Supplementary Figure Legends

Figure S1. Auto-phosphorylation of EGFR-A289V and EGFR-G598V can be inhibited by EGFR TKIs. (A) Murine Ink4a/Arf-/- Pten -/- astrocytes transduced with control, EGFR-A289V, EGFR-G598V or lung cancer mutant EGFR-L858R were serum starved for 24 hours followed by 4-hour treatment of vehicle (DMSO), 250 nM erlotinib or 50 nM gefitinib. Cell lysates were prepared and subjected to immunoblot analysis. (B) Cell lysates prepared from the EGFR-A289V and EGFR-G598V transduced cells treated as in (A) were immunoprecipitated with anti-EGFR antibody and subjected to immunoblot analysis with anti-phospho-tyrosine antibody (4G10).

Figure S2. *EGFR** expression is strongly induced in *hGFAP-tTA tetO-EGFR** off-Dox mouse brains. (A) Total RNAs were isolated from brain, skin, pancreas, intestine or liver of 12-week-old control *hGFAP-tTA* or *hGFAP-tTA tetO-EGFR** mice maintained either off- (-) or on- (+) Dox (n = 2 for each), and were subjected to qPCR for EGFR* and ß-Actin. Results were normalized with ß-Actin expression and shown as mean ± SD. (B) *EGFR** expression was induced in Gfap-positive cells along subventriclular zone (SVZ) and brain parenchyma of off-Dox *hGFAP-tTA tetO-EGFR** bitransgenic mice. Shown are representative co-immunostaining of anti-EGFR and anti-Gfap on brain sections of 12-week-old *hGFAP-tTA tetO-EGFR** bitransgenic animals kept off- or on-Dox. Scale bar represents 100 µm. (C) *EGFR** induction promoted SVZ neural precursor cell (NPC) expansion. Shown are representative images of brain sections of off- and on-Dox 12-week-old bitransgenic mice stained with H&E or anti-EGFR. The arrows indicate EGFR*-expressing cells that were migrating into adjacent sub-striatum of cortical white matter. Scale bar represents 50 µm.

Figure S3. Gfap and Nestin protein expression are overlapped in a subpopulation of NPCs along the lateral ventricles of mouse brain neurogenic SVZ regions but not in parenchymal astrocytes. Scale bar represents 50 μm.

Figure S4. Representative H&E images of a Grade IV malignant glioma show secondary structures of Scherer - perineuronal (arrow heads), perivascular (arrows) satellitosis and supial collections in the cerebral cortex.

Figure S5. Malignant glioma cells do not express terminally differentiated CNS cell lineage markers. Representative sections from normal brains and malignant gliomas were stained with H&E or antibodies against mature oligodendrocyte marker Mbp or neuronal marker NeuN. Scale bar represents 50 μm.

Figure S6. *iEIP* glioma subcutaneous transplants are sensitive to genetic suppression of *EGFR** induction but not to EGFR TKI. (A) Shown are representative images of tumor sections from the indicated treatment stained with H&E, anti-EGFR, or anti-phospho-EGFR. Note, EGFR* phosphorylation but not EGFR* protein levels were diminished in tumors subjected to erlotinib treatment. Scale bar represents 50 μm. (B) Growth curve of subcutaneously grafted *iEIP* glioma cells treated with vehicle (n = 4), Dox (2 g/L in drinking water; n = 5) or erlotinib (50 mg/kg/d; n = 5). Day 0 represents the day when treatment was initiated. Tumor growth was measured at indicated time and calculated by relative change to the initial tumor volume. The data are presented as mean ± SD. (C) Immunoblot analysis using tumor lysates prepared from indicated treatment groups revealed efficient inhibition of EGFR* phosphorylation by erlotinib treatment.

