Science Advances
AAAS

Supplementary Materials for

Lactate supports a metabolic-epigenetic link in macrophage polarization

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Published 12 November 2021, *Sci. Adv.* **7**, eabi8602 (2021)
DOI: 10.1126/sciadv.abi8602

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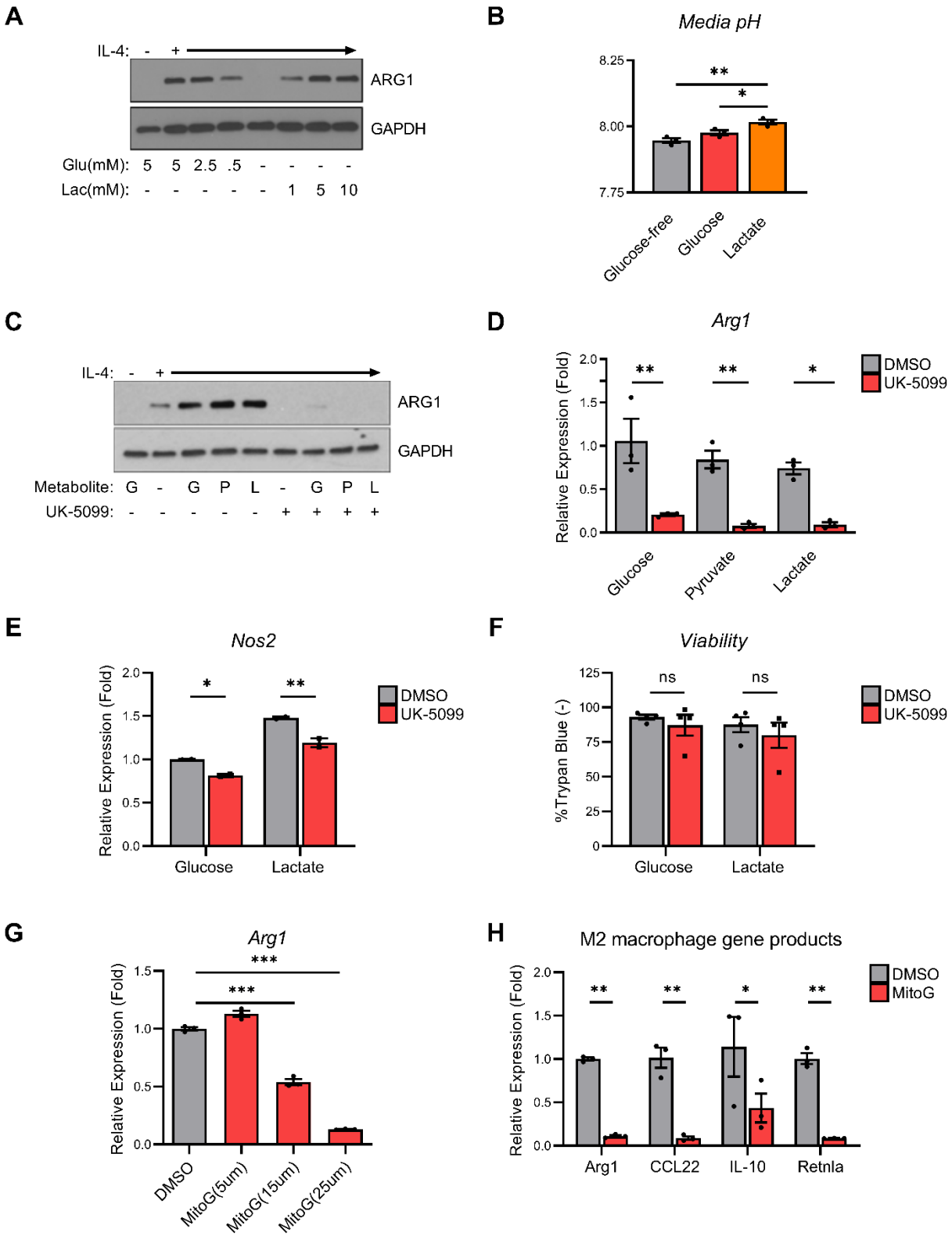
A
IL-4: - +
ARG1
GAPDH
Glu(mM): 5 5 2.5 .5 - - - -
Lac(mM): - - - - - 1 5 10
B
Media pH
8.25
8.00
7.75
**
*
Glucose-free
Glucose
Lactate
C
IL-4: - +
ARG1
GAPDH
Metabolite: G - G P L - G P L
UK-5099: - - - - - + + + +
D
Arg1
Relative Expression (Fold)
2.0
1.5
1.0
0.5
0.0
**
**
*
DMSO
UK-5099
Glucose
Pyruvate
Lactate
E
Nos2
Relative Expression (Fold)
2.0
1.5
1.0
0.5
0.0
*
**
DMSO
UK-5099
Glucose
Lactate
F
Viability
%Trypan Blue (-)
125
100
75
50
25
0
ns
ns
DMSO
UK-5099
Glucose
Lactate
G
Arg1
Relative Expression (Fold)
1.5
1.0
0.5
0.0
***
***
DMSO
MitoG(5um)
MitoG(15um)
MitoG(25um)
H
M2 macrophage gene products
Relative Expression (Fold)
2.0
1.5
1.0
0.5
0.0
**
**
*
**
DMSO
MitoG
Arg1
CCL22
IL-10
Retnla

