Supplemental Data

Regulators of the protein phosphatase PP1γ2, PPP1R2, PPP1R7, and PPP1R11, are involved in epididymal sperm maturation.

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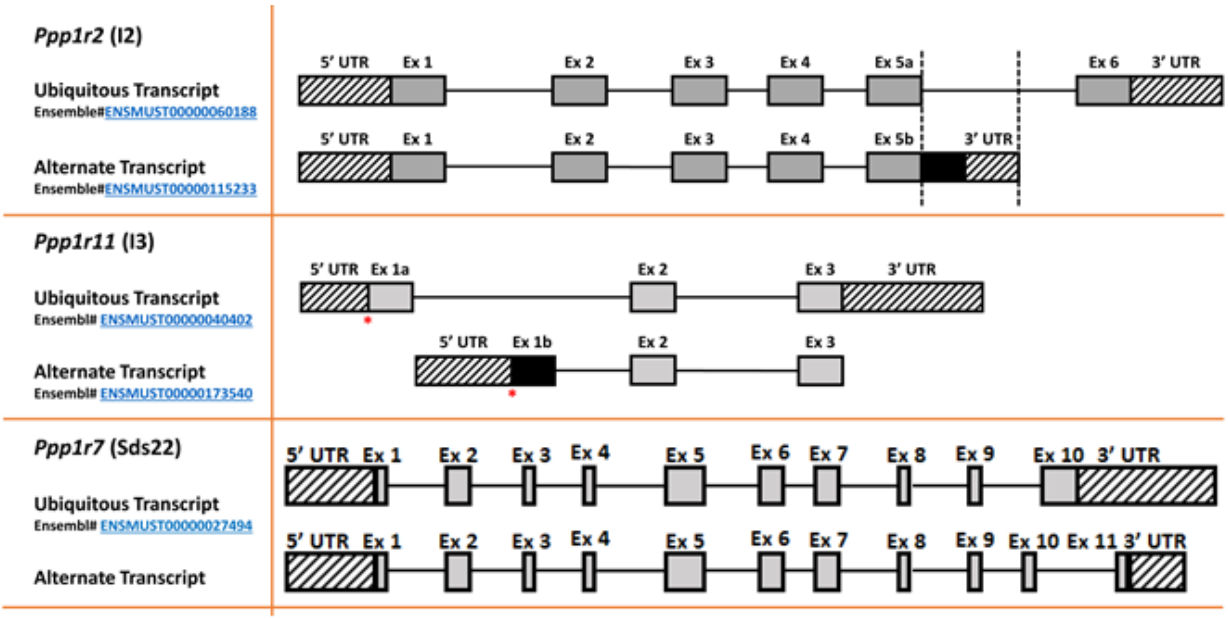
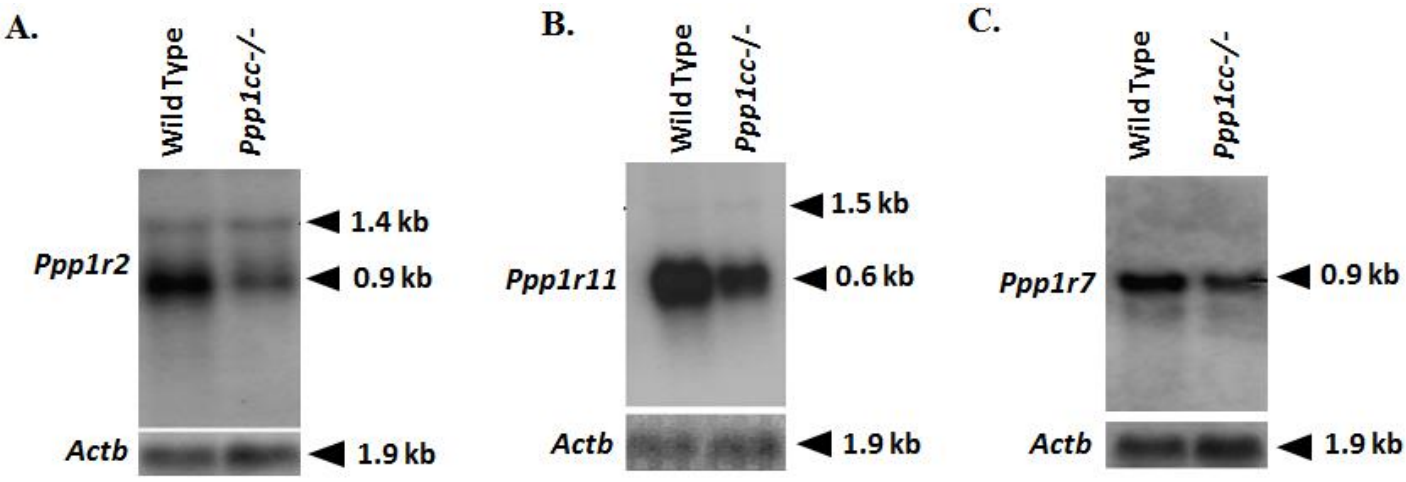
Supplemental Figure 1.



