SUPPLEMENTARY MATERIALS AND METHODS

Cell lines. NB4 cells (ATCC) were maintained in RPMI + 8% FBS. A549 cells were maintained in F12 (Kaign's) + 8% FBS. LnCAP cells were maintained in RPMI 1640 + 10% FBS and 1X sodium pyruvate and nonessential amino acids, and were plated on cell bind cell culture vessels (Corning).

Surface GLUT1 Measurement and Flow Cytometry. Cells were analyzed by a FACscan (BD Biosciences, San Jose, CA) and FlowJo software (Tree Star, Inc., Ashland, OR). To determine FLAG surface expression, cells were washed once in phosphate-buffered saline (PBS)/2% fetal bovine serum (FBS). Cells were blocked with anti-FcIII/II (BD Biosciences PharMingen, San Diego, CA) and incubated with 5% rat serum and rabbit anti-FLAG (Sigma-Aldrich) followed by R-Phycoerythrin donkey anti-rabbit (Jackson ImmunoResearch, West Grove, PA) for analysis. Surface FLAG-GLUT1 levels were normalized to total FLAG expression by division of the mean fluorescence of surface FLAG by the average pixel density from respective immunoblot. To determine TfR (CD71) surface expression, cells were incubated with anti-mouse CD71-PE (BD Biosciences PharMingen). For pulse-chase assays, cells were blocked with anti-FcIII/II (BD Biosciences PharMingen) for 5 min followed by a pulse with anti-FLAG antibody (Sigma-Aldrich) at room temperature for 10 min, washed, and cultured for various periods at 37°C before addition of secondary stain on ice. Mean fluorescence of cell surface GLUT1 levels were normalized to the starting value.

SUPPLEMENTARY FIGURE LEGENDS

Supplementary Figure 1: HDACI alter levels of acyl carnitines and amino acids in NCI-H929 cells. H929 cells were treated with VPA (1 2, or 4mM) or SAHA (1, 2.5 or 5uM) for 48 hours. Following analysis of protein concentration, lysates were deproteinated and acylcarnitines and amino acids were measured by MS-MS analysis. Acylcarnitine and amino acid levels were determined by normalization to lysate protein concentration. Results are representative of two independent experiments and values are calculated mean +/- SD of triplicate samples.

Supplementary Figure 2: HDACI do not affect expression or persistence at the cell membrane of retrovirally expressed FLAG-GLUT1. A) OPM2 parent or OPM2-GLUT1 cells were treated 24 or 44 hours with VPA (1mM) or SAHA (2.5uM) prior to staining with anti-FLAG antibody and FACS analysis. Surface FLAG-GLUT1 levels were normalized to total FLAG expression by division of the mean fluorescence of surface FLAG by the average pixel density from respective immunoblot (B).

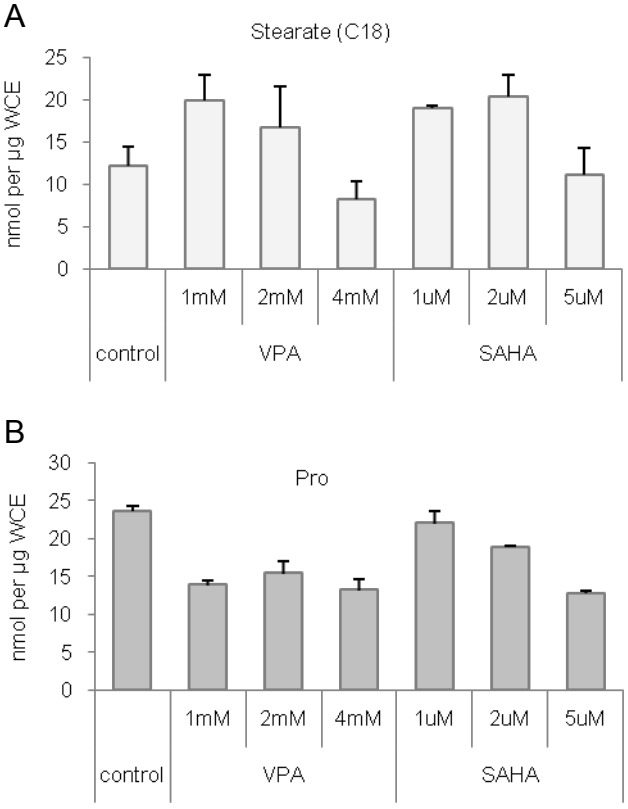
Supplementary Figure 3: VPA inhibits glucose uptake in NB4 APL cells. $2x10^5$ live cells (per trypan blue exclusion) were incubated 10 min with ^{3}H-2-DOG prior to washing and lysis of the cells. Retained radioactivity was detected by analysis in scintillation fluid.

