Supplemental Materials

Molecular Biology of the Cell

Hwang et al.

Supplemental Figure Legends

Supplemental Figure S1: Conditional mutation of *Smc5* via germ cell-specific Ddx4-Cre recombinase causes decreased protein levels of SMC5/6 complex components and partial germ cell loss.

(A) Table demonstrating that the proportion of Smc5 flox/del, Ddx4-Cre cKO mice obtained (4.48%) is more than 5x less than expected (25%) from the breeding strategy used. (B) Protein extracts from crude germ cell isolates from control and *Smc5 flox/del*, *Ddx4-Cre*cKOmice were loaded on a 4-15% SDS PAGE gradient gel and assessed for SMC5, SMC6, and NSMCE2protein levels. α-Tubulin was used as the loading control. Extracts were all from mice ≥ 12 weeks old. Protein levels of SMC5/6 components are reduced in all *Smc5*cKO extracts compared to controls. (C) Assessment of adult testis to body weight ratio (mg/mg), whereby*Smc5 flox/del*, *Ddx4-Cre*cKO(n=5, mean=0.27) ratios are decreased compared to control (n=7, mean= 0.36). Bars indicate mean and standard error. The *P* value (Mann-Whitney, two-tailed) for the indicated comparison is significant, *P*<0.0001 (***). (D) Periodic acid-Schiff staining of tubule cross sections from control and *Smc5 flox/del*, *Ddx4-Cre*cKOtestes. Markers: (Ser) Sertoli cell, (pL) pre-leptotene stage,(P) pachytene stage, (R) round spermatid, (E) elongated spermatid. Roman numerals correspond to the seminiferous tubule stage. The Δ symbol indicates absence of marked cell type.Scale bar: 50 μm.

Supplemental Figure S2: Conditional mutation of *Smc5* via germ cell-specific Cre recombinases causes decreased protein levels of SMC5/6 complex components

(A) Bar graph of relative protein levels for each tested SMC5/6 complex component from crude germ cell isolates from control, *Smc5*cKO (*Stra8-Cre*), Smc5 cKO (*Spo11-Cre*), and *Smc5* cKO (*Hspa2-Cre*) loaded on 4-15% SDS PAGE gradient gels shown in Figure 1C. Protein band signal intensities were normalized against α-Tubulin loading control. Protein levels of SMC5/6 components are reduced in all *Smc5*cKO extracts compared to controls. (B) Bar graph of relative protein levels for each tested SMC5/6 complex component from STA-PUT purified germ cell isolates from control and *Smc5*cKO (*Stra8-Cre*) loaded on 4-15% SDS PAGE gradient gels shown in Figure 1E. Spermatocytes were purified via STA-PUT into specified prophase substages: leptotene/zygotene (L/Z, ~85% enrichment), pachytene (P, ~90% enrichment), and round spermatid (RS, ~95% enrichment). Spermatocytes were isolated from *Smc5 flox/del* (control) and *Smc5 flox/del, Stra8-cre*$^{tg/0}$(*Smc5*cKO). Protein levels of SMC5 SMC6 and NSMCE2 are reduced in all *Smc5*cKO extracts compared to controls.

Supplemental Figure S3: *Smc5 flox/del, Spo11-Cre* or *Smc5 flox/del, Hspa2-Cre*cKO mice did not show a difference in testis weight compared to controls

(A) Testis of adult control (*Smc5 +/flox, Spo11-cre*$^{tg/0}$)and *Smc5* cKO (*Smc5 flox/del, Spo11-cre*$^{tg/0}$)mice. (B) Assessment of testis to body rate ratio (mg/mg) of adult control (n=4, mean= 0.39) and *Smc5*cKO (*Spo11-Cre*) (n=4, mean= 0.40) mice. Bars indicate average and standard error. The *P* value (Mann-Whitney, two-tailed) for the indicated comparison is not significant (n.s.). (C) Examples of tubule cross sections of testes from adult control and *Smc5*cKO (*Spo11-Cre*) mice stained with hematoxylin and eosin. *Smc5*cKO (*Spo11-Cre*) tubules are indistinguishable from controls. Scale bar: 500 μm. (D) Testis of adult control (*Smc5 +/flox, Hspa2-cre*$^{tg/0}$) and *Smc5*cKO (*Smc5 flox/del, Hspa2-cre*$^{tg/0}$) mice. (E) Assessment of testis to body rate ratio (mg/mg) of adult control (n= 8, average= 0.35) and *Smc5*cKO (*Hspa2-Cre*) (n=6, average= 0.33) mice. Bars indicate average and standard error. The *P* value (Mann-Whitney, two-tailed) for the indicated comparison is not significant (n.s.). (F) Examples of tubule cross sections of testes from adult control and *Smc5*cKO (*Hspa2-Cre*) mice stained with hematoxylin and eosin. *Smc5*cKO (*Hspa2-Cre*) tubules are indistinguishable from controls. Scale bar: 500 μm.

