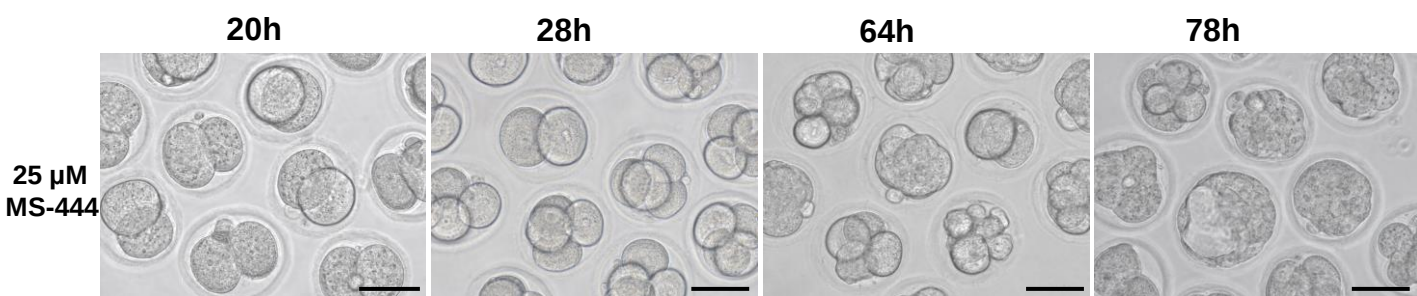
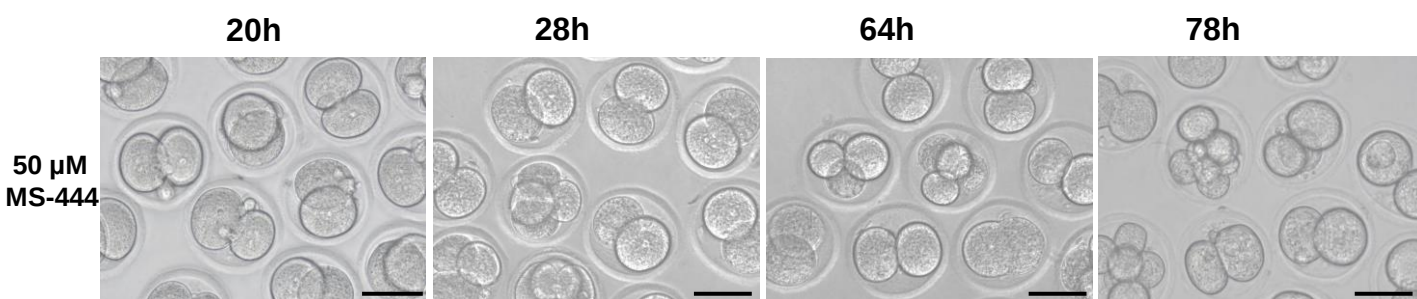
