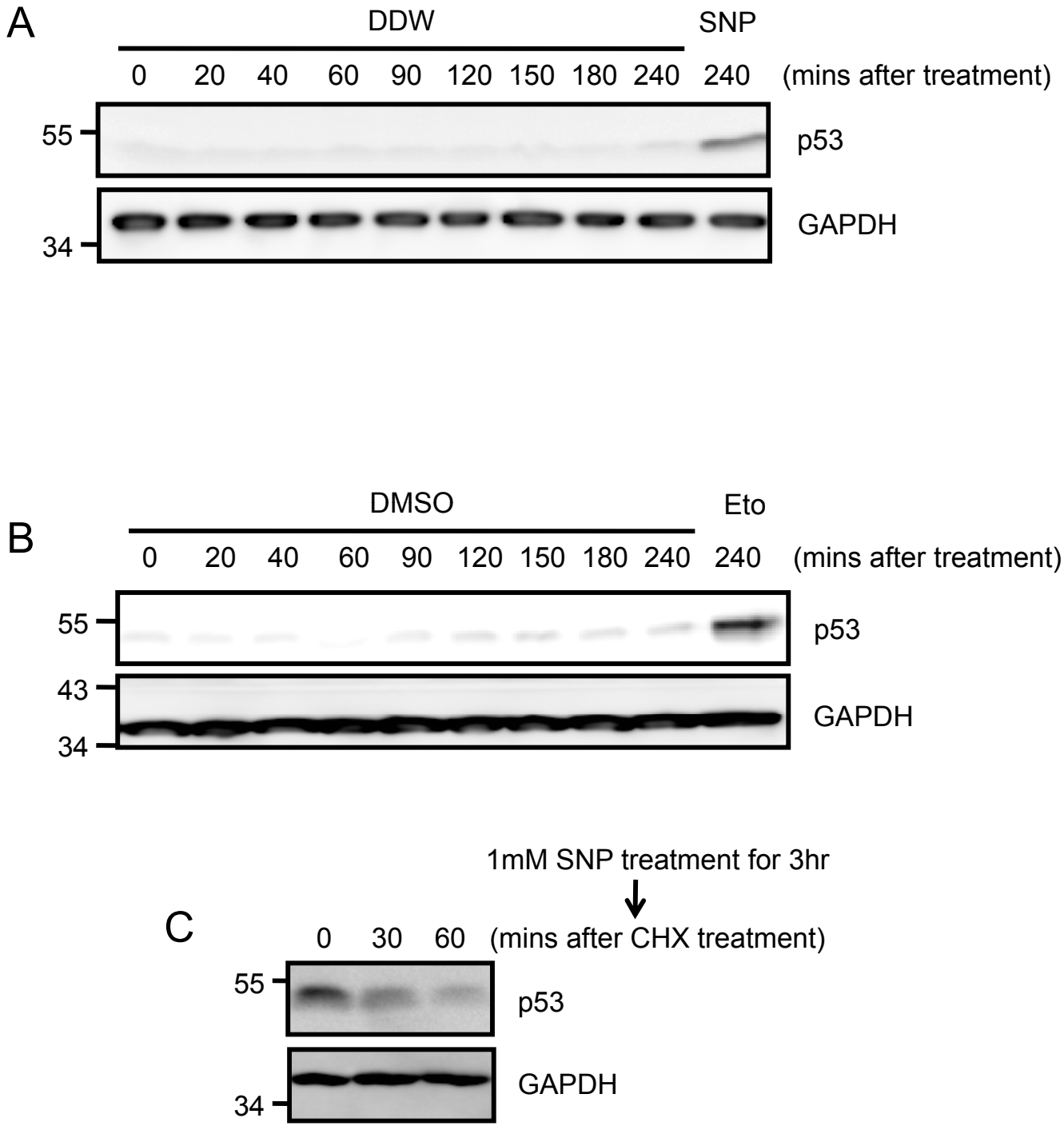
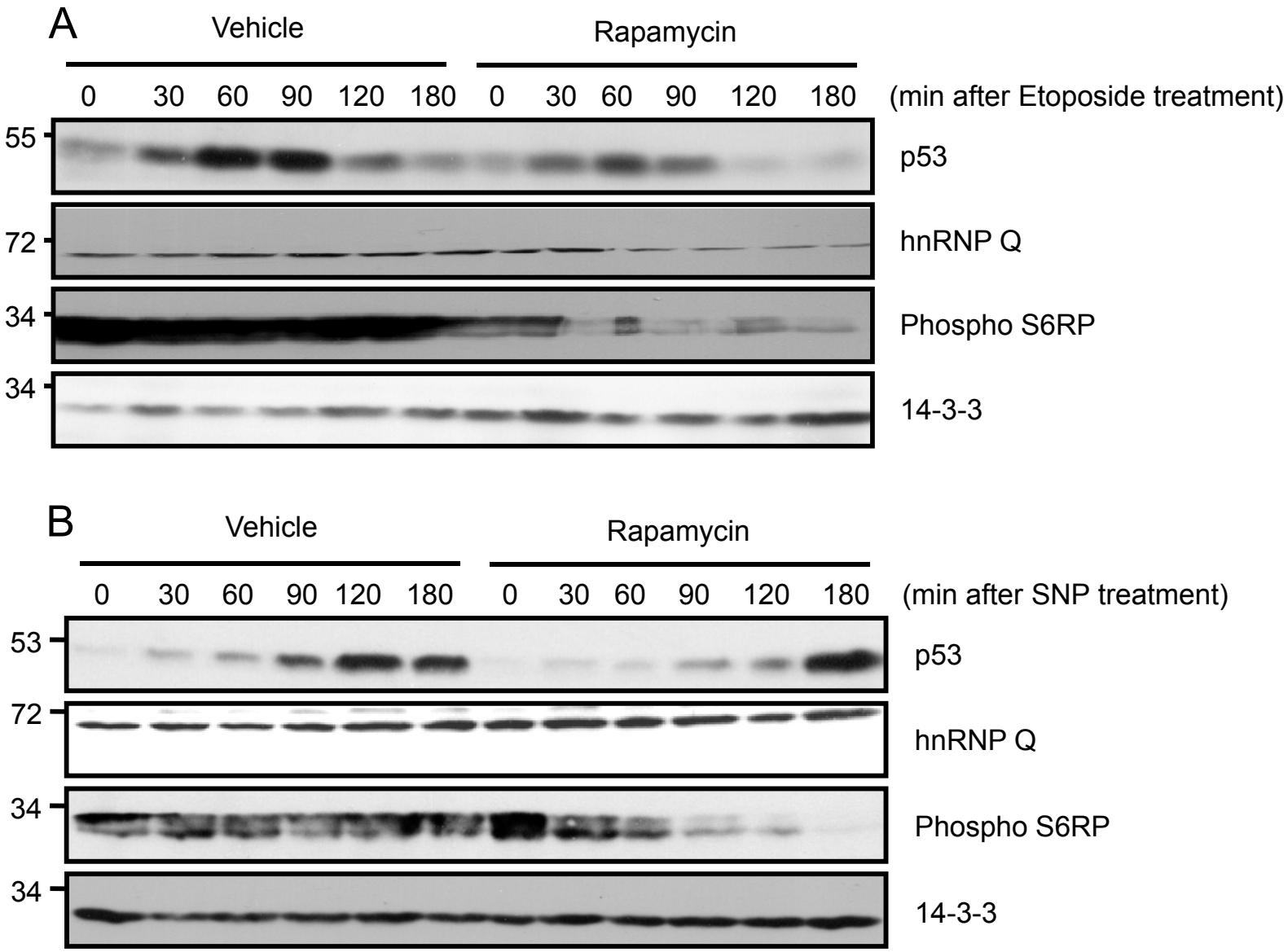
Supplementary Fig 1.



