SUPPLEMENTARY DATA

Supplementary Figure 1

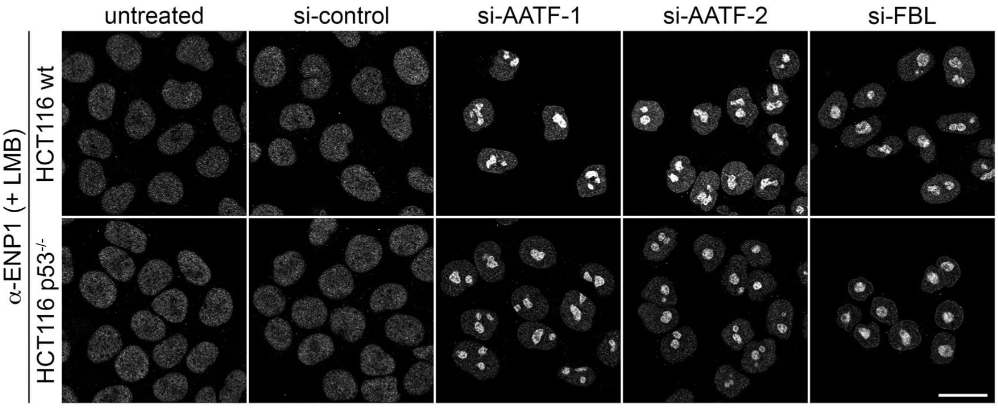
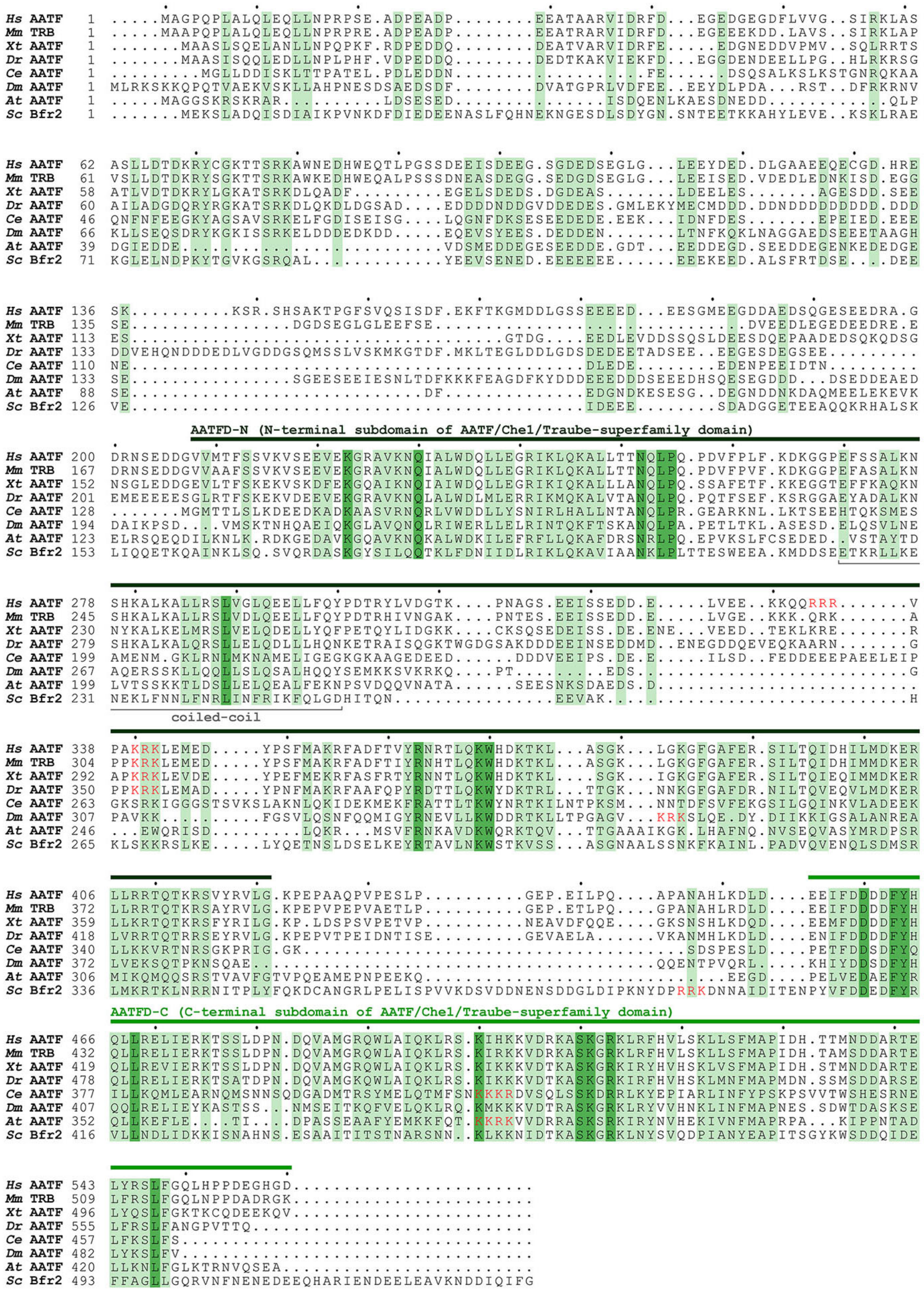


