Supplemental data

Inventory of Supplemental Items

Supplemental Items include 7 Supplemental Figures, Supplemental Methods and 5 Supplemental Tables.

(1) Supplemental Figure 1 is related to Figure 2.

(2) Supplemental Figure 2 is related to Figure 3B.

(3) Supplemental Figure 3 is related to Figure 3D.

(4) Supplemental Figure 4 is related to Figure 3, D and E.

(5) Supplemental Figure 5 is related to Figure 6A.

(6) Supplemental Figure 6 is related to Figure 6C.

(7) Supplemental Figure 7 is related to Figure 7, A-C.

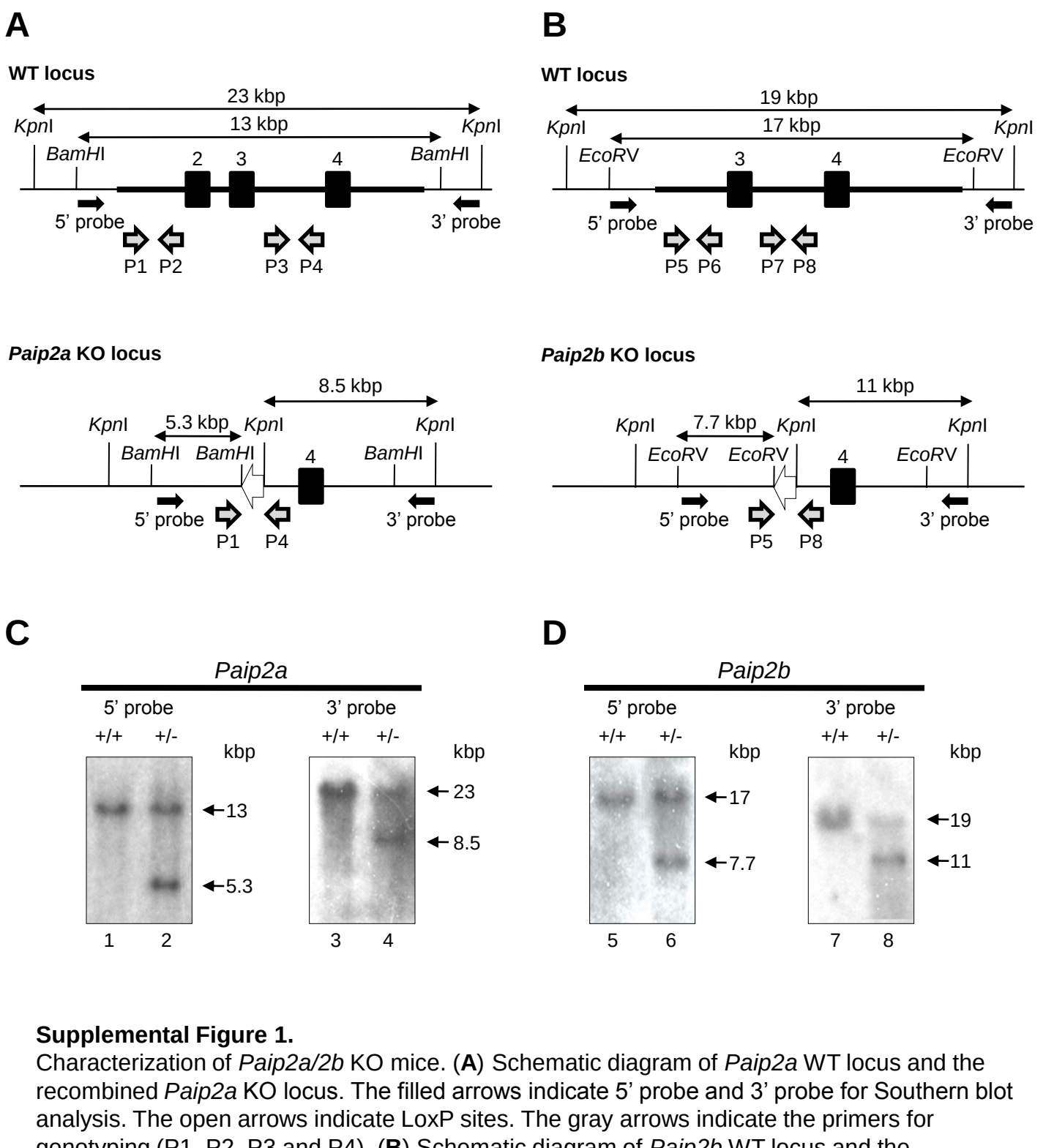
(8) Supplemental Table 1 is related to Figure 2A.

