Supplementary Figures

Legends to Supplementary Figures

Figure S1. Design of the preliminary experiment. Four monkeys were evaluated before and periodically while receiving no treatment, or after exposure to radiation, treatment with hormone suppression, or both radiation and hormone suppression (one monkey per group). Evaluation included sampling of serum and semen, measurements of testis volume and weight, and testis biopsies as indicated. No germ cell transplantation was performed.

Figure S2. Determination of GnRH-ant dose for appropriate hormone suppression in monkeys. (A) Serum testosterone levels in the four individual monkeys used in the preliminary study are shown. One unirradiated monkey was initially given daily subcutaneous injections of Acyline at 50 μg/kg/day for 2 weeks, followed by twice-weekly injections, at doses of 200 μg/kg (Monday) and 300 μg/kg (Thursday) during weeks 3 and 4 and 300 and 450 μg/kg during weeks 5 through 8. One irradiated monkey was initially given a bolus injection of 600 μg/kg and then twice-weekly injections at doses of 200 μg/kg and 300 μg/kg from weeks 3 through 8. The other two monkeys, one unirradiated and one irradiated, received no GnRH-ant treatment, only bacteriostatic water. The shaded area represents the period of hormone suppression. LOD represents the limit of detection of the testosterone assay. (B) Intratesticular testosterone levels in the same four monkeys at week 8.

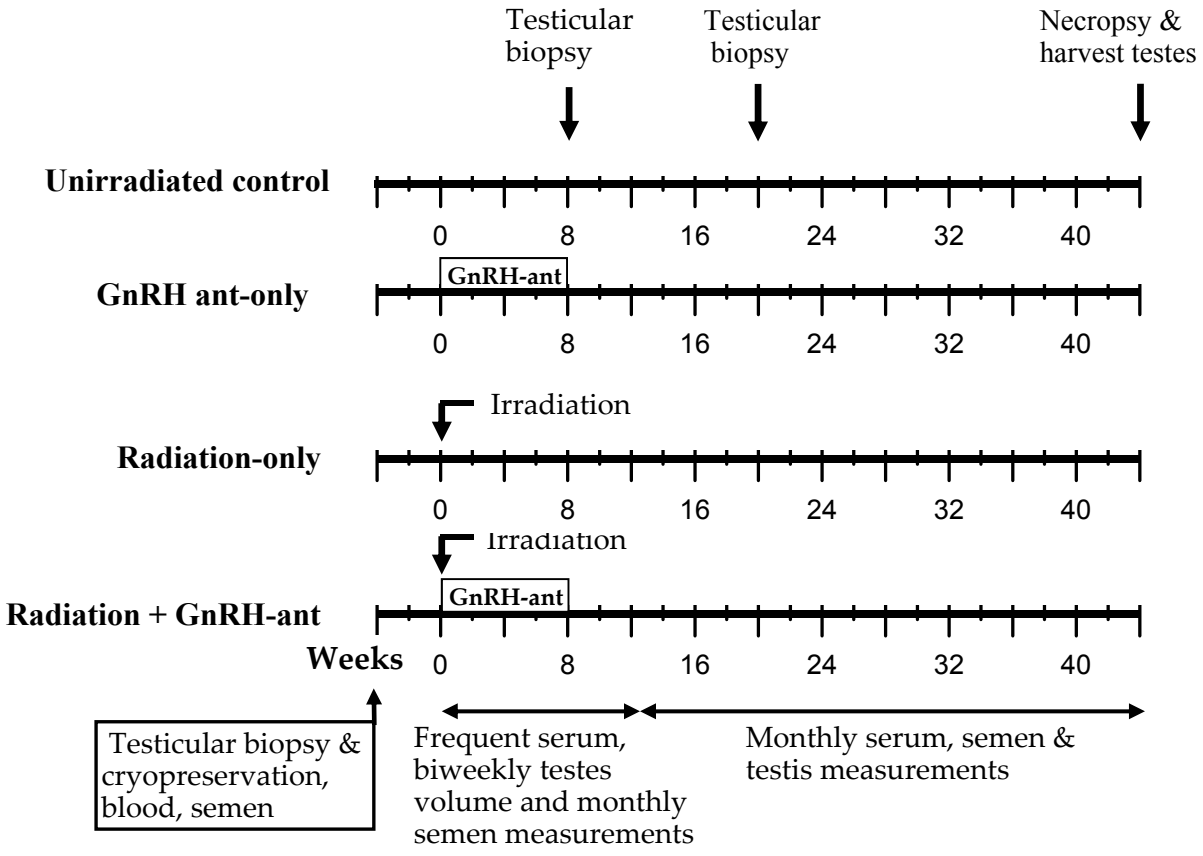
Figure S3. Hormone suppression alone transiently reduces testicular volume. The shaded area represents the period of hormone suppression. (A) Testicular volume was transiently reduced and subsequently restored in the GnRH-ant–treated, unirradiated monkey. (B) Concurrent histologic examination shows shrunken tubules with sloughed germ cells at the end the treatment (8 weeks). (C) Histology was normal at the next biopsy 12 weeks later. Bar represents 50 μm.

