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Supplemental Information

Glutamine Anabolism Plays a Critical Role in Pancreatic Cancer by Coupling Carbon and Nitrogen Metabolism

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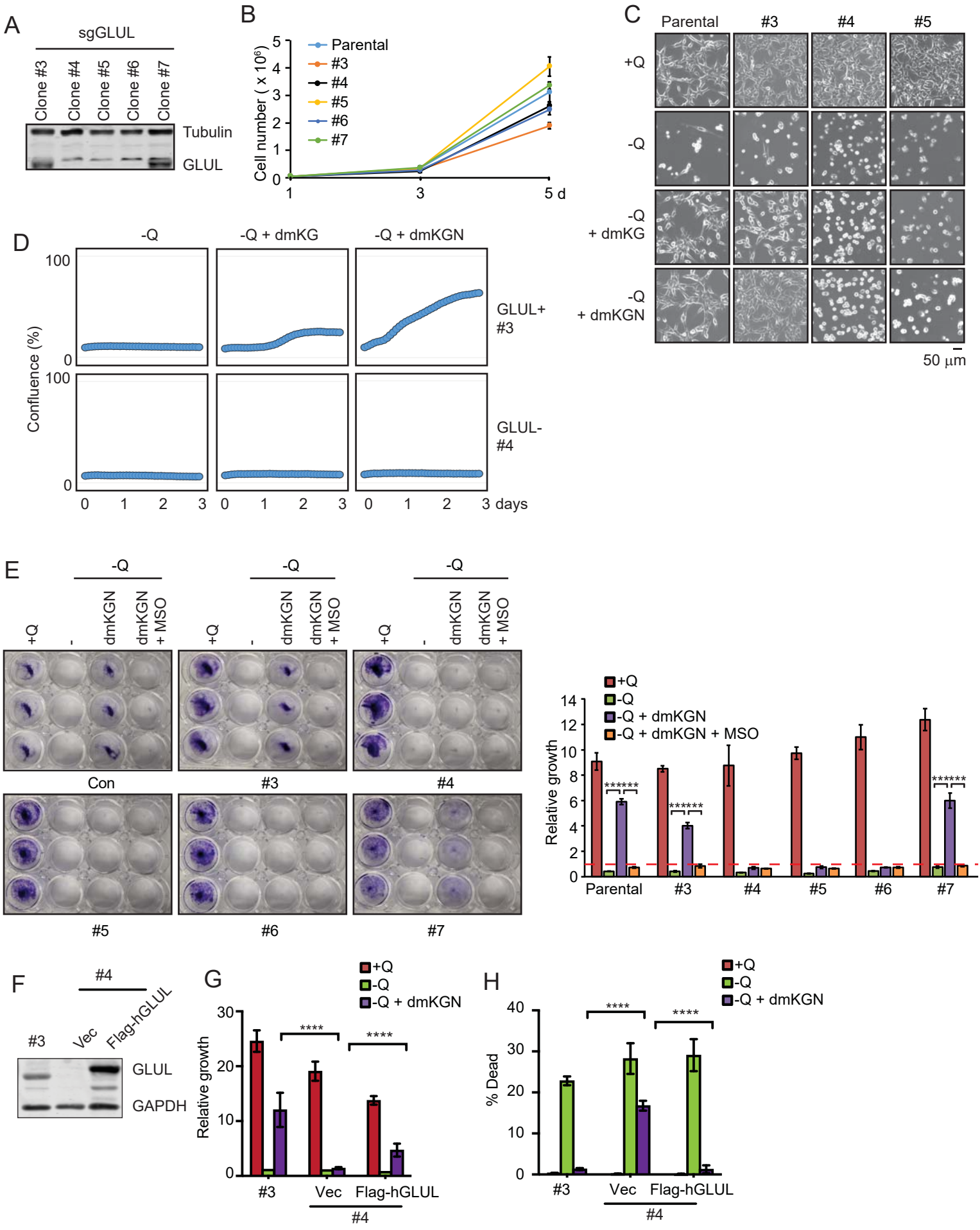

