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Supplementary Information for

Leptin in Hippocampus Mediates Benefits of Mild Exercise by an Antioxidant on Neurogenesis and Memory

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Supplementary Information Text

Detailed Materials and Methods

Animals: Male C57BL/6J mice (11-weeks-old) and male leptin-deficient (*ob/ob*) mice (5-weeks-old) were obtained from SLC (Shizuoka, Japan). All mice were housed in standard polycarbonate cages under a controlled room temperature (21-23°C), relative humidity (50%), and a 12-h light-dark cycle (lights on at 07:00 AM). The C57BL/6J mice (12-weeks-old) were randomly divided into 4 groups as follows: (1) sedentary with placebo (SE+PL), (2) mild exercise with placebo (ME+PL), (3) sedentary with AX supplementation (SE+AX), and (4) mild exercise with AX supplementation (ME+AX). The *ob/ob* mice (12 to13-weeks-old) were randomly divided into 3 groups as follows: (1) SE+PL, (2) ME+PL, and (3) ME+AX. Animal care and experiments were performed in accordance with procedures approved by the University of Tsukuba Animal Experiment Committee.

Mild exercise and Astaxanthin intervention: Based on the ventilatory threshold (VT), we determined the intensity of treadmill running for both the WT and *ob/ob* mice (Fig. S6). Their running speed at VT was found to be 12.29 m/min and 8.66 m/min for the WT and *ob/ob* mice, respectively. The mice from the exercise groups (ME+PL and ME+AX) were submitted to mild treadmill exercise at intensity below their VT, and the sedentary groups (SE+PL and SE+AX) remained on the treadmill for the same amount of time without running. For chronic exercise training (4 weeks), C57BL/6J and *ob/ob* mice were habituated on a rodent treadmill (KN-73; Natsume, Japan) for 10 min before each running session, and then they ran following the appropriated running protocol, including 30 min/day and 5 times/week, as described in Table S1. Slight electric shocks at the rear of the treadmill were occasionally used during the running period. During the first week of acclimatization, all mice were fed a non-purified basal diet (MF powder diet, Oriental Yeast, Japan) *ad libitum.* Drinking water was also available *ad libitum.* During the 4 weeks of intervention, mice received a non-purified basal diet (MF powder; Oriental Yeast, Tokyo, Japan) with either AX or a placebo powder (AstaReal powder 20F based on 2% content of AX derived from *Haematococcus pluvialis*; AstaReal Co. Ltd, Tokyo, Japan) at concentrations of 0.5% (wt:wt), as reported in our previous study (1). Placebo

powder for AstaReal 20F contained an equivalent amount of medium chain triglycerides instead of AX. The composition of the experimental diets is shown in Table S8. Individual food intake and body weight were measured twice per a week.

Surgical implantation of osmotic pump: For the rescue experiment with chronic LEP administration, osmotic pump implantation was performed, as described previously (2). A micro-osmotic pump (0.11 μl/h; Alzet Model 1004, DURECT, USA) was filled with recombinant mouse leptin (0.1 μg/d; R&D Systems, USA) or aCSF (recipe can be found at www.alzet.com) 48 hours before surgery. The filled pump was then connected to a cannula *via* a plastic catheter using Alzet Brain Infusion Kit 3 (DURECT). The *ob/ob* mice were anesthetized with isoflurane and placed on a heating pad set on the stereotaxic frame (Narishige, Japan). The pump was inserted into a subcutaneous pocket that was generated on the back of the mice and the cannula was stereotaxically implanted in the right lateral ventricle at the following coordinates: AP = -0.5 mm, ML = -1.1 mm, DV = -2.5 mm. The cannula was secured with super glue (Loctite 454; Henkel, Düsseldorf, Germany). The mouse remained on the heating pad until regaining consciousness and hosed individually in polycarbonate cages before the combined intervention (ME+AX).

Morris water maze: To evaluate spatial learning and memory, the WT and *ob/ob* mice were tested using the MWM test during the final week of the intervention (1). White, non-toxic paint was added to a black plastic circular pool (150 cm in diameter, 45 cm depth, Bio Research Center, Japan) containing opaque water (20-22°C), and a round platform was hidden 0.5 cm below the surface and positioned in one of the four quadrants. The WT and *ob/ob* mice were trained for 4 and 5 consecutive days, respectively. All mice received four trials per day with 10-min inter-trial intervals during the acquisition period. As described elsewhere, one of four starting positions was randomly selected for each trial based on a semi-random set of start locations. In each acquisition trial, mice were trained to find the hidden platform with a maximum time limit of 60 sec, and then they were allowed to remain on the platform for 10 sec. If a mouse was still swimming after a given trial it was gently placed on the platform by the experimenter and allowed to rest for 10 sec prior to being returned to the home cage. Mice were submitted to the probe test

24 hours after the last training session, in which the platform was removed from the pool and the mice, were allowed to search for it during a 60-sec time interval. The total amount of time spent in each quadrant was recorded. The parameters of behavior for each acquisition trial and probe test were recorded with a video tracking system (SMART, Polyvalent video-tracking system, 35C67-AC5, PANLAB, Spain). The time the animals took to reach the platform was recorded (latency, path length, and swim speed) with Ethovision 3.0 software (Noldus, Netherlands).

Sample collection: To evaluate AHN, mice were deeply anaesthetized with sodium pentobarbital (100 mg/kg, *i.p.*) and transcardially perfused with 0.9% saline. After perfusion, the brains were immediately removed from the skull, and fixed in a commercial 4% paraformaldehyde (PFA) solution (Nacalai Tesque, Kyoto, Japan) at 4°C for 24 hours. After the fixation step, brains were sequentially equilibrated in a 10%, 20%, and 30% sucrose gradient contained in a 0.1 M phosphate buffer (PB), and then frozen on powdered dry ice. Coronal sections of 50 μm were sequentially cut through the entire hippocampus using a cryostat (HM505E; MICROM) and stored in antifreeze solution (30% glycerol and 30% ethylene glycol in 0.1 M PB) at -30°C until the next analysis for immunohistochemistry. For DNA microarray, semi-quantitative RT-PCR, and western blot analyses, the brains were removed from the skull and the hippocampi were rapidly dissected upon cold petri dishes, and immediately flash-frozen in liquid nitrogen. The samples were stored at -80°C before being further processed. Stored hippocampi were ground to a very fine powder using a precooled mortar and pestle with liquid nitrogen, as described previously (3). The powdered samples were stored at -80°C until further analyses. The samples were collected one day after the last day of the intervention period to avoid acute effects of the intervention.

DNA microarray analysis: Total RNA was extracted from the finely powdered hippocampal tissue of C57BL/6J mice (n = 8/group) using the QIAGEN RNeasy Mini Kit (QIAGEN, USA). The quantity and quality of extracted total RNA was determined with spectrophotometric analysis (NanoPhotometer^{TM}; IMPLEN, Germany) (Fig. S1A) and the subunits were visualized using formaldehyde-agarose gel electrophoresis (Fig.

S1B). In order to identify differential gene expressions, we performed DNA microarray analysis using the method described in a previous study with a slight modification (4). An equal amount of total RNA (800 ng) that extracted from eight randomly chosen powdered tissues (*n*=8/group) was pooled in each group (SE+PL, ME+PL, and ME+AX) prior to DNA microarray analysis. The rest of individual RNAs stored at -80°C to validate the DNA microarray-based gene expressions using semi-quantitative RT-PCR. Total RNAs (600 ng; each replicate pooled together) were labeled with Cy3 and Cy5 dye using an Agilent Low RNA Input Fluorescent Linear Amplification Kit (Agilent Technologies, USA). A SurePrint G3 Mouse GE 8X60K Microarray Kit (G4858AF, Agilent Technologies, USA) was used for global gene expression analysis with two-color dye labeling on a single microarray chip (Fig. S1C). Fluorescently labeled targets of control (SE+PL) as well as interventions (ME+PL and ME+AX) were hybridized to the same microarray slide with 60-mer probes. The microarray was then scanned using an Agilent Microarray scanner G2505C after wash processes according to the manufacturer's instructions. The slide image was measured by Agilent Feature Extraction software (version 11.0.1.1) to gain significantly differentially expressed genes. In brief, this program measured Cy3 and Cy5 signal intensities of whole probes. Dye-bias tends to be signal intensity dependent, and therefore the software selected probes using a set by rank consistency filter for dye normalization. The normalization was performed by LOWESS (locally weighted linear regression), which calculates the log ratio of dye-normalized Cy3 and Cy5 signals, as well as the final error of the log ratio. The significance *p*-value was based on the propagate error and universal error models. The threshold of significance for differentially expressed genes was < 0.01 (for the confidence that the feature was not differentially expressed), and erroneous data generated owing to artifacts were eliminated before data analysis using the software. The outputs of microarray analysis used in this study are available under the series number GSE 107314 at the NCBI Gene Expression Omnibus (GEO) public functional genomics data repository (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE107314).

Ingenuity Pathways Analysis: To identify biological function and network of hippocampus-specific genes, we applied Ingenuity Pathways Analysis (IPA, Ingenuity®

Systems, www.ingenuity.com) with tissue-specific gene expression. The dataset from the DNA microarray, which comprises the differentially expressed (≧/≦ 1.5/0.75-fold compared to SE+PL group) genes, and their corresponding fold-change values were uploaded as an Excel data sheet to the web-based IPA software. To obtain gene networks, genes were overlaid onto a global molecular network developed from information contained in the Ingenuity Knowledge Base. The functional analysis identified the biological functions that were most significant to the data set ($P < 0.05$) according to a right-tailed Fisher's exact test. A functional annotation of the up- and down-regulated genes is summarized in Table S2-S5.

Semi-quantitative RT-PCR: To validate changed gene expression in the DNA microarray analysis, semi-quantitative (sq) RT-PCR was used in this study. Briefly, cDNA synthesized from independent total RNA (800 ng) samples using an Affinity Script QPCR cDNA Synthesis Kit (Agilent Technologies, USA) according to the manufacturer's protocol. The reaction mixture contained 0.6 μL of the first-strand cDNA, 7 pmols of each primer set and 6.0 μL of the Emerald Amp PCR Master Mix (2×premix) (TaKaRa Shuzo, Japan) in a total volume of 12 μL. Thermal-cycling (C1000^{TM} Thermal Cycler; Bio-Rad Laboratories, USA) parameters were as follows: after an initial denaturation at 97°C for 5 min, samples were applied to a cycling regime with 22-32 cycles. After completion of the PCR, the total reaction mixture was spun down and mixed (3 μL) before being loaded into the wells of a 1.6% agarose (Nacalai Tesque, Japan) gel. Electrophoresis was then performed for 20 min at 100 volts in a 1×TAE buffer (Wako, Japan) using a Mupid-exU electrophoresis system (ADVANCE, Japan). The gels were stained for 7 min with ethidium bromide, and the stained bands were visualized using the Image Quant LAS 4000 system (GE Healthcare, Japan). Primer sequences for each primer are shown in the list of primer design for sqRT-PCR.

