6. Association of novel miRNA with mitochondria: miR-293m80059

The deep sequencing of small RNA from mitochondria approach elucidated specific novel miRNAs from mitochondria. However the functional significance is not well understood. In this chapter the novel miRNAs are elaborated, their association with mitochondria is validated and role of a novel miRNA. Further, the role of miR-293m80059 in ND2 processing and regulation of mitochondrial genome function has been characterized.

6.1 Analysis of novel miRNAs associated with mitochondria from HEK293 and HeLa

In principle, deep sequencing of sRNA generates sequences from as yet unannotated regions of the genome. A significant number of reads from unannotated region of chromosome were found in the small RNA libraries.

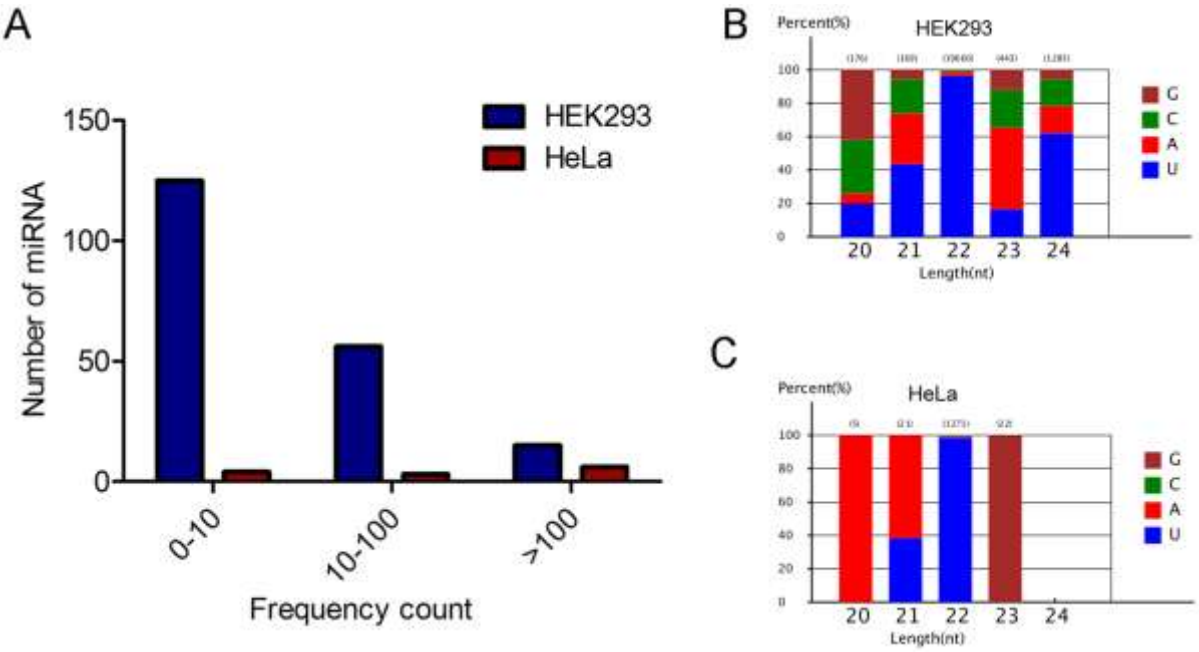

Figure 6.1. Analysis of putative novel miRNAs associated with human mitochondria*. The putative novel miRNAs were predicted from unannotated clean reads using Mireap software. (A) The distribution of putative novel miRNAs levels with respect to frequency. Numbers of sequence reads are taken as miRNA levels and the values are represented in the form of range of values in both HEK293 and HeLa mitochondrial sRNA libraries.(B) The percentage of first nucleotide base bias of 18–23 nt putative novel*

miRNAs from mitochondria-associated sRNA library of HEK293. (C) The percentage of first nucleotide base bias of 18–23 nt putative novel miRNAs from mitochondria-associated sRNA library of HeLa.

The unannotated sequences were analyzed through computational pipeline described in methods. In total, 196 and 13 novel miRNAs were identified by virtue of their ability to form miRNA-like single hairpins and other criteria of miRNA from the mitochondria of HEK293 and HeLa, respectively and has been summarized in Table 6.1. The naming convention ‘‘293m8-miRX’’ and ‘‘HM3-miRX’’ was specified for each novel miRNAs from the mitochondrial libraries of HEK293 and HeLa. The frequency count of novel mitomiRs ranged from 5 - 3132 in HEK293 and 5 - 208 in HeLa, respectively (Figure 6.1A). Interestingly, some novel miRNA were equally expressed, similar in sequence and structure but mapped to different genomic location. Analyses of the first nucleotide bias of the 18-30nt miRNAs candidates revealed that uridine (U) was the most common base (more than 90%) at positions 1, 19, 20, 21, 22 of 22 nt long predicted novel miRNAs. The analysis of 20 – 24 nt miRNA showed that first nucleotide of 40% of 20 nt long miRNA started with G, 40% of 21 nt long miRNA with U, >90% 22nt long miRNA with U, >50% of 23 nt long miRNA with A and >60% of 24 nt long miRNA with U in HEK293 (Figure 6.1B). Interestingly, in HeLa 100% 20 nt long miRNA began with A, 60% 21 nt long miRNA with A, 40% 21 nt long miRNA with U, >95% 22 long miRNA with U and 100% 23 nt long miRNA with G (Figure 6.1 C).

6.2 Putative targets of novel miRNAs associated with mitochondria from HEK293 and HeLa

An individual miRNA may regulate several mRNAs in a pathway thus fine-tuning the cellular processes. Hence, we determined the targets of all the novel miRNA enriched in mitochondria of both cell lines. The targets of putative novel miRNAs was analysed and clustered as described in chapter 1. The targets of novel miRNAs of HEK293 also showed enrichment for positive/negative regulation transcription (GO:0006350~transcription, GO:0051252~regulation of RNA metabolic process, GO:0010629~negative regulation of gene expression). However many important nucleic acid, protein metabolic and catabolic

related GO terms were also found to be enriched (GO:0051254~positive regulation of RNA metabolic process, GO:0045935~positive regulation of nucleic acid metabolism, GO:0030163~protein catabolic process, GO:0019220~regulation of phosphate metabolic process, GO:0032268~regulation of cellular protein metabolic process) (Table 6.2). The KEGG analysis of the identified targets of novel miRNA of HEK293 showed the involvement of neurotrophin signaling pathways, cell cycle and phosphatidyl inositol signaling system (Table 6.3). Similarly, target analysis of novel miRNA of mitochondria from HeLa cells was also done. The GO term related to apoptosis, cell cycle, stress response (GO:0042981~regulation of apoptosis, GO:0051726~regulation of cell cycle cellular processes, GO:0000079~regulation of cyclin-dependent protein kinase activity, GO:0001938~positive regulation of endothelial cell proliferation) were enriched clusters from novel miRNA of HeLa (Table 6.4). KEGG analysis of identified targets of novel miRNA of mitochondria from HeLa showed the gene network involved in endocytosis, p53 signaling, adherence junction, dilated cardiomyopathy and cancer (Table 6.5).

6.3 Association of novel miRNA with mitochondria

The putative miRNAs with high count were selected from HEK293 libraries and their association with mitochondria and mitoplast was further determined by qPCR (Figure 6.1A). The enrichment of miR-293m80056, miR-293m80099, miR-293m80134, miR-293m80165 and miR-293m80195 increased in mitoplast preparations whereas miR-293m80022 appeared to surface enriched. This suggests that novel miRNA follows differential association patterns at mitochondria. The role of putative novel miRNA with highest count, miR-293m80134 was determined on mitochondrial functions and cellular viability by transfecting its mimic. Its transfection did not influence cellular ATP production (Figure 6.1B) or cellular viability (Figure 6.1C) and neither induced ROS production (Figure 6.1D). These observations suggest that saturating levels of miR-293m80134 may not influence the mitochondrial function and downstream effect on cell death. Hence, its impact on mitochondrial association needs to be further elucidated.

