

1 **Supplementary Information**

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3 **hnRNPM cooperates with BCAS2 to modulate alternative splicing during oocyte** 4 **development**

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12 **The PDF file includes**

13 Supplementary Figure 1 | hnRNPM is a conserved protein expressed in the female germline.

14 Supplementary Figure 2 | *Hnrnpm* deficiency disrupts cytoplasmic maturation in oocytes.

15 Supplementary Figure 3 | SCAN-seq reveals isoform profiling and novel transcript functions.

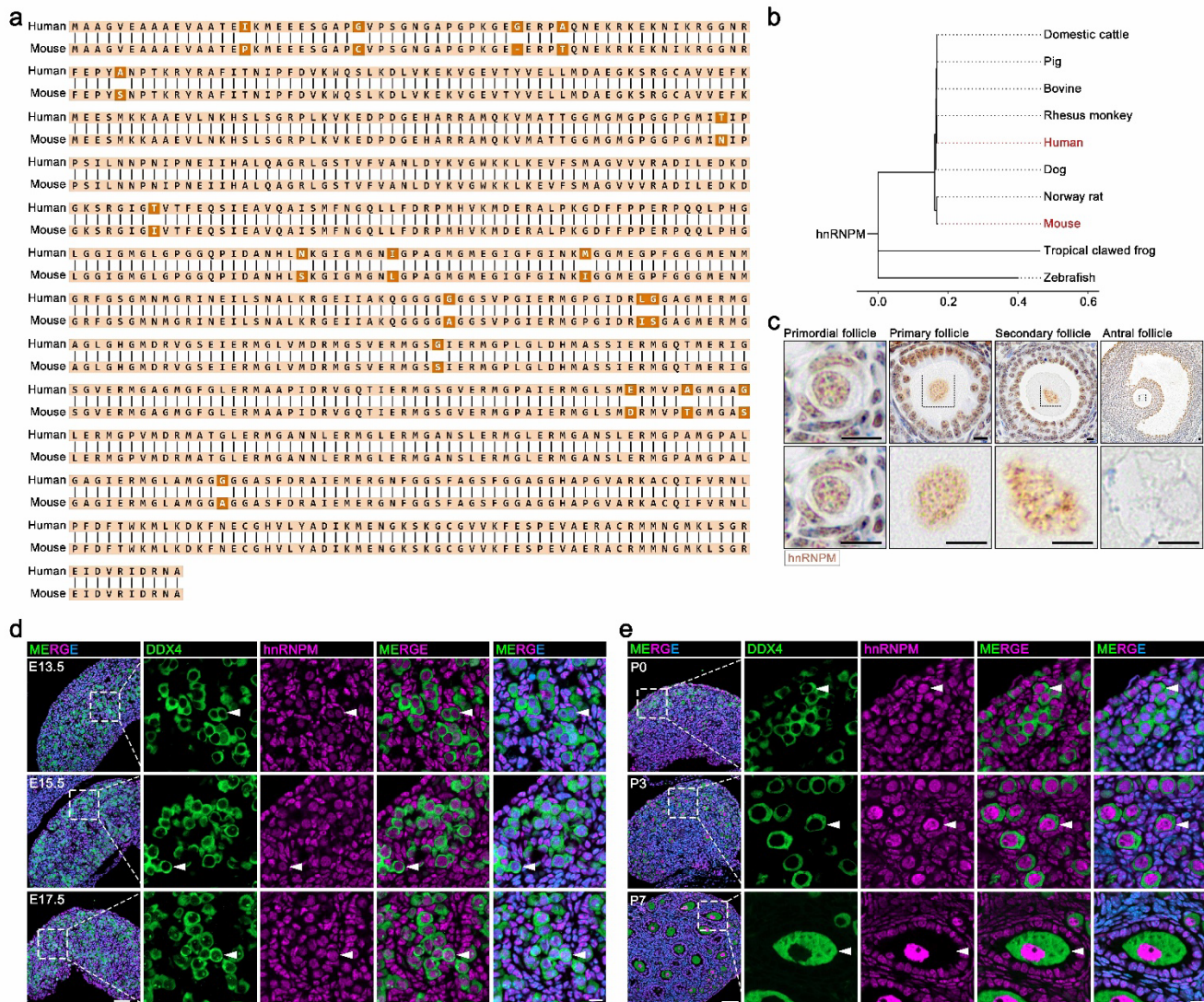
16 Supplementary Figure 4 | Genetic ablation of *Hnrnpm* alters the alternative splicing in oocytes.

