

Supplemental Data

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Supplemental Figure S6. Occupancy Input, H2AK119ub, and BMI1 around TSSs in wild-type GS cells.

Supplemental Figure S7. Localization of SALL4 and RNF2 during spermatogenesis.

Supplemental Figure S8. ChIP-seq enrichment and gene expression profiles of 6 clusters of genes identified by k-means clustering.

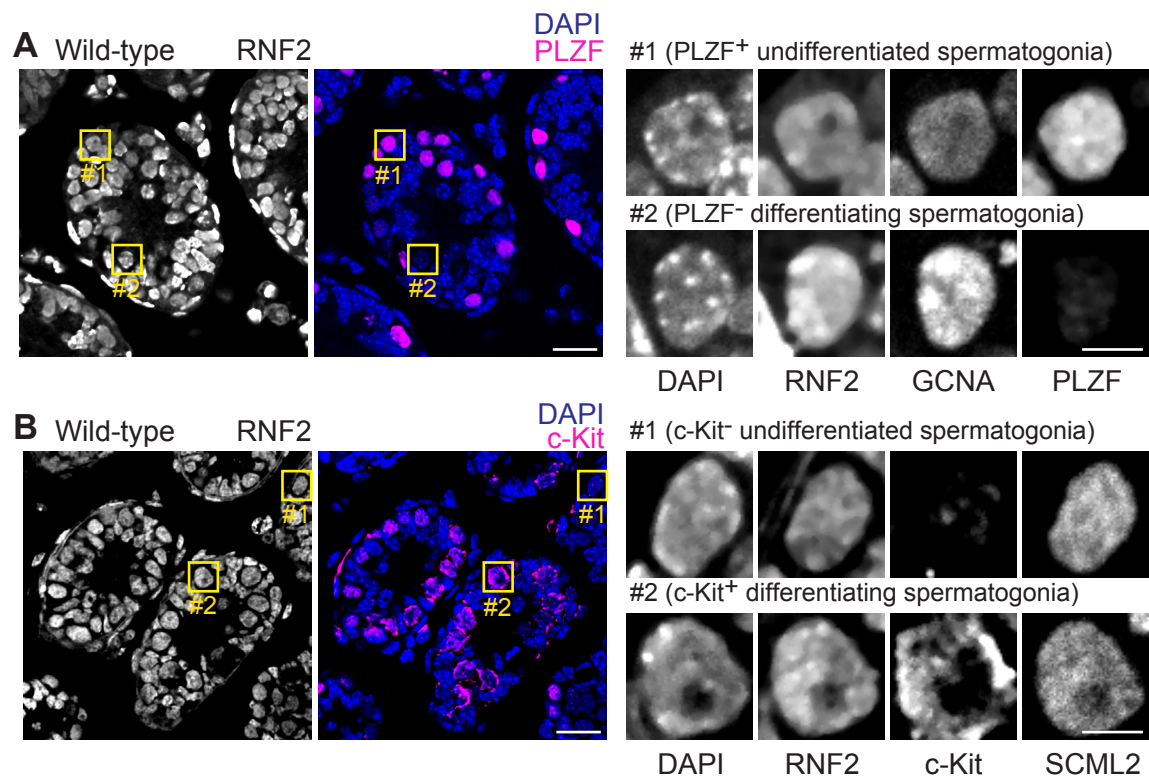
Antibody list

Supplemental Table 1 (separate file)

This table contains the gene list of PRC1-regulated genes based on RNA-seq analysis. This list also contains RNA-seq reads and *P*-values between PRC1ctrl and PRC1cKO at Thy1⁺ spermatogonia and c-Kit⁺ spermatogonia.

