## 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. Pvalue is generated by DAVID Functional Annotation Tool and is a modified Fisher exact pvalue.



### 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 eroltinib and U0126 for 24 hours inhibits hnRNPA1 expression in U87-EGFRvIII cells. А





С

В

Max	10 MSDNDDIEVESDEF	20 SQPRFQSAADKF	30 RAHHNALERK	40 RRDHIKDSFH: ::::::::::	50 Slrdsvpslqo	60 GEKASR
Deita Max	MSDNDDIEVESDEF 10	20 20	30	RRDHIKDSFH 40	SLRDSVPSLQ0 50	JEKASR 60
Max	70 AQILDKATEYIQYN	80 IRRKNHTHQQDI	90 DDLKRQNAL	100 LEQQVRALEK 	110 ARSSAQLQTNY	120 ZPSSDN
Delta Max	AQILDKATEYIQYN 70	1RRKNHTHQQDI 80	DDLKRQNAL 90	LEQQ <mark>GESES</mark> - 100		
Max	130 Slytnakgstisaf	140 FDGGSDSSESE	150 Speepqsrkk	160 LRMEAS		
Delta Max						

# 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 wildtype (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<u>+</u>SD).

(E) RT-qPCR for hnRNPA1 with or without Myc and with or without Delta Max overexpression (n=3; shown is the mean+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 S1. Alternative sp	plicing events includ	led in each	GO	enrichment f	term.

<ol> <li>positive regulatio</li> </ol>	a of means allocate matchell'		Molecular Function	later estivity	
Transcript	n or macromolecule metabolic process Description	Exon Coordinates	1) transcription regu Transcript	Description	Exon Coordinates
SMAD5	SMAD family member 5	chr5:135516263-135516346	AFF4	AF4/FMR2 family, member 4	chr5:132301240-132301331
APP DOCK7	amyloid beta (A4) precursor protein dedicator of cytokinesis 7	chr21:26291546-26291602 chr1:62783206-62783298	CNOT2 SETDA	CCR4-NO I transcription complex, subunit 2 SET domain containing (lysine methyltransferase) 8	chr12:68974284-68974341 chr12:122439933-122440054
ECE1	endothelin converting enzyme 1	chr1:21420827-21420922	SMAD5	SMAD family member 5	chr5:135516263-135516346
GTF2H1	general transcription factor IIH, polypeptide 1, 62kDa	chr11:18313877-18314069	CUTA	cutA divalent cation tolerance homolog (E. coli)	chr6-33492675-33492724
LEF1 MED21	lymphoid enhancer-binding factor 1 mediator complex subunit 21	chr4:109222191-109222274 chr12:27070620-27070734	GTF2H1 GTF2H2	general transcription factor IIH, polypeptide 1, 62kDa general transcription factor IIH, polypeptide 2, 44kDa	chr11:18313877-18314069 chr5:69205247-69205280
MLL5	myeloid/tymphoid or mixed-lineage leukemia 5 (trithorax homolog, Drosophila)	chr7:104465809-104465882	LEF1	lymphoid enhancer-binding factor 1	chr4:109222191-109222274
NFAT5 PSMP3	nuclear factor of activated T-cells 5, tonicity-responsive	chr16:68159899-68159952 chr17:34170210-34170387	MED21 MTA1	mediator complex subunit 21 metastasis sesociated 1	chr12:27070620-27070734 chr14:104986741-104986704
PSMB7	proteasome (prosome, macropain) subunit, beta type, 7	chr9:126207417-126207532	MLL5	myeloid/lymphoid or mixed-lineage leukemia 5 (trithorax homolog, Drosophila)	chr7:104465809-104465882
RAG1AP1	recombination activating gene 1 activating protein 1	chr1:153376661-153376822	NFE2L1	nuclear factor (erythroid-derived 2)-like 1	chr17:43489393-43489482
RNF14 TNFRSF1A	ring finger protein 14 tumor necrosis factor receptor superfamily, member 1A	chr5:141338052-141338579 chr12:6310280-6310353	BNF14	nuclear factor of activated T-cells 5, tonicity-responsive ring finger protein 14	chr16:68159899-68159952 chr5:141338052-141338579
