Supplemental Data

Table S1. Heterogeneity of rRNA 3′ ends
Calculations are based on at least 96 clones for each PCR product shown in Fig. 3. For each sequence category, values are in percentile versus total number of sequenced clones from a specific PCR product.

3′ rRNA sequence categories	9S, mock	9S, RET1 RNAi	12S, mock	12S, RET1 RNAi
3′ deletions extending into mature rRNA sequence	0	3	0	1.6
No U-tail	0	3	0	0
1-8 Us	0	9	60	35
9-20 Us	100	28	40	40
3′ lengthening, no U- tail	0	11	0	0
3′ lengthening, plus 1-8 Us	0	30	0	3
3′ lengthening plus 9-20 Us	0	16	0	20

Total RNA isolation

Cells were grown in SDM 79 with 10 % serum (Clontech) and 0.01 mg/ml hemin chloride (Calbiochem) to 4-6×10^6/ ml. Unless otherwise specified, 50 ml of culture was centrifuged at 5000g for 10 min. Pellets were washed with ice-cold PBS, spun down under same conditions and stored at – 80^0C. Frozen cell pellets were immediately re-suspended in 4 ml of ice-cold Buffer D (4M guanidine isothiocyanate, 25 mM sodium citrate (pH 7.0), 0.5 % sarcosyl, 0.1 M β-mercaptoethanol). Cell lysate was mixed with 0.5 ml of 2M sodium acetate (pH 4.0) and 5 ml of water-saturated phenol. 1.5 ml

chloroform/isoamyl alcohol 49:1 was added and mixture incubated for 15 min with gentle rocking at ambient temperature. After centrifugation at 10,000 g for 15 min, the supernatant was transferred into fresh tube and extracted with 5 ml of chloroform/isoamyl alcohol. RNA was precipitated with 5 ml of isopropanol at -20^{0}C for 1 hour, collected by centrifugation at 10,000 g for 15 min and washed with 80 % ethanol. The pellet was re-dissolved in 400 µl water and precipitated with ethanol by standard techniques. Expected yield is 600-800 µg of RNA with $OD_{260}/_{280}$ ratio of 1.6-1.8. For Northern blotting, primer extension and capping with guanylyltransferase experiments, RNA was treated with DNase I (Invitrogen) following the company's protocol. For real-time PCR and cRT-PCR, additional purification step with RNase Easy (Qiagen) columns was included.

Northern blotting

The 20 cm-long, 8 mm-thick 1.6 % agarose formaldehyde gels were prepared in 1x MOPS buffer as described (Sambrook *et al.* 1989) and run for 3 hr at 100 V. The polyacrylamide/8M urea gels (5% for mRNA blotting and 10% for gRNA blotting) were prepared in 20x20x0.15 cm format, and run at 20 W for 2.5 hr. For agarose gels, 15 µg of RNA was resuspended in 20 µl of 1x loading buffer (Ambion). For acrylamide gels, 10 µg of RNA was resuspended in 95 % formamide, 10 mM EDTA, 0.05 % xylene cyanol, 0.05 % Bromphenol Blue buffer. Samples were heated at 65 ^{0}C for 5 min prior to loading. To visualize molecular mass markers on the membrane, 1 µg of the 0.1-2 kb RNA ladder (Invitrogen) was labeled with T4 RNA ligase and [α-^{32}P]pCp. RNA on

agarose gel was transferred on to the BrightStar-Plus membrane (Ambion) in 10 × SSC, 10 mM NaOH buffer for 2 hours using vacuum blotter (model 785, Bio-Rad). RNA from the acrylamide gel was transferred to the same membrane in ½ TBE buffer for 2 hours at 90 V using a cooling blotter unit (TE series, Hoefer). The membranes were exposed twice to UV light at 120 mJ/cm^2 using HL 2000 HibriLinker (UVP) and stained with 0.04 % of Methylene Blue in 0.5 M Na Acetate (pH 5.0) to check transfer.