Supplemental Figure S4: *Smc5 flox/del*, *Stra8-Cre*$^{tg/0}$ mutants do not have aberrant localization of proteins related to sex body formation and transcriptional silencing.

Pachytene stage chromatin spread preparations from control and *Smc5*cKO (*Stra8*-Cre) mice immunolabeled with antibodies against CEN (blue, kinetochore/centromere marker), the SC lateral element protein SYCP3 (red) and proteins required for sex body formation (green in merge and white in lower panels) including (A) ATR, (B) TOPBP1, (C) HORMAD1, (D) SUMO1, (E) RNA polymerase II (RNA POLII). In (E), the sex body is circled to show lack of RNA polymerase II signal. All proteins assessed did not show an abnormal localization pattern in *Smc5*cKO chromatin spreads compared to the control. Scale bar: 10 μm.

Supplemental Figure S5: Whole body γ-irradiation causes DNA damage in control and *Smc5*cKO (*Stra8-Cre*) testes

(A,B) Tubule cross sections in testes from control and *Smc5*cKO (*Stra8-Cre*) adult mice that were not irradiated (A) and from testes 6 days post whole body γ-irradiation (B). Cross sections immunolabeled with antibodies against the SC lateral element protein, SYCP3 (red), γH2AX (green), and counterstaining of chromatin with DAPI (blue, DNA). Magnified images of single tubule in the bottom row. γH2AX, a

marker for DNA damage, signal is not exclusive to the sex body in pachytene stage spermatocytes after whole body γ-irradiation in both the control and *Smc5*cKO (*Stra8-Cre*) testes, indicating γ-irradiation caused exogenous DNA damage to tubules. Scale bar: 50 μm.

Supplemental Figure S6: Conditional mutations of *Smc5* viagerm cell specific-Cre recombinases causes increased sensitivity to γ-irradiation resulting in increased spermatids abnormal morphology in tubules compared to control mice

Bar graph assessing the percentage of spermatids with enlarged and normal morphology in control (n=2,291), *Smc5*cKO (*Stra8-Cre*, n=513), Smc5 cKO (*Spo11-Cre*, n=2,408), and *Smc5* cKO (*Hspa2-Cre*, n=1,252) testes 10 days post-irradiation. While all *Smc5*cKO mutants have increased percentages of enlarged spermatids to the controls, *Smc5*cKO (*Stra8-Cre*) mice have the highest percentage, which is likely due to *Stra8-Cre* recombinase being expressed earlier that *Spo11-Cre* and *Hspa2-Cre*. Bars indicate standard error. The *P* values (Mann Whitney two-tailed t test) for the indicated comparisons are significant, $P<0.0001$ (***) and $P<0.05$ (*).

Supplemental Table S1: Analysis of fertility, litter size and Cre excision efficiency

At least three of each *Smc5*cKO mutant were assessed for fertility and compared to littermate controls. All mice were bred with female C57BL6/J mice. The genotypes of all pus were recorded to determine Cre excision efficiency of the *Smc5flox* allele to produce progeny with the *Smc5 del* allele. The same colored columns represent the results from each *Smc5*cKO and littermate controls.

Supplemental Table S2: Primary Antibodies used in this study

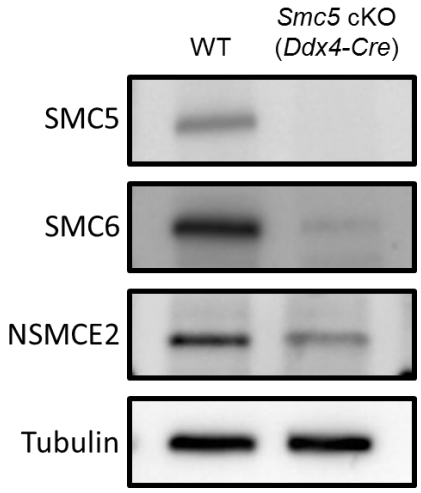
Target protein for each primary antibody, host of antibody production, source of antibody, catalogue number and dilution factors are listed.

A

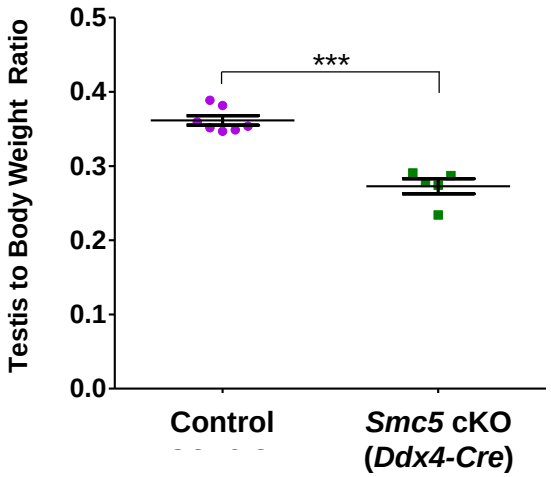
Cross: Smc5+/del, Ddx4-Cre tg/0 x Smc5flox/flox