17 Supplementary Figure 5 | LACE-seq reveals hnRNPM binding landscape in oocytes.

18 Supplementary Figure 6 | hnRNPM cooperates with BCAS2 to regulate alternative splicing.

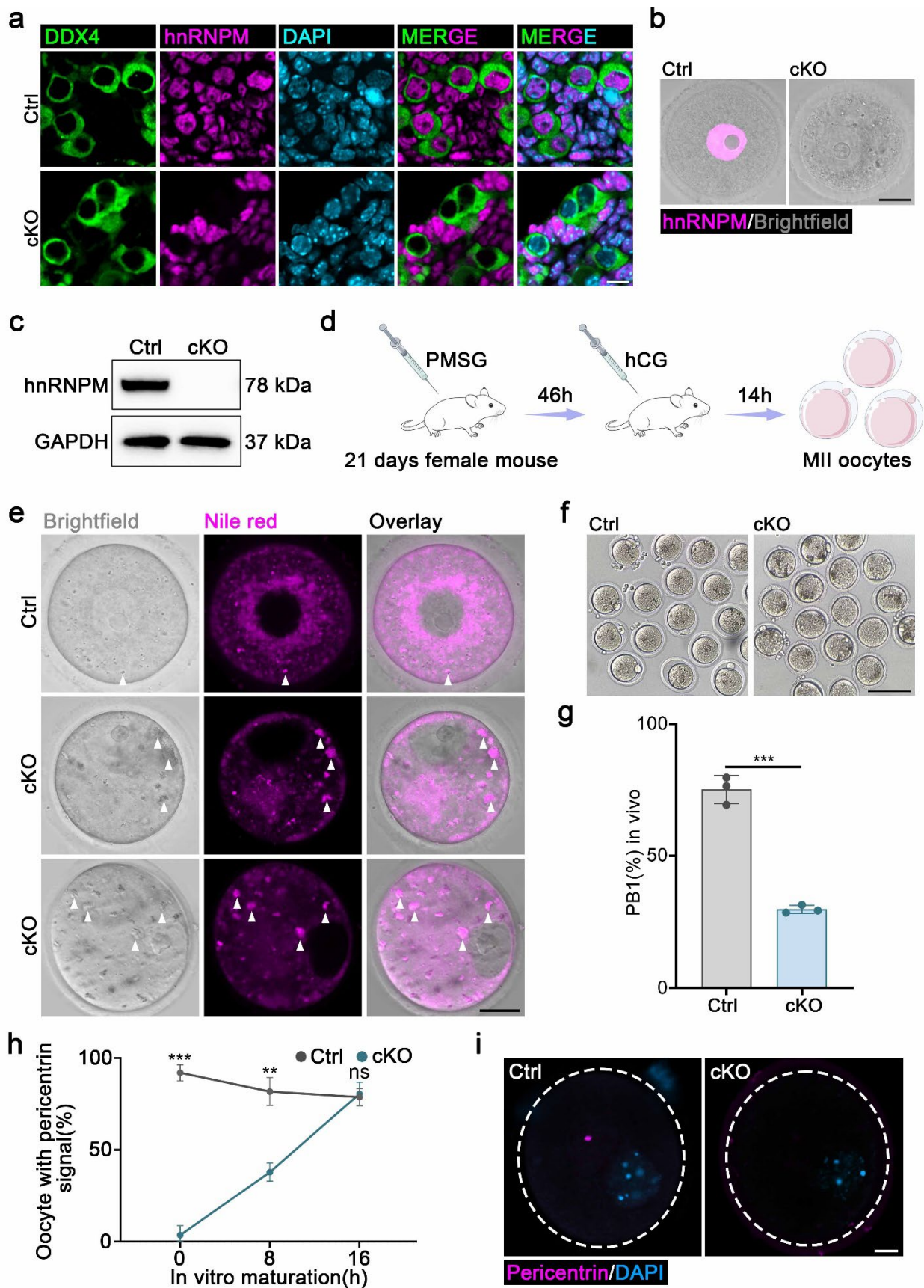
19 Supplementary Figure 7 | Loss of hnRNPM impairs the enrichment of BCAS2 on pre-mRNA.

