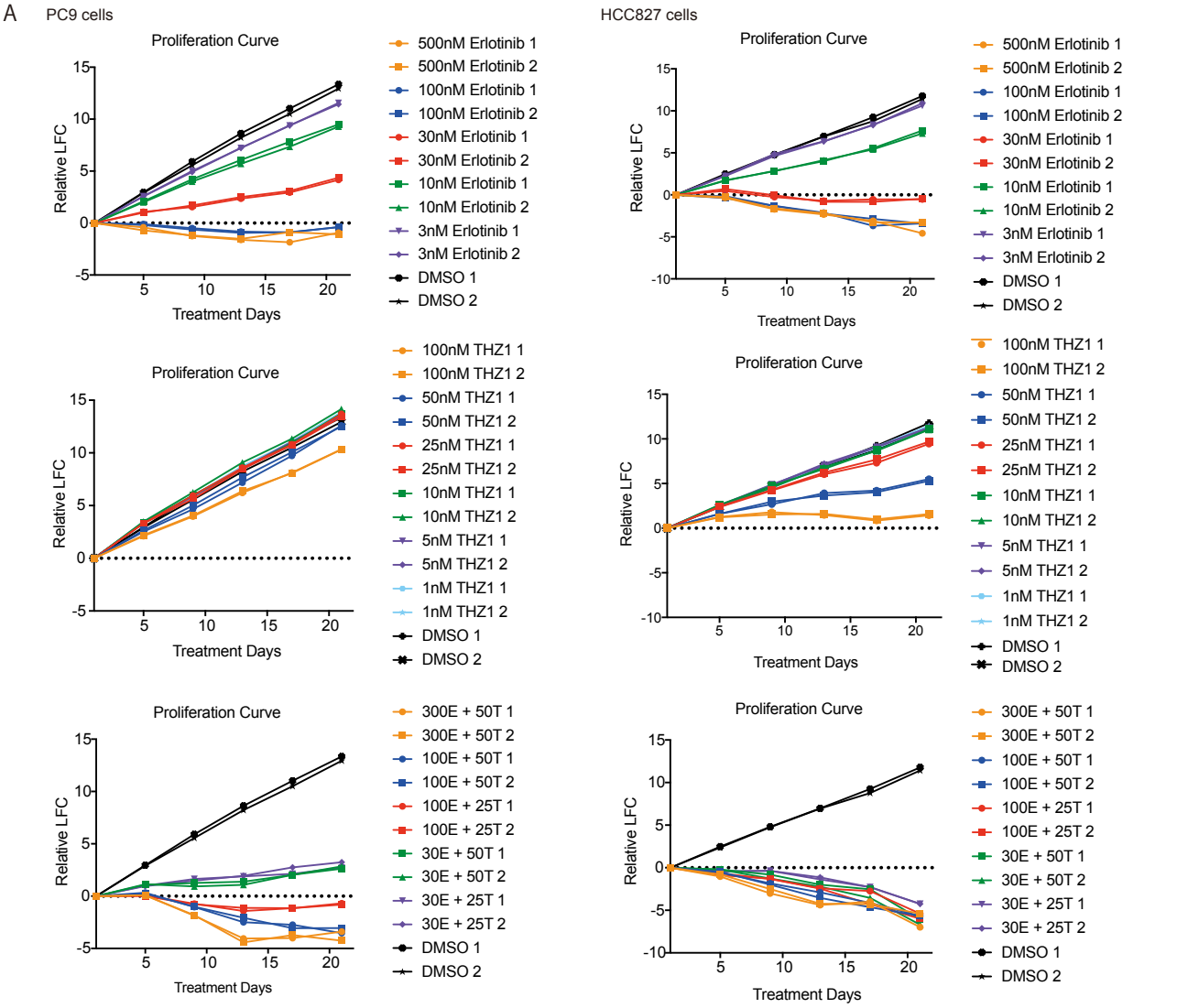
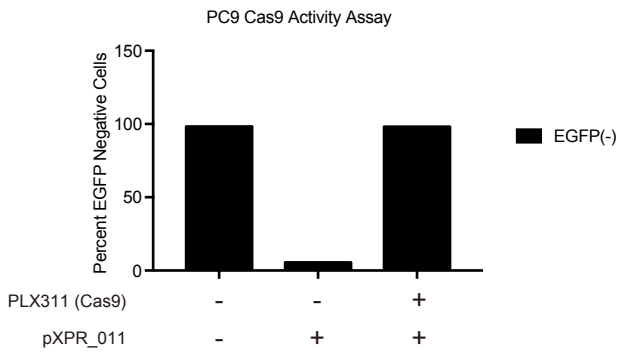
A