Genotypes	Smc5+/flox	Smc5del/flox	Smc5+/flox, Ddx4-Cre tg/0	Smc5del/flox, Ddx4-Cre tg/0	**TOTAL**
Expected %	25%	25%	25%	25%	100%
Number of pups	109	112	133	18	372
Actual %	29.30%	30.11%	35.75%	4.84%	100%

B



C



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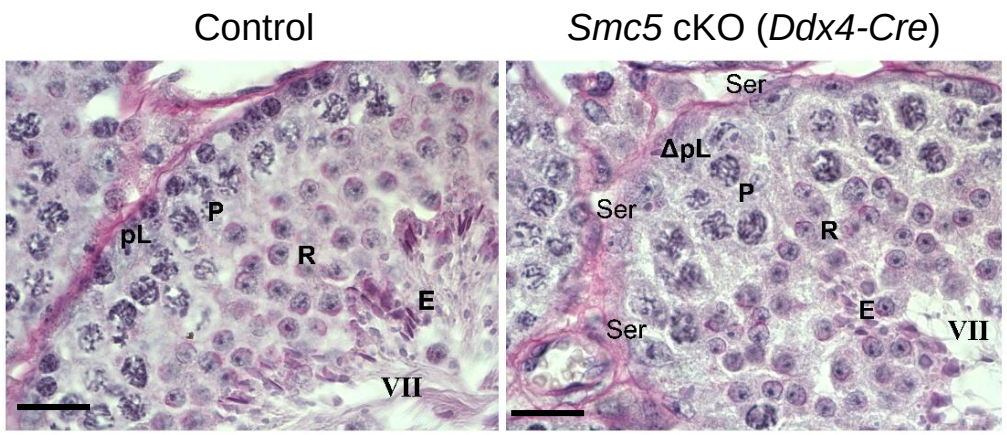
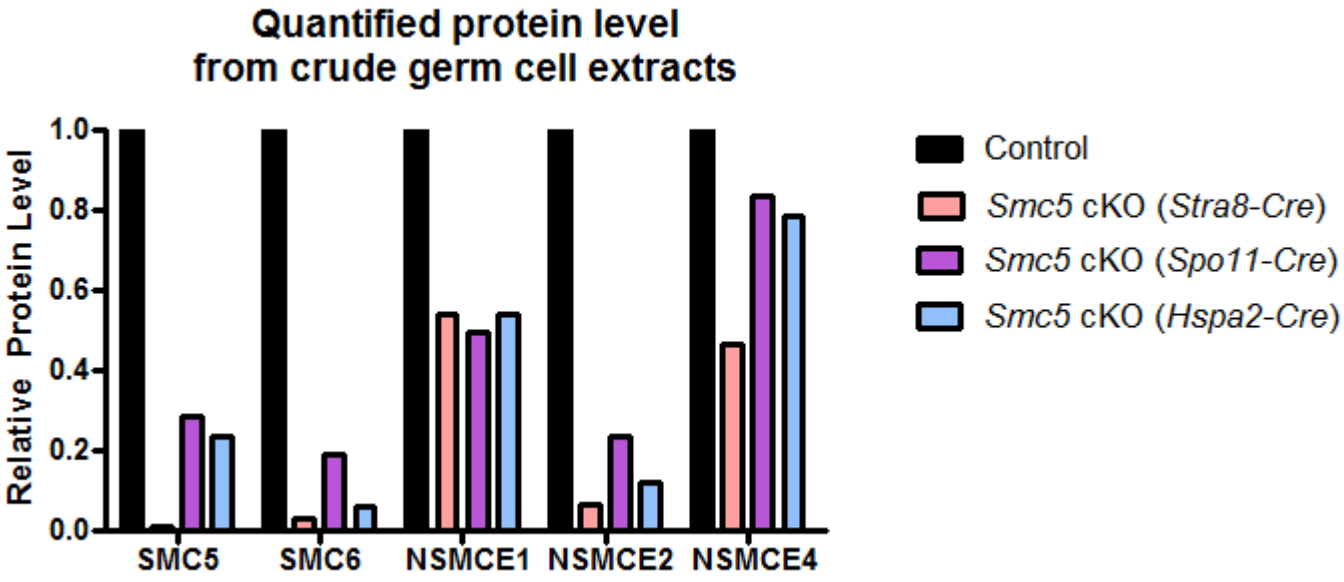
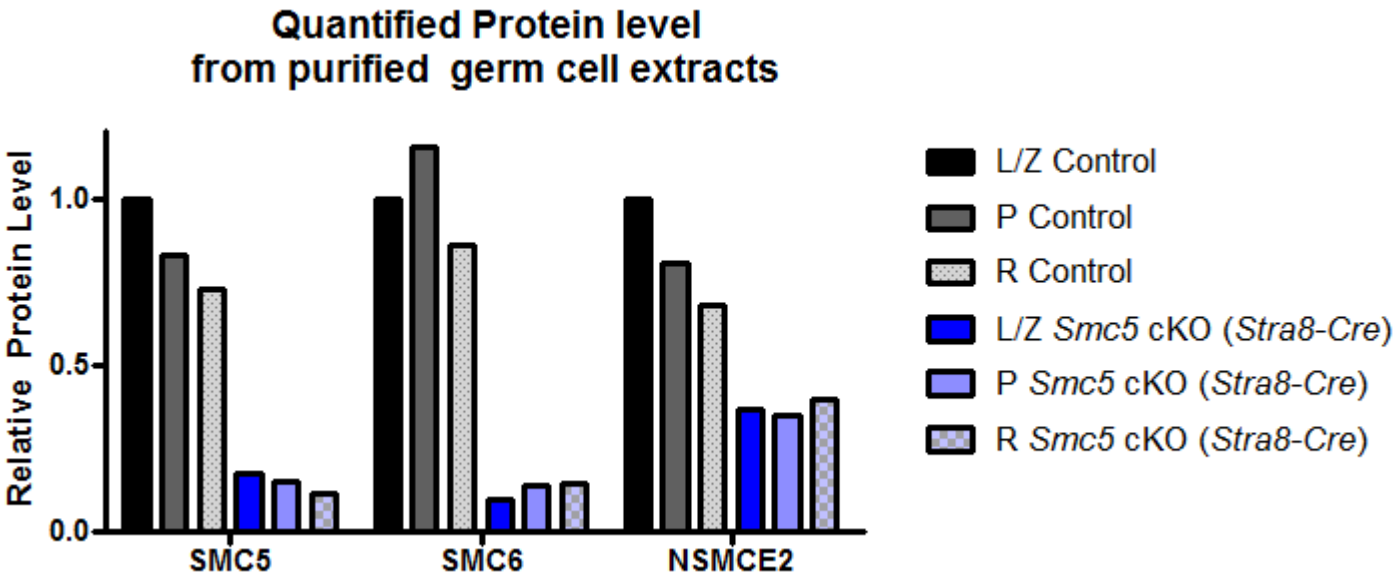
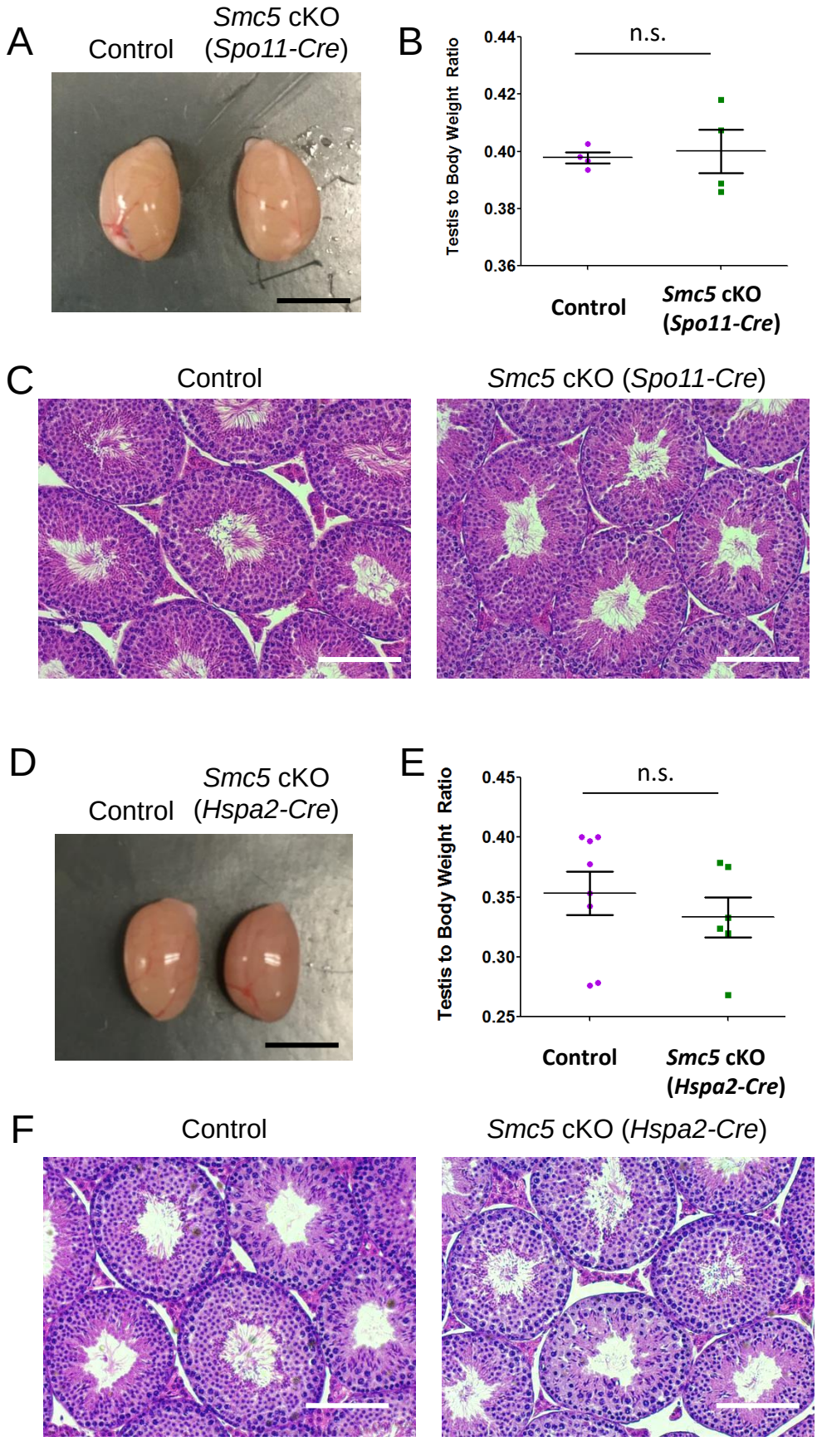