USF2	upstream transcription factor 2, c-fos interacting	chr19:40452546-40452746	STAT1	signal transducer and activator of transcription 1, 91kDa	chr2:191583338-191583420
2) macromologulo or	stabolic azossa		SSBP4	single stranded DNA binding protein 4	chr19:18403163-18403228
Transcript	Description	Exon Coordinates	SFRS2	splicing factor, arginine/serine-rich 2	chr17:72243449-72243552
FBX022	FBXO22 opposite strand (non-protein coding); F-box protein 22	chr15:73983887-73984025	USF2	upstream transcription factor 2, c-fos interacting	chr19:40452546-40452746
SENP1	SUMO1/sentrin specific peptidase 1	chr12:46769213-46769343	2111-207	zinc inger protein 207	Chr17:27721132-27721214
UPF3A	UPF3 regulator of nonsense transcripts homolog A (yeast)	chr13:114066414-114066520	2) RNA polymerase I	I transcription factor activity	
ASB8 ARIH2	ankyrin repeat and SOCS box-containing 8 ariadoe homolog 2 (Drosophila)	chr12:46830137-46830190 chr3:48957573-48957618	CNOT2	CCR4-NOT transcription complex, subunit 2	Exon Coordinates chr12:68974284-68974341
CHID1	chitinase domain containing 1	chr11:883427-883519	GTF2H1	general transcription factor IIH, polypeptide 1, 62kDa	chr11:18313877-18314069
GTF2H1	general transcription factor IIH, polypeptide 1, 62kDa	chr11:18313877-18314069	LEF1	lymphoid enhancer-binding factor 1	chr4:109222191-109222274
GTF2H2 PSMB3	general transcription factor IIH, polypeptide 2, 44kDa proteasome (prosome, macropain) subunit, beta type, 3	chr5:69205247-69205280 chr17:34170210-34170387	NED21 NEAT5	mediator complex subunit 21 nuclear factor of activated T-cells 5. tonicity-responsive	chr12:27070620-27070734 chr16:68159899-68159852
PSMB7	proteasome (prosome, macropain) subunit, beta type, 7	chr9:126207417-126207532	SF1	splicing factor 1	chr11:64297478-64297553
RNF14 PNE34	ring finger protein 14	chr5:141338052-141338579 chr12:120324928-120324993	USF2	upstream transcription factor 2, c-fos interacting	chr19:40452546-40452746
USP8	ubiquitin specific peptidase 8	chr15:48563764-48563850	3) transcription cofa	ctor activity	
			Transcript	Description	Exon Coordinates
3) regulation of trans	slation		CUTA	cutA divalent cation tolerance homolog (E. coli)	chr6:33492675-33492724
Transcript	Description	Exon Coordinates	MED21	mediator complex subunit 21	chr12:27070620-27070734
APP ANKHD1	amyloid beta (A4) precursor protein ankyrin repeat and KH domain containing 1	chr21:26291546-26291602 chr5:139895131-139895307	MLL5 NFE2L1	nyeioid/iymphoid or mixed-lineage leukemia 5 (trithorax nomolog, Drosophila) nuclear factor (ervthrold-derived 2)-like 1	chr17:104465809-104465882 chr17:43489393-43489482
EIF4G2	eukaryotic translation initiation factor 4 gamma, 2	chr11:10779784-10779897	RNF14	ring finger protein 14	chr5:141338052-141338579
EIF4H IGE2BP2	eukaryotic translation initiation factor 4H	chr7:73242513-73242572 chr3:186873023.186873151	SF1 SERS2	splicing factor 1 eplicing factor, aminine/serine.rich 2	chr11:64297478-64297553
RPS5	ribosomal protein S5	chr19.63596155-63596364			
(a) collular macro	ecule catabolic process		Cellular Comportment		
Transcript	Description	Exon Coordinates	1) eukaryotic transla	tion initiation factor 4F complex	
