# Supporting Information

## Vilborg et al. 10.1073/pnas.0900862106

### SI Materials and Methods

**Plasmid Information.** ***Construct generation.*** For exogenous expression of Wig-1, the pCMVtag2b plasmid was used either alone or with indicated constructs. WT and mutant Wig-1 constructs were subcloned into pCMVtag2b (Stratagene) using BamHI as described (1) or into pEGFP-C1 (Clontech) for the heterokaryon assay using the same strategy. For overexpression of p53, the pCS2+ plasmid with indicated inserts was used. Full-length p53 cDNA from pCMVp53 was subcloned into pCS2+ by using BamHI. The 5′ UTR and ORF constructs were made from pCMVp53 with the following primers: p53 R reverse primer (same for both) and forward primers ORF F or 5′ UTR F. The forward primers contains an EcoRI site, the reverse primer contains a PinA1 site. PCR products were ligated into TEasy and then subcloned into pCS2+ by using EcoRI. The 3′ UTR construct was made by cloning the 3′ UTR of p53 into the pCS2+ORF construct (directly downstream of ORF) using primers 3′ UTR F and R, containing *PinA1* and *NotI* sites, respectively. The 3′ UTR Δ was cloned by using Sp53aPinA1 and 3′ UTR R as above. To make the +U and −U deletion mutants, the primers p53+UClaI or p53−UClaI, respectively, were used together with 3′ UTR F, and p53del2 (also with a ClaI site) was used in combination with 3′ UTR R. This strategy amplified regions upstream and downstream of the desired deletion. Products were then cleaved with ClaI and ligated together, and the resulting product was amplified by using 3′ UTR F and 3′ UTR R and cloned into the pCS2+ORF as above for the 3′ UTR construct. For Luciferase assays, the p53 3′ UTR fl (Fig. 3*A*) was PCR amplified with primers p53 a and p53 b, the p53 3′ UTR + U-rich was PCR amplified with p53+U a and p53 b, and the p53 3′ UTR Δ was PCR amplified with primers Sp53 a and p53 b, and inserted downstream of luciferase in the pGL3C vector (Promega) using the XbaI and HpaI sites. GUS reporter constructs were provided by Helen Blau (2).


1. Mendez Vidal C, Prahl M, Wiman KG (2006) The p53-induced Wig-1 protein binds double-stranded RNAs with structural characteristics of siRNAs and miRNAs. *FEBS Lett* 580:4401–4408.
2. Rossi FM et al. (1998) Tetracycline-regulatable factors with distinct dimerization domains allow reversible growth inhibition by p16. *Nat Genet* 20:389–393.
3. Michael WM, Choi M, Dreyfuss G (1995) A nuclear export signal in hnRNP A1: a signal-mediated, temperature-dependent nuclear protein export pathway. *Cell* 83:415–422.

