# Supporting Information

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### SI Materials and Methods

**Plasmid Constructs.** The CMV promoter of the pIRES2-EGFP vector (Clontech Laboratories) was replaced with the tetO fragment of the pRevTRE vector (Clontech) to make pΔCMV/(tetO)-IRES2-EGFP. ΔEGFR or kinase-defective ΔEGFR (DK) genes (1) were then cloned into the multiple-cloning site downstream of the ΔCMV/(tetO) promoter.

The human SΔE-1/KLHDC8A and mouse klhdc8a were PCR amplified by using the primers forward (F) 5′-GTCGACGCCGCC-ATGGAGGTGCCTAACGTCAAGGACT-3′, reverse (R) 5′-G-CGGCCGC-CTAGGAGTCAGAGACACACAGGGCC-3′ and the primers forward (F) 5′-GTCGACGCCGCC-ATGGAAGT-GCCCAATGTCAAGGAC-3′, reverse (R) 5′- GCGGCCGC-CT-AGGAGTCAGAGACAAACAGTGCCTCTAC-3, respectively, and cloned into the pCR2.1-TOPO vector. After sequence verification, targeted fragments were excised from these vectors with SalI-NotI digestion, and subcloned into the pCI-neo vector (Promega), into which we had inserted a FLAG-epitope upstream of the multiple cloning site, thus creating FLAG-tagged SΔE1.

**shRNA Expression Plasmid Construction.** The shRNA-expressing $^{1}$H retroviral system used for DNA vector-based shRNA synthesis, pSuper.retro.blasticidin, is derived from pSuper.retro.puro (Oligoengine) by replacing the puromycin resistance gene with blasticidin S deaminase. shRNA sequences incorporated into pSuper.retro.blasticidin were designed using the online design tool, si*DESIGN* Center (Dharmacon, www.dharmacon.com/DesignCenter/DesignCenterPage.aspx), or were based on published sequences and are as follows: *ΔEGFR* 5′-GAAAGGTAATTATGTGGTG -3′ (2); human *KLHDC8A*, No. 1, 5′-GCAGCAGCACAATGATTAA-3′ or No. 2, 5′-AGCGAGAATTGGACATGAA-3′; human *GFP*, 5′-GCTGACCCTGAAGTTCATC-3′; negative control No. 1, 5′-AA TTCTCTGAACGTGTCAC-3′ or No. 2, 5′-TTCTCTGAACGT-GTCACGT-3′ (3). The retroviral vector was digested with BglII and HindIII and ligated with the annealed oligos.

**Retroviral Infection.** The 293T packaging cell line was cotransfected with the retroviral constructs and pCL10A1 by using Lipofectamine 2000 (Invitrogen), following the manufacturer's instructions. Viral supernatants were harvested at 48 and 72 h after transfection, filtered (0.45 μm), then used for overnight infections of U373MG cells in the presence of 8 μg/mL polybrene. Cells were allowed to recover in fresh media and were then selected in media containing 400 μg/mL neomycin (G418) at 50% activity, 1,100 ng/mL puromycin, or 2 mg/mL blasticidin S according to the drug resistant type of the retroviral plasmid.

**Cell Proliferation Assay.** Cell proliferation was assessed by measuring the conversion of the triazolium salt, WST-1, to formazan according to the manufacturer's instructions (TaKaRa Bio). Briefly, cells were seeded at 10,000 cells per well in 96-well microtiter plates in 100 μL of growth medium. At indicated days, 10 μL of WST-1 solution was added to each well, and the plates were incubated at 37 °C for 4 h, followed by absorbance measurement at 450 and 900 nm by using a GENios Pro microplate reader (Tecan).

**Generation and Purification of Rabbit Polyclonal Antibodies to SΔE-1/KLHDC8A.** Antibodies against SΔE-1/KLHDC8A were produced by using a synthesized peptide corresponding to amino acid residues Ile102 to Ala116 C(IDEGKWKKRSMLREA) coupled to carrier protein KLH. Rabbits were immunized with the peptide-KLH conjugate by following standard procedures, and polyclonal antibodies were purified by affinity chromatography using the synthetic antigen peptide.

**Western Blotting.** Western blotting was performed as described (1). Briefly, 10–30 μg of protein samples were separated through polyacrylamide/SDS gels and electroblotted onto nitrocellulose membranes (Trans-Blot Transfer medium; Bio-Rad Laboratories). Primary antibodies used in this study were as follows, Anti-phosphotyrosine, clone 4G10 (Upstate/Millipore); EGFR, clone 13 (BD Transduction Laboratories); GFP, FL (Santa Cruz Biotechnology); Akt, 5G3 (Cell Signaling) pSer473 Akt (Cell Signaling); Stat1 (Cell Signaling); pTyr701 Stat1 (Upstate); Stat3 (Cell Signaling); pTyr705 Stat3 (Cell Signaling); CyclinA (Santa Cruz Biotechnology); CyclinE (Santa Cruz Biotechnology); CDK2 (BD Transduction Labs); CDK4 (Santa Cruz Biotechnology); CDK6 (Santa Cruz Biotechnology); p27 (BD Transduction Labs); Bcl-$x_L$ (Cell Signaling); pT180/pY182 p38 MAPK (BD Transduction Labs); p38 MAPK (Santa Cruz Biotechnology); phospho-p42/44 MAPK (Cell Signaling); p42/44 MAPK (Cell Signaling); β-Actin, clone AC-15 (Sigma) was used as a loading control for each result.

