SUPPLEMENTARY FIGURE LEGENDS

Figure S1. Mitochondrial one-carbon enzymes are induced by TGF-β. (A-D) Quantification of relative (A) MTHFD2, (B) ALDH1L2, (C) MTHFD1L, and (D) MTHFD1 protein levels after treatment of normal human lung fibroblasts (NHLFs) treated with TGF-β for the indicated intervals. **(E-H)** Quantification of relative (E) MTHFD2, (F) ALDH1L2, (G) MTHFD1L, and (H) MTHFD1 protein levels after treatment of NHLFs transfected with siRNA targeting ATF4 or nontargeting siRNA. Cells were treated with TGF-β for the indicated intervals. **(I-L)** Quantification of relative (I) MTHFD2, (J) ALDH1L2, (K) MTHFD1L, and (L) MTHFD1 protein levels after treatment of NHLFs with TGF-β for the indicated intervals in the presence or absence of Rapalink-1. Bar graphs represent mean ± SEM, n=3 independent experimental replicates. **P*<0.05, ***P*<0.01, ****P*<0.001.

Figure S2. Mitochondrial one-carbon metabolism is required for TGF-β-induced collagen protein production. (A-B) Western blot analysis of collagen and α-smooth muscle actin (α-SMA) protein expression in normal human lung fibroblasts (NHLFs) transfected with siRNAs targeting *MTHFD2* or nontargeting siRNA. Cells were treated with TGF-β for the indicated intervals in the presence of the indicated doses of formate. **(C)** Western blot analysis of collagen and α-SMA protein expression in NHLFs treated TGF-β for the indicated intervals in the presence of the indicated doses of methotrexate (MTX). **(D-E)** Western blot analysis of collagen and α-SMA protein expression in NHLFs transfected with siRNAs targeting *MTHFD2* or nontargeting siRNA. Cells were treated with TGF-β for the indicated intervals in the presence of the indicated doses of 5-methyl-tetrahydrofolate (mTHF).

Figure S3. MTHFD2 is required for increased cellular glycine levels downstream of TGF-β. (A) Schematic representation of metabolite labeling downstream of ^{13}C2-Glycine. Normal human lung fibroblasts (NHLFs) were transfected with siRNA targeting *MTHFD2* or nontargeting siRNA. Cells were labeled with ^{13}C2-Glycine and treated with TGF-β for 48 hours or left untreated. **(B)** Analysis of cellular glycine after labeling with ^{13}C2-Glycine in NHLFs treated with TGF-β or left untreated. **(C)** Relative total glycine levels from (B). **(D)** Relative levels of M+0 (*de novo* synthesized) glycine from (B). **(E)** Analysis of cellular serine after labeling with ^{13}C2-Glycine in NHLFs treated with TGF-β or left untreated. **(F)** Relative total serine levels from (E). **(G)** Relative levels of M+2 serine from (E). Bar graphs represent mean ± SEM, n=3. **P*<0.05, ***P*<0.01, ****P*<0.001.

Figure S4. SHMT2 is required for increased cellular glycine levels downstream of TGF-β. Normal human lung fibroblasts (NHLFs) were transfected with siRNA targeting *SHMT2* or nontargeting siRNA. Cells were labeled with ^{13}C2-Glycine and treated with TGF-β for 48 hours or left untreated. **(A)** Analysis of cellular glycine after labeling with ^{13}C2-Glycine in NHLFs treated with TGF-β or left untreated. **(B)** Relative total glycine levels from (A). **(C)** Relative levels of M+0 (*de novo* synthesized) glycine from (A). **(D)** Analysis of cellular serine after labeling with ^{13}C2-Glycine in NHLFs treated with TGF-β or left untreated. **(E)** Relative total serine levels from (D). **(F)** Relative levels of M+2 serine from (D). Bar graphs represent mean ± SEM, n=3. **P*<0.05, ***P*<0.01, ****P*<0.001.

Figure S5. Analysis of cellular metabolite levels in *MTHFD2* knockdown human lung fibroblasts. Normal human lung fibroblasts (NHLFs) were transfected with siRNA targeting *MTHFD2* or nontargeting siRNA. Cells were labeled with 2,3,3-D3-Serine and treated with TGF-β for 48 hours or left untreated. **(A)** Relative total glycine levels from Figure 3B. **(B)** Relative total serine levels from Figure 3D. **(C)** Relative total proline levels from Figure 3F. Bar graphs represent mean ± SEM, n=3. **P*<0.05, ***P*<0.01, ****P*<0.001.

Figure S6. Analysis of cellular metabolite levels in *ALDH1L2* knockdown human lung fibroblasts. Normal human lung fibroblasts (NHLFs) were transfected with siRNA targeting *ALDH1L2* or nontargeting siRNA. Cells were labeled with 2,3,3-D3-Serine and treated with TGF-β for 48 hours or left untreated. **(A)** Relative total glycine levels from Figure 4A. **(B)** Relative levels of M+1 glycine from Figure 4A. **(C)** Relative total serine levels from Figure 4B. **(D)** Relative levels of M+1 and M+2 serine from Figure 4B. **(E)** Relative total proline levels from Figure 4C. **(F)** Relative levels of M+1 proline from Figure 4C. Bar graphs represent mean ± SEM, n=3. **P*<0.05, ***P*<0.01, ****P*<0.001.

Figure S7. Analysis of cellular metabolite levels in *MTHFD1L* knockdown human lung fibroblasts. Normal human lung fibroblasts (NHLFs) were transfected with siRNA targeting *MTHFD1L* or nontargeting siRNA. Cells were labeled with 2,3,3-D3-Serine and treated with TGF-β for 48 hours or left untreated. **(A)** Relative total glycine levels from Figure 4E. **(B)** Relative levels of M+1 glycine from Figure 4E. **(C)** Relative total serine levels from Figure 4F. **(D)** Relative levels of M+1 and M+2 serine from Figure 4F. **(E)** Relative total proline levels from Figure 4G. **(F)** Relative levels of M+1 proline from Figure 4G. Bar graphs represent mean ± SEM, n=3. **P*<0.05, ***P*<0.01, ****P*<0.001.