A
sgGLUL
Clone #3
Clone #4
Clone #5
Clone #6
Clone #7
Tubulin
GLUL
B
Parental
#3
#4
#5
#6
#7
Cell number (x 10^6)
5 d
C
Parental
#3
#4
#5
+Q
-Q
-Q + dmKG
-Q + dmKGN
50 μm
D
-Q
-Q + dmKG
-Q + dmKGN
Confluence (%)
GLUL+ #3
GLUL- #4
3 days
E
-Q
+Q
-
dmKGN
dmKGN + MSO
Con
#3
#4
#5
#6
#7
+Q
-Q
-Q + dmKGN
-Q + dmKGN + MSO
Relative growth
******
Parental
F
#4
#3
Vec
Flag-hGLUL
GLUL
GAPDH
G
+Q
-Q
-Q + dmKGN
Relative growth
****
#3
Vec
Flag-hGLUL
#4
H
% Dead

Figure S1. GLUL is essential for aKG to rescue KC cells under glutamine limitation, Related to Figure 2.

(A) Tumor cells isolated from the LSL-Kras$^{G12D/+}$; Pdx1-Cre (KC) mice were stably transfected with a plasmid containing Cas9 and three independent sgRNAs targeting GLUL. Single cell clones were isolated via limited dilution and screened for GLUL expression. We chose to use clone #3 as GLUL-proficient and #4 as GLUL-deficient cells for the following studies. **(B)** Cells were seeded at equal density and cultured in complete medium. Cell growth was determined by hemocytometer counting. Shown are the mean of a representative triplicate experiment plus S.D. **(C** and **D).** Cells were cultured under indicated conditions for 72 h. Images were captured **(C)**, and cell growth was determined via Satorious IncuCyte **(D)**. **(E)** Cells were cultured under indicated conditions for 72 h. Relative cell growth was determined by crystal violet staining and normalized to the O.D. of the initially seeded cells (red dashed line). Shown are the mean of a representative triplicate experiment plus S.D. ***p<0.001. **(F-H)** Flag-GLUL was reconstituted in GLUL-deficient KC cell line (clone #4) **(F)**. Cells were cultured under indicated conditions for 72 h. Relative growth **(G)** and cell death by propidium iodide (PI) staining **(H)** were measured by Celigo cell imager. Shown is the mean of a representative experiment performed in triplicate plus S.D. ****p<0.0001.

