Supplementary information, Data S1 Materials and Methods

Animal ethics statement

The use and care of animals complied with the guideline of the Animal Advisory Committee at the Shanghai Institute of Biological Science, CAS. The ethic application entitled "Reproductive physiology of cynomolgus monkey and establishment transgenic monkey" #ER-SIBS-221106P, were approved by Shanghai Institute of Biological Science, Chinese Academy of Sciences.

Procedures for Xenografting

One testis each was obtained from a 14-month and a 27-month monkey that was sedated with ketamine hydrochloride. The testis was cut into small fragments (1-2 mm in diameter). As the hosts, 16 immunodeficiency male nude mice were anesthetized and castrated. Six or eight monkey testis fragments were grafted into the subcutaneous tissue on the back of the mice. Eight recipient mice were grafted for each donor testis. Some fragments were fixed immediately for histology.

Isolation and storage of sperm from excised grafts

The testis xenografts were recovered from nude mice after incubation for more than 10

months and was minced and dispersed in TALP/HEPES medium supplemented with 0.3% fatty acid-free BSA (TH3) medium. Motile sperms were seen in the suspension after repeatedly gentle pipetting of the minced tissue. The suspension was centrifuged for 3 min at 1200g, the pellet was re-suspended in TH3 medium for ICSI or in frozen medium for storage in liquid nitrogen.

Sperm cryopreservation and thawing

For sperm cryopreservation, sperms were obtained from xenografts by gentle teasing with forceps and pipetting, and then transfered to TH3 medium for washing twice by centrifugation at 1800rpm. The supernatant was removed and the pellet was re-suspended in human sperm freezing medium (No.90128, Irvine Scientific) and divided into cryo tubes for liquid nitrogen preservation.

For sperm thawing, cryo tubes with sperm were incubated in 37^{o}C for 5 min, and transferred into TH3 medium for washing twice to remove the freezing medium. The supernatant was removed and the pellet was re-suspended in TH3 for ICSI.

Oocytes collection, ICSI, and embryo transfer

The oocytes were collected by laparoscopy. In brief, cycling females were subjected to follicular stimulation using twice-daily intramuscular injections of 25 IU of recombinant

human FSH (rhFSH) for 8 d. Then 1000 IU of human chorionic gonadotropin (hCG) were injected on day 9. Thirty-thirty six hours after the hCG injection, the female was anesthetized. Recovery of the oocytes was performed with a laparoscope (Olympus) attached to a video system. The oocytes were aspirated from follicles 2–8 mm in diameter into a container with warmed Hepes-buffered Tyrode's lactate medium supplemented with 100 IU/ml penicillin and 100g/ml streptomycin (TH3 medium) with 10 IU/ml heparin using a 19-gauge needle attached to the Cook aspiration system. All oocytes treated with 1 mg/ml hyaluronidase (Sigma) were retrieved from aspirates using a 70μm mesh size filter (BD). The collected oocytes were cultured in pre-equilibrated maturation medium. For ICSI, a single picked sperm was aspirated tail first and injected into the ooplasm using a piezo-actuated micromanipulator. After ICSI, embryos were cultured in pre-equilibrated hamster embryo culture medium 9 (HECM9) at 37°C under 5% CO_2, and high-quality zygotes were selected for embryo transfer to menstruation-synchronized recipients on the next day. The pregnancies of recipients were confirmed by B ultrasonography examination 30 days after embryo transfer.

Genotyping PCR

A small piece of ear tissue collected from newborn monkey pups were used to extracted DNA. Samples were digested by proteinase K overnight at 65 degree and precipitated for DNA. PCR with specific primers again GFP were used for initial genotyping analysis. Primers: GFP-F: ATGGTGAGCAAGGGCGAGGA. GFP-R: TTACTTGTACAGCTCGTCCATGC

Western Blotting

Monkey brain tissues were homogenized in RIPA buffer (containing 50 mM Tris-HCl pH 7.4, 150 mM NaCl, 1% TritonX-100, 0.1% SDS, 1% sodium deoxycholate, protease inhibitor cocktail, phosphatase inhibitor cocktail) on ice and then centrifuged at 1000 g for 10 min at 4^{o}C. The supernatant was stored at -80 ^{o}C until use. 30 μg protein of each sample was loaded in 10% SDS-PAGE and run at 120V constant voltage, with 0.36 mA constant current was used for transblotting. Blots were probed with primary antibodies (1:1000) overnight at 4^{o}C. After washing for three times blots were then incubated with goat anti-rabbit secondary antibody (1:3000) at room temperature for 2 h. Chemiluminescence was used to visualize protein bands. Antibodies used: GFP antibody (Invitrogen #A11122).

Artificial embryo activation

Embryos obtained by ICSI were activated by exposure to 5 mM ionomycin for 5 min in TALP/HEPES medium supplemented with 0.3% fatty acid-free BSA, and then cultured in HECM-9 medium containing 7.5 μg/ml CHX at 37 °C in 6% CO_2 for 5 h.

Histological analysis

The testis xenografts were fixed in Bouin solution for 48 h and processed for paraffin section

and hematoxylin-eosin staining.

Statistical calculation of the growth of seminiferous tubule in xenografted testicular tissue

The cross section area of seminiferous tubule is calculated by the three stipple round quantitative provided by the software cellSens. Diameter obtained with area divided by 3.14.

Short tandem repeats (STR) analysis

Locus-specific primers each containing a fluorescent dye (FAM/HEX/TMR) were used for PCR amplification in batches. FAM, HEX or TMR-labeled STR amplicons were diluted and mixed with internal size standard ROX500 and deionized formamide, followed by capillary electrophoresis on ABI PRISM 3730 genetic analyzer to obtain raw data.

Sequenator-genetated raw data were analyzed with the program Gene Marker 2.2.0, which produces wave plots, Excel documents (including information such as size and genotype), and DNA profiles.

Single nucleotide polymorphism (SNP) analysis

Skin sample collected from monkeys were used to extract DNA. PCR with specific primers against 22 SNP were performed. DNA was amplified with 35 cycles of 95°C for 30 sec, 55°C

for 30 sec, and 72°C for 1 min, followed by a 5-min extension at 72°C. The PCR products were used for sequencing and the result were used for the SNP analysis.