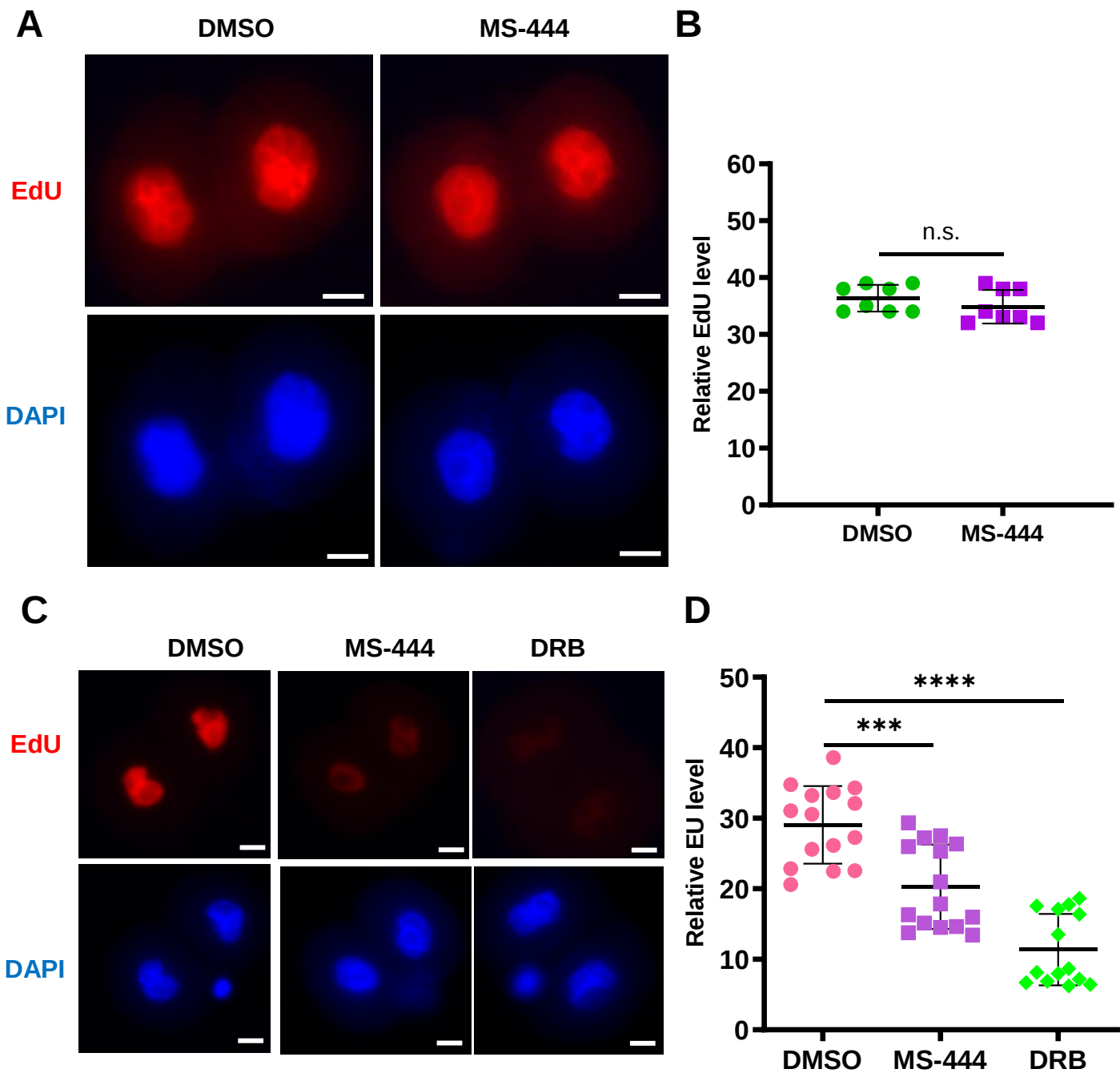