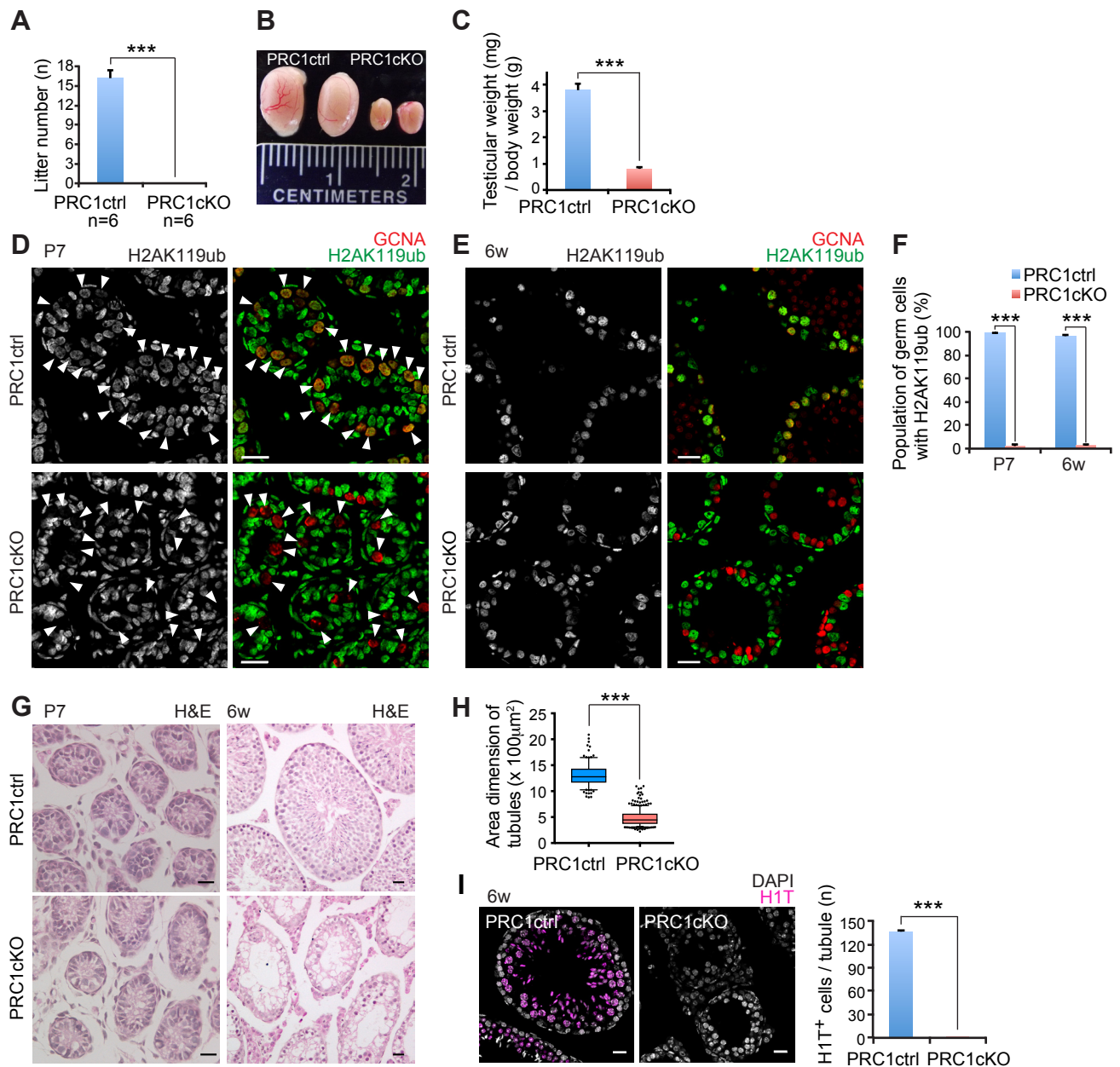
Supplemental Table 2 (separate file)

This table contains the gene list of each cluster identified by k-means clustering analysis of PRC1-regulated genes based on ChIP-seq analysis. This list also contains ChIP-seq reads (± 2 kb around TSS) in GS cells (RNF2, BMI1, H2Ak119ub) and cultured Thy1⁺ spermatogonia (SALL4).



