A novel *Amh*-Treck transgenic mouse line allows toxin-dependent loss of supporting cells in gonads

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Abstract

Cell ablation technology is useful for studying specific cell lineages in a developing organ *in vivo*. Herein, we established a novel anti-Müllerian hormone (*AMH*)-toxin receptor-mediated cell knockout (Treck) mouse line, in which the diphtheria toxin (*DT*) receptor was specifically activated in Sertoli and granulosa cells in postnatal testes and ovaries respectively. In the postnatal testes of *Amh*-Treck transgenic (Tg) male mice, DT injection induced a specific loss of the Sertoli cells in a dose-dependent manner, as well as the specific degeneration of granulosa cells in the primary and secondary follicles caused by DT injection in Tg females. In the testes with depletion of Sertoli cell, germ cells appeared to survive for only several days after DT treatment and rapidly underwent cell degeneration, which led to the accumulation of a large amount of cell debris within the seminiferous tubules by day 10 after DT treatment. Transplantation of exogenous healthy Sertoli cells following DT treatment rescued the germ cell loss in the transplantation sites of the seminiferous epithelia, leading to a partial recovery of the spermatogenesis. These results provide not only *in vivo* evidence of the crucial role of Sertoli cells in the maintenance of germ cells, but also show that the *Amh*-Treck Tg line is a useful *in vivo* model of the function of the supporting cell lineage in developing mammalian gonads.

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Introduction

In mammalian embryos at early organogenic stages, undifferentiated genital ridges start to develop into the testis or ovary dependent on their genetic sex. As the first morphological sign of testis differentiation, germ cells are surrounded by differentiating Sertoli cells, leading to the formation of testis cords (future seminiferous tubules), a tubular structure of the germ and Sertoli cells packed within the basal lamina sheath (Harikae *et al.* 2013a, Svingen & Koopman 2013). In developing ovaries, both germ and pre-granulosa cells also aggregate to form the poorly defined ovarian cords (ovigerous cord), subsequently resulting in the formation of ovarian cysts, a closed sack-like structure of several oocytes and their surrounding pre-granulosa cells (Pepling 2006, Hummitzsch *et al.* 2013). After birth, the testis cords develop into the seminiferous tubules associated with active spermatogenesis, while in the ovary the ovarian

cyst breaks down into each individual primordial follicle, an oocyte surrounded by a single layer of granulosa cells. A part of the primordial follicle continuously grows into primary and subsequent secondary follicle stages at the medullary side during the initial round of folliculogenesis (Mork *et al.* 2012, Harikae *et al.* 2013b).

From the fetal to adult stages, both Sertoli and granulosa cells secrete various growth factors to support the proliferation, differentiation and maintenance of the germ cells (Matsui 1998, Choi & Rajkovic 2006, Buratini & Price 2011, Zhang *et al.* 2011, Feng *et al.* 2014). Moreover, the germ cells physically contact and interact intimately with the supporting cells inside the basal lamina sheath. These histological and physiological findings indicate the crucial roles of the gonadal supporting cells in the maintenance and survival of germ cells in mammalian gonads. However, the duration

for which the germ cells are maintained after the specific loss of supporting cells *in vivo* remains unclear.

A conditional *in vivo* depletion of lineage-specific cells can be achieved through administration of diphtheria toxin (DT) to toxin-receptor-mediated cell-knockout (Treck)-transgenic (Tg) mice that carry a human DT receptor (DTR; *HBEGF*-mut) cDNA transgene driven by a cell lineage-specific promoter (Saito *et al.* 2001). In this Treck system, the administration of DT to the Treck Tg mice causes depletion in only DTR-expressing cell lineages and specifically eliminates these target cells *in vivo* (Takada *et al.* 2008, Matsuoka *et al.* 2013).

In mice, anti-Müllerian hormone (*Amh*) is expressed specifically in the gonadal-supporting cell lineages: Sertoli and granulosa cells of postnatal immature gonads in both males and females (Lécureuil *et al.* 2002). Herein, we established three *Amh*-Treck Tg lines carrying the *Amh*-promoter-fused DTR cassette, in which conditional *in vivo* deletion of the gonadal-supporting cells was induced by DT injection at postnatal timepoints. Here we show a great potential in the *Amh*-Treck line as an *in vivo* functional assay system of the supporting cell lineage in mammalian gonads.

