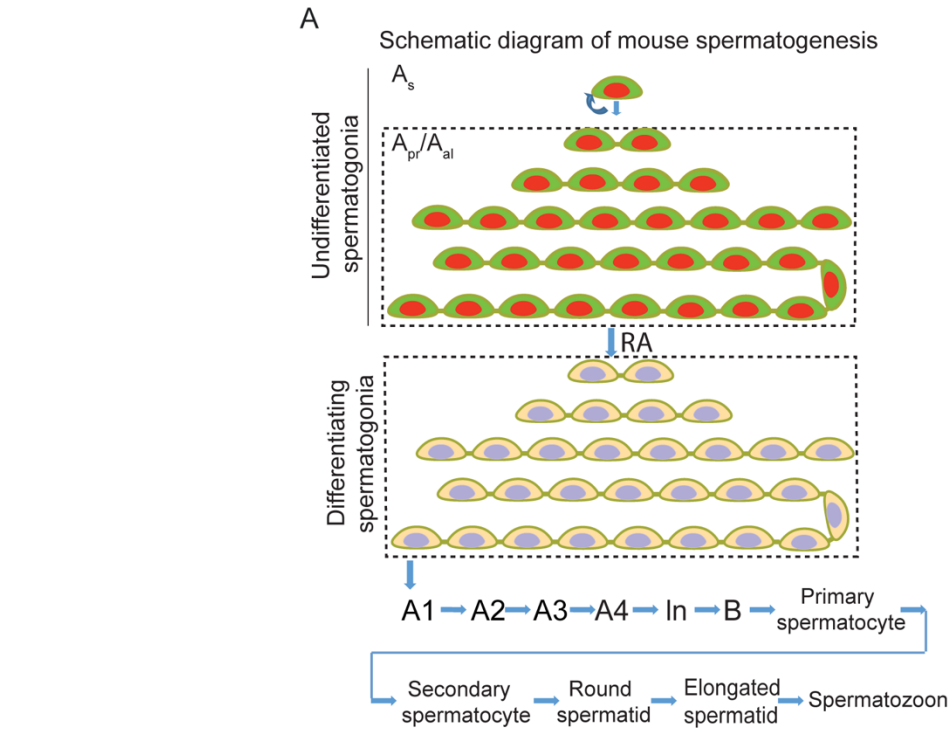


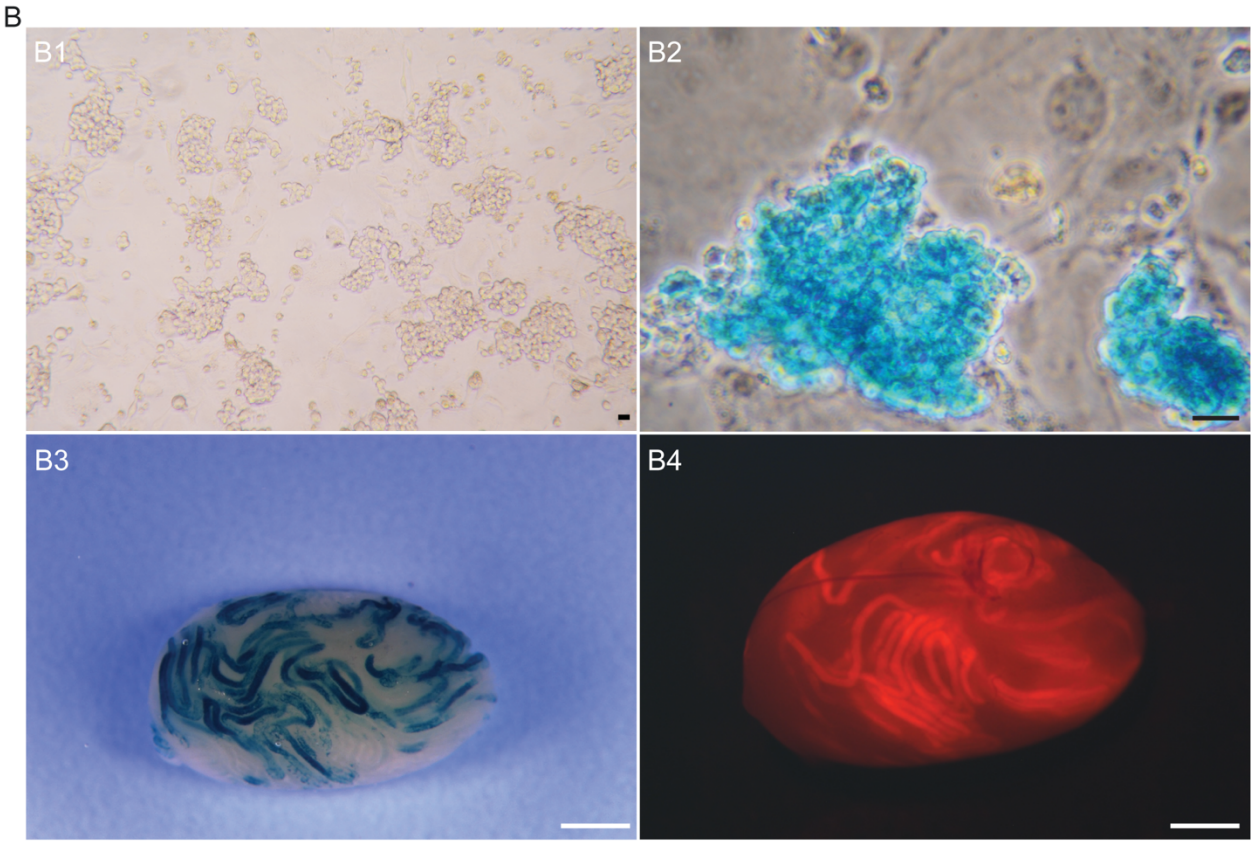
