Supporting Information

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SI Materials and Methods

Animals. For electroconvulsive seizure (ECS) and voluntary exercise experiments, male Sprague–Dawley rats (weight 200–250 g) were used. Rats were habituated under our laboratory conditions for 4–6 d before initiation of the experiments, and food and water were provided ad libitum. *Mif*$^{-/-}$ animals in the C57/BL6 (B6) mouse background were bred at Pohang University of Science and Technology's animal facilities from a breeding pair provided by R.B. (Yale University School of Medicine, New Haven, CT). The animal handling procedure was based on the National Institutes of Health guidelines for animal studies. The experimental procedure was approved by the Animal Ethical Review Boards of Pohang Institute of Science and Technology and Seoul National University Hospital. Each animal experiment used the minimum number of animals possible.

Voluntary Exercise. After an adaptation period of 4–6 d, the exercise group was given free access to a running wheel (diameter, 34.5 cm; width, 9.5 cm for rats; diameter, 10.2 cm; width, 5.1 cm for mice) with a counter that monitored its revolutions. The revolutions of each wheel were recorded automatically. Other groups of rats and mice were housed in similar cages without exercise wheels. Because of individual variations in running behavior, only animals that had run more than 50 km and 112 km, respectively (~1.8 km/night for rats; 4 km/night for mice), by the end of the fourth week were chosen for subsequent experiments.

ECS Treatments. Male Sprague–Dawley rats (200–250 g) were used in all of the experiments. The rats were randomly divided into two groups: sham (sham treatment for 10 d) and E10× (daily ECS for 10 d). ECS was administered via ear-clip electrodes (Ugo Basile ECT Unit-57800–001; frequency, 100 pulses/s; pulse width, 0.5 ms; shock duration, 0.5 s; current, 55 mA). Sham-treated control animals were handled identically but without ECS treatment. ECS-induced seizures were validated by the presence of a general convulsion consisting of tonic and clonic phases. Animals that underwent general convulsion for more than 30 s were included for further analysis. The rats were decapitated 24 h after the last treatment, and their hippocampal tissues were dissected.

Recombinant Migration Inhibitory Factor Protein Purification. Recombinant mouse and human migration inhibitory factor (MIF) vectors (kindly provided by R.B.) were expressed in *Escherichia coli* and purified by anion exchange and reversed-phase chromatography as described previously (1, 2). For increasing purity, MIF-containing fractions were injected into Mono S cation-exchange columns (GE Healthcare) that had been equilibrated with 50 mM phosphate buffer (pH 6.2) containing 50 mM NaCl and eluted with 10 mM phosphate buffer (pH 6.2) containing 1 M NaCl. Refolding of MIF was initiated from 8 M urea and acid-denatured states and dialyzed using a 10,000-molecular weight cutoff dialysis tube (Sigma-Aldrich). Using SDS/PAGE, estimated fractions were pooled, and the identity of MIF was confirmed by MALDI-TOF mass spectrometry. MIF was then applied to a detoxification column (Pierce Biotechnology) to remove endotoxins. The amount of MIF was measured using a MIF ELISA kit (USCN Life Science).

Cell Culture and siRNA Transfection. Neuro-2A cells were cultured in DMEM (Lonza) supplemented with 10% heat-inactivated FBS and penicillin (final concentration, 100 U/mL). Grown cells were reseeded in six-well plates at a density of 10^4 cells/well at 1 d before experiments. RBL-2H3 cells were grown in monolayers in MEM with 2 mM L-glutamine and Earle's balanced salts, 15% 150 μL/ml FCS, 100 IU/L penicillin, and 10 μg/mL of streptomycin. For experiments, cells were placed at a density of 2×10^6 cells/well in six-well plates. After 24 h, fresh medium was added to the cells; for secretion experiments, phenol red-free medium was added. This time point was considered time 0. Cultures were maintained at 37 °C in a 95% air/5% CO_2 humidified atmosphere. CD74 siRNA (catalog no. sc-35024), and control scramble siRNA (catalog no. sc-37007) were purchased from Santa Cruz Biotechnology. A total of 2×10^4 cells/well for Neuro-2A and 2×10^6 cells/well for RBL-2H3 were plated for 24 h, and siRNA transfection was conducted using Lipofectamine 2000 (Invitrogen) according to the manufacturer's instructions.