Primer design for semi-quantitative RT-PCR

Gene	Nucleotide sequence	Nucleotide sequence	Product

symbol	forward	reverse	size (bp)
ActinB	5′-acgttgacatccgtaaagacct-3′	5′-ggtgtaaaacgcagctcagtaa-3′	302
Lep	5′-gttagccctgaatgctgaagtt-3′	5′-ggatgggatggctcttatctct-3′	278
Stat3	5′-agaaaatgaaggtggtggagaa-3′	5′-cgtactccattgctgacaagag-3′	213
Mapk1	5′-gatcacacagggttcttgacag-3′	5′-gcgggagagaaagcaaatagtt-3′	271
Akt	5′-gttcacagctcagatgatcacc-3′	5′-acagtagaaacatcctcccctg-3′	273
Igf1r	5′-tcctagctgatgttgtcctacc-3′	5′-catatgtgggtaactggcagtg-3′	317
Lepra	5′-gtgctgtggagtcactcagtg-3′	5′-agtcattcaaaccattagtttagg-3′	595
Leprb	5′-gatatttggtcctcttcttctgg-3′	5′-agttgtggtgaaatcatggtggg-3′	437

Western blotting: The frozen hippocampi were placed in liquid nitrogen, and ground to a very fine powder with a mortar and pestle. The tissue powder was subsequently solubilized in an appropriate lysis buffer containing thiourea and Tris (LB-TT) for extraction of hippocampal protein (5). The total protein solution was precipitated using the ProteoExtract Protein Precipitation Kit (Calbiochem, Germany) for the concentration and clean up. Protein concentration was determined with a Bio-Rad Protein Assay Dye Reagent Concentrate (Bio-Rad, USA) using bovine serum albumin (BSA) as a standard and a NanoPhotometer^{TM} spectrophotometer (IMPLEN). The proteins in SDS were heated to 98°C for 10 min, rapidly cooled to 4°C for 1 min, and then were loaded on an appropriate percentage of polyacrylamide gel (ATTO, Japan) by electrophoresis. The gel was then transferred to a PVDF membrane using the Trans-Blot Turbo^{TM} Transfer System (Bio-Rad). The membranes were blocked for 1 h at room temperature with 5% non-fat skim milk in 1×Tris-buffered saline containing 0.1% Tween-20 (TBS) to reduce non-specific binding and then incubated for overnight at 4°C with one of the following diluted primer antibodies: rabbit polyclonal anti-LEPR (1:500, ab5593, Abcam), rabbit polyclonal anit-phospho-IGF-1 Receptor β (1:500, #3024, Cell Signaling), rabbit

polyclonal anti-IGF-1 Receptor β (1:500, #3027, Cell Signaling), rabbit polyclonal anti-LEP (1:500, ab3583, Abcam), rabbit polyclonal anti-AKT (1:2,000, #9272, Cell Signaling), rabbit polyclonal anit-phospho-AKT (1:2,000, #9271, Cell Signaling), mouse monoclonal anti-STAT3 (1:1,000, #9139, Cell Signaling), rabbit polyclonal anti-PI3K (1:2,000, #4292, Cell Signaling), rabbit polyclonal anti-phospho-PI3K (1:2,000, #4228, Cell Signaling), mouse monoclonal anti-STAT3 (1:10,000, #9139, Cell Signaling), mouse monoclonal anti-pho-STAT3 (1:500, sc-136193, Santa Cruz Biotechnology), or mouse monoclonal β-actin (1:50,000, A5441, Sigma-Aldrich). After the membranes were washed with 1×TBS buffer, they were incubated for 1 hour at room temperature with secondary antibodies: horseradish peroxidase (HRP)-conjugated goat anti-rabbit (1:10,000, sc-2004, Santa Cruz Biotechnology) was used for LEP, LEPR, pho-IGF1R, IGF1-R, pho-AKT, AKT, PI3K, and pho-PI3K, and HRP-conjugated goat anti-mouse (sc-2005, Santa Cruz Biotechnology) was used for pho-STAT3, STAT3 (1:10,000) and β-actin (1:50,000). After washing with 1×TBS, the membranes were developed using a Chemi-Lumi One Super Kit (Nacalai Tesque, Japan) following the manufacturer's instructions. The density of the developed bands was scanned and then quantified using image analysis software (Image Quant LAS 4000 system).

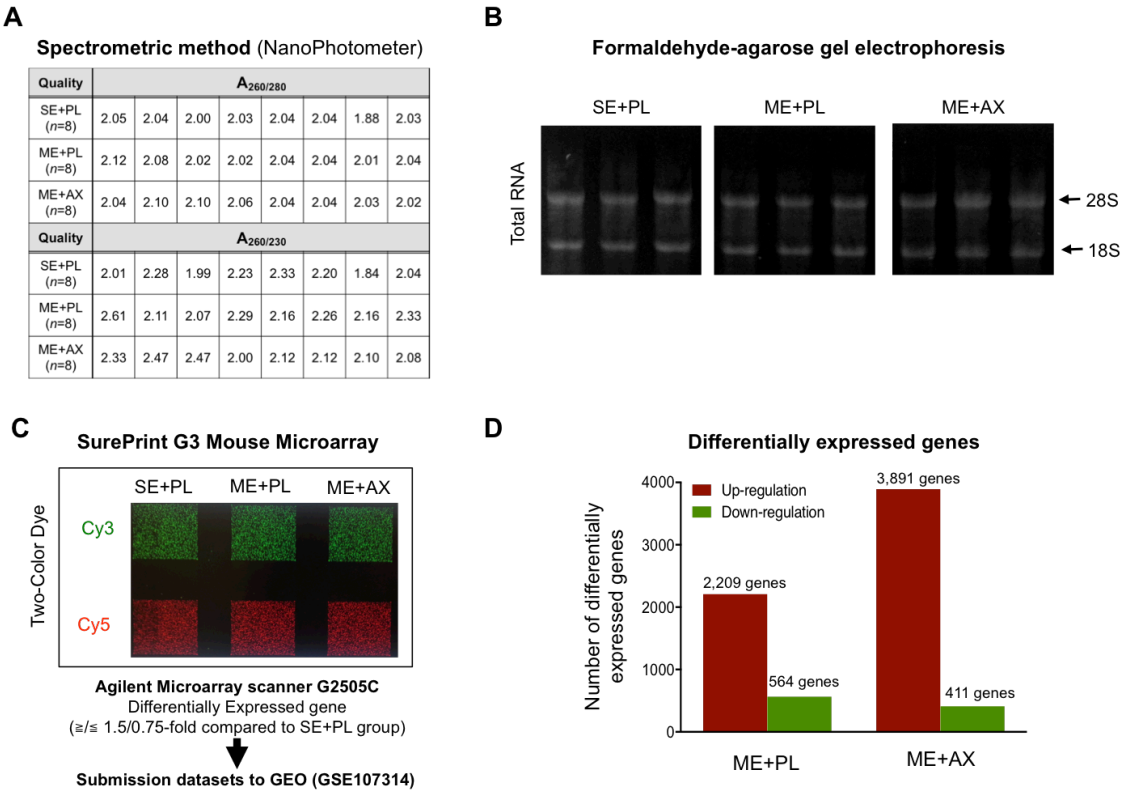
ELISA assays: Blood was collected in heparinized tubes by cardiac puncture and immediately placed on ice and centrifuged at 3,000 rpm for 15 min at 4°C. The plasma samples were stored at -80°C until further analysis. Leptin concentrations in plasma were measured using a Mouse/Rat Leptin Quantikine ELISA Immunoassay Kit (SMOB00, R&D Systems) according to the manufacture's protocol. Briefly, frozen samples were

defrosted and then diluted 1:20 with Calibrator Diluent RD5-3. Samples were loaded in duplicate on the plate and incubated according to manufacturer's assay procedure. The plate was measured with a fluorescence microplate reader (ARVO X4, PerkinElmer, Groningen, Netherlands).

Cell cultures of AX: SH-SY5Y cells were incubated with Dulbecco's modified Eagle's medium (DMEM) containing 15% fetal bovine serum at 37°C in 5% CO_2 in an incubator. Cells were plated into 96-well plates at a density of 2~3 × 10^4 cells/dish. The experimental cells were treated with various concentrations of AX (5, 10, and 20 μM) or DMSO for 1 hour. After removal of AX-treated medium, the cells were re-incubated in Opti-MEM without H_2O_2 for 1 hour, 6 hours, and 24 hours. The cell viability was measured by LDH assay (LDH-Cytotoxic Test Wako for Cytotoxicity; Wako Pure Chemical Industries) that monitored the reduction of pyruvic acid. The absorbance of each sample was determined at 570 nm with a microplate reader. For western blot analysis, AX or DMSO-treated SH-SY5Y cells were washed with PBS, and then collected the cells by PBS including 10 mM EDTA. After centrifugation for 10 min at 3,000 rpm, the pellets were dissolved in RIPA buffer containing protease inhibitor (Nacalai Tesque). For western blot analysis, the experimental cells were treated to fresh Opti-MEM containing various concentrations of astaxanthin (5, 7.5, 10, and 20 μM) or 0.5% DMSO as a vehicle for 1 hour. DMSO and AX-treated cells were lysed in RIPA lysis buffer (Nacalai Tesque) containing phosphatase inhibitor cocktail and protease inhibitor cocktail. Lysates were clarified by centrifugation at 3,000 rpm for 5 min, and the total protein content was determined using a Bio-Rad Protein Assay Dye Reagent

Concentrate (Bio-Rad, USA). Equal amounts (20 μg) of protein extracts were subjected to 15% of polyacrylamide gel (ATTO, Japan) and a PVDF membrane using the Trans-Blot Turbo^{TM} Transfer System (Bio-Rad); rabbit polyclonal anti-LEP (1:4,000, PA1-052, Invitrogen) for primary antibody and HRP-conjugated goat anti-rabbit (1:10,000, sc-2004, Santa Cruz Biotechnology) for secondary antibody.

Statistical analyses: All data are presented as mean ± standard error (SEM). Escape latency, escape length and swimming speed for the MWM were analyzed with a repeated-measures two-way ANOVA, followed by Bonferroni's multiple comparison tests for *post-hoc* analysis. The results of the immunohistochemistry, probe test, western blots, and ELISA were analyzed using two-way ANOVA followed by Fisher's LSD *post-hoc* test. The results for sqRT-PCR were analyzed using one-way ANOVA followed by Fisher's LSD *post-hoc* test. One-way ANOVA with Fisher's LSD *post-hoc* test was used for the probe test and western blots of the *ob/ob* mice study. Correlations between spatial memory and the expression of protein were calculated using Pearson's correlation analysis. Statistically significant differences were evaluated at $P < 0.05$.



Quality	$A_{260/280}$							
SE+PL (*n*=8)	2.05	2.04	2.00	2.03	2.04	2.04	1.88	2.03
ME+PL (*n*=8)	2.12	2.08	2.02	2.02	2.04	2.04	2.01	2.04
ME+AX (*n*=8)	2.04	2.10	2.10	2.06	2.04	2.04	2.03	2.02
Quality	$A_{260/230}$							
SE+PL (*n*=8)	2.01	2.28	1.99	2.23	2.33	2.20	1.84	2.04
ME+PL (*n*=8)	2.61	2.11	2.07	2.29	2.16	2.26	2.16	2.33
ME+AX (*n*=8)	2.33	2.47	2.47	2.00	2.12	2.12	2.10	2.08

Fig. S1. Assessment of RNA integrity and experimental design for DNA microarray. (A) The quality of total RNA was determined using a spectrometric measurement of absorbance with a NapnoPhometer™ (IMPLEN, Germany). The $A_{260/280}$ and $A_{260/230}$ ratio in all samples (n = 8/group) was greater than 1.8, respectively, which is a commonly accepted value for high RNA quality. (B) The integrity of RNA was assessed by inspection of the 28S and 18S ribosomal RNA bands using formaldehyde-agarose gel electrophoresis stained with ethidium bromide. (C) A SurePrint G3 Mouse GE 8X60K Microarray Kit (Agilent Technologies, G4858A) was used for the global gene expression profiling in the hippocampus, with two-color labeling method on a single microarray chip. The data set from the microarray consisted of the differentially expressed genes (≧/≦ 1.5/0.75-fold compared to SE+PL group). (D) DNA microarray analysis using cut-off values (≧/≦ 1.5/0.75-fold) revealed 2,209 and 564 up- and down-regulated genes in ME+PL group, respectively. Interestingly, a much higher number of changes appeared in the combined intervention (ME+AX group), where 3,891 and 411 up- and down-regulated genes were altered.