Figure S4. Testicular histology of individual monkeys showing that germ cell transplantation in irradiation-only monkeys (monkeys #1 to 6) did not appreciably increase the percentage of tubules with differentiated germ cells. Representative photomicrographs of the sham-transplanted and the respective contralateral transplanted testes are placed side by side. Tubules containing germ cells are marked with *. The sham-transplanted tests in monkey #5 had a necrotic testis and not considered for analysis. Bar: 200 μm.

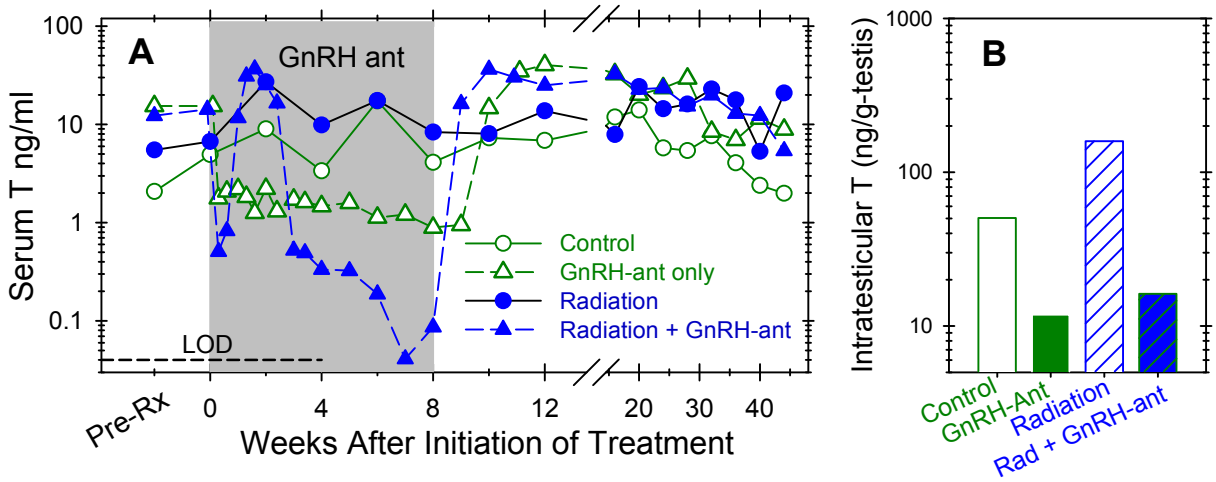
Figure S5. Individual percentages of tubules showing differentiated germ cells (tubule differentiation indices) at 24 weeks after irradiation, in radiation-only and irradiated GnRH-ant–treated monkeys that received autologous transplantation of testicular cells to one of the testes. The individual monkey numbers are indicated.