(9) Supplemental Table 2 is related to Figure 2A.

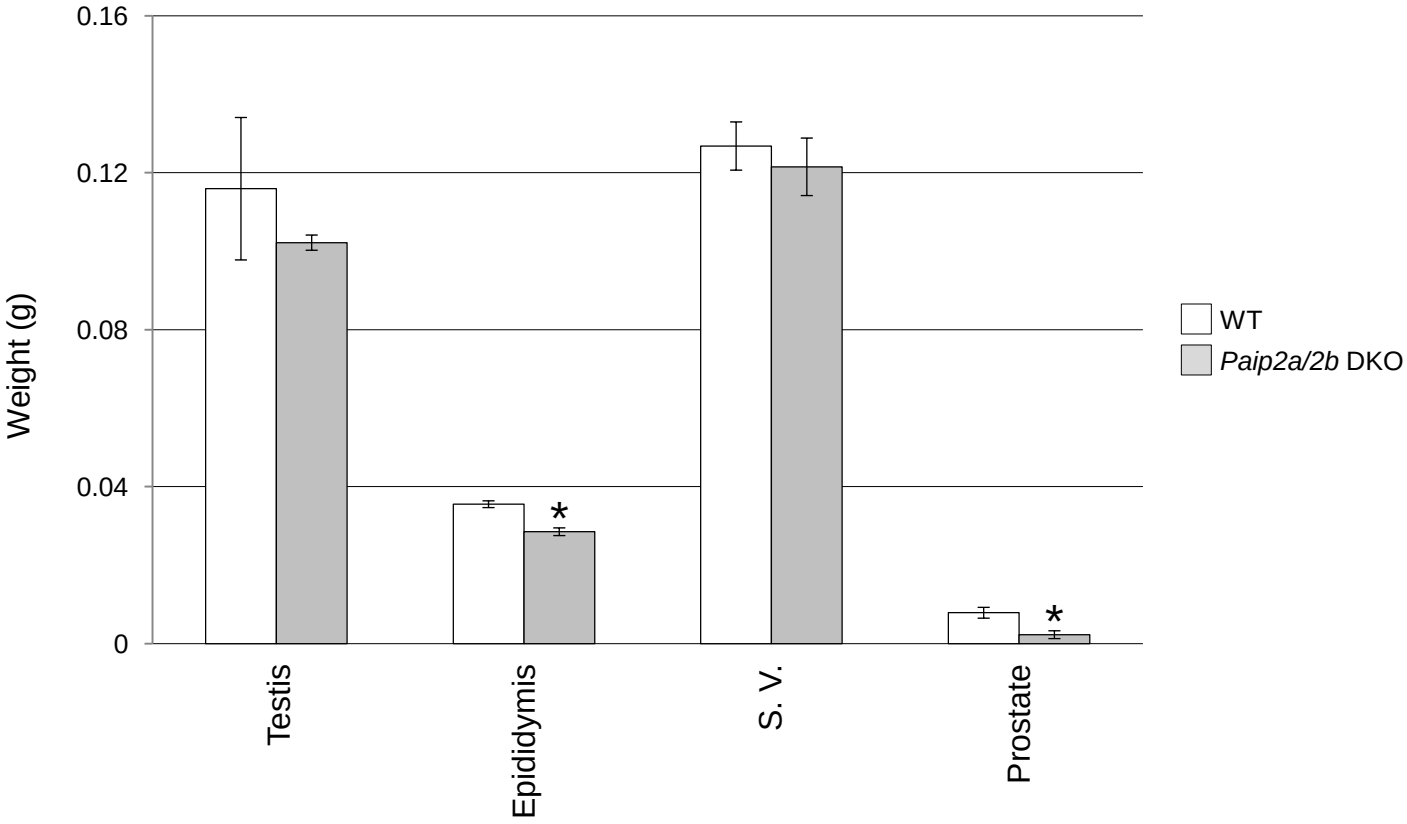
(10) Supplemental Table 3 is related to Figure 2B.