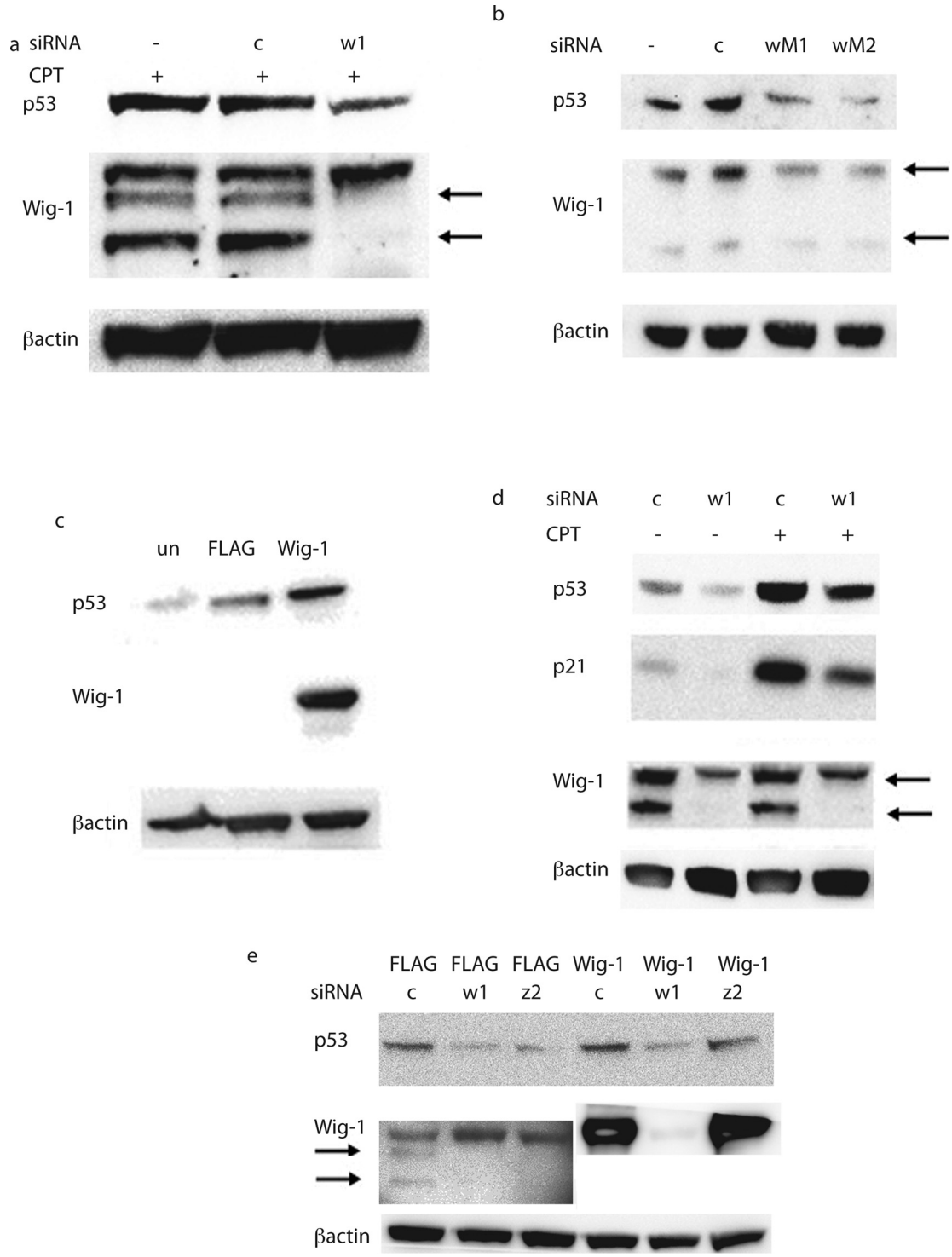


**Fig. S1.** Wig-1 positively regulates p53 protein levels. (*a*) Wig-1 knockdown by siRNA leads to decreased p53 protein levels in IMR-90 primary lung fibroblasts treated with camptothecin (CPT). IMR-90 were cultured in Eagle's minimal essential medium (Sigma–Aldrich). (*b*) Wig-1 knockdown by siRNA leads to decreased p53 protein levels also in unstressed mouse fibroblasts NIH 3T3. (*c*) Overexpression of Wig-1 causes increased p53 levels in U2OS cells. (*d*) The decreased p53 protein expression after Wig-1 knockdown results in lower levels of the p53 target p21, both in unstressed (by 89% compared with control siRNA) and CPT-treated U2OS cells (by 76% compared with control siRNA). (*e*) To confirm that the effect on p53 is due to the presence or absence of the Wig-1 protein, Wig-1 was knocked down in U2OS cells by using one siRNA (w1) targeting the ORF and one (z2) targeting the 3′ UTR. Subsequently, the cells were transfected with the Wig-1 ORF or empty vector. c, control siRNA; w1, Wig-1 siRNA 1; z2, Wig-1 siRNA 3′ UTR; wM1, mouse Wig-1 siRNA 1; wM2, mouse Wig-1 siRNA 2. Arrows indicate the two major Wig-1 protein species. When transfected with both siRNA and plasmid, cells were transfected with siRNA 24 h after plating, transfected with plasmid 24 h after siRNA transfection, and cultured for another 24 h before harvesting.

