Expanded View Figures

Figure EV1. TGFβ's effect on nutrient uptake and secretion, on glycolytic and oxidative metabolism, and on translation.

A Western blot of NIH-3T3 cells treated with TGFβ or mock for 24 h and incubated with puromycin for the last 10 min. The translation inhibitor cycloheximide (CHX) was added during puromycin incubation as a control.

B Growth curve of IMR90 cells treated with TGFβ or mock for the indicated time. The cell number at the indicated days relative to the number at the start of the treatment (d0) is shown. The dotted line represents the cell number at d0.

C ECM was produced from IMR90 cells grown in the presence or absence of TGFβ, and collagen abundance was measured by picrosirius red staining, normalized to the packed cell volume of cells grown on a separate plate under identical experimental conditions, and expressed relative to mock-treated cells.

D NIH-3T3 cells were treated with TGFβ or mock for 48 h, and the glycolytic rate (glycoPER) was measured at baseline and after subsequent injection of inhibitors using the Seahorse bioanalyzer. Rot/Anti-A, rotenone/antimycin; 2-DG, 2-deoxyglucose; PCV, packed cell volume.

E NIH-3T3 cells were treated with TGFβ or mock for 48 h, and lactate secretion into the medium was measured for the last 12 h of the experiment.

F NIH-3T3 cells were treated with TGFβ or mock for 48 h, and the extracellular acidification rate (ECAR) was measured at baseline. Data were normalized to the packed cell volume.

G–I NIH-3T3 cells were treated with TGFβ or mock for 3, 12, 24 or 48 h, and the consumption of glucose from the media (G) or the secretion of lactate into the media (H) was measured in the last 12 h of the experiment. For each time point, values of TGFβ-treated cells were normalized to the respective mock-treated controls. Glucose uptake and lactate secretion of TGFβ-treated relative to mock-treated cells for each time point were analyzed by Pearson's correlation (I).

J Respiratory coupling, calculated by dividing the lactate secretion values from (E) through the glucose consumption values from Fig 1G.

K NIH-3T3 cells were treated with TGFβ or mock for 2, 4, 8, 24, or 48 h and incubated with O-propargyl-puromycin (OPP) for the last 60 min. The translation rate was determined by flow cytometry for OPP incorporation into proteins. For each time point, values of TGFβ-treated cells were normalized to the respective mock-treated controls.

L NIH-3T3 cells were treated with TGFβ or mock for 2, 4, 8, 24 or 48 h, and the oxygen consumption rate (OCR) before and after treatment with mitochondrial inhibitors was measured using the Seahorse bioanalyzer. From these data, the mitochondrial ATP production rate (J_{ATP}ox) was calculated. For each time point, values of TGFβ-treated cells were normalized to the respective mock-treated controls.

M NIH-3T3 cells were treated with TGFβ or mock for 3, 12, 24 or 48 h, and the consumption of glutamine from the media was measured in the last 12 h of the experiment. For each time point, values of TGFβ-treated cells were normalized to the respective mock-treated controls.

N, O NIH-3T3 cells were serum-deprived (0.5% FBS) and (N) treated with TGFβ or mock for 24 h and incubated with O-propargyl-puromycin (OPP) for the last 60 min followed by flow cytometry for OPP incorporation to measure the translation rate, or (O) treated with TGFβ for 48 h, and the oxygen consumption rate (OCR) before and after treatment with mitochondrial inhibitors was measured using the Seahorse bioanalyzer. Oligo, oligomycin. Values in (N) are relative to mock-treated cells.

Data information: *P*-values were calculated by two-sided unpaired *t*-test with Welch's correction (C, E, F, J, N) or by Pearson's correlation (I). Bars in (C, E, F, J, N) represent the mean + SD; data in (B, D, G–I, K–M, O) represent the mean ± SD; and line in (I) represents linear regression with the SD shown as dotted lines. $n = 3$ (B, C, E, G, H, J, K, M, N); $n = 8$ (D, F, O), $n = 4$ (I), $n = 5$–6 (L). A representative experiment is shown (A, N).