Fig. S1: Mitochondrial pyruvate metabolism is required for maximal M2 macrophage polarization. (A) *Arg1* expression of BMDMs starved in GF media + 10% dFBS (4 hours) before supplementation with glucose or lactate (as indicated), and then polarization with IL-4 (20 ng/mL) for forty-eight hours. **(B)** pH of glucose-free RPMI that was supplemented with or without glucose (5 mM) or sodium lactate (10 mM) **(C)** Immunoblot of ARG1 expression of BMDMs starved in GF media + 10% dFBS (4 hours) and then pre-treated with UK-5099 (25 μM) before supplementation with glucose (G; 5 mM), pyruvate (P; 10 mM) or lactate (L; 10 mM), and then polarization with IL-4 (20 ng/mL) for forty-eight hours. **(D)** qPCR analysis of *Arg1* expression of BMDMs starved in GF media + 10% dFBS (4 hours) and then pre-treated with UK-5099 (25 μM) before supplementation with glucose (5 mM), pyruvate (10 mM), or lactate (10 mM) and then polarization with IL-4 (20 ng/mL) for forty-eight hours. **(E)** *Nos2* expression of BMDMs starved in GF media + 10% dFBS (4 hours) and then pre-treated with UK-5099 (25 μM) before supplementation with glucose (5 mM) or lactate (10 mM) and then polarization with LPS/IFN-γ for twenty-four hours. **(F)** Trypan blue exclusion analysis of BMDMs starved in GF media + 10% dFBS (4 hours) and then pre-treated with UK-5099 (25 μM) before supplementation with glucose (5 mM) or lactate (10 mM) and then polarization with IL-4 (20 ng/mL) for twenty-four hours. **(G)** qPCR expression of *Arg1* of BMDMs starved in GF media + 10% dFBS (4 hours) and then pre-treated with increasing concentrations of Mitoglitazone (MitoG) and then polarized with IL-4 (20 ng/mL) for forty-eight hours. **(H)** qPCR expression of M2 gene products of BMDMs starved in GF media + 10% dFBS (4 hours) and then pre-treated with Mitoglitazone (MitoG; 25 μM) and then polarized with IL-4 (20 ng/mL) for forty-eight hours. Data are shown as mean ± SEM and are representative of at least 3 independent experiments. *$p \leq 0.05$, **$p \leq 0.01$, ***$p \leq 0.001$ by two-way ANOVA (D-H) or one-way ANOVA (B) with Tukey's post-test. Immunoblots are representative of three replicates.