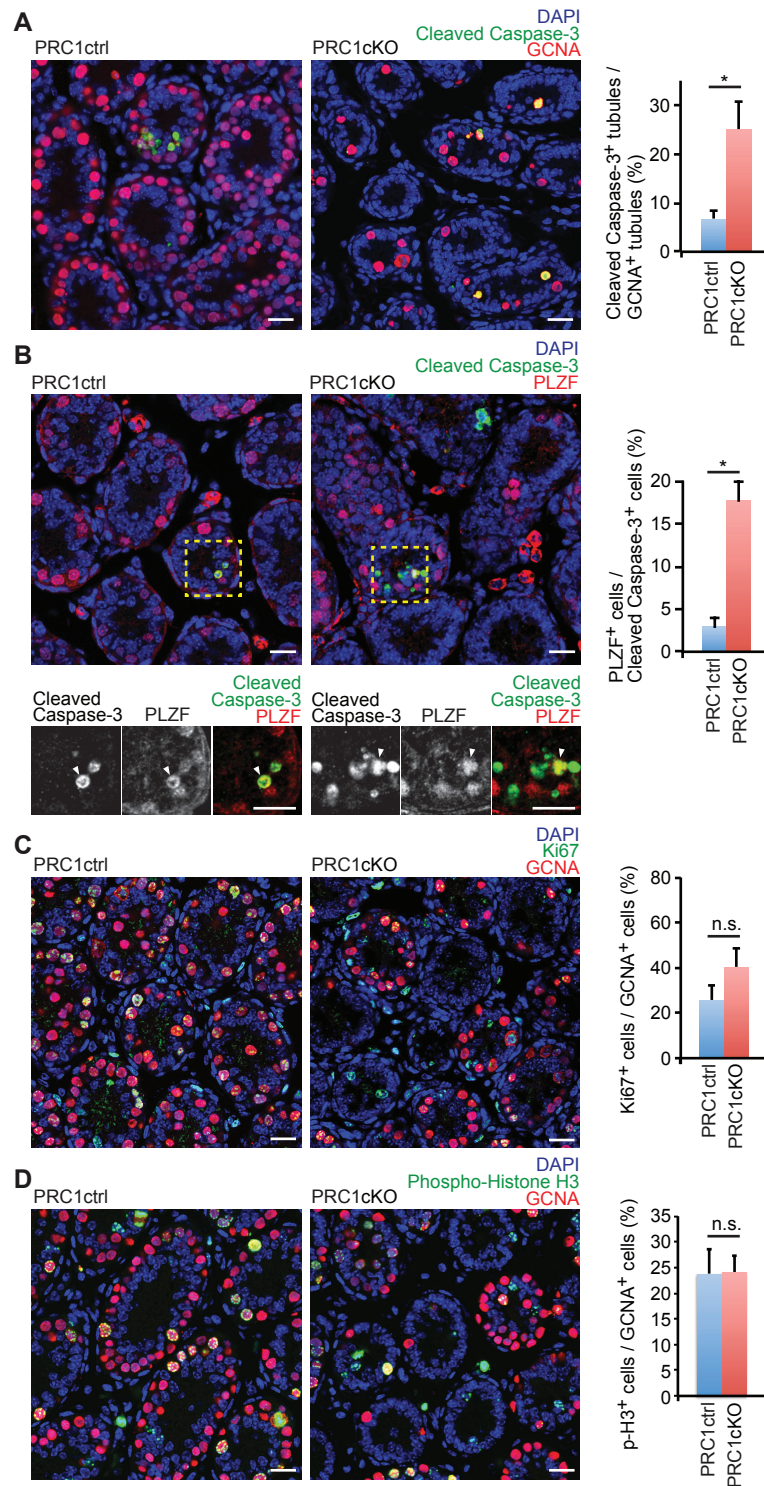
Supplemental Figure S1. Localization of RNF2 in spermatogenesis.

(A, B) Immunostaining of testicular sections at P7. Scale bars, 20 μm . The regions with yellow squares are magnified in panels shown on the right. Scale bars in magnified panels, 5 μm .



