

[10.1071/RD23006](https://doi.org/10.1071/RD23006)

*Reproduction, Fertility and Development*

### Supplementary Material

#### **Melatonin improves the ability of spermatozoa to bind with oocytes in the mouse**

*YaNan Liu<sup>A</sup>, YuSheng Zhang<sup>A</sup>, ZeLin Wang<sup>B</sup>, Zi Teng<sup>B</sup>, Peng Zhu<sup>B</sup>, MeiNa Xie<sup>A,\*</sup>, FuJun Liu<sup>A,B,\*</sup>, and XueXia Liu<sup>A,B,\*</sup>*

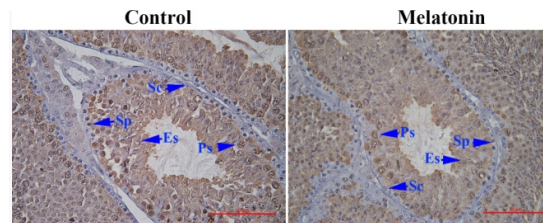
<sup>A</sup>School of Bioscience and Technology, Weifang Medical University, Weifang, China.

<sup>B</sup>Shandong Stem Cell Engineering Technology Research Center, Affiliated Yantai Yuhuangding Hospital of Qingdao University, Yantai, China.

\*Correspondence to: MeiNa Xie School of Bioscience and Technology, Weifang Medical University, Weifang, China Email: xiemeina@wfmc.edu.cn FuJun Liu School of Bioscience and Technology, Weifang Medical University, Weifang, China Email: lfjyt@126.com XueXia Liu School of Bioscience and Technology, Weifang Medical University, Weifang, China Email: xue6er6@163.com

**Supplementary Figure S1. Immunohistochemical analysis of the expression of HSPA2 in mice testis of control and melatonin group.**

Sp, Spermatogonia; Ps, Pachytene spermatocytes; Es, Elongated spermatid; Sc, Sertoli cell. Scale bars=50  $\mu$ m.



**Supplementary Table S1. Lists of differentially expressed proteins in melatonin-treated mice sperm**

Accession	Protein name	Gene name	Fold of wt/mlt	P.Value
G5E8C3	G protein-coupled receptor, family C, group 5, member A	Gprc5a	0.44	5.43E-08
Q91ZB5	Mas-related G-protein coupled receptor member G	Mrgprg	0.47	6.73E-08
Q64523	Histone H2A type 2-C	Hist2h2ac	0.48	6.96E-08
Q80ZX2	Zfp790 protein	Zfp790	0.48	7.24E-08
Q05769	Prostaglandin G/H synthase 2	Ptgs2	0.50	2.21E-03
A0A140T8M5	Immunoglobulin kappa variable 6-15 (Fragment)	Igkv6-15	0.51	8.36E-08
F7C950	Predicted gene 9573	Gm9573	0.51	8.81E-08
F6RYE9	Seminal vesicle secretory protein 5 (Fragment)	Svs5	0.52	9.30E-08
P11087	Collagen alpha-1(I) chain	Col1a1	0.53	1.74E-02
P50608	Fibromodulin	Fmod	0.55	1.11E-07
Q8BNP8	Putative uncharacterized protein	Ptges	0.55	1.03E-02
Q01149	Collagen alpha-2(I) chain	Col1a2	0.55	1.78E-02
Q9CPU3	Protein 4930549C01Rik	4930549C01Rik	0.55	1.16E-07
Q3UK41	Putative uncharacterized protein	Nelfa	0.55	1.18E-07
Q60847	Collagen alpha-1(XII) chain	Col12a1	0.56	9.70E-04
P97477	Aurora kinase A	Aurka	0.56	1.22E-07
A0A0R4J0B6	ATP-binding cassette sub-family G member 2	Abcg2	0.56	1.33E-02
Q3U962	Collagen alpha-2(V) chain	Col5a2	0.57	4.16E-02
B2RU69	CUB and zona pellucida-like domain-containing protein 1	Cuzd1	0.57	1.36E-03
P13634	Carbonic anhydrase 1	Ca1	0.57	1.24E-02
Q8BV79	TPR and ankyrin repeat-containing protein 1	Trank1	0.58	1.39E-07
P28653	Biglycan	Bgn	0.58	5.59E-02
Q5M9J9	Aldo-keto reductase family 1, member B7	Akr1b7	0.58	3.76E-02
Q3TSV1	Putative uncharacterized protein	Dcn	0.59	2.73E-02
Q61469	Phospholipid phosphatase 1	Plpp1	0.59	1.50E-07
A0A140LHQ2	Ankyrin-repeat and fibronectin type III domain-containing 1	Ankfn1	0.59	1.51E-07
Q64462	Cytochrome P450 4B1	Cyp4b1	0.63	2.46E-02
Q04857	Collagen alpha-1(VI) chain	Col6a1	0.63	2.51E-02
Q08EA5	Serine (Or cysteine) peptidase inhibitor, clade A, member 1F	Serpina1f	0.63	3.12E-02
A0A140T8T7	Collagen alpha-5(VI) chain	Col6a5	0.63	2.35E-02
Q920S1	Granzyme N	Gzmn	0.64	1.84E-01
Q99JH8	ER lumen protein-retaining receptor 1	Kdelr1	0.64	2.24E-07
A0A068BGR9	NADH dehydrogenase	Ndufa7	0.64	1.24E-02
A0A1Y7VJW9	Fibulin-5	Fbln5	0.64	2.31E-07
Q7TQ95	Protein lunapark	Lnp	0.65	2.43E-07
Q91YL3	Uridine-cytidine kinase-like 1	Uckl1	0.65	2.46E-07
K3W4R4	Collagen alpha-1(XIV) chain	Col14a1	0.65	3.64E-02
B7ZNX2	Anaphase promoting complex subunit 1	Anapc1	0.65	2.56E-07
O55022	Membrane-associated progesterone receptor component 1	Pgrmc1	0.66	8.36E-02

