Upregulation of BDNF and hippocampal functions by a hippocampal ligand of PPARα

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Running title: Improving hippocampal functions by a hippocampal drug

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SUPPLEMENTAL INFORMATION

MATERIALS AND METHODS

Isolation of mouse hippocampal neurons: Dissociated hippocampal neuronal cultures were prepared from fetuses (E18) of pregnant *Ppara*-null mice and strain-matched C57BL6 littermate mice using methods similar to those described earlier with few modifications **(1-4).** Briefly, hippocampi from fetal pups (n > 10) were isolated as a thin slice of tissue near the cortical edge of the medial temporal lobe and placed together in the Dulbecco's Modified Eagle Medium/Nutrient Mixture F-12 (DMEM/F12) media supplemented with 10% heat-inactivated fetal bovine serum. Cells were dissociated by trituration and single cell suspension was plated in a poly-D-lysine pre-coated 6 wells plate containing complete DMEM/F12 media. After cell attachment (5 mins after plating), the DMEM/F12 media was replaced with Neurobasal Medium supplemented with B27 supplements *(Life Technologies).* Next day, 10μM AraC was added to remove glial contamination in the neuronal culture. Experiments were done in 9-10 day old pure hippocampal neuronal cultures. Immediately before experimental treatment, the medium was replaced with Neurobasal Medium without B27 supplements.

Immunofluorescence analyses of primary hippocampal neurons: After hexadecanamide treatment, primary hippocampal neurons were washed three times with 1X PBS, fixed in 4% paraformaldehyde for 10 min or with chilled methanol overnight, washed again with 1X PBS and incubated first with monoclonal or polyclonal primary antibodies **(Table S1)** and then with the Cy2 or Cy5 conjugated secondary antibodies. Following secondary antibody incubation, coverslips were rinsed in 1X PBS, mounted on slides in Fluoromount *(Sigma)* and imaged using an *Olympus BX41* fluorescent microscope equipped with a *Hamamatsu ORCA-03G* camera.

Semi-quantitative RT-PCR: Total RNA was isolated from the primary hippocampal neurons using *Quick-RNA^{TM} MiniPrep kit (ZYMO RESEARCH Inc, Catalog Nos. R1054 & R1055)* following the manufacturer's protocol. Isolated total RNA was digested with DNase and RT-PCR was carried out as described earlier **(1, 5, 6)** using the RT-PCR kit from *Clontech* and the primers **(Table S2)**. GAPDH (glyceraldehyde-3-phosphate dehydrogenase) was used to ascertain that an equivalent amount of cDNA was synthesized from different samples.

Real-time PCR: Real-time PCR analysis was performed in the *ABI-Prism7700* sequence detection system *(Applied Biosystems)* as described earlier **(1, 6)**. Complementary DNA (cDNA) was created using *TaqMan Universal Master Mix* and amplified with *SYBR Green-conjugated PCR master mix (Applied Biosystems)* and the primers listed in **Table S2**. Data were processed by the *ABI Sequence Detection System 1.6* software and analyzed by the relative $2^{-\Delta\Delta CT}$ *method*.

Immunoblotting and densitometric analyses: For whole cell and tissue lysates, samples were homogenized in RIPA buffer + protease and phosphatase inhibitors *(Sigma)*, rotated end over end for 30 min at 4°C and centrifuged for 10 min at 15,000g. The supernatant was aliquoted and stored at -80°C until use. Protein concentrations were determined using a *NanoDrop 2000* (*Thermo Fisher*), and 15-30 µg sample was heat-denatured and resolved on 12% or 15% polyacrylamide-SDS gels in MES buffer (50 mM MES, 50 mM Tris base, 0.1% SDS, 1 mM EDTA, pH 7.3) or 1X SDS Running Buffer. Proteins were transferred to 0.45µm nitrocellulose membranes in Towbin Buffer (25 mM Tris, 192 mM glycine, 20% (w/v) methanol) under wet conditions (40V for 120 mins). Membranes were blocked for 1 hr with blocking buffer *(Li-Cor)*, incubated with primary antibodies (Table S1) overnight at 4ºC under shaking conditions, washed, incubated with IR-dye labeled secondary antibodies (1:5,000; *Li-Cor*) for 45 min at room temperature, washed and visualized with the

Odyssey Infrared Imaging System (Li-Cor). Blots were converted to binary, analyzed using ImageJ (NIH) and normalized to the loading control (β-actin).