Materials and methods

Amh-Treck Tg mouse line

The *Amh*-Treck transgene was prepared by inserting the entire 5'-flanking promoter region of the *Amh* gene (Guerrier *et al.* 1990) upstream of DT receptor (DTR; I117V/L148V mutant of human HB-EGF tagged with EGFP; Furukawa *et al.* 2006). We successfully established three *Amh*-Treck Tg lines (#36, #85, #94) with testis-specific expression of DTR in male pups (Supplementary Figure S1, see section on supplementary data given at the end of this article). The C57BL/6-Tg (CAG-EGFP) mice (GFP mice; SLC) and C57BL/6-R26-H2B-mCherry knock-in mice (Cherry mice; Abe *et al.* 2011) were used as transplant donors for the rescue experiment. The ICR and C57BL/6 male mice (E12.5–10 weeks old; SLC) were also used for the immunohistochemical analyses. The animal experiments were carried out in accordance with the Guidelines for Animal Use and Experimentation of the University of Tokyo (approval IDs: P12-660 and P12-764).

Quantitative RT-PCR

Total RNA was reverse-transcribed using random primer with a Superscript-III cDNA Synthesis Kit (Invitrogen). PCR was carried out using an Applied Biosystems Step-One Real-Time PCR System with the primers and probes of *Egfp* (Mr04329676_mr) and *Gapdh* (Taqman control reagents).

DT treatment

Amh-Treck Tg mice (P3–21) were treated with DT (Sigma-Aldrich, 0.04–40.0 µg/kg, i.p.) as described previously (Saito *et al.* 2001, Takada *et al.* 2008, Matsuoka *et al.* 2013).

All tissues were subjected to histological and immunohistochemical analyses as described below.

Transplantation of EGFP or Cherry-positive Sertoli cells

A single-cell suspension (including gonocytes and Sertoli cells) (1.0×10^8 cells/ml) was prepared from the testes of 1-week-old GFP/Cherry mice (C57BL6) by a two-step enzymatic digestion procedure (Shinohara *et al.* 2003), and then transplanted into the testes of 3-week-old recipient Tg males pre-treated with DT (0.4–4.0 µg/kg). On days 10 and 45 after transplantation, all recipient Tg testes were removed from the tunica albuginea and roughly dissected in cold PBS under an Olympus fluorescence microscope (BX51N-34-FL2) and stereomicroscope (SZX16 plus U-LH100HG) (Nagai *et al.* 2012). The tissues were processed using the immunohistochemical techniques described below.

Histology and immunohistochemistry

The tissues were fixed in 4% paraformaldehyde (PFA) or Bouin's solution and routinely embedded in paraffin. The sections (4 µm thickness) were subjected to conventional histological (hematoxylin and eosin; H&E) and immunohistochemical staining as described previously (Hiramatsu *et al.* 2010, Harikae *et al.* 2013b).

In brief, for immunohistochemical staining, the sections were incubated with anti-AMH (1:100 dilution; sc-6886; Santa Cruz), anti-3β-HSD (1:2000 dilution; sc-30821; Santa Cruz), anti-Cherry (1:200 dilution; ab125096; Abcam, Cambridge, United Kingdom), anti-E-cadherin (1:500 dilution; 610181; BD-Pharmingen, Franklin Lakes, NJ, USA), anti-FOXL2 (1:200 dilution; Uda *et al.* (2004); 1:200 dilution, ab5096; Abcam), anti-GATA4 (1:250 dilution; sc-1237; Santa Cruz), anti-GFP (1:200 dilution; 598; MBL, Nagoya, Japan), anti-GFRα1 (1:100 dilution; AF560; R&D Systems, Minneapolis, MN, USA), anti-HuB/Elav12 (1:1000 dilution; H1538; Sigma), anti-laminin (1:400 dilution; 10765; ICN Pharmaceuticals, Santa Ana, CA, USA), anti-MVH/DDX4 (1:5000 dilution; Toyooka *et al.* (2000); 1:1000 dilution; ab13840; Abcam), anti-PLZF (1:500 dilution; OP128, Calbiochem, Darmstadt, Germany), anti-α-smooth muscle actin (anti-SMA; 1:5000 dilution, A2547; Sigma), or anti-SOX9 (1:250 dilution; Kidokoro *et al.* (2005)) antibody at 4 °C for 12 h. The reaction was visualized using a biotin-conjugated secondary antibody in combination with the Elite ABC Kit (Vector Laboratories) or by secondary antibodies conjugated with Alexa-488/594. TUNEL assays were also performed by using the Apoptotic Detection Kit (MK500; TaKaRa, Shiga, Japan).