Q9CQI6	Coactosin-like protein	Cotl1	0.66	1.10E-02
P15209	BDNF/NT-3 growth factors receptor	Ntrk2	0.66	2.69E-07
A0A0J9YUN0	CutA divalent cation tolerance homolog-like	Cutal	0.66	2.69E-07
P58404	Striatin-4	Strn4	0.66	2.76E-07
A0A1W2P6F0	Beta-1,4 N-acetylgalactosaminyltransferase 1	B4galnt1	1.50	2.89E-07
P51432	1-phosphatidylinositol 4,5-bisphosphate phosphodiesterase beta-3	Plcb3	1.50	2.85E-07
Q3TXR9	Beta-hexosaminidase	Hexb	1.50	4.46E-02
Q9D8Z2	TP53-regulated inhibitor of apoptosis 1	Triap1	1.51	8.85E-04
Q8BYB9	Protein O-glucosyltransferase 1	Poglut1	1.51	2.73E-07
A2AQ39	Formin-binding protein 1 (Fragment)	Fnbp1	1.51	2.72E-07
B1AR69	Myosin, heavy polypeptide 13, skeletal muscle	Myh13	1.51	2.69E-07
Q8BZR0	Probable G-protein coupled receptor 82	Gpr82	1.52	4.09E-02
Q9Z1B8	PHD finger protein 1	Phf1	1.52	2.69E-07
Q9ERU3	Zinc finger protein 22	Znf22	1.52	2.66E-07
P29788	Vitronectin	Vtn	1.52	7.70E-02
Q05020	Apolipoprotein C-II	Apoc2	1.52	2.62E-07
C7G3P2	MKIAA0536 protein (Fragment)	Prpf4b	1.52	2.62E-07
Q8QZY9	Splicing factor 3B subunit 4	Sf3b4	1.53	2.56E-07
Q8R1I1	Cytochrome b-c1 complex subunit 9	Uqcr10	1.53	2.56E-07
Q7TNG8	Probable D-lactate dehydrogenase, mitochondrial	Ldhd	1.53	7.31E-02
O08539	Myc box-dependent-interacting protein 1	Bin1	1.53	2.53E-07
E4NKG5	MCG117362, isoform CRA_a	Caprin2	1.54	2.46E-07
K4DI77	WD repeat-containing protein 81 (Fragment)	Wdr81	1.54	2.42E-07
Q8R0W6	NEDD4 family-interacting protein 1	Ndfip1	1.54	2.42E-07
A0A1L1SUG9	Cadherin EGF LAG seven-pass G-type receptor 3	Celsr3	1.55	2.38E-07
O35290	Eosinophil cationic-type ribonuclease 3	Ear3	1.55	5.64E-02
Q3UN88	Mast cell protease 4	Mcpt4	1.56	7.36E-02
Q14DH7	Acyl-CoA synthetase short-chain family member 3, mitochondrial	Acss3	1.57	2.25E-07
E9QAB6	Protein prenyltransferase alpha subunit repeat-containing 1	Ptar1	1.57	2.24E-07
Q61878	Bone marrow proteoglycan	Prg2	1.57	3.51E-02
E9PWD5	Vomer nasal 2, receptor 110	Vmn2r110	1.58	2.18E-07
P15089	Mast cell carboxypeptidase A	Cpa3	1.59	5.84E-04
A4QPC5	Chymase	Cma1	1.60	1.92E-02
Q9D7N3	28S ribosomal protein S9, mitochondrial	Mrps9	1.61	7.46E-03
O09043	Napsin-A	Napsa	1.61	1.95E-07
D3YZ16	Uroplakin 3B-like	Upk3bl	1.62	1.90E-07
Q8BMX2	Putative uncharacterized protein (Fragment)	Pla2g12a	1.63	1.84E-07
Q2LKU9	NACHT, LRR and PYD domains-containing protein 1a	Nlrp1a	1.63	1.82E-07
A0A1L1STC6	Nesprin-1	Syne1	1.64	1.79E-07
H3BL50	Ceramide synthase 4	Cers4	1.64	1.76E-07
Q80Y17	Lethal(2) giant larvae protein homolog 1	Llg1	1.65	1.75E-07
F6QS57	Protein Gm6792 (Fragment)	Gm6792	1.65	1.72E-07

