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SUPPLEMENTARY INFORMATION

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AMP kinase promotes glioblastoma bioenergetics and tumour growth

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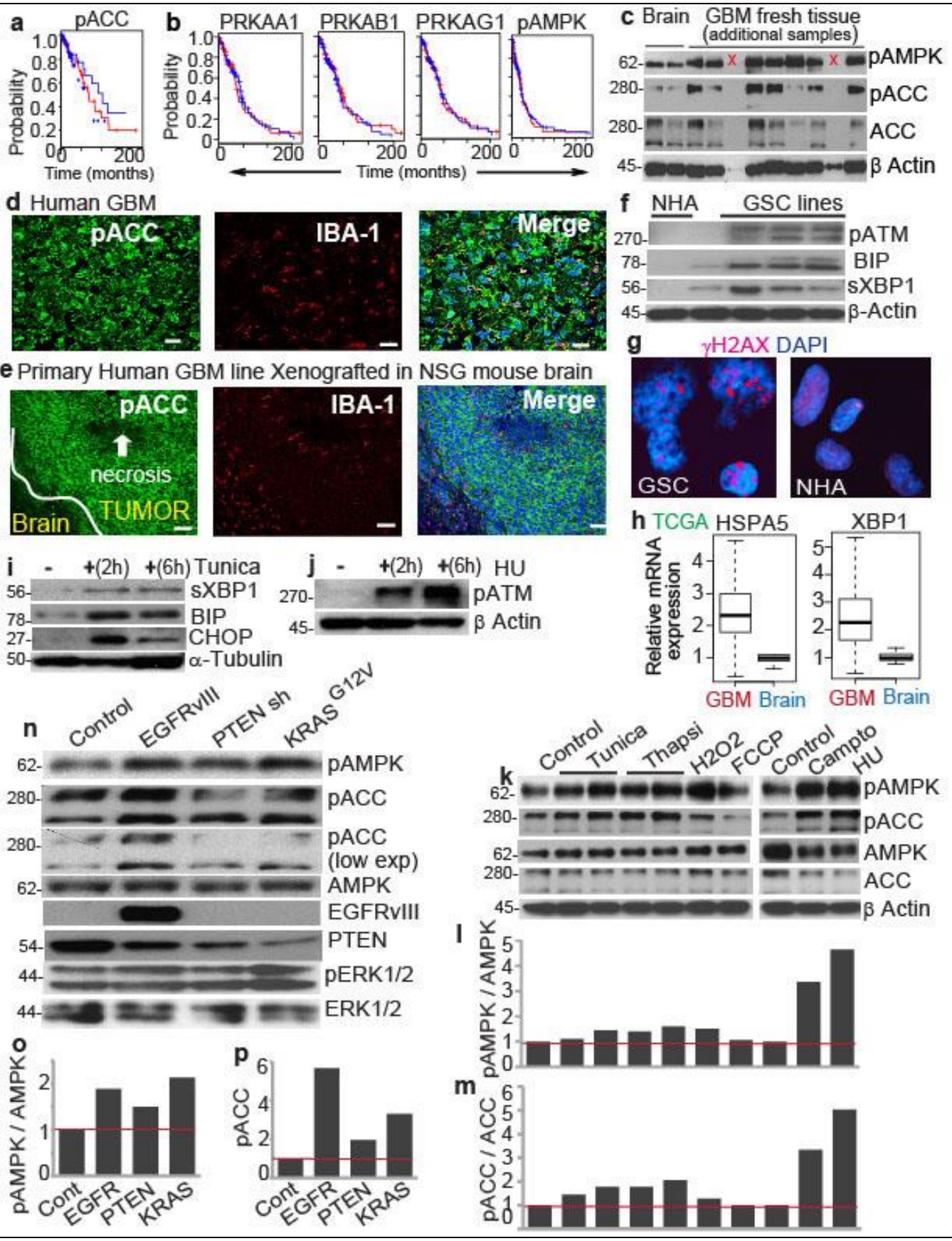
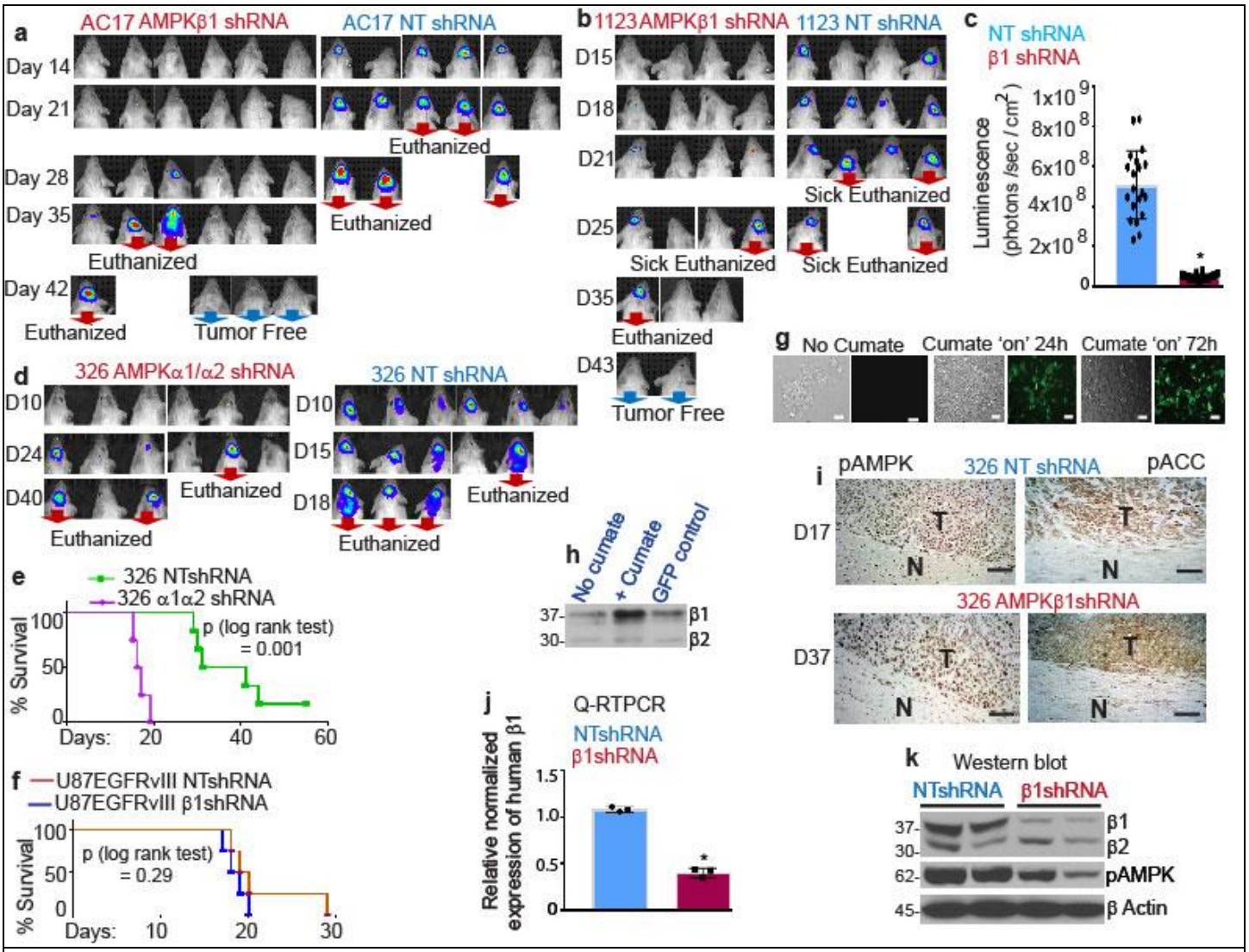

a
pACC
Probability
Time (months)
b
PRKAA1
PRKAB1
PRKAG1
pAMPK
Time (months)
c Brain
GBM fresh tissue
(additional samples)
pAMPK
pACC
ACC
β Actin
d Human GBM
pACC
IBA-1
Merge
e Primary Human GBM line Xenografted in NSG mouse brain
pACC
necrosis
TUMOR
Brain
IBA-1
Merge
f
NHA
GSC lines
pATM
BIP
sXBP1
β-Actin
g
γH2AX DAPI
GSC
NHA
h TCGA
HSPA5
XBP1
Relative mRNA expression
GBM Brain
GBM Brain
i
Tunica
sXBP1
BIP
CHOP
α-Tubulin
j
HU
pATM
β Actin
n
Control
EGFRvIII
PTEN sh
KRAS G12V
pAMPK
pACC
pACC (low exp)
AMPK
EGFRvIII
PTEN
pERK1/2
ERK1/2
k
Control
Tunica
Thapsi
H2O2
FCCP
Control
Campto
HU
pAMPK
pACC
AMPK
ACC
β Actin
l
pAMPK / AMPK
m
pACC / ACC
o
pAMPK / AMPK
Cont
EGFR
PTEN
KRAS
p
pACC
Cont
EGFR
PTEN
KRAS

Supplementary Figure 1

Oncogenic stress-associated high expression of AMPK in glioblastoma.

a, Kaplan-Meier survival plots of LGG patients expressing phosphorylated ACC (Source: TCGA). **b,** Kaplan-Meier survival plots of GBM patients expressing AMPK subunits (Source: TCGA). **c,** Western blot (WB) of pAMPK and pACC in Human GBM and normal human brain tissue. X indicates tissue degradation. Actin was used as loading control. **d, e,** IHC of pACC, DAPI (nuclei) and the microglia marker IBA-1 in human GBM tissue (d), and xenograft of the human GBM line 326 in NSG mice (e). Scale bar = 50 μm (d) and 100μM (e). **f,** Oncogenesis-associated stress markers are high in primary GSC lines relative to normal human astrocytes (NHA). WB showing markers of genotoxic stress (pATM), and endoplasmic reticulum (ER) stress (BIP and sXBP1). Actin was used as loading control. **g,** IHC of γH2AX foci in GSC and NHA. Scale bar 100 μm**.** Nuclei were stained with DAPI. Scale bar = 20 μm **h,** Box plots Source: (TCGA) showing expression of ER stress transcripts in GBM and normal brain (HSPA5 is the gene name for BIP). The edges on the boxplots indicate the first and 3rd quartile (25th- and 75th percentile) of the data, with the line in the middle being the median. The whiskers on the boxplots extend another 1.5x of the inter-quartile range (between 25%-75% range of data) from the edges of the boxes, respectively. **i, j,** WB of sXBP1, BIP, CHOP, pATM in NHA treated with tunicamycin and hydroxyurea (HU). **k,** WB of pAMPK, AMPK, pACC and ACC in NHA treated with indicated reagents. Actin was used as loading control. **l, m,** Quantification of band intensities in the same order as in (k). **n,** WB of indicated proteins in NHA expressing oncogenic EGFRvIII, constitutively active KRAS^{G12V} or PTEN shRNA. Note: PTEN appears to be significantly downregulated in EGFRvIII and KRAS^{G12V} cells. **o, p,** Quantification of band intensities in (n). All western blots represent data from 2 (f, n, k), or 3 (c, l, j) independent repeats. Scanned images of unprocessed blots are shown in Supplementary Fig. 9.