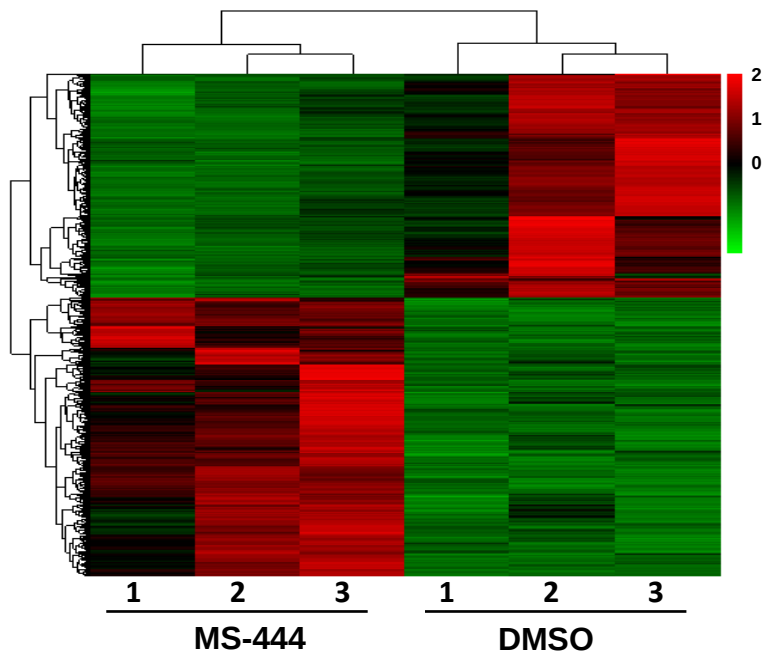
A**B**

Supplementary Figure 1. Effects of 25 μ M and 50 μ M MS-444 on early embryonic development. A) Morphology of embryos at 20, 28, 64, and 78 h following culture from late zygotes treated with 25 μ M MS-444. B) Morphology of embryos at 20, 28, 64, and 78 h following culture from late zygotes treated with 50 μ M MS-444. Scale bar represents 50 μ m.



Supplementary Figure 2. HuR dimerization is required for transcriptional activity in 2-cell stage embryos. A) Nuclear staining showing DNA replication levels by EdU staining in 2-cell stage embryos following culture with DMSO or MS-444. B) Quantification of the fluorescence in DNA replication levels in DMSO- or MS-444-treated 2-cell stage embryos by EdU incorporation. Eight embryos were observed for each group with three replicates. n.s., not significant. C) Nuclear staining showing newly synthesized RNA by EU staining in 2-cell stage embryos following culture with DMSO or MS-444. D) Quantification of the fluorescence in newly synthesized RNA in DMSO- or MS-444-treated 2-cell stage embryos by EU incorporation. More than 14 embryos were observed for each group with three replicates. Scale bar, 10 μ m. *** $p < 0.001$, **** $p < 0.0001$.

A



Supplementary Figure 3. Heatmap of gene expression differences between 2-cell stage embryos from the two groups.