**TUNEL Assay.** Apoptotic cells were detected by TUNEL using the In Situ Cell Death Detection Kit–Fluorescein (Roche Diagnostics) as described (1).

**Oligonucleotide Array Experiments.** For microarray analysis, U373MG cells engineered to express ΔEGFR or DK under regulation by doxycycline were serum starved for 36 h before sample collection. Indicated samples were cultured in medium containing 1 μg/mL doxycycline for at least five days. Total RNA was isolated by using RNeasy Plus Mini Kit (Qiagen) according to the manufacturer's instructions. High-density oligonucleotide arrays (GeneChip Human HG-U133 Plus 2.0 array, Affymetrix), which contain >47,000 transcripts and variants, were used. Biotin-labeled cRNA was synthesized from aliquots (5 μg) of total RNA from each sample, and hybridization, washing, and detection of signals were carried out according to the vendor's protocol. Scanned images were visually inspected and analyzed by Microarray Analysis Suite (MAS) 5.0 software (Affymetrix) to generate the CEL files and define absent/present calls. CEL files were imported into DNA-Chip Analyzer (dChip 2006) (4) to compute a model-based expression index, which was normalized against the median intensity array with the perfect match minus mismatch method. For replicated arrays, expression values were pooled by dChip implementation.

**Quantitative Real-Time PCR.** RNA was extracted by using the RNeasy Plus Mini Kit (Qiagen). cDNA was synthesized from 2 μg of total RNA by using an oligo-dT primer and the SuperScript first-strand synthesis system for RT-PCR (Invitrogen) according to the manufacturer's instructions. Quantitative real-time PCR (qPCR) was performed by using the iCycler iQ Real-Time Detection System (Bio-Rad). Aliquots of cDNA were amplified for 40 cycles consisting of 10 seconds of denaturing at 95 °C, 45 seconds of annealing and extension at 60–68 °C and monitoring of the SYBR Green I dye intercalation signal. Each PCR was performed in triplicate. For each sample, relative expression of a gene to expression in a reference cDNA mixture of several cell lines and tissues was calculated, and the expression of each gene was then normalized using β-*actin* expression as an internal control. The following primer sets and annealing temperatures (Tm) were used: forward (F) 5′-AGAAGGAGATCACTGCCCTGGCACC-3′,

reverse (R) 5′-CCTGCTTGCTGATCCACATCTGCTG-3′ and Tm 68 °C for β-*actin*; (F) 5′-GGGGATGGGGCGGAAGG-GTGTCT-3′, (R) 5′-GTGGGGCAACTGGTGGTGGCTGTG-3′ and Tm 66 °C for *KLHDC8A*. The specificity of the amplification products was validated by using postamplification melt curve analysis. Differences of gene expression were tested by Kruskal-Wallis analysis.

**Northern Blotting.** Northern blotting was performed as described with minor modification (1). Briefly, 10 μg of total RNA were electrophoresed on a 1% agarose formaldehyde gel and transferred onto a nylon filter (Hybond $N^+$; Amersham). The filter was hybridized with $^{32}$P-labeled human *KLHDC8A* 470-bp-long DNA fragment amplified by PCR. The primer sets, forward (F) 5′-GCCCAGGCCATGGCAGGAAGAATC-3′, reverse (R) 5′-GTGGGGCAACTGGTGGTGGCTGTG-3′, were used for probe production.

**Immunohistochemistry.** Tumor cell proliferation was assessed by $K_i$-67 (clone MIB-1; Dako) immunohistochemistry by using formalin-fixed paraffin-embedded tissues and angiogenesis was evaluated by CD31 (rat anti-mouse CD31 monoclonal antibody, BD Pharmingen) staining as described with minor modification (5). Microvessel areas (MVA) were determined by measuring the total amount of staining in each section. Captured digital images of the sections were analyzed with IMAGE PRO PLUS (version 4.5) software (Media Cybernetics) as described (5). Tissue microarray slides of human glioblastoma were obtained from US Biomax (array no. GL802). The TMA contains duplicate cores from 33 glioblastoma patients (30 adult, 3 pediatric). Immunohistochemistry for SΔE1/KLHDC8A was performed using rabbit anti-SΔE1 antibody to human SΔE protein at a concentration of 1:1000 with 10' proteinase K retrieval. Detection was performed by using the Envision+ system (DAKO Cytomation). Testing of antibody specificity was performed by using Cos7 cells engineered to express SΔE1 as well as SΔE1-positive escaper clones. Scoring of staining was performed manually (K.L.L.) in the TMAJ application (Johns Hopkins) from scanned images of the TMA slides (Ariol slide scanning system, Applied Imaging Systems Inc.). Scoring was performed by using the following scale: 0, no staining; 1, <10% of tumor cells positive; 2, 10–40% of tumor cells; 3, 40–70% of tumor cells positive; 4, >70% of tumor cells positive. Intensity of staining was also measured by pathologist visual estimation (0-no stain, 1 low, 2 med, 3 high) in addition to the Ariol scanning analysis.

