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MS TITLE: A 1.1 Mb Segmental Deletion on the X Chromosome Causes Meiotic Failure in Male Mice

Supplemental information

Table S1. Genes in the *Nxf2-Nxf3* genomic interval on the X chromosome

Table S2. RT-PCR primer sequences

FIG. S1. Dynamic expression and subcellular localization of PRAMEL3 in male germ cells. **A-C**) Expression of PRAMEL3 in representative seminiferous tubules. Testis sections from 8-week-old mice were immunostained with anti-PRAMEL3 antibody (green). Nuclei were stained with DAPI. The morphology of nuclei and the association of various types of germ cells are used to determine the stages of tubules. An early stage tubule (I-IV) is shown in panel A. Stage IX tubules (**B**) are characterized by the presence of elongating spermatids and leptotene spermatocytes. Stage XII tubules (**C**) contain metaphase spermatocytes. Bottom panels show nuclear staining to visualize the distinct nuclear morphology of germ cells at different stages. **D**) Graphic of PRAMEL3 protein (green) localization during spermatogenesis. Stages (I-XII) of spermatogenesis are indicated below. Abbreviations: SG, spermatogonium; PL, preleptotene spermatocytes; L, leptotene spermatocytes; Z, zygotene spermatocytes; Pa, pachytene spermatocytes; M, metaphase spermatocytes; RS, round spermatids; ES, elongating/elongated spermatids. Scale bar, 50 μm.

FIG. S2. Diagram of the conditional (floxed) and recombined alleles. Rectangles represent *Nxf2* and *Nxf3* exons. Triangles indicate the location and direction of the loxP sites contained within the previously generated *Nxf2*^{fl} and *Nxf3*^{fl} alleles [21, 22]. The loxP sites in *Nxf2*^{fl} and *Nxf3*^{fl} alleles are in the same orientation, permitting Cre recombinase-mediated deletion of the 1.1 Mb floxed segment.

FIG. S3. Analyses of 4-month-old mice. **A**) Body weight was not significantly different between wild type and mutant mice. **B**) Significantly reduced testis weight in *Nxf*$^{fl/Y}$ *Ddx4*-Cre males. **C-F**) Histological analysis of testes from 4-month-old mice. Sections were stained with hematoxylin and eosin. **C**, **E**) wild type. **D**, **F**) *Nxf*$^{fl/Y}$ *Ddx4*-Cre. Pa, pachytene spermatocytes; RS, round spermatids; ES, elongating/elongated spermatids. Scale bar, 50 μm.

FIG. S4. Expression analysis of genes in the 1.1 Mb Nxf region and genes on either side of this region in testes from wild type and *Nxf*$^{fl/Y}$ *Ddx4*-Cre males of various ages. Semi-quantitative RT-PCR was performed on total RNAs from wild type and mutant testes. Three genes on either side of the Nxf region were analyzed: *Armcx6*, *Armcx3*, and *Armcx2* map close to *Nxf2*, whereas *5730412P04Rik*, *Bex4*, and *Tceal8* map close to *Nxf3*. *Gapdh* served as a positive control. Primer sequences are listed in supplemental Table S2. Reactions without reverse transcriptase were negative (data not shown). Notably, *Nxf2* was completely deleted in testes from *Nxf*$^{fl/Y}$ *Ddx4*-Cre males of all ages. Because the *Nxf2* gene harbors two closely located loxP sites (See supplemental Fig. S2), the deletion of *Nxf2* alone is more efficient and thus its deletion is not a good indicator of complete deletion of the 1.1 Mb Nxf segment. In contrast, deletion of genes such as *Pramel3* reflects the deletion of the entire Nxf segment.

