

Neuromedin S Regulates Steroidogenesis through Maintaining Mitochondrial Morphology and Function via NMUR2 in Goat ovarian Granulosa Cells

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Table S1. The nucleotide of amplified sequence and NCBI reference sequence blast results.

Amplified sequence genes	NCBI reference sequence Accession	Max Score	Total Score	Query Cover	Per. Ident	Acc. Len
NMS	XM_005686290.2	907	907	98%	99.20%	708
NMUR1	XM_005676958.2	2370	2370	100%	98.94%	1800
NMUR2	XM_005683202.3	1816	1816	99%	99.50%	2150

Table S2. Details of primer sequences, expected product sizes and Genebank accession numbers of gene cloning.

Items	Primer sequence (5'-3')	Genebank No.	Fragment size (bp)
NMS	F: GGTGTCTTTGCCGGTGGAA	XM_005686290.2	577
	R: ATTGCCTGATCCAGACTCGG		
NMUR1	F: GAGCGGATCCAGGAACCAAG	XM_005676958.2	1383
	R: TTGGGGTGAAGCAGCATGAG		
NMUR2	F: GTCTTGCATTTTAATAGCAGTTATGGA	XM_005683202.3	1270
	R: AGAGTACAGAACCACACAGGGA		

Table S3. Accession number of amino acid sequence.

Items	NMS Genebank No.	NMUR1Genebank No.	NMUR2 enebank No.
Goat	ACS32212.1	XP_005677015.2	XP_005683259.1
Cattle	NP_001070990.1	XP_002685694.3	NP_001030220.1
Pig	XP_020944053.1	NP_001302643.1	NP_001302544.1
Human	NP_001011717.1	AAH36543.1	NP_064552.3
Mouse	NP_001298717.1	AAI37776.1	AAI37612.1

Table S4. Details of siRNA sequences used for GCs transfection.

Gene	Sense (5'-3')	Antisense (5'-3')
NMUR1-goat-974	GGACACAGGUGACCAAGAUTT	AUCUUGGUCACCUGUGUCCTT
NMUR1-goat-774 (siNMUR1)	CCCGAGAGCCAUCUACAAATT	UUUGUAGAUGGCUCUCGGGTT
NMUR1-goat-1100	UCCAGUACGUGCACGUCAUTT	AUGACGUGCACGUACUGGATT
NMUR2-goat-650	GCAUCAAGCUCCACUACUUTT	AAGUAGUGGAGCUUGAUGCTT
NMUR2-goat-844 (siNMUR2)	GCAGAUAAAGUGACUGCAATT	UUGCAGUCACUUUAUCUGCTT
NMUR2-goat-844 (siNMUR2)	CCAGAAAGGAACUCUCAATT	UUUGAGAGUCCUUUCUGGTT
YAP1-goat-1817 (siYAP1)	CCGCCACCAAGCUAGAUAAATT	UUAUCUAGCUUGGUGGCGGTT
Negative control	UUCUCCGAACGUGUCACGUTT	ACGUGACACGUUCGGAGAATT

Table S5. Details of primer sequences, expected product sizes and Genbank accession numbers of genes used for qPCR.

Items	Primer sequence (5'-3')	Genebank No.	Fragment size (bp)
NMS	F: ATTGTGAAGTTTGAGCGGATGG	XM_005686290.2	205
	R: CTGCCAGACGCATTAGAGGG		
NMUR1	F: GAGCGGATCCAGGAACCAAG	XM_005676958.2	210
	R: AACAGAGAGGAACCTGAGCC		

