

Supplementary figures 1-9

Supplementary Table 1

Long Noncoding RNA MALAT1 regulates cancer glucose metabolism by enhancing mTOR-mediated TCF7L2 translation.

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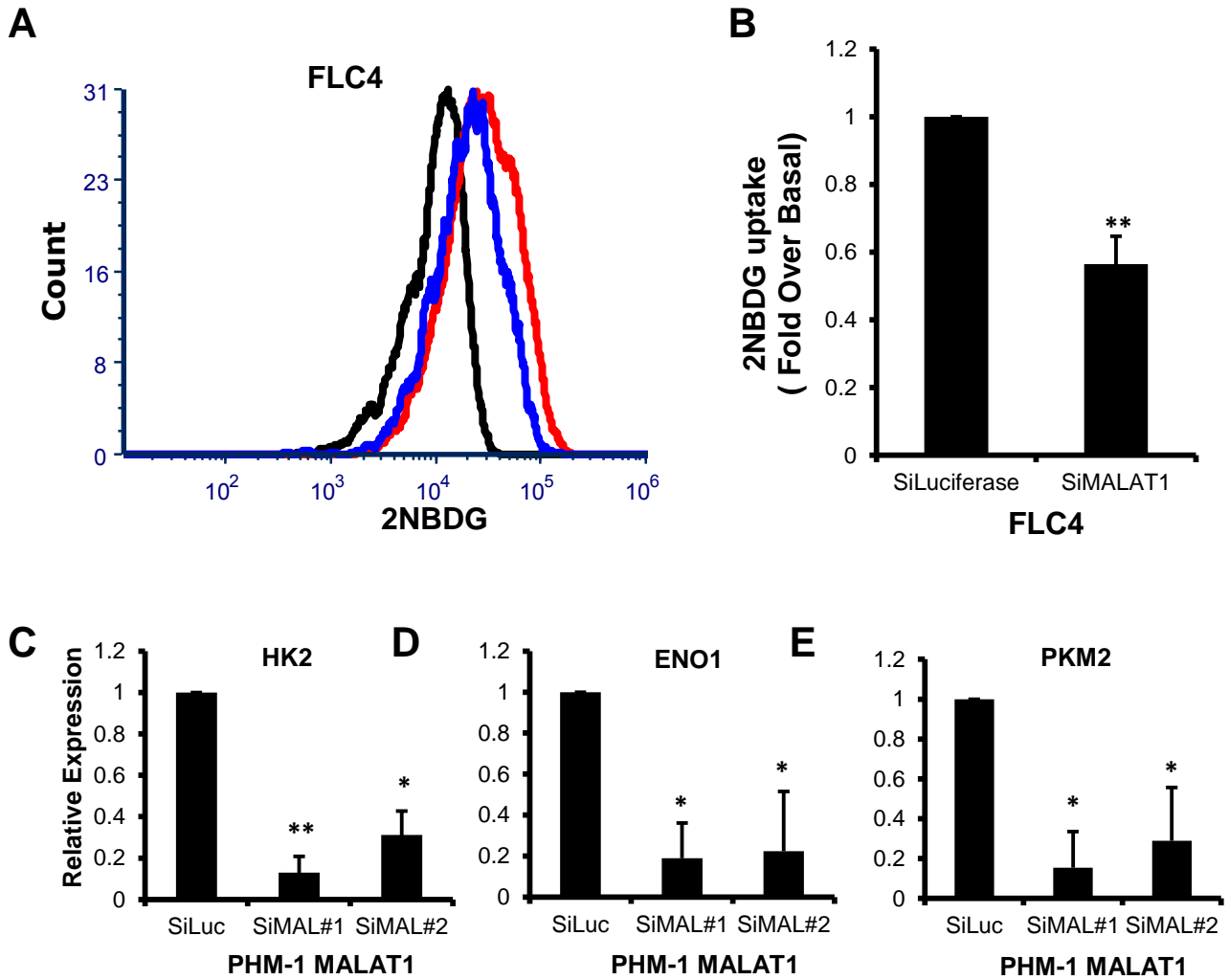


Figure S1: MALAT1 affects cancer glucose metabolism. **A.** FLC4 cells were treated with siRNAs targeted against either luciferase or MALAT1 for 48 hours. Cells were then placed in medium without glucose for 16 hours and then exposed to 2NBDG for 30 minutes. 2NBDG fluorescence was recorded using flow cytometry. A histogram of 2NBDG fluorescent intensities is shown. Black: cells without 2NBDG treatment; Red: cells treated with SiLuciferase and exposed to 2NBDG; Blue: cells treated with SiRNAs against MALAT1 and exposed to 2NBDG. **B.** Quantification of 2NBDG uptake from two independent experiments of cells described in (A). **C-E.** qRT-PCR analysis of glycolytic pathway genes (HK2, ENO1, PKM2) in PHM-1 MALAT1 cells transfected with MALAT1 siRNAs (SiMAL#1,2) or Control siRNA (SiLuc). All samples were normalized to GAPDH mRNA levels. The error bars indicate S.D. (n=2). Student T-Test was used. *, $P < 0.05$, **, $P < 0.01$, *** $P < 0.001$.

