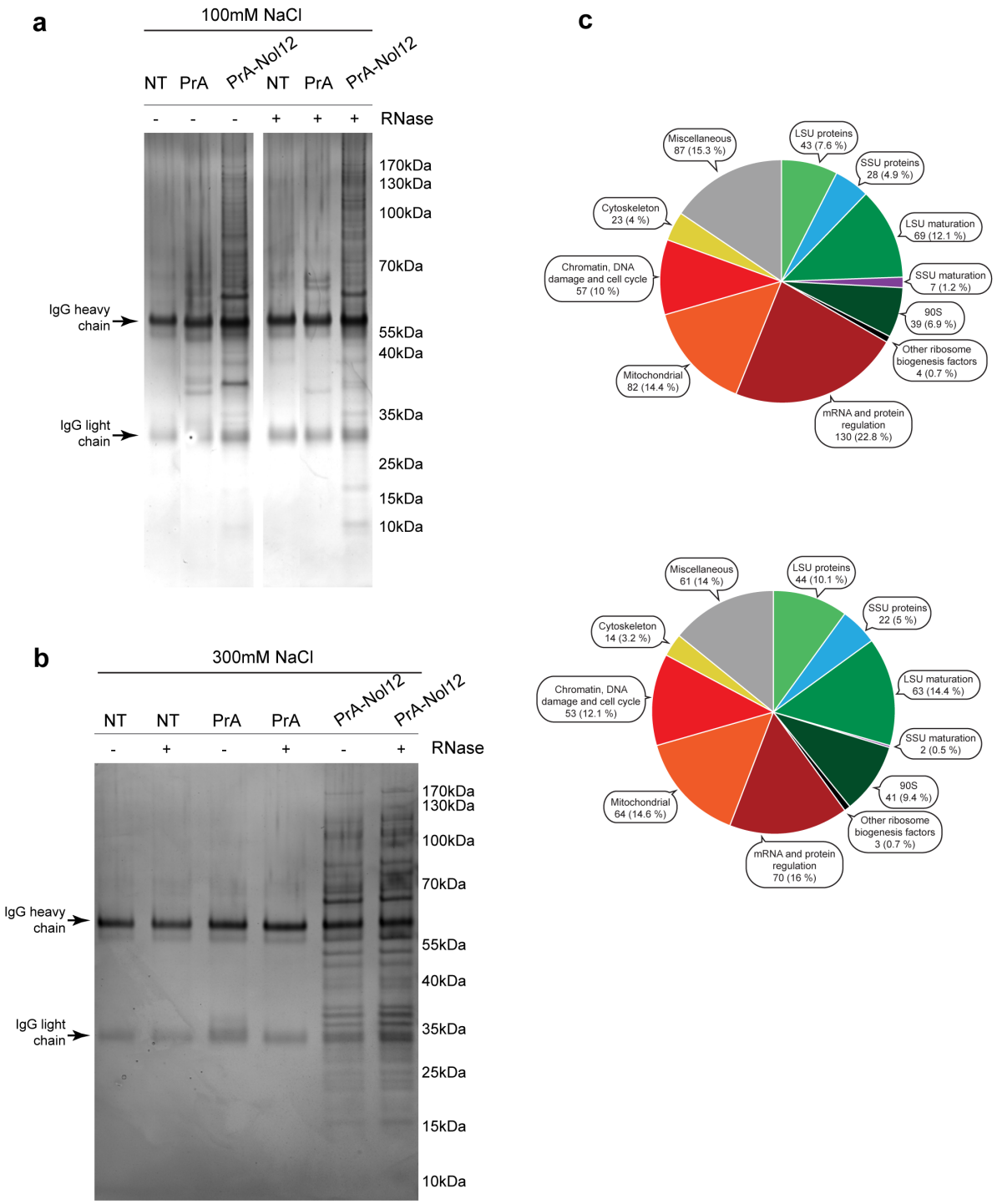
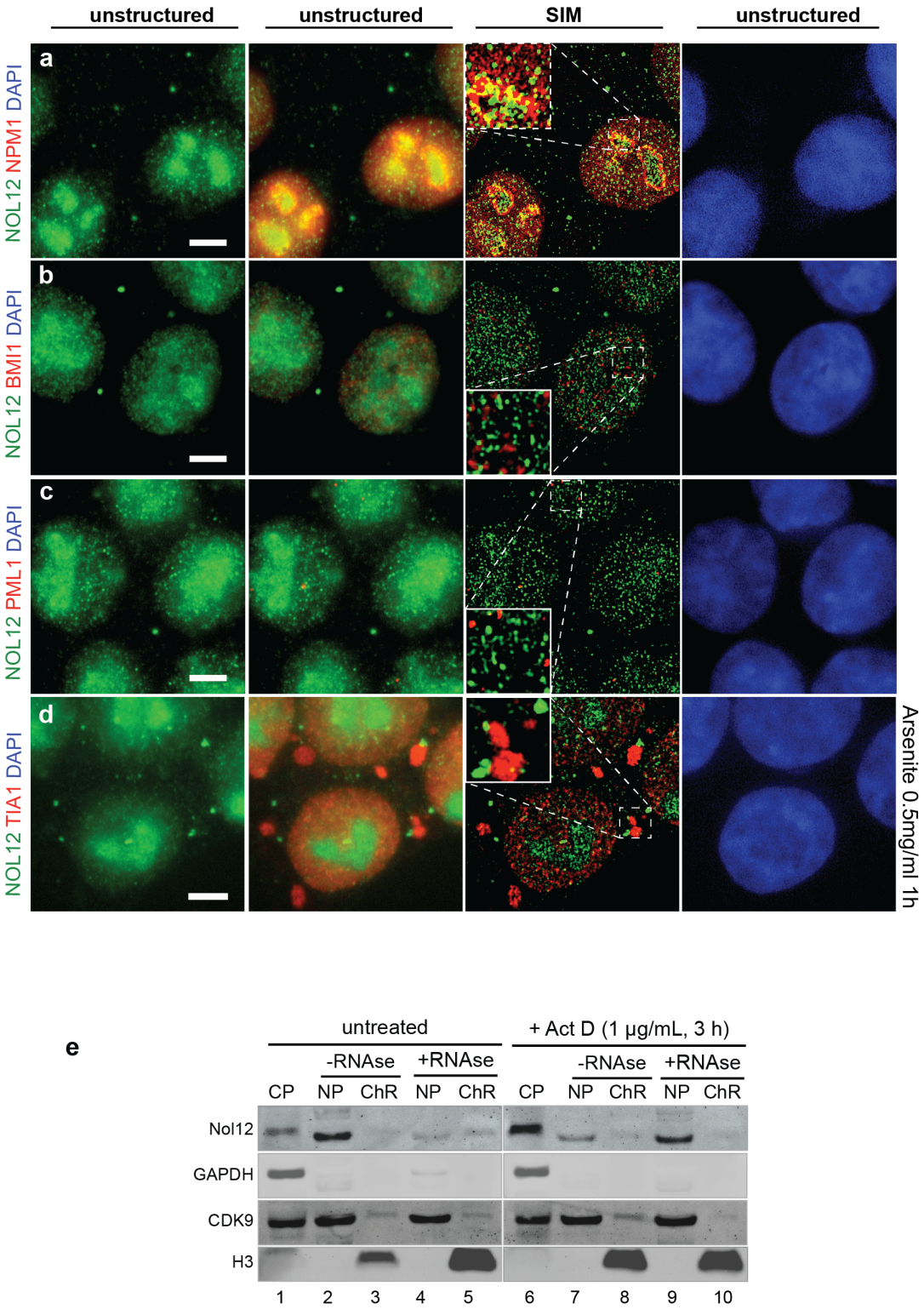
SUPPLEMENTAL FIGURES AND LEGENDS

