Supplementary Figures

Architectural Control of Metabolic Plasticity in Epithelial Cancer Cells

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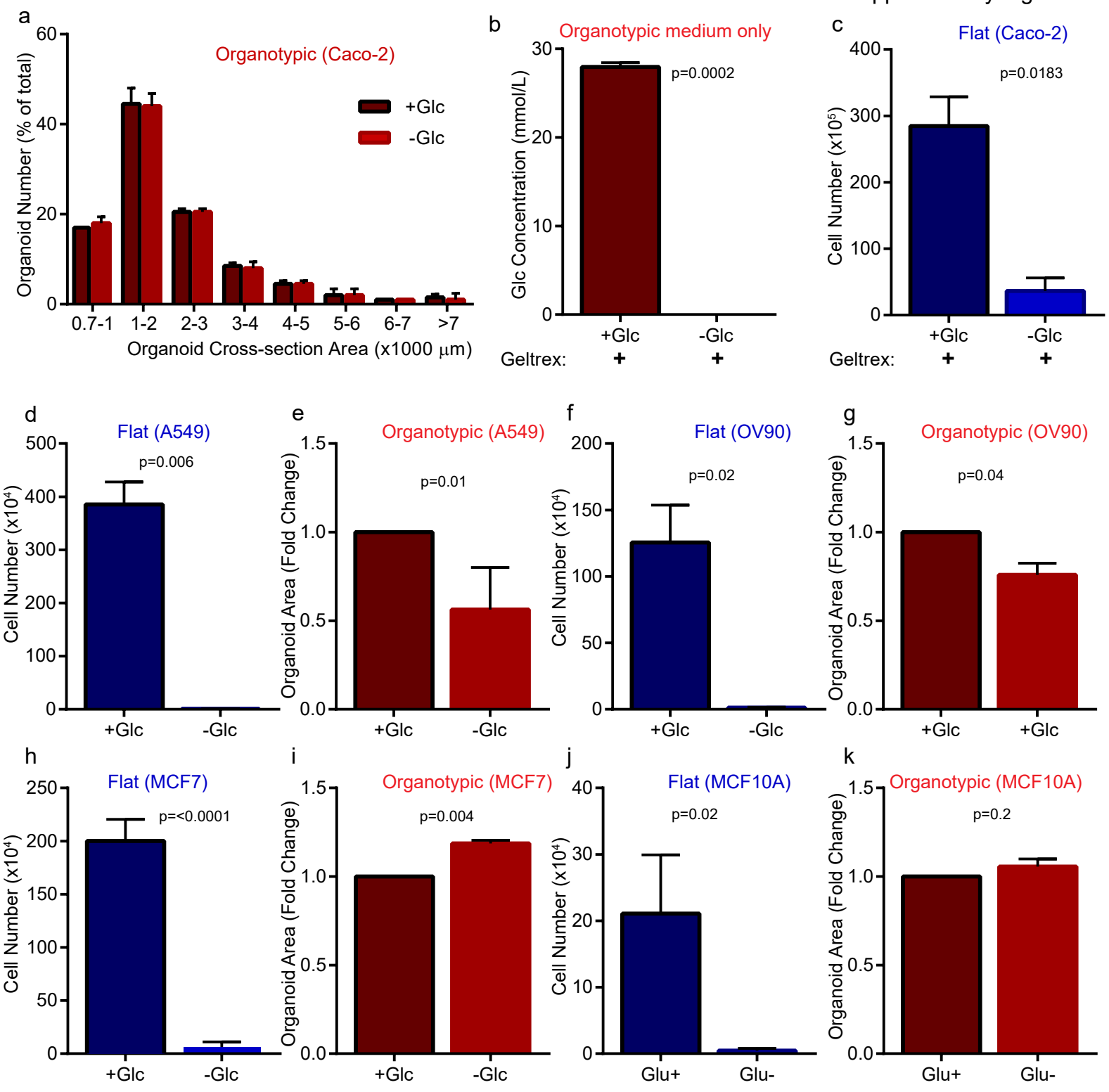
Supplementary Figure S1



Supplementary Figure S1 - Epithelial architecture is associated with glucose-dependent growth
a) Histogram showing the distribution of Caco-2 organoid cross-section areas after 6-days of organotypic culture in the presence (+Glc) or absence (-Glc) of glucose (n=2677 (+Glc), n=2663 (-Glc)) (r=2 independent experimental replicates).

b) Bar chart showing the glucose concentration cell-free culture media containing Geltrex in the presence (+Glc) or absence (-Glc) of glucose (r=2 independent experimental replicates).

c) Bar chart showing Caco-2 cell number after 6 days of flat culture in media containing soluble basement membrane extract (BME) in the presence (+Glc) or absence (-Glc) of glucose (r=2 independent experimental replicates).

d) Bar chart showing A549 cell number after 6 days of flat culture in the presence (+Glc) or absence (-Glc) of glucose (r=2 independent experimental replicates).

e) Bar chart showing cross-section area of A549 clusters after 6 days of organotypic culture in the presence (+Glc) or absence (-Glc) of glucose (n=3048 (+Glc), n=3435(-Glc)) (r=4 independent experimental replicates).

f) Bar chart showing OV90 after 6 -days of flat culture in the presence (+Glc) or absence (-Glc) of glucose (r=2 independent experimental replicates).

g) Bar chart showing cross-section area of OV90 clusters after 6 days of organotypic culture in the presence (+Glc) or absence (-Glc) of glucose (n=859 (+Glc), n=592 (-Glc)) (r=2 independent experimental replicates).

h) Bar chart showing MCF7 cell number after 6 days of flat culture in the presence (+Glc) or absence (-Glc) of glucose (r=3 independent experimental replicates).