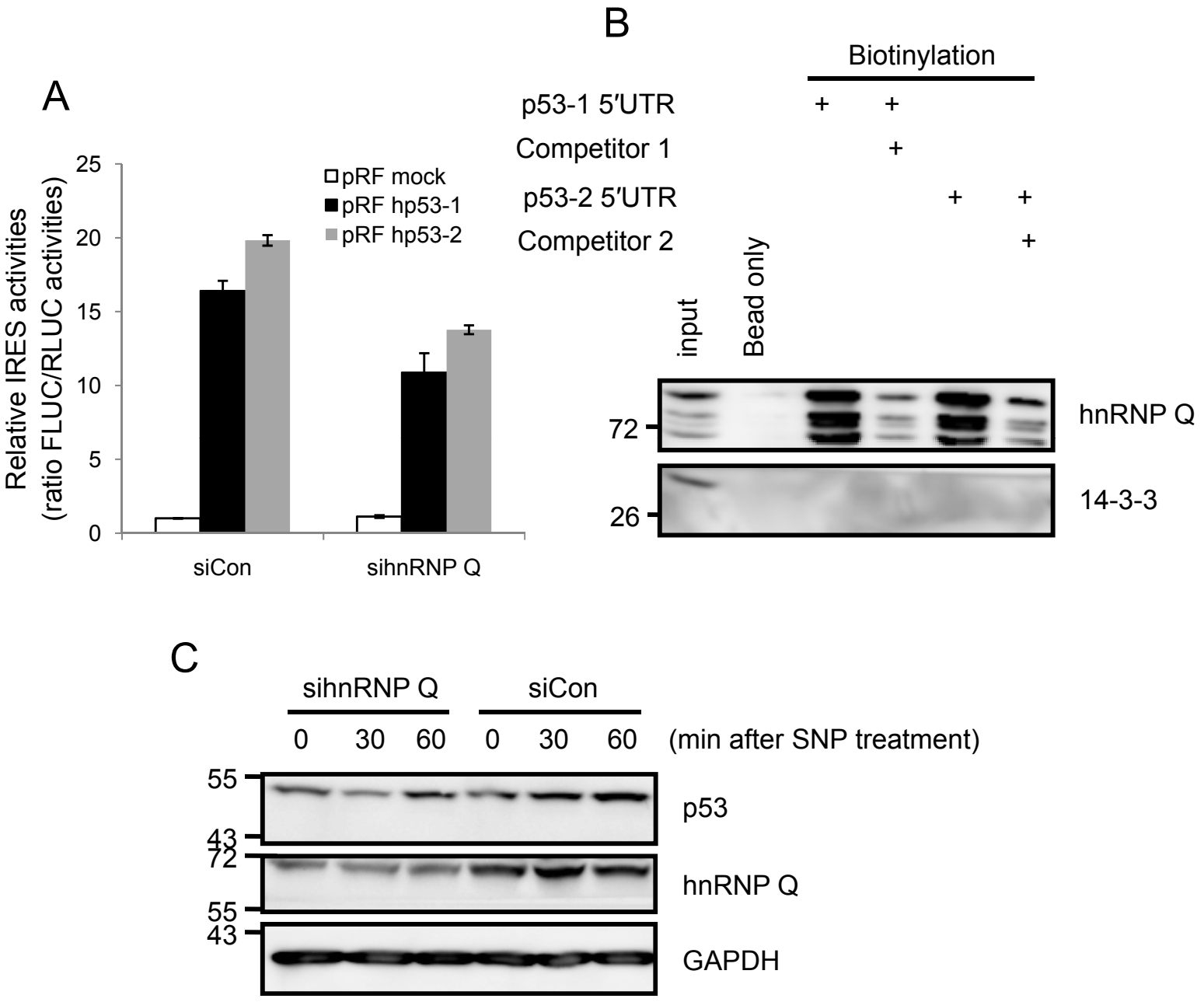
Supplementary Fig 1. Vehicle treatment does not induce p53 protein.
(A and B) DDW (A) or DMSO (B) treatment did not induce p53 protein accumulation. GAPDH was used as a loading control. SNP (A) or etoposide (B) treated cell extract was used for positive control. (C) p53 protein stability was analyzed. After treatment of SNP for 3hrs, cells were treated with cycloheximide. p53 protein level was assessed at indicated time points. GAPDH was used as a loading control.

Supplementary Fig 2.



