# Supplemental Information

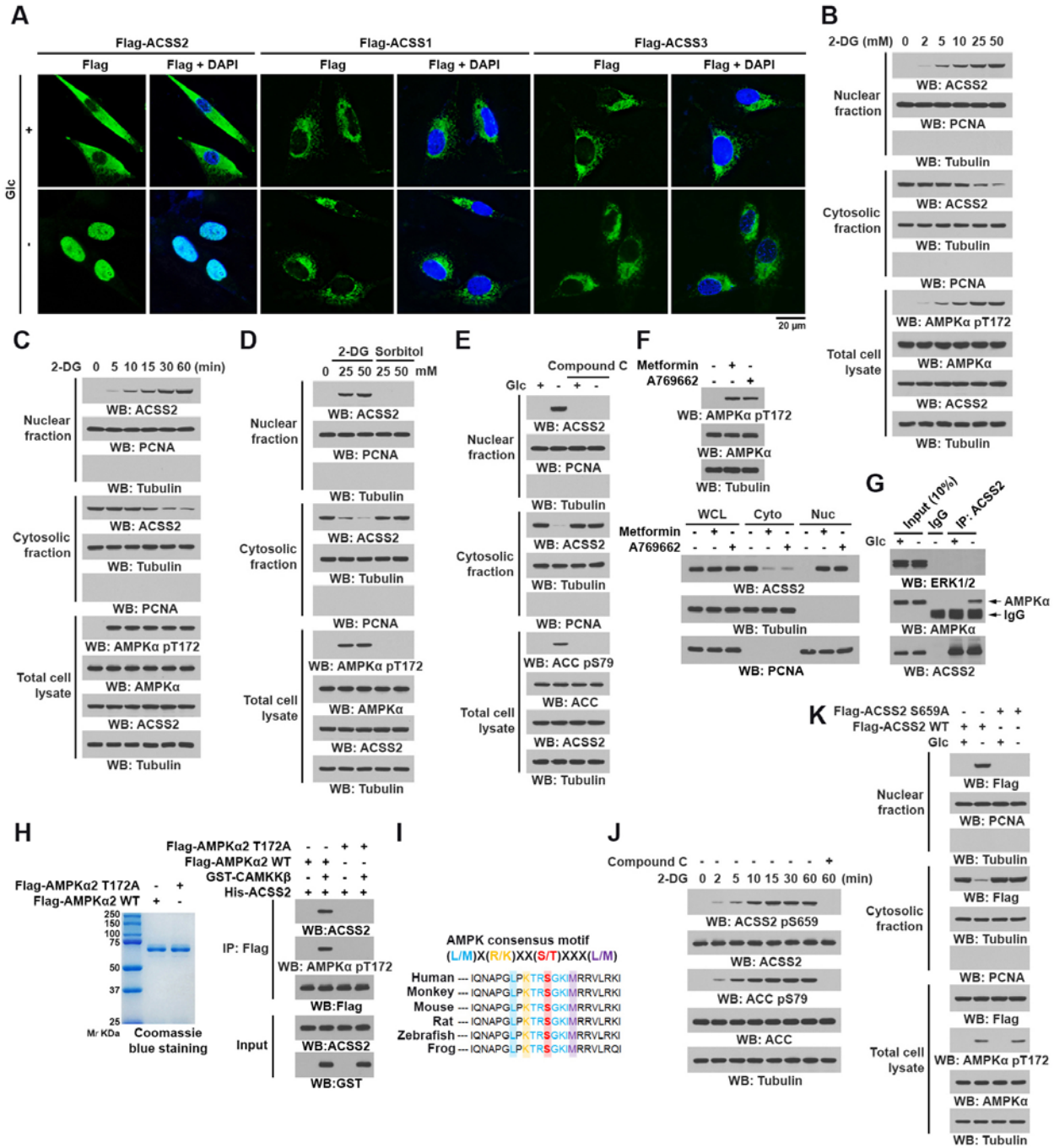


**Figure S1. ACSS2 S659 phosphorylation by AMPK induces nuclear translocation of ACSS2. Related to Figure 1.**

(**B, C, D, E, F** and **K**) total cell lysates and cytosolic and nuclear fractions were prepared. Immunoblot analyses were performed with the indicated antibodies. WB, Western blot.

**(A)** U87 cells expressing the indicated Flag-tagged proteins were deprived of glucose for 1 h. Immunofluorescent analyses were performed with an anti-Flag antibody. DAPI (blue) was used to stain the nuclei.

