

Supplementary information

miR-8-3p regulates *mitoferrin* in the testes of *Bactrocera dorsalis* to ensure normal spermatogenesis.

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Supplementary Table

Table S1. miRNA mimics and antagomiRs used in this study.

Supplementary Figure

Figure S1: The putative *Bactrocera dorsalis* mitoferrin (bmfrn) amino acid sequence contains conserved histidine residues required for mitoferrin iron transport function. Amino acid sequences from mitoferrins of *B. dorsalis* (GI: 751779949, NCBI), fruit fly (*dmfrn*, flybase), yeast (GI: 1008338 and GI: 486507, NCBI) and human (GI: 82775373 and GI: 149274643, NCBI) were aligned using CLC Main Workbench 6.9 (CLC bio, Denmark) using the “very accurate” setting. Conserved histidine residues required for mitoferrin iron transport function¹⁹ are indicated by asterisks.

miRNA	Sequence
Mimics	
miR-279-3p-sense strand	UGACUAGAUCCACACUCAU
miR-279-3p-antisense strand	GAUGAGUGUGGAUCUAGUCA
miR-8-3-p-sense strand	UAAUACUGUCAGGUAAGAUGUC
miR-8-3-p-antisense strand	ACAUCUUUACCUGACAGUAUUAU
miR-275-3p-sense strand	UCAGGUACCUGAAGUAGCGCGCG
miR-275-3p-antisense strand	CGCGCUACUUCAGGUACCUGAUU
miR-34-3p-sense strand	CAGCCACUAUCUUCACUGCCGCC
miR-34-3p-antisense strand	CGGCAGUGAAGAUAGUGGCUGUU
miR-304-5p-sense strand	UAAUCUCAAUUUGUAAACUGUGA
miR-304-5p-antisense strand	ACAGUUACAAAUUGAGAUUAUU
N.C mimc-sense strand	UUCUCCGAACGUGUCACGUTT
N.C mimc-antisense strand	ACGUGACACGUUCGGAGAATT
antagomiRs	
miR-8-3-p	AGACAUCUUUACCUGACAGUAUUA
N.C antagomiR	CAGUACUUUUGUGUAGUACAA

Table S1. miRNA mimics and antagomiRs used in this study.

bmfrn ----- MNM 3
 dmfrn ----- MNM 3
 mfrn2 MELEGRGAGGVAGGPAAGPGRSPGESALLDGLQRFVGRGAGGGAGACRPPVQRDPDSG 60
 mfrn1 ----- MELRSGSV - - GSAVARRMGDSDGGGGKDATGS 33
 MRS3 ----- MVENSSSNSTRIPIAIPMDL 21
 MRS4 ----- MNTSELSIAEEI - - - - - 12

Sequence logo
 4.3bits
 0.0bits MELEGRGAGGVAGGPAAGPGRSPGESALLDGLQRFVGRGAGGGAGACRPPVQRDPDSG

bmfrn DDYESLPT - TSVSINMTAGATAGVLEHCVMPYLDVSKTRMQSLSP - - MTSNYDISTTFKN 60
 dmfrn DDYESLPT - TSVGNMTAGATAGVLEHVVMPYLDVSKTRMQSLSP - - PTKNMNIIVSTLRT 60
 mfrn2 PDYEALPAGATVTTHMVAGAVAGLEHCVMPYLDVSKTRMQSLQDPAAARYRNVLEALWR 120
 mfrn1 EDYENLPTSAVSTHMTAGAMAGLEHSMVMPYLDVSKTRMQSLSPDKAQYTSIYGAIKK 93
 MRS3 PDYEALPTHAPLYHQLIAGAFAGIMEHSMVFPYLDVSKTRMQSLSPDKAQYTSIYGAIKK 80
 MRS4 - DYEALPSHAPLHSQLLAGAFAGIMEHSLMFPYLDVSKTRVQAAGLN - KAASTGMIISQISK 70

Sequence logo
 4.3bits
 0.0bits DDYESLPT - TSVSINMTAGATAGVLEHCVMPYLDVSKTRMQSLSP - - MTSNYDISTTFKN

bmfrn MIKKEGIMRPIFGVSAVVAGAGPAHALYFGSYEMTKELTKFT - - TNNHVNYMASGATA 117
 dmfrn MITREGLRPIFGASAVVAGAGPAHSLYFAAYEMTKELTAKFT - - SVNRLNVIYISGAVA 117
 mfrn2 IIRTEGLWRPMEGLNVITAGAGPAHALYFACYEKKKTLSDVTHPGNSHIANGAAGCVA 180
 mfrn1 IMRTEGLWRPMEGLNVIMGAGPAHAMYFACYENMKFTLNDVFHHQGNSHLANGIAGSMA 153
 MRS3 ISTEGLTALWKGVSQSVILGAGPAHAYVFGTYEFCCKNLIDSSDTQTHHPFKTAISGACA 140
 MRS4 ISTEGLTALWKGVSQSVILGAGPAHAYVFGTYEFCCKNLIDSSDTQTHHPFKTAISGATA 130

