

Supplemental Information

**A New Long Noncoding RNA ALB Regulates
Autophagy by Enhancing the Transformation of
LC3BI to LC3BII during Human Lens Development**

**Qiuli Fu, Zhenwei Qin, Lifang Zhang, Danni Lyu, Qiaomei Tang, Houfa Yin, Zhijian
Chen, and Ke Yao**

Figure.S1

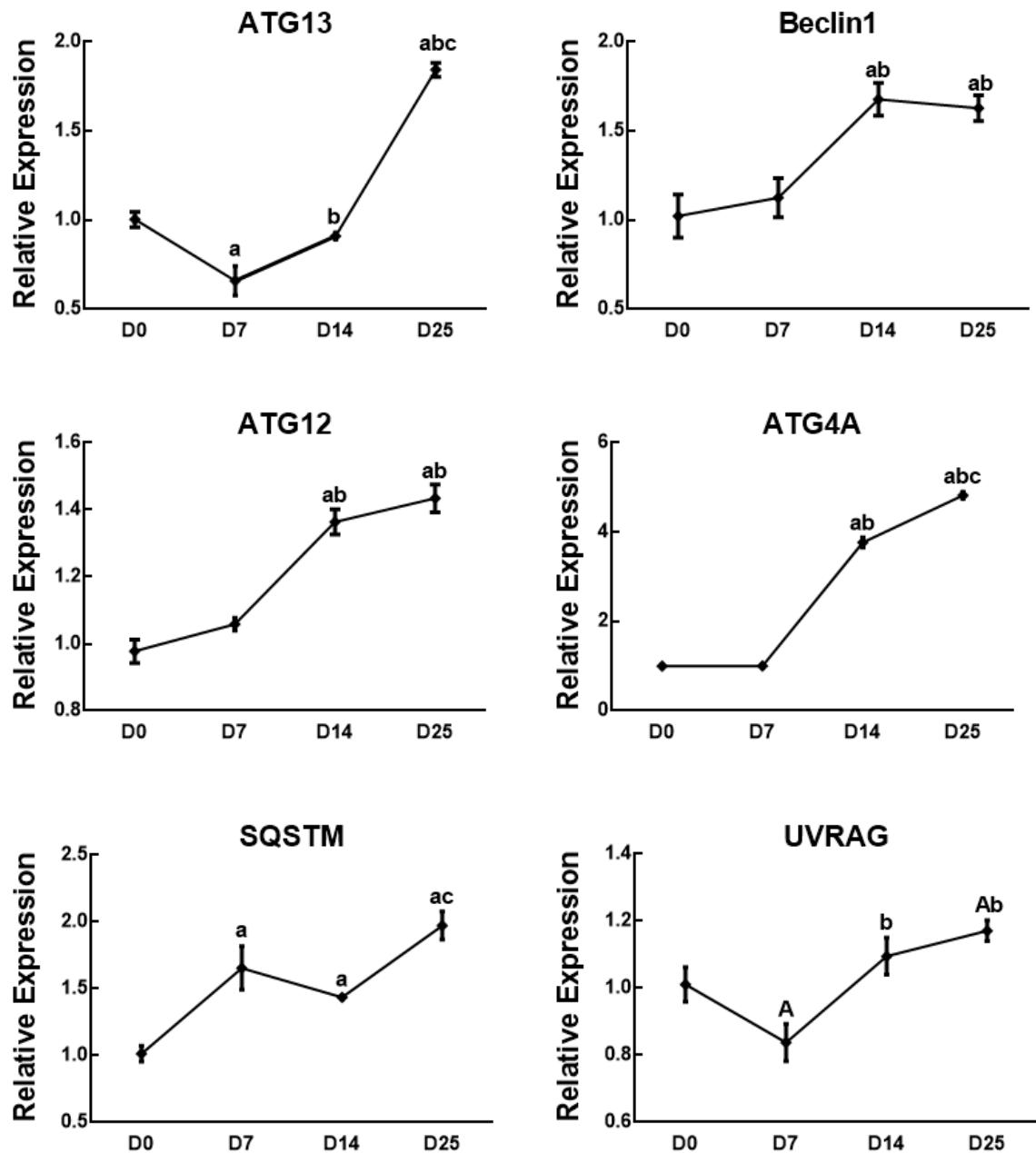
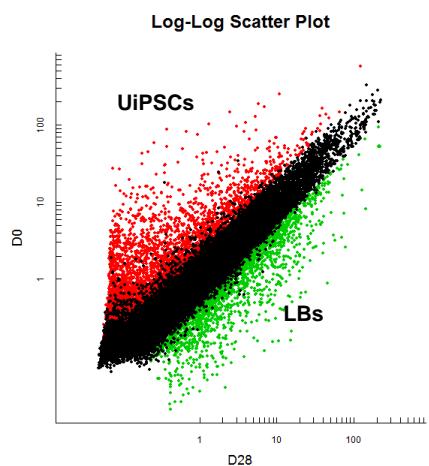
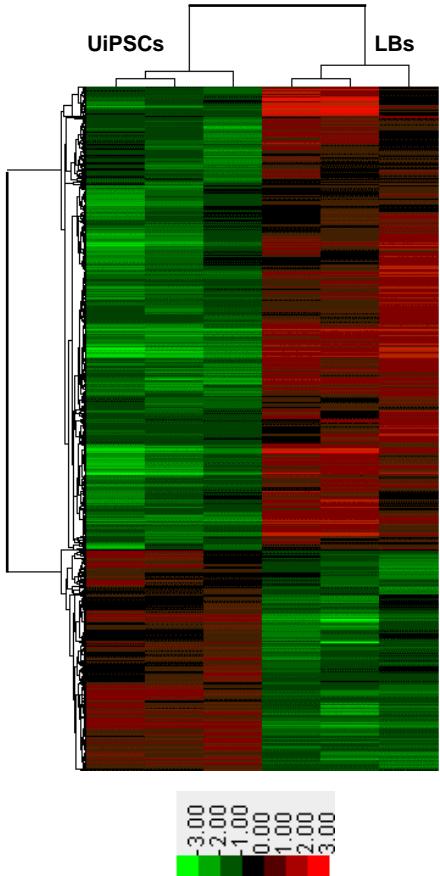


