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Supplemental Information

lncRNA *Neat1* regulates neuronal dysfunction post-sepsis via stabilization of hemoglobin subunit beta

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Supplemental figure

Figure S1

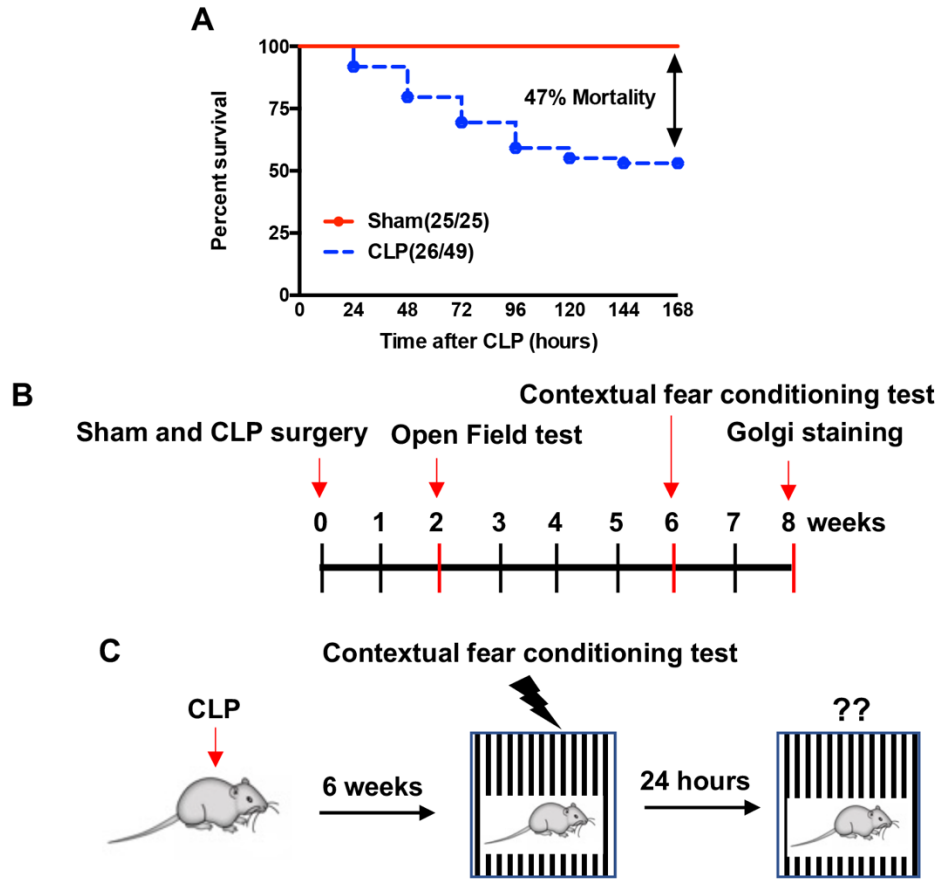

Figure S1. The survival rate of CLP-induced sepsis model and timeline of the experimental design.

(A) Cecal ligation and puncture (CLP)-induced sepsis resulted in 47% mortality over 7 days. (B) Diagram of the timeline for the experiments in this study. Mice were subjected to sham or CLP surgery. The open field (OF) test was performed at 2 weeks after CLP, and contextual fear conditioning (CFC) test was performed at 6 weeks after CLP. Mice were sacrificed at 8 weeks after CLP and dendritic spine density were determined. (C) Graphic depiction of CFC paradigm. Mice were subjected to a foot shock at 6 weeks after CLP and freezing behavior was monitored 24 hours after the foot shock.

