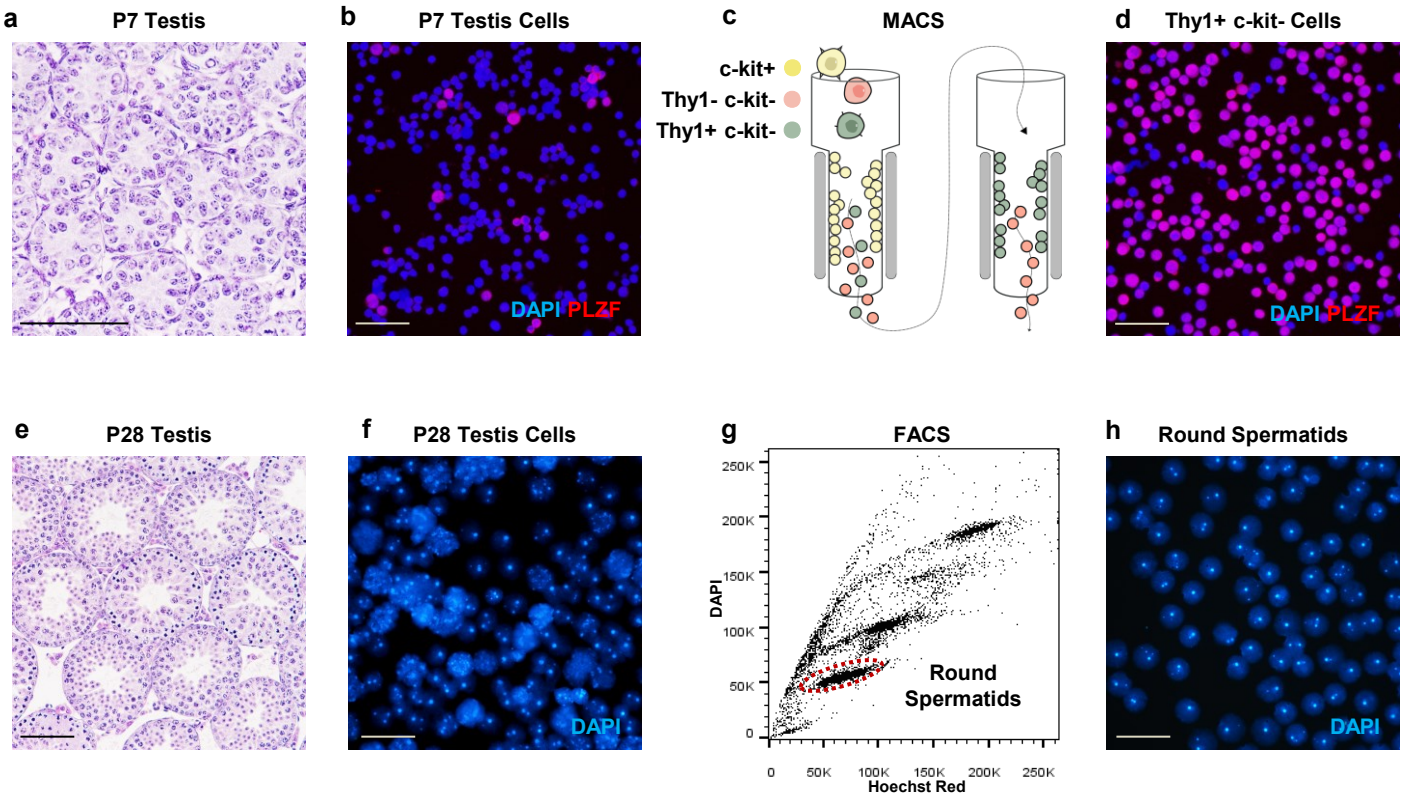


**Fig. S1 Isolation of undifferentiated spermatogonia (Aundiff) and round spermatids (RS).**
**a** Cross section of H&E stained testes from P7 mouse. **b** DAPI (blue) and anti-PLZF (red) immuno-staining of digested P7 testes cells before MACS purification. **c** Illustration of magnetic activated cell sorting (MACS) strategy to isolate THY1+ c-KIT- Aundiff cells. Differentiated spermatogonia and other testis somatic cells expressed c-KIT (cKIT+ cells, shown as yellow cells), which were depleted first by binding to the anti-c-KIT antibody and magnetic columns. The unbound c-KIT- cells were subsequently separated by MACS to enrich the THY1+ c-KIT- cells (showed as green cells). **d** DAPI (blue) and anti-PLZF (red) immuno-staining of THY1+ c-KIT- cells after MACS purification. **e** Cross section of H&E stained testes of P28 mouse. **f** DAPI staining of digested P28 testis cells before FACS purification. **g** FACS plot of digested testes cells stained with Hoechst 33,342. Gate for sorting haploid RS is circled. **h** DAPI staining of RS after FACS purification. All scale bars = 50 μm.

Fig S2

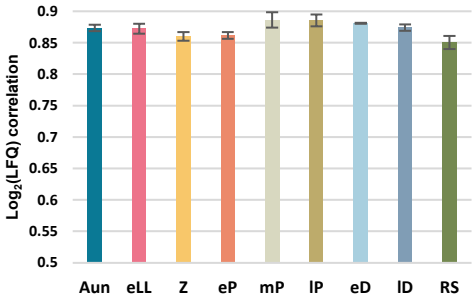


**Fig. S2 The Pearson correlations coefficients of the proteomes between the triplicate techniques.**

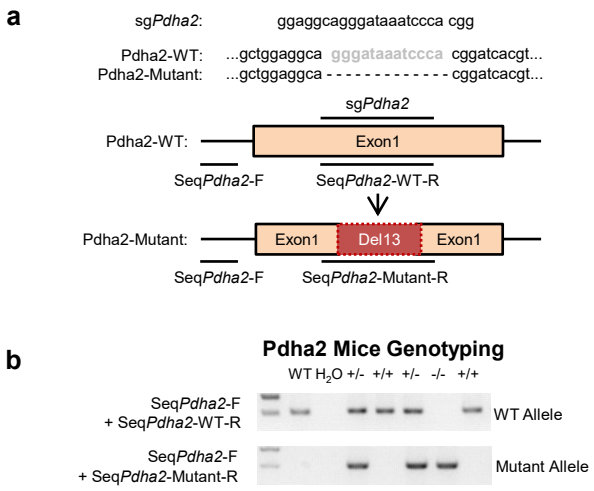





**Fig. S3 Construction and genotyping of Pdha2 knockout mice.**
**a** The strategy to construct Pdha2 knockout mice.
**b** Genotyping of Pdha2 knockout mice verified by PCR.

