Figure S1

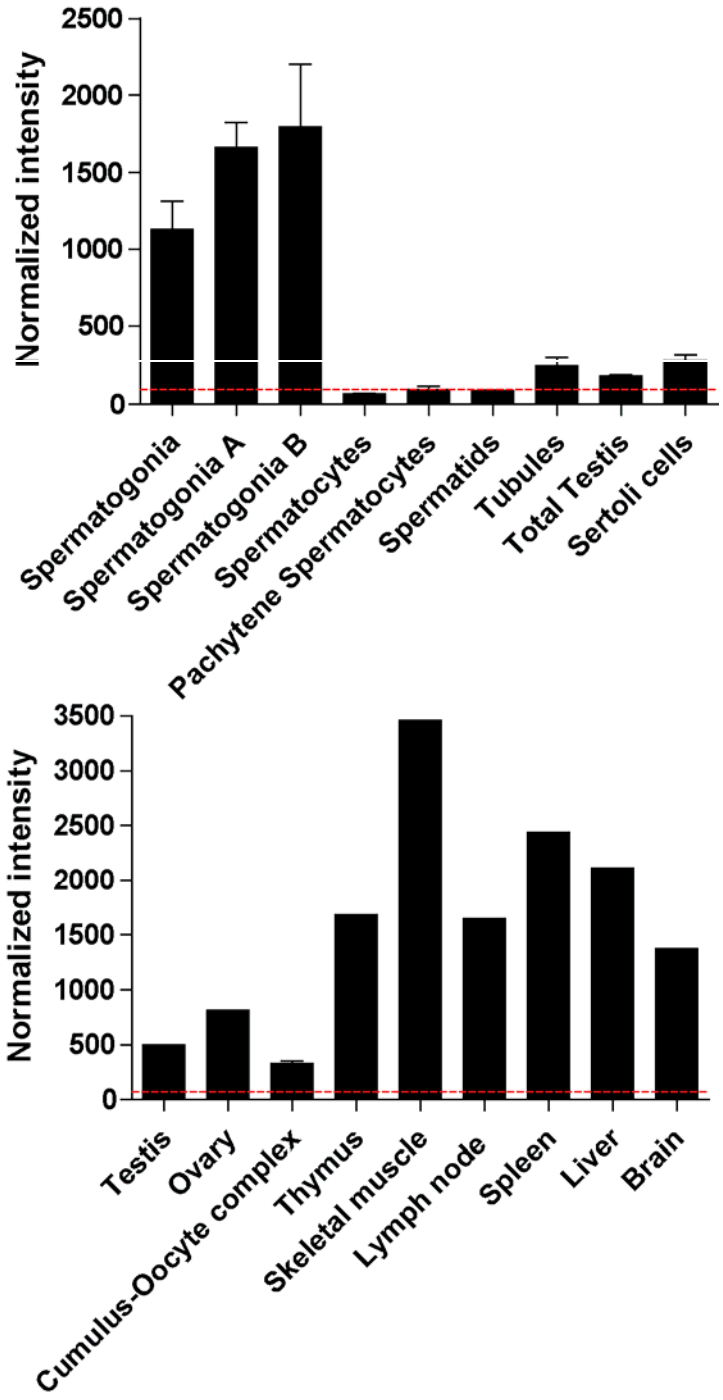

Fig. S1. Levels of *Gilz* transcripts present in male germ cells, Sertoli cells, testis and other tissues determined by Affymetrix microarray analysis. Expression profiles for the transcript *Tsc22d3* (*Gilz*, probeset 1420772) were determined by microarrays as determined by Chalmel et al. [2007] and Lardenois et al. [2010]. The expression data were extracted from the website www.germonline.org. Note in testis the high levels of *Tsc22d3* transcripts in spermatogonia. The red dash line represents the normalized median intensity of the samples, a value that is commonly assimilated to the background level.