B

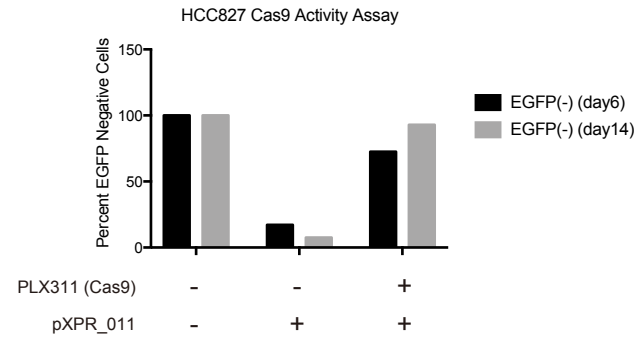
PC9 cells (day6)

	GFP (+)	GFP (−)
parental	1.72	98.3
PLX311(Cas9) (−), pXPR_011	94.4	5.61
PLX311(Cas9) (+), pXPR_011	1.81	98.2



HCC827 cells (day6)

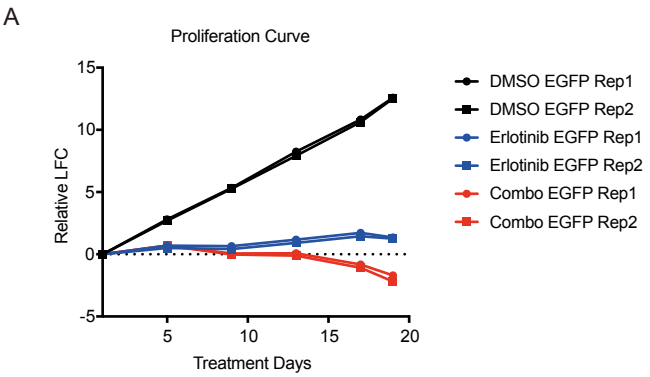
	GFP (+)	GFP (−)
parental	0	100
PLX311(Cas9) (−), pXPR_011	82.8	17.2
PLX311(Cas9) (+), pXPR_011	27.4	72.6

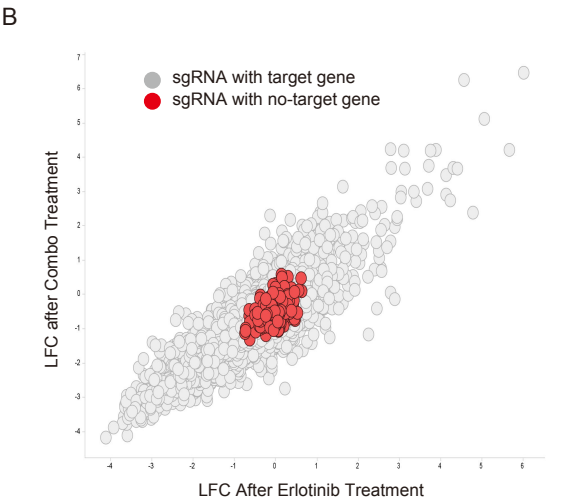


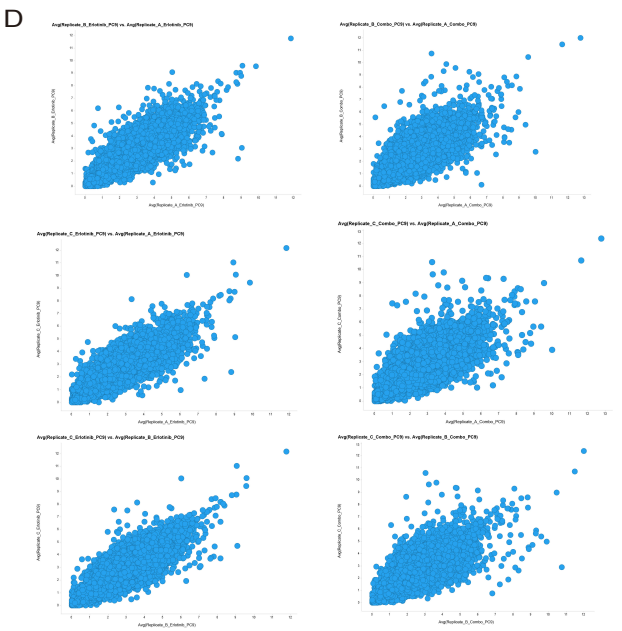
HCC827 cells (day14)