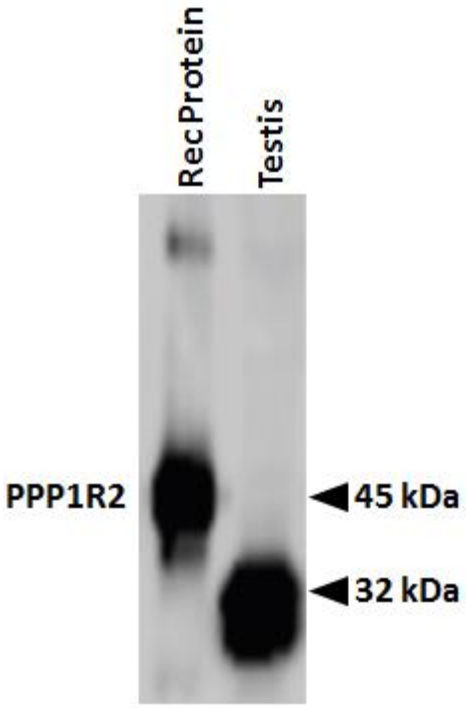
Supplemental Figure S1. Schematic diagrams of the PP1γ2 testis-enriched binding partners' pre-mRNAs. The schematic diagrams show the arrangements of the exons and introns in the pre-mRNAs of PP1γ2 binding partners in mice. PPP1R2 (I2): the predicted testis specific *Ppp1r2* transcript differs from the ubiquitous transcript by the retention of part of intron 5 region after splicing of exon 6. PPP1R11 (I3): on the contrary, the testis specific *Ppp1r11* transcript has a different N-terminus where the Exon 1b presence resulted due to an alternate start site (the asterisk mark show the difference in start site). PPP1R7 (sds22): the *Ppp1r7* testis specific isoform has an extra exon in the C-terminus (the exons and introns are not drawn to scale).

Supplemental Figure 2.



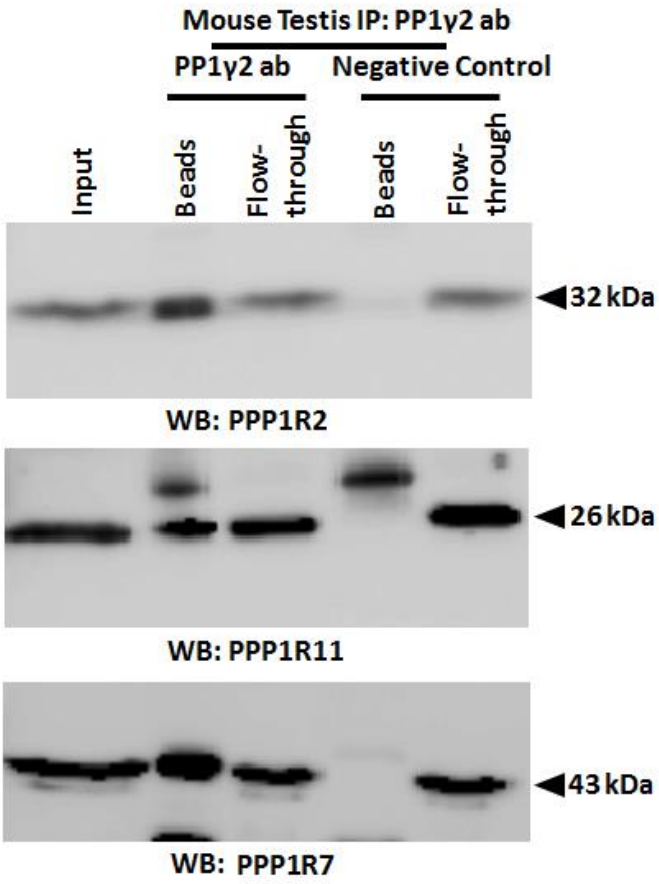
Supplemental Figure S2. mRNA abundance of PP1γ2 testis-enriched binding partners in *Ppp1cc* knockout mice is reduced. (**A, B, C and D**) Northern blot analyses of total RNA (25 μg) from mouse testis of wild type compared with *Ppp1cc* homozygous knockouts demonstrating the reduced expression of testis-specific isoform messages of *Ppp1r2* (**A**), *Ppp1r11* (**B**) and *Ppp1r7* (**C**). The lower panel represents the corresponding actin (loading control) blots

