

Tables

Table S1. the primers are used in this study

Primers	Sequence (5'-3')
gene-MIR6531 F	GCAGAGCGTGTCTTAGTTA
gene-MIR6531 R	CCGTCAGGTACTCCAACA
bta-miR-6531 (cDNA synthesis)	GTCGTATCCAGTGCAGGGTCCGAGGTATTTCGCACT GGATACGACTCTCTC
bta-miR-6531 (qPCR)	GCGCGTAGACTTGGTGTACTTT
URP	AGTGCAGGGTCCGAGGTATT
U6 F	CTCGCTTCGGCAGCACA
U6 R (cDNA synthesis)	AACGCTTCACGAATTTGCGT
Bta-miR-6531 mimics	Sense UAGACUUGGUGUACUUUGAGAGA Antisense UCUCAAAGUACACCAAGUCUAUU
Bta-miR-6531 inhibitor	UCUCUCAAAGUACACCAAGUCUA
NC mimics	Sense UUCUCCGAACGUGUCACGUTT Antisense ACGUGACACGUUCGGAGAATT
NC inhibitor	CAGUACUUUUGUGUAGUACAA
miR6531-F-BamH I	cgGGATCCAGAGCGTGTCTTAGTTA
miR6531-R-EcoR I	cGAATTCCTCCGTCAGGTACTCCAACA
ATP2A2-seed-F	acgcGTCGACggcaggcatcagagtcacat
ATP2A2-seed-R	attGCGGCCCGCgtgtgtatatgccccgtcct
ATP2A2-mut-F	CAGgggggggAAAATTGTAGAATTTCTTCAGTCGTTTG
ATP2A2-mut-R	ACAATTTTcccccccTGGAAGGTTCAACTCGAGCA
GAPDH-F	GATGGTGAAGGTCGGAGTGAAC
GAPDH-R	GTCATTGATGGCGACGATGT
ATP2A2-F	CAGGCATCAGAGTCATCAT
ATP2A2-R	GGTGGAGAAGTTGTCATCA
siATP2A2	Sense CAGCUAUGACUGGAGACGGUGUAAA Antisense UUUACACCGUCUCCAGUCAUAGCUG
siRNA control	Sense CAGAGUAGGUCCAGAGUGGUUCAA Antisense UUUGAACCACUCUGGACCUACUCUG

Table S2 the sperms samples used in WB analysis

	Bulls' ID	Fresh motility (%)	Frozen motility (%)
Low motility	1	62.4	36.25
	2	62.4	28.33
	3	62.75	35.12
Mean±SEM		62.15±0.16	33.24±3.50
High motility	4	73.46	44
	5	73.63	49.44
	6	73.72	46.11
Mean±SEM		73.60±0.11	46.52±2.24

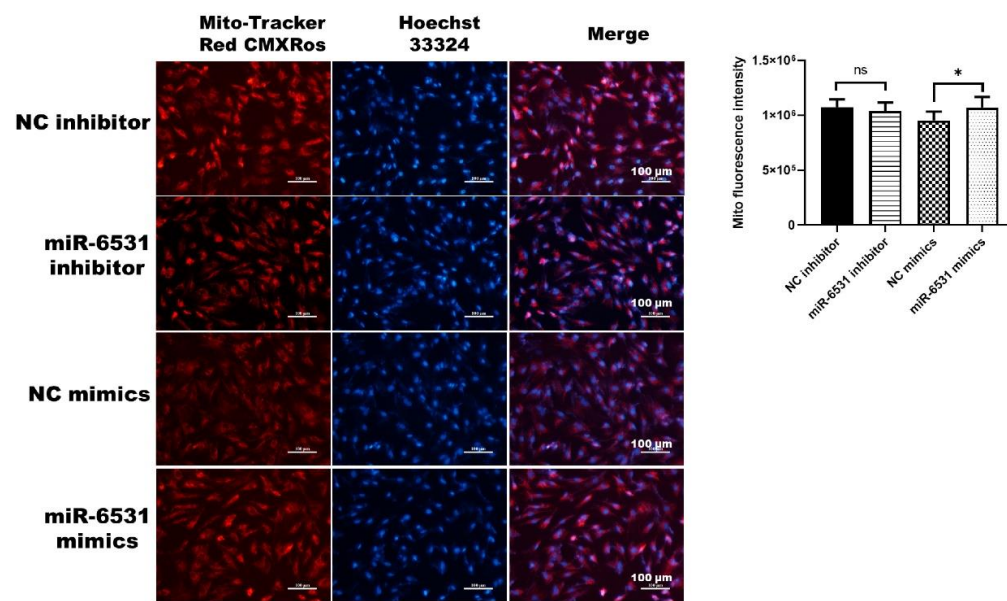


Figure S1. Measurement of MMP using fluorescent probe in Leydig cell.

Bovine Leydig cells were transfected with NC mimics or bta-miR-6531 mimics, and NC inhibitor or bta-miR-6531 inhibitors over 48 h, the cell MMP were measured. The mitochondria are marked by MitoTracker Red CMXRos, and nuclear is stained by Hoechst 33342.