SUPPLEMENTAL FIGURES AND TABLES

Scott,Trahan et al., Figure S1

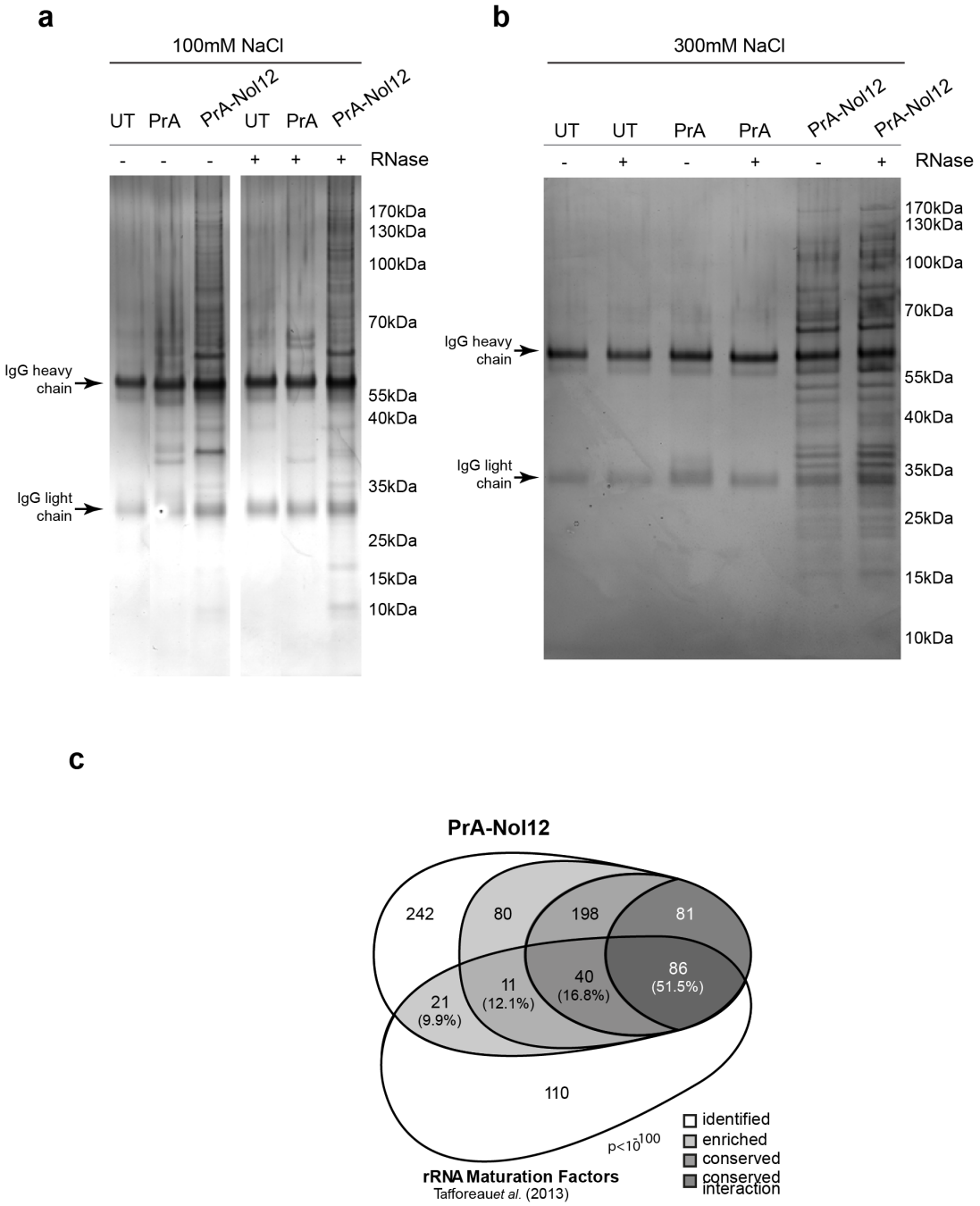
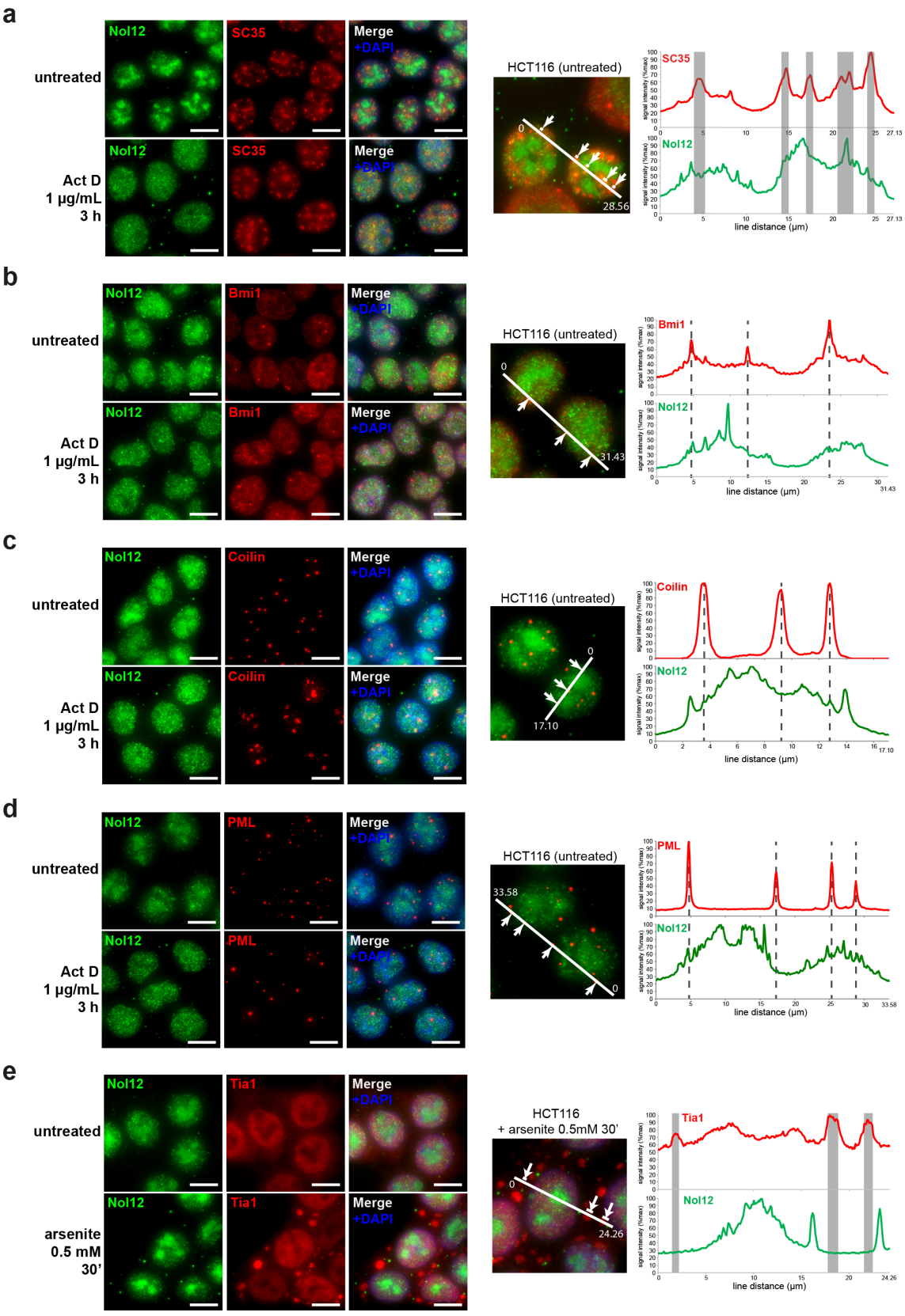


