Supplementary Information

Exploration of CCA-added RNAs revealed the expression of mitochondrial non-coding RNAs regulated by CCA-adding enzyme

Supplementary figure legends

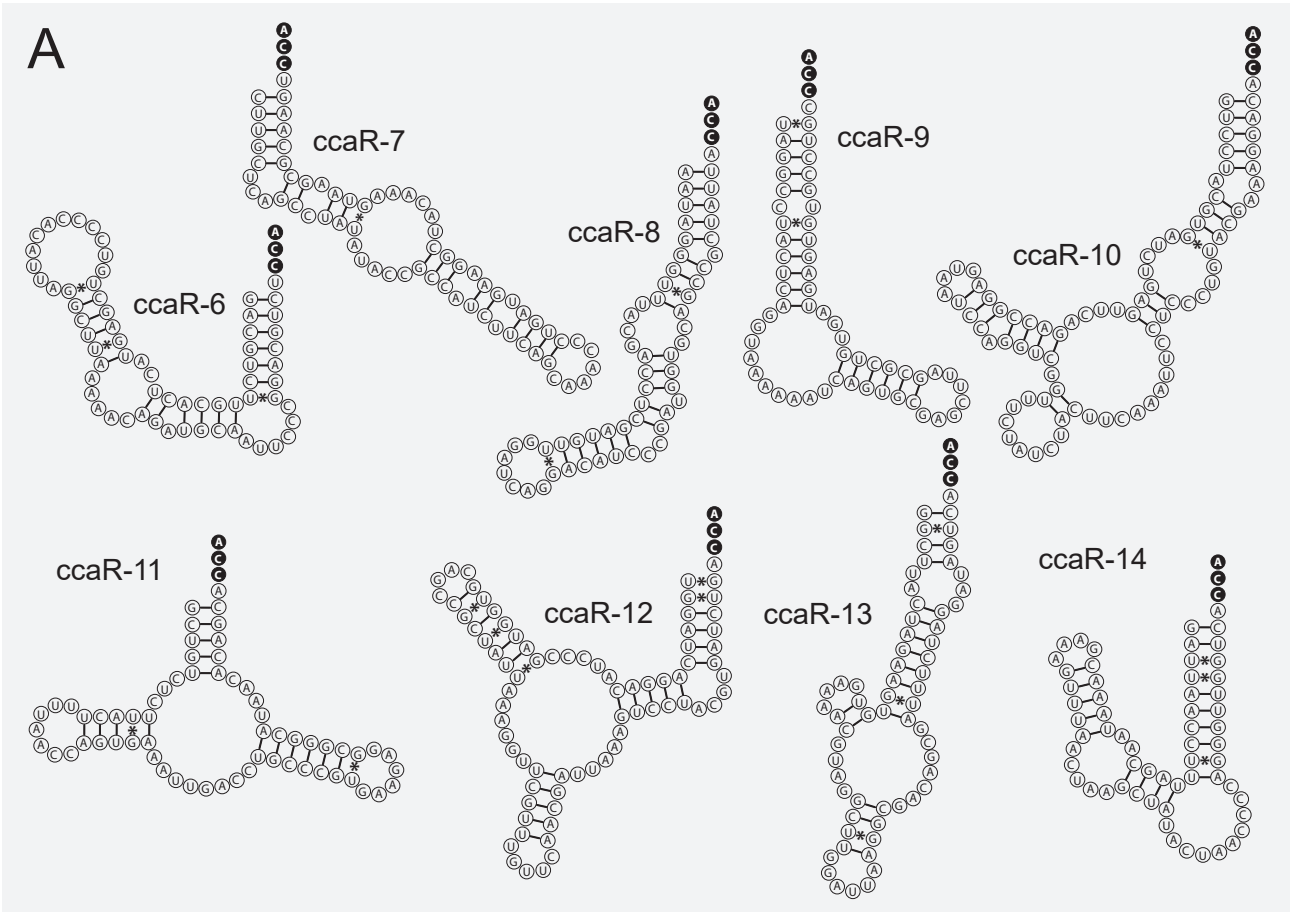
Figure S1. Sequences and secondary structures of identified CCA-RNAs (A) and NCCA-RNAs (B).

