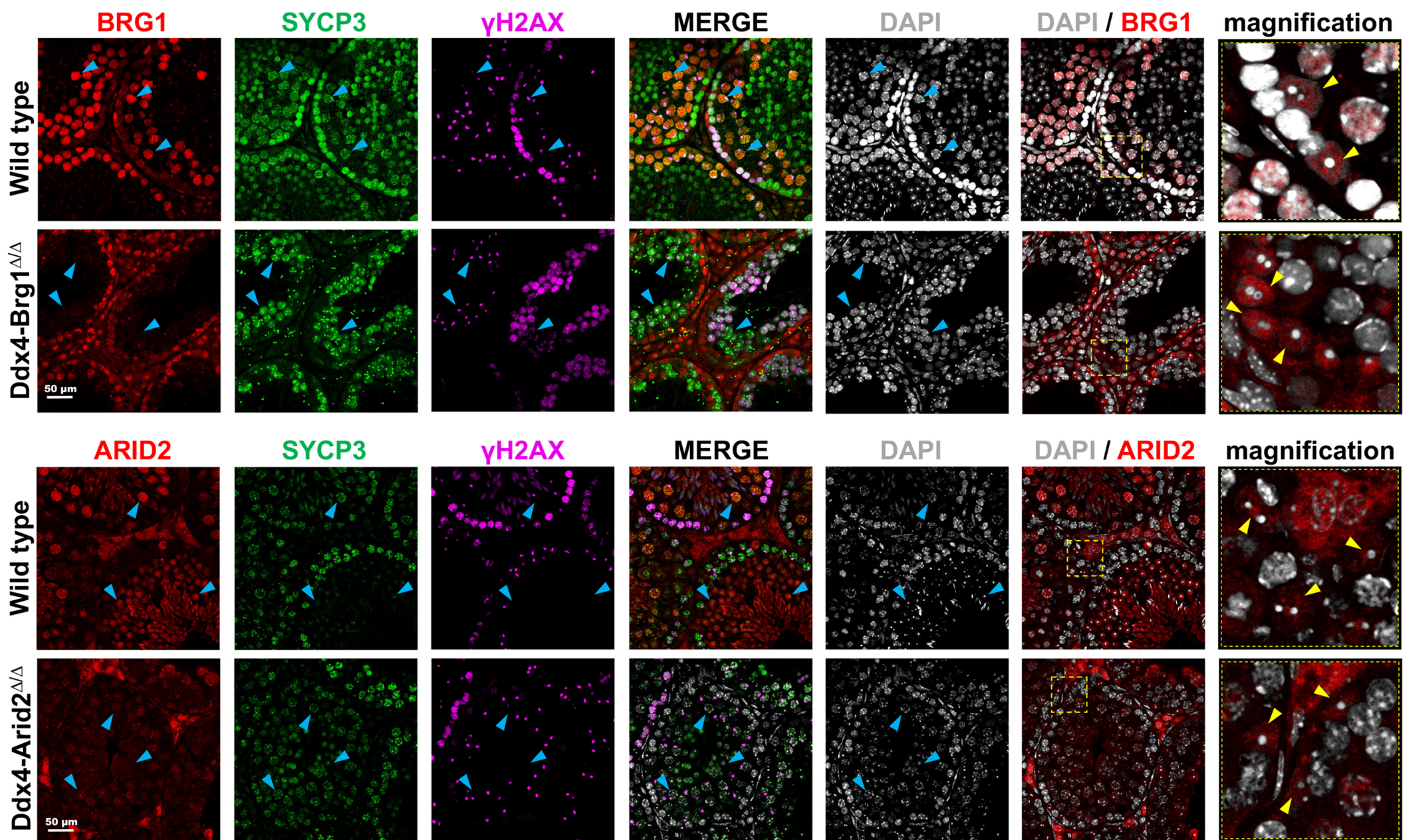

Fig. S1. Immunolocalization of BRG1 and ARID2 in paraffin embedded mouse testis sections. SYCP3 and γH2AX immunostaining serve to classify primary spermatocytes at different stages of prophase I in 2-month-old testis. Blue arrowheads mark examples of positive primary spermatocytes in wild type and examples of primary spermatocytes with negative immunostaining for BRG1 and ARID2 in knockout testis. Magnifications in the far-right panels correspond to the yellow square inserts. Yellow arrowheads show examples of Sertoli cells still expressing BRG1 or ARID2 proteins in both wild type and knockout mice.