FBX022	FBXO22 opposite strand (non-protein coding); F-box protein 22	chr15:73983887-73984025	Transcript	Description	Exon Coordinates
PCNP SENP1	Pto r proteorytic signal containing nuclear protein SUMO1/sentrin specific peptidase 1	chr3:102/81376-102781538 chr12:46769213-46769349	ANKHD1 EIF4G2	anxyrm repeat and KH domain containing 1 eukaryotic translation initiation factor 4 damma, 2	cnrb:139895131-139895307 chr11:10779784-10779897
UPF3A	UPF3 regulator of nonsense transcripts homolog A (yeast)	chr13:114066414-114066520	EIF4H	eukaryotic translation initiation factor 4H	chr7:73242513-73242572
ASB8	ankyrin repeat and SOCS box-containing 8	chr12:46830137-46830190	21 macromology	stabolic process	
GTF2H1	general transcription factor IIH, polypeptide 1, 62kDa	chr3.4685/5/3-4695/618 chr11:18313877-18314069	Transcript	Description	Exon Coordinates
GTF2H2	general transcription factor IIH, polypeptide 2, 44kDa	chr5:69205247-69205280	SMAD5	SMAD family member 5	chr5:135516263-135516346
PSMB3 DSMD7	proteasome (prosome, macropain) subunit, beta type, 3	chr17:34170210-34170387	GTF2H1 GTF2H2	general transcription factor IIH, polypeptide 1, 62kDa	chr11:18313877-18314069
RNF14	ring finger protein 14	chr5:141338052-141338579	LEF1	lymphoid enhancer-binding factor 1	chr4:109222191-109222274
RNF34	ring finger protein 34	chr12:120324928-120324993	MED21	mediator complex subunit 21	chr12:27070620-27070734
USP8	ubiquitin specific peptidase 8	chr15:48563764-48563850	MTA1 MU5	metastasis associated 1 myeloid/tymphoid or mixed-lineage laukemia 5 (trithorax homolog, Drosophila)	chr14:104986741-104986791 chr7:104465809-104465882
5) transcription, DN	A-dependent		RNF34	ring finger protein 34	chr12:120324928-120324993
Transcript	Description	Exon Coordinates	SRRM1	serine/arginine repetitive matrix 1	chr1:24846157-24846286
ADRM1	adhesion regulating molecule 1	chr20:60316819-60316921	3FN32	spicing lactor, arginitersenile-rich z	GIE 17:72243445-72243502
GTF2H1	general transcription factor IIH, polypeptide 1, 62kDa	chr11:18313877-18314069	3) membrane-enclos	ed lumen	
GTF2H2 NFE2L1	general transcription factor IIH, polypeptide 2, 44kDa nuclear factor (enthroid-derived 2)-like 1	chr5:59205247-69205280 chr17:43489393-43489482	FKBP10	EK506 binding protein 10, 65 kDa	Exon Coordinates chr17:37226836-37226981
NFAT5	nuclear factor of activated T-cells 5, tonicity-responsive	chr16:68159899-68159952	SMAD5	SMAD family member 5	chr5:135516263-135516346
STAT1	signal transducer and activator of transcription 1, 91kDa	chr2:191583338-191583420	AK2	adenylate kinase 2	chr1:33250262-33250310
05F2	upstream transcription factor 2, c-tos interacting	cnr19.40452546-40452746	FN1	fibronectin 1	chr2:215953779-215954048
6) RNA biosynthetic	process		FXR1	fragile X mental retardation, autosomal homolog 1	chr3:182171557-182171637
AFF4	AF4/FMR2 family, member 4	chr5:132301240-132301331	GTF2H1 GTF2H2	general transcription factor IIH, polypeptide 1, 62kDa general transcription factor IIH, polypeptide 2, 44kDa	chr5 69205247-69205280
ADRM1	adhesion regulating molecule 1	chr20:60316819-60316921	LEF1	lymphoid enhancer-binding factor 1	chr4:109222191-109222274
GTF2H1 GTF2H2	general transcription factor IIH, polypeptide 1, 62kDa	chr11:18313877-18314069	MED21	mediator complex subunit 21	chr12:27070620-27070734 chr14:104996741-104996701
