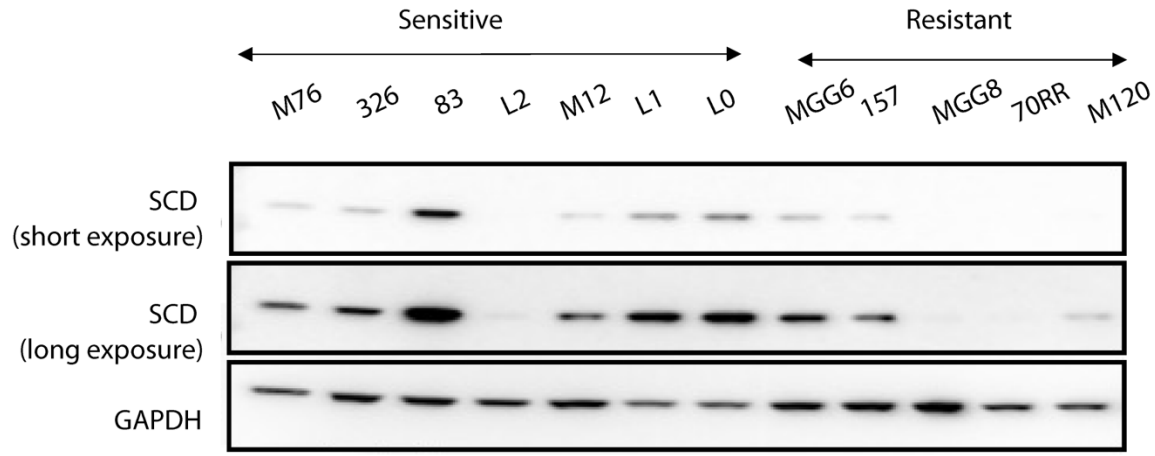
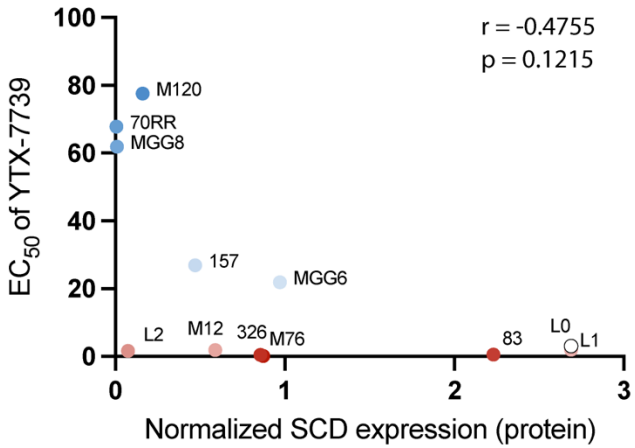
Supplementary Materials

Divergent MEK/ERK and AMPK signaling dictate lipogenic plasticity and dependence on fatty acid synthesis in Glioblastoma

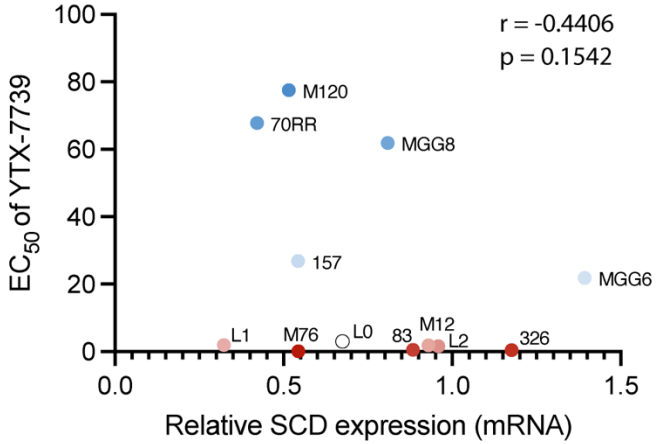
Katharina M. Eyme[1,2#], Alessandro Sammarco[1,3,4#], Roshani Jha[1], Hayk Mnatsakanyan[1], Caline Pechdimaljian[1], Litia Carvalho[1,8], Rudolph Neustadt[1], Charlotte Moses[1], Ahmad Alnasser[1], Daniel Tardiff[5], Baolong Su[6,7], Kevin J Williams[6,7], Steven J. Bensinger[4,7], Chee Yeun Chung[5], Christian E. Badr[1,8*]

A


Sensitive
Resistant
M76
326
83
L2
M12
L1
L0
MGG6
157
MGG8
70RR
M120
SCD
(short exposure)
SCD
(long exposure)
GAPDH


r = -0.4755
p = 0.1215
EC50 of YTX-7739
Normalized SCD expression (protein)
M120
70RR
MGG8
157
MGG6
L2
M12
326
M76
83
L0
L1

B


r = -0.4406
p = 0.1542
EC50 of YTX-7739
Relative SCD expression (mRNA)
M120
70RR
MGG8
157
MGG6
L1
M76
L0
83
M12
L2
326

C

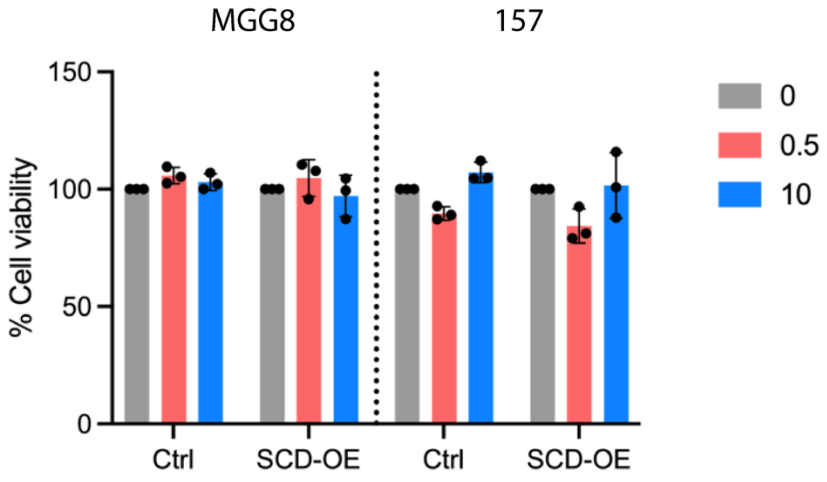

MGG8
157
% Cell viability
Ctrl
SCD-OE
Ctrl
SCD-OE
0
0.5
10

Figure S1 (A) Immunoblot analysis of SCD expression in YTX-7739-sensitive and -resistant GSCs lines. Also shown is the correlation analysis between the EC_{50} of YTX-7739 and SCD expression in all 12 GSCs lines. Western-blot bands of SCD normalized to GAPDH were quantified using Image J software. Spearman nonparametric correlation r=-0.4755; p=0.1215. (B) Correlation analysis between the EC_{50} of YTX-7739 and relative mRNA expression of SCD determined by qPCR. The normalized mRNA expression of SCD in GSCs was expressed relative to that of NHA, with the latter set to 1. Spearman nonparametric correlation r=-0.4406; p=0.1215. (C) Cell viability of GSCs ectopically expressing a control vector (Ctrl) or SCD1 (SCD1-OE) treated with YTX-7739 for 96h.

