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Supplemental Information

Retinoic Acid Is Sufficient for the In Vitro Induction of Mouse Spermatocytes

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Supplemental Information

Supplemental Figures and Legends

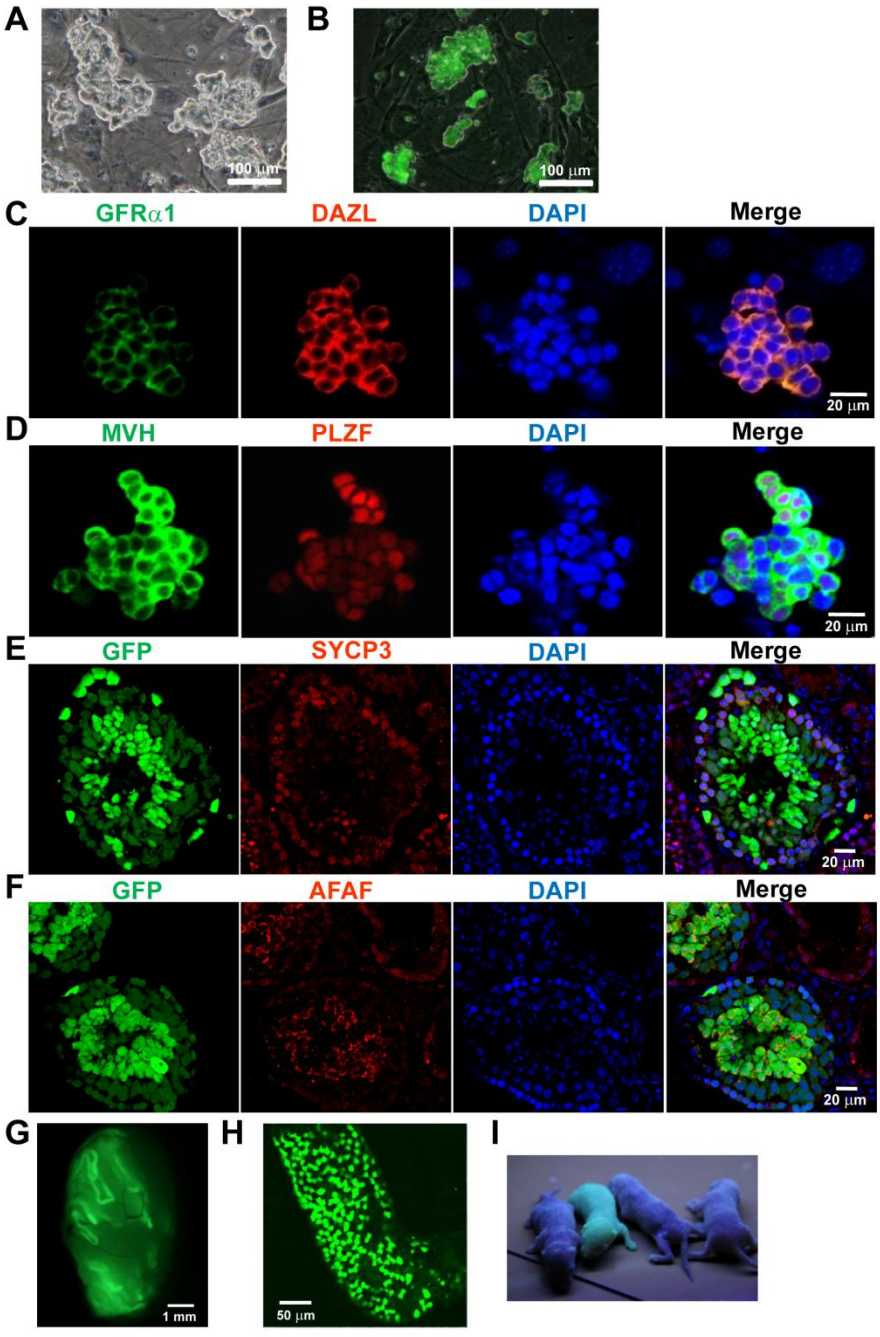

Fig. S1. Characterization of cultured mSSCs.

(A) The morphology of *in vitro* cultured mSSCs.

(B) GFP-labeled mSSCs were obtained by infection of mSSCs with GFP-expressed lentivirus.

(C-D) Characterization of mSSCs by examining the expression of different marker genes. mSSCs were positive for the undifferentiated spermatogonia marker proteins GFRα1 and PLZF, and positive for germ cell marker proteins DAZL and MVH.

(E-H) The GFP-labeled mSSCs were transplanted into the testes of busulfan-pretreated recipient mice. GFP-labeled mSSCs could colonize the recipient seminiferous tubules after one month (H). Two months later, GFP-labeled seminiferous tubules colonized by the transplanted GFP-mSSCs were observed in the testes of recipient mice (G). Spermatogenesis was recovered indicated by appearance of SYCP3-positive spermatocytes (E) and AFAF-positive spermatids (F), all of which derived from injected GFP-labeled mSSCs. AFAF is an acrosome marker protein.

(I) Three months later, live GFP-transgenic offspring was sired by the recipient mice after transplantation.