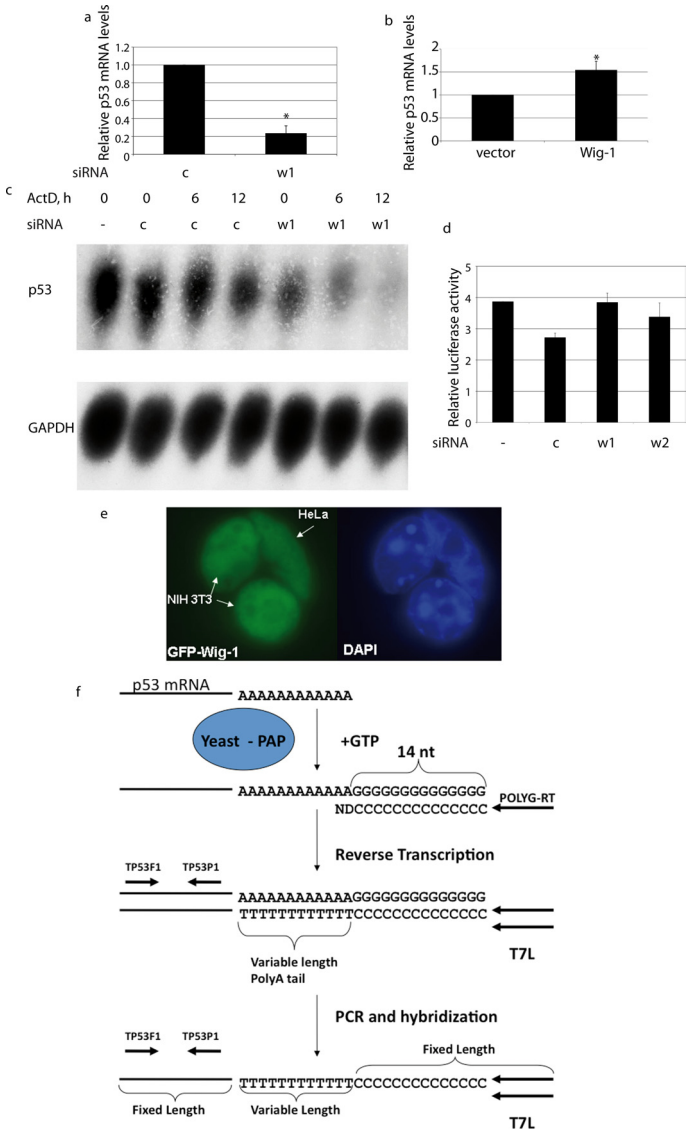


**Fig. S2.** Wig-1 positively regulates p53 mRNA, but has no effect on transcription from the p53 promoter. (*a*) Real-time PCR shows down-regulation of p53 mRNA levels in MCF7 cells after Wig-1 knockdown by 65% ($P = 0.013$). (*b*) Up-regulation of p53 mRNA in U2OS cells after exogenous expression of Wig-1 with 52% ($P = 0.036$). The data shown are the average of three (siRNA) and four (overexpression) individual experiments, and bars indicate SEM. Real-time PCR on RNA from MCF7 cells was carried out as described for HCT116 cells in *Materials and Methods*, and real-time PCR in U2OS cells was carried out as described for HaCaT cells in *Materials and Methods* with p53 primers p53RTa and p53RTb and GAPDH probes GAPDHa and GAPDHb (Table S2). (*c*) MCF7 cells were treated with siRNA and subsequently with the transcriptional inhibitor actinomycin (Act) D and harvested at 6 or 12 h. Total RNA was analyzed by Northern blotting using probes for p53 and GAPDH. The levels were quantified by using densitometry, and the experiment was performed three independent times. One representative Northern blotting is shown. (*d*) To assess whether Wig-1 affects transcription of p53, the p53 promoter region from transcript start site P1 and including 1 kb of upstream sequences was cloned upstream of luciferase in the pGL3 Basic vector (Promega). The effect of Wig-1 knockdown on luciferase activity was analyzed. Primers used to make the constructs p53 Promoter F and R are listed in Table S2. For transfections, 600 ng of plasmid DNA and 20 ng of *Renilla* luciferase (as internal control) were used for each well in a 24-well plate. The graphs show the average luciferase activity from three independent experiments normalized to *Renilla*. Bars are shown with SEM. No effect of Wig-1 on the p53 promoter was