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Supplemental Information

miR-202-3p Regulates Sertoli Cell Proliferation, Synthesis Function, and Apoptosis by Targeting LRP6 and Cyclin D1 of Wnt/β-Catenin Signaling

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Supplemental Figures

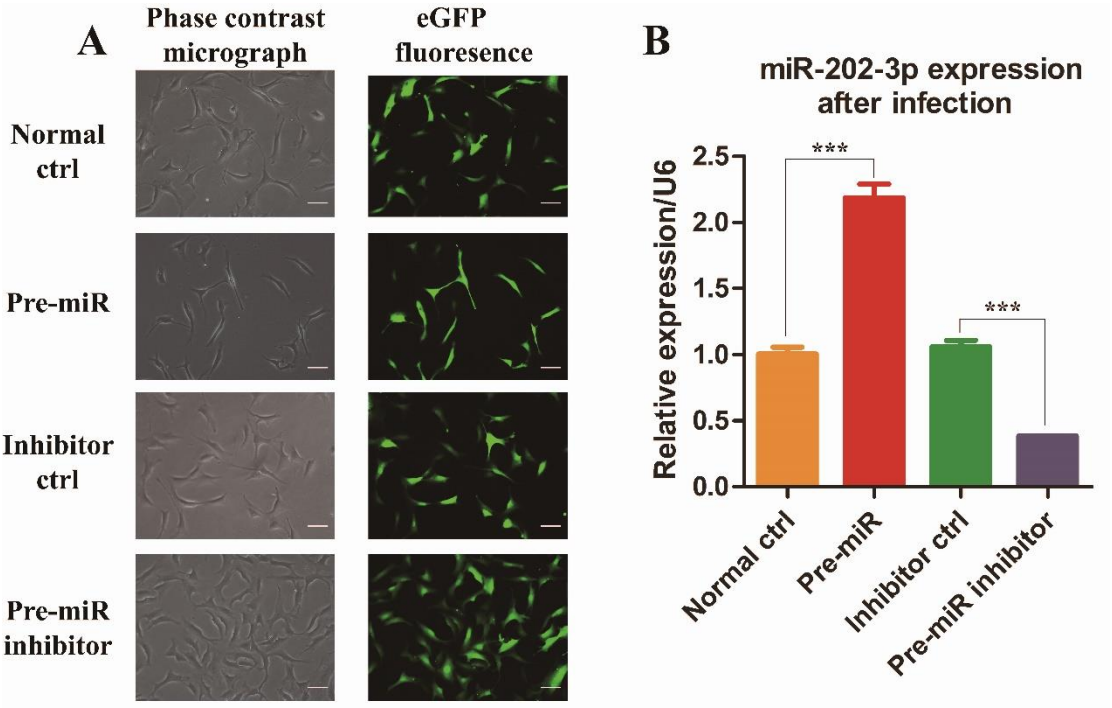

Figure S1. Establishment of stable cell strains with upregulated or downregulated miR-202-3p. Stable cell strains with upregulated or downregulated miR-202-3p and corresponding controls were established in OA Sertoli cells using virus infection and 2 μg/ml puromycin screening for about 120 hours. Cell strains that expressed pGMLV-MA2, pGMLV-MA2-miR-202-3p, pGMLV-SC5, and pGMLV-SC5-miR-202-3p inhibitor stably were named Normal ctrl, Pre-miR, Inhibitor ctrl, and Pre-miR inhibitor, respectively. **(A)** eGFP fluorescence after virus infection and puromycin screening. Scale bars in A=20 μm. **(B)** Different miR-202-3p expression was normalized to U6 among the four cell strains. Notes: *P<0.05.