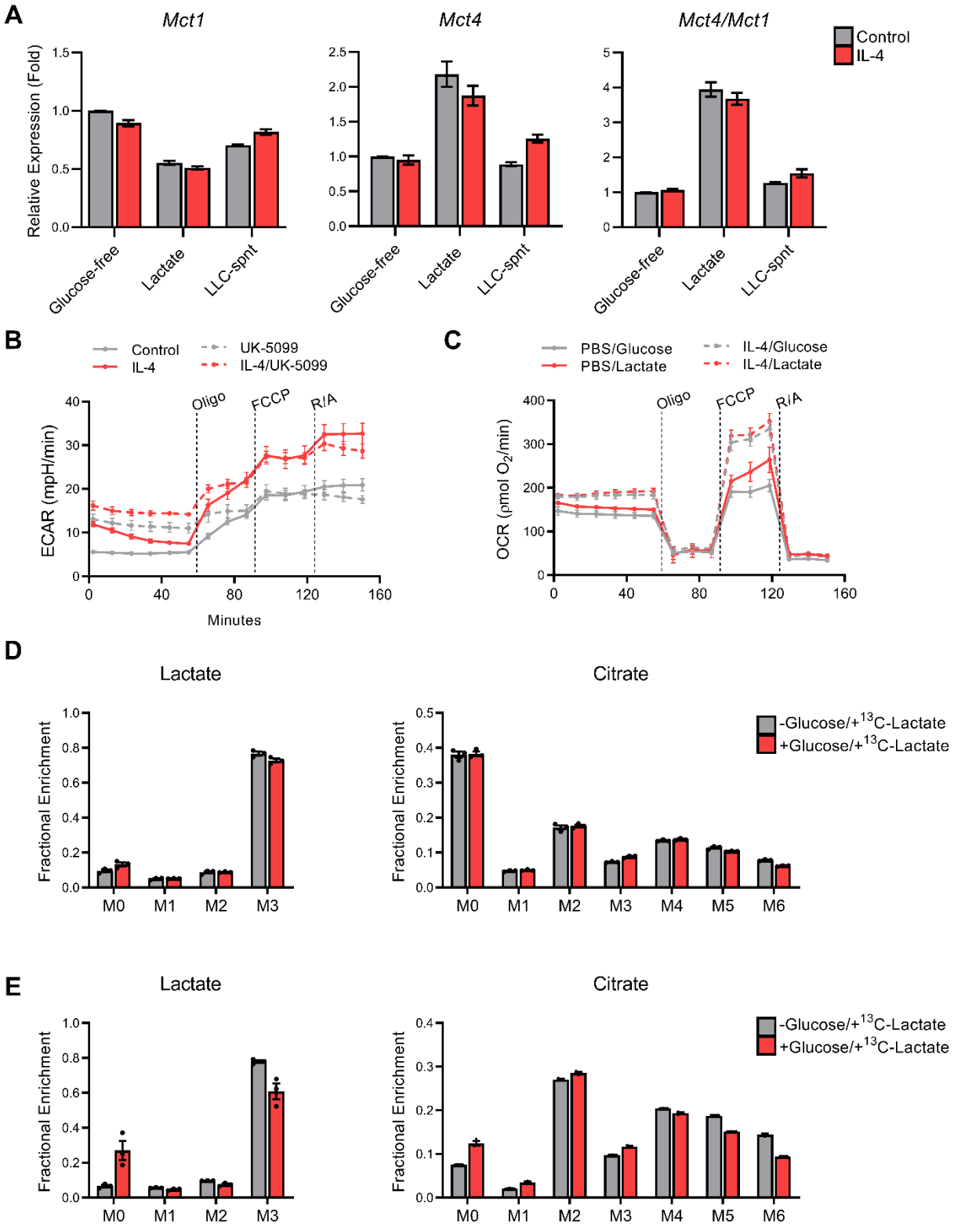

Fig. S2: Metabolic analyses of IL-4 induced M2 polarized macrophages (A) qPCR analysis of *Mct1* and *Mct4* expression of BMDMs starved in GF media + 10% dFBS (4 hours) before

supplementation with lactate (10 mM) or LLC cell-conditioned supernatants (LLC-spnt) and then polarization ± IL-4 (20 ng/mL) for forty-eight hours. **(B)** Trace of extracellular acidification rate (ECAR) of BMDMs pre-treated ± UK-5099 (25 μM) and then polarized with IL-4 (20 ng/mL) or vehicle control (PBS) for twenty-four hours before extracellular flux analysis with oligomycin (oligo), FCCP, and rotenone plus antimycin A (Rot/Ant). **(C)** Trace of oxygen consumption rate (OCR) of BMDMs supplemented with glucose (5 mM) or lactate (10 mM) and then polarized with IL-4 (20 ng/mL) or vehicle control (PBS) for twenty-four hours before extracellular flux analysis with oligomycin (oligo), FCCP, and rotenone plus antimycin A (Rot/Ant). Fractional enrichment of lactate **(left)** and citrate **(right)** in BMDMs starved in GF media + 10% dFBS (4 hours) before supplementation with ^{13}C-lactate (10 mM) in the presence or absence of unlabeled ^{12}C-glucose, and then polarization with IL-4 (20 ng/mL) for **(D)** three or **(E)** six hours. Data are shown as mean ± SEM of five (B, C) or three (A, D, E) replicates.

