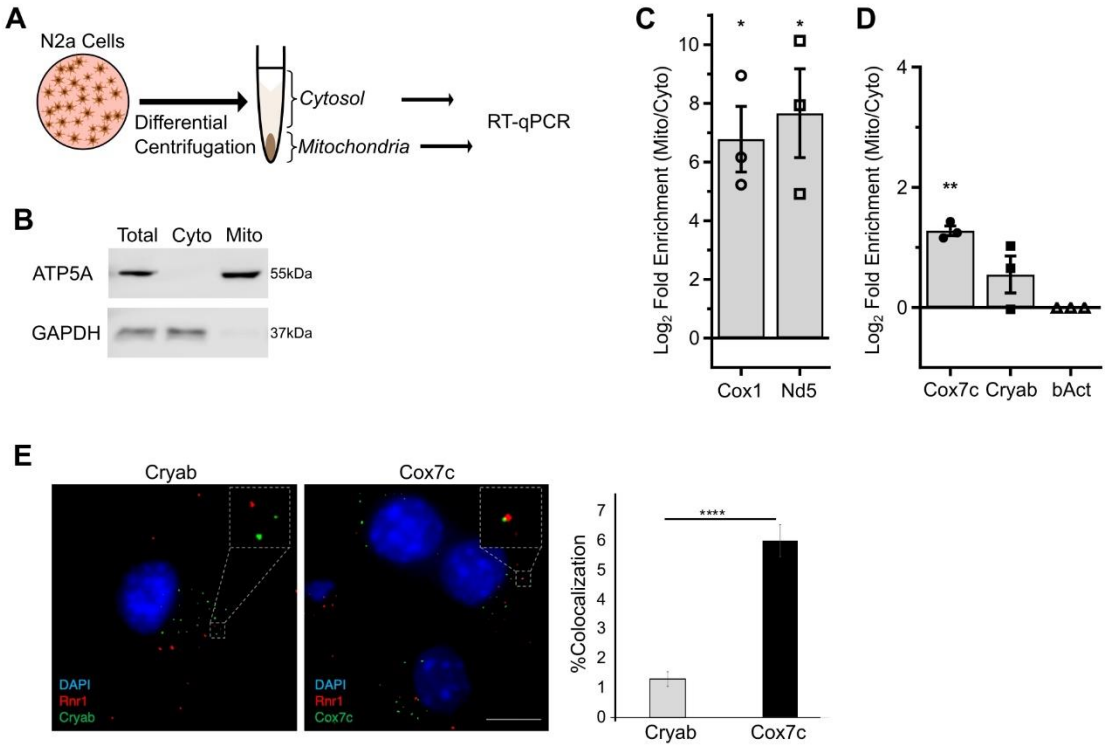

Fig. S1. Cox7c mRNA is associated with mitochondria from N2a cells. A) Schematic workflow: N2a cells were collected and fractionated by differential centrifugation into cytosolic and mitochondrial fractions followed by RNA purification and RT-qPCR. B) Western analysis of the unfractionated total sample, cytosolic (Cyto) and mitochondrial (Mito) fractions with a mitochondria marker (ATP5A) and cytosol marker (GAPDH), demonstrating fractionation purity. C) RT-qPCR analysis of mitochondrial and cytosolic fraction for mRNAs encoded inside the mitochondria. All values are normalized to β-actin transcript levels and presented as log2 fold enrichment of mitochondria and cytosolic Ct signals. Error bars are SEM. Two-way ANOVA, *p<0.05. n = 3 independent biological repeats. D) RT-qPCR analysis of mitochondrial and cytosolic fraction for mRNAs encoded in the nucleus, separated to those that their proteins are destined to mitochondria or cytosol. Quantification as in panel C. Error bars are SEM. Two-way ANOVA, **p<0.01. n = 3 independent biological repeats. E) Representative images of smFISH done on N2a cells with the mRNA (green) of Cryab (left) or Cox7c (right). Probes targeting the 21S mitochondrial rRNA (Rnr1-red) were used to detect mitochondria signal. The histogram presents the percent of spots of each mRNA that colocalize with Rnr1 signals. Student's t-test, ****p<0.0001. n=82, 69 cells for Cox7c and Cryab, respectfully, in three independent experiments. To minimize ambiguity, only spots with overlapping signals were considered as colocalized. Scale bar = 5 μm.

