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Supplemental Information

Stearoyl CoA Desaturase Is Essential for Regulation of Endoplasmic Reticulum Homeostasis and Tumor Growth in Glioblastoma Cancer Stem Cells

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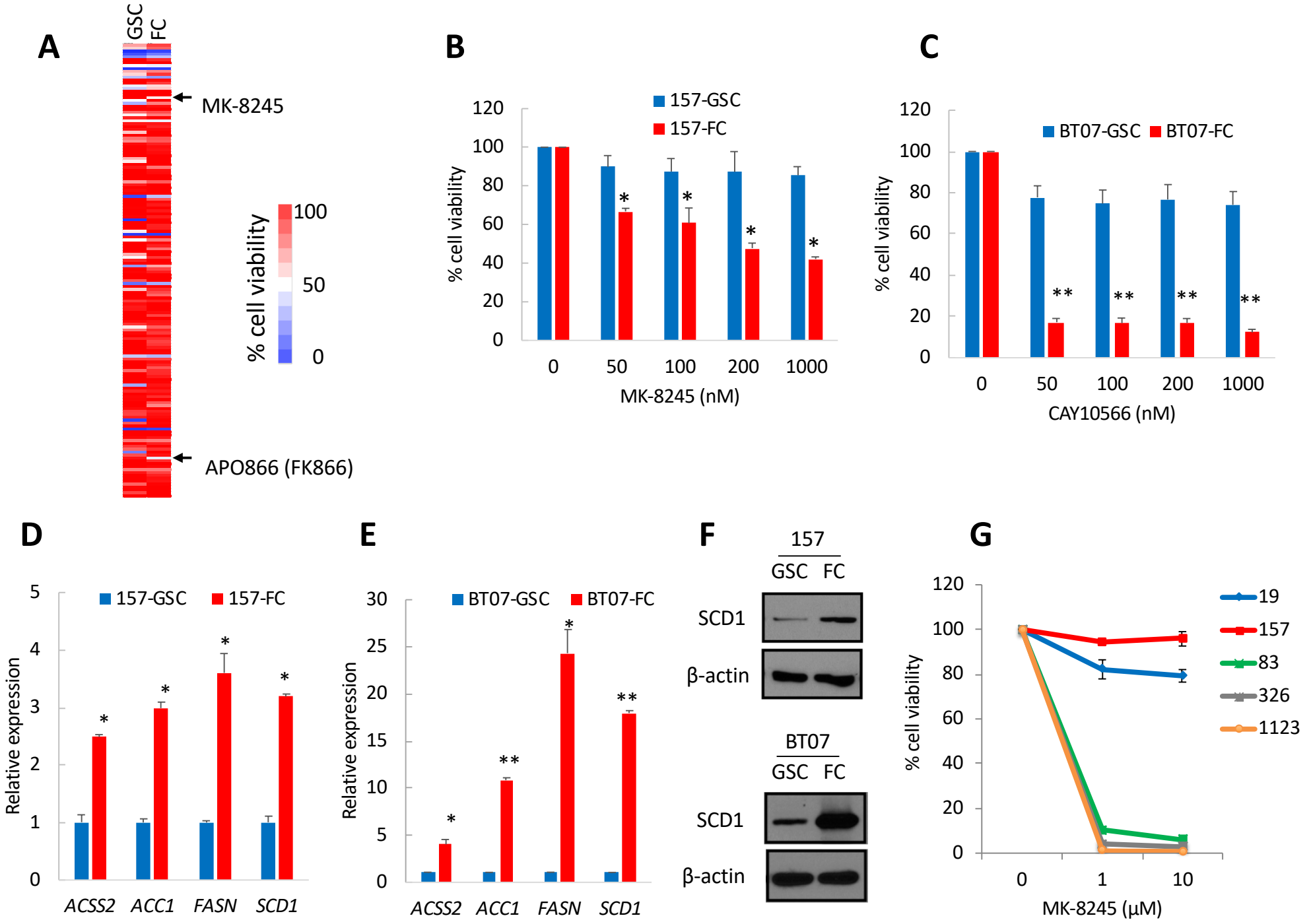
A
GSC
FC
MK-8245
APO866 (FK866)
% cell viability
100
50
0
B
157-GSC
157-FC
% cell viability
MK-8245 (nM)
0
50
100
200
1000
C
BT07-GSC
BT07-FC
% cell viability
CAY10566 (nM)
D
157-GSC
157-FC
Relative expression
ACSS2
ACC1
FASN
SCD1
E
BT07-GSC
BT07-FC
Relative expression
F
157
GSC
FC
SCD1
β-actin
BT07
G
% cell viability
MK-8245 (μM)
19
157
83
326
1123

Figure S1: SCD1 is a therapeutic target in highly proliferative GSC populations. Related to first paragraph in Results section. **A.** Targeted inhibitors screening in MGG23-GSCs and MGG23-FC. Heat map representing cell viability expressed at % of DMSO treated control, measured after 3 days of treatment. As shown, only two inhibitors for SCD1 (MK-8245) and NAMPT (APO866) resulted in increased cell death in FC compared to the parental GSCs. **B-C.** Two GSCs (157 and BT07) and their matching FC were treated with different doses of the indicated SCD1 inhibitors. Cell viability was measured 5 days after treatment. **D-E.** Relative mRNA expression of the indicated SREBP1 target genes in 157 and BT07 GSCs and their matching FC. **F.** Protein expression of SCD1 in 157 and BT07 GSCs and FC. **G.** Cell viability in proneural GSCs (19;157) and mesenchymal GSCs (83; 326; 1123) treated with MK-8245 for 4 days. *$p<0.05$; **$p<0.001$; Student t-test.