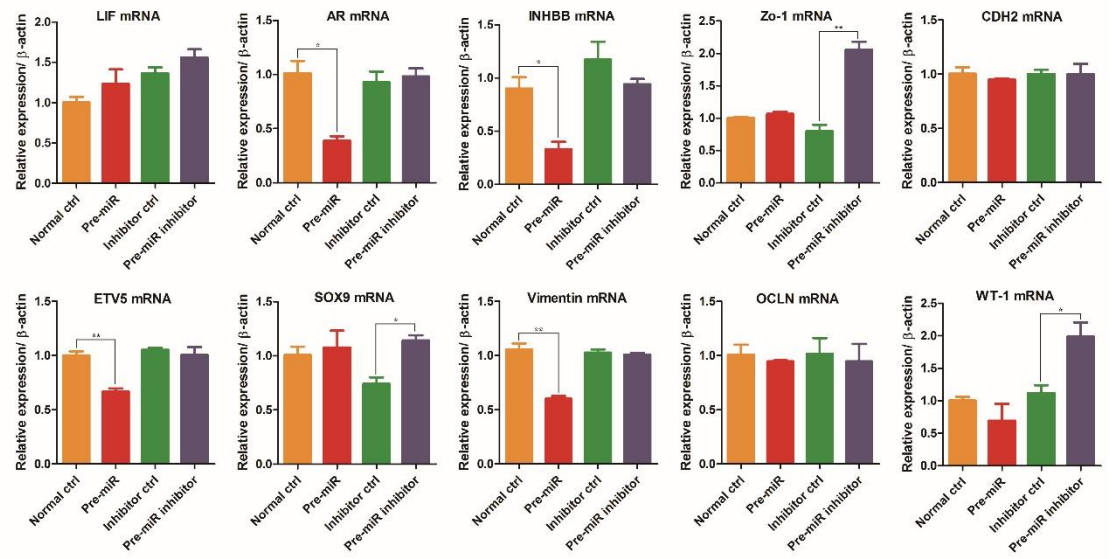

Figure S2. The mRNA levels of genes related to Sertoli cell functions that showed no significant difference among the four cell strains.

