Supplementary Legend

Figure S1. TRAIL and ABT-737 induced cleavage of caspases and Bid. A498 renal cancer cells were treated with DMSO, TRAIL, ABT-737 or the combination for 6h (**A**; *left panel*) or 24h (**B**; *right panel*). Western blots were accomplished to measure the level of caspase-8, Bid, caspase-9, caspase-3 and GAPDH. Arrows denote procaspase-8 (p55 and p53), first cleavage fragments (p47 and p43) and the active p18 form of caspase-8; procaspase-9 (p47) and the cleavage products (p37 and p35); procaspase-3 (p32) and the cleavage products (p21, p17); and full length Bid (p22) and the truncated Bid (p15).

Figure S2. Apoptosis induced by TRAIL and ABT-737 requires activation of both caspase-8 and caspase-9. **(A)** Bax expression levels in HCT116 and DU145 cell lines demonstrated by western blot analysis. The GAPDH antibody probe serves as a loading control. **(B)** Bax-proficient (Bax+/-) and -deficient (Bax-/-) HCT116 cells were treated with or without ABT-737 and 50 ng/ml TRAIL for 16 hr. Cell viability (% change from the control) was determined by acid phosphatase assay (Mean+/-SD, n=4). **(C)** Western blots demonstrate the role of caspase cleavage to induction of apoptosis. PV10 cells were pretreated with DMSO, z-LEHD-fmk (20 μM; caspase-9 inhibitor), or z-IETD-fmk (20 μM; caspase-8 inhibitor) followed by ABT-737, TRAIL or combination therapy for 3 hr. **(D)** PV-10 cells were treated as in Figure S2C for 16 hr with ABT-737, TRAIL, z-LEHD, z-IETD-fmk or the combination and changes in cell viability was assessed by an acid phosphatase assay (Mean+/-SD, n=4).

Figure S3. The effect of an inactive ABT-737 enantiomer on cell viability and synergism with TRAIL. PV-10 cells were treated with 10 μM inactive enantiomer or ABT-737 with or without TRAIL (100 ng/ml). The acid phosphatase assay was done to assess cell viability and the mean +/- SD (n=4) is shown.

Figure S4. The role of Bak in TRAIL and ABT-737-induced apoptosis. **(A)** Western blot analysis of PV10 cells transfected with either control or Bak siRNA for 48hr. **(B)** PV10 cells transfected with either control or Bak siRNA duplexes were treated with DMSO, 100 ng/ml TRAIL, ABT-737 10 μM or the combination for 24 hr. Cell viability was measured by the acid phosphatase assay (Mean +/- SD, n=4). **(C)** DU145 and HCT116 cell lines were transfected with a Bak expressing cDNA or empty control vector for 48 hr. The overexpression of Bak was examined by western blotting. **(D)** Bak-overexpressing DU145 and HCT116 Bax-/- cells were treated with DMSO, 100 ng/ml TRAIL, ABT-737 10 μM or the combination for 24 hr. Cell viability was measured by the acid phosphatase assay (Mean +/- SD, n=4).

Figure S5. The synergism of ABT-737 and TRAIL in the induction of apoptosis requires DR5. **(A)** Tumor cell lines were treated with either DMSO (-) or 10 μM ABT-737 (+) for 24 hr and western blots carried out to measure DR5 levels. **(B)** PV10 cells were treated with either 10 μM ABT-737 or an inactive enantiomer in the presence or absence of z-VAD-fmk (20 μM) for 16 hr. Cell extracts were used for western blotting with DR5, DR4 and GAPDH antibodies. **(C)** 786-O cells were transfected with pcDNA3.1 expressing DR5 (0, 0.1, 0.5, 1 or 2 μg) for 36 hr and extracts of these cells were subjected to western blotting with antibodies to DR5 and GAPDH. PV10 cells treated with ABT-737 were used as a control. **(D)** 786-O cells expressing DR5 (1 μg DNA), which produced a similar level of DR5 expression to that seen in the PV10 cells, were treated with DMSO, TRAIL (100 ng/ml), ABT-737 or the combination for 16hr. Cell viability was assessed by acid phosphatase assay (Mean +/- SD, n=4).