NMUR2	F: GTGTTTGCTATCTGCTGGGC	XM_005683202.3	320
	R: GGTAGAAGAAGACACCTGACACC		
STAR	F: GGTCCCCGAGACTTTGTGAG	XM_013975437.2	262
	R: AATCCACTTGGGTCTGCGAG		
CYP11A1	F: CACTTTCGCCACATCGAGAAC	NM_001287574.1	217
	R: AGGCTCCTGACTTCTTAAACAGG		
3BHSD	F: AGACCAGAAGTTCGGGAGGAA	XM_013962473.2	292
	R: TCTCCCTGTAGGAGTTGGGC		
CYP19A1	F: TGGTGTCCGAAGTTGTGCCTATTG	XM_013967046.2	393
	R: AAGGTCGAACAGCTTCCAGAGTG		
MST1 (STK4)	F: TCAGATCAACAGCTTCGGCA	XM_013968939.2	105
	R: ACCGTCCAGCTCTTCAGAAAC		
MST2 (STK3)	F: CAGCGCCCAAGAGTAAGCTA	XM_018058284.1	148
	R: GCCACAACCTTGACCAGATTCC		
LATS1	F: CTCATCAGCAACGTCTACATCG	XM_018053335.1	70
	R: TCTTGAGATAATCCAACCCGCA		
LATS2	F: AGTGCTCCTCCGAAAGGTTA	XM_018056548.1	146
	R: GCGTGCTCTCCCAGTTAATC		
YAP1	F: TGACCCTCGTTTTGCCATGA	XM_018059884.1	128
	R: TCTGTTGCTGCTGGTTGGAA		
MFN1	F: ATCAGGGAGGTTACAGAGGAGGTTG	XM_018047540.1	226

	R: GGGATTGAAGCATTGAAGCGTTGAC		
MFN2	F: GTTGTTGGAGGAGTGGTGTGGAAG R: TCTGGTTCATGGCGGCGATTTC3	XM_018060333.1	301
OPA1	F: GAACGCAGCATTGTTACAGACTTGG R: AGCCTGTTGTTCAACTGACTCTCG	XM_005675105.2	363
DNM1L	F: TTCCAATTATGCCAGCCAGTCCAC R: GTGCCATGTCCTCAGATTCAGTCAG	XM_018048322.1	294
FIS1	F: CTGAACGAGTTGGTGTCTGTGGAG R: GGTTGTTCTGCGGCTCTGTCTG	XM_005697811.3	304
ClpP	F: GGTGGAACAGACGGGTCG R: GGGCTTCTTGTTGCTTTCCG	XM_018050927.1	157
PKR	F: CCAAGCAACCAAAGAACCAGC R: CTTTGATGCCCCCTTCCAGT	XM_018055124.1	179
JNK	F: ATGGGCTACAAAGAGAACGTTGATA R: ATTCCTCACAGTTGGCTGAAGT	XM_018051622.1	177
JUN	F: GGAAACGACCTTCTACGACGATGC R: CCGTTGCTGGACTGTATGATGAGG	XM_018044742.1	243
HSP60	F: CGTTGCTGTGTTGAAGGTTGG R: AAGGCTGGAATGCACCGAAG	XM_018061271.1	153
GAPDH	F: CCGTTCGACAGATAGCCGTAA R: CCGTTCGACAGATAGCCGTAA	XM_005680968.3	296

Table S6. Details of antibodies.

Antibodies name	Cat NO.	Source	Dilutions used	Dilutions used	Dilutions used
			in IHC	in WB	in IF
NMS antibody	DF4237	LTD (USA)	1:250	1:800	1:200
Anti-NMUR1 antibody	AF9133	LTD (USA)	1:250	1:1000	1:200
NMUR2 polyclonal antibody	bs-11421R	Bioss (Beijing, China)	1:250	1:500	1:200
StAR polyclonal antibody	bs-3570R	Bioss (Beijing, China)	–	1:1000	–
CYP11A1 polyclonal antibody	bs-10099R	Bioss (Beijing, China)	–	1:1000	–
Anti-HSD3B1 antibody	NB110-78644SS	Novus (USA)	–	1:200	–
Cytochrome P450 19A1 antibody	DF6884	LTD (USA)	–	1:2000	–
STK4/MST1 polyclonal antibody	22245-1-AP	ProteinTech (IL, USA)	1:200	1:2000	1:100
STK3/MST2 polyclonal antibody	12097-1-AP	ProteinTech (IL, USA)	1:200	1:2000	1:200

Anti-MST1/2(Phospho-Thr183) antibody	D155304	BBI (Shanghai, China)	–	1:500	–
LATS1 polyclonal antibody	17049-1-AP	ProteinTech (IL, USA)	1:200	1:2000	1:100
Anti-LATS2 antibody	D260914	Sangon Biotech (Shanghai, China)	1:200	1:1000	1:100
Anti-LATS1/2(Phospho-Ser909/872) antibody	D155305	BBI (Shanghai, China)	–	1:1000	–
YAP1 polyclonal antibody	13584-1-AP	ProteinTech (IL, USA)	1:200	1:2000	1:100
Anti-YAP1(Phospho-Ser127) antibody	D151452	BBI (Shanghai, China)	–	1:1000	–
PGC1 alpha polyclonal antibody	NBP1-04676SS	Novus (USA)	–	1:500	–
Phospho-PGC1 α (S571) polyclonal antibody	AF6650-SP	R&D systems (USA)	–	1:1000	–
NFN1 polyclonal antibody	13798-1-AP	ProteinTech (IL, USA)	–	1:1000	–
NFN2 polyclonal antibody	12186-1-AP	ProteinTech (IL, USA)	–	1:4000	–
OPA1 polyclonal antibody	27733-1-AP	ProteinTech	–	1:2000	–