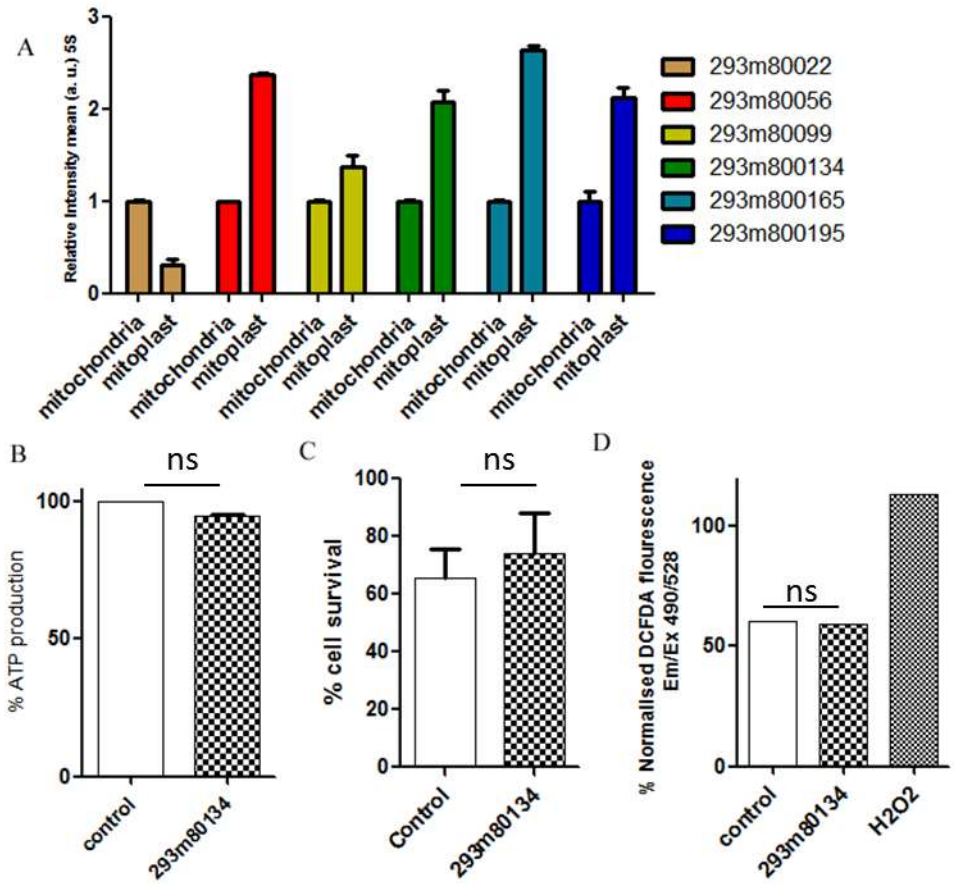

Figure 6.2. Association of novel miRNAs with high count at mitochondria*. A. The association of novel miRNA with mitochondria and mitoplast was performed by qPCR .The role of mmiR-293m80134 on mitochondrial ATP Production levels B. Cellular Viability C. and ROS generation D. was performed.*

6.4 Novel miRNA aligns at mitochondrial genome

The novel miRNAs were enriched in mitochondria; hence their impact on mitochondrial gnome regulation was elucidated. The seed sequences of novel miRNAs were aligned using BLASTN version 2.2.20 program to. The alignment of first 11 bases of 24 putative novel miRNAs at different positions of mitochondrial genome was observed (Figure 6.3A). The 7 putative novel miRNAs aligned to non coding region, 5 to ATP6, 3 to tRNA, 2 to 12S rRNA, 2 to ND2 and 1 to HVRI, COI, CytB and ND1 gene region.

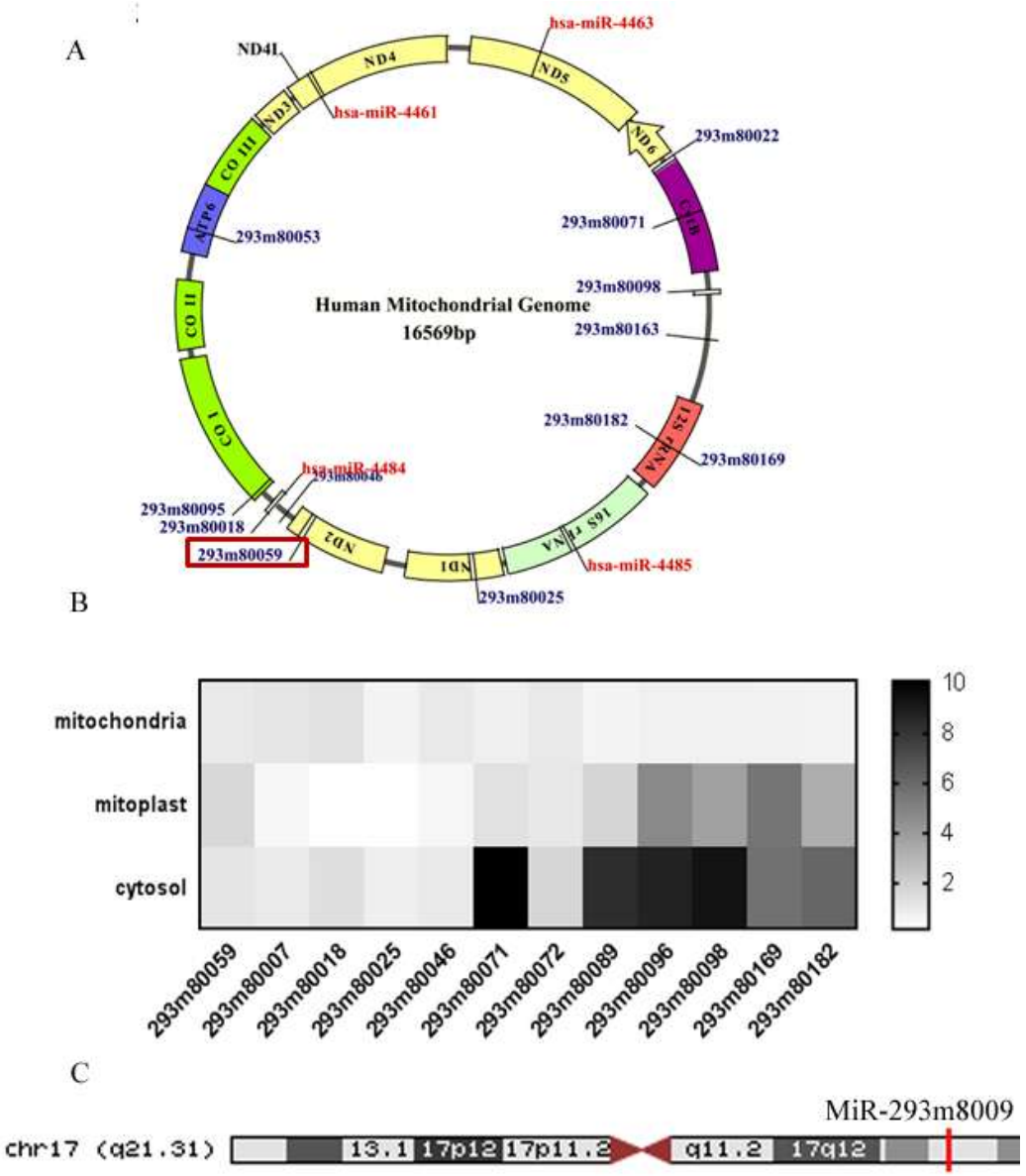

Figure 6.3. Association of novel miRNAs targeting mitochondrial genome*. A. The hypothetical miRNA seed sequence binding sites of novel miRNA were identified by BLASTN tool. B. The association of novel miRNA with mitochondria, cytosol and mitoplast fractions was performed by qPCR. C. Genomic location obtained from UCSC genome browser.*

The enrichment of these miRNA in mitochondria, mitoplast and cytosol was performed by qPCR (Figure 6.3B). miR-293m80071, miR-293m80089, miR-293m8072, miR-293m80096, miR-293m8098, miR-293m80169 and miR-293m80182 were enriched in mitoplast as well as abundant in cytosol; whereas miR-293m80046, miR-293m80025,

miR-293m80018 and miR-293m80007 appeared to mitochondrial surface associated. The enrichment of miR-293m80059 in mitoplast was higher than mitochondria and cytosol, suggesting its specific enrichment inside mitochondria.

6.5 miR-293m80059 associates with mitochondria

miR-293m80059 is encoded from chromosome 17 in nuclear genome and enriched in mitoplasts preparation (Figure 6.3C). To further analyze its specific import, mitochondria were treated with RNaseA (removes non specific RNAs at the surface of mitochondria) and Proteinase K (removes surface bound RNA binding proteins). In RNase A protection assay the levels were unaltered while the levels of miR-293m80059 enriched in mitochondria after treatment of cells with proteinase K (Figure 6.4A). The functional characterization was done in HEK293 using miR-293m80059 mimic. The seed sequence of miR-293m80059 aligned to ND2 region of mitochondrial genome hence its role in and effect on mitochondrial function was analyzed. The sequence similarity was observed in ND2 region of mitochondrial genome hence the levels of miR-293m80059 were determined in mitochondrial DNA depleted cells. The enrichment of miR-293m80059 was not significantly altered in rho0 cells (Figure 6.4B) suggesting that it is nuclear encoded miRNA. To further validate it, the cells were treated with leptomycinB, nuclear pore inhibitor. Leptomycin B treatment decreased miR-293m80059 at mitochondria, confirming its canonical origin. Further, DICER inhibition by poly-L-Lysine decreased miR-293m80059, suggesting its canonical processing. The treatment with Trypaflavin, Ago2 and TRBP binding inhibitor and mitochondrial transcriptional inhibitor, trypaflavin increased its accumulation (Figure 6.4C). This suggest two possibilities, it's mode of action may be Ago2 independent. Secondly it may be involved in the processing of pre-mRNA and not in its production. This was further supported by estimating its relative levels in rho0 cells as its levels marginally increased. Since, the level are high in mitoplast preparation and sequence alignment was observed in mitochondrial genome, its mimic was transfected to determine its effect on mitochondrial function.

