

ORIGINAL RESEARCH ARTICLE

Multiple PDZ domain protein regulates sperm motility through CatSper channel

Supplementary Files

(A) Supporting information

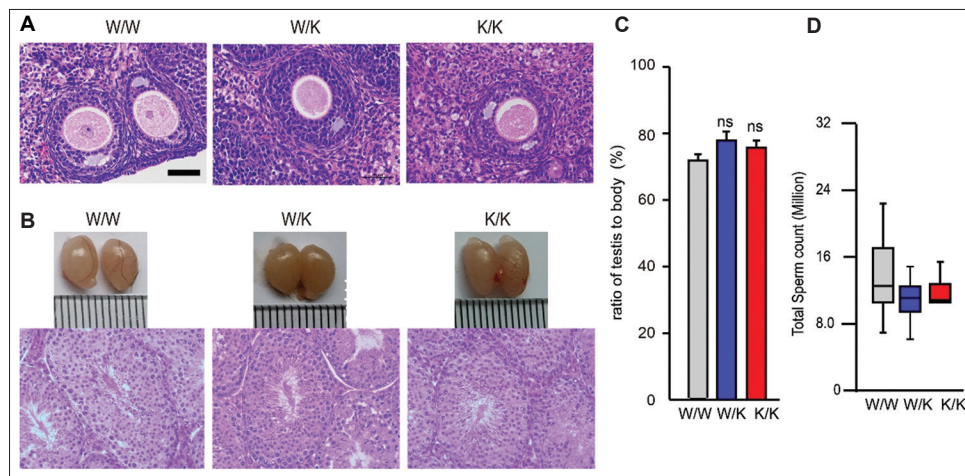


Figure S1. The pathological morphology of mouse ovaries and testes. Hematoxylin and eosin (H&E) staining photographs were used to detect the effect of MPDZ on ovaries (A) and testes (B) (scale bar: 50 μ m). Comparative analysis of ratios of the testis to the body of three genotypes (C). Total sperm count was comparatively analyzed among three genotypes (D). Abbreviation: MPDZ: Multiple PDZ domain protein.

