Cell Metabolism, Volume *32*

Supplemental Information

Targeting DGAT1 Ameliorates Glioblastoma by Increasing Fat Catabolism and Oxidative Stress

Xiang Cheng, Feng Geng, Meixia Pan, Xiaoning Wu, Yaogang Zhong, Chunyan Wang, Zhihua Tian, Chunming Cheng, Rui Zhang, Vinay Puduvalli, Craig Horbinski, Xiaokui Mo, Xianlin Han, Arnab Chakravarti, and Deliang Guo

Fig. S1

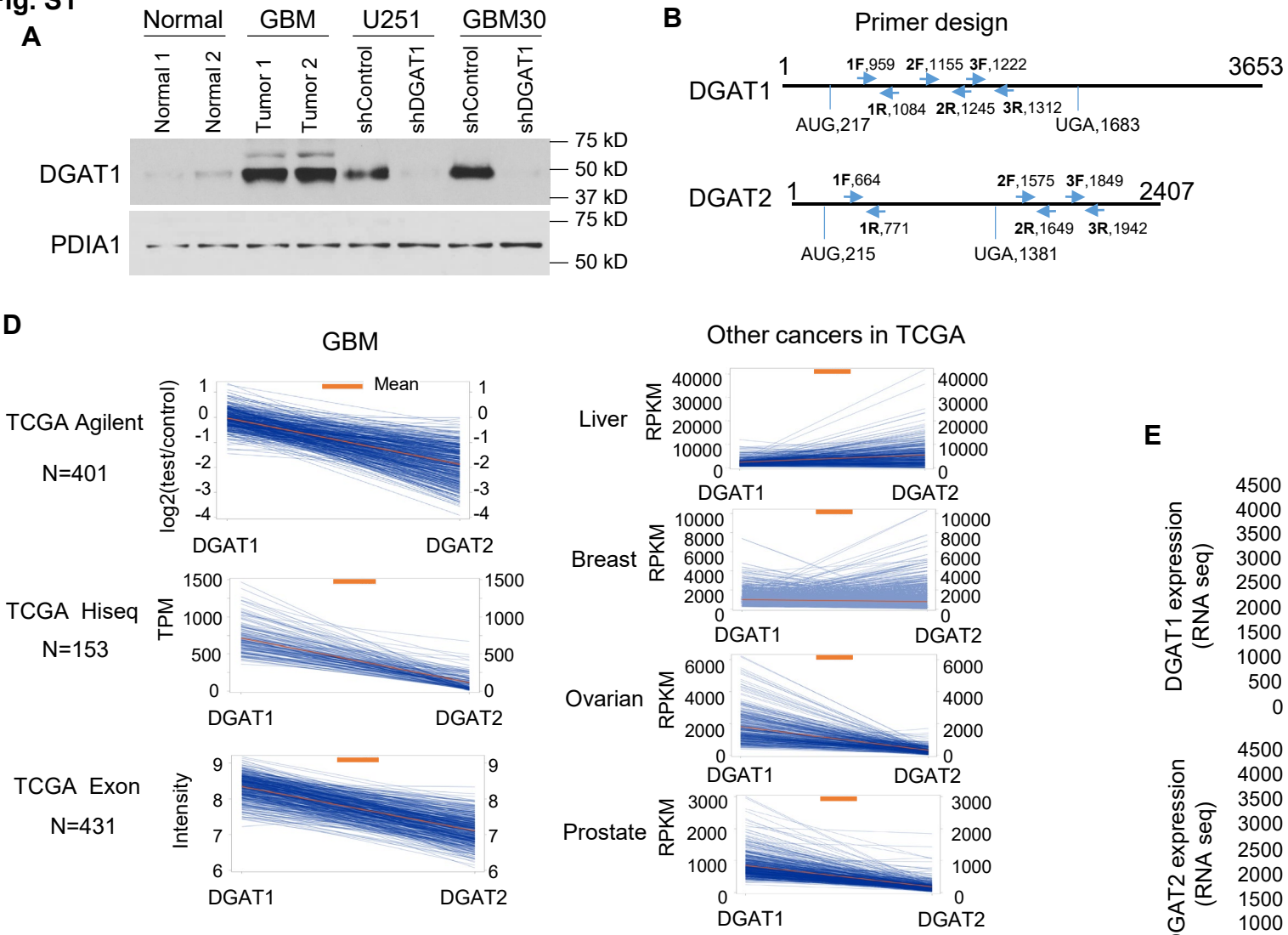

A
Normal
GBM
U251
GBM30
Normal 1
Normal 2
Tumor 1
Tumor 2
shControl
shDGAT1
shControl
shDGAT1
DGAT1
PDIA1
75 kD
50 kD
37 kD
75 kD
50 kD
B
Primer design
DGAT1
1
3653
1F,959
2F,1155
3F,1222
1R,1084
2R,1245
3R,1312
AUG,217
UGA,1683
DGAT2
1
2407
1F,664
2F,1575
3F,1849
1R,771
2R,1649
3R,1942
AUG,215
UGA,1381
C
GBM
Relative mRNA
1
2
3
4
5
6
7
8
9
10
DGAT1
DGAT2
D
GBM
Other cancers in TCGA
TCGA Agilent
N=401
log2(test/control)
Mean
TCGA Hiseq
N=153
TPM
TCGA Exon
N=431
Intensity
DGAT1
DGAT2
Liver
Breast
Ovarian
Prostate
RPKM
E
DGAT1 expression (RNA seq)
DGAT2 expression (RNA seq)
ACC
AML
Bladder
Breast
Cervical
Cholangiocarcinoma
Colorectal
DLBC
GBM
Glioma
Head & neck
Liver
Lung adeno
Lung squ
Melanoma
Mesothelioma
ovarian
PCPG
Pancreas
Prostate
Sarcoma
Testicular germ cell
Thymoma
Thyroid
Uterine
Uterine CS
Uveal melanoma
ccRCC
chRCC
pRCC
F
TCGA Proneural
Low, N=31
High, N=14
P = 0.0235
TCGA Classical
Low, N=31
High, N=28
P = 0.018
TCGA Mesenchymal
Low, N=37
High, N=15
P = 0.0151
Survival Probability
Months
G
Rembrandt GBM total population
Low, N=99
High, N=82
P = 0.0094

Figure S1. DGAT1 is highly expressed in GBM and is inversely correlated with patient overall survival (Related to Figure 1)

(A) A representative Western blot (n = 2 blots in total) of DGAT1 expression in human normal brain, tumor tissues from individuals with GBM, and GBM cell lines that were infected with shControl or shDGAT1 lentivirus (48 hr). PDIA1 (protein disulfide isomerase family A, member 1), an ER-resident protein, was used as an internal control.

(B) Schematic diagram showing the primer design for detection of *DGAT1* and *DGAT2* gene expression using RT-qPCR.

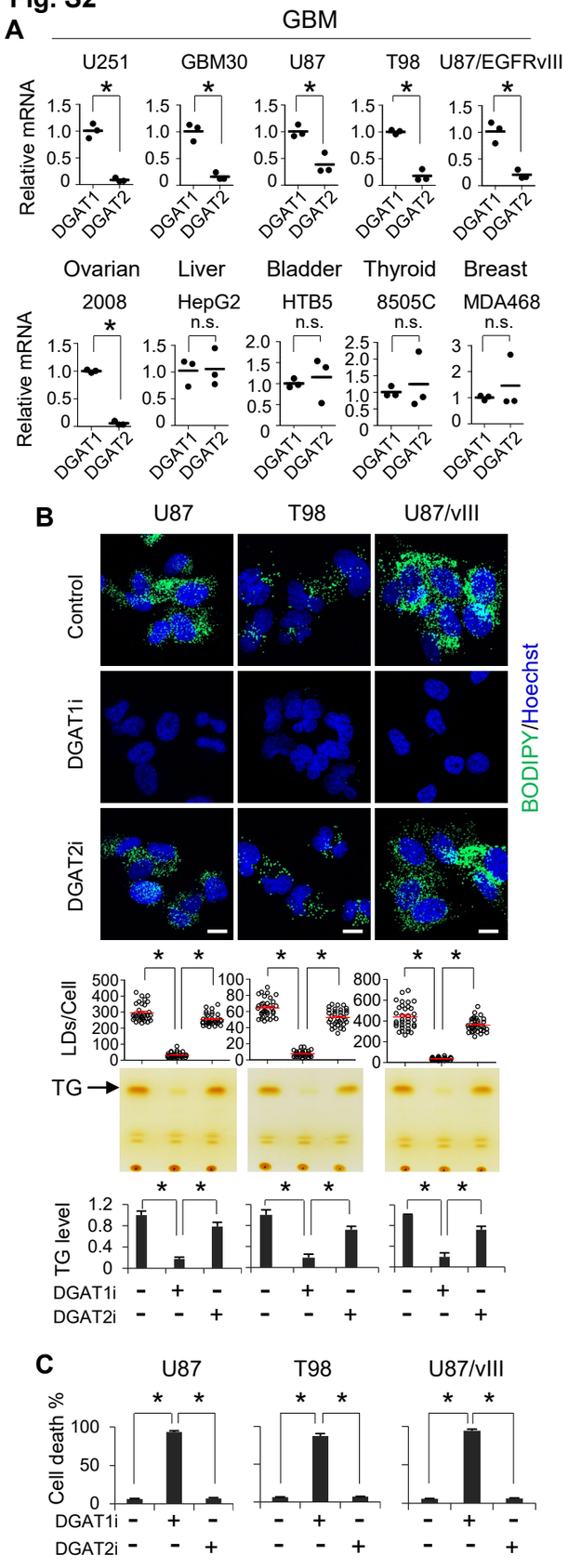
(C) RT-qPCR analysis of *DGAT1* vs. *DGAT2* mRNA levels in individuals with GBM (n = 10) using three separate primers as shown in panel B. The expression was normalized to the average DGAT1 expression. *$P < 0.001$

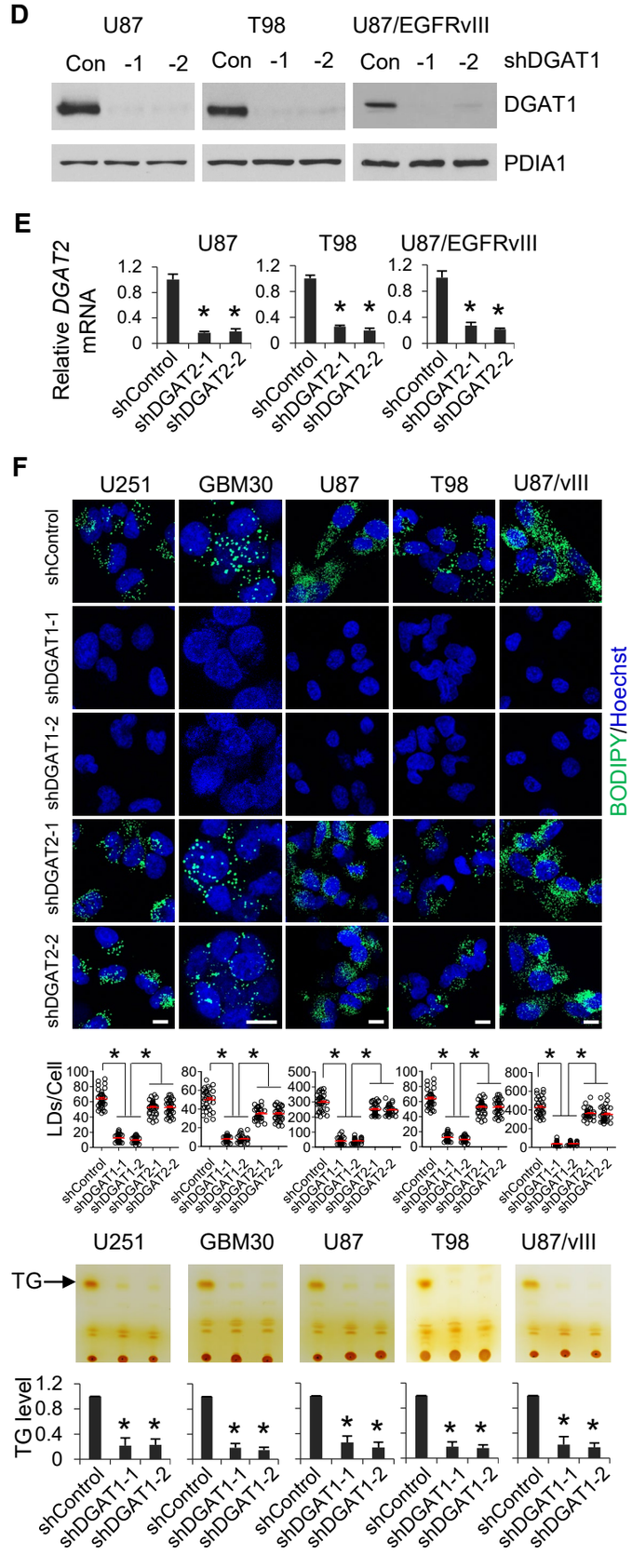
(D) Paired profiles of *DGAT1* vs. *DGAT2* gene expression in tumor tissues from individuals with GBM and other cancers in the TCGA database. TPM, transcripts per million; FPKM, fragments per kilobase million; RPKM, reads per kilobase million.

