Supplemental Data

Supplemental Figure 1: Caspase 9 minigene mutants having no effect on the alternative splicing of caspase 9 pre-mRNA. RT-PCR analysis of caspase 9 minigene-derived transcripts from A549s transfected with the WT caspase 9 minigene and the indicated caspase 9 minigene mutants (specific sequence site mutations are described in Table I). The caspase 9a/9b mRNA ratio was determined by densitometric analysis of RT-PCR fragments. Data represent 3 separate determinations on 3 separate occasions.

Supplemental Figure 2: The caspase 9a/9b ratio is dysregulated in transformed lung epithelial cells. Total RNA was isolated from non-transformed, immortalized HBEC-3KT cells and normal human bronchial epithelial (NHBE) cells, and transformed lung epithelial cells, A549 and H2030 cells. RNA was subjected to RT-PCR analysis for caspase 9 splice variants. . All ratios of Casp9a/9b were determined by densitometric analysis of RT-PCR fragments. Data are representative of three separate determinations on two separate occasions.

Supplemental Figure 3: Daunarubicin, Carboplatin, and paclitaxol do not affect the caspase 9a/9b mRNA ratio in NSCLC cells. A549 cells were treated with DNR (0.1 nM to 100 nM), Cap (0.1 nM to 500 nM) or Pac (0.1 nM to 500 nM) for 48 h. Total RNA was subjected to RT-PCR analysis for caspase 9 splice variants after treatment. Data are representative of four separate determinations on four separate occasions.

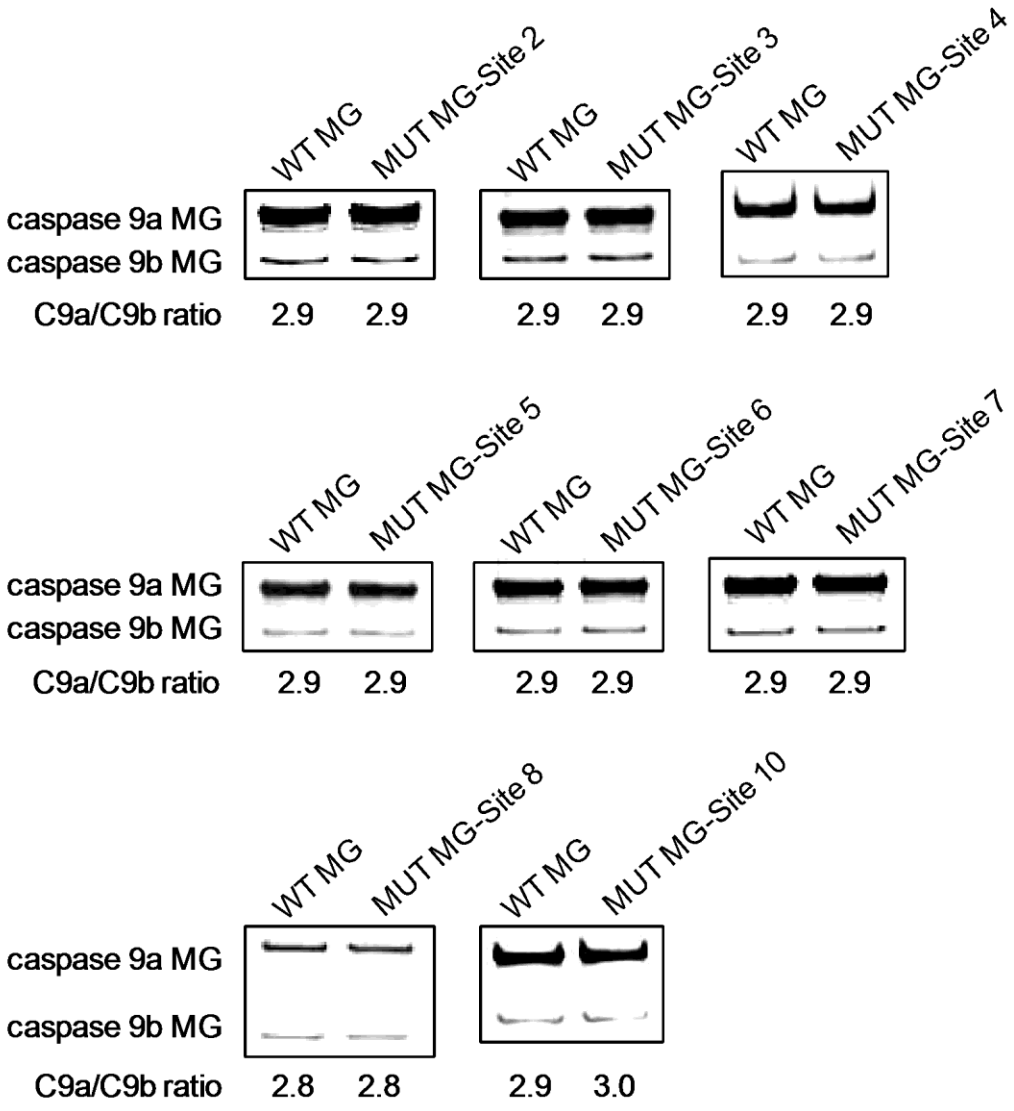
Supplemental Figure 4: Manipulation of the alternative splicing of caspase 9 using an E4 ASRO increases the chemotherapy resistance of A549 cells to Cisplatin and Paclitaxel. A549 cells were transfected with control ASRO (CON) or E4 ASRO (E4). Twenty-four hours post-transfection cells were re-plated and treated with **A)** cisplatium (Cp) (0.1-0.7 ug/ml) or **B)** paclitaxel (Pac) (0.5 nM to 3.0 nM) for 48 h. Cells were then allowed to form colonies for 10 days. The colonies were scored and percentage survival was calculated with respect to the control treated cells. Data are expressed as mean ±SE and are representative of three separate determinations on four separate occasions.

