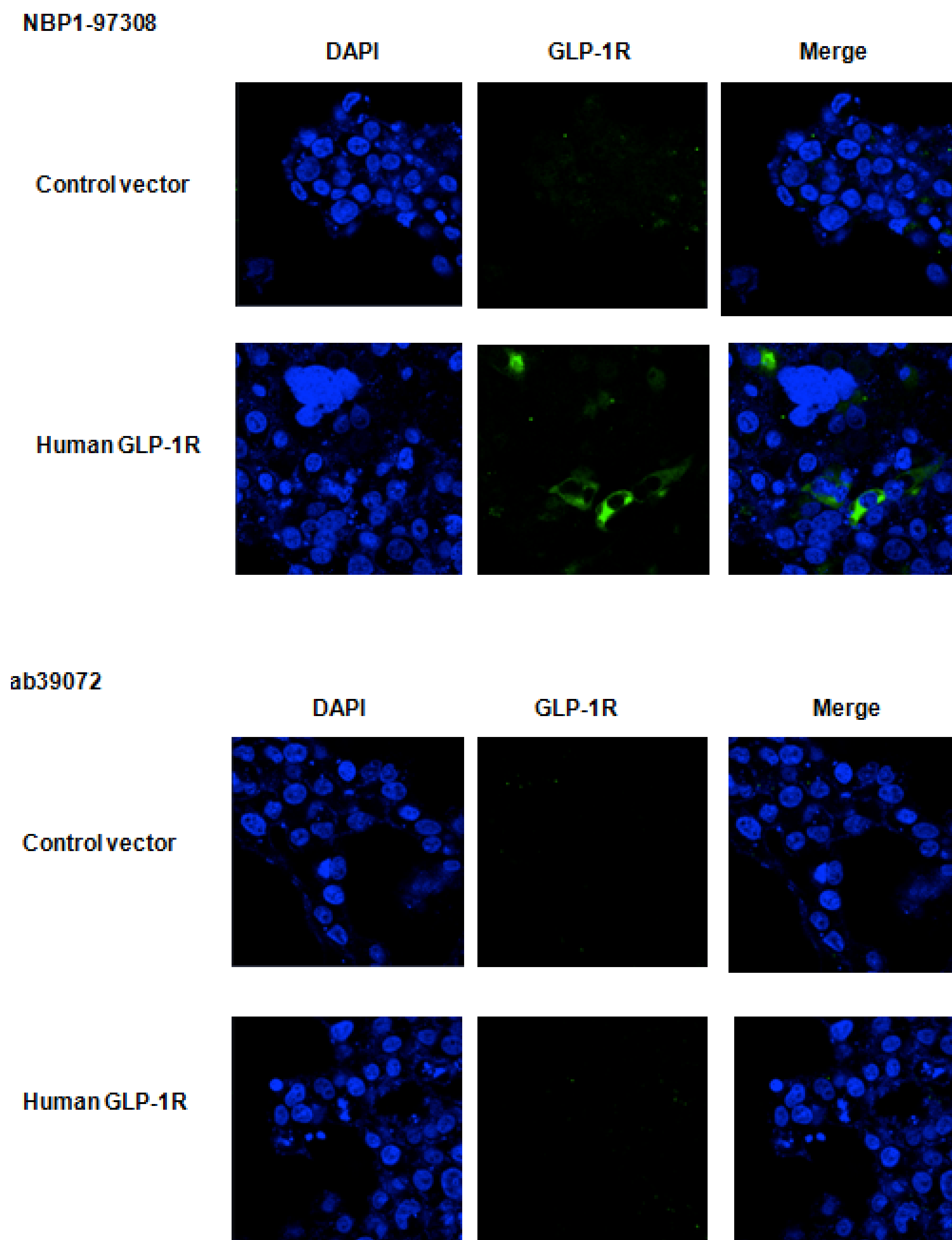
SUPPLEMENTARY DATA

Supplementary Figure 1. Evaluation of different antibodies against GLP-1R. COS-7 cells transiently transfected with either an expression vector for GLP-1R (hGLP1R-pFN21AB7198, Kazusa DNA Research Institute, Promega) or a control vector (pcDNA3.1; Promega) were used to test the specificity of two different anti-GLP-1R antibodies, NBP1-97308 (Novus Biologicals) and ab39072 (Abcam), by immunofluorescent labeling. Sections were counterstained with DAPI and visualized by confocal microscopy.