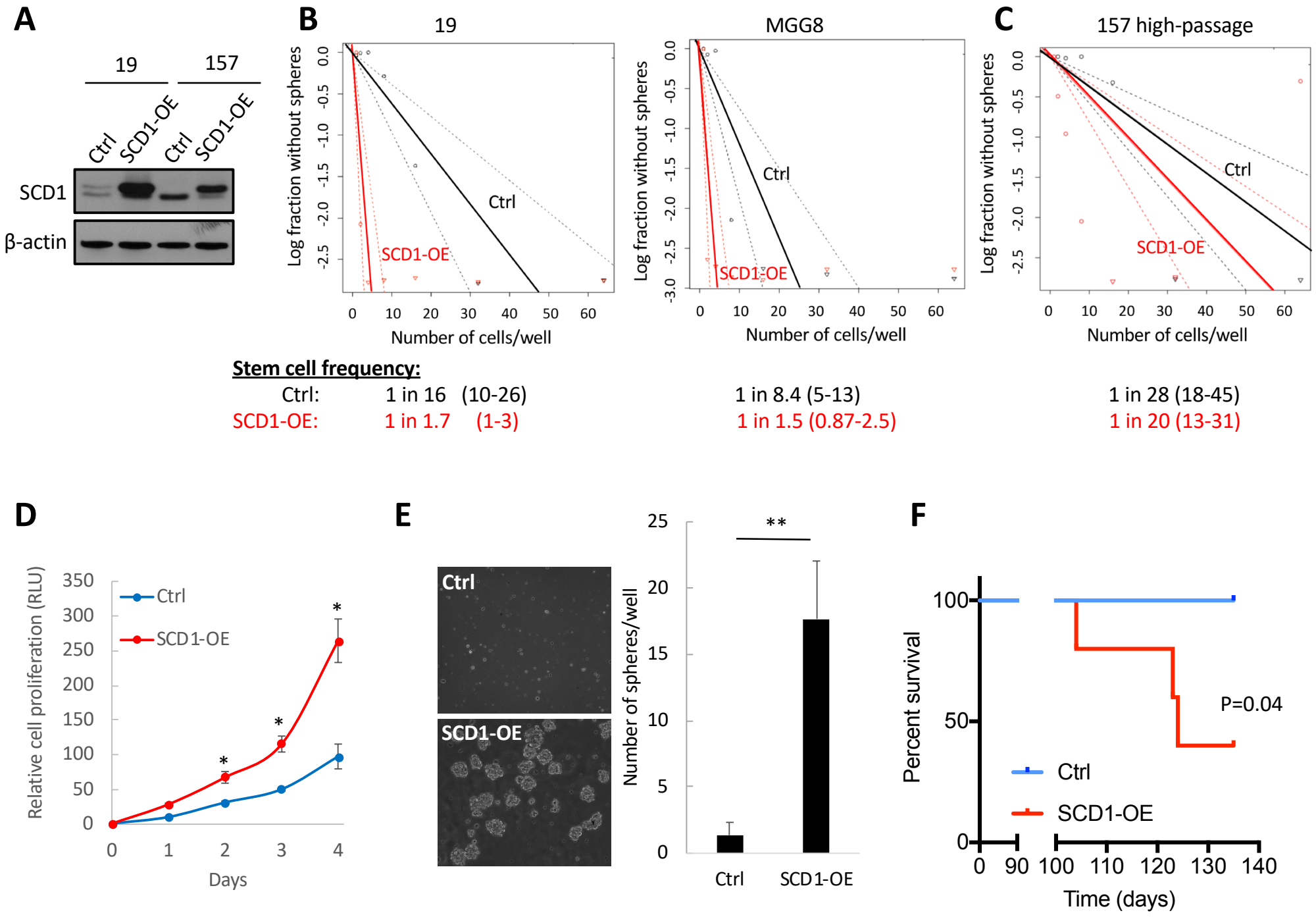
A
19
157
Ctrl
SCD1-OE
Ctrl
SCD1-OE
SCD1
β-actin
B
19
MGG8
C
157 high-passage
Log fraction without spheres
Number of cells/well
Ctrl
SCD1-OE
Stem cell frequency:
Ctrl: 1 in 16 (10-26) 1 in 8.4 (5-13) 1 in 28 (18-45)
SCD1-OE: 1 in 1.7 (1-3) 1 in 1.5 (0.87-2.5) 1 in 20 (13-31)
D
Relative cell proliferation (RLU)
Days
Ctrl
SCD1-OE
E
Number of spheres/well
**
F
Percent survival
Time (days)
P=0.04

Figure S2: SCD1 is essential for GSC maintenance and tumor initiation. Related to Figure 1. **A.** Immunoblot showing upregulated expression of SCD1 after transduction of two GSCs with lentivirus control or SCD1-OE. **B-C** Stem cell frequency in 19 and MGG8 GSCs (A) or high-passage 157 GSCs (B) transduced with control (Ctrl) or SCD1-expressing lentivirus (SCD1-OE) determined using the limited dilution analysis algorithm. **D.** Cell proliferation in high-passage 157 GSCs expressing Ctrl or SCD1-OE. **E.** The same cells were dissociated into single cells and plated at a density of 1 cell/µl. Secondary spheres were counted after 10 days. **F.** High-passage 157-Fluc GSCs ($5x10^4$) expressing a control lentivirus (Ctrl) or SCD1 (SCD1-OE) were implanted in the brain of nude mice (n=5/group). Kaplan-Meier curves showing median survival in both groups. (P values, two-sided log-rank test). *$p<0.05$; **$p<0.001$; Student t-test.

