Supplementary Figure 1: USP42 depletion and depletion with siRNA

(A) Western blot of U2OS cell extracts with a specific antibody against USP42, showing the presence of several isoforms and the depletion of these bands following transfection with a pool of exon 13-targeting USP42 siRNAs.

(B) Depletion of USP42 mRNA by individual siRNAs targeting exon 13 in U2OS cells.
Supplementary Figure 2: effects of USP42 depletion

(A) Western blot of U2OS cell extracts after depletion of USP42 followed by incubation for up to 24 hours with vehicle or 5nM actinomycin D.

(B) mRNA expression of p53 was determined by qRT-PCR with specific primers. The results were normalized against two different standard genes and the graphs represent the mean of 3 independent experiments.

(C) Western blot of RPE, RKO and HCT116 cell extracts with antibodies against USP42, MDM2, p53 and actin as a loading control. Cells were transfected with siRNAs targeting USP42 or a scrambled control for the indicated time.

(D) Western blot of HCT116 cell extracts after depletion of USP42 with the deconvoluted pool of siRNAs targeting exon 13, followed by incubation for 6 hours with vehicle or 5nM actinomycin D.

(E) Rescue experiment reverting the USP42 knockdown effects by expression of USP42. Cells were treated for 7h with 5nM ActD
Supplementary Figure 3: USP42 depletion delays activation of several p53 target genes in response to p53 activation

(A) U2OS cells were transfected with siRNAs against USP42 or non-targeting siRNA. Cells were then treated for the indicated time with 5nM actinomycin D to activate p53. mRNA expression of the indicated genes was determined by qRT-PCR with specific primers. The results were normalized against two different standard genes and the graphs represent the mean of 3 independent experiments.

(B) U2OS cells treated as in (A) were fixed and chromatin was immunoprecipitated with an antibody recognizing only the elongating form of the RNA polymerase II. Polymerase II bound DNA was then analysed by qPCR with specific primers amplifying the indicated regions of the p21 genes. The results are expressed as a percentage of input and represent the mean of three experiments.