Figure S1. Nol12 interactome overlaps partially with pre-ribosome complexes. (a) Analysis of ssAP samples by gel electrophoresis, comparing UT (untagged) PrA-, or PrA-Nol12 expressing HEK293 cells in different salt condition +/- RNase. (b) Overlap of PrA-Nol12 purification classes from (Figure 1 a) were compared to the ribosome-regulatory list of Tafforeau *et al*. (Tafforeau et al., 2013) and statistical significance calculated by hypergeometric testing.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untreated
Act D
1 µg/mL
3 h
Nol12
SC35
Merge
+DAPI
HCT116 (untreated)
0
28.56
SC35
Nol12
signal intensity (%max)
line distance (µm)
b
untreated
Act D
1 µg/mL
3 h
Nol12
Bmi1
Merge
+DAPI
HCT116 (untreated)
0
31.43
Bmi1
Nol12
signal intensity (%max)
line distance (µm)
c
untreated
Act D
1 µg/mL
3 h
Nol12
Coilin
Merge
+DAPI
HCT116 (untreated)
0
17.10
Coilin
Nol12
signal intensity (%max)
line distance (µm)
d
untreated
Act D
1 µg/mL
3 h
Nol12
PML
Merge
+DAPI
HCT116 (untreated)
33.58
0
PML
Nol12
signal intensity (%max)
line distance (µm)
e
untreated
arsenite
0.5 mM
30'
Nol12
Tia1
Merge
+DAPI
HCT116
+ arsenite 0.5mM 30'
0
24.26
Tia1
Nol12
signal intensity (%max)
line distance (µm)

