Current Biology

Sexual Cell-Fate Reprogramming in the Ovary by DMRT1

Cell PRESS

刘慧芬

2016.8.13

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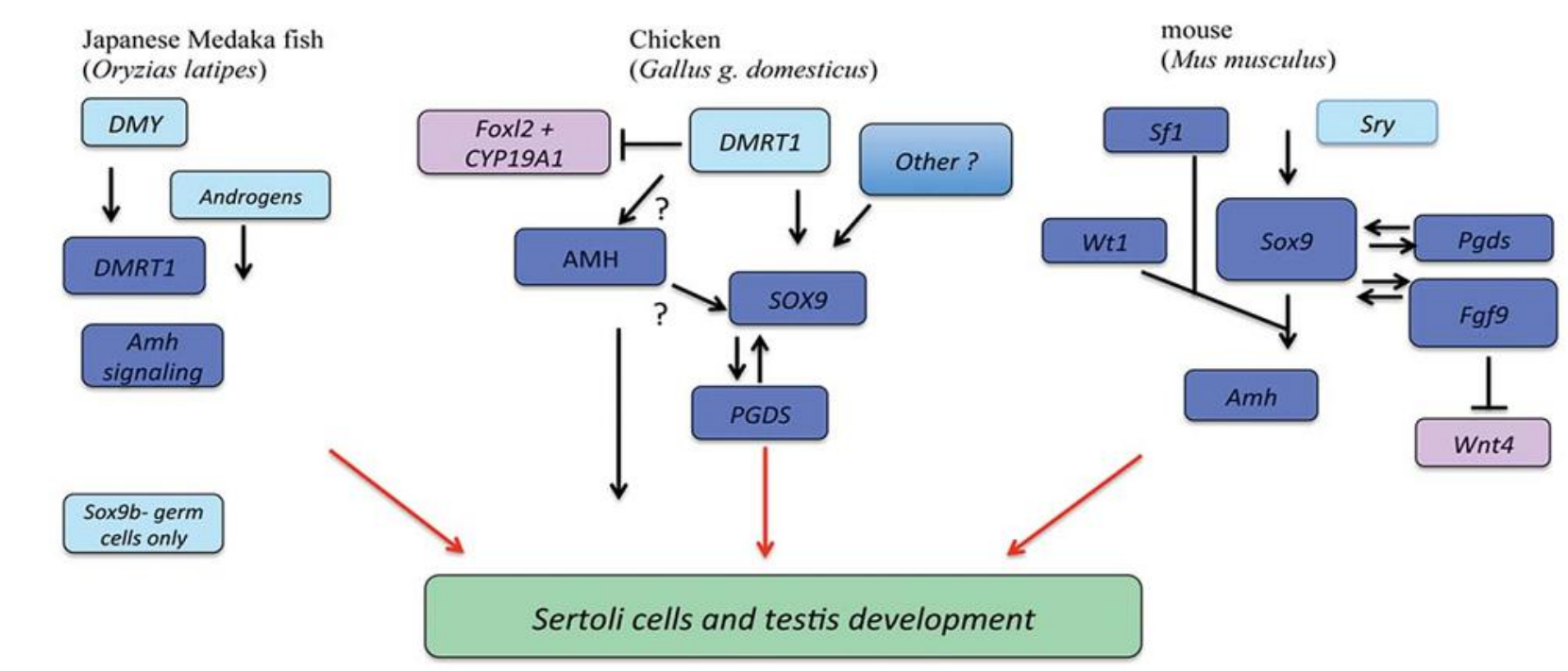
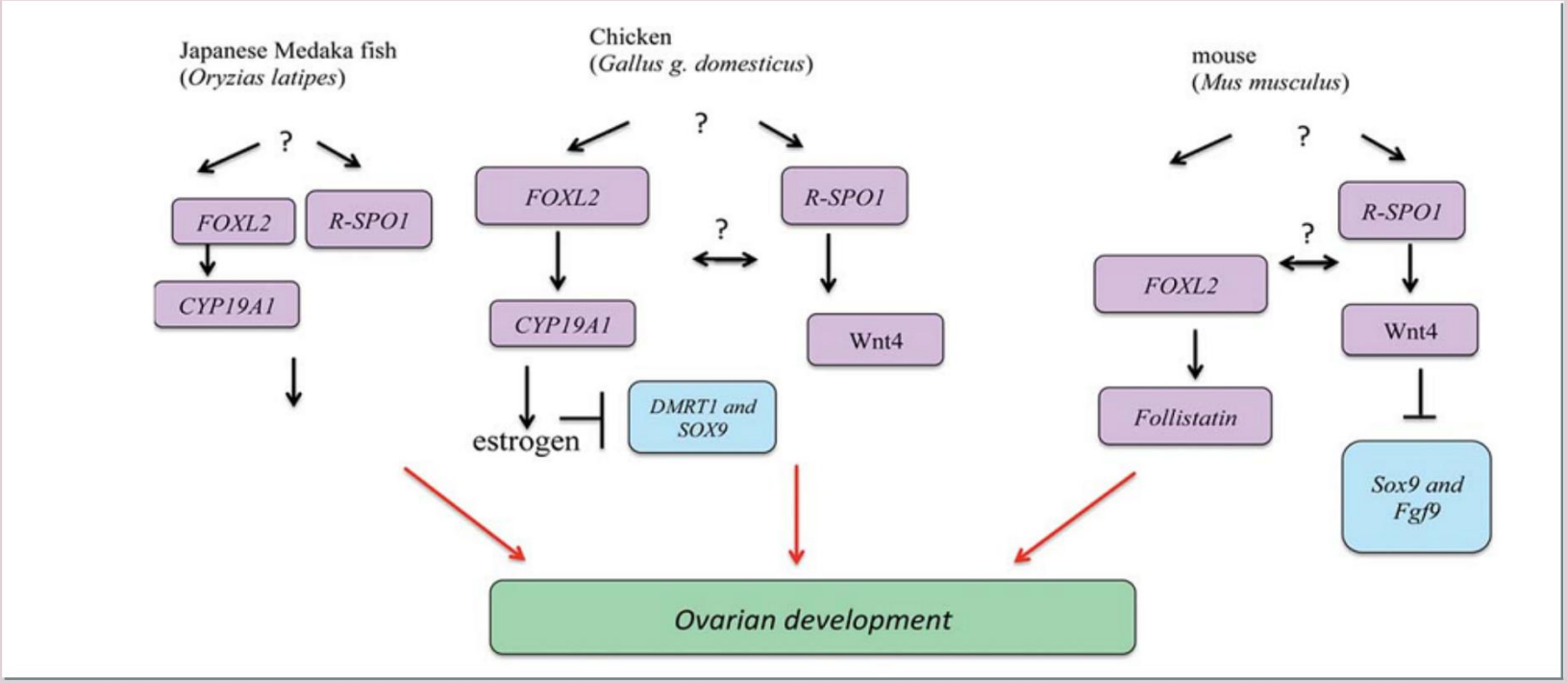


(Cutting et al. 2013)

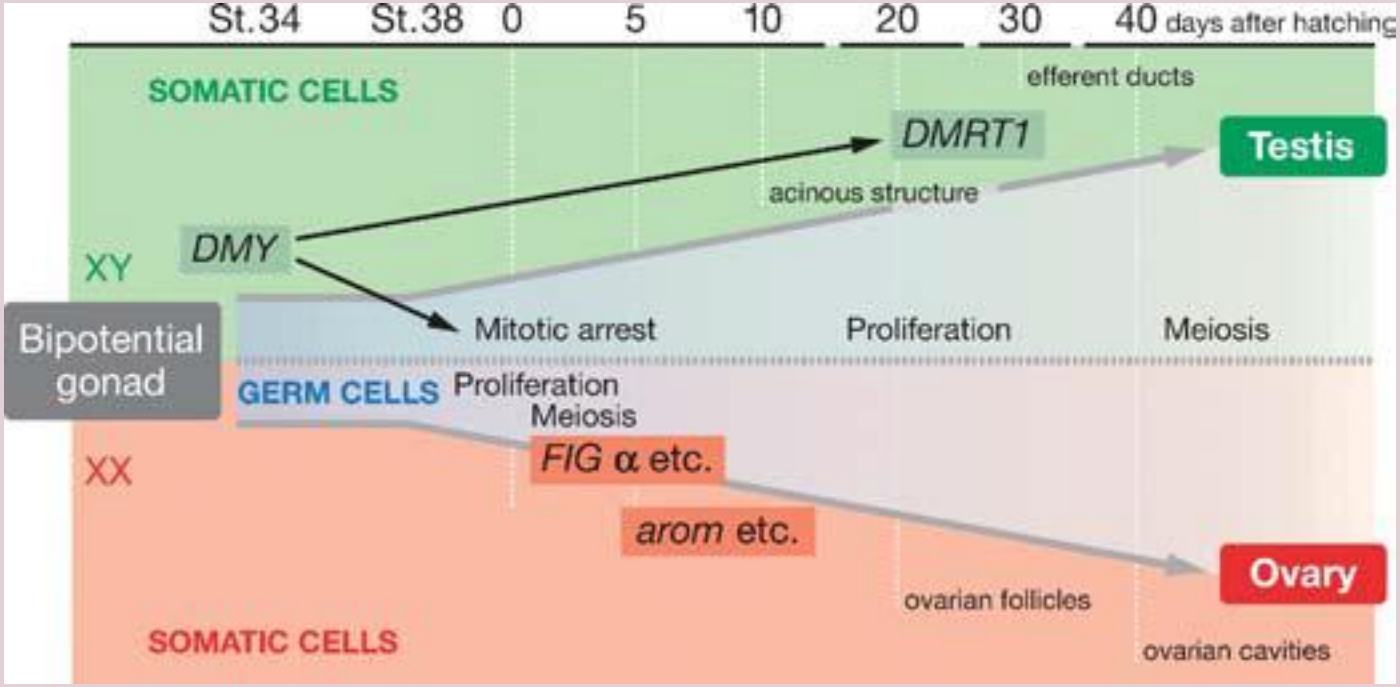
Introduction



(Cutting et al. 2013)