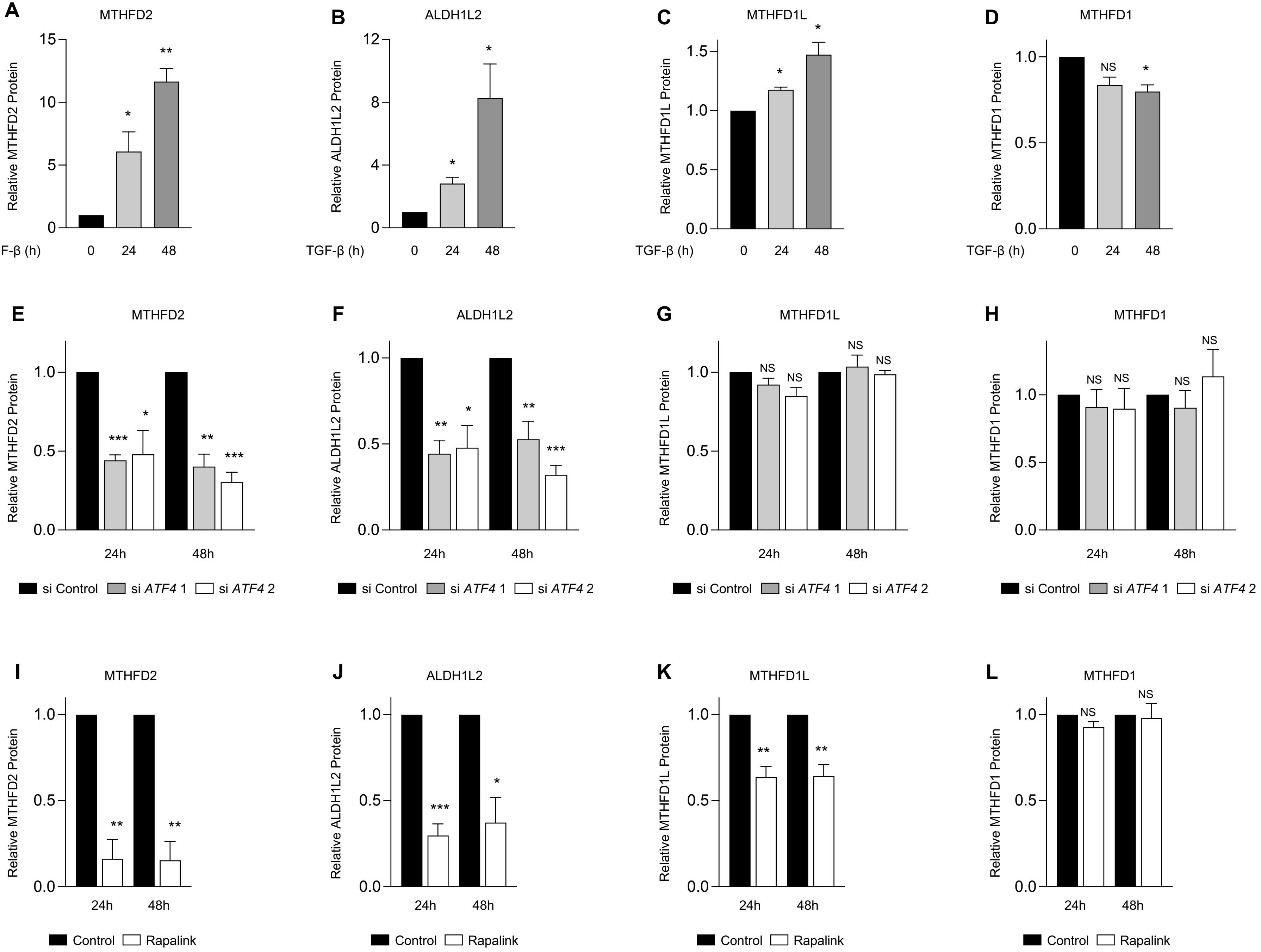
Figure S8. Analysis of cellular metabolite levels in human lung fibroblasts with knockdown of both *ALDH1L2* and *MTHFD1L*. Normal human lung fibroblasts (NHLFs) were transfected with siRNA targeting both *ALDH1L2* and *MTHFD1L* or nontargeting siRNA. Cells were labeled with 2,3,3-D3-Serine and treated with TGF-β for 48 hours or left untreated. **(A)** Relative total glycine levels from Figure 4I. **(B)** Relative levels of M+1 glycine from Figure 4I. **(C)** Relative total serine levels from Figure 4J. **(D)** Relative levels of M+1 and M+2 serine from Figure 4J. **(E)** Relative total proline levels from Figure 4K. **(F)** Relative levels of M+1 proline from Figure 4K. Bar graphs represent mean ± SEM, n=3. **P*<0.05, ***P*<0.01, ****P*<0.001.

Figure S9. *MTHFD1* is not required for increased cellular glycine levels downstream of TGF-β. Normal human lung fibroblasts (NHLFs) were transfected with siRNA targeting *MTHFD1* or nontargeting siRNA. Cells were labeled with 2,3,3-D3-Serine and treated with TGF-β for 48 hours or left untreated. **(A)** Analysis of cellular glycine after labeling with 2,3,3-D3-Serine in NHLFs treated with TGF-β or left untreated. **(B)** Relative total glycine levels from (A). **(C)** Relative levels of M+1 glycine from (A). **(D)** Analysis of cellular serine after labeling with 2,3,3-D3-Serine in NHLFs treated with TGF-β or left untreated. **(E)** Relative total serine levels from (D). **(F)** Relative levels of M+1 and M+2 serine from (D). **(G)** Analysis of cellular proline after labeling with 2,3,3-D3-Serine in NHLFs treated with TGF-β or left untreated. **(H)** Relative total serine levels from (G). **(I)** Relative levels of M+1 and M+2 serine from (G). Bar graphs represent mean ± SEM, n=3. **P*<0.05, ***P*<0.01, ****P*<0.001.

Figure S10. Pharmacologic inhibition of MTHFD2 inhibits fibrotic responses. (A) Relative total glycine levels from Figure 5E. **(B)** Relative levels of M+1 glycine from Figure 5E. **(C)** Relative total serine levels from Figure 5F. **(D)** Relative levels of M+1 and M+2 serine from Figure 5F. **(E)**

Relative total proline levels from Figure 5G. **(F)** Relative levels of M+1 proline from Figure 5G. Bar graphs represent mean ± SEM, n=3. **(G)** Representative transverse microCT images of mice prior to bleomycin instillation (Day 0), 7 days after bleomycin instillation (Day 7), and 14 days after bleomycin instillation (Day 14). Mice began receiving DS18561882 (125 mg/kg) or vehicle 8 days after bleomycin instillation. **(H)** Percent fibrotic area of lung from mice 21 days after bleomycin instillation. Mice received either vehicle or DS18561882 beginning on day 8. Bar graphs represent mean ± SEM, n=5 independent mice per condition. $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$.