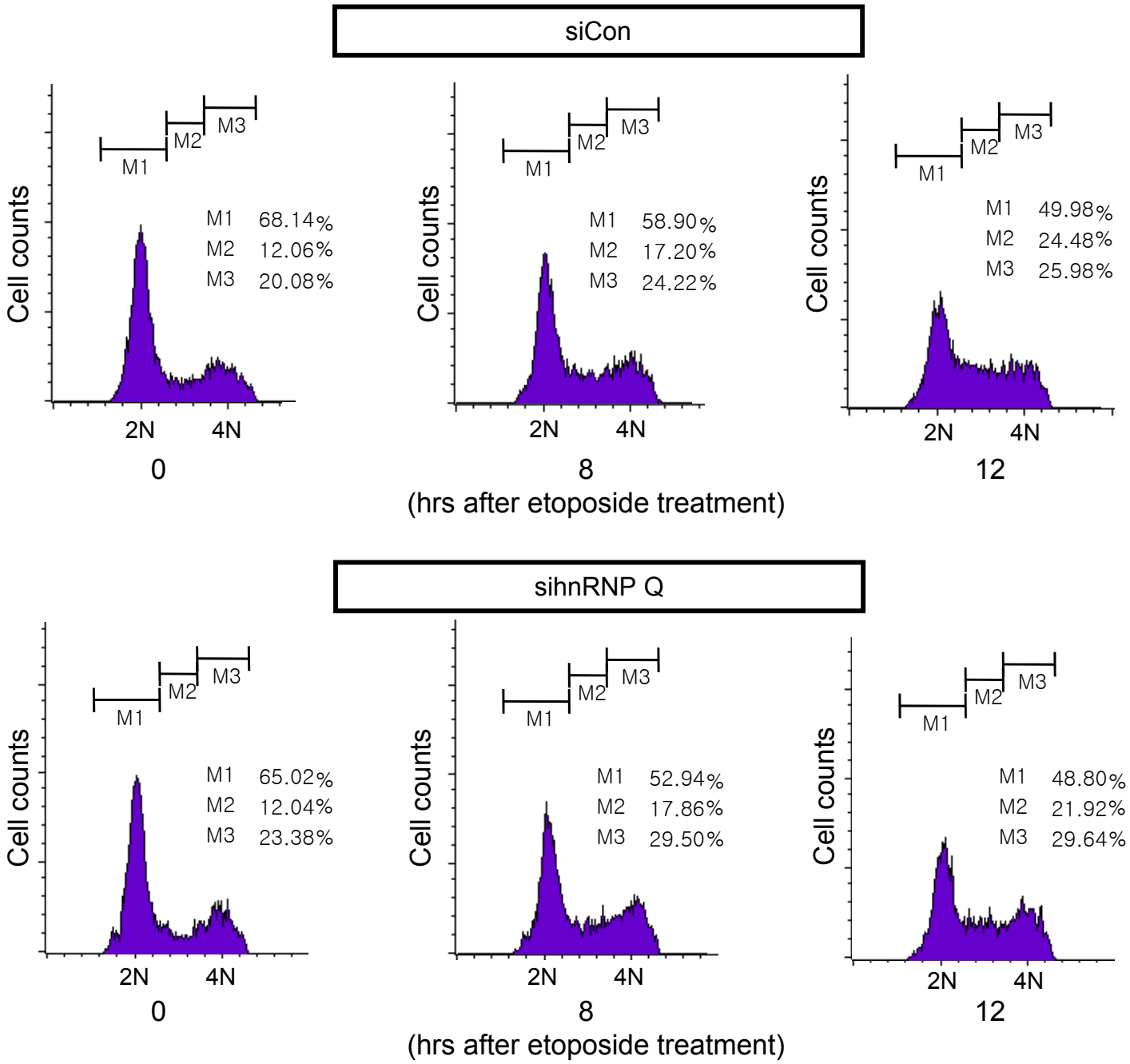
Supplementary Fig 2. Protein synthesis step is necessary for rapid p53 accumulation.
NIH3T3 cells were treated with etoposide (A) or SNP (B) with vehicle or rapamycin. Rapamycin inhibits the function of mTOR, leading to inhibition of the cap-dependent translation by interfering both phosphorylation of S6 ribosomal proteins and 4E-BPs The activity of rapamycin was measured by the phosphorylation status of S6 ribosomal proteins. 14-3-3 was used as a loading control. Overall, rapamycin inhibited full accmulation of p53. This data suggested that protein synthesis step is required for p53 induction.
Also, importantly, rapamycin did not block p53 induction completely. This data indicated that there remained the possibility that cap-independent translation is utilized in p53 protein synthesis.

Supplementary Fig 3.



Supplementary Fig 3. hnRNP Q regulates translation of p53 mRNA in SH-SY5Y cells.
(A) The suppression of IRES activity of human p53 when hnRNP Q was reduced is shown. The results are expressed as the mean ± SD of two different experiments. The IRES activity of the pRF mock vector in control siRNA-transfected cells was set as 1. Accession number: NM_000546.5 (hp53-1), NM_001126115.1 (hp53-2) (B) Confirmation of the interaction between the 5′UTR of human p53 mRNA and human hnRNP Q by RNA affinity purification followed by Western blotting. (C) 53 protein accumulation was suppressed when hnRNP Q was downregulated in the early phase after SNP treatment. Knockdown of hnRNP Q was confirmed by Western blotting. GAPDH protein was used as a loading control.

