

Fig. S1. RT-PCR analysis of RNA isolated from cytoplasmic fraction and pure mitochondria/mitoplast fraction. *GAPDH* served as cytoplasmic marker, *MT-CYB* served as mitochondrial marker (C: Cytoplasmic fraction; M: Mitochondria fraction; NC: Negative control).

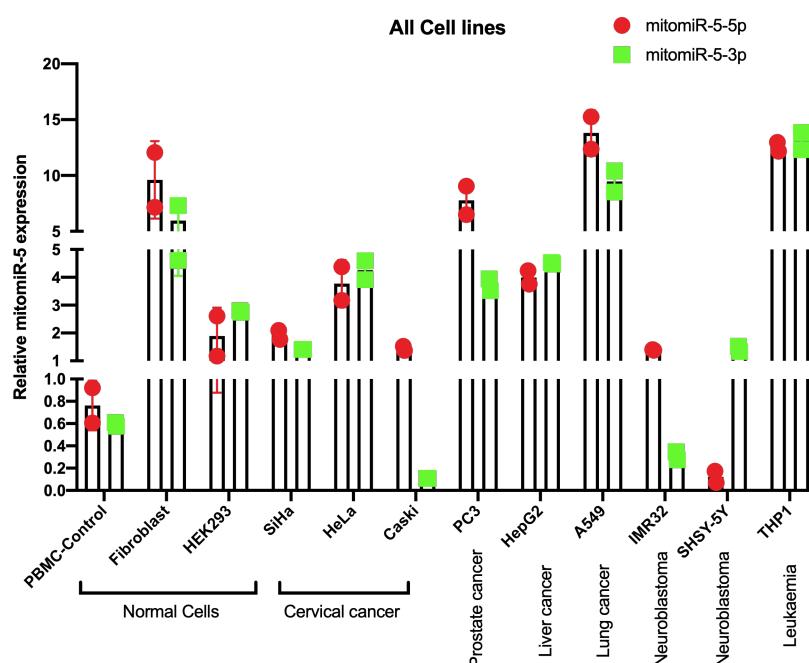


Fig. S2. MitomiR-5 expression profile in cancer cell lines such as SiHa, HeLa, Caski (cervical cancer), PC3 (prostate cancer), HepG2 (hepatocellular cancer), A549 (lung cancer), IMR32 and SHSY-5Y (neuroblastoma), THP1 (leukemia) and normal cell line Hek293 and primary cells fibroblasts and PBMCs. 1×10^6 cells were used for RNA isolation from each cell lines and qRT-PCR analysis was performed as mentioned in the methodology section. Relative quantity was calculated using the formula $2^{-\Delta\Delta Ct}$ upon normalization with endogenous control RNU6B.