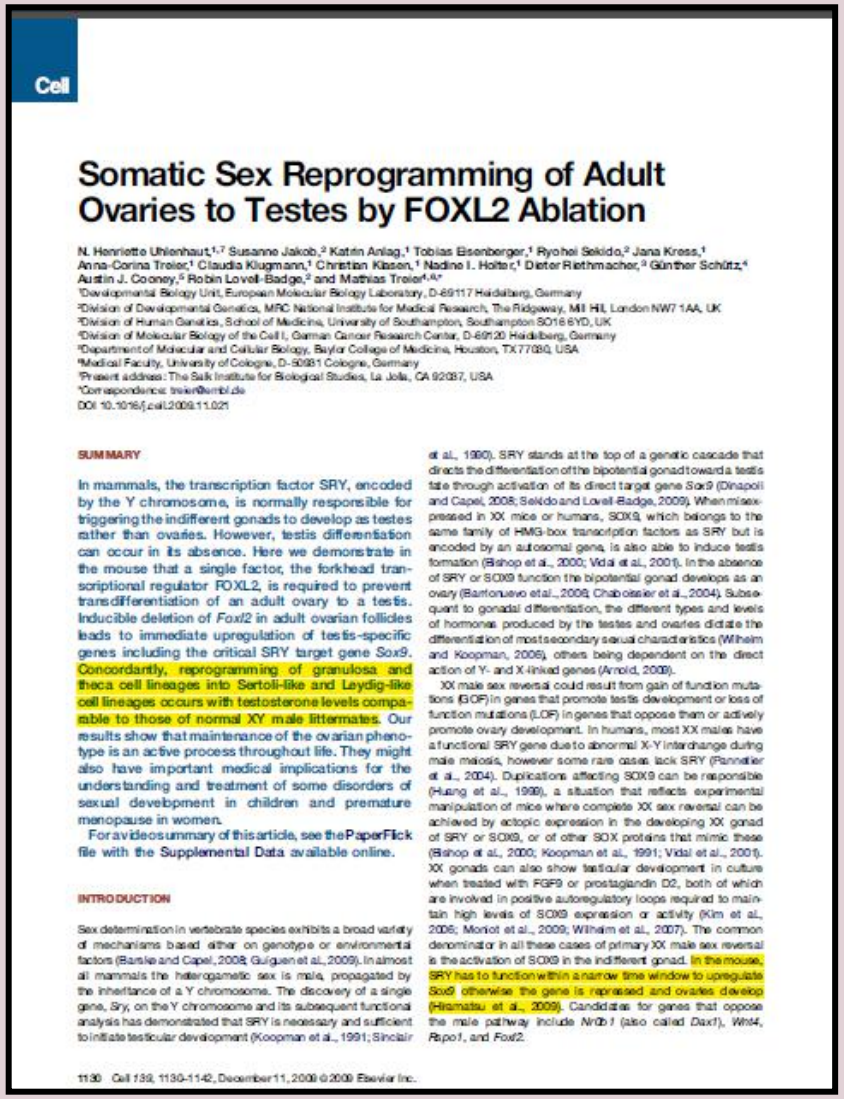
Introduction

Sex Determination in the Teleost Medaka, *Oryzias latipes*



(Matsuda, M. et al. 2013)

Introduction



Cell

Somatic Sex Reprogramming of Adult Ovaries to Testes by FOXL2 Ablation

N. Henriette Uhlenhaut,[1,7] Susanne Jakob,[2] Katrin Anlag,[1] Tobias Eisenberger,[1] Ryohei Sekido,[2] Jana Kress,[1] Anna-Corina Treier,[1] Claudia Klugmann,[1] Christian Klasen,[1] Nadine I. Holter,[1] Dieter Riethmacher,[3] Günther Schütz,[4] Austin J. Cooney,[5] Robin Lovell-Badge,[2] and Mathias Treier[1,6,*]
[1]Developmental Biology Unit, European Molecular Biology Laboratory, D-69117 Heidelberg, Germany
[2]Division of Developmental Genetics, MRC National Institute for Medical Research, The Ridgeway, Mill Hill, London NW7 1AA, UK
[3]Division of Human Genetics, School of Medicine, University of Southampton, Southampton SO16 6YD, UK
[4]Division of Molecular Biology of the Cell I, German Cancer Research Center, D-69120 Heidelberg, Germany
[5]Department of Molecular and Cellular Biology, Baylor College of Medicine, Houston, TX 77030, USA
[6]Medical Faculty, University of Cologne, D-50931 Cologne, Germany
[7]Present address: The Salk Institute for Biological Studies, La Jolla, CA 92037, USA
[*]Correspondence: treier@embl.de
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SUMMARY

In mammals, the transcription factor SRY, encoded by the Y chromosome, is normally responsible for triggering the indifferent gonads to develop as testes rather than ovaries. However, testis differentiation can occur in its absence. Here we demonstrate in the mouse that a single factor, the forkhead transcriptional regulator FOXL2, is required to prevent transdifferentiation of an adult ovary to a testis. Inducible deletion of *Foxl2* in adult ovarian follicles leads to immediate upregulation of testis-specific genes including the critical SRY target gene *Sox9*. Concordantly, reprogramming of granulosa and theca cell lineages into Sertoli-like and Leydig-like cell lineages occurs with testosterone levels comparable to those of normal XY male littermates. Our results show that maintenance of the ovarian phenotype is an active process throughout life. They might also have important medical implications for the understanding and treatment of some disorders of sexual development in children and premature menopause in women.

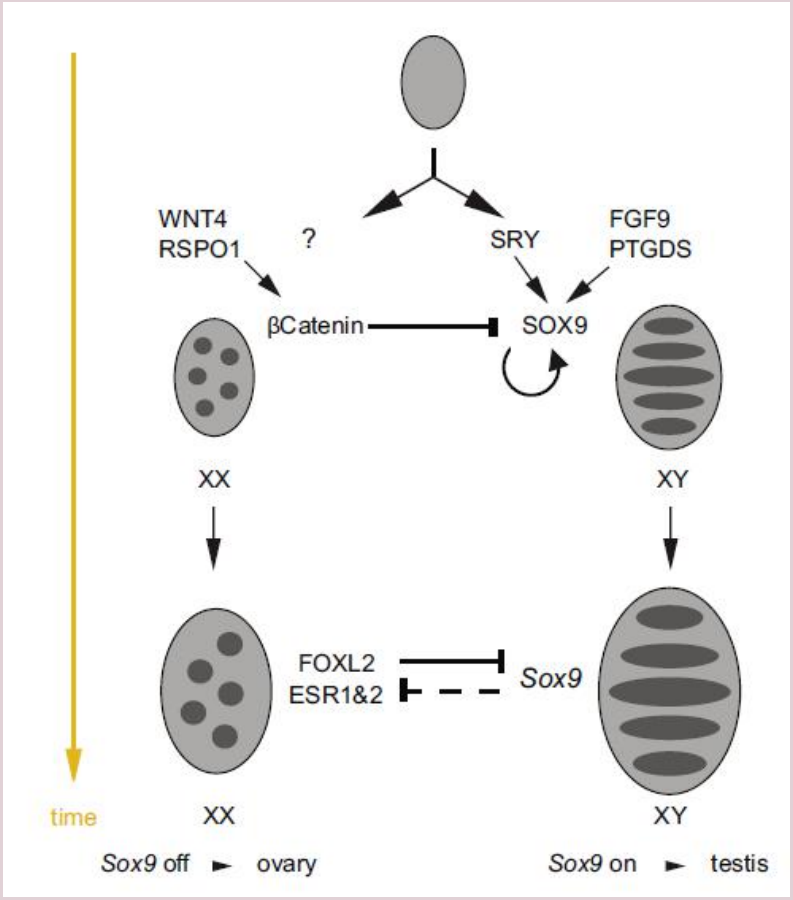
For a video summary of this article, see the PaperFlick file with the Supplemental Data available online.

INTRODUCTION

Sex determination in vertebrate species exhibits a broad variety of mechanisms based either on genotype or environmental factors (Barske and Capel, 2008; Guiguen et al., 2009). In almost all mammals the heterogametic sex is male, propagated by the inheritance of a Y chromosome. The discovery of a single gene, *Sry*, on the Y chromosome and its subsequent functional analysis has demonstrated that SRY is necessary and sufficient to initiate testicular development (Koopman et al., 1991; Sinclair et al., 1990). SRY stands at the top of a genetic cascade that directs the differentiation of the bipotential gonad toward a testis fate through activation of its direct target gene *Sox9* (Dinapoli and Capel, 2008; Sekido and Lovell-Badge, 2009). When misexpressed in XX mice or humans, SOX9, which belongs to the same family of HMG-box transcription factors as SRY but is encoded by an autosomal gene, is also able to induce testis formation (Bishop et al., 2000; Vidal et al., 2001). In the absence of SRY or SOX9 function the bipotential gonad develops as an ovary (Bartonuevo et al., 2006; Chaboissier et al., 2004). Subsequent to gonadal differentiation, the different types and levels of hormones produced by the testes and ovaries dictate the differentiation of most secondary sexual characteristics (Wilhelm and Koopman, 2006), others being dependent on the direct action of Y- and X-linked genes (Arnold, 2009).

XX male sex reversal could result from gain of function mutations (GOF) in genes that promote testis development or loss of function mutations (LOF) in genes that oppose them or actively promote ovary development. In humans, most XX males have a functional SRY gene due to abnormal X-Y interchange during male meiosis, however some rare cases lack SRY (Pannetier et al., 2004). Duplications affecting SOX9 can be responsible (Huang et al., 1999), a situation that reflects experimental manipulation of mice where complete XX sex reversal can be achieved by ectopic expression in the developing XX gonad of SRY or SOX9, or of other SOX proteins that mimic these (Bishop et al., 2000; Koopman et al., 1991; Vidal et al., 2001). XX gonads can also show testicular development in culture when treated with FGF9 or prostaglandin D2, both of which are involved in positive autoregulatory loops required to maintain high levels of SOX9 expression or activity (Kim et al., 2006; Moniot et al., 2009; Wilhelm et al., 2007). The common denominator in all these cases of primary XX male sex reversal is the activation of SOX9 in the indifferent gonad. In the mouse, SRY has to function within a narrow time window to upregulate *Sox9* otherwise the gene is repressed and ovaries develop (Hiramatsu et al., 2009). Candidates for genes that oppose the male pathway include *Nr0b1* (also called *Dax1*), *Wnt4*, *Rspo1*, and *Foxl2*.

Model of Sox9 Regulation Required for Maintenance of Gonadal Phenotype in Mammals



(Uhlenhaut. et al. 2009)

Introduction

LETTER

doi:10.1038/nature10239

DMRT1 prevents female reprogramming in the postnatal mammalian testis

Clinton K. Matson[1,2], Mark W. Murphy[1], Aaron L. Sarver[3], Michael D. Griswold[4], Vivian J. Bardwell[1,2,3] & David Zarkower[1,2,3]

Sex in mammals is determined in the fetal gonad by the presence or absence of the Y chromosome gene *Sry*, which controls whether bipotential precursor cells differentiate into testicular Sertoli cells or ovarian granulosa cells[1]. This pivotal decision in a single gonadal cell type ultimately controls sexual differentiation throughout the body. Sex determination can be viewed as a battle for primacy in the fetal gonad between a male regulatory gene network in which *Sry* activates *Sox9* and a female network involving WNT/β-catenin signalling[2]. In females the primary sex-determining decision is not final: loss of the FOXL2 transcription factor in adult granulosa cells can reprogram granulosa cells into Sertoli cells[3]. Here we show that sexual fate is also surprisingly labile in the testis: loss of the DMRT1 transcription factor[4] in mouse Sertoli cells, even in adults, activates *Foxl2* and reprograms Sertoli cells into granulosa cells. In this environment, the cells form, oestrogen is produced and germ cells appear feminized. Thus *Dmrt1* is essential to maintain mammalian testis determination, and competing regulatory networks maintain gonadal sex long after the fetal choice between male and female. *Dmrt1* and *Foxl2* are conserved throughout vertebrates[4,5] and *Dmrt1*-related sexual regulators are conserved throughout metazoans[4]. Antagonism between *Dmrt1* and *Foxl2* for control of gonadal sex may therefore extend beyond mammals. Reprogramming due to loss of *Dmrt1* also may help explain the aetiology of human syndromes linked to *DMRT1*, including disorders of sexual differentiation[6] and testicular cancer[7].