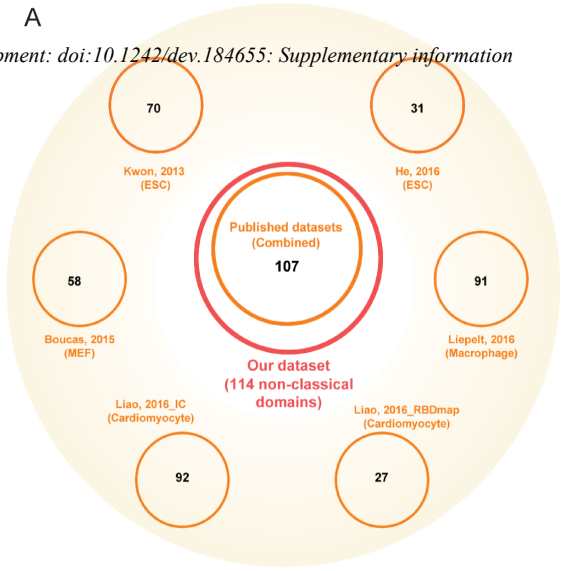
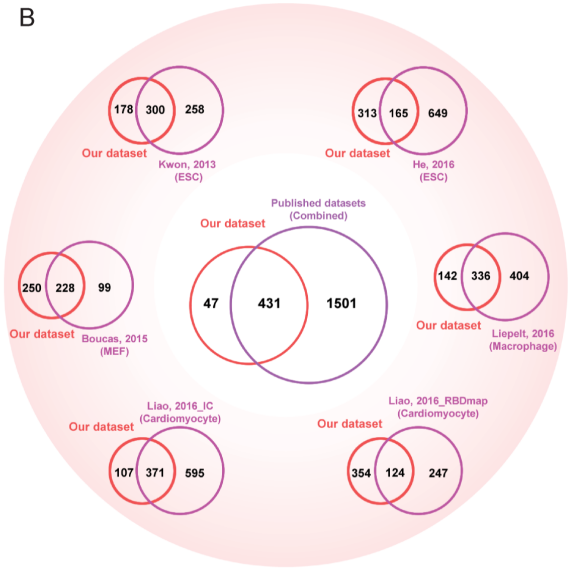


