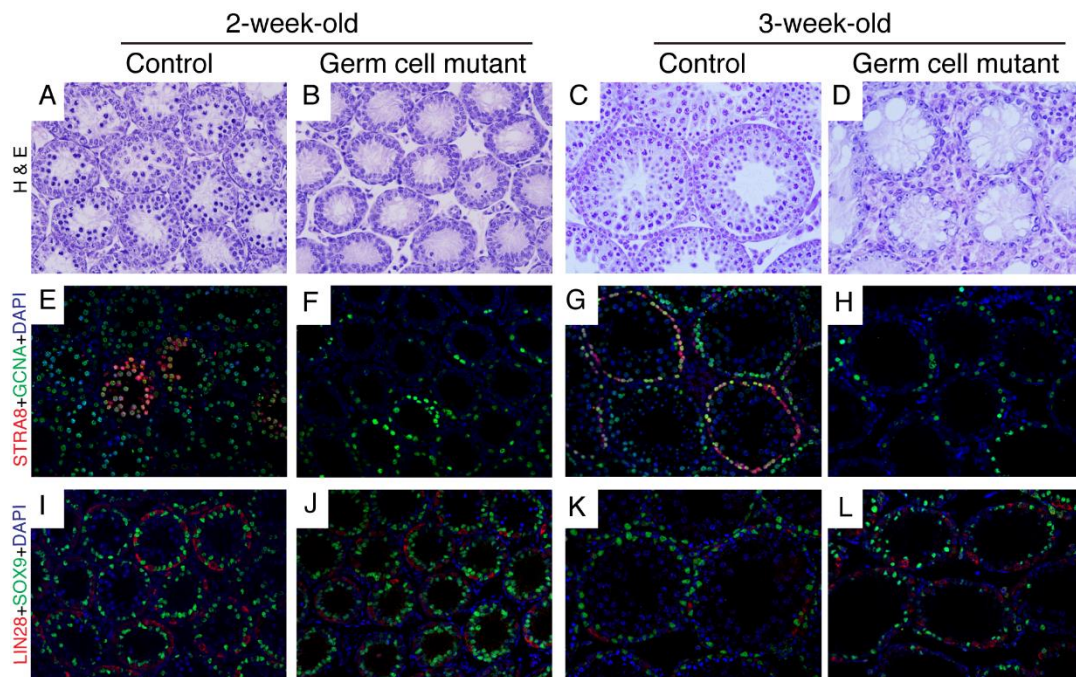
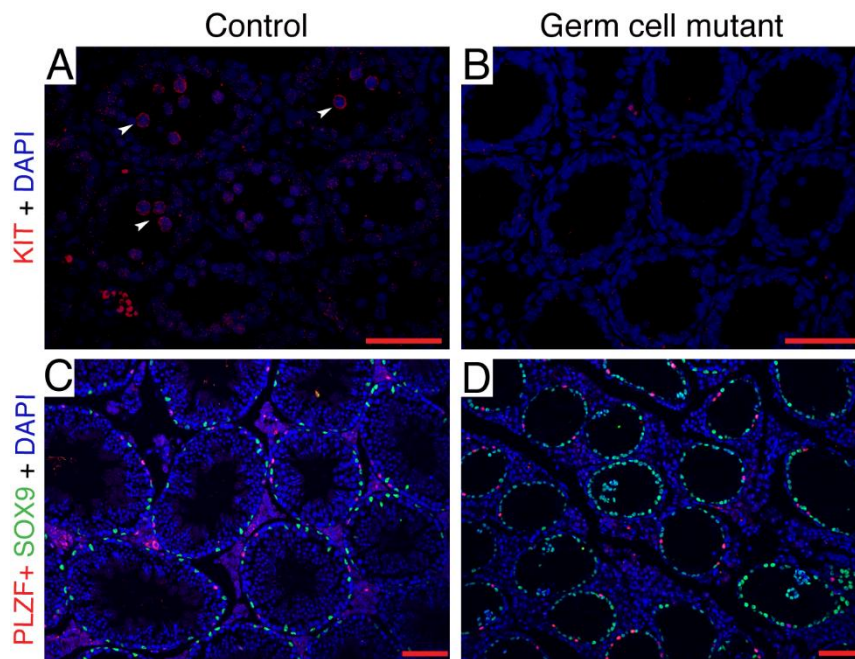