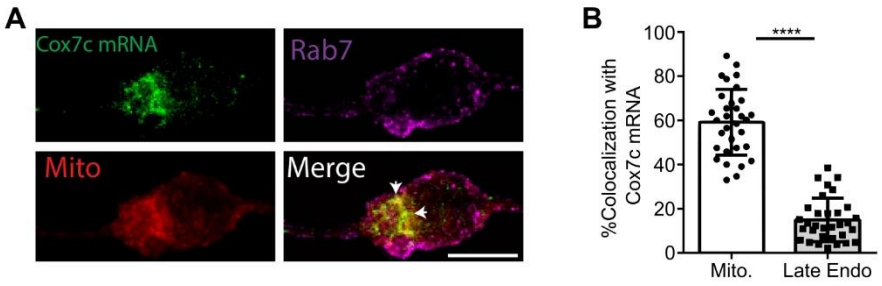

Fig. S2. Cox7c mRNA is associated with mitochondria in motor neurons cell body. A) Representative images of smFISH done on primary motor neurons for Cox7c mRNA (green) along with immunostaining for late endosomes (Rab7 marker, magenta) and mitochondria staining (MitoTracker, red). Arrows indicate areas of colocalization between mitochondria and Cox7c mRNA. Scale bar =10µm. B) Colocalization analysis of Cox7c mRNA with mitochondria and late endosomes signals. n=33 cells from 3 repeats. Error bars are SD. Unpaired-t-test ****p<0.0001.

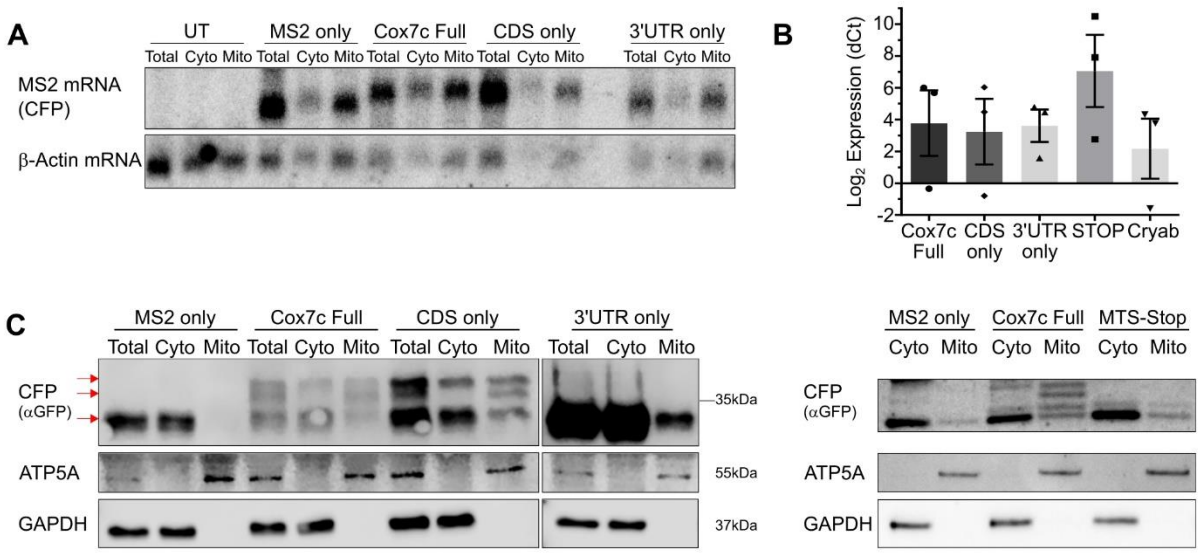

Fig. S3. MS2 constructs are expressed in N2a cells. A) Northern analysis to verify transcripts at the expected size, in unfractionated total sample (Total), cytosolic (Cyto) and mitochondrial (Mito) N2a fractions either from untransfected cells (UT) or transfected with the indicated constructs. Probes indicating MS2 transcripts expression (CFP) and endogenous mRNA expression (β-Actin) were used. B) RT-qPCR analysis of total MS2 mRNAs levels, in N2a cells transfected with different MS2 constructs. CFP levels are used as indicators for mRNA levels. All values are normalized to β-actin transcript and presented as log2 expression of Ct signals. Error bars are SEM. n = 3 independent biological repeats. C) Western blot analysis of cytosolic (Cyto) and mitochondrial (Mito) fractions from cells transfected with the indicated constructs, to verify translation of the Cox7c-CFP fusion proteins. Mitochondria marker (ATP5A) and cytosol marker (GAPDH), demonstrating fractionation sufficiency. Upper, middle and lower red arrows to the left, indicate the precursor Cox7c-CFP, mature Cox7c-CFP and CFP alone, respectively.

