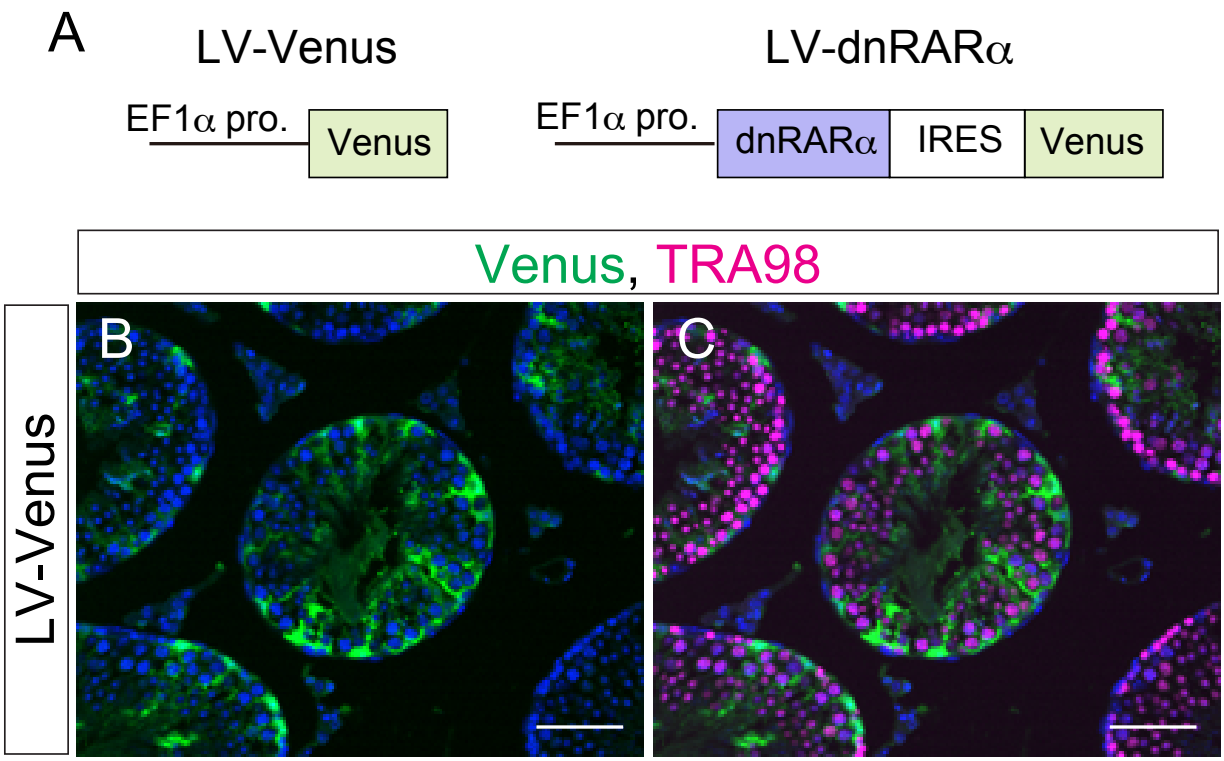

Fig. S1. Confirmation of cell type-specific infection of lentivirus. (**A**) Construction of LV-Venus and LV-dnRARα vectors. (**B**,**C**) At day 5 after the injection of LV-Venus, expression of broad germ cell marker TRA98 (magenta) and Venus (Green) were examined with immunohistochemistry. Scale bars: 80 μm.

