SUPPLEMENTAL FIGURE AND TABLE LEGENDS

Figure S1: Acetate rescues histone acetylation but not AMPK or UPR activation in low glucose. (A) Western blot detecting p-AMPKα in whole cell lysate after LN229 cells were treated for 24 hours under indicated conditions. (B) Relative mRNA levels of ER stress related genes as detected by RT-qPCR in LN229 cells. (***, $p<0.001$; ****, $p<0.0001$). (C) Detection of histone acetylation as determined by western blot of acid extracted histones after 24 hours of indicated treatment. (D) Relative adhesion onto 1% fibronectin after 24 hours of treatment with C646 or vehicle control (DMSO) (***, $p<0.001$).

Figure S2: Multiple sources of acetyl-CoA can promote adhesion. (A) Relative levels of citrate in LN229 cells. After treating overnight in 1 mM glucose, medium was replaced with indicated glucose and acetate concentrations. Cells were harvested and citrate measured by LC-MS at indicated timepoints. Significance of acetate conditions compared to 1 mM glucose (##, $p<0.01$; ####, $p<0.0001$). Significance of 10 mM glucose conditions compared to 1 mM glucose (*, $p<0.05$; **, $p<0.01$; ***, $p<0.001$). (B) Percent enrichment of citrate from ^{13}C-acetate under high glucose and low glucose conditions. Cells were starved in 1 mM glucose for 16 hours followed by re-addition of 1 mM or 10 mM glucose for 6 hours, in the presence of ^{13}C-acetate. (C) Adhesion assay on 1% fibronectin after treatment with indicated concentrations of pyruvate. (*, $p<0.05$; **, $p<0.01$; ***, $p<0.001$). (D) Adhesion assay on 1% fibronectin after treatment with BSA conjugated palmitic acid: oleic acid (FA). (**, $p<0.01$; ***, $p<0.001$; ****, $p<0.0001$. (E) mRNA expression in U251 cells after 24 hours treatment with 50 μM ACLYi. ***, $p<0.001$; ****, $p<0.0001$). (F) Tumor volume of tumors formed from parental LN229 cells (LCV2) and ACLY KO clone (sg3.8). For each condition, n = 7. (*, $p<0.05$; ***, $p<0.001$) (G) Tumor weight (***, $p<0.001$). (H) Relative mRNA expression of acetyl-CoA up-regulated genes in xenograft derived tumors. (*, $p<0.05$; ***, $p<0.001$; ****, $p<0.0001$).

Figure S3: ChIP-Rx reveals quantitative changes in H3K27 acetylation. (A) Summary of peak regions (left) and summary of distance of peaks to nearest gene transcription start site (TSS) (right). (B) Ratio of total aligned *Homo sapiens* reads: total aligned *Drosophila melanogaster* reads in H3K27ac and H3 ChIP samples, as well as in input, under each condition. (C) Venn diagram of the genes nearest to differential H3K27ac peaks (regions that gained acetylation over 1 mM glucose conditions) and acetyl-CoA up-regulated genes defined by RNA-seq. (D) Features of the regions that DiffBind defined as differentially H3K27ac bound in each indicated condition, as compared to 1 mM glucose condition (FDR < 0.01).

Figure S4: Examples of H3K27ac ChIP-seq tracks, comparing traditional normalization and reference genome normalization. (A) Tracks for *ITGA9*, an acetyl-CoA upregulated gene that exhibits differential H3K27ac peaks at multiple intronic loci (exons displayed as vertical solid blue

line). (B) Tracks for *MMP11*, an acetyl-CoA upregulated gene that exhibits reduced H3K27ac peak height surrounding the TSS. (C) Tracks for *RPL19*, a gene that is not responsive to acetyl-CoA.

