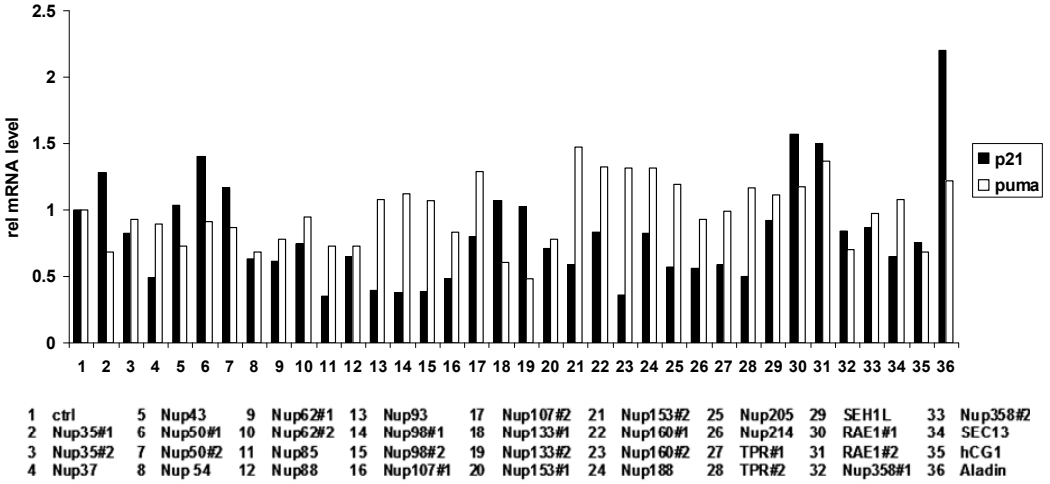
Supplemental Information

Figure S1

A



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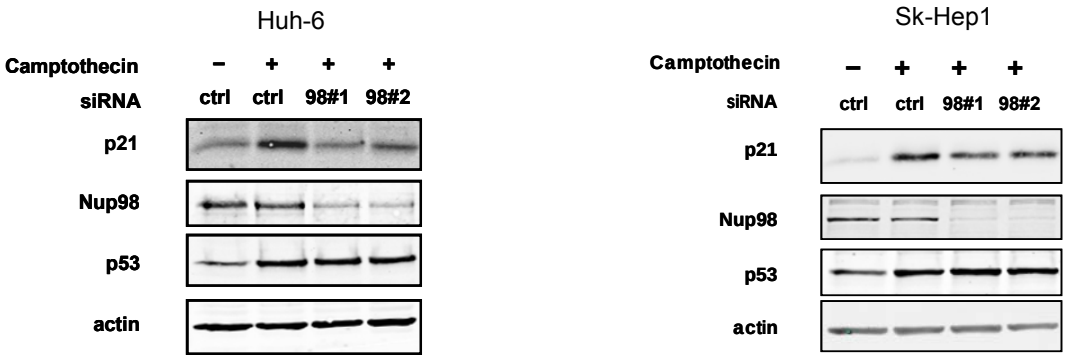


