BRG1 is dispensable for Sertoli cell development and functions in mice

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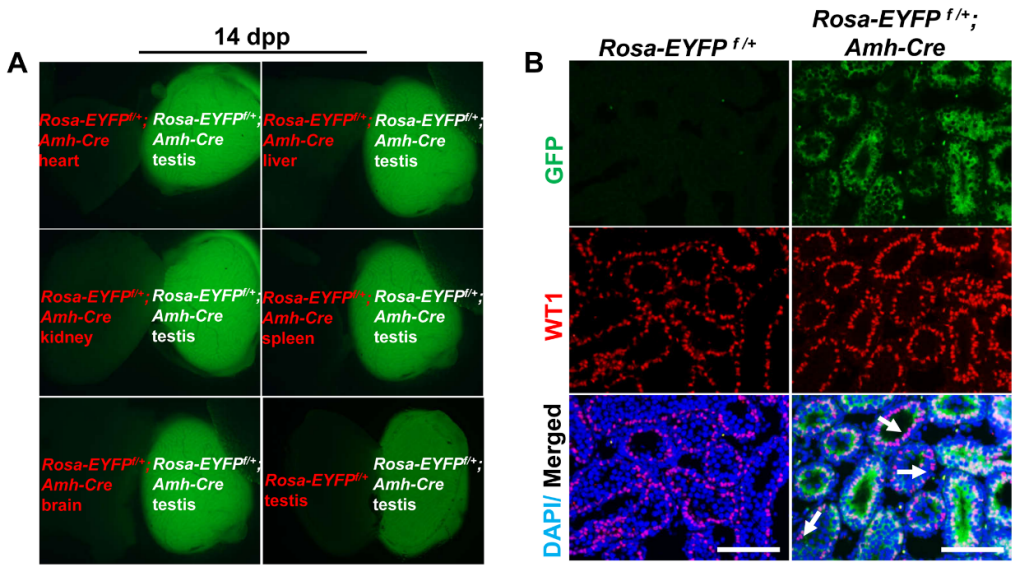

Figure S1. Specific but inefficient CRE recombinase activity in *Amh-Cre* mice from Jackson Lab.

(A) GFP fluorescence in different organs from *Rosa-EYFP*$^{f/+}$*; Amh-Cre* and *Rosa-EYFP*$^{f/+}$mice.

(B) GFP and WT1 expression was examined with IHF in testes from *Rosa-EYFP*$^{f/+}$*; Amh-Cre* and *Rosa-EYFP*$^{f/+}$mice. CRE in Sertoli cells excised the strong transcriptional termination sequence (triple SV40 polyadenylation sequence) before *EYFP* coding sequences, which led to expression of EYFP (detected by a GFP antibody because of high homologous sequences between GFP and EYFP proteins). GFP protein was not expressed in a fraction of Sertoli cells (white arrows), suggesting that *Amh-Cre* recombinase activity was inefficient. Scale bars, 200 μm.