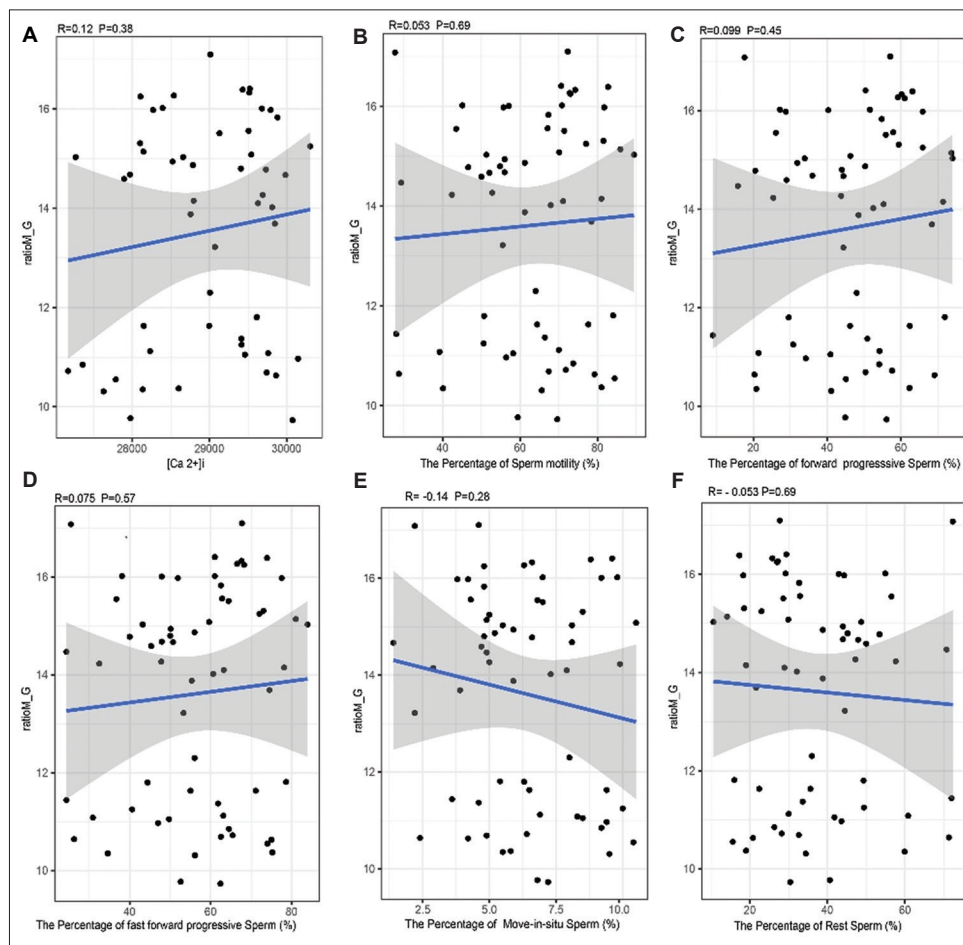


Figure S2. The relationship between MPDZ expression and sperm parameters. Positive correlations were found between MPDZ expression and the level of Ca^{2+} signal ($[Ca^{2+}]_i$) (A), sperm motility (B), the fractions of forward progressive sperms (C), and fast-forward progressive (D). Negative correlations were found between MPDZ expression and the fractions of move-in-situ sperms (E) and rest sperms (F) in human sperm samples using the R language. The ratioM_G represents the Ct value of MPDZ divided by that of GAPDH.

Abbreviations: MPDZ: Multiple PDZ domain protein; GAPDH: Glyceraldehyde-3-phosphate dehydrogenase.

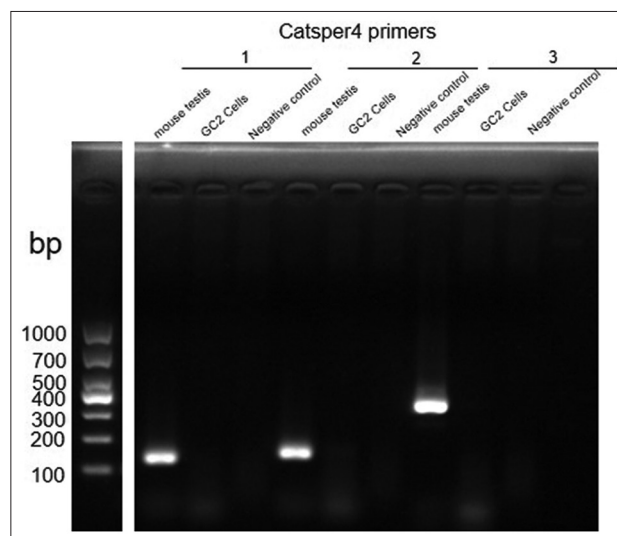


Figure S3. CatSper4 did not express in GC2 cells. We designed multiple pairs of primers and used mouse testis cDNA as a positive control. Primers are listed in Table S1.