Human chromosome 9p deletions removing *DMRT1* are associated with XY male-to-female sex reversal, and *Dmrt1* homologues determine sex in several non-mammalian vertebrates[8–10]. In mice, *Dmrt1* is expressed and required in both germ cells and Sertoli cells of the testis[11–13]. XY *Dmrt1*-null mutant mice are born as males with testes, although these gonads later undergo abnormal differentiation[14]; hence the role of *Dmrt1* in mammalian sex determination has been unclear (for overview of mammalian sex determination see Supplementary Fig. 1). Here we examine *Dmrt1* mutant testes during postnatal development, asking whether loss of *Dmrt1* causes postnatal feminization in mice.

We first examined gonads of *Dmrt1*-null mutant males ($Dmrt1^{-/-}$) for the presence of FOXL2, a female-specific transcription factor expressed in granulosa cells and theca cells[15,16], the two somatic cell types of the ovarian follicle (Fig. 1a). Four weeks after birth, abundant FOXL2-positive cells were present within mutant seminiferous tubules (Fig. 1b), which in control testes contain only germ cells and Sertoli cells (Fig. 1c). To establish the origin of the FOXL2-positive cells, we deleted *Dmrt1* either in germ cells (using *Nanos3-cre*) or in Sertoli cells (using *Dhh-cre* or *Sf1-cre*) (Supplementary Fig. 2a–l and Supplementary Table 1). Loss of *Dmrt1* in fetal Sertoli cells (*SCDmrt1KO*) but not in fetal germ cells (*GCDmrt1KO*) induced FOXL2 expression (Fig. 1d–f). *SCDmrt1KO* gonads retained small numbers of germ cells, which appeared to arrest in meiotic prophase on the basis of SYCP3 localization (Supplementary Fig. 3). These results demonstrate that DMRT1 expression in Sertoli cells prevents FOXL2 expression and suggest that *Dmrt1* mutant testes become feminized during the first postnatal month.

Next we examined the timing of FOXL2 induction. At postnatal day (P)7, *SCDmrt1KO* testes had seminiferous tubules in which all Sertoli cells expressed SOX9 normally (Supplementary Fig. 2m–r), but at P14

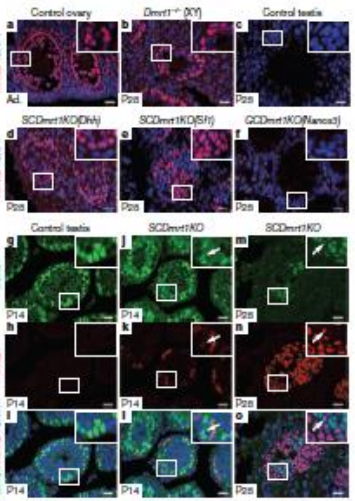
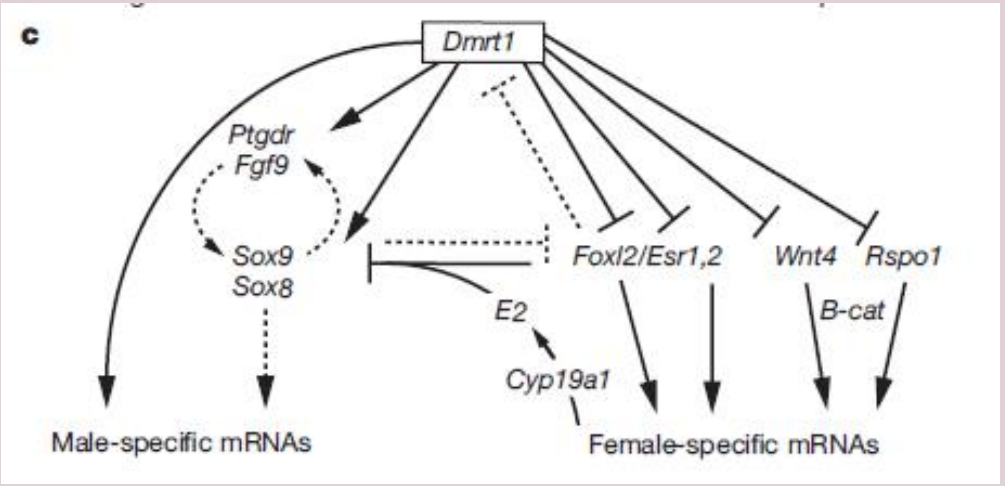


Figure 1 | DMRT1 maintains SOX9 and suppresses FOXL2 expression in postnatal Sertoli cells. a–c, FOXL2 expression detected by immunofluorescence in adult (Ad) granulosa and theca cells of control ovary (a) and intratubular cells of *Dmrt1*-null testicat P28 (b), but not in control testis (c). DAPI, 4′,6-diamidino-2-phenylindole. d–f, FOXL2 is robustly expressed when *Dmrt1* is mutated in fetal Sertoli cells with *Dhh-cre* (d) or *Sf1-cre* (e) but not when *Dmrt1* is mutated in fetal germ cells with *Nanos3-cre* (f). g–o, Timing of FOXL2 expression. FOXL2 is absent from control testis at P14 (g–i). Cells expressing FOXL2 or FOXL2 and SOX9 (arrowheads) are present in *SCDmrt1KO* testis at P14 (j–l). FOXL2-positive cells are abundant in *SCDmrt1KO* testis at P28 and most cells no longer express SOX9 (m–o). Scale bars, 20 μm.

[1]Developmental Biology Center and Department of Genetics, Cell Biology and Development, University of Minnesota, Minneapolis 55455, Minnesota, USA. [2]Molecular, Cellular, Developmental Biology and Genetics Graduate Program, University of Minnesota, Minneapolis 55455, Minnesota, USA. [3]University of Minnesota Masonic Cancer Center, Minneapolis, Minnesota 55455, USA. [4]School of Molecular Biosciences, Washington State University, Pullman, Washington 99164, USA.

Model for regulation by postnatal sex maintenance by DMRT1.



(Matson. et al. 2011)

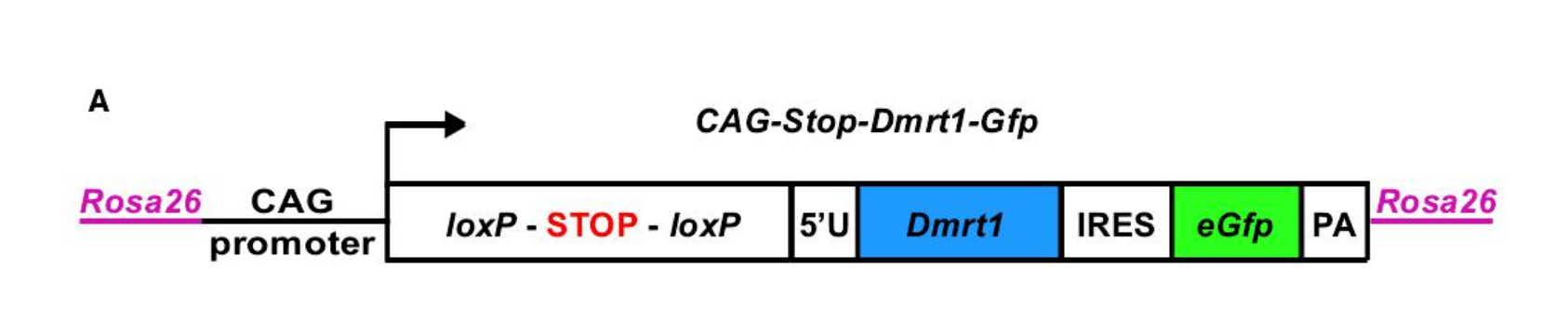
Introduction

sexually antagonistic functions???