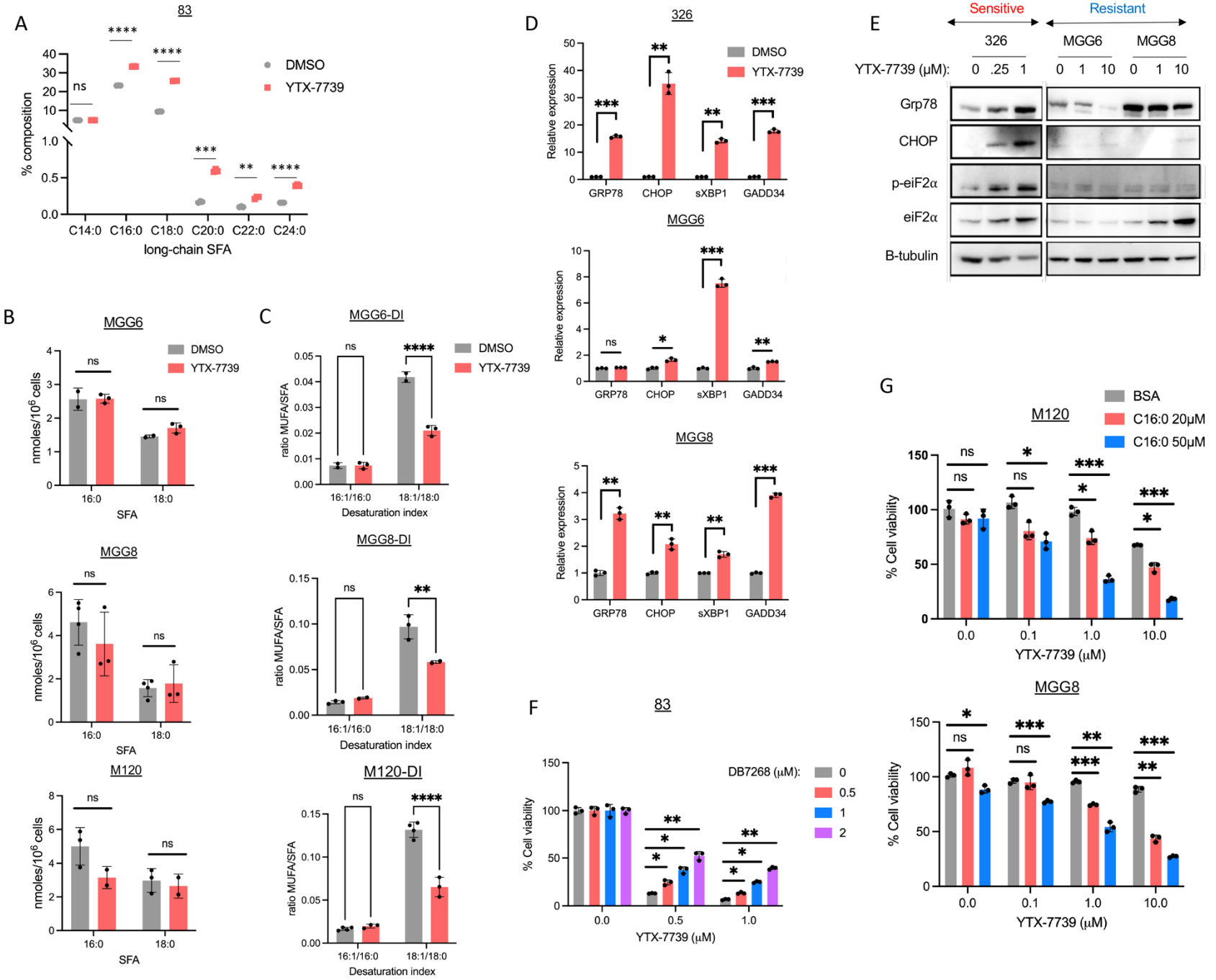

A
83
DMSO
YTX-7739
% composition
long-chain SFA
C14:0
C16:0
C18:0
C20:0
C22:0
C24:0
B
MGG6
MGG8
M120
nmoles/10^6 cells
SFA
16:0
18:0
C
MGG6-DI
MGG8-DI
M120-DI
ratio MUFA/SFA
Desaturation index
16:1/16:0
18:1/18:0
D
326
MGG6
MGG8
Relative expression
GRP78
CHOP
sXBP1
GADD34
E
Sensitive
Resistant
326
MGG6
MGG8
YTX-7739 (μM):
Grp78
CHOP
p-eiF2α
eiF2α
B-tubulin
F
83
DB7268 (μM):
% Cell viability
YTX-7739 (μM)
G
M120
MGG8
BSA
C16:0 20μM
C16:0 50μM
% Cell viability
YTX-7739 (μM)

Figure S2 (A) Quantification of individual fatty acids in 83 treated with YTX-7739 (1µM) for 72h. (B-C) Quantification of C16:0 and C18:0 (B) as well as the C16 and C18 desaturation index (C) in MGG6, MGG8 and M120 treated with YTX-7739 (1µM) for 72h. (D) Relative mRNA expression of ER stress markers in 326, MGG6, and MGG8 GSCs treated with DMSO or YTX-7739 (1µM) for 48h (326) or 72h (MGG6 and MGG8). (E) Immunoblot analysis of 326, MGG6 and MGG8 GSCs treated with DMSO or YTX-7739 (1µM) for 48h (326) or 72h (MGG6 and MGG8). (F) Cell viability of 83 treated with YTX-7739 (0.5-1µM) or its combination with the JNK1 inhibitor DB07268 for 4 days. (G) Cell viability of YTX-7739 resistant M120 and MGG8 GSCs treated with the indicated doses of YTX-7739 or its combination with BSA (Control) or C16:0 (20-50µM) for 96h. ****P<0.0001; *** P<0.001; **P<0.01; *P<0.05

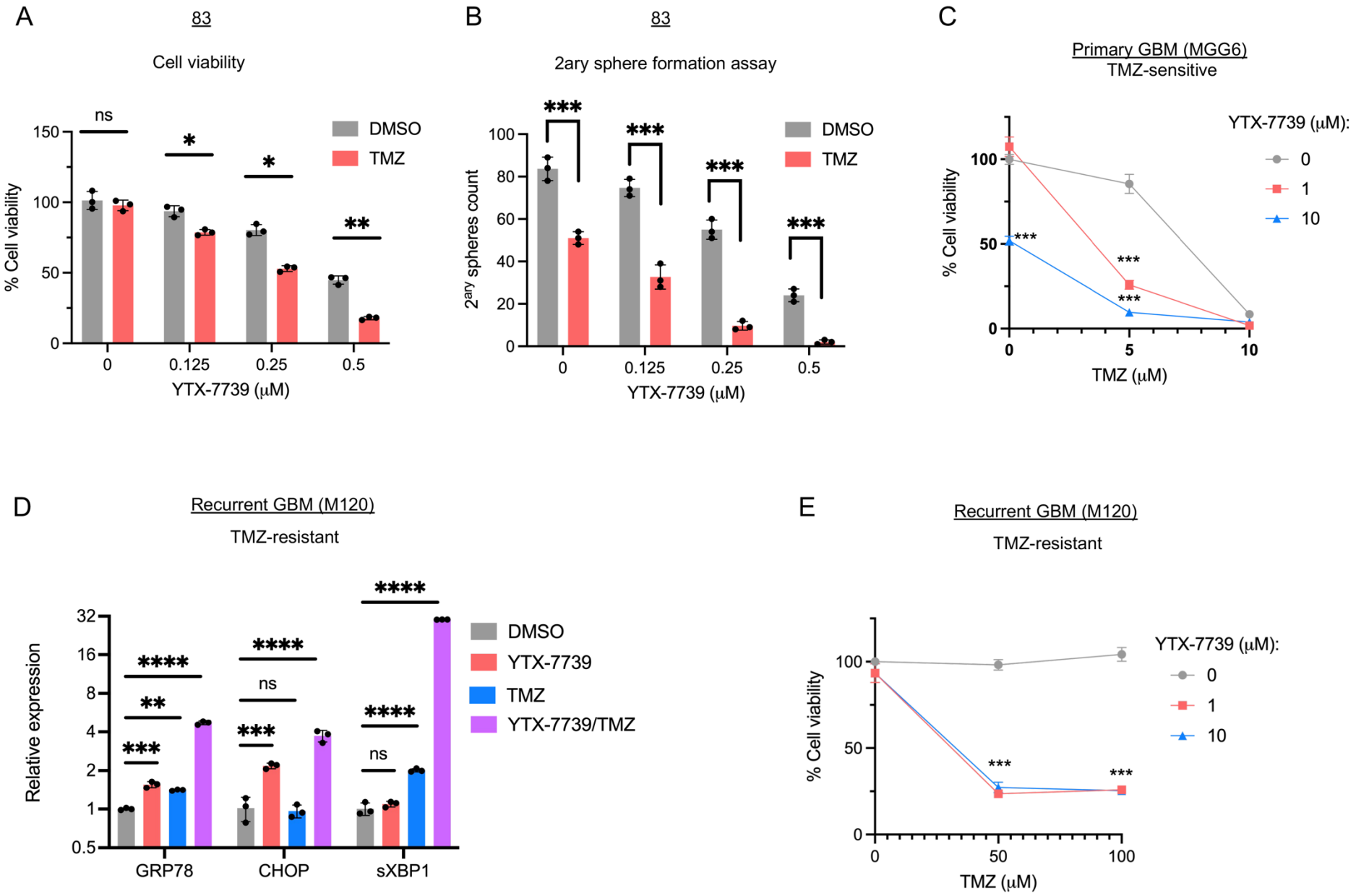

A
83
Cell viability
% Cell viability
150
100
50
0
ns
*
*
**
DMSO
TMZ
0
0.125
0.25
0.5
YTX-7739 (μM)
B
83
2ary sphere formation assay
2ary spheres count
100
80
60
40
20
0
***
***
***
***
DMSO
TMZ
0
0.125
0.25
0.5
YTX-7739 (μM)
C
Primary GBM (MGG6)
TMZ-sensitive
% Cell viability
100
50
0
***
***
***
YTX-7739 (μM):
0
1
10
0
5
10
TMZ (μM)
D
Recurrent GBM (M120)
TMZ-resistant
Relative expression
32
16
8
4
2
1
0.5
****
**
***
****
ns
***
****
ns
DMSO
YTX-7739
TMZ
YTX-7739/TMZ
GRP78
CHOP
sXBP1
E
Recurrent GBM (M120)
TMZ-resistant
% Cell viability
100
50
0
***
***
YTX-7739 (μM):
0
1
10
0
50
100
TMZ (μM)

Figure S3 (A-B) 83 were treated with the indicated doses of YTX-7739 and TMZ (100µM). Cell viability was measured 96h after treatment (A). Secondary spheres were counted on day 8 after treatment (B). (C) Cell viability of MGG6 (primary) or M120 (recurrent) pretreated with YTX-7739 followed by TMZ for 96h. (D) Relative mRNA expression of ER stress markers in M120 (recurrent) pretreated with YTX-7739 (10µM) followed by TMZ for 72h. (E) Cell viability of M120 pretreated with YTX-7739 followed by TMZ for 96h. ****P<0.0001; *** P<0.001; **P<0.01; *P<0.05