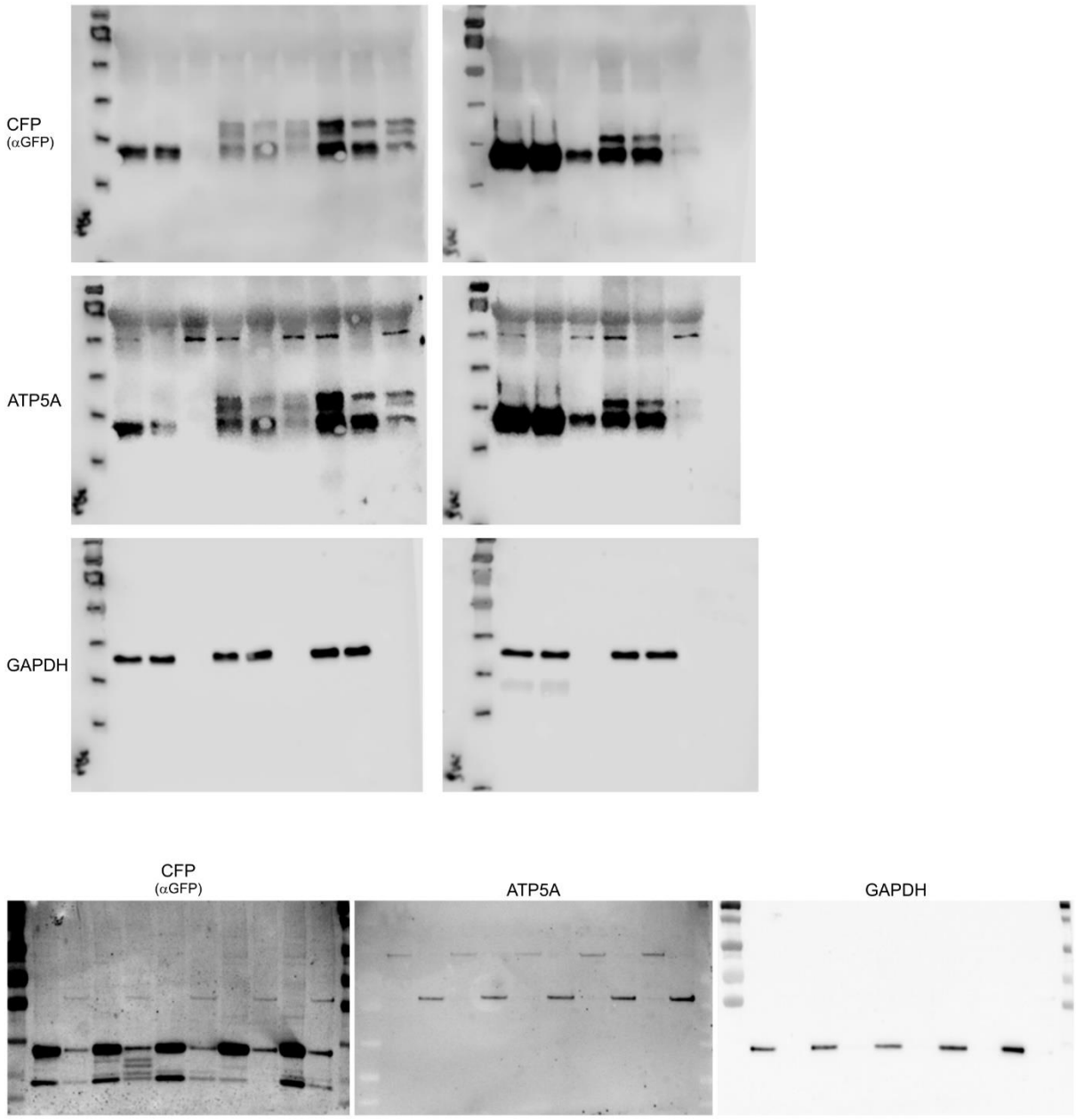

Fig. S4. Blot transparency. Full, uncropped western blots corresponding to western blots in figure S3 C.