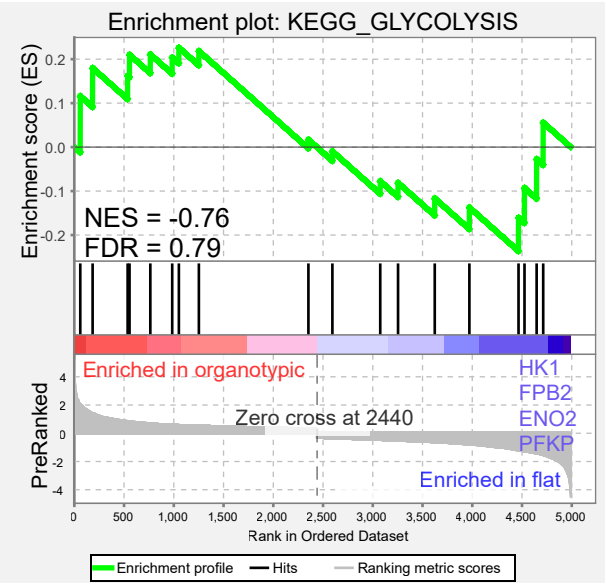
i) Bar chart showing cross-section area of MCF7 clusters after 6 days of organotypic culture in the presence (+Glc) or absence (-Glc) of glucose (n=680 (+Glc), n=944 (-Glc)) (r=2 independent experimental replicates).

j) Bar chart showing MCF10A cell number after 6 days of flat culture in the presence (+Glc) or absence (-Glc) of glucose (r=3 independent experimental replicates).

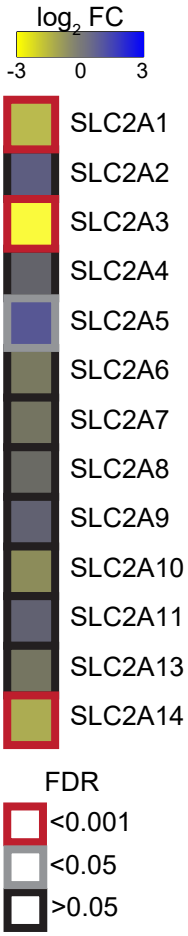
k) Bar chart showing cross-section area of MCF10A spheroids after 6 days of organotypic culture in the presence (+Glc) or absence (-Glc) of glucose (n=313 spheroids (+Glc), n=294 spheroids (-Glc)), (r=2 independent experimental replicates).

All error bars are standard deviation.

a


Enrichment plot: KEGG_GLYCOLYSIS
Enrichment score (ES)
0.2
0.1
0.0
-0.1
-0.2
NES = -0.76
FDR = 0.79
Enriched in organotypic
HK1
FPB2
ENO2
PFKP
Zero cross at 2440
Enriched in flat
PreRanked
4
2
0
-2
-4
0
500
1,000
1,500
2,000
2,500
3,000
3,500
4,000
4,500
5,000
Rank in Ordered Dataset
Enrichment profile
Hits
Ranking metric scores