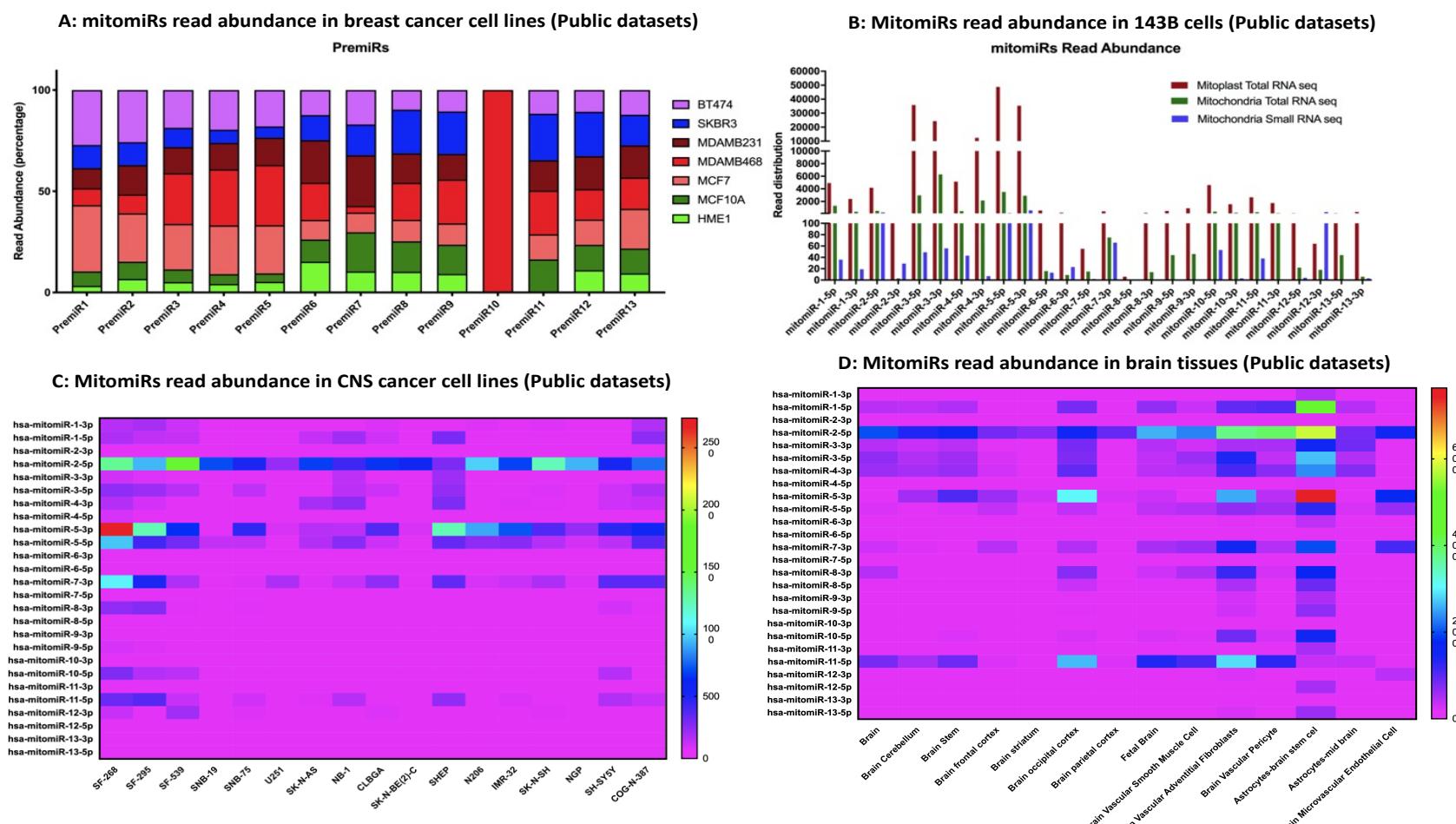


Fig. S3. A. Read abundance of mitomiRs in breast cancer cell lines and **(B)** in previously published mitochondrial transcriptome and small RNA datasets (Mercer et al., 2011). **(C)** MitomiRs read abundance in CNS cancer cell lines and **(D)** brain tissue sample data from RNA atlas datasets(Lorenzi et al., 2021). Scale for both the heat maps on **C** & **D** are provided on the right side of each figure. Read count extraction was performed as described in the methodology section. Data were downloaded from ENA and GEO databases, processed in European galaxy server and aligned using human hg38 annotation GTF files. Bam files were used for read count extraction using rCRS mitochondrial reference genome coordinates as well as pre-mitomiR coordinates.

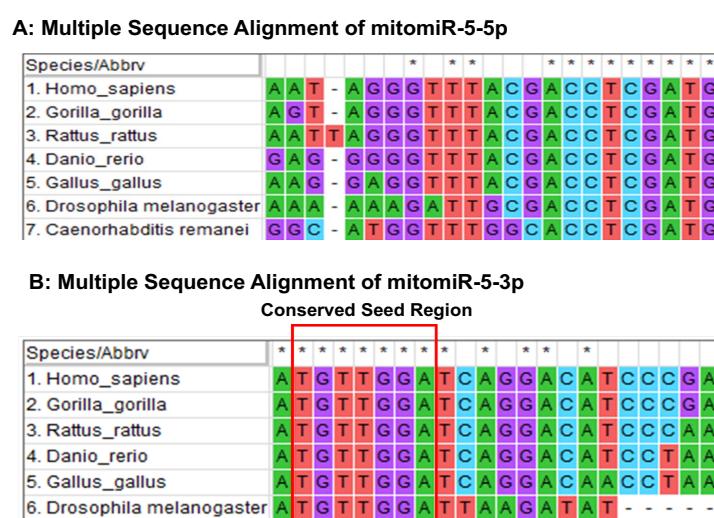


Fig. S4. Multiple Sequence Alignment of mitomiRs: MitomiR-5 mature sequences were aligned by Clustal W, using MEGA 7.0 software. A. Multiple Sequence Alignment of mitomiR-5-5p. B. Multiple Sequence Alignment of mitomiR-5-3p with conserved seed region.

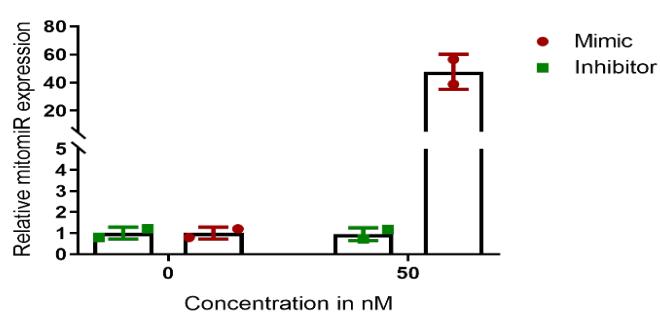


Fig. S5. qRT-PCR expression of mitomiR-5-5p levels in control oligos, mitomiR-5-5p mimic and inhibitor (50nM) transfected in MDA-MB-468 cell lines after 24 hours. Relative quantity was calculated upon normalization of mitomiR-5-5p mimic and inhibitor groups with the formula $2^{-\Delta\Delta Ct}$ to plot fold changes compared to their respective control oligos.

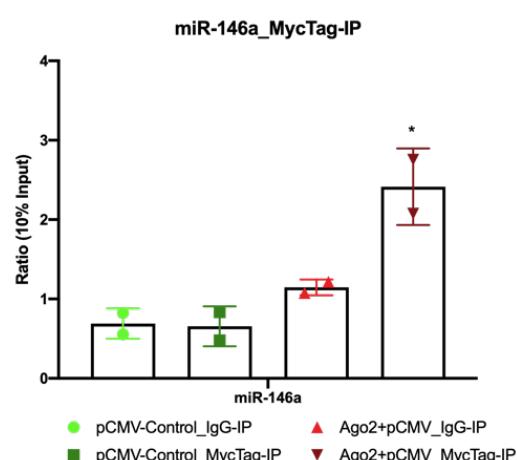


Fig. S6. qRT-PCR analysis of nuclear miRNA hsa-miR-146a abundance in Myc-Ago2-IP elutes of Ago2/myc/mito cells compared to respective IgG-IP elutes. Relative quantity (RQ) of each IP elutes were normalized to 10% input controls to calculate the ratio. P-value <0.05.

