Figure S1

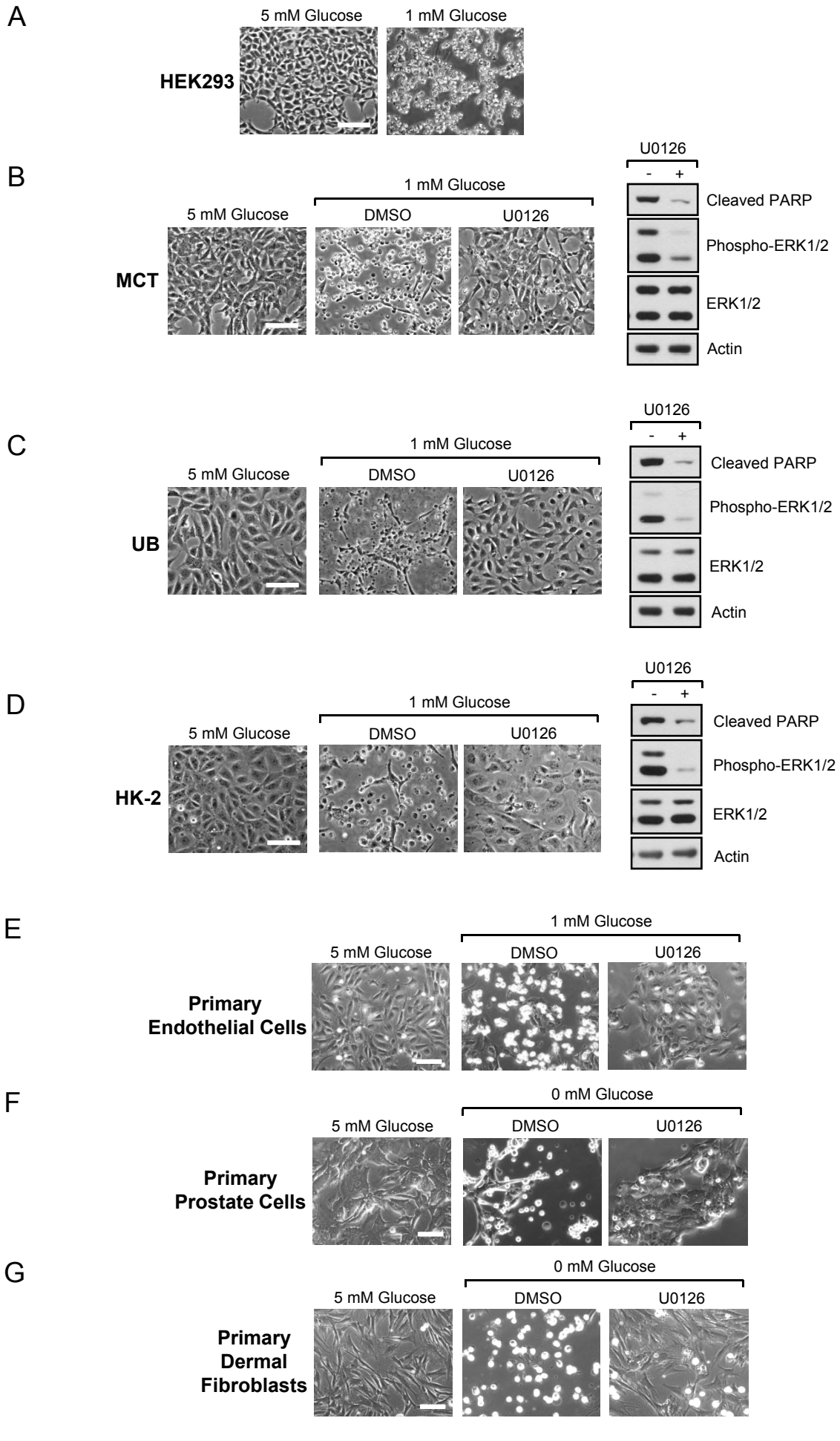

A
5 mM Glucose
1 mM Glucose
HEK293
B
U0126
- +
1 mM Glucose
5 mM Glucose
DMSO
U0126
MCT
Cleaved PARP
Phospho-ERK1/2
ERK1/2
Actin
C
U0126
- +
1 mM Glucose
5 mM Glucose
DMSO
U0126
UB
Cleaved PARP
Phospho-ERK1/2
ERK1/2
Actin
D
U0126
- +
1 mM Glucose
5 mM Glucose
DMSO
U0126
HK-2
Cleaved PARP
Phospho-ERK1/2
ERK1/2
Actin
E
1 mM Glucose
5 mM Glucose
DMSO
U0126
Primary Endothelial Cells
F
0 mM Glucose
5 mM Glucose
DMSO
U0126
Primary Prostate Cells
G
0 mM Glucose
5 mM Glucose
DMSO
U0126
Primary Dermal Fibroblasts

Fig. S1, related to Fig. 1. ERK regulates low glucose-induced apoptosis. (A) HEK293 cells were incubated in the presence of 5 mM or 1 mM glucose for 54 h, and cell images were taken. Scale bar, 50 μm. **(B-G)** MCT (B), UB (C), HK-2 (D), primary endothelial cells (E), primary prostate cells (F), or primary fibroblast cells (G) were incubated in the presence of 5 mM, 1 mM, or 0 mM glucose with/without 10 μM U0126. Cell images were taken or immunoblot analysis was performed. Scale bar, A-D: 50 μm; E-G: 100 μm.