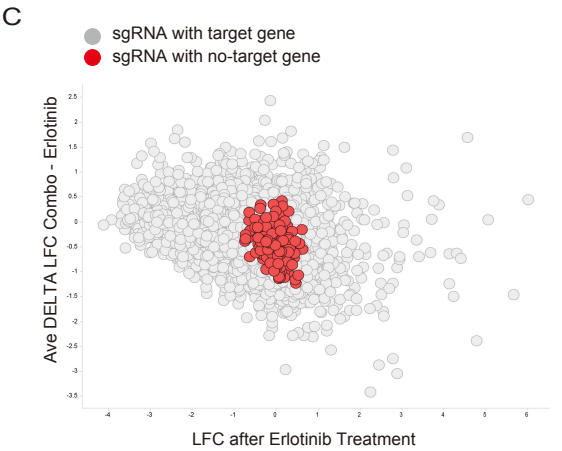
	GFP (+)	GFP (−)
parental	0	100
PLX311(Cas9) (−), pXPR_011	92.5	7.5
PLX311(Cas9) (+), pXPR_011	7.1	92.9

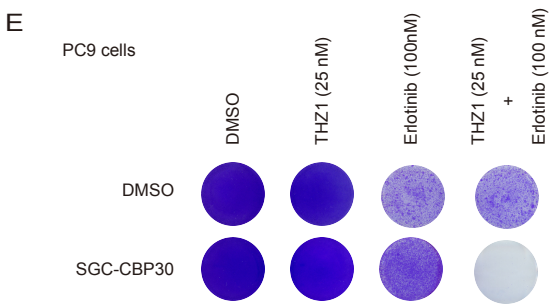
Figure S1


A
Proliferation Curve
Relative LFC
Treatment Days
DMSO EGFP Rep1
DMSO EGFP Rep2
Erlotinib EGFP Rep1
Erlotinib EGFP Rep2
Combo EGFP Rep1
Combo EGFP Rep2


B
sgRNA with target gene
sgRNA with no-target gene
LFC after Combo Treatment
LFC After Erlotinib Treatment


D


C
sgRNA with target gene
sgRNA with no-target gene
Ave DELTA LFC Combo - Erlotinib
LFC after Erlotinib Treatment


E
PC9 cells
DMSO
THZ1 (25 nM)
Erlotinib (100nM)
THZ1 (25 nM) + Erlotinib (100 nM)
DMSO
SGC-CBP30

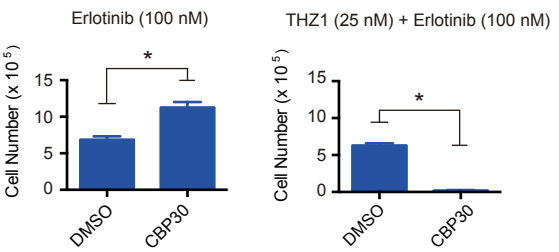

Erlotinib (100 nM)
THZ1 (25 nM) + Erlotinib (100 nM)
Cell Number (x 10 5)
DMSO
CBP30