Western Blot Analysis. Whole-cell lysates were prepared in lysis buffer [1% Triton X-100, 10% glycerol, 150 mM NaCl, 50 mM Hepes (pH 7.3), 1 mM EGTA, 1 mM sodium orthovanadate, 1 mM sodium fluoride, 1 mM phenylmethysulfonyl fluoride, 10 mg/mL leupeptin, and 10 mg/mL aprotinin]. Lysates were then centrifuged at 14,000 × *g* for 10 min at 4 °C. Electrophoresis was performed using 8% polyacrylamide gels, and proteins were electrotransferred to nitrocellulose membranes. Membranes were immunoblotted with rabbit anti-MIF (sc-20121; Santa Cruz Biotechnology), anti–brain-derived neurotrophic factor (BDNF; sc-546; Santa Cruz Biotechnology), or goat anti- tryptophan hydroxylase 2 (TPH2; sc-48955; Santa Cruz Biotechnology) in Tween 20/Tris-buffered saline containing 5% skim milk. After incubation with the appropriate peroxidase-conjugated secondary antibody, proteins were detected using an enhanced chemiluminescence system (Habersham).

Immunohistochemistry. Immunohistochemical analyses with an anti-MIF antibody were performed at 1 d after the last stimulation. Rats were anesthetized with CO_2 gas, and their brains were removed and postfixed with paraformaldehyde solution at 4 °C overnight. Sections were fixed and rinsed, followed by incubation in blocking solution (2.5% BSA in PBS), rinsing in PBS, and then incubation in primary antibody solution (containing 0.1% Triton-X 100 and 1% BSA) overnight at 4 °C. The antibody used was rabbit anti-MIF (1:200; Santa Cruz Biotechnology).

RT-PCR, Real-Time RT-PCR, and PCR Array. Hippocampi were dissected and stored at −80 °C. Frozen tissues or cell lysates were homogenized using the easy-BLUE Total RNA Extraction Kit (iNtRON Biotechnology), and total RNA was extracted. First-strand cDNA was synthesized by reverse-transcription using oligo dT primers and SuperScript II reverse transcriptase (Invitrogen). cDNA was amplified for 25–30 cycles using mouse or rat gene-specific primers (Table S2). For real-time RT-PCR, total RNA (100 ng) was amplified using the One-Step SYBR RT-PCR Kit and Light Cycler 2.0 PCR System (Roche Diagnostics). This experiment was repeated twice. For PCR array, after total RNA was extracted from the samples, the PCR arrays for neurogenesis and neuronal stem cell-related genes were evaluated with the RT^{2}Profiler PCR array (PAMM-404; SABiosciences) in accordance with the manufacturer's instructions.

Intracerebroventricular Cannula Placement and MIF Injection. Intracerebroventricular (ICV) cannulation was performed as described previously (3). The rats were anesthetized with an ketamine (40 mg/kg i.p.; Yuhan) and xylazine (18.64 mg/kg; Bayer), and

mounted on a stereotaxic apparatus (Narishige). The skull was exposed, and a hole was drilled to place a guide cannula (21 gauge; PlasticsOne) into the lateral ventricle [Bregma coordinates: AP, −1 mm; ML, 2.5 mm; DV, 3.5 mm; Paxinos et al.; see ref. 4). Cannula placement was verified by visual observation during brain dissection; only verified samples were included for analysis. MIF or artificial cerebrospinal fluid (Harvard Apparatus) was injected into the ICV using a syringe pump (KD Scientific) for 1 min. At 1 h or 4 h later, rats were subjected to the forced swim test (FST) for 5 min and then decapitated for brain dissection.