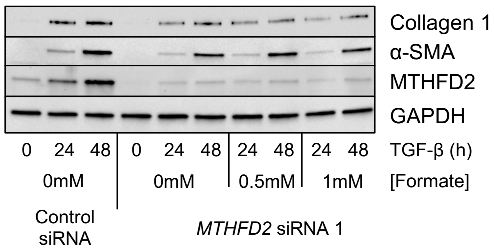
Figure S1



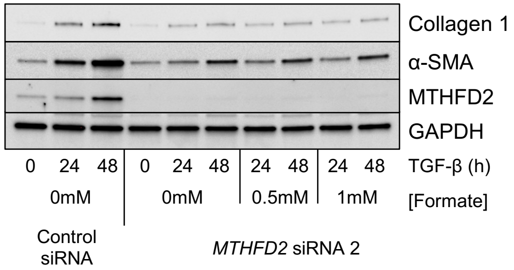
A
MTHFD2
Relative MTHFD2 Protein
15
10
5
0
*
**
TGF-β (h)
0
24
48
B
ALDH1L2
Relative ALDH1L2 Protein
12
8
4
0
*
*
TGF-β (h)
0
24
48
C
MTHFD1L
Relative MTHFD1L Protein
1.5
1.0
0.5
0.0
*
*
TGF-β (h)
0
24
48
D
MTHFD1
Relative MTHFD1 Protein
1.0
0.5
0.0
NS
*
TGF-β (h)
0
24
48
E
MTHFD2
Relative MTHFD2 Protein
1.0
0.5
0.0
***
*
**
***
24h
48h
si Control
si ATF4 1
si ATF4 2
F
ALDH1L2
Relative ALDH1L2 Protein
1.0
0.5
0.0
**
*
**
***
24h
48h
si Control
si ATF4 1
si ATF4 2
G
MTHFD1L
Relative MTHFD1L Protein
1.0
0.5
0.0
NS
NS
NS
NS
24h
48h
si Control
si ATF4 1
si ATF4 2
H
MTHFD1
Relative MTHFD1 Protein
1.0
0.5
0.0
NS
NS
NS
NS
24h
48h
si Control
si ATF4 1
si ATF4 2
I
MTHFD2
Relative MTHFD2 Protein
1.0
0.5
0.0
**
**
24h
48h
Control
Rapalink
J
ALDH1L2
Relative ALDH1L2 Protein
1.0
0.5
0.0
***
*
24h
48h
Control
Rapalink
K
MTHFD1L
Relative MTHFD1L Protein
1.0
0.5
0.0
**
**
24h
48h
Control
Rapalink
L
MTHFD1
Relative MTHFD1 Protein
1.0
0.5
0.0
NS
NS
24h
48h
Control
Rapalink

