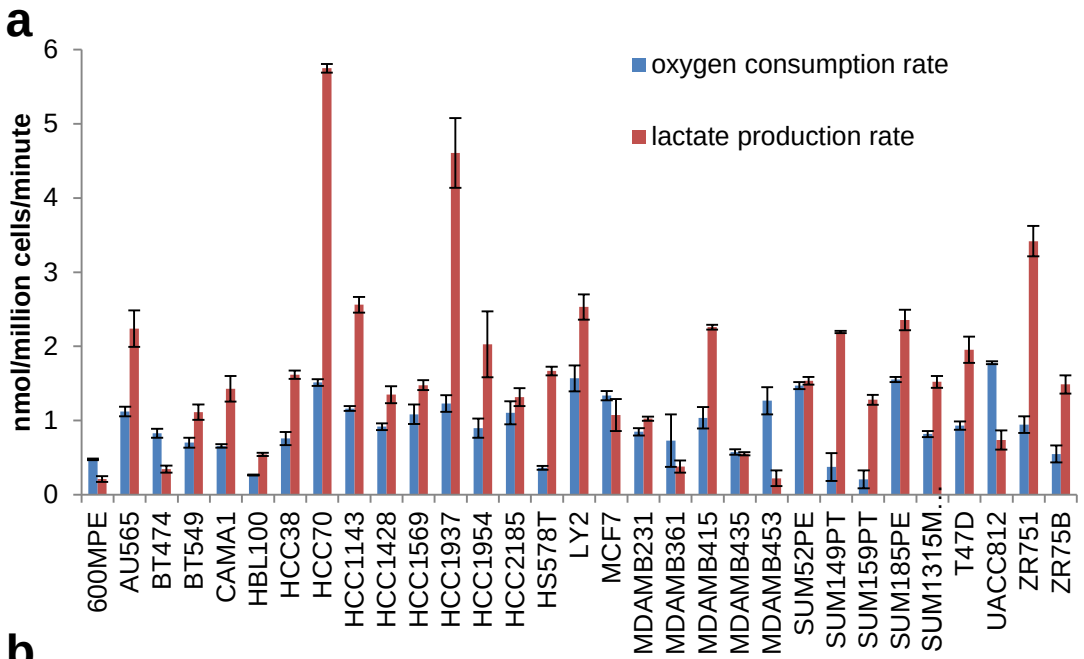
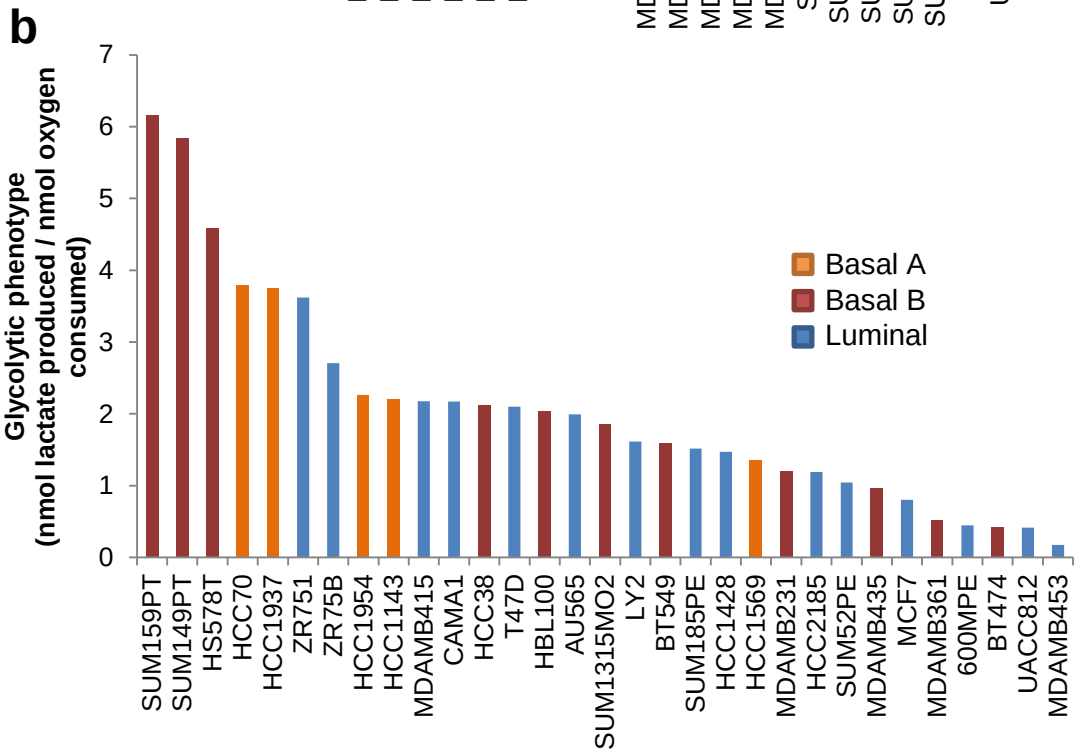
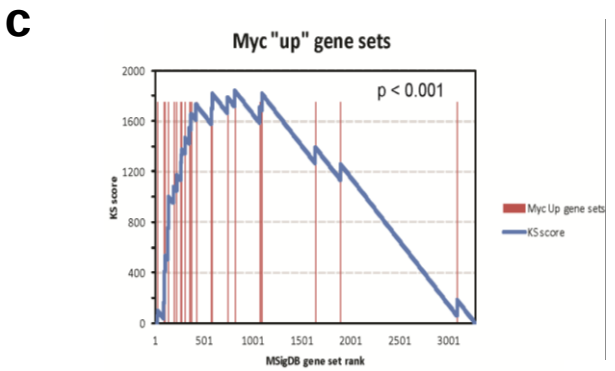
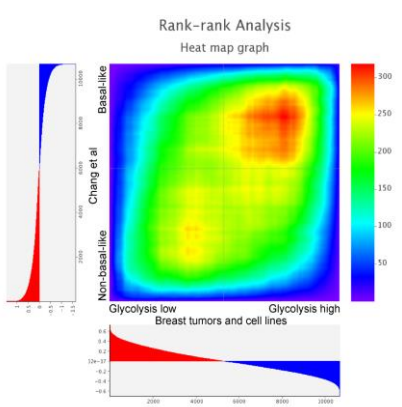
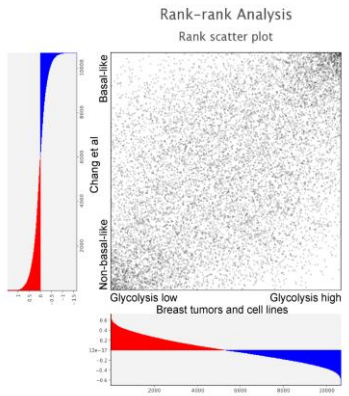
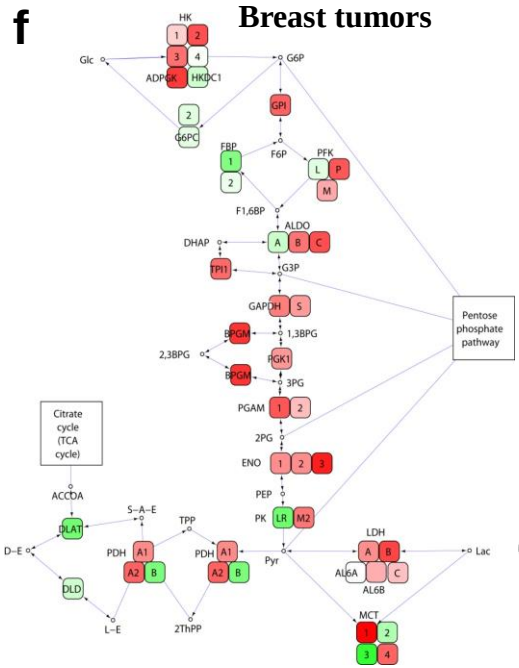