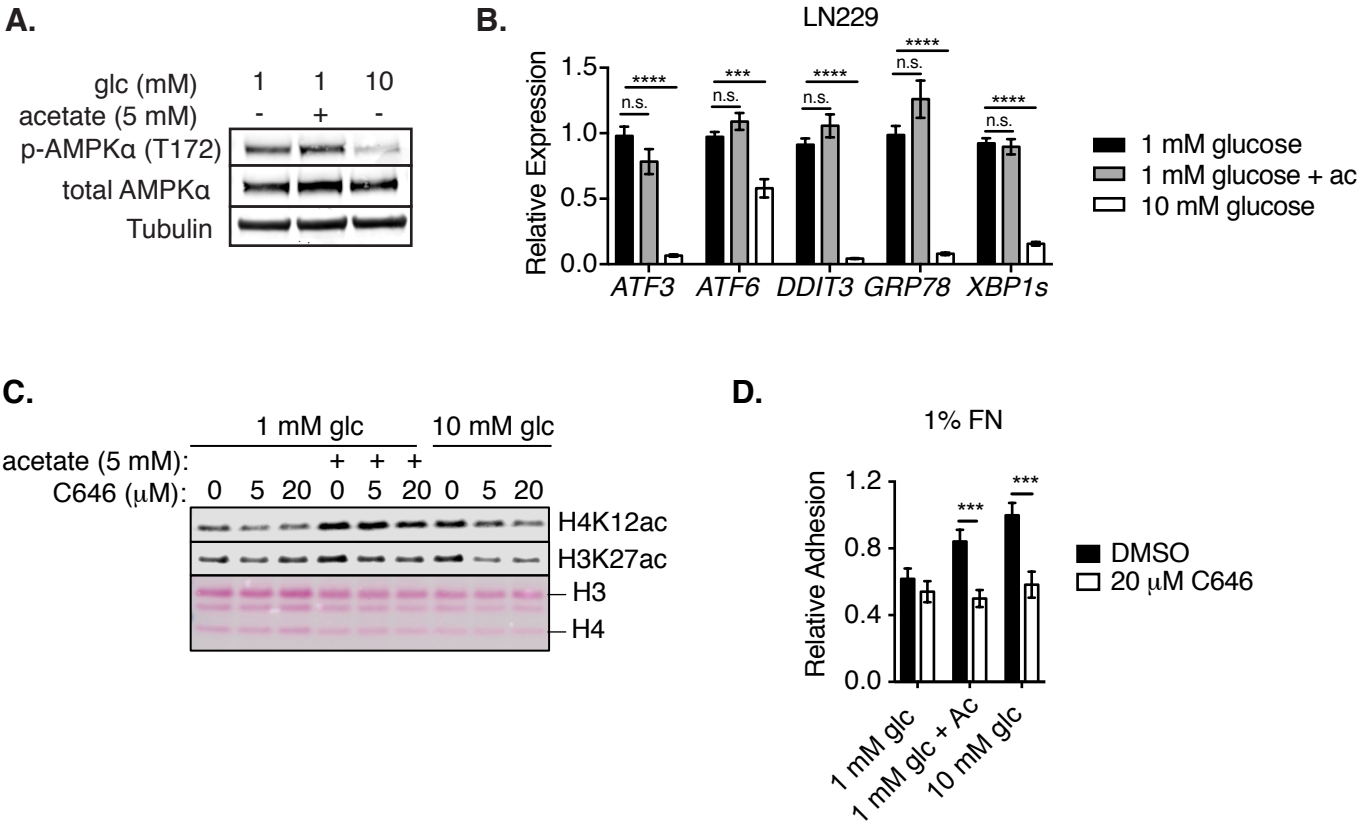
Figure S5: **NFAT family proteins are predicted to bind near promoters of acetyl-CoA upregulated genes**. (A) Top motifs present within 2 Kb of the TSS of acetyl-CoA upregulated genes. (B) Heatmap representation of genes in NFAT signature in Figure 4A.

