Supplementary materials and methods

Animals

R26-mTmG female mice (double-fluorescent Cre reporter mice that express membrane-targeted tandem dimmer TOMATO prior to Cre-mediated excision and membrane-torgeted green fluorescent protein (GFP) after excision (Muzumdar *et al.* 2007)) were crossed with *Vasa-Cre* male mice. Male offspring from this cross that inherited the *Vasa-Cre* allele were collected at newborn stage and immunostained as described below in order to verify the Cre recombinase activity.

The animal experiments were carried out in accordance with the Guidelines for Animal Use and Experimentation of the University of Tokyo (approval IDs: P13-764, P14-876, PM15-085, and PH16-084) and the Tokyo Medical and Dental University (approval IDs: 0140007A, 0150259C2, 0160024C2, and 0170248C2).

Whole-mount immunostaining

Testes and epididymides of newborn mice were fixed in 4% paraformaldehyde. After washing in phosphate-buffered saline, the samples were treated with CUBIC solution 1 (Susaki *et al.* 2014) for 1 week at 4°C to facilitate the visualization of immunoreactive signals, and then blocked with Tris-buffered blocking solution. Subsequently, the samples were incubated for 12 hours at 4°C with anti-VASA (1:1,000 dilution; Abcam, Cambridge, United Kingdom) antibody. The immunoreactive signals were visualized by using Alexa-680 conjugated secondary antibody (Molecular Probes) in combination with the nuclear counterstaining of 4',6-diamidino-2-phenylindore (DAPI). The stained samples were then transferred to CUBIC solution 2 (Susaki *et al.* 2014) and analyzed using Leica TCS SP8 (Leica Microsystems GmbH) confocal laser microscope as

25 described previously (Aiyama *et al.* 2015).

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28 **Supplementary references**

29 **Aiyama Y, Tsunekawa N, Kishi K, Kawasumi M, Suzuki H, Kanai-Azuma M,**
30 **Kurohmaru M & Kanai Y** 2015 A Niche for GFRalpha1-Positive Spermatogonia in
31 the Terminal Segments of the Seminiferous Tubules in Hamster Testes. *Stem cells* **33**
32 2811-2824.
33 **Muzumdar MD, Tasic B, Miyamichi K, Li L & Luo L** 2007 A global double-
34 fluorescent Cre reporter mouse. *Genesis* **45** 593-605.
35 **Susaki EA, Tainaka K, Perrin D, Kishino F, Tawara T, Watanabe TM, Yokoyama**
36 **C, Onoe H, Eguchi M, Yamaguchi S, Abe T, Kiyonari H, Shimizu Y, Miyawaki A,**
37 **Yokota H & Ueda HR** 2014 Whole-brain imaging with single-cell resolution using
38 chemical cocktails and computational analysis. *Cell* **157** 726-739.
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