

Data S1.

Supplementary materials and methods

Lasers scanning confocal microscopy. Primary cultured Sertoli cells (SCs) were fixed by 4% paraformaldehyde at 24°C for 30 min and then sealed with 10% goat serum (cat. no. C0265; Beyotime Institute of Biotechnology) at 24°C for 30 min. These SCs were then incubated with Wilms tumor 1 primary antibody (1:200; cat. no. ab89901; Abcam) at 4°C for 16 h. After washing three times with PBS, the cells were further incubated with goat anti-rabbit IgG H&L secondary antibody (1:500; Alexa Fluor® 647; cat. no. ab150079; Abcam) in a water bath at 37°C for 30 min. After the nuclei were stained with DAPI, the images were captured by laser scanning confocal fluorescence microscope (Olympus FV1000; Olympus Corporation).

Electron microscopy. The collected testes were cut into 1-mm³ pieces and fixed with glutaraldehyde at 4°C for 4 h. After dehydration with 50, 70, 80, 90 and 100% alcohol at 4°C for 15 min each, the testes tissues were immersed with acetone containing 50% EPON (cat. no. 90529-77-4; Structure Probe, Inc.) at 24°C for 12 h and then were embedded with EPON at 60°C for 48 h. The embedded testes were sliced into 70-nm tissues sections with an ultra-thin slicer (EMUC7; Leica Microsystems, Inc.) and then stained with 2% uranium acetate at 24°C for 15 min, followed by staining with 3% lead citrate at 24°C for 10 min. Tissue ultrastructure was observed using a transmission electron microscope (magnification x9,300; HITACHI HT7800; Hitachi, Ltd.).

Figure S1. Electron microscopy of BTB structures in the testes from 8-week-old and 18-month-old mice. The BTB structures in the testes from 8-week-old and 18-month-old mice were observed by a transmission electron microscope. Red boundary lines indicate the BTB. Scale bar, 1 μ m. BTB, blood-testis barrier.

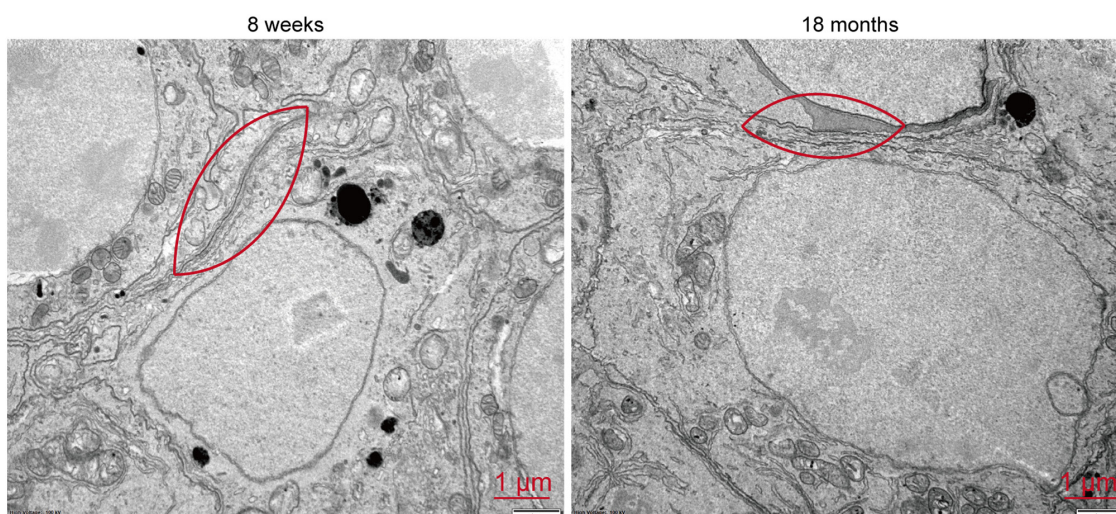


Figure S2. Expression of Wt1 in primary SCs. The expression of Wt1 in primary SCs was detected by immunofluorescent-staining, and the images were captured by laser scanning confocal fluorescence microscope. Scale bar, 25 μ m. SCs, Sertoli cells; Wt1, Wilms tumor 1.