Sequence logo
 4.3bits
 0.0bits MIKKEGIMRPIFGVSAVVAGAGPAHALYFGSYEMTKELTKFT - - TNNHVNYMASGATA

bmfrn TLIHDAVSNPTFVIKQRMQMYNSKYNVSVISCMKDYRTEGLIQAFYRSYSTQLVMNIPLYQT 177
 dmfrn TLIHDAVSSPTFVIKQRMQMYNSPYTSVSVSCVRFYKREGFKAIFYRAYGTQLVMNIPLYQT 177
 mfrn2 TLLHDAAMNPAEVVKQRMQMYNSPYHRVTDVRAVWQNEGAGAFYRSYTTQLTMNYPQA 240
 mfrn1 TLLHDAVMNPAEVVKQRMQMYNSQHRSAISCI RTVWRTEGLGAFYRSYTTQLTMNYPQS 213
 MRS3 TIASDALMNPFDTIKQRIQLNTSA - - SVWQTTKQIYQSEGLAAFYYSYPTTLVMNIPFAA 198
 MRS4 TIAADALMNPFDTVKQRQLQDNTNL - - RVWNVTKQIYQNEGFAAFYYSYPTTLAMNIPFAA 188

Sequence logo
 4.3bits
 0.0bits TLIHDAVSNPTFVIKQRMQMYNSKYNVSVISCMKDYRTEGLIQAFYRSYSTQLVMNIPLYQT

bmfrn IHFATYEFFQNMNLHERRYSPVHMVAGGAAGATAAAFTTPLDVVKTLLNTQEN - - - - - 231
 dmfrn IHFTTYEFFQNMNLERKYNPPVHMAAGAAAGACAAAFTTPLDVVKTLLNTQET - - - - - 231
 mfrn2 IHLTHITGMASAFRTVYQVGGVTAYFRGVCARVLYQITPSTAIAWSVYEFFKYLITKRQEE 299
 mfrn1 IHFTTYEFFQEQVNPHTYNSQSHLISGGLAGALAAAFTTPLDVVKTLLNTQENALSLA 273
 MRS3 INFVIYESSTKFLNPSNEYNPLIHCLCGSISGSTCAAITTPLDCKITVLLQIRGSQTVSL 257
 MRS4 INFMIYESASKFFNPQNSYNPLIHCLCGSISGATCAAITTPLDCKITVLLQVRGSETVSL 247

Sequence logo
 4.3bits
 0.0bits IHFATYEFFQNMNLHERRYSPVHMVAGGAAGATAAAFTTPLDVVKTLLNTQEN - - - - -

bmfrn - - - GLTKMGFEAIRQIYAVAGVKGFFFRGMLARVLYSMPATAICWSTYEFFKFYLAGSNHS 288
 dmfrn - - - GLTRGMIEASRKIYHMAGPVGFFFRGTTARVLYSMPATAICWSTYEFFKFYLCGLDAD 288
 mfrn2 HITGHTIGMASAFRTVYQVGGVTAYFRGVCARVLYQITPSTAIAWSVYEFFKYLITKRQEE 359
 mfrn1 NISGRLSGMANAFRTVYQLNGLAGYFKGIQARVLYQOMPSTAI SWSVYEFFKYFLTKRQEE 333
 MRS3 EIMRKADTFSKAASAIYQVYGWKGFWRGWKPRIVANMPATAISWTAYECAKHFMTY - - - 314
 MRS4 EIMKDANTFGRASRAILEVHGWKGFWRGLKPRIVANIPATAISWTAYECAKHFMTY - - - 304

Sequence logo
 4.3bits
 0.0bits HITGHTIGMASAFRTVYQVGGVTAYFRGVCARVLYQITPSTAIAWSVYEFFKYLITKRQEE

bmfrn TYKSSIITGKNALQRRDDMVEPAEKTAYVLPVITADAVEELPTSPSATTATAPSSSSAGGA 348
 dmfrn QYKSSIITGSSE - PRKADYVLPRTTDEEQID - QEREAAKEKDTT - - ATLHSAPTSVNASGA 344
 mfrn2 WRAGK - - - - - 364
 mfrn1 NRAPY - - - - - 338
 MRS3 - - - - - 314
 MRS4 - - - - - 304

Sequence logo
 4.3bits
 0.0bits TYKSSIITGKNALQRRDDMVEPAEKTAYVLPVITADAVEELPTSPSATTATAPSSSSAGGA

bmfrn TAGLAPSSATAIKSVCELPSNVTTALNLHTRHTDVKSTRPFERGFYSSP 397
 dmfrn - - - - - IKTVCELSTRPAGPTINLHTRHTDVKS - - PYERGFST - 379
 mfrn2 - - - - - 364
 mfrn1 - - - - - 338
 MRS3 - - - - - 314
 MRS4 - - - - - 304

Sequence logo
 4.3bits
 0.0bits TAGLAPSSATAIKSVCELPSNVTTALNLHTRHTDVKSTRPFERGFYSSP

Figure S1: The putative *Bactrocera dorsalis* mitoferrin (bmfrn) amino acid sequence contains conserved histidine residues required for mitoferrin iron transport function. Amino acid sequences from mitoferrins of *B. dorsalis* (GI: 751779949, NCBI), fruit fly (*dmfrn*, flybase), yeast (GI: 1008338 and GI: 486507, NCBI) and human (GI: 82775373 and GI: 149274643, NCBI) were aligned using CLC Main Workbench 6.9 (CLC bio, Denmark) using the “very accurate” setting. Conserved histidine residues required for mitoferrin iron transport function¹⁹ are indicated by asterisks.