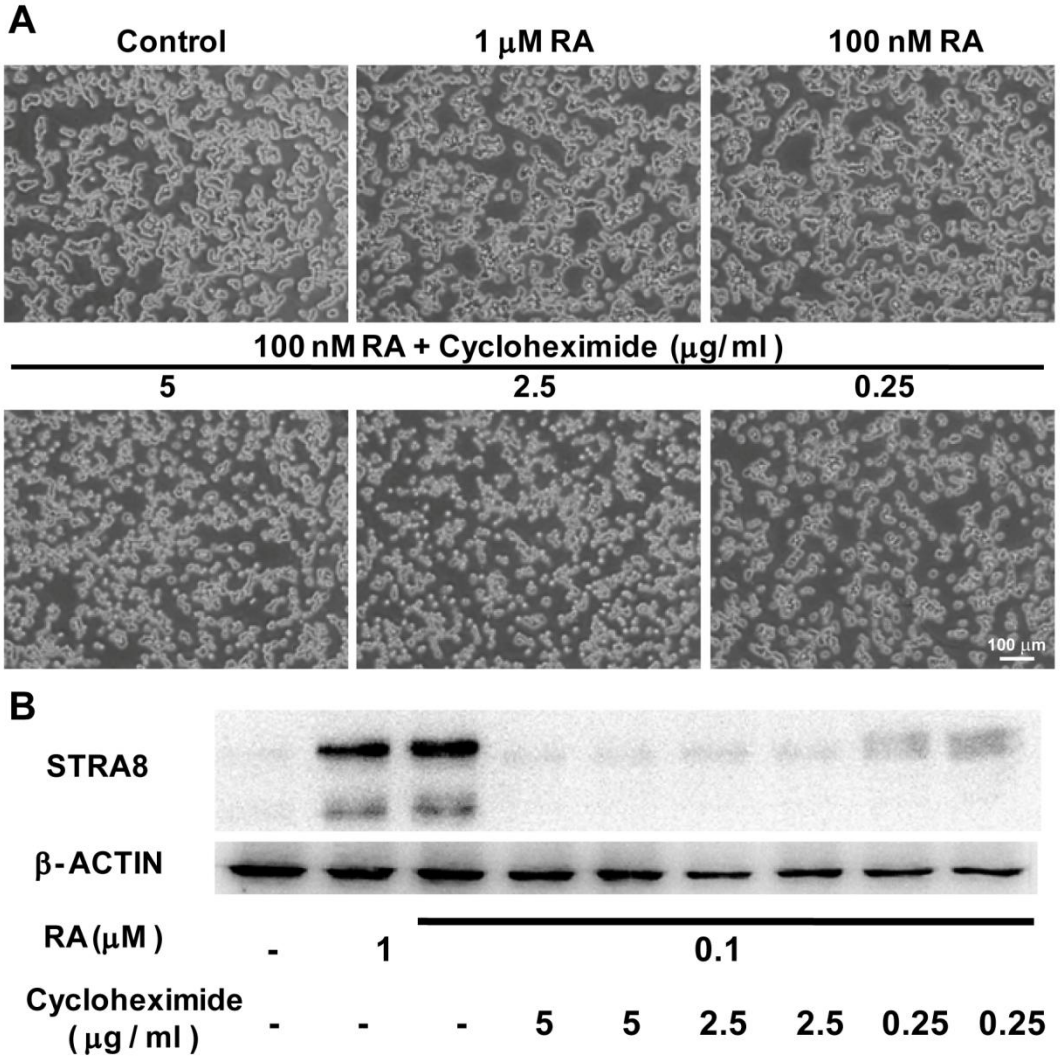

Fig. S2. Determination of the optimal concentration of cycloheximide. (Related to Figure 2)

(A) The effects of different concentrations of cycloheximide on the growth of mSSCs. In the absence of cycloheximide, feeder-free mSSCs proliferated to form chain-like structures while in its presence, especially at high concentration, mSSCs stopped proliferation and most of them adopted single cell morphology. 0.25 μg/ml cycloheximide had the minimum negative effect on the growth of mSSCs treated with 100 nM RA.

(B) Inhibition of protein production by cycloheximide. STRA8 protein in mSSCs in response to RA was detected by Western blotting. Cycloheximide of concentration of 0.25 μg/ml and above reduced the protein levels significantly.

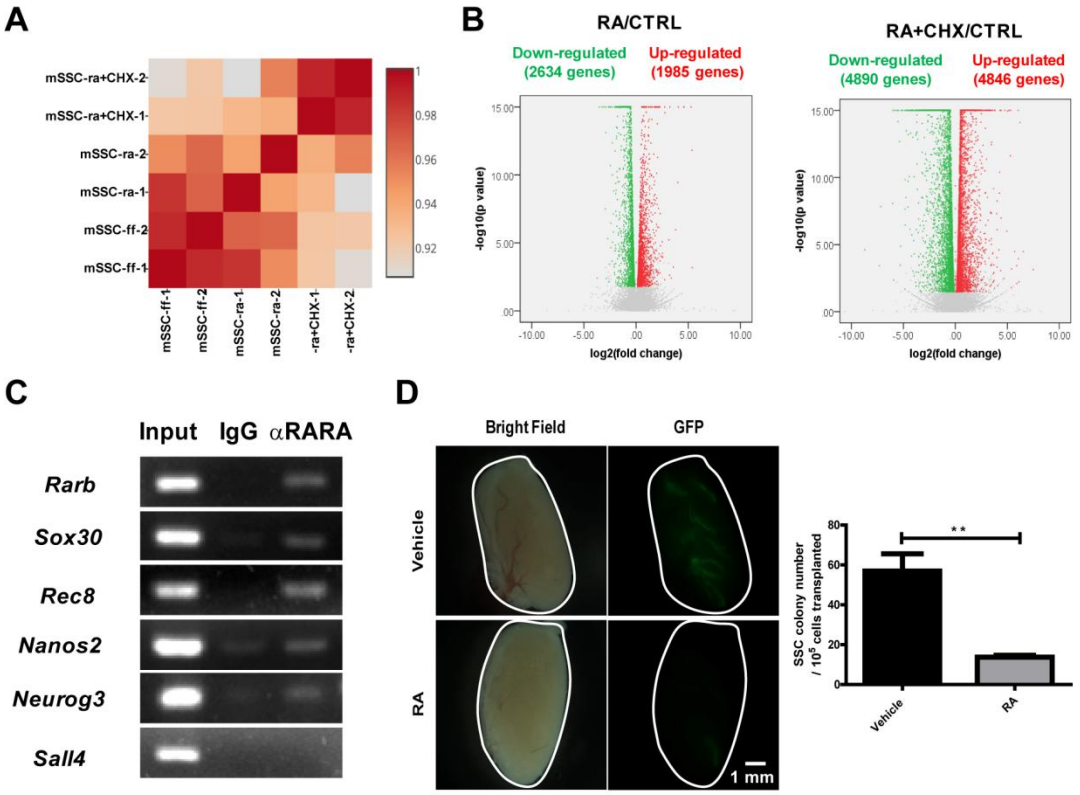

Fig. S3. Analysis of RA-regulated genes and function evaluation of RA-treated cells. (Related to Figure 1-2)

(A) Pearson correlation coefficients between two biological duplicates were calculated based on the FPKM values of RefSeq genes.

(B) Up- and down-regulated genes by RA and RA plus cycloheximide were shown by volcano plot. Feeder-free and serum-free mSSCs were treated with vehicle or RA or RA plus cycloheximide (RA+CHX) for 24 h and then total RNA from different samples was extracted for RNA sequencing.