(11) Supplemental Table 4 is related to Figure 2B.

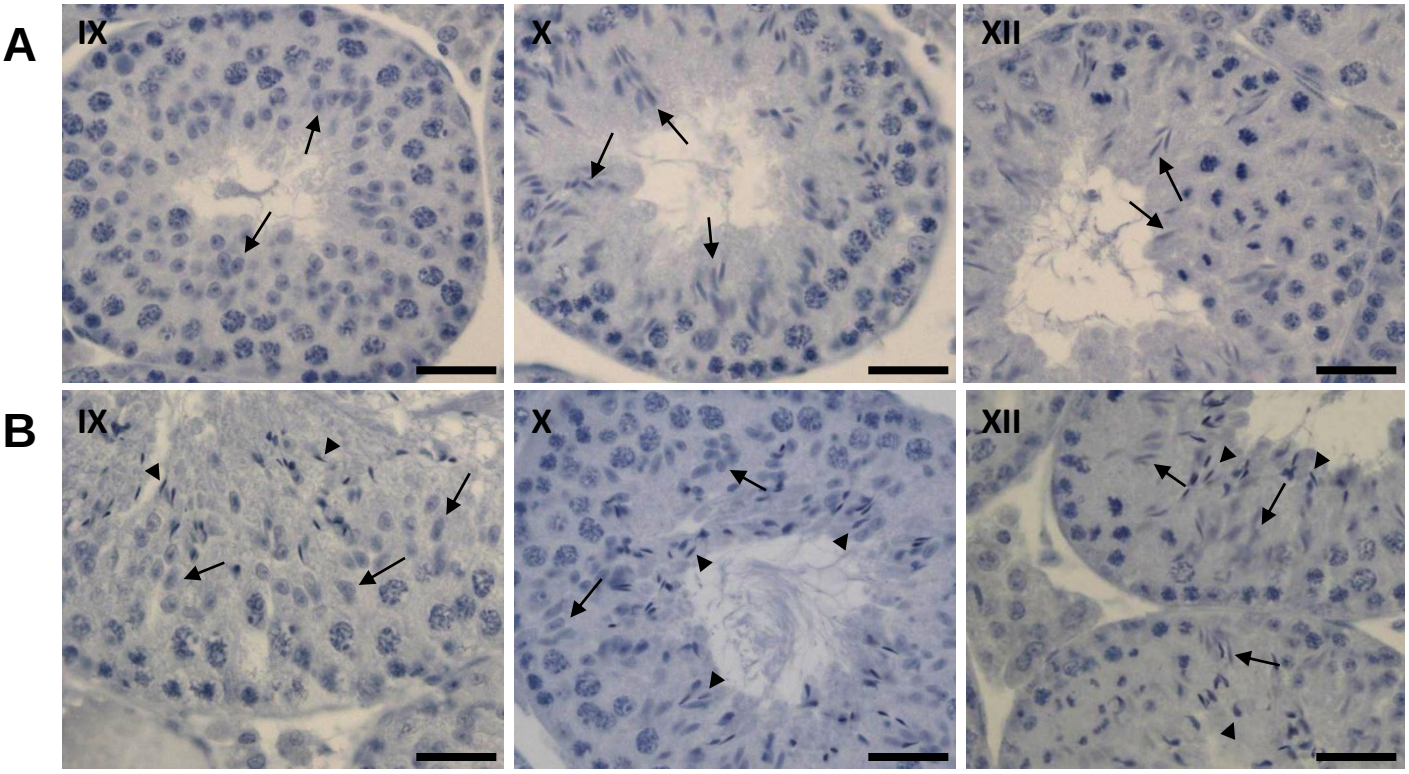
(12) Supplemental Table 5 is related to Supplemental Figure 5.