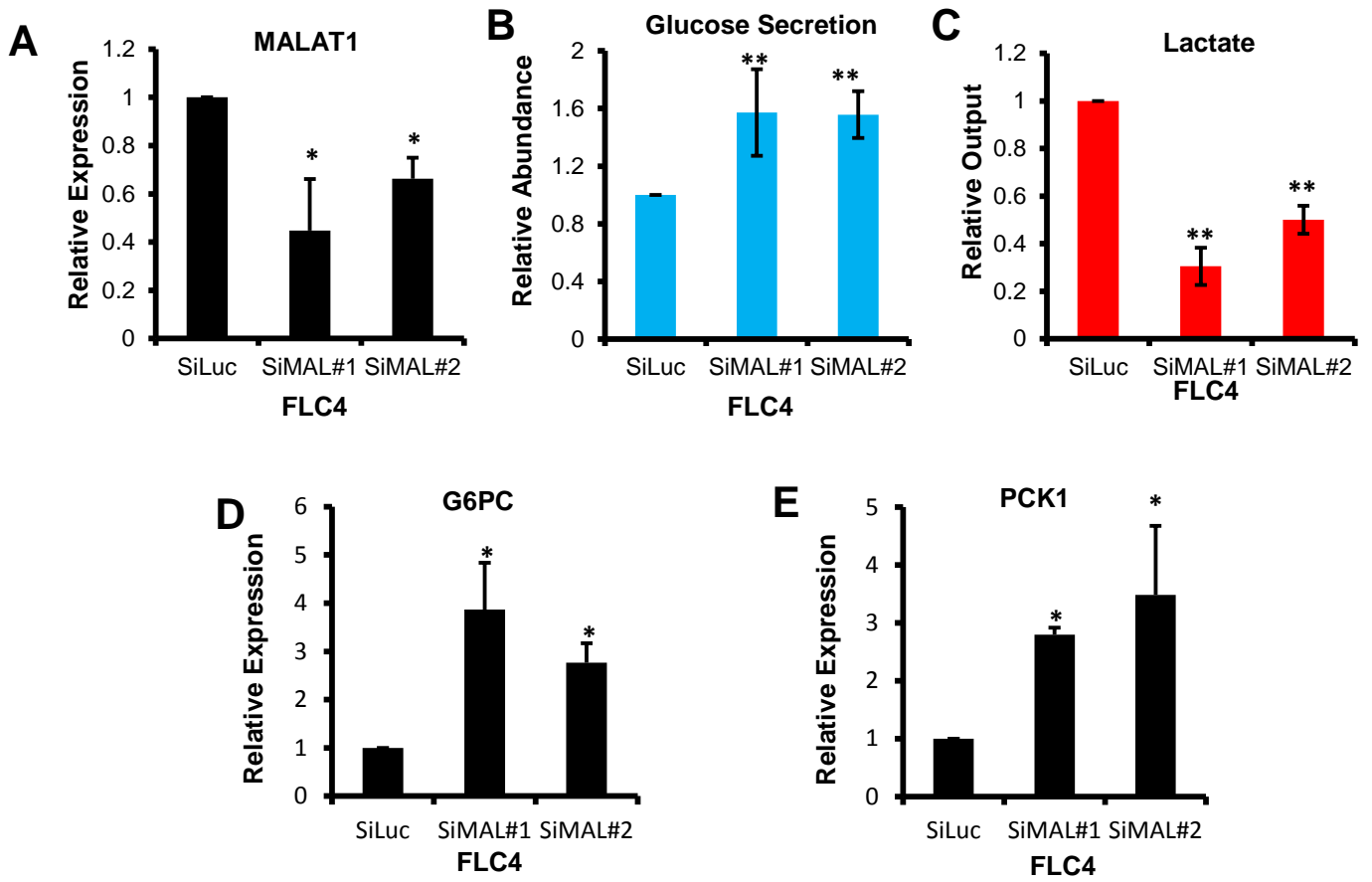


Figure S2: Glucose metabolism in HCC cell lines with MALAT1 knockdown. **A.** qRT-PCR analysis of MALAT1 expression in FLC4 cells transfected with MALAT1 siRNAs (siMAL#1,2) or control siRNA (SiLuc). **B.** Cellular glucose secretion was measured for cells described in (A) using a glucose assay kit (n=3). **C.** Extracellular lactate production was measured for cells described in (A) using a lactate assay kit (n=2). **D and E.** qRT-PCR analysis of gluconeogenesis pathway genes (G6PC, PCK1) in FLC4 cells transfected with MALAT1 siRNAs (siMAL#1,2) or control siRNA (SiLuc). The error bars indicate S.D. (n=2). Student T-Test was used. *, P<0.05, **, P<0.01, *** P<0.001