**Selection of Escaper-Specific Genes.** Probes with less than two present calls (detected) in all samples, determined by the expression algorithm in MAS 5.0 software, were excluded because of low confidence of scarcely expressed genes. The variation filter selected for a coefficient of variation >0.5 across all samples, and yielded 1,024 genes. For selecting differentially expressed genes, Significance Analysis of Microarrays (SAM version 3.0) was used (6). SAM estimates the percentage of genes identified by chance as the false discovery rate (FDR) by using permutations of the repeated measurements. SAM also identifies genes with statistically significant changes and score $q$ value, which is similar to the familiar "$p$ value". The output criteria selected for SAM included genes with a fold change exceeding 2.5-fold between escapers and other samples, which have no ΔEGFR expression, and $q$ value <0.05 (Delta value = 0.802). Estimated FDR in this analysis was 4.2%. Hierarchical clustering was performed by using the dChip software with centroid method for 48 probes selected by SAM analysis.

**Clinical Samples.** Tumor samples, kindly provided by Ryo Nishikawa, were obtained at the time of surgery after written informed consent from the Department of Neuro-Oncology, International Medical Center, Saitama Medical University, Japan. Frozen tumor samples were ground in liquid nitrogen and lysed in RIPA buffer for immunoblot analysis. Total RNA was isolated from frozen tumor samples by using RNAeasy kit (QIAGEN), following the manufacturer's instructions. Use of commercially available glioma tumor microarrays is as described above.


1. Nagane M, et al. (1996) A common mutant epidermal growth factor receptor confers enhanced tumorigenicity on human glioblastoma cells by increasing proliferation and reducing apoptosis. *Cancer Res* 56:5079–5086.
2. Fan QW, Weiss WA (2005) RNA interference against a glioma-derived allele of EGFR induces blockade at G2M. *Oncogene* 24:829–837.
3. Lee EJ, et al. (2005) Insulin-like growth factor binding protein 2 promotes ovarian cancer cell invasion. *Mol Cancer* 4:7.
4. Li C, Wong WH (2001) Model-based analysis of oligonucleotide arrays: Expression index computation and outlier detection. *Proc Natl Acad Sci USA* 98:31–36.
5. Mishima K, et al. (2001) Growth suppression of intracranial xenografted glioblastomas overexpressing mutant epidermal growth factor receptors by systemic administration of monoclonal antibody (mAb) 806, a novel monoclonal antibody directed to the receptor. *Cancer Res* 61:5349–5354.
6. Tusher VG, Tibshirani R, Chu G (2001) Significance analysis of microarrays applied to the ionizing radiation response. *Proc Natl Acad Sci USA* 98:5116–5121.

