SUPPLEMENTARY FIGURE LEGENDS

Supplementary Figure 1. NPM enhances p53 half-life. (A) Kinetics of p53 degradation in the presence of cycloheximide (20 μg/ml) in JB6 cells transiently transfected with pcDNA3.1 vector. Cycloheximide was added to the medium 1 h after TPA treatment and cells were collected at the indicated times. Nuclear extract was prepared and subjected to 10% SDS-polyacrylamide gel electrophoresis. p53 proteins were detected by Western blotting using antibody specific to p53 (Ab-11). The membrane was re-blotted with GAPDH antibody which was used as loading control. (B) A similar kinetic study was performed as stated in (A) following transient transfection of JB6 cells with NPM expression vector pcDNA3.1/NPM. p53. GAPDH bands were densitometrically scanned and normalized with GAPDH band intensity, and p53 half-life was determined using half-life calculator software (http:/www.users.med.cornell.edu/~spon/picu/calc/halfcalc.htm) (Table 1).

Supplementary Figure 2. Alteration of mitochondrial pro-apoptotic and anti-apoptotic proteins. JB6 cells were transfected with pcDNA3.1 or pcDNA3.1/NPM expression vector and were treated with TPA for 1 h. Specific proteins were detected by Western blotting. Bcl2 and Bax levels in (a) total cell lysate and (b) in isolated mitochondria are shown. Nuclear specific marker PCNA was detected as mitochondria purity and the GAPDH was used as loading control. (c) Bcl2 and Bax ratio in mitochondrial fraction was calculated. Each data point represents the mean ± SD of three experiments. * $p<0.05$ compared with corresponding DMSO group; #$p<0.05$ compared with pcDNA3.1-DMSO group. JB6 cells were co-transfected with NPM-expression vector with either control siRNA or Bcl2 siRNA (20 nM final concentration) for 48 h and then the cells were treated with DMSO or TPA for an additional 24 h. The effects of NPM over-expression on Bcl2 siRNA mediated apoptotic cell death were determined by TUNEL assay. (d) The TUNEL positive cells were counted from a defined number of cells (3 x 300) and the fold changes of TUNEL positive cells were compared. Each data point represents the mean ± SD of three samples. Duplicate experiment was performed to verify reproducibility. **$p<0.01$ corresponding DMSO group vs corresponding TPA group; #$p<0.01$ Bcl2 siRNA TPA group vs Bcl2 siRNA-NPM TPA group; [a] $p<0.05$ control siRNA DMSO group vs control siRNA-NPM DMSO group; [b] $p<0.01$ control siRNA TPA group vs control siRNA-NPM TPA group; c $p< 0.05$ Bcl2 siRNA DMSO group vs Bcl2 siRNA-NPM DMSO group. (e) Western analysis of Bcl2 verified that transfection of Bcl2 siRNA in JB6 cells suppressed Bcl2 expression level.

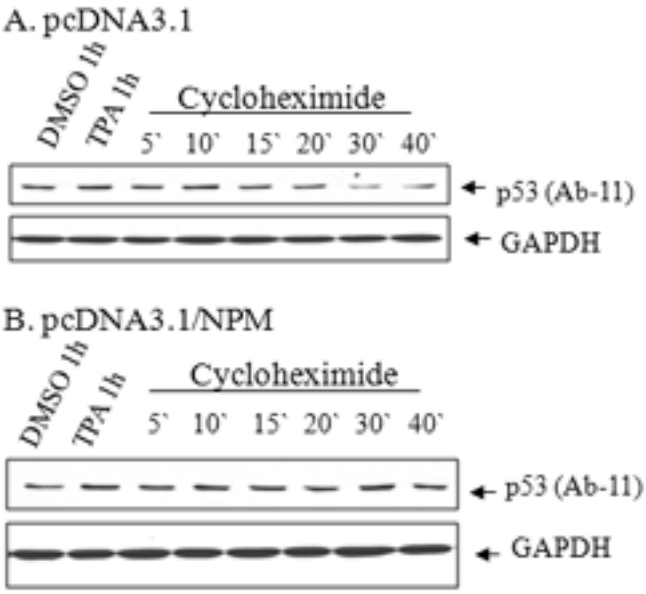
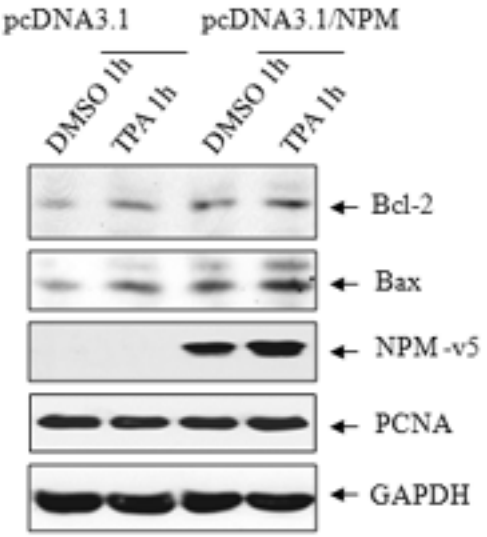


