SUPPLEMENTARY DATA

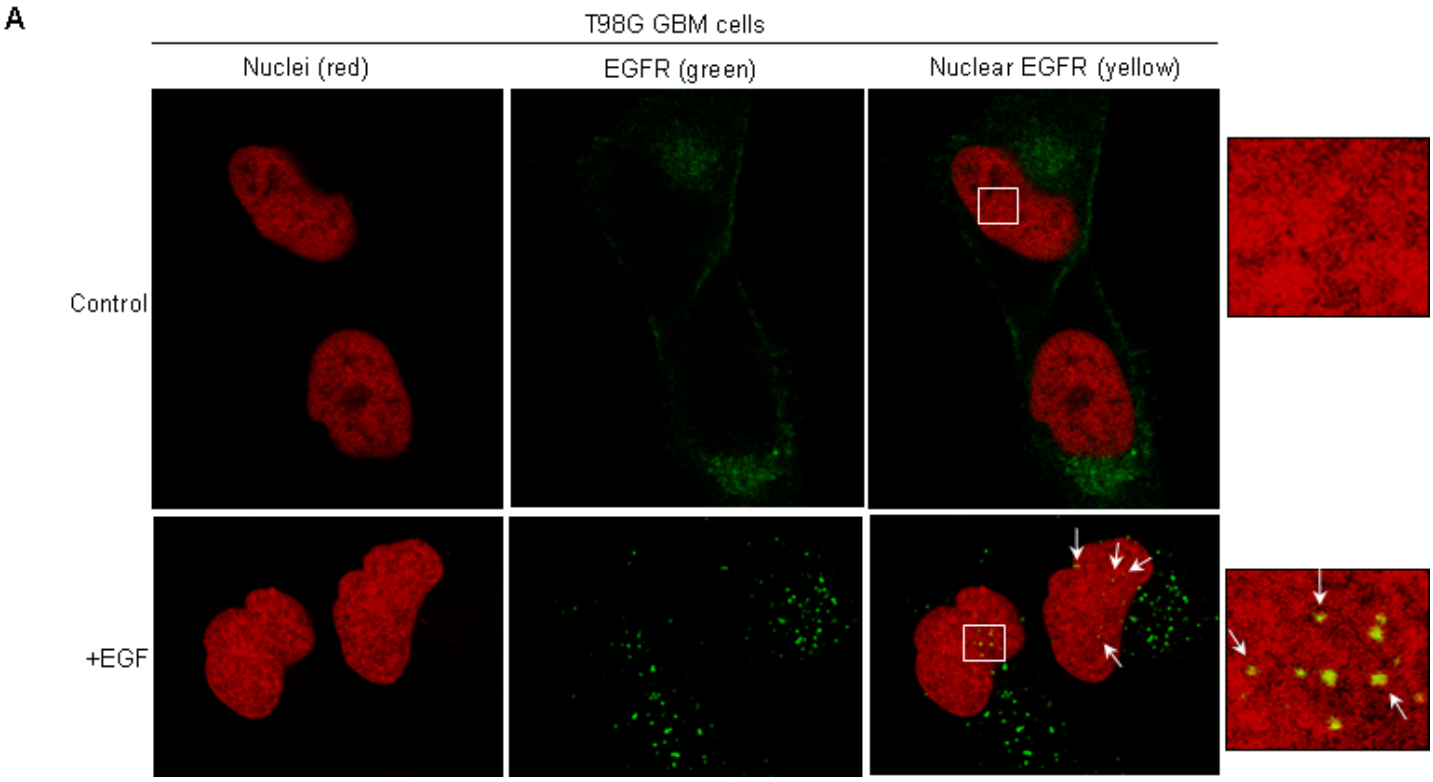
COX-2 is a Novel Transcriptional Target of the Nuclear EGFR-STAT3 and EGFRvIII-STAT3 Signaling Axes