Table S1. List of primers used for real-time PCR

Primer name	Primer sequence
β-actin Fw	ATGGATGACGATATCGCTG
β-actin Rv	GTTGGTAACAATGCCATGTTC
Cox1 Fw	TCCAACTCATCCCTTGACATC
Cox1 Rv	TCCTGCTATGATAGCAAACACT
Cox7c Fw	AGCATGTTGGGCCAGAGT
Cox7c Rv	ACTGAAAACGGCAAATTCTT
Cryab Fw	ACTTCCCTGAGCCCCTTCTA
Cryab Rv	TGCTTCACGTCCAGATTCAC
Nd5 Fw	AACCACACCTAGCATTCCTAC
Nd5 Rv	CAGGCGTTGGTGTTGCAGGTA
CFP Fw	CGTGACCACCCTGACCTGG
CFP Rv	TCCTGGACGTAGCCTTCGG

Table S2. List of primers used for plasmid cloning

Primer name	Primer sequence
Cox7c CDS Fw	ATATGCGGCCGCATGTTGGGCCAGAGTAT
Cox7c CDS Rv	CAGCACCGGTCGTTTTTTAAGTAGCTGGTGT
Cox7c 3’ UTR Fw	CCCGATCGATGGATATTTAATTCATCCCT
Cox7c 3’ UTR Rv	GCCGATCGATCAGAGACGAGGCATTG
Cox7c CDS no-MTS Fw	ATATGCGGCCGCCACCATGGTTAGCCACTATGAGGAG
Cox7c CDS stop-MTS Rv	ATATGCGGCCGCCACCATGGTTTTGGGCTAGAGTAT
Cryab CDS Fw	ATTTGCGGCCGCATGGACATCGCCATCC
Cryab CDS Rv	CAATACCGGTACCTTCTTAGGGGCTGCG
Cryab 3’ UTR Fw	CCCGATCGATATCCCCTTTCCTCATTG
Cryab 3’ UTR Rv	GCCGATCGATCAGAGACGAGGCATTG

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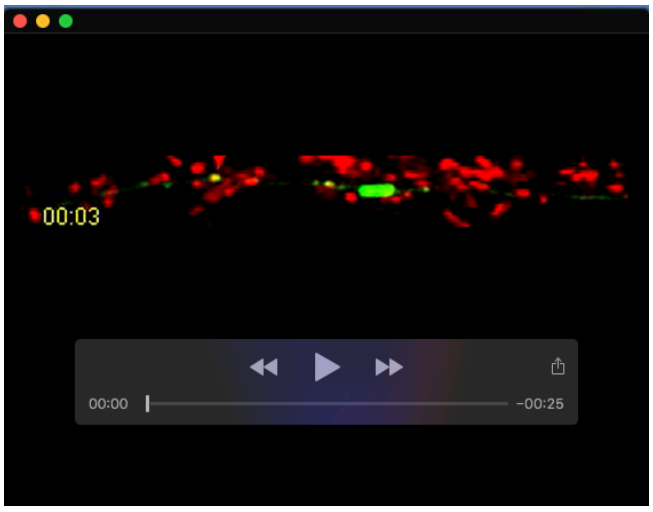
Movie 1. mRNA of Cox7c-MS2 visualized with MCP-GFP construct and displays complete co-localization and co-transport with mitochondria in axons. Live-cell imaging of mRNA particles (green) together with mitotracker marked mitochondria (red) in a motor neuron axon. The red arrows indicate Cox7c mRNA particles co-transported with mitochondria. Movie speed is 10 frames per second, at 3 seconds per frame.



Movie 2. mRNA of Cryab-MS2 visualized with MCP-GFP construct and displays low co-localization and co-transport with mitochondria in axons. live-cell imaging of mRNA particles (green) together with mitotracker marked mitochondria (red) in a motor neuron axon. The red arrows indicate Cryab mRNA particles colocalized with static mitochondria. Note that moving mRNA puncta are not colocalized with mitochondria. Movie speed is 10 frames per second, at 3 seconds per frame.