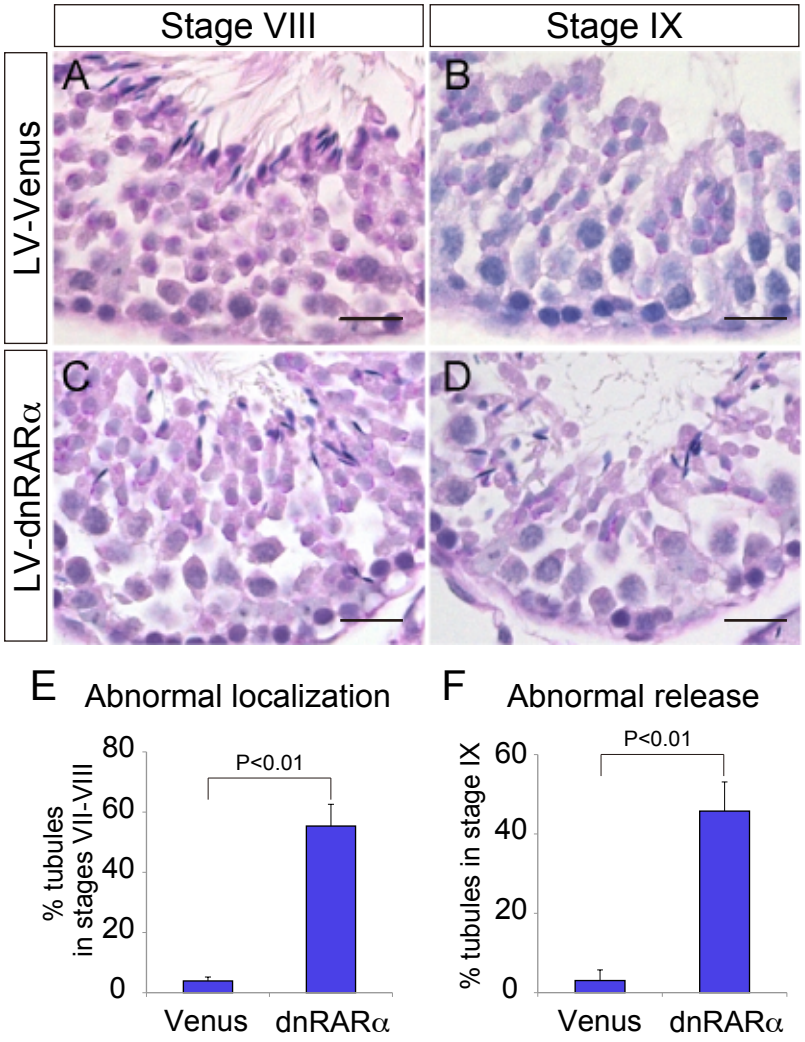

Fig. S2. Abnormal transition and release of elongated spermatids upon the overexpression of dnRARα. (**A-D**) Histological sections derived from mouse testes injected with LV-Venus or LV-dnRARα at stage VIII or stage IX. (**E**) Proportion of Venus-positive tubules at stages VII-VIII showing abnormal alignment of elongated spermatids (*n*=4). (**F**) Proportion of Venus-positive tubules at stage IX containing abnormally retained elongated spermatids (*n*=4). Only Venus-positive tubules were counted. Error bars, s.d. Scale bars: 20 μm.

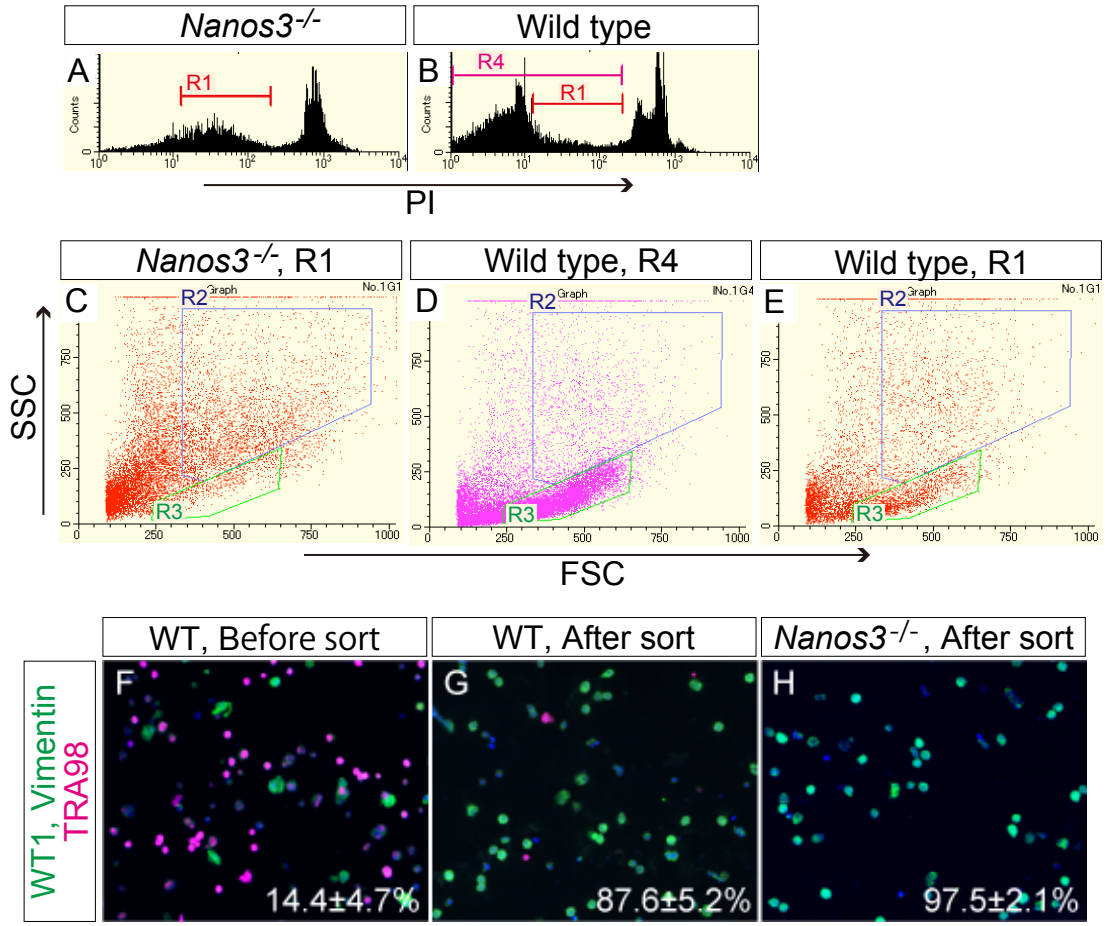

Fig. S3. Direct isolation of Sertoli cells from adult testes by FACS. (**A**,**B**) Testicular cells obtained from *Nanos3*$^{-/-}$ or wild-type mice were analyzed by FACS. Propidium iodide (PI)-negative cells (R4) or cells showing high intrinsic fluorescence (R1) were selected for flow sorting. PI-positive dead cells were removed. (**C-E**) R1 fractions from *Nanos3*$^{-/-}$ or wild-type testicular cells and an R4 fraction from wild-type testicular cells. R2 and R3 fractions represent Sertoli cells and germ cells, respectively. (**F-H**) Wild-type testicular cells before and after sorting and *Nanos3*$^{-/-}$ testicular cells after sorting were immunostained with antibodies against WT1, vimentin (green) and TRA98 (magenta). The proportions of WT1 and vimentin-positive Sertoli cells are indicated at the bottom-right of the lower panels. The values are the mean ± s.d.