***Mif* KO Mice.** Behavioral characterization of these KO mice was done using $Mif^{-/-}$ (male) in the B6 background and WT B6 (male) as respective controls. All animals were genotyped by PCR. Genomic DNA was amplified for 28 cycles using the following primers: *Mif*, 5′-ACGACATGAACGCTGCCAAC-3′ (sense), 5′-ACCGTGGTCTCTTATAAACC-3′ (antisense); *Neo*, 5′-GAATGAACTGCAGGACGAGG-3′ (sense), 5′-GCTCTTCGTCCAGATCATCC-3′ (antisense).

FST. At 1 d before the FST, the rats were placed in a cylindrical water tank for 15 min. The water temperature was 24 °C, and the diameter and depth of the tank were 25 and 60 cm for rats and 10 and 25 cm for mice (5–7). On the test day, the rats and mice were placed in the water tank for 5 min, and their behavior was videotaped. Three independent raters who were blinded to the groups of rats or mice individually rated times for immobility, swimming, and climbing, and the average values of these raters served as the final results. Immobility was defined as floating or no active movements made other than those necessary to keep the nose above water. Swimming was defined as active motions throughout the swim tank and crossing into another quadrant. Climbing was evaluated as upward-directed movements of the forepaws against the wall.

Novelty-Suppressed Feeding Test. The novelty-suppressed feeding test was performed as described previously (8) with some modifications. In brief, food-deprived mice (24 h with water available ad libitum) were placed in one corner of open arena (50 × 45 cm) containing clean bedding. A food pellet weighing 3.5~4 g was placed in the center of the arena. The mice, which had been placed in a holding cage for 30 min before being introduced were observed, and their latency to approach the pellet and begin feeding was recorded (maximum allowed time, 5 min).

Open Field Test. For this test, mice were placed in the central part of a square-shaped arena (38.5 cm × 38.5 cm × 25 cm) and allowed to explore it for 10 min. The total distance traveled (locomotion activity) was recorded for 10 min and analyzed using a custom-made Visual Basic application. The tests were performed under dim illumination.

Serotonin Measurement. Following a previously described method (9), the culture media and cell lysates were collected and treated with 0.4 M perchloric acid and centrifuged. Both samples were then subjected to HPLC using a C18 column (Novapak) in the mobile phase [consisting of 0.1 M citric acid, 0.1 M sodium acetate (pH 4.1), 0.2 mM EDTA, and 5% ethanol], and serotonin was detected electrochemically using a Waters 460 detector. The serotonin concentration was calculated using a Waters 991 computerized integrator system, and a curve was prepared each time. Background serotonin in the culture medium was subtracted from all control and experimental values for released serotonin. The total amount of serotonin was calculated as the sum of the intracellular and released serotonin.

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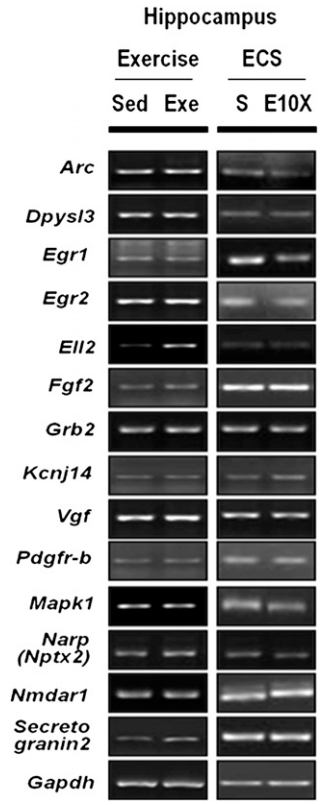


Fig. S1. Validation of expression profiles of candidate genes with RT-PCR using rat hippocampal tissues acquired after long-term voluntary exercise (28 d) and repeated ECS treatments (10 d).