C

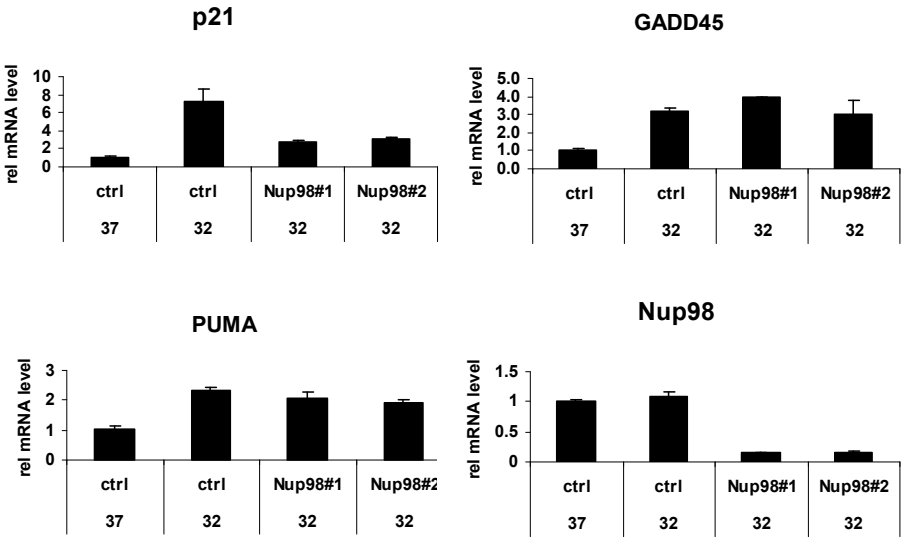
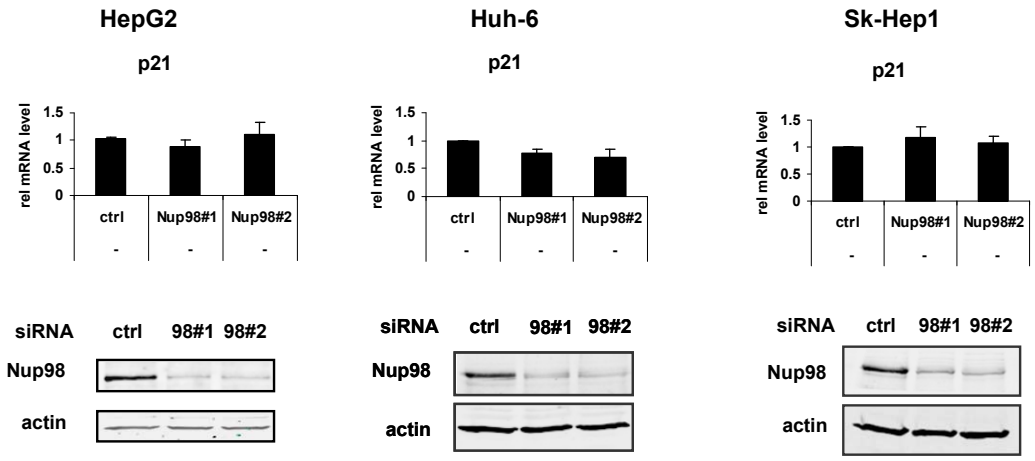


Figure S1 cont.

