

Supplemental Information

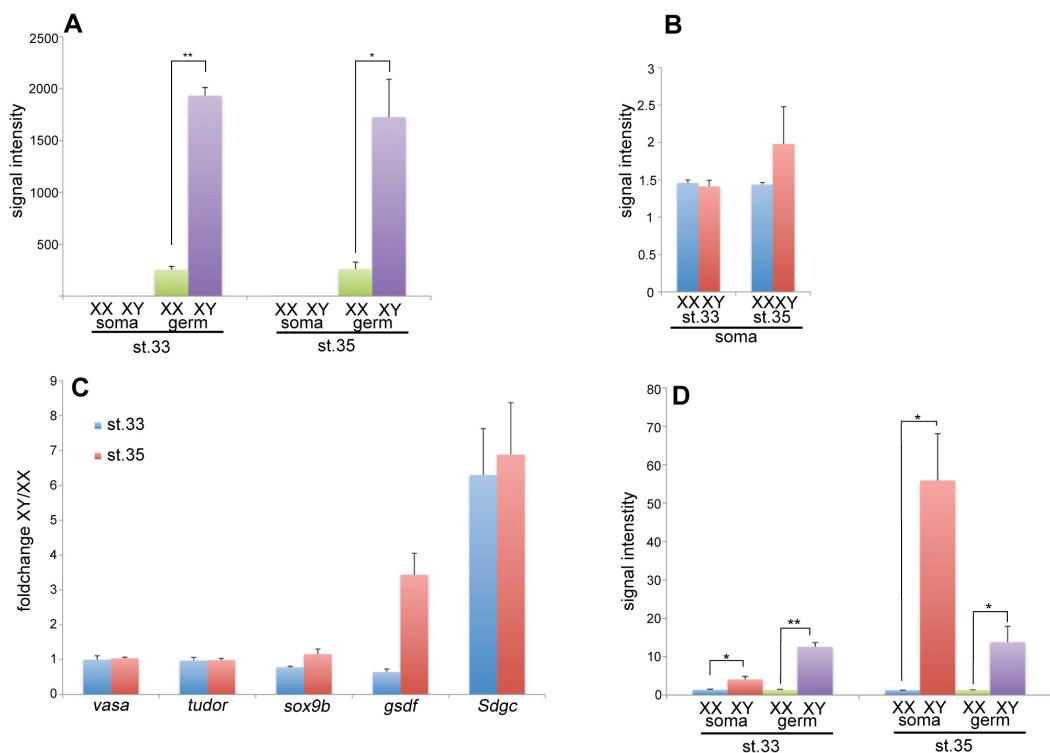


Figure S1. *Sdgc* is enriched and *DMY/dmrt1bY* transcripts are detected in XY germ cells as determined by microarray analysis. (A, B, D) Microarray analysis ($n=3$) (A) Signal from the novel gene, *Sdgc*, is highly enriched in XY germ cells as compared to XX germ cells at both st.33 and st.35. (B) In the gonadal somatic cells, the signal intensity of *Sdgc* is extremely low, and no sexual difference is present. (C) qPCR analysis of *Sdgc* and gonadal marker expression during gonad formation. The y-axis indicates the fold change of expression levels in XY compared to XX embryos. The expression levels were normalized to β -actin ($n=3$). (D) *DMY/dmrt1bY* is detected in XY germ cells at st.33. By the time the gonad forms as bilateral structures at st.35, *DMY/dmrt1bY* expression is abundant in XY gonadal somatic cells but still persists in germ cells. The y-axis indicates the raw signal intensity of microarray data. * $p<0.05$, ** $p<0.01$, Student's *t*-test, $n = 3$. Values are expressed as the mean \pm s.e.m.