Scott,Trahan et al., Figure S1



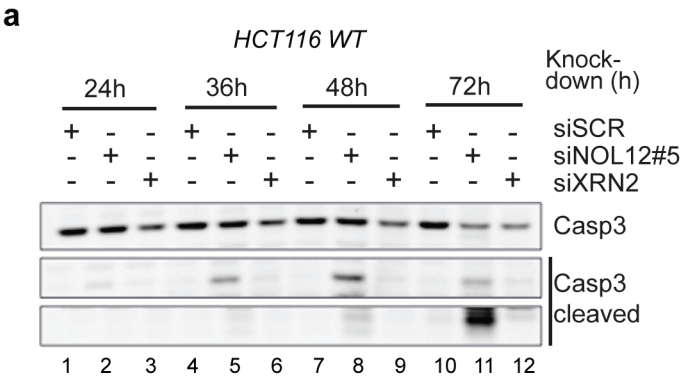
Supplementary Figure 1. Nol12 Interactome. (a, b) Silver stained 4-12% Bis-Tris PAGE gels of affinity-purified samples from non-tagged HEK 293 cells (NT), HEK 293 cells expressing Protein A alone (PrA), or HEK 293 cells expressing Protein A-Nol12 (PrA-Nol12) from lysates using either 100 mM NaCl (a) or 300 mM NaCl (b), with and without RNAseA treatment. (c) Pie chart representations of preys compiled

from Supplementary Table 3 that were co-purified by AP-MS with NOL12 using 100 mM NaCl and RNase A (top) and 300 mM NaCl and RNase A (bottom).

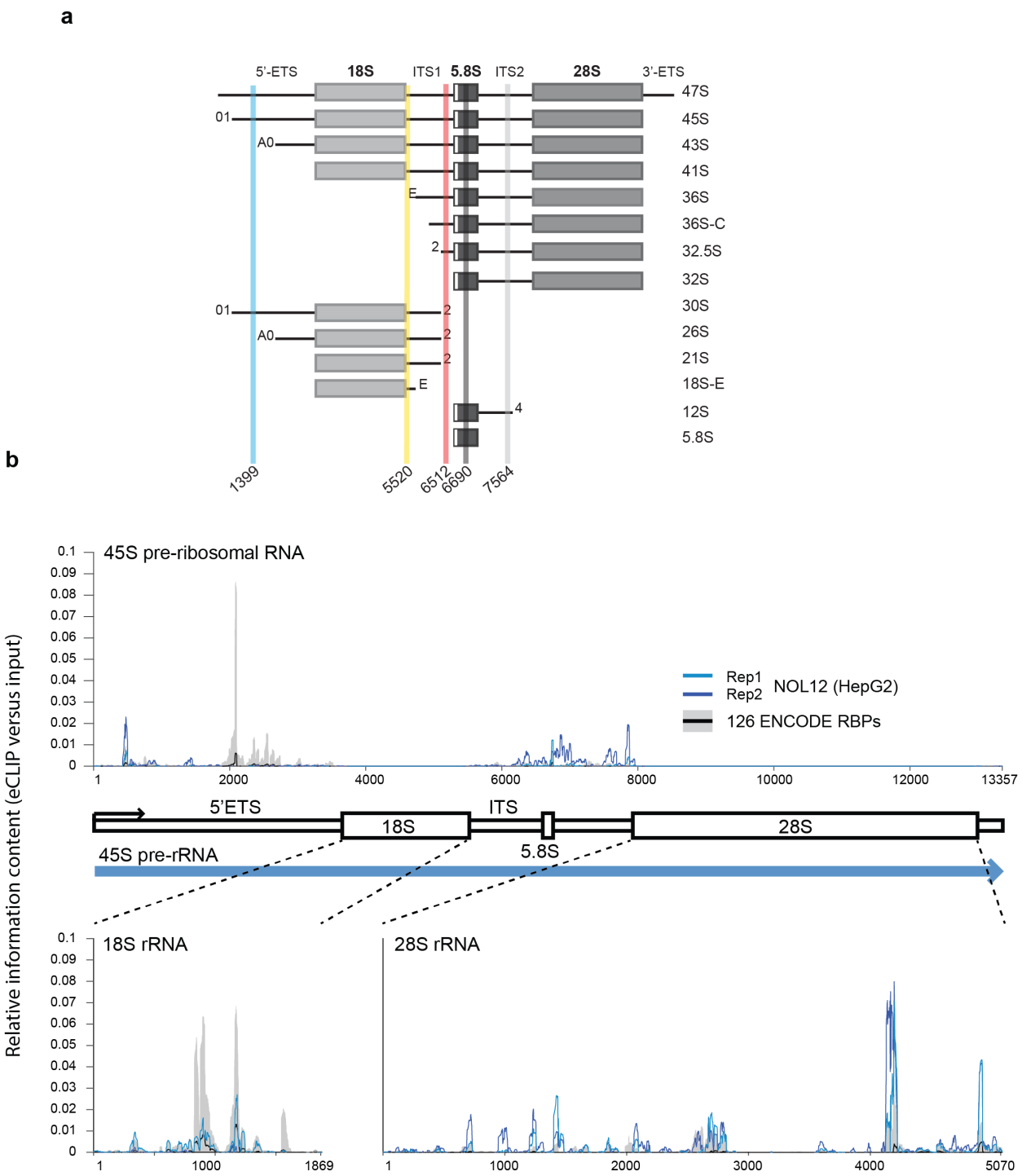


Supplementary Figure 2. Co-localization of Nol12 with subcellular structures. Localization of endogenous Nol12 in HCT116 WT cells. HCT116 cells were grown on poly-L-lysine-coated glass coverslips, fixed, permeabilized, and hybridized for immunofluorescence (IF) with antibodies against the