**(B)** U87 cells were treated with or without the indicated concentrations of 2-DG for 1 h.

**(C)** U87 cells were treated with or without 2-DG (50 mM) for the indicated periods of time.

**(D)** U87 cells were treated with indicated concentrations of 2-DG or sorbitol for 1 h.

**(E)** U87 cells pretreated with or without compound C (5 μM) for 30 min were deprived of glucose for 1 h.

**(F)** U87 cells were treated with or without Metformin (1 mM) or A769662 (100 μM) for 1 h.

**(G)** U87 cells were deprived of glucose for 10 min. Immunoprecipitation with an anti-ACSS2 antibody was performed.

**(H)** Purified WT Flag-AMPKα2 or Flag-AMPKα2 T172A proteins (left panel) on agarose beads were incubated with or without active GST-CaMKKβ protein for a kinase assay and were then washed by PBS three times and incubated with purified His-ACSS2 proteins. A Flag pull-down assay was performed (right panel).

**(I)** 654-LPKTRSGKIM-663 of human ACSS2 containing an AMPK phosphorylation motif is evolutionally conserved in the indicated species.

**(J)** U87 cells pretreated with or without compound C (5 μM) for 30 min were treated with 2-DG (50 mM) for the indicated periods of time. Total cell lysates were prepared.

**(K)** U87 cells expressing the indicated Flag-tagged ACSS2 proteins were deprived of glucose for 1 h.

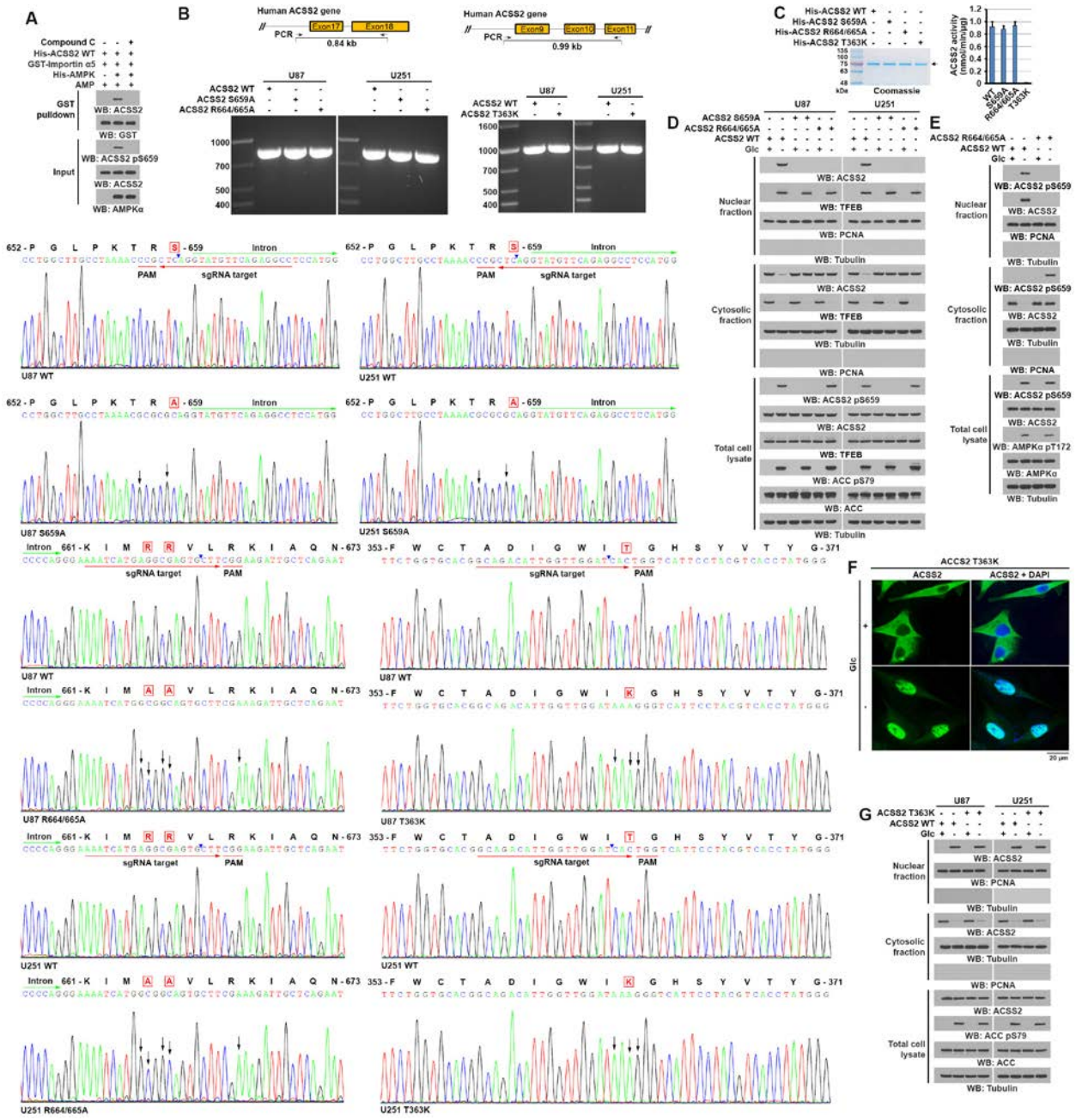


**Figure S2. ACSS2 S659A or R664/665A expression blocks the nuclear translocation of ACSS2. Related to Figure 2.**

**(A)** Purified His-ACSS2 proteins was incubated with or without active His-AMPK proteins in the presence or absence of compound C (5 μM) for a kinase assay, which was followed by incubation with purified GST-importin α5 proteins for a GST pull-down assay.