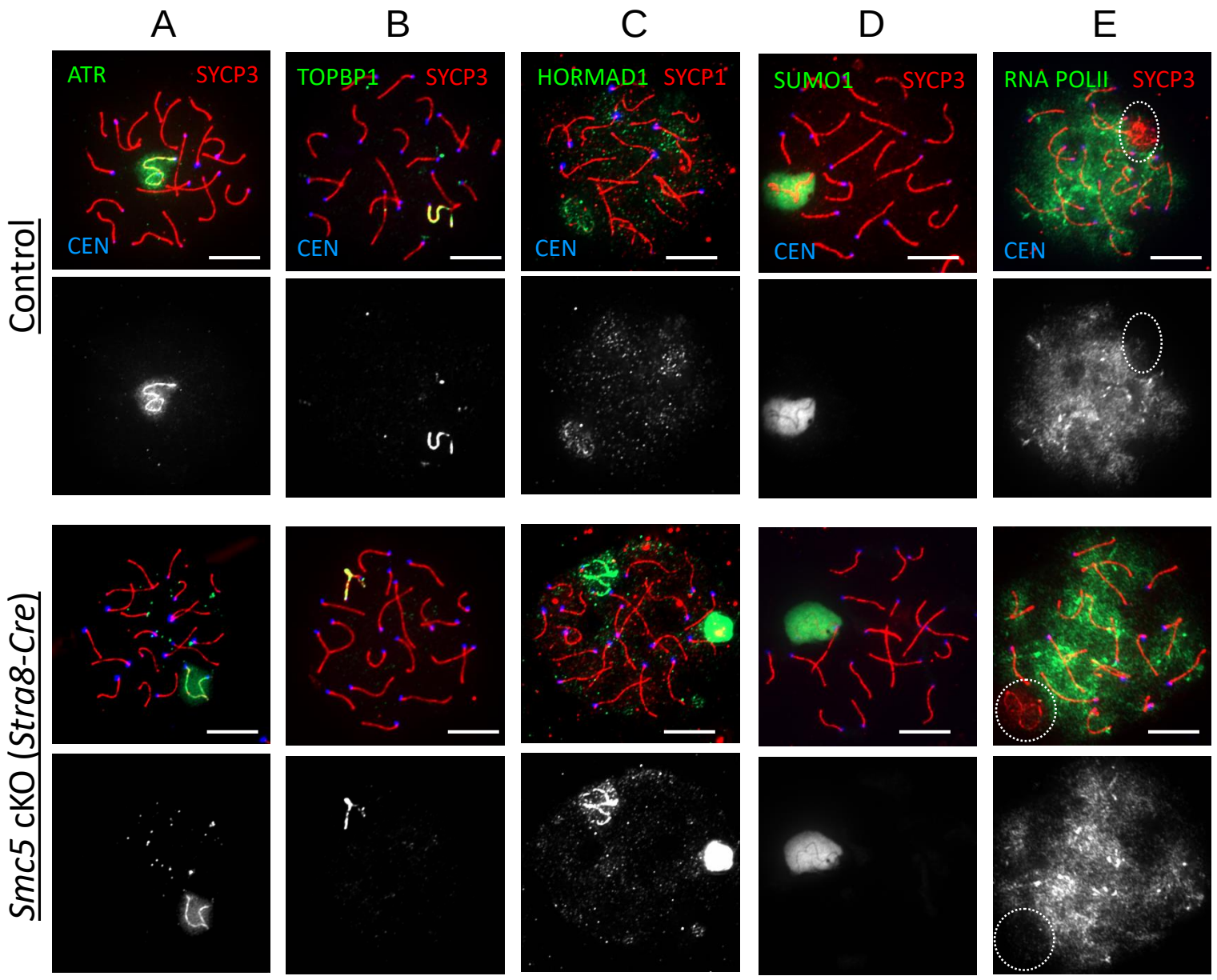
A


Quantified protein level
from crude germ cell extracts
Relative Protein Level
1.0
0.8
0.6
0.4
0.2
0.0
SMC5
SMC6
NSMCE1
NSMCE2
NSMCE4
Control
Smc5 cKO (Stra8-Cre)
Smc5 cKO (Spo11-Cre)
Smc5 cKO (Hspa2-Cre)

