Supplementary Figures

Fig. S1 Precursor of miR-542-3p induces p53 expression. U2OS cells were transfected with either empty vector or genomic sequence that expresses precursor of miR-542-3p (pre-miR-542). After two days, cells were harvested for RNA and protein extraction. (A) The expression of miR-542-3p was determined by quantitative real-time RT-PCR. (B) The expression of p53 was assayed by western blotting.

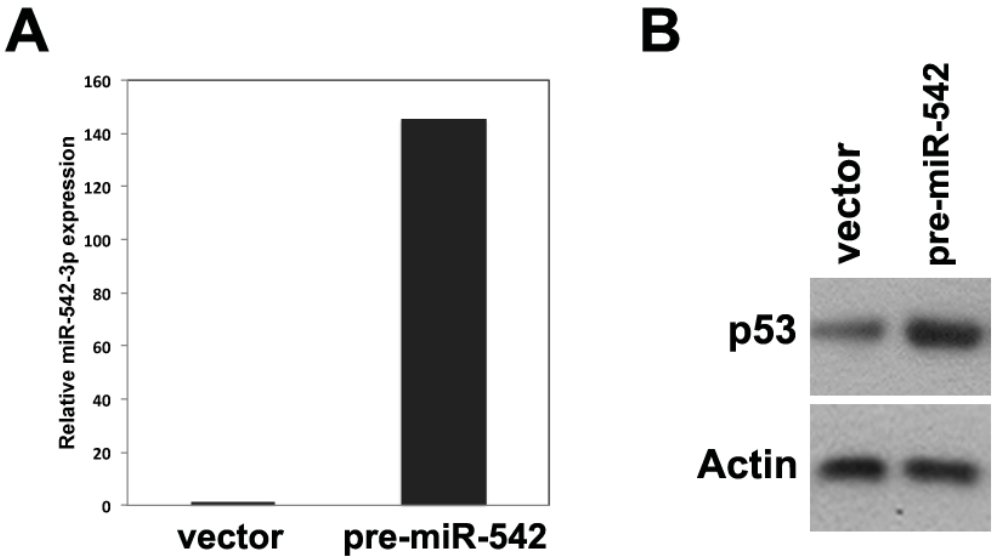

Fig. S2 MiR-542-3p has no effect on p53 mRNA level. U2OS cells were transfected with negative or miR-542-3p mimics for 48 hr. Cells were then harvested for RNA extraction and p53 mRNA level quantitation by real-time RT-PCR.

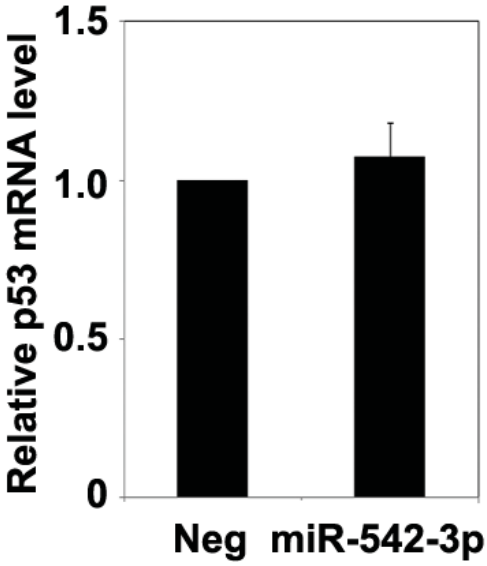

Fig. S3 Effects of miR-542-3p on p53 expression in multiple cell lines. Several cell lines, including normal human foreskin fibroblasts (HF) and tumor cell lines HCT116, T98G, U118 and MDA-MB-231, were transfected with negative or miR-542-3p mimics. Two days later, cells were harvested for western blot analysis of p53 expression.