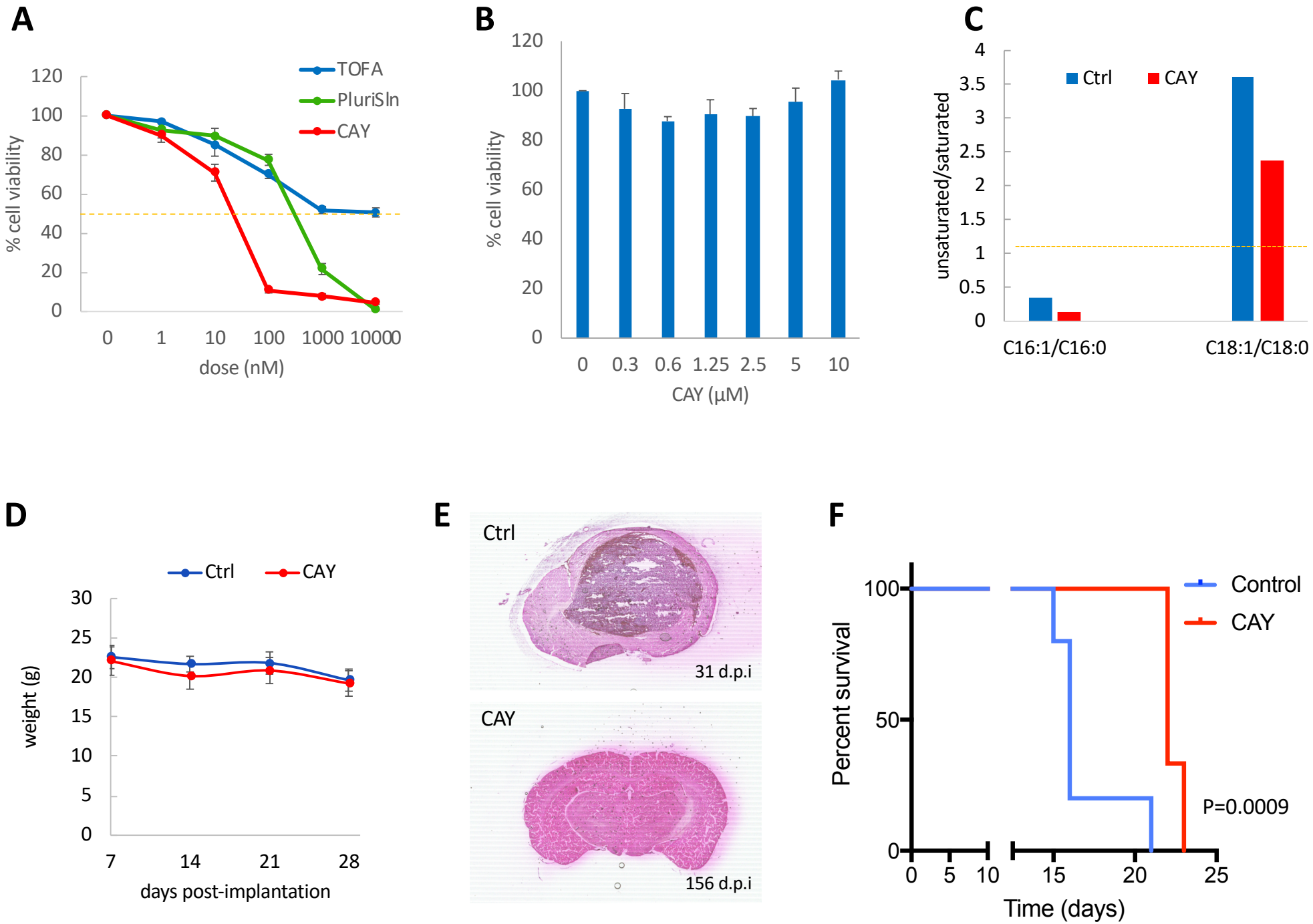
A
120
100
80
60
40
20
0
% cell viability
TOFA
PluriSln
CAY
0
1
10
100
1000
10000
dose (nM)
B
120
100
80
60
40
20
0
% cell viability
0
0.3
0.6
1.25
2.5
5
10
CAY (µM)
C
4
3.5
3
2.5
2
1.5
1
0.5
0
unsaturated/saturated
Ctrl
CAY
C16:1/C16:0
C18:1/C18:0
D
Ctrl
CAY
30
25
20
15
10
5
0
weight (g)
7
14
21
28
days post-implantation
E
Ctrl
31 d.p.i
CAY
156 d.p.i
F
100
50
0
Percent survival
Control
CAY
P=0.0009
0
5
10
15
20
25
Time (days)

Figure S3: Pharmacological targeting of SCD1. Related to Figures 2 and 3. **A.** GSCs were treated with the indicated inhibitors. Cell viability was measured 4 days after treatment. **B.** Cell viability in NHA treated with CAY in serum-free medium at the indicated doses for 4 days. **C.** Ratios of C16:1/C16:0 and C18:1/C18:0 in GSCs treated with CAY (200nM) for 48h. This comparison shows an abundance of C18:1 as compared to C16:1 prior to CAY treatment (since C18:1/C18:0 >1) and depicts a decrease in unsaturated fatty acids (C16:1 and C18:1) after treatment. **D.** Average weight of mice from Control and CAY-treated groups during and post-treatment shows no significant change. **E.** Brains from mice from the control group were isolated at day 25-31 post-implantation (dpi). Similarly brains of the 2 remaining mice from the CAY treated group were isolated at day 156 post-implantation. Brain sections were analyzed by H&E staining. Micrographs from one representative mouse per group are shown. **F.** Mice bearing 83-Fluc tumors (n=5/group) were treated with Ctrl (n=5) or CAY (n=6; 1.5 mg/kg) for 14 days starting at day 6 after implantation. Kaplan-Meier curves showing median survival for Control (16 days) and CAY10566 treated mice (22 days) with P=0.009; two-sided log-rank test. *$p<0.05$; Student t-test.

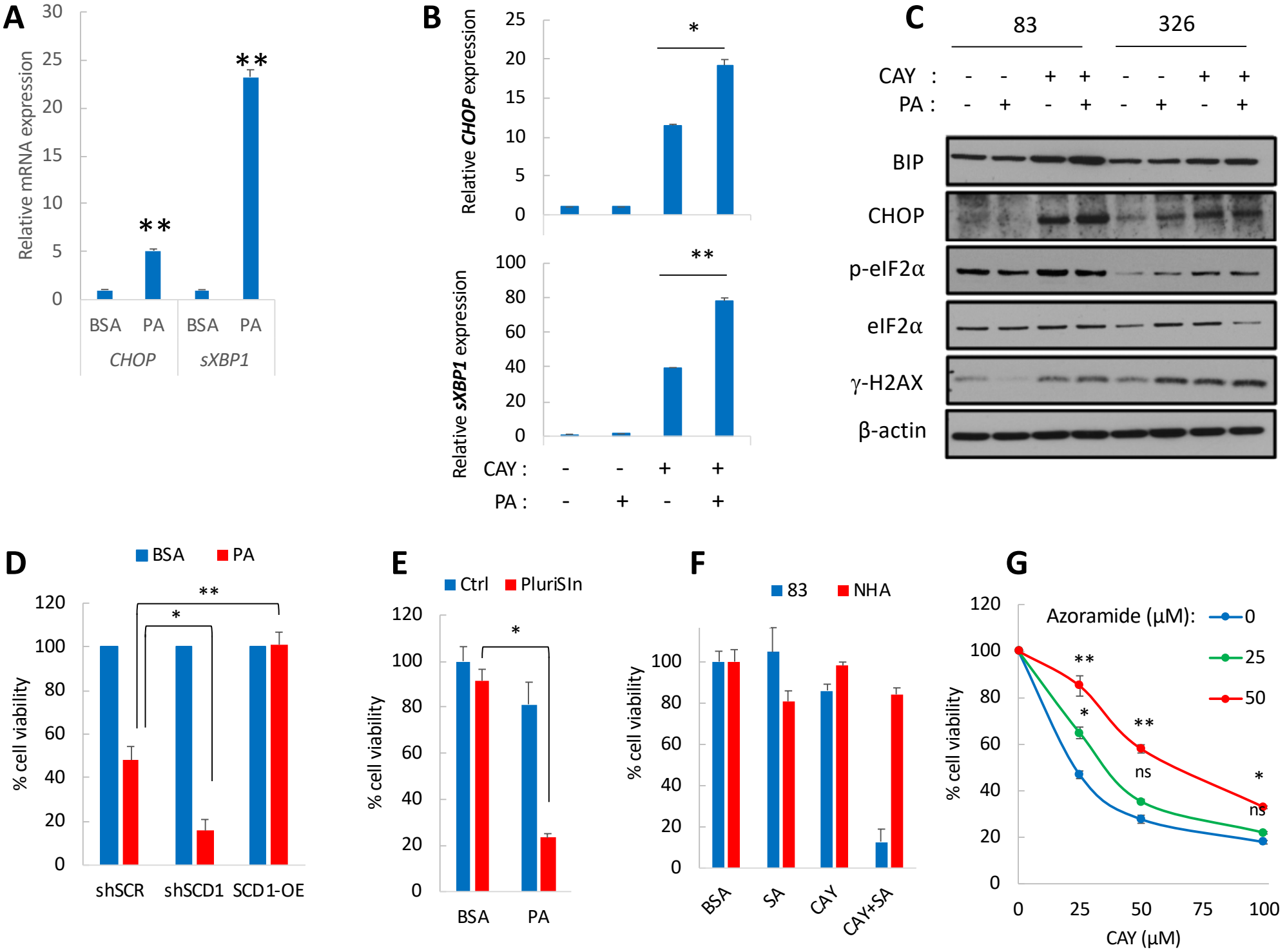
A
Relative mRNA expression
30
25
20
15
10
5
0
**
**
BSA
PA
BSA
PA
CHOP
sXBP1
B
Relative CHOP expression
25
20
15
10
5
0
*
Relative sXBP1 expression
100
80
60
40
20
0
**
CAY : - - + +
PA : - + - +
C
83
326
CAY : - - + + - - + +
PA : - + - + - + - +
BIP
CHOP
p-eIF2α
eIF2α
γ-H2AX
β-actin
D
BSA
PA
% cell viability
120
100
80
60
40
20
0
**
*
shSCR
shSCD1
SCD1-OE
E
Ctrl
PluriSIn
% cell viability
120
100
80
60
40
20
0
*
BSA
PA
F
83
NHA
% cell viability
100
80
60
40
20
0
BSA
SA
CAY
CAY+SA
G
Azoramide (μM): 0
25
50
% cell viability
120
100
80
60
40
20
0
**
*
**
ns
*
ns
0
25
50
75
100
CAY (μM)

