

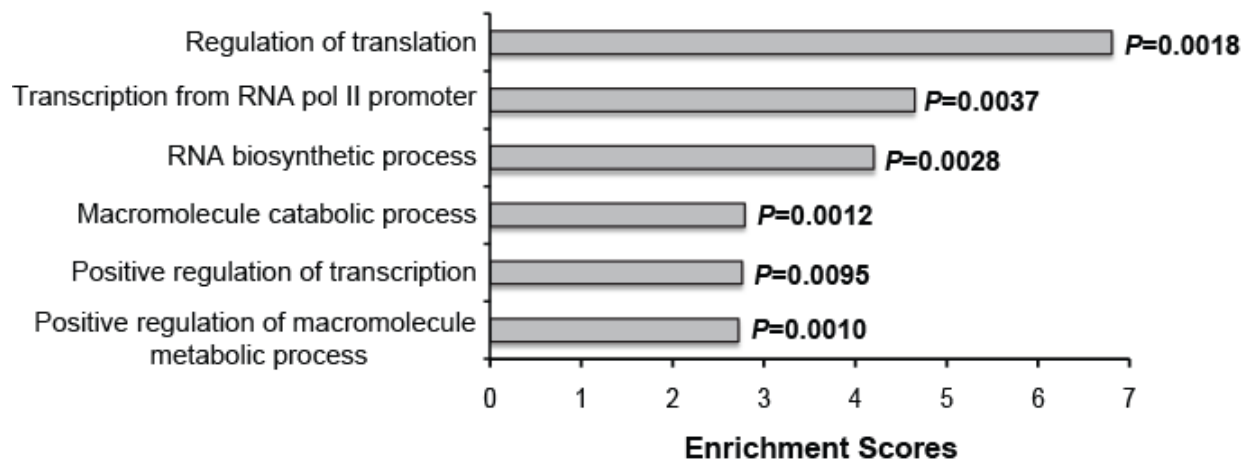
Alternative splicing in EGFRvIII-expressing tumours - Gene Ontology Category

Figure S1. Gene Ontology (GO) analysis of transcripts with altered splicing induced by EGFRvIII.

GO analysis of transcripts that contain MADS+ identified changes in alternative splicing. P-value is generated by DAVID Functional Annotation Tool and is a modified Fisher exact p-value.