D



E

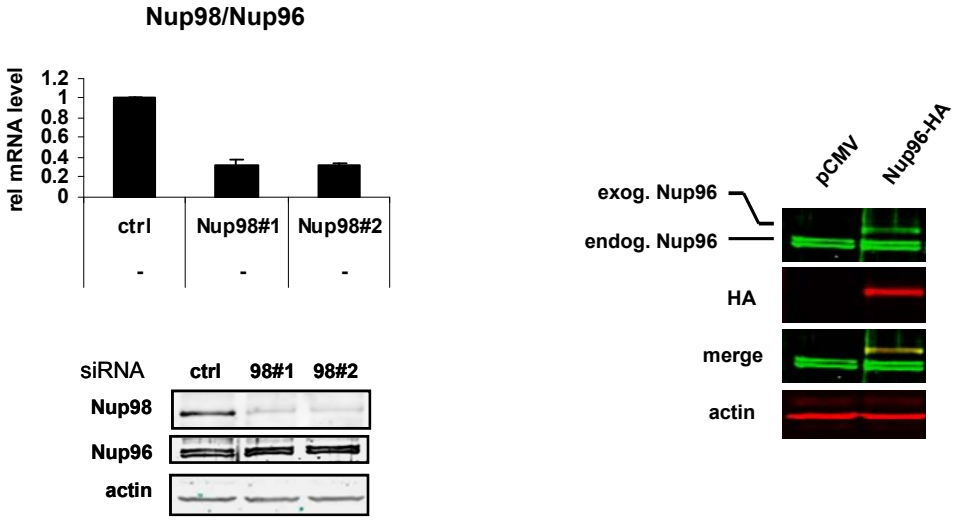


Figure S2

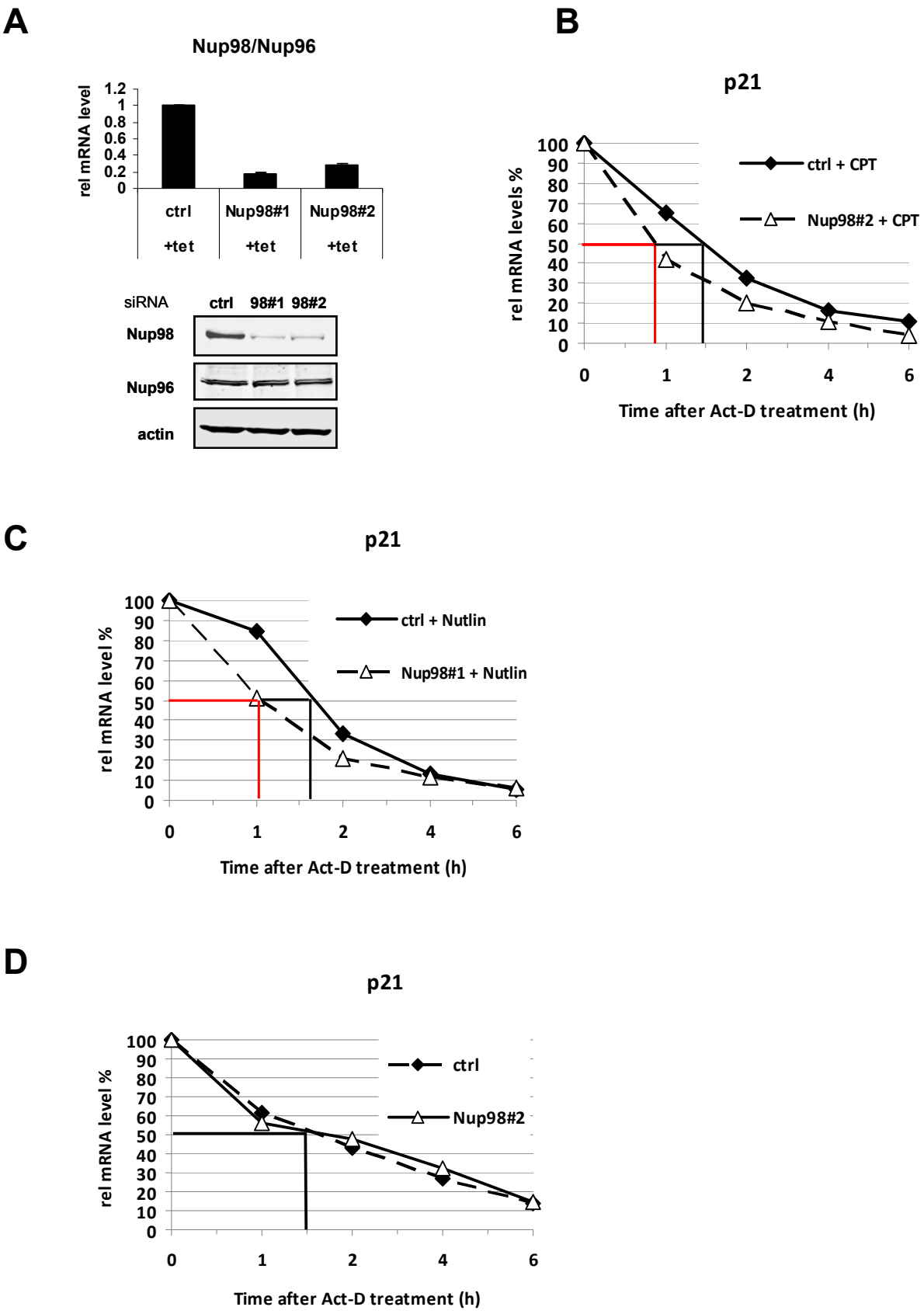

A
Nup98/Nup96
rel mRNA level
1.2
1
0.8
0.6
0.4
0.2
0
ctrl +tet
Nup98#1 +tet
Nup98#2 +tet
siRNA
ctrl
98#1
98#2
Nup98
Nup96
actin
B
p21
rel mRNA levels %
ctrl + CPT
Nup98#2 + CPT
Time after Act-D treatment (h)
C
p21
rel mRNA level %
ctrl + Nutlin
Nup98#1 + Nutlin
Time after Act-D treatment (h)
D
p21
rel mRNA level %
ctrl
Nup98#2
Time after Act-D treatment (h)