Results and discussion

Establishment of *Amh*-Treck Tg mouse lines

The transgene was constructed as the *Amh* promoter-driven DT receptor (DTR; *HBEGF*-mut) (I117V/L148V mutant of human HB-EGF) tagged with EGFP (Furukawa *et al.* 2006; Fig. 1A). We obtained three Tg lines (healthy and fertile in both sexes) by pronuclear injection of the plasmid-based transgene into the C57BL6 background. In Wt mice, endogenous *Amh* expression is found in the

Figure 1 Characterization of AMH-Treck Tg lines (#94). (A) Schematic representation showing the transgene of AMH promoter-driven diphtheria toxin receptor (DTR; *HBEGF*-mut) fused with EGFP. (B) H&E staining and anti-SOX9 immunostaining of the testes on day 4 after DT treatment, showing a dose-dependent degeneration of SOX9-positive Sertoli cells in Tg males treated with DT at 0, 0.04, 0.4, and 4.0 $\mu\text{g/kg}$. (C) H&E and TUNEL staining showing a time-course pattern of the Sertoli cell and germ cell degeneration in the Tg males treated with DT at 4.0 $\mu\text{g/kg}$. (D) Anti-GFR α 1 and HuB immunostaining of the testes on day 4 (two left-handed rows) and day 10 (most right-handed row) after DT treatment, showing the dynamics of undifferentiated (GFR α 1+) and differentiated (HuB+) spermatogonia in the Wt and Tg littermates treated with DT at 4.0 $\mu\text{g/kg}$. Both GFR α 1+ and HuB+ populations of the spermatogonia disappear by day 10 after DT treatment. Scale bars represent 20 μm .

Sertoli cells of the testes from embryonic day (E) 12.5 to postnatal day (P) 14 (Supplementary Figure S2, see section on supplementary data given at the end of this article) and then almost repressed by P24 (Al-Attar *et al.* 1997). In the ovary, *Amh* expression is defined in the growing follicles after P3 and later stages (Lécureuil *et al.* 2002). The preliminary experiments carried out with DT treatment (4.0 µg/kg; Matsuoka *et al.* 2013) in the postnatal testes at P3–P10 showed degeneration in the majority of Sertoli cells in all of the three lines as well as no appreciable defects in other organs (Fig. 1 and Supplementary Figure S3). Among these three lines, the EGFP-tagged *DTR* expression was, although fluorescent signals are very weak, detectable in the seminiferous tubules and the primordial follicles of the testes and ovaries in the #94 line (Supplementary Figure S4), in addition to the gonad-specific high-level expression of the *DTR* transgene by qPCR (Supplementary Figure S1). Moreover, even a low-dose DT treatment (0.04 µg/kg) can affect the nuclear shape of Sertoli cells and their arrangement in the #94 line (see Fig. 1B), and thus we used mainly the #94 line for subsequent experiments.

DT injection induces Sertoli cell degeneration in postnatal testes of Tg males in a dose-dependent manner

First, we examined the dose-dependent effects of DT on the Sertoli cell degeneration in the *Amh*-Treck line. The Tg males and their Wt littermates at P21 (post-weaning) were treated with DT at various dosages, from 0.04 to 40.0 µg/kg, and their organs including testes were histopathologically examined on day 4 after DT treatment (Fig. 1B and Supplementary Figure S5, see section on supplementary data given at the end of this article). In the Tg males treated with 0.04 µg/kg DT, most of Sox9-positive Sertoli cells appeared morphologically healthy but the nucleus position was arranged irregularly in the seminiferous epithelia as compared with the non-treated control testes (Fig. 1B). DT treatment at either 0.4 or 4.0 µg/kg clearly induced the loss of the Sox9-positive Sertoli cells in the seminiferous tubules by day 4 after DT treatment (Fig. 1B). In contrast, no appreciable defects were found in the Wt mice treated with DT at 4.0–40 µg/kg (Supplementary Figure S5). In addition, no gross-anatomical and histological defects were observed in the other organs of both Wt and Tg males treated with 4.0–40 µg/kg DT (Supplementary Figure S5; data not shown).

