Supplementary information

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

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Supplementary Figures

1ggcatcaggacagaagcaccgcaggtgtgcgtacctgtttcatacacgtacacatcaatg \mathbf{B} 1tatgcttggacacagcactctgtcaacagccagcctccttaactataaacttttgaggtt A $\underbrace{ggtattaggaaaggaaaaggaagaaaaccccgttgtttaatcgatttttttaagtgacggtagaa \underline{M} V T M E V P V E E L M S$ 61 61 tacccatcctatggagggtatcaatgatggtctcctgggcacttgtcatgtctgcagtct 121 tccccatattgaaccgaactgggacaattgaaccagaccaaagcaattaaataacacctg 121 atattttgtcattgttattataATGGTTACAATGGAGGTTCCGGTGGAGGAATTAATGAG 14 D V L K R V T G E S A L P M F S N I E K 181 gggaaacctgtgcaggtgctttgagtttagtagatgattggtgtgtgacactcagtttaa 181 CGACGTGTTGAAGCGTGTGACGGGCGAGTCTGCTCTGCCCATGTTCAGTAACATAGAGAA 34 F K R A K D G L Y F V A E D F N E T V K 241 GTTCAAACGAGCCAAAGACCGTTTGTATTTCGTAGCCGAAGACTTCAATGAAACGGTGAA 361 gattgagttgtggcccatgaatctatgaatatacaggggtttggctttctgaatggaatt 421 gtggaggttgaacagctttttccgcgatgttctgattttttggaaaggatctgtagttcac 54 K R E L V N A K E R L R I R N L N T M F 301 AAAGAGGGAATTGGTTAACGCCAAGGAACGACTCAGGATTCGTAACTTGAACACAATGTT 481 tttcctttctttcctttgatgcatggagggtagtagcagcagcagtagcagaagcaacag 541 gtggagtagtggcagaagcatgctagcctgccttttttcttccaactgaactgctggatg 601 cacagttcacaaatgtctaagttatttgtggaccctgcttgatatgagagcttcacttta RR M VPLMRP D 361 CTCCCGTCTGAGGCGAATGGTGCCCCTTATGAGACCTGACAGGAAGCCCAGTAAAGTAGA 661 caaattctactgtgctttcatgttgtcatccaaatgctacttatttttctgttttcactc 861 caaattetaurgrgunnuargrupusususus 721 attittgeaceaeaeaectetegtetteaeteetteetteageggtattaececeg 解 F C R I. R R M V Т A A Ε R A A 421 CACACTAAAAGCTGCTACCGAATATATCCGATTGCTGCTGCAGTTTTGCAAGATACTGA S R RR 781 ccccttcctgctttgtgattcgtaacttgaacacaATGTICTCCCGTCTGAGGCGAATGG 10 P L M R P D R K P S K V D T L K A A T E DHDSSG SDFLNSAI 114 Τ 0 481 TGATCATGACAGCAGTGGGTCTGATTTCCTAAATAGTGCAATCACTTATGGCCAGACTGA 841 TGCCCCTTATGAGACCTGACAGGAAGCCCAGTAAAGTAGACACACTAAAAAGCTGCTACCG GL GNDLWRLDD IL N Т D Ε S 30 Y I R L L A A V L Q D T D D H D S S G S 901 AATATATCCGATTGCTGCTGCTGCAGTTTTGCAAGATACTGATGATCATGACAGCAGTGGGT 541 AGGCCTAGGGAATGACCTATGGAGATTGGATGATATACTAAACACATCAGATGAGTGCAT 154 D D G F M L S P G P V P E D G E M T R L 601 CGATGACGGATTCATGTTATCCCCTGGCCCAGTCCCAGAGGATGGAGAAATGACCAGGCT 50 D F L N S A I T Y G Q T E G L G N D L W 961 CTGATTTCCTAAATAGTGCAATCACTTATGGCCAGACTGAAGGCCTAGGGAATGACCTAT 174 M L Q H C V M P A Y Q V I I Q V A P D Q 661 GATGTTACAGCACTGTGTGATGCCTGCGTATCAGGTCATCCAAGTGGCTCCTGATCA SDEC ILN Т I D G 1021 GGAGATTGGATGATATACTAAACACATCAGATGAGTGCATCGATGACGGATTCATGTTAT 194 Т v . 721 AACTACGGTC<u>TGA</u>tcctgtgaagacttgcatctcatagaaagaaattttctttgtctaat 781 ttctttataattattgtttgagttaattaatgtggaactgcttttattttcatttttaag P E D G E M TRL M Q H 1081 CCCCTGGCCCAGTCCCAGAGGATGGAGAAATGACCAGGCTGATGTTACAGCACTGTGTGA AYQVIIQVAPDQTT 110 1141 IGCCIGCGTATCAGGTCATCATCCAAGTGGCTCCTGATCAAACTACGGTCIGAtcctgtg 1201 aagacttgcatctccatagaaagaaattttctttgtctaatttctttataattattgtttg 901 ttgatggtttttatgttctcaacaaaagtccagcacgaattatatcgatacacgtggcag 961 atggtatcaagatttatgtttgtaataaatgtagtcact<u>aataaa</u>ttacctcttccaatt agttaattaatgtggaactgcttttattttcatttttaagccacatttacagaaggtcta 1261 1321 gacatttctaatgggcctctaatttatttaaagtttattttgatggtttttatgttctc 1381 aacaaaagtccagcacgaattatatcgatacacgtggcagatggtatcaagatttatgtt 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.

