

Supplementary material

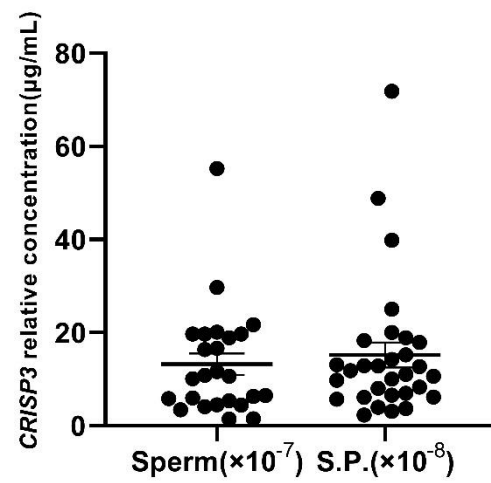


Figure S1. Boar sperm and seminal plasma protein CRISP3 relative content determination.

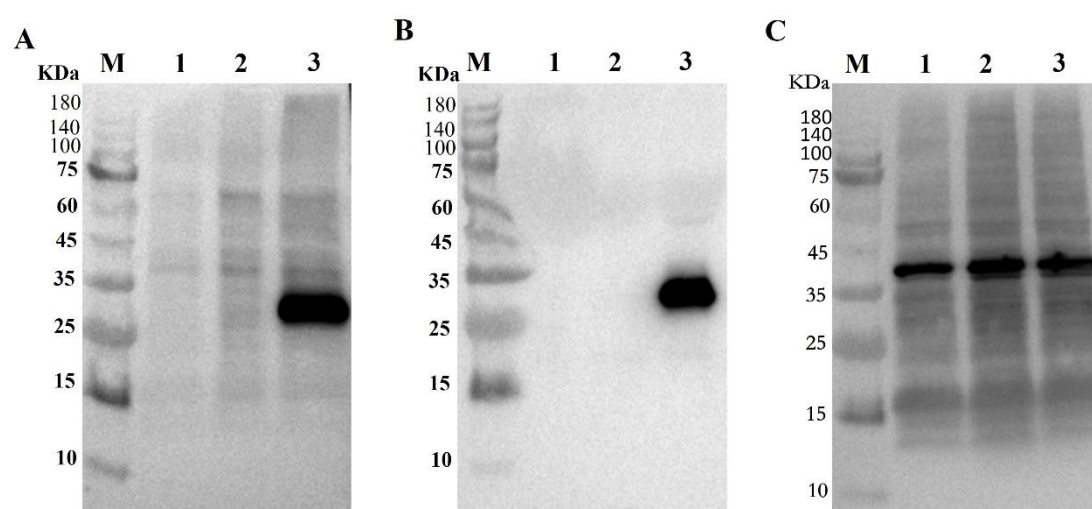


Figure S2. Identification of CRISP3 protein expression in CHO-K1 cells and supernatant. (A-B) Western blot analysis of His tag protein in CHO-K1 cell lysate and supernatant, respectively. (C) CHO-K1 Western blot analysis of GAPDH internal reference protein in cell lysates. Lane 1: untreated CHO-K1 cells; Lane 2: CHO-K1 cells transfected with empty vector; Lane 3: CHO-K1 cells carrying the CRISP3 gene vector were transfected.