Figure S1: The function of AATF in ribosome synthesis is independent of p53. AATF and FBL RNAi was performed in HCT116 wt and $p53^{-/-}$ cell lines for 72 h. Localization of ENP1 was visualized by IF after LMB treatment (2 h, 20 nM). Scale bar, 20 μm.

Supplementary Figure 2



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Hs AATF    1  .....MAGPQPLALQLEQLLNPRPSE.ADPEADP........EEATAARVIDRFD...EGEDGEGDFLVVG..SIRKLAS
Mm TRB      1  .....MAAPQPLALQLEQLLNPRPRE.ADPEADP........EEATRARVIDRFD...EGEEEKDD.LAVS..SIRKLAP
Xt AATF    1  .......MAASLSQELANLLNPQPKF.RDPEDDQ........DEATVARVIDRFE.....EDGNEDDVPMV..SQLRRTS
Dr AATF    1  .......MAASISQQLEDLLNPLPHF.VDPEDDQ........DEDTKAKVIEKFD...EGGDENDEELLPG..HLRKRSG
Ce AATF    1  .........MGLLDDISKLTTPATEL.PDLEDDN......................FE......DSQSALKSLKSTGNRQKAA
Dm AATF    1  MLRKSKKQPQTVAEKVSKLLAHPNESDSAEDSDF........DVATGPRLVDFEE..EEYDLPDA...RST..DFRKRNV
At AATF    1  .....MAGGSKRSKRAR..........LDSESED...............ISDQENLKAESDNEDD............QLP
Sc Bfr2    1  .......MEKSLADQISDIAIKPVNKDFDIEDEENASLFQHNEKNGESDLSDYGN.SNTEETKKAHYLEVE..KSKLRAE

Hs AATF   62  ASLLDTDKRYCGKTTSRKAWNEDHWEQTLPGSSDEEISDEEG.SGDEDSEGLG...LEEYDED.DLGAAEEQECGD.HRE
Mm TRB     61  VSLLDTDKRYSGKTTSRKAWKEDHWEQALPSSSDNEASDEGG.SEDGDSEGLG...LEEISED.VDEDLEDNKISD.EGG
Xt AATF   58  ATLVDTDKRYLGKATSRKDLQADF.........EGELSDEDS.DGDEAS.......LDEELES.......AGESDD.SEE
Dr AATF   60  AILADGDQRYRGKATSRKDLQKDLDGSAD....EDDDDNDDGVDDEDES.GMLEKYMECMDDD.DDNDDDDDDDDD.DDD
Ce AATF   46  QNFNFEEGKYAGSAVSRKELFGDISEISG....LQGNFDKSESEEDEDE.EEK...IDNFDES.......EPEIED.EEE
Dm AATF   66  KLLSEQSDRYKGKISSRKELDDDEDKDD.....EQEVSYEES.DEDDEN.......LTNFKQKLNAGGAEDSEEETAAGH
At AATF   39  DGIEDDE.........................VDSMEDDEGESEEDDE.GDT...EEDDEGD.SEEDDEGENKEDEDGE
Sc Bfr2   71  KGLELNDPKYTGVKGSRQAL.............YEEVSENED.EEEEEEE......EEEKEED.ALSFRTDSE....DEE