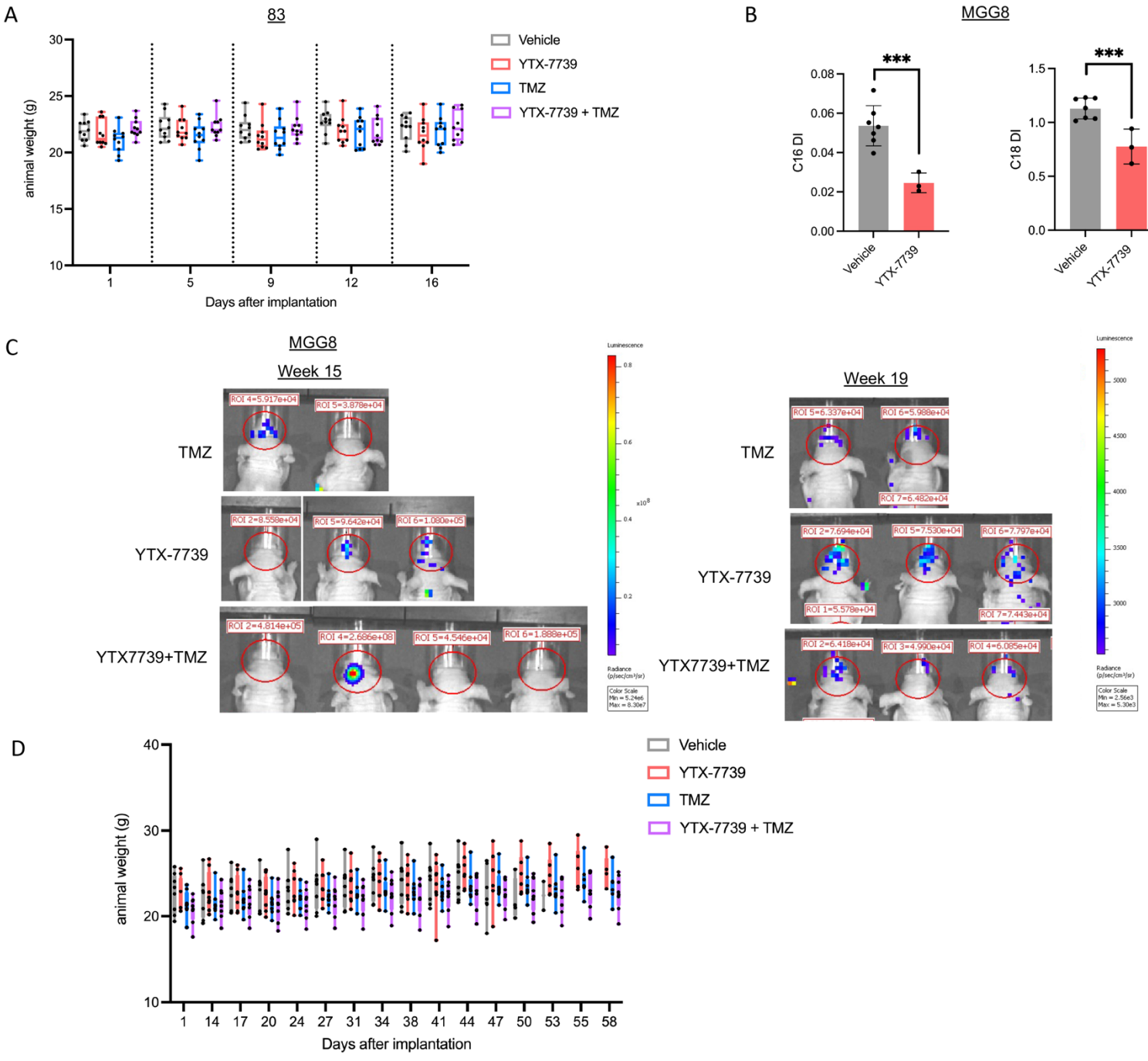

A
83
Vehicle
YTX-7739
TMZ
YTX-7739 + TMZ
animal weight (g)
Days after implantation
B
MGG8
***
***
C16 DI
C18 DI
Vehicle
YTX-7739
C
MGG8
Week 15
Week 19
TMZ
YTX-7739
YTX7739+TMZ
Luminescence
Radiance (p/sec/cm²/sr)
D
Vehicle
YTX-7739
TMZ
YTX-7739 + TMZ
animal weight (g)
Days after implantation

Figure S4 (A) Average weight of mice bearing 83-Fluc and treated with vehicle, YTX-7739, TMZ, or YTX-7739+TMZ. Overtime monitoring of animal weight in all four treatment groups. (B) DI of C16 and C18 in plasma from mice bearing MGG8 tumors and treated with YTX-7739 (30mg/kg). Plasma was collected on day 10 after treatment. (C) Bioluminescence imaging in surviving mice bearing MGG8 tumors from TMZ, YTX-7739, and YTX-7739+TMZ treatment groups at weeks 15 and 19 post tumor implantation. (D) Average weight of mice bearing MGG8-Fluc GSCs and treated with vehicle, YTX-7739, TMZ, or YTX-7739+TMZ. Monitoring of animal weight over 58 days shows no significant change among all four treatment groups. ****P<0.0001; *** P<0.001; **P<0.01; *P<0.05

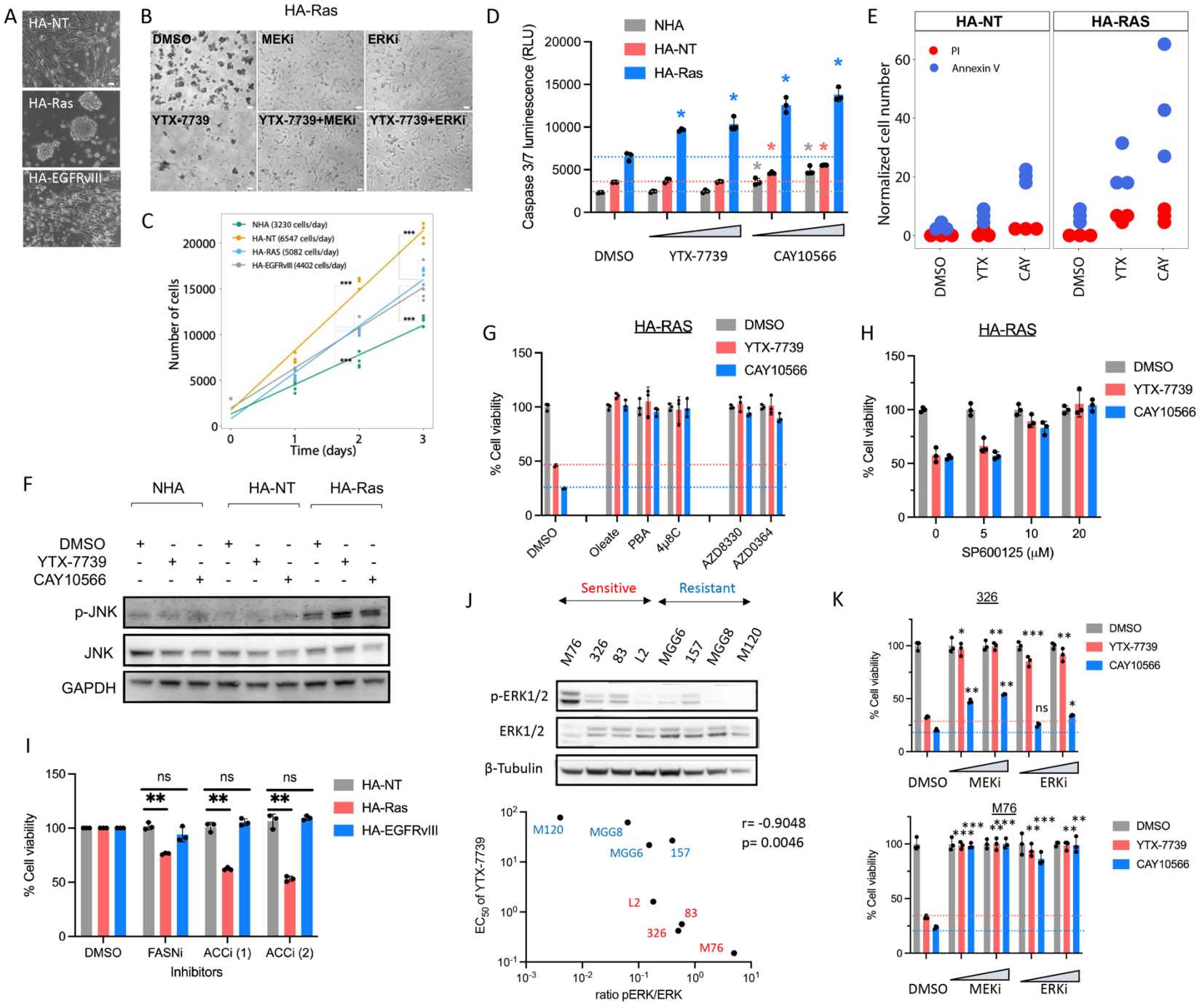

A
HA-NT
HA-Ras
HA-EGFRvIII
B
HA-Ras
DMSO
MEKi
ERKi
YTX-7739
YTX-7739+MEKi
YTX-7739+ERKi
C
NHA (3230 cells/day)
HA-NT (6547 cells/day)
HA-RAS (5082 cells/day)
HA-EGFRvIII (4402 cells/day)
Number of cells
Time (days)
D
NHA
HA-NT
HA-Ras
Caspase 3/7 luminescence (RLU)
DMSO
YTX-7739
CAY10566
E
HA-NT
HA-RAS
PI
Annexin V
Normalized cell number
DMSO
YTX
CAY
F
NHA
HA-NT
HA-Ras
DMSO
YTX-7739
CAY10566
p-JNK
JNK
GAPDH
G
HA-RAS
DMSO
YTX-7739
CAY10566
% Cell viability
DMSO
Oleate
PBA
4μ8C
AZD8330
AZD0364
H
HA-RAS
DMSO
YTX-7739
CAY10566
% Cell viability
SP600125 (μM)
I
HA-NT
HA-Ras
HA-EGFRvIII
% Cell viability
ns
DMSO
FASNi
ACCi (1)
ACCi (2)
Inhibitors
J
Sensitive
Resistant
M76
326
83
L2
MGG6
157
MGG8
M120
p-ERK1/2
ERK1/2
β-Tubulin
r= -0.9048
p= 0.0046
EC50 of YTX-7739
ratio pERK/ERK
K
326
DMSO
YTX-7739
CAY10566
% Cell viability
DMSO
MEKi
ERKi
M76
DMSO
YTX-7739
CAY10566
% Cell viability
DMSO
MEKi
ERKi

