Supporting Information

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SI Materials and Methods

Embryonic Stem (ES) Cell Culture, Manipulations, and Derivation. ES cells were cultured on irradiated mouse embryonic fibroblasts in DMEM containing 10% FBS, leukemia inhibiting factor (LIF), penicillin/streptomycin, L-glutamine, and nonessential amino acids. For ES cell derivation, zona pellucidae were removed using acidic tyrode (AT) solution and blastocysts were explanted on irradiated feeders in ES medium supplemented with MEK1 inhibitor (PD98059; Cell Signaling Technology).

Genotyping of *mir-290–295* Embryos. Midday of the day of the vaginal plug was considered as 0.5 dpc in the timing of embryo collection. Embryos were dissected from the decidua at various timepoints during development. Blastocysts were flushed from the ovaries at 3.5 dpc and dissociated in 30 μL lysis buffer (50 mM Tris pH 8.8, 1 mM EDTA, 0.5% Tween-20, 200 μg/mL Proteinase K) for 4 h at 55 °C. Samples were then heated to 95 °C for 10 min to inactivate Proteinase K. Successful genotyping of blastocysts required nested PCRs. For first-round PCR, 5 μL of lysed blastocyst was used and then 2 μL of first-round PCR was used for the second round of amplification. The following primers were used: endogenous allele first-round PCR, 5′-CTACAATGCACCTGGACTCA-3′ (forward) and 5′-AGAGGCGAAAGTAGATCCAG-3′ (reverse); endogenous allele second-round PCR, 5′-CGGTTTGGCTGGGTTTACTA-3′ (forward) and 5′-AACGACCACCTCAGTTACCG-3′ (reverse); targeted allele first-round PCR, 5′-AGAGGCCACTTGTGTAGCGC-3 (forward) and 5′-AGAGGCGAAAGTAGATCCAG-3′ (reverse); and targeted allele second-round PCR, 5′-CGGTTTGGCTGGGTTTACTA-3′ (forward) and 5′-CAGACTGCCTTGGGAAAAGT-3′ (reverse). For embryos E7.5 and older, one round of amplification was sufficient for genotyping.

Antibodies, Histological, and Immunohistochemical Analysis of Adult and Embryonic Gonads. For immunohistochemistry and histology, gonads were fixed in either 4% paraformaldehyde or Bouin's solution overnight, embedded in paraffin, and sectioned. For immunohistochemistry, slides were dewaxed, rehydrated, and steamed in 10 mM sodium citrate buffer, pH 6.0 for 24 min. Rabbit polyclonal anti-mouse vasa homolog (MVH) (Abcam) was used at a dilution of 1/200, mouse monoclonal anti-proliferating cell nuclear antigen (PCNA) (Abcam) was used at a dilution of 1/1,000, polyclonal rabbit anti-cleaved caspase-3 (Cell Signaling Technology) was used at a dilution of 1/200, rat monoclonal anti-K_i-67 (Dako) was used at a dilution of 1/200, mouse monoclonal SSEA-1 (Developmental Studies Hybridoma Bank) was used at a dilution of 1/20, and rat anti-GCNA1 (a gift from George Enders, University of Kansas Medical Center, Kansas City, KS) was used as undiluted supernatant. Images were captured with a Nikon E800 upright microscope or a PerkinElmer Ultraview Spinning Disk Confocal microscope.

To determine the germ cell density in control and mutant E13.5 gonads, the following methods were used. For E13.5 males, the number of MVH^+ cells in each tubule was counted for at least three nonconsecutive histological sections for each gonad from each animal. The analysis program ImageJ (1) was used to determine the total relative tubule area for each section. The total number of MVH germ cells counted for all sections was then divided by the total tubule area for all sections to calculate a relative germ cell density for each testis. The two germ cell densities for each testis were then averaged to obtain the average germ cell density for each E13.5 male. For females, the total number of MVH^+ cells in at least two nonconsecutive histological sections was counted and ImageJ was used to calculate the relative area of each ovarian section. Then the total number of MVH^+ germ cells counted for all sections for each ovary was divided by the total relative area. The two germ cell densities for each ovary were then averaged to obtain the average germ cell density for each E13.5 female. For E12.5, the middle longitudinal section for each gonad was used for counting.

Whole-Mount Alkaline Phosphatase Staining. Alkaline phosphatase staining was performed according to the procedure outlined in Hara et al. (2). Briefly, E9.5 embryos were dissected into PBS, fixed 6 h overnight at 4 °C in 4% paraformaldehyde then washed for 12 h in TBS-T. The samples were then stored in 70% ethanol for at least 3 d. After several washes in NTMT buffer (100 mM NaCl, 100 mM Tris-HCl (pH 9.5), 50 mM $MgCl_2$, 0.1% Tween-20) the samples were stained with BCIP-NBT (Roche Applied Science). The hindgut and developing hindlimb areas were dissected away from the embryo and flattened under a coverslip to facilitate counting of alkaline phosphatase positive cells.

1. Abramoff MD, et al. (2004) Image processing with Image J. *Biophoton Int* 11:7.

2. Hara K, et al. (2009) Evidence for crucial role of hindgut expansion in directing proper migration of primordial germ cells in mouse early embryogenesis. *Dev Biol* 330: 427–439.