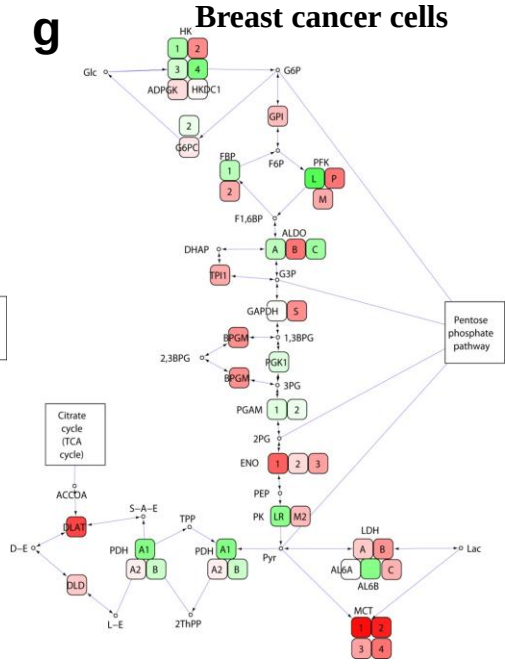










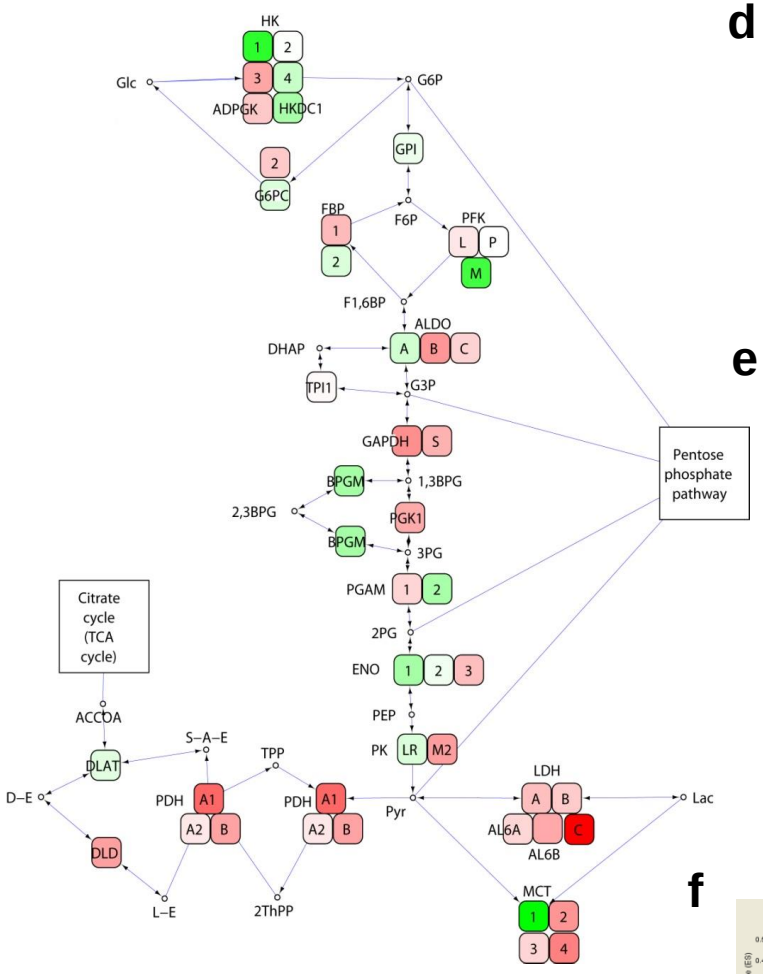


S1

Red = HIGH in the MCT1 knockdown cells