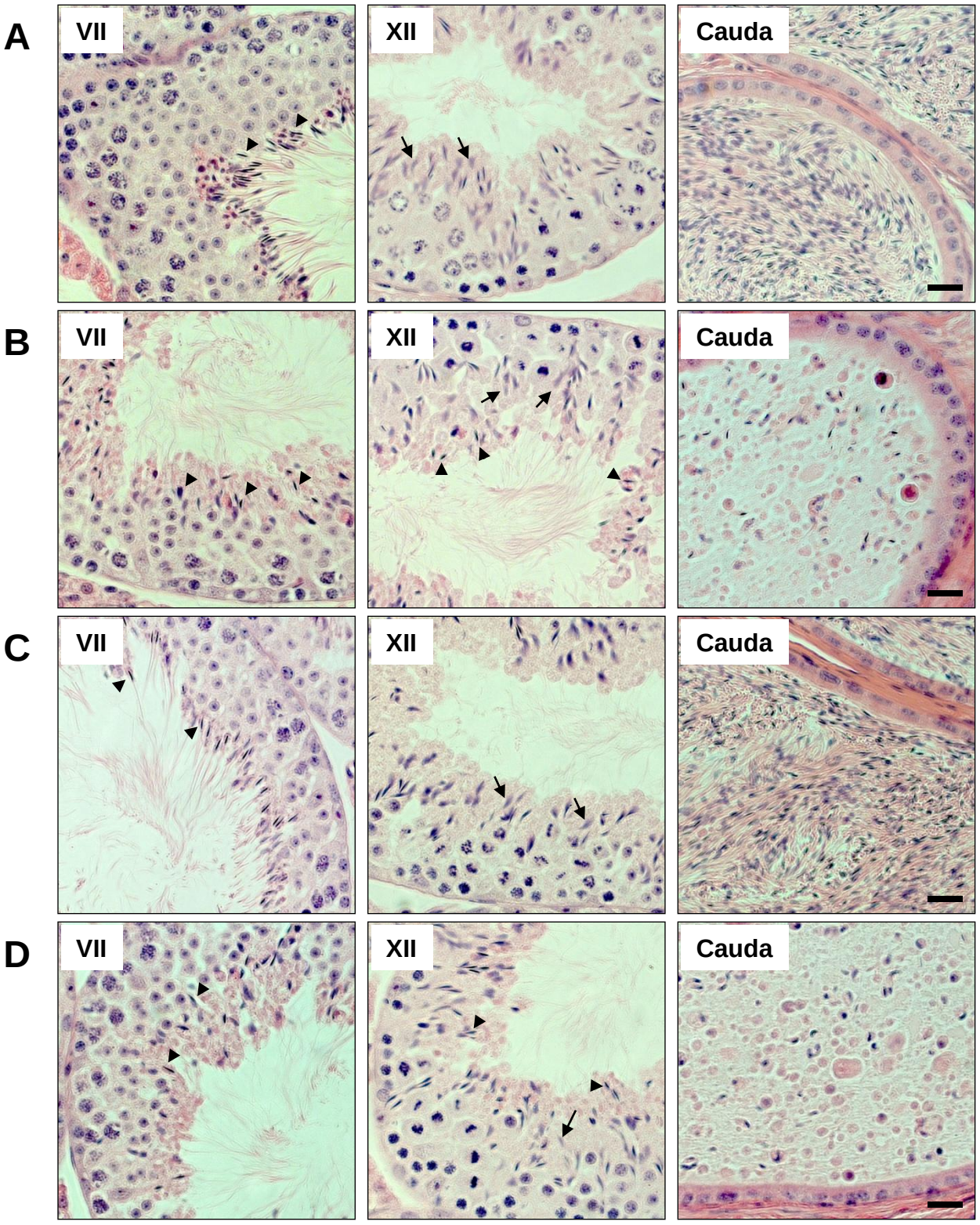
Supplemental Figure 1.
Characterization of *Paip2a/2b* KO mice. (**A**) Schematic diagram of *Paip2a* WT locus and the recombined *Paip2a* KO locus. The filled arrows indicate 5' probe and 3' probe for Southern blot analysis. The open arrows indicate LoxP sites. The gray arrows indicate the primers for genotyping (P1, P2, P3 and P4). (**B**) Schematic diagram of *Paip2b* WT locus and the recombined *Paip2b* KO locus. The filled arrows indicate 5' probe and 3' probe for Southern blot analysis. The open arrows indicate LoxP sites. The gray arrows indicate the primers for genotyping (P5, P6, P7 and P8). (**C**) Southern blot analysis of WT and *Paip2a* heterozygote. Mouse genomic DNA was digested by *BamH*I for 5' probe or digested by *Kpn*I for 3' probe. (**D**) Southern blot analysis of WT and *Paip2b* heterozygote. Mouse genomic DNA was digested with *EcoR*V for 5' probe or digested with *Kpn*I for 3' probe.