Next, we examined the temporal changes in the testicular phenotype in DT-treated Tg testes (4.0 µg/kg) between days 1 and 10 after DT treatment (Fig. 1C). On day 1, DT treatment showed onset of Sertoli cell degeneration; however, histological loss of the nucleus was observed on day 4 after DT treatment. Moreover, histological analysis revealed that the germ cells appeared to undergo germ cell degeneration with visible accumulation of cell debris at the luminal side on day 4.

Finally, by day 10 after DT treatment, all Sertoli and germ cells were degenerated in the seminiferous tubules and consequently, a large amount of cell debris had accumulated inside the seminiferous tubules (Fig. 1C). TUNEL staining also confirmed the appearance of apoptotic cell death in the germ cells at luminal side by day 4 after DT treatment (lower plates in Fig. 1C; Supplementary Figure S6, see section on supplementary data given at the end of this article). Anti-GFR α 1 and anti-HuB immunostaining revealed that the GFR α 1 + undifferentiated spermatogonia (including spermatogenic stem cells; Sato *et al.* 2011) and HuB + differentiated spermatogonia (including pre-leptotene spermatocytes; Supplementary Figure S7) are detectable in the basal compartment of the seminiferous tubules on day 4 after DT treatment. However, both spermatogonial populations were depleted by day 10 after DT treatment (Fig. 1D). These findings, therefore, confirmed that loss of Sertoli cells led to rapid cell death of germ cells within several days. This is consistent with previous reports involving Sertoli cell-specific deletion of *Wt1* (Gao *et al.* 2006) or *Sox9/Sox8* (Barrionuevo *et al.* 2009) gene(s), which demonstrated a drastic reduction in germ cell number in the postnatal testes, together with defective Sertoli cell function.

Sertoli cell-specific loss of the seminiferous tubules in DT-treated Tg testes at P3 and P21

The specificity of the cell depletion in DT-treated testes was examined on day 4 after DT treatment. The male Tg pups and their Wt littermates at P3 and P21 were treated with DT (4.0 µg/kg) and the expression patterns of various somatic cell markers were examined immunohistochemically (Fig. 2A). In P3 testes treated with DT (upper plates in Fig. 2B), the almost complete depletion of the *Amh*-positive or Sox9-positive Sertoli cells was found throughout the entire testis area. This is in sharp contrast with E-cad-positive rete testis epithelia, which appeared histologically normal (Fig. 2B). Immunohistochemical analyses of P21 testes on day 4 after DT treatment also confirmed that GATA4-positive cells appeared intact in the peritubular and interstitial regions. However, no GATA4-positive signals were detectable in the seminiferous epithelia, leading to MVH-positive germ cells occupying the inside of the tubules (Fig. 2C). The laminin-positive basal lamina, SMA-positive peritubular myoid cells, and 3 β -HSD-positive Leydig cells appeared intact in the interstitial region (Fig. 2C). The complete degeneration of cells inside the tubules by day 10 after DT treatment indicates that Sertoli cell-specific depletion occurs by day 4; germ cells then undergo rapid degeneration, which may result in the accumulation of a large amount of cell debris at the luminal side by day 10 after DT treatment.

Figure 2 A specific loss of the Sertoli cells in postnatal testes of DT-treated Tg males. (A) Schematic representation of DT treatment. Both Tg and Wt littermates at P3 and P21 were treated with DT at 4.0 µg/kg and their testes were examined histopathologically on day 4 after DT treatment. (B) Anti-E-cadherin (E-CAD), anti-AMH and anti-SOX9 immunostaining of Tg and Wt testes treated with DT at P3. The DT-treated testis shows a reduced size of the testicular parenchyma only in the Tg line, in which AMH-/SOX9-positive Sertoli cells are almost completely missing throughout the testicular parenchyma. In contrast, E-cad-positive rete testis epithelia are normally found in the same Tg testis (the plate farthest to the right in B). No histopathological defects in the DT-treated testis are detectable in the Wt littermate (lower plates in B). (C) Anti-GATA4, anti-MVH, anti-laminin, anti-SMA and anti-3β-HSD immunostaining of Tg and Wt testes treated with DT at P21. GATA4-positive nuclear signals are missing inside the seminiferous tubules. No appreciable differences in anti-laminin-/SMA-/3β-HSD-staining patterns are detectable between Tg and Wt testes. Scale bars represent 100 µm in B; 20 µm in C.

