Supporting Information

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SI Discussion

Our previous study showed that *AT-rich interaction domain 4a* (*Arid4a*) and *Arid4b* control genomic imprinting in the Prader–Willi/Angelman syndrome (PWS/AS) domain through regulation of epigenetic modifications on the PWS/AS imprinting center (1). We and other groups have generated several PWS/AS mouse models with deletions of the PWS/AS imprinting center or imprinting genes (2). No apparent male fertility defects have been reported from these PWS/AS mouse models. Therefore, loss of male fertility in the *Arid4a*$^{-/-}$*Arid4b*$^{+/-}$ mice is not directly linked to dysregulation of the PWS/AS imprinting genes.

Arid4a$^{-/-}$*Arid4b*$^{+/-}$ mice developed acute myeloid leukemia with an onset between 7 and 15 mo of age (3). In the *Arid4a*$^{-/-}$*Arid4b*$^{+/-}$ mice, leukemic phenotype does not seem to coincide with male infertility. Specifically, not all infertile males developed leukemia. Conversely, some leukemic *Arid4a*$^{-/-}$*Arid4b*$^{+/-}$ males remained fertile and produced a similar size of pups as the wild-type counterpart. In addition, although modifications of histones H3 and H4 were disturbed in bone marrow of the leukemic *Arid4a*$^{-/-}$*Arid4b*$^{+/-}$ mice (3), no such changes in histone modifications can be found in the testicular cells of *Arid4a*$^{-/-}$*Arid4b*$^{+/-}$ mice (Fig. S5). These observations suggest that hematologic malignancy is not the major cause for male infertility in the *Arid4a*$^{-/-}$*Arid4b*$^{+/-}$ mice.

Some of the infertile *Arid4a*$^{-/-}$*Arid4b*$^{+/-}$ males exhibit defects in seminal vesicles. Seminal vesicles secrete a large amount of fluid that ultimately becomes a part of semen. Seminal vesicle fluid provides nutrients for spermatozoa and neutralizes the acidity of the vaginal tract to prolong the life span of sperm. Function of the seminal vesicle is important for male fertility. Therefore, in the *Arid4a*$^{-/-}$*Arid4b*$^{+/-}$ mice, defects in seminal vesicles may also contribute to the observed infertility, but independently of the abnormal Sertoli cell function.

Within seminiferous tubules of testes, Sertoli cells provide structural support for the movement of developing germ cells migrating from the basal compartment to the adluminal compartment. Histological analysis showed that the integrity of the seminiferous epithelium in the *Arid4a*$^{-/-}$*Arid4b*$^{+/-}$ testes was compromised, mostly from the apical compartment (Fig. 3 *B*, *b*). This defect could disrupt the interactions between Sertoli cells and spermatocytes/round spermatids, resulting in spermatogenic failure at the meiosis II and the postmeiotic spermatid stages.

Abundant expression of *Arid4a* and *Arid4b* was detected in Sertoli cells, which implies their roles in Sertoli cell function. The *Arid4a*$^{-/-}$*Arid4b*$^{+/-}$ testes showed decreased width of seminiferous tubules as well as vacuolization within the tubules, both of which are hallmarks of unhealthy Sertoli cells. Accordingly, we found spermatogenic failures as evidenced by differentiation arrest at the transition between early to late spermatocytes and during the maturation of postmeiotic haploid spermatids in the *Arid4a*$^{-/-}$*Arid4b*$^{+/-}$ mice. In addition, the permeability of seminiferous tubules in the *Arid4a*$^{-/-}$*Arid4b*$^{+/-}$ testes was increased because the blood–testis barrier formed by tight junctions between Sertoli cells was impaired. Interestingly, these phenotypes of Sertoli cell dysfunction found in the *Arid4a*$^{-/-}$*Arid4b*$^{+/-}$ mice bear resemblance to that shown in the Sertoli cell-specific androgen receptor (AR) knockout mice and the Sertoli cell-specific retinoblastoma (RB) knockout mice. Specifically, the Sertoli cell-specific AR knockout mice lost the integrity of the blood–testis barrier (4) and demonstrated spermatogenesis arrest before first meiotic division and during the transition from round to elongated spermatids (5–7). These AR mutant males are infertile (5–7). Similarly, the Sertoli cell-specific RB knockout mice also showed the impaired blood–testis barrier and loss of elongating spermatids and spermatozoa (8). These RB mutant males showed progressive infertility (8).

The extensive phenotypic similarities between our *Arid4a*$^{-/-}$Arid*4b*$^{+/-}$ mice, the Sertoli cell-specific AR knockout mice, and the Sertoli cell-specific RB knockout mice imply that ARID4A and ARID4B might participate in the AR and RB pathways to regulate male reproductive function. Therefore, Sertoli cell dysfunction caused by deficiency of *Arid4a* and *Arid4b* may be, in part, due to dysregulation of the *AR*-responsive and/or *Rb*-responsive genes. Interestingly, four AR-responsive genes, including claudin 3 (*Cldn3*), prostaglandin D2 synthase (*Ptgds*), inhibin alpha (*Inha*), and embigin (*Emb*), were identified and confirmed to be downstream targets of *Arid4a* and *Arid4b*. *Ptgds* encodes prostaglandin D2 synthase, an enzyme that produces prostaglandin D2 and contributes to Sertoli cell differentiation (9). Down-regulation of *Ptgds* in the *Arid4a*$^{-/-}$Arid*4b*$^{+/-}$ mice might affect Sertoli cell development. *Inha* encodes the α-subunit of inhibin, a gonadal glycoprotein hormone. Inhibin and activin are members of the transforming growth factor-β superfamily. In males, activin enhances spermatogenesis, and inhibin functions as an antagonist of activin (10). Up-regulation of *Inha* in the *Arid4a*$^{-/-}$Arid*4b*$^{+/-}$ mice might be involved in spermatogenic failures. Inhibin is also a marker of Sertoli cell dysfunction with impaired spermatogenesis (11). *Cldn3* encodes a transient component of newly formed tight junctions in the blood–testis barrier (4). *Cldn3* is also a RB-responsive gene (8). Down-regulation of *Cldn3* was suggested to be the main cause for the increased permeability of the blood–testis barrier in the Sertoli cell-specific AR knockout mice (4) and in the Sertoli cell-specific *Rb* knockout mice (8). Interestingly, decreased expression of *Cldn3* accompanied by increased permeability of seminiferous tubules was also found in the *Arid4a*$^{-/-}$*Arid4b*$^{+/-}$ testes. *Emb* encodes embigin, a cell adhesion molecule that mediates interactions between cells and extracellular matrix (12). Increased expression of *Emb* might perturb the network of adhesion junctions between Sertoli cells or between Sertoli cells and germ cells. Further investigation is required to determine whether these genes collectively mediate the actions of *Arid4a* and *Arid4b* on Sertoli cell function with respect to spermatogenesis or the impermeable blood–testis barrier.