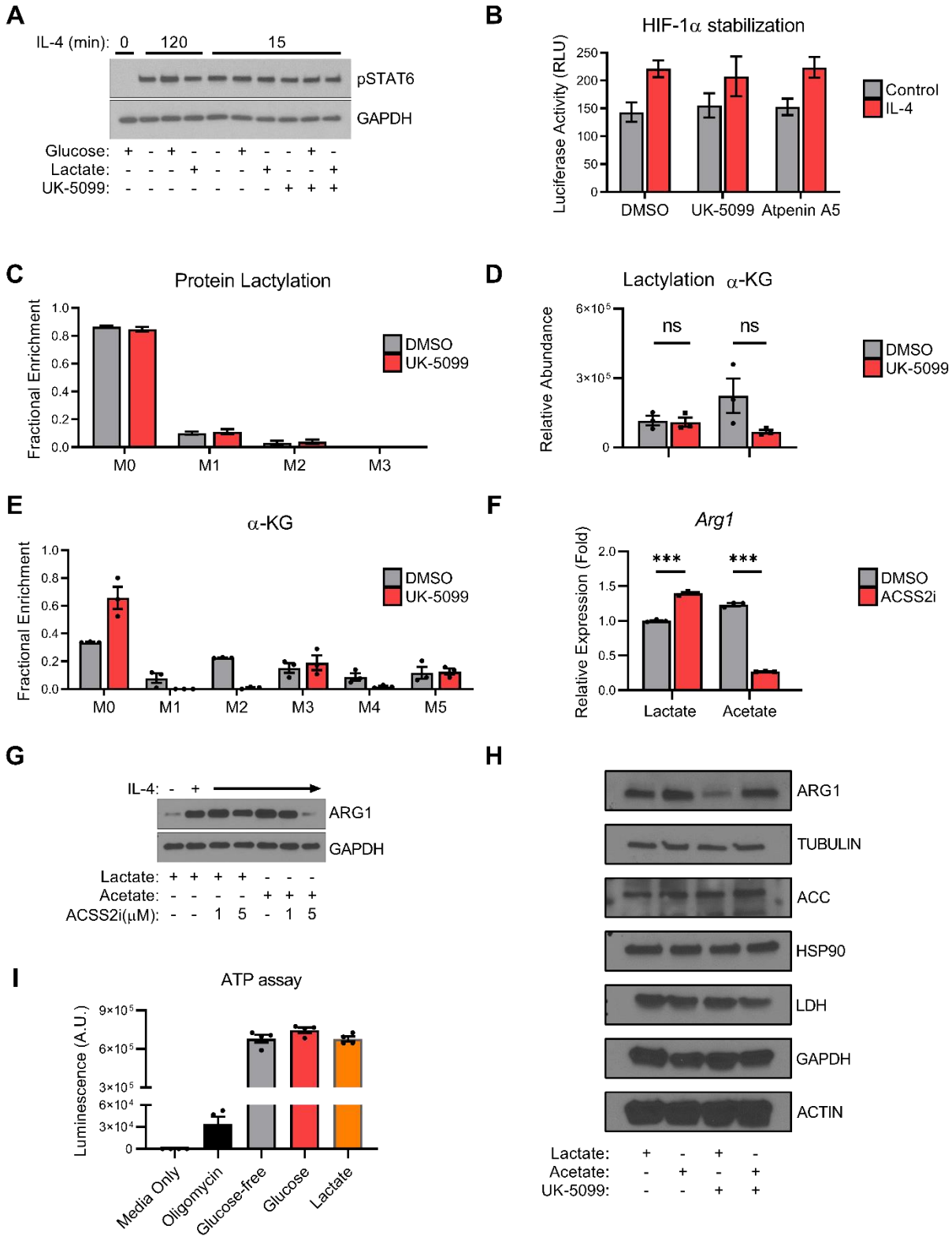
A
IL-4 (min): 0 120 15
pSTAT6
GAPDH
Glucose: + - + - - + - - + -
Lactate: - - - + - - + - - +
UK-5099: - - - - - - - + + +
B
HIF-1α stabilization
Luciferase Activity (RLU)
250
200
150
100
50
0
DMSO
UK-5099
Atpenin A5
Control
IL-4
C
Protein Lactylation
Fractional Enrichment
1.0
0.8
0.6
0.4
0.2
0.0
M0
M1
M2
M3
DMSO
UK-5099
D
Lactylation α-KG
Relative Abundance
6×10⁵
3×10⁵
0
ns
ns
DMSO
UK-5099
E
α-KG
Fractional Enrichment
1.0
0.8
0.6
0.4
0.2
0.0
M0
M1
M2
M3
M4
M5
DMSO
UK-5099
F
Arg1
Relative Expression (Fold)
2.0
1.5
1.0
0.5
0.0
***
***
Lactate
Acetate
DMSO
ACSS2i
G
IL-4: - +
ARG1
GAPDH
Lactate: + + + + - - -
Acetate: - - - - + + +
ACSS2i(μM): - - 1 5 - 1 5
H
ARG1
TUBULIN
ACC
HSP90
LDH
GAPDH
ACTIN
Lactate: + - + -
Acetate: - + - +
UK-5099: - - + +
I
ATP assay
Luminescence (A.U.)
9×10⁵
6×10⁵
3×10⁵
6×10⁴
3×10⁴
0
Media Only
Oligomycin
Glucose-free
Glucose
Lactate

