

Supplementary Materials for

hnRNPH1 recruits PTBP2 and SRSF3 to cooperatively modulate alternative pre-mRNA splicing in germ cells and is essential for spermatogenesis and oogenesis

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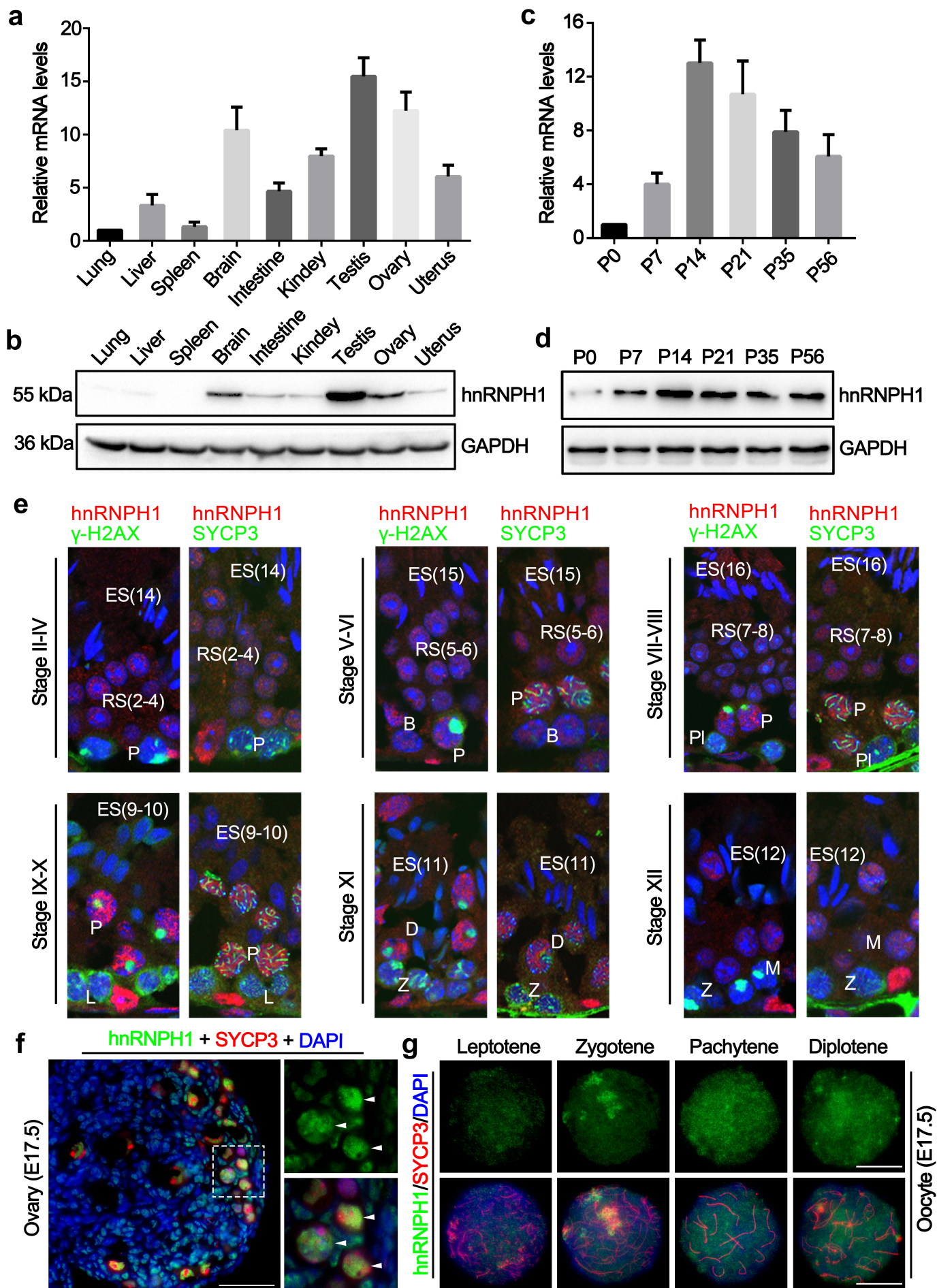
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Supplementary Fig.8. hnRNPH1 cKO ovaries show defective morphology and more apoptotic oocyte and elevated hnRNPH1 and PTBP2 in oocytes.

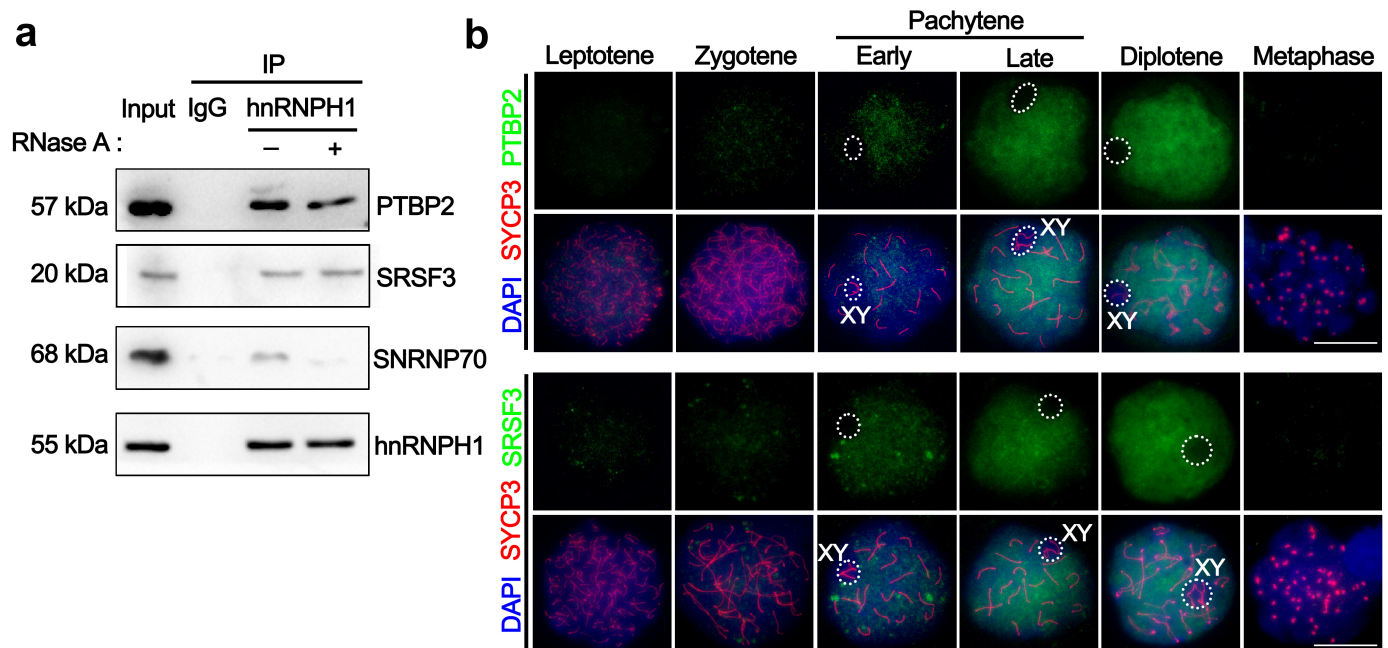
Supplementary Fig.9. GO term analyses of AS changed genes that bound by hnRNPH1 and comparison of hnRNPH1-regulated AS genes and PTBP2-bound genes.

