



Fig. S4. *Dazl* is necessary for germ cell commitment in diverse strains of mice.

(A) Gating strategy for flow cytometric analysis and collection of germline cells from an embryo carrying both *Oct4:EGFP* and *Dazl:tdTomato* reporter alleles, collected at E12.5. Germline expression of *DAZL:tdTomato* in *Oct4:EGFP*-positive cells is presented in lower panel. (B) Flow cytometric counting of cells expressing *Nanog:GFP*, *Dazl-tdTomato*, or both reporters, from isolated urogenital ridges. Each point represents one embryo. Data was then averaged across

intervals of three tail somites, and presented in Fig. 2A. (C) Immunofluorescence of E15.5 ovary and testis in *Nanog:GFP* controls (upper panels) and *Nanog:GFP; Dazl*-deficient gonad (lower panels) in 129S4 embryo. *Nanog:GFP*-reporter (stained with an antibody to GFP, green) was expressed in germline cells of *Dazl*-deficient gonad (stained with DDX4, magenta, marked by arrows). Asterisks show GFP-negative germline cells. DNA stained with DAPI (blue). (Scale bar, 50 μ m.) (D) Heatmap showing expression of naïve and general pluripotency factors in control, *Stra8*-deficient, and *Dazl*-deficient ovaries at E14.5. * denotes expression from exons retained in *Dazl* mutant allele. (E) Derivation of EG cells from 129SB6F1 control and *Dazl*-deficient embryos. Cells were collected by FACS at the embryonic age indicated on x-axis, and cultured under defined conditions. After 10 days, the number of EG cell colonies per 100 cells plated was counted, and the rate of EG cell derivation determined. The number of embryos tested is listed in each column, mean + SD, ** *P* value < 0.01, *** < 0.001, ns = not significant, using t-test or Fishers exact test as appropriate. (F) Morphology of EG cell line derived from E15.5 F1 *Dazl*-deficient testis cultured on feeder cells (left). Cells derived from PGCs carry *Oct4:EGFP* transgene, which is expressed in EG cell colonies (right). (G) Two chimeric mice at 30 days of age, derived from injection of E15.5 F1 *Dazl*-deficient EG cells (which contain the white-bellied Agouti coat color allele from the maternal 129S4 background) into B6 blastocysts. High contribution of EG cells to the epidermis can be observed by the brown (Agouti) coat color. (H) Flow cytometric analysis of control and chimeric testes for EG cell contribution to the germline, revealed by expression from the *Oct4:EGFP* transgene. (I) Heatmap of expression of germ cell factors (identified in Fig. 1D) from sorted control and *Dazl*-deficient migratory (E10.3, left) and gonadal (E11.5, right) germlines; *n* = 3 embryos at each time point. * denotes expression from exons retained in *Dazl* mutant allele.