(E) *DGAT1* (top) vs. *DGAT2* (bottom) gene expression pattern in 30 cancer types in the TCGA database from cBioPortal.

(F and G) Kaplan-Meier plot of patient survival in GBM subtypes (F) or total patient population (G) based on *DGAT1* expression levels in the GBM TCGA RNAseq (F) or Rembrandt (G) databases using optimal cutoff. The cutoff is 9.711 for the proneural subtype, 9.436 for the classical and 9.471 for the mesenchymal subtypes. Rembrandt GBM cutoff is 8.109. Significance was analyzed by Log-rank test.

Fig. S2


A
GBM
U251
GBM30
U87
T98
U87/EGFRvIII
Relative mRNA
DGAT1
DGAT2
Ovarian
Liver
Bladder
Thyroid
Breast
2008
HepG2
HTB5
8505C
MDA468
n.s.
B
U87
T98
U87/vIII
Control
DGAT1i
DGAT2i
BODIPY/Hoechst
LDs/Cell
TG
TG level
DGAT1i
DGAT2i
C
Cell death %


D
U87
T98
U87/EGFRvIII
Con
-1
-2
shDGAT1
DGAT1
PDIA1
E
Relative DGAT2 mRNA
shControl
shDGAT2-1
shDGAT2-2
F
U251
GBM30
U87
T98
U87/vIII
shControl
shDGAT1-1
shDGAT1-2
shDGAT2-1
shDGAT2-2
BODIPY/Hoechst
LDs/Cell
TG
TG level

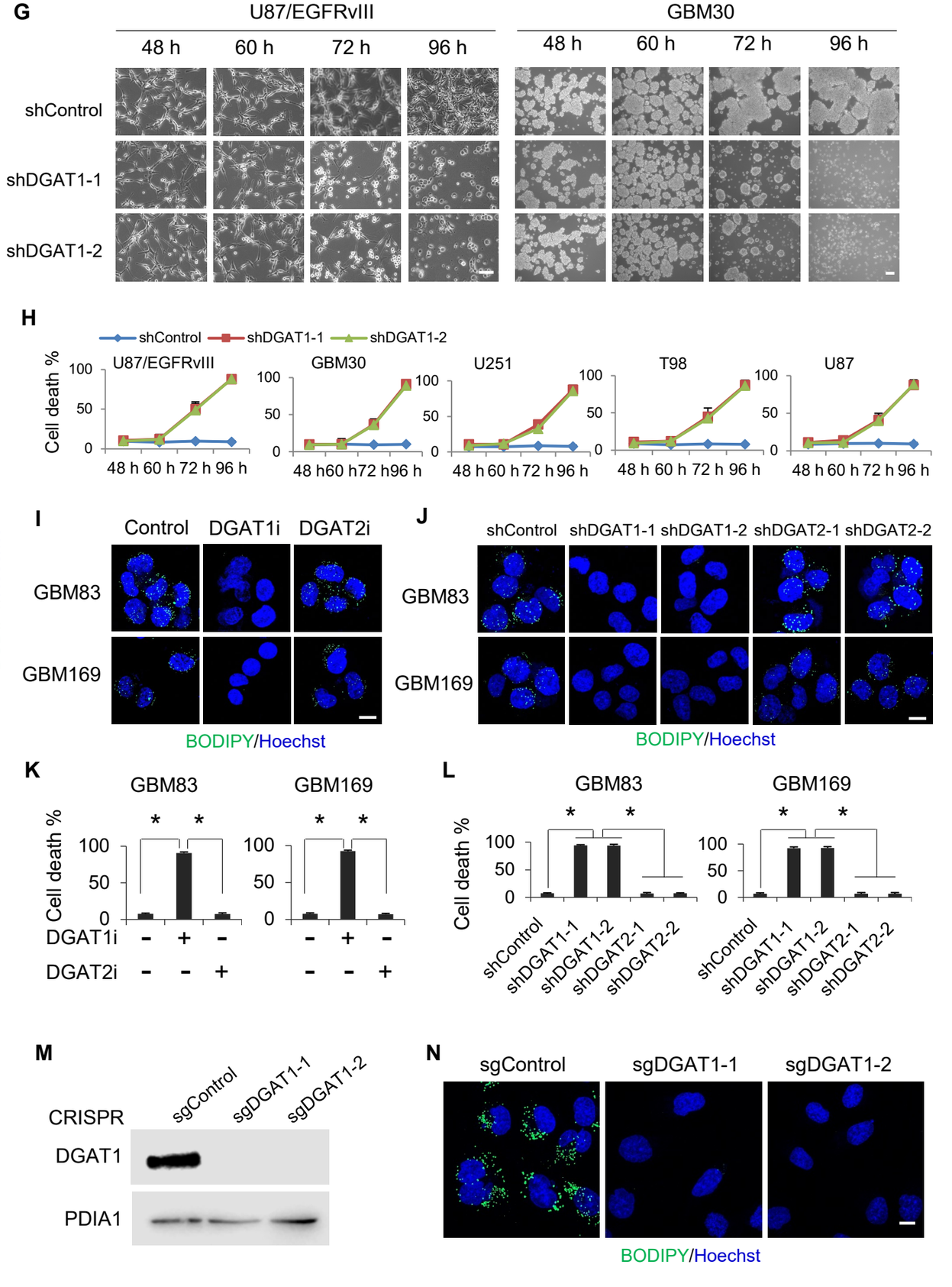

G
U87/EGFRvIII
GBM30
48 h
60 h
72 h
96 h
shControl
shDGAT1-1
shDGAT1-2
H
shControl
shDGAT1-1
shDGAT1-2
U87/EGFRvIII
GBM30
U251
T98
U87
Cell death %
I
Control
DGAT1i
DGAT2i
GBM83
GBM169
BODIPY/Hoechst
J
shControl
shDGAT1-1
shDGAT1-2
shDGAT2-1
shDGAT2-2
GBM83
GBM169
BODIPY/Hoechst
K
GBM83
GBM169
Cell death %
DGAT1i
DGAT2i
L
GBM83
GBM169
Cell death %
M
CRISPR
sgControl
sgDGAT1-1
sgDGAT1-2
DGAT1
PDIA1
N
sgControl
sgDGAT1-1
sgDGAT1-2
BODIPY/Hoechst

Figure S2. Inhibition of DGAT1, but not DGAT2, significantly suppresses TG and LD formation and induces GBM cell death (Related to Figure 2)

(A) RT-qPCR analysis of *DGAT1* vs. *DGAT2* mRNA levels in multiple types of cancer cell lines using 3 pairs of different primers showing in Figure S1B. The expression was normalized to the average DGAT1 expression. **P* < 0.01.

(B) Representative fluorescence imaging (*n* = 6 images in total) of LDs stained with BODIPY 493/503 (green) (upper panels) and TLC analysis (*n* = 3) of TG levels (lower panels) in GBM cells after treatment with DGAT1 inhibitor A-922500 (20 μg/ml) or DGAT2 inhibitor PF-06424439 (20 μg/ml) for 24 hr. Nuclei were stained with Hoechst 33342 (blue). Scale bar: 10 μm. LDs were quantified by the ImageJ software in more than 30 cells in each treatment group. TG levels were quantified by the ImageJ software and normalized to control group (mean ± SD, *n* = 3). **P* < 0.001; #*P* < 0.05.

(C) Percentage of dead GBM cells after treatment for 3 days with the DGAT1 or DGAT2 inhibitors as in panel B. **P* < 0.0001.

(D) A representative Western blot (*n* = 3 blots in total) of DGAT1 expression in GBM cancer cells after infection with shRNA lentivirus against DGAT1 for 48 hr.

(E) RT-qPCR analysis of *DGAT2* mRNA levels in GBM cells after infection with shDGAT2 lentivirus for 48 hr. The expression level was normalized to shControl and shown as mean ± SD (*n* = 3). **P* < 0.001.

(F) Representative fluorescence imaging (*n* = 6 images in total) of LDs stained with BODIPY 493/503 (green) (upper panels) and TLC analysis (*n* = 3) of TAG levels (lower panels) in GBM cells after infection with two independent clones of shDGAT1 or shDGAT2 lentivirus for 48 hr. Nuclei were stained with Hoechst 33342 (blue). * *P* < 0.0001. TLC analysis of TG levels in GBM cells infected with two independent clones of shDGAT1 lentivirus for 48 hr. **P* < 0.01.

(G and H) Percentage of dead GBM cells after infection with shDGAT1 lentivirus at 48, 60, 72, and 96 hr. The significance was determined by one-way ANOVA (mean ± SD, *n* = 3). **P* < 0.0001.

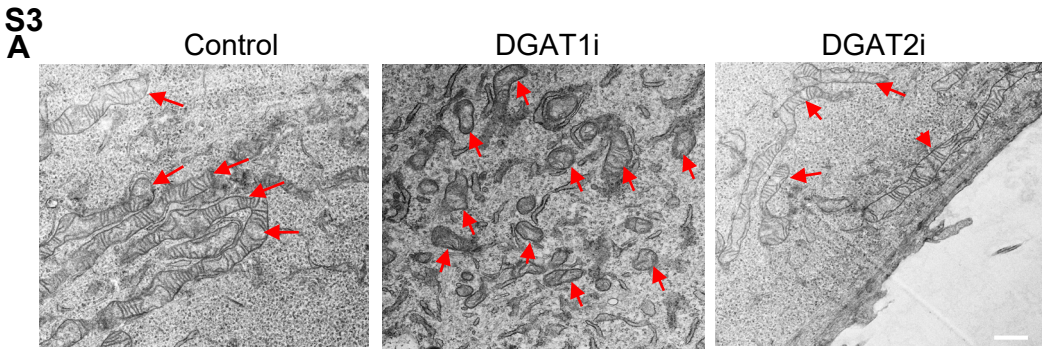
(I and J) Representative fluorescence imaging (*n* = 6 images in total) of LDs stained with BODIPY 493/503 (green) in primary GBM83 and GBM169 cells after treatment with DGAT1 or DGAT2 inhibitors as in panel B for 24 hr (I), or infected with two independent clones of shDGAT1 or shDGAT2 lentivirus for 48 hr (J). Nuclei were stained with Hoechst 33342 (blue). Scale bar, 10 μm.