10	20	30	40	50	60
GAGAAAGCGGCAAAGCAGCAACAAGTGACAACCCGAAGCAGCATCGTTGAGCAAAG <u>ATGC</u>					
scaffold804: 41833-41858			scaffold2884: 11492-11521 M Q		
scaffold4140: 5809-5834					
70	80	90	100	110	120
AAAACCAACCCGAGCAGGGGCCGCCAGGGCGGCAGGGGCCGCCAGGGGCCAGGGGCC					
N Q P E <u>Q G P P A Q G R</u>	<u>Q G P P A Q G R</u>	<u>Q G P P A Q G R</u>	<u>Q G P P A Q G R</u>	<u>Q G P P A Q G R</u>	<u>Q G P P A Q G R</u>
130	140	150	160	170	180
GGCAGGGGCCGCCGCCAGGGCGGCAGGGGCCGCCAGGGCGGCAGGGGCCGCCAGGGCGGCAGGGGCC					
<u>Q G P P A Q G R</u>	<u>Q G P P A Q G R</u>	<u>Q G P P A Q G R</u>	<u>Q G P P A Q G R</u>	<u>Q G P P A Q G R</u>	<u>Q G P P A Q G R</u>
190	200	210	220	230	240
CGGCCAGGGCGGCAGGGGCCGCCAGGGCGGCAGGGGCCGCCAGGGCGGCAGGGGCCGCCAGGGCGC					
<u>A Q G R</u>	<u>Q G P P A Q G R</u>	<u>Q G P P A Q G R</u>	<u>Q G P P A Q G R</u>	<u>Q G P P A E G R</u>	<u>Q G P P A E G R</u>
250	260	270	280	290	300
GGCAGGGGCCGCCGCCAGGGCGGCAGGGGCCGCCAGGGCGGCAGGGGCCGCCAGGGCGGCAGGGGCC					
<u>Q G P P A Q G R</u>	<u>Q G P P A Q G R</u>	<u>Q G P P A Q G R</u>	<u>Q G R</u>	<u>Q G P P</u>	<u>Q G P P</u>
310	320	330	340	350	360
CGGCCAGGGCGGCAGGGGCCGCCAGGGCGGCCACTTGTGGCACAGTGCTG					
<u>A Q G R</u>	<u>Q G P P A Q G R</u>	<u>Q G R</u>	<u>P L V W H S A V</u>		
370	380	390	400	410	420
TCAACAGAGCTCGTGAAGAACAGCGTAAGGCTTCATGAATAAGTTATCGTCGGAGGC					
N R A R E E Q R K A S M N K V I V R R L					
430	440	450	460	470	480
TGTTGCCCTCGCACCTCGGAGGAGGAGCGGGCCCGCCTCATCATGGTTATGGGCC					
L P L A T S E E E R A R L I M V Y G P P					
490	500	510	520	530	
CAT <u>TAA</u> AAAAATAAAAGAAATTGTATAAGGTATGTTCTGTGACAAAAGTTGAATG					

Figure S2. Structure and characterization of *Sdgc* cDNA. Nucleotide sequence and deduced amino acid sequence of *Sdgc*. Putative initiation ATG and stop TAA codons are boxed. Polypeptide repeats (QGPPAQ(E)GR) are underlined. The 5'UTR regions mapped on medaka genome are double underlined.

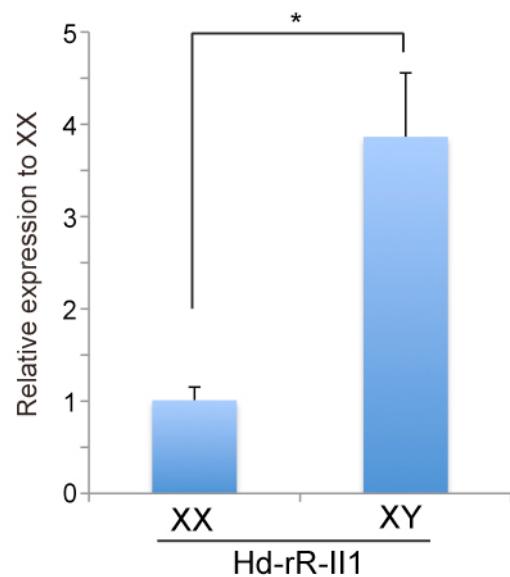


Figure S3. qPCR analysis of *Sdgc* in Hd-rR-III1 strain. The expression level of *Sdgc* is higher in XY compared to XX embryos at st.30. The expression levels were normalized to *olvas* (n=2). * $p<0.01$, Student's *t*-test. Values are expressed as the mean \pm s.e.m.