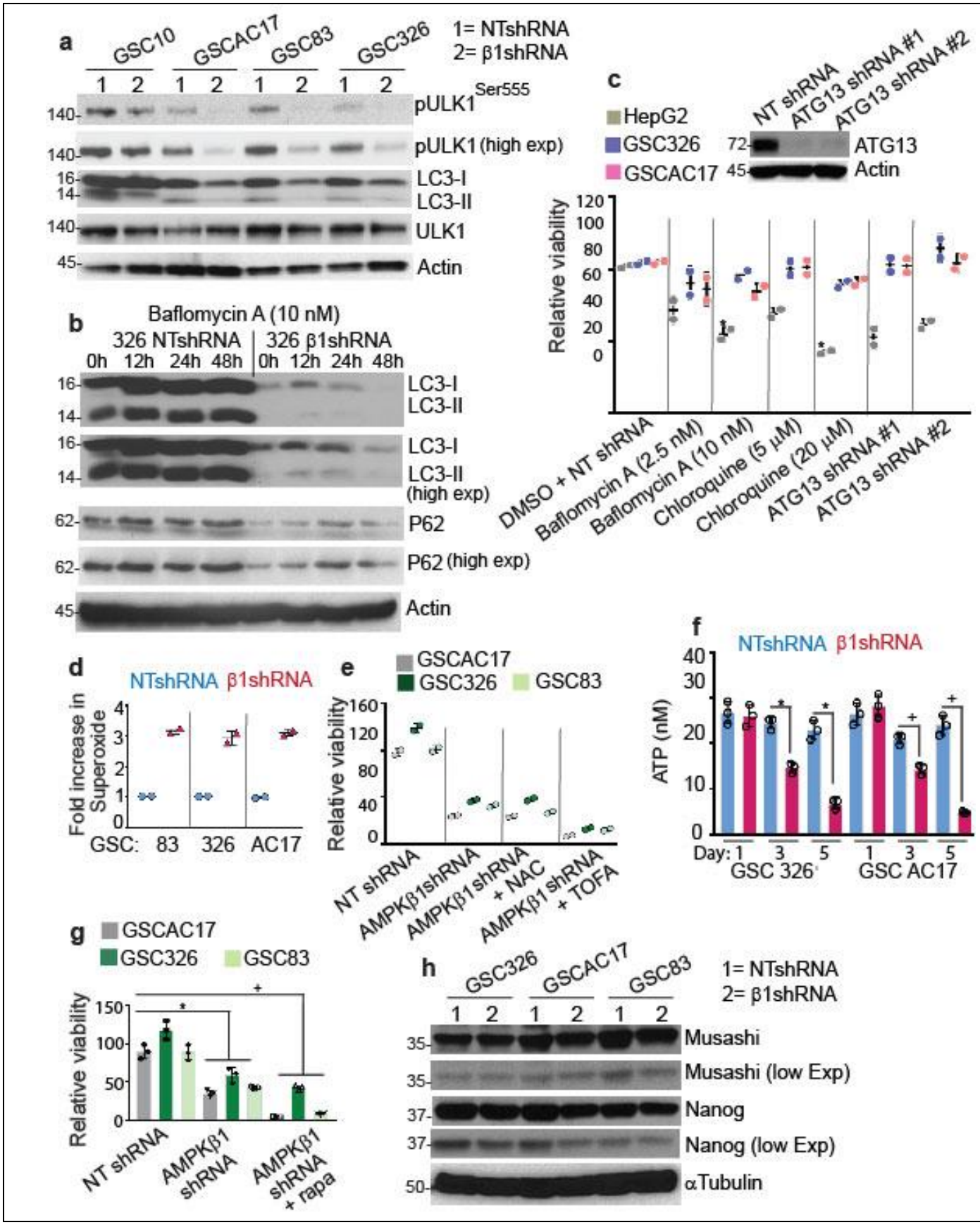


Supplementary Figure 2

AMPK is essential for optimal growth of primary GBM orthotopic xenografts in mice.

a, b, Luciferase imaging of mice to monitor tumor growth of GSC lines expressing AMPKβ1 or nontarget (NT) shRNA at indicated days. (n = 8-15 mice per line). Primary GSC lines AC17, 1123 were infected with NT or AMPKβ1 shRNA for two days and 10,000 Trypan blue negative live cells were transplanted into the cortex of NSG mice 48 hours after *in vitro* lentiviral transduction (when AMPK β1shRNA had negligible effect on viability). **c**, Quantification of tumor luminescence. Total range of tumor luminescence of mice was quantified three weeks after cell transplantation. (n = 20 mice / genotype). *p ≤ 0.0001. **d,** Luciferase imaging of mice to monitor tumor growth of a GSC line expressing AMPK α1α2 shRNA or nontarget (NT) shRNA at indicated days. (n = 6 mice per genotype). **e,** Kaplan-Meier survival data of mice in (d). **f,** Kaplan-Meier survival data of mice transplanted with U87EGFRvIII GBM serum line expressing NT or AMPK β1 shRNA. (n = 4 mice per genotype). **g,** Expression of cumate-inducible GFP in 293T cells. Scale bar 100μm. **h,** WB showing expression of cumate-inducible mouse AMPKβ1 (subcloned in the SparQ vector) in 293T cells. **i**, IHC pf pAMPK and pACC in NT and AMPKβ1 shRNA expressing tumors at indicated days. (T = tumor; N = normal brain). Scale bar 100 μm. **j,** Q-RTPCR of human AMPK β1 (using human-specific primers) in tumor cells isolated from NT shRNA or AMPKβ1 shRNA

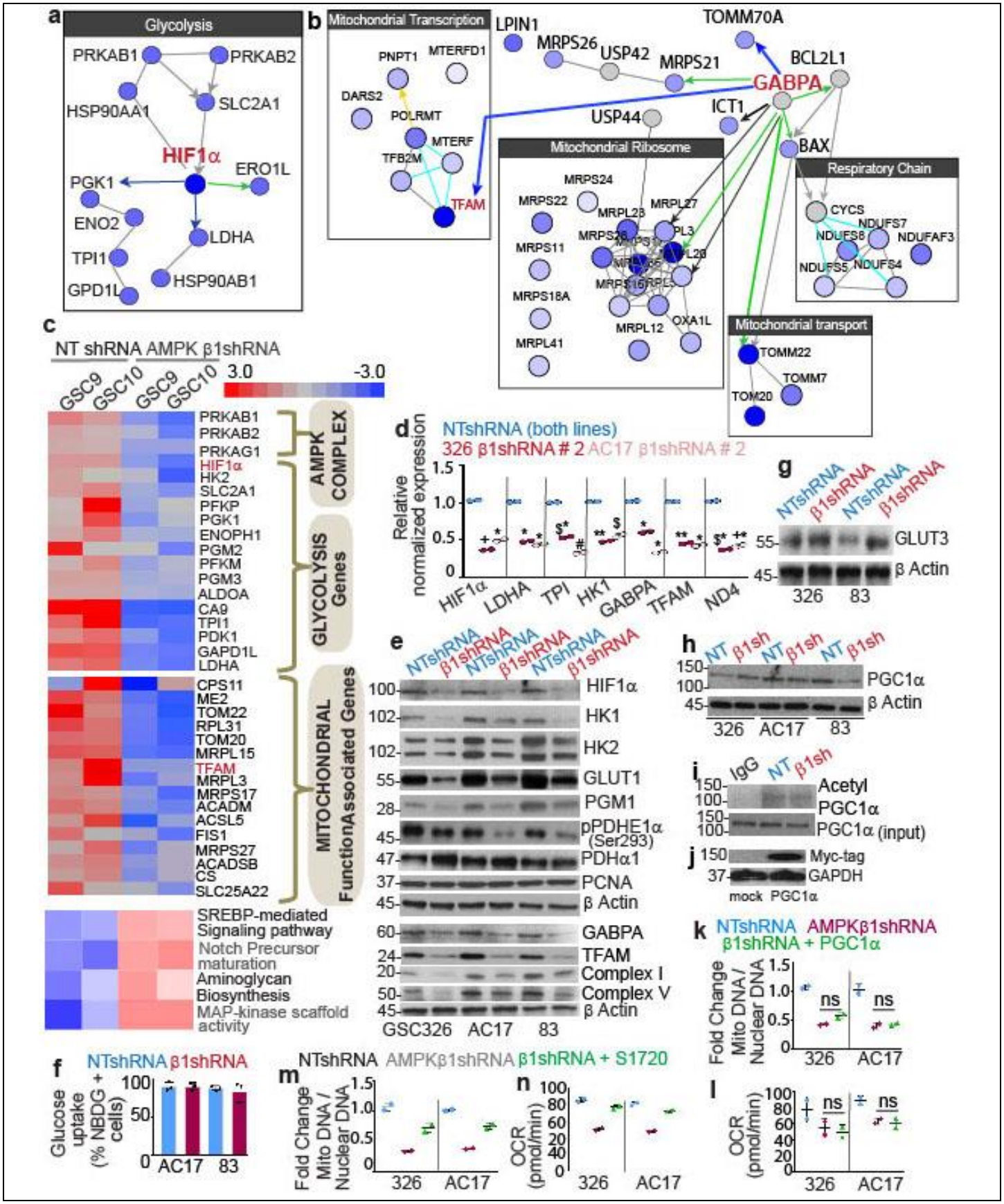
tumors. (n = 3). *p = 0.0007. **k,** WB of AMPKβ1/β2 and pAMPK in tumor-derived cells as in (j). Error bars; mean +/- S.D. Statistical significance in (c, j); two-tailed t-test. n values represent independent experiments. Source data are available in Supplementary Table 4.All western blots represent data from 2 (h) or 3 (k) independent repeats. Unprocessed blots are shown in Supplementary Fig. 9.


a
GSC10
GSCAC17
GSC83
GSC326
1= NTshRNA
2= β1shRNA
1 2 1 2 1 2 1 2
140-
pULK1 Ser555
140-
pULK1 (high exp)
16-
14-
LC3-I
LC3-II
140-
ULK1
45-
Actin
b
Baflomycin A (10 nM)
326 NTshRNA
326 β1shRNA
0h 12h 24h 48h 0h 12h 24h 48h
16-
14-
LC3-I
LC3-II
16-
14-
LC3-I
LC3-II (high exp)
62-
P62
62-
P62 (high exp)
45-
Actin
c
HepG2
GSC326
GSCAC17
NT shRNA
ATG13 shRNA #1
ATG13 shRNA #2
72-
ATG13
45-
Actin
Relative viability
120
100
80
60
40
20
0
DMSO + NT shRNA
Baflomycin A (2.5 nM)
Baflomycin A (10 nM)
Chloroquine (5 μM)
Chloroquine (20 μM)
ATG13 shRNA #1
ATG13 shRNA #2
d
NTshRNA β1shRNA
Fold increase in Superoxide
4
3
2
1
0
GSC: 83 326 AC17
e
GSCAC17
GSC326
GSC83
Relative viability
160
120
80
40
0
NT shRNA
AMPKβ1shRNA
AMPKβ1shRNA + NAC
AMPKβ1shRNA + TOFA
f
NTshRNA β1shRNA
ATP (nM)
40
30
20
10
0
Day: 1 3 5 1 3 5
GSC 326
GSC AC17
g
GSCAC17
GSC326
GSC83
Relative viability
150
100
50
0
NT shRNA
AMPKβ1 shRNA
AMPKβ1 shRNA + rapa
h
GSC326
GSCAC17
GSC83
1= NTshRNA
2= β1shRNA
1 2 1 2 1 2
35-
Musashi
35-
Musashi (low Exp)
37-
Nanog
37-
Nanog (low Exp)
50-
αTubulin

Supplementary Figure 3

GSC death occurs independent of AMPK-regulated mTOR and autophagy pathways.