(C) Identification of potential direct target genes of RA/RAR by ChIP-PCR. Putative RAREs in the promoters of the *Rarb, Sox30, Rec8, Nanos2, Neurog3* were bound by RARA.

(D) GFP mSSCs cultured on laminin-coated dishes were treated with vehicle or RA for 24 h, and the induced germ cells were collected on day 3 of induction for transplantation assay. One month later, the recipient testes were colonized by the cells with stem cell activity, and the colony number was counted and analyzed. Error bars indicate mean ± SD (n=4, ** $p < 0.01$).

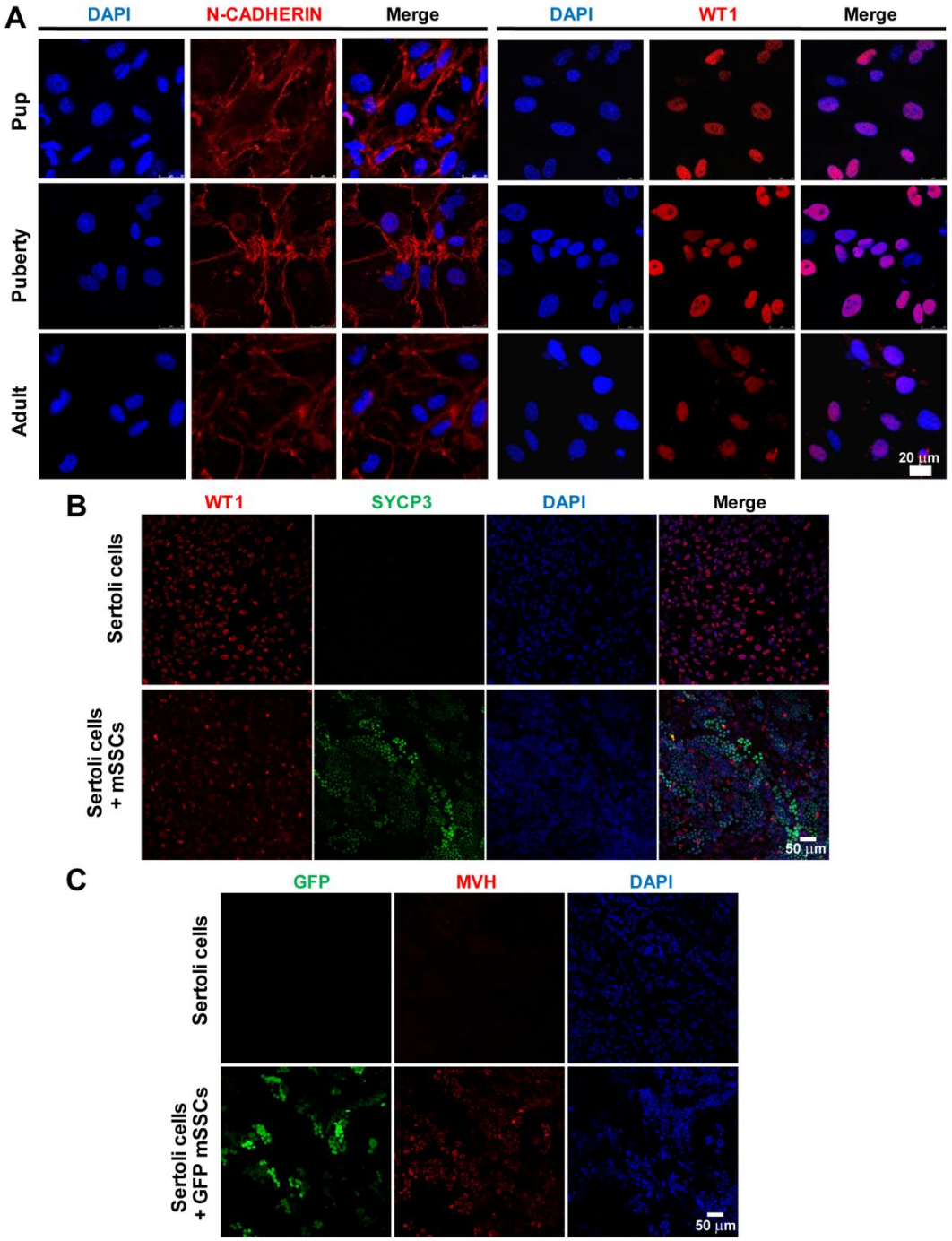

Fig. S4. Characterization of Sertoli cells from pup, puberty and adult mice. (Related to Figure 3-4)

(A) Characterization of Sertoli cells from pup, puberty, adult mice by immunostainings of WT1 and N-CADHERIN. WT1 is a specific marker for Sertoli cells among all testicular cells.

(B) The induction assay was conducted in the presence or absence of mSSCs on mitomycin C-inactivated pup Sertoli cells. After a 6-day induction, immunostainings of SYCP3 and WT1 were performed. Note that the Sertoli cells were free of germ cell contamination as indicated by the absence of SYCP3 signal.

(C) The induction assay was conducted in the presence or absence of GFP mSSCs on mitomycin C-inactivated pup Sertoli cells. After the same 6-day induction, immunostainings of GFP and MVH were conducted. Note that the Sertoli cells were free of germ cell contamination as indicated by the absence of MVH signal.