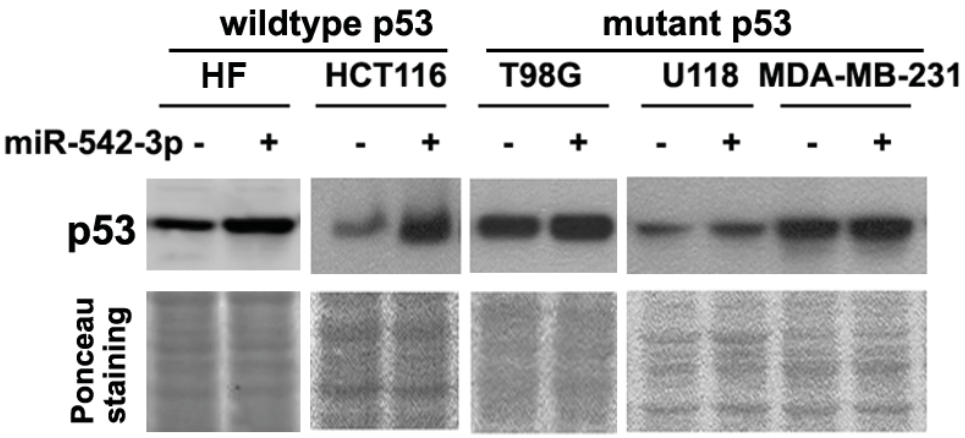

Fig. S4 MiR-542-3p has no effect on expression levels of various modulators of p53 degradation. U2OS cells were transfected with negative or miR-542-3p mimics for 48 hr. Cells were pelleted for analysis of various p53 modulators by western blotting.

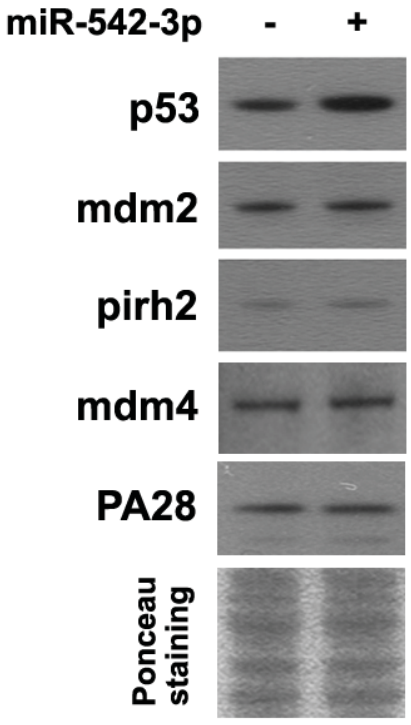

Fig. S5 Actinomycin D induces p53 expression. U2OS cells were treated with actinomycin D at 5 nM for 24 h. Cells were then harvested for western blot analysis of p53 and RPS23 expression.

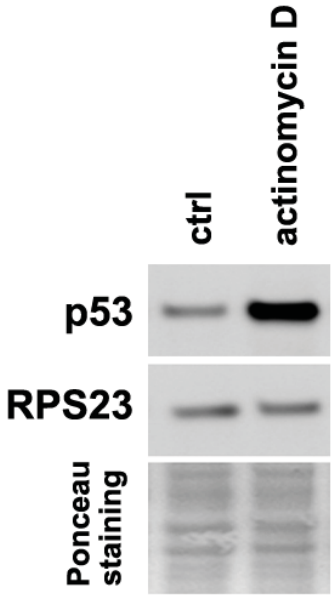

Fig. S6 Amplification of RPS23 transcript. (A) Scheme of RPS23 mRNA deposited in the NCBI database. (B) Total RNA extracted U2OS cells was reverse transcribed into cDNA using oligo-dT primers. Since RPS23 gene is organized into four exons (), RPS23 transcript fragments were then amplified with PCR using primers designed to target different exons to verify the presence of the full RPS23 transcript. (C) The region spanning miR-542-3p binding site in RPS23 3'UTR was validated using Sanger sequencing with the PCR product (84-3281) from (B).

