# Supplementary Materials: IL-13Rα2 Status Predicts GB-13 (IL13.E13K-PE4E) Efficacy in High-Grade Glioma

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**Table S1.** Details regarding the cell lines used in this study.

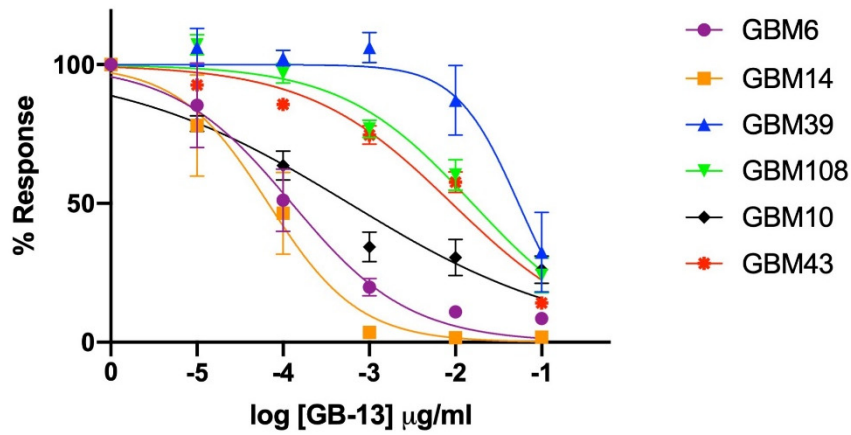| Cell line ID | Age/Sex | Institution | Molecular Status | Tests | Date of Tissue Collection | Culture Media |
|---|---|---|---|---|---|---|
| GBM6 | 65/M | Mayo Clinic | GBM, IDH WT | WES, Sanger, STR | 2000 | FBS or Stem |
| GBM8 | 75/F | Mayo Clinic | GBM, IDH WT | WES, Sanger, STR | 2000 | FBS or Stem |
| GBM10 | 40/M | Mayo Clinic | GBM, IDH WT | WES, Sanger, STR | 2001 | FBS or Stem |
| GBM12 | 69/M | Mayo Clinic | GBM, IDH WT | WES, Sanger, STR | 2001 | Stem |
| GBM14 | 58/M | Mayo Clinic | GBM, IDH WT | WES, Sanger, STR | 2001 | FBS or Stem |
| GBM26 | 49/M | Mayo Clinic | GBM, IDH WT | WES, Sanger, STR | 2002 | FBS |
| GBM39 | 51/M | Mayo Clinic | GBM, IDH WT | WES, Sanger, STR | 2003 | FBS or Stem |
| GBM43 | 69/M | Mayo Clinic | GBM, IDH WT | WES, Sanger, STR | 2003 | FBS or Stem |
| GBM59 | 83/F | Mayo Clinic | GBM, IDH WT | WES, Sanger, STR | 2004 | FBS |
| GBM64 | 63/M | Mayo Clinic | GBM, IDH WT | WES, Sanger, STR | 2006 | FBS or Stem |
| GBM108 | 62/M | Mayo Clinic | GBM, IDH WT | WES, Sanger, STR | 2009 | FBS or Stem |
| GBM118 | 56/F | Mayo Clinic | GBM, IDH WT | WES, Sanger, STR | 2009 | FBS |
| GBM123 | 62/F | Mayo Clinic | GBM, IDH WT | WES, Sanger, STR | 2009 | FBS or Stem |
| GBM134 | 42/F | Mayo Clinic | GBM, IDH WT | WES, Sanger, STR | 2009 | FBS |
| GBM161 | 78/M | Mayo Clinic | Glioscarcoma, IDH WT | WES, Sanger, STR | 2012 | FBS |
| PED17 | 12/F | Mayo Clinic | DMG, H3.3 K27M | Sanger, STR | 2015 | MHM |
| SU-DIPG XIII-P | 6/F | Stanford University | DMG, H3.3 K27M | Sanger, STR | Received 2016 | TSM |
| SU-DIPG XVII | 8/M | Stanford University | DMG, H3.3 K27M | Sanger, STR | Received 2016 | MHM |
| SF8628 | 3/F | Millipore | DMG, H3.3 K27M | Sanger, STR | Purchased 2019 | dBT |
| SF8628-B23 | – | Hormel Institute | DMG, H3.3 K27M KO | Sanger, STR | Created 2019 | dBT |

Abbreviations: GBM = glioblastoma, IDH = isocitrate dehydrogenase, DMG = diffuse midline glioma, WES = whole exome sequencing, STR = short tandem repeat, WT = wildtype.