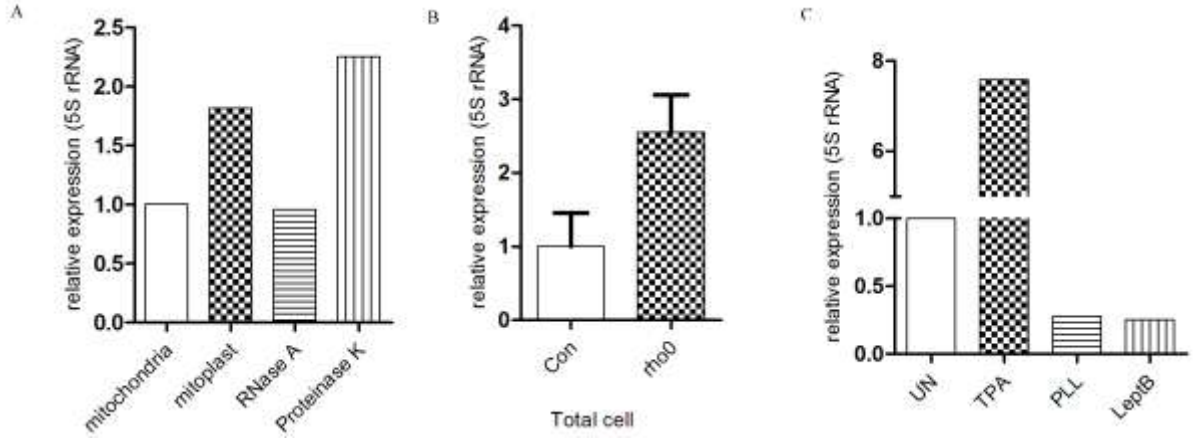

Figure 6.4. miR-293m80059 undergoes canonical processing and associates with mitochondria. *A. The extent of miR-293m80059 associations with mitochondrial compartments was determined by preparing mitoplasts, RNase A and Proteinase K (PK) protection assays. B. The levels of miR-293m80059 were determined in mitochondrial DNA depleted cells. C. The cells were treated with trypaflavine (TPA), poly-L-lysine (PLL) and LeptomycinB (LeptB) to inhibit the canonical miRNA processing and the levels of 293m80059 were determined in mitochondrial fractions.*

6.6. miR-293m80059 affects mitochondrial functions

The expression of miR-293m80059 decreased the ATP levels (Figure 6.5A) as compared to control. We further analyzed its effect on mitochondrial transmembrane potential and ROS in presence of miR-293m80059. The mitochondrial membrane potential (Figure 6.5B) decreased whereas ROS (Figure 6.5C) increased in miR-293m80059 mimic transfected cell as compared to control. Mitochondrial complex-I is site of entry of NADH and alteration in complex-I activity is source of ROS generation (Grivennikova et. al., 2006). The activity of mitochondrial complex-I activity and the assembly of ETC complexes was monitored. The in gel activity showed increased activity in the presence of miR-293m80059 mimic transfected cells. The accumulation of smaller subcomplexes was also observed in the miR-293m80059 mimic transfected cells as compared to control (Figure 6.6A) whereas complex-IV activity remains unaltered (Figure 6.6B). However, the effect on accumulation of individual sub-complexes was not observed (Figure 6.6C).

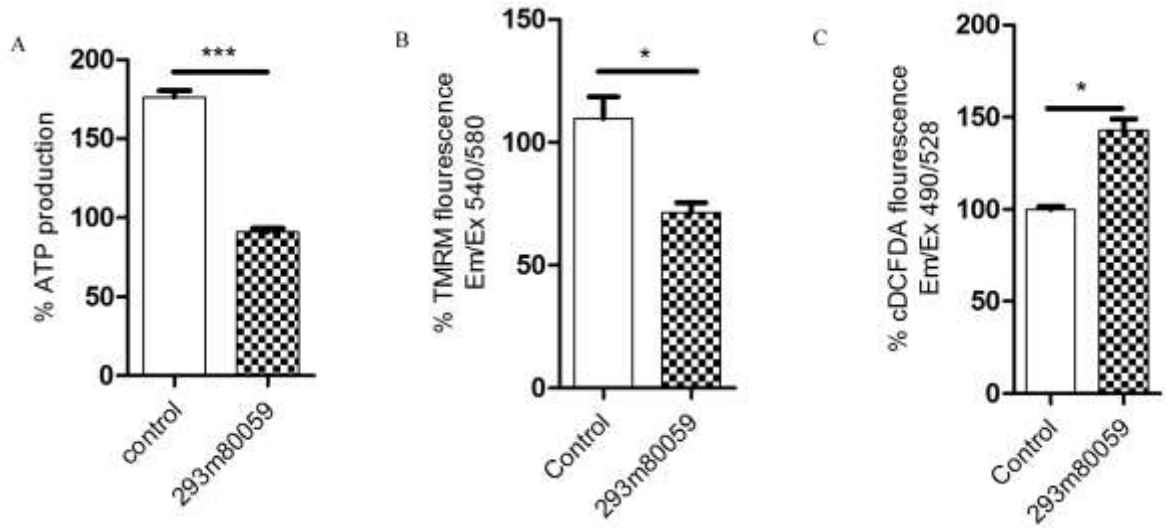

Figure 6.5 miR-293m80059 regulates mitochondrial function. *The mimic of miR-293m80059 was transfected along with control in HEK293 and effect on mitochondrial functions was analyzed. (A) Levels of total cellular ATP (oligomycin was added as control) and (B) Mitochondrial membrane potential by pre-incubating cells with TMRM. (C) Total cellular ROS produced was quantified by staining with CM-H_2DCFDA fluorescence using flourimeter.* $^{*}P < 0.01$, $^{***}P < 0.0001$.

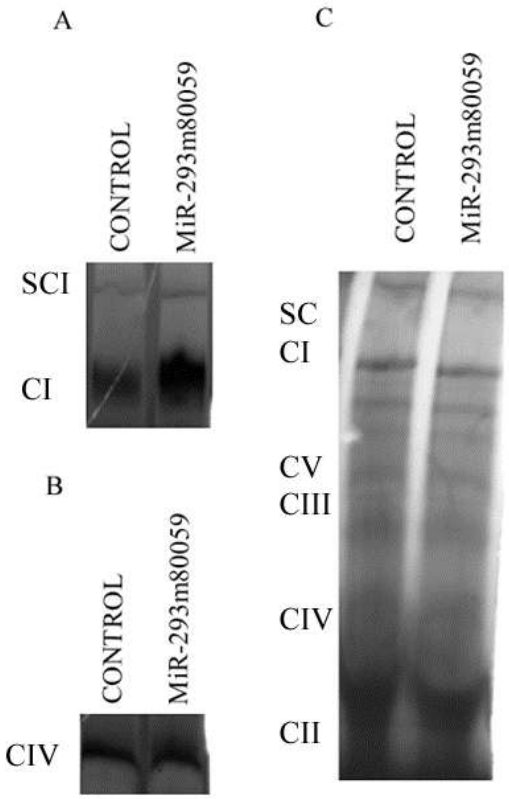

Figure 6.6. miR-293m80059 regulates mitochondrial complex-I assembly. *293m80059 mimic was transfected in HEK293, mitochondria were isolated and in gel activity of A. complex-I and B. complex-IV. C The assimilation of individual mitochondrial complex-I was analysed by staining Blue native gels with coomassie brilliant blue.*

This suggests its specific effect on Complex-1 super accumulation and not on the assembly of individual super complexes. Hence, we further determined the levels of mt-ND2 and the adjacent transcript levels in miR-293m80059 mimic transfected cells as compared to the control cells. Interestingly mt-ND1 and mt-ND2 and adjacent transcripts increased significantly replicates (Figure 6.7A).

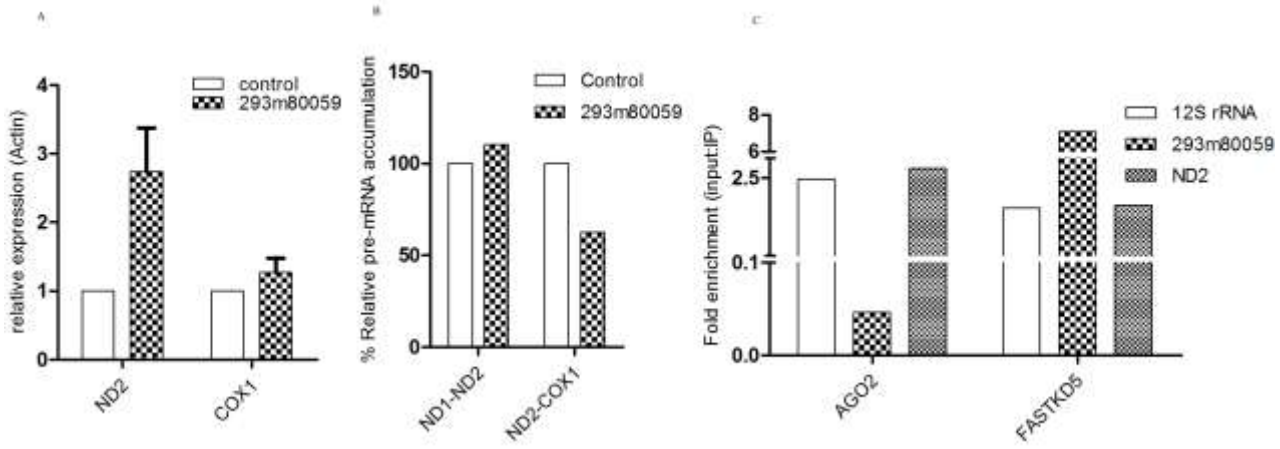

Figure 6.7. miR-293m80059 regulates ND2 processing. *A. The levels of mature mitochondrial genome-encoded transcripts were determined by qPCR in HEK293 cells. B. Its role in mitochondrial RNA processing was analyzed as described in methods. C. The cells were transfected with Flag tagged FASTKD5 and Ago2 and immunoprecipitated as described in methods. The association of 293-m80059 and ND2 were determined by qPCR in input and pull down extracts. *p < 0.01, ***p < 0.0001*

The mitochondrial heavy chain is transcribed into single polycistronic mRNA. The mature transcripts are processed by cleaving off the interspaced tRNA, caping and polA tailing of individual transcript. Emerging reports suggests the processes occur in specialized structure called mitochondrial RNA granules with the involvement of numerous RNA binding proteins (Attonicka et al., 2015). We have recently demonstrated the involvement of miRNA in processing of the mitochondrial pre-mRNA. Hence, we next questioned if

miR-293m80059 regulates the processing of the precursor transcript. Interestingly, the processing intermediate of ND2-COX1 and COX1-COX2 junctions decreased suggesting its role in enhancing the pre-processing (Figure 6.7B). The FASTK family members, DHX family proteins and GRSF were demonstrated to be part of mitochondrial RNA granules (Antonicka et al., 2015).