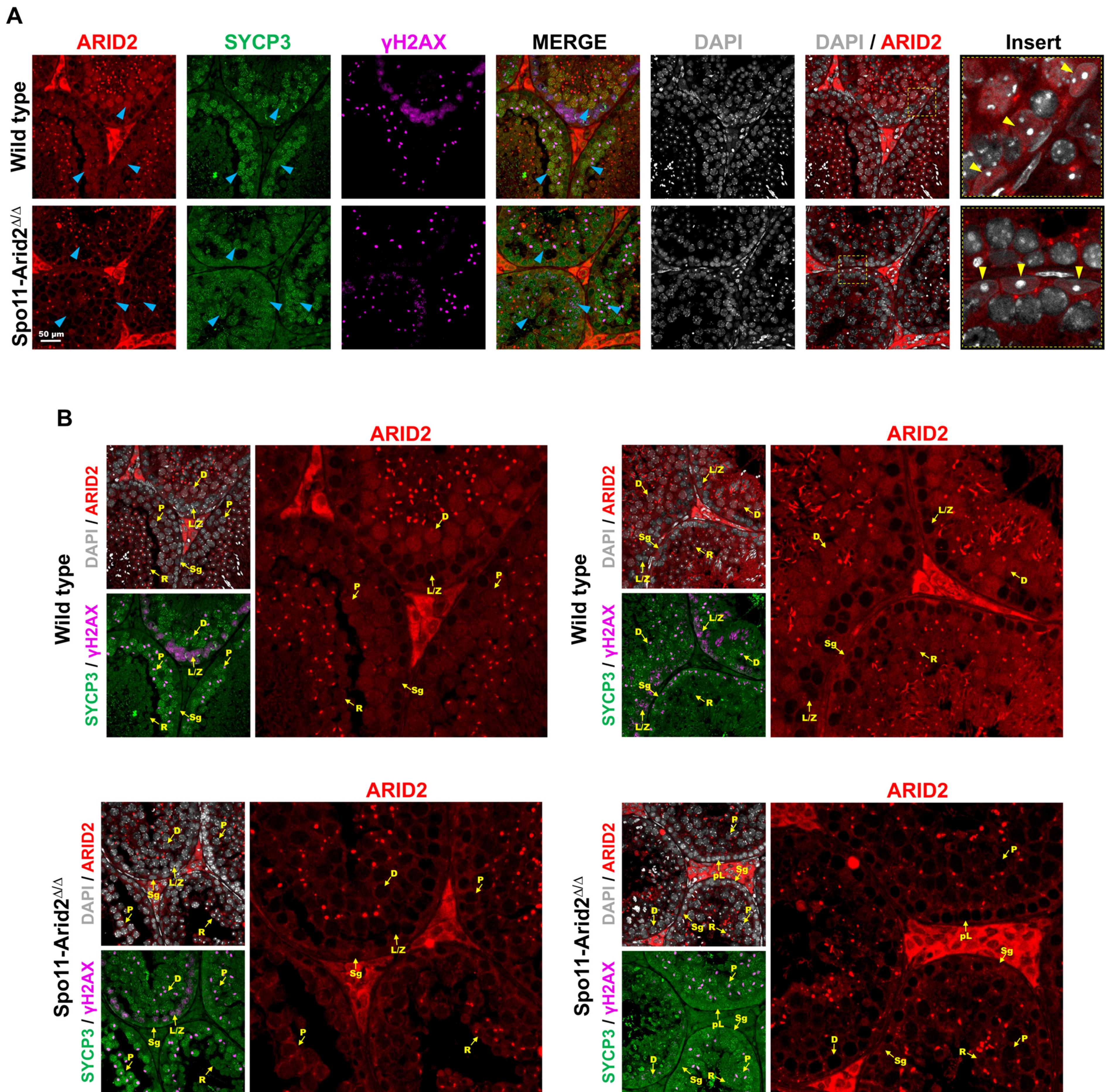

Fig. S2. Analysis of ARID2 immunosignal in testis cuts of wild type and Spo11-Arid2 knockout. A. Paraffin embedded cuts from Spo11-Arid2 knockout and wild type control stained with SYCP3, ARID2, and γH2AX. Blue arrowheads mark examples of primary spermatocytes immunostained with ARID2 in wild type and Arid2 knockout testis. Magnifications in the far-right panels correspond to the yellow square inserts. Yellow arrowheads show examples of Sertoli cells still expressing ARID2 protein in both wild type and knockout mice. **B.** High magnification images showing cells at different stages of spermatogenesis immunostained with the indicated markers. Sg: spermatogonia, pL: pre-leptotene, L/Z: leptotene/zygotene, P: pachytene, D: diplotene, and R: rounded. In these images we observed red foci in both wild type and knockout seminiferous tubules, possibly caused by a different antibody lot used in these experiments.

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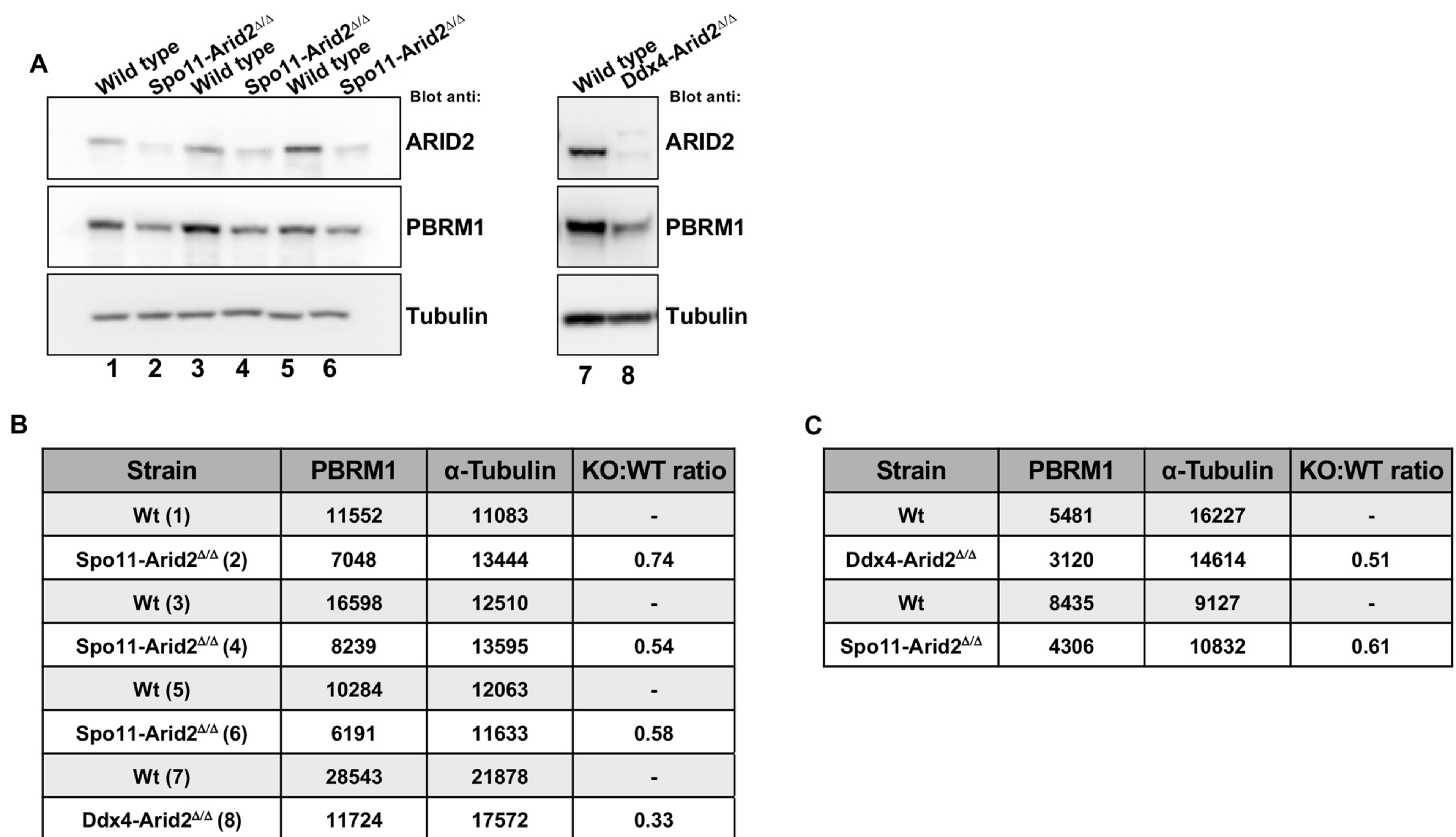