Immunofluorescence analyses for hippocampal tissue sections: After 1 month Hex treatment, mice were anesthetized and perfused with PBS (pH 7.4) and then with 4% (w/v) paraformaldehyde solution in PBS followed by hemisection of the brain and isolation of hippocampus from each dissected brains for immunofluorescence microscopy **(6-8).** Briefly, hemisected brains were incubated in PBS containing 0.05% Tween 20 (PBST) and 10% sucrose for 3 h and then 30% sucrose overnight at 4°C. Hemisected brains was then embedded in O.C.T (Tissue Tech) at -80°C and processed for conventional cryosectioning. Frozen hippocampal sections (40 micron thick) were treated with cold ethanol (-20°C) followed by two rinses in PBS, blocking with 3% bovine serum albumin in PBST and double labeling with two antibodies **(Table S1)**. After three washes in PBST, sections were further incubated with Cy2 and Cy5 *(Jackson ImmunoResearch Laboratories, Inc.).* The samples were mounted and observed under the *Olympus BX41* fluorescent microscope equipped with a *Hamamatsu ORCA-03G* camera. Captured images were calibrated with the scale bar and then opened in *ImageJ* software for further quantification analysis. For PSD95 puncta numbers assessment, a touch count tool was used to quantify the number of PSD95 + MAP2-ir signals (yellow colored punctas) in the specific regions of hippocampus (CA1 and DG region). 10 independent images per group were included in the counting. While measuring BDNF MFI, a closed square tool was used to draw the boundary around BDNF and MAP2-ir signals followed by monitoring MFI using *ImageJ* software. The final MFI was analyzed after subtracting the value with the background signal of respective images.

Barnes maze and T maze: Maze experiments were performed as described by us **(1, 5, 6).** Briefly, for Barnes maze, mice were trained for 2 consecutive days followed by examination on day 3. During

training, the overnight food-deprived mouse was placed in the middle of the maze in a 10 cm high cylindrical start chamber. After 10 s, the start chamber was removed to allow the mouse to move around the maze to find out the color food chips in the baited tunnel. The session was ended when the mouse entered the baited tunnel. The tunnel was always located underneath the same hole (stable within the spatial environment). After each training session, maze and escape tunnel were thoroughly cleaned with a mild detergent to avoid instinctive odor avoidance due to mouse's odor from the familiar object. On day 3, a video camera *(Basler Gen I Cam - Basler acA 1300-60)* connected to a *Noldus* computer system was placed above the maze and was illuminated with high wattage light that generated enough light and heat to motivate animals to enter into the escape tunnel. The performance was monitored by the video tracking system (*Noldus System*). Cognitive parameters were analyzed by measuring latency (duration before all four paws were on the floor of the escape box) and errors (incorrect responses before all four paws were on the floor of the escape box). For T-maze, mice were also habituated in the T-maze for two days under food-deprived conditions so that animals can eat food rewards at least five times during 10 minutes period of training. During each trial, mice were placed in the start point for 30 s and then forced to make a right arm turn which was always baited with color food chips. On entering the right arm, they were allowed to stay there for 30-45 s, then returned to the start point, held for 30 s and then allowed to make right turn again. As described above, after each training session, T-maze was thoroughly cleaned with a mild detergent. On day 3, mice were tested for making positive turns and negative turns. The reward side is always associated with a visual cue. The number of times the animal eats the food reward would be considered as a positive turn.

Novel object recognition task (NORT): Novel object recognition task was performed to monitor the short-term memory as described by others (9) and us (1). Briefly, during training, mice were

placed in the NORT testing apparatus comprised of a wooden floor square arena of 40 cm long and 40 cm wide, with walls 30 cm high. Two plastic toys (between 6 and 7 cm) that varied in color, shape, and texture were placed in specific locations in the environment 30 cm away from each other. The mice were able to explore freely the environment and objects for 15 min and then were placed back into their individual home cages. After 30 min, mice were placed back into the environment with two objects in the same locations, but now one of the familiar objects was replaced with a third novel object. The mice were then again allowed to explore freely both objects for 15 min. The objects were thoroughly cleaned with a mild detergent. A video camera (*Basler Gen I Cam - Basler acA 1300-60*) connected to a *Noldus computer system* was placed above the box. Each mouse was placed individually on the center of the NORT arena and the performance was monitored by the live video tracking system (*Noldus System*).