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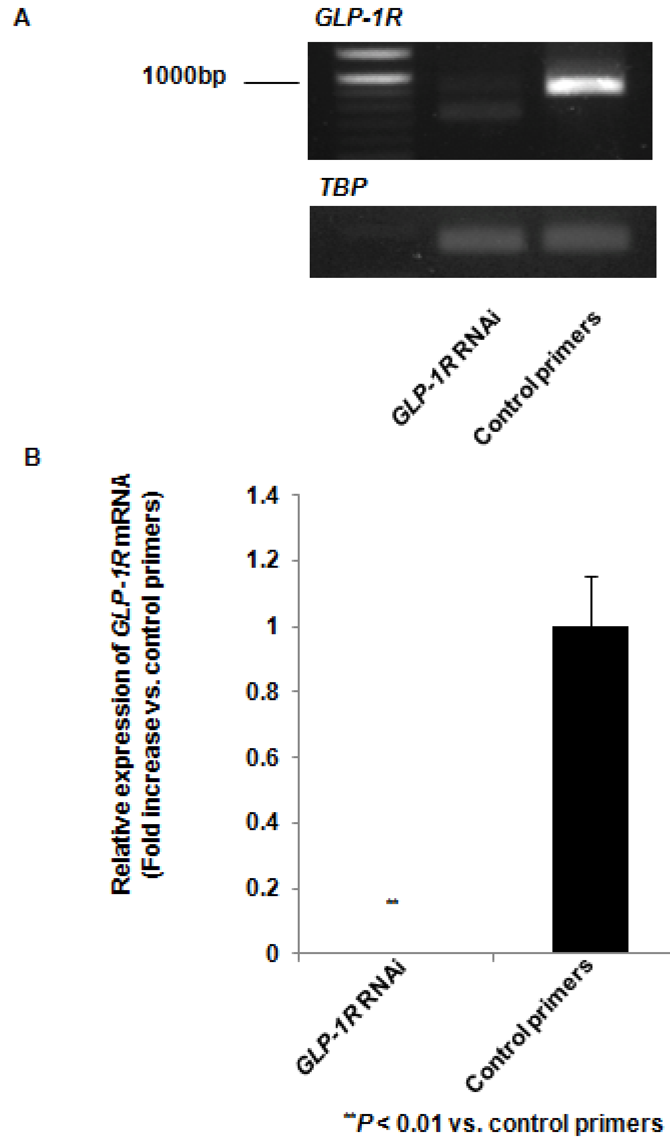
Supplementary Figure 2.Validation of *GLP-1R* siRNA. *GLP-1R* mRNA expression in LNCaP cells transiently transfected with siRNA directed against *GLP-1R* (*GLP-1R* RNAi) or control primers was examined by RT-PCR. *TBP* mRNA levels were determined as an input control. (A) RT-PCR of the 890 bp coding sequence of human *GLP-1R* was performed as previously reported (26). (B) Quantitative RT-PCR of human *GLP-1R* was performed as described in RESEARCH DESIGN AND METHODS. Data are shown as a ratio of the control primers.



