BT-IgSF is essential for male fertility in mice

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SUPPLEMENTAL MATERIAL

Figure S1: Characterization of the BT-IgSF non-conditional knockout mouse

Figure S2: Tubuli diameter, expression of ZO-2 at transcript and protein level, background activity of the goat-anti mouse antibody, images of BT-IgSF and γH2AX staining at higher magnification and electron microscopy images showing “new” and “old” BTBs behind and ahead of the pachytene spermatocytes.

Figure legends

Figure S1: Characterization of the BT-IgSF non-conditional knockout mouse

(A) Scheme of the targeting-construct of the BT-IgSF non-conditional knockout mouse. The binding region of a probe for Southern blotting is shown as red bar. (B) Southern blot analysis using BstEII-digested DNA and a sequence-specific DNA probe; (C) Genotyping of BT-IgSF wildtype, heterozygous and knockout mice by PCR, wildtype fragment 197 bp, knockout fragment: 343 bp; (D) Scheme of the targeting-construct of the BT-IgSF non-conditional knockout mouse, primers for RT-qPCR are shown in red; (E) Primer for the RT-qPCR amplifies a fragment coding for the cytoplasmic segment of BT-IgSF, illustrated by the black arrows. (F) Although mRNA of BT-IgSF is still detectable in minor amounts by qRT-PCR in testes of BT-IgSF knockout males (n=4 per genotype) BT-IgSF is not present at the protein level in Western blots of testes (G) or cultivated astrocytes (H) prepared from early postnatal cortices of BT-IgSF knockout animals.

Figure S2: Tubuli diameter, expression of ZO-2 at transcript and protein level, background activity of the goat-anti mouse secondary antibody, images of BT-IgSF and γH2AX staining and electron microscopy images showing “old” and “new” BTBs.

(A) Tubuli diameter of BT-IgSF knockout males are significantly smaller compared to wildtype tubuli; data are shown as mean ± SD; **=p<0.01, n= 3 per genotype, 20-60 tubuli were analyzed. (B) ZO-2 transcript expression is not changed in BT-IgSF knockout testes as analyzed by RT-qPCR. (C) Immunofluorescence staining against ZO-2 of cryostat sections, expression and localization is normal in knockout testis, although less organized; scale bar: 50 µm. (D) Higher magnification of BT-IgSF immunofluorescence stainings of WT testes; arrowheads: strong BT-IgSF expression at Sertoli cells in the basal compartment; outlining in white indicates the nucleus of Sertoli cells; scale bar: 10µm. (E) Immunofluorescence staining against γH2AX. In the wildtype meiotic cells with and without γH2AX expression are present (see arrow head in the insert); in contrast in the KO only γH2AX positive meiotic
cells are present (see arrow head in the insert), indicating that all of those are in meiosis I; scale bar: 50µm. (F) Immunofluorescence staining control with goat anti-mouse-Alexa488 only, showing an unspecific signal in the lamina propria of the seminiferi tubuli (compare with Fig. 1E and 1F); scale bar: 50 µm. (G) Electron microscopy of adult testes showing the “old” BTB ahead of the pachytene spermatocyte (Pl) and the “new” BTB behind the pachytene spermatocyte, suggesting a normal assembly and disassembly of the BTB; S: Sertoli cells, arrowhead: BTB, scale bar left panel WT: 2 µm, right panel WT: 500 nm, left panel KO: 10 µm, right panel KO: 1 µm.
Fig. S1 Pelz et al., 2017
Fig. S2 Pelz et al., 2017