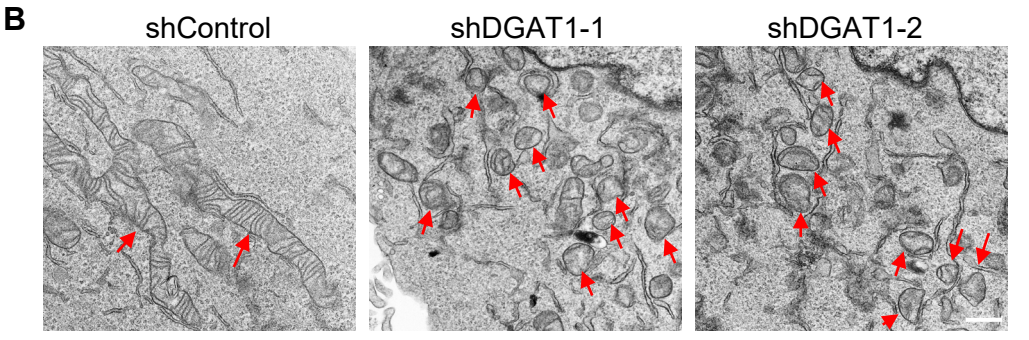
(K and L) Dead cell percentage primary GBM83 and GBM169 after treatment for 3 days with DGAT1 or DGAT2 inhibitors (K) or infection with shRNA lentivirus for 4 days (L). **P* < 0.0001.

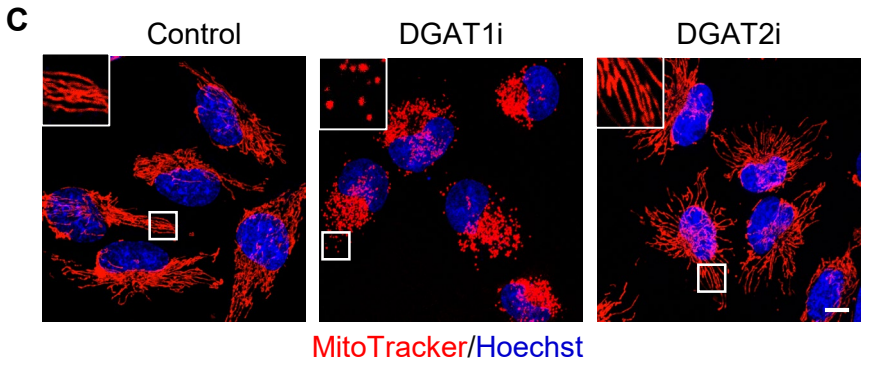
(M) A representative Western blot (*n* = 2 blots in total) of DGAT1 in U251 cells after infection with sgControl- or sgDGAT1 CRISPR lentivirus for 6 days.

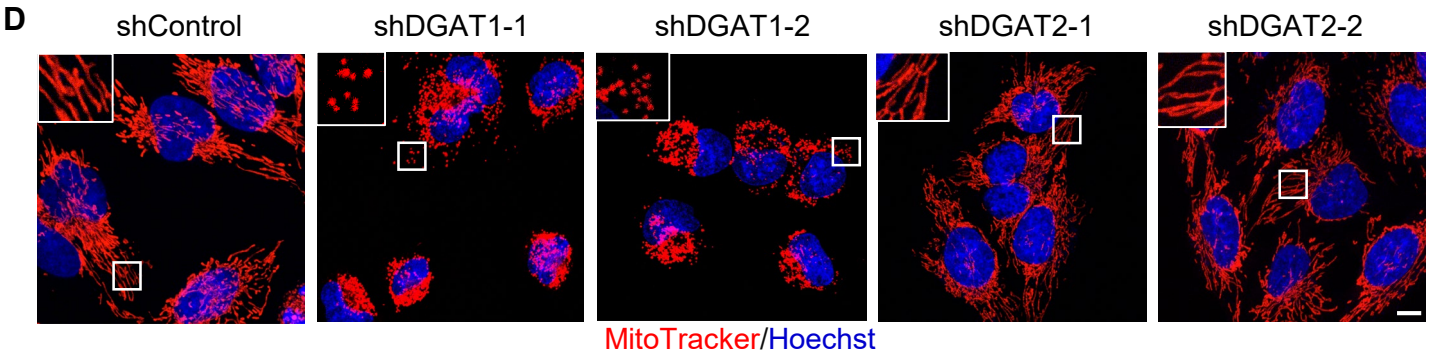
(N) Representative fluorescence imaging (*n* = 6 images in total) of LDs stained with BODIPY 493/503 (green) in U251 cells infected with sgControl or sgDGAT1 for 7 days. Nuclei were stained with Hoechst 33342 (blue). Scale bar, 10 μm.