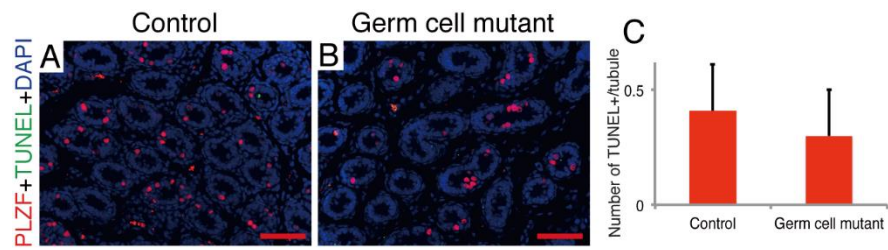
**Figure S1.** (A) Schematic diagram of dnRAR transgene allele. (B) X-Gal staining of testis from germ cell mutants ( $dnRAR^{lox/lox}$ ,  $Stra8-Cre^+$ ,  $RARE/lacZ$ ) (A'), controls ( $dnRAR^{lox/lox}$ ,  $RARE/lacZ$ ) (B'), and heterozygous germ cell mutants ( $dnRAR^{lox/+}$ ,  $Stra8-Cre^+$ ,  $RARE/lacZ$ ) (C') at 6-week-old. (C) A lacZ-stained section from a control, heterozygous germ cell mutant, and germ cell mutant testes. Cells with a positive signal are shown in blue. Nuclei were counterstained with fast red. Scale bar: 20 $\mu$ m.



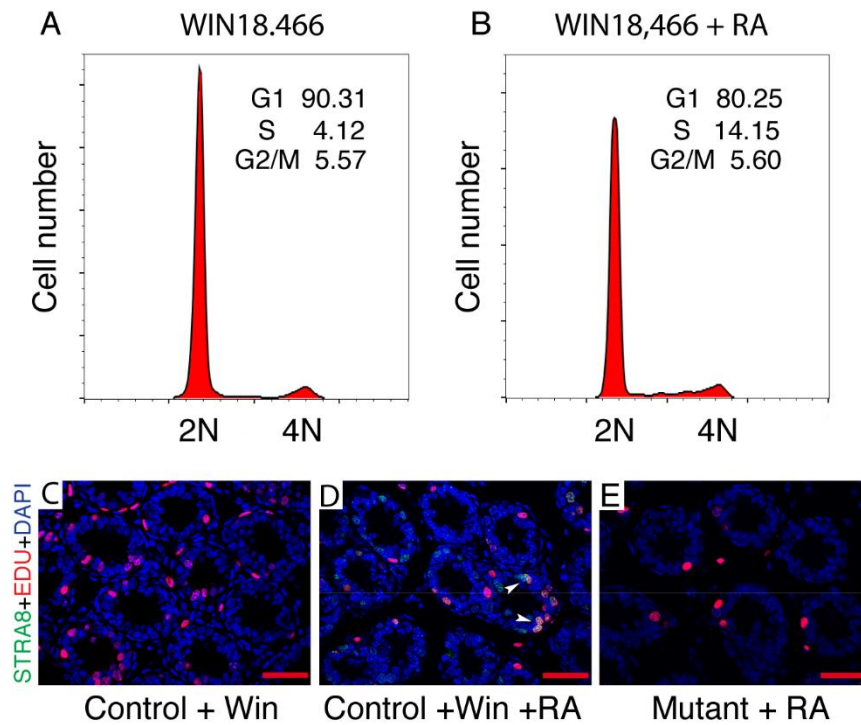
**Figure S2.** Complete blockage of spermatogonial differentiation in germ cell mutants at 2- and 3-week-old. (A-D) Hematoxylin and eosin staining of control (A and C) and germ cell mutant (B and D) testes. (E-H) Immunohistochemical staining for STRA8 (red) in sections of 2- and 3-week-old control (E and G) and germ cell mutant (F and H) testes. Costaining for a germ cell marker, GCNA (green). (I-L) Immunohistochemical staining for LIN28 (red) and SOX9 (green) in sections of 2- and 3-week-old control (I and K) and germ cell mutant (J and L) testes.



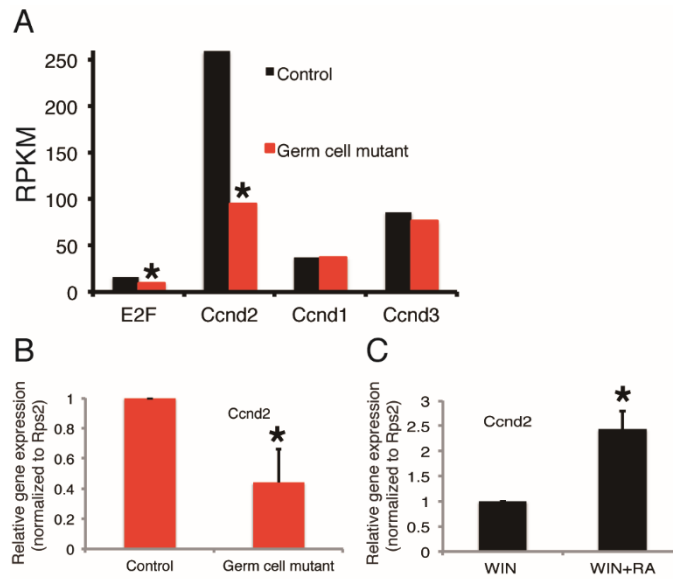
**Figure S3.** Impaired spermatogonial differentiation in germ cell mutant testes. (A and B) Immunofluorescence staining for KIT (red) in sections of control (A), germ cell mutant (B). (C and D) Immunofluorescence staining for PLZF (red) and SOX9 (green) in sections of control (C), germ cell mutant (D). Costaining with DAPI (blue). White arrowheads indicate representative germ cells (differentiating spermatogonia). *Scale bar*, 20µm.