**(B)** Knock-in expression of ACSS2 S659A, R664/665A, and T363K was made in U87 and U251 cells. Genomic DNA was extracted from the indicated cells. PCR products for the indicated DNA fragments were separated on an agarose gel and sequenced. The red arrows indicate the sgRNA-targeting sequences. Each red line indicates the protospacer-adjacent motif (PAM). The blue triangles indicate the cutting sites for hSpCas9. The black arrows point to the mutated nucleotides. Mutated amino acids and their WT counterparts are highlighted in rectangles. Silent mutations of the indicated nucleotides were introduced into the sgRNA-targeting sequences to avoid repeat cutting by hSpCas9.

**(C)** Bacterially purified ACSS2 proteins were stained with Coomassie Brilliant Blue (left). The catalytic activities of the indicated ACSS2 proteins were measured (right). The data are presented as the mean ± SD for triplicate samples.

**(D)** Parental and U87 cells with knock-in of ACSS2 S659A or R664/665A were deprived of glucose for 1 h. Total cell lysates and cytosolic and nuclear fractions were prepared. Immunoblot analyses were performed with the indicated antibodies. WB, Western blot.

**(E)** Parental and U87 cells with knock-in of ACSS2 R664/665A were deprived of glucose for 1 h. Total cell lysates and cytosolic and nuclear fractions were prepared. Immunoblot analyses were performed with the indicated antibodies.

**(F)** U87 cells with knock-in of ACSS2 T363K were deprived of glucose for 1 h. Immunofluorescent analyses were performed with an anti-ACSS2 antibody. DAPI (blue) was used to stain the cell nuclei.

**(G)** U87 and U251 cells with knock-in of ACSS2 T363K were deprived of glucose for 1 h. Total cell lysates and cytosolic and nuclear fractions were prepared. Immunoblot analyses were performed with the indicated antibodies.