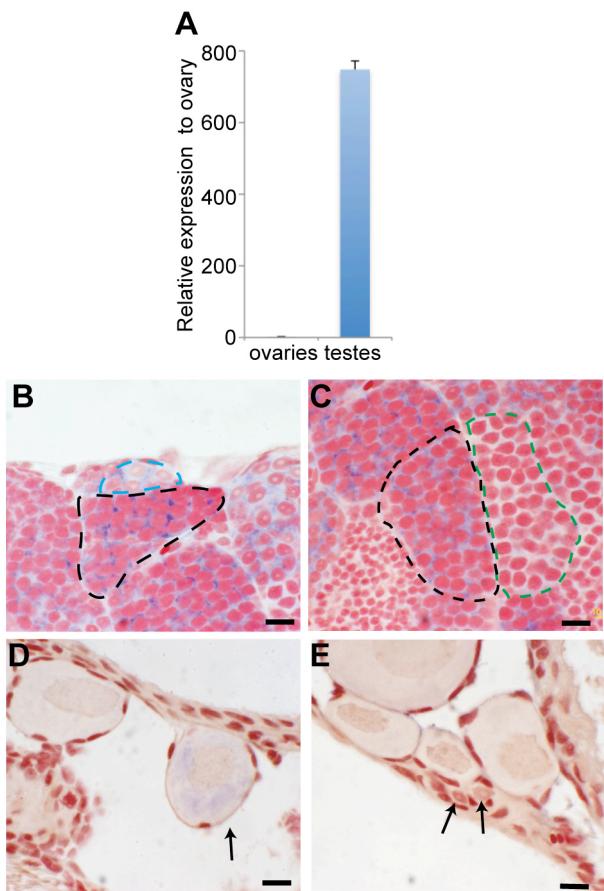


Figure S4. Expression of *Sdgc* in adult gonads. (A) *Sdgc* is highly enriched in testes as compared to ovaries. The y-axis indicates the expression levels of *Sdgc* in testes relative to ovaries normalized by *olvas* expression ($n = 3$). Values are expressed as the mean \pm s.e.m. (B) *Sdgc* expression is detected in type A (blue dotted line) and type B spermatogonia (black dotted line) but not in spermatocytes (C, green dotted line). (D) In ovaries, *Sdgc* is weakly detected in the early stage of oocytes (arrow). (E) *Sdgc* signal is not detected in oogonia (arrows). Bars represent 10 μ m.

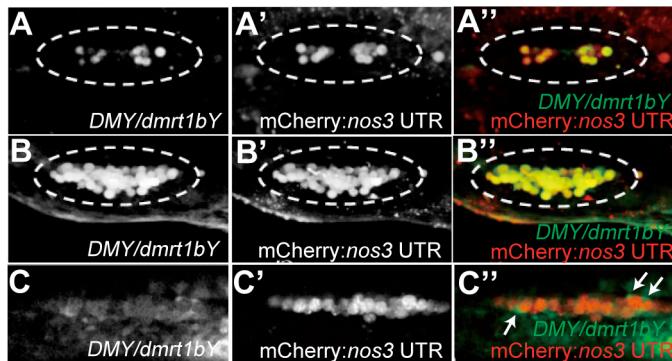


Figure S5. Detection of *DMY/dmrt1bY* expression in germ cells of *DMY/dmrt1bY*-EGFP reporter transgenic medaka. (A) *DMY/dmrt1bY* expression is first detected in clustered germ cells located within the lateral plate mesoderm at st.28. Expression is co-localized with the fluorescence of the germ cell-specific marker (mCherry:*nos3*-3'UTR). (B) Germ cell-specific expression of *DMY/dmrt1bY* is observed until st.30–31. (C) By st.33, while germ cell expression becomes weaker, additional *DMY/dmrt1bY* expression is detected in the somatic cells directly surrounding the germ cells (arrows).

