Supplementary Figure Legends

Figure S1. Lactate, but not acetate, modulates the viability of GBM cells in a manner dependent on MCT1. Related to Figure 1.

(A) U87-EGFRvIII, U251, and DBTRG cells were cultured in different media conditions: physiological media (5 mM glucose, 1 mM glutamine), starvation media (0.5 mM glucose, 0.5 mM glutamine), 5 mM lactate media (0.5 mM glucose, 0.5 mM glutamine, 5 mM lactate), 10 mM lactate media (0.5 mM glucose, 0.5 mM glutamine, 10 mM lactate), 15 mM lactate media (0.5 mM glucose, 0.5 mM glutamine, 15 mM lactate) for 72h and cell viability was assessed by using CellTiter-Glo (n=4). FC: fold change.

(B) KNS42, U251, GBM22, and GBM43 cells were cultured in different media condition for 24h or 96h and cell viability analysis was performed by using Crystal violet staining. Media condition: 0.5-0.5-0: 0.5 mM glucose, 0.5 mM glutamine, 0 mM lactate; 0.5-0.5-5: 0.5 mM glucose, 0.5 mM glutamine, 5 mM lactate; 0.5-0.5-10: 0.5 mM glucose, 0.5 mM glutamine, 10 mM lactate; 0.5-0.5-15: 0.5 mM glucose, 0.5 mM glutamine, 15 mM lactate; 0.5-0.5-20: 0.5 mM glucose, 0.5 mM glutamine, 20 mM lactate (n=5).

(C) KNS42 cells were cultured in standard media (25 mM glucose, 5 mM glutamine) and physiological media (5 mM glucose, 1 mM glutamine), exposed to different concentration of lactate, and cell viability analysis was performed (n=5).

(D) KNS42, GBM22, and GBM43 cells were cultured in different media condition and cell viability analysis was performed. Media condition: 5-1-0: 5 mM glucose, 1 mM glutamine, 0 mM lactate; 0.5-0.5-0: 0.5 mM glucose, 0.5 mM glutamine, 0 mM lactate; 0.5-0.5-10: 0.5 mM glucose, 0.5 mM glutamine, 10 mM lactate; 0.5-0.08-0: 0.5 mM glucose, 0.08 mM glutamine, 0 mM lactate; 0.5-0.08-10: 0.5 mM glucose, 0.08 mM glutamine, 10 mM lactate; 0.5-0.08-20: 0.5 mM glucose, 0.08 mM glutamine, 20 mM lactate (n=3).

(E) KNS42 cells were cultured in different media condition containing lactic acid or sodium lactate and cell viability analysis was performed. Media condition: 0.5-0.5-0: 0.5 mM glucose, 0.5 mM glutamine, 0 mM lactate acid/ sodium lactate; 0.5-0.5-5: 0.5 mM glucose, 0.5 mM glutamine, 5 mM lactate acid/ sodium lactate; 0.5-0.5-10: 0.5 mM glucose, 0.5 mM glutamine, 10 mM lactate acid/ sodium lactate; 0.5-0.5-15: 0.5 mM glucose, 0.5 mM glutamine, 15 mM lactate acid/ sodium lactate; 0.5-0.5-20: 0.5 mM glucose, 0.5 mM glutamine, 20 mM lactate acid/ sodium lactate; 0-0-0: 0 mM glucose, 0 mM glutamine, 0 mM lactate acid/ sodium lactate; 0-0-10: 0 mM glucose, 0 mM glutamine, 10 mM lactate acid/ sodium lactate; 5-1-0: 5 mM glucose, 1 mM glutamine, 0 mM lactate acid/ sodium lactate; 5-5-1: 5 mM glucose, 5 mM glutamine, 1 mM lactate acid/ sodium lactate; 0.5-0.08-0: 0.5 mM glucose, 0.08 mM glutamine, 0 mM lactate acid/ sodium lactate; 0.5-0.08-10: 0.5 mM glucose, 0.08 mM glutamine, 10 mM lactate acid/ sodium lactate (n=3).

(F) KNS42 and GBM22 cells were cultured in physiological media (5 mM glucose, 1 mM glutamine) or starvation media (0.5 mM glucose, 0.5 mM glutamine), treated with increasing concentration of acetate for 24h, and cell viability analysis were performed (n=4).

(G) U251, KNS42, and GBM22 cells were exposed in starvation media (0.5 mM glucose, 0.5 mM glutamine) or lactate media (0.5 mM glucose, 0.5 mM glutamine, 10 mM lactate) overnight and were stained with propidium iodide staining for cell cycle analysis.

(H) KNS42 and GBM22 cells were transfected with non-targeting siNT or MCT1 specific siRNA, exposed in starvation media (0.5 mM glucose, 0.5 mM glutamine) or lactate media (0.5 mM glucose, 0.5 mM glutamine, 10 mM lactate) for 24h, and cell viability analysis was performed (n=3). *the statistic was compared between starvation media and lactate media, +the statistic was compared between siNT and siMCT1.

(I) Standard western blot of KNS42 and GBM22 transduced with non-targeting shNT or MCT1 specific shRNA. Actin is used as a loading control.

(J) KNS42 and GBM22 cells were transfected with non-targeting siNT or MCT1 specific siRNA, were exposed to starvation media (0.5 mM glucose, 0.5 mM glutamine) or lactate media (0.5 mM glucose, 0.5 mM glutamine, 10 mM lactate), and were stained with annexin V/propidium iodide staining (n=3).

Statistical significance was assessed by ANOVA with Dunnett's multiple comparison test. Data are shown as mean ± SD. *p<0.05, **p<0.01,***/ ****p<0.001.

Figure S2. Lactate promotes an oxidative metabolic phenotype in glioblastoma cell lines. Related to Figure 2.

(A) GBM22, GBM43, and KNS42 cells were exposed to the starvation media (0.5 mM glucose, 0.5 mM glutamine) or lactate media (0.5 mM glucose, 0.5 mM glutamine, 10 mM lactate) overnight and were subjected to transcriptomic analysis and followed by GSEA. Shown are the enrichment plots of REACTOME_CELL_CYCLE_CHECKPOINTS and KEGG_DNA_REPLICATION. NES: normalized enrichment score. FDR: false discovery rate.

(B) GBM22, GBM43, and KNS42 cells were exposed to the starvation media (0.5 mM glucose, 0.5 mM glutamine) or lactate media (0.5 mM glucose, 0.5 mM glutamine, 10 mM lactate) overnight and were subjected to transcriptomic analysis. Shown is the heat map of cellular respiration.

(C, D) KNS42 cells were cultured in three different cell media conditions; starvation (0 mM glucose, 0 mM glutamine), lactate media (0 mM glucose, 0 mM glutamine, 10 mM lactate), or physiological media (5 mM glucose, 1 mM glutamine) for 24h. Cells were then harvested and processed for polar metabolite LC/MS analysis. Shown is a heat map of the metabolites in (C) and relative intensity of metabolites in (D). Data are shown as mean ± SD.

Figure S3. Lactate mediated rescue of GBM viability is depend on a functional electron transport chain/oxidative phosphorylation. Related to Figure 2.

(A) KNS42, GBM22, and GBM43 cells were exposed to physiological media (5 mM glucose, 1mM glutamine) or lactate media (0 mM glucose, 0 mM glutamine, 10 mM lactate), treated with indicated concentration of IACS-010759, Oligomycin, or Etoposide for 24h and cell viability analysis was performed (n=3).

(B) KNS42 and GBM22 cells were exposed in physiological media (5 mM glucose, 1mM glutamine) or lactate media (0 mM glucose, 0 mM glutamine, 10 mM lactate), treated with 1nM Oligomycin for 7h and the cells were stained with annexin V/propidium iodide staining (n=3).

(C) Gene expression of OXPHOS related genes were analyzed by RT-qPCR in the KNS42 and GBM22 cells in starvation media (0.5 mM glucose, 0.5 mM glutamine) or lactate media (0.5 mM glucose, 0.5 mM glutamine, 10 mM lactate) for 24h overnight in presence or absence of 1nM of IACS-010759. 18S is used as a house keeping gene (n=4).

(D) KNS42 and GBM22 cells were cultured in different media condition and were analyzed for the NAD/NADH ratio. Media condition: 0-0-0: 0 mM glucose, 0 mM glutamine, 0 mM lactate; 0-0-10: 0 mM glucose, 0 mM glutamine, 10 mM lactate; 5-1-0: 5 mM glucose, 1 mM glutamine, 0 mM lactate; 5-1-10: 5 mM glucose, 1 mM glutamine, 10 mM lactate; 5-1-20: 5 mM glucose, 1 mM glutamine, 20 mM lactate; 0.5-0.5-0: 0.5 mM glucose, 0.5 mM glutamine, 0 mM lactate; 0.5-0.5-10: 0.5 mM glucose, 0.5 mM glutamine, 10 mM lactate; 0.5-0.5-20: 0.5 mM glucose, 0.5 mM glutamine, 20 mM lactate (n=3).

Statistical significance was assessed by a two-tailed student's t-test. Data are shown as mean ± SD. *p<0.05, **p<0.01,***/ ****p<0.001.

Figure S4. U-^{13}C lactate substantially labels the intermediary metabolism and PDHA1 is involved in its pro-survival effect. Related to Figure 3.

(A) Established GBM cells (U87, U87-EGFRVIII, and U87-IDH1-R132H), pediatric GBM cells (KNS42), and patient-derived GBM cells (GBM12, GBM43, and GBM22) were incubated in DMEM depleted of phenol red, glucose, and glutamine, supplemented with 1.5% dialyzed FBS in presence or absence of 10 mM U-^{13}C-labelled lactate for 16 h and were processed for LC/MS polar metabolite analysis. Shown are fraction of the isotopologues for each metabolite (n = 3).

(B) KNS42 cells were exposed to different media conditions containing U-^{13}C-lactate overnight and were performed LC/MS analysis. Media condition: 0-0-10: 0 mM glucose, 0 mM glutamine, 10 mM lactate, 0.5-0.5-10: 0.5 mM glucose, 0.5 mM glutamine, 10 mM lactate, 5-0-10: 5 mM glucose, 0 mM glutamine, 10 mM lactate, 5-1-10: 5 mM glucose, 1 mM glutamine, 10 mM lactate. Shown are fraction of the isotopologues for each metabolite (n=3).

(C) Standard western blot of KNS42, GBM22, and GBM43 with indicated antibodies. Actin is used as a loading control.

(D) Gene expression of PC and PCK1 analyzed by RT-qPCR in astrocyte, KNS42, GBM22, and GBM43 cells. 18S is used as a house keeping gene. FC: fold change over astrocyte.

(E) KNS42 cells were incubated overnight in DMEM depleted of phenol red, glucose, and glutamine containing 10 mM U-^{13}C-labelled and 1.5% dialyzed FBS lactate. Thereafter cells were processed for LC/MS polar metabolite analysis. Shown is the relative intensity of palmitic acid isotopologues.

(F) KNS42 and GBM22 cells were transduced with non-targeting shRNA or PDHA specific shRNA, exposed to physiological media (5 mM glucose, 1 mM glutamine), starvation media (0.5 mM glucose, 0.5 mM glutamine) or lactate media (0.5 mM glucose, 0.5 mM glutamine, 10 mM/ 20 mM lactate) for 72h, and cell viability analysis was performed (n=4).

(G) KNS42 cells were transfected with non-targeting siRNA, PC targeting siRNA, or PDHA targeting siRNA and were exposed to starvation media (0.5 mM glucose, 0.5 mM glutamine) or lactate media (0.5 mM glucose, 0.5 mM glutamine, 10 mM lactate) overnight, and cell viability analysis was performed (n=3). *the statistic was compared between starvation media and lactate media, +the statistic was compared between siNT, siPC, and siPDHA1.

(H) Standard western blot of KNS42 cells transfected with non-targeting siRNA, PC targeting siRNA, or PDHA1 targeting siRNA.

(I, J) KNS42 cells were transfected with non-targeting siRNA, PC targeting siRNA, or PDHA1 targeting siRNA and were exposed to starvation media (0.5 mM glucose, 0.5 mM glutamine) or lactate media (0.5 mM glucose, 0.5 mM glutamine, 10 mM lactate) for 48h, and the cells were stained with annexin V/propidium iodide staining (n=3). The quantification was shown in (J).
Statistical significance was assessed by a two-tailed student's t-test or ANOVA with Dunnett's multiple comparison test. Data are shown as mean ± SD. *p<0.05, **p<0.01,***/ ****p<0.001.

Figure S5. Lactate carbons label the TCA-cycle intermediates in orthotopic GBM PDX models. Related to Figure 4.

(A) Schematic representation of subcutis of immunocompromised Nu/Nu mice. After the tumors were established, the mice were injected with 3-^{13}C-labelled lactate one hour prior to tumor collection.
(B) U87-EGFRvIII were implanted into the subcutis of immunocompromised Nu/Nu mice. After the tumors were established, the mice were injected with 3-^{13}C-labelled lactate one hour prior to tumor collection for LC/MS analysis to assess the labelling pattern of the TCA cycle.

(C) U87-EGFRvIII were implanted into the subcutis of immunocompromised Nu/Nu mice. Serum, normal brain, liver, and GBM tumor were collected for the LC/MS to analyze the absolute lactate level.

(D) U87-EGFRvIII cells were implanted into the right striatum of Nu/Nu mice for three weeks. An equal amount of both 3-^{13}C-labelled lactate (m+1) and U-^{13}C-labelled glucose (m+6) were injected i.p 30 min prior to brain tumor collection for LC/MS analysis to assess the labelling pattern of the TCA cycle intermediates in the normal brain and tumor brain.

(E) Shown are the levels of TCA cycle-intermediates isotopologue m+1 (derived from lactate, blue dot graph) and TCA cycle-intermediates isotopologue m+2 (derived from glucose, red dot graphs) of experiments in (D).

(F) GBM22 cells were implanted into the right striatum of Nu/Nu mice for three weeks. An equal amount of both 3-13C-labelled lactate (m+1) and U-13C-labelled glucose (m+6) were injected i.p 30 min prior to brain tumor collection for LC/MS analysis. Shown are the levels of TCA cycle-intermediates isotopologue m+1 (derived from lactate, blue dot graph) and TCA cycle-intermediates isotopologue m+2 (derived from glucose, red dot graphs).

(G) Two groups of mice implanted with GBM22 cells were randomly assigned: vehicle and CPI613 (50mg/kg) treatment after seven days of the implantation. Mice were treated four times per week by i.p. injection. Shown is the tumor size of representative MRI images using a Bruker BioSpecTM, 9.4 Tesla MR Imager.