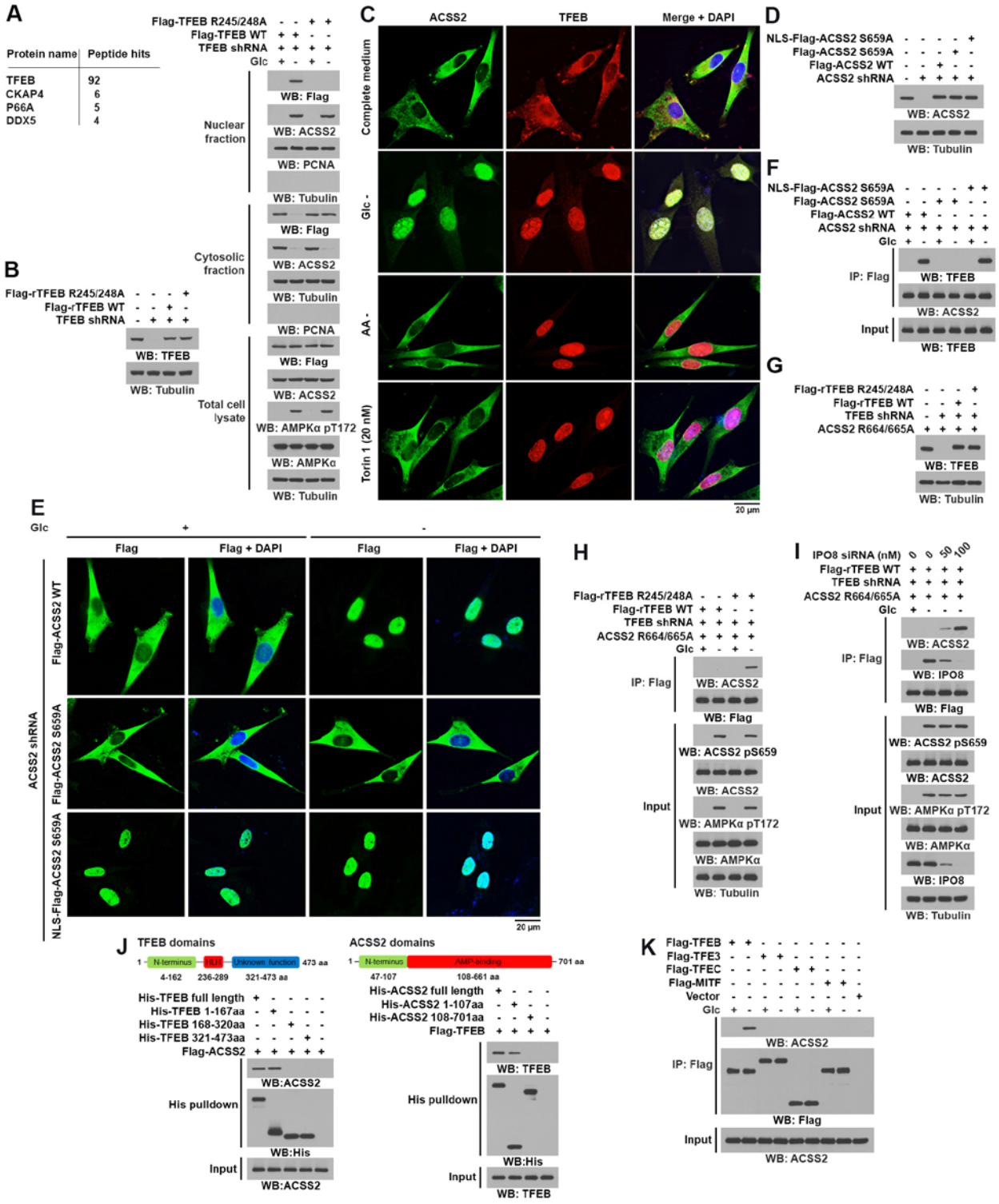


**Figure S3. TFEB and ACSS2 separately translocate into the nucleus where they form a complex. Related to Figure 3.**

**(A)** The peptide hits of TFEB and other proteins associated with immunoprecipitated nuclear ACSS2 were identified using mass spectrometry.

**(B)** Endogenous TFEB-depleted U87 cells with reconstituted expression of WT rTFEB or rTFEB R245/248A were deprived of glucose for 1 h. Total cell lysates and cytosolic and nuclear fractions were prepared. Immunoblot analyses were performed with the indicated antibodies. WB,Western blot.

**(C)** U87 cells were deprived of glucose or amino acids for 1 h with or without Torin 1 (20 nM) pretreatment for 30 min. Immunofluorescent analyses were performed with anti-ACSS2 and anti-TFEB antibodies. DAPI (blue) was used to stain the cell nuclei.

**(D and E)** Endogenous ACSS2-depleted U87 cells with reconstituted expression of WT ACSS2, ACSS2 S659A or NLS-ACSS2 S659A were deprived of glucose for 1 h. Immunoblot analyses were performed with the indicated antibodies (**D**). Immunofluorescent analyses were performed with an anti-ACSS2 antibody (**E**). DAPI (blue) was used to stain the cell nuclei. NLS, nuclear localization sequence.

**(F)** Endogenous ACSS2-depleted U87 cells with reconstituted expression of the indicated Flag-ACSS2 proteins were deprived of glucose for 1 h. Immunoprecipitation with an anti-Flag antibody was performed. Immunoblot analyses were performed with the indicated antibodies.

**(G, H)** U87 cells with knock-in expression of ACSS2 R664/665A and with or without expression of TFEB shRNA were reconstituted with expression of the indicated Flag-rTFEB proteins (**G**). These cells were deprived of glucose for 1 h. Immunoprecipitation with an anti-Flag antibody was performed (**H**). Immunoblot analyses were performed with the indicated antibodies.

**(I)** U87 cells with knock-in expression of ACSS2 R664/665A and with or without expression of TFEB shRNA were reconstituted with expression of the indicated Flag-rTFEB proteins. These cells were transfected with indicated concentration of IPO8 siRNA for 48 h and were deprived of glucose for 10 min thereafter. Immunoprecipitation with an anti-Flag antibody was performed. Immunoblot analyses were performed with the indicated antibodies.

**(J)** Purified indicated truncation mutants of His-TFEB were mixed with the purified Flag-ACSS2 proteins (left panel). Purified indicated truncation mutants of His-ACSS2 proteins were mixed with the purified Flag-TFEB proteins (right panel). A His pull-down assay was performed. Immunoblot analyses were performed with the indicated antibodies.

**(K)** U87 cells expressing the indicated Flag-tagged proteins were deprived of glucose for 1 h. Immunoprecipitation with an anti-Flag antibody was performed. Immunoblot analyses were performed with the indicated antibodies.