Figure S2

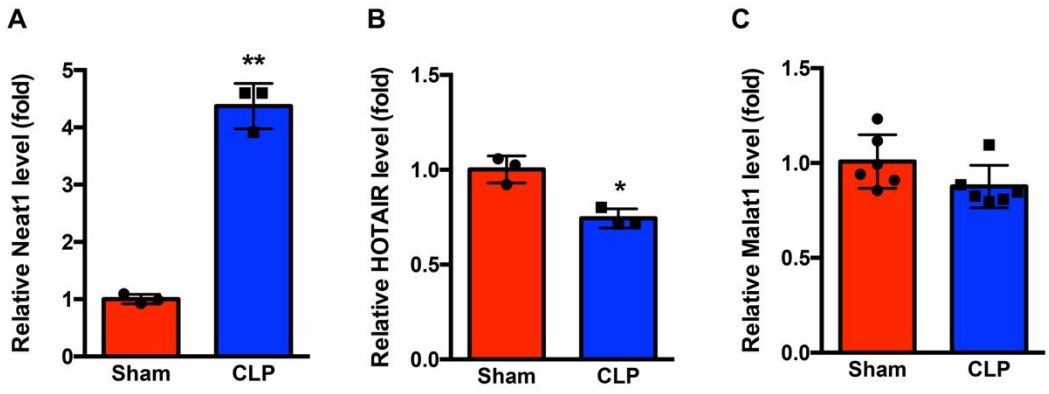

Figure S2. The LncRNA expression levels in mouse brain tissues after CLP.

The expression levels of *Neat1* (A), *HOTAIR* (B) and *Malat1* (C) in brain tissue were assessed 24h after sham or CLP (*P < 0.05, **P < 0.01, n = 3-6 mice/group)

Figure S3

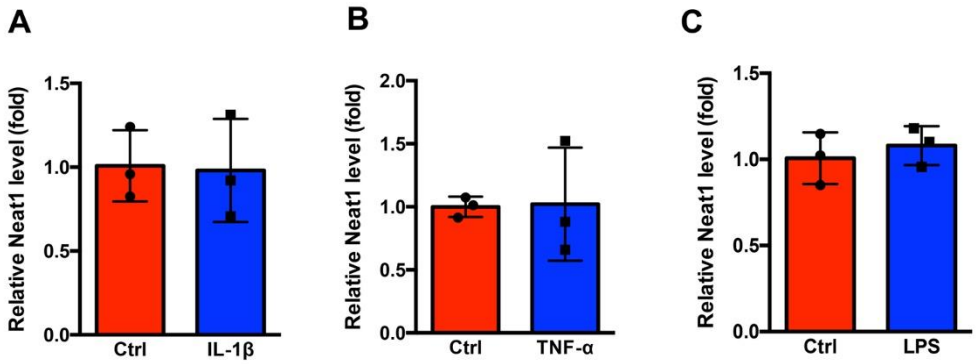

Figure S3. Inflammatory cytokines IL-1β, TNFα or LPS do not induce *Neat1* expression in N2a cell.

N2a cells were treated with IL-1β (40 ng/ml, A), TNF-α (20 ng/ml, B) or LPS (100 ng/ml, C) for 16h. *Neat1* expression levels were determined by RT-PCR (n = 3)