Fig S4

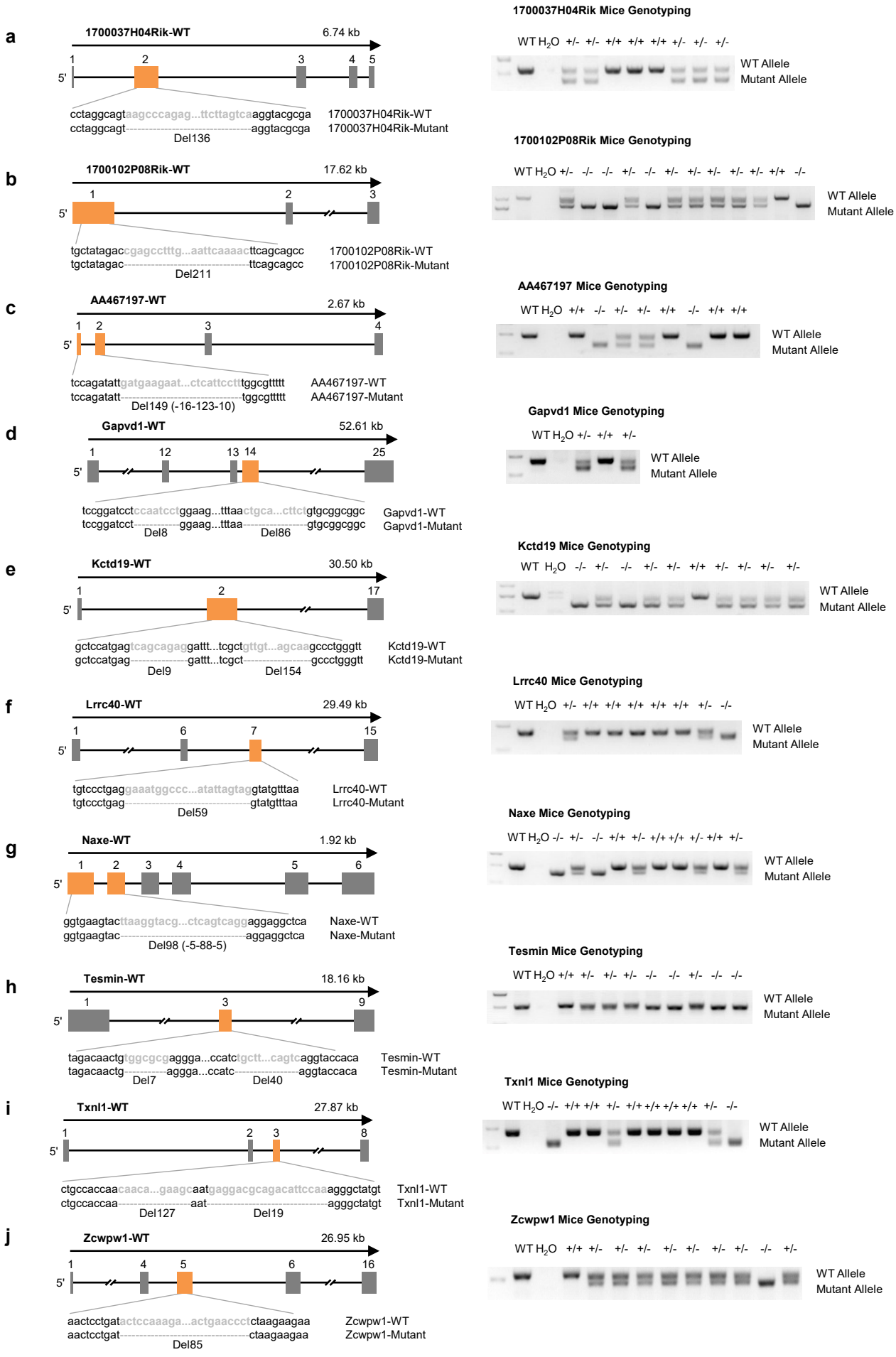


**Fig. S4 Construction and genotyping of ten knock out mice of the top ten RBF-ranked candidates.** **a-j** Strategy to construct knockout mice of the top 10 RBF-ranked candidates (left panel) and the genotyping of knockout mice verified by PCR (right panel).