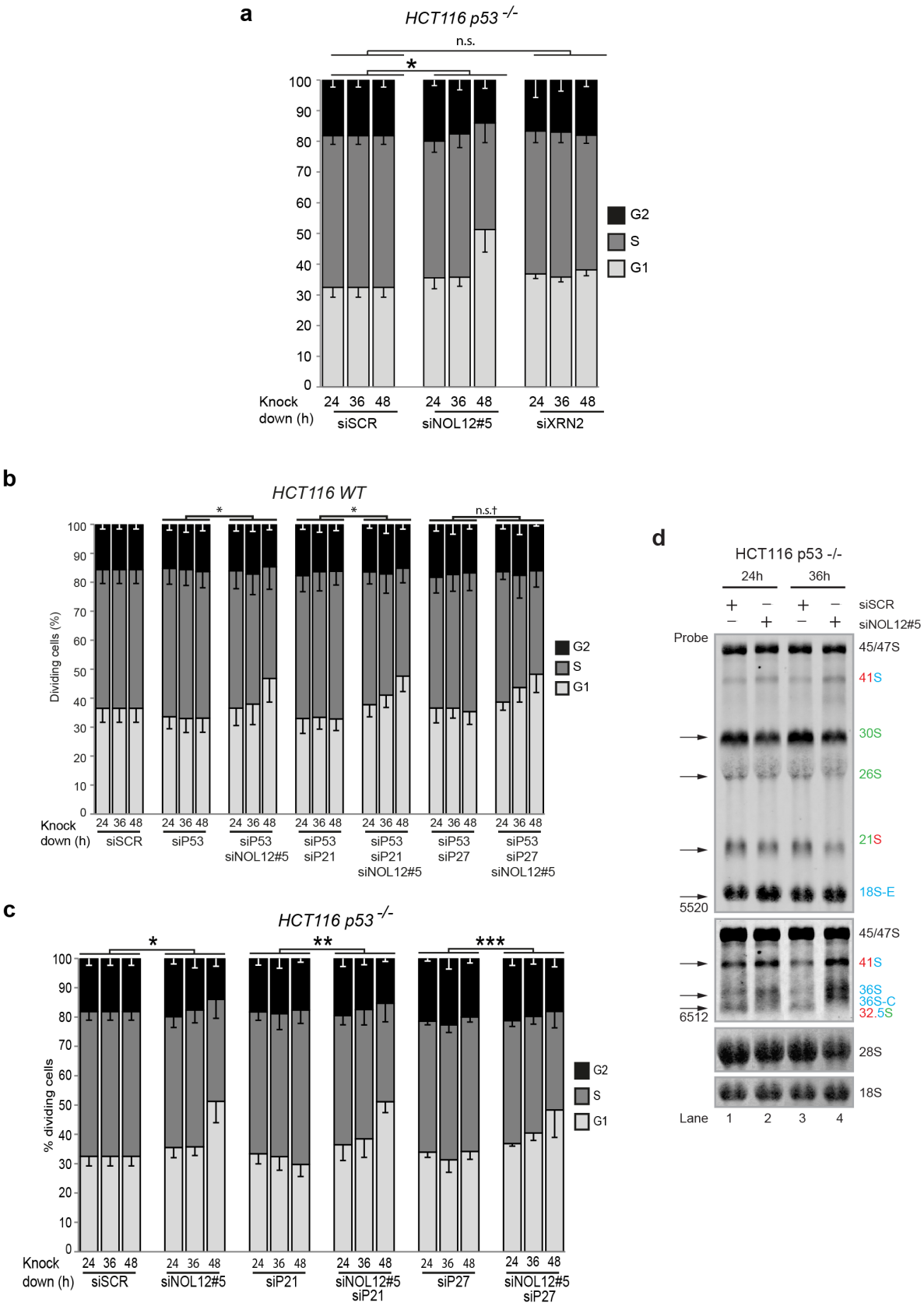
indicated proteins (Supplementary Table 7). Endogenous Nol12 localization was compared to that of the nucleolar protein NPM1 (a), the polycomb body marker BMI1 (b), the PML body marker PML (c), and the stress granule marker TIA1 in arsenite-treated (0.5mg/ml 1h) cells (d). DNA was visualized by DAPI staining. Cells were imaged using Super-Resolution-Structured Illumination Microscopy (SIM) and raw (unstructured) and SIM images were computed and processed using Zen SP1 Black Software and the Fiji package of ImageJ. Scale bar = 5µm. (e) 3 x 10^6 HCT116 cells treated or not with actinomycin D (1 µg/mL) were fractionated into cytoplasmic, nucleoplasmic and chromatin fractions; the latter were treated or not with RNAse A (100µg/mL). 20% of each fraction was resolved by SDS-PAGE and the indicated proteins were detected by western blotting to designate cytoplasmic (GAPDH), cytoplasmic/nucleoplasmic (CDK9) and chromatin (H3) fractions (see Supplementary Table 7 for antibody details), and determine presence of Nol12 in each fraction.



Supplementary Figure 3. NOL12 kd causes apoptosis. HCT116 WT cells were transfected with 10nM of silencing RNAs, siSCR (control), siNOL12#5 or siXRN2, for the times indicated. Whole cell extracts (WCEs) of siRNA-treated cells were resolved on 4-12% Bis-Tris gradient gels, transferred to PVDF membrane and blotted with the indicated primary antibodies. (a) To determine apoptosis in Nol12-depledet cells, caspase3 cleavage was determined by western blotting.



Supplementary Figure 4. Nol12 eCLIP mapping to 18S and 28S ribosomal RNA. (a) Scheme denoting the relative positions of probes used with respect to different rRNA processing intermediates. (b) Nol12 enrichment at rRNA. Lines indicate relative information in IP versus input at each position along (top) the 45S pre-ribosomal RNA or (bottom) the mature 18S and 28S rRNA transcripts. Blue lines indicate biological replicates of Nol12 in HepG2, whereas black line indicates the mean observed across 181 eCLIP experiments in K562 and HepG2 cell lines (profiling 126 total RBPs) generated by the ENCODE consortia (with standard deviation indicated in grey).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HCT116 p53 -/-
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18S-E
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36S-C
32.5S
6512
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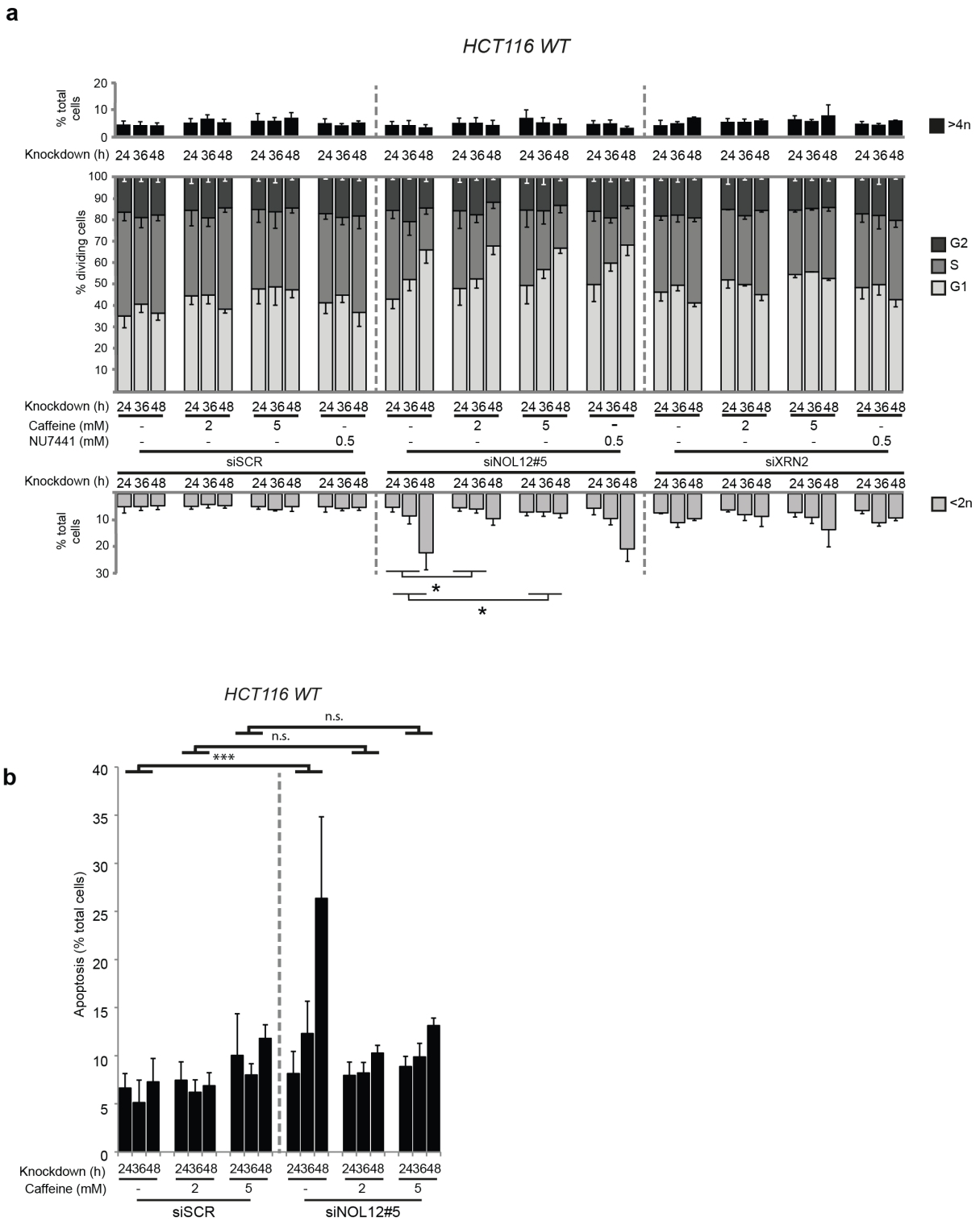
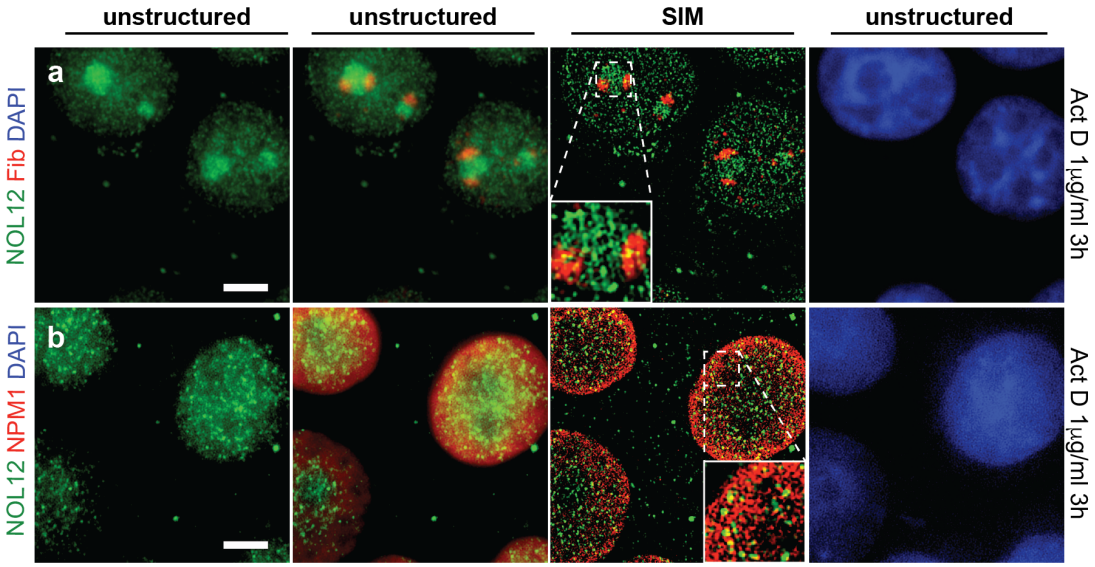
18S
Lane 1 2 3 4