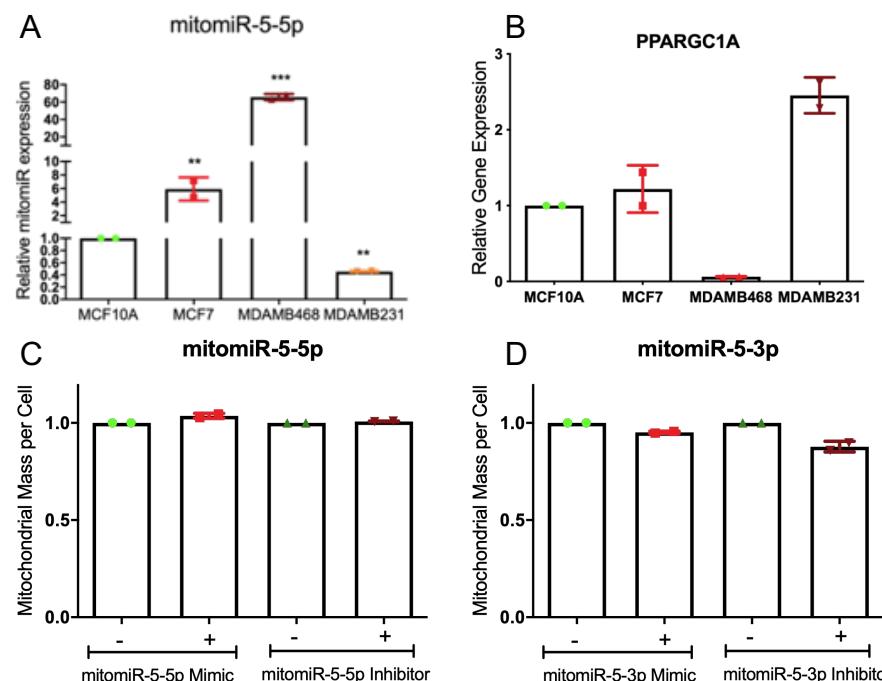


Fig. S7. (A&B) *PPARGC1A* gene expression analysis in breast cancer cell lines showing decreased expression in MDA-MB-468 compared to other cell lines, whereas mitomiR-5-5p expression is high in MDA-MB-468. (C&D) Mitochondrial mass by FACS analysis in MDA-MB-468 cell lines transfected with mitomiR-5-5p and mitomiR-5-3p mimic and inhibitor at 48 hours. P-value <0.05.

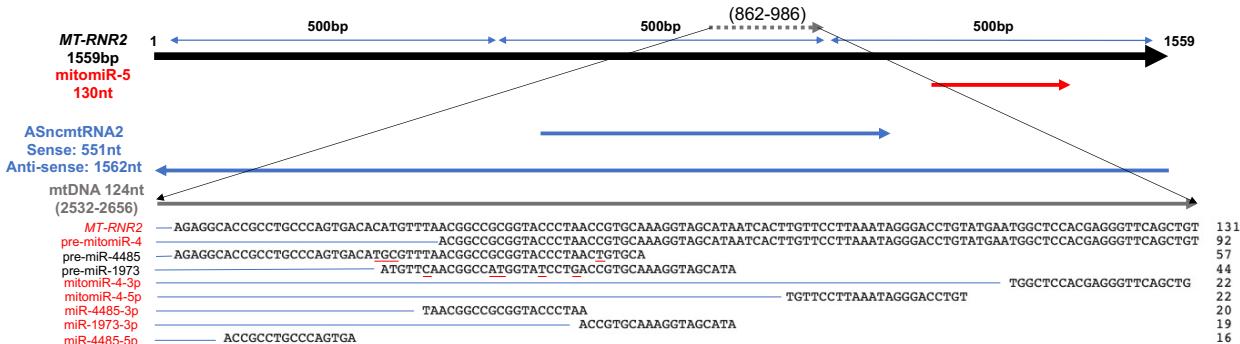


Fig. S8. Analysis of mitomiRs sequence similarity with reported nuclear miRNAs and antisense mitochondrial small non-coding RNAs (ASncmtRNA) showed overlapping patterns of predicted mitomiR-4 with reported miRNAs miR-4485 and miR-1973.

Raw western blot images:

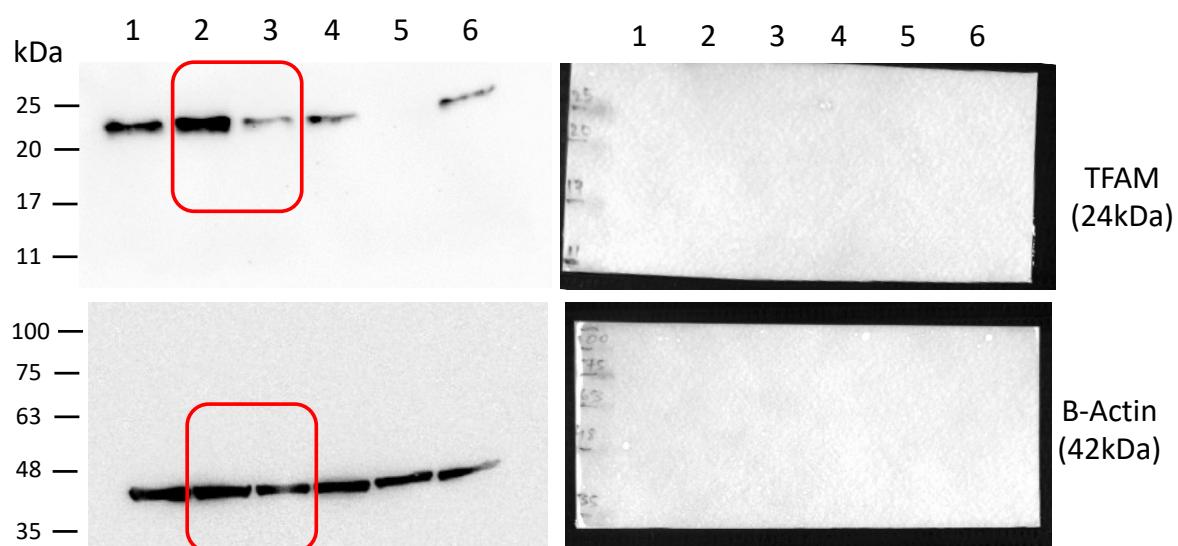


Fig. S9. Western blot images showing TFAM expression in TFAM-KD cells (Fig. 3G);