Hs AATF  136  SK..........KSR.SHSAKTPGFSVQSISDF.EKFTKGMDDLGSSEEEED...EESGMEEGDDAEDSQGESEEDRA.G
Mm TRB    135  SE................DGDSEGLGLEEFSE..............................DVEEDLEGEDEEDRE.E
Xt AATF  113  ES..................................GTDG....EEDLEVDDSSQSLDEESDQEPAADEDSQKQDSG
Dr AATF  133  DDVEHQNDDDEDLVGDDGSQMSSLVSKMKGTDF.MKLTEGLDDLGDSDEDEETADSEE..EEGESDEGSEE.........
Ce AATF  110  NE......................................DLEDE...........EDENPEEIDTN.........
Dm AATF  133  SE................SGEESEEIESNLTDFKKKFEAGDFKYDDDEEEDDDSEEEDHSQESEGDDD...DSEDDEAED
At AATF   88  SE..............................DF..............EDGNDKESESG..DEGNDDNKDAQMEELEKEVK
Sc Bfr2  126  VE.........................................IDEEE........SDADGGETEEAQQKRHALSK

                    AATFD-N (N-terminal subdomain of AATF/Che1/Traube-superfamily domain)
Hs AATF  200  DRNSEDDGVVMTFSSVKVSEEVEKGRAVKNQIALWDQLLEGRIKLQKALLTTNQLPQ.PDVFPLF.KDKGGPEFSSALKN
Mm TRB    167  DRNSEDDGVVAAFSSVKVSEEVEKGRAVKNQIALWDQLLEGRIKLQKALLTTNQLPQ.PDVFPVF.KDKGGPEFASALKN
Xt AATF  152  NSGLEDDGEVLTFSKEKVSKDFEKGQAIKNQIAIWDQLLEGRIKIQKALLLANQLPQ.SSAFETF.KKEGGTEFFKAQKN
Dr AATF  201  EMEEEEESGLRTFSKEKVDEEVEKGRAVKNQLALWDLMLERRIKMQKALVTANQLPQ.PQTFSEF.KSRGGAEYADALKN
Ce AATF  128  .......MGMTTLSLKDEEDKADKAASVRNQRLVWDDLLYSNIRLHALLNTANQLPR.GEARKNL.LKTSEEHTQKSMES
Dm AATF  194  DAIKPSD....VMSKTNHQAEIQKGLAVQNQLRIWERLLELRINTQKFTSKANQLPA.PETLTKL.ASESD.ELQSVLNE
At AATF  123  ELRSQEQDILKNLK.RDKGEDAVKGQAVKNQKALWDKILEFRFLLQKAFDRSNRLPQ.EPVKSLFCSEDED..VSTAYTD
Sc Bfr2  153  LIQQETKQAINKLSQ.SVQRDASKGYSILQQTKLFDNIIDLRIKLQKAVIAANKLPLTTESWEEA.KMDDSEETKRLLKE

Hs AATF  278  SHKALKALLRSLVGLQEELLFQYPDTRYLVDGTK....PNAGS.EEISSEDD.E.....LVEE..KKQQRRR.......V
Mm TRB    245  SHKALKALLRSLVDLQEELLFQYPDTRHIVNGAK....PNTES.EEISSEDD.E.....LVGE..KKK.QRK.......A
Xt AATF  230  NYKALKELMRSLVELQDELLYQFPETQYLIDGKK....CKSQSEDEISS.DE.ENE...VEED..TEKLKRE.......R
Dr AATF  279  SHKALKALQRSLLELQDLLLHQNKETRAISQGKTWGDGSAKDDDEEINSEDDMD..ENEGDDQEVEQKAARN.......G
Ce AATF  199  AMENM.GKLRNLMKNAMELIGEGKGKAAGEDEED......DDDVEEIPS.DE.E.....ILSD..FEDDEEEPAEELEIP
Dm AATF  267  AQERSSKLLQQLLSLQSALHQQYSEMKKSVKRKQ....PT..........EDS.........................G
At AATF  199  LVTSSKKTLDSLLELQEALFEKNPSVDQQVNATA......SEESNKSDAEDS.D.........................
Sc Bfr2  231  NEKLFNNLFNRLINFRIKFQLGDHITQN.................EEVAK.............................H
               coiled-coil