Figure S6. Xenotransplantation of monkey testicular cells into nude mice verifies the presence of spermatogonial stem cells. A representative donor colony that originated from the monkey stem spermatogonia transplanted into a nude mouse was identified by the anti-rhesus antibody in a whole-mount preparation of the seminiferous tubules. Dotted lines show the outline of the seminiferous tubule. Bar represents 50 μm.

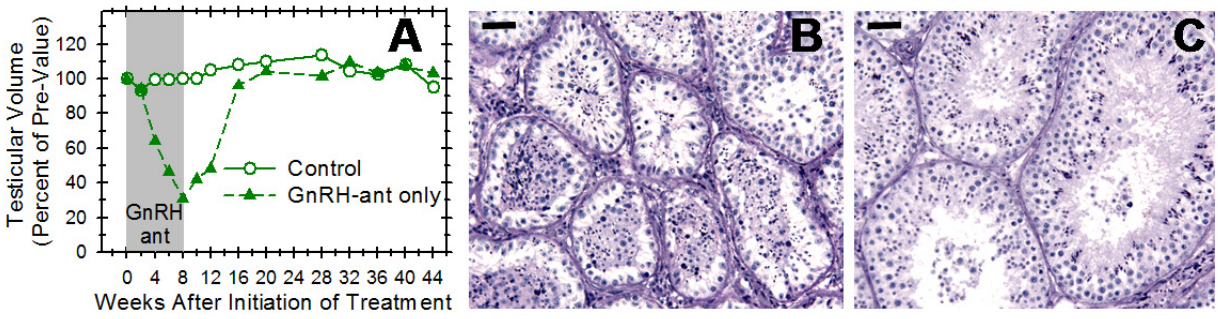
Figure S7. Testicular histology of individual irradiated monkeys showing that combined hormone suppression and germ cell transplantation (monkeys #7 to 12) increased the percentage of tubules with differentiated germ cells in some monkeys (#11 and #12). Representative photomicrographs of the sham-transplanted testes and the respective contralateral transplanted testes are placed side by side. Tubules containing germ cells are marked with *. Bar: 200 μm.