Figure S5 (A) HA-NT cells stably expressing a control vector, HRASG12V or EGFRvIII were cultured under serum-free conditions for 7 days. Representative micrographs showing cell growth as a monolayer or as fully suspended spheroids. Scale bar, 10µm. (B) Representative micrographs of HA-Ras treated with YTX-7739 (0.5µM), MEKi (AZD8330; 1µM) and ERKi (AZD0364; 1µM) for 72h. Scale bar, 10µm. (C) Cell proliferation rate of primary NHA and the immortalized astrocytes HA-NT, HA-Ras, and HA-EGFRvIII. (D) Fold change in caspase-3/7 activation in primary (NHA) and transformed astrocytes (HA-NT and HA-Ras) treated with YTX-7739 (0.5-1µM) or CAY10566 (0.25-0.5µM) for 48 h. (E) HA-NT and HA-Ras were treated with YTX-7739 (0.5µM) or CAY10566 (0.25µM) for 48h before staining with Propidium iodide (PI) and Annexin V. Cells with positive PI or Annexin V staining were counted, and three experimental replicates are represented. (F) Immunoblot analysis of JNK activation in NHA, HA-NT, and HA-Ras treated with YTX-7739 (1µM) or CAY10566 (0.5µM) for 48 h. (G) Cell viability of HA-Ras treated with YTX-7739 (1µM) or CAY10566 (0.5µM) alone or in combination with Oleate (C18:1n9), or inhibitors of ER stress (PBA; 4mM), IRE1 (4µ8C: 25µM,) MEKi (AZD8330; 1µM) and ERKi (AZD0364; 1µM) for 96h. (H) Cell viability of HA-Ras treated with YTX-7739 (1µM) or CAY10566 (0.5µM) alone or in combination with the JNK inhibitor SP600125 for 72h. (I) Cell viability of HA-NT, HA-Ras, and HA-EGFRvIII treated with inhibitors of FASN (GSK2194069; 50nM) or ACC (CP-640186; 20µM and ND-646; 1 µM) for 96h. (J) Immunoblot analysis of ERK activity in YTX-7739 sensitive and resistant GSCs lines. Also shown is the correlation analysis between the EC_{50} of YTX-7739 and the ratio of p-ERK1/2 to total ERK1/2 (western-blot bands were quantified using Image J software) in all 8 GSCs lines. Spearman nonparametric correlation r=-0.9048; **p=0.0046. (K) Cell viability of 326 and M76 treated with YTX-7739 (1µM), CAY10566 (0.5 µM), or their combination with MEKi and ERKi (0.25-0.5µM) for 96 hours. ****P<0.0001; *** P<0.001; **P<0.01; *P<0.05

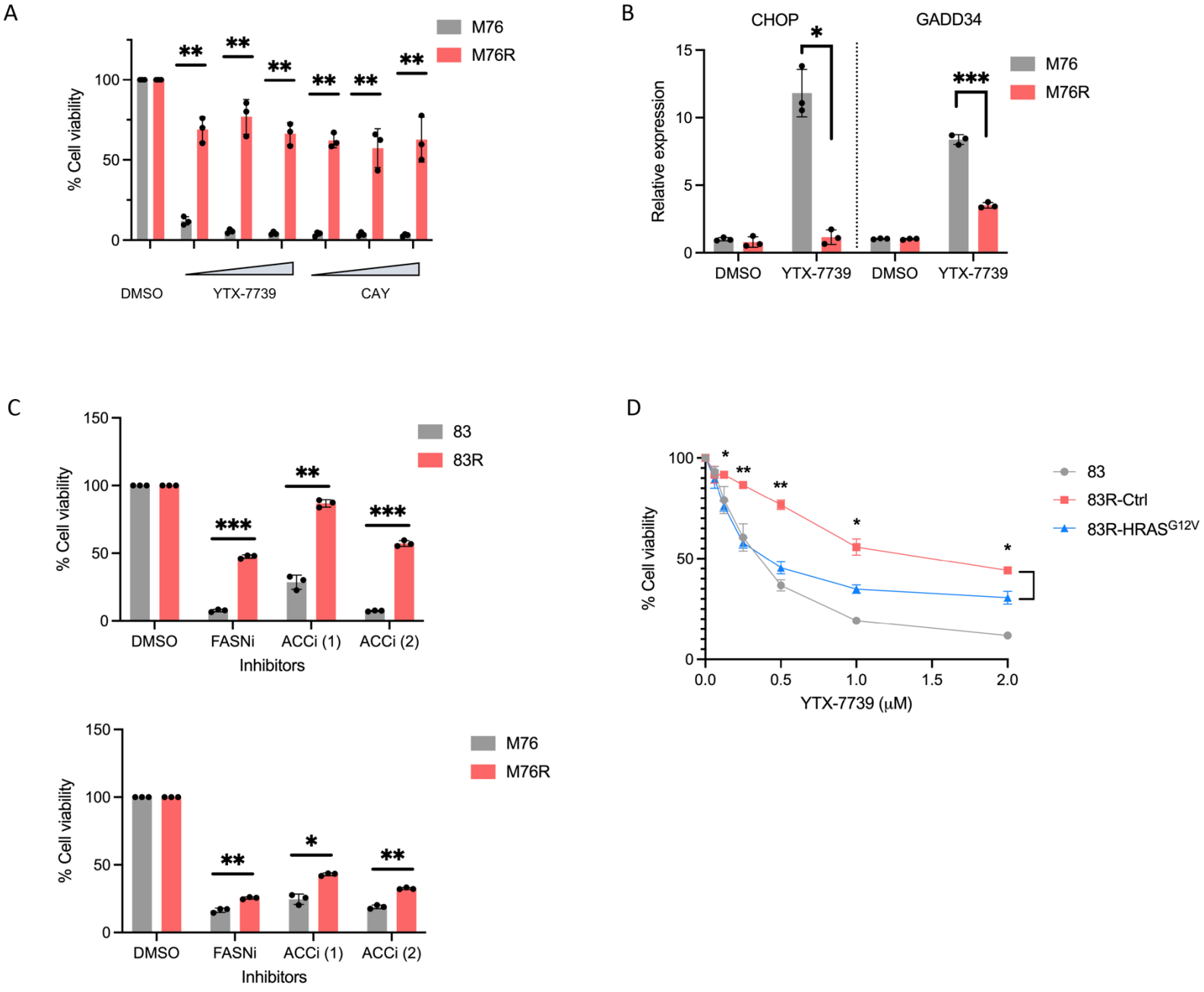

A
M76
M76R
% Cell viability
100
50
0
DMSO
YTX-7739
CAY
B
CHOP
GADD34
Relative expression
15
10
5
0
M76
M76R
DMSO
YTX-7739
DMSO
YTX-7739
C
83
83R
% Cell viability
150
100
50
0
DMSO
FASNi
ACCi (1)
ACCi (2)
Inhibitors
M76
M76R
% Cell viability
150
100
50
0
DMSO
FASNi
ACCi (1)
ACCi (2)
Inhibitors
D
% Cell viability
100
50
0
0.0
0.5
1.0
1.5
2.0
YTX-7739 (μM)
83
83R-Ctrl
83R-HRASG12V

Figure S6 (A) Cell viability of M76 and M76R treated with YTX-7739 (0.25-1μM) and CAY10566 (0.125-0.5μM) for 96h. (B) Relative mRNA expression of UPR markers CHOP and GADD34 in M76 and M76R GSCs treated with DMSO (control) or YTX-7739 (1μM) for 72h. (C) Cell viability of 83/83R treated with inhibitors of FASN (GSK2194069; 0.5 μM) or ACC (CP-640186; 20μM and ND-646; 0.5μM) and M76/M76R treated with inhibitors of FASN (GSK2194069; 0.025 μM) or ACC (CP-640186; 2.5μM and ND-646; 0.025μM) for 96h. (D) Cell viability of 83 and 83R expressing a control vector or $HRAS^{G12V}$ and treated with YTX-7739 for 96h.