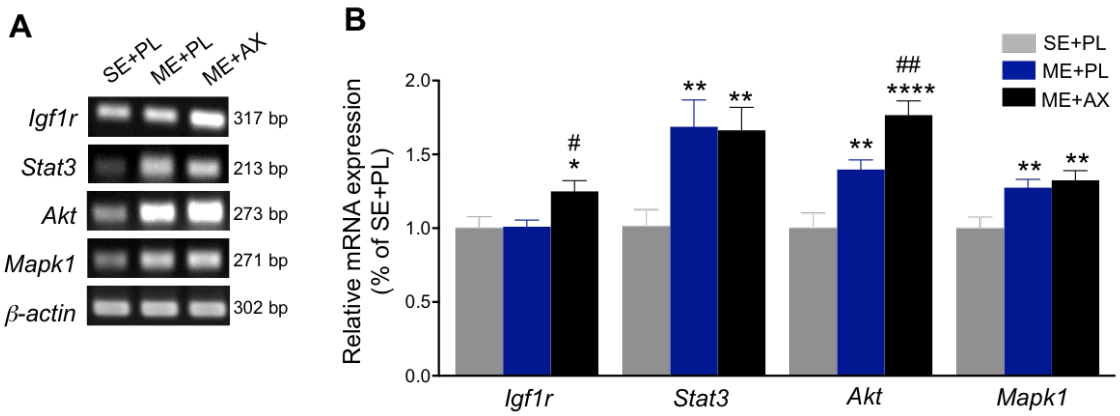


Fig. S2. Validation of specific gene expression by sqRT-PCR. (A) Gel-images on top present the representative PCR product bands. (B) Both the ME alone and the combined ME with AX significantly increased *Stat3* ($F_{(2, 20)} = 5.37$, $P < 0.05$), *Akt* ($F_{(2, 20)} = 18.37$, $P < 0.0001$) and *Mapk1* mRNA expression ($F_{(2, 20)} = 6.89$, $P < 0.01$). *Igf1r* mRNA ($F_{(2, 20)} = 6.89$, $P < 0.01$) was only increased in the combined intervention. Data are shown as protein levels relative to SE+PL group, presented the means ± SEM (n = 6-8/group). $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.0001$ vs. SE+PL. $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ vs. ME+PL.

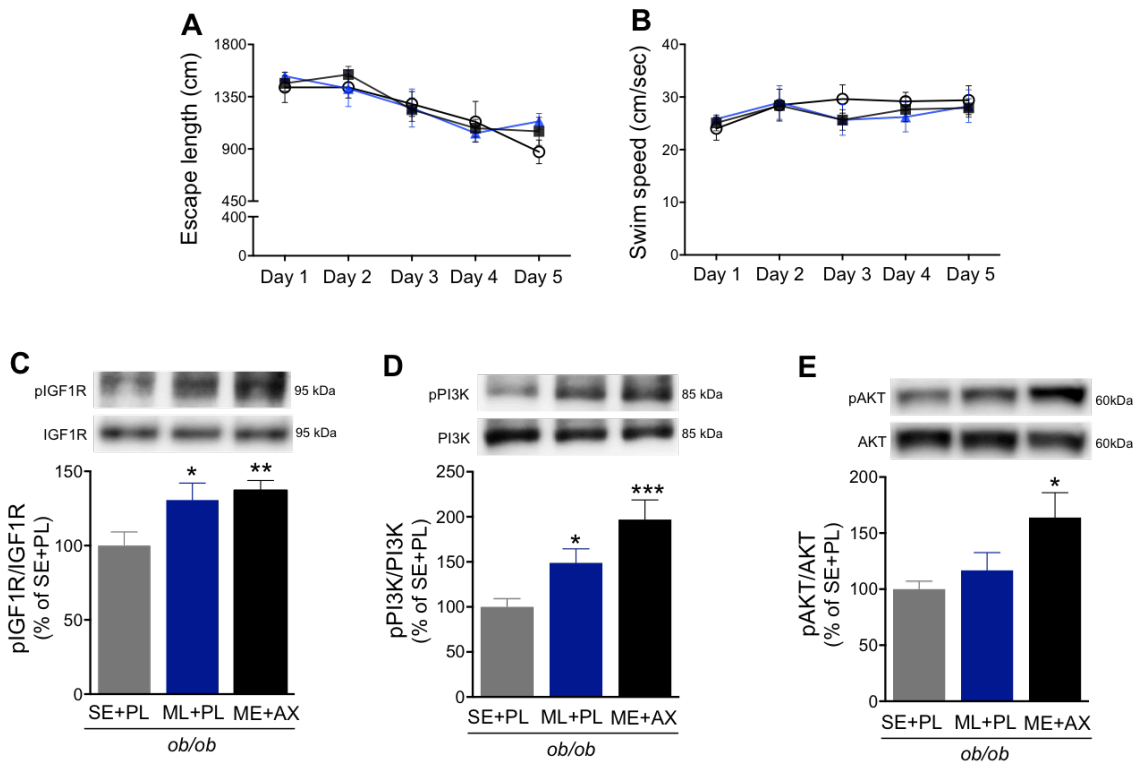


Fig. S3. Spatial learning and IGF1R signaling on the hippocampus of *ob/ob* mice. (A and B) There was no difference between any of the groups in the escape latency of the acquisition period. ME alone increased pIGF1R in the hippocampus of WT mice (Fig 3 D and E). Therefore, we checked whether hippocampal IGF1R signaling is involved in the ME alone-enhanced memory functions in the *ob/ob* mice. (C and D) Both ME+PL and ME+AX groups significantly increased pIGF1R ($F_{(2, 18)} = 4.56$, $P < 0.05$) and pPI3K ($F_{(2, 18)} = 8.83$, $P < 0.01$) protein abundances, compared to SE+PL group. (E) The level of pAKT protein was only increased in the ME+AX group ($F_{(2, 18)} = 4.17$, $P < 0.05$). Protein levels were normalized to the band intensity of β-actin. All data are presented as mean ± SEM. One-way ANOVA with Fisher's LSD *post-hoc* test were performed. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ vs. SE+PL.

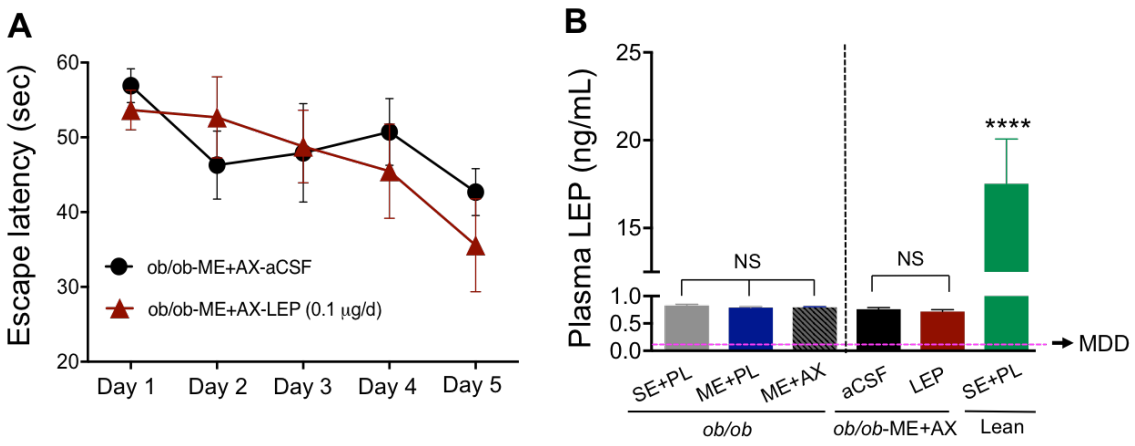


Fig. S4. Spatial learning and plasma LEP of *ob/ob* mice by administration of LEP. (A) The *ob/ob* mice were continuously administered with LEP (0.1 μg/day) or aCSF (control) during the 4 weeks of combined intervention (n = 5/group). There was no significant difference in escape latency of the Morris water maze test ($F_{(1, 8)} = 0.12$, P = 0.73). Repeated-measures two-way ANOVA with Bonferroni's *post-hoc* test was performed. (B) Plasma LEP in *ob/ob* mice was almost undetectable compared to lean mice with SE+PL (n = 3) and there were no significant increases in response to chronic LEP administration and intervention. The minimum detectable dose (MDD) of ELISA kit is 0.022 ng/mL (Pink dotted line). One-way ANOVA with Tukey's *post-hoc* test was performed ($^{****}P < 0.0001$ vs. aCSF or LEP group). All data are presented as mean ± SEM. NS: not significant.

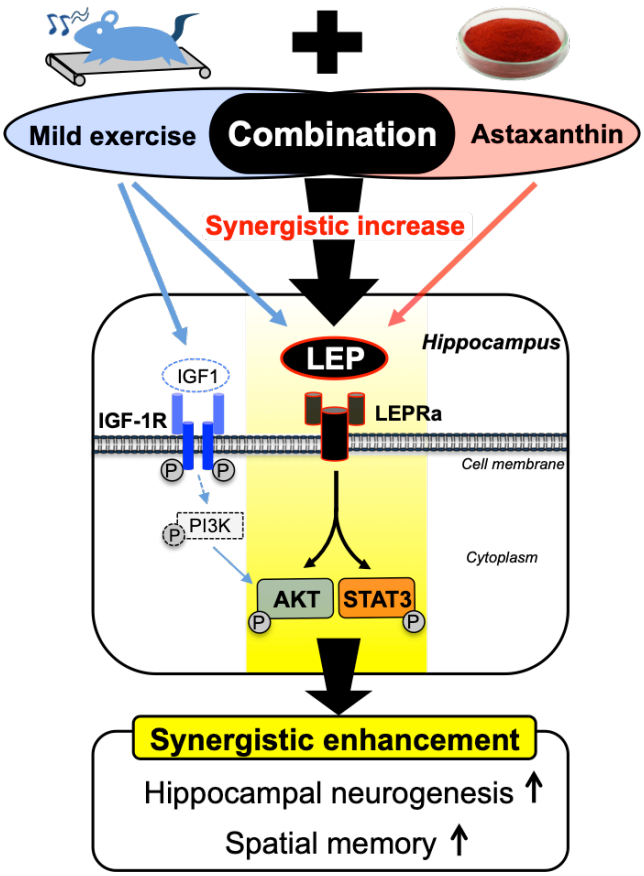
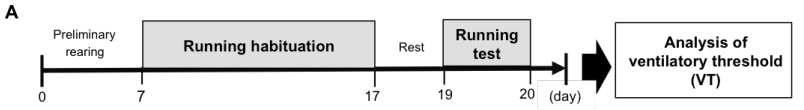


Fig. S5. A hypothetical molecular mechanism by which the ME combined with AX can induce synergistic effects on AHN and spatial memory. Activation of IGF1R/AKT pathway is involved in the ME-alone-enhanced hippocampal neurogenesis and memory functions (blue line). By extension, ME combined with AX induces synergistic effects on hippocampal neurogenesis and spatial memory performance. The respective ME- and the AX-increased LEP expressions in the hippocampus are further enhanced by their combination (red line), suggesting that the synergistic increase of hippocampal leptin (h-LEP) mediates the combined-intervention-induced strong synergistic benefits on the hippocampus-based neurogenesis and spatial memory. Activation of the AKT/STAT3 pathway is also involved in the combined-intervention-enhanced hippocampal functions.