a.WB of pULK1 (AMPK target) and autophagy substrate LC3 in AMPK-silenced GSCs. ULK1 and actin are used as loading controls. **b,** WB of autophagy substrates LC3 and P62 in NT or AMPKβ1shRNA expressing cells treated with Bafilomycin A showing autophagy flux. **c,** Viability of GSCs and HepG2 liver cancer line treated with autophagy inhibitors Bafilomycin and Chloroquine, or infected with NT or ATG13 shRNAs. (Average of two independent experiments). **d,** Superoxide detection by flow cytometry in NT or AMPKβ1 shRNA expressing GSCs using MitoSox Red. (Average of two independent experiments). **e**, Viability of GSCs expressing NT or AMPKβ1 shRNA treated with the reducing agent N-acetylcysteine (NAC) or the ACC inhibitor TOFA. (Average of two independent experiments). **f,** ATP levels in GSC lines expressing AMPKβ1 shRNA or NT shRNA at indicated times. ATP value was normalized to protein. (n = 3). *$p \leq 0.002$; $^{+} \leq 0.004$. **g,** Viability of GSCs expressing NT or AMPKβ1 shRNA treated with the mTORC1 inhibitor rapamycin (5nM). (n = 3). *$p \leq 0.01$; $^{+} \leq 0.004$. **h,** WB of stem cell markers Musashi and Nanog in GSCs expressing NT and AMPKβ1 shRNA. Error bars; mean +/- S/d. Statistical significance in above experiments was assessed using two-tailed t-test. n values represent independent experiments. Source data are available in Supplementary Table 4. All western blots represent data from 2-3 independent repeats. Unprocessed blots are shown in Supplementary Fig. 9.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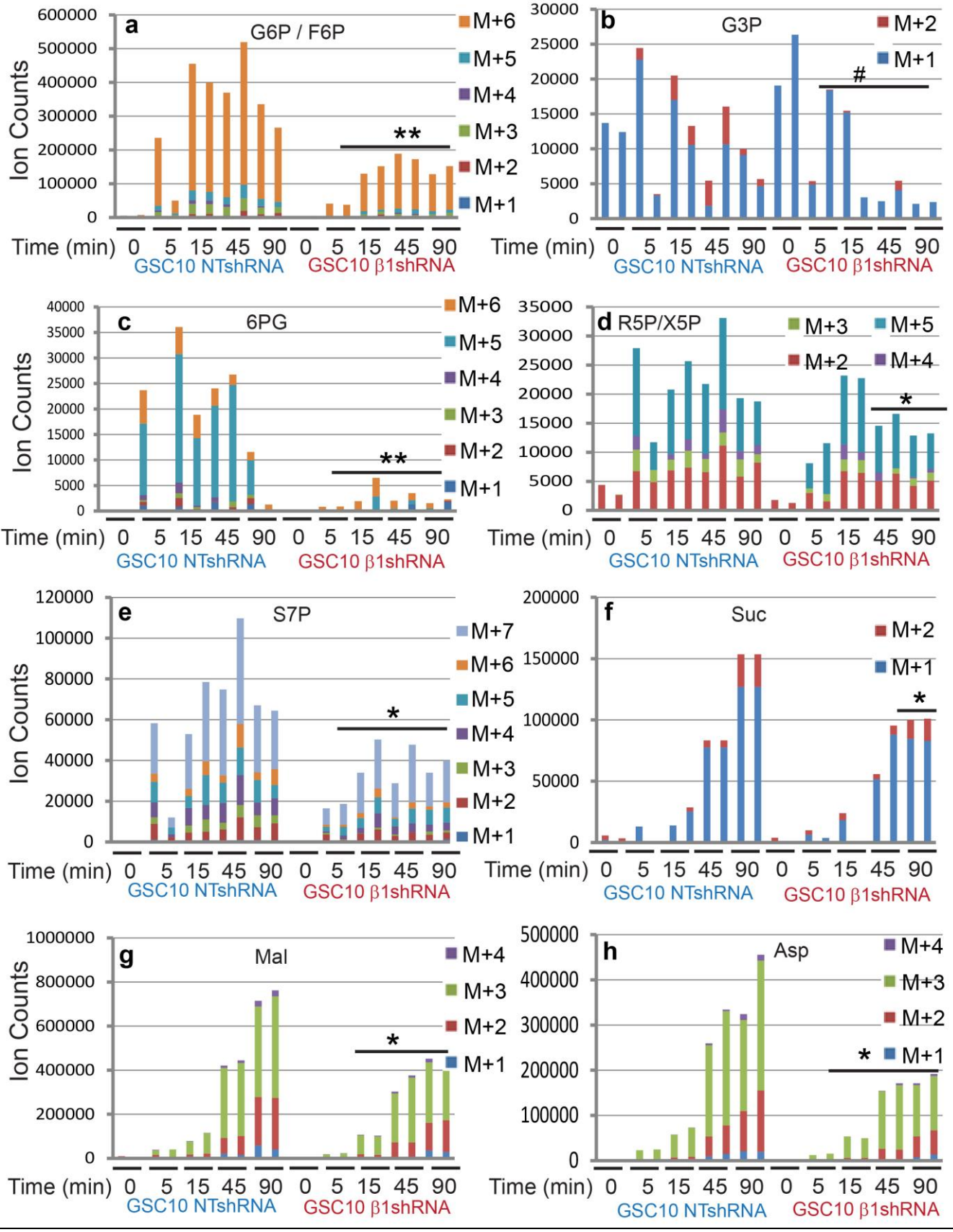
Glycolysis
PRKAB1
PRKAB2
HSP90AA1
SLC2A1
HIF1α
ERO1L
PGK1
ENO2
LDHA
TPI1
HSP90AB1
GPD1L
b
Mitochondrial Transcription
PNPT1
MTERFD1
DARS2
POLRMT
MTERF
TFB2M
TFAM
LPIN1
MRPS26
USP42
MRPS21
TOMM70A
BCL2L1
GABPA
ICT1
USP44
BAX
Mitochondrial Ribosome
MRPS24
MRPS22
MRPL23
MRPL27
MRPS11
MRPS18A
MRPL41
MRPL12
OXA1L
Respiratory Chain
CYCS
NDUFS7
NDUFS8
NDUFAF3
NDUFS5
NDUFS4
Mitochondrial transport
TOMM22
TOMM7
TOM20
c
NT shRNA AMPK β1shRNA
GSC9 GSC10 GSC9 GSC10
3.0
-3.0
PRKAB1
PRKAB2
PRKAG1
HIF1α
HK2
SLC2A1
PFKP
PGK1
ENOPH1
PGM2
PFKM
PGM3
ALDOA
CA9
TPI1
PDK1
GAPD1L
LDHA
CPS11
ME2
TOM22
RPL31
TOM20
MRPL15
TFAM
MRPL3
MRPS17
ACADM
ACSL5
FIS1
MRPS27
ACADSB
CS
SLC25A22
AMPK COMPLEX
GLYCOLYSIS Genes
MITOCHONDRIAL FunctionAssociated Genes
SREBP-mediated Signaling pathway
Notch Precursor maturation
Aminoglycan Biosynthesis
MAP-kinase scaffold activity
d
NTshRNA (both lines)
326 β1shRNA # 2 AC17 β1shRNA # 2
Relative normalized expression
1.5
1.0
0.5
0
HIF1α
LDHA
TPI
HK1
GABPA
TFAM
ND4
g
NTshRNA β1shRNA NTshRNA β1shRNA
55-
45-
GLUT3
β Actin
326 83
e
NTshRNA β1shRNA NTshRNA β1shRNA NTshRNA β1shRNA
100-
102-
102-
55-
28-
45-
47-
37-
45-
60-
24-
20-
50-
45-
HIF1α
HK1
HK2
GLUT1
PGM1
pPDHE1α (Ser293)
PDHα1
PCNA
β Actin
GABPA
TFAM
Complex I
Complex V
β Actin
GSC326 AC17 83
h
NT β1sh NT β1sh NT β1sh
150-
100-
45-
PGC1α
β Actin
326 AC17 83
i
IgG NT β1sh
150-
100-
150-
100-
Acetyl PGC1α
PGC1α (input)
j
150-
37-
Myc-tag
GAPDH
mock PGC1α
k
NTshRNA AMPKβ1shRNA
β1shRNA + PGC1α
Fold Change Mito DNA / Nuclear DNA
1.5
1.0
0.5
0
ns
ns
326 AC17
f
NTshRNA β1shRNA
Glucose uptake (% NBDG + cells)
100
50
0
AC17 83
m
NTshRNA AMPKβ1shRNA β1shRNA + S1720
Fold Change Mito DNA / Nuclear DNA
1.5
1.0
0.5
0
326 AC17
n
OCR (pmol/min)
100
80
60
20
40
0
326 AC17
l
OCR (pmol/min)
100
80
60
40
20
0
ns
ns
326 AC17

Supplementary Figure 4

GSC bioenergetics is downregulated in AMPK-silenced GSCs.

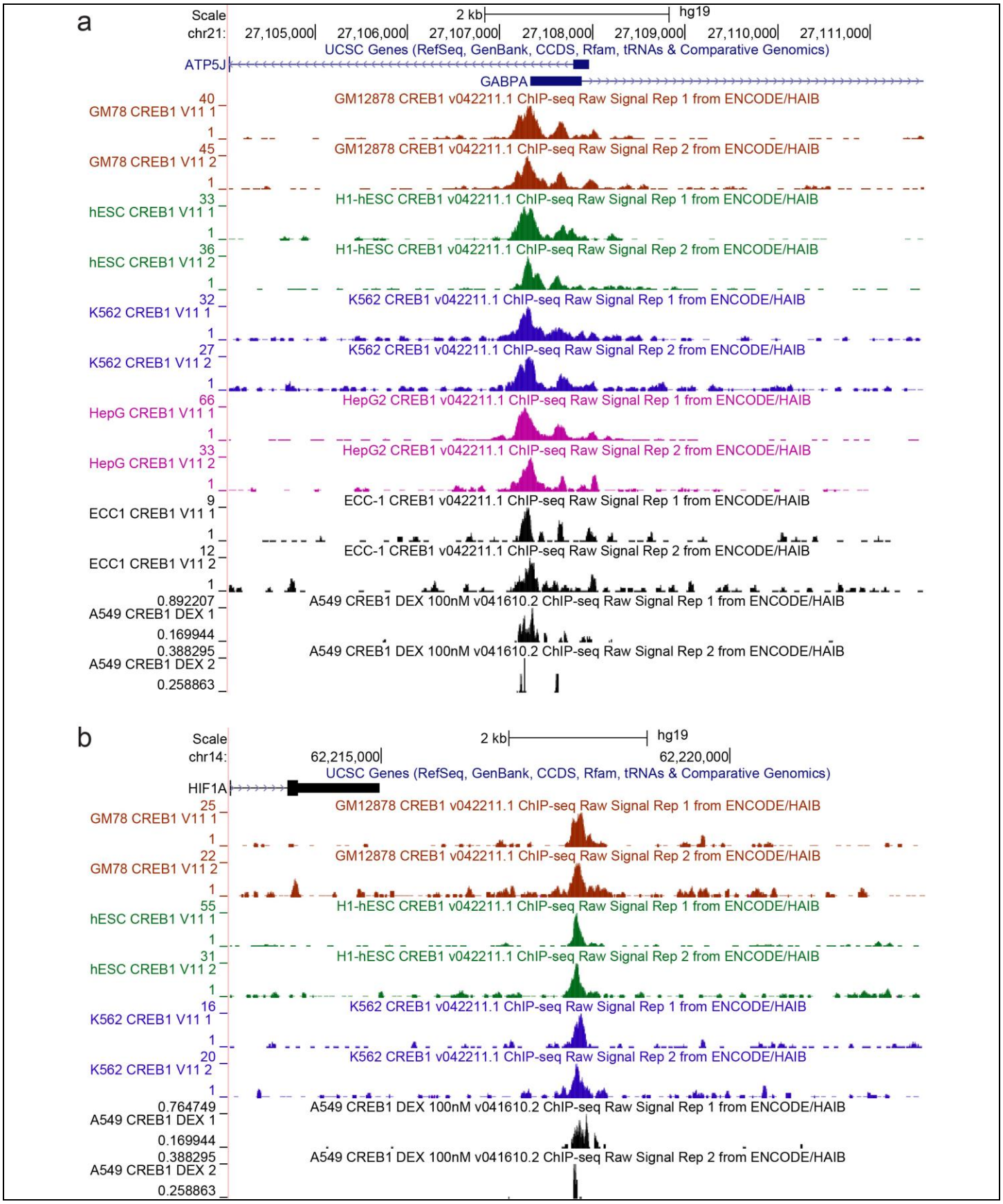
a, b, Molecular network of most downregulated glycolysis genes (a) and mitochondrial genes (b) in two AMPKβ1-sienced GSCs. Node color denotes extent of downregulation (darker = greater downregulation). Ingenuity Pathway was also used to confirm NetWalker analysis. **c,** Heatmap showing differentially expressed genes in indicated pathways in AMPKβ1 shRNA GSCs relative to NT shRNA expressing GSCs. **d.** Q-RTPCR analysis of HIF1α, GABPA and their target genes in GSCs using a second AMPKβ1 shRNA (Average of two independent experiments). $^{+}$p = 0.001; * 0.01; $^{\$}$* 0.002; $^{\#}$ 0.006; ** 0.003; $^{\$}$0.02; $^{+}$* 0.05. **e,** WB of indicated proteins in GSCs expressing NT or AMPKβ1shRNA. **f,** Glucose uptake (at 30 min) in GSCs expressing NT or AMPKβ1shRNA. . (n = 3). **g,** WB of GLUT3 in GSCs expressing NT or AMPKβ1shRNA. **h,** WB of PGC1α in nontarget and AMPKβ1 shRNA expressing GSCs. **i,** WB using anti-acetyl antibody following immunoprecipitation with PGC1α antibody in control (NT) and AMPK silenced GSC. **j**, WB showing overexpression of Myc-tagged PGC1α in GSCs. **k,** Q-PCR analysis of ND4 and β actin DNA in control and AMPK-silenced GSCs overexpressing PGC1α. (Average of two independent experiments). ns = nonsignificant. **l**, Oxygen consumption rate (OCR) in control and AMPK-silenced GSCs overexpressing PGC1α. (Average of two independent experiments. **m,** Q-RT-PCR analysis of ND4 and β actin RNA in control and AMPK-silenced GSCs treated with SIRT1720 for 48h. (Average of two independent experiments). **n**, OCR in control and AMPK-silenced GSCs treated with SIRT1720. (Average of two independent experiments). Error bars; mean +/- S.D. Statistical significance in above experiments was assessed using two-tailed t-test. n values represent independent experiments. Source data are available in Supplementary Table 4. All western blots represent data from 2 (e, h, I, j) or 3 (e, g) independent repeats. Unprocessed blots are shown in Supplementary Fig. 9.


