Table S1. PCR primer sequence

Genes	Primer sequence
Stra8-GFPcre	F:ACTCCAAGCACTGGGCAGAA
	R1:GCCACCATAGCAGCATCAAA
	R2:CGTTTACGTCGCCGTCCAG
Vasa-Cre	F:AAGAACCTGATGGACATGTTCAG
	R:CTGATCCTGGCAATTTCGG
Rpn1	R:GGCAGGGAGATATATCAGTGGGTAAA
	F1:GGGTTCTCTGTGAAATTGTTCATGTTC
	F2:GGACCATTTAGGAAGTGAATGTGGA

PCR Reaction Component

Reaction component	Volume (μl)
2 × Taq Master Mix, Dye Plus, (Vazyme P112-03)	12.5
ddH2O	9.5
Primer F(10pmol/μl)	1
Primer R(10pmol/μl)	1
Template	1

PCR program

Temp.	Time	Cycle
95℃	5min	
98℃	30s	20×
65℃（-0.5℃/cycle）	30s	
72℃	45s	
98℃	30s	20×
55℃	30s	
72℃	45s	

72℃	5min	
10℃	hold	

RT-qPCR primer sequence

Rpn1	F:GCTCCACATCACGAGCCAG
	R:CAGTTTCCACAACGACCGAGA
Xbp1s	F:TGCTGAGTCCGCAGCAGGTG
	R:GCTGGCAGGCTCTGGGGAAG
Perk	F:TGGTGTCATCCAGCCTTAGCAA
	R:CATGCTTTCACGGTCTTGGTCC
Ire1α	F:ACTTTGTCATCGGCCTTTGCAG
	R:AGTGAGGCCGCATAGTCAAAGT
Bip	F:TCTGTGCAGCAGGACATCAAGT
	R:GTCTTTGTTTGCCCACCTCCAA
Chop	F:GGGAAACAGCGCATGAAGGA
	R:GCGTGATGGTGCTGGGTACA
Atf6	F:TGGGCAGGACTATGAAGTAATG
	R:CAACGACTCAGGGATGGTGCTG
Atf4	F:ATGGCGCTCTTCACGAAATC
	R:ACTGGTCGAAGGGGTCATCAA
Traf2	F:AGAGAGTAGTTCGGCCTTTCC
	R:GTGCATCCATCATTGGGACAG
β-Actin	F:GGGTCAGAAGGATTCCTATG
	R:GGTCTCAAACATGATCTGGG
Gapdh	F:GTCATTGAGAGCAATGCCAG
	R:GTGTTGCTACCCCCAATGTG

Table S2. Antibody information

Primary antibodies	Vendor	Dilution	Source
RPN1 (IF/WB)	Santa Cruz Biotechnology (SC48367)	1:200/1:1000	Mouse
γH2AX(Ser139) (IF/WB)	Millipore (05-636)	1:1000	Mouse
β-Actin (WB)	Proteintech(66009-1-Ig)	1:1000	Rabbit
MVH (IF/WB)	Abcam (ab13840)	1:1000	Rabbit
DMC1 (IF)	Santa Cruz Biotechnology (sc-22768)	1:500	Rabbit
RAD51 (IF)	Thermo Fisher Scientific (PA5-27195)	1:500	Rabbit
SYCP3 (IF)	Abcam (ab97672)	1:500	Mouse
SYCP3 (IF)	Abcam (ab15093)	1:500	Rabbit
SYCP1 (IF)	Abcam (ab15090)	1:500	Rabbit
LAMIN B1 (WB)	Proteintech(12987-1-AP)	1:1000	Rabbit
PLZF (IF/WB)	Santa Cruz Biotechnology (SC28319)	1:500	Mouse
c-Kit (IF)	Proteintech (18696-1-AP)	1:500	Rabbit
SOX9 (IF/WB)	Millipore (3389351)	1:500	Rabbit
STRA8 (IF/WB)	Abcam (ab49602)	1:500	Mouse
H1t (IF)	Abcam (ab81498)	1:500	Rabbit
MLH1 (IF)	Abcam (ab92312)	1:500	Rabbit
RPA2 (IF)	Abcam (ab76420)	1:500	Rabbit
BIP (WB)	Proteintech (11587-1-AP)	1:1000	Rabbit
XBP1s (WB)	Proteintech (24868-1-AP)	1:1000	Rabbit
ATF6 (WB)	Proteintech (24169-1-AP)	1:1000	Rabbit
CHOP (WB)	Proteintech (15204-1-AP)	1:1000	Rabbit
p-eIF2α (WB)	Cell Signaling Technology	1:1000	Rabbit

	(D9G8)		
p-PERK (WB)	Affinity (DF7576)	1:1000	Rabbit
PERK (WB)	Abmart (MA8131S)	1:1000	Rabbit
HSP90B1 (WB)	Santa Cruz Biotechnology (sc-517405)	1:1000	Mouse
HYOU1 (WB)	Santa Cruz Biotechnology (sc-398224)	1:1000	Rabbit
ERO1A (WB)	Proteintech (12007-1-AP)	1:1000	Rabbit
BSG (WB)	Proteintech (11989-1-AP)	1:1000	Rabbit
STT3A (WB)	Proteintech (12034-1-AP)	1:1000	Rabbit
STT3B (WB)	Proteintech (15323-1-AP)	1:1000	Rabbit
DAD1 (WB)	Abclonal (A14723)	1:1000	Rabbit
DDOST (WB)	Abclonal (A9056)	1:1000	Rabbit
BAX (WB)	Proteintech (50599-2-Ig)	1:1000	Rabbit
BCL2 (WB)	Proteintech (26593-1-AP)	1:1000	Rabbit
Caspase 3 (WB)	Cell Signaling Technology (9662)	1:1000	Rabbit
Cleaved Caspase 3 (WB)	Cell Signaling Technology (9664)	1:1000	Rabbit
Alexa Fluor 488 Goat anti-Mouse IgG	Abcam (ab150117)	IF (1:500)	Goat
Alexa Fluor 594 Goat anti-Rabbit IgG	Abcam (ab150080)	IF (1:500)	Goat
Alexa Fluor 594 Goat anti-Mouse IgG	Abcam (ab150120)	IF (1:500)	Goat
Alexa Fluor 488 Goat anti-Rabbit IgG	Abcam (ab150077)	IF (1:500)	Goat
HRP-conjugated goat anti- Mouse IgG	Beyotime (A0216)	WB (1:1000)	Goat