FOXL2  **DMRT1**

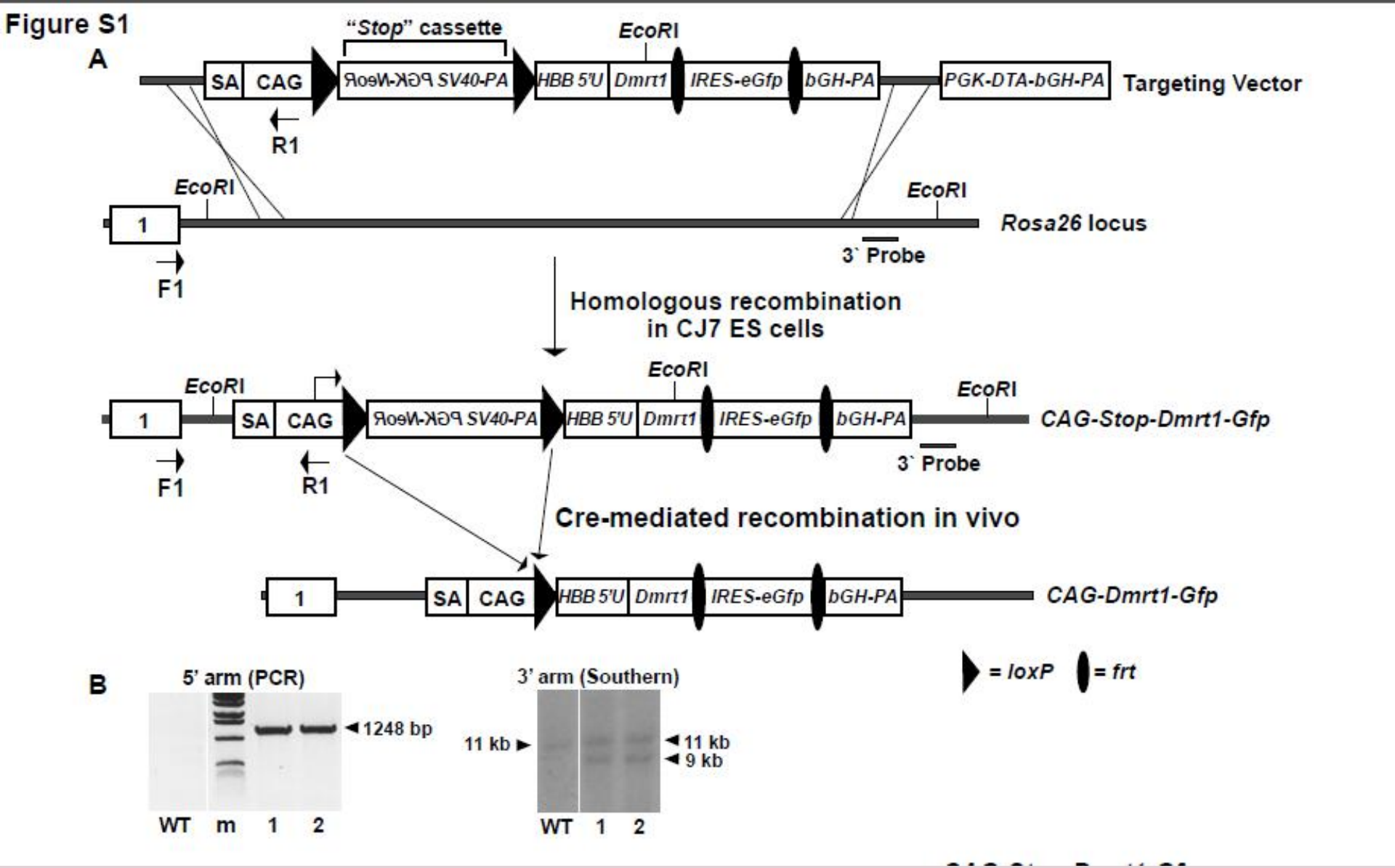
Experimental Procedures

Ectopic DMRT1 Induces Formation of Sertoli-like Cells in the Ovary

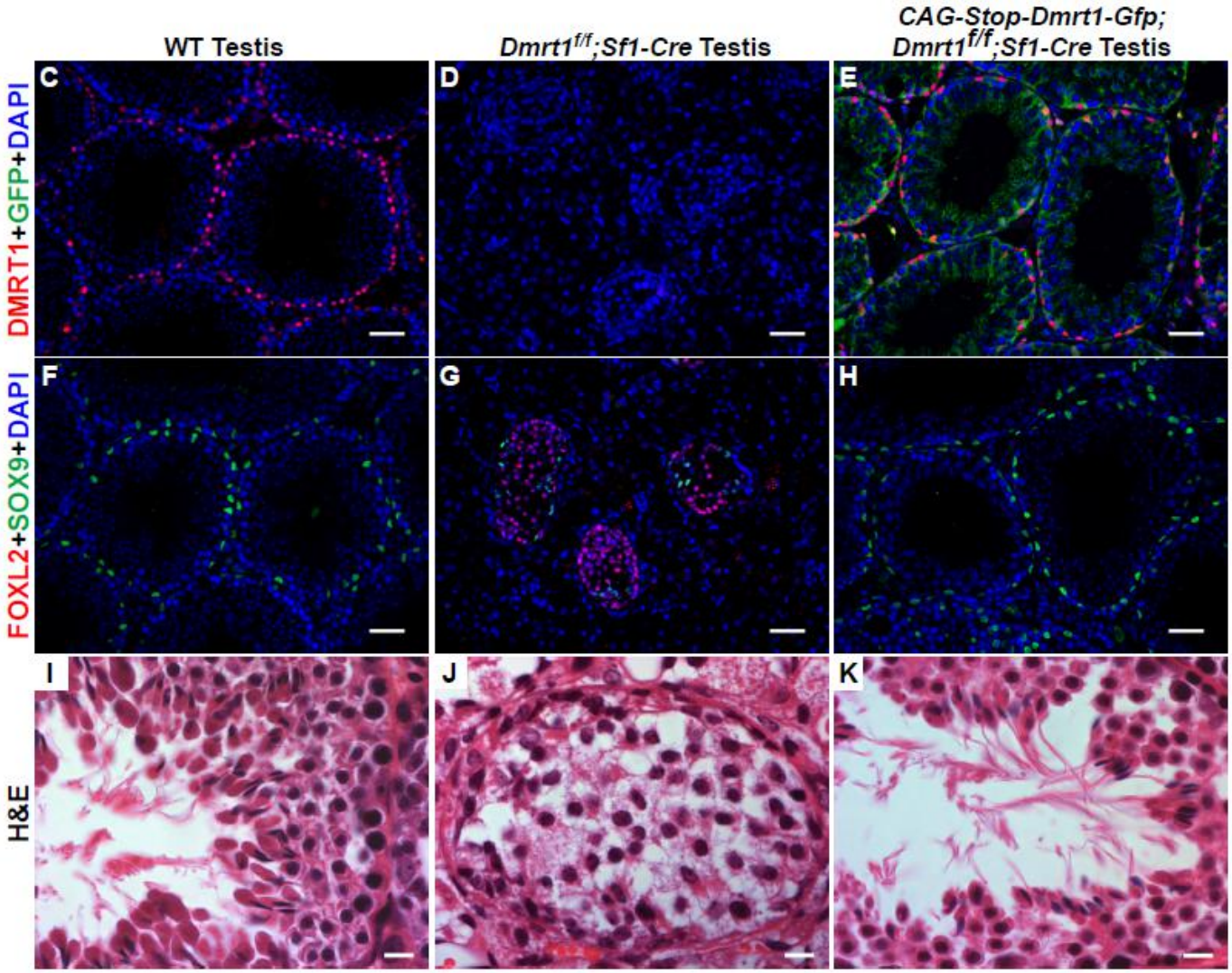


Schematic diagram of conditional DMRT1 expression transgene CAG-Stop-Dmrt1-Gfp, which is transcribed to express DMRT1 and GFP upon Cre mediated deletion of a floxed "STOP" cassette.

Cre/loxP 系统与基因打靶技术相结合的条件性基因打靶,可允许靶基因的缺失/突变仅发生在小鼠发育的某一阶段或和特定的组织器官,成为研究基因功能及动物模型的重要工具。Cre/LoxP系统属于传统的同源重组载体, 但是具有了时空调控的功能。它由Cre重组酶和LoxP位点两部分组成。Cre是1个重组酶蛋白,它可以介导LoxP的34 bp重复序列的位点特异性重组。通过诱导表达Cre重组酶将Loxp位点间的基因切除, 实现特定基因在特定时间或组织中的失活。另外, Cre/LoxP系统还可以用于染色体间基因重排。为了条件性表达DMRT1, 作者构建了CAG-STOP-DMRT1-GFP, 通过在特异性位点同源重组导入含有LoxP序列的5′ DMRT1框, 然后随机整合含有LoxP序列的3′ GFP框,在Cre酶的作用下, 两个LoxP位点之间发生重组,形成具有功能的DMRT1微小基因,使重组后的ES细胞在选择培养基中存活下来。



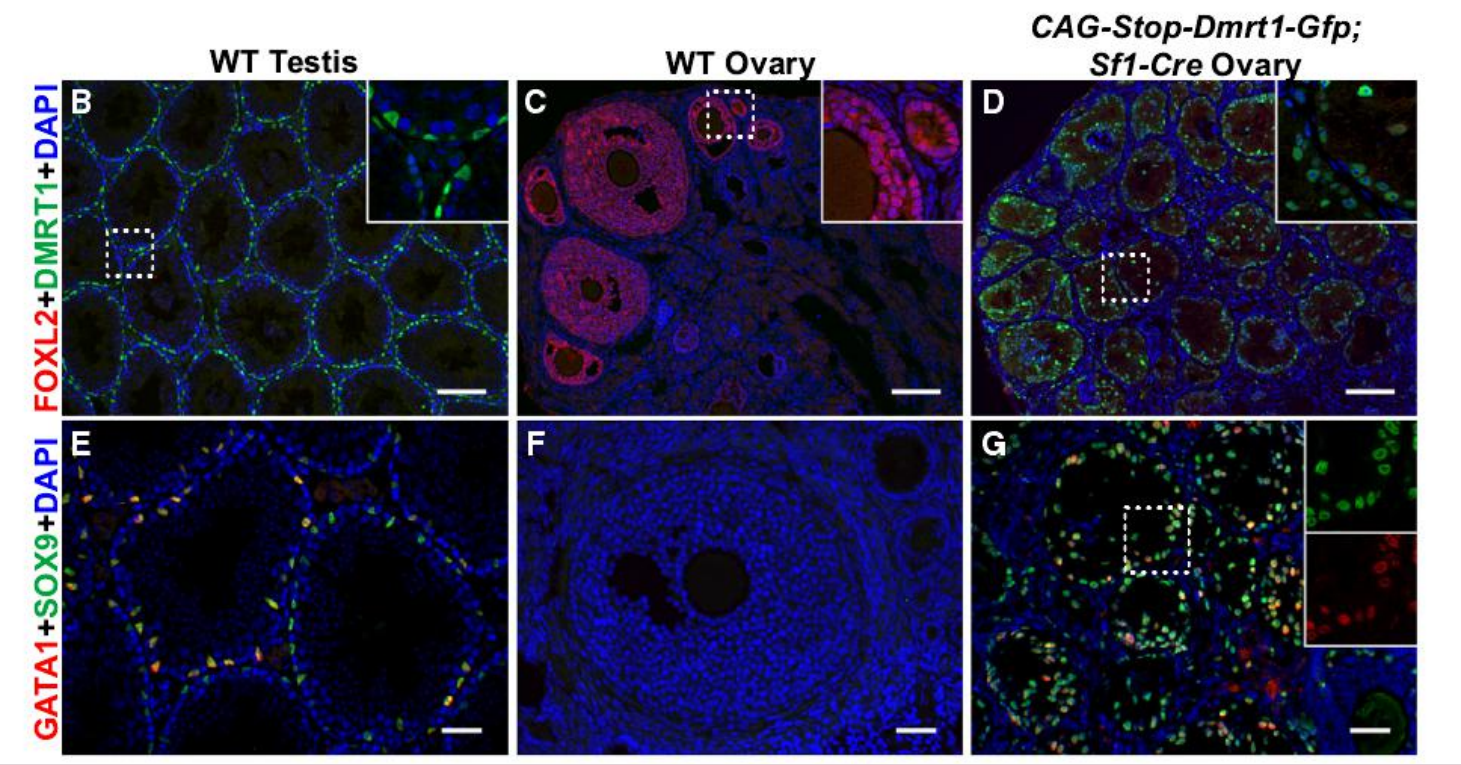
DMRT1 conditional expression transgene. (A)Targeting vector (top) used to insert conditional expression construct for Dmrt1 into the Rosa26 locus in ES cells. Between the two Rosa26 homology regions, the vector contains a splice acceptor sequence transcriptional stop cassette containing a reverse-oriented Pgk-Neo selectable marker and the SV40 terminator/polyA sequence



(C,F,I) WT testis expresses DMRT1 in Sertoli cells and spermatogonia and SOX9 in Sertoli cells does not express FOXL2, and is actively undergoing spermatogenesis. (D,G,J) Conditional deletion of Dmrt1 in somatic cells of the fetal gonad using Sf1-Cre eliminates DMRT1 expression in Sertoli cells and leads to germ cell death. Most mutant Sertoli cells transdifferentiate into FOXL2-positive granulosa-like cells. (E,H,I) Activation of CAG-Stop-Dmrt1-Gfp together with deletion of Dmrt1 using Sf1-Cre rescues Sertoli cell differentiation.