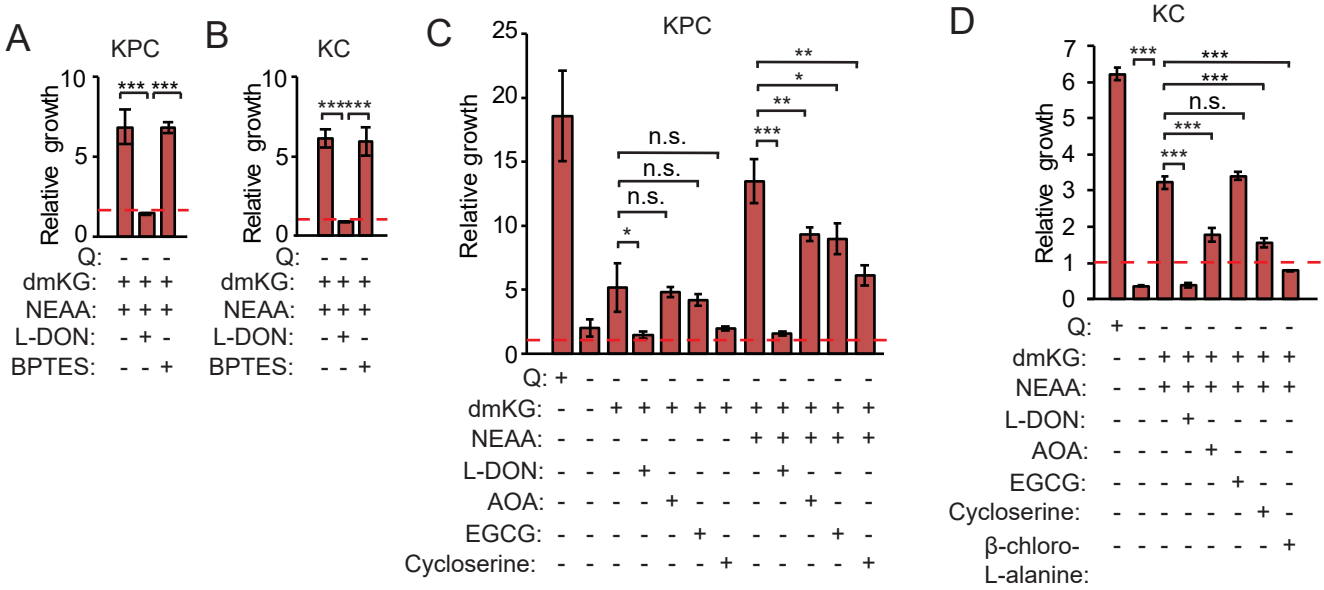

A
KPC
Relative growth
10
5
0
***
***
Q: - - -
dmKG: + + +
NEAA: + + +
L-DON: - + -
BPTES: - - +
B
KC
Relative growth
10
5
0
******
Q: - - -
dmKG: + + +
NEAA: + + +
L-DON: - + -
BPTES: - - +
C
KPC
Relative growth
25
20
15
10
5
0
n.s.
n.s.
n.s.
*
**
*
**
***
Q: + - - - - - - - - - - -
dmKG: - - + + + + + + + + + +
NEAA: - - - - - - - + + + + +
L-DON: - - - + - - - - + - - -
AOA: - - - - + - - - - + - -
EGCG: - - - - - + - - - - + -
Cycloserine: - - - - - - + - - - - +
D
KC
Relative growth
7
6
5
4
3
2
1
0
***
***
***
n.s.
***
***
Q: + - - - - - - - -
dmKG: - - + + + + + + +
NEAA: - - + + + + + + +
L-DON: - - - + - - - - -
AOA: - - - - + - - - -
EGCG: - - - - - + - - -
Cycloserine: - - - - - - + - -
β-chloro-
L-alanine: - - - - - - - + -

Figure S2. Characterization of aKG rescue of glutamine-deprived cells, Related to Figure 2.

KPC **(A, C)** and KC cells **(B, D)** were seeded at same density, then cultured under QKGN conditions in the presence of L-DON or BPTES **(A, B)** or transaminase inhibitors **(C, D)** for 72 h. Cell growth was determined by crystal violet staining. Shown are the mean of representative experiments performed in triplicates plus S.D. Red line indicates initial cell density at time of starting different culturing conditions. *p<0.05; **p<0.01; ***p<0.001; ****p<0.0001.