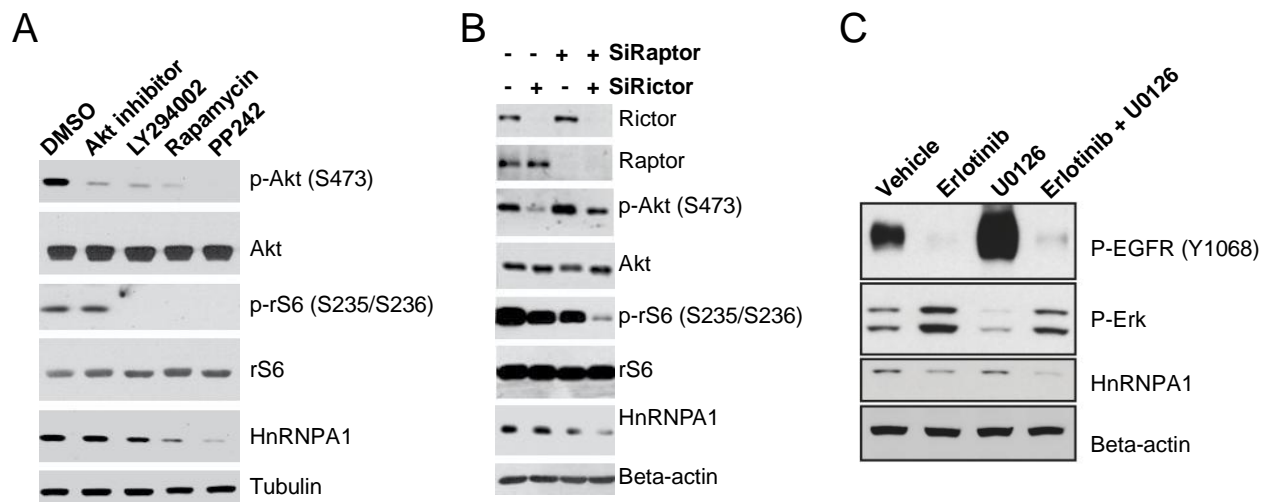


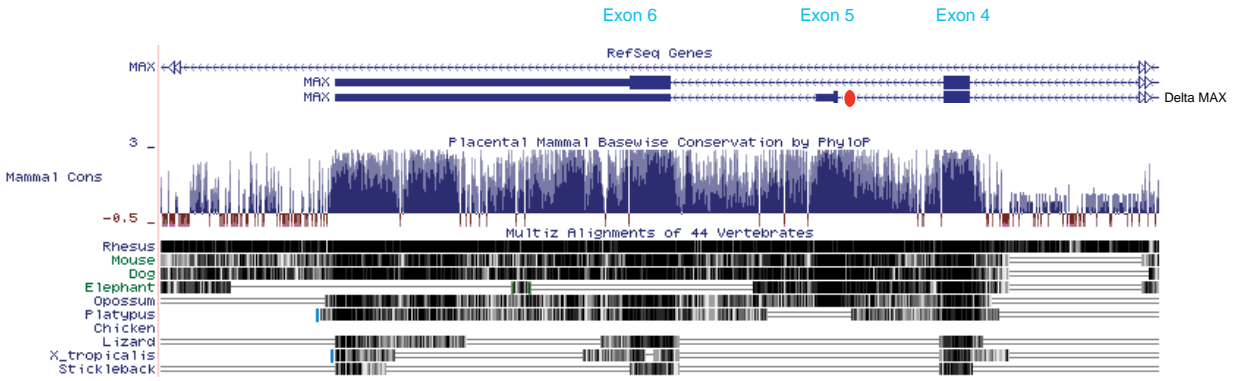
Figure S2. Inhibition of mTOR decreases hnRNPA1 expression in U87-EGFRvIII cells.

(A) Pharmacological inhibition of mTOR in U87-EGFRvIII cells inhibits expression of hnRNPA1.

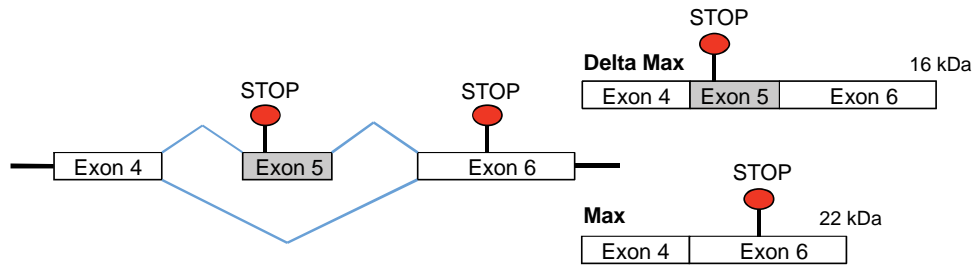
(B) Genetic inhibition of mTOR by rictor and raptor knockdown inhibits hnRNPA1 expression.

(C) Combination of erlotinib and U0126 for 24 hours inhibits hnRNPA1 expression in U87-EGFRvIII cells.

A



B



C

	10	20	30	40	50	60
Max	MSDNDDIEVESDEEQPRFQSAADKRAHNNALERKRRDH IKDSFHSRLRDSVPSLQGEKASR					
Delta Max	MSDNDDIEVESDEEQPRFQSAADKRAHNNALERKRRDH IKDSFHSRLRDSVPSLQGEKASR					
	70	80	90	100	110	120
Max	AQILDKATEYIQYMRKNHHTHQDIDDLKRONALLEQQVRALEKARSSAQLQTNYPSSDN					
Delta Max	AQILDKATEYIQYMRKNHHTHQDIDDLKRONALLEQQGESES-----					
	130	140	150	160		
Max	SLYTNAGSTISAFDGGSDSSSESEPEEPQSRKKLRMEAS					
Delta Max	-----					

Figure S3

Figure S3. Data from the UCSC genome browser web site shows high conservation within the intronic region upstream of Max exon 5.

(A) The red spot shows the location of the potential hnRNPA1 binding site within the highly conserved intronic region.

(B) Schematic illustrating Max exon 5 splicing generating the C-terminal truncated Delta Max.

(C) The amino acids highlighted in gray are unique to Delta Max carboxy-terminus and are encoded by exon 5.