Supplementary Fig.10. RT-PCR analyses of AS changed genes regulated RNA splicing and RIP-qPCR assay of enrichment of *Rbm5* target genes to hnRNPH1.

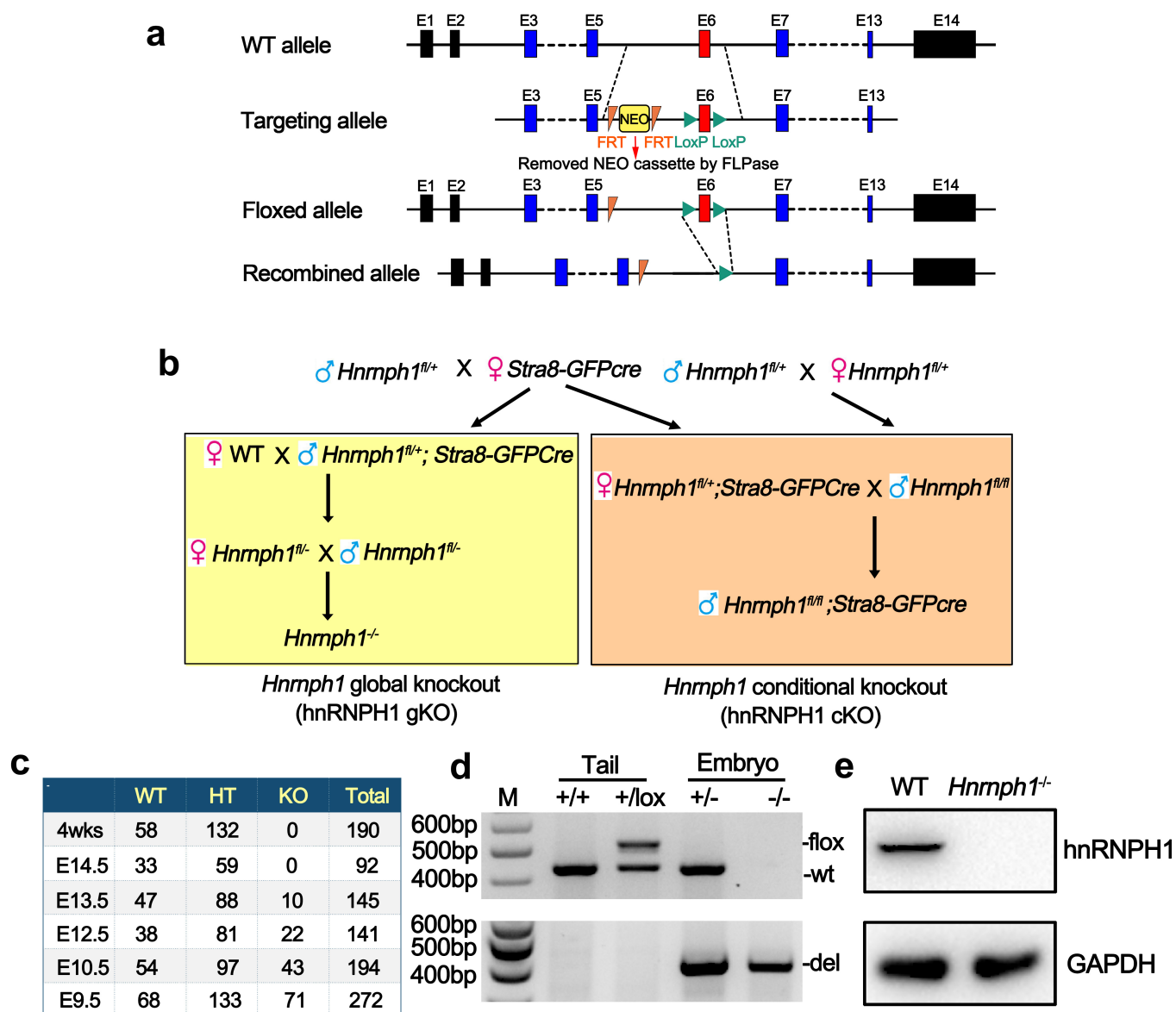
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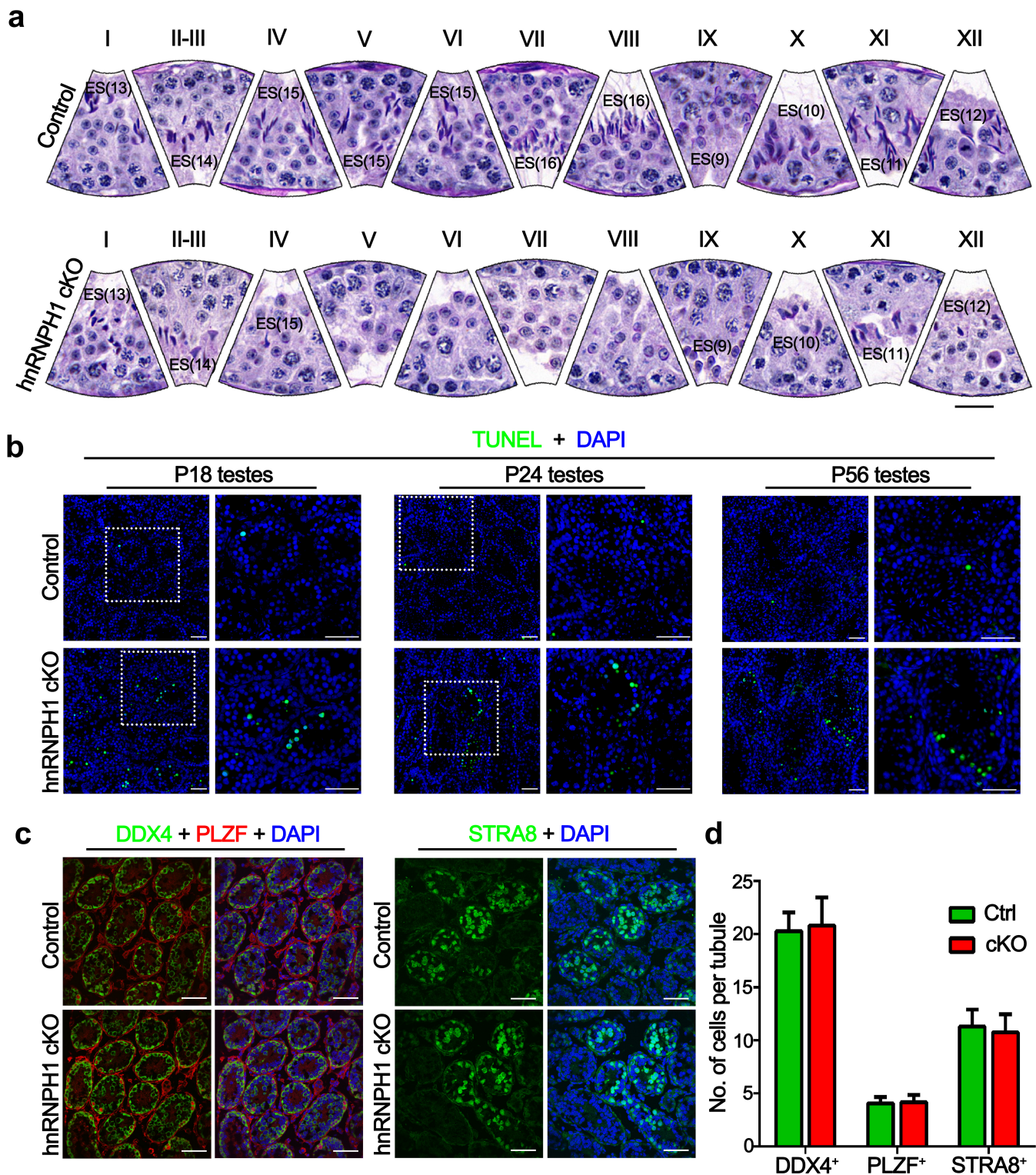
Supplementary Fig.1. Expression profiles of hnRNPH1 during spermatogenesis in mice. (a) RT-qPCR analyses of *Hnrnp1* mRNA levels in multiple organs from WT adult mice. Data are presented as mean $\pm$ SD, n = 3. (b) Expression of hnRNPH1 protein levels in adult WT multiple organs by western blot analyses. GAPDH serves as the loading control. (c) RT-qPCR analyses of *Hnrnp1* mRNA levels in developing testes at postnatal day 0 (P0), P7, P14, P21, P35, and P56. Data are presented as mean $\pm$ SD, n = 3. (d) Expression of hnRNPH1 protein in developing testes by western blot analyses. GAPDH serves as the loading control. (e) Co-immunofluorescence staining of anti-hnRNPH1 with anti- γ H2AX and anti-SYCP3 on staged WT adult testis sections are shown. Nuclei were stained with DAPI. (f) Co-immunofluorescence staining of hnRNPH1 with SYCP3 in ovaries from WT mice at E17.5 is shown. Scale bar = 50 μ m. (g) Double immunostaining with hnRNPH1 and SYCP3 on surface-spread oocytes from WT mice at E17.5 are shown. Scale bars = 5 μ m.