Transplantation of healthy immature Sertoli cells can rescue the germ cell degeneration in DT-treated Tg testes, leading to a partial recovery of the spermatogenesis

The intratubular injection method of the cell suspension prepared from immature testes previously showed that

immature dividing Sertoli cells selectively colonize and support the recipient-derived spermatogenesis in the empty seminiferous tubules pre-treated with the Buslfan and cadmium (Shinohara *et al.* 2003). In order to rescue the germ cell degeneration, healthy immature Sertoli cells (around P7) labeled by cytoplasmic/nuclear EGFP

Figure 3 A rescue experiment by transplantation of the healthy Sertoli cells into DT-treated Tg testes. (A) Schematic representation of Sertoli cell transplantation on day 4 and day 45 after DT injection (0.4–4.0 $\mu\text{g}/\text{kg}$). (B) Various degrees of contribution of donor-derived (EGFP/Cherry-positive) Sertoli cells in each testis on day 10 and day 45 after Sertoli cell transplantation. (C, D, E, and F) Donor-derived cytoplasmic/nuclear EGFP and nuclear Cherry signals in the transplanted tubules (lower magnified images; C and E) and EGFP/Cherry signals (green/red) and anti-SOX9 (brown in D, green in F) immunostaining images of the same testicular section (D and F), showing the contribution of donor-derived healthy Sertoli cells (arrowheads) in the DT-treated testis on day 10 after transplantation. In the most right-handed images of plates D and F, transplanted Sertoli cells directly support the host-derived germ cells (asterisks) in DT-treated Tg testes. (G and H) Anti-Cherry (red) and anti-SOX9 (green in H) double immunostaining images showing spermatogenic patches derived from both endogenous (asterisks) and exogenous (open arrow) germ cells in the transplanted DT-treated Tg testis on Day 45 after transplantation. Transplanted Sertoli cells directly support spermatogenesis of the host-derived germ cells (asterisks). In the host, plates C and D are the testis pre-treated with DT at 0.4 $\mu\text{g}/\text{kg}$, while plates E, F, G, and H are the testes pre-treated with DT at 4.0 $\mu\text{g}/\text{kg}$. Scale bars represent 200 μm in C, E, and G; 10 μm in D; 20 μm in F, H.

Figure 4 Specific depletion of the granulosa cells of AMH-positive presumptive primary and secondary follicles in developing ovaries. (A and B) Anti-AMH immunostaining and H&E staining images of the P7 ovaries of Tg and Wt littermates (DT; 40.0 µg/kg treatment at P3). In B, the ovarian surface region including an AMH-negative primordial follicle pool (indicated by bars) is shown on the left side. (C) Higher magnified images of H&E, anti-FOXL2, anti-MVH, anti-laminin and anti-SMA staining patterns in the presumptive primary and secondary follicles located in the centromedullary region of DT-treated ovaries (Tg: upper plates, Wt: lower plates). FOXL2-positive granulosa cells are missing in the affected follicles of the DT-treated Tg ovary, despite no differences in anti-laminin- /anti-SMA-immunostaining patterns between Tg and Wt ovaries. (D) Gross-anatomical, H&E staining and anti-3β-HSD/anti-AMH immunostaining images of the P28 ovaries of Tg and Wt littermates treated with DT (40.0 µg/kg) at P3 (day 25 after DT treatment). The new recruitment of AMH-positive primary and secondary follicles is shown in the DT-treated Tg ovary, and both the ovary size and the 3β-HSD-positive area are reduced in the Tg ovary. Nuclei are counterstained with hematoxylin in blue. In A and D, each inset shows a higher-magnification image of the area indicated by the broken rectangle. Scale bars represent 100 µm in A, B, and D; 20 µm in C.