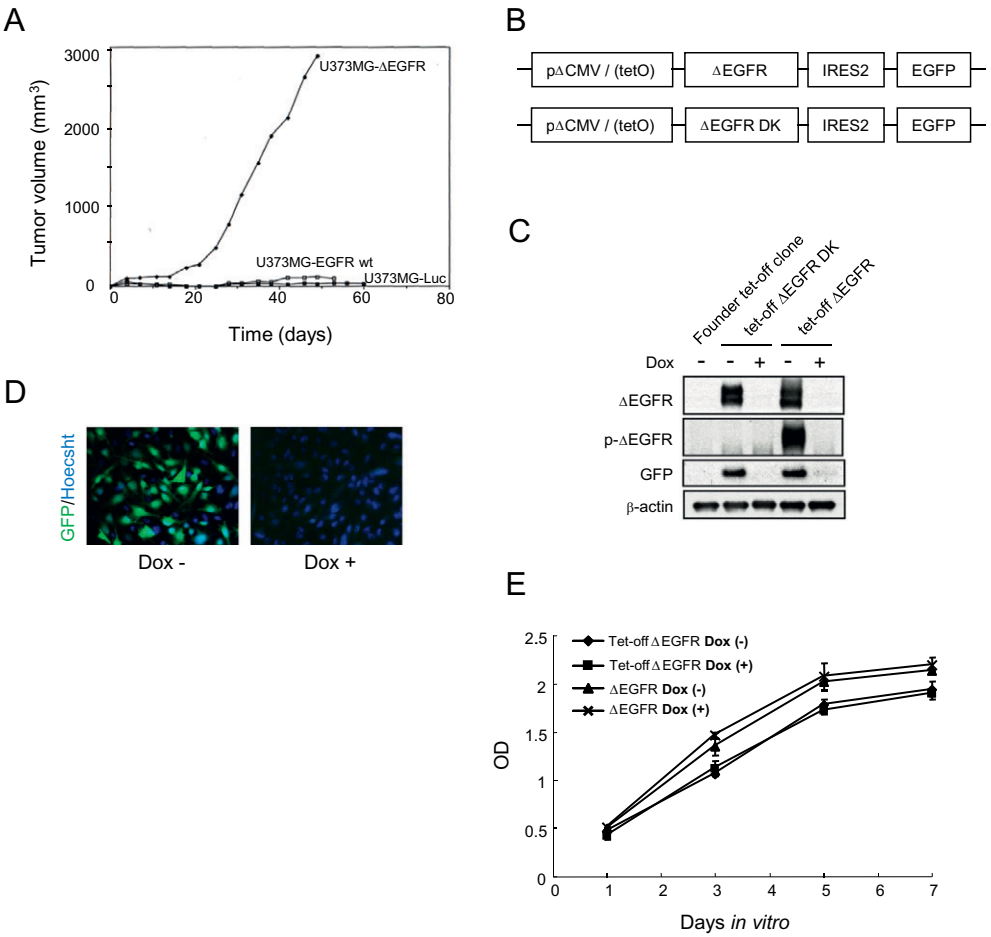


**Fig. S1.** In vitro analysis of U373MG cells engineered with doxycycline-repressible ΔEGFR. (*A*) Constitutive ΔEGFR expression conveys tumorigenic potential to human glioma cell line U373MG. U373MG cells were infected with viruses carrying ΔEGFR, wild-type (wt) EGFR, or control luciferase gene (*Luc*), and the stable cell lines were established. Two million cells in 0.1 mL of PBS of the indicated cell lines were injected s.c. into the right flank of nude mice, and tumor volumes were measured at the indicated times. (*B*) Illustration of pΔCMV/(tetO)-ΔEGFR-IRES2-EGFP or pΔCMV/(tetO)-ΔEGFR DK-IRES2-EGFP cassette that allow for ΔEGFR (or DK) and enhanced green fluorescent protein (EGFP) to be translated from single bicistronic mRNA under the regulation of doxycycline. (*C*) Western blot analysis of proteins from whole-cell lysates of engineered U373MG cells with indicated antibodies. The cells were cultured either in medium containing 1 μg/mL doxycycline for >5 days or in medium without doxycycline. (*D*) Microscopic images of tet-regulatable ΔEGFR-expressing U373MG cells after culturing in medium with or without 1 μg/mL doxycycline for >5 days. Nuclei were visualized with Hoechst dye. (*E*) In vitro cell proliferation assay (WST assay) of U373MG cells expressing constitutive ΔEGFR or tetracycline-regulatable ΔEGFR. The cells were cultured in medium containing 2% serum with or without 1 μg/mL of doxycycline for 7days. WST assays were performed in triplicate at indicated days for each sample. Each experiment was carried out independently and repeated at least twice with similar results. Error bars represent standard deviation.

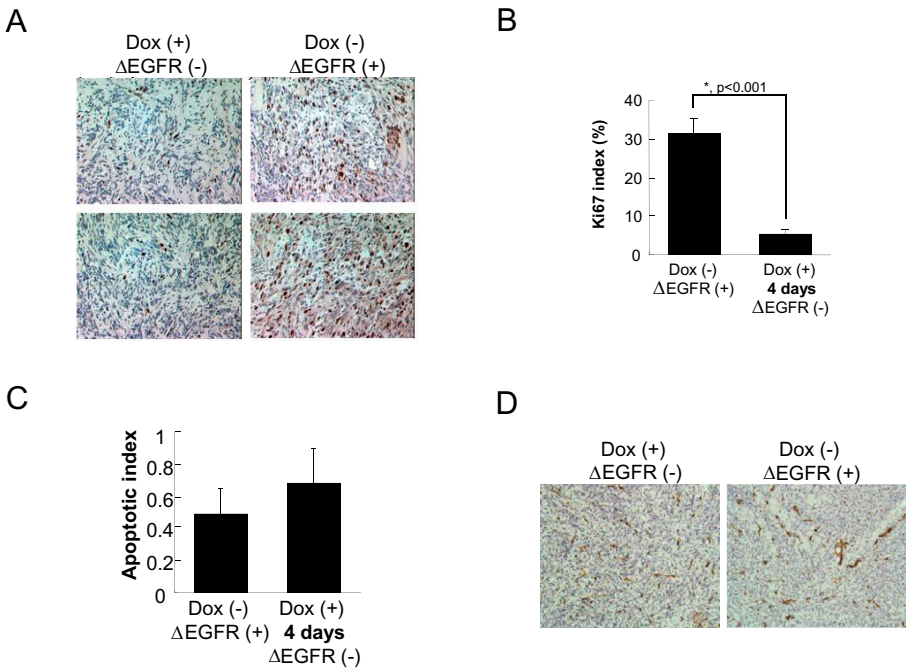


**Fig. S2.** Short-term effects on tumor proliferation, apoptosis, and vessel density in response to ΔEGFR silencing in vivo. U373MG cells expressing tetracycline-regulatable ΔEGFR were injected s.c. into mice, and tumors were allowed to establish. At 4 or 8 days after doxycycline addition to the drinking water, tumors were collected. (*A*) U373MG tumor tissues with (Dox -) or without (Dox +) ΔEGFR expression for 4 days were stained with anti-$K_i$-67 antibody and counter-stained with hematoxylin. (*B*) Proportions of $K_i$-67 positive cells after 4 days were counted, and error bars are presented with standard deviation. (*, $P < 0.001$, Student's *t* test). (*C*) Percentage of TUNEL-positive cells (apoptotic index) was assessed in each tumor and is shown as mean. Error bars represent standard deviation. At 4 days, N.S., $P = 0.064$. (*D*) U373MG tumor tissues stained with anti-CD31 antibody and counterstained with hematoxylin.