Figure S2

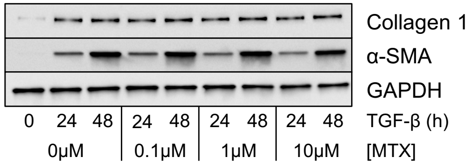
A



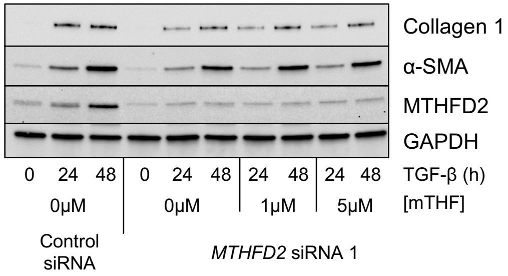
B



C



D



E

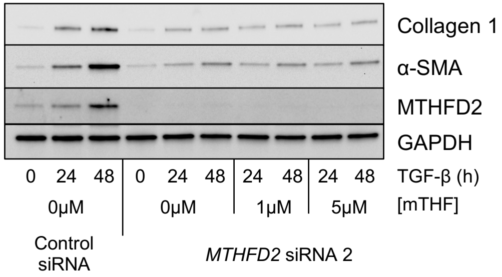


Figure S3

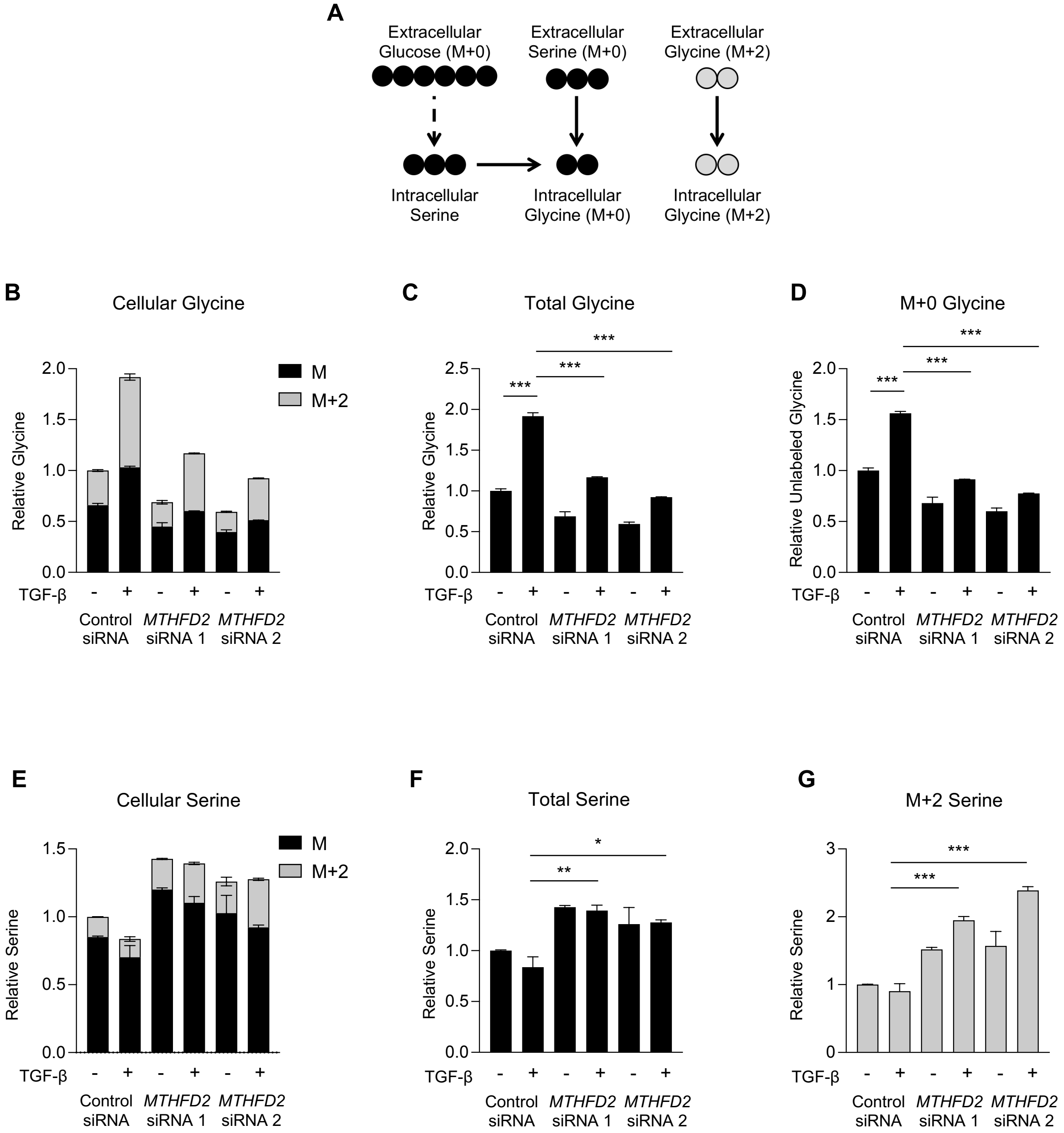

A
Extracellular Glucose (M+0)
Extracellular Serine (M+0)
Extracellular Glycine (M+2)
Intracellular Serine
Intracellular Glycine (M+0)
Intracellular Glycine (M+2)
B
Cellular Glycine
Relative Glycine
0.0
0.5
1.0
1.5
2.0
M
M+2
C
Total Glycine
Relative Glycine
0.0
0.5
1.0
1.5
2.0
2.5
***
***
***
D
M+0 Glycine
Relative Unlabeled Glycine
0.0
0.5
1.0
1.5
2.0
***
***
***
E
Cellular Serine
Relative Serine
0.0
0.5
1.0
1.5
M
M+2
F
Total Serine
Relative Serine
0.0
0.5
1.0
1.5
2.0
**
*
G
M+2 Serine
Relative Serine
0
1
2
3
***
***
TGF-β
-
+
-
+
-
+
Control siRNA
MTHFD2 siRNA 1
MTHFD2 siRNA 2

Figure S4

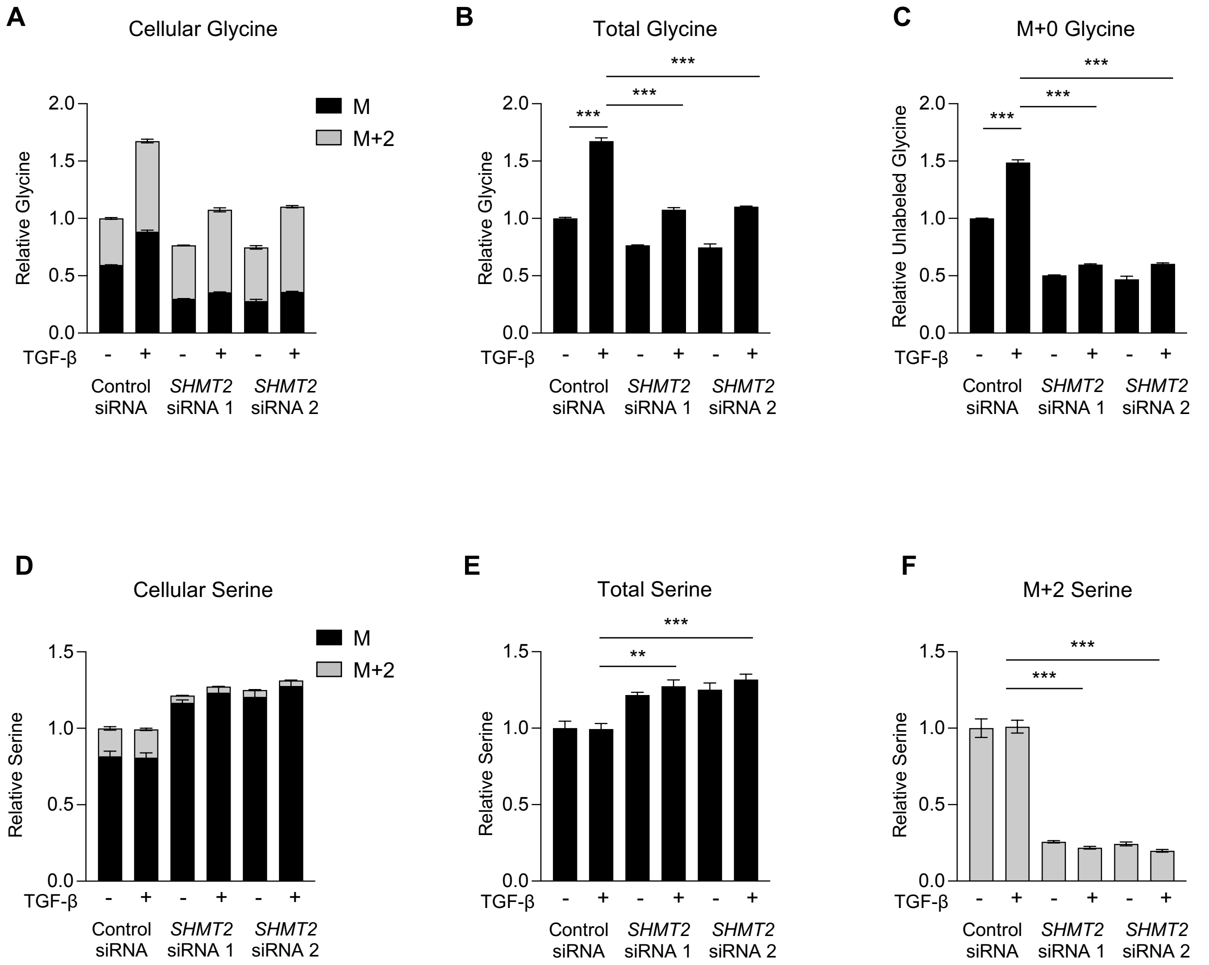

A
Cellular Glycine
Relative Glycine
2.0
1.5
1.0
0.5
0.0
M
M+2
TGF-β
-
+
-
+
-
+
Control siRNA
SHMT2 siRNA 1
SHMT2 siRNA 2
B
Total Glycine
Relative Glycine
***
***
***
C
M+0 Glycine
Relative Unlabeled Glycine
***
***
***
D
Cellular Serine
Relative Serine
1.5
1.0
0.5
0.0
M
M+2
E
Total Serine
Relative Serine
**
***
F
M+2 Serine
Relative Serine
***
***