Interestingly, GRSF1 was previously demonstrated to bind to miRNAs (Antonicka H et al., 2015). Hence we questioned if miR-293m80059 forms a part of the RNA granule. The cells were transfected with Flag tagged FASTKD5 and Ago2 (was taken as control) and immuno-precipitated from mitochondria to analyze miR-293m80059 levels. Interestingly, miR-293m80059 was enriched with FASTKD5 (Figure 6.7C) as compared to Ago-2. These results suggest that miR-293m80059 regulates of mitochondrial genome encoded polycistronic mRNA processing with the mitochondrial RNA granules.

6.7 miR-293m80059 effects cell survival and proliferation

Mitochondria had been considered as central executioner of apoptosis (Shakeri R et al., 2017). Many mitochondrial proteins are released during different oxidative stress and in condition of mitochondrial dysfunction (Saelens et al., 2004). Therefore we monitored the cell survival in the presence of miR-293m80059. The transfection of its miR-293m80059 mimic decreased the cell survival (Figure 6.8A) and increased cellular cytotoxicity (Figure 6.8B) as compared to control mimic transfected cells. In consonance to it we observed a significant increase in activity of executioner caspase, caspase 3/7 (Figure 6.8C) suggesting potentiation of mitochondria mediated apoptosis. Further the role of ROS mediated effect was established by treating cells with NAC and Mitotempo post transfection.

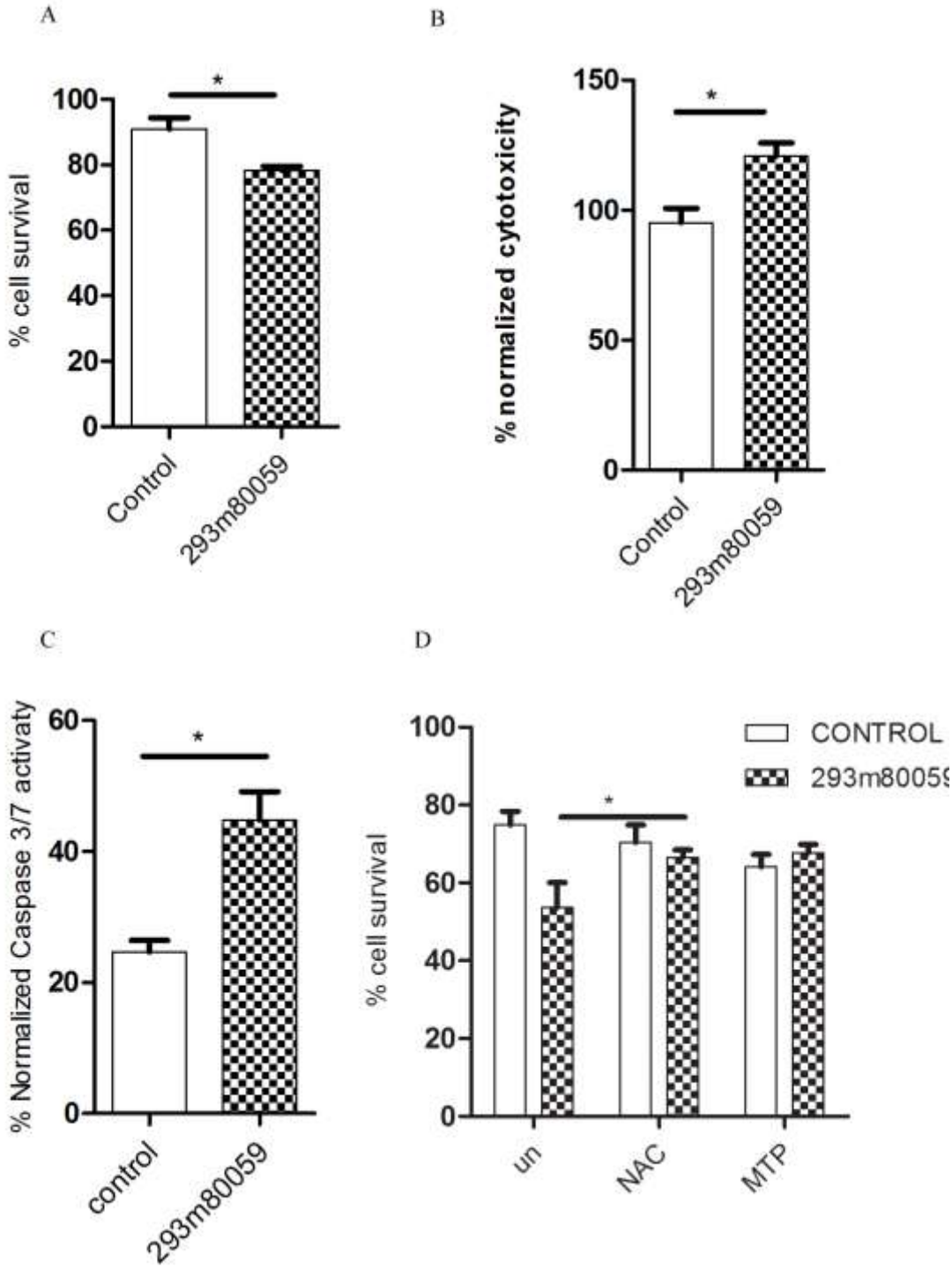

Figure 6.8. miR-293m80059 potentiates cell death. *The mimic of miR-293m80059 was transfected in HEK293 and cellular viability was assessed by A. MTT assay, B. Caspase 3/7 activity (was determined using Caspase-GloR 3/7 Assay kit) and C. cytotoxic LDH release assay (was determined using Cytotoxicity detection Kit) and D. The role of ROS in cell death signals was assessed by treating cells with ROS scavengers, NAC and Mitotempo.*

6.8 Discussion

The differential regulation of mitochondrial function in different tissue and patho-physiological conditions is not understood. The studies using high throughput tools of genomics and proteomics have identified major constituents of mitochondria (Mercer et. al, 2011; Pinto et. al, 2016; Liu et. al, 2013). It had been observed that level of nuclear encoded mitochondrial proteins in mitochondria modulates the capacity of mitochondrial functions (Rackham O et. al, 2012). The post transcriptional regulation of mRNA by small non coding RNA specifically miRNAs plays important role in the regulation of mRNA copy number. Here role of novel miRNA in regulation of the assembly of mitochondrial complex-I by targeting ND2 and mitochondrial cell death pathway has been demonstrated. The role of miRNAs in regulation of mRNA degradation and translation in cytoplasm is now well understood now. The presence of miRNAs in mitochondria further suggests its role in regulation of mitochondrial genome encoded transcripts. Recently, miR-181c was shown to be present inside the mitochondria and regulated differentially the mitochondrial genome encoded transcripts in ventricular cardiac cells (Das et. al, 2014). Interestingly the novel miRNA miR-293m80059 is expressed at low level in the HEK293 cells and mature form predominantly associated with mitochondria. The genomic locus of 293m80059 corresponds to chromosome 17 and the levels are not altered in the rho 0 cells suggesting that it nuclear genome transcribed miRNA and translocate to mitochondria. As its seed sequence similarity is in ND2 region of mitochondrial genome. The expression of miR-293m80059 increased the level of ND2 mature transcript and a decrease in processing intermediate suggesting its role in regulation of processing of mitochondrial genome transcribed transcripts. The two strands of mtDNA contain different mitochondrial genes and are transcribed from dedicated promoters; designated the light- and heavy-strand promoters (LSP and HSP). The light encodes 1 transcript and 8 of the 22 tRNAs whereas heavy chain codes for polycistronic mRNA coding for 13 mRNAs, 2 rRNAs and 22tRNAs. This polycistronic pre-RNA processing regulation is complex and yields different levels of steady state individual components (Wolf et. al, 2016). The process occurs in catalytic core composed of distinct nuclear genome transcribed RNA binding proteins forming distinct from nucleoid called mitochondrial RNA granule. GSRF1 was

the first protein identified as a component of this interactome however further FASKTD series proteins were identified. The knockdown of FASTKD5 showed decreased level of ND2 and its processing intermediate (Anotnicka et. al, 2015). The RNA-IP experiments were performed using different RNA binding proteins present in mitochondria. Interestingly enrichment of miR-293m80059 was only observed in FASKD5 suggesting that it may form part of RNA granules and regulate the processing from its intermediate specifically from heavy chain.