Figure.S2

A



C



B

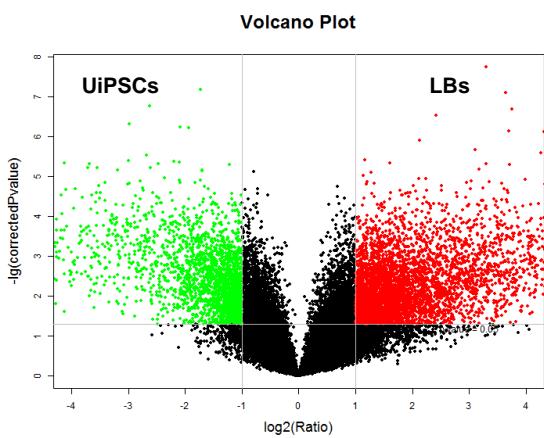


Figure.S3

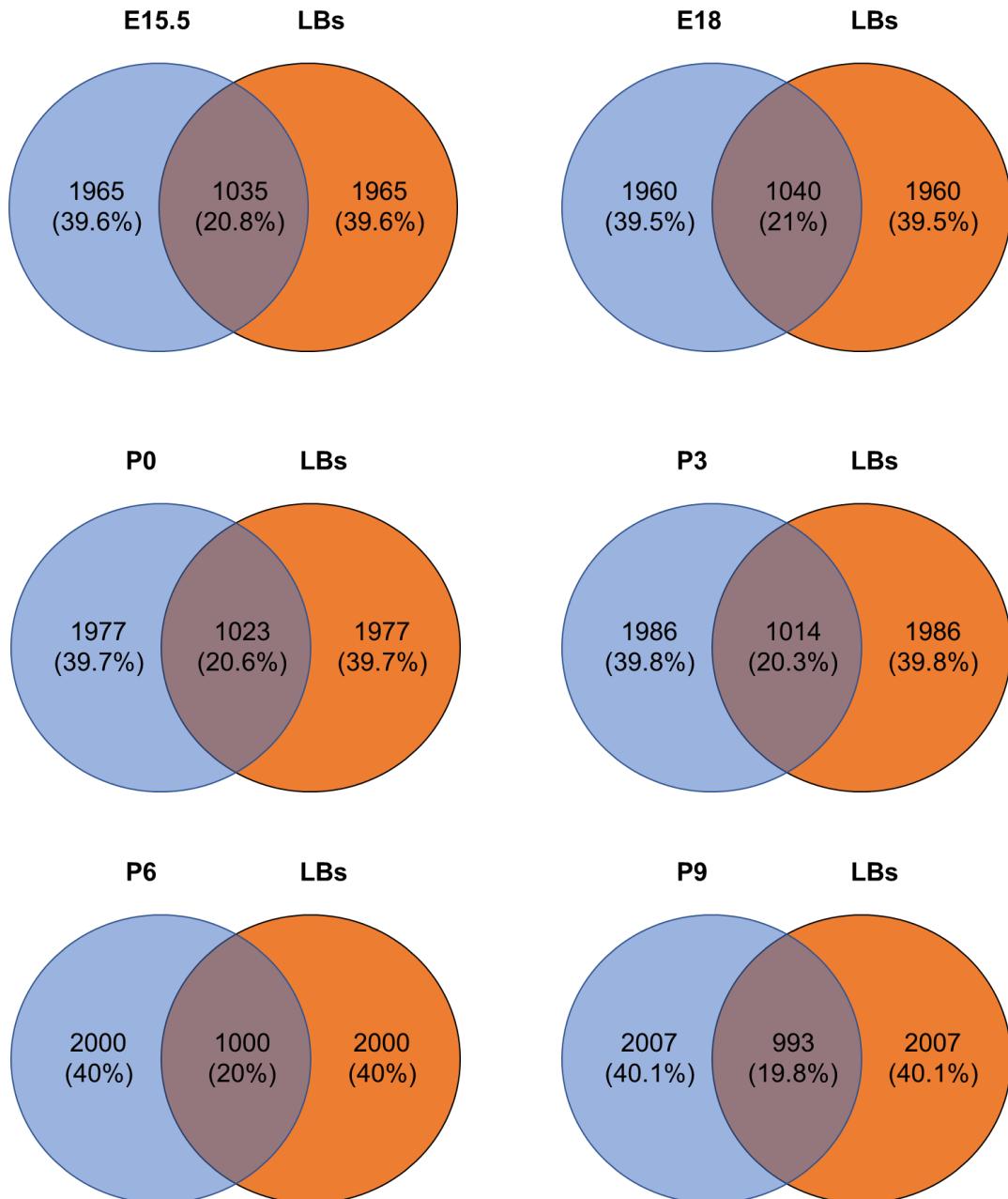


Figure.S4

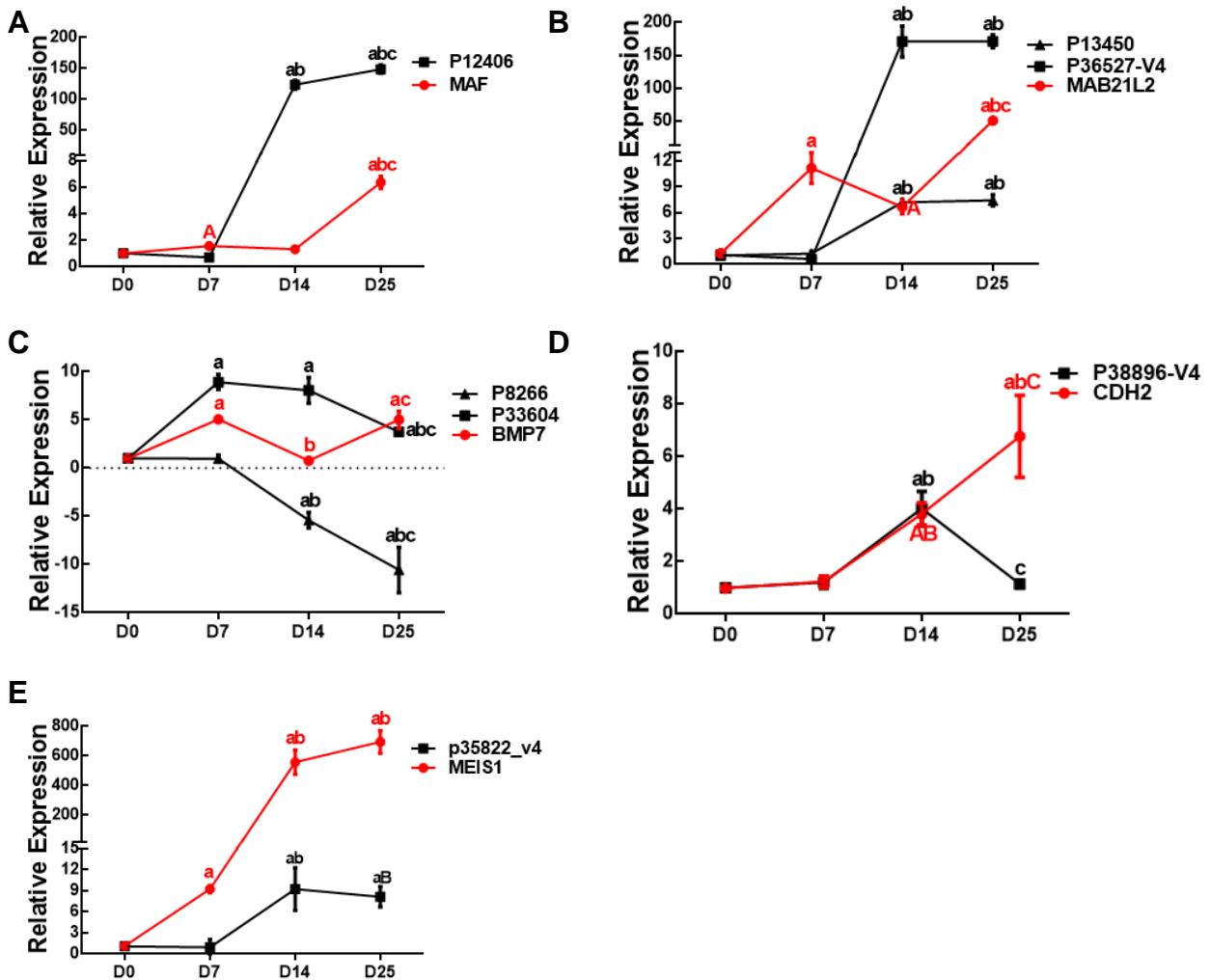
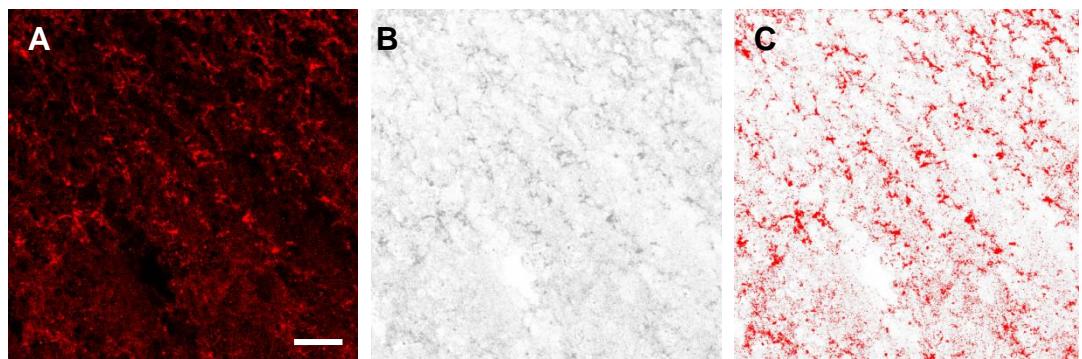


Figure.S5



SUPPLEMENTAL FIGURE LEGENDS

Figure. S1 Expression pattern of other autophagy associated genes during LB differentiation

qRT-PCR analysis showed the increased expression of other autophagy associated genes during LB differentiation process including ATG13, Beclin1, ATG12, ATG4A, SQSTM and UVRA. The bar represents mean \pm SEM (n=3 independent experiments). a = p<0.01 vs D0; A = p<0.05 vs D0; b = p<0.01 vs D7; c = p<0.01 vs D14.

Figure. S2 Differential expression of mRNA between LBs and UiPSCs.

(A-B) Log-Log Scatter plot and Volcano plot show the differential expressed mRNAs between LBs and UiPSCs. (C) Heat map shows the different expression profiling of mRNAs between LBs and UiPSCs.

Figure. S3 Global bioinformatic comparison of the gene expression patterns between LBs and mouse embryonic and postnatal lens.

Global bioinformatic comparison of top 3000 expressed genes identified

in LBs with that of mouse embryonic and postnatal lens published by others using second generation sequencing.

Figure. S4 Expression pattern of lens development associated mRNAs and their corresponding lncRNAs during LB differentiation process.