B

Strain	PBRM1	α-Tubulin	KO:WT ratio
Wt (1)	11552	11083	-
Spo11-Arid2$^{\Delta/\Delta}$ (2)	7048	13444	0.74
Wt (3)	16598	12510	-
Spo11-Arid2$^{\Delta/\Delta}$ (4)	8239	13595	0.54
Wt (5)	10284	12063	-
Spo11-Arid2$^{\Delta/\Delta}$ (6)	6191	11633	0.58
Wt (7)	28543	21878	-
Ddx4-Arid2$^{\Delta/\Delta}$ (8)	11724	17572	0.33

C

Strain	PBRM1	α-Tubulin	KO:WT ratio
Wt	5481	16227	-
Ddx4-Arid2$^{\Delta/\Delta}$	3120	14614	0.51
Wt	8435	9127	-
Spo11-Arid2$^{\Delta/\Delta}$	4306	10832	0.61

Fig. S3. Reduction of PBRM1 protein levels in Arid2 knockout mice. A. Western blots showing results for three 2-month-old independent mouse of each wild type and Spo11-Arid2 knockout genotype (lines 1-6). The right panel contain western blot corresponding to an additional example of wild type and Ddx4-Arid2 knockout (lines 7, 8). **B.** Quantitation corresponding to PBRM1 and tubulin band density in western blots shown in A. To determine PBRM1 expression changes, PBRM1 immunosignal intensity was normalized to the corresponding tubulin values, followed by KO/Wt ratio calculati on. **C.** Quantitation corresponding to band density in western blots shown Figure 1A. PBRM1 expression changes were calculated as describe in B.

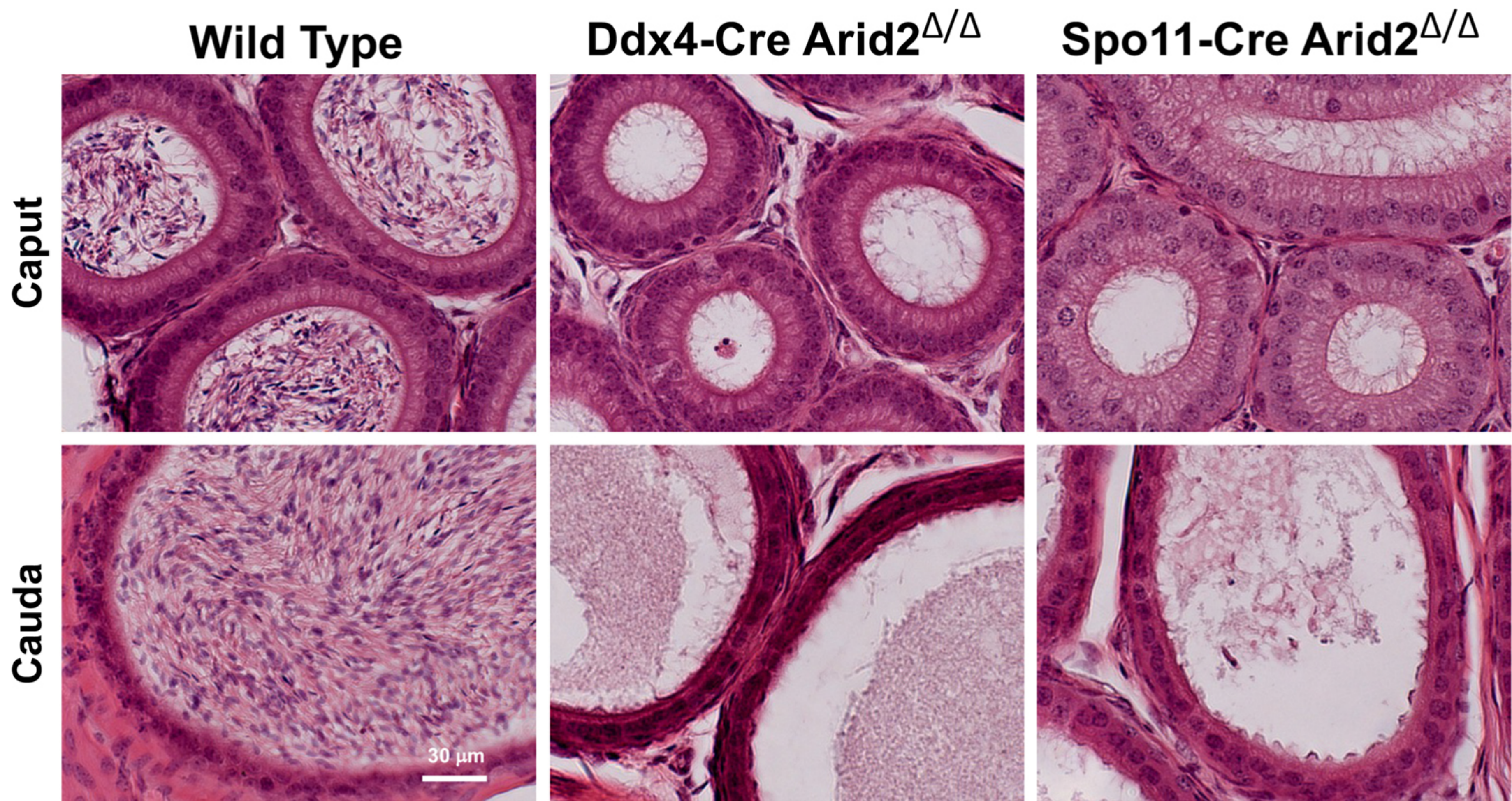

Fig. S4. Images corresponding to H&E stained epididymis tubules. Shown are the caput and cauda sections of the epididymis. Note the absent of spermatozoa in Arid2 knockout samples.