(H) H&E staining of the vehicle or CPI613 treated GBM22 tumors treatment in (G) in different organ systems. Scale bar: 120 μm.

(I) The graphs show the relative intensity of MALDI-TOF imaging of the brain tissues treated with vehicle or CPI613.

Statistical significance was assessed by a two-tailed student's t-test or ANOVA with Dunnett's multiple comparison test. Data are shown as mean ± SD. *$p<0.05$, **$p<0.01$.

Figure S6. Lactate affects the localization of histone marks on the DNA and thereby modulates the epigenome. Related to Figure 5 and Figure 6.

(A) Standard western blot of H3K27ac protein level in KNS42 cells cultured in different media condition.

(B) Real-time PCR analysis of genes related to histone acetylation mRNA levels of KNS42, GBM22, and GBM43 cells exposed to starvation media (0.5 mM glucose, 0.5 mM glutamine) or lactate media (0.5 mM glucose, 0.5 mM glutamine, 10 mM lactate) overnight. FC: fold change.

(C) KNS42 were exposed to the media conditions as indicated: starvation media (0.5 mM glucose, 0.5 mM glutamine), lactate media (0.5 mM glucose, 0.5 mM glutamine, 10 mM lactate), or physiological media (5 mM glucose, 1mM glutamine) overnight and harvested for ChIP with H3K27ac antibody followed by massive parallel DNA-sequencing (ChIP-seq). Shown are heatmaps for all peaks detected (+/- 7kb surrounding the peak center) for the different media conditions.

(D-F) KNS42 were exposed to starvation media or lactate media overnight, and chromatin immunoprecipitation with H3K27ac and H3K9ac antibodies were conducted followed by massive parallel DNA-sequencing (ChIP-seq.) Shown are ChIP-seq. tracks for three different categories of gene promoters: OXPHOS-related gene promoters (NDUFB7, NDUFV2 and ATP5MC1), proliferation-related gene promoters (MYC) and MCTs related gene promoters (SLC16A1).

(G) The PDGFRA promoting region was analyzed via qPCR after ChIP-seq analysis and ChIP-seq tracks upon starvation media or lactate media (n = 3).

(H) KNS42 were exposed to starvation media or lactate media overnight, and chromatin immunoprecipitation with H3K27ac and H3K9ac antibodies were conducted followed by massive parallel DNA-sequencing (ChIP-seq.) Shown are ChIP-seq. tracks for three different categories of gene promoters: histone acetyltransferase related gene promoters (HAT1 and KAT2A).

Statistical significance was assessed by a two-tailed student's t-test in (B) and ANOVA with Dunnett's multiple comparison test in (G). Data are shown as mean ± SD. **p<0.01, ***/****p<0.001.

Figure S7. Oxidative energy metabolism (cellular respiration and PDHA1) and ATP-citrate lyase (ACLY) are required for lactate mediated histone acetylation. Related to Figure 7.

(A) Stably transduced shNT or shACLY (3'UTR) KNS42 cells were transduced with empty vector (EV) or ACLY cDNA, exposed to different media culture: physiological media (5 mM glucose, 1mM glutamine), starvation media (0.5 mM glucose, 0.5 mM glutamine), and lactate media (0.5 mM glucose, 0.5 mM glutamine, 10 mM/ 20 mM lactate), and cell viability analysis was performed (n=4).

(B) Standard western blot of stably transduced shNT or shACLY (3'UTR) KNS42 cells were transduced with empty vector (EV) or ACLY cDNA, exposed to lactate media (0.5 mM glucose, 0.5 mM glutamine, 10 mM lactate), and cell viability analysis was performed.

(C) KNS42 cells transfected with non-targeting siNT and ACLY targeting siRNA, exposed to lactate media (0.5 mM glucose, 0.5 mM glutamine, 10 mM lactate), and cell lysates were analyzed with indicated antibodies. Actin is used as a loading control.

(D) KNS42 and GBM22 cells were exposed to physiological media (5 mM glucose, 1 mM glutamine) or lactate media (0.5 mM glucose, 0.5 mM glutamine, 10 mM lactate) and were treated with increasing concentration of IACS-010759 for KNS42 overnight/ GBM22 7h. Thereafter, the cell lysates were collected and analyzed with indicated antibodies.

(E) KNS42 and GBM22 cells were transduced with empty vector (EV) or over-expressed NDl1 protein and were exposed to lactate media (0.5 mM glucose, 0.5 mM glutamine, 10 mM lactate)

in the presence or absence of 1 nM IACS-010759 overnight. Thereafter, the cell lysates were collected and analyzed with indicated antibodies.

(F) KNS42 and GBM22 cells were exposed to physiological media (5 mM glucose, 1 mM glutamine) or lactate media (0.5 mM glucose, 0.5 mM glutamine, 10 mM lactate) and were treated with increasing concentration of CPI613 for 24h. Thereafter, the cell lysates were collected and analyzed with indicated antibodies.

(G) Stably transduced shNT or shPDHA in KNS42 and GBM22 cells were exposed to lactate media (0.5 mM glucose, 0.5 mM glutamine, 10 mM lactate), and analyzed by western blot.

(H) Stably transduced non-targeting shNT or PDHA specific shRNA KNS42 cells were transduced with empty vector (EV), PDHA-R wild-type (PDHA cDNA resistant against PDHA shRNA), or PDHA-R S293E, cultured in the presence or absence of 10 mM lactate media, and analyzed by western blot.

Statistical significance was assessed by a two-tailed student's t-test. Data are shown as mean ± SD. ***$p<0.001$.

Supplementary Table 2. Related to Figure 3, Figure S3, Figure S4.

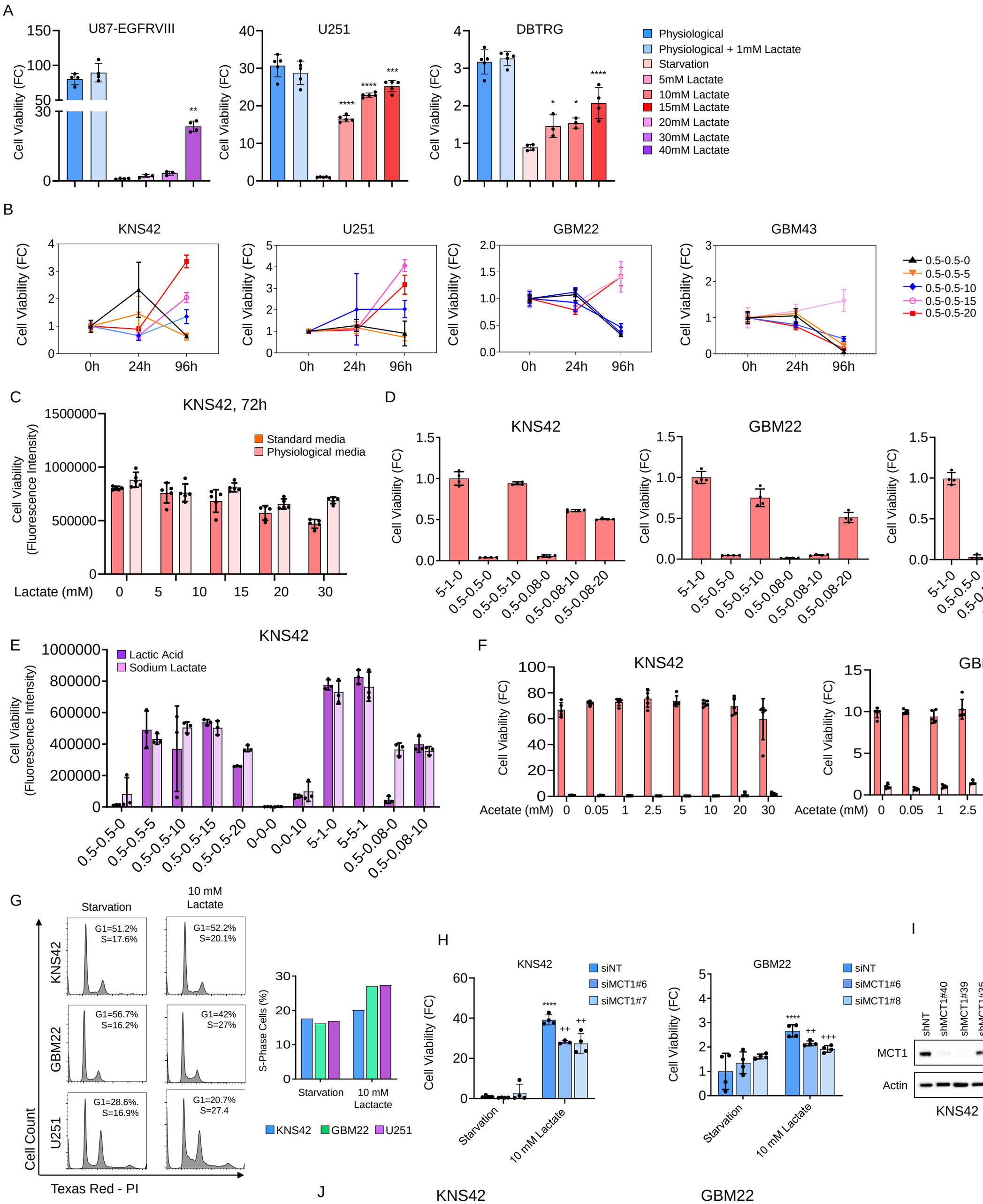
Primer sequences for real time PCR and chromatin immunoprecipitation qPCR

Gene Name	**Fw primer**	**Rv primer**
18S	ACCCGTTGAACCCCATTCGTGA	GCCTCACTAAACCATCCAATCGG
SLC16A1(MCT1)	TTGTTGGTGGCTGCTTGTCAGG	TCATGGTCAGAGCTGGATTCAAG
NDUFB7	CTGCTCAAGTGCAAGCGTGACA	CGCTCAAACTCCTTCATGCGCA
NDUFV2	ACTCTGACAGCATACTGGAGGC	ACCATTGGTGCGTTCACACAGG
NDUFB3	CCAGAAGAAGCTGGCTGCAAAAG	GAATACATCAGAAAAGGAAACACTC
NDUFA3	CTCGCTGTAATTCTGCCCCCAT	GCATGTTCCCATCATCACGGAC
NDUFV1	TGTGTGAGACGGTGCTGATGGA	CGATGGCTTTCACGATGTCCGT
NQO2	GTATGCCATGAACCTTGAGCCG	GCTCATCAGTGATGTCGCTAGC
PC (PCB)	GCCATGTCATGGTAAACGGTCC	GCAGGATGTCTCTGAAACCAGC
PCK1	CATTGCCTGGATGAAGTTTGACG	GGGTTGGTCTTCACTGAAGTCC
HAT1	GGTAGCCTGTCAACAATGTTCCG	CGTGTTTGTGCAAAATCCAGGTG
KAT2B	GCACCATCTCAACGAAGACTGC	GTGTGGTTTCGTACCGAGGTAG
KAT3A	AGTAACGGCACAGCCTCTCAGT	CCTGTCGATACAGTGCTTCTAGG
KAT3B(EP300)	GATGACCCTTCCCAGCCTCAAA	GCCAGATGATCTCATGGTGAAGG
KAT5	GGAACTCACCACATTGCCTGTC	CTCATTGCCTGGAGGATGTCGT
KMT2A	GTGCTTTGTGGTCAGCGGAAGT	TGTGAGACAGCAACCCACGGTG
ACSS1	ATGGGCAGTGAGGACATGCTCT	CACAGCCAAAGATGTCACCTGG
ACSS2	GGTGACCAAGTTCTACACAGCAC	GTTCACCCACTGTGCCTAACAC
PDGFRA -0.5kb	CATATTGGACTCAACAGTTTGCC	TCTACAAACTCGGCCCAAC

Site directed mutagenesis

Gene Name	Fw primer	Rv primer
PDHA1-S293E	CTGACTCCAGGGTCACTCATCTC G TGTCCGTGGTAACGGTAAG	CTTACCGTTACCACGGACACGAGATGA GTGACCCTGGAGTCAG
shPDHA#602_P DHA1-R	CTCTCTCAACAGACGTTCCCA TGCCGTAACGGTTATTCTCAC AGATGAAAATACAAGGT	ACCTTGTATTTTCATCTGTGAGAATAA CCGTTACGGCATGGGAACGTCTGTTGA GAGAG

Figure S1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U87-EGFRVIII
U251
DBTRG
Cell Viability (FC)
Physiological
Physiological + 1mM Lactate
Starvation
5mM Lactate
10mM Lactate
15mM Lactate
20mM Lactate
30mM Lactate
40mM Lactate
B
KNS42
U251
GBM22
GBM43
0.5-0.5-0
0.5-0.5-5
0.5-0.5-10
0.5-0.5-15
0.5-0.5-20
C
KNS42, 72h
Cell Viability (Fluorescence Intensity)
Standard media
Physiological media
Lactate (mM)
D
KNS42
GBM22
GBM43
5-1-0
0.5-0.5-0
0.5-0.5-10
0.5-0.5-20
0.5-0.08-0
0.5-0.08-10
0.5-0.08-20
E
KNS42
Lactic Acid
Sodium Lactate
0-0-0
0-0-10
5-1-0
5-5-1
F
KNS42
GBM22
Physiological
Starvation
Acetate (mM)
G
Starvation
10 mM Lactate
KNS42
GBM22
U251
S-Phase Cells (%)
Cell Count
Texas Red - PI
H
siNT
siMCT1#6
siMCT1#7
siMCT1#8
I
shNT
shMCT1#40
shMCT1#39
shMCT1#35
MCT1
Actin
43 kDa
42 kDa
J
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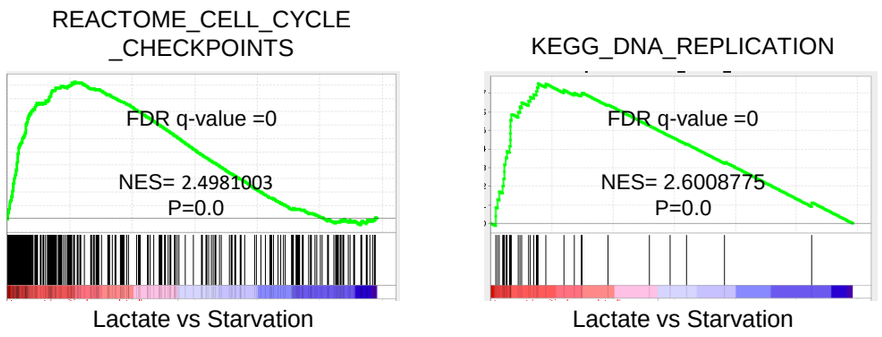
Starvation
PE-PI
FITC-Annexin V