Figure S2. Co-localization of Nol12 with subcellular structures. HCT116 WT cells were grown on poly-L-lysine-coated coverslips for 48h, with or without treatment with either (a-d) actinomycin D (1ug/ml, 3h), or (e) sodium arsenite (0.5 mM, 30') prior to fixation and immunofluorescence analysis. Nol12 localization was compared to that of markers for various cellular granules including splicing speckles (a), polycomb bodies (b), Cajal bodies (c), PML bodies (d), and stress granules (e). Linear profiles of fluorescence density were created from representative images of each co-immunofluorescence experiment and normalized against the maximum observed fluorescence along the line segment. Dotted lines indicate the location of local fluorescence maxima in the marker channel, and colocalization was analysed using the Fiji software Coloc2 module.

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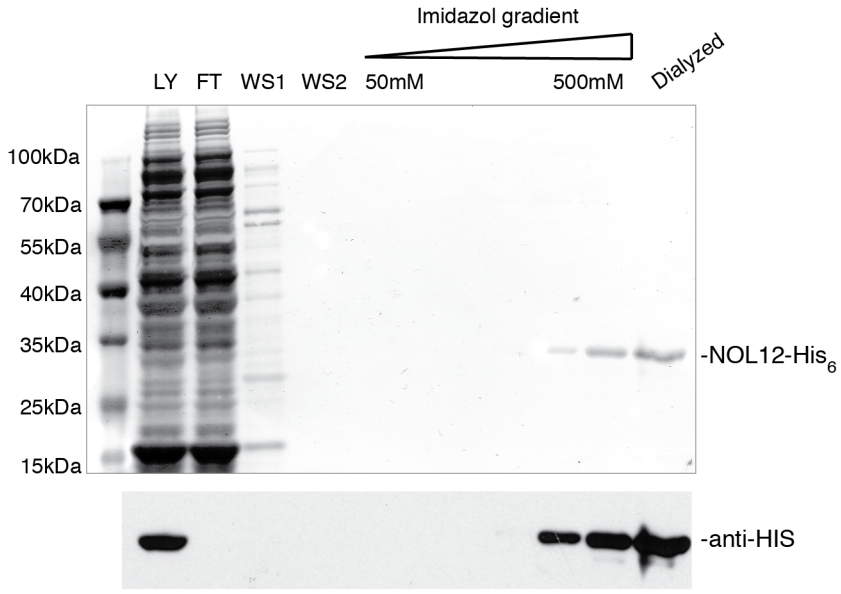
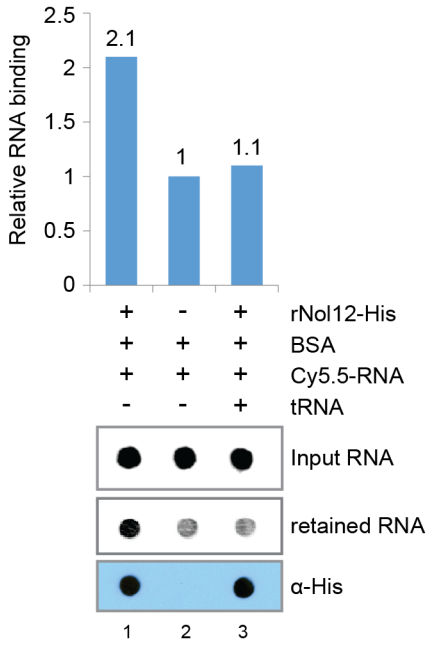
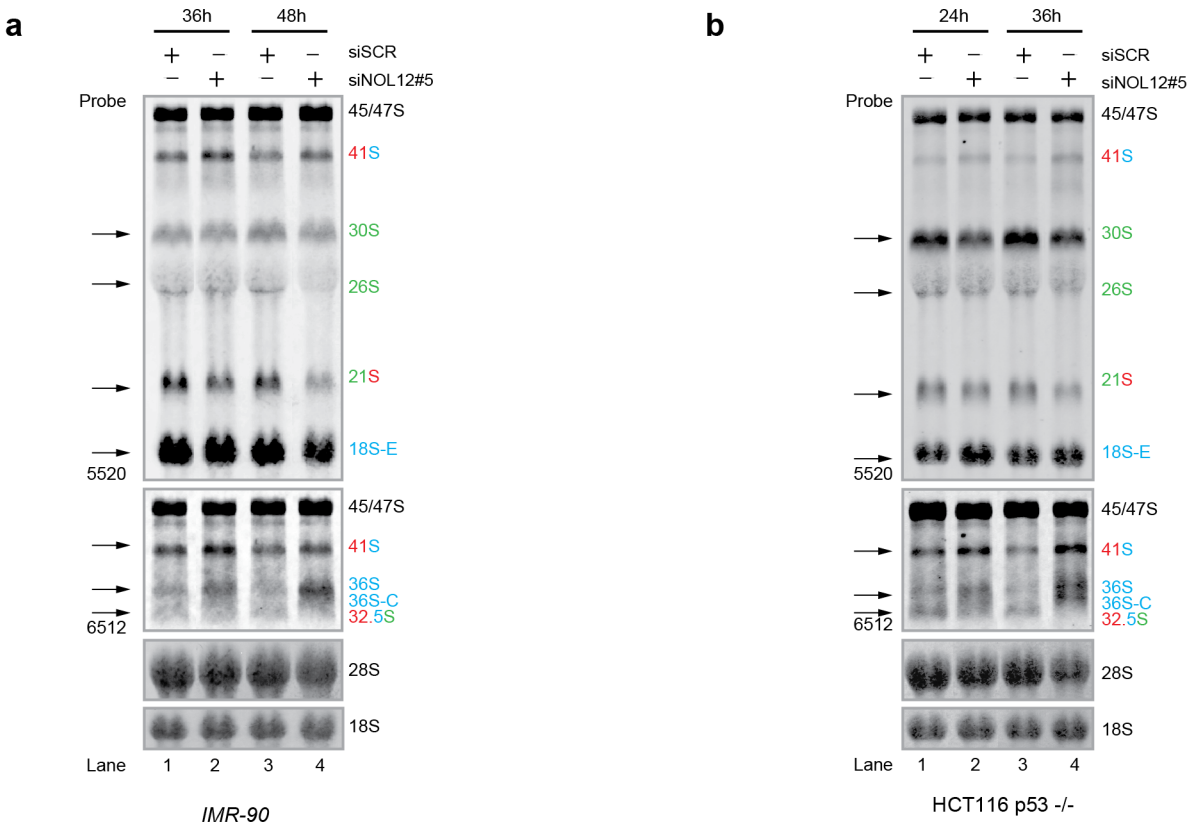
a