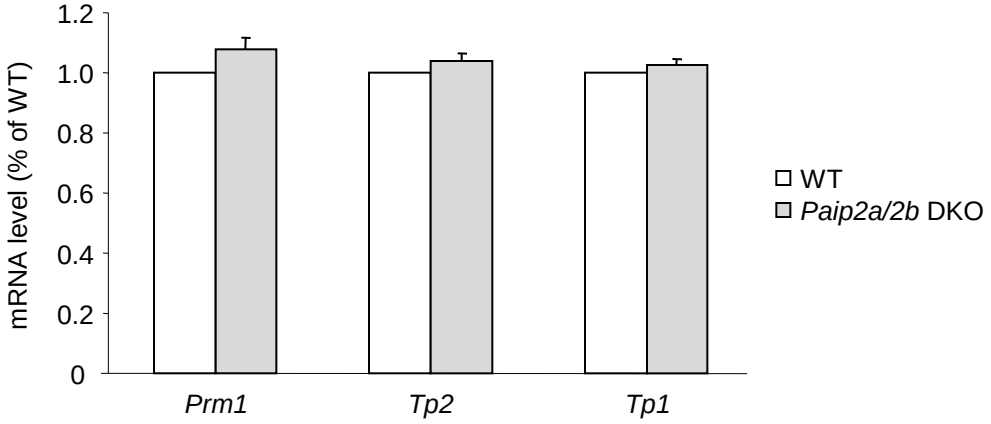
Supplemental Figure 2.
Organ weight of reproductive tissues. S.V., seminal vesicle.



