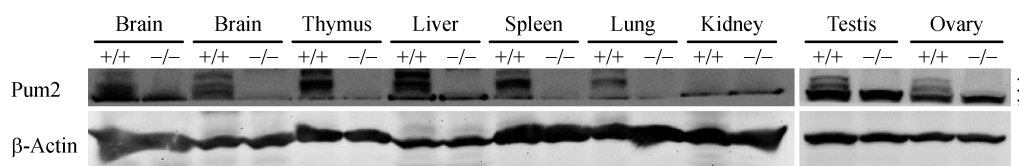


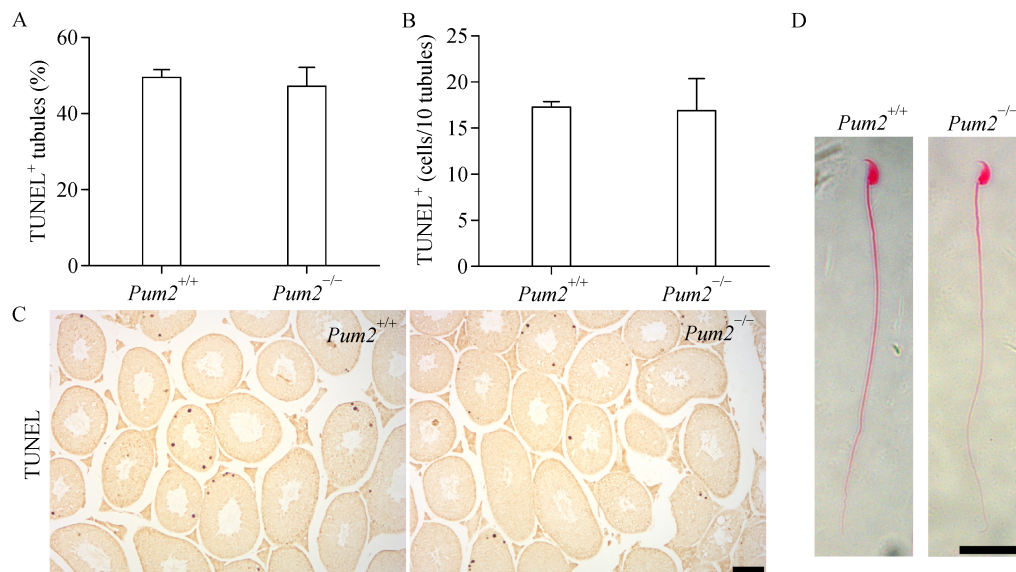
Generation and functional characterization of a conditional *Pumilio2* null allele

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Supplementary Fig. 1 PUM2 is absent in all *Pum2*^{-/-} tissues examined. Western blot analysis of PUM2 in various tissues of wildtype and *Pum2*^{-/-} mutants, showing PUM2 was indeed absent in various tissues. PUM2 bands are indicated by arrowhead in Fig. S1 and in all the tissues PUM2 was detected except the kidney where it was barely detectable. Asterisk indicates non-specific band on Pum2 Western blot.



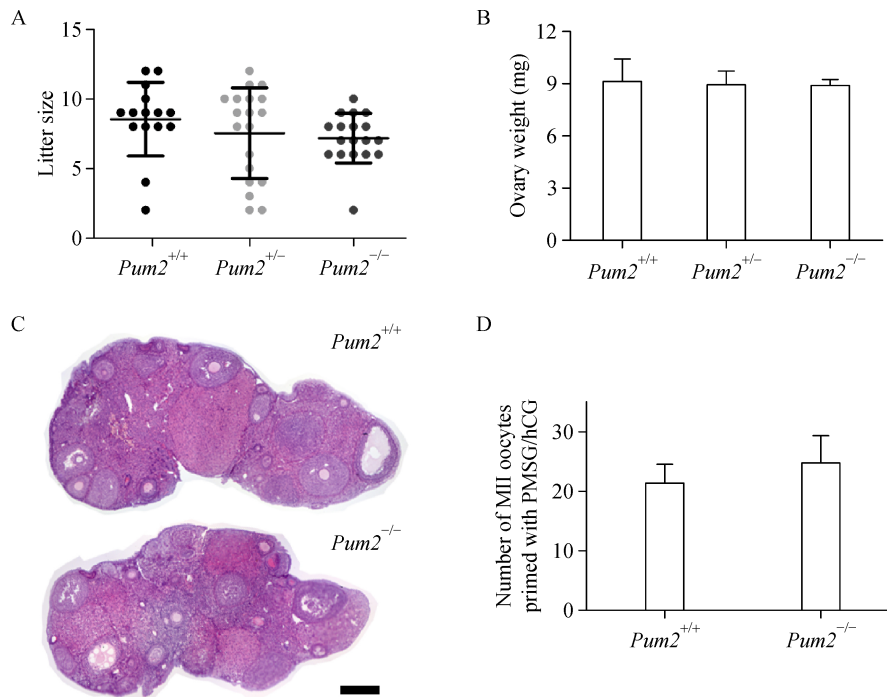
Supplementary Fig. 2 Apoptosis analysis in *Pum2* knockout testis. A, B: The percentage of TUNEL positive tubules(A) and number of TUNEL positive cells per 10 seminiferous tubules (B) in *Pum2*^{+/+} and *Pum2*^{-/-} testis (Data are presented as mean±SEM. *Pum2*^{+/+}, n = 3; *Pum2*^{-/-}, n = 3). C: TUNEL staining of *Pum2*^{+/+} and *Pum2*^{-/-} testis. Scale bar = 100 μm. D: Sperm morphology analysis by eosin staining showed that mutant sperm appear normal. Scale bar = 20 μm.

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Supplementary Fig. 3 Fertility of $Pum2$ null females is not different from that of wild type mice. A: Results of fertility assay for $Pum2^{+/+}$, $Pum2^{+/-}$, and $Pum2^{-/-}$ females (Data are presented as mean±SD. $Pum2^{+/+}$, $n=3$; $Pum2^{+/-}$, $n=4$; $Pum2^{-/-}$, $n=6$). B: Ovary weight measurement in $Pum2^{+/+}$, $Pum2^{+/-}$, and $Pum2^{-/-}$ (Data are presented as mean±SEM. $Pum2^{+/+}$, $n=4$; $Pum2^{+/-}$, $n=7$; $Pum2^{-/-}$, $n=8$). C: H&E staining of adult $Pum2^{+/+}$ and $Pum2^{-/-}$ ovary. Scale bar = 200 μ m. (D) Number of MII oocytes retrieved from the oviducts of adult $Pum2^{+/+}$ and $Pum2^{-/-}$ female mice after superovulation (Data are presented as mean±SEM. $Pum2^{+/+}$, $n=5$; $Pum2^{-/-}$, $n=4$).

Apoptosis assays

TdT-mediated dUTP nicked-end labeling (TUNEL) staining was performed by using the In Situ Cell Death Detection Kit (Roche, Cat#11684817910) according to the manufacturer's instructions. Three discontinuous cross sections of testis were counted and at least 3 animals per genotype were assessed.

Oocyte collection

Both $Pum2^{+/+}$ and $Pum2^{-/-}$ females at the age of 3-month were first superovulated with 10IU PMSG and followed 48 hours later with 10IU hCG via intraperitoneal injection. At 14-16 h post hCG treatment, MII stage oocytes with cumulus cells from oviduct were collected and counted.