Hs AATF  338  PAKRKLEMED.....YPSFMAKRFADFTVYRNRTLQKWHDKTKL...ASGK...LGKGFGAFER.SILTQIDHILMDKER
Mm TRB    304  PPKRKLEMED.....YPSFMAKRFADFTIYRNHTLQKWHDKTKL...ASGK...LGKGFGAFER.SILTQIDHIMMDKER
Xt AATF  292  APKRKLEVDE.....YPEFMEKRFASFRTYRNNTLQKWHDKTKL....SGK...IGKGFGAFER.SILTQIEQIMMDKER
Dr AATF  350  PPKRKLEMAD.....YPNFMAKRFAAFQPYRDTTLQKWYDKTRL...TTGK...NNKGFGAFDR.NILTQVEQVLMDKER
Ce AATF  263  GKSRKIGGGSTSVKSLAKNLQKIDEKMEKFRATTLTKWYNRTKILNTPKSM...NNTDFSVFEKGSILGQINKVLADEEK
Dm AATF  307  PAVKK...........FGSVLQSNFQQMIGYRNEVLLKWDDRTKLLTPGAGV...KRKSLQE.DY.DIIKKIGSALANREA
At AATF  246  ...EWQRISD.........LQKR...MSVFRNKAVDKWQRKTQV...TTGAAAIKGK.LHAFNQ.NVSEQVASYMRDPSR
Sc Bfr2  265  KLSKKRSLKE.....LYQETNSLDSELKEYRTAVLNKWSTKVSS...ASGNAALSSNKFKAINL.PADVQVENQLSDMSR

Hs AATF  406  LLRRTQTKRSVYRVLG.KPEPAAQPVPESLP..........GEP.EILPQ....APANAHLKDLD....EEIFDDDDFYH
Mm TRB    372  LLRRTQTKRSAYRVLG.KPEPVPEPVAETLP..........GEP.ETLPQ....GPANAHLRDLD....EEIFDDDDFYH
Xt AATF  359  LLKRTQTKRSFYRILG.KP.LDSPSVPETVP..........NEAVDFQQE....GKSNSHLKDQD....EEMFDDDDFYH
Dr AATF  418  LVRRTQTRRSEYRVLG.KPEPVTPEIDNTISE.........GEVAELA......VKANMHLKDLD....ENIFDDDDFYH
Ce AATF  340  LLKKVRTNRSGKPRIG.GK..................................SDSPESLD....PETFDDSDFYQ
Dm AATF  372  LVEKSQTPKNSQAE..............................QQENTPVQRL.....KHIYDDSDFYH
At AATF  306  MIKQMQQSRSTVAVFGTVPQEAMEPNPEEKQ..........................EEGD....PELVEDAEFYR
Sc Bfr2  336  LMKRTKLNRRNITPLYFQKDCANGRLPELISPVVKDSVDDNENSDDGLDIPKNYDPRRKDNNAIDITENPYVFDDEDFYR
               AATFD-C (C-terminal subdomain of AATF/Che1/Traube-superfamily domain)
Hs AATF  466  QLLRELIERKTSSLDPN.DQVAMGRQWLAIQKLRS.KIHKKVDRKASKGRKLRFHVLSKLLSFMAPIDH.TTMNDDARTE
Mm TRB    432  QLLRELIERKTSSLDPN.DQVAMGRQWLAIQKLRS.KIRKKVDRKASKGRKLRFHVLSKLLSFMAPIDH.TAMSDDARTE
Xt AATF  419  QLLREVIERKTSSLDPN.DQVAMGRQWLAIQKLRS.KIKKKVDTKASKGRKIRYHVHSKLVSFMAPIDH.STMNDDARTE
Dr AATF  478  QLLRELIERKTSATDPN.DQVAMGKQWLAIQKLRS.KIKKKVDTKASKGRKIRFHVHSKLMNFMAPMDN.SSMSDDARSE
Ce AATF  377  ILLKQMLEARNQMSNNSQDGADMTRSYMELQTMFSNKKKRDVSQLSSKDRRLKYEPIARLINFYPSKPSVVTWSHESRNE
Dm AATF  407  QQLRELIEYKASTSS...NMSEITKQFVELQKLRQ.KMKKKVDTRASKGRKLRYVVHNKLINFMAPNES.SDWTDASKSE
At AATF  352  QLLKEFLE....TI....DPASSEAAFYEMKKFQT.KKRKVVDRRASKSRKIRYNVHEKIVNFMAPRPA...KIPPNTAD
Sc Bfr2  416  VLLNDLIDKKISNAHNS.ESAAITITSTNARSNN..KLKKNIDTKASKGRKLNYSVQDPIANYEAPITSGYKWSDDQIDE