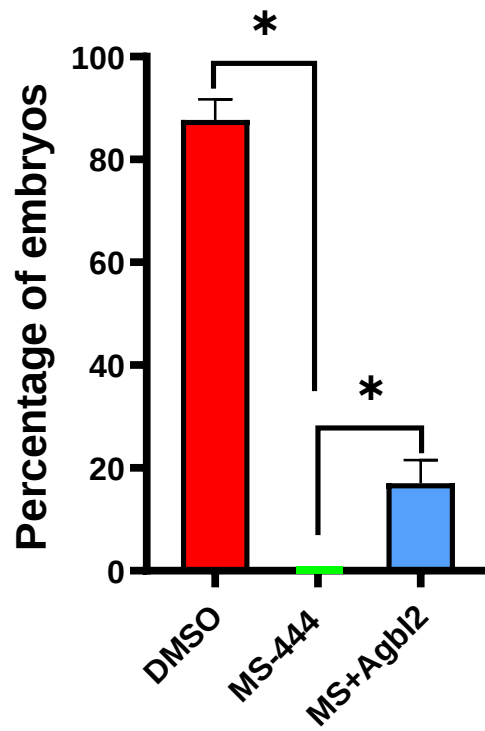
A

2798	uukruuu	cugcgacacacagcugcuuccucca aggguu ugggguaguuuuugguuuuuuuug	3.231	6.17e-04
2804	uukruuu	cacacagcugcuuccuccaaaggu uugggua guuuuuugguuuuuuuugaugca	3.099	9.71e-04
2808	uukruuu	cagcugcuuccuccaaagguuuugg uaguuu guuugguuuuuuuugaugcaaugc	3.495	2.37e-04
2809	uukruuu	agcugcuuccuccaaagguuuugg uaguuu guuugguuuuuuuugaugcaaugcu	3.253	5.71e-04
2812	uukruuu	ugcuuccuccaaagguuuuggguag uuuuuu ggguuuuuuuugaugcaaugcuggg	3.154	8.05e-04
2813	uukruuu	gcuuccuccaaagguuuuggguagu uuuguu ggguuuuuuuugaugcaaugcugggg	3.473	2.57e-04
2817	uukruuu	ccuccaaagguuuuggguaguuuug uugguu guuuuuugaugcaaugcuggggacca	4.176	1.48e-05
2821	uukruuu	caaagguuuuggguaguuuug uuuguu ugaugcaaugcugggggaccaaacc	4.055	2.51e-05
2822	uukruuu	aaagguuuuggguaguuuug uuguuu ugaugcaaugcugggggaccaaaccu	3.945	3.99e-05
2826	uukruuu	guuuuggguaguuuug uuugu ugaugcaaugcugggggaccaaaccucuag	3.385	3.56e-04
3069	uukruuu	cuuagaugcaugccuuguuaccucg ugaauu guuagggauuuuuuuuuccuuaaga	2.615	4.46e-03
3073	uukruuu	gaugcaugccuuguuaccucguga uuuguu agggauuuuuuuuuuccuuaugagag	3.033	1.21e-03
3083	uukruuu	uuguuaccucgugaauuuguuaggg auuuuuu uuuccuuaugagagauuauuuuu	2.934	1.67e-03
3084	uukruuu	uguuaccucgugaauuuguuaggg auuuuuu uuuccuuaugagagauuauuuuug	3.319	4.52e-04
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3086	uukruuu	uuaccucgugaauuuguuagggau uuuuuuu uccuuaugagagauuauuuuugau	3.319	4.52e-04
