IRE1α RNase-dependent lipid homeostasis promotes survival in Myc-transformed cancers

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Supplementary Material Index

Supplementary Figure Legends

Figure S1. **ER stress and IRE1α-XBP1 signaling are increased in human and murine c-Myc transformed cancers.** (**A**) Correlation between *HSPA5* and an established Myc signature (see Methods for description). (**B**) Comparison of RIDD targets between BL and CB. Data were obtained from the Oncomine database. $P<0.05$ was regarded as significant (two tailed Student *t* test). (**C**) mRNA was extracted from liver tumors and paired normal liver tissues from the *LAP/MYC* mouse model. *MYC* and *Xbp1s/Xbp1t* examined with qRT-PCR. *Actb* was used as an endogenous control gene. Relative mRNA expression was determined by normalizing to levels in each normal sample. Three technical triplicates were used in each sample.

Figure S2. **c-Myc engages ER stress and regulates the IRE1α-XBP1 pathway.** (**A**) Growth curve and immunoblot analysis for P493 cells with different levels of c-Myc expression (annotated as “No Myc”, “Low Myc”, and “High Myc”, n=3). (**B**) Quantification of relative cell size from **Figure 2A** (n=3). (**C**) Total RNA content per million cells determined by Nanodrop 1000 (n=3). (**D**) c-Myc target *LDHA* mRNA expression analyzed in P493 cells with different c-Myc levels (n=3). (**E**) qRT-PCR analysis of *MYC* and *LDHA* from experiment in **Figure 2F**. (**F**) Western blot comparing protein levels between P493 cells and 4 bona fide BL cell lines: Raji, Daudi, Ramos, and EB-2. (**G**) ChIP-seq analysis shows binding of c-Myc to the promoters of *ERN1*, *HSPA5*, and *XBP1* in P493 cells, canonical (CACGTG) or non-canonical (CACGCG) E-boxes were found in the binding regions. (**H**) Immunoblots of IRE1α decay in P493 cells with different c-Myc levels after treatment with 100μg/mL cycloheximide for the indicted times. The graph represents the quantification of IRE1α protein levels. (**I** and **J**) Immunoblots for DDRGK1 (**I**), SEL1L1 and HRD1 (**J**) in P493 cells. (**K**) Protein expression of SEL1L and HRD1 in experiment of **Figure 2G**. (**L** and **M**) qRT-PCR (**L**) and Western blot (**M**) analysis of BL cell lines treated with 500nM JQ1 for 24 hours or 48 hours. (**N**) qRT-PCR analysis of Ramos cells treated with 0.1μg/mL CHX for 4 hours. For qRT-PCR, *ACTB* was utilized as the endogenous control gene; data are representative of 3 independent experiments, if not specified elsewhere. Two-way ANOVA with Bonferroni correction was used to determine significance. *, $P<0.05$, **, $P<0.01$, ***, $P<0.001$.

Figure S3. **Synthetic lethality between c-Myc overexpression and IRE1α RNase inhibition.** (**A**) Chemical structure of B-I09. (**B**) Western blot shows IRE1α phosphorylation (phos-tag SDS-PAGE) of P493 High Myc cells treated with indicated concentrations of B-I09, in the presence of DMSO or 5μg/mL tunicamycin for 6 hours. (**C**) Representative contour plots of P493 High Myc, Low Myc, and No Myc cells treated with indicated concentrations of B-I09 for 48 hours. (**D**) Immunoblots of XBP1s and c-Myc in P493 cells treated with 10μM B-I09 for 48 hours. (**E**) P493 cells treated with indicated concentrations of Doxorubicin or JQ1 for 48 hours. Viability was examined and relative viability determined by normalizing to cells treated with Control or DMSO. (**F**) Growth curve of CLL cell lines (MEC1, MEC2, and WaC3) (n=3); Immunoblots show expression of c-Myc and XBP1s in each. (**G**) CLL cells treated with 20μM B-I09 for 72 hours, relative cell growth (n=3) and viability were determined. Immunoblots show expression of PARP cleavage upon B-I09 treatment. (**H**) Cells cultured with different concentrations of 4μ8c, and counted at indicated times (n=3). (**I**) Cells treated with indicated concentrations of 4μ8c for 48 hours. Viability was examined and relative viability determined by normalizing to viability of cells treated with DMSO. (**J**) Body weight of mice bearing P493 High Myc xenografts treated with Control or B-I09 (n=5 for Control, n=4 for B-I09). (**E** and **I**) Results are representative of 3 independent experiments. *, "High Myc" vs. "Low Myc"; #, "High Myc" vs. "No Myc". Two-way ANOVA with Bonferroni correction was used to determine significance. **, $P<0.01$; ***, $P<0.001$. ###, $P<0.001$.

Figure S4. **B-I09 has no effect on IRE1α phosphorylation.** (**A**) Western blot shows IRE1α phosphorylation (phos-tag SDS-PAGE) of Ramos cells treated with indicated concentrations of B-I09, in the presence of DMSO or 5μg/mL tunicamycin for 6 hours.

Figure S5. B-I09 treatment responses are dependent on SCD1 loss in P493 cells. (**A**) P493 High Myc cells treated with 10μM B-I09 for 24 hours and 48 hours. Phospho-JNK and total JNK protein levels analyzed. (**B**) P493 cells treated with 10μM B-I09 for 48 hours. Autophagy markers

including p62 and LC3 analyzed. (**C**) Schematic model of U-^{13}C-glucose tracing and fatty acid labeling. (**D**) Western blot analysis of P493 cells treated with 20μM 4μ8c for 48 hours. (**E**) CLL cell lines treated with 20μM B-I09 for 48 hours; c-Myc and SCD1 expression monitored. (**F**) P493 High Myc cells transfected with non-targeting (NT) siRNA or siRNA targeting XBP1 (siXBP1) for 48 hours, XBP1s and SCD1 were analyzed. (**G**) Immunoblots of experiment from **Figure 5F**. (**H**) P493 High Myc cells treated with DMSO or 20μM B-I09 for 48 hours, with OA, or Palm, or the combination (1:1 ratio). (**I**) P493 High Myc cells cultured in media with normal lipid concentration (100%) or delipidated condition (10%), treated with 10μM B-I09, and rescued with BSA or OA for 48 hours. Contribution ratios of viability restoration of BSA and OA in 100% or 10% lipid media were calculated (see Methods). (**J** and **K**) P493 cells treated with 0.5μM SCDi for 72 hours, cell growth (n=3) and viability were determined. For viability assays, data are representative of 3 independent experiments. Two-way ANOVA with Bonferroni correction was used to determined significance. ***, P<0.001. n.s., not significant.

