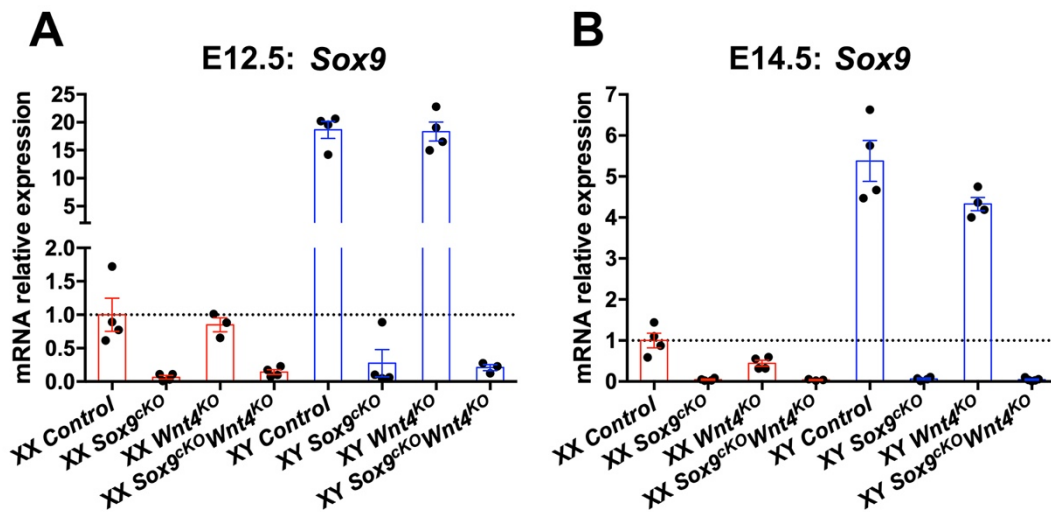


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Mouse gonad development in the absence of the pro-ovarian factor WNT4 and the pro-testis factor SOX9

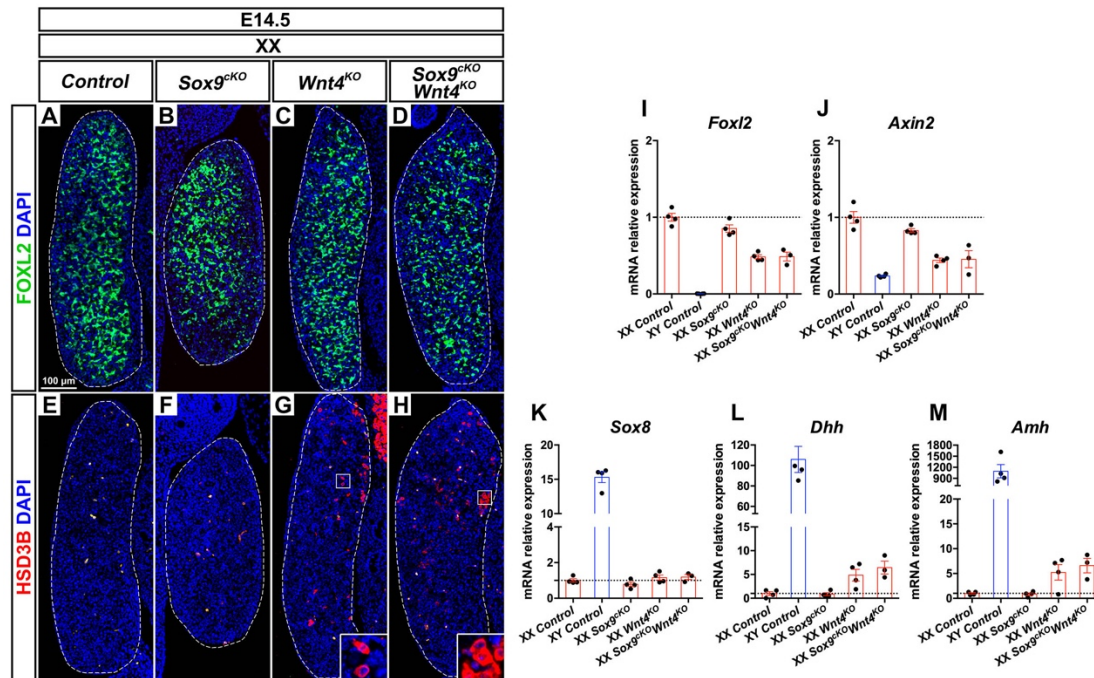
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Supplementary Materials