Following RNA transcripts were detected with single-stranded DNA probes produced by asymmetric PCR: COI, ND1, ND4, MURF1, MURF5 and RPS12 (edited and unedited). The template was generated with oligonucleotides used for real-time RT-PCR analysis and gel-purified. The PCR reactions were set up with 10 pmol of 5′ labeled oligonucleotides and ~ 0.5 pmol of template. Membranes were pre-hybridized for 2 hours at 55 ^{0}C temperature using HL 2000 HibriLinker (UVP) in 20 ml of 6 × SSPE, 5 × Denhardt's solution, 0.5 % SDS, 0.2 mg/ml of denatured salmon sperm DNA. Hybridization was performed overnight in 4-5 ml of 6 × SSPE, 5 × Denhardt's solution, 0.5 % SDS, 20 µg/ml of yeast tRNA(Sigma), 3-4 × 10^6 cpm/ml of DNA probe. Membrane was washed 3 times with 2 × SSC, 0.1 % SDS solution at 55^{0}C and 1 time with 1 × SSC, 0.5 % SDS at 55^{0}C – 65^{0}C (temperature was increased if background appeared or near-melting point stringency was required, as in the case of 5′ pre-edited RPS12 mRNA). Membranes was stripped by boiling for 20 min in 0.1 % SDS and stored in plastic bag at -20^{0}C. Guide RNA and tRNA hybridizations with oligonucleotides were performed at 42^{0}C in ULTRAhyb -Oligo Hybridization solution as recommended by the manufacturer (Ambion).

gRNA labeling

Total DNase-treated RNA (5 μg) was re-suspended in 10 μl of water, incubated at 85^0 for 2 min and placed on ice for 2 min. RNA samples were mixed with 10 μl of reaction mix (60 mM Tris-HCl pH 8.0, 6 mM $MgCl_2$, 5 mM DTT, 20 U of RNaseout (Invitrogen), 10 μCi of [α-^{32}P] GTP) and 1 μg of guanylyltransferase, and incubated at 37^0C for 30 min. Vaccinia virus guanylyltransferase was purified from *E. coli* carrying pET-HisD1/D12 plasmid ((Luo *et al.* 1995), kind gift of Stuart Shuman, MSKCC). Reaction was stopped with equal volume of 0.2 % SDS plus 1 μg of proteinase K, and incubation continued for 20 min. The reaction mix was extracted with phenol (pH 5.0), re-extracted with phenol/chloroform (pH 8.0), and precipitated with ethanol. Products were separated on 9 % polyacrylamide/urea gel.

Quantitative RT-PCR

cDNA was synthesized with Applied Biosystems RT-PCR kit as follows:

Components	**Volume (μl)**
RNA (6 μg)	x
10x RT Buffer	4
25mM MgCl2	8.8
dNTP	8
Random Hexamer	2
RNAse Inhibitor	0.8
H2O	up to 39.5 μl

Reaction was split in two and 0.25 µl (12.5U) of Multiscribe Reverse Transcriptase (50U/ul) was added to one tube and 0.25 µl H20 to the other serving as a No-RT control. Samples were placed into a thermocycler for 1 cycle of: 25°C 10min, 48°C 30min., 95°C 5min., 4°C hold. For the qRT- PCR reaction, cDNA was diluted with 20 µl H2O.

For each PCR reaction in the 96-well plate:

Components	**Volume (µl)**
SYBR Green Mix	12.5
1.5 µM Primer 1	5
1.5 µM Primer 2	5
cDNA products	2.5

cDNA produced from the one reaction was used to analyze up to 15 transcripts. Samples were run in triplicate and the Ct values from each sample were averaged. Nuclearly encoded β-tubulin mRNA and 18s rRNA were used as internal normalization controls.

Reactions were carried out in the Eppendorf Realplex2 S cycler under following conditions: 50°C for 2 min., 95°C for 10 min., followed by 45 cycles of 95°C for 15 sec. and 60°C for 1 min. Thermal dissociations were used to confirm generation of a single amplicon. PCR products were also analyzed by electrophoresis in 2.0% agarose gel. Oligonucleotide primers were designed with Beacon Designer 3 and purchased from Invitrogen. Relative changes in target amplicons were determined by using the Pfaffl method with PCR efficiencies calculated by linear regression using LinRegPCR (Ramakers *et al.* 2003)