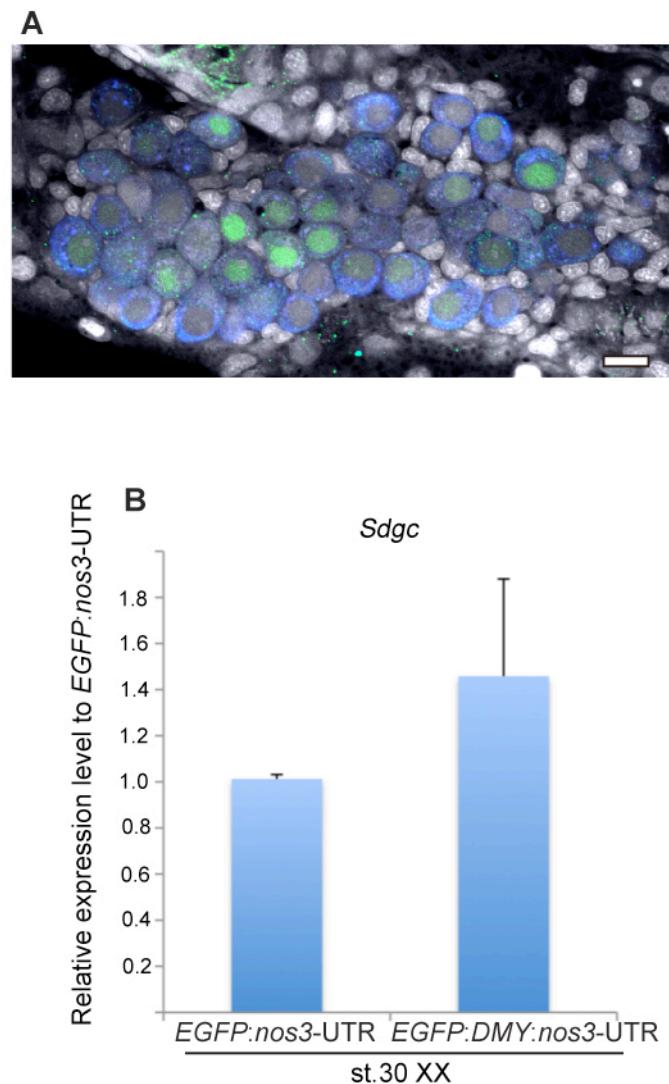


Figure S6. Overexpression of *DMY/dmrt1bY* in XX germ cells did not alter the expression level of *Sdgc*. (A) Immunohistochemistry of EGFP and DMY fusion protein (EGFP:DMY) using *EGFP:DMY:nos3-3'UTR* injected embryos at st.33. Note that EGFP:DMY signal (green) was localized to the nuclei of germ cells (blue). Bar represents 10 μ m. (B) qPCR analysis of embryos injected with *EGFP:nos3-3'UTR* (control) and *EGFP:DMY:nos3-3'UTR*. The expression of *Sdgc* was not altered by *DMY/dmrt1bY* expression in XX germ cells (n=3). Values are expressed as the mean \pm s.e.m.