Figure S4: SCD1 inhibition promotes ER stress. Related to Figure 4. **A.** Relative mRNA expression of the ER stress markers *CHOP* and *sXBP1* in 83 GSCs treated with PA (400 μM) for 24h. **B.** Relative mRNA expression of *CHOP* and *sXBP1* in 83 GSCs treated with CAY (50nM) and PA (50 μM) for 24h. **C.** Immunoblot analysis of the indicated ER stress and DNA damage markers in two GSCs treated with CAY (50nM) and PA (50 μM) for 24h. **D.** Cell viability in GSCs expressing shSCR, shSCD1 or SCD1-OE and treated with PA (200 μM) for 4 days. **E.** Cell viability in GSCs treated with PluriSIn (1 μM) in the presence or absence of PA (50 μM) for 3 days. **F.** 83 GSCs and NHA co-treated with CAY (10nM for 83 and 1 μM for NHA) and Stearic Acid (SA; 200 μM). Cell viability was measured after 4 days. **G.** Treatment of GSCs with Azoramide (25-50 μM) protects against CAY-induced cell death. *$p<0.05$; **$p<0.001$; ns: not significant; Student t-test.

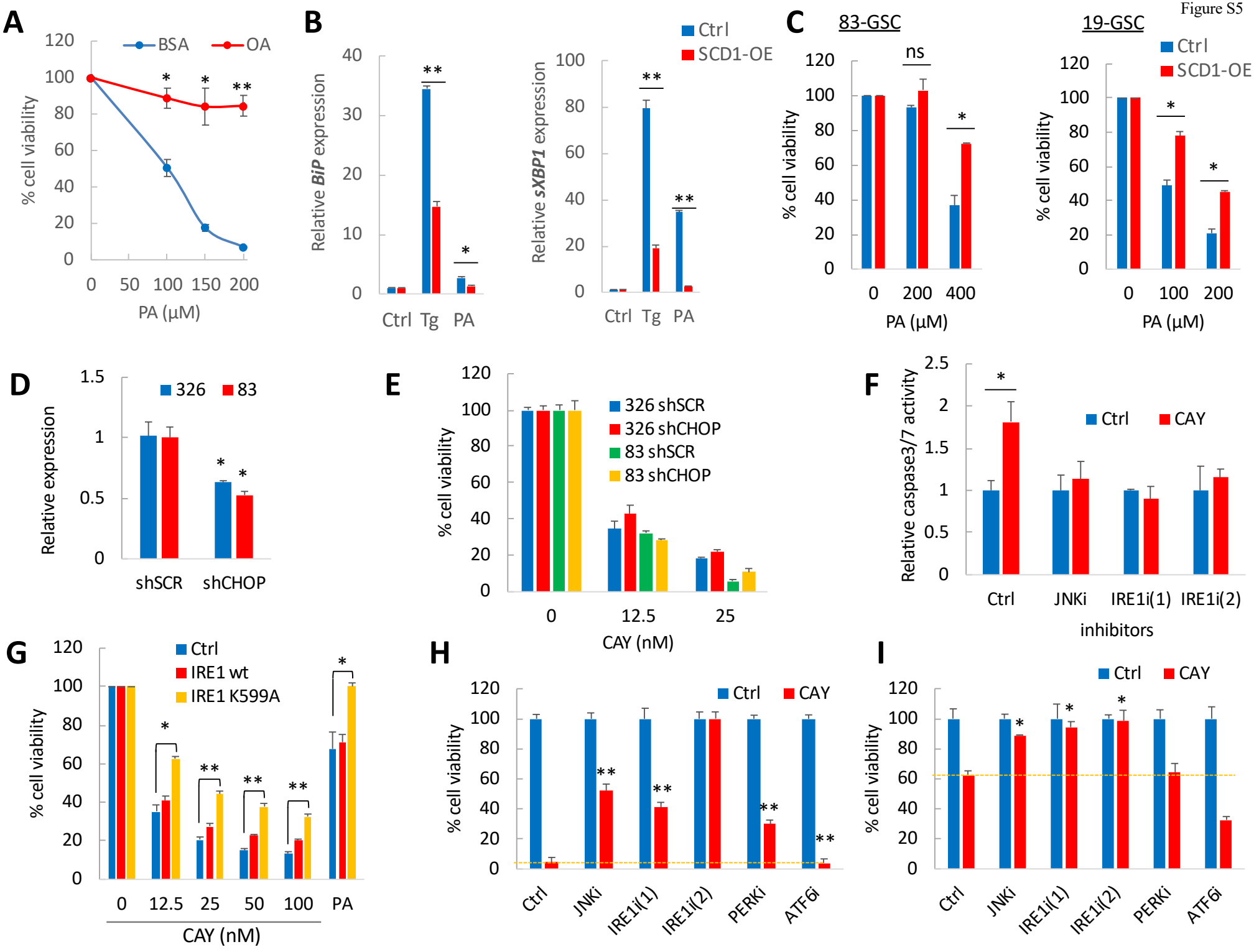
A
BSA
OA
% cell viability
PA (μM)
B
Relative BiP expression
Relative sXBP1 expression
Ctrl
SCD1-OE
Ctrl Tg PA
C
83-GSC
19-GSC
ns
% cell viability
PA (μM)
D
326
83
Relative expression
shSCR shCHOP
E
326 shSCR
326 shCHOP
83 shSCR
83 shCHOP
% cell viability
CAY (nM)
F
Relative caspase3/7 activity
Ctrl
CAY
Ctrl JNKi IRE1i(1) IRE1i(2)
inhibitors
G
Ctrl
IRE1 wt
IRE1 K599A
% cell viability
CAY (nM)
H
Ctrl
CAY
% cell viability
Ctrl JNKi IRE1i(1) IRE1i(2) PERKi ATF6i
I
Ctrl
CAY
% cell viability
Ctrl JNKi IRE1i(1) IRE1i(2) PERKi ATF6i

