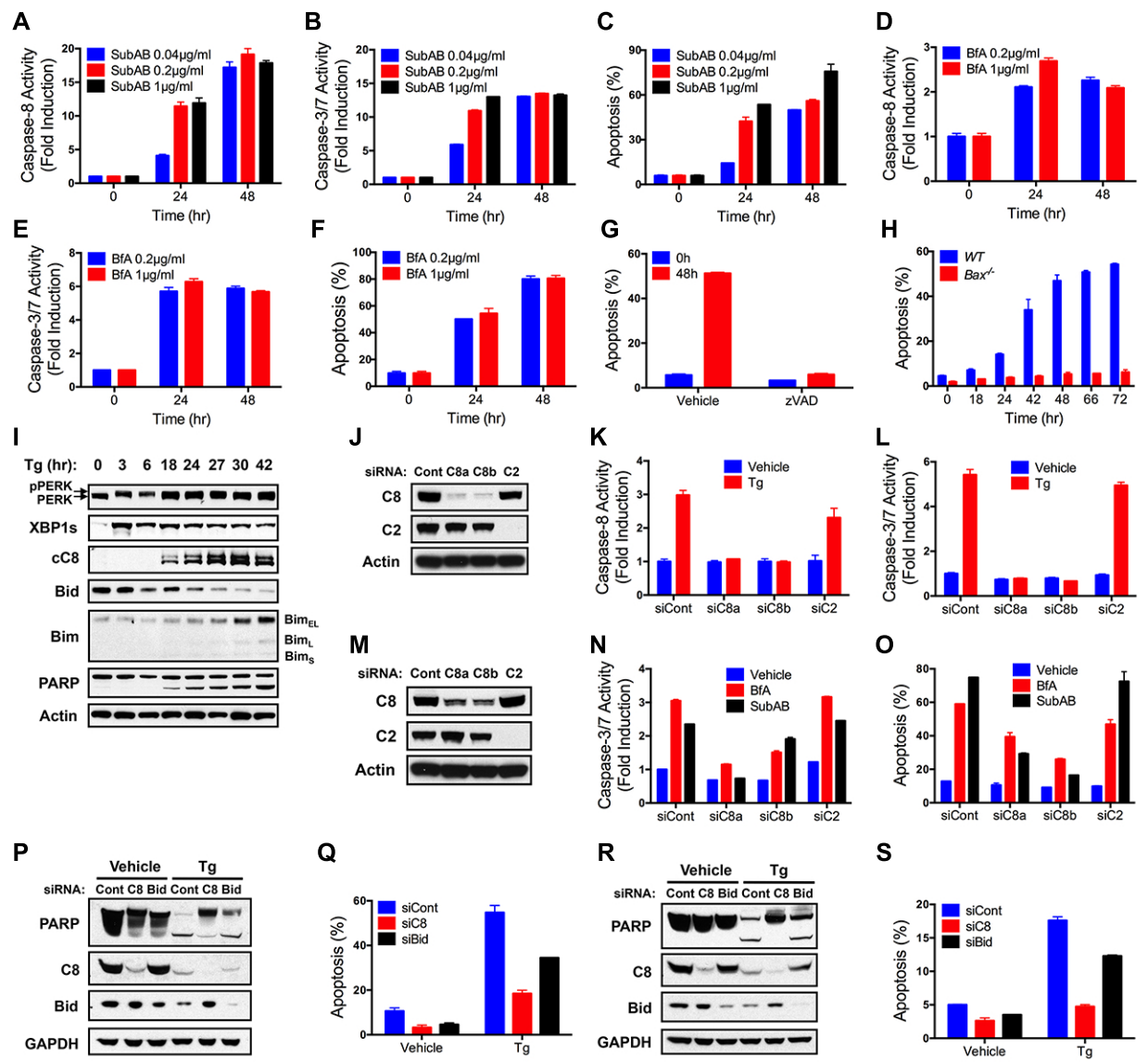
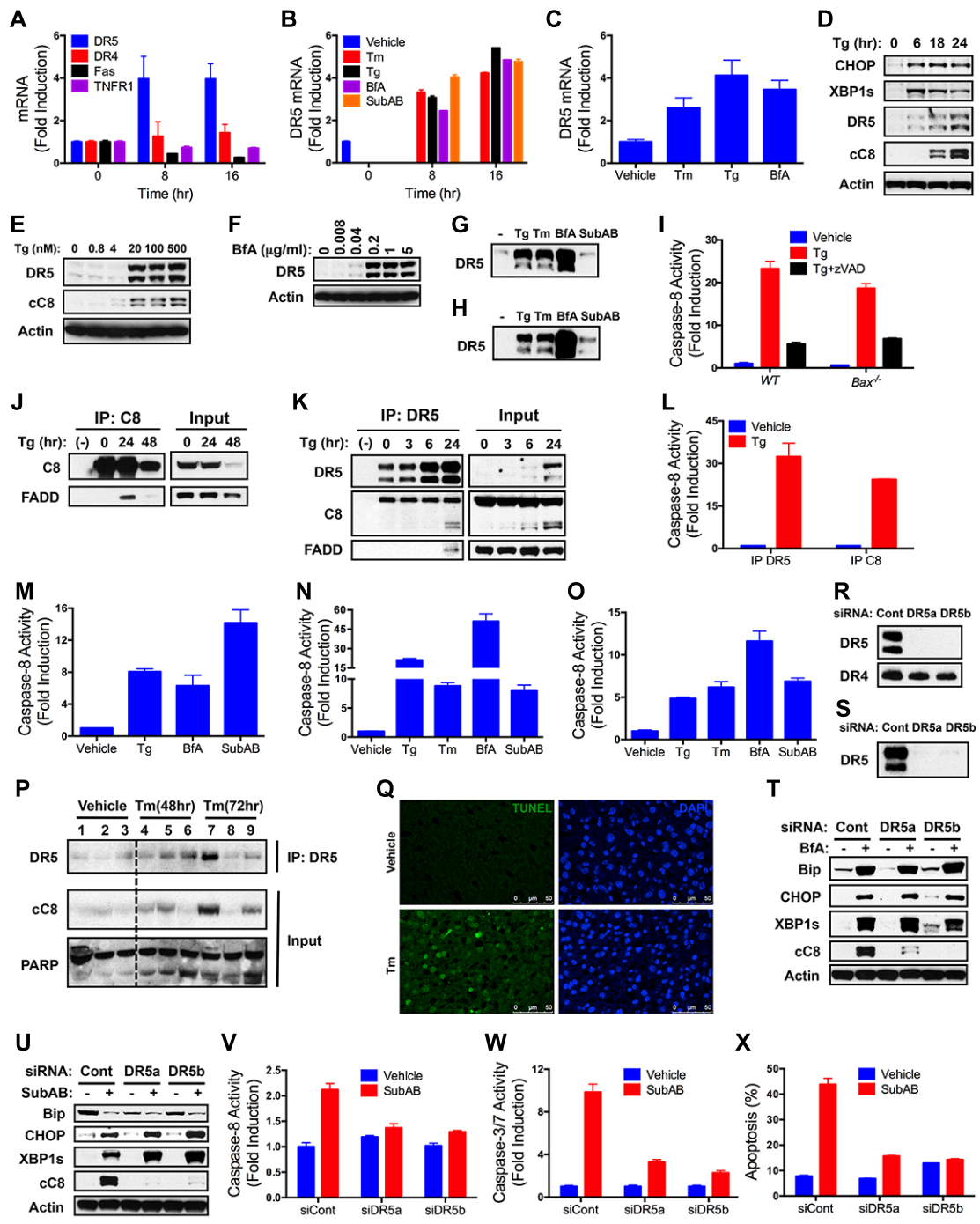
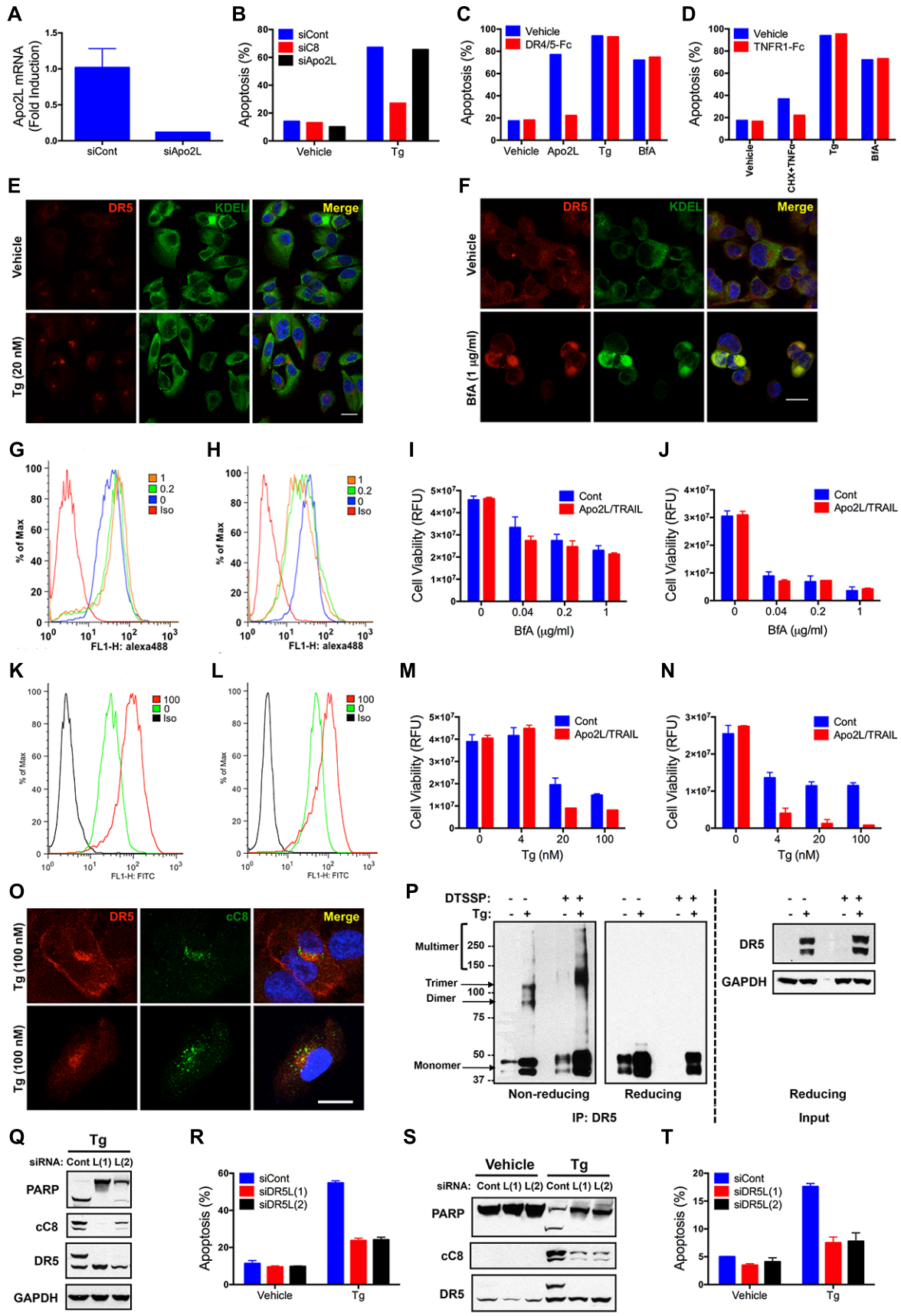
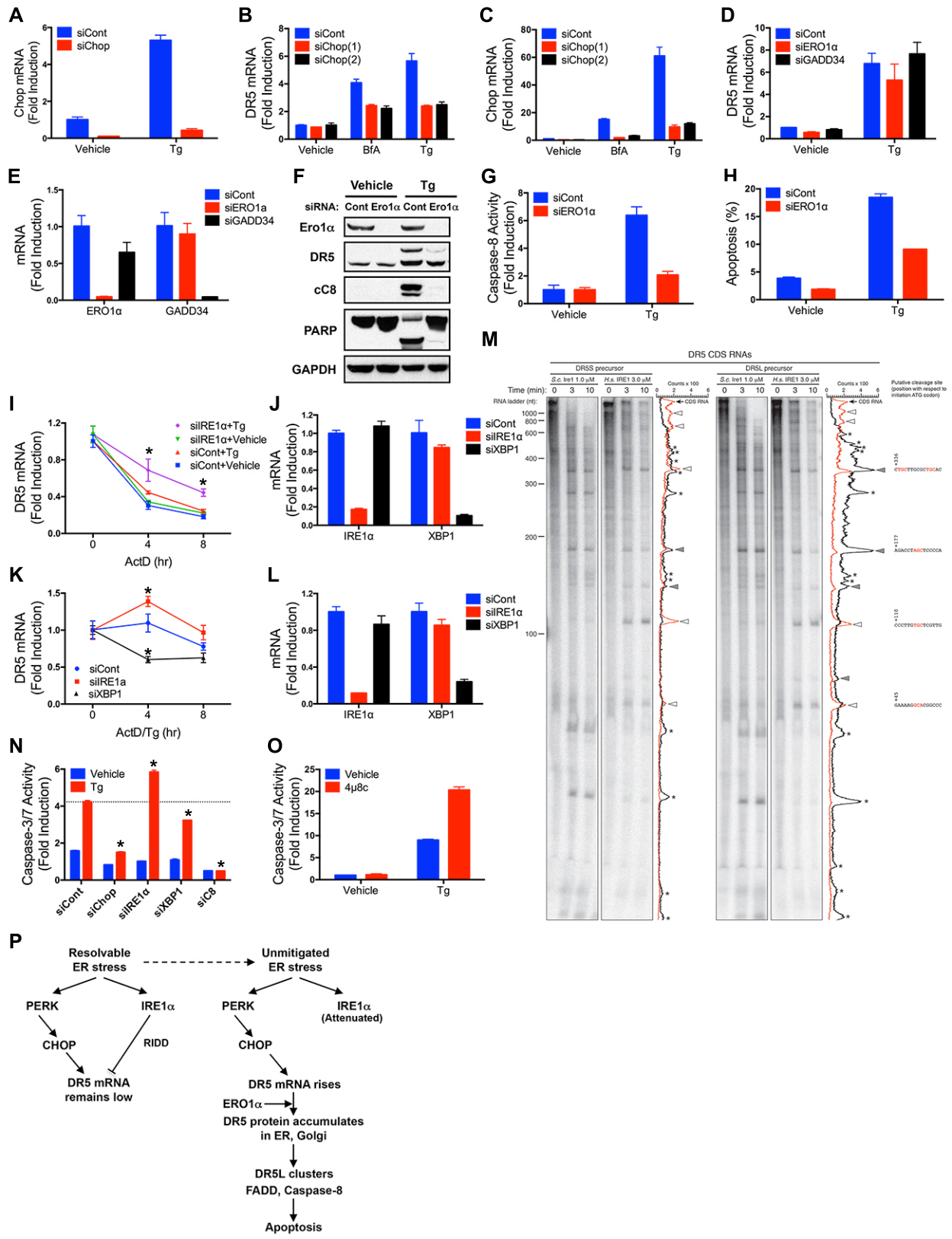

A
Caspase-8 Activity (Fold Induction)
SubAB 0.04μg/ml
SubAB 0.2μg/ml
SubAB 1μg/ml
Time (hr)
B
Caspase-3/7 Activity (Fold Induction)
C
Apoptosis (%)
D
BfA 0.2μg/ml
BfA 1μg/ml
E
F
G
0h
48h
Vehicle
zVAD
H
WT
Bax-/-
I
Tg (hr): 0 3 6 18 24 27 30 42
pPERK
PERK
XBP1s
cC8
Bid
Bim
BimEL
BimL
BimS
PARP
Actin
J
siRNA: Cont C8a C8b C2
C8
C2
Actin
K
Vehicle
Tg
siCont
siC8a
siC8b
siC2
L
M
N
BfA
SubAB
O
P
Vehicle
Tg
siRNA: Cont C8 Bid Cont C8 Bid
PARP
C8
Bid
GAPDH
Q
siCont
siC8
siBid
R
S


A
mRNA (Fold Induction)
DR5
DR4
Fas
TNFR1
Time (hr)
B