Figure S6. B-I09 treatment responses are dependent on SCD1 loss in BL cells. (**A**) Immunoblots of experiment from **Figure 6B**. (**B**) Ramos cells treated with DMSO or 20μM B-I09 for 48 hours, with OA, or Palm, or the combination (1:1 ratio). (**C** and **D**) Ramos or Daudi cells cultured in media with normal lipid concentration (100%) or delipidated condition (25%), treated with 10μM B-I09, and rescued with BSA or OA for 48 hours. Contribution ratios of viability restoration of BSA and OA in 100% or 25% lipid media were calculated (see Methods). (**E**) Body weight of mice bearing Ramos xenografts treated with Control or SCDi. (**F**) mRNA was extracted from Ramos cells in experiment of **Figure 6D**; genes representing three UPR pathways examined with qRT-PCR. ATF6 pathway: *HERPUD1*; IRE1α-XBP1 pathway: *XBP1s*, *DNAJB9*; PERK-ATF4 pathway: *ATF3*, *DDIT3*. *TBP* and *ACTB* were utilized as endogenous control genes. *, comparison of SCDi and DMSO treatment. #, comparison of SCDi+OA and SCDi. For qRT-PCR and viability assay, data are representative of 3 independent experiments. Two-way ANOVA with Bonferroni correction was used to determined significance. **, P<0.01, ***, P<0.001. #, P<0.05; ###, P<0.001. n.s., not significant.

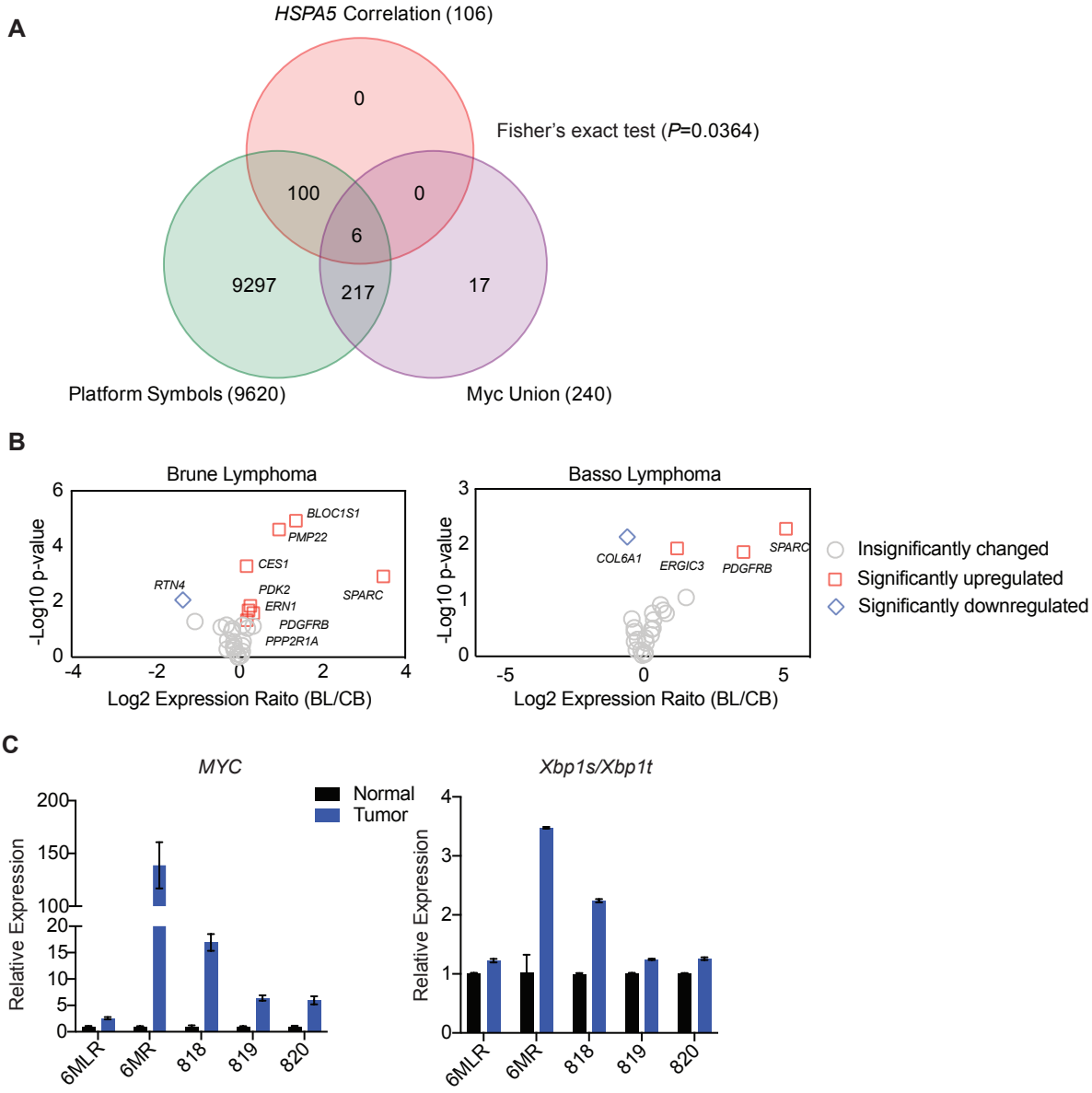
Figure S7. **N-Myc activation engages the IRE1α-XBP1 pathway, rendering cells vulnerable to XBP1s loss.** (**A**) N-Myc target *ODC1* examined with qRT-PCR (n=3). (**B**) Control and 4-OHT SHEP cells were exposed to 100, 50, 25, 12.5, 6.25, 3.125, 1.5625μM B-I09 for 72 hours, IC_{50} was then determined (n=3). (**C** and **D**) Control and 4-OHT SHEP cells treated with indicated concentrations of B-I09 for different times, viability was then measured. Relative viability was determined by normalizing to viability of cells with DMSO treatment. (**E**) Control or N-Myc SHEP cells treated with DMSO or 30μM B-I09, rescued with BSA or OA for 72 hours (n=6). Absorbance was measured using WST-1 reagents. *, comparison of B-I09 and DMSO treatment. #, comparison of B-I09+BSA or B-I09+OA and B-I09 treatment. (**F**) Control or N-Myc SHEP cells treated with 0.5μM SCDi for indicated times, viability was examined. Relative viability was determined by normalizing to viability of cells with DMSO treatment. (**G**) Control or N-Myc SHEP cells treated with siNT or siSCD for 72 hours, and viability was determined. Relative viability was determined by normalizing to viability of cells with siNT treatment. (**H**) N-Myc SHEP cells treated with 0.5μM SCDi, rescued with BSA control or OA for 72 hours, and viability was determined. Relative viability was determined by normalizing to viability of cells with DMSO treatment. For viability assays, data are representative of 3 independent experiments. Two-way ANOVA with Bonferroni correction was used to determined significance. **, $P<0.01$, ***, $P<0.001$. ###, $P<0.001$. n.s., not significant.