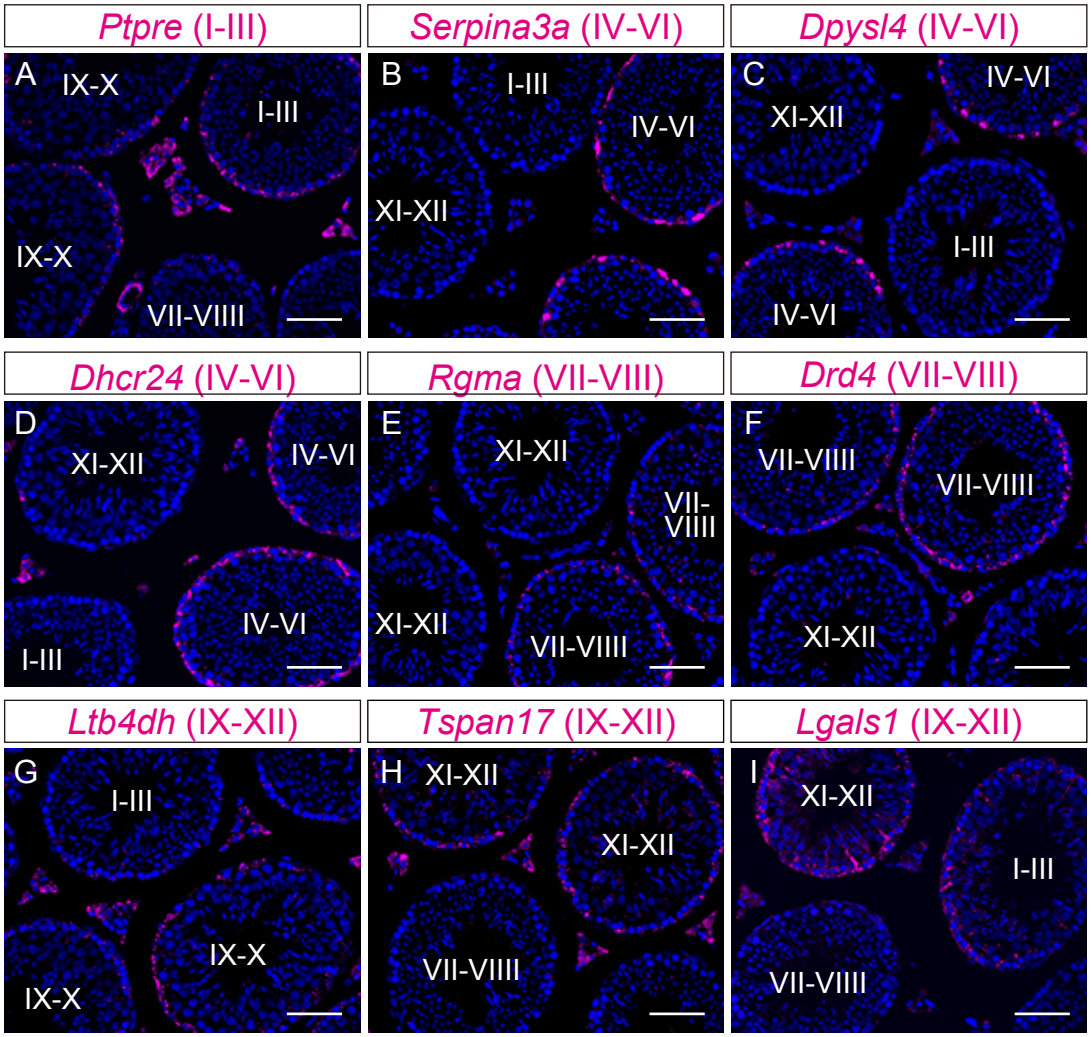

Fig. S4. Validation of the microarray results. (**A-I**) The spatial expression patterns of nine genes identified as stage-dependent and showing peaks during stages I-III (*Ptpre*), IV-VI (*Serpina3a*, *Dpysl4*, *Dhcr24*), VII-VIII (*Rgma*, *Drd4*) and IX-XII (*Ltb4dh, Tspan17*, *Lgals1*) were examined by in situ hybridization. The seminiferous epithelial stages were determined by serial section staining with PAS and Hematoxylin. Scale bars: 80 μm.