a
G6P / F6P
M+6
M+5
M+4
M+3
M+2
M+1
Ion Counts
600000
500000
400000
300000
200000
100000
0
**
Time (min) 0 5 15 45 90 0 5 15 45 90
GSC10 NTshRNA
GSC10 β1shRNA
b
G3P
M+2
M+1
30000
25000
20000
15000
10000
5000
0
#
Time (min) 0 5 15 45 90 0 5 15 45 90
GSC10 NTshRNA
GSC10 β1shRNA
c
6PG
M+6
M+5
M+4
M+3
M+2
M+1
Ion Counts
40000
35000
30000
25000
20000
15000
10000
5000
0
**
Time (min) 0 5 15 45 90 0 5 15 45 90
GSC10 NTshRNA
GSC10 β1shRNA
d
R5P/X5P
M+3
M+5
M+2
M+4
35000
30000
25000
20000
15000
10000
5000
0
*
Time (min) 0 5 15 45 90 0 5 15 45 90
GSC10 NTshRNA
GSC10 β1shRNA
e
S7P
M+7
M+6
M+5
M+4
M+3
M+2
M+1
Ion Counts
120000
100000
80000
60000
40000
20000
0
*
Time (min) 0 5 15 45 90 0 5 15 45 90
GSC10 NTshRNA
GSC10 β1shRNA
f
Suc
M+2
M+1
200000
150000
100000
50000
0
*
Time (min) 0 5 15 45 90 0 5 15 45 90
GSC10 NTshRNA
GSC10 β1shRNA
g
Mal
M+4
M+3
M+2
M+1
Ion Counts
1000000
800000
600000
400000
200000
0
*
Time (min) 0 5 15 45 90 0 5 15 45 90
GSC10 NTshRNA
GSC10 β1shRNA
h
Asp
M+4
M+3
M+2
M+1
500000
400000
300000
200000
100000
0
* *
Time (min) 0 5 15 45 90 0 5 15 45 90
GSC10 NTshRNA
GSC10 β1shRNA

Supplementary Figure 5

Glucose metabolism is diminished in AMPK-silenced GBM cells.

a-h, GSC10 cells were fed with $U^{13}C$ glucose and ^{13}C carbon incorporation in the metabolites of glycolysis and TCA cycle was quantitated by HPLC/Mass spectrometry. Shown are several metabolites of glycolysis (G6P/F6P, G3P), pentose phosphate pathway (6PG, R5P/X5P, S7P) and TCA cycle (Suc, Mal) that show reduced incorporation of 13C glucose carbon at indicated times. Note: Aspartate is generated from the TCA cycle metabolite oxaloacetate. G6P/F6P, glucose 6-phosphate/fructose 6-phosphate; G3P, glucose 3-phosphate; 6-PG, 6-phosphogluconate; R5P/X5P, ribulose 5-phosphate/xylulose 5-phosphate; S7P, sedoheptulose 7-phosphate; Suc; succinate; Mal, malate; Asp, aspartate. Duplicate samples were processed. ** $p = 0.003$ (M+6 G6P/F6P; M+6 and M+5 6PG); # $p = 0.001$ (M+ 2 G3P); * $p \leq 0.05$ (M+5 R5P/X5P; M+7 and M+6 S7P; M+2 Suc; M+3 Mal; M+3 and M+2 Asp). Source data are available in Supplementary Table 4.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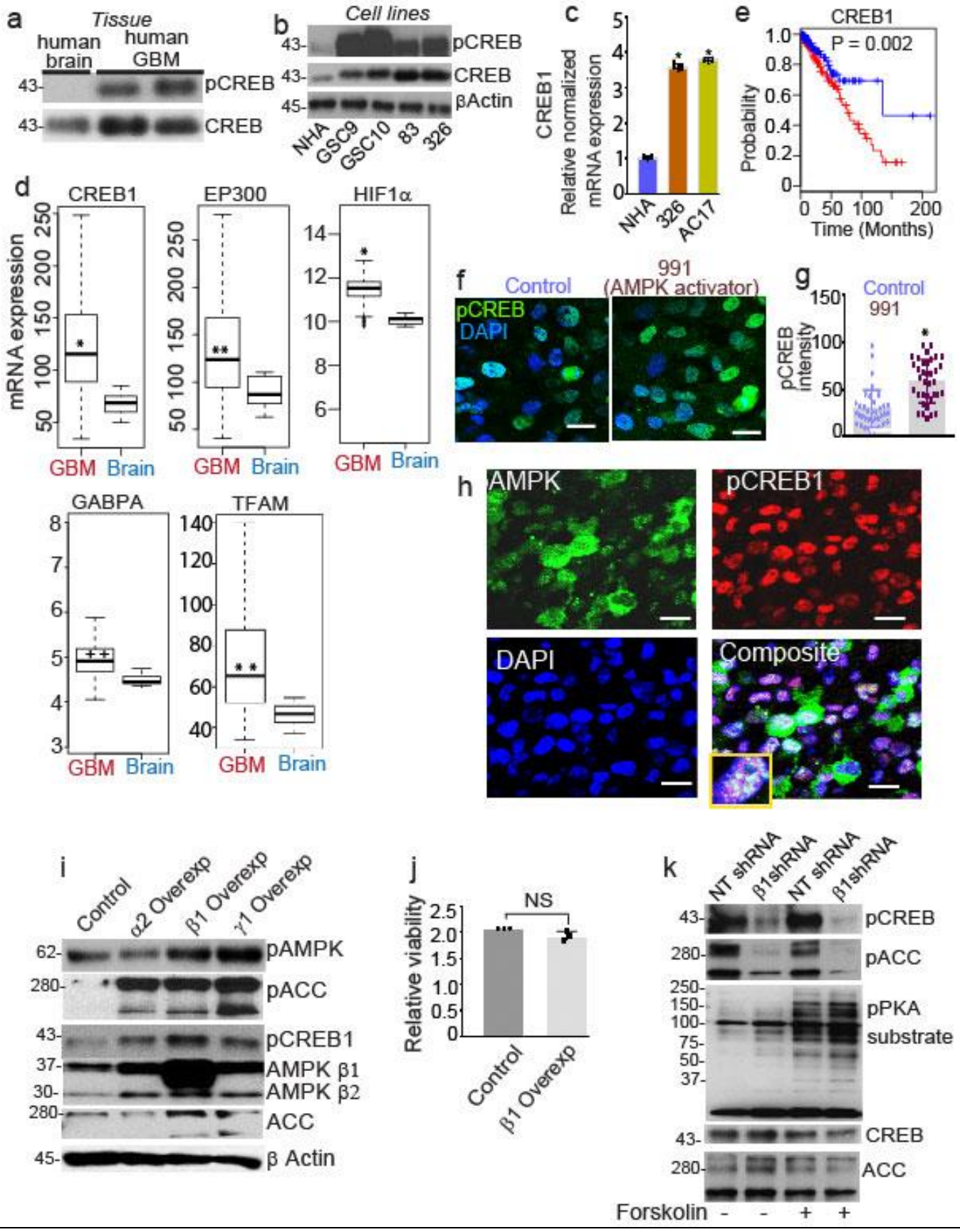
Scale
chr21:
2 kb
hg19
27,105,000
27,106,000
27,107,000
27,108,000
27,109,000
27,110,000
27,111,000
UCSC Genes (RefSeq, GenBank, CCDS, Rfam, tRNAs & Comparative Genomics)
ATP5J
GABPA
40
GM78 CREB1 V11 1
GM12878 CREB1 v042211.1 ChIP-seq Raw Signal Rep 1 from ENCODE/HAIB
1
45
GM78 CREB1 V11 2
GM12878 CREB1 v042211.1 ChIP-seq Raw Signal Rep 2 from ENCODE/HAIB
1
33
hESC CREB1 V11 1
H1-hESC CREB1 v042211.1 ChIP-seq Raw Signal Rep 1 from ENCODE/HAIB
1
36
hESC CREB1 V11 2
H1-hESC CREB1 v042211.1 ChIP-seq Raw Signal Rep 2 from ENCODE/HAIB
1
32
K562 CREB1 V11 1
K562 CREB1 v042211.1 ChIP-seq Raw Signal Rep 1 from ENCODE/HAIB
1
27
K562 CREB1 V11 2
K562 CREB1 v042211.1 ChIP-seq Raw Signal Rep 2 from ENCODE/HAIB
1
66
HepG CREB1 V11 1
HepG2 CREB1 v042211.1 ChIP-seq Raw Signal Rep 1 from ENCODE/HAIB
1
33
HepG CREB1 V11 2
HepG2 CREB1 v042211.1 ChIP-seq Raw Signal Rep 2 from ENCODE/HAIB
1
9
ECC1 CREB1 V11 1
ECC-1 CREB1 v042211.1 ChIP-seq Raw Signal Rep 1 from ENCODE/HAIB
1
12
ECC1 CREB1 V11 2
ECC-1 CREB1 v042211.1 ChIP-seq Raw Signal Rep 2 from ENCODE/HAIB
1
0.892207
A549 CREB1 DEX 1
A549 CREB1 DEX 100nM v041610.2 ChIP-seq Raw Signal Rep 1 from ENCODE/HAIB
0.169944
0.388295
A549 CREB1 DEX 2
A549 CREB1 DEX 100nM v041610.2 ChIP-seq Raw Signal Rep 2 from ENCODE/HAIB
0.258863
b
Scale
chr14:
2 kb
hg19
62,215,000
62,220,000
UCSC Genes (RefSeq, GenBank, CCDS, Rfam, tRNAs & Comparative Genomics)
HIF1A
25
GM78 CREB1 V11 1
GM12878 CREB1 v042211.1 ChIP-seq Raw Signal Rep 1 from ENCODE/HAIB
1
22
GM78 CREB1 V11 2
GM12878 CREB1 v042211.1 ChIP-seq Raw Signal Rep 2 from ENCODE/HAIB
1
55
hESC CREB1 V11 1
H1-hESC CREB1 v042211.1 ChIP-seq Raw Signal Rep 1 from ENCODE/HAIB
1
31
hESC CREB1 V11 2
H1-hESC CREB1 v042211.1 ChIP-seq Raw Signal Rep 2 from ENCODE/HAIB
1
16
K562 CREB1 V11 1
K562 CREB1 v042211.1 ChIP-seq Raw Signal Rep 1 from ENCODE/HAIB
1
20
K562 CREB1 V11 2
K562 CREB1 v042211.1 ChIP-seq Raw Signal Rep 2 from ENCODE/HAIB
1
0.764749
A549 CREB1 DEX 1
A549 CREB1 DEX 100nM v041610.2 ChIP-seq Raw Signal Rep 1 from ENCODE/HAIB
0.169944
0.388295
A549 CREB1 DEX 2
A549 CREB1 DEX 100nM v041610.2 ChIP-seq Raw Signal Rep 2 from ENCODE/HAIB
0.258863

Supplementary Figure 6

Evidence for CREB1 regulation of GABPA and HIF1α.

