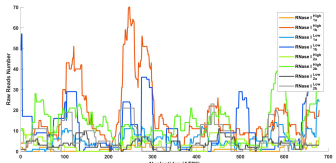
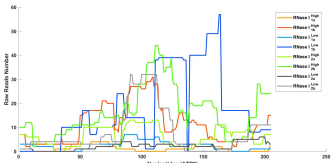
Type of resource	Source	ID
Deposited Data		
Human brain tissues (BA9)	Hoss AG. *et al.* 2015	PRJNA272617
Human liver tissues (CHC)	Butt AM. *et al.* 2016	PRJNA347838
HeLa cell culture	Zhang K. *et al.* 2018	PRJNA473925
Neuronal progenitor human cell culture	Young-Soo Kwon	PRJNA438936
teratoma-derived fibroblast human cell culture	Young-Soo Kwon	PRJNA438936
Mouse brain tissues	Viljetic B. *et al.* 2017	PRJNA309689
Mouse liver tissues	Jee D. *et al.* 2018	PRJNA395699
Mouse embryonic stem cell culture (mESC)	Zamudio JR. *et al.* 2014	PRJNA218026
Mouse kidney tissues	Woo YM. *et al.*2017	PRJNA342099
Chicken brain and liver tissues	Warnefors M. *et al.* 2017	PRJNA396511
Zebrafish brain and liver tissues	Vaz C. *et al.* 2015	PRJNA245824
Huh7.5 human hepatoma cell culture	Moore MJ. *et al.* 2015	PRJNA296130
N2A mouse neuroblastoma cell culture	Moore MJ. *et al.* 2015	PRJNA296129
HCT116 cell culture	Golden RJ. *et al.* 2017	PRJNA354540
HeLa cell culture (Ago2-KOs)	Schuster S. *et al.* 2019	PRJNA550031
Experimental Models: Cell Lines		
HeLa cell culture	Xiang-Dong Fu	GSM3168220
Neuronal progenitor human cell culture	Young-Soo Kwon	GSM3052811
teratoma-derived fibroblast human cell culture	Young-Soo Kwon	GSM3052815
Mouse embryonic stem cell culture (mESC)	Jesse R Zamudio	GSM1224442
Huh7.5 human hepatoma cell culture	Moore MJ. *et al.* 2015	PRJNA296130
N2A mouse neuroblastoma cell culture	Moore MJ. *et al.* 2015	PRJNA296129
HCT116 human cell culture	Golden RJ. *et al.* 2017	GSE89942
HeLa cell culture (Ago2-KOs)	Schuster S. *et al.* 2019	PRJNA550031
Experimental Models: Organisms/Strains		
Mouse Brain tissues from mix of strains 129 and C57BL/6	The Jackson laboratory	No:002448 No:000664
Mouse liver tissues from strain B6.Cg-Tg(Vav1-icre)A2Kio/J vav1-cre	The Jackson laboratory	No:008610
kidney tissues from mice with floxed Pkd1	Stefan Somlo	NA
Chicken brain and liver tissues	Peter Jensen	NA
Zebrafish brain and liver tissues from Singapore strain	Obtained from individuals in the original study	NA
Software and Algorithms		
BowTie2	Langmead and Salzberg 2012	http://bowtie-bio.sourceforge.net/bowtie2/index.shtml
Bedtool	Quinlan and Hall 2010	https://bedtools.readthedocs.io/en/latest/
Ncbi Blast	Altschul *et al.*1997	https://blast.ncbi.nlm.nih.gov/Blast.cgi?CMD=Web&PAGE_TYPE=BlastHome
R package *circlize*	Gu *et al.* 2014	https://jokergoo.github.io/circlize_book/
MATLAB	The MathWorks, Inc.	https://au.mathworks.com/
Vsearch	Rognes *et al.* 2016	https://github.com/torognes/vsearch
RNAfold	Lorenz *et al.* 2011	http://rna.tbi.univie.ac.at/cgi-bin/RNAWebSuite/RNAfold.cgi
AliView	Larsson *et al.* 2014	https://ormbunkar.se/aliview/
VaRNA	Darty *et al.* 2009	http://varna.lri.fr/

Gene-by-gene analysis of samples treated with different contrations of RNase I

The **figure S1** highlights the lack of effect from different concentrations of RNase I, across multiple mitochondrial genes.

In the following figure, we show the small RNAs transcriptional signature of all the mitochondrial genes, divided by gene. The genes order is protein-coding, rRNAs, tRNAs, and within each group, the genes are in alphabetic order.