Figure S7. *iEIP* gliomas are sensitive to acute EGFR* ablation. Tumor bearing animals grafted with GFP-expressing *iEIP* glioma cells were switched to Dox and sacrificed at indicated time-points. H&E and IHC staining against EGFR and phospho-Akt (p-Akt) revealed rapid

downregulation of EGFR* protein expression as well as gradually decreased tumor cellularity and phospho-Akt levels in response to Dox treatment. Scale bars represent 50 µm.

Figure S8. Ingenuity pathway analysis comparing gene expression profiling of untreated control tumors (n = 3) with Dox-treated relapsed tumors (n = 3) reveals pathway enrichment related to hepatic fibrosis/hepatic stellate cell activation.

Figure S9. Combined treatment of Dox and Bez-235 inhibits *EGFR** induction and Akt activation. Mice with subcutaneously grafted *iEIP* glioma cells were treated with vehicle, Bez-235 (45 mg/kg/day), Dox (2 g/L in drinking water), or Dox + Bez-235. Note, Bez-235 inhibited Akt activation in tumors treated with Bez-235 and Dox + Bez-235, and focally patched Met activation was detected in relapsed tumors from Dox and Dox + Bez-235 treatment. Scale bars represent 50 µm.

Supplementary Experimental Procedures

Complete Materials and Methods

Mice

The *hGFAP-tTA tetO-EGFR** mice were intercrossed with *cInk4a/Arf*$^{Lox/Lox}$ *cPten*$^{Lox/Lox}$ *Nestin-CreER*^{T2} and maintained under Dox (2 g/L, in drinking water) during breeding period until 4-week-old age. Genotyping was done using standard PCR using following primers: for detection of *cInk4a/Arf*$^{Lox/Lox}$ primer 5'-TTG TTG GCC CAG GAT GCC GAC ATC-3' and 5'-CCA AGT GTG CAA ACC CAG GCT CC-3' resulting in ~180bp Lox allele or ~140bp Wild type allele; *cPten*$^{Lox/Lox}$ 5'-CTT CGG AGC ATG TCT GGC AAT GC-3', 5'- CTG CAC GAG ACT AGT GAG ACG TGC-3' and 5'-GGT AGG TCT TGG ATG TTC TCA G-3' resulting in a ~1000bp

Lox allele or 811 bp wild type allele; *Nestin-CreER^{T2}* 5'- CCT GGA AAA TGC TTC TGT CCG-3' and 5'- CAG GGT GTT ATA AGC AAT CCC-3' resulting in a ~400bp allele; *hGFAP-tTA* 5'- CGC TGT GGG GCA TTT TAC TTT AG-3' and 5'- CAT GTC CAG ATC GAA ATC GTC -3' resulting in a ~450bp allele; *tetO-EGFR** 5'- GGGCTCTGGAGGAAAAGAAA-3' and 5'- AGGCCCTTCGCACTTCTTAC-3' resulting in a ~150bp.

4-6 week-old immunocompromised Nu/Nu mice were purchased from Charles River. All procedures for mouse experiments were reviewed and approved by the Cold Spring Harbor Laboratory Institutional Animal Care and Use Committee (IACUC).

Drug Formulation

Tamoxifen (TMX) (Sigma Aldrich, T5648) was solved in 10% ethanol 90% sun flower oil. 4-week-old experimental mice were administered with TMX (124 mg/kg body weight) intraperitoneal for 5 consecutive days. Doxcycline was purchased from Research Products International (D43020) and solved in tap water with 2% Sucrose and sterile filtered. Erlotinib (LC Laboratories, E-4007) was solved in phosphate buffer saline (PBS) with 0.5% Methylcellulose (Sigma Aldrich, M7027) and 0.4% TWEEN80 (Sigma Aldrich, P4780) at a concentration of 10 mg/ml. Gefitinib (LC Laboratories, G-4408) was solved in PBS with 1% TWEEN80 at a concentration of 25 mg/ml. Crizotinib (LC Laboratories, C-7900) was solved in sterile water at a concentration of 10 mg/ml (1). Bez-235 (LC Laboratories, N-4288) was prepared fresh daily in 10% 1-Methyl-2-pyrrolidinone (Sigma Aldrich, 49449) and 90% Polyethylene Glycol 300 (Sigma Aldrich, 90878) (2). Erlotinib (50 mg/kg/day), gefitinib (150 mg/kg/day), crizotinib (25mg/kg/day) and Bez-235 (45 mg/kg/day) were administered through oral gavage.