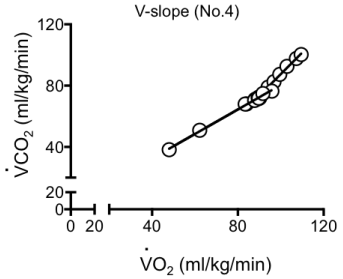
B

Protocol of treadmill running habituation for C57BL/6J mice	
Day 1	5 m/min (5 min) + 10 m/min (15 min)
Day 2	10 m/min (10 min) + 12.5 m/min (20 min)
Day 3	Rest
Day 4	10 m/min (10 min) + 12.5 m/min (10 min) + 15 m/min (10 min)
Day 5	12.5 m/min (10 min) + 15 m/min (10 min) + 17.5 m/min (10 min)
Day 6	Rest
Day 7	15 m/min (10 min) + 17.5 m/min (10 min) + 20 m/min (10 min)
Day 8	17.5 m/min (10 min) + 20 m/min (10 min) + 22.5 m/min (10 min)
Day 9	Rest
Day 10	20 m/min (10 min) + 22.5 m/min (10 min) + 25 m/min (10 min)

C

Protocol of treadmill running habituation for ob/ob mice	
Day 1	2.5 m/min (5 min) + 5 m/min (15 min)
Day 2	5 m/min (10 min) + 6.3 m/min (20 min)
Day 3	Rest
Day 4	5 m/min (10 min) + 6.2 m/min (10 min) + 7.5 m/min (10 min)
Day 5	6.2 m/min (10 min) + 7.5 m/min (10 min) + 8.7 m/min (10 min)
Day 6	Rest
Day 7	7.5 m/min (10 min) + 8.7 m/min (10 min) + 10 m/min (10 min)
Day 8	8.7 m/min (10 min) + 10 m/min (10 min) + 11.2 m/min (10 min)
Day 9	Rest
Day 10	10 m/min (10 min) + 11.2 m/min (10 min) + 12.5 m/min (10 min)

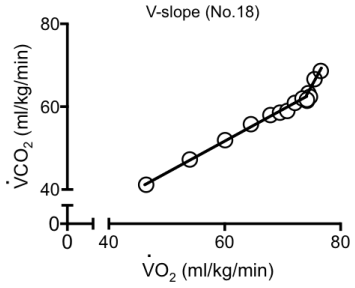
D



E

Subjects (n=8)	Speed at VT (m/min)	RER at VT
No.1	17.06	0.924
No.2	9.23	0.805
No.3	13.51	0.813
No.4	10.46	0.825
No. 5	9.56	0.829
No.6	11.58	0.822
No.7	17.31	0.850
No. 8	9.59	0.777
Mean	12.29	0.831
SEM	1.18	0.015

F



G

Subjects (n=7)	Speed at VT (m/min)	RER at VT
No.7	7.88	0.830
No.8	9.20	0.820
No.9	8.33	0.832
No.17	7.80	0.864
No. 18	9.29	0.856
No.19	9.49	0.841
No.20	8.63	0.836
Mean	8.66	0.840
SEM	0.26	0.001

Fig. S6. The measurement of ventilatory threshold in C57BL/6J and *ob/ob* mice. (A) For a running test, C57BL/6J (WT) and *ob/ob* mice initially had an exercise habituation for 10 days, followed by a rest period for 2 days. (B and C) To measure the level of ventilatory threshold (VT), the mice had a running habituation including a total of 7 bouts of training under graded speed, from 5 to 25 m/min for WT mice and from 2.5 to 12.5 m/min for *ob/ob* mice, on an isolated treadmill containing chamber system (Metabolic Modular Treadmill; Columbus Instruments, Columbus, OH, USA). During the test, the gas flow such as oxygen consumption ($\dot{V}O_2$) and carbon dioxide expiration ($\dot{V}CO_2$) every 60 sec was collected (Oxymax Software, Columbus Instruments). (D and F) The VT is a point at which the slope of the relative rate of increase in $\dot{V}CO_2$ relative to $\dot{V}O_2$ changes by the V-slope method. A representative VT profile for a WT mouse (No. 4) and *ob/ob* mouse (No. 18) is indicated. (E) The mean of treadmill speed and RER at VT from eight WT mice was found to be 12.29 m/min and 0.831, respectively. (G) The mean of treadmill speed and RER at VT from seven *ob/ob* mice was found to be 8.66 m/min and 0.840, respectively.

Table S1. Morris Water Maze (MWM) results for different intervention.

Group	Day 1	Day 2	Day 3	Day 4
WT mice		**Escape latency (sec)**		
SE+PL	58.62 ± 1.12	42.58 ± 3.08††	34.55 ± 3.18††††	28.99 ± 2.89††††
ME+PL	52.41 ± 2.84	31.57 ± 4.41††††	20.14 ± 1.02††††	19.25 ± 2.79††††
SE+AX	51.48 ± 3.59	39.13 ± 5.10†	25.62 ± 2.81††††	14.52 ± 2.27††††
ME+AX	57.00 ± 1.77	38.50 ± 6.09††††	19.19 ± 3.61††††	17.95 ± 2.54††††
		Escape length (cm)		
SE+PL	2579.79 ± 94.34	2103.72 ± 149.21	1598.90 ± 149.85††††	1371.02 ± 133.15††††
ME+PL	2564.58 ± 111.63	1721.29 ± 219.70†††	1133.36 ± 74.04††††	1037.73 ± 167.93††††
SE+AX	2367.30 ± 175.59	1889.75 ± 171.53	1330.58 ± 111.52††††	944.12 ± 127.77††††
ME+AX	2524.84 ± 75.57	1853.07 ± 253.42††	934.87 ± 185.38††††	976.12 ± 156.17††††

Repeated two-way ANOVA (group × day) showed a main effect of day on escape latency and escape length ($F_{(3, 99)} = 109.55$, $P < 0.0001$ for latency; $F_{(3, 99)} = 75.95$, $P < 0.0001$ for length). $^{\dagger}P < 0.05$, $^{\dagger\dagger}P < 0.01$, $^{\dagger\dagger\dagger\dagger}P < 0.0001$ compared to day 1. Values are expressed as mean ± SEM.

Table S2. Up-regulated genes in the hippocampus by the ME alone.

Probe name	Molecules	ME-upregulated genes	Fold change
	Gene symbol	Description	
A_51_P104939	Klra4	killer cell lectin-like receptor, subfamily A, member 4	37.17
A_55_P2157799	MYO7B	myosin VIIB	19.51
A_55_P2007447	ABHD3	abhydrolase domain containing 3	6.76
A_51_P519301	IL17F	interleukin 17F	5.33
A_55_P2041086	TYW5	tRNA-yw synthesizing protein 5	5.18
A_51_P1978860	TP53BP2	tumor protein p53 binding protein 2	4.77
A_55_P1979536	HRAS	Harvey rat sarcoma viral oncogene homolog	4.36
A_52_P374882	LEP	leptin	4.18
A_51_P104718	GPR12	G protein-coupled receptor 12	4.18
A_55_P2116621	CITED2	Cbp/p300-interacting transactivator, with Glu/Asp-rich carboxy-terminal domain, 2	4.08
A_51_P448478	SLC10A4	solute carrier family 10, member 4	4.81
A_52_P337246	ISL1	ISL LIM homeobox 1	3.99
A_55_P2005838	TICRR	TOPBP1-interacting checkpoint and replication regulator	3.97
A_52_P548680	PIK3C2A	phosphatidylinositol-4-phosphate 3-kinase, catalytic subunit type 2 alpha	3.79
A_55_P2115141	LCOR	ligand dependent nuclear receptor corepressor	3.77
A_55_P2029289	SMAD1	SMAD family member 1	3.76
A_52_P498086	SPI1	Spi-1 proto-oncogene	3.75
A_66_P120736	BNIP3	BCL2/adenovirus E1B 19kDa interacting protein 3	3.63
A_51_P345649	PDGFRA	platelet-derived growth factor receptor, alpha polypeptide	3.62
A_55_P2041784	GNA13	guanine nucleotide binding protein (G protein), alpha 13	3.56
A_55_P2128492	RAP1A	RAP1A, member of RAS oncogene family	3.50
A_55_P2026889	MED10	mediator complex subunit 10	3.47
A_52_P532033	HLTF	helicase-like transcription factor	3.42
A_52_P284821	SLCO4C1	solute carrier organic anion transporter family, member 4C1	3.38
A_51_P116007	HDAC2	histone deacetylase 2	3.34
A_52_P506493	DCX	doublecortin	3.28
A_52_P474636	STK4	serine/threonine kinase 4	3.26
A_52_P343690	KPNA4	karyopherin alpha 4 (importin alpha 3)	3.19
A_52_P535052	RCOR1	REST corepressor 1	3.09
A_66_P121459	CENPA	centromere protein A	3.07
A_52_P336825	MATR3	matrin 3	3.06
A_52_P227445	PARP2	poly (ADP-ribose) polymerase 2	3.04
A_51_P230934	CAB39	calcium binding protein 39	3.03

A_51_P275101	CHST11	carbohydrate (chondroitin 4) sulfotransferase 11	3.01
A_55_P2071447	IL21R	interleukin 21 receptor	2.99
A_55_P2023114	HSD3B1	hydroxy-delta-5-steroid dehydrogenase, 3 beta- and steroid delta-isomerase 1	2.97
A_66_P106716	AKAP8	A kinase (PRKA) anchor protein 8	2.97
A_52_P329398	ATP12A	ATPase, H+/K+ transporting, nongastric, alpha polypeptide	2.95
A_52_P228236	TFRC	transferrin receptor	2.92
A_55_P2058923	WBSCR22	Williams Beuren syndrome chromosome region 22	2.90
A_55_P2079761	GGPS1	geranylgeranyl diphosphate synthase 1	2.90
A_55_P2055107	AGPAT1	1-acylglycerol-3-phosphate O-acyltransferase 1	2.88
A_51_P468126	TRIM39	tripartite motif containing 39	2.86
A_52_P406482	MBTPS1	membrane-bound transcription factor peptidase, site 1	2.84
A_55_P1994907	SENP2	SUMO1/sentrin/SMT3 specific peptidase 2	2.84
A_55_P1961736	RCAN2	regulator of calcineurin 2	2.81
A_51_P158922	PRKAA1	protein kinase, AMP-activated, alpha 1 catalytic subunit	2.81
A_52_P49014	SHH	sonic hedgehog	2.78
A_52_P549827	MGST1	microsomal glutathione S-transferase 1	2.78
A_55_P2408588	ARNTL	aryl hydrocarbon receptor nuclear translocator-like	2.78
A_65_P10351	ATP2B1	ATPase, Ca++ transporting, plasma membrane 1	2.78
A_51_P430766	IL10	interleukin 10	2.76
A_51_P470328	SEPP1	selenoprotein P, plasma, 1	2.75
A_51_P480320	USP24	ubiquitin specific peptidase 24	2.74
A_51_P124535	MEST	mesoderm specific transcript	2.74
A_55_P2057040	PPP1R16B	protein phosphatase 1, regulatory subunit 16B	2.73
A_66_P129769	SIK2	salt-inducible kinase 2	2.73
A_51_P374900	P2RY13	purinergic receptor P2Y, G-protein coupled, 13	2.71
A_52_P307739	SOX2	SRY (sex determining region Y)-box 2	2.71
A_55_P2186205	TRAPPC2	trafficking protein particle complex 2	2.68
A_51_P121302	THEM4	thioesterase superfamily member 4	2.68
A_55_P2151646	MCM9	minichromosome maintenance complex component 9	2.68
A_52_P585124	CXCR4	chemokine (C-X-C motif) receptor 4	2.67
A_51_P511015	FZD9	frizzled class receptor 9	2.65
A_66_P116154	CSTF2	cleavage stimulation factor, 3' pre-RNA, subunit 2, 64kDa	2.64
A_51_P433026	PPAPDC2	phosphatidic acid phosphatase type 2 domain containing 2	2.64
A_51_P254855	PTGS2	prostaglandin-endoperoxide synthase 2 (prostaglandin G/H synthase and cyclooxygenase)	2.61
A_51_P513392	AZI2	5-azacytidine induced 2	2.60