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3088	uukruuu	accucgugaauuuguuagggau uuuuuuu ccuuaugagagauuauuuuugaugu	2.659	3.92e-03
3107	uukruuu	gauuuuuuuuuuccuuaugagagau uaauuu ugaugauagaaaagccuuauuga	2.736	3.11e-03
3108	uukruuu	auuuuuuuuuuccuuaugagagau uaauuu ugaugauagaaaagccuuauugaa	2.824	2.37e-03
3113	uukruuu	uuuuuuuuuuuccuuaugagagauuauu uugaugu auagaaaagccuuauugaacgcug	2.396	8.29e-03
3131	uukruuu	uuauuuuuuugauguauagaaaagcc uuuaug aacgcugaagccuguacacauaaaa	2.165	1.52e-02
3183	uukruuu	uaaaaagacagaacaguguauauac uucuuu uaaccauagauuucuuuuuaag	2.275	1.15e-02
3226	uukruuu	uguucucuuaaaaguuagaugcaaa ucaguuu uaaaagaucgagcuguuuuaagugu	2.121	1.70e-02
3245	uukruuu	ugcaaaucaguuuuaaaagaucgag cuguuuu aaguguguuuugggaugugccaua	2.560	5.23e-03
3255	uukruuu	uuuuuuuagaucgagcuguuuuaag uguguuu ugggaugugccauaugaaaaaguc	2.363	9.06e-03
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3327	uukruuu	caaugggugacugaccagucagacu gugguu auuuaggguuugcuuaaaguuaggua	2.418	7.80e-03
3336	uukruuu	acugaccagucagacugugguuaua uuagguu ugcuuaaaguuagguaauuccuacau	2.648	4.05e-03
3337	uukruuu	cugaccagucagacugugguuaua uuagguu ugcuuaaaguuagguaauuccuacaua	2.758	2.91e-03
3341	uukruuu	ccagucagacugugguuauauuagg uuugcuu aaaguuagguaauuccuacauaggaa	2.681	3.67e-03
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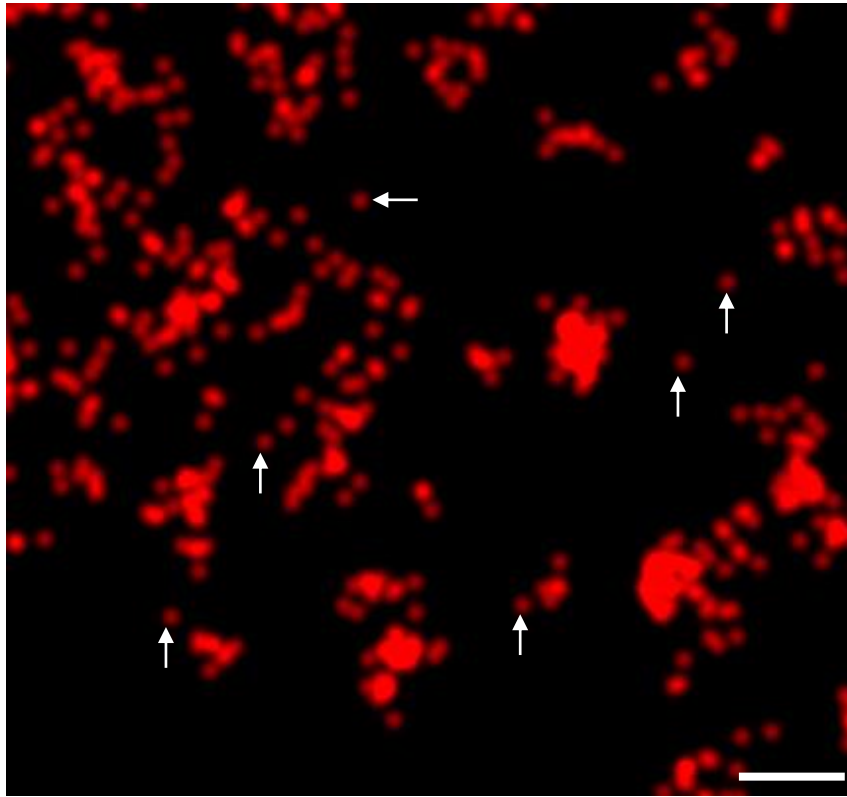
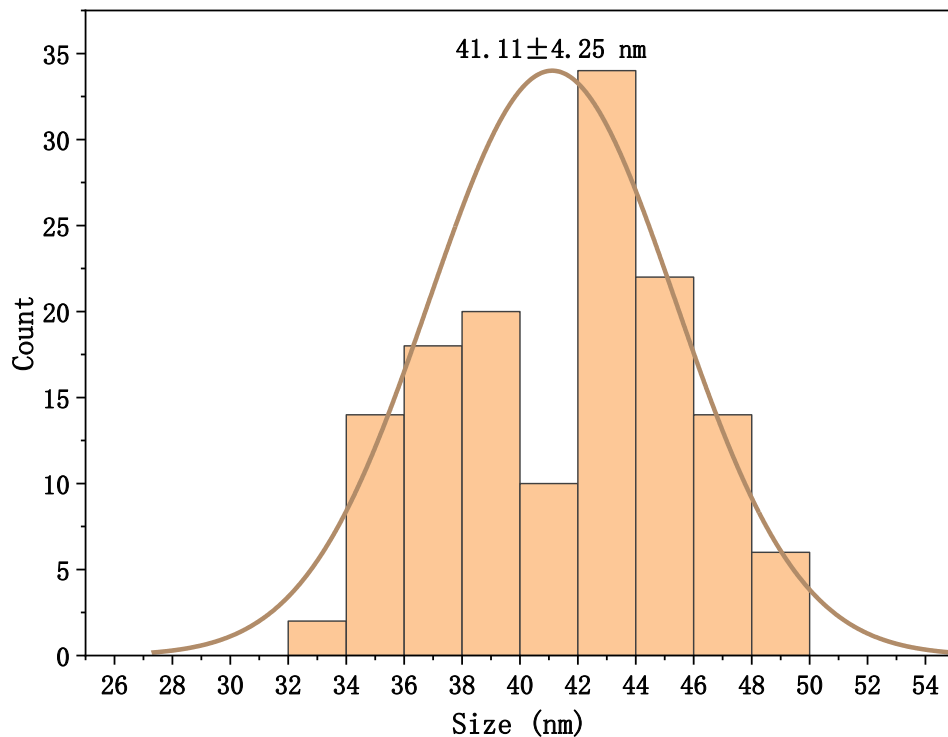
Agbl2 3'-UTR区域: 2762-3479