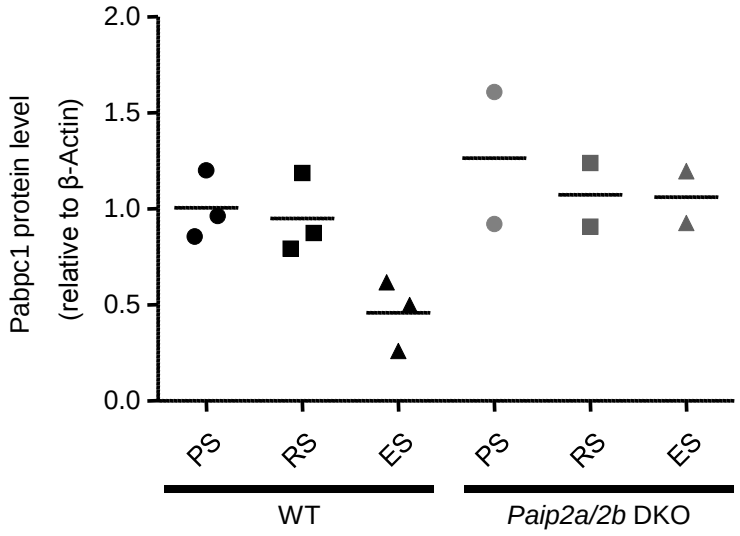
Supplemental Figure 3.
Histology of testes from WT (A) and *Paip2a/2b* DKO (B) mice at stages IX, X and XII using hematoxylin staining. Arrows point at elongating spermatids. Arrowheads in *Paip2a/2b* DKO show aberrant sperm retained in the epithelium of the seminiferous tubule at stage VII and later stages. Scale bar indicates 25 µm.



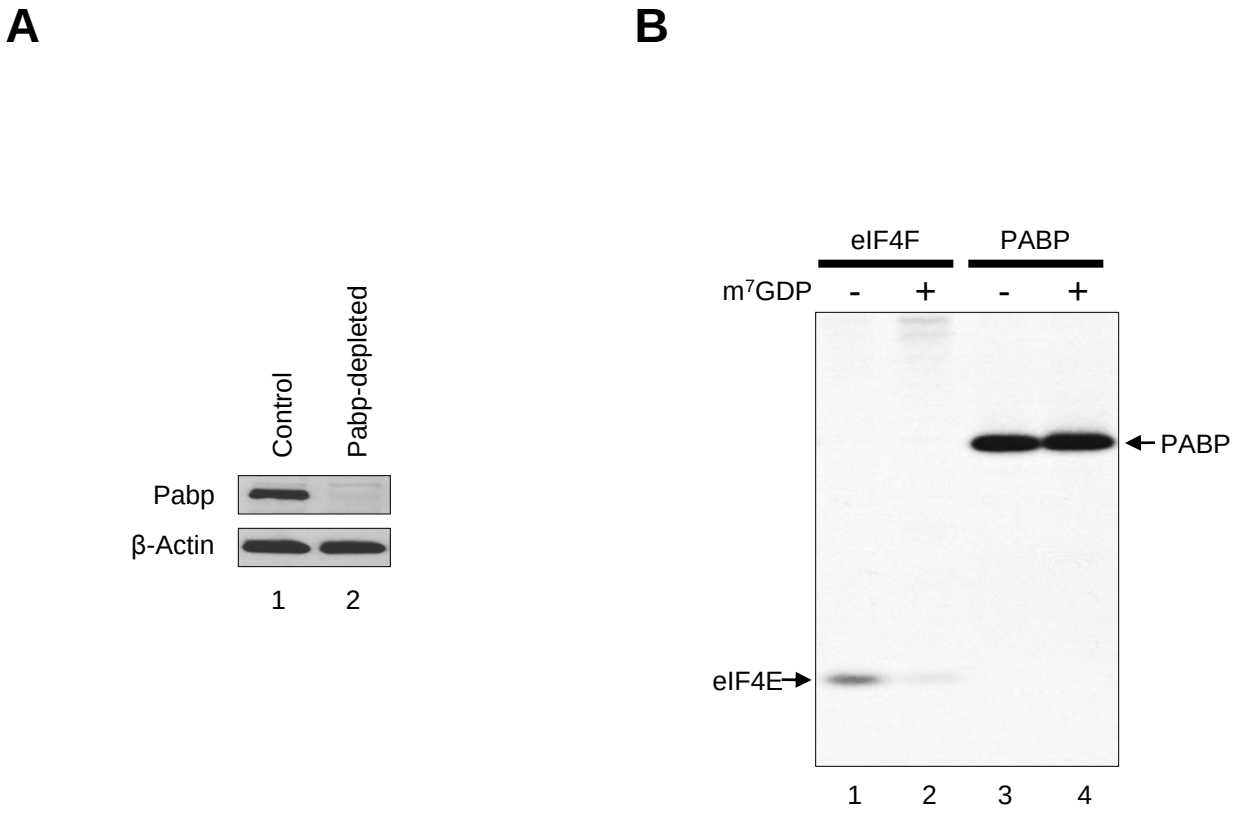
Supplemental Figure 4.
Aberrant spermiogenesis in *Paip2a* KO and *Paip2a/2b* DKO mice, related to Figure 3, D and E. Sections stained with HE staining from WT (**A**), *Paip2a* KO (**B**), *Paip2b* KO (**C**) and *Paip2a/2b* DKO (**D**) mice are shown. Stage VII and XII in testes and cauda epididymides are shown. Arrows indicate elongating spermatids and arrowheads indicate sperm. Scale bar indicates 20 μm.