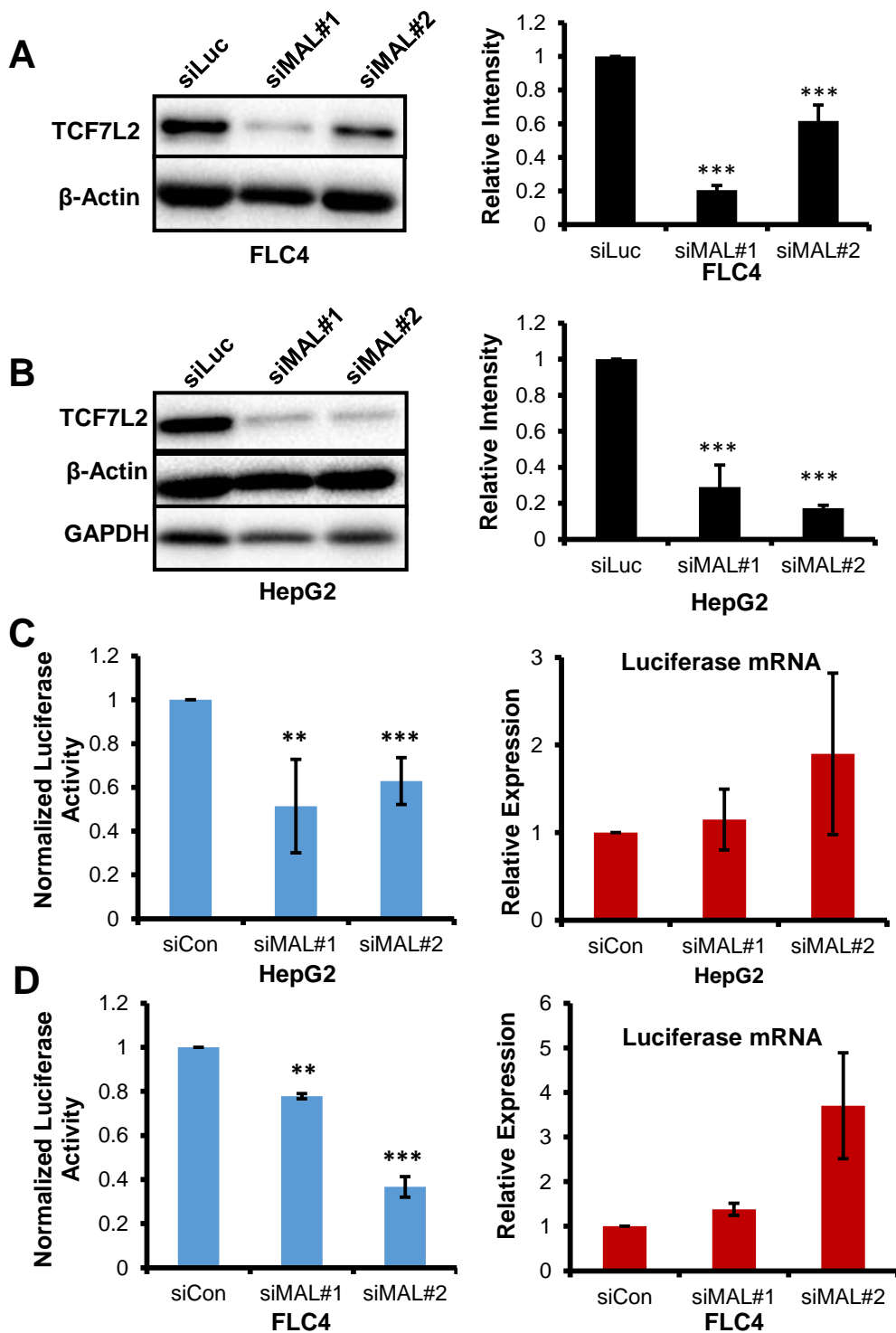


Figure S3: Regulation of TCF7L2 protein expression by MALAT1 in HCC cell lines. **A.** Western blot analysis of TCF7L2 protein levels in FLC4 cells transfected with MALAT1 siRNAs (siMAL#1,2) or control siRNA (siLuc) (left panel). Quantification of TCF7L2 protein levels (right panel) (n=3). **B.** Western blot analysis of TCF7L2 protein levels in HepG2 cells transfected with MALAT1 siRNAs (siMAL#1,2) or control siRNA (siLuc) (left panel). Quantification of TCF7L2 protein levels (n=3) (right panel). **C and D.** HepG2 (C) and FLC4 (D) cells were transfected with MALAT1 siRNAs (siMAL#1, #2) or control siRNA (siCon) along with a luciferase construct containing the WT TCF7L2 5'UTR. Fold change of luciferase activity (compared to siCon) is shown (left panels). Luciferase activity was normalized using Renilla expression. qRT-PCR analysis of luciferase mRNA levels in the same experiments (right panels). All bars show the average of 2-3 experiments. Student T-Test was used. *, P<0.05, **, P<0.01, *** P<0.001.

