

Supplemental_Fig_S1. An integrative computational pipeline for the systematic identification of ncRNAs in Chinese tongue sole gonad.



Supplemental_Fig_S2. Distribution and the interactions of lncRNAs, circRNAs, miRNAs along each chromosome. The points in chromosomes indicate the ncRNAs (circRNAs, miRNAs and lncRNAs in blue, green and red, respectively). Each line connects the circRNA and miRNA or lncRNA and miRNA based on the miRNA binding site predicted by miRnada.



Supplemental_Fig_S3. Genomic features of ncRNAs and mRNAs in Chinese tongue sole gonad. (A) Length distribution of predicted lncRNAs and mRNAs observed in each phenotype. (B) Distribution of detected circRNAs lengths observed in each phenotype. (C) Exon number distribution of lncRNAs and mRNAs observed in each phenotype. (D) Distribution of the number of exons per detected circRNAs observed in each phenotype. (E) Distribution of detected miRNA lengths in each data set. (F) Distribution of the number of circRNA reads observed in each phenotype for each parental gene.



Supplemental_Fig_S4. Expression analysis of circRNAs and miRNAs in Chinese tongue sole gonad. (A) Expression density distribution map of circRNAs. (B) Expression density distribution map of miRNAs. The x-axis indicates the log_2 (RPM) and log_2 (TPM) values of circRNAs and miRNAs, respectively. The y-axis indicates the density values.



Supplemental_Fig_S5. Three dimensional plots of the PCA of 15 gonadal samples using the three principal components of the expression levels of lncRNAs (A) and miRNAs (B) and circRNAs (C)



Supplemental_Fig_S6. Representative images of confocal micrographs of the subcellular localization and expression of *circdmrt1* (green) and *dmrt1* (red) in tongue sole testis. Nuclei were counterstained with DAPI (blue). Scale Bars are 20 µm.



Supplemental_Fig_S7. Representative images of confocal micrographs of *circdmrt1*, *AMSDT*, cse-miR-196 in tongue sole testis and liver. (A) Representative merged images of confocal micrographs of tongue sole testis stained with *circdmrt1* sense probe, cse-miR-196 as negative control, and the nuclear stain DAPI. (B) Representative merged images of confocal micrographs of tongue sole testis stained with *AMSDT* sense probe, cse-miR-196 as negative control, and DAPI. (C) Representative merged images of confocal micrographs of tongue sole testis stained with *AMSDT* sense probe, cse-miR-196 as negative control, and DAPI. (C) Representative merged images of confocal micrographs of tongue sole liver stained with *circdmrt1* anti-sense probe, cse-miR-196 antisense probe as negative control, and DAPI. (E) Representative merged images of confocal micrographs of tongue sole liver stained with *AMSDT* anti-sense probe, cse-miR-196 antisense probe as negative control, and DAPI. (E) Representative merged images of confocal micrographs of tongue sole liver stained with *AMSDT* anti-sense probe, cse-miR-196 antisense probe as negative control, and DAPI. (E) Representative merged images of confocal micrographs of tongue sole liver stained with *AMSDT* anti-sense probe, cse-miR-196 antisense probe as negative control, and DAPI. (E) Representative merged images of confocal micrographs of tongue sole liver stained with *AMSDT* anti-sense probe, cse-miR-196 antisense probe as negative control, and DAPI. Scale bars represent 20 μm.



Supplemental_Fig_S8. RIP experiments carried out using an Ago2 antibody and Chinese tongue sole testis extracts, followed by qRT-PCR to detect *gsdf*. *p < 0.05; **p < 0.01; ***p < 0.001, two-tailed *t*-test.



Supplemental_Fig_S9. Sequence comparisons of *C. semilaevis circdmrt1* (A), the cse-miR-196 precursor (B) with other teleosts. Sequences were aligned using Mega and analyzed online via MEME. Different colored boxes represent the motifs. The letters S, R, Y, M, and B designate strong hydrogen bonding (G or C), purine (A or G), pyrimidine (C or T), amino group at common position (A or C), and not A (G, C, or T) ribonucleotides, respectively.



Supplemental_Fig_S10. Expression levels of *gsdf* in tongue sole testis treated with *dmrt1* siRNA and a *circdmrt1* overexpression vector. The transcription levels were normalized to *Actb1* levels. Vector+nc represents the empty vector without *dmrt1* and is the negative control of the siRNA co-transfection group. The error bars represent SD. (n = 3). *p < 0.05; **p < 0.01; ***p < 0.001, two-tailed *t*-test.