Supplementary Materials

Zebrafish establish female germ cell identity by advancing cell proliferation and meiosis

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This supplement contains two Supplementary Tables and two Supplementary Figures. The RNAseq data sets has been uploaded to the Gene Expression Ominbus database with the accession number: GSE196899 (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE196899).

Supplementary Table S1. Key Resources. All key resources used in this article are listed here.

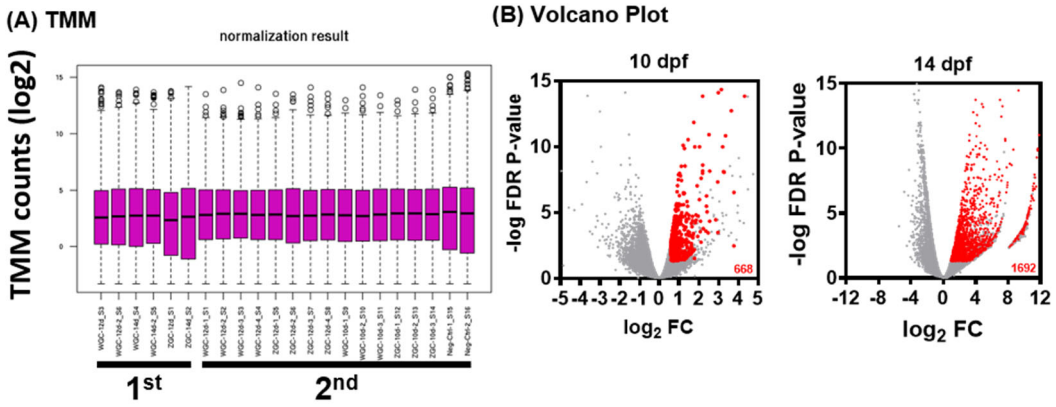
Reagent or resource	Source	Identifier	Note
Experiment animal			
Zebrafish/TL	Zebrafish International Resource Center	https://zfin.org/ZDB-GENO-990623-2	
Zebrafish/Nadia	From Dr. John H. Postlethwait	(Wilson, High et al. 2014)	
Antibodies			
Vasa Rabbit polyclonal against recombinant protein encompassing a sequence within the center region of zebrafish Vasa.	GeneTex	GTX128306	IF 1：500
Sycp3 Rabbit polyclonal against synthetic peptide corresponding to Human SCP3 aa 200 to the C-terminus (C terminal) conjugated to keyhole limpet haemocyanin	Abcam	ab150292	IF 1：200
Rad51 Rabbit polyclonal	Abcam	ab137323	IF 1：200
PCNA Mouse monoclonal Rat PCNA-protein A fusion protein	Dako	M0879	IF 1：500
Casp3 Cleaved Caspase-3 (Asp175) Rabbit polyclonal	Cell Signaling Technology	Cat.#9661	IF 1：400
Oligonucleotides			
Genotype primer			
NA-F	CCGGCCCTCAAGGACCGAAA		
NA-R	GGTTGCTCAAGTGTTGGTGAGA		
sycp3 sgRNA-sequence-F	CGCAAAATACTCCTCAAAAACC		
sycp3 sgRNA-sequence-R	CAGCACACAAGGGTCAAATAAA		
sgRNA primer			
sycp3 sgRNA	gcgtaatacgactcactataCGGTGTGGTGACAGACCTGAgttttagagctagaaatagc		
Guide-constant oligo	AAAGCACCGACTCGGTGCCACTTTTTCAAGTTGATAACGGACTAGCCTTATTTTAACTTGCTATTTCTAGCTCTAAAAC		
qPCR primer			
EEF1al1-F	AGC AGC AGC TGA GGA GTG AT		

EEF1al1-R	TGG TTC TCT TGT CGA TTC CA		
Q-CU207311.2-F	CAGACATTTTCAGGGCTGCCA		
Q-CU207311.2-R	CAGACGACACAAACAGAGCAA		
Q-zgc:100951-F	ACATGTGATCAGTGTCTGAAGAGT		
Q-zgc:100951-R	GGGAAACCGTTTTGCTGCAT		
Q-FO704836.1-F	TGCGGCAAAAATTTTAGGGCTT		
Q-FO704836.1-R	CACACCAGTGTGGATCTTCTGA		
Q-FO834825.3-F	CACAAATGTGATCAATGCGGC		
Q-FO834825.3-R	TGTGTTCTTTAATTTGTTCCGTGTA		
QuantiFast SYBR Green RT-PCR Kit (2000)	QIAGEN	204156	
DyLight™ 488 Microscale Antibody Labeling Kit	Thermo Scientific	53025	
PCR Purification Kit	QIAquick	Cat. #28106	
MEGAshortscript T7 Transcription Kit	Thermo Scientific	AM1354	
TRUECUT CAS9 PROTEIN V2	Thermo Scientific	A36498	
Mob II	BioLabs	Cat.#R0148	For tp53 fish genotyping
DNA Screening Kit	QIAxcel	Cat. #929004	For sycp3 genotyping
Normal Goat Serum	Jason Immuno Research	Cat.#005-000-121	
Collagenase Type 4	Worthington	Cat. #LS004188	
Transcriptome			
SMART-seq HT Kit	Clontech (Takara Bio)	Cat. 634437 (96 rxns)	
Nextera XT DNA Library Prep Kit	Illumina	FC-131-1096 (96 rxns)	
Qubit dsDNA HS assay Kit	Invitrogen	Q32854	
Agilent High Sensitivity DNA Kit	Agilent	5067-4626	
Agilent 2100 bioanalyzer	Agilent	G2938B	
Ampure XP	Agencount	A63881	
NetSeq500 (equipment)	Illumina	NB501708	
NetSeq500 High Output kit v.2	Illumina	FC-404-2001	
NetSeq500 System Suite Version	Illumina	2.2.0	
NetSeq500 Control Software Version	Illumina	2.2.0	