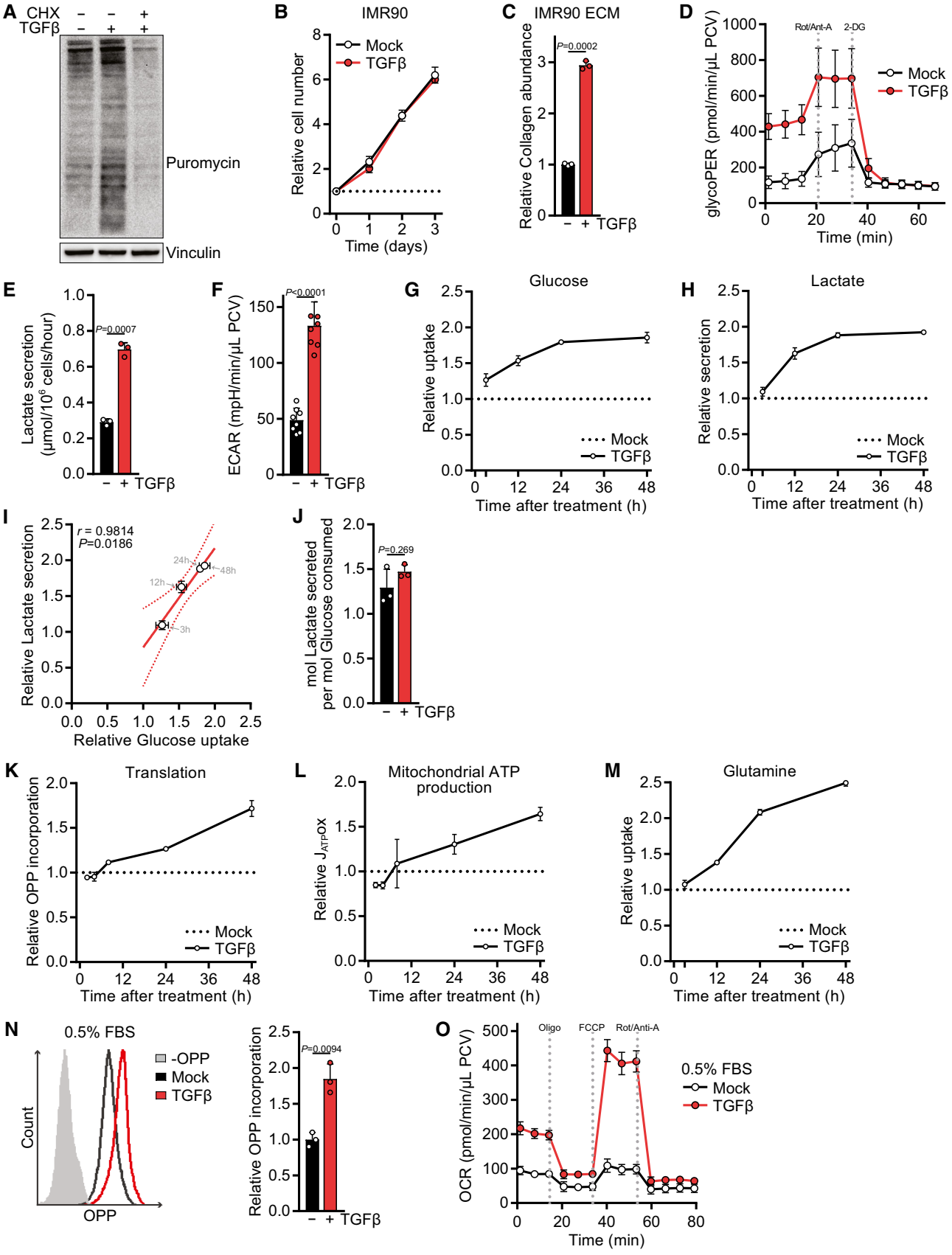

Figure EV1.