A_55_P12124582	Xlr4a (includes others)	X-linked lymphocyte-regulated 4A	2.60
A_55_P2034864	TUBB2B	tubulin, beta 2B class IIb	2.59
A_55_P2094955	GRM1	glutamate receptor, metabotropic 1	2.55
A_51_P234881	ELOVL4	ELOVL fatty acid elongase 4	2.54
A_51_P475501	NDUFA8	NADH dehydrogenase (ubiquinone) 1 alpha subcomplex, 8, 19kDa	2.54
A_55_P2038212	ATP13A4	ATPase type 13A4	2.54
A_52_P350750	CHRNA4	cholinergic receptor, nicotinic, alpha 4 (neuronal)	2.53
A_66_P108791	CAMK4	calcium/calmodulin-dependent protein kinase IV	2.52
A_51_P227392	RHOU	ras homolog family member U	2.51
A_51_P268697	SLC1A3	solute carrier family 1 (glial high affinity glutamate transporter), member 3	2.48
A_51_P501550	UXS1	UDP-glucuronate decarboxylase 1	2.47
A_51_P488092	PDE6D	phosphodiesterase 6D, cGMP-specific, rod, delta	2.47
A_52_P214630	SOX9	SRY (sex determining region Y)-box 9	2.44
A_66_P139167	SAFB	scaffold attachment factor B	2.42
A_55_P1960901	TGFBR1	transforming growth factor, beta receptor 1	2.42
A_52_P596008	ZEB2	zinc finger E-box binding homeobox 2	2.42
A_55_P1961436	PRKCA	protein kinase C, alpha	2.40
A_52_P317393	ADGRG1	adhension G protein-coupled receptor 1	2.26
A_66_P120555	GRIK3	glutamate receptor, ionotropic, kainate 3	2.25
A_55_P2121466	NCOR1	nuclear receptor corepressor 1	2.25
A_51_P371279	ICOS	inducible T-cell co-stimulator	2.23
A_51_P258768	PAQR5	progestin and adipoQ receptor family member V	2.23
A_51_P484289	SLC7A6	solute carrier family 7 (amino acid transporter light chain, y+L system), member 6	2.23
A_55_P2086692	NFIB	nuclear factor I/B	2.23
A_51_P261107	OGT	O-linked N-acetylglucosamine (GlcNAc) transferase	2.23
A_52_P38011	RALYL	RALY RNA binding protein-like	2.22
A_52_P552665	FZD7	frizzled class receptor 7	2.22
A_55_P2160682	SET	SET nuclear proto-oncogene	2.22
A_51_P289392	Naip1 (includes others)	NLR family, apoptosis inhibitory protein 1	2.21
A_66_P118592	ATRX	alpha thalassemia/mental retardation syndrome X-linked	2.20
A_55_P1981040	DDX6	DEAD (Asp-Glu-Ala-Asp) box helicase 6	2.19
A_55_P2015238	DCTN4	dynactin 4 (p62)	2.19
A_55_P2058340	PRDX4	peroxiredoxin 4	2.18
A_66_P134405	AXIN2	axin 2	2.18
A_51_P261164	F2RL2	coagulation factor II (thrombin) receptor-like 2	2.18
A_55_P2015113	ATG5	autophagy related 5	2.18
A_51_P349878	TXNDC17	thioredoxin domain containing 17	2.17

A_55_P2164070	GRIP1	glutamate receptor interacting protein 1	2.17
A_55_P1968778	RASA1	RAS p21 protein activator (GTPase activating protein) 1	2.17
A_55_P2089645	TUB	tubby bipartite transcription factor	2.16
A_52_P369581	ATM	ATM serine/threonine kinase	2.15
A_55_P1960601	SLC4A10	solute carrier family 4, sodium bicarbonate transporter, member 10	2.15
A_51_P374772	ARFGEF1	ADP-ribosylation factor guanine nucleotide-exchange factor 1 (brefeldin A-inhibited)	2.14
A_52_P658122	ETS2	v-ets avian erythroblastosis virus E26 oncogene homolog 2	2.13
A_51_P220896	CHMP2B	charged multivesicular body protein 2B	2.13
A_51_P100787	SNW1	SNW domain containing 1	2.12
A_51_P104891	EPT1	ethanolaminephosphotransferase 1 (CDP-ethanolamine-specific)	2.12
A_55_P2024580	DTNBP1	dystrobrevin binding protein 1	2.11
A_51_P285916	RAPGEF4	Rap guanine nucleotide exchange factor (GEF) 4	2.10
A_55_P2119897	ERBB3	v-erb-b2 avian erythroblastic leukemia viral oncogene homolog 3	2.10
A_65_P09090	ARIH2	ariadne RBR E3 ubiquitin protein ligase 2	2.10
A_51_P464588	DNAJC28	DnaJ (Hsp40) homolog, subfamily C, member 28	2.09
A_55_P2167249	CCDC112	coiled-coil domain containing 112	2.08
A_51_P155763	ASCC3	activating signal cointegrator 1 complex subunit 3	2.08
A_52_P258546	NLGN1	neuroligin 1	2.07
A_55_P2039771	ITGAV	integrin, alpha V	2.07
A_51_P487360	HPCAL1	hippocalcin-like 1	2.07
A_51_P142923	CHKA	choline kinase alpha	2.07
A_55_P1968129	TCEB1	transcription elongation factor B (SIII), polypeptide 1 (15kDa, elongin C)	2.07
A_55_P2164724	GNAQ	guanine nucleotide binding protein (G protein), q polypeptide	2.07
A_55_P2167769	ARID1A	AT rich interactive domain 1A (SWI-like)	2.07
A_52_P565051	CPNE3	copine III	2.07
A_55_P2021149	CLTB	clathrin, light chain B	2.06
A_55_P2062274	RAD23B	RAD23 homolog B (S. cerevisiae)	2.06
A_55_P1952056	TP63	tumor protein p63	2.06
A_51_P383991	04-Sep	septin 4	2.05
A_52_P206881	PRPF18	pre-mRNA processing factor 18	2.05
A_51_P232281	PLA2G2D	phospholipase A2, group IID	2.04
A_55_P2106922	ARFGAP3	ADP-ribosylation factor GTPase activating protein 3	2.03
A_55_P1972923	POLR1D	polymerase (RNA) I polypeptide D, 16kDa	2.03
A_55_P2154835	AP1G1	adaptor-related protein complex 1, gamma 1 subunit	2.02
A_55_P1960994	NLGN3	neuroligin 3	2.02

A_51_P100997	Serpinb3b/Serpin b3c	serine (or cysteine) peptidase inhibitor, clade B (ovalbumin), member 3B	2.02
A_55_P2125774	OLA1	Obg-like ATPase 1	2.01
A_51_P144249	UBR1	ubiquitin protein ligase E3 component n-recognin 1	2.01
A_55_P2070936	TRA2B	transformer 2 beta homolog (Drosophila)	2.01
A_52_P39157	COMMD3-BMI1	COMMD3-BMI1 readthrough	2.00
A_66_P117838	DHX29	DEAH (Asp-Glu-Ala-His) box polypeptide 29	2.00
A_52_P327402	CDS1	CDP-diacylglycerol synthase (phosphatidate cytidylyltransferase) 1	2.00
A_66_P109220	METTL20	methyltransferase like 20	1.99
A_55_P1980543	UBE2I	ubiquitin-conjugating enzyme E2I	1.99
A_55_P2030186	IFNA4	interferon, alpha 4	1.99
A_52_P200617	IARS	isoleucyl-tRNA synthetase	1.98
A_66_P113760	ACVR2A	activin A receptor, type IIA	1.98
A_55_P2130535	DNMT3B	DNA (cytosine-5-)-methyltransferase 3 beta	1.97
A_52_P338110	CHMP1B	charged multivesicular body protein 1B	1.97
A_55_P2027117	RGS3	regulator of G-protein signaling 3	1.97
A_65_P08507	ADAM23	ADAM metallopeptidase domain 23	1.96
A_55_P2042071	SLC25A3	solute carrier family 25 (mitochondrial carrier; phosphate carrier), member 3	1.96
A_55_P2451183	PDPK1	3-phosphoinositide dependent protein kinase 1	1.96
A_55_P1975420	DHX40	DEAH (Asp-Glu-Ala-His) box polypeptide 40	1.95
A_55_P2038947	LIG3	ligase III, DNA, ATP-dependent	1.95
A_55_P2108171	GLUD1	glutamate dehydrogenase 1	1.94
A_55_P2105371	DLK1	delta-like 1 homolog (Drosophila)	1.93
A_51_P431772	NDUFA3	NADH dehydrogenase (ubiquinone) 1 alpha subcomplex, 3, 9kDa	1.92
A_55_P2080225	PLAA	phospholipase A2-activating protein	1.91
A_52_P453785	CTHRC1	collagen triple helix repeat containing 1	1.91
A_55_P2235931	CACNA1C	calcium channel, voltage-dependent, L type, alpha 1C subunit	2.29
A_51_P221031	SLC16A12	solute carrier family 16, member 12	2.14
A_55_P1994462	AVPR2	arginine vasopressin receptor 2	1.88
A_51_P295410	NAP1L5	nucleosome assembly protein 1-like 5	1.87
A_51_P121818	PRKAG1	protein kinase, AMP-activated, gamma 1 non-catalytic subunit	1.87
A_55_P2176802	HIPK1	homeodomain interacting protein kinase 1	1.87
A_55_P1969481	HIVEP3	human immunodeficiency virus type I enhancer binding protein 3	1.86
A_52_P867058	KCNC1	potassium voltage-gated channel, Shaw-related subfamily, member 1	1.86
A_52_P140394	MTMR1	myotubularin related protein 1	1.85
A_51_P452820	RPL31	ribosomal protein L31	1.85
A_55_P2051254	PVT1	Pvt1 oncogene (non-protein coding)	1.85
A_66_P129564	NGB	neuroglobin	1.85

A_55_P2107715	COL25A1	collagen, type XXV, alpha 1	1.84
A_51_P108581	ADRBK2	adrenergic, beta, receptor kinase 2	1.84
A_55_P2158921	HTR2C	5-hydroxytryptamine (serotonin) receptor 2C, G protein-coupled	1.84
A_52_P30920	SRR	serine racemase	1.84
A_51_P307243	PPP5C	protein phosphatase 5, catalytic subunit	1.84
A_55_P2040026	ITGA4	integrin, alpha 4 (antigen CD49D, alpha 4 subunit of VLA-4 receptor)	1.84
A_51_P119039	NPY5R	neuropeptide Y receptor Y5	1.84
A_55_P1993708	LRP12	low density lipoprotein receptor-related protein 12	1.83
A_51_P131561	PLET1	placenta expressed transcript 1	1.83
A_51_P432937	NPAS3	neuronal PAS domain protein 3	1.83
A_55_P2089942	XPO1	exportin 1	1.83
A_51_P397375	GATB	glutamyl-tRNA(Gln) amidotransferase, subunit B	1.82
A_52_P68221	GRIA3	glutamate receptor, ionotropic, AMPA 3	1.81
A_52_P38627	EGF	epidermal growth factor	1.81
A_55_P1982245	PHACTR2	phosphatase and actin regulator 2	1.81
A_51_P122141	MAMSTR	MEF2 activating motif and SAP domain containing transcriptional regulator	1.81
A_52_P265002	PIK3CA	phosphatidylinositol-4,5-bisphosphate 3-kinase, catalytic subunit alpha	1.80
A_55_P1954196	C1QL3	complement component 1, q subcomponent-like 3	1.80
A_52_P524895	EIF3A	eukaryotic translation initiation factor 3, subunit A	1.80
A_55_P2090330	KCNMB4	potassium large conductance calcium-activated channel, subfamily M, beta member 4	1.80
A_55_P2065389	CAMSAP1	calmodulin regulated spectrin-associated protein 1	1.79
A_51_P323842	FAM96B	family with sequence similarity 96, member B	1.77
A_55_P2009622	S100PBP	S100P binding protein	1.77
A_51_P100309	OPRM1	opioid receptor, mu 1	1.77
A_52_P311104	BRD7	bromodomain containing 7	1.77
A_55_P2058240	IPO5	importin 5	1.75
A_52_P238468	GIPR	gastric inhibitory polypeptide receptor	1.75
A_51_P444052	SCP2	sterol carrier protein 2	1.74
A_51_P293989	SENP6	SUMO1/sentrin specific peptidase 6	1.74
A_51_P109840	VTN	vitronectin	1.73
A_52_P623226	FUT2	fucosyltransferase 2 (secretor status included)	1.73
A_55_P2005135	TARDBP	TAR DNA binding protein	1.73
A_55_P2078247	PPP1R10	protein phosphatase 1, regulatory subunit 10	1.72
A_51_P367162	PSMB1	proteasome (prosome, macropain) subunit, beta type, 1	1.72
A_55_P2013793	ZNF654	zinc finger protein 654	1.72
A_52_P452787	PDCD6IP	programmed cell death 6 interacting protein	1.72