Table S1. Genes in the *Nxf2-Nxf3* genomic interval on the mouse X chromosome

Gene symbol	Gene name	Comments
Nxf2	nuclear RNA export factor 2	reduced fertility in *Nxf2*-null males; germ cell-specific
Zmat1	zinc finger, matrin type 1	a C2H2-type zinc finger protein
3632454L22Rik	transcribed sequence	small putative coding region with homology to ZMAT1; a putative lincRNA
Gm6207	predicted gene 6207	pseudogene
Gm15023	predicted gene 15023	nearly identical to *Pramel3*
Tceal6	transcription elongation factor A (SII)-like 6	contains the BEX (Brain-expressed X-linked) domain
Pramel	preferentially expressed antigen in melanoma-like	LRR (leucine-rich repeat)-containing protein
Pramel3	preferentially expressed antigen in melanoma-like 3	germ cell-specific in testis
Gm6215	predicted gene 6215	lincRNA
Gm5128	predicted gene 5128	nearly identical to *Pramel3*
Gm6221	predicted gene 6221	nearly identical to *Gm6215*
Gm7903	predicted gene 7903	nearly identical to *Pramel3*
Gm7905	predicted gene 7905	nearly identical to *Gm6215*
AV320801	expressed sequence AV320801	nearly identical to *Pramel3*
Gm6228	predicted gene 6228	pseudogene
Nxf7	nuclear RNA export factor 7	NXF7 localizes to cytoplasmic granules such as stress granules and P bodies
Prame	preferentially expressed antigen in melanoma	member of *Prame* gene family
Tcp11l3	t-complex 11 (mouse) like 3	RIKEN cDNA 1700008I05 gene with extensive homology to *Tcp11*
Tmsb15a	thymosin beta 15a	member of thymosin beta-4 family
Armcx5	Armcx5 armadillo repeat containing, X-linked 5	the armadillo-like repeats (Arm2) domain mediates protein interactions; 5' exons are shared with *Gprasp1*
Gprasp1	G protein-coupled receptor associated sorting protein 1	decreased behavioral responses to cocaine in *Gprasp1*-null mice; contains an Arm2 domain
mmu-mir-1970	microRNA	mapped within the *Gprasp1* gene
Gprasp2	G protein-coupled receptor associated sorting protein 2	contains an Arm2 domain
Bhlhb9	basic helix-loop-helix domain containing, class B9	contains an Arm2 domain
Gm15016	predicted gene 15016	pseudogene
Arxes2	adipocyte-related X-chromosome expressed sequence 2	signal peptidase subunit
Arxes1	adipocyte-related X-chromosome expressed sequence 1	signal peptidase subunit
Bex2	brain expressed X-linked 2	a putative tumor suppressor in human glioma; contains the BEX domain
Nxf3	nuclear RNA export factor 3	normal fertility in *Nxf3*-null males; Sertoli cell-specific

Notes: 1) The "20 genes" in this region do not include three predicted pseudo genes: *Gm6207*, *GmGm6228*, *Gm15016 (in red)*.

2) *Pramel3* and its four close homologues *(Gm15023*, *Gm5128*, *Gm7903*, and *AV320801 in green)* are counted once.

3) *Gm6215*, *Gm6221*, and *Gm7905 (in blue)* are nearly identical and are counted once.