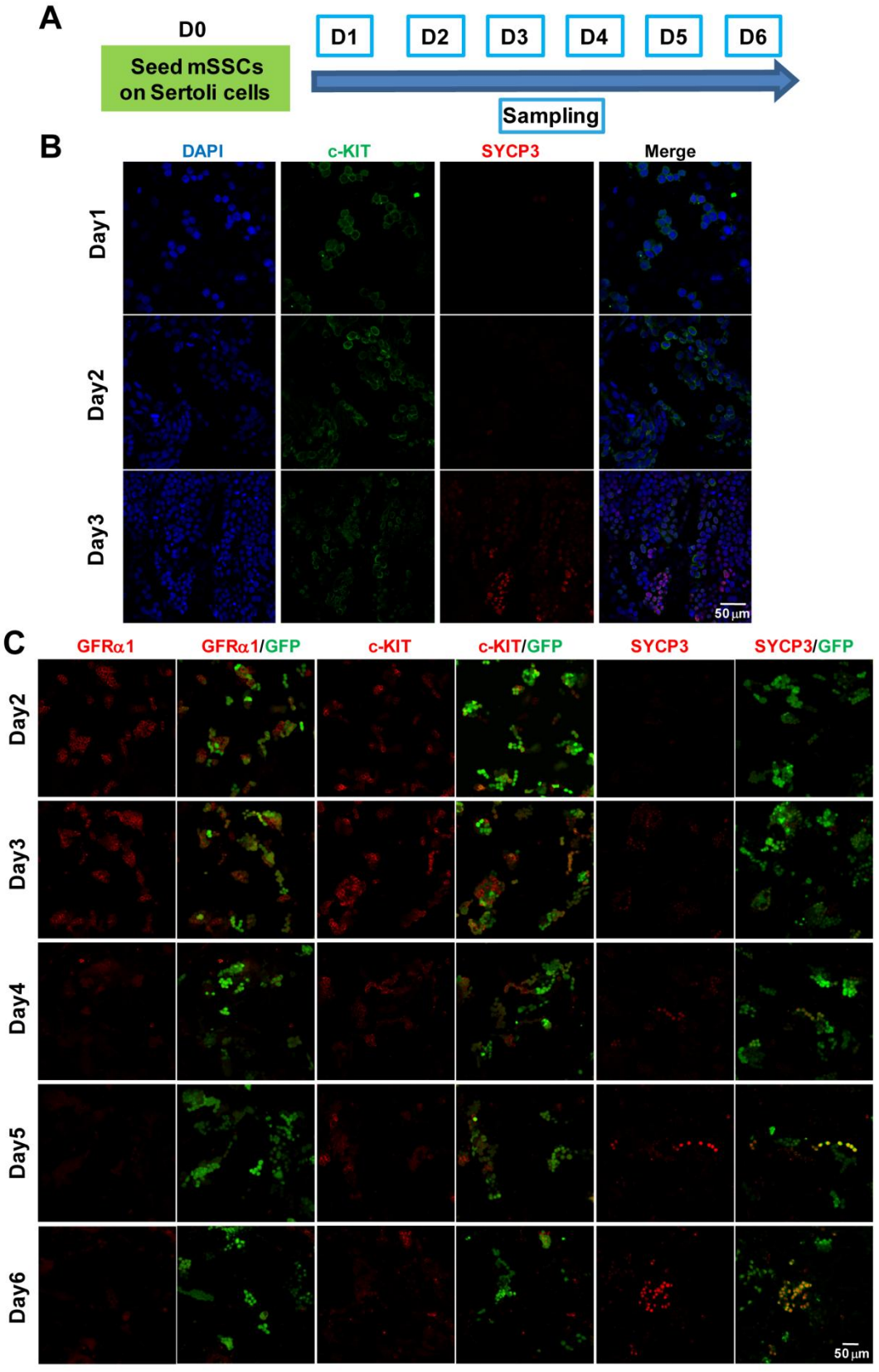

Fig. S5. Dynamics of different marker proteins during the induction of mSSCs on the pup Sertoli cells. (Related to Figure 3)

(A) The illustration of the germ cells induction procedure.

(B) Co-immunostaining of c-KIT and SYCP3 showing that SYCP3 expression followed the expression of c-KIT.

(C) Dynamics of GFRα1, c-KIT and SYCP3 from day 2 to day 6 of induction.

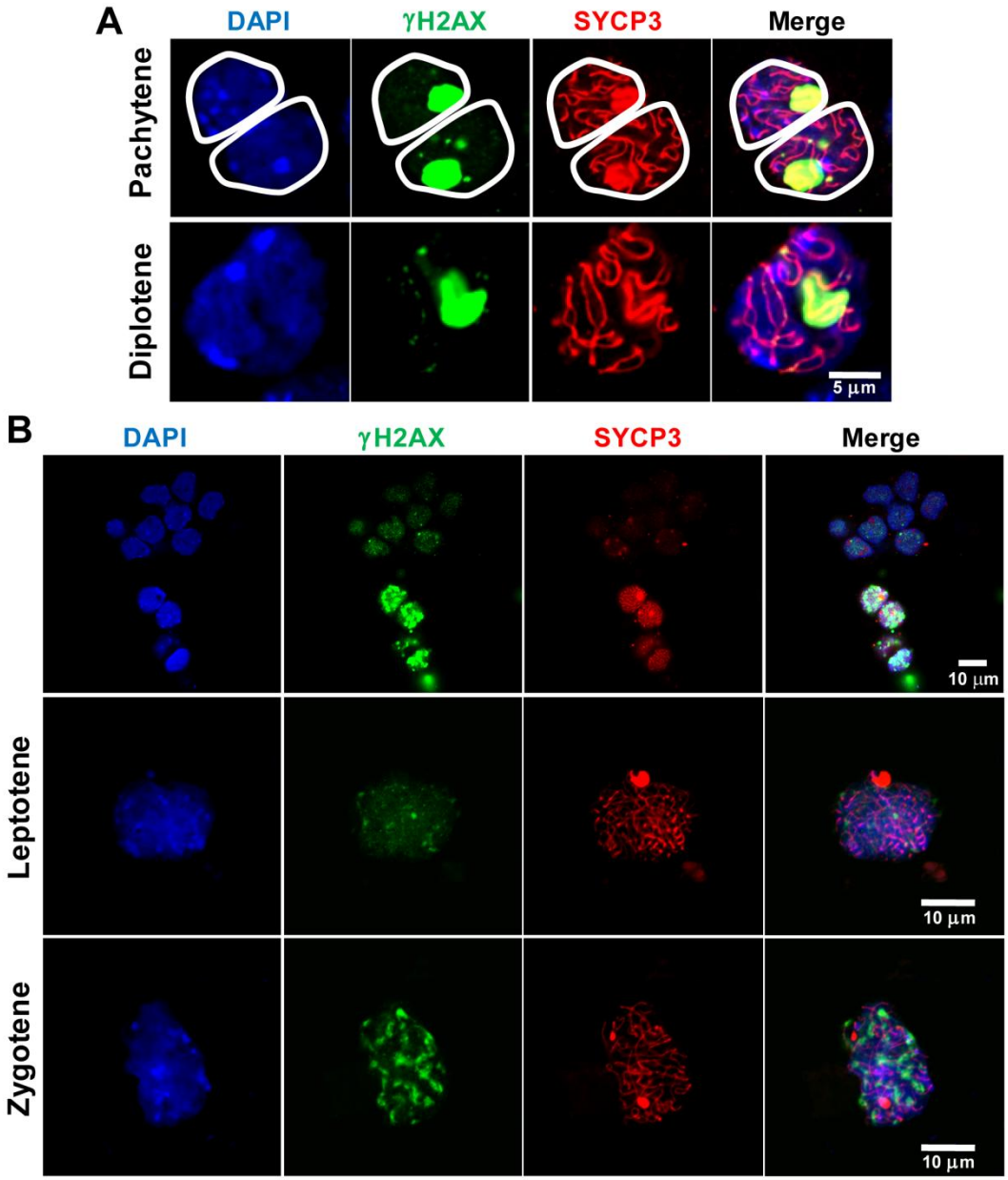

Fig. S6. Induction of pup and adult mSSCs using RA/pup Sertoli cells (RA/pSC) meiosis model. (Related to Figure 5)