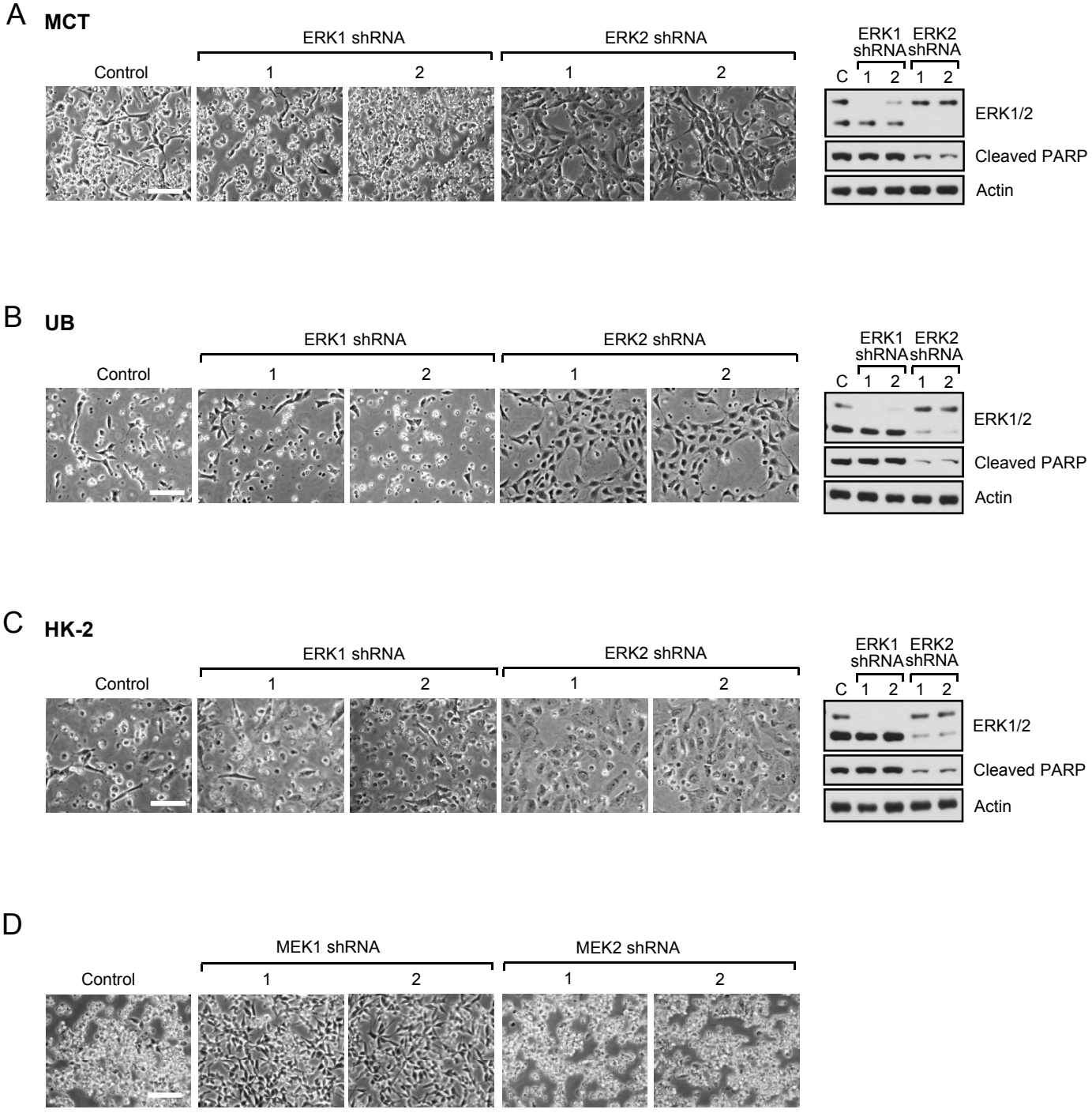
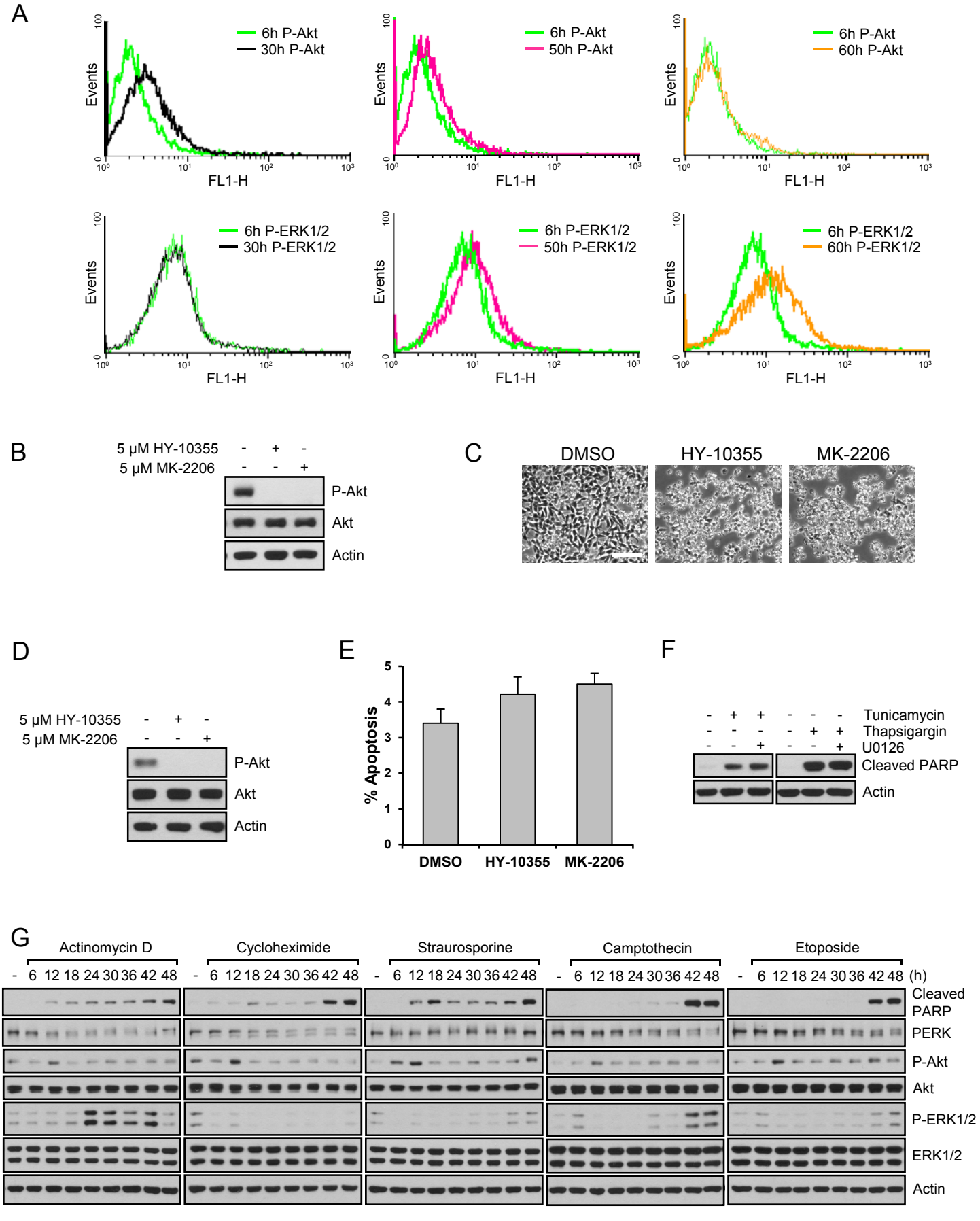