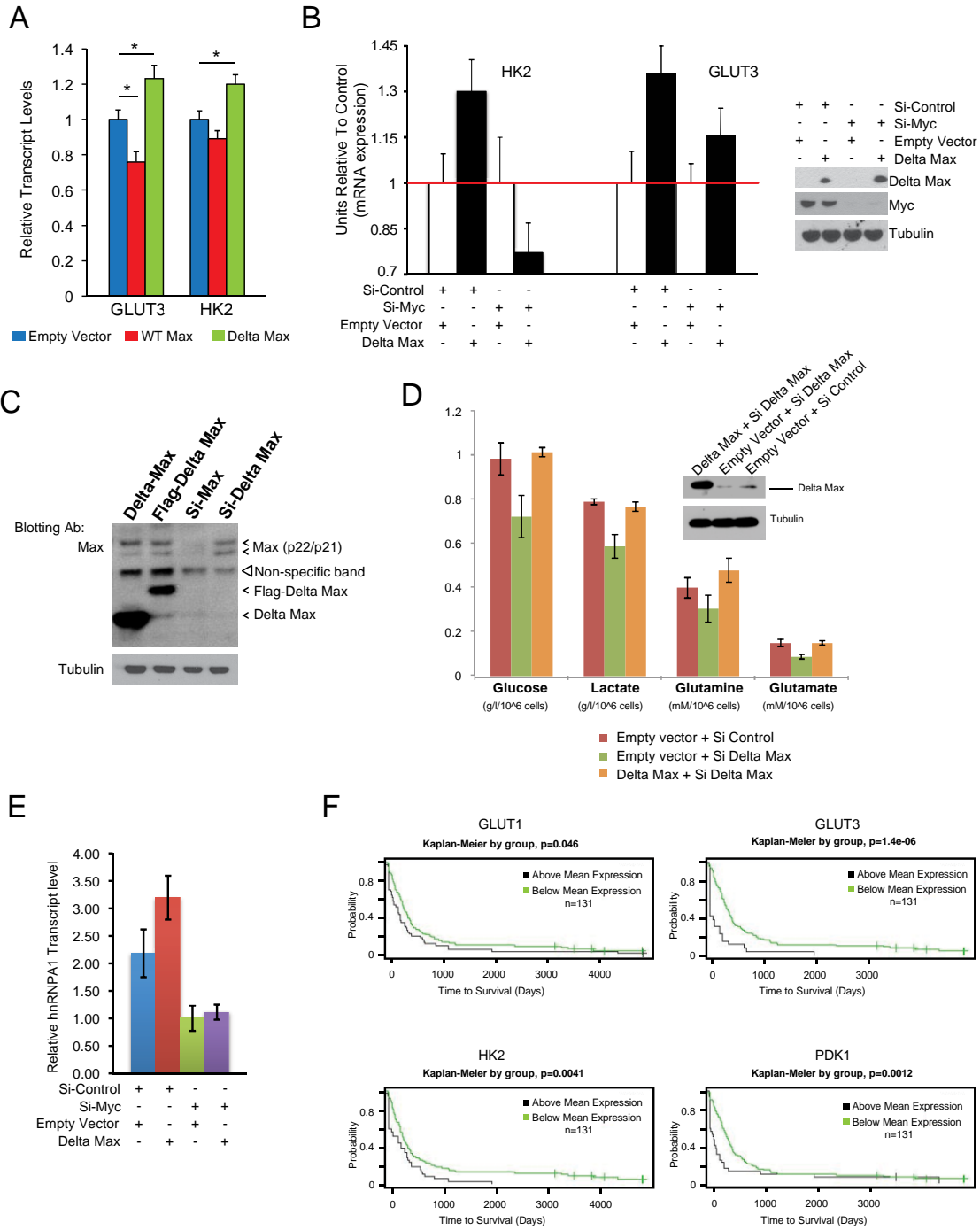


Figure S4

Figure S4. Delta Max overexpression augments Myc-dependent gene expression and promotes aerobic glycolysis.

(A) Relative transcript level of GLUT3 and HK2 in U87 cells overexpressing Delta Max or wild-type (WT) Max.

(B) HK2 and GLUT3 transcript levels in U87-EGFRvIII cells overexpressing Delta Max with or without Myc.

(C) Immunoblot demonstrating specific knockdown of Delta Max in U87-EGFRvIII cells.

(D) Overexpression of siRNA resistant Delta Max in U87 cells transfected with siRNA to Delta Max rescues glycolysis as measured by Nova BioProfile (n=3; shown is the mean \pm SD).

(E) RT-qPCR for hnRNPA1 with or without Myc and with or without Delta Max overexpression (n=3; shown is the mean \pm SD).

(F) Survival plots of 131 primary GBM patients stratified by expression of the indicated glycolytic genes. The single p-value is based on the difference of the two curves, and was calculated using the log-rank test.

SUPPLEMENTAL TABLES

Table S1. Alternative splicing events included in each Gene Ontology (GO) enrichment term. Listed are the transcripts that are grouped into each of the terms listed in Figure 1D. Coordinates for the EGFRvIII regulated alternative exon are listed for each transcript. P-value for each term was generated by a modified Fisher exact test using the DAVID Bioinformatics Tool. Terms shown all displayed p-values < 0.01 and are ranked from most significant to least.

Table S2. Analysis of TCGA dataset identifies a correlation between EGFRvIII and hnRNPA1 in GBM. Analysis was restricted to the classical, mesenchymal and neural subtypes of GBM, and data analyzed for hnRNPA1 expression in tumors definitively defined by the TCGA as EGFRvIII+ vs. those for which EGFRvIII expression could not be clearly identified. HnRNPA1 expression was higher in the EGFRvIII+ tumors (p=0.00881). Correlations were computed with Pearson correlation coefficient.