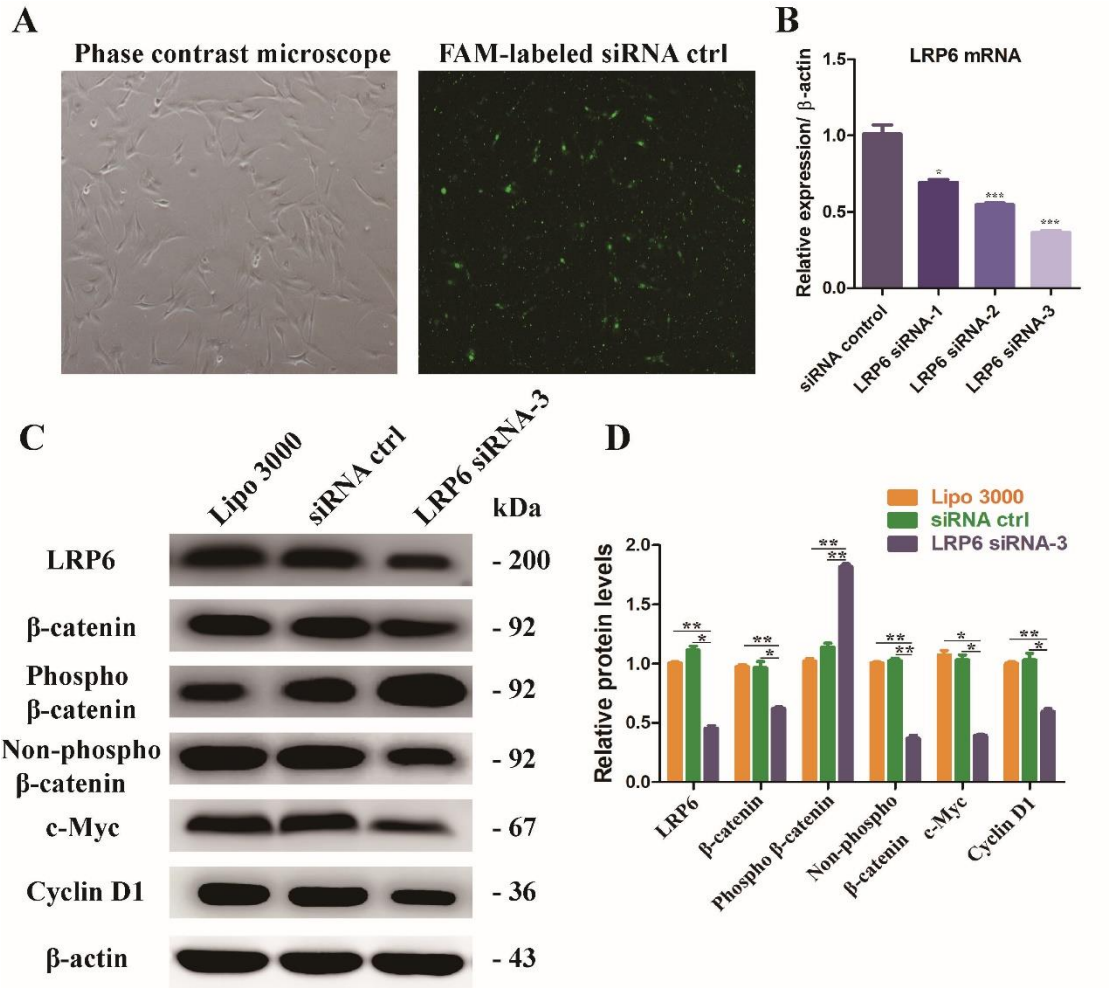

Figure S3. Knockdown of LRP6 using siRNA in Sertoli cells. (A) The transfection with FAM-labeled siRNA at 6 hours showed the transfection efficiency of LRP6 siRNAs. **(B)** qRT-PCR showed the transcription of LRP6 in human Sertoli cells at 24 hours after transfection with LRP6 siRNAs and control siRNA. **(C-D)** Western blots demonstrated the LRP6, β-catenin, Phospho-β-catenin, Non-phospho β-catenin, c-Myc, and Cyclin D1 protein expressions in human Sertoli cells at 48 hours after transfection with control siRNA or LRP6 siRNA-3. β-actin served as a loading control of proteins.

Supplemental Tables

Table S1. Primer sequences used for quantitative RT-PCR

Genes	Primer sequences (5'-3')	Product size (bp)	Tm (℃)
GDNF	F:GGCAGTGCTTCCTAGAAGAGA	111	60.9
	R:AAGACACAACCCCGGTTTTTG		61.3
SCF	F:AATCCTCTCGTCAAAACTGAAGG	163	60.2
	R:CCATCTCGCTTATCCAACAATGA		60.4
BMP4	F:ATGATTCCTGGTAACCGAATGC	93	60.4
	R:CCCCGTCTCAGGTATCAAACT		60.9
WT1	F:CACAGCACAGGGTACGAGAG	133	61.9
	R:CAAGAGTCGGGGCTACTCCA		62.8
GATA4	F:CGACACCCCAATCTCGATATG	117	60.0
	R:GTTGCACAGATAGTGACCCGT		62.1
SOX9	F:AGCGAACGCACATCAAGAC	85	60.7
	R:CTGTAGGCGATCTGTTGGGG		62.0
Zo-1	F:ACCAGTAAGTCGTCCTGATCC	128	60.6
	R:TCGGCCAAATCTTCTCACTCC		61.8
OCLN	F:ACAAGCGGTTTTATCCAGAGTC	89	60.3
	R:GTCATCCACAGGCGAAGTTAAT		60.4
EGF	F:TCCTCACCCGATAATGGTGGA	80	62.1
	R:CCAGGAAAGCAATCACATTCCC		61.5
LIF	F:CCAACGTGACGGACTTCCC	82	62.6
	R:TACACGACTATGCGGTACAGC		61.6
IGF1	F:GCTCTTCAGTTCGTGTGTGGA	133	62.3
	R:GCCTCCTTAGATCACAGCTCC		61.7
INHBB	F:CGGGTCCGCCTATACTTCTTC	97	61.7
	R:CGTAGGGCAGGAGTTTCAGG		61.9
CXCL12	F:ATTCTCAACACTCCAAACTGTGC	88	61.2
	R:ACTTTAGCTTCGGGTCAATGC		60.6
ETV5	F:TCAGCAAGTCCCTTTTATGGTC	119	60.0
	R:GCTCTTCAGAATCGTGAGCCA		62.1
FSHR	F:TCTGTCACTGCTCTAACAGGG	131	60.9
	R:TGCACCTTTTTGGATGACTCG		60.8
Vimentin	F:AGTCCACTGAGTACCGGAGAC	98	62.4
	R:CATTTCACGCATCTGGCGTTC		62.5
AR	F:GACGACCAGATGGCTGTCATT	106	62.1
	R:GGGCGAAGTAGAGCATCCT		60.8
GJA1	F:TGGTAAGGTGAAAATGCGAGG	123	60.3
	R:GCACTCAAGCTGAATCCATAGAT		60.2
CDH2	F:TGCGGTACAGTGTAACTGGG	123	61.5
	R:GAAACCGGGCTATCTGCTCG		62.7