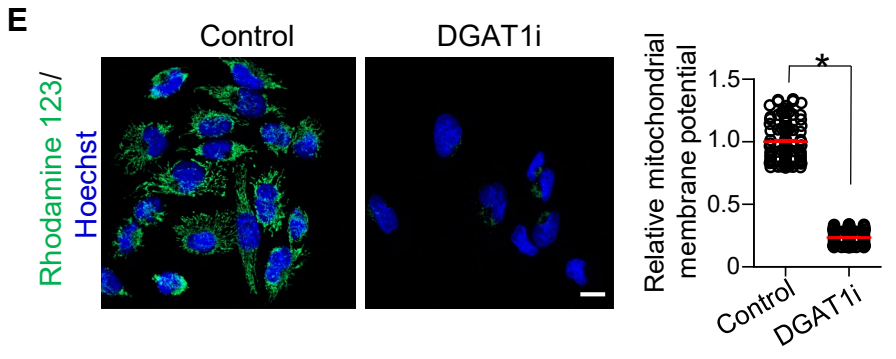
Fig. S3

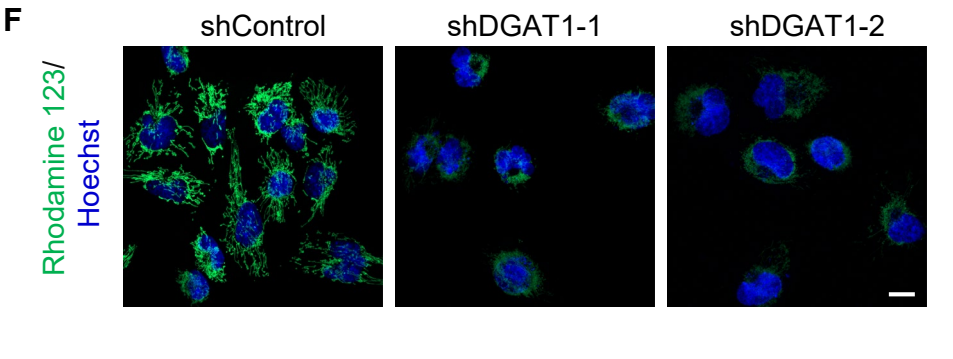

A
Control
DGAT1i
DGAT2i

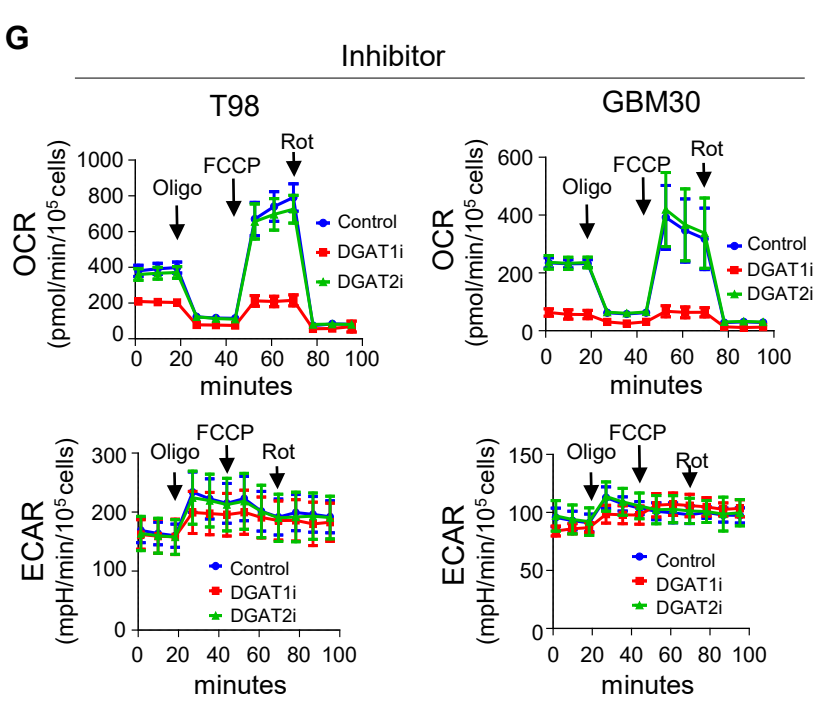

B
shControl
shDGAT1-1
shDGAT1-2

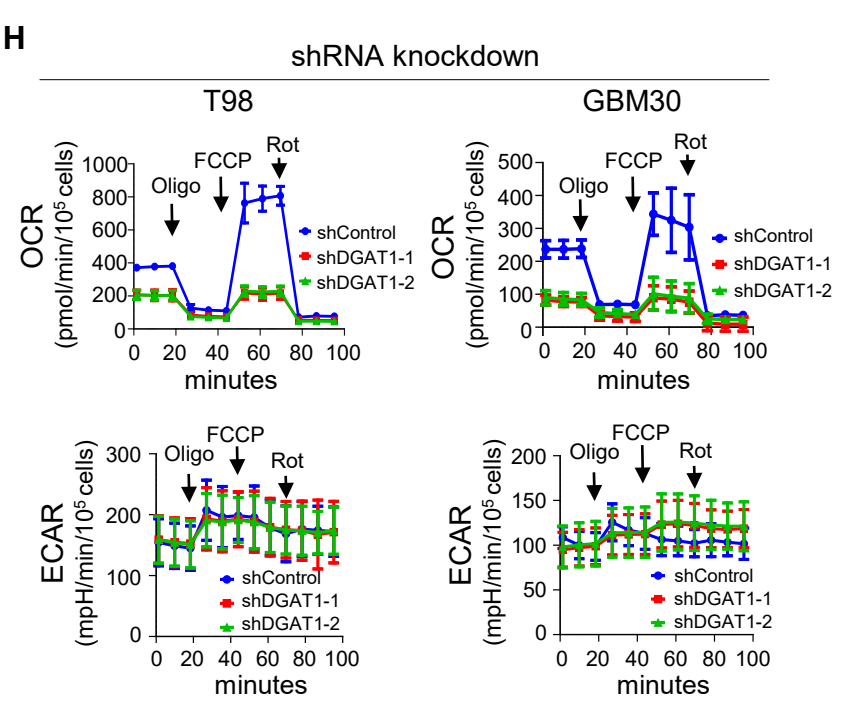

C
Control
DGAT1i
DGAT2i
MitoTracker/Hoechst

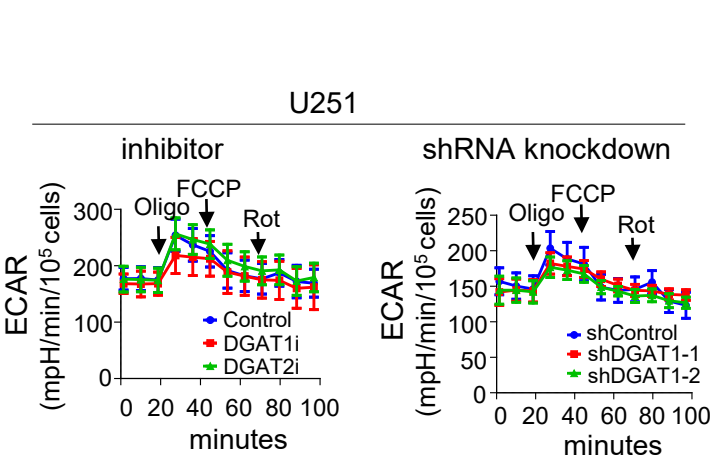

D
shControl
shDGAT1-1
shDGAT1-2
shDGAT2-1
shDGAT2-2
MitoTracker/Hoechst

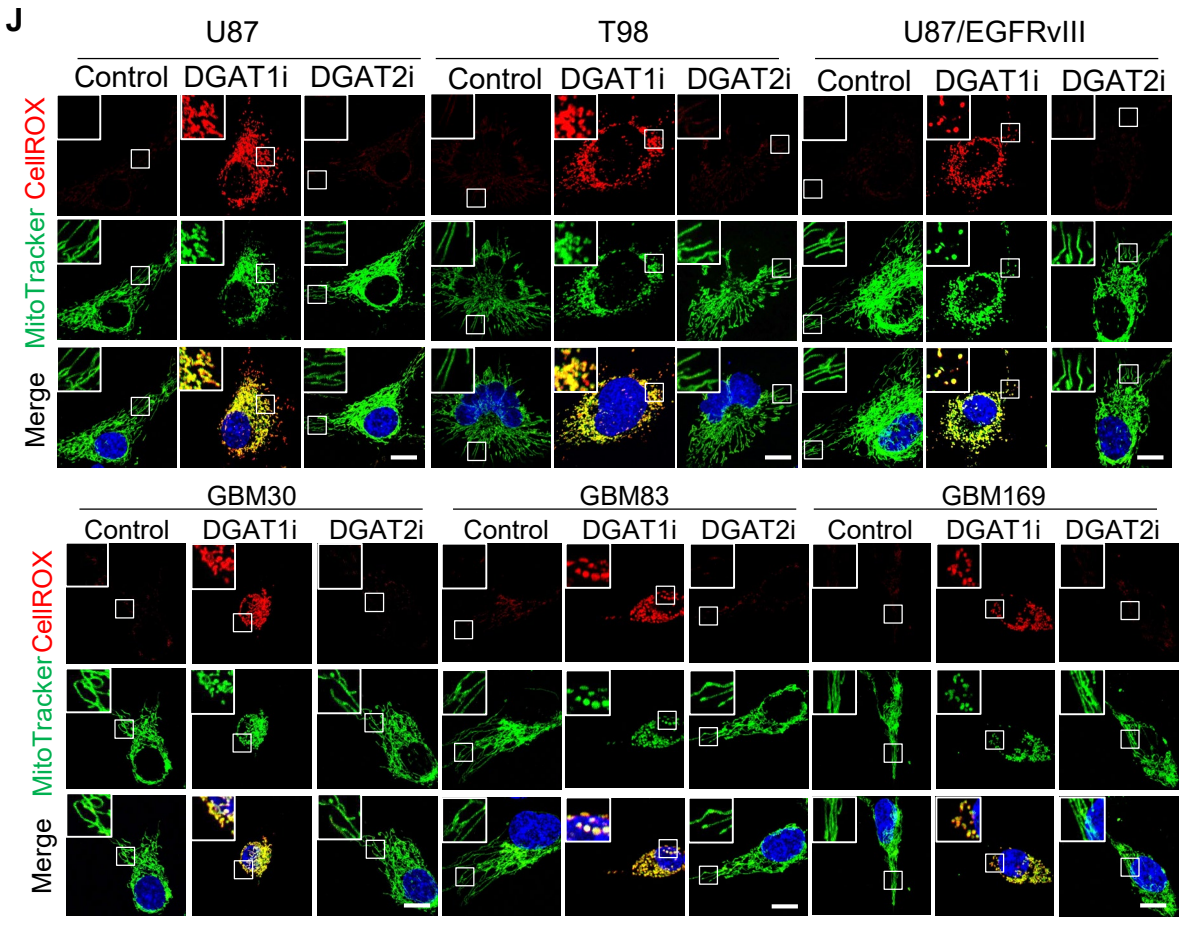

E
Control
DGAT1i
Rhodamine 123/
Hoechst
Relative mitochondrial membrane potential
1.5
1.0
0.5
0
*
Control
DGAT1i

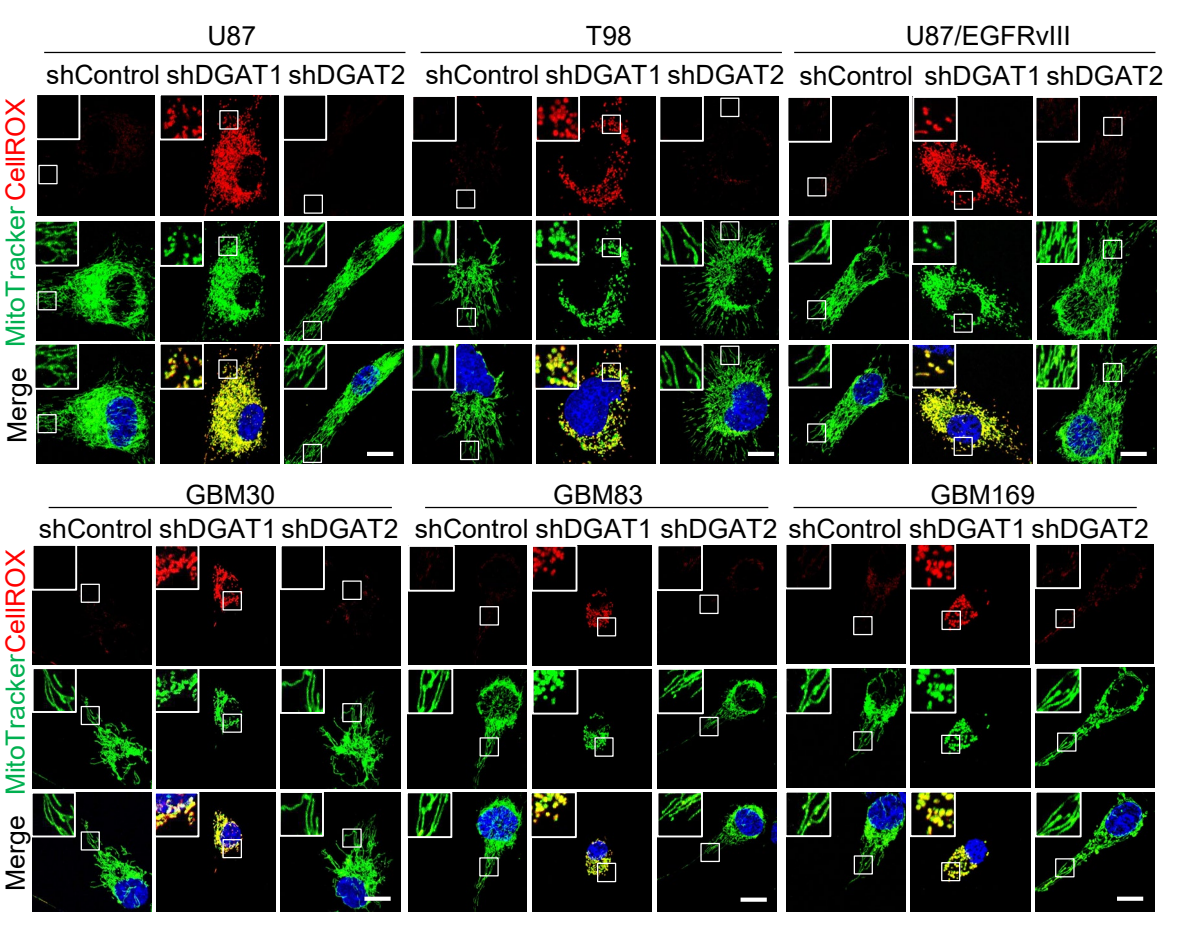

F
shControl
shDGAT1-1
shDGAT1-2
Rhodamine 123/
Hoechst
Relative mitochondrial membrane potential
1.5
1.0
0.5
0
*
*
shControl
shDGAT1-1
shDGAT1-2

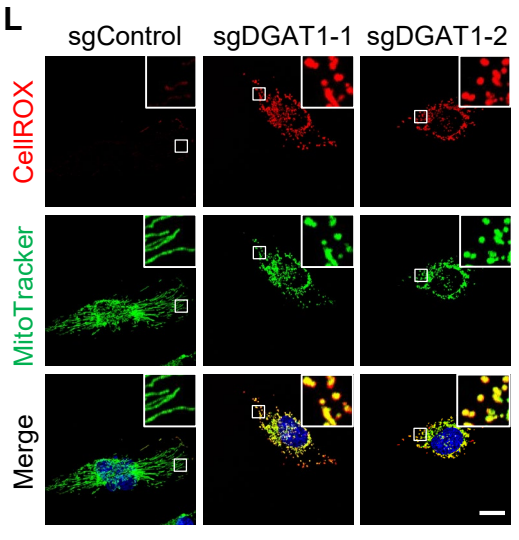

G
Inhibitor
T98
GBM30
OCR
(pmol/min/10^5 cells)
Oligo
FCCP
Rot
Control
DGAT1i
DGAT2i
minutes
ECAR
(mpH/min/10^5 cells)

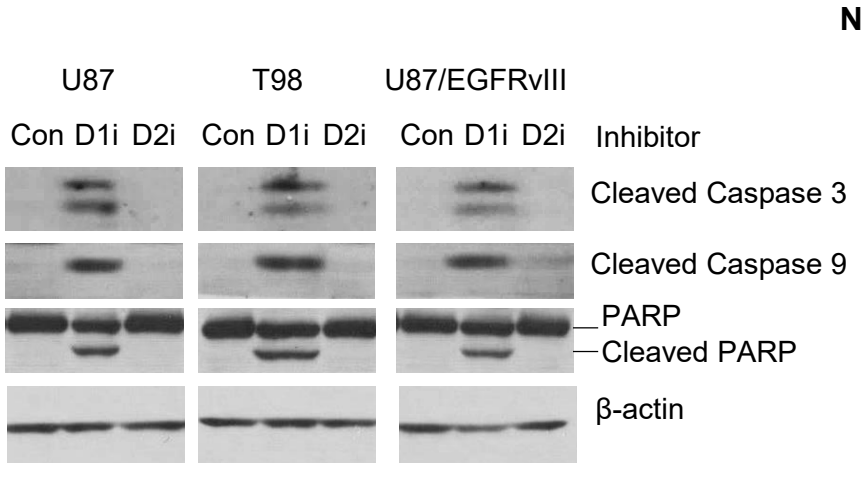

H
shRNA knockdown
T98
GBM30
OCR
(pmol/min/10^5 cells)
Oligo
FCCP
Rot
shControl
shDGAT1-1
shDGAT1-2
minutes
ECAR
(mpH/min/10^5 cells)


I
U251
inhibitor
shRNA knockdown
ECAR
(mpH/min/10^5 cells)
Oligo
FCCP
Rot
Control
DGAT1i
DGAT2i
shControl
shDGAT1-1
shDGAT1-2
minutes


J
U87
T98
U87/EGFRvIII
Control
DGAT1i
DGAT2i
CellROX
MitoTracker
Merge
GBM30
GBM83
GBM169