(A) Co-immunostaining of SYCP3 and γH2AX indicated the presence of pachytene and diplotene spermatocytes after a 6-day induction. The white lines mark two individual pachytene spermatocytes.

(B) Induction of adult mSSCs on pup Sertoli cells. After a 6-day induction, leptotene and zygotene spermatocytes can be derived as indicated by the co-immunostaining of γH2AX and SYCP3.

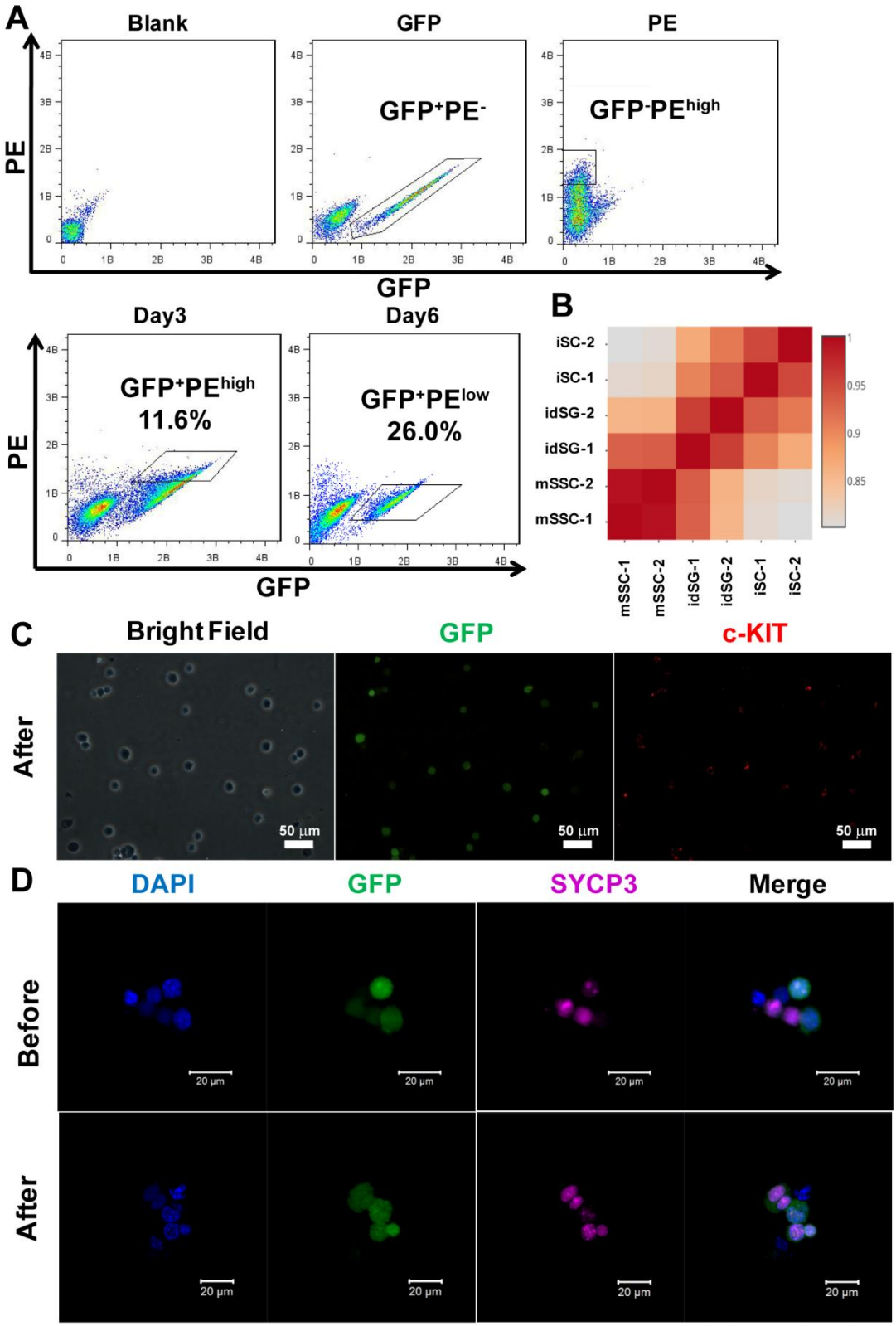

Fig. S7. FACS of the induced germ cells for RNA-sequencing. (Related to Figure 6)

(A) The idSG cells were collected by FACS as the GFP^{+} c-KIT^{high} cells on day 3 of induction while iSC were collected as the GFP^{+} c-KIT^{low} cells on day 6 of induction.

(B) Pearson correlation coefficients between biological duplicates were shown.

(C) Validation of FACS-sorted idSG by immunofluorescence of GFP and c-KIT signals.
(D) Validation of FACS-sorted iSCs by immunostaining of SYCP3. The pictures of cells before and after FACS were shown in the upper, bottom rows, respectively.

Supplemental Table

Table S3. The putative RAREs in the promoters of RA potential target genes.

The red marks refer to the potential RARE sequences scanned by motif analysis tool.

[a] ND means "not determined".

[b] Expression change folds were the ratio of FPKM values of RA-treated sample to control sample.