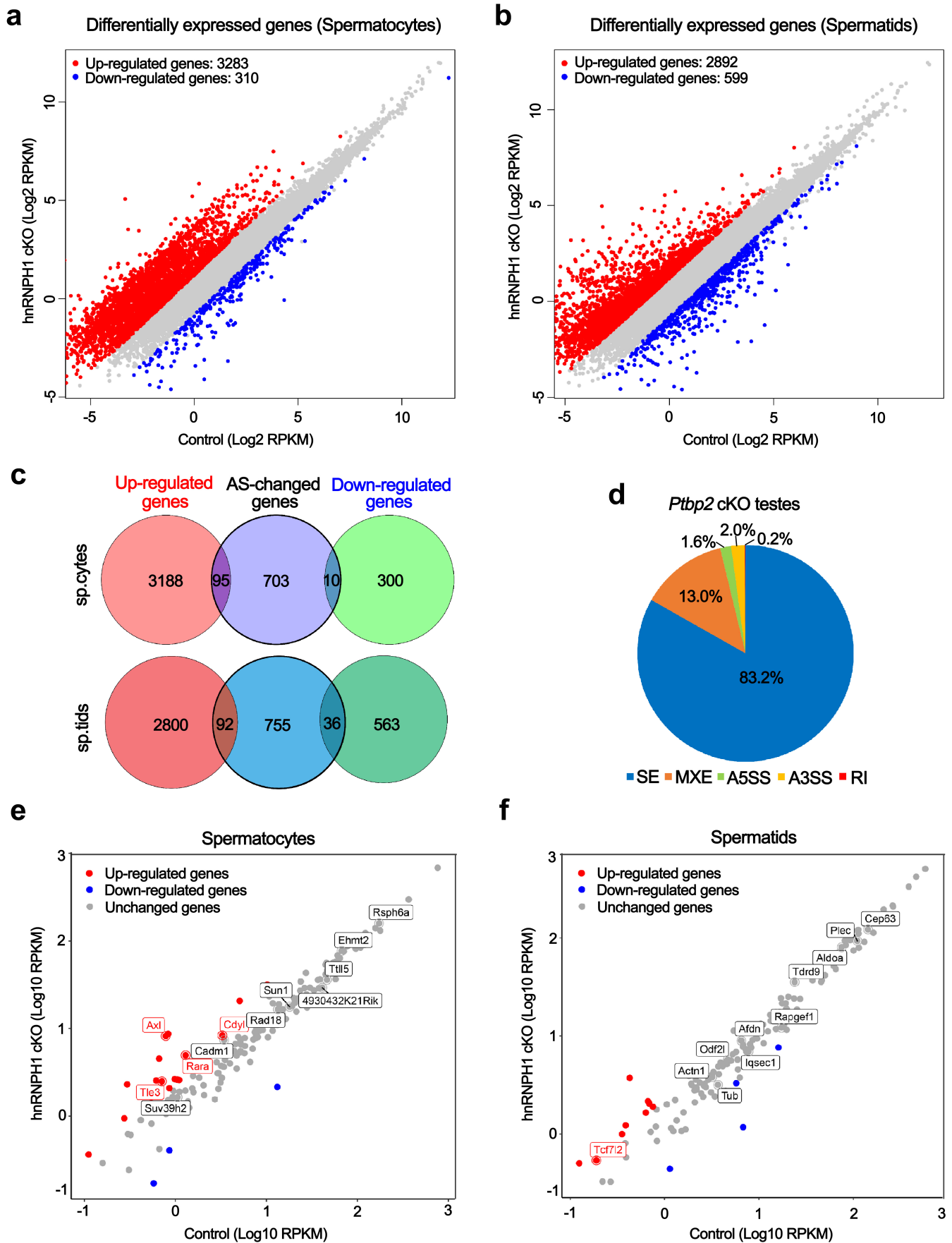
Supplementary Fig.2. The interaction between hnRNPH1 and its bound proteins and their localization in spermatocytes. (a) Immunoprecipitation of hnRNPH1 on WT adult mouse testis lysate with or without RNase A treatment followed by western blot using indicated antibodies are shown. IgG was used as a negative control. (b) Co-immunofluorescence staining of SYCP3 with PTBP2 (upper) and SRSF3 (lower) on surface-spread spermatocytes from WT mice are shown. The dotted lines outline XY-body. Scale bar = 5 μ m.