A_55_P1999691	USP27X	ubiquitin specific peptidase 27, X-linked	1.71
A_52_P579640	RRS1	RRS1 ribosome biogenesis regulator homolog (S. cerevisiae)	1.70
A_51_P438527	CTB5R1	cytochrome b5 reductase 1	1.70
A_55_P2015687	PHF11	PHD finger protein 11	1.70
A_52_P144310	GAD1	glutamate decarboxylase 1 (brain, 67kDa)	1.70
A_55_P2011286	HOPX	HOP homeobox	1.69
A_55_P2141306	SYNJ2BP	synaptojanin 2 binding protein	1.69
A_51_P320614	TXNRD1	thioredoxin reductase 1	1.69
A_51_P106144	ZFP64	ZFP64 zinc finger protein	1.69
A_55_P2078960	Tdh	L-threonine dehydrogenase	1.68
A_55_P2006118	RBP4	retinol binding protein 4, plasma	1.67
A_51_P128336	SERINC5	serine incorporator 5	1.67
A_55_P1988033	ATP6V1G1	ATPase, H+ transporting, lysosomal 13kDa, V1 subunit G1	1.67
A_55_P2113256	PARP11	poly (ADP-ribose) polymerase family, member 11	1.67
A_55_P2033120	SRXN1	sulfiredoxin 1	1.66
A_51_P114005	GSTM2	glutathione S-transferase mu 2 (muscle)	1.65
A_55_P1972981	PHACTR3	phosphatase and actin regulator 3	1.64
A_55_P2029366	ABHD6	abhydrolase domain containing 6	1.64
A_52_P657435	C16orf91	chromosome 16 open reading frame 91	2.88
A_55_P2087671	ABHD12	abhydrolase domain containing 12	1.63
A_52_P376214	CSTF3	cleavage stimulation factor, 3' pre-RNA, subunit 3, 77kDa	1.62
A_55_P2078463	NRL	neural retina leucine zipper	1.62
A_55_P1999829	THOC7	THO complex 7 homolog (Drosophila)	1.62
A_55_P2090943	LARP4	La ribonucleoprotein domain family, member 4	1.61
A_51_P481788	MAPK1	mitogen-activated protein kinase 1	1.61
A_55_P2003115	RNF7	ring finger protein 7	1.61
A_55_P2140711	TUFM	Tu translation elongation factor, mitochondrial	1.60
A_51_P196127	PAPSS1	3'-phosphoadenosine 5'-phosphosulfate synthase 1	1.60
A_66_P126836	ZBTB44	zinc finger and BTB domain containing 44	1.59
A_51_P487501	MOB1A	MOB kinase activator 1A	1.59
A_55_P2137887	MPDZ	multiple PDZ domain protein	1.59
A_55_P2076489	RAC1	ras-related C3 botulinum toxin substrate 1 (rho family, small GTP binding protein Rac1)	1.58
A_51_P415126	TGFA	transforming growth factor, alpha	1.58
A_55_P2133280	AP4B1	adaptor-related protein complex 4, beta 1 subunit	1.58
A_55_P2044045	YBX1	Y box binding protein 1	1.57
A_51_P296448	CASP2	caspase 2, apoptosis-related cysteine peptidase	1.57
A_51_P107362	SOCS2	suppressor of cytokine signaling 2	1.57

A_52_P135469	NDST1	N-deacetylase/N-sulfotransferase (heparan glucosaminyl) 1	1.57
A_55_P2350022	AKAP6	A kinase (PRKA) anchor protein 6	1.56
A_55_P1991219	STAT3	signal transducer and activator of transcription 3 (acute-phase response factor)	1.56
A_55_P2076159	WRB	tryptophan rich basic protein	1.54
A_55_P2054082	CHST9	carbohydrate (N-acetylgalactosamine 4-0) sulfotransferase 9	1.53
A_55_P2074326	NEU4	sialidase 4	1.52
A_55_P2148844	BEND4	BEN domain containing 4	1.51

Table S3. Down-regulated genes in the hippocampus by the ME alone.

Probe name	Molecules	ME-induced downregulation	Fold Change
	Gene Symbol	Description	
A_55_P1966239	Podxl	podocalyxin-like	-4.17
A_52_P15388	LTF	lactotransferrin	-3.23
A_55_P2063107	INADL	InaD-like (Drosophila)	-3.13
A_52_P334562	VDR	vitamin D (1,25- dihydroxyvitamin D3) receptor	-3.03
A_55_P2071349	TRH	thyrotropin-releasing hormone	-2.70
A_51_P385099	TNF	tumor necrosis factor	-2.70
A_51_P104687	CSHL1	chorionic somatomammotropin hormone-like 1	-2.63
A_55_P2115044	NEURL3	neuralized E3 ubiquitin protein ligase 3	-2.50
A_51_P186574	THEG	theg spermatid protein	-2.08
A_55_P2000379	PDE10A	phosphodiesterase 10A	-1.85
A_55_P2143923	SLC13A2	solute carrier family 13 (sodium-dependent dicarboxylate transporter), member 2	-1.82
A_51_P212308	CXADR	coxsackie virus and adenovirus receptor	-1.82
A_55_P2068306	POLR1B	polymerase (RNA) I polypeptide B, 128kDa	-1.79
A_55_P2184434	EOMES	eomesodermin	-1.75
A_55_P2201345	HOXC8	homeobox C8	-1.70
A_55_P2266178	IL2RB	interleukin 2 receptor, beta	-1.67
A_66_P116635	IGF2-AS	IGF2 antisense RNA	-1.61
A_51_P150302	CRTAM	cytotoxic and regulatory T cell molecule	-1.61
A_51_P484880	BCL2L11	BCL2-like 11 (apoptosis facilitator)	-1.61
A_55_P2005655	NTSR1	neurotensin receptor 1 (high affinity)	-1.56
A_51_P155565	C3orf20	chromosome 3 open reading frame 20	-1.54
A_55_P2148204	PTGDR	prostaglandin D2 receptor (DP)	-1.52
A_55_P2080870	CMTM7	CKLF-like MARVEL transmembrane domain containing 7	-1.50
A_55_P2124736	COL14A1	collagen, type XIV, alpha 1	-1.49
A_51_P125205	AQP1	aquaporin 1 (Colton blood group)	-1.47
A_51_P256747	ADCY10	adenylate cyclase 10 (soluble)	-1.45
A_55_P2005585	TRPS1	trichorhinophalangeal syndrome I	-1.43
A_55_P2069435	ZFP82	ZFP82 zinc finger protein	-1.41
A_55_P2241299	OTUD7B	OTU deubiquitinase 7B	-1.41
A_55_P1997696	PLBD1	phospholipase B domain containing 1	-1.39
A_52_P173580	LRRIQ3	leucine-rich repeats and IQ motif containing 3	-1.37
A_66_P108138	IMPACT	impact RWD domain protein	-1.37
A_52_P532355	LIN28B	lin-28 homolog B (C. elegans)	-1.37
A_51_P242967	PIWIL2	piwi-like RNA-mediated gene silencing 2	-1.35

Table S4. Up-regulated genes in the hippocampus by the combined the ME and AX.

Probe name	Molecules	ME+AX-induced upregulation	Fold Change
	Gene Symbol	Description	
A_51_P104939	Klra4	killer cell lectin-like receptor, subfamily A, member 4	48.44
A_55_P1952091	CA10	carbonic anhydrase X	20.15
A_55_P2094149	LHX6	LIM homeobox 6	14.06
A_51_P519301	IL17F	interleukin 17F	8.14
A_55_P2007447	ABHD3	abhydrolase domain containing 3	7.5
A_52_P374882	LEP	leptin	6.64
A_52_P498086	SPI1	Spi-1 proto-oncogene	5.61
A_55_P2023114	HSD3B1	hydroxy-delta-5-steroid dehydrogenase, 3 beta- and steroid delta-isomerase 1	4.85
A_55_P2116621	CITED2	Cbp/p300-interacting transactivator, with Glu/Asp-rich carboxy-terminal domain, 2	4.63
A_55_P2029289	SMAD1	SMAD family member 1	4.57
A_55_P1978860	TP53BP2	tumor protein p53 binding protein 2	4.5
A_52_P406482	MBTPS1	membrane-bound transcription factor peptidase, site 1	4.46
A_55_P2063755	Naip1	NLR family, apoptosis inhibitory protein 1	4.31
A_51_P371279	ICOS	inducible T-cell co-stimulator	4.29
A_52_P548680	PIK3C2A	phosphatidylinositol-4-phosphate 3-kinase, catalytic subunit type 2 alpha	4.05
A_51_P104718	GPR12	G protein-coupled receptor 12	3.93
A_55_P1952985	RPS6KA3	ribosomal protein S6 kinase, 90kDa, polypeptide 3	3.91
A_51_P345649	PDGFRA	platelet-derived growth factor receptor, alpha polypeptide	3.7
A_66_P120736	BNIP3	BCL2/adenovirus E1B 19kDa interacting protein 3	3.58
A_52_P532033	HLTF	helicase-like transcription factor	3.5
A_52_P350750	CHRNA4	cholinergic receptor, nicotinic, alpha 4 (neuronal)	3.39
A_55_P1976204	CDKN1A	cyclin-dependent kinase inhibitor 1A (p21, Cip1)	3.38
A_52_P174915	GJA1	gap junction protein, alpha 1, 43kDa	3.37
A_51_P116007	HDAC2	histone deacetylase 2	3.35
A_51_P261164	F2RL2	coagulation factor II (thrombin) receptor-like 2	3.34
A_55_P2128492	RAP1A	RAP1A, member of RAS oncogene family	3.31
A_66_P134405	AXIN2	axin 2	3.31
A_51_P275101	CHST11	carbohydrate (chondroitin 4) sulfotransferase 11	3.3
A_52_P474636	STK4	serine/threonine kinase 4	3.29
A_51_P468126	TRIM39	tripartite motif containing 39	3.25
A_52_P228236	TFRC	transferrin receptor	3.18
A_55_P2026889	MED10	mediator complex subunit 10	3.18
A_66_P121459	CENPA	centromere protein A	3.15
A_55_P2041784	GNA13	guanine nucleotide binding protein (G protein), alpha 13	3.13
A_52_P343690	KPNA4	karyopherin alpha 4 (importin alpha 3)	3.13