b

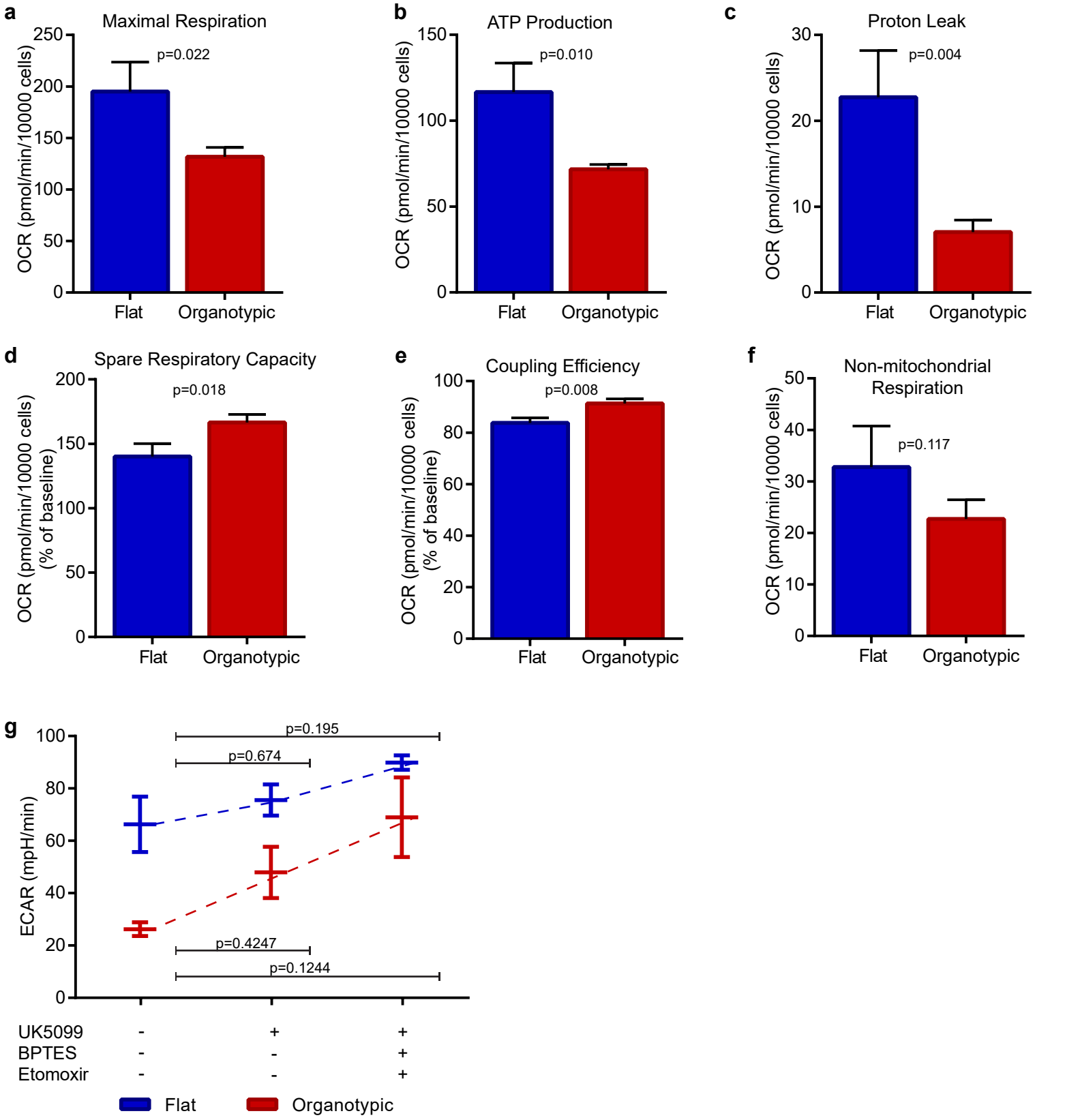

log2 FC
-3
0
3
SLC2A1
SLC2A2
SLC2A3
SLC2A4
SLC2A5
SLC2A6
SLC2A7
SLC2A8
SLC2A9
SLC2A10
SLC2A11
SLC2A13
SLC2A14
FDR
<0.001
<0.05
>0.05

Supplementary Figure S2 – Enrichment of genes related to glycolysis

a) Gene Set Enrichment plot for KEGG_GLYCOLYSIS_GLUCONEOGENESIS gene set. Four genes in blue represente genes enriched (false discovery rate (FDR) < 0.01) in flat cultures.

b) Heatmap of gene expression for glucose transporters from RNA-Seq showing log2 fold change (FC) of organotypic compared to flat cultures. Outlines indicate the FDR for each gene. (Supplementary Table S1).