Supplementary Fig.3. Generation of both conventional and conditional *Hnrnp1* knockout mouse models. (a) Schematic diagram of the targeting strategy used to generate floxed *Hnrnp1* allele by homologous recombination in mouse embryonic stem cells. Exons 6 was deleted after Cre-mediated recombination. (b) Schematic diagram of the generation of both global and germline conditional *Hnrnp1* knockout mice. (c) The number of embryonic offspring with different genotypes produced by mating heterozygous *Hnrnp1* male and female mice are shown. (d) PCR-based genotyping of the mouse tails and E12.5 embryos is shown. (e) Western blotting of the hnRNPH1 in the lysates of WT and *hnRNPH1^{-/-}* embryos at E12.5. GAPDH was blotted as a loading control.

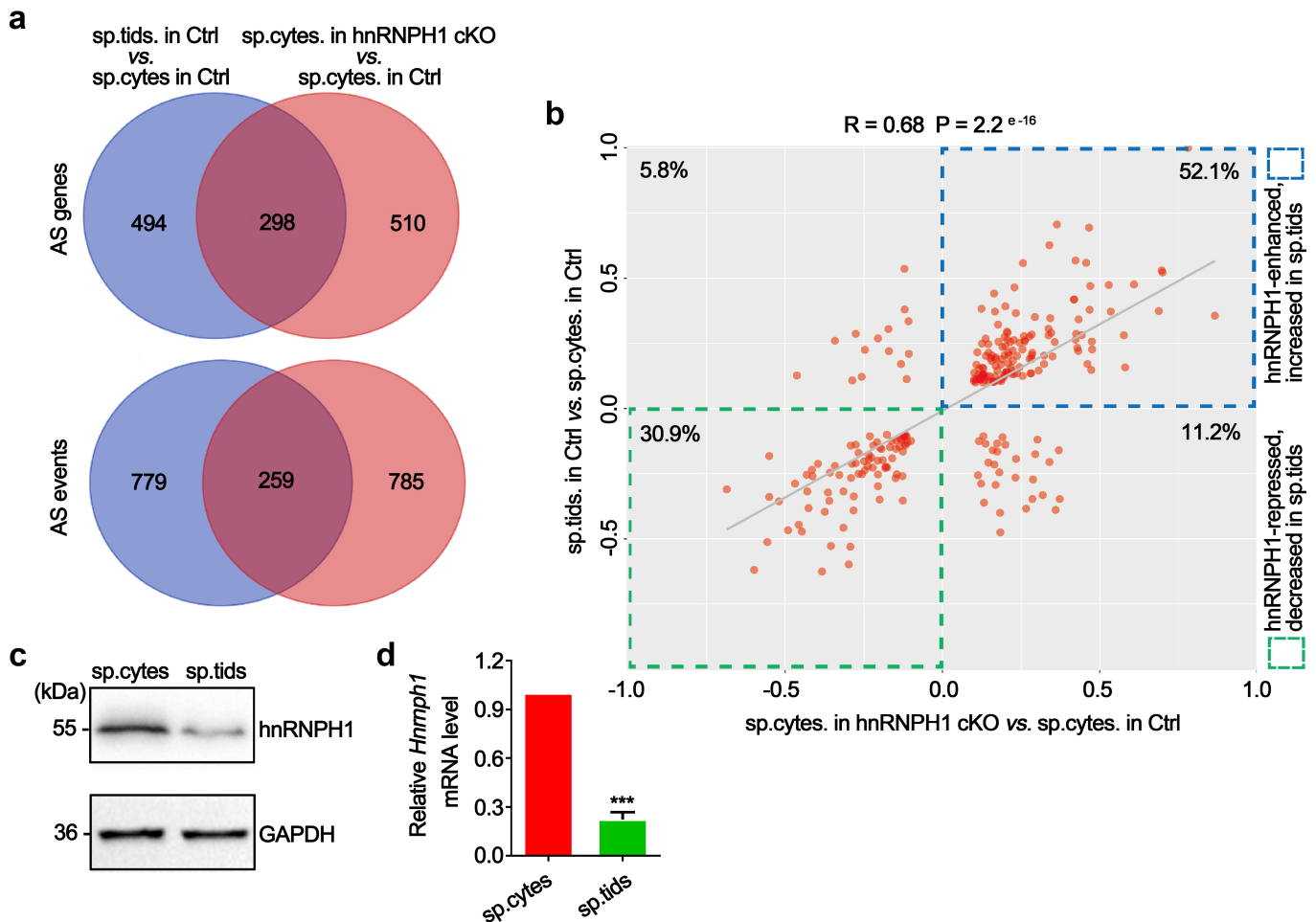


Supplementary Fig.4. Conditional deletion of hnRNPH1 in male germ cells causes defective spermatogenesis. (a) Periodic acid-Schiff (PAS) staining of staged testis sections from control and hnRNPH1 cKO mice at P56. Scale bar = 25µm. (b) TUNEL staining of testes from control and hnRNPH1 cKO mice at P18, P24, and P56. The DNA was stained with DAPI. Scale bars = 50µm. (c) Immunofluorescence staining of DDX4/PLZF (left) and STRA8 (right) on testis sections from control and hnRNPH1 cKO mice at P10. The DNA was stained with DAPI. Scale bars = 50µm. (d) The quantification of DDX4⁺, PLZF⁺, and STRA8⁺ cells per tubule for (c). Data are presented as mean ± SD, n = 3.

