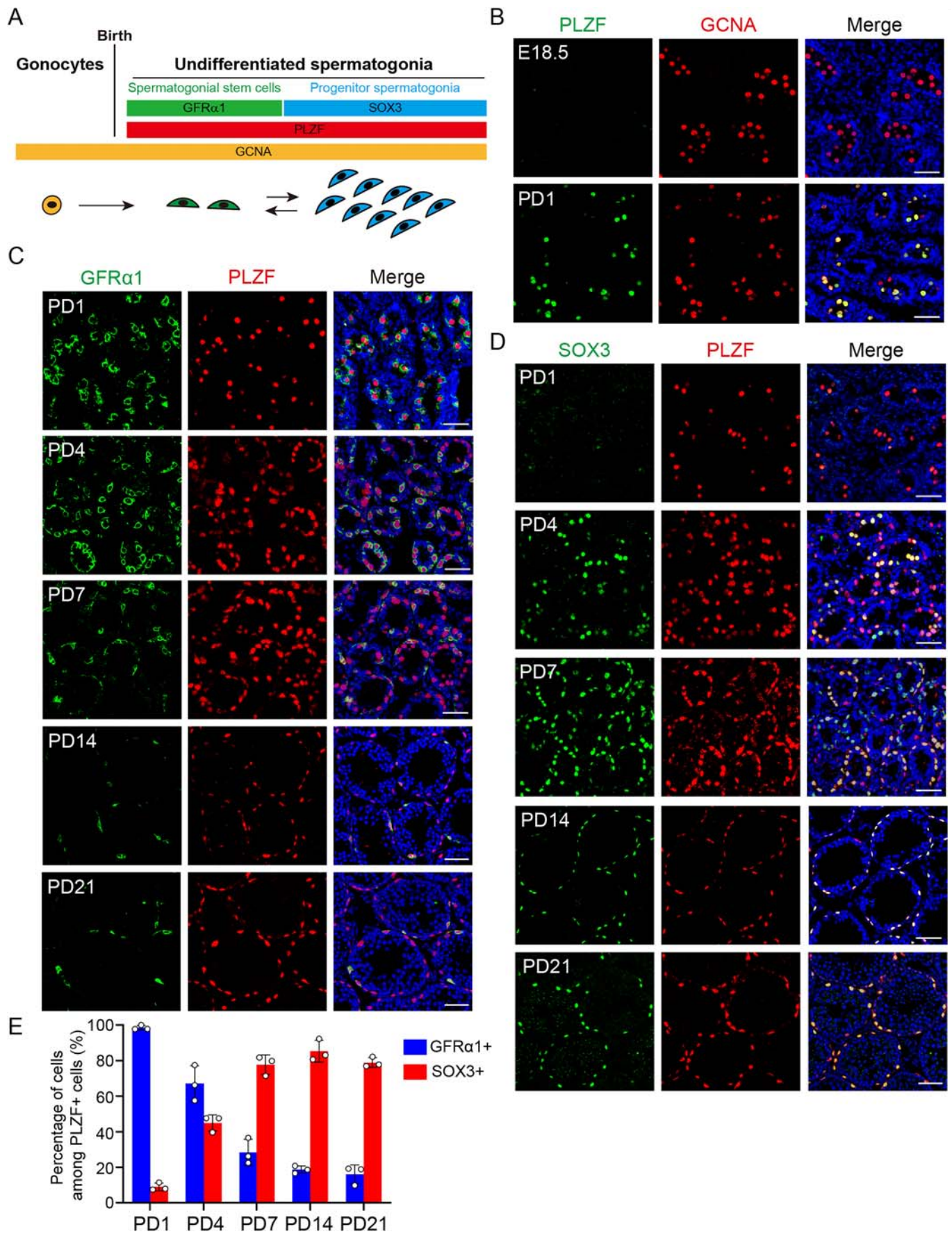


Expanded View Figures

Figure EV1. Spermatogonial stem cells and progenitor spermatogonia achieve homeostasis when undifferentiated spermatogonia is formed after birth.