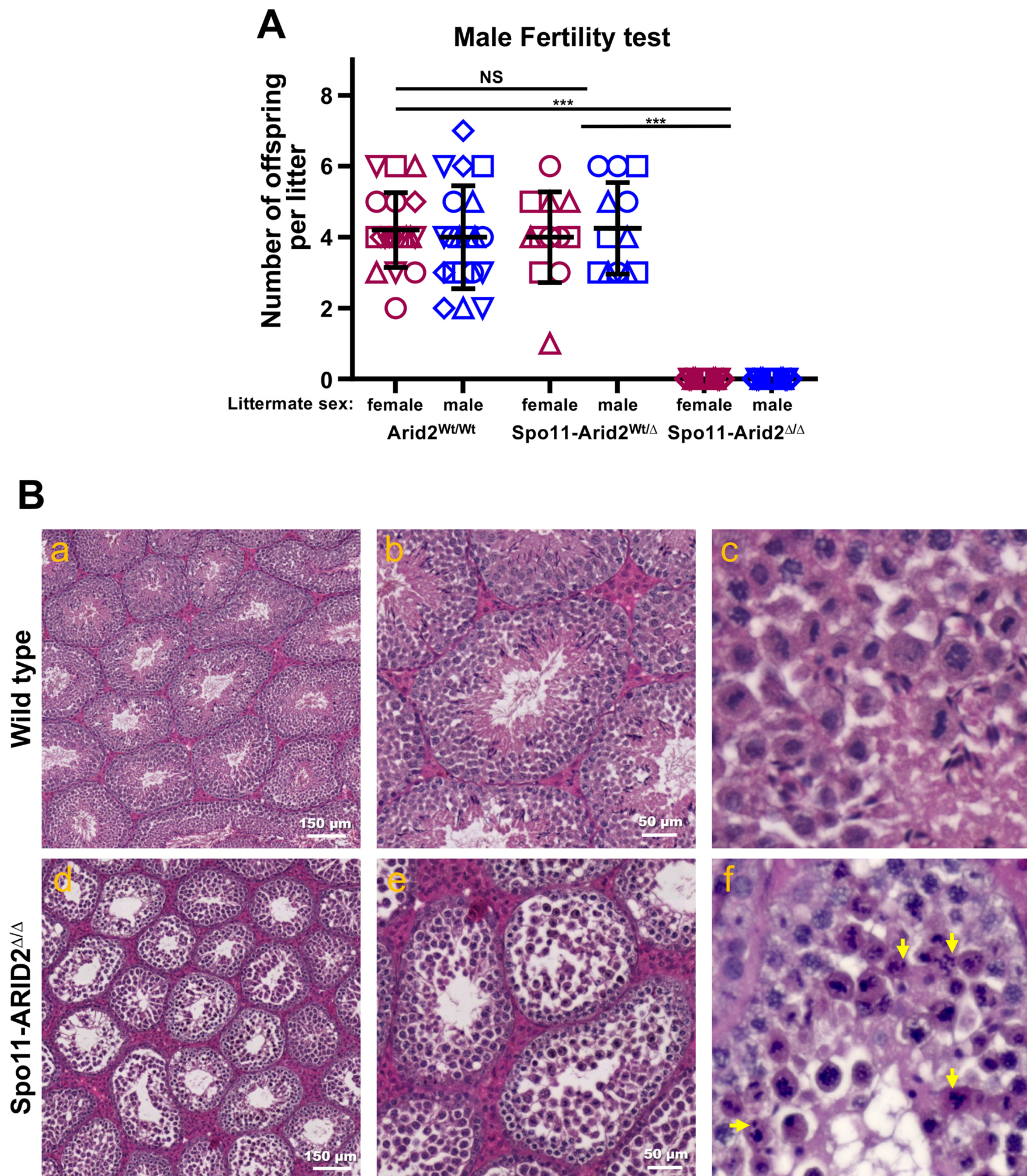

Fig. S5. Fertility assessment and testis tissue analysis of 4-months-old wild type and Arid2 knockout mice. A. The graph represents the number of male and female offspring resulting from mating the indicated male Arid2 genotype crossed with Arid2$^{wt/wt}$ females. NS, P=0.95. ***, P > 0.0001. Details of crosses are in Table S1. Experiments were conducted for a total of approximately 4 months. **B.** wild type and Spo11-Arid2$^{\Delta/\Delta}$ 4-months-old testis cuts stained with Hematoxylin & Eosin. Note meiosis arrest at meiosis exit in Spo11-Arid2 knockout. Arrows indicate examples of metaphase cells with lagging chromosomes.