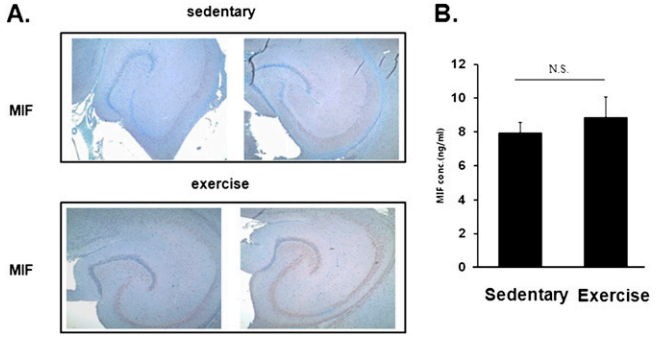
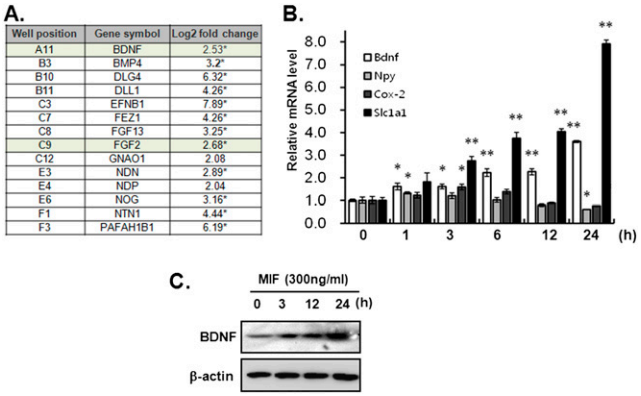


Fig. S2. (*A*) Immunohistochemistry staining in horizontal sections of the rat hippocampus showing that exercise increases MIF (brown). DG, dentate gyrus. (*B*) Protein MIF concentration in the plasma from in mice exercise groups compared with sedentary mice groups (n = 7–8). The MIF level was determined using ELISA. Conc, concentration; NS, not significant.



Well position	Gene symbol	Log2 fold change
A11	BDNF	2.53*
B3	BMP4	3.2*
B10	DLG4	6.32*
B11	DLL1	4.26*
C3	EFNB1	7.89*
C7	FEZ1	4.26*
C8	FGF13	3.25*
C9	FGF2	2.68*
C12	GNAO1	2.08
E3	NDN	2.89*
E4	NDP	2.04
E6	NOG	3.16*
F1	NTN1	4.44*
F3	PAFAH1B1	6.19*

Fig. S3. (*A*) The list of selected genes based on its fold change from PCR array (above twofold, listed genes were all $P < 0.05$ vs. vehicle-treated group). (*B*) Expression of selected candidate genes (*BDNF*, *NPY*, *COX-2*, and *SLC1a1*) of long-term exercise and ECSs were analyzed by real-time RT-PCR after treatment with vehicle or MIF in the neuronal cell line Neuro-2A. Error bars show mean ± SD. $*P < 0.05$; $**P < 0.01$ vs. vehicle treatment. (*C*) BDNF proteins were induced in a time-dependent manner in Neuro-2A cells ($n = 3$).

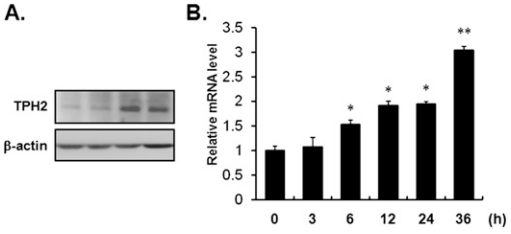


Fig. S4. (*A*) TPH2 proteins were induced in a time-dependent manner in Neuro2A cells ($n = 3$). Blotting with anti–β-actin was conducted as a protein loading control. (*B*) Increased expression of TPH2 from MIF treatment was detected by real-time RT-PCR in the RBL-2H3 cell line. Error bars show mean ± SD. $*P < 0.05$; $**P < 0.01$ vs. vehicle treatment.