Although AR and RB have been shown to regulate expression of *Cldn3* from several published literatures (4, 8, 13, 14), so far no evidence that AR or RB were recruited to the *Cldn3* promoter can be found. After confirming that expression of *Cldn3* was regulated by ARID4A, ARID4B, and AR, we examined whether these proteins can be recruited to the *Cldn3* promoter. Chromatin immunoprecipitation (ChIP) analyses were performed using antibodies against endogenous AR or ARID4B in the wild-type testes or anti-HA for ectopically expressed HA-ARID4A in TM4 cells (no ChIP grade anti-ARID4A antibodies are available). Our ChIP results showed that no recruitment of AR, ARID4A, and ARID4B to the *Cldn3* promoter region (from −972 to +229 bp) can be detected (Figs. S6 and S7), even though this region of the *Cldn3* promoter can be activated by these proteins in luciferase reporter gene assays (Fig. 6*C*). As a positive control for the ChIP analysis, the AR was found to be recruited to the promoter region of reproductive homeobox 5 (*Rhox5*), a known AR target gene (15) (Fig. S6). In addition, ARID4A and ARID4B were associated with the promoter of small nuclear ribonucleoprotein N (*Snrpn*) (1) (Fig. S7).

Microarray analysis showed decreased expression of both *Arid4a* and *Arid4b* in the *Arid4a*$^{-/-}$*Arid4b*$^{+/-}$ testes (Table S1). Quantitative RT-PCR (qRT-PCR) analyses also independently confirmed a threefold difference of the *Arid4a* transcripts and a twofold difference of the *Arid4b* transcripts between the *Arid4a*$^{-/-}$*Arid4b*$^{+/-}$ and wild-type mice (Fig. S2, *A* and *C*, respectively). The *Arid4a* null mutation was generated by a partial deletion of exon 1, which includes the start codon for protein translation, and deletion of the entire exon 2 (1). Although it remains possible that truncated or alternatively spliced *Arid4a* mRNA could still be generated, no ARID4A protein was detected in the *Arid4a*$^{-/-}$*Arid4b*$^{+/-}$ mice (Fig. S2*B*).

SI Materials and Methods

Mouse Lines. *Arid4a* null (*Arid4a*$^{-/-}$) mice, mice heterozygous for the *Arid4b* deletion (*Arid4b*$^{+/-}$), and mice null for *Arid4a* and heterozygous for the *Arid4b* deletion (*Arid4a*$^{-/-}$*Arid4b*$^{+/-}$) have been described previously (1). These mutant mice are maintained on a hybrid C57BL6/J and 129/SvEv genetic background. All of the mice were bred and maintained according to a protocol (protocol A208) approved by The George Washington University Institutional Animal Care and Use Committee at the institution's specific pathogen-free mouse facility. The facility is approved by the American Association for Accreditation of Laboratory Animal Care and is operated in accordance with current regulations and standards of the Department of Agriculture and the Department of Health and Human Services.

Histological Analysis, TUNEL Assays, and Immunofluorescence. Testes and epididymis were dissected from male mice and fixed in Bouin's fluid [75% (vol/vol) saturated picric acid, 5% (vol/vol) glacial acetic acid, 9.3% (vol/vol) formaldehyde]. Histology was performed on 5-μm thick paraffin-embedded tissue sections by hematoxylin and eosin staining.

For analysis of apoptosis, testes sections were stained by an in situ cell death detection kit (Roche Diagnostics) using the TUNEL-based assays.

For immunofluorescence analysis, antigen retrieval was perform by boiling the testes sections in citric acid-based antigen unmasking solution (H3300;Vector Laboratories). Samples were blocked with blocking solution (5% goat serum, 2% BSA, and 0.02% triton-X 100 in PBS), followed by incubation with primary antibodies against acetylated histone H3 and H4 or trimethylated histone H3K4, H3K9, and H4K20 (06-599 for H3Ac, 06–866 for H4Ac, 07–442 for H3K9me3, 05–745 for H3K4me3, and 07–463 for H4K20me3; Millipore) at a dilution of 1:200 in blocking solution. Then, samples were washed with PBS and incubated with Alex 488-conjugated goat anti-rabbit secondary antibody (Molecular Probes and Invitrogen).

Analysis of Spermatozoa. Epididymides were removed from mice, cut open, and incubated in Dulbecco modified Eagle medium (DMEM) with 10% FCS at 37 °C for 10 min to allow dispersion of the contents. A homogeneous suspension was spread over a hemacytometer, and then the number of mature spermatozoa containing both a head and a tail were counted.