Table S2. RT-PCR primer sequences

Gene symbol	Primer sequence	PCR product size (bp)
Armcx6	GACTTGAGAAGAAGCAACCCG TGCCATCCAACCCATTTCC	334
Armcx3	GGAAGGAAATTAACCAGATCGG CCCAGAACAGTCCCCAGCA	480
Armcx2	GTATTCCTGCCCTTCAACCC GCACTCGCCCCAATCACTA	397
Nxf2	CTGAACTGTTGTCCTTGAACT AAGGAACTGACAAGGAGAAGC	657
Zmat1	ATGGAGAACAAAGCGAAGTGC TTCAGGAAGGGTGGAGGTGC	274
3632454L22Rik	TGCCAAACCTTCCCTCCCA TGTGAAGGCACCCATAGAGCTG	340
Tceal6	TGGGTCTCCTCAGGTCTCTGC CTCATCTTGCCCCTGCTCAT	292
Pramel	GTCTACACAGATTGCTCAGAGTCCT TTTCAGCTCCATTAGTGGTAGTAAGT	377
Pramel3	TATGATGGACACCAAGGACCC CAGCATCCTGCCTTAAATCTAGG	335
Gm6215	GATCTTTTCCTTGATGGCTCCT GAAAGACCACAGAATGGCAGATAC	434
Nxf7	ATTTTTGTCAATCCCTCGGTT TTCAGCCCTTTCATCTTCTCC	383
Prame	GAGGTCTGCGTCAACTGGG CAAAAGCGAGGATGGGTCTC	498
Tcp11l3	AACTGTCACAAACCCCTCCTG TCTGTGGCAGCTCTAGTCATCC	466
Tmsb15a	ACTGCAAGGGCAGCATCAGT CCAGTGTTGGCATTTATTTGTTG	263
Armcx5	GCTATGTAGTCGCTTCGTGAGG TGTGGAGGGTGGTGGTCTTC	440
Gprasp1	AGATCTTGGAGGACTCCCTGC TGACAATTCAGCACTCAGTGAGG	273
Gprasp2	AGATCTTGGAGGACTCCCTGC TAAATGACCAACTAATCCAGTACCA	281
Bhlhb9	GGTTTTGGGAAGGCGATGA GCAAGCGAAGGGCTTGATG	386
Arxes	GGCTTCATCACCTTCCACATC ATCCCAGCAATCGGTATCACT	278
Bex2	GCGAGCGGGACAGATTGACT TGTCTCACATCATCCCCAAACC	375
Nxf3	CAGCATCCCTTTCACTCCG AGAGTTCACCTGGGTCATATCC	334
5730412P04Rik	CACGGTTAGACCTTATGCTGG GGAGGATTGCCTTATCTTGGA	384
Bex4	AGAAAGAGGAGAAGGCAAGGAT GAAACCGTTAAGTGGAAACACC	498
Tceal8	GCTACTTCCAGCACTCTTGATCT CTCTCCTCTATGGCTCCCTTC	267