Supplemental Figure 3.



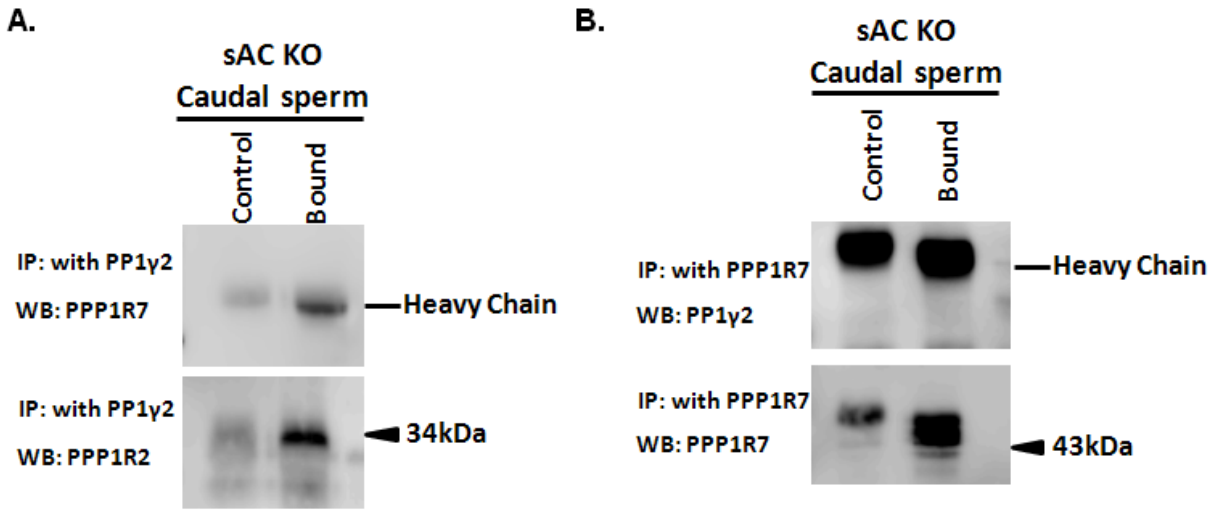
Supplemental Figure S3. PPP1R2 antibody validation. Validation of a new PPP1R2 antibody. PPP1R2 antibody was tested against recombinant His-protein (RecProtein) and in mice testis extract.