cDNA preparation from Quartz-Seq			
10x TaKaRa Taq buffer (Mg2+)	TaKaRa	9151A	
Rnasin Plus Ribonuclease Inhibitor	Promega	N2611, N2615	
SuperScript III Rnase H- Reverse Transcriptase	Life Technologies	18080-044	
0.1 M DTT (supplied with Enzyme)	Life Technologies	18080-044	
10mM dNTP Mix	Life Technologies	18427-013	
Exonuclease I	TaKaRa	2650A	
10x Exo I bufffer (supplied with Enzyme)	TaKaRa	2650A	
Rnase H	Life Technologies	18021-014	
100 mM dATP	Life Technologies	10216-019	
Ter minal Transferase(Tdt), recombinant	Roche	3333574	
10 % NP-40	Thermo Scientific	28324	
MightyAmp DNA Polymerase Ver.2	TaKaRa	R071A, R071B	
2x MightyAmp Buffer Ver.2 (supplied with Enzyme)	TaKaRa	R071A, R071B	
DNA Clean & Concentrator -5	ZYMO RESEARCH	D4013, D4014	
Agencourt AMPure XP	BEKMAN COULTER	A63880, A63881	
Software			
BD FACSDiva software	Becton Dickinson biosciences	v6.2	
GraphPad Prism	https://www.graphpad.com/		
Imaris x64 9.8	https://imaris.oxinst.com/packages		
Zeiss Zen 2009 (Black edition)	https://www.zeiss.com/microscopy/int/products/microscope-software/zen.html		
CHOPCHHOP	https://chopchop.cbu.uib.no/		
Benchling	https://benchling.com/editor		

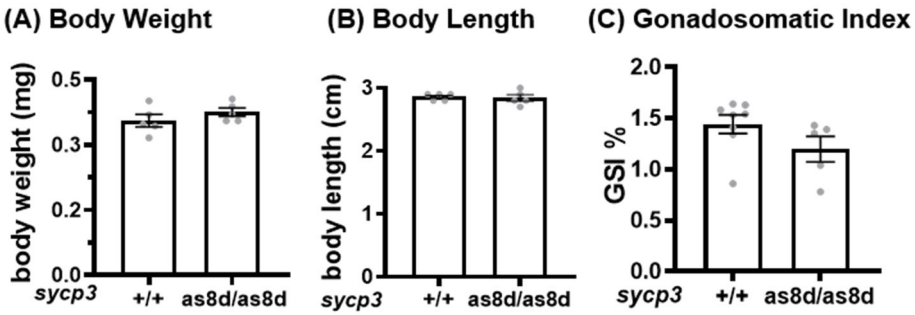
Supplementary Table S2. List of samples batches used for RNA-seq analysis. Germ cells from the W- and Z-hybrids were collected in different batches and cDNA libraries prepared using two different methods in two different sets of experiments. In first sequencing experiment, there are two batches of W and one batch of Z samples at 12- and 14 dpf, and their cDNA were performed by Quartz-seq. In second sequencing experiment, there are triplicated batches W and Z at 10 dpf, and quadruplicated batches W and Z at 12 dpf. The cDNAs of second sequencing were prepared by Smart-seq HT. The trunk tissues are used as a negative control.

#	Stage	Sample	no. of Sample Batches	Method cDNA Preparation
1st	12 dpf	W	2	Quartz-seq
		Z	1	
	14 dpf	W	2	
		Z	1	
2nd	10 dpf	W	3	Smart-seq HT
		Z	3	
	12 dpf	W	4	
		Z	4	
	Neg. Ctrl (trunk)		2	



Supplementary Fig. S1. The Quality control of the transcriptomic analysis and volcano plots of 14- and 10-dpf transcriptomes

A box plot showing the normalization of RNA-seq results by the method of Trimmed Mean of the M-values (TMM) from the edgeR package R program. The X-axis shows all the sample batches used in RNA-seq. All these batches of samples have similar TMM counts, therefore can be compared. (B) The volcano plots show differentially expressed genes (DEGs) at 10- and 14-dpf. FC: fold change of gene expression in W- and Z-hybrid. FDR: false discovery rate. There are 668 genes upregulated in the W-hybrid compared to the Z-hybrid at 10 dpf and 1692 genes in 14 dpf (upregulated genes labeled in red dots, others genes labeled in gray).



Supplementary Fig. S2. The gonad morphology of *sycp3* mutants.

(A) The body weight, (B) body length, and (C) gonadosomatic index (GSI%) of 6-month *sycp3* deficient mutants (as8d/as8d) are not changed.