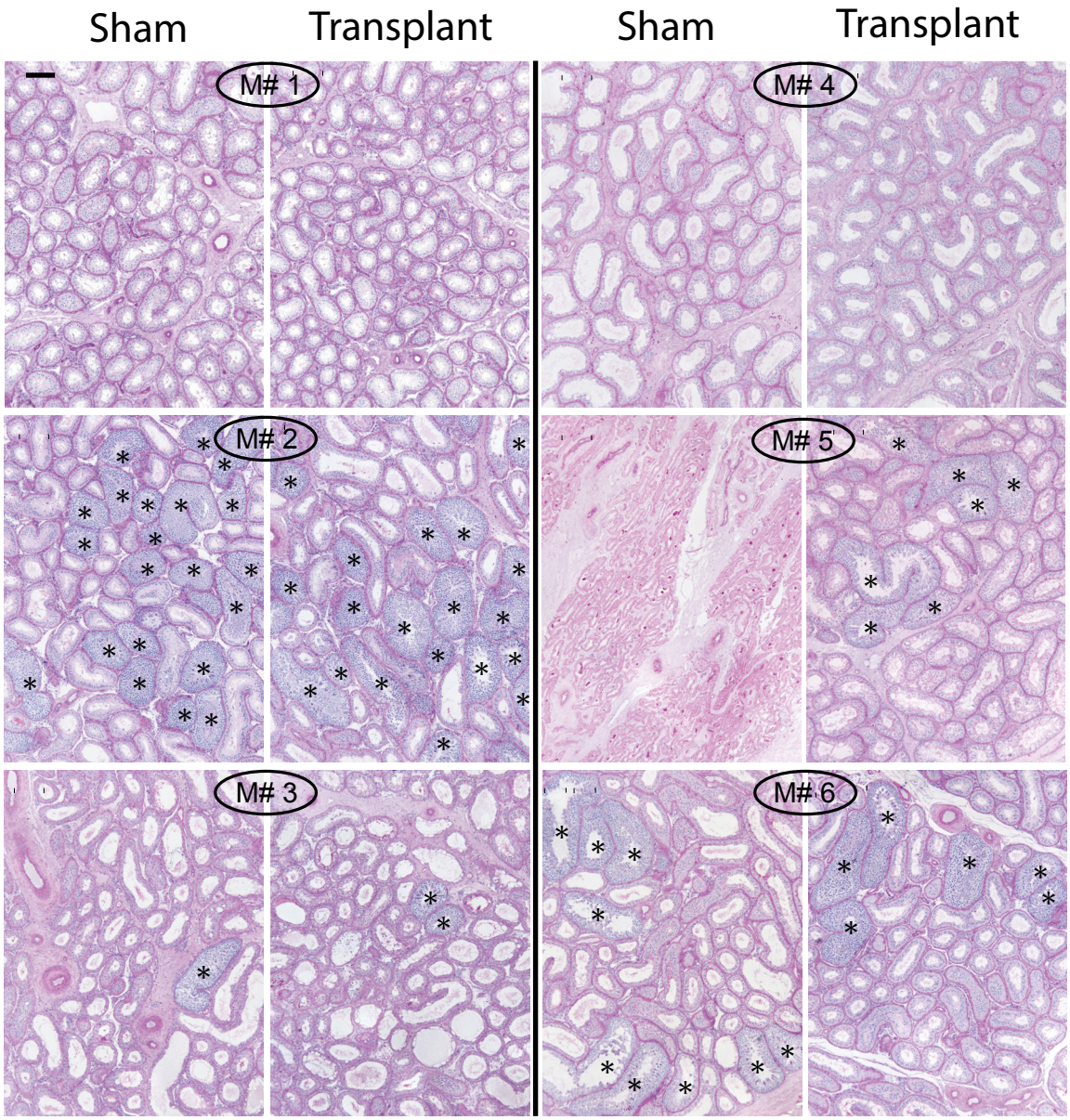
Supplementary Fig. 1



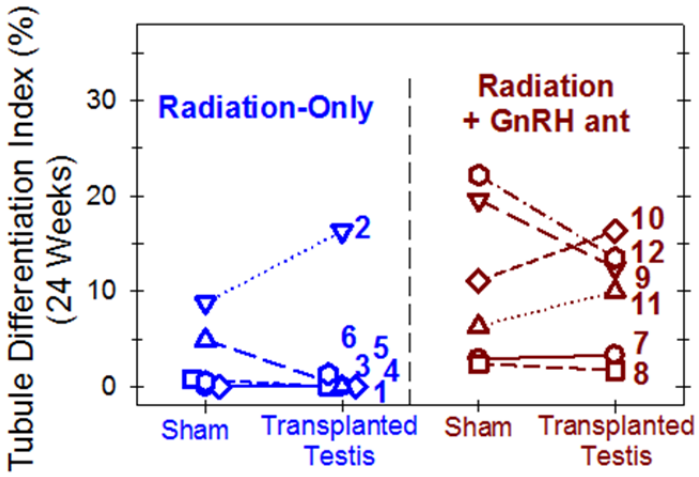
Supplementary Fig. 2



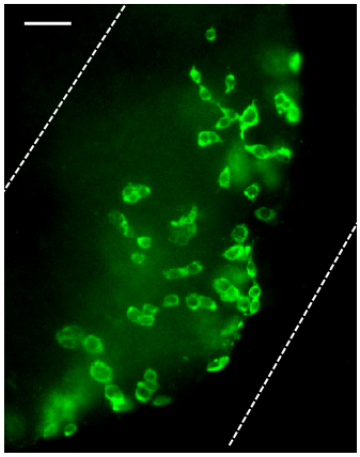