HRP-conjugated goat anti- rabbit IgG (WB)	Beyotime (A0258)	WB (1:1000)	Goat

12

13 **Table S3. Alterations in Protein Glycosylation Site**

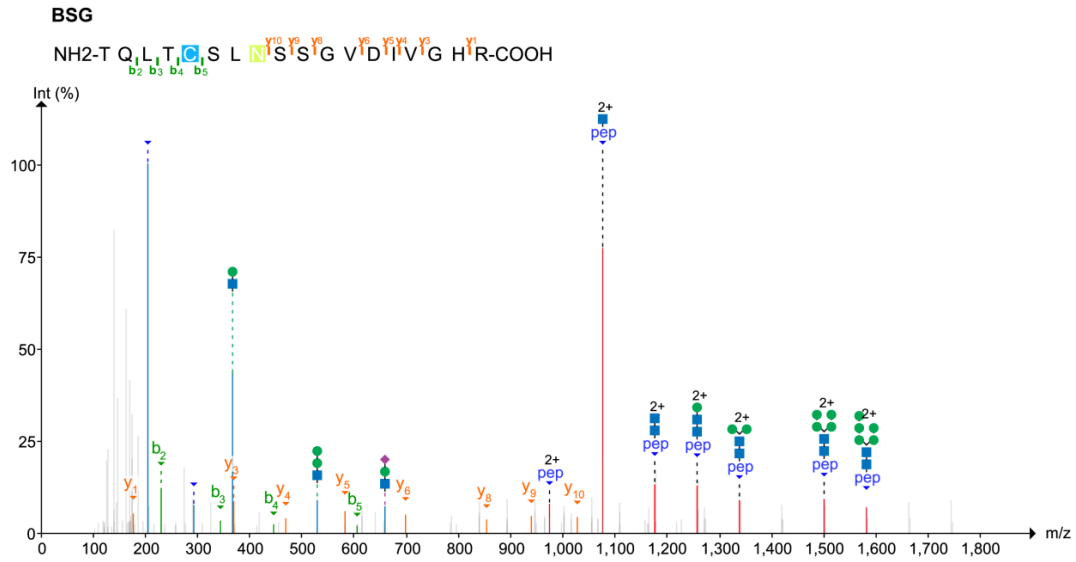
Protein name	Glycosylation Sites	Observed Modification	Assigned Modification	SKO/WT Ratio	Regulated Type
ITGB5	N[347]	HexNAc(2)Hex(8)	1702.5813	0.615999028	Down
HSP90B1	N[62]	HexNAc(2)Hex(6)	1378.4757	0.372636373	Down
HSP90B1	N[217]	HexNAc(2)Hex(8)	1702.5813	0.650323658	Down
ITGB1	N[669]	HexNAc(2)Hex(6)	1378.4757	0.635035799	Down
SLC3A2	N[385]	HexNAc(2)Hex(7)	1540.5285	0.660697221	Down
SLC3A2	N[385]	HexNAc(2)Hex(9)	1864.6341	0.623229373	Down
LAMP2	N[322]	HexNAc(2)Hex(6)	1378.4757	0.507568378	Down
CTSD	N[134]	HexNAc(2)Hex(7)	1540.5285	0.256563492	Down
BSG	N[160]	HexNAc(2)Hex(9)	1864.6341	0.476613385	Down
LAMA1	N[45]	HexNAc(2)Hex(9)	1864.6341	0.262010587	Down
ABCA1	N[1637]	HexNAc(2)Hex(9)	1864.6341	0.12599944	Down
STT3A	N[537]	HexNAc(2)Hex(9)	1864.6341	0.23900947	Down
LAMA4	N[308]	HexNAc(4)Hex(5)Fuc(1)	1768.6395	0.570055025	Down
COl6A2	N[912]	HexNAc(4)Hex(5)Fuc(1)	1768.6395	0.127530896	Down
HSPG2	N[3098]	HexNAc(3)Hex(5)Fuc(1)	1565.5601	0.129190029	Down
STT3B	N[613]	HexNAc(2)Hex(9)	1864.6341	0.240063169	Down
HSPA13	N[184]	HexNAc(2)Hex(8)	1702.5813	0.361818703	Down
COLGALlT1	N[376]	HexNAc(4)Hex(4)	1460.5288	0.585103856	Down
ERO1A	N[276]	HexNAc(2)Hex(8)	1702.5813	0.550938518	Down
TMED4	N[117]	HexNAc(5)Hex(3)Fuc(1)	1647.6132	0.38151888	Down
MCAM	N[58]	HexNAc(5)Hex(4)Fuc(1) NeuAc(1)	2100.7615	0.528611452	Down
LRP1	N[1512]	HexNAc(2)Hex(6)	1378.4757	0.614586827	Down

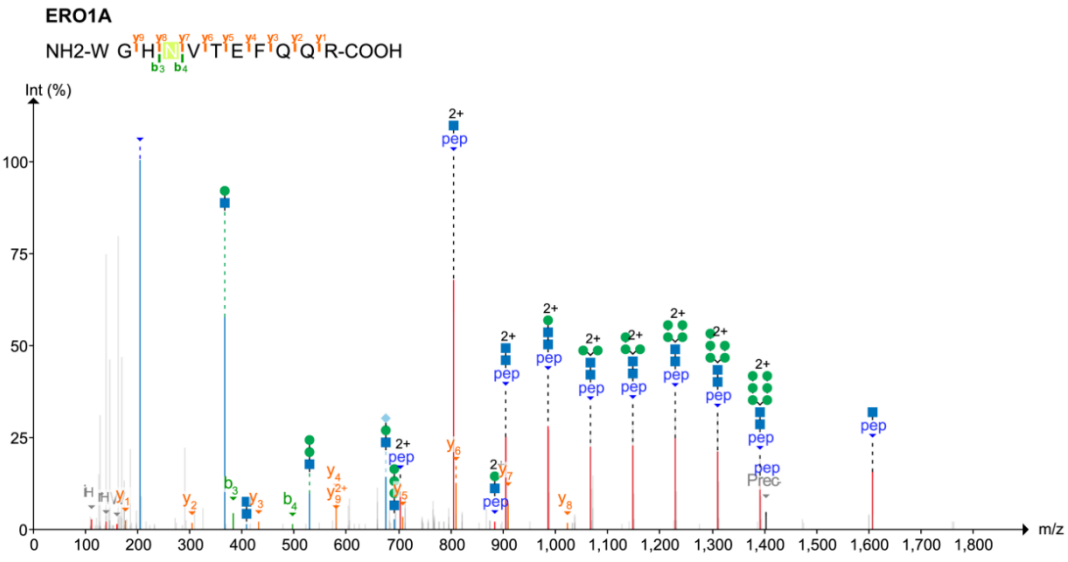
TMED9	N[125]	HexNAc(4)Hex(3)Fuc(1)	1444.5338	0.66556221	Down
CRTAP	N[362]	HexNAc(2)Hex(8)	1702.5813	0.340694912	Down
DPEP3	N[240]	HexNAc(4)Hex(4)Fuc(1)	1606.5867	0.251143261	Down
HYOU1	N[515]	HexNAc(2)Hex(9)	1864.6341	0.656782783	Down
HYOU1	N[830]	HexNAc(2)Hex(7)	1540.5285	0.502468366	Down
HYOU1	N[830]	HexNAc(2)Hex(8)	1702.5813	0.485332445	Down
TEX101	N[45]	HexNAc(4)Hex(4)Fuc(1)	1606.5867	0.301223764	Down
TEX101	N[45]	HexNAc(4)Hex(5)Fuc(1)	1768.6395	0.142273473	Down
TEX101	N[134]	HexNAc(2)Hex(7)	1540.5285	0.346869026	Down
TEX101	N[134]	HexNAc(2)Hex(8)	1702.5813	0.361086511	Down
TEX101	N[160]	HexNAc(2)Hex(8)	1702.5813	0.484853608	Down
NUP210	N[405]	HexNAc(2)Hex(9)	1864.6341	0.482313481	Down
PLOD2	N[696]	HexNAc(2)Hex(7)	1540.5285	0.494405884	Down