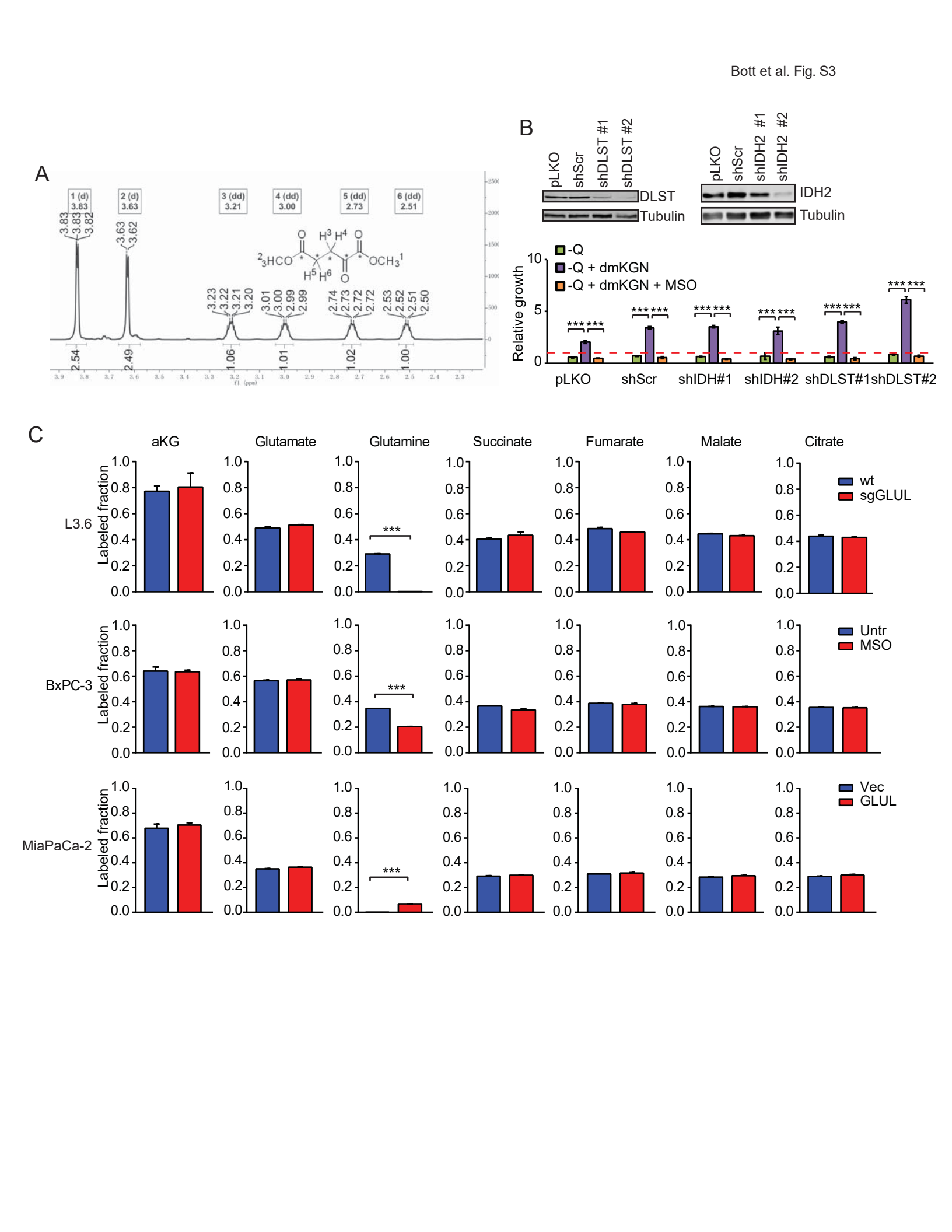

A
1 (d) 3.83
2 (d) 3.63
3 (dd) 3.21
4 (dd) 3.00
5 (dd) 2.73
6 (dd) 2.51
2.54
2.49
1.06
1.01
1.02
1.00
B
pLKO
shScr
shDLST #1
shDLST #2
DLST
Tubulin
shIDH2 #1
shIDH2 #2
IDH2
-Q
-Q + dmKGN
-Q + dmKGN + MSO
Relative growth
pLKO
shScr
shIDH#1
shIDH#2
shDLST#1
shDLST#2
C
aKG
Glutamate
Glutamine
Succinate
Fumarate
Malate
Citrate
L3.6
BxPC-3
MiaPaCa-2
Labeled fraction
wt
sgGLUL
Untr
MSO
Vec
GLUL

Figure S3. GLUL expression does not affect the TCA cycle, Related to Figure 3.