- Lane 1: Scramble control 1
- Lane 2: Scramble control 2
- Lane 3: siTFAM 1
- Lane 4: siTFAM 2
- Lane 5: siTFAM 3
- Lane 6: siTFAM 4

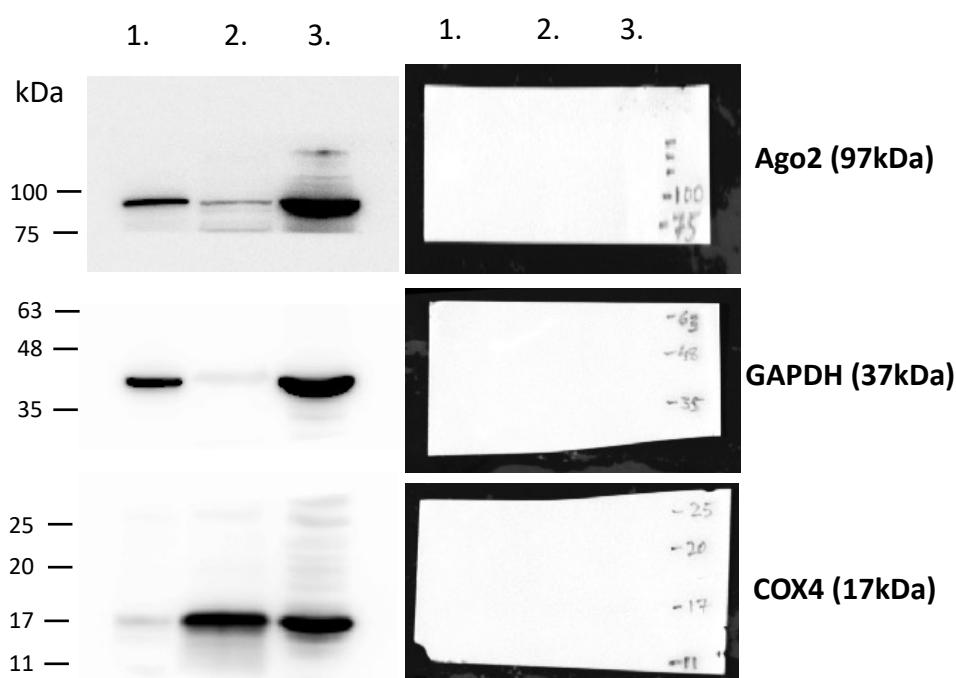


Fig. S10. Western blot images showing the purity of mitoplast isolated (Fig. 4B); Lane 1: cytoplasmic fraction; Lane 2: Mitoplast fraction; Lane 3: Whole cell lysate (WCL)

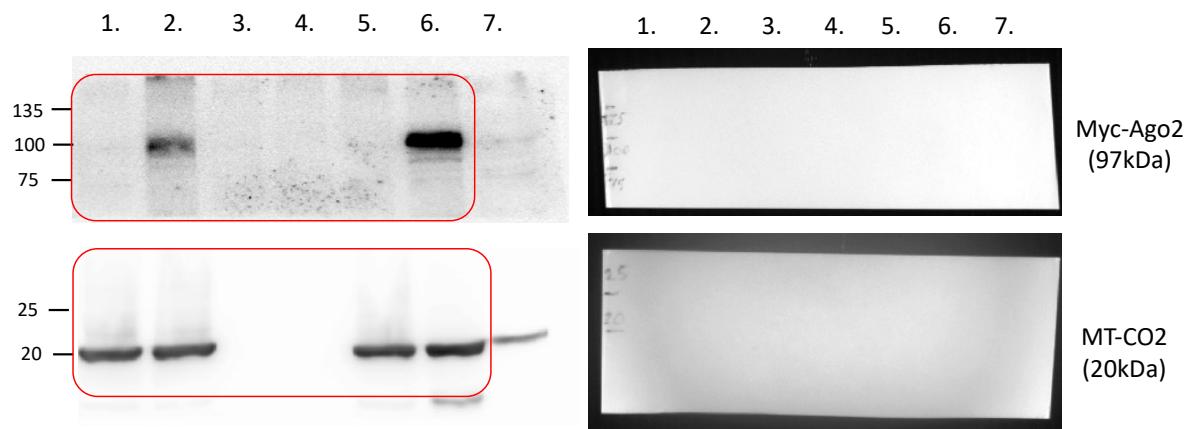


Fig. S11. Western blot images showing the localization of Ago2/myc/mito protein localization to mitochondria (Fig. 4C);
Lane 1: pCMV-Control_WCL;
Lane 2: Ago2+pCMV_WCL;
Lane 3: pCMV-Control_cytoplasmic fraction;
Lane 4: Ago2+pCMV_cytoplasmic fraction;
Lane 5: pCMV-Control_Mitochondrial fraction;
Lane 6: Ago2+pCMV_Mitochondria fraction.
Lane 7: Un-transfected cells