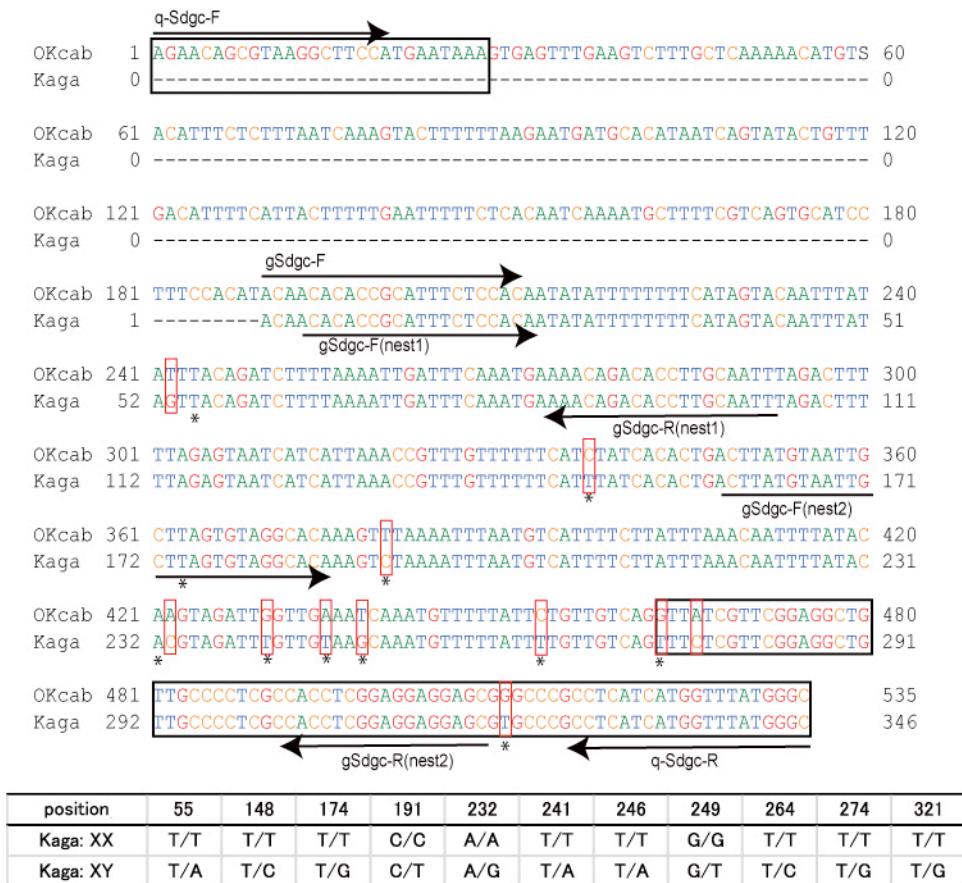


Figure S7. Identification of SNPs in the genomic region of *Sdgc* and primer sets for linkage analysis. (A) 14 SNPs (red boxes and asterisks) were identified between OKcab and Kaga strains when the genomic amplicon was sequenced with gSdgc-F and q-Sdgc-R primers. The alignment is based on the sequence of XX/XY OKcab and XX Kaga. Asterisks indicate the heterozygous SNPs found in XY Kaga (see the table below). The position of the heterozygous SNPs in the table is based on the Kaga sequence. The purpose of each primer is indicated in Table S3. Exons are boxed (black). For linkage analysis, PCR products were amplified by gSdgc-F and q-Sdgc-R primers, and then used as templates for nested PCR. Next, two PCR products amplified by nested primers (nest1 and nest2) were genotyped via High-Resolution Melting analysis.