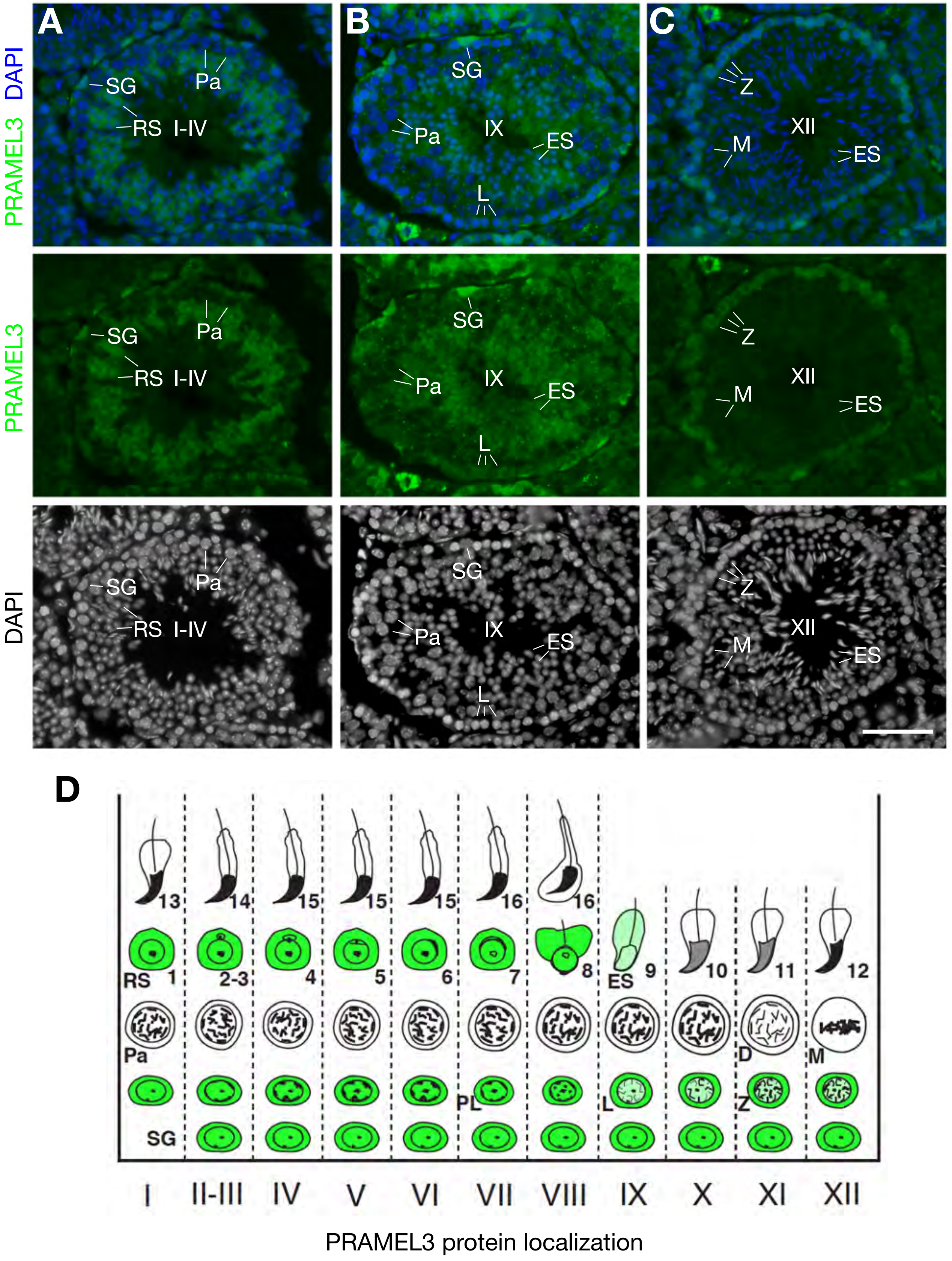
