

Supplementary information

Two *Figla* homologues have disparate functions during sex differentiation in half-smooth tongue sole (*Cynoglossus semilaevis*)

**Hailong Li^{1,2}, Wenteng Xu^{1,2}, Ning Zhang^{1,2}, Changwei Shao^{1,2}, Ying Zhu^{1,2},
Zhongdian Dong^{1,2}, Na Wang^{1,2}, Xiaodong Jia^{1,2}, Hao Xu^{1,2} & Songlin Chen^{1,2*}**

¹Key Lab of Sustainable Development of Marine Fisheries, Ministry of Agriculture;
Yellow Sea Fisheries Research Institute, Chinese Academy of Fishery Sciences,
Qingdao 266071, China

²Laboratory for Marine Fisheries Science and Food Production Processes, Qingdao
National Laboratory for Marine Science and Technology, Qingdao 266273, China

*Corresponding author:

E-mail: chensl@ysfri.ac.cn

Supplementary Figures

A

```

1  ggcatcaggacagaagcaccgcaggtgtgcgtacctgtttcatcacagtacacatcaatg
61  agaaaggaaggaagaaacccgttgtttaatcagatTTTTTaaagtgacggtagaa
1  M V T M E V P V E E L M S
121 atattttgctattgtattataATGTTACAATGGAGGTTCGGTGGAGGAATTAATGAG
14  D V L K R V T C E S A L P M F S N I E K
181 CGACGTGTGAAGCGTGTGACGGCGAGTCTGCTGCCCCATGTTCACTAACATAGAGAA
34  F K R A K D G L Y F V A E D F N E T V K
241 GTTCAAACGAGCCAAAGACGGTTTGATTTCGTAGCCGAAAGACTTCAATGAAACGGTGAA
54  K R E L V N A K E R L R I R N L N T M F
301 AAAGAGGGAATTGGTTAACGCCAAGGAACGACTCAGGATTGCTAACCTGAACACAATGTT
74  S R L R R M V P L M R P D R K P S K V D
361 CTCCCGTCTGAGGCCAATGGTGCCTTATGAGACCTGACAGGAAGCCAGTAAAGTAGA
94  T L K A A T E Y I R L L A A V L Q D T D
421 CACACTAAAAGCTGCTACCGAATATATCCGATTGCTGCTGAGTTTTCGAAGATACTGA
114  D H D S S G S D F L N S A I T Y G Q T E
481 TGATCATGACAGCAGTGGTCTGATTTCCCTAAATAGTCAATCACTTATGGCCAGACTGA
134  G L G N D L W R L D D I L N T S D E C I
541 AGGCCTAGGGAATGACCTATGGAGATTGGATGATATACTAAACACATCAGATGAGTGCAT
54  D D G F M L S P G P V P E D G E M T R L
601 CGATGACGGATTATGTTATCCCTGGCCAGTCCAGAGGATGGAGAAATGACCAGGCT
174  M L Q H C V M P A Y Q V I I Q V A P D Q
661 GATGTTACAGCACTGTGTGCTGCTGATCAGGTCATCAATCAAGTGGCTCCTGATCA
194  T T V *
721 AACTACGGTCTGAtcctgtgaagacttgcatctcatagaagaatTTTctttgtctaat
781 ttctttataatTattgtttgagtttaattaatgtggaactgcttttattttcatttttaag
841 ccacatttacagaaggtctagacatttctaatggcctctaatTTTaaagtttattt
901 ttgatggTTTTatgttctcaacaaaagtcagcagcaattatcagatcacagtgccag
961 atggtatcaagatttatgtttgtaataaagtgtcactaataaattacctctccaatt
1021 tgcgaaaaaaaaaaaaaaaaaaaaaaaaa