Purification of the GRBC1/2 complex

GRBC1 and *2* genes lacking the 5′ regions corresponding to predicted mitochondrial importation signals (amino acids 24-460 and 21-491, respectively) were cloned into pDUET-1 vector. GRBC2 was expressed as N-terminally 6His-tagged protein. Six liters of bacterial culture were grown in 2YT media at 37°C to 0.3 OD^{600} and the temperature was lowered to 10^0C over 30-60 min. Protein expression was induced with 1 mM of IPTG and cultivation continued for 3 hours. All chromatographic steps were performed on AKTA purifier (GE) at 4 ^{0}C. The cell pellet (~40 g) was resuspended in 200 ml of 50 mM HEPES buffer (pH 7.8), 50 mM NaCl and passed through a French pressure cell at 12000 Psi. Sodium chloride was adjusted to 300 mM and the extract centrifuged at 200,000 g for 45 min. The supernatant was filtered thorough a low-protein binding 0.45 micron filter and loaded onto a 10 ml Talon metal affinity column (Clontech), washed with 100 ml of 50 mM HEPES (pH 8.0), 300 mM NaCl and with 100 ml of 50 mM HEPES (pH 8.0), 300 mM NaCl and 10 mM imidazole. Protein was eluted with the same buffer containing 200 mM of imidazole, diluted four-fold and immediately loaded on 15 ml column with Poros HS20 (Roche), which had been pre-equilibrated with 50 mM KCl, 50 mM HEPES (pH 8.0), 1 mM DTT. The column was developed with linear 50-500 mM KCl, 20 column volume gradient at 10 ml/min. Fractions eluting at ~ 250 mM KCl were pooled, diluted 3 fold (~ 50 ml) and loaded on 5 ml HiTrap HQ column (GE Healthcare). Linear 50-500 mM gradient in 25 mM Tris-HCl, pH 7.8, (20 column volumes was applied at 1 ml/min; GRBC1/2 eluted as a single symmetrical peak at ~ 300 mM KCl. The obtained protein was typically >95% pure as estimated by Sypro Ruby staining of the SDS-acrylamide gel. The final fraction was supplemented with glycerol to 10%, flash frozen in liquid nitrogen and stored at -80 ^{0}C.

For size exclusion chromatography the protein sample (50 µl, 5 mg/ml) was loaded onto a Superose 12 column (GE Healthcare) in 20 mM HEPES, pH 7.5, 150 mM KCl at 0.1 ml/min. Molecular mass standards used to estimate Stokes' radius were catalase, aldolase, albumin and ovalbumin.

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3′ lengthening, plus 1-8 Us	0	30	0	3
3′ lengthening plus 9-20 Us	0	16	0	20

Table S2. Oligonucleotides used in this study

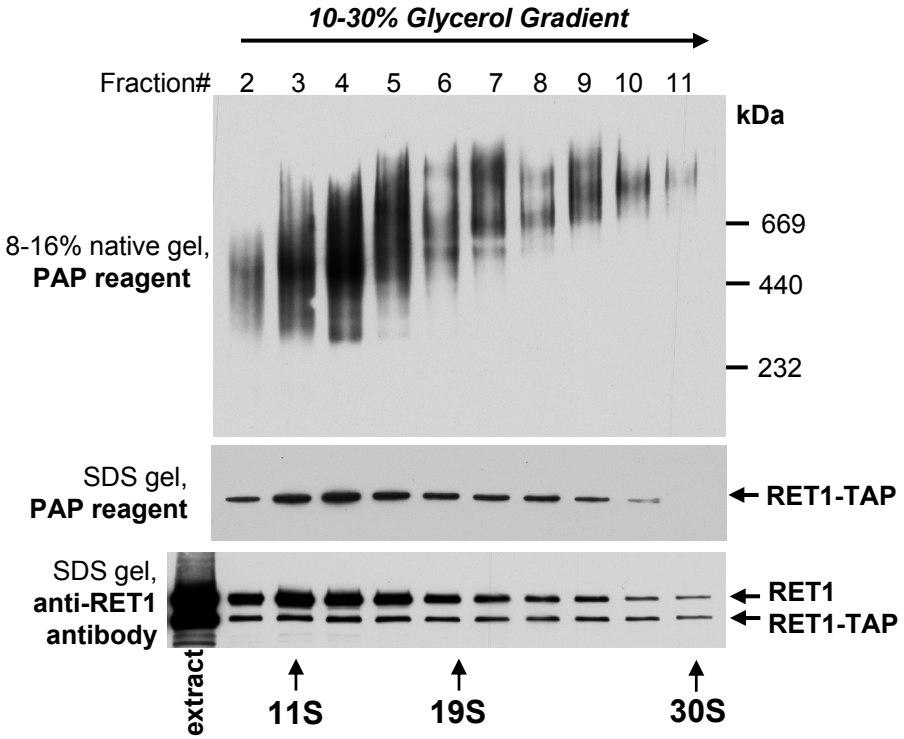
Name	Sequence, 5′-3′	Polarity	Application
Northern Blotting			
A304	TGAACAATCAATCATGGTAATAAGTAGACGATG		oligo probe 12S rRNA
A504	ACGGCTGGCATCCATTTC		oligo probe 9S rRNA
A 850	CAAATCGGACGGGATATGGTGCATTCGGCCAAGTATGGTCG		oligo probe 5S
A662	CATTCAATTACTCTAATTTAATTTTATTTTTGTGC		oligo probe Murf2 (II) gRNA
A872	GGGGACCATTCGGACTGCAGCCG		$tRNA^{Cys}$
A798	TAATTAAATCTTCTCATTGTCACTGTCTTATACTACGATTGAGTTTGTAT		Oligo probe CO3 gRNA [147]
A661	TATTTACTCACTTTATCTCACTACATAAATCCATGAT		Oligo probe RPS12 gRNA [64]
A663	TAATTATCATATCACTGTCAAAATCTGATTCGTTAT		Oligo probe A6 gRNA [14]
A343	TGGTAAAGTTCCCCGTGTTGA		Oligo probe 18S rRNA
A359	CGACGGAGAGCTTCTTTTGAATA	sense	unedited RPS12 mRNA
A360	CCCCCCACCCAAATCTTT	anti	unedited RPS12 mRNA
A357	CGTATGTGATTTTTGTATGGTTGTTG	sense	edited RPS12 mRNA
A358	ACACGTCGGTTACCGGAACT	anti	edited RPS12 mRNA
A297	CAATCTGACCATTCCATGTGTGACTACC	sense	ND4 mRNA