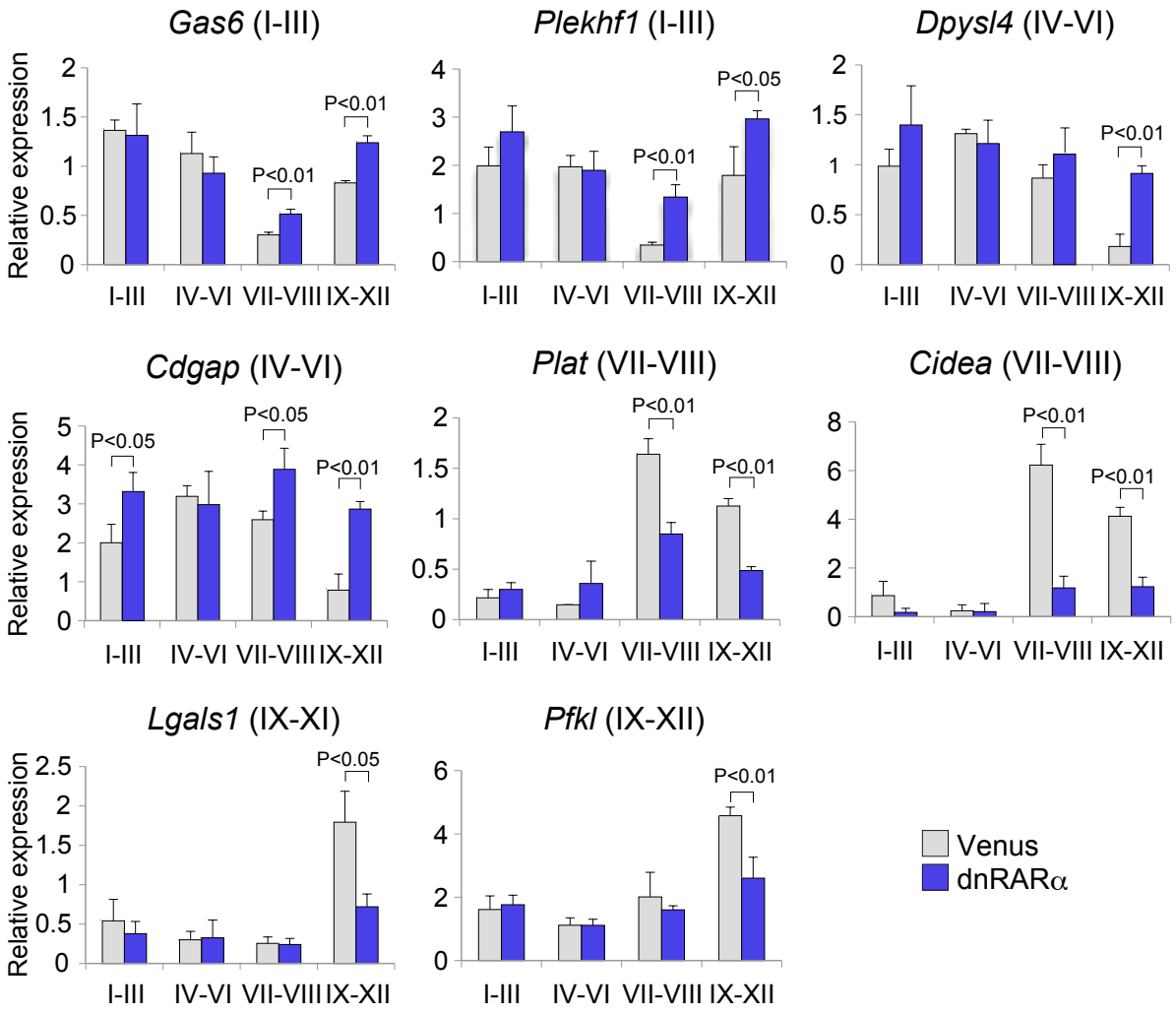

Fig. S5. Validation of the gene expression changes induced by the overexpression of dnRARα. At day 5 after the injection of LV-dnRARα or LV-Venus, stage-specific Venus$^+$ tubules were isolated and the expression of the indicated stage-dependent genes showing peaks during stages I-III (*Gas6*, *Plekhf1*), IV-VI (*Dpysl4*, *Cdgap*), VII-VIII (*Plat*, *Cidea*) and IX-XII (*LgalsI*, *Pfkl*) were quantified by qRT-PCR (n=3). *Gapdh* was used as internal control. Error bars, s.d.