(A) $^{13}C_5$-dmKG was synthesized from $^{13}C_5$-aKG. The chemical shift (δ), coupling constant (*J*), integration and splitting patterns of $^{13}C_5$-dmKG were characterized in ^{1}H-NMR spectrum. Chemical shifts are expressed in parts per million (ppm) and reported as δ value (chemical shift δ). Coupling constants are reported in units of hertz (*J* value, Hz; Integration and splitting patterns: where s = singlet, d = double, t = triplet, q = quartet, brs = broad singlet). The NMR result shows a complete conversion of $^{13}C_5$-aKG to $^{13}C_5$-dmKG. **(B)** KPC cells were stably transduced with 2 independent shRNAs targeting either IDH2 or DLST. Cells were cultured under indicated conditions for 72 h, and relative cell growth was determined by crystal violet staining. Shown are the mean of a triplicate experiment plus S.D. **(C)** L3.6 cells stably infected with sgGLUL, BxPC-3 cells treated with MSO, or MiaPaCa-2 cells stably expressing GLUL were seeded at equal density. After attachment, cells were washed three times with PBS, and cultured in glutamine-free media supplemented with 1.5 mM $^{13}C_5$-dmKG for 24 h. Polar metabolites were extracted and subjected to LC-MS. Total labeling of indicated metabolite is shown as means from a representative triplicate experiment plus S.D.