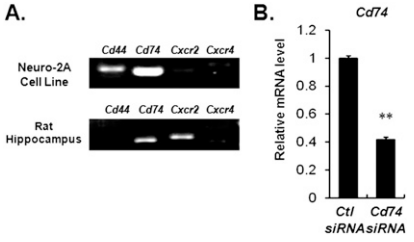


Fig. S5. (*A*) Expression level of receptors (CXCR2, CXCR4, CD44, and CD74) of MIF in rat hippocampus and Neuro-2A cell lines were detected by RT-PCR. (*B*) CD74 siRNA reduced the expression level of CD74. $**P < 0.01$ vs. control siRNA.

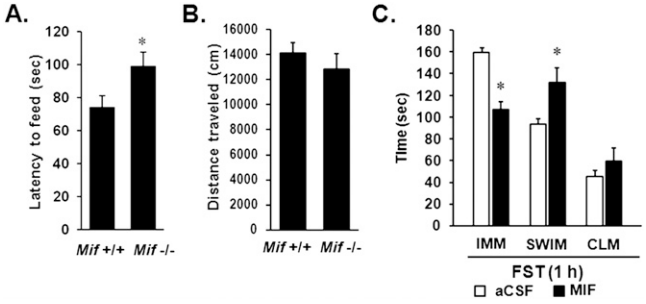


Fig. S6. (*A*) Latency to feed increased in $Mif^{-/-}$ mice compared with WT littermates ($n = 11$–14 for each group). $*P < 0.05$. (*B*) Locomotor activity levels were similar between $Mif^{-/-}$ mice and WT littermates ($n = 6$–8 for each group). (*C*) ICV injection of recombinant MIF protein into rats significantly reduced immobility time in the FST 1 h after injection ($n = 8$–10 for each group). IMM, immobility; SWM, swimming; CLM, climbing. Error bars show mean ± SEM. $*P < 0.05$.

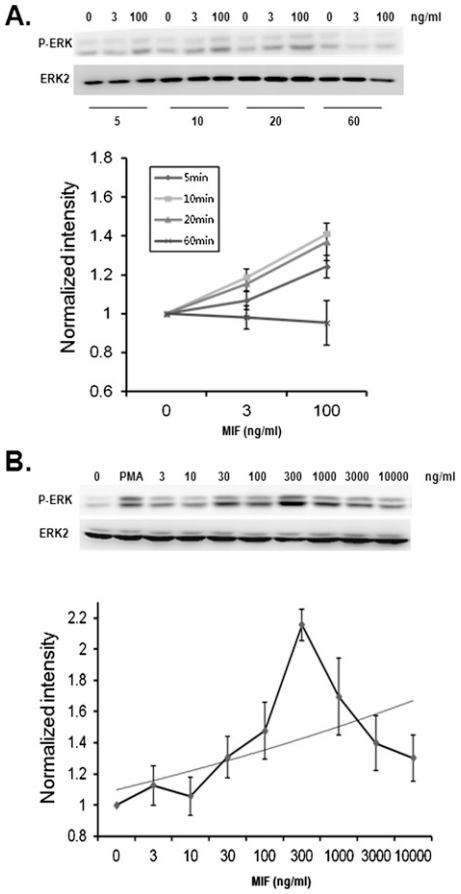


Fig. S7. Western blot analysis of ERK phosphorylation in response to MIF treatment. (*A*) For time course experiments, serum-starved cells were treated with 3 or 100 ng/mL of MIF and harvested at 5, 10, 20, and 60 min. Samples were analyzed for phosphorylated (*Upper*) and total ERK levels (*Lower*). (*B*) The expanded dose–response data were collected when the time course response was the most linear at 10 min. Phorbol 12-myristate 13-acetate (PMA) (100 nm) was used as a positive control. Data were normalized to net intensity values for vehicle-treated controls at each time point. Error bars show mean ± SD of three replicates.