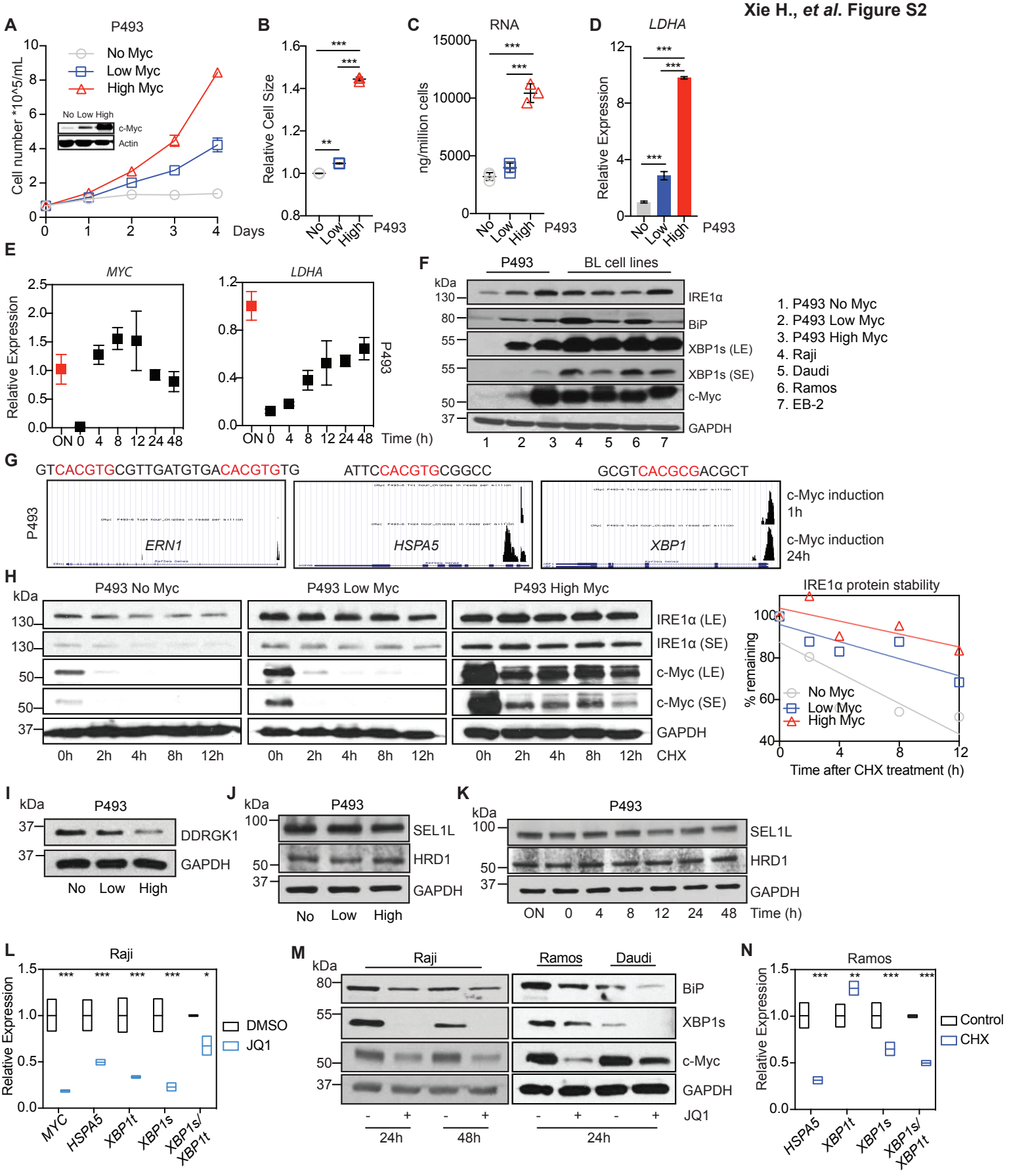
Figure S8. XBP1 depletion in neuroblastoma cells. (**A**) qRT-PCR comparing 3 neuroblastoma cell lines SK-N-AS, BE2C, and Kelly in terms of expressions of *MYCN*, *ODC1*, *HSPA5*, and *XBP1s*. (**B**) Three neuroblastoma cell lines were tested for IC_{50} of B-I09 treatment for 72 hours. (**C**) Validation of *XBP1* knockdown in Kelly xenograft tumors by qRT-PCR (two-tailed paired t test). (**D**) Body weight of mice bearing Kelly xenograft tumors (n=10). For qRT-PCR, *TBP* and *ACTB* were used as endogenous control genes, 3 technical triplicates were used in each sample, and data are representative of 3 independent experiments. ***, $P<0.001$.

