Supplemental Methods

Cell culture: Primary human pediatric DMG/diffuse intrinsic pontine glioma (DIPG) cell lines (DIPG 4 and DIPG 6, derived from previously irradiated DIPGs at autopsy, provided by Dr. Michelle Monje, Stanford University; DIPG7, provided by Dr. Angel Montero Carcaboso, Hospital Sant Joan de Deu; and SF7761, derived from a biopsy sample, provided by Dr. Nalin Gupta, University of California-San Francisco) were grown in neurosphere (suspension) culture conditions in ultra-low attachment flasks (Corning) (DIPG4, DIPG6 and DIPG7) or in tissue culture-treated flasks (Falcon/Corning) (SF7761). DIPG4 cells were also grown adherently in tissue-culture treated plates. DIPG lines were maintained in TSM medium prepared from Neurobasal-A medium mixed 1:1 with Dulbecco's modified Eagle's medium/F-12 supplemented 1:100 by volume with: HEPES [4-(2-hydroxyethyl)-1-piperazine ethanesulfonic acid] 1 M, sodium pyruvate 100 mM, MEM Non-Essential Amino Acids 10 mM, Glutamax-I, and antibiotic-antimycotic (all Gibco/Life Technologies); B27-A supplement 50x (1:50, Invitrogen); heparin (2 μg/mL, Stemcell Technologies); and human EGF, FGFb, and platelet derived growth factor–AB (PDGF) (all 20 ng/mL, Shenandoah Biotech). For SF7761 cells, N2 supplement was also added (1:100, Life Technologies). A thalamic diffuse midline glioma cell line (BT245, provided by Dr. Keith Ligon, Dana-Farber Cancer Institute) was grown in neurosphere conditions in ultra-low attachment flasks (Corning) in Neuro-Cult medium (StemCell Technologies) supplemented with EGF, FGFb and PDFG and Pen-Strep (1%).[11] A supratentorial pediatric glioblastoma cell line (GBM1, provided by Dr. Angel Montero Carcaboso) was grown in neurosphere conditions in TSM as described above for the DIPG cell lines. The identity of all lines was validated by microsatellite DNA profiling and mycoplasma testing performed prior to and during this project.

Selinexor preparation and treatment: Selinexor (Karyopharm Therapeutics) was dissolved in dimethyl sulfoxide (DMSO, Sigma) to a concentration of 10 mM and stored in accordance with manufacturer instructions. Selinexor was diluted with PBS or medium to its final concentration and a DMSO level of 0.1% by volume for all experiments. DMSO at 0.1% by volume was used as the vehicle control for *in vitro* experiments involving drug treatment. In experiments involving measurements of cell viability following treatment by selinexor, cells were plated at 10,000 (DIPG4), 20,000 (BT245, DIPG7 and GBM1) or 30,000 (SF7761) cells per well in 100 μL medium in a 96-well cell culture plate. Three replicates per condition were utilized. Viability assays were 120h in length. At the conclusion of each experiment, cell viability was evaluated using CellTiter 96 Aqueous One Solution Cell Proliferation Assay (Promega) according to manufacturer instructions. Plates were imaged for absorbance at 490 nm using a Synergy 2 plate reader (Biotek) at incubation times ranging from 1h to 7h depending upon the cells used and conditions of the assay. Results were adjusted by subtracting background absorbance observed in medium treated with CellTiter 96. IC_{50} values were calculated using Prism 7 (Graphpad) or CompuSyn as noted in the text.

Apoptosis assays: Cells to be evaluated for apoptosis levels were plated in triplicate at 10,000-20,000 cells per well in 100 uL medium in white 96-well plates. Caspase Glo 3/7 (Promega) was prepared and added to cells per manufacturer instructions and incubated for 30-60 minutes. Plates were imaged for luminescence using a GloMax Multi-Detection System (Promega).

ELISA: Approximately 4 million cells were plated into 3 mL of medium in a 6-well plate and treated with DMSO (0.1%) or selinexor (1μM) for 16h, after which the cells were exposed to 50 ng/mL TNFα (to activate NF-κB transcription) for 4h and then immediately lysed in Pierce RIPA Buffer containing 1% HALT protease inhibitor (Thermo Scientific). The transcriptional activity of NF-κB in the cells was measured using the Chemiluminescent Transcription Factor Assay kit (Thermo Scientific Catalog #89859) according to the manufacturer's recommendations. Briefly, RIPA-lysed whole cell extract was placed in a streptavidin-coated 96 well plate to which the biotinylated κB-consensus sequence was attached. Only NF-κB

molecules that are free of their inhibitor (IκB-α) can bind to the κB-consensus sequence; ELISA therefore provides a direct measure of transcriptionally active NF-κB levels. Following incubation to allow active NF-κB to bind to the κB-consensus sequence, primary antibody to the p65 subunit of NF-κB was added followed by a secondary HRP-conjugated antibody. A chemiluminescent substrate was then added to the wells and the resulting signal detected using a luminometer.