Hs AATF  543  LYRSLFGQLHPPDEGHGD.........................
Mm TRB    509  LFRSLFGQLNPPDADRGK.........................
Xt AATF  496  LYQSLFGKTKCQDEEKQV.........................
Dr AATF  555  LFRSLFANGPVTTQ.............................
Ce AATF  457  LFKSLFS....................................
Dm AATF  482  LYKSLFV....................................
At AATF  420  LLKNLFGLKTRNVQSEA..........................
Sc Bfr2  493  FFAGLLGQRVNFNENEDEEQHARIENDEELEAVKNDDIQIFG
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Figure S2: AATF is conserved in eukaryotes.
Alignment of AATF orthologs generated using MUSCLE (1). The two conserved subdomains as annotated in Pfam are marked by a dark and light green line, respectively. Predicted NLS sequences of each ortholog are shown in red. A predicted coiled-coil helix is highlighted with a grey line. Residues that are fully conserved are marked with dark green boxes, while residues with more than 70% similarity between orthologs are indicated with light green. Species are abbreviated as follows: *Homo sapiens* (*Hs*), *Mus musculus* (*Mm*), *Xenopus tropicalis* (*Xt*), *Danio rerio* (*Dr*), *Caenorhabditis elegans* (*Ce*), *Drosophila melanogaster* (*Dm*), *Arabidopsis thaliana* (*At*), *Saccharomyces cerevisiae* (*Sc*).

Supplementary Figure 3

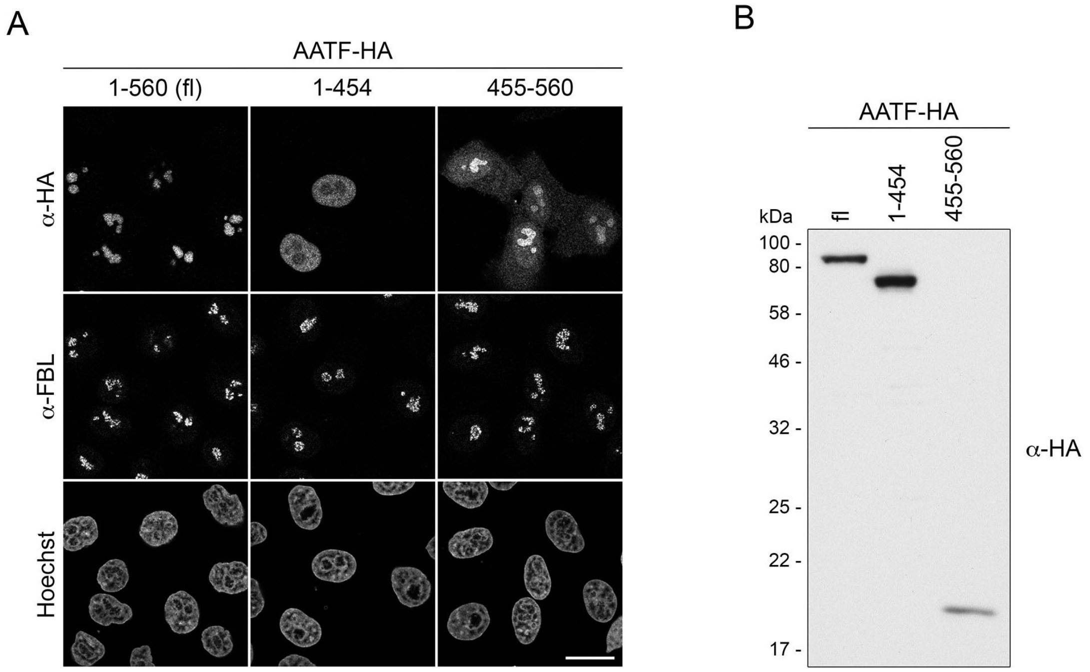

Figure S3: Localization and expression levels of AATF constructs.
(A) HeLa cells were transiently transfected with constructs encoding for HA-tagged full-length (fl) AATF, AATF(1-454) or AATF(455-560). After 48 h, localization of AATF-derivatives was analyzed by IF using an anti-HA antibody and compared to FBL localization. Nuclei were stained with Hoechst. Scale bar, 20 μm.
(B) AATF constructs were expressed as in (A). Protein levels and integrity were examined by immunoblotting.