Figure S4

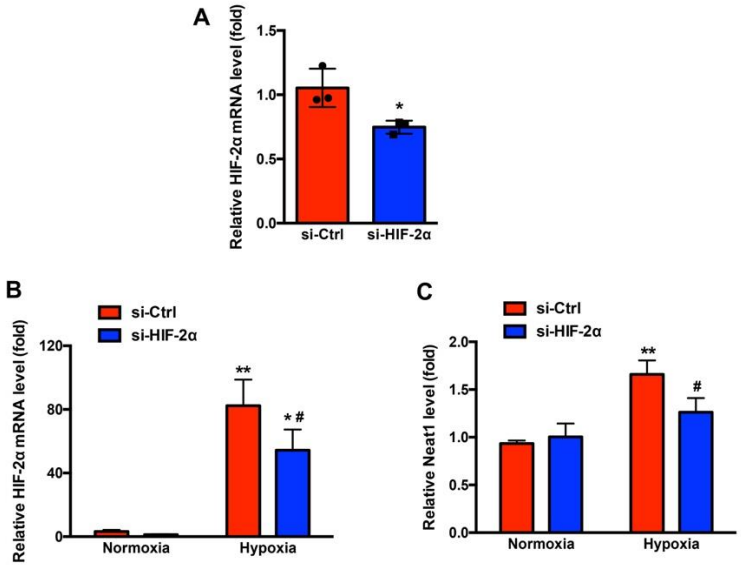

Figure S4. Hypoxia induced increases of *Neat1* levels were mediated through HIF-2*α* dependent signaling pathway.

(A) N2a cells were treated with siRNA against the HIF-2α and *HIF-2α* mRNA levels were analyzed by RT-PCR (*P < 0.05, n = 3). N2a cells were transfected with control or HIF-2α siRNA and expression levels of *HIF-2α* (B) and *Neat1* (C) in the normoxia and hypoxia condition were determined by RT-PCR (*P < 0.05, **P < 0.01 compared with normoxia group, $^{\#}$P < 0.05 compared with si-Ctrl hypoxia group, n = 3).

Figure S5

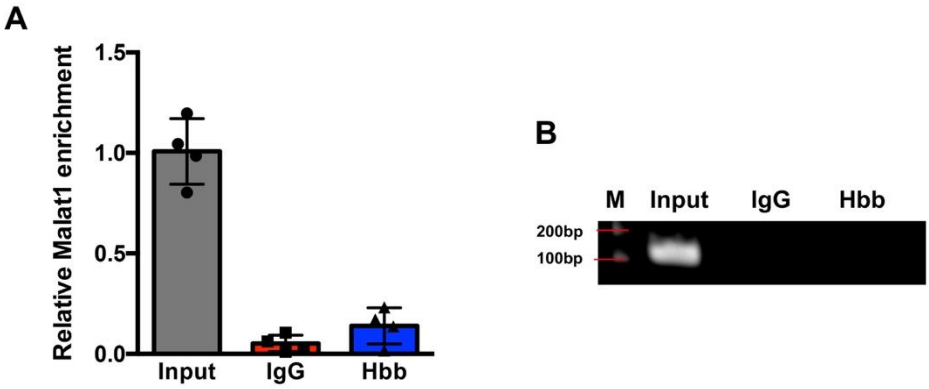

Figure S5. Hbb protein was not associated with Malat1

RNA immunoprecipitation (RIP) assays were performed in N2a cells. Protein-RNA complexes immunoprecipitated by anti-Hbb or control IgG were determined by qRT-PCR using primer for *Malat1* (A) and the qRT-PCR products were analyzed by electrophoresis (B) (M: marker).