DR5 mRNA (Fold Induction)
Vehicle
Tm
Tg
BfA
SubAB
Time (hr)
C
DR5 mRNA (Fold Induction)
Vehicle
Tm
Tg
BfA
D
Tg (hr): 0 6 18 24
CHOP
XBP1s
DR5
cC8
Actin
E
Tg (nM): 0 0.8 4 20 100 500
DR5
cC8
Actin
F
BfA (μg/ml): 0 0.008 0.04 0.2 1 5
DR5
Actin
G
- Tg Tm BfA SubAB
DR5
H
- Tg Tm BfA SubAB
DR5
I
Caspase-8 Activity (Fold Induction)
Vehicle
Tg
Tg+zVAD
WT
Bax-/-
J
IP: C8
Input
Tg (hr): (-) 0 24 48 0 24 48
C8
FADD
K
IP: DR5
Input
Tg (hr): (-) 0 3 6 24 0 3 6 24
DR5
C8
FADD
L
Caspase-8 Activity (Fold Induction)
Vehicle
Tg
IP DR5
IP C8
M
Caspase-8 Activity (Fold Induction)
Vehicle
Tg
BfA
SubAB
N
Caspase-8 Activity (Fold Induction)
Vehicle
Tg
Tm
BfA
SubAB
O
Caspase-8 Activity (Fold Induction)
Vehicle
Tg
Tm
BfA
SubAB