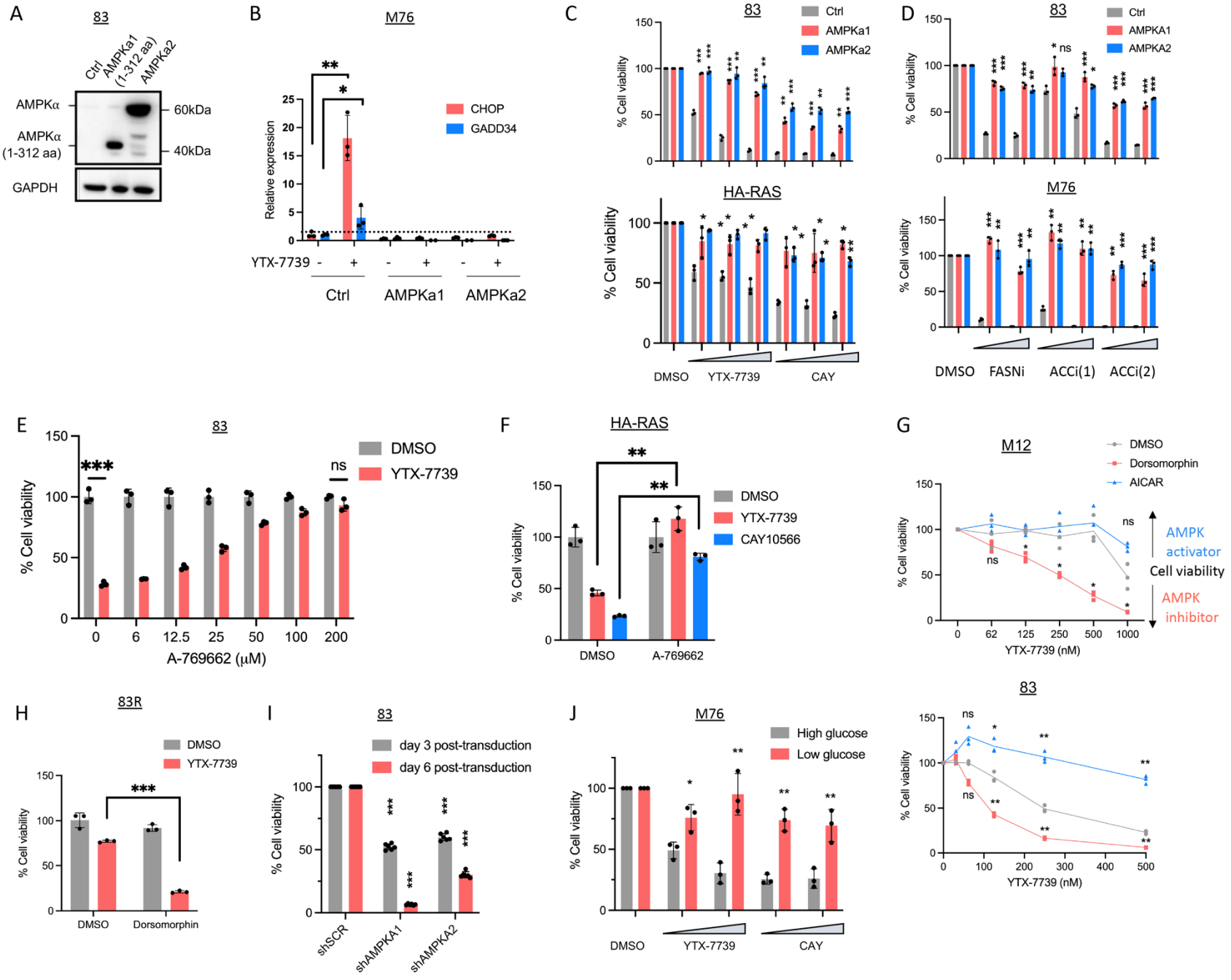
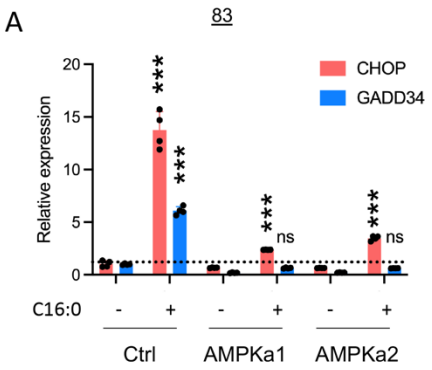
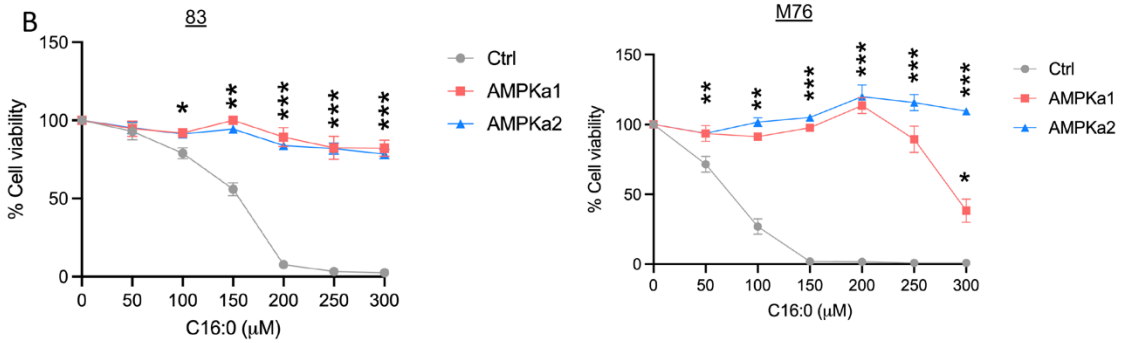
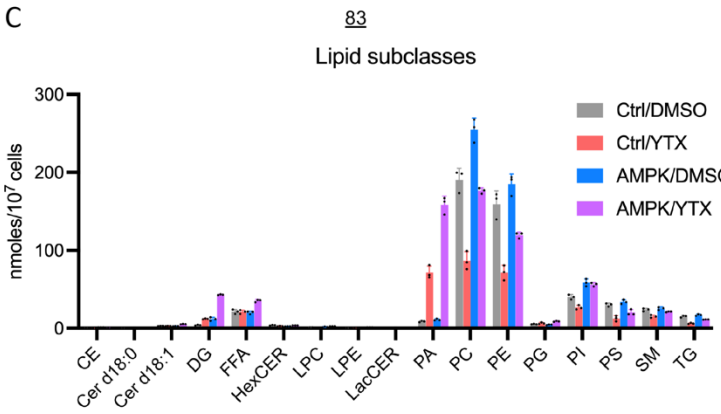

A
83
Ctrl
AMPKa1 (1-312 aa)
AMPKa2
AMPKα
AMPKα (1-312 aa)
GAPDH
60kDa
40kDa
B
M76
CHOP
GADD34
Relative expression
YTX-7739
Ctrl
AMPKa1
AMPKa2
C
83
Ctrl
AMPKa1
AMPKa2
% Cell viability
HA-RAS
DMSO
YTX-7739
CAY
D
83
M76
DMSO
FASNi
ACCi(1)
ACCi(2)
E
83
DMSO
YTX-7739
A-769662 (μM)
F
HA-RAS
DMSO
YTX-7739
CAY10566
A-769662
G
M12
DMSO
Dorsomorphin
AICAR
AMPK activator
Cell viability
AMPK inhibitor
YTX-7739 (nM)
H
83R
DMSO
YTX-7739
Dorsomorphin
I
83
day 3 post-transduction
day 6 post-transduction
shSCR
shAMPKA1
shAMPKA2
J
M76
High glucose
Low glucose
DMSO
YTX-7739
CAY
83
YTX-7739 (nM)

Figure S7 (A) Immunoblot analysis of 83 expressing a control lentivirus (Ctrl) or a constitutively active AMPKa1 (truncated; 1-312 aa; 40 kDa) or AMPKa2. (B) Relative mRNA expression of UPR markers CHOP and GADD34 in M76 Ctrl, AMPKa1, and AMPKa2 treated with DMSO (control) or YTX-7739 (1μM) for 72h. (C) Cell viability of 83 and HA-Ras expressing Ctrl, AMPKa1, or AMPKa2 after treatment with YTX-7739 (0.25-1μM) or CAY10566 (0.125-0.5μM) for 96h. (D) Cell viability of 83 and M76 expressing Ctrl, AMPKa1, or AMPKa2. 83 cells were treated with inhibitors of FASN (GSK2194069; 0.25-0.5μM) or ACC (CP-640186; 20-30μM and ND-646; 0.25-0.5μM), while M76 cells were treated with GSK2194069; 0.1-0.2μM) or ACC (CP-640186; 5-10μM and ND-646; 0.1-0.2μM) for 96h. (E) Cell viability of 83 treated with YTX-7739 (0.5 μM) and increasing concentration of the AMPK activator A-769662 for 96h. (F) Cell viability of HA-Ras co-treated with YTX-7739 (2μM) or CAY10566 (1μM) and A-769662 (100μM) for 96h. (G) Cell viability of 83 and M12 co-treated with YTX-7739 and the AMPK activator AICAR (100μM) or the AMPK inhibitor Dorsomorphin (2μM) for 96h. (H) Cell viability of 83R co-treated with YTX-7739 (0.5μM) and Dorsomorphin (3μM) for 96h. (I) Cell viability of 83 transduced with shSCR (control), shAMPKa1or shAMPKa2 was measured on day 3 and day 6 post-transduction. (J) Cell viability of M76 cultured in high (17.5mM) or low (5mM) glucose and concomitantly treated with YTX-7739 (0.25-1μM) or CAY10566 (0.125-0.5μM) for 72h.







D

	PC1
PG 16:0_18:2	0.05277195
PG 18:0_18:2	0.05246415
PG 16:0_18:0	0.05236412
PE 18:0_18:3	0.05233498
PE O-16:0/16:0	0.05228855
PC 16:0_18:0	0.05224292
PE 16:0_18:3	0.05198232
TG 54:2-FA18:2	0.05193921
PE O-18:0/18:2	0.05172799
PE 18:0_18:2	0.05149444
PC 18:2_20:0	0.05144699
PC 18:0_18:2	0.05126975
TG 52:2-FA18:2	0.05119111
PC 16:0_18:3	0.05115294
TG 50:0-FA14:0	0.05110445
TG 52:0-FA18:0	0.05105448
PG 14:0_16:0	0.05071409
TG 52:0-FA16:0	0.05067294
PI 18:0_20:0	0.0506364
PG 16:1_18:2	0.05047584
PC 18:0_20:4	0.05043719
TG 50:2-FA18:2	0.05043481
PI 18:0_20:4	0.05036117
PE O-16:0/18:2	0.05019472
PE O-16:0/18:0	0.05017597
PG 14:0_18:0	0.05006667
TG 54:0-FA16:0	0.05004131
TG 54:0-FA18:0	0.04998885
PE P-18:0/18:3	0.0498157
PE P-16:0/18:0	0.04976795
TG 50:0-FA18:0	0.04955088
TG 48:0-FA18:0	0.04953886
TG 48:0-FA14:0	0.04932727
PG 18:2_18:2	0.04931375
PE 18:0_18:0	0.04883941
TG 50:0-FA16:0	0.04883488
PG 18:1_18:2	0.04868545
Cer d18:1/20:0	0.04852943
TG 51:0-FA18:0	0.04851049
TG 52:0-FA20:0	0.04849929
FA 22:4	0.0484839
TG 46:0-FA18:0	0.04842093
TG 52:7-FA16:0	0.04840456
TG 46:0-FA12:0	0.04816754
PC 18:0_20:3	0.04801499
FA 22:5	0.04795985
PE O-18:0/20:4	0.0478621
PI 18:0_18:0	0.04764286
PA 36:0	0.04749278
PE 18:0_20:4	0.04745877

E

	PC2
PE P-18:0/16:0	0.06715333
PE P-16:0/16:0	0.05925258
PE P-18:1/18:0	0.05370419
PE O-18:0/18:0	0.04107441
PE O-18:0/16:0	0.03979228
PE 16:0_18:0	0.03785558
PE P-18:1/16:0	0.03658964
PE P-18:0/18:0	0.03591061
PG 18:0_18:0	0.03472441
PG 18:1_20:1	0.03242445
PG 18:0_20:0	0.03124097
PE 14:1_18:2	0.03045473
TG 46:2-FA16:1	0.02680686
PS 18:0_22:4	0.02658466
TG 50:3-FA14:1	0.02556925
TG 55:2-FA18:1	0.02442121
CE 20:1	0.02313447
PC 18:0_18:0	0.02160814
PS 18:0_18:0	0.02106952
PE 18:0_18:0	0.01832105
TG 48:2-FA14:1	0.01794033
PG 16:0_16:0	0.01768565
PI 18:1_20:4	0.0168361
TG 46:2-FA14:1	0.01660793
CE 16:1	0.01583873
TG 54:4-FA16:0	0.01491992
PE P-18:0/22:1	0.01491615
DG 18:1_20:4	0.01456446
PE P-16:0/18:0	0.01403008
PE 15:0_18:1	0.01378161
TG 55:3-FA18:1	0.01338905
PE 18:1_22:2	0.01334005
TG 50:3-FA20:3	0.01290603
TG 52:3-FA18:3	0.01245433
DG 18:1_20:2	0.01242085
TG 52:3-FA20:1	0.01239198
TG 46:2-FA18:1	0.01214091
PC 18:1_22:4	0.0119844
TG 45:1-FA18:1	0.0115229
TG 52:7-FA18:1	0.01084446
TG 56:8-FA16:0	0.01058887
PE 18:1_22:1	0.01005994
TG 56:5-FA22:4	0.00981921
PS 18:0_20:3	0.00946119
PC 12:0_16:1	0.00942276
TG 58:6-FA18:1	0.00906472
DG 16:1_16:1	0.00906279
TG 54:2-FA18:2	0.0090392
TG 56:4-FA18:0	0.00891969
PG 18:2_20:0	0.00874716