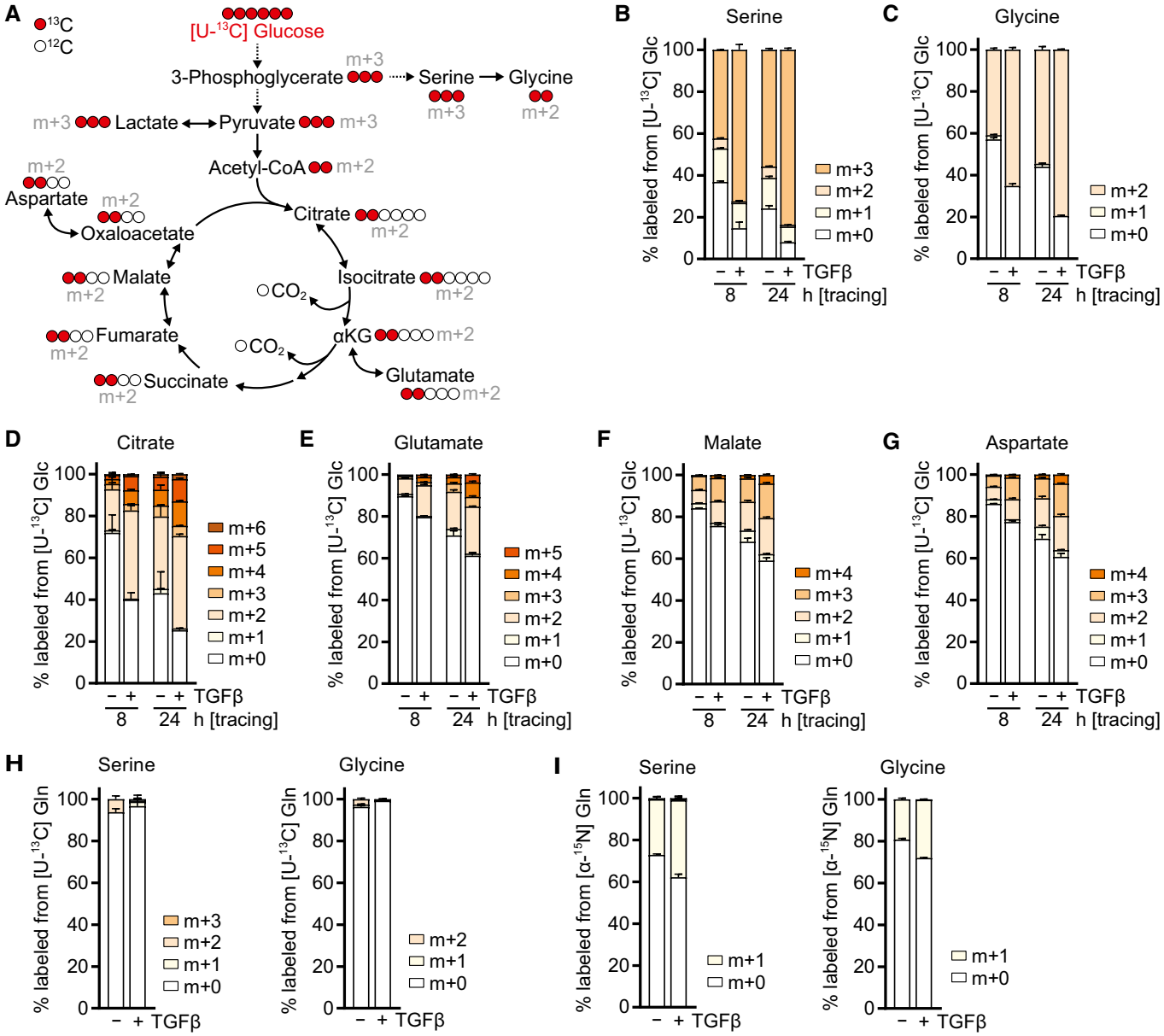

Figure EV2. TGFβ promotes serine and glycine biosynthesis and glucose oxidation.

A Schematic of stable isotope tracing using D-glucose labeled with [^{13}C] at all six carbons ([U-^{13}C] glucose, shown in red).

B–G Tracing of [U-^{13}C] glucose ([U-^{13}C] Glc) into indicated metabolites. NIH-3T3 cells were treated with TGFβ or mock for 48 h in DMEM lacking L-serine and glycine in the presence of 0.5% dialyzed FBS, and the medium was replaced (including all treatments) with DMEM lacking D-glucose, L-serine, and glycine and supplemented with [U-^{13}C] Glc for the last 24 or 8 h in the presence of 0.5% dialyzed FBS. Metabolites were measured by LC-MS.

H, I Tracing of (H) [U-^{13}C] L-glutamine ([U-^{13}C] Gln) or (I) [α-^{15}N] L-glutamine ([α-^{15}N] Gln) into serine and glycine. NIH-3T3 cells were treated with TGFβ or mock for 48 h in DMEM lacking L-serine and glycine in the presence of 0.5% dialyzed FBS, and the medium was replaced (including all treatments) with DMEM lacking L-glutamine, L-serine, and glycine and supplemented with [U-^{13}C] Gln (H) or [α-^{15}N] Gln (I) for the last 8 h in the presence of 0.5% dialyzed FBS. Metabolites were measured by GC-MS.

Data information: Bars represent the mean + SD. $n = 3$.

Figure EV3. TGFβ promotes proline biosynthesis and glutamine oxidation.

A Schematic of stable isotope tracing using L-glutamine labeled with [^{13}C] at all five carbons ([U-^{13}C] glutamine, shown in red).

B–D Kinetic labeling curves of the indicated isotopomers after tracing with [U-^{13}C] glutamine ([U-^{13}C] Gln). NIH-3T3 cells were treated with TGFβ or mock for 48 h, and the medium was replaced (including all treatments) with DMEM lacking L-glutamine and supplemented with [U-^{13}C] Gln for the last 2, 8, or 24 h. Metabolites were measured by LC-MS. For other isotopomers, see Fig 2C–H.

E Western blot of NIH-3T3 cells treated with TGFβ or mock for the indicated time in the presence of 0.5% FBS. KGA (kidney type) and GAC (glutaminase C) denote the two isoforms of GLS1.

F IMR90 cells were treated with TGFβ or mock for 48 h, and abundance of proline was measured by GC-MS. Values are relative to mock-treated cells.