Fig. S2, related to Fig. 2. ERK2 regulates cell apoptosis induced by low glucose. (A-C) Cell images were taken or immunoblot analysis was performed using ERK1 or ERK2 knockdown MCT (A), UB (B), or HK-2 (C) cells grown in the presence of 1 mM glucose. Scale bar, 50 μm. **(D)** Stable HEK293 cells were generated using MEK1 or MEK2 shRNAs. Cells were grown in the presence of 1 mM glucose for 54 h and cell images were taken. Scale bar, 50 μm.

Figure S3


A
6h P-Akt
30h P-Akt
50h P-Akt
60h P-Akt
6h P-ERK1/2
30h P-ERK1/2
50h P-ERK1/2
60h P-ERK1/2
Events
FL1-H
B
5 μM HY-10355
5 μM MK-2206
P-Akt
Akt
Actin
C
DMSO
HY-10355
MK-2206
D
5 μM HY-10355
5 μM MK-2206
P-Akt
Akt
Actin
E
% Apoptosis
DMSO
HY-10355
MK-2206
F
Tunicamycin
Thapsigargin
U0126
Cleaved PARP
Actin
G
Actinomycin D
Cycloheximide
Straurosporine
Camptothecin
Etoposide
6 12 18 24 30 36 42 48 (h)
Cleaved PARP
PERK
P-Akt
Akt
P-ERK1/2
ERK1/2
Actin