Figure S1: Stem cell identity and functional properties of primary undifferentiated spermatogonial cultures. (A) Basic schematic of spermatogenesis. (B) Primary cultures of undifferentiated spermatogonia established from C57BL/6 mice, B6;129S-Gt (ROSA) 26Sor/J (also known as Rosa-lacZ), or Gt (ROSA)26Sortm4(ACTB-tdTOMATO (also known as Rosa-tdTomato) transgenic mice were used for RBP capture experiments and exhibited typical grape-shaped morphology (B1, C57BL/6 mice), stained for beta-galactosidase reporter activity following X-gal incubation (B2, Rosa-lacZ transgenic mice), and generated colonies for donor-derived spermatogenesis following transplantation into recipient testes (B3-B4, Rosa-lacZ and Rosa-tdTomato transgenic mice). (Scale bars are 20 μm for cells and 1 mm for testes).

A



B



C

List of preferential cRBPs of spermatogonial progenitor cells

Protein ID	Gene name	Description	Class I	Class II
A2AWN8	*Ythdf1*	YTH domain family 1, isoform CRA_a	I	
E0CYD7	*Unkl*	Putative E3 ubiquitin - protein ligase UNKL	I	
F6QX82	*Drosha*	Ribonuclease 3	I	
J3QNW0	*Dnmt1*	DNA (cytosine - 5) - methyltransferase	I	
Q3TKR3	*Nlrp4c*	NACHT, LRR and PYD domains - containing protein 4C	I	
Q6VY05	*Dnd1*	Dead end protein homolog 1	I	
Q8R499	*Nxf2*	Nuclear RNA export factor 2	I	
E9PVX6	*Mki67*	Protein Mki67		II
G3UYU0	*Mex3a*	Mex3 RNA– binding family member A		II
J3QK52	*Noc2l*	Nucleolar complex protein 2 homolog		II
Q1PSW8	*Trim71*	E3 ubiquitin - protein ligase TRIM71		II
Q64368	*Dazl*	Deleted in azoospermia - like		II
Q80Y44	*Ddx10*	Probable ATP- dependent RNA helicase DDX10		II
Q8K3Y3	*Lin28a*	Protein lin - 28 homolog A		II
Z4YMQ5	*Esrp1*	Epithelial - splicing regulatory protein 1		II

Class I: preferentially expresses in undifferentiated spermatogonia
Class II: expresses both in ESCs and undifferentiated spermatogonia

D

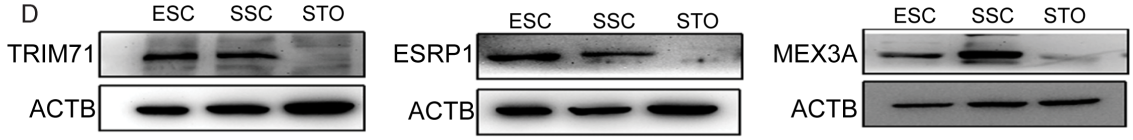


Figure S2: RBPs captured by mRBPome analysis that are preferentially expressed by primary cultures of undifferentiated spermatogonia. (A) Venn diagram analysis of non-classical RBP domains identified in the undifferentiated spermatogonial mRBPome compared to other mouse RBP datasets. (B) Venn diagram analysis of proteins identified in the undifferentiated spermatogonial mRBPome compared to other mouse RBP datasets. (C) Summary of 15 RBP genes that are preferentially expressed in mouse spermatogonia. (D) Validation of TRIM71, ESRP1, and MEX3A expression in ESC and undifferentiated spermatogonia (labeled as SSC here) by Western blot analysis. STO feeder cells were used as a negative control.