Supplementary Figure 4

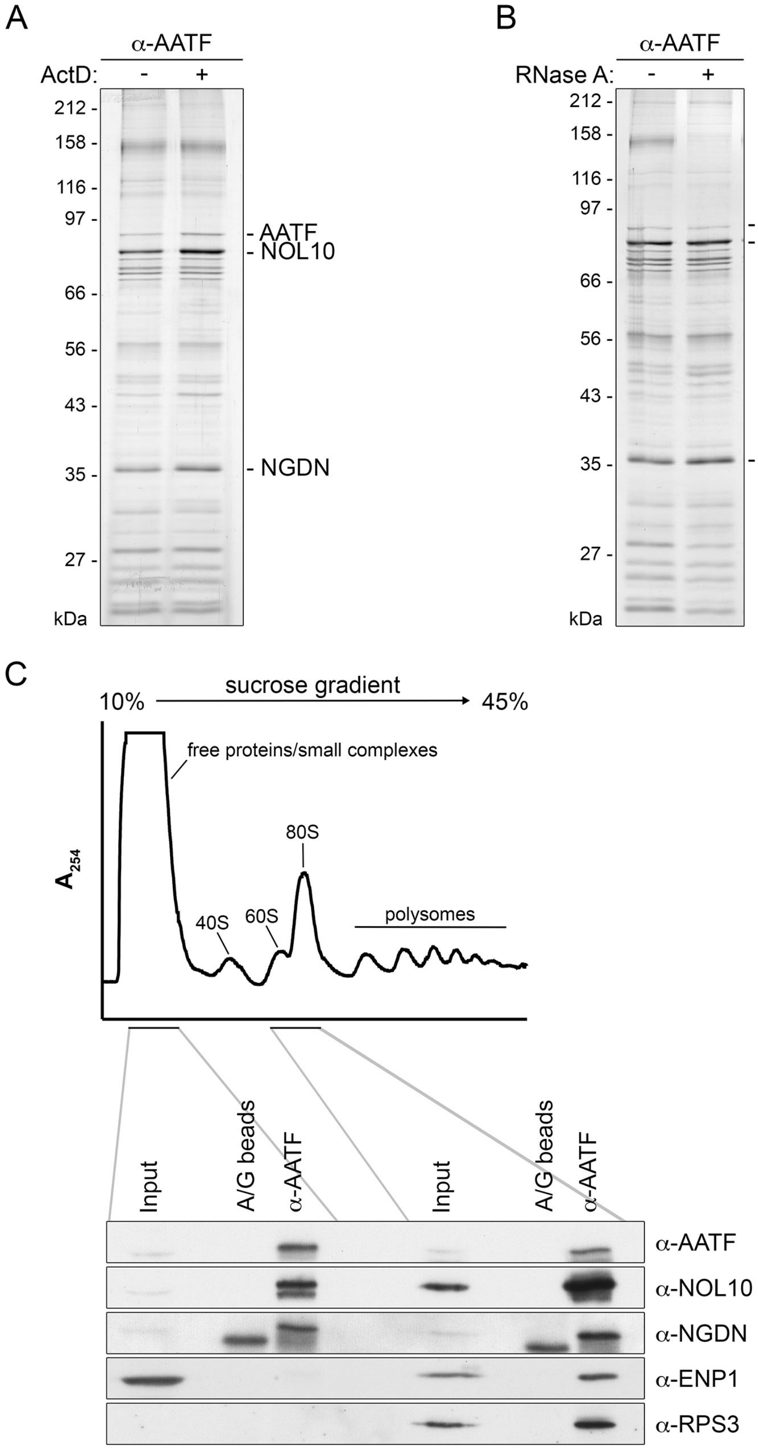

Figure S4: The ANN complex is resistant to actinomycin D and RNase A treatment.

(A) Purification of AATF from HeLa cells by IP after incubating the cells with actinomycin D (ActD, 2 µM, 37°C) for 2.5 h or (B) incubating the cell extract with RNase A (100 µg/ml, 4°C) for 30 min. Isolated proteins were analyzed by SDS-PAGE, followed by silver staining. Members of the ANN complex are marked next to the gels. Note that the activities of ActD and of RNase A were controlled by pre-rRNA FISH and analysis of RNA digestion in the extract, respectively (data not shown).

(C) Sucrose gradient analysis performed as in Figure 3D, except that the centrifugation was done using an SW41 rotor at 32’000 rpm for 3 h 45 min. Gradients were analyzed with a Foxy Jr. gradient collector at OD_{254}. Fractions containing either free proteins/small complexes or 60S/80S particles were pooled and used as separate inputs for AATF IPs. A/G beads without antibody were used as a negative control. The indicated proteins were detected in the inputs and eluates by immunoblotting. Note that the anti-NGDN antibody recognizes an unspecific band running below the NGDN protein in the A/G beads negative control.