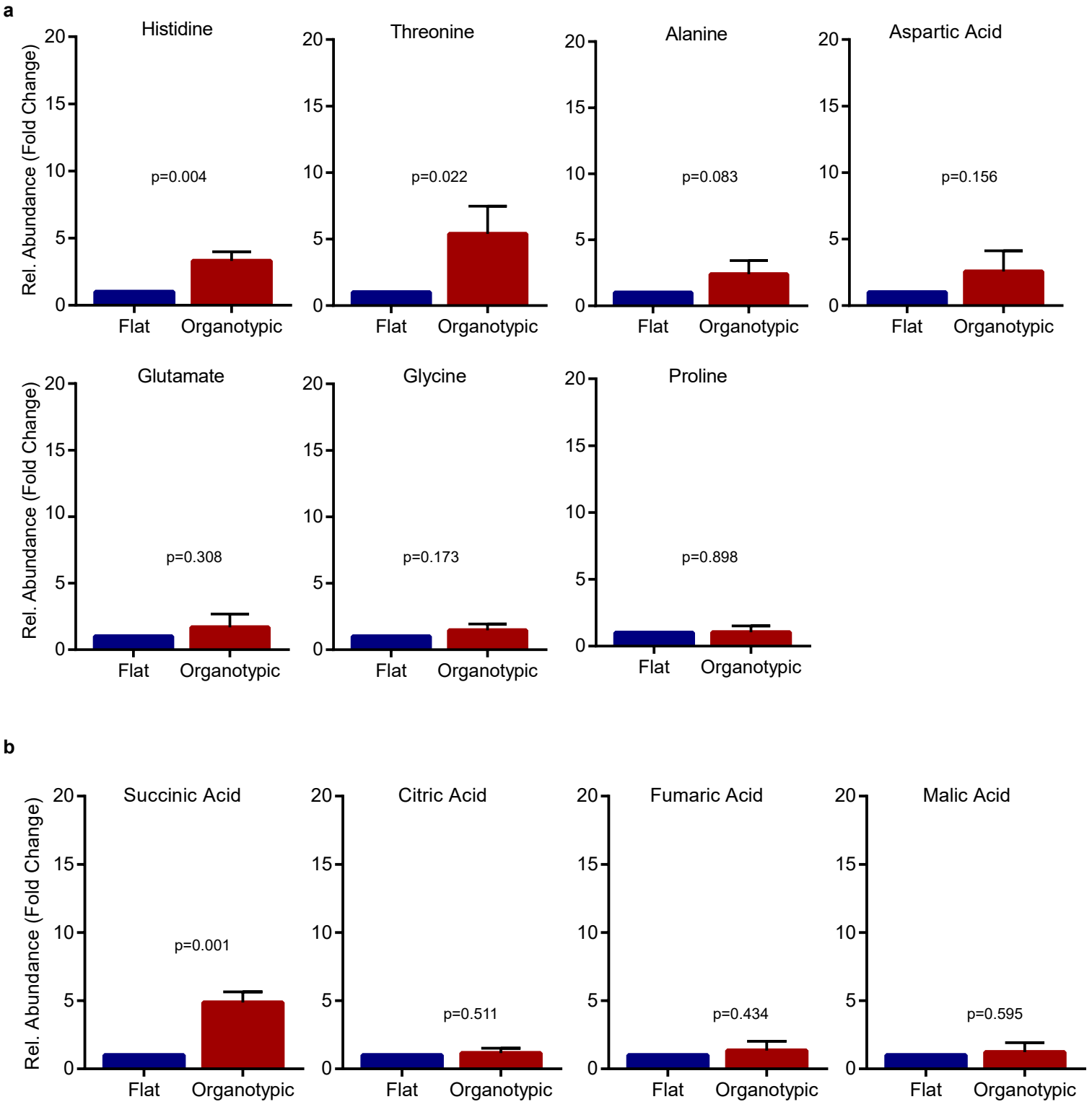
Supplementary Figure S3



Supplementary Figure S3 – Epithelial architecture is associated with energetic stress responses
a-f) Bar charts showing maximal respiration **(a)**, ATP production **(b)**, proton leak **(c)**, spare respiratory capacity **(d)**, mitochondrial coupling efficiency **(e)**, and non-mitochondrial respiration **(f)** for flat and organotypic Caco-2 cultures (r=3 independent experimental replicates).

g) Waterfall chart showing ECARs at baseline and following sequential addition of UK5099 followed by BPTES and Etomoxir for flat and organotypic Caco-2 cultures (r=2 independent experimental replicates).
All error bars are standard deviation.

Supplementary Figure S4

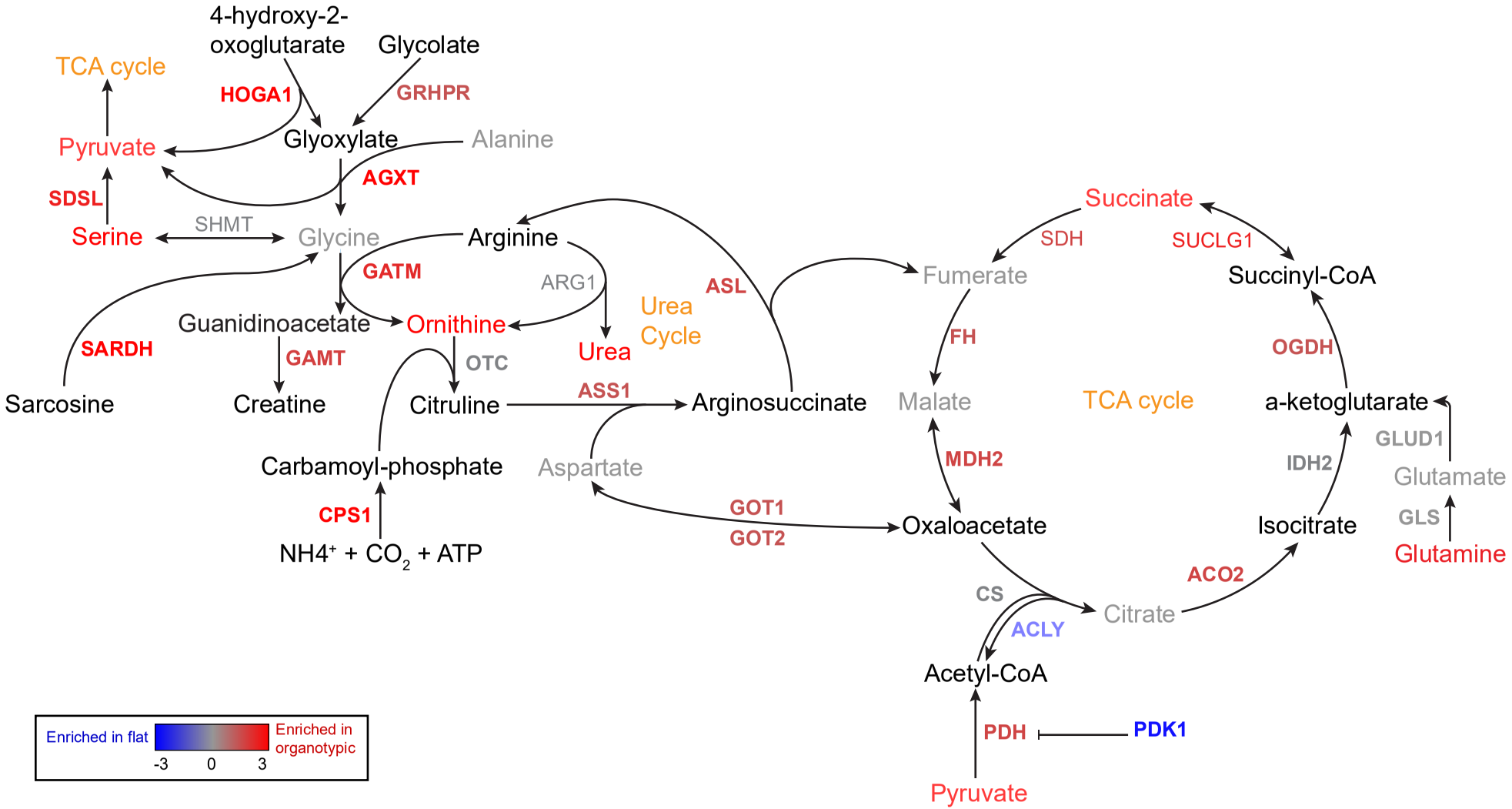


Supplementary Figure S4 – Amino acid metabolism is enriched in organotypic cultures