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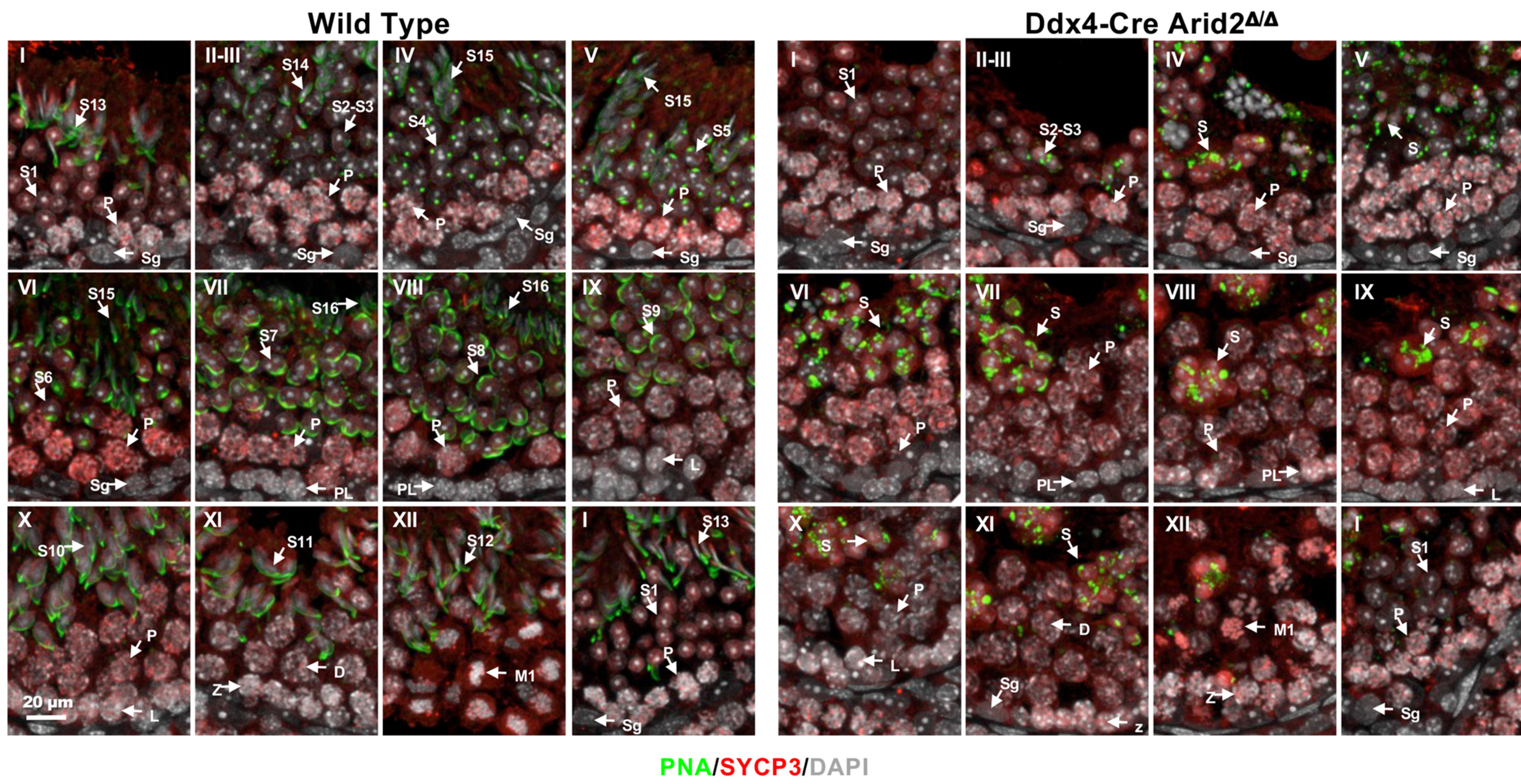


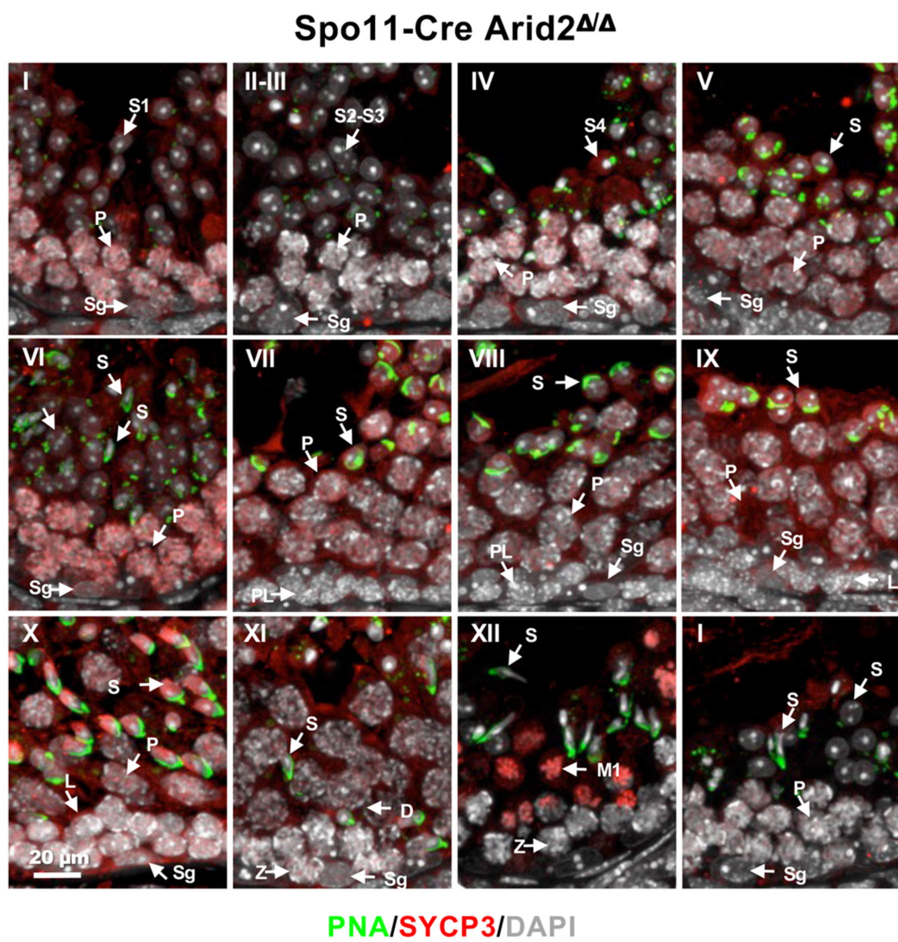
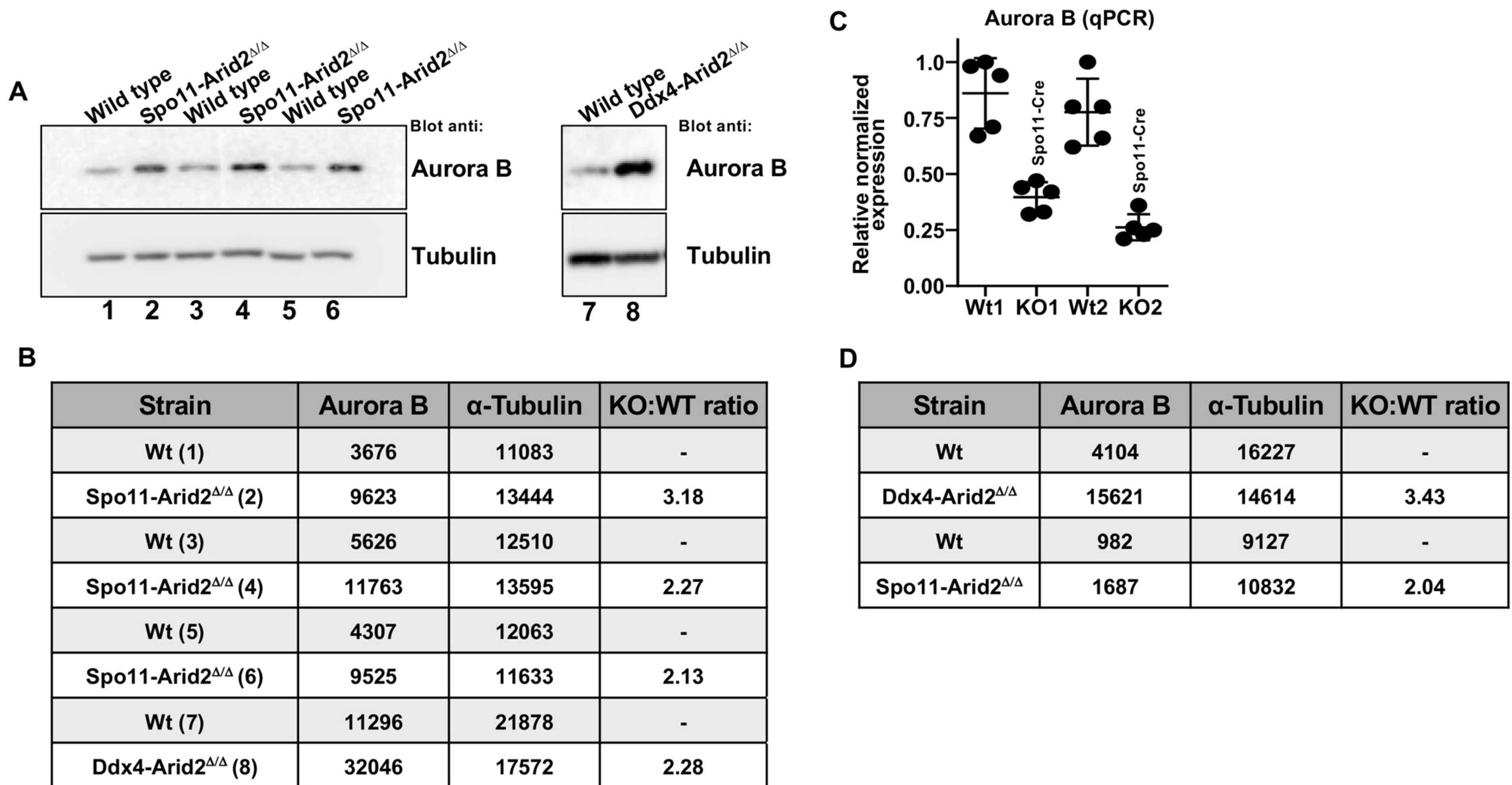