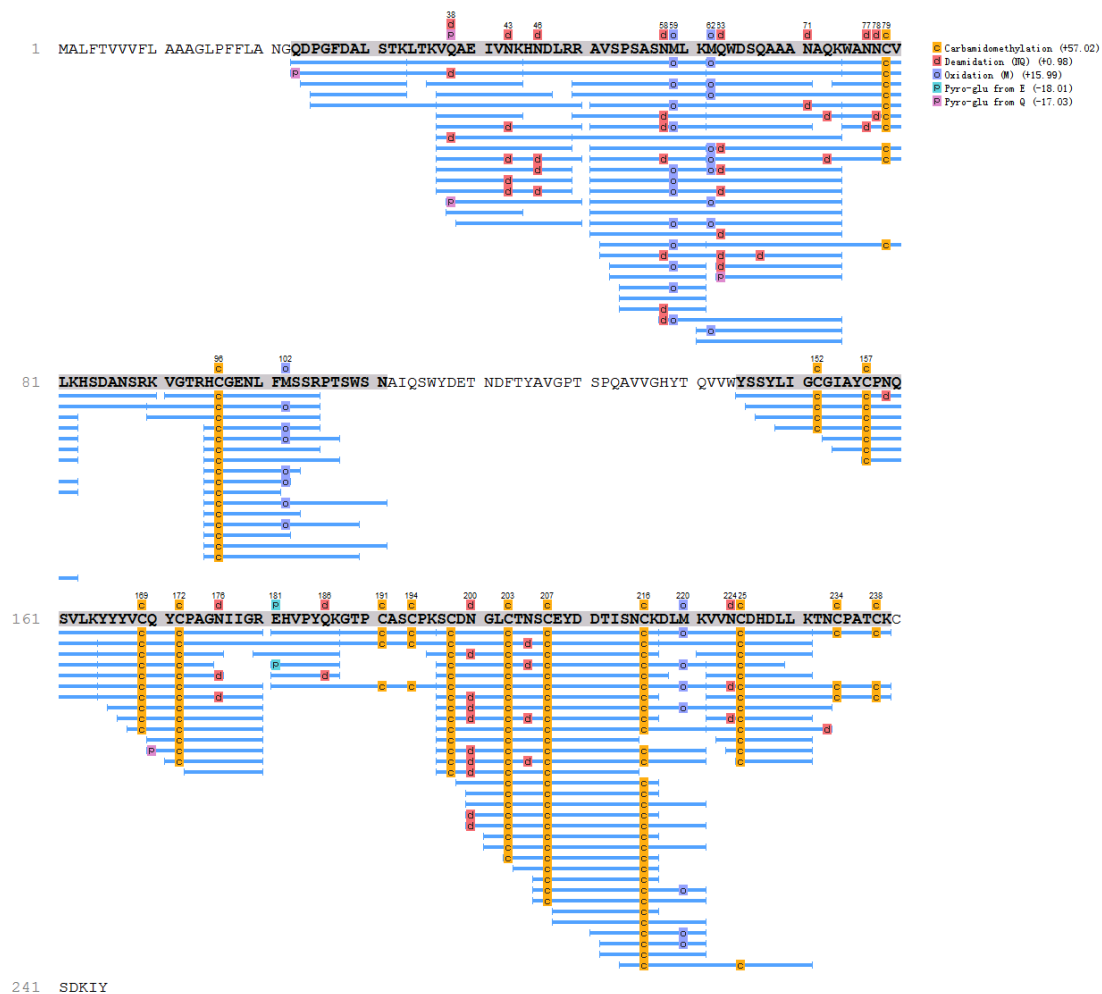


Figure S3. Peptide coverage map of the porcine CRISP3 recombinant protein by LC-MS/MS mass spectrometry.

