

The conditional deletion of steroidogenic factor 1 (*Nr5a1*) in *Sox9-Cre* mice compromises testis differentiation

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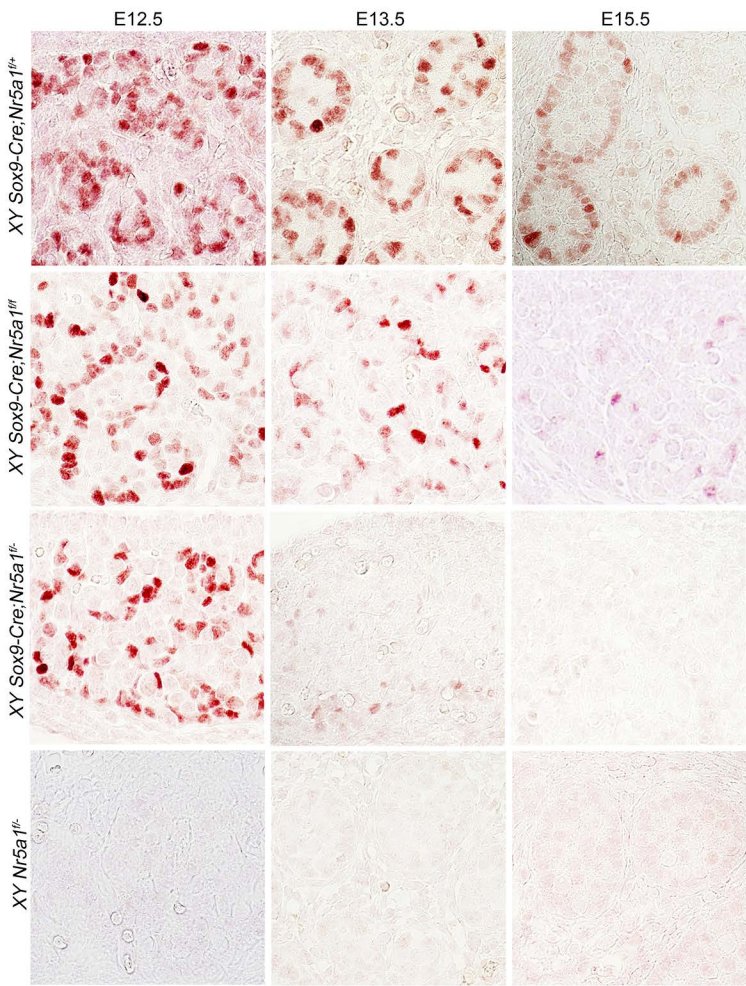
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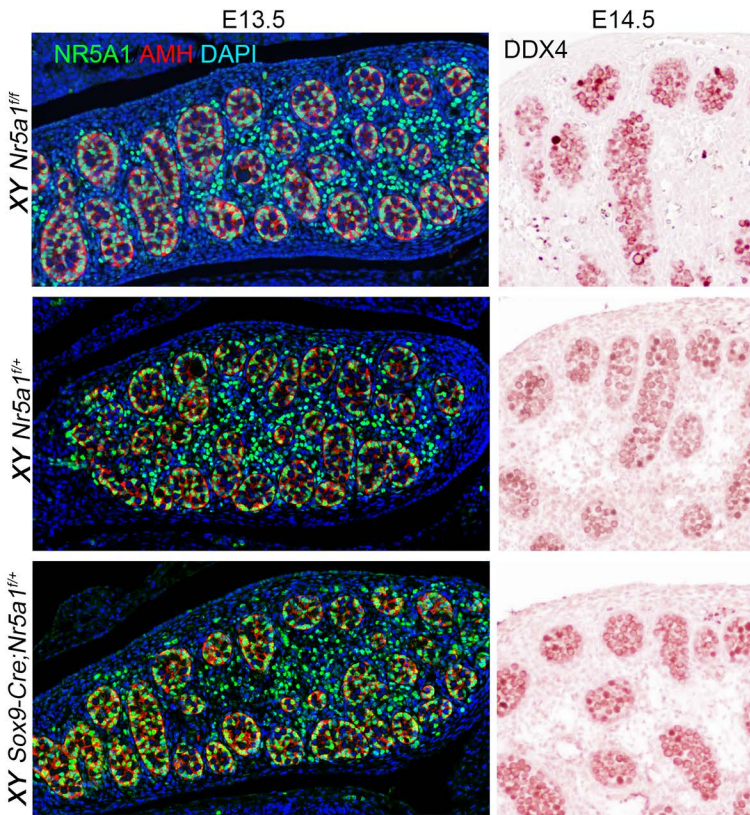
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Supplementary Figure 1