(A) Cell identity and marker gene expression before and after the formation of undifferentiated spermatogonia, which contain spermatogonial stem cells and progenitor spermatogonia that achieve homeostasis. (B) IF staining of PLZF and GCNA in frozen sections of testes from WT mice at E18.5 and PD1. (C) IF staining of GFR α 1 and PLZF in frozen sections of testes from WT mice at PD1, PD4, PD7, PD14, and PD21. (D) IF staining of SOX3 and PLZF in frozen sections of testes from WT mice at PD1, PD4, PD7, PD14, and PD21. (E) Statistical analysis of the percentage of GFR α 1⁺ and SOX3⁺ cells among PLZF⁺ cells in testes from WT mice at PD1, PD4, PD7, PD14, and PD21. Data information: Data are presented as mean $\pm$ SD. Three mice at each timepoint were analyzed. Scale bar, 50 μ m. n.s., not significant.



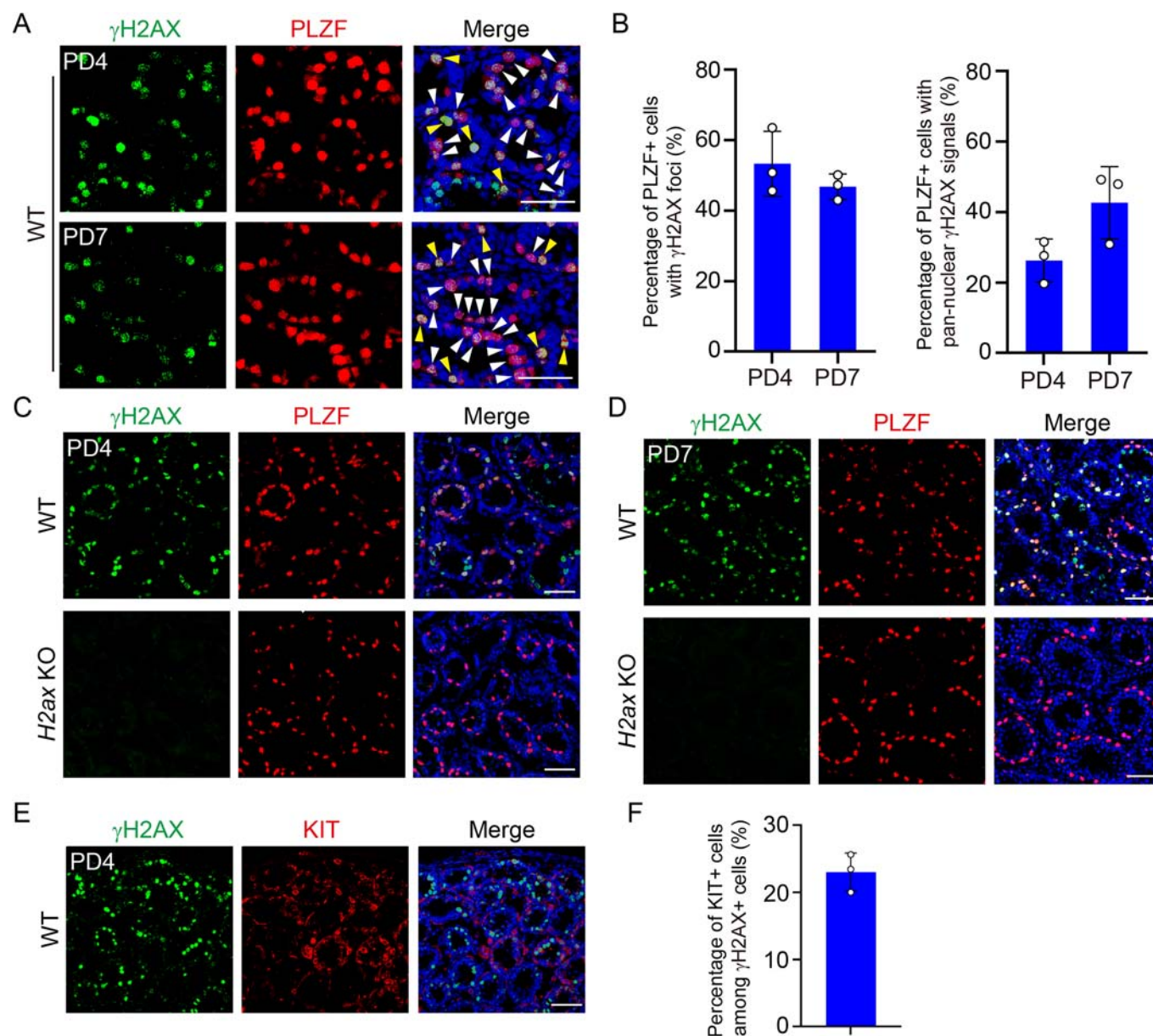


Figure EV2. γ H2AX signals are not observed during the formation of undifferentiated spermatogonia in *H2ax* KO mice.

(A) Representative images of γ H2AX and PLZF staining in frozen sections of testes from WT mice at PD4 and PD7. PLZF+ cells with γ H2AX+ foci were labeled by white arrowhead and PLZF+ cells with pan-nuclear γ H2AX+ signals were labeled by yellow arrowhead. (B) Statistical analysis of the percentage of PLZF+ cells with γ H2AX+ foci or with pan-nuclear γ H2AX+ signals in WT mice at PD4 and PD7. (C, D) IF staining of γ H2AX and PLZF in frozen sections of testes from WT and *H2ax* KO mice at PD4 (C) and PD7 (D). (E, F) IF staining of γ H2AX and KIT in frozen sections of testes from WT mice at PD4 and statistical analysis of the percentage of KIT+ cells among γ H2AX+ cells. Data information: Data are presented as mean $\pm$ SD. 3 mice were analyzed. Scale bar, 50 μ m.

