Α

TTGGTAACAGTCTACAGCCA

miR-132 gRNA *miR-132* locus

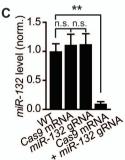
ATTTTTGG<u>TAACAGTCTACAGCCATGGTCG</u>CTAGTGG

mature miR-132

В

ATTTTTGGTAACAGTCTACAGCCATGGTCGCTAGTGG miR-132

- ATTTTTGGTAACAGTCT----AG-----TGGTCGCTAGTGG -7, +2
- ATTTTTGGTAACAGTCTACAGC----TAGTGG -9
- ATTTTTGGTAACAGTCTACA-----ATGGTCGCTAGTGG -3
- ATTTTTGGTAACAGTCTACA----TGGTCGCTAGTGG -4
- ATTTTTGGTAACAGTC-----CATGGTCGCTAGTGG -6
- ATTTTTGGTAACAGTCTACAGGTCGCTATCCATGGTC +8 GCTAGTGG



Supplementary Information, Figure S4. Mutation of *miR-132* by CRISPR/Cas9. (A) Design of *miR-132* gRNA. The gRNA targets to the mature sequence of *miR-132* (marked by the line), and the PAM sequence is in green. (B) Sequence analysis of *miR-132* mutations caused by co-injection of zebrafish codon-optimized *Cas9* mRNA and *miR-132* gRNA. The red fonts show the target site of *miR-132* gRNA, yellow blanks show mutated sequences, and the green fonts show the PAM sequence. (C) Reduced expression of *miR-132* by co-injection of *Cas9* mRNA and *miR-132* gRNA. The experiments were repeated 5 times. Error bars, SEM. n.s., no significant; ***P* < 0.01 (one-way ANOVA with post-hoc Tukey's multiple comparison test for (C)).