Figure S2

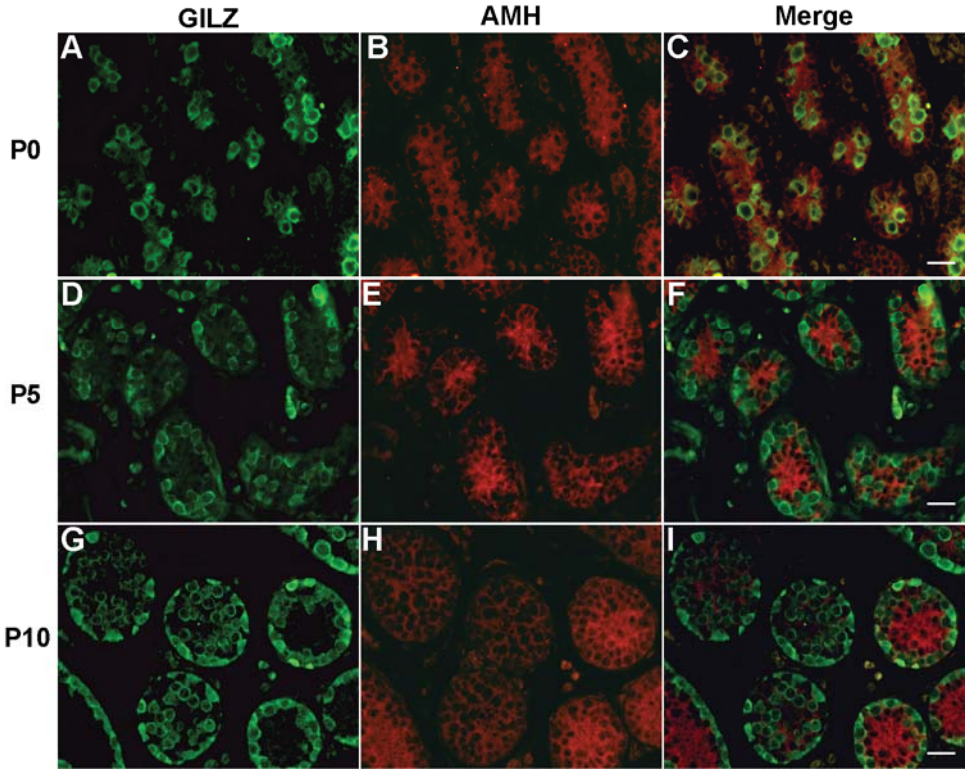

Fig. S2. GILZ is not expressed in Sertoli cells. Double immunolabelling for GILZ (green, **A**, **D**, **G**) and the Sertoli cell marker AMH (red, **B**, **E**, **H**) in the testis at P0 (**A**–**C**), P5 (**D**–**F**) and P10 (**G**–**I**). A merged image of green and red is shown in the right panel (**C**, **F**, **I**). GILZ is present at the vicinity of the germ cell nucleus but not present in the Sertoli cell. Scale bars = 20 μm.