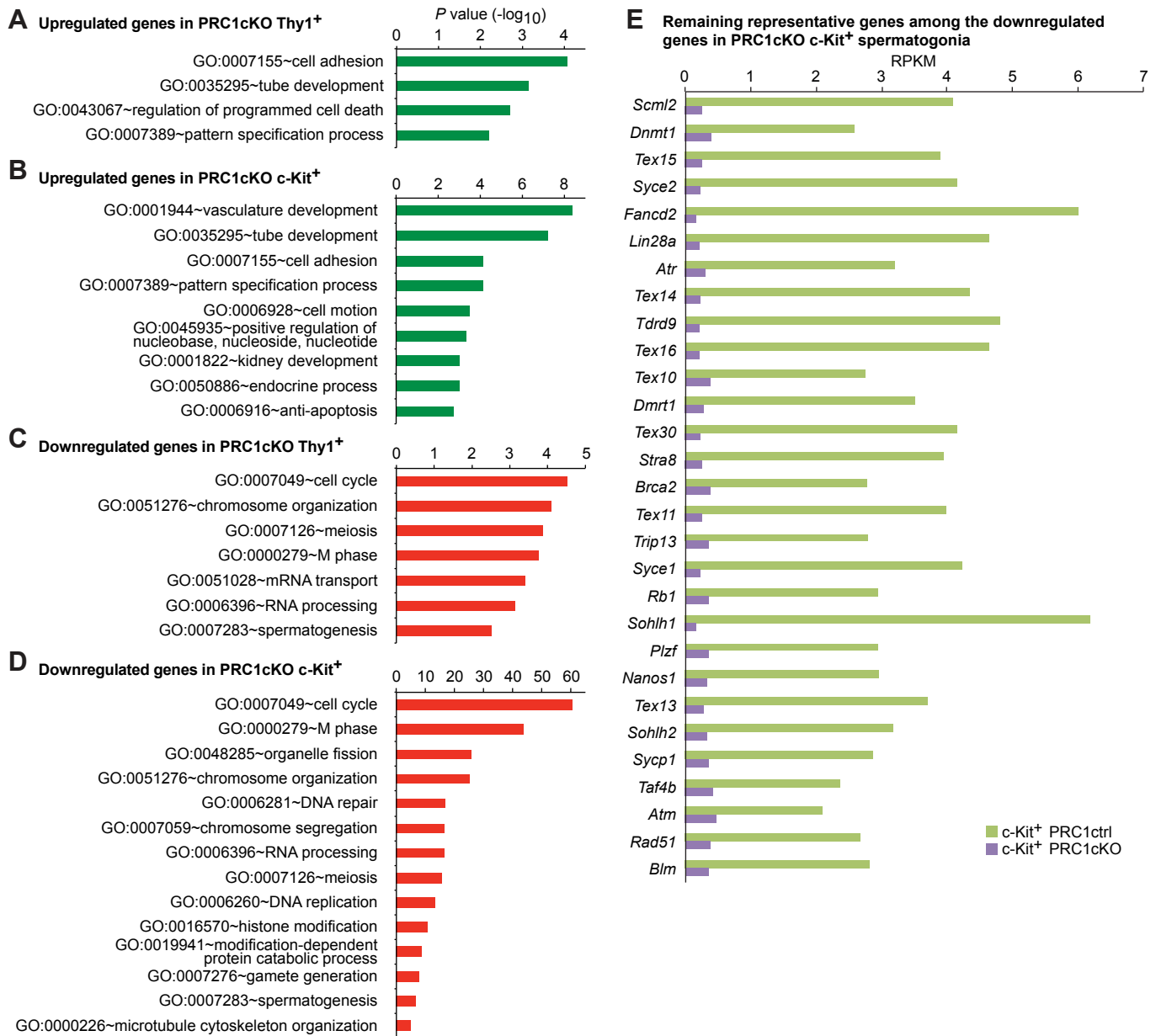
Supplemental Figure S2. Spermatogenic phenotypes of PRC1cKO testes.

(A) Litter sizes when PRC1ctrl or PRC1cKO males were mated with wild-type females. Data are represented as mean \pm s.e.m. Six independent mice were examined. (B) Picture of PRC1ctrl and PRC1cKO testes at 6 months. (C) Testicular weight per body weight of adult testes at the ages of 2-9 months. Data are represented as mean \pm s.e.m. Five and four independent mice were scored for the PRC1ctrl and PRC1cKO, respectively. (D, E) Immunostaining of H2AK119ub and GCNA, a germ cell marker, in testicular sections at postnatal day 7 (P7) and at 6 weeks old (6w). GCNA-positive cells are shown with arrowheads. Scale bar, 20 μ m. (F) Quantitative data of the percentage of germ cells with H2AK119ub at P7 and 6w. Data are represented as the mean \pm s.e.m. Three independent mice were examined for each time point. (G) Histological testicular sections stained with hematoxylin and eosin. Scale bars, 20 μ m. (H) Area dimension of tubules in testes at 6 weeks. Center bars are medians; bottom and top bars are, respectively, 10% and 90% of data points; and bottom and top boxes are, respectively, 25% and 75% of data points. Three independent mice were examined. (I) Immunostaining of H1T in testicular sections at 6 weeks. Scale bars, 20 μ m. Quantitative data are shown in right-hand panels. Data are represented as mean \pm s.e.m. Three independent mice were examined for each antibody. *** $P < 0.0001$, unpaired t-test.