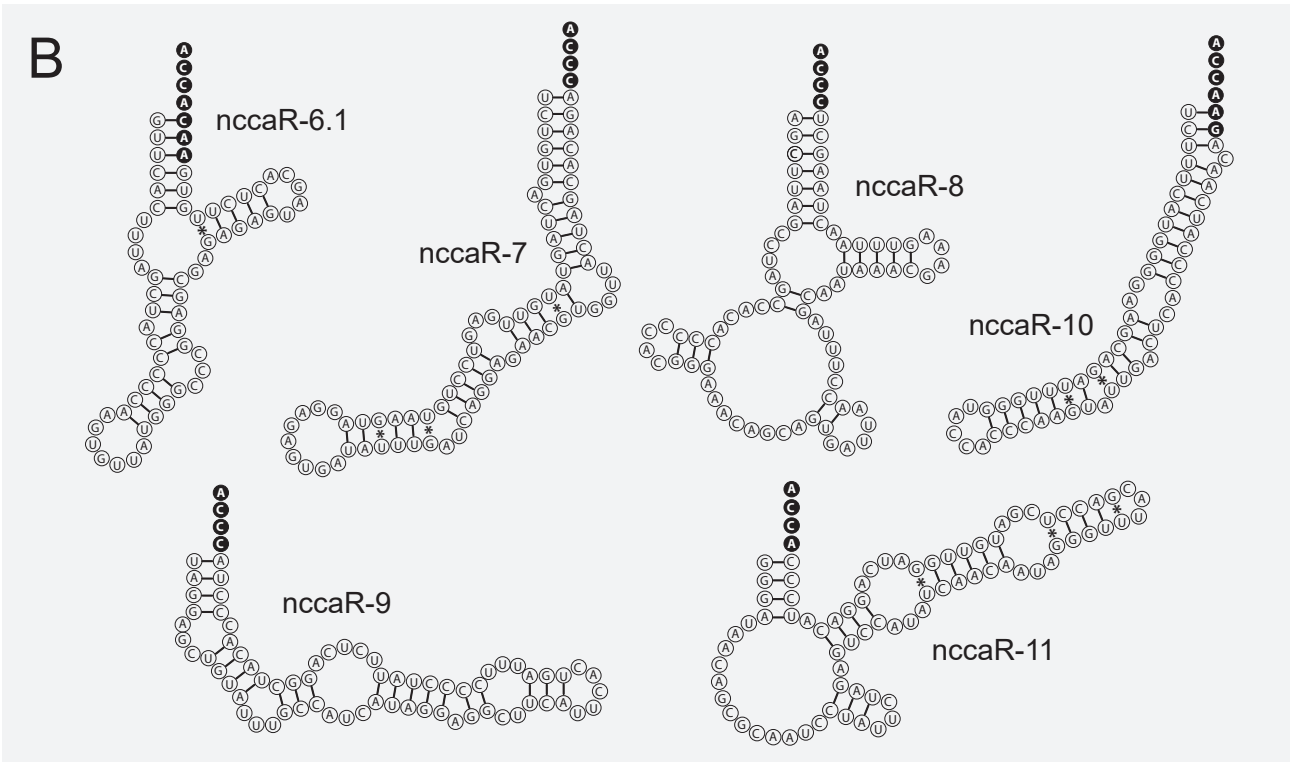
Figure S2. A possible mechanism for the non-template addition of 3′-terminal nucleotides of nccaR-1

