Supplementary Figure Legends

Supplementary Figure 1. Human GBM tumors that overexpress EGFR also express EGFR ligands. A, Table of known EGFR ligands used in the Gene Set Enrichment Analysis (GSEA). B, GSEA of the distribution of EGFR ligands (as listed in A) in a list of genes ordered from high expression in GBM tumors with the highest levels of EGFR expression to high expression in tumors with the lowest levels of EGFR expression from the TCGA database. GSEA is a computational method that determines if the expression of a set of genes correlates with one biological state or another. The enrichment score (ES) reflects the degree to which a gene set is positively or negatively correlated. EGF is the EGF ligand showing the highest degree of correlation with the high EGFR expression phenotype class. Differential expression analysis using limma shows that EGF is expressed at a significantly higher level ($p=0.000076$) in the high EGFR class. C, Graphs plotting the EGFR gene copy number and the relative EGFR expression levels from the EGFR high expressing and EGFR low expressing tumors used to perform the GSEA in (B). The red line in the upper graph delineates the 2 gene copy number level and the red asterisks indicate EGFR genes with detected single substitution point mutants.

Supplementary Figure 2. Titering and testing the lenti-Cre viruses. A, Functional titering of viral preparations by measuring iCre activity. A 3T3-LacZ reporter cell line was used to determine viral titer for pTyf TGFα-IRES-iCre and pTyf eGFP-IRES-iCre. Infected cultures were fixed and stained with X-gal and number of cells staining positive for X-gal staining are reported as percentage and used to calculate viral concentrations reported as transducing units per volume (TU/mL). Volumes were matched prior to intracranial injection based on these titers. B, TGFα expressed from pTyf TGFα-IRES-iCre is secreted and activates $EGFR^{WT}$. Conditioned media (C.M.) from 3T3-LacZ cells infected with pTyf TGFα-IRES-iCre or pTyf eGFP-IRES-iCre was added to starved cultures of mouse cells that express human $EGFR^{WT}$. As a positive control, cells were stimulated with 50ng/ml of EGF for 10 min. Immunoblot analysis of total cell lysates for phosphotyrosine indicates that EGFR is activated in response to EGF and TGFα-containing conditioned media but not from eGFP control virus conditioned media.

Supplementary Figure 3. EGFR expression levels from TGFα-$EGFR^{WT}$;Inkβ2/3$^{-/-}$ tumors are similar to those observed in human GBMs. Anti EGFR immunoblot analysis of total cell lysates from TGFα-$EGFR^{WT}$;Inkβ2/3$^{-/-}$ tumor cultures and from human GBM xenograft samples (8, 9) demonstrating similar levels of EGFR expression. GBM-12, GBM-34 and GBM-38 all have an amplified EGFR genomic locus. Dynamin is used as a loading control.

Supplementary Figure 4. TGFα-$EGFR^{WT}$;Inkβ2/3$^{-/-}$ GBM cells grow when orthotopically allografted in immunocompromised animals. A, representative photomicrographs of the same NOD-SCID mouse injected intracranially with 5 X 10^5 TGFα-$EGFR^{WT}$;Inkβ2/3$^{-/-}$ GBM cell culture T3 and monitored using bioluminescence 7, 14 and 23 days after injection. B, photomicrograph of an H&E stained paraffin-embedded brain section from a TGFα-$EGFR^{WT}$;Inkβ2/3$^{-/-}$ GBM cell-injected mouse. Scale bar: 1 mm.

Supplementary Figure 5. $EGFR^{WT}$ GBM primary tumor cells proliferate in low serum conditions ex vivo. A, Cell cultures from three independent tumors (T1-3) were seeded in triplicate at equal density. Cells were incubated overnight in culture media supplemented

with 0.1% fetal bovine serum (FBS) and viable cells counted using trypan blue exclusion. The total number of viable cells recovered at time 0 hour (seeded number) and after 24 hours are plotted. (mean ± SD; n=3 in each group, *p<0.05, **p<0.005, two-tailed t-test).

Supplementary Figure 6. Blockade of TGFα inhibits TGFα-EGFR^{WT};InkΔ2/3$^{-/-}$ tumor cell growth. A, quantitation of TGFα protein measured by ELISA from 1) the conditioned media of TGFα-EGFR^{WT};InkΔ2/3$^{-/-}$ tumor cells and naïve media (control) and 2) total cell lysates from the same cells and from GBM-15 (8, 9) as negative control. B, Cell cultures from three independent tumors (T1-3) were seeded in quadruplicate at equal density. Cells were incubated overnight in culture media supplemented with 0.1% fetal bovine serum (FBS) with or without gefitinib (10 μM) or the indicated concentrations of anti TGFα antibody and viable cells counted using XTT assay (mean ± SD; n=4 in each group, *p<0.0001, two-tailed t-test).

Supplementary Figure 7. Lack of Akt signaling in TGFα-EGFR^{WT};InkΔ2/3$^{-/-}$ tumor cultures. Immunoblot analysis of total cell lysates from the indicated cultures for the presence of phospho Akt^{Thr308} and Akt^{Ser473}. Control cells are EGFR^{WT} expressing mouse GBM cultures (1) starved overnight and stimulated with 50 ng/mL of exogenous EGF for 15 min. Cells were lysed and the levels of phospho and total Akt were detected.

Supplementary Figure 8. Sensitivity of TGFα-EGFR^{WT};InkΔ2/3$^{-/-}$ tumor cells to gefitinib. Tumor cell cultures (T1-T3) were treated with a range of concentrations (0-10 μM) of gefitinib and subjected to an XTT assay. The viability of each sample is normalized to that of vehicle-treated cells. The IC_{50} values for each line are indicated.

Supplementary Figure 9. Dynamics of apoptotic response upon EGFR kinase inhibition in TGFα-EGFR^{WT};InkΔ2/3$^{-/-}$ GBM tumor cells. Immunoblot of total cell extracts from vehicle- and gefitinib-treated cultures at the indicated time point for two TGFα-EGFR^{WT};InkΔ2/3$^{-/-}$ GBM tumor cell cultures analyzed for presence of the apoptotic marker, cleaved PARP. β-tubulin is used as an internal loading control.

Supplementary Figure 10. Selection of a potent sh-RNA against mouse PTEN. sh-RNA sequences against mouse PTEN were extracted from TRC (The RNAi Consortium) as well as a scrambled sequence with no target in the mouse genome were introduced in the pLKO.1 pgk-puro lentiviral expression system according to TRC online protocols. Viruses were produced and used to infect a mouse GBM tumor cell culture. Following selection, total cell lysates from the indicated sh-RNA expressing cells were analyzed for relative PTEN expression levels by western blot. sh-PTEN-2 sequence mediates a robust knock down of PTEN expression.