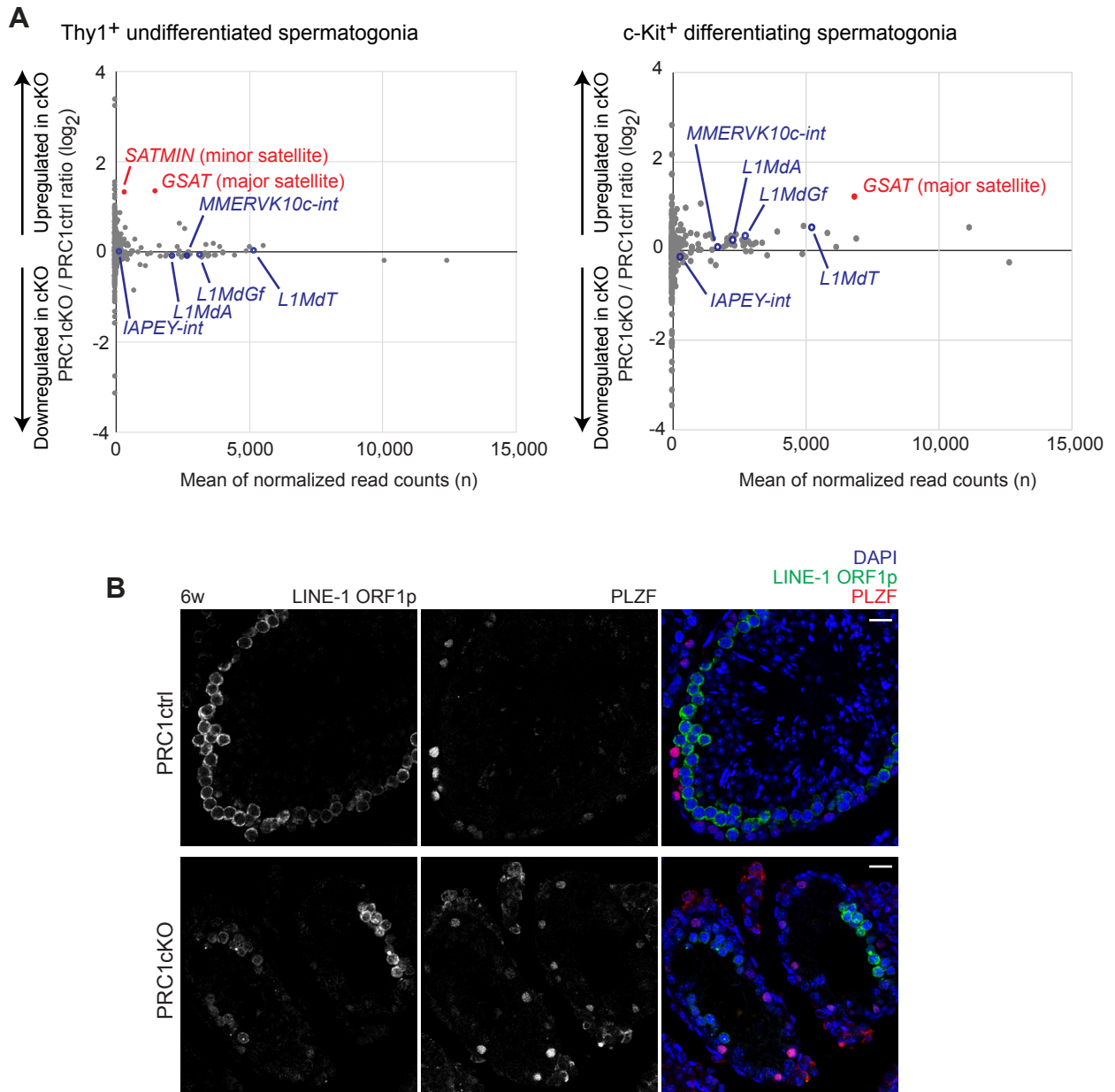
Supplemental Figure S3. Increased apoptosis in spermatogonia of PRC1cKO testes.