(A) qRT-PCR analysis of MAF and its corresponding lncRNA p12406. (B) qRT-PCR analysis of MAB21L2 and its corresponding lncRNAs including lncRNA p13450 and lncRNA p36527_v4. (C) qRT-PCR analysis of BMP7 and its corresponding lncRNAs including lncRNA p8266 and lncRNA p33604. (D) qRT-PCR analysis of CDH2 and its corresponding lncRNA p38896_v4. (E) qRT-PCR analysis of MEIS1 and its corresponding lncRNA p35882_v4. The bar represents mean \pm SEM ($n=3$ independent experiments). a = $p < 0.01$ vs D0; A = $p < 0.05$ vs D0; b = $p < 0.01$ vs D7; B = $p < 0.05$ vs D7; c = $p < 0.01$ vs D14; C = $p < 0.05$ vs D14.

Figure. S5 Example for algorithm of LC3B fluorescence.

(A) The original image of LC3B staining. (B) The transformed image

after turning into 8-bit type and inverted by Image J software. (C) The calculated image after adjusting its threshold to manifest the LC3B dots.
Scale bars: 25 μ m (630X).

Table S1. Differentially expressed autophagy related genes in LBs versus UiPSCs

mRNA	FC (abs) in microarray	FC (abs) in qRT-PCR	Regulation
SH3GLB1	3.96	2.78	up
WIPI1	3.4	9.81	up
MAP1LC3B	2.33	1.71	up
VAMP7	2.33	1.84	up
VMP1	2.45	1.87	up
BNIP3L	4.18	5.71	up
PTEN	1.86	2.06	up
ATG5	1.4	1.81	up
PINK1	1.97	4.18	up

“up” means the expression of mRNA is higher in LBs both in microarray and qRT-PCR analysis.

Table S2. Differentially expressed lens development related lncRNA and their correlated genes in LBs versus iPSCs

LncRNA ProbeName	LncRNA ID	Database	FC (abs) in microarray	FC (abs) in qRT-PCR	Regulation	Correlated mRNA
p12406	ENST00000513690.1	ENSEMBL	2.31	149.01	up	MAF
p36527_v4	ENST00000509463.1	ENSEMBL	5.05	171.24	up	MAB21L2
p13450	ENST00000517660.1	ENSEMBL	4.99	7.44	up	
p33604	ENST00000523759.1	ENSEMBL	4.4	3.76	up	BMP7
p8266	ENST00000567877.1	ENSEMBL	6.62	10.56	down	
p38896_v4	ENST00000571328.1	ENSEMBL	2.41	1.15	up	CDH2
p35822_v4	TCONS_00025230	HumanLincRNACatalog	5.64	8.15	up	MEIS1
p34737_v4	ENST00000444301.1	ENSEMBL	2.11	1.02	up/—	CTNNB1

“down” means the expression of lncRNA is lower in LBs both in microarray and qRT-PCR analysis. “up” means the expression of lncRNA is higher in LBs both in microarray and qRT-PCR analysis. “up/—” means the expression of lncRNA is higher in LBs in microarray analysis but shows no difference in qRT-PCR analysis.

Table S3. Differentially expressed lens development related genes in LBs *versus* UiPSCs

mRNA	FC (abs) in microarray	FC (abs) in qRT-PCR	Regulation
MAF	4.24	6.38	up
MAB21L2	4.97	50.46	up
BMP7	4.68	5.03	up
CDH2	4.89	6.78	up
MEIS1	12.18	694.94	up
CTNNB1	2.1	0.99	up/—

“up” means the expression of mRNA is higher in LBs both in microarray and qRT-PCR analysis. “up/—” means the expression of mRNA is higher in LBs in microarray analysis but shows no difference in qRT-PCR analysis.