Figure S6. Identification of the region of the DR5 promoter regulated by ABT-737. **(A)** PV10 cells were transfected with the pGVB2-based reporter plasmids (4 μg DNA) containing various lengths of 5'- human DR5 promoter gene. Cells were treated with DMSO or ABT-737 for 24 hr. Luciferase activity was measured as described in Methods and the activity was normalized to *Renilla*-luc as described (Mean +/- SD, n=3). **(B)** PV10 cells were co-transfected with siRNA duplexes targeting CHOP and PGVB2-DR5-luc and then treated with DMSO, ABT-737 or tunicamycin (1 μM). Luciferase activity was measured as described in Methods and the activity was normalized to *Renilla*-luc as described (Mean +/-SD, n=3).

Figure S1

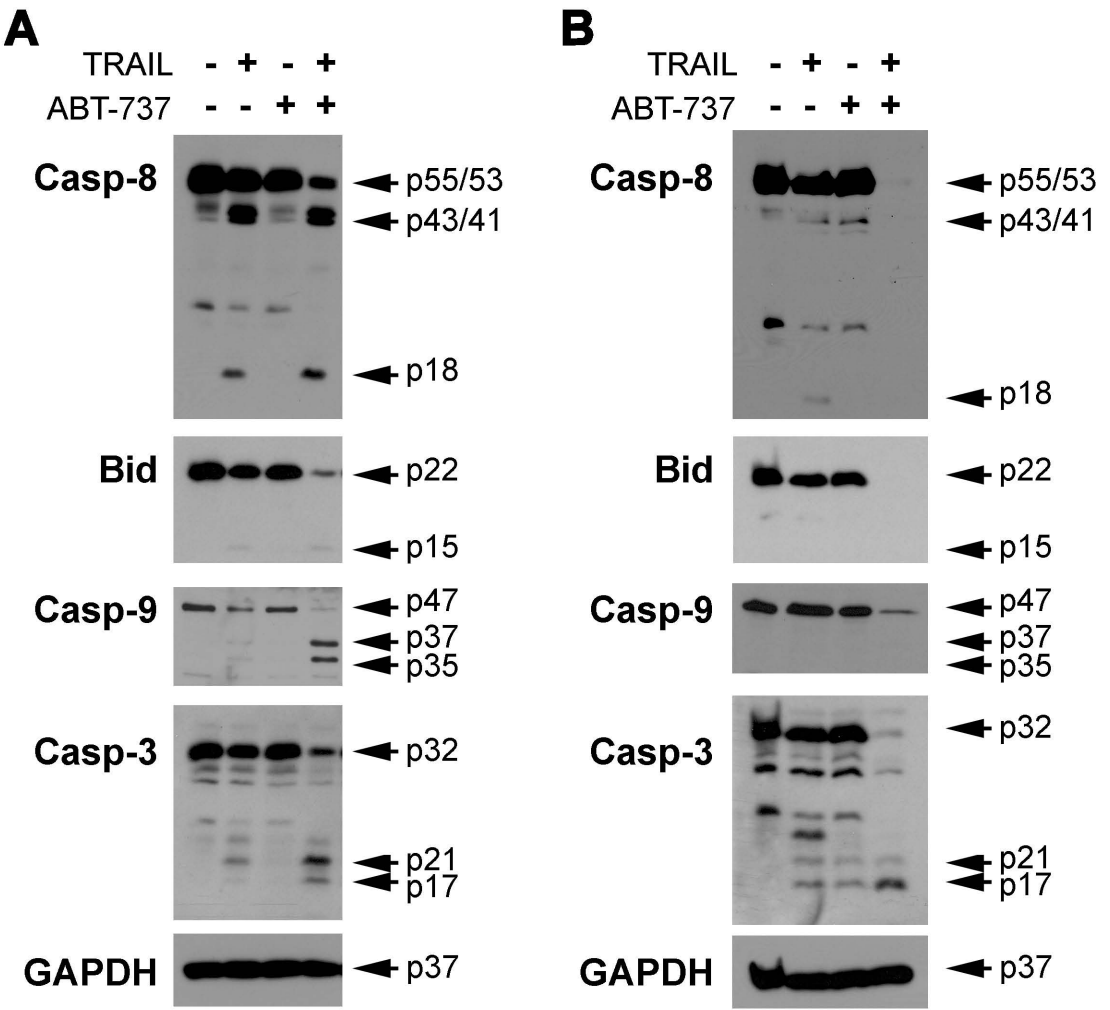
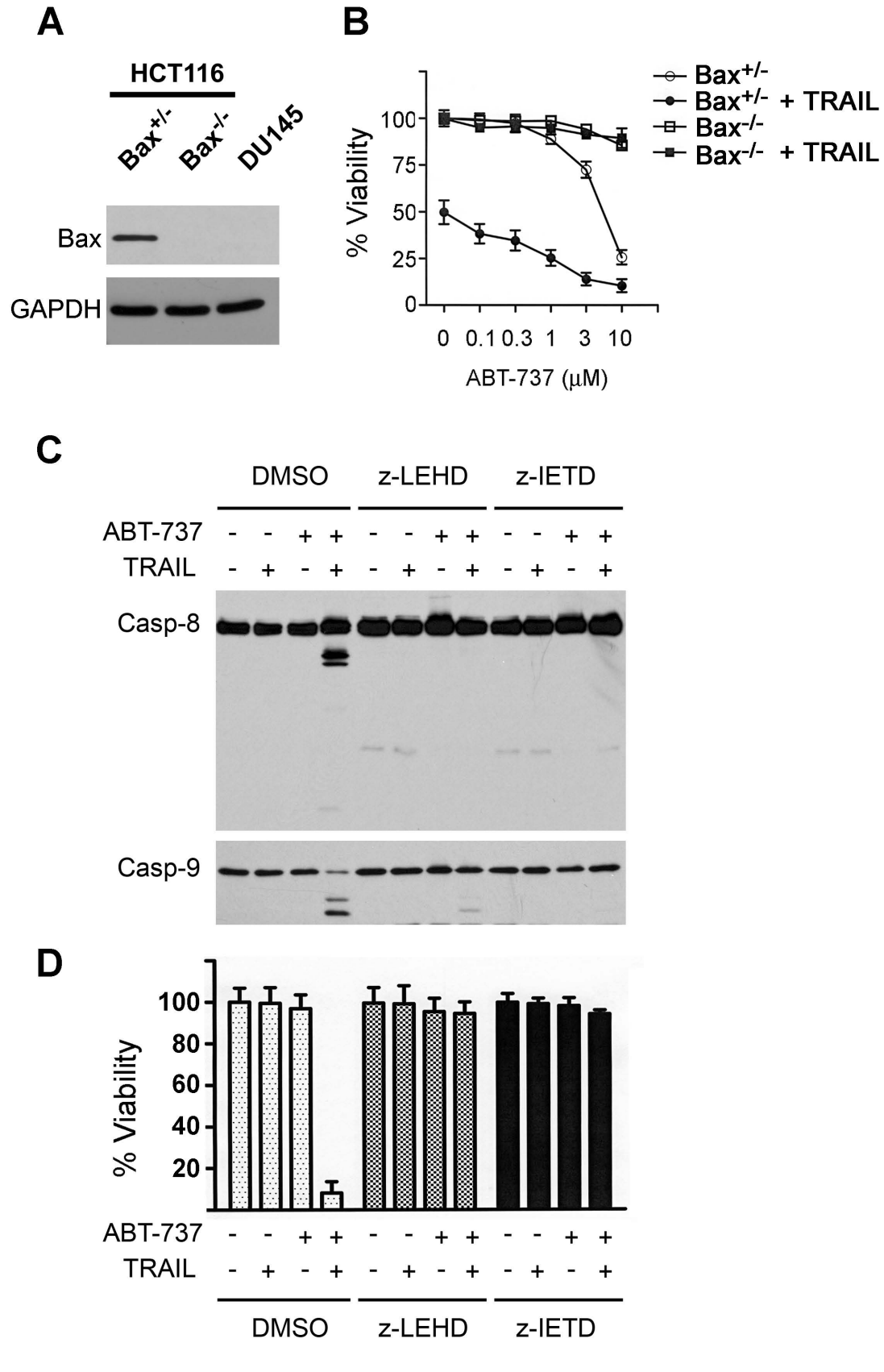

A
TRAIL - + - +
ABT-737 - - + +
Casp-8
p55/53
p43/41
p18
Bid
p22
p15
Casp-9
p47
p37
p35
Casp-3
p32
p21
p17
GAPDH
p37
B
TRAIL - + - +
ABT-737 - - + +
Casp-8
p55/53
p43/41
p18
Bid
p22
p15
Casp-9
p47
p37
p35
Casp-3
p32
p21
p17
GAPDH
p37