Figure S3

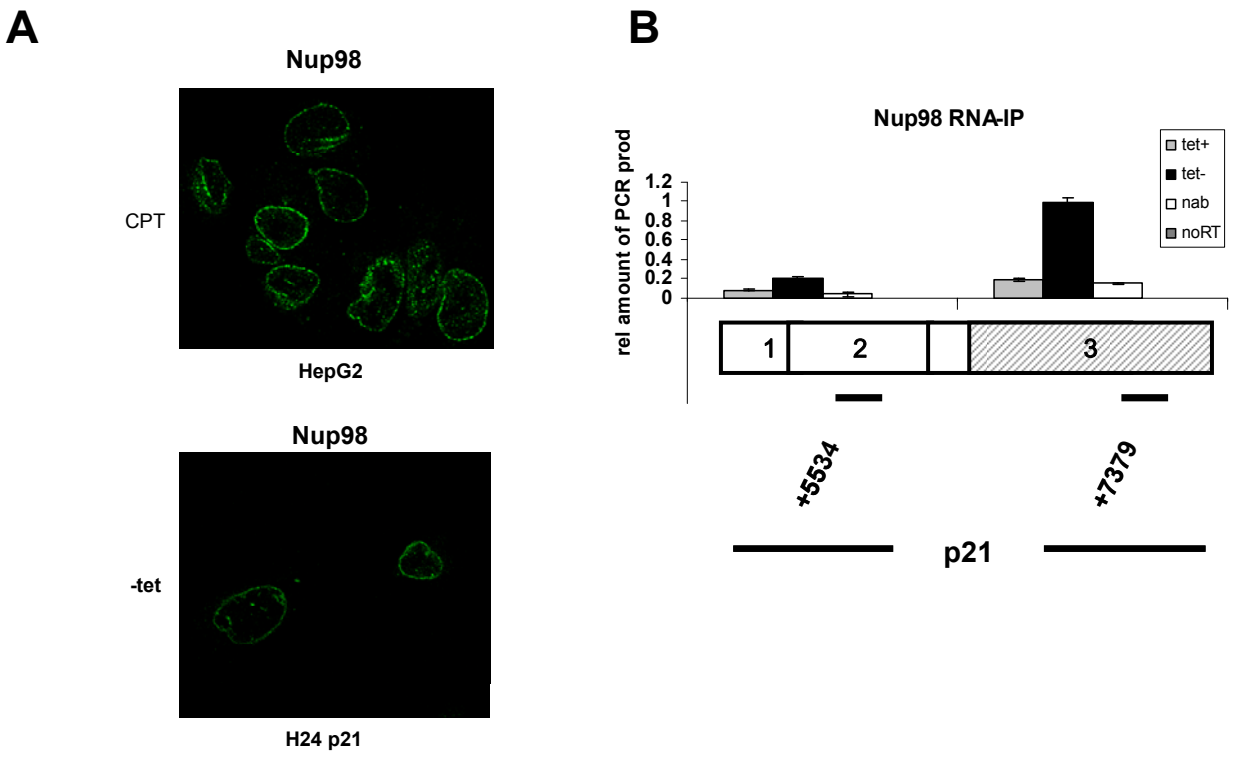

A
Nup98
CPT
HepG2
Nup98
-tet
H24 p21
B
Nup98 RNA-IP
rel amount of PCR prod
1.2
1
0.8
0.6
0.4
0.2
0
tet+
tet-
nab
noRT
1
2
3
+5534
+7379
p21
C
µg
0.75 1.25
0.75 1.25
HA
actin

Figure S4

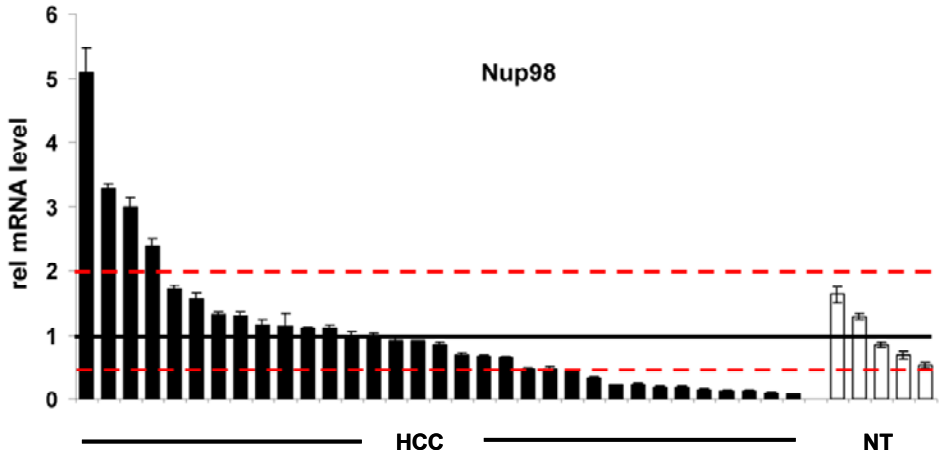


Figure S1, related to main Figure 1

(A) *An RNAi screen reveals selective impact on p21 and/or PUMA induction by subsets of Nucleoporins*. HepG2 cells were treated either with control (ctrl) or specific small interfering RNAs (siRNA) against a variety of nucleoporins (Nup) or other nuclear pore components for 72 h. In cases where two siRNAs were used, they are designated as #1 and #2. Camptothecin (CPT; 300 nM) was added for the final 24 h before RNA extraction. qRT-PCR was used to measure the levels of p21 (black bars) and PUMA (white bars) mRNA accumulation. The ctrl-siRNA plus CPT condition is normalized to 1.

(B) *Reduced p21 protein accumulation upon Nup98 knockdown.* Huh6 (left panel) and Sk-Hep1 cells (right panel) were treated either with control (ctrl) or two Nup98 specific small interfering RNAs (siRNA Nup98#1 and Nup98#2) for 72 h. Camptothecin (CPT; 300 nM) was added for the final 24 h before extraction of cells for protein analysis. Proteins were detected by immunoblotting with indicated antibodies.