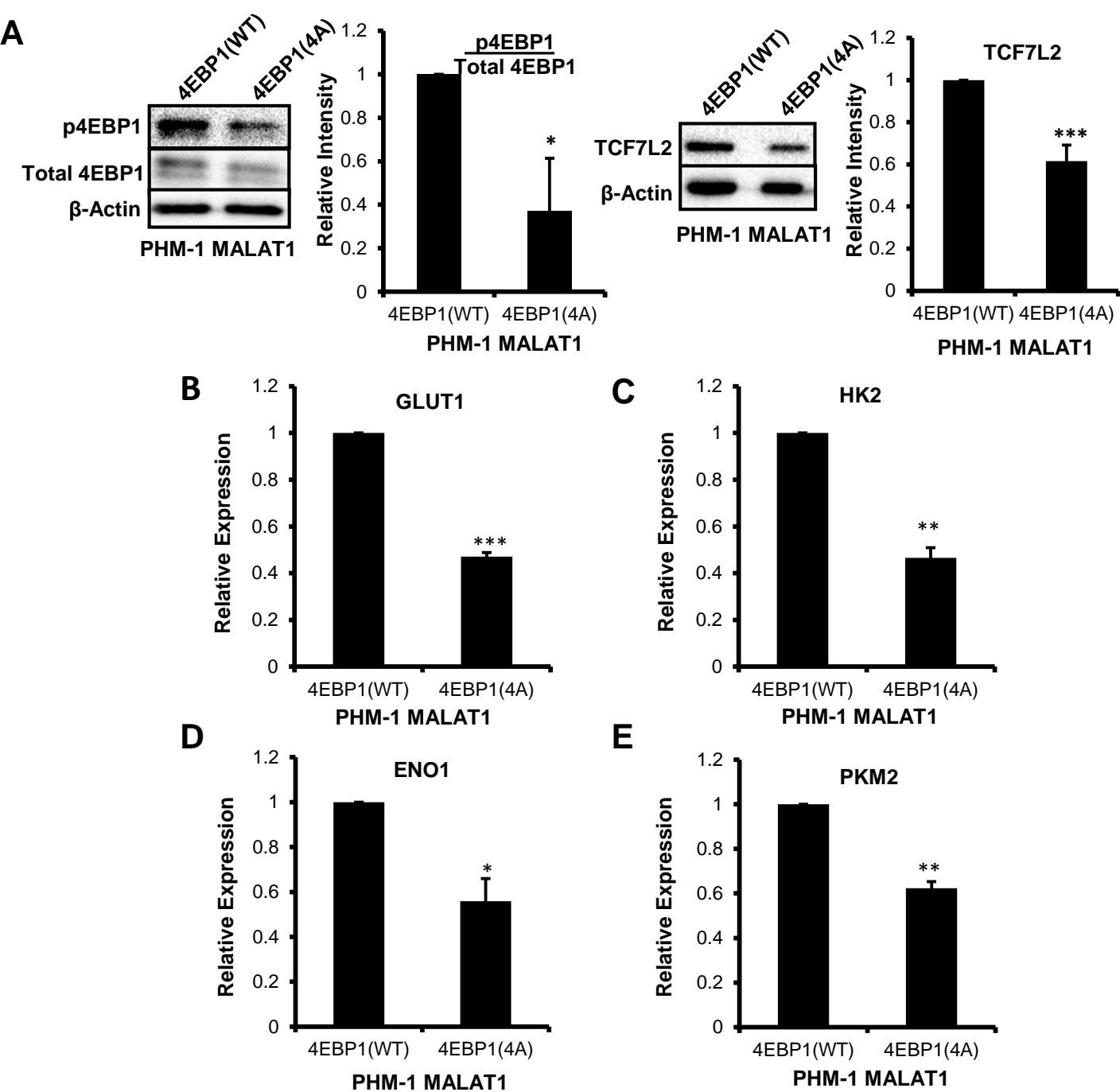


Figure S4

Figure S4: A non-phosphorylatable mutant of 4EBP1 inhibits TCF7L2 protein expression and expression of glycolytic genes. **A.** PHM-1 cells stably expressing hMALAT1 were transduced with retroviruses encoding for wildtype 4EBP1 (4EBP1(WT)) or 4EBP1 phosphorylation defective mutant in which all four phosphorylation sites were mutated to alanine (4EBP1(4A)). Total protein from stable pools was extracted for Western blot analysis. Western blot analysis of phosphorylation of 4EBP1 and quantification of inhibition of phosphorylation of 4EBP1 (n=2) (left panels). Western blot analysis of TCF7L2 protein levels and quantification of TCF7L2 protein levels (n=2) (right panels). **B, C, D and E.** mRNA expression of the glycolytic genes (GLUT1, HK2, ENO1, PKM2) were determined by qRT-PCR using cells described in (A). The error bars indicate S.D. (n=2). Student T-Test was used. *, P<0.05, **, P<0.01, *** P<0.001.

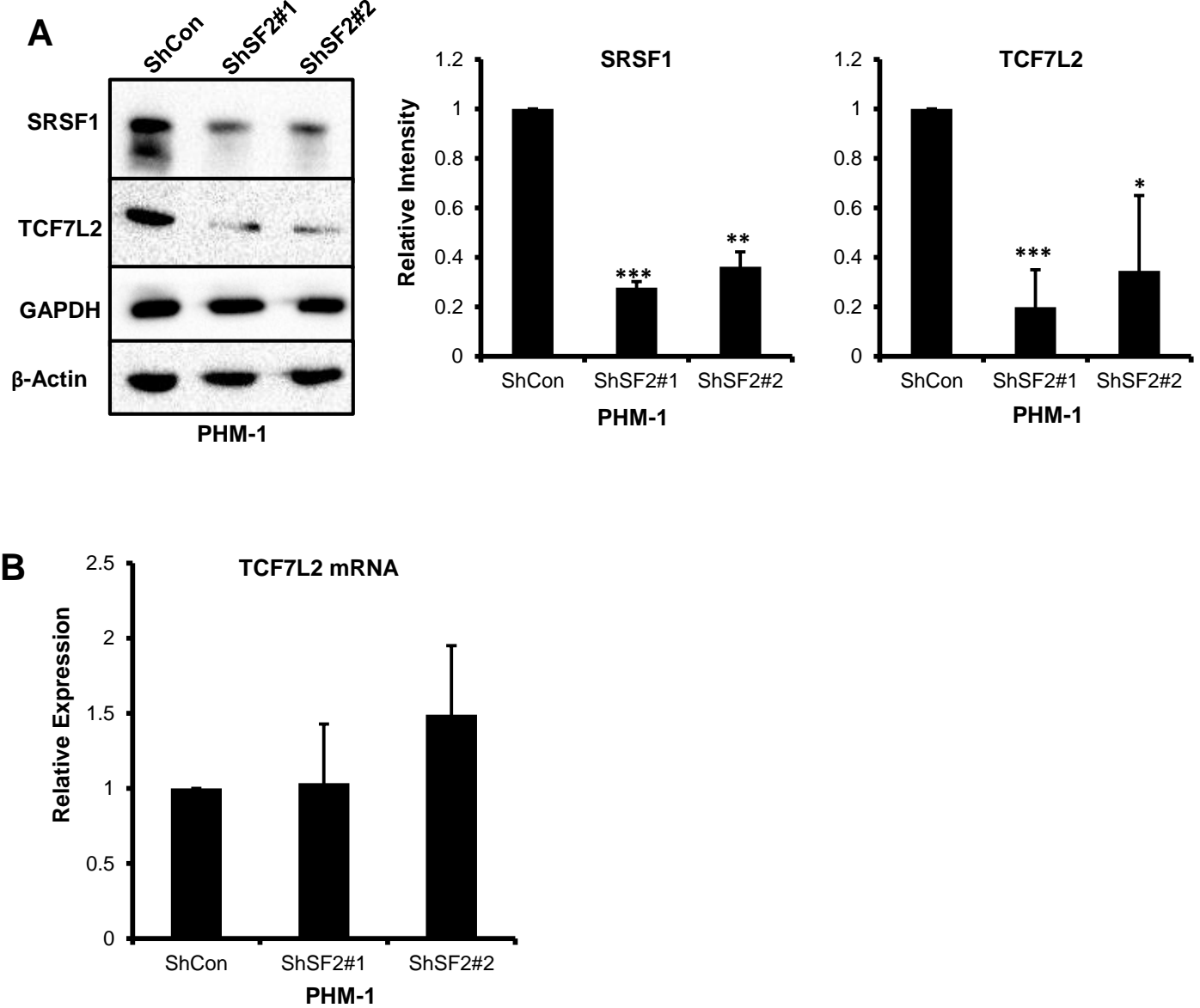


Figure S5: SRSF1 regulates TCF7L2 levels post-transcriptionally.

A. Western blot analysis of PHM-1 cells transduced with lentiviruses containing shRNAs against SRSF1 (shSRSF1#1, #2) or an empty vector (shControl). GAPDH and β -Actin were used as loading controls. Quantification of SRSF1 and TCF7L2 protein levels upon shRNA knockdown (n=3) (right panels). **B.** qRT-PCR of TCF7L2 mRNA levels in cells described in (A). Error bars indicate standard deviation. Student T-Test was used. *, $P < 0.05$, **, $P < 0.01$, *** $P < 0.001$.