Fig. S3: Lactate supports IL-4 induced M2 polarization independent of STAT6 phosphorylation, HIF-1α stabilization, protein lactylation, α-ketoglutarate production, and bioenergetic metabolism. (A) Immunoblotting analysis of phosphorylated STAT6 (pSTAT6) of BMDMs starved in GF media + 10% dFBS (4 hours) before pre-treatment with UK-5099 (25 μM) and supplementation with glucose (5 mM) or lactate (10 mM) and then polarization ± IL-4 (20 ng/mL) for fifteen or one hundred twenty minutes. **(B)** Luciferase assay of ODD-Luc BMDMs treated UK-5099 (25 μM) or Atpenin A5 (25 μM) before polarization ± IL-4 (20 ng/mL) for twenty-four hours. **(C-E)** Relative abundance and fractional enrichment of protein lactylation and alpha-ketoglutarate (α-KG) in BMDMs starved in GF media + 10% dFBS (4 hours) before pre-treatment with UK-5099 (25 μM), supplementation with ^{13}C-lactate (10 mM), and then polarization with IL-4 (20 ng/mL) for six hours. **(F, G)** Arg1 expression in BMDMs starved in GF media + 10% dFBS (4 hours) before pre-treatment with ACSS2i, supplementation with lactate (10 mM) or acetate (10 mM) and then polarization with IL-4 (20 ng/mL) for twenty-four hours. **(H)** Immunoblotting analysis of "house-keeping" gene products of BMDMs starved in GF media + 10% dFBS (4 hours) before pre-treatment with UK-5099 (25 μM) and supplementation with lactate (10 mM) or acetate (10 mM) and then polarization ± IL-4 (20 ng/mL) for twenty-four hours. **(I)** ATP assay of BMDMs starved in GF media + 10% dFBS (4 hours) before pre-treatment ± Oligomycin A (Oligo; 10 nM) and supplementation with glucose (5 mM) or lactate (10 mM) and then polarization ± IL-4 (20 ng/mL) for twenty-four hours. Data are shown as mean ± SEM of two (B), three (C-F), or four (I) replicates. Immunoblots are representative of three replicates.