(C) *Nup98 depletion selectively reduces p21 mRNA accumulation induced by p53 only when in wild-type conformation.* Hep3B-4Bv cells harboring a temperature sensitive p53 mutant (135Val) were treated either with control (ctrl) or Nup98#1or Nup98#2 siRNA for 72 h. 24 h prior to RNA extraction cells were either maintained at 37°C (37) with inactive p53 or shifted to 32°C (32) with activated p53 in wild-type conformation. qRT-PCR was used to measure the levels of p21, GADD45, PUMA or Nup98 mRNA expression as indicated. Data are presented as mean ± SD.

(D) *Basal p21mRNA levels are not affected by Nup98 knockdown* HepG2 (right panel), Huh-6 (middle panel), and Sk-Hep1 (right panel) cells were treated with ctrl or Nup98#1 and Nup98#2 siRNA for 72 h before RNA and protein extraction. Bar diagrams show relative p21 mRNA expression assessed by using qRT-PCR analysis. Data are presented as mean ± SD derived from two independent experiments. Immunoblots in lower panels from the same experimental conditions show Nup98 protein expression with actin as loading control.

(E) *Nup96 protein levels are unaltered in HepG2 cells harboring Nup98 siRNAs.*

Left panel: HepG2 cells were treated either with control (ctrl) or two Nup98 siRNAs (Nup98#1 and Nup98#2) for 72 h and harvested for RNA (upper panel) or protein (lower panel) extraction. Bar diagram in upper panel depicts relative mRNA

expression of the Nup98/Nup96 precursor mRNA measured by qRT-PCR by using primers annealing to the Nup96 part of the Nup98/96 precursor mRNA. Data are presented as mean ± SD. Immunoblot (lower panel) from the same experimental condition shows Nup98 and Nup96 protein expression detected with respective antibodies. Actin serves as loading control.

Right panel: H1299 cells were transfected with HA-tagged Nup96 (2 µg) or the corresponding empty vector (pCMV 2 µg) and harvested 24 h after transfection for protein extraction. Immunoblot shows endogenous and exogenous Nup96 detected either with anti-Nup96 (green channel) or anti-HA (red channel). Merge shows migration positions of ectopic HA-tagged and endogenous Nup96 polypeptides. Actin serves as loading control (red channel). The LiCor-Odessey System was used for signal detection.

Figure S2, **related to main Figure 2**

(A) Nup96 *protein levels are unaltered in H24-p21 cells harboring Nup98 siRNAs*

H24-p21 cells (as in Figure 2B) were treated either with control (ctrl) or two Nup98 siRNAs (Nup98#1 and Nup98#2) for 72 h and harvested for protein and RNA extraction. The bar diagram depicts relative mRNA expression of the Nup98/96 precursor mRNA measured by qRT-PCR by using primers annealing to the Nup96 part of the Nup98/96 precursor mRNA. Data are presented as mean ± SD. Immunoblot from the same experimental condition shows Nup98 and Nup96 protein expression detected with respective antibodies. Actin serves as loading control.

(B) *Nup98 stabilizes mature p21 mRNA induced by CPT treatment.* HepG2 cells were treated either with control (ctrl) or (Nup98#2) for 72 h and CPT (300 nM) was added 24 h before blocking mRNA synthesis by Actinomycin-D (Act-D). Cells were harvested at indicated time points and mature p21 mRNA decay was measured by qRT-PCR with the exon-spanning primer pairs described in Figure 2A. The black and red line indicate the p21 mRNA half-life of the ctrl-siRNA or the Nup98#2 conditions, respectively.

(C) *Nup98 stabilizes mature p21 mRNA induced by Nutlin treatment.* HepG2 cells were treated either with control (ctrl) or (Nup98#1) for 72 h and Nutlin-3 (20 M) was added 24 h before blocking mRNA synthesis by Actinomycin-D (Act-D). Cells were harvested at indicated time points and mature p21 mRNA decay was measured by qRT-PCR with the exon-spanning primer pairs described in Figure 2A. The black and

red lines indicate the p21 mRNA half-life of the ctrl-siRNA or the Nup98#1 conditions, respectively

(D) *Nup98 depletion does not affect the half-life of basal p21 mRNA.* HepG2 cells were treated either with control (ctrl) or Nup98#2siRNA (Nup98#2) for 72 h before Actinomycin-D (Act-D) treatment as in Figure 2 C. Cells were harvested at indicated time points and mature p21 mRNA decay was measured by qRT-PCR with the exon-spanning primer pairs described in Figure 2A. The black and red lines indicate the p21 mRNA half-life of the ctrl-siRNA or the Nup98#2 conditions, respectively.