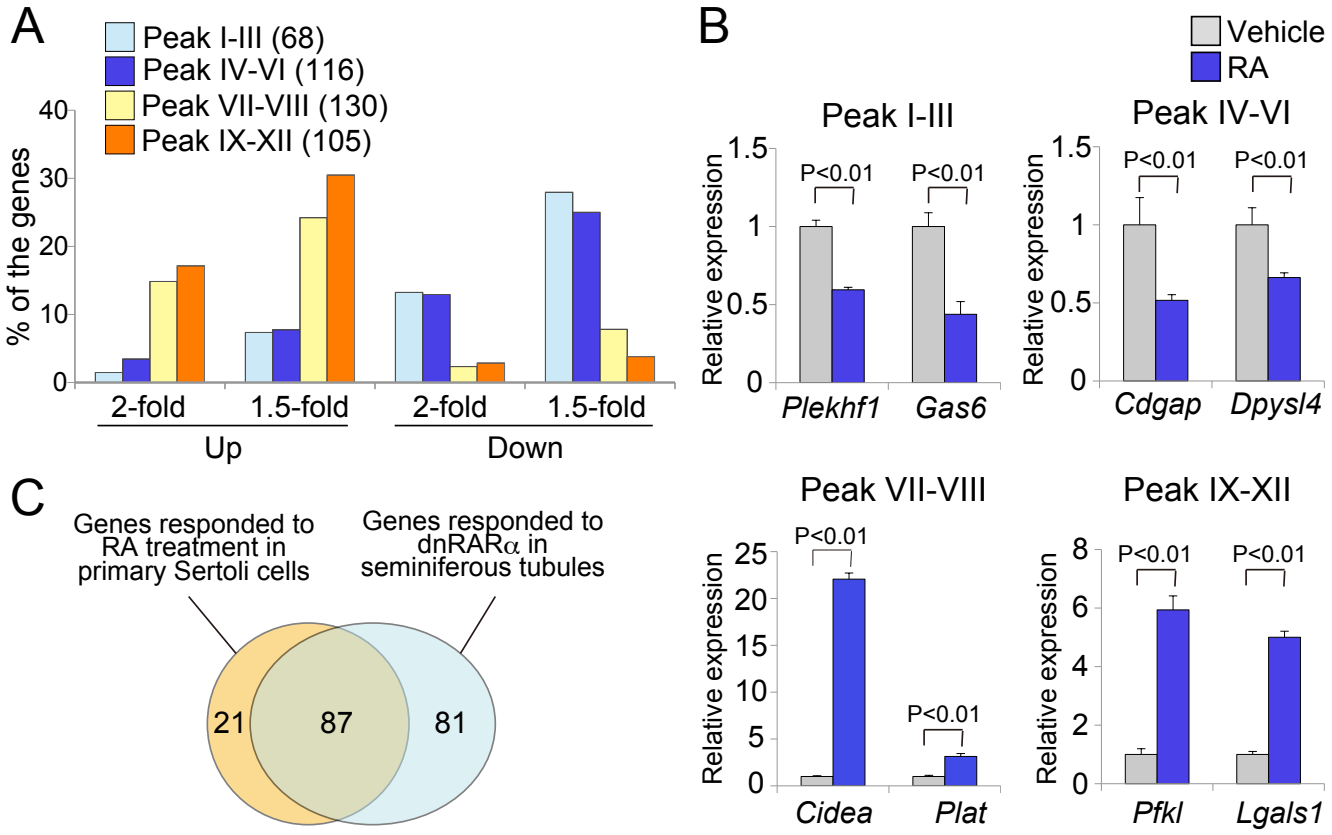

Fig. S6. Expression changes of the stage-dependent genes upon the activation of RA signaling in primary Sertoli cells. (**A**) Cultured Sertoli cells were incubated with 1 μM RA for 24 hours and the expression changes of stage-dependent genes were measured by microarray (*n*=2). *y*-axis represents total percentage of genes that showed up- or downregulation. (**C**) Comparison of genes that responded to RA treatment in primary Sertoli cells (108 genes) and to overexpression of dnRARa in seminiferous tubules (168 genes). Between those, 87 genes were common. (**B**) To validate the microarray results, the expression of stage-dependent genes showing peaks during stages I-III *(Plekhf*, *Gas6*), IV-VI (*Cdgap*, *Dpysl4*), VII-VIII (*Cidea*, *Plat*) and IX-XII (*Pfkl*, *Lgals1*) in cultured Sertoli cells treated with RA were quantified using qRT-PCR (*n*=3). *Gapdh* was used as internal control. Error bars, s.d.

