

Appendix

miR-181a/b downregulation exerts a protective action on Mitochondrial Disease models

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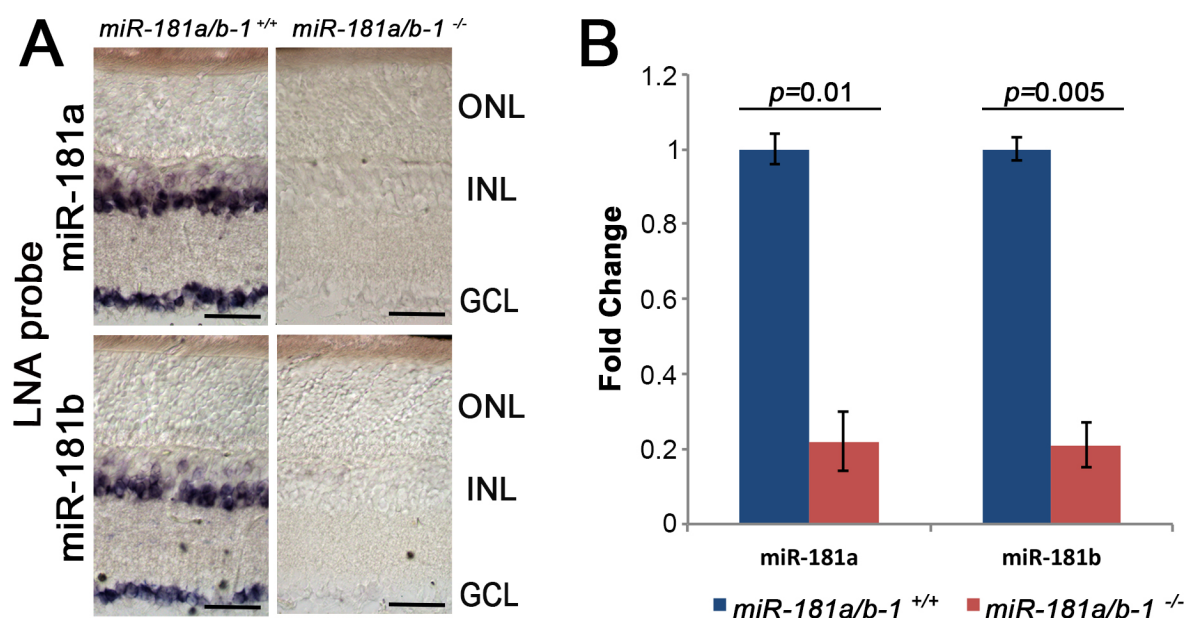
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Appendix Figure S1. The *miR-181a/b-1* cluster accounts for most of miR-181a and miR-181b expression in mouse eye. A) RNA in-situ hybridization with miR-181a and miR-181b probes in retina sections of *miR-181a/b-1*^{+/+} and *miR-181a/b-1*^{-/-} mice. Scale bars are 50μm. B) Expression analysis of miR-181a and miR-181b in *miR-181a/b-1*^{+/+} and *miR-181a/b-1*^{-/-} eyes by Taqman assays. Targeted deletion of the miR-181a/b cluster-1 leads to a striking reduction of the expression levels of the mature forms of miR-181a and miR-181b. N=3; *p*-values were calculated by one tailed Student's t-test; Error bars are SEM.

Appendix Table S1: *p*-values Figure 5G calculated by Two-ANOVA Repetaed Measures with post-hoc analysis