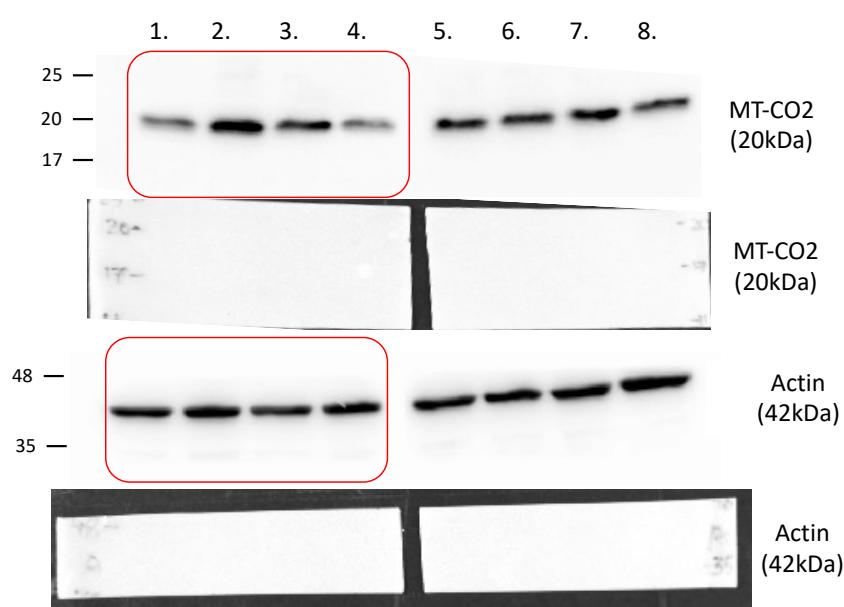


Fig. S12. Western blot images for MT-CO2 expression in mitomiR-5-5p mimic and inhibitor transfected cells (Fig. 5I)
Lane 1: Scramble mimic control
Lane 2: mitomiR-5-5p mimic
Lane 3: Scramble inhibitor control
Lane 4: mitomiR-5-5p inhibitor
Lane 5: mitomiR-5-3p inhibitor
Lane 6: Scramble inhibitor control
Lane 7: mitomiR-5-3p mimic
Lane 8: Scramble mimic control

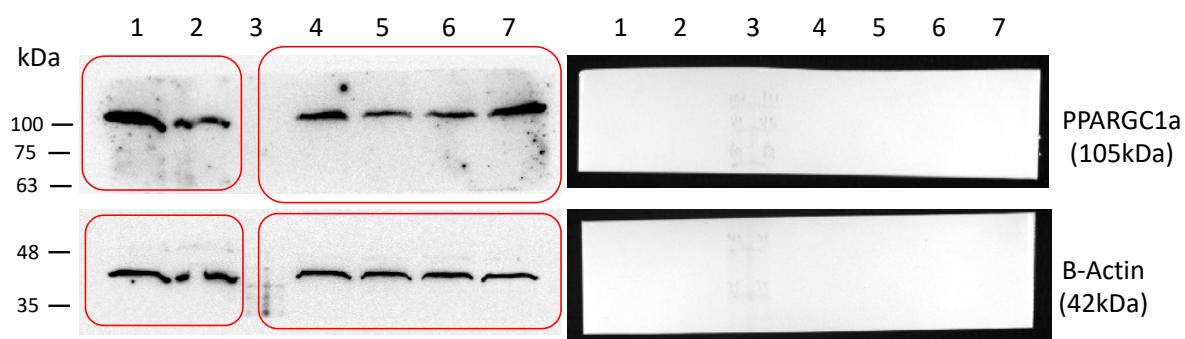


Fig. S13. Western blot images showing the expression of PPARGC1a protein in mitomiR-5-5p mimic & inhibitor transfected cells as well as mitomiR-5 sponge cells. (Fig 6A & 6I)

Lane 1: mitomiR-5 sponge;
Lane 2: Sponge control
Lane 3: Protein marker
Lane 4: Scramble mimic control
Lane 5: mitomiR-5-5p mimic
Lane 6: Scramble inhibitor control
Lane 7: mitomiR-5-5p inhibitor

Table S1. Pre-mitomiR Sequences

	Precursor mitomiR sequences	Sequence length
hsa-pre-mitomiR-1	GTTAGGTCAAGGTGTAGCCCATGAGGTGGCAAGAAATGGGCTACATTTCTAC CCCAGAAAACTACGATAGCCCTATGAAACTTAAGGGTGAAGGTGGATTAGC	107
hsa-pre-mitomiR-2	TAGAGGAGACAAGTCGTAACATGGTAAGTGTACTGGAAAGTGCACTTGGACG AACCAGAGTGTAGCTAACACAAAGCACCCAACCTACACTTAGGAGATTCAA CTTAACCTGACCGCTCTG	123
hsa-pre-mitomiR-3	GAGCTACCTAAGAACAGCTAAAAGAGCACACCCGCTATGTAGCAAAATAGT GGGAAGATTATAGGTAGAGGCACAAACCTACCGAGCCTGGTGTAGCTGG TTGTCCAAGATAGAATCTTAGTTC	128
hsa-pre-mitomiR-4	ACGCCCGGGTACCTAACCGTGCAAAGGTAGCATAATCACTTGTTCCTTAAA TAGGGACCTGTATGAATGGCTCACGAGGGTTAGCTGT	92
hsa-pre-mitomiR-5	AACGGAACAAGTTACCCCTAGGGATAACAGCGCAATCCTATTCTAGAGTCATA TCAACAATAGGGTTACGACCTCGATGTTGGATCAGGACATCCGATGGTGCA GCCGCTATTAAAGGTTGTTGTT	130
hsa-pre-mitomiR-6	AGCAGAGACCAACCGAACCCCCCTCGACCTTGCGAAGGGGAGTCCGAACTA GTCTCAGGCT	62
hsa-pre-mitomiR-7	CCTAGCATTACTTATGATATGTCCTACCCATTACAATCTCCAGCATTCCC CCTCAAACCTAAGAAATATGTCGATAAAAGAGTTACTTGATAGAGTAAATA ATAGGAGCTTAAACCCCTTATTCTAGG	137
hsa-pre-mitomiR-8	GTGGAGGCCGGAGCAGGAACAGGTTAACAGTCTACCCCTCCCTAGCAGGGA ACTACTCCCACCCCTGGAGCCTCCGTAGACCTAACCATCTCTCCTACACCTAGC AGGTGTCTCCTCTAT	122
hsa-pre-mitomiR-9	ACCTGAAGTTATATTCTTACCCAGGCTCGGAATAATCTCCATATTGT AACTTACTACTCCGGAAAAAAAGAACCATTTGGATACATAGGTATGGTCTGAG CTATGATATCAATTGGCTCTAGGGT	135
hsa-pre-mitomiR-10	TATTGTAACTACTCCGGAAAAAAAGAACCATTTGGATACATAGGTATGG TCTGAGCTATGATATCAATTGGCTCTAGGGTTATCGTGTGAGCACACCATA TATTACAGTA	118
hsa-pre-mitomiR-11	ACGGAAGCAATATGAAATGATCTGCTGCAGTGCTCTGAGCCCTAGGATTCA TTCTTTTACCGTAGGTGGCCTGACTGGCATTGTATTAGCAAACACTCATCACTA GACATCGT	115
hsa-pre-mitomiR-12	TCTTAGCCAATATTGTGCCTATTGCCATACTAGTCTTGCCGCCTGCGAAGCAG CGGTGGGCCTAGCCCTACTAGTCTCAATCTCAACACATATGGCCTAGA	103
hsa-pre-mitomiR-13	CTTAGTTACCGCTAACAAACCTATTCCAACGTGTTCATCGGCTGAGAGGGCGTAG GAATTATATCCTCTTGCTCATCAGTTGATGATACGCCGAGCAGATGCCAAC CAGCAGGCCATTCAAG	122

