Supplementary Material

Supplementary Figures

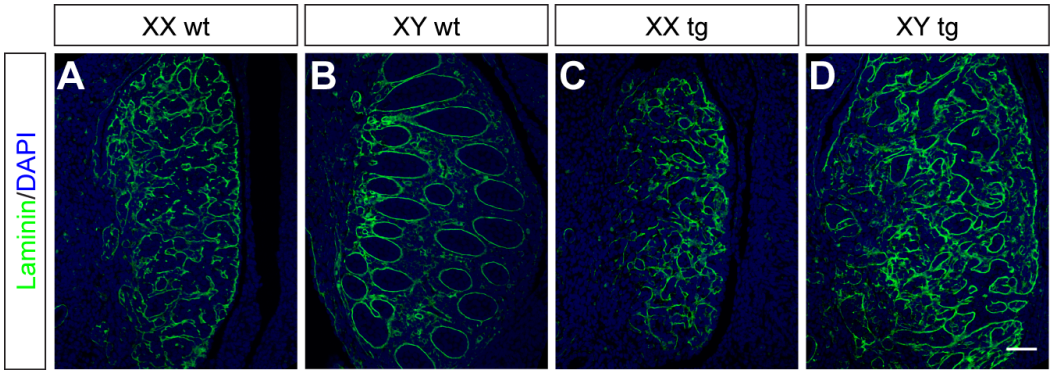

Fig. S1. Lack of cord formation in XX/XY fetal gonads expressing the *Dmrt1* transgene.

(A-D) Immunofluorescence staining for laminin (green) on sagittal sections of fetal gonads at 14.5 dpc. Testis cords were observed in wild type testes but not in XX or XY testes transgenic for *Dmrt1*. Nuclei were counterstained with DAPI (blue). Scale bar, 50 μm.

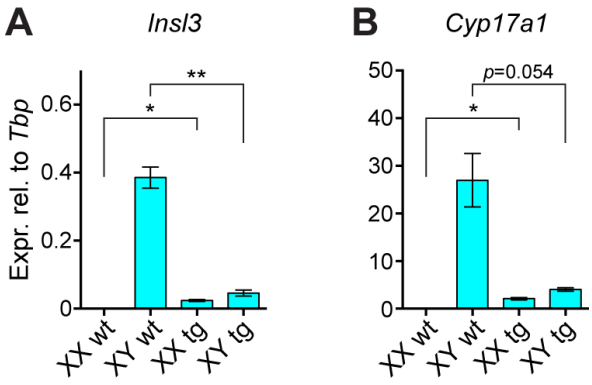

Fig. S2. Quantitative gene expression analyses of steroidogenic marker genes in fetal mouse gonads at 14.5 dpc using qRT-PCR.

(A-B) Both *Insl3* and *Cyp17a1* were up-regulated in XX transgenic gonads, compared with XX wild-type ovaries. Mean ± s.e.m., n=3. Statistical significance was determined for two-way comparisons indicated by brackets. *, $p < 0.05$; **, $p < 0.01$: Student's t test.