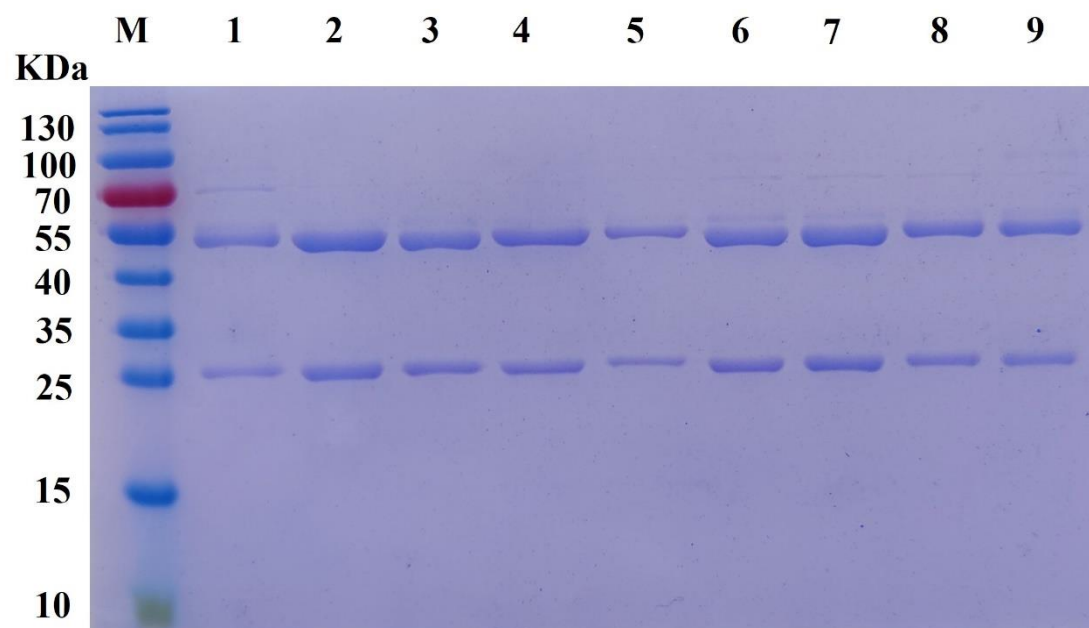


Figure S4. Identification by SDS-PAGE of CRISP3 monoclonal antibodies. M: 180 KDa protein marker; Lanes 1-9: Nine monoclonal antibodies.

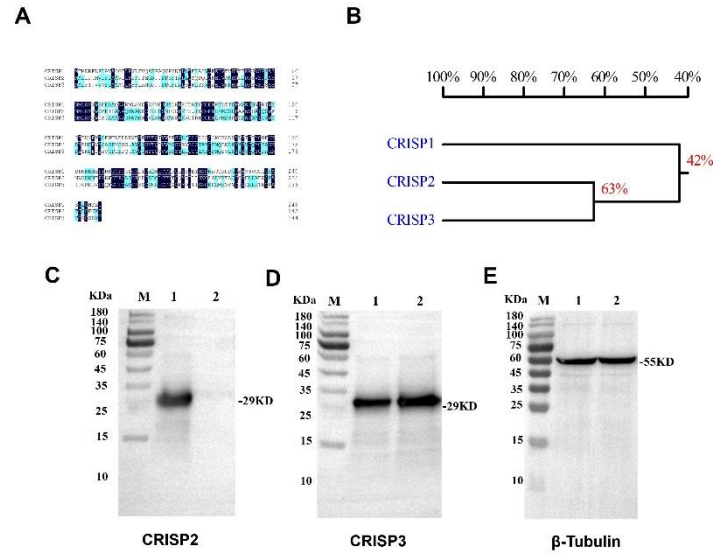


Figure S5. Comparison of amino acid homology among porcine CRISP1, CRISP2, and CRISP3 and specificity identification of antibodies against CRISP3 in boars. (A) Amino acid sequence alignment of porcine CRISP1, CRISP2, and CRISP3. (B) Homologous tree of porcine CRISP1, CRISP2, and CRISP3. (C) Western blot analysis of boar testis and bulbourethral gland bands recognized by anti-CRISP2 antibody. M: Protein Marker; 1: Boar testis; 2: Boar bulbourethral gland. (D) Western blot analysis of boar testis and bulbourethral gland bands recognized by anti-CRISP3 antibody. M: Protein Marker; 1: Boar testis; 2: Boar bulbourethral gland. (E) Western blot analysis of β -tubulin reference proteins in boar testis and bulbourethral glands. M: Protein Marker; 1: Boar testis; 2: Boar bulbourethral gland.

