# Expanded View Figures

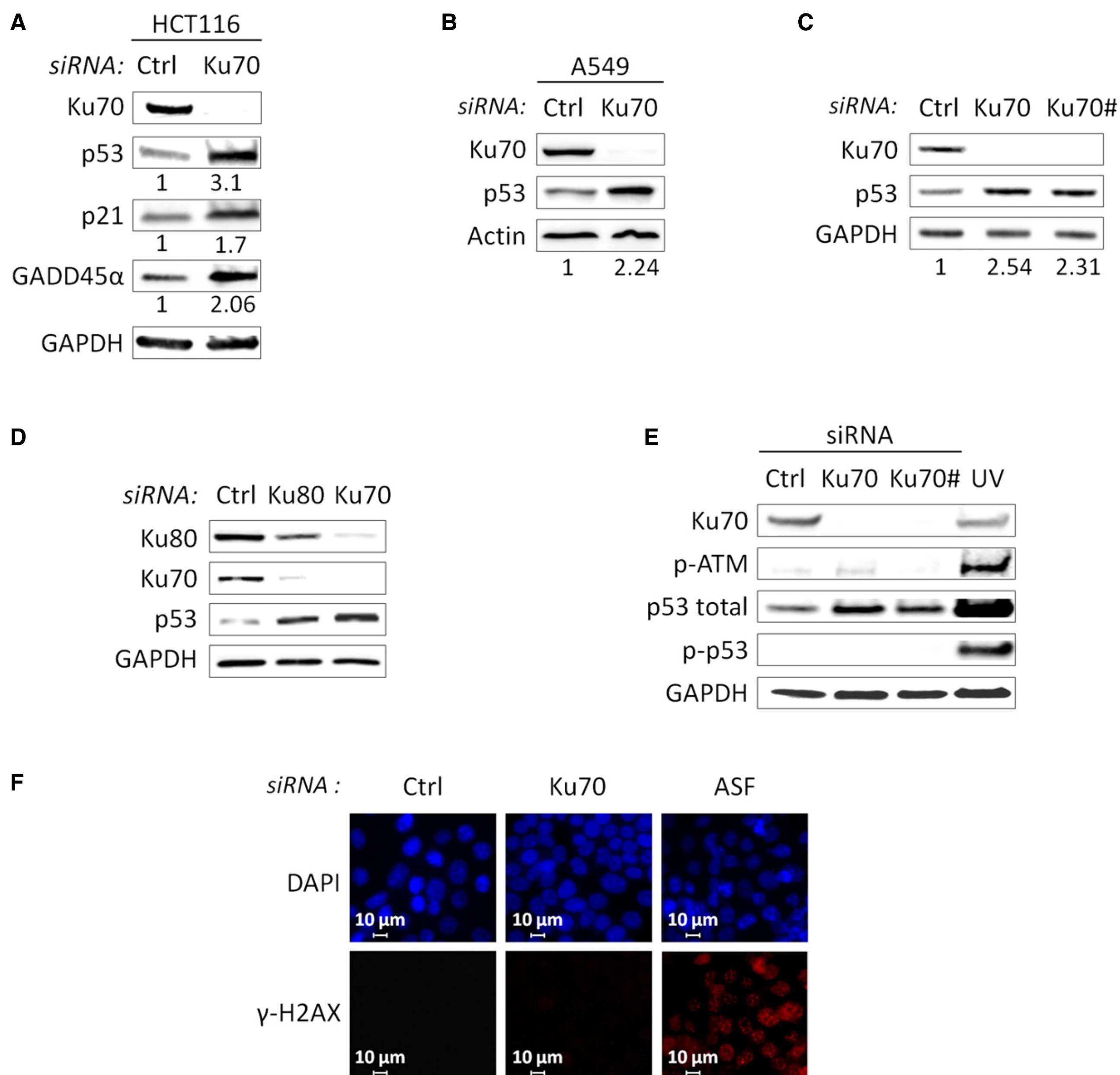


**Figure EV1. Ku knockdown induces p53 accumulation.**

A Western blot analysis of HCT116 cells after Ku70 depletion. The basal levels of p53, p21, and GADD45α normalized to GAPDH were arbitrarily set at 1.0, and the fold change is shown below each lane.

B Western blot analysis of A549 cells after siRNA-mediated Ku70 or control depletion. The basal levels of p53 normalized to actin were arbitrarily set at 1.0, and the fold change is shown below each lane.