Figure S2

A



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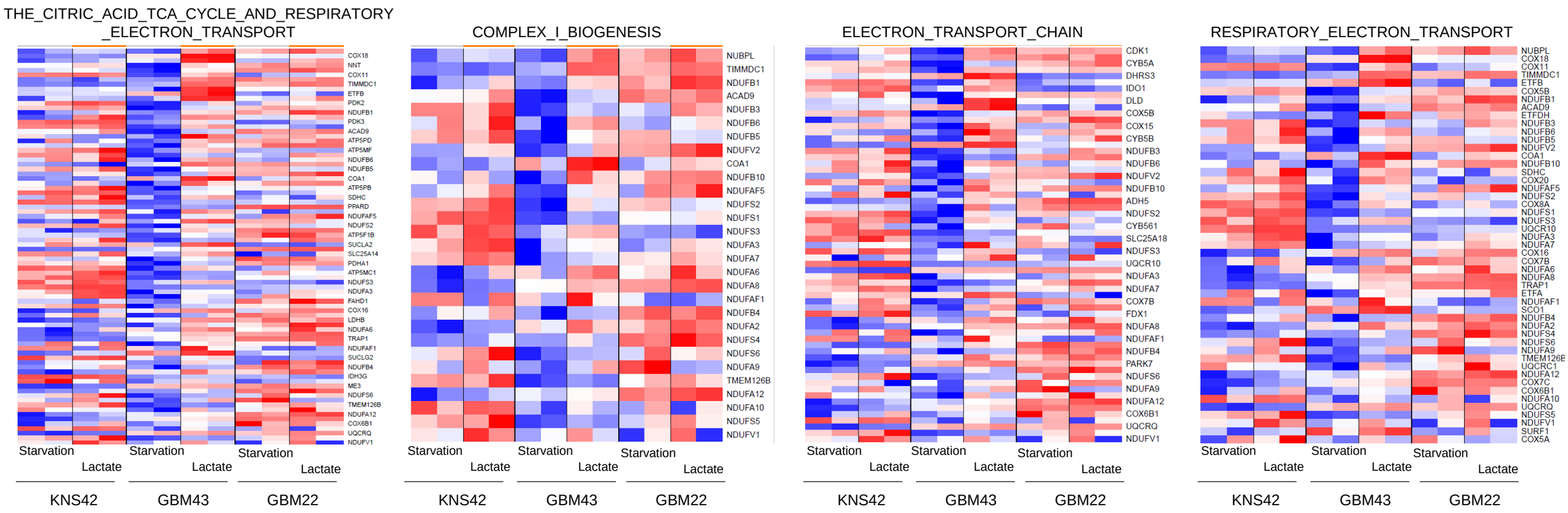

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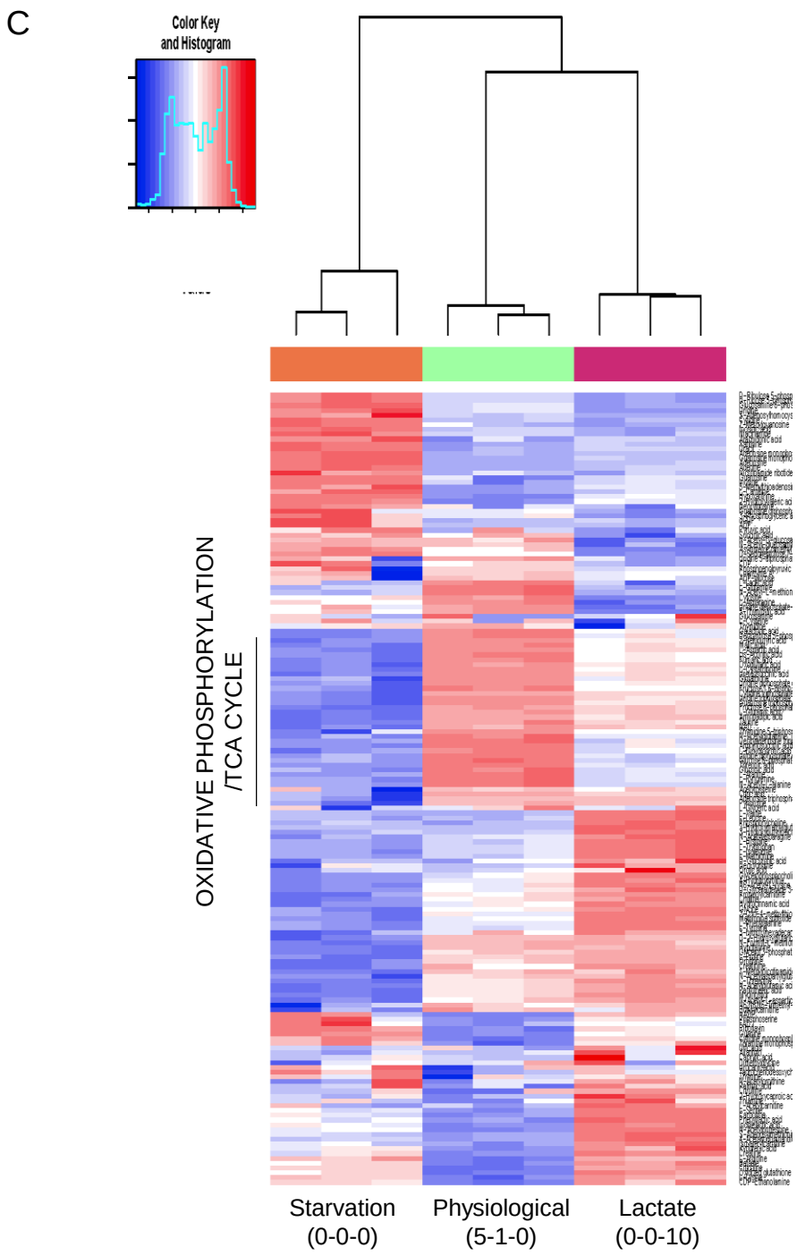

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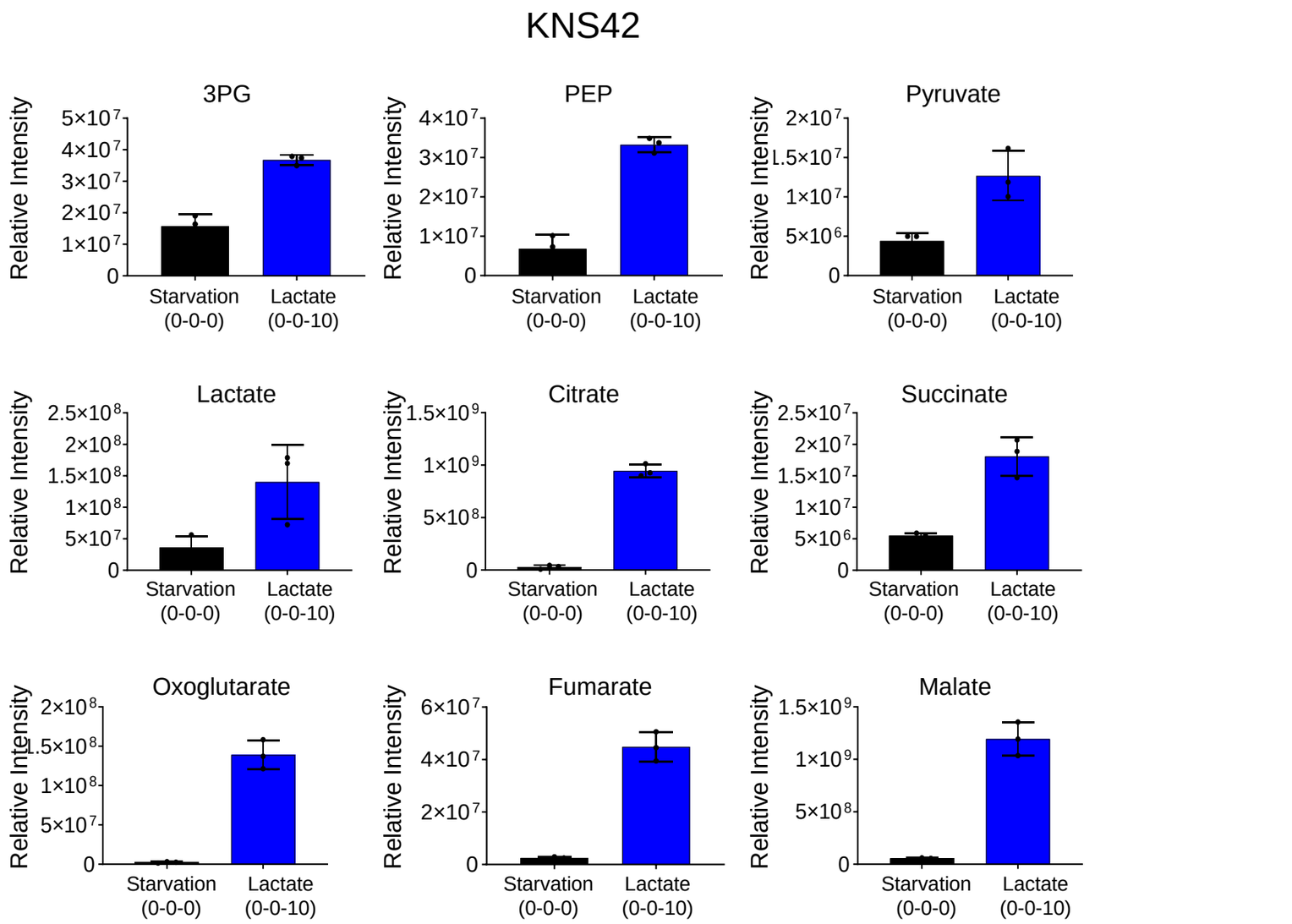
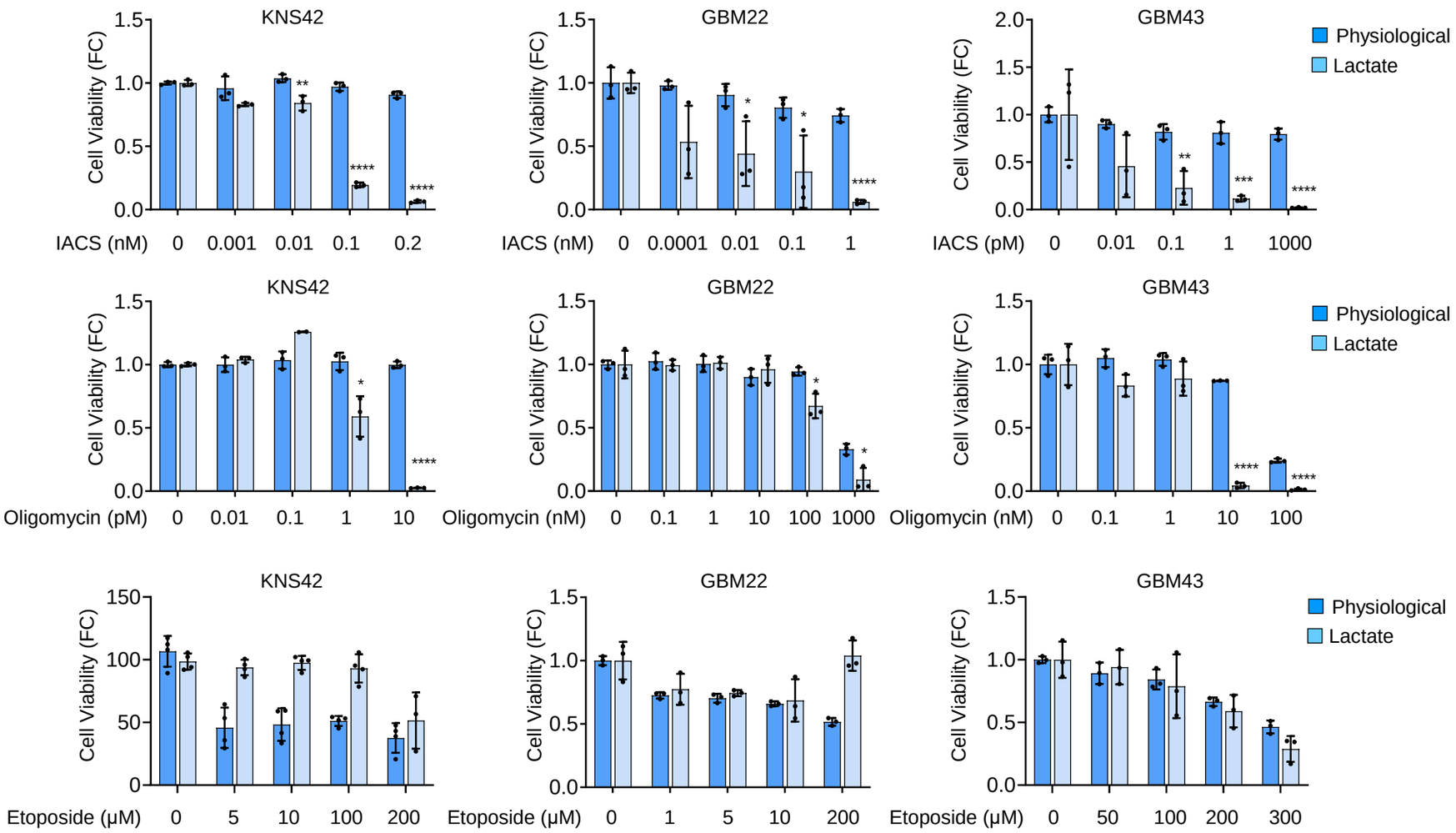