Figure S5: SCD1-mediated UFA synthesis mitigates ER stress and protects from UPR-induced apoptotic signaling. Related to Figure 4. **A.** Treatment of GSCs with OA (50 μM) protects against PA-induced cell death assessed 4 days after treatment. **B.** Relative mRNA expression of *sXBP1* and *BiP* in GSCs expressing an empty vector (Ctrl) or SCD1 and treated with Thapsigargin (Tg; 300nM) or PA (400 μM). **C.** SCD1 overexpression protects against PA-mediated cell death as shown in 2 GSCs specimens. **D.** mRNA expression of CHOP following shRNA mediated knockdown of this gene in 2 GSCs. **E.** Silencing of CHOP fails to alter CAY-mediated cytotoxicity in 2 GSCs as detected by measuring cell viability 4 days after treatment. **F.** Fold change in caspase 3/7 activation after treatment with CAY (50nM) in the presence of JNK inhibitor (SP 600125; 20 μM) or IRE1 inhibitors (4μ8C: 25 μM and KIRA6: 5 μM). **G.** 83 GSCs stably expressing a control vector, IRE1 (wild-type) or mutant IRE1 K599A were treated with CAY or PA (300 μM). Cell viability was assessed after 3 days. **H-I.** Cell viability of 83 (H) and L0 (I) GSCs treated with CAY (50nM) or the combination of CAY with: JNK inhibitor (SP 600125; 20 μM), IRE1 inhibitors (4μ8C: 25 μM and KIRA6: 5 μM), PERK inhibitor (GSK2656157; 10 μM) and ATF6 inhibitor (Ceapin A7: 20 μM) for 4 days. *$p<0.05$; **$p<0.001$; ns: not significant; Student t-test.

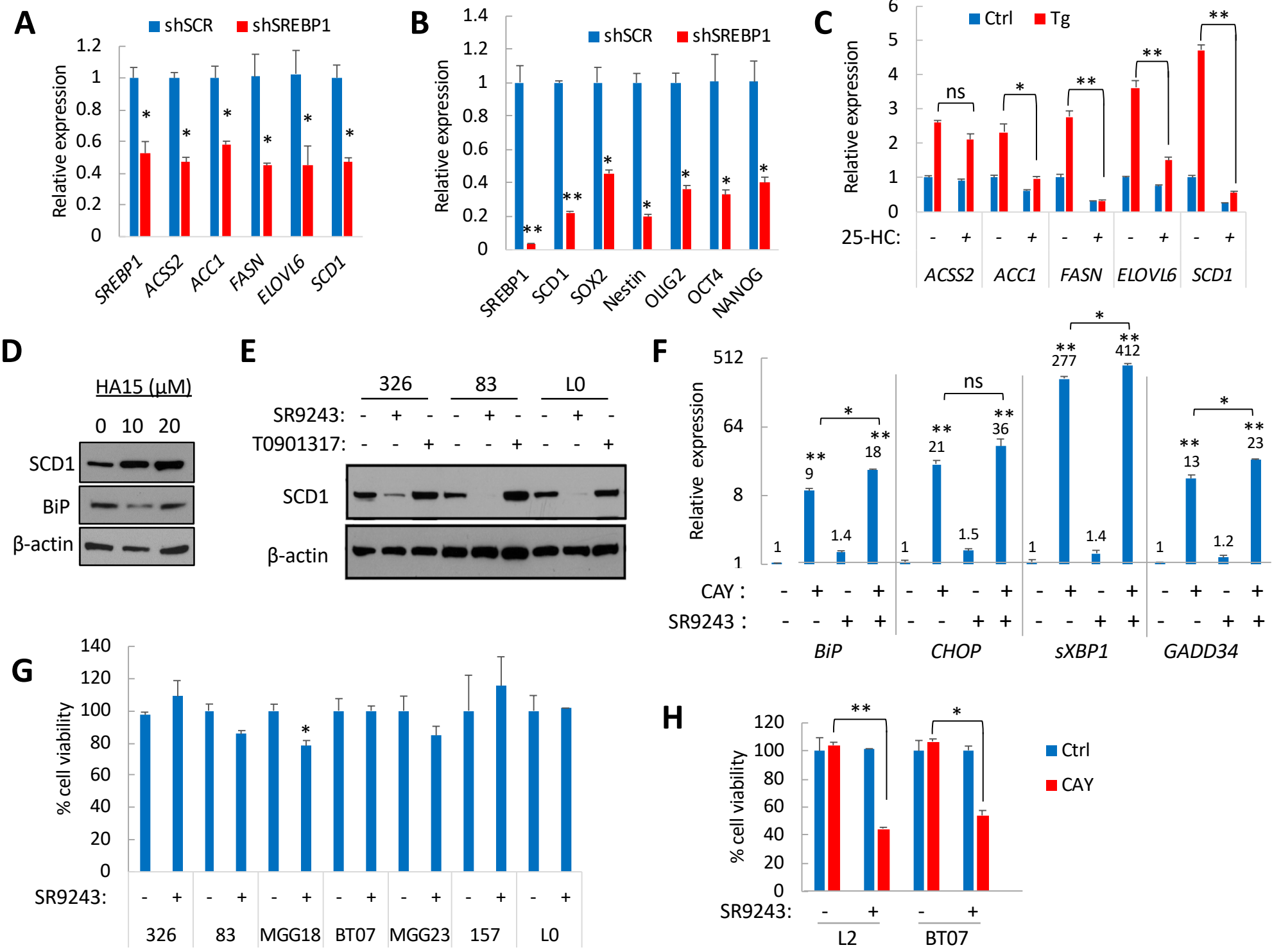
A
shSCR
shSREBP1
Relative expression
SREBP1
ACSS2
ACC1
FASN
ELOVL6
SCD1
B
shSCR
shSREBP1
Relative expression
SREBP1
SCD1
SOX2
Nestin
OLIG2
OCT4
NANOG
C
Ctrl
Tg
Relative expression
25-HC:
ACSS2
ACC1
FASN
ELOVL6
SCD1
D
HA15 (μM)
0 10 20
SCD1
BiP
β-actin
E
326
83
L0
SR9243:
T0901317:
SCD1
β-actin
F
Relative expression
CAY :
SR9243 :
BiP
CHOP
sXBP1
GADD34
G
% cell viability
SR9243:
326
83
MGG18
BT07
MGG23
157
L0
H
% cell viability
Ctrl
CAY
SR9243:
L2
BT07