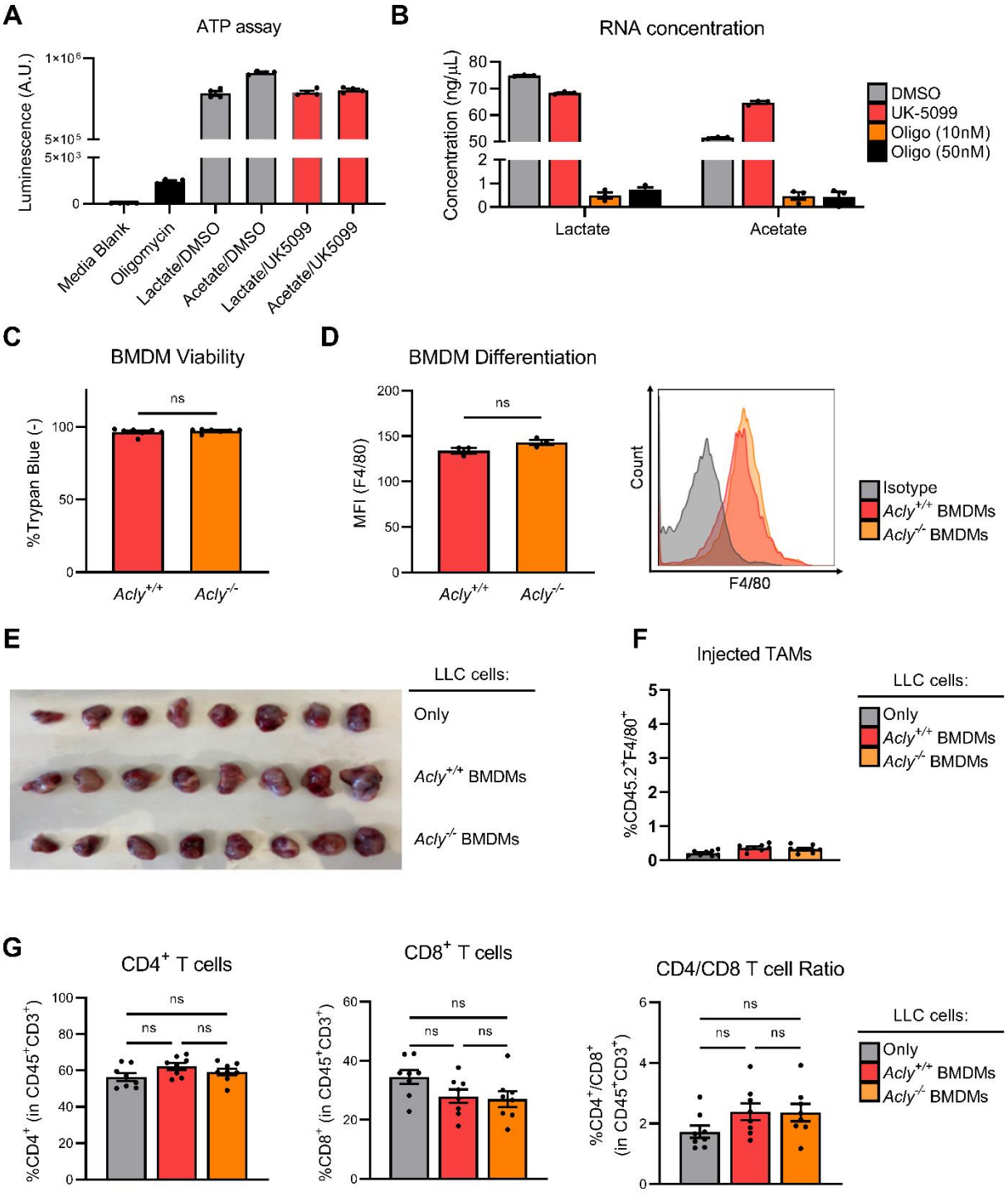

Figure S4: ACLY-deficiency abrogates M2 macrophage polarization and tumor progression **(A)** ATP assay of BMDMs starved in GF media + 10% dFBS (4 hours) before pre-treatment ± Oligomycin A (10 nM) or UK-5099 (25 µM) and supplementation with lactate (10 mM) or acetate (10 mM) and then polarization ± IL-4 (20 ng/mL) for twenty-four hours. **(B)** RNA concentration of BMDMs starved in GF media + 10% dFBS (4 hours) before pre-treatment ± Oligomycin A (Oligo) or UK-5099 (25 µM) and supplementation with lactate (10 mM) or acetate (10 mM) and

then polarization ± IL-4 (20 ng/mL) for twenty-four hours **(C)** Viability and **(D)** F4/80 expression in *Acly*$^{+/+}$ and *Acly*$^{-/-}$ M0 BMDMs following differentiation. **(E)** Gross dissection of tumors from mice injected with LLC cells alone, LLC cells with M2 polarized *Acly*$^{+/+}$ BMDMs, or LLC cells with M2 polarized *Acly*$^{-/-}$ BMDMs. Flow cytometric analysis of **(F)** CD45.2^{+} TAMs and **(G, left)** CD4^{+} T cells, **(G, middle)** CD8^{+} T cells and **(G, right)** CD4^{+}/CD8^{+} T cell ratio of the respective tumors at endpoint. Data are shown as mean ± SEM of three (A, B), seven (C, D), and eight (E-G) replicates. *p≤0.05, **p≤0.01, ***p≤0.001 by student's t test (C, D), or one-way ANOVA (F, G) with Tukey's post-test. Photo Credit: Jordan Noe, University of Louisville.