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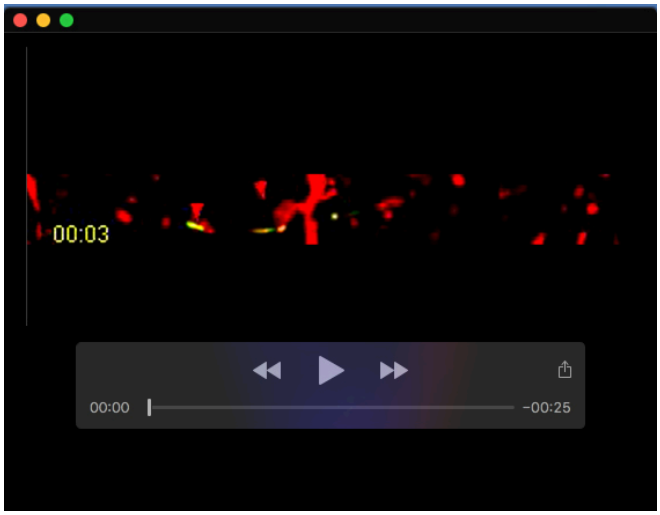
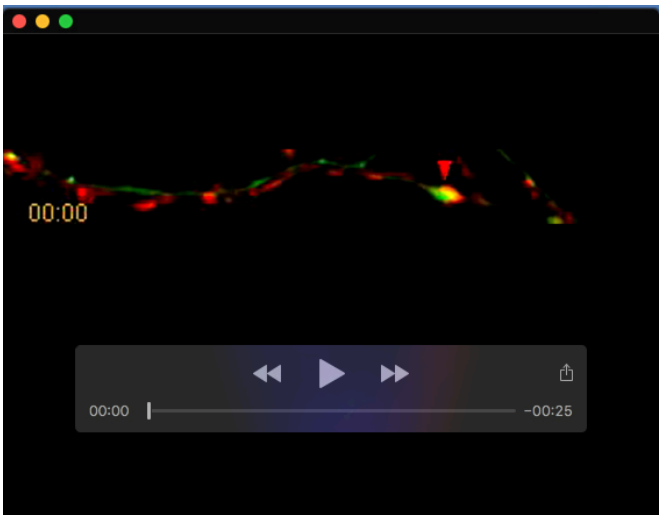
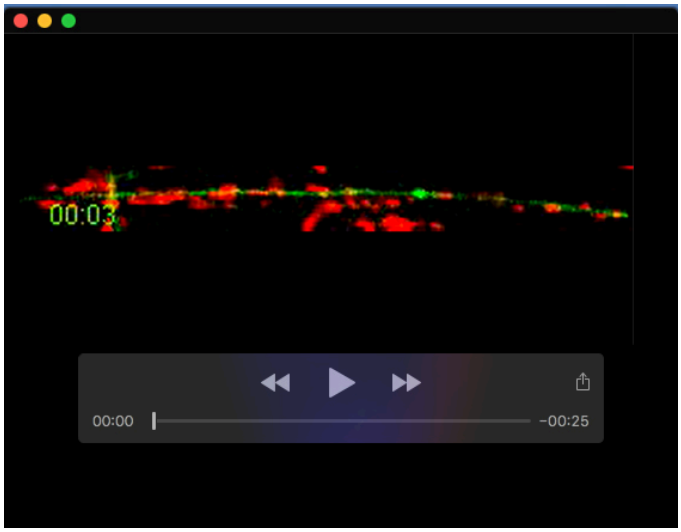


Movie 3. mRNA of Cox7c-CDS-only MS2 visualized with MCP-GFP construct displays complete co-localization and co-transport with mitochondria in axons. Live-cell imaging of mRNA particles (green) together with mitotracker-marked mitochondria (red) in a motor neuron axon. The red arrows indicate Cox7c CDS-only mRNA particles co-localized with static mitochondria and co-transported with moving mitochondria. Movie speed is 10 frames per second, at 3 seconds per frame.



Movie 4. mRNA of Cox7c 3'UTR-only MS2 visualized with MCP-GFP construct and displays low frequency of co-localization with mitochondria in axons. Live-cell imaging of mRNA particles (green) together with mitotracker-marked mitochondria (red) in a motor neuron axon. The red arrows indicate Cox7c 3'UTR-only mRNA particles co-localized with static mitochondria. Note that moving mRNA puncta are not co-localized with mitochondria. Movie speed is 10 frames per second, at 3 seconds per frame.



Movie 5. mRNA of Cox7c-MTS STOP MS2 visualized with MCP-GFP construct and displays low frequency of co-localization with mitochondria in axons. Live-cell imaging of mRNA particles (green) together with mitotracker-marked mitochondria (red) in a motor neuron axon. The red arrow indicates Cox7c-MTS-STOP mRNA particles co-localizes with static mitochondria. Note that moving mRNA puncta are not colocalized with mitochondria. Movie speed is 10 frames per second, at 3 seconds per frame.