Figure S6. ER stress promotes SCD1 expression through SREBP1 activation. Related to Figures 5. **A.** Relative expression of SREBP1 target genes after shRNA-mediated silencing of SREBP1 in 326-GSCs. **B.** Relative expression of SREBP1, SCD1 as well as the stem cell markers *SOX2*, *NESTIN*, *OLIG2*, *OCT4* and *NANOG* in BT07 GSCs expressing shSREBP1. **C.** Relative expression of SREBP1 target genes in GSCs treated with Tg (300nM) in the presence or absence of 25-HC (50 μM). **D.** Immunoblot analysis of SCD1 and BiP expression in GSCs treated with HA15 at the indicated doses. **E.** Immunoblot analysis of SCD1 expression in GSCs treated with SR9243 (20 μM) or T0901317 (25 μM) for 24h. **F.** mRNA expression of ER stress markers following treatment with CAY (100nM) and SR9243 (20 μM) for 48h. **G.** Cell viability of the indicated GSCs specimens after treatment with SR9243 (20 μM) for 5 days. **H.** Two GSCs were treated with CAY (25nM) and SR9243 (10 μM), and cell viability was assessed after 4 days. *$p<0.05$; **$p<0.001$; Student t-test.

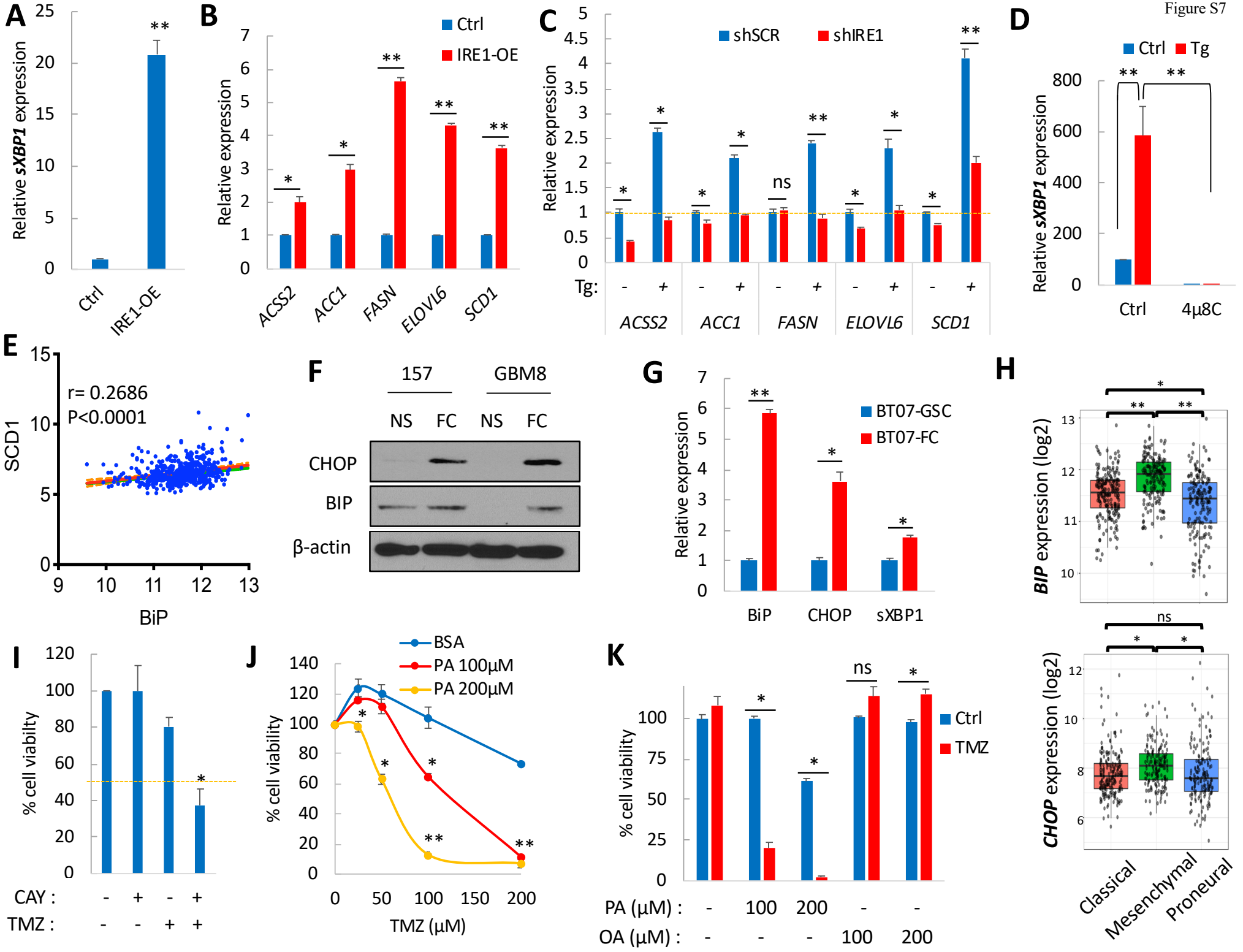
A
Relative sXBP1 expression
Ctrl
IRE1-OE
B
Relative expression
Ctrl
IRE1-OE
ACSS2
ACC1
FASN
ELOVL6
SCD1
C
Relative expression
shSCR
shIRE1
Tg:
ACSS2
ACC1
FASN
ELOVL6
SCD1
D
Relative sXBP1 expression
Ctrl
Tg
Ctrl
4μ8C
E
r= 0.2686
P<0.0001
SCD1
BiP
F
157
GBM8
NS
FC
NS
FC
CHOP
BIP
β-actin
G
Relative expression
BT07-GSC
BT07-FC
BiP
CHOP
sXBP1
H
BIP expression (log2)
CHOP expression (log2)
ns
Classical
Mesenchymal
Proneural
I
% cell viability
CAY :
TMZ :
J
BSA
PA 100μM
PA 200μM
% cell viability
TMZ (μM)
K
% cell viability
Ctrl
TMZ
PA (μM) :
OA (μM) :
ns