In Situ Hybridization. Testes were dissected from mice and immediately frozen on dry ice. Cryosections of testes were used for the in situ hybridization analysis performed by the RNA in situ hybridization core at the Intellectual and Developmental Disabilities Research Center at Baylor College of Medicine. Primer sequences used to amplify the *Arid4a* and *Arid4b* cDNA templates for generating the probes are as follows: *Arid4a* forward primer, 5′-*GCG*TAATACGACTCACTATAGGCATGGAAATGACAACTGGAGTGTC-3′; *Arid4a* reverse primer, 5′-*GCG*ATTTAGGTGACACTATAGCATCACCTGCACTCTACAGCAAG-3′; *Arid4b* forward primer, 5′-*GCG*TAATACGACTCACTATAGGGCTCTGACTCGGAGACT-3′; and *Arid4b* reverse primer, 5′-*GCG*ATTTAGGTGACACTATAGGCACTCAACTGACATTCC-3′. The T7 and SP6 RNA polymerase promoters (underlined) were added to the 5′ end of the forward and reverse primers, respectively. Three extra bases (italicized) were attached to each of the promoters at the 5′ end to allow better binding by the RNA polymerase.

Biotin Tracer Assay. To examine the permeability of the blood–testis barrier, the biotin tracer assay was performed as described previously (4). Briefly, male mice at 3 mo of age were anesthetized, and their testes were exposed. The interstitium of testes was injected with 50 μL of EZ-Link Sulfo-NHS-LC-Biotin (10 mg/mL; Pierce Chemical Co.) freshly prepared in PBS containing 1 mM $CaCl_2$. After 30 min, the testes were dissected out and fixed in Bouin's fluid (75% saturated picric acid, 5% glacial acetic acid, 9.3% formaldehyde). The biotin tracer was detected in testis sections with Alexa Fluor 488 streptavidin (Molecular Probes).

DNA Content Analysis. Testes were excised from mice, dissociated into single cells, and filtered through 70-μm cell strainers. Testicular cells were fixed in Bouin's fluid. Cells were treated with RNase A (1 μg/mL; Sigma) and stained with propidium iodide (50 μg/mL; Sigma). DNA content was analyzed by flow cytometry (Beckman-Coulter EPICS XL-MCL).

Whole Genome-Wide Expression Analysis. Testes were dissected from three infertile *Arid4a*$^{-/-}$*Arid4b*$^{+/-}$ and three wild-type males at 10 mo of age. Total RNA was purified using an RNeasy plus kit (Qiagen) and was treated by DNase I (Qiagen). Microarray was performed by the microarray core facility at Baylor College of Medicine using Affymetrix MOE430-2 gene chips (Affymetrix). Gene expression arrays were analyzed using GeneChip Command Console software (Affymetrix). Results were analyzed by applying an unpaired *t* test ($P \leq 0.05$) on genes differently expressed in the *Arid4a*$^{-/-}$*Ardi4b*$^{+/-}$ testes with a more than 1.8-fold difference from the wild-type testes.

qRT-PCR Analysis. Total RNA was purified from mouse testes using an RNeasy plus kit (Qiagen). Two micrograms of DNase I-treated total RNA was used for reverse transcription to synthesize the first-strand cDNA by the SuperScript III first-strand synthesis system (Invitrogen). qPCR was performed on the ABI StepOne Plus machine using TaqMan Gene expression assays (Applied Biosystems). The *Gapdh* transcripts were amplified as an internal control to normalize gene expression. For quantification experiments, at least three sets of mice from every genotype were used. The levels of gene expression were normalized against the levels of an endogenous control in each sample. In each set of experiments, the normalized level of gene of interest from the wild-type mouse was always set as 1.

Cell Lines and Culture Conditions. TM4 cells were purchased from the American Type Culture Collection and were routinely maintained in a 1:1 mixture of DMEM and F12 media (Invitrogen) supplemented with 5% horse serum and 2.5% FBS (HyClone) at 37 °C in the presence of 5% CO_2. When transiently transfected with the Flag-AR plasmid, the TM4 cells were cultured in phenol red-free DMEM/F12 media (Invitrogen) containing 5% charcoal-treated FBS (HyClone). Approximately 30 h after transfection, R1881 or vehicle was added at a final concentration of 100 nM. The cells were incubated for an additional 18 h and were used for the luciferase reporter gene assay.

Plasmids. The mammalian expression plasmids for Flag-ARID4A and ARID4B-V5 have been described previously (1). To generate the plasmid expressing HA-ARID4A, the coding region of human

ARID4A cDNA from the Flag-ARID4A plasmid was subcloned into the pSG5-HA vector with a HA epitope in frame at the N terminus. The pSG5-HA vector was modified from the pSG5 vector (Stratagene). The plasmid expressing Flag-AR was generated by subcloning the *AR* cDNA into a modified pCR3.1 vector (Invitrogen) containing a Flag tag at the N terminus. The RB expression plasmid was kindly provided by Goberdhan Dimri at The George Washington University. The mouse *Cldn3* promoter (−972 to +229 bp) was amplified by PCR from genomic DNA prepared from mouse testis and cloned into the pGL3-basic vector (Promega). For amplification of the *Cldn3* promoter, the following primers were used: the forward primer, 5′-CCGCTCGAGGCTTCTGCACAGGAGTTGTGAG-3′; the reverse primer, 5′-GAAGATCTGGACTTGAACCGGCTCGGCCGGG-3′.

Transfection and Luciferase Reporter Gene Assay. Plasmid transfection by FuGene HD (Promega) was carried out according to the manufacturer's instructions. Transfection of siRNAs was performed using the TransIT TKO reagents (Mirus). All of the ON-TARGET*plus* siRNA reagents were purchased from Dharmacon. Forty-eight hours after transfection, whole-cell lysates were prepared and the luciferase activity was determined by the luciferase assay system as instructed by the manufacturer (Promega).