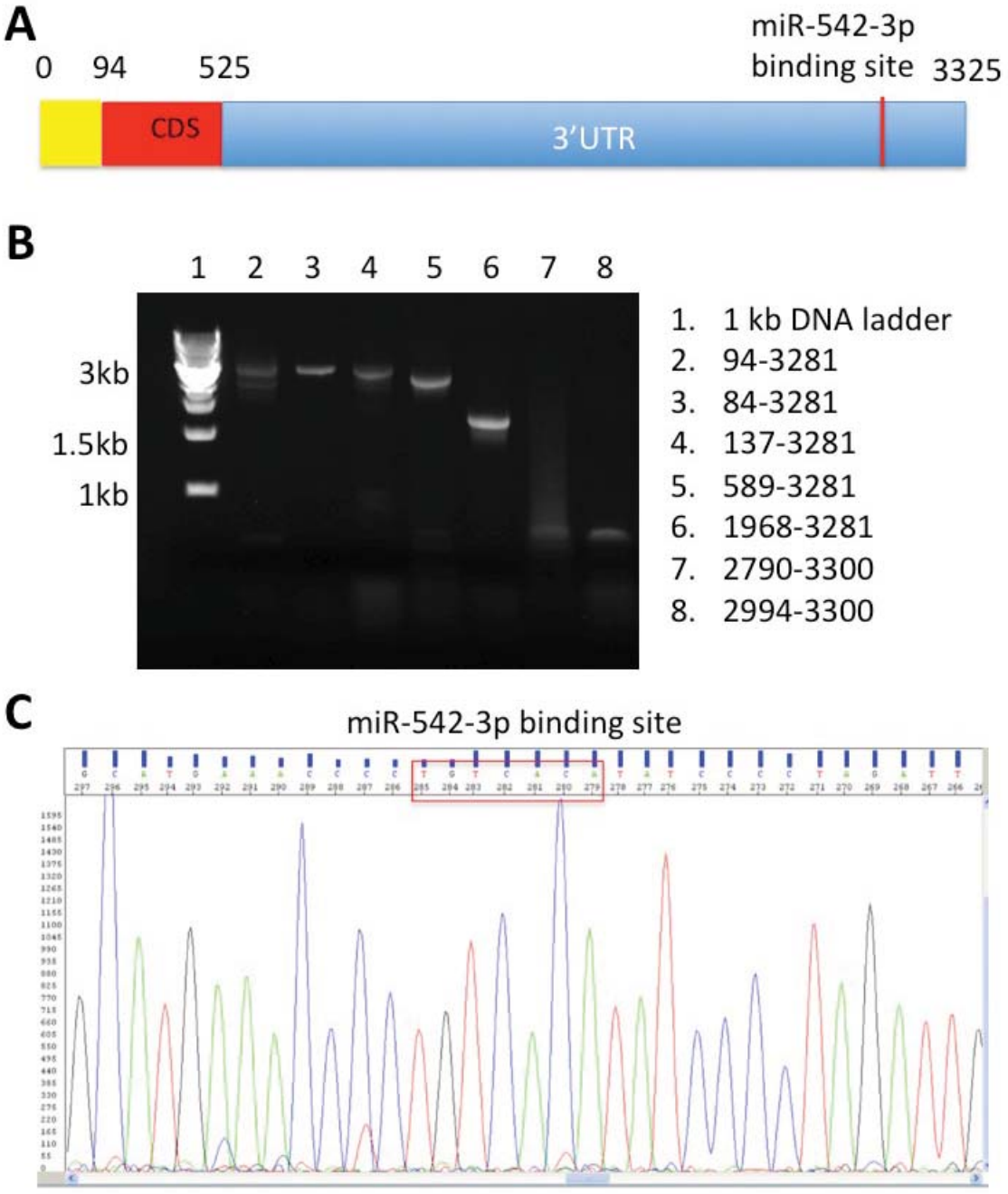

Fig. S7 Effects of miR-542-3p and siRPS23 on cell growth. U2OS cells were transfected with negative control, miR-542-3p mimics or siRPS23. Images (phase contrast) were taken 48 h following the transfection.

