Supplemental Information:

Methods:

mRNA expression profiling. Affymetrix Mouse Genome 439 2.0 arrays were normalized by GC-RMA normalization[1] using GeneData Refiner. The Raw .cel files and the normalized data are deposited in GEO[2] as GSE27261. GSE9954 was obtained from the GEO database. The arrays with the highest sample IDs were removed from the tissue dataset to select 22 tissue types, each with three experimental replicates. When multiple probe sets were mapped to the same gene symbol, these values were averaged to obtain one value for each gene symbol. Direct Pearson Correlation R-values were calculated using all array data following reduction to gene symbols, and these values are shown in Figure 2b.

Each experiment in our dataset was divided by the average expression value from control testis tissue. GSE9954 data were separately divided by the average signal obtained from the GSE9954 testis samples. This was done separately for each dataset to determine how samples from each dataset differed from a baseline "testis" expression state. Cluster 3.0 software[3] was used to: i) log base 2 transform the data; ii) filter the dataset for genes that showed at least three observations with abs(val) >= 3 (8-fold) which resulted in 5030 genes passing the filter using both datasets combined; and iii) cluster the data on the gene-axis using average linkage hierarchial clustering. The experimental axis was defined by order of decreasing correlation to the mutant testes calculated as described above. Javatreeview Software[4] was used to generate heatmap images.

Oestadiol assays: Serum oestradiol was assayed using a clinical electrochemiluminescence immunoassay (Roche Estradiol II, 03000079 122) according to manufacturer's instructions. Three of three males assayed had levels below the detection limit, whereas two of three females had measurable oestradiol (5.0 and 19.7 pg/dl). Two of three *SCDmrt1KO(Dhh)* mutant males had measurable oestradiol (5.6 and 21.2 pg/dl).

Gonadotropin treatment: 6-8 week old mutant males, control males, and control females were treated with 5 units of pregnant mare serum by intraperitoneal injection and gonads were harvested 48 hours later.

Supplemental Tables:

Supplemental Table 1. Tissue-specific cre recombinases.

Name	Promoter	Cell type mutated for *Dmrt1*	Reference	PMID
Dhh-cre	desert hedgehog	Foetal Sertoli	Lindeboom et al. 2003	12954777
Nanos3-cre	nanos homolog 3 (Drosophila)	Foetal germ	Suzuki et al. 2008	18281459
Sf1-cre	steroidogenic factor 1	Foetal Sertoli	Bingham et al. 2006	16937416
UBC-cre/ERT2	ubiquitin C (Tamoxifen inducible)	Adult germ, adult Sertoli	Ruzankina et al. 2007	18371340

Supplemental Table 2. Granulosa cell genes elevated in *SCDmrt1KO* mutant gonads versus control testes.

Gene Name	Gene Symbol	Fold Change	P Value
forkhead box L2 opposite strand transcript	*Foxl2os*	414.5	2.36E-05
secreted frizzled-related protein 4	*Sfrp4*	139.0	0.0065
luteinizing hormone/choriogonadotropin receptor	*Lhcgr*	95.8	5.30E-05
insulin-like growth factor binding protein 5	*Igfbp5*	26.3	0.0040
prolactin receptor	*Prlr*	21.3	7.97E-06
forkhead box L2	*Foxl2*	18.6	0.0063
follistatin	*Fst*	10.2	0.0009
inhibin beta-B	*Inhbb*	8.7	0.0001
follicle stimulating hormone receptor	*Fshr*	8.3	0.0016
inhibin alpha	*Inha*	6.4	2.57E-06
nuclear receptor subfamily 5, group A, member 2/liver receptor homolog 1	*Nr5a2/Lrh1*	5.4	0.0348
LFNG O-fucosylpeptide 3-beta-N-acetylglucosaminyltransferase/lunatic fringe	*Lfng*	5.4	7.67E-05
wingless-related MMTV integration site 4	*Wnt4*	5.3	0.0036

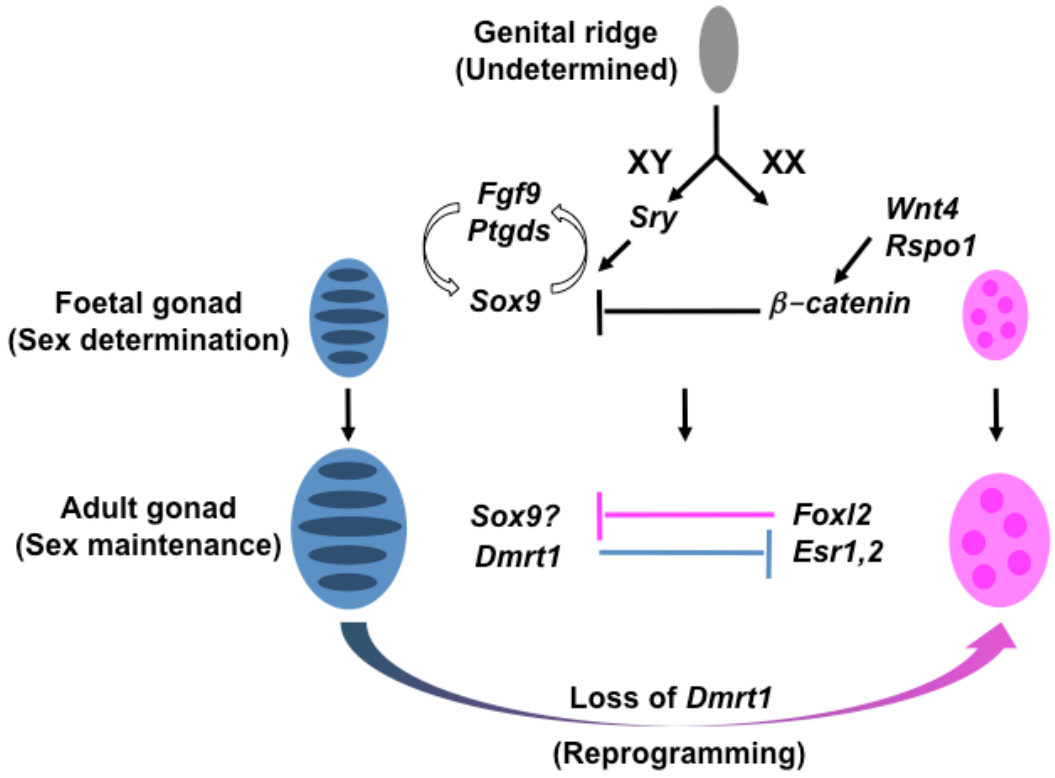
Supplemental Table 3. Antibodies.