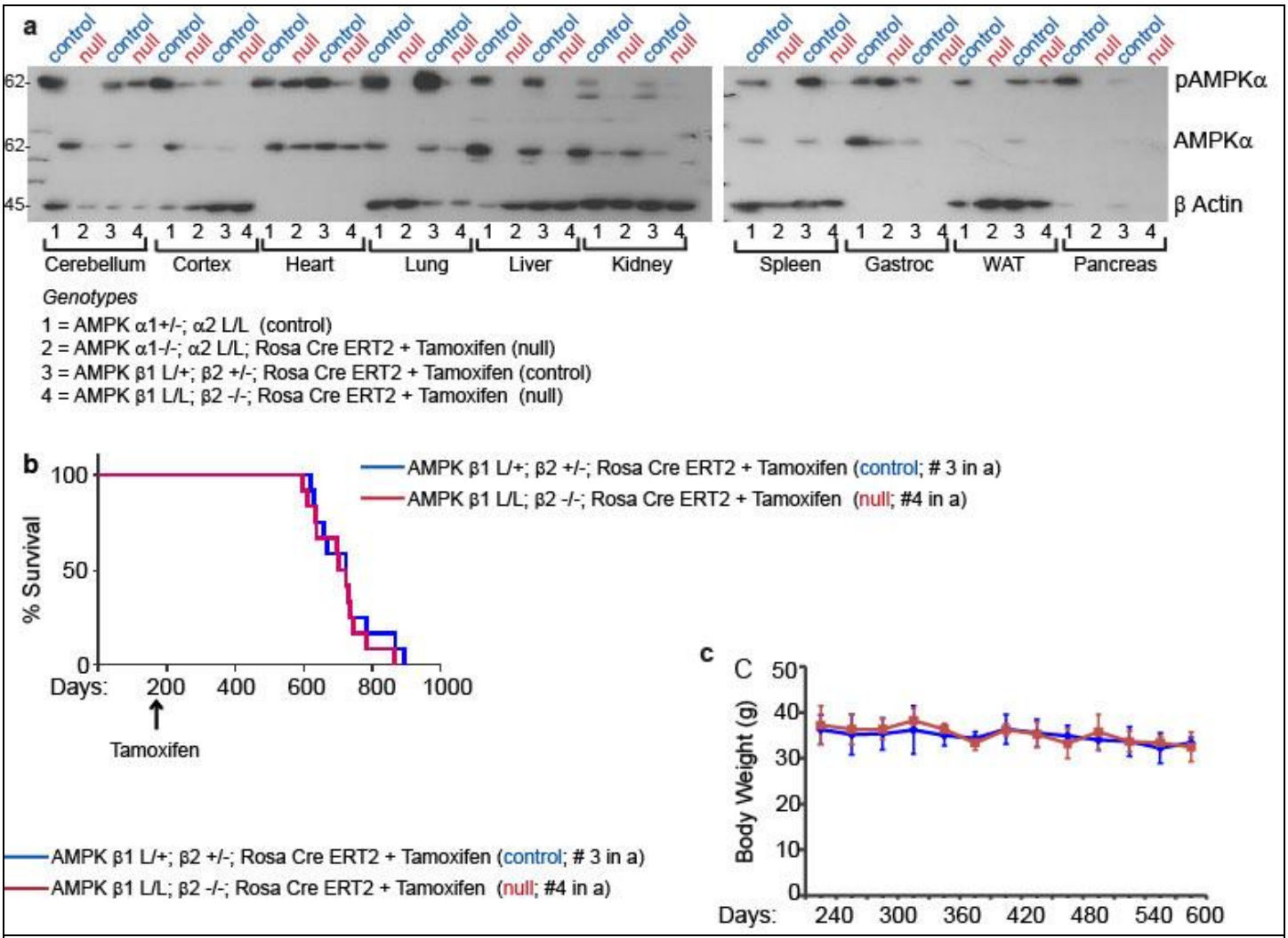
a, b, UCSC Genome Browser screenshot showing multiple ENCODE CREB1 ChIP-seq datasets in a variety of cell types. "UCSC Genes" track at the top shows the genomic location of GABPA (a) and HIF1α (b). Thick bars indicate UTRs and coding regions; thin bars indicate introns (arrows show direction of transcription). Colored tracks below indicate CREB1 ChIP-seq binding signals in various cell types. Higher peaks indicate genomic locations bound by CREB1 in the indicated cell type.


a
Tissue
human brain
human GBM
43-
pCREB
43-
CREB
b
Cell lines
43-
pCREB
43-
CREB
45-
βActin
NHA
GSC9
GSC10
83
326
c
CREB1
Relative normalized mRNA expression
NHA
326
AC17
e
CREB1
P = 0.002
Probability
Time (Months)
d
CREB1
EP300
HIF1α
mRNA expression
GBM
Brain
GABPA
TFAM
f
Control
991 (AMPK activator)
pCREB
DAPI
g
Control
991
pCREB intensity
h
AMPK
pCREB1
DAPI
Composite
i
Control
α2 Overexp
β1 Overexp
γ1 Overexp
62-
pAMPK
280-
pACC
43-
pCREB1
37-
AMPK β1
30-
AMPK β2
280-
ACC
45-
β Actin
j
Relative viability
NS
Control
β1 Overexp
k
NT shRNA
β1shRNA
NT shRNA
β1shRNA
43-
pCREB
280-
pACC
250-
150-
100-
75-
50-
37-
pPKA substrate
43-
CREB
280-
ACC
Forskolin - - + +

Supplementary Figure 7

Active CREB1 is highly expressed in GBM and is regulated by AMPK.

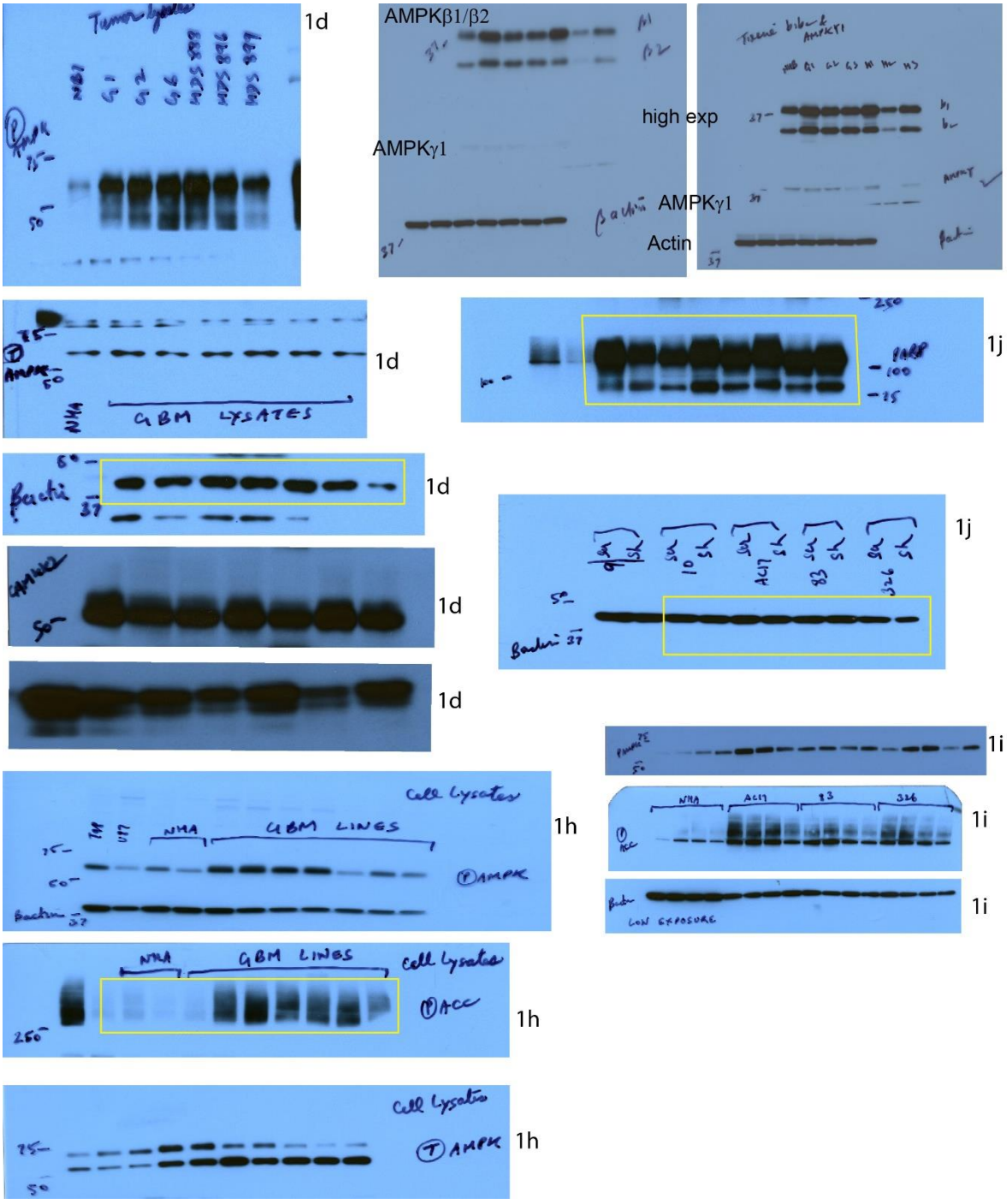
a,b, WB of CREB1 and pCREB1 in human GBM compared to normal brain tissue (a), and in primary GSC lines compared to normal human astrocytes (NHA) (b). **c,** Q-RTPCR analysis of CREB1 in NHA and GSC lines. (n = 3). *p ≤ 0.0005. **d,** High expression of CREB1, CREB1-binding protein EP300, HIF1α, GABPA and TFAM in GBM relative to normal human brain tissue (Source: TCGA). * p ≤ 0.001;** ≤ 0.005; $^{++}$ = 0.01. The edges on the boxplots indicate the first and 3rd quartile (25th- and 75th percentile) of the data, with the line in the middle being the median. The whiskers on the boxplots extend another 1.5x of the inter-quartile range (between 25%-75% range of data) from the edges of the boxes, respectively. **e,** Kaplan-Meier survival plots of LGG patients expressing CREB1 (Source: TCGA). **f**, Immunocytochemistry using pCREB1 antibody in NHA treated with the AMPK activating small molecule 991 for 30 minutes. Nuclei were detected with DAPI. Scale bar 50 μm**. g,** Quantification of nuclear pCREB1 signal intensity in vehicle or 991-treated NHA. *p < 0.0001. (n=36 cells/condition). **h,** IHC of pAMPK and pCREB in GSC326 tumors. Nuclear staining is shown using DAPI. Scale bar 50 μm**.** Note: Mainly nuclear pCREB but both nuclear and cytoplasmic pAMPK; nuclear pAMPK co-localizes with pCREB (inset). **i,** WB of pAMPK and pACC and pCREB1 in AMPK α2, β1, γ1 overexpressing NHA. Note: Stabilization of β1 and β2 is increased in cells overexpressing α2 and γ1. **j,** Viability of NHA overexpressing AMPK β1 subunit. (n = 3). NS = nonsignificant. **k,** WB of pCREB1, pACC, pPKA substrates, CREB1 and ACC in NT and AMPKβ1 shRNA expressing GSCs treated with the PKA activator forskolin. Error bars; mean +/- S.D. Statistical significance in above experiments was assessed using Student's two-tailed t-test, except (d) where Welch's t test was used. n values present independent experiments unless stated otherwise. Source data are available in Supplementary Table 4. All western blots represent data from 2 (i, k) or 3 (a, b) independent repeats. Scanned images of unprocessed blots are shown in Supplementary Fig. 9.



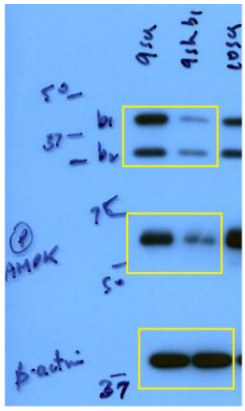
Supplementary Figure 8

Adult mice with global AMPK deletion has normal lifespan.

a, WB showing efficiency of Cre-recombination in the indicated tissues. Expression of total and active AMPK in control and AMPK-deleted tissues in mice are shown. β actin was used as loading control. Note that AMPK α subunits are unstable in the absence of β subunits. **b**, Kaplan-Meier survival curve of control and AMPK β subunit-deleted mice. (n = 12 mice/genotype). **c**, Body weight of control and AMPK β subunit-deleted mice are shown. Note: AMPK α subunit deleted mice (genotype #2) also had normal lifespan and bodyweight. (n = 12 mice/genotype). Error bars; mean +/- S.D. Statistical significance in above experiments was assessed using two-tailed t-test.



Supplementary Figure 9 Unprocessed scans of all western blot data.