Orthotopic and Subcutaneous Grafting Experiments

Low passage iEIP glioma cells were maintained under proliferation conditions in neural basal media containing 20 ng/ml EGF (Sigma Aldrich, E4127) and 10 ng/ml bFGF (Gibco, PMG0035) and retrovirally transduced with either luciferase and/or GFP. The orthotopic transplantation was performed as described previously (3). In brief, immunocompromised Nu/Nu mice were anesthetized and restraint using a stereotaxic instrument (Stoelting, 51725), a small hole was drilled into the skull with a dental drill 0.5 mm anterior and 1.5 mm lateral from the bregma. 10,000 *iEIP* glioma cells in Hank's balanced salt solution (Gibco, 24020) were injected into the right caudate nucleus 3 mm below the surface of the brain using a 10-µl Hamilton syringe with an unbeveled 30 gauge needle. The scalp was closed using a 9-mm Autoclip Applier. Animals were followed daily for development of neurological deficits. For subcutaneous grafting, *iEIP* glioma cells were resuspended in 30% BD Matrigel (BD Biosciences, 354234) in Hank's balanced salt solution and ~200,000 cells were injected in each flank of immunocompromised Nu/Nu mice. Tumor growth was monitored and measured every three days by Caliper measurement, and tumor volume was calculated by the following formula: $V = \frac{4\pi}{3} * \frac{a}{2} * \frac{b}{2} * \frac{c}{2}$ (V - tumor volume; a- tumor length; b - tumor width; c - tumor height). Relative tumor volume change was calculated by dividing tumor volume by initial tumor volume.

Immunohistochemical and Immunofluorescence Staining

Paraffin embedded mouse brains or subcutaneously grafted tumors were deparaffinized and rehydrated. Endogenous peroxidases were blocked by incubation with 3% H_2O_2 in PBS followed by antigen retrieval using heat induced pH 6 citrate buffer protocol. Antibodies used were EGFR (Bethyl, IHC0005, 1:250), EGFR (Upstate, 06-129, 1:300), p-EGFR (Y1173, Cell Signaling, 4407, 1:100), Ki67 (Vector Laboratories, VP-K452, 1:100), Gfap (Dako, Z0334, 1:2000), Gfap (BD Bioscience, BD556330, 1:50), Pten (Cell Signaling, 9559, 1:100), activated Caspase 3 (Cell

Signaling, 9661, 1:150), p-Akt (Cell Signaling, 4060, 1:200), p-Mapk (Cell Signaling, 4370, 1:500), p-Met (Cell Signaling, 3077, 1:100), pStat3 (Cell Signaling, 9145, 1:200), Nestin (Millipore, MAB353, 1:100), Olig2 (Chemicon, AB9610, 1:400), Tuj1 (Covance, MMS-43-435P, 1:400), GFP (Abcam, AB13970, 1:300), Mbp (Abcam, 7349, 1:200), O4 (Millipore, MAB345, 1:100), NeuN (Millipore, MAB377, 1:100). The secondary antibodies used for IHC staining were: goat anti-rabbit (Vector Laboratories ABC Elite kit rabbit PK-6101), goat-anti chicken (Vector Laboratories, BA9010). For mouse primary antibodies, Vector Laboratories MOM kit (Vector Laboratories, PK-2200) was used according to manufactures recommendations. Antibody staining was visualized with Vector Laboratories DAB kit (SK-4100) according to manufacturer's recommendations. The secondary antibodies used for IF staining were: Alexa 488 nm anti-rabbit (Invitrogen, A10042, 1:500), Alexa 568 nm anti-mouse (Invitrogen, A-21202, 1:500), Alexa 488 nm anti-chicken (Invitrogen, A-11039, 1:500), Alexa 568 nm anti-sheep (Invitrogen, A-21099, 1:500).