Supplementary Figure 5. Cell cycle arrest and pre-rRNA processing delays upon NOL12-knockdown are p53 independent. (a) HCT116 *p53*$^{-/-}$ cells were transfected with siSCR, siXRN2, or siNOL12#5 (10nM) for the times indicated. Propidium Iodide (PI)-stained DNA content was quantified by FACS, and G_1 (2n), S (2n-4n) and G_2 (4n) populations determined. Data represent mean±s.d. for five independent experiments. * $p<0.05$ by Student's two-tailed *t*-test on area under the curve (AUC) of G_1 or AV^+ PI^- populations as appropriate. (b) *Nol12*, *p21*$^{WAF1/CIP1}$, *p27*^{KIP1} and *p53* were depleted alone or in concert in HCT 116 WT cells for 24, 36 and 48h and G_1, S and G_2 populations determined by Propidium Iodide (PI)-stained DNA content and quantified by FACS. (c) *Nol12*, *p21*$^{WAF1/CIP1}$ and *p27*^{KIP1} were depleted alone or in concert in HCT 116 *p53*$^{-/-}$ cells for 24, 36 and 48h and G_1, S and G_2 populations determined by Propidium Iodide (PI)-stained DNA content and quantified by FACS. Data represent mean±s.d. for 3-5 independent experiments. *, $p<0.05$ by Student's two-tailed *t*-test on G_1 AUCs; †, $0.05<p<0.1$ (borderline significance). (d) Total RNA extracted at the indicated time points post-siRNA transfection from HCT116 *p53*$^{-/-}$ cells was analyzed by Northern blotting using the indicated probes. RNA loading was monitored by methylene blue staining of mature 18S/28S rRNAs.



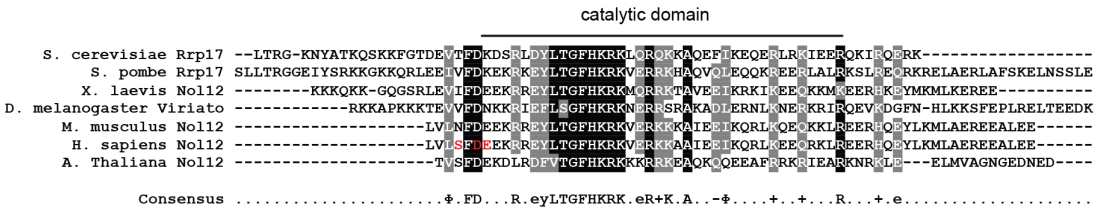
Supplementary Figure 6. NOL12-knockdown induced apoptosis is linked to DNA damage response pathways. (a) HCT116 WT cells transfected with siSCR or siNOL12 (10nM) and grown in the presence of caffeine (2 or 5mM) or NU7441 (0.5μM) for the indicated times prior to analysis as described

in Figure 4A except that <2n and >4n populations were also scored. Data represents mean±s.d. for >4 independent experiments. * $p<0.5$, ** $p<0.01$, *** $p<0.005$ by Student's two-tailed *t*-test on AUCs of AV^+ PI^- or <2n populations as appropriate. (b) HCT116 WT cells were transfected with siSCR or siNOL12 (10nM) as indicated and grown in the presence of either 0, 2 or 5mM caffeine for the indicated times prior to quantification of annexin V-FITC-positive, PI-negative (apoptotic) populations by FACS.

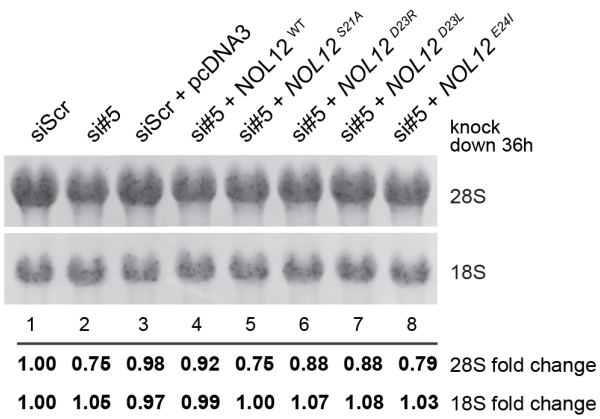


Supplementary Figure 7. Localization of Nol12 and nucleolar markers upon induction of DNA damage. HCT116 WT cells were grown on poly-L-lysine-coated glass coverslips, fixed, permeabilized, and hybridized for immunofluorescence (IF) with antibodies against the indicated proteins (Supplementary Table 7). Endogenous Nol12 localization was compared to that of the of the nucleolar proteins Fibrillarin (a) and NPM1 (b) in actinomycin D-treated (1μg/ml 3h) cells. DNA was visualized by DAPI staining. Images are representative of three independent experiments. Cells were imaged using Super-Resolution-Structured Illumination Microscopy (SIM) and raw (unstructured) and SIM images were computed and processed using Zen SP1 Black Software and the Fiji package of ImageJ. Scale bar = 5μm.

a



b



Supplementary Figure 8. Nol12 contains a highly conserved domain. (a) Alignment of Nol12 with fungal and higher eukaryotic homologs using a ClustalW alignment algorithm (1). Individual residues with more than 80% identity across the whole alignment are shown in a black box and as capital letters on the consensus line. The identified putative catalytic domain is indicated (2). Red letters indicate sites of mutated amino acids in Figure 8d and e. (b) Northern blotting of rRNA using total RNA extracted from HCT116 WT cells transfected for 36h with either siSCR or siNOL12 (10nM; lanes 1 and 2), or for 12h with siSCR or siNOL12 (10nM), followed by a 24h transfection of empty pcDNA3 in the case of siSCR (lane 3), or with Nol12 WT, Nol12-S21A, Nol12-D23R, NOL12-D23L, or NOL12-E24I in the case of siNOL12 (lanes 4-8). RNA loading was monitored by methylene blue staining of mature 18S and 28S rRNAs (large RNAs) and changes in levels of mature rRNAs (18S and 28S) were quantified and normalized against 47S pre-rRNA for each lane.