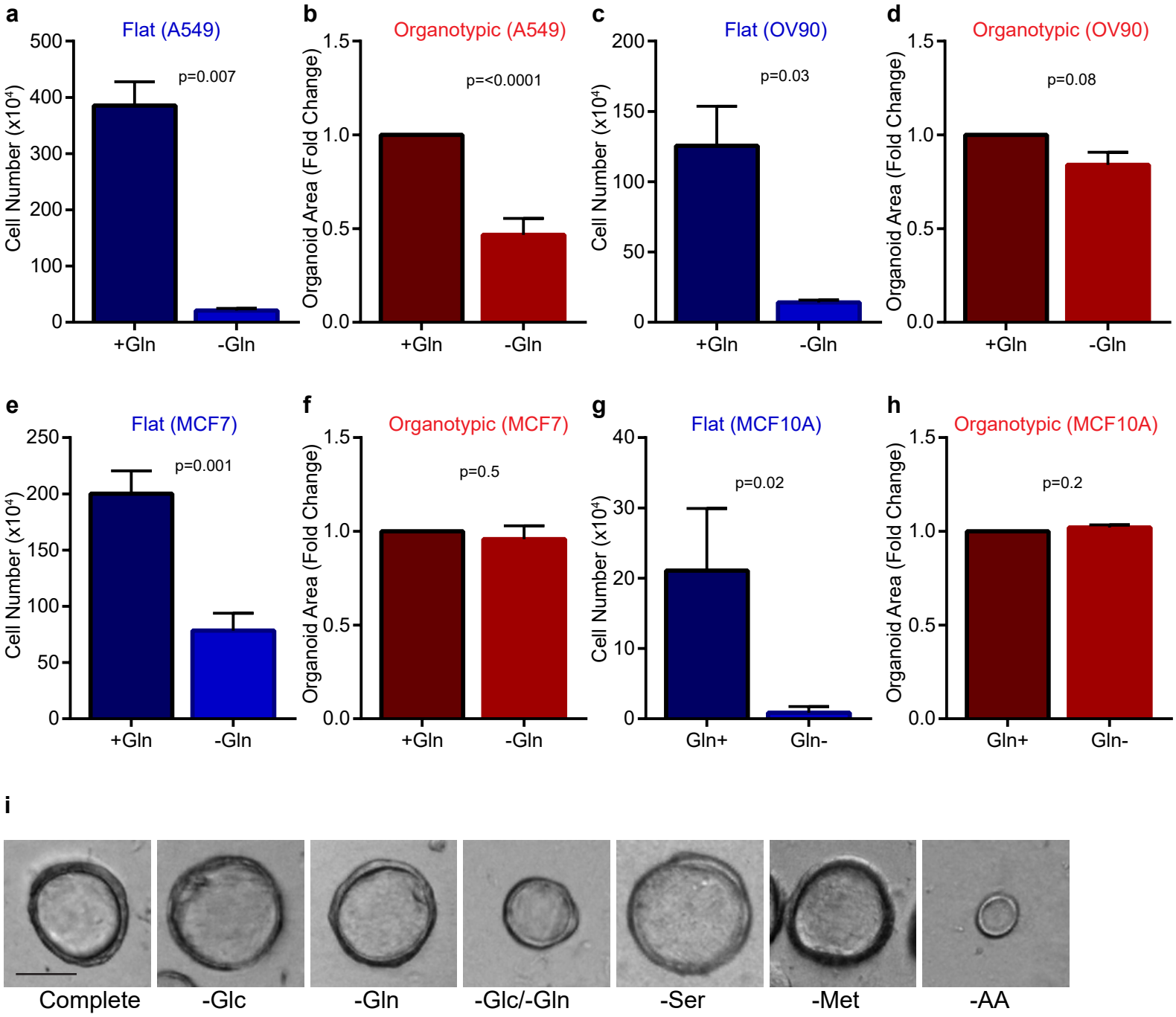
a) Bar graphs showing abundance of histidine, threonine, alanine, aspartic acid, glutamic acid, glycine, and proline from flat and organotypic Caco-2 cells. Cells were cultured for 5 days, then cellular extracts were analyzed by GC/MS. Data available in Supplementary Table S2. (n=5 sample replicates for each of r=3 independent experimental replicates).

b) Bar graphs showing normalized levels of succinic acid, citric acid, fumaric acid, and malic acid from flat and organotypic Caco-2 cells. (n=5 sample replicates for each of r=3 independent experimental replicates).

All error bars are standard deviation.


4-hydroxy-2-oxoglutarate
Glycolate
TCA cycle
HOGA1
GRHPR
Pyruvate
Glyoxylate
Alanine
AGXT
SDSL
Serine
SHMT
Glycine
Arginine
GATM
ARG1
ASL
Urea Cycle
Guanidinoacetate
Ornithine
Urea
SARDH
GAMT
OTC
Sarcosine
Creatine
Citruline
ASS1
Arginosuccinate
Carbamoyl-phosphate
Aspartate
CPS1
NH4+ + CO2 + ATP
GOT1
GOT2
Oxaloacetate
Succinate
SDH
SUCLG1
Fumerate
Succinyl-CoA
FH
OGDH
Malate
TCA cycle
a-ketoglutarate
MDH2
IDH2
GLUD1
Glutamate
GLS
Glutamine
Isocitrate
ACO2
CS
ACLY
Citrate
Acetyl-CoA
PDH
PDK1
Pyruvate
Enriched in flat
Enriched in organotypic
-3
0
3