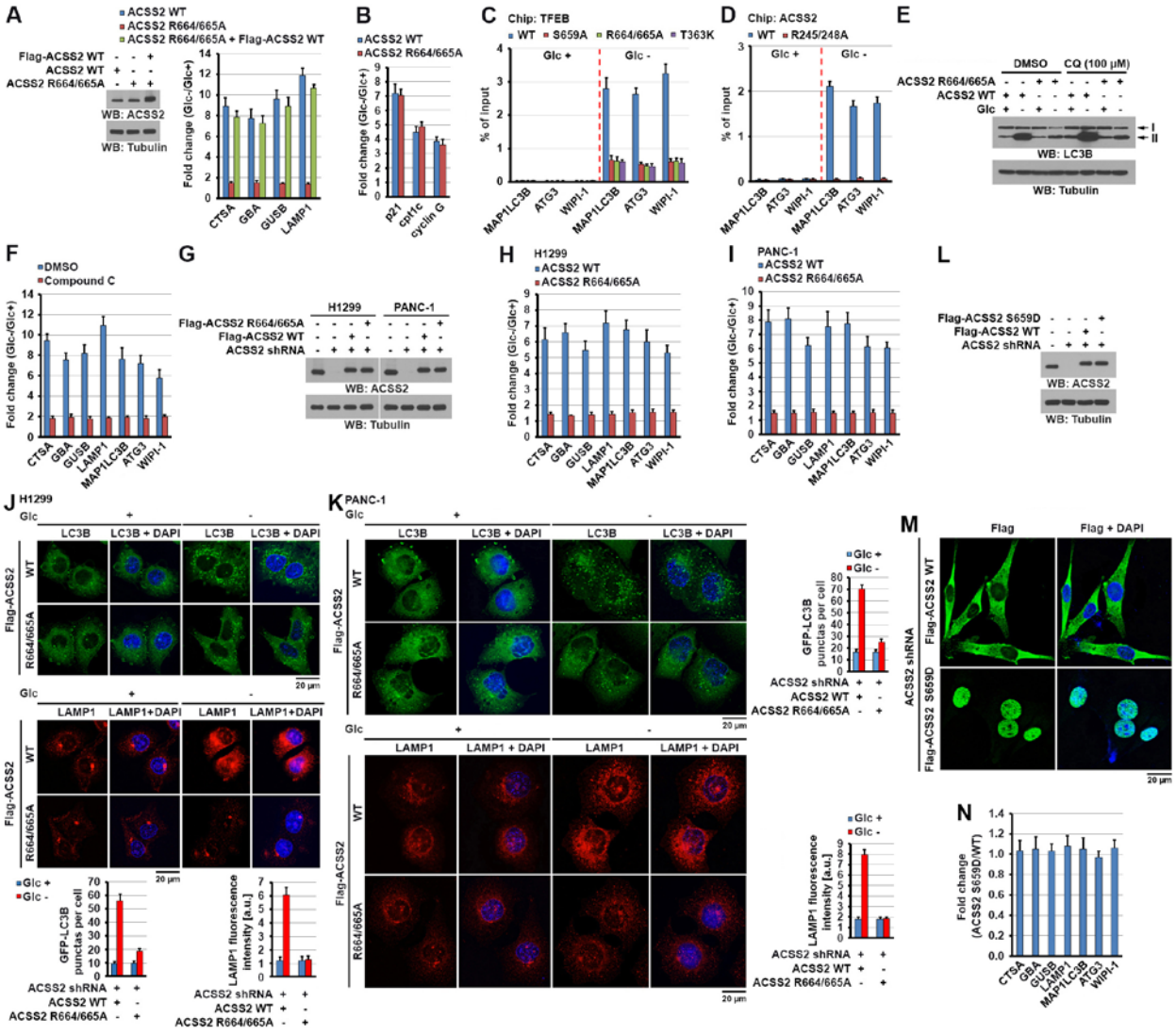


**Figure S4. Nuclear ACSS2 mediates local generation of acetyl-CoA and subsequent histone H3 acetylation in the promoter regions of lysosomal and autophagy genes. Related to Figure 3.**

**(A)** U87 cells expressing the indicated ACSS2 proteins were deprived of glucose for 10 h. mRNA levels of the indicated genes were determined using quantitative polymerase chain reaction (qPCR) analysis. Data were normalized for β-actin mRNA levels and presented as fold changes induced by glucose deprivation. The data are presented as the mean ± SD for triplicate samples.

**(B)** Parental and the indicated U87 cells with knock-in expression of ACSS2 R664/665A mutant were deprived of glucose for 10 h. mRNA levels of the indicated genes were determined using qPCR analysis. Data were normalized for β-actin mRNA levels and presented as fold changes induced by glucose deprivation. The data are presented as the mean ± SD for triplicate samples.

**(C)** Parental and the indicated U87 cells with knock-in expression of ACSS2 S659A, R664/665A, or T363K mutant were deprived of glucose for 1 h. ChIP analyses using an anti-TFEB antibody were performed. The histogram shows the amount of immunoprecipitated DNA expressed as a percentage of the total input DNA. The data are presented as the mean ± SD for triplicate samples.

**(D)** Endogenous TFEB-depleted U87 cells with reconstituted expression of WT rTFEB or rTFEB R245/248A were treated with or without glucose starvation for 1 h. ChIP analyses were performed using an anti-ACSS2 antibody. The histogram shows the amount of immunoprecipitated DNA expressed as a percentage of the total input DNA. The data are presented as the mean ± SD for triplicate samples.

**(E)** Parental U87 and the U87 cells with knock-in of ACSS2 R664/665A mutant were deprived of glucose for 10 h in the absence or presence of 100 μM chloroquine (CQ) for 4 h (prior to the end of glucose-deprivation treatment). Immunoblot analyses with the indicated antibodies were performed.