As ND2 levels were modulated by miR-293m80059 hence the complex-I activity may be regulated.The mitochondrial NADH ubiquinone oxidoreductase (complex-I) is composed of 45 subunits, 7 of which are encoded by mitochondrial genome. The 1MDa complex has hydrophobic base at inner membrane with proton pumping module P and ubiquinone module Q at the base of arm and N module in the hydrophilic arm protruding inside mitochondrial matrix involved in NADH oxidation. The assembly pathway comprises of sequential addition of different subunits assembled. The proteins encoded by mitochondrial genome forms early subunit forming part of P module. ND2 forms the core (460 kDa complex) of second entry point of assembling pathway, arm b (Guerrero-Castillo et. al, 2017). Here we observed that novel miRNA, miR-293m80059 increases both super complex and sub complexes, suggesting overall enhancement of complex 1 assembly. On the other hand the levels of miR-293m80059 are slightly increased in rho0 cells suggesting it might be utilized by cells as response mechanism to maintain the complex-I activity in the mitochondrial DNA deficient cells.

Generally miRNAs are known to negatively regulate the target however the reports of increased mRNA stability as well as translation are emerging (Valinezhad et. al, 2014; Vasudevan et. al, 2007). Postive regulation of target of miRNA has also been shown in mitochondria, where during myogenic reprogramming, miR-1 is translocated in mitochondria binds to Ago-2, associate with polysomes and enhance the translation of its target ND1 and COX1 (Zhang et. al, 2014). In the current study however miR-293m80059 may enhance the processing and hence the level of its target transcript and protein. These evidences are suggesting that miRNA may act at different steps during transcription, processing and translation of mitochondrial genome encoded transcripts.

In conclusion, miR-293m80059 enhances complex-1 subunit processing with mitochondrial RNA granule. The mitochondrial complex-I is also site of leakage of electron leading to ROS leading to cell death. The evidences here suggest that novel miRNA, miR-293m80059 is present in the mitochondria which may regulate its level in required narrow physiological levels to regulate mitochondrial functions, potentiate cell death. This may provide tissue specific differential bioenergetic capacity to the mitochondria. Thus emerging concept of mitochondria specific miRNAs needs to be further elucidated in different patho-physiological conditions to explore specificity of mitochondrial functions. This study as well as other recent report also suggests that there is specific set of miRNAs which is specific cell type to modulate the processing of mitochondrial genome encoded transcripts. Hence, the alignment of all identified small RNA sequences to mitochondrial genome was performed.

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Table 6.4 The GO term of predicted targets of putative novel miRNAs associated with mitochondria of HeLa.
Table 6.5 KEGG pathways enriched for targets of putative novel miRNAs associated with mitochondria of HeLa.

Table 6.1 Putative novel miRNAs associated with mitochondria of HEK293 and HeLa

Id [1]	Location [2]	Strand [3]	MFE [4]	Count [5]	Sequence [6]
293m8-m0001	chr10:102989932:102990017	+	-33.5	5	CCTGATGATGGGCGTCTGAAG
293m8-m0002	chr10:104409126:104409215	+	-34.4	6	CGCTGTTGTGTTGGGTGCAGTCTG
293m8-m0003	chr10:114468962:114469045	+	-32.5	6	AACACCTCAGAAAAGGGACAGA
293m8-m0004	chr10:133771005:133771105	+	-30.8	26	CCCCTGTGGAGATGAAGGTGTGTG
293m8-m0005	chr10:133771139:133771232	+	-33.1	21	CCCCTGTGGAGATGAAGGTGTGTG
293m8-m0006	chr10:133772961:133773061	+	-26.3	13	CCCCTGTGGAGATGAAGGTGTGTG
293m8-m0007	chr10:687634:687712	-	-49.7	37	ACAAGGAAGGACAAGAGGTGTGA
293m8-m0008	chr10:6008343:6008420	-	-24.2	5	AGGCACCCCTAGGATAAGCTGGA
293m8-m0009	chr11:71210711:71210792	+	-32.3	5	ACGAGAGGAGAGAGCCGGCCAG
293m8-m0010	chr11:16984501:16984581	-	-27.1	42	TGGGAGGAACAAGTATGCATT
293m8-m0011	chr11:65319869:65319956	-	-45.2	6	AGCAGCTGGGCAGAGGGACGTGG
293m8-m0012	chr11:65950890:65950975	-	-19.5	5	AGCAAGGCATTTTCCTCTCTGTG
293m8-m0013	chr11:125757934:125758033	-	-49.1	22	AGGGGCGCGGCCCAGGAGCTCAGA
293m8-m0014	chr12:656312:656383	+	-20.7	39	GGGCGTTGCTGGGCGTTGCT
293m8-m0015	chr12:56473391:56473478	+	-30.5	5	GTCTGGGAGAAACTGAGGTGGC
293m8-m0016	chr12:57906466:57906537	+	-26.9	15	TAGAGAGGGGAAGGATGTGATGTG
293m8-m0017	chr12:107487823:107487908	+	-19	5	AGTGAGGGGAGGATGGAGTG
293m8-m0018	chr12:111882758:111882836	+	-37.2	5	CCAGGCTGGCTGTGGGTGAGGAG
293m8-m0019	chr12:121352226:121352320	+	-27.2	19	CGTGAAGGCTGGCAGTGATGGGG
293m8-m0020	chr12:27134835:27134909	-	-21.4	7	AGGCTATGGGGCTGCAGTGTACAG
293m8-m0021	chr12:112082398:112082469	-	-18.9	6	GAGCAGAAAGGGGACTGTGTCAGC
293m8-m0022	chr12:113552380:113552453	-	-22.6	93	ATCAGGCTGTGTAGTATAGGGGTT
293m8-m0023	chr13:103498671:103498747	+	-24	5	GCCGCGTTGGGACTTGGGGTGC
293m8-m0024	chr13:111102941:111103018	+	-25.7	86	AGCTGGGGATGGAAGCTGAAGCC
293m8-m0025	chr13:101240819:101240906	-	-48.2	7	AGGGAGGAGGCCTGGGGCGCCG
293m8-m0026	chr14:65937573:65937649	+	-33.1	8	TGCACAGAGGGCGGCGGGCTGTT
293m8-m0027	chr14:105364251:105364331	+	-39.6	5	CAGGGAGGACTGTGGGTAAGGCC
293m8-m0028	chr14:24550739:24550823	-	-31.6	6	TGGCGCTGACCCGGTTTCTGCATT
293m8-m0029	chr14:91406423:91406511	-	-26	5	AACTCTCAGGGCTCAGGTAGG
293m8-m0030	chr15:45316328:45316424	+	-33.9	5	GGGATGGCAGTGGGTATGTGGGA
293m8-m0031	chr15:75446532:75446624	+	-29.4	5	CATGGAAGAGGACAACAGGG
293m8-m0032	chr15:82647742:82647826	+	-32.9	6	GGTAGCCGGGCGGCGCGGGGACC
293m8-m0033	chr15:82945229:82945313	+	-32.9	6	GGTAGCCGGGCGGCGCGGGGACC
293m8-m0034	chr15:83024229:83024313	+	-32.9	6	GGTAGCCGGGCGGCGCGGGGACC
293m8-m0035	chr15:92623052:92623123	+	-34.3	5	TTGGGTGTGGACAGCAGGGAGCCT