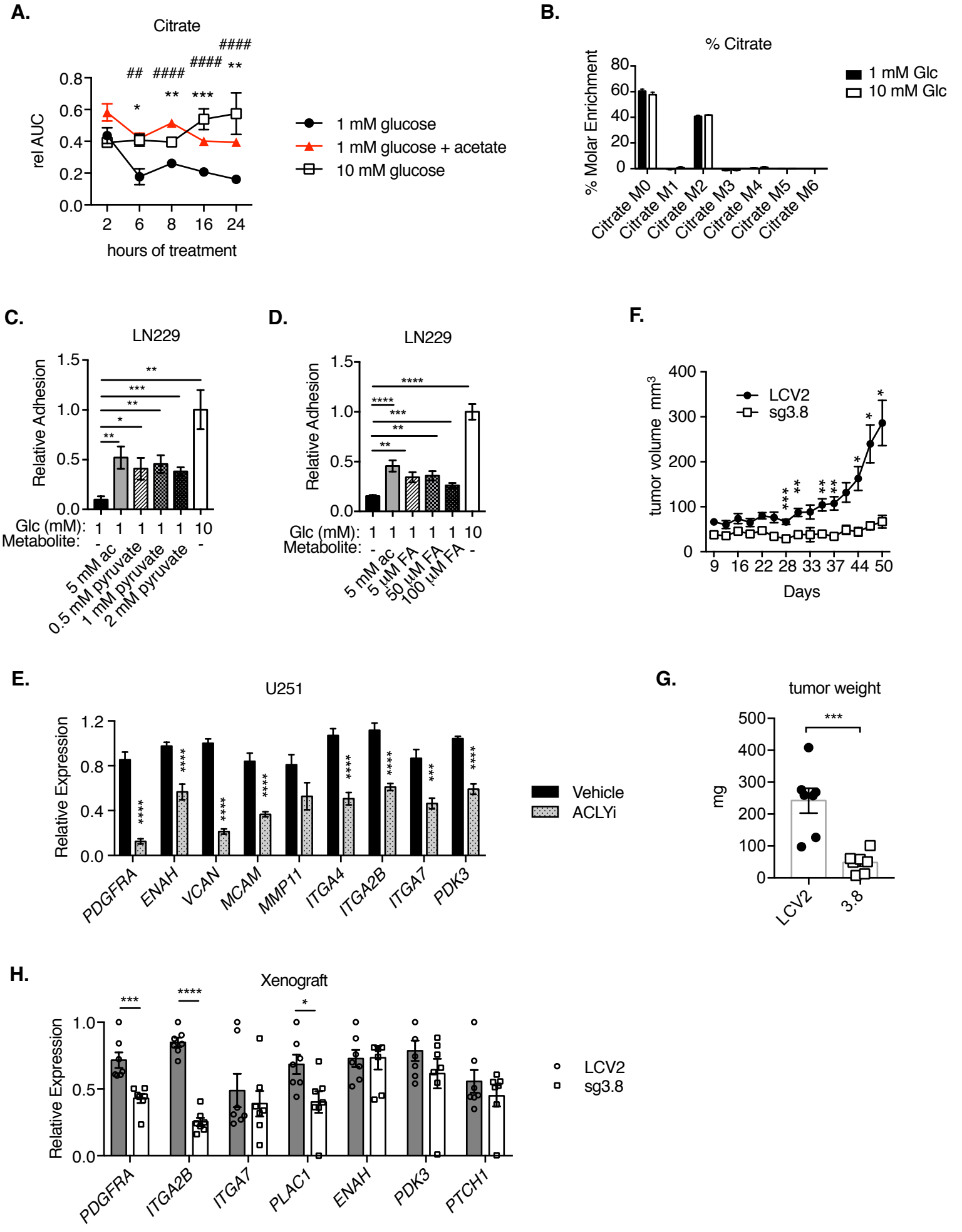
Figure S6: NFAT inhibition reduces cell adhesion. (A) Relative adhesion of U251 cells onto 1% fibronectin after 24 hour treatment with CsA (20 μM). (****, $p<0.0001$). (B) Relative mRNA levels of each NFAT family member in LN229. (C) Representative image of immunofluorescent staining of NFAT1 demonstrating knockdown efficiency. (D) Relative expression of genes after knockdown of NFAT1 with siRNA in LN229 cells. (*, $p<0.05$; **, $p<0.01$; ****, $p<0.0001$).

Figure S7. NFAT1 regulation by glucose and ACLY. (A) Western blot analyzing exogenously expressed HA-tagged NFAT1 after 16 hour of treatment in indicated glucose and acetate conditions followed by 15 minutes of treatment with 1 μM ionomycin or vehicle before harvest. (B) Western blot of exogenously expressed HA-tagged NFAT1 after 16 hours of treatment in 10 mM glucose and indicated concentrations of ACLYi followed by 15 minutes of treatment with 1 μM ionomycin or vehicle before harvest.