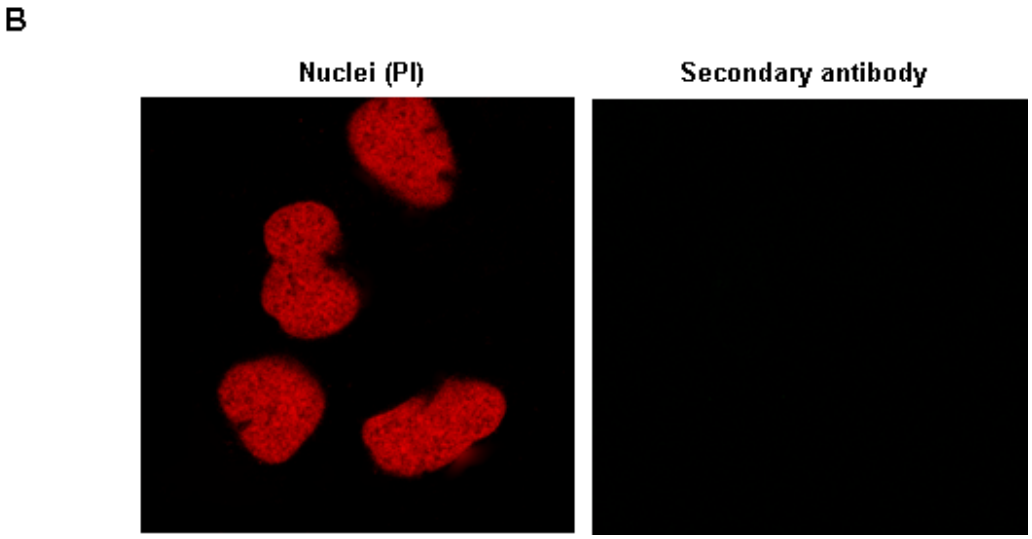
Supplementary Table I Genes significantly up-regulated by EGF in U87MG-EGFR cells compared to U87MG-EGFRdNLS and U87MG-vector cells. DNA microarray data were subjected to ANOVA analysis to identify genes that are expressed in U87MG-EGFR cells at a significantly higher level, namely, at least 1.9-fold and $p<0.05$, compared to U87MG-EGFRdNLS and U87MG-vector cells. The analysis revealed 19 known human genes.

Probe Set ID	Gene Name	Gene Symbol	EGF/control U87MG-EGFR	p-value EGF/control U87MG-EGFR	EGF/control U87MG-EGFRdNLS	p-value EGF/control U87MG-EGFRdNLS
231577_s_at	guanylate binding protein 1, interferon inducible, 67kDa	GBP1	8.06	0.007	1.00	0.99
1554997_a_at	cyclooxygenase-2	COX-2	5.00	0.007	0.98	0.96
209719_x_at	serpin peptidase inhibitor, clade B (ovalbumin), member 3	SERPINB3 SCCCA1	4.48	0.045	1.02	0.96
229450_at	interferon induced protein with tetratricopeptide repeats 3	IFIT3	4.26	0.002	1.04	0.81
208370_s_at	Down syndrome critical region gene 1	DSCR1	3.45	0.044	0.97	0.62
239445_at	RAB6A, member RAS oncogene family	RAB6A	3.12	0.016	0.98	0.92
226757_at	interferon-induced protein with tetratricopeptide repeats 2	IFIT2	3.06	0.004	1.04	0.82
206432_at	hyaluronan synthase 2	HAS2	3.03	0.029	1.02	0.94
208389_s_at	solute carrier family 1 (glial high affinity glutamate transporter), member 2	SLC1A2	2.80	0.003	1.01	0.98
216588_at	ribosomal protein L7	RPL7	2.80	0.046	1.01	0.99
206133_at	XIAP associated factor-1	BIRC4BP	2.63	0.031	0.99	0.97
237209_s_at	nuclear factor related to kappaB binding protein	NFRKB	2.50	0.048	1.05	0.91
234986_at	Glutamate cysteine ligase, modifier	GCLM	2.12	0.019	0.96	0.82