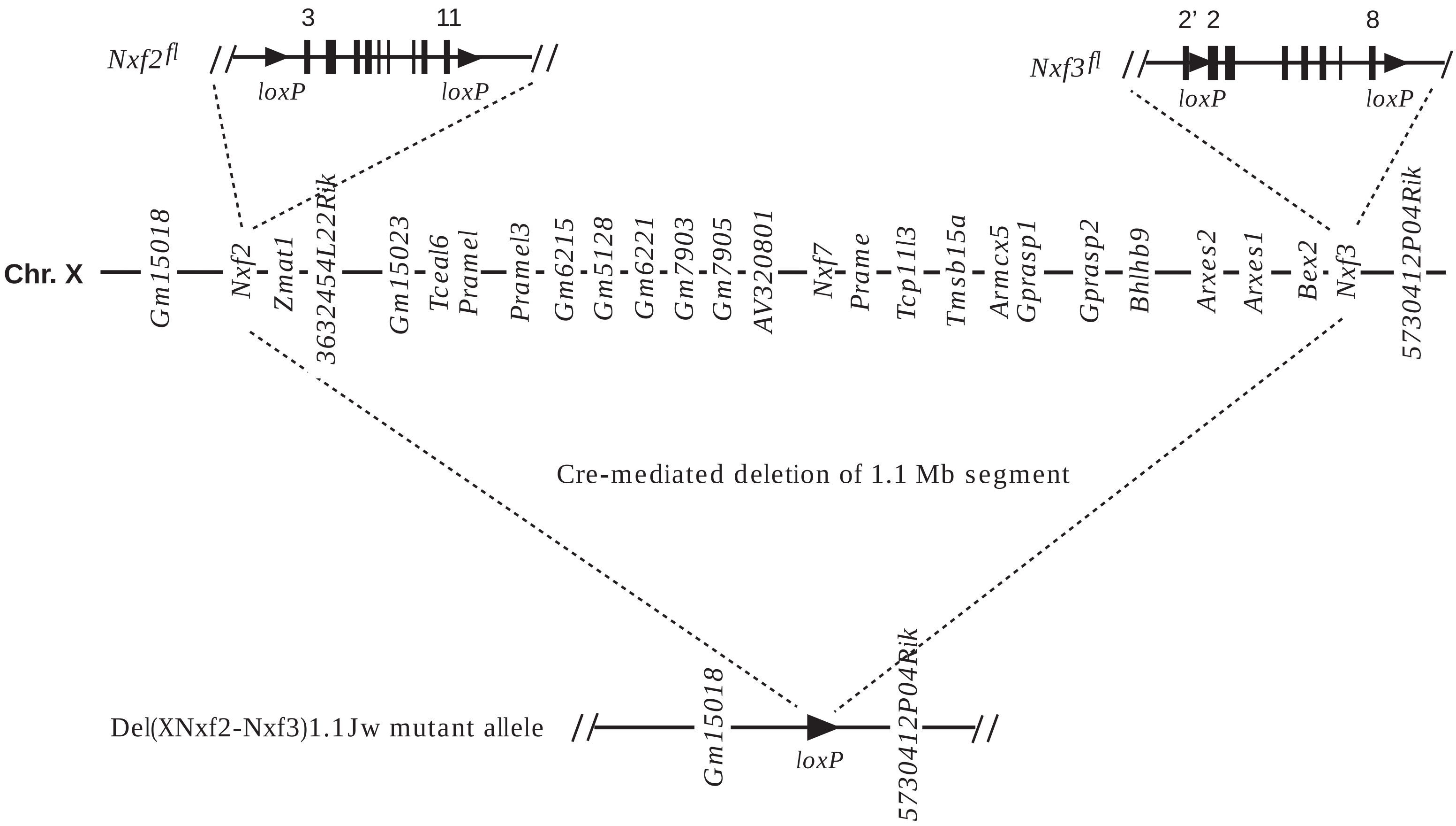