R
siRNA: Cont DR5a DR5b
DR5
DR4
S
siRNA: Cont DR5a DR5b
DR5
P
Vehicle Tm(48hr) Tm(72hr)
1 2 3 4 5 6 7 8 9
DR5
IP: DR5
cC8
Input
PARP
Q
TUNEL
DAPI
Vehicle
Tm
T
siRNA: Cont DR5a DR5b
BfA: - + - + - +
Bip
CHOP
XBP1s
cC8
Actin
U
siRNA: Cont DR5a DR5b
SubAB: - + - + - +
Bip
CHOP
XBP1s
cC8
Actin
V
Caspase-8 Activity (Fold Induction)
Vehicle
SubAB
siCont
siDR5a
siDR5b
W
Caspase-3/7 Activity (Fold Induction)
Vehicle
SubAB
siCont
siDR5a
siDR5b
X
Apoptosis (%)
Vehicle
SubAB
siCont
siDR5a
siDR5b


A
Apo2L mRNA (Fold Induction)
siCont
siApo2L
B
Apoptosis (%)
siCont
siC8
siApo2L
Vehicle
Tg
C
Apoptosis (%)
Vehicle
DR4/5-Fc
Vehicle
Apo2L
Tg
BfA
D
Apoptosis (%)
Vehicle
TNFR1-Fc
Vehicle
CHX+TNFα
Tg
BfA
E
DR5
KDEL