Figure S2

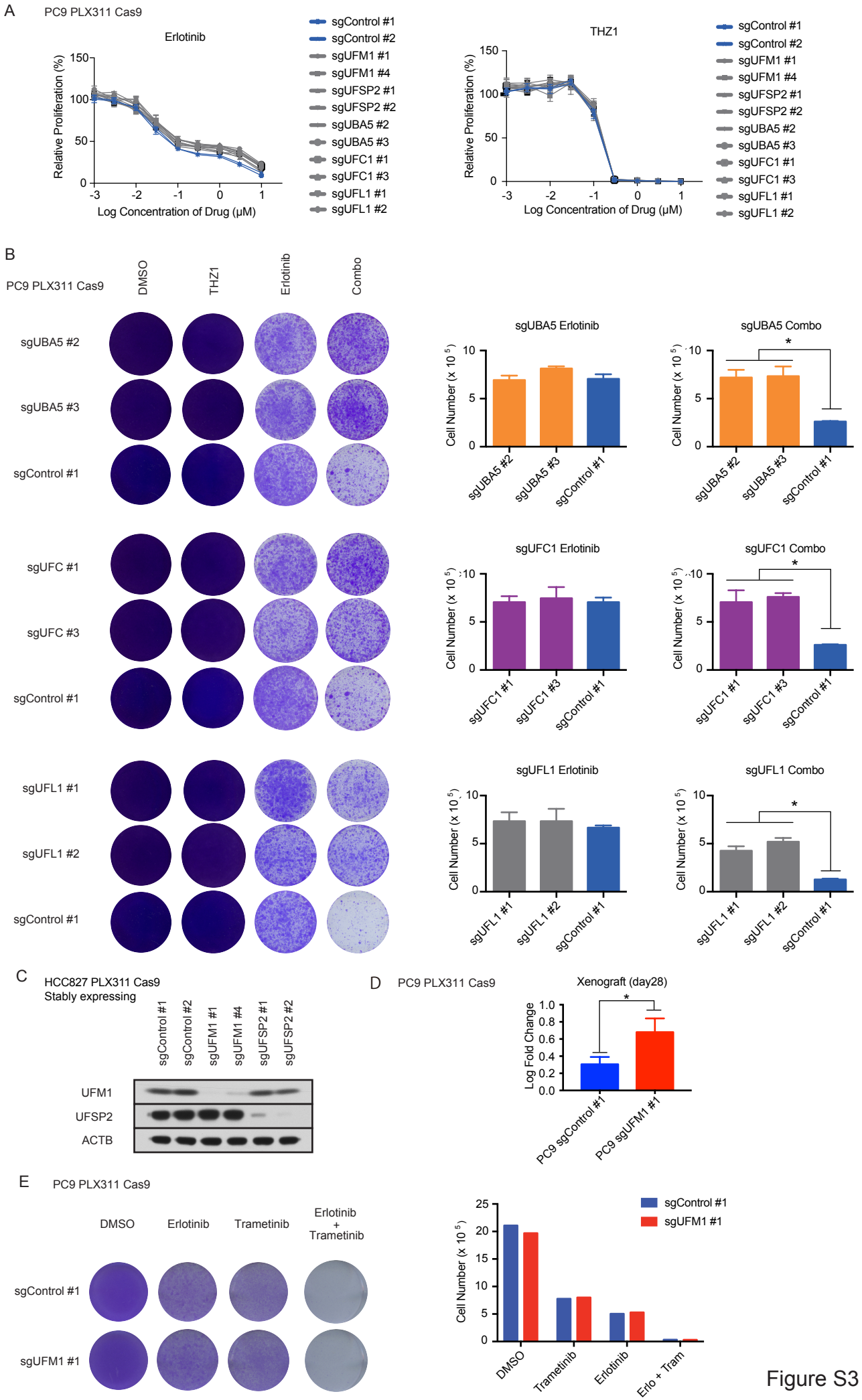

A
PC9 PLX311 Cas9
Erlotinib
Relative Proliferation (%)
Log Concentration of Drug (μM)
THZ1
sgControl #1
sgControl #2
sgUFM1 #1
sgUFM1 #4
sgUFSP2 #1
sgUFSP2 #2
sgUBA5 #2
sgUBA5 #3
sgUFC1 #1
sgUFC1 #3
sgUFL1 #1
sgUFL1 #2
B
PC9 PLX311 Cas9
DMSO
THZ1
Erlotinib
Combo
sgUBA5 #2
sgUBA5 #3
sgControl #1
sgUFC #1
sgUFC #3
sgUFL1 #1
sgUFL1 #2
sgUBA5 Erlotinib
sgUBA5 Combo
sgUFC1 Erlotinib
sgUFC1 Combo
sgUFL1 Erlotinib
sgUFL1 Combo
Cell Number (x 10 5)
C
HCC827 PLX311 Cas9
Stably expressing
UFM1
UFSP2
ACTB
D
PC9 PLX311 Cas9
Xenograft (day28)
Log Fold Change
PC9 sgControl #1
PC9 sgUFM1 #1
E
PC9 PLX311 Cas9
DMSO
Erlotinib
Trametinib
Erlotinib + Trametinib
Erlo + Tram