Gene Symbol	Potential Binding Site(s)	Confirmed by ChIP-PCR	Expression Change Folds (RA/CTRL)		PCR Products
			RNA-Seq [b]	qRT-PCR	
Rarb	atgtcagactggttg ggtcatttgaaggtt agcagcccgggaa ggttcaccgaaag ttca	Yes	40.89	ND[a]	cgctgtgagaatcctgggagttggtgat gtcagactggttgggtcatttgaaggtt agcagcccgggaag*ggttcaccgaaa gttca*ctcgcatatattaggcaattcaat ctttcattccgtgtgacagaagtggtag gaagtgagctgctcc
Sox30	aggtccagtaagat ca	Yes	1.60	2.13±0.44	gtggatgaatctgggtcgcatcttggaa gcagactgaaaattcaactagtaaaat agttatacctagtcaaaaaccaggtcca gtaagatcagttataagttccaaggtgt ccccatacttgagaatgactagaagac cctatcagactccg
Rec8	tgcacctgatccgc tggagggcgacag gacctagagcaagg tccagaagctca	Yes	4.34	4.79±0.27	ccaggagtctttgagtttcttctggcgcg gcttctcattggctgtcggtggtacaaa gccttggagagtgtgcacctgatccgc tggagggcgacaggacctagagcaag gtccagaagctcaacg
Nanos2	tgccctctgacct	Yes	0.09	0.11±0.02	gattggagccagccaaggatctttagtg gaagtgggccctttaaatctaagctcca cctttgcttagaatggtctctaggagagt atttataaggggtgtgcctcctctctgc tccacaaaactcctgctcctgccctctga cctcacagcccctgctccccagtccctct ccagtgcccacagccacattta
Neurog3	tgccccctctgcact	Yes	0.12	0.33±0.06	tccctccttgacctttccctatcacctgcc tctcgggtcaggccttcccgatagcatc catagtggggcggggcgtgatgagatg ccccctctgcactctctctacaaccaccc tcgcctccggaatagaac
Sall4	aggtcttggacccca ggcca	No	0.89	ND[a]	

Table S5. qPCR primers used in this study.

Gene	Sense	Antisense
Aldh1a3	GGGTCACACTGGAGCTAGGA	CTGGCCTCTTCTTGGCGAA
Clgn	CCAGGGTGTTGGACTATGTTTG	CCCCGAGGAAGGTTCATCTTTA
Cyp26b1	TCATCGGAGAGACTGGTCACT	GGTGCTCACTAGCTGGTGTTC
Dmc1	CCCTCTGTGTGACAGCTCAAC	GGTCAGCAATGTCCCGAAG
Foxc2	AACCCAACAGCAAACTTTCCC	GCGTAGCTCGATAGGGCAG
Foxf1a	ACGCCGTTTACTCCAGCTC	CGTTGTGACTGTTTTGGTGAAG
Id4	CAGTGCGATATGAACGACTGC	GACTTTCTTGTTGGGCGGGAT
Prdm9	CTGAATACAAGTGGCTCAGAACA	CCTCATAGGCAAGGCCCTTTC
Rec8	TATGTGCTGGTAAGAGTGCAAC	TGTCTTCCACAAGGTACTGGC
Rxrg	CATGAGCCCTTCAGTAGCCTT	CGGAGAGCCAAGAGCATTGAG
Sall3	TGACATCGACGAGAACTCCAT	AAGTGGTTTAGACGAGTCGCT
Smad6	GCAACCCCTACCACTTCAGC	GTGGCTTGTACTGGTCAGGAG
Sox13	TACTCACAGACTGATCCCCAG	AAGTGCAGTTTCACCCATCAC
Tgif1	GACAGTCCCCTGGACCTTTC	ATACAGCCAGTCTCGCAGAAT
Fgf9	CTATCCAGGGAACCAGGAAAGA	CTCGTTCATGCCGAGGTAGAG
Ngn3	GCCTCATTGGAGGAATTCC	AGATGCTTGAGAGCCTCCAC
Nanos2	CCATATGCAACTTCTGCAAGC	TGAGTGTATGAGCCTGGTCG
Sox30	TCCCATTCCACACTCACACG	GAGGCCGACTATAAGGGCAC
Sall1	CTCAACATTTCCAATCCGACCC	GGCATCCTTGCTCTTAGTGGG
c-Kit	TGATTGTGCTGGATGATGGATGG	ATCTGCTCTGCGTCTGTTGGT
Lin28a	AGACCAACCATTTGGAGTGC	AATCGAAACCCGTGAGACAC
Stra8	ACAAGAGTGAGGCCCAGCAT	CCTCTGGATTTTCTGAGTTGCA
Sycp3	ATGATGGAAACTCAGCAGCAAGAGA	TTGACACAATCGTGGAGAGAACAAC
Ret	AGGACTGGGTAGTTGCATCC	CATATATTGAGCCGAGGACAGC
Nanos3	CACTACGGCCTAGGAGCTTGG	TGATCGCTGACAAGACTGTGG
Zbtb16	ATTTACTGGCTCATTCAGCG	CCAGTATGGGTCTGTCTGTG
β-Actin	CAGCCTTCCTTCTTGGGTAT	TGGCATAGAGGTCTTTACGG
Agpat3	CTTTACCACGGCAGTCCA	GCCTCATATTTCTTCCCATAC
Wdr91	GCACGGCTTTCAATCAC	TGGGCCTTCCAGCTCAT
Fam57a	CGAGGAGACCTTGGAGA	TGCTGCTTTAGCTGGAT

Table S6. ChIP-PCR primers used in this study.

Gene	Sense	Antisense
Rarb	CGCTGTGAGAATCCTGGGAG	GGAGCAGCTCACTTCCTACC
Nanos2	GATTGGAGCCAGCCAAGGAT	TAAATGTGGCTGTGGGCACT
Sox30	GTGGATGAATCTGGGTCG	CGGAGTCTGATAGGGTCT
Rec8	CCAGGAGTCTTTGAGTTTCTTC	CGTTGAGCTTCTGGACCTT
Ngn3	TCCCTCCTTGACCTTTCCCT	GTTCTATTCCGGAGGCGAGG
Sall4	TAGATGAACACGCCTTTG	GCGCATGTCCCAGTAATT

Table S7. Antibodies used in the study.