Coimmunoprecipitation and Western Blot Analysis. Transfections were carried out as described above. The transfected cells were lysed in lysis buffer [20 mM Tris·HCl (pH 8.0), 125 mM NaCl, 0.5% Nonidet P-40, 2 mM EDTA, 0.2 mM NaF, 0.2 mM Na_3VO_4, protease inhibitor mixture] for 30 min, followed by centrifugation at 13,400 × *g* for 20 min at 4 °C to clear the debris. For coimmunoprecipitation experiments, the lysates were incubated with 0.5 μg of anti-Flag antibody (Sigma) with constant rotation at 4 °C overnight. The antibody was allowed to bind to Protein A beads for 1 h and then washed extensively with lysis buffer. For Western blot analysis, the samples were resolved by SDS/PAGE and transferred to nitrocellulose membranes (Bio-Rad). After blocking with 5% milk in TBST, the primary antibodies diluted in TBST buffer [50 mM Tris·HCl, 150 mM NaCl (pH 7.5), 0.1% Tween 20] with 5% milk were added to the membranes for overnight at 4 °C, followed by incubation with the appropriate horseradish peroxidase-conjugated secondary antibodies for 1 h at room temperature. All blots were developed with Supersignal substrate (Pierce) and visualized by chemiluminescence. Subsequent probing with different antibodies was made possible by stripping the membranes with buffer [62.5 mM Tris·HCl (pH 6.8), 2% SDS, 100 mM β-mercaptoethanol] at 55 °C for 30 min.

The primary antibodies used for Western blotting were as follows: anti-Cldn3 (34-1700, Z23.JM; Invitrogen), anti-ARID4A (A302-231A for Western blot and A302-232A for immunoprecipitation; Bethyl Laboratories), anti-ARID4B (A302-233A; Bethyl Laboratories), anti-AR (sc-816, *N*-20; Santa Cruz Biotechnology), and goat anti-actin (ac-1616; Santa Cruz Biotechnology).

ChIP. Whole testes dissected from wild-type mice at 3 mo of age were used for ChIP assays as described by Millipore (available at www.millipore.com). Chromatin extracted from mouse testis was immunoprecipitated with anti-ARID4B (A302-233A; Bethyl Laboratories) and anti-AR (sc-816, *N*-20; Santa Cruz Biotechnology) antibodies.

When using TM4 cells, transfection was carried out as described above. Chromatin extracted from the transfected cells was incubated with anti-HA (12CA5; Roche) antibody to immunoprecipitate the HA-ARID4A protein.

DNA from immunoprecipitated chromatin was analyzed by qPCR analyses using the primer sets from *Cldn3*, *Rhox5*, and *Snrpn*. The primer sets used to amplify the *Cldn3* promoter were as follows: primer pair 1: 5′-GCTTCTGCACAGGAGTTGTGAG-3′ (forward) and 5′-CACGGAGGTTCTCTAGATGGAG-3′ (reverse); primer pair 2: 5′- GGCATTTGAGCTAAGCCATGTAG-3′ (forward) and 5′-CTCCAAGTCCCTCAGGCTTTG-3′ (reverse); primer pair 3: 5′-CAGGTGCTACAATTCCATCCAC-3′ (forward) and 5′-CGAATTCTCTAGAGCTATACAGAG-3′ (reverse); primer pair 4: 5′-CGTTATGCTTAAGGAGCTTGGG-3′ (forward) and 5′-GGACTTAGCTTGGTGCCTAGAG-3′ (reverse); and primer pair 5: 5′-CCAGTCTCAGAAGCCAGTCTC-3′ (forward) and 5′-CTGCTGGACTTGAACCGGCTC-3′ (reverse). The primer sets used to amplify the *Rhox5* promoter were as follows: primer pair 6: 5′-GGAGTCAGCTGAGCTGTAACTG-3′ (forward) and 5′-GATGTGCTTGCAAGATGGACACA-3′ (reverse); and primer pair 7: 5′-CCAAACTGCTATCACTTGTGTACC-3′ (forward) and 5′-GGAACAGAATGAGATCTGTGATGC-3′ (reverse). The primer sets used to amplify *Snrpn* exons 1 and 7 have been described previously (1).

Steroid Hormone Assays. Mice were anesthetized, and blood was obtained from retro-orbital venous plexus. Serum was separated by centrifugation. Measurement of serum levels of testosterone, luteinizing hormone (LH), and follicle-stimulating hormone (FSH) was performed by University of Virginia Ligand Assay and Analysis Core.

Statistical Analysis. Means were calculated from at least three independent experiments. All results are shown as the mean ± SD. The comparison of different groups was carried out by two-tailed unpaired Student *t* test. *P* values less than 0.05 were considered to be statistically significant.

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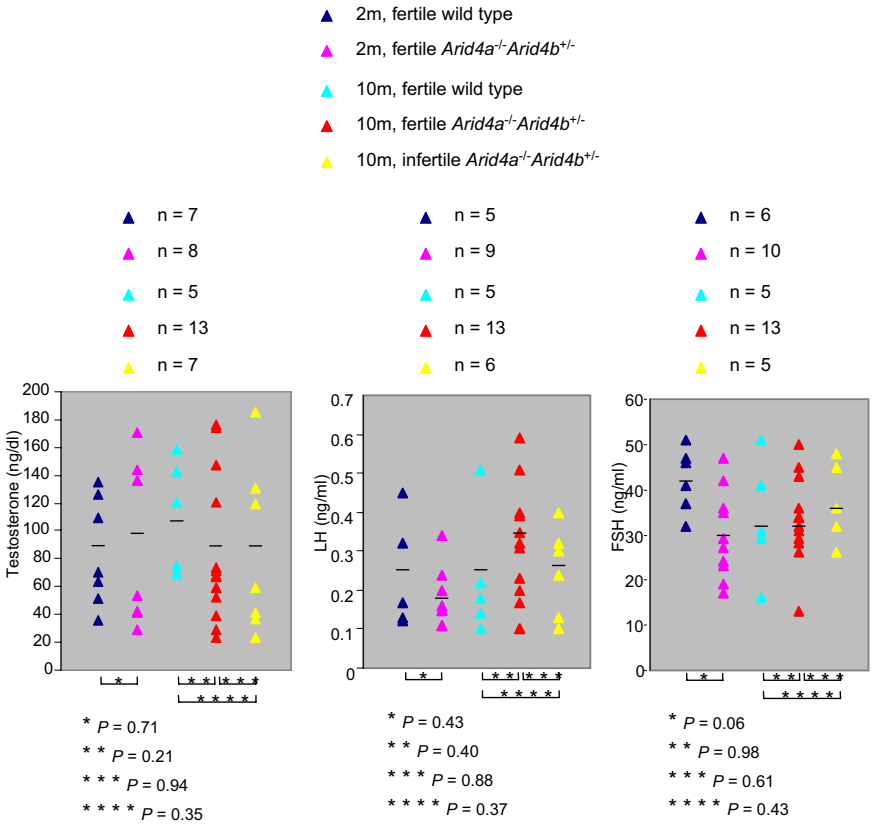