A
B
C
PRAMEL3 DAPI
PRAMEL3
DAPI
SG
Pa
RS
I-IV
IX
ES
L
Z
M
XII
D
13
14
15
15
15
16
16
RS
1
2-3
4
5
6
7
8
ES
9
10
11
12
Pa
D
M
PL
L
Z
SG
I
II-III
IV
V
VI
VII
VIII
IX
X
XI
XII
PRAMEL3 protein localization

Fig. S1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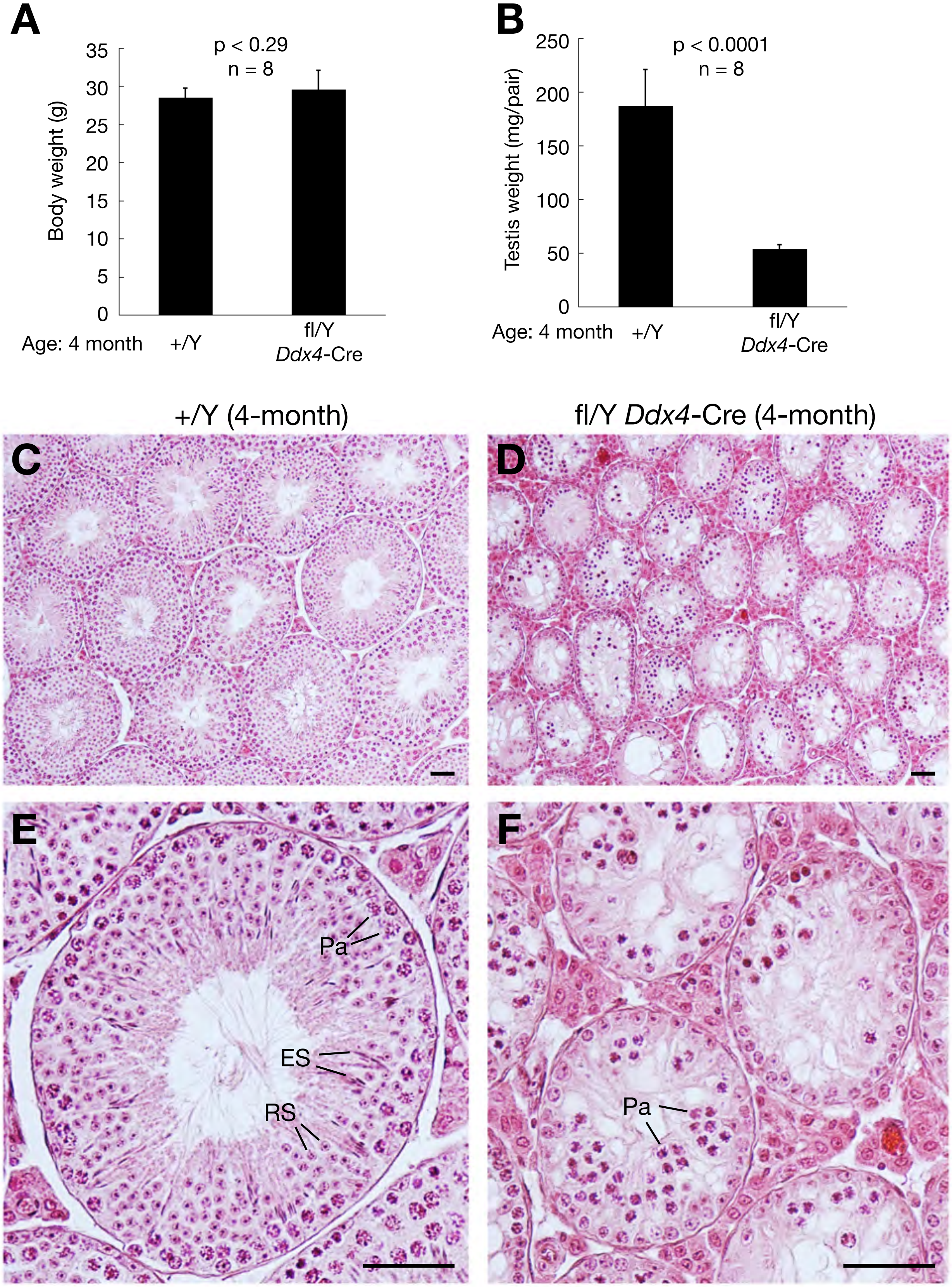
3
11
loxP
loxP
Nxf3fl
2' 2
8
loxP
loxP
Chr. X
Gm15018
Nxf2
Zmat1
3632454L22Rik
Gm15023
Tceal6
Pramel
Pramel3
Gm6215
Gm5128
Gm6221
Gm7903
Gm7905
AV320801
Nxf7
Prame
Tcp11l3
Tmsb15a
Armcx5
Gprasp1
Gprasp2
Bhlhb9
Arxes2
Arxes1
Bex2
Nxf3
5730412P04Rik
Cre-mediated deletion of 1.1 Mb segment
Del(XNxf2-Nxf3)1.1Jw mutant allele
Gm15018
loxP
5730412P04Rik

Fig. S2


A
p < 0.29
n = 8
Body weight (g)
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30
25
20
15
10
5
0
Age: 4 month
+/Y
fl/Y
Ddx4-Cre
B
p < 0.0001
n = 8
Testis weight (mg/pair)
250
200
150
100
50
0
Age: 4 month
+/Y
fl/Y
Ddx4-Cre
+/Y (4-month)
fl/Y Ddx4-Cre (4-month)
C
D
E
Pa
ES
RS
F
Pa