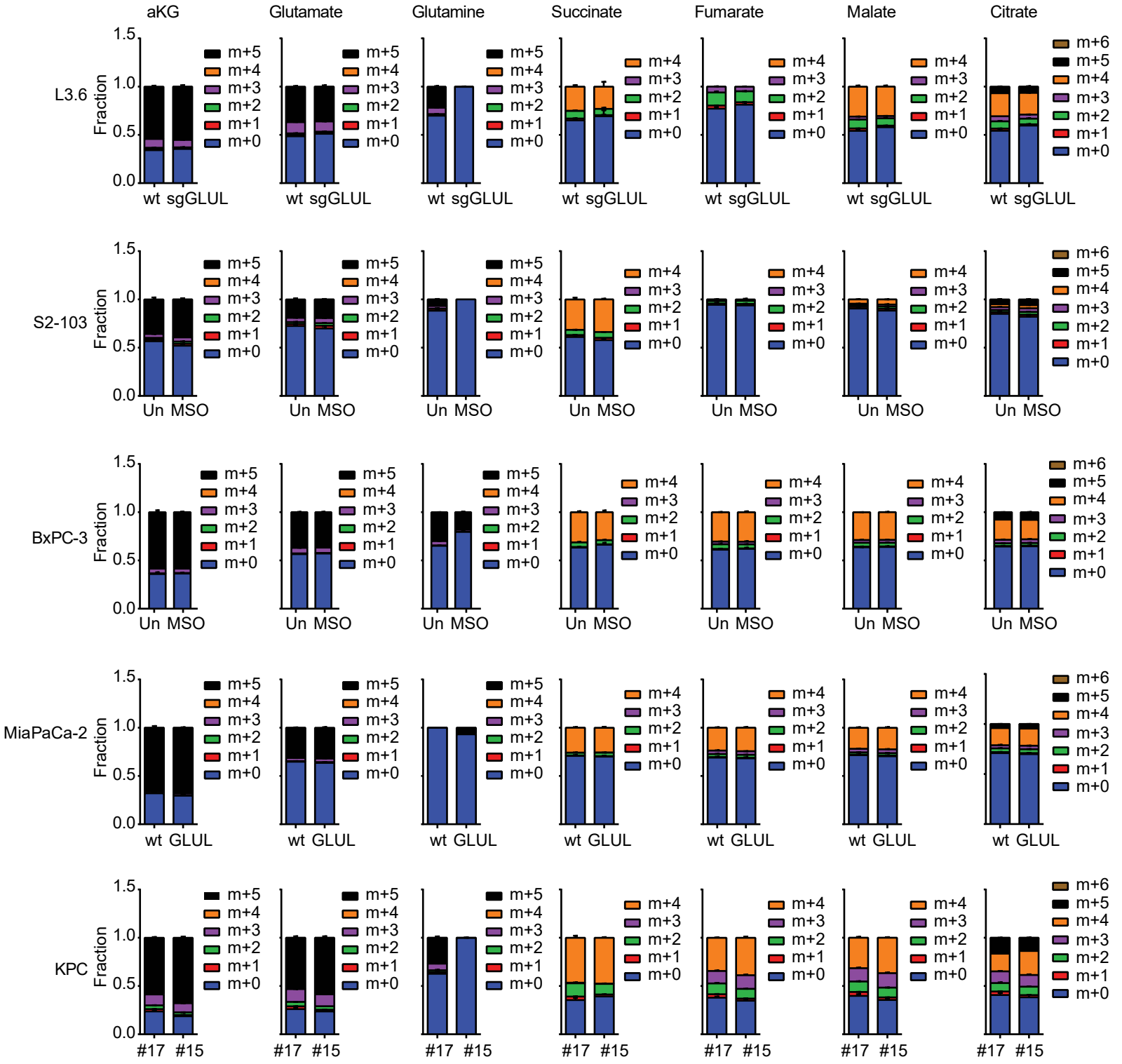

aKG
Glutamate
Glutamine
Succinate
Fumarate
Malate
Citrate
L3.6
S2-103
BxPC-3
MiaPaCa-2
KPC
Fraction
1.5
1.0
0.5
0.0
m+6
m+5
m+4
m+3
m+2
m+1
m+0
wt sgGLUL
Un MSO
wt GLUL
#17 #15

Figure S4. Isotopologue distribution of $^{13}C_5$-dmKG labeled cells, Related to Figure 3. L3.6 (GLUL-proficient or deficient), S2-103 (untreated vs MSO-treated), BxPC-3 (untreated vs MSO-treated), MiaPaCa-2 (wt vs GLUL-overexpressing), and KPC (GLUL-proficient or deficient) were cultured in glutamine-free media supplemented with 1.5 mM ^{13}C-dmKG for 24 h. Polar metabolites were extracted and subjected to LC-MS. Fractional labeling of indicated metabolite is shown, with each isotopologue shown. Shown are the means of a representative triplicate experiment plus S.D.