Genotype	log cd.s/m2	<i>p</i>-value
<i>Ndufs4</i> ^{+/+} / <i>miR-181a/b-1</i> ^{+/+} vs. <i>miR-181a/b-1</i> ^{+/+}	0.0001	0.99
<i>Ndufs4</i> ^{+/+} / <i>miR-181a/b-1</i> ^{+/+} vs. <i>Ndufs4</i> ^{+/+}	0.0001	0.99
<i>Ndufs4</i> ^{+/+} / <i>miR-181a/b-1</i> ^{+/+} vs. WT	0.0001	0.99
<i>miR-181a/b-1</i> ^{+/+} vs. <i>Ndufs4</i> ^{+/+}	0.0001	0.99
<i>miR-181a/b-1</i> ^{+/+} vs. WT	0.0001	0.99
<i>Ndufs4</i> ^{+/+} vs. WT	0.0001	0.99
<i>Ndufs4</i> ^{+/+} / <i>miR-181a/b-1</i> ^{+/+} vs. <i>miR-181a/b-1</i> ^{+/+}	0.1	0.003
<i>Ndufs4</i> ^{+/+} / <i>miR-181a/b-1</i> ^{+/+} vs. <i>Ndufs4</i> ^{+/+}	0.1	0.91
<i>Ndufs4</i> ^{+/+} / <i>miR-181a/b-1</i> ^{+/+} vs. WT	0.1	0.000003
<i>miR-181a/b-1</i> ^{+/+} vs. <i>Ndufs4</i> ^{+/+}	0.1	0.003
<i>miR-181a/b-1</i> ^{+/+} vs. WT	0.1	0.16
<i>Ndufs4</i> ^{+/+} vs. WT	0.1	0.000006
<i>Ndufs4</i> ^{+/+} / <i>miR-181a/b-1</i> ^{+/+} vs. <i>miR-181a/b-1</i> ^{+/+}	1	0.01
<i>Ndufs4</i> ^{+/+} / <i>miR-181a/b-1</i> ^{+/+} vs. <i>Ndufs4</i> ^{+/+}	1	0.00004
<i>Ndufs4</i> ^{+/+} / <i>miR-181a/b-1</i> ^{+/+} vs. WT	1	0.000002
<i>miR-181a/b-1</i> ^{+/+} vs. <i>Ndufs4</i> ^{+/+}	1	<0.0E-7
<i>miR-181a/b-1</i> ^{+/+} vs. WT	1	0.07
<i>Ndufs4</i> ^{+/+} vs. WT	1	<0.0E-7
<i>Ndufs4</i> ^{+/+} / <i>miR-181a/b-1</i> ^{+/+} vs. <i>miR-181a/b-1</i> ^{+/+}	10	0.000002
<i>Ndufs4</i> ^{+/+} / <i>miR-181a/b-1</i> ^{+/+} vs. <i>Ndufs4</i> ^{+/+}	10	0.0002
<i>Ndufs4</i> ^{+/+} / <i>miR-181a/b-1</i> ^{+/+} vs. WT	10	0.000002
<i>miR-181a/b-1</i> ^{+/+} vs. <i>Ndufs4</i> ^{+/+}	10	<0.0E-7
<i>miR-181a/b-1</i> ^{+/+} vs. WT	10	0.93
<i>Ndufs4</i> ^{+/+} vs. WT	10	<0.0E-7
<i>Ndufs4</i> ^{+/+} / <i>miR-181a/b-1</i> ^{+/+} vs. <i>miR-181a/b-1</i> ^{+/+}	20	<0.0E-7
<i>Ndufs4</i> ^{+/+} / <i>miR-181a/b-1</i> ^{+/+} vs. <i>Ndufs4</i> ^{+/+}	20	0.0008
<i>Ndufs4</i> ^{+/+} / <i>miR-181a/b-1</i> ^{+/+} vs. WT	20	0.0009
<i>miR-181a/b-1</i> ^{+/+} vs. <i>Ndufs4</i> ^{+/+}	20	<0.0E-7
<i>miR-181a/b-1</i> ^{+/+} vs. WT	20	0.94
<i>Ndufs4</i> ^{+/+} vs. WT	20	<0.0E-7
<i>Ndufs4</i> ^{+/+} / <i>miR-181a/b-1</i> ^{+/+} vs. <i>miR-181a/b-1</i> ^{+/+}	PHOTOPIC	0.39
<i>Ndufs4</i> ^{+/+} / <i>miR-181a/b-1</i> ^{+/+} vs. <i>Ndufs4</i> ^{+/+}	PHOTOPIC	0.04
<i>Ndufs4</i> ^{+/+} / <i>miR-181a/b-1</i> ^{+/+} vs. WT	PHOTOPIC	0.31
<i>miR-181a/b-1</i> ^{+/+} vs. <i>Ndufs4</i> ^{+/+}	PHOTOPIC	0.004
<i>miR-181a/b-1</i> ^{+/+} vs. WT	PHOTOPIC	0.98
<i>Ndufs4</i> ^{+/+} vs. WT	PHOTOPIC	0.04