Figure S6

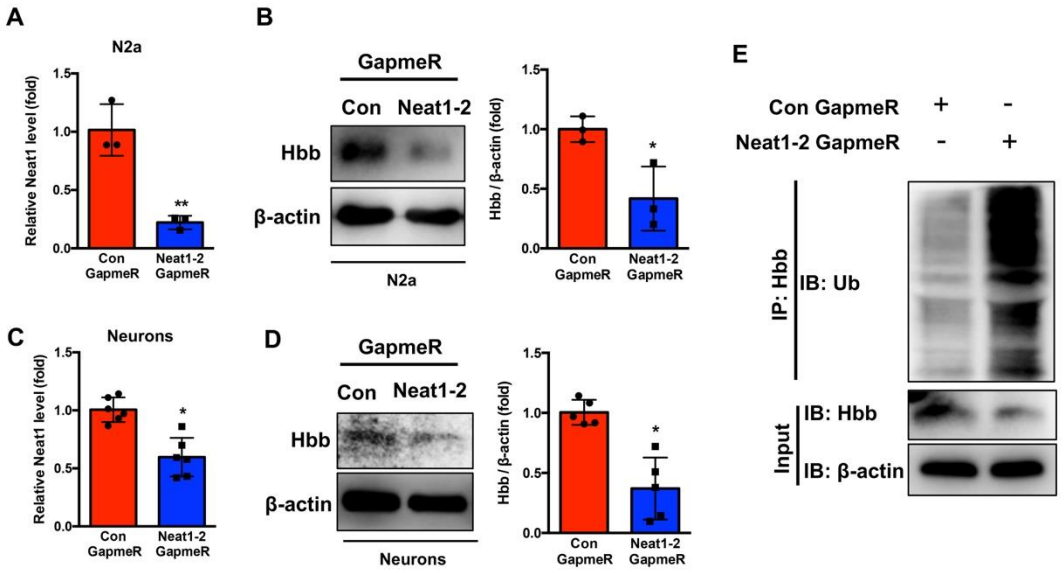

Figure S6. Neat1 stabilizes Hbb via inhibiting Hbb ubiquitination.

(A) The *Neat1* levels were measured in N2a cells transfected with Neat1 GapmeR #2 (**P<0.01, n = 3). (B) The Hbb protein levels in N2a cells after transfection with *Neat1* GapmeR #2 (*P<0.05, n =3). (C) The *Neat1* levels were determined in primary neuronal cells transfected with *Neat1* GapmeR #2 for 24h (*P<0.05, n = 6). (D) The Hbb protein levels in primary neuronal cells after transfection with *Neat1* GapmeR #2 for 24h (*P<0.05, n = 5). (E) N2a cells transfected with control or *Neat1* GapmeR #2 were treated with MG-132 (5 μM) for 16h. Cell lysates were immunoprecipitated with antibodies against Hbb or IgG. The levels of ubiquitination were analyzed by western blot. Lower panel, input from cell lysates. IB, immunoblot.

Figure S7

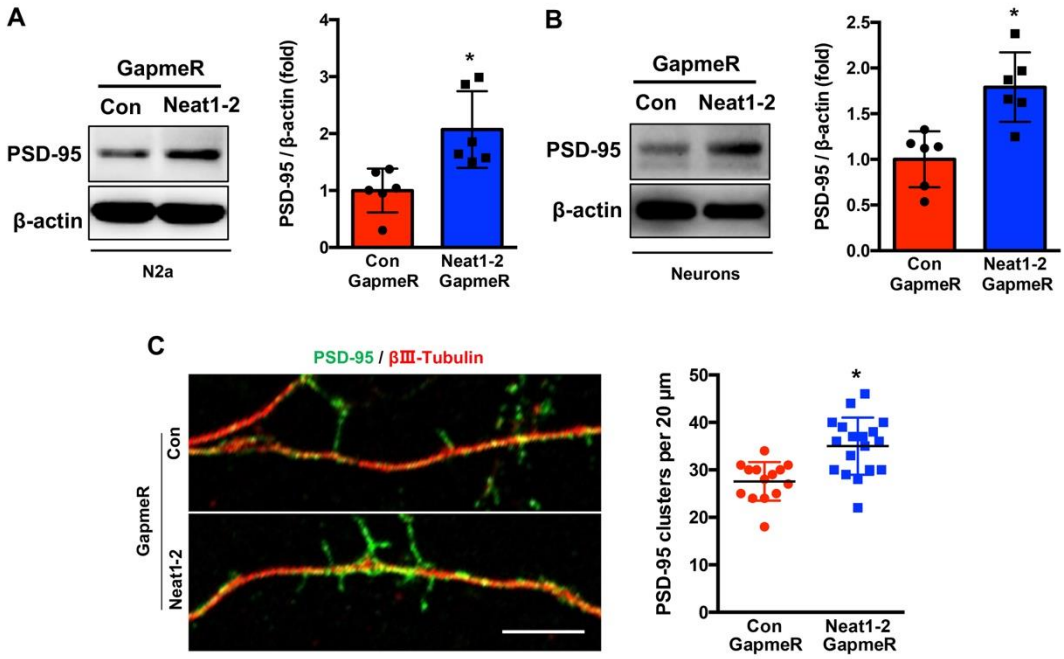

Figure S7. Inhibition of Neat1 by GapmeR Neat1 #2 increases PSD-95 expression and dendritic spine density.