Merge
Vehicle
Tg (20 nM)
F
DR5
KDEL
Merge
Vehicle
BfA (1 μg/ml)
G
% of Max
1
0.2
0
Iso
FL1-H: alexa488
H
% of Max
1
0.2
0
Iso
FL1-H: alexa488
I
Cell Viability (RFU)
Cont
Apo2L/TRAIL
BfA (μg/ml)
J
Cell Viability (RFU)
Cont
Apo2L/TRAIL
BfA (μg/ml)
K
% of Max
100
0
Iso
FL1-H: FITC
L
% of Max
100
0
Iso
FL1-H: FITC
M
Cell Viability (RFU)
Cont
Apo2L/TRAIL
Tg (nM)
N
Cell Viability (RFU)
Cont
Apo2L/TRAIL
Tg (nM)
O
DR5
cC8
Merge
Tg (100 nM)
Tg (100 nM)
P
DTSSP:
Tg:
Multimer
Trimer
Dimer
Monomer
Non-reducing
Reducing
IP: DR5
DR5
GAPDH
Reducing
Input
Q
Tg
siRNA: Cont L(1) L(2)
PARP
cC8
DR5
GAPDH
R
Apoptosis (%)
siCont
siDR5L(1)
siDR5L(2)
Vehicle
Tg
S
Vehicle
Tg
siRNA: Cont L(1) L(2) Cont L(1) L(2)
PARP
cC8