G Tracing of [U-^{13}C] glucose ([U-^{13}C] Glc) into proline. NIH-3T3 cells were treated with TGFβ or mock for 48 h in DMEM lacking L-serine and glycine in the presence of 0.5% dialyzed FBS, and the medium was replaced (including all treatments) with DMEM lacking D-glucose, L-serine, and glycine and supplemented with [U-^{13}C] Glc for the last 8 or 24 h in the presence of 0.5% dialyzed FBS. Proline was measured by LC-MS.

H Representative MS1 spectra of the CO1A1 peptide in ECM generated with unlabeled ([^{12}C] Gln, left) and fully labeled glutamine ([U-^{13}C] Gln, right). Also see Fig 2K–M.

Data information: *P*-values were calculated by two-sided unpaired *t*-test with Welch's correction (F). Bars in (F, G) represent the mean + SD; data in (B–D) represent the mean ± SD. $n = 3$ (B–D, G); $n = 4$ (F). A representative experiment is shown (E, H).

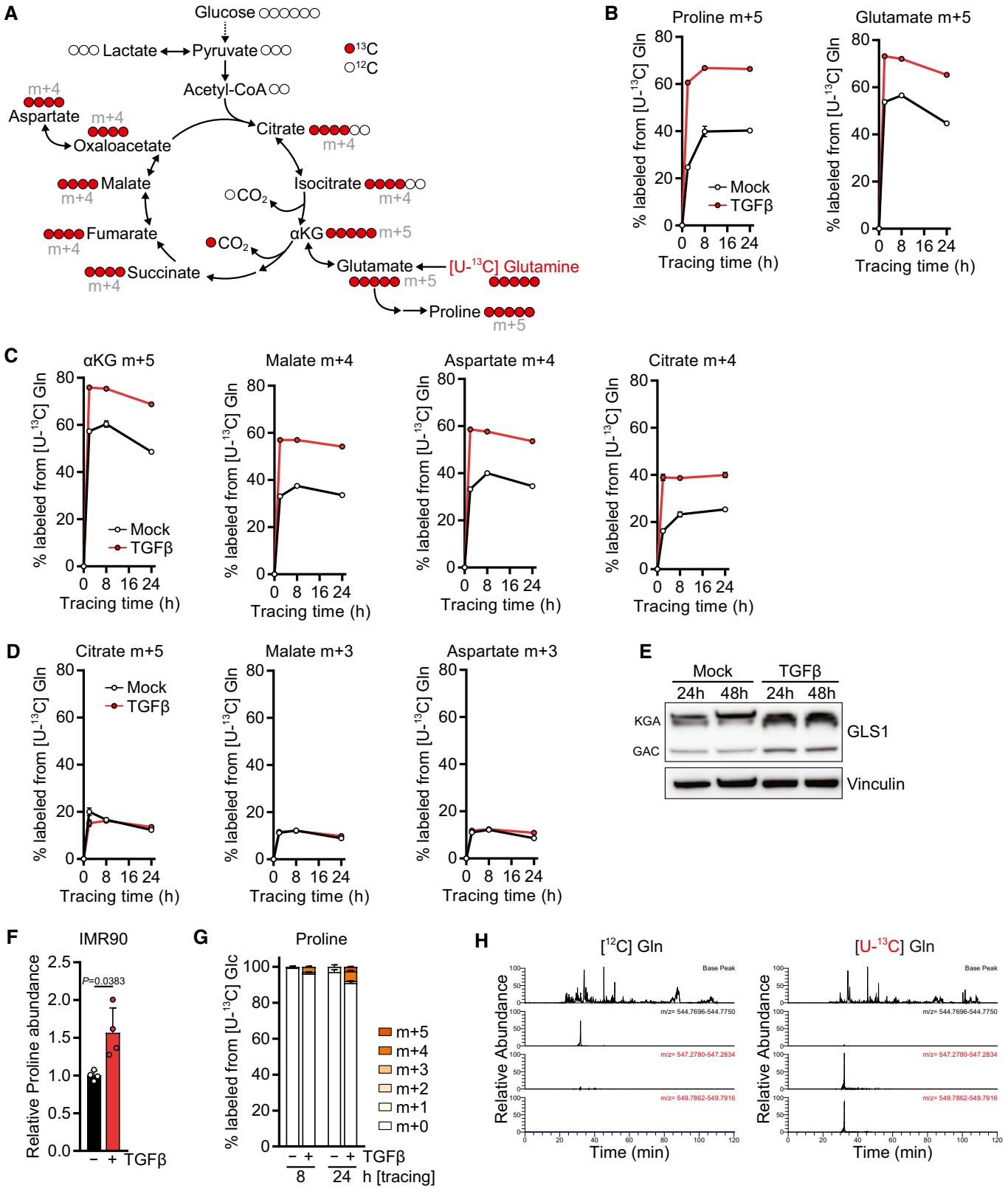
A
Glucose
Lactate
Pyruvate
13C
12C
Acetyl-CoA
Aspartate m+4
Oxaloacetate
Citrate m+4
Malate m+4
Isocitrate m+4
CO2
Fumarate m+4
αKG m+5
Succinate m+4
Glutamate m+5
[U-13C] Glutamine
Proline m+5
B
Proline m+5
Glutamate m+5
% labeled from [U-13C] Gln
Mock
TGFβ
Tracing time (h)
C
αKG m+5
Malate m+4
Aspartate m+4
Citrate m+4
D
Citrate m+5
Malate m+3
Aspartate m+3
E
Mock
TGFβ
24h 48h 24h 48h
KGA
GAC
GLS1
Vinculin
F
IMR90
Relative Proline abundance
P=0.0383
TGFβ
G
Proline
% labeled from [U-13C] Glc
m+5
m+4
m+3
m+2
m+1
m+0
8 24 h [tracing]
H
[12C] Gln
[U-13C] Gln
Relative Abundance
Base Peak
m/z= 544.7696-544.7750
m/z= 547.2780-547.2834
m/z= 549.7862-549.7916
Time (min)