(A, B) Immunostaining of testicular sections at P7. Cleaved Caspase-3 is an apoptosis marker, GCNA is a germ-cell marker, and PLZF is an undifferentiated spermatogonia marker. Scale bars, 20 μ m. Quantitative data are shown in right-hand panels. Data are represented as mean \pm s.e.m. Three independent mice were examined. * $P < 0.05$, unpaired t-test. (C, D) Immunostaining of testicular sections at P7. Scale bars, 20 μ m. Quantitative data are shown in right-hand panels. Three independent mice were examined. Data are represented as mean \pm s.e.m. n.s. not significant, unpaired t-test.



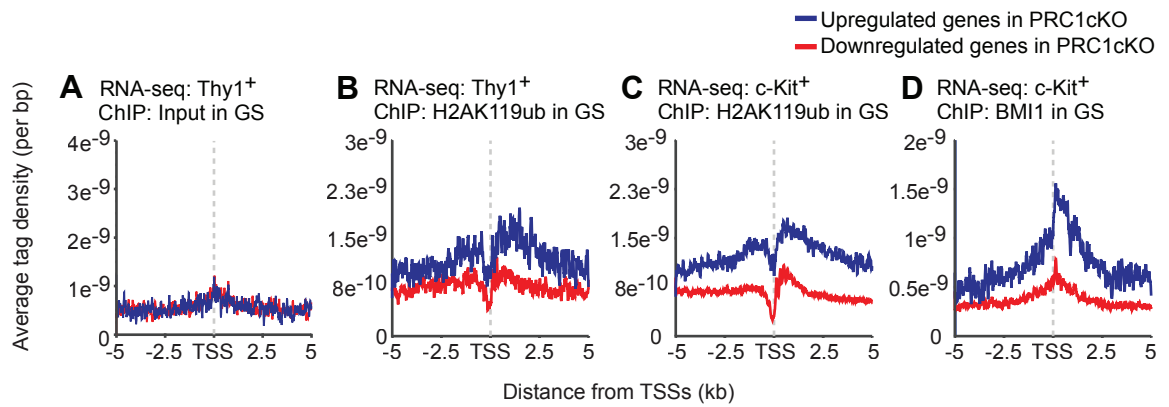
Supplemental Figure S4. Gene ontology analysis and examples of differentially expressed genes in PRC1cKO spermatogonia.

(A, B) Gene ontology analysis of genes upregulated in PRC1cKO spermatogonia. (C, D) Gene ontology analysis of genes downregulated in PRC1cKO spermatogonia. *P* value represents modified Fisher Exact *P* Value. (E) Expression profiles of representative genes downregulated in PRC1cKO c-Kit⁺ spermatogonia. 15 germline genes among the top 50 downregulated genes in c-Kit⁺ spermatogonia with the lowest *P* values are shown in Figure 2D, the remaining representative genes are shown here.