Supplemental Figure 5: Sequence specific silencing of caspase 9b using siRNA sensitizes A549 cells to chemotherapy. A549 cells were transfected with control siRNA (CON) or caspase 9b siRNA (9b-si) (200 nM). Twenty-four hours post-transfection cells were replated (500 cells/ well of a 6-well dish) and treated **A)** cisplatium (Cp) (0.1-0.7 ug/ml) or **B)** paclitaxel (Pac) (0.5 nM to 3.0 nM) for 48 h. Cells were then allowed to form colonies for 10 days. The colonies were scored and percentage survival was calculated with respect to the control siRNA treated cells. Data are expressed as mean ± SE and are representative of three separate determinations on four separate occasions.

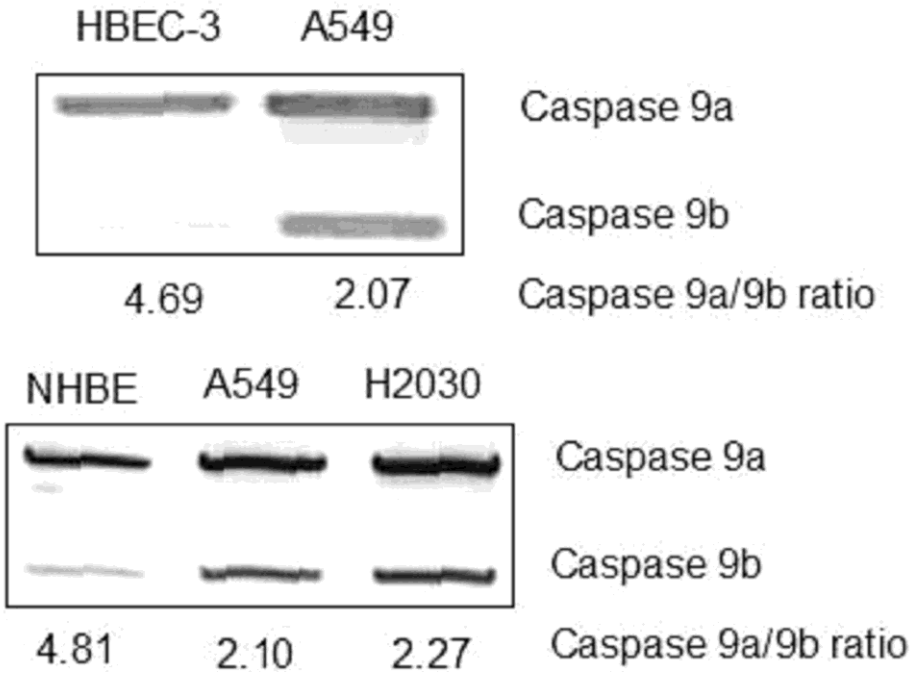
Supplemental Figure 6: The E4-ASRO treatment increases the chemotherapy resistance of H2030 cells. H2030 cells were transfected with control ASRO (CON) or E4 ASRO (E4). Twenty-four hours post-transfection cells were re-plated and treated with **A)** daunorubicin (DNR) (0.1-20 nM) or **B)** paclitaxel (Pac) (0.5 nM to 3.0 nM) for 48 h. Cells were then allowed to form colonies for 10 days. The colonies were scored and percentage survival was calculated with respect to the control treated cells. Data are expressed as mean ±SE and are representative of three separate determinations on four separate occasions.