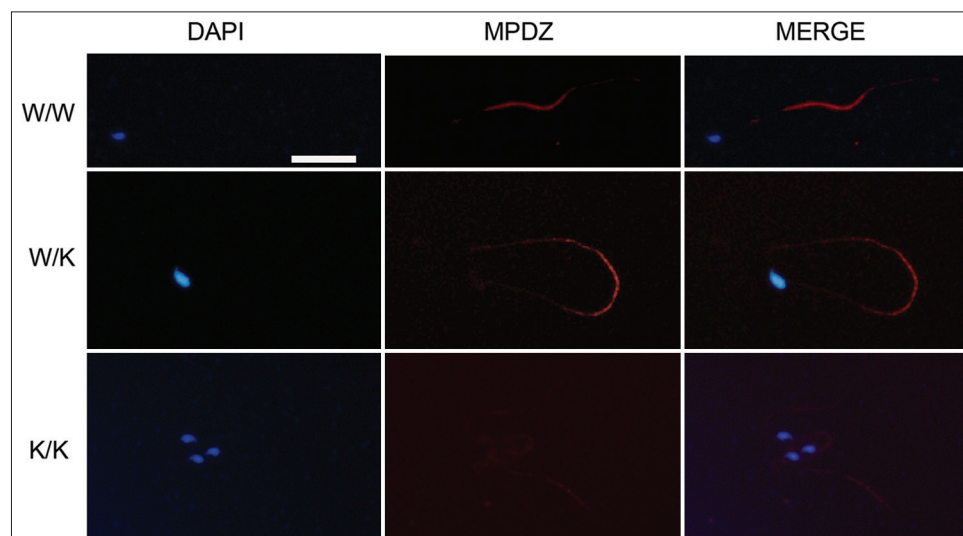


Figure S4. Laser scanning confocal microscopy analysis of the location of MPDZ in spermatozoa. Spermatozoa were obtained from the mouse epididymides. A 10- μ L aliquot of sperm suspension was evenly loaded onto a glass slide, air dried, fixed in 4% paraformaldehyde for 30 min, air dried again, and stored at -80°C . The glass slides were rewarmed to room temperature for 20 min. Following washing in PBS, antigen retrieval was performed with a quick antigen retrieval solution for frozen sections (P0090, Beyotime), after which the slides were blocked in 3% bovine serum albumin for 1 h at room temperature. The cells were then incubated with the rabbit anti-MPDZ antibody (1:70, ab302621, abcam) overnight at 4°C at which point they were incubated with the appropriate Alexa Fluor[®] 555 goat anti-rabbit IgG (H+L) antibody (A-21424, Invitrogen) for 2 h at room temperature. Cells were counterstained in DAPI staining solution (C1005, Beyotime) for 10 min at room temperature to visualize the nuclei. All digital images were acquired using a ZEISS LSM800 confocal laser scanning microscope (ZEISS, Germany). Scale bar: 20 μm .
Abbreviation: MPDZ: Multiple PDZ domain protein.

Table S1. Primers used in qRT-PCR and PCR

Species	Gene symbol	Forward primer (5' - 3')	Reverse primer (5' - 3')	Product size (bp)	Purpose	Primer source
Mouse	MPDZ	GCAGACTCTCCGTCTTCCAC	CCGTGCTCAGTTAGGCTTTC	201	qRT-PCR	Designed in NCBI
Mouse	GAPDH	CTTGCTCTGGTGTGACAG	TCTGAGCTAGCCACCAGAG	316	qRT-PCR/RT-PCR	Designed in NCBI
Mouse	Stat3	TGGTGTCCAGTTTACCACGA	TGTTTCGTGCCAGAATGTTA	149	RT-PCR	Designed in NCBI
Mouse	CatSper1	AGGCGCCTGGTACATCATA	TACCAGGACAGCAATCACCA	88	qRT-PCR/RT-PCR	Designed in NCBI
Mouse	CatSper2	AGCCGTGTCTTTAGCAGCAT	GGCTCATCTCCTCACTCAGC	133	qRT-PCR/RT-PCR	Designed in NCBI
Mouse	CatSper3	AGTCAAGTCGGGCTCACTGT	AGCCTGGCACTCCTTATCCT	91	qRT-PCR/RT-PCR	Designed in NCBI
Mouse	CatSper3	TTCACCACAACCCTGTACGA	AGCCTGGCACTCCTTATCCT	110	qRT-PCR/RT-PCR	Designed in NCBI
Mouse	CatSper3	CCACGGTTGATGGCTGGAC	GTGCATGATCATCACACCCAC	140	qRT-PCR/RT-PCR	Referred to an article
Mouse	CatSper4	GAGAGTACGCGATGGAGGTC	TTGTTCCAGGTTTGTGGTCA	113	qRT-PCR/RT-PCR	Designed in NCBI
Mouse	CatSper4	AGCAGGTGGAGAACATCGAC	ATCCCAGGCATCCTTCTTCT	128	qRT-PCR/RT-PCR	Designed in NCBI
Mouse	CatSper4	TGCCAAGCATTTCAGAAC	ACAATGCACCAAGTGGCAGCT	317	qRT-PCR/RT-PCR	Referred to an article
Human	MPDZ	AAGCCTTACCCCTTCACC	CCTGAAGAGGGTTTGTCAG	114	qRT-PCR	Designed in NCBI

Abbreviations: MPDZ: Multiple PDZ domain protein; GAPDH: Glyceraldehyde-3-phosphate dehydrogenase; RT-PCR: Reverse transcription-polymerase chain reaction; qRT-PCR: Quantitative reverse transcription-polymerase chain reaction.