Supplementary Figure S5 – Differences in metabolic networks in flat and organotypic cultures
Metabolic map showing integrated gene-expression and GC/MS data for metabolic pathways enriched in organotypic Caco-2 cultures. Red indicates enrichment in organotypic cultures and blue indicates enrichment in flat cultures. Grey indicates no enrichment and black indicates the gene/metabolite was not measured.


a
Flat (A549)
p=0.007
Cell Number (x10⁴)
500
400
300
200
100
0
+Gln
-Gln
b
Organotypic (A549)
p=<0.0001
Organoid Area (Fold Change)
1.5
1.0
0.5
0.0
+Gln
-Gln
c
Flat (OV90)
p=0.03
Cell Number (x10⁴)
200
150
100
50
0
+Gln
-Gln
d
Organotypic (OV90)
p=0.08
Organoid Area (Fold Change)
1.5
1.0
0.5
0.0
+Gln
-Gln
e
Flat (MCF7)
p=0.001
Cell Number (x10⁴)
250
200
150
100
50
0
+Gln
-Gln
f
Organotypic (MCF7)
p=0.5
Organoid Area (Fold Change)
1.5
1.0
0.5
0.0
+Gln
-Gln
g
Flat (MCF10A)
p=0.02
Cell Number (x10⁴)
40
30
20
10
0
Gln+
Gln-
h
Organotypic (MCF10A)
p=0.2
Organoid Area (Fold Change)
1.5
1.0
0.5
0.0
Gln+
Gln-
i
Complete
-Glc
-Gln
-Glc/-Gln
-Ser
-Met
-AA

Supplementary Figure S6 - Organotypic cells exhibit plasticity to Amino Acid nutrient stress

a) Bar chart showing A549 cell number after 6 days of flat culture in the presence (+Gln) or absence (-Gln) of glutamine (n=2 independent experimental replicates).

b) Bar chart showing cross-section area of A549 cell clusters after 6 days of organotypic culture in the presence (+Gln) or absence (-Gln) of glutamine (n=3048(+Gln), n=2872(-Gln)) (r=4 independent experimental replicates).

c) Bar chart showing OV90 cell number after 6 days of flat culture in the presence (+Gln) or absence (-Gln) of glutamine (n=2 independent experimental replicates).

d) Bar chart showing cross-section area of OV90 cell clusters after 6 days of organotypic culture in the presence (+Gln) or absence (-Gln) of glutamine (n=859(+Gln), n=821(-Gln)) (r=2 independent experimental replicates).

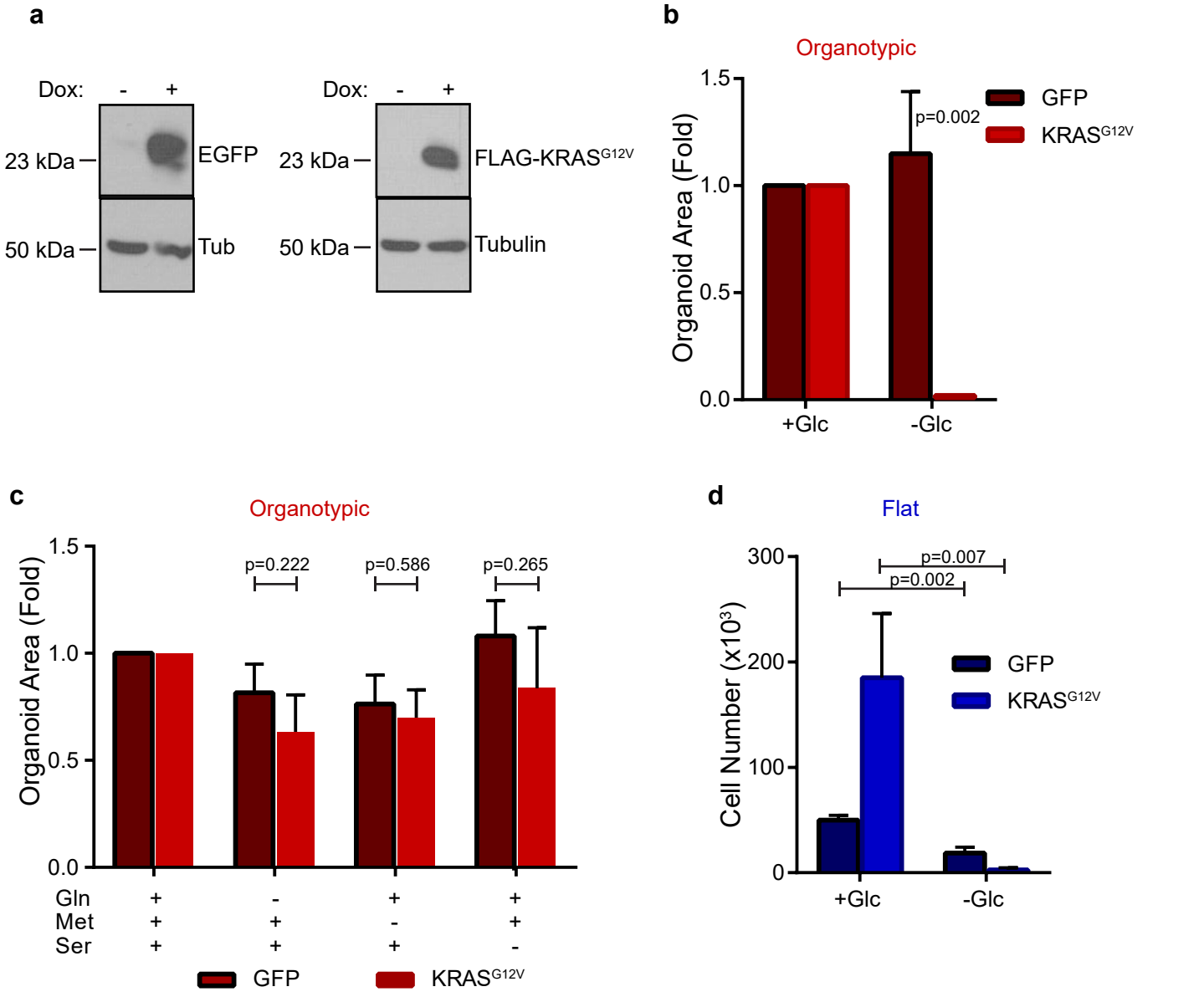
e) Bar chart showing MCF7 cell number after 6 days of flat culture in the presence (+Gln) or absence (-Gln) of glutamine (n=2 independent experimental replicates).