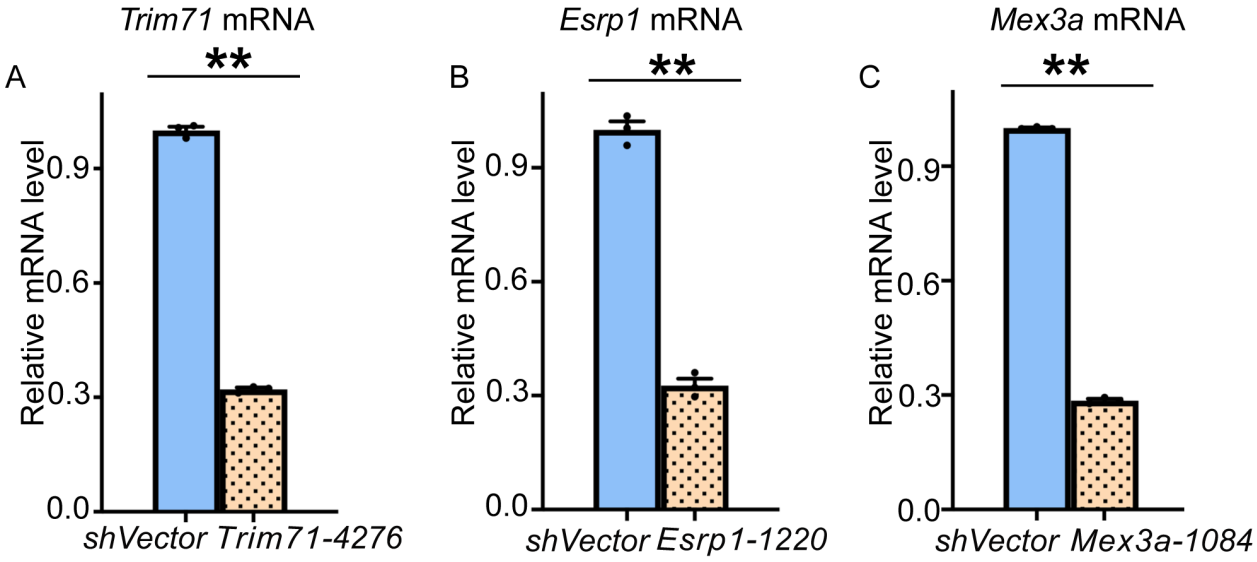


Figure S3: Knockdown efficiency of *Trim71*, *Esrp1*, and *Mex3a* in primary cultures of undifferentiated spermatogonia. (A-C) Quantitative real-time RT-PCR analysis of *Trim71* mRNA (A), *Esrp1* mRNA (B), and *Mex3a* mRNA (C) levels at 7 days after treatment with shRNA.