Figure S3

A



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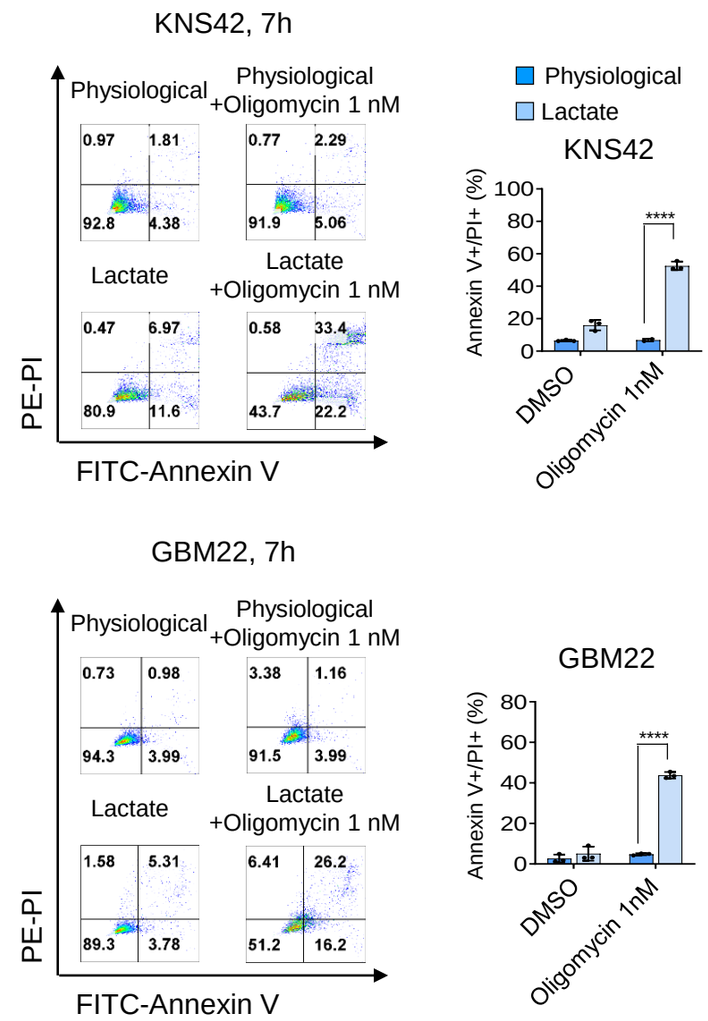

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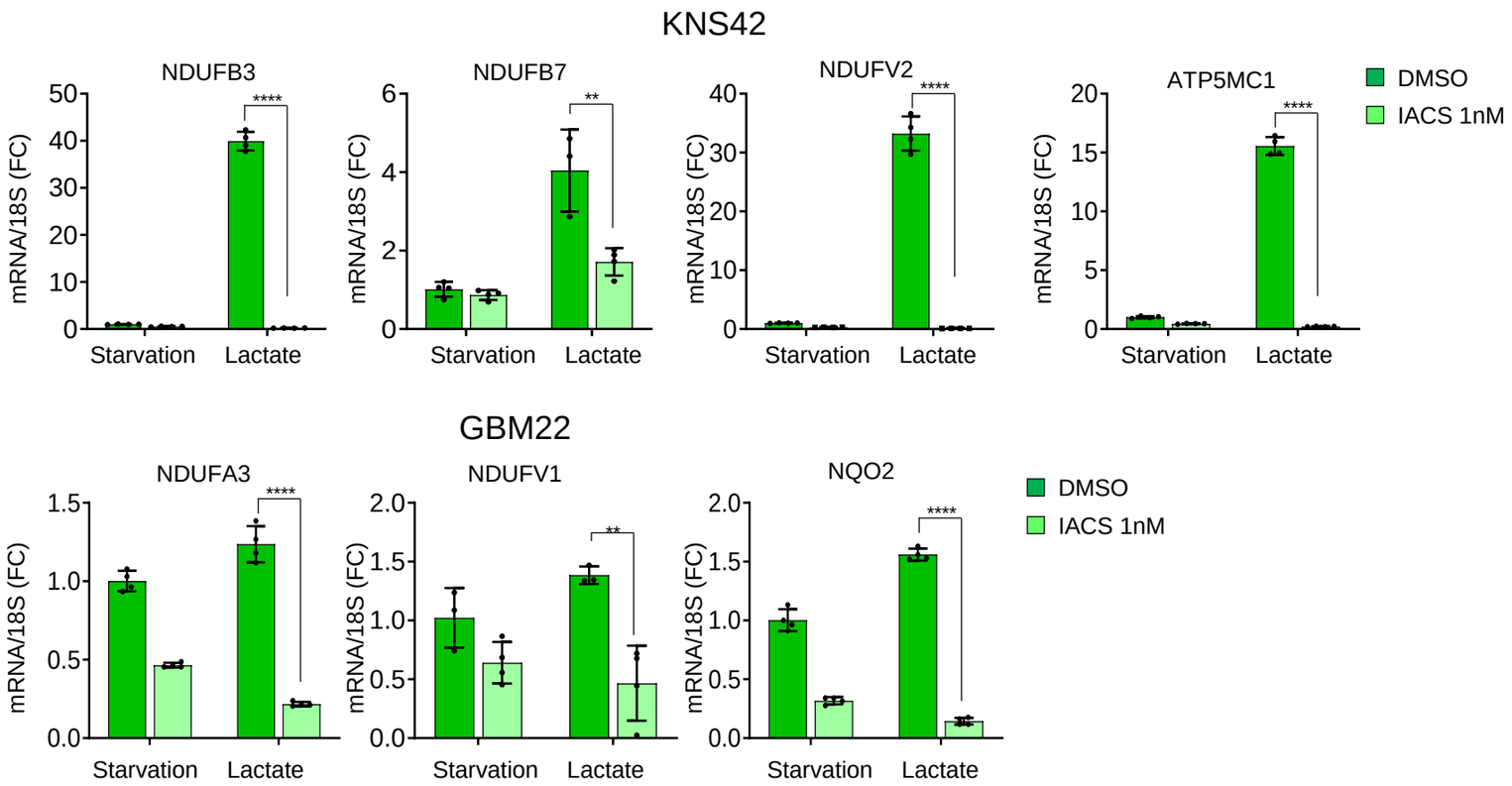

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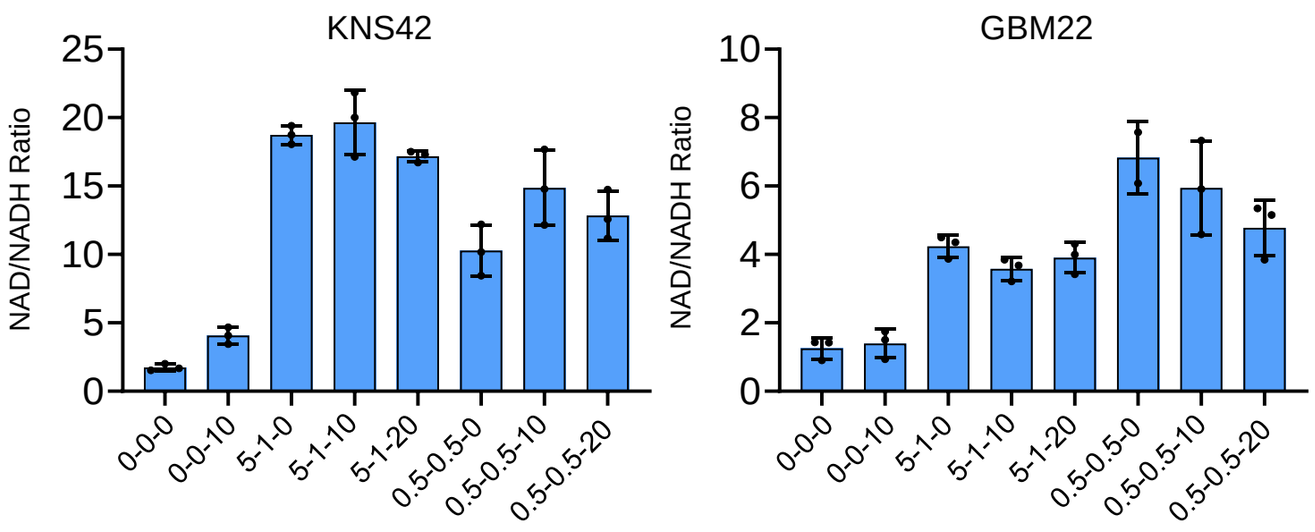
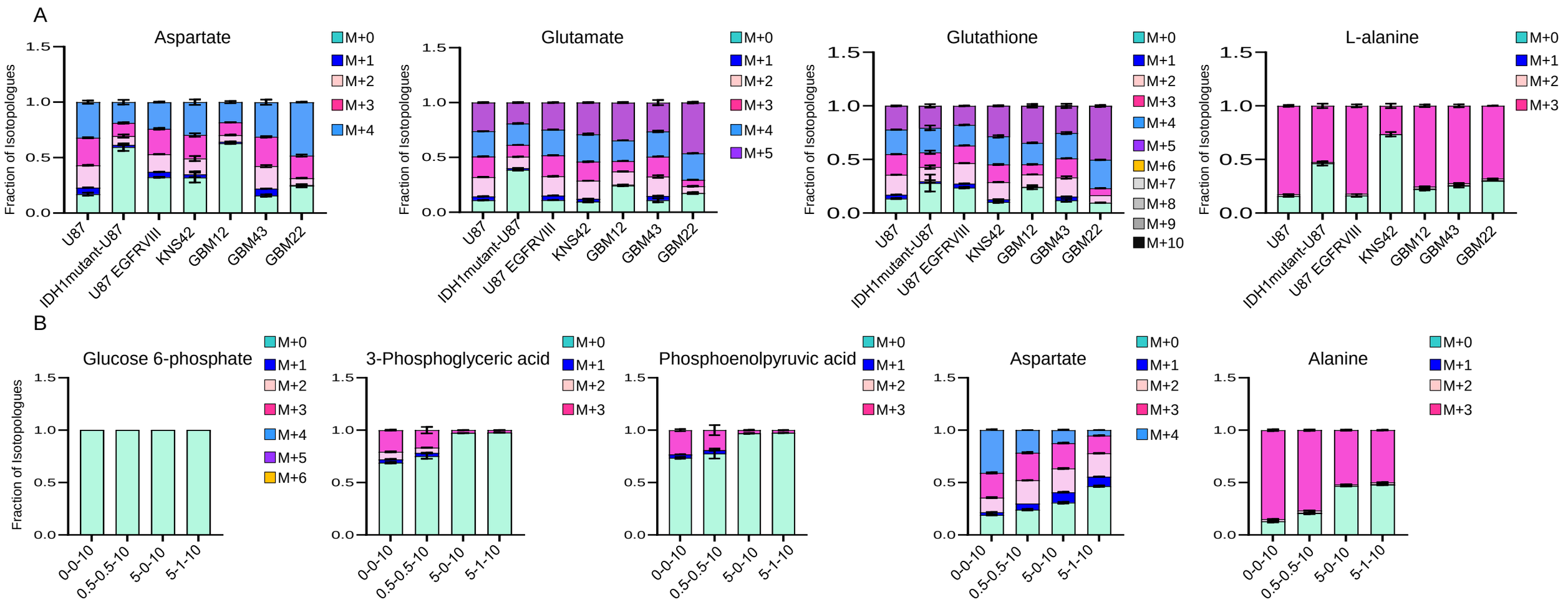


Figure S4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Glutamate
Glutathione
L-alanine
Fraction of Isotopologues
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M+1
M+2
M+3
M+4
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M+6
M+7
M+8
M+9
M+10
U87
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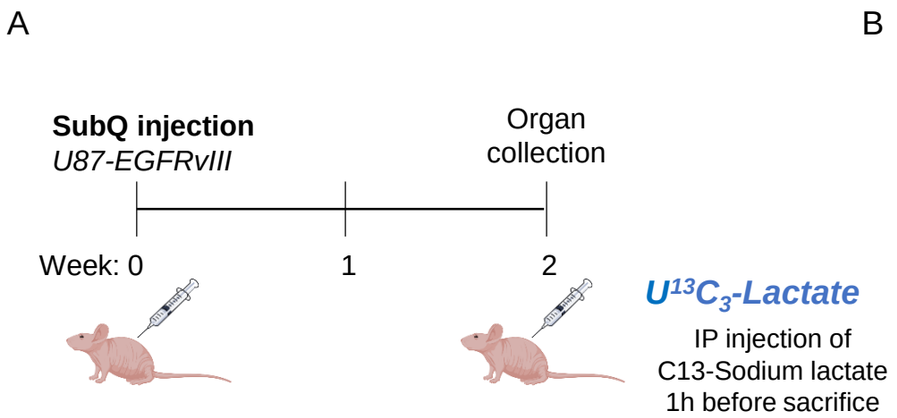
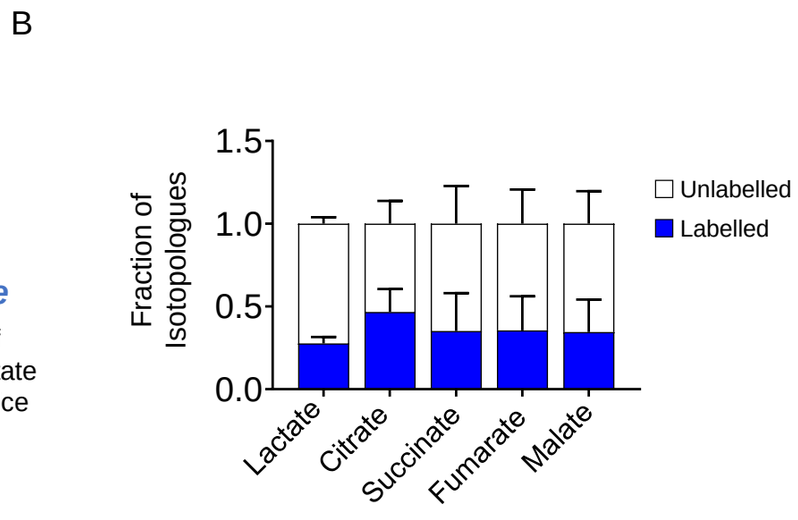
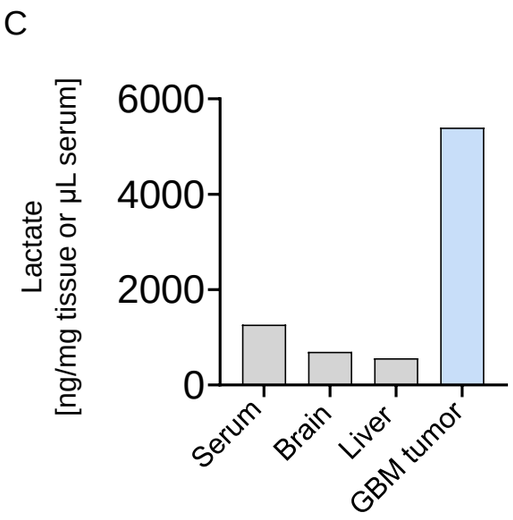
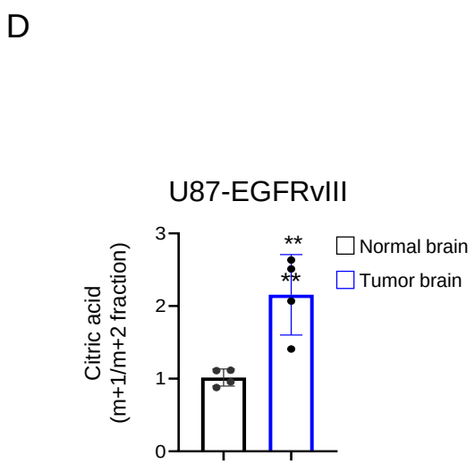
U87 EGFRVIII
KNS42
GBM12
GBM43
GBM22
B
Glucose 6-phosphate
3-Phosphoglyceric acid
Phosphoenolpyruvic acid
Aspartate
Alanine
0-0-10
0.5-0.5-10
5-0-10
5-1-10
C
KNS42
GBM22
GBM43
PC
PCK1
Actin
130 kDa
63 kDa
42 kDa
D
mRNA/18S (FC)
PC
PCK1
Astrocytes
KNS42
GBM22
GBM43
E
Palmitic Acid
Relative intensity
M+1
M+2
M+3
M+4
M+5
M+6
M+7
M+8
M+9
M+10
M+11
M+12
M+13
M+14
M+15
M+16
F
GBM22, 72h
KNS42, 72h
Cell Viability (FC)
Physiological
Starvation
10 mM Lactate
20 mM Lactate
shNT
shPDHA1#582
shPDHA1#590
shPDHA1#602
G
siNT
siPC
siPDHA1
Starvation
Lactate
H
siNT
siPDHA1
siPC
PC
PDHA1
Actin
130 kDa
43 kDa
42 kDa
KNS42
I
siNT
siPDHA1
siPC
10mM Lactate
Starvation
Standard Media
PE-PI
FITC-Annexin V
0.99
6.31
88.6
4.13
1.05
53.2
34.7
11.1
3.02
31.0
57.5
8.49
0.93
64.3
20.0
14.8
0.78
88.3
3.58
7.31
3.80
64.3
6.89
25.0
2.98
5.29
86.0
5.71
2.22
4.44
85.3
8.04
2.91
16.2
57.4
23.5
J
KNS42
Annexin V+/PI+ (%)
siNT
siPC
siPDHA1
Starvation
Lactate