Figure S3, **related to main Figure 4**

(A) *Nup98 is localized at the nuclear membrane and in the nucleoplasm.* HepG2 cells treated with Camptothecin (CPT; 300 nM) for 24 h (upper panel) or H24 p21 cells 72 h after tetracycline (Tet) removal (lower panel) were processed for immunofluorescent staining for Nup98.

(B) *Nup98 associates with the 3'UTR of ectopic p21 in H1299 cells*. Nup98 was immunoprecipitated from 0.1% formaldehyde cross-linked samples of H24-p21 treated with Tet or 48 hour after Tet removal as in Figure 4A. p21 mRNA that co-immunoprecipitated with cross-linked Nup98 was reverse-transcribed and amplified by qRT-PCR. Rectangles below graph represent exons in p21 cDNA and shaded rectangle indicates the 3'UTR. The numbers indicate the position of each amplicon within the *p21* gene locus for which primer pairs specific for the two different regions of p21 mRNA were derived. Data are presented as mean ± SD.

(C) *Levels of transfected HA-Nup98 protein*. Hep3B cells were transfected with indicated concentrations of HA-tagged full length Nup98 24 h before protein extraction. Protein levels of the ectopically expressed HA-Nup98 in samples corresponding to those shown in Figure 4B were analyzed by Western blotting with anti-HA or actin antibodies as indicated.

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Figure S4, related to main Figure 7

Nup98 expression is downregulated in human hepatocellular carcinoma. The relative Nup98 mRNA expression in 33 human hepatocellular carcinoma (HCC) tissue samples (black bars) was assessed by qRT-PCR and compared to the similarly assessed averaged expression (horizontal line) of 5 non-tumorous (NT) liver tissues (white bars) normalized to 1. Red dotted lines indicate thresholds of 2-fold higher or

lower expression than the averaged expression in non-tumorous liver tissue. 18s-rRNA was used as a housekeeping gene for internal normalization. Data are presented as mean ± SD.

Table S1, related to main Figure 3 *Decreased transcript numbers of cytoplasmic and nuclear p21 mRNA upon Nup98 knockdown.* Table lists values for p21 mRNA molecules in the analyses described in Figure 3 A-E. In the first column N_{mRNA} is the average number of molecules per cell. The average of mRNA molecules present within the nucleus (nux) and cytoplasm (cyto) are listed in the next two columns. The median, together with the minimum and maximum number of p21 mRNAs, given in parentheses, reports the cell to cell variability seen in the histograms in A-E. The Ratio is the average number of cytoplasmic mRNAs divided by the average number of nuclear mRNAs. In the last column the number of analyzed cells per condition pooled from three repeats of the experiment is listed. Statistical significance (p_{Nut}) was determined from a two tailed t-test comparing each condition with Nup98 siRNA plus Nutlin (Nut) treatment to the condition with control siRNA (ctrl) plus Nutlin treatment.

Condition	N_{mRNA}	$N_{mRNAnux}$	$N_{mRNAcyto}$	$Median_{nux}$	$Median_{cyto}$	Ratio	N_{cells}
ctrl DMSO	8.4± 5.5	1.8± 1.8	6.6 ±4.8	2 (0-9)	6 (0-20)	3.7	48
ctrl Nut 20µM	59.6± 23.4	8.5± 4.1	51 ±21.7	8 (3-18)	47 (14-117)	6	58
Nup98#1 Nut 20µM	28.2± 9.4 p_{Nut}<0.001	6.4± 3.9 p_{Nut} =0.025	21.8 ±8.9 p_{Nut}<0.001	6 (1-17)	22 (6-45)	3.4	29
Nup98#3 Nut 20µM	24.2± 12.9 p_{Nut}<0.001	5.6± 3.8 p_{Nut}<0.001	18.7 ±11.8 p_{Nut}<0.001	5 (0-20)	17 (3-61)	3.3	55
p21siRNA Nut 20µM	19.6± 12.8 p_{Nut}<0.001	5.6± 3.5 p_{Nut}<0.001	14 ±11.5 p_{Nut}<0.001	5 (0-15)	12 (0-53)	2.5	59

Table S2, related to main Figure 5 *14-3-3 scores highest of all bona fide p53 target genes based on the relative enrichment of a C-rich motif in its 3'UTR*

Bona fide p53 target genes are stratified based on their relative enrichment of a C-rich motif shown in Figure 5A. The name of each gene, the motif score, length of the 3' untranslated region (UTR) in bps, the motif score divided by 3'UTR length (= normalized motif score), and the rank are given in the respective columns.

For Table S2 see separate Excel sheet

Table S3

siRNA Sequences for all transfection experiments are listed below.