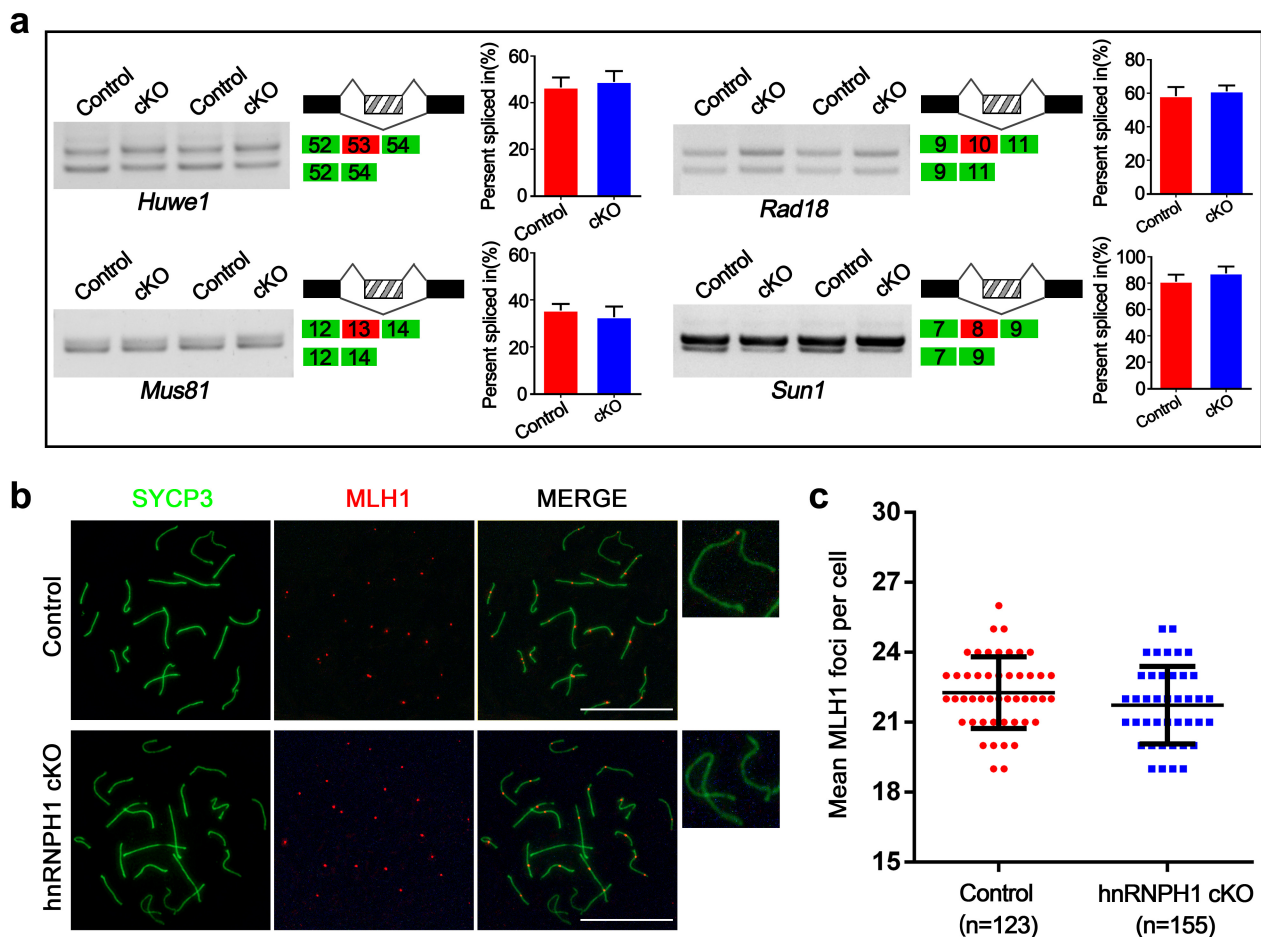


Supplementary Fig.5. Analysis of RNA-seq data and the comparison of hnRNPH1- and PTBP2-regulated genes. (a-b) Volcano plot showing the differentially expressed genes determined by RNA-seq analysis of hnRNPH1 cKO spermatocytes (a) and spermatids (b). (c) Venn diagrams