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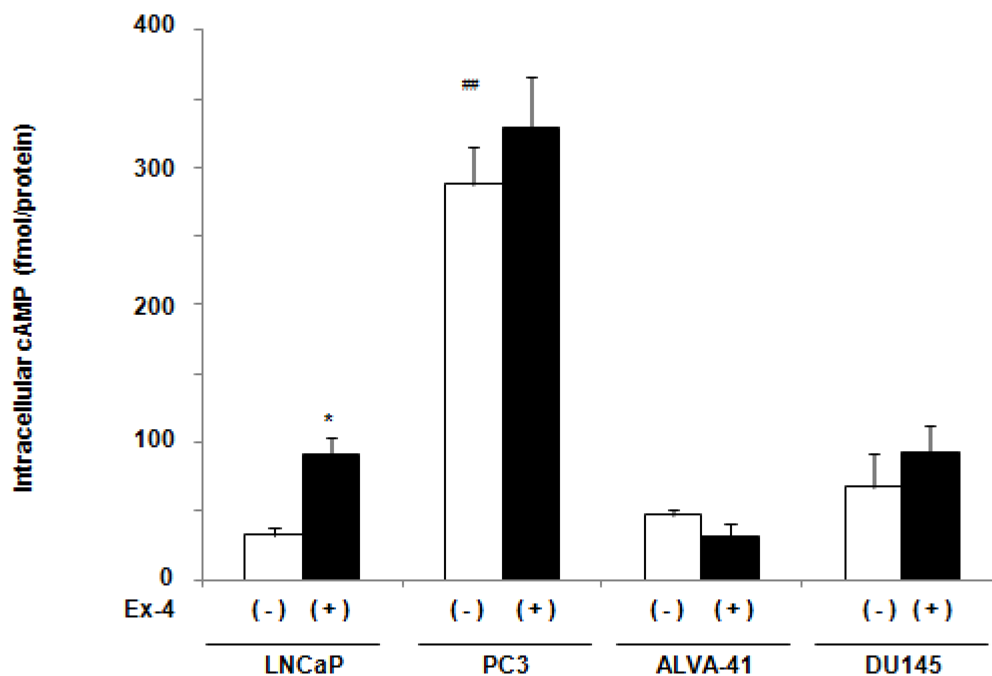
Supplementary Figure 3. Induction of intracellular cAMP concentration by Ex-4 was examined in prostate cancer cells as described in RESEARCH DESIGN AND METHODS. After 24 h of serum deprivation cells were treated with 10 nM Ex-4 for 60 min. $^{*}P < 0.05$ vs. 0 min, $^{\#\#}P < 0.01$ vs. 0 min of the other cells.



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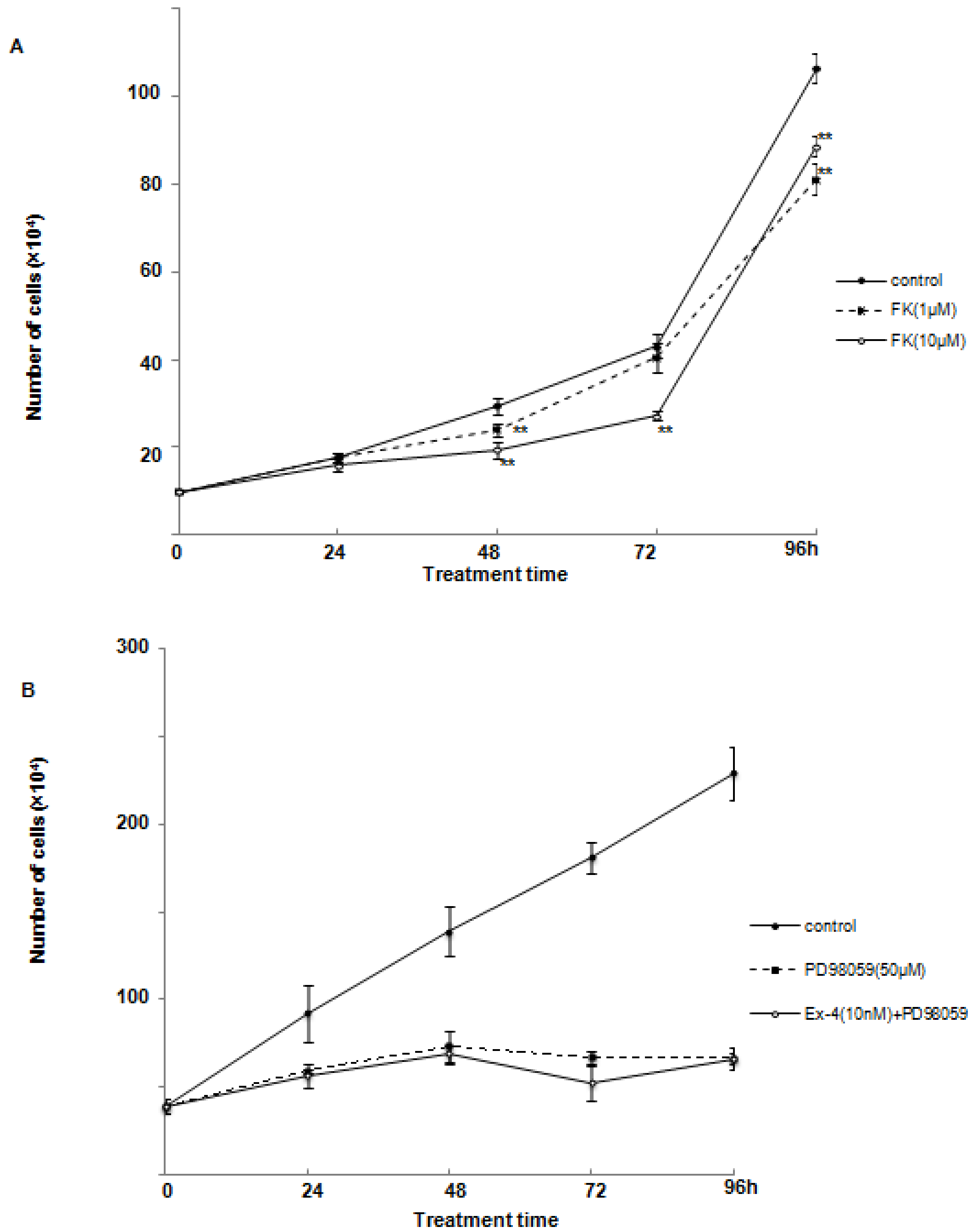
Supplementary Figure 4. Cell proliferation assay was performed with LNCaP cells treated with (A) forskolin (0, 1 or 10 mM) and (B) PD98059 as described in RESEARCH DESIGN AND METHODS.