Primary antibodies	Name	Description	Dilution	Reference
AROMATASE (Cyp19a1)	cytochrome P450, family 19, subfamily a, polypeptide 1	Rabbit polyclonal	1 to 200	Abcam (ab18995)
BETA-CATENIN (Ctnnb1)	catenin (cadherin associated protein), beta 1	Mouse monoclonal (IgG)	1 to 200	DB Transduction (610153)
DMRT1 (Dmrt1)	doublesex and mab-3 related transcription factor 1	Rabbit polyclonal	1 to 200	Raymond et al. 2000, PMID: 11040213
FOXL2 (Foxl2)	forkhead box L2	Goat polyclonal	1 to 200	Abcam (ab5096)
FOXL2 (Foxl2)	forkhead box L2	Rabbit polyclonal	1 to 200	Cocquet et al. 2002, PMID: 12471206
GATA4 (Gata4)	GATA binding protein 4	Goat polyclonal	1 to 50	Santa Cruz (sc-1237)
LRH1 (Nr5a2)	nuclear receptor subfamily 5, group A, member 2	Goat polyclonal	1 to 50	Santa Cruz (sc-21132)
MATER (Nlrp5)	NLR family, pyrin domain containing 5	Rabbit polyclonal	1 to 100	Hoodbhoy et al. 2006, PMID: 17047254
SCC (Cyp11a1)	cytochrome P450, family 11, subfamily a, polypeptide 1	Rabbit polyclonal	1 to 200	Chemicon (AB1294)
SF1 (Nr5a1)	nuclear receptor subfamily 5, group A, member 1	Rabbit polyclonal	1 to 200	Morohashi et al. 1993, PMID: 8247022
SMA (Acta2)	actin, alpha 2, smooth muscle, aorta	Mouse monoclonal (IgG)	1 to 200	Sigma (A2547)
SOX9 (Sox9)	SRY-related protein 9	Rabbit polyclonal	1 to 200	Millipore (AB5535)
SYCP3 (Sycp3)	synaptonemal complex protein 3	Rabbit polyclonal	1 to 200	Abcam (ab15092)
TRA98		Rat monoclonal (IgG)	1 to 200	Bio Academia (73-003)
ZP2 (Zp2)	zona pellucida glycoprotein 2	Rat monoclonal (IgG)	1 to 50	Hoodbhoy et al. 2006, PMID: 17047254
Secondary antibodies		**Description**		**Reference**
Anti-goat IgG (Alexa Fluor 594)		Donkey polyclonal	1 to 500	Invitrogen (A11058)
Anti-rabbit igG (Alexa Fluor 488)		Goat polyclonal	1 to 500	Invitrogen (A11073)
Anti-rabbit igG (Alexa Fluor 488)		Donkey polyclonal	1 to 500	Invitrogen (A21206)
Anti-rat IgG (Alexa Fluor 594)		Goat polyclonal	1 to 500	Invitrogen (A11007)

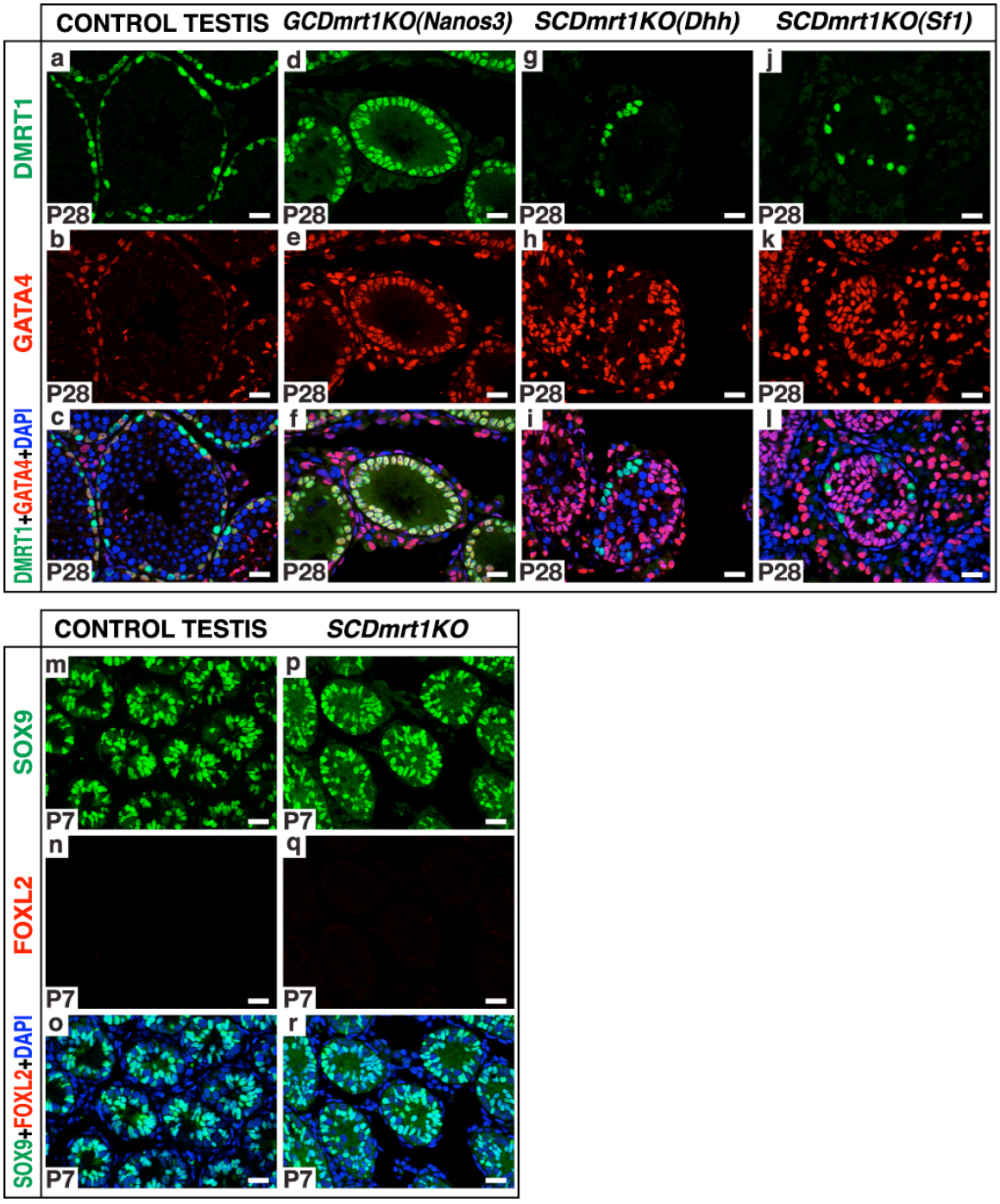
Supplemental Table 4. qRT-PCR and qChIP primers.