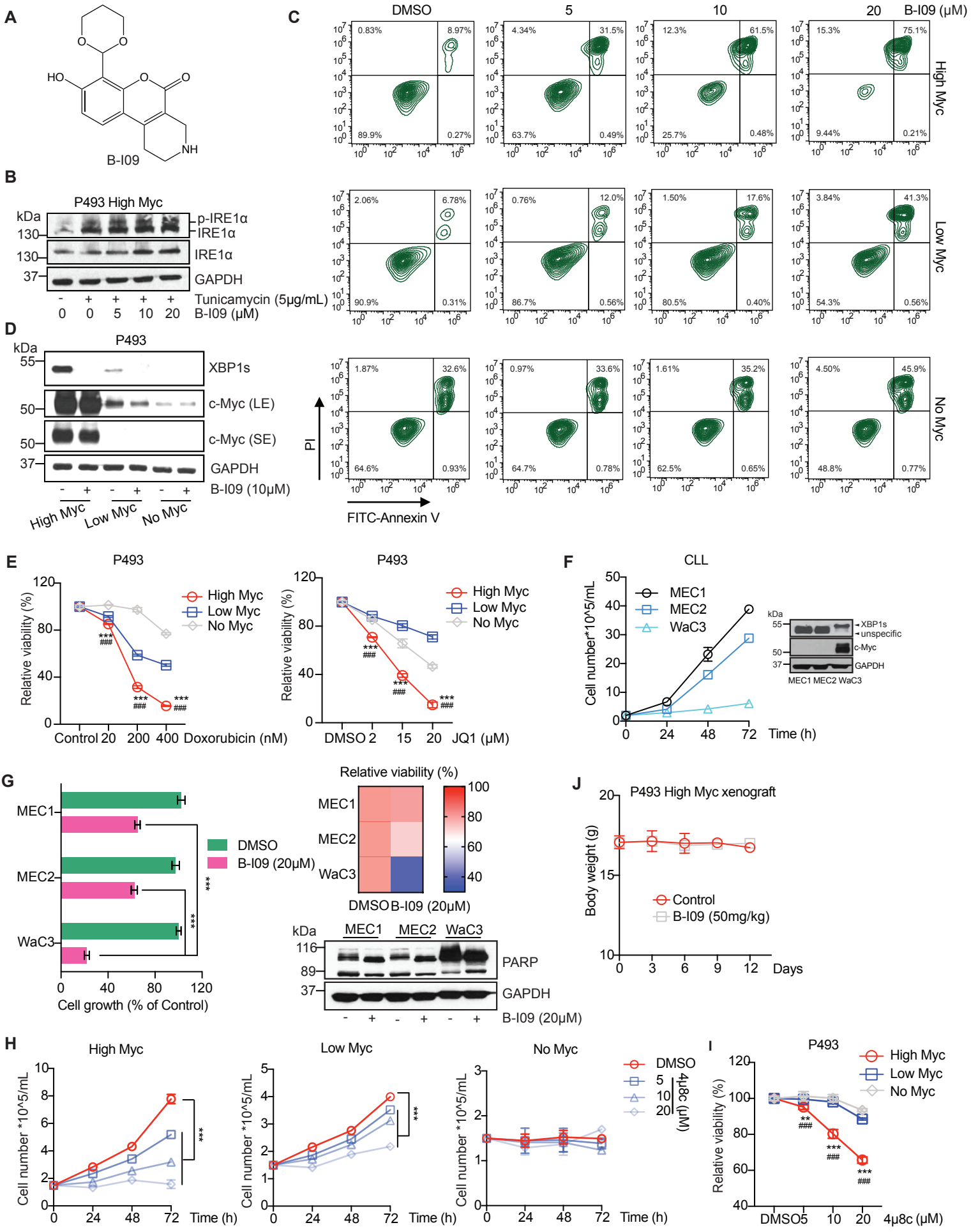
Figure S9. Combinational treatment between B-I09 and Vincristine. (**A**) Western blot examining SCD1 expression after treatment with 10μM B-I09 or 0.5μM Vincristine alone, or the combination of both in Daudi and Ramos cells after 48 hours. (**B**) Daudi or Ramos cells treated with 10μM B-I09 or 0.5μM Vincristine alone, or the combination of both, rescued with OA for 48 hours, viability was determined. Data are representative of 3 independent experiments.

Supplementary Table 1. Fisher's exact test to determine correlation between *HSPA5* and Myc signature.

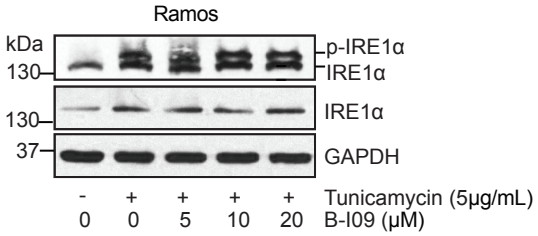
	Myc Union	Not Myc Union	Total
HSPA5 correlation	6	100	106
HSPA5 no correlation	217	9297	9514
Total	223	9397	9620