Figure S3

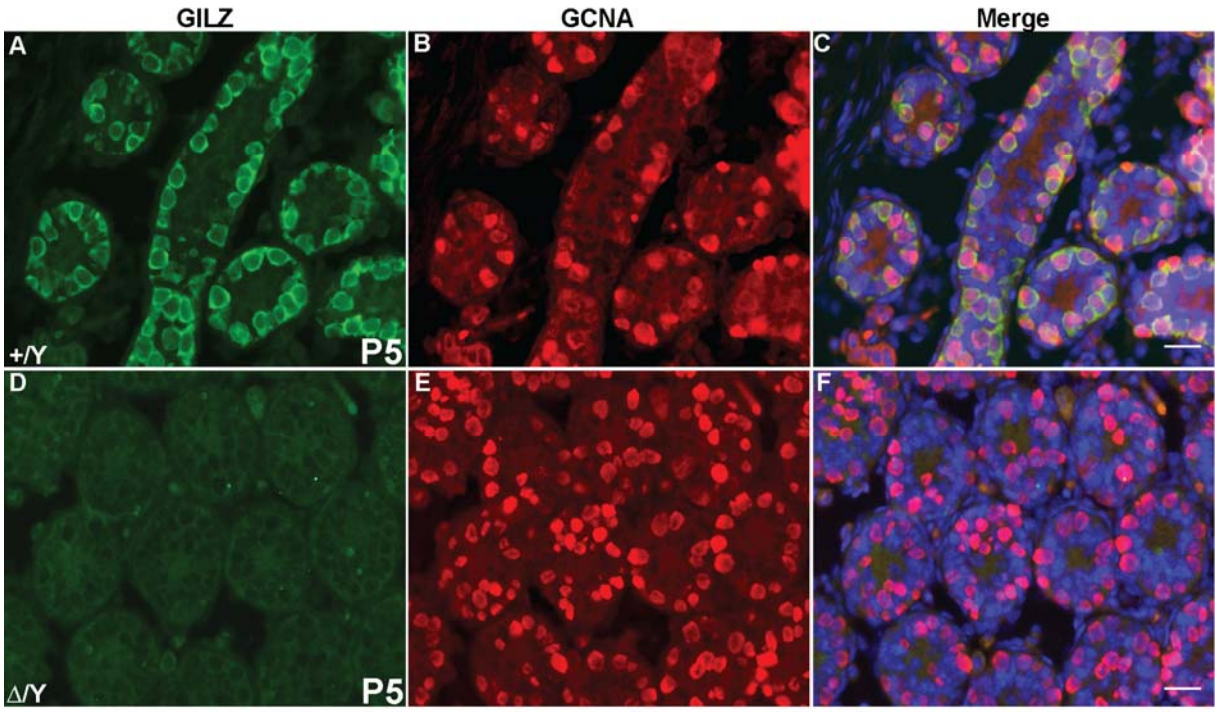

Fig. S3. GILZ is absent in *Gilz*$^{\Delta/y}$ mutant testes. Double immunolabelling for GILZ (green, **A**, **D**) and the germ cell marker GCNA (red, **B**, **E**) in control and mutant testes at P5. A merged image of green and red is shown in the right panel (**C**, **F**). Note the complete absence of GILZ protein in mutant individuals (Δ/Y). Scale bars = 20 μm.