Fig. S6. Characterization of gametogenesis arrest in Arid2$^{\Delta/\Delta}$ knockout mice. Immunofluorescence of mouse seminiferous tubules with SYCP3 and lectin PNA showing representative stages I to XII. Note the spermatids differentiation failure in Ddx4-Arid2$^{\Delta/\Delta}$ and Spo11-Arid2$^{\Delta/\Delta}$ mice when compared to wild type. Sg-spermatogonia; PL-preleptotene; f and S-spermatids.



B

Strain	Aurora B	α-Tubulin	KO:WT ratio
Wt (1)	3676	11083	-
Spo11-Arid2$^{\Delta/\Delta}$ (2)	9623	13444	3.18
Wt (3)	5626	12510	-
Spo11-Arid2$^{\Delta/\Delta}$ (4)	11763	13595	2.27
Wt (5)	4307	12063	-
Spo11-Arid2$^{\Delta/\Delta}$ (6)	9525	11633	2.13
Wt (7)	11296	21878	-
Ddx4-Arid2$^{\Delta/\Delta}$ (8)	32046	17572	2.28

D

Strain	Aurora B	α-Tubulin	KO:WT ratio
Wt	4104	16227	-
Ddx4-Arid2$^{\Delta/\Delta}$	15621	14614	3.43
Wt	982	9127	-
Spo11-Arid2$^{\Delta/\Delta}$	1687	10832	2.04

Fig. S7. Aurora B expression analysis in 2-months-old wild type and Arid2 knockout testis. A. Western blot showing increase in Aurora B in three different Spo11-Arid2$^{\Delta/\Delta}$ mice compared to wild type controls. Note that the tubulin panel is same as in figure S3A. **B.** Quantitation corresponding to Aurora B and tubulin band density in western blots shown in A. o` determine Aurora B expression changes, Aurora B immunosignal intensity was normalized to the corresponding tubulin values, followed by KO/Wt ratio calculation. **C** he` graph shows values obtained from q-PCR assessment of Aurora B gene expression monitored using two independent exon boundaries in .. month-old wild type and Spo11-Arid2 knockouts. **D.** Quantitation corresponding to band density in western blots shown in figure 5A. Aurora B expression changes were calculated as described in B.