Figure EV3.

Figure EV4. Regulation of proline biosynthesis and controls for metabolic manipulations.

A Western blot of IMR90 cells treated with TGFβ or mock for 48 h in the presence of 0.5% FBS.

B Left: Western blot of NIH-3T3 cells expressing sgCtrl, sg*Pycr1*-1, sg*Pycr1*-2, sg*Pycr2*-1, or sg*Pycr2*-2. Right: NIH-3T3 cells expressing sgCtrl, sg*Pycr1*-1, sg*Pycr1*-2, sg*Pycr2*-1, or sg*Pycr2*-2 were treated with TGFβ or mock for 48 h, and proline abundance was measured by GC-MS. Values are relative to mock-treated sgCtrl-expressing cells.

C Pearson's correlation of *P5CS* mRNA level and diffusing capacity for carbon monoxide (DLCO) in IPF patients from GSE32537 as a percentage of what was predicted for each patient. AU, arbitrary units.

D Western blot of NIH-3T3 cells expressing sgCtrl, sg*Smad4*-1, or sg*Smad4*-2 and treated with TGFβ or mock for 48 h in the presence of 0.5% FBS.

E NIH-3T3 cells were treated with TGFβ or mock for 1, 6, 24 or 48 h, and abundance of the indicated metabolites was measured by GC-MS. Values are relative to mock-treated cells for each time point.

F NIH-3T3 cells expressing empty vector, mito*TP*NOX, or mito*Lb*NOX were treated with TGFβ or mock for 48 h, and abundance of succinate, lactate, and pyruvate was measured by GC-MS. The lactate/pyruvate ratio was calculated as a surrogate for the cytosolic NADH/NAD^+ ratio. Values are normalized to mock-treated empty vector-expressing cells.

G NIH-3T3 cells were treated with rotenone, antimycin, myxothiazol, or vehicle control for 1 h, and abundance of the indicated metabolites was measured by GC-MS. Values are normalized to control-treated cells.

H NIH-3T3 cells were treated with cobalt chloride ($CoCl_2$) or vehicle control for 6 h, and abundance of the indicated metabolites was measured by GC-MS. Values are normalized to control-treated cells.

I NIH-3T3 cells were treated with TGFβ or mock for 48 h, and oligomycin or vehicle control was added for the last 1 or 6 h of the treatment. Abundance of the indicated metabolites was measured by GC-MS. Values are normalized to mock and control-treated cells.

Data information: *P*-values were calculated by two-way ANOVA with Holm–Sidak multiple comparison test (B) or by Pearson's correlation (C). Bars represent the mean + SD (B, F–I); line in (C) represents linear regression with the SD shown as dotted lines; and data in (E) represent the mean ± SD. $n = 2$ (sg*Pycr2*-2), $n = 3$ (all others) (B); $n = 99$ (C); $n = 3$ (E–I). A representative experiment is shown (A, B, D).