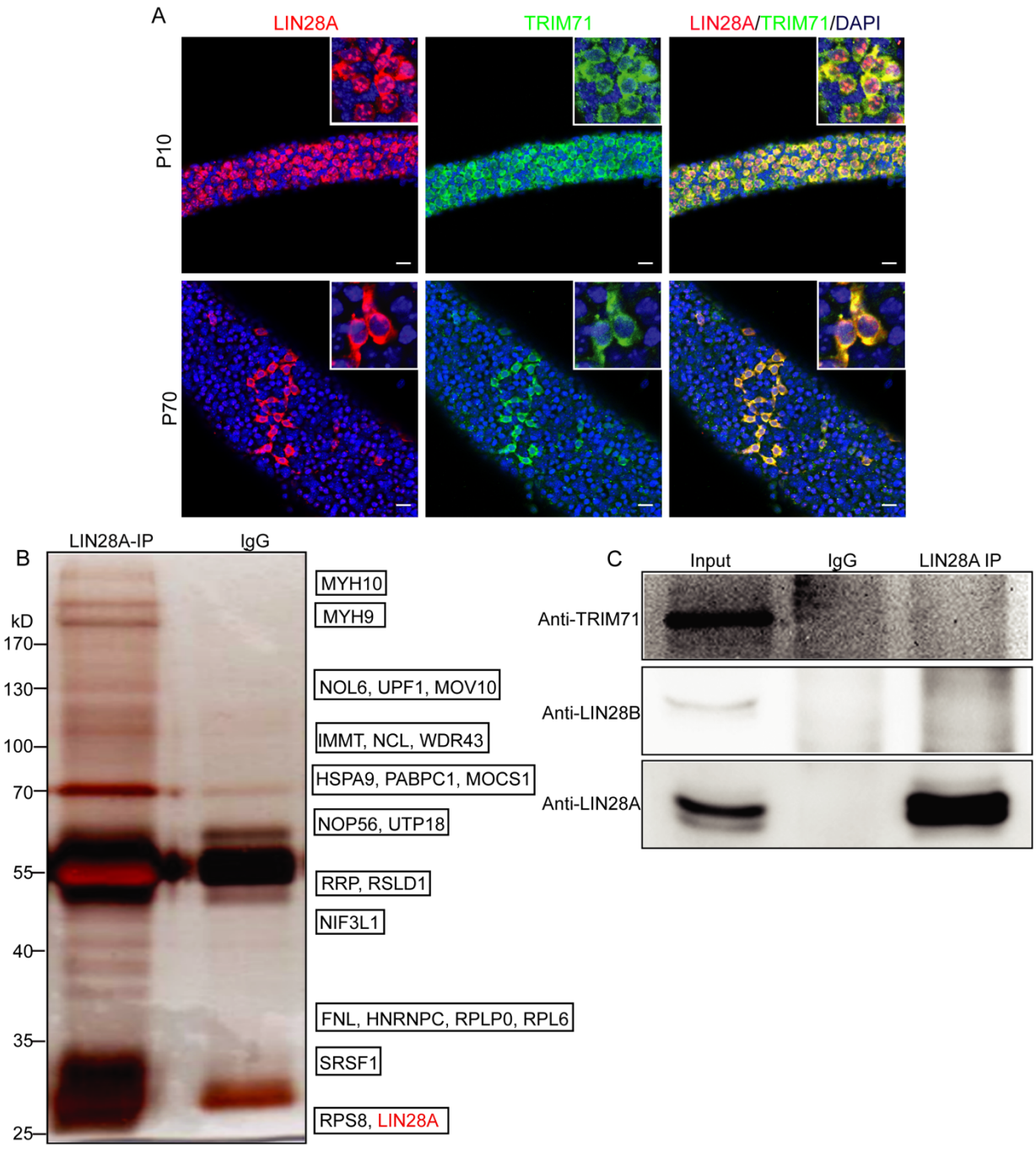


Figure S4: TRIM71 does not interact with LIN28A in mouse testes. (A) Whole-mount immunofluorescent staining for TRIM71 and LIN28A in seminiferous tubules of prepubertal (P10) and adult (P70) mice. (B) Silver staining of proteins isolated from P14 testis lysate following LIN28A IP and profiled using MS analysis. (C) Western blot analysis of TRIM71, LIN28A, and LIN28B in LIN28A IP. Scale bar: 20 µm.