Figure S7: SCD1 is activated through ER stress-mediated IRE1 signaling and its inhibition sensitizes to TMZ. Related to Figures 6 and 7. **A.** Relative expression of *sXBP1* after doxycycline-induced expression of IRE1. **B.** Relative expression of SREBP1 target genes after IRE1 expression. **C.** Relative mRNA expression of SREBP1 target genes in GSCs transduced with shSCR or shIRE1 and subsequently treated with Tg (300nM). **D.** Relative expression of *sXBP1* after treatment with Tg (300nM) and the IRE1 inhibitor 4μ8C (25 μM) for 24h. **E.** SCD1 mRNA expression in the TCGA GBM dataset (n= 528 patients; platform: HG-133A) positively correlates with BiP expression. **F.** Immunoblot analysis for BiP and CHOP expression in two GSCs and their matching FC. **G.** mRNA expression of BIP, CHOP and sXBP1 in BT07 GSC and their FC. **H.** mRNA expression of *BiP* and *CHOP* in GBM subtypes from the TCGA dataset and plotted using Gliovis data portal. **I.** Cell viability in TMZ-resistant MGG23 GSCs pretreated with CAY (50 nM) for 24h followed by TMZ (200 μM) for 5 days. **J.** Treatment of L0 GSCs with PA increases the cytotoxicity of TMZ (200 μM). Cell viability was measured 5 days after treatment. **K.** Cell viability of L1 GSCs treated with TMZ (200 μM) in combination with PA or OA at the indicated doses for 4 days. *$p<0.05$; **$p<0.001$; ns: not significant; Student t-test.

SUPPLEMENTAL EXPERIMENTAL PROCEDURES

Cell culture

Primary GBM cells used in this study 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 or provided by Dr. Ichiro Nakano (GSCs: 157, 19, 326, 83, 1123) and Dr. Brent Reynolds (GSCs: L0, L1, L2). All specimens used in this study have been previously characterized (see table below) (Wakimoto et al., 2009; Wakimoto et al., 2012) (Mao et al., 2013) (Siebzehnrubl et al., 2013) (Teng et al., 2017). 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). ReNcell VM (Millipore) were expanded under similar conditions. Primary human astrocytes were purchased from ScienCell and cultured in complete astrocyte medium (ScienceCell). All cells were maintained at 37°C in humidified 5% CO_2 incubators and regularly tested for potential mycoplasma contamination.

Specimen	diagnosis	SOX2	CD44	EGFR	Subtype	Reference
MGG18	GBM		+	ND	PN	Wakimoto et al.,2009, 2012
MGG8	GBM	+	-	ND	PN	Wakimoto et al.,2009, 2012
MGG23	GBM	-	+	ND	M	Wakimoto et al.,2009, 2012
BT07	GBM	+	-	ND	PN	Teng et al., 2017
83	GBM	-	+	+	M	Mao et al., 2013
326	N/A	-	+	+	M	Mao et al., 2013
1123	Gr III Oligo,1p19q del	-	+	+	M	Mao et al., 2013
157	GBM	+	-	-	PN	Mao et al., 2013
19	GBM	+	-	-	PN	Mao et al., 2013
L0	GBM	+	-	+	M-like	Siebzehnrubl et al., 2013
L1	GBM	+	+	+	M-like	Siebzehnrubl et al., 2013
L2	GBM	+	-	+	M-like	Siebzehnrubl et al., 2013

Patient derived GSCs were classified into Proneural (PN), Mesenchymal (M) or Mesenchymal-like based on the predominant expression of PN markers or M markers. The expression profile of SOX2, CD44 and EGFR is indicated as follows: High (+), low (-) or Intermediate (+/-). ND: Not determined.

Floating cells subpopulations

Floating cells (FC) collection and culture was performed as previously described (Teng et al., 2017). In brief, GSCs were cultured as monolayer in the presence of 10% fetal bovine serum (FBS) for 7-10 days. Non-adherent cells (FC) were collected from the supernatant and pelleted by centrifugation. Single FC were then expanded in serum-free stem cell medium.

Chemical reagents and Antibodies

CAY10566 was purchased from Cayman and Glixx Labs. Ceapin A7 (ATF6i) was provided by Dr. Peter Walter (Gallagher et al., 2016). The following compounds were obtained from Cayman Chemicals: PluriSIn; 4µ8C; KIRA6; GSK2656157; SP 600125; T0901317 and SR9243. MK-8245 and APO866 were obtained from SelleckChem, HA15, 25-Hydroxycholesterol and Thapsigargin from Sigma, Temozolomide from MedChemExpress. All fatty acids were obtained from Cayman and dissolved in DMSO to yield a stock concentration of 500mM- 3M. Fatty acids stocks were

subsequently complexed to fatty acid-free Bovine Serum Albumin (BSA; 2.5mM; Gold Biotechnology) and added to the culture medium.
The following antibodies were purchased from Cell Signaling Technologies: SCD1 (2438); BiP (3177); phospho SAPK/JNK (9255); SAPK/JNK (9252); phospho-eIF2α (3597); γ-H2AX (9718); Nestin (4760); β-actin (3700); Anti-rabbit IgG, HRP-linked Antibody (7074) and Anti-mouse IgG, HRP-linked Antibody. Rad51 (BSM-51402M) and IRE1 (BS-8680R) were obtained from BIOSS Antibodies, CHOP (sc-7351) from Santa Cruz, SOX2 (ab97959), Nestin (ab22035) and eIF2α (ab169528) from AbCam.