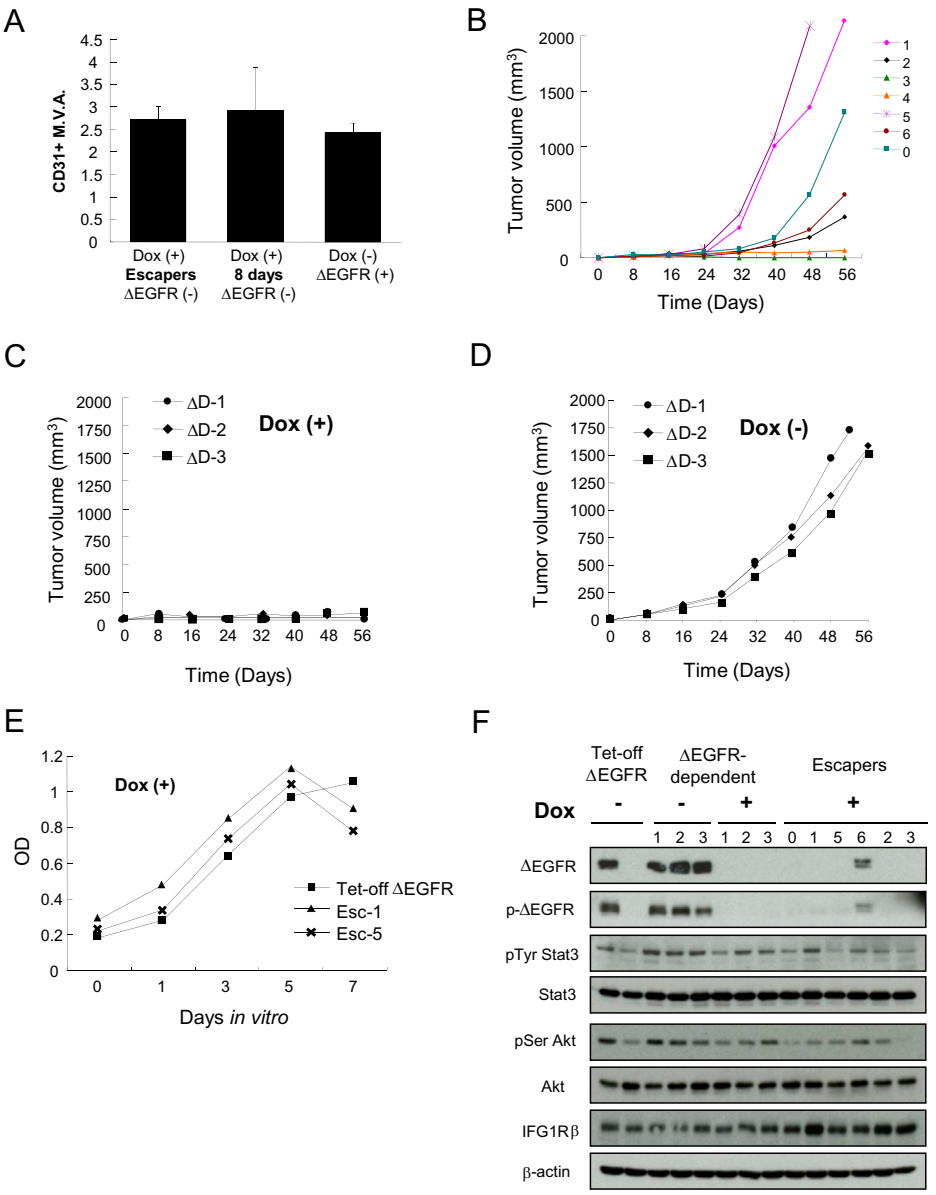


**Fig. S3.** Analysis of stable cell lines established from ΔEGFR-dependent and ΔEGFR independent escaper tumors. (*A*) CD31-positive microvessel area was determined in escapers (Dox+) and ΔEGFR-dependent (with Dox for 8 days from day 32 or without Dox) tumors and results are presented as mean ± SD. No significant difference in microvessel area for escapers was detected ($P > 0.1$, ANOVA). (*B*) Tetracycline-regulatable U373MG cells ($3 \times 10^6$) established from ΔEGFR-independent escaper tumors were injected s.c. into the flanks of nude mice that were fed doxycycline-containing water. Each line represents an individual growth curve of a s.c. tumor developed in 1 or 2 mice per group. One mouse per group was tested for escaper tumors 1, 2, and 0; 2 mice per group were tested for escaper tumors 3, 4, 5, and 6. Standard error was not calculated due to the small sample number per group. (*C*) Tetracycline-regulatable U373MG cells ($3 \times 10^6$) established from ΔEGFR-dependent tumors in the presence of dox were injected s.c. into the flanks of nude mice. Mice were fed with doxycycline containing water. Each line represents an individual growth curve of a s.c. tumor developed in a mouse. (*D*) Tetracycline-regulatable U373MG cells ($3 \times 10^6$) established from ΔEGFR-dependent tumors in the absence of dox were injected s. c. into the flanks of nude mice. Mice were fed normal drinking water. Each line represents an individual growth curve of a s.c. tumor developed in a mouse. (*E*) In vitro cell proliferation assay (WST assay) comparing the original U373MG cells expressing tetracycline-regulatable ΔEGFR and cells established from ΔEGFR-independent tumors (escapers) under ΔEGFR silenced condition. Cells were cultured in medium containing 0.5% serum with 1 μg/mL of doxycycline for 7 days. WST assays were performed in triplicate at indicated days for each sample. Similar experiments were done at least two separate times. (*F*) Western blot analysis of proteins from whole-cell lysates prepared from cell lines propagated 24 h in serum-free growth conditions by using the indicated antibodies used to interrogate EGFR and potential escape-associated pathway signaling; β-actin was used as a loading control.

