IL13-PE in U251 cells (engineered with constructs containing Firefly luciferase under control of DR4 or DR5 promoters). (B) Western blot analysis of apoptosis in U251 cells treated with 25 ng/ml IL13-PE or ENb-PE for 24 h and co-treated with 100 ng/ml S-TRAIL for an additional 24 h. Samples were probed with antibodies against cleaved PARP, caspase-8 and cleaved caspase-9 and β-actin. Plots indicate (C) Cell viability and (D) caspase-3/7 activity in U251 cells pre-treated with 0-50 ng/ml IL13-PE for 24 h and then treated with S-TRAIL (100 ng/ml). Data represent the means ± SEM and P-values were determined by one-way ANOVA and a post hoc test (Bonferroni) ($^{*}P<0.05$ and $^{**}P<0.001$ compared to control). TRAIL, tumor necrosis factor-related apoptosis-inducing ligand; GBM, glioblastoma; c-Casp, cleaved caspase.