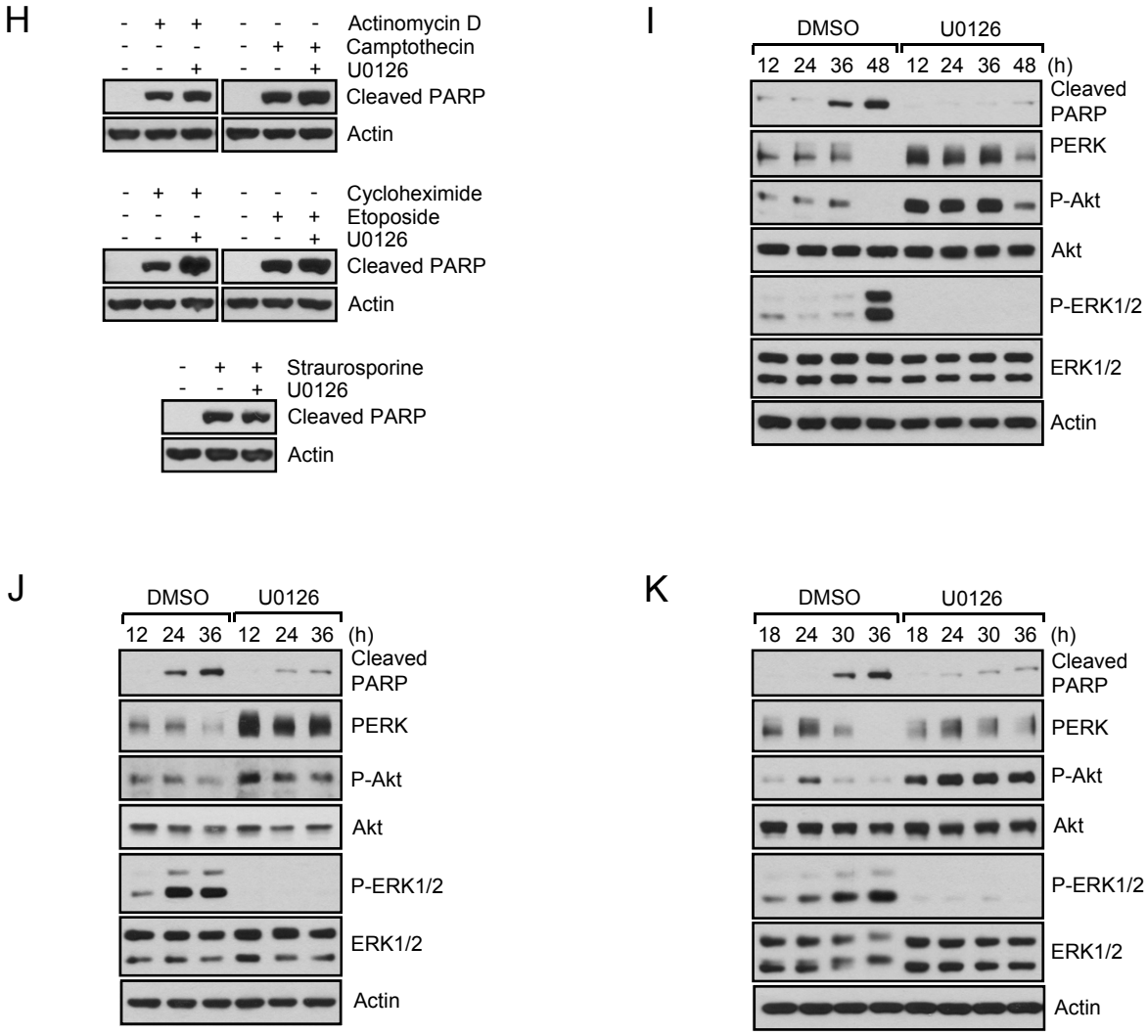


Fig. S3, related to Fig. 3. Akt is involved in cell survival under low glucose condition. (A) HEK293 cells were grown in the presence of 1 mM glucose for the indicated time, and flow cytometry was performed to measure phosphorylated forms of Akt and ERK. **(B-C)** HEK293 cells growing in the presence of 1 mM glucose for 24 h were treated with 5 μM MK-2206 or 5 μM HY-10355 for additional 20 h. Immunoblot analysis was performed (B) or cell images were taken (C). Scale bar, 50 μm. **(D-E)** HEK293 cells growing in the presence of 5 mM glucose were treated with 5 μM MK-2206 or 5 μM HY-10355 for 20 h. Immunoblot analysis was performed (D) or cell apoptosis was measured (E). **(F)** HEK293 cells treated with tunicamycin or thapsigargin with/without U0126 were incubated in the complete media. Cells were lysed and immunoblot analysis was performed. **(G)** HEK293 cells treated with actinomycin D, cycloheximide, staurosporine, camptothecin, or etoposide were incubated in the complete media. Cells were lysed at the indicated time points and immunoblot analysis was performed. **(H)** HEK293 cells treated with actinomycin D, cycloheximide, staurosporine, camptothecin, or etoposide with/without U0126 were incubated in the complete media and immunoblot analysis was performed. **(I-K)** Primary endothelial cells (I), primary prostate cells (J), or primary fibroblast cells (K) were incubated in the presence of 1 mM glucose (endothelial cells) or 0 mM glucose (prostate cells and fibroblasts) with/without 10 μM U0126. Cells were lysed at the indicated time points and immunoblot analysis was performed.

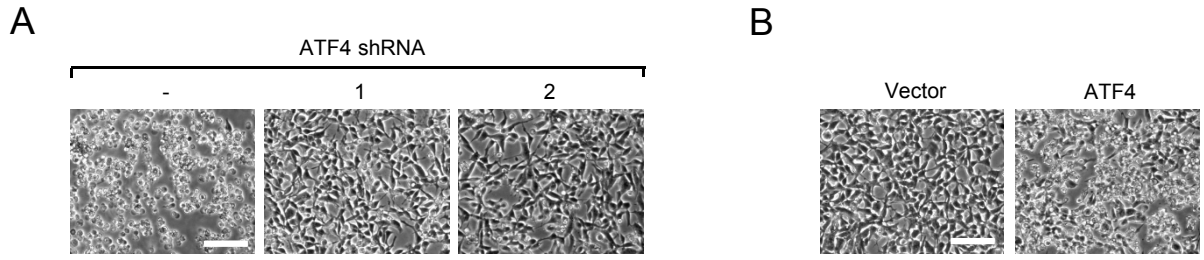


Fig. S4, related to Fig. 4. ATF4 regulates cell death induced by low glucose. (A) Control or ATF4 knockdown cells were grown in the presence of 1 mM glucose for 54 h and cell images were taken. Scale bar, 50 μm. **(B)** Control or ATF4 overexpressing cells were grown in the presence of 1 mM glucose for 30 h and cell images were taken. Scale bar, 50 μm.