	*	20	*	40	*	60	*	80	*	100	
Figla_tvl: Figla_tv2:	TATGCTTGGA	CACAGCACTCTGT	CAACAGCCAG	CCTCCTTAAC	TATAAACTTT	TGAGGTTTACC	CATCCTATG	GAGGGTATCAA	TGATGGTCTC	CTGGGC :	100
Figla tvl ·	*	120	*	140	*	160	*	180	*	200 .	
Figla_tv2 :	ACTTGTCATO	TCTGCAGTCTTCC	CCATATTGAA	CCGAACTGGG	ACAATTGAAC	CAGACCAAAGC	AATTAAATA	ACACCTGGGGA	AACCTGTGCA	GGTGCT :	200
Figla_tvl:	*	220	*	240	*	260	*	280	*	300 :	-
Figla_tv2 :	TTGAGTTTAC	TAGATGATTGGTG	TGTGACACTC.	AGTTTAAAAC	ATTCATGCCC	FGATGATTTCT	TCACAGTAT	CTGATTTGTT	GAGGTCCTGT	TTTTGT :	300
Figla_tvl:	*	320	*	340	*	360	*	380	*	400 :	-
Figla_tv2 :	GGGTTTTATC	AGCTGGAGGCCAA	AATTATGTAA	AAAATAAACA/	AATAAATACC	TGAAATTGATT	GAGTTGTGGG	CCATGAATCT	ATGAATATAC	CAGGGGT :	400
Figla_tvl:	*	420	*	440 GGCATCAGGA	* AGAACCACC	460 CAGGIGICC-	* CTACCTG	480 TTCA TACA	* CGTACACATO	500 AATGAG :	62
Figla_tv2 :	TIGGCITICI	GAAIGGAAIIGIG	GAGGI I GAAC.	GC T	C GA G C	G T TG	G A CTG	AG <mark>TTCA</mark> CITI TTCA		T T	499
Figla_tvl:	* AAAGGAAAAA TCCATCCACC	520 CGAAGAAAACCCC	* TTGTTTAATA	540 CCATTTTTTTT	* FAAGTGACGC	560 TAGAAATAT	* TT TC TC/	580	* AATGCTTACA	600 ATGGAG :	157
rigiu_ivz .	A	G A A A C G	GT A A	G T	AGT GG	AGAA AT	TG C	TT TT TT	AA G	TGGA	399
Figla_tvl: Figla_tv2:	* CTTCCCCTGC CAC-ACTC	620 AGGAATTAATGAG ACAAATGTCTAAG	* CGACGTGTTG TTATTTGTGG	640 AACC- CTGTG ACCOTCCTTG	* ACGCCCCACT ATATCAGACC	660 CT- G CT CTGCC TTCACTTTACA	* CATGTT	680 CAGTAA <mark>CAT</mark> A TGCUTTCATG	* GACAAGTTCA TTCTCATCCA	700 AACCAG : AATCCT :	251
	G C GT	A AAT T AG	A TGT G	A C G TG	A G GAG	TCTTC	AT T	T CAT	G T CA	AA G	0,70
Figla_tvl: Figla_tv2:	CCAAAGA ACTTATTTT	720 CGGTTTGTA CTGTTTTCACTCA	- TTTCCTAGC TTTTTCCACC	CGAAGACTTC/ ACACAACCTC	AA TGA ICGTCTTCAC	760 AACGGTGAAAA FCCTCUCTCCT	AGAGGGAAT TCAGCCGTA	780 GGTTAA <mark>CGCC</mark> TACCCC <mark>CGCC</mark>	AAGGAACCAC	800 TICAGGA : TITGTGA :	338 798
	<u></u>	C GTTT A	TTT G A C	A AC TC	*		AG G 1	CGCC	G *	T GA	
Figla_tvl: Figla_tv2:	TTCGTAACTT TTCGTAACTT	GAACACAATGTTC GAACACAATGTTC	TCCCGTCTGA TCCCGTCTGA	GGCGAATGGT GGCGAATGGT	GCCCCTTATG. GCCCCTTATG.	AGACCTGACAG AGACCTGACAG	GAAGCCCAG GAAGCCCAG	AAAGTAGACA AAAGTAGACA	CACTAAAAGO CACTAAAAGO	TGCTAC : TGCTAC :	438 898
	TTCGTAACTT	GAACACAATGTTC 920	TCCCGTCTGA	GGCGAATGGT 940	GCCCCTTATG. *	AGACCTGACAG 960	GAAGCCCAG7 *	AAAGTAGACA	CACTAAAAGC *	1000	
