SI Discussion

Comparison of *Dazl*-deficient mice on four genetic backgrounds

Given numerous reports that germline phenotypes vary according to genetic background, we considered whether the *Dazl*-dependent restriction of developmental potential is unique to the B6 strain. We approached this question amidst conflicting reports regarding *Dazl*'s role in germ cell viability. Specifically, in *Dazl*-deficient mice on an inbred B6 background, PGCs that colonize the nascent gonads subsequently die; no germ cells remain in the post-natal gonads of either sex (1). By contrast, on a mixed genetic background, some *Dazl*-deficient germline cells survive embryogenesis in males [although the animals are sterile (2), with defects in spermatogonial differentiation (3) and meiosis (4)]. These seemingly conflicting observations, albeit in different genetic backgrounds, raised the possibility that *Dazl* might be necessary to restrict developmental potential in B6 mice, but not in other inbred mouse strains.

To address whether our findings in B6 mice are unique to that strain, and to clarify the role of *Dazl* in germline survival, we backcrossed the $Dazl^{tm1Hjc}$ null allele to several *M. m. domesticus* strains of western European origin, and examined the testes of *Dazl*-deficient mice for the presence of germ cells. In juvenile males of the *M. m. domesticus* strains B6 and FVB/NTac (FVB), no germ cells were observed in *Dazl*-deficient testes at post-natal day 5, indicating that *Dazl* is necessary for embryonic germline survival in these strains (SI Appendix, Fig. S5A). However, in males of the *M. m. domesticus* strain 129S4, some *Dazl*-deficient germline cells survive embryogenesis, and a few commence but fail to complete spermatogenesis (SI Appendix, Fig. S5A and B). Analysis of 129.*Dazl*-deficient spermatocytes by meiotic spreads shows that many chromosomes remain un-synapsed, with no cells progressing beyond the zygotene stage (SI Appendix, Fig. S5C). Looking further afield, we backcrossed the $Dazl^{tm1Hjc}$ null allele to the *M. m. molossinus* strain (MSM/MsJ; MSM) derived from wild-trapped Japanese mice; the MSM and *M. m. domesticus* lineages diverged more than a million years ago. On an inbred MSM background, far fewer germline cells were observed in the post-natal testes of *Dazl*-deficient mice (SI Appendix, Fig. S5A). These findings demonstrate that expression of *Dazl*, beginning shortly after PGCs colonize the nascent gonads, is critical for germline survival in the testis, not only in multiple *M. m. domesticus* strains, but also in the distant outgroup *M. m. molossinus*. By contrast, no oocytes are observed in the post-natal ovary of *Dazl*-deficient females in any strain (SI Appendix, Fig. S5D).

We next considered the testicular phenotype of the *Dazl*-deficient germline prior to germline loss. In embryos, newly arrived PGCs rapidly proliferate, ceasing shortly after the commencement of male sexual differentiation. In B6.*Dazl* embryos, these newly arrived PGCs

remain proliferative for an extended period, until at least E15.5 (5). To test if the germline of *Dazl*-deficient embryos retains an extended period of mitosis in other genetic backgrounds, we injected pregnant mice with EdU, a thymidine analog that incorporates into the DNA of proliferative cells. As anticipated, EdU was detected in the proliferative somatic lineages of the testis in control and *Dazl*-deficient embryos at E17.5 (SI Appendix, Fig. S5E). By contrast, germline cells in control embryos did not incorporate EdU, whereas the germline of *Dazl*-deficient littermates was regularly EdU positive, indicating DNA synthesis and retained mitotic activity, regardless of genetic background.

Given the extended proliferation of the *Dazl*-deficient germline in the embryonic testes of all strains examined, we asked whether this ongoing proliferation is sufficient for teratoma formation in other strains (6). To test this, we examined post-natal testes for the occurrence of spontaneous testicular teratomas in each strain. While *Dazl*-deficient mice on a 129 strain background regularly produced teratomas (SI Appendix, Table S4), we did not observe testicular teratomas in any non-129 strain background (SI Appendix, Fig. S5F).

When these results are considered in conjunction with the retained *Nanog:GFP* positivity in the *Dazl*-deficient germline of B6 and 129S4 embryos (Fig. 2B), we conclude that the embryonic requirement for *Dazl* is consistent in all strains examined. Further, the extended proliferative activity and failure to restrict the expression of pluripotency markers in the *Dazl*-deficient germline reflects the retention of a PGC-like state on arrival at the gonad. In teratoma-resistant mouse strains, these PGC-like cells usually undertake apoptosis prior to birth. We note, however, that despite the abnormal germline development of the 129S4.*Dazl*-deficient germline, these cells may survive beyond birth, although mice are sterile with a failure to complete meiosis in both sexes.