Table S1. Common genes regulated by both exercise and ECS

Gene	Gene description	Function/class
Nmdar1	*N*-methyl-D-aspartate glutamate receptor a1	Receptors
Grb2	Growth factor receptor-bound protein 2	Miscellaneous
Eaac1/Slc1a1	Solute carrier (high-affinity glutamate transporter), member 1	Miscellaneous
Ell2	Elongation factor, RNA polymerase II, 2	Miscellaneous
Mif	Macrophage migration inhibitory factor	Immune response
Cox-2	Cyclooxygenase isoform 2	Immune response
Fgf2	Fibroblast growth factor2	Hormones/growth factors
Pdgfr-b	Platelet-derived growth factor-associated protein	Hormones/growth factors
Secretogranin2	Neuroendocrine protein 7b2	Neurotrophin/neuropeptide
Egr1	Early growth response 1	Transcriptional factor
Egr2	Early growth response 2	Transcriptional factor
Bdnf	Brain-derived neurotrophic factor	Hormones/growth factors
Arc	Activity-regulated cytoskeletal-associated protein	Cytoskeletalprotein binding
Npy	Neuropeptide Y	Hormones/growth factors
Vgf	VGF nerve growth factor inducible	Hormones/growth factors
Mapk1	Mitogen-activated protein kinase	Neural processing
Dpysl3	Dihydropyrimidinase-like	Metabolic enzymes
Kcnj14	Potassium inwardly rectifying channel, subfamily J, member 14	Ion transport
Narp (Nptx2)	Neuro activity-regulated petaxin	Protein coding