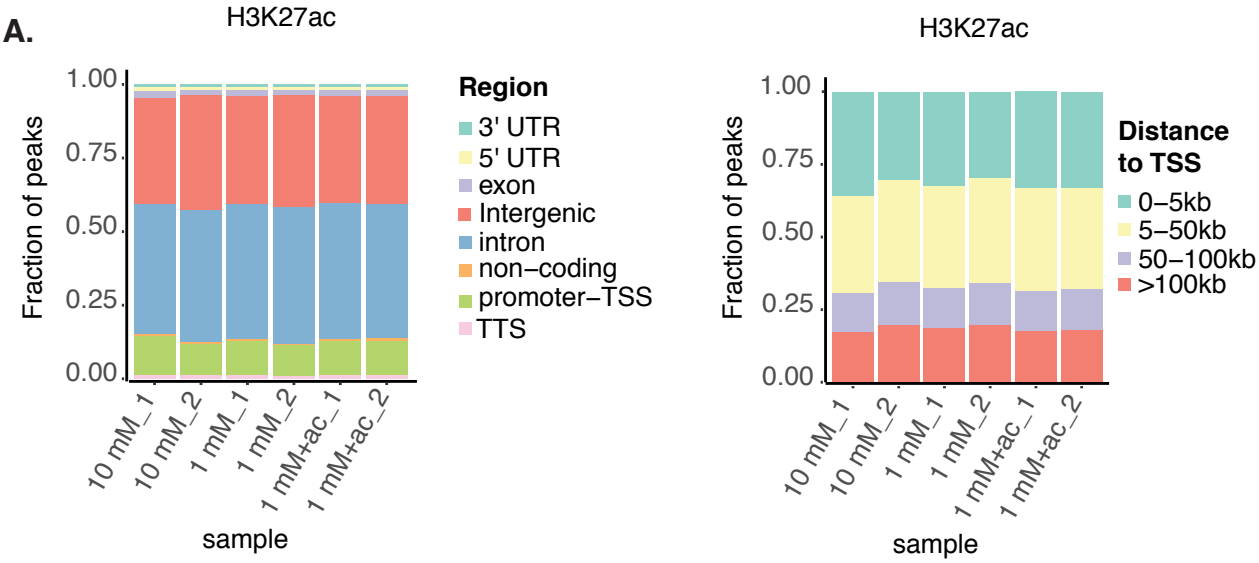
Figure S8: **Mitochondrial function is needed for NFAT1 nuclear localization**. (A) LC-MS quantification of phosphoenolpyruvate (PEP). (****, $p<0.0001$, Mean +/- SD). (B) LN229 cells expressing HA-NFAT1 (WT) were treated +/- rotenone (0.5 μM), piericidin (0.5 μM), or both and nuclear and cytosolic fractions prepared and analyzed by western blot.

Table S1: List of primer sequences.

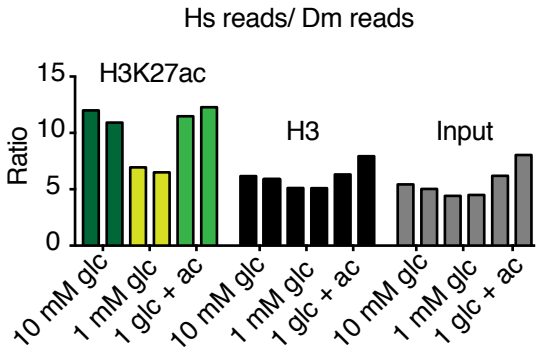

A.
glc (mM) 1 1 10
acetate (5 mM) - + -
p-AMPKα (T172)
total AMPKα
Tubulin
B.
LN229
Relative Expression
1.5
1.0
0.5
0.0
****
n.s.
***
****
****
n.s.
****
n.s.
ATF3
ATF6
DDIT3
GRP78
XBP1s
1 mM glucose
1 mM glucose + ac
10 mM glucose
C.
1 mM glc
10 mM glc
acetate (5 mM): + + + + + +
C646 (μM): 0 5 20 0 5 20 0 5 20
H4K12ac
H3K27ac
H3
H4
D.
1% FN
Relative Adhesion
1.2
0.8
0.4
0.0
***
***
1 mM glc
1 mM glc + Ac
10 mM glc
DMSO
20 μM C646


A.
Citrate
rel AUC
hours of treatment
1 mM glucose
1 mM glucose + acetate
10 mM glucose
B.
% Citrate
% Molar Enrichment
1 mM Glc
10 mM Glc
Citrate M0
Citrate M1
Citrate M2
Citrate M3
Citrate M4
Citrate M5
Citrate M6
C.
LN229
Relative Adhesion
Glc (mM):
Metabolite:
5 mM ac
0.5 mM pyruvate
1 mM pyruvate
2 mM pyruvate
D.
LN229
Relative Adhesion
5 mM ac
5 µM FA
50 µM FA
100 µM FA
F.
tumor volume mm3
LCV2
sg3.8
Days
E.
U251
Relative Expression
Vehicle
ACLYi
PDGFRA
ENAH
VCAN
MCAM
MMP11
ITGA4
ITGA2B
ITGA7
PDK3
G.
tumor weight
mg
LCV2
3.8
H.
Xenograft
Relative Expression
LCV2
sg3.8
PDGFRA
ITGA2B
ITGA7
PLAC1
ENAH
PDK3
PTCH1

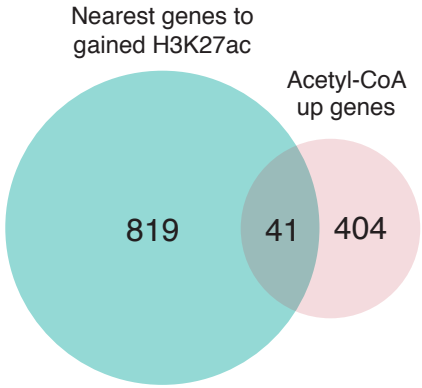
A.