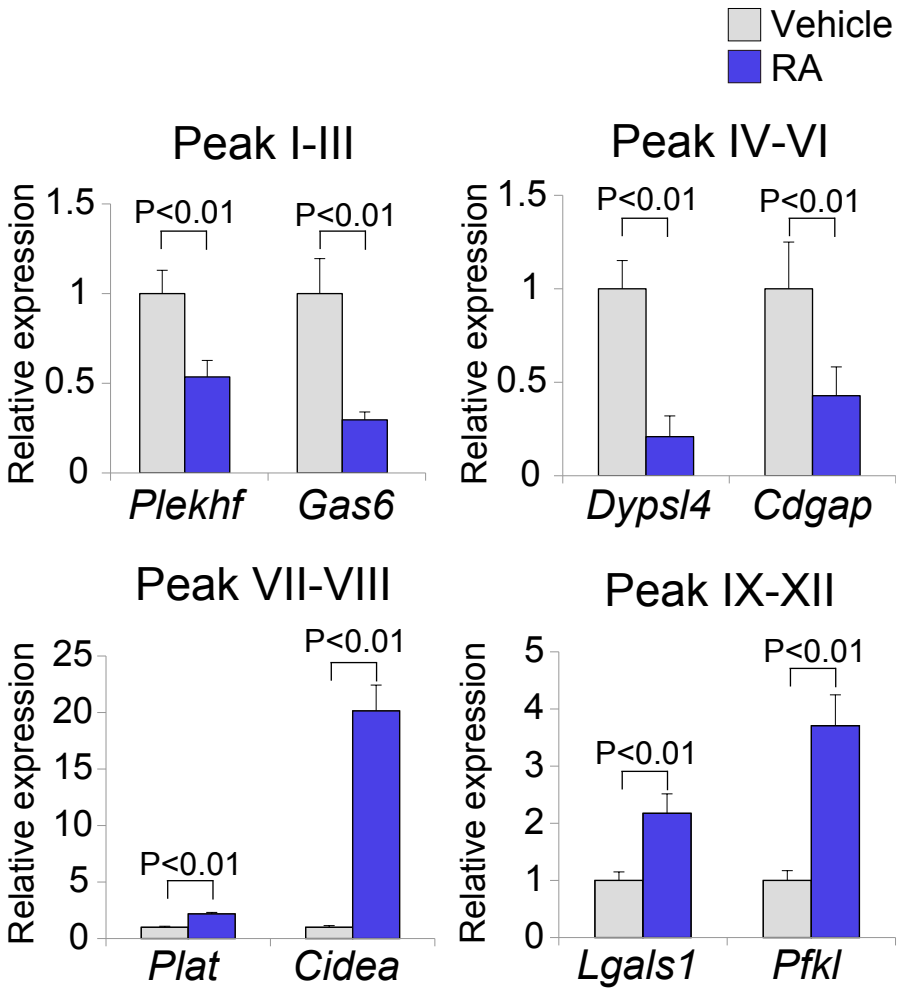

Fig. S7. Expression changes of stage-dependent genes in VAD mice following retinol injections. At 24 hours post-injection, genes showing peak expression during stages I-III (*Plekhf1*, *Gas6*), IV-VI (*Dpysl4*, *Cdgap*), VII-VIII (*Plat*, *Cidea*) and IX-XII (*Lgals1*, *Pfkl*) in whole mouse testes were assayed by qRT-PCR (*n*=3). *Gapdh* was used as internal control. Error bars, s.d.