(A) The PSD-95 protein levels were measured in N2a cells after transfection with *Neat1* GapmeR #2 for 48h (*P<0.05, n = 6). (B) The protein levels of PSD-95 were detected after transfection of the primary neuronal cells with *Neat1* GapmeR #2 for 24h (*P<0.05, n = 6). (C) Primary neurons were transfected with control or *Neat1* GapmeR #2 for 24h. The dendritic spine numbers were analyzed by immunostaining to label PSD-95 puncta and axons (*P<0.05, n = 6, PSD-95: green, βIII-Tubulin: red, Scale bar=5 μm).

Figure S8

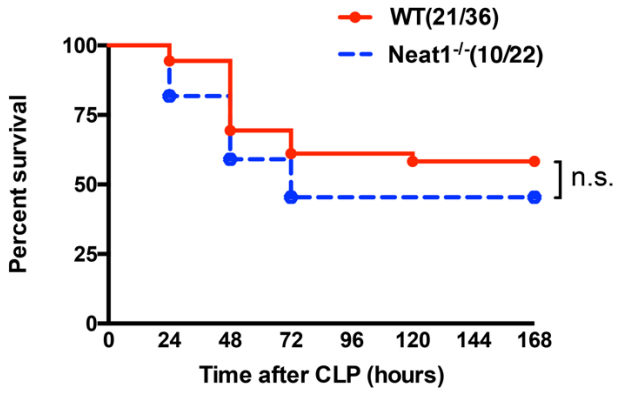

Figure S8. The survival rate of CLP-induced sepsis model in wild-type and Neat1$^{-/-}$ mice

Survival curves of WT and *Neat1*$^{-/-}$ mice after cecal ligation and puncture (CLP) over 168 hours. Mortality rate for WT mice was 42% and for *Neat1*$^{-/-}$ mice was 55%.