20 Uncropped scans of blots.



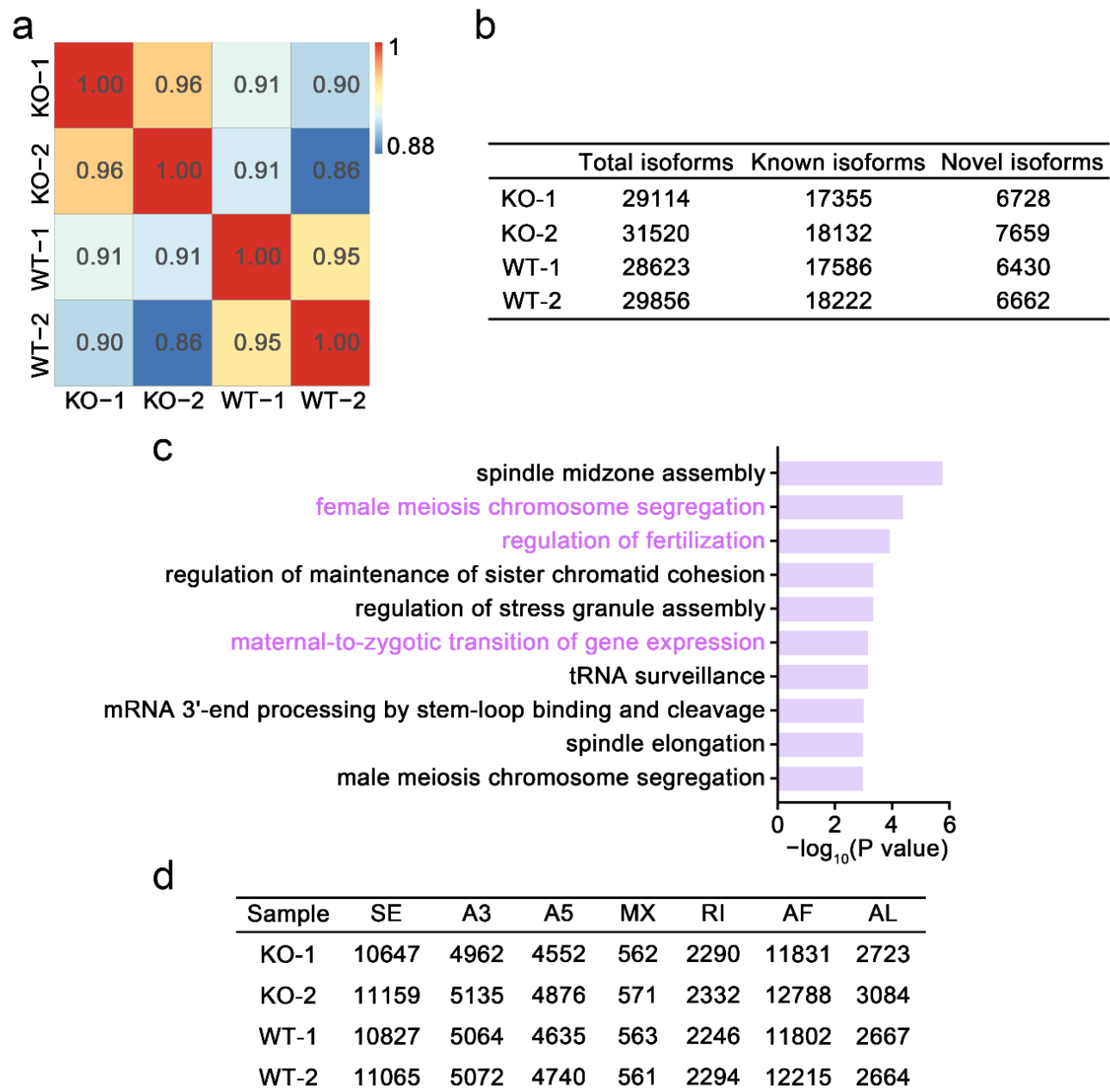
Supplementary Fig. 1 hnRNPM is a conserved protein expressed in the female germline.

a Alignment of the human and mouse hnRNPM protein sequences. **b** Phylogenetic analysis of hnRNPM proteins based on the Maximum Likelihood method. **c** Immunohistochemical analysis of hnRNPM expression in wild-type (WT) ovaries. Insets represent higher-magnification views of the boxed regions in the upper panels. Scale bars: 10 μ m. **d-e** Immunofluorescence staining of hnRNPM in mouse ovaries at perinatal stages. White arrowheads indicate oocytes with hnRNPM (Purple) and DDX4 (Green) signal. Scale bars: 50 μ m (left), 10 μ m (right). Representative results shown in panels (c-e) were obtained from at least three independent experiments, with consistent findings observed in all cases.