Supplementary Figure 4: Glycolytic inhibitors do not recapitulate all metabolic activities of VPA. A) OPM2 cells were treated 72 hours with 2-DOG (2.5mM) or maltose (75mM) in the presence of absence of either supplemental non-essential amino acids (0.2mM) or VPA (0.75mM). Apoptosis was analyzed using 7AAD and Annexin V PE staining followed by FACS. Values indicate the average percent remaining live (unstained) cells per treatment in duplicate samples. B) OPM2 cells were treated with VPA (1mM), 2-DOG (5mM), maltose (100mM), or Dox (100nM) for 24 hours. C) OPM2 cells were treated with VPA (2mM), 2-DOG (5mM), maltose (100mM) or Dox (100nM) for 48 hours. B-C) Following analysis of protein concentration, lysates were deproteinated and acylcarnitines and amino acids were measured by MS-MS analysis. Acylcarnitine and amino acid levels were determined by

normalization to lysate protein concentration. D) Apoptosis in samples from (B) was analyzed as in (A) prior to lysis.

Supplementary Figure 5: Metabolic effects of VPA differ in models of malignancy. A) A549 lung carcinoma cells were treated 48 hours with VPA (2 or 4mM) or 2-DOG (5mM) prior to lysis and analysis of HXK activity, normalized to protein input. B) RNA isolated from cells treated in (A) was reverse transcribed prior to analysis by RTqPCR; detected levels of HXK1 mRNA were normalized to the similarly detected housekeeping gene 36B4. Fold induction over control was determined by setting HXK1 levels in the untreated control equal to 1. C-D) A549 cells were treated with VPA or 2-DOG for 48 hours prior to lysis. Following analysis of protein concentration, lysates were deproteinated and acylcarnitines (C) and amino acids (D) were measured by MS-MS analysis. Acylcarnitine and amino acid levels were determined by normalization to lysate protein concentration. E) HXK activity in LnCAP prostate cancer cells treated with VPA (2 or 4mM) or 2-DOG (5mM) was measured as in (A). F) HXK1 mRNA levels from (E) were measured as in (B). G-H) LnCAP cells were treated for 48 hours with VPA (0-5mM) prior to harvest. Acylcarnitines (G) and amino acids (H) were analyzed in lysates of treated cells as described above.

H929


A
Stearate (C18)
nmol per µg WCE
25
20
15
10
5
0
1mM
2mM
4mM
1uM
2uM
5uM
control
VPA
SAHA
B
Pro
nmol per µg WCE
30
25
20
15
10
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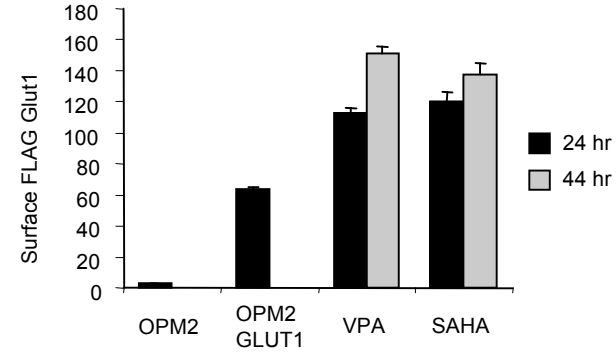
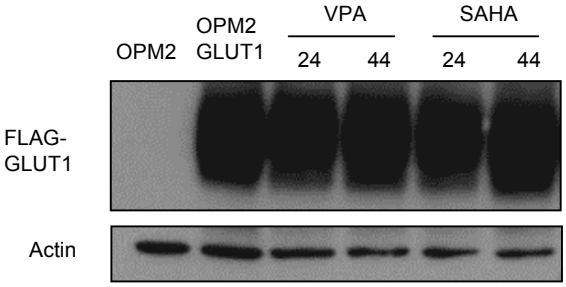
SAHA