Figure S5

Name	Function
ABL1	Pro-apoptotic factor
AIFM1	Caspase activator
AIFM2	Apoptosis induction
BAD	Apoptosis induction
BAK1	Apoptosis induction
BAX	Caspase activator
BBC3	Caspase activator
BCL2	Anti-apoptosis
BCL2L1	Anti-apoptosis
BCL2L11	Apoptosis induction
BCLAF1	Apoptosis induction
BID	Apoptosis induction
BIK	Apoptosis induction
BNIP3	Apoptosis induction
BNIP3L	Apoptosis induction
CD40	Apoptosis induction
CD70	Apoptosis induction
CDKN1A	Apoptosis induction - intracellular signal
CFLAR	Apoptosis induction - extracellular signal
CIDEA	Apoptosis induction - DNA damage
CIDEB	Apoptosis induction - DNA damage
CRADD	Caspase
CUL1	Apoptosis induction - intracellular signal
CUL2	Apoptosis induction - intracellular signal
CUL3	Apoptosis induction - intracellular signal
DAPK1	Apoptosis induction - extracellular signal
DDIT3	Apoptosis induction - stress
FADD	Apoptosis induction - death domain receptor
FAS	Apoptosis induction - TNFR domain
FOS	Pro-survival or pro-apoptotic factor
FOSL1	Pro-survival or pro-apoptotic factor
GPX1	Apoptosis induction - oxidative stress
HRK	Apoptosis induction
HSPA5	Anti-apoptosis - stress
LTA	Apoptosis induction
LTBR	Apoptosis induction
MCL1	Anti-apoptosis - Bcl2 domain
MYC	Capase activator
PRODH	Apoptosis induction - oxidative stress
RNF7	Apoptosis induction - oxidative stress
TNF	Pro-apoptotic or anti-apoptotic factor
TNFRSF10A	Apoptosis induction - death domain
TNFRSF10B	Apoptosis induction - death domain
TNFRSF11B	Apoptosis induction - death domain
TNFRSF21	Death domain protein
TNFRSF25	Apoptosis induction - extracellular signal
TNFRSF9	Apoptosis induction - TNFR domain
TNFSF15	Caspase activator - TNF domain
TNFSF8	Apoptosis induction - TNF domain
TP53	Apoptosis induction
TP73	Pro-apototic or anti-apoptotic
TRAF2	Apoptosis induction
TRAF3	Apoptosis induction
TRIB3	Anti-survival
YBX1	Anti-apoptosis

Fig. S5, related to Fig. 5. Summary of pro-apoptotic or anti-apoptotic genes assessed via qPCR.

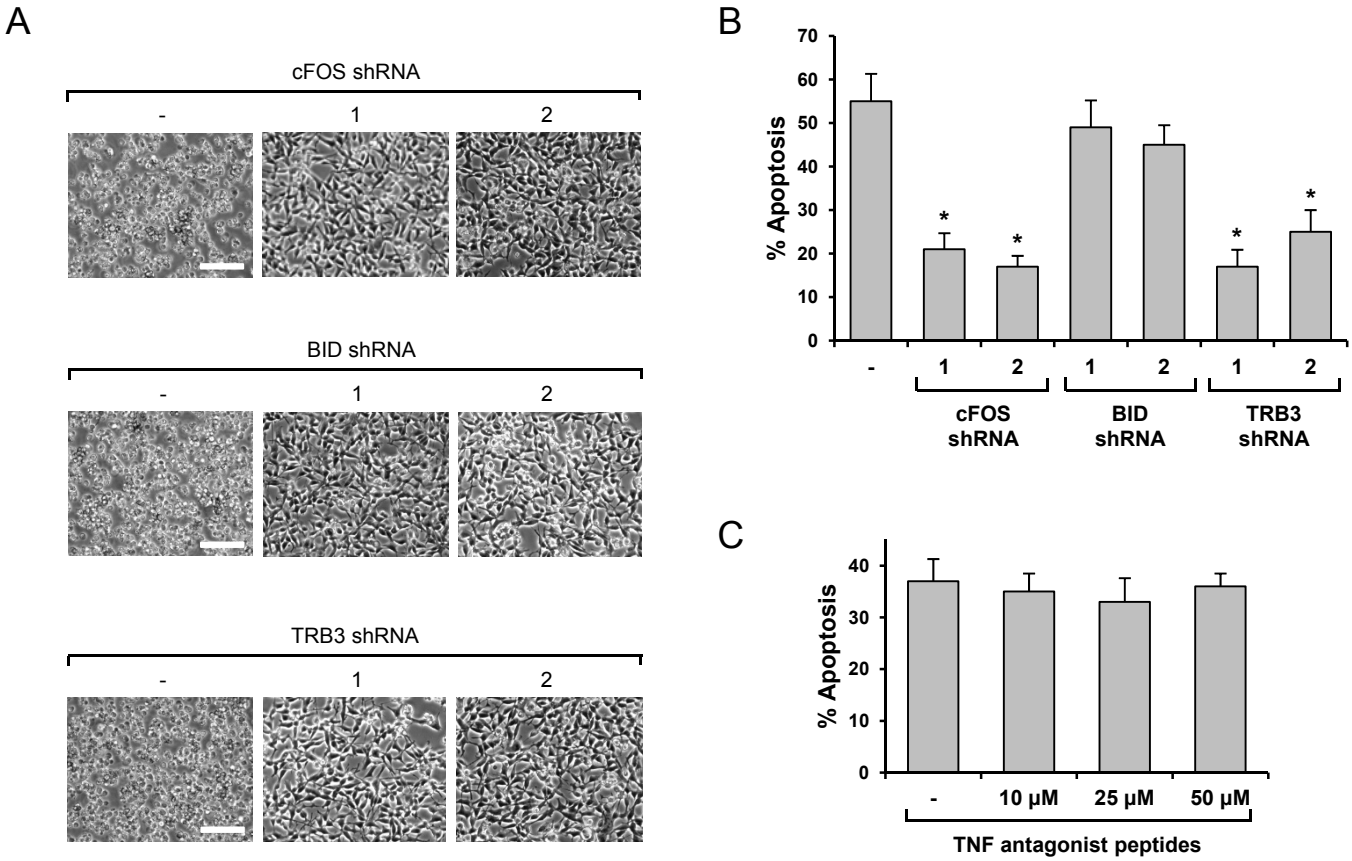
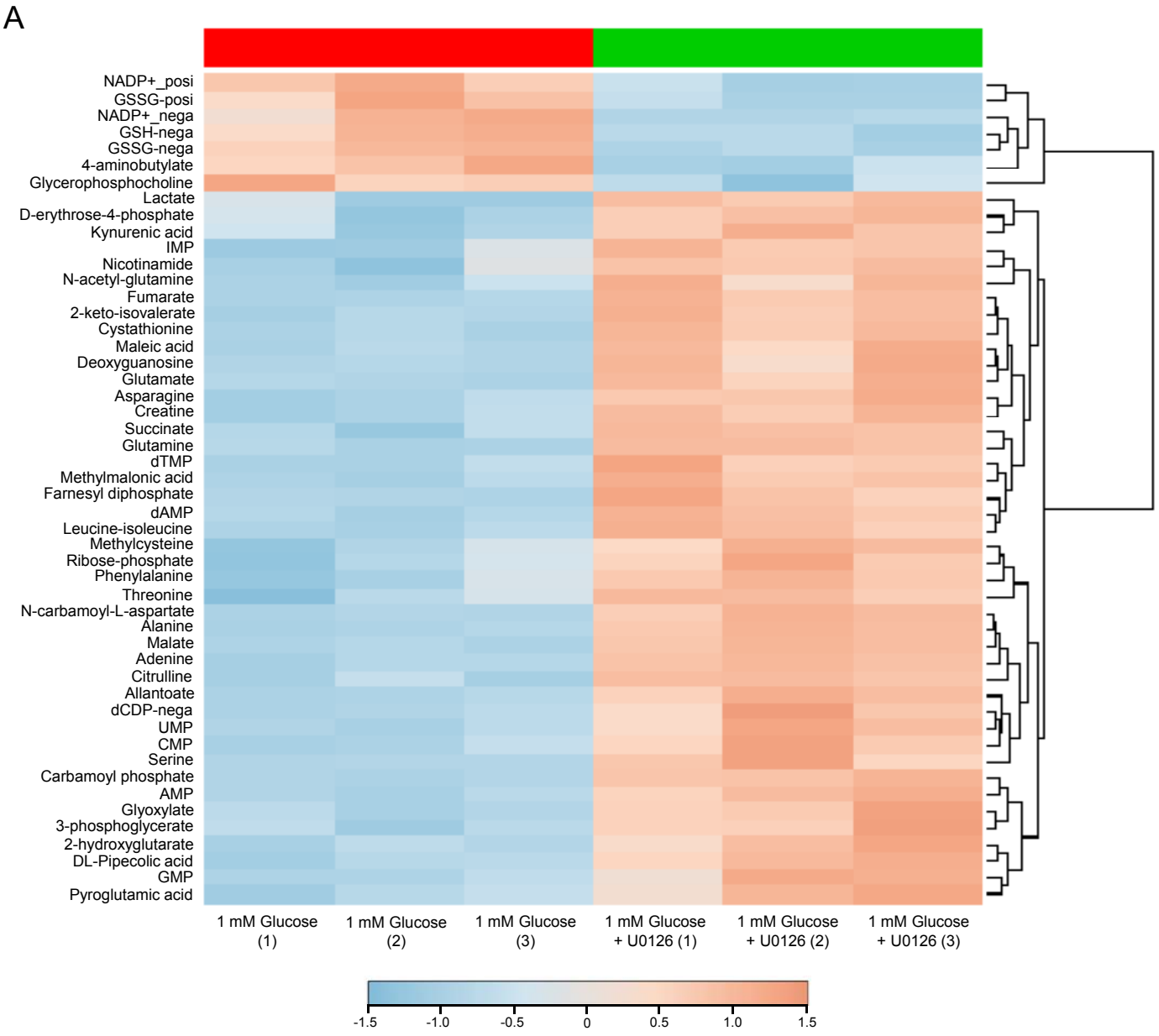