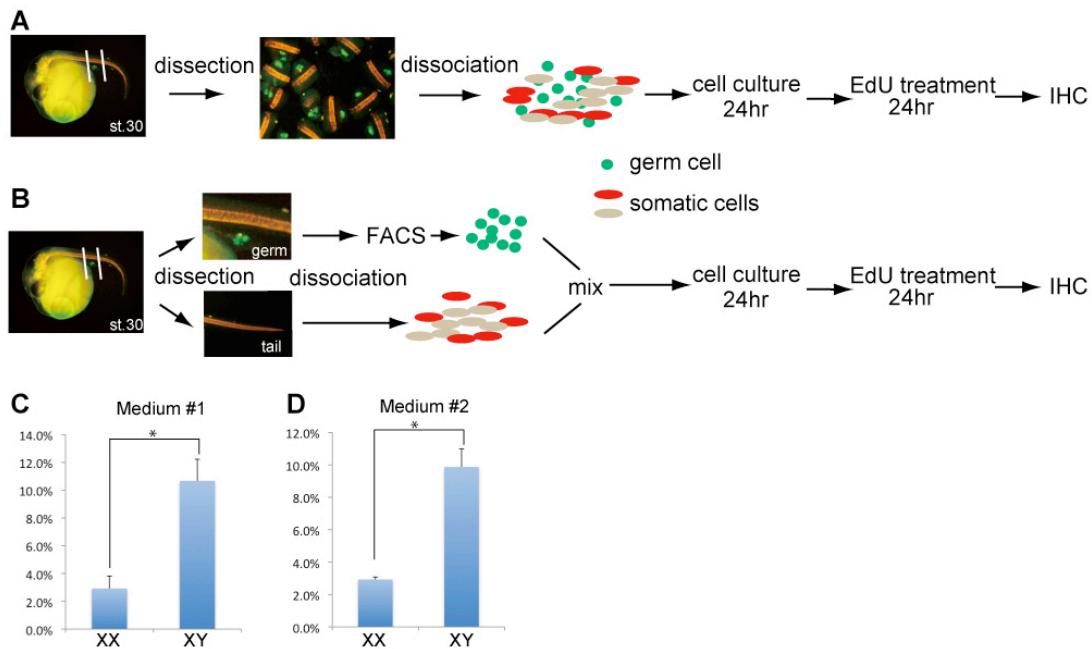


Figure S8. Sexually different germ cell mitotic activity in XX and XY germ cells *in vitro*. (A) Schematic representation of the *in vitro* culture experiment. (B) Experimental procedure of the *in vitro* culture using FACS isolated germ cells. (C,D) The EdU incorporation rate of germ cells cultured in Medium #1 (C, n = 4) or Medium #2 (D, n = 3). See Table S2 for the components of each medium. *p < 0.05, Student's t-test. Values are expressed as the mean ± s.e.m.