Raw Reads Number
Nucleotides (ATP6)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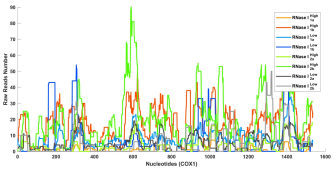
Nucleotides (ATP8)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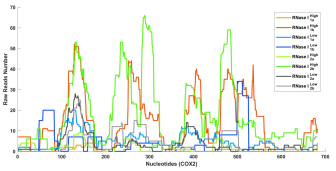
Nucleotides (COX1)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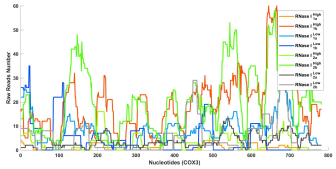
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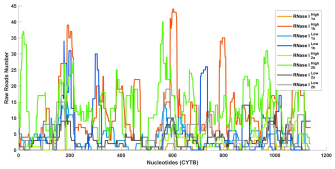
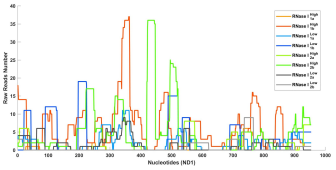
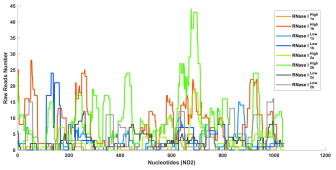
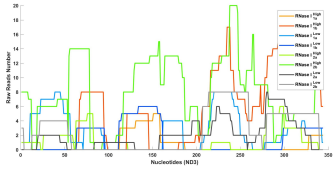
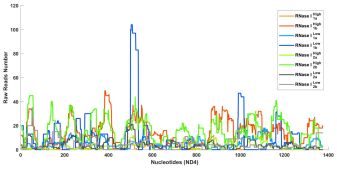
Nucleotides (COX3)

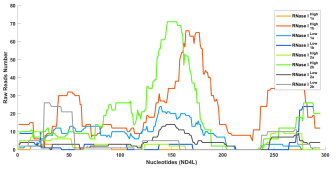

Raw Reads Number
Nucleotides (CYTB)

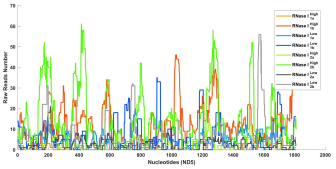

Raw Reads Number
Nucleotides (ND1)


Raw Reads Number
Nucleotides (ND2)


Raw Reads Number
Nucleotides (ND3)


Raw Reads Number
Nucleotides (ND4)


Raw Reads Number
Nucleotides (ND4L)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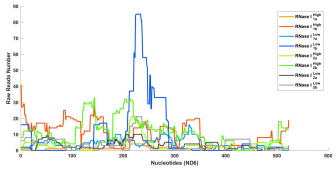
Nucleotides (ND5)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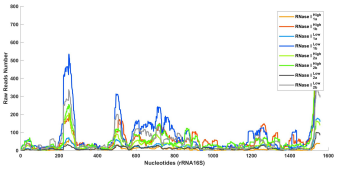
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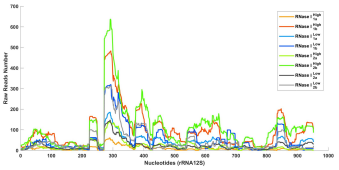
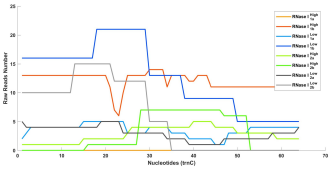
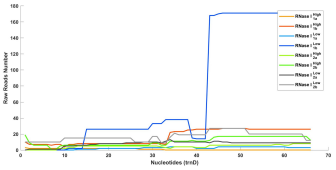
Nucleotides (rRNA16S)


Raw Reads Number
Nucleotides (rRNA12S)