Figla_tvl: Figla_tv2:	CGAATATATC CGAATATATC	CGATTGCTTGCTG CGATTGCTTGCTG	CAGTTTTGCA CAGTTTTGCA	AGATACTGATO AGATACTGATO	GATCATGACA GATCATGACA	GCAGTGGGTCT GCAGTGGGTCT	GATTTCCTA GATTTCCTA	ATAGTGCAAT ATAGTGCAAT	CACTTATGGC CACTTATGGC	CAGACT : CAGACT :	538 998
	CGAATATATC *	1020	*	1040	JATCATGACA *	1060	4111CC1A	1080	*	1100	
Figla_tvl: Figla_tv2:	GAAGGCCTAC GAAGGCCTAC	GGAATGACCTATG GGAATGACCTATG	GAGATTGGAT GAGATTGGAT	GATATACTAA/ GATATACTAA/ GATATACTAA/	ACACATCAGA ACACATCAGA	IGAGTGCATCG IGAGTGCATCG	ATGACGGATT ATGACGGATT	CATGTTATCC CATGTTATCC	CCTGGCCCAC CCTGGCCCAC	TCCCAG :	$\begin{array}{r} 638 \\ 1098 \end{array}$
	*	1120	*	1140	*	1160	*	1180	*	1200	
Figla_tvl: Figla_tv2:	AGGATGGAGA AGGATGGAGA AGGATGGAGA	AATGACCAGGCTG AATGACCAGGCTG AATGACCAGGCTG	ATGTTACAGC. ATGTTACAGC. ATGTTACAGC	ACTGTGTGATO ACTGTGTGATO ACTGTGTGTGATO	SCCTGCGTAT SCCTGCGTAT SCCTGCGTAT	CAGGTCATCAT CAGGTCATCAT CAGGTCATCAT	CCAAGTGGCT CCAAGTGGCT CCAAGTGGCT	CCTGATCAAA CCTGATCAAA CCTGATCAAA	CTACGGTCTC CTACGGTCTC CTACGGTCTC	GATCCTG : GATCCTG : GATCCTG	738 1198
F: 1 1	*	1220	*	1240	*	1260	*	1280	*	1300	0.2.0
Figla_tv1 : Figla_tv2 :	TGAAGACTTC TGAAGACTTC TGAAGACTTC	CATCICATAGAAA CATCTCATAGAAA CATCTCATAGAAA	GAAATTTTCT GAAATTTTCT GAAATTTTCT	TTGTCTAATT TTGTCTAATT TTGTCTAATT	I CITTATAAT I CTTTATAAT I CTTTATAAT	TATIGITIGAG TATTGTTTGAG TATTGTTTGAG	TTAATTAATO TTAATTAATO TTAATTAATO	TGGAACTGCT TGGAACTGCT TGGAACTGCT	TTTATTTCA TTTATTTTCA TTTATTTTCA	TTTTTA : TTTTTTA :	838
Figla tyly	*		*	1340	*	1360	*	1380	*	1400	028
Figla_tv2 :	AGCCACATTT AGCCACATTT AGCCACATTT	ACAGAAGGTCTAG ACAGAAGGTCTAG	ACATTTCTAA ACATTTCTAA ACATTTCTAA	TGGGCCTCTA/ TGGGCCTCTA/	ATTTATTTAA ATTTATTTAA ATTTATTTAA	AGTTTATTTTT AGTTTATTTTT	GATGGTTTTT GATGGTTTTT	ATGTTCTCAA ATGTTCTCAA	CAAAAGTCCA CAAAAGTCCA CAAAAGTCCA	GCACGA : GCACGA :	1398
Figla tyl ·	*		* TGGTATCAAG	1440	* STAATAAATG		* AAATTACCT(1480	*	1500	1038
Figla_tv2:	ATTATATCGA ATTATATCGA	TACACGTGGCAGA TACACGTGGCAGA	TGGTATCAAG TGGTATCAAG	ATTTATGTTTO	GTAATAAATG GTAATAAATG	ГАСТСАСТААТ ГАСТСАСТААТ	AAATTACCTO	TTCCAATTTC TTCCAATTTG	CGAAAAAAAA	AAAAAA AAAAAAA	1498
Figlatvl:	* AAAAAAAAAAA	AA : 1050									
$Figla_tv2$:	АААААААААА ААААААААААА	AA : 1510 AA									