Figure S3. Probes for northern blots of CCA-RNAs

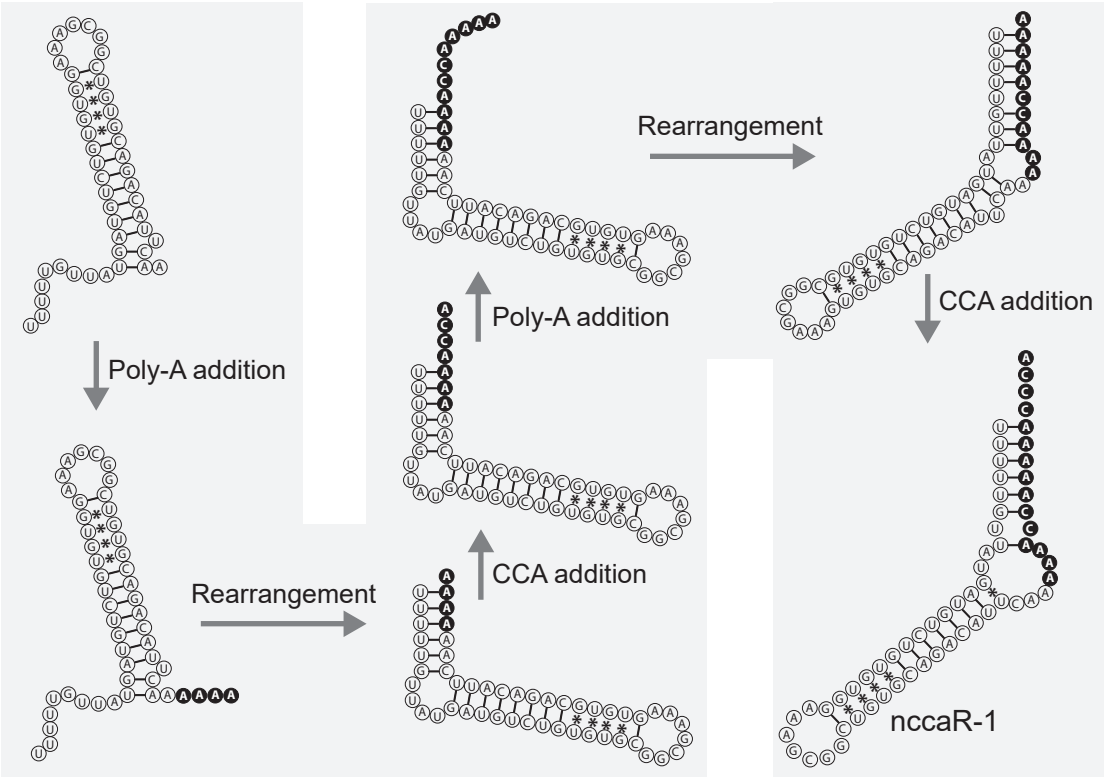
Figure S4. Quantification of northern blots

Northern blots from TRNT1 knockdown experiments (A, from Fig. 5B) and cellular treatments (B, from Fig. 6) were quantified and relative intensities are presented.