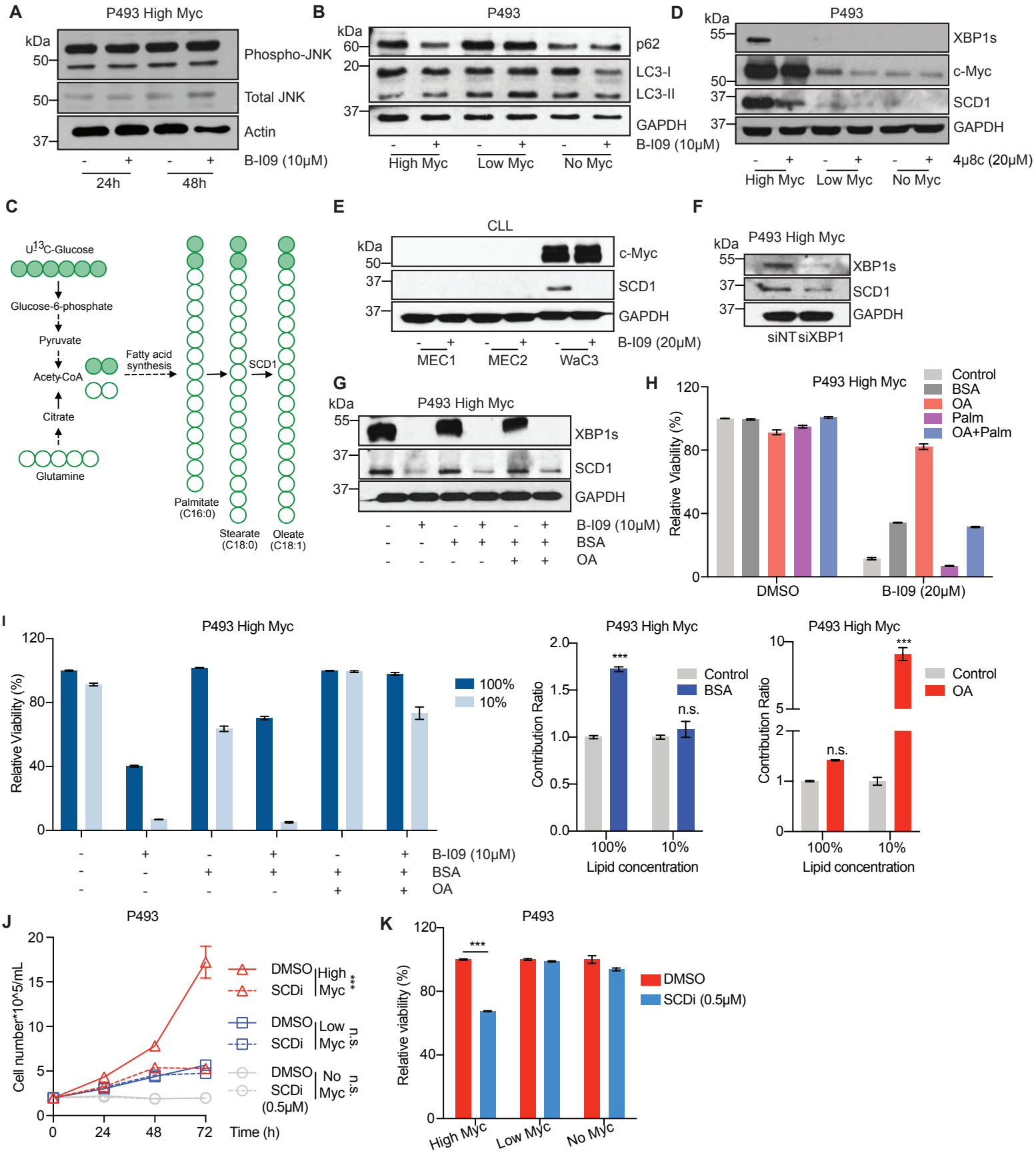

A
HSPA5 Correlation (106)
0
100
0
6
9297
217
17
Fisher's exact test (P=0.0364)
Platform Symbols (9620)
Myc Union (240)
B
Brune Lymphoma
-Log10 p-value
Log2 Expression Raito (BL/CB)
BLOC1S1
PMP22
CES1
RTN4
PDK2
ERN1
SPARC
PDGFRB
PPP2R1A
Basso Lymphoma
COL6A1
ERGIC3
PDGFRB
SPARC
Insignificantly changed
Significantly upregulated
Significantly downregulated
C
MYC
Xbp1s/Xbp1t
Normal
Tumor
Relative Expression
6MLR
6MR
818
819
820