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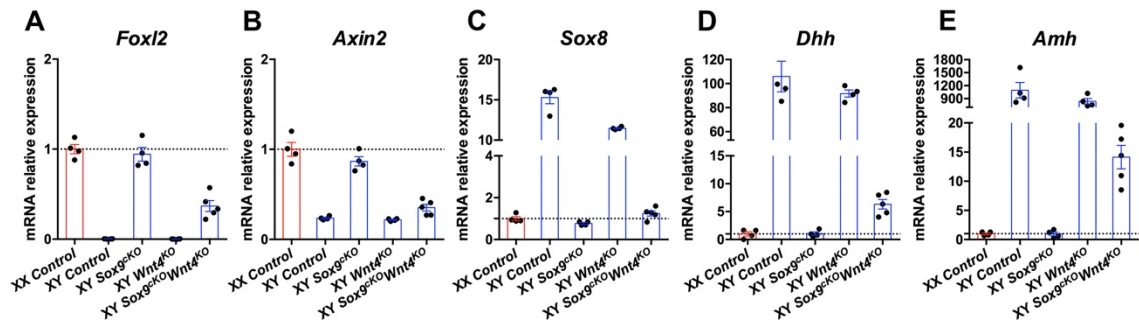
Figure S1. *Sox9* expression in control and mutant gonads at E12.5 and E14.5. RT-quantitative PCR analysis of *Sox9* expression in E12.5 (A) and E14.5 (B) XX and XY gonads of Control, *Sox9*^{KO}, *Wnt4*^{KO} and *Sox9*^{KO}*Wnt4*^{KO} genotypes (n = 3 to 5 embryos for each genotype). Expression level in XX controls is 1. Graphs show individual values of the relative normalized expression levels (dots), and the mean fold-change (bars) ± SEM.



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25 **Figure S2. Phenotype of E14.5 XX control and mutant gonads.** (A-D) Immunodetection of the pre-
 26 granulosa cell marker FOXL2 (green) in E14.5 XX gonads of Control (A), *Sox9^{cKO}* (B), *Wnt4^{KO}* (C) and
 27 *Sox9^{cKO}Wnt4^{KO}* (D) genotypes. (E-H) Immunodetection of the steroidogenic enzyme HSD3B (red) in
 28 E14.5 XX gonads of Control (E), *Sox9^{cKO}* (F), *Wnt4^{KO}* (G) and *Sox9^{cKO}Wnt4^{KO}* (H) genotypes. (I-M) RT-
 29 quantitative PCR analysis of *Foxl2* (I), *Axin2* (J), *Sox8* (K), *Dhh* (L) and *Amh* (M) expression in E14.5
 30 gonads of XX Control, XY Control, XX *Sox9^{cKO}*, XX *Wnt4^{KO}* and XX *Sox9^{cKO}Wnt4^{KO}* genotypes (n = 3 to 4
 31 embryos for each genotype). Expression level in XX controls is 1. Graphs show individual values
 32 (dots), and the mean fold-change (bars) ± SEM. Nuclei labeled with DAPI are shown in blue.
 33 Magnification is the same in all panels. Scale bar = 100 μm. Gonads are outlined with broken white
 34 lines.

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Figure S3. Quantitative PCR analysis of gene expression in XY control and mutant gonads at E14.5.