SUPPLEMENTARY TABLES.

Supplementary Tables 1-6 are included in a separate .xls file

Supplementary Table 1a-c. Raw MS data of PrA-Nol12 ssAP-MS analysis.

Supplementary Table 2. List of proteins enriched by PrA-Nol12 ssAP-MS. PrA-Nol12 ssAP-enriched proteins were defined as in Figure 1. Mean spectral counts of the two replicates of each condition were normalized against the average spectral count for the bait (PrA-Nol12) for that condition as a means of semi-quantitative analysis based on spectral counting (Oeffinger et al. 2007).

Supplementary Table 3. Functional grouping of proteins enriched with PrA-Nol12 ssAP-MS. Proteins defined and quantified in Table S1 were subjected to functional classification as described in Figure S3. Within each functional class, proteins are sorted according to their distribution across the homology/purification patterns as defined in Table S1. The bait protein is highlighted in red. Proteins with multiple functions, thus appearing in multiple functional class lists, are marked with purple text.

Supplementary Table 4: Nol12-interactome CORUM complex analysis.

Supplementary Table 5: DNA repair proteins identified in the Nol12-interactome based on gene ontology.

Supplementary Table 6 Nol12 eCLIP Repeat Elements.

Supplementary Table 7. List of antibodies used in this study. CST, Cell Signalling Technologies; TBS+T, Tris-buffered saline with 0.2% Tween-20; PBS+T, Phosphate-Buffered Saline with 0.2% Tween-20; WB, western blot; IF, immunofluorescence.

Target	Supplier	Code	Use	Dilution	Blocking Solution
β-actin	Abcam	AB8224	WB	1/2000	5 % milk in TBS+T
53BP1	Millipore	MAB3802	IF	1/500	1 % BSA in PBS+T
Bmi1	Abcam	AB38295	IF	1/100	1% BSA in PBS+T
BRCA1	Santa Cruz	SC-642	WB	1/200	5 % milk in TBS+T
c-Myc	CST	5605	WB	1/1000	5% BSA in TBS+T
Caspase 3	CST	9662	WB	1/3000	5 % milk in TBS+T
Cdk9	Santa Cruz	SC-484	WB	1/1000	5% milk in TBS+T
Chk1	Abcam	AB40866	WB	1/1000	5 % milk in TBS+T
Chk1-pS345	CST	2348	WB	1/1000	5% BSA in TBS+T
Chk2	CST	6334	WB	1/1000	5% BSA in TBS+T
Chk2-pS19	CST	2666	WB	1/1000	5% BSA in TBS+T
Chk2-pT68	CST	2197	WB	1/1000	5% BSA in TBS+T
Coilin	GeneTex	GTX11822	IF	1/200	1% BSA in PBS+T
Dcp1a	Santa Cruz	SC100706	IF	1/50	1% BSA in PBS+T
Ddx1	GeneTex	GTX105205	WB	1/1000	5% milk in TBS+T
Ddx21	MBLInternat.	RN090PW	WB	1/1000	5% milk in TBS+T
Dhx9	Abcam	AB54593	IF, WB	1/500 (IF), 1/1000 (WB)	1% BSA in PBS+T (IF) 5% milk in TBS+T (WB)
Dhx30	Bethyl	A302-218A	WB	1/2000	5% milk in TBS+T
Fibrillarin	Abcam	AB4566	IF	1/500	1 % BSA in PBS+T
GAPDH	Abcam	AB125427	WB	1/1000	5% milk in TBS+T
H2A.X	CST	7631	WB	1/1000	5 % milk in TBS+T
H2A.X-pS139	CST	9718	WB, IF	1/1000 (WB) 1/400 (IF)	5 % milk in TBS+T (WB) 1 % BSA in PBS+T (IF)
H3	Abcam	AB39655	WB	1/1000	5% milk in TBS+T
HA (rabbit)	CST	3724	IF	1/3200	1 % BSA in PBS+T
HA (mouse)	Genscript	A01244	WB, IF	1/2000 (WB) 1/500 (IF)	5 % milk in TBS+T (WB) 1 % BSA in PBS+T (IF)
Mdm2	Abcam	AB16895	WB	1/100	5 % milk in TBS+T

Mdm2-pS166	CST	3521	WB	1/1000	5% BSA in TBS+T
NOL12	Bethyl Laboratories	A160-100P	WB	1/2000	5 % milk in TBS+T
NOL12	Sigma	HPA003547	IF	1/700	1% BSA in PBS+T
p21	CST	2947	WB	1/1000	5 % milk in TBS+T
p27	CST	3686	WB	1/1000	5 % milk in TBS+T
p53	Santa Cruz	SC6243	WB	1/200	5 % milk in TBS+T
$p54^{nrb}$/NONO	Bethyl	A300-587A	WB	1/2000	5% milk in TBS+T
PML	Santa Cruz	SC966	IF	1/33	1% BSA in PBS+T
PSF/SFPQ	Santa Cruz	SC374502	IF	1/500	1% BSA in PBS+T
RPL5	Abcam	AB86863	WB	1/1000	5 % milk in TBS+T
RPL11	Abcam	AB79352	WB, IF	1/500 (WB), 1/250 (IF)	5 % milk in TBS+T (WB) 1 % BSA in PBS+T (IF)
SC35	GeneTex	GTX11826	IF	1/200	1% BSA in PBS+T
Tia1	Santa Cruz	SC1751	IF	1/100	1% BSA in PBS+T
Xrn2	Abcam	AB74799	WB	1/1000	5 % milk in TBS+T
2° anti-goat-800	LI-COR	926-32214	Dot Blot	1/15000	1 % casein in TBS+T
2° anti-mouse-AF-488	Life Technologies	A11001	IF	1/500	1 % BSA in PBS+T
2° anti-mouse DyLight® 680	Li-Cor	926-68022	WB	1/20000	Same as primary antibody
2° anti-rabbit-AF-555	Life Technologies	A21428	IF	1/500	1 % BSA in PBS+T
2° anti-rabbit-DyLight® 800	Li-Cor	926-32213	WB	1/5000	Same as primary antibody

Supplementary Table 8. siRNAs used in this study. For NOL12, XRN2, SCR and P53 siRNAs, individual strands were annealed at 37°C, 1 h prior to use.

Target	Sequence (sense)	Sequence (antisense)	Supplier
NOL12 (#5)	5'-GGUCGAGCGAAAGAAGGCAC-dTdT-3'	5'-GUGCCUUCUUUCGCUCGACC-dTdT-3'	Sigma
NOL12-2 (#8)	5'- GACGGAGUCGGUGCAGUAU-dTdT-3'	5'- AUACUGCACCGACUCCGUC-dTdT-3'	Sigma
XRN2	5'- AAGAGUACAGAUGAUCAUGUU-dTdT-3'	5'-AACAUCAUCAUCUGUACUCUU-dTdT-3'	Sigma
SCR	5'- UAAGGUUAAGUCGCCCUCG-dTdT-3'	5'- CGAGGGCGACUUAACCUUA-dTdT-3'	Sigma
p21	SignalSilence® p21 Waf1/Cip1 siRNA I (Cell Signalling Technologies, Cat. No. #6456)		
p27	SignalSilence® p27 Kip1 siRNA II (Cell Signalling Technologies, Cat. No. #12410)		
p53	5'-GCAUCUUAUCCGAGUGGAA-dTdT-3'	5'-UUCCACUCGGAUAAGAUGC-dTdT-3'	Sigma

Supplementary Table 9. Northern Probes used in this study. Probes were labeled as described in the Materials and Methods prior to use. Positions are defined relative to the 5' end of the 35S primary transcript.