Supplementary Figure 5

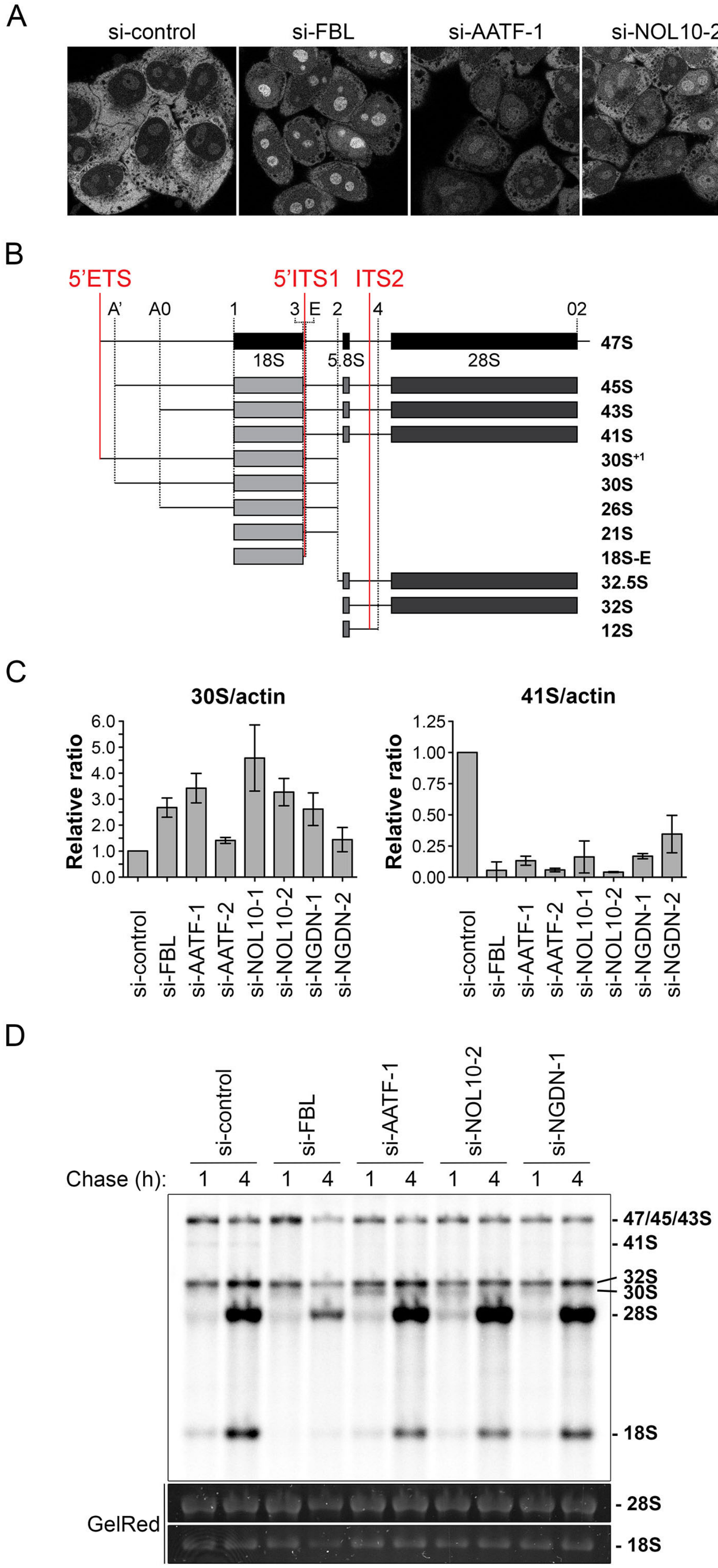

Figure S5: The ANN complex supports pre-18S rRNA processing.
(A) Indicated proteins were depleted by RNAi for 48 h in RPS2-YFP expressing HeLa cells as in Figure 1. Cells were analyzed by confocal microscopy. Scale bar, 20 µm.
(B) Schematic representation of major rRNA precursors in human cells, including endonucleolytic cleavage sites (dashed lines) (2). The binding sites for the 5'ETS, 5'ITS1 and ITS2 probes are indicated (red lines). Each probe recognizes a specific subset of rRNA precursors.
(C) Quantification of three independent experiments as shown in Figure 8B. Mean ± SD (error bars).
(D) Pulse-labeling experiment performed as in Figure 8E. RNAi-induced depletion of the indicated proteins was for 48 h. Levels of newly synthesized 18S and 28S rRNA after a chase period of 4 h were quantified from three independent experiments and are depicted in Figure 8F.