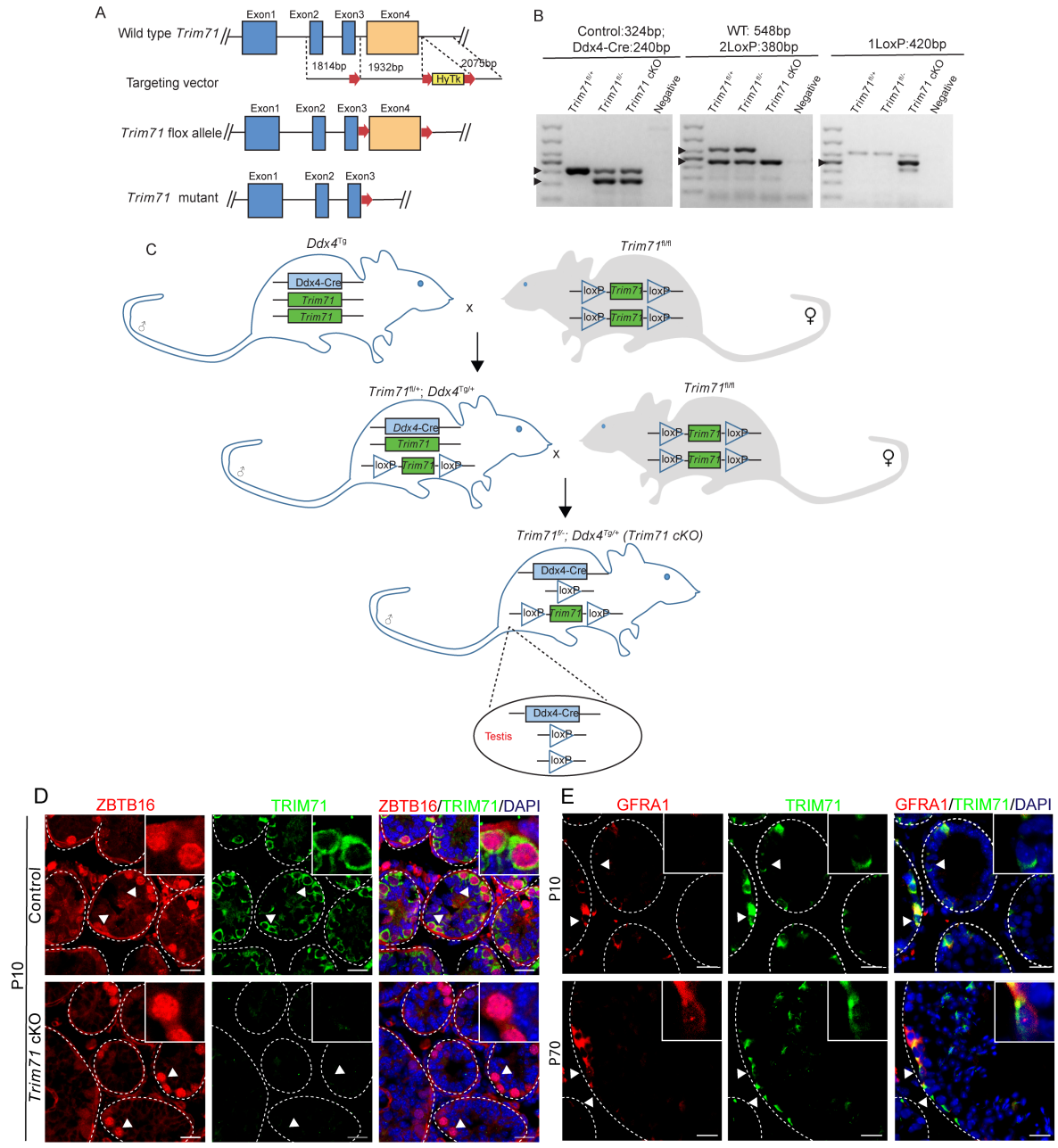


Figure S5: Generation of a *Trim71* cKO mouse model. (A) Schematic of the gene targeting strategy to produce a mouse line harboring a *Trim71* floxed allele. (B) PCR genotyping to confirm recombination of the *Trim71* flowed allele following introduction of the *Ddx4*-Cre transgene via backcrossing. (C) Schematic of the breeding scheme to produce males with germ cell conditional knockout of *Trim71*. (D) Immunofluorescent staining for ZBTB16 and TRIM71 in cross-sections of testes from control and *Trim71* cKO mice at P10. (E) Co-localization of TRIM71 and the primitive spermatogonial marker GFRA1 in cross-sections of seminiferous tubules from prepubertal (P10) and adult (P70) mice. Scale bars: 20 µm.