293m8-m0036	chr15:45153111:45153207	-	-37.7	5	GGGATGGCAGTGGGTATGTGGGA
293m8-m0037	chr15:82554827:82554895	-	-35.1	5	CTCGCTGCGGCTCTGAGGCGGG
293m8-m0038	chr15:100736923:100737019	-	-42.2	7	GTTGCTGGGCTGTGGTTGCAGGCT
293m8-m0039	chr16:4729550:4729637	+	-33.7	5	GTTGGTGATAAGTGAGCGGCGGTG
293m8-m0040	chr16:15025091:15025183	+	-34.5	5	CCCTGAGGACTAGGACAGCT
293m8-m0041	chr16:15027124:15027207	+	-41	15	GTGGGTGCCGTAGGGGTCGGGA
293m8-m0042	chr16:16425611:16425694	+	-41	15	GTGGGTGCCGTAGGGGTCGGGA
293m8-m0043	chr16:16466875:16466967	+	-34.5	5	CCCTGAGGACTAGGACAGCT
293m8-m0044	chr16:16468904:16468987	+	-41	15	GTGGGTGCCGTAGGGGTCGGGA
293m8-m0045	chr16:22335764:22335833	+	-56.7	9	TGGAGGGGAGGGGGCTGGCTGT
293m8-m0046	chr16:28858254:28858329	+	-33.5	7	GGGTAGGAGTCAGTGTTGGG
293m8-m0047	chr16:85588491:85588569	+	-37.3	5	CTTCGGAGCGAAGGGGAGCA
293m8-m0048	chr16:88535341:88535424	+	-46.7	9	TCTGGGCACAGGCGGATGGACAG
293m8-m0049	chr16:2151811:2151903	-	-34.5	5	CCCTGAGGACTAGGACAGCT
293m8-m0050	chr16:2166112:2166199	-	-46.4	6	CGGGTAGGGGGAGTCTGGGCTTC
293m8-m0051	chr16:15221470:15221553	-	-41	15	GTGGGTGCCGTAGGGGTCGGGA
293m8-m0052	chr16:18430634:18430717	-	-41	15	GTGGGTGCCGTAGGGGTCGGGA
293m8-m0053	chr16:18432654:18432746	-	-34.5	5	CCCTGAGGACTAGGACAGCT
293m8-m0054	chr16:18470736:18470819	-	-41	15	GTGGGTGCCGTAGGGGTCGGGA
293m8-m0055	chr16:21831653:21831733	-	-37.4	6	ACGTTGTTTGTCGGGAGATGCG
293m8-m0056	chr17:5015327:5015401	+	-41.9	114	CGACTGGACTGGAGCGGCCGGGTG
293m8-m0057	chr17:18138666:18138751	+	-25.6	16	TCGGGGTGAGAGGGAGAAGATGT
293m8-m0058	chr17:27075452:27075535	+	-38.1	5	TGGGTTGCAGGGTAGGTGACA
293m8-m0059	chr17:39976834:39976931	+	-29.8	8	CAGGGGAAGGGAAGGTGAAGC
293m8-m0060	chr17:42277217:42277301	+	-40.7	5	GATGGATGGATGGAGCGAGCGTGA
293m8-m0061	chr17:45028000:45028070	+	-36.3	5	CAAGGGCTGACAGGATGGGGC
293m8-m0062	chr17:64657849:64657944	+	-21.4	5	ATTGTGGAACAAGTGGCTCAG
293m8-m0063	chr17:74341881:74341956	+	-23.5	50	AAGAGAAAGGCTGAAGGGGATG
293m8-m0064	chr17:80551877:80551966	+	-40.7	8	TGGCGGTGGCAGTGGAGCAGCT
293m8-m0065	chr17:80887324:80887412	+	-47.5	12	AGGAGGCGTCGGGCTGGCTGGGG
293m8-m0066	chr17:4873372:4873451	-	-25.5	16	ATAGGTGGCAGAGGAGGGACTTCA
293m8-m0067	chr17:7255242:7255336	-	-47.4	7	GACGCTGGCGGGACGGGGTG
293m8-m0068	chr17:18194399:18194498	-	-31	6	TGCAGGACATTAGGCTAAGGCTAC
293m8-m0069	chr17:26890915:26891000	-	-30.8	13	TCTGGGAAGGTGGGCAGAGGCA
293m8-m0070	chr17:35453721:35453802	-	-24.3	5	TGTTAGAAGAGGTGGTAGGGCA
293m8-m0071	chr17:42940266:42940357	-	-32.2	5	AGGAAGATGGCGGGGAGTAG
293m8-m0072	chr17:73500622:73500700	-	-30	5	GTAGGATGATGGGGTCACGGA
293m8-m0073	chr17:80343748:80343815	-	-23	5	AAGGGCAGGGGGGCAGCGGCTC

293m8-m0074	chr18:33160868:33160957	-	-40.9	6	CAGAACGGGGACTGTTTGGGG
293m8-m0075	chr19:1253351:1253436	+	-35.6	10	CTGGGAGGCGGTCGGTTCTG
293m8-m0076	chr19:2246431:2246519	+	-42.1	7	TGAAGGGCTCTGGGGATTGGGA
293m8-m0077	chr19:3538149:3538218	+	-37.1	7	CAAGGGCAGGGGAAGCGGAGACCA
293m8-m0078	chr19:13005002:13005098	+	-26.1	13	CTGGGTGAAGAATGGAAGGGTT
293m8-m0079	chr19:41919193:41919291	+	-38.2	5	TTTGGGAGAAGGGAACTGGGCA
293m8-m0080	chr19:49606363:49606451	+	-33.6	11	ACTCGGGGATTGGTGAGGGCGGA
293m8-m0081	chr19:56135910:56135997	+	-43.7	6	CTGAAAGGGGGTGGGGCTCGAA
293m8-m0082	chr19:2762628:2762702	-	-35.7	9	TCCTGGAGCTGGGCAGATGGGA
293m8-m0083	chr19:16752390:16752479	-	-25.6	5	AAAGTTGTACTGGGACCGGGCA
293m8-m0084	chr19:42926413:42926490	-	-28.1	51	GAGCTGTGATGTTCTGGGCAGGAG
293m8-m0085	chr19:44237877:44237969	-	-28.7	13	TTTGGGGTGAGAAAGGGGAG
293m8-m0086	chr19:49865598:49865672	-	-40	14	TAGGAAGGCGCCGGCGGGAGT
293m8-m0087	chr1:16188231:16188296	+	-29.9	7	AAATAACAGGACTGGGCCGGG
293m8-m0088	chr1:27084023:27084121	+	-25.5	6	GAGGGCAACAGAAGGACAGCTG
293m8-m0089	chr1:27179867:27179958	+	-29.2	5	CTAGAGGGGAAGGGAACATAGGAA
293m8-m0090	chr1:161196975:161197053	+	-26.5	68	TGGGATGAGGGATTGAAGTGGA
293m8-m0091	chr1:182992444:182992536	+	-35.7	7	CGCGGGAGTTGCTGAGAGGAGACA
293m8-m0092	chr1:202203624:202203712	+	-18.1	5	ATGATAGATGCTGTGGTGTAATGG
293m8-m0093	chr1:227969441:227969520	+	-39.3	5	GCAGGAAAACTGGGAAGCAG
293m8-m0094	chr1:1247909:1248006	-	-39.6	5	CTCGGGGCAGGCGGCTGGGAGCG
293m8-m0095	chr1:27676426:27676498	-	-32.7	5	CGGGAGTGGGTGGGAGTCAG
293m8-m0096	chr1:40723546:40723627	-	-28.1	7	TGGGCTAGTGAACGCGGCGAAGT
293m8-m0097	chr1:43830310:43830390	-	-33.5	27	TGAGGGGAGAATGAGGTGGAGA
293m8-m0098	chr1:45811409:45811507	-	-29.9	5	AAATGGGAGGGGAAGGAGATGATG
293m8-m0099	chr20:3194751:3194835	+	-20.4	165	CAAAATGATGAGGTACCTGATA
293m8-m0100	chr20:327811:327886	-	-37.8	5	CGGCCCGGGCTGAGGATGCGGG
293m8-m0101	chr20:5049486:5049569	-	-32.8	6	TGCTGAAGAGACTGGGATGCT
293m8-m0102	chr21:46895578:46895674	+	-59.6	7	ACAGGGATGCTGGGCTGGGCAGA
293m8-m0103	chr21:47850248:47850347	+	-42.8	11	CACGTGTGGGACCTGGCAGGGCT
293m8-m0104	chr22:20129389:20129477	+	-24.1	5	ACTTTGGAAGAATTTGAGACGTG
293m8-m0105	chr22:20388787:20388887	-	-36.8	6	AGGGAACTGTCCAGGGGTTGGGTG
293m8-m0106	chr22:20818898:20818990	-	-33.5	7	ATAAGAGAAGGGGACATGTGGAT
293m8-m0107	chr22:21067261:21067361	-	-36.8	6	AGGGAACTGTCCAGGGGTTGGGTG
293m8-m0108	chr22:21832566:21832666	-	-36.8	6	AGGGAACTGTCCAGGGGTTGGGTG
293m8-m0109	chr22:31556037:31556127	-	-45.3	26	GGAGGAACCTTGGAGCTTCGGCA
293m8-m0110	chr22:42004840:42004940	-	-26	6	AGTGGGTGATGTTTGCTGACACTC
293m8-m0111	chr22:42519490:42519567	-	-28.4	11	AAGGAGAGAGAACAGGCTGAGG

