

B gonadal histology of mice with the conditional-ready (Dazl-2L) or recombined (Dazl-1L) allele C immuno-fluorescence of Ddx4-Cre mediated recombination of



D teratoma incidence in 129S4.*Dazl-1L/1L* mice male female



Fig. S7. Generation of conditional *Dazl* allele in mice.

(A) CRISPR/Cas9-mediated strategy to insert *loxP* sites to intron 3 and intron 7 of *B6N-Dazl* (upper panels). Correct targeting on a single allele confirmed by Sanger sequencing, and designated as B6N-*Dazl*^{em1Dcp} (carrying 2 *loxP* sites, referred to as *Dazl-2L*, central panels). This conditional allele was recombined by *Ddx4-Cre*, and designated as B6N-*Dazl*^{em1.1Dcp} (containing a single *loxP* site, referred to as *Dazl-1L*, lower panel). When recombined, the *Dazl-1L* is predicted to introduce a frame-shift mutation (*p.Tyr82Cys*) resulting in a premature stop codon. (*B*)

Histology of mice homozygous for the conditional allele (B6.*Dazl-2L/2L*, left panels), show complete gametogenesis at two months of age in both sexes. The histology of mice zygotically homozygous for the recombined allele (B6.*Dazl-1L/1L*) show complete germline loss in both sexes (right panels), phenocopying the established allele (GCNA; green, SOX9; magenta). Primordial follicle (arrowhead) and secondary follicle (chevron) marked in ovary (*C*) Immunofluorescence of P1 ovary (left) and testis (right) in mice carrying *Ddx4-Cre;Oct4:EGFP*; and *LSL-tdTomato* alleles. tdTomato expression (stained with an antibody to tdTomato protein, red) is restricted to Oct4:EGFP-positive cells (stained with an antibody to EGFP protein, green). DNA stained with DAPI (blue). (Scale bar, 100 μ m.) (*D*) Incidence of gonadal teratomas in control mice, or mice zygotically homozygous for the recombined *Dazl* allele (129S4.*Dazl-1L/1L*). Females were dissected at two months of age, and males between one and two months of age. *n* = number of animals examined, * *P* value < 0.05, ns = not significant, using Fishers exact test.