(Table S2 is a separate Excel File)

Table S3. Analysis of TCGA dataset identifies a correlation between hnRNPA1 and glycolytic genes. Expression of glycolytic genes correlated with hnRNPA1 expression in GBM from TCGA dataset. Correlations were computed with Pearson correlation coefficient.

	HNRNPA1	SLC2A3 (Glut3)	HK2	SLC2A1 (Glut1)	PDHK1 (PDK1)
HNRNPA1	1	0.179484606	0.238654	0.080826701	0.027049
SLC2A3 (Glut3)	0.179485	1	0.570375	0.589632526	0.580685
HK2	0.238654	0.57037536	1	0.510702251	0.483486
SLC2A1 (Glut1)	0.080827	0.589632526	0.510702	1	0.727501
PDHK1 (PDK1)	0.027049	0.580685165	0.483486	0.727500884	1

SUPPLEMENTAL EXPERIMENTAL PROCEDURES

UV-crosslinking and immunoprecipitation (CLIP)

U87-EGFRvIII cells in 15 cm plates were washed twice with cold PBS, added 12.5 mls PBS then UV irradiated in a UV stratalinker 1800 UV cross-linker (Stratagene) on ice with lid off. UV crosslinking was with either 100 or 500 J/m². Cells were collected by scraping, pelleted 5 min 500xg, resuspended in 1 ml PBS, transferred to eppy tubes and pelleted at 4000rpm 4 min at 4°C. PBS was removed and cell pellets frozen and stored at -80°C. Cells were lysed by adding 300 ul RIPA buffer (Boston BioProducts) containing 2x half protease inhibitor cocktail (Thermo Scientific), vortexed, and placed on ice for 20 min. Lysates were clarified by centrifuging 13 min 14000rpm at 4°C. Supernatant lysates were pre-cleared with 100 ul Pro A Dynabeads (Invitrogen) for 45 min on rotator 4°C. Immunoprecipitation (IP) was for 2 hrs at 4°C with either anti-HA Control antibody (6E2) (Cell Signaling) or with anti-hnRNPA1 9H10 clone (Sigma) with 250 ug/ml yeast tRNA. IP beads were washed 4X with RIPA buffer, resuspended in water, Dnase treated with DNase I (Ambion) for 1 hr and subsequently proteinase K (Roche) treated 1 hr, followed by phenol/Chloroform extraction, and RNA ethanol precipitated overnight at -20°C. RNA was pelleted, washed with 70% ethanol, air dried and resuspended in 14 ul water, and used for cDNA synthesis with Superscript VILO (Invitrogen). Primers used for RT-PCR were: Max Intron (Forward), 5'-GCTCAGTAGCAGGCTTGGTC-3', Max Intron (Reverse), 5'-AGAGGTCAGGCCAGAAAAGA-3'. Reverse primers were ³²P end-labeled and 22 cycle PCR reactions were resolved by denaturing PAGE. Radioactive signal was detected using a Typhoon phosphorimager (GE).

Primers used for Real-Time (RT) quantitative PCR

Primers used for Real-Time PCR were: Vinculin-F 5'-ctttgctgctacaggggaag-3'; Vinculin-R 5'-ggatatgggacgggaagttt-3'; GLUT1-F 5'-gtggagactaagccctgtcg-3'; GLUT1-R 5'-aggggcaaatacctaatggag-3'; GLUT3-F 5'-tcctggacatcctcttctgc-3'; GLUT3-R 5'-agtctgaggttgggggaact-3'; HK2-F 5'-agagaggaccccactggact-3'; HK2-R 5'-ccaaggtgaagcaaccgtat-3'; PDK1-F 5'-ggttggaaccactctttca-3'; PDK-R 5'-gcttggttacgtggcattt-3'; hnRNPA1-F 5'-ttgtgaactcagccaagcac-3'; hnRNPA1-R 5'-cagcgtcacgatcagactgt-3'.

Lactate production, glucose/glutamine consumption, glutamate assay

For NOVA measures of lactate production, glucose consumption, glutamine consumption, and glutamate secretion U87-EGFRvIII cells were grown in 10 cm plates with DMEM and 5% FBS. Media changed to 5 ml for 24 hr before measuring glucose, lactate, glutamine, and glutamate in the media by NOVA BioProfile automatic analyzer (Nova Biomedical). Glucose uptake, lactate production, glutamine uptake, and glutamate production were calculated by comparing to control plates without cells. Cells were collected after measurement and cell count were used to normalize results. Experiments were performed in triplicate.