f) Bar chart showing cross-section area of MCF7 cell clusters after 6 days of organotypic culture in the presence (+Gln) or absence (-Gln) of glutamine (n=680(+Gln), n=829(-Gln)) (r=2 independent experimental replicates).

e) Bar chart showing MCF10A cell number after 6 days of flat culture in the presence (+Gln) or absence (-Gln) of glutamine (n=2 independent experimental replicates).

f) Bar chart showing cross-section area of MCF10A cell clusters after 6 days of organotypic culture in the presence (+Gln) or absence (-Gln) of glutamine (n=359 (+Gln), n=316 (-Gln)); (r=2 independent experimental replicates).

i) Representative phase contrast images from Caco-2 organotypic cultures showing architecture of cell cysts grown in complete medium, or medium free of glucose (-Glc), glutamine (-Gln), glucose and glutamine (-Glc/Gln), serine (-Ser), methionine (-Met), and amino acids (-AA).
All error bars are standard deviation.


a
Dox: - +
23 kDa
EGFP
50 kDa
Tub
Dox: - +
23 kDa
FLAG-KRASG12V
50 kDa
Tubulin
b
Organotypic
Organoid Area (Fold)
1.5
1.0
0.5
0.0
p=0.002
GFP
KRASG12V
+Glc
-Glc
c
Organotypic
Organoid Area (Fold)
1.5
1.0
0.5
0.0
p=0.222
p=0.586
p=0.265
Gln + - + +
Met + + - +
Ser + + + -
GFP
KRASG12V
d
Flat
Cell Number (x10³)
300
200
100
0
p=0.007
p=0.002
GFP
KRASG12V
+Glc
-Glc

Supplementary Figure S7 – Organotypic cultures have reduced metabolic plasticity with Ras-driven glucose addiction

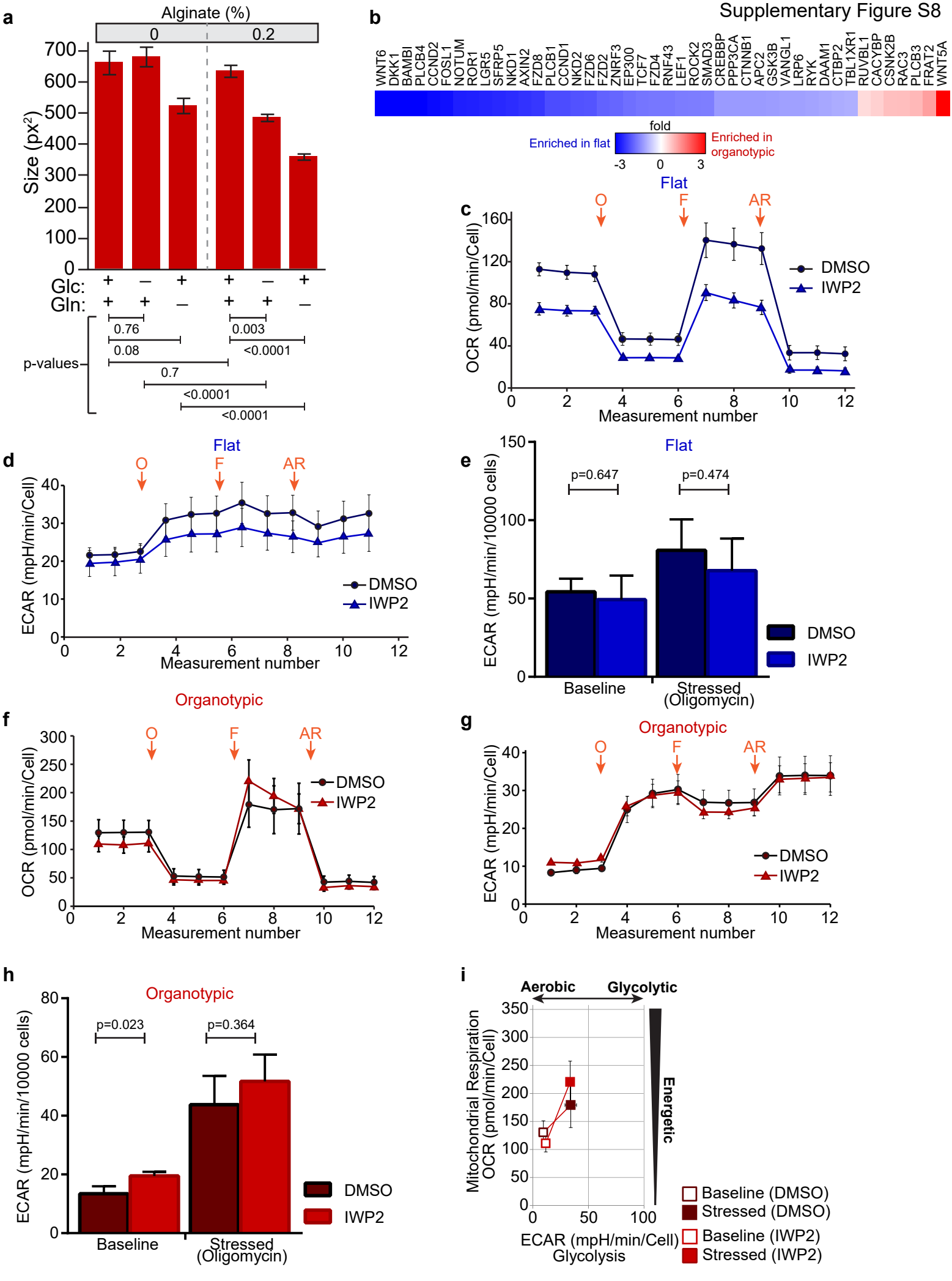
a) Immunoblot showing doxycycline-inducible (+/- Dox) expression of GFP and FLAG-tagged $KRAS^{G12V}$ in Caco-2 cells using anti-GFP and anti-FLAG antibodies, respectively. Tubulin (Tub) was included as a loading control. Molecular weights (kDa) are indicated on the left.