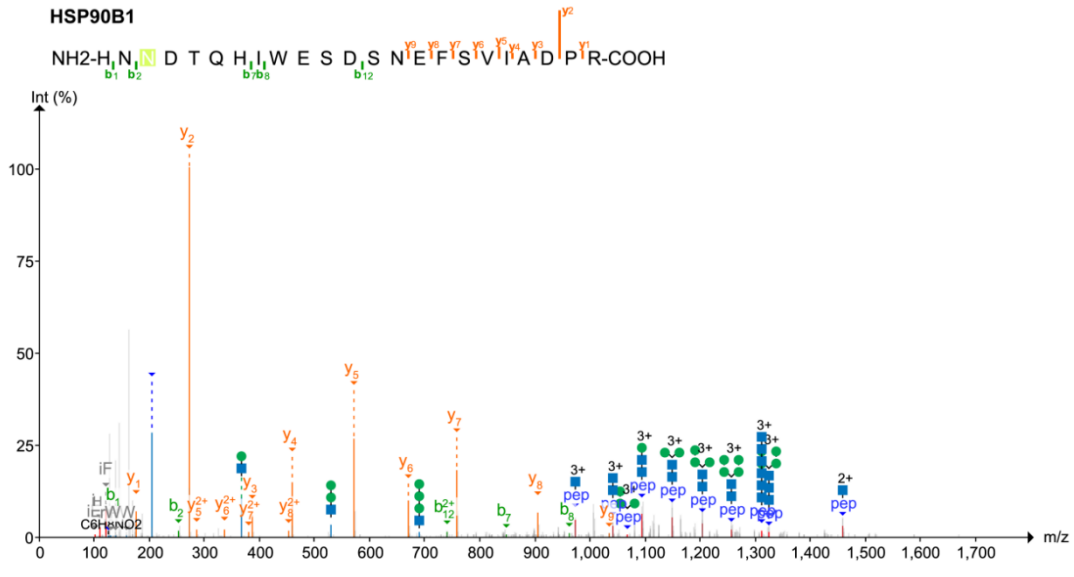
Protein name	Glycosylation Sites	Observed Modification	Assigned Modification	SKO/WT Ratio	Regulated Type
HSP90B1	N[445]	HexNAc(2)Hex(8)	1702.5813	3.792752041	Up
HSP90B1	N[481]	HexNAc(2)Hex(8)	1702.5813	2.282822295	Up
HSP90B1	N[481]	HexNAc(2)Hex(9)	1864.6341	2.999030985	Up
HSP90B1	N[502]	HexNAc(2)Hex(7)	1540.5285	3.904399469	Up
ACP2	N[267]	HexNAc(2)Hex(9)	1864.6341	2.075285066	Up
BGN	N[312]	HexNAc(5)Hex(5)Fuc(1)	1971.7189	1.704358073	Up
LUM	N[127]	HexNAc(4)Hex(6)NeuAc(1)	2075.7298	2.042297464	Up
GXYlT1	N[201]	HexNAc(2)Hex(9)	1864.6341	1.622291229	Up
ERLIN2	N[106]	HexNAc(2)Hex(9)	1864.6341	11.24429231	Up
BTD	N[326]	HexNAc(2)Hex(6)	1378.4757	7.710842273	Up
LRP1	N[1826]	HexNAc(2)Hex(7)	1540.5285	1.535336334	Up
LRP1	N[3840]	HexNAc(2)Hex(7)	1540.5285	1.888928898	Up
TEX101	N[45]	HexNAc(5)Hex(4)Fuc(1)	1809.666	1.983835733	Up

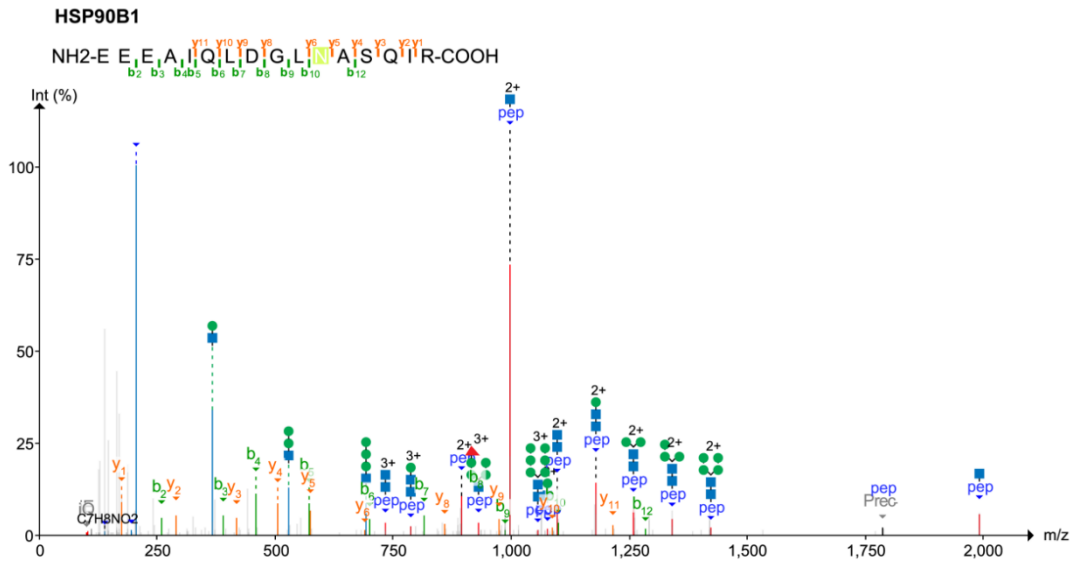
TEX101	N[160]	HexNAc(2)Hex(10)	2026.6869	2.046098645	Up
SEMA7A	N[256]	HexNAc(2)Hex(8)	1702.5813	16.49727389	Up

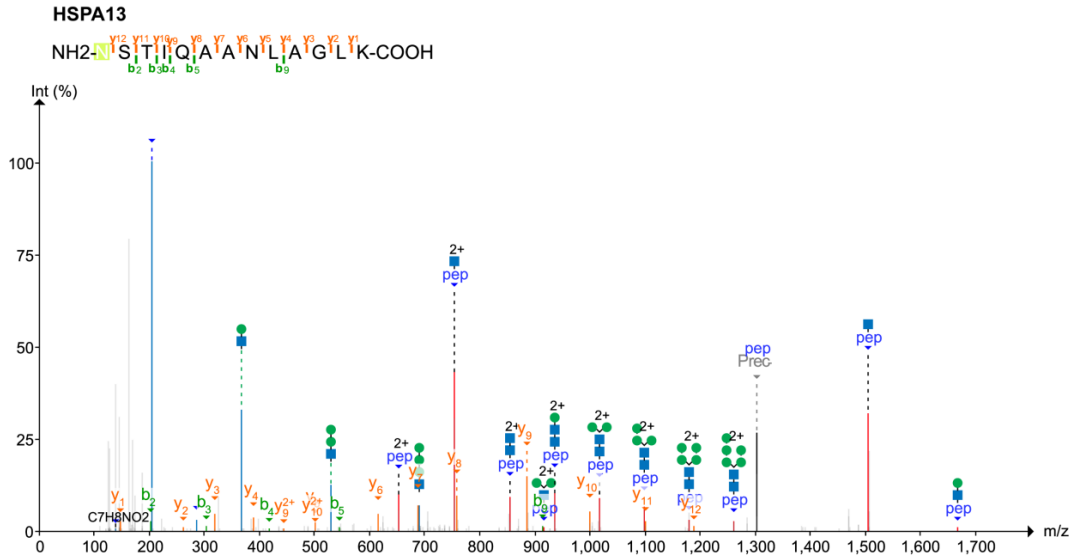
Table S4. The secondary spectra of proteins.