Supplementary Figure 4. The HuR binding site of Agbl2 mRNA was predicted by RBPmap analysis.

A



Supplementary Figure 5. Percentage of embryos reaching the blastocyst stage following microinjection of Agbl2 mRNA in late zygotes treated with MS-444. MS, MS-444. *p < 0.05.

A**B**

Supplementary Figure 6. The diameter of a Alexa 647-conjugated HuR protein. A) Representative STORM images of Alexa 647-conjugated HuR proteins. Arrows represent scattered single molecules. Scale bar, 300 nm. B) The distribution of the diameter of single molecule.

Table S1. Agbl2 RNA-FISH and quantitative PCR primers.

Sequences for Agbl2 RNA-FISH primers used in the study		
Agbl2	probe1	CCAC+TATCACGCCA+TCTGGGT+TTAACA+T
	probe2	GACAAA+TCTCGCCCT+TCCTGGGG
	probe3	CCG+TG TAGAGG+TCAGTCCGCAAGG+T
For PCR		
HuR	Forward (5'-3')	GGATGACATTGGGAGAACGAAT
	Reverse (5'-3')	TGTCCTGCTACTTTATCCCGAA
For qRT-PCR		
hRluc	Forward (5'-3')	CAAGGAGAAGGGCGAGGTT
	Reverse (5'-3')	AGGCGTTGTAGTTGCGGAC
luc	Forward (5'-3')	TTGAGGTGGACATCACCTATGC
	Reverse (5'-3')	CGCTCGTTGTAAATGTCGTTAG
Smarchb1(v2)	Forward (5'-3')	AGCTGCGAGACGCTTTTACC
	Reverse (5'-3')	CTCAATCTGCTGTCGAATGGC
stxbp1	Forward (5'-3')	GTGGACCAGTTAAGCATGAGG
	Reverse (5'-3')	GCTCTCGGCGCTTGTTGAT
PLK2	Forward (5'-3')	CCTGCGGACTATCACCTACCA
	Reverse (5'-3')	CTGCCCATCTTCAGAAGGCT
Aspscr1	Forward (5'-3')	GAGGGGCTGAGAACATAGTT
	Reverse (5'-3')	CTCCCTGGTCTGTGCAAAATG
VPS8	Forward (5'-3')	CCTCGTCTCAGGTAAAAATGTCG
	Reverse (5'-3')	CACCTCGGTTCTGGGAGAG
Agbl2	Forward (5'-3')	ATGAATGTCCTGCTTGAGATGG
	Reverse (5'-3')	CAAACGCGCTGATGAGTGC
Bicc1	Forward (5'-3')	AACACGCAGATTGCATGGC
	Reverse (5'-3')	TGACGCGGTTGCTTTTTGTG
Bcat2	Forward (5'-3')	CAGCCACACTAGGACAGGTCT
	Reverse (5'-3')	CAGCCTTGTTATTCCACTCCAC
Ctdspl	Forward (5'-3')	CCAGCGCAACATCAGCCTAA
	Reverse (5'-3')	GGCTTGGTACGGGAATGACC
Pdss2	Forward (5'-3')	CGCTTGTCGGTTACCTCG
	Reverse (5'-3')	GGGTAGCCACGATCTTCTC
Pitrm1	Forward (5'-3')	AGAGTATGGAGGGAGAAGAGTGA
	Reverse (5'-3')	GGACAGGAGTGACCTGGTTTAC
Prkg1	Forward (5'-3')	ATCCGAGAGGTCGAAGGATCT
	Reverse (5'-3')	ATTCCACGGGGTACATACAGT
Rufy2	Forward (5'-3')	GCAAACCTCTGCGAATCGAAAC
	Reverse (5'-3')	ACAGTGGGTTGCATCTTTGTC
Id2	Forward (5'-3')	ATGAAAGCCTTCAGTCCGGTG
	Reverse (5'-3')	AGCAGACTCATCGGGTTCGT
Manba	Forward (5'-3')	GACGTGAACTCCTTAAAGCTGC
	Reverse (5'-3')	GCCTTTCGGATGAAGTTGACG