Results 1

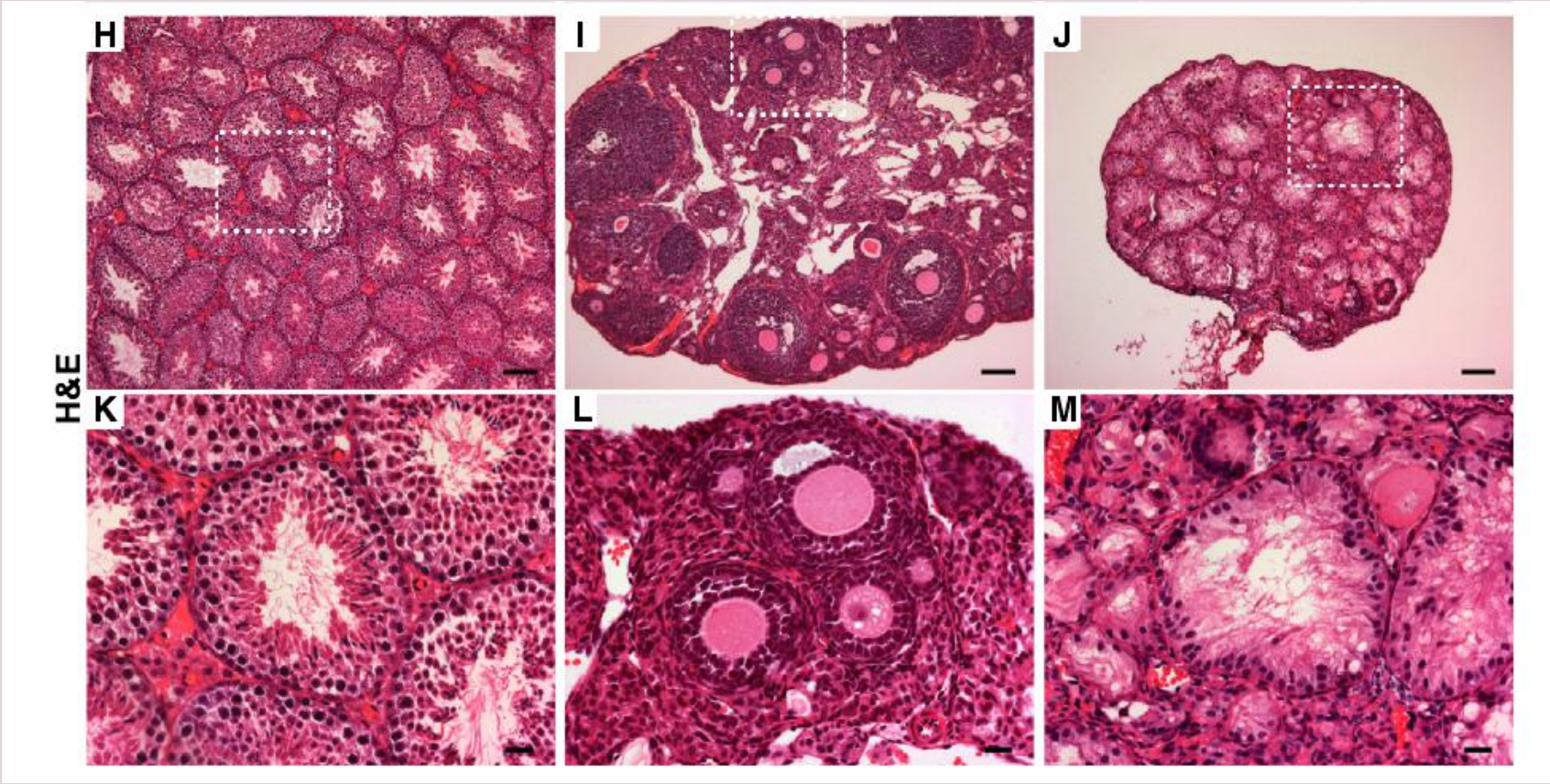
Ectopic DMRT1 Induces Formation of Sertoli-like Cells in the Ovary



Immunofluorescence (IF) of gonads from 8- to 10-week-old mice showing that activation of CAG-Dmrt1-Gfp in somatic cells of the fetal ovary by Sf1-Cre activates DMRT1, silencing the ovarian granulosa cell transcription factor FOXL2.

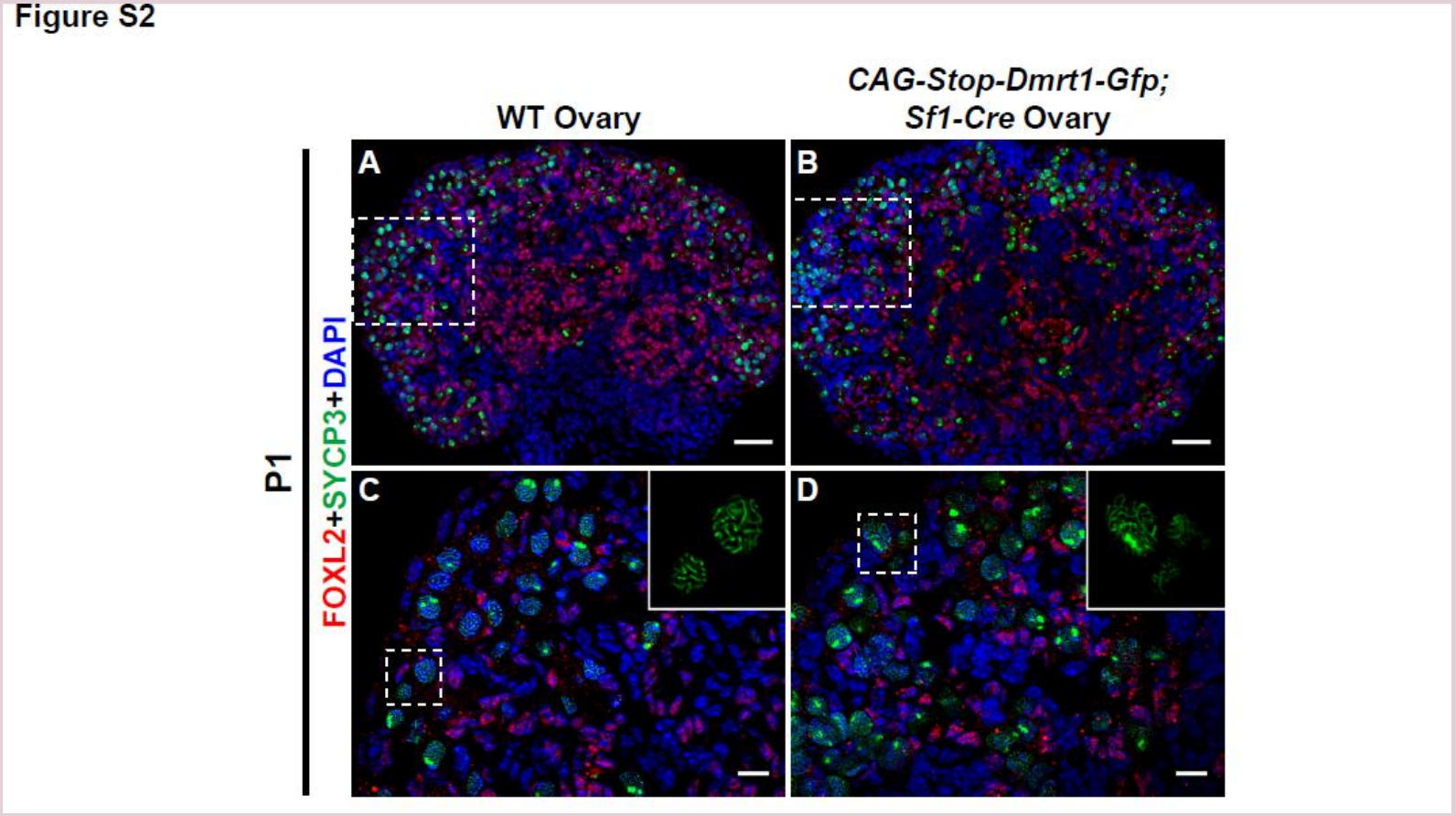
Results

Ectopic DMRT1 Induces Formation of Sertoli-like Cells in the Ovary



H&E-stained sections of adult testes (H), ovaries (I), and CAG-Dmrt1-Gfp expressing ovaries (J
at low and high magnification (dashed boxes indicate magnified areas shown in K–M).

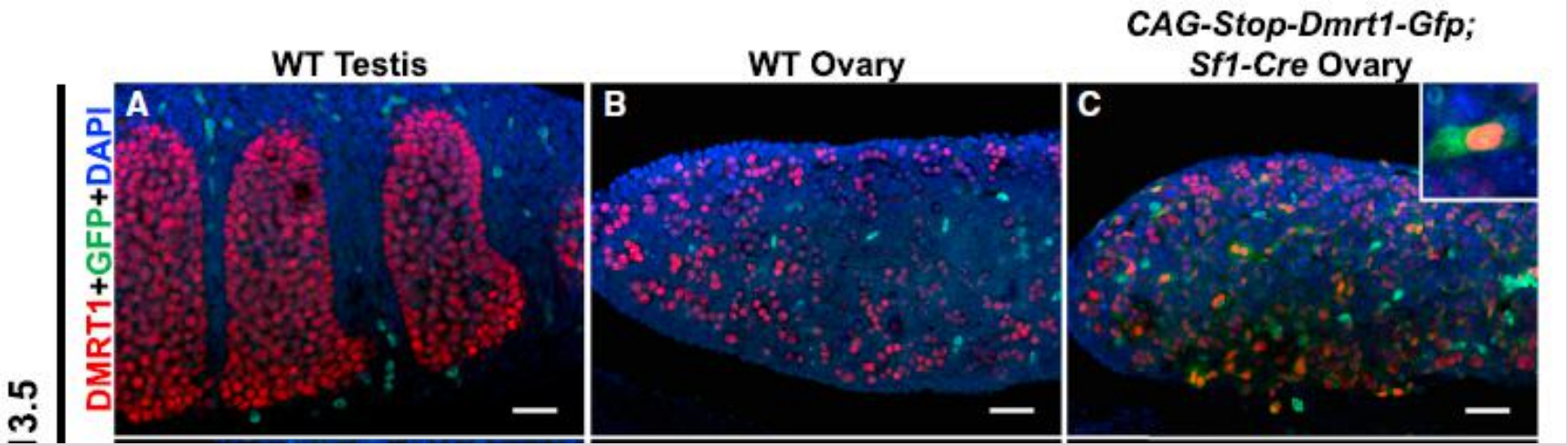
Results 2



Early activation of DMRT1 does not masculinize the fetal ovary. (A-D) IF showing that activation of CAG-Stop-Dmrt1 with Sf1-Cre in the fetal gonad does not disrupt specification of oocytes, meiotic initiation, or the normal diplotene arrest (indicated by accumulation of SYCP3 on synapsed chromosomes) at birth.

Results 2

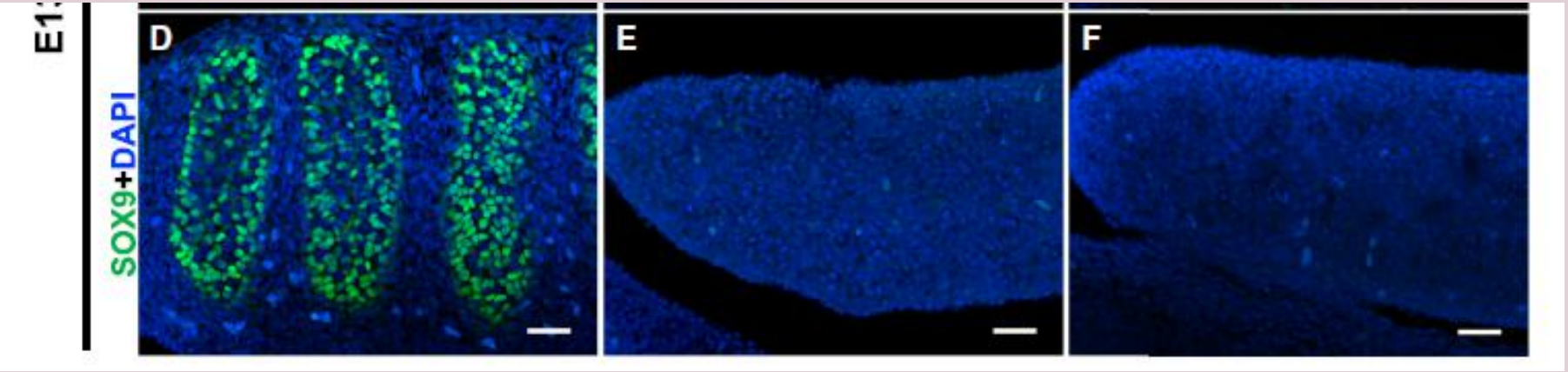
DMRT1 Induces Postnatal Sexual Transdifferention



(A–C) Activation of CAG-Dmrt1-Gfp in the fetal gonad. Confocal images of whole mount IF on E13.5 gonads show normal expression of DMRT1 in testis (A) and ovarian germ cells (B) and activation of CAG-Dmrt1-Gfp in ovarian somatic cells (C) as indicated by cytoplasmic GFP (example is shown in the highermagnification inset). Dispersed green cells lacking DMRT1 in wild-type gonads are autofluorescent cells of unknown type.