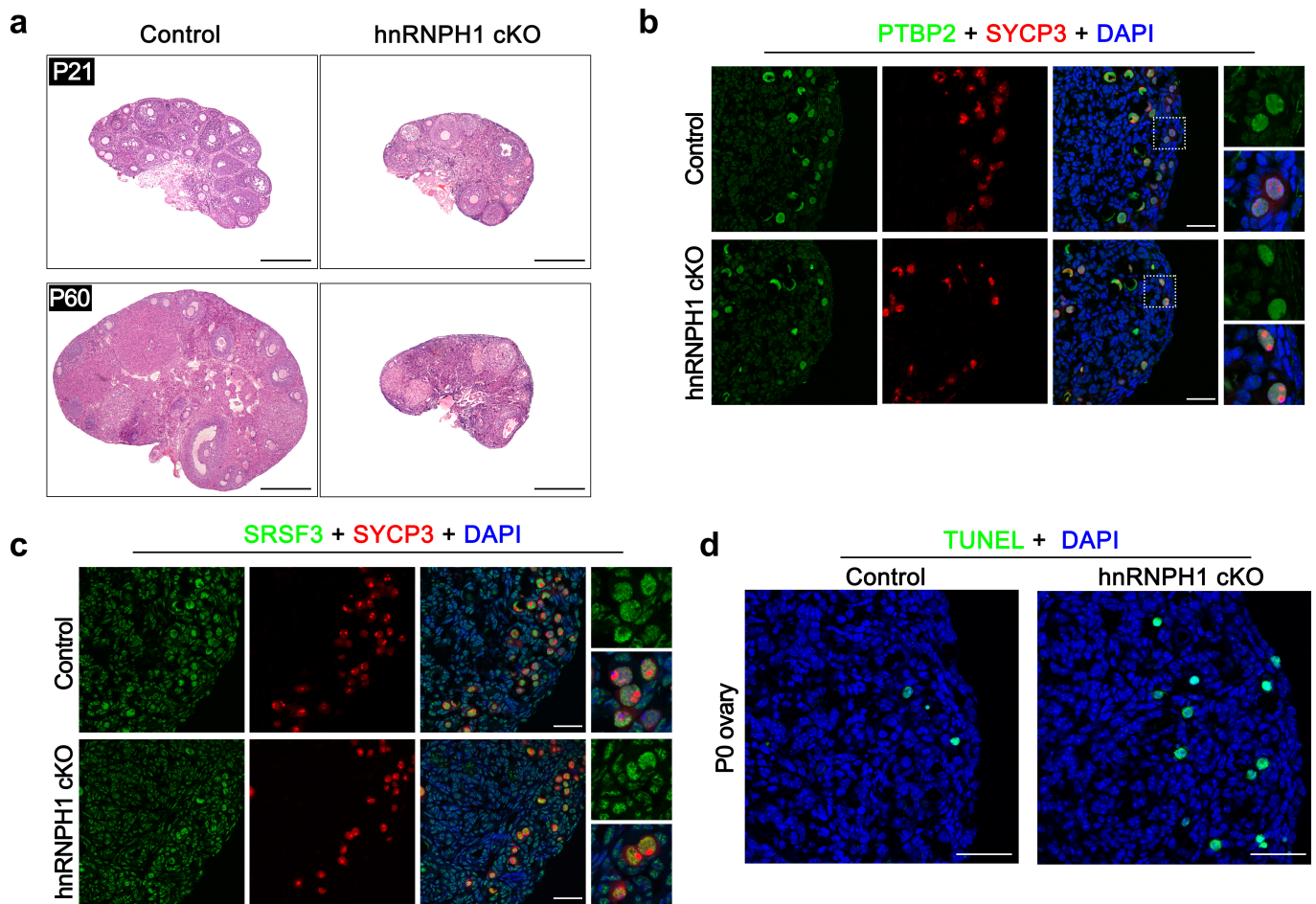
showing overlap of splicing-changed genes, up-regulated genes, and down-regulated genes in hnRNPH1 cKO spermatocytes (*upper*) and spermatids (*lower*). (**d**) The pie chart represents the distribution of regulated splicing events among different types of splicing events in *Ptbp2* cKO testes. (**e-f**) Volcano plots depicting the differentially expressed genes with the same abnormal AS events caused by hnRNPH1 and PTBP2 ablation in spermatocytes (*e*) and spermatids (*f*).



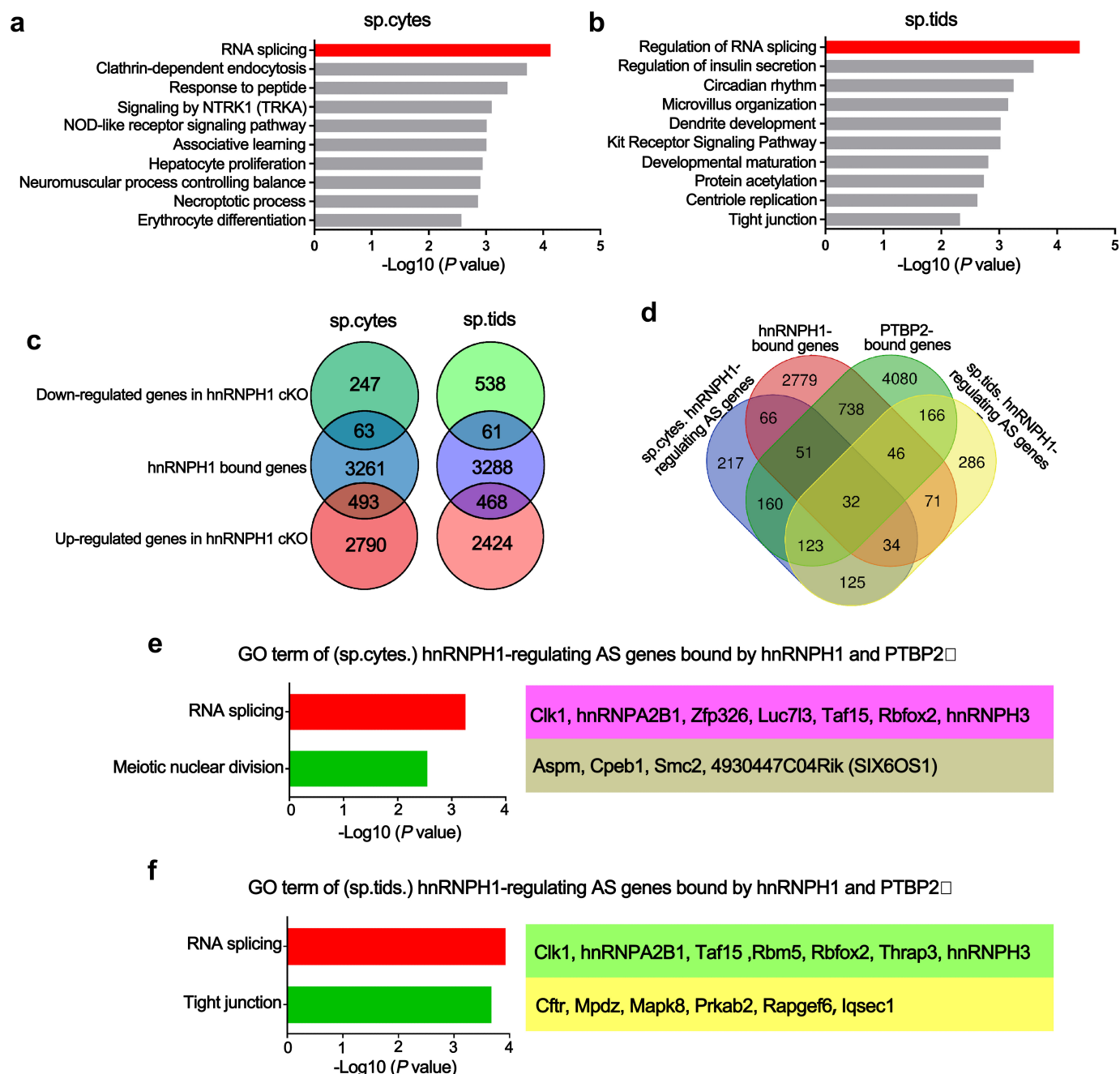
Supplementary Fig.6. AS events that occurred during the transition from spermatocytes to spermatids are related to the hnRNPH1 dynamic expression. (**a**) Venn diagrams showing the overlaps of AS genes (upper) and events (down) in the indicated comparisons. (**b**) Distribution of ΔPSI values for genes with AS events that abnormally appear in hnRNPH1 cKO spermatocytes and also normally occur during the transition from spermatocytes to round spermatids. (**c**) Expression of hnRNPH1 protein in purified spermatocytes and spermatids isolated from P25 WT mice by western blot analyses. GAPDH serves as the loading control. (**d**) RT-qPCR analyses of *Hnmp1* mRNA levels in purified spermatocytes and spermatids. Data are presented as mean $\pm$ SD, $n = 3$. *** $P < 0.001$ by Student's *t*-test.