Western Blotting

Total protein was isolated from mouse brains or subcutaneously grafted iEIP tumors using RIPA buffer (150 mM NaCl, 50 mM Tris, 0.5% Na-Deoxycholate, 0.1% SDS and 1% NP-40). Protein concentration was determined by Bradford Assay (Bio-Rad Protein Assay Dye Reagent Concentrate, 500-0006). Proteins were separated on Nupage Novex 4-12% Bis-Tris Gel and blotted on PVDF membrane (Bio-Rad, 162-0177). Blots were blocked with 5% dry milk powder in Tris buffered saline supplemented with 0.2% TWEEN20 (TBST). Primary antibodies were diluted in TBST as: β-Actin (Santa Cruz, sc-1615) 1:1000, p-EGFR 1:1000 (Y1068, Cell Signaling, 3777), EGFR 1:2000 (Santa Cruz, sc-03), p-Akt 1:2000, Akt (Cell Signaling, 9272) 1:1000; p-S6 (Cell Signaling, 5364) 1:2000), S6 (Cell Signaling, 2317) 1:500. Secondary

antibodies donkey anti-rabbit HRP (Santa Cruz, sc-2077), donkey anti-mouse HRP (Santa Cruz, sc-2096) and donkey anti-goat HRP (SantaCruz, sc-2056) were diluted 1:2000 in 5% dry milk/TBST and incubated for 45 minutes at room temperature, antibody signal was detected by Western Lightning ECL Pro (Perkin Elmer, NEL120001EA)

Immunoprecipitation

Total protein lysates were prepared from murine astrocytes using IP buffer (150mM NaCL, 20mM HEPES, 1% NP-40) supplemented with complete mini protease inhibitor cocktail (Roche, 1836153), PhosStop phosphatase inhibitor (Roche, 4906845001) and 200µM Na_3VO_4. Protein concentration was determined by Bradford Assay. To immunoprecipitate EGFR protein, EGFR antibody (Santa Cruz, sc-03, 1:200) was added to total protein lysate and incubated for 2 hours at 4°C, then 50µl protein A/G PLUS-agarose beads (Santa Cruz, sc-220) were added and incubated at 4°C for 2 hours. For p-Y immunoprecipitation, 30µl pre-conjugated anti-phosphotyrosine agarose beads (Sigma Aldrich, A1806) were added to total protein lysate and rotated overnight at 4°C. Precipitates were subjected to SDS-PAGE and Western Blot analysis as described earlier. Phospho-tyrosine proteins were detected by using monoclonal mouse primary anti-phospho-tyrosine antibody 4G10 (Millipore, 05-321) 1:5000. EGFR protein was detected with primary anti-EGFR antibody (Santa Cruz, sc-03) 1:1000.

Quantitative Real Time PCR

Total RNA was isolated using RNeasy Mini kit (Qiagen, 74104) and concentration was determined by NanoDrop followed by reverse transcription of 2 µg total RNA using SuperScript VILO cDNA Synth Kit (Applied Biosystems, 4453650). Real time qPCR was performed on Applied Biosystems StepOne plus 96-well PCR System according to manufacturer's recommendations. The primer sequences are:

Gene	Forward 5’→3’	Reverse 5’→3’	Amplicon bp
β-Actin	GGCTGTATTCCCCTCCATCG	CCAGTTGGTAACAATGCC ATGT	241
Hgf	ACTTCTGCCGGTCCTGTTG	CCCCTGTTCCTGATACACC T	222
Met	GTGAACATGAAGTATCAGC TCCC	TGTAGTTTGTGGCTCCGAG AT	100
EGFR*	GGGCTCTGGAGGAAAAGA AA	AGGCCCTTCGCACTTCTTA C	150

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