Figure S4

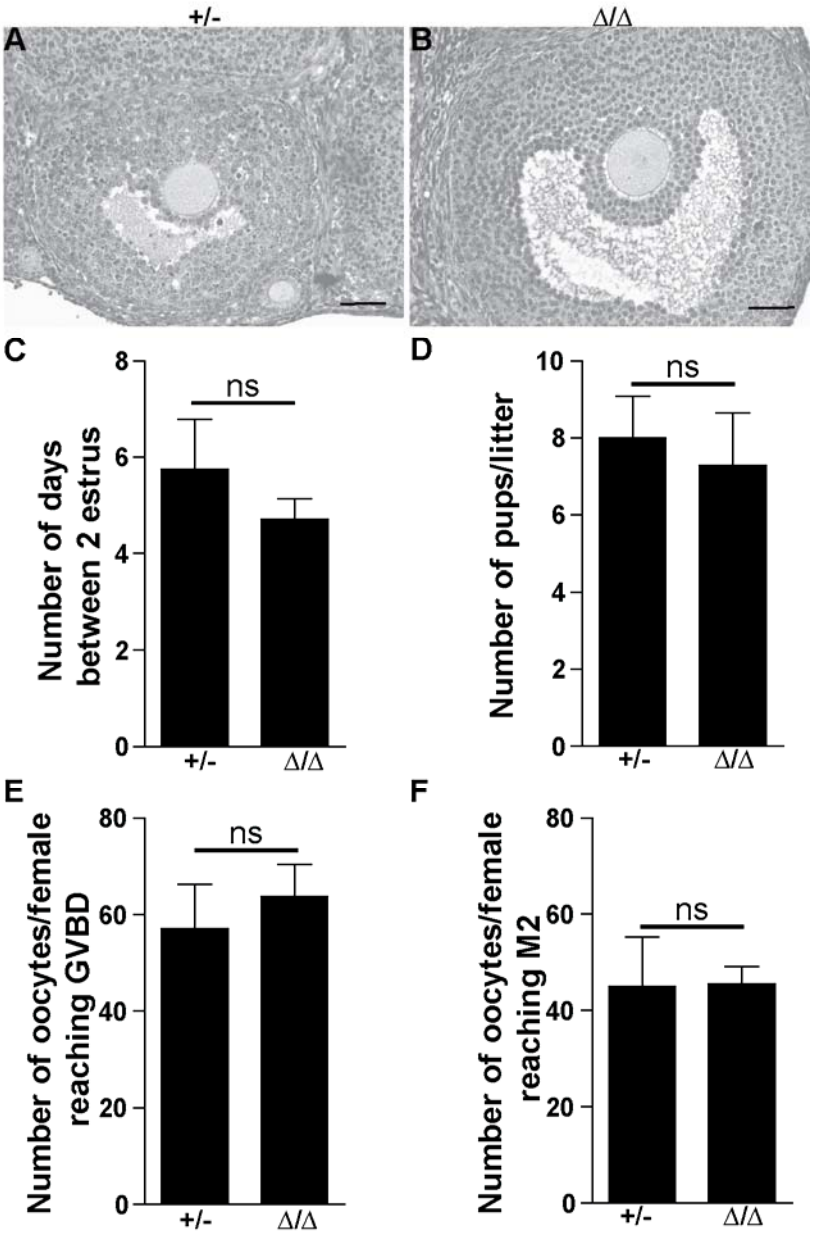

Fig. S4. Absence of reproductive defects in female mice lacking *Gilz*. Histological sections show control (**A**, +/–) and *Gilz* mutant (**B**, Δ/Δ) tertiary oocytes. Oestrous cyclicity assessments show no differences between control and mutant individuals (**C**). Fertility tests demonstrate that the number of pups produced by either control or mutant female individuals is similar (**D**). The number of maturing oocytes into GVBD (Germinal Vesicle Break-Down) and M2 (Metaphase II) is similar between control and mutant females (**E**, **F**). Results are mean ± SEM, n = 3–4, ns = not significant. Scale bars = 50 μm.