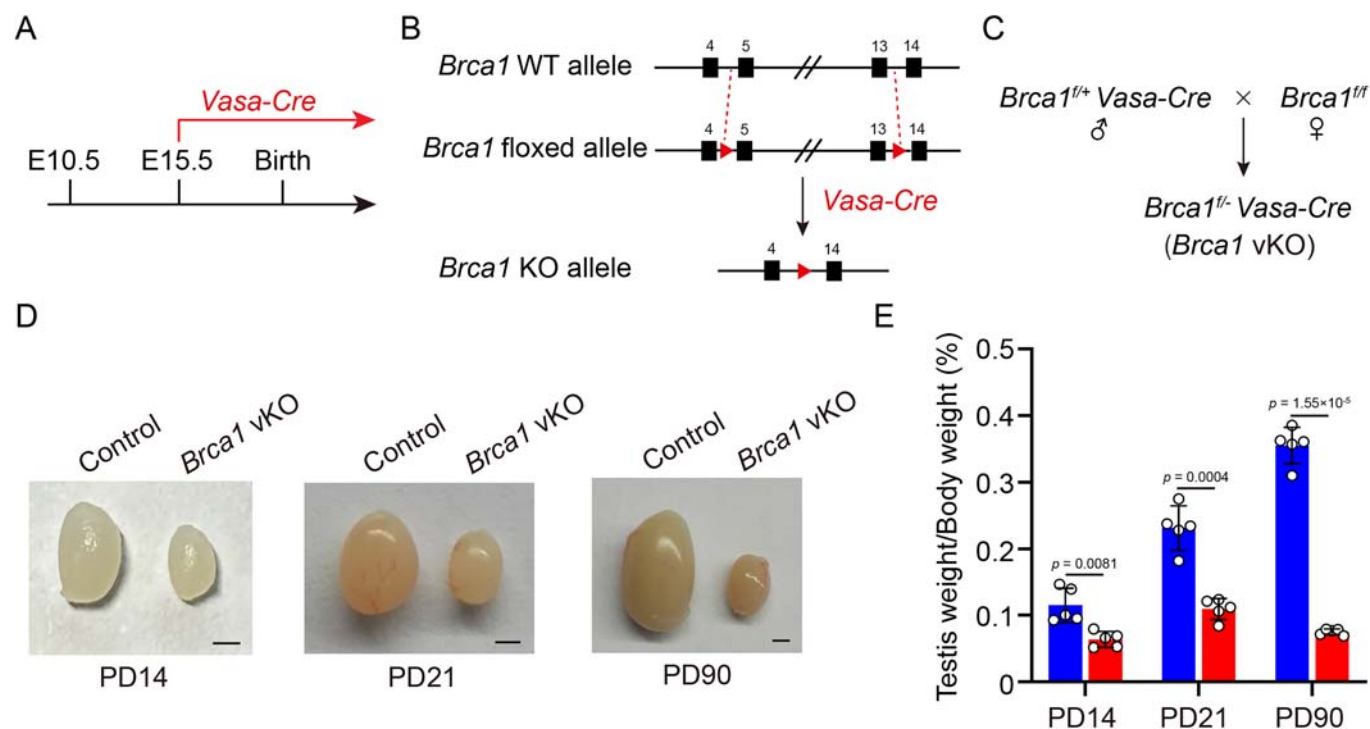