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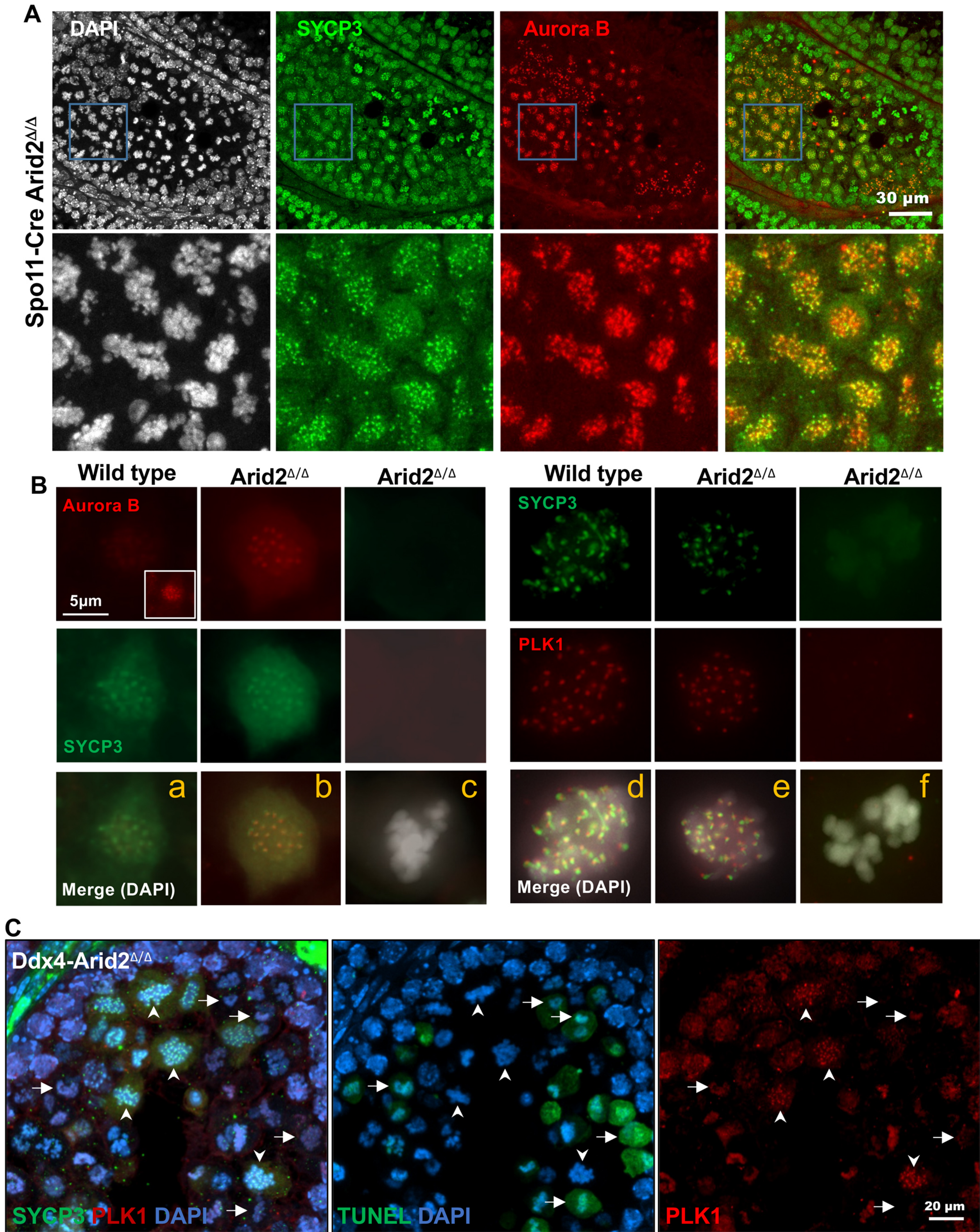
A
DAPI
SYCP3
Aurora B
30 µm
Spo11-Cre Arid2$^{\Delta/\Delta}$
B
Wild type
Arid2$^{\Delta/\Delta}$
Arid2$^{\Delta/\Delta}$
Aurora B
5µm
SYCP3
a
b
c
Merge (DAPI)
Wild type
Arid2$^{\Delta/\Delta}$
Arid2$^{\Delta/\Delta}$
SYCP3
PLK1
d
e
f
Merge (DAPI)
C
Ddx4-Arid2$^{\Delta/\Delta}$
SYCP3 PLK1 DAPI
TUNEL DAPI
PLK1
20 µm

Fig. S8. A. Representative images of paraffin embedded testis cuts immunostained with SYCP3 and Aurora B in 2-month-old Spo11-Arid2 knockout mice (wild type control is shown in figure 5C top panel). The magnification (within the blue frame) shows cells transitioning metaphase-anaphase I immunostained with SYCP3 (which at this stage marks centromeres) and Aurora B. **B.** Representative images of cells at metaphase- anaphase I obtained from squashed seminiferous tubules immunostained with SYCP3, Aurora B, or PLK1 in wild type and Ddx4-Arid2 knockout mice. Note that the insert shows the cell with a longer exposure for Aurora B to allow comparison to knockout. **C.** Seminiferous tubule of 2-month-old Ddx4-Arid2$^{\Delta/\Delta}$ mice showing that PLK1 negative cells are also positive for TUNEL (marking apoptotic cells). Arrowheads indicate cells with PLK1 positive signal and negative for TUNEL. Arrows indicate cells positive for TUNEL and showing no signal for PLK1.