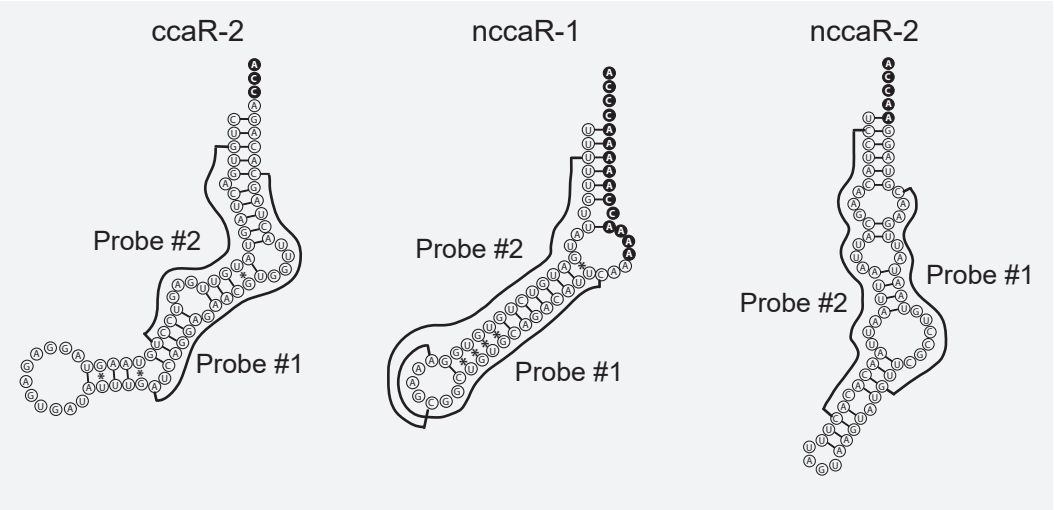




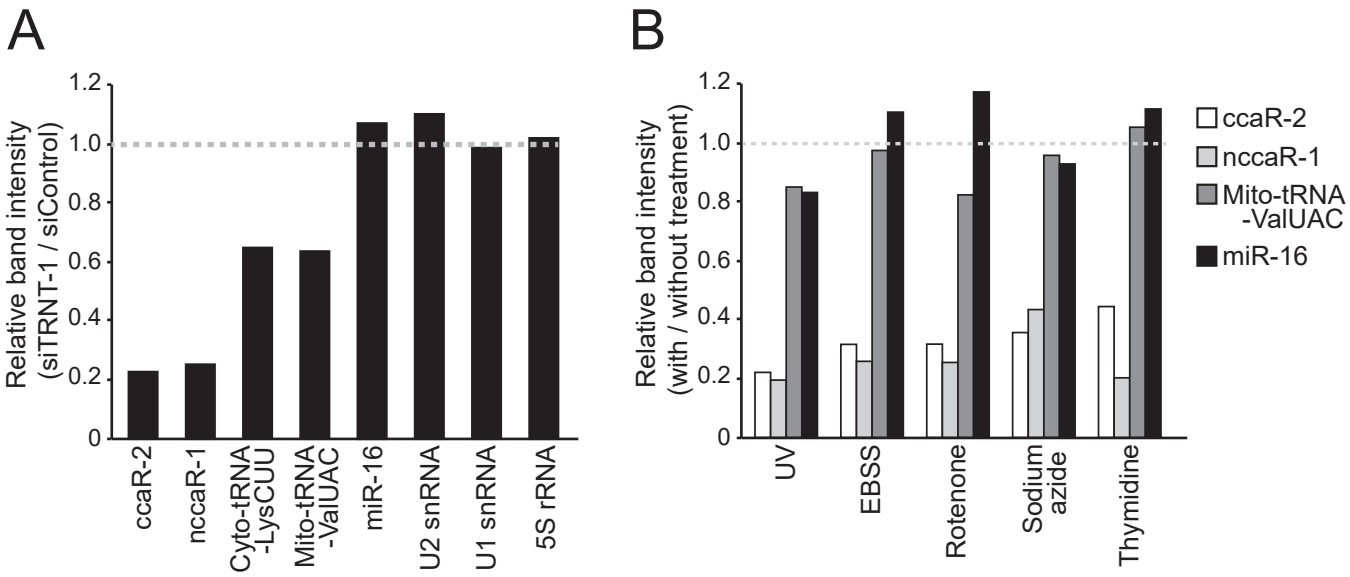
Pawar et al. Figure S1



Pawar et al. Figure S2



Pawar et al. Figure S3



Pawar et al. Figure S4

Table S1. Identified sequences of CCA-RNAs and NCCA-RNAs

Name	Variant	%	Sequence (*red characters are not encoded on the genome*)	Length (nt)	Genome
CCA-RNAs					
mascRNA		68.69	GATGCTGGTGGTTGGCACTCCTGGTTTCCAGGACGGGGTTCAGATCCCTGCGGCGTCTCCA	61	Nuc
ccaR-1		9.45	CCTGATGAAGGCTACAAAGTAAGCGCAAGTACCCACGTAAAGACGTTAGGTCAAGGTGTAGCCCATGAGGTCCA	74	Mito
ccaR-2		5.95	CTGTGACTAGTATGTTGAGTCCTGTAAGTAGGAGAGTGATATTTGATCAGGAGAACGTGGTTACTAGCACAGACCA	76	Mito
ccaR-3		3.16	GGGATAACAGCGCAATCCTATTCTAGAGTCCATATCAACAATAGGGTTTACGACCTCGATGTTGGATCAGGACATCCCGCCA	82	Mito
ccaR-4		1.75	GCTTAGCCTAGCCACACCCCCACGGGAAACAGCAGTGATTAACCTTTAGCAATAAACGAAAGTTTAACTAAGCTCCA	77	Mito
ccaR-5		1.36	AGTGACACATGTTTAACGGCCGCGGTACCCTAACCGTGCAAAGGTAGCATAATCACTTCCA	61	Mito
ccaR-6		1.29	GACGTCTTGCACTCATGAGCTGTCCCCACATTAGGCTTAAAAACAGATGCAATTCCCGGACGTCTCCA	68	Mito
ccaR-7		0.76	CTTGCTCAGCCTATATACCGCCATCTTCAGCAAACCCTGATGAAGGCTACAAAGTAAGCGCAAGTCCA	68	Mito
ccaR-8		0.46	AATAGGGTTTACGACCTCGATGTTGGATCAGGACATCCCGATGGTGCAGCCGCTATTACCA	61	Mito
ccaR-9		0.34	TAGGCCTACTCAGGTAAAAAATCAGTGCGAGCTTAGCGCTGTGATGAGTGTGCCTGCCCA	60	Mito
ccaR-10		0.25	GTCCTACGTGATCTGAGTTCAGACCGGAGTAATCCAGGTCGGTTTCTATCTACTTCAAATTCCTCCCTGTACGAAAGGACACCA	84	Mito
ccaR-11		0.19	GCTGTCTCTTACTTTTAACCAGTGAAATTGACCTGCCCGTGAAGAGGCGGGCATAACACAGCACCA	66	Mito
ccaR-12		0.08	TGGATCAGGACATCCCGATGGTGCAGCCGCTATTAAAGGTTCGTTTGTTCAACGATTAAAGTCCTACGTGATCTGACCA	79	Mito
ccaR-13		0.07	GGCTTACTAGAAGTGTGAAAACGTAGGCTTGGATTAAGGCGACAGCGATTTCTAGGATAGTCACCA	66	Mito
ccaR-14		0.03	GATTAACCTTTAGCAATAAACGAAAGTTTAACTAAGCTATACTAACCCCAGGGTTGGTCACCA	63	Mito
NCCA-RNAs					
nccaR-1	1	0.73	TTTTTGTTATGATGTCTGTGTGGAAAGCGGCTGTGCAGACATTCAAAAAACCAAAAACCCA	61	Mito
	2	0.19	TTTTTTGTTATGATGTCTGTGTGGAAAGCGGCTGTGCAGACATTCAAAAAAACCAAAAAACCCA	64	Mito