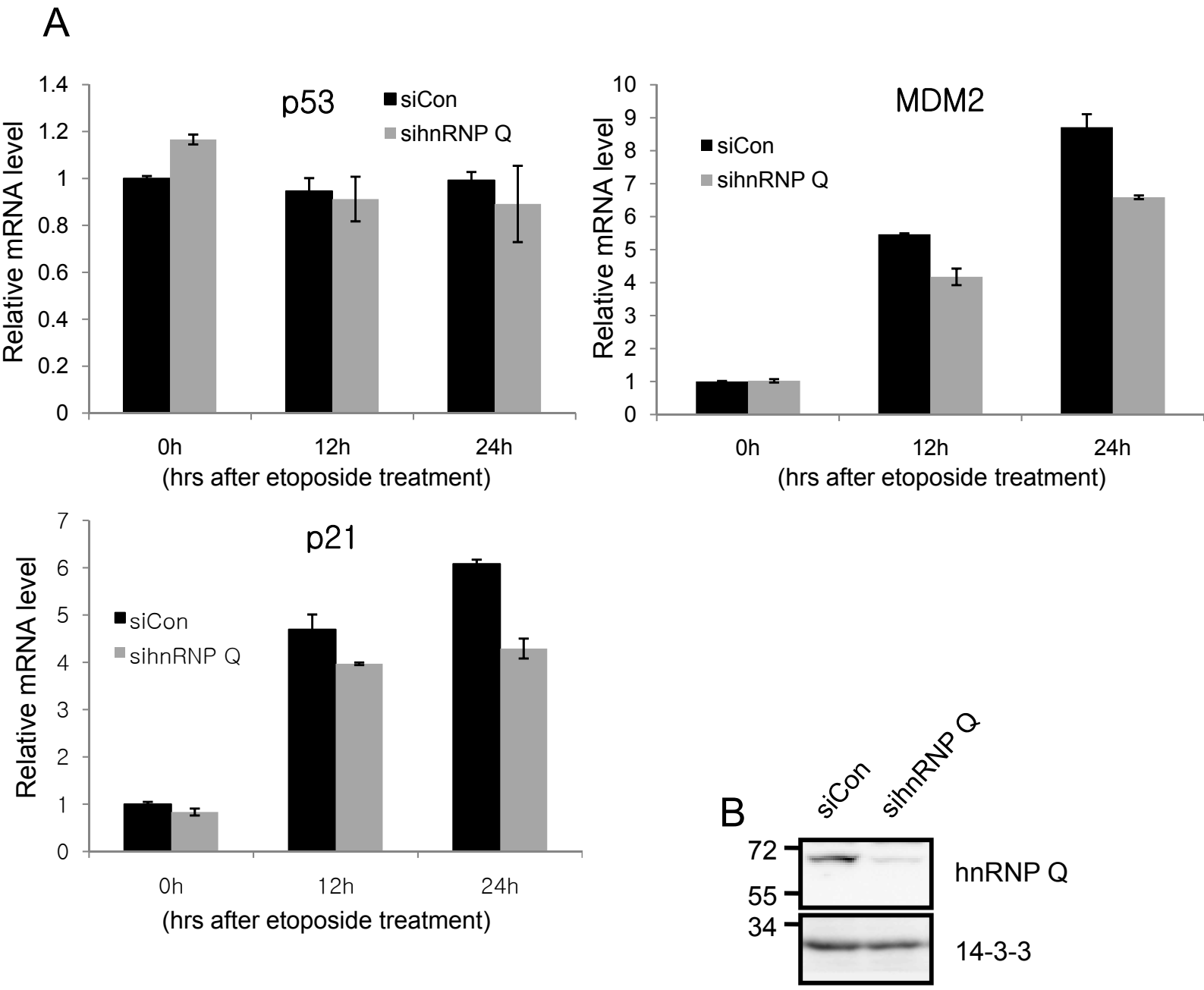
Supplementary Fig 4.



Supplementary Fig 4. The effect of hnRNP Q on cell cycle is analyzed.

24hrs after control siRNA (upper panel) or hnRNP Q siRNA (lower panel) transfection, cells were treated with etoposide for 0, 8, 12hrs. Cell cycle was analyzed by nuclei staining with propidium iodide (PI) followed by flow-cytometric analysis.

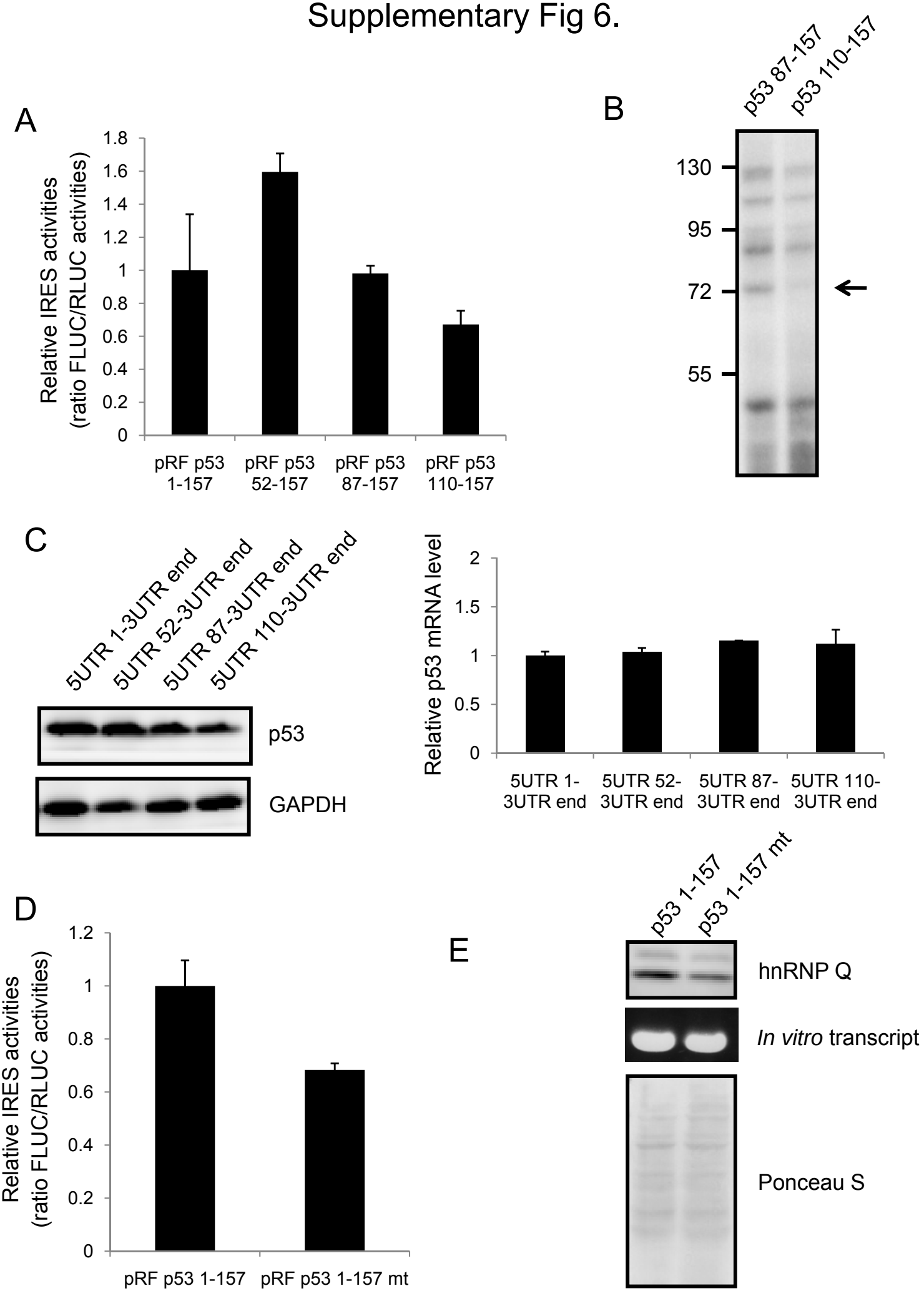
Supplementary Fig 5.