H3K27ac
Fraction of peaks
1.00
0.75
0.50
0.25
0.00
10 mM_1
10 mM_2
1 mM_1
1 mM_2
1 mM+ac_1
1 mM+ac_2
sample
Region
3' UTR
5' UTR
exon
Intergenic
intron
non−coding
promoter−TSS
TTS
H3K27ac
Fraction of peaks
sample
Distance to TSS
0−5kb
5−50kb
50−100kb
>100kb

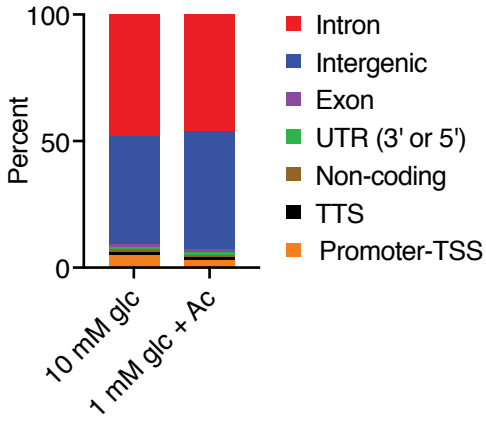
B.

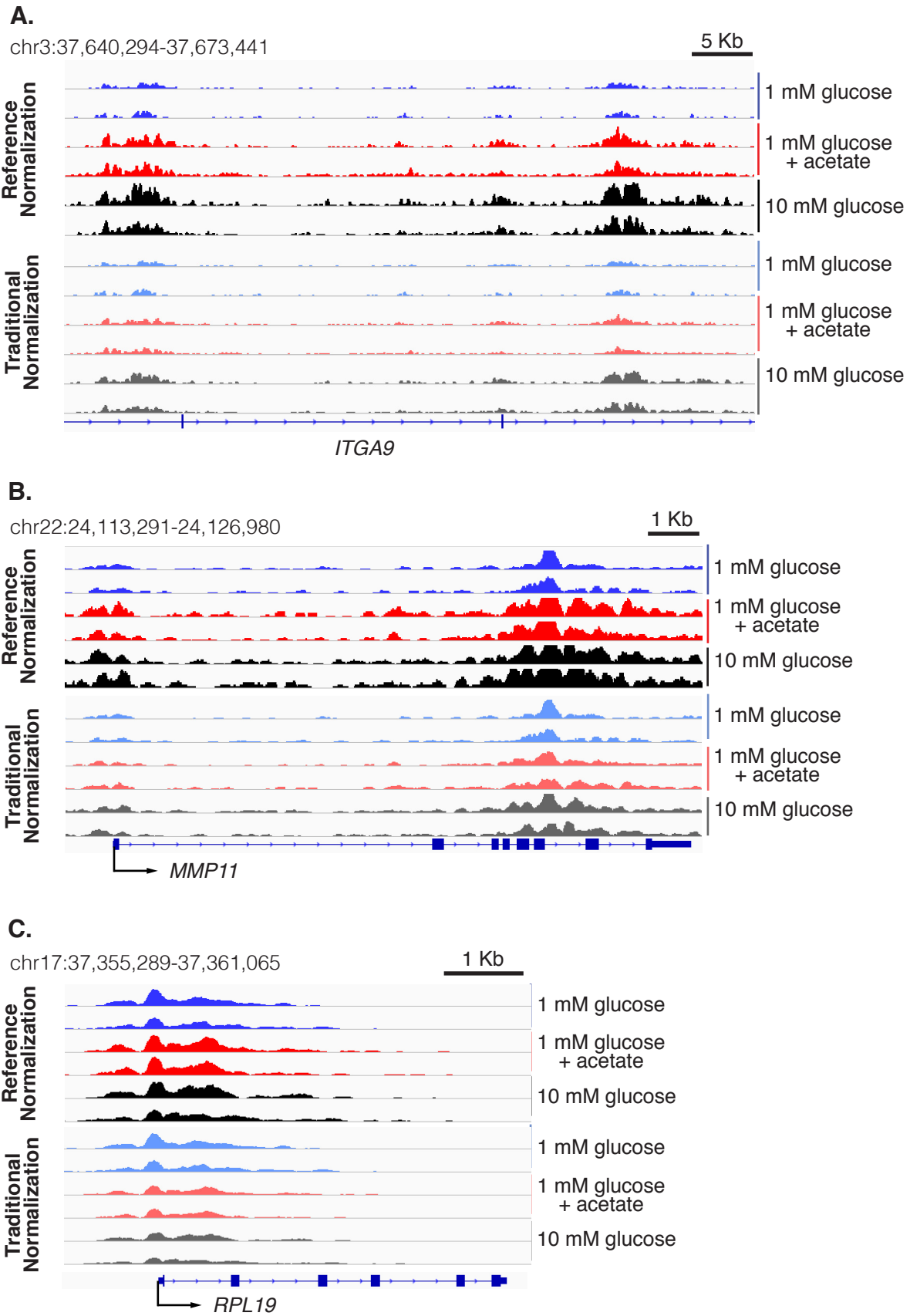