Nup98#1	5'-CTGGAGTTAGCACTAACATAA-3'
Nup98#2	5'-CAGTGTATTACTGCTATGAAA-3'
Nup98#3	5'-AACCC-TATTGCCAAACCTATT-3'
Exosc3 #1	5'-CCGGAACAGGAGGACGCGGAA-3'
Exosc3#2	5'-CTGGGTGGACTCTCAGCAGAA-3'
CDKN1A #1	5-CAGTTTGTGTGTCTTAATTAT-3
CKDN1A #2	5-CTGGCATTAGAATTA-TTTAAA-3
Luc	5-TCGAAGTACTCAGCGTAAG-3

Table S4

Primer Sequences for all RNA-IP/qRT-PCR reactions are listed below.

RPL32 for	TTCCGGTCCACAACGTCAAG
RPL32 rev	TGTGAGCGATCTCGGCAC
p21 for	GGCGGCAGACCAGCATGACAGATT
p21 rev	GCAGGGGGCGGCCAGGGTAT
PUMA for	CCTGGAGGGTCCTGTACAATCT
PUMA rev	GCACCT-AATTGGGCTCCATCT
ATF3 for	CAGGGTGTTTGCAGCTGTGA
ATF3 rev	GACAGTAGCCAGCGTCCTTGT
GADD45 for	GATGTGGCTCTGCAGATCCA
GADD45 rev	ATGTCGTTCTCGCAGCAAAA
14-3-3σ for	GCCGAACGCTATGA-GGACAT
14-3-3σ rev	CTTCTCCACGGCGCCTT
Nup98 for	CCATCTATGGATGACCTTGCTAAA
Nup98 rev	TCCGACCAATAGTGAAAT-CAGAGA
Nup98/96 for	AAAATGGCAGGTTGGGTAACC
Nup98/96 rev	GGGTGGGAAATGCTTTGGA

Luc for	TACTGGGACGAAGACGAACAC
Luc rev	GTTCACCGGCGTCATCGTCG
18s for	AAACGGCT-ACCACATCCAAG
18s rev	CCTCCAATGGATCCTCGTTA3
p21 + 182 for	CGTGTTCGCGGGTGTGT
p21 + 182 rev	CATTCACCTGCCGCAGAAA
p21 + 4001 for	AGTCACTCAGCCCTGGAGTCAA
p21 + 4001 rev	GGAGAGTGAGTTTGCCCATGA
p21 + 5534 for	GGCGGGCTGCATCCA
p21 + 5534 rev	AGTGGTGTCTCGGTGACAAAGTC
p21 + 7011 for	CCTGGCTGACTTCTGCTGTCT
p21 + 7011 rev	CGGCGTTTGGAGTGGTAGA
p21 + 7379 for	CTCATCCCGTGTTCTCCTTTTC
p21 + 7379 rev	GGAGGAAGTAGCTGGCATGAA
p21 + 8281 for	AGCGGAACAAGGAGTCAGACAT
p21 + 8281 rev	TGGCATGCCCTGTCCATAG
p21 + 8566 for	CCTCCCACAATGCTGAATATACAG
p21 + 8566 rev	AGTCACTAAGAATCATTTATTGAGCACC
PUMA +160 for	GAAACGGAATGGAAAGCTATGAGA
PUMA +160 rev	GCAGACCCCATGCCAAATT
PUMA +4341 for	CCGCGCACGCCAGGAGGGCA
PUMA +4341 rev	GCGGGCCAGGCCCTCTACG
PUMA +10855 for	CCAGAGACAAGAGGAGCAGCA
PUMA +10855 rev	CGCTGCTGCTCCTCTTGTC
PUMA +11632 for	ATCCCATTGCATAGGTTTAGAGAGA
PUMA +11632 rev	GCGGCTTCAGCCAAAATCT
SFN +70 for	CCATGGAGAGAGCCAGTCTGA
SFN +70 rev	GGCTGCCATGTCCTCATAGC
SFN +710 for	CAAAGACAGCACCCTCATCATG
SFN +710 rev	TCGGCCGTCCACAGTGT
SFN +1232 for	GCCAGTGCAAGACCGAGATT
SFN +1232 rev	CTTTATTGAGAGGAAACATGGTCACA

Table S5
Antibodies used for Immunoblotting:

Antibody	**Company/Source**
Nup98 mouse monocl.	Santa-Cruz (USA)
p21 rabbit polycl.	Santa-Cruz (USA)
PUMA polycl	Calbiochem (USA)
Actin rabbit polycl.	Sigma (USA)
Exosc3 mouse monocl.	Santa-Cruz (USA)
GFP mouse monocl.	Santa-Cruz (USA)
HA mouse monocl.	Covance

p53 mouse moncl.	supernatants of hybridoma cultures

Supplemental Experimental Procedures

Fluorescence In Situ Hybridization (FISH)