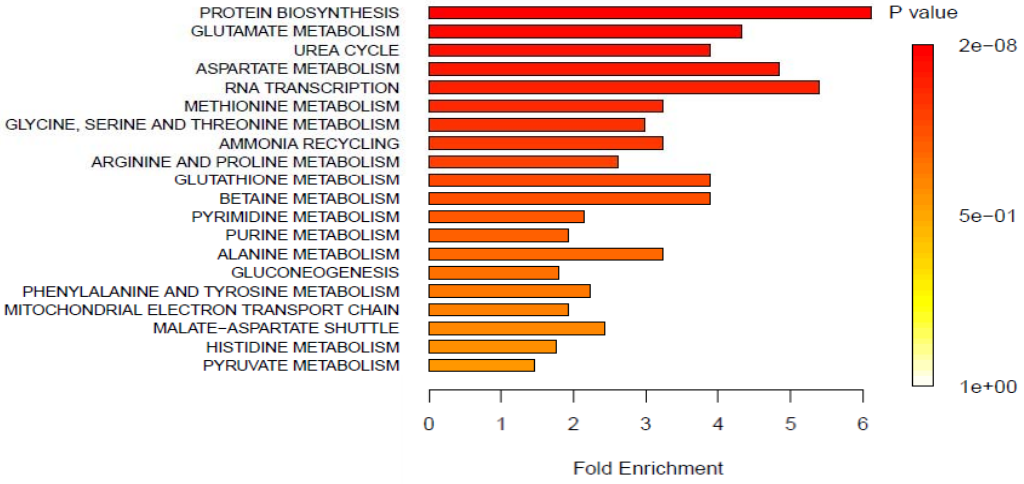
Fig. S6, related to Fig. 6. c-Fos, Bid, and Trb3 are involved in cell death induced by low glucose. (A) Stably knocked down HEK293 cells with c-Fos, Bid, or Trb3 shRNAs were grown in the presence of 1 mM glucose for 54 h and cell images were taken. Scale bar, 50 μm. **(B)** Stably knocked down HEK293 cells with c-Fos, Bid, or Trb3 shRNAs were grown in the presence of 1 mM glucose for 66 h and cell apoptosis analysis was performed. Results were statistically significant (*, $p < 0.01$) as assessed by using the Student's t test. **(C)** HEK293 cells grown in the presence of 1 mM glucose for 42 h were treated with TNFα antagonist, WP9QY, for additional 12 h and cell apoptosis rate was measured.