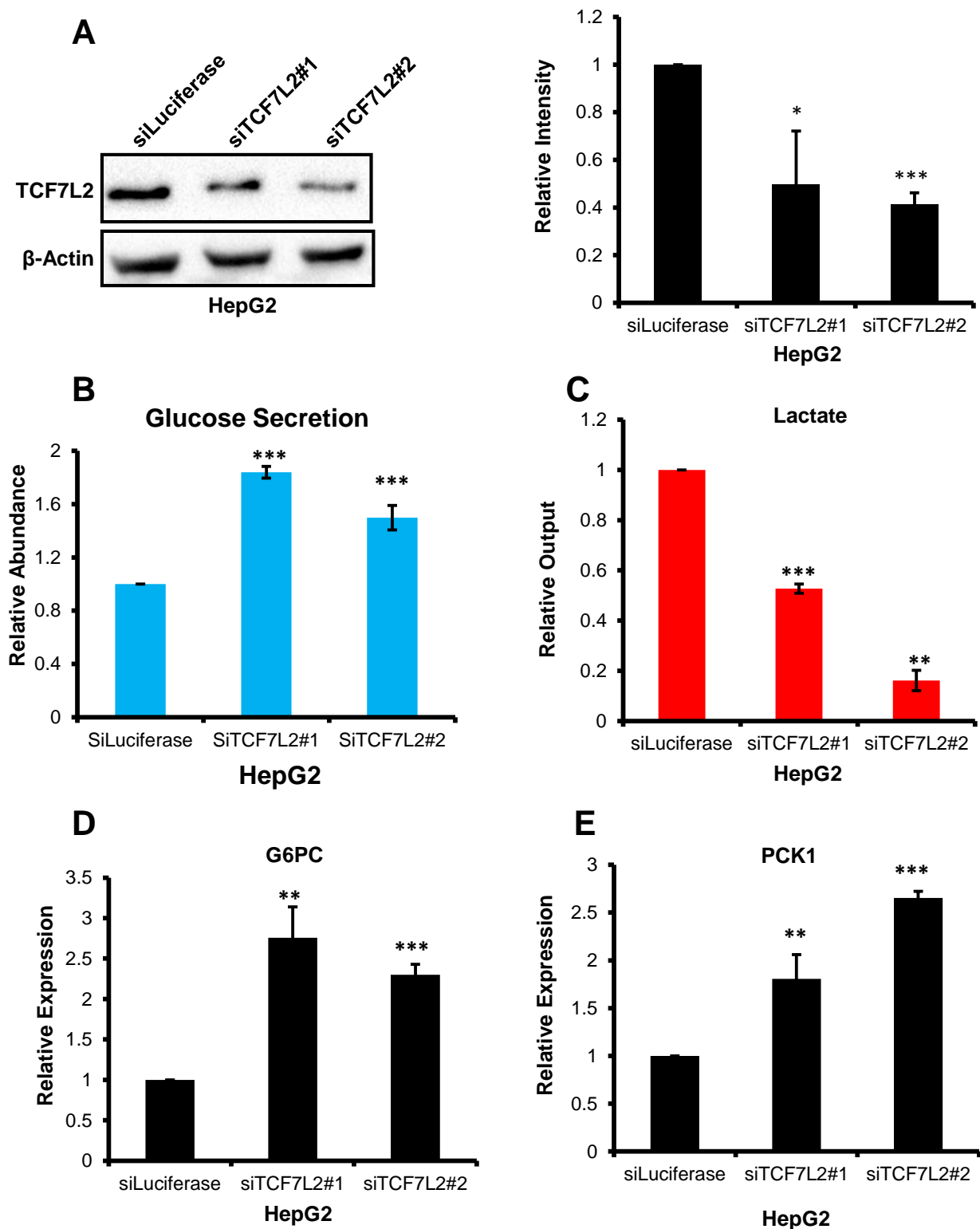


Figure S6: TCF7L2 modulates glucose metabolism in a HCC cell line. **A.** Western blot analysis of HepG2 cells transfected with siRNAs against TCF7L2 (siTCF7L2 #1,2) or control siRNA (siLuciferase) (left panel). Quantification of TCF7L2 protein levels (n=2) (right panel). **B.** Cellular glucose secretion was measured for cells described in (A) using a glucose assay kit (n=3). **C.** Extracellular lactate production was measured in cells described in (A) using a lactate assay kit (n=2). **D and E.** mRNA expression of genes in the gluconeogenesis pathway (G6PC, PCK1) were determined by qRT-PCR using cells described in (A). The error bars indicate S.D. (n=3). Student T-Test was used. *, P<0.05, **, P<0.01, *** P<0.001