Green = LOW in the MCT1 knockdown cells

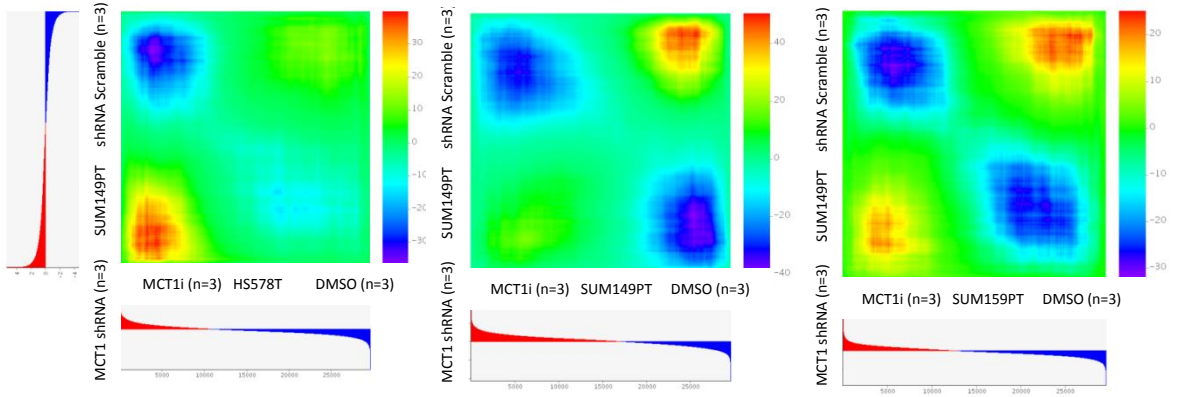
a

b

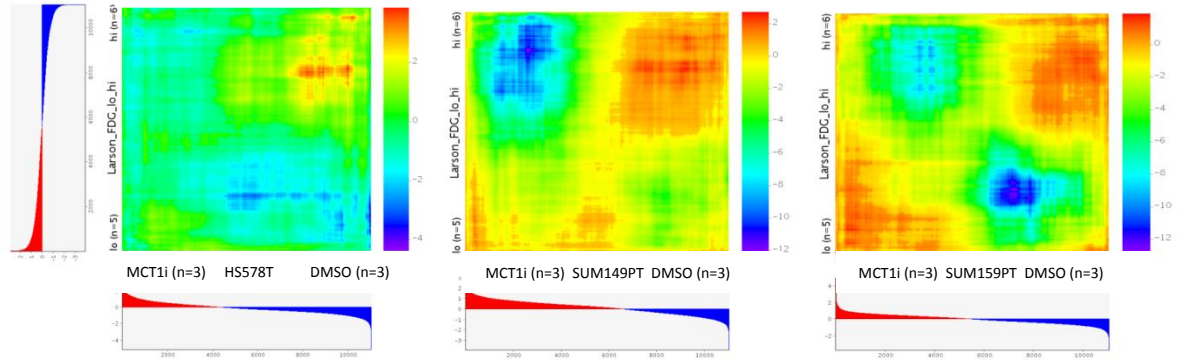


Red = HIGH in the MCT1 knockdown cells