Table S2. The predicted binding sites of Stat3

Matrix ID	Name	Score	Relative score	Sequence ID	Start	End	Strand	Predicted sequence
MA0144.1	Stat3	11.4906	0.899267854	Catsper1	2015	2024	-	TTCCAGGGAG
MA0144.1	Stat3	11.1701	0.893384618	Catsper1	914	923	+	TTTCAGGAAA
MA0144.1	Stat3	10.7852	0.886316614	Catsper1	2016	2025	+	TCCCTGGAAG
MA0144.1	Stat3	10.0192	0.872251769	Catsper1	913	922	-	TTCTGAAAG
MA0144.1	Stat3	8.99703	0.853483553	Catsper1	81	90	-	TGCCAGCAAG
MA0144.1	Stat3	7.40004	0.824160908	Catsper2	1684	1693	-	TTCTAGAAAA
MA0144.1	Stat3	6.7854	0.812875296	Catsper2	1036	1045	+	TGCTAGGCAA
MA0144.1	Stat3	10.0163	0.872199464	Catsper3	1213	1222	-	GGCCAGGAAA
MA0144.1	Stat3	11.1701	0.893384618	Catsper4	1710	1719	-	TTTCAGGAAA
MA0144.1	Stat3	10.0192	0.872251769	Catsper4	1711	1720	+	TTCTGAAAG

(B) Raw images

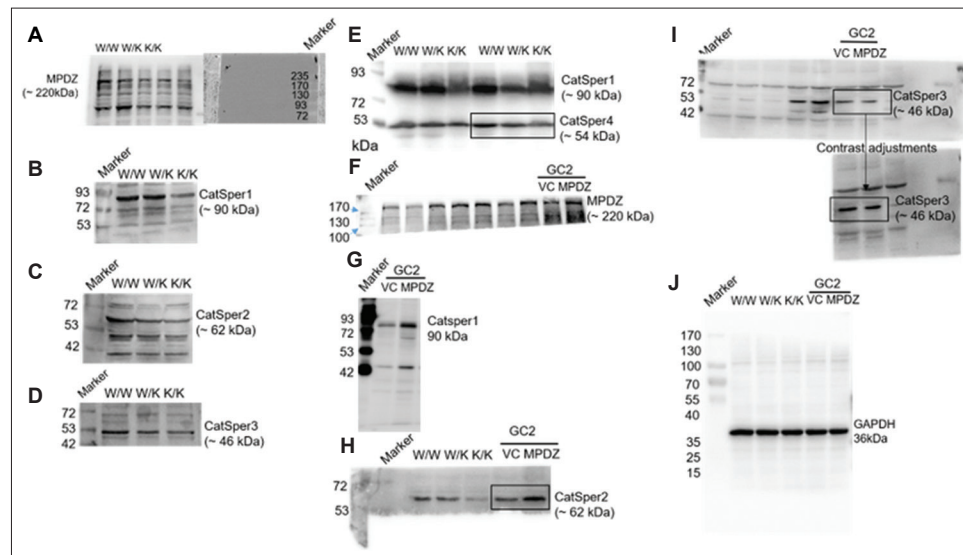


Figure S5. Original images of western blotting for MPDZ, CatSper1-4, and GAPDH in three genotypes and GC2 cells in Figure 3B and F, respectively. (A) western blotting of MPDZ (220 kDa); (B) western blotting of CatSper1 (90 kDa); (C) western blotting of CatSper2 (62 kDa); (D) western blotting of CatSper 3 (46 kDa); and (E) western blotting of CatSper4 (54 kDa) in sperms of three genotypes. (F) Western blotting of MPDZ (220 kDa); (G) western blotting of CatSper1 (90 kDa); (H) western blotting of CatSper2 (62 kDa); and (I) western blotting of CatSper3 (46 kDa) in GC2 cells. (J) Western blotting of GAPDH (36 kDa) in sperms of three genotypes and GC2 cells.