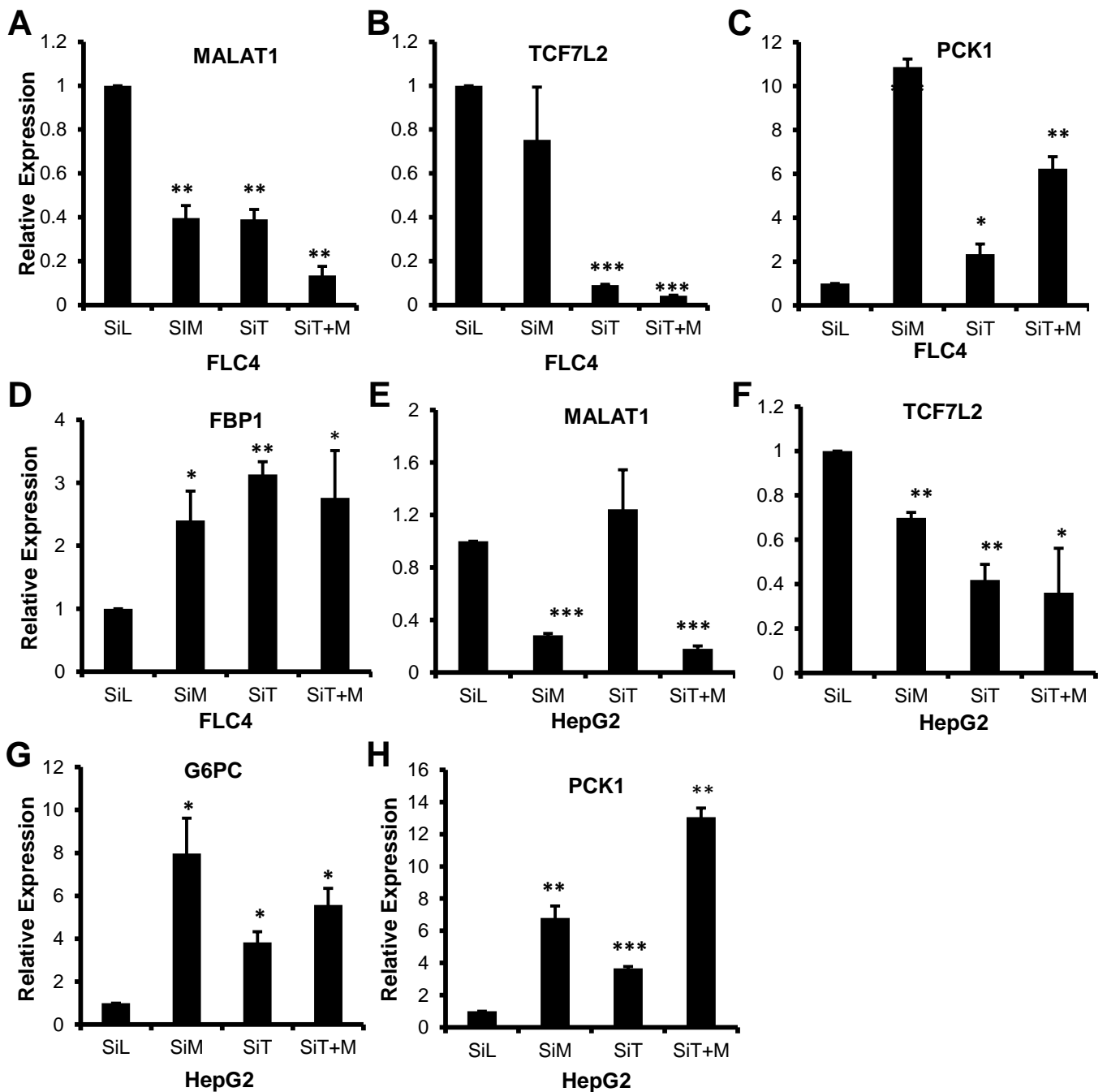


Figure S7: MALAT1 and TCF7L2 regulate gluconeogenesis through the same pathway.

A. qRT-PCR analysis of MALAT1 expression in FLC4 cells transfected with siRNAs against MALAT1(SiM) or TCF7L2(SiT) or both (SiT+M). SiRNA against Luciferase (SiL) was used as a control. **B.** qRT-PCR analysis of TCF7L2 expression for cells described in (A). **C** and **D.** qRT-PCR analysis of gluconeogenesis pathway genes (PCK1 and FBP1) for cells described in (A). (n=2). **E.** qRT-PCR analysis of MALAT1 expression in HepG2 cells transfected with siRNAs against MALAT1(SiM) or TCF7L2(SiT) or both (SiT+M). SiRNA against Luciferase (SiL) was used as control. **F.** qRT-PCR analysis of TCF7L2 expression for cells described in (A). **G** and **H.** qRT-PCR analysis of gluconeogenesis pathway genes (G6PC and PCK1) for cells described in (E). n=2. Error bars indicate standard deviation. Student T-Test was used. *, P<0.05, **, P<0.01, *** P<0.001.