observed, indicating that Wig-1 does not affect p53 mRNA transcription. (*e*) To investigate whether Wig-1 is a shuttling protein, interspecies heterokaryons of human HeLa and mouse NIH 3T3 cells were generated as described (3). Briefly, HeLa cells grown in 6-well plates were transfected with 1 $\mu$g pEGFP-hWig-1 (see plasmid information in *SI Materials and Methods*) per well using PEI as described in *Materials and Methods*, incubated for 24 h, then trypsinized and seeded on glass coverslips. The day after, untransfected mouse NIH 3T3 cells were added to the HeLa culture. The coculture was incubated for 1.5 h followed by 30-min incubation in the presence of 75 mg/mL cycloheximide. Cells were thereafter fused by using 200 $\mu$L 50% polyethylene glycol 3350 for 2 min, washed three times in PBS, and incubated in medium containing 100 mg/mL cycloheximide for 4 h. Cells were then fixed with 4% paraformaldehyde, permeabilized with 0.5% Triton X-100, mounted by using Vectashield H-1000 containing DAPI stain (Vector Laboratories), and analyzed by fluorescence microscopy as described in *Materials and Methods*. Human and mouse nuclei can be distinguished by their differential DAPI staining (human cells exhibit the ''holes'' characteristic of nucleoli, whereas mouse cells stain in a pattern of brighter dots). The presence of GFP-Wig-1 in mouse nuclei after fusion is a proof of the ability of the protein previously present in the human cell to shuttle between cytoplasm and nuclei; because protein synthesis is being blocked by cyloheximide, the GFP-Wig-1 in the mouse nuclei cannot arise from de novo synthesis after cell fusion. (*e*) One human HeLa cell fused with two mouse NIH 3T3 cells, and the presence of GFP-Wig1 in all three nuclei is evidence of the ability of Wig-1 to shuttle. (*f*) Schematic representation of deadenylation assay.

