Supplemental Material

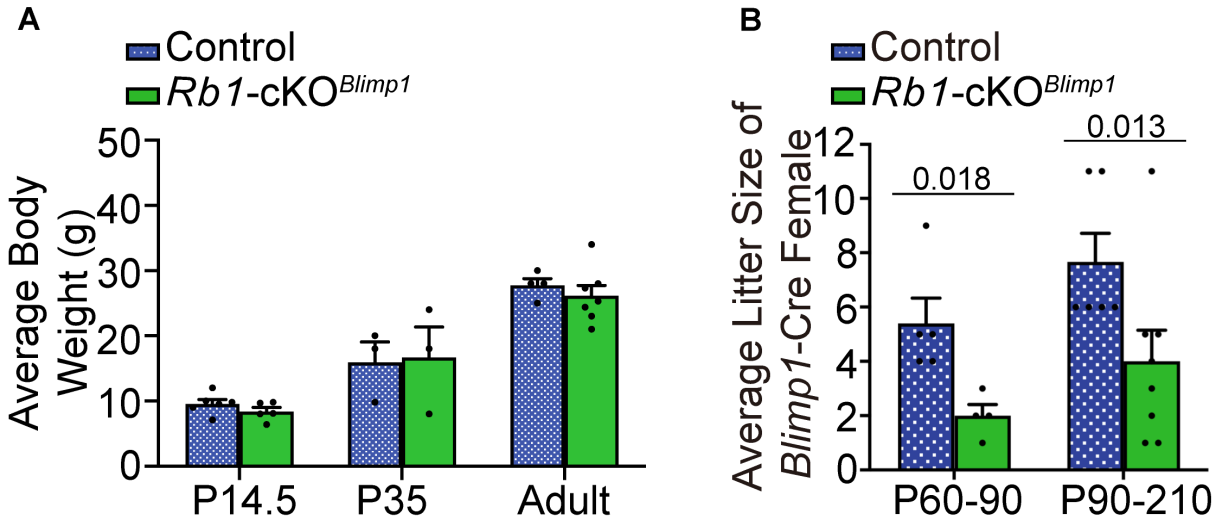

Fig. S1. Phenotypic features of control and *Rb1-cKO*^{Blimp1} mice at different stages of development. (*A*) Body weights for control and *Rb1-cKO*^{Blimp1} male mice. Data are mean±SEM for n=3-7 different males at each age point and dots represent values of individual mice. (*B*) Fertility Assessment of *Rb1*-cKO^{Blimp1} females. Litter sizes for control and *Rb1*-cKO^{Blimp1} females at the ages of P60-90 and P90-210 following mating with wild-type males. Data are presented as mean±SEM from n=4-8 litters and * denotes significantly different at $P \leq 0.05$. For both A and B, statistical comparisons were made using Mann-Whitney U tests and * denotes significantly different at $P \leq 0.05$.