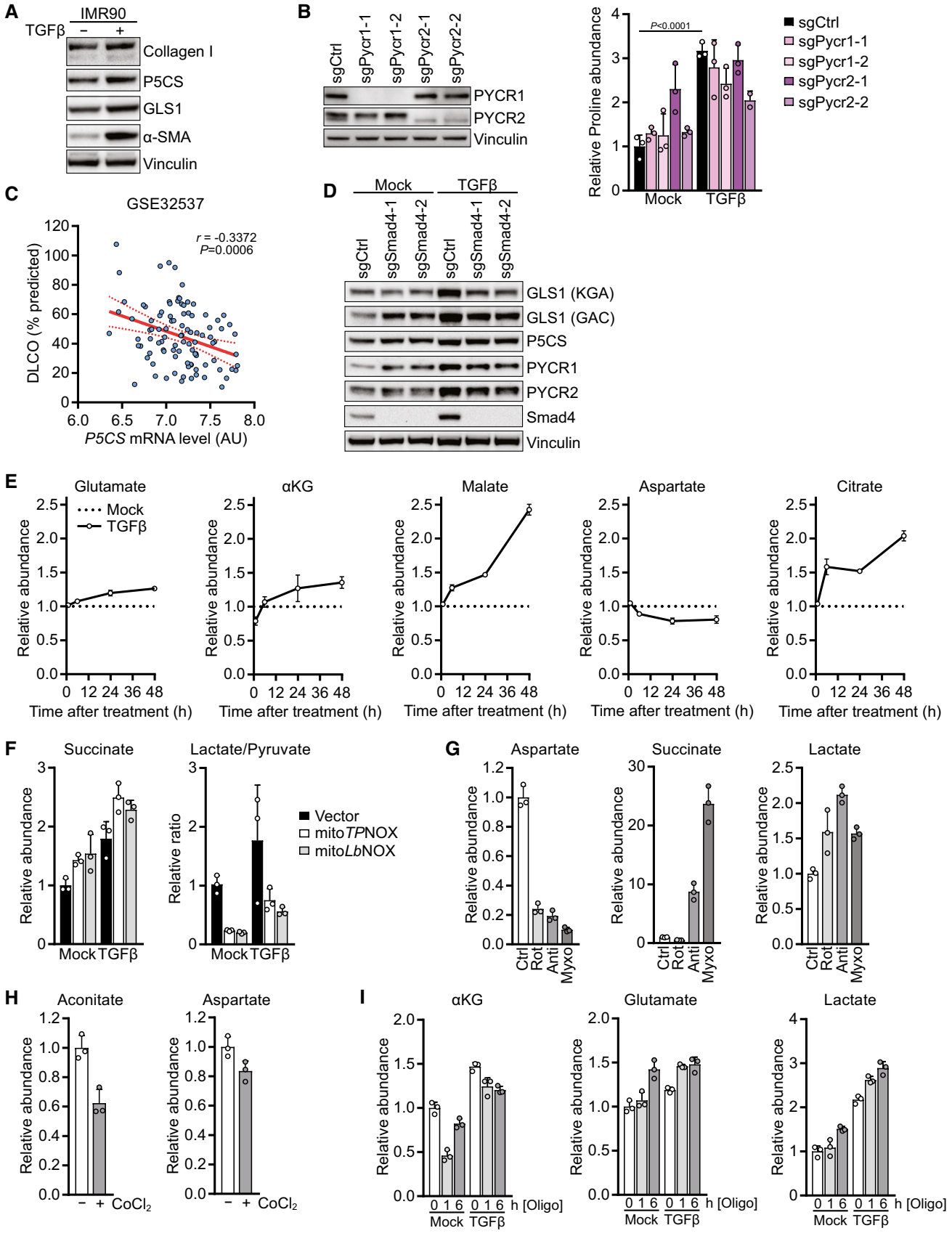
A
IMR90
TGFβ – +
Collagen I
P5CS
GLS1
α-SMA
Vinculin
B
sgCtrl
sgPycr1-1
sgPycr1-2
sgPycr2-1
sgPycr2-2
PYCR1
PYCR2
Vinculin
P<0.0001
Relative Proline abundance
Mock
TGFβ
sgCtrl
sgPycr1-1
sgPycr1-2
sgPycr2-1
sgPycr2-2
C
GSE32537
r = -0.3372
P=0.0006
DLCO (% predicted)
P5CS mRNA level (AU)
D
Mock
TGFβ
sgCtrl
sgSmad4-1
sgSmad4-2
GLS1 (KGA)
GLS1 (GAC)
P5CS
PYCR1
PYCR2
Smad4
Vinculin
E
Glutamate
αKG
Malate
Aspartate
Citrate
Mock
TGFβ
Relative abundance
Time after treatment (h)
F
Succinate
Lactate/Pyruvate
Relative abundance
Relative ratio
Vector
mitoTPNOX
mitoLbNOX
Mock TGFβ
G
Aspartate
Succinate
Lactate
Relative abundance
Ctrl
Rot
Anti
Myxo
H
Aconitate
Aspartate
Relative abundance
– + CoCl2
I
αKG
Glutamate
Lactate
Relative abundance
0 1 6 h [Oligo]
Mock TGFβ

Figure EV4.