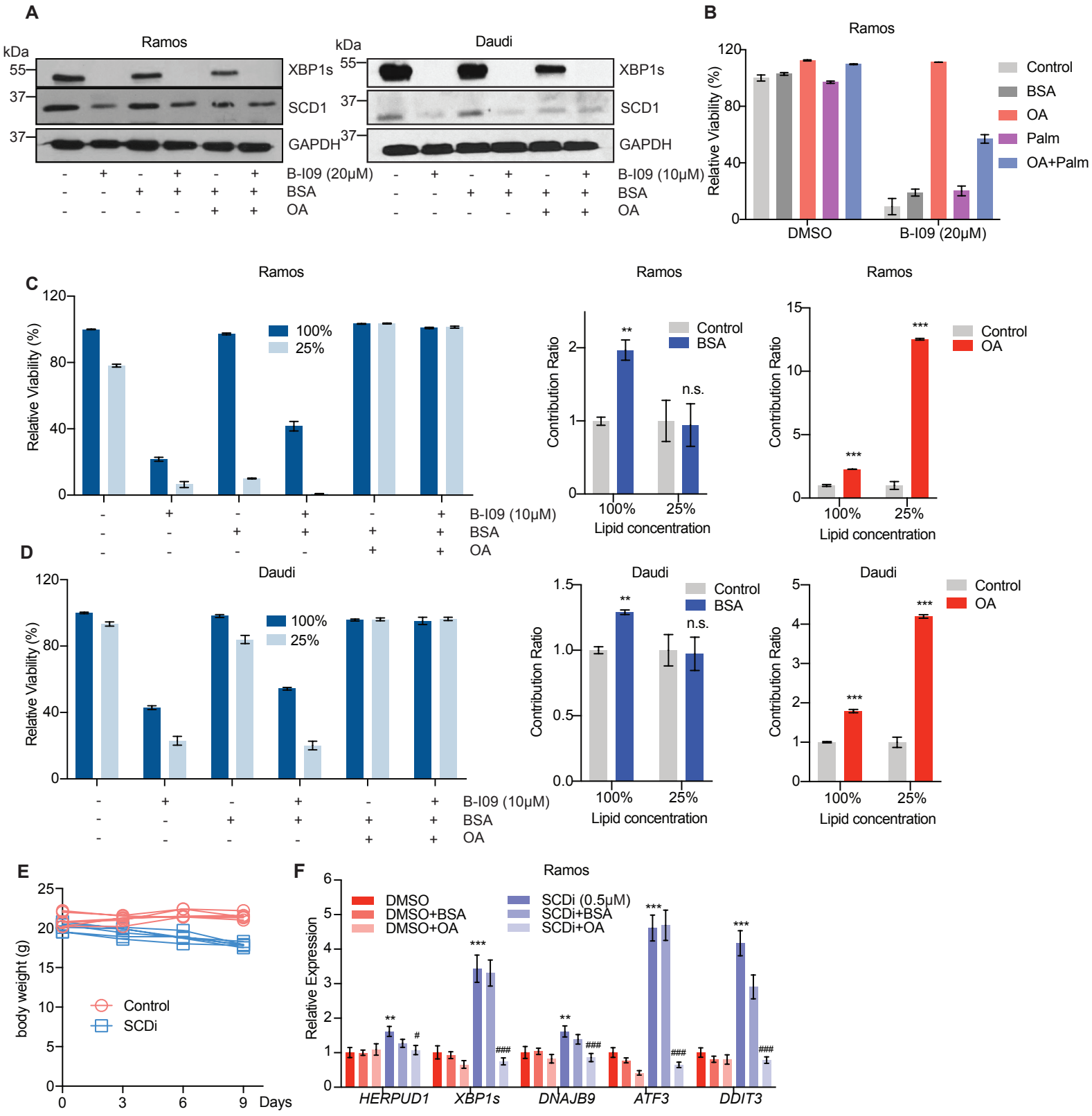

A
P493
No Myc
Low Myc
High Myc
No Low High
c-Myc
Actin
Cell number *10^5/mL
Days
B
Relative Cell Size
No Low High P493
C
RNA
ng/million cells
No Low High P493
D
LDHA
Relative Expression
No Low High P493
E
MYC
LDHA
Relative Expression
ON 0 4 8 12 24 48 Time (h)
P493
F
P493
BL cell lines
kDa
IRE1α
BiP
XBP1s (LE)
XBP1s (SE)
c-Myc
GAPDH
1 2 3 4 5 6 7
1. P493 No Myc
2. P493 Low Myc
3. P493 High Myc
4. Raji
5. Daudi
6. Ramos
7. EB-2
G
GTCACGTGCGTTGATGTGACACGTGTG
ATTCCACGTGCGGCC
GCGTCACGCGACGCT
P493
ERN1
HSPA5
XBP1
c-Myc induction 1h
c-Myc induction 24h
H
P493 No Myc
P493 Low Myc
P493 High Myc
kDa
IRE1α (LE)
IRE1α (SE)
c-Myc (LE)
c-Myc (SE)
GAPDH
0h 2h 4h 8h 12h CHX
IRE1α protein stability
% remaining
No Myc
Low Myc
High Myc
Time after CHX treatment (h)
I
P493
kDa
DDRGK1
GAPDH
No Low High
J
P493
kDa
SEL1L
HRD1
GAPDH
No Low High
K
P493
kDa
SEL1L
HRD1
GAPDH
ON 0 4 8 12 24 48 Time (h)
L
Raji
Relative Expression
DMSO
JQ1
MYC
HSPA5
XBP1t
XBP1s
XBP1s/XBP1t
M
Raji
Ramos
Daudi
kDa
BiP
XBP1s
c-Myc
GAPDH
JQ1
24h
48h
24h
N
Ramos
Relative Expression
Control
CHX
HSPA5
XBP1t
XBP1s
XBP1s/XBP1t