C Western blot analysis of HCT116 cells after Ku70 depletion using two different siRNAs (Ku70 and Ku70#) or after control depletion. The basal levels of p53 normalized to GAPDH were arbitrarily set at 1.0, and the fold change is shown below each lane.

D Western blot analysis of HCT116 cells after Ku70 or Ku80 depletion or after control depletion.

E Western blot analysis of HCT116 cells after siRNA-mediated Ku70 depletion using two different siRNAs (Ku70 and Ku70#) or control depletion, or after UV irradiation (positive control for phosphorylation of ATM and p53).

F Immunofluorescence experiments were performed using γ-H2AX antibody and DAPI staining on HCT116 cells treated with siRNA targeting Ku70, ASF (positive control for γ-H2AX activation [29]) or control. Images of a single representative field were shown.

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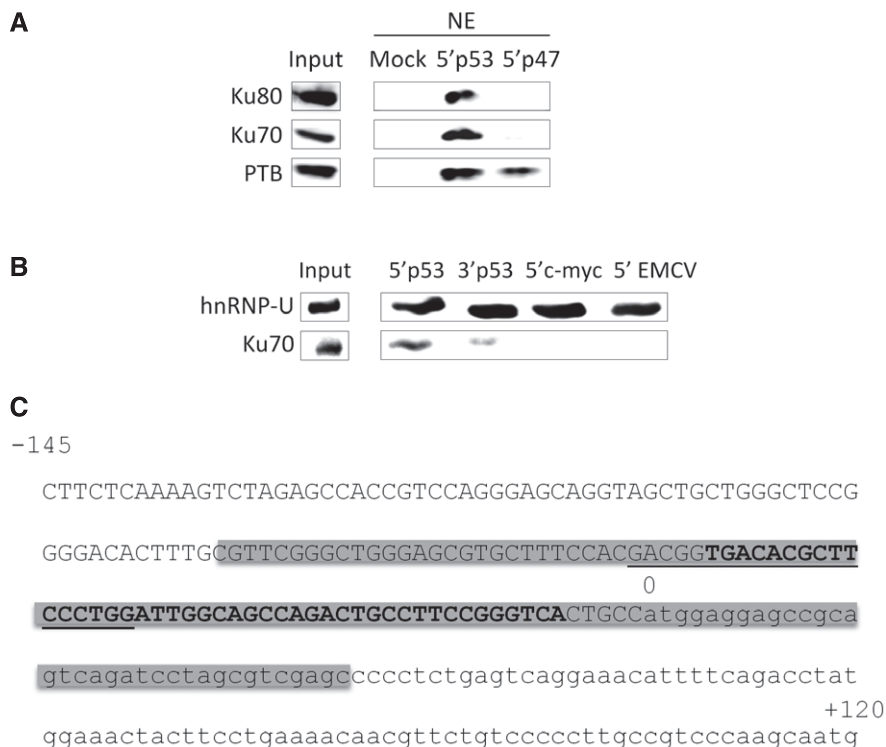
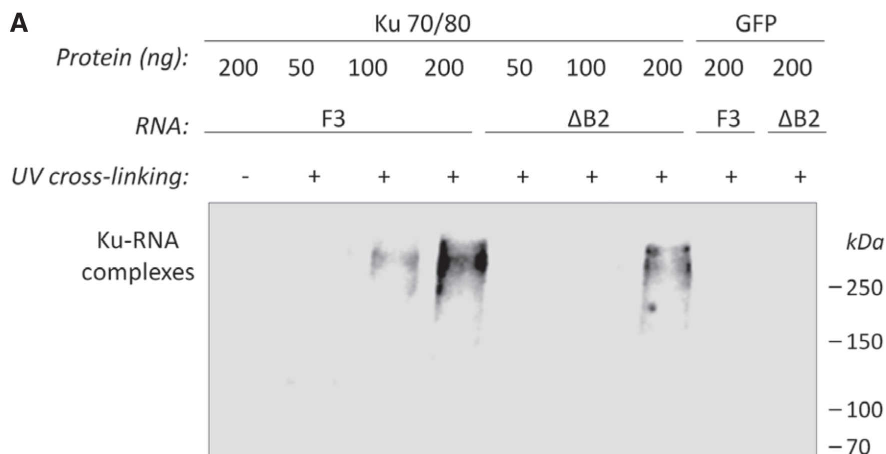



**Figure EV2. Ku specifically interacts with the p53 mRNA.**

A RNA affinity chromatography using the p53 5′ UTR WT (5′p53) or a portion of the p47 5′ UTR (5′p47) (depicted in Fig 2Ai) and HeLa nuclear extracts (NE), followed by Western blot analysis with the Ku70, Ku80, or PTB antibodies.