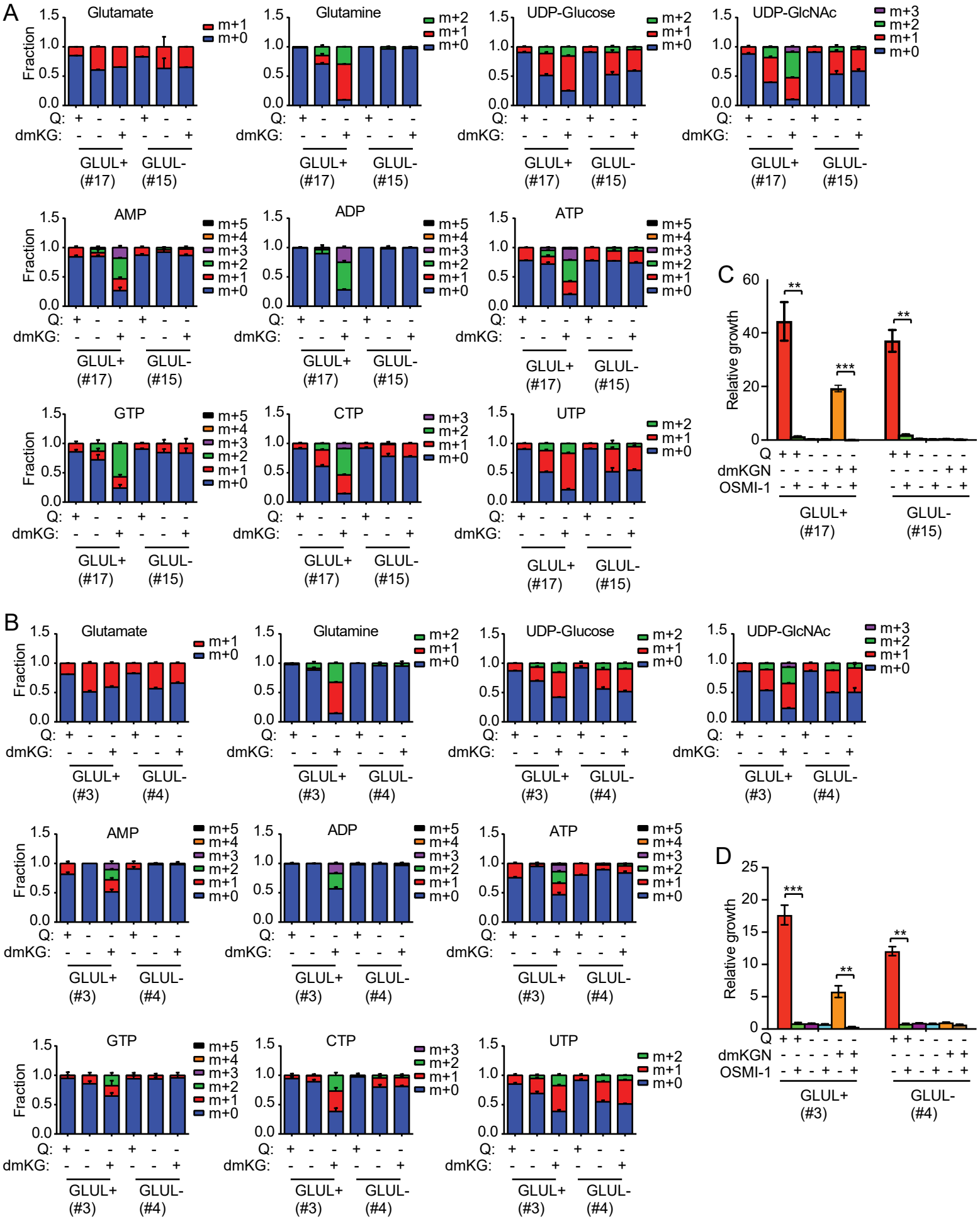
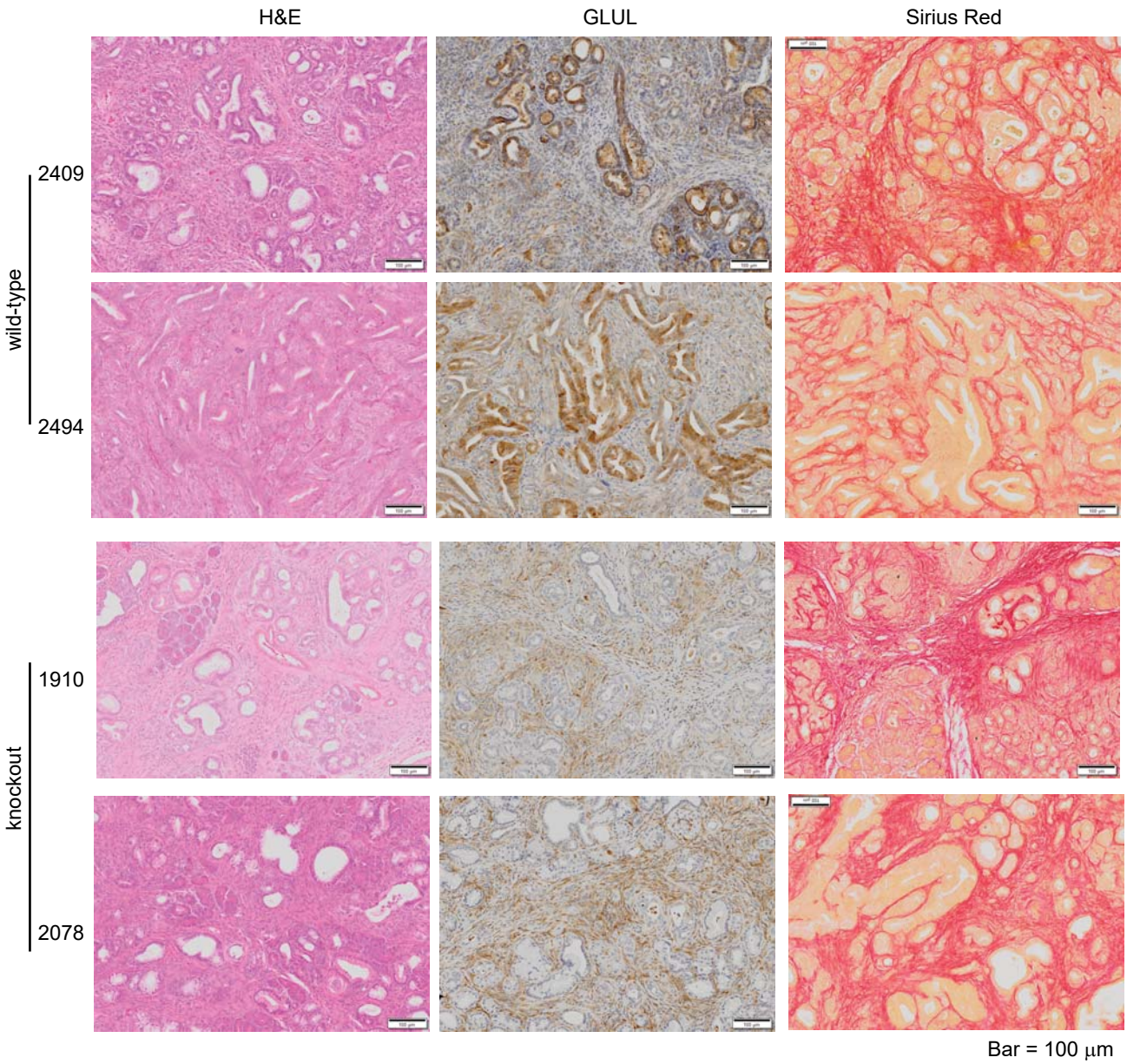