Figure S5

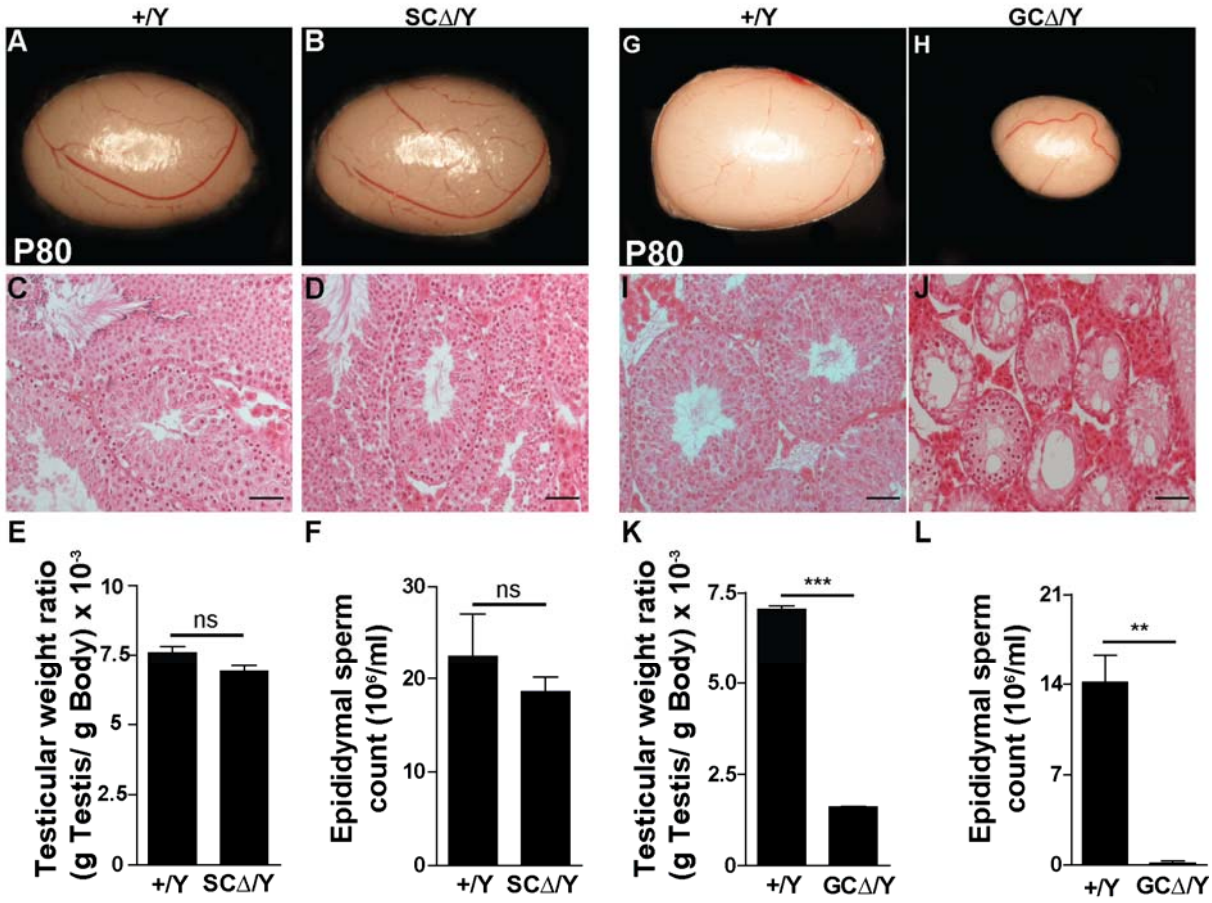

Fig. S5. Specific depletion of *Gilz* in Sertoli and germ cells. A–F Testicular function is not affected when *Gilz* is specifically deleted in Sertoli cells. Testis parameters comparison between control (*Gilz*$^{fx/Y}$, abbreviated +/Y, n = 4) and Sertoli-specific mutant (*Amh:Cre;Gilz*$^{fx/Y}$ abbreviated SCΔ/Y, n = 9) animals revealed no differences in global testis morphology (**A**, **B**), histology (**C**, **D**), testicular weight (**E**) and epididymal sperm count (**F**). **G–L** Specific ablation of *Gilz* in germ cells recapitulates the reproductive phenotype observed in the constitutive mutant (*Gilz*$^{\Delta/y}$). Similarly to *Gilz*$^{\Delta/y}$ testes, adult testis from *Ddx4:Cre;Gilz*$^{fx/Y}$ (abbreviated GCΔ/Y, n = 3; **H**) mice exhibited a 80% reduction (**K**) in weight compared to control *Gilz*$^{fx/Y}$ individuals (abbreviated +/Y, n = 6; **G**). H&E staining of testis sections (**I**, **J**) revealed complete absence of mature spermatozoa and elongated spermatids in GCΔ/Y testes. **L** Epididymal sperm count analysis shows a ~100% decrease in GCΔ/Y mutants. Results are mean ± SEM, ns = not significant, ** $p < 0.01$, *** $p < 0.001$ versus controls. Scale bars = 50 μm.

Figure S6

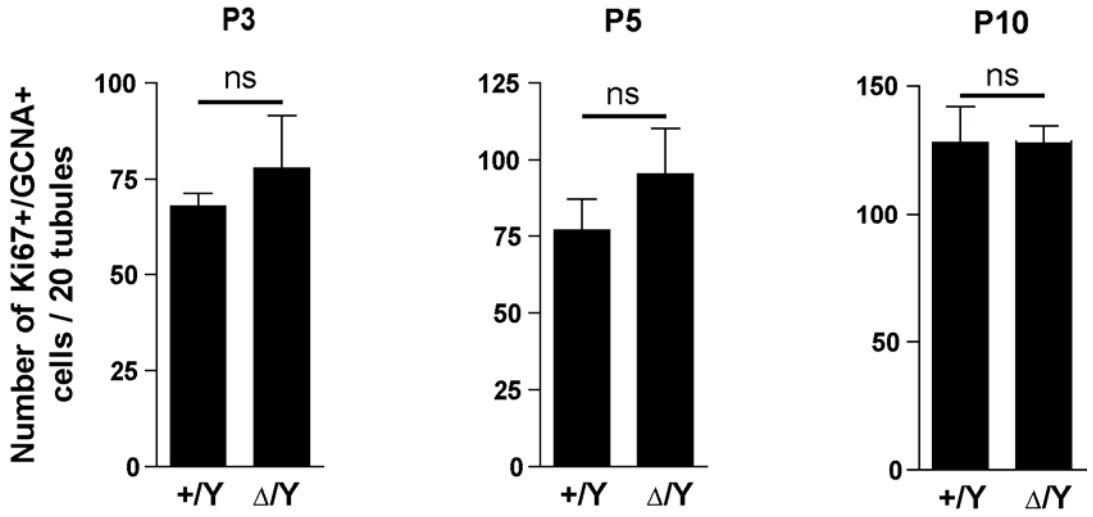

Fig. S6. Spermatogonial proliferation is not affected in P3, P5 and P10 *Gilz*$^{\Delta/y}$ mutant testes. Graphs showing count of double-positive cells for both GCNA and Ki-67-specific markers. Cell counts were performed on paraffin-embedded sections at P3, P5 and P10. No differences were observed between control (+/Y) and mutant (Δ/Y) individuals. Results are mean ± SEM, ns = not significant.