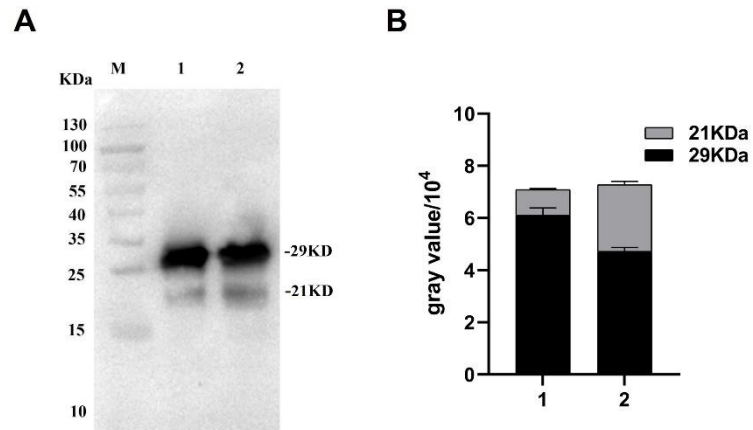


Figure S6. Western blot analysis of glycosylation of porcine CRISP3 recombinant protein, (A) M: protein Marker; 1: without glycosidase treatment of pig CRISP3 recombinant protein; 2: Glycosidase-treated porcine CRISP3 recombinant protein. (B) Western blot analysis of CRISP3 protein expression before and after glycosidase F treatment was performed using Image J software; 1: lane 1; 2: lane 2. The bar graphs show the gray values at 29 KDa and 21 KDa in lane 1 and 2, respectively.

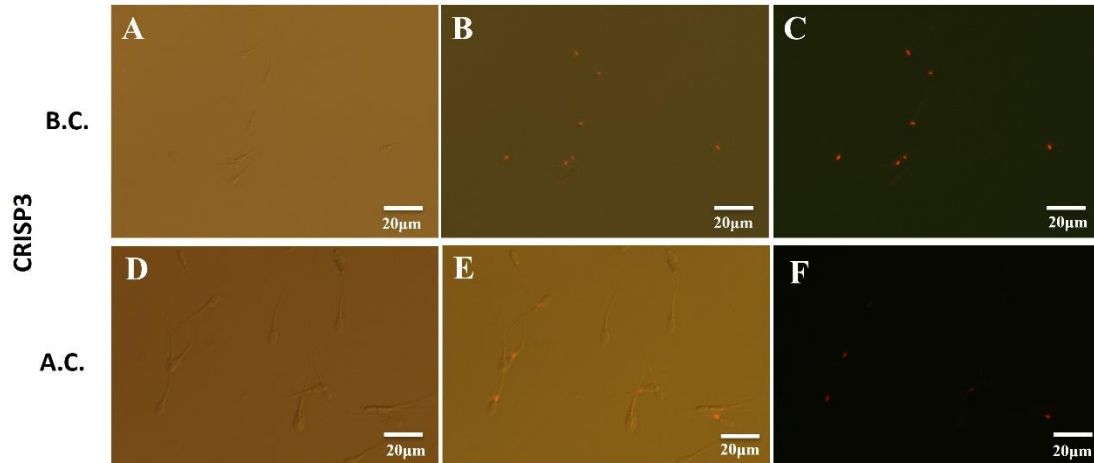


Figure S7. Immunofluorescent staining of CRISP3 in sperm before and after capacitation. (A-C) Representative images of the immunofluorescent staining of CRISP3 in sperm before capacitation; (A) images taken under light microscope, (B) images taken under fluorescent scope; (C) merged images. (D-F) Representative images of the immunofluorescent staining of CRISP3 in sperm after capacitation; (D) images taken under light microscope, (E) images taken under fluorescent scope; (F) merged images. B.C., before capacitation; A.C., after capacitation. The arrow indicates the distribution of the target proteins.