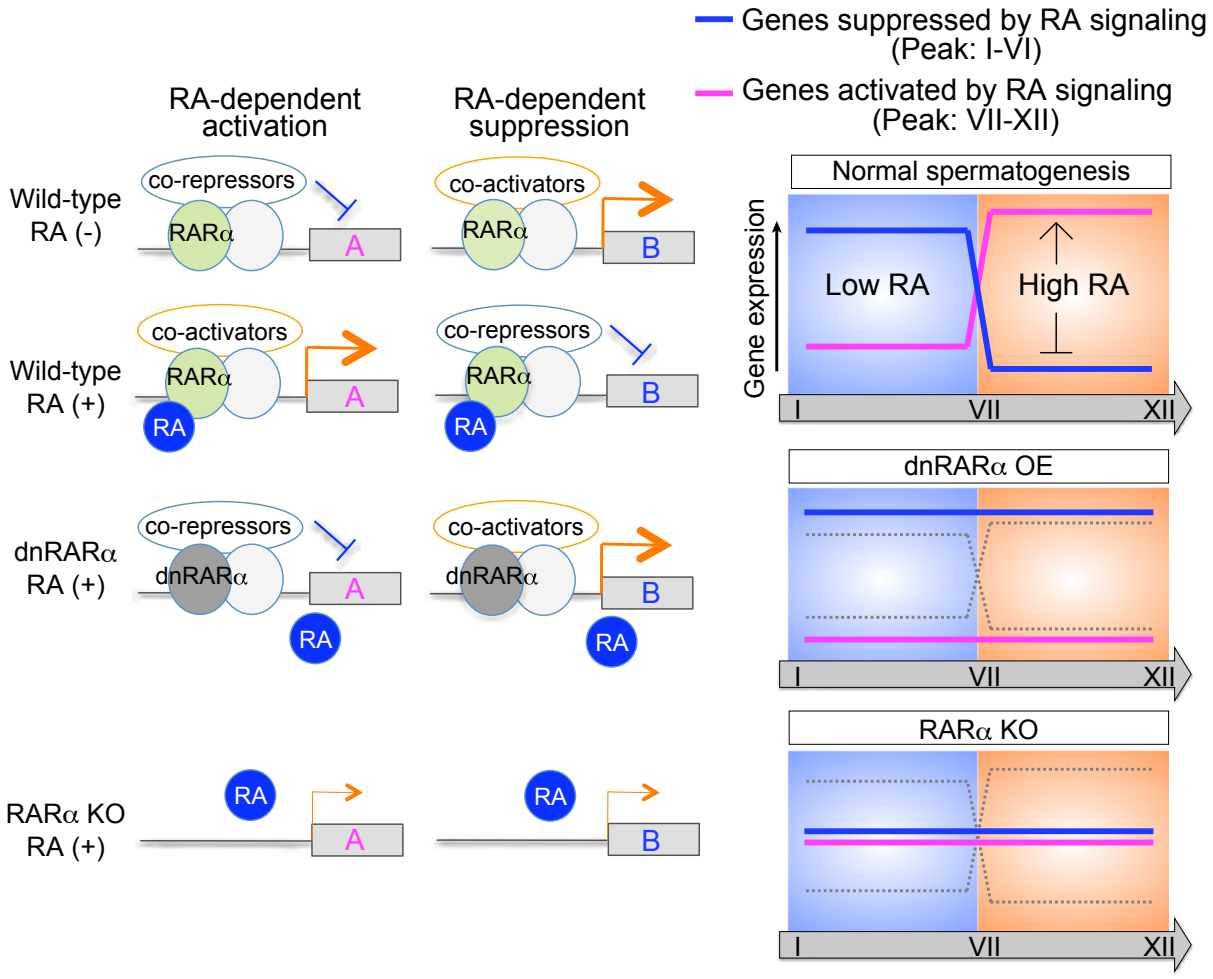

Fig. S8. Proposed model for regulation of stage-dependent gene expression in Sertoli cells by RA signaling. RARα usually binds to DNA and activates or suppresses the corresponding target genes, even in the absence of RA. Upon the binding of RA, RARα exchanges the co-regulators and alters the transcription status of the target genes. During normal spermatogenesis, RA signaling is maintained at low and high levels during stages I-VI and VII-XII, respectively, and thereby creates two patterns of gene expression in Sertoli cells through the regulation of RA-responsive genes. Genes suppressed and activated after the binding of RA to RARα show expression peaks during stages I-VI and VII-XII, respectively. In the case of the overexpression (OE) of dnRARα, the exchange of co-regulators would not occur even in the presence of RA. As a result, the genes controlled by RA signaling will remain in a stage I-VI-like expression state. In the case of a RARα KO, both RA-dependent and -independent activation or suppression might be diminished.

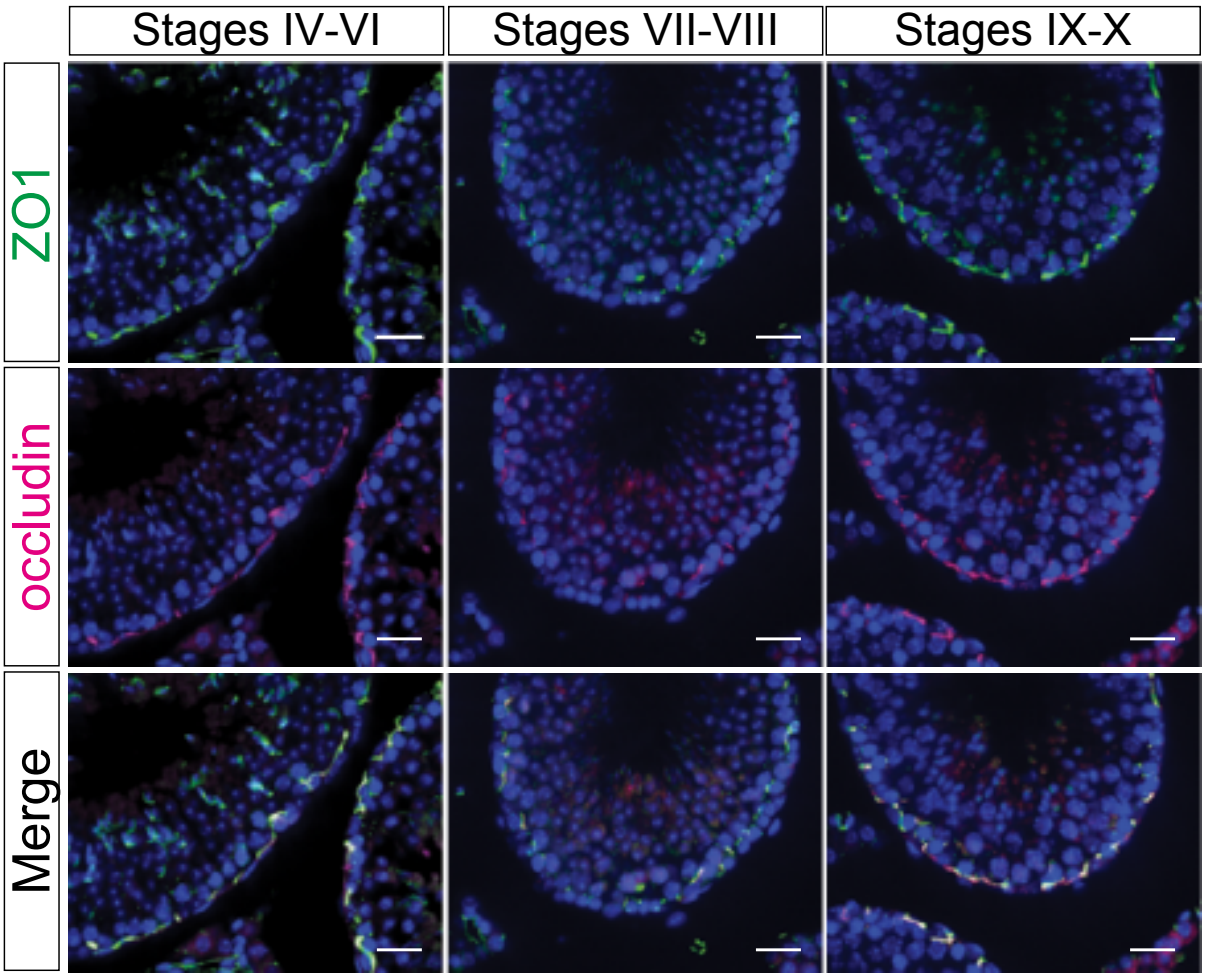

Fig. S9. Stage-dependent expression change of occludin. Immunostaining for ZO1 (green) and occludin (magenta) in stage-specific tubules. Seminiferous epithelial stages were determined with serial sections with PAS and Hematoxylin. Scale bars: 20 μm.