Gene Symbol	RT Forward Primer 5' to 3'	RT Reverse Primer 5' to 3'
Cyp19a1	TAGCGCAAGATGTTCTTGGA	GCCCAATTCCCAGACAGTAG
Dmrt1	TGGGTTCTGGAAGCAAGAAG	CTGTCTTCTCAGGGCCACCT
Esr1	TGCAATGACTATGCCTCTGG	CTCCGGTTCTTGTCAATGGT
Esr2	TGTGCTATGGCCAACTTCTG	AGTAACAGGGCTGGCACAAC
Foxl2	TCATAGCCAAGTTCCCGTTC	CCCTTCTCGAACATGTCCTC
Hprt	TCCTCCTCAGACCGCTTTT	CCTGGTTCATCATCGCTAATC
Hsd17b1	TGTTCGCCTAGCTTCTGACC	AGCAGCCACAGATTTGGAGT
Hsd17b3	ATGGCATCGGGAAAGCCTAT	CTCTTCTGCAATGGTCTGTAGC
Ptgdr	AGGAGCTGGACCACTTTGTG	TCACAGACAGGAAACGCAAG
Rspo1	AGAAGGCATTGAGCAACTGG	GAATCCACCTCAGAGGGTCA
Sox9	AGGAAGCTGGCAGACCAGTA	CGTTCTTCACCGACTTCCTC
Wnt4	CCTGCGACTCCTCGTCTTC	AAGGTTCCGTTTGCACATCT
Gene Symbol	**ChIP Forward Primer 5' to 3'**	**ChIP Reverse Primer 5' to 3'**
Esr1	TTGGAAGACGGCAAGGATAC	GTATTGGCAATGGGGTGAAG
Esr2	GGCTCCACTACCCTATTCTGC	GGTTCTTGTGGGCTGTTGTT
Foxl2	GCTTCAAAGTCAACCCAAGC	CTGGGTTGCAGGTTCTCATT
Ptgdr	AGGGCAGAGTTAACGGGAGT	GGCCCGAATCATCACTCTAA
Rspo1	CCAGGGAATGTGATGAAAGG	GGCTGACGAATTGGAAACAT
Sox8	TACTCACCCCTGGAAACCAC	TGGATGAACCCTAAGACCTCA
Sox9	TGTTTGAAGGCTGACAGCAG	CTGAGCCACTTCTCCAGCTC
Wnt4	GCAGACTGGAGGCGTTACAT	CCCCTTCTTGTTTCCTAGCC