A296	AGATAATTCAGTAACAAGGCCAGCAACAAG	anti	ND4 mRNA
A296	AGATAATTCAGTAACAAGGCCAGCAACAAG	anti sense	ND1
A295	GGACTGCTTCTTGATGGATTACGTTTACC	sense	ND1
A294	AAAGCCAATACAAATACAAAGGTAACTTAG	anti	MURF1
A293	GTTTACTACTTGCATGTCTCTTTCTTTG	sense	MURF1
A302	ACTAAGCAACCAAATCCTCCAATAAACATTC	sense	CO1 mRNA
A301	TGCCTATAACTATGGGTGGGTTTACAAAC	anti	CO1 mRNA
B105	ATATACTATAACTCCGATAACGAATCAGATTTTGACAGTGATATGATAATTAT	anti	gA6[14]
B108	ATAATTATCATATCACTGTCAAAATCTGATTCGTTATCGGAGTTATAGTATAT	sense	gA6[14]
qRT-PCR			
A345	TGACGCCGGACACAACAG	anti	β-tubulin
A344	TTCCGCACCCTGAAACTGA	sense	β-tubulin
A343	TGGTAAAGTTCCCCGTGTTGA	anti	18s rRNA
A342	CGGAATGGCACCACAAGAC	sense	18s rRNA
A304	TGAACAATCAATCATGGTAATAAGTAGACGATG	anti	12S rRNA
A303	GGGCAAGTCCTACTCTCCTTTACAAAG	sense	12S rRNA
A302	ACTAAGCAACCAAATCCTCCAATAAACATTC	anti	CO1
A301	TGCCTATAACTATGGGTGGGTTTACAAAC	sense	CO1
A300	GCGTGTATTAATGCTGATACTGGGATAGG	anti	ND5
A299	TTTCTATATGTTTGTTAGTAGGATGTGCGTTC	sense	ND5
A298	TGCTATAAATACTAAACCCAACACAATTACACTATC	anti	ND4
A297	CAATCTGACCATTCCATGTGTGACTACC	sense	ND4
A296	AGATAATTCAGTAACAAGGCCAGCAACAAG	anti	ND1
A295	GGACTGCTTCTTGATGGATTACGTTTACC	sense	ND1
A294	AAAGCCAATACAAATACAAAGGTAACTTAG	anti	Murf1
A293	GTTTACTACTTGCATGTCTCTTTCTTTG	sense	Murf1
A281	TCCGCACACGCAACGACTTC	anti	RET1
A280	TGAGCAGTGGTGGTGGAGGTAG	sense	RET1