Results 2

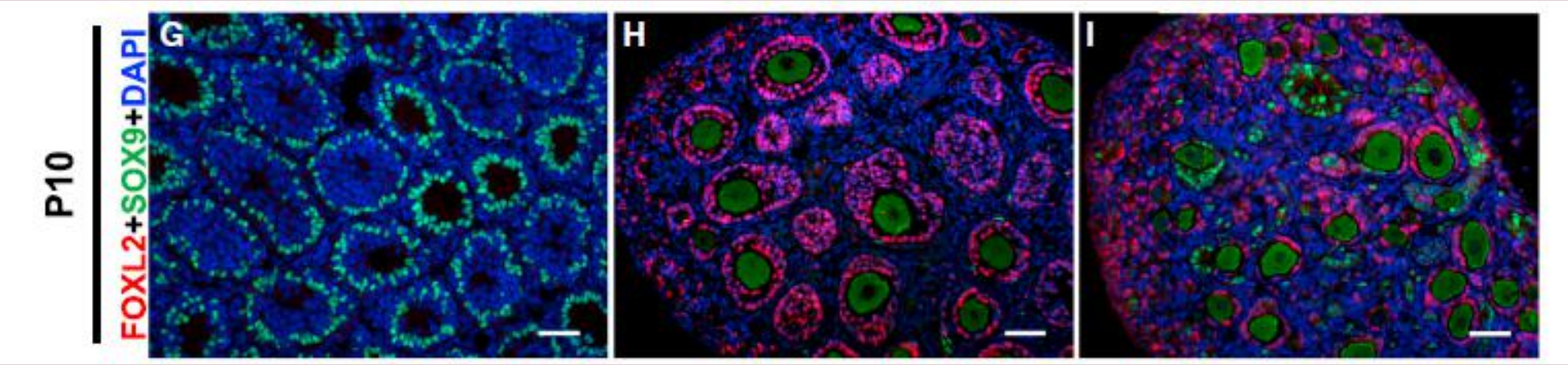
DMRT1 Induces Postnatal Sexual Transdifferention



(D–F) SOX9 expression in the fetal gonad. IF shows that SOX9 is strongly expressed in pre-Sertoli cells of wild-type testes at E13.5 (D) but is not detected in wild-type fetal ovaries (E) or CAG-Stop-Dmrt1-Gfp;Sf1-Cre transgenic ovaries (F).

Results 2

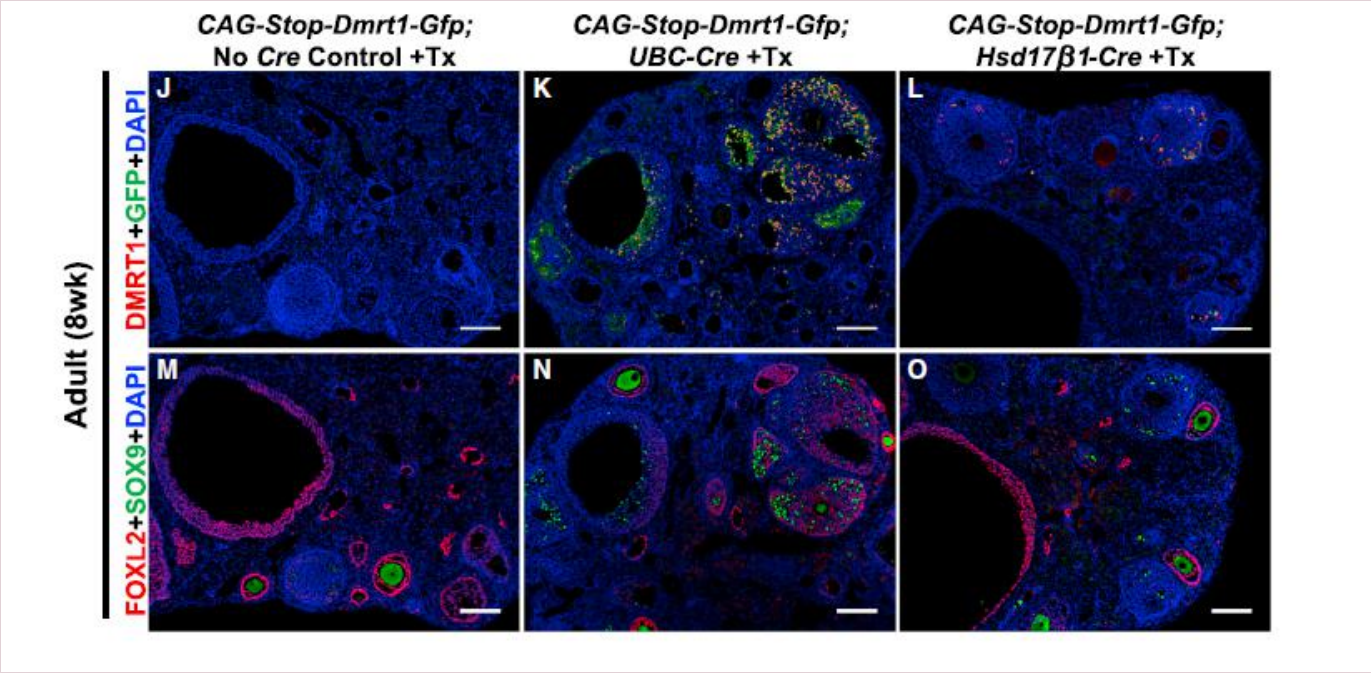
DMRT1 Induces Postnatal Sexual Transdifferention



(G–I) Postnatal expression of SOX9 and FOXL2. IF shows that wild-type testes at P10 express SOX9 and not FOXL2 (G), wild-type ovaries express FOXL2 and not SOX9 (H), and CAG-Stop-Dmrt1-Gfp;Sf1-Cre transgenic ovaries have cells expressing each protein (I), indicating the onset of transdifferentiation.

Results 3

DMRT1 Induces Postnatal Sexual Transdifferention



(J–O) Transdifferentiation in the adult ovary. Control tamoxifen-injected ovaries from adults carrying CAG-Stop-Dmrt1-Gfp but lacking a Cre transgene do not express DMRT1 or GFP (J), but ovaries from animals also containing UBC-CreERT2 or Hsd17b1-Cre have cells expressing both proteins (K and L). Somatic cells from control adult ovaries express FOXL2, but not SOX9 (M), whereas animals with UBC-CreERT2 (N) or Hsd17b1-Cre (O) have cells expressing each protein (SOX9 IF in adult oocytes is thought to be a non-specific antibody artifact).

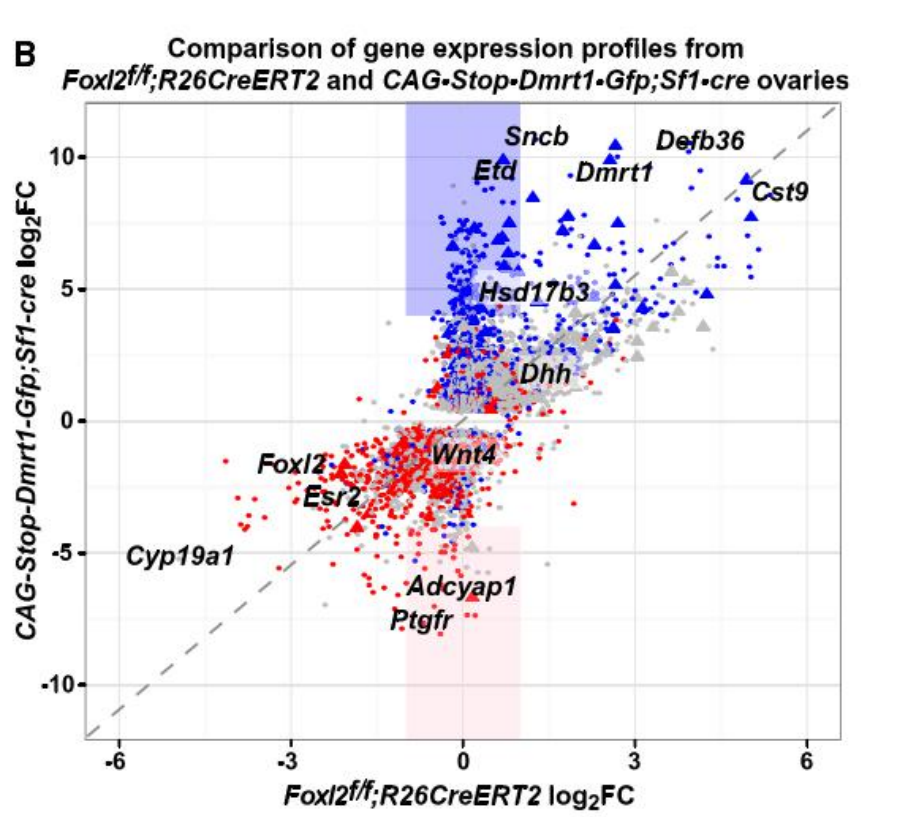
Results 4

DMRT1 Expression Masculinizes the Ovarian Transcriptome

(A) Heatmap comparing mRNA expression in adult wild-type testis and ovary with CAG-Dmrt1-Gfp;Sf1-Cre ovaries. Columns are from RNA-seq of two gonads (rep1, rep 2) of each genotype. Genes differentially expressed in wild-type ovary and DMRT1-expressing ovary (>4-fold ; $p < 0.05$; Table S1, part A) are shown in rows that are sorted based on high expression in the wild-type ovary (top) to high expression in the testis (bottom). Each gene was normalized to a range of 22 (violet) to +2 (green).