	3	0.19	TTTTTGTTATGATGTCTGTGTGGAAAGCGGCTGTGCAGACATTCAAAAAACAAAAACCCA	60	Mito
	4	0.12	TTTTTGTTATGATGTCTGTGTGGAAAGCGGCTGTGCAGACATTCAAAAAACCAAAAAACCA	61	Mito
	5	0.08	TTTTTGTTATGATGTCTGTGTGGAAAGCGGCTGTGCAGACATTCAATAAAAAACCAAAAACCCA	64	Mito
	6	0.08	TTTTGTTATGATGTCTGTGTGGAAAGCGGCTGTGCAGACATTCAAAAAAAAACAAAACCCA	62	Mito
	7	0.08	TTTTTGTTATGATGTCTGTGTGGAAAGCGGCTGTGCAGACATTCAAAAAACCAAAAACCCA	62	Mito
	8	0.08	TTTTGTTATGATGTCTGTGTGGAAAGCGGCTGTGCAGACATTCAAAAAAAACAAAACCCA	61	Mito
	9	0.08	TTTTTTGTTATGATGTCTGTGTGGAAAGCGGCTGTGCAGACATTCAATTAAAAAAACCCA	60	Mito
	10	0.07	TTTTTGTTATGATGTCTGTGTGGAAAGCGGCTGTGCAGACATTCAAAAAACAAAAACCCA	61	Mito
	11	0.05	TTTTGTTATGATGTCTGTGTGGAAAGCGGCTGTGCAGACATTCAATTGAAAACAAAACCCA	61	Mito
	12	0.05	TTTTTGTTATGATGTCTGTGTGGAAAGCGGCTGTGCAGACATTCAAAAAACAAAACCCA	60	Mito
	13	0.05	TTTTTGTTATGATGTCTGTGTGGAAAGCGGCTGTGCAGACATTCAAAAAACCAAAAACCA	60	Mito
	14	0.05	TTTTTGTTATGATGTCTGTGTGGAAAGCGGCTGTGCAGACATTCAATAAACAAAAACCCA	60	Mito
	15	0.03	TTTTTTGTTATGATGTCTGTGTGGAAAGCGGCTGTGCAGACATTCAAAAAAACCAAAAAACCCA	65	Mito
	16	0.03	TTTTTGTTATGATGTCTGTGTGGAAAGCGGCTGTGCAGACATTCAATTGAAAAACAAAAACCCA	64	Mito
	17	0.03	TTTTGTTATGATGTCTGTGTGGAAAGCGGCTGTGCAGACATTCAAAAAAAAACAAAACCCA	63	Mito
	18	0.03	TTTTTTGTTATGATGTCTGTGTGGAAAGCGGCTGTGCAGACATTCAAAAACCAAAAAACCCA	63	Mito
	19	0.03	TTTTGTTATGATGTCTGTGTGGAAAGCGGCTGTGCAGACATTCAATTAAAAACCAAAAACCA	62	Mito
	20	0.03	TTTTGTTATGATGTCTGTGTGGAAAGCGGCTGTGCAGACATTCAATTGAAAAACAAAACCCA	62	Mito
	21	0.03	TTTTTGTTATGATGTCTGTGTGGAAAGCGGCTGTGCAGACATTCAATAAAAAAAACCCA	60	Mito
	22	0.03	TTTTTTGTTATGATGTCTGTGTGGAAAGCGGCTGTGCAGACATTCAAAAAAAAAACCCA	60	Mito
	23	0.03	TTTTTTGTTATGATGTCTGTGTGGAAAGCGGCTGTGCAGACATTCAATAAAAAAAACCCA	60	Mito
nccaR-2	1	1.16	TCCTACAAGCATTAATTAATTAACACACTTTAGTAAGTATGTTCGCCTGTAATATTGAACGTAGGAACCA	70	Mito
	2	0.58	TTCCTACAAGCATTAATTAATTAACACACTTTAGTAAGTATGTTCGCCTGTAATATTGAACGTAGGAACCCA	72	Mito
	3	0.19	TCCTACAAGCATTAATTAATTAACACACTTTAGTAAGTATGTTCGCCTGTAATATTGAACGTAGGACCCA	70	Mito