Supplemental Figures

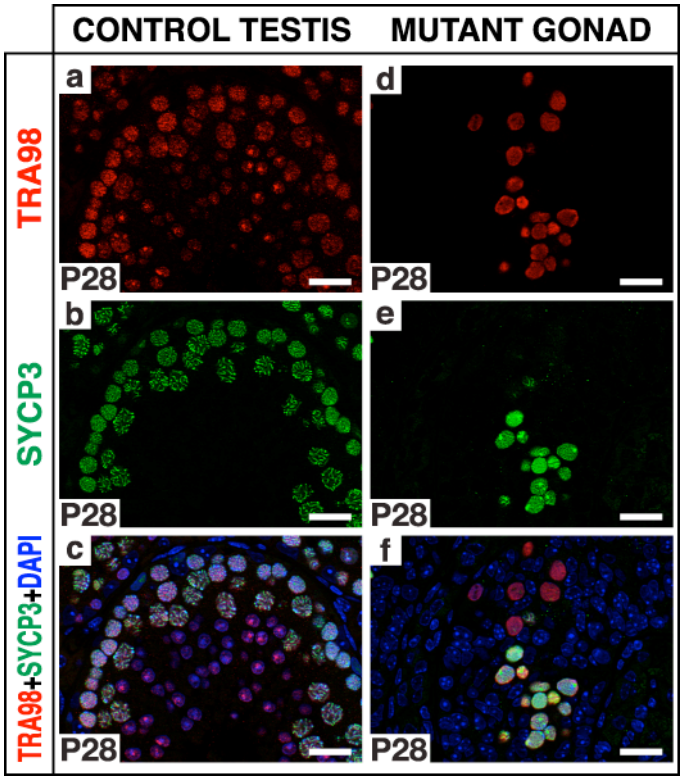


Supplemental Figure 1. DMRT1 prevents male-to-female sex reversal in the postnatal testis. Somatic sex determination occurs in the bipotential gonad, or genital ridge, around embryonic day 11 (E11), in cells with potential to form either male Sertoli cells or female granulosa cells. In males the Y-linked *Sry* gene is transiently expressed and activates *Sox9* transcription, which also is activated and maintained by the action of *Fgf9* and *Ptgds*. In XX gonads, lack of *Sry* permits activity of an ovarian gene network activated by *Wnt4* and *Rspo1*, in which *β-catenin* and *Dax1* (not shown) repress *Sox9* expression and promote female differentiation. After birth *Foxl2* and the oestrogen receptors *Esr1* and *Esr2* jointly suppress transcription of *Sox9* to maintain female fate. In this paper we show that male fate must be actively maintained postnatally in the testis. Loss of *Dmrt1* causes reprogramming of Sertoli cells into their female equivalent, granulosa cells, and induces the expression of many ovarian mRNAs. Based on mRNA and protein expression and in vivo DNA binding analysis we propose that DMRT1 controls a male sex maintenance program that promotes expression of *Sox9* and other testicular genes and prevents expression of *Foxl2* and other ovarian genes. (Figure modified from Uhlenhaut et al. 2009[5].)

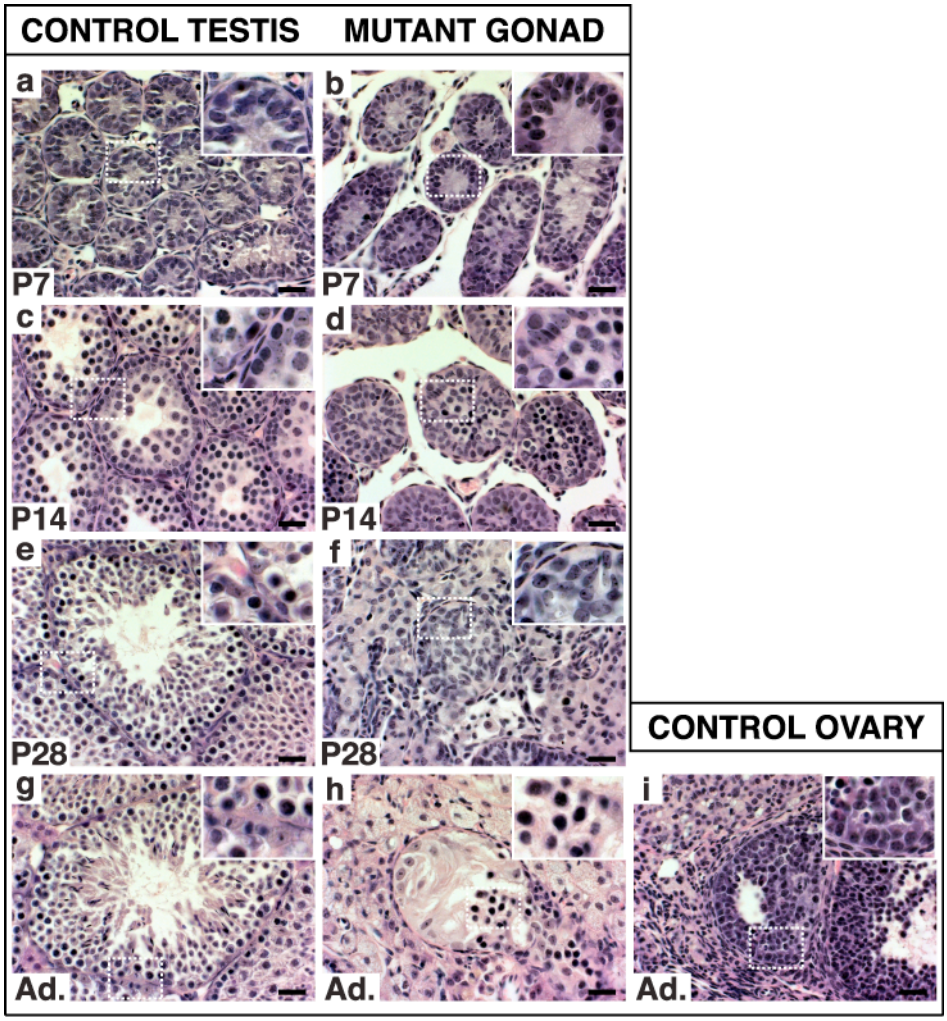


Supplemental Figure 2. (a-l) Cell type-specific deletion of *Dmrt1*. Immunofluorescence (IF) detection of DMRT1 in mitotic spermatogonia (GATA4-negative) and Sertoli cells (GATA4-positive) of control testis (a-c). Deletion of *Dmrt1* in germ cells with *Nanos3-cre* (*GCDmrt1KO*) eliminates DMRT1 expression in spermatogonia but not in Sertoli cells (d-f). Deletion of *Dmrt1* with *Dhh-cre* or *Sf1-cre*