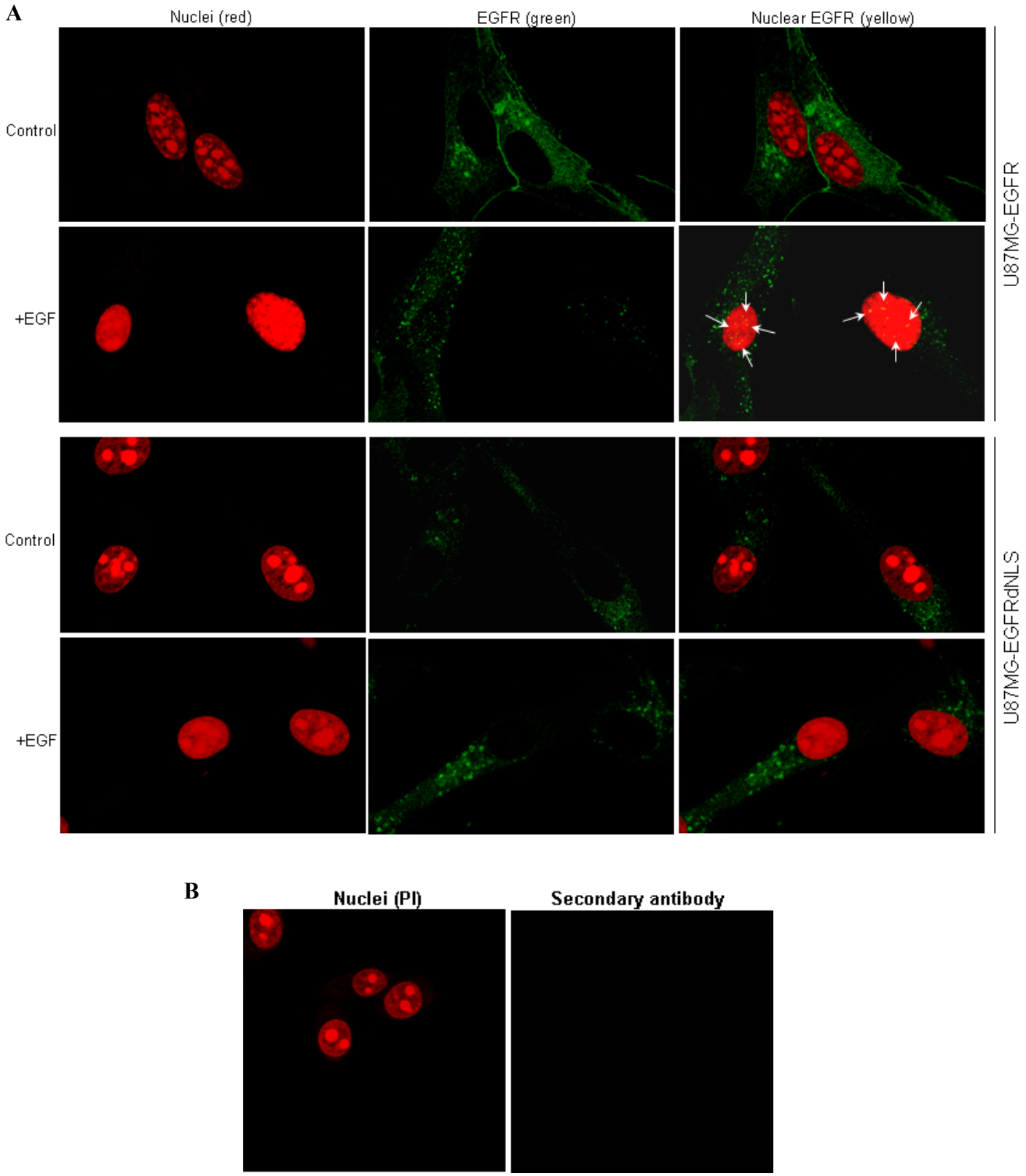
	subunit					
209969_s_at	signal transducer and activator of transcription 1,	STAT1	2.00	0.046	1.02	0.80
219209_at	interferon induced with helicase C domain 1	IFIH1	2.00	0.019	1.01	0.97
227609_at	epithelial stromal interaction 1 (breast)	EPSTI1 BRESI1	1.94	0.017	0.96	0.78
202948_at	interleukin 1 receptor, type I	IL1R1	1.93	0.045	0.99	0.97
223690_at	latent transforming growth factor beta binding protein 2	LTBP2	1.90	0.001	1.03	0.93
230511_at	cAMP responsive element modulator	CREM	1.88	0.047	0.99	0.94