Figure S5

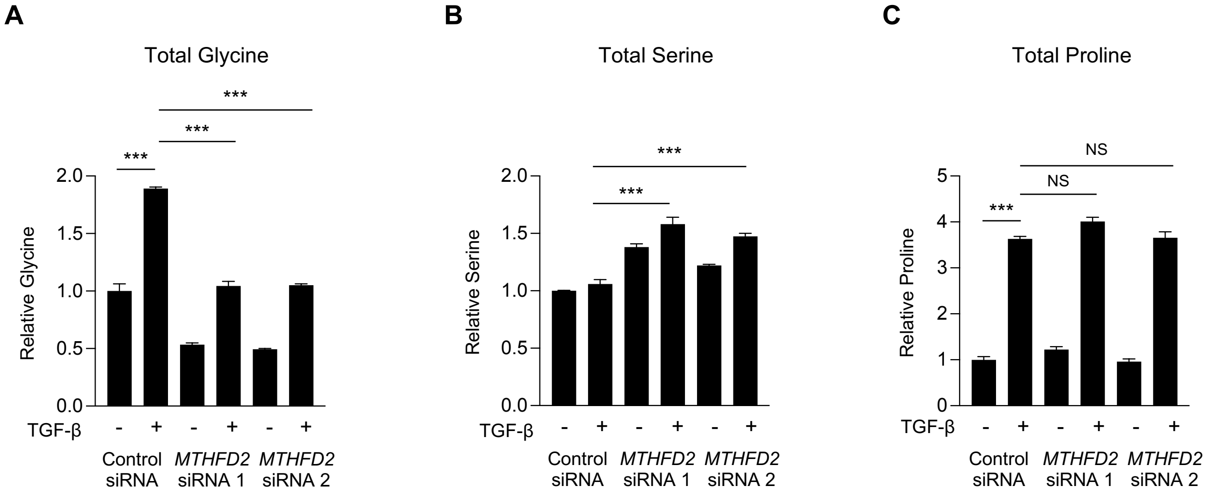

A
Total Glycine
Relative Glycine
2.0
1.5
1.0
0.5
0.0
***
***
***
TGF-β
- + - + - +
Control siRNA
MTHFD2 siRNA 1
MTHFD2 siRNA 2
B
Total Serine
Relative Serine
2.0
1.5
1.0
0.5
0.0
***
***
TGF-β
- + - + - +
Control siRNA
MTHFD2 siRNA 1
MTHFD2 siRNA 2
C
Total Proline
Relative Proline
5
4
3
2
1
0
***
NS
NS
TGF-β
- + - + - +
Control siRNA
MTHFD2 siRNA 1
MTHFD2 siRNA 2

Figure S6

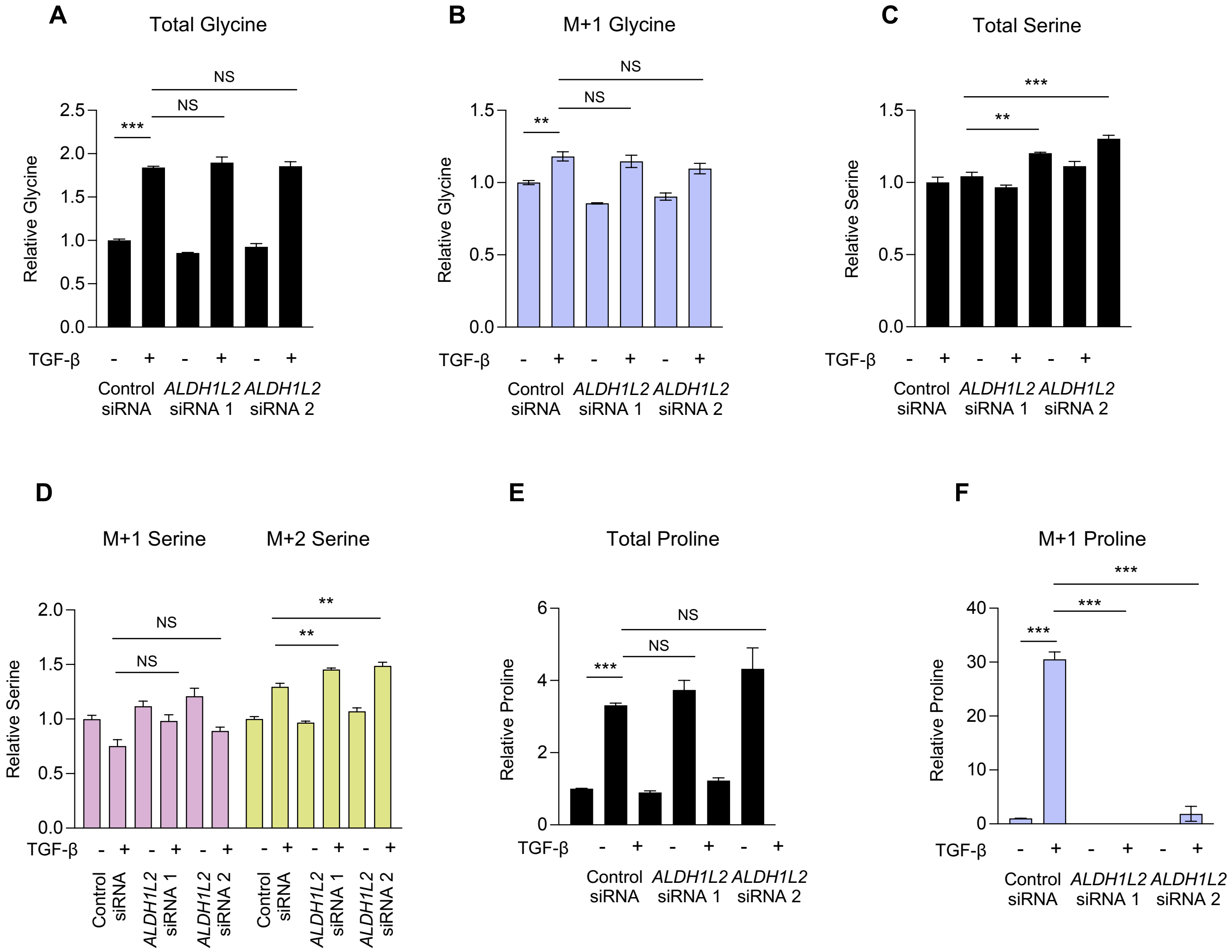

A
Total Glycine
Relative Glycine
2.5
2.0
1.5
1.0
0.5
0.0
***
NS
NS
TGF-β - + - + - +
Control siRNA
ALDH1L2 siRNA 1
ALDH1L2 siRNA 2
B
M+1 Glycine
Relative Glycine
1.5
1.0
0.5
0.0
**
NS
NS
TGF-β - + - + - +
Control siRNA
ALDH1L2 siRNA 1
ALDH1L2 siRNA 2
C
Total Serine
Relative Serine
1.5
1.0
0.5
0.0
**
***
TGF-β - + - + - +
Control siRNA
ALDH1L2 siRNA 1
ALDH1L2 siRNA 2
D
M+1 Serine
M+2 Serine
Relative Serine
2.0
1.5
1.0
0.5
0.0
NS
NS
**
**
TGF-β - + - + - + - + - + - +
Control siRNA
ALDH1L2 siRNA 1
ALDH1L2 siRNA 2
Control siRNA
ALDH1L2 siRNA 1
ALDH1L2 siRNA 2
E
Total Proline
Relative Proline
6
4
2
0
***
NS
NS
TGF-β - + - + - +
Control siRNA
ALDH1L2 siRNA 1
ALDH1L2 siRNA 2
F
M+1 Proline
Relative Proline
40
30
20
10
0
***
***
***
TGF-β - + - + - +
Control siRNA
ALDH1L2 siRNA 1
ALDH1L2 siRNA 2