293m8-m0112	chr22:42531646:42531723	-	-28.4	11	AAGGAGAGAGAACAGGCTGAGG
293m8-m0113	chr22:47094991:47095064	-	-36.9	6	AACGTGGCAGGGTCTGGACTGGAA
293m8-m0114	chr2:86420148:86420231	+	-57.4	14	TAGGAGGGAACAGTAAAAGCAGT
293m8-m0115	chr2:102342302:102342377	+	-23.9	5	AGGCTCTGTAGTTGATGTGGTG
293m8-m0116	chr2:121717568:121717636	+	-18.1	7	ATGGACAGCCAAGGAGAACAGG
293m8-m0117	chr2:137063588:137063680	+	-31.2	17	AGTGTAGCGGTTAAGAGCATG
293m8-m0118	chr2:233438716:233438793	+	-32.3	7	GAGGGAACAGGGGCAGACTTCT
293m8-m0119	chr2:234346473:234346566	+	-36.2	6	TCTGGCTGGGACATGGTGGCA
293m8-m0120	chr2:236960394:236960483	+	-37.9	5	CCAAGAGGACTAAAGGCAGCCAG
293m8-m0121	chr2:20864925:20864988	-	-21.1	6	TGTGTAGAGCAGAGAGCGGA
293m8-m0122	chr2:97302580:97302657	-	-18.7	5	GCTTCTAAGGGCTGCTGGTA
293m8-m0123	chr3:40740792:40740886	-	-20.6	50	AAGAGAAAGGCTGAAGGGGATG
293m8-m0124	chr3:127294107:127294179	-	-46.3	20	TGGGGAGGTGTGGAGTCAGCAT
293m8-m0125	chr3:149657211:149657285	-	-19.2	10	ACTTAGTAGCTGTGGAGGAAGATG
293m8-m0126	chr4:8878565:8878644	+	-40.8	15	CAGCTGTGGAGATGTGGATCGGA
293m8-m0127	chr4:8622640:8622710	-	-32	6	CAATGGAACGAGGCTGAGGGTG
293m8-m0128	chr4:8628278:8628348	-	-32	6	CAATGGAACGAGGCTGAGGGTG
293m8-m0129	chr4:8633916:8633986	-	-32	6	CAATGGAACGAGGCTGAGGGTG
293m8-m0130	chr4:169929813:169929901	-	-32.3	5	CCATCAGTGTTTGCTGGACTGG
293m8-m0131	chr5:141315097:141315181	+	-30.7	6	CGTTGAACTCCTGGGCATCAGAAT
293m8-m0132	chr5:179219456:179219524	+	-32.7	6	AGGAAGGAGGCTGAGTGGGCAG
293m8-m0133	chr5:137503764:137503837	-	-25.9	5	TGGGAAATGGTAGTGAAGGA
293m8-m0134	chr6:28918819:28918903	+	-22.2	3132	TCGGGCGGGAGTGGTGGCTTTT
293m8-m0135	chr6:31633499:31633580	+	-34.9	12	AGGGGAACGTGAGGAGAGCTGC
293m8-m0136	chr6:1390549:1390646	-	-39.6	9	CGCGCCTGCAGGAACTGGTAGA
293m8-m0137	chr6:24719707:24719787	-	-24.9	8	AATGTGGGTGATGGGGAAGTTG
293m8-m0138	chr6:26634385:26634482	-	-34.2	11	CCGAGGCAGAGAGGAACAGAAGG
293m8-m0139	chr6:28950453:28950526	-	-20.2	5	ATCCGAGGAAGATAGAGTAGGTG
293m8-m0140	chr6:32137807:32137893	-	-30.1	109	TGGGCAGGGGCTTATTGTAGGAGT
293m8-m0141	chr6:42071607:42071696	-	-31.9	17	TCGGGGAGATGAGAGACGTGTT
293m8-m0142	chr6:70377908:70377989	-	-22.3	12	AGAAATGATGGACAAACTGATC
293m8-m0143	chr6_apd_hap1:253855:253928	-	-20.2	5	ATCCGAGGAAGATAGAGTAGGTG
293m8-m0144	chr6_apd_hap1:3452548:3452634	-	-30.1	109	TGGGCAGGGGCTTATTGTAGGAGT
293m8-m0145	chr6_cox_hap2:437555:437639	+	-22.2	3132	TCGGGCGGGAGTGGTGGCTTTT
293m8-m0146	chr6_cox_hap2:3143119:3143200	+	-34.9	12	AGGGGAACGTGAGGAGAGCTGC
293m8-m0147	chr6_cox_hap2:469256:469329	-	-20.2	5	ATCCGAGGAAGATAGAGTAGGTG
293m8-m0148	chr6_cox_hap2:3608488:3608574	-	-30.1	109	TGGGCAGGGGCTTATTGTAGGAGT
293m8-m0149	chr6_dbb_hap3:2919078:2919159	+	-34.9	12	AGGGGAACGTGAGGAGAGCTGC

293m8-m0150	chr6_dbb_hap3:253864:253937	-	-20.2	5	ATCCGAGGAAGATAGAGTAGGTG
293m8-m0151	chr6_mann_hap4:222224:222308	+	-22.2	3132	TCGGGCGGGAGTGGTGGCTTTT
293m8-m0152	chr6_mann_hap4:2976389:2976470	+	-34.9	12	AGGGGAACGTGAGGAGAGCTGC
293m8-m0153	chr6_mann_hap4:253856:253929	-	-20.2	5	ATCCGAGGAAGATAGAGTAGGTG
293m8-m0154	chr6_mann_hap4:3480631:3480717	-	-30.1	109	TGGGCAGGGGCTTATTGTAGGAGT
293m8-m0155	chr6_mcf_hap5:222372:222456	+	-22.2	3132	TCGGGCGGGAGTGGTGGCTTTT
293m8-m0156	chr6_mcf_hap5:3013179:3013260	+	-34.9	12	AGGGGAACGTGAGGAGAGCTGC
293m8-m0157	chr6_mcf_hap5:3517636:3517722	-	-30.1	109	TGGGCAGGGGCTTATTGTAGGAGT
293m8-m0158	chr6_qbl_hap6:222219:222303	+	-22.2	3132	TCGGGCGGGAGTGGTGGCTTTT
293m8-m0159	chr6_qbl_hap6:2927142:2927223	+	-34.9	12	AGGGGAACGTGAGGAGAGCTGC
293m8-m0160	chr6_qbl_hap6:253850:253923	-	-20.2	5	ATCCGAGGAAGATAGAGTAGGTG
293m8-m0161	chr6_qbl_hap6:3398827:3398913	-	-30.1	109	TGGGCAGGGGCTTATTGTAGGAGT
293m8-m0162	chr6_ssto_hap7:259726:259810	+	-22.2	3132	TCGGGCGGGAGTGGTGGCTTTT
293m8-m0163	chr6_ssto_hap7:2964304:2964385	+	-34.9	12	AGGGGAACGTGAGGAGAGCTGC
293m8-m0164	chr6_ssto_hap7:291424:291497	-	-20.2	5	ATCCGAGGAAGATAGAGTAGGTG
293m8-m0165	chr6_ssto_hap7:3485535:3485621	-	-30.1	109	TGGGCAGGGGCTTATTGTAGGAGT
293m8-m0166	chr7:72043554:72043643	+	-24.3	5	ACTGAGCCTGGGGTGACTGACATG
293m8-m0167	chr7:74010282:74010364	+	-31.1	6	TGACTATGATGTGCACCTGATT
293m8-m0168	chr7:127676484:127676582	+	-26.2	6	TAAGGAAGTCAGAAGGGAGTGG
293m8-m0169	chr7:2876826:2876917	-	-33.6	6	CTAGGGAATGGGAGGGGGTAGCA
293m8-m0170	chr7:12443171:12443245	-	-25.3	6	GCGGTAGAGGGACTAGGCTA
293m8-m0171	chr7:66700353:66700442	-	-24.3	5	ACTGAGCCTGGGGTGACTGACATG
293m8-m0172	chr7:75362475:75362566	-	-31.8	5	ACGGGTGAGTGGAAGGGCCAG
293m8-m0173	chr7:100421719:100421795	-	-35.6	5	CTGAGGGAGGAGAGGGCGCCTGTG
293m8-m0174	chr7:132606758:132606842	-	-21.5	6	AGCTGTAGAAGTGAGGCAGCTC
293m8-m0175	chr8:42020578:42020678	+	-21.2	5	CAGAATTTGTAGAAGCCAGTCATG
293m8-m0176	chr8:79679467:79679541	+	-20.3	6	TGAGTGTGTGTGTGTGAGTGTGA
293m8-m0177	chr8:97281042:97281128	+	-31.9	6	CAAGGGGAGAGGGAAGTCGCTG
293m8-m0178	chr8:145102566:145102634	+	-28.1	5	GGGGTGCTGGGAAGGGCTTG
293m8-m0179	chr8:145196607:145196676	+	-25.5	5	GCCAGAAGGTGGGACCGCGGTG
293m8-m0180	chr8:145441815:145441884	+	-25.5	5	GCCAGAAGGTGGGACCGCGGTG
293m8-m0181	chr9:95862071:95862170	+	-36.2	9	CCAGATGATGGAGTGGCTGATGG
293m8-m0182	chr9:135927378:135927447	+	-35	13	AGGGCCGAAGGGTGGAAGCTG
293m8-m0183	chr9:21559796:21559871	-	-37.4	5	GTGGATGGGGCGAAGGTGCGG
293m8-m0184	chr9:35710651:35710743	-	-39	5	CCCTGGGGTTCTGAGGACATG
293m8-m0185	chr9:108203991:108204067	-	-19.1	7	CAGACTCTGGTGCCAGACTG
293m8-m0186	chr9:111880902:111880974	-	-23	11	TTGTTGTGCAGGGACTGGTG
293m8-m0187	chr9:136600367:136600454	-	-35.3	5	CAAGGGAGCAGAGCCGGGACA