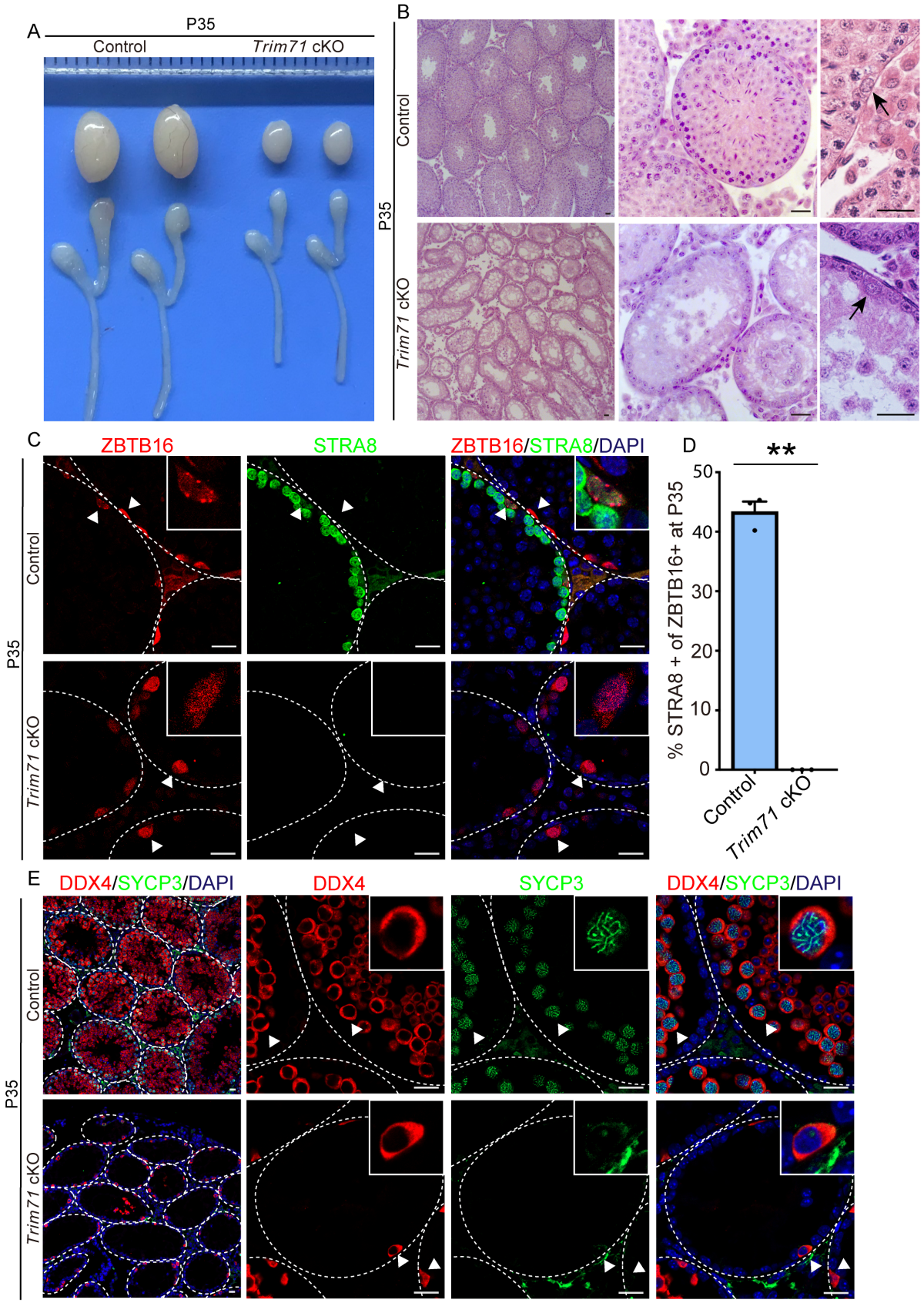


Figure S6: Male mice with germ cell conditional knockout of *Trim71* lack a first round of spermatogenesis. (A) Morphology of testes and epididymis from P35 control and *Trim71* cKO mice. (B) Representative images of H&E stained seminiferous tubule cross-sections from testes of P35 control and *Trim71* cKO mice. (C-D) Representative images (C) and quantification (D) from immunofluorescence staining for ZBTB16 and STRA8 in cross-sections of testes from control and *Trim71* cKO mice at P35. (E) Representative images of immunofluorescence staining for DDX4 and SYCP3 in cross-sections of testes from control and *Trim71* cKO mice at P35. Quantification in D is presented as mean±*SEM* from n = 3 biologically independent mice of each genotype, dots represent average values of individual mice, ** denotes significantly different at $p<0.01$. Scale bars: 20 µm.