Open field test (OFT): The Open Field tests were performed as described earlier **(10, 11)** with slight modifications and used to assess spontaneous exploratory activity. Briefly, each mouse was allowed to freely explore an open field arena for 5 min. The testing apparatus was a classic open field (i.e., a wooden floor square arena, 40 × 40 cm, with walls 30 cm high). A video camera (*Basler Gen I Cam - Basler acA 1300-60*) connected to a Noldus computer system was placed above the box. Each mouse was placed individually on the center of the arena and the performance was monitored by the live video tracking system (*Noldus System*). Exploratory parameters in OFT that were analyzed include total distance traveled by the mice during 5 min of free exploration in the open field arena and the velocity with which this distance was covered.

Table S1. Antibodies, sources, applications and dilutions used in this study.

Protein	Antibody (Clone)	Epitope/Immunogen	Application - Dilution	Source (Catalog)
BDNF	Sheep polyclonal	Synthetic peptide from human mature BDNF	WB – 1:1000 IF – 1:200 IF – 1:200	Abcam (ab75040)
TrkB	Rabbit monoclonal (80E3)	Synthetic peptide surrounding Pro50 of human TrkB	WB – 1:1000	Cell Signaling (#4603)
MAP2	Rabbit polyclonal	Purified rat MAP-2	IF – 1:1000	Millipore (AB5622)
NR2A	Rabbit monoclonal (EPR7063)	Recombinant protein fragment corresponding to a region within Human NMDAR2A (Q12879)	WB – 1:1000	Abcam (ab133265)
GLUR1	Rabbit polyclonal	Synthetic peptide corresponding to Human Glutamate Receptor 1 (AMPA subtype) aa 850 to the C-terminus	WB – 1:1000	Abcam (ab31232)
NT3	Chicken polyclonal	Synthetic peptide based on the human NT3 protein	IF – 1:100	ThermoFisher (PA1-9520)
PSD95	Mouse monoclonal (6G6-1C9)	Recombinant full-length protein	WB – 1:1000 IF – 1:200	Abcam (ab2723)
β actin	Mouse monoclonal (AC-15)	a.a. 1-15 of *Xenopus laevis* β actin	WB – 1:5000	Abcam (ab6276)

Table S2. Primers used for RT-PCR and real-time PCR analyses.

Gene	Forward Primer (5′ to 3′)	Reverse Primer (5′ to 3′)
Bdnf	AGGCAACTTGGCCTACCCAGGTG TG	TACTGTCACACACGCTCAGCTCCCC
Gapdh	GCACAGTCAAGGCCGAGAAT	GCCTTCTCCATGGTGGTGAA
NT3	GAGAGGCCACCAGGTCAGAGTTC CA	GTCATCAATCCCCCTGCAACCGTTT
NT4	AGCGTTGCCTAGGAATACAGC	GGTCATGTTGGATGGGAGGTATC
$p75^{NTR}$	CAGACGATGCCGTGTGCCGA	CAGGGCGTGCACTCGCGTAA
TrkB	CCGGCACTGTCCTGCTACCG	CCTTCCCTGCGCCCTCTTGC

Table S3. Primers used for mRNA-based microarray analysis of plasticity-associated genes.

