

## Supporting Online Material

### Materials and Methods

**Mice.** Mice carrying the *Dazl*<sup>TM1Hgu</sup> allele (1) were generously provided by Howard Cooke, MRC Human Genetics Unit, Western General Hospital, Edinburgh, UK. As described previously (2), we crossed *Dazl*<sup>TM1Hgu</sup> /+ mice to C57BL/6 mice (Taconic Farms Inc., Germantown, NY). All experiments were conducted using mice backcrossed to C57BL/6 between 7 and 16 generations, when 99.2% to >99.9% of the genome is expected to be of C57BL/6 origin; all Y chromosomes and mitochondria are of C57BL/6 origin. *Dazl*-deficient embryos were generated by mating heterozygotes. *Dazl* genotypes were assayed by PCR as previously described (1). All experiments involving mice were approved by the Committee on Animal Care at the Massachusetts Institute of Technology.

**Embryo Collection and Sexing.** To establish timed matings, female mice were housed with male mice overnight. Noon of the day when a vaginal plug was evident was considered E0.5. Embryonic gonads and mesonephroi were dissected into cold phosphate buffered saline. We determined the sex of the tissues by scoring the presence or absence of testicular cords.

**Alkaline phosphatase staining.** Dissected embryonic gonads and mesonephroi were fixed in 4% paraformaldehyde overnight and then treated for 4 minutes with methanol to permeabilize the tissue. Samples were then stained with NBT (nitroblue tetrazolium chloride) and BCIP (5-bromo-4-chloro-3'-indolylphosphate p-toluidine salt) (Roche).

**Immunohistochemistry.** Embryonic gonads were fixed in 4% paraformaldehyde overnight, embedded in paraffin and sectioned. Slides were de-waxed, rehydrated and autoclaved in 10 mM Sodium Citrate buffer, pH 6.0 for 5 minutes. Mouse monoclonal anti- $\gamma$ H2AX (Upstate Biotech) was used at a dilution of 1:1000. Rabbit anti-SCP3 was a gift from Christa Heyting (Department of Genetics, Agricultural University, Wageningen, The Netherlands) and was used at a dilution of 1:700. Rabbit anti-MVH was a gift from Toshiaki Noce (Mitsubishi Kagaku Institute of Life Sciences (MITILS), Tokyo, Japan) and was used at a 1:1000 dilution. Texas Red-conjugated secondary antibodies (Jackson ImmunoResearch Laboratories Inc.) were used at a dilution of 1:200.

**Histology.** Embryonic gonads were fixed in Bouin's solution at 4°C overnight, embedded in paraffin and sectioned. Slides were then stained with hematoxylin and eosin.

**Whole-mount *in situ* hybridization.** Embryonic gonads and mesonephroi were fixed in 4% paraformaldehyde at 4°C overnight. Whole-mount *in situ* hybridizations were performed as previously described (3, 4). Riboprobes for *Stra8* and *Dmc1* were prepared as previously described (5).

**Quantitative RT-PCR.** Embryonic gonads were dissected away from mesonephroi, placed in Trizol (Invitrogen), and stored at -80°C. Following genotyping, total RNAs were prepared according to the manufacturer's protocol. Total RNAs were then DNase

treated using DNA Free Turbo (Ambion). 200 ng of total RNA was reverse transcribed using a RETROScript kit (Ambion). Quantitative PCR was performed using SYBR Green Core PCR Reagents (Applied Biosystems) on an ABI9700 Fast Real-time PCR machine (Applied Biosystems). Results were analyzed using the delta-delta Ct method using *Hprt* (hypoxanthine-guanine phosphoribosyltransferase) as a normalization control. RT-PCR primers for all genes were picked from the PrimerBank web site (6), and their sequences are listed in Supplementary Table S1.