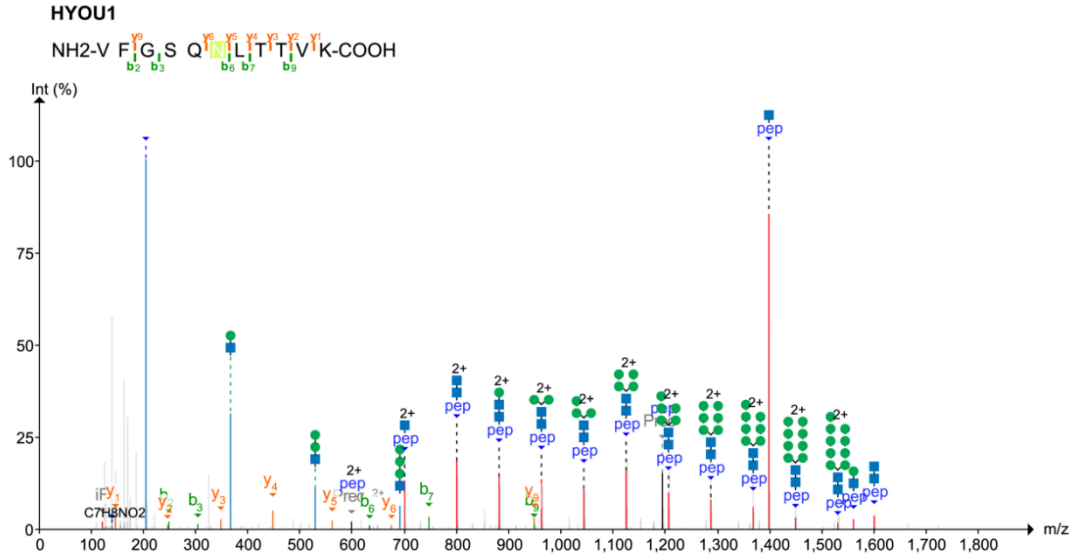


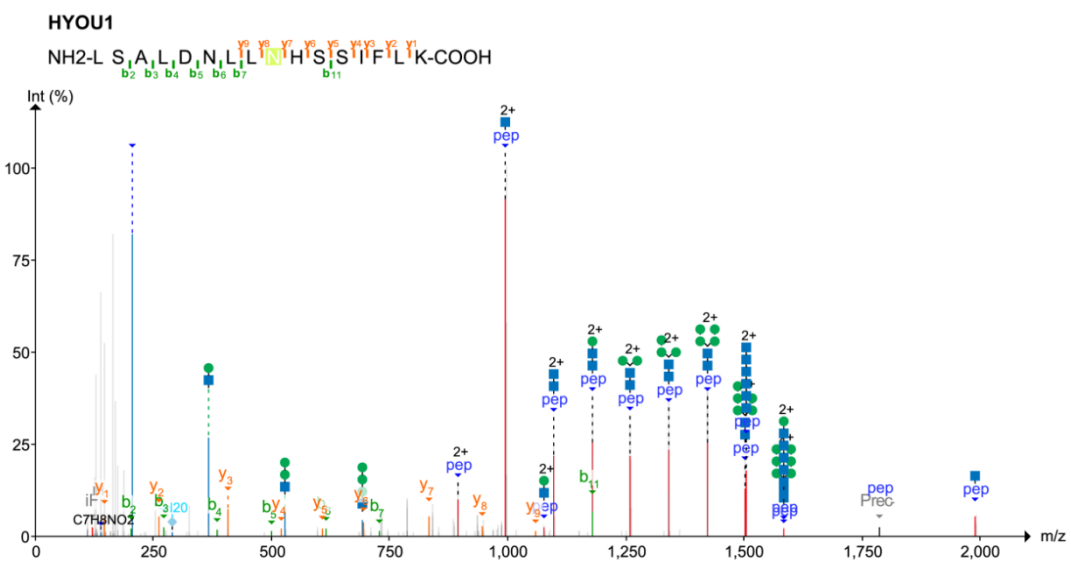


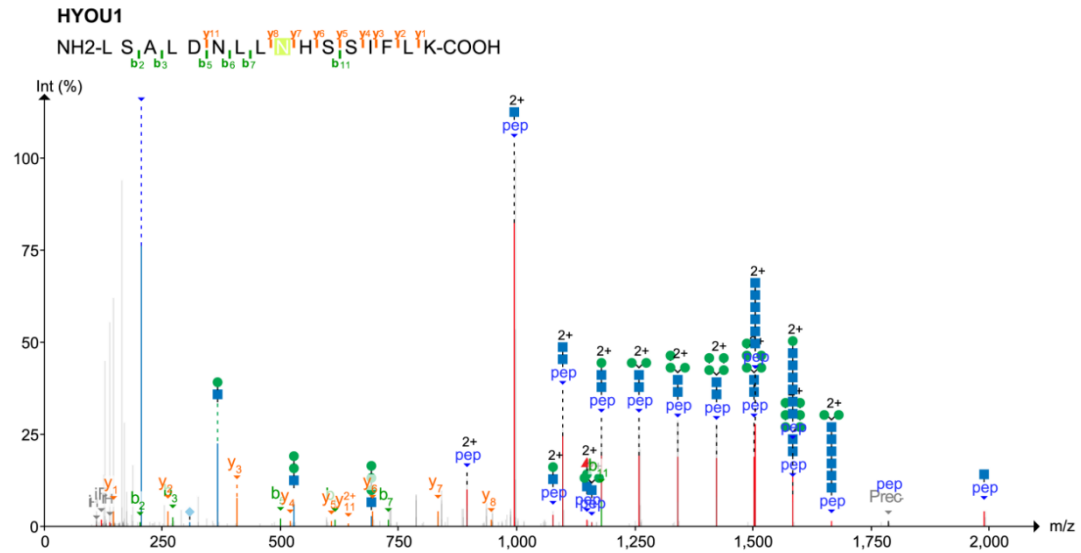

HSP90B1
NH2-H N N D T Q H I W E S D S N E F S V I A D P R-COOH
Int (%)
m/z


HSP90B1
NH2-E E A I Q L D G L N A S Q I R-COOH
Int (%)
m/z

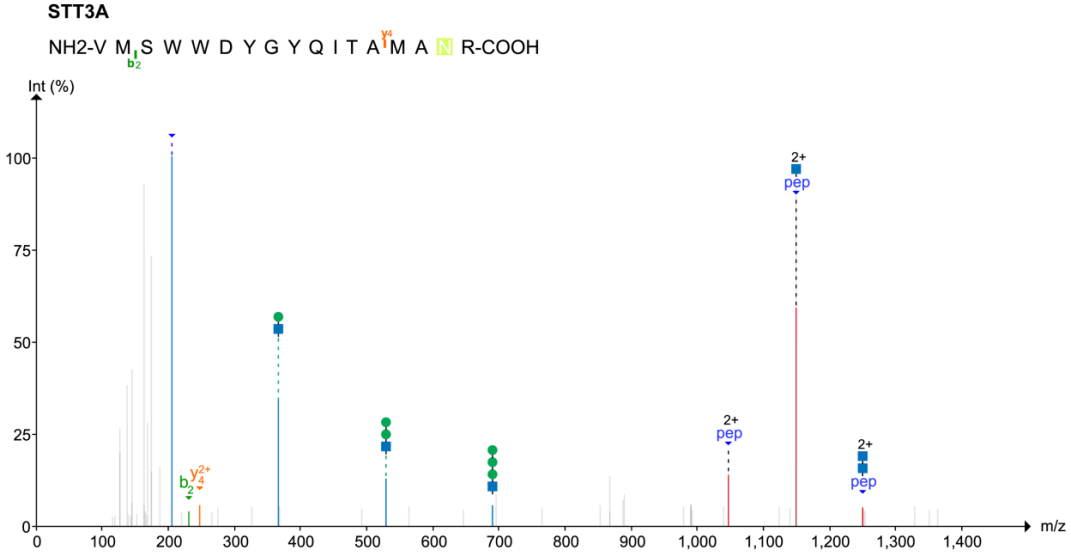

HSPA13
NH2-NSTIIQAANLAGLK-COOH
Int (%)
m/z
C7H8NO2
pep
Prec


HYOU1
NH2-V FGS QNLTTVK-COOH
Int (%)
m/z
C7H8NO2
pep
Prec


HYOU1
NH2-L S A L D N L L N H S S I F L K-COOH
Int (%)
m/z
C7H8NO2
pep
Prec


HYOU1
NH2-L S A L D N L L N H S S I F L K-COOH
Int (%)
m/z
pep
Prec

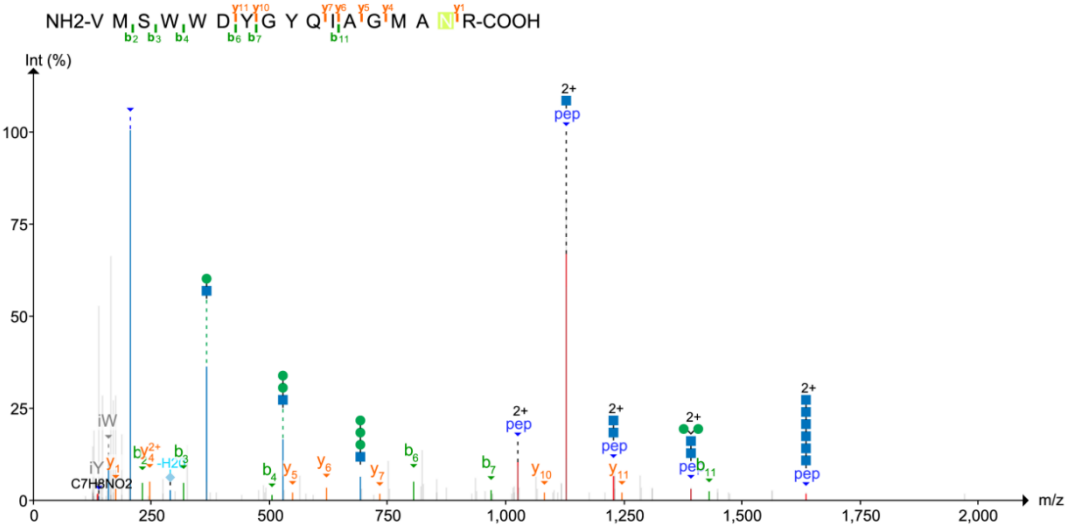
STT3A



100

101

102 **STT3B**



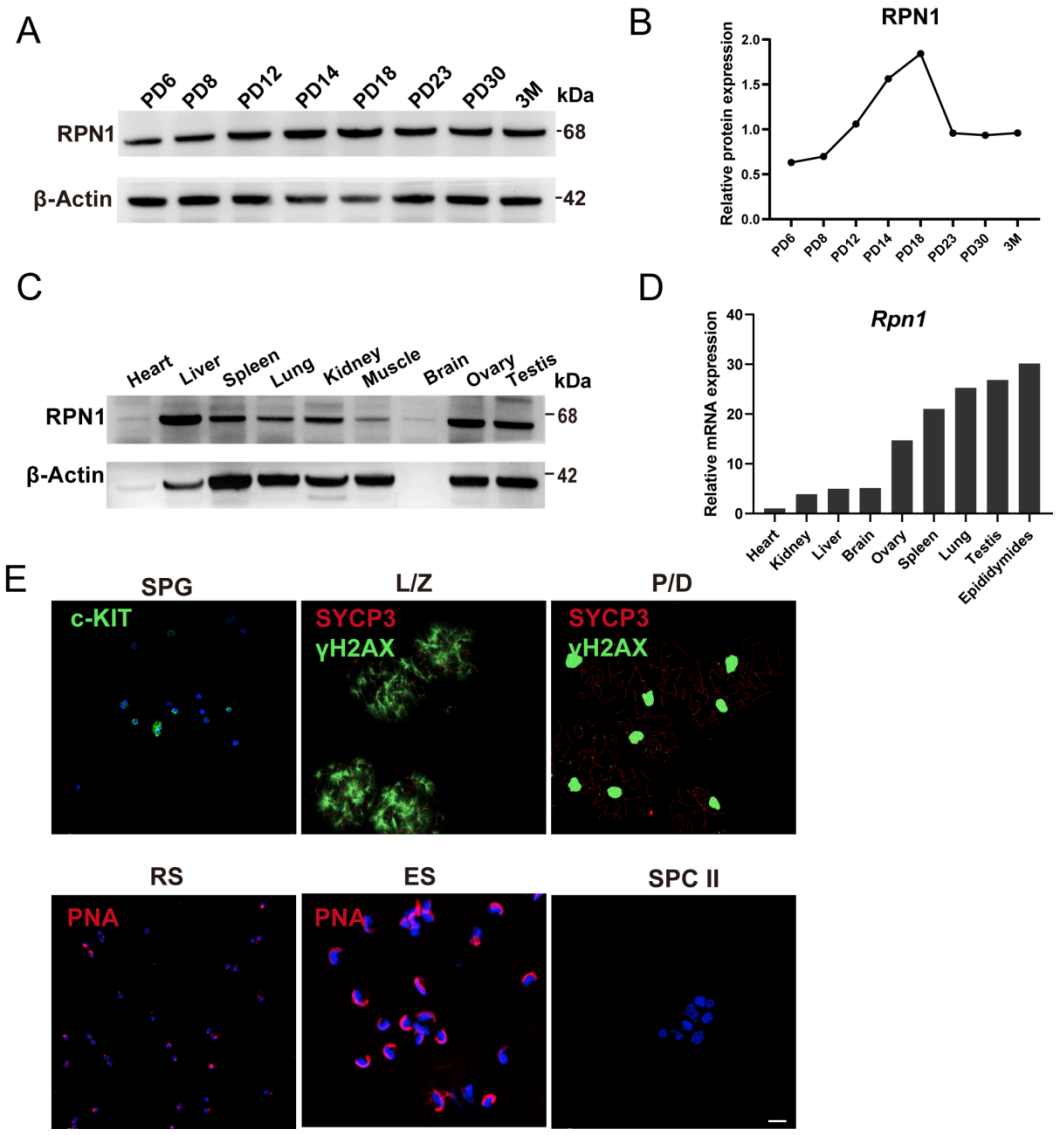


Figure S1. Expression pattern of RPN1 during spermatogenesis

A. WB detection of RPN1 at different developmental stages in mice testes. β-Actin served as the loading control. **B.** Quantification of the relative expression levels of RPN1 at different ages. The relative expression level of the RPN1 protein was calculated by quantifying the gray value of RPN1/β-Actin for each sample. **C.** WB analysis of RPN1 in various organs of adult mice. β-Actin served as the loading control. **D.** Quantification of the relative expression levels of *Rpn1* at various organs. *Gapdh* served as loading control. **E.** Label and stain the different cells obtained through FACS. Scale bar, 20 μm.