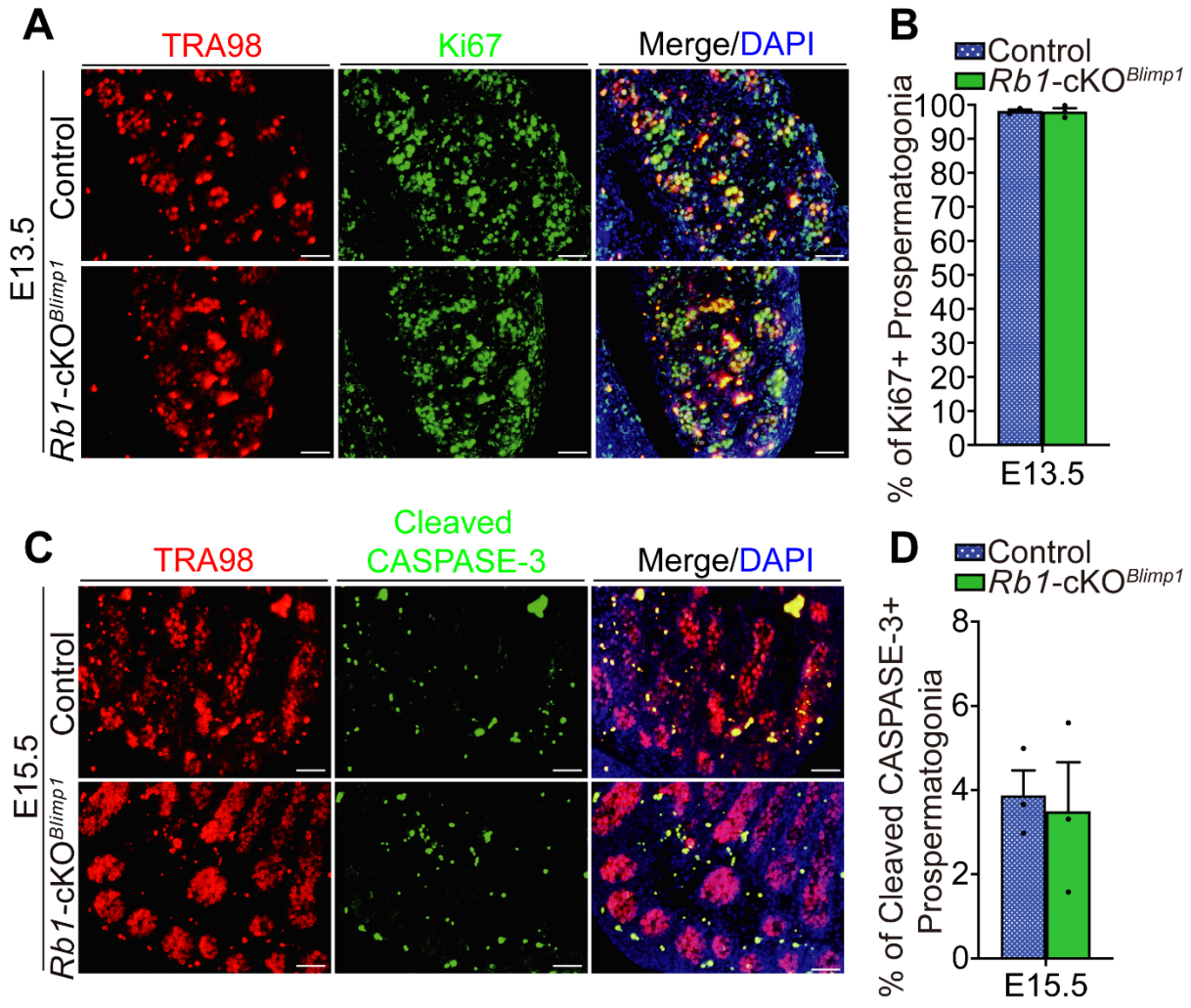

Fig. S2. Impacts of prospermatogonial development following *Rb1* inactivation at the PGC stage. (*A and B*) Representative images of immunofluorescence staining (*A*) for the germ cell marker TRA98 (red) and proliferation marker Ki67 (green), and quantification of the percentage of proliferating (Ki67+) prospermatogonia (*B*) in cross-sections of gonads from control and *Rb1*-cKO^{Blimp1} fetuses at E13.5. (*C and D*) Representative images of immunofluorescence staining (*C*) for the apoptotic marker cleaved CASPASE-3 (green) and TRA98 (red) and quantification of the percentage of apoptotic (Cleaved CASPASE3+) prospermatogonia (*D*) in cross-sections of gonads from control and *Rb1*-cKO^{Blimp1} fetuses at E15.5. For *A* and *C*, cell nuclei are labeled by DAPI staining (blue) and scale bars = 50 µm. For B and D, data are mean±SEM for n=3 different fetuses of each genotype and statistical comparisons were made using unpaired Student's *t*-tests.