Name	Manufacturer	Cat. Number	Working Conc.
anti-SYCP3	Abcam	Ab97672	1:300

anti-SYCP1	Abcam	Ab175191	1:200
anti-MVH	Abcam	Ab13840	1:200
anti-GFP	Product Description	50430-2-AP	1:200
anti-γH2AX	Upstate Biotechnology	05-636	1:300
anti-GFRα1	R&D	AF560	1:100
anti-WT1	Epitmics	2797-1	1:300
anti-N-CADHERIN	Abcam	Ab18203	1:200
anti-c-KIT	R&D	AF1356	1:200
anti-STRA8	Abcam	Ab-49405	1:1000
anti-RARα	Santa Cruz	sc-551x	1:100
anti-RARG	Santa Cruz	sc-7387x	1:100
anti-β-ACTIN	Abmart	M20010	1:3000
anti-DMC1	Santa Cruz	sc-8973	1:500
anti-PLZF	Santa Cruz	sc-28319	1:500

Supplemental Experimental Procedures

RNA-sequencing and Data Analysis

mSSCs were cultured on laminin-coated dishes overnight and then treated with vehicle, 100 nM RA, and 100 nM RA plus 0.25 μg/ml cycloheximide for 24 h, respectively. For RNA-sequencing of induced germ cells, GFP-mSSCs were cultured on mitomycin C-inactivated pup Sertoli cells and induced for different days. After a 3-day induction, GFP^+ c-KIT^{high} differentiating spermatogonia (idSG) were collected by FACS. Induced spermatocytes (iSC) were collected after a 6-day induction by sorting the GFP^+ c-KIT^{low} cell population. Two biological replicates were included for each treatment. Total RNA was isolated with TRIZOL (Invitrogen) reagent and purified with RNase-free DNase I to remove contaminated DNA if any. The libraries were generated using NEBNext Ultra Directional RNA Library Prep Kit for Illumina (NEB, Ispawich, USA) following manufacturer's recommendations. Briefly, Oligo (dT) beads were used to isolate poly (A) mRNAs. Fragmentation was carried out using divalent cations under elevated temperature in NEB proprietary fragmentation buffer. The mRNAs was digested into short fragments, from which the first strand cDNAs were synthesized using random oligonucleotides and M-MuLV Reverse Transcriptase (RNase H-). The second strand cDNAs were synthesized using dNTPs mixture containing dUTP, DNA Polymerase I and RNase H. Next, NEBNext adaptor oligonucleotides were ligated to cDNA fragments. The second strand cDNA that contains dUTP was digested using USER enzyme. The first strand DNA fragments with ligated adaptor were selectively enriched by PCR reaction. The library preparations were sequenced on an Illumina Hiseq 2000 platform and 100 bp paired-end reads were generated. The numbers of reads of the samples ranged from 24 to 32 million. The reads were mapped to the mouse genome (mm9) by using the TopHat package(Kim et al., 2013). RefSeq genes downloaded from UCSC were used as the reference for assembling transcription units using the Cufflinks software package. The RNA expression level of a gene was represented by fragments per kilobase of transcript per million mapped reads (FPKM) calculated using the Cufflinks package (Trapnell et al., 2010). The differentially expressed genes were identified using the Cuffdiff package. Pearson correlation coefficients between biological duplicates,

calculated from the FPKM values of RefSeq genes were 0.93~0.99 (Fig. S3A, S7B), indicating satisfying reproducibility of our experiments. Accordingly, data of duplicates were pooled for further analysis. Clustering analyses were conducted using the Cluster 3.0 software based on the expression values of the 20632 protein coding genes. To normalize the data of different samples, multiply values in a row by a factor S so that the sum squares of the values in each row is 1.0. Samples were clustered using the hierarchical clustering method. Euclidean distance similarity metric was used for clustering.

Quantitative RT-PCR

Total RNA from mSSC samples was extracted using standard protocol. Potential DNA contaminants were eliminated by RNase-free DNase digestion. Reverse transcription (RT) of purified RNA was conducted using Reverse Transcription System (Promega) by following the manufacturer's instructions. qPCRs were performed with UltraSYBR Mixture (Beijing CoWin Biotech) according to the manufacturer's instructions on a LightCycler® 480 platform (Roche). *β-Actin* gene was used as the internal control and the relative expression values were determined using the ΔΔCt method. The primer pairs of selected genes were listed in Table S5.

Motif Analysis

Promoter sequences of human (hg18) and mouse (mm9) protein coding genes spanning from -10 K bp to 5 K bp of the transcription start sites were aligned to identify conserved regions. A maximal 2 K bp accumulated conserved region of each gene was then scanned for the Retinoic Acid Response Element (RARE). If the promoter of a gene contains at least two consensus direct repeats ((a/g)g(g/t)(g/t)(g/c)a), and the gap between the two direct repeats was less than 10 bp, the direct repeats was considered to be a predicted RARE, and the gene was regarded as a potential direct target gene of RA (Balmer and Blomhoff, 2005).

Chromatin Immunoprecipitation (ChIP)-PCR

ChIP assay was performed with a kit (Upstate Cell Signaling Solutions, NY, USA) according to manufacturer's instruction with slight modifications. Briefly, 10^7 mSSCs were cultured on MEF for four days. Then cells were digested and re-suspended in MEF medium, and plated on 10-cm dish for 30 min to remove the MEF cells. The remaining floating mSSCs were collected and fixed with 1% formaldehyde in PBS for 10 min at 37℃ to crosslink DNA and protein. The cells were then washed with iced-cold phosphate buffered saline (PBS) and lysed with 1 ml lysis buffer [1% SDS, 10 mM EDTA, 50 mM Tris, pH 8.1, 1 mM PMSF, protease inhibitor cocktail (Sigma)]. Lysates were incubated on ice for 10 min and sonicated on ice with an ultrasonic sonicator (Omni-Ruptor 250) at 20% power and 40% pulse for 7 min. The resulting DNA fragments were between 0.2 to 1 kb in length. The sonicated cell lysates were centrifuged at 13,000 rpm for 10 min at 4℃ to remove cell debris. 200 μl supernatant was diluted 10-fold in dilution buffer (0.01% SDS, 1.1% Triton X-100, 1.2 mM EDTA, 16.7 mM Tris-HCl, pH 8.1, 167 mM NaCl) and then incubated with anti-RARG/RARα antibody or normal rabbit IgG as a negative control at 4℃ overnight. The immuno complexes were collected with 50 μl Protein A/G sepharose beads at 4℃ for 3 h and precipitated at 2000 rpm at 4℃ for 2 min. The precipitated beads were washed sequentially with each immunoprecipitation buffers. RARG/RARA-bound DNA fragments were eluted and subjected to PCR reaction. PCR reactions were

conducted using EasyTaq DNA polymerase (TransGen Biotech, China). The PCR reaction started at 94℃ for 5 min and followed by 32 cycles at 94℃ for 30 sec, 58℃ for 30 sec, and 72℃ for 30 sec. The primers for chosen potential RARE-containing genes were designed and listed in Table S6. PCR products were separated by electrophoresis on 1.5 % agarose gels. The gels were analyzed with the ChemiDoc XRS+ system (Bio-Rad).