Supplemental Figure 7: Sequence specific silencing of caspase 9b using siRNA sensitizes H2030 cells to chemotherapy. H2030 cells were transfected with control siRNA (CON) or caspase 9b siRNA (9b-si) (200 nM). Twenty-four hours post-transfection cells were replated (500 cells/ well of a 6-well dish) and treated **A)** daunorubicin (DNR) (0.1-20 nM) or **B)** paclitaxel (Pac) (0.5 nM to 3.0 nM) for 48 h. Cells were then allowed to form colonies for 10 days. The colonies were scored and percentage survival was calculated with respect to the control siRNA treated cells. Data are expressed as mean ± SE and are representative of three separate determinations on four separate occasions.

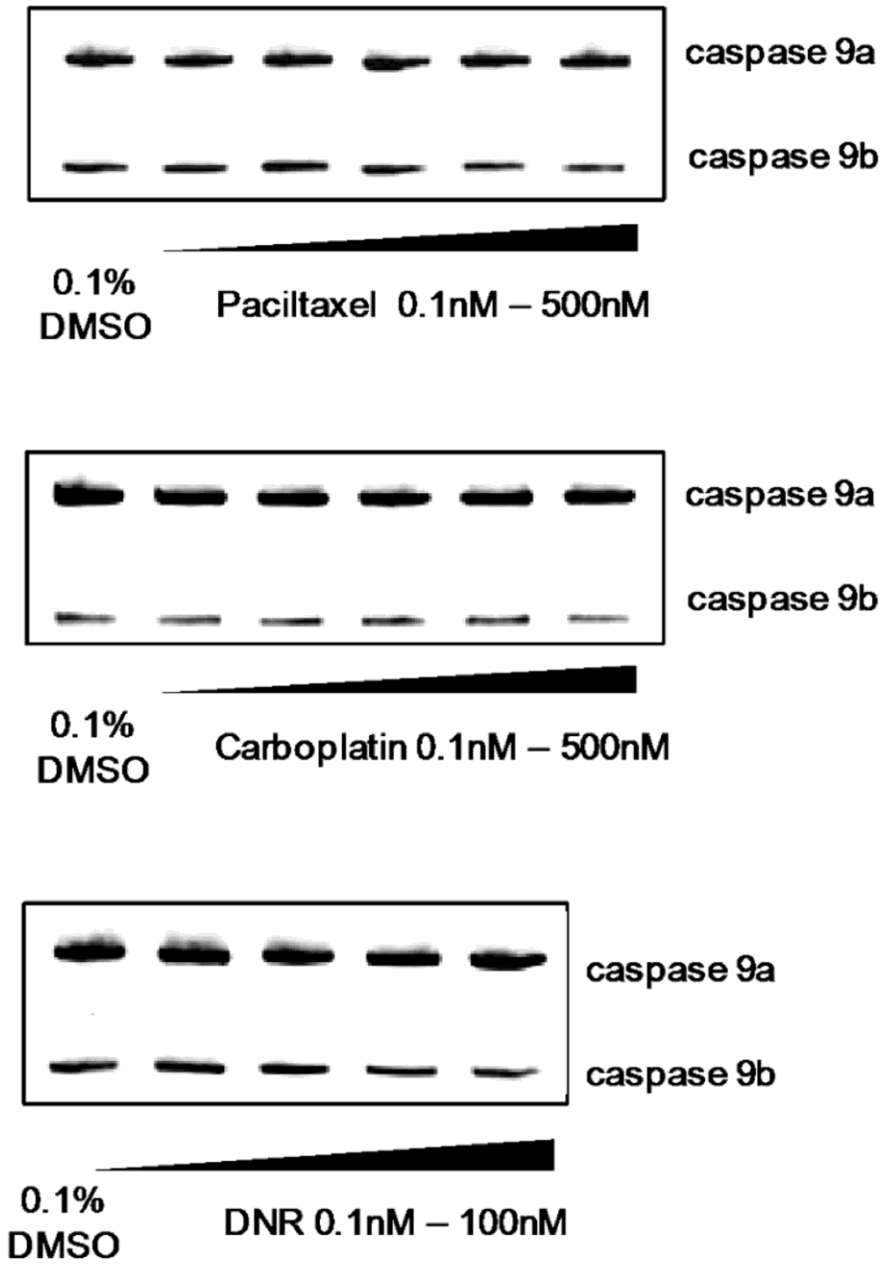
Supplemental Figure 1.