Abbreviations: MPDZ: Multiple PDZ domain protein; GAPDH: Glyceraldehyde-3-phosphate dehydrogenase.

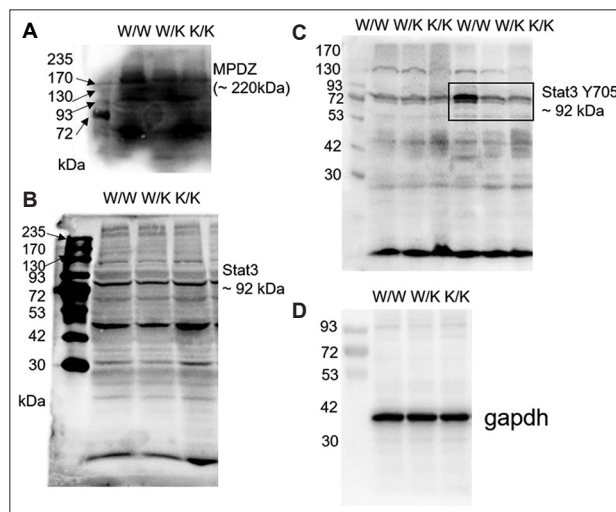


Figure S6. Original images of western blotting for MPDZ, Stat3, Stat3Y705, and GAPDH in Figure 4A. (A) Western blotting of MPDZ (220 kDa); (B) western blotting of Stat3 (92 kDa); (C) western blotting of Stat3 Y705 (92 kDa); and (D) western blotting of GAPDH (36 kDa). Abbreviations: MPDZ: Multiple PDZ domain protein; GAPDH: Glyceraldehyde-3-phosphate dehydrogenase.

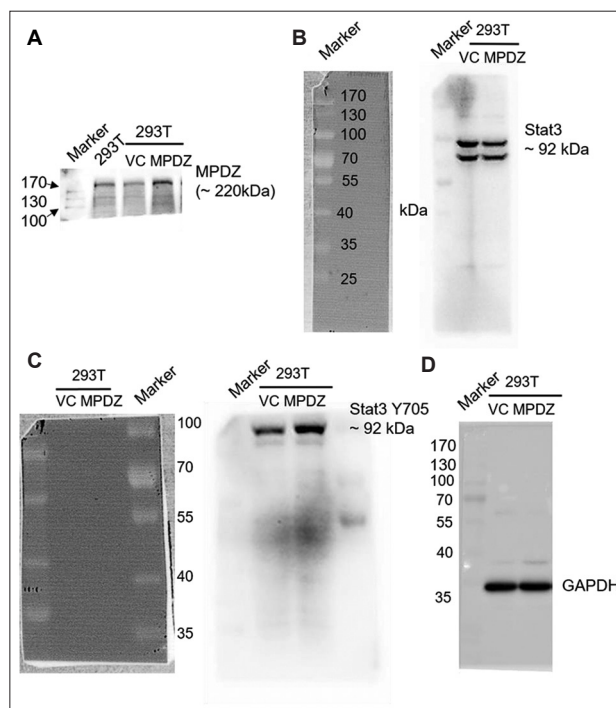


Figure S7. Original images of western blotting for MPDZ, Stat3, Stat3Y705, and GAPDH in HEK-293T cells in Figure 4C. (A) Western blotting of MPDZ (220 kDa); (B) western blotting of Stat3 (92 kDa); (C) western blotting of Stat3 Y705 (92 kDa); and (D) western blotting of GAPDH (36 kDa). Abbreviations: MPDZ: Multiple PDZ domain protein; GAPDH: Glyceraldehyde-3-phosphate dehydrogenase.