**Figure S4.** (A and B) Detection of apoptotic cells (TUNEL assays, green signal) from testis of (A) control and germ cell mutant (B) testes. *Scale bar*, 10 $\mu$ m. (C) Quantification of apoptotic spermatogonia (PLZF positive cells) in control and germ cell mutant testes. n=3-4, P>0.1; Student t test.



**Figure S5.** (A and B) FACS analysis showing stimulation of RA on cell cycle progression in spermatogonia. The data (*inset*) shows one of the representative FACS. (C-E) Immunostaining for STRA8 (green) and EdU (red) in sections of WIN18,466-treated control (C), RA-injected WIN18,466-treated control (D), and RA-injected germ cell mutant (E) testes. Arrowheads indicate both STRA8 and EdU positive representative spermatogonia. *Scale bar*, 10 $\mu$ m.



**Figure S6.** Altered expression of genes for cell cycle progression in germ cell mutant spermatogonia. (A) Differential expression of genes for cell cycle progression between controls and germ cell mutants. (B) RT-qPCR analysis of mRNA levels of *Ccnd2* in control and germ cell mutant spermatogonia. (C) RT-qPCR analysis of mRNA levels of *Ccnd2* in WIN18,466-treated and RA-injected WIN18,466-treated mouse spermatogonia. Data (B and C) are expressed as fold differences compared with controls or WIN18,466-treated, respectively, normalized *Rps2* (mean  $\pm$  s.d.,  $n=3$ ;  $*P < 0.05$ , Student *t*-test).

**Table S1.** Summary of differentially expressed genes in control and germ cell mutant THY1<sup>+</sup> spermatogonia.

[Click here to Download Tables S1](#)

**Table S2.** Primers used for quantitative RT-PCR.

Gene	Primer sequence	
<i>Ccnd2</i>	Forward:	5'-GAACCTGGCCGCGAGTCACCC-3'
	Reverse:	5'-GGCTGCTCCCACGCTTCCAG-3'
<i>Hist1h1b</i>	Forward:	5'-TCCAAAGAAAGCGAAGAAGC-3'
	Reverse:	5'-GCCTTAGGCTTAGCGGTCTT-3'
<i>Hist1h2ai</i>	Forward:	5'-GCGACAACAAGAAGACGCGCAT-3'
	Reverse:	5'-CTGGATGTTGGGCAGGACGCC-3'
<i>Hist1h2bl</i>	Forward:	5'-AAGAAGGACGGCAAGAAGCGCA-3'
	Reverse:	5'-CGCTCGAAGATGTCGTTCCACGA-3'
<i>Hist1h2bg</i>	Forward:	5'-TCGTGAACGACATCTTCGAG-3'
	Reverse:	5'-AGTGACAGCCTTGGTGCCCT-3'
<i>Hist1H3i</i>	Forward:	5'-TACCAGAAGTCGACCGAGCTG-3'
	Reverse:	5'-AGCCGAGCTCTGGAAGCG-3'
<i>Hist1H2bf</i>	Forward:	5'-AAGGAGAGCTACTCGGTGTACG-3'
	Reverse:	5'-AAACGAGTTCATGATGCCCA-3'
<i>Hist1H2bn</i>	Forward:	5'-GTGTACGTGTACAAGGTGCTGAAG-3'
	Reverse:	5'-TGTGATGGTCGAGCGCTTGT-3'
<i>Hist1h4k</i>	Forward:	5'-AACATCCAGGGCATCACCAAGC-3'
	Reverse:	5'-GTTCTCCAGGAACACCTTCAGC-3'
<i>Kit</i>	Forward:	5'-GCCACGTCTCAGCCATCTG-3'
	Reverse:	5'-GTCGGGATCAATGCACGTCA-3'
<i>Rps2</i>	Forward:	5'-CTGACTCCCGACCTCTGGAAA-3'
	Reverse:	5'-GAGCCTGGGTCCCTCTGAACA-3'
<i>Stra8</i>	Forward:	5'-GTTTCCTGCGTGTCCACAAG-3'
	Reverse:	5'-CACCCGAGGCTCAAGCTTC-3'

**Table S3.** Primers used for ChIP quantitative-PCR.

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<i>Stra8</i>	Forward:	5'-ACCTCAAGTGACCTCCGTTT-3'
	Reverse:	5'-CACCACATCAGTTTGGCACA-3'
<i>Hist1</i>	Forward:	5'-TTTCACTTCCCCTTGGCCTT-3'
	Reverse:	5'-ACAAGAAGACGCGCATCATC-3'
<i>Control</i>	Forward:	5'-GGCCAAGAAGCTGGTTGTAG-3'
	Reverse:	5'-ACTGCTCAGCTATCCAGTCC-3'