b) Bar chart showing cross-section area of control (GFP) and $KRAS^{G12V}$-expressing organotypic Caco-2 cells in the presence (+Glc) or absence (-Glc) of glucose (GFP: n=888 (+Glc), n= 898 (-Glu); $KRAS^{G12V}$: n=975 (+Glc) n=0 (-Glc)); (r=3 independent experimental replicates).

c) Bar chart showing cross-section area of control (GFP) and $KRAS^{G12V}$-expressing organotypic cells in the presence (+) or absence (-) of glutamine (Gln), methionine (Met), or serine (Ser) (GFP: n=888 (+Gln, +Met, +Ser), n= 916 (-Gln), n= 585 (-Met), n=759 (-Ser); $KRAS^{G12V}$: n=975 (+Gln, +Met, +Ser) n=1106 (-Gln), n=1103 (-Met), n=999 (-Ser)); (r=3 independent experimental replicates).

d) Bar chart showing cell number for GFP and $KRAS^{G12V}$-expressing Caco-2 flat cells in the presence (+Glc) or absence (-Glc) of glucose); (r=3 independent experimental replicates).
All error bars are standard deviation.

Supplementary Figure S8



Supplementary Figure S8 – Mechanical regulation of metabolic plasticity correlates with Wnt signaling

a) Bar chart showing the sizes of Caco-2 spheroids in organotypic cultures cells in complete (Glc/Gln+), glucose-depleted (Glc-), or glutamine-depleted (Gln-) medium. Cells were cultured embedded in 50% basement membrane extract (BME) in the presence of 0 or 2% alginate for 5 days. For 0% alginate: n=319 (Glc+/Gln+), n=254 (Glc-), and n=127 (Gln-) spheroids were measured; for 0.2% alginate: n=234 (Glc+/Gln+), n=254 (Glc-), and n=127 (Gln-) (r=2 independent replicates).

b) Heat map showing enrichment of canonical Wnt-associated genes in flat cultures based on differential gene expression data (Supplementary Table S1). Wnt-associated genes are annotated from the KEGG_WNT_SIGNALING_PATHWAY gene set.

c) Representative line graph showing OCR measurements from control (DMSO) and IWP2-treated flat Caco-2 cultures in response to mitochondrial stressors (Oligomycin (O), FCCP (F), and Antimycin A with Rotenone (AR).

d) Representative line graph showing ECAR measurements from control (DMSO) and IWP2-treated flat Caco-2 cultures in response to mitochondrial stressors (Oligomycin (O), FCCP (F), and Antimycin A with Rotenone (AR).

e) Bar chart showing ECAR measurements from control (DMSO) and IWP2-treated flat Caco-2 cultures at baseline and following oligomycin-induced mitochondrial stress (r=3 independent experimental replicates).

f) Representative line graph showing OCR measurements from control (DMSO) and IWP2-treated organotypic Caco-2 cultures in response to mitochondrial stressors (Oligomycin (O), FCCP (F), and Antimycin A with Rotenone (AR).

g) Representative line graph showing ECAR measurements from control (DMSO) and IWP2-treated organotypic Caco-2 cultures in response to mitochondrial stressors (Oligomycin (O), FCCP (F), and Antimycin A with Rotenone (AR).

h) Bar chart showing ECAR measurements from control (DMSO) and IWP2-treated organotypic Caco-2 cultures at baseline and following oligomycin-induced mitochondrial stress (r=3 independent experimental replicates).

i) Plot showing the bioenergetic profile of control or IWP2-treated organotypic Caco-2 cultures at baseline activity (open symbols) and in response to mitochondrial oxidative phosphorylation uncoupler, FCCP (closed symbols) (r=3 independent experimental replicates).