Green = LOW in the MCT1 knockdown cells

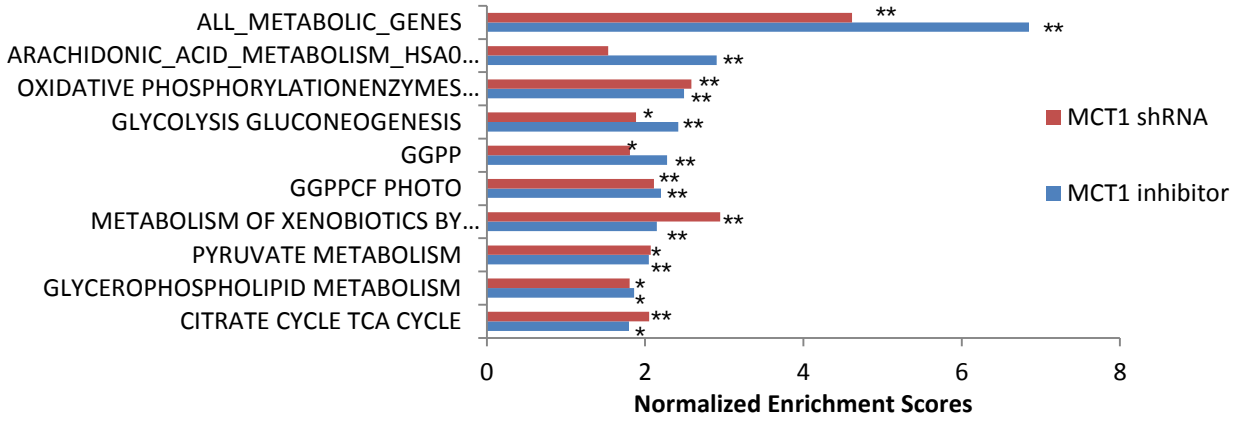
c



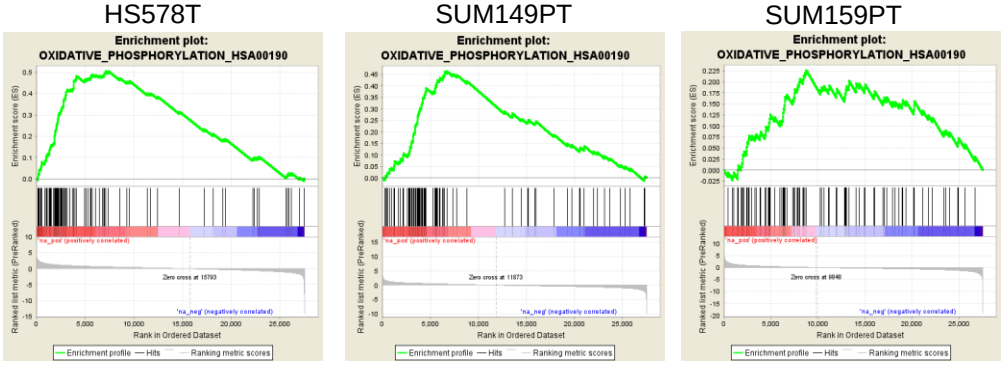
d



e