Results 5

Foxl2 Deletion and DMRT1 Expression Cause SimilarRemodeling of the Ovarian Transcriptome

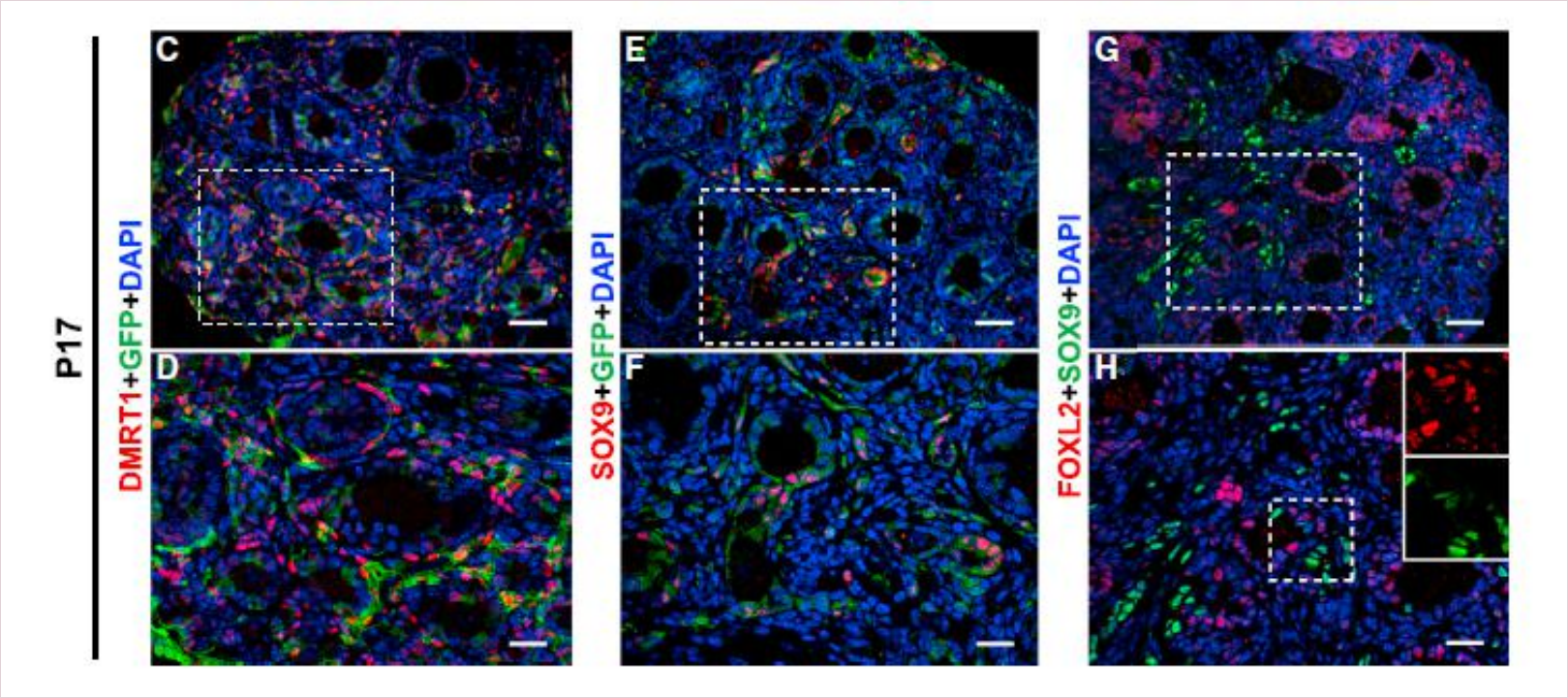


- Blue indicates mRNAs with 4-fold or greater expression in wild-type testis versus wild-type ovary,
- Red indicates those with 4-fold or greater expression in wild-type ovary versus wild-type testis.
- Gray indicates mRNAs not differing significantly between testis and ovary.
- Triangles denote X-linked genes, and blue and pink boxes highlight mRNAs strongly up- or downregulated, respectively, in CAG-Dmrt1-Gfp-expressing ovaries, but not in Foxl2-mutant ovaries.

(B) Scatterplot comparing gene expression in adult Foxl2 conditionally mutant ovaries and CAG-Stop-Dmrt1-Gfp;Sf1-Cre ovaries.

Results 6

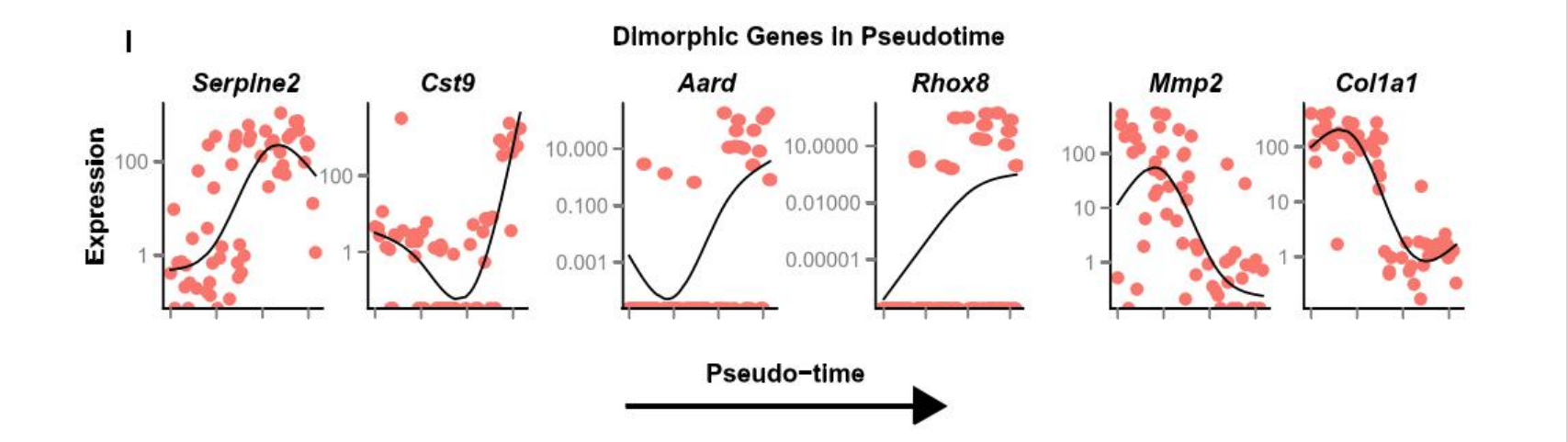
Single Cell Transcriptome Profiling Identifies CandidateMediators of Transdifferentiation



(C–H) IF showing that P17 CAG-Stop-Dmrt1-Gfp;Sf1-Cre transgenic ovaries have a mix of GFP+ cells expressing DMRT1 (C and D), SOX9 (E and F), or FOXL2 (G and H). Scale bars represent 40 mm (C, E, and G) and 20 mm (D, F, and H).

Results 6

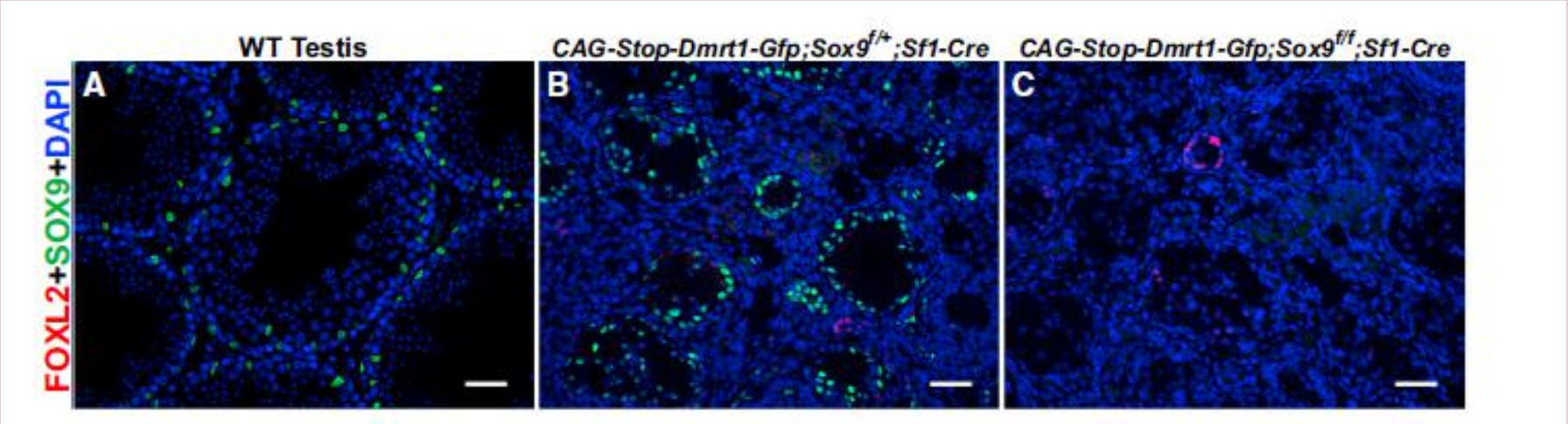
Single Cell Transcriptome Profiling Identifies CandidateMediators of Transdifferentiation



(I)Expression levels (FPKM)of selectmRNAsin single cells fromP17CAG-Stop-Dmrt1-Gfp;Sf1-Cre transgenic ovaries, orderedby pseudotime alongthe x axis.

Results 7

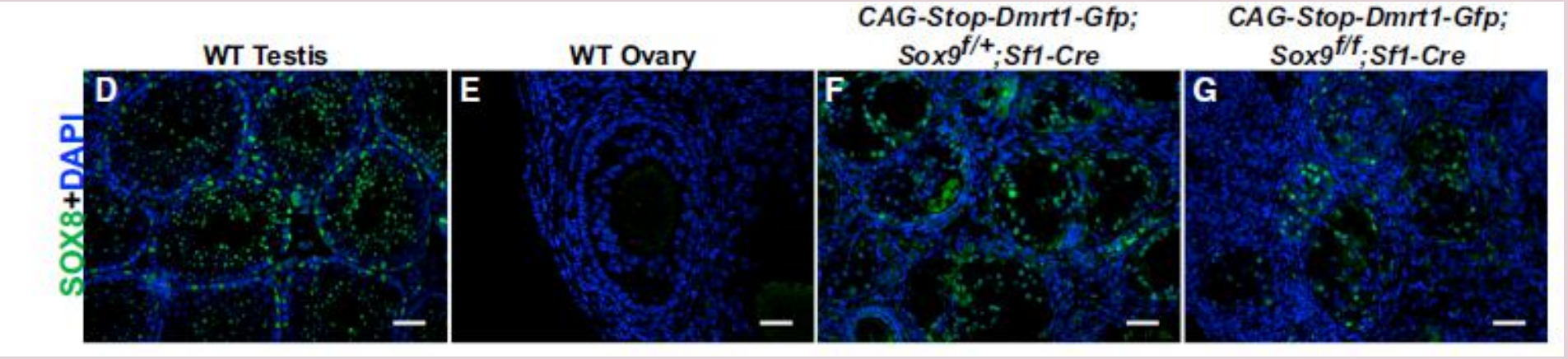
DMRT1 Can Silence Foxl2 without SOX8 and SOX9



(A–C) CAG-Stop-Dmrt1-Gfp can silence FOXL2 in Sox9 conditional mutant ovaries. IF shows that SOX9 is expressed in Sertoli cells of wild-type testes (A)
and in Sertoli-like cells of control conditional Sox9/+ DMRT1-expressing ovaries (B).
FOXL2 is almost completely silenced in DMRT1-expressing ovaries
conditionally deleted for one (B) or both (C) copies of Sox9 with Sf1-Cre.