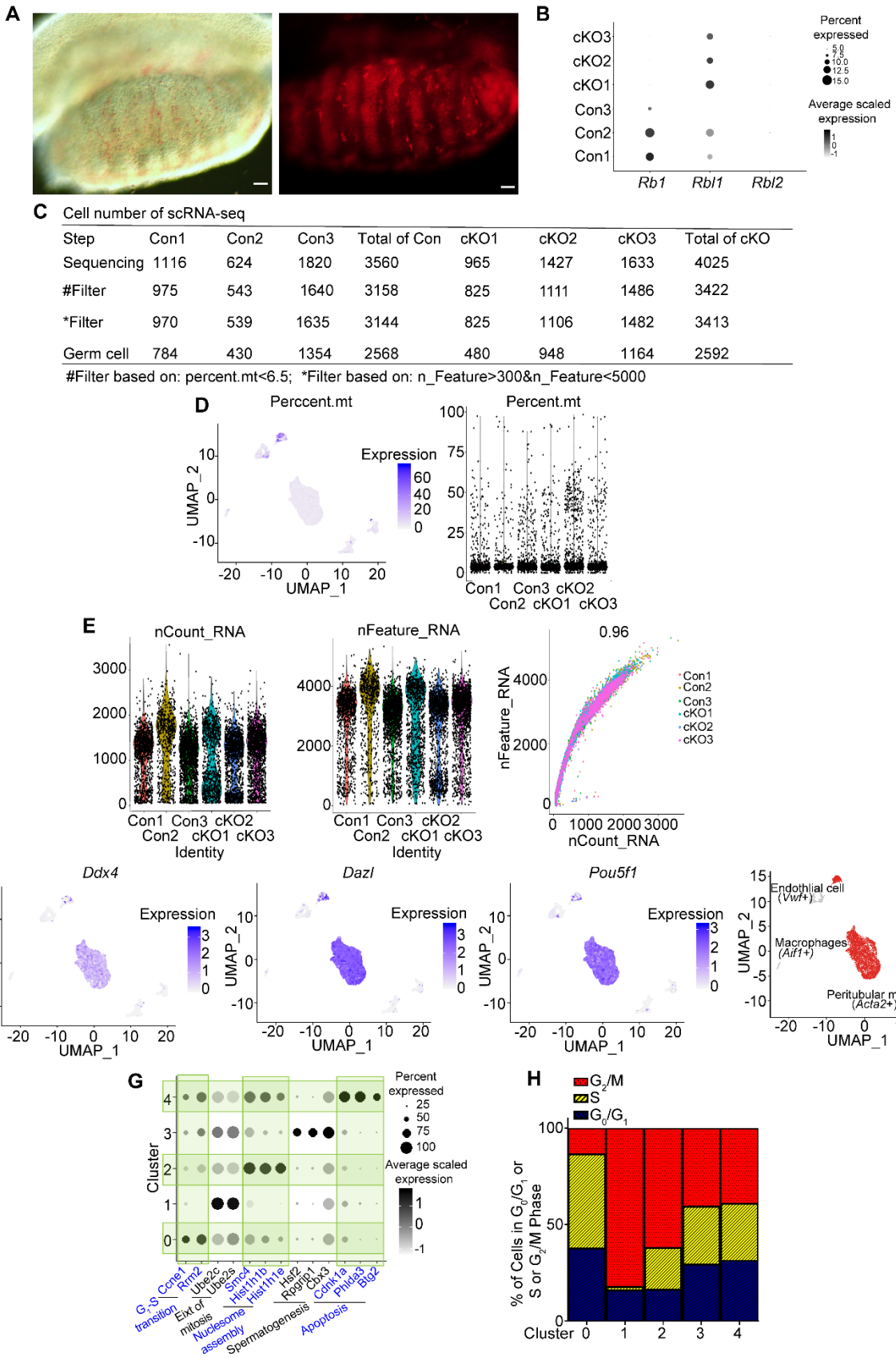

Fig. S3. Quality control and downstream analyses from single-cell RNA-sequencing of isolated E14.5 germ cells from control and *Rb1*-cKO^{Blimp1} mice. (*A*) Representative images of *tdTomato* fluorescent germ cells in multi-transgenic mouse gonads. Scale bar = 50 µm. (*B*) Dotplot representation of *Rb1*, *p107*, and *p130* gene expression for each control and *Rb1-cKO*^{Blimp1} prospermatogonial library. Data are average scaled expression (color gradient) and the percentage of cells with detectable expression (dot size). (*C*) Table of metrics for single cell RNA-seq (scRNA-seq) analysis with prospermatogonia isolated from *Rb1-cKO*^{Blimp1} and control mice at E14.5. (*D*) Uniform manifold approximation and projection (UMAP) and Violin plot representation for percent mitochondrial gene (percent.mt) representation of six prospermatogonial libraries from *Rb1-cKO*^{Blimp1} and control mice. (*E*) Violin plot and FeatureScatter representations for the number of unique molecular identifiers (nCount_RNA) and genes (nFeature_RNA) of six prospermatogonial libraries from *Rb1-cKO*^{Blimp1} and control mice. (*F*) UMAP representation for expression of germ cell marker genes (*Ddx4*, *Dazl* and *Pou5f1*) or Somatic cell marker genes across six prospermatogonial libraries from *Rb1-cKO*^{Blimp1} and control mice. (*G*) Dotplot representation of average scaled expression (color gradient) and the percentage of cells within each UMAP defined cluster with detectable expression (dot size) for marker genes associated with cellular processes. (*H*) Percentage of prospermatogonia in different UMAP defined clusters of control and *Rb1-cKO*^{Blimp1} mice that were determined to be in G_0/G_1, S or G_2/M phases of the cell cycle.