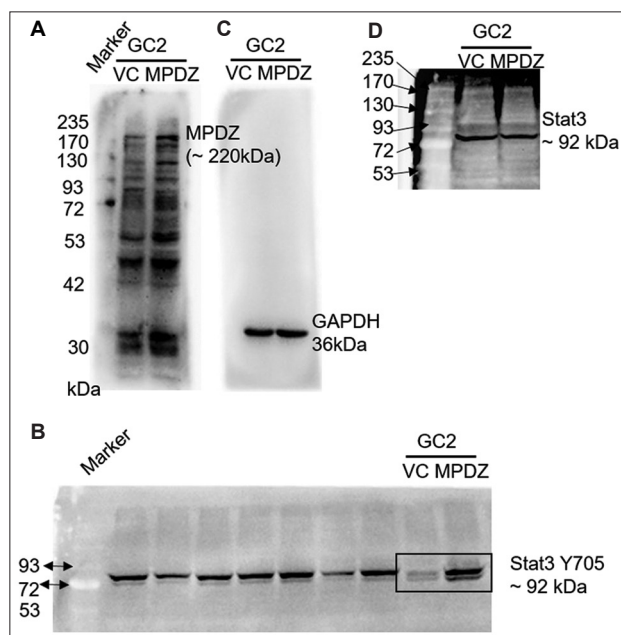


Figure S8. Original images for Western blotting for MPDZ, Stat3, Stat3Y705, and GAPDH in GC2 cells in Figure 4C. (A) Western blotting of MPDZ (220 kDa); (B) western blotting of Stat3 Y705 (92 kDa); (C) western blotting of GAPDH (36 kDa); and (D) western blotting of Stat3 (92 kDa). Abbreviations: MPDZ: Multiple PDZ domain protein; GAPDH: Glyceraldehyde-3-phosphate dehydrogenase.

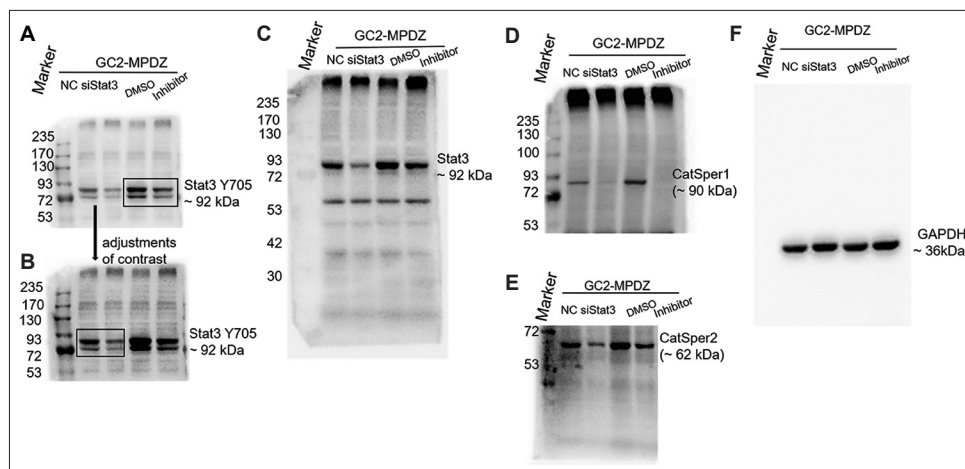


Figure S9. Original images for Western blotting for Stat3, Stat3Y705, CatSper1, CatSper2, and GAPDH in GC2 cells in Figure 4E and G. (A) Western blotting of Stat3 Y705 (92 kDa, Figure 4G); (B) western blotting of Stat3 Y705 (92 kDa, Figure 4F); (C) western blotting of Stat3 (92 kDa); (D) western blotting of CatSper1 (90 kDa); (E) western blotting of CatSper2 (62 kDa); and (F) western blotting of GAPDH (36 kDa). Abbreviations: MPDZ: Multiple PDZ domain protein; GAPDH: Glyceraldehyde-3-phosphate dehydrogenase.