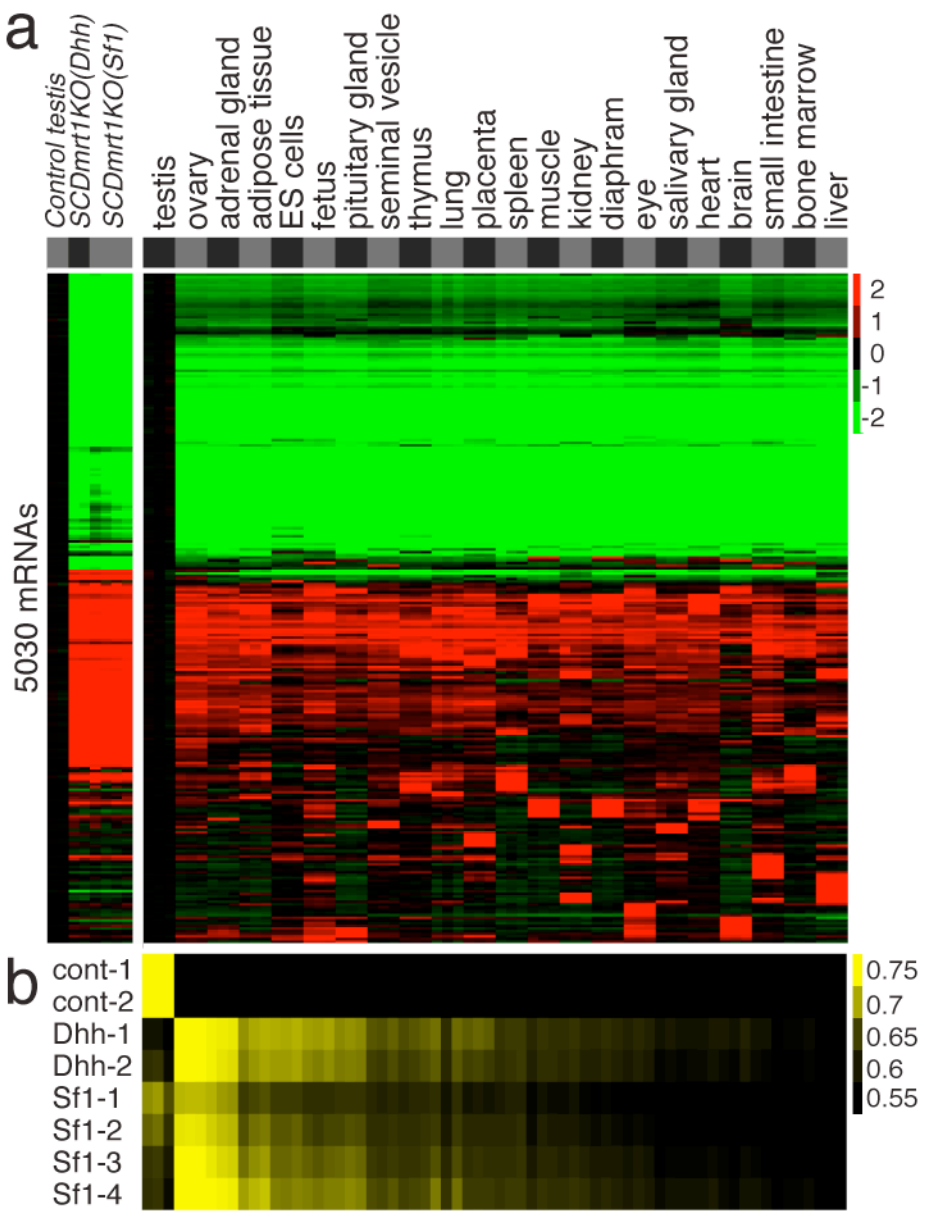
(*SCDmrt1KO*) eliminates expression of DMRT1 in most Sertoli cells but not in germ cells of conditional mutant testis (g-l). Cells inside tubules that are negative for both DMRT1 and GATA4 are germ cells that have initiated meiosis. The mutant somatic cells in *SCDmrt1KO* gonads retain GATA4 expression, which is found in somatic supporting cells of both testis and ovary, and these mutant cells have granulosa-like morphology. **(m-r)** IF of SOX9 and FOXL2 at P7. Sertoli cells of control (m-o) and *SCDmrt1KO(Dhh)* (p-r) testes strongly express SOX9, whereas FOXL2 is undetectable. Scale bars: 20 μm.



Supplemental Figure 3. SYCP3 expression in mutant gonads. IF showing expression of the germ cell marker TRA98 and meiotic prophase marker SYCP3. Some germ cells in the mutant testis have strong nuclear SYCP3 expression, consistent with meiotic prophase, but the protein is not localized to condensed and paired chromosomes characteristic of cells in pachynema as seen in the control. Most SYCP3-negative germ cells in the mutant have nuclear morphology typical of spermatogonia.