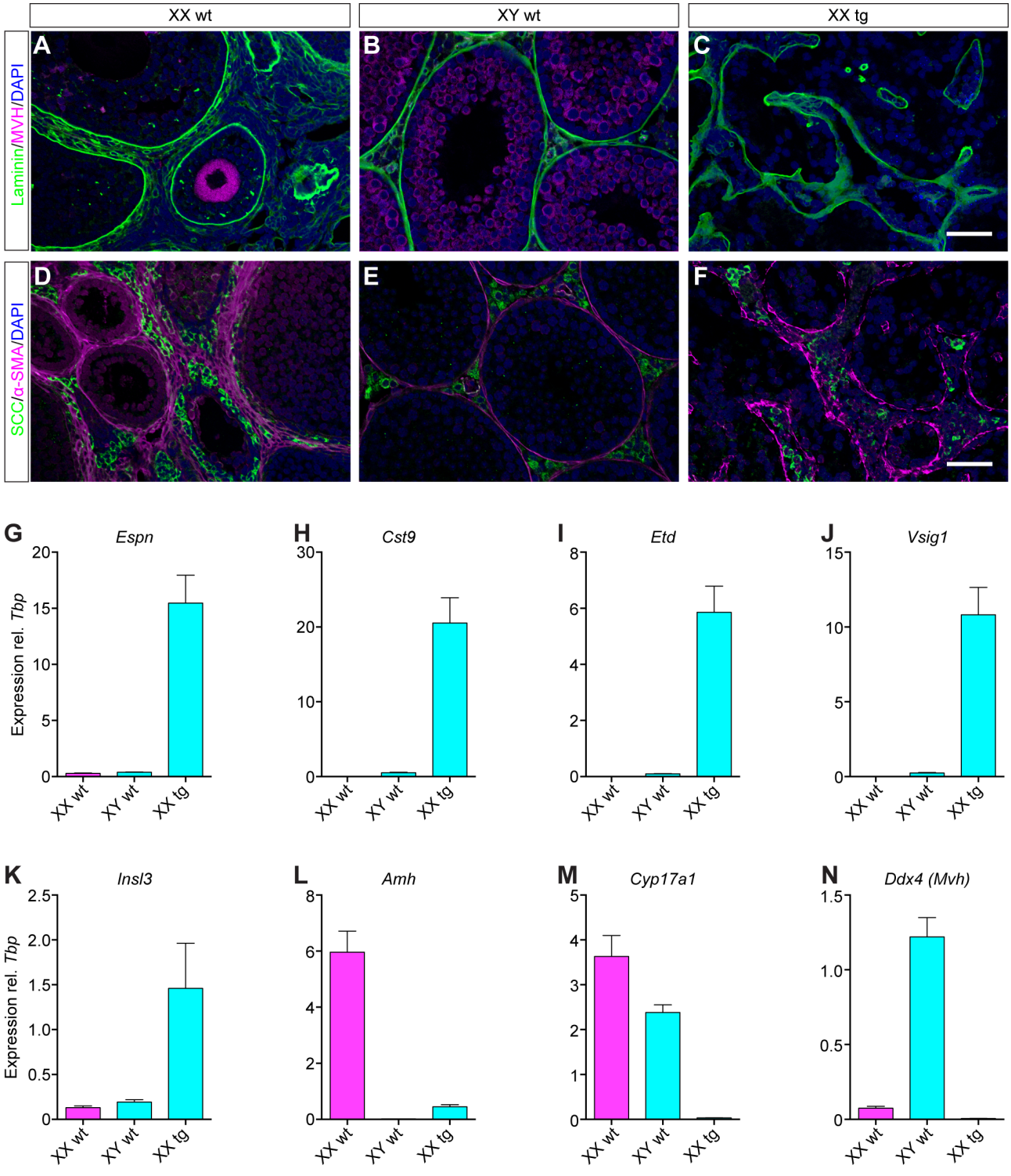

Fig. S3. Expression analyses of marker genes in adult mouse gonads.

(A-F) Immunofluorescence analyses of sections of adult mouse gonads at 4 weeks. Sertoli cell clusters in sex-reversed transgenic testes (C) were outlined by laminin (green), a marker for basal

membrane. These resembled neither ovarian follicles (A) nor testis cords (B). Germ cells (marked by MVH, magenta) were absent in XX transgenic testes. (D-F) In the wild type (E) and transgenic testes (F), interstitial Leydig cells stained positive for SCC, whereas in wild type ovaries, theca cells surrounding the follicles were positive for SCC (D). Sections were also analysed for alpha-smooth muscle actin (α-SMA); staining for α-SMA in combination with flattened cell morphology marks peritubular myoid cells. Scale bar, 50 μm. (G-N) Quantitative gene expression analyses in adult gonads at 4 weeks using qRT-PCR. Pink bars represent phenotypic female samples and blue bars phenotypic male samples, mean ± s.e.m. of three technical replicates.