Hs reads/ Dm reads
H3K27ac
H3
Input
Ratio
15
10
5
0
10 mM glc
1 mM glc
1 glc + ac

C.


Nearest genes to gained H3K27ac
Acetyl-CoA up genes
819
41
404

D.

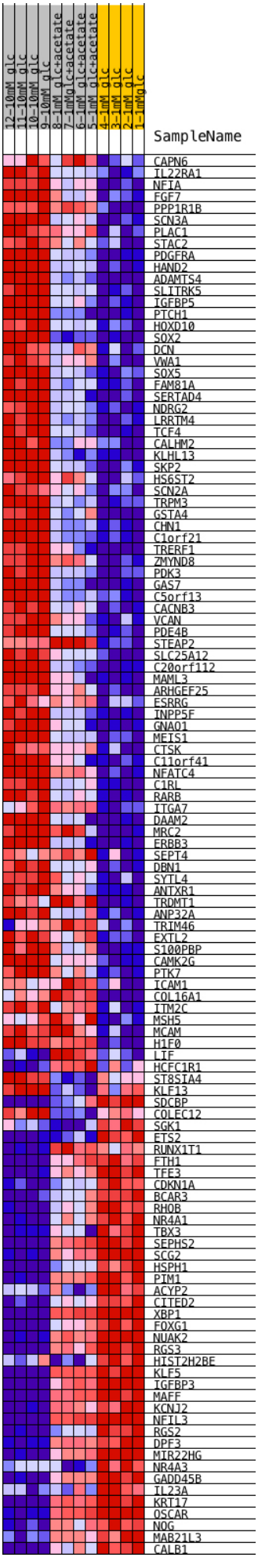

Features
Percent
100
50
0
10 mM glc
1 mM glc + Ac
Intron
Intergenic
Exon
UTR (3' or 5')
Non-coding
TTS
Promoter-TSS