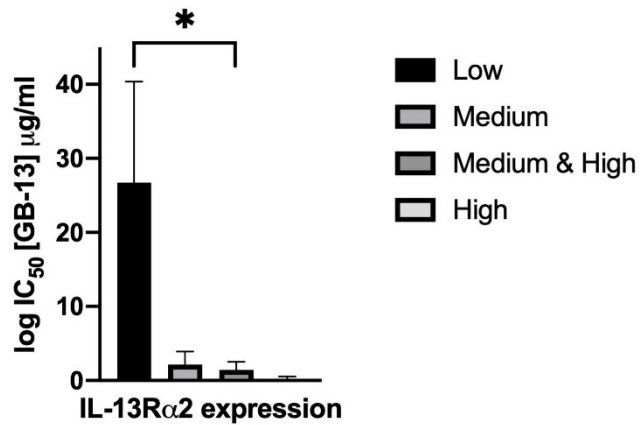**Table S2.** Media composition for culture of patient-derived cell lines.

| Cell Lines | Media Name | Media Contents |
|---|---|---|
| GBM6, GBM8, GBM10, GBM14, GBM26, GBM39, GM43, GBM59, GBM64, GBM108, GBM118, GBM123, GBM134, GBM161 | FBS-containing medium (FBS) | DMEM (Thermo Fisher), 10% FBS (Atlanta Biolocials), and 1% penicillin/streptomycin (Thermo Fisher). |
| GBM6, GBM8, GBM10,GBM12, GBM14, GBM39, GM43, GBM64, GBM108, GBM123 | Stem cell medium (Stem) | KnockOut DMEM/F12 (Thermo Fisher), StemPro NSC SFM supplement (Thermo Fisher), 20ng/mL FGF basic recombinant human (Thermo Fisher), 20ng/mL EGF recombinant human(Thermo Fisher), 200mM L-glutamine (Corning), and 1% penicillin/streptomycin (Thermo Fisher). |
| PED17, SU-DIPG XVII | Serum-free complete medium (MHM) | DMEM/F12 (HyClone), 25 mM glucose (Sigma-Aldrich), 8.9 mM sodium bicarbonate (Life Technologies), 2 mM glutamine (Life Technologies), 4 mM HEPES (Life Technologies), 1% penicillin/streptomycin (Life Technologies), N2 supplement (Life Technologies), 4 μg/mL heparin (Sigma Aldrich), 20 ng/mL human EGF (PeproTech US), 20 ng/mL human b-FGF (PeproTech US), and 20 ng/mL human PDGF AA with 20 ng/mL human PDGF BB (Shenandoah Biotechnology). |
| SU-DIPG XIII | Tumor stem cell medium (TSM) | Neurobasal(-A) (Life Technologies), DMEM/F12 (Life Technologies) B27(-A) (Life Technologies), 4 μg/mL heparin (Sigma Aldrich), 20 ng/mL human EGF (PeproTech US), 20 ng/mL human b-FGF (PeproTech US), 20 ng/mL human PDGF AA with 20 ng/mL human PDGF BB (Shenandoah Biotechnology), and 1% penicillin/streptomycin (Life Technologies). |
| SF8628, SF8628-B23 | dBT medium | DMEM/F12 (HyClone), 10% FBS, and 1% penicillin/streptomycin (Life Technologies). |