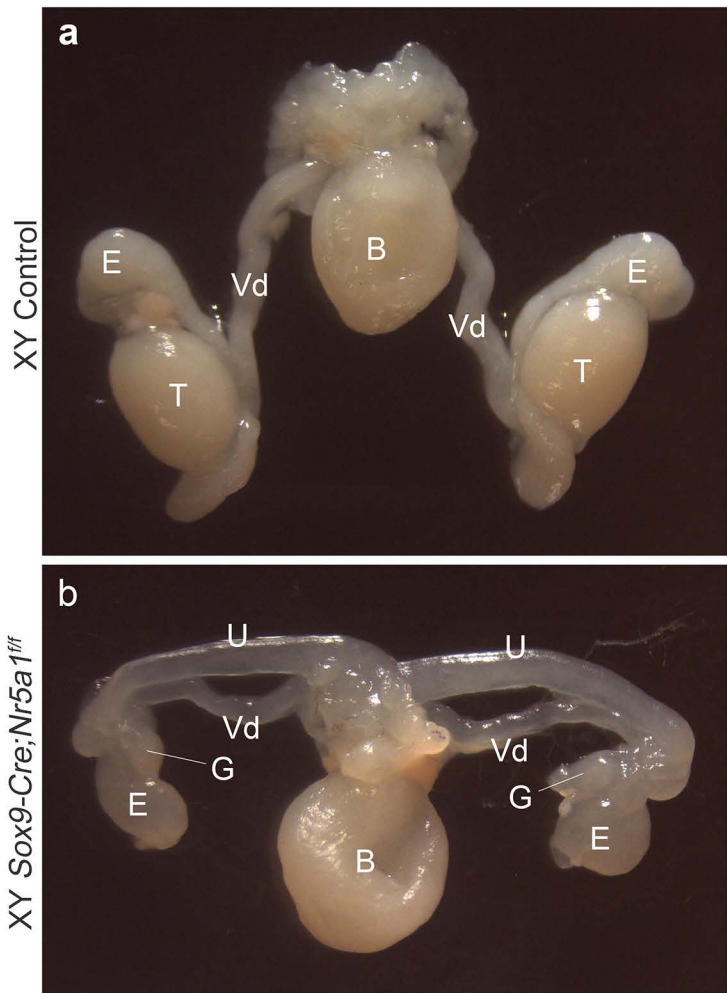
Supplementary Figure 1. Cre expression in XY *Sox9-Cre* gonads. IHC for Cre in gonads of XY *Sox9-Cre*;Nr5a1^{fl/+}, XY *Sox9-Cre*;Nr5a1^{fl/fl}, XY *Sox9-Cre*;Nr5a1^{fl/+}, and XY *Nr5a1*^{fl/-} mice at E12.5, E13.5, and E15.5 (left, middle, and right panels, respectively). Scale bar, 50 μ m.

Supplementary Figure 2



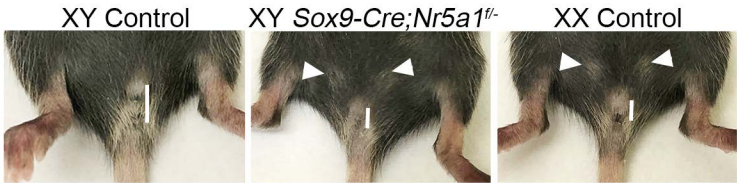
Supplementary Figure 2. IHC in three genotypes of XY control testes. Double IHC for NR5A1 and AMH at E13.5 and IHC for DDX4 at E14.5 in the testis of XY *Nr5a1*^{fl/fl}, XY *Nr5a1*^{fl/+}, and XY *Sox9-Cre;Nr5a1*^{fl/+} mice. Scale bar, 100 μ m.