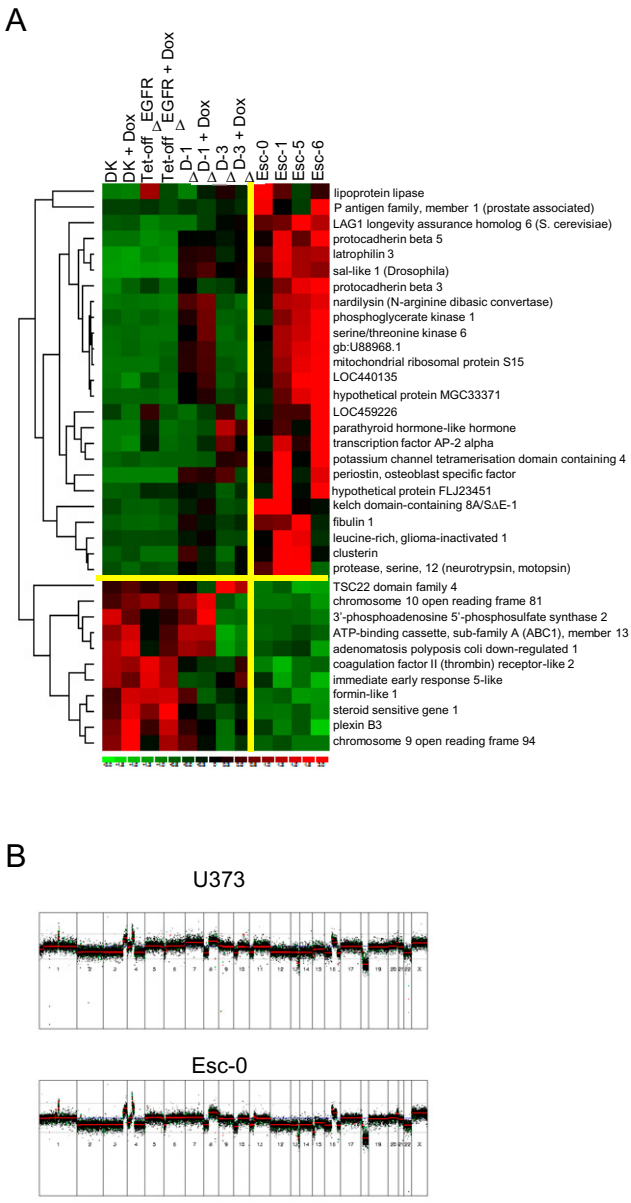


**Fig. S4.** Differential gene expression and copy number analysis in ΔEGFR-dependent and ΔEGFR-independent escaper tumors and cells. (*A*) Differentially expressed genes in ΔEGFR-independent, ΔEGFR dependent cell lines. Each column represents a cell line sample and each row represents a gene. Expression of each gene in four ΔEGFR-independent (escapers), together with original U373MG tet-regulatable ΔEGFR and DK cells, as well as cell lines from ΔEGFR-dependent U373MG tumors (control) is presented in color gradation after being normalized and mean-centered. Red indicates increased expression, and green indicates decreased gene expression. Redundant probe sets representing identical genes are masked. Samples marked as Dox were cultured with 1 μg/mL doxycycline in medium for more than 5 days before RNA isolation. (*B*) Representative array CGH performed with original U373 and one escaper cell line.