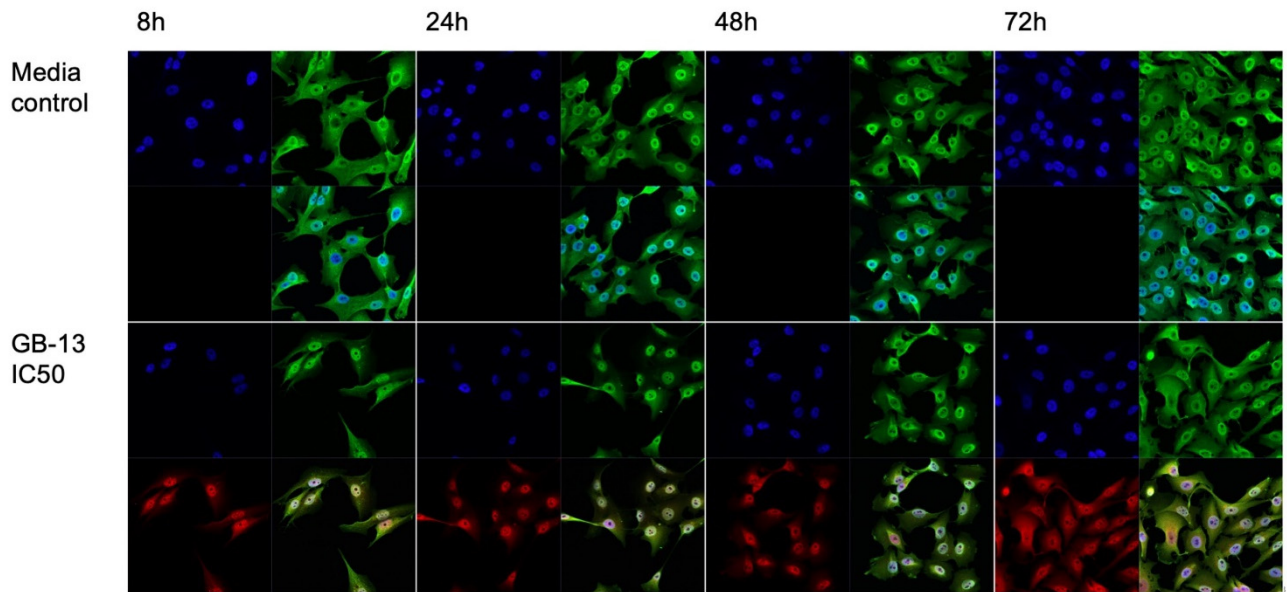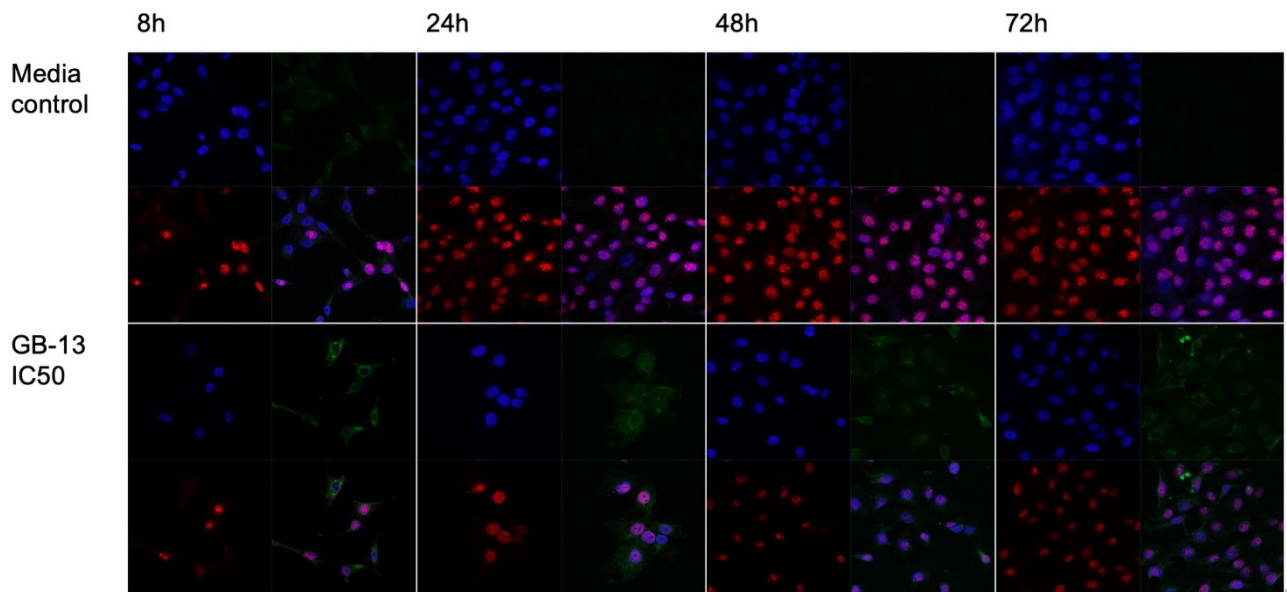**Table S3.** Antibodies used for immunoblotting, immunofluorescence and immunohistochemistry.