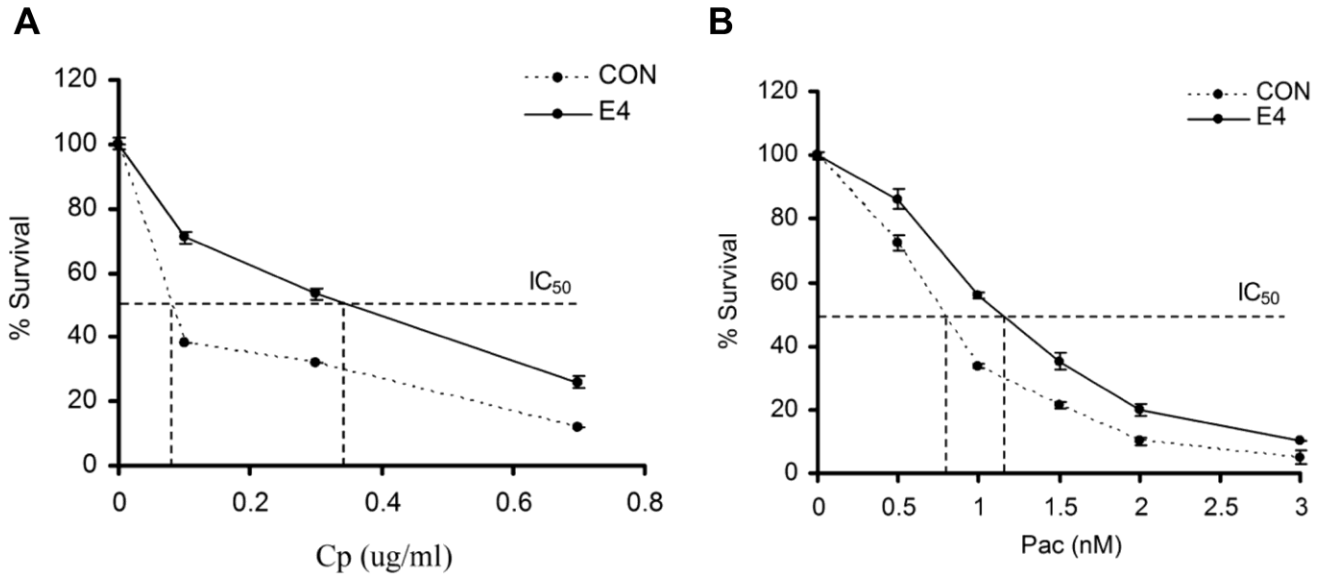
Supplemental Figure 2.



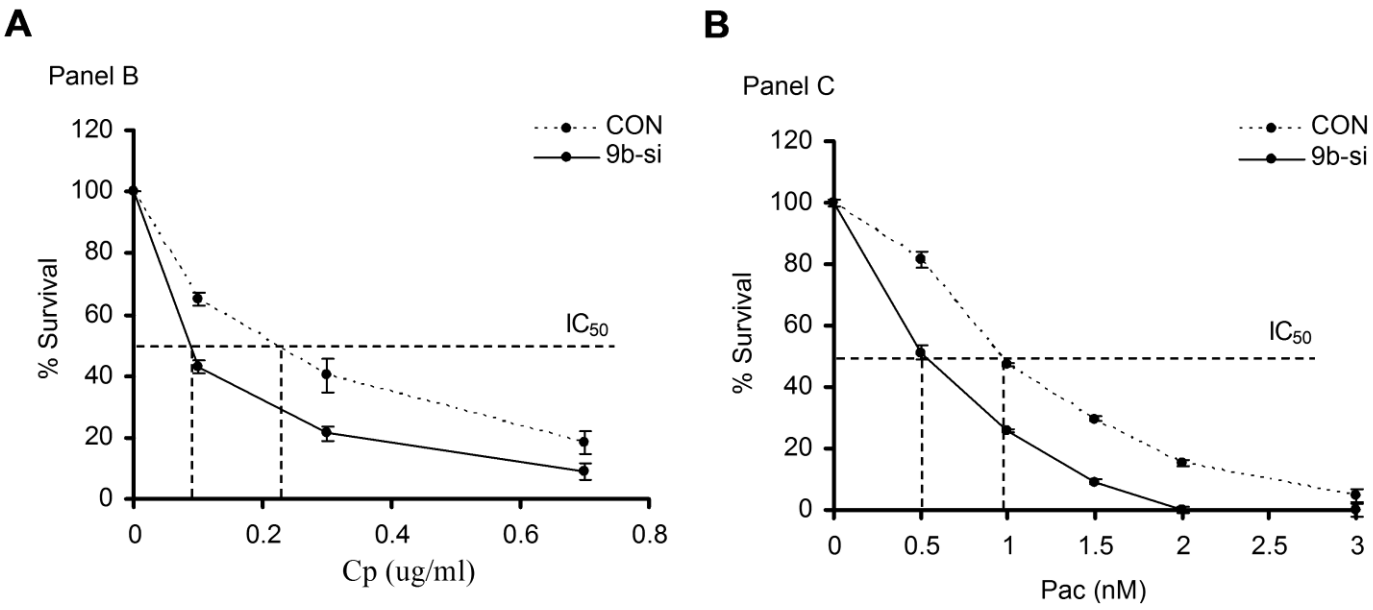
Supplemental Figure 3.