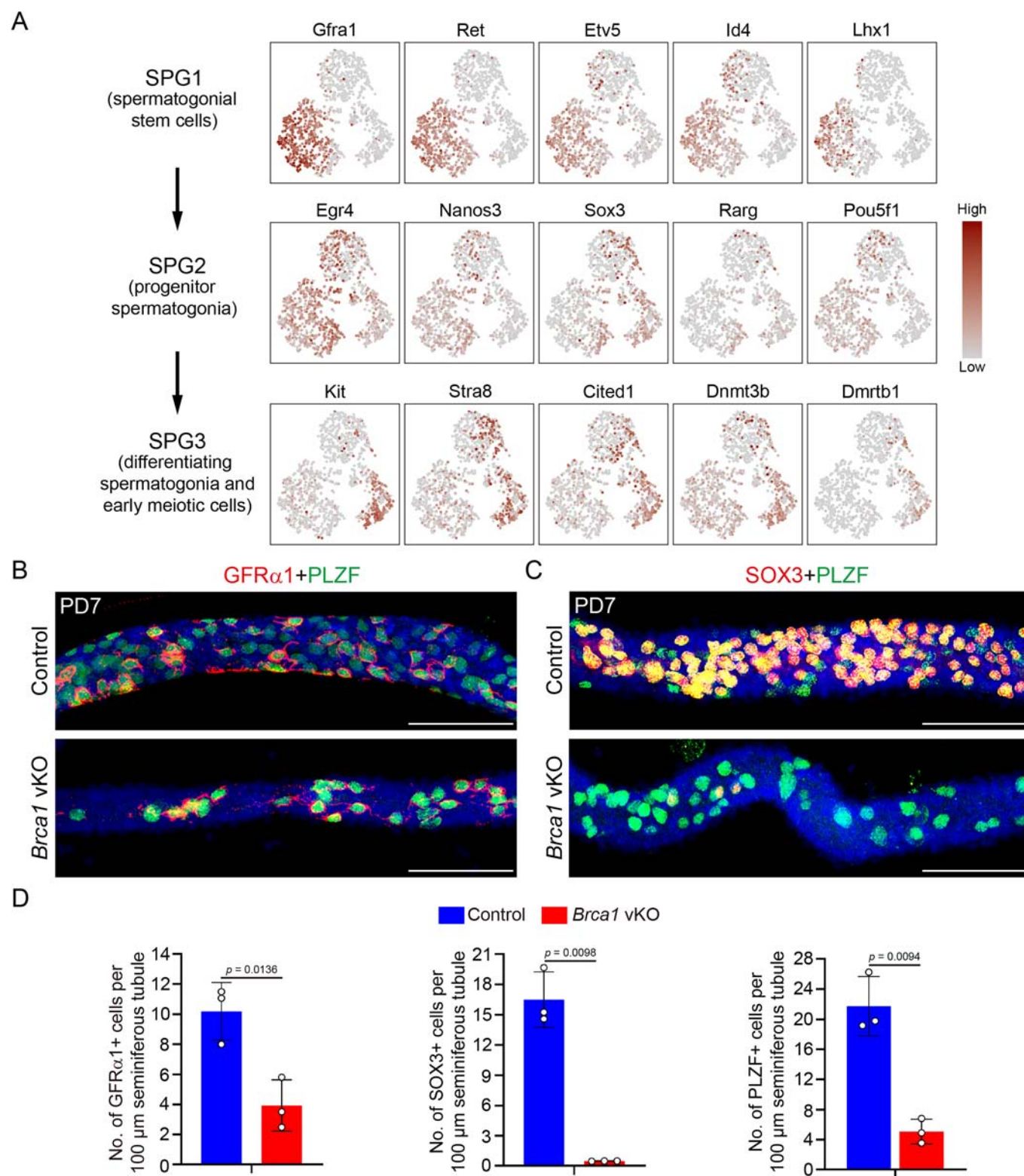