		(IL, USA)			
DRP1(C-terminal)	12957-1-AP	ProteinTech	–	1:2000	–
polyclonal antibody		(IL, USA)			
FIS1 polyclonal antibody	10956-1-AP	ProteinTech	–	1:2000	–
		(IL, USA)			
CLPP polyclonal antibody	15698-1-AP	ProteinTech	–	1:4000	–
		(IL, USA)			
Anti-EIF2AK2(PKR)	bs-1493R	Bioss	–	1:500	–
antibody		(Beijing, China)			
JNK1/2/3 polyclonal	51153-1-AP	ProteinTech	–	1:1000	–
antibody		(IL, USA)			
Phospho-					
JNK1/2/3(Thr183+Tyr185)	AF3318	LTD	–	1:500	–
polyclonal antibody		(USA)			
Phospho-JUN(Ser73)	28891-1-AP	ProteinTech	–	1:1000	–
polyclonal antibody		(IL, USA)			
HSP60 antibody	AF5374	LTD	–	1:1000	–
		(USA)			
GAPDH Mouse	60004-1-Ig	ProteinTech	–	1:8000	–
Monoclonal antibody		(IL, USA)			

HRP-conjugated					
Affinipure Goat Anti-	SA00001-2	ProteinTech	-	1:5000	-
Rabbit IgG(H+L)		(IL, USA)			
HRP-conjugated					
Affinipure Goat Anti-	SA00001-1	ProteinTech	-	1:5000	-
Mouse IgG(H+L)		(IL, USA)			
Goat Anti- Rabbit IgG	ab96886	Abcam	1:100	-	-
H&L (DyLight® 650)		(IL, USA)			

IHC: immunohistochemistry, WB: Western blot, IF: immunocytochemistry, (-): absent.