Figure S7

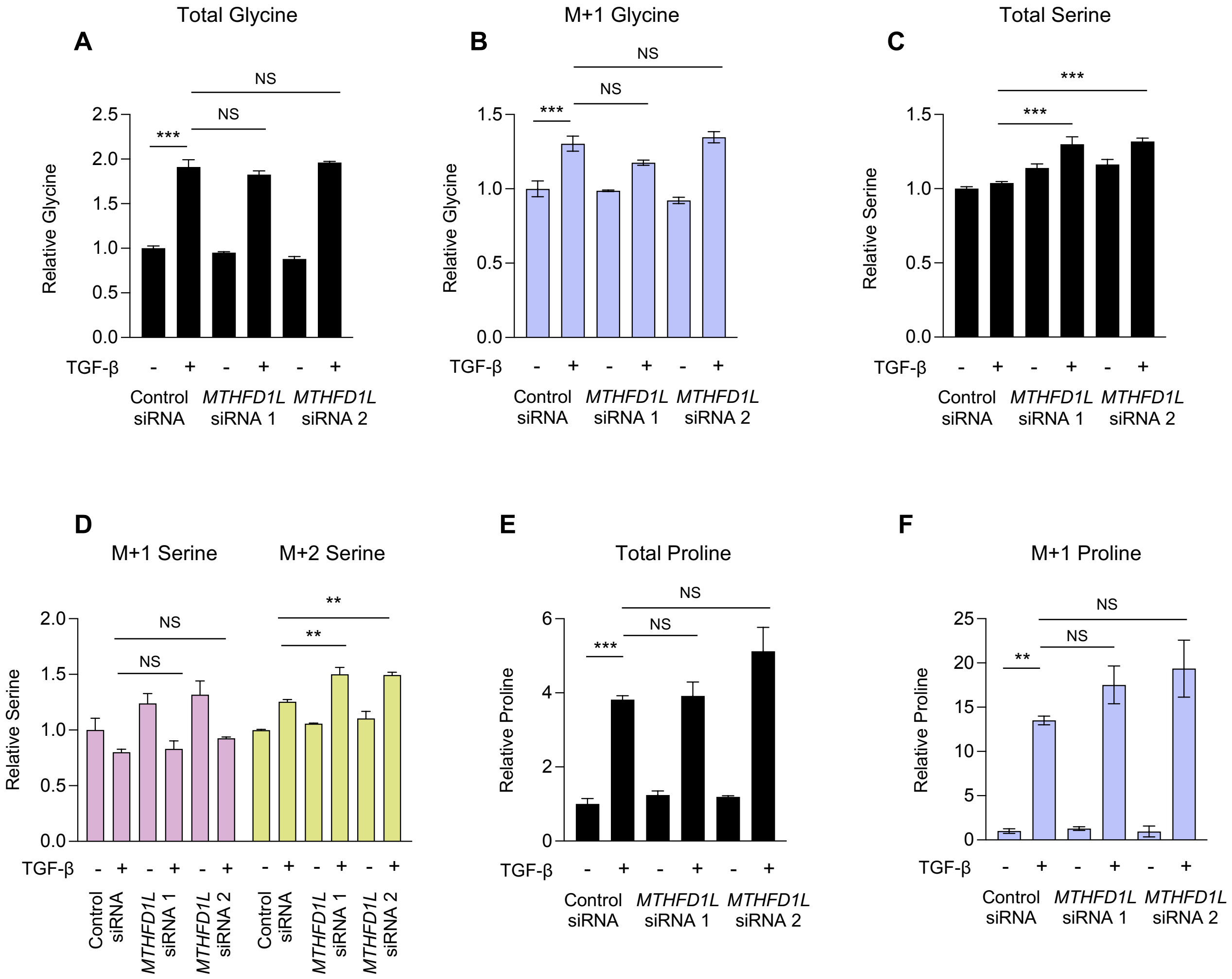

A
Total Glycine
Relative Glycine
0.0
0.5
1.0
1.5
2.0
2.5
***
NS
NS
TGF-β
-
+
-
+
-
+
Control siRNA
MTHFD1L siRNA 1
MTHFD1L siRNA 2
B
M+1 Glycine
Relative Glycine
0.0
0.5
1.0
1.5
***
NS
NS
C
Total Serine
Relative Serine
0.0
0.5
1.0
1.5
***
***
D
M+1 Serine
M+2 Serine
Relative Serine
0.0
0.5
1.0
1.5
2.0
NS
NS
**
**
E
Total Proline
Relative Proline
0
2
4
6
***
NS
NS
F
M+1 Proline
Relative Proline
0
5
10
15
20
25
**
NS
NS