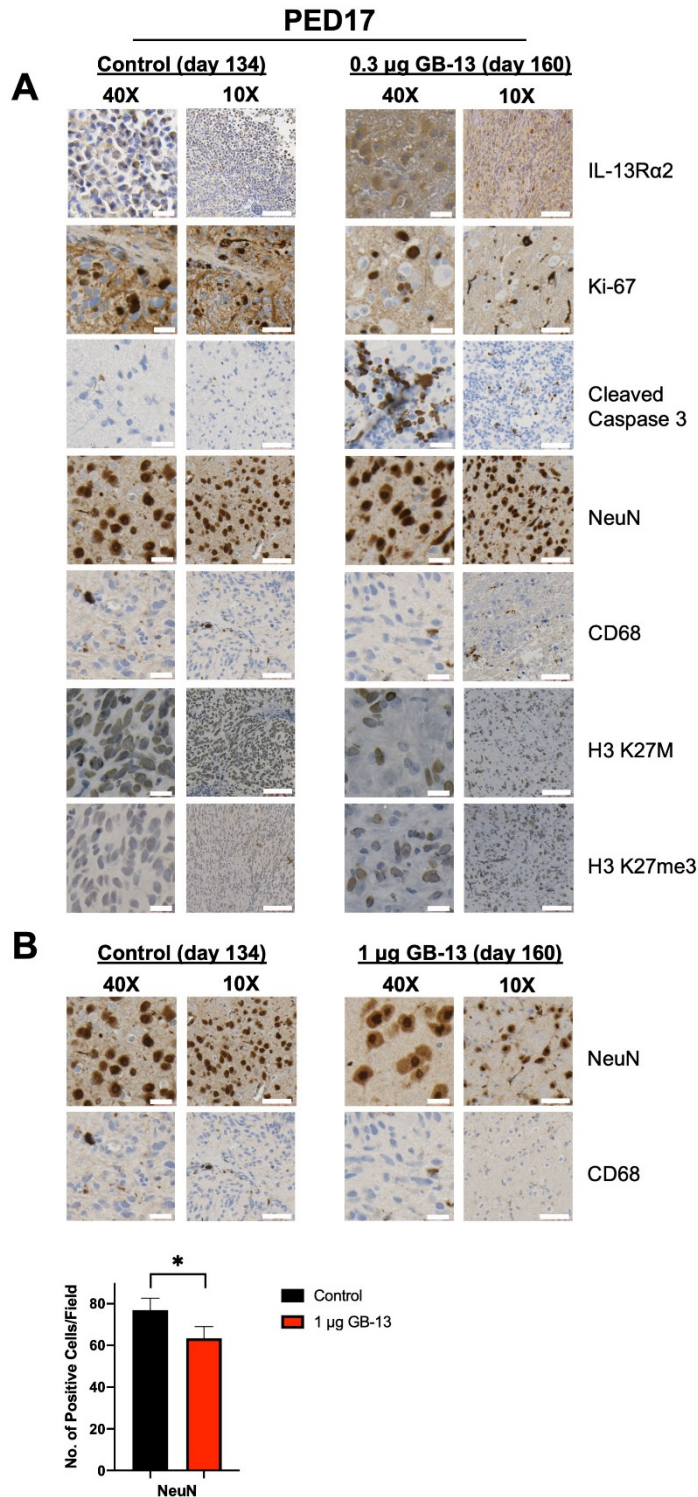| Antibodies | Source | Identifier | Dilution for WB | Dilution for IF | Dilution for IHC |
|---|---|---|---|---|---|
| Cleaved PARP | Cell Signaling | 5625S | 1:1000 | | |
| Cleaved Caspase 3 | Cell Signaling | 9664S | 1:1000 | 1:500 | 1:200 |
| IL-13R$\alpha$1 | Abcam | Ab79277 | 1:1000 | | |
| IL-13R$\alpha$2 | Cell Signaling | 85677 | 1:1000 | 1:200 | |
| H3K27M | Abcam | 190631 | 1:3000 | | 1:500 |
| H3K27me3 | Cell Signaling | 9733S | 1:1000 | | 1:100 |
| Histone H3 | Cell Signaling | 3638S | 1:1000 | | |
| $\alpha$-Tubulin | Sigma | T9026 | 1:20,000 | | |
| Vinculin | Cell Signaling | 13901S | 1:1000 | | |
| Pseudomonas Exotoxin A | Sigma | P2318 | | 1:500 | |
| Ki-67 | Invitrogen | 14-5698-82 | | 1:200 | |
| IL-13R$\alpha$2 | R&D | AF146 | | | 1:250 |
| Ki-67 | Dako | GA626 | | | 1:400 |
| NeuN | Sigma | MAB377 | | | 1:2400 |
| CD68 | Abcam | Ab125212 | | | 1:800 |