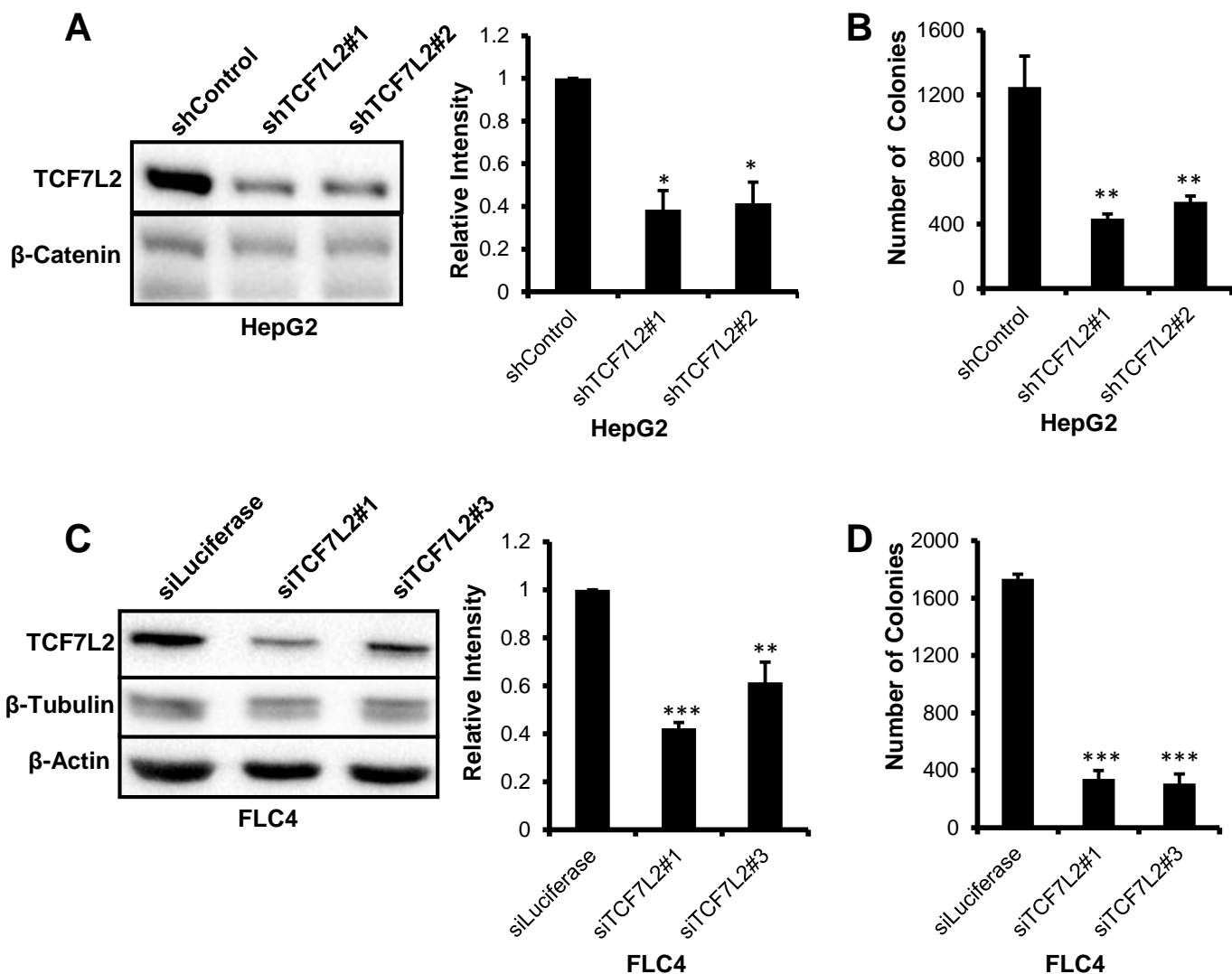


Figure S8: Oncogenic properties of HCC cell lines with TCF7L2 knockdown. **A.** Western blot analysis of HepG2 cells transduced with lentiviruses encoding shRNAs against TCF7L2 (shTCF7L2 #1,2) or an empty vector (shControl). The graph on the right shows quantification of TCF7L2 protein levels (n=2). **B.** Quantification of growth in soft agar assay of cells described in (A). **C.** Western blot analysis of FLC4 cells treated with siRNAs against TCF7L2 (siTCF7L2 #1,2) or control (siLuciferase). The graph on the right shows the quantification of TCF7L2 protein levels (n=3). **D.** Quantification of growth in soft agar assay of cells described in (C). Student T-Test was used. *, P<0.05, **, P<0.01, *** P<0.001.

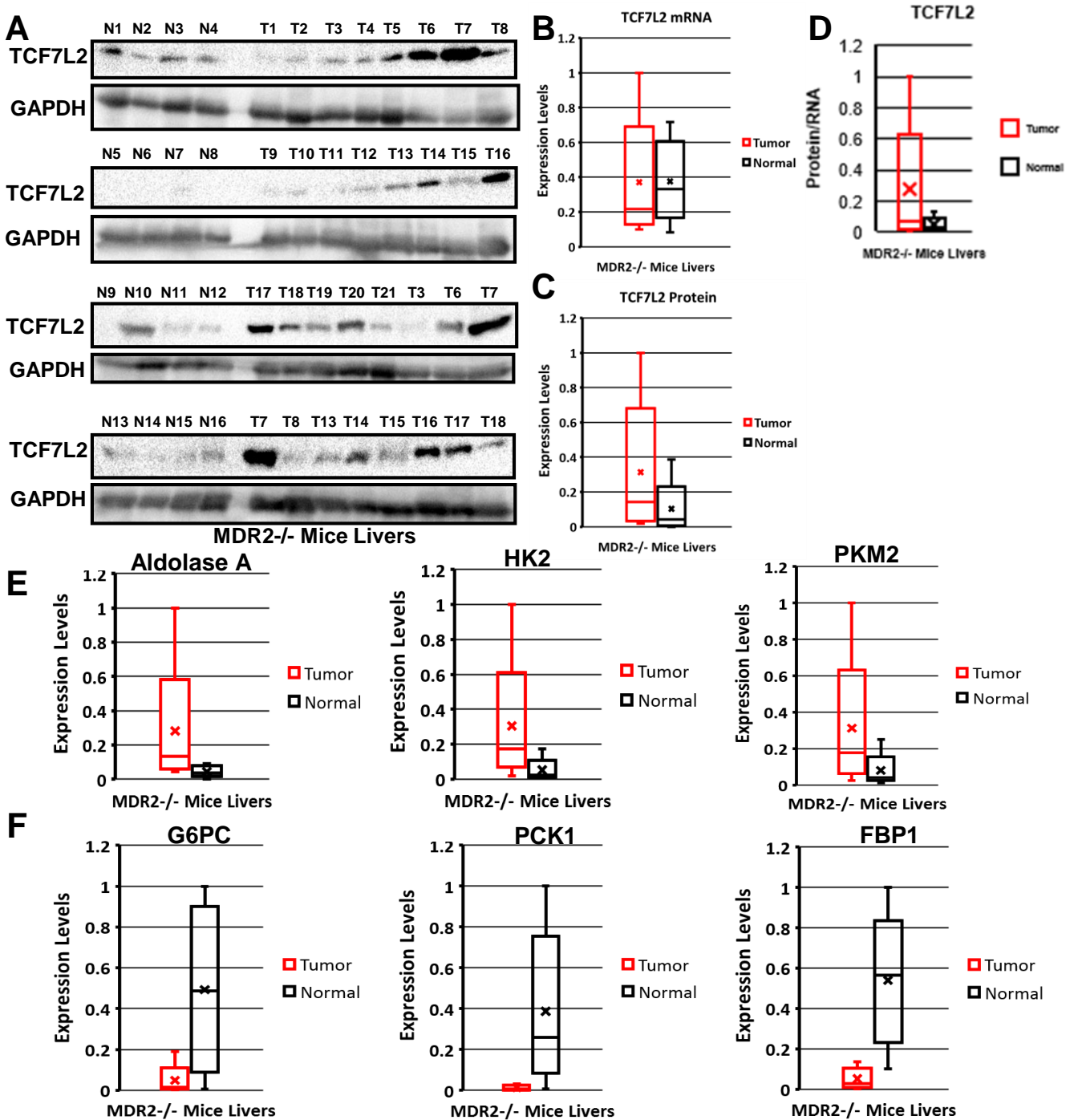


Figure S9: TCF7L2 protein, Gluconeogenesis and Glycolytic enzyme expression in livers from mouse HCC model *Mdr2*^{-/-}. **A.** Western blot analysis using protein isolated from 16 adjacent inflamed liver parenchyma (N) and 20 tumor liver samples (T) from *Mdr2*^{-/-}-mice. Some tumor samples were analysed twice to have both normal and tumor samples on the same blot for western blot analysis. **B.** Box plot representation of TCF7L2 mRNA levels derived from qRT-PCR analysis of RNA isolated from 16 adjacent inflamed liver parenchyma and 20 tumor liver samples from *Mdr2*^{-/-}-mice. **C.** Box plot representation of TCF7L2 protein levels derived from western blot analysis of protein isolated from 16 adjacent inflamed liver parenchyma and 20 tumor liver samples from *Mdr2*^{-/-}-mice. **D.** Box plot representation of TCF7L2 protein/TCF7L2 mRNA levels derived from samples described in B and C. **E.** Box plot representation of mRNA levels of glycolytic genes (Aldolase A, HK2, PKM2) from qRT-PCR analysis of RNA isolated from 11 adjacent inflamed liver parenchyma (Normal) and 19 tumor liver samples (Tumor) from *Mdr2*^{-/-} mice. All samples were normalized to GAPDH mRNA levels. **F.** Box plot representation of mRNA levels of gluconeogenic genes (G6PC, PCK1, FBP1) from qRT-PCR analysis of RNA isolated from 12 adjacent inflamed liver parenchyma and 16 tumor liver samples from *Mdr2*^{-/-} mice. All samples were normalized to GAPDH mRNA levels.