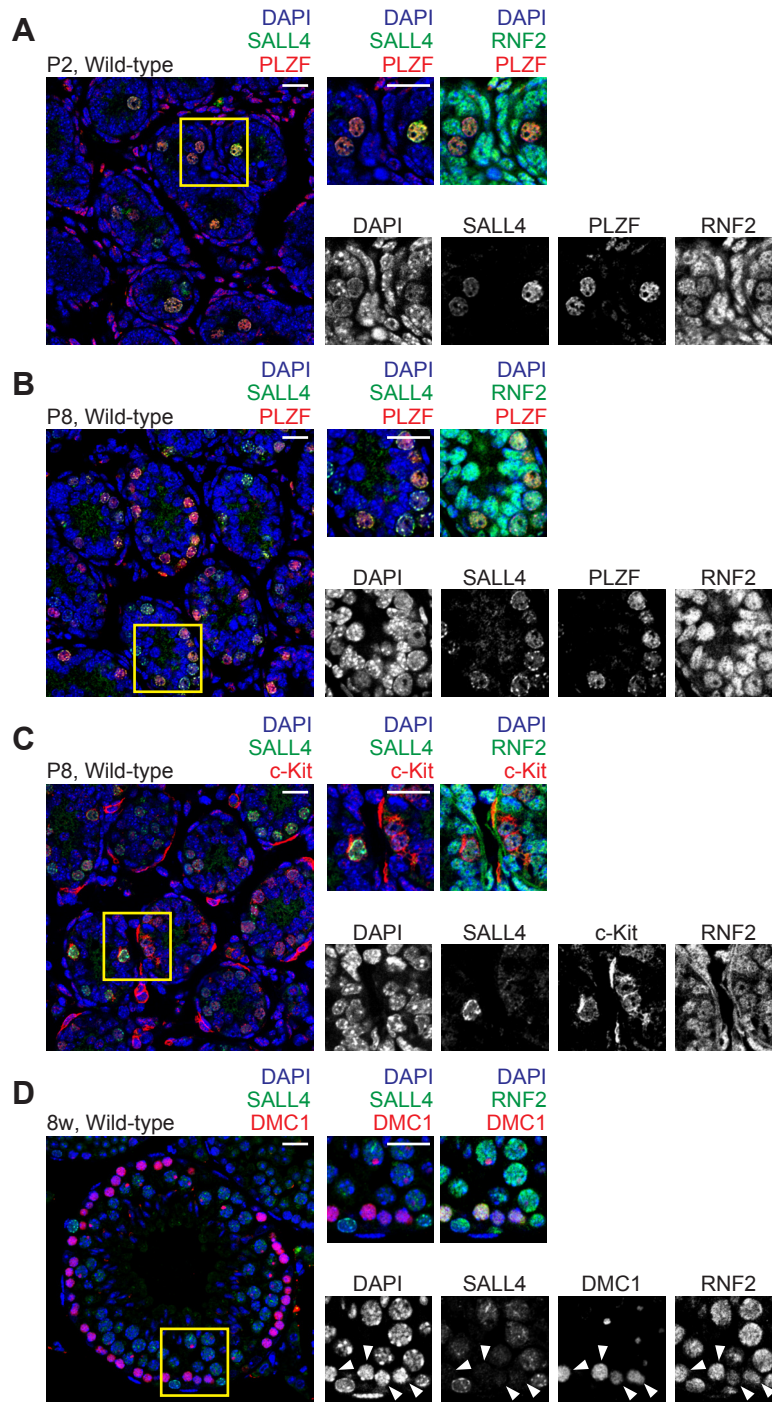
Supplemental Figure S5. Retrotransposons were not derepressed in PRC1cKO spermatogonia.

(A) RNA-seq analysis using Rebase. *GSAT* (major satellite) and *SATMIN* (minor satellite) were modestly derepressed in Thy1⁺ undifferentiated spermatogonia, and *GSAT* was modestly derepressed in c-Kit⁺ undifferentiated spermatogonia of the PRC1cKO (shown as red dots; $P < 0.05$, Wald test and > 2 fold change). Retrotransposons such as LINE, IAP, and ERVK10c were not derepressed in PRC1cKO spermatogonia (shown as blue dots). (B) Immunostaining of testicular sections at 6 weeks (6w) with LINE1 ORF1 protein (ORF1p) and PLZF, an undifferentiated spermatogonia marker. Scale bars, 20 μ m.



Supplemental Figure S6. Occupancy Input, H2AK119ub, and BMI1 around TSSs in wild-type GS cells.

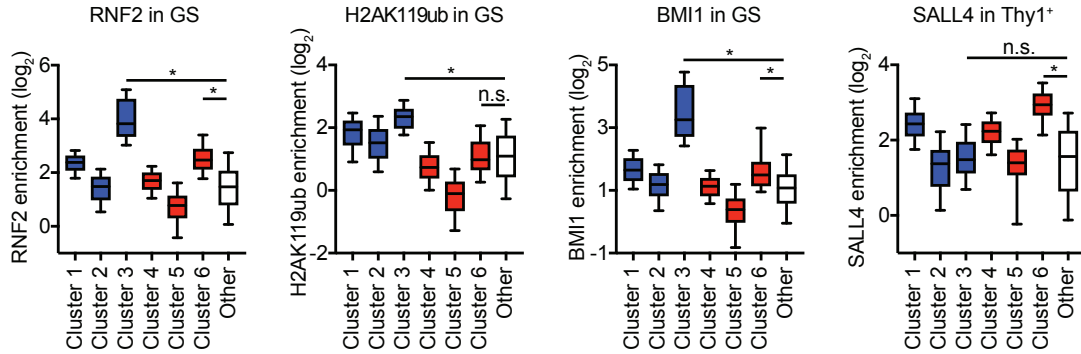
(A-D) Occupancy of Input, H2AK119ub, and BMI1 around TSSs in wild-type GS cells. ChIP-seq data of GS are shown for indicated genes based on RNA-seq analyses.