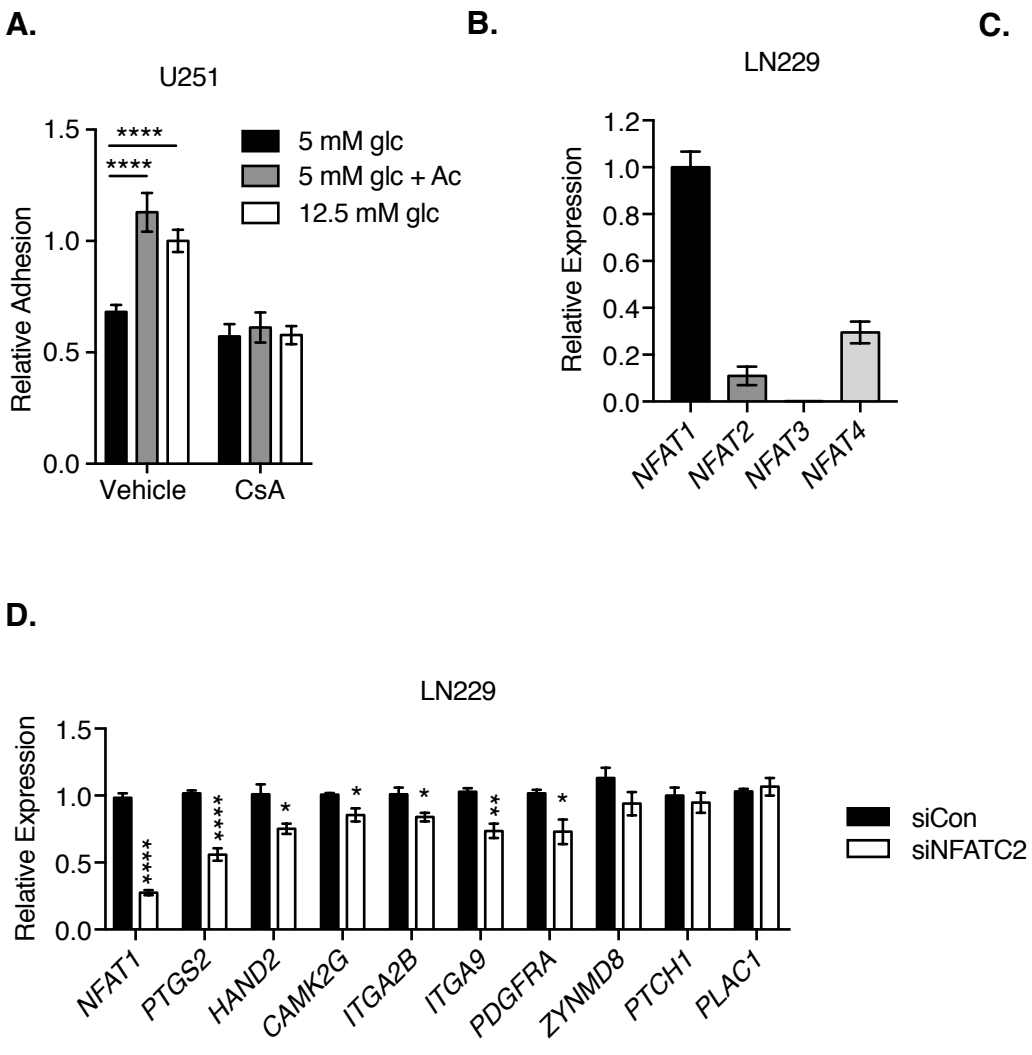

A.
chr3:37,640,294-37,673,441
5 Kb
Reference Normalization
1 mM glucose
1 mM glucose + acetate
10 mM glucose
Traditional Normalization
1 mM glucose
1 mM glucose + acetate
10 mM glucose
ITGA9
B.
chr22:24,113,291-24,126,980
1 Kb
Reference Normalization
1 mM glucose
1 mM glucose + acetate
10 mM glucose
Traditional Normalization
1 mM glucose
1 mM glucose + acetate
10 mM glucose
MMP11
C.
chr17:37,355,289-37,361,065
1 Kb
Reference Normalization
1 mM glucose
1 mM glucose + acetate
10 mM glucose
Traditional Normalization
1 mM glucose
1 mM glucose + acetate
10 mM glucose
RPL19

A.

TF Motif	FDR q-value
TGGAAA_V$ **NFAT** _Q4_01	9.52E-24
CTTTGT_V$ **LEF1** _Q2	5.93E-21
GGGTGGRR_V$ **PAX4** _03	7.81E-19
GGGAGGRR_V$ **MAZ** _Q6	9.94E-18
TTGTTT_V$ **FOXO4** _01	1.04E-17
CAGGTG_V$ **E12** _Q6	1.63E-17
AACTTT_UNKNOWN	2.60E-17
GGGCGGR_V$ **SP1** _Q6	1.19E-14
CTGCAGY_UNKNOWN	6.09E-14
CAGCTG_V$ **AP4** _Q5	1.33E-13

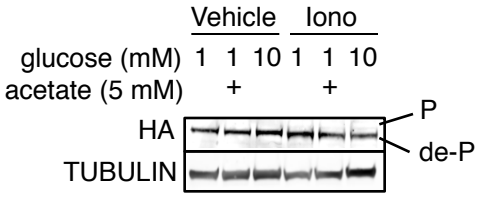
B.



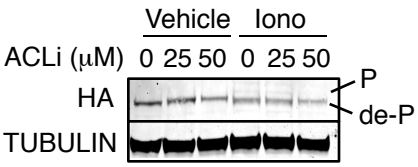

A.
U251
Relative Adhesion
1.5
1.0
0.5
0.0
****
****
5 mM glc
5 mM glc + Ac
12.5 mM glc
Vehicle
CsA
B.
LN229
Relative Expression
1.2
1.0
0.8
0.6
0.4
0.2
0.0
NFAT1
NFAT2
NFAT3
NFAT4
C.
shCon
αNFAT1 DAPI
shNFAT1 #47
αNFAT1 DAPI

D.
LN229
Relative Expression
1.5
1.0
0.5
0.0
****
****
*
*
*
**
*
NFAT1
PTGS2
HAND2
CAMK2G
ITGA2B
ITGA9
PDGFRA
ZYNMD8
PTCH1
PLAC1
siCon
siNFATC2

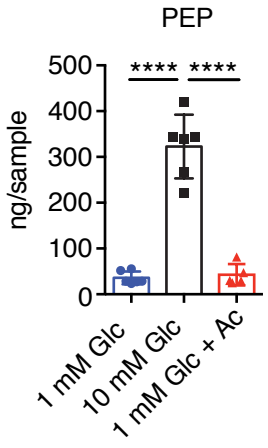
A.