Table S2. Mature mitomiR sequences

Sl. No.	Mature mitomiRs	Sequences	Sequence length
1	hsa-mitomiR-1-5p	GGTCAAGGTGTAGCCCATGAGG	22
2	hsa-mitomiR-1-3p	AATGGGCTACATTTCTACCCC	22
3	hsa-mitomiR-2-5p	AAGTGTACTGGAAAGTGCACCT	22
4	hsa-mitomiR-2-3p	ACTTGGACGAACCAGAGTGTAG	22
5	hsa-mitomiR-3-5p	AGCTAAAAGAGCACACCCGTCT	22
6	hsa-mitomiR-3-3p	TGGGAAGATTATAGGTAGAGG	22
7	hsa-mitomiR-4-5p	TGTTCTTAAATAGGGACCTGT	22
8	hsa-mitomiR-4-3p	TGGCTCCACGAGGGTCAGCTG	22
9	hsa-mitomiR-5-5p	AATAGGGTTTACGACCTCGATG	22
10	hsa-mitomiR-5-3p	ATGTTGGATCAGGACATCCGA	22
11	hsa-mitomiR-6-5p	CCCCTTCGACCTGCCGAAGGG	22
12	hsa-mitomiR-6-3p	GGGAGTCCGAACTAGTCTCAGG	22
13	hsa-mitomiR-7-5p	TGTCTCCATACCCATTACAATC	22
14	hsa-mitomiR-7-3p	AGTAAATAATAGGAGCTAAC	22
15	hsa-mitomiR-8-5p	GAACAGGTTGAACAGTCTACCC	22
16	hsa-mitomiR-8-3p	AGACCTAACCATCTTCTCCTTA	22
17	hsa-mitomiR-9-5p	CGGAATAATCTCCATATTGTA	22
18	hsa-mitomiR-9-3p	ATTGGATACATAGGTATGGTC	22
19	hsa-mitomiR-10-5p	CGGAAAAAAAGAACCATTTGGA	22
20	hsa-mitomiR-10-3p	TGAGCACACCATAATTACAG	22
21	hsa-mitomiR-11-5p	TGATCTGCTGCAGTGCTCTGAG	22
22	hsa-mitomiR-11-3p	AGGTGGCCTGACTGGCATTGTA	22
23	hsa-mitomiR-12-5p	TGCGAAGCAGCGGTGGGCCTAG	22
24	hsa-mitomiR-12-3p	CGGTGGCCTAGCCCTACTAGT	22
25	hsa-mitomiR-13-5p	AGTTACCGCTAACAACCTATT	22
26	hsa-mitomiR-13-3p	TCTTGCTCATCAGTTGATGATA	22

Table S3. In-silico target gene prediction

Sl. No.	Targeting mitomiR	Target Genes	Target gene/protein Functions	Prediction Tools
1	MitomiR-1-5p	MAPK14	p38 MAPKs belonging to serine/threonine kinase which are involved in the activation of transcription factors such as <i>CREB</i> etc. which in turn phosphorylates <i>PGC1A</i> .	RNA22, RNAhybrid
2	MitomiR-1-3p	PSMD10	Facilitates stress induced autophagy during nutrient depletion on chemotherapies to ensure tumor cell survival.	miRDB, RNA22
3	MitomiR-1-3p	MAPK14	p38 MAPKs belonging to serine/threonine kinase which are involved in the activation	miRDB