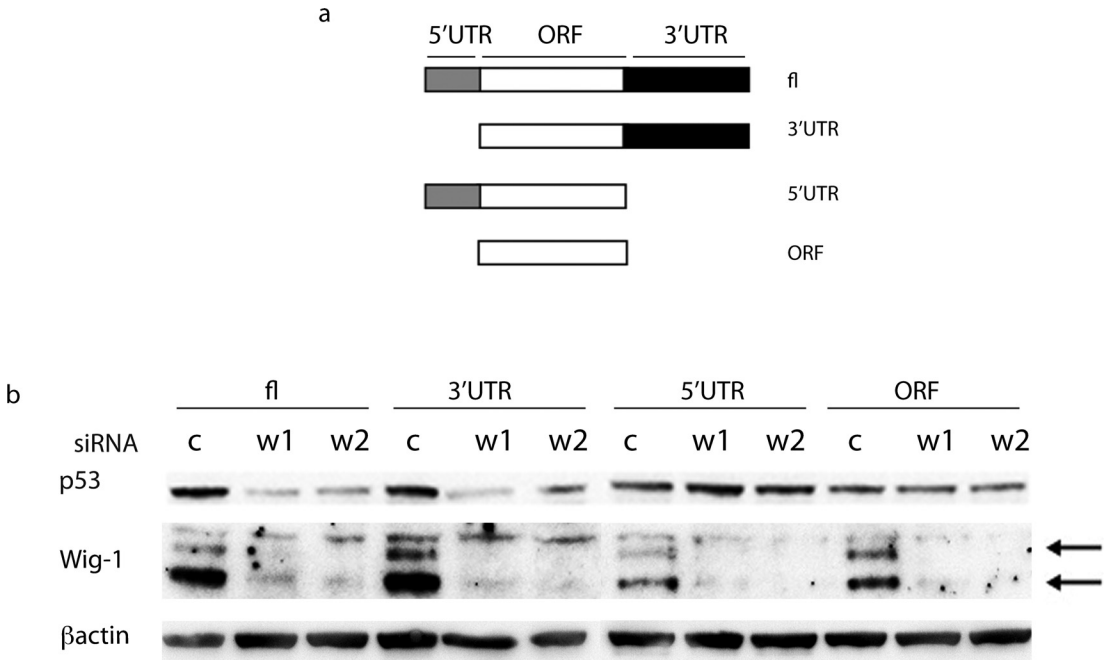


**Fig. S3.** The effect of Wig-1 on p53 depends on the 3′ UTR of p53. Saos-2 cells (p53 null) were transfected with Wig-1 or control siRNA and subsequently with the p53 constructs (*a*). Constructs containing the 3′ UTR (''fl'' and ''3′ UTR''), but not constructs lacking the 3′ UTR (''5′ UTR'' and ''ORF''), were affected by Wig-1 knockdown, as shown by Western blotting (*b*). Cells were transfected with siRNA 24 h after plating, transfected with plasmid 24 h after siRNA transfection, and cultured for another 24 h before harvesting. Arrows indicate the major Wig-1 species.