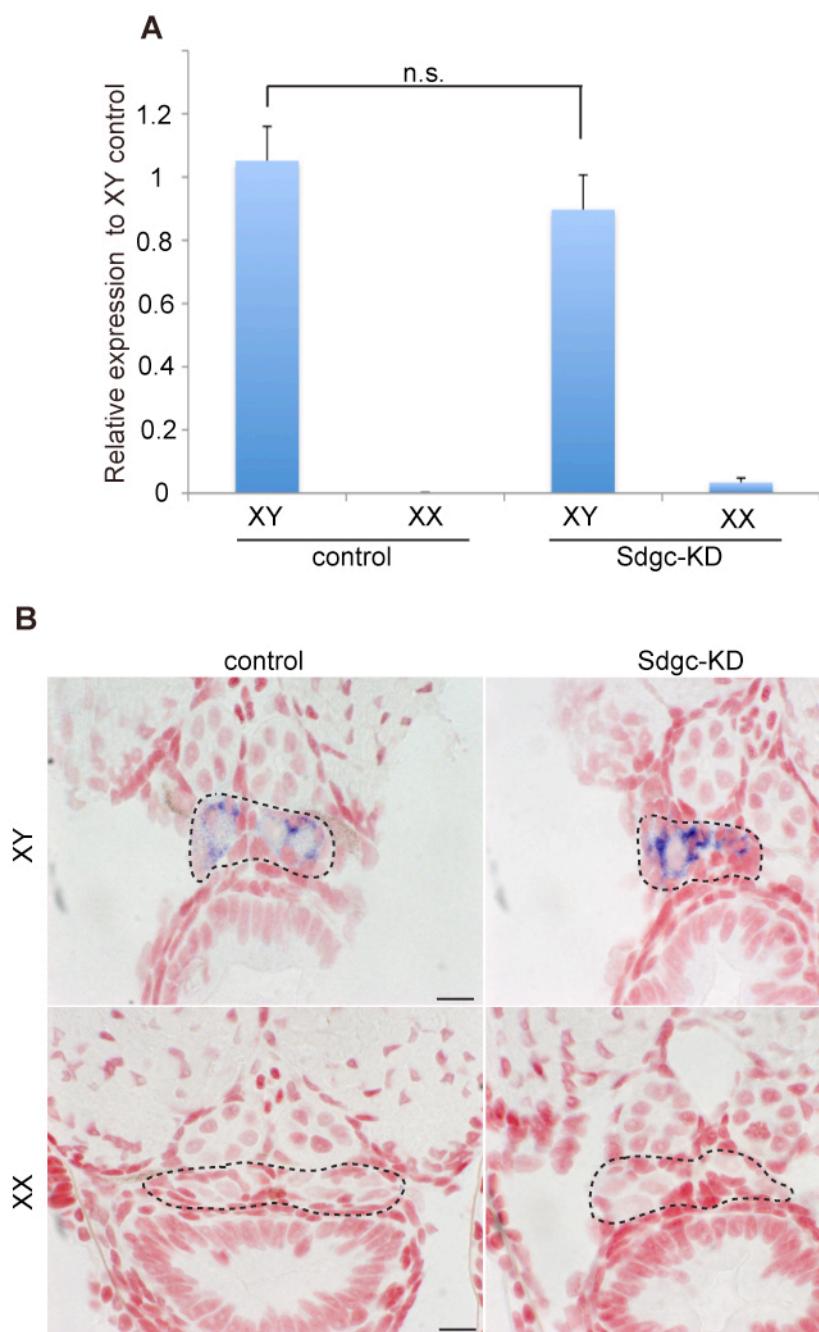


Figure S9. Knockdown of *Sdgc* did not alter the expression of *DMY/dmrt1bY* during gonadal sex differentiation. (A) qPCR analysis of *DMY/dmrt1bY* using *Sdgc*-knockdown (Sdgc-KD) XY embryos at st.35. The expression level of *DMY/dmrt1bY* did not differ significantly from that of control XY embryos. The expression levels were normalized to β -actin (n=3). Values are expressed as the mean \pm s.e.m. (B) *DMY/dmrt1bY* expression was normally detected in gonadal somatic cells of *Sdgc*-knockdown XY embryos at st.35 by *in situ* hybridization (purple signals in dotted line). Bars represent 10 μ m.

Table S1. The genotyping panel of *Sdgc*

data type f2 intercross A=HdrR/HdrR B=Kaga/Kaga H=HdrR/Kaga

94 148 0

*Sdgc H A H A A H H H A H A H H B H H B H B V A H H B H A H H B H H A - A H H H B H H A H B H B H B B B H H B B V A H H B A H A H A H B A A H H H H
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Table S2. Components of culture media.

Components	Medium#1	Medium#2
Leibovitz L15	+	+
Hepes (10mM, pH 7.9)	+	+
Penicillin (50U/ml)	+	+
Streptomycin (50µg/ml)	+	+
Kanamycin (100µg/ml)	+	+
Glutamax (2mM)	+	+
Fetal bovine serum	10%	5.0%
Bovine serum albumine		0.5%
Embryonic Extract		3 embryos/ml

Table S3. Primers used in this study.

Primer Name	Sequence (5'-3')	Purposes
q-vasa-F	GATTCCGCTCAGGCAAGTG	q-PCR
q-vasa-R	GTCAATGGTGGTGGGCAGGT	q-PCR
q-beta-actin-F	TGGCGCTTGACTCAGGATT	q-PCR
q-beta-actin-R	GCAGATGCCTGGGTGTTA	q-PCR
q-tudor-F	GCGTCTGTTGCAGCTTCCTT	q-PCR
q-tudor-R	ACCGAAACACCTGCTGCACT	q-PCR
q-sox9b-F	TTGCCAGACAGCCAATGTT	q-PCR
q-sox9b-R	TCTCTGTTGACCCTGTTGGCTT	q-PCR
q-gsdf-F	TCCATGGCCACCGAGGTCTT	q-PCR
q-gsdf-R	CCGAGGAATTGCAGAGAGCACA	q-PCR
q-dmy-F	ACCTGACCTACCGCTCCAT	q-PCR
q-dmy-R	CGCAGCTTCCTCATTTGG	q-PCR
q-Sdgc-F	AGAACAGCGTAAGGCTCCA	q-PCR, genomic DNA sequence
q-Sdgc-R	GCCCATAAACCATGATGAGG	q-PCR, genomic DNA sequence, HRM
gSdgc-F	ACAAACACACCGCATTCTCC	genomic DNA sequence, HRM
gSdgc-F(nest1)	ACACACCGCATTCTCCA	HRM, genotyping
gSdgc-R(nest1)	AATTGCAAGGTGTCTGTT	HRM, genotyping
gSdgc-F(nest2)	ACTTATGTAATTGCTTAGTGTAGGC	HRM, genotyping
gSdgc-R(nest2)	GCTCCTCCTCCGAGGTG	HRM, genotyping
MID0123-F	ATCGTCTTGATCTATTAGAAAAGC	genotyping
MID0123-R	TCCTGCTCTGTGCTTGG	genotyping

F: forward, R: reverse, nest: primers used for nested PCR.