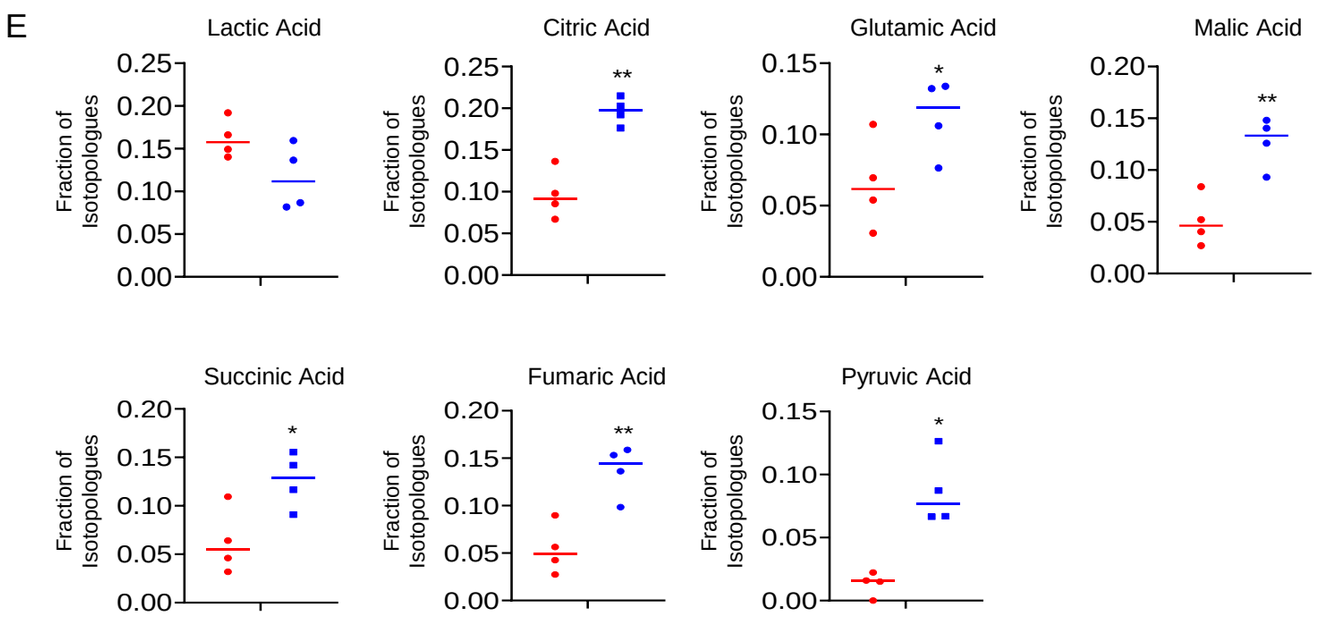
Figure S5

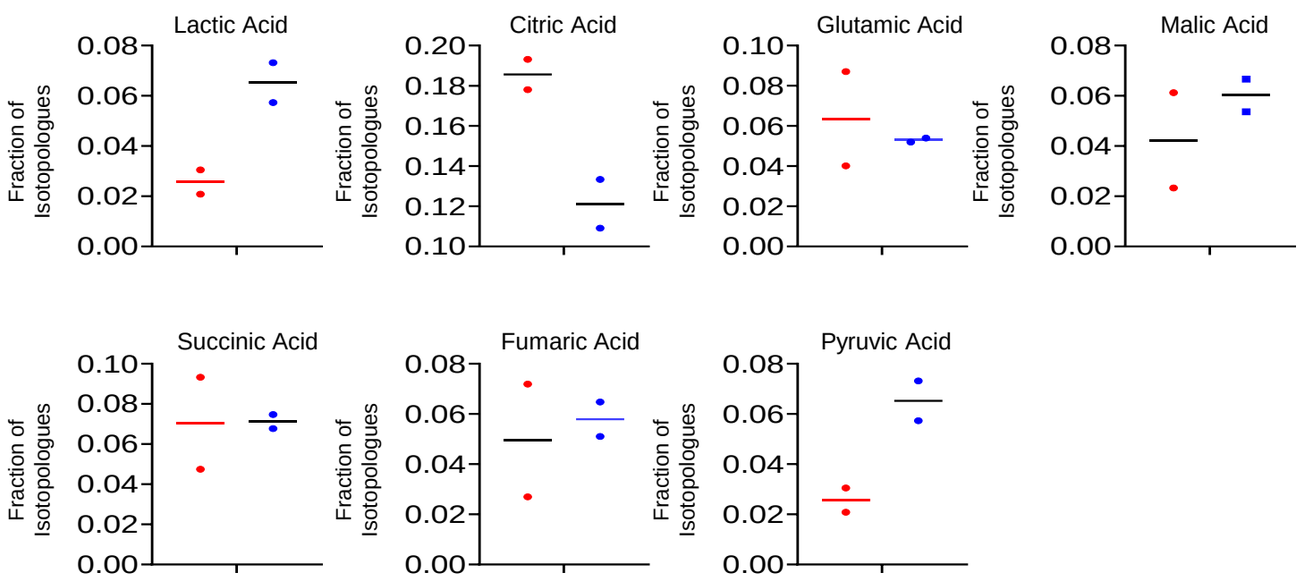

A
SubQ injection
U87-EGFRvIII
Organ collection
Week: 0
1
2
U13C3-Lactate
IP injection of C13-Sodium lactate 1h before sacrifice

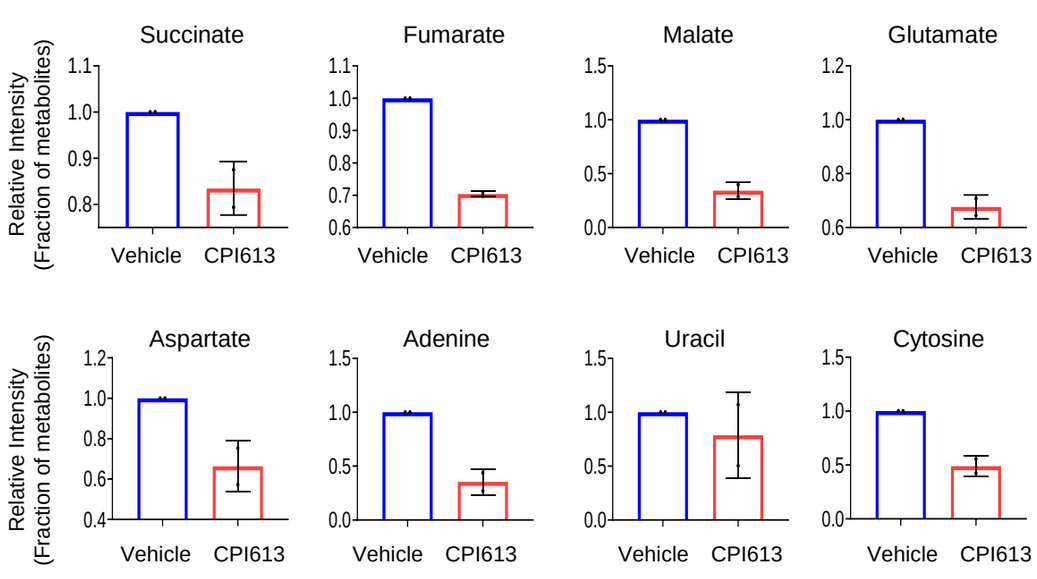

B
Fraction of Isotopologues
1.5
1.0
0.5
0.0
Unlabelled
Labelled
Lactate
Citrate
Succinate
Fumarate
Malate

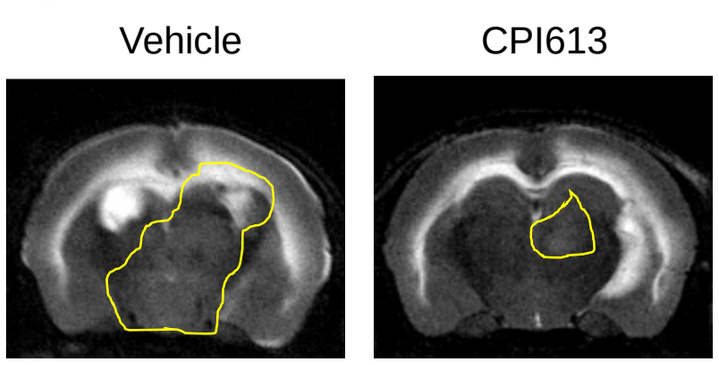

C
Lactate [ng/mg tissue or µL serum]
6000
4000
2000
0
Serum
Brain
Liver
GBM tumor


D
U87-EGFRvIII
Citric acid (m+1/m+2 fraction)
3
2
1
0
**
**
Normal brain
Tumor brain


E
Lactic Acid
Citric Acid
Glutamic Acid
Malic Acid
Succinic Acid
Fumaric Acid
Pyruvic Acid
Fraction of Isotopologues
0.25
0.20
0.15
0.10
0.05
0.00
**
*
**
*
**
*


F
Lactic Acid
Citric Acid
Glutamic Acid
Malic Acid
Succinic Acid
Fumaric Acid
Pyruvic Acid
Fraction of Isotopologues


I
Succinate
Fumarate
Malate
Glutamate
Aspartate
Adenine
Uracil
Cytosine
Relative Intensity (Fraction of metabolites)
Vehicle
CPI613