Supplementary Figure 3



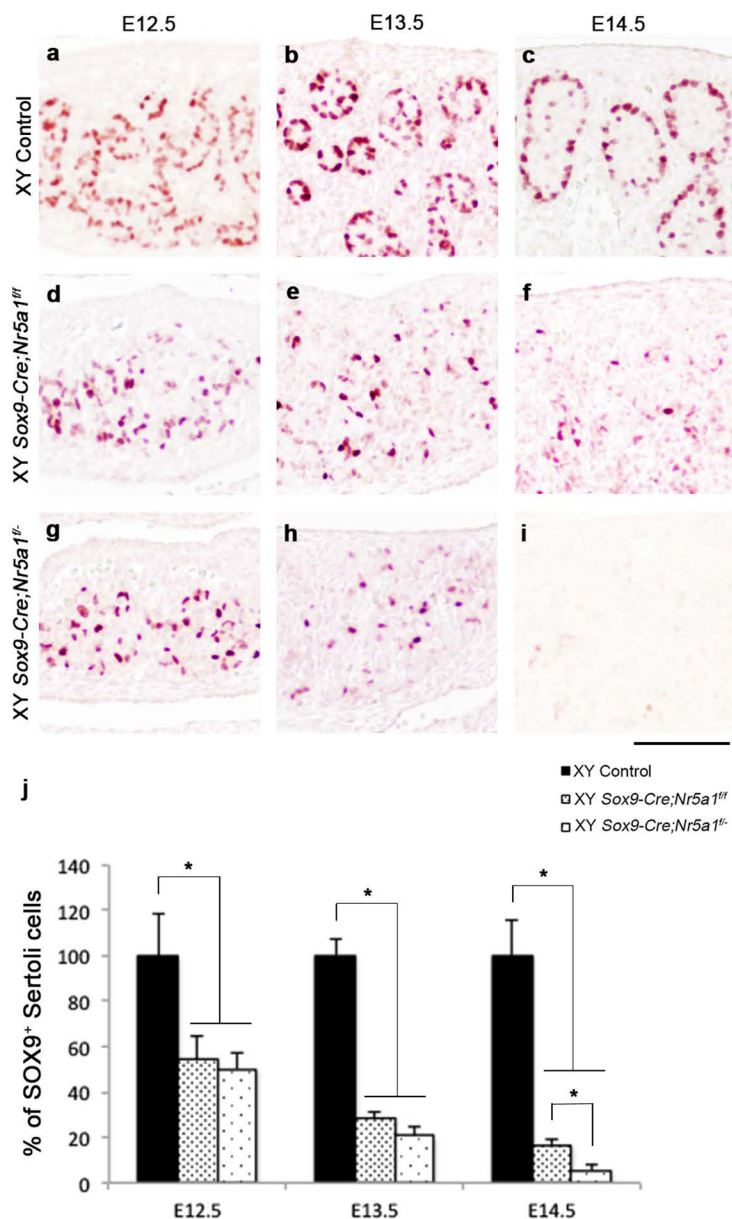
Supplementary Figure 3. Internal reproductive organs of XY *Sox9-Cre;Nr5a1^{ff}* mice. Stereomicroscopy images of internal reproductive organs in XY control (upper panel) and XY *Sox9-Cre;Nr5a1^{ff}* (lower panel) mice at two week-old. B, bladder; E, epididymis; G, gonad; T, testis; U, uterus; Vd, vas deferens. Scale bar, 1 mm.