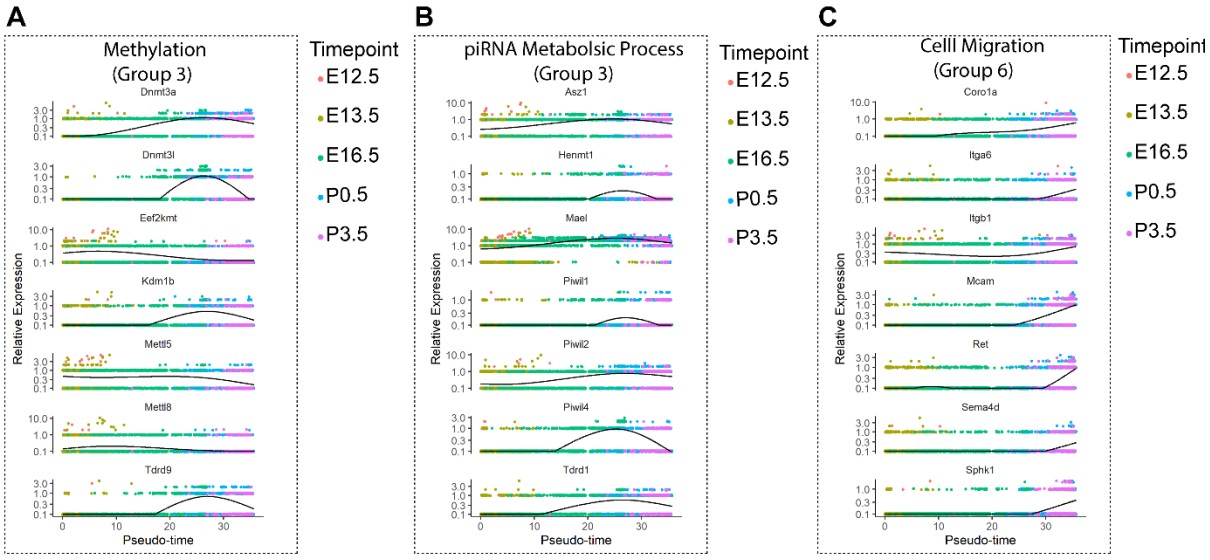

Fig. S4. Trajectory analysis of well-described cellular processes during quiescence. (*A-C*) Temporal expression of genes associated with DNA methylation (*A*), piRNA metabolism (*B*), and cell migration (*C*) along the normal developmental trajectory in mice organized as pseudo-time.

