Additional file 1

Additional file legends

Figure S1 – Potent augmentation of ABT-737-killing by chemotherapeutic drugs requires caspases

Cells from RCC cell lines 21, 30, 26A and Caci-2 were treated with 1 µM ABT-737, 100 nM vinblastine, 200 nM paclitaxel, 200 µM etoposide, 1 mM 5-FU or with the combination of ABT-737 plus chemotherapeutic drugs. Cell death was quantified by propidium iodide (A) or Annexin V (B) staining at 24 h. (C) RCC cell line 26A was treated for 6 h and 12 h with ABT-737 + vinblastine or ABT-737 + etoposide and stained for activated caspase-3. (D) RCC cell line 26A was treated with 1 µM ABT-737, 1 mM 5-FU or with the combination of ABT-737 plus 5-FU. 100 µM zVAD-fmk was added 1 h prior to treatment with drugs in (A). Values are the mean/SEM of at least three separate experiments (* P < 0.03, single treatment versus combination treatment).

Figure S2 – Efficiency of the targeting of Mcl-1 or A1 by RNAi

(A), expression levels of Mcl-1 or A1 protein (B) in cells from RCC cell lines 21, 26A, 30, 26A shLuciferase, 26A shMcl-1 and a human melanoma cell line WM35 were analyzed by Western blotting 48 h post transfection with siRNA. Tubulin served as a loading control. As a specificity control, the levels of Mcl-1 mRNA upon A1-knock-down were analyzed (A). Data are means/SEM from three separate experiments. An A1 control lysate from human Burkitt lymphoma cell line Raji has been included in (B). Mcl-1 protein levels are shown for the line WM35 upon A1-knock-down. The immunoblots shown here are representatives of three separate experiments. (C), mRNA levels of A1 in cells from RCC cell lines 21, 26A and 30
were determined by quantitative RT-PCR 48 h post transfection with siRNA. Mean values of not transfected controls were normalized to 100%. Results are means/SEM of three independent transfections.

**Figure S3 – A1-targeting by two different siRNAs sensitizes RCC-26A cells to apoptosis induced by ABT-737**

Cells from the RCC cell line 26A were transfected with two different siRNA sequences targeting A1 (nucleotides 511 to 530 or nucleotides 441 to 460). 48 h post transfection cells were treated with 5 µM ABT-737 for 24 h and cell death was quantified by staining for propidium iodide (A) or activated caspase-3 (B). Data obtained with siA1 441 are the same as in Figure 4 and are here reproduced for comparison. Data represents the mean/SEM of three experiments. (* P < 0.01, ** P < 0.02, control siRNA versus A1 siRNA).

**Figure S4 – Synergism between ABT-737 and etoposide or vinblastine requires Noxa**

Expression levels of Puma (A), Bim (B) and Noxa (C) protein in the RCC cell lines 26A and 30 transfected with specific or control siRNA. Cells were assayed by Western blot analysis 48 h post transfection (A and B) or were treated with 200 µM etoposide for 24 h (C) to induce Noxa. As a specificity control, levels of Bim and Puma were also tested upon treatment with Noxa-specific siRNA. The asterisk indicates an unspecific band. (D), Expression levels of Bcl-2 family proteins in the RCC cell lines 26A and 30. Tubulin served as a loading control. The immunoblots shown are representatives of three separate experiments. A1-levels were analyzed by quantitative RT-PCR (mean/SEM of three separate experiments).
Figure S5 – MG-132 increases the levels of Mcl-1 and Noxa in the RCC-26A cell line and sensitizes for ABT-737 induced apoptosis

(A), expression levels of Noxa and Mcl-1 were analysed by Western blotting in RCC-26A cells treated with 10 µM MG-132 and 200 µM etoposide for 16 h. (B), expression levels of Noxa and Mcl-1 in RCC-26A cells treated with 10 µM MG-132, 200 µM etoposide and 200 µM etoposide plus 100 µM zVAD-fmk for 6 h, 12 h and 24 h. Tubulin served as a loading control. (C), cells from the RCC-26A cell line were treated for 6 h and 12 h with 1 µM ABT-737 plus 10 µM MG-132 or 200 µM etoposide. Apoptosis was measured by staining for activated caspase-3. Data represent the means of three independent experiments/SEM. (D), cells from the RCC-26A cell line were transfected with control or p53-specific siRNA and were treated with 200 µM etoposide 24 h later. Shown are the expression levels of Noxa, Mcl-1 and p53 at 48 h post transfection with siRNA. Tubulin served as a loading control. The immunoblots shown are representatives of three independent experiments.
Figure S1

A

RCC-26A
- No ABT-737
- ABT-737
- ABT-737 + zVAD

Untreated  Vinblastine  Paclitaxel  Etoposide  5-FU

B

RCC-26A
- No ABT-737
- ABT-737

Untreated  Vinblastine  Paclitaxel  Etoposide  5-FU

C

RCC-26A
- ABT-737 + Vinblastine
- ABT-737 + Etoposide

Untreated  6h  12h

D

RCC-26A
- 24h
- 48h

Untreated  ABT-737  5-FU  ABT-737 + 5-FU
Figure S2

A. Protein expression levels in RCC-21, RCC-26A, and RCC-30 cells treated with siControl or siA1, and treated with siMcl-1 or shLuciferase. Western blot images show the expression of Tubulin, A1, and Mcl-1. The bar graphs show the normalized expression levels of Mcl-1 mRNA in RCC-21, RCC-26A, and RCC-30 cells under different conditions.

B. Protein expression levels in WM35, RCC-26A, and Raji cells treated with siControl or siA1. Western blot images show the expression of Tubulin and A1. The bar graphs show the normalized expression levels of A1 mRNA in WM35, RCC-26A, and Raji cells under different conditions.

C. mRNA expression levels of A1 in RCC-21, RCC-26A, and RCC-30 cells treated with siControl or siA1. The bar graphs show the normalized expression levels of A1 mRNA in RCC-21, RCC-26A, and RCC-30 cells under different conditions.

Legend:
- +: Transfected with target siRNA
- -: Not transfected

siA1: Small interfering RNA targeting A1
siMcl-1: Small interfering RNA targeting Mcl-1
shLuciferase: ShRNA targeting Luciferase

 normalized units (%)

Not transfected

A1 mRNA expression

### Figure S4

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#### A

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#### Tubulin

- RCC-26A: 50 kDa
- RCC-30: 50 kDa

#### Puma

- RCC-26A: 25 kDa
- RCC-30: 25 kDa

#### Bim

- RCC-26A: 25 kDa
- RCC-30: 25 kDa

#### Bcl-2

- RCC-26A: 25 kDa
- RCC-30: 20 kDa

#### Bcl-XL

- RCC-26A: 25 kDa
- RCC-30: 25 kDa

#### Bcl-w

- RCC-26A: 20 kDa
- RCC-30: 20 kDa

#### Mcl-1

- RCC-26A: 37 kDa
- RCC-30: 37 kDa

#### A1 mRNA expression

- RCC-26A: 200 relative units
- RCC-30: 800 relative units

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*Puma* indicates the presence of Puma protein.