Figure S9

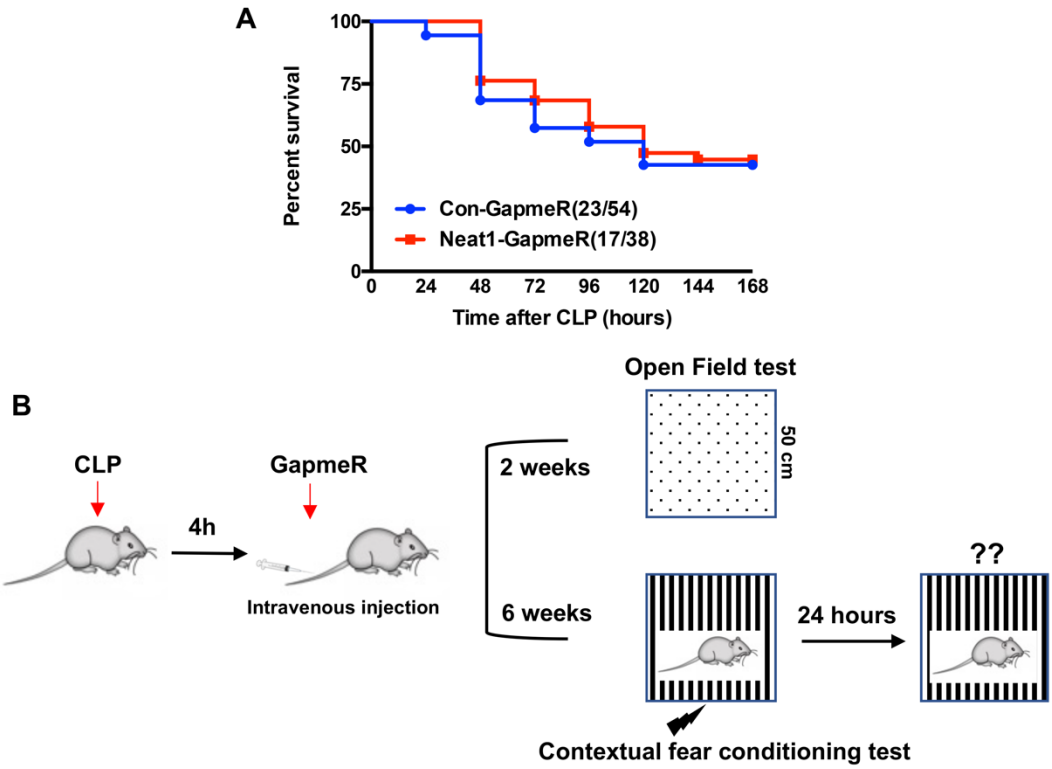

Figure S9. The survival rate of CLP-induced septic mice treated with GapmeRs and the experimental design for behavior tests after Neat1 GapmeR treatment.

(A) Cecal ligation and puncture (CLP) sepsis resulted in 57% mortality after treatment with control GapmeR over 7 days, and CLP mice treatment with *Neat1* GapmeR resulted in 55% mortality. (B) Graphic depiction of open field test and single-pairing CFC paradigm in the GapmeR treated septic mice.

Table. S1: The details of proteins that bound to *Neat1* in lysed neuronal cells and their expression levels were altered 2-fold after CLP by LC-MS/MS analysis

Protein IDs	Majority protein IDs	Protein names	Gene names	LFQ intensity CLP	LFQ intensity Sham	CLP-Sham	Fold
P01942;P06467	P01942	Hemoglobin subunit alpha	Hba	32.82627106	30.12743378	2.69883728	6.4928
P84244;P02301	P84244;P02301	Histone H3.3;Histone H3.3C	H3f3a;H3f3c	32.06814194	29.47618484	2.59195709	6.0292
P08551	P08551	Neurofilament light polypeptide	Nefl	31.68420029	29.39054871	2.29365158	4.9030
P02088;P02089;CON__Q3SX09;CON__P02070;P02104	P02088;P02089	Hemoglobin subunit beta-1;Hemoglobin subunit beta-2	Hbb-b1;Hbb-b2	31.23020363	28.47119331	2.75901031	6.7693
P08553	P08553	Neurofilament medium polypeptide	Nefm	31.1278019	29.23288155	1.89492035	3.7190
P60202;P60202-2	P60202;P60202-2	Myelin proteolipid protein	Plp1	31.0664444	28.93438339	2.132061	4.3834
P15864;Q07133	P15864	Histone H1.2	Hist1h1c	30.32345581	32.5653801	-2.2419243	0.2114
P19246	P19246	Neurofilament heavy polypeptide	Nefh	29.55605316	27.56347275	1.99258041	3.9795
Q9Z2D6-2;Q9Z2D6	Q9Z2D6-2;Q9Z2D6	Methyl-CpG-binding protein 2	Mecp2	29.26391792	30.37181473	-1.1078968	0.4640
P10922	P10922	Histone H1.0;Histone H1.0, N-terminally processed	H1f0	29.03351784	31.00309753	-1.9695797	0.2553
P21844	P21844	Chymase	Cma1	28.78441048	27.57708359	1.20732689	2.3091
Q9JHU4	Q9JHU4	Cytoplasmic dynein 1 heavy chain 1	Dync1h1	28.70833015	30.03826141	-1.3299313	0.3978
P16330-2;P16330	P16330-2;P16330	2,3-cyclic-nucleotide 3-phosphodiesterase	Cnp	28.70454216	26.92953873	1.77500343	3.4224
P43274	P43274	Histone H1.4	Hist1h1e	28.62505531	30.4685936	-1.8435383	0.2786
P0CW02;P0CW03	P0CW02;P0CW03	Lymphocyte antigen 6C1;Lymphocyte antigen 6C2	Ly6c1;Ly6c2	28.29535294	27.24780464	1.04754829	2.0670
O54962	O54962	Barrier-to-autointegration factor;Barrier-to-autointegration factor, N-terminally processed	Banf1	27.51638031	25.75121117	1.76516914	3.3991
P43277	P43277	Histone H1.3	Hist1h1d	27.27746201	29.79197311	-2.5145111	0.1750
P63276	P63276	40S ribosomal protein S17	Rps17	26.50084686	27.81860542	-1.3177586	0.4012
Q02257	Q02257	Junction plakoglobin	Jup	26.47374916	25.28665543	1.18709373	2.2769
O08599;O08599-2	O08599;O08599-2	Syntaxin-binding protein 1	Stxbp1	26.42356682	27.54510117	-1.1215343	0.4596
Q9R069	Q9R069	Basal cell adhesion molecule	Bcam	26.11221313	24.4571991	1.65501404	3.1493
P47955	P47955	60S acidic ribosomal protein P1	Rplp1	25.9355526	27.59199333	-1.6564407	0.3172
P63085;Q63844	P63085	Mitogen-activated protein kinase 1	Mapk1	25.71988869	26.82699203	-1.1071033	0.4642
P21619;P21619-2	P21619;P21619-2	Lamin-B2	Lmnb2	25.57346153	28.06002617	-2.4865646	0.1784
P60229	P60229	Eukaryotic translation initiation factor 3 subunit E	Eif3e	25.12986565	26.43552208	-1.3056564	0.4045
Q8CHT1-2;Q8CHT1	Q8CHT1-2;Q8CHT1	Ephexin-1	Ngef	24.10876846	25.27255821	-1.1637897	0.4463