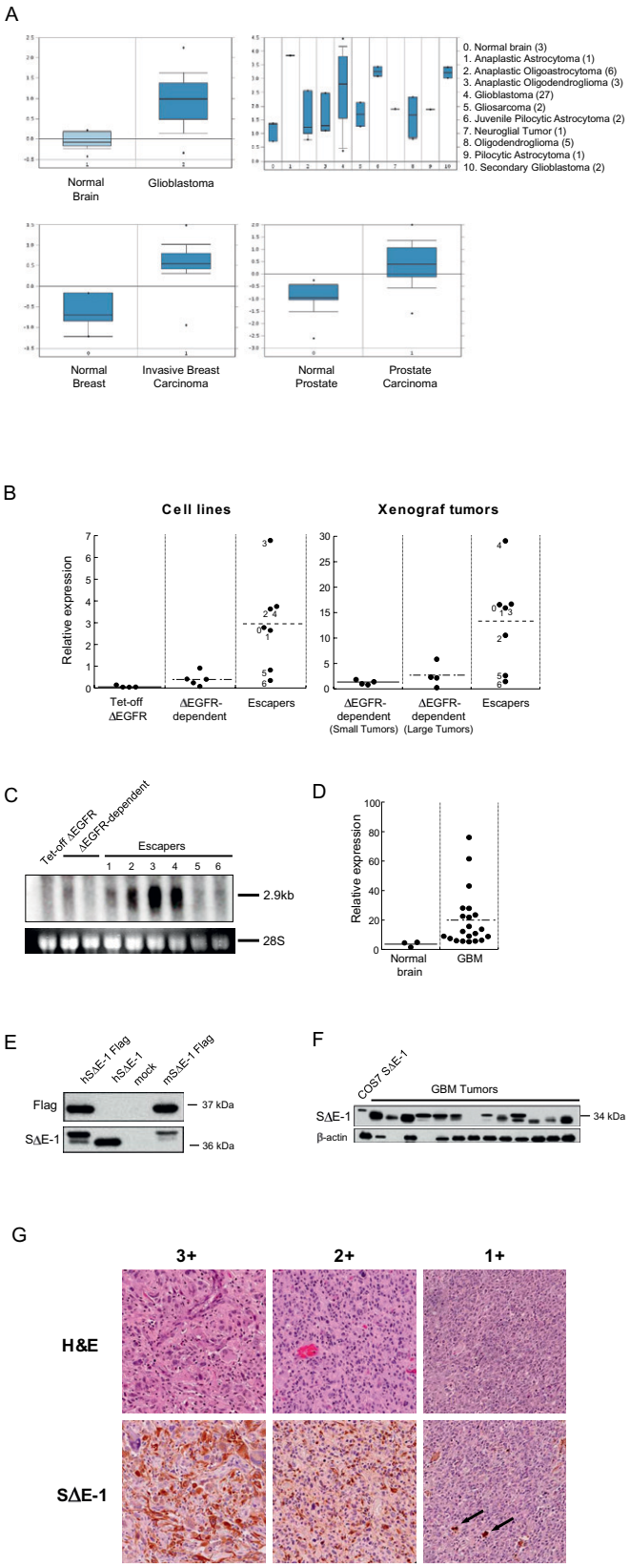


**Fig. S5.** *SΔE-1/KLHDC8A* expression in ΔEGFR-dependent and escaper tumors and human primary glioblastoma. (*A*) Analysis of *SΔE-1/KLHDC8A* gene expression in various solid tumors using the Oncomine database. (*B*) qPCR analysis of *SΔE-1 / KLHDC8A* gene expression in the original tetracycline-regulatable U373MG cells and cell lines established from either ΔEGFR-independent or -dependent tumors (left), and in mouse tumor xenografts (right). Each bar represents the mean ±SD of three replicates. Y-axis represents the relative gene expression level ($p<0.01$, Kruskal-Wallis test). (*C*) Northern blotting of *SΔE-1 / KLHDC8A* expression in escaper cell lines and control cells. Ten μg of total RNA was size fractionated in an agarose-formaldehyde gel, transferred to nylon membrane, and probed with a

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human *SΔE-1 / KLHDC8A*. Ethidium bromide staining of 28S rRNA demonstrates similar loading of RNA in each lane. (*D*) qPCR analysis of *SΔE-1 / KLHDC8A* gene expression in normal brains and human glioblastomas. Each bar in *A-C* represents the mean ±SD of three replicates. Y-axis represents the relative gene expression level ($p<0.01$, Kruskal-Wallis test). (*E*) Western blot analysis using a rabbit polyclonal anti- SΔE-1 / KLHDC8A antibody. Cos7 cells were transfected with plasmid vector containing full length human SΔE-1 / KLHDC8A with/without Flag-tag or with mouse SΔE-1 / KLHDC8A. Overexpressed proteins were detected with anti-Flag antibody. (*F*) Western blot analysis of whole cell lysates from GBM clinical samples. Antibodies used were as in panel D. (*G*) Immunohistochemistry for SΔE-1 / KLHDC8A using rabbit polyclonal anti- SΔE-1 / KLHDC8A antibody demonstrates expression within representative human GBM samples. Staining was noted to be heterogeneous with respect to percentage of tumor cells estimated to express SΔE-1 / KLHDC8A as well as the levels of expression within positive tumor cells. Each panel is an example of SΔE-1 / KLHDC8A expression, from low (+1) to high (+3) positivity. Arrows highlight rare cells with immunoreactivity.