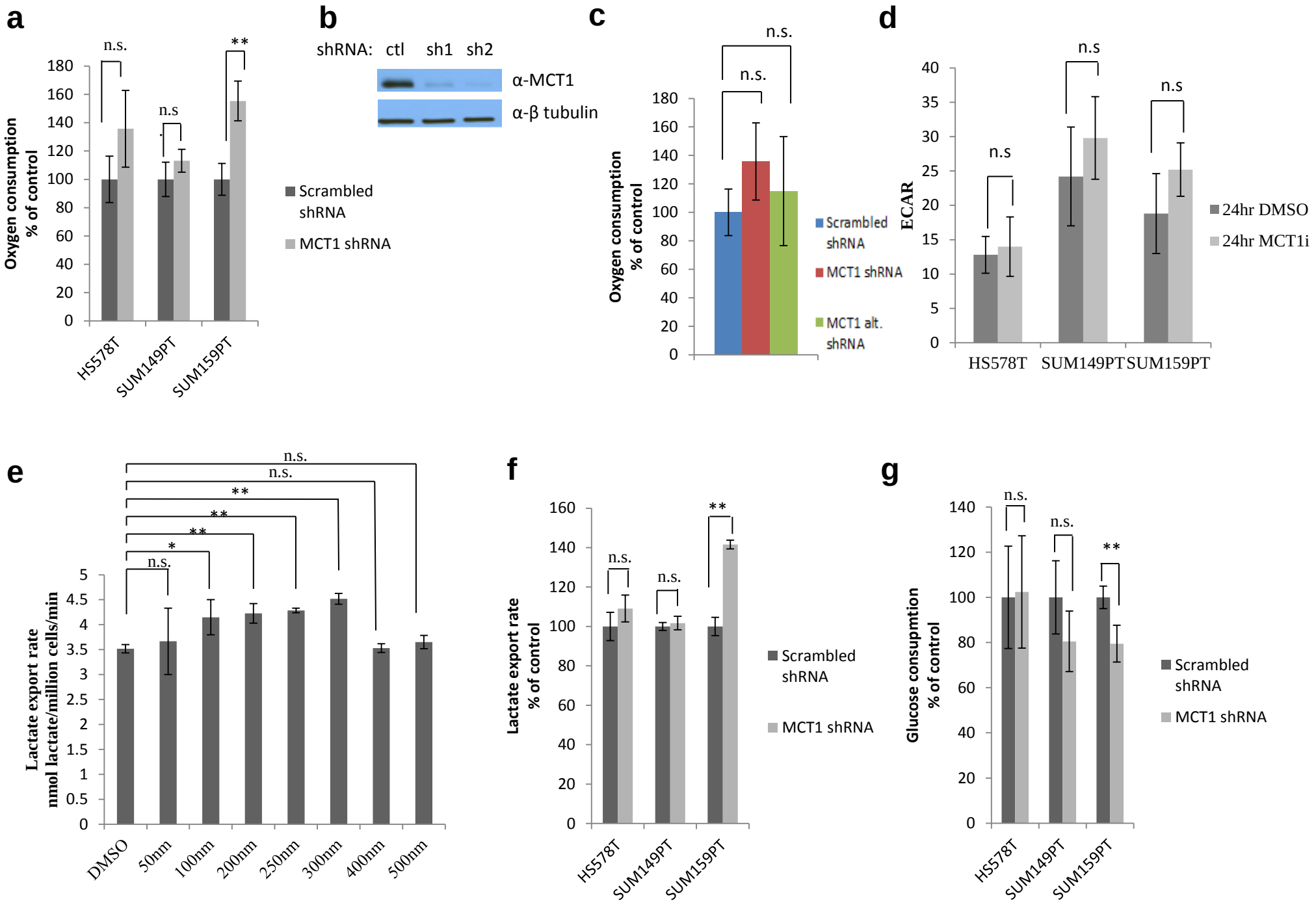


f

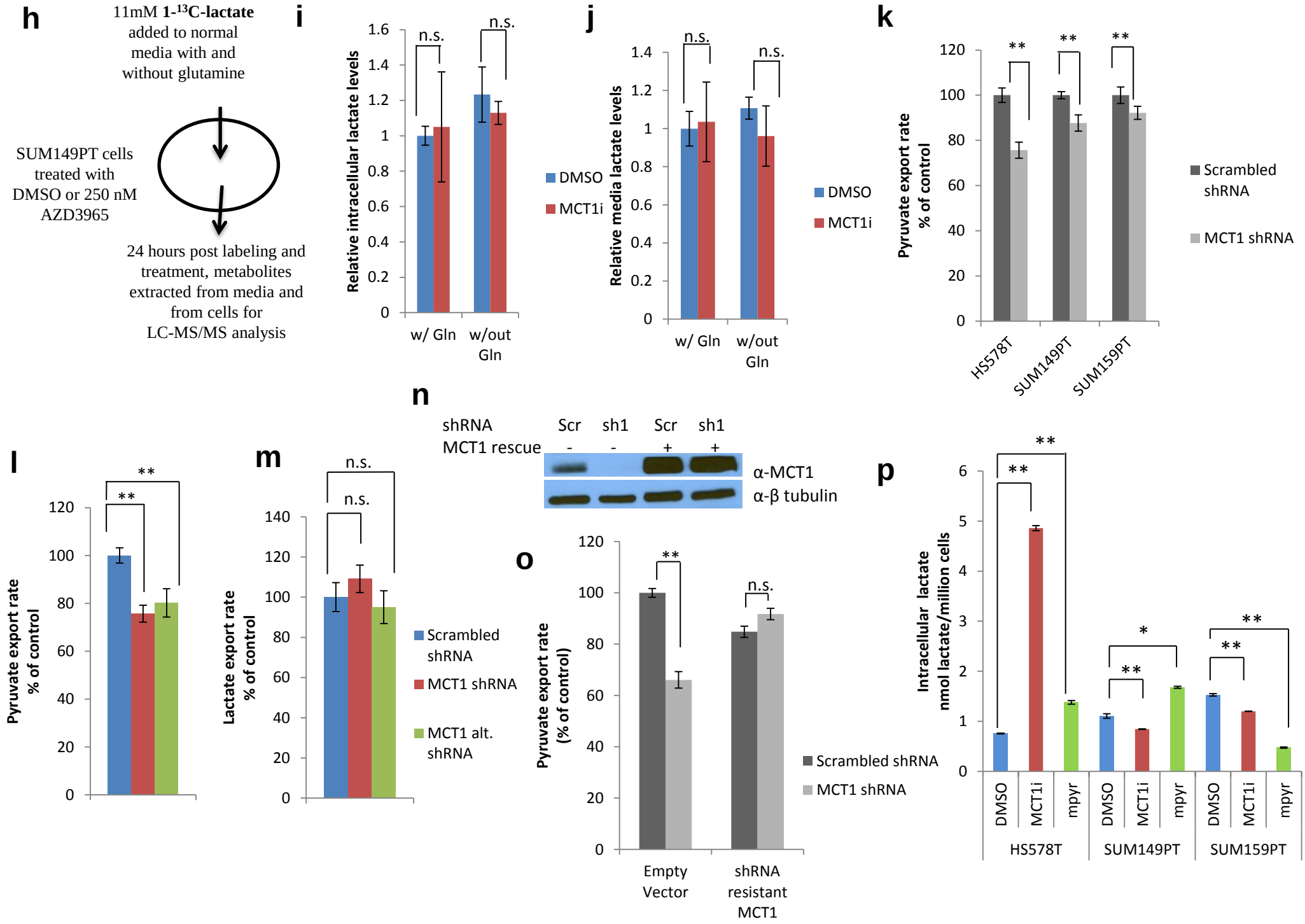


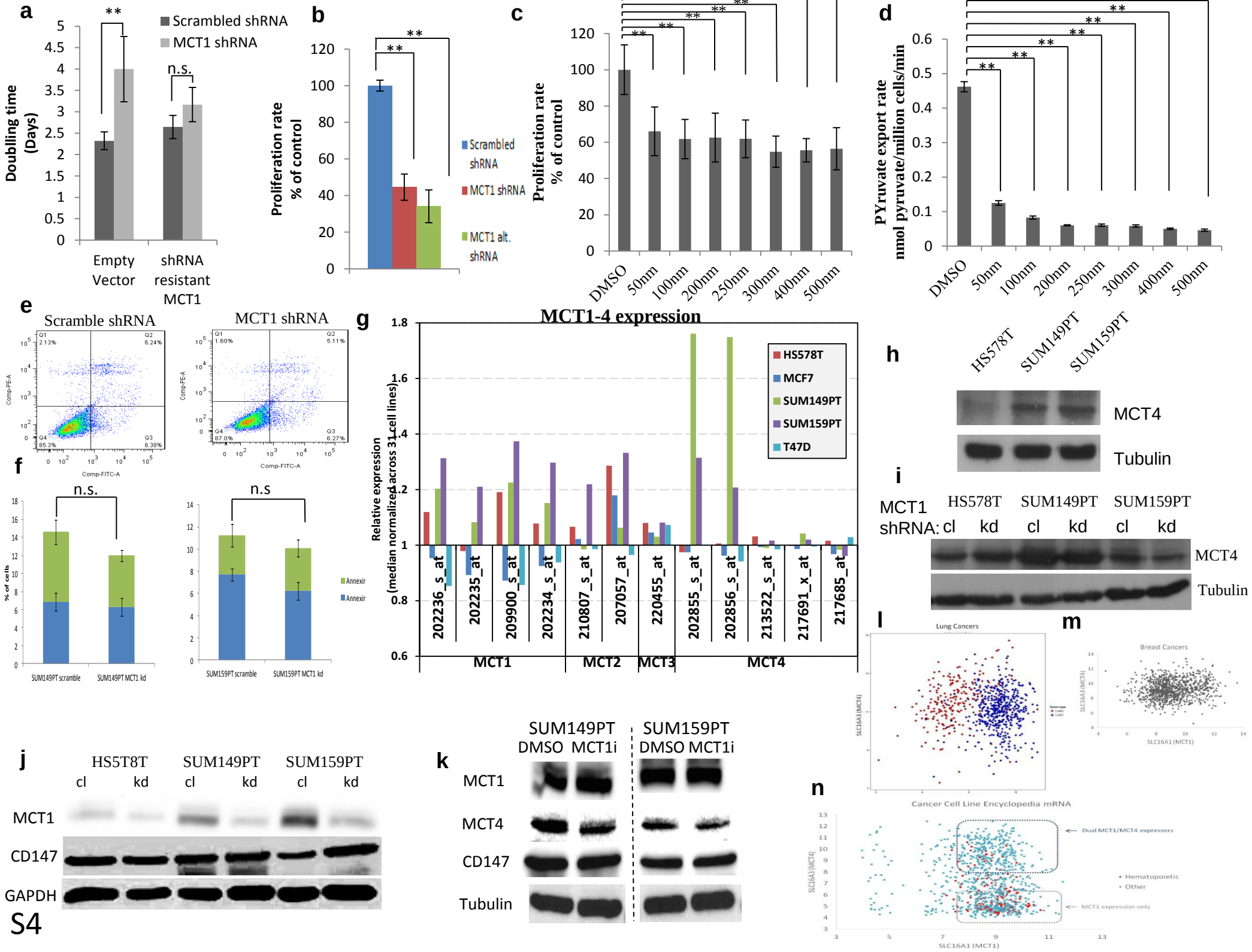
p –value < 0.001
for all three cell lines