Figure S8

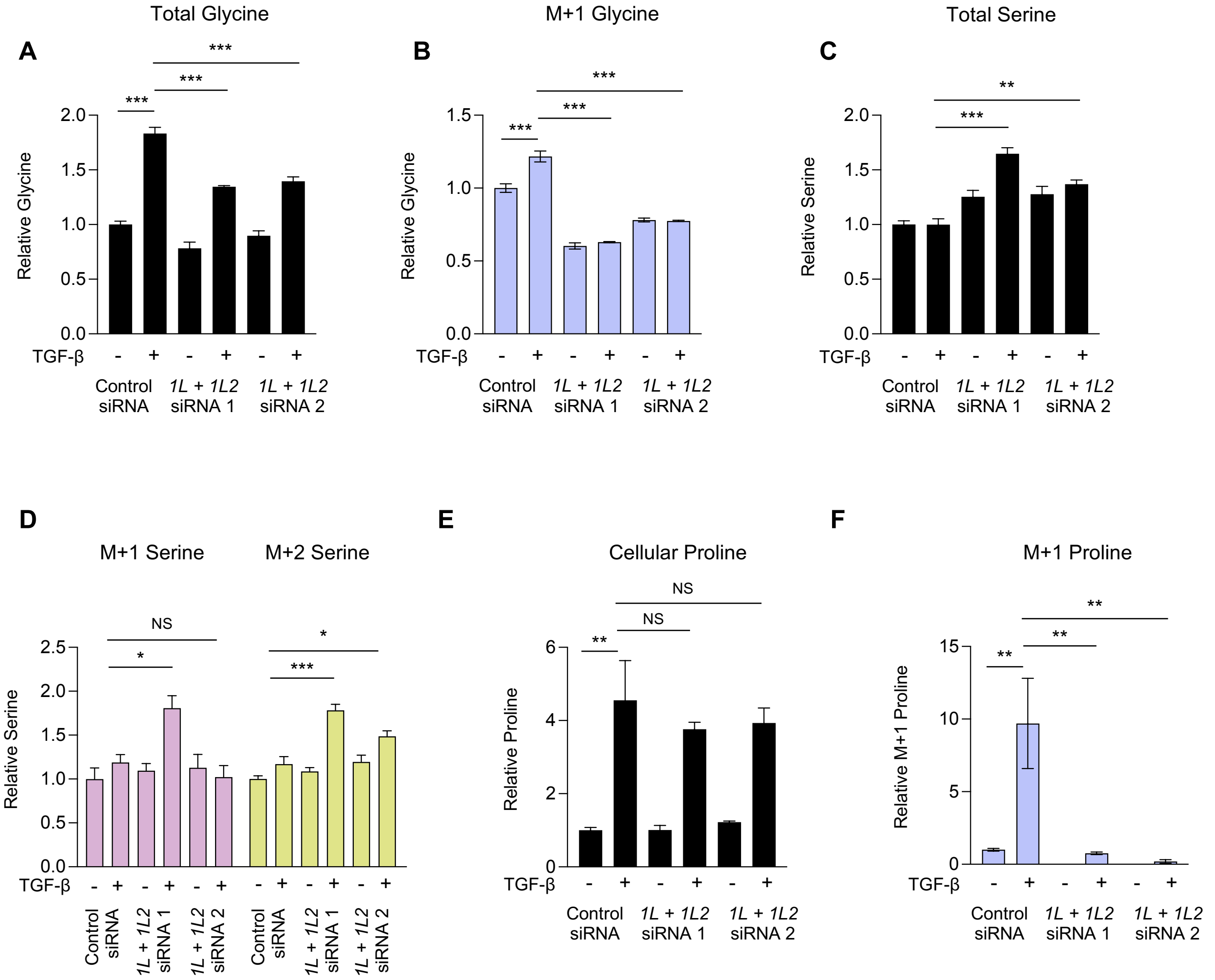

Total Glycine
A
Relative Glycine
2.0
1.5
1.0
0.5
0.0
***
***
***
TGF-β
- + - + - +
Control siRNA
1L + *1L2* siRNA 1
1L + *1L2* siRNA 2
M+1 Glycine
B
Relative Glycine
1.5
1.0
0.5
0.0
***
***
***
Total Serine
C
Relative Serine
2.0
1.5
1.0
0.5
0.0
***
**
D
M+1 Serine
M+2 Serine
Relative Serine
2.5
2.0
1.5
1.0
0.5
0.0
*
NS
***
*
E
Cellular Proline
Relative Proline
6
4
2
0
**
NS
NS
F
M+1 Proline
Relative M+1 Proline
15
10
5
0
**
**
**

Figure S9

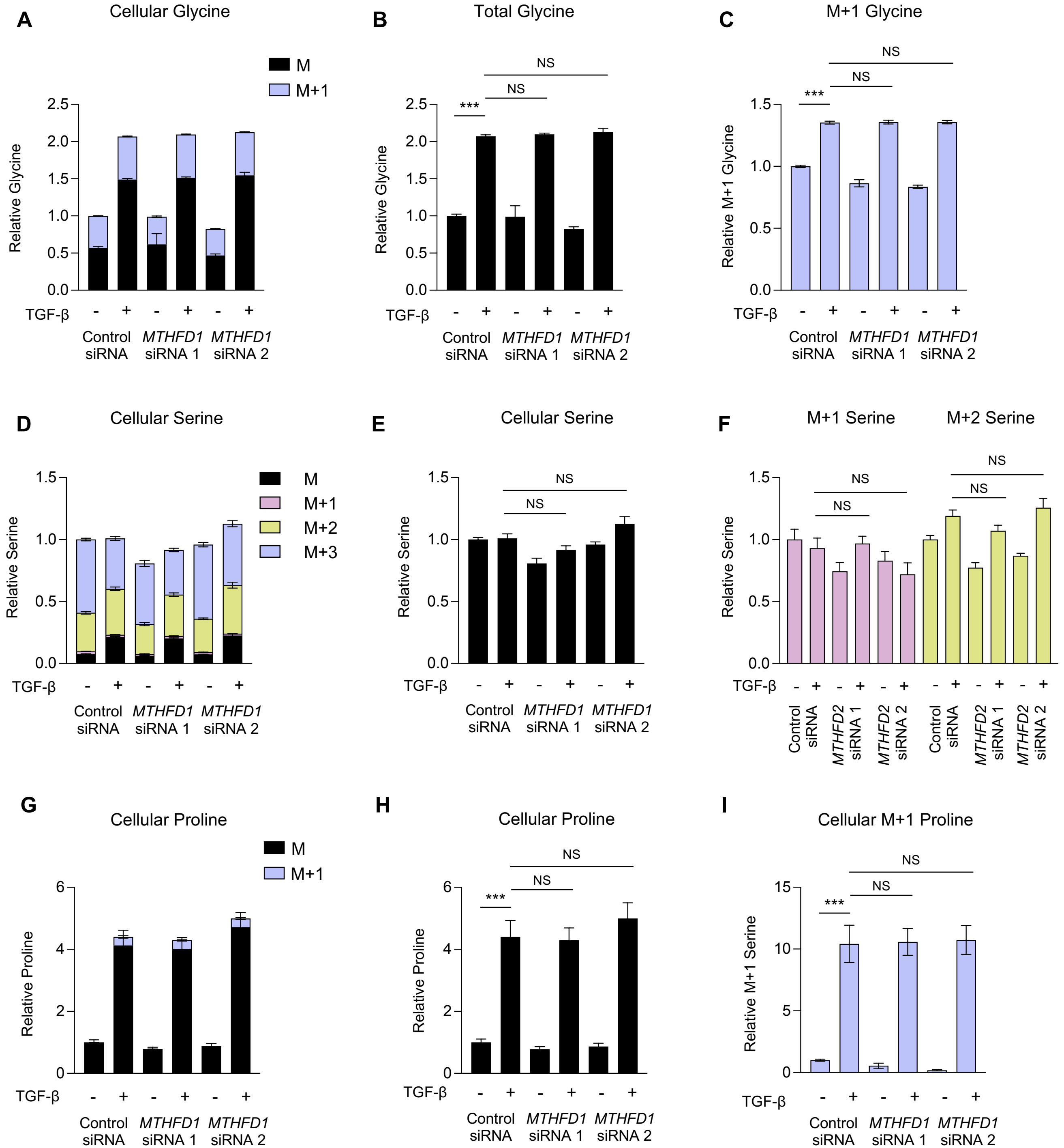
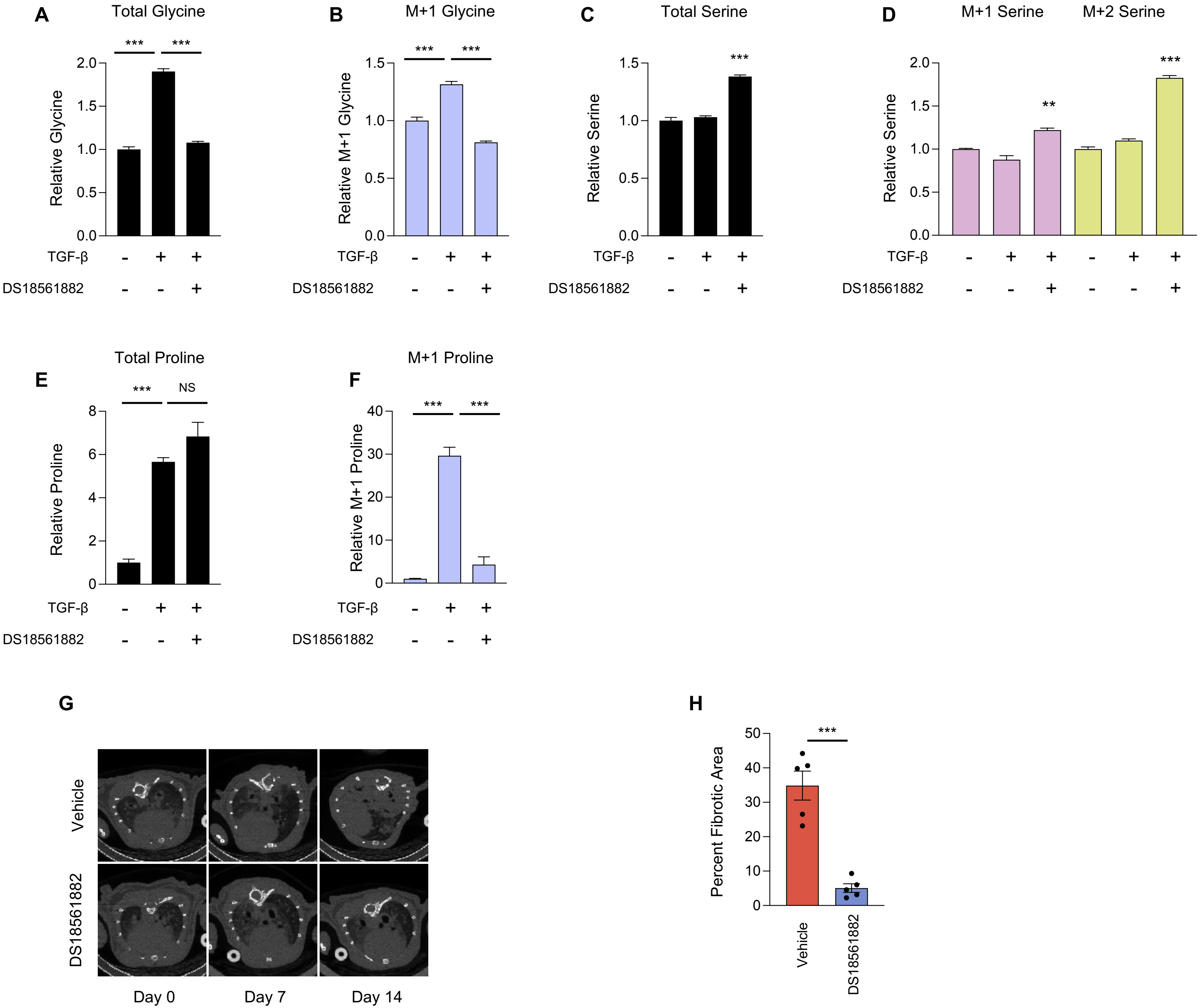