**Table S1. Genes up-regulated in escapers**

| Gene symbol | Gene | Mean fold change (±SD) | *q* value |
|---|---|---|---|
| | **Regulation of transcription** | | |
| *LASS6* | *LAG1 homolog, ceramide synthase 6* | 3.1 (0.1414) | 0 |
| *SALL1* | *sal-like 1* | 2.7 | 0.03 |
| *TFAP2A* | *transcription factor activating enhancer binding protein 2* alpha | 2.6 | 0 |
| | **G protein coupled receptor activity/protein signaling pathway** | | |
| *LPHN3* | *latrophilin 3* | 2.7 (0.1000) | 0 |
| *PTHLH* | *parathyroid hormone-like hormone* | 2.5 | 0.03 |
| | **Cell adhesion** | | |
| *PCDHB3* | *protocadherin beta 3* | 4 | 0 |
| *POSTN* | *periostin, osteoblast specific factor* | 3.3 (0.1414) | 0.03 |
| *PCDHB5* | *protocadherin beta 5* | 2.8 | 0 |
| | **Glycolysis** | | |
| *PGK1* | *phosphoglycerate kinase 1* | 2.7 | 0.03 |
| | **Anti-apoptosis** | | |
| *CLU* | *clusterin* | 2.6 | 0.03 |
| | **Lipid metabolism** | | |
| *LPL* | *lipoprotein lipase* | 2.6 | 0.03 |
| | **Mitotic cell cycle, spindle organization** | | |
| *AURKA* | *aurora kinase A* | 3.7 | 0 |
| | **Translation** | | |
| *MRPS15* | *mitochondrial ribosomal protein S15* | 2.7 | 0.03 |
| | **Potassium ion transport** | | |
| *KCTD4* | *potassium channel tetramerisation domain containing 4* | 4.55 (1.768) | 0.03 |
| | **Nervous system development, cell proliferation** | | |
| *LGI1* | l*eucine-rich, glioma inactivated 1* | 6.4 | 0.04 |
| | **Extracellular matrix structural constituent** | | |
| *FBLN1* | *fibulin 1* | 2.95 (0.495) | 0.03 |
| | **Cellular defense response** | | |
| *PAGE1* | *P antigen family, member 1 (prostate associated)* | 4.2 | 0.03 |
| | **Other** | | |
| *FAM76B* | *family with sequence similarity 76, member B* | 3.4 | 0.03 |
| *KLHDC8A* | *kelch domain containing 8A* | 4.6 | 0.03 |

**Table S2. Genes down-regulated in escapers**

| Gene symbol | Gene | Mean fold change (±SD) | *q* value |
|---|---|---|---|
| | ATP binding | | |
| *PAPSS2* | *3′-phosphoadenosine 5′-phosphosulfate synthase 2* | 0.24 (0.010) | 0.03 |
| *ABCA13* | *ATP-binding cassette, sub-family A (ABC1), member 13* | 0.37 | 0.03 |
| | Transcription repressor activity | | |
| *TSC22D4* | *TSC22 domain family, member 4* | 0.37 | 0 |
| | G-protein coupled receptor activity/protein signaling pathway | | |
| *F2RL2* | *coagulation factor II (thrombin) receptor-like 2* | 0.39 | 0 |
| | Receptor activity, signal transduction | | |
| *PLXNB3* | *plexin B3* | 0.38 | 0 |
| | Rho GTPase binding, actin cytoskeleton organization | | |
| *FMNL1* | *formin-like 1* | 0.39 | 0.03 |
| | Metallopeptidase activity | | |
| *ADAMTSL1* | *ADAMTS-like 1* | 0.37 | 0.03 |
| | Fibronectin binding, extracellular matrix organization | | |
| *CCDC80* | *coiled-coil domain containing 80* | 0.29 | 0.03 |
| | Other | | |
| *APCDD1* | *adenomatosis polyposis coli down-regulated 1* | 0.39 | 0.03 |
| *IER5L* | *immediate early response 5-like* | 0.37 | 0.03 |

**Table S3. SΔE-1 expression in primary human glioblastoma**

| Patient | Tumor cells positive, % | Manual intensity | Automated intensity |
|---|---|---|---|
| 1 | 4.0 | 3.0 | 135 |
| 2 | 4.0 | 2.5 | 156 |
| 3 | 3.5 | 2.5 | 132 |
| 4 | 3.0 | 3.0 | 147 |
| 5 | 3.0 | 3.0 | 131 |
| 6 | 3.0 | 3.0 | 146 |
| 7 | 3.0 | 2.5 | 154 |
| 8 | 2.5 | 3.0 | 145 |
| 9 | 2.5 | 1.5 | 160 |
| 10 | 2.0 | 3.0 | 153 |
| 11 | 2.0 | 3.0 | 117 |
| 12 | 2.0 | 2.5 | 149 |
| 13 | 2.0 | 2.5 | 160 |
| 14 | 2.0 | 2.5 | 154 |
| 15 | 2.0 | 2.0 | 150 |
| 16 | 2.0 | 2.0 | 156 |
| 17 | 2.0 | 1.5 | 156 |
| 18 | 1.5 | 2.5 | 150 |
| 19 | 1.5 | 2.5 | 162 |
| 20 | 1.5 | 2.5 | 155 |
| 21 | 1.5 | 2.0 | 150 |
| 22 | 1.5 | 2.0 | 162 |
| 23 | 1.5 | 1.5 | 164 |
| 24 | 1.5 | 0.5 | 155 |
| 25 | 1.0 | 3.0 | 143 |
| 26 | 1.0 | 1.5 | 157 |
| 27 | 1.0 | 1.0 | 160 |
| 28 | 1.0 | 1.0 | 160 |
| 29 | 0.5 | 1.5 | 148 |
| 30 | 0.5 | 1.0 | 163 |
| 31 | 0.5 | 0.5 | 169 |
| 32 | 0.5 | 0.5 | 159 |
| 33 | 0.5 | 0.5 | 155 |
| Median | 2.0 | 2.5 | 155 |

Summary of staining pattern in human GBM with respect to percentage of tumor cells positive, manual intensity of staining, and automated intensity.