A525	TGTTCCTTATTCATTTTGTGCATTAC	sense	Murf5
A256	TGTGTATAATGTTAAGTCAAATTAAAATGC	anti	Murf5
3′ End Analysis – cRT-PCR			
A863	CAAAATTATAAATTATTATTAACTATTCC		9S cDNA
A861	TATACAAAAAATCTTTCAAAAATAAACC		9S
A862	GAAATTAAAAAGGTATTGTTGCCCAC		9S
A866	CTATAAATATGTATCTAAATATAAAACAAC		12S cDNA
A864	AAATAATACAAATAATAAATTTACTACACGG		12S
A865	TTTGAAATTATAAAAGGTTCGAGCAGG		12S
A957	ACGTTCACATAAACTAACATACC		ND1 cDNA
A955	TTATTTACAATGGGATTCATTAATTTTTC		ND1
A956	CATAAGACAGATAAAACGAGTATAAATATAAC		ND1

A

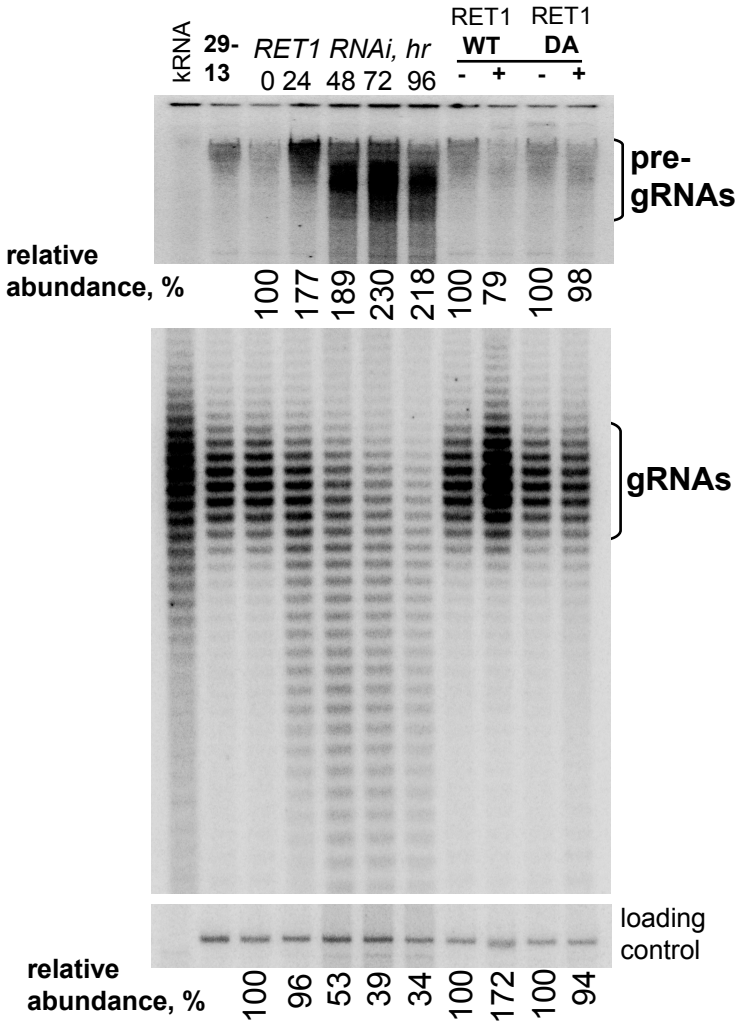
TAP-tagged RET1 is incorporated into heterogeneous high-molecular mass complexes