Table S4. Primer sequences used in qRT-PCR

Gene	Direction	Primer sequence 5'→3'
SH3GLB1	Forward	ATTACCAAGACTTCTGCTAGAGGG
	Reverse	GGATGGAAAACCTCCCAGTTGTT
WIPI1	Forward	AGTCAGTCACACAAAACCACG
	Reverse	AGAGCACATAGACCTGTTGGG
MAP1LC3B	Forward	AAGGCCTTACAGCTCAATG
	Reverse	CTGGGAGGCATAGACCATGT
VAMP7	Forward	GAGGTTCCAGACTACTTACGGT
	Reverse	GACACTTGAGAACTCGCTATTCA
VMP1	Forward	GACCAGAGACGTGTAGCAATG
	Reverse	ACAATGCTTGACGATGCCATAA
BNIP3L	Forward	TTGGATGCACAACATGAATCAGG
	Reverse	TCTTCTGACTGAGAGCTATGGTC
PTEN	Forward	TTTGAAGACCATAACCCACCAC
	Reverse	ATTACACCAGTTCGTCCCTTTC
ATG5	Forward	AAAGATGTGCTTCGAGATGTGT
	Reverse	CACTTGTCAAGTACCAACGTCA
PINK1	Forward	GCCTCATCGAGGAAAAACAGG
	Reverse	GTCTCGTGTCCAACGGGTC
MAF	Forward	AGTCCTGCCGCTTCAAGAG
	Reverse	CCGCTGCTCACCAACTTCT
MAB21L2	Forward	GTCTCTCTGGGTCGAGTTCAT
	Reverse	CACATCCCGATAGCTGCACTT
BMP7	Forward	TCAACCTCGTGGAACATGACA
	Reverse	CTTGGAAAGATCAAACCGGAAC
CDH2	Forward	AGCCAACCTTAACTGAGGAGT
	Reverse	GGCAACTTGATTGGAGGGATG
MEIS1	Forward	TACCCGCACACAGCTCATAC
	Reverse	CATTGAATGACTCTGACGAGCA
CTNNB1	Forward	CATCTACACAGTTGATGCTGCT
	Reverse	GCAGTTTGTCAAGTCAGGGA
Beclin1	Forward	GGTGTCTCTCGCAGATTATC
	Reverse	TCAGTCTCGGCTGAGGTTCT
UVRAG	Forward	ATGCCAGACCGTCTTGATACA
	Reverse	TGACCCAAGTATTCAGCCCCA
SQSTM	Forward	GCACCCCAATGTGATCTGC
	Reverse	CGCTACACAAAGTCGTAGTCTGG
ATG12	Forward	TAGAGCGAACACGAACCATCC

		Reverse	CACTGCCAAAACACTCATAGAGA
ATG4A		Forward	TGCTGGTTGGGGATGTATGC
		Reverse	GCGTTGGTATTCTTGGGTTGT
ATG13		Forward	AGACAGTCGTGTTGGGACAG
		Reverse	CTCAAATTGCCTGGTAGACATGA
LncRNA p4790		Forward	AGAGCCACCTACGACCTCA
		Reverse	CTCCATCTCCTGTCTTCCT
LncRNA p13116		Forward	GCCTGTTGGTGGTCTCTTC
		Reverse	CTGTTCCTGTGTTAGTTGCTGA
LncRNA p38664_v4		Forward	TCAATAGTCAAGGAGGACAAAGAC
		Reverse	TTCGGTGATGCTGTAGATGG
LncRNA p19101		Forward	GCACTCAAGACCCTCTACGG
		Reverse	GCCATCCCTGTTCACTCCT
LncRNA p13515		Forward	CTGCTCTTGCTCCGTGAAA
		Reverse	GGCTGTGAGGTGTAGAAGTCT
LncRNA p34310_v4		Forward	ATGAATCTCGCTGTGTTGTC
		Reverse	GGTATTAGGTGTCCAAGAAGAA
LncRNA ALB		Forward	TGGGAGAGGAGAACAGAGATGC
		Reverse	CTGTAGGTTGCTGGTGGTCA
LncRNA p35822_v4		Forward	GCTGCGAGAGAACATTTGTTTC
		Reverse	AAACTCACCGTGTGATGA
LncRNA p13839		Forward	GACAGCAACATTCCCTTCAG
		Reverse	TACCTTCACAGCCTCCAA
LncRNA p36469_v4		Forward	TCTTGCCTCTGCCTCCTGAGT
		Reverse	CCTGTGGTTGCCTCTGTGCTTA
LncRNA p12406		Forward	CCACCTCGGTTCCACTCAAC
		Reverse	TCTCACAAGGCTGTCAACTCA
LncRNA p36527-V4		Forward	CGCTTCGGGATGAAATGACCAG
		Reverse	GCTGCTTCTGCCTCTGTCTTCT
LncRNA p13450		Forward	TCCTGCCTGTCCCTACTCA
		Reverse	GCTCTTATCTTGCTGCTGATGG
LncRNA p33604		Forward	TTCCTGGTTCTGGCTGGCATC
		Reverse	TGGATTCCCTGAGGT CGCACTG
LncRNA p8266		Forward	TCCTGCCACCTCAACCTCACA
		Reverse	TCCACCACATTGCTGCCTCTG
LncRNA p38896-v4		Forward	ACCGCCAGACTATGATGAGAG
		Reverse	GGGAAGGAGAGATGGAGTTAT
LncRNA p34737-v4		Forward	CCAGCAGACCACGCTCATTACT
		Reverse	GGCAGCACAGCAGTCACCTT