			of transcription factors such as <i>CREB</i> etc. which in turn phosphorylates <i>PGC1A</i> .	
4	MitomiR-2-5p	<i>SIRT1</i>	Deacetylates <i>PGC1A</i> and facilitates mitochondrial biogenesis, which in turn activates downstream partners for mitochondrial transcriptional activation.	miRDB, RNAhybrid, Diana tools
5	MitomiR-2-5p	<i>PPARGC1A</i>	Master regulator of mitochondrial biogenesis, phosphorylates upon energy demand and thereby interacts with transcription factors like <i>TFAM</i> to initiate mitochondrial transcription.	miRDB, RNAhybrid, Diana tools
6	MitomiR-2-5p	<i>SLC2A2</i>	Glucose transporter facilitating bidirectional transport across plasma membrane to be up taken by beta cells.	miRDB, RNAhybrid
7	MitomiR-2-3p	<i>YWHAQ</i>	Phosphoserine/threonine binding proteins with protective role against mitochondria mediated apoptosis during type 2 Diabetes Mellitus.	miRDB, RNA22
8	MitomiR-2-3p	<i>NDUFS4</i>	Nuclear encoded subunit of complex I, involved in the transfer of electrons from NADH to respiratory chain.	Diana tools, RNAhybrid
9	mitomiR-3-5p	<i>SLC4A4</i>	Sodium bicarbonate cotransporter involved in the regulation of bicarbonate secretion, absorption and intracellular pH.	miRDB, Diana tools
10	mitomiR-3-3p	<i>ESRRG</i>	Belongs to nuclear hormone receptor superfamily and acts as transcription activator in the presence of bound receptor.	miRDB, Diana tools
11	mitomiR-3-3p	<i>UCP2</i>	Involved in the transport of proton across the inner and outer mitochondrial membrane, also involved in the uncoupling of OXPHOS from ATP synthesis.	miRDB, RNA22
12	mitomiR-3-3p	<i>KRAS</i>	Proto-oncogene with intrinsic GTPase activity, regulates mitochondrial metabolism and ROS production during cell proliferation and tumorigenesis.	RNA22, RNAhybrid
13	mitomiR-4-3p	<i>BNIP3L</i>	Bcl2 family proto-apoptotic protein, directly targets mitochondria, causing loss of membrane potential and release of cytochrome c.	miRDB, Diana tools
14	mitomiR-4-3p	<i>YWHAE</i>	Phosphoserine/threonine binding proteins involved in cellular processes such as metabolism, protein trafficking, signal transduction etc.	miRDB, Diana tools

15	mitomiR-5-5p	<i>PPARGC1A</i>	Master regulator of mitochondrial biogenesis, phosphorylates upon energy demand and thereby interacts with transcription factors like <i>TFAM</i> to initiate mitochondrial transcription.	RNA22, RNAhybrid, miRanda, FindTar3
16	mitomiR-5-5p	<i>MAPK14</i>	p38 MAPKs belonging to serine/threonine kinase which are involved in the activation of transcription factors such as <i>CREB</i> etc. which in turn phosphorylates <i>PGC1A</i> .	RNA22, RNAhybrid, Diana tools
17	mitomiR-5-5p	<i>MT-CO1</i>	Major mitochondrially encoded complex IV protein involved in OXPHOS.	miRanda, FindTar3
18	mitomiR-5-5p	<i>MT-CO2</i>	Major mitochondrially encoded complex IV protein involved in OXPHOS.	miRanda, FindTar3
19	mitomiR-5-3p	<i>SIRT3</i>	Proteins with NAD dependent deacetylase activity regulating mitochondrial transcription.	RNA22, RNAhybrid
20	mitomiR-5-3p	<i>MIEF1</i>	Mitochondrial elongation factor, recruits <i>DNM1L</i> to mitochondrial outer membrane to facilitate mitochondrial fission.	miRDB, RNA22, FindTar3
21	mitomiR-5-3p	<i>DNM1L</i>	Dynamin like protein majorly involved in mitochondrial fission.	RNA22, RNAhybrid
22	mitomiR-5-3p	<i>PPP3R1</i>	Serine/threonine phosphatase, which is an isoform of calcineurin B, dephosphorylates transcription factors like NFATs to initiate transcription.	miRDB, Diana tools
23	mitomiR-6-3p	<i>SLC25A41</i>	Belongs to the mitochondrial carrier proteins family.	miRDB, Diana tools
24	mitomiR-6-3p	<i>WNT2</i>	Receptor targeting ligand protein involved in wnt signaling pathway.	miRDB, Diana tools
25	mitomiR-7-5p	<i>SIRT2</i>	Proteins with NAD dependent deacetylase activity regulating major cellular processes.	miRDB, Diana tools
26	mitomiR-7-5p	<i>PTEN</i>	Tumor suppressor protein involved in mitochondrial energy metabolism by promoting COX activity and ATP production.	miRDB, Diana tools,
27	mitomiR-7-5p	<i>NDUFS1</i>	Nuclear encoded subunit of complex I, involved in the transfer of electrons from NADH to respiratory chain.	Diana tools, RNAhybrid
28	mitomiR-7-3p	<i>NDUFS1</i>	Nuclear encoded subunit of complex I, involved in the transfer of electrons from NADH to respiratory chain.	miRDB, RNA22
29	mitomiR-7-3p	<i>PPP1R14C</i>	Serine/threonine phosphatase inhibitor, involved in signal transduction processes of metabolism and cell division etc.	miRDB, Diana tools