Figure EV5. Deletion of HIF-1α, P5CS, and Slc25a1, and tracing PPP activity.

A IMR90 cells were treated with TGFβ or mock for 6 h, and ROS levels were measured by flow cytometry for CM-H_2DCFDA after incubation with CM-H_2DCFDA for the last 30 min of the treatment. Values are relative to mock-treated cells.

B Western blot of NIH-3T3 cells treated with TGFβ or mock for 6 h in the presence or absence of mitoquinol (MitoQ).

C Western blot of NIH-3T3 cells expressing sgCtrl, sg*HIF-1α*-1, or sg*HIF-1α*-2 and treated with TGFβ or mock for 6 h in the presence of 0.5% FBS.

D NIH-3T3 cells expressing sgCtrl, sg*HIF-1α*-1, or sg*HIF-1α*-2 were treated with TGFβ or mock for 48 h, and abundance of the indicated metabolites was measured by GC-MS. Values are relative to mock-treated sgCtrl-expressing cells.

E–G Tracing of [1,2-^{13}C] D-glucose ([1,2-^{13}C] Glc) into (E) G6P and (F) lactate. NIH-3T3 cells were treated with TGFβ or mock for 48 h in DMEM lacking L-serine and glycine in the presence of 0.5% dialyzed FBS, and the medium was replaced (including all treatments) with DMEM lacking D-glucose, L-serine, and glycine and supplemented with [1,2-^{13}C] Glc for the last 1 or 8 h in the presence of 0.5% dialyzed FBS. Metabolites were measured by LC-MS. (G) The ratio of m+1- versus m+2-labeled lactate was calculated as a measure of PPP activity.

H NIH-3T3 cells expressing sgCtrl, sg*P5CS*-1, or sg*P5CS*-2 were treated with TGFβ or mock for 48 h, and abundance of the indicated metabolites was measured by GC-MS. Values are relative to mock-treated sgCtrl-expressing cells.

I NIH-3T3 cells were treated with TGFβ, LDHAi (GSK 2837808A), PKM2a (DASA), CTPi, or vehicle control for 6 h, and ROS levels were measured by flow cytometry for CM-H_2DCFDA after incubation with CM-H_2DCFDA for the last 30 min of the treatment. Values are relative to control-treated cells. *P*-values represent comparison of individual samples to the control.

J NIH-3T3 cells expressing sgCtrl, sg*Slc25a1*-1, or sg*Slc25a1*-2 were treated with TGFβ or mock for 48 h, and abundance of citrate was measured by GC-MS. Values are relative to mock-treated sgCtrl-expressing cells.

K NIH-3T3 cells expressing sgCtrl or sg*Slc25a1*-1 were treated with TGFβ or mock for 48 h, and the oxygen consumption rate (OCR) before and after treatment with mitochondrial inhibitors was measured using the Seahorse bioanalyzer. Oligo, oligomycin; Rot/Anti-A, rotenone/antimycin; PCV, packed cell volume.

L NIH-3T3 cells expressing sgCtrl, sg*Slc25a1*-1, or sg*Slc25a1*-2 were treated with TGFβ or mock for 48 h, and abundance of the indicated metabolites was measured by GC-MS. Values are relative to mock-treated sgCtrl-expressing cells.

M, N Pearson's correlation of *Slc25a1* and *P5CS* mRNA levels in mice (M) or patients (N) from the indicated datasets, as described in Fig 3. AU, arbitrary units.

Data information: *P*-values were calculated by two-sided unpaired *t*-test with Welch's correction (A), two-way ANOVA with Holm–Sidak multiple comparison test (D, H, J, L), by one-way ANOVA with Holm–Sidak multiple comparison test (I), or by Pearson's Correlation (M, N). Bars in (A, D–J, L) represent the mean + SD; data in (K) represent the mean ± SD; and line in (M, N) represents linear regression with the SD shown as dotted lines. A representative experiment is shown (B, C). $n = 3$ (A, D–J, L); $n = 8$ (K, M); $n = 33$ (N).