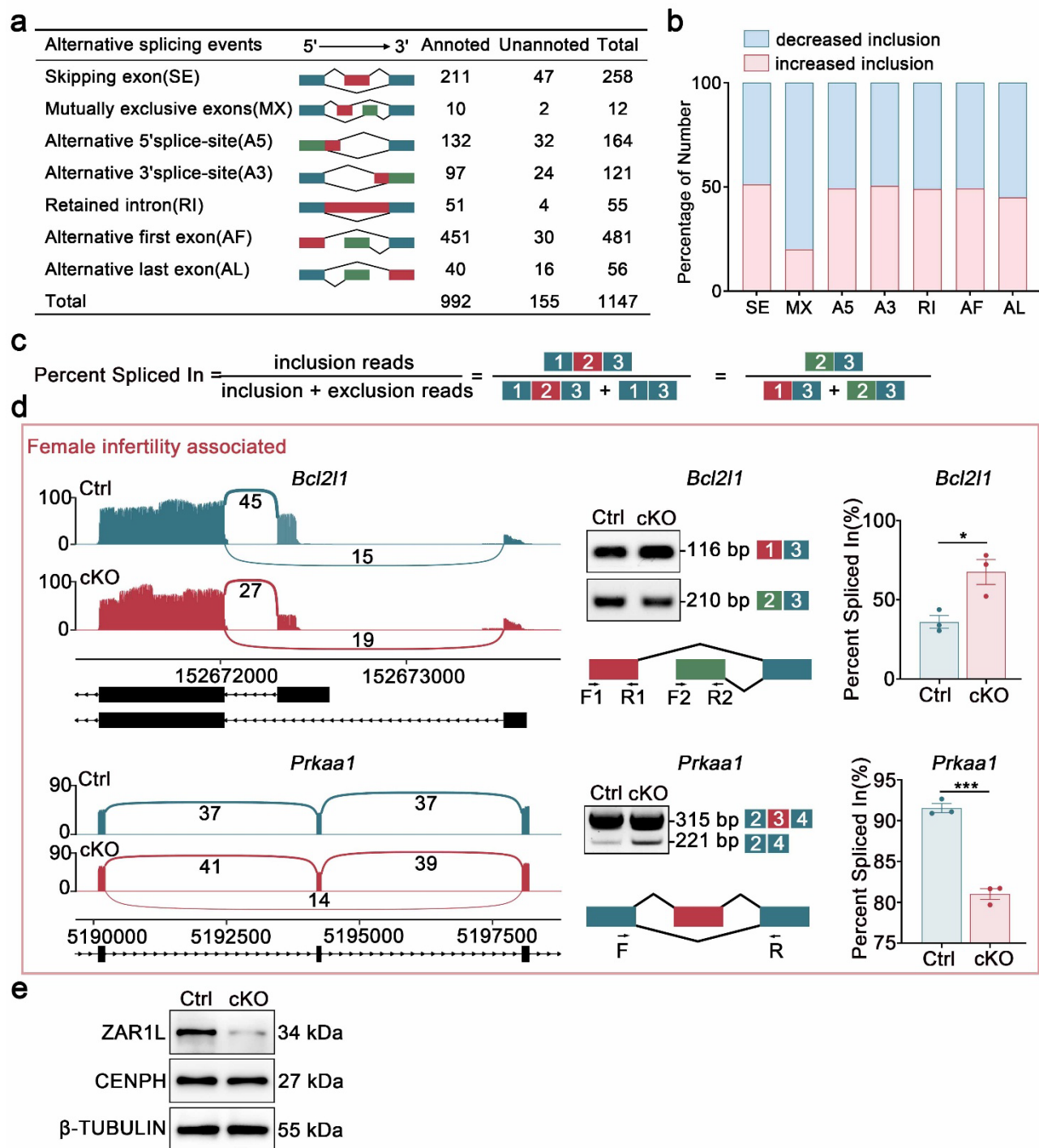
32 **Supplementary Fig. 2 *Hnrnpm* deficiency disrupts cytoplasmic maturation in oocytes.**
33 **a-b** Representative immunofluorescence images showing the knockout efficiency of hnRNPM in
34 P0 ovaries and GV oocytes. hnRNPM immunofluorescence signal (purple) is undetectable in
35 cKO oocytes, in contrast to robust staining observed in controls. Scale bars: 10 μ m (a), 20 μ m
36 (b). **c** Western blot showing the knockout efficiency of hnRNPM in control and cKO oocytes. All
37 samples presented within this figure panel derive from the same experiment and were processed
38 in parallel. **d** The workflow illustrating the key steps for collecting metaphase II (MII) oocytes.
39 Schematic illustration of mouse was adapted from SciDraw. The oocyte diagram is a modified
40 version of an original Servier Medical Art resource. All modified images are used under a CC BY
41 4.0 license (<https://creativecommons.org/licenses/by/4.0/>). **e** Representative confocal images of
42 control and *Hnrnpm* cKO oocytes stained with Nile Red (lipid droplets, purple) and DAPI (nuclei,
43 blue). White arrowheads indicate lipid aggregates in oocytes. Scale bars: 20 μ m. **f**
44 Representative images of ovulated oocytes from control and *Hnrnpm* cKO mice after hCG
45 injection. Scale bars: 50 μ m. **g** Quantification of the first polar body extrusion rate in these
46 ovulated oocytes. Data are presented as mean $\pm$ SEM. n = 3. $p=0.000139$. **h** Quantitative
47 analysis of oocytes with pericentrin signal during the in vitro maturation process. Data are
48 presented as mean $\pm$ SEM. n = 3. $p = 0.000021$ (0), 0.001119(8), 0.710192(16). **i** Representative
49 images showing the expression of pericentrin (purple) in control and *Hnrnpm* cKO GV oocytes.
50 Scale bars: 10 μ m. Representative results shown in panels (**a-c**, **e-f**, **i**) were obtained from at
51 least three independent experiments, with consistent findings observed in all cases. Source data
52 (**g**, **h**) are provided as a Source Data file.

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Supplementary Fig. 3 SCAN-seq reveals isoform profiling and novel transcript functions.