**(F)** U87 cells pretreated with or without compound C (5 μM) for 30 min were deprived of glucose for 10 h. mRNA levels of the indicated genes were determined using qPCR analysis. Data were normalized for β-actin mRNA levels and presented as fold changes induced by glucose deprivation. The data are presented as the mean ± SD for triplicate samples.

**(G-I)** Endogenous ACSS2-depleted H1299 or PANC-1 cells with reconstituted expression of the indicated Flag-ACSS2 proteins (**G**) were deprived of glucose for 10 h. mRNA levels of the indicated genes were determined in H1299 (**H**) or PANC-1 (**I**) cells using qPCR analysis. Data were normalized for β-actin mRNA levels and presented as fold changes induced by glucose deprivation. The data are presented as the mean ± SD for triplicate samples.

**(J and K)** Endogenous ACSS2-depleted H1299 or PANC-1 cells with reconstituted expression of the indicated Flag-ACSS2 proteins were deprived of glucose for 10 h. Immunofluorescent analyses were performed in H1299 (**J**) or PANC-1 (**K**) cells with anti-LC3B and anti-LAMP1 antibodies. The numbers of LC3B puncta in 20 cells and the immunofluorescence intensity in 100 cells were quantitated using the ImageJ software program.

**(L-N)** Endogenous ACSS2-depleted U87 cells were reconstituted with expression of WT ACSS2 or ACSS2 S659D (**L**). Immunofluorescent analyses were performed with an anti-Flag antibody (**M**). DAPI (blue) was used to stain the cell nuclei. mRNA levels of the indicated genes were determined using qPCR analysis **(N)**. Data were normalized for β-actin mRNA levels and presented as fold changes induced by ACSS2 S659D mutation. The data are presented as the mean ± SD for triplicate samples.