Cells were fixed in 4% paraformaldehyde for 20 min at room temperature. After three washes in PBS the cells were stored in 70% (v/v) ethanol at 4 °C for 24 h, rehydrated, and inverted onto 40 µl of hybridization solution containing the 14 Cy5 labeled probes for the *p21* (*CDKN1a*) gene. Hybridization was done for 2 h at 37 °C and followed by several PBS washes. After staining the nuclei with DAPI, coverslips were mounted with ProLong Gold antifade reagent (Invitrogen). Cells stained for p21 mRNA by FISH and DNA using DAPI were imaged on an inverted Olympus IX81 microscope equipped with a 150x, 1.45 N.A. oil objective, an Andor iXon 897 EMCCD, an Andor Metal Halide Lamp, a Prior scanning stage with z-axis piezo inset and zero-shift fluorescence filter sets for Cy5 and DAPI from Semrock. Camera cooling was set to -80ºC using the internal fan of the camera, an EMGain of 1000 and light intensity was adjusted for 400-microsecond integration time per frame. The FISH signal was recorded first, followed by a second z stack for the DNA signal to minimize pre-bleaching of the FISH signal. Stacks were acquired using Nyquist sampling. The imaging protocol was automated using Andor iQ 2 software. Data processing was performed offline. All image stacks were visually inspected for staining artifacts and high background levels, inherent to liver cell lines. Image stacks with either separate cells or a small number of separable cells were cut to the minimal possible size to reduce computing time. Image stacks were then loaded into Diatrack (Semasopth) 3D analysis software and mRNA signals counted per stack. A constant threshold was applied for particle identification within Diatrack. This threshold was initially verified on a test sample of ten cells from the control (ctrl)

siRNA plus DMSO condition and ctrl siRNA plus Nutlin condition. Analysis of a total of 16 cells that were processed for FISH but not exposed to label gave a false positive count of 0.8 ± 1 mRNA signal per cell. For thresholding, a masked image stack with only nuclear signal was produced using the DNA stain. Nuclear mRNA counts were based on maximum projections of the masked image stacks. FISH images were filtered with a Laplacian of Gaussian (LoG) filter (Daniel Sage, ImageJ) using a width of 1.9. Contrast and brightness were adjusted for display.

RNA Immunoprecipitation

HepG2 and H24-p21 cells were treated with 0.1% formaldehyde for 8 min at room temperature, followed by the addition of 0.25 M glycine and incubation for 5 min. Cells were then lysed in RIPA buffer (50 mM Tris-HCl [pH 7.4], 1% NP-40, 0.5% Na deoxycholate, 0.1% SDS, 5 mM EDTA, and 150 mM NaCl). After sonication cell lysates were centrifuged for 10 min with 13000 RPM to spin out debris. Cell lysates were adjusted to equal protein concentrations (2 mg/mL) and pre-cleared with protein A/G beads for 1 h. For immunoprecipitation of Nup98 blocked protein A/G beads pre-incubated with anti-Nup98 serum (provided by Dr. G. Blobel) were added to the lysates and the mixture was incubated overnight at 4°C. After 3 washes with high salt RIPA buffer (containing 500 mM NaCl) crosslinking was reversed by adding 100 µl elution buffer (50 mM Tris-HCl [pH 6.5], 5 mM EDTA, 1% SDS, and 10 mM DTT) and heating for 45 min at 70°C. RNA was purified with the RNeasy MiniKit (Qiagen), and then DNAse treated and reverse-transcribed by using the Quantitect Reverse Transcription Kit (Qiagen). “No-RT” reactions in which RNA was subjected to the conditions of cDNA synthesis without reverse transcriptase enzyme served as a negative control to confirm the purity of RNA samples. PCR was performed on an ABI 7300 real-time PCR instrument with primer pairs listed in Supplementary Table 2.

Absolute quantification was done with standard curves using 0.1–9 ng of genomic DNA run along with the cDNA from the RNA-IP samples.

Animals and Immunohistochemical Staining

MDR2 -/- mice were housed under specific pathogen-free conditions. The procedures for performing animal experiments were in accordance with the principles and guidelines of the Arbeitsgemeinschaft der Tierschutzbeauftragten in Baden-Württemberg and were approved by the Regierungspräsidium of Karlsruhe, Germany. Staining intensity of non-tumorous and tumorous tissue was evaluated by a pathologist using a semiquantitative score: 0=no, 1=weak, 2=moderate, 3=strong, and 4= very strong staining. The median score out of up to 5 High Power Fields was calculated.