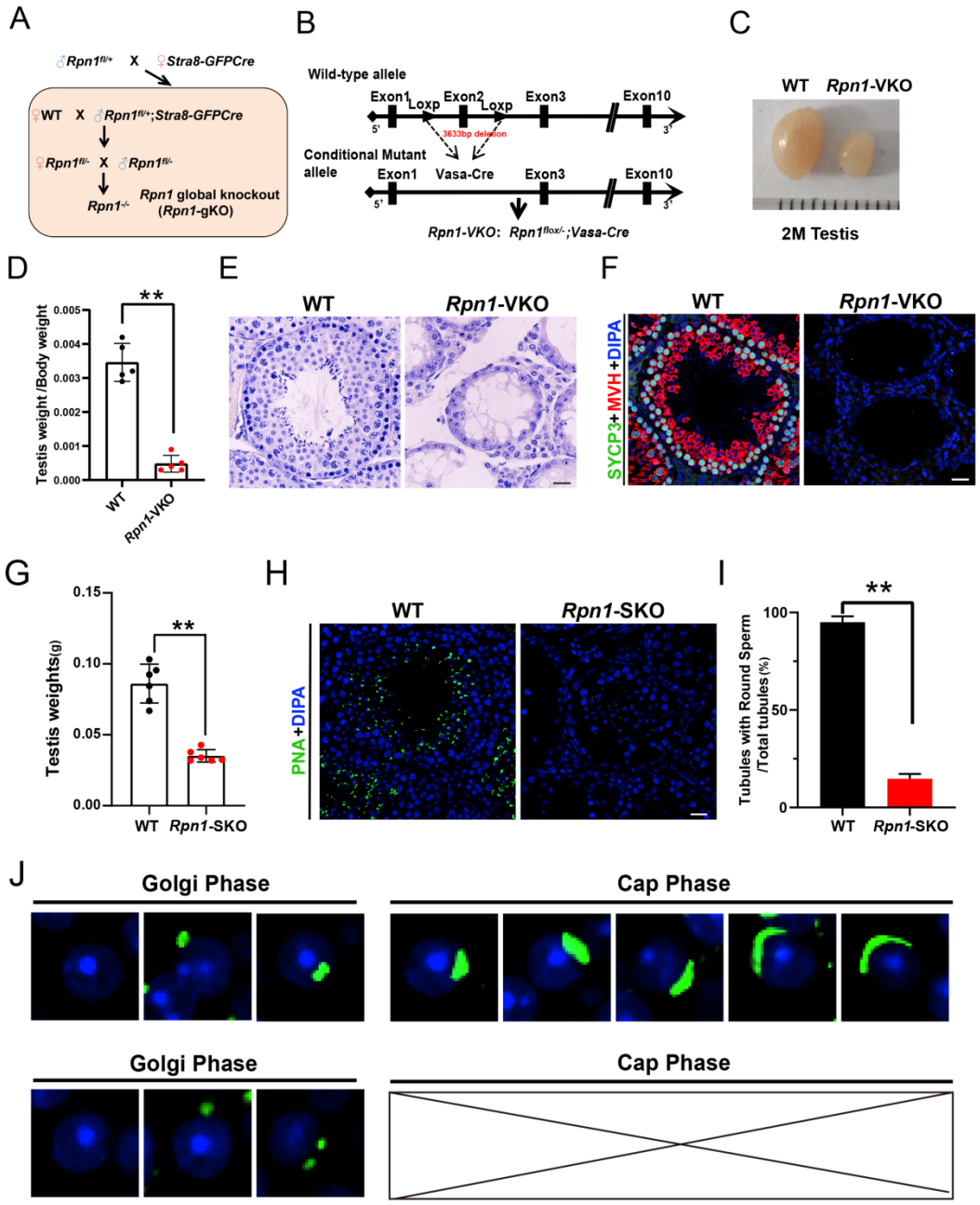


Figure S2 *Rpn1*-VKO mouse phenotype

A. Schematic diagram of the generation of global *Rpn1* knockout mice. **B.** Schematic of *Rpn1* conditional knockout strategy in germ cells. **C.** Morphology of testes from 2M WT and *Rpn1*-VKO mice. **D.** Testis weight/body weight ratios in WT and *Rpn1*-VKO mice. Data represent means ± SEM. $^{**}p < 0.01$ by Student's t-test. **E.** Morphological analysis of WT and *Rpn1*-VKO testes by hematoxylin staining. Scale bar, 20 μm. **F.** Immunofluorescence co-staining of SYCP3 (green) and MVH (red) from WT and *Rpn1*-VKO mice. Scale bar, 30 μm. **G.** Testis weight of WT and *Rpn1*-SKO mice. Data

represent means ± SEM. **$p < 0.01$ by Student's t-test. **H.** Analysis of spermiogenesis and acrosome biogenesis in WT and *Rpn1*-SKO mice by fluorescence imaging of spermatids labeled with PNA lectin (green). Scale bar, 30 μm. **I.** Quantification of seminiferous tubules containing round spermatids. **J.** IF staining of PNA (green) in the phases of acrosome biogenesis in WT and *Rpn1*-SKO spermatids. The phases of acrosome biogenesis and corresponding spermiogenesis steps are the Golgi phase (steps 1-3), cap phase (steps 4-7).