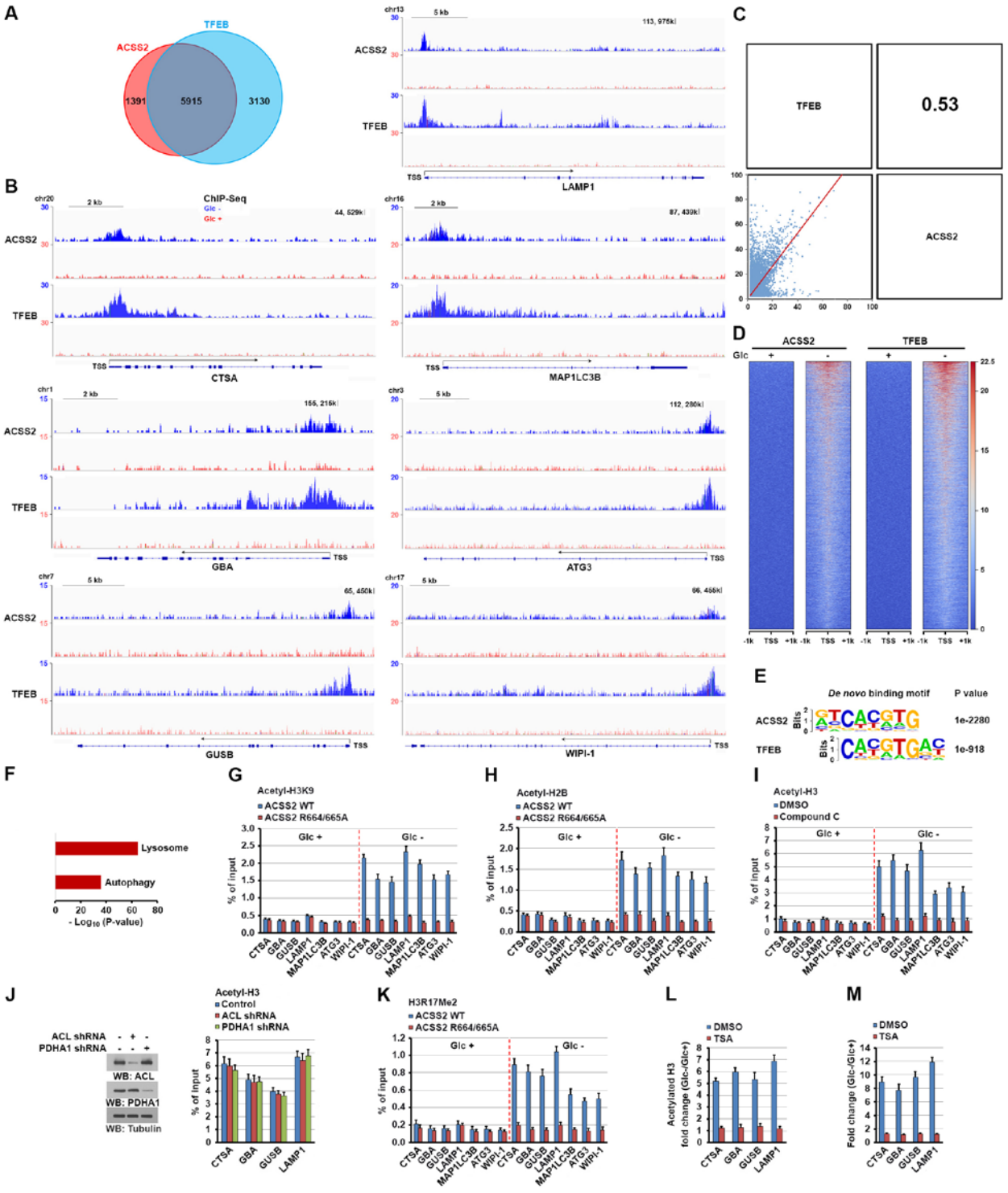


**Figure S5. Nuclear ACSS2 co-localizes with TFEB and enhances histone acetylation in the promoter regions of lysosomal and autophagosomal genes. Related to Figures 3 and 4.**

(**G-L**) ChIP analyses were performed using the indicated antibodies. The histogram shows the amount of immunoprecipitated DNA expressed as a percentage of the total input DNA. The data are presented as the mean ± SD for triplicate samples.

(**A**) The Venn diagram of ACSS2 and TFEB target genes in U87 cells deprived of glucose for 1 h. Target genes of ACSS2 and TFEB were determined by the peaks located within canonical chromosomes, within -500 to +100 bp of TSS and with a peak-score ≥ 2.

**(B)** Peaks visualization of selected gene regions of ACSS2 and TFEB ChIP using Integrated Genome Browser. Blue plots represent treatment condition where U87 cells were cultured under glucose deprivation for 1 h and red plots represent control condition where U87 cell were cultured under normal glucose conditions.

(**C**) The peak score scatter plot of the union between ACSS2 and TFEB binding sites (blue dots) and the corresponding regression line (red line). Correlation between the paired peak scores was calculated using Pearson correlation (Pearson correlation coefficient = 0.53) and the significance of the correlation was calculated using permutation test at 12 million iterations ($P < 0.001$).

(**D**) Heatmaps of normalized read depth for all binding sites corresponding to ACSS2 and TFEB ChIP. We consider +/- 1kbp of TSSs of canonical chromosomes to be potential binding sites for both treatment (Glc -) and control (Glc +) conditions using U87 cells.

**(E)** Alignment of the highest ranked *de novo* binding motif found within +/-50bp of ACSS2 (top) or TFEB (bottom) ChIP peak positions.

**(F)** Lysosomal and autophagosomal genes were significantly enriched in the ACSS2 target gene set where U87 cells were deprived of glucose for 1h. P-values were calculated using the hyper-geometic test.

**(G, H, and K)** Parental and the indicated U87 cells with knock-in expression of ACSS2 R664/665A mutant were deprived of glucose for 1 h.

**(I)** U87 cells pretreated with or without compound C (5 μM) for 30 min were deprived of glucose for 1 h.

**(J)** U87 cells with or without expression of ACL shRNA or PDHA1 shRNA were deprived of glucose for 1 h.

**(L)** U87 cells were deprived of glucose for 1 h in the absence or presence of TSA (500 nM).

**(M)** U87 cells were deprived of glucose for 10 h in the absence or presence of TSA (500 nM). mRNA levels of the indicated genes were determined using qPCR analysis. Data were normalized for β-actin mRNA levels and presented as fold changes induced by glucose deprivation. The data are presented as the mean ± SD for triplicate samples.