Fig. S1. Levels of serum testosterone, LH, and FSH in wild-type mice and *Arid4a*$^{-/-}$*Arid4b*$^{+/-}$ mice at 2 and 10 mo of age. Each triangle represents the hormone level of a single mouse. The mean of hormone levels at each genotype is designated by a horizontal line.

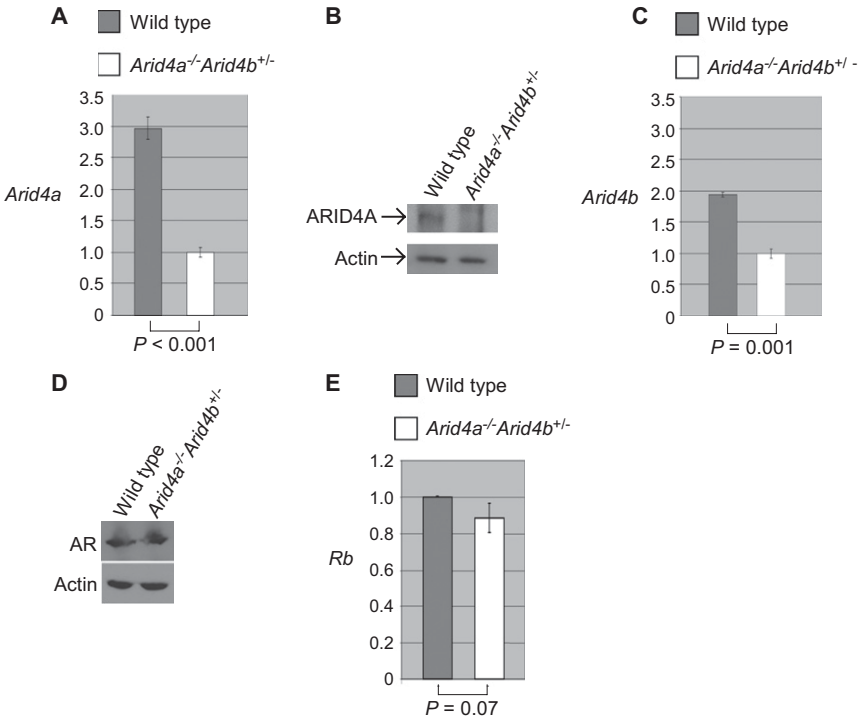

Fig. S2. Gene expression analyses of *Arid4a*, *Arid4b*, *AR*, and *Rb* in the *Arid4a*$^{-/-}$*Arid4b*$^{+/-}$ testes. (*A*) Levels of the *Arid4a* transcripts in testes from wild-type mice and *Arid4a*$^{-/-}$*Arid4b*$^{+/-}$ mice were compared by qRT-PCR analysis. (*B*) ARID4A protein was detected in the wild-type testes but was not in the *Arid4a*$^{-/-}$*Arid4b*$^{+/-}$ testes by immunoprecipitation combined with Western blot analysis. Actin from cell extracts was assayed by Western blot analysis as an input control. ARID4A, 200 kDa; actin, 47 kDa. (*C*) Levels of the *Arid4b* transcripts in testes from wild-type mice and *Arid4a*$^{-/-}$*Arid4b*$^{+/-}$ mice were compared by qRT-PCR analysis. (*D*) Levels of AR protein in testes from wild-type mice and *Arid4a*$^{-/-}$*Arid4b*$^{+/-}$ mice were compared by Western blot analysis. Actin was used as a loading control. AR, 110 kDa; actin, 47 kDa. (*E*) Levels of the *Rb* transcripts in testes from wild-type mice and *Arid4a*$^{-/-}$*Arid4b*$^{+/-}$ mice were compared by qRT-PCR analysis. Wild-type mice and *Arid4a*$^{-/-}$*Arid4b*$^{+/-}$ mice were 8 mo of age. Three mice for each genotype were analyzed.

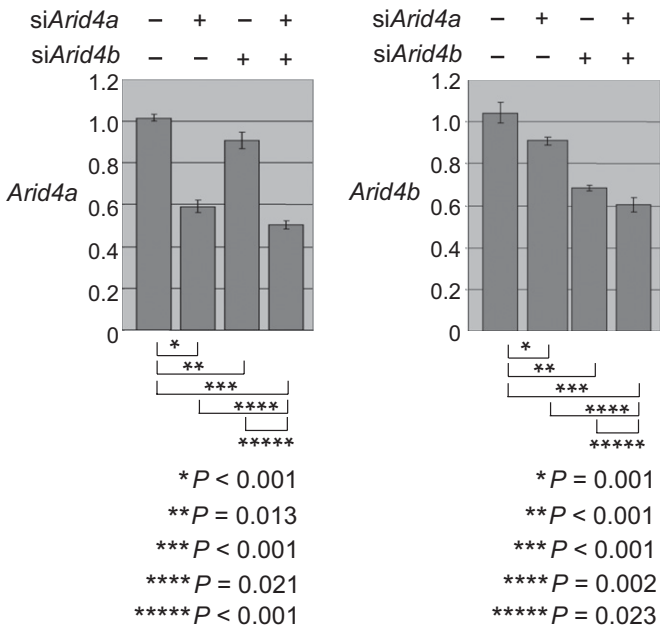

Fig. S3. Knockdown of *Arid4a* and *Arid4b* by si*Arid4a* and si*Arid4b*, respectively. TM4 cells were transfected with siRNAs (si*Arid4a* and si*Arid4b*) to knock down *Arid4a* and *Arid4b* alone or combined as indicated. qRT-PCR analyses showed decreased expression of *Arid4a* (*Left*) and *Arid4b* (*Right*). Shown are means ± SD from three experiments performed in triplicate. The mean of gene expression from the first control sample was set as 1.