B RNA affinity chromatography using the p53 5′ UTR WT (5′p53), p53 3′ UTR WT (3′p53), c-myc 5′ UTR (5′c-myc) or the encephalomyocarditis virus (EMCV) 5′ UTR (5′EMCV), and HeLa cytoplasmic extracts, followed by Western blot analysis with the hnRNP-U (loading control) and Ku70 antibodies.

C 5′-terminal sequence of p53 mRNA. Gray box: large hairpin domain reported in [19] (−80/+34). Capital letter: 5′ UTR of p53 (5′p53; −145/0). Small letter: sequence in between AUG1 and AUG2 (5′p47; 0/+120). Bold: stem-loop sequence in the large hairpin domain bound by Ku and represented in Fig 2E (−49/−6). Underlined: sequence involved in 5′-3′ base-pairing [13].



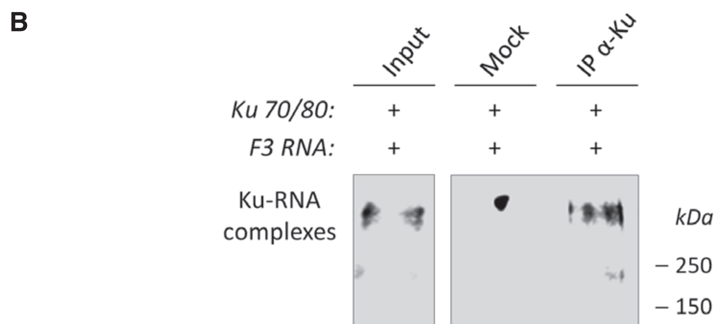




**Figure EV3. Ku directly interacts with the p53 mRNA.**

A Increasing amounts of purified recombinant Ku [32] (or GFP, negative control) were incubated with the p53 RNA fragments containing the stem-loop WT (F3) or mutated in the loop proximal bulge (ΔB2), followed or not by UV cross-linking. After SDS–PAGE analysis, transfer on membrane, and UV cross-linking, the position of Ku-RNA complexes was revealed by chemiluminescence.

B UV cross-linked complexes processed as in (A) were immunoprecipitated with an antibody recognizing the conformational epitope of the Ku heterodimer (clone 162) and processed as above.

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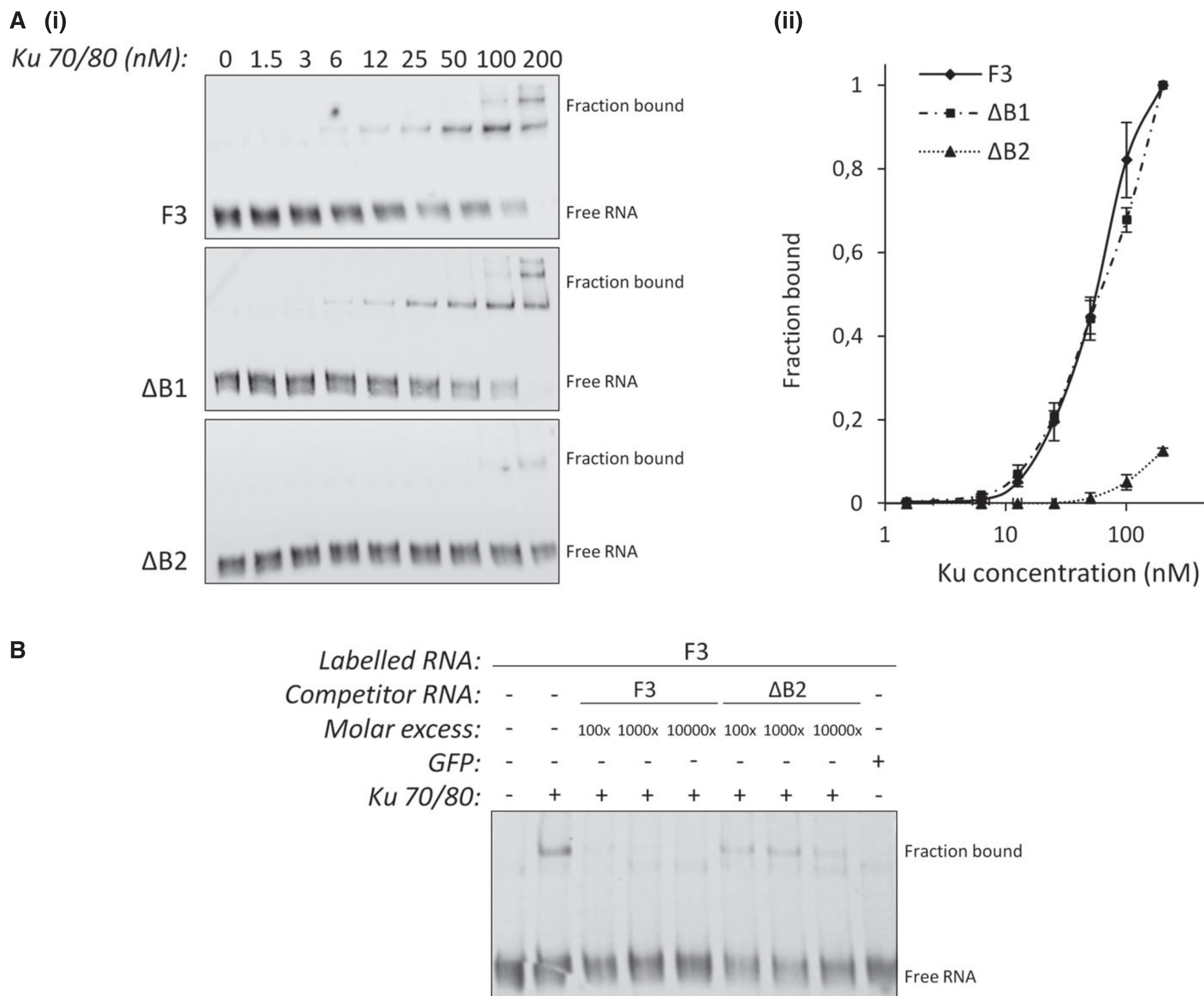