	4	0.03	TCCTACAAGCATTAATTAATTAACACACTTTAGTAAGTATGTTCGCCTGTAATATTGAACGTAGGAACCCA	71	Mito
	5	0.03	TCCTACAAGCATTAATTAATTAACACACTTTAGTAAGTATGTTCGCCTGCAATATTGAACGTAGGAACCA	70	Mito
nccaR-3	1	0.41	ACCTGATGAAGGCTACAAAGTAAGCGCAAGTACCCACGTAAAGACGTTAGGTCAAGGTGTAGCCCATGAGGTCCCA	76	Mito
	2	0.07	GCCTGATGAAGGCTACAAAGTAAGCGCAAGTACCCACGTAAAGACGTTAGGTCAAGGTGTAGCCCATGAGGTCCCA	76	Mito
	3	0.03	CCCTGATGAAGGCTACAAAGTAAGCGCAAGTACCCACGTAAAGACGTTAGGTCAAGGTGTAGCCCATGAGGTACCA	76	Mito
nccaR-4	1	0.27	GGTTTACGACCTCGATGTTGGATCAGGACATCCCGATGGTGCAGCCGCTATTAAAGGTTCGTTTGTAAAACCACCA	76	Mito
	2	0.12	GTTTACGACCTCGATGTTGGATCAGGACATCCCGATGGTGCAGCCGCTATTAAAGGTTCGTTTGTAAACACCA	73	Mito
	3	0.12	TTTACGACCTCGATGTTGGATCAGGACATCCCGATGGTGCAGCCGCTATTAAAGGTTCGTTTGTAAAACCA	71	Mito
nccaR-5	1	0.14	GTTTGGGCTACTGCTCGCAGTGCGCCGATCAGGGCGTAGTTTGAGTTTGATGCTCACCCTGATCACAAAAAACACCA	77	Mito
	2	0.05	GTTTGGGCTACTGCTCGCAGTGCGCCGATCAGGGCGTAGTTTGAGTTTGATGCTCACCCTGATCACCAAAACCCCA	76	Mito
	3	0.05	GTTTGGGCTACTGCTCGCAGTGCGCCGATCAGGGCGTAGTTTGAGTTTGATGCTCACCCTGATACCAAAACACCA	75	Mito
	4	0.03	GTTTGGGCTACTGCTCGCAGTGCGCCGATCAGGGCGTAGTTTGAGTTTGATGCTCACCCTGATAAAAACACCA	73	Mito
	5	0.03	GTTTGGGCTACTGCTCGCAGTGCGCCGATCAGGGCGTAGTTTGAGTTTGATGCTCAACCCCA	62	Mito
	6	0.03	GTTTGGGCTACTGCTCGCAGTGCGCCGATCAGGGCGTAGTTTGAGTTTGATGCTCACCACCA	62	Mito
nccaR-6	1	0.12	GTTCACTTTAGCTACCCCCAAGTGTTATGGGCCCGGAGCGAGGAGAGTAGCACTCTTGTGAACACCA	67	Mito
	2	0.08	GTTCACTTTAGCTACCCCCAAGTGTTATGGGCCCGGAGCGAGGAGAGTAGCACTCTTGTGAACCCCA	67	Mito
nccaR-7		0.14	TCTGTGACTAGTATGTTGAGTCCTGTAAGTAGGAGAGTGATATTTGATCAGGAGAACGTGGTTACTAGCACAGACCCA	78	Mito
nccaR-8		0.12	AGCTTAGCCTAGCCACACCCCCACGGGAAACAGCAGTGATTAACCTTTAGCAATAAACGAAAGTTTAACTAAGCTCCCA	79	Mito
nccaR-9		0.05	TAGGAGCTGTATTTGCCATCATAGGAGGCTTCATTCACTGATTTCCCCTATTCTCAGGCTACACCCTACCCA	72	Mito
nccaR-10		0.03	TCTTTCATGGGGAAGCAGATTTGGGTACCACCCAAGTATTGACTCACCCATCAACAGAACCA	62	Mito
nccaR-11		0.03	GGGATAACAGCGCAATCCTATTCTAGAGTCCATATCAACAATAGGGTTTACGACCTCGATGTTGGATCAGGACATCCCACCA	82	Mito