Results 7

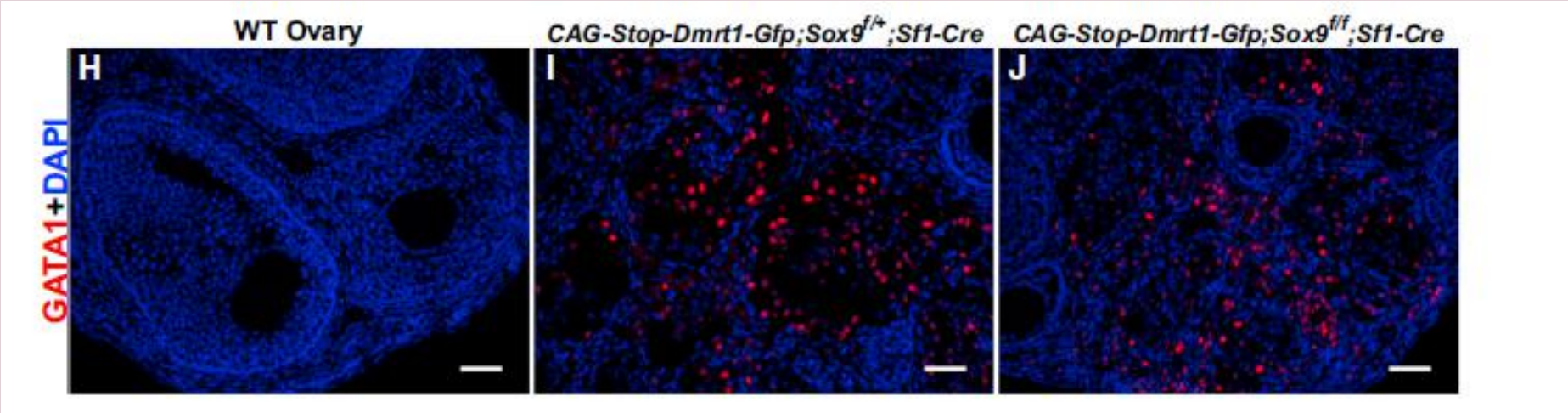
DMRT1 Can Silence Foxl2 without SOX8 and SOX9



(D–G) CAG-Dmrt1-Gfp can activate SOX8 in SOX9-mutant granulosa cells. IF shows that SOX8 is expressed in Sertoli cells in wild-type adult testes (D) and is not detectable in wild-type adult ovaries (E). CAG-Dmrt1-Gfp can activate SOX8 in ovaries conditionally deleted for one copy (F) or both copies (G) of Sox9 in somatic cells using Sf1-Cre.

Results 7

DMRT1 Can Silence Foxl2 without SOX8 and SOX9



(H–J) CAG-Dmrt1-Gfp can activate the mature Sertoli cell marker GATA1 in Sox9-mutant granulosa cells. IF shows that GATA1 is not expressed in wild-type
adult ovaries (H) but is expressed in ovaries conditionally deleted for one (I) or two (J) copies of Sox9 in somatic cells using Sf1-Cre.

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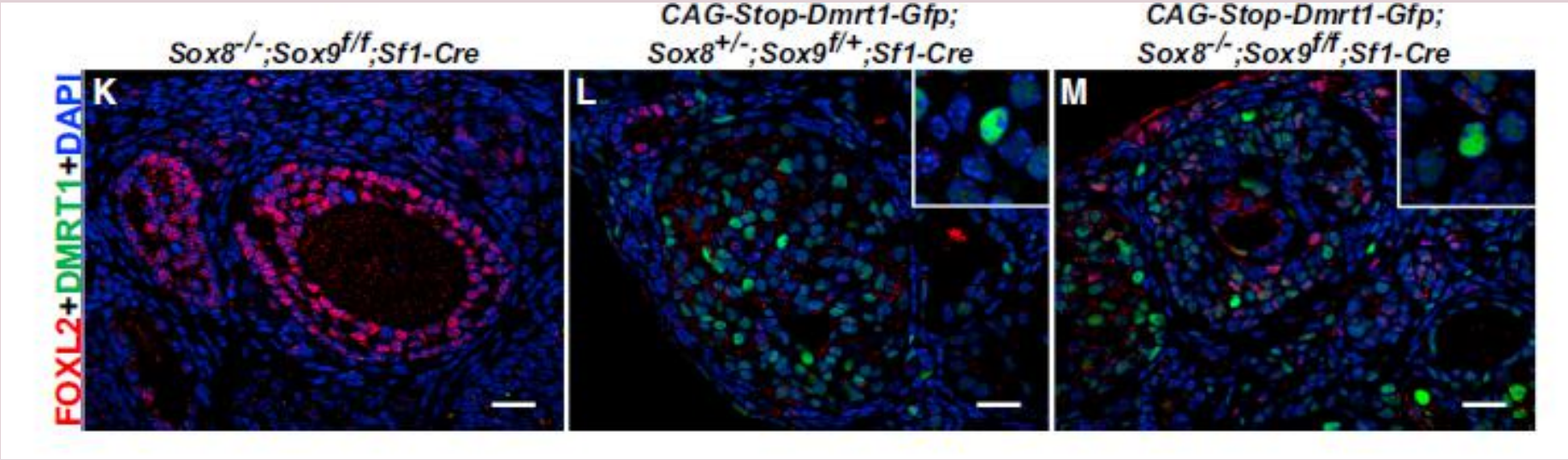
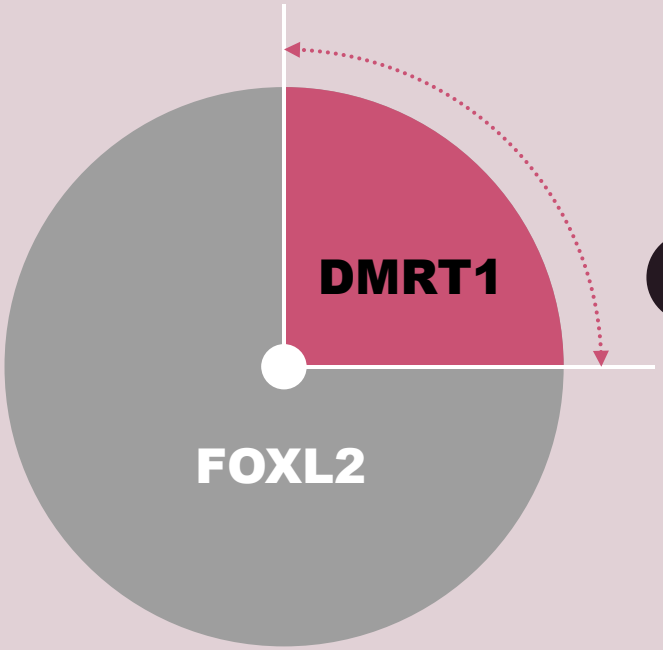


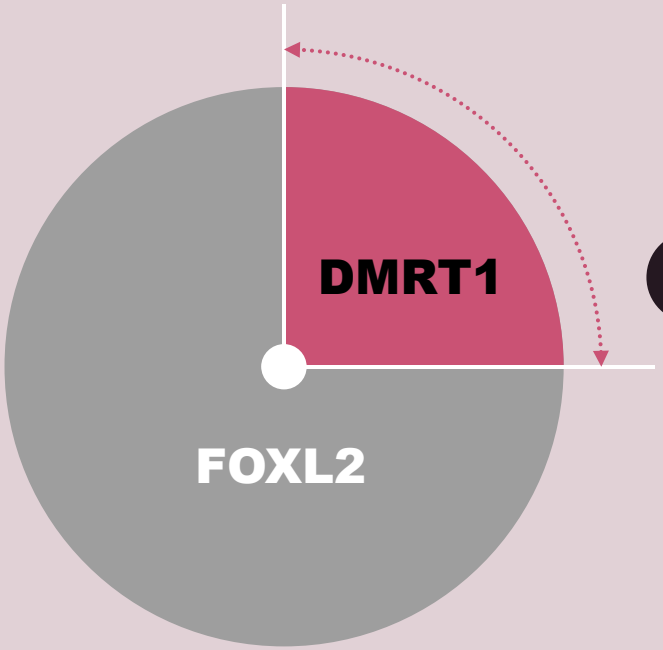
(K–M) DMRT1 silences FOXL2 in granulosa cells lacking both Sox8 and Sox9. IF shows that Sox8;Sox9 double-mutant ovaries have normal FOXL2 expression and normal morphology and lack DMRT1 (K). Activation of CAG-Dmrt1-Gfp in ovaries heterozygous for Sox8 and Sox9 (L) or homozygous mutant for both genes in somatic cells (M) can induce DMRT1 expression and silence FOXL2.

Discussion



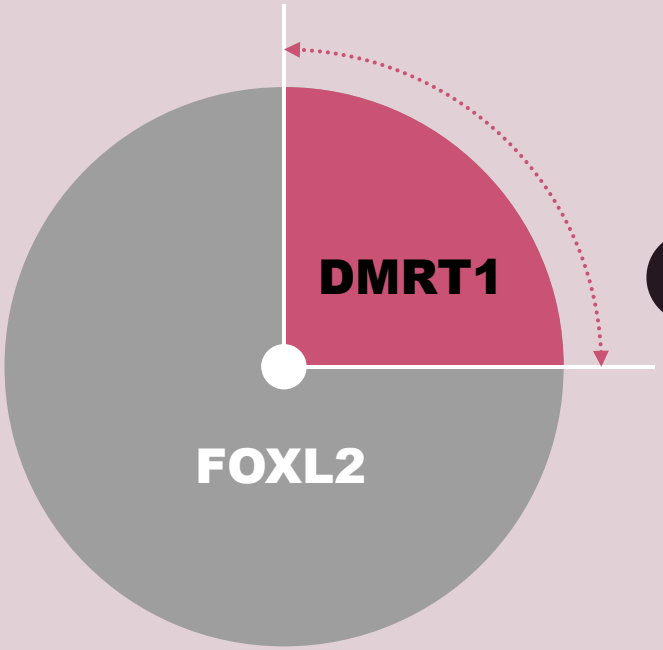
Ectopic DMRT1 activity can account for virtually all transcriptome changes resulting from deletion of Foxl2, suggesting that silencing of Dmrt1, directly or indirectly, is the primary means by which FOXL2 blocks sexual transdifferentiation.

Discussion



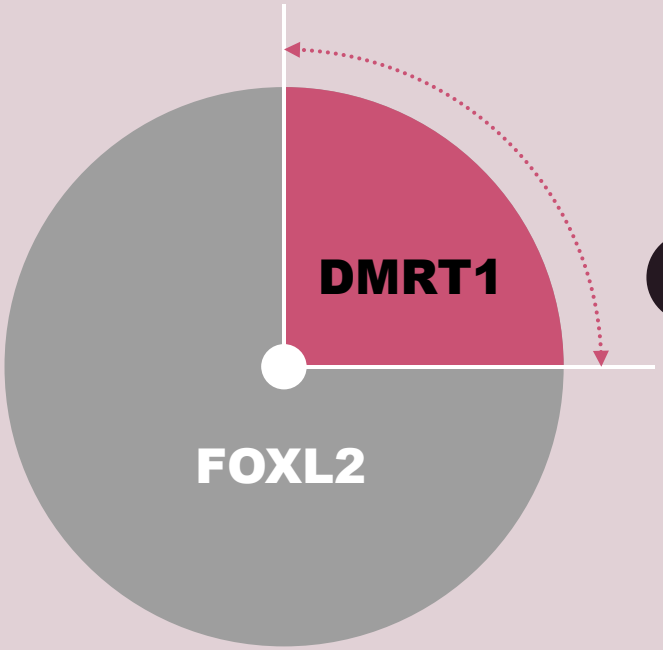
We found that even in mice, the presence or absence of DMRT1 can toggle a switch between the Sertoli and granulosa cell fates.

Discussion



The ability of ectopic DMRT1 expression to specify Sertoli cell fates also has potential significance for understanding evolution of genetic sex-determining mechanisms.

Discussion



DMRT1, with other testicular transcription factors (SOX9, GATA4, NR5A1/SF1, and WT1), can reprogram cultured induced pluripotent stem cells into Sertoli-like cells in vitro, but cannot reprogram them on its own.

Highlights

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- DMRT1 expression can masculinize the mammalian ovary
- DMRT1 expression causes sexual transdifferentiation
- Loss of FOXL2 and gain of DMRT1 similarly affect the ovarian transcriptome
- DMRT1 activity can toggle Sertoli and granulosa cell fates

THANK YOU !