Supplemental Figure 4



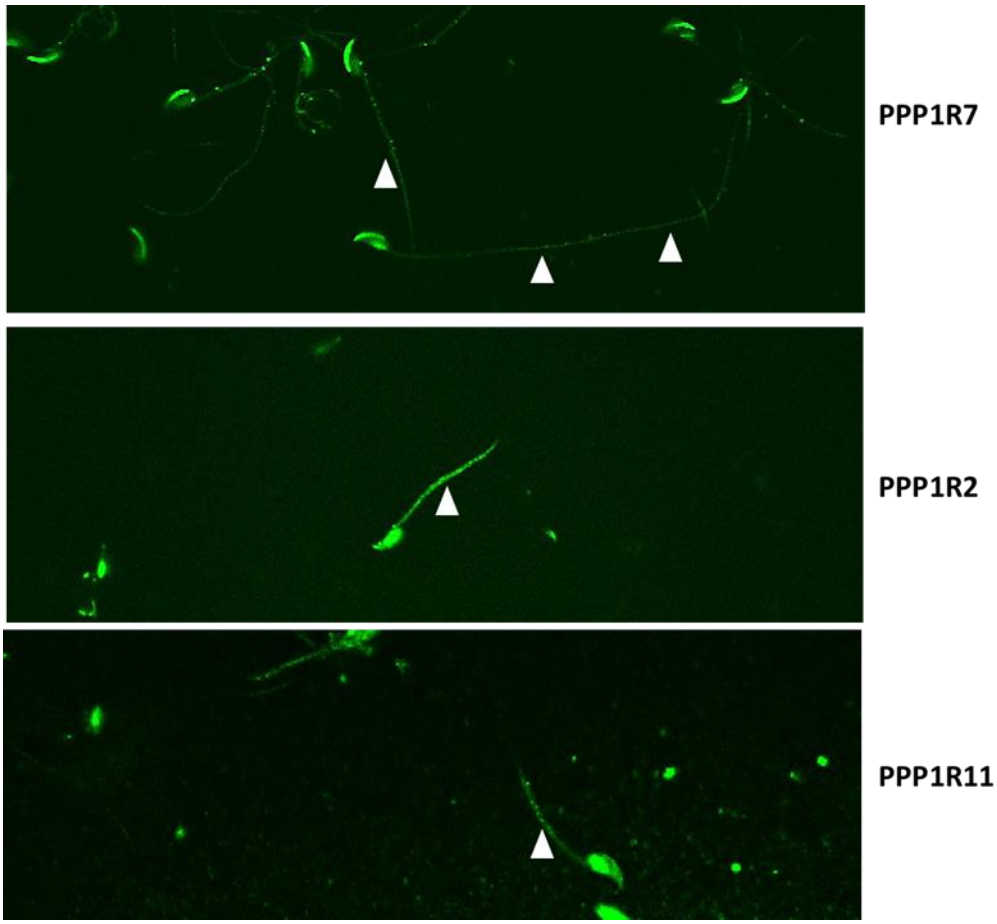
Supplemental Figure S4. PP1γ2 binds with PPP1R2, PPP1R11 and PPP1R7 in testis. Mouse testis co-immunoprecipitations were done with PP1γ2 antibody. The input, beads and flow-through extracts were probed with PPP1R2 (32 kDa), PPP1R11 (26 kDa) and PPP1R7 (43 kDa) antibodies. All the three regulators in testis show up in bead fraction. In the negative control mostly they were observed in the flow-through fraction.

Supplemental Figure S5



Supplemental Figure S5. Binding of PPP1R7 is different with PP1γ2 in sAC KO caudal sperm extracts. (A)Immunoprecipitation was done with PP1γ2 antibody using caudal sperm extracts. Immunoblots probed with PPP1R7 and PPP1R2 show that PPP1R7 is not bound to PP1γ2 whereas PPP1R2 is bound to PP1γ2. (B) Reciprocal IP was done in caudal sperm extracts using PPP1R7 and the immunoblots were probed with PP1γ2 to confirm that it is not bound to PPP1R7. The same blot was reprobed with PPP1R7 antibody as a control to show that the immunoprecipitation worked.

Supplemental Figure S6



Supplemental Figure S6: PPP1R2, PPP1R7 and PPP1R11 staining in mouse spermatozoa. (A) rabbit anti-PPP1R7 (B) rabbit anti-PPP1R2 (C) rabbit anti-PPP1R11 (D) antibodies. Specific secondary antibodies conjugated with Alexa Fluor 488 were used. PPP1R2, PPP1R11 and PPP1R7 are localized in mouse spermatozoa flagellum just like PP1γ2.