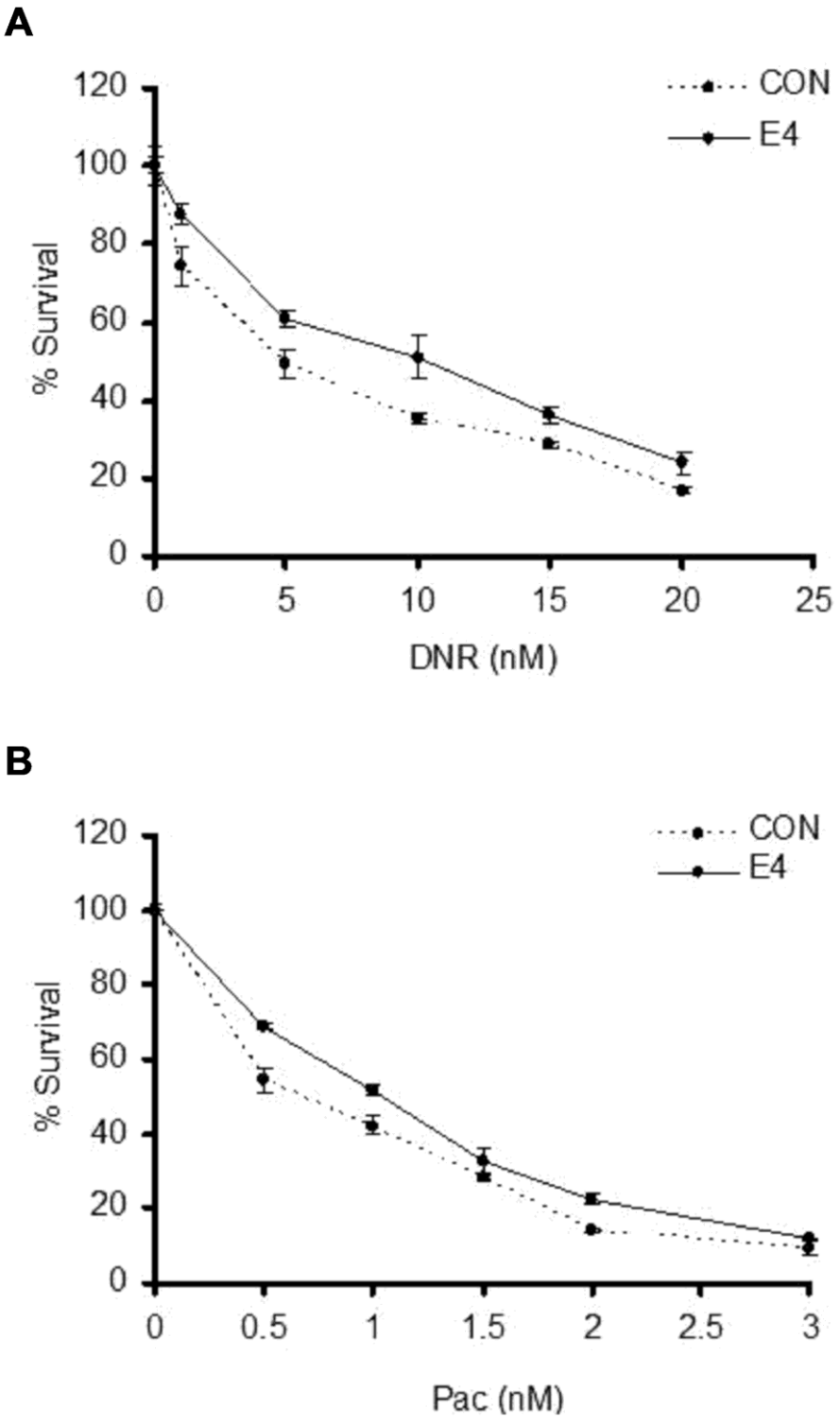
Supplemental Figure 4



Supplemental Figure 5



Supplemental Figure 6



Supplemental Figure 7