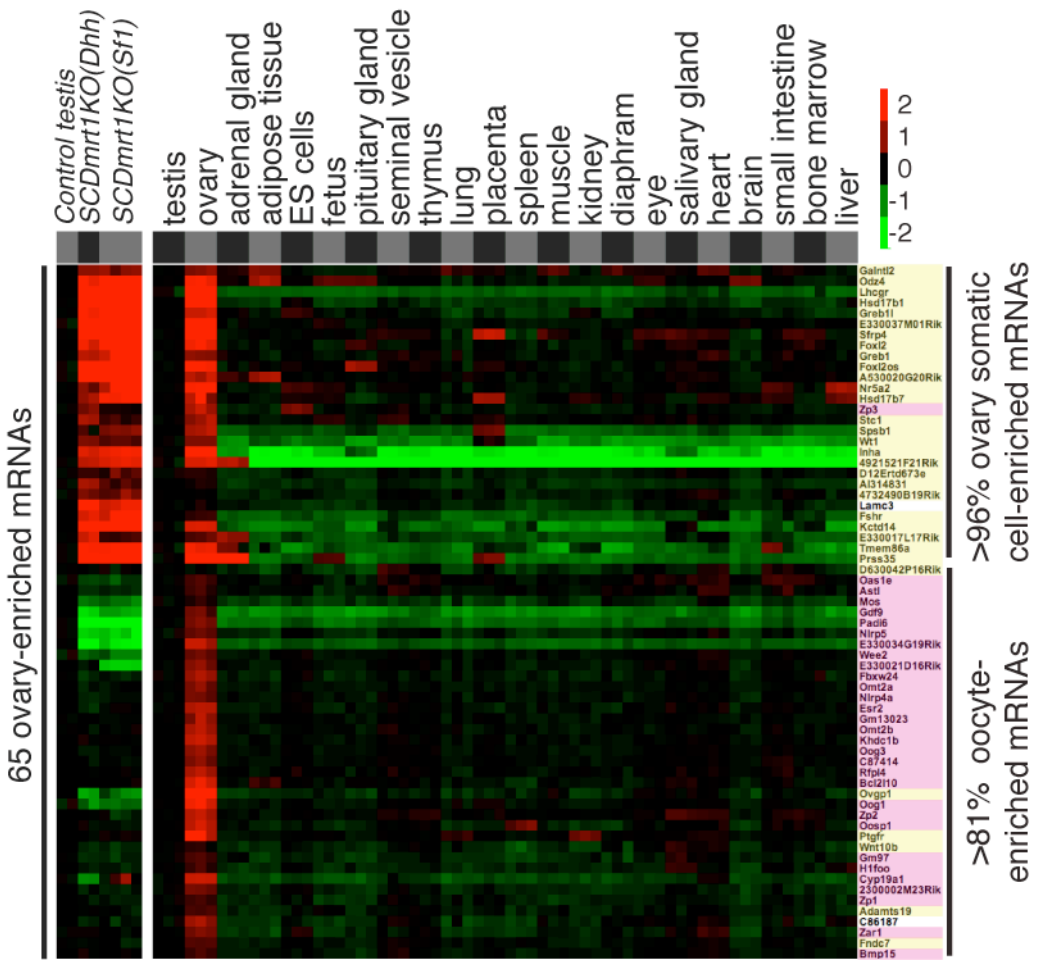


Supplemental Figure 4. Histology of *SCDmrt1* mutant gonads. Hematoxylin and eosin staining of mutant gonads compared to control testis and ovary. Mutant gonads were *SCDmrt1KO(Dhh)*. Scale bars: 50 μm.

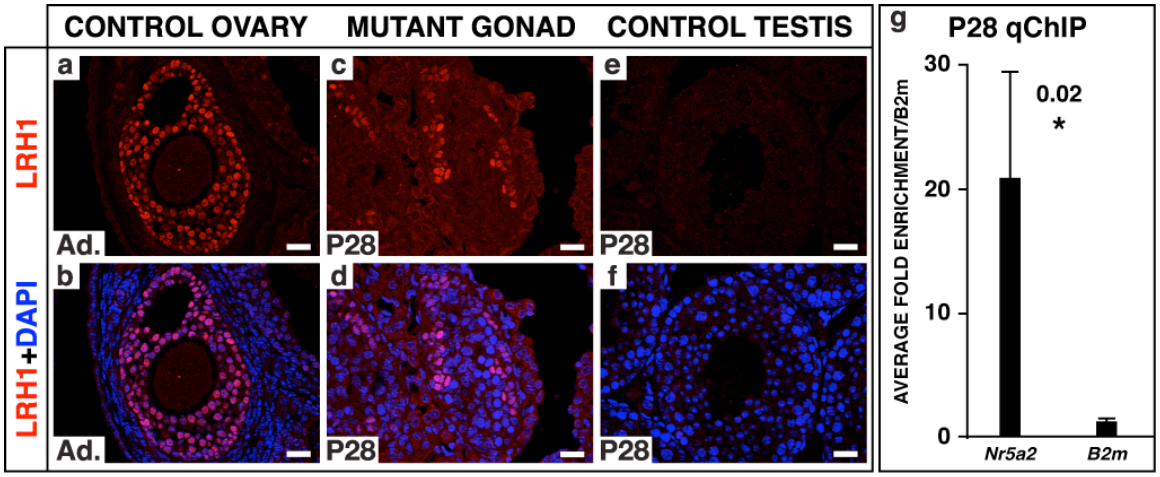


Supplemental Fig. 5. Testis-to-ovary reprogramming of mRNA expression in *SCDmrt1KO* gonads.

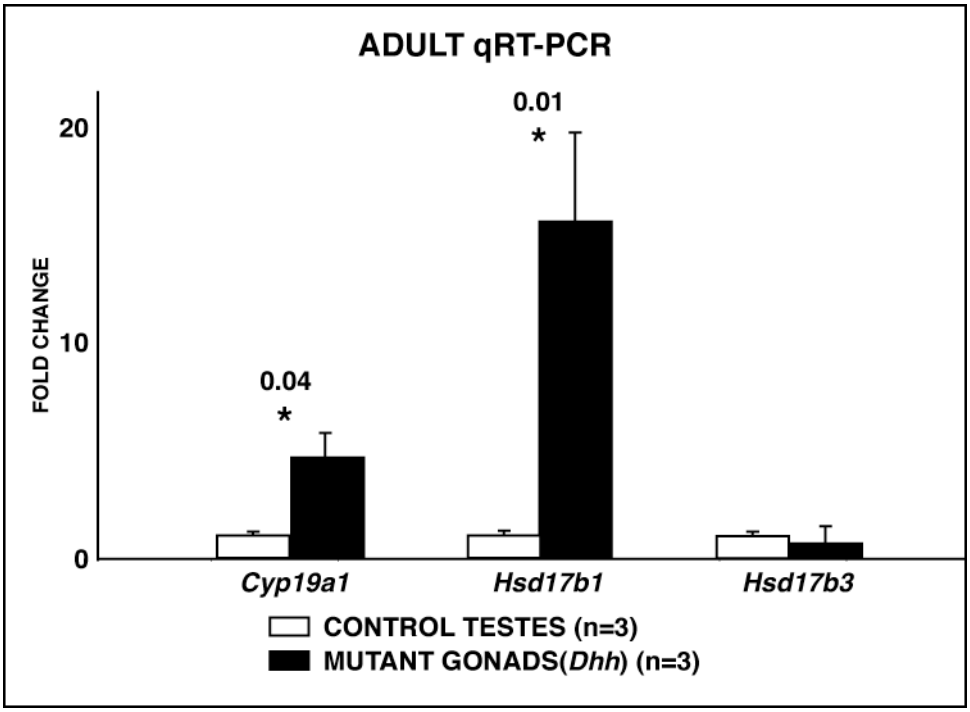
(a) Heat map of gene expression profiles from control testes and *SCDrmt1KO* gonads at p28. Data are shown in context of GSE9954, which contains data from 22 tissue types including ovary and testes. For each independent dataset, transcript level is shown relative to expression patterns observed in normal testes. Only the 5030 genes with >8-fold expression change relative to testis in at least 3 experiments are shown. **(b)** Heat map showing pair-wise Pearson correlation coefficients comparing control testes and *SCDmrt1KO* gonads to ovaries and other tissues, using absolute expression values.