Probe	Position	Sequence
ETS1 between 01-A0 sites	1399	$^{+}H_4N$-5'-cgctagagaaggcttttctc-3'-NH_4^{+}
5' of ITS1	5520	$^{+}H_4N$-5'-cctcgccctccgggctccgttaatgatc-3'-NH_4^{+}
ITS1 3' of site 2	6512	$^{+}H_4N$-5'-cgcccaagaggagaggggttgcctcag-3'--NH_4^{+}
3' of ITS1	6603	$^{+}H_4N$-5'-cgtacgaggtcgatttggcgag-3'-NH_4^{+}

5.8S	6690	$^{+}H_4N$-5'-caatgtgtcctgcaattcac-3'-NH_4^{+}
ITS2 5' of site 4	7564	$^{+}H_4N$-5'-gcgcgacggcggacgacaccgcggcgtc-3'-NH_4^{+}
7SL	N/A	$^{+}H_4N$-5'-gctccgtttccgacctgggcc-3'-NH_4^{+}

Supplementary Table 10. Oligonucleotides used for cDNA cloning and mutagenesis in this study.

NdeI-Nol12-F	5'-CCCCATATGGGCCGCAACAAGAAGAAGAA-3'
XhoI-Nol12-R	5'-GGGCTCGAGCGGGCCCTGAAACAGCACTTCCAGGCCGCTGCTCTCCCCGC TGTGCCGTGC-3'
siRNA5-mut	5'- CTGACAGGCTTCCACAAGCGGAAAGTTGAAAGGAAAAAAGCTGCCATTGAG GAGATTAAGCAGCGG-3'
siRNA3-8-mut	5'-GAGAGAGAAGAGGCTCTGGAGGAGGCTGACGAACTCGATAGACTGGTGAC AGCAAAAACTGAAAGTGTCCAATACGACCACCCCAACCACACAGTCACC-3'
siRNA6-mut	5'-GGAGGAGGCGTCATCCACGGAAAAGCCTACAAAGGCTCTGCCCAGGAAGTC CAGAGACCC-3'
Link+HA	5'-CAAAGCACGGCACAGCGGGGAGGGAGGAGGAGGAAGCGGAGGAGGAGGA AGCTATCCTTATGATCTCCCTGATTATGCACACCACCACTGAGATCCGGCTGC-3'
PmeI-Nol12-F	5'-CCCGTTTAAACCCACCATGGGCCGCAACAAGAAGAAGAAGC-3'
PmeI-2HA-Nol12-R	5'-CCCGTTTAAACTCAAGCGTAGTCTGGCACGTCGTATGGGTAGCTGCCGCCA GCGTAGTCTGGCACGTCGTATGGGTAGCTGCCGCCTGCATAATCAGGGAGAT CATAAGGATAGCTTC-3'
Nol12-S21A-F	5'-CGTTCTTGCCTTCGACGAGGAGAAGAGGCG-3'
Nol12-S21A-R	5'-CGTCGAAGGCAAGAACGAGCCTCGGCCG-3'
Nol12-D23R-F	5'-CTTAGCTTCCGCGAGGAGAAGAGGCGGGAG-3'
Nol12-D23R-R	5'-CTCCTCGCGGAAGCTAAGAACGAGCCTCGGC-3'
Nol12-D23L-F	5'-CTTAGCTTCCTGGAGGAGAAGAGGCGGGAGTACC-3'
Nol12-D23L-R	5'-CTCCTCCAGGAAGCTAAGAACGAGCCTCGGC-3'
Nol12-E24I-F	5'-GCTTCGACATCGAGAAGAGGCGGGAGTACCTGA-3'
Nol12-E24I-R	5'-CTTCTCGATGTCGAAGCTAAGAACGAGCCTCG-3'
NcoI-Nol12-F	5'-GGAATCAGGACCATGGGAACCGGCCGCAACAAGAAGAAGAAGCGAGATGG TGA-3'
NotI-Nol12-R	5'-GGAATCAGGAGCGGCCGCCTCCCCGCTGTGCCGTGCTTTGCCTGTGAGAC GGCGGCGCT-3'
HindIII-PrA-F	5'-GCTAGCAAGCTTGAATTCATGGTTGGTACTTTCTATCG-3'
HindIII-PrA-R	5'-CCTGATAAGCTTGAATTCAGGATCGTCTTTAAGGCTTT-3'

Supplementary Table 11. Plasmids used and generated in this study.

pRS414-3xHA-NOL12	(2)
pET21a-NOL12-6xHis	This study
pWPI-NOL12	This study
pWPI-NOL12-siRESIST-WT	This study
pWPI-NOL12-siRESIST-S21A	This study
pWPI-NOL12-siRESIST-D23R	This study
pWPI-NOL12-siRESIST-D23L	This study
pWPI-NOL12-siRESIST-E24I	This study
pcDNA3-siRESIST-WT	This study
pcDNA3-NOL12-S21A	This study
pcDNA3-NOL12-D23R	This study
pcDNA3-NOL12-D23L	This study
pcDNA3-NOL12-E24I	This study

pENTR4	Invitrogen
pFRT-TO-DEST-GFP	Kindly received from M. Lanthaler
pFRT-TO-DEST-PrA	This study
pBXA-PrA-HIS5	(3, 6)
pENTR4-NOL12	This study
pFRT-TO-PrA-NOL12	This study
pOG44	Addgene

SUPPLEMENTAL EXPERIMENTAL METHODS

DNA constructs

NOL12 cDNA was amplified by PCR from pRS414-3xHA-Nol12 (42) with oligonucleotides NdeI-Nol12-F and XhoI-Nol12-R. The PCR product was cloned into NdeI-XhoI sites of pET21a to generate plasmid pET21a-Nol12-6xHis, which was used as template to create a siRNA-resistant cDNA of Nol12 by introducing silent mutations by site-directed mutagenesis using the QuickChange Multi Site Mutagenesis Kit (Agilent Technologies) and oligos siRNA5-mut, siRNA3-8-mut, siRNA6-mut and Link+HA. The siRNA resistant Nol12 cDNA was then amplified with oligos PmeI-Nol12-F and PmeI-2HA-Nol12-R, and inserted into the PmeI site of pWPI to generate pWPI-Nol12-siRESIST-WT. Mutants S21A, D23R, D23L and E24I were generated with respective oligonucleotide pairs using pWPI-Nol12-siRESIST-WT as template according to the QuickChange II XL Site-Directed Mutagenesis Kit instructions (Agilent Technologies). Finally, siRNA resistant Nol12-WT and mutants were subcloned from the PmeI site of pWPI and inserted into the EcoRV site in pcDNA3 to generate pcDNA3-siRESIST-WT, pcDNA3-Nol12-S21A, pcDNA3-Nol12-D23R, pcDNA3-Nol12-D23L, and pcDNA3-Nol12-E24I.

In order to construct a PrA-NOL12 encoding plasmid for Flip-in recombination Flp-In T-REx system (see cell culture section), the Nol12 cDNA was amplified by PCR from the same template as above with oligonucleotides NcoI-Nol12-F and NotI-Nol12-R. The amplicon was cloned into NcoI-NotI sites of pENTR4 to generate pENTR4-Nol12. Protein A was amplified by PCR from pBXA (53) with oligonucleotides HindIII-PrA-F and HindIII-PrA-R and swapped with GFP into HindIII site of pFRT-TO-DEST-GFP to generate pFRT-TO-DEST-PrA. pENTR4-Nol12 and pFRT-TO-DEST-PrA were recombined with LR clonase to generate pFRT-TO-PrA-Nol12 according to the Gateway system instructions (Life Technologies). All DNA constructs in this study were sequenced. Oligos used for cloning and mutagenesis are shown in Supplementary Table 10. Plasmids are listed in Supplementary Table 11.