**Figure S1.** Cell viability and dose-response curve of adult GBM cell models. Similar to other HGG cell lines tested, GB-13 treatment of adult GBM cell lines elicits potent anti-tumor responses in an IL-13Rα2-dependent fashion. IC$_{50}$ values were determined using nonlinear least-squares curve-fitting analysis. GB-13 was tested in triplicate with 3 independent experiments ($n$ = 9) in each cell line. Analysis was performed after 72 h of drug exposure.



**Figure S2.** DMG and adult GBM cell lines demonstrate similar sensitivity towards GB-13 dependent on IL-13Rα2 status. High and medium expressors are similarly sensitive ($p$ = 0.43) and together have significantly different IC$_{50}$ values compared to IL-13Rα2-low cell models ($p$ = 0.009).
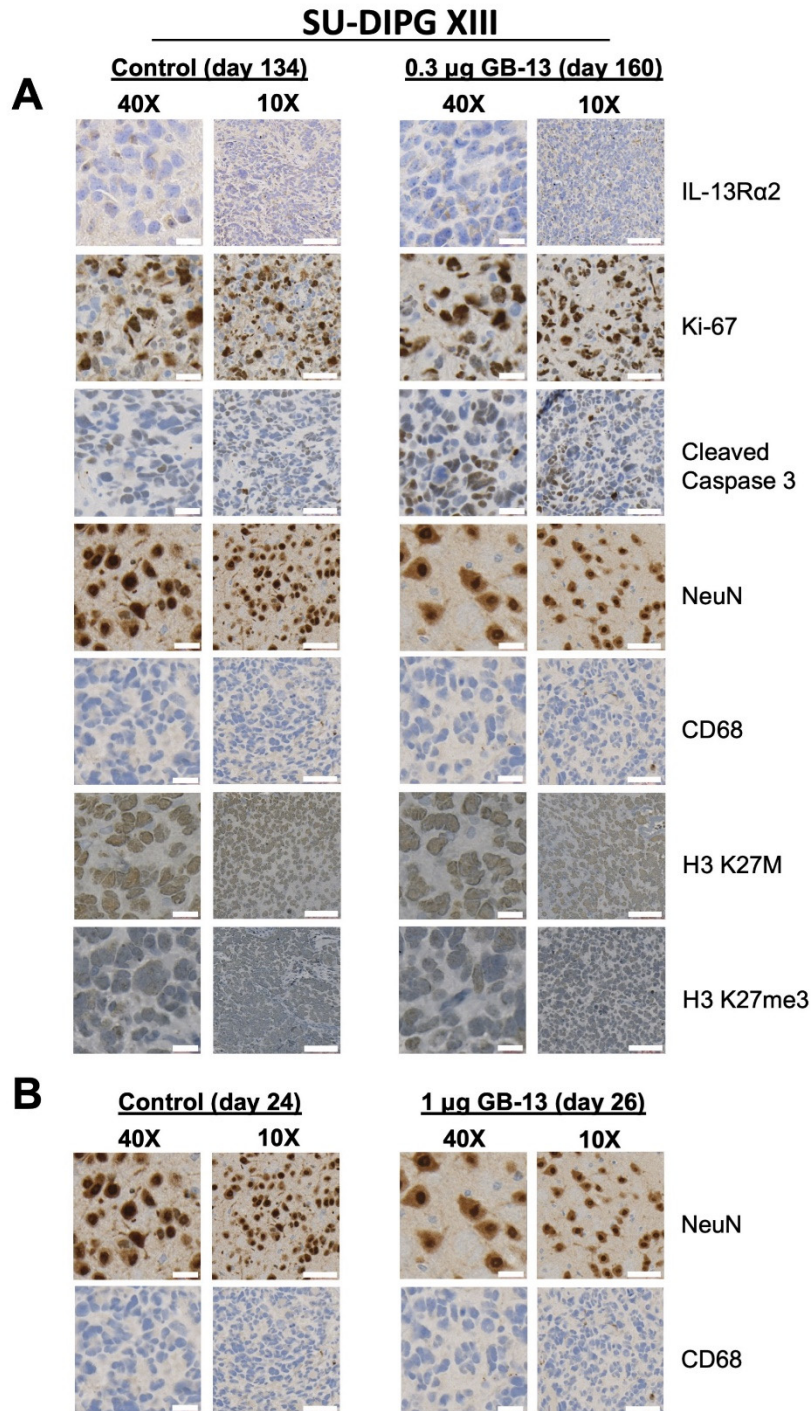
**A**