REFERENCES

1. Edgar,R.C. (2004) MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Research*, **32**, 1792–1797.

2. Henras,A.K., Plisson-Chastang,C., O'Donohue,M.-F., Chakraborty,A. and Gleizes,P.-E. (2015) An overview of pre-ribosomal RNA processing in eukaryotes. *WIREs RNA*, **6**, 225–242.

Supplementary Table 1

Band	Gene name	Unique peptides [#]	Sequence coverage [%]	Gene ID	Molecular weight [kDa]
1	THRAP3	41	34	9967	109 kDa
	PPP1R12A	14	14	4659	115 kDa
2	MATR3	21	23	9782	95 kDa
	NAT10	18	17	55226	116 kDa
	HNRNPU	11	11	3192	89 kDa
3	PPP1R13L	29	37	10848	89 kDa
	PML	14	14	5371	98 kDa
	HNRNPUL2	13	17	221092	85 kDa
4	AATF	13	20	26574	63 kDa
5	NOL10	26	31	79954	80 kDa
	HSPA5	18	30	3309	72 kDa
	DDX3X	8	12	1654	73 kDa
	SLC30A1	7	14	7779	55 kDa
6	YBX1	10	34	4904	40 kDa
	RPL4	9	18	6124	53 kDa
	SEPT7	4	16	989	51 kDa
7	EIF4A3	15	29	9775	47 kDa
	RPL3	14	27	6122	49 kDa
	ACTB	11	30	60	41 kDa
	WDR18	7	15	57418	49 kDa
	PAK1IP1	6	14	55003	44 kDa
8	NGDN	15	43	25983	36 kDa
	ANXA2	8	23	302	39 kDa
	RPL5	6	21	6125	34 kDa
	CENPV	6	28	201161	30 kDa
	RPLP0	5	17	6175	31 kDa
	PPP1CA	5	14	5499	39 kDa
	RPL6	4	14	6128	33 kDa
9	PGAM5	9	27	192111	32 kDa
	RPS3A	7	26	6189	30 kDa
	GNB2L1	7	17	10399	43 kDa
	HIST1H1E	2	11	3008	22 kDa
10	FHL2	15	46	2274	32 kDa
	RPL8	12	29	6132	28 kDa
	RPS3	12	43	6188	27 kDa
	RPS6	11	33	6194	29 kDa
	RPL7A	10	31	6130	30 kDa
	RPS4X	8	17	6191	43 kDa
	SLC25A5	8	24	292	33 kDa
	RPS2	7	23	6187	31 kDa
	RPL7	6	24	6129	29 kDa
	NSA2	4	16	10412	30 kDa
	FHL3	3	10	2275	31 kDa
	SLC25A3	3	11	5250	26 kDa
11	RPL13	9	41	6137	24 kDa
	RPS8	5	23	6202	27 kDa
	MRPL24	5	18	79590	25 kDa
	HSPB1	2	10	3315	23 kDa
12	RPL19	9	31	6143	26 kDa
	PCMT1	8	28	5110	25 kDa
	POLR2E	4	20	5434	25 kDa
	RPL14	3	17	9045	24 kDa
	MRPS34	3	13	65993	26 kDa
13	RPL10	11	41	6134	25 kDa
	RPL13A	9	35	23521	23 kDa
	MAD2L1	5	21	4085	24 kDa
	RPL15	4	15	6138	24 kDa

14	RPS9	17	58	6203	23 kDa
	RPS5	4	13	6193	23 kDa
	RPL18	2	11	6141	19 kDa
15	RPL17	10	42	6139	21 kDa
	RPL18A	5	26	6142	21 kDa
	MRPL47	4	16	57129	27 kDa
	CAV1	3	15	857	20 kDa

Supplementary Table 1
MS analysis of the AATF immunoprecipitation. All proteins that have been identified with at least 2 peptides and a sequence coverage of ≥ 10% are listed. The band numbers correspond to the labeling in Figure 3A.