Raw Reads Number
Nucleotides (trnC)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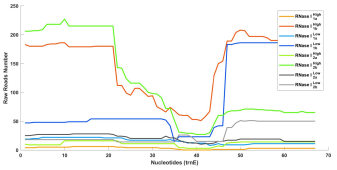
Nucleotides (trnD)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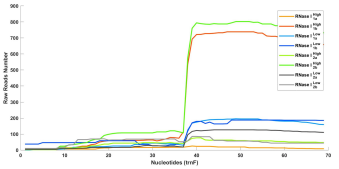
Nucleotides (trnE)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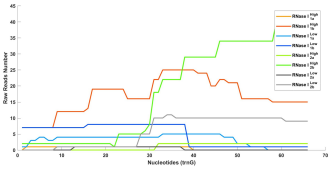
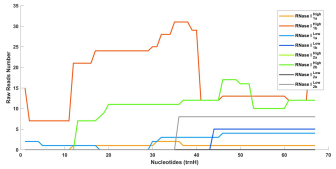
Nucleotides (trnF)


Raw Reads Number
Nucleotides (trnG)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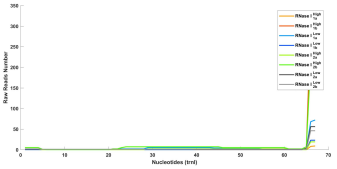
Nucleotides (trnH)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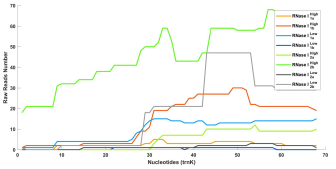
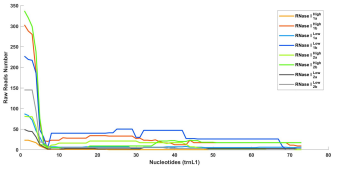
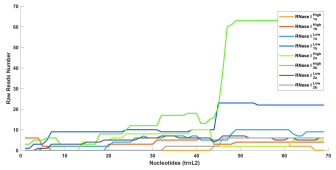
Nucleotides (trnI)


Raw Reads Number
Nucleotides (trnK)


Raw Reads Number
Nucleotides (trnL1)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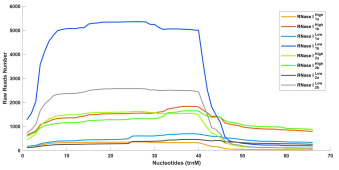
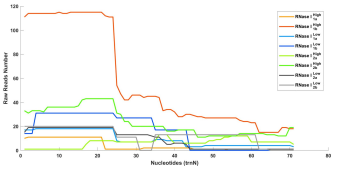
Nucleotides (trnL2)


Raw Reads Number
Nucleotides (trnM)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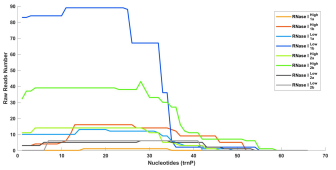
Nucleotides (trnN)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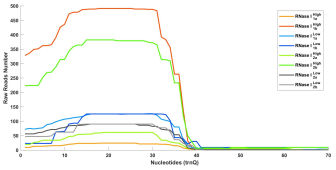
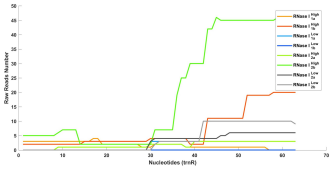
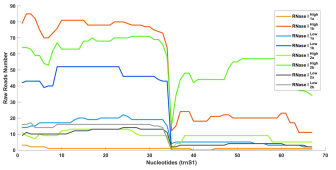
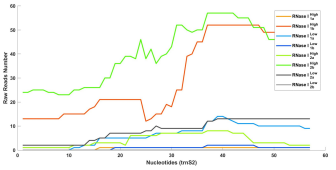
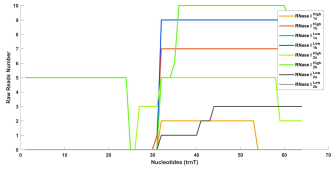
Nucleotides (trnP)

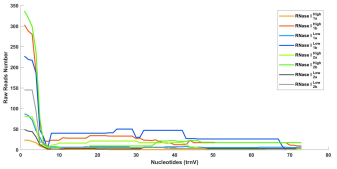

Raw Reads Number
Nucleotides (trnQ)

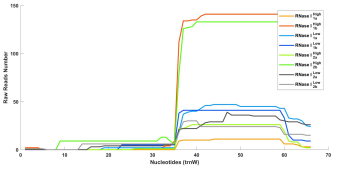

Raw Reads Number
Nucleotides (trnR)


Raw Reads Number
Nucleotides (trnS1)


Raw Reads Number
Nucleotides (trnS2)


Raw Reads Number
Nucleotides (trnT)