Figure S3

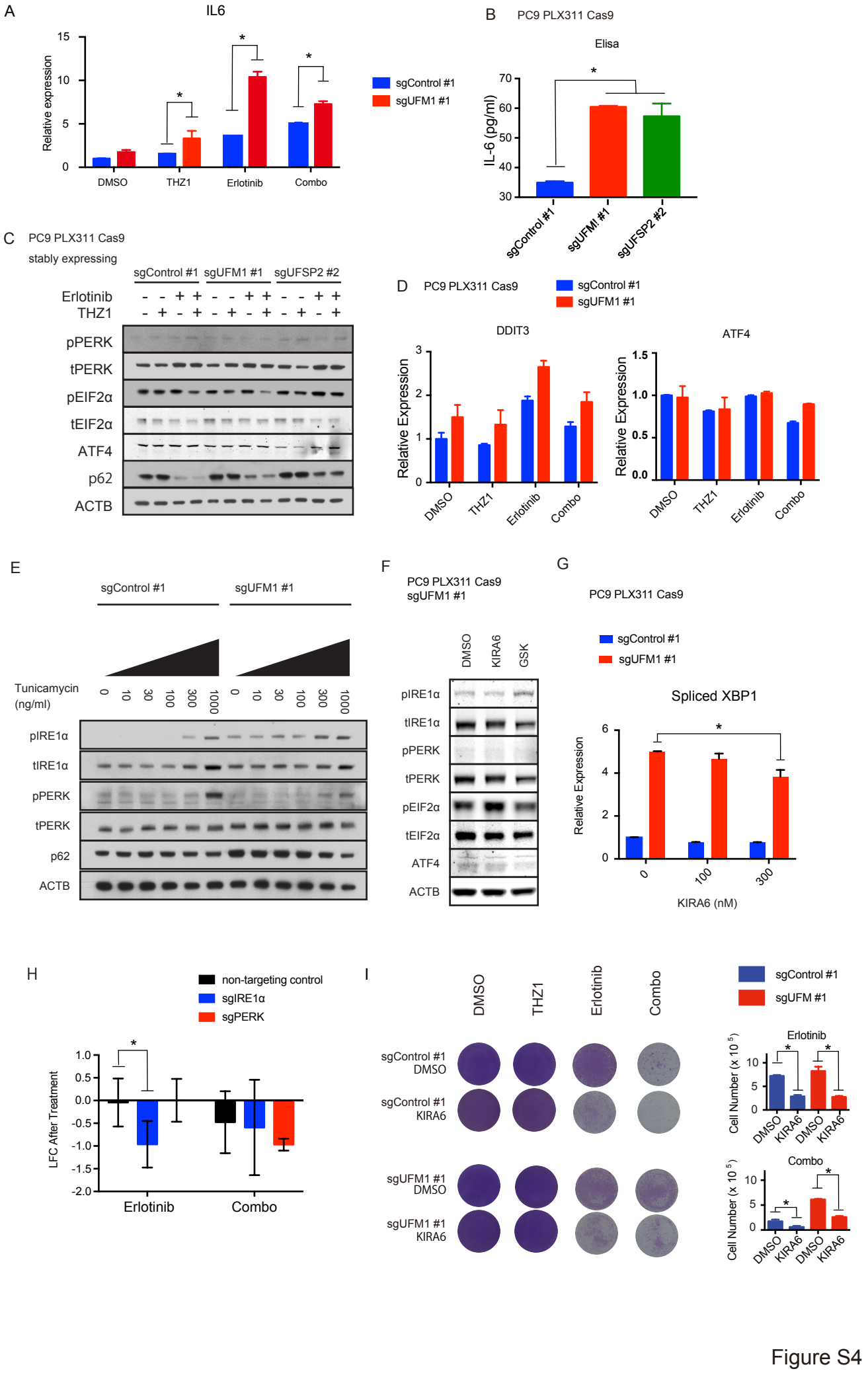

A
IL6
Relative expression
15
10
5
0
DMSO
THZ1
Erlotinib
Combo
sgControl #1
sgUFM1 #1
B
PC9 PLX311 Cas9
Elisa
IL-6 (pg/ml)
70
60
50
40
30
sgControl #1
sgUFM1 #1
sgUFSP2 #2
C
PC9 PLX311 Cas9
stably expressing
sgControl #1
sgUFM1 #1
sgUFSP2 #2
Erlotinib
THZ1
pPERK
tPERK
pEIF2α
tEIF2α
ATF4
p62
ACTB
D
PC9 PLX311 Cas9
sgControl #1
sgUFM1 #1
DDIT3
ATF4
Relative Expression
DMSO
THZ1
Erlotinib
Combo
E
sgControl #1
sgUFM1 #1
Tunicamycin
(ng/ml)
0
10
30
100
300
1000
pIRE1α
tIRE1α
pPERK
tPERK
p62
ACTB
F
PC9 PLX311 Cas9
sgUFM1 #1
DMSO
KIRA6
GSK
pIRE1α
tIRE1α
pPERK
tPERK
pEIF2α
tEIF2α
ATF4
ACTB
G
PC9 PLX311 Cas9
sgControl #1
sgUFM1 #1
Spliced XBP1
Relative Expression
KIRA6 (nM)
H
non-targeting control
sgIRE1α
sgPERK
LFC After Treatment
Erlotinib
Combo
I
DMSO
THZ1
Erlotinib
Combo
sgControl #1
sgUFM #1
sgControl #1
DMSO
sgControl #1
KIRA6
sgUFM1 #1
DMSO
sgUFM1 #1
KIRA6
Erlotinib
Combo
Cell Number (x 10 5)