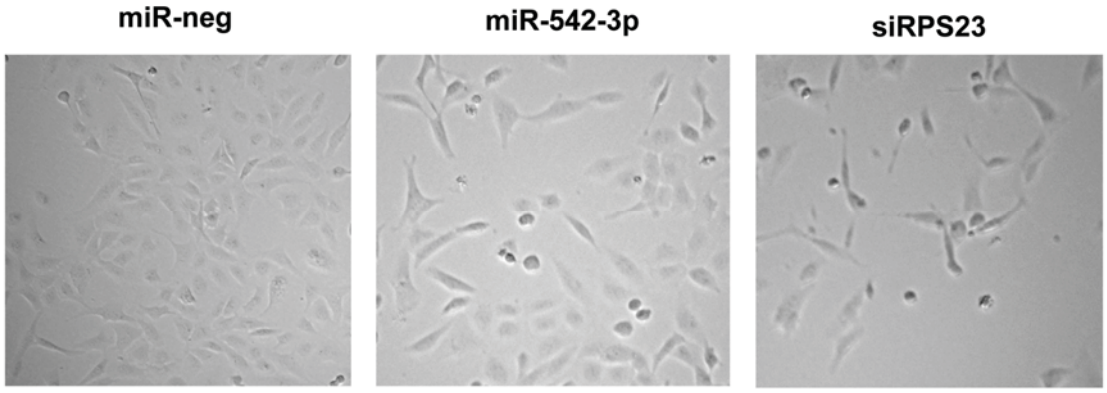

Fig. S8 MiR-542-3p Inhibits cell growth independent of RPS23. U2OS cells expressing empty vector or V5-tagged RPS23 were transfected with negative or miR-542-3p mimics for 2 days. Cells were then reseeded at $1x10^4$ cells/well in 12-well for 5-days before being fixed and stained. Relative cell growth was calculated after re-solubilizing the plates.

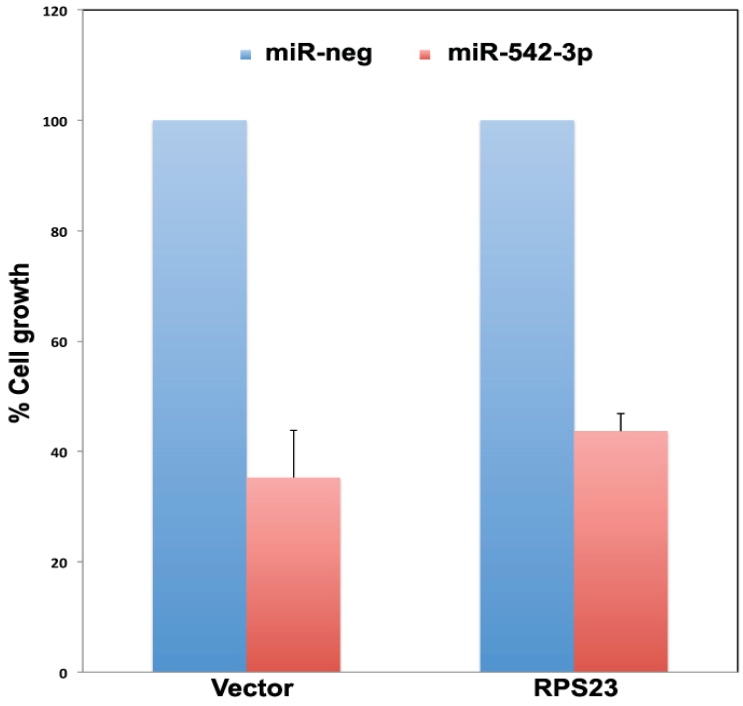

Fig. S9 miR-542-3p inhibits cell growth independent of p53. U2OS cells (A) stably expressing scramble shRNA or p53 shRNA and HCT116 cells (B) with wildtype (WT) p53 or without p53 (p53-/-) were transfected as indicated for two days. Cells were then reseeded at $1x10^4$ cells/well in 12-well for 5-days before being fixed and stained. Relative cell growth was calculated after re-solubilizing the plates.