```

B

```

1  tatgcttgacacagcactctgtcaacagccagcctcottaactataaaactttgaggtt
61  taccatcctatggagggtacaatgatggtctcctgggcaactgtcatgtctgcagctt
121 tccccatattgaaccgaactgggacaattgaaccagaccaagcaattaaataaacactg
181 gggaaaactgtcaggtgctttgagtttagtagatgattggtgtgacactcagtttaa
241 aacattcatgcctgatgatttcttcacagattctgattgttgaggtcctgttttgt
301 ggttttatgagctggaggccaaaattatgtaaaaaataacaaaataaataactgaaatt
361 gattgagttgtgcccataatctatgaatatacaggggtttggctttctgaatggaatt
421 gtggagttgaacagcttttgcgcatgttctgatttttggaaaggatctgtatgtcac
481 tttcctttcttctttgatgcatggagggtagtagcagcagcagtagcagaagcaacag
541 gtggagtagtgagcaagcagctagcctgctttttcttccaactgaactgctggatg
601 cacagttcacaaatgtctaatgattttgagccctgottgatagagcttcaattta
661 caaattctactgtctttcatgtgtcacaatgctacttattttctgttttctactc
721 atttttgaccacacaaacctctgctctcaactctctctcctcagcggattaccoccg
1  M F S R L R R M V
781 cccctctcgtttgtgattcgtaactgaaacacATGTTTCCTCCGTCGAGGCCAATGG
10  P L M R P D R K P S K V D T L K A A T E
841 TGCCCTTATGAGACCTGACAGGAAGCCAGTAAAGTAGACACACTAAAAGCTGCTACCG
30  Y I R L L A A V L Q D T D D H D S S G S
901 AATATATCCGATTGCTTGTGCGATTGCAAGATCTGATGATCATGACAGCAGTGGGT
50  D F L N S A I T Y G Q T E G L G N D L W
601 CTGATTTCCCTAAATAGTCAATCACTTATGCCCAGACTGAAGGCCATGGGAATGACCTAT
70  R L D D I L N T S D E C I D D G F M L S
1021 GGAGATTGGATGATATACTAAACACATCAGATGATGATGATGACGGATTTCATGTTAT
90  P G P V P E D G E M T R L M L Q H C V M
1081 CCCCCTGGCCAGTCCCAGAGGATGGAGAAATGACCAGGCTGATGTTACAGCACTGTGTA
110  P A Y Q V I I Q V A P D Q T T *
1141 TGCTGCGTATCAGGTCATCAATCAAGTGGCTCCTGATCAAACACTACGGTCTGAtcctgtg
1201 aagactgcatctcatagaagaatTTTctttgtctaatTTTctttataaattattgtttg
1261 agttaattaatgtggaactgcttttattttcatttttaagccacatttacagaaggtcta
1321 gacatttctaatggcctctaatTTTaaagttattttgatggtttttatgttctc
1381 aacaaaagtcagcagcaattatcagatcacagtgccagatggtatcaagatttatgtt
1441 tghtaataatgtagtcactaataaattacctctccaatttgcgaaaaaaaaaaaaaaaaa
1501 aaaaaaaaaa

```

Figure S1 Full-length cDNA sequences and deduced amino acid sequences of *Figla_tv1* (A) and *Figla_tv2* (B) from *C. semilaevis*. Lower case letters indicate the 5' and 3' UTR sequences. Upper case letters indicate the coding sequence. The predicted start codon (ATG) is boxed, and the predicted stop codon (TGA) is double underlined. The polyadenylation signal (aataaa) is underlined.