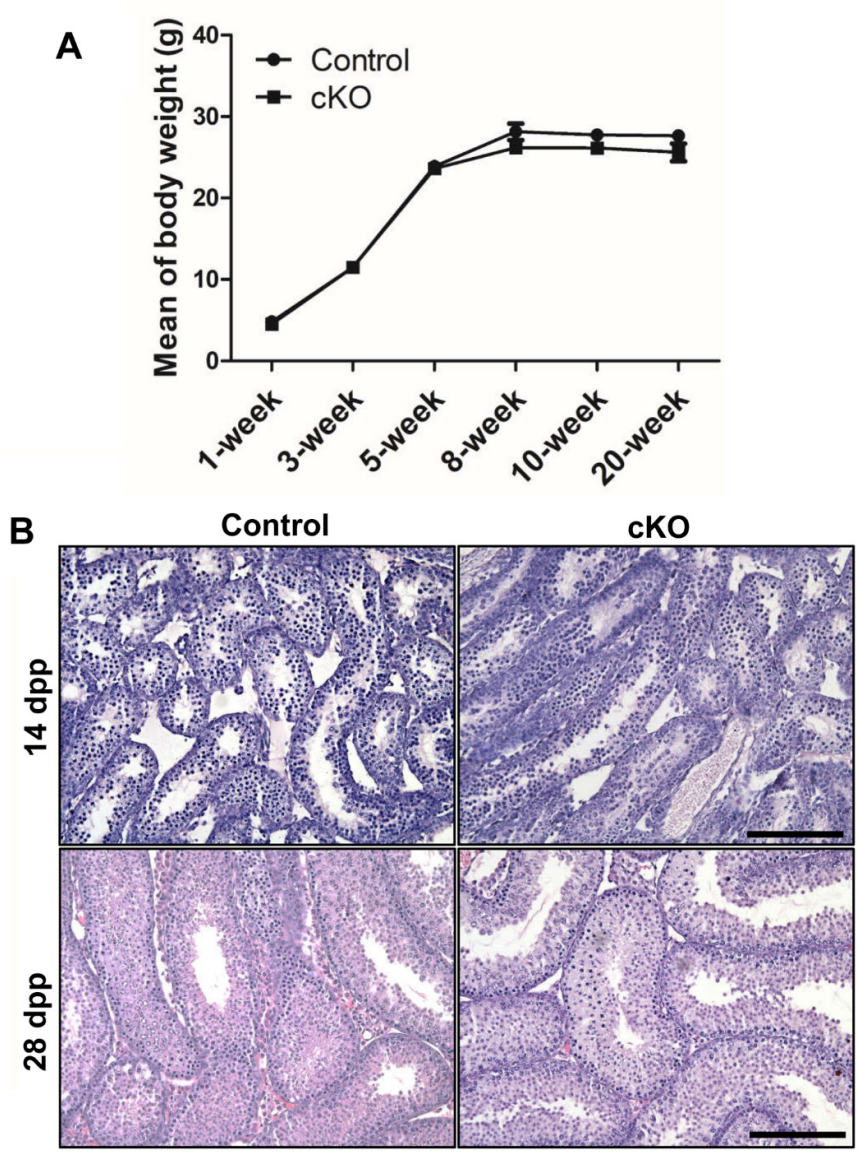

Figure S2. Normal spermatogenesis and body weights upon partial *Brg1* deletion in Sertoli cells mediated by *Amh-Cre* (Jackson).

(A) Mean body weight of mice at different ages, body weights were recorded for 3–10 animals per group. Error bars represent ± SEM. Statistical analyses were performed using Student's t-test. (B) Histology study on testes from control and *Brg1*-cKO mice at 14 dpp and 28 dpp. Normal spermatogenesis and germ cell layout in seminiferous tubules were observed in control and cKO testes. Scale bars, 200 μm.

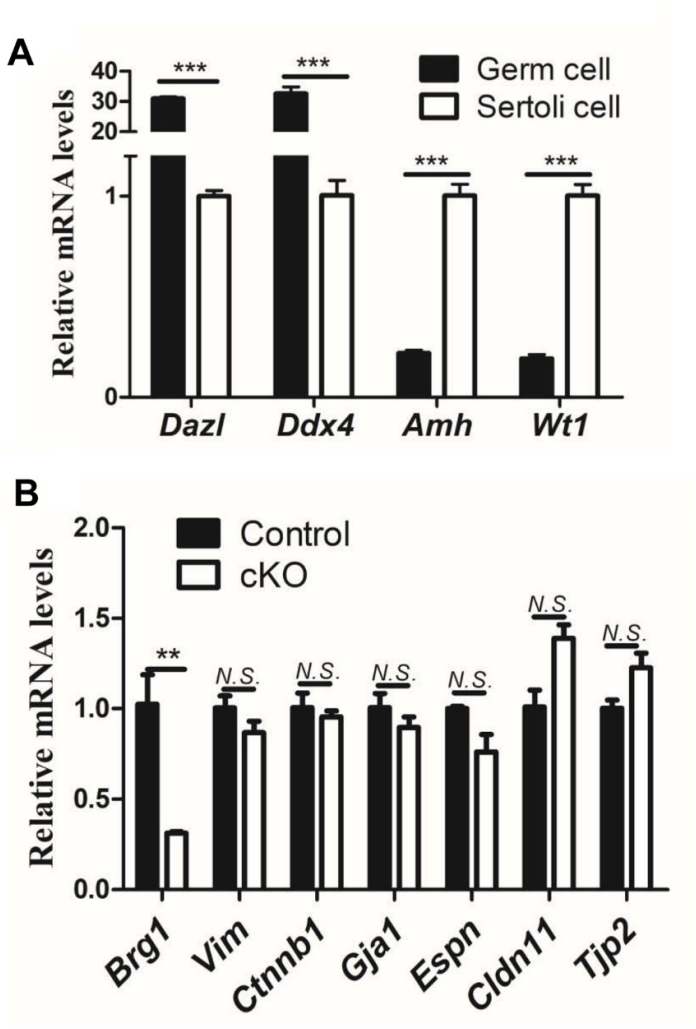

Figure S3. High purity of the isolated Sertoli cells and BTB related-gene expression analyses.

(A) Expression levels of germ cell markers (*Dazl* and *Ddx4*) and Sertoli cell genes (*Amh* and *Wt1*) were examined by real-time RT-PCR in isolated germ cells from 14 dpp wildtype mice. Gene expression was normalized to *Gapdh* and relative expression levels were calculated to those from Sertoli cells.

(B) *Brg1* and BTB related-genes (*Vim, Ctnnb1, Gja1, Cldn11, Tjp2* and *Espn*) were determined for their transcript levels by real-time RT-PCR in isolated Sertoli cells from 14 dpp control and cKO mice. Gene expression was normalized to *Gapdh* and calculated relatively to $Brg1^{f/f}$ control group.

(A-B) Data are presented as mean ± SEM from three independent experiments. Statistical analyses were performed using Student's t-test. **: $p < 0.01$; ***: $p < 0.001$; *N.S.*: no significance.