Appendix Table S2: Primer sequences for q-RT-PCR analysis.

Transcript	Forward	Reverse
hsa- <i>HPRT</i>	AACACCCTTTCCAAATCCTCA	TGGCGTCGTGATTAGTGATG
hsa- <i>GAPDH</i>	ATGTTTCGTCATGGGTGTGAA	AGGGGTGCTAAGCAGTTGGT
hsa- <i>PPARGC1A</i>	GCTTGGCCCTCTCAGACTCT	GCCACTACAGACACCGCACG
hsa- <i>NRF1</i>	GAGTGATGTCCGCACAGAAG	GTTTGCGTTTGCTGATCTTC
hsa- <i>COX11</i>	GCTGCCGTACCCCTTTATCG	CACTGGAGACTTGCATGCAC
hsa- <i>COQ10</i>	GGCGTTTTAGCCAGGTCTTC	GCTACCATCTGCTTCACAAC
hsa- <i>PRDX3</i>	CCCGAGACTACGGTGTGCTG	CACTGGGAGATCGTTGACGC
mmu- <i>Hprt</i>	AGCTTGCTGGTGAAAAGGAC	GTCAAGGGCATATCCACAAC
mmu- <i>Gapdh</i>	GGTGCTGAGTATGTCGTGGA	CTAAGCAGTTGGTGGTGCAG
mmu- <i>Bcl2</i>	ACAACATCGCCCTGTGGATG	GTTTGTCGACCTCACTTGTG
mmu- <i>Mcl1</i>	GCTTCATCGAACCATTAGCAG	CCAGCAGCACATTTCTGATG
mmu- <i>Xiap</i>	GGTCCTGATTGCAGATCTTG	GTCAACTGCTTCTGCACAC
mmu- <i>Atg5</i>	GCCTATATGTACTGCTTCATC	CAACGTCAAATAGCTGACTC
mmu- <i>Erk2</i>	CACCAACCTCTCGTACATCG	GTGCCCGGATGATGTCATTG
mmu- <i>Park2</i>	CCATCAAGAAGACCACCAAG	CAAGTGACATCTCTCTCTAC
mmu- <i>Nrf1</i>	CTTACTGGAGTCCAAGATGC	GGAGCCAACAGAATCCTTTC
mmu- <i>Cox11</i>	GAATCCTACTGACAAACCAG	GAGGTCGACATTCAACATTC
mmu- <i>Coq10</i>	GATGATCATGGCAGCTCGGA	CTCGCACAGATCTCTTTAGG
mmu- <i>Prdx3</i>	GGAGTATTTCTGCCTCAACAG	CTCTCCATTGACAACAGCAG
mmu- <i>Ppargc1a</i>	GGAATGCACCGTAAAATCTGC	TTCTCAAGAGCAGCGAAAGC
mmu- <i>Sqstm1</i>	TTCTTTTCCCTCCGTGCTC	GGATCCGAGTGTGAATTTCC
mmu- <i>Map1lc3b</i>	TTATAGAGCGATACAAGGGGGAG	CGCCGTCTGATTATCTTGATGAG
ol- <i>hprt</i>	CTGAACAGGAACAGCGACC	TGAGGAGCTCCAATAACGTC
ol- <i>gapdh</i>	CGGCAAGCTGATAGTCGATG	AGAAACACTCCGGTGGACTC
ol- <i>bcl2</i>	GGATGACGGAGTATTTAAACG	GGCCGAAGACAGTCTTGATG
ol- <i>mcl1</i>	CGCGAAAAGAGTGGTGAAC	CCTTCAAGGACTGACACAC
ol- <i>atg5</i>	CAGATGACAAAGACGTGCTG	CTCCACATCTTCTGTCCTCA
ol- <i>erk2</i>	GCAGCGACAGCAGATAGTTC	GCCGAGATGTTGTCCAACAG
ol- <i>cox11</i>	CACCGACAAACCCATCATC	GAATTCGGGTGCGATGTAG
ol- <i>coq10</i>	GTTCTGCAAGGCCAAACTG	CGTCTCCAAGTGGTTGAAG
ol- <i>prdx3</i>	GTCCTGTTCTTCTACCCTC	AGTGAGAATCCACAGACACC
ol- <i>ppargc1a</i>	GGACGGATTGCCTTCATTG	GTTTGCAGGTGCCAGAAGG