Vehicle
Iono
glucose (mM) 1 1 10 1 1 10
acetate (5 mM) + +
HA
TUBULIN
P
de-P

B.


Vehicle
Iono
ACLi (μM) 0 25 50 0 25 50
HA
TUBULIN
P
de-P

A.


PEP
ng/sample
500
400
300
200
100
0
****
****
1 mM Glc
10 mM Glc
1 mM Glc + Ac

B.

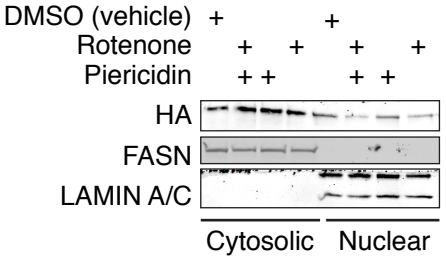

DMSO (vehicle) + +
Rotenone + + + +
Piericidin + + + +
HA
FASN
LAMIN A/C
Cytosolic
Nuclear

Table S1: List of primers

cDNA Primers	**5' -> 3'**
*ENAH*_F	GGACCATCAGGTCGTGATAAA
*ENAH*_R	CCATACACCTGTCTAGCATCTC
*FGF7*_F	GGCCTCCATCCCTCTTACTC
*FGF7*_R	AGCTGCGTGACCTTAGGTGT
*ICAM1*_F	GTCATCATCACTGTGGTAGC
*ICAM1*_R	GGCCTGTTGTAGTCTGTATTT
*MMP11*_F	GGCAGAGGCCCTAAAGGTAT
MMP11 _R	CGAAGTCGATCATGATGTCAG
*VCAN*_F	GGCACCTGTTATCCTACTGAAA
*VCAN*_R	GCTCCATTACGACAGGGATTAG
*ITGA2B*_F	CCCTGGAAGAAGATGATGAAGAG
*ITGA2B*_R	GGAGGCAACTTGTTGGAGAA
*ITGA4*_F	GCCACCCTGAGTCAGTAAATAG
*ITGA4*_R	CTGGAACTTCCTTGCCCTTAT
*ITGA9*_F	ACCAGGAATTTCTTGCCTAAC
*ITGA9*_R	CTGTGTTCAGCAGCATGTA
*PDGFRA*_F	GAAGAAGAGAGCTCCGATGTG
*PDGFRA*_R	TAGCAAGTGTACAACCCTGTG
*MCAM*_F	CTGTTGGAGACAGGTGTTGAA
*MCAM*_R	CTGGTGTGAGGGTGGTTAAAT
*PDK3*_F	ATACCAACCGCATCTCTTTC
*PDK3*_R	GGTGGGATCGATACTTCCTA
*ZMYND8*_F	CGCAGGACACATCAACAA
*ZMYND8*_R	GTGAGTGGCTGCTTCATATAG
*PTCH1*_F	GTTGTGGGCCTCCTCATATT
*PTCH1*_R	GACTTACTCGTCCTCCAACTTC
*PLAC1*_F	GCCCAGAAGGATGAGAAATG
*PLAC1*_R	ACCTGGGTATGCTCTTCTT
*HAND2*_F	TACATCGCCTACCTCATGGA
*HAND2*_R	TCCTTCTTCCTCTTCTCCTCTT
*CAMK2G*_F	CGCAGGTGTGTGAAGAAA
*CAMK2G*_R	CGTTCTAGTTTCTGGTGATCC
*RPL19*_F	CAAGAAGGAGGAGATCATCAAG
*RPL19*_R	ATCACAGAGGCCAGTATGTA
*PTGS2*_F	TTGACAGTCCACCAACTTAC
*PTGS2*_R	GGAGGAAGGGCTCTAGTATAA
*NFATC2*_F	GTGGCAGAATCGTCTCTTTAC

*NFATC2*_R		GCTGTCTGTGTCTTGTCTTT
*GPC6*_F		GTCAGCATTACCCTACACTATC
*GPC6*_R		AGGCAAGTATCTGGCTTTG

ChIP primers		**5' -> 3'**
PDGFRA	TSS_F	ATCCCATCTGGTCTGCTTCT
	TSS_R	AGATTGCGCCTTCGCTTT
	-1Kb_F	GGGAGAAGGATGAAGGATGAC
	-1Kb_R	GATGCTCCAGGAACCAGAC
	+1Kb_F	CTGCGGGACAAGCAGAG
	+1Kb_R	CACCTCACTGACCCAACAAA
	-0.5Kb_F	CATATTGGACTCAACAGTTTGCC
	-0.5Kb_R	TCTACAAACTCGGCCCAAC