Supplementary Figure 4



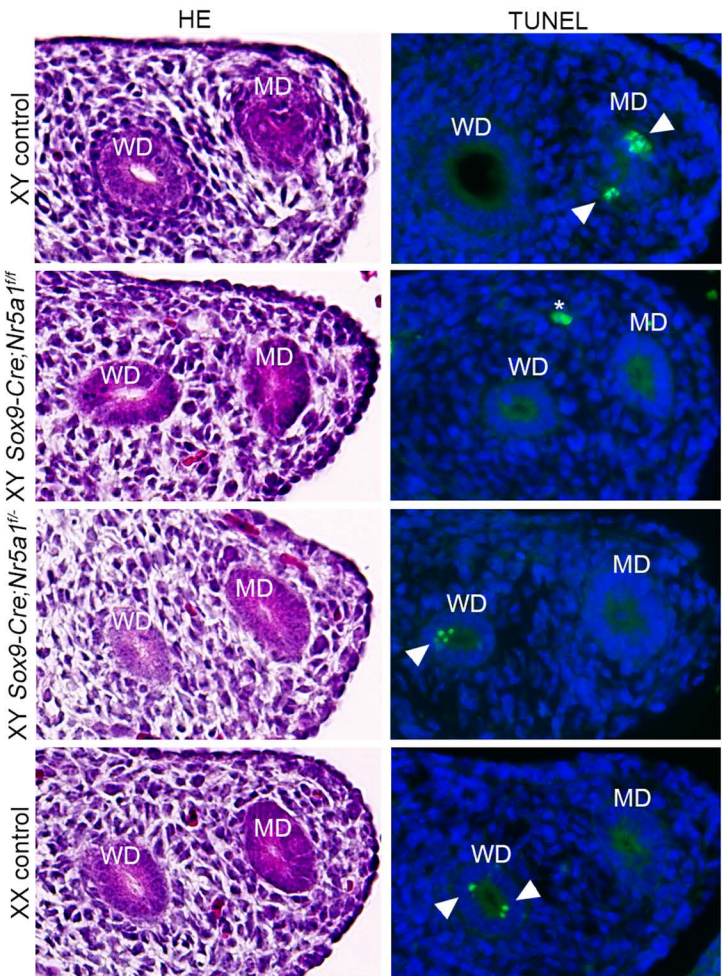
Supplementary Figure 4. External genitalia of XY *Sox9-Cre;Nr5a1^{f/-}* mice. Images of external genitalia in XY control, XY *Sox9-Cre;Nr5a1^{f/-}*, and XX control mice (left, middle, and right panels, respectively) at two week-old. White bars and white arrowheads indicate anogenital distances and mammary teats, respectively. Scale bar, 1 cm.

Supplementary Figure 5



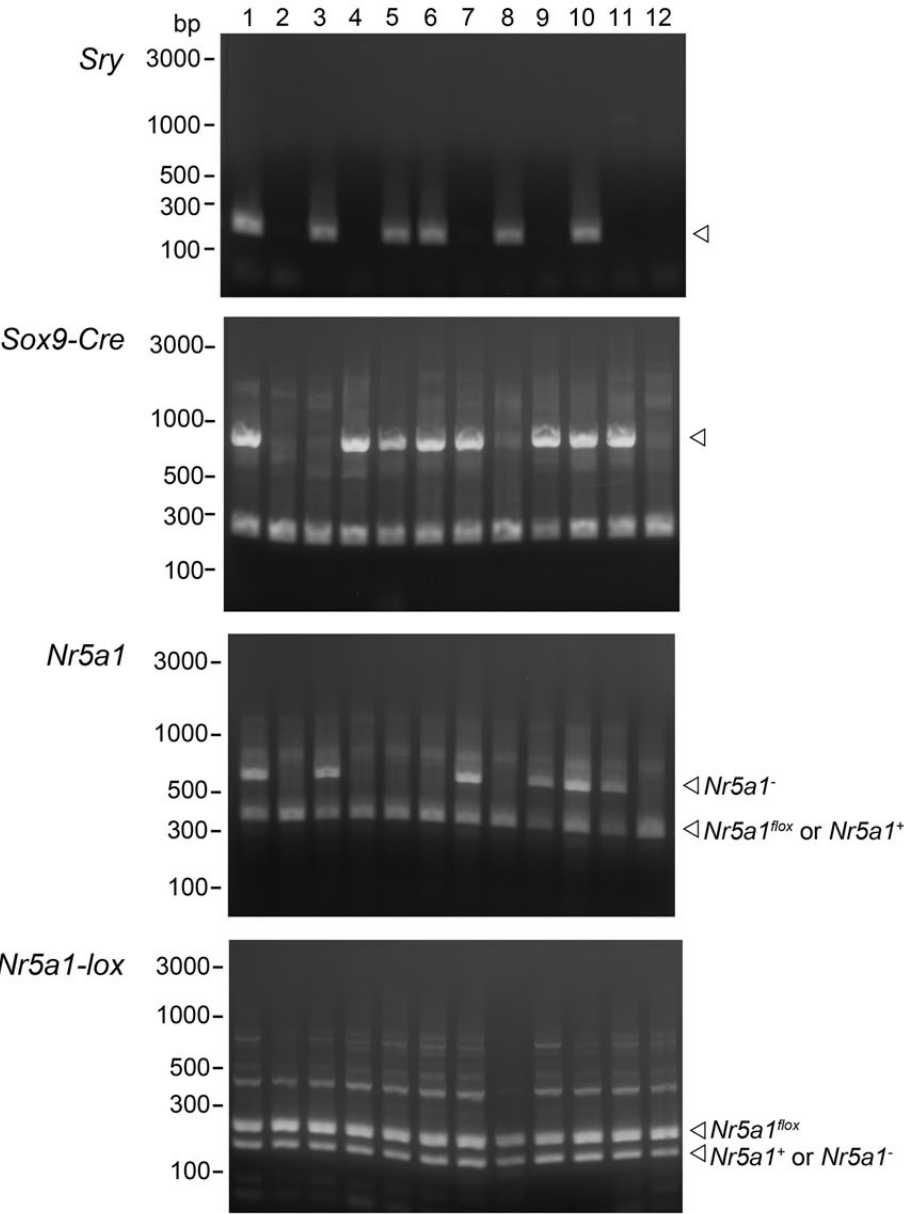
Supplementary Figure 5. SOX9 expression in XY *Sox9-Cre-Nr5a1* cKO gonads. IHC for SOX9 in gonads of XY control (a-c), XY *Sox9-Cre;Nr5a1^{ff}* (d-f), and XY *Sox9-Cre;Nr5a1^{fl-}* (g-i) mice at E12.5, E13.5, and E14.5 (left, middle, and right panels, respectively). Scale bar, 100 μ m. (j) Ratios (%) of SOX9⁺ cells. (Daily values for XY control were set at 100%). Data are shown as means $\pm$ SEM (n = 3). *P < 0.05 vs. XY control.