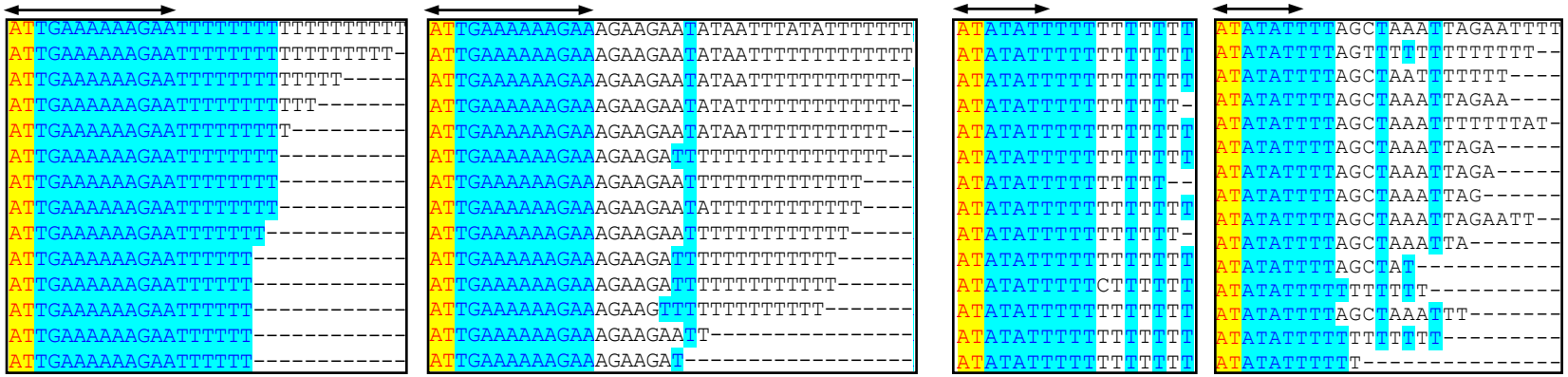
Mitochondrial extract was fractionated on 10-30% glycerol gradients (SW41 rotor, for 20 hr at 100,000g). Fractions (0.65 ml) were analyzed for the presence of tagged (PAP reagent) and endogenous (anti-RET1 antibody) RET1 proteins by electrophoresis in 8-16% PAGE. Tris-glycine gels were run under native or denaturing (SDS) conditions and electroblotted onto nitrocellulose membranes for immunodetection.

C

Overexpression of active RET1 increases the overall abundance of fully-processed gRNAs and stimulates precursor processing

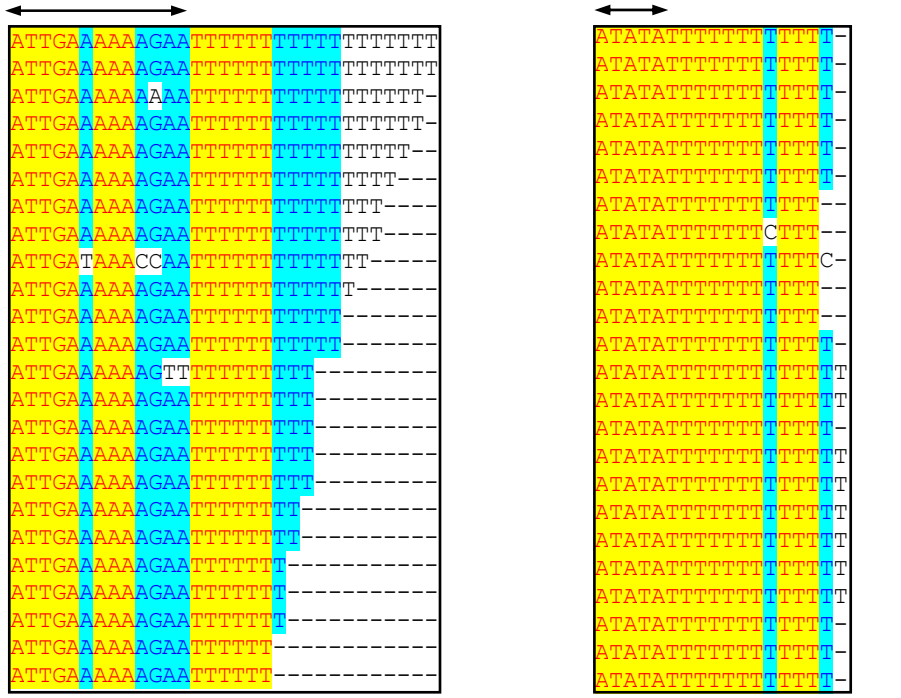


A cRT-PCR analysis of ribosomal RNAs in RET1-depleted cells

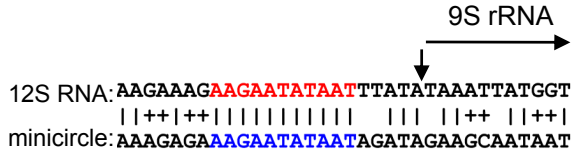


Partial multiple sequence alignment of 3′-ends from representative clones. Double-headed arrows show encoded 3′-ends.
Nucleotides Identical in all sequenced clones are red on yellow; nucleotides that varied in at least a single clone are black on cyan background.