DR5
T
Apoptosis (%)
siCont
siDR5L(1)
siDR5L(2)
Vehicle
Tg


A
siCont
siChop
Chop mRNA (Fold Induction)
Vehicle
Tg
B
siCont
siChop(1)
siChop(2)
DR5 mRNA (Fold Induction)
Vehicle
BfA
Tg
C
siCont
siChop(1)
siChop(2)
Chop mRNA (Fold Induction)
Vehicle
BfA
Tg
D
siCont
siERO1α
siGADD34
DR5 mRNA (Fold Induction)
Vehicle
Tg
E
siCont
siERO1a
siGADD34
mRNA (Fold Induction)
ERO1α
GADD34
F
Vehicle
Tg
siRNA: Cont Ero1α Cont Ero1α
Ero1α
DR5
cC8
PARP
GAPDH
G
siCont
siERO1α
Caspase-8 Activity (Fold Induction)
Vehicle
Tg
H
siCont
siERO1α
Apoptosis (%)
Vehicle
Tg
I
siIRE1α+Tg
siIRE1α+Vehicle
siCont+Tg
siCont+Vehicle
DR5 mRNA (Fold Induction)
ActD (hr)
J
siCont
siIRE1α
siXBP1
mRNA (Fold Induction)
IRE1α
XBP1
K
siCont
siIRE1a
siXBP1
DR5 mRNA (Fold Induction)
ActD/Tg (hr)
L
siCont
siIRE1α
siXBP1
mRNA (Fold Induction)
IRE1α
XBP1
M
DR5 CDS RNAs
DR5S precursor
DR5L precursor
S.c. Ire1 1.0 μM
H.s. IRE1 3.0 μM
Time (min): 0 3 10 0 3 10 0 3 10 0 3 10
RNA ladder (nt):
Counts x 100
CDS RNA
Putative cleavage site (position with respect to initiation ATG codon)
N
Vehicle
Tg
Caspase-3/7 Activity (Fold Induction)
siCont
siChop
siIRE1α
siXBP1
siC8
O
Vehicle
4μ8c
Caspase-3/7 Activity (Fold Induction)
Vehicle
Tg
P
Resolvable ER stress
Unmitigated ER stress
PERK
IRE1α
PERK
IRE1α (Attenuated)
CHOP
RIDD
CHOP
DR5 mRNA remains low
DR5 mRNA rises
ERO1α
DR5 protein accumulates in ER, Golgi
DR5L clusters
FADD, Caspase-8
Apoptosis