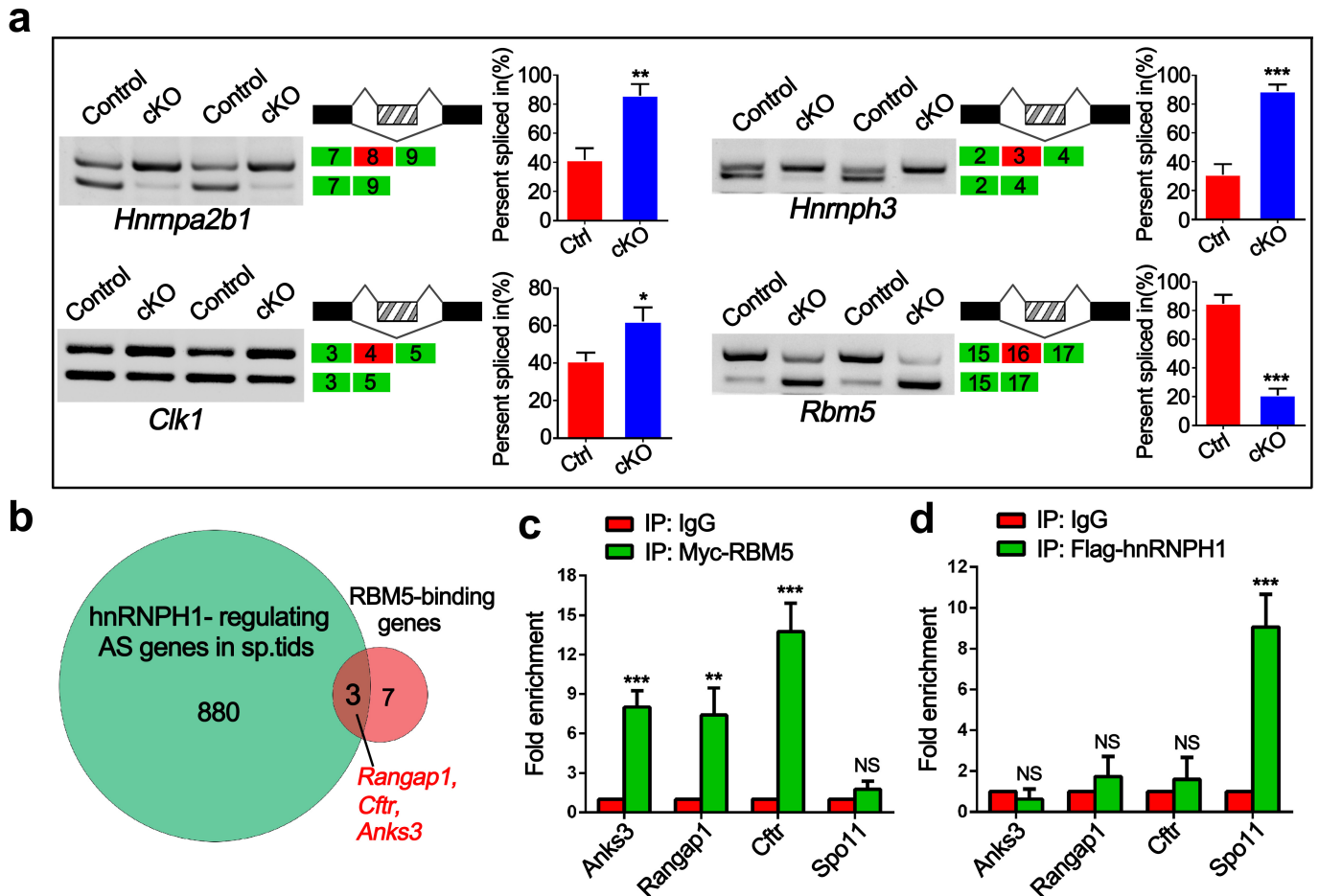
Supplementary Fig.7. Analyses of AS changes and MLH1 localization in hnRNPH1 cKO spermatocytes. (a) Representative examples of RT-PCR analyses for indicated AS events differentially regulated between control and hnRNPH1 cKO spermatocytes isolated from P25 mice are shown. Middle panels represent the schematic diagram of AS exons detected by RNA-Seq analysis. Right panels show the quantification of percent spliced in (PSI). Data are presented as mean $\pm$ SD, $n = 3$. (b) Co-immunofluorescence staining of SYCP3 with MLH1 in spermatocyte spreads at pachytene stage from control and hnRNPH1 cKO mice. Scale bar = 5 μ m. (c) Mean MLH1 foci per cell in pachytene spermatocytes from the control and hnRNPH1 cKO mice. Data are presented as mean $\pm$ SD. Biologically independent mice ($n = 3$) for each genotype were examined.