B


Quantified Protein level
from purified germ cell extracts
Relative Protein Level
1.0
0.5
0.0
SMC5
SMC6
NSMCE2
L/Z Control
P Control
R Control
L/Z Smc5 cKO (Stra8-Cre)
P Smc5 cKO (Stra8-Cre)
R Smc5 cKO (Stra8-Cre)


A
Control
Smc5 cKO (Spo11-Cre)
B
n.s.
Testis to Body Weight Ratio
0.44
0.42
0.40
0.38
0.36
Control
Smc5 cKO (Spo11-Cre)
C
Control
Smc5 cKO (Spo11-Cre)
D
Control
Smc5 cKO (Hspa2-Cre)
E
n.s.
Testis to Body Weight Ratio
0.45
0.40
0.35
0.30
0.25
Control
Smc5 cKO (Hspa2-Cre)
F
Control
Smc5 cKO (Hspa2-Cre)

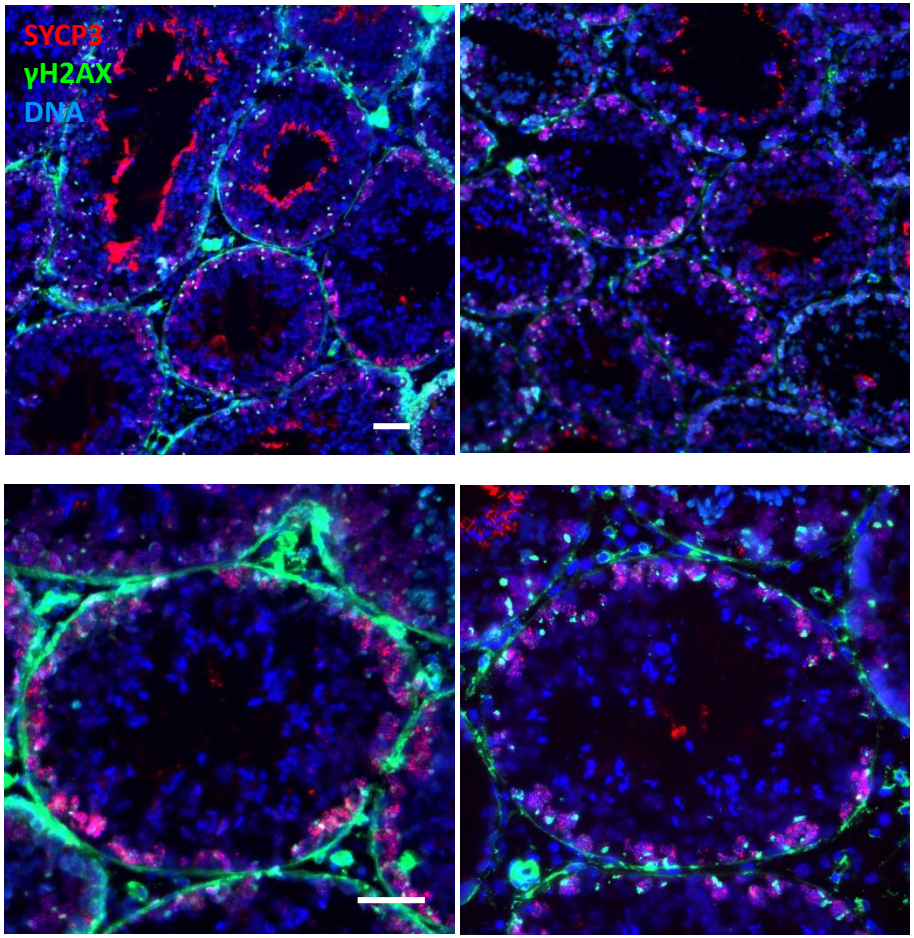

A
B
C
D
E
Control
Smc5 cKO (Stra8-Cre)
ATR
SYCP3
CEN
TOPBP1
SYCP3
CEN
HORMAD1
SYCP1
CEN
SUMO1
SYCP3
CEN
RNA POLII
SYCP3
CEN

