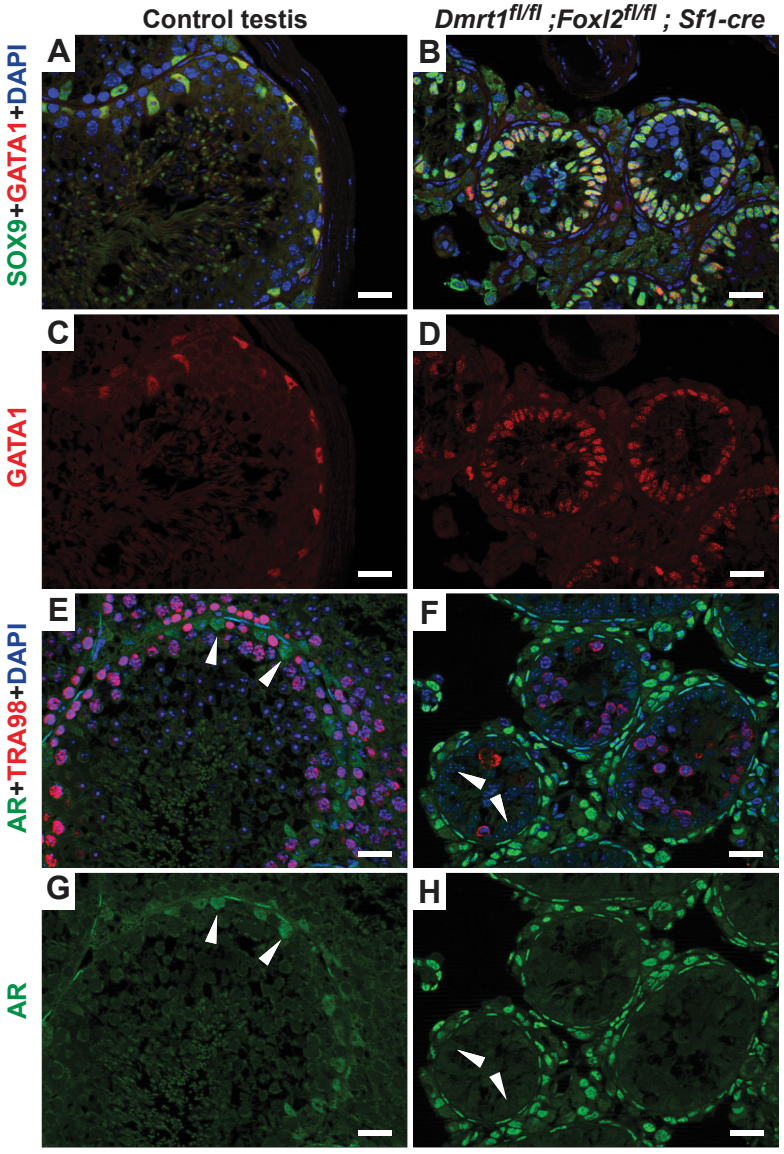
SUPPLEMENTAL INFORMATION

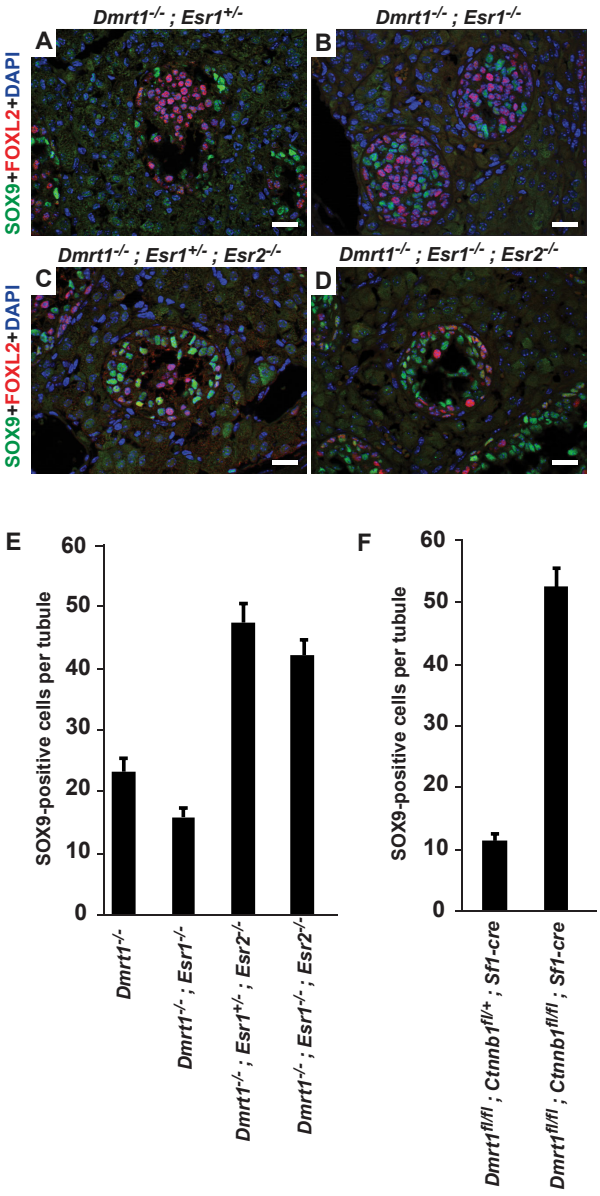
Inventory of supplemental information: Three supplemental figures (related to Figs 1, 2, and 6) and one supplemental table of antibodies used in this study.