NFE2L1	nuclear factor (erythroid-derived 2)-like 1	chr17:43489393-43489482	MLL5	myeloid/tymphoid or mixed-lineage leukemia 5 (trithorax homolog, Drosophila)	chr7:104465809-104465882
NFAT5	nuclear factor of activated T-cells 5, tonicity-responsive	chr16:68159899-68159952	PTBP1	polypyrimidine tract binding protein 1	chr19:750413-750443
USF2	signal transducer and activator of transcription 1, 91kDa upstream transcription factor 2, c-tos interacting	chr2:191583338-191583420 chr19:40452546-40452746	PTBP1 BNE34	polypyrimidine tract binding protein 1 ring finger protein 34	chr19:756513-756569 chr12:120324928-120324993
			SRRM1	serine/arginine repetitive matrix 1	chr1 24846157-24846286
7) positive regulatio	n of transcription, DNA-dependent	Even Coordinates	STAT1	signal transducer and activator of transcription 1, 91kDa	chr2:191583338-191583420
SMAD5	SMAD family member 5	chr5:135516263-135516346	SFRS2	splicing factor 1 splicing factor, arginine/serine-rich 2	chr11:04297478-04297003 chr17:72243449-72243552
APP	amyloid beta (A4) precursor protein	chr21:26291546-26291602	ZNF207	zinc finger protein 207	chr17:27721132-27721214
GTF2H1	general transcription factor IIH, polypeptide 1, 62kDa hepphoid enhancer binding factor 1	chr11:18313877-18314069			
MED21	mediator complex subunit 21	chr12:27070620-27070734			
MLL5	myeloid/lymphoid or mixed-lineage leukemia 5 (trithorax homolog, Drosophila)	chr7:104465809-104465882			
DNFA15	nuclear factor of activated 1-cells 5, tonicity-responsive				
ALC: 120		chr5:141338052-141338579			
TNFRSF1A	tumor necrosis factor receptor superfamily, member 1A	chr5:141338052-141338579 chr12:6310280-6310353			
TNFRSF1A USF2	umor necrosis factor receptor superfamily, member 1A upstream transcription factor 2, c-fos interacting	chr15:0615959-06159952 chr5:141338052-141338579 chr12:6310280-6310353 chr19:40452546-40452746			
TNFRSF1A USF2 8) positive regulatio	In gringer protein 19 tumor necrosis factor receptor superfamily, member 1A upstream transcription factor 2, c-fos interacting n of RNA metabolic process	chr5:141338052-141338579 chr5:141338052-141338579 chr12:6310280-6310353 chr19:40452546-40452748			
8) positive regulatio	Ing may poule tumor necross factor receptor superfamily, member 1A upstream transcription factor 2, o-fos interacting n of RNA mstabolic process Description	chr10.00139059-0015952 chr12:6310280-631038579 chr12:6310280-6310353 chr19:40452546-40452746			
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WH FRSF1A     USF2     USF2     USF2     Transcript     SMAD5     APP     GTF2H1     LEF1     MED21     MLL5     NFAT5     NFAT5     NFAT5     NFRSF1A     USF2     USF2     USF2     USF2     USF2     USF2     USF2	tumo recrossi factor receptor superfamily, member 1A upstream transcription factor 2, e-fosi interacting <b>Description</b> SMAD family member 5 amyloid beta (A4) precursor protein general transcription factor III; poypeptide 1, 62KDa lymphold enhance-shrinding factor 1 myeloid/shrphohol of cm xxx4c1reage leukenia 5 (triftorax homolog, Drosophila) muclear factor of activatel 7-cells 5 (triftorax homolog, Drosophila) muclear factor of activated 7-cells 5 (triftorax homolog, Dro	hins 14 138022.2 41 138273 chr128 1020-2110353 chr19.40452546-40452746 Exon Coordinates chr6.136516263-135516346 chr6.136516263-135516346 chr6.126170262174-052146 chr6.1262707620-27070734 chr7.146455809-104455882 chr6.14138098-66150952 chr6.14138098-66150953 chr12.6131088-6613033 chr12.614386-613133 chr12.40452546-40452746			