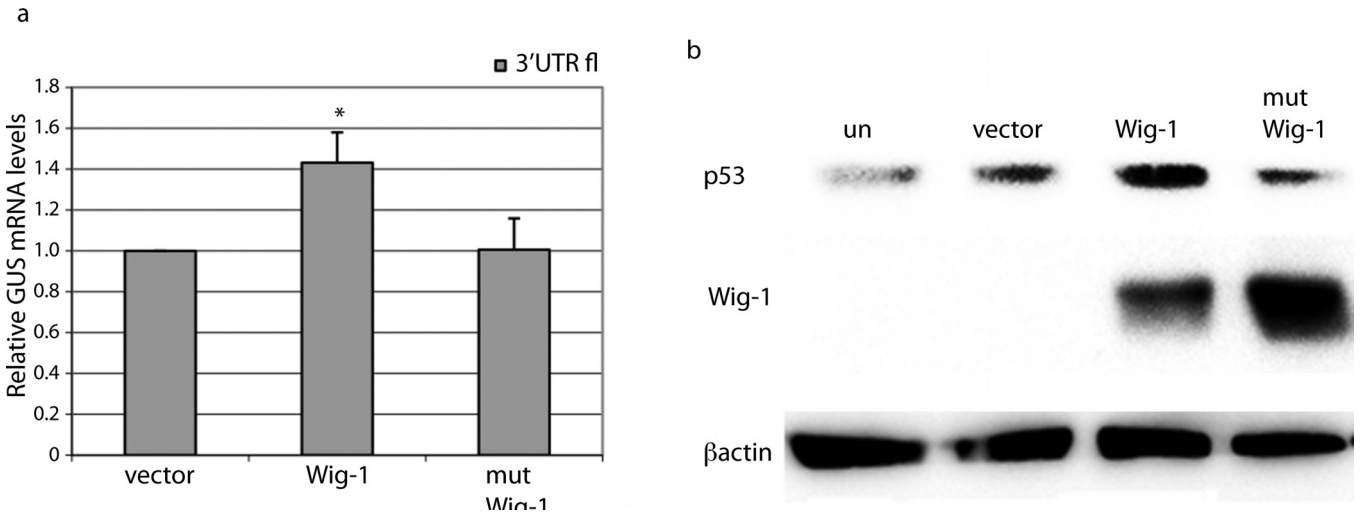


**Fig. S4.** Wig-1 binding to the p53 3′ UTR is required for Wig-1-mediated stabilization of p53 mRNA. To determine whether Wig-1 RNA binding is required for its effect on p53, we expressed the zinc finger 1 point mutant Wig-1 (mutWig-1) that does not bind to RNA or WT Wig-1 in cells. Overexpression of the mutWig-1 did not affect the p53 3′ UTR, in contrast to overexpression of WT Wig-1, as shown by the GUS reporter assay in stably transfected HaCaT cells (*a*), and by Western blotting in U2OS cells (*b*).