Figure EV3. Generation of *Brca1* germ cell-specific KO mice.

(A) Schematic diagram showing the expression timepoint of *Vasa-Cre* during mouse germ cell development. (B) Schematic illustrations of *Vasa-Cre* recombinase-mediated deletion of *Brca1* floxed allele. (C) Schematic illustrations of mating strategies to obtain *Brca1* vKO mice. (D) Representative images of testes from Control and *Brca1* vKO male mice at PD14, PD21, and PD90. Scale bar, 1 mm. (E) Statistical analysis of ratios of testis weight to body weight of Control and *Brca1* vKO male mice at PD14, PD21, and PD90. 5 mice of each genotype were analyzed. Data information: Data are presented as mean $\pm$ SD. *P* value, two-tailed unpaired Student's *t* test.



**Figure EV4. Progenitor spermatogonia have a much more dramatic reduction than spermatogonial stem cells after BRCA1 loss.**

(A) Gene expression patterns of selected marker genes for each subtype of germ cells. The tSNE plot is in reference to all germ cells (control + *Brca1* vKO). (B) Whole-mount IF staining of GFR α 1 and PLZF in seminiferous tubules of testes from Control and *Brca1* vKO male mice at PD7. (C) Whole-mount IF staining of SOX3 and PLZF in seminiferous tubules of testes from Control and *Brca1* vKO male mice at PD7. (D) Statistical analysis of the number of GFR α 1+, SOX3+ cells, and PLZF+ cells per 100 μ m seminiferous tubule in Control and *Brca1* vKO male mice at PD7. Three mice of each genotype were analyzed. Data information: Data are presented as mean $\pm$ SD. *P* value, two-tailed unpaired student's *t* test. Scale bar, 100 μ m.