**B**



**Figure S3.** Immunofluorescence (IF) of SF8628 cells, a DMG cell line, after 8, 24, 48, and 72 h of GB-13 exposure at $IC_{50}$. (**A**) The PE moiety of GB-13 (red) colocalizes to IL-13R$\alpha$2 (green) while receptor levels are maintained over prolonged durations of treatment. DAPI shown in blue. Overlay in bottom right of each image. (**B**) Cells demonstrate increased levels of apoptosis (cleaved caspase 3; green) and decreased cell proliferation (Ki-67; red) after exposure to GB-13. DAPI shown in blue. Overlay in bottom right of each image. Images are representative of 3 independent experiments.
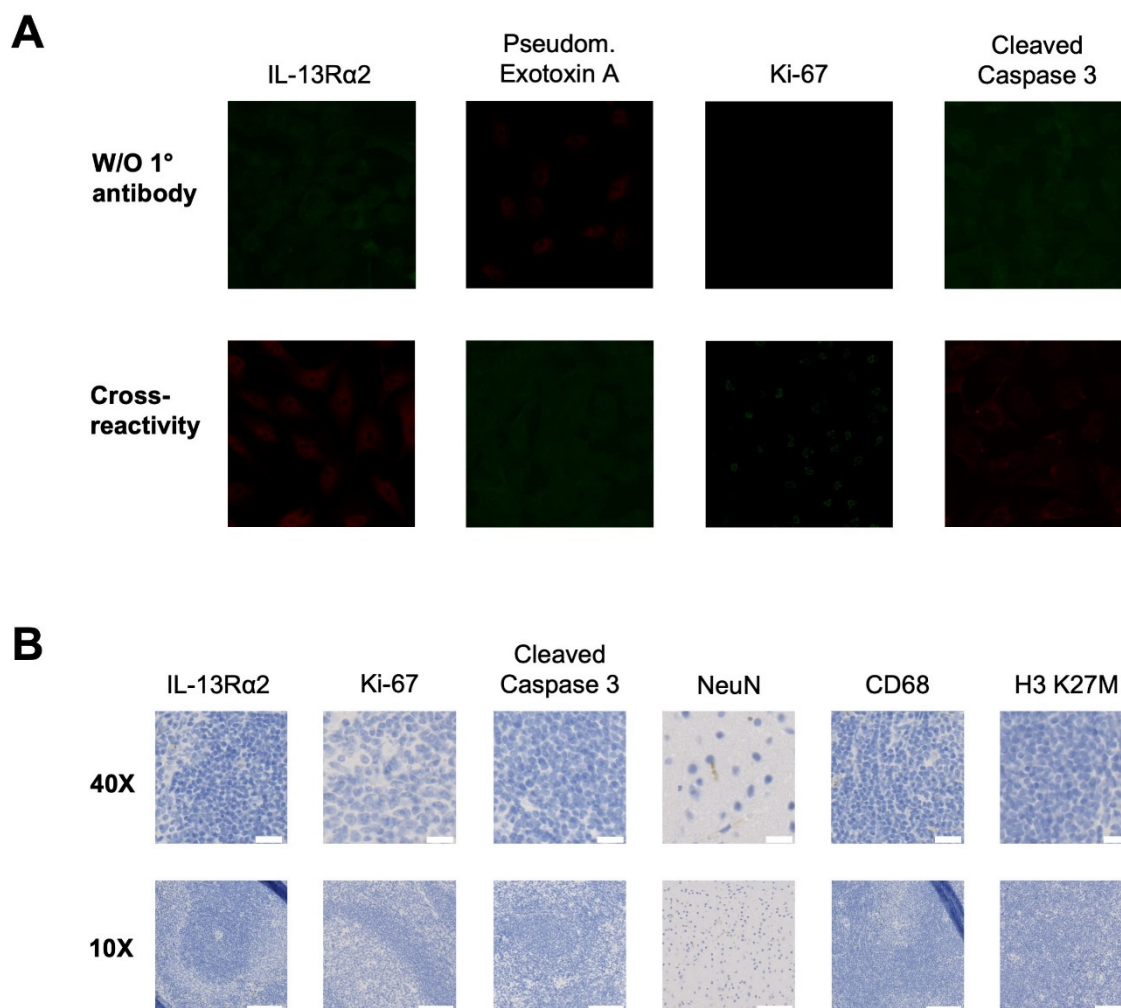
**Figure S4.** Immunohistochemistry (IHC) of PED17-bearing mouse brains harvested at endpoint. Images are representative of 5 mice in each group. (**A**) Animals were euthanized at day 134 and 160 (treatment with vehicle and GB-13 at a dose of 0.3 mg, respectively). IL-13Rα2 status retained after a single CED infusion of 0.3 mg GB-13. Treatment leads to decreased cell proliferation and increased levels of apoptosis. The density of NeuN+ cells is retained and infiltrative CD68+ monocytes are absent in the infused brainstem. Staining for H3 K27M decreases and H3 K27me3 increases in GB-13 treated tumors. (**B**) A 1 mg CED infusion of GB-13 decreases NeuN+ cell density in the brainstem as compared to lower GB-13 doses or vehicle control. CD68+ monocyte infiltrate is not evidenced following exposure to 1 mg GB-13. Scale bars: 40×: 20 mm, 10×: 100 mm.