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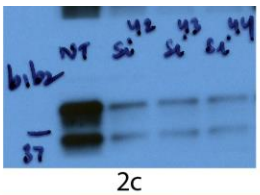

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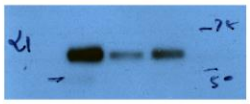

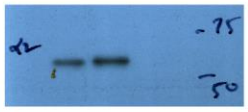

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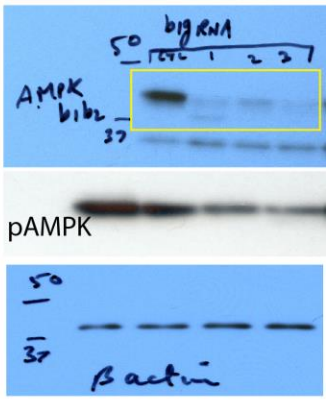

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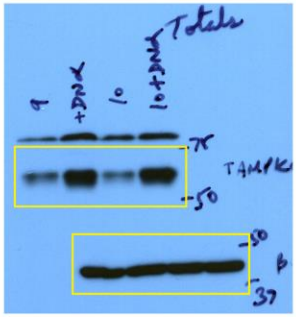

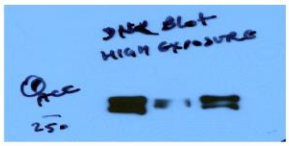

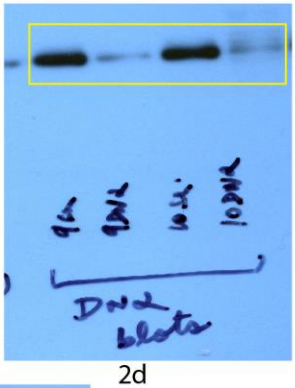

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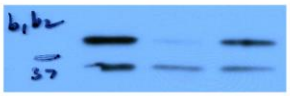

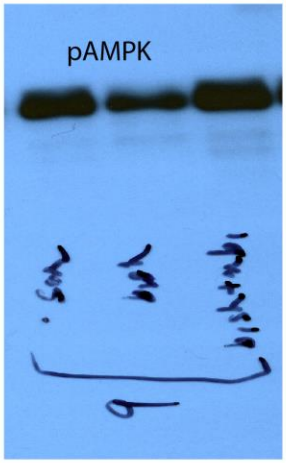

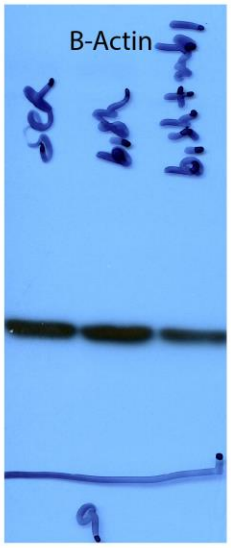

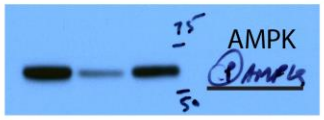

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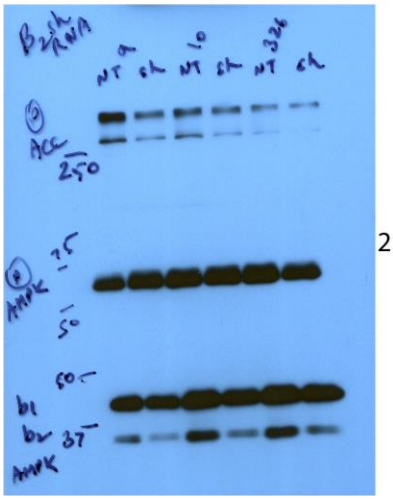

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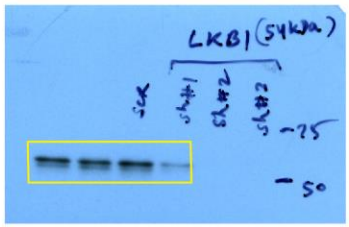

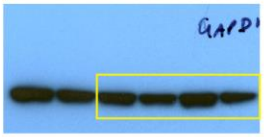

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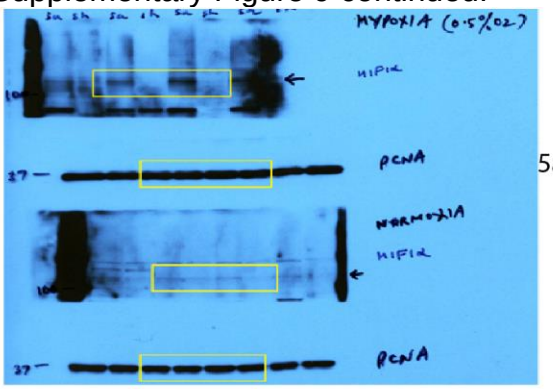

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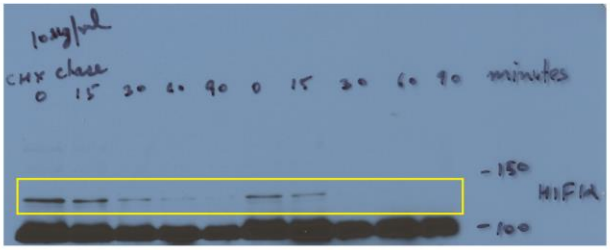

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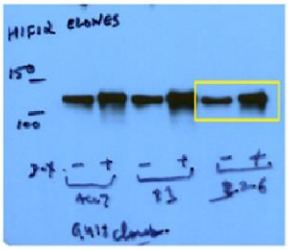

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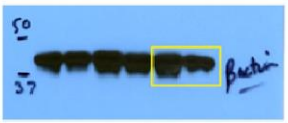

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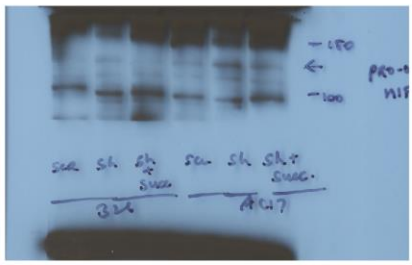

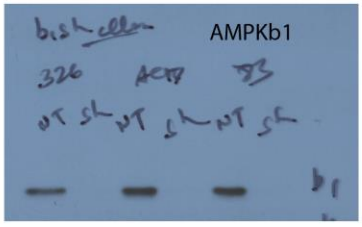

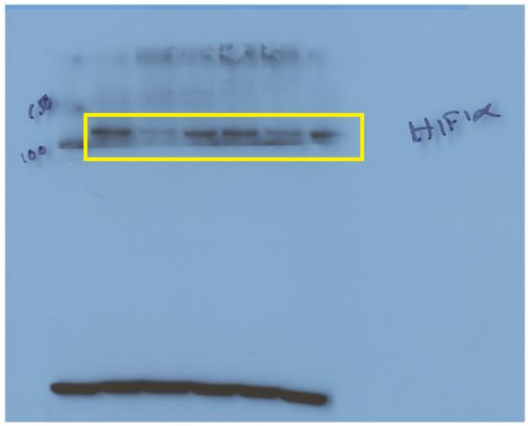

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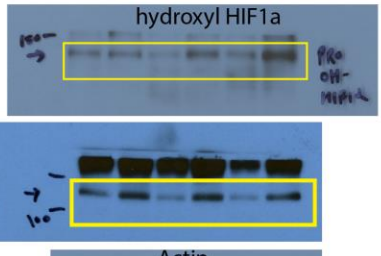

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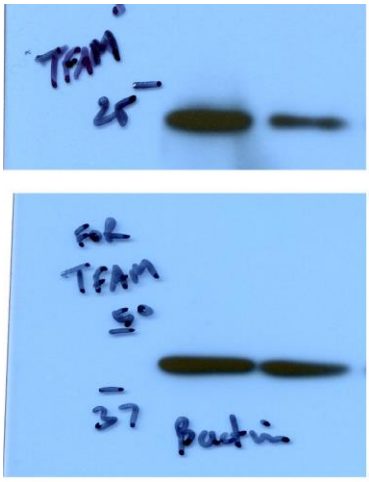

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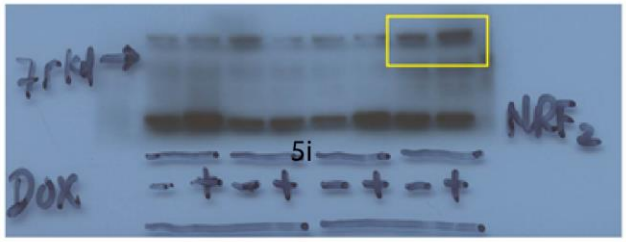

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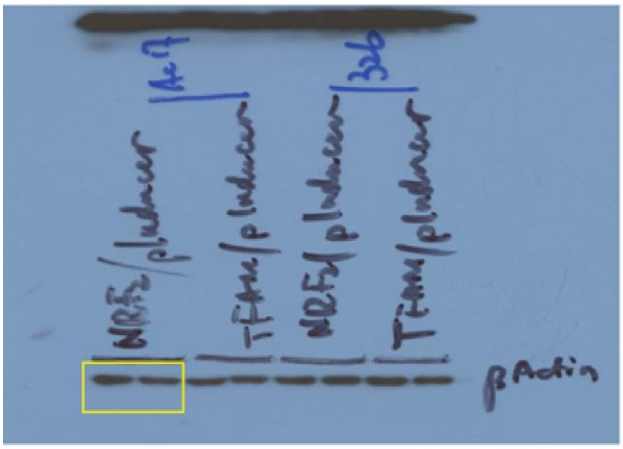

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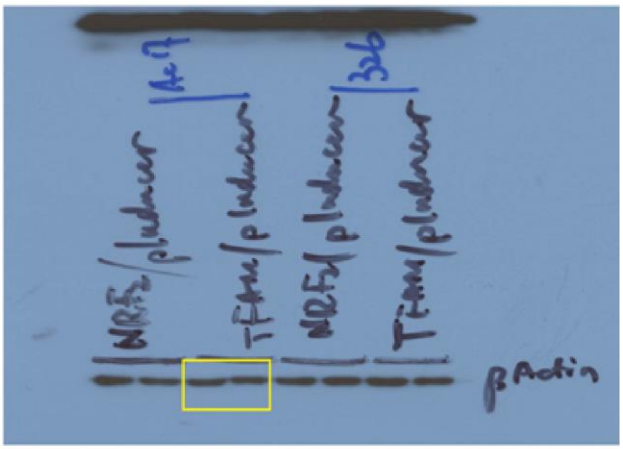

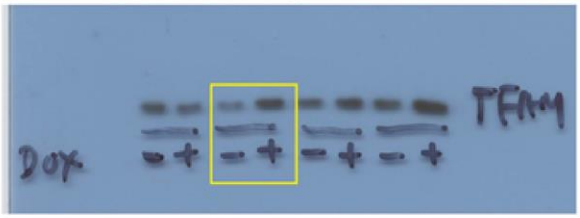

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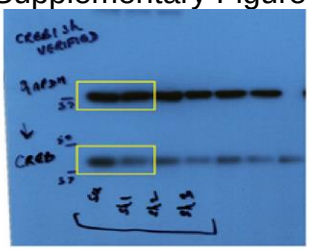

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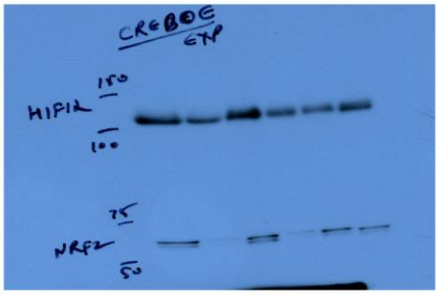

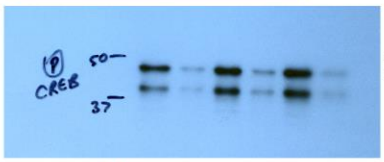

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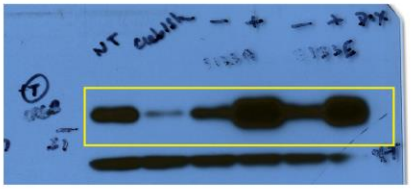

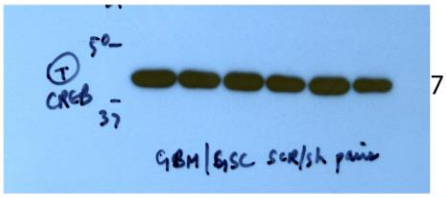

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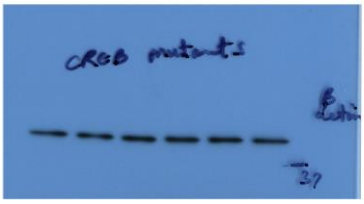

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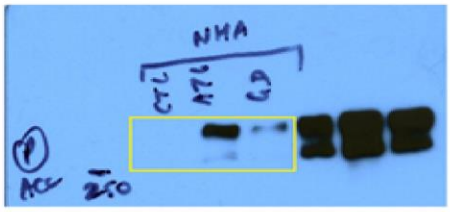

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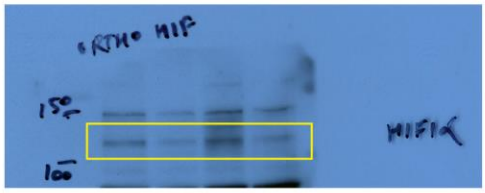

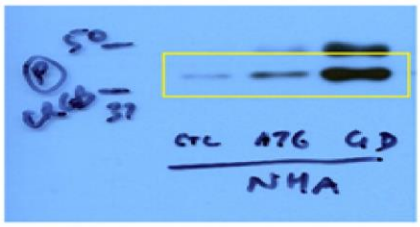

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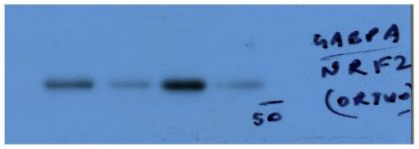

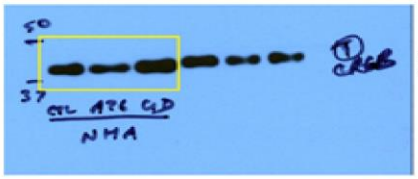

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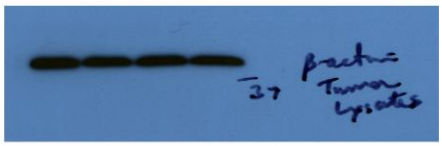