Supplementary Fig. 3



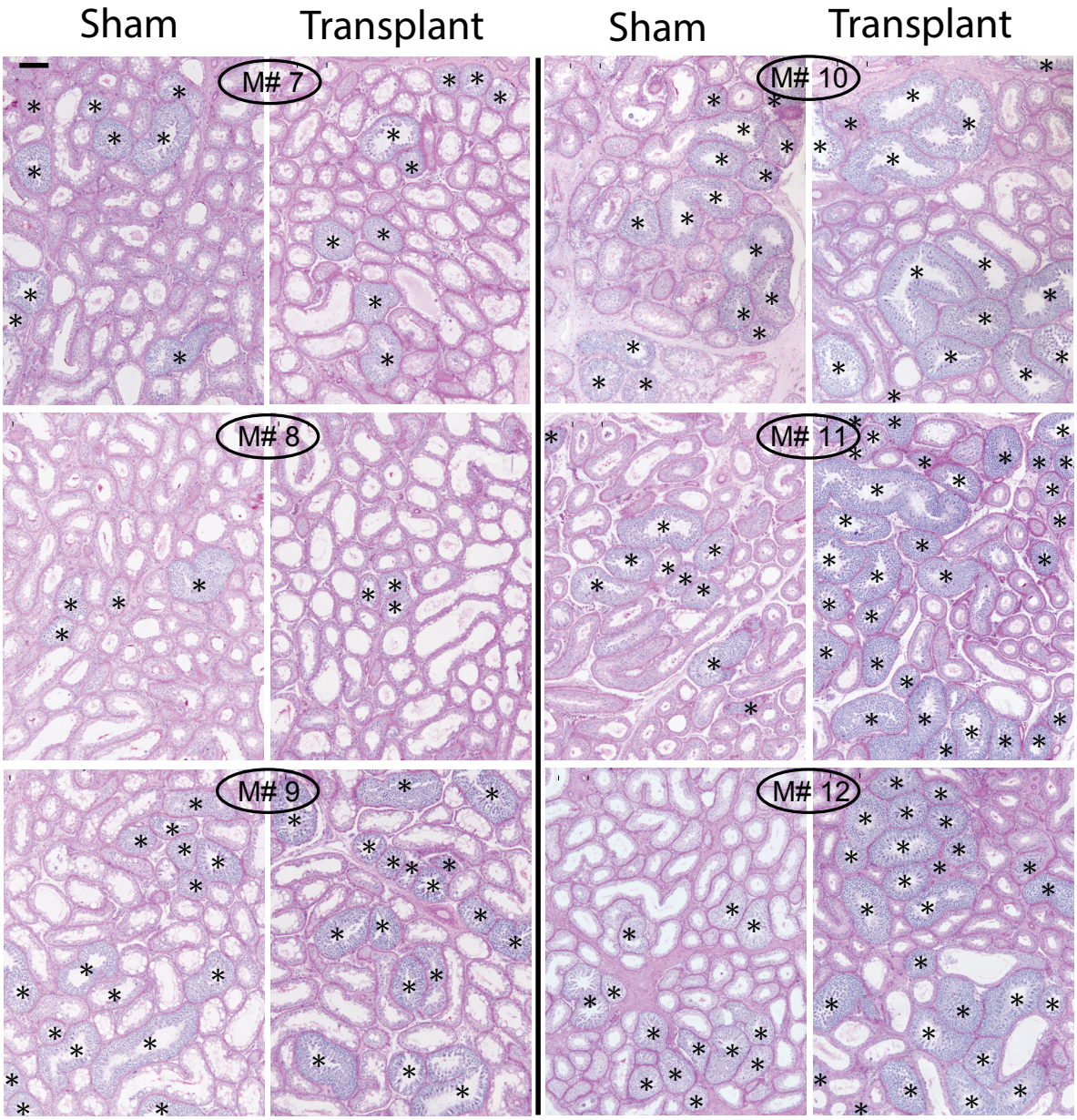
Supplementary Fig. 4



Supplementary Fig. 5



Supplementary Fig. 6



Supplementary Fig. 7