Supplemental Figure 5.
Relative expression levels of *Prm1*, *Tp1* and *Tp2* mRNAs in WT (white) and *Paip2a/2b* DKO (gray) mice. Testicular RNA was extracted and reverse-transcribed. The resultant cDNA was used for real-time PCR. Values indicate the relative mRNA levels in *Paip2a/2b* DKO mice compared with those in WT mice, which were set as 1. Values are mean±SEM of 4 mice. No statistical difference in *Prm1*, *Tp1* and *Tp2* mRNA levels was observed in *Paip2a/2b* DKO mice compared with WT mice.



Supplemental Figure 6.
Pabpc1 protein level in pachytene spermatocytes (PS) (circles), round spermatids (RS) (squares) and elongating/elongated spermatids (ES) (triangles) in WT (black) and *Paip2a/2b* DKO (gray) mice. PS, RS and ES were isolated, and Pabpc1 protein level was evaluated by Western blotting (Figure 6C). Quantification of the Pabpc1 bands were performed using NIH Image J software. Each value was normalized by that of β-Actin (n=3 in WT and n=2 in *Paip2a/2b* DKO mice). A statistically significant decrease in the amount of Pabpc1 was observed in ES compared with PS in WT mice using an unpaired Student's *t* test (*P*=0.02).



Supplemental Figure 7.
Pabp depletion of Krebs-2 extract and non-specific binding of recombinant PABP to mRNA, related to Figure 7, A-C. (**A**) For the removal of Pabp, Krebs-2 cell extract was incubated with GST-PAIP2A protein immobilized onto glutathione-Sepharose beads. For control Krebs-2 extract, GST alone was used. Pabp depletion was confirmed by Western blot analysis. β-Actin was used as a loading control. (**B**) eIF4F purified from RRL (35 µg/ml) and recombinant PABP (10 µg/ml) were incubated with the ^{32}P-cap-labeled Luc(A_{98}) mRNA for chemical crosslinking assay. m^7GDP (1 mM) was added as indicated. eIF4E exhibits specific cross-linking to the cap structure (lanes 1 and 2), while PABP does not (lanes 3 and 4).

Supplemental Tables

Supplemental Table 1.

PCR primers for *Paip2a*.

Primer	Sequence
Paip2a 5' Fwd	5'-GAGGCATGCAGCAAGTGTAGTTTG-3'
Paip2a 5' Rev	5'-ACTAGAGCCTCTCCTTTCTGACCT-3'
Paip2a 3' Fwd	5'-GTATTCTCAGCCTGGTGGTGCTAA-3'
Paip2a 3' Rev	5'-CAGGCATTCCTGAAGTGGATCAGA-3'
Paip2a P1	5'-GGAAAACAACCCACTCAGGAAAATC-3'
Paip2a P2	5'-GTTCCAGGACAGCTGAGGCTATACA-3'
Paip2a P3	5'-GGCTGCCCTAGAATTTGTGGTAATC-3'
Paip2a P4	5'-TAATCAAGGCATTGAGTTGCAGGTT-3'

Supplemental Table 2.