Figure S7

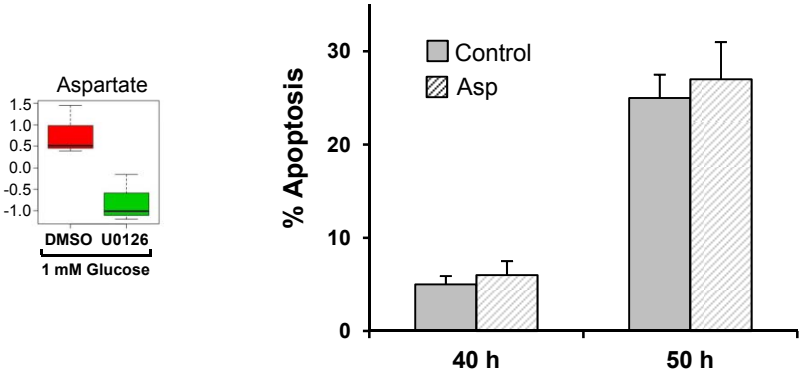
A


NADP+_posi
GSSG-posi
NADP+_nega
GSH-nega
GSSG-nega
4-aminobutylate
Glycerophosphocholine
Lactate
D-erythrose-4-phosphate
Kynurenic acid
IMP
Nicotinamide
N-acetyl-glutamine
Fumarate
2-keto-isovalerate
Cystathionine
Maleic acid
Deoxyguanosine
Glutamate
Asparagine
Creatine
Succinate
Glutamine
dTMP
Methylmalonic acid
Farnesyl diphosphate
dAMP
Leucine-isoleucine
Methylcysteine
Ribose-phosphate
Phenylalanine
Threonine
N-carbamoyl-L-aspartate
Alanine
Malate
Adenine
Citrulline
Allantoate
dCDP-nega
UMP
CMP
Serine
Carbamoyl phosphate
AMP
Glyoxylate
3-phosphoglycerate
2-hydroxyglutarate
DL-Pipecolic acid
GMP
Pyroglutamic acid
1 mM Glucose (1)
1 mM Glucose (2)
1 mM Glucose (3)
1 mM Glucose + U0126 (1)
1 mM Glucose + U0126 (2)
1 mM Glucose + U0126 (3)
-1.5
-1.0
-0.5
0
0.5
1.0
1.5

B



C



D

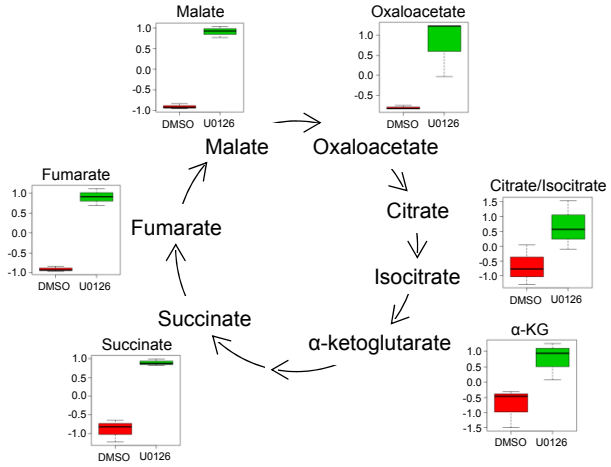


Fig. S7, related to Fig. 7. Inhibition of ERKs reprograms cellular metabolites (A) Top 50 metabolites that were altered by UO126 treatment were selected from LC-MS/MS data and heat map was generated. **(B)** Metabolic Set Enrichment Analysis of metabolites that were changed significantly by U0126 treatment. **(C)** Metabolites from HEK293 cells grown in the presence of 1 mM glucose for 54 h with or without U0126 were analyzed. The box plot shows change of aspartate levels. HEK293 cells grown in the presence of 1 mM glucose for 36 h were treated with 2 mM aspartate. Cell death was measured at the indicated time points. **(D)** The levels of TCA cycle intermediates from HEK293 cells grown in the presence of 1 mM glucose for 54 h with or without U0126 were analyzed.