Table S1. List of siRNAs, shRNAs and primer sequences.

SiRNAs (Sigma):

Mouse MALAT1 siRNAs

SASI_Mm02_00352691

SASI_Hs02_00377093

Human MALAT1 siRNAs

SASI_Hs02_00377093

SASI_Hs02_00377095

Human TCF7L2 siRNAs

SASI_Hs01_00197690

SASI_Hs01_00197692

Mouse TCF7L2 siRNAs

SASI_Hs01_00197690

SASI_Hs01_00197692

Mouse ShRNAs

ShTCF7L2#1

Sequence:CCGGGCGCCAACGACGAACTGATTTCTCGAGAAATCAGTTCGTCGTTGGCGCTTTTTTG

ShTCF7L2#2

Sequence:CCGGAGAGAAGAGCAAGCGAAATACCTCGAGGTATTTGCTTGCTCTTCTTTTTTG

ShSF2#1

Sequence:CCGGGCAACCACGAAACCTGTAATACTCGAGTATTACAGGTTTCGTGGTTGCTTTTTTG

ShSF2#2

Sequence:CCGGAGATCTCGCTCTCGTACATAACTCGAGTTATGTACGAGAGCGAGATCTTTTTTG

Human ShTCF7L2

ShTCF7L2#1

Sequence:CCGGCCGAAAGTTTCCGAGACAAATCTCGAGATTTGTCTCGGAAACTTTCGGTTTTTG

ShTCF7L2#2

Sequence:CCGGCCTTTCACCTCCTCCGATTACCTCGAGGTAATCGGAGGAAGTGAAAGGTTTTTG

ShTCF7L2#3

Sequence:CCGGAGAGAAGAGCAAGCGAAATACCTCGAGGTATTTGCTTGCTCTTCTTTTTTG

ShSF2#1

Sequence:CCGGACTTACCTCCAGACATCCGAACTCGAGTTCGGATGTCTGGAGGTAAGTTTTTTG

ShSF2#2

Sequence:CCGGGCAACCACGAAACCTGTAATACTCGAGTATTACAGGTTTCGTGGTTGCTTTTTG

ShSF2#3

Sequence:CCGGGAAGCAGGTGATGTATGTTATCTCGAGATAACATACATCACCTGCTTCTTTTTG

PCR Primers

Human Actin For GGCACCCAGCACAATGAAGA

Human Actin Rev AGGATGGAGCCCGCCGATC

Human PGK1 For CTTGGGACAGCAGCCTTAAT

Human PGK1 Rev CAAGCTGGACGTAAAGGGA

Human Eno1 For GAATCCCACTGTTGAGGTTGA

Human Eno1 Rev ATCATTGTCCCGGAGCTCTA

Human PKM For GGAATGAATGTGGCTCGTCT

Human PKM Rev TAGAGGATGGGGTCAGAAGC

Mouse Eno1 For GGAATCCCACTGTTGAGGTC

Mouse Eno1 Rev CCATGAAGCGGGTCTTATCA

Mouse PGK1 For CAGCCTTGATCCTTTGGTTG

Mouse PGK1 Rev CTGACTTTGGACAAGCTGGA

Mouse PKM For CCATGCAGAGACCATCAAGA
Mouse PKM Rev GAGTCCAGTCCGGATCTCAG
Human GAPDH For TTAAAAGCAGCCCTGGTGAC
Human GAPDH Rev CTCTGCTCCTCCTGTTTCGAC
Human PCK1 For GACCTGCTGGTGTCCCTCTA
Human PCK1 Rev GCATGGCAAAGGGGTCAT
Human FBP1 For TGACCCAGCTGCTCAACTC
Human FBP1 Rev TGATCACCTGTCACGTTGGT
18 S For ATCAACTTTCGATGGTAGTCG
18 S Rev TCCTTGGATGTGGTAGCCG
Mouse Slc2a1 For GAGTGTGGTGGATGGGATG
Mouse Slc2a1 Rev AACACTGGTGTTCATCAACGC
Mouse Hk2 For GGAACCGCCTAGAAATCTCC
Mouse HK2 Rev GGAGCTCAACCAAACCAAG
Mouse LDHA For TTCAGCGCGGTTCCGTTAC
Mouse LDHA Rev CCGGCAACATTCACACCAC
Mouse Aldolase A For GGTGATCCTCTTCCACGAGA
Mouse Aldolase A Rev GGGCACCACACCCTTATCTA
Human MALAT1 For GACGGAGGTTGAGATGAAGC
Human MALAT1 Rev ATTCGGGGCTCTGTAGTCCT

Mouse MALAT1 For AGCTTTTGAGGGCTGACTGC
Mouse MALAT1 Rev CCATTCATTCCCCTCTGAGC
Mouse GAPDH For ATCAAGAAGGTGGTGAAGCAG
Mouse GAPDH Rev GCTGTTGAAGTCGCAGGAGA
Mouse G6PC For CAGTGGTCCGAGACTGGTTC
Mouse G6PC Rev ACAGGTGACAGGGAAGTCTGCT
Mouse PCK1 For CTGGATGAAGTTTGTATGCC

Mouse PCK1 Rev TGTCTTCACTGAGGTGCCAG

Mouse Fbp1 For CGATCAAAGCCATCTCGTCT

Mouse Fbp1 Rev ACTTGATCCCCAGTCACATTG

Mouse TCF7L2 For TAAATCCCGGGAAAGTTTGG

Mouse TCF7L2 Rev GGGATCATGATGAAGGGGTA

Human G6PC For CTACAGCAACACTTCCGTGC

Human G6PC Rev GTATACACCTGCTGTGCCCAT

Human TCF7L2 For ATGGCCACTGCTTGATGTC

Human TCF7L2 Rev GCACCACTGGCACTTTGTTA