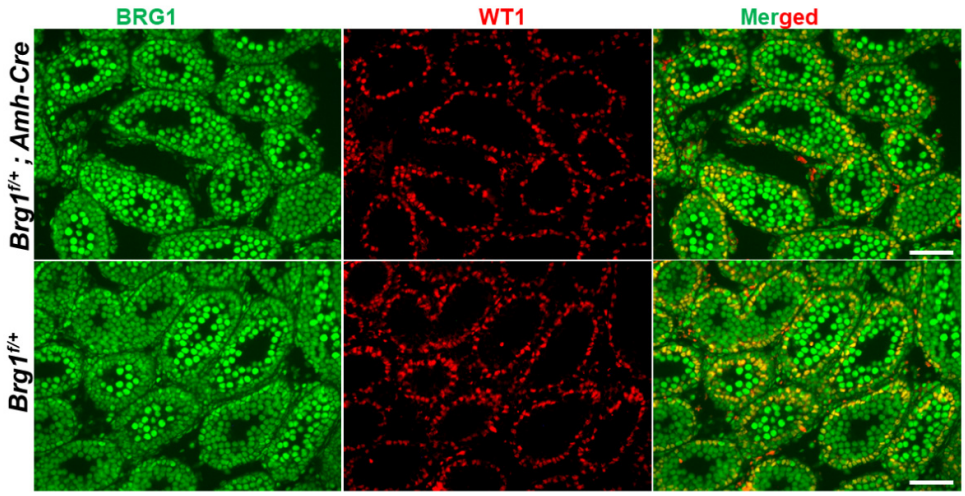

Figure S4. Heterozygote deletion of *Brg1* does not affect its expression in Sertoli cells and germ cells.

IHF were performed with BRG1 and WT1 antibodies on testis sections from wild-type mice or mice with heterozygote *Brg1* deletion in Sertoli cells mediated by *Amh-Cre* (EMMA). No obvious alteration was observed in spermatogenesis and in expression of WT1 and BRG1 from mice upon heterozygote *Brg1* deletion in Sertoli cells. Scale bars, 100 μm.

Table S1. Primers used in genotyping

Gene name	Forward primers	Reverse primers	Amplicon Size
Brg1	GTCATACTTATGT CATAGCC	GCCTTGTCTCAAA CTGATAAG	Homozygous:380bp Wildtype:250bp
Amh (Jackson)	GCGGTCTGGCAGT AAAAACTATC	GTGAAACAGCATT GCTGTCACTT	100bp
Amh (EMMA)	TCCTGGAAAATGC TTCTGTCCG	CAGGGTGTTATAA GCAATCCC	400bp

Table S2. Primers used in real-time PCR

Gene name	Forward primers	Reverse primers
Brg1	GGTTCTGCCCACAGCATGAT	GGACTCCATAGGCTTGTGCAT
Brm	CTCCTGGACCAATTCTGGGG	CATCGTTGACAGAGGATGTG AG

Dazl	ATGTCTGCCACAACTTCTGAG	CTGATTTCGGTTTCATCCATCCT
Ddx4	GCTTCATCAGATATTGGCGAGT	GCTTGGAAAACCCTCTGCTT
Plzf	CTGCGGAAAACGGTTCCTG	GTGCCAGTATGGGTCTGTCT
Gfra1	CACTCCTGGATTTGCTGATGT	AGTGTGCGGTACTTGGTGC
Sycp3	AGCCAGTAACCAGAAAATTGAGC	CCACTGCTGCAACACATTCATA
Stra8	ACAACCTAAGGAAGGCAGTTTAC	GACCTCCTCTAAGCTGTTGGG
Kit	GCCACGTCTCAGCCATCTG	GTCGCCAGCTTCAACTATTAACT
Tnp1	ACCAGCCGCAAGCTAAAGAC	TTTCCTACTTTTCAGGACGCTC
Prm1	CCGTCGCAGACGAAGATGTC	CACCTTATGGTGTATGAGCGG
Amh	CCACACCTCTCTCCACTGGTA	GGCACAAAGGTTCAGGGGG
Wt1	GAGAGCCAGCCTACCATCC	GGGTCCTCGTGTTTGAAGGAA
Vim	CGGCTGCGAGAGAAATTGC	CCACTTTCCGTTCAAGGTCAAG
Ctnnb1	ATGGAGCCGGACAGAAAAGC	CTTGCCACTCAGGGAAGGA
Gja1	ACAGCGGTTGAGTCAGCTTG	GAGAGATGGGGAAGGACTTGT
Espn	CCACAGGCTACCTCTCTTGC	AGCAGCCACTTCACCACATC
Cldn11	ATGGTAGCCACTTGCCTTCAG	AGTTCGTCCATTTTTCGGCAG
Tjp2	ATGGGAGCAGTACACCGTGA	TGACCACCCTGTCATTTTCTTG
Gapdh	AGGTCGGTGTGAACGGATTTG	TGTAGACCATGTAGTTGAGGTCA