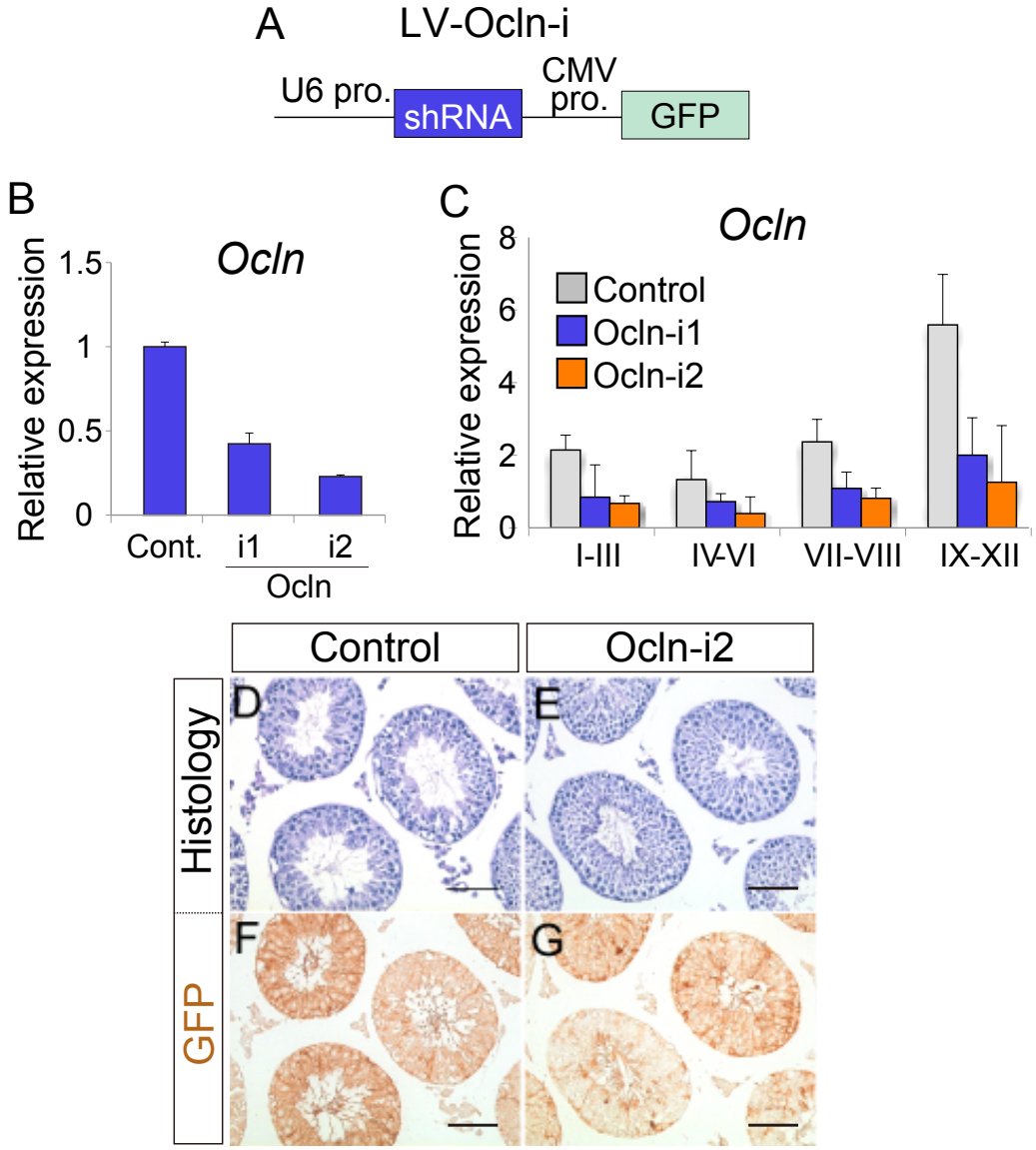

Fig. S10. Efficiency of RNAi in suppressing *Ocln* expression. (**A**) Construction of the LV-Ocln-i vector. Cells infected with this lentiviral construct start to express both shRNA from the U6 promoter and GFP from the CMV promoter. (**B**) Cultured Sertoli cells were infected with LV-Ocln-i1 or -i2 and the expression of *Ocln* was subsequently quantified by qRT-PCR (n=3). (**C**) Adult mouse testes were injected with LV-Ocln-i1 or i2 and changes in the stage-dependent expression of *Ocln* were then quantified by qRT-PCR (n=3). *Gapdh* was used as internal control. (**D-G**) Histological analysis of mouse testes at two weeks after injection of LV-control or LV-Ocln-i2 vectors. Tissue sections were stained with PAS and Hematoxylin. Error bars, s.d. Scale bars: 40 μm.