Vehicle
CPI613
GBM22

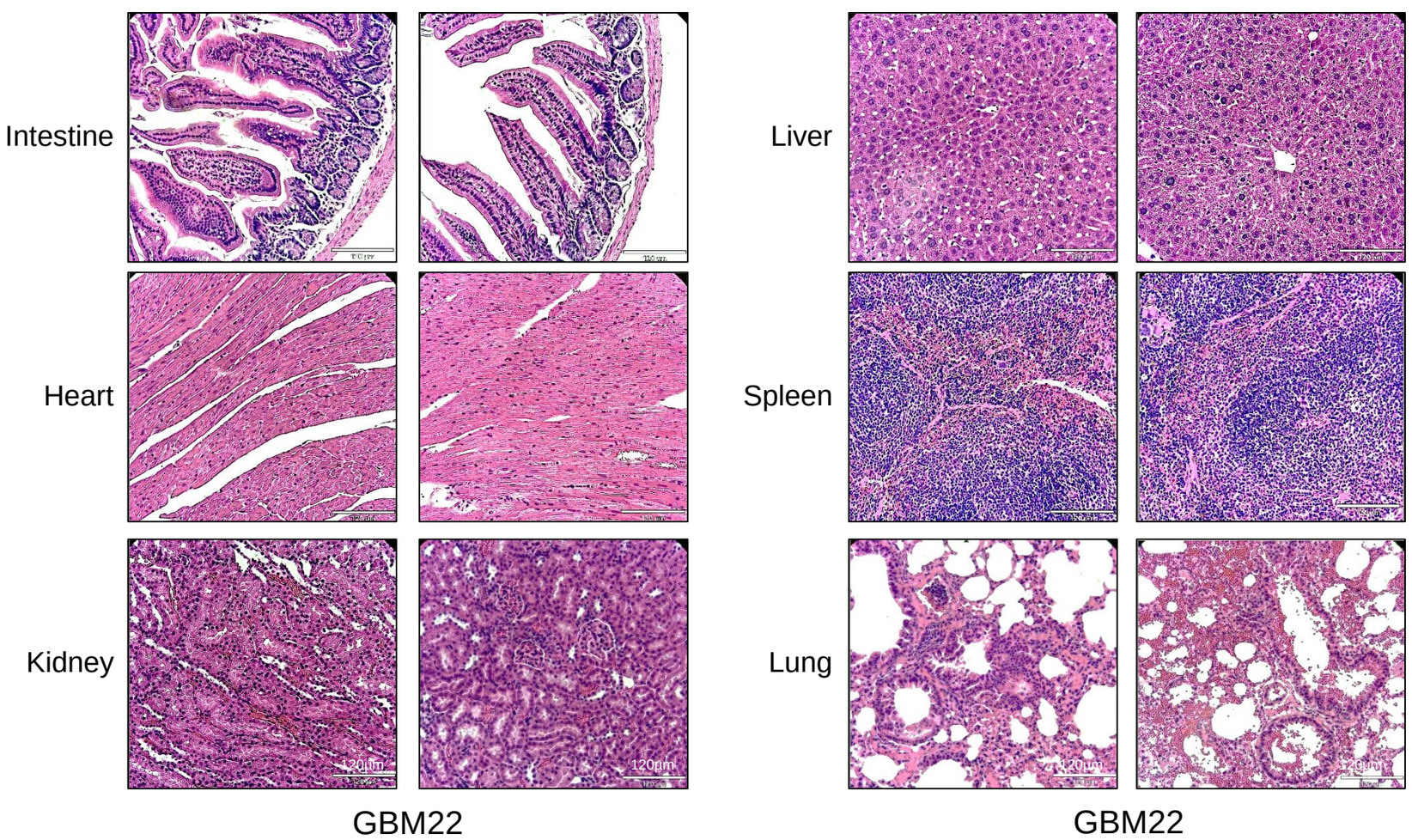
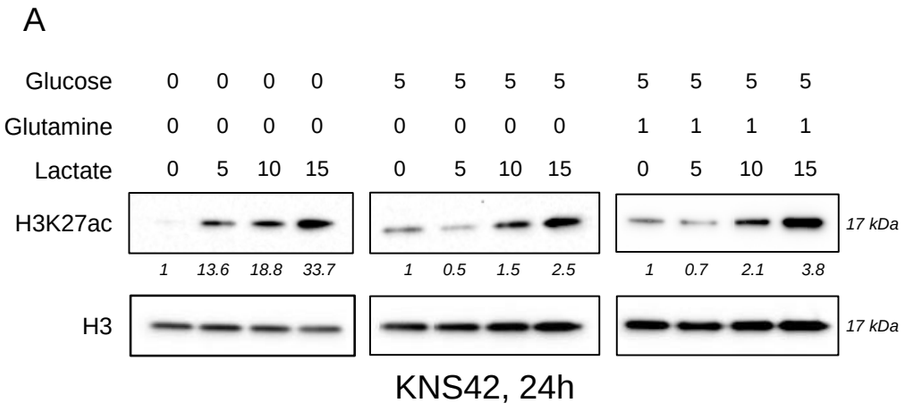
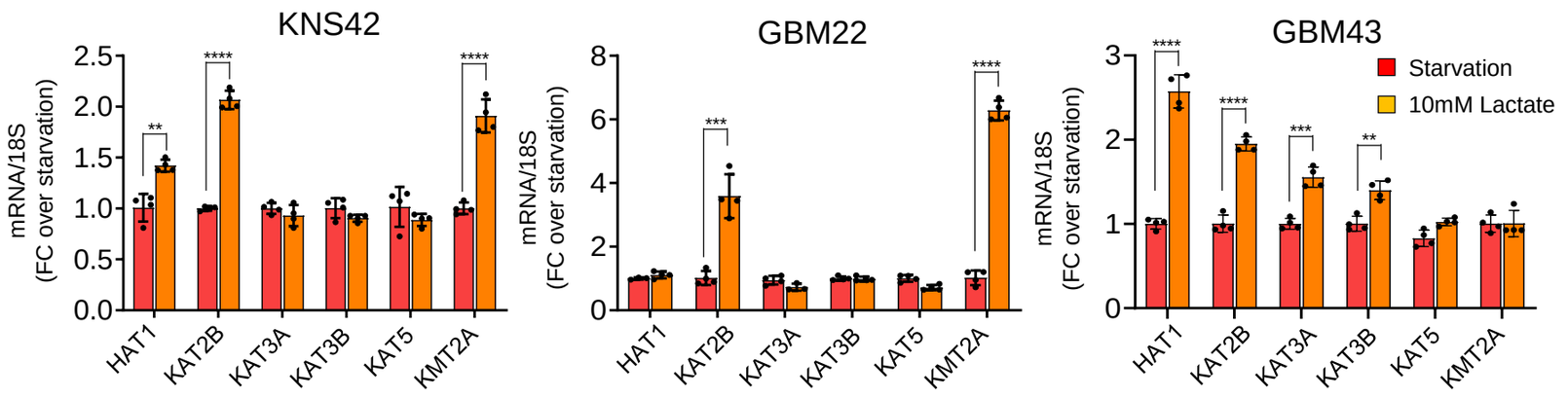
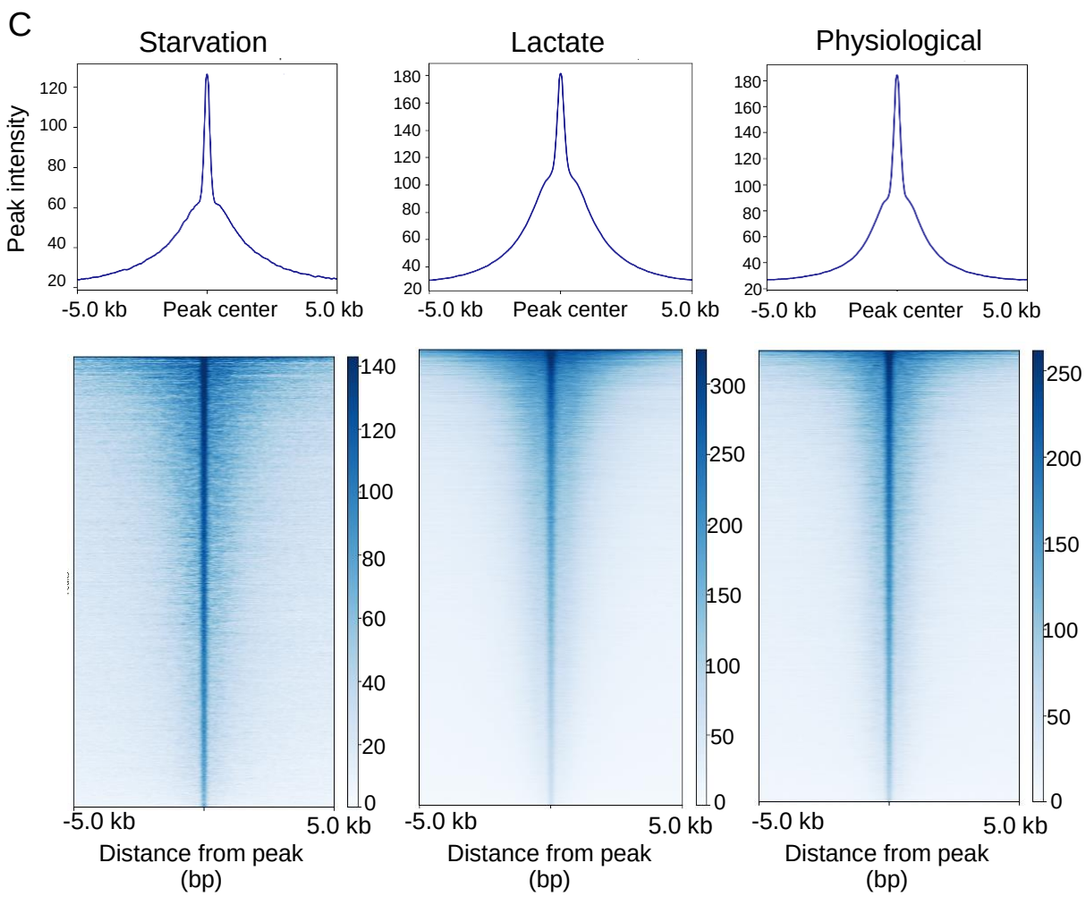
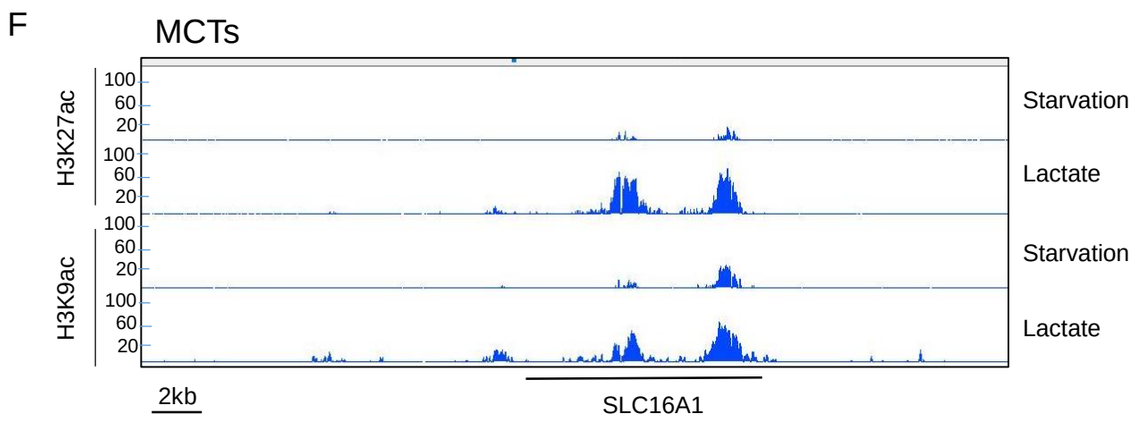

H
Vehicle
CPI613
Vehicle
CPI613
Intestine
Liver
Heart
Spleen
Kidney
Lung
120µm
GBM22
GBM22

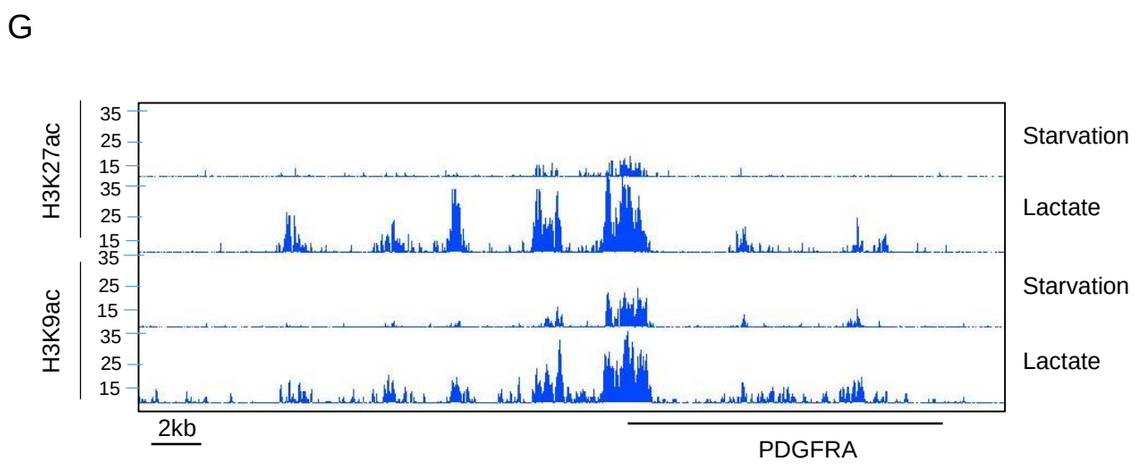
Figure S6

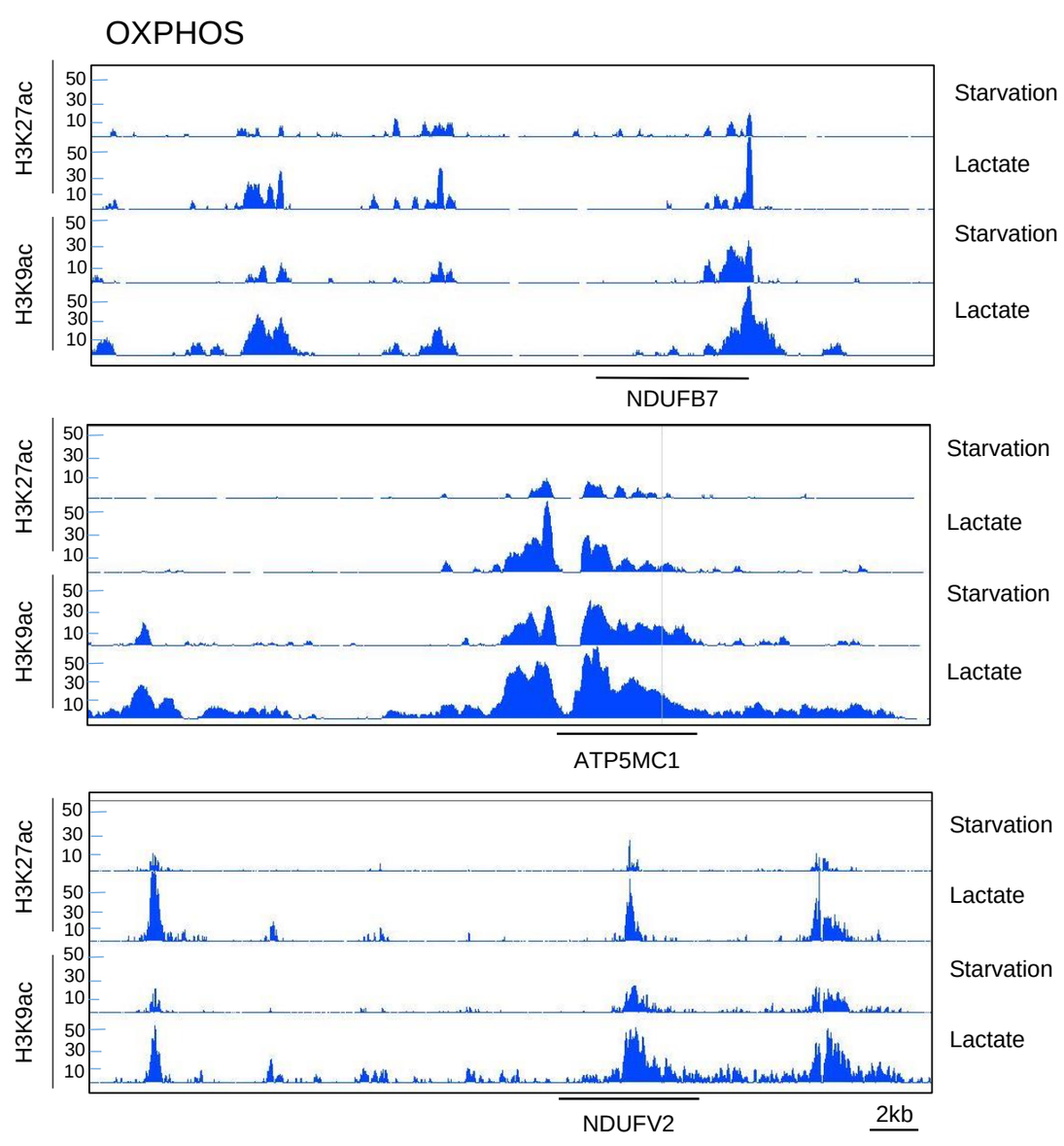

A
Glucose 0 0 0 0 5 5 5 5 5 5 5 5
Glutamine 0 0 0 0 0 0 0 0 1 1 1 1
Lactate 0 5 10 15 0 5 10 15 0 5 10 15
H3K27ac
1 13.6 18.8 33.7 1 0.5 1.5 2.5 1 0.7 2.1 3.8
17 kDa
H3
17 kDa
KNS42, 24h