Figure S4

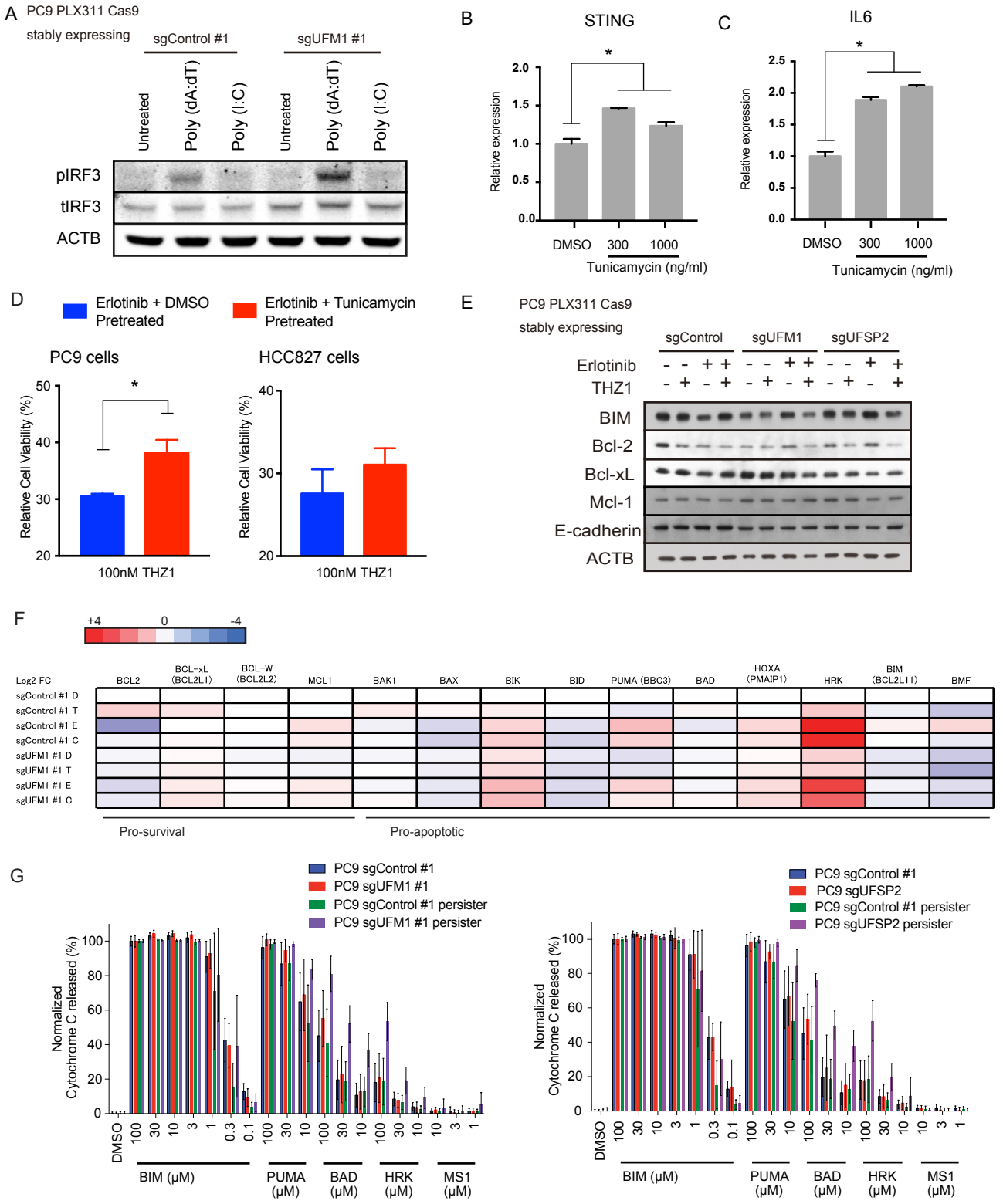
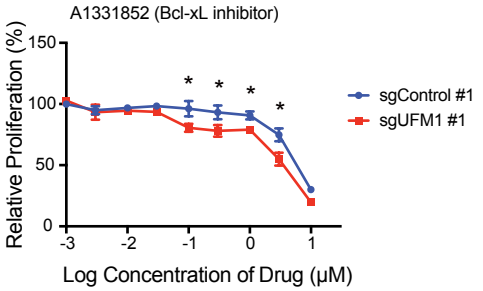
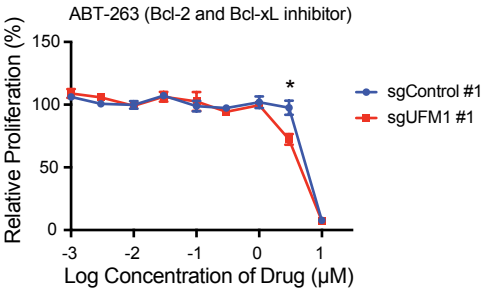