PCNA	F:GCGTGAACCTCACCAGTATGT	76	62.1
	R:TCTTCGGCCCTTAGTGTAATGAT		60.9
β-actin	F:CATGTACGTTGCTATCCAGGC	250	60.8
	R:CTCCTTAATGTCACGCACGAT		60.2
Ki-67	F:AGAAGAAGTGGTGCTTCGGAA	202	61.3
	R:AGTTTGCGTGGCCTGTACTAA		61.7
LRP6	F:ACGATTGTAGTTGGAGGCTTG	95	60.0
	R:ATGGCTTCTTCGCTGACATCA		61.8
β-catenin	F:CATCTACACAGTTTGATGCTGCT	150	60.9
	R:GCAGTTTTGTCAGTTCAGGGA		60.4
c-Myc	F:GTCAAGAGGCGAACACACAAC	162	61.7
	R:TTGGACGGACAGGATGTATGC		61.9
Cyclin D1	F:GCTGCGAAGTGGAAACCATC	135	61.6
	R:CCTCCTTCTGCACACATTTGAA		60.8

Table S2. The sequences of oligonucleotides for human LRP6 siRNAs

siRNA	siRNA sequences (5'-3')		Knockdown rate
LRP6 siRNA-1	Sense	CCACAAAUCCAUGUGGAAUTT	69.3%±1.7%
	Antisense	AUUCCACAUGGAUUUGUGGTT	
LRP6 siRNA-2	Sense	GGUUCUGACCGUGUAGUAUTT	54.6%±1.3%
	Antisense	AUACUACACGGUCAGAACCTT	
LRP6 siRNA-3	Sense	GCAGAUAUCAGACGAAUUUTT	36.4%±1.4%
	Antisense	AAAUUCGUCUGAUAUCUGCTT	
Negative control	Sense	UUCUCCGAACGUGUCACGUTT	
	Antisense	ACGUGACACGUUCGGAGAATT	
FAM control	Sense	UUCUCCGAACGUGUCACGUTT	
	Antisense	ACGUGACACGUUCGGAGAATT	

Table S3. Primary antibodies used for Western blots and immunocytochemistry

Antibodies	Sources	Vendors	Working dilutions
GATA4	Mouse	Santa Cruz	ICC: 1:200
WT1	Rabbit	Santa Cruz	ICC: 1:200
SOX9	Rabbit	Millipore	ICC: 1:500
GDNF	Rabbit	Santa Cruz	ICC: 1:300 WB:1:300
SCF	Rabbit	Santa Cruz	ICC: 1:300 WB:1:300
VIM	Rabbit	Cell Signaling Technology	ICC: 1:100
OCLN	Rabbit	Abcam	ICC: 1:200
α-SMA	Rabbit	Abcam	ICC: 1:200
CYP11A1	Rabbit	Abcam	ICC: 1:200
VASA	Goat	Santa Cruz	ICC: 1:100
Ki-67	Mouse	Santa Cruz	ICC: 1:200
PCNA	Mouse	Santa Cruz	WB:1:200
Cyclin A2	Rabbit	Proteintech	WB:1:2000
Cyclin B1	Rabbit	Cell Signaling Technology	WB:1:1000
Cyclin D1	Mouse	Proteintech	WB:1:1000
Cyclin E1	Mouse	Proteintech	WB:1:1000
β-actin	Mouse	Proteintech	WB:1:5000
cleaved-PARP	Rabbit	Cell Signaling Technology	WB:1:1000
Bcl2	Mouse	Santa Cruz	WB:1:500
Bax	Mouse	Santa Cruz	WB:1:500
BMP4	Mouse	Cell Signaling Technology	WB:1:1000
FGF2	Rabbit	Cell Signaling Technology	WB:1:1000
CXCL12	Rabbit	Cell Signaling Technology	WB:1:1000
LRP6	Rabbit	Cell Signaling Technology	WB:1:1000
c-Myc	Rabbit	Cell Signaling Technology	WB:1:1000
β-catenin	Mouse	Santa Cruz	WB:1:300
Phospho-β-catenin	Rabbit	Cell Signaling Technology	WB:1:1000
Non-phospho β-catenin	Rabbit	Cell Signaling Technology	WB:1:1000