F

	PC2
PE P-18:0/20:4	-0.0782112
PG 16:1_18:1	-0.0766582
SM d18:1/20:1	-0.0766237
PC 12:0_18:1	-0.0763073
PC 18:2_18:2	-0.0761946
PC 18:1_20:0	-0.0756637
PC 14:0_18:2	-0.0755357
PG 14:0_18:1	-0.0752573
PE P-16:0/18:2	-0.0748288
PE 18:0_18:1	-0.0745887
Cer d18:1/22:1	-0.0745296
PI 16:0_20:1	-0.0743774
DG 18:0_18:1	-0.0742535
TG 54:1-FA20:1	-0.0741287
PC 18:0_18:1	-0.073802
PC 16:0_16:0	-0.0734181
PC 12:0_16:0	-0.0733065
PC 16:0_20:3	-0.0732285
PC 16:1_18:0	-0.0729335
PI 15:0_22:1	-0.0719524
TG 53:1-FA18:0	-0.0719077
TG 42:0-FA16:0	-0.0717248
PE P-18:1/18:2	-0.0714212
TG 50:1-FA16:1	-0.0713568
FA 16:1	-0.071302
CE 18:0	-0.0710891
FA 20:1	-0.0706141
TG 42:0-FA12:0	-0.0704093
LPC 16:0	-0.0697938
PC 14:0_20:3	-0.0695461
PI 18:0_18:1	-0.0694371
DG 16:1_18:0	-0.0693072
TG 51:1-FA18:0	-0.068574
PA 38:3	-0.0681236
PE P-18:0/18:1	-0.0680365
PE O-18:0/16:1	-0.0678745
PE 16:1_18:0	-0.0670289
PI 16:0_18:1	-0.0668858
FA 15:0	-0.0665815
CE 16:0	-0.0661202
FA 14:0	-0.0661174
PC 14:0_20:4	-0.0660881
FA 18:1	-0.0658117
TG 52:1-FA16:0	-0.0653712
PA 32:1	-0.0647386
PE 15:0_18:0	-0.0645778
DG 16:0_16:1	-0.064451
FA 16:0	-0.0644235
PA 34:2	-0.064391
TG 52:1-FA16:1	-0.0641119

Figure S8 (A) Relative mRNA expression of UPR markers CHOP and GADD34 in 83 supplemented with BSA or C16:0 (300 µM) for 24h. (B) Cell viability of 83 and M76-GSCs cultured in medium supplemented with BSA or increased concentrations of C16:0 for 96h. (C) Barplot representing the amount, expressed in nanomoles/10^7 cells, of lipid subclasses in untreated (DMSO) and treated /YTX) Ctrl/AMPK GSCs, measured by shotgun lipidomics. (D-F) Principal Component Analysis (PCA). Top 50 lipids (D) positively contributing to PC1, (E) positively contributing to PC2, and (F) negatively contributing to PC2 in untreated (DMSO) and treated /YTX) Ctrl/AMPK GSCs, measured by shotgun lipidomics.

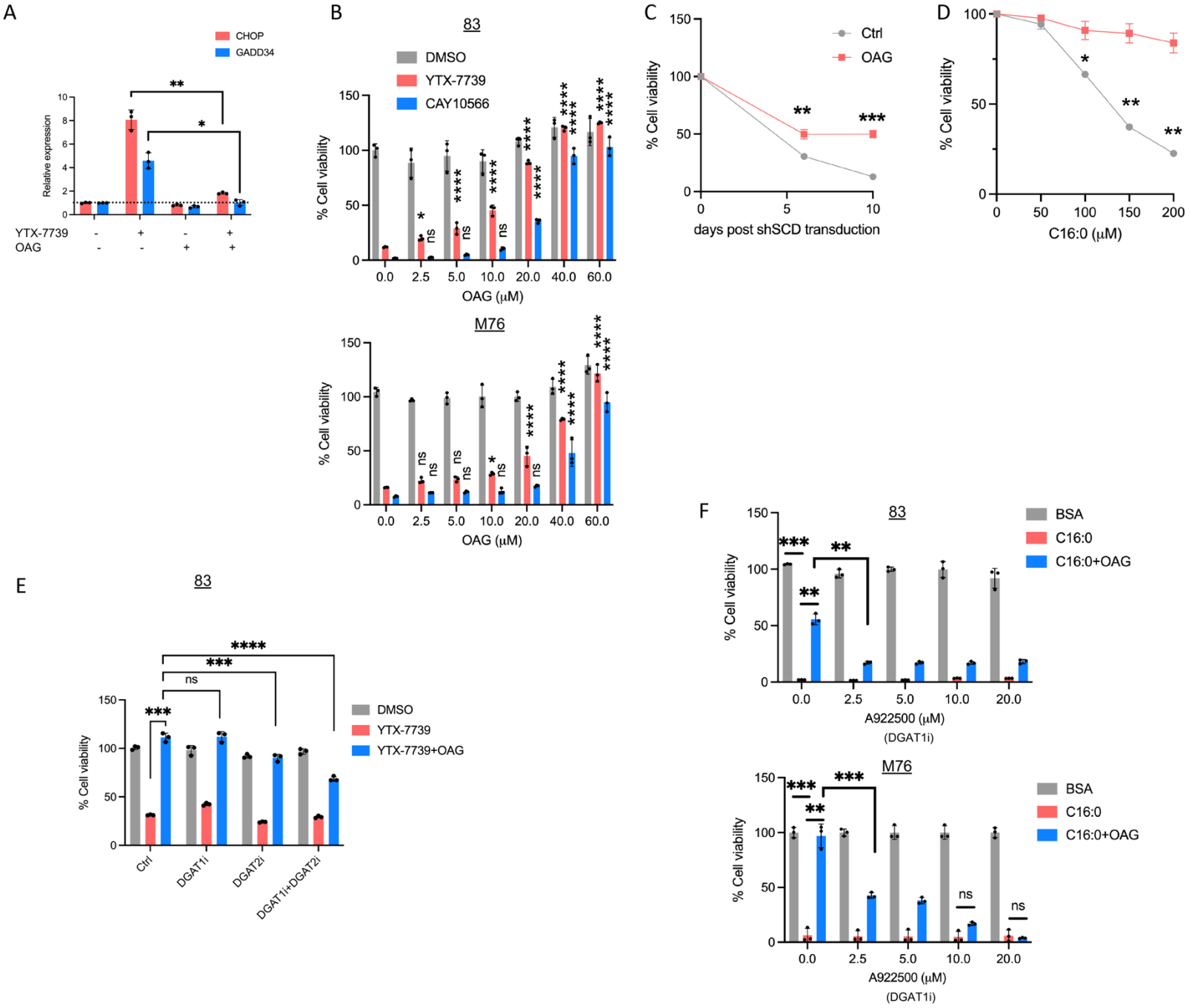

A
CHOP
GADD34
Relative expression
YTX-7739
OAG
B
83
DMSO
YTX-7739
CAY10566
% Cell viability
OAG (μM)
M76
C
Ctrl
OAG
% Cell viability
days post shSCD transduction
D
% Cell viability
C16:0 (μM)
E
83
DMSO
YTX-7739
YTX-7739+OAG
% Cell viability
Ctrl
DGAT1i
DGAT2i
DGAT1i+DGAT2i
F
83
BSA
C16:0
C16:0+OAG
% Cell viability
A922500 (μM)
(DGAT1i)
M76

Figure S9 (A) Relative mRNA expression of UPR markers CHOP and GADD34 in 83 treated with YTX-7739 (1µM) for 24h with or without OAG (60µM). (B) Cell viability of 83 and M76 co-treated with YTX-7739 (0.5µM) or CAY10566 (0.25 µM) and the indicated doses of OAG for 96h. (C) 83 cells were transduced with shSCD1 and subsequently treated with OAG at 24h and day 96h post-transduction; Cell viability was measured on days 6 and 10 after transduction with shSCD. (D) Cell viability of 83 treated with C16:0 at the indicated doses in the presence or absence of OAG (60µM) for 72h. (E) Cell viability of 83 co-treated with YTX-7739 (0.5µM) and OAG (60µM) in addition to DGAT1i (A922500; 5µM) and/or DGAT2i (PF-06424439; 50 µM) for 96h. (F) Cell viability of 83 and M76 co-treated with C16:0 (300µM) and OAG (60µM) in addition to DGAT1i (A922500) at the indicated doses for 96h. ****P<0.0001; *** P<0.001; **P<0.01; *P<0.05

Table S1: Clinical information and key genetic alterations of patient-derived GSCs used in this study. GSCs were classified into the following subtypes: Proneural (PN), Mesenchymal/Mesenchymal-like (M), or Classical (C). (+) Gain/amplification; (m) Mutation; MGMT promoter methylation: (M) methylated; (U): unmethylated. The TMZ response was defined as follows: Sensitive (S) EC_{50}< 50µM; Semi-sensitive (S/R) EC_{50} : 50-300µM; Resistant (R) EC_{50}>300µM. ND: not determined; NA: not available.