Raw Reads Number
Nucleotides (trnV)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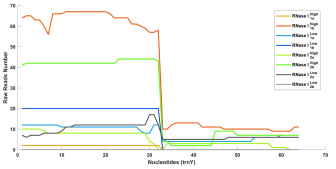
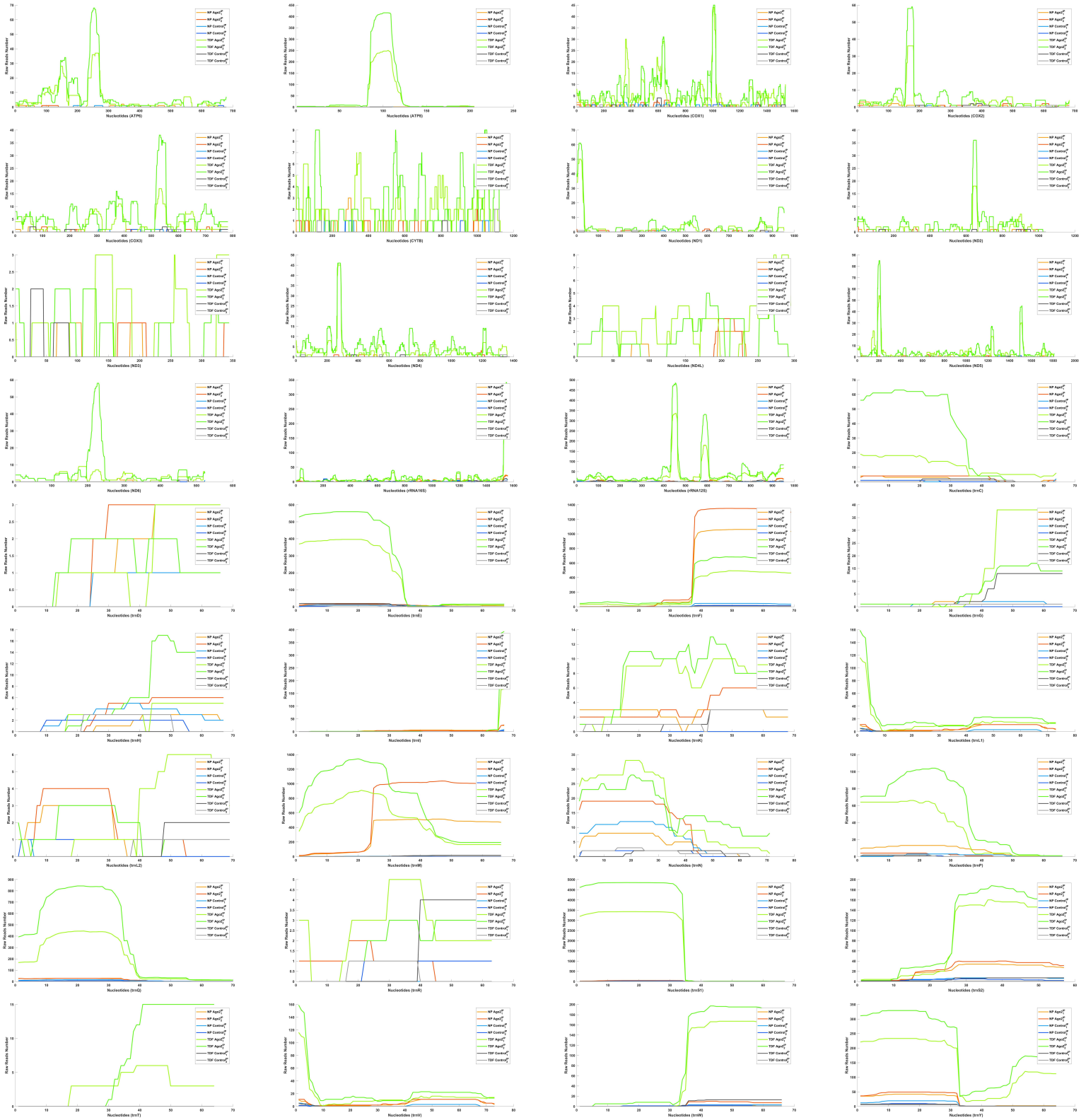
Nucleotides (trnW)


Raw Reads Number
Nucleotides (trnY)

Gene-by-gene analysis of Ago2-IP and mock-IP samples

The **figure S2** highlights the enrichment of Ago2-IP samples compared to IgG-IP samples, across multiple mitochondrial genes.