Q9Z2Z9	Glutamine--fructose-6-phosphate aminotransferase [isomerizing] 2	Gfpt2	1.66	1.69E-07
A0A1W2P7C6	Proto-oncogene tyrosine-protein kinase ROS	Ros1	1.69	1.57E-07
P32848	Parvalbumin alpha	Pvalb	1.69	1.54E-07
E9Q046	T-complex protein 10c	Tcp10c	1.70	1.02E-01
Q5HZJ0	Ribonuclease 3	Drosha	1.70	1.52E-07
P03975	IgE-binding protein	Iap	1.71	1.49E-07
Q6P3A4	V-set and immunoglobulin domain-containing protein 8	Vsig8	1.71	1.49E-07
A2AEX6	Four and a half LIM domains protein 1	Fhl1	1.71	1.92E-02
Q91XC9	Peroxisomal membrane protein PEX16	Pex16	1.72	1.44E-07
B7ZNU3	Uncharacterized protein	Gzmd	1.73	1.41E-07
P19426	Negative elongation factor E	Nelfe	1.74	1.37E-07
Q8BPG0	Putative uncharacterized protein	Ybx3	1.78	1.25E-07
Q8C9S4	Coiled-coil domain-containing protein 186	Ccdc186	1.79	1.21E-07
P15864	Histone H1.2	Hist1h1c	1.80	1.18E-07
B1Q450	Hemoglobin beta chain subunit	HBB1	1.82	1.14E-07
Q6NXL5	ArfGAP with coiled-coil, ankyrin repeat and PH domains 3	Acap3	1.83	1.11E-07
Q9CWG8	Protein arginine methyltransferase NDUFAF7, mitochondrial	Ndufaf7	1.85	1.07E-07
Q08ED0	Bcl-2-like protein 15	Bcl2l15	1.85	1.80E-02
Q3TAV1	Putative uncharacterized protein	Gpnmb	1.87	1.02E-07
Q9R097	Kunitz-type protease inhibitor 1	Spint1	1.88	1.01E-07
Q9JJT0	RNA 3'-terminal phosphate cyclase-like protein	Rcl1	1.88	9.96E-08
Q1W5W7	Putative gag-pol protein	gag-pol	1.91	9.56E-08
Q32M04	Prps1l1 protein	Prps1l1	1.93	1.61E-02
Q8CDG5	CREB3 regulatory factor	Crebrf	1.94	8.92E-08
P50446	Keratin, type II cytoskeletal 6A	Krt6a	1.99	8.22E-08
Q7TPD0	Integrator complex subunit 3	Ints3	2.01	7.98E-08
O54825	Bystin	Bysl	2.01	7.92E-08
A2AUC9	Kelch-like protein 41	Klhl41	2.05	7.47E-08
P04247	Myoglobin	Mb	2.06	7.33E-08
Q7TQ39	Gag protein	gag	2.12	6.64E-08
Q9D4D3	MCG113882	4933402D24Rik	2.13	6.61E-08
P04939	Major urinary protein 3	Mup3	2.19	2.40E-02
E9Q1Z0	Keratin 90	Krt90	2.24	5.62E-08
B5X0G2	Major urinary protein 17	Mup17	2.32	5.09E-08
P02762	Major urinary protein 6	Mup6	2.37	4.83E-08
Q3U2X7	Putative uncharacterized protein	Nov	2.39	4.68E-08
Q4FZE8	Major urinary protein 1	Gm21320	2.45	7.75E-03
A0A0R4J0X7	Glyceraldehyde-3-phosphate dehydrogenase	Gapdhs	2.53	1.08E-01
P04938	Major urinary protein 11	Mup11	2.63	3.68E-08
Q9Z2K1	Keratin, type I cytoskeletal 16	Krt16	2.76	3.28E-08
Q8BWT1	3-ketoacyl-CoA thiolase, mitochondrial	Acaa2	2.90	1.72E-02
O88312	Anterior gradient protein 2 homolog	Agr2	3.07	2.60E-08
P97783	Protein AF1q	Mllt11	3.35	1.17E-03
Q91X17	Uromodulin	Umod	3.50	2.00E-08
Q8C7M3	E3 ubiquitin-protein ligase TRIM9	Trim9	6.63	7.59E-09