Supplemental Experimental Procedures

Cells and Reagents

Human primary prostate epithelial cells were obtained from ATCC and were maintained with prostate epithelial growth media kit (ATCC). Human primary umbilical vein endothelial cells and human primary dermal fibroblasts were purchased from Lonza and were maintained with endothelial growth media-2 kit (Lonza) and fibroblast growth media-2 kit (Lonza), respectively. Human embryonic kidney 293 (HEK293), HEK293TD, mouse cortical tubular (MCT), and mouse ureteric bud (UB) cells were maintained in DMEM containing 10% FBS. Human tubular epithelial (HK-2) cells were cultured in K-SFM supplemented with bovine pituitary extract and recombinant epidermal growth factor (Invitrogen). To incubate cells at 5 mM glucose or 1 mM glucose as starting levels of glucose, cells were first plated onto 6 well plates. The following day, cells were washed with 3 ml of PBS and 2.5 ml of 5 mM or 1 mM glucose media containing 10% dialyzed serum were added. To incubate cells at constant glucose levels, cells were washed with 3 ml of PBS and then 10 ml of media with indicated concentrations of glucose containing 10% dialyzed serum were added. Media were changed every 12 h. Anti-phospho-ERK, anti-ERK, anti-cleaved PARP (human), anti-cleaved PARP (mouse), anti-cleaved caspase 3, anti-actin, anti-MEK1, anti-MEK2, anti-MEK1/2, anti-phospho-4E-BP1, anti-4E-BP1, anti-phospho-Akt, anti-Akt, anti-HA, anti-PERK, anti-phospho-eIF2α, anti-eIF2α, anti-GCN2, anti-Bid, anti-c-fos, anti-phospho-RSK, anti-RSK, and anti-ATF4 antibodies were purchased from Cell Signaling Technology. Anti-TRB3, anti-phospho-GCN2, and Anti-ATF4 antibodies were obtained from Millipore, Genetex, and Santa Cruz, respectively. UO126, GSK2606414, Z-VAD-FMK, HY10355, thapsigargin, actinomycin D, cycloheximide, camptothecin, etoposide, and TNFα antagonist WP9QY were purchased from EMD Millipore. AZD6244 and MK2206 were obtained from Selleckchem. TNFα ELISA kit, Amplex Red glucose assay kit, and dialyzed serum were purchased from Life Technologies. Glucose, all amino acids, α-ketoglutarate, glutathione reduced ethyl ester, A-Raf siRNA, tunicamycin, and glutamate assay kit were purchased from Sigma. Glutamine assay kit was obtained from Abnova.

Plasmids and generation of stable cells

pcDNA3-myc-p110α-CAAX was a gift from Julian Downward (Addgene plasmid # 39490). Lentiviral short hairpin RNA (shRNA) plasmids targeting human or mouse genes were obtained from Sigma or DF/HCC DNA resource core. 293TD cells, lentiviral packaging and envelope plasmids were a generous gift from Dr. Andrew L. Kung (Dana-Farber Cancer Institute, MA) and Dr. David Baltimore (California Institute of Technology, CA). To generate lentiviruses, shRNA plasmids or overexpression plasmids were transfected into 293TD cells with the expression plasmids for packaging (Δ8.9) and envelope (VSVG), and medium was changed next day. After 30 h, viral supernatants were harvested, and new medium was added. Viral supernatants were collected again after 24 h. Cells were infected with viral supernatants in the presence of a serum-containing medium supplemented with 8 μg/ml polybrene. After 24 h, viral-containing medium was removed and cells were grown in serum-containing medium for 24 h. Cells were treated with puromycin (2 μg/ml puromycin for HEK293, MCT, and UB cell lines, and 6 μg/ml puromycin for HK-2 cell line) for selection. The knockdown or overexpression of target protein was confirmed by immunoblot analysis. For knockout of ERK1, ERK2, MEK1, and MEK2, target sequences were obtained from Human GeCKO lentiviral sgRNA v2 library and following oligonucleotides were annealed and ligated into lentiCRISPR V2 (gift from Feng Zhang - Addgene plasmid # 52961) cut with BSMBI.

ERK1-1: caccg GCGTAGCCACATACTCCGTC
ERK1-2: aaac GACGGAGTATGTGGCTACGCc
ERK2-1: caccg TGCAAACAGATATATAGTAC
ERK2-2: aaac GTACTATATATCTGTTTGCAc
MEK1-1: caccg TATGGTGCGTTCTACAGCGA
MEK1-2: aaac TCGCTGTAGAACGCACCATA c
MEK2-1: caccg TCTCTGCAGGGCAAACCTGG
MEK2-2: aaac CCAGGTTTGCCCTGCAGAGAc

To generate lentiviruses for knockout, the same method was used as described in generation of knockdown cells except lentiCRISPR V2, pCMV-Pax2, and pVSVg plasmids were used for transfection into 293TD cells.

Transfection

Cells were transfected with LipofectAMINE 2000 reagent (Invitrogen) in the presence of 10% serum without antibiotics according to the manufacturer's protocol.

Immunoblot analysis

For immunoblot analysis, the cells were extracted in lysis buffer (20 mM HEPES (pH 7.5), 150 mM NaCl, 10 mM EDTA, 1 mM EGTA, 10 mM β-glycerophosphate, 1 mM sodium orthovanadate, 5 mM NaF, 2 mM phenylmethylsulfonyl fluoride, 2 mg/ml aprotinin, 2 mg/ml leupeptin, and 1mg/ml pepstatin) containing 1% Triton X-100, and samples were resuspended in reducing buffer (6X: 60 mM Tris-HCl pH 6.8, 25% glycerol, 2% SDS, 14.4 mM 2-mercaptoethanol, 0.1% bromophenol blue). Samples were boiled for 5 min and electrophoresed by SDS-PAGE. Proteins were then transferred to nitrocellulose membranes (Whatman). The membranes were blocked with TBST (25 mM Tris-HCl, pH 7.5, 150 mM NaCl, 0.05% Tween 20) containing 5% nonfat dried milk or 5% BSA, and probed overnight with primary antibodies, followed by 1h incubation with secondary antibodies coupled to peroxidase. Blots were developed by using enhanced chemiluminescence. Immunoblot data are representative of at least three independent experiments. Grouped immunoblot data in each figure were obtained from duplicate membranes of the same experiment, stripped membranes, or separate experiments under the same conditions. To strip the membranes, wet membranes were washed with TBST and incubated with stripping buffer (62.5 mM Tris, pH 6.8, 2% SDS, 100 mM β-mercaptoethanol) for 30 min at 55°C under agitation. The membranes were washed three times with TBST.

Glucose measurement

Glucose levels in the media were measured using Amplex Red glucose assay kit (Life Technologies) according to the manufacturer's instruction.