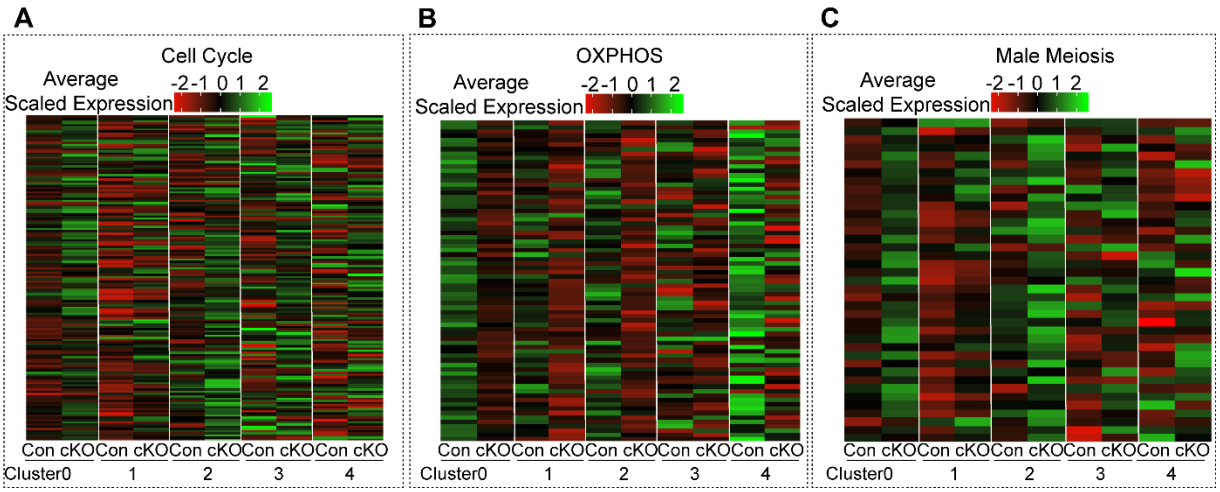

Fig. S5. Effects of disrupted entry into quiescence on prospermatogonial metabolism and meiosis. (*A*-*C*) Heatmap representation of scaled differentially expressed genes that positively regulate cell cycle progression (*A*), oxidative phosphorylation (OXPHOS) (*B*), and male meiosis (*C*) in prospermatogonial clusters (defined by scRNA-seq analysis) of control and *Rb1*-cKO^{Blimp1} mice at E14.5. Data are summarized in Fig. 5C-E.

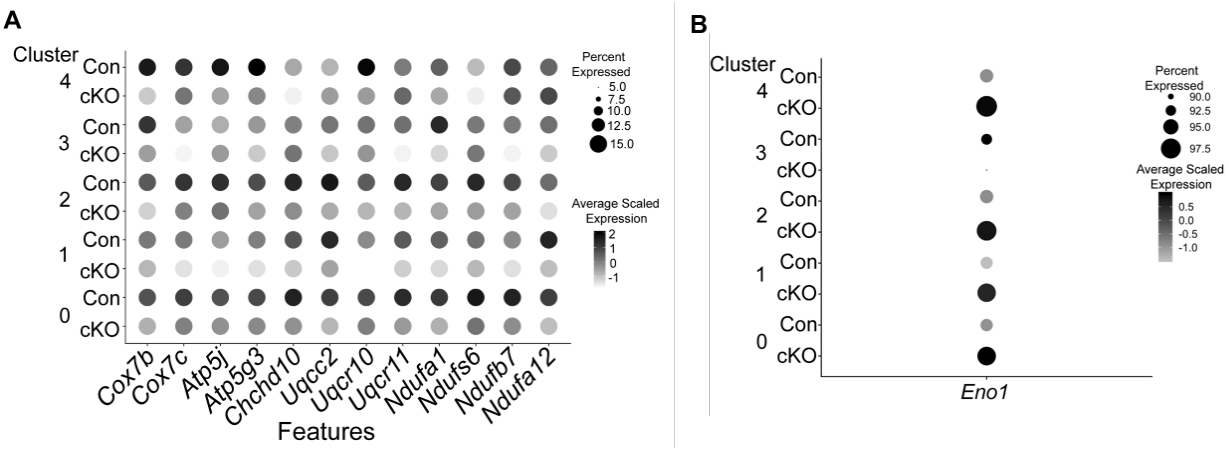

Fig. S6. Dotplot expression profiles for genes associated with OXPHOS (*A*) and the glycolytic enzyme gene *Eno1* (*B*) in *Rb1*-cKO^{Blimp1} and control prospermatogonia at E14.5. Data are derived from scRNA-seq analysis of 3 different control (Con) and *Rb1*-cKO^{Blimp1} (cKO) libraries each.