Immunofluorescent Staining and Quantification of SYCP3-positive Cells

For immunofluorescent staining, the cultured cells were fixed with 4% paraformaldehyde in PBS. The cells were washed with PBS and permeabilized with 0.1% Triton X-100 for 25 min. Blocking was conducted with 5 % BSA for 1 h at RT. The primary antibodies were added and incubated for overnight at 4℃ (See Table S7 for their dilutions). After 3 washes in PBS, the secondary antibodies were added and incubated for 1 h at RT. Images were captured using a confocal laser microscope (Nikon Eclipse TE200). For the quantification of S-cells, images of immunostained slides of induced cells under different conditions were taken using a laser confocal microscope with the same set of parameters. The Image J software was then used for the quantification in two steps. First, the training set of S-cells was identified by visual inspection of the images and the minimum signal intensity was determined to be 20. Second, any cell with intensity above this threshold value (> 20) was identified as an S-cell. Each quantification assay was conducted in three independent experiments, and the statistical analyses were based on the *t*-test.

Flow Cytometric Analysis

The cultured cells were treated with 0.25 % trypsin/1 mM EDTA for 5 min at 37℃ to dissociate cells. Cell suspension was passed through a cell strainer of 40 μm pore size to remove any clumps. For analysis based on immunostaining, the cells were re-suspended with 5 % BSA for 30 min, and then incubated with primary antibody at 37℃ for 1 h. The flow cytometric analysis was conducted using the BD Influx Sorter.

Chromosomal Spread Immunostaining

After a six-day induction, the germ cells on the pup Sertoli cells were digested with typsin into single cells. After a brief wash with PBS, 10^5 cells were suspended in 500 μl hypotonic extraction buffer [30 mM Tris, 17 mM trisodium citrate dihydrate, 5 mM EDTA, 50 mM sucrose, 0.5 mM DTT and 0.5 mM phenylmethyl-sulphonyl fluoride (PMSF), pH 8.2], and were incubated on ice for 30 - 60 min. The cells were collected by centrifugation at 600 g for 6 min and resuspended in buffer containing 2 % PFA and 100 mM surose, pH 8.2. The cell suspension was placed on the clean glass slides and the nuclei were dried for at least 2 h in a closed box with high humidity. The dried slides were stored at -20℃ or - 80℃ for future use (Peters et al., 1997). Subsequent steps followed the same procedure as for the immunofluorescent staining.

Transplantation of mSSCs/ induced germ cells

The mSSCs were infected with lentivirus through which GFP was expressed under the control of the ubiquitin promoter. 2×10^5 GFP-labelled mSSCs or induced germ cells were transplanted into each testis of busulfan-pretreated recipient mice. One month later, GFP positive cells colonized to the

recipient seminiferous tubules and the SSC colony number was counted and analyzed. Two months later, the recipient testes were fixed for histological section analysis. After two to three months, the recipient mice were mated to female mice for derivation of GFP offspring.

Western Blotting

mSSCs cultured on laminin-coated dish were treated with vehicle, 1 μM RA, 100 nM RA, 100 nM RA plus different concentration of cycloheximide: 5, 2.5, 0.25 μg/ml for 24 h. Then the protein from various samples was extracted with 2 × SDS-PAGE loading buffer. The lysates were separated by PAGE and transferred to nitrocellulose membranes. The blots were blocked with 5 % non-fat milk/PBST for 2 h, followed by incubation with primary antibodies at the appropriate dilution (Table S7) in blocking buffer overnight at 4℃. After three washes with PBST, the membranes were incubated with HRP-conjugated secondary antibodies at RT for 2 h followed by three washes in PBST. The proteins were detected using the Western Blotting SuperSignal West Pico Chemiluminescent Substrate (Thermo) and analyzed with the ChemiDoc XRS+ system (Bio- Rad).

RNA Interference of *Stra8, Agpat3, Sox30, Wdr91* and *Fam57a* by shRNAs

The shRNAs were introduced into mSSCs by lentivirus which was produced from the 293T cells by co-transfection with the transfer vector pLL3.7 and packaging plasmids psPAX and pMD2.G. The sense and antisense oligos against the coding sequence of *Stra8, Agpat3, Sox30, Wdr91* and *Fam57a* genes
(*Stra8*-sense:TCTACATACAGATCATTTTCAAGAGAAATGATCTGTATGTAGAGCTTTTTTC,
antisense: CGAGAAAAAAGCTCTACATACAGATCATTTCTCTTGAAAATGATCTGTATGT;
Agpat3-sense:TGCGGGTTAATTATCAACTTTTCAAGAGAAAGTTGATAATTAACCCGCTTTTTTC,
antisense:TCGAGAAAAAAGCGGGTTAATTATCAACTTTCTCTTGAAAAGTTGATAATTAACCCGCA;
Sox30-sense:TGCGACCCATGAATGCATTTTTCAAGAGAAAATGCATTCATGGGTCGCTTTTTTC,
antisense:TCGAGAAAAAAGCGACCCATGAATGCATTTTCTCTTGAAAAATGCATTCATGGGTCGCA;
Wdr91-sense:TGCACGGCTTTCAATCACAATTCAAGAGATTGTGATTGAAAGCCGTGCTTTTTTC,
antisense:TCGAGAAAAAAGCACGGCTTTCAATCACAATCTCTTGAATTGTGATTGAAAGCCGTGCA;
Fam57a-sense:TGCAGCAGCATACGCTTCTTTTCAAGAGAAAGAAGCGTATGCTGCTGCTTTTTTC,
antisense:TCGAGAAAAAAGCAGCAGCATACGCTTCTTTCTCTTGAAAAGAAGCGTATGCTGCTGCA) were annealed, and inserted into the lentiviral transfer vector pLL3.7, respectively. To produce shRNA lentivirus particles, 293T cells were co-transfected with transfer plasmid and packaging plasmids by Lipofectamine 2000 transfection reagent (Invitrogen). Viral particle-containing supernatants were harvested 48 h and 72 h later and were concentrated by ultra centrifugation (20,000 g for 2.5 h). mSSCs were infected with lentivirus and the transgenetic cells were purified by FACS.

Supplemental Reference

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