K
U87
T98
U87/EGFRvIII
shControl
shDGAT1
shDGAT2
CellROX
MitoTracker
Merge
GBM30
GBM83
GBM169


L
sgControl
sgDGAT1-1
sgDGAT1-2
CellROX
MitoTracker
Merge


M
U87
T98
U87/EGFRvIII
Con D1i D2i
Inhibitor
Cleaved Caspase 3
Cleaved Caspase 9
PARP
Cleaved PARP
β-actin

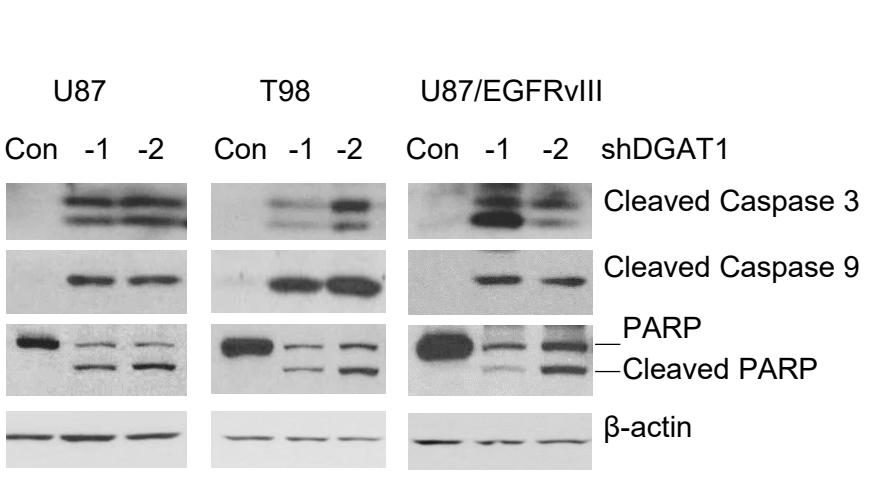

N
U87
T98
U87/EGFRvIII
Con -1 -2
shDGAT1
Cleaved Caspase 3
Cleaved Caspase 9
PARP
Cleaved PARP
β-actin

Figure S3. Inhibition of DGAT1 causes severe mitochondria damage, membrane potential reduction, high levels of ROS and apoptosis in GBM cells (Related to Figure 3)

(A and B) Transmission electron microscopy analysis (n = 20 images in total) of the mitochondrial structure in U251cells treated with DGAT1 inhibitor A-922500 (20 μg/ml) or DGAT2 inhibitor PF-06424439 (20 μg/ml) for 24 hr (A) or infected with shDGAT1 lentivirus for 48 hr (B). Scale bar, 500 nm. Mitochondria are indicated by red arrows.

(C and D) Representative fluorescence imaging (n = 6 images in total) of mitochondria (red) stained with MitoTracker Red in U251 cells treated with DGAT1 or DGAT2 inhibitors (C) or infected with shRNA lentivirus (D). Nuclei were stained with Hoechst 33342 (blue). Scale bar, 10 μm.

(E and F) Representative fluorescence image (n = 12 images in total) of mitochondrial membrane potential (green) stained with Rhodamine123 in U251 cells treated with DGAT1 inhibitor for 24 hr or infected with shDGAT1 lentivirus for 48 hr. Nuclei were stained with Hoechst 33342 (blue). Scale bar, 10 μm. Rhodamine123 staining was quantified by ImageJ software in more than 100 cells. *$P < 0.0001$.

(G-I) Analysis of mitochondrial activity by measuring oxygen consumption rate (OCR) and extracellular acidification rate (ECAR) in GBM cells treated with DGAT1 inhibitor or DGAT2 inhibitor as in panel A for 24 hr, or infected with shDGAT1 or shDGAT2 lentivirus for 48 hr. Data represent the mean ± SD from three independent experiments. Oligo, oligomycin; FCCP, carbonyl cyanide 4-trifluoromethoxy-phenylhydrazone; Rot, rotenone.

(J and K) Representative fluorescence imaging (n = 6 images in total) of ROS production (red) stained with CellROX Deep Red and mitochondria (green) stained with MitoTracker Green in GBM cells treated with DGAT1 inhibitor or DGAT2 inhibitor as in panel A for 24 hr (J) or infected with shDGAT1 or shDGAT2 lentivirus for 48 hr (K). Nuclei were stained with Hoechst 33342 (blue). Scale bar, 10 μm.

(L) Representative fluorescence imaging (n = 6 images in total) of ROS production (red) stained with CellROX Deep Red and mitochondria (green) stained with MitoTracker Green in U251 cells infected with sgControl or sgDGAT1 CRISPR lentivirus for 7 days. Nuclei were stained with Hoechst 33342 (blue). Scale bar, 10 μm.

(M and N) A Western blot (n = 3 blots in total) of apoptosis markers in multiple GBM cells treated with DGAT1 inhibitor or DGAT2 inhibitor as in panel A for 24 hr (M) or shDGAT1 lentivirus for 72 hr.

Fig. S4

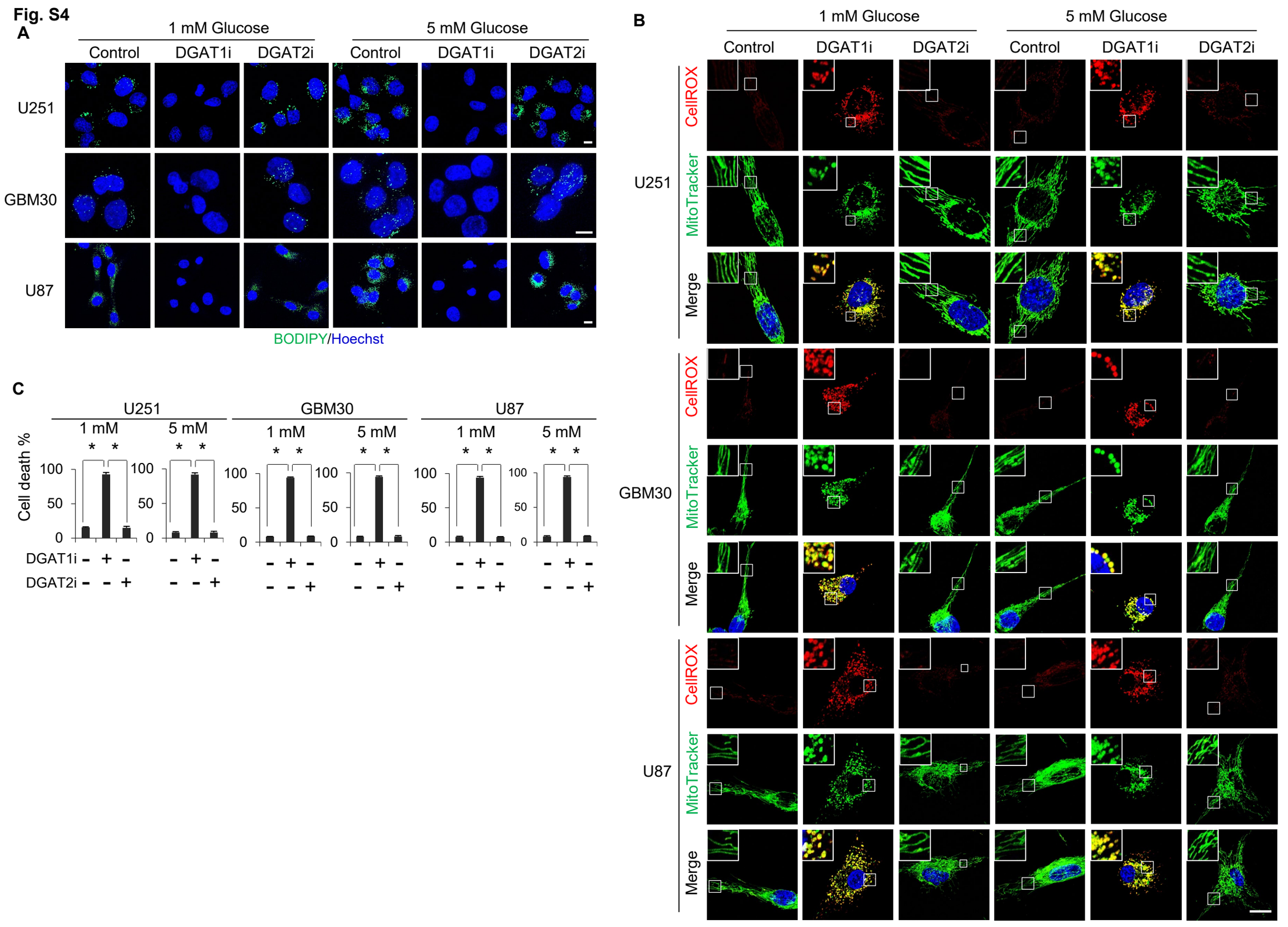

A
1 mM Glucose
5 mM Glucose
Control
DGAT1i
DGAT2i
U251
GBM30
U87
BODIPY/Hoechst
B
CellROX
MitoTracker
Merge
C
U251
GBM30
U87
1 mM
5 mM
Cell death %
DGAT1i
DGAT2i

Figure S4. DGAT1 inhibition blocks LD formation, induces high level of ROS, triggers mitochondria damage and GBM cell death under low glucose condition (Related to Figure 3)

(A) Representative fluorescence imaging (n = 6 images in total) of LDs stained with BODIPY 493/503 (green) in GBM cells after treatments with DGAT1 inhibitor A-922500 (20 μg/ml) or DGAT2 inhibitor PF-06424439 (20 μg/ml) in 1 mM or 5 mM glucose for 24 hr. Nuclei were stained with Hoechst 33342 (blue). Scale bar, 10 μm.

(B) Representative fluorescence imaging (n = 6 images in total) of ROS (red) detected by CellROX Deep Red and mitochondria (green) detected by MitoTracker Green in GBM cells treated with DGAT1 inhibitor or DGAT2 inhibitor as in panel A in 1 mM or 5 mM glucose. Nuclei were stained with Hoechst 33342 (blue). Scale bar, 10 μm.

(C) Percentage of dead GBM cells after treatment with DGAT1 inhibitor or DGAT2 inhibitor as in panel A in 1 mM or 5 mM glucose. *P < 0.0001.

