Supplementary Figure Legends

Supplementary Fig. 1 – Analysis of the half-life of p53 after Pdcd4 knock-down. A. Hela-wt cells and two stable Pdcd4 knock-down clones (K11 and K12) were treated with 50 µg/ml cycloheximide and incubated for the indicated times. Total cell extracts were then analyzed by western blotting for the expression of p53 and β-actin. B. HepG2 cells were transfected with the indicated siRNAs. 48 hours later 50 µg/ml cycloheximide was added and the cells were analyzed as described in A.

Supplementary Fig. 2 – Effect of Pdcd4 on truncated versions of the p53 5'-UTR. A. QT6 fibroblasts were transfected with the indicated luciferase reporters together with or without expression vector for human Pdcd4. Cells were additionally transfected with the β-galactosidase expression vector pCMVβ to normalize for transfection efficiencies. 24 hours after transfection the cells were harvested and luciferase and β-galactosidase activities were determined. The luciferase activities were then first normalized to the β-galactosidase activities. The normalized luciferase activities of each reporter gene in the presence of Pdcd4 was normalized to the activity of the same construct in the absence of Pdcd4. The numbering indicates the part of the UTR that is retained in each construct with the last nucleotide before the start codon designated as -1. B. Predicted secondary structures of the truncated UTRs. RNA secondary structure predictions were performed using the M-fold web server (http://rna.tbi.univie.ac.at/cgi-bin/RNAfold.cgi). The minimum free energy of each structure is indicated.

Supplementary Fig. 3 – UV-treatment of HCT116 cells. HCT116 cells were irradiated with approximately 100 J/m² UV light, followed by further incubation for the indicated times and western blot analysis of total cellular protein extracts with antibodies against Pdcd4, p53 and β-actin.

Supplementary Experimental Procedures

Luciferase reporter genes: pCMV-Luc was generated by cloning the CMV promoter into the polylinker of pGL3 basic. p53 5'-UTR sequences from -195 to -1 were then inserted upstream of the luciferase coding region using HindIII and XmaI sites of the polylinker to generate the reporter gene pCMV-Luc(-195/-1), containing the full-length UTR. Truncated versions of the p53 5'UTR were generated by PCR and inserted into pCMV-Luc at the same position to obtain plasmids pCMV-Luc(-137/-1) and pCMV-Luc(-67/-1). UTR sequences are numbered as described in the legend to supplementary Fig. 2.