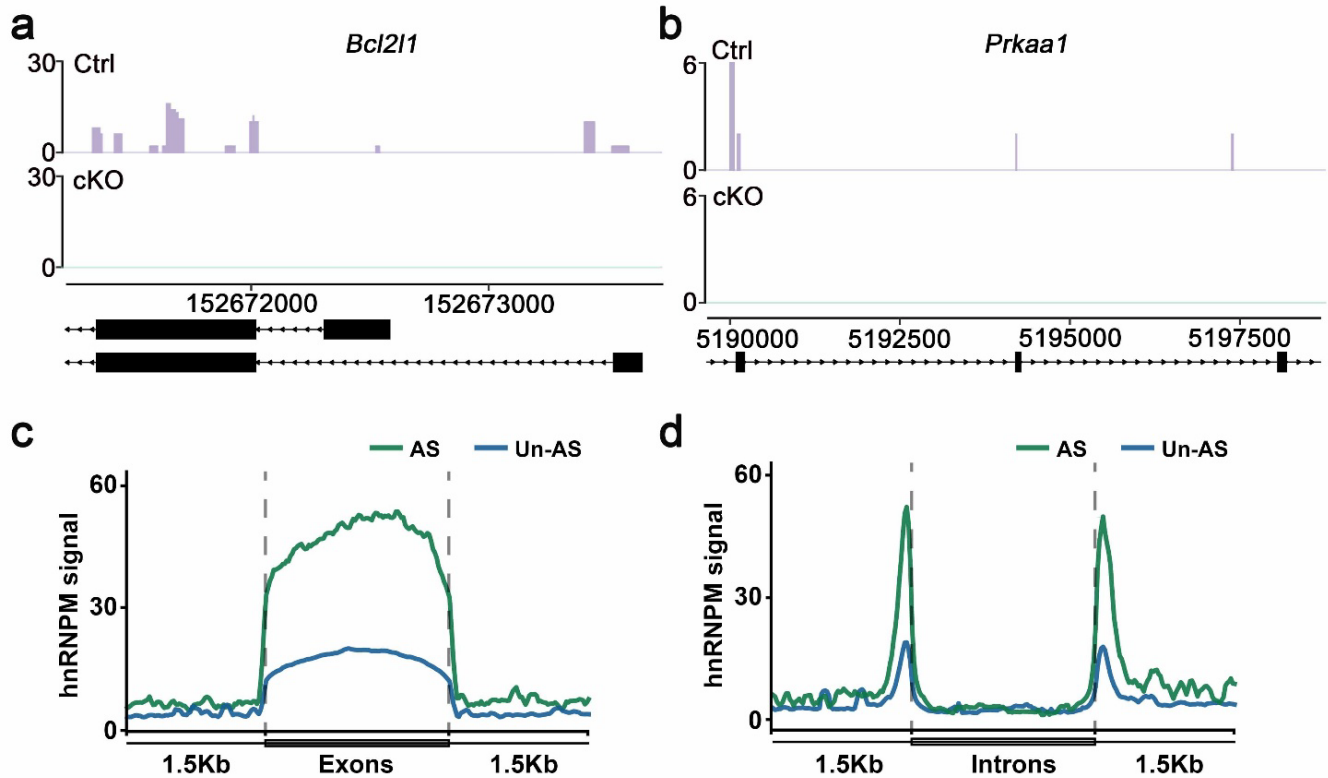
a Correlation heatmap of gene isoform expression in control and cKO GV oocytes. **b** Quantification of total, known and novel isoforms that identified in control and cKO GV oocytes. **c** Gene Ontology (GO) enrichment analysis using the international standard database (<https://www.geneontology.org/>) to investigate the biological processes involving novel transcripts. Source data are provided as a Source Data file. **d** Numbers of each category of annotated differential alternative splicing (AS) events detected in control and cKO GV oocytes.



Supplementary Fig. 4 Genetic ablation of *Hnrnp* alters the alternative splicing in oocytes.

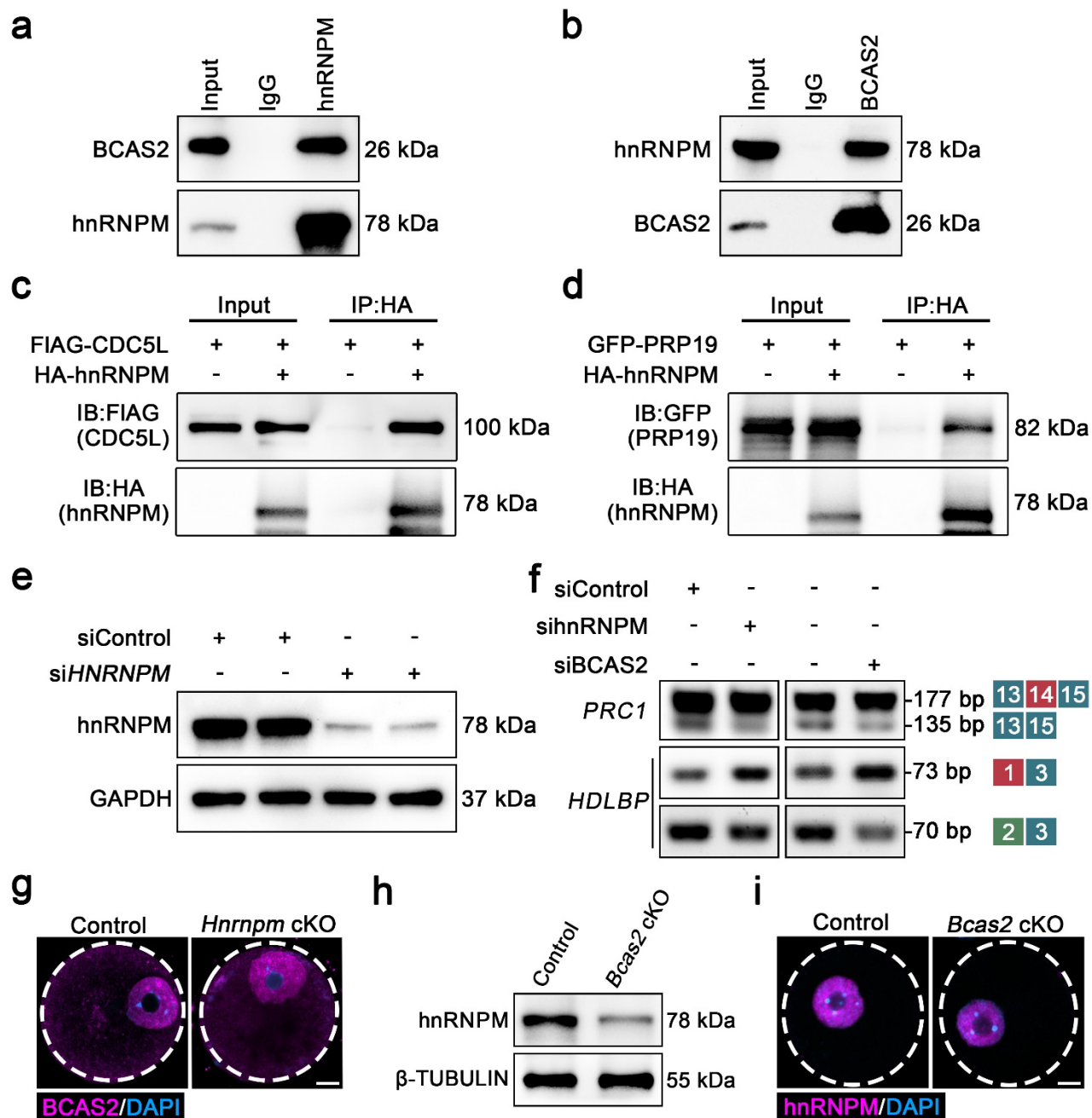
a Counts and categories of differential AS events (annotated, unannotated, and total) detected in GV oocytes after hnRNPM depletion. **b** The percentage of alternative splicing events with increased or decreased inclusion. **c** Diagrammatic representation of alternative splicing