Appendix Table S3: Primer sequences for human 3'UTR amplification and mutagenesis

Transcript	Primer sequences
hsa- <i>NRF1</i> -3'UTR	Fw-GCTCTAGAGACTTCTTTCTGCGGAAATG Rv-GCTCTAGACTGTTTTCTATGGCCAGGTG
hsa- <i>NRF1</i> -3'UTR mutagenesis	Fw-CCACAGGCAGATGCGCGTCTTGAAAGCTCCCGGGCC Rv-GGCCCCGGGAGCTTTCAAGACGCGCATCTGCCTGTGG
hsa- <i>PPARGC1A</i> - 3'UTR	Fw-GCTCTAGAGCACTACAGATATCATATTGAGG Rv-GCTCTAGATAGATTTGAAACATTCGTTTCCC
hsa- <i>PPARGC1A</i> - 3'UTR mutagenesis	Fw-CTGAGCTAATAAAGGGAAACGCCGGTTTCAAATCTCTAGGTCGGG Rv-CCCGACCTAGAGATTTGAAACCGGCGTTTCCCTTTATTAGCTCAG
hsa- <i>COX1</i> -3'UTR	Fw-GCTCTAGAGCCTAGCTAGAATATATGAC Rv-GCTCTAGACACAGGCTTCCTACATTTAG
hsa- <i>COX11</i> -3'UTR mutagenesis	Fw-CAAGTCCATGCGCGTTAAAATGTACAGGTGGGATTG Rv-CAATCCCACCTGTACATTTTAACGCGCATGGACTTG
hsa- <i>COQ10</i> -3'UTR	Fw-GCTCTAGAGCCACCTGCTTCTGACTTTAG Rv-GCTCTAGACAACTTCAGTCCTTACATTG
hsa- <i>COQ10</i> -3'UTR mutagenesis	Fw-GAAGATAAGTTGGTTGGGCGTCTCCAGCACTATGCATCCC Rv-GGGATGCATAGTGCTGGAGACGCCCAACCAACTTATCTTC
hsa- <i>PRDX3</i> -3'UTR	Fw-GCTCTAGACTGAGAGAAGAACCACAGTTG Rv-GCTCTAGACCCTGGATTTGATAAATATCC
hsa- <i>PRDX3</i> -3'UTR mutagenesis site I	Fw-CGGTCCTGAAATTTTCATCTTGCCGGTCTTTGTATTAACTGAATTTTC Rv-GAAAATTCAGTTTAATACAAAGACCGGCAAGATGAAAATTTTCAGGACCG
hsa- <i>PRDX3</i> -3'UTR mutagenesis site II	Fw-GCCGTGTAACCTCCTGCAATGCCGGTTTATGTGATTGAAGC Rv-GCTTCAATCACATAAACCGGCATTGCAGGAGTTACACGGC