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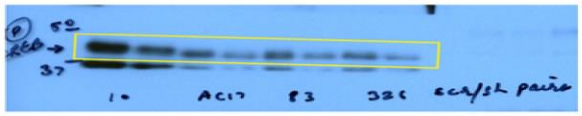

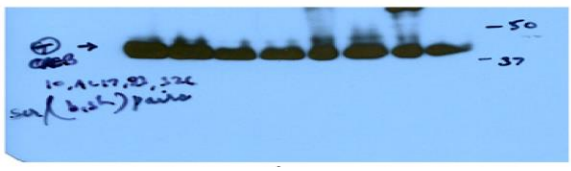

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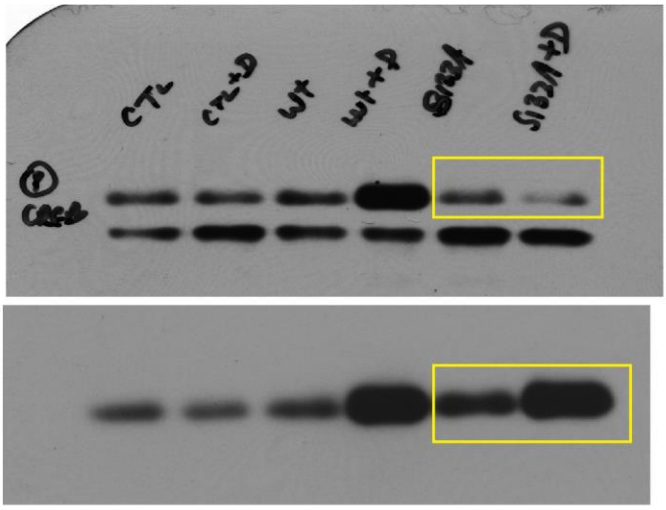

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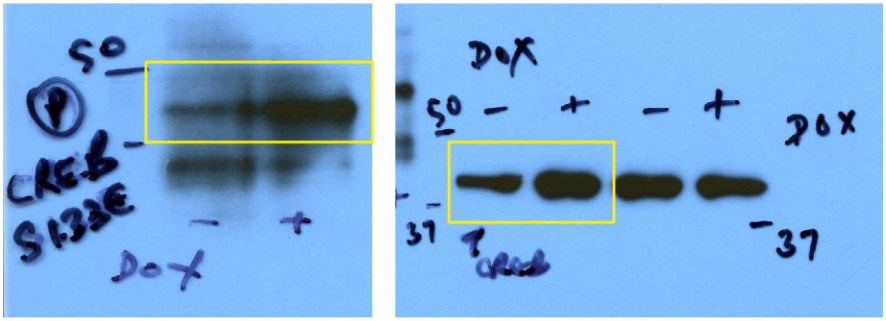

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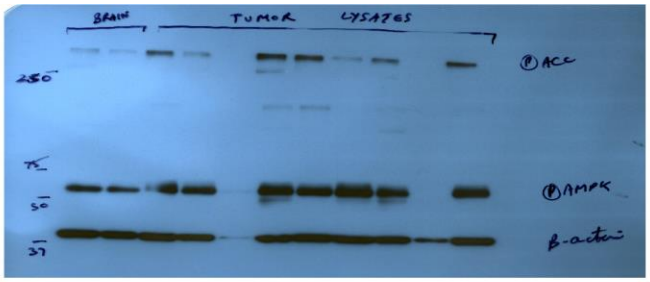
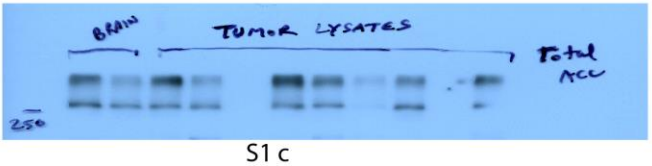
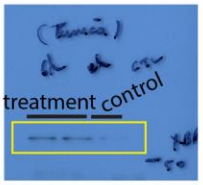
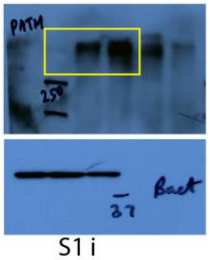




S1 c

S1 f





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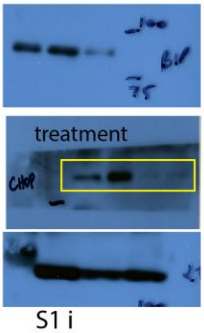
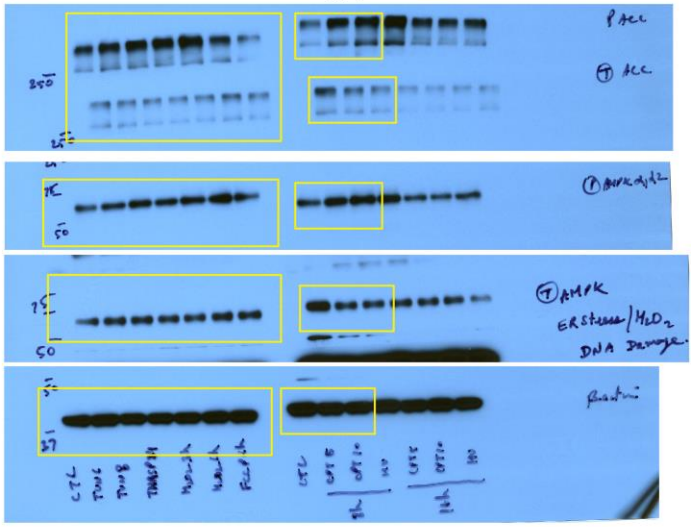
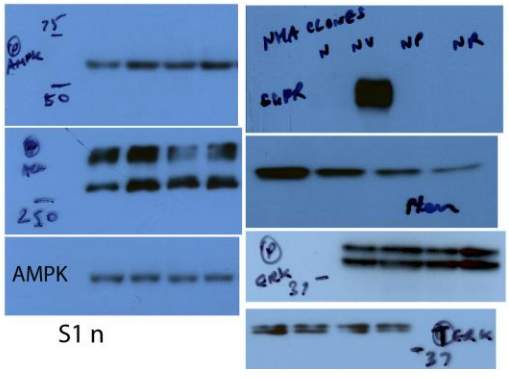


S1 i



S1 k



S1 n

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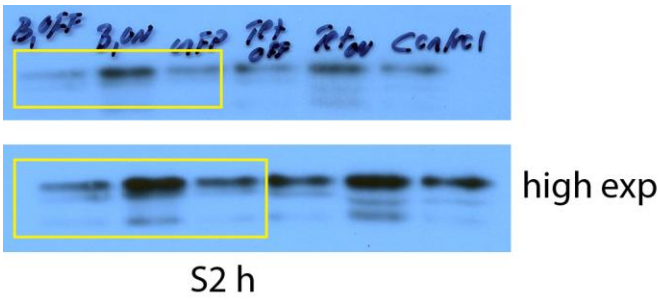
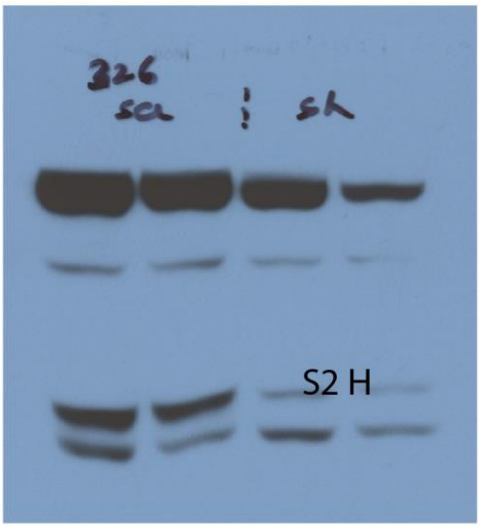


S2 h



S2 k

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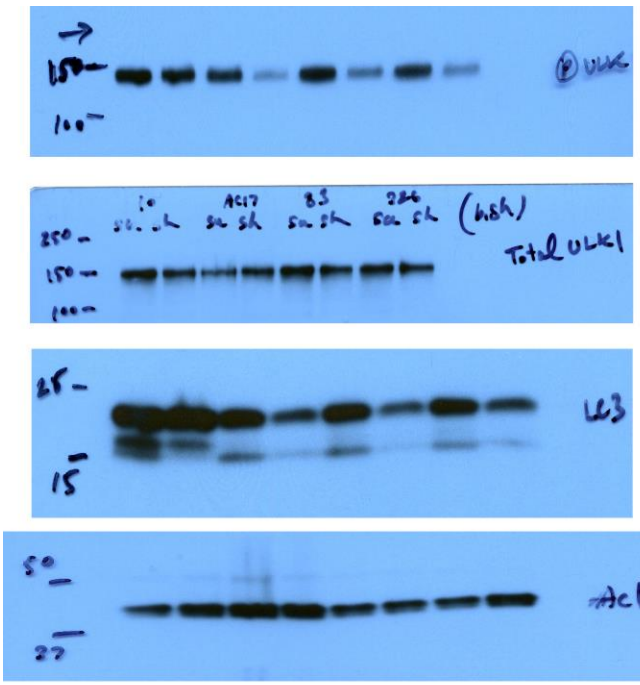
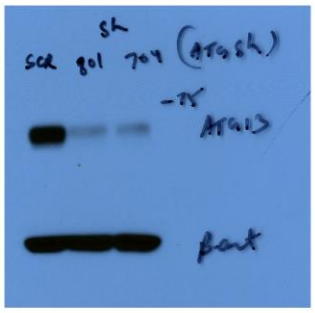
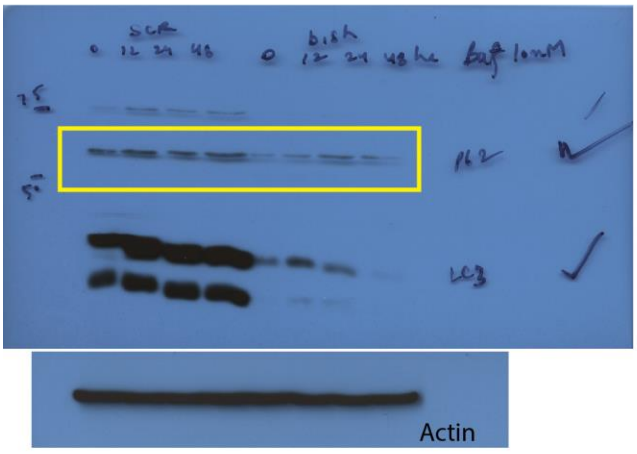


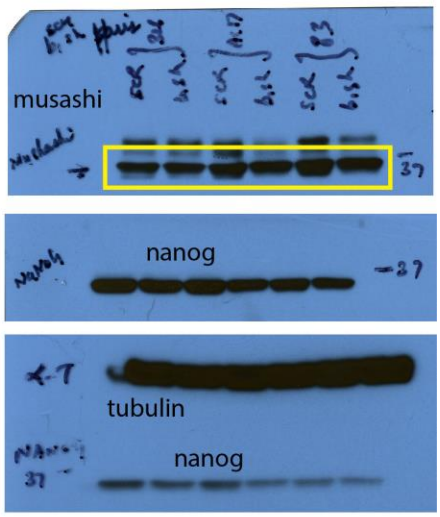
S3 a



S3 c

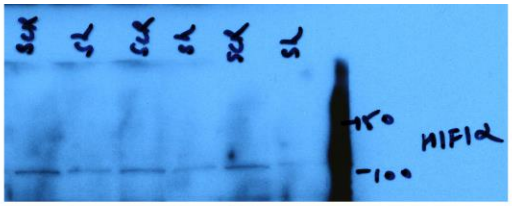


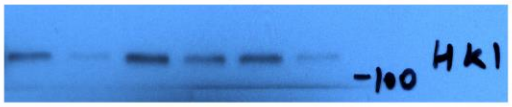
S3 b

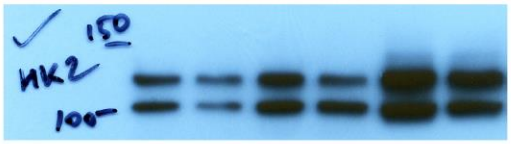


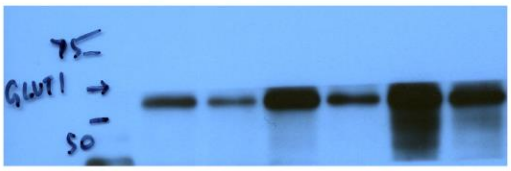
S3 h

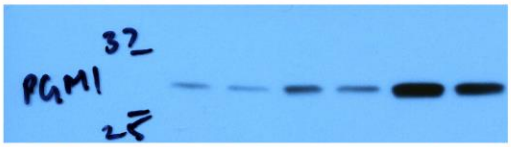
Supplementary Figure 9 continued.