67 quantification methodology using Percent Spliced In (PSI) values. PSI (Percent Spliced In)
68 represent the relative abundance of transcript containing a specific exon or splice site. **d** *Hnrnp*
69 deletion inducing aberrant splicing of female fertility-related transcripts (*Bcl2l1* and *Prkaa1*).
70 Two-sided student's t-test. Data are presented as mean $\pm$ SEM. n = 3. $p = 0.023338$ (*Bcl2l1*),
71 0.000259 (*Prkaa1*). **e** Western blot showing the expression of CENPH and ZAR1L in control and
72 cKO GV oocytes. All samples presented within a single figure panel (**d**, **e**) derive from the same
73 experiment and were processed in parallel. Representative results shown in panels (**d-e**) were
74 obtained from at least three independent experiments, with consistent findings observed in all
75 cases. Source data (**b**, **d**) are provided as a Source Data file.
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Supplementary Fig. 5 LACE-seq reveals hnRNPM binding landscape in oocytes.

a–b LACE-seq tracks reveal hnRNPM binding at the transcripts of female fertility-related genes (*Bcl2l1* and *Prkaa1*) in control (purple) oocytes, with disappeared signal in *Hnrnpm* cKO (green) oocytes. **c–d** hnRNPM binding signals on exons and introns that are alternatively spliced compared to those unaffected by hnRNPM depletion. AS, differentially alternative spliced transcripts. Un-AS, unaffected alternative spliced transcripts.