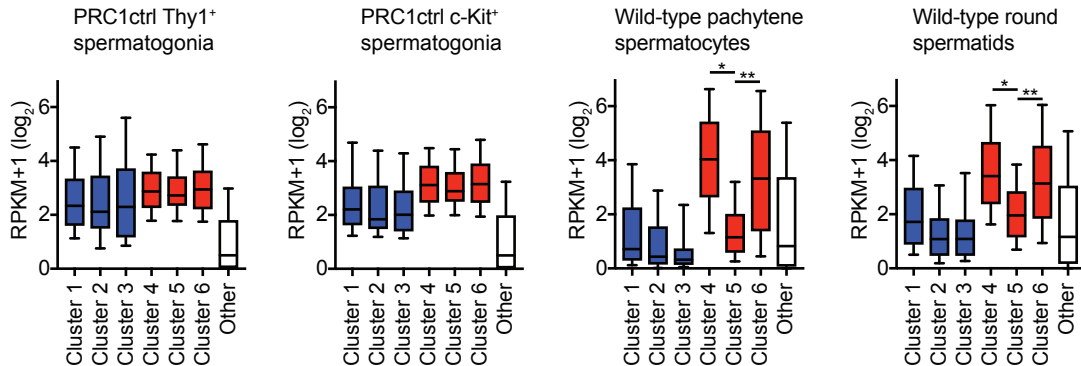
Supplemental Figure S7. Localization of SALL4 and RNF2 during spermatogenesis.

(A-D) Immunostaining of testicular sections at P2 (A), P8 (B, C), and 8 weeks (8w: D). DMC1-positive leptotene and zygotene spermatocytes are shown with arrowheads. Scale bars, 20 μ m. The regions with yellow squares are magnified in right panels. Scale bars in magnified panels, 20 μ m.

A ChIP-seq enrichment in GS or cultured Thy1⁺ spermatogonia



B Gene expression during spermatogenesis



Supplemental Figure S8. ChIP-seq enrichment and gene expression profiles of 6 clusters of genes identified by k-means clustering.

(A) Distribution of ChIP-seq enrichment in each cluster of genes identified by k-means clustering shown in Figure 5B. “Other” represents all other genes that do not belong to these clusters. Center bars are medians; bottom and top bars are, respectively, 10% and 90% of data points; and bottom and top boxes are, respectively, 25% and 75% of data points. (B) Distribution of RNA-seq RPKM enrichment during representative stages of spermatogenesis in each cluster of genes identified by k-means clustering shown in Figure 5B. Other represents all other genes that do not belong to these clusters. * $P < 0.05$, ** $P < 0.001$, unpaired t-test.

Antibody list

Name	Origin	Applications
Rabbit anti-H2AK119ub	Cell Signaling	IHC
Rat anti-GCNA	Abcam	IHC
Rabbit anti-PLZF	Santa Cruz	IHC
Mouse anti-PLZF	Santa Cruz	IHC
Mouse anti- γ H2AX	Millipore	IHC
Rabbit anti-SALL4	Abcam	IHC, IP
Guinea Pig anti-H1T	Gift from Dr. Mary Ann Handel	IHC
Goat anti-c-Kit	R&D systems	IHC
Mouse anti-RNF2	MBL	IHC, IP
Rabbit anti-Ki67	Millipore	IHC
Rabbit anti-phospho-H3	Cell Signaling	IHC
Rabbit anti-LINE1-ORF1p	Gift from Dr. Alex Bortvin	IHC
Goat anti-DMC1	Santa Cruz	IHC
Rabbit anti-Cleaved Caspase-3	Cell Signaling	IHC