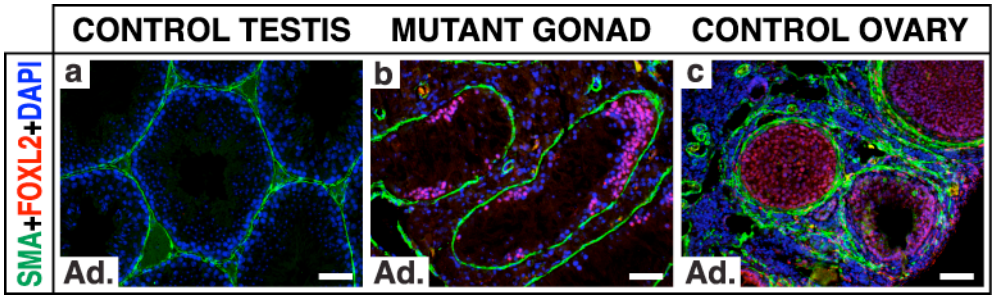
Supplemental Fig. 6. Expression of ovary-enriched mRNAs in *SCDmrt1KO* gonads relative to other tissues. Ovary-enriched mRNAs were identified using BioGPS[6] (biogps.gnf.org), identifying genes with expression similar to that of *Foxl2* (correlation 0.75). Based on expression levels in the GeneAtlas GNF1M dataset or GEO dataset GDS1266, mRNA names shaded yellow are primarily enriched in somatic ovarian cells and mRNA names shaded pink had highly enriched expression in oocytes.



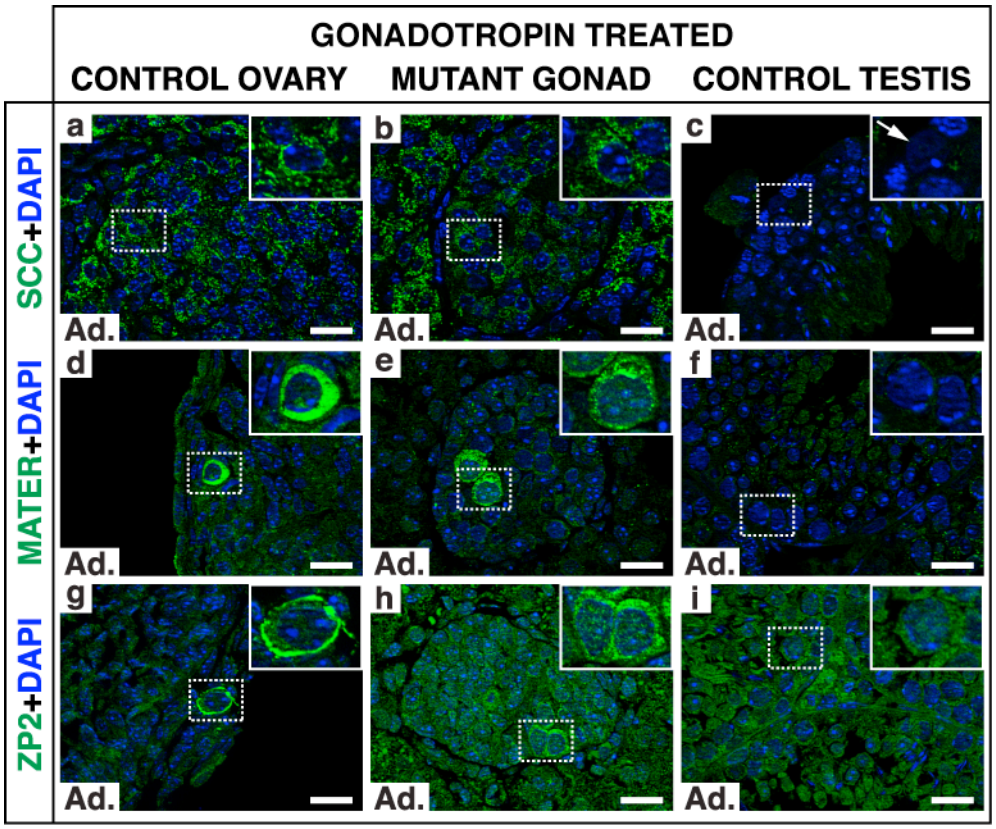
Supplemental Figure 7. LRH1 expression in *SCDmrt1KO* testis. (a-f) IF detects LRH1 expression in granulosa cells of adult ovary (**a,b**) and ectopically in intratubular cells of mutant testis (**c,d**) but not in control testis (**e,f**). (**g**) qChIP analysis of DMRT1 association with *Nr5a2/Lrh1* in P28 testes showing strong enrichment relative to *B2m* negative control. Mutant gonads were from *SCDmrt1KO(Dhh)* males. Scale bar: 20 μm.