Figure S2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HCT116
Bax+/-
Bax-/-
DU145
Bax
GAPDH
B
% Viability
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75
50
25
0
0 0.1 0.3 1 3 10
ABT-737 (μM)
Bax+/-
Bax+/- + TRAIL
Bax-/-
Bax-/- + TRAIL
C
DMSO
z-LEHD
z-IETD
ABT-737
TRAIL
Casp-8
Casp-9
D
% Viability
100
80
60
40
20
ABT-737
TRAIL
DMSO
z-LEHD
z-IETD

Figure S3

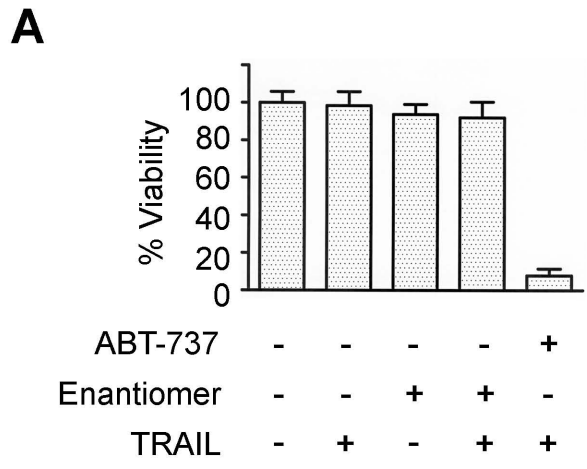
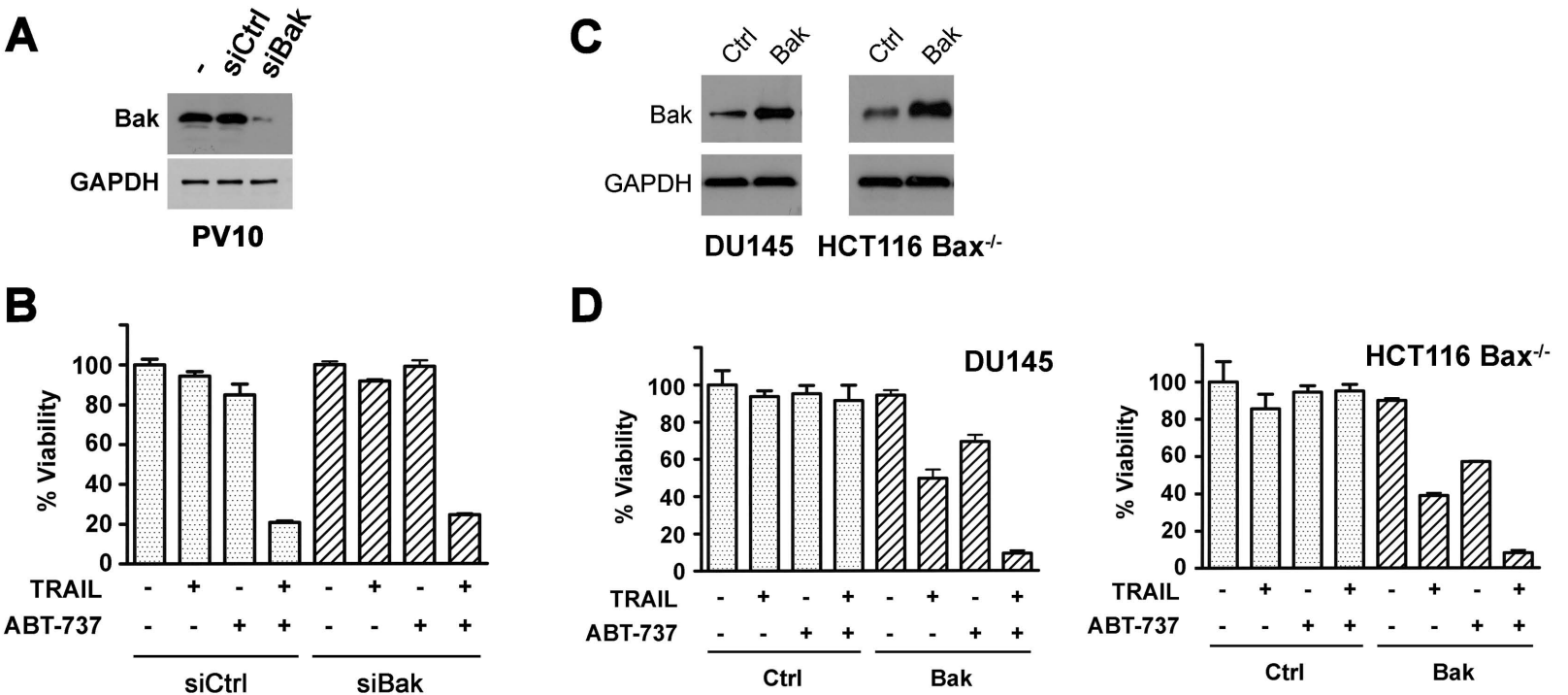