** $P < 0.01$ vs. control



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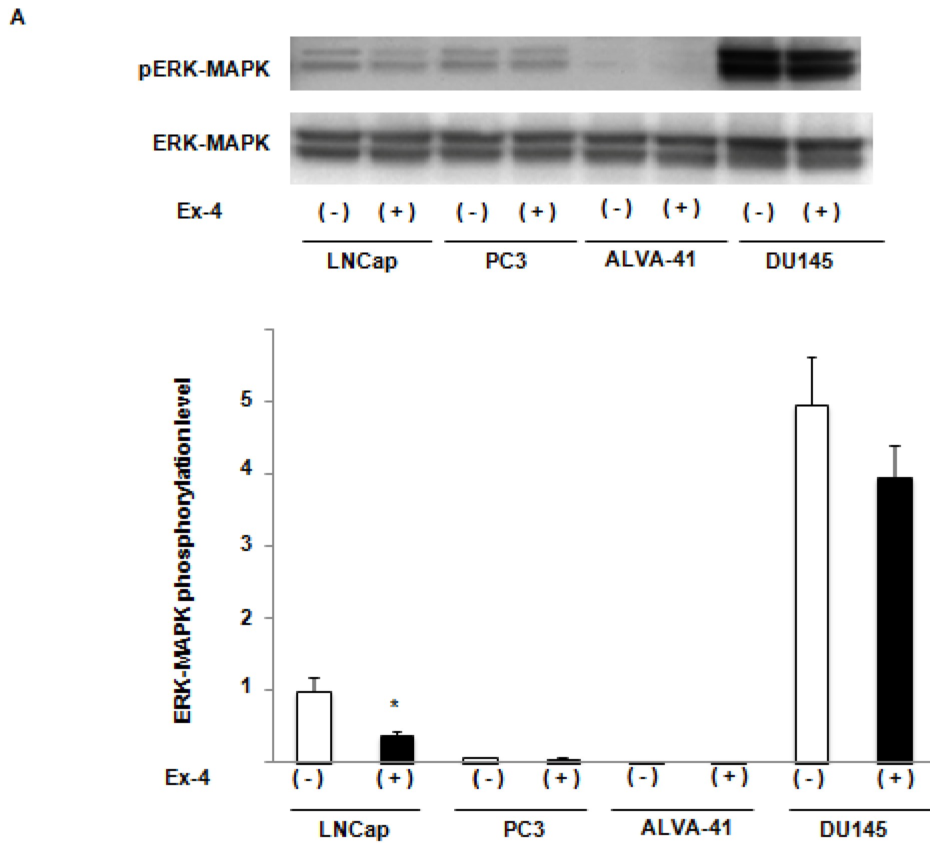
Supplementary Figure 5. Reduction in phosphorylation of ERK-MAPK was examined in prostate cancer cells. (A) Cells maintained in media with 10% FBS were stimulated with 10 nM Ex-4 or saline for 15 min. (B) LNCaP cells maintained in media with 10% FBS were stimulated with 10 mM forskolin or DMSO for 15 min. Cell lysates were harvested and subjected to western blotting to assess phosphorylated ERK-MAPK and ERK-MAPK expression. Phospho-ERK-MAPK/ERK-MAPK