A

Non-irradiated

Control

Smc5 cKO

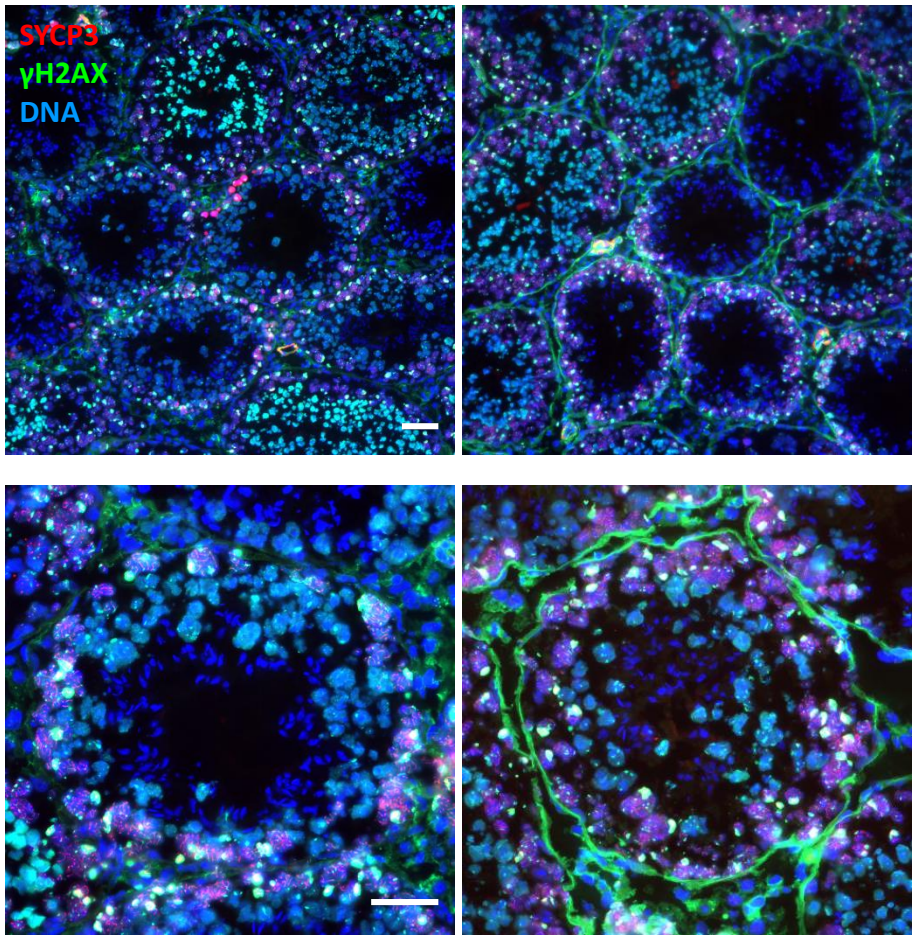


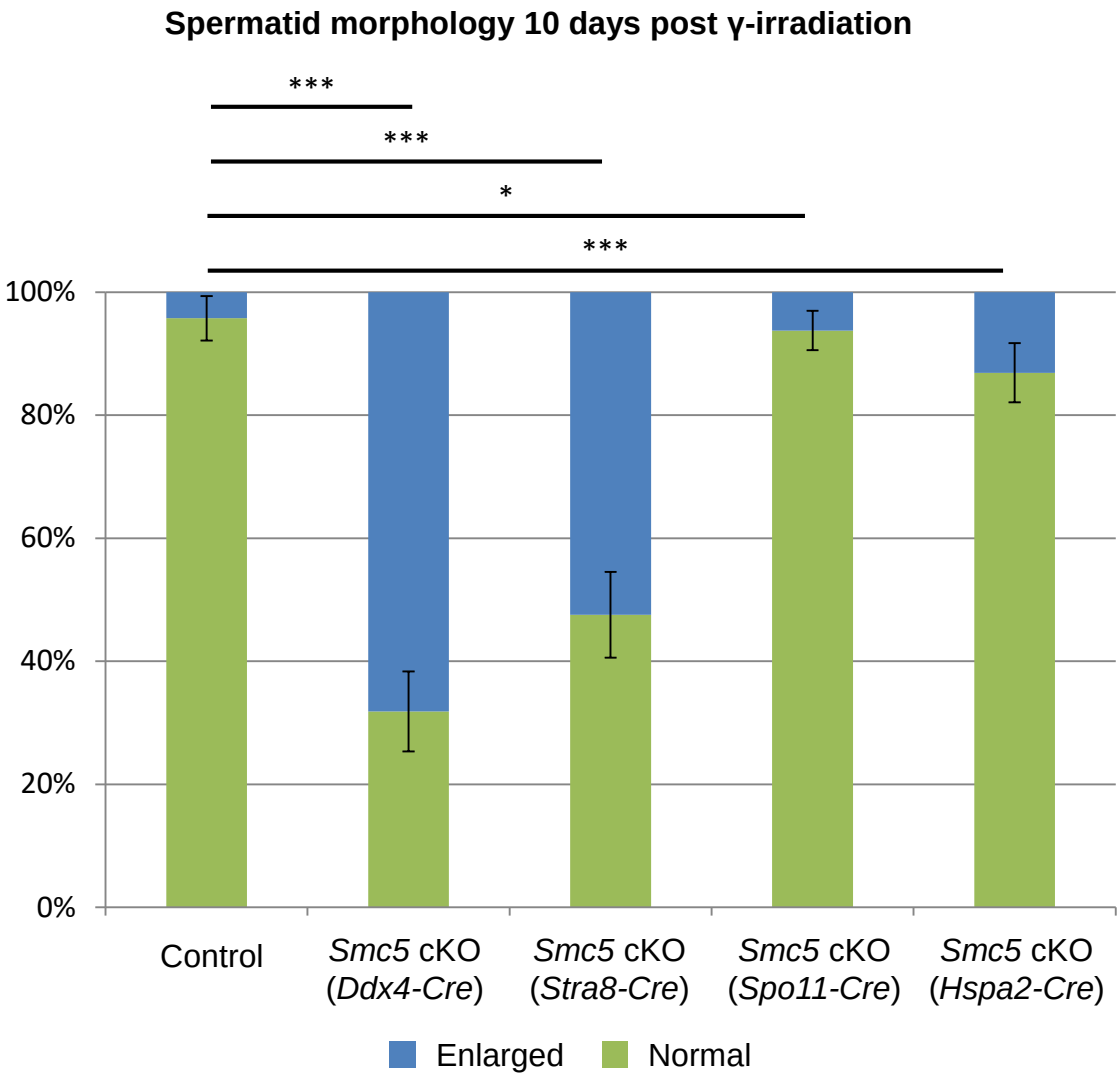
B

Irradiated

Control

Smc5 cKO




Spermatid morphology 10 days post γ-irradiation
***
***
*
***
100%
80%
60%
40%
20%
0%
Control
Smc5 cKO (Ddx4-Cre)
Smc5 cKO (Stra8-Cre)
Smc5 cKO (Spo11-Cre)
Smc5 cKO (Hspa2-Cre)
Enlarged
Normal

Supplemental Table S1: Analysis of fertility and Cre excision efficiency

	Offspring genotype distribution		
Strain	***Smc5+/flox***	***Smc5+/del***	**Total**
♂ ***Smc5flox/del*** **x ♀ wild-type**	48% (29 pups)	52% (32 pups)	61 pups
♂ ***Smc5flox/del, Ddx4-Cre*** **x ♀ wild-type**	3% (1 pup)	95% (38 pups)	39 pups
♂ ***Smc5flox/del*** **x ♀ wild-type**	51% (97 pups)	49% (94 pups)	191 pups
♂ ***Smc5flox/del, Stra8-Cre*** **x ♀ wild-type**	6% (6 pups)	94% (88 pups)	94 pups
♂ ***Smc5flox/del*** **x ♀ wild-type**	50% (26 pups)	50% (26 pups)	52 pups
♂ ***Smc5flox/del, Spo11-Cre*** **x ♀ wild-type**	15% (6 pups)	85% (34 pups)	40 pups
♂ ***Smc5flox/del*** **x ♀ wild-type**	49% (51 pups)	51% (53 pups)	104 pups