A
PC9 PLX311 Cas9
stably expressing
sgControl #1
sgUFM1 #1
Untreated
Poly (dA:dT)
Poly (I:C)
pIRF3
tIRF3
ACTB
B
STING
Relative expression
DMSO
300
1000
Tunicamycin (ng/ml)
C
IL6
Relative expression
DMSO
300
1000
Tunicamycin (ng/ml)
D
Erlotinib + DMSO Pretreated
Erlotinib + Tunicamycin Pretreated
PC9 cells
HCC827 cells
Relative Cell Viability (%)
100nM THZ1
E
PC9 PLX311 Cas9
stably expressing
sgControl
sgUFM1
sgUFSP2
Erlotinib
THZ1
BIM
Bcl-2
Bcl-xL
Mcl-1
E-cadherin
ACTB
F
Log2 FC
BCL2
BCL-xL (BCL2L1)
BCL-W (BCL2L2)
MCL1
BAK1
BAX
BIK
BID
PUMA (BBC3)
BAD
HOXA (PMAIP1)
HRK
BIM (BCL2L11)
BMF
sgControl #1 D
sgControl #1 T
sgControl #1 E
sgControl #1 C
sgUFM1 #1 D
sgUFM1 #1 T
sgUFM1 #1 E
sgUFM1 #1 C
Pro-survival
Pro-apoptotic
G
PC9 sgControl #1
PC9 sgUFM1 #1
PC9 sgControl #1 persister
PC9 sgUFM1 #1 persister
PC9 sgUFSP2
PC9 sgUFSP2 persister
Normalized Cytochrome C released (%)
BIM (μM)
PUMA (μM)
BAD (μM)
HRK (μM)
MS1 (μM)

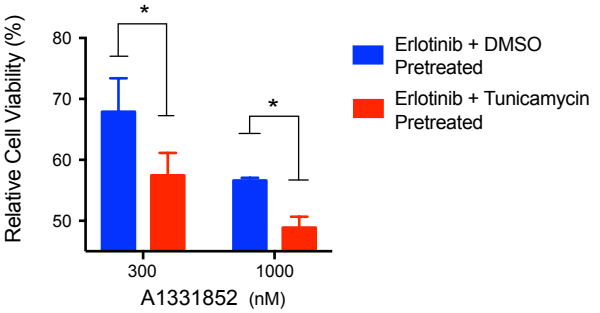
Figure S5

A HCC827 cell





B PC9 cells



C HCC827 cell

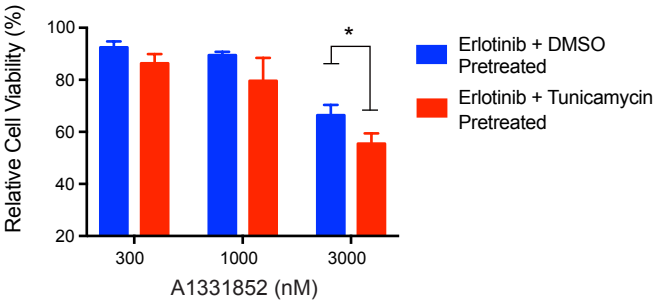


Figure S6

Figure legends for supplementary figures

Figure S1 Preparation experiments for CRIPSR screening

A) Proliferation curves of PC9 cells or HCC827 cells infected with EGFP expressing plasmid under the indicated concentration of drugs (DMSO (D), erlotinib (E), THZ1 (T)) treatment. Experiments have been performed in duplicate. Cell number was counted by Vi-cell counter every 4 days. B) Cas9 activity assay with PLX311 Cas9 expressing plasmid infected PC9 cell and HCC827 cell. Each bar indicates the percentage of the EGFP negative cell population.

Figure S2 Quality control of the result of CRISPR screening.

A) Proliferation curves of PC9 cells infected with EGFP expressing plasmid. Experiments were performed simultaneously with genome wide CRISPR screen experiment. Cells were treated with DMSO, 100 nM erlotinib or 100 nM erlotinib + THZ1 (combo). Experiments were performed in duplicate. B, D) Scatter plots for the results of CRISPR screen. The dots indicate non-targeting control sgRNAs were emphasized with dots in red color. C) Correlation plots between each replicate in the indicated treatment condition. E) Colony formation assay results with EP300 inhibitor SGC-CBP30 (5μM). PC9 cells were incubated with DMSO, low dose THZ1 (25nM), Erlotinib (100 nM) or Combo with or with out sub-lethal dose of SGC-CBP30 for 18 days.