Table. S2: Primers used for quantitative RT-qPCR (F: forward; R: reverse)

Gene Name	Species	Sequence (5' - 3')
c-fos	Mouse	F: CGGGTTTCAACGCCGACTA
	Mouse	R: TTGGCACTAGAGACGGACAGA
Egr1	Mouse	F: TATACTGGCCGCTTCTCCCT
	Mouse	R: AGAGGTCGGAGGATTGGTCA
Arc	Mouse	F: AAGTGCCGAGCTGAGATGC
	Mouse	R: CGACCTGTGCAACCCTTTC
Bdnf	Mouse	F: TCATACTTCGGTTGCATGAAGG
	Mouse	R: AGACCTCTCGAACCTGCCC
Homer1	Mouse	F: CCCTCTCTCATGCTAGTTCAGC
	Mouse	R: GCACAGCGTTTGCTTGACT
Nrn1	Mouse	F: GCGGTGCAAATAGCTTACCTG
	Mouse	R: CGGTCTTGATGTTCGTCTTGTC
Hbb-b1	Mouse	F: GCACCTGACTGATGCTGAGAA
	Mouse	R: TTCATCGGCGTTCACCTTTCC
Neat1	Mouse	F: GCTCTGGGACCTTCGTGACTCT
	Mouse	R: CTGCCTTGGCTTGGAAATGTAA
GAPDH	Mouse	F: GGCAAATTCAACGGCACAGT
	Mouse	R: GGGTCTCGCTCCTGGAAGAT
Malat1	Mouse	F: GGGAGTGGTCTTAACAGGGAGGAG
	Mouse	R: GTGCCAACAGCATAGCAGTACACG
HOTAIR	Mouse	F: TCCAGATGGAAGGAACTCCAGACA
	Mouse	R: ATAGATGTGCGTGGTCAGATCGCT
PVT1	Mouse	F: CCTGGATGCCCACTGAAAAC
	Mouse	R: GATAGACTGCTTGCCAGGGG
HIF-2α	Mouse	F: CTGAGGAAGGAGAAATCCCGT
	Mouse	R: TGTGTCCGAAGGAAGCTGATG