30	mitomiR-7-3p	<i>DKK2</i>	Dickkopf family protein, inhibits wnt signaling pathway and involved in vertebrate development.	miRDB, Diana tools
31	mitomiR-8-5p	<i>BNIP2</i>	Cell death suppressor, protects cells from virally induced cell death.	miRDB, Diana tools
32	mitomiR-8-5p	<i>BCL11A</i>	Transcription factor associated with SWI/SNF chromatin remodeling.	miRDB, Diana tools, RNA22
33	mitomiR-9-5p	<i>IGF1R</i>	Insulin like growth factor I receptor functions as anti-apoptotic agent in malignant tissues for their survival.	miRDB, Diana tools, RNA22
34	mitomiR-9-5p	<i>ESRRG</i>	Belongs to nuclear hormone receptor superfamily and acts as transcription activator in the presence of bound receptor.	miRDB, Diana tools, RNA22
35	mitomiR-9-5p	<i>GABRB2</i>	Multi-subunit chloride channel that mediates major inhibitory neurotransmitter in the CNS.	miRDB, RNA22
36	mitomiR-9-3p	<i>ESR1</i>	Ligand activated transcription factor with domains for DNA, hormone binding and transcription activation.	miRDB, RNA22
37	mitomiR-10-5p	<i>MAPK6</i>	Belongs to serine/threonine protein kinase family and closely related to MAPKs.	miRDB
38	mitomiR-10-5p	<i>MAP3K7</i>	Belongs to serine/threonine protein kinase family and mediates TGF β induced signal transduction and involved in transcription regulation and apoptosis.	miRDB, RNA22
39	mitomiR-11-5p	<i>MTERF1</i>	Mitochondrial transcription terminator, binds downstream of <i>MT-RNR2</i> and terminates the transcription.	miRDB, RNA22
40	mitomiR-11-5p	<i>CDH8</i>	Encodes integral membrane proteins, mediating calcium dependent cell-cell adhesion.	miRDB, RNA22
41	mitomiR-11-3p	<i>PCDH17</i>	Belongs to proto-cadherin gene family involved in calcium dependent cell adhesion protein.	miRDB, RNA22
42	mitomiR-12-5p	<i>PDCD5</i>	Programed Cell Death protein, involved in apoptosis.	miRDB, Diana tools
43	mitomiR-12-5p	<i>AATK</i>	Induced during apoptosis and involved in neuronal differentiation.	miRDB, Diana tools
44	mitomiR-12-5p	<i>PPARGC1B</i>	Parologue and interacting partner of <i>PGC1A</i> involved in the mitochondrial transcription initiation.	RNA22, RNAhybrid

45	mitomiR-12-3p	<i>CABP2</i>	Calcium binding protein, similar to calmodulins.	miRDB, RNAhybrid
46	mitomiR-12-3p	<i>CAMK2A</i>	Belongs to serine/threonine protein kinase family and calmodulin dependent protein kinase subfamily, involved in neurotransmitter release in CNS.	miRDB, RNA22
47	mitomiR-13-3p	<i>PPARGC1B</i>	Parologue and interacting partner of <i>PGC1A</i> involved in the mitochondrial transcription initiation.	miRDB, RNA22
48	mitomiR-13-3p	<i>DNM3</i>	GTP binding protein, involved in vesicular transport and essential for signal transduction.	miRDB, RNAhybrid

Table S4. Clinico-pathological details of breast cancer patient samples.

Breast Cancer Clinicopathological Details	
Number of patients	40
Age	
<50	19
>50	21
Menopausal Status	
Pre	18
Post	22
Intrinsic subtypes	
Luminal A (ER+, PR+, Her2-)	15
Triple Negative (ER-, PR-, Her2-)	15
Her2 Positive (ER-, PR-, Her2+)	10
Tumor grade	
Grade 1	5
Grade 2	19
Grade 3	16
Lymph Node Status	
Positive	29
Negative	11

Table S5. List of primers used in the study

Sl. No.	Primers	Sequences
1	<i>PPARGC1A</i> -F	CTGCTAGCAAGTTGCCTCA
	<i>PPARGC1A</i> -R	AGTGGTCAGTGACCAATCA
2	<i>MT-CO1</i> -F	CTGCCATAACCCAATACC
	<i>MT-CO1</i> -R	GAAGAAGGTGGTGTGAG
3	<i>MT-CO2</i> -F	CCGACTACGGCGGACTAAC
	<i>MT-CO2</i> -R	CGCCTGGTTCTAGGAATAATGG
4	<i>GAPDH</i> -F	CTTCTCTGCTGTAGGCTCATT
	<i>GAPDH</i> -R	AGGCTGTTGTACACTTCTCAT
5	<i>MT-CYB</i> -F	TGAAACTTCGGCTCACTCCT
	<i>MT-CYB</i> -R	AATGTATGGATGGCGGATA
6	<i>ACTB</i> -F	GACGACATGGAGAAAATCTG
	<i>ACTB</i> -R	ATGATCTGGGTCATCTTCTC

Table S6. List of cloning primers used in the study

Sl. No.	Gene	Primer sequence	Restriction enzyme
1	<i>PPARGC1A</i>	FP: CACCGAGCTCAGCATATTGTGTTCTGAGTG	Sacl
		RP: CCAACCTGCAGGACATCATACCCCAATAGTGC	Sbfl
2	<i>MT-CO1</i>	FP: CCGAGAGCTCCGTTGACTATTCTCTACAA	Sacl
		RP: AGTGCCTGCAGGAATGAAGCCTCCTATGATGG	Sbfl
3	<i>MT-CO2</i>	FP: CTACGAGCTCAAGACGCTACTTCCCCTATC	Sacl
		RP: CTAGCCTGCAGGGACGATGGCATGAAACTGT	Sbfl

References:

- Lorenzi, L., Chiu, H.-S., Avila Cobos, F., Gross, S., Volders, P.-J., Cannoodt, R., Nuytens, J., Vanderheyden, K., Anckaert, J., Lefever, S., et al. (2021). The RNA Atlas expands the catalog of human non-coding RNAs. *Nat. Biotechnol.* 39, 1453–1465.
- Mercer, T. R., Neph, S., Dinger, M. E., Crawford, J., Smith, M. A., Shearwood, A. M., Haugen, E., Bracken, C. P., Rackham, O., Stamatoyannopoulos, J. A., et al. (2011). The human mitochondrial transcriptome. *Cell* 146, 645–58.