Fig. S5

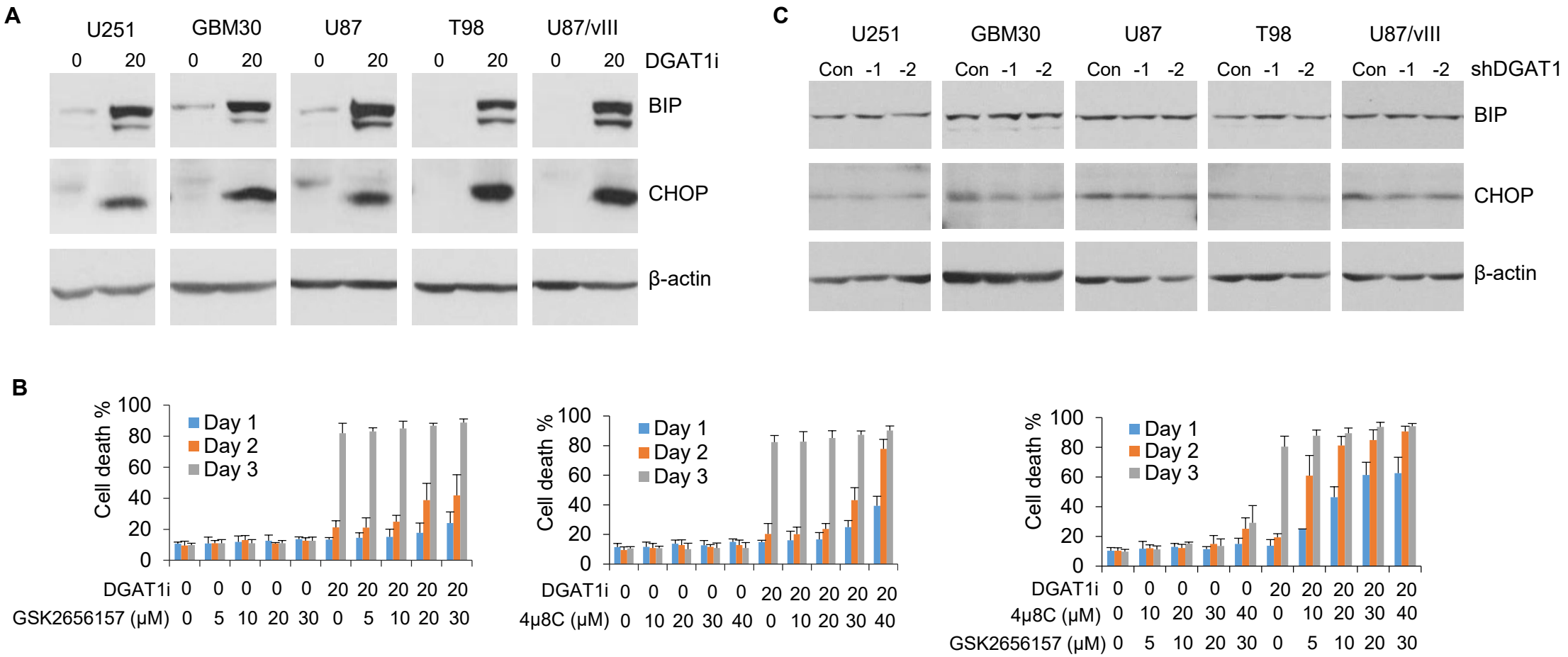


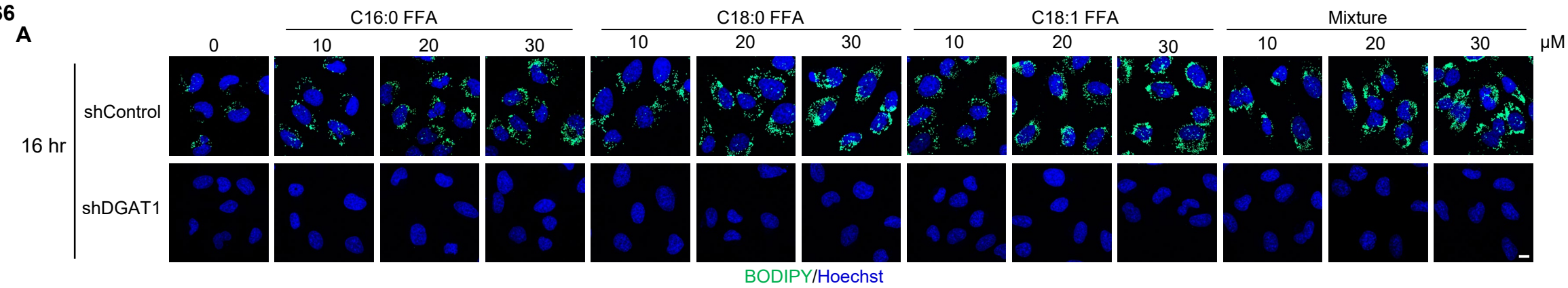
Figure S5. Pharmacological inhibition of DGAT1 induces ER stress (Related to Figure 3)

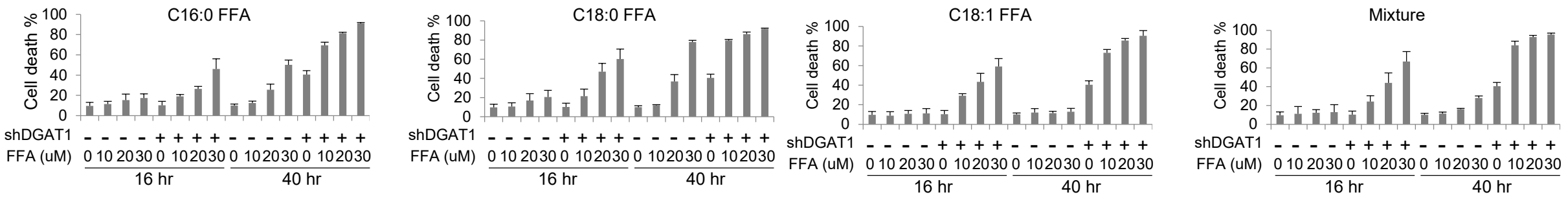
(A) A representative Western blot (n = 3 blots in total) of ER stress protein markers BIP and CHOP expression in GBM cells treated with the DGAT1 inhibitor A-922500 (20 μg/ml) for 24 hr.

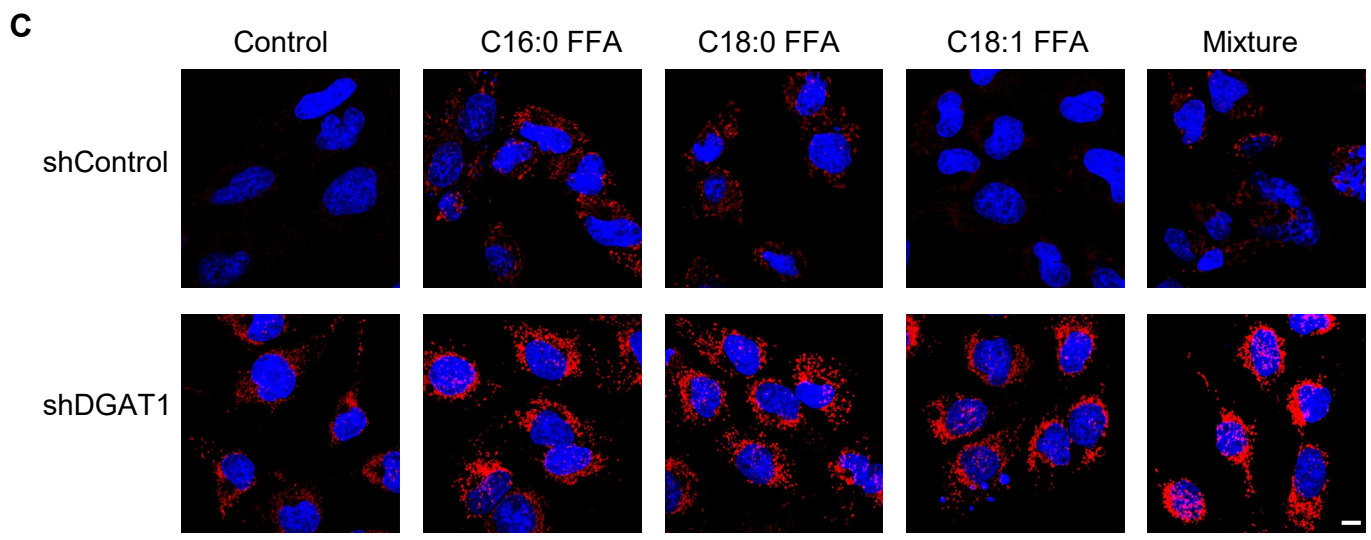
(B) Percentage of cell death in U251 cells treated with the ER stress pathway PERK inhibitor GSK2656157 or IRE1a inhibitor 4μ8C at indicated concentration, alone or in combination with the DGAT1 inhibitor A-922500 (20 μg/ml) for 3 days.

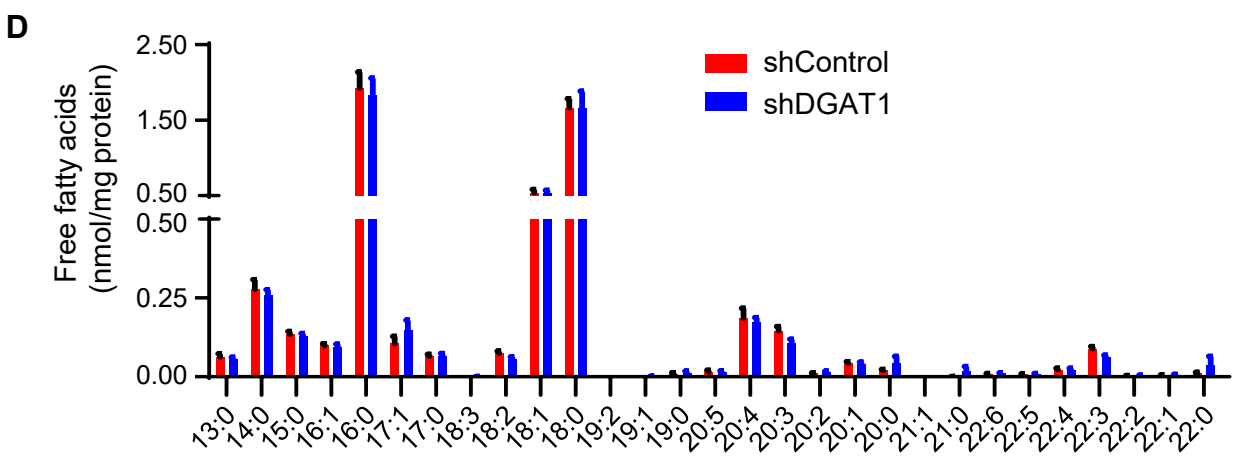
(C) A representative Western blot (n = 3 blots in total) of expression of ER stress protein markers BIP and CHOP in GBM cells infected with shDGAT1 lentivirus for 48 hr.

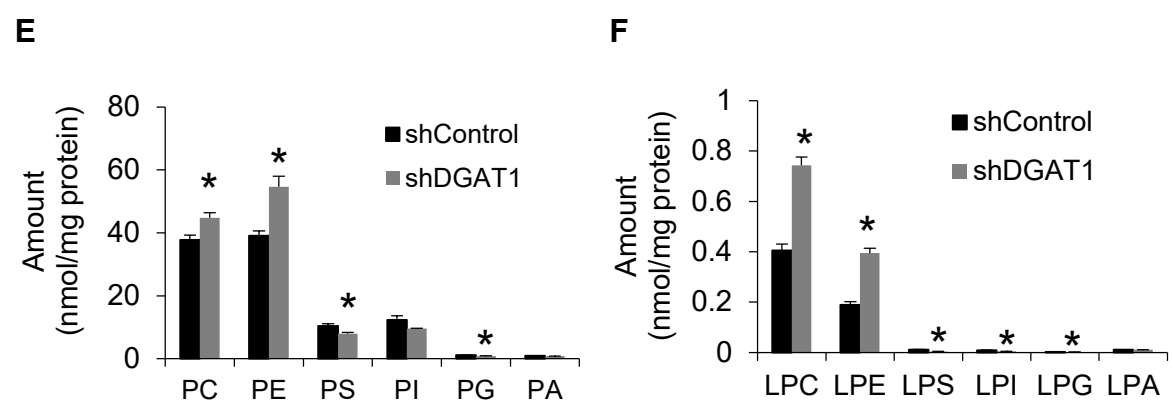
Fig. S6


A
C16:0 FFA
C18:0 FFA
C18:1 FFA
Mixture
0
10
20
30
μM
16 hr
shControl
shDGAT1
BODIPY/Hoechst
B
40 hr


Cell death %
shDGAT1
FFA (uM)
16 hr
40 hr


C
Control
C16:0 FFA
C18:0 FFA
C18:1 FFA
Mixture
shControl
shDGAT1
CellROX/Hoechst