DNA constructs and Lentiviral vectors
To generate a lentiviral vector expression SCD1, the human SCD1 open reading frame was amplified by PCR (plasmid template obtained from the DNA Resource Core at Harvard Medical School, HMS) and subcloned into a CSCW-IRES-CFP lentivirus backbone thus generating CSCW-SCD1-IRES-CFP. This construct was verified by restriction digestion and sequencing.
CSCW-Fluc-IRES-mCherry lentivirus vector carrying an expression cassette for firefly luciferase (Fluc) and mCherry fluorescent protein was used for in vivo imaging. CSCW-IRES-CFP or pHAGE-CMV-MCS-IRES-ZsGreen (obtained from DNA Resource core, HMS) were used as control lentivirus vectors in all experiments. Doxycycline-inducible IRE1 wild-type (IRE1) or a kinase/endoribonuclease-inactive dominant-negative IRE1 (IRE1-K599A) were a gift from Fumihiko Urano (Addgene plasmids 20744 and 20745(Lipson et al., 2008)). Transcriptional activity was induced by treatment with Doxycycline (10 μM; Cayman) for at least 24h.
The following shRNA bacterial glycerol stocks: shSCD1 (TRCN0000327814), shSREBF1 (TRCN0000434619), shCHOP (TRCN0000364393), shIRE1 (TRCN0000000529) as well as a non-targeting control shRNA (pLKO.1-puro Non-Target shRNA Control) were obtained from Sigma (MISSION® shRNA Library), amplified and packaged into lentivirus vectors. GSCs were stably transduced with shRNA lentivirus and selected using puromycin (1 μg/mL). Knockdown efficiency was determined using immunoblotting and/or qRT-PCR.

Mass spectrometry profiling of Fatty acids
Fatty acids were analyzed as methyl ester derivatives (FAME) using gas chromatography/mass spectrometry (GC/MS) analysis. Sample preparation and fatty acid analysis were performed at the Small Molecule Mass Spectrometry Facility (Harvard University). GSCs were treated with CAY for 48 hours, cells were counted and fixed with methanol then dehydrated using a freeze-dry cycle. For extraction and preparation of fatty acids, samples were resuspended in methanol (500 μl) prior to adding the internal standard d27C14 (2mg/mL), hexane (400 μl) and acetyl chloride (100 μl). Samples were then vortexed, incubated in an ultrasound water bath with glass beads for 10 min followed by a 15 min incubation at 105°C. After an overnight incubation at 4 °C, hexane (500 μl) and water (1mL) were added and mixed. The hexane phase was separated by 10 min centrifugation (4000 rpm) and evaporated to dryness under N2 flow. Sample were resuspended in hexanes (100 μl) and ran on a Thermo Q exactive GC Orbitrap GC-MS/MS System. A FAME standard (Sigma) was run after all the samples to confirm ID of the FAME detected in the samples.
To determine an approximate comparison of concentrations of different fatty acids, we calculated an estimated normalized response as follows: 1. Since C14 is constant between samples, each area is divided by the area of C14 in the same sample. This should correct for biomass/extraction differences. This is a normalized area. 2. Using the standard reference, we calculated the ratio between normalized area and the known concentration of those fatty acids in the standard. 3. For fatty acids not present in the reference standard, the normalization factors are estimated by taking the factor corresponding to the fatty acid in the standard that is the closest (in number of carbon and insaturation). 4. Estimated normalized response is calculated for all the fatty acid. This is only an estimation, so small difference in response between fatty acids within a sample are not significant. 5. Difference between samples for each individual fatty acid are not dependent on this estimated response factor and are therefore significant.

ELDA assay
Stem cell frequency was calculated using the extreme limiting dilution analysis (ELDA) algorithm(Hu and Smyth, 2009) (http://bioinf.wehi.edu.au/software/elda/index.html). GSCs were dissociated then cells were counted and seeded into a 96-well plate at different cell numbers in 8-replicates. After 7-10 days, neurospheres of > 50 μm in diameter were counted. To determine secondary sphere formation, GSCs were dissociated into single cells and plated in eight replicates at a density of 1 cell per μl. Neurospheres were counted after 7-10 days.

Quantitative RT-PCR
Total RNA isolation was performed with RNeasy kit (Qiagen) followed by reverse transcription with OneScript cDNA synthesis Kit (ABM). qPCR reactions were performed using PowerUp SYBR Green Master Mix (Thermo Fisher) in a QuantStudio 3 Real-Time PCR system (AppliedBiosystems). All primer sequences used in this study are listed in

the table below. Oligonucleotides were synthesized by the CCIB DNA Core Facility at the Massachusetts General Hospital. qPCR runs included both human β-actin and HPRT as internal normalization controls.

GENE	Forward primer 5'	Reverse primer 5'	Source/Reference
SCD1	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
SREBP1	ACAGTGACTTCCCTGGCCTAT	GCATGGACGGGTACATCTTCAA	MGH primer bank
NAMPT	CGGCAGAAGCCGAGTTCAA	GCTTGTGTTGGGTGGATATTGTT	MGH primer bank
FASN	AAGGACCTGTCTAGGTTTGATGC	TGGCTTCATAGGTGACTTCCA	MGH primer bank
ACC1	GCAGGTCACACGTCTCTTTAT	CCAGCCTGTCATCCTCAATATC	This paper
ACSS2	AAAGGAGCAACTACCAACATCTG	GCTGAACTGACACACTTGGAC	MGH primer bank
ELOVL6	AACGAGCAAAGTTTGAACTGAGG	TCGAAGAGCACCGAATATACTGA	MGH primer bank
SOX2	AACCCCAAGATGCACAACTC	GCTTAGCCTCGTCGATGAAC	MGH primer bank
NESTIN	AACAGCGACGGAGGTCTCTA	TTCTCTTGTCCCGCAGACTT	MGH primer bank
OCT4	AGTGAGAGGCAACCTGGAGA	ACACTCGGACCACATCCTTC	MGH primer bank
NANOG	TTCCTTCCTCCATGGATCTG	TCTGCTGGAGGCTGAGGTAT	MGH primer bank
OLIG2	CTCCTCAAATCGCATCCAGA	AGAAAAAGGTCATCGGGCTC	MGH primer bank
ACTB	CATGTACGTTGCTATCCAGGC	CTCCTTAATGTCACGCACGAT	MGH primer bank
HPRT	CCTGGCGTCGTGATTAGTGAT	AGACGTTCAGTCCTGTCCATAA	MGH primer bank

TCGA analysis

Datasets for mRNA expression and survival of GBM patients were obtained from the TCGA using Gliovis data portal (Bowman et al., 2017). Data plotting and statistical analysis were performed using GraphPad Prism.

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