Table S2. Primer sequences used for quantitative RT-PCR

Gene	Sequence	Direction	Species
Npy	AGAGATCCAGCCCTGAGACA	Sense	*Musculus*
Npy	TTTCATTTCCCATCACCACA	Antisense	*Musculus*
Tph-1	GGACTGAGAGGAGAGGAAGG	Sense	*Musculus*
Tph-1	TTTCGGAGGAATGGTCTTT	Antisense	*Musculus*
Tph-2	AAAAGCGAGGACAAGAAAAGC	Sense	*Musculus*
Tph-2	ACCAGCCCACCAACTTCAT	Antisense	*Musculus*
Sert	TTTCCTCCTGTCTGTCATTGG	Sense	*Musculus*
Sert	AAGATGGCCATGATGGTGTAA	Antisense	*Musculus*
Maoa	GTAGCTCTGCCAGCTCGTTC	Sense	*Musculus*
Maoa	CCTGAATGGATTCGTTCGTC	Antisense	*Musculus*
Bdnf	AAAATGCTCACACTCCAC	Sense	*Musculus*
Bdnf	GAACAAATGCTGGTCTTT	Antisense	*Musculus*
Ffg-2	GACCCCAAGCGGCTCTACTGC	Sense	*Musculus*
Fgf-2	GTGCCACATACCAACTGGAGT	Antisense	*Musculus*
Bdnf	GCGGCAGATAAAAAGACTGC	Sense	*Rattus*
Bdnf	GCAGCCTTCCTTCGTGTAAC	Antisense	*Rattus*
Mif	GTGCCAGAGGGGTTTCTCTC	Sense	*Rattus*
Mif	TTGCTGTAGTTGCGGGTTCTG	Antisense	*Rattus*
Tph2	TTACACTGAGAACCCCAAATCC	Sense	*Rattus*
Tph2	GCAGCACAAGTCAACAGACCT	Antisense	*Rattus*
Cox-2	TGGTGCCGGGTCTGATGATG	Sense	*Rattus*
Cox-2	GCAATGCGGTTCTGATACTG	Antisense	*Rattus*
Npy	GCAGAGGCGCCCAGAGCAGA	Sense	*Rattus*
Npy	GCATTGGGTGGGACAGGCAGAC	Antisense	*Rattus*
Gapdh	AAGGTGGAAGAATGGGAGTTG	Sense	*Rattus*
Gapdh	CATCAAGAAGGTGGTGAAGCA	Antisense	*Rattus*
Rrna	CTGCTGAGACGACTTGTTCG	Sense	*Rattus*
Rrna	CCTGGGTCTGCAGTCTTCTC	Antisense	*Rattus*
Grb2	TCAATGGGAAAGATGGCTTC	Sense	*Rattus*
Grb2	CTGCTGTGGCACCTGTTCTA	Antisense	*Rattus*
Nmdar1	ACTCCCAACGACCACTTCAC	Sense	*Rattus*
Nmdar1	AGCAGAGCCGTCACATTCTT	Antisense	*Rattus*
Eaac1/Slc1a1	ACCCACTTCACAAGGCTGTC	Sense	*Rattus*
Eaac1/Slc1a1	TTTGAAACAGCAGTGGCTTG	Antisense	*Rattus*
Ell2	TGCCTGTCTCCAATCCTACC	Sense	*Rattus*
Ell2	CTCCTTTTCCTCCCTGCTCT	Antisense	*Rattus*
Fgf2	AGCGGCTCTACTGCAAGAAC	Sense	*Rattus*
Fgf2	CCGTTTTGGATCCGAGTTTA	Antisense	*Rattus*
Pdgfr-b	ACACATCAAATACGCGGACA	Sense	*Rattus*
Pdgfr-b	CCACTTCAGAGGCAGGAAAG	Antisense	*Rattus*
Secretogranin2	AAGCTGGGGAGAAAACCAAAT	Sense	*Rattus*
Secretogranin2	TGTCTGCTTTCAACGTCTGG	Antisense	*Rattus*
Egr1	AACACTTTGTGGCCTGAACC	Sense	*Rattus*
Egr1	AGGTCTCCCTGTTGTTGTGG	Antisense	*Rattus*
Egr2	CATCTCTGCGCCTAGAAACC	Sense	*Rattus*
Egr2	CCAGAGAGGAGGTGGACTC	Antisense	*Rattus*
Arc	GGTGAGCCACTTGACCAGTT	Sense	*Rattus*
Arc	TATTCAGGCTGGGTCCTGTC	Antisense	*Rattus*
Vgf	AGAACGAGAGGGAGGA	Sense	*Rattus*
Vgf	AGGTGGAGTTTGGTGGACAG	Antisense	*Rattus*
Mapk1	GCTAATGTTCTGCACCGTGA	Sense	*Rattus*
Mapk1	ACGGCACCTTATTTTTGTGC	Antisense	*Rattus*
Dpysl3	ATCAAGGGAGGGAGAATCGT	Sense	*Rattus*
Dpysl3	GGTGATGTCCACATGCAAAG	Antisense	*Rattus*
Kcnj14	GGTGCAGTTATGGCCAAGAT	Sense	*Rattus*
Kcnj14	CTGTGGCCTCAACCATACCT	Antisense	*Rattus*
Narp (Nptx2)	GGCAAGATCAAGAAGACGTTG	Sense	*Rattus*
Narp (Nptx2)	TCCAGGTGATGCAGATATGGT	Antisense	*Rattus*
Dcx	AAGTGACCAACAAGGCTATT	Sense	*Musculus*
Dcx	TCATTGTGTTTTCCCGGA	Antisense	*Musculus*
Pax6	GCTTGGTGGTGTCTTTGTCA	Sense	*Musculus*
Pax6	TCACACAACCGTTGGATACC	Antisense	*Musculus*