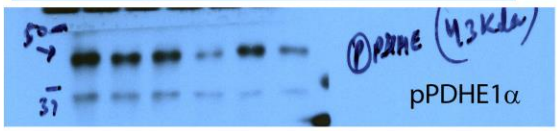


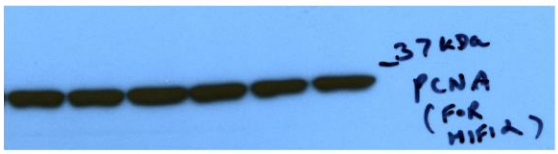


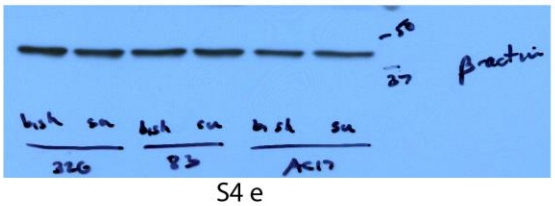




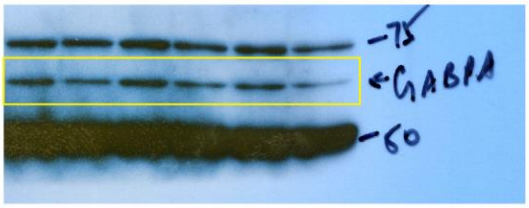


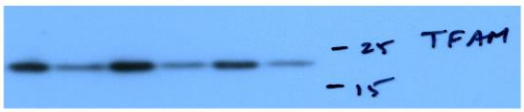


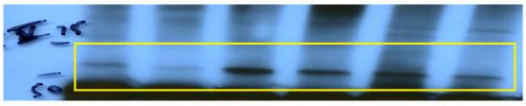


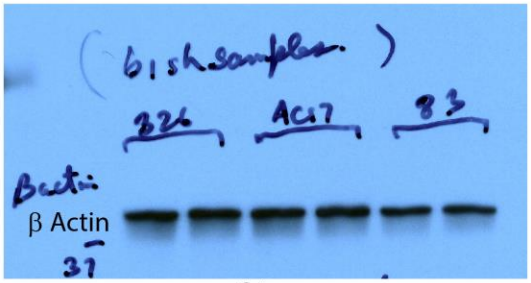


S4 e









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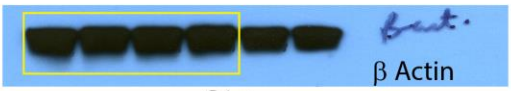
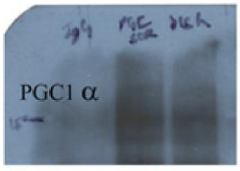
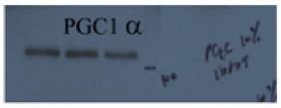
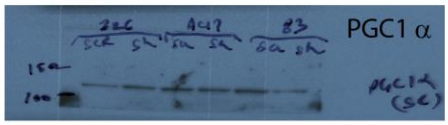


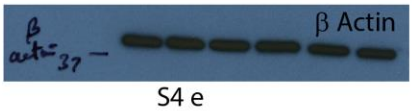
S4 g





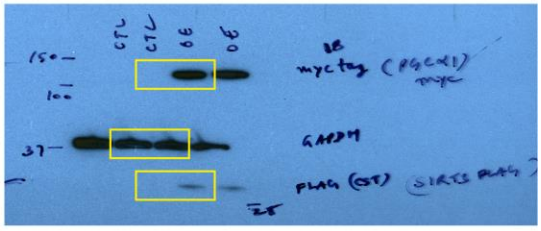
S4 i





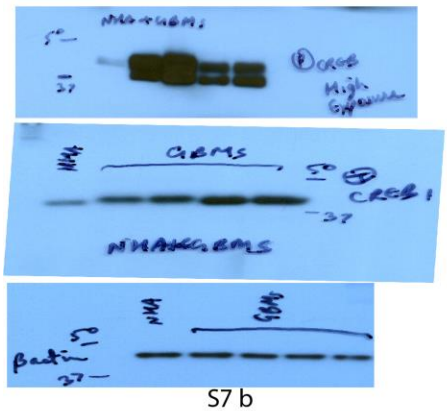
S4 e

S4 j



Supplementary Figure 9 continued.

S7 a



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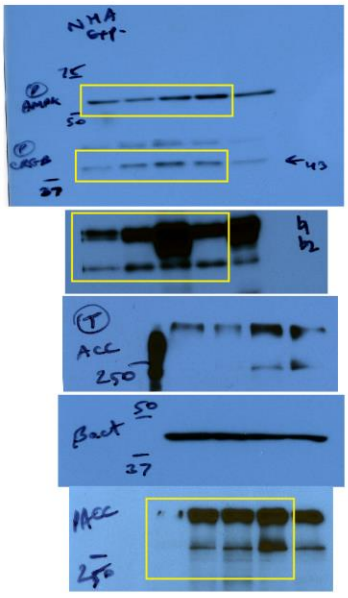

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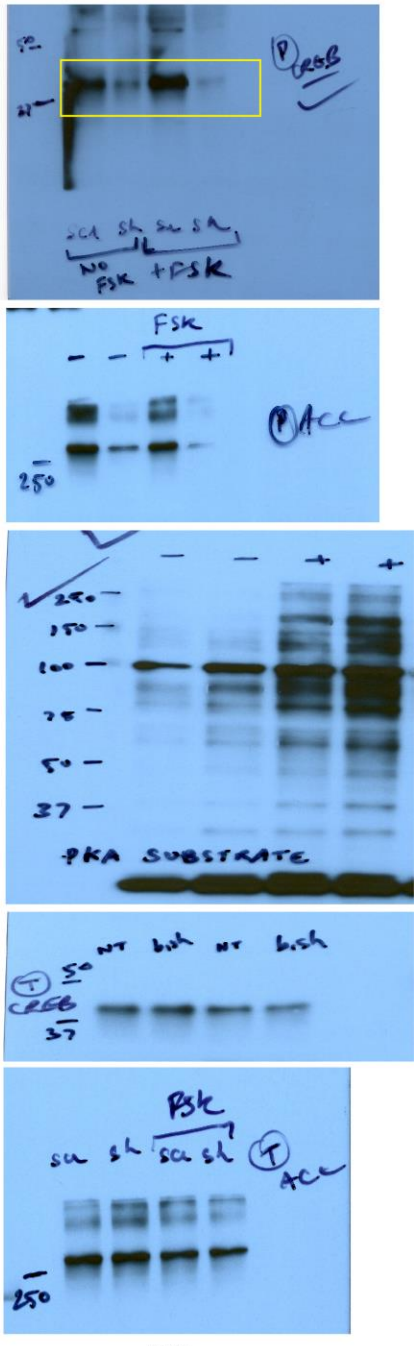
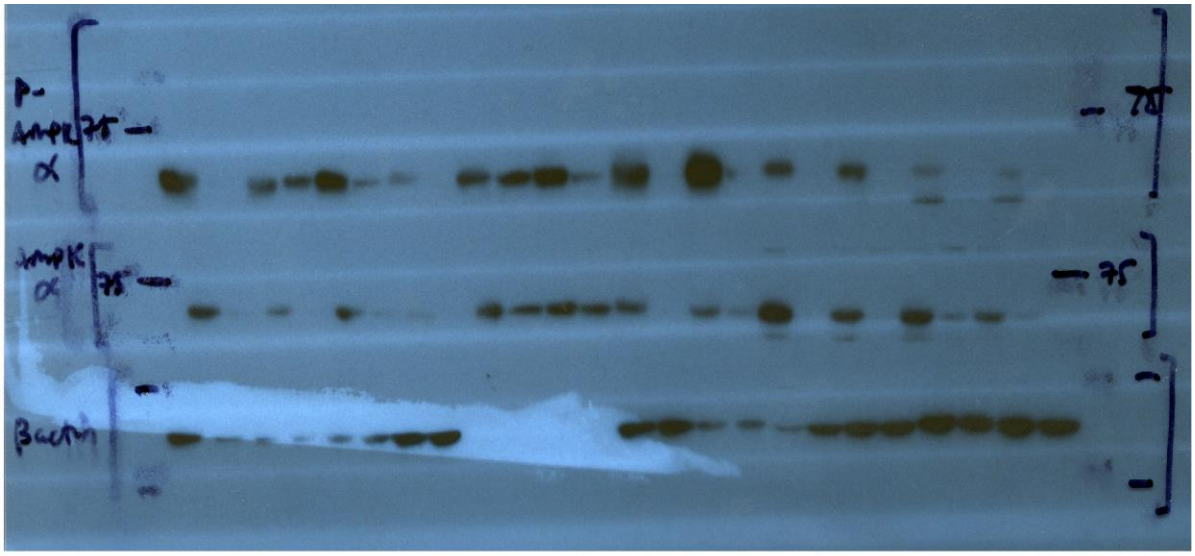
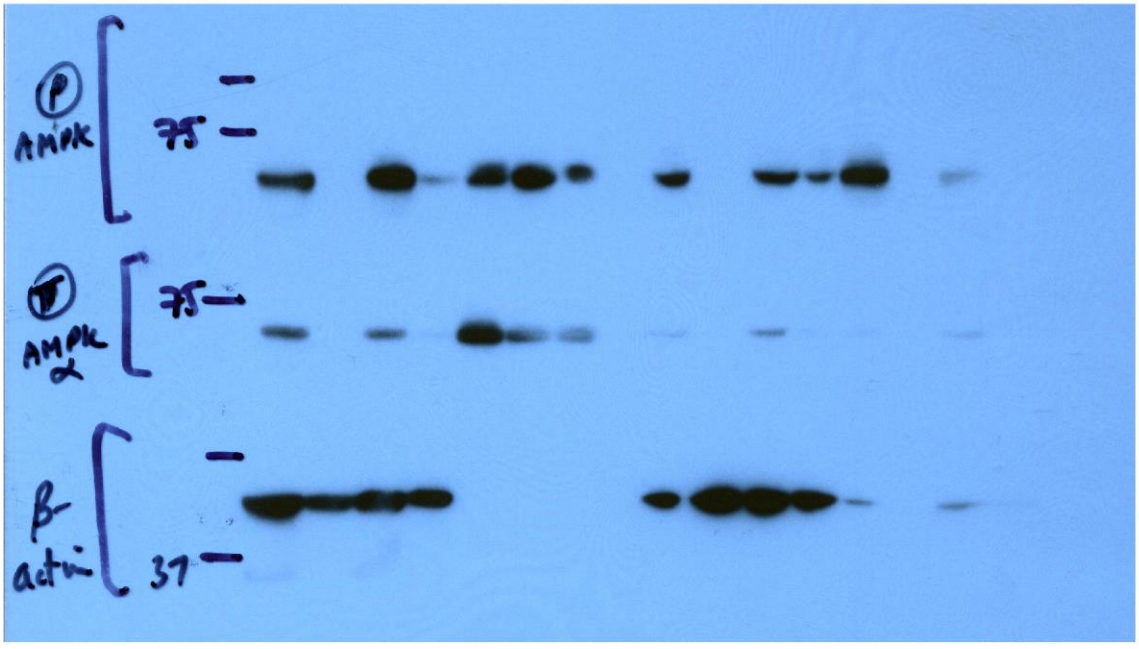


S7 k

Supplementary Figure 9 continued.





S8 a

Supplementary Figure 9 continued.

SUPPLEMENTARY VIDEO 1

Normal proliferation of GSC10 line in the presence of nontarget shRNA. GSC10 cells were infected with nontarget (NT) shRNA and after 3 days of infection, cultured cells were visualized with Zeiss microscope fitted with Hamamatsu digital camera placed on Kinetic Systems Vibraplane platform (with controlled temperature chamber). 12 frames/hr were captured for 10 hours and stitched together by using ImageJ and Micro-Manager 1.4.12 software.

SUPPLEMENTARY VIDEO 2

Apoptosis of GSC10 line in the presence of AMPKβ1shRNA. GSC10 cells were infected with AMPK β1 shRNA and after 3 days of infection, cultured cells were visualized with Zeiss microscope fitted with Hamamatsu digital camera placed on Kinetic Systems Vibraplane platform (with controlled temperature chamber). 12 frames/hr were captured for 10 hours and stitched together by using ImageJ and Micro-Manager 1.4.12 software.

SUPPLEMENTARY TABLE LEGEND

Table1. Antibody name, vendor, catalog number and dilution information.

Table2. Silencing RNA ID and sequence information.

Table3. Primer sequence information.

Table4. Supplementary Source data.