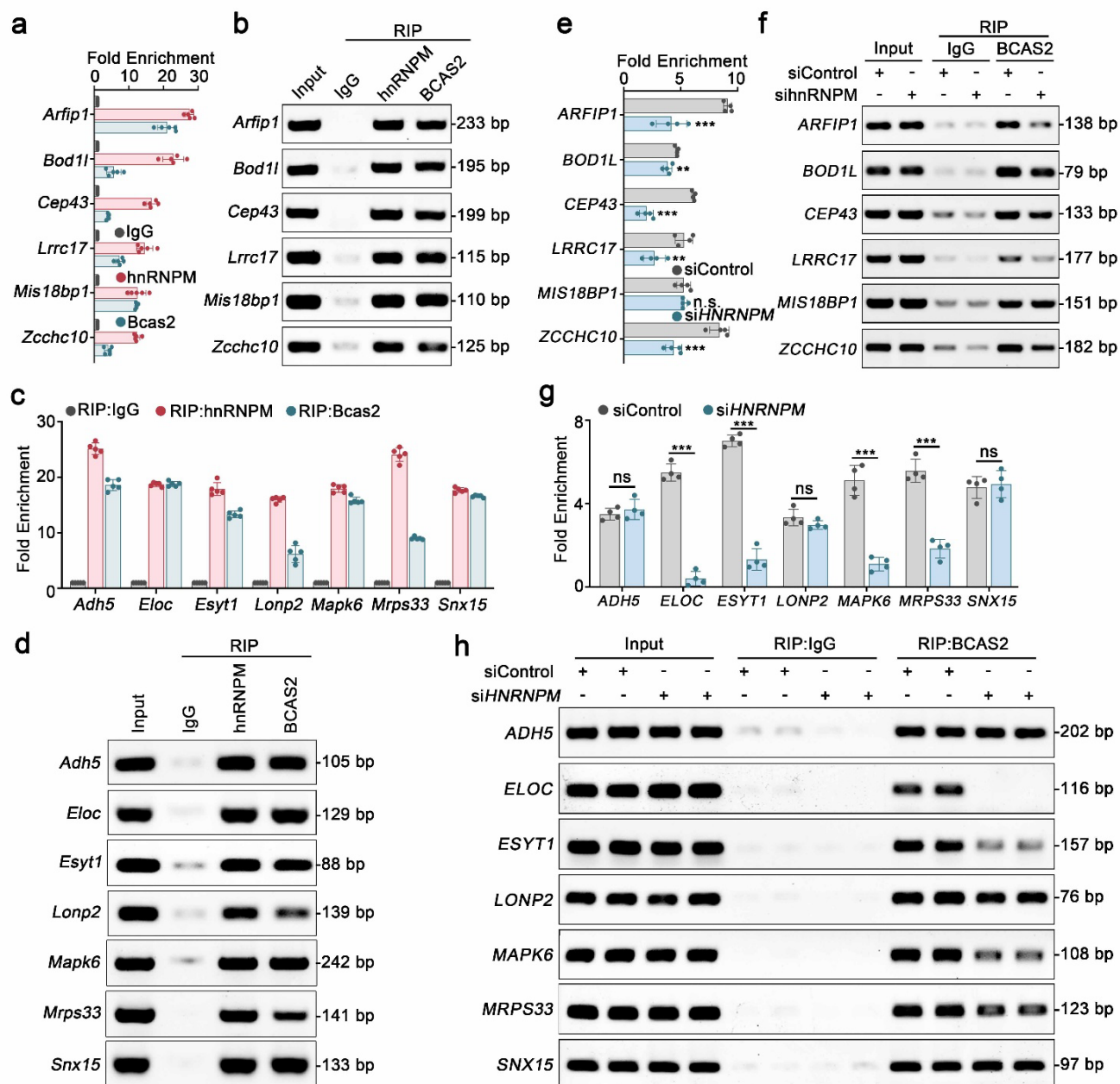


Supplementary Fig. 6 hnRNPM cooperates with BCAS2 to regulate alternative splicing.

a–b Co-immunoprecipitation (Co-IP) assays validating the interaction between hnRNPM and BCAS2 in mouse oocytes, with IgG serving as a negative control. **c–d** co-IP assays show that HA-tagged hnRNPM interacts with both FLAG-tagged CDC5L and GFP-tagged PRP19. **e** Western blotting confirmed the high knockdown efficiency of *HNRNPM* in HEK293T cells. **f** PCR validation of *Prc1* and *Hdlbp* splicing events in control and *HNRNPM*-knockdown 293T cells.

93 Schematic showing the alternatively spliced exons. **g** Representative images showing the
94 expression of BCAS2 (purple) in control and *Hnrnpm* cKO GV oocytes. Scale bars: 10 μ m. **h**
95 Western blot showing that protein level of hnRNPM in control and *Bcas2*-deficient oocytes. **i**
96 Representative images showing the expression of hnRNPM (purple) in control and *Bcas2* cKO
97 GV oocytes. Scale bars: 10 μ m. All samples presented within a single figure panel (**e-f, h**) derive
98 from the same experiment and were processed in parallel. Representative results shown in
99 panels (**a-i**) were obtained from at least three independent experiments, with consistent findings
100 observed in all cases.

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Supplementary Fig. 7 Loss of hnRNPM impairs the enrichment of BCAS2 on pre-mRNA.

a–d RIP-PCR and qPCR analysis of hnRNPM and BCAS2-interacted transcripts in P21 ovaries. Transcripts with abnormal exon skipping (**a–b**) and alternative first exon splicing (**c–d**) in both *Hnrnpm* cKO, and *Bcas2* cKO oocytes were examined. **e–h** RIP-PCR and qPCR analysis of BCAS2-RNA interactions of abnormal exon skipping genes (**e–f**) and alternative first exon splicing genes (**g–h**) in control and hnRNPM knockdown 293T cells. Two-sided student's t-test. Data are presented as mean $\pm$ SEM. $n = 5$. For Fig.7e, $p = 0.000395$ (*ARFIP1*), 0.006951

110 (*BOD1L*), 0.000011 (*CEP43*), 0.005575 (*LRRC17*), 0.884893 (*MIS18BP1*), 0.000337
111 (*ZCCHC10*). For Fig.7g, $p = 0.459562$ (*ADH5*), 0.000001 (*ELOC*), 0.000001 (*ESYT1*), 0.163727
112 (*LONP2*), 0.000051 (*MAPK6*), 0.000044 (*MRPS33*), 0.715035 (*SNX15*). Representative results
113 shown in panels (**b, f, d, h**) were obtained from at least three independent experiments, with
114 consistent findings observed in all cases. Source data (**a, e, c, g**) are provided as a Source Data
115 file.
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Fig.S6f