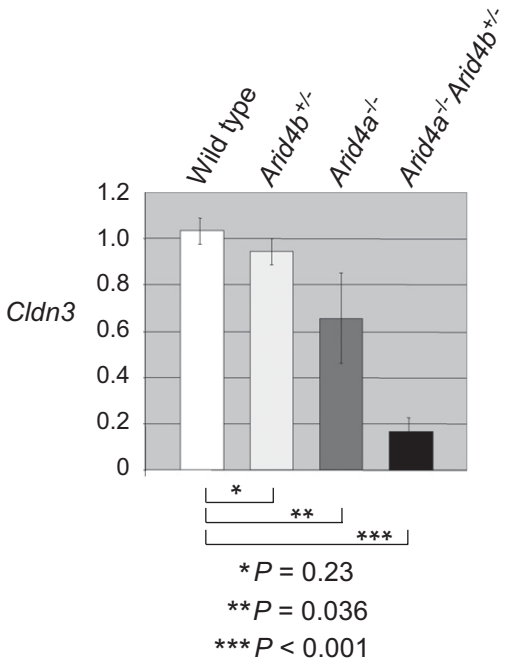

Fig. S4. Markedly decreased expression of *Cldn3* in *Arid4a*$^{-/-}$*Arid4b*$^{+/-}$ mice and partially decreased expression of *Cldn3* in *Arid4a*$^{-/-}$ mice. Levels of the *Cldn3* transcripts in testes from wild-type mice, *Arid4b*$^{+/-}$ mice, *Arid4a*$^{-/-}$ mice, and *Arid4a*$^{-/-}$*Arid4b*$^{+/-}$ mice at 2 mo of age were compared by qRT-PCR analysis. Three mice for each genotype were used. The level of gene expression from one wild-type mouse was set as 1.

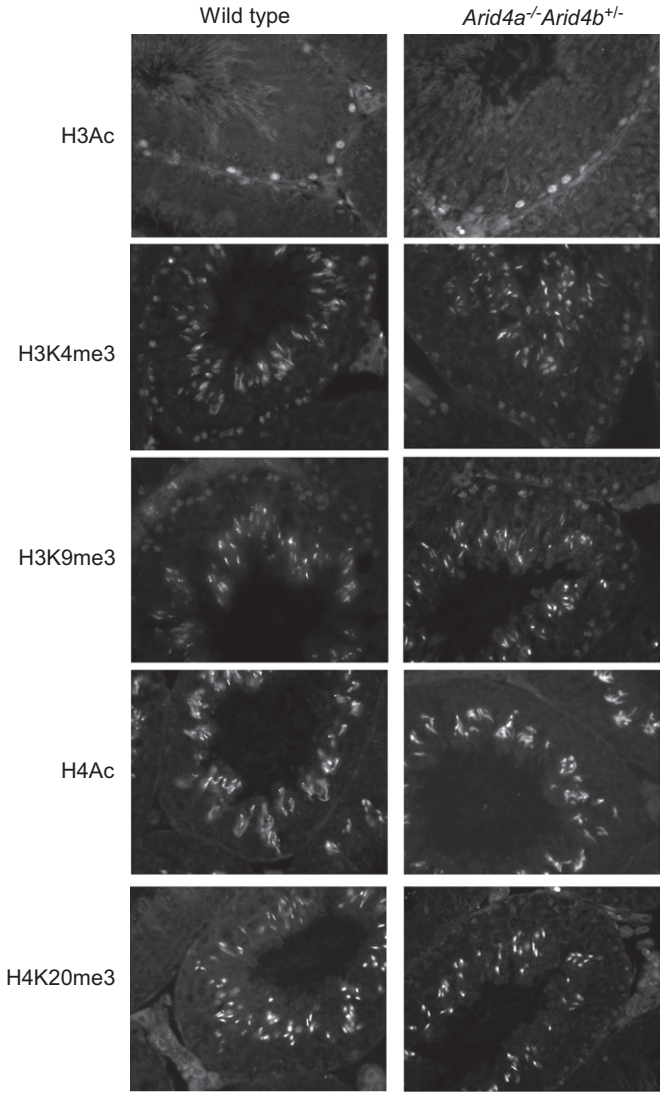

Fig. S5. Chromatin modification analyses of wild-type testes and *Arid4a*$^{-/-}$*Arid4b*$^{+/-}$ testes. Immunofluorescence analyses were performed on paraffin sections of seminiferous tubules of wild-type testes and *Arid4a*$^{-/-}$*Arid4b*$^{+/-}$ testes using antibodies against H3Ac, H3K4me3, H3K9me3, H4Ac, and H4K20me3.