NGFR knockdown: We utilized MISSION shRNA plasmid DNA against NGFR (TRCN0000058153 and TRCN0000058154) or scrambled controls (SHC007 and SHC216), purchased from the University of Colorado Cancer Center Functional Genomics Facility, which we packaged into lentivirus using second generation packaging and envelope plasmids. Knockdown of expression was confirmed with both NGFR plasmids. For transduction, cells were plated in 6-well plates at a density of 500,000 cells per well in 1.9 mL of antibiotic-free medium, incubated for 30-60 minutes with polybrene at a concentration of 8 μg/mL, and then incubated with 100 μL of virus-containing medium for 16h. At 16h, the virus-containing medium was removed and fresh medium containing antibiotics was added. At 48h – 72h, selection with puromycin was begun at 4x the IC_{90} for the wild-type cell line; the puromycin concentration was reduced to the IC_{90} after 72h. Transduced cells were maintained in puromycin for the duration of the study. Clonal NGFR KD and scrambled shRNA control cell lines were prepared from cells grown in neurosphere dilution assays described below.

NGFR overexpression: Cells to be transfected were plated in a 6-well plate at a density of 500,000 cells per well in 2 mL medium. To transfect cells growing in suspension, we used 2.5 μg of plasmid DNA and 9 μL of Lipofectamine 2000 per well, according to manufacturer recommendations. For cells growing adherently, we used 2 μg of plasmid DNA and 4 μL of JetPRIME reagent (Polyplus), according to manufacturer recommendations.

Cell proliferation, neurosphere dilution, and methycellulose assays: Cell proliferation assays - were commenced by plating 100,000 cells per well into a 6-well plate in 2 mL medium per well. Cells were counted at approximately weekly intervals and, where counts exceeded 100,000 cells per well, were replated to 100,000 cells per well. Counting was performed in trypan blue using a TC20 Automated Cell Counter (Bio-Rad). Counts were verified periodically using a hemacytometer. Neurosphere dilution (self-renewal) assays were performed by plating 20 or 30 replicates of 1 or 10 cells per well into a 96-well cell tissue culture plate in 100 μL medium. Medium was replenished weekly. Cells were grown until neurospheres were established in at least some wells, at which point the wells with neurospheres were counted and the size of the neurospheres measured. Imaging was performed using an IncuCyte System (Essen BioScience) at 4x. For the methycellulose (colony formation) assay, 5,000 cells were plated in a 1:1 mixture of 2x medium and methylcellulose and 7.5 mL of 2x medium, after which approximately 7 ml of methylcellulose was added. After thorough mixing, 3 mL of the mixture was plated in triplicate into 6-well cell culture plates. When visible colonies began to form, the cells were stained by adding 700 μL per well of nitrotetrazolium blue chloride dissolved in PBS (1.5 mg/mL, Sigma). Imaging was performed by scanning at 600 dpi; counting was performed in IMAGEJ by converting images to 16-bit black and white, inverting the image, thresholding at 3%, and counting as colonies images comprising 16 or more pixels.

Immunofluorescence staining and quantification in vitro: Cells were plated at a density of 20,000 cells per well in BioCoat chamber slides coated with poly-D-lysine or poly-D-lysine and laminin (Corning) and allowed approximately 24-48 hours in which to develop adhesion before subjecting them to