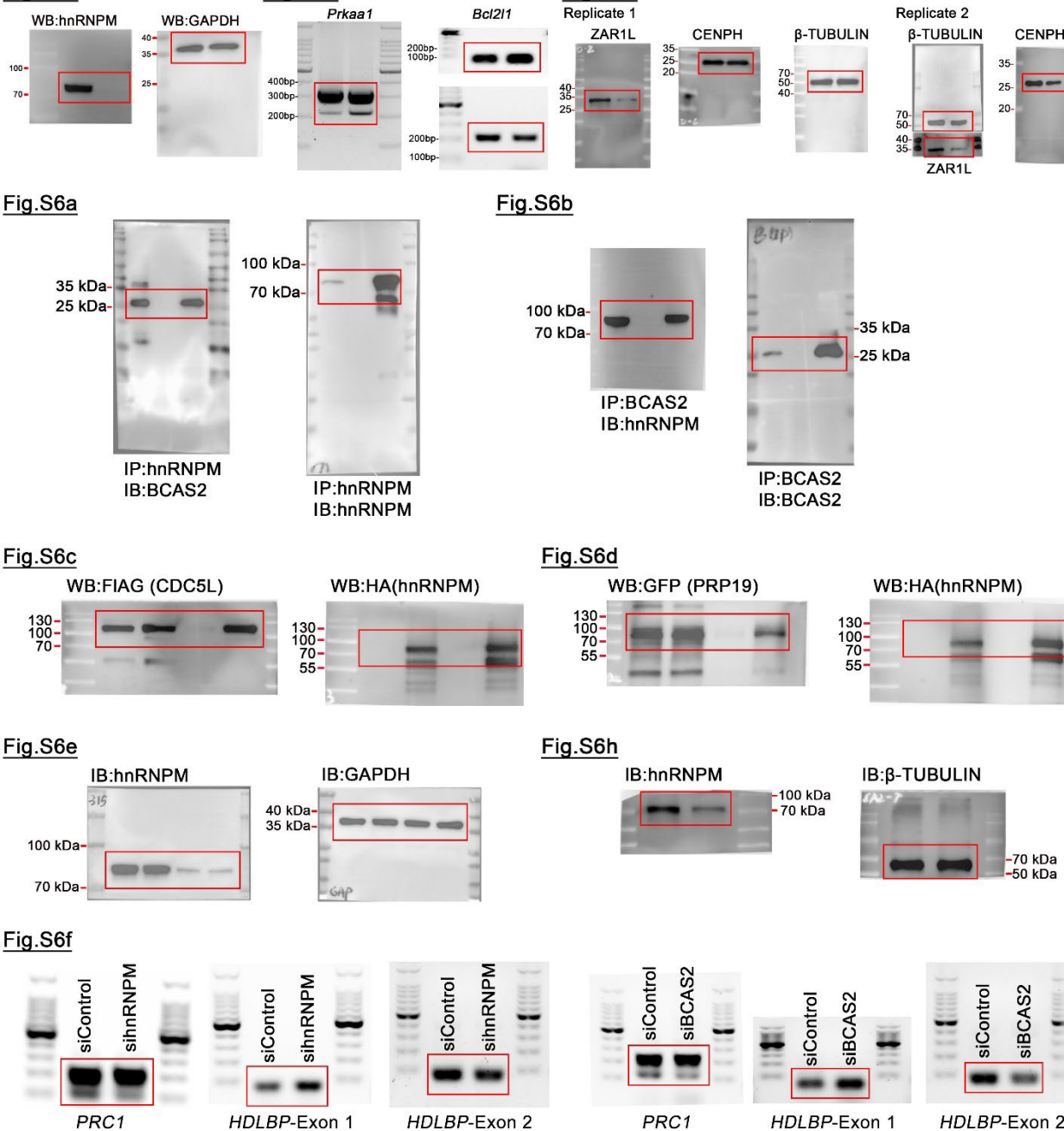


Fig.S7b

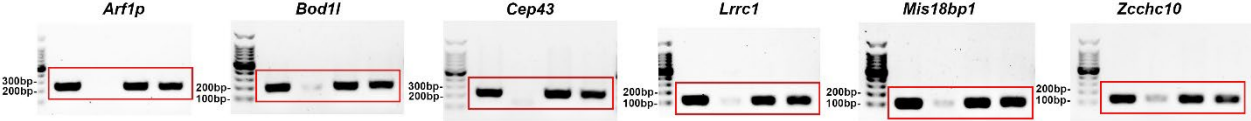


Fig.S7f

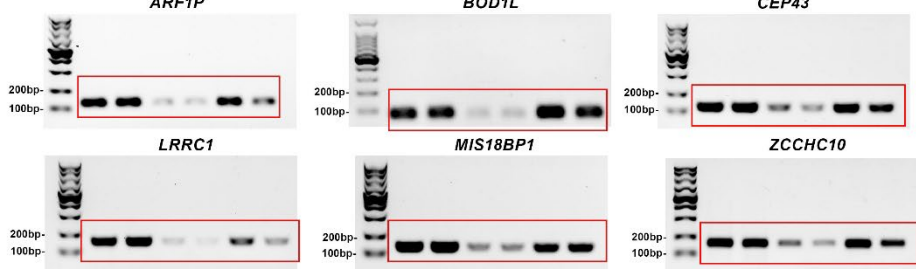


Fig.S7d

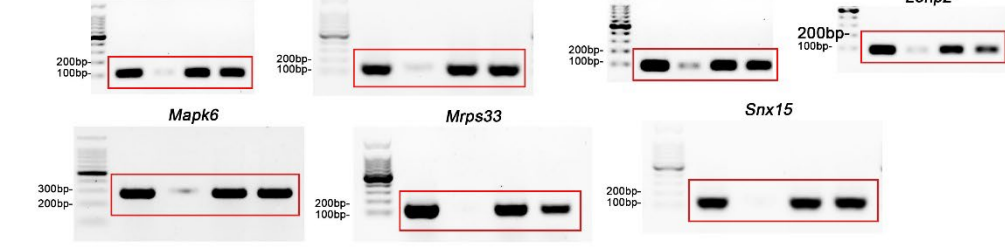


Fig.S7h