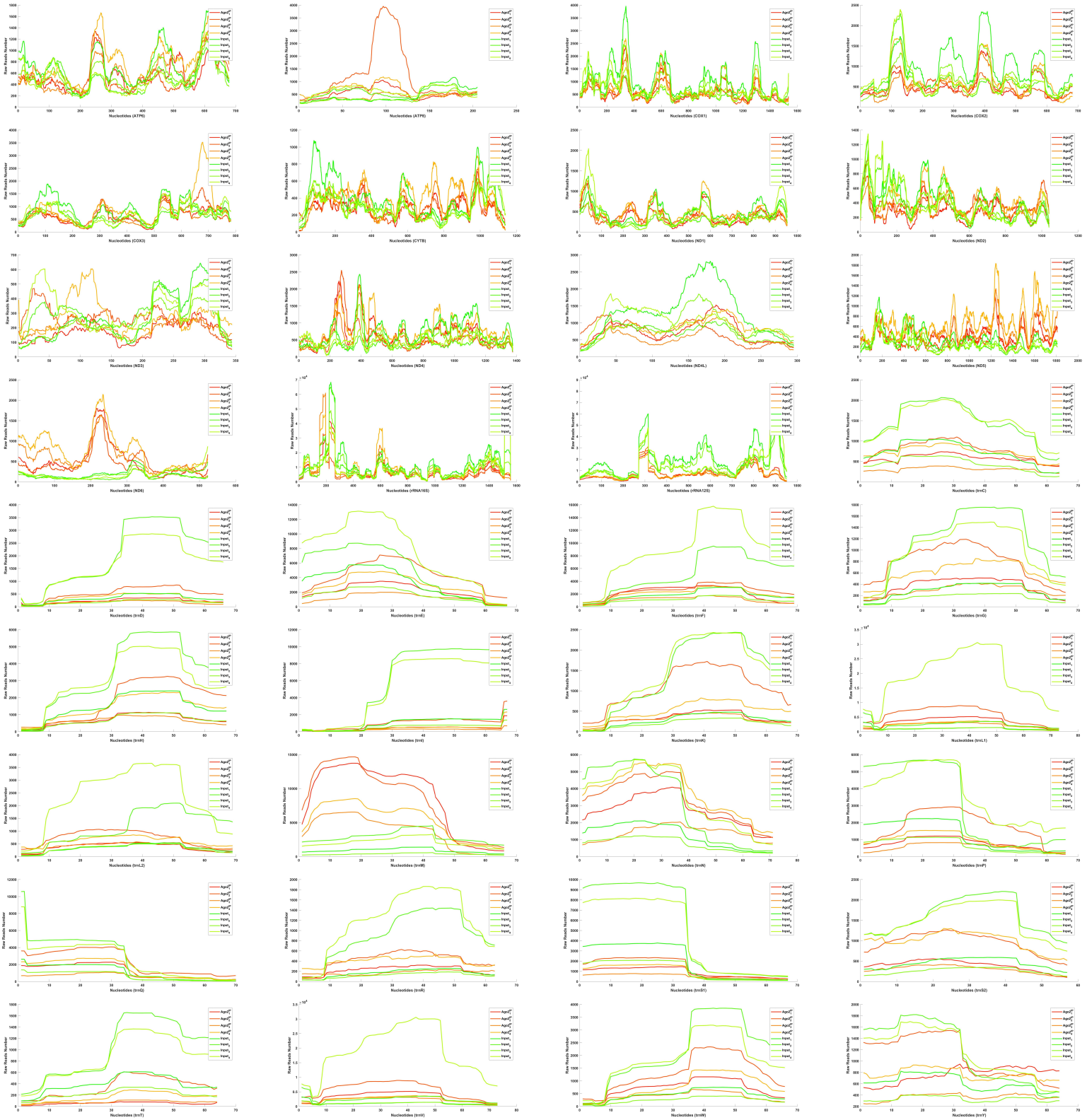
In the following figure, we show the small RNAs transcriptional signature of all the mitochondrial genes, divided by gene for neuronal progenitors (NP) and teratoma-derived fibroblast (TDF). The genes order is protein-coding, rRNAs, tRNAs, and within each group, the genes are in alphabetic order.