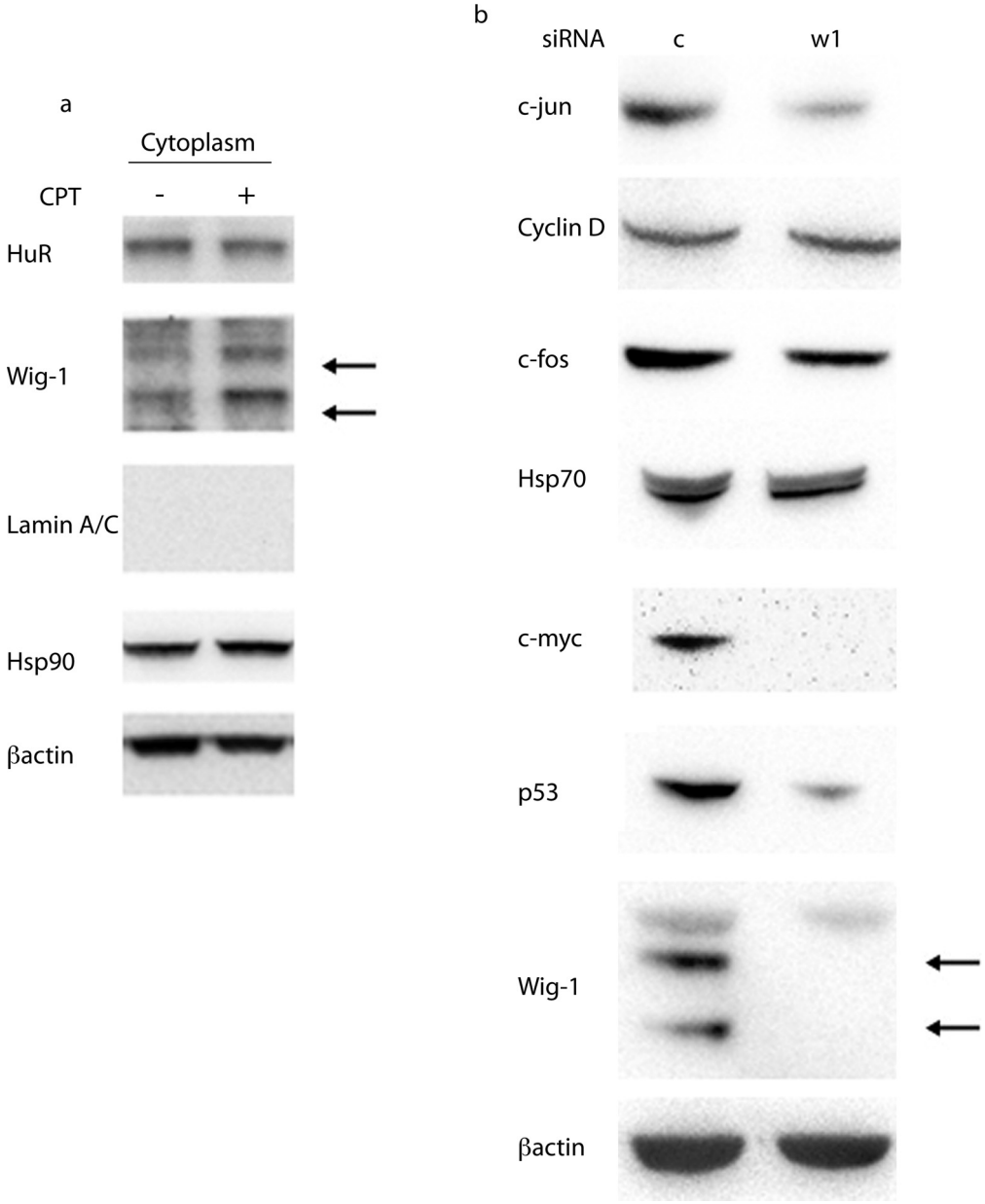


**Fig. S5.** Differential regulation of Wig-1 and HuR and putative previously undescribed Wig-1 targets. (*a*) To investigate the effect of camptothecin on Wig-1 and HuR protein expression in the cytoplasm, HCT116 p53 +/+ cells were treated with 600 nM CPT and subjected to mild lysis to generate cytoplasmic fractions (see *Materials and Methods*) that were analyzed by Western blotting with indicated antibodies. Lamin A/C is a nuclear marker and Hsp90 a cytoplasmic marker. Cytoplasmic levels of Wig-1, but not HuR, are increased on CPT treatment. (*b*) To examine whether Wig-1 can affect expression of other ARE regulated mRNA/proteins, U2OS cells were treated with siRNA against Wig-1 or control siRNA, and analyzed by Western blotting with indicated antibodies. Arrows indicate the two major Wig-1 protein species.

**Table S1. Drug treatments**

| Drug | Company | Concentration | Duration, h |
|---|---|---|---|
| Actinomycin D | Sigma–Aldrich | 1 μg/mL | Indicated time points |
| Camptothecin | Sigma–Aldrich | 600 nM | 16 |
| Camptothecin, colony formation assay | Sigma–Aldrich | 60 nM | 16 |
| Mitomycin C | Sigma–Aldrich | 6 μg/mL | 24 |