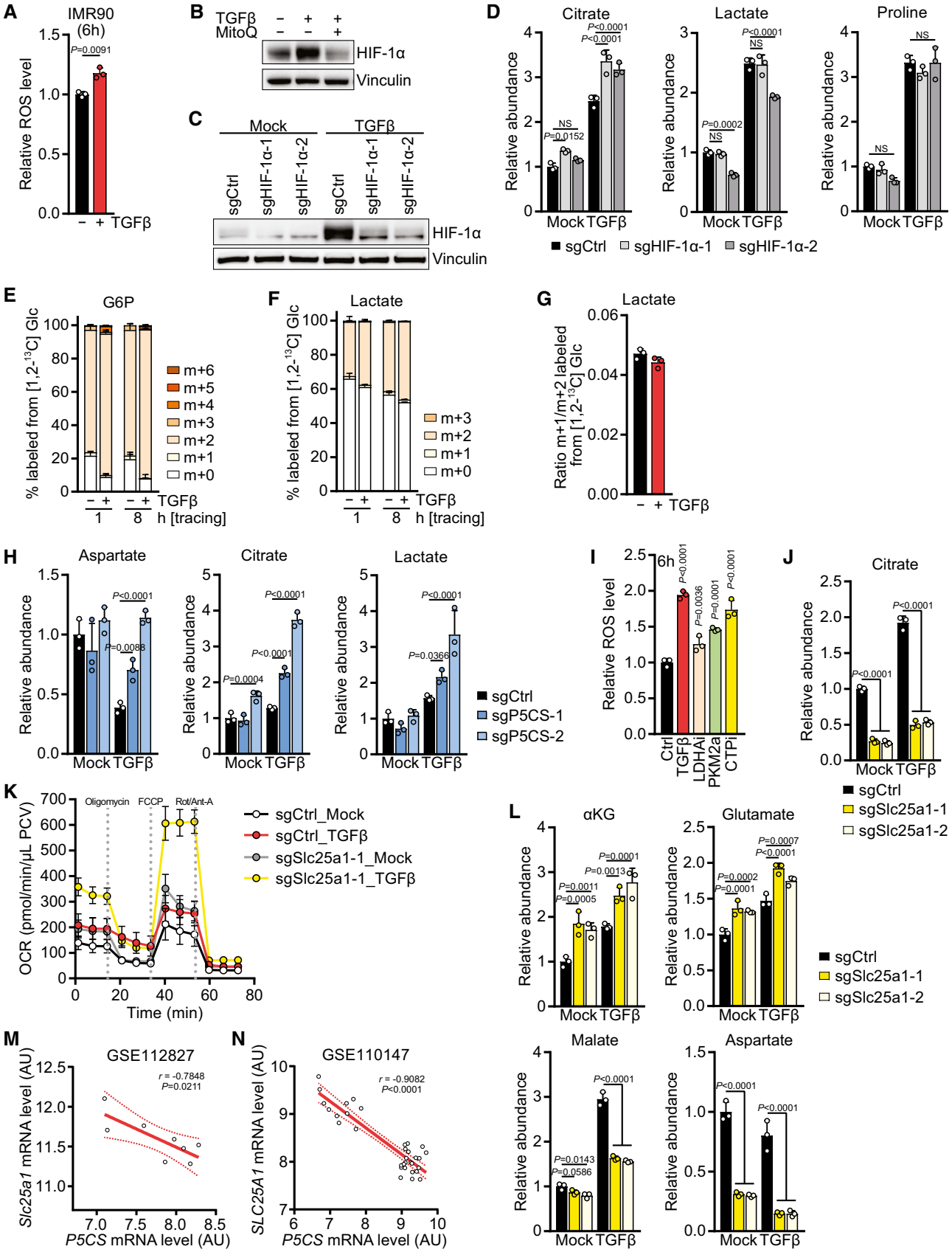
A
IMR90 (6h)
Relative ROS level
P=0.0091
− + TGFβ
B
TGFβ
MitoQ
HIF-1α
Vinculin
C
Mock
TGFβ
sgCtrl
sgHIF-1α-1
sgHIF-1α-2
HIF-1α
Vinculin
D
Citrate
Lactate
Proline
Relative abundance
Mock TGFβ
sgCtrl
sgHIF-1α-1
sgHIF-1α-2
E
G6P
% labeled from [1,2-13C] Glc
m+6
m+5
m+4
m+3
m+2
m+1
m+0
TGFβ
h [tracing]
F
Lactate
% labeled from [1,2-13C] Glc
G
Lactate
Ratio m+1/m+2 labeled from [1,2-13C] Glc
H
Aspartate
Citrate
Lactate
Relative abundance
sgCtrl
sgP5CS-1
sgP5CS-2
I
6h
Relative ROS level
Ctrl
TGFβ
LDHAi
PKM2a
CTPi
J
Citrate
Relative abundance
sgCtrl
sgSlc25a1-1
sgSlc25a1-2
K
Oligomycin
FCCP
Rot/Ant-A
OCR (pmol/min/µL PCV)
Time (min)
sgCtrl_Mock
sgCtrl_TGFβ
sgSlc25a1-1_Mock
sgSlc25a1-1_TGFβ
L
αKG
Glutamate
Malate
Aspartate
Relative abundance
M
GSE112827
r = -0.7848
P=0.0211
Slc25a1 mRNA level (AU)
P5CS mRNA level (AU)
N
GSE110147
r = -0.9082
P<0.0001
SLC25A1 mRNA level (AU)
P5CS mRNA level (AU)

Figure EV5.