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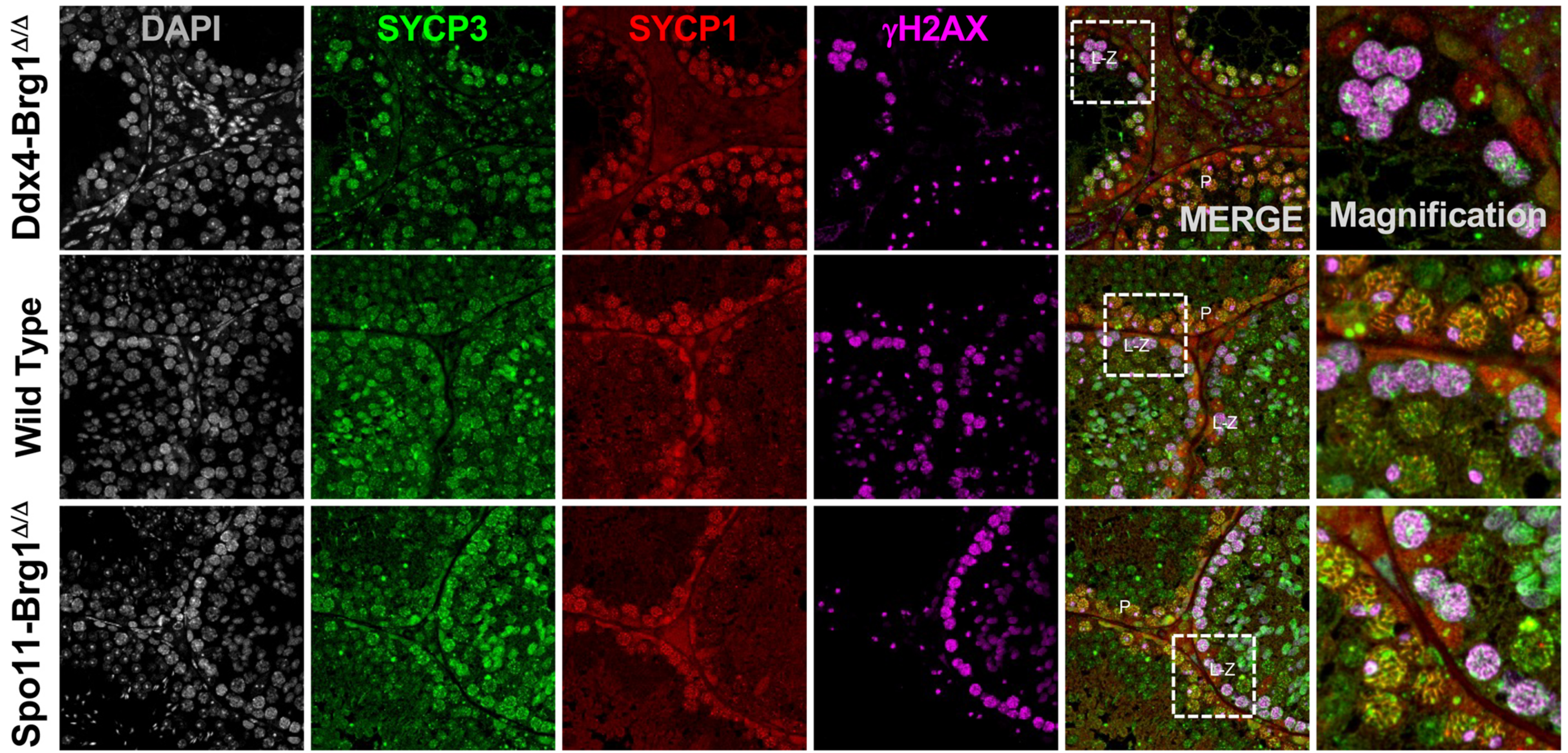

Fig. S9. Immunohistochemical analysis of 2-months-old testis cuts from Ddx4-Brg1 knockout, Spo11-Brg1 knockout, and wild type mice. Testis cuts were immunostained with SYCP1, SYCP3 and γH2AX. Magnifications in the far-right panels correspond to the white square inserts. L-Z, leptotene/zygotene and P-pachytene

Table S1. Fertility assay of wild type, heterozygous, and Spo11-Arid2 knockout mice.

Primer sequences

Primer name	Sequence (5' → 3')
ARID2 forward	GAAACTCAAACAGGGCACAAAG
ARID2 reverse	AACAGCTGCTCTCTGTGTAAG
ARID2 Δ reverse	TTGGCCTTTCAGGATAGACTTAC
LacZ forward	ATCACGACGCGCTGTATC
LacZ reverse	ACATCGGGCAAATAATATCG
BRG1 forward	GTCATACTTATGTCATAGCC
BRG1 reverse	GCCTTGTCTCAAACTGATAAG
BRG1 Δ allele forward	GATCAGCTCATGCCCTAAGG
Ddx4-Cre forward	CACGTGCAGCCGTTTAAGCCGCGT
Ddx4-Cre reverse	TTCCCATTCTAAACAACACCCTGAA
Spo11-Cre forward	CCATCTGCCACCAGCCAG
Spo11-Cre reverse	TCGCCATCTTCCAGCAGG

Table S2. Sequence of primers and PCR products for mouse genotyping.

PCRs primer pairs and expected size bands

PCR type	Forward primer	Reverse primer	Expected size band (bp)
ARID2 Wt/floxed alleles	ARID2 forward	ARID2 reverse	506 (floxed allele) 288 (Wt allele)
ARID2 Δ allele	ARID2 forward	ARID2 Δ reverse	452
LacZ Cassette	LacZ forward	LacZ reverse	108
BRG1 Wt/floxed alleles	BRG1 forward	BRG1 reverse	387 (floxed allele) 241 (Wt allele)
BRG1 Δ allele	BRG1 Δ allele forward	BRG1 reverse	268
Ddx4-Cre	Ddx4-Cre forward	Ddx4-Cre reverse	240
Spo11-Cre	Spo11-Cre forward	Spo11-Cre reverse	281

Table S3. List of antibodies and dilutions used in this study.

Antibody	Source	Western blot dilution	Immuno-labeling dilution
ARID2/BAF200	Sigma, #SAB2702507	1:2500	1:200
ARID2/BAF200	Our lab: ref: J Biol Chem. 2017 May 19; 292(20): 8459–8471. PMC5437250	1:3000	
AurkB/AIM-1	BD Biosciences, 611082	1:500	1:50
BRG1	AbCam, 110641	1:3000	
BRG1	Proteintech, 21634-1-AP		1:200
PLK1	AbCam, 17056	1:1500	1:200
PBRM1/BAF180	Bethyl, A301-591A	1:3000	
SMARCB1/BAF47	Cell Signaling Tech., #91735	1:3000	
SMARCC1/BAF155	Cell Signaling Tech., #11956	1:500	
SMARCC2/BAF170	Cell Signaling Tech., #12760	1:1000	
Survivin	NOVUS Biologicals, NB500-201	1:1000	
SMARCE1/BAF57	Cell Signaling Tech., #33360	1:1000	
SYCP3	Our lab: ref: PLoS Genet. 2018 May 9;14(5):e1007381. PMC5962103		1:300
SYCP3	AbCam, ab97672	1:1000	
SYCP1	Novus, NB300-229		1:300
γH2AX	Millipore, 05-636		1:1000
Lamin B	AbCam, 16048	1:2500	
α-tubulin	Proteintech, 66031-1-Ig	1:5000	
Lectin PNA-Alexa488	Invitrogen L21409		1:200