Fig. S3

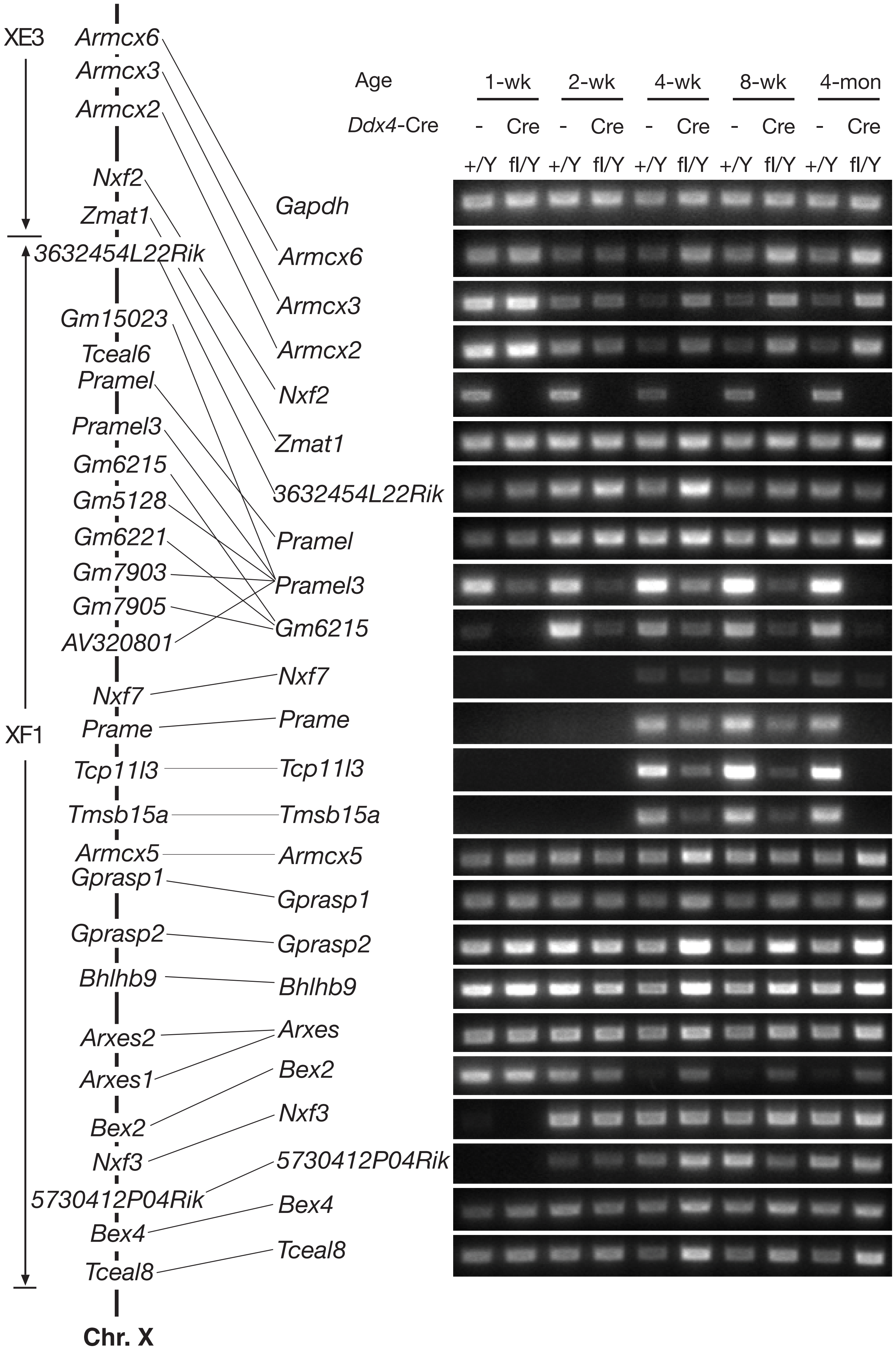
XE3
XF1
Chr. X
Armcx6
Armcx3
Armcx2
Nxf2
Zmat1
3632454L22Rik
Gm15023
Tceal6
Pramel
Pramel3
Gm6215
Gm5128
Gm6221
Gm7903
Gm7905
AV320801
Nxf7
Prame
Tcp11l3
Tmsb15a
Armcx5
Gprasp1
Gprasp2
Bhlhb9
Arxes2
Arxes1
Bex2
Nxf3
5730412P04Rik
Bex4
Tceal8
Age
1-wk
2-wk
4-wk
8-wk
4-mon
Ddx4-Cre
- Cre - Cre - Cre - Cre - Cre
+/Y fl/Y +/Y fl/Y +/Y fl/Y +/Y fl/Y +/Y fl/Y
Gapdh
Armcx6
Armcx3
Armcx2
Nxf2
Zmat1
3632454L22Rik
Pramel
Pramel3
Gm6215
Nxf7
Prame
Tcp11l3
Tmsb15a
Armcx5
Gprasp1
Gprasp2
Bhlhb9
Arxes
Bex2
Nxf3
5730412P04Rik
Bex4
Tceal8

Fig. S4