

SUPPLEMENTARY MATERIAL

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Supplementary Tables Description

Supplementary Table S1. Zebrafish pre-miRNA sequences downloaded from miRBase.

Supplementary Table S2. Zebrafish pre-miRNA sequences downloaded from Ensembl.

Supplementary Table S3. Zebrafish pre-miRNA PQSs predicted with QGRMapper, containing two G-tetrads and scores predicted by G4RNAScreener.

Supplementary Table S4. Zebrafish pre-miRNA PQSs predicted with QGRMapper, containing three G-tetrads and scores predicted by G4RNAScreener.

Supplementary Table S5. PQSs predicted with QGRMapper in pre-miR-150 orthologs from 38 vertebrate species downloaded from miRBase and scores predicted by G4RNAScreener.

Supplementary Table S6. RNA and DNA oligonucleotide sequences.

Supplementary Table S7. P values of pairwise comparisons from Figures 3, 5 and S3.

Supplementary Table S8. Descriptive statistics for RT-qPCR and phenotype analyses shown in Figures 3, 5 and S3.

Phylogenetic tree showing relationships between various species and their corresponding DNA sequences. The tree is rooted on the left and branches out to the right. Species names are listed on the left, and their corresponding DNA sequences are listed on the right. The sequences are color-coded: red for 'A', green for 'C', blue for 'G', and black for 'T'. The sequences are aligned in columns, with each column representing a specific position in the genome. The tree structure indicates the evolutionary relationships between the species, with branches representing the divergence of lineages. The DNA sequences are provided for each species, allowing for comparison and analysis of genetic differences and similarities.

G4RNAScreener prediction

- Three G4RNAScreener predictors above threshold
- At least one G4RNAScreener predictor above threshold
- No G4RNAScreener predictor above threshold

Supplementary Figure S1. Conservation of the PQSs in pre-miR-150 among vertebrate species. Multiple sequence alignments were performed using Clustal Omega (Madeira et al. 2019, doi:10.1093/nar/gkz268) of annotated pre-miR-150 sequences from 38 vertebrate species downloaded from miRBase [46] (Kozomara et al. 2019, doi:10.1093/nar/gky1141) database. The phylogenetic tree is represented on the left. The mature miR-150 sequences are shaded in blue. PQSs found by QGRS Mapper (<https://bioinformatics.ramapo.edu/QGRS/analyze.php>, Kikin et al. 2006, doi:10.1093/nar/gkl253; max length: 45, Min G-Group Size: 2, Loop size: from 1 to 15) are shaded in grey. Dots on the right indicate the predicted G4 folding probability according to G4RNAScreener predictors [59] (Garant et al. 2018, doi:10.1016/j.biochi.2018.06.002) (see Supplementary Table S5 for score values). PQSs that were found with QGRS Mapper and whose scores for the three G4RNAScreener predictors were over the defined thresholds were classified with a high probability to form G4 (green dots). Those PQSs that were found with QGRS Mapper and whose score for at least one G4RNAScreener predictor was over the defined thresholds were classified with medium probability to form G4 (yellow dots). Those PQSs that were found with QGRS Mapper and whose scores for the three G4RNAScreener predictors were below the defined thresholds were classified with low probability to form G4 (red dots). *S. harrissi* and *G. gorilla* pre-miR-150 contain two predicted PQS, where the first and second probability dots correspond to the first and second PQS, respectively. The asterisks at the bottom represent 100% nucleotide conservation among all species.