S2


a
Oxygen consumption
% of control
n.s.
n.s
**
HS578T
SUM149PT
SUM159PT
Scrambled shRNA
MCT1 shRNA
b
shRNA: ctl sh1 sh2
α-MCT1
α-β tubulin
c
Oxygen consumption
% of control
n.s.
n.s.
Scrambled shRNA
MCT1 shRNA
MCT1 alt. shRNA
d
ECAR
n.s
n.s
n.s
HS578T
SUM149PT
SUM159PT
24hr DMSO
24hr MCT1i
e
Lactate export rate
nmol lactate/million cells/min
n.s.
*
**
DMSO
50nm
100nm
200nm
250nm
300nm
400nm
500nm
f
Lactate export rate
% of control
n.s.
**
HS578T
SUM149PT
SUM159PT
Scrambled shRNA
MCT1 shRNA
g
Glucose consupmtion
% of control
n.s.
**
HS578T
SUM149PT
SUM159PT
Scrambled shRNA
MCT1 shRNA

S3


h
11mM 1-13C-lactate added to normal media with and without glutamine
SUM149PT cells treated with DMSO or 250 nM AZD3965
24 hours post labeling and treatment, metabolites extracted from media and from cells for LC-MS/MS analysis
i
Relative intracellular lactate levels
n.s.
n.s.
w/ Gln
w/out Gln
DMSO
MCT1i
j
Relative media lactate levels
n.s.
n.s.
w/ Gln
w/out Gln
DMSO
MCT1i
k
Pyruvate export rate % of control
**
**
**
HS578T
SUM149PT
SUM159PT
Scrambled shRNA
MCT1 shRNA
n
shRNA
Scr
sh1
Scr
sh1
MCT1 rescue
-
-
+
+
α-MCT1
α-β tubulin
l
Pyruvate export rate % of control
**
**
m
Lactate export rate % of control
n.s.
n.s.
Scrambled shRNA
MCT1 shRNA
MCT1 alt. shRNA
o
Pyruvate export rate (% of control)
**
n.s.
Empty Vector
shRNA resistant MCT1
Scrambled shRNA
MCT1 shRNA
p
Intracellular lactate nmol lactate/million cells
**
**
*
**
**
**
DMSO
MCT1i
mpyr
HS578T
SUM149PT
SUM159PT


a
Doubling time (Days)
Scrambled shRNA
MCT1 shRNA
n.s.
Empty Vector
shRNA resistant MCT1
b
Proliferation rate % of control
Scrambled shRNA
MCT1 shRNA
MCT1 alt. shRNA
c
Proliferation rate % of control
DMSO
50nm
100nm
200nm
250nm
300nm
400nm
500nm
d
PYruvate export rate nmol pyruvate/million cells/min
e
Scramble shRNA
MCT1 shRNA
f
n.s.
n.s
% of cells
Annexin
SUM149PT scramble
SUM149PT MCT1 kd
SUM159PT scramble
SUM159PT MCT1 kd
g
MCT1-4 expression
Relative expression (median normalized across 31 cell lines)
HS578T
MCF7
SUM149PT
SUM159PT
T47D
202236_s_at
202235_at
209900_s_at
202234_s_at
210807_s_at
207057_at
220455_at
202855_s_at
202856_s_at
213522_s_at
217691_x_at
217685_at
MCT1
MCT2
MCT3
MCT4
h
HS578T
SUM149PT
SUM159PT
MCT4
Tubulin
i
MCT1 shRNA:
HS578T
SUM149PT
SUM159PT
cl
kd
MCT4
Tubulin
j
HS5T8T
SUM149PT
SUM159PT
cl
kd
MCT1
CD147
GAPDH
k
SUM149PT
SUM159PT
DMSO
MCT1i
MCT1
MCT4
CD147
Tubulin
l
Lung Cancers
SLC16A3 (MCT4)
m
Breast Cancers
SLC16A1 (MCT1)
n
Cancer Cell Line Encyclopedia mRNA
Dual MCT1/MCT4 expressers
Hematopoietic
Other
MCT1 expression only
SLC16A1 (MCT1)

S4

Supplemental Figure Legends

Supplemental Figure 1. MCT1 is amongst other metabolic genes elevated in glycolytic breast tumors and cell lines, related to Figure 1. a, Oxygen consumption rates (blue) and lactate production rates (red) of 31 breast cancer cell lines. Error bars denote standard deviation (n = 3). **b,** Stratification of the breast cancer cell lines based on glycolytic phenotype (nmol lactate produced / nmol oxygen consumed). Cell lines with gene expression profiles that cluster with Basal A breast cancers are indicated by orange bars, those that cluster with Basal B breast cancers are indicated by red bars, and those that cluster with Luminal breast cancers are indicated by blue bars. **c,** RNA expression data sets from i) human breast cancers with measured FDG uptake and ii) human breast cancer cell lines with measured glycolytic phenotype were analyzed individually by GSEA. Pathways from the Molecular Signatures Database (MSigDB) C2 collection were ranked by the average normalized enrichment score (NES) across the two data sets. Within this ranked list, gene sets associated with upregulated Myc activity (Myc "up") were significantly enriched in the highly glycolytic breast tumors and cell lines ($p < 0.001$). Myc "down" gene sets were not significantly enriched in either direction. **d, e,** A ranked list of transcripts correlating with glycolytic phenotype in breast tumors and cell lines was compared to a ranked list of transcripts correlating with basal phenotype in breast cancer using the rank-rank hypergeometric overlap (RRHO) algorithm. The resulting overlap from the ranked lists, represented as a hypergeometric heat map (**d**) and a scatter plot (**e**), indicates significant correlation between the gene signatures for glycolytic and basal phenotypes in breast cancer (p-value = 10^{-300}; -log p-value = 300). **f, g,** Highly glycolytic breast tumors (**f**) and cell lines (**g**) demonstrate coordinate upregulation of glycolysis genes and MCT1. Genes within the glycolysis pathway are colored red or green to denote high and low average correlation coefficients with glycolytic phenotype, respectively.