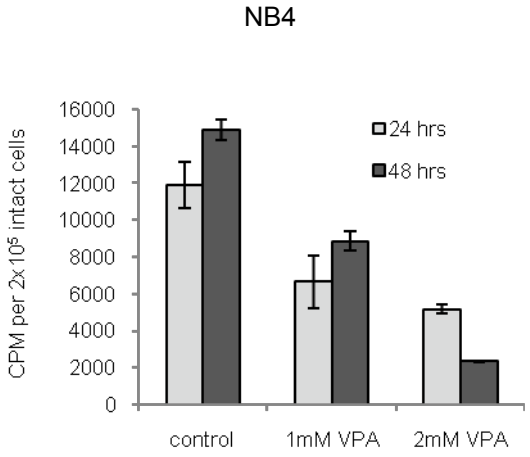
A


Surface FLAG Glut1
180
160
140
120
100
80
60
40
20
0
OPM2
OPM2 GLUT1
VPA
SAHA
24 hr
44 hr

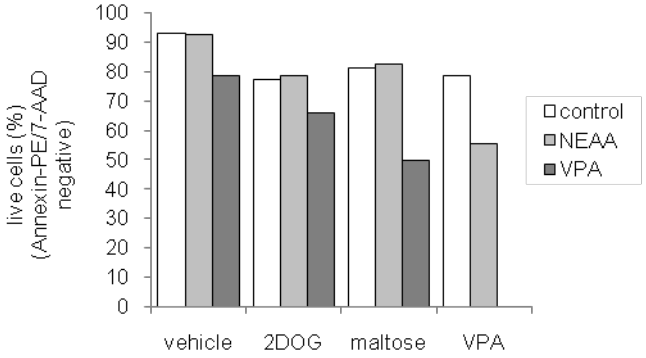
B


OPM2
OPM2 GLUT1
VPA
SAHA
24
44
24
44
FLAG-GLUT1
Actin

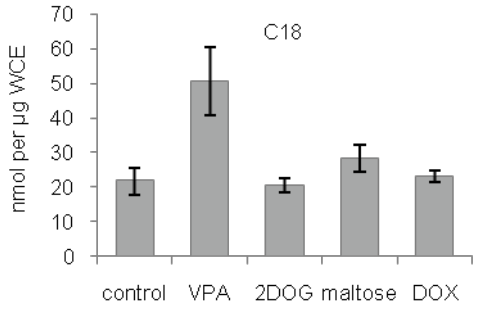
A


NB4
CPM per 2x10^5 intact cells
16000
14000
12000
10000
8000
6000
4000
2000
0
24 hrs
48 hrs
control
1mM VPA
2mM VPA

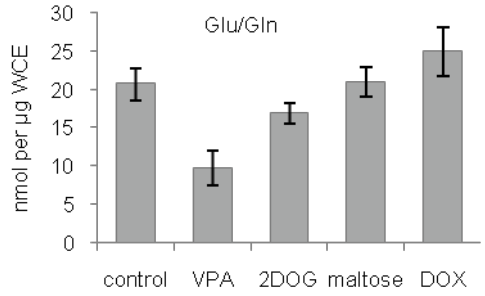
A


live cells (%)
(Annexin-PE/7-AAD
negative)
100
90
80
70
60
50
40
30
20
10
0
vehicle
2DOG
maltose
VPA
control
NEAA
VPA