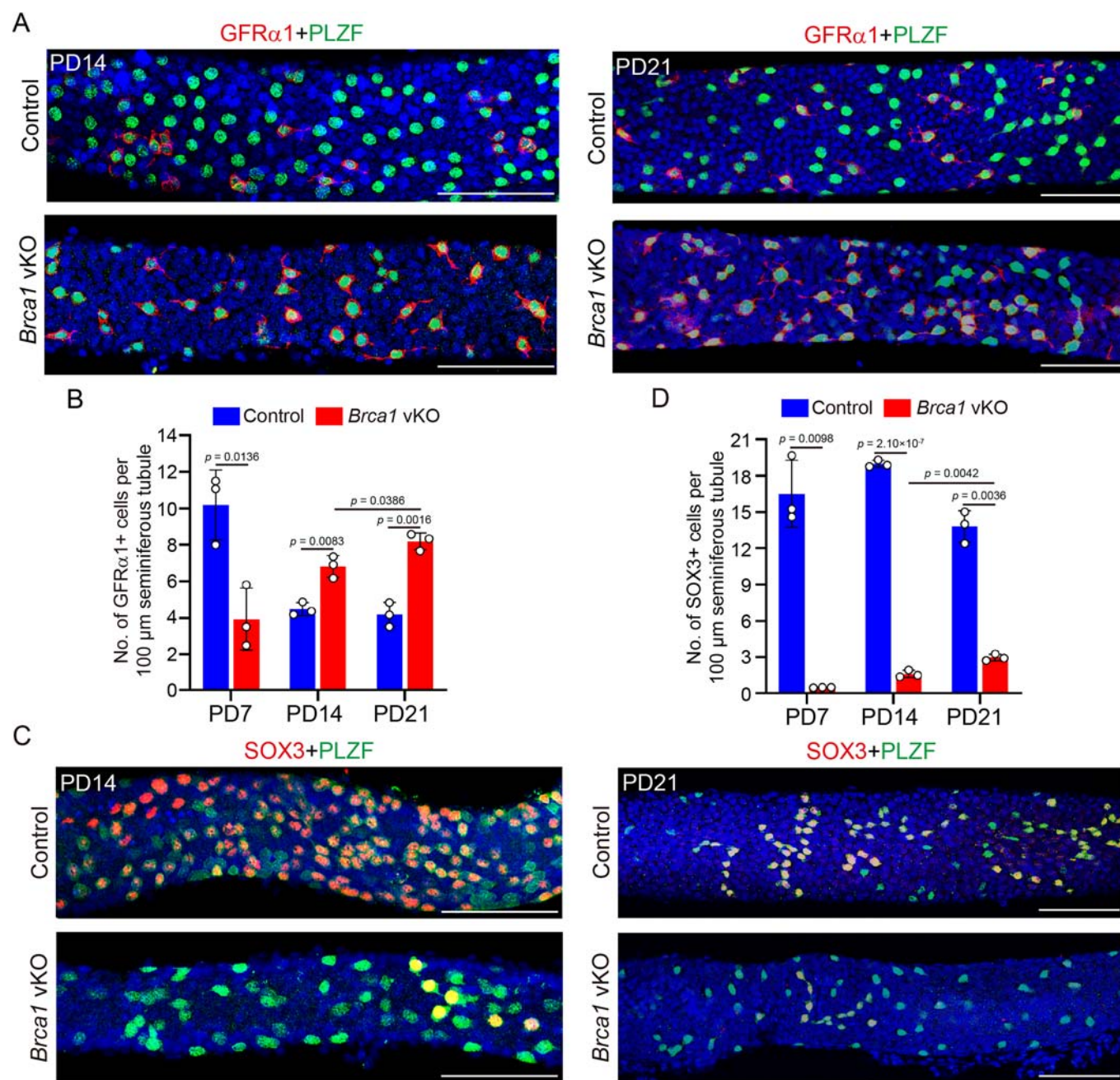


Figure EV5. The number of spermatogonial stem cells gradually increases in *Brca1* vKO testes.

(A) Whole-mount IF staining of GFR α 1 and PLZF in seminiferous tubules of testes from Control and *Brca1* vKO male mice at PD14 and PD21. (B) Statistical analysis of the number of GFR α 1+ cells per 100 μ m seminiferous tubule in testes from Control and *Brca1* vKO male mice at PD7, PD14, and PD21. Three mice of each genotype were analyzed. (C) Whole-mount IF staining of SOX3 and PLZF in seminiferous tubules of testes from Control and *Brca1* vKO male mice at PD14 and PD21. (D) Statistical analysis of the number of SOX3+ cells per 100 μ m seminiferous tubule in testes from Control and *Brca1* vKO male mice at PD7, PD14, and PD21. Three mice of each genotype were analyzed. Data information: Data are presented as mean $\pm$ SD. *P* value, two-tailed unpaired Student's *t* test. Scale bar, 100 μ m.