**Figure S5.** IHC of SU-DIPG XIII-P-bearing mouse brains harvested at endpoint. Images are representative of 5 mice in each group. (**A**) Animals were euthanized at day 24 and 26 (treatment with vehicle and GB-13 at a dose of 0.3 mg, respectively). IL-13Rα2 levels were undetectable after a single CED infusion of vehicle or 0.3 mg GB-13. Treatment did not lead to decreased cell proliferation, and levels of apoptosis were equally low following treatment with vehicle or GB-13. NeuN+ cells density is retained and infiltrative CD68+ monocytes are absent in the infused brainstem. The staining pattern for H3 K27M and H3 K27me3 remains constant following GB-13 treatment. (**B**) A 1 mg CED infusion of GB-13 decreases NeuN+ cell density in the brainstem as compared to lower GB-13 doses or vehicle control. CD68+ monocyte infiltrate is not evidenced following exposure to 1 mg GB-13. Scale bars: 40×: 20 mm, 10×: 100 mm.

**A**



**B**



**Figure S6.** Negative staining controls for IF and IHC experiments. (**A**) A negative control lacking primary antibody was included to confirm the specificity of IF staining (upper panel). Since multi-color IF using two primary antibodies from different hosts was performed, one control per primary antibody condition was included by applying the other secondary antibody to the primary antibody to test for cross-reactivity (lower panel). (**B**) Sections from the following tissues known not to express the target antigen were stained to check for non-specific signal and false positive results in IHC staining: Mouse spleen (IL-13Rα2), normal human tonsil (Ki-67, cleaved caspase 3, H3 K27M), mouse brain (NeuN), and mouse spleen (CD68). Untreated DMG samples served as negative control for H3 K27me3 (see Figures S4 and S5.). Scale bars: 40×: 20 mm, 10×: 100 mm.