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Transcript     SMAD5     SMAD5     AMD5	Iumor necrosas factor recordor superfamily, member 1A upstream transcription factor 2, c-fos interacting <b>n of RNA metabolic process</b> <b>Description</b> SMAD family (Alt) personano crosinin general transcription factor 1, 63XDa ymphod enhance-briefing factor metabolic ormoles aubunit 21 myebiol/mybbiol or mixed-freege liskemia 5 (trithorax homolog, Dresophila) nuclear factor of activited T-cells 5, tonicly-responsive myebiol/mybbiolic mixed-freege liskemia 5 (trithorax homolog, Dresophila) nuclear factor of activited T-cells 5, tonicly-responsive myebiol/mybbiolic mixed-freege liskemia 5 (trithorax homolog, Dresophila) nuclear factor of activited T-cells 5, tonicly-responsive <b>n of gene expression</b> <b>Description</b> <b>MOD</b> family (inclusion: activity 2, o-fos interacting ymphotid enhance-briefing factor 1 myebial/mybbiols factor 12, o-fos interacting ymphotid enhance-briefing factor 1 myebial/mybbiols factor 10, spopeptids 1, 63XDa ymphotid enhance-briefing factor 1 myebial/mybiols factor 10, spopeptids 1, 63XDa general transcription factor 11, spopeptids 1, 63XDa general transcription factor 111, spopeptids 1, 63XDa general transcription factor 111, spopeptids 1, 63XDa general transcription factor 111, spopeptids 1, 53XDa general transcription factor 111, spopeptids 1, 53XDa generat transcription factor 111, spopeptids 1, 53XDa <b>moder factor</b>	Exon Coordinates           christ 3193622-261 39383           christ 31936262-3133551           christ 319516223-135516346           christ 319516223-135516346           christ 319516223-135516346           christ 319516223-135516346           christ 319516223-135516346           christ 319516223-135516346           christ 319516223-13516336           christ 319516223-14-10922274           christ 3198062-24707034           christ 31980623-135516346           christ 31980623-135516346           christ 31980623-135516346           christ 31980623-135516346           christ 3198079           chrit 3198079           chrit 3198079 <th></th> <td></td> <td></td>			
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**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. HnRNAPA1 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	HK2	SLC2A1	PDHK1
		(Glut3)		(Glut1)	(PDK1)
HNRNPA1	1	0.179484606	0.238654	0.080826701	0.027049
SLC2A3	0.179485	1	0.570375	0.589632526	0.580685
(Glut3)					
HK2	0.238654	0.57037536	1	0.510702251	0.483486
SLC2A1	0.080827	0.589632526	0.510702	1	0.727501
(Glut1)					
PDHK1	0.027049	0.580685165	0.483486	0.727500884	1
(PDK1)					

### 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  $J/m^2$ . 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 <sup>32</sup>P 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'aggggcaaatcctaatggag-3'; GLUT3-F 5'-tcctggacatcctcttctgc-3'; GLUT3-R 5'agtctgaggttgggggaact-3'; HK2-F 5'-agagaggaccccactggact-3'; HK2-R 5'-ccaaggtgaagcaaccgtat-3'; PDK1-F 5'-ggttgggaaccactctttca-3'; PDK-R 5'-gctttggttacgtggcattt-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.