A_52_P337246	ISL1	ISL LIM homeobox 1	3.11
A_52_P49014	SHH	sonic hedgehog	3.08
A_66_P106716	AKAP8	A kinase (PRKA) anchor protein 8	3.06
A_55_P2094955	GRM1	glutamate receptor, metabotropic 1	3.03
A_55_P2139201	HMGCLL1	3-hydroxymethyl-3-methylglutaryl-CoA lyase-like 1	3.03
A_52_P227445	PARP2	poly (ADP-ribose) polymerase 2	3.01
A_55_P1963232	MEF2B	myocyte enhancer factor 2B	3
A_55_P2053404	LILRB3	leukocyte immunoglobulin-like receptor, subfamily B (with TM and ITIM domains), member 3	2.97
A_55_P2055107	AGPAT1	1-acylglycerol-3-phosphate O-acyltransferase 1	2.97
A_51_P100327	TAP1	transporter 1, ATP-binding cassette, sub-family B (MDR/TAP)	2.94
A_55_P1952056	TP63	tumor protein p63	2.94
A_51_P158922	PRKAA1	protein kinase, AMP-activated, alpha 1 catalytic subunit	2.91
A_55_P2079761	GGPS1	geranylgeranyl diphosphate synthase 1	2.91
A_51_P448856	ELF4	E74-like factor 4 (ets domain transcription factor)	2.91
A_52_P585124	CXCR4	chemokine (C-X-C motif) receptor 4	2.85
A_55_P2408588	ARNTL	aryl hydrocarbon receptor nuclear translocator-like	2.84
A_55_P2100770	USP48	ubiquitin specific peptidase 48	2.82
A_51_P374900	P2RY13	purinergic receptor P2Y, G-protein coupled, 13	2.82
A_55_P2131672	MICAL2	microtubule associated monooxygenase, calponin and LIM domain containing 2	2.81
A_52_P549827	MGST1	microsomal glutathione S-transferase 1	2.8
A_51_P480320	USP24	ubiquitin specific peptidase 24	2.8
A_55_P1994907	SENP2	SUMO1/sentrin/SMT3 specific peptidase 2	2.79
A_66_P116154	CSTF2	cleavage stimulation factor, 3' pre-RNA, subunit 2, 64kDa	2.78
A_55_P1961736	RCAN2	regulator of calcineurin 2	2.75
A_51_P403564	LHX5	LIM homeobox 5	2.74
A_51_P433026	PPAPDC2	phosphatidic acid phosphatase type 2 domain containing 2	2.73
A_65_P10351	ATP2B1	ATPase, Ca++ transporting, plasma membrane 1	2.7
A_51_P245414	KLK3	kallikrein-related peptidase 3	2.68
A_51_P511015	FZD9	frizzled class receptor 9	2.67
A_51_P513392	AZI2	5-azacytidine induced 2	2.66
A_55_P2057040	PPP1R16B	protein phosphatase 1, regulatory subunit 16B	2.65
A_52_P307739	SOX2	SRY (sex determining region Y)-box 2	2.64
A_51_P470328	SEPP1	selenoprotein P, plasma, 1	2.63
A_55_P1999888	CHST4	carbohydrate (N-acetylglucosamine 6-O) sulfotransferase 4	2.59
A_51_P234881	ELOVL4	ELOVL fatty acid elongase 4	2.58
A_51_P430766	IL10	interleukin 10	2.58
A_51_P488092	PDE6D	phosphodiesterase 6D, cGMP-specific, rod, delta	2.56
A_66_P108791	CAMK4	calcium/calmodulin-dependent protein kinase IV	2.55

A_66_P139167	SAFB	scaffold attachment factor B	2.55
A_51_P294095	PRKAR1A	protein kinase, cAMP-dependent, regulatory, type I, alpha	2.54
A_52_P651034	TMX1	thioredoxin-related transmembrane protein 1	2.53
A_51_P432937	NPAS3	neuronal PAS domain protein 3	2.53
A_55_P2094034	NRG2	neuregulin 2	2.51
A_51_P479914	PIK3CB	phosphatidylinositol-4,5-bisphosphate 3-kinase, catalytic subunit beta	2.51
A_55_P2235931	CACNA1C	calcium channel, voltage-dependent, L type, alpha 1C subunit	2.5
A_66_P120555	GRIK3	glutamate receptor, ionotropic, kainate 3	2.5
A_52_P535052	RCOR1	REST corepressor 1	2.5
A_51_P268697	SLC1A3	solute carrier family 1 (glial high affinity glutamate transporter), member 3	2.48
A_52_P214630	SOX9	SRY (sex determining region Y)-box 9	2.48
A_51_P475501	NDUFA8	NADH dehydrogenase (ubiquinone) 1 alpha subcomplex, 8, 19kDa	2.48
A_52_P327402	CDS1	CDP-diacylglycerol synthase (phosphatidate cytidylyltransferase) 1	2.48
A_51_P124535	MEST	mesoderm specific transcript	2.46
A_52_P67440	IRAK4	interleukin-1 receptor-associated kinase 4	2.45
A_66_P118592	ATRX	alpha thalassemia/mental retardation syndrome X-linked	2.43
A_51_P464918	MEFV	Mediterranean fever	2.42
A_55_P1999691	USP27X	ubiquitin specific peptidase 27, X-linked	2.42
A_51_P285916	RAPGEF4	Rap guanine nucleotide exchange factor (GEF) 4	2.42
A_51_P227392	RHOU	ras homolog family member U	2.42
A_52_P369581	ATM	ATM serine/threonine kinase	2.41
A_55_P2063311	MGLL	monoglyceride lipase	2.39
A_55_P1961436	PRKCA	protein kinase C, alpha	2.37
A_52_P317393	ADGRG1	adhesion G protein-coupled receptor G1	2.37
A_55_P2026669	ATP5L	ATP synthase, H+ transporting, mitochondrial Fo complex, subunit G	2.37
A_51_P100997	Serpinb3b/Serpinb3c	serine (or cysteine) peptidase inhibitor, clade B (ovalbumin), member 3B	2.37
A_52_P284821	SLCO4C1	solute carrier organic anion transporter family, member 4C1	2.36
A_51_P484289	SLC7A6	solute carrier family 7 (amino acid transporter light chain, y+L system), member 6	2.35
A_52_P35384	DRD5	dopamine receptor D5	2.35
A_51_P254855	PTGS2	prostaglandin-endoperoxide synthase 2 (prostaglandin G/H synthase and cyclooxygenase)	2.35
A_66_P129769	SIK2	salt-inducible kinase 2	2.31
A_55_P2089645	TUB	tubby bipartite transcription factor	2.3
A_52_P596008	ZEB2	zinc finger E-box binding homeobox 2	2.3
A_55_P2160682	SET	SET nuclear proto-oncogene	2.3
A_52_P237232	THPO	thrombopoietin	2.3
A_55_P2034864	TUBB2B	tubulin, beta 2B class IIb	2.29

A_52_P552665	FZD7	frizzled class receptor 7	2.27
A_55_P2121466	NCOR1	nuclear receptor corepressor 1	2.27
A_52_P38011	RALYL	RALY RNA binding protein-like	2.26
A_65_P08507	ADAM23	ADAM metallopeptidase domain 23	2.26
A_52_P658122	ETS2	v-ets avian erythroblastosis virus E26 oncogene homolog 2	2.25
A_55_P1976395	NSDHL	NAD(P) dependent steroid dehydrogenase-like	2.24
A_55_P1968778	RASA1	RAS p21 protein activator (GTPase activating protein) 1	2.23
A_51_P487360	HPCAL1	hippocalcin-like 1	2.23
A_51_P100787	SNW1	SNW domain containing 1	2.22
A_51_P142923	CHKA	choline kinase alpha	2.22
A_55_P2086692	NFIB	nuclear factor I/B	2.19
A_55_P1981040	DDX6	DEAD (Asp-Glu-Ala-Asp) box helicase 6	2.18
A_55_P1999057	CPS1	carbamoyl-phosphate synthase 1, mitochondrial	2.18
A_51_P144249	UBR1	ubiquitin protein ligase E3 component n-recognin 1	2.18
A_55_P2021149	CLTB	clathrin, light chain B	2.17
A_55_P2059904	CHSY1	chondroitin sulfate synthase 1	2.17
A_55_P2154835	AP1G1	adaptor-related protein complex 1, gamma 1 subunit	2.15
A_55_P2015238	DCTN4	dynactin 4 (p62)	2.14
A_51_P131942	DPH5	diphthamide biosynthesis 5	2.14
A_51_P104891	EPT1	ethanolaminephosphotransferase 1 (CDP-ethanolamine-specific)	2.14
A_55_P1972842	KLF3	Kruppel-like factor 3 (basic)	2.13
A_55_P2164070	GRIP1	glutamate receptor interacting protein 1	2.13
A_55_P2133032	KIRREL3	kin of IRRE like 3 (Drosophila)	2.11
A_51_P383991	04-Sep	septin 4	2.11
A_55_P1960601	SLC4A10	solute carrier family 4, sodium bicarbonate transporter, member 10	2.11
A_51_P212068	AOC1	amine oxidase, copper containing 1	2.09
A_55_P1980543	UBE2I	ubiquitin-conjugating enzyme E2I	2.08
A_55_P2167769	ARID1A	AT rich interactive domain 1A (SWI-like)	2.08
A_55_P2181602	CALB1	calbindin 1, 28kDa	2.08
A_51_P466910	CACNA1G	calcium channel, voltage-dependent, T type, alpha 1G subunit	2.07
A_55_P2038947	LIG3	ligase III, DNA, ATP-dependent	2.05
A_52_P152219	RPGRIP1L	RPGRIP1-like	2.05
A_52_P671676	RASGRF1	Ras protein-specific guanine nucleotide-releasing factor 1	2.05
A_52_P93422	CABIN1	calcineurin binding protein 1	2.05
A_55_P1955497	DHCR7	7-dehydrocholesterol reductase	2.04
A_55_P2015113	ATG5	autophagy related 5	2.04
A_55_P2164724	GNAQ	guanine nucleotide binding protein (G protein), q polypeptide	2.04
A_55_P1960994	NLGN3	neuroligin 3	2.04

A_51_P108581	ADRBK2	adrenergic, beta, receptor kinase 2	2.04
A_52_P200617	IARS	isoleucyl-tRNA synthetase	2.04
A_52_P529650	DRD2	dopamine receptor D2	2.03
A_51_P232281	PLA2G2D	phospholipase A2, group IID	2.03
A_51_P124254	COL4A1	collagen, type IV, alpha 1	2.02
A_52_P304858	GNB1	guanine nucleotide binding protein (G protein), beta polypeptide 1	2.01
A_51_P220993	PFN1	profilin 1	2
A_55_P2120089	ZMYND11	zinc finger, MYND-type containing 11	1.99
A_51_P121818	PRKAG1	protein kinase, AMP-activated, gamma 1 non-catalytic subunit	1.99
A_51_P109840	VTN	vitronectin	1.99
A_52_P225898	KCNJ8	potassium inwardly-rectifying channel, subfamily J, member 8	1.98
A_52_P38627	EGF	epidermal growth factor	1.98
A_55_P2170409	DOCK3	dedicator of cytokinesis 3	1.97
A_52_P399054	TMED2	transmembrane emp24 domain trafficking protein 2	1.97
A_52_P305658	NTNG1	netrin G1	1.97
A_52_P82701	SLC9A3	solute carrier family 9, subfamily A (NHE3, cation proton antiporter 3), member 3	1.96
A_51_P401343	CLDN14	claudin 14	1.95
A_55_P2078040	CACNA1B	calcium channel, voltage-dependent, N type, alpha 1B subunit	1.95
A_51_P415126	TGFA	transforming growth factor, alpha	1.95
A_51_P307944	S1PR4	sphingosine-1-phosphate receptor 4	1.95
A_55_P2087458	OPRM1	opioid receptor, mu 1	1.94
A_52_P288614	RARS	arginyl-tRNA synthetase	1.94
A_55_P1968129	TCEB1	transcription elongation factor B (SIII), polypeptide 1 (15kDa, elongin C)	1.94
A_52_P238468	GIPR	gastric inhibitory polypeptide receptor	1.93
A_51_P307243	PPP5C	protein phosphatase 5, catalytic subunit	1.93
A_52_P452787	PDCD6IP	programmed cell death 6 interacting protein	1.93
A_51_P507051	BHLHE22	basic helix-loop-helix family, member e22	1.93
A_55_P1968068	TP53	tumor protein p53	1.92
A_52_P140394	MTMR1	myotubularin related protein 1	1.92
A_55_P2108171	GLUD1	glutamate dehydrogenase 1	1.91
A_52_P68221	GRIA3	glutamate receptor, ionotropic, AMPA 3	1.91
A_51_P396643	NCSTN	nicastrin	1.9
A_51_P257550	MARCKSL1	MARCKS-like 1	1.88
A_51_P397375	GATB	glutamyl-tRNA(Gln) amidotransferase, subunit B	1.88
A_51_P279552	CAV2	caveolin 2	1.87
A_55_P2027117	RGS3	regulator of G-protein signaling 3	1.86
A_55_P1972923	POLR1D	polymerase (RNA) I polypeptide D, 16kDa	1.85
A_55_P2130535	DNMT3B	DNA (cytosine-5-)-methyltransferase 3 beta	1.85
A_55_P1994068	RBP1	retinol binding protein 1, cellular	1.84