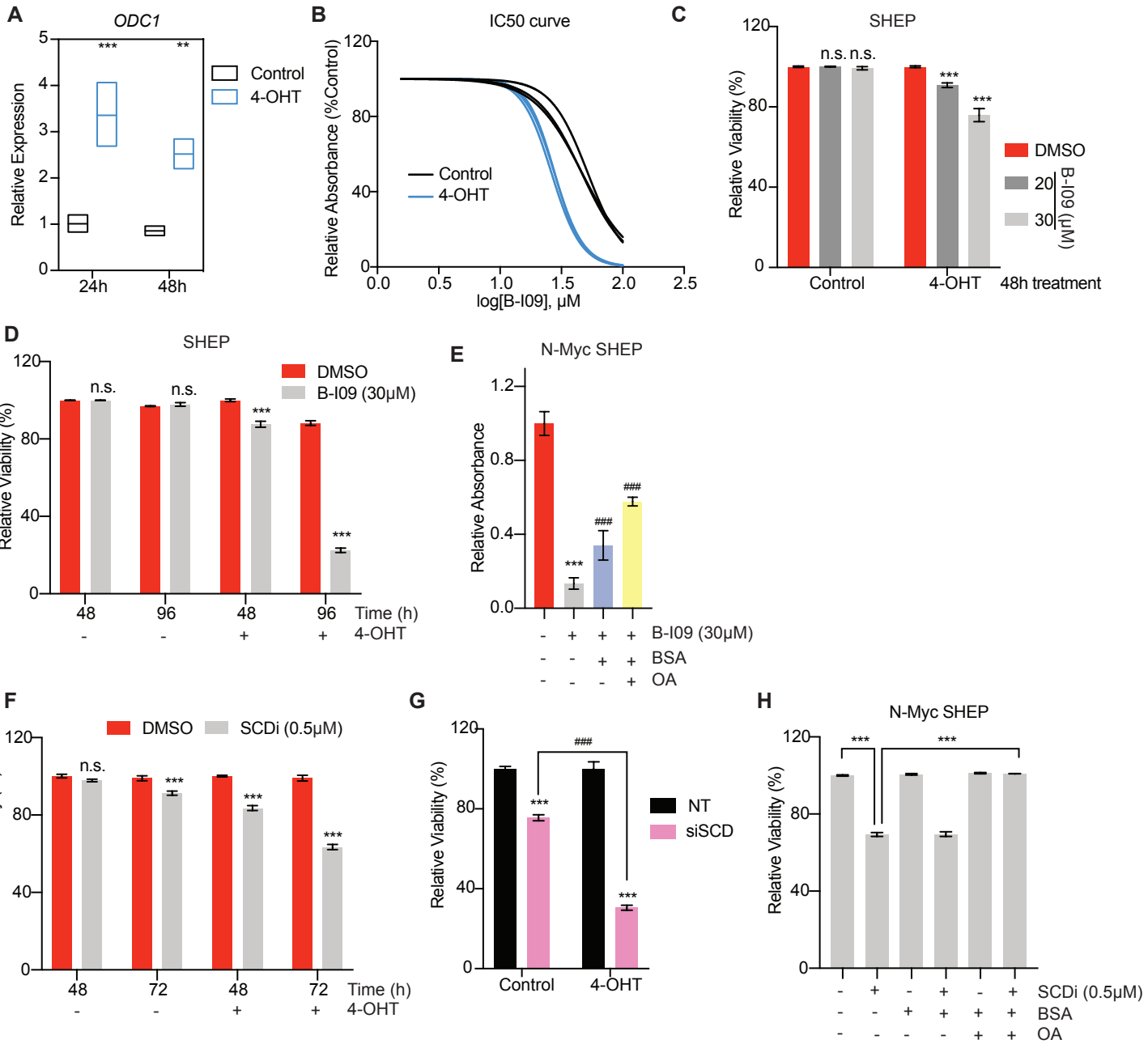

A
B-I09
B
P493 High Myc
kDa
p-IRE1α
IRE1α
GAPDH
Tunicamycin (5μg/mL)
B-I09 (μM)
C
DMSO
5
10
20
B-I09 (μM)
High Myc
Low Myc
No Myc
D
P493
XBP1s
c-Myc (LE)
c-Myc (SE)
GAPDH
B-I09 (10μM)
PI
FITC-Annexin V
E
P493
High Myc
Low Myc
No Myc
Relative viability (%)
Doxorubicin (nM)
JQ1 (μM)
F
CLL
MEC1
MEC2
WaC3
Cell number*10^5/mL
Time (h)
XBP1s
unspecific
c-Myc
GAPDH
G
Cell growth (% of Control)
DMSO
B-I09 (20μM)
Relative viability (%)
PARP
J
P493 High Myc xenograft
Body weight (g)
Control
B-I09 (50mg/kg)
Days
H
High Myc
Low Myc
No Myc
Cell number *10^5/mL
Time (h)
4μ8c (μM)
I
P493
Relative viability (%)
4μ8c (μM)

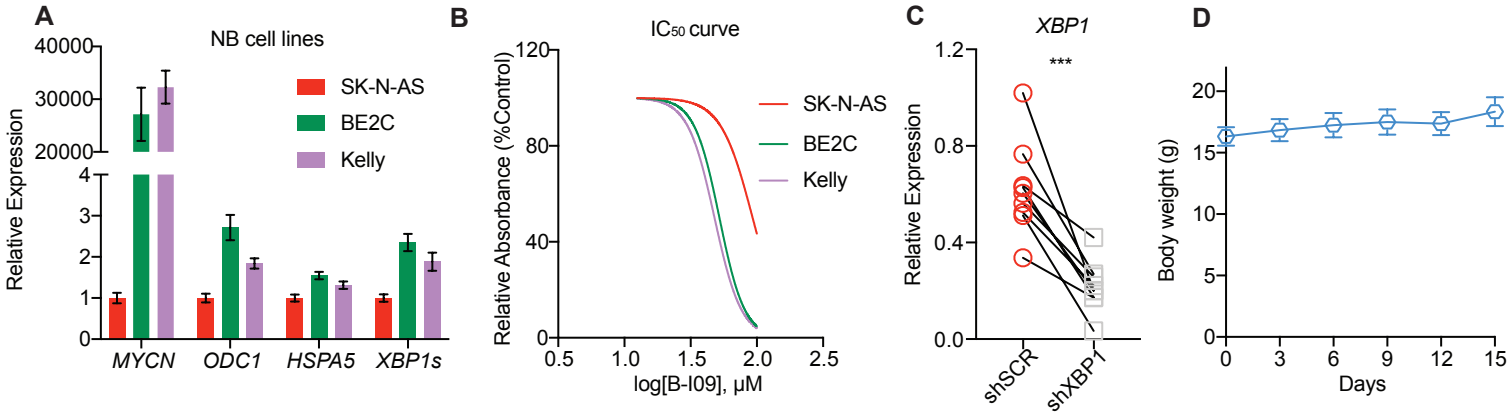
A

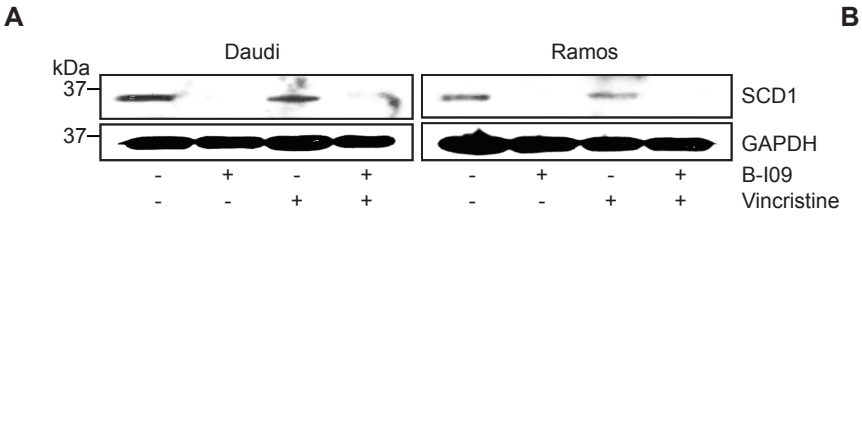

Ramos
kDa
130
p-IRE1α
IRE1α
130
IRE1α
37
GAPDH
- + + + +
0 0 5 10 20
Tunicamycin (5μg/mL)
B-I09 (μM)