Cell Culture and Drug Treatment

HCT116 WT, HCT116 $p53^{-/-}$ and HEK293T cells were grown in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10 % Fetal Calf Serum (FCS) and Penicillin/Streptomycin (1000 IU, 1 mg/mL respectively; Wisent) at 37 °C with 5 % CO_2.

To create the PrA-NOL12 Flp-in cell line, pFRT-TO-PrA-NOL12 and pOG44 expressing the Flp recombinase were co-transfected in HEK293T Flp-In™ cells containing a single FRT recombination site according to the Flp-In™ T-REx™ core kit manufacturer's instructions (Life Technologies). Successful transformants were identified by a combination of hygromycin resistance and zeocin sensitivity and doxycycline-inducible expression of PrA- or PrA-Nol12 was verified by western blotting. PrA and PrA-Nol12 expression was induced in the relevant cell lines with 1µg/mL doxycycline for 24 h and monitored by western blotting using anti-PrA antibodies.

Synthetic siRNAs against NOL12 (#5 and #8), XRN2, p21, p27 and p53 and a scrambled (SCR) control were supplied by Sigma-Aldrich. siRNAs were transfected at a concentration of 10nM using Lipofectamine RNAiMAX (Life Technologies) in a reverse transfection protocol according to the manufacturer's instructions. Between $7.5x10^4$ and $5.4x10^5$ cells/mL were transfected, depending on the duration of knockdown. Successful silencing of siRNA targets was monitored by western blotting (Figure 3a). All siRNA sequences are listed in Supplementary Table 8. Caffeine (2 or 5mM), VE822 (1µM), KU55933 (1µM), ChiR-124 (100nM), NSC109555 (5µM) or NU7441 (0.5µM) were added to the above growth media for the duration of the time course; these supplemented media were changed every 24 h throughout the time course to maintain an effective dose. Actinomycin D (0.05 or 1µg/mL) was applied to

cells for 3 hours, arsenite (0.5mM), hydroxyl urea (1mM; HU) for 1 hour, and etoposide (25 or 50 μM) for 3 hours or 1 hour, immediately prior to downstream processing.

Western Blotting

siRNA-transfected cells were harvested at the indicated time points by trypsin-treatment, washed with PBS and lysed by sonication (5 min 30" on/off, 4°C) in RIPA buffer (50mM Tris, 150mM NaCl, 0.1% SDS, 0.5 % sodium deoxycholate, 1 % Nonidet P-40, pH 7.5) supplemented with cOmplete protease inhibitor, EDTA-free (Roche). Soluble fractions were resolved on a 4-12% gradient Bis-Tris NuPAGE® gel according to the manufacturer's instructions, transferred to a PVDF membrane which was blocked with 5 % milk or BSA in TBS+T (50mM Tris, 150mM NaCl, 0.05% Tween-20, pH 7.6) and probed with the indicated primary and secondary antibodies according to Supplementary Table 7. Secondary antibody-conjugated fluorescence was detected using the Li-Cor Odyssey Infrared scanner. Representative images of biological replicates are shown.

Sucrose gradient

HCT 116 cells transfected with either scramble or Nol12#5 siRNAs were harvested 36h post-transfection. Cells were lysed in 250 μl of 50mM Tris pH 7.5, 100 mM NaCl, 5 mM $MgCl_2$, 1 mM DTT and 100 μl of acid washed glass beads by vortexing the samples 3 times 2 min with 1 minute intervals on ice in the cold room. Cell lysates were cleared using the hot needle method and centrifuged at 600 x g for 30 seconds. 6 O.D. at 260 nm were loaded onto 10-45% (w/v) gradient and spun at 22.3 kRPM for 16h at 4 °C in a SW 41 Ti rotor. Sucrose gradients were fractionated in 500 μl fractions using a piston gradient fractionator (BioComp), and whole fractions were precipitated by TCA. Briefly, each fraction was supplemented with 5 μl of 2 % deoxycholate, vortexed, and left at room temperature for 30 min. One tenth volume of 100 % TCA was then added to each fraction, vortexed, and left overnight at 4 °C. Next day, the samples were centrifuged at 16k x g for 45 min at 4 °C, the supernatants were removed, and the samples were given a quick spin in order to remove the remaining traces of supernatants. Pellets were directly resuspended in 2X Leammli buffer, the pH was adjusted by adding 1 μl of a 1 M Tris pH 8.8 solution, and the samples were entirely loaded on a 4-12% NuPAGE Bis-Tris gels. The gels were transferred to PVDF membranes, and probed with both RPL5/uI18 and RPL11/uL5 antibodies (Supplementary Table 7). To assess the efficiency of Nol12 knockdown, the equivalent of 1/30th of the sucrose gradient inputs were also analyzed by western blotting using Nol12 antibody.

ssAP-MS/MS and western blotting analysis of Nol12-containing complexes

For ssAP purification of Nol12-containing complexes, HEK293T Flp-In T-REx PrA-Nol12 cells were grown to ~50% confluency, induced for 24h with 1μg/mL doxycycline and were lysed by cryogrinding (3). Purifications form PrA-Nol12cells and negative controls (PrA-containing or untagged) purifications were performed in duplicate with five volumes of extraction buffer (20mM HEPES-KOH pH 7.4, 0.5% Triton, 1x mini complete EDTA-free protease inhibitor cocktail, 1:5000 antifoam A) supplemented with 100 mM NaCl (+/- 100μg/mL RNAse A) or 30 mM NaCl (+100 μg/mL RNAse A) as previously described (3). Briefly, 0.2 g of cryo-lysed cell powder was slightly thawed (1 minute on ice) and vortex mixed in extraction buffer. The samples were then sonicated (20W, 2 seconds, on ice). RNAse-treated samples were incubated for 10 min at room temperature. All samples were then centrifuged at 16000 x g for 10 min at 4 °C. The supernatant was then incubated for 30 min at 4°C on a nutator, with 7.5mg of rabbit IgG conjugated Dynabeads M270 epoxy (160μg IgG/mg beads) pre-washed with extraction buffer.

Complexes bound to the beads were quickly washed ten times 1 ml with extraction buffer, and once with 100mM NH_4OAc, 0.1mM $MgCl_2$, 0.5 % tritonX-100 for 5 min on a nutator. To eliminate detergents, beads were washed four times with the same buffer without detergent (three quick washes, and one 5 min wash). One tenth of each affinity purification sample was eluted with 0.5M NH_4OH and resolved on 4-12% gradient Bis-Tris NuPAGE® gels for silver staining. The remaining samples were on-bead digested with 750ng of trypsin in 50μl of 20mM Tris-HCl pH 8.0 at 37 °C overnight in a thermomixer set at 900 RPM (4). Next day, the supernatants were recovered, spiked with an additional 250ng of trypsin and incubated for four additional hours as described above. Samples were adjusted to 3 % formic acid, frozen at -80 °C until analyzed by LS-MS as described previously (5).

For ssAP-MS/MS analysis, MS data generated by Q-Exactive were analyzed using the ProHits software package (6). Raw files were first converted to mzML format using ProteoWizard (v3.0.9322) and the AB SCIEX MS Data Converter (v1.3 beta), and then searched using Mascot (version) and Comet (v2014.02 rev. 2) search engines. The searches were performed against the RefSeq database release 57 including a decoy set. Two missed cleavages were allowed in the search parameters for +2 to +4precursor ions with a 10ppm error tolerance, and a 0.6 Da error tolerance on fragmented ions. The output from each search engine were analyzed through the Trans-Proteomic Pipeline (7) (v4.7 POLAR VORTEX rev 1) by means of the iProphet pipeline using a 5 % FDR (8).