A_51_P432403	FGFR2	fibroblast growth factor receptor 2	1.84
A_55_P2167339	CNOT3	CCR4-NOT transcription complex, subunit 3	1.83
A_66_P123282	ZDHHC23	zinc finger, DHHC-type containing 23	1.83
A_51_P388696	NDUFS4	NADH dehydrogenase (ubiquinone) Fe-S protein 4, 18kDa (NADH-coenzyme Q reductase)	1.82
A_52_P232648	SLC38A1	solute carrier family 38, member 1	1.82
A_55_P2040026	ITGA4	integrin, alpha 4 (antigen CD49D, alpha 4 subunit of VLA-4 receptor)	1.82
A_52_P546459	UBE2D1	ubiquitin-conjugating enzyme E2D 1	1.81
A_52_P558382	MAPK8	mitogen-activated protein kinase 8	1.79
A_55_P2107715	COL25A1	collagen, type XXV, alpha 1	1.79
A_51_P230987	PI4K2B	phosphatidylinositol 4-kinase type 2 beta	1.78
A_55_P2095663	PGR	progesterone receptor	1.78
A_52_P144310	GAD1	glutamate decarboxylase 1 (brain, 67kDa)	1.78
A_55_P2176802	HIPK1	homeodomain interacting protein kinase 1	1.78
A_52_P456640	FGR	FGR proto-oncogene, Src family tyrosine kinase	1.78
A_51_P452820	RPL31	ribosomal protein L31	1.77
A_52_P379337	RTN4	reticulon 4	1.76
A_55_P1987979	PLRG1	pleiotropic regulator 1	1.76
A_55_P2089942	XPO1	exportin 1	1.76
A_55_P2161410	SERPINA3	serpin peptidase inhibitor, clade A (alpha-1 antiproteinase, antitrypsin), member 3	1.76
A_51_P377528	OPRK1	opioid receptor, kappa 1	1.75
A_65_P01319	PDE4B	phosphodiesterase 4B, cAMP-specific	1.75
A_52_P311104	BRD7	bromodomain containing 7	1.74
A_55_P2061064	GGT5	gamma-glutamyltransferase 5	1.74
A_51_P115159	FAM162A	family with sequence similarity 162, member A	1.74
A_52_P246165	PTPRK	protein tyrosine phosphatase, receptor type, K	1.74
A_51_P410744	DGKE	diacylglycerol kinase, epsilon 64kDa	1.73
A_66_P127411	ACAT2	acetyl-CoA acetyltransferase 2	1.73
A_55_P2049196	IFNA4	interferon, alpha 4	1.73
A_55_P2113550	NUDT3	nudix (nucleoside diphosphate linked moiety X)-type motif 3	1.73
A_52_P507214	MMP9	matrix metallopeptidase 9 (gelatinase B, 92kDa gelatinase, 92kDa type IV collagenase)	1.72
A_55_P2125947	AGPAT2	1-acylglycerol-3-phosphate O-acyltransferase 2	1.72
A_52_P584279	PNLIP	pancreatic lipase	1.72
A_51_P115005	EDN1	endothelin 1	1.72
A_55_P2140711	TUFM	Tu translation elongation factor, mitochondrial	1.7
A_55_P2090330	KCNMB4	potassium large conductance calcium-activated channel, subfamily M, beta member 4	1.7
A_66_P105801	IGF1R	insulin-like growth factor 1 receptor	1.69
A_52_P537545	SMPD3	sphingomyelin phosphodiesterase 3, neutral membrane (neutral sphingomyelinase II)	1.69
A_52_P612803	CCNG1	cyclin G1	1.68

A_66_P125538	GPC5	glypican 5	1.68
A_52_P229709	UBE2D3	ubiquitin-conjugating enzyme E2D 3	1.67
A_51_P367162	PSMB1	proteasome (prosome, macropain) subunit, beta type, 1	1.66
A_55_P2168588	CDK9	cyclin-dependent kinase 9	1.66
A_51_P440460	HIP1R	huntingtin interacting protein 1 related	1.65
A_51_P117604	RAB5A	RAB5A, member RAS oncogene family	1.62
A_55_P2008687	NLK	nemo-like kinase	1.62
A_55_P1978511	HLA-A	major histocompatibility complex, class I, A	1.62
A_51_P464576	PSEN1	presenilin 1	1.62
A_55_P2002893	PFKFB1	6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 1	1.61
A_55_P2054897	RND1	Rho family GTPase 1	1.6
A_55_P2041592	RDH16	retinol dehydrogenase 16 (all-trans)	1.6
A_51_P471025	ABLIM1	actin binding LIM protein 1	1.59
A_51_P132013	CYSLTR2	cysteinyl leukotriene receptor 2	1.58
A_55_P2107854	HNRNPK	heterogeneous nuclear ribonucleoprotein K	1.58
A_55_P2076861	SEMA6D	sema domain, transmembrane domain (TM), and cytoplasmic domain, (semaphorin) 6D	1.58
A_55_P2144110	MINK1	misshapen-like kinase 1	1.57
A_55_P2083654	PRMT2	protein arginine methyltransferase 2	1.51

Table S5. Down-regulated genes in the hippocampus by the combination of the ME and AX.

Probe name	Molecules	ME+AX-induced downregulation	Fold Change
	Gene Symbol	Description	
A_51_P412348	SCNN1B	sodium channel, non-voltage-gated 1, beta subunit	-5.00
A_52_P334562	VDR	vitamin D (1,25- dihydroxyvitamin D3) receptor	-4.76
A_55_P1960683	F2	coagulation factor II (thrombin)	-4.17
A_66_P136095	CTNNA3	catenin (cadherin-associated protein), alpha 3	-2.22
A_55_P2071349	TRH	thyrotropin-releasing hormone	-2.08
A_51_P317176	CSF3	colony stimulating factor 3 (granulocyte)	-1.89
A_51_P212308	CXADR	coxsackie virus and adenovirus receptor	-1.82
A_55_P2184434	EOMES	eomesodermin	-1.70
A_52_P641132	SOX14	SRY (sex determining region Y)-box 14	-1.67
A_55_P2149020	RPL35	ribosomal protein L35	-1.61
A_55_P2077188	STXBP1	syntaxin binding protein 1	-1.61
A_55_P2030433	GPI	glucose-6-phosphate isomerase	-1.56
A_55_P2052166	AKAP13	A kinase (PRKA) anchor protein 13	-1.54
A_55_P2027225	FAU	Finkel-Biskis-Reilly murine sarcoma virus (FBR-MuSV) ubiquitously expressed	-1.54
A_55_P1984695	IP6K3	inositol hexakisphosphate kinase 3	-1.49
A_55_P2100705	AUH	AU RNA binding protein/enoyl-CoA hydratase	-1.45
A_52_P581594	HTR4	5-hydroxytryptamine (serotonin) receptor 4, G protein-coupled	-1.39

Table S6. Representative top molecules in the hippocampus by AX only.

Gene name	Description	Fold change SE+AX
UP-REGULATED GENES		
Klra4	killer cell lectin-like receptor, subfamily A, member 4	38.97
IL17F	interleukin 17F	7.87
ABHD3	α/β-hydrolase domain containing 3	7.32
HRAS	Harvey rat sarcoma viral oncogene homolo	5.30
SMAD1	SMAD family member 1	5.21
TP53BP2	tumor protein p53 binding protein 2	4.93
CHST4	Carbohydrate (N-acetylglycosamine 6-0) sulforansferase 4	4.61
BTK	Bruton agammaglobulinemia tyrosine kinase	4.56
CITED2	Cbp/p300-interacting transactivator, with Glu/Asp-rich	4.32
GPR12	G protein-coupled receptor 12	4.21
USO24	ubiquitin specific peptidase 24	3.86
LEP	leptin	3.82
DOWN-REGULATED GENES		
CSF3	colony stimulating factor 3 (granulocyte)	-4.64
TRH	thyrotropin-releasing hormone	-3.15
VDR	vitamin D (1,25-dihydroxyvitamin D3) receptor	-3.08
HOXC8	homeobox C8	-2.83
LTF	lactotransferrin	-2.58
CAV2	caveolin 2	-2.24
ACVR2B	activin A Receptor, Type IIB	-2.19
PDE3A	phosphodiesterase 3A, CGMP-Inhibited	-2.09
IL4	interleukin 4	-2.01
TNF	tumor Necrosis Factor	-1.78
CDKN1A	cyclin-Dependent Kinase Inhibitor 1A (P21, Cip1)	-1.73
OPRM1	Opioid Receptor Mu 1	-1.73

Table S7. Protocol of mild exercise training.

Exercise protocol			
		Speed and time for C57BL/6J mice	**Speed and time for *ob/ob* mice**
Week 1	Day 1	3 m/min (10 min) + 5 m/min (20 min)	2.5 m/min (10 min) + 4 m/min (20 min)
	Day 2	5 m/min (30 min)	4 m/min (30 min)
	Day 3	Rest	Rest
	Day 4	5 m/min (20 min) + 7 m/min (10 min)	4 m/min (20 min) +5 m/min (10 min)
	Day 5	5 m/min (10 min) +7 m/min (20 min)	4 m/min (10 min) +5 m/min (20 min)
	Day 6	7 m/min (30 min)	5 m/min (30 min)
	Day 7	Rest	Rest
Week 2	Day 8	7 m/min (30 min)	5 m/min (30 min)
	Day 9	7 m/min (30 min)	5 m/min (30 min)
	Day 10	Rest	Rest
	Day 11	7 m/min (30 min)	5 m/min (30 min)
	Day 12	7 m/min (30 min)	5 m/min (30 min)
	Day 13	7 m/min (30 min)	5 m/min (30 min)
	Day 14	Rest	Rest
Week 3	Day 15	7 m/min (30 min)	5 m/min (30 min)
	Day 16	7 m/min (30 min)	5 m/min (30 min)
	Day 17	Rest	Rest
	Day 18	7 m/min (30 min)	5 m/min (30 min)
	Day 19	7 m/min (30 min)	5 m/min (30 min)
	Day 20	7 m/min (30 min)	5 m/min (30 min)
	Day 21	Rest	Rest
Week 4	Day 22	7 m/min (30 min)	5 m/min (30 min)
	Day 23	7 m/min (30 min)	5 m/min (30 min)
	Day 24	Rest	Rest
	Day 25	7 m/min (30 min)	5 m/min (30 min)
	Day 26	7 m/min (30 min)	5 m/min (30 min)
	Day 27	7 m/min (30 min)	5 m/min (30 min)
	Day 28	Rest	Rest

Table S8. Composition of experimental diets.

Ingredients	Placebo (without AX)	Astaxanthin (0.5% of AX)
	g/ kg of diet	
MF powder		
Moisture	63.75	63.75
Crude protein	174.75	174.75
Crude fat	42.00	42.00
Crude fiber	25.50	25.50
Crude ash	43.50	43.50
Nitrogen-free extract	400.50	400.50
AstaReal powder 20F		
Starch sodium octenyl succinate	35.00	35.00
Arabic gum	18.50	18.50
Mixed-tocopherol	7.00	7.00
Silicon dioxide	2.50	2.50
Glycerin-fatty acid ester	2.00	2.00
Carboxymethyl cellulose sodium	2.00	2.00
L-Ascorbyl palmitate ester	1.25	1.25
Dextrin	80.00	80.00
Astaxanthin	NA	101.75
Medium chain triglycerides	101.75	NA

All mice received the MF powder with either AX or a placebo powder (AstaReal power 20F based on 2% content of astaxanthin derived from *Haematococcus pluvialis*; AstaReal Co. Ltd, Tokyo, Japan) at concentrations of 0.5% (wt:wt). Placebo powder for AstaReal 20F contained an equivalent amount of medium chain triglycerides instead of AX. NA: not available.

References

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