293m8-m0188	chrUn_gl000220:122580:122664	+	-29.2	5	CAGGGAGCTCTGAGGCGGATGCG
293m8-m0189	chrX:84501906:84502004	+	-22	7	AAGAGAATGTAGTAGAGTGAGG
293m8-m0190	chrX:123414681:123414755	+	-18.4	10	ACTTAGTAGCTGTGGAGGAAGATG
293m8-m0191	chrX:153688352:153688448	+	-50.8	5	CTTTGGCGGGGTGGTGGGTG
293m8-m0192	chrX:584371:584461	-	-35.6	5	AGGACCTTGGAGGCTGGAGGCT
293m8-m0193	chrX:46460949:46461042	-	-21.3	13	AATGTCCTCTGTGGTTTGGCTG
293m8-m0194	chrX:53228054:53228153	-	-44.7	12	TCTGGAGGAAGTGGGGCAAGAAG
293m8-m0195	chrX:152220738:152220825	-	-20.6	50	AAGAGAAAGGCTGAAGGGGATG
293m8-m0196	chrY:534371:534461	-	-35.6	5	AGGACCTTGGAGGCTGGAGGCT
HM3-m0001	chr11:122022801:122022877	-	-36.6	5	AAAAGGGGGCTGAGGTGGAG
HM3-m0002	chr20:3194751:3194835	+	-20.4	7	CAAAATGATGAGGTACCTGATA
HM3-m0003	chr22:31556037:31556127	-	-45.3	22	GGAGGAACCTTGGAGCTTCGGCA
HM3-m0004	chr3:45883723:45883820	+	-40.5	5	AAGGTAGCGGGAGGGTTGGGCT
HM3-m0005	chr3:43527166:43527246	-	-18.1	13	ATTGTCTACTGTGGGCAGGTG
HM3-m0006	chr6:28918819:28918903	+	-22.2	209	TCGGGCGGGAGTGGTGGCTTTT
HM3-m0007	chr6_cox_hap2:437555:437639	+	-22.2	209	TCGGGCGGGAGTGGTGGCTTTT
HM3-m0008	chr6_mann_hap4:222224:222308	+	-22.2	209	TCGGGCGGGAGTGGTGGCTTTT
HM3-m0009	chr6_mcf_hap5:222372:222456	+	-22.2	209	TCGGGCGGGAGTGGTGGCTTTT
HM3-m0010	chr6_qbl_hap6:222219:222303	+	-22.2	209	TCGGGCGGGAGTGGTGGCTTTT
HM3-m0011	chr6_ssto_hap7:259726:259810	+	-22.2	209	TCGGGCGGGAGTGGTGGCTTTT
HM3-m0012	chr8:101733539:101733629	-	-50.7	8	TCGGGCGGCGGCGGCGGGCGG
HM3-m0013	chr8:144897778:144897866	-	-46.8	11	GCGGGCGGACGAGCGGGCGGGA

Table 6.1 Putative novel miRNAs associated with mitochondria of HEK293 and HeLa. *Features of putative novel miRNAs associated with mitochondria of HEK293 and HeLa as determined by miReap. 1 identification code assigned to each putative novel miRNA, 2 genomic location of each putative novel miRNA, 3 orientation of putative novel miRNA on chromosome (+/2), 4 MFE energy score (,218 kcal/mol) of each miRNA, 5 number of sequence reads.*

Table 6.2 The GO term of predicted targets of putative novel miRNAs associated with mitochondria of HEK293.

Cluster [1]	Term [2]	No. of target [3]	P-Value [4]	Benjamini [5]
Cluster 1 (ES : 8.07)	GO:0006350~transcription	133	1.17E-10	2.59E-07
	GO:0051252~regulation of RNA metabolic process	99	4.78E-05	0.007055
Cluster 2 (ES : 4.62)	GO:0010629~negative regulation of gene expression	42	1.69E-06	0.001252
	GO:0010605~negative regulation of macromolecule metabolic process	54	1.97E-06	0.001091
Cluster 3 (ES : 3.62)	GO:0042981~regulation of apoptosis	54	2.71E-05	0.00546
Cluster 4 (ES : 3.61)	GO:0006417~regulation of translation	17	4.46E-05	0.007042
Cluster 5 (ES : 2.77)	GO:0051254~positive regulation of RNA metabolic process	34	4.59E-04	0.045298
	GO:0045935~positive regulation of nucleic acid metabolism	40	9.04E-04	0.054227
Cluster 6 (ES : 2.61)	GO:0016568~chromatin modification	23	5.31E-04	0.050008
Cluster 7 (ES : 2.00)	GO:0007049~cell cycle	44	0.00486	0.191083
Cluster 8 (ES : 1.90)	GO:0016310~phosphorylation	39	0.064638	0.68618
Cluster 9 (ES : 1.68)	GO:0046907~intracellular transport	43	3.65E-04	0.039726
	GO:0015031~protein transport	42	0.009569	0.307903
Cluster 10 (ES : 1.65)	GO:0001568~blood vessel development	17	0.01961	0.430881
Cluster 11 (ES : 1.61)	GO:0016570~histone modification	13	0.001818	0.091723
	GO:0016569~covalent chromatin modification	13	0.00239	0.109053
Cluster 12 (ES : 1.59)	GO:0051726~regulation of cell cycle	27	2.42E-04	0.03115
Cluster 13 (ES : 1.39)	GO:0030163~protein catabolic process	35	0.014118	0.380141
Cluster 14 (ES : 1.15)	GO:0043549~regulation of kinase activity	23	0.013162	0.368459
	GO:0019220~regulation of phosphate metabolic process	28	0.022043	0.449085
Cluster 15 (ES : 1.14)	GO:0016071~mRNA metabolic process	23	0.01909	0.430511
	GO:0000398~nuclear mRNA splicing, via spliceosome	12	0.026275	0.47403
Cluster 16 (ES : 1.13)	GO:0048259~regulation of receptor-mediated endocytosis	4	0.019525	0.433631

Table 6.2 The GO term of predicted targets of putative novel miRNAs associated with mitochondria of HEK293*.The targets of novel miRNAs associated with mitochondria of HEK293 were determined by StarBase and clustered into GO terms using the DAVID gene annotation tool. 1, 2, 3, 4, 5 same as table 5.4*

Table 6.3 KEGG pathways enriched for targets of putative novel miRNAs associated with mitochondria of HEK293

Term [1]	No. of target [2]	P-Value [3]	Benjamini [4]
hsa04722: Neurotrophin signaling pathway	12	0.00653	0.60831
hsa04110: Cell cycle	12	0.00693	0.39196
hsa04520: Adherens junction	9	0.00782	0.31216
hsa04070: Phosphatidylinositol signaling system	8	0.02031	0.51984
hsa04120: Ubiquitin mediated proteolysis	11	0.03245	0.61075
hsa05120: Epithelial cell signaling in Helicobacter pylori infection	7	0.04134	0.63436
hsa04144: Endocytosis	13	0.04288	0.59155
hsa05211: Renal cell carcinoma	7	0.0466	0.57384

Table 6.3 KEGG pathways enriched for targets of putative novel miRNAs associated with mitochondria of HEK293. *The targets of putative novel miRNAs associated with mitochondria of HEK293 were determined by StarBase and clustered into KEGG pathways using the DAVID gene annotation tool. 1, 2, 3, 4 same as table 5 .5.*

Table 6.4 The GO term of predicted targets of putative novel miRNAs associated with mitochondria of HeLa.

Cluster [1]	Term [2]	No. of target [3]	P-Value [4]	Benjamini [5]
Cluster 1 (ES : 4.81)	GO:0045449~regulation of transcription	44	2.16E-07	2.35E-04
	GO:0051252~regulation of RNA metabolic process	35	4.23E-07	1.54E-04
Cluster 2 (ES : 2.70)	GO:0010629~negative regulation of gene expression	14	1.39E-04	0.03715
	GO:0045934~negative regulation of nucleic acid metabolic process	12	0.00206	0.18457
Cluster 3 (ES : 1.67)	GO:0045893~positive regulation of transcription, DNA-dependent	11	0.00391	0.22217
	GO:0045935~positive regulation of nucleic acid metabolic process	12	0.00901	0.24556
	GO:0031328~positive regulation of cellular biosynthetic process	12	0.01713	0.29914
Cluster 4 (ES : 1.57)	GO:0022403~cell cycle phase	11	0.0014	0.17372
	GO:0051329~interphase of mitotic cell cycle	5	0.00842	0.25029
Cluster 5 (ES : 1.54)	GO:0006915~apoptosis	11	0.0184	0.30342
	GO:0042981~regulation of apoptosis	12	0.04722	0.49577
Cluster 6 (ES : 1.53)	GO:0009725~response to hormone stimulus	10	0.0022	0.181

	GO:0006468~protein amino acid phosphorylation	13	0.00549	0.21322
	GO:0007254~JNK cascade	4	0.01078	0.2558
	GO:0001932~regulation of protein amino acid phosphorylation	6	0.01116	0.25272
	GO:0032268~regulation of cellular protein metabolic process	10	0.01136	0.24659
	GO:0031098~stress-activated protein kinase signaling pathway	4	0.01288	0.25971
	GO:0051174~regulation of phosphorus metabolic process	10	0.01305	0.25794
	GO:0044093~positive regulation of molecular function	11	0.01554	0.28441
	GO:0007257~activation of JUN kinase activity	3	0.01718	0.29514
Cluster 7 (ES : 1.17)	GO:0040029~regulation of gene expression, epigenetic	5	0.003	0.19637
	GO:0005694~chromosome	8	0.02196	0.41461

Table 6.4 The GO term of predicted targets of putative novel miRNAs associated with mitochondria of HeLa. *The targets of putative novel miRNAs associated with mitochondria of HeLa were determined by StarBase and clustered into GO term using the DAVID gene annotation tool. 1, 2, 3, 4, 5 same as table 5.4.*

Table 6.5 KEGG pathways enriched for targets of putative novel miRNAs associated with mitochondria of HeLa.

Term [1]	**No. of target [2]**	**P-Value [3]**	**Benjamini [4]**
hsa04144: Endocytosis	6	0.01383	0.65304
hsa04115: p53 signaling pathway	4	0.01588	0.45568
hsa04520: Adherens junction	4	0.02208	0.43199
hsa05414: Dilated cardiomyopathy	4	0.03497	0.49153
hsa05200: Pathways in cancer	7	0.04116	0.47213

Table 6.5 KEGG pathways enriched for targets of putative novel miRNAs associated with mitochondria of HeLa. *The targets of putative novel miRNAs associated with mitochondria of HeLa were determined by StarBase and clustered into KEGG pathways using the DAVID gene annotation tool. 1, 2, 3, 4 same as table 5.5.*