A
Cellular Glycine
M
M+1
Relative Glycine
0.0
0.5
1.0
1.5
2.0
2.5
TGF-β
- + - + - +
Control siRNA
MTHFD1 siRNA 1
MTHFD1 siRNA 2
B
Total Glycine
***
NS
NS
Relative Glycine
0.0
0.5
1.0
1.5
2.0
2.5
TGF-β
- + - + - +
Control siRNA
MTHFD1 siRNA 1
MTHFD1 siRNA 2
C
M+1 Glycine
***
NS
NS
Relative M+1 Glycine
0.0
0.5
1.0
1.5
TGF-β
- + - + - +
Control siRNA
MTHFD1 siRNA 1
MTHFD1 siRNA 2
D
Cellular Serine
M
M+1
M+2
M+3
Relative Serine
0.0
0.5
1.0
1.5
TGF-β
- + - + - +
Control siRNA
MTHFD1 siRNA 1
MTHFD1 siRNA 2
E
Cellular Serine
NS
NS
Relative Serine
0.0
0.5
1.0
1.5
TGF-β
- + - + - +
Control siRNA
MTHFD1 siRNA 1
MTHFD1 siRNA 2
F
M+1 Serine
M+2 Serine
NS
NS
NS
NS
Relative Serine
0.0
0.5
1.0
1.5
TGF-β
- + - + - + - + - + - +
Control siRNA
MTHFD2 siRNA 1
MTHFD2 siRNA 2
Control siRNA
MTHFD2 siRNA 1
MTHFD2 siRNA 2
G
Cellular Proline
M
M+1
Relative Proline
0
2
4
6
TGF-β
- + - + - +
Control siRNA
MTHFD1 siRNA 1
MTHFD1 siRNA 2
H
Cellular Proline
***
NS
NS
Relative Proline
0
2
4
6
TGF-β
- + - + - +
Control siRNA
MTHFD1 siRNA 1
MTHFD1 siRNA 2
I
Cellular M+1 Proline
***
NS
NS
Relative M+1 Serine
0
5
10
15
TGF-β
- + - + - +
Control siRNA
MTHFD1 siRNA 1
MTHFD1 siRNA 2

Figure S10


A
Total Glycine
*** ***
Relative Glycine
0.0 0.5 1.0 1.5 2.0
TGF-β - + +
DS18561882 - - +
B
M+1 Glycine
*** ***
Relative M+1 Glycine
0.0 0.5 1.0 1.5
TGF-β - + +
DS18561882 - - +
C
Total Serine
***
Relative Serine
0.0 0.5 1.0 1.5
TGF-β - + +
DS18561882 - - +
D
M+1 Serine
M+2 Serine
** ***
Relative Serine
0.0 0.5 1.0 1.5 2.0
TGF-β - + + - + +
DS18561882 - - + - - +
E
Total Proline
*** NS
Relative Proline
0 2 4 6 8
TGF-β - + +
DS18561882 - - +
F
M+1 Proline
*** ***
Relative M+1 Proline
0 10 20 30 40
TGF-β - + +
DS18561882 - - +
G
Vehicle
DS18561882
Day 0
Day 7
Day 14
H
***
Percent Fibrotic Area
0 10 20 30 40 50
Vehicle
DS18561882