**Figure EV4. Quantitative binding of Ku to the p53 mRNA.**

A Increasing concentrations of purified recombinant Ku were incubated with trace amounts of p53 RNA fragments containing the stem-loop WT (F3) or mutated in the loop proximal bulge (ΔB1 or ΔB2). After acrylamide gel electrophoresis, transfer on membrane, and UV cross-linking, the position of Ku-RNA complexes was revealed by chemiluminescence (i). The fraction of RNA (F3, ΔB1 or ΔB2) bound to Ku [bound/(bound + free)] was quantitated and plotted as a function of the Ku concentration ($n$ = 3) (ii). All error bars reflect SEM.

B EMSA analysis as described in (A) with a constant amount of Ku (100 nM) and trace amounts of labeled F3 RNA in the presence of increasing concentrations of unlabeled F3 or ΔB2 competitors. The specificity of binding was demonstrated further by adding purified recombinant GFP (100 nM).

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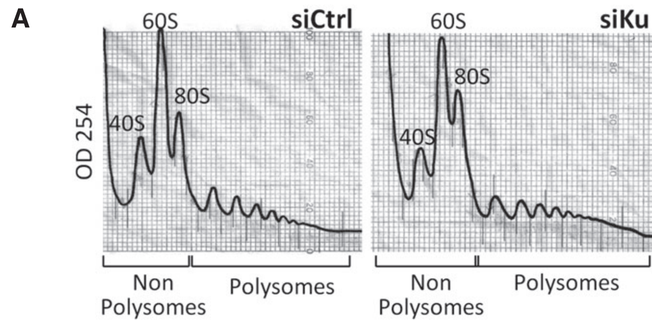