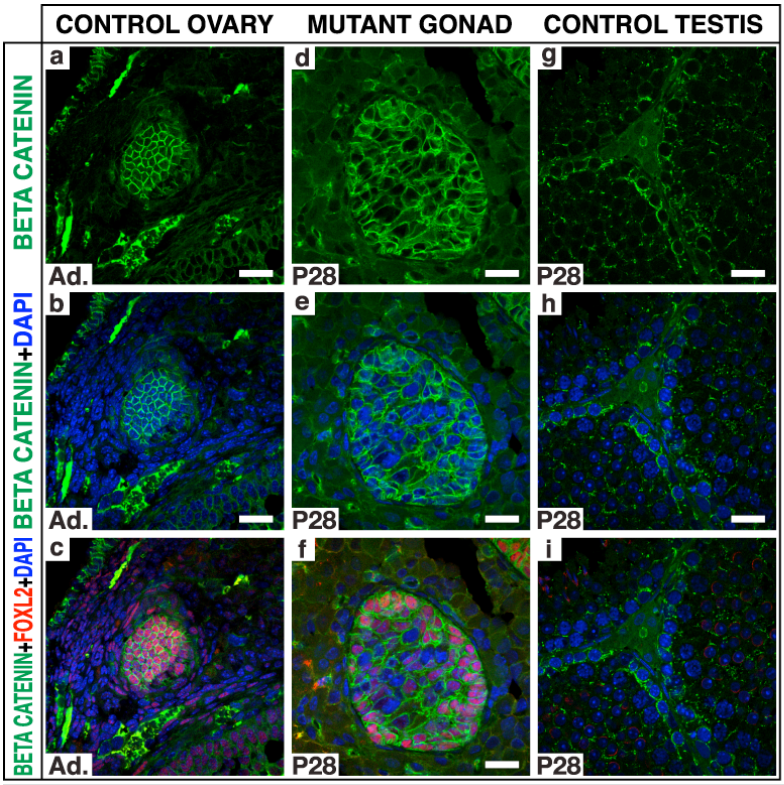
Supplemental Fig. 8. Elevated expression of *Cyp19a1* and *Hsd17b1* in adult mutant gonad. qRT-PCR analysis in adult gonads. Significance of each expression change in mutant gonad (Students t-test) is indicated. Mutant gonads were *SCDmrt1KO(Dhh)*.



Supplemental Fig. 9. Loss of seminiferous tubule integrity in mutant gonads. IF staining for smooth muscle actin (SMA) shows that control testes have a contiguous ring of SMA-positive peritubular myoid cells surrounding each seminiferous tubule (**a**), whereas mutant gonads do not. (**b**). SMA-positive cells surround the follicles of the control ovary (**c**); these are primarily theca cells (see Fig. 3 for higher magnification).



Supplemental Fig. 10. Luteinized granulosa cells and feminized germ cells after gonadotropin stimulation. (a-c) SCC-positive granulosa-like cells in treated control ovary and mutant gonad but not in control testis (arrow indicates Sertoli cell). (d-f) MATER-positive germ cells in gonadotropin-treated control ovary and mutant gonad but not treated control testis. (g-i) ZP2-positive germ cells in treated control ovary and mutant gonad but not control testis. Scale bar: 20 micron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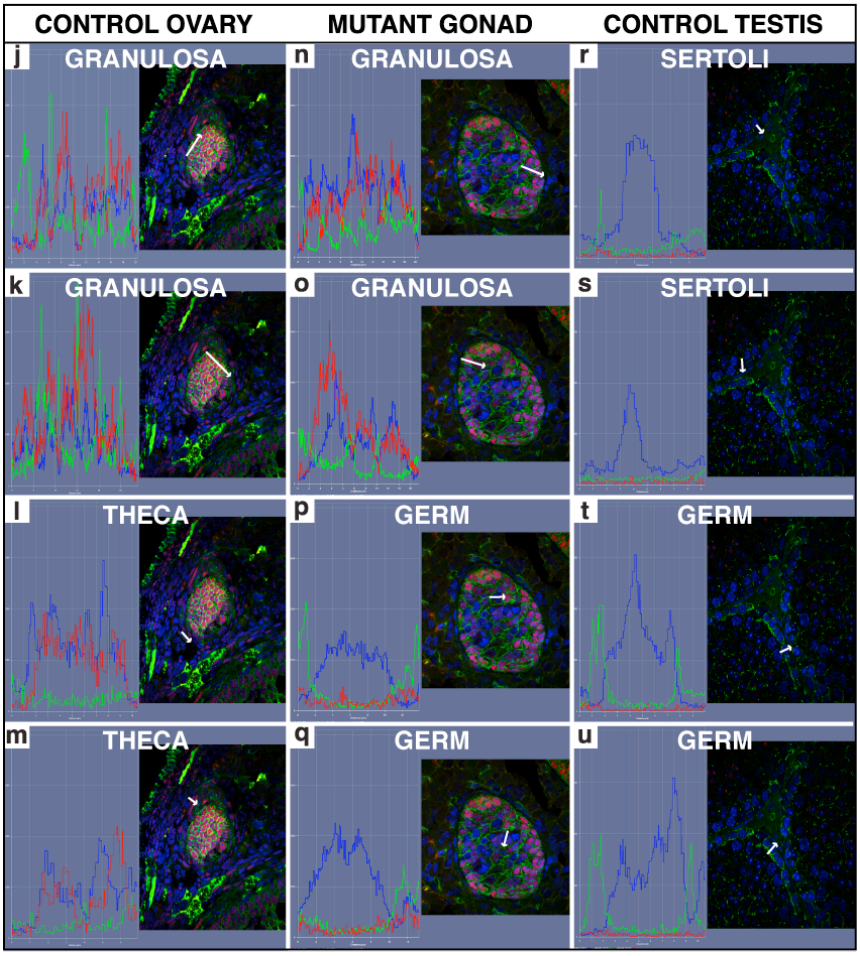
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Supplemental Figure 11. Elevated Wnt/β-catenin signaling in *SCDmrt1KO* gonads. (**a-c**) Control ovary stained for β-catenin and FOXL2. (**d-f**) Mutant gonad stained for β-catenin and FOXL2. (**g-i**) Control testis stained for active β-Catenin and FOXL2. (**j-u**) Pixel density tracings across areas indicated by arrows, showing higher nuclear β-catenin in granulosa cells of ovary and in granulosa-like cells of mutant gonad relative to theca cells, control Sertoli cells, and germ cells of control testis and mutant gonad. β-Catenin tracing is green, FOXL2 is red, DAPI is blue. Mutant gonads were from *SCDmrt1KO(Dhh)* males. Images are 0.3 μm optical sections captured on a Zeiss LSM710 laser-scanning confocal microscope.

Supplemental References:

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