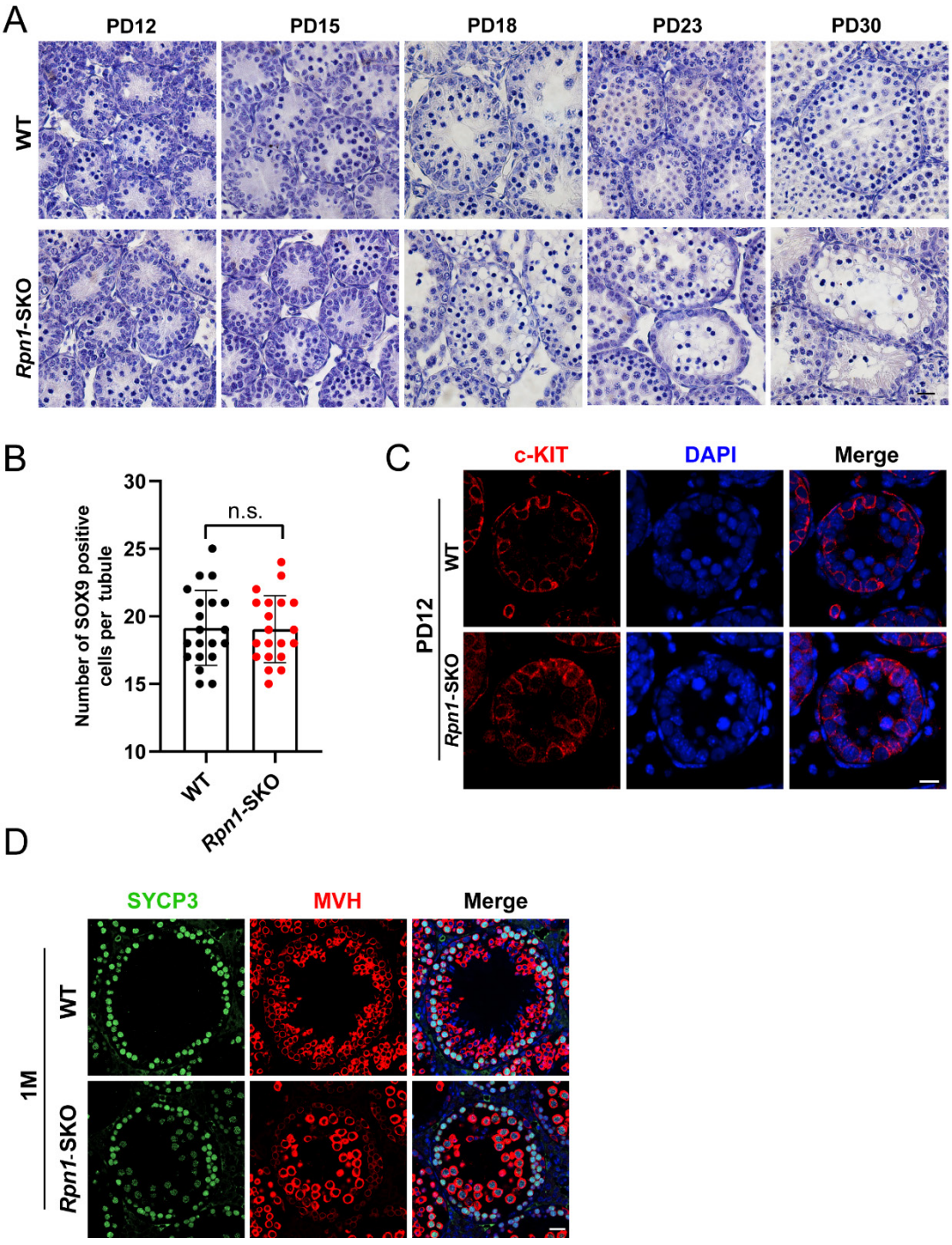


Figure S3. RPN1 is required for spermatogenesis

A. Hematoxylin staining of WT and *Rpn1*-SKO testes at different ages. Scale bar, 20 μm. **B.** Quantification of $SOX9^{+}$ cells per tubule in PD12 WT and *Rpn1*-SKO mice. Data represent means ± SEM. n.s., not significant. **C.** IF staining for c-KIT (red) in PD12 WT and *Rpn1*-SKO mice. Scale bar, 30 μm. **D.** IF co-staining for SYCP3 (green) and MVH (red) in 1M WT and *Rpn1*-SKO mice. Scale bar, 30 μm.

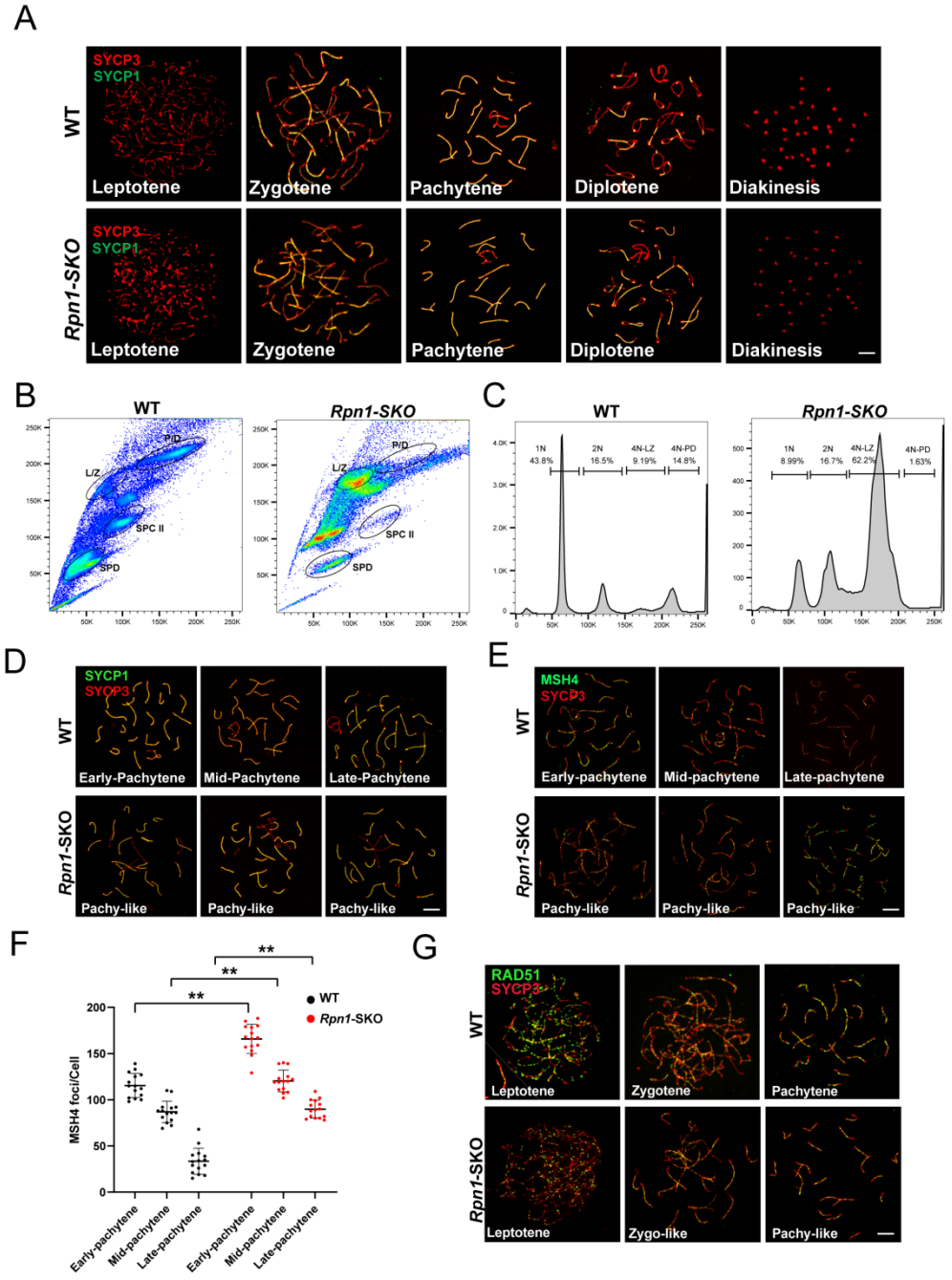


Figure S4. Abnormal meiosis in spermatocytes with conditional knockout of *Rpn1*

A. SYCP3 (red) and SYCP1 (green) co-staining in spermatocyte spreads from 1M WT and *Rpn1*-SKO mice. Scale bar, 5 μm. **B-C.** Representative FACS profiles of distinct spermatogenic populations based on Hoechst fluorescence from adult WT and *Rpn1*-SKO male mice. Histogram peaks correspond to the N, 2N, and 4N cell populations. **D.** Representative images of pachy-like spermatocytes of *Rpn1*-SKO mice. Scale bar, 5 μm. **E.** IF co-staining for SYCP3 (red) and MSH4 (green) in WT and *Rpn1*-SKO mice. Scale bar, 5μm. **F.** Quantification of MSH4 foci per nucleus in WT and *Rpn1*-SKO

mice. Data represent means ± SEM, *$p < 0.05$, **$p < 0.01$ by Student's t-test. **G.** IF co-staining for SYCP3 (red) and RAD51 (green) in WT and *Rpn1*-SKO mice. The dashed line separates the target cell from the adjacent unrelated cell. Scale bar, 5 μm.