Table 1: NPM increases the half-life of p53

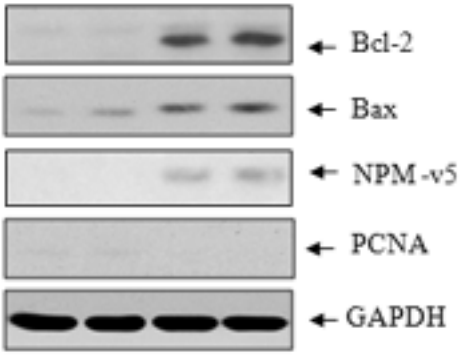
Cells	Transfect ion	$t_{1/2}$ (min)	R^2
JB6	pcDNA3.1	18.51	0.968
JB6	pcDNA3.1/NPM	35.94	0.915

Supplementary Figure 1

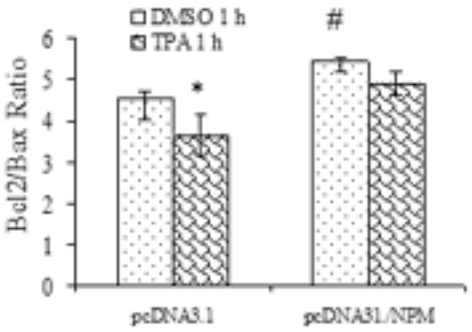
(a) Total cell extract



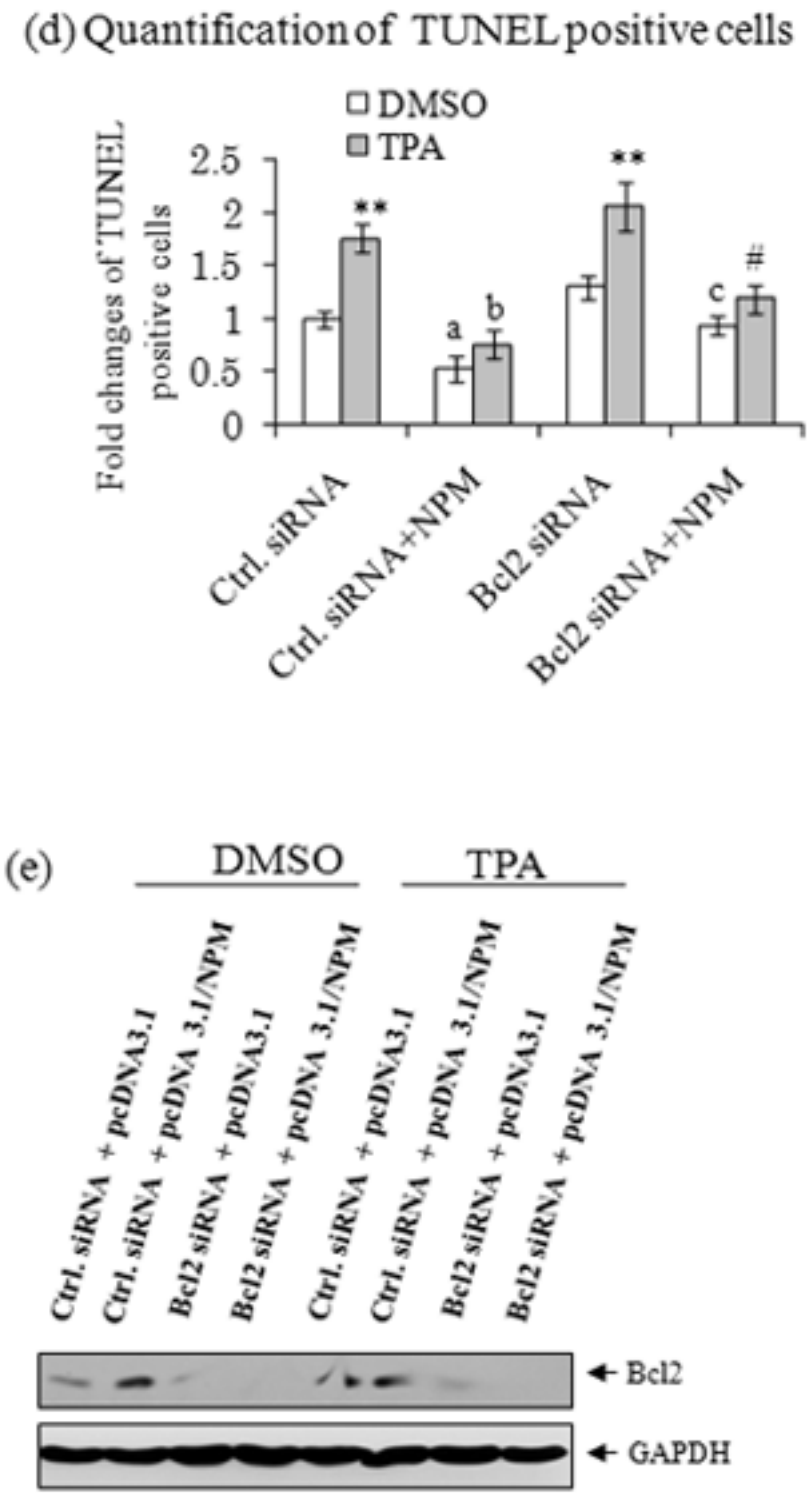
(b) Mitochondria



(c) Mitochondrial Bcl2/Bax ratio



Supplementary Figure 2



Supplementary Figure 2