Supplementary Fig 5. p53 downstream gene expression was suppressed when hnRNP Q was downregulated.

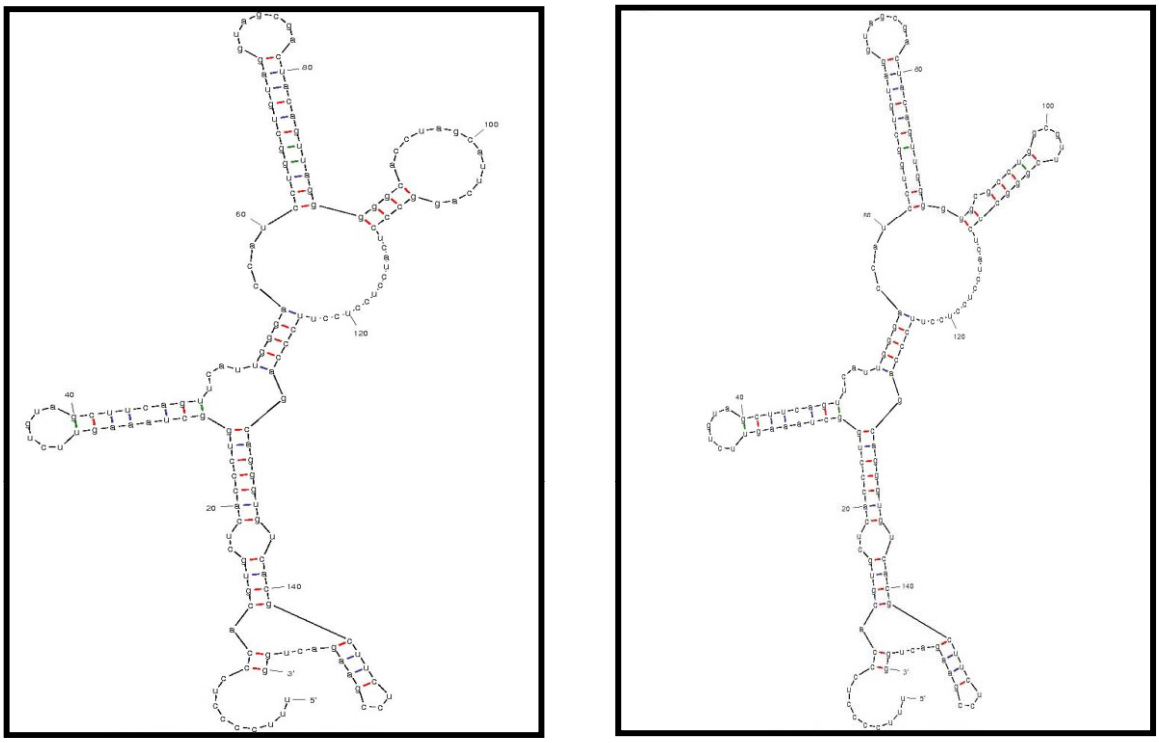
(A) 12hrs after control siRNA or hnRNP Q siRNA transfection, cells were treated with etoposide. At indicated time points, endogenous mRNA level of p53, Mdm2, p21 was assessed by quantitative real-time RT-PCR and normalized to mRPL32 mRNA levels. The mRNA level of each gene in control siRNA transfected cells at zero time was set to 1.0. (B) hnRNP Q downregulation was confirmed by Western blotting.

Supplementary Fig 6.



Supplementary Fig 6.