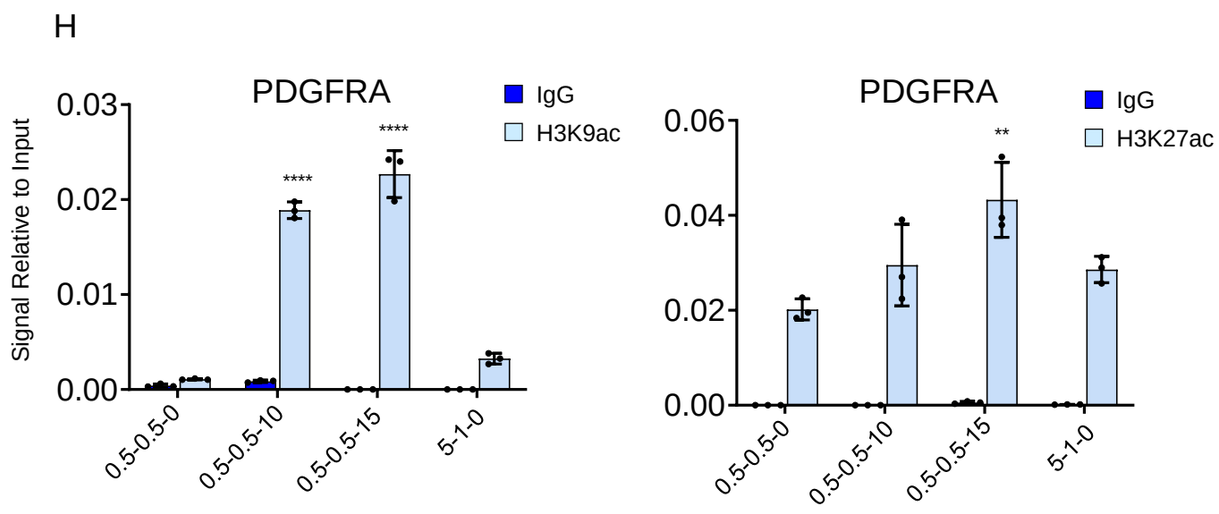

B
KNS42
GBM22
GBM43
mRNA/18S (FC over starvation)
Starvation
10mM Lactate
HAT1
KAT2B
KAT3A
KAT3B
KAT5
KMT2A

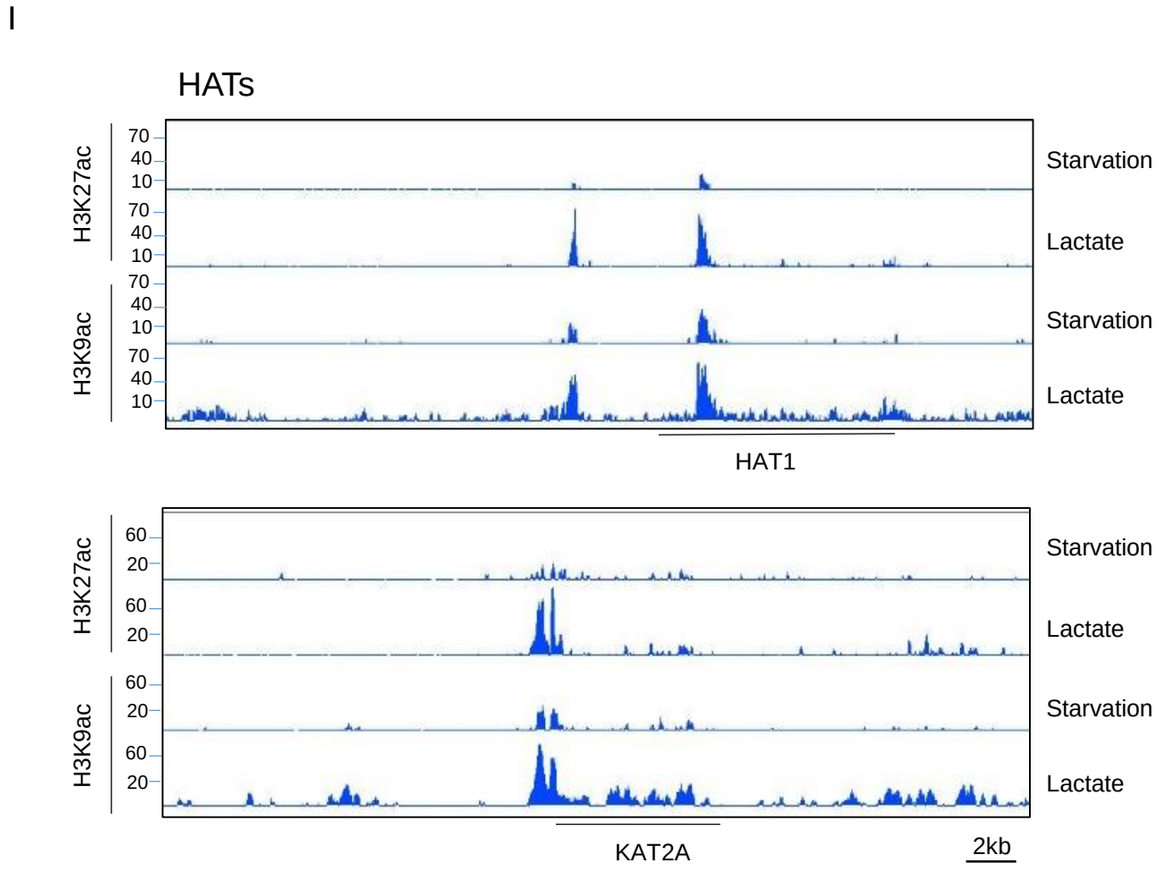

C
Starvation
Lactate
Physiological
Peak intensity
-5.0 kb
Peak center
5.0 kb
Distance from peak (bp)


F
MCTs
H3K27ac
H3K9ac
Starvation
Lactate
2kb
SLC16A1


G
H3K27ac
H3K9ac
Starvation
Lactate
2kb
PDGFRA


D
OXPHOS
H3K27ac
H3K9ac
Starvation
Lactate
NDUFB7
ATP5MC1
NDUFV2
2kb


H
PDGFRA
IgG
H3K9ac
H3K27ac
Signal Relative to Input
0.5-0.5-0
0.5-0.5-10
0.5-0.5-15
5-1-0


I
HATs
H3K27ac
H3K9ac
Starvation
Lactate
HAT1
KAT2A
2kb

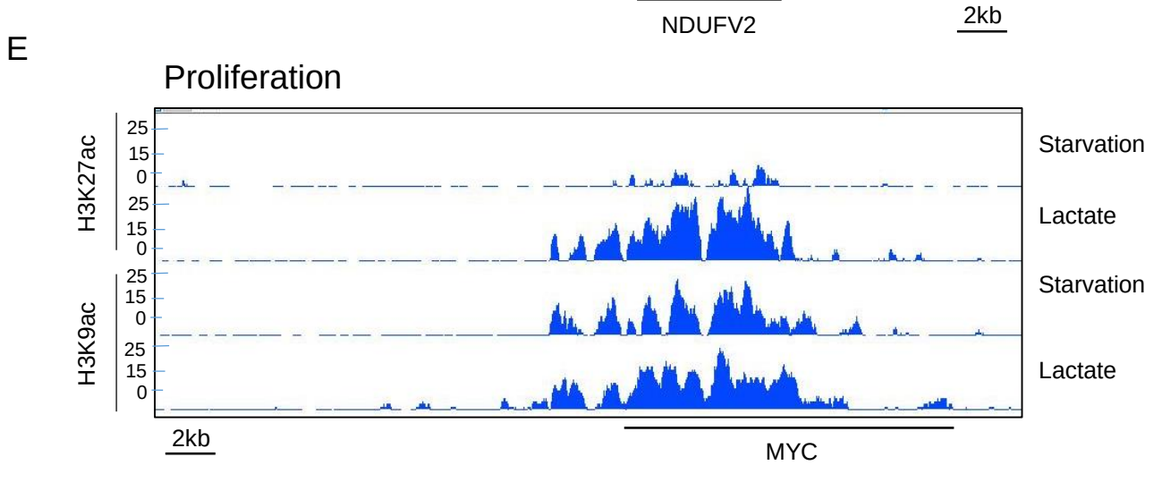
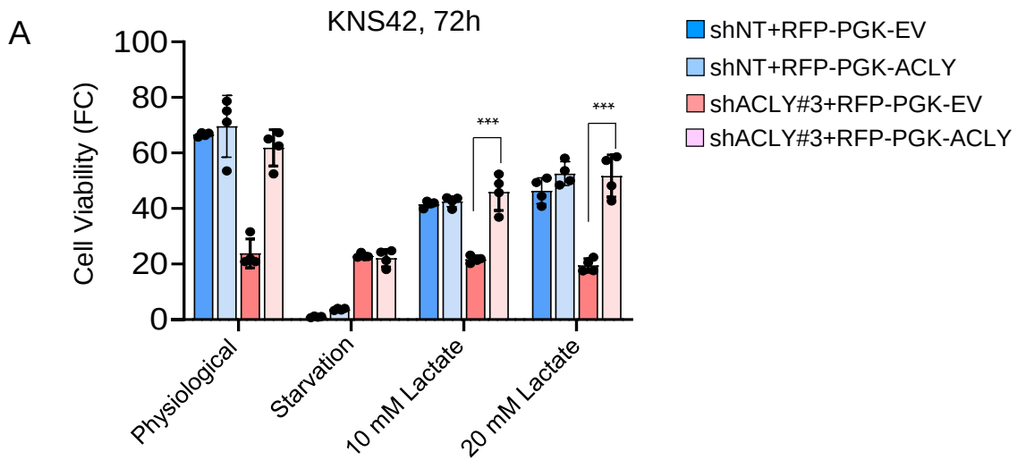
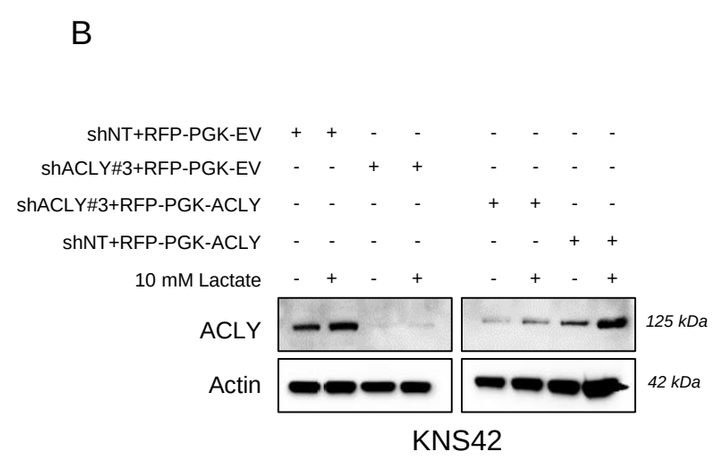
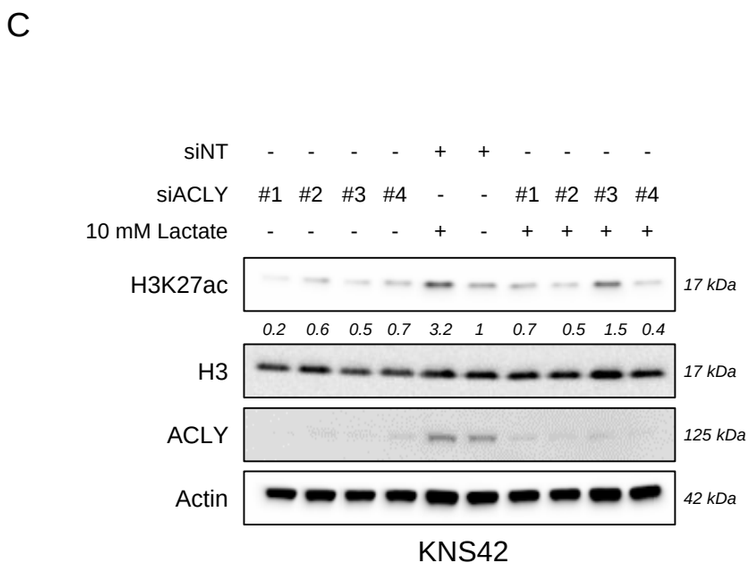
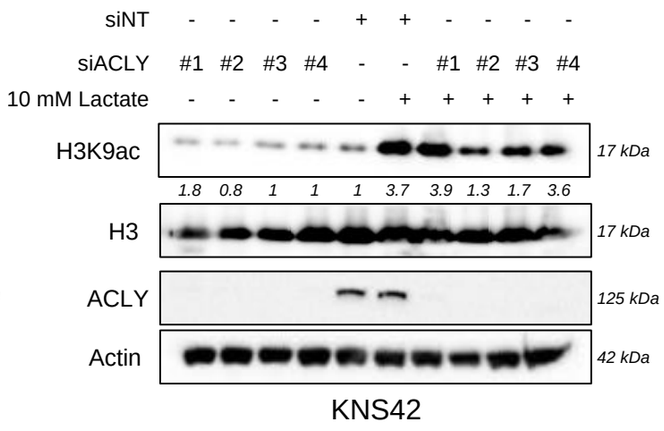