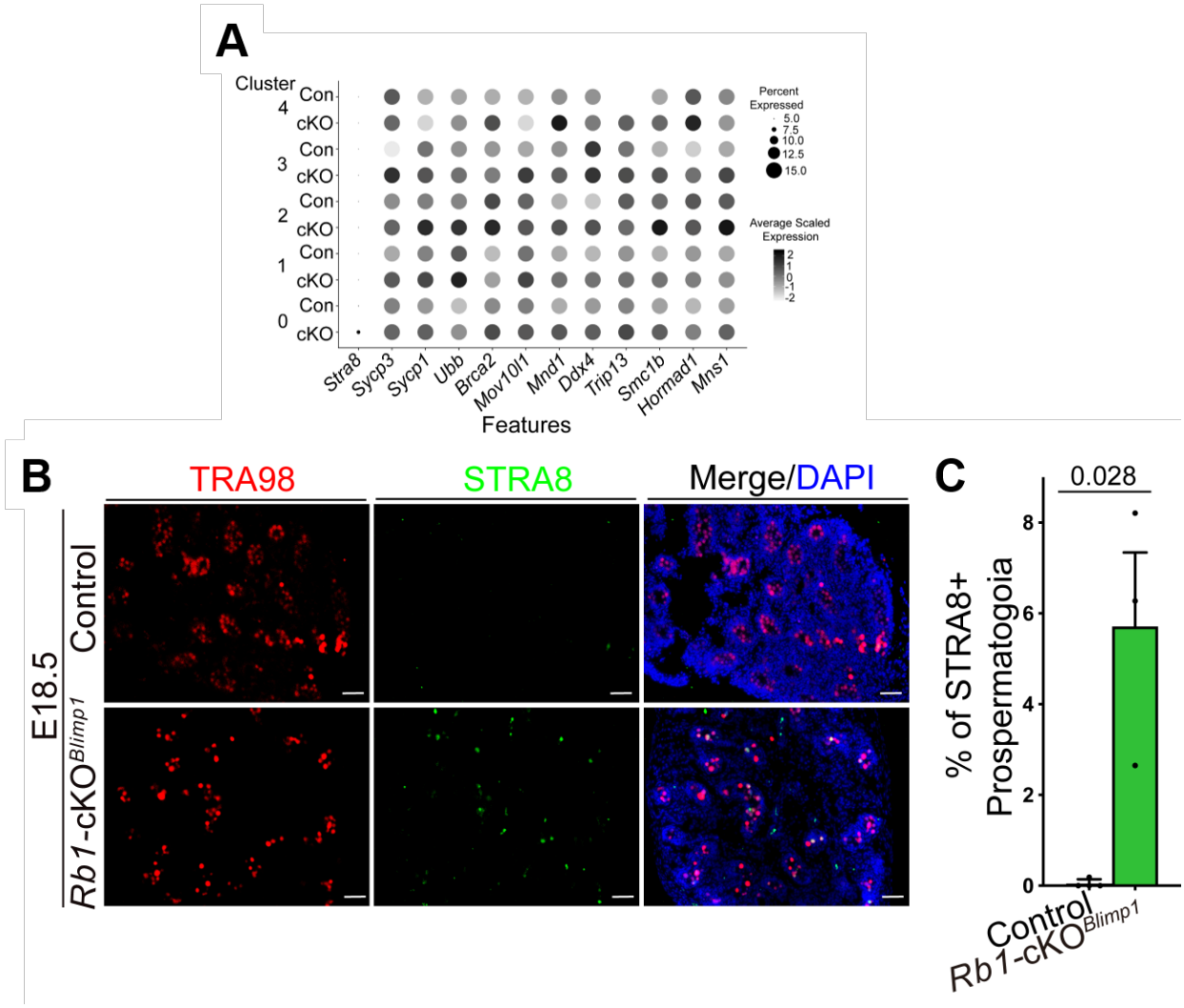

Fig. S7. Assessment of STRA8+ germ cells in cross-sections of testes from control and *Rb1-cKO*^{Blimp1} mice at E18.5. (*A*) Dotplot expression profile of genes associated with meiosis in *Rb1*-cKO^{Blimp1} and control prospermatogonia at E14.5. Data are derived from scRNA-seq analysis of 3 different control (Con) and *Rb1*-cKO^{Blimp1} (cKO) libraries each. (*B*) Representative images of immunofluorescence staining for TRA98+ germ cells (red) and the meiotic marker STRA8 in cross-sections of tests from control and *Rb1-cKO*^{Blimp1} at E18.5. Cell nuclei are labeled with DAPI (blue) and scale bars = 50 µm. (*C*) Quantification of the percentage of STRA8+ germ cells in cross-sections of testes from control and *Rb1-cKO*^{Blimp1} at E18.5. Data are mean±SEM for n=3 different mice of each genotype and dots represent data points of individual mice. Statistical comparisons were made using an unpaired Student's *t*-test and * denotes significantly different at $P \leq 0.05$.

Table S1. Gene lists of heatmaps.

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Table S2. Differentially expressed genes (DEGs) between control and *Rb1*-cKO^{Blimp1} mice at E14.5 for each cluster defined by scRNA-seq analysis.

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Table S3. Mitochondrial RNA reads across scRNA-seq transcriptome libraries generated from prospermatogonia of Rb1-cKOBlimp1 and control mice at E14.5.

Condition_Cluster	Mean±Std
0_Con	3.82±0.73
0_cKO	3.87±0.75
1_Con	3.75±0.71
1_cKO	3.89±0.74
2_Con	3.86±0.77
2_cKO	4.01±0.82
3_Con	3.78±1.10
3_cKO	3.96±0.89
4_Con	3.88±0.91
4_cKO	4.15±0.76

Table S4. Primers sequence used RT-PCR and genotyping analyses.

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