LoxP sites for *Paip2a*.

LoxP site	Sequence
Paip2a first LoxP	5'-GAATTCAGAGATATGCCTGCTTCTGCCTCCTAAGTGCTGGGATTAAA AGCCTACACCACTACCACCAGACTTTCAAACATAAAATTCATGAGTTTG TTTT-3'
Paip2a second LoxP	5'-TTGGATCCCCTCGAGGGACCTAATAACTTCGTATAGCATACATTAT ACGAAGTTATATTAAGGGTTATTGAATATGATCGGAATTGGGCTGCAGG AATTCGGTACCA-3'

Supplemental Table 3.

PCR primers for *Paip2b*.

Primer	Sequence
*Paip2b 5'*Fwd	5'-CTGTTGTGGCATGGCTGACATCAT-3'
*Paip2b 5'*Rev	5'-TCTGCTAGAGATTCCAGCTCCTTG-3'
*Paip2b 3'*Fwd	5'-AGAGAGATGAAGGTGCAGGCAAAC-3'
*Paip2b 3'*Rev	5'-AGGGTCCCTACTTCACATCAGCAT-3'
Paip2b P5	5'-GCATGGCATACACTCATACAGATGC-3'
Paip2b P6	5'-AAGAATTGAGAGGAATGGGACTTGG-3'
Paip2b P7	5'-CATCTGTGGGTCTCTGTCTTCCCTA-3'
Paip2b P8	5'-GTGGATGTCAGATGACAACTTGTGG-3'

Supplemental Table 4.

LoxP sites for *Paip2b*.

LoxP site	Sequence
Paip2b first LoxP	5'-GGATATCGGATCCCCTCGAGGGACCTAATAACTTCGTATAGCATACA TTATACGAAGTTATATTAAGGGTTATTGAATATGATCGGAATTGGGCTG CAGGAATTCGAGCTCCTAG -3'
Paip2b second LoxP	5'-TGGATCCCCTCGAGGGACCTAATAACTTCGTATAGCATACATTATAC GAAGTTATATTAAGGGTTATTGAATATGATCGGAATTGGGCTGCAGGAA TTCTCTAGAGGTACCA-3'

Supplemental Methods

Real-time PCR. Testicular RNA was extracted using TRIZOL Reagent (Invitrogen) and reverse-transcribed by SuperScript III RT (Invitrogen), following the manufacturer's instruction. The resultant cDNA was used for real-time PCR using the gene specific primers for mouse *Prm1*, *Tp1*, *Tp2* (Supplemental Table 5). The SYBR Green PCR Master Mix (Applied Biosystems) was used for real-time PCR using the first-strand cDNA as a template, and real-time PCR was performed in triplicate. The *Gapdh* mRNA level in each sample was determined to normalize the differences of total RNA amount. Values indicate the relative mRNA levels in *Paip2a/2b* DKO mice compared with those in WT mice, which were set as 1.

Supplemental Table 5.

PCR primers for real-time PCR.

Primer	Sequence
Prm1 Fwd	5'- TCACAGGTTGGCTGGCTCGAC-3'
Prm1 Rev	5'- GCATCGCCTCCTCCGTCTGC-3'
Tp1 Fwd	5'- CCGAGCTCCTCACAAGGGCG -3'
Tp1 Rev	5'- CCTCATGCTCCTGCCCCGTG -3'
Tp2 Fwd	5'- GTGCACCTGCAGCCACCACT-3'
Tp2 Rev	5'- TTTCCGCCTCCTGACGGCCT-3'