Supplementary Materials and Methods

Genotyping

Genomic DNA was extracted from mouse tissue using QuickExtract solution (Epicentre). Genotyping PCRs were performed using GoTaq (Promega) to determine genetic sex and presence of the transgene. Sexing primers were as described (McFarlane et al., 2013). Primers (5'-3') for the *Wt1*:*Dmrt1*-IRES-EGFP transgene were 5'Wt1-F (GTGCGTCCAGCAGCCGGAGCAA) and Dmrt1-R (ATCAGGCTGCACTTCTTGCACTG).

qRT-PCR

qRT-PCR analyses were performed as described (Zhao et al., 2014). Expression was normalized to *Tbp* (Svingen et al., 2009) or *Ddx4* in the cases of *Nanos2* and *Dnmt3l*. Statistical analyses were performed using a two-tailed Student's *t* test. Primers or TaqMan probes (Life Tech) used are listed in Table S1.

Table S1. Primers and probes

Primer	Sequence or TaqMan probe ID
Tbp.F	ACGGACAACTGCGTTGATTTT
Tbp.R	GAAGGGAGGAGGGGTGAG
Sox9.F	AGTACCCGCATCTGCACAAC
Sox9.R	TACTTGTAATCGGGGTGGTCT
Dmrt1.F	CCTACTCAGAAGCCAAAGCCAGTG
Dmrt1.R	CCGAGGACGCAGACTCACATTC
Sox8.F	GCAAGACCCTAGGCAAGCTGT
Sox8.R	TCTGGGTGGTCTTTCTTGTGC
Sox10.F	CAAGGAGGGGCTGCTGCTAT
Sox10.R	ATGGCTCTGGCCTGAGGGGT
Amh.F	CGAGCTCTTGCTGAAGTTCCA
Amh.R	GAAGTCCACGGTTAGCACCAA
Wt1.F	CGGTCCGACCATCTGAAGAC
Wt1.R	GTTGTGATGGCGGACCAATT
Ptdgs.F	GCTCTTCGCATGCTGTGGAT

Ptdgs.R	GCCCCAGGAACTTGTCTTGTT
Dhh.F	CCCGACATAATCTTCAAGGATGA
Dhh.R	GCGATGGCTAGAGCGTTCAC
Cyp26b1.F	TGGACTGTGTCATCAAGGAGGT
Cyp26b1.R	GTCGTGAGTGTCTCGGATGCTA
Star.F	AAACTCACTTGGCTGCTCAGTATTGAC
Star.R	CAGGTGGTTGGCGAACTCTATCTG
Insl3.F	TCCTCGGCAGGCTTCTCAGC
Insl3.R	GCTCCTTCAGTGGGGACACAGAC
Cyp11a1.F	CAGTGATGACCTATTCCGCTTTTCC
Cyp11a1.R	TCTGGTAGACAGCATTGATGAACCG
Hsd3b.F	GGACAAAGTATTCCGACCAGAAACC
Hsd3b.R	CAGCAGTGTGGATGACAACAGAGATG
Cyp17a1.F	CATCCCACACAAGGCTAACA
Cyp17a1.R	CAGTGCCCAGAGATTGATGA
Hsd17b1.F	TGTTCGCCTAGCTTCTGACC
Hsd17b1.R	AGCAGCCACAGATTTGGAGT
Hsd17b3.F	ATGGCATCGGGAAAGCCTAT
Hsd17b3.R	CTCTTCTGCAATGGTCTGTAGC
Foxl2.F	AGGGAGAGAATAAAACATTCATGG
Foxl2.R	GCAAACTCCAAGGCCATTAC
Wnt4.F	CTGGACTCCCTCCCTGTCTTT
Wnt4.R	CATGCCCTTGTCACTGCAA
Rspo1.F	CGACATGAACAAATGCATCA
Rspo1.R	CTCCTGACACTTGGTGCAGA
Fst.F	GCAGCCGGAACTAGAAGTACA
Fst.R	ACACAGTAGGCATTATTGGTCTG
Stra8.F	TTCAGCTCTACATACAGATCATTGAG
Stra8.R	ATCTGGGGGCTCTGGTTC
Sycp3.F	AAATCTGGGAAGCCACCTTTGG
Sycp3.R	TGGAGCCTTTTCATCAGCAACATC
Cyp19a1.F	CCTGGACGAAAGTGCTATTGTGAAG
Cyp19a1.R	GAAGATGTTTGGTTTGATGAGGAGAGC
Ddx4.F	CAGGAATGCCATCAAAGGAACAAC
Ddx4.R	CCCAACAGCGACAAACAAGTAACTG
Espn.F	TTACATGCAGACCAAGAACAAGCT
Espn.R	CCACCTTGGGCTCCTTGAG
Cst9.F	TGAATATGCCTACAGGATGGAA
Cst9.R	CATGGAATACACGGTTGGAA