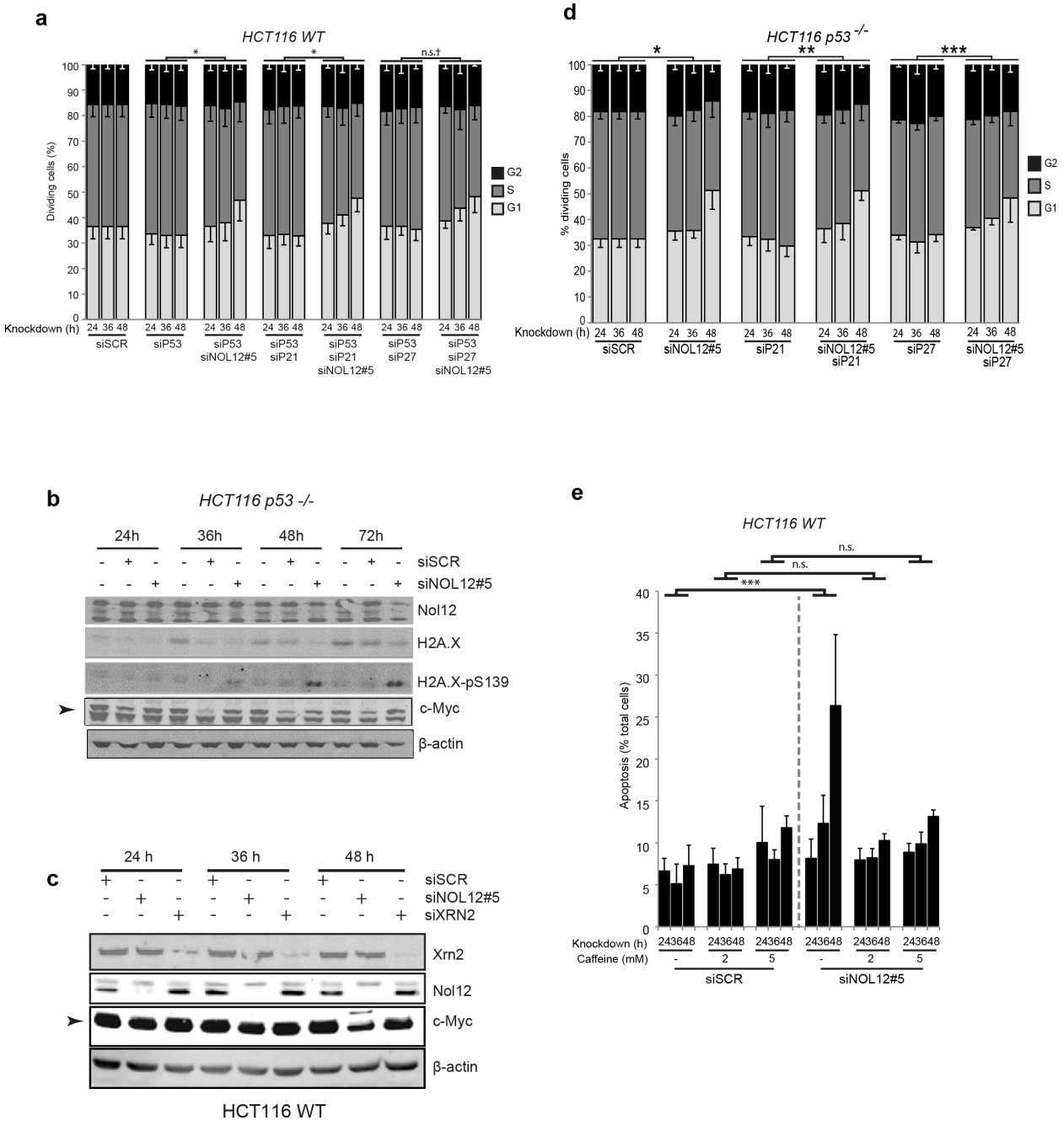
b



Supplementary Figure 3. Nol12 is a non-specific RNA binding protein. (a) PAGE Analysis of rNOL12. (b)Nol12 binds to ssRNA *in vitro*. RNA binding was determined by resolving the complexes by retention on a nylon membrane. RNA signals were resolved on a Li-Cor Odyssey Infrared scanner. Retained RNAs were normalized on the signal observed for the BSA only control.