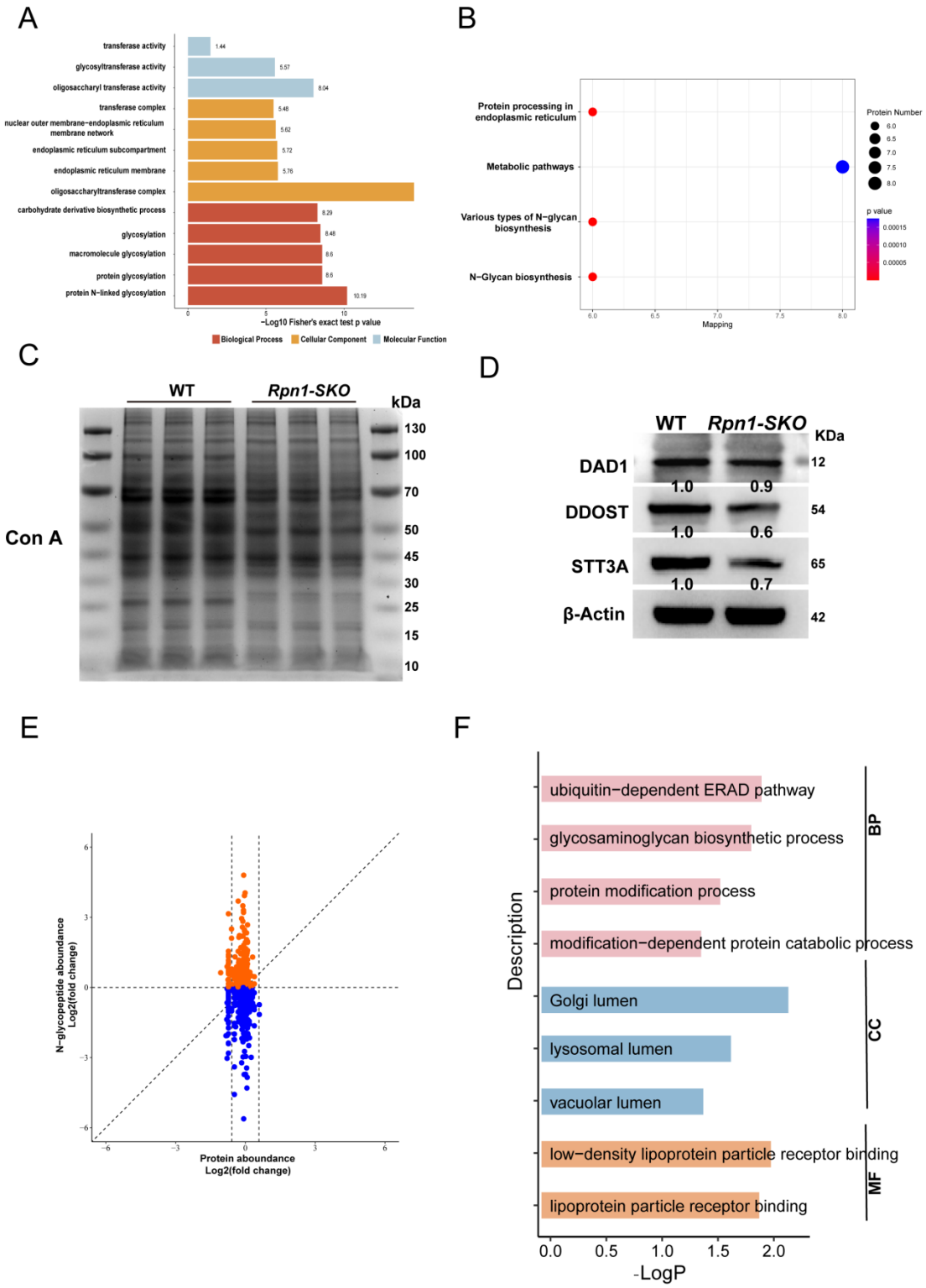


Figure S5. Proteomics analysis of WT and *Rpn1*-SKO testes

A. GO analysis of significantly downregulated proteins. **B.** The bubble chart demonstrating KEGG pathway enrichment results of downregulated proteins. **C.** WB with Con A lectin in WT and *Rpn1*-SKO testes, staining by Coomassie Brilliant Blue. **D.** The proteomic results were verified by WB, β-Actin served as the loading control.

E. Nine-quadrant diagram showing that glycopeptide abundance changes in *Rpn1*-SKO testes are not due to altered protein abundance. **F.** GO analysis of significantly upregulated glycoproteins.

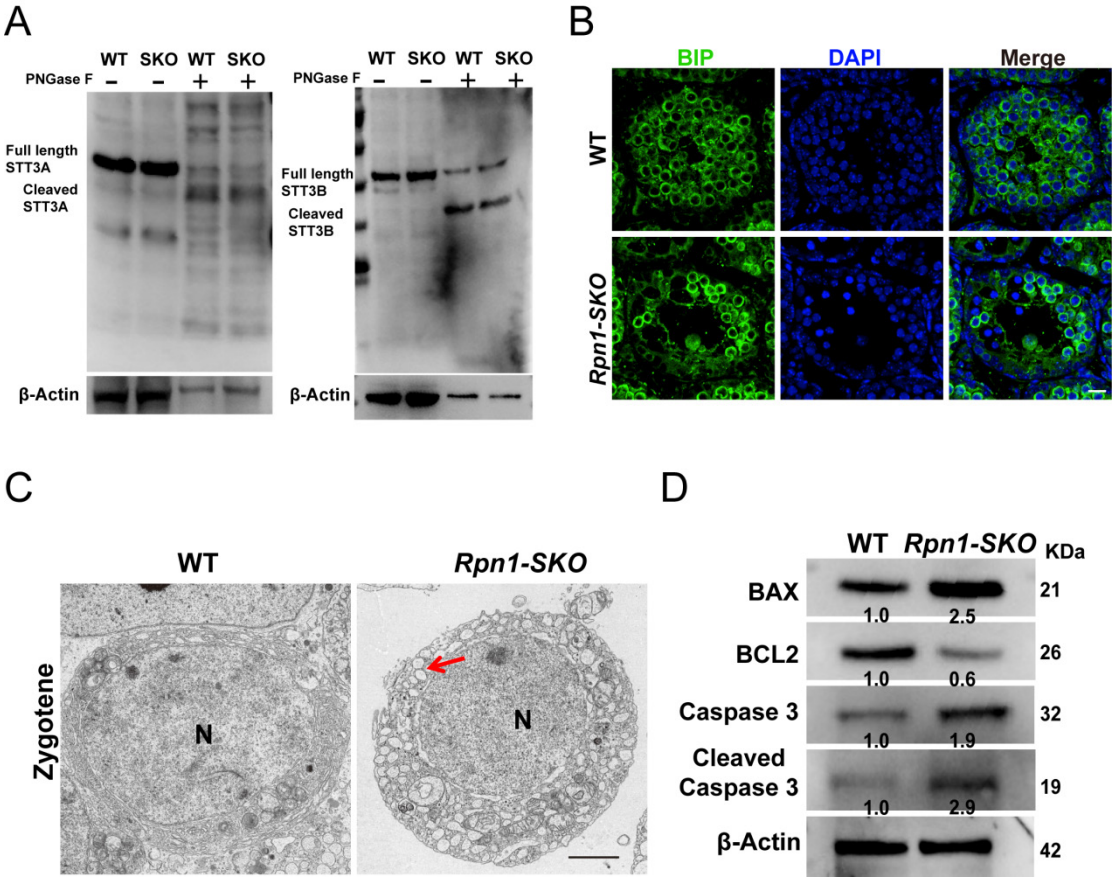


Figure S6. RPN1 deficiency induces ERS and apoptosis of spermatocytes

A. WB detection of key protein glycosylation levels in adult WT and *Rpn1*-SKO testes. β-Actin served as the loading control. **B.** IF staining for BIP in testes of WT and *Rpn1*-SKO testes. Scale bar, 30 μm. **C.** TEM imaging of ER architecture in testicular sections from 2M WT and *Rpn1*-SKO mice. N, nucleus. Scale bar, 5 μm. **D.** WB detection of apoptosis-related proteins levels in adult WT and *Rpn1*-SKO testes. β-Actin served as the loading control.