Etd.F	CTTCTCTCACCGCACAACAA
Etd.R	CTCAAGGCAATGGAGAAAGC
Vsig1.F	GGTGTTTGCATTTTGGAAGG
Vsig1.R	TGAAAGCTTTTCCAGGGACT
Ddx4	TaqMan probe Mm00802445_m1
Nanos2	TaqMan probe Mm02525720_s1
Dnmt3l	TaqMan probe Mm00457635_m1

Immunofluorescence

Immunofluorescence staining was performed on paraffin sections of mouse embryos or adult gonads. Antibodies and dilutions are listed in Table S2.

Table S2. Antibodies

Antibody	**Source/Reference**	**Dilution**
Goat anti-GFP	Abcam (Ab5450)	1:500
Mouse anti-SOX9	Abnova (H00006662-M01)	1:200
Rabbit anti-FOXL2	Polanco et al. (Polanco et al., 2010)	1:500
Rabbit anti-DMRT1	Santa Cruz (H-240; SC-98341)	1:100
Rabbit anti-MVH	Abcam (Ab13840)	1:800
Mouse anti-MVH	Abcam (ab27591)	1:500
Goat anti-AMH	Santa Cruz (C-20; SC-6886)	1:300
Rabbit anti-HSD3β	Transgenic Inc (KAL-KO607)	1:500
Mouse anti-SCP3	Abcam (ab97672)	1:200
Rabbit anti-Laminin	Sigma (L9393)	1:200
Mouse anti-α-SMA	Sigma (A2547)	1:200
Rabbit anti-SCC	Svingen et al. (Svingen et al., 2012)	1:300

Supplementary References

McFarlane, L., Truong, V., Palmer, J. S. and Wilhelm, D. (2013). Novel PCR assay for determining the genetic sex of mice. *Sex. Dev.* **7**, 207-211.

Polanco, J. C., Wilhelm, D., Davidson, T.-L., Knight, D. and Koopman, P. (2010). Sox10 gain-of-function causes XX sex reversal in mice: implications for human 22q-linked disorders of sex development. *Hum. Mol. Genet.* **19**, 506-516.

Svingen, T., François, M., Wilhelm, D. and Koopman, P. (2012). Three-dimensional imaging of Prox1-EGFP transgenic mouse gonads reveals divergent modes of lymphangiogenesis in the testis and ovary. *PLoS ONE* **7**, e52620.

Svingen, T., Spiller, C. M., Kashimada, K., Harley, V. R. and Koopman, P. (2009). Identification of suitable normalizing genes for quantitative real-time RT-PCR analysis of gene expression in fetal mouse gonads. *Sex. Dev.* **3**, 194-204.

Zhao, L., Ng, E. T., Davidson, T.-L., Longmuss, E., Urschitz, J., Elston, M., Moisyadi, S., Bowles, J. and Koopman, P. (2014). Structure–function analysis of mouse Sry reveals dual essential roles of the C-terminal polyglutamine tract in sex determination. *Proc. Natl. Acad. Sci. USA* **111**, 11768-11773.