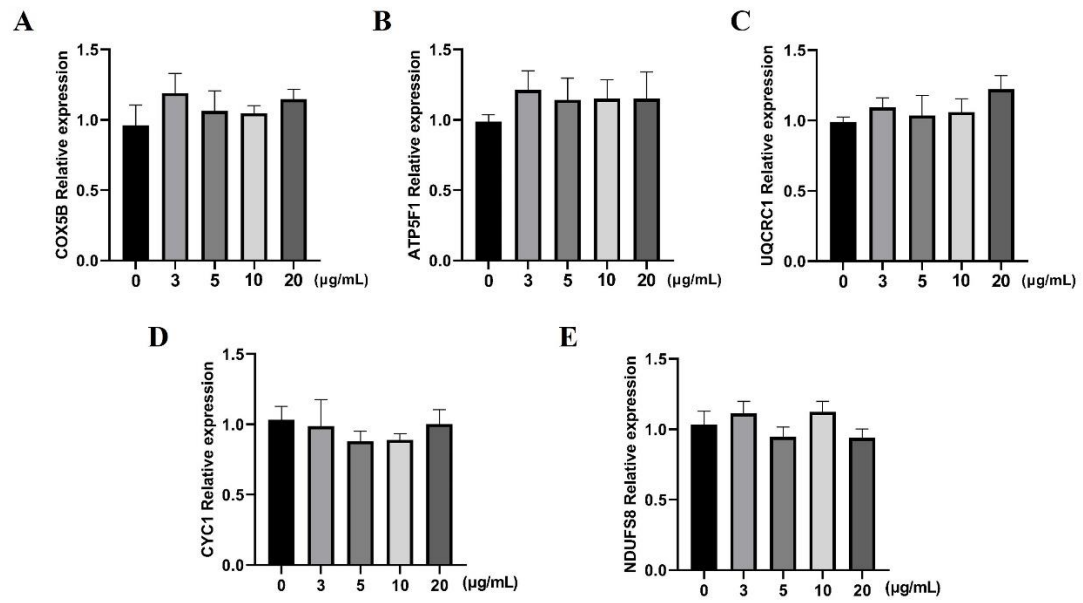


Figure S8. Effects of CRISP3 recombinant protein on COX5B, ATP5F1 and UQCRC1 mRNA expression in sperm. (A) COX5B mRNA expression level; (B) ATP5F1 mRNA expression level; (C) UQCRC1 mRNA expression level. (D) CYC1 mRNA expression level. (E) NDUFS8 mRNA expression level. GAPDH was included in the standardization of all samples as an internal reference gene, and the data were expressed as the mean \pm SEM of three independent experiments.

Table S1. RT-PCR and qRT-PCR primers

	Genes	Forward (5' to 3')	Reverse (5' to 3')	Product size (bp)
RT-PCR	<i>β-actin</i>	CCAAAGCCAACCGTGAGA	CTCGTTGCCGATGGTGAT	425
	<i>CRISP3</i>	CCCCACTGAAGAGTAACCCC	GACCGCAGTCCAGGATTCAT	143
	<i>GAPDH</i>	GAGATCCCGCCAACATCAAAT	G TTCACGCCCATCACAAACAT	170
	<i>CRISP3</i>	CCCCACTGAAGAGTAACCCC	GACCGCAGTCCAGGATTCAT	168
	<i>IL-1α</i>	AAGCAGCCTTATTTTCGGGAG	TATCATATGTCGGGGTGGCT	187
	<i>IL-1β</i>	GCAACTGTTCTGAACTCAACT	ATCTTTTGGGGTCCGTCAACT	89
	<i>IL-6</i>	GGCCTTCCCTACTTCACAAG	GCAAGTGCATCATCGTTGTT	183
	<i>GAPDH</i>	TCCAAGGAGTAAGAAACCCTGGAC	GTTATTATGGGGGTCTGGGATGG	128
qRT-PCR	<i>COX5B</i>	TATGGCATCTGGAGGTGGTG	CAGCCCACTATCCGCTTGTT	183
	<i>ATP5F1</i>	CCAAGACCGTTCTGTGGACG	TCCTTGTTGCCTGCAGTACC	127
	<i>UQCRC1</i>	CGCCTTGTTCACTTTACGCA	GAGACCTCAGCAAGGCCG	144
	<i>CYC1</i>	CTGAACCCGAACACGACCAT	AGTTTCCGGCTCTTGAGGAC	124
	<i>NDUFS8</i>	CTCTTGAATCCAGCTCGGCG	TCCGCGCTGACTCTTCATAG	141