Supplemental Figure 2. MCT1 loss-of-function alters expression of glycolytic genes and increases expression of oxidative phosphorylation genes in breast cancer cell lines that co-express MCT1 and MCT4, related to Figure 3. a, SUM159PT cells were infected with lentivirus containing the pLKO vector with scrambled shRNA or shRNA that knocks down MCT1 expression. After selection in puromycin for 5 days, mRNA was extracted and transcript levels were measured using an Affymetrix microarray. **b,** A comparison of glycolysis gene transcript levels in the MCT1 knockdown cells versus scrambled shRNA cells is depicted. Red and green indicate increased and decreased mRNA levels, respectively in the MCT1 knockdown cells relative to cells infected with scrambled shRNA. mRNA was extracted from HS578T, SUM149PT, and SUM159PT cells treated for 24 hours with an MCT1 inhibitor (AZD3965) and transcript levels were measured using an Affymetrix microarray. These gene expression profiles were compared to mRNA from (a) SUM149PT cells expressing either scrambled (control) or MCT1 shRNA and (b) breast tumors with high or low FDG uptake by rank-rank hypergeometric analysis (Plaisier et al., 2010). The resulting hypergeometric heat maps indicate the degree of overlap between the two gene expression signatures where the direction-signed $\log_{10}$-transformed hypergeometric p-values are represented by the accompanying color scale. **c,** MCT1 inhibition rendered the gene expression profiles of HS578T cells more similar to SUM149PT cells with stable MCT1 knockdown. In SUM149PT and SUM159PT cells, MCT1 inhibition abrogated the similarity to SUM149PT cells expressing the scrambled shRNA. **d,** MCT1 inhibition rendered the gene expression profiles of all three glycolytic cell lines less similar to tumors with high FDG uptake. **e,** Gene set enrichment (GSEA) of MCT1 knockdown (red) SUM149PT cells, as well as MCT1i (AZD3965) (blue) treated SUM149PT cells, shows enrichment in oxidative phosphorylation enzymes, pyruvate metabolism and TCA cycle KEGG pathways. **f,** HS578T, SUM149PT, SUM159PT were treated with vehicle (DMSO) versus 250 nM MCT1 inhibitor (AZD3965) for 24hrs. mRNA was extracted (Qiagen) and transcript levels were measured using Affymetrix (U133plus2.0). Mountain plots showing the enrichment of the oxidative phosphorylation gene sets in each of the cell lines is depicted.

Supplemental Figure 3. Lactate export rates are not consistently altered upon MCT1 inhibition, but pyruvate export and proliferation rates are reduced even at low concentrations of AZD3965, related to figure 3. a, Oxygen consumption rates of the indicated cell lines stably expressing scrambled shRNA or shRNA towards MCT1 (MCT1 shRNA). **b,** Immunoblotting of lysates from HS578T cells stably expressing shRNA constructs and used in used in **c,l,m**. HS578T cells were infected with lentivirus containing the pLKO vector with scrambled shRNA (cl), an shRNA that knocks down MCT1 expression (sh1), or an alternate shRNA that knocks down MCT1 expression (sh2). **c,** Oxygen consumption rates of HS578T cells stably expressing scrambled shRNA (ctl, blue), MCT1 shRNA (sh1, red), or an alternate MCT1 shRNA (sh2, green). **d,** Extracellular acidification rates (ECAR) of the indicated cell lines 24 hrs post treatment with DMSO or 250 nm AZD3965 (MCT1i). **e,** Lactate export rates of HS578T cells treated with the indicated doses of AZD3965 or DMSO for 4 hours. **f,** Lactate export rates of the indicated cell lines

stably expressing scrambled shRNA or shRNA towards MCT1 (MCT1 shRNA). **g**, Glucose consumption rates of the indicated cell lines stably expressing scrambled shRNA or shRNA towards MCT1 (MCT1 shRNA). **h,** Schematic depicting experimental setup for Figure 3f and Supplemental Figure 3i-j. Briefly, 11mM 1-13C-lactate was added to normal culture media at the same time as addition of DMSO or 250 nm AZD3965 (MCT1i). Metabolites were extracted from the cells and media 24 hrs post labeling and treatment, and analyzed by LC-MS/MS. **i,** Relative intracellular lactate levels as measured by LC-MS/MS. **j,** Relative media lactate levels as measured by LC-MS/MS. For **i** and **j**, values shown are relative to lactate levels found in DMSO-treated cells grown in the presence of glutamine. **k,** Pyruvate export rates of the indicated cell lines stably expressing scrambled shRNA or shRNA towards MCT1 (MCT1 shRNA). **l,** Pyruvate export rates of HS578T cells stably expressing scrambled shRNA (ctl, blue), MCT1 shRNA (sh1, red), or an alternate MCT1 shRNA (sh2, green). **m,** Lactate export rates of HS578T cells stably expressing scrambled shRNA (ctl, blue), MCT1 shRNA (sh1, red), or an alternate MCT1 shRNA (sh2, green). For **n-o**, HS578T cells were infected with lentivirus containing the empty M4 vector or an M4 vector containing an MCT1 cDNA construct resistant to shRNA (MCT1 rescue), and selected for 5 days with blasticidin. Both cell lines were then infected with a lentivirus containing a pLKO vector with scrambled shRNA (Scramble shRNA) or shRNA that knocks down MCT1 expression (MCT1 shRNA) and selected with media containing blasticidin and puromycin. **n,** Immunoblotting of the breast cancer cell line HS578T stably expressing empty M4 vector or the M4-MCT1 rescue vector and shRNA constructs. **o,** Pyruvate export rates of the indicated cells. **p,** Intracellular lactate levels from the indicated cell lines treated with DMSO, 250 nM MCT1i, or 5 mM methyl-pyruvate for 30min. For a, c-g, i-m, n-o error bars denote standard deviation (n = 3). * denotes $p < 0.05$; ** denotes $p < 0.01$.