experimental conditions. Cells to be stained were fixed for 20 minutes in formaldehyde diluted to 3.7% in PBS (Sigma), permeabilized in 0.1% Triton-X in PBS for 10 minutes, and blocked for 45 minutes in 4% BSA in PBS supplemented with 0.05% Triton-X. Cells were incubated in primary antibody diluted with 4% BSA (in PBS and 0.05% Triton-X) for 1h at room temperature or overnight at 4C. In experiments with cells unable to develop sufficient adhesion in chamber slides, the cells were fixed and permeabilized in suspension in Foxp3/ Transcription Factor Fixation/ Permeabilization Concentrate and Diluent (Affymetrix EBioscience) according to manufacturer recommendations and incubated in primary antibody diluted with Permeabilization Buffer (Affymetrix Ebioscience) supplemented with 2% BSA. After rinsing, cells were incubated in secondary antibody (Alexa Fluor 488 or 555, Life Technologies) for 1h at room temperature. Suspension cells were affixed to slides using a Cytospin 4 Centrifuge (Thermo Fisher Scientific) at 2000 rpm for 10 minutes. Slides were fixed using ProLong antifade reagent with DAPI (Life Technologies). Confocal imaging was performed at 400x using 405 nM (DAPI), 488 nM (Alexa Fluor 488) and 561 nM (Alexa Fluor 555) lasers on a 3I Marianas imaging system (Intelligent Imaging Innovations). Images were obtained using an Evolve 16-bit EMCCD camera (Photometrics). Primary antibodies and concentrations utilized for adherent and suspended cells were: NGFR (Santa Cruz sc-8317, 1:200), IκB-α (Santa Cruz sc-371, 1:200), NF-κB (p65) (Cell Signaling #6956, 1:400), phosphorylated-NF-κB (S536) (Cell Signaling #3033, 1:200). Nuclear expression was quantified in ImageJ by creating a threshold mask in the DAPI channel and using the analyze particles function to select regions of interest (ROIs) consisting of cell nuclei. The ROIs were then applied to the corresponding antibody-stained channels and quantified using the individual measure function eliminating particles occupying less than 200 pixels. Mean values for treated samples were computed and evaluated for statistical significance versus control using Student's t test ($p<0.05$).

Immunofluorescence staining of in vivo samples: Histologic sections prepared from brains of mice implanted with orthographic xenografts of BT-145, an adult patient-derived glioblastoma cell line, were utilized for immunofluorescence staining. Tumor samples were obtained at the death of the mouse from the tumor-related effects or euthanasia. Slides were de-paraffinized and antigen retrieval performed in BORG solution (Biocare Medical) for 10 minutes at 110C in a Decloaking Chamber (Biocare). Cells were permeabilized and stained as described above. Confocal imaging and image analysis were performed as above.

Western blotting: We isolated nuclear and cytoplasmic fractions using the Nuclear Extraction Kit (abcam, ab113474), and quantified protein amounts by Pierce BCA Protein Assay Kit (Thermo, 23225). We loaded 20 ug of protein per lane into 4 – 20% Mini-PROTEAN SDS-PAGE gels (BioRad, #456-8094) and performed electrophoresis at 125V, followed by wet transfer to Immobilon-P membrane (Millipore, #IPVH00010) at 75V for two hours. We blocked in 4% BSA, immunoblotted using the antibodies also utilized for immunofluorescence staining at a concentration of 1:1000. Actin (Cell Signaling, #3700, 1:2000) and lamin A-C (abcam, ab108595, 1:2000) were used as loading controls. Blots were imaged using horseradish peroxidase conjugated secondary antibodies and Luminata Forte Western HRP Substrate (Millipore) in a G:Box imaging system (Syngene).

RNA-Seq: RNA was extracted from approximately 1 million cells per condition following vehicle (0.1% DMSO) or selinexor treatment for 16h at five times the cell-specific IC_{50} concentration. RNA integrity was confirmed using RIN scores (all of which exceeded 9.5) calculated using TapeStation Analysis Software (Agilent). Library preparation was performed using the TruSeq Library Preparation Kit v2 (Agilent), and sequencing was performed at the University of Colorado's Genomics and Sequencing Core Facility on a HiSeq 2500 sequencing system (Illumina) using single pass 125 bp reads (1x125) with approximately 50

million reads per sample. The resulting data were mapped to the human genome (hg19) by gSNAP, expression (FPKM) was derived by Cufflinks, and differential expression analyzed with ANOVA in R.[13-15] The output for analysis files contained read-depth data (FPKM) for approximately 66,000 transcripts. Relative gene expression levels versus control were computed for each transcript in log2 format, and the data were analyzed using Geneset Enrichment Analysis (GSEA, Broad Institute) using specific NF-κB genesets (Table 1b). GSEA results were evaluated using the net enrichment score (NES), in which increased expression results in a positive NES and reduced expression in a negative NES. Scores were considered potentially informative from a statistical perspective, in accordance with Broad Institute recommendations, if the false discovery rate (FDR) was less than 0.25.