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RT-quantitative PCR analysis of *Foxl2* (A), *Axin2* (B), *Sox8* (C), *Dhh* (D) and *Amh* (E) expression in

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E14.5 gonads of XX Control, XY Control, XY Sox9^{KO}, XY Wnt4^{KO} and XY Sox9^{KO}Wnt4^{KO} genotypes (n =

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4 to 5 embryos for each genotype). Expression level in XX controls is 1. Graphs show individual values

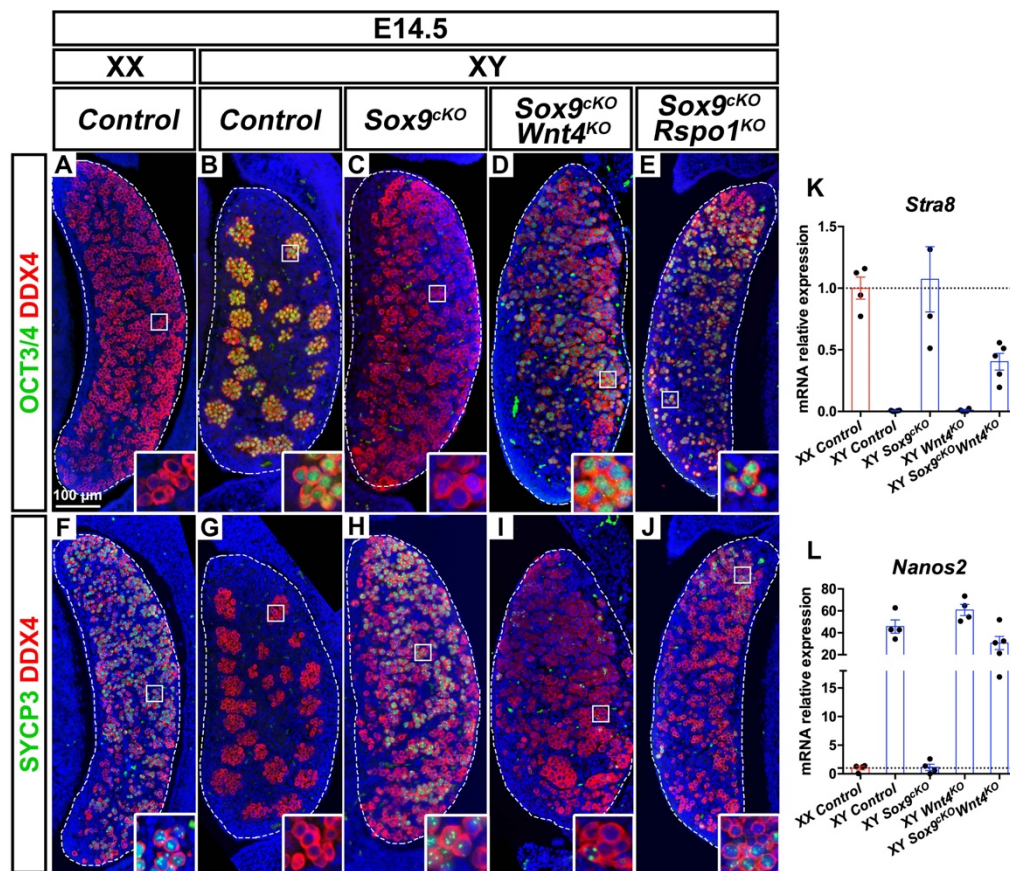
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of the relative normalized expression levels (dots), and the mean fold-change (bars) ± SEM.