Supplementary Fig. S1. EGF induces EGFR nuclear transport in a GBM cell line expressing endogenous EGFR, as shown by immunofluorescence staining and confocal microscopy.
A: T98G human GBM cells were serum-starved for 24 hrs and stimulated with EGF (100 ng/ml) for 0 and 15 min. The cells were fixed and subjected to immunofluorescence staining and confocal microscopy to determine the extent of EGFR nuclear translocalization. EGFR is indicated by green fluorescence whereas nuclei are marked by propedium iodine (PI) in red. Nuclear EGFR is shown as yellow merged signals (arrows).
B: Negative control with secondary antibody only did not yield any non-specific signal.

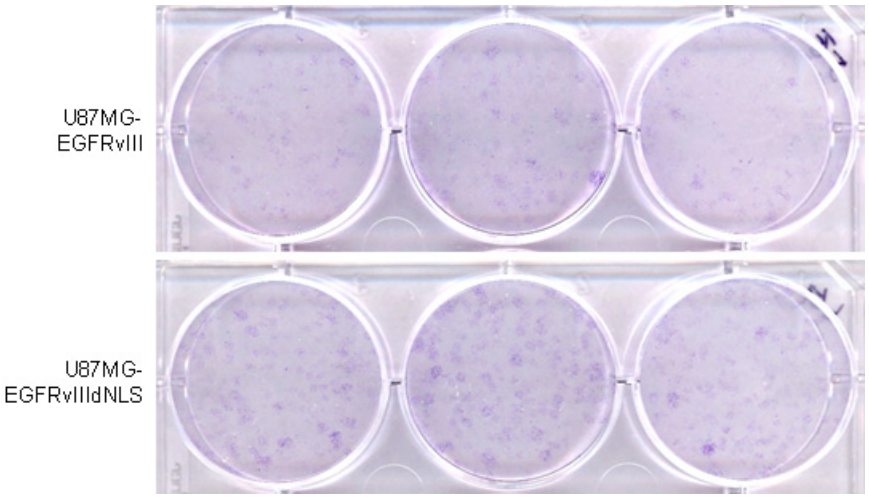




Supplementary Fig. S2 EGFR-NLS fails to undergo nuclear translocalization.
A: Serum-starvedU87MG-EGFR and U87MG-EGFRdNLS cells were stimulated with EGF (100 ng/ml) for 0 and 15 min and subjected to immunofluorescence staining and confocal microscopy. EGFR staining is indicated by green fluorescence while nuclei stained red for propidium iodide. Nuclear EGFR is indicated by yellow fluorescence, merged signals of green and red (arrows).
B: Negative control with secondary antibody only did not yield any non-specific signal.



Supplementary Fig. S3. U87MG-EGFRvIIIdNLS cells formed colonies to the extent similar to U87MG-EGFRvIII cells.



Supplementary Materials and Methods

Detection of nuclear EGFR/EGFRvIII via immunofluorescence staining and confocal microscopy. Tumor cells were seeded in 8-well Lab-Tek chamber slides (Nunc Inc., Rochester, NY) for 24 hrs. After washing with ice-cold PBS, the cells were fixed in 4% paraformaldehyde for 15 min and permeablized with 0.2% Triton-X100 for 5 min. Following treatment with 10% normal goat serum/1% BSA for 60 min, the cells were incubated with anti-Myc monoclonal antibody overnight at 4°C. After three washes with PBS, the cells were incubated with goat anti-mouse secondary antibody (1:200, Vector Lab) tagged with Texas Red or fluorescein and after which, they were mounted with VECTASHIELD Mounting Medium containing DAPI or propidium iodide (for nuclei detection) and then examined under a Zeiss LSM 510 confocal microscope.