♂ ***Smc5flox/del, Hspa2-Cre*** **x ♀ wild-type**	2% (1 pups)	98% (64 pups)	65 pups

Supplemental Table S2: Primary Antibodies used in this study

Primary Antibodies					
Target Protein	**Host**	**Source**	**Cat. Number**	**IHC Dilution**	**WB Dilution**
ATR	Goat	Santa Cruz	sc-18887	1:50	
CREST (CEN)	Human	Antibodies Incorporated	15-235	1:50	
HORMAD1	Rabbit	Abcam	ab155176	1:100	
Lectin PNA-AF488 conjugate	NA	Molecular Probes	L21409	1:700	
LIN28	Rabbit	Abcam	ab46020	1:5000	
MLH1	Mouse	Thermo-Fisher	MA51531	1:100	
NSE1	Mouse	Abcam	ab168578		1:1000
NSE2	Mouse	Novus Biologicals	H00286053-B01		1:500
NSE4a	Rabbit	Sigma	HPA037459		1:100
PCNA	Rabbit	Abcam	ab29	1:30,000	
RAD51	Rabbit	Thermo	PA527195	1:100	
RNA Polymerase II (RNA POLII)	Mouse	Millipore	05-623	1:400	
SMC5	Rabbit	Novus Biologicals	NB100-469		1:400
SMC6	Rabbit	Abcam	ab155495		1:500
SYCP3	Mouse	Santa Cruz	sc-74569	1:50	
SYCP3	Goat	Santa Cruz	sc-20845	1:50	
SYCP3	Goat	Novus Biologicals	af3750	1:100	
TOPBP1	Rabbit	Abcam	ab2402	1:100	
α tubulin (αTUB)	Mouse	Sigma	T9026	1:1000	1:10,000
γH2AX	Mouse	Thermo	MA1-2022	1:500	
γ tubulin (TUB)	Mouse	Sigma	T6557	1:1000	