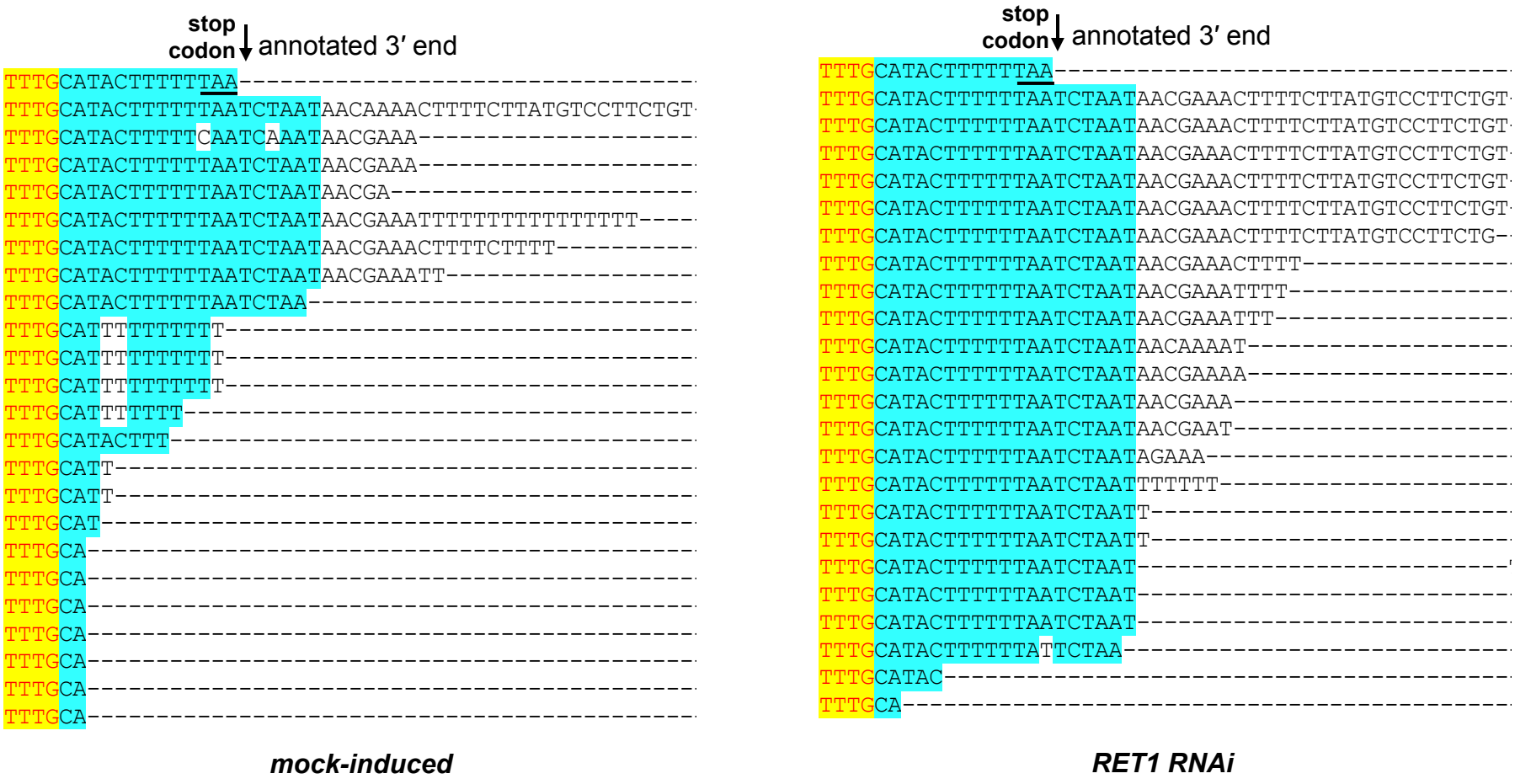
B cRT-PCR analysis of ribosomal RNAs in MEAT1-depleted cells



C



cRT-PCR analysis of the 3′ end in ND1 mRNA



Partial multiple sequence alignment of randomly selected clones (out of 120 and 96 clones from mock-induced and RET1 RNAi cells, respectively).