Table S2. Sequences of probes for northern blot

Probe	Sequence (5′–3′)
ccaR-2 (probe #1)	GCTAGTAACCACGTTCTCCTGAT
ccaR-2 (probe #2)	AGGACTCAACATACTAGTCAC
nccaR-1 (probe #1)	GAATGTCTGCACAGCCGCTTT
nccaR-1 (probe #2)	GCTTTCCACACAGACATCATAACAAA
nccaR-2 (probe #1)	GTTCAATATTACAGGCGAAC
nccaR-2 (probe #2)	GTGTGTTAATTAATTAATGCTTGTAGG
Cyto $tRNA^{LysCUU}$	GTCTCATGCTCTACCGACT
Mito $tRNA^{ValUAC}$	GTGTTAAGCTACACTCTG
miR-16	GCCAATATTTACGTGCTGCTA
U2 snRNA	CCAACTCCTAGTTCCAAAAATCC
U1 snRNA	GTGATCATGGTATCTCCCCTGCCAG
5S rRNA	GTTCAGGGTGGTATGGCCGT

Table S3. Sequences of primers for standard RT-qPCR

Target	Primer	Sequence (5′–3′)
TNRT1	Forward	GATTCTAGGGAACCTGATGC
	Reverse	GATGTCATGGCCACTTACAG
RPLP0	Forward	CTATCATCAACGGGTACAAACGAG
	Reverse	CAGATGGATCAGCCAAGAAGG
GAPDH	Forward	GTCTTCACCACCATGGAGAAGG
	Reverse	ATGATCTTGAGGCTGTTGTCAT