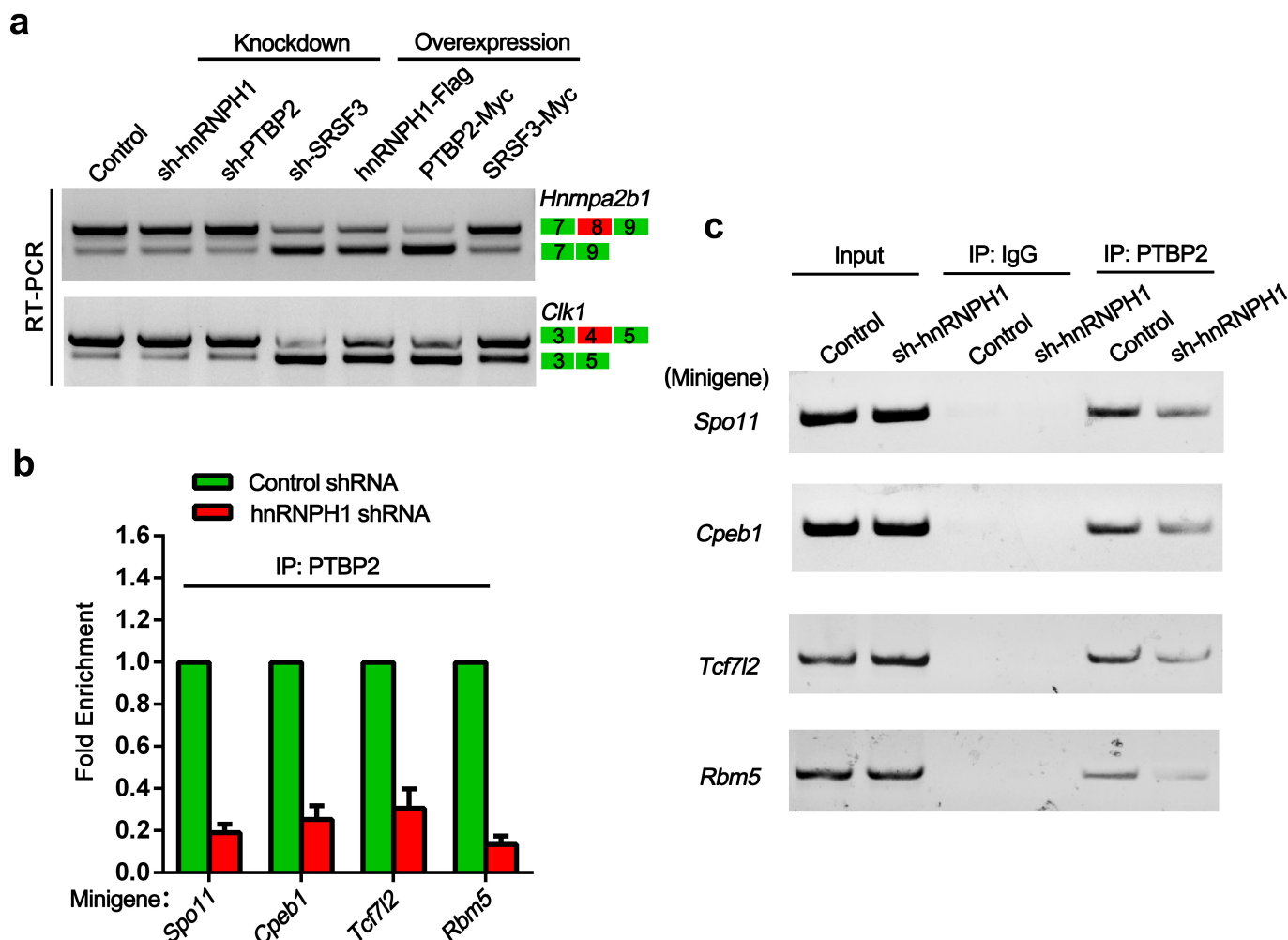
Supplementary Fig.8. hnRNPH1 cKO ovaries show defective morphology and more apoptotic oocyte and elevated hnRNPH1 and PTBP2 in oocytes. (a) Representative micrographs of ovarian sections stained with hematoxylin and eosin (HE) from control and hnRNPH1 cKO mice at P21 and P60 are shown. Scale bars = 250µm. (b-c) Co-immunofluorescence staining of SYCP3 with PTBP2 (b) and SRSF3 (c) in ovaries from control and hnRNPH1 cKO mice at P0. The DNA was stained with DAPI. Scale bar = 50µm. (d) Terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling (TUNEL) staining of the ovaries derived from control and hnRNPH1 cKO mice at P0. The DNA was stained with DAPI. Scale bars = 50µm.



Supplementary Fig.9. GO term analyses of AS changed genes that bound by hnRNPH1 and comparison of hnRNPH1-regulated AS genes and PTBP2-bound genes. (a-b) GO term enrichment analyses of the overlapped genes between the hnRNPH1-bound and abnormal AS genes in hnRNPH1 cKO spermatocytes (a) or spermatids (b). **(c)** Venn diagrams showing overlap of hnRNPH1-bound genes, down-regulated genes, and up-regulated genes in hnRNPH1 cKO versus control spermatocytes (*left*) and spermatids (*right*). **(d)** Venn diagrams showing overlap of hnRNPH1-regulating AS genes in spermatocytes, hnRNPH1-regulating AS genes in spermatids, hnRNPH1-bound genes, and PTBP2 bound genes. **(e-f)** GO term enrichment analyses of hnRNPH1-regulating AS genes bound by both hnRNPH1 and PTBP2 in spermatocytes (e) and spermatids (f).



Supplementary Fig.10. RT-PCR analyses of AS changed genes regulated RNA splicing and RIP-qPCR assay of enrichment of *Rbm5* target genes to hnRNPH1. (a) Representative examples of RT-PCR analyses for indicated AS events differentially regulated between control and hnRNPH1 cKO spermatocytes. Middle panels represent the schematic diagram of alternatively spliced exons detected by RNA-Seq analysis. Right panels show the quantification of percent spliced in (PSI). Data are presented as mean $\pm$ SD, $n = 3$. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ by Student's t -test. (b) Venn diagrams showing overlap of hnRNPH1-regulating AS genes in spermatids and RBM5-binding genes. (c-d) RIP-qPCR analyses for indicated mRNA of 4 genes co-precipitated by anti-Myc-RBM5 (c) and anti-Flag-hnRNPH1 (d) antibodies and control IgG in RIP experiments performed from purified germ cells isolated from WT mice at P25. Data are presented as mean $\pm$ SD, $n = 3$. *** $P < 0.001$ by Student's t -test.



Supplementary Fig.11. RT-PCR analyses of AS upon overexpression and knockdown of indicated genes and the enrichment assay of 4 target minigenes to PTBP2. (a) RT-PCR analysis of splicing assay performed in HEK293T cells transfected with the indicated minigenes and knockdown/overexpression related vectors for hnRNP1, PTBP2, and SRSF3. (b-c) RIP-qPCR (b) and RT-PCR (c) analyses for indicated mRNA of 4 genes co-precipitated by PTBP2-specific antibody in RIP experiments performed in HEK293T cells transfected with the indicated minigenes and knockdown related vectors for *Hnnp1*.