F

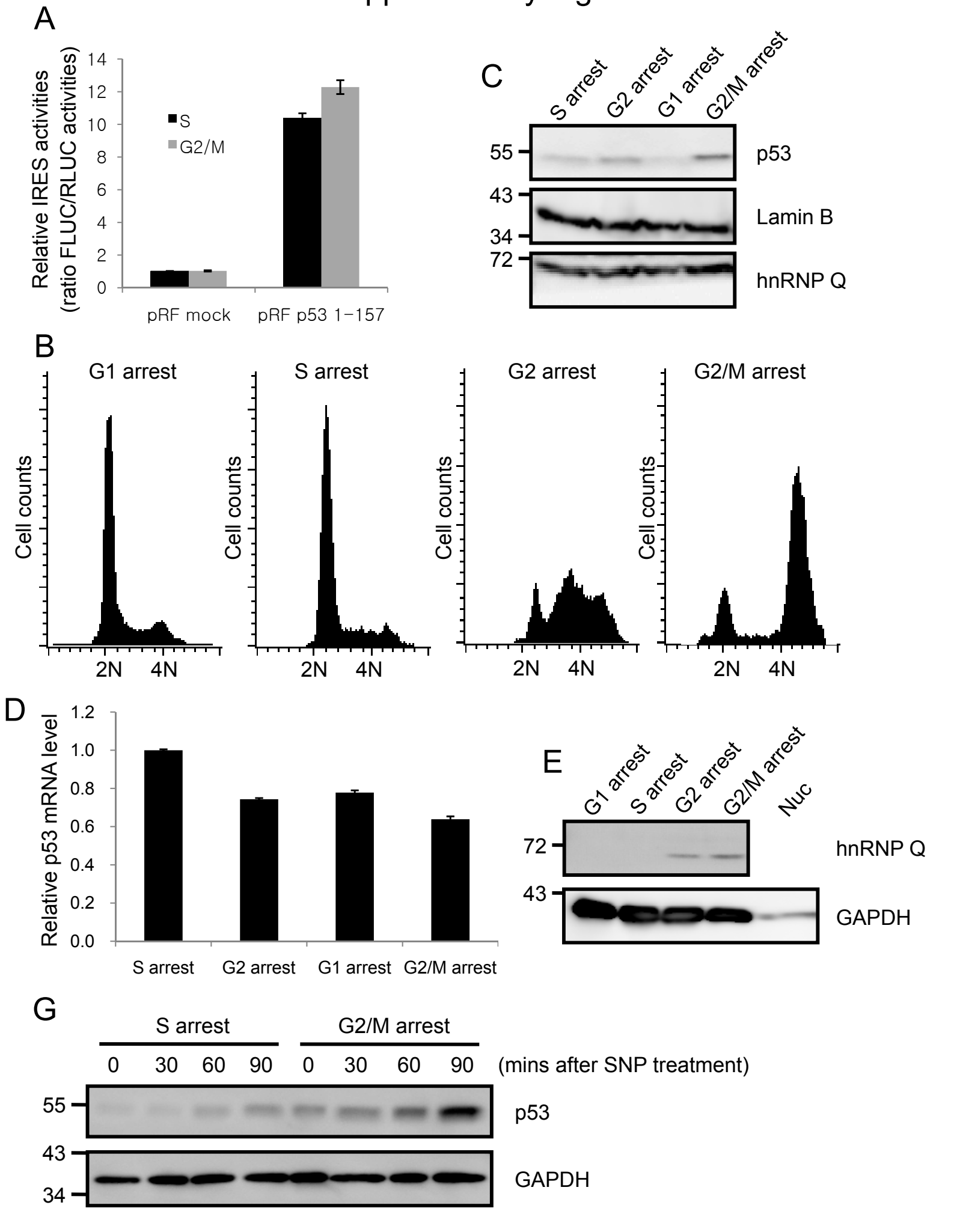


p53 1-157 p53 1-157 mt

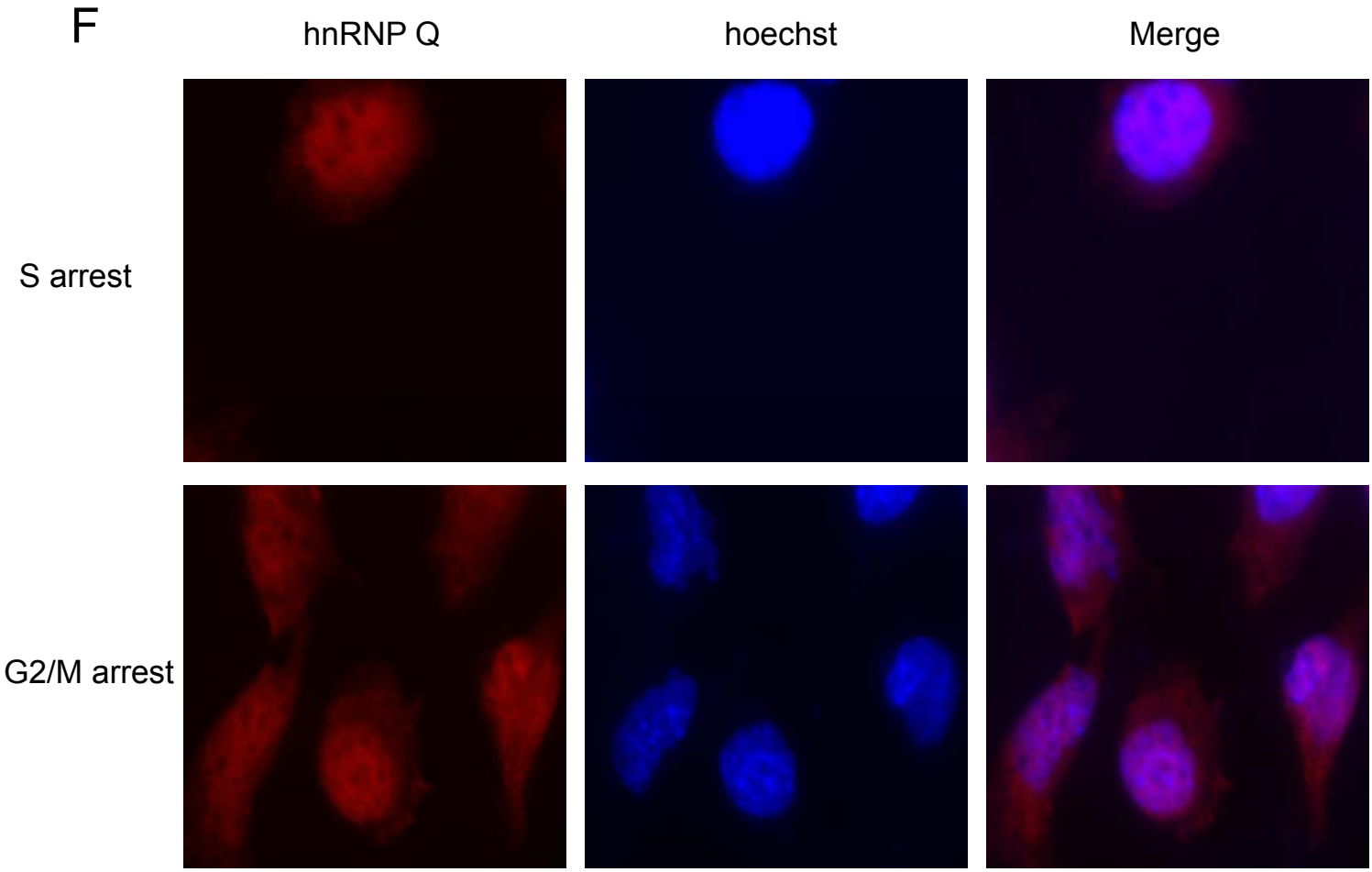
Supplementary Fig 6. Cis-acting element resides between nucleotide 87 and 109 in 5′UTR of mouse p53 mRNA.