**Table S2. Primer and probe sequences**

| Oligonucleotide | Sequence |
|---|---|
| Ambion siRNA Wig-1 oligo | 5′-AAGCCCAGG-CTCATTATCAGG-3′ |
| Ambion siRNA control oligo | 5′-AAACGTGACACGTTCGGAGAA-3′ |
| Qiagen human Wig-1 siRNA 1 (w1) | Hs_WIG1_1_HP |
| Qiagen human Wig-1 siRNA 2 (w2) | Hs_WIG1_2_HP |
| Qiagen human Wig-1 siRNA 3′ UTR (z2) | Hs_ZMAT3_2_HP |
| Qiagen mouse Wig-1 siRNA 1 (wM1) | Mm_Wig1_1_HP |
| Qiagen mouse Wig-1 siRNA 1 (wM2) | Mm_Wig1_2_HP |
| Qiagen siRNA control | AllStars Negative Control siRNA |
| p53 R | 5′-ATGACCGGTTCAGTCTGAGTCAGG-3′ |
| ORF F | 5′-GAATT-CATGGAGGAGCCGCAG-3′ |
| 5′ UTR F | 5′-GAATTCTCTAGAGC-CACCGTC-3′ |
| 3′ UTR F | 5′-GCATGGACACCGGTCATTCTCCACTTCT TGTTCCCCACTGAC-3′ |
| 3′ UTR R | 5′-GCATGGACGCGGCCGCCTCACTCA CCCCTGCAC-3′ |
| Sp53aPinAI | 5′-GCATGGAACCGGTGAGACTGGGTCTCG CTTTGTTGCCC-3′ |
| p53 + UClaI | 5′-GCATGGACATCGATAAAGAAAAAGAA AAAAAAAAAAAAAAAAGAAAAAAGAAAT TGACCCTGAGCATAAAACAAGTCTTGGTG |
| p53-UClaI | 5′-GCATGGACATCGATGGATCCAGATCAT CATATAC-3′ |
| p53del2 | 5′-ACCCTTCCCCTCCTTCTCCCATCGATCT CTTATTTTACAATAAAACTTTG |
| p53 a | 5′-CATGGACTCTAGACATTCTCCACTTCTT GTTCCCCACTGAC-3′ |
| p53 b | 5′-GCATGGACGTTAACTCCTCACTCAC CCCTGCACCTGCTGACC-3′ |
| p53 + U a | 5′-GTTTTATGCTCAGGGTC-3′ |
| Sp53 a | 5′-TGAGACTGGGTCTCGCTTTGTTGCCC-3′ |
| GUS F | 5′-GTGATGATAATCGGCTGATG-3′ |
| GUS R | 5′-CCTGCGTCAATGTAATGTTC-3′ |
| PURO F | 5′-AGGGCAAGGGTCTGGGCA-3′ |
| PURO R | 5′-TCGGCGGTGACGGTGAAG-3′ |
| p53 Promoter F | 5′-GGTACCGGCTCTAGACTTTTGAGAAGC TCA-3′ |
| p53 RTa | 5′-ATAGTGTGGTGGTGCCCTATGA-3′ |
| p53 RTb | 5′-TGTGATGATGGTGAGGATGG-3′ |
| GAPDHa | 5′-GAAGGTGAAGGTCGGAGTC-3′ |
| GAPDHb | 5′-GAAGATGGTGATGGGATTTC-3′ |
| p53 Promoter R | 5′-CTCGAGGGAGTAGGCAGAAGACTC CCG-3′ |
| PGRT | 5′- GGCCAGTGAATTGTAATACGACTCACT ATAGGACCCCCCCCCCCCCDN-3′ |
| T7L | 5′-CAGTGAATTGTAATACGACTCACTATA GG −3′ |
| P53FOR1 | 5′-CTGCATTTTCACCCCACCCTTCC-3′ |
| TP53P1 | 5′-GTTTTATTGTAAAATAAGAGATCGATA-3′ |
| P53 TaqMan | Hs00153340_m1 |
| β-Actin TaqMan | Hs99999903_m1 |
| GAPDH TaqMan | Hs99999905_m1 |

**Table S3. Buffers**

| Name | Composition |
|---|---|
| WBLB | 100 mM Tris, pH 8.0/150 mM NaCl/1% NP40/1% Protease Inhibitor Cocktail (Sigma–Aldrich) |
| PLB | 100 mM KCl/5 mM $MgCl_2$/10 mM Hepes, pH 7.0/0.5% NP40/1 mM DTT/1% Protease Inhibitor Cocktail (Sigma–Aldrich)/0.5% RNAseOUT (Invitrogen) |
| CBB | 20 mM Hepes, pH 7.5/20 mM KCl/5 mM DTT/1 mM $MgCl_2$/10 $\mu$M $ZnCl_2$/100 $\mu$g/mL BSA/0.1% NP40/1% Protease Inhibitor Cocktail (Sigma–Aldrich)/0.5% RNAseOUT (Invitrogen) |
| Blocking buffer | 2% (wt/vol) BSA/0.05% glycerol/0.1% $NaN_3$/0.002% Tween 20 |
| RIP buffer A | 150 mM KCl/25 mM Tris·HCl, pH 7.5/10 $\mu$M $ZnCl_2$ |
| RIP buffer B | 150 mM KCl/25 mM Tris·HCl, pH 7.5/10 $\mu$M $ZnCl_2$/1% NP40/1% Protease Inhibitor Cocktail (Sigma–Aldrich)/0.5 mM DTT/100 U/mL RNaseOUT (Invitrogen) |