Figure S4


A
-
siCtrl
siBak
Bak
GAPDH
PV10
C
Ctrl
Bak
Ctrl
Bak
Bak
GAPDH
DU145
HCT116 Bax-/-
B
% Viability
100
80
60
40
20
0
TRAIL - + - + - + - +
ABT-737 - - + + - - + +
siCtrl
siBak
D
DU145
% Viability
100
80
60
40
20
0
TRAIL - + - + - + - +
ABT-737 - - + + - - + +
Ctrl
Bak
HCT116 Bax-/-
% Viability
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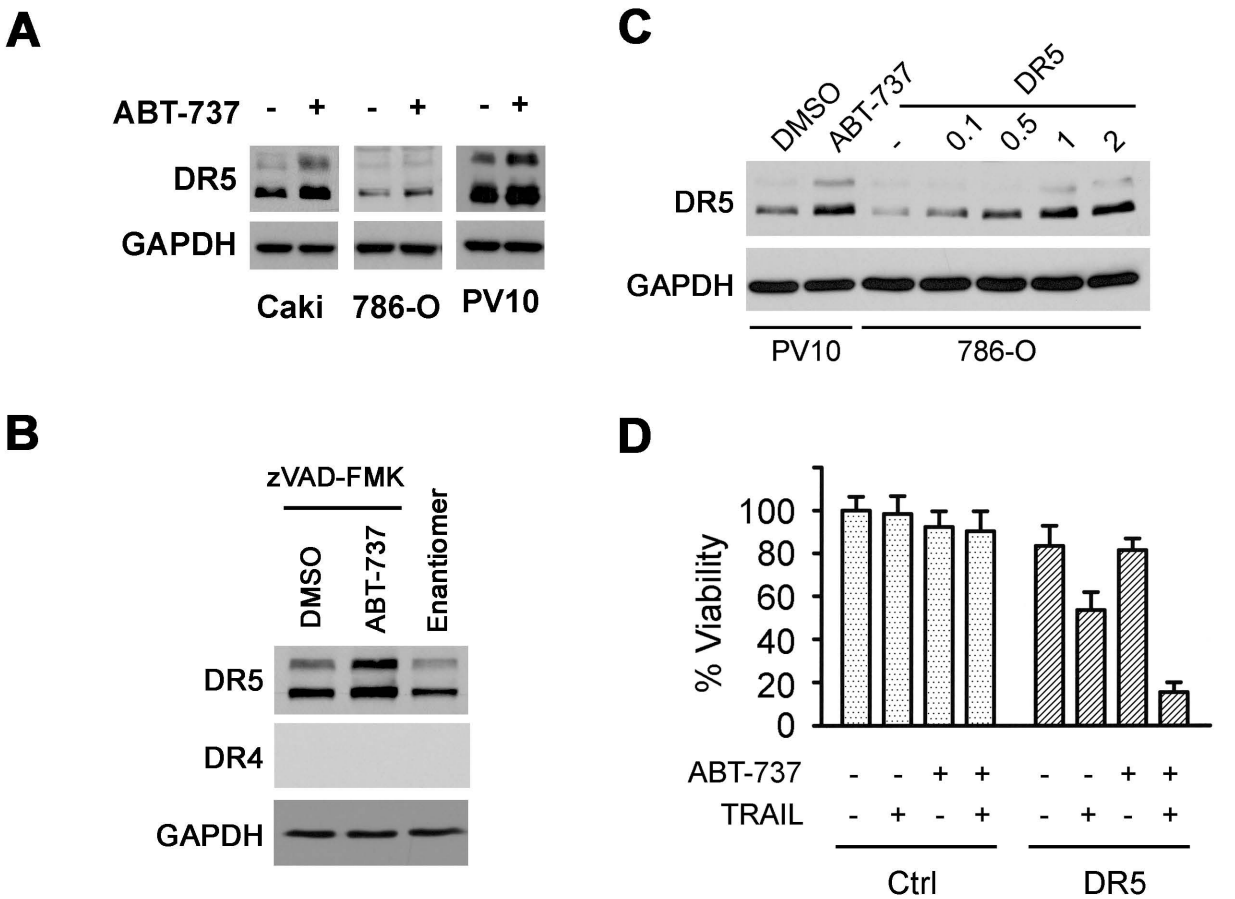
60
40
20
0
TRAIL - + - + - + - +
ABT-737 - - + + - - + +
Ctrl
Bak