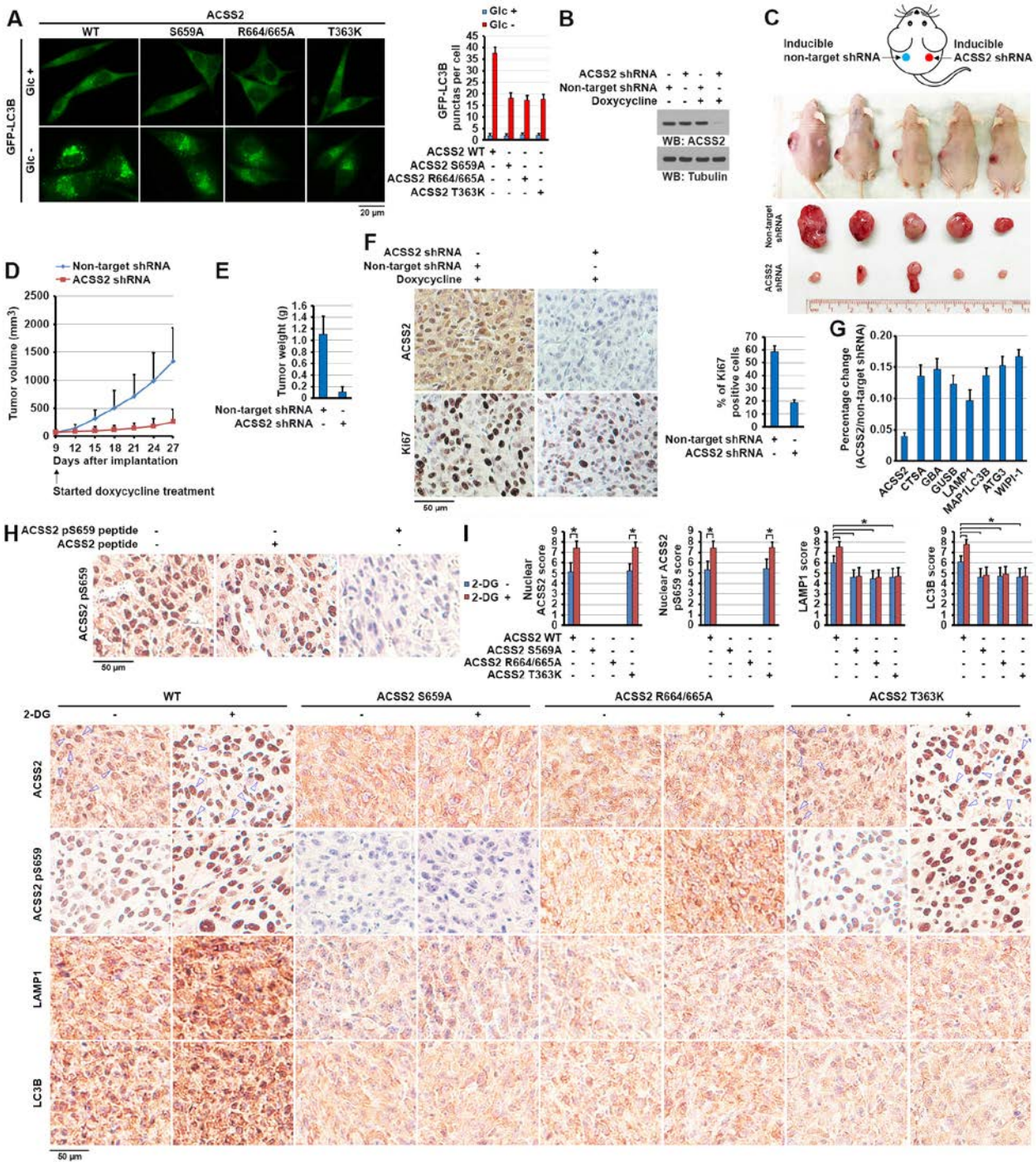


**Figure S6. ACSS2 is required for tumorigenesis. Related to Figures 5 and 6.**

**(A)** Parental U87 cells and U87 cells with knock-in expression of the indicated proteins stably expressed GFP-LC3B protein. These cells were deprived of glucose for 10 h. The numbers of GFP-LC3B puncta in 20 cells were quantitated using the ImageJ software program.

**(B)** U87 cells expressing the indicated shRNAs were treated with or without doxycycline (1 μg/ml)) for 24 h. Immunoblot analyses were performed with the indicated antibodies.

**(C-E)** U87 cells expressing the inducible non-target shRNAs or ACSS2 shRNA were subcutaneously injected into the left and right flank of athymic nude mice, respectively. Nine days after tumor-cell injection, doxycycline (2 mg/ml in 5% sucrose) was delivered to the mice via drinking water for 18 days. Photographs (**C**), growth curves (**D**), and weigh (**E**) of indicated tumors dissected from athymic nude mice ($n$ = 5) were shown. The data represent the mean ± SD.

**(F)** Immunohistochemical analyses of the indicated tumor sections were performed with the indicated antibodies. Ki67-positive cells were quantified in 10 microscope fields.

**(G)** mRNA levels of the indicated genes were determined in tumor tissues derived from U87 cells expressing the inducible non-target shRNAs or ACSS2 shRNA using qPCR analysis. Data were normalized with β-actin mRNA levels and presented as percentage changes induced by ACSS2 shRNA expression relative to non-target shRNA expression (defined as 100%). The data are presented as the mean ± SD for triplicate samples.

**(H)** U87 cells were intracranially injected into athymic nude mice. Two weeks after tumor-cell injection, 0.2 ml of 2-DG (500 mg/kg) was delivered to the mice via intraperitoneal administration daily for 14 days. Immunohistochemical analysis of glioblastoma tissue was performed with an anti-ACSS2 pS659 antibody in the presence or absence of specific blocking peptides.

**(I)** Parental and the indicated U87 cells with knock-in expression of ACSS2 S659A, ACSS2 R664/665A, or T363K mutant were intracranially injected into athymic nude mice. Two weeks after tumor-cell injection, 0.2 ml of 2-DG (500 mg/kg) was delivered to the mice via intraperitoneal administration daily for 14 days. Immunohistochemical analyses of the indicated

brain tumor sections were performed with the indicated antibodies. Blue arrows indicated the nuclear ACSS2 staining. Representative photos of tumors are shown. Stainings were quantified in 10 microscope fields. A two tailed Student's t test was used. $*$ represents $P < 0.001$.