Supplementary Figure 4. Knockdown of Nol12 causes rRNA processing defects in primary cells and in the absence of p53. Total RNA extracted at the indicated time points post-siRNA transfection from (a) IMR-90 or (b) HCT116 *p53*$^{-/-}$ cells was analyzed by Northern blotting using the indicated probes (refer to Figure 4c for a schematization of precursors detected by each probe). RNA loading was monitored by methylene blue staining of mature 18S/28S rRNAs.



Supplementary Figure 5. Nol12 depletion causes upregulation of DDR pathway proteins in a p53-, p21-, and p27-independent manner and signals via ATM/ATR. (a) *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. (b) HCT116 *p53*$^{-/-}$ cells were either mock transfected, or siSCR-/siNOL12#5-transfected and harvested at the indicated times post-transfection for western blotting of whole cell lysates. Beta-actin was used as a loading control. c) HCT116 WT cells were transfected with siSCR, siNOL12#5 or siXRN2 and harvested at the indicated times post-transfection for western blotting of whole cell lysates. Beta-actin was used as a loading control.

(d) *Nol12*, $p21^{WAF1/CIP1}$ and $p27^{KIP1}$ were depleted alone or in concert in HCT 116 $p53^{-/-}$ cells for 24, 36 and 48hs 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). (e) HCT116 WT cells were transfected with siSCR or siNOL12#5 (10μM) 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 TABLES.

Supplementary Tables 1-5 are included as separate .xls file

Supplementary Table 1. List of proteins identified in Untagged and PrA-expressing HE293 control cell lines by ssAP-MS.

Supplementary Table 2. List of proteins enriched by PrA-Nol12 ssAP-MS analysis. 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 analysis. Proteins defined and quantified in Table S2 were subjected to functional classification as described in Figure 1. Within each functional class, proteins are sorted according to their distribution across the homology/purification patterns as defined in Table S2. 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. Proteins identified, but not enriched, with PrA-Nol12 ssAP. List of proteins for which one or more enriched peptides were identified in the PrA-Nol12 ssAP analysis, but which did not meet the criteria of protein "enrichment" (≥3 peptides enriched in both replicates of one or more condition). Human-yeast homology pairs were defined as for Table S2. Functional classes were defined as described in Figure 1.

Supplementary Table 5: Proteins identified with recombinant Nol12.

Supplementary Table 6. List of antibodies used in this study. CST, Cell Signalling Technologies; TBS+T, Tris-buffered saline with Tween-20; PBS+T, Phosphate-Buffered Saline with 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
8-OHdG	Millipore	AB5830	Dot Blot	1/1000	1 % casein in TBS+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
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
Dhx9	Abcam	AB54593	IF	1/500	1% BSA in PBS+T
Fibrillarin	Abcam	AB4566	IF	1/500	1 % BSA in PBS+T
H2A.X	CST	7631	WB	1/1000	5 % milk in TBS+T

H2A.X-pS139	CST	9718	WB, IF	1/400 (IF), 1/1000 (WB)	5 % milk in TBS+T (WB) 1 % BSA in PBS+T (IF)
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		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
PML	Santa Cruz	SC966	IF	1/33	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
SfpQ	Santa Cruz	SC374502	IF	1/500	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 7. 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 8. 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'-cctcgccctccgggctccgttaatgatc-3'-NH_4^{+}
5' of ITS1	5520	$^{+}H_4N$-5'-cgcccaagaggagaggggttgcctcag-3'-NH_4^{+}
ITS1 3' of site 2	6512	$^{+}H_4N$-5'-gcgcgacggcggacgacaccgcggcgtc-3'-NH_4^{+}
3' of ITS1	6603	$^{+}H_4N$-5'-cgtacgaggtcgatttggcgag-3'-NH_4^{+}
5.8S	6690	$^{+}H_4N$-5'-ctgcgagggaacccccagccgcgca-3'-NH_4^{+}
ITS2 5' of site 4	7564	$^{+}H_4N$-5'-caatgtgtcctgcaattcac-3'-NH_4^{+}
7SL	N/A	$^{+}H_4N$-5'-gctccgtttccgacctgggcc-3'-NH_4^{+}