NP Ago2+
NP Ago2-
NP Control+
NP Control-
TDF Ago2+
TDF Ago2-
TDF Control+
TDF Control-
Raw Reads Number
Nucleotides (ATP6)
Nucleotides (ATP8)
Nucleotides (COX1)
Nucleotides (COX2)
Nucleotides (COX3)
Nucleotides (CYTB)
Nucleotides (ND1)
Nucleotides (ND2)
Nucleotides (ND3)
Nucleotides (ND4)
Nucleotides (ND4L)
Nucleotides (ND5)
Nucleotides (ND6)
Nucleotides (rRNA16S)
Nucleotides (rRNA12S)
Nucleotides (trnC)
Nucleotides (trnD)
Nucleotides (trnE)
Nucleotides (trnF)
Nucleotides (trnG)
Nucleotides (trnH)
Nucleotides (trnI)
Nucleotides (trnK)
Nucleotides (trnL1)
Nucleotides (trnL2)
Nucleotides (trnM)
Nucleotides (trnN)
Nucleotides (trnP)
Nucleotides (trnQ)
Nucleotides (trnR)
Nucleotides (trnS1)
Nucleotides (trnS2)
Nucleotides (trnT)
Nucleotides (trnV)
Nucleotides (trnW)
Nucleotides (trnY)

Gene-by-gene analysis of HeLa cells with Ago2-IP and without

The **figure S3** highlights the enrichment in small mitochondrial RNAs of specific genes after Ago2-IP treatment, across multiple mitochondrial genes.

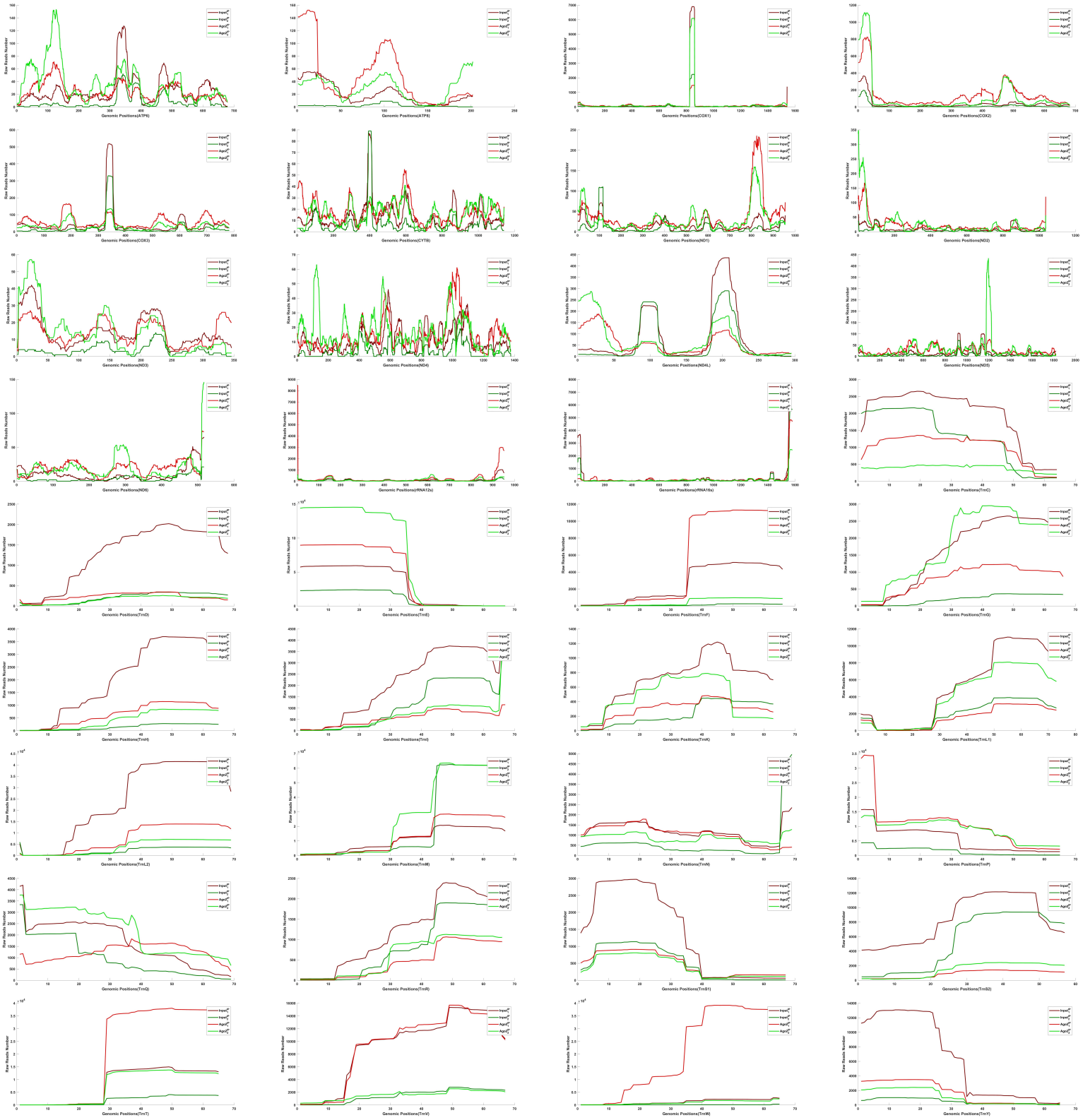
In the following figure, we show the small RNAs transcriptional signature of all the mitochondrial genes, divided by gene. The genes order is protein-coding, rRNAs, tRNAs, and within each group, the genes are in alphabetic order. The samples are paired, thus the samples of each treatment have to be compared to their corresponding number. For example, $Input_1$ should be compared to $Ago2^{IP}{}_1$ and not to $Ago2^{IP}{}_2$.