protein levels were quantified by densitometry. Data were calculated from triplicate independent experiments and are shown as a ratio of LNCaP(-). Experiments were repeated at least three times. Unpaired t-tests were performed to calculate statistical significance ($^{*}P < 0.05$ vs. control).



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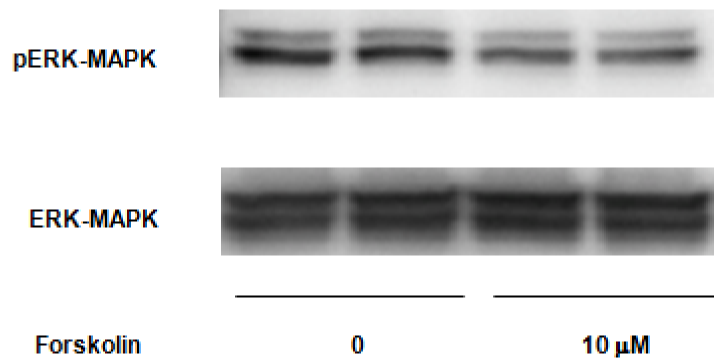

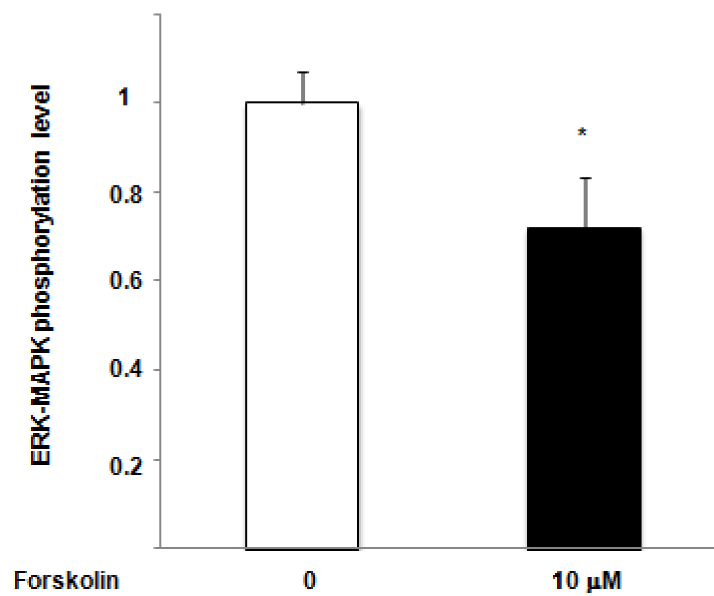

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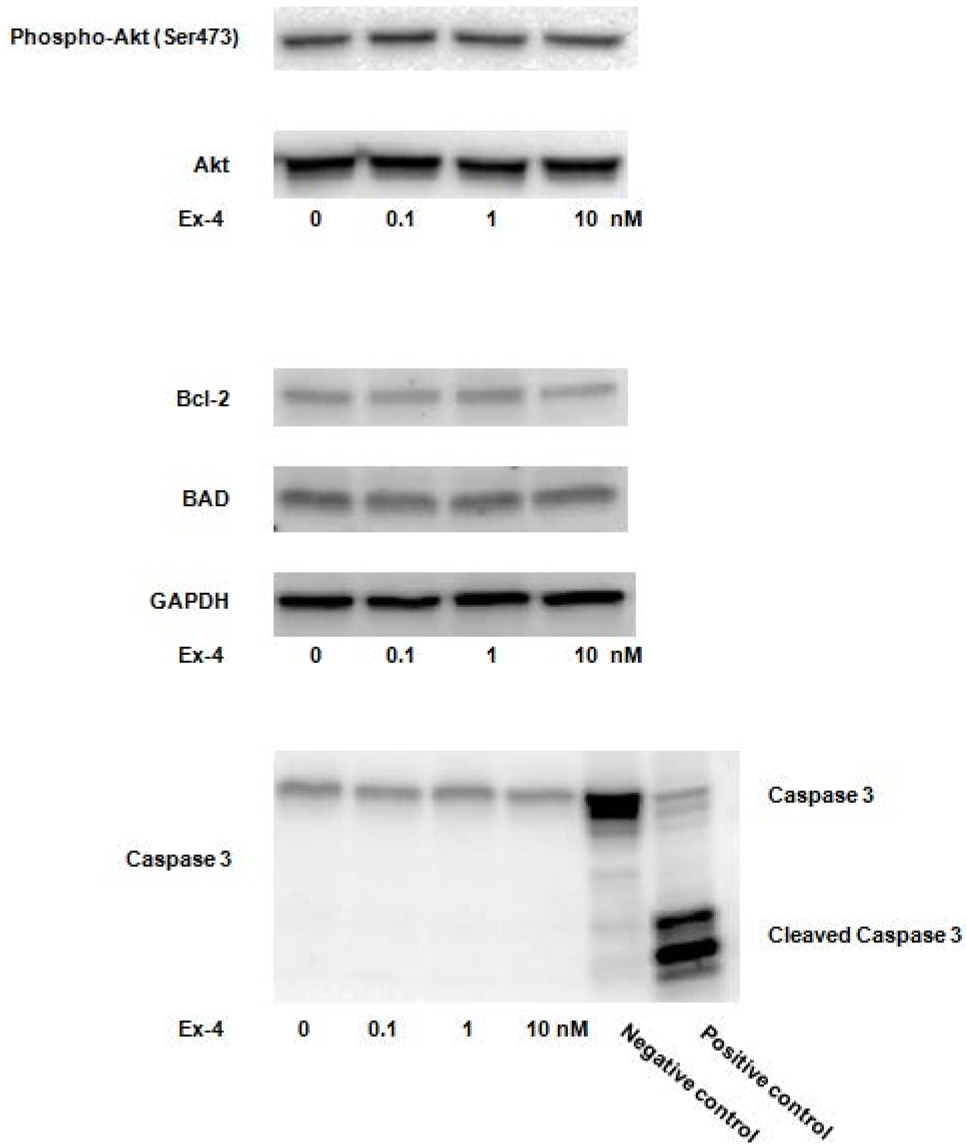
Supplementary Figure 6. LNCaP cells maintained in media with 10% FBS were treated with Ex-4 (0.1–10 nM) or saline for 15 min in the case of Akt activation detection, or 24 h for the examination of Caspase 3, Bcl-2, and BAD protein expression levels by western blotting. The following antibodies were used: phospho-Akt (Cell Signaling #4051) and Akt (Cell Signaling, #9272), Capspase 3 (Cell Signaling, #9662), Bcl-2 (Santa Cruz, sc-7382), BAD (Cell Signaling, #9292) and GAPDH (Santa Cruz, sc-20357). Caspase 3 control cell extracts (Cell Signaling, #9663) were used as a positive control for Caspase 3. Experiments were repeated at least three times.



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