(A) To identify cis-acting element for translation initiation by hnRNP Q, luciferase assay was performed with deletion construct. The numbers indicate the remaining nucleotides in the mouse p53 mRNA 5′UTR. The results are expressed as the mean ± SD of two different experiments. The IRES activity of the full length of mouse p53 5′UTR-containg pRF construct was set as 1. (B) Cellular proteins that bound to the cis-acting region in the p53 5′UTR were analyzed with the in vitro binding followed by UV-crosslinking assay. The sizes of proteins are indicated to the left of the panels. Arrow is the band size of hnRNP Q. (C) The 5′UTR of p53 mRNA affects the level of p53 protein. Four constructs have coding sequences and 3'UTR of mouse p53, and we inserted serially deleted sequence of 5'UTR upstream of coding region. At 24 hours after transfection in p53/Mdm2 double knockout MEF, p53 protein level was measured (left). Transfection efficiency was confirmed by mRNA level of p53 (right). (D) After substitution of all adenosine which reside between nucleotide 87 and 109 to guanosine and luciferase assay was performed. The results are expressed as the mean ± SD of two different experiments. The IRES activity of the full length of mouse p53 5′UTR-containg pRF construct was set as 1. (E) Interaction between hnRNP Q and wildtype or mutant 5′UTR of mouse p53 mRNA. (F) Comparison of predicted secondary structure between wildtype and mutant 5′UTR of mouse p53.

Supplementary Fig 7.



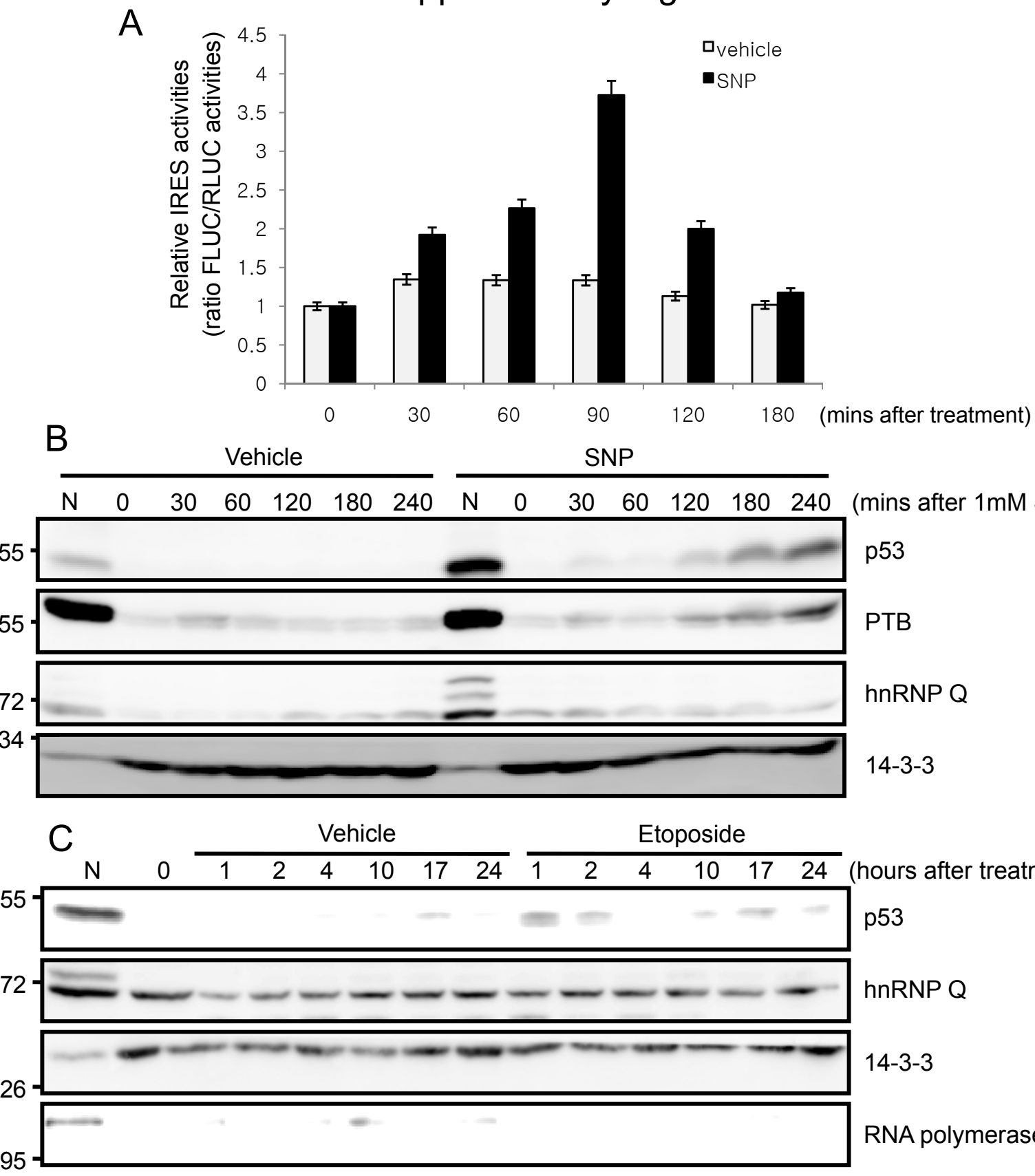
Supplementary Fig 7.