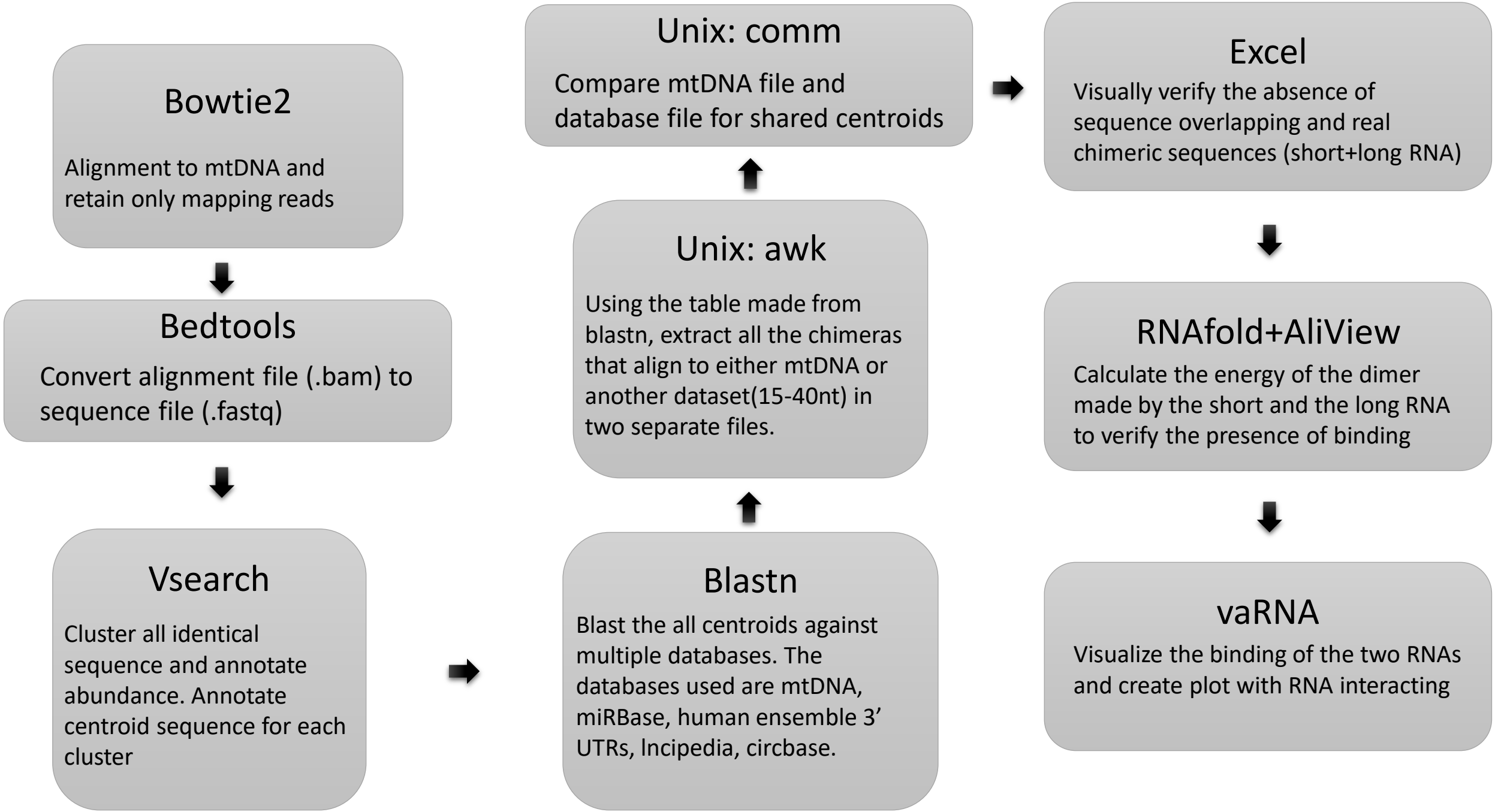

Raw Reads Number
Nucleotides (ATP6)
Nucleotides (ATP8)
Nucleotides (COX1)
Nucleotides (COX2)
Nucleotides (COX3)
Nucleotides (CYTB)
Nucleotides (ND1)
Nucleotides (ND2)
Nucleotides (ND3)
Nucleotides (ND4)
Nucleotides (ND4L)
Nucleotides (ND5)
Nucleotides (ND6)
Nucleotides (rRNA16S)
Nucleotides (rRNA12S)
Nucleotides (trnC)
Nucleotides (trnD)
Nucleotides (trnE)
Nucleotides (trnF)
Nucleotides (trnG)
Nucleotides (trnH)
Nucleotides (trnI)
Nucleotides (trnK)
Nucleotides (trnL1)
Nucleotides (trnL2)
Nucleotides (trnM)
Nucleotides (trnN)
Nucleotides (trnP)
Nucleotides (trnQ)
Nucleotides (trnR)
Nucleotides (trnS1)
Nucleotides (trnS2)
Nucleotides (trnT)
Nucleotides (trnV)
Nucleotides (trnW)
Nucleotides (trnY)

Gene-by-gene analysis of mouse embryonic stem cells with Ago2-IP and without

The **figure S4** highlights the enrichment in small mitochondrial RNAs of specific genes after Ago2-IP treatment, across multiple mitochondrial genes.

In the following figure, we show the small RNAs transcriptional signature of all the mitochondrial genes, divided by gene. The genes order is protein-coding, rRNAs, tRNAs, and within each group, the genes are in alphabetic order. The samples are paired, thus, the samples of each treatment have to be compared to their corresponding number. For example, $InputIP_1$ should be compared to $Ago2^{IP}{}_1$ and not to $Ago2^{IP}{}_2$.


Raw Reads Number
Genomic Positions(ATP6)
Genomic Positions(ATP8)
Genomic Positions(COX1)
Genomic Positions(COX2)
Genomic Positions(COX3)
Genomic Positions(CYTB)
Genomic Positions(ND1)