Patient	EGFR	TP53	PDGFRA	PTEN	CDKN2 A/B	MYC/ MYCN	MGMT methylation	TMZ response	GBM	Subtype	Sex	Age	Reference
M76	+/vIII			m			M	S	Recurrent	C	M	36	Vaubel et al., 2020
326	vIII						ND	S/R	Primary	M	M	50	Mao et al., 2013
83	vIII						ND	S/R	Primary	M	F	72	Mao et al., 2013
L2	+						ND	R	Primary	M	F	30	Siebzehnrubl et al., 2013
M12	+						M	S	Primary	M	M	69	Vaubel et al., 2020
L1	+						ND	S	Primary	M	F	45	Siebzehnrubl et al., 2013
L0	+						ND	R	Primary	M	M	43	Siebzehnrubl et al., 2013
MGG6	+			+	+		M	S	Primary	PN	NA	NA	Wakimoto et al., 2009, 2012
157			+				ND	S	Primary	M	F	55	Mao et al., 2013
MGG8			+	m		+	M	S	Primary	PN	NA	NA	Wakimoto et al., 2009, 2012
MGG70RR			m				ND	R	Recurrent	ND	M	62	Tanaka et al., 2019
M120		m					U	R	Recurrent	PN	M	57	Vaubel et al., 2020

Table S2: Pharmacokinetics of YTX-7739 in the plasma and brain of mice following IV or IP administration

Parameter	IV (1 mg/kg, PLASMA)	IP (3 mg/kg, PLASMA)	IP (10 mg/kg, PLASMA)	IP (30 mg/kg, PLASMA)	IP (10 mg/kg, BRAIN)
Clearance (mL/min/kg)	4.06	N/A	N/A	N/A	N/A
Tmax (hr)	ND	0.5	0.25	0.5	0.25
Cmax (ng/mL)	ND	636	2817	9780	2820
T1/2 (hr)	4.12	7.76	5.87	6.01	4.27
AUClast (ng.hr/mL)	4045	6685	23836	80150	11212
Bioavailability (%)	N/A	61.1	61.2	68.9	N/A

Supplementary Material and Methods

Primary cells and cell lines

Primary GBM cells were derived from surgical specimens obtained from GBM patients at the Massachusetts General Hospital (provided by Dr. Hiroaki Wakimoto) under the appropriate Institutional Review Board approval (MGG6, MGG8, 70RR) or provided by Dr. Ichiro Nakano (157, 83, 326), Dr. Brent Reynolds (L0, L1, L2) and Dr. Jann Sarkaria at The Brain Tumor PDX National Resource at Mayo Clinic (M76, M12, M120). All GBM cells used in this study have been previously characterized (*39-43*). Cells were expanded as neurospheres and maintained in DMEM/F12 medium supplemented with B27 without vitamin A (1:50; Life Technologies), heparin (2 μg/mL; Sigma Aldrich), human recombinant EGF (20 ng/mL; ABM), and human recombinant bFGF-2 (10ng/mL; ABM). DMEM-F12 w/o L-Glutamine w/o Hepes w/o Glucose (Biowest) was used to control glucose concentrations in the culture medium. NHAs were obtained from Sciencell Research Laboratories and grown in Astrocyte Medium (Sciencell). Human astrocytes transformed with E6/E7/hTERT were provided by Dr. Anna Krichevsky and Dr. Erik Uhlmann and generated as previously described (*44*). Ionizing radiation of GSCs was performed using a ^{137}Cs irradiator.

Preparation of YTX-7739

For cell culture studies, YTX-7739 was solubilized in DMSO. For in vivo studies, YTX-7739 was solubilized by vortexing (1 min), sonication (3-5 min), and heating (3-5 min at 60°C) in 0.5% methylcellulose + 0.2% Tween80 in sterile saline solution.

Mouse Orthotopic Brain Tumor Models

All animal experiments were approved by the Massachusetts General Hospital Subcommittee on Research Animal Care and conformed to the guidelines of the NIH Guide for the Care and Use of Laboratory Animals. GSCs (1-5 x 10^4) expressing Firefly luciferase (Fluc) were implanted into the left forebrain of male and female athymic nude mice (1.0 mm anterior and 2.0 mm laterally to bregma at a depth of 2.5 mm from the skull surface) using a stereotactic frame. Brain tumors

were detected and monitored over time using Fluc bioluminescence imaging using a Xenogen IVIS 200 imaging system (PerkinElmer) following IP injections of D-luciferin (150 mg/kg body weight) (Gold Biotechnology). Signal intensity was quantified with Living Image software 4.3.1 (PerkinElmer). For all in vivo studies, YTX-7739 was administered by IP injections. Control groups were administered with 100 µl of vehicle (0.5% methylcellulose + 0.2% Tween80 in sterile saline solution) by IP injections. TMZ was dissolved in DMSO (100 mM stock solution) and freshly resuspended in 5% dextrose before IP administration in mice.

Lentiviral production

For lentivirus packaging, 293T cells (5×10^6) were seeded in 150 mm plates with Opti-MEM (Gibco; 51985091). After 24h, 30 µL of chloroquine (25 mM; Sigma-Alrich; C6628) was added to the fresh culture medium, and cells were co-transfected with 15 µg plasmids encoding the gene or shRNA of interest, 3.75 µg PMD2.G (a kind gift from Didier Trono, Addgene plasmid 12259) and 11.25 µg psPAX2 (a kind gift from Didier Trono, Addgene plasmid 12260) using PEI (Polysciences, Inc; 23966100) in a 1:3 ratio (total DNA:PEI). At 72 h post-transfection, the conditioned medium was centrifuged at 500 × g for 10 min to remove cell debris. The supernatant was filtered through a 0.45 µm pore size polyethersulfone (PES) filter (Cell Treat; 229749). The filtrate was then centrifuged at 70,000 x g for 90 min at 4°C. Virus pellets were resuspended in 200-500µL PBS/1% BSA, aliquoted, and stored at -80°C. To transduce GSCs or astrocytes, cells ($2\text{-}5 \times 10^5$) were seeded in a six-well plate in the presence of polybrene (10 µg/ml; Sigma-Aldrich; TR-1003G), and 25 µl of lentivirus was added to the well. To generate stable cell lines, cells were subsequently selected using Puromycin (1 µg/ml; Invivogen; ant-pr), G418 (400 µg/ml; Invivogen; ant-gn) or Blasticidin (5-10 µg/ml; Invivogen; ant-bl).

DNA constructs and Lentiviral vectors

For experiments involving ectopic expression of different genes, a control lentivirus vector pHAGE-CMV-MCS-IRES-ZsGreen (obtained from the DNA Resource Core at Harvard Medical School, HMS) was used as a control vector (Ctrl). pLenti6-AMPK alpha1 (1-312), referred to as AMPKa1, was a gift from Boyi Gan (Addgene plasmid 162131). pLenti-XI-Neo-GST-Constitutively Active AMPK, referred to as AMPKa2, was a gift from Jacob Corn (Addgene plasmid 139843). CSCW-Fluc-IRES-mCherry lentivirus vector carrying an expression cassette for firefly luciferase (Fluc) and mCherry fluorescent protein was used for in vivo imaging. The following shRNA bacterial glycerol stocks: shSCD1 (TRCN0000327814), shAMPKa1 (TRCN0000000861), shAMPKa2 (TRCN0000000861), and non-targeting control shRNA (pLKO.1-puro non-Target shRNA Control; referred to as shCtrl) were obtained from Sigma (MISSION® shRNA Library), amplified, and packaged into lentivirus vectors. GSCs were stably transduced with shRNA lentivirus and, when applicable, selected using puromycin (1 μg/mL), Blasticidin (5-10 μg/mL), or G418 (200-400 μg/mL). Knockdown efficiency was determined using immunoblotting and/or qRT-PCR.

Chemical Reagents

The following compounds were obtained from Cayman Chemicals: CAY10566; 4μ8C; NSC95682; SP 600125; DB07268; ND-646; GSK2194069; CP-640186; AZD8330; AZD0364; Azoramide. Salubrinal, Phenylbutyrate, and 1-Oleoyl-2-acetyl-sn-glycerol were obtained from Sigma, Temozolomide, and PF-06424439 from MedChemExpress. A922500 was purchased from Apexbio Technology. Oleate and Palmitate (Cayman Chemicals) were dissolved in DMSO to yield a stock concentration of 0.5-1M. Fatty acid stocks were subsequently complexed to fatty acid-free Bovine Serum Albumin (BSA; 2.5mM; Gold Biotechnology) and added to the culture medium.

Immunoblot analysis

Cell lysis was performed using RIPA buffer (Boston BioProducts) supplemented with protease and phosphatase inhibitors. Proteins were quantified using the Bradford protein determination assay (Bio-Rad), and 20–40 μg of protein were loaded and resolved on 10% NuPAGE Bis-Tris gels (Life Technologies),

transferred to nitrocellulose membranes (Bio-Rad), then incubated with the indicated antibodies. Proteins were detected with SuperSignal West Pico Chemiluminescent Substrate (Pierce). The following antibodies were purchased from Cell Signaling Technologies: SCD (2438); GRP78 (3177); phospho SAPK/JNK (9255); SAPK/JNK (9252); phospho c-Jun (3270); phospho-eIF2α (3597); γ-H2AX (9718); CHOP (2895); PARP (9532); phospho AMPKα (2531); AMPKα (2532); phospho P44/42 MAPK (9101); P44/42 MAPK (4695); Phospho-Acetyl-CoA Carboxylase (11818); Phospho-EGF Receptor (3777); Phospho-AKT (4060); AKT (9272); β-actin (3700); Anti-rabbit IgG, HRP-linked Antibody (7074) and Anti-mouse IgG, HRP-linked Antibody. Phospho IRE1α (AB5700519) and phospho RSK1 (ABS1849) were obtained from Signa-Aldrich. Rad51 (BSM-51402M) and IRE1 (BS-8680R) were obtained from BIOSS Antibodies, GAPDH (sc-47724) from Santa Cruz and eIF2a (ab169528) from AbCam.