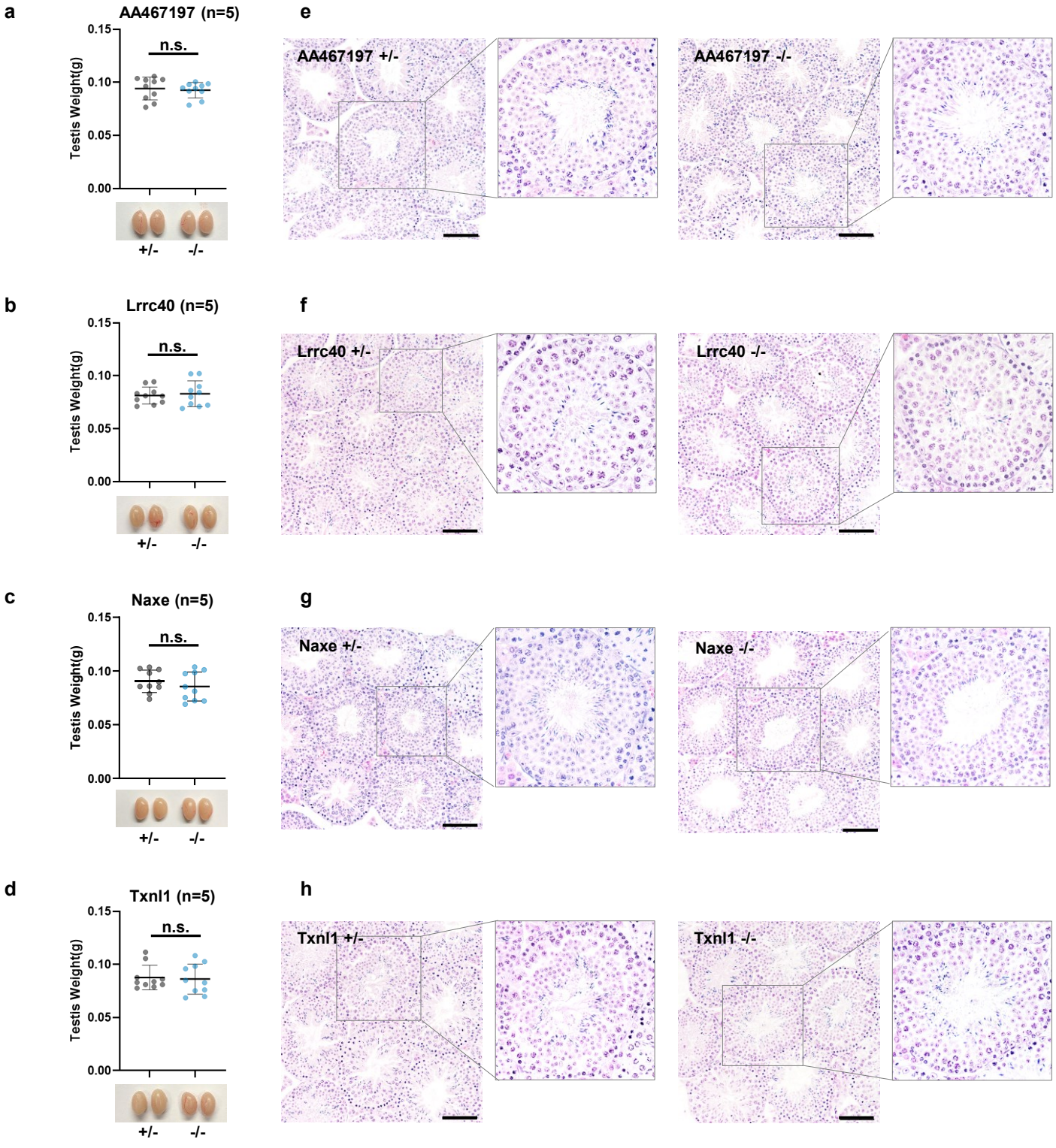


**Fig. S5 Phenotypic validation of the meiosis non-essential proteins predicted by RBF.**
**a-d** Comparison of testis weights derived from 8-week-old Txnl1+/- and Txnl1-/- mice (**a**), AA467197+/- and AA467197 -/- mice (**b**), Lrrc40+/- and Lrrc40-/- mice (**c**), and Naxe+/- and Naxe -/- mice (**d**). n.s. means no significance in unpaired two-tailed t-test. **e-h** Cross-sections of H&E stained seminiferous tubules from the heterozygous and homozygous knockout mice of the four genes above, insets denote the specific seminiferous tubule under higher magnification. All scale bars = 50 μm.