Supplemental Figure 4. MCT1 and MCT4 are coexpressed in patient tumors and cell lines, related to Figure 4. a, Doubling time of HS578T cells stably expressing empty M4 vector or the M4-MCT1 rescue vector and shRNA constructs. **b,** Proliferation rate of HS578T cells stably expressing scrambled shRNA (ctl, blue), MCT1 shRNA (sh1, red), or an alternate MCT1 shRNA (sh2, green). **c,** Proliferation rates of HS578T cells treated with the indicated doses of AZD3965 or DMSO for 5 days. **d**, Pyruvate export rates of HS578T cells treated with indicated doses of AZD3965 or DMSO for 4 hours. Error bars in (**c**) denote standard deviation (n=5), and in (**d**) denote standard deviation (n=3). * denotes $p < 0.05$; ** denotes $p < 0.01$. **e,f,** SUM149PT and SUM159PT cells were infected with lentivirus containing the pLKO vector with scrambled shRNA (Scramble shRNA) or shRNA that knocks down MCT1 expression (MCT1 kd shRNA). After 5 days of puromycin selection, 10^5 resistant cells were incubated with Annexin V and propidium iodide (PI) for 15 minutes and then analyzed by flow cytometry. **e,** Representative FACS plots showing percentage of cells in early apoptosis indicated by Annexin V positive, PI negative staining (lower right quadrant) and percentage of cell in late apoptosis marked by Annexin V/PI double positive staining (upper right quadrant). **f,** Percentage of early apoptotic cells are shown in blue, and percentage of late apoptotic cells are shown in green. Error bars denote standard deviation (n = 3). **g,** MCT1-4 transcript levels determined by different microarray probes in HCC1937, HS578T, SUM149PT, and SUM159PT cells. **h,** Immunoblotting of whole cell lysates from the indicated breast cancer cell lines using antibodies towards MCT4 and tubulin. SUM149PT and SUM159PT cells express relatively higher amounts of MCT4. **i,** Immunoblotting of whole cell lysates from the indicated cell lines stably expressing scrambled shRNA (cl) or shRNA that knocks down MCT1 expression (kd). Whole cell lysates were probed with antibodies towards MCT4 and tubulin. MCT4 protein levels are reduced upon MCT1 knockdown in multiple cell lines including HCC1937 and HS578T cells. **j,** Immunoblotting of whole cell lysates from indicated breast cancer cell lines stably expressing scrambled shRNA (cl) or shRNA that knocks down MCT1 expression (kd). Whole cell lysates were probed with antibodies towards MCT1, CD147 and GAPDH. CD147 protein levels remain unchanged in HS578T and SUM149PT cells. SUM159PT cells express higher amounts of CD147 upon MCT1 knockdown. **k,** Immunoblotting of whole cell lysates from indicated breast cancer cell lines treated with DMSO or 250 nm AZD3965 (MCT1i) for 24hrs. Whole cell lysates were probed with antibodies towards MCT1, MCT4, CD147 and Tubulin. Levels of indicated proteins remain unchanged after MCT1i treatment. **l**, Scatter plot showing relative SLC16A1 mRNA (encoding MCT1) and SLC16A3 mRNA (encoding MCT4) levels from patient lung adenocarcinomas (red dots) and lung squamous cell carcinomas (blue dots). **m**, Scatter plot showing relative SLC16A1 and SLC16A3 mRNA levels in breast cancers. **n**, Scatter plot showing relative levels of SLC16A3 and SLC16A1 in cancer cell lines.

Supplementary Table S1: Gene Set Enrichment Analysis of KEGG pathways in glycolytic breast tumors and cell lines. Related to Figure 1

Supplementary Table S2: Gene Set Enrichment Analysis of MSigDB C2 pathways in glycolytic breast tumors and cell lines. Related to Figure 1

Supplemental Methods

Stable Cell Line Construction

For stable cell line construction, breast cancer cell lines were infected with lentivirus containing scrambled or MCT1 shRNA, and cells were selected in 2 μg ml^{-1} puromycin for 5 days before experimentation.

Cell Proliferation Measurements

For cell proliferation measurements, $1x10^4$– $2x10^4$ cells were seeded in triplicate in 6-well plates and accurate cell counts were obtained using a Coulter particle analyser after a 5-day period.

Intracellular pyruvate and lactate measurements

To measure the intracellular pyruvate and lactate levels cells were seeded in triplicate in 10cm plates at 50-70% confluency. Tweny-four hours post seeding cells were treated with DMSO (ctl), MCT1i or methyl-lactate/pyruvate for 30min. Cells were lysed in NP40 lysis buffer and the lysates were spun in 10K filter columns for 10min at 4°C. Aliquots of the filtered lysate were assess for amount of pyruvate and lactate present.

FACS Analysis

Cell cycle analysis was performed as described previously (Krishan, 1975), and cell viability was assessed using an apoptosis detection kit (BD Pharmingen).