Cell-based Assays

CellTiter-Glo (Promega) was used to measure cell viability. Caspase 3/7 activity was detected using Caspase-Glo 3/7 (Promega). Annexin V/Propidium Iodide staining was performed using Alexa Fluor 488 Annexin V/Dead Cell Apoptosis Kit (Invitrogen). These reagents were used as recommended by the manufacturer. Each data point in the treated samples was normalized to its respective vehicle or pretreatment control for data analysis.

Cell proliferation assay

Cells were seeded on poly-D-lysine-coated 96 well plates at a density of 3000 cells/well. At days 1, 2, and 3, the medium was replaced by astrocytes medium supplemented with Hoechst (1/1000). Cells were incubated with Hoechst for 20 minutes and then imaged using a fluorescence microscope. The number of cells per well was quantified using the ImageJ tool Find Maxima. The number of cells was plotted against time and the estimated growth rates were determined using linear regression.

RNA extraction and PCR

RNA was extracted using the Zymo Quick-RNA MiniPrep kit. RT-PCR was performed with abm 5X AllInOne RT MasterMix. qPCR was run with LUNA universal qPCR master mix with three to four replicates using QuantStudio™ 3 System. All reagents were used as recommended by the manufacturers. Relative mRNA expression was calculated using the $2^{-\Delta\Delta CT}$ method. qPCR runs included human β-actin and/or HPRT as internal normalization controls. Primer sequences used in this study are listed below. Oligonucleotides were synthesized by the CCIB DNA Core Facility at the Massachusetts General Hospital.

Primer sequences used for qPCR analysis

GENE	Forward primer 5'	Reverse primer 5'	Source/Reference
SCD	TCTAGCTCCTATACCACCACCA	TCGTCTCCAACTTATCTCCTCC	MGH primer bank
BiP (GRP78)	CATCACGCCGTCCTATGTCG	CGTCAAAGACCGTGTTCTCG	MGH primer bank
CHOP	GGAAACAGAGTGGTCATTCCC	CTGCTTGAGCCGTTCATTCTC	MGH primer bank
sXBP1	GGTCTGCTGAGTCCGCAGCAGG	GGGCTTGGTATATATGTGG	Hirota et al., 2006
GADD34	AGCCACGGAGGATAAAAGAACA	CTGAACGATACTCCCAGGACC	MGH primer bank
IRE1	CATCCCCATGCCGAAGTTCA	CTGCTTCTCTCCGGTCAGGA	MGH primer bank
PRKAA1 (AMPKα1)	TTGAAACCTGAAAATGTCCTGCT	GGTGAGCCACAACTTGTTCTT	MGH primer bank
PRKAA2 (AMPKα2)	GTGAAGATCGGACACTACGTG	CTGCCACTTTATGGCCTGTTA	MGH primer bank
ACTB	CATGTACGTTGCTATCCAGGC	CTCCTTAATGTCACGCACGAT	MGH primer bank
HPRT	CCTGGCGTCGTGATTAGTGAT	AGACGTTCAGTCCTGTCCATAA	MGH primer bank

Fatty acid profiling

Quantitative measurements on FA for cultured GSCs, plasma, and tissue were performed by OmegaQuant (Sioux Falls, SD) using gas chromatography (GC) with flame ionization detection. Homogenized tissues were extracted with a modified Folch extraction, and portions of the organic layer were used for analysis. Dried cell pellets or tissue extracts were transferred into glass vials and dried in a speed-vac before adding methanol containing 14% boron trifluoride, toluene, methanol; 35:30:35 v/v/v) (Sigma-Aldrich, St. Louis, MO). Vials were vortexed and heated at 100ºC for 45 min. Hexane (EMD Chemicals, USA) and HPLC grade water were added to the cooled samples, followed by vortexing and centrifugation to separate layers. An aliquot of the hexane layer was then transferred to a GC vial. GC was carried out using a GC-2010 Gas Chromatograph (Shimadzu Corporation, Columbia, MD) equipped with an SP-2560, 100-m fused silica capillary column (0.25-mm internal diameter, 0.2-um film thickness; Supelco, Bellefonte, PA). A standard mixture of fatty acids characteristic of RBC (GLCOQ-A,

NuCheck Prep, Elysian, MN) was used to identify fatty acids and determine individual fatty acid calibration curves. The following 24 fatty acids classes were detected: saturated (14:0, 16:0, 18:0, 20:0, 22:0 24:0); cis monounsaturated (16:1, 18:1, 20:1, 24:1); trans [16:1,18:1*, 18:2*); cis n-6 polyunsaturated (18:2, 18:3, 20:2, 20:3, 20:4, 22:4, 22:5); cis n-3 polyunsaturated (18:3, 20:5, 22:5, 22:6). The fatty acid composition was expressed as a percent of the identified fatty acids. SCD-mediated desaturation was determined by measuring the DI as follows: C16 DI= C16:1n-7/C16:0; C18 DI = C18:1n-9/C18:0.

Shotgun Lipidomics Analysis

83 cells were seeded at a density of $1x10^5$ cells/well in a 6-well plate and treated with DMSO (Control) or YTX-7739 (1 μM) in four replicates. At 72h after treatment, cells were counted and collected for lipid extraction. Shotgun lipidomics analysis was performed as previously described (*45*). Briefly, after the addition of an internal standard mixture consisting of 70 lipid standards across 17 subclasses (AB Sciex, 5040156, Avanti 330827, Avanti 330830, Avanti 330828, Avanti 791642), and two successive extractions, organic layers were vacuum dried in a Thermo SpeedVac SPD300DDA machine, using ramp setting 4 at 35ºC for 45 minutes with a total run time of 90 minutes. Samples were then resuspended in methanol/dichloromethane (1:1 ratio) with 10 mM Ammonium Acetate and transferred to robovials (Thermo 10800107) for subsequent lipidomics analysis. Samples were analyzed by direct infusion using the Sciex 5500 with Differential Mobility Device (DMS) (comparable to Sciex Lipidyzer platform) for quantitative measurement of 1450 lipid species belonging to 17 subclasses. The DMS was tuned with EquiSPLASH LIPIDOMIX (Avanti 330731). Quantitative values of lipid species were normalized to cell counts. Heatmaps and PCA plots were generated using Clustvis (https://biit.cs.ut.ee/clustvis/). The color scale on heatmaps refers to the row Z score. Nipals PCA was utilized to calculate principal components (PC) on PCA. X and Y axis show PC1 and PC2, which refer to the percentage of the total variance, respectively.

Immunocytochemistry and Lipid Droplet staining

Cells were fixed with cold acetone for 20 min. Cells were mounted on slides, dried, permeabilized with 0.1% Triton X-100, and blocked with 5% BSA for 1h at room temperature. Cells were then incubated overnight at 4°C with a p-ERK1/2 antibody (1:400). Fluorophore-conjugated secondary antibodies (Life Technologies, 1:100) were added for 1h. Nuclei were counterstained with DAPI (Life Technologies), mounted on a microscope slide, and analyzed by fluorescence microscopy. BODIPY 493/503 dissolved in ethanol (1mg/ml stock) was used to stain lipid droplets. Cells were washed 2 times with PBS and incubated with BODIPY 493/503 at 1µg/ml for 30 minutes before mounting and analysis with fluorescent microscopy. The number of lipid droplets per cell was manually counted in 100 cells for each condition.

Histological Analysis

Brains were directly collected after euthanasia, fixed in 4% paraformaldehyde, and cryoprotected in sucrose solution. Brain sections were prepared from fresh-frozen brains. Immunostaining for cleaved caspase 3 was performed by the Specialized Histopathology Core at the Dana-Farber/Harvard Cancer Center. Brain sections were then mounted on a microscope slide, and analyzed using LSM 710, AxioObserver microscope (Zeiss).

Bioluminescence BLRR assay to monitor DNA damage repair

The BLRR system (14) was used to track DNA damage repair through Homology directed repair (HDR) and non-homologous end-joining (NHEJ), as previously described (*46*). In brief, GSCs were sequentially transduced to express the three components of this reporter: BLRR, trGluc, and I-SceI. To detect BLRR activity, GSCs were seeded in a 96-well plate and treated with YTX-7739. On day 3 post-treatment, an aliquot of the conditioned medium (25µl) was collected and transferred into a 96-well white plate to measure bioluminescence using a multimode reader (Biotek). Gaussia luciferase (Gluc; HDR) and Vargula luciferase (Vluc; NHEJ) activities were measured by adding coelenterazine (20µM; Nanolight) and Vargulin (5ng/mL; Nanolight), respectively. All bioluminescence results were normalized to cell viability measured using CellTiter-Glo.

Mouse Pharmacokinetics and Pharmacodynamics studies

Mouse PK studies were performed by ChemPartner (Shanghai, China) in an IACUC-accredited facility, following the guidelines of their ethics committee. Male CD1 mice were used in these studies with n=3mice/group. YTX-7739 was administered at 1 mg/kg intravenously (IV) in the foot dorsal vein (formulated in 10% DMSO/10% Solutol HS 15/80% HP-β-CD in saline) or IP at 3, 10, and 30 mg/kg (formulated in 0.5% methylcellulose in water). Approximately 150 µL blood/time point was collected at each time point into K_2EDTA tubes via the tail vein. Blood samples were prepared and processed as previously described (8). Samples were analyzed using standard LCMS/MS methods (API6500, Qtriple) and compared to a reference standard. Brain levels of YTX-7739 were determined from homogenized tissue prior to LC-MS/MS analysis. YTX-7739 concentrations were compared to a standard curve of YTX-7739 spiked into naïve brain homogenate. Pharmacokinetic (PK) parameters were determined using WinNonlin.

To measure the PD of YTX-7739 in plasma, brain, and tumor tissue, female athymic nude mice bearing 83 GSCs-Fluc brain tumors (confirmed using Fluc imaging) were treated for 11 days with solvent control or YTX-7739 (30mg/kg). Mice were sacrificed 4h after the last YTX-7739 injection. Plasma and brain tissue samples which include the brain tumor as well as the contralateral hemisphere (brain), were collected for fatty acid profiling.