A
Glutamate
Glutamine
UDP-Glucose
UDP-GlcNAc
AMP
ADP
ATP
GTP
CTP
UTP
Fraction
Q:
dmKG:
GLUL+ (#17)
GLUL- (#15)
B
GLUL+ (#3)
GLUL- (#4)
C
Relative growth
dmKGN
OSMI-1
D

Figure S5. GLUL-deficiency leads to defective nitrogen metabolism, Related to Figure 3. KPC **(A)** and KC **(B)** cells with stable sgGLUL expression were seeded at equal density. After attachment, cells were washed three times with PBS, and cultured in media supplemented with 1 mM ^{15}N-NH_4Cl in the presence of 2 mM glutamine, glutamine-free, or glutamine-free supplemented with dmKG. Polar metabolites were extracted and subjected to LC-MS. Fractional labeling of indicated metabolite is shown, with each isotopologue shown. Shown are the mean of a representative triplicate experiment plus S.D. KPC **(C)** and KC **(D)** cells were cultured under indicated conditions for 72 h. Relative growth was measured by the Celigo Cell Imager. Shown is the mean of a representative experiment performed in triplicate plus S.D. **p<0.01; ***p<0.001. Note that OGT inhibitor OSMI-1 (50 mM) profoundly inhibited cell growth and dmKGN rescue in GLUL-proficient cells.

Bott et al. Fig. S6



Bar = 100 μm

Figure S6. GLUL expression is induced in stromal cells in GLUL$^{f/f}$ mice, Related to Figure 4.

Endpoint GLUL wild-type (WT) and knockout (KO) mice in the Kras$^{G12D/+}$;p53$^{f/f}$;Pdx1-Cre background were sacrificed. H&E, GLUL IHC, and Sirius Red (for fibrosis) staining were performed. Invasive pancreatic ductal carcinoma was developed in both WT and KO mice albeit with much latency in KO mice. Note in mice #1910 and #2078, while the ductal lesions were negative for GLUL expression, the stromal cells were strongly positive for GLUL and fibrosis.