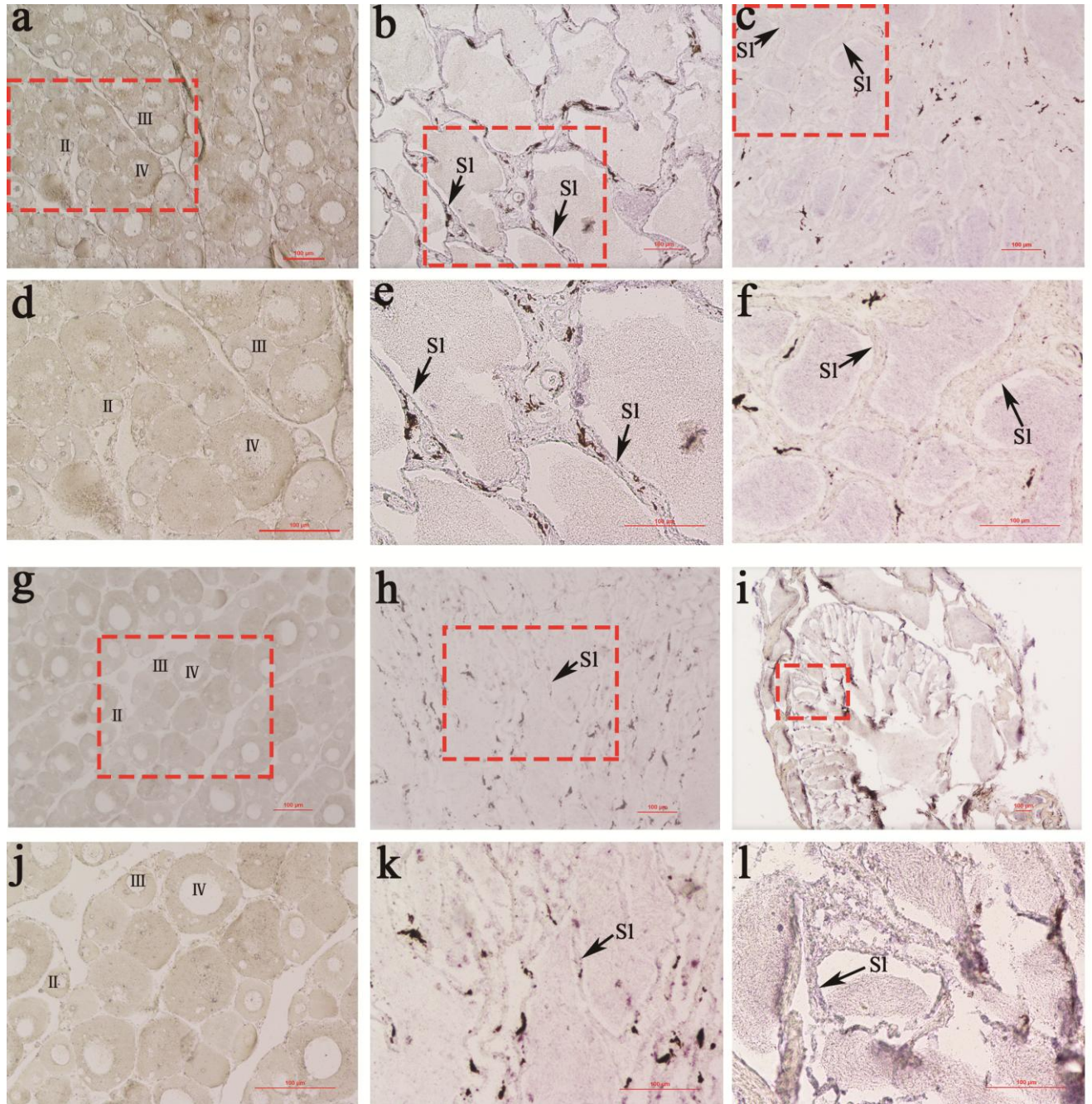


Figure S3 Cyto-locations of *Figla_tv1* and *Figla_tv2* mRNAs in 1 yah gonads with sense RNA probes. The figure shows the ovaries (left hand column), testes (middle column) and pseudomale testes (right hand column), labelled with *Figla_tv1* sense (a-f) and *Figla_tv2* sense (g-l) probes. a-c and g-i show the architecture with low magnification, while d-f and j-l indicate the red framed areas in (a-c, g-i) with large magnification. Oocytes at different developmental stages are marked by II, III and IV.

Sl: seminal lobule. Scale bars: 100 μm .

Supplementary Tables

Table S1 Primers used in the PCR amplifications.

Group	Primers name	Sequence (5'-3')
Sex identification primers	CseF382F	ATTCACTGACCCCTGAGAGC
	CseF382R	TGGCACCATCATTGTAAAATA
RACE primers	Figla_tv1-5'R	CTGGTCATTTCTCCATCCTCTGGGACTGG
	Figla_tv1-5'N	GGGGCACCATTTCGCTCAGACGGGAGAAC
	Figla_tv1-3'R	TGTTCTCCCGTCTGAGGGCAATGGTGCC
	Figla_tv1-3'N	CCCAGTCCCAGAGGATGGAGAAATGACC
	Figla_tv2-5'R	AGTTACGAATCACAAAGCAGGAAGGGGC
	Figla_tv2-5'N	CGCTGAAGGAGAGAGGAGTGAAGACGAG
	Figla_tv2-3'R	TATGGCCAGACTGAAGGCCTAGGGAAT
	Figla_tv2-3'N	GGCTGATGTTACAGCACTGTGTGATGC
	UPM-long	CTAATACGACTCACTATAGGGCAAGCAG TGGTATCAACGCAGAGT
	UPM-short	CTAATACGACTCACTATAGGG
qRT-PCR primers	NUP	AAGCAGTGGTATCAACGCAGAGT
	Figla_tv1-qRT-F	ACATAGAGAAGTTCAAACGAGCC
	Figla_tv1-qRT-R	CGGTAGCAGCTTTTAGTGTGTCT
	Figla_tv2-qRT-F	AACCTCTCGTCTTCACTCCTCTCTC
	Figla_tv2-qRT-R	TCCCTAGGCCTTCAGTCTGG
	StAR-F	AGGACGGCTGGACCACTGAAAT
	StAR-R	ACCTCGTGGGTGACCATCGTGT
	P450scc-F	GGATACGGGCGTGGTGAA
	P450scc-R	TGAATGGCCGGGTGCTTA
	β -actin-F	GCTGTGCTGTCCCTGTA
	β -actin-R	GAGTAGCCACGCTCTGTC
	Rpl13 α -F	GTTTGCCCTCCTTGGTGC
	Rpl13 α -R	TGCCTGCTTTGTCAGCTTGA
	<i>in situ</i> hybridization primers	Figla_tv1-ISH-F
Figla_tv1-ISH-R		<u>GATGATATCTGTCTACTTTACTGGGCTTC</u>
Figla_tv2-ISH-F		<u>GAAGGATCCCATGCCCTGATGATTTCT</u>
Figla_tv2-ISH-R		<u>GATGATATCTCTGCCACTACTCCACCT</u>