Supplementary Figure 6



Supplementary Figure 6. Lack of Müllerian duct regression in XY *Sox9-Cre-Nr5a1* cKO mice. Haematoxylin and eosin (HE) staining and TUNEL assay in the mesonephroi in XY control, XY *Sox9-Cre;Nr5a1^{ff}*, XY *Sox9-Cre;Nr5a1^{fl/-}*, and XX control mice at E14.5. In TUNEL assay, nuclei are counterstained blue with DAPI. White arrowheads indicate TUNEL-positive apoptotic cells. White asterisk indicates nonspecific autofluorescence. MD, Müllerian duct; WD, Wolffian duct. Scale bar, 50 μ m.

Supplementary Figure 7



No	Sry	Sox9	Nr5a1	Genotype
1	+	Cre/+	f/-	cKO ♂
2	-	+/+	f/+	control ♀
3	+	+/+	f/-	hetero ♂
4	-	Cre/+	f/+	control ♀
5	+	Cre/+	f/+	control ♂
6	+	Cre/+	f/+	control ♂
7	-	Cre/+	f/-	cKO ♀
8	+	+/+	f/+	control ♂
9	-	Cre/+	f/-	cKO ♀
10	+	Cre/+	f/-	cKO ♂
11	-	Cre/+	f/-	cKO ♀
12	-	+/+	f/+	control ♀

Supplementary Figure 7. PCR genotyping and sexing of *Sox9-Cre;Nr5a1^{f/-}* mice. A result of genotyping and sexing of 12 mice is shown.

Supplementary Table 1. Primers for genotyping

Gene	Primer name	Primer sequence
<i>Sry</i>	Sry-Forward	AAGCGCCCCATGAATGCATT
	Sry-Reverse	CGATGAGGCTGATATTTATA
<i>Sox9-Cre</i>	Sox9-Forward	CCAGATGGACCCACCAGTATCAG
	Sox9-Reverse	GGGACACTCTTGAACTAGGAGTAG
	CW-Cre2	TCCGGTTATTCAACTTGCACCATGC
<i>Nr5a1</i>	Nr5a1-Forward	ACAAGCATTACACGTGCACC
	Nr5a1-Reverse	TGACTAGCAACCACCTTGCC
	Neo Nr5a1	AGGTGAGATGACAGGAGATC
<i>Nr5a1-lox</i>	Nr5a1-lox-Forward	AGACAAGTGCACCCCATTTC
	Nr5a1-lox-Reverse	ACCATCACCAACCGCTAAAC