(CAG-EGFP) or nuclear Cherry (R26-H2B-mCherry) were transplanted (i.e. intratubular injection) in P21 Tg males, on day 4 after DT treatment (0.4–4.0 µg/kg). The transplanted testes were examined on day 10 after Sertoli cell transplantation (Fig. 3A). In the DT-treated Tg testes transplanted with exogenous healthy testis cells, the transplantation sites were visualized by means of EGFP- or Cherry-positive signals under a dissecting microscope (number of testes with donor cells per total testes injected: 10/18 testes (EGFP, 4/8 testes; Cherry, 6/10 testes); Fig. 3B). Histological analysis revealed that a majority of transplanted cells were positive for *Sox9*, a Sertoli cell marker, suggesting selective settlement of the exogenous healthy Sertoli cells inside the DT-treated seminiferous tubules (Fig. 3D). Moreover, exogenous healthy Sertoli cells were shown to maintain both endogenous and exogenous spermatogenic cells at the transplantation sites (Fig. 3C, D, E, and F). In some areas, the transplanted Sertoli cells had replaced the degenerated Sertoli cells and contributed to supporting the host-derived germ cells on day 10 (most right plates in Fig. 3D and F). Next, we examine the phenotypes of the DT-treated Tg testes on day 45 after the Sertoli cell transplantation. In one sample (Cherry, 1/3 testes), the transplanted Sertoli cells were observed to survive in the DT-treated testes (Fig. 3B, G, and Supplementary Figure S8, see section on supplementary data given at the end of this article). Moreover, in some areas, it was shown that the transplanted Sertoli cells partially restore spermatogenesis of endogenous spermatogenic cells on day 45 after the transplantation (Fig. 3H and Supplementary Figure S8). These findings show that the transplanted immature Sertoli cells could replace some degenerated Sertoli cells and rescue in part the germ cell degeneration, albeit locally, in DT-treated Tg testes. These data also suggest this AMH-Treck system is useful for transplantation studies in terms of evaluating Sertoli cell function. Indeed, this new Treck system is more simple and effective than the previous method of the Sertoli cell transplantation, which used an intratubular injection of cadmium to deplete Sertoli cells (Shinohara *et al.* 2003).

DT treatment allows the loss of granulosa cells under high-dose DT treatment in postnatal ovaries

Finally, we examined the phenotype of DT-induced cell depletion in the initial round of folliculogenesis in the Tg females at P7 (on day 4 after DT treatment at P3). Since the preliminary experiments using DT treatment at 4.0 µg/kg resulted in no appreciable histological defects in the developing ovaries (data not shown), we applied DT treatment (40.0 µg/kg) to the Tg ovaries; the phenotype was examined histopathologically on day 4 or day 25 after DT injection at P3 (Fig. 4). A high-dose DT treatment induced almost complete loss of the AMH-positive follicles in the centromedullary region of the ovaries (Fig. 4A and B). At this DT concentration, both histological

and immunohistochemical analyses confirmed the lack of granulosa cells around the growing oocyte in the majority of presumptive primary and secondary follicles (Fig. 4B and C). The FOXL2-positive presumptive theca cells were found in the ovarian interstitium around the growing follicles. Both laminin-positive basal lamina and SMA-positive perifollicular cells appeared intact and were directly connected to the growing oocyte with a diameter similar to those of the primary and secondary follicle stages (Fig. 4C). No appreciable defects were detected in the ovaries of the littermates treated with DT at the same dose ('Wt' in Fig. 4B and C). The absence of any histological abnormality in the ovarian-surface primordial follicle pool of the same DT-treated ovaries (Fig. 4B) indicated a specific loss of the granulosa cells in growing follicles. Moreover, this suggested that the sensitivity to DT treatment of the granulosa cells of female pups was lower than that of the Sertoli cells of male pups in this *Amh*-Treck line. On day 30 after DT treatment (40 µg/kg), the DT-treated ovaries displayed a recovery of folliculogenesis in the cortex region, although the ovary size and the 3β-HSD-positive interstitial region appeared to be reduced (Fig. 4D). The DT-treated ovaries contained many healthy *Amh*-positive primordial and secondary follicles (the plate farthest to the right in Fig. 4D), suggesting new recruitment of primary and secondary follicles in these DT-treated ovaries. This was also consistent with the absence of any appreciable defects in the primordial follicle pool after the high-dose DT treatment.

In conclusion, we have established a novel Treck system that permits the specific loss of gonadal supporting cells at the postnatal stages using DT treatment. The present data confirm the essential role of Sertoli cells in germ cell survival *in vivo*. This model system is also useful not only for *in vivo* analyses of structural and endocrine roles of the supporting cell lineage at the postnatal stages, but also potentially for the *in vivo* functional assay of defective supporting cells in stem cell niche and spermatogenesis.

Supplementary data

This is linked to the online version of the paper at <http://dx.doi.org/10.1530/REP-14-0171>.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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Author contribution statement

M Shinomura and K Kishi contributed equally to this work as first authors. M Kanai-Azuma and Y Kanai contributed equally to this work for last authors.

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