D
Free fatty acids (nmol/mg protein)
shControl
shDGAT1


E
F
Amount (nmol/mg protein)
PC
PE
PS
PI
PG
PA
LPC
LPE
LPS
LPI
LPG
LPA

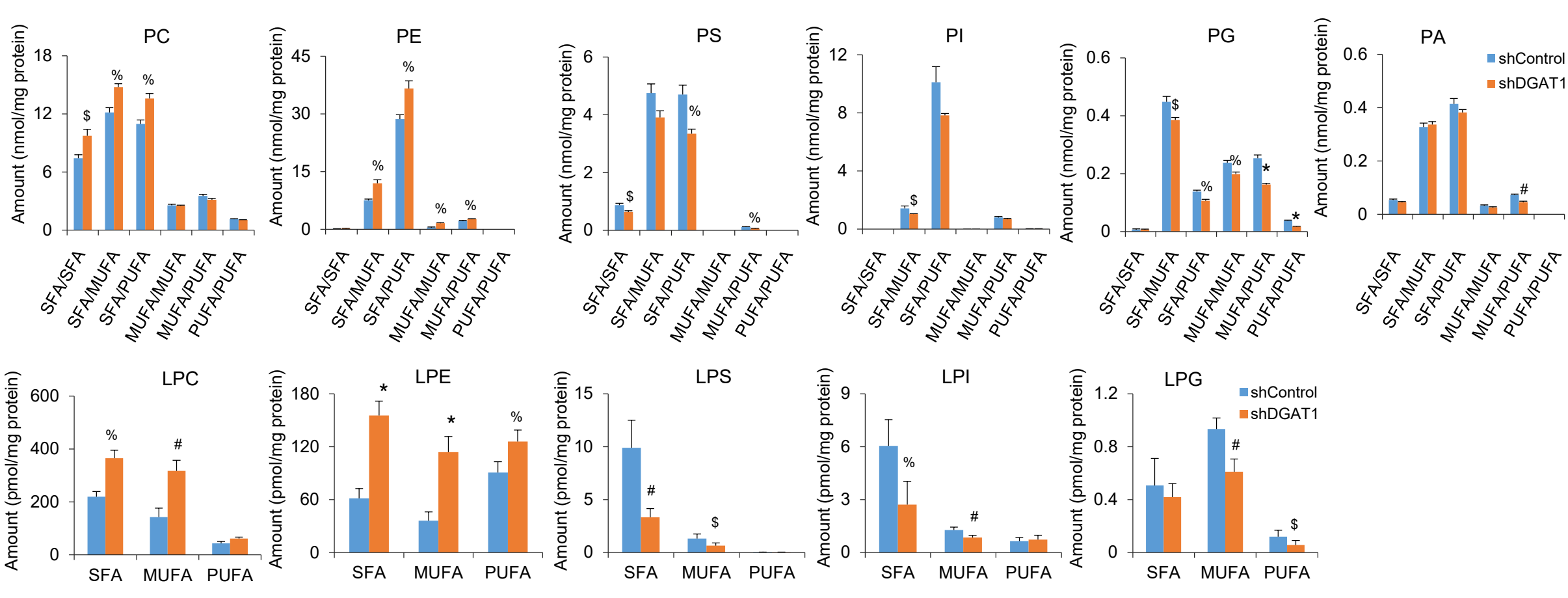

G
H
Amount (nmol/mg protein)
Amount (pmol/mg protein)
SFA/SFA
SFA/MUFA
SFA/PUFA
MUFA/MUFA
MUFA/PUFA
PUFA/PUFA
SFA
MUFA
PUFA
shControl
shDGAT1

Figure S6. Blocking the storage of free fatty acids upon DGAT1 inhibition disrupts lipid homeostasis and triggers marked cytotoxicity in GBM cells (Related to Figure 4)

(A) Representative fluorescence imaging (n = 6 images in total) of LDs in U251 cells cultured in charcoal-stripped FBS media supplemented with the indicated free fatty acids alone or in mixture for 16 hr after DGAT1 shRNA knockdown for 32 hr. Nuclei were stained with Hoechst 33342 (blue). Scale bar, 10 μm.

(B) Representative cell morphology (n = 6 images in total) and percentage of cell death in U251 cells cultured in charcoal-stripped FBS media supplemented with the indicated free fatty acids alone or in mixture for 16 hr and 40 hr after DGAT1 shRNA knockdown for 32 hr. Scale bar, 50 μm.

(C) Representative fluorescence imaging (n = 6 images in total) of ROS stained by CellROX Deep Red in U251 cells cultured in charcoal-stripped FBS media supplemented with the indicated free fatty acids alone or in mixture for 16 hr after DGAT1 shRNA knockdown for 32 hr. Nuclei were stained with Hoechst 33342 (blue). Scale bar, 10 μm.

(D) The profiling of free fatty acids in U251 cells after shRNA knockdown (60 hr) of shDGAT1 vs. shControl cells (n = 5).

(E and F) The levels of different phospholipids (E) or lysophospholipids (F) (n = 5) in U251 cells with shDGAT1 knockdown in comparison with shControl cells. Statistical significance was determined by Student's t-test. *$P < 0.05$. Phosphatidylcholine (PC), Phosphatidylethanolamine (PE), Phosphatidylserine (PS), Phosphatidylinositol (PI), Phosphatidylglycerol (PG), Phosphatidic acid (PA), Lysophosphatidylcholine (LPC), Lysophosphatidylethanolamine (LPE), Lysophosphatidylserine (LPS), Lysophosphatidylinositol (LPI), Lysophosphatidylglycerol (LPG), Lysophosphatidic acid (LPA).

(G and H) Comparison of the components of fatty acids with saturated and unsaturated species in membrane structural lipids, i.e., glycerolphospholipids (PS, PG, PI, PC, PE and PA) and lysophospholipids (LPS, LPG, LPI, LPC and LPE) (n = 5) in U251 cells after shRNA knockdown of DGAT1 vs. control cells. SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acids. *$P < 0.0001$; #$P < 0.001$; %$P < 0.01$; $$P < 0.05$.

Fig. S7

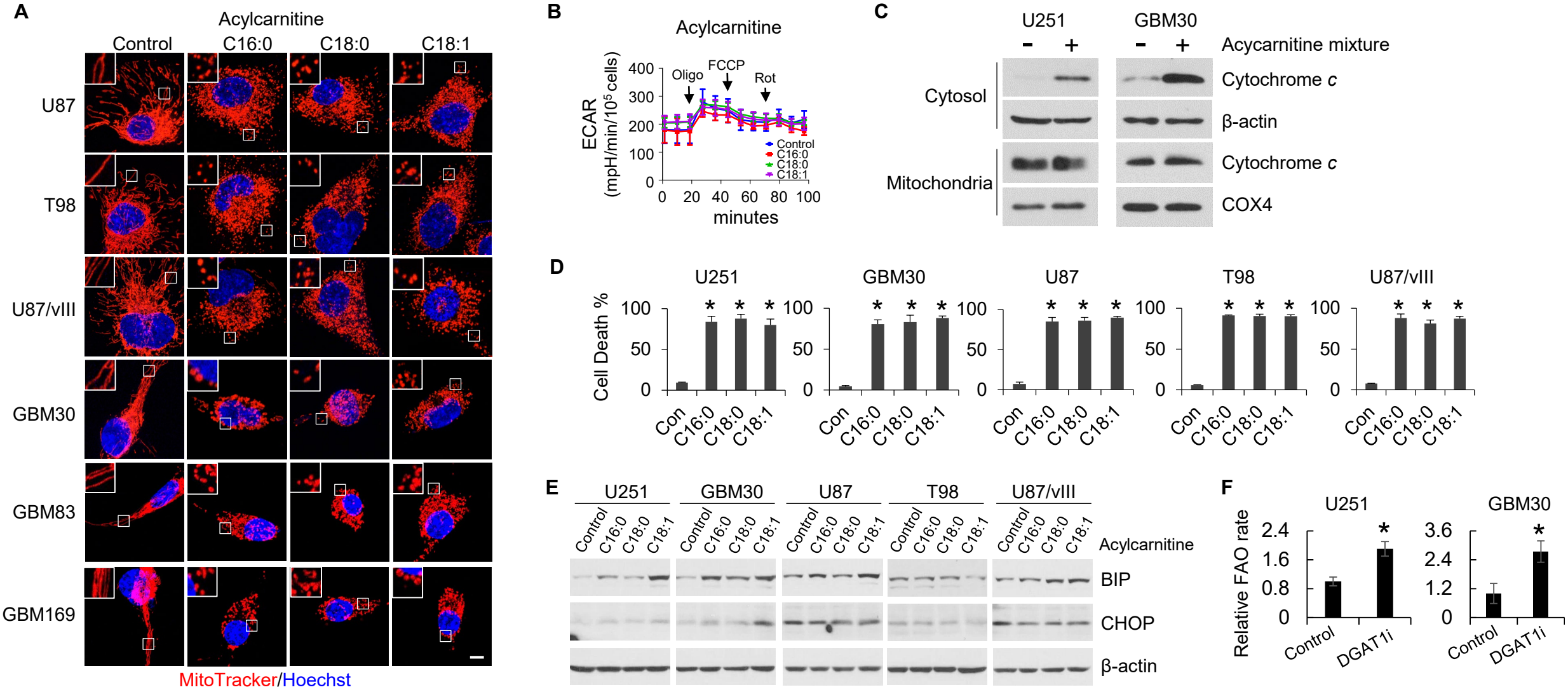

A
Acylcarnitine
Control
C16:0
C18:0
C18:1
U87
T98
U87/vIII
GBM30
GBM83
GBM169
MitoTracker/Hoechst
B
Acylcarnitine
ECAR (mpH/min/10^5 cells)
Oligo
FCCP
Rot
Control
C16:0
C18:0
C18:1
minutes
C
U251
GBM30
Acycarnitine mixture
Cytosol
Mitochondria
Cytochrome c
β-actin
Cytochrome c
COX4
D
U251
GBM30
U87
T98
U87/vIII
Cell Death %
Con
C16:0
C18:0
C18:1
E
U251
GBM30
U87
T98
U87/vIII
Control
C16:0
C18:0
C18:1
Acylcarnitine
BIP
CHOP
β-actin
F
U251
GBM30
Relative FAO rate
Control
DGAT1i

Figure S7. Supplementing GBM cells with acylcarnitines results in mitochondria damage and marked GBM cells death, but had only minor effects on ER stress (Related to Figure 5)

(A) Representative fluorescence imaging (n = 6 images in total) of mitochondria (red) stained with MitoTracker Red in GBM cells treated with different acylcarnitines (20 μM) for 24 hr. Nuclei were stained with Hoechst 33342 (blue). Scale bar, 10 μm.

(B) Analysis of ECAR (n = 3) by Seahorse in U251 cells treated with different acylcarnitines (20 μM) for 24 hr. Oligo, oligomycin; FCCP, carbonyl cyanide 4-trifluoromethoxy-phenylhydrazone; Rot, rotenone.

(C) A representative Western blot (n = 3 blots in total) of cytochrome c levels in the cytosol and mitochondria in GBM cells treated with an acylcarnitine mixture (C16:1-, C18:0- and C18:1-carnitines; 20 μM) for 24 hr.

(D) Percentage of dead GBM cells (n = 3) after supplementation with C16:1-, C18:0-, or C18:1-carnitine (20 μM) for 48 hr. *$P < 0.0001$.

(E) A representative Western blot (n = 3) of ER stress protein markers BIP and CHOP expression in GBM cells supplemented with different acylcarnitines as in panel A for 24 hr.