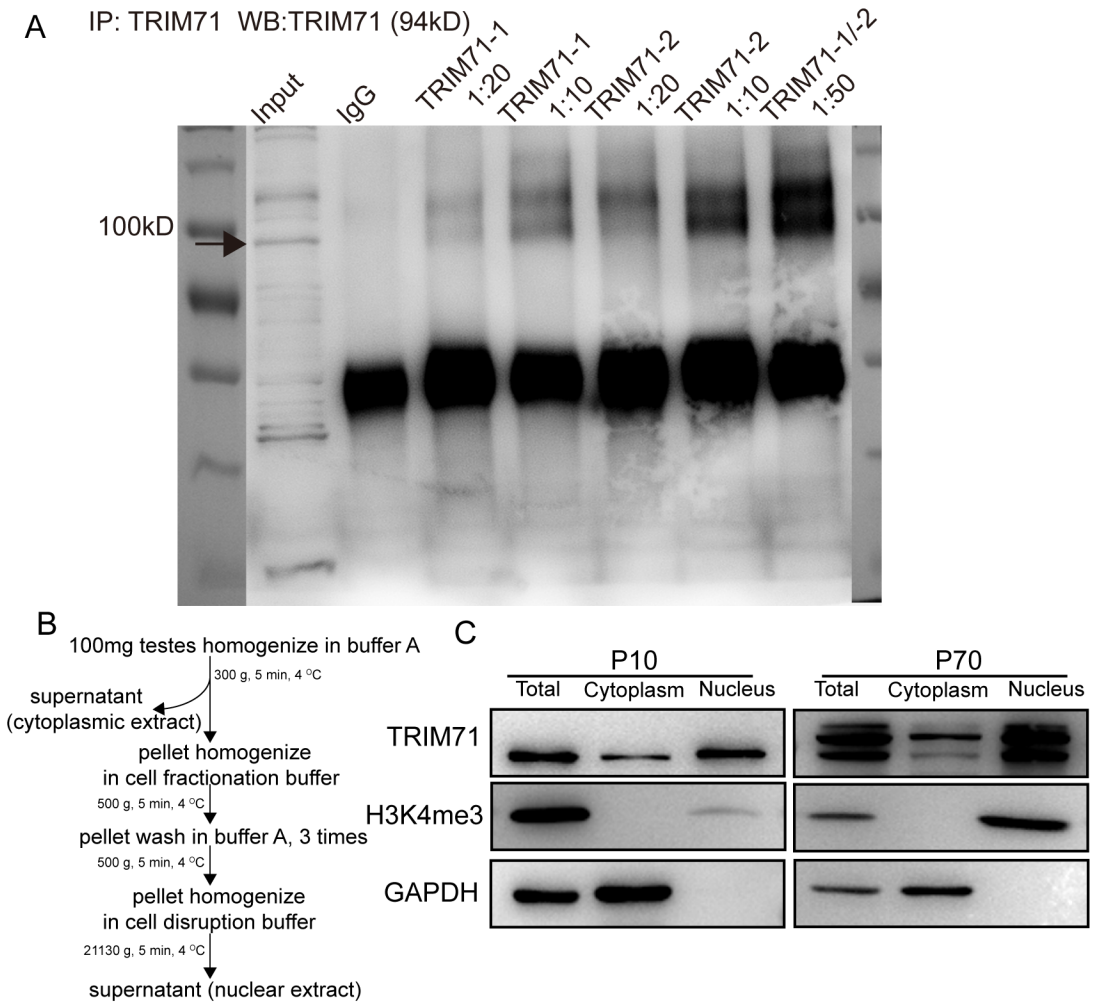


Figure S7: TRIM71 in nuclear and cytoplasmic fractions (A) Western blot (WB) analysis of TRIM71 confirms the generated TRIM71 antibodies work in IP. (B) Purification of cytoplasm and nucleus of testes and (C) expression of TRIM71 in cytoplasm and nucleus of testes at P10 and P70.

Supplementary Tables

Table S1. Characteristics of 473 proteins identified in the mRBPome capture from primary cultures of undifferentiated spermatogonia.

Click here to Download Table S1

Table S2. Protein mass spectrometry from three independent TRIM71 immunoprecipitation in the mouse testes.

Click here to Download Table S2

Table S3. Primers and shRNA sequences used in this study.

Click here to Download Table S3

Table S4. Primary antibodies and secondary antibodies used in this study.

Click here to Download Table S4