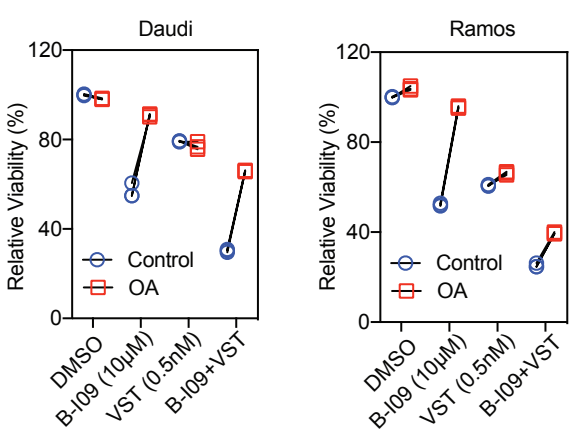

A
P493 High Myc
kDa
50
Phospho-JNK
50
Total JNK
37
Actin
- + - +
B-I09 (10μM)
24h
48h
B
P493
kDa
60
p62
20
LC3-I
LC3-II
37
GAPDH
B-I09 (10μM)
High Myc
Low Myc
No Myc
D
P493
kDa
55
XBP1s
c-Myc
50
37
SCD1
37
GAPDH
High Myc
Low Myc
No Myc
4μ8c (20μM)
C
U13C-Glucose
Glucose-6-phosphate
Pyruvate
Acety-CoA
Citrate
Glutamine
Fatty acid synthesis
SCD1
Palmitate (C16:0)
Stearate (C18:0)
Oleate (C18:1)
E
CLL
kDa
50
c-Myc
37
SCD1
37
GAPDH
MEC1
MEC2
WaC3
B-I09 (20μM)
F
P493 High Myc
kDa
55
XBP1s
37
SCD1
37
GAPDH
siNT siXBP1
G
P493 High Myc
kDa
55
XBP1s
37
SCD1
37
GAPDH
B-I09 (10μM)
BSA
OA
H
P493 High Myc
Control
BSA
OA
Palm
OA+Palm
Relative Viability (%)
120
80
40
0
DMSO
B-I09 (20μM)
I
P493 High Myc
Relative Viability (%)
120
80
40
0
100%
10%
B-I09 (10μM)
BSA
OA
P493 High Myc
Contribution Ratio
2.0
1.5
1.0
0.5
0.0
***
n.s.
Control
BSA
100%
10%
Lipid concentration
P493 High Myc
Contribution Ratio
10
5
2
1
0
***
n.s.
Control
OA
100%
10%
Lipid concentration
J
P493
Cell number*10^5/mL
20
15
10
5
0
0
24
48
72
Time (h)
DMSO
SCDi
High Myc
***
DMSO
SCDi
Low Myc
n.s.
DMSO
SCDi (0.5μM)
No Myc
n.s.
K
P493
Relative viability (%)
120
80
40
0
***
DMSO
SCDi (0.5μM)
High Myc
Low Myc
No Myc


A
Ramos
kDa
55
37
37
XBP1s
SCD1
GAPDH
B-I09 (20μM)
BSA
OA
Daudi
B-I09 (10μM)
B
Ramos
Relative Viability (%)
Control
BSA
OA
Palm
OA+Palm
DMSO
B-I09 (20μM)
C
Ramos
100%
25%
Contribution Ratio
Control
BSA
n.s.
Lipid concentration
OA
D
Daudi
E
body weight (g)
Control
SCDi
Days
F
Ramos
DMSO
DMSO+BSA
DMSO+OA
SCDi (0.5μM)
SCDi+BSA
SCDi+OA
Relative Expression
HERPUD1
XBP1s
DNAJB9
ATF3
DDIT3


A
ODC1
Relative Expression
Control
4-OHT
24h
48h
B
IC50 curve
Relative Absorbance (%Control)
Control
4-OHT
log[B-I09], μM
C
SHEP
Relative Viability (%)
n.s. n.s.
DMSO
B-I09 (μM)
Control
4-OHT
48h treatment
D
SHEP
Relative Viability (%)
DMSO
B-I09 (30μM)
Time (h)
4-OHT
E
N-Myc SHEP
Relative Absorbance
B-I09 (30μM)
BSA
OA
F
DMSO
SCDi (0.5μM)
Relative Viability (%)
Time (h)
4-OHT
G
Relative Viability (%)
NT
siSCD
Control
4-OHT
H
N-Myc SHEP
Relative Viability (%)
SCDi (0.5μM)
BSA
OA


A
NB cell lines
SK-N-AS
BE2C
Kelly
Relative Expression
MYCN
ODC1
HSPA5
XBP1s
B
IC50 curve
SK-N-AS
BE2C
Kelly
Relative Absorbance (%Control)
log[B-I09], μM
C
XBP1
***
Relative Expression
shSCR
shXBP1
D
Body weight (g)
Days


A
Daudi
Ramos
kDa
37
37
SCD1
GAPDH
B-I09
Vincristine


B
Daudi
Ramos
Relative Viability (%)
120
80
40
0
Control
OA
DMSO
B-I09 (10μM)
VST (0.5nM)
B-I09+VST