Nol12-interacting proteins were identified as follows. Preys having an average of less than 2 unique peptide count from the Nol12 pullout conditions were first filtered out (avgTSC). The highest total spectrum counts from all negative controls (hbgTSC from untagged and PrA) were then subtracted from the average total spectrum of each prey (avgTSC – hbgTSC) under their respective conditions. The resulting average prey total spectrum count containing less than two total spectrum counts were finally filtered out (Tables S1a, S1b and S1c). Normalization of the data was done as follows. To take into account the variable sizes of all proteins identified in normalizing the preys on the bait, all proteins from Tables S1a-c were *in silico* digested with trypsin using MS-digest (http://prospector.ucsf.edu) using zero missed cleavages and no variable modifications to determine how many theoretical peptides each protein would generate upon trypsin digestion (*in silico* pept). Average total spectrum counts of each protein were then divided by its theoretical number of peptides (avgTSC/*in silico* pept) before normalizing each preys values on PrA-Nol12 values (Supplementary Table 2; (prey avg TSC/*in silico* pept)/ (Nol12 avg TSC/*in silico* pept)).

For western blotting of Nol12-interacting proteins, 0.2g doxycycline-induced HEK293T FlpIn TREx PrA and HEK293T FlpIn TREx PrA-Nol12 cryogrindates were resuspended in extraction buffers containing 100 µg/mL RNAse A and either 100mM or 300mM NaCl. These lysates were analysed by ssAP as described above except that: a) prior to addition of Dynabeads, 1/100 volume of each clarified lysate was collected denatured with 6x Laemmli buffer and as the "input" sample; and b) instead of being trypsin digested on beads, PrA-Nol12-associated proteins were eluted twice with 0.5M NH_4OAc, 0.5mM EDTA for 20 min with rocking, vacuum concentrated and resuspended in 20µL 1x Laemmli buffer ("eluate" samples). 50µg of the "input" samples, and 0.25 volumes of each "eluate" sample, were resolved on a 4-12% gradient Bis-Tris NuPAGE® gels and analyzed by western blotting as described elsewhere.

Northern blotting

Total RNA from scramble and NOL12 siRNA transfected cells was extracted with TRIzol (Invitrogen) according to the manufacturer's protocol. For long RNA analysis, total RNA were separated on 1% agarose-formaldehyde gels in Tricine-Triethanolamine and transferred on nylon membrane in 10x SSC by capillarity as described by Mansour and Pestov (9). Small RNAs were separated on 8% acrylamide-urea gels and transferred on nylon membrane in 0.5x TBE overnight at 12 V. For long rRNA analysis, 3µg of total RNA extracted from HCT116 WT and HCT116 $p53^{-/-}$ cells were used. A total of 8µg of RNA was used for small RNA analysis. All probes used for hybridization are shown in Supplementary Table 9. Probes identified with terminal amines were fluorescently labeled with DyLight™ 800 NHS Ester as previously described (10), while other probes were radiolabeled with [γP^{32}]ATP (3000 Ci/mmol; Perkin-Elmer).

eCLIP analysis

NOL12 eCLIP data (accession ENCSR820DQJ), including raw reads as well as standard genomic mapping of reads, was obtained from the ENCODE consortia (*https://www.encodeproject.org/*). Binding to ribosomal rRNA was quantified using a family-aware repeat element mapping pipeline (Van Nostrand, E.L., *et al. in preparation*). To summarize relative enrichment between IP and input, relative information was defined as the Kullback-Leibler divergence (relative entropy): $p_i \times log_2 (\frac{p_i}{q_i})$, where p_i is the fraction of total reads in IP that map to a queried element *i* (peak, gene, or repetitive element), and q_i is the fraction of total reads in input for the same element.

Cell cycle profiling and quantification of apoptosis

To profile cell cycle distribution, siRNA- and/or drug-treated cells were harvested by trypsinization at the indicated time points, washed twice with PBS and fixed with 70% ethanol at -20°C for >1 h. Fixed cells were rehydrated 2x in PBS then permeabilized, RNAse-treated and stained for DNA content with a modified Krishan Buffer (20µg/mL propidium iodide, 0.1 % Triton X-100, 0.2mg/mL RNAse A in PBS) for 30 min at RT. Apoptosis was measured using the Annexin V-FITC apoptosis detection kit (Sigma-Aldrich) according to the manufacturer's instructions. Briefly, siRNA- and/or drug-treated cells were harvested at the indicated time points and resuspended at $5x10^5$ cells/ml. Cells were treated with Annexin V-FITC (250ng/ml) and propidium iodide (500ng/ml). In both cases, staining was quantified by flow cytometry and results were analyzed using the FlowJo software.

Cellular Fractionation

HCT116 cells (~$3x10^6$ per condition) treated or not with Actinomycin D (1µg/mL, 3 h) were collected by scraping followed by centrifugation at 500x*g* for 5 min. To collect the cytoplasmic and nucleoplasmic fractions, the Nuclear Extract Kit from Active Motif® was used according to the manufacturer's instructions, except that the nuclear fractions were split in two and either treated or not with 100µg/mL RNAse A for 30 min at 4 °C in Complete Lysis Buffer (Active Motif). To prepare the chromatin fraction, pelleted untreated/RNAse A-treated chromatin yielded by the Nuclear Extract Kit above was washed once with 250µL Buffer B (3mM EDTA, 0.2mM EGTA, 1mM DTT, 1x cOmplete Protease Inhibitor Cocktail (Roche)) then resuspended in 250µL Buffer B and disrupted using a Branson 102C probe sonicator (30% amplitude, 3 cycles of 10" on followed by 30" on ice) prior to addition of SDS-PAGE loading buffer. 0.2 volumes of the total volume of each fraction was run on a 10% SDS-PAGE gel and protein content analyzed by western blotting.

Hypersensitivity and Recovery assays

$9x10^5$ HCT116 WT cells were transfected with 10nM siSCR or siNOL12#5 using Lipofectamine RNAiMAX (ThermoFisher) according to the manufacturer's instructions for reverse transfection. Transfected cells were cultured in 6-well dishes for 24h, then media was replaced with fresh DMEM + 10% FBS containing the indicated concentration(s) of H_2O_2 for 30 min. After treatment with either 0.1 mM H_2O_2 or 50 µM etoposide for 30 min, cells were washed with PBS and allowed to recover in regular DMEM + 10% FBS for the indicated times at 37°C. Cells were harvested and lysed in RIPA-PIC buffer prior to running on 10% or 12% SDS-PAGE gels and analysis by western blotting as described elsewhere.

Nol12 phenotype rescue

$2x10^5$ HCT116 WT cells were reverse-transfected with 10nM siSCR or siNOL12#5 using Lipofectamine RNAiMAX (ThermoFisher) according to the manufacturer's instructions. After 12h, cells were forward-transfected with 1µg of the plasmids expressing either wild-type of mutant Nol12 using Lipofectamine LTX (ThermoFisher) according to the manufacturer's instructions. 24 h after plasmid transfection, cells were harvested and lysed in RIPA-PIC buffa prior to running on a 10% SDS-PAGE gel and analysis by western blotting as described elsewhere.

Super-Resolution Structured Illumination Microscopy (SR-SIM)

HCT116 WT cells were grown on poly-L-lysine-coated glass coverslips, fixed, permeabilized, and incubated for immunofluorescence (IF) with antibodies against the indicated proteins (Supplementary Table 7). SR-SIM was performed on a Zeiss Axio Observer.Z1 coupled to a Zeiss Elyra PS.1 using a Plan-APOCHROMAT 63x/1.4 Oil DIC M27 objective lens (NA = 1.4). Three angles of the excitation grid, each with five phases, were acquired for each channel and z-stack slice using an Andor iXon3^{EM}+ 885 EMCCD camera. SIM images were processed using the Zen SP1 Black 2011 software and analyzed using the Fiji package of ImageJ.

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