Figure S5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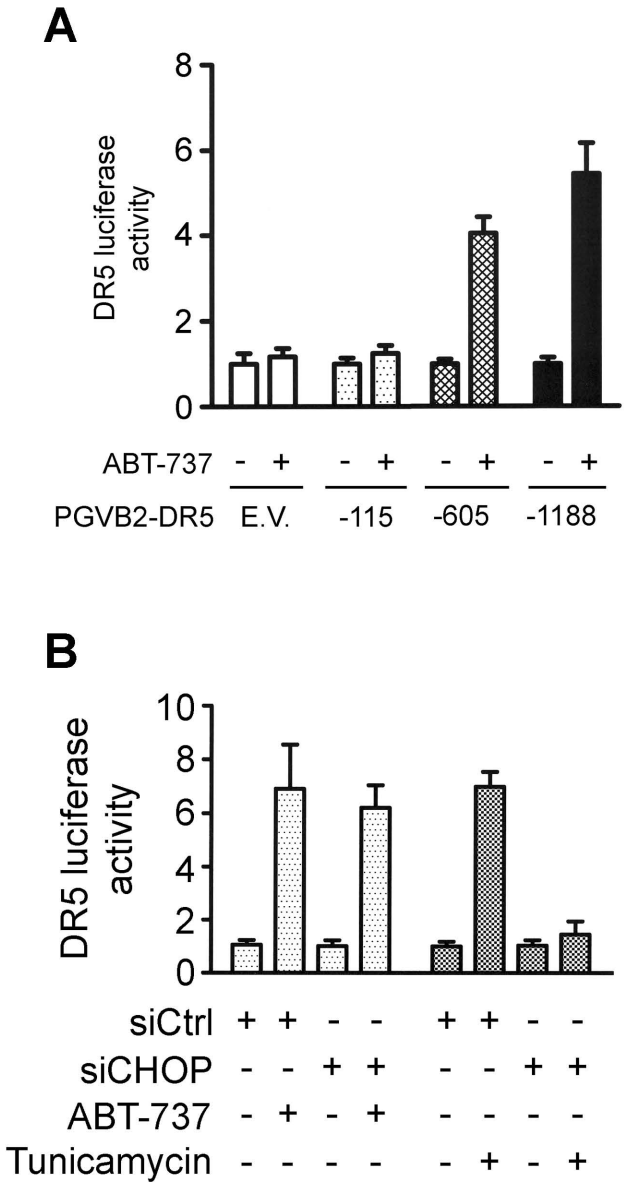
ABT-737 - + - + - +
DR5
GAPDH
Caki 786-O PV10
B
zVAD-FMK
DMSO
ABT-737
Enantiomer
DR5
DR4
GAPDH
C
DMSO
ABT-737
DR5
- 0.1 0.5 1 2
DR5
GAPDH
PV10
786-O
D
% Viability
100
80
60
40
20
0
ABT-737 - - + + - - + +
TRAIL - + - + - + - +
Ctrl
DR5

Figure S6


A
DR5 luciferase activity
8
6
4
2
0
ABT-737 - + - + - + - +
PGVB2-DR5 E.V. -115 -605 -1188
B
DR5 luciferase activity
10
8
6
4
2
0
siCtrl + + - - + + - -
siCHOP - - + + - - + +
ABT-737 - + - + - - - -
Tunicamycin - - - - - + - +