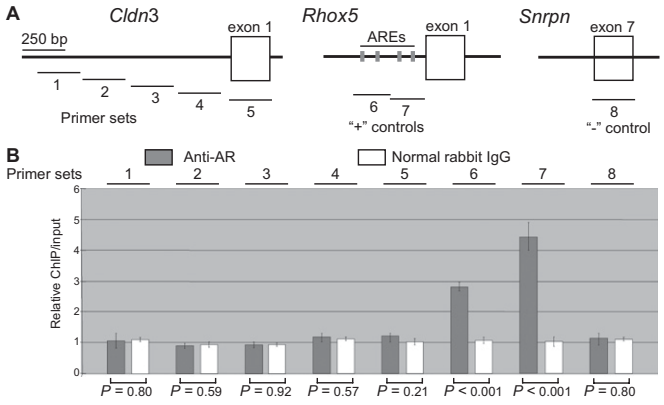

Fig. S6. AR did not bind to the *Cldn3* promoter region (−972 to +229 bp) by ChIP analyses. (*A*) The promoter regions of *Cldn3*, *Rhox5*, and *Snrpn* are shown with primer sets indicating the amplified region of each PCR product in ChIP analysis. The amplified products from primer sets of 1, 2, 3, 4, and 5 were within the *Cldn3* promoter (−972 to +229 bp). The amplified products from primer sets 6 and 7 as ChIP-positive controls were within the *Rhox5* promoter containing AR response elements (AREs). Primer 8 overlaps the *Snrpn* exon 7 as a negative control. (*B*) Cross-linked chromatin of testes from wild-type mice was immunoprecipitated with anti-AR antibody or normal rabbit IgG. DNA from immunoprecipitated chromatin was subjected to qPCR analysis using the primer sets for the sites shown in *A*.

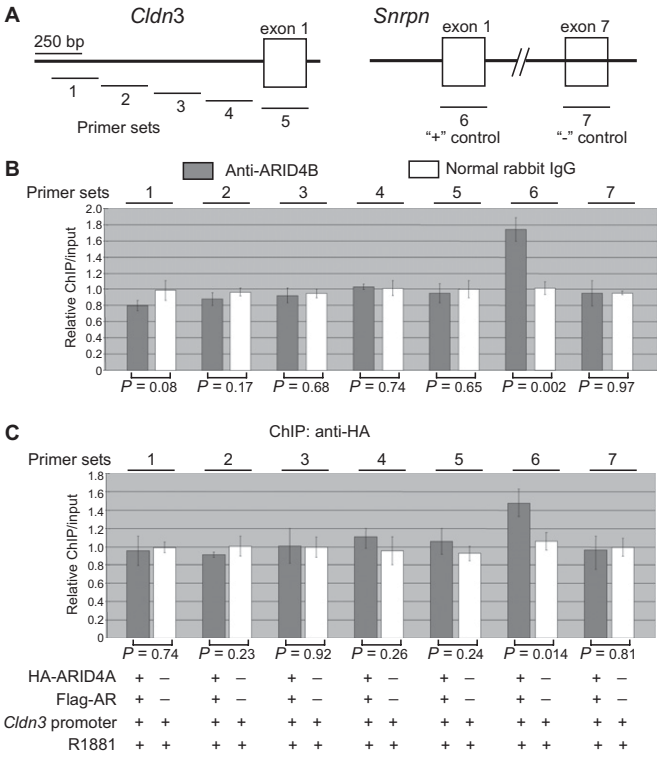

Fig. S7. Neither ARID4A nor ARID4B bind to the *Cldn3* promoter region (−972 to +229 bp) by ChIP analyses. (*A*) The promoter regions of *Cldn3* and *Snrpn* are shown with primer sets indicating the amplified region of each PCR product used in ChIP analysis. The amplified products from primer sets of 1, 2, 3, 4, and 5 were within the *Cldn3* promoter (−972 to +229 bp). Primer sets 6 and 7 overlap the *Snrpn* exons as positive and negative controls, respectively. (*B*) Cross-linked chromatin of testes from wild-type mice was immunoprecipitated with anti-ARID4B antibody or normal rabbit IgG. DNA from immunoprecipitated chromatin was subjected to qPCR analysis using the primer sets for the sites shown above. (*C*) Cross-linked chromatin was immunoprecipitated with anti-HA antibody using the TM4 cells transiently transfected with the plasmid containing the *Cldn3* promoter (−972 to +229 bp) along with or without the expression vectors for Flag-tagged AR and HA-tagged ARID4A as indicated. All cells were treated with R1881. DNA from immunoprecipitated chromatin was subjected to qPCR analysis using the primer sets for the sites shown in *A*.

Table S1. Forty-two genes decreasingly expressed in *Arid4a*$^{-/-}$*Arid4b*$^{+/-}$ (MU) testes compared with wild-type (WT) testes

Gene name	Gene symbol	Fold change (WT vs. MU)
solute carrier family 25, member 37	*Slc25a37*	15.4
ribosomal protein S9	*Rps9*	5.2
ectonucleoside triphosphate diphosphohydrolase 4	*Entpd4*	4.1
EG231885	—	2.9
chloride intracellular channel 6	*Clic6*	2.8
4921528I07Rik	—	2.8
2210011C24Rik	—	2.8
geranylgeranyl diphosphate synthase 1	*Ggps1*	2.5
receptor (G protein-coupled) activity modifying protein 1	*Ramp1*	2.5
family with sequence similarity 83, member E	*Fam83e*	2.3
1700112E06Rik	—	2.3
AT rich interactive domain 4A	***Arid4a***	**2.3**
TBC1 domain family, member 9	*Tbc1d9*	2.2
1810046K07Rik	—	2.2
geranylgeranyl diphosphate synthase 1	*Ggps1*	2.2
mitogen-activated protein kinase kinase 7	*Map2k7*	2.1
regulator of chromosome condensation (RCC1) and BTB (POZ) domain containing protein 2	*Rcbtb2*	2.1
zinc finger protein 93	*Zfp93*	2.1
prostaglandin D2 synthase	***Ptgds***	**2.0**
AT rich interactive domain 4B	***Arid4b***	**2.0**
spastic paraplegia 20	*Spg20*	2.0
ERO1-like beta	*Ero1lb*	2.0
claudin 3	***Cldn3***	**1.9**
actin filament associated protein 1	*Afap1*	1.9
4933430N04Rik	—	1.9
EG545391	—	1.9
RAS p21 protein activator	*Rasa1*	1.9
glycoprotein Ib	*Gp1bb*	1.9
apolipoprotein A-II	*Apoa2*	1.9
GATA binding protein 3	*Gata3*	1.9
tumor necrosis factor receptor superfamily, member 10b	*Tnfrsf10b*	1.9
GDNF family receptor alpha 2	*Gfra2*	1.9
MAM domain containing glycosylphosphatidylinositol anchor 2	*Mdga2*	1.8
exportin 7	*Xpo7*	1.8
granzyme N	*Gzmn*	1.8
1700031C06Rik	—	1.8
choroideremia-like	*Chml*	1.8
AT rich interactive domain 4B	***Arid4b***	**1.8**
trafficking protein particle complex 2-like	*Trappc2l*	1.8
adenomatosis polyposis coli down-regulated 1	*Apcdd1*	1.8
DnaJ (Hsp40) homolog, subfamily C, member 2	*Dnajc2*	1.8
adenomatosis polyposis coli down-regulated 1	*Apcdd1*	1.8

