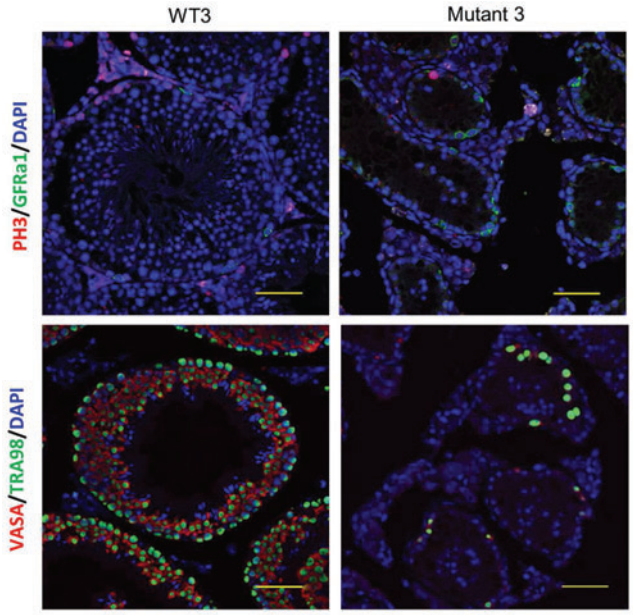
Supplementary Data

Supplementary Materials and Methods

Immunohistochemical analysis

Four percent of PFA-fixed paraffin-embedded testes were sliced into 7-μm-thick sections. Immunohistochemical analysis was conducted as described previously [16] using the following antibodies: a rat anti-TRA98 (a gift from Y. Nishimune, Osaka University), a rabbit anti-VASA antibody (Abcam), a goat anti-GFRα1 antibody (Neuromics), and a rabbit anti-phospho-histone H3 antibody (Ser10; Cell Signaling). For the detection of GFRα1, we used the Can Get Signal immunostain (Toyobo). The resulting signals were detected using incubation with Alexa 488- or Alexa 594-conjugated anti-IgG antibodies (Molecular Probes).



SUPPLEMENTARY FIG. S1. Immunohistochemistry of the *Eif2s3y* knockout mouse. Immunohistochemical analyses using staining for PH3 (*red*)/GFRα1 (*green*)/DAPI (*blue*) and VASA (*red*)/TRA98 (*green*)/DAPI (*blue*). Mutant 3 showed an increased GFRα1 signal, a lesser TRA98 signal, and a lesser VASA signal, suggesting that the differentiation of undifferentiated spermatogonia was arrested. GFRα1 is a marker of undifferentiated spermatogonia, and TRA98/VASA are markers of testicular germ cells. The scale bar is 50 μm.