Figure S1, Related to Figure 1. Loss of *Foxl2* rescues feminization but not maturation of *Dmrt1* mutant Sertoli cells.



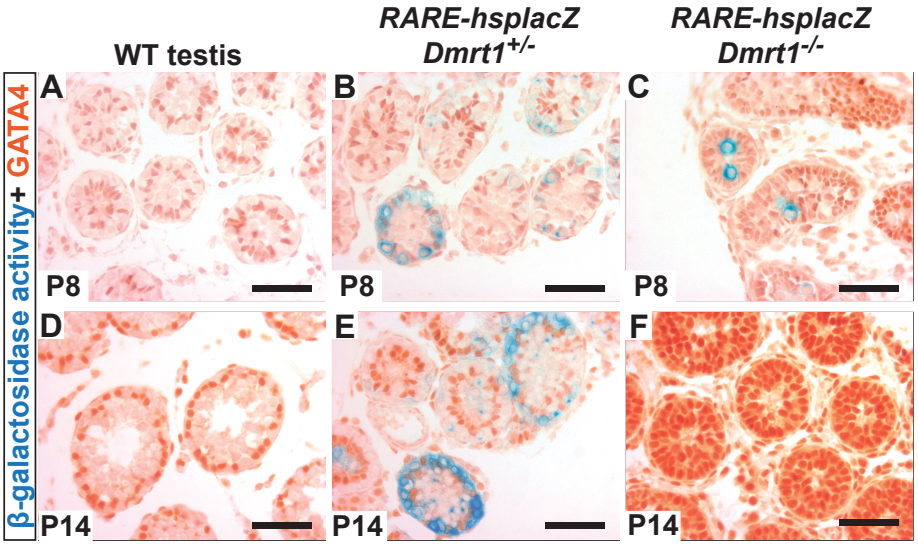
A-D, SOX9 and GATA1 protein expression in adult XY gonads. Like control testis from floxed animals lacking *cre* (**A,C**), conditional *Dmrt1;Foxl2* double mutant testes (**B,D**) express the mature Sertoli cell marker GATA1 in SOX9-positive cells with elongated nuclei typical of Sertoli cells. **E-H**, Androgen receptor (AR) and TRA98 protein expression in adult XY gonads. Control testes (**E,G**) express AR in Sertoli cells (cells near the tubule periphery and negative for the spermatogonial marker TRA98; arrowheads), whereas conditional *Dmrt1;Foxl2* double mutant testes (**F,H**) lack AR in Sertoli cells (arrowheads) and express AR only in peritubular myoid cells surrounding the tubules and in interstitial Leydig cells. Scale bars, 20 um.

Figure S2, Related to Figure 2. Estrogen and Wnt/beta-catenin signaling.



A-D, Estrogen signaling drives transdifferentiation primarily via the receptor *Esr2*. SOX9 and FOXL2 protein expression in adult XY gonads. *Dmrt1* null mutant XY testes are strongly feminized when one or two copies of *Esr1* is deleted (**A,B**). Deletion of *Esr2* in *Dmrt1* mutants suppresses feminization to a similar extent when *Esr1* is heterozygous (**C**), or homozygous null (**D**). Thus the *Esr2* genotype strongly influences transdifferentiation of *Dmrt1* mutant testes, whereas loss of *Esr1* does not, although we cannot exclude some functional redundancy between *Esr1* and *Esr2*. Scale bars, 20 mm. **E,F**, Effect of estrogen and Wnt/beta-catenin signaling on transdifferentiation. Y axis: number of SOX9-positive, FOXL2-negative cells per tubule, based on counts of at least 34 tubule cross-sections from two animals (17 tubules/animal). SOX9/FOXL2 double positive cells were excluded. Error bars indicate standard error of the mean. **E**, Effect of *Esr1* and *Esr2* on transdifferentiation. Loss of *Esr1* does not increase the number of SOX9-positive cells but loss of *Esr2* does. **F**, Effect of *Ctnnb1/beta-catenin* on transdifferentiation. Loss of *Ctnnb1* in Sertoli cells increases the number of SOX9-positive cells.

Figure S3, Related to Figure 6. Transdifferentiation of *Dmrt1* mutant Sertoli cells does not cause elevated RA-dependent reporter expression in spermatogonia.



A-F, Colorimetric detection of RA-responsive beta-galactosidase reporter *RARE-hsplacZ* (blue). Wild type testes lacking the reporter transgene show no background staining at P8 or P14 (**A,D**). At P8 LacZ staining is detected in a subset of GATA4-negative germ cells when reporter transgene is present (**B,C**), whether *Dmrt1* is heterozygous or homozygous mutant. No staining above background was detected in Sertoli cells. Many germ cells in the mutant (large round cells) are negative for reporter expression even though they are surrounded by mutant Sertoli cells. (Germ cell numbers are reduced but still present in *Dmrt1* mutants at P8). At P14 LacZ is again expressed strongly only in a subset of germ cells in *Dmrt1* heterozygous control (**E**) but by this stage germ cells have died in *Dmrt1* homozygote (**F**), showing clearly that no expression is detectable in mutant Sertoli cells. Scale bars, 20 um.