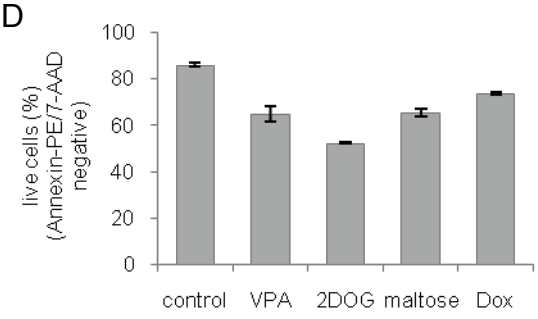
B

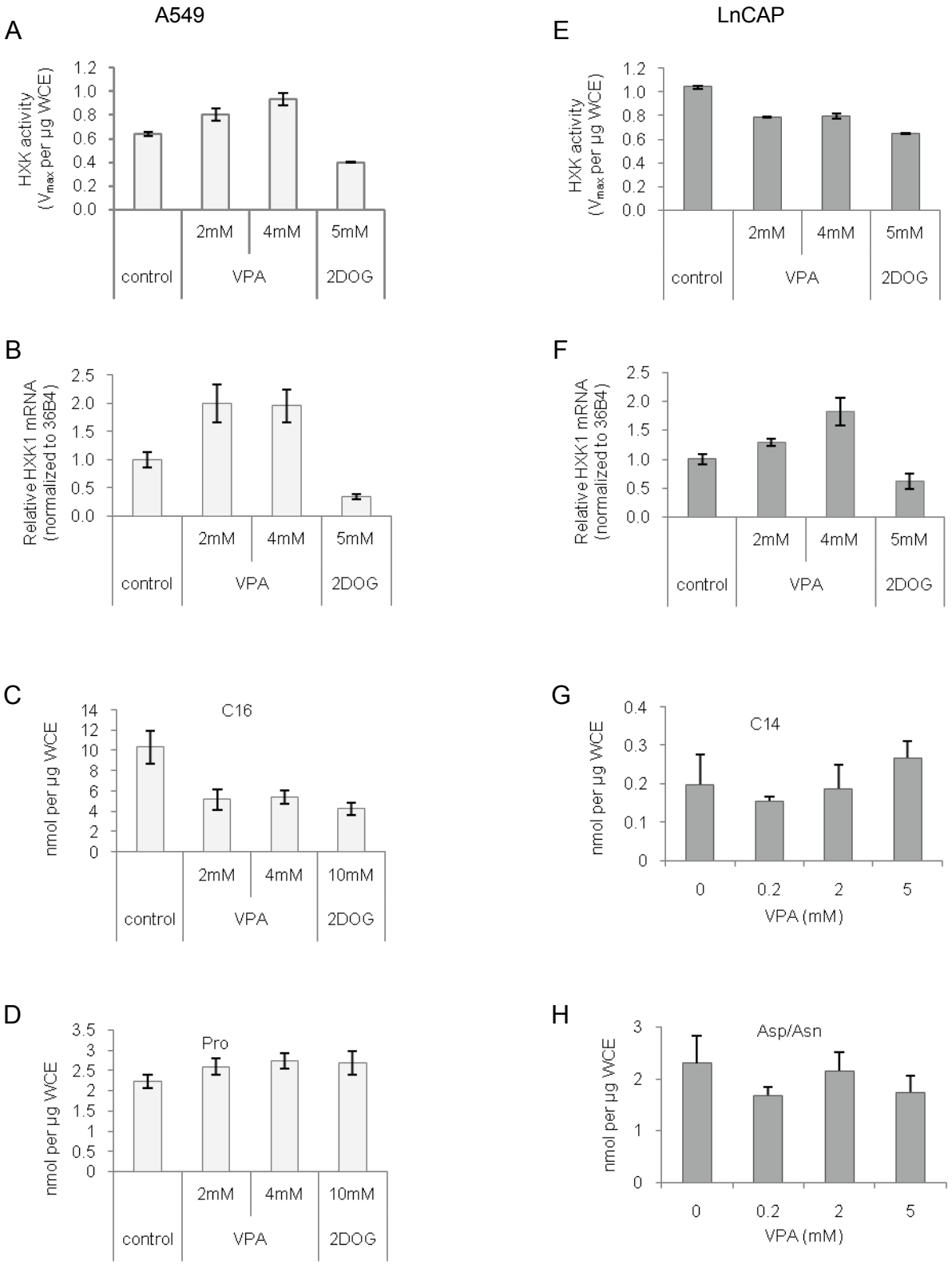

C18
nmol per µg WCE
70
60
50
40
30
20
10
0
control
VPA
2DOG
maltose
DOX

C


Glu/Gln
nmol per µg WCE
30
25
20
15
10
5
0
control
VPA
2DOG
maltose
DOX

D


live cells (%)
(Annexin-PE/7-AAD
negative)
100
80
60
40
20
0
control
VPA
2DOG
maltose
Dox


A549
LnCAP
A
E
HXK activity
(V_{max} per µg WCE)
1.2
1.0
0.8
0.6
0.4
0.2
0.0
2mM
4mM
5mM
control
VPA
2DOG
B
F
Relative HXK1 mRNA
(normalized to 36B4)
2.5
2.0
1.5
1.0
0.5
0.0
C
G
C16
C14
nmol per µg WCE
14
12
10
8
6
4
2
0
10mM
0.4
0.3
0.2
0.1
0
0.2
2
5
VPA (mM)
D
H
Pro
Asp/Asn
3.5
3
2.5
1.5
1
0.5