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46 **Figure S4. Germ cell development in XY control and mutant gonads at E14.5. (A-E)**
 47 Immunodetection of germ cell marker DDX4 (red) and the pluripotent cell marker OCT3/4 (green) in
 48 E14.5 gonads of XX Control (A), XY Control (B), XY *Sox9^{ckO}* (C), XY *Sox9^{ckO}Wnt4^{ckO}* (D) and XY
 49 *Sox9^{ckO}Rspo1^{ckO}* (E) genotypes. (F-J) Immunodetection of germ cell marker DDX4 (red) and the meiosis
 50 marker SYCP3 (green) in E14.5 gonads of XX Control (F), XY Control (G), XY *Sox9^{ckO}* (H), XY
 51 *Sox9^{ckO}Wnt4^{ckO}* (I) and XY *Sox9^{ckO}Rspo1^{ckO}* (J) genotypes. The majority of germ cells in XY *Sox9^{ckO}Wnt4^{ckO}*
 52 and XY *Sox9^{ckO}Rspo1^{ckO}* express OCT3/4 like in XY controls. Only a few meiotic germ cells expressing
 53 SYCP3 can be observed at this stage in double mutant gonads. (K-L) RT-quantitative PCR analysis of
 54 the meiosis marker *Stra8* (K) and the male germ cell marker *Nanos2* (L) in E14.5 gonads of XX Control,
 55 XY Control, XY *Sox9^{ckO}*, XY *Wnt4^{ckO}* and XY *Sox9^{ckO}Wnt4^{ckO}* genotypes (n = 4 to 5 embryos for each
 56 genotype). Expression level in XX controls is 1. Graphs show individual values of the relative
 57 normalized expression levels (dots), and the mean fold-change (bars) ± SEM. Nuclei labeled with
 58 DAPI are shown in blue. Magnification is the same in all panels. Scale bar = 100 μm. Gonads are
 59 outlined with broken white lines.

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Table S1: Antibodies for immunodetection.

Antibody	Species	Supplier	Reference	Dilution
DDX4	Rabbit	Abcam	ab13840	200X
TRA98	Rat	Abcam	82527	200X
AMH	Mouse	Bio-Rad Laboratories	MCA2246	30X
AMH	Goat	Santa Cruz Biotechnology	sc6886	200X
OCT3/4	Mouse	BD Transduction Laboratories	611202	200X
FOXL2	Goat	Novus Biologicals	NB100-1277SS	200X
NR2F2	Mouse	R&D Systems	PP-H7147-00	200X
SOX9	Rabbit	Merck KGaA	HPA001758	250X
P27	Rabbit	Santa Cruz Biotechnology	sc-52	200X
DMRT1	Mouse	Santa Cruz Biotechnology	SC-377167	200X
HSD3B	Goat	Santa Cruz Biotechnology	sc-30820	200X
SYCP3	Mouse	Santa Cruz Biotechnology	Sc-74569	200X
SRY	Rabbit	Dagmar Wilhelm's Lab		200X
SDMG1	Rabbit	Ian Adams's Lab		600X

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Table S2: quantitative-PCR primers.

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Gene	Forward 5' - 3'	Reverse 5' - 3'
<i>Axin2</i>	GCAAGTCCAAGCCCCATA	CGGCTGACTCGTTCTCCT
<i>Foxl2</i>	ATCGGGGTTCCTCAACAAC	CATCTGGCAGGAGGCGTA
<i>Fst</i>	GCCTATGAGGGAAAGTGTATCAA	TGGAATCCCATAGGCATTTT
<i>Sox9</i>	GCGGAGCTCAGCAAGACTCTG	ATCGGGGTGGTCTTTCTTGTC
<i>Sox8</i>	GACCCTAGGCAAGCTGTGG	CTGCACACGGAGCCTCTC
<i>Egf9</i>	TGCAGGACTGGATTTCAATTTAG	CCAGGCCCACTGCTATACTG
<i>Dhh</i>	GGACCTCGTACCCAATACTACAA	CGATGGCTAGAGCGTTCACC
<i>Amh</i>	GGGGAGACTGGAGAACAGC	AGAGCTCGGGCTCCCATA
<i>Nanos2</i>	AGGTCCCCGATCTCAAC	CAGCATTTCCAGTGTTTCAG
<i>Stra8</i>	TGACGTGGCAAGTTTCCTG	GTTGCAGGTGGCAAACATAG