Figure S3 Validation experiments with PC9 or HCC827 cells transduced sgRNAs targeting ufmylation

A, Drug sensitivity assay was performed with ufmlyation genes depleted PC9 cells. Relative proliferation was measured by cell titer glo assay on day 4 after indicated drug treatment. B) UBA5, UFC1 or UFL1 depleted or control sgRNA infected PC9 cells were treated with DMSO, 50nM THZ1 (THZ1), 100nM erlotinib (Erlo) or 50nM THZ1 + erlotinib (Combo) for 18 days. C) Immunobloting analysis showed the protein expression of UFM1 or UFSP2 of UFM1 or UFSP2 deleted HCC827 cells. D) The fold change of combo treated PC9 xenograft tumors compared with untreated xenograft tumors at day 28th. PC9 cells were transduced indicated sgRNAs. E) PC9 cells transduced sgUFM1 #1 or sgControl #1 were incubated with DMSO, trametinib (30nM), erlotinib (100nM) or combo for 18 days. After treatment, cell number was counted by Vi-cell counter at day 18 to quantify the proliferation.

Figure S4 ER stress pathway response involve in the phenotypic change of the PC9 cells in the abcense of ufmylation

A) RNA-seq result of IL-6 expression levels in PC9 cells transduced indicated sgRNAs incubated with indicated drugs for 48 hr. B) Elisa experiment of IL6 expression in UFM1 or UFSP2 deleted PC9 cells and control PC9 cells. C) Immunobloting analysis of indicated sgRNA infected PC9 cells. Lysates were extracted from the cells after 72 hr indicated drug treatment. D) qRT-PCR of DDIT3 or ATF4 in PC9 cells transduced indicated sgRNAs. E) Immunobloting analysis with UFM1 depleted or control PC9 cells. Cells were incubated with indicated concentration of Tunicamycin for 24 hr before protein extraction. F) Immunoblotting analysis of downstream pathway gene proteins of unfolded protein pathway. Cells were treated with DMSO, IRE1α kinase inhibitor KIRA6 (100 nM) or PERK inhibitor GSK2606414 (300 nM). G qRT-PCR or SQSTM1 in UFM1 or control sgRNA infected PC9 cells after incubated with KIRA6. H) Curated results of CRISPR screen of non-targeting control sgRNAs (n=1000), sgIRE1a (n=4) and sgPERK (n=4). Each bar indicates the log fold change

after indicated treatment. I) PC9 cells were treated with DMSO, THZ1, erlotinib or combo ± KIRA6 (100 nM) for 18 days. After treatment, cell number was counted by Vi-cell counter at day 18 to quantify the proliferation.

Figure S5 Inflammation pathway activation affect the status of cancer cells through modifying the apoptosis pathway.

A) Immunobloting analysis of indicated proteins. Cells were incubated 3 hr with 1 μg/ml of poly (dA:dT) or poly (I:C) before protein extraction. B), C) qRT-PCR of STING or IL6 in PC9 cells after 24 hr tunicamycin treatment. D) Relative cell viability after 96 hr of 100nM THZ1 treatment in PC9 cells or HCC827 cells. Cells were pre-treated with 100nM erlotinib plus DMSO or 30 ng/ml tunicamycin for 24 hr. E) Immunobloting analysis of UFM1 or UFSP2 deleted PC9 cells. Proteins were extracted after 72hr incubation with media contains indicated drugs. F) E) Curated RNA-seq data about pro-apoptotic and anti-apoptotic pathway genes. Cells were treated with DMSO (D), 50nM THZ1 (T), 100nM erlotinib (E) or 50nM THZ1 plus 100nM erlotinib (C) for 48 hr. Values shown are log2 fold change after each treatment normalized by DMSO treatment. G) BH3 profiling was performed to show the alteration of mitochondrial permability of PC9 cells after variety of BH3 peptide incubation.

Figure S6 Bcl-xL dependency in the cells with increased ER stress

A) Drug sensitivity assay of indicated drugs in PC9 cells transduced indicated sgRNAs. B), C) Relative cell viability after 96 hr of indicated concentration of A1331852 treatment in PC9 cells or HCC827 cells. Cells were pre-treated with 100nM erlotinib plus DMSO or

30 ng/ml tunicamycin for 24 hr.