E
Proliferation
H3K27ac
H3K9ac
Starvation
Lactate
2kb
MYC

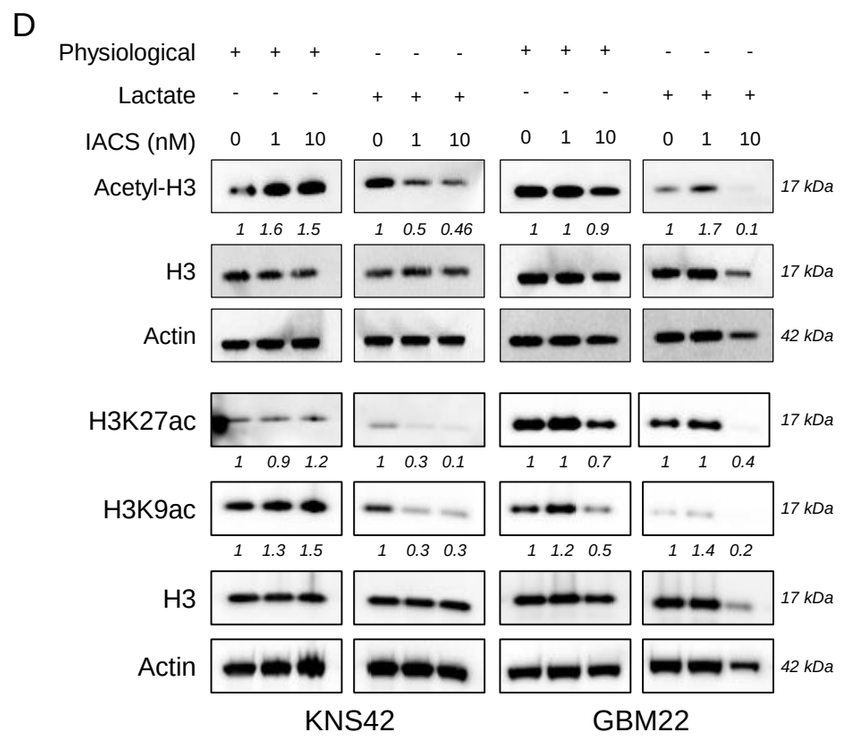
Figure S7

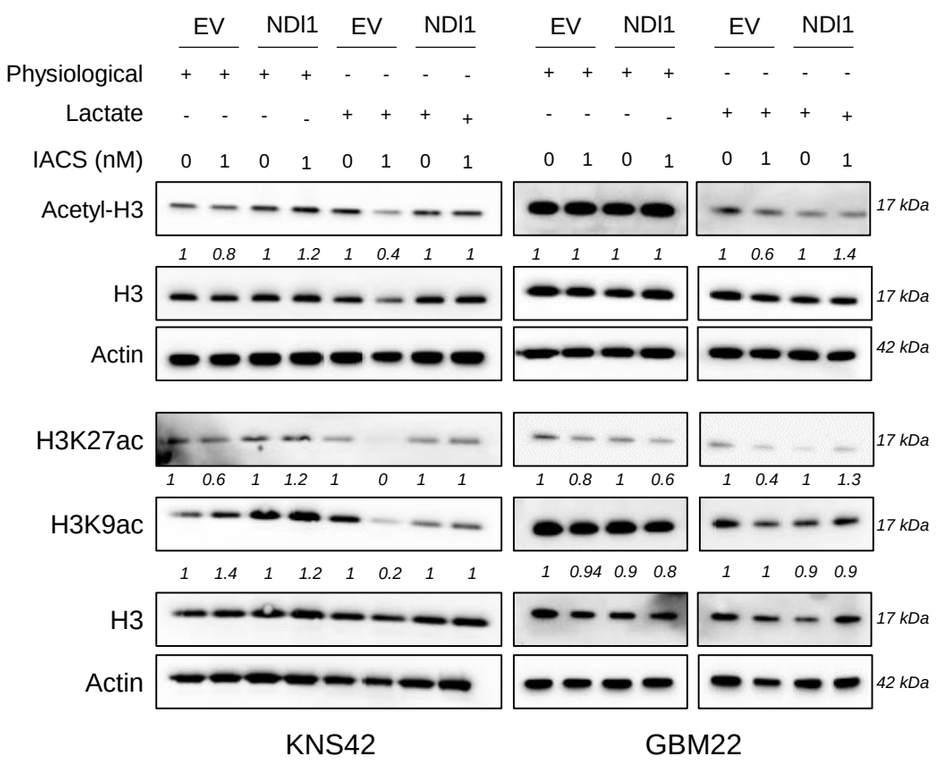

A
KNS42, 72h
shNT+RFP-PGK-EV
shNT+RFP-PGK-ACLY
shACLY#3+RFP-PGK-EV
shACLY#3+RFP-PGK-ACLY
Cell Viability (FC)
100
80
60
40
20
0
***
***
Physiological
Starvation
10 mM Lactate
20 mM Lactate

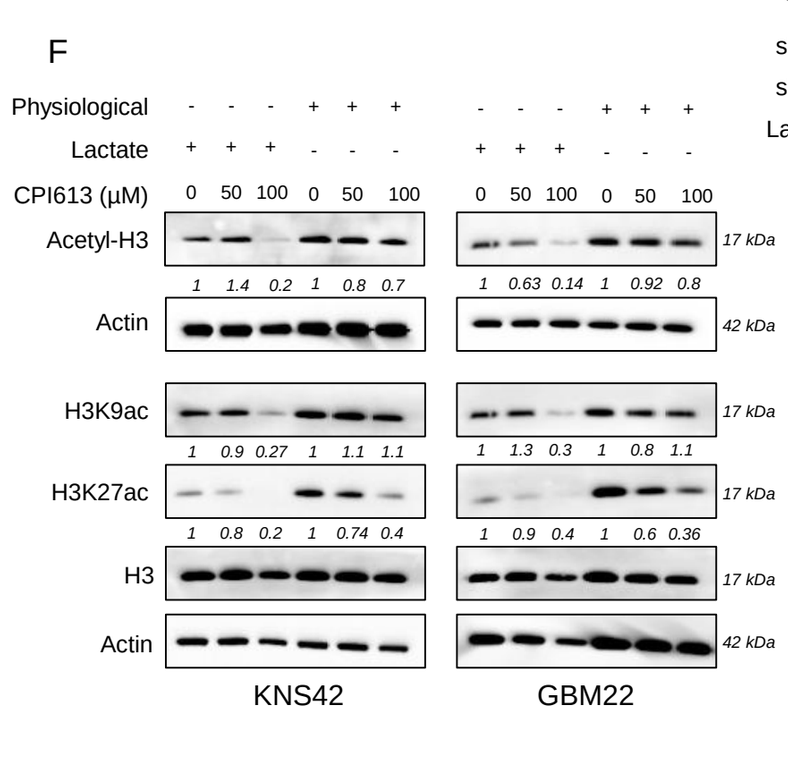

B
shNT+RFP-PGK-EV
shACLY#3+RFP-PGK-EV
shACLY#3+RFP-PGK-ACLY
shNT+RFP-PGK-ACLY
10 mM Lactate
ACLY
Actin
125 kDa
42 kDa
KNS42

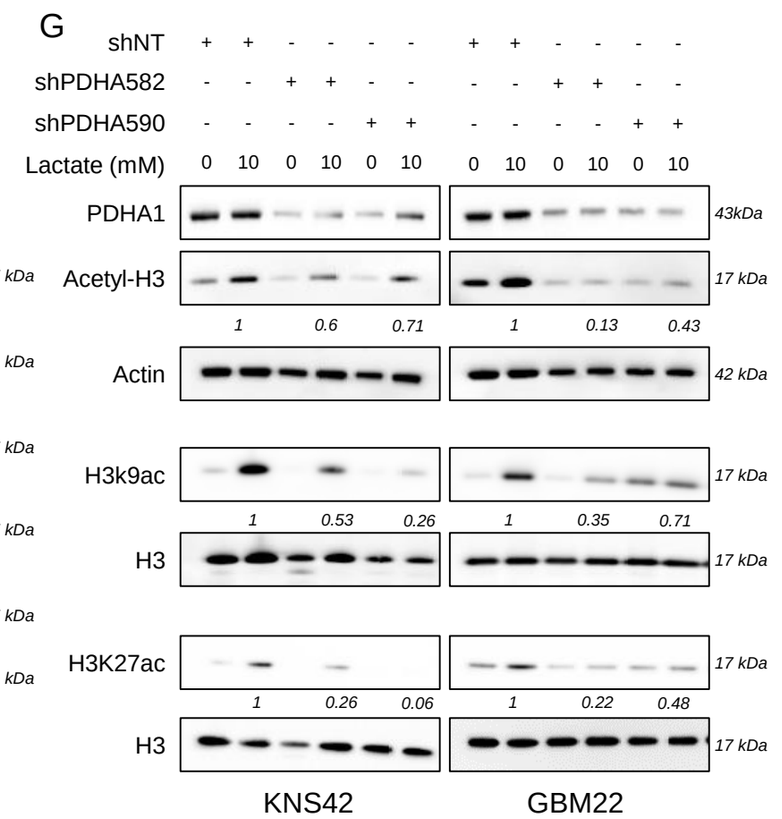

C
siNT
siACLY
10 mM Lactate
H3K27ac
0.2 0.6 0.5 0.7 3.2 1 0.7 0.5 1.5 0.4
H3
Actin
17 kDa
125 kDa
42 kDa
KNS42

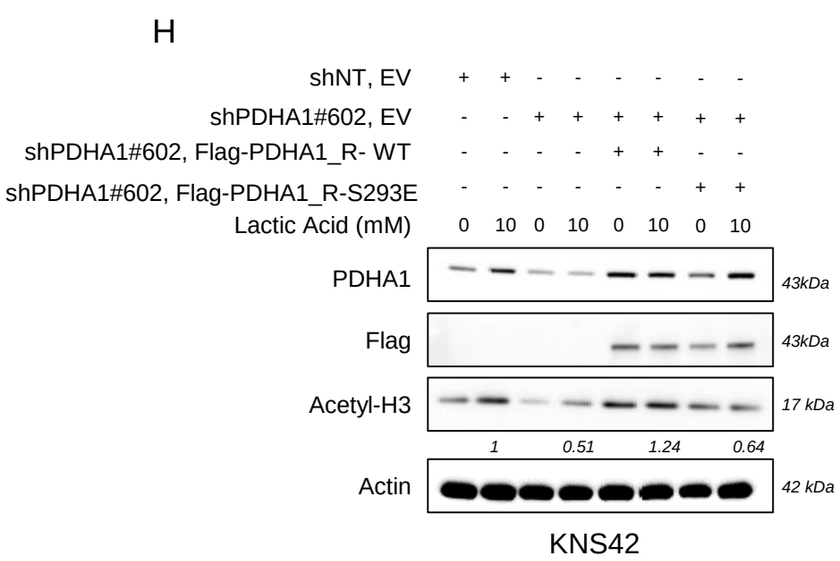

siNT
siACLY
10 mM Lactate
H3K9ac
1.8 0.8 1 1 1 3.7 3.9 1.3 1.7 3.6
H3
ACLY
Actin
17 kDa
125 kDa
42 kDa
KNS42

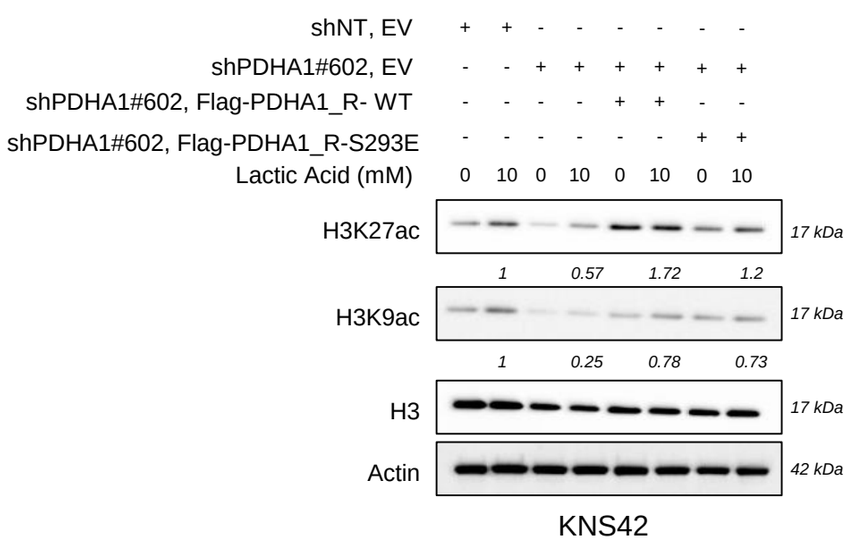

D
Physiological
Lactate
IACS (nM)
Acetyl-H3
1 1.6 1.5 | 1 0.5 0.46 | 1 1 0.9 | 1 1.7 0.1
H3
Actin
H3K27ac
1 0.9 1.2 | 1 0.3 0.1 | 1 1 0.7 | 1 1 0.4
H3K9ac
1 1.3 1.5 | 1 0.3 0.3 | 1 1.2 0.5 | 1 1.4 0.2
17 kDa
42 kDa
KNS42
GBM22


E
EV
NDI1
Physiological
Lactate
IACS (nM)
Acetyl-H3
1 0.8 1 1.2 1 0.4 1 1 | 1 1 1 1 | 1 0.6 1 1.4
H3
Actin
H3K27ac
1 0.6 1 1.2 1 0 1 1 | 1 0.8 1 0.6 | 1 0.4 1 1.3
H3K9ac
1 1.4 1 1.2 1 0.2 1 1 | 1 0.94 0.9 0.8 | 1 1 0.9 0.9
17 kDa
42 kDa
KNS42
GBM22


F
Physiological
Lactate
CPI613 (μM)
0 50 100 0 50 100 | 0 50 100 0 50 100
Acetyl-H3
1 1.4 0.2 1 0.8 0.7 | 1 0.63 0.14 1 0.92 0.8
Actin
H3K9ac
1 0.9 0.27 1 1.1 1.1 | 1 1.3 0.3 1 0.8 1.1
H3K27ac
1 0.8 0.2 1 0.74 0.4 | 1 0.9 0.4 1 0.6 0.36
H3
Actin
17 kDa
42 kDa
KNS42
GBM22


G
shNT
shPDHA582
shPDHA590
Lactate (mM)
0 10 0 10 0 10 | 0 10 0 10 0 10
PDHA1
Acetyl-H3
1 0.6 0.71 | 1 0.13 0.43
Actin
H3k9ac
1 0.53 0.26 | 1 0.35 0.71
H3
H3K27ac
1 0.26 0.06 | 1 0.22 0.48
43kDa
17 kDa
42 kDa
KNS42
GBM22


H
shNT, EV
shPDHA1#602, EV
shPDHA1#602, Flag-PDHA1_R- WT
shPDHA1#602, Flag-PDHA1_R-S293E
Lactic Acid (mM)
0 10 0 10 0 10 0 10
PDHA1
Flag
Acetyl-H3
1 0.51 1.24 0.64
Actin
43kDa
17 kDa
42 kDa
KNS42


shNT, EV
shPDHA1#602, EV
shPDHA1#602, Flag-PDHA1_R- WT
shPDHA1#602, Flag-PDHA1_R-S293E
Lactic Acid (mM)
0 10 0 10 0 10 0 10
H3K27ac
1 0.57 1.72 1.2
H3K9ac
1 0.25 0.78 0.73
H3
Actin
17 kDa
42 kDa
KNS42