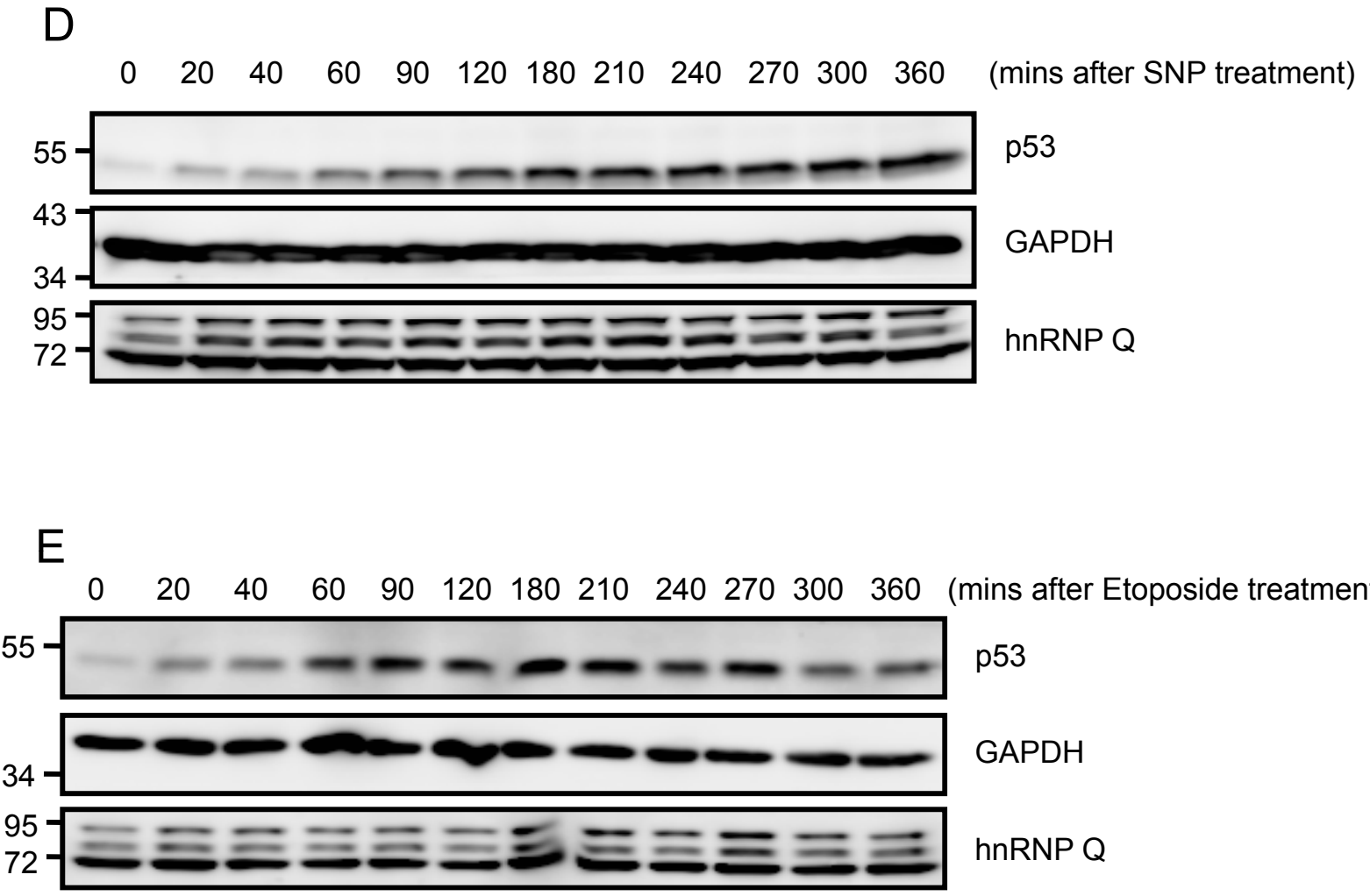
Supplementary Fig 7. Cell cycle-dependent cytosolic hnRNP Q protein level correlates with the amount of p53 protein.

(A) 12hrs after pRF mock or pRF p53 1-157 construct transfection, cells were treated with hydroxyl urea or nocodazole. 16hrs after cell cycle synchronization, luciferase activity was measured. The IRES activity of the pRF mock vector in S phase-arrested cells was set as 1. The results are expressed as the mean ± SD of two different experiments. (B) Cells were arrested at G1, S, G2, and G2/M phases, by treating with mimosine, hydroxyl urea, irinotecan, and nocodazole, respectively. Cell cycle arrest was assessed by nuclei staining with propidium iodide (PI) followed by flow-cytometric analyses. (C) The p53 protein level was enhanced in G2 or G2/M arrested cells. The levels of each protein were analyzed by Western blotting from total cell lysates. (D) The endogenous p53 mRNA levels were determined by quantitative real-time RT-PCR and normalized to mRPL32 mRNA levels. The level of p53 mRNA in S phase arrested cells was arbitrarily set to 1.0. The results are presented as the mean ± SD of three experiments. (E) Cytosolic hnRNP Q levels were determined by Western blotting. GAPDH protein was used as a cytosolic marker. Nuc, nuclear lysate. (F) Cytosolic hnRNP Q level was monitored by immunocytochemistry after cell cycle arrest. (G) p53 protein induction rates in S and G2/M-arrested cells were compared. Sixteen hours after synchronization by hydroxyl urea (S arrest) or nocodazole (G2/M arrest), cells were treated with 1mM SNP. The levels of each protein were analyzed by Western blotting.

Supplementary Fig 8.



Supplementary Fig 8.



Supplementary Fig 8. Both of IRES activity of p53 and cytosolic hnRNP Q level are enhanced especially in early phase of stress condition.

(A) 24hrs after NIH3T3 cells were transfected with pRF p53 1-157 construct, vehicle or SNP was treated. Luciferase assay was performed at indicated time points. The IRES activity of the full length of mouse p53 5′UTR-containg pRF construct at zero time was set as 1. (B and C) Cytosolic hnRNP Q level was assessed by Western blotting. 14-3-3 was used as a loading control. PTB was used as positive control in (B). N, nuclear lysate 6hr after vehicle or 1mM SNP (B) or 100μM etoposide (C) treatment. (D and E) The levels of mouse p53 protein and hnRNP Q were analyzed by Western blotting after 1mM SNP (D) or 100 μM etoposide (E) treatment at the indicated time points. GAPDH protein was used as a loading control