Decreased expression of *Arid4a* (dark shading) and *Arid4b* (light shading) in the *Arid4a*$^{-/-}$ *Arid4b*$^{+/-}$ testes was confirmed. Decreased expression of genes (boldface) in the *Arid4a*$^{-/-}$ *Arid4b*$^{+/-}$ testes was verified by qRT-PCR. —, gene symbol not available.

Table S2. Sixty-five genes increasingly expressed in *Arid4a*$^{-/-}$*Arid4b*$^{+/-}$ (MU) testes compared with wild-type (WT) testes

Gene name	Gene symbol	Fold change (MU vs. WT)
lymphocyte antigen 6 complex, locus D	*Ly6d*	4.5
C-type lectin domain family 4, member G	*Clec4g*	3.3
dentin matrix protein 1	*Dmp1*	2.7
1110021L09Rik	—	2.5
tetraspanin 7	*Tspan7*	2.4
C85492	—	2.3
potassium voltage-gated channel, shaker-related subfamily, beta member 1	*Kcnab1*	2.3
syntaxin binding protein 6	*Stxbp6*	2.3
immunoglobulin superfamily, member 8	*Igsf8*	2.3
integrin, alpha 6	*Itga6*	2.2
tweety homolog 2	*Ttyh2*	2.1
milk fat globule-EGF factor 8 protein	*Mfge8*	2.1
glucosidase, beta, acid	*Gba*	2.1
aminoadipate-semialdehyde synthase	*Aass*	2.1
glucosidase, beta, acid	*Gba*	2.1
family with sequence similarity 3, member C	*Fam3c*	2.1
glycogenin	*Gyg*	2.1
exostoses (multiple)-like 2	*Extl2*	2.1
fibroblast growth factor 13	*Fgf13*	2.0
1700025G04Rik	—	2.0
fatty acid binding protein 5	*Fabp5*	2.0
embigin	***Emb***	**2.0**
TSC22 domain family, member 1	*Tsc22d1*	2.0
serine (or cysteine) peptidase inhibitor, clade A, member 3A	*Serpina3a*	2.0
integrin, beta 5	*Itgb5*	2.0
Eph receptor B1	*Ephb1*	2.0
2810468N07Rik	—	2.0
sulfatase 1	*Sulf1*	2.0
B-cell receptor-associated protein 31	*Bcap31*	2.0
golgi membrane protein 1	*Golm1*	2.0
integrin, beta 5	*Itgb5*	2.0
maternally expressed 3	*Meg3*	1.9
aminolevulinate, delta-, synthase 2	*Alas2*	1.9
AU015836	—	1.9
tubby-like protein 3	*Tulp3*	1.9
peroxisomal biogenesis factor 19	*Pex19*	1.9
T cell receptor alpha chain	*Tcra*	1.9
zinc finger, DHHC-type containing 9	*Zdhhc9*	1.9
tweety homolog 3	*Ttyh3*	1.9
yippee-like 2	*Ypel2*	1.9
lipoma HMGIC fusion partner	*Lhfp*	1.9
ATP-binding cassette, sub-family A (ABC1), member 3	*Abca3*	1.9
matrix Gla protein	*Mgp*	1.9
mediator complex subunit 12-like	*Med12l*	1.9
integrin, beta 5	*Itgb5*	1.9
ceramide synthase 5	*Lass5*	1.9
inhibin, alpha	***Inha***	**1.9**
collagen, type XXV, alpha 1	*Col25a1*	1.9
transmembrane protein 114	*Tmem114*	1.9
fibronectin leucine rich transmembrane protein 1	*Flrt1*	1.8
Rho GTPase activating protein 26	*Arhgap26*	1.8
monocyte to macrophage differentiation-associated	*Mmd*	1.8
glypican 4	*Gpc4*	1.8
sulfatase 1	*Sulf1*	1.8
major facilitator superfamily domain containing 1	*Mfsd1*	1.8
integrin, beta 5	*Itgb5*	1.8
cleft lip and palate associated transmembrane protein 1	*Clptm1*	1.8

Table S2. Cont.

Gene name	Gene symbol	Fold change (MU vs. WT)
secernin 1	*Scrn1*	1.8
SH3-domain GRB2-like 2	*Sh3gl2*	1.8
zinc finger protein 704	*Zfp704*	1.8
cytohesin 1 interacting protein	*Cytip*	1.8
integrin, alpha 6	*Itga6*	1.8
RNA imprinted and accumulated in nucleus	*Rian*	1.8
G protein-coupled receptor, family C, group 5, member B	*Gprc5b*	1.8
adenomatosis polyposis coli	*Apc*	1.8

Increased expression of *Emb* (boldface) and *Inha* (boldface) in the *Arid4a*$^{-/-}$*Arid4b*$^{+/-}$ testes was verified by qRT-PCR. —, gene symbol not available.