Genomic Positions(ND2)
Genomic Positions(ND3)
Genomic Positions(ND4)
Genomic Positions(ND4L)
Genomic Positions(ND5)
Genomic Positions(ND6)
Genomic Positions(rRNA12s)
Genomic Positions(rRNA16s)
Genomic Positions(TrnC)
Genomic Positions(TrnD)
Genomic Positions(TrnE)
Genomic Positions(TrnF)
Genomic Positions(TrnG)
Genomic Positions(TrnH)
Genomic Positions(TrnI)
Genomic Positions(TrnK)
Genomic Positions(TrnL1)
Genomic Positions(TrnL2)
Genomic Positions(TrnM)
Genomic Positions(TrnN)
Genomic Positions(TrnP)
Genomic Positions(TrnQ)
Genomic Positions(TrnR)
Genomic Positions(TrnS1)
Genomic Positions(TrnS2)
Genomic Positions(TrnT)
Genomic Positions(TrnV)
Genomic Positions(TrnW)
Genomic Positions(TrnY)


Bowtie2
Alignment to mtDNA and retain only mapping reads
Bedtools
Convert alignment file (.bam) to sequence file (.fastq)
Vsearch
Cluster all identical sequence and annotate abundance. Annotate centroid sequence for each cluster
Blastn
Blast the all centroids against multiple databases. The databases used are mtDNA, miRBase, human ensemble 3' UTRs, lncipedia, circbase.
Unix: awk
Using the table made from blastn, extract all the chimeras that align to either mtDNA or another dataset(15-40nt) in two separate files.
Unix: comm
Compare mtDNA file and database file for shared centroids
Excel
Visually verify the absence of sequence overlapping and real chimeric sequences (short+long RNA)
RNAfold+AliView
Calculate the energy of the dimer made by the short and the long RNA to verify the presence of binding
vaRNA
Visualize the binding of the two RNAs and create plot with RNA interacting