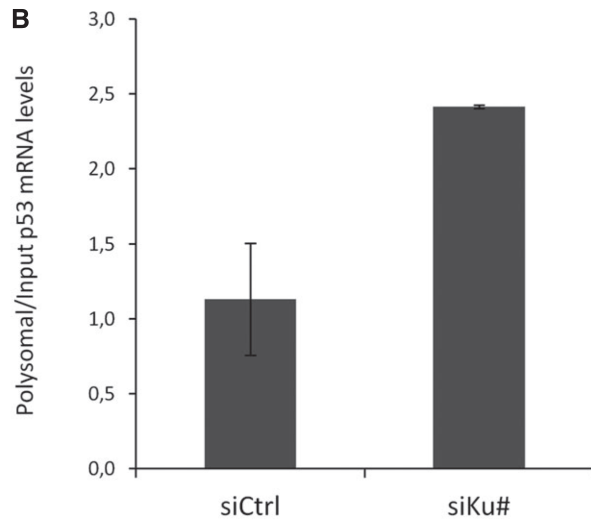


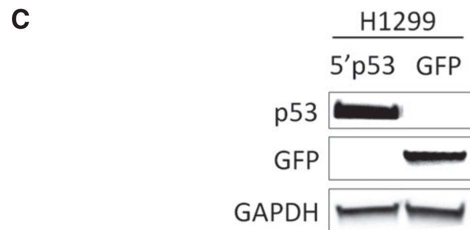


**Figure EV5. Ku decreases p53 mRNA translation.**

A Polysome distribution profile of cytoplasmic lysates after siRNA-mediated Ku or control depletion. The positions of the 40S, 60S, and 80S ribosomal subunits and polysomal peaks are indicated, as well as fractions and polysomal fractions.

B Non-polysomal and polysomal fractions were extracted from HCT116 cells treated with the siRNA Ku70# and quantitative RT–qPCR was performed using specific primers for p53 mRNAs. The p53 mRNA levels in P and NP were normalized to the input and HPRT mRNA. The height in bar graphs represents the mean and the bars indicate single data points of two independent experiments.

C Western blot from H1299 (p53-null) cells transfected with p53 or GFP reporters, showing that p53 can be detected in H1299 cells only when ectopically expressed. GAPDH: loading control.

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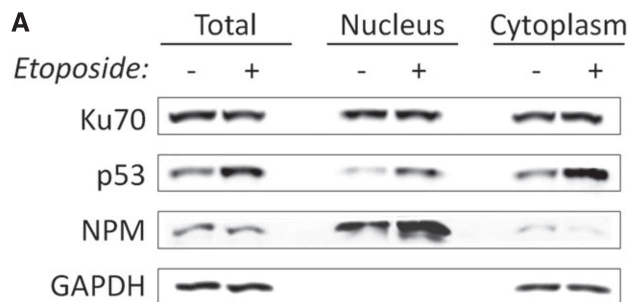


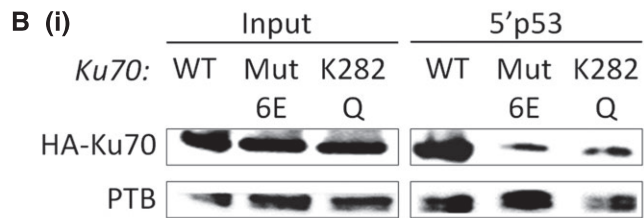


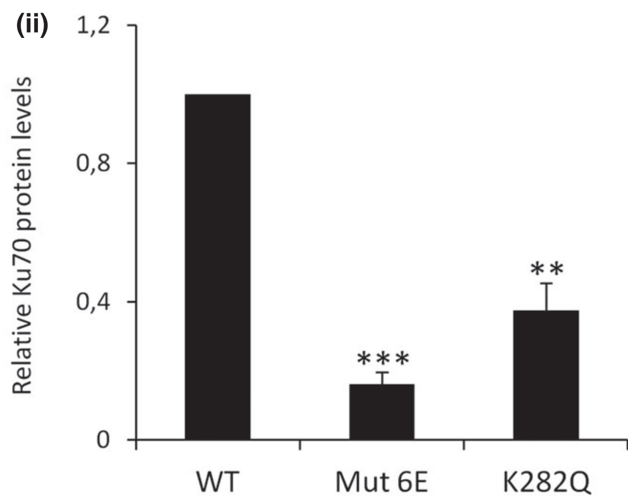


**Figure EV6. Ku acetylation relieves repression of p53 mRNA translation.**

A Western blot analysis after treatment with ETO for 16 h, followed or not by nuclear/cytosolic fractionation. NPM (nucleophosmin) and GAPDH are used as markers of the nuclear and cytosolic fractions, respectively.

B RNA affinity chromatography using the 5′p53 RNA and total extracts from cells transfected with HA-tagged wild-type (WT) or mutated (Mut6E or K282Q) Ku70 for 48 h, followed by Western blot analysis of Ku70 (i) quantified and normalized to the input and to the loading control, PTB (ii, $n = 3$).

Data information: All the experiments were performed with HCT116 cells. GAPDH: loading control in (A). PTB: RNA affinity chromatography control in (B). Statistical analysis by $t$-test (**$P < 0.01$; ***$P < 0.001$). All error bars reflect SEM.

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