Figure S2 Nucleotide sequence alignment of two *Figla* **cDNAs from** *C. semilaevis.* Sequences were aligned by Clustal X and edited by GeneDoc. Identical amino acids are indicated by black shading. Gaps introduced in the sequences to optimize the alignment are indicated by dashes.



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.

SI: seminal lobule. Scale bars: 100 $\mu m.$

Supplementary Tables

Table S1 Primers used in the PCR amplifications.

Group	Primers name	Sequence (5'-3')					
Sex identification primers	CseF382F	ATTCACTGACCCCTGAGAGC					
	CseF382R	TGGCACCATCATTGTAAAACTA					
RACE primers	Figla_tv1-5'R	CTGGTCATTTCTCCATCCTCTGGGACTGG					
	Figla_tv1-5'N	GGGGCACCATTCGCCTCAGACGGGAGAAC					
	Figla_tv1-3'R	TGTTCTCCCGTCTGAGGCGAATGGTGCC					
	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					
	LIPM long	CTAATACGACTCACTATAGGGCAAGCAG					
	Of WI-long	TGGTATCAACGCAGAGT					
	UPM-short	CTAATACGACTCACTATAGGG					
	NUP	AAGCAGTGGTATCAACGCAGAGT					
qRT-PCR primers	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					
	Rpl13a-F	GTTTGCCCTCCTTGGTCG					
	Rpl13a-R	TGCCTGCTTTGTCAGCTTGA					
in situ hybridization primers	Figla_tv1-ISH-F	GAAGGATCCATGGTTACAATGGAGGTTC					
	Figla_tv1-ISH-R	GATGATATCTGTCTACTTTACTGGGCTTC					
	Figla_tv2-ISH-F	GAAGGATCCCATGCCCTGATGATTTCT					
	Figla_tv2-ISH-R	<u>GATGAT</u> ATCTCTGCCACTACTCCACCT					