(F) Relative fatty acid β-oxidation (FAO) rate (n = 3) in GBM cells treated with control or DGAT1 inhibitor for 24 hr. *$P < 0.01$.

Fig. S8

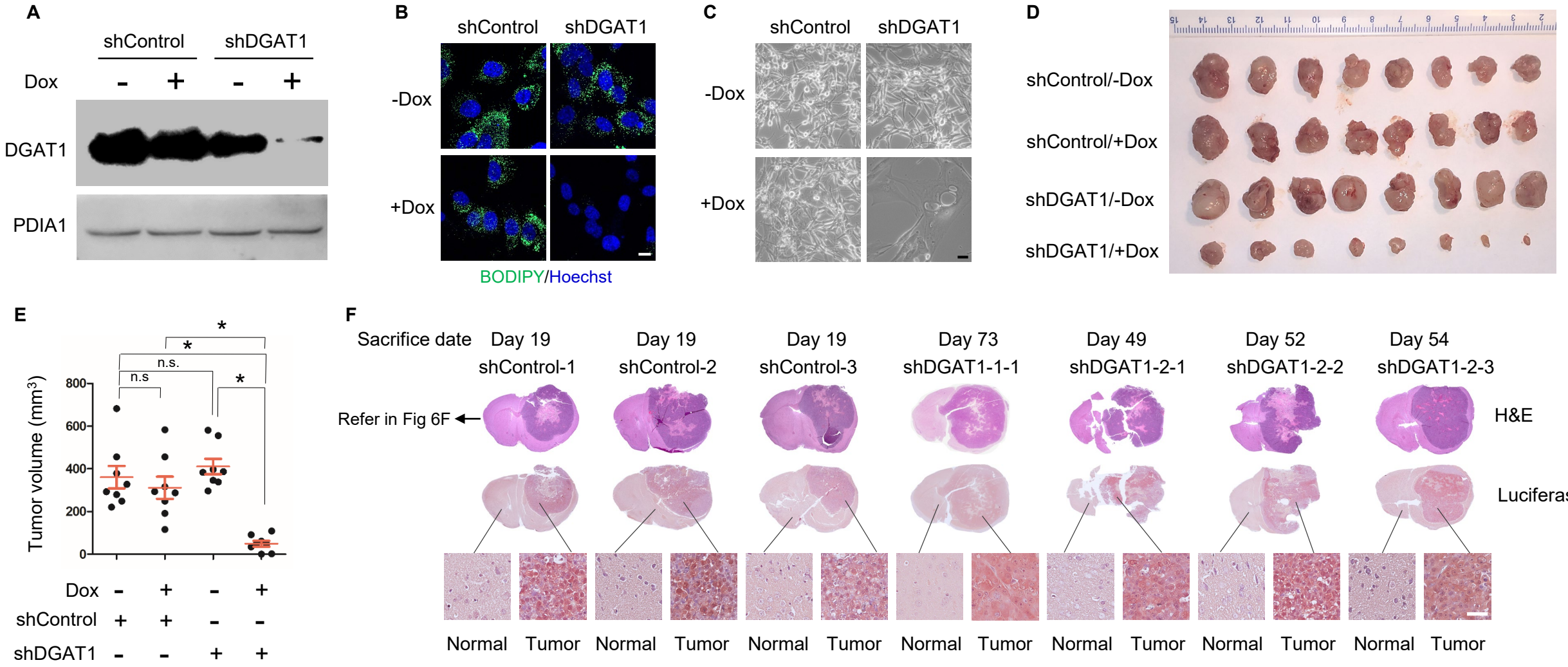

A
shControl
shDGAT1
Dox
DGAT1
PDIA1
B
shControl
shDGAT1
-Dox
+Dox
BODIPY/Hoechst
C
shControl
shDGAT1
-Dox
+Dox
D
shControl/-Dox
shControl/+Dox
shDGAT1/-Dox
shDGAT1/+Dox
E
Tumor volume (mm3)
n.s
n.s.
*
*
*
Dox
shControl
shDGAT1
F
Sacrifice date
Day 19
shControl-1
Day 19
shControl-2
Day 19
shControl-3
Day 73
shDGAT1-1-1
Day 49
shDGAT1-2-1
Day 52
shDGAT1-2-2
Day 54
shDGAT1-2-3
Refer in Fig 6F
H&E
Luciferase
Normal
Tumor

Figure S8. Doxycycline-inducible shRNA knockdown of DGAT1 significantly inhibits GBM growth *in vitro* and *in vivo (*Related to Figure 6)

(A) A representative Western blot ($n = 3$) of knockdown efficiency of inducible shDGAT1 in U87/EGFRvIII cells after treatment with doxycycline (Dox, 5 μg/mL) for 4 days.

(B) Representative fluorescence imaging ($n = 6$ images in total) of LDs stained with BODIPY 493/503 (green) in U87/EGFRvIII Dox-inducible shControl or shDGAT1cells after treatment with Dox (5 μg/mL) for 7 days. Nuclei were stained with Hoechst 33342 (blue). Scale bar, 10 μm.

(C) Representative cell morphology images ($n = 6$ images in total) of U87/EGFRvIII Dox-inducible shControl or shDGAT1cells after treatment with Dox (5 μg/mL) for 7 days. Scale bar, 100 μm.

(D and E) Dox-inducible knockdown of DGAT1 dramatically suppressed tumor growth in U87/EGFRvIII cells-derived subcutaneous model as compared with control group ($n = 8$). Tumors were excised from mice at day 18 post implantation. $*P < 0.001$.

(F) H&E and luciferase staining ($n = 5$) of whole brain sections from GBM intracranial xenograft mice. Gross image of the H&E staining and luciferase staining of mouse brains from GBM30-luciferase intracranial xenografts with shControl or shDGAT1 knockdown after implantation. Mouse brains were stained for luciferase by IHC. The image of H&E staining of shControl-1 is used as the representative shControl in Figure 6F. Scale bar, 50 μm.

Fig. S9

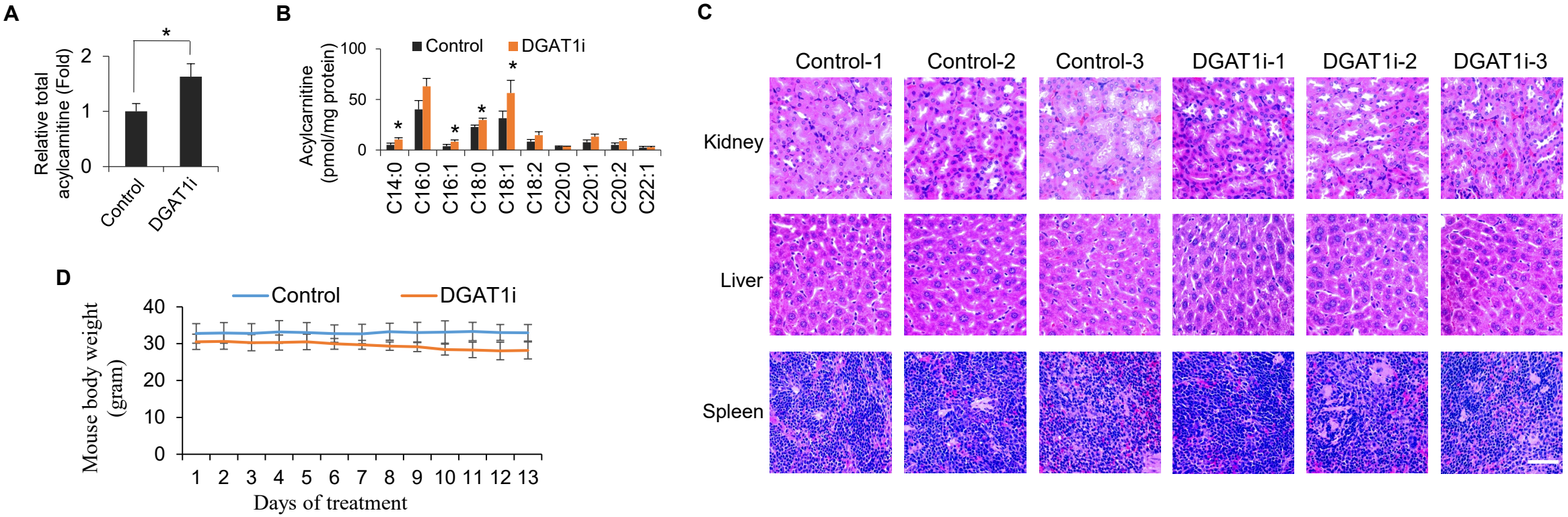


Figure S9. No obvious toxic effects in kidney, liver and spleen or weight loss are observed in mice treated with DGAT1 inhibitor A-922500 (Related to Figure 7)

(A and B) The relative total acylcarnitine levels in tumor tissues (n = 3) from the GBM30 xenograft model treated with the DGAT1 inhibitor A-922500 (120 mg/kg/day, oral gavage) for 13 days as compared with vehicle treatment group (control) (A). The levels of different types of acylcarnitine are shown in B. *$P < 0.05$.

(C) The H&E staining (n = 5) of the liver, kidney and spleen of mice treated with the vehicle (Control) or DGAT1 inhibitor A-922500 for 13 days. Scale bar, 50 μm.

(D) Body weight of mice (n = 6) treated with vehicle (Control) or DGAT1 inhibitor A-922500 (120 mg/kg/day, oral gavage) for 13 days.

Table S1. Primers for RT-qPCR (Related to Figures 1, 2, S1 and S2).

Gene	Primer name	Sequence
DGAT1	DGAT1-1F	5'-CCTACCGCGATCTCTACTACTT-3'
DGAT1	DGAT1-1R	5'-GGGTGAAGAACAGCATCTCAA-3'
DGAT1	DGAT1-2F	5'-CATGGACTACTCACGCATCAT-3'
DGAT1	DGAT1-2R	5'-GTGGAAGAGCCAGTAGAAGAAG-3'
DGAT1	DGAT1-3F	5'-CATGGACTACTCACGCATCAT-3'
DGAT1	DGAT1-3R	5'-GTGGAAGAGCCAGTAGAAGAAG-3'
DGAT2	DGAT2-1F	5'-GCTGACCACCAGGAACTATATC-3'
DGAT2	DGAT2-1R	5'-GGGAACTTCTTGCTCACTTCT-3'
DGAT2	DGAT2-2F	5'-CTGTTCTAGGTGGTGGCTAAAT-3'
DGAT2	DGAT2-2R	5'-CACTTCAGGAAGGGAAGAAGAG-3'
DGAT2	DGAT2-3F	5'-AACTGCAGGACCAGTTTCTC-3'
DGAT2	DGAT2-3R	5'-GAGCATTCCAGATGCCTACTAC-3'
36B4	36B4-F	5'-AATGGCAGCATCTACAACCC-3'
36B4	36B4-R	5'-TCGTTTGTACCCGTTGATGA-3'

Table S2. Synthesized oligos for constructing inducible shRNA plasmids (Related to STAR Methods and Figure S8).

Oligo name	Sequence
shDGAT1-top	5’-CCGGTGTGCTACGAGCTCAACTTTCCTCGAGGAAAGTTGAGCTCGTAGCACATTTTT-3’
shDGAT1-bottom	5’-AATTAAAAATGTGCTACGAGCTCAACTTTCCTCGAGGAAAGTTGAGCTCGTAGCACA-3’
shControl-top	5’-CCGGCAACAAGATGAAGAGCACCAACTCGAGTTGGTGCTCTTCATCTTGTTGTTTTT-3’
shControl-bottom	5’-AATTAAAAACAACAAGATGAAGAGCACCAACTCGAGTTGGTGCTCTTCATCTTGTTG-3'