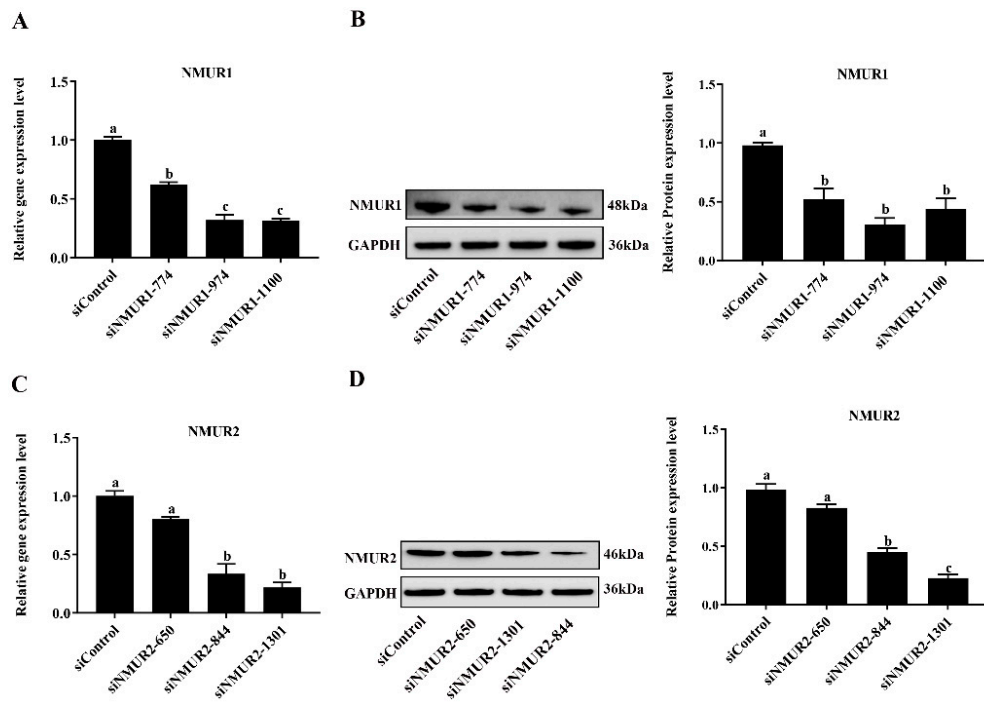


Figure S2. NMS receptors interference efficiency verification in goat granulosa cells. (A) The mRNA expression of NMUR1 in goat GCs under siNMUR1-774, siNMUR1-974, and siNMUR1-1100 treatment was determined by qPCR. (B) The protein expression of NMUR1 in goat CSs under siNMUR1-774, siNMUR1-974, and siNMUR1-1100 treatment was shown by western blot. (C) The mRNA expression of NMUR2 in goat GCs under siNMUR2-650, siNMUR2-844, and siNMUR2-1301 treatment was determined by qPCR. (D) The protein expression of NMUR2 in goat GCs under siNMUR2-650, siNMUR2-844, and siNMUR2-1301 treatment was shown by western blot. The data are expressed as fold change. Data are presented as mean \pm S.E.M. of the results obtained in at least three independent experiments. Different letters indicate significant differences in the expressions between the groups ($P < 0.05$).

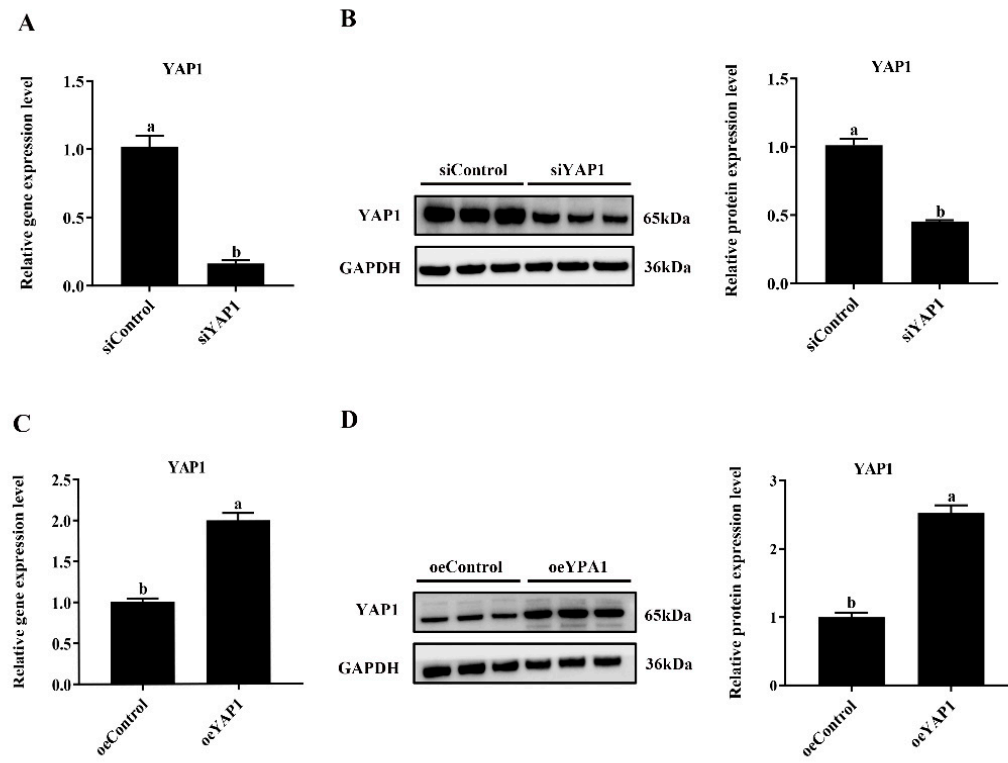


Figure S3. YAP1 interference and overexpression efficiency verification in goat granulosa cells. (A) The mRNA expression of YAP1 in goat GCs under siYAP1-1817 treatment was determined by qPCR. (B) The protein expression of YAP1 in goat GCs under siYAP1-1817 treatment was determined by western blot. (C) The mRNA expression of oeYAP1 treatment in goat GCs was determined by qPCR. (D) The protein expression of oeYAP1 treatment in goat GCs was determined by western blot. Data are presented as mean \pm S.E.M. value from at least three individuals. Different letters indicate significant differences in the expressions between the groups ($P < 0.05$).