UniGene	GenBank	Symbol	Description	Primer Sequence	
				Forward	Reverse
Mm.347452	NM_010623	*Kif17*	Kinesin family member 17	CGAGAGTCGAGCTGGGATGAG	TCTCACTGTCGCTTCGGCTG
Mm.4292	NM_013692	*Klf10*	Kruppel-like factor 10	CATGCTCAACTTCGGCGCTT	TCCAGTCGCAGCTCATGGAC
Mm.196581	NM_011949	*Mapk1*	Mitogen-activated protein kinase 1	GCTCTGGCCCACCCATACCT	GTCCTCTGAGCCCTTGTCCTGA
Mm.4406	NM_013599	*Mmp9*	Matrix metallopeptidase 9	TCCAGACCAAGGGTACAGCC	AGGCCTTGGGTCAGGCTTAG
Mm.4974	NM_010875	*Ncam1*	Neural cell adhesion molecule 1	TGGGCCTGAAACCTGAGACG	AGCTTGGGTGCACTGGGTTC
Mm.256765	NM_008689	*Nfkb1*	Nuclear factor of kappa light polypeptide gene enhancer in B-cells 1, p105	GGTGCGCCTCATGTTCACAG	AATCTCCTCCCCTCCCGTCA
Mm.220333	NM_010908	*Nfkbib*	Nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, beta	AGAGAACGAAGAGGAGCCGC	AGCCGGACCATCTCTGCATC
Mm.1259	NM_013609	*Ngf*	Nerve growth factor	GGAGCGCATCGAGTGACTT	GGTGAGCTTGGGTCCAGCAT
Mm.283893	NM_033217	*Ngfr*	Nerve growth factor receptor (TNFR superfamily, member 16)	GCCCAGAGGGCACATACTCA	GGGGGCGTAGACCTTGTGAT
Mm.44249	NM_008712	*Nos1*	Nitric oxide synthase 1, neuronal	GGAGGAGGATGCTGGTGTGT	GGAGGATCCAGTTAGGAGCTG
Mm.10099	NM_016789	*Nptx2*	Neuronal pentraxin 2	GGTACGCCATTCTCCTACGCT	TCTCCCCATCCTGGAACGCT
Mm.119	NM_010444	*Nr4a1*	Nuclear receptor subfamily 4, group A, member 1	TGCAGGAGGCTGCGAAAGTTG	GGCTCGTTGCTGGTGTTCCAT

Mm.267570	NM_008742	*Ntf3*	Neurotrophin 3	CCCCCGTCAG CCAGGATAAT	GCTGTTGCCTT GGATGCCAC
Mm.20344	NM_198190	*Ntf5*	Neurotrophin 5	CAGCCGGGG AGCAGAGAA	AGCACCTCCAC CTCCTCACT
Mm.130054	NM_008745	*Ntrk2*	Neurotrophic tyrosine kinase, receptor, type 2	TCGACCCTCC GGAATCATCT C	CCGCTAAACC GGCACGAAT
Mm.390715	NM_021543	*Pcdh8*	Protocadherin 8	CGACGTGCTC ACCTTCCCTG	AGCCTGGAGA GGCACCGTAA
Mm.259464	NM_008837	*Pick1*	Protein interacting with C kinase 1	TCCGACCGGA AGTGACGC	GGTGACCTTCC CAGGCACAG
Mm.405293	NM_008842	*Pim1*	Proviral integration site 1	CTCCACCGCG ACATCAAGG A	CTGCCGTGGTA GCGATGGTA
Mm.154660	NM_008872	*Plat*	Plasminogen activator, tissue	AAGAGGAGC CCGGTCCTAC A	GGTTCGCTGCA ACTTCGGAC
Mm.44463	NM_021280	*Plcg1*	Phospholipase C, gamma 1	ACTCCAAGAA GTCGCAGCGG	TGGTAGCGGTC AAAGTCCCG
Mm.1970	NM_031868	*Ppp1ca*	Protein phosphatase 1, catalytic subunit, alpha isoform	TGTGCCAGCA TCAACCGCAT	GTGGGCCGCA TAATACGCCT
Mm.280784	NM_013636	*Ppp1cc*	Protein phosphatase 1, catalytic subunit, gamma isoform	TCTGCCTCTT GCTGGCCTAC	CTCGTCCACGA TGGCTGCTA
Mm.2343	NM_026731	*Ppp1r14a*	Protein phosphatase 1, regulatory (inhibitor) subunit 14A	GTGGATCGAC GGATGCTTGG	GTTTGTGCAGG CCCCGAAG
Mm.260288	NM_019411	*Ppp2ca*	Protein phosphatase 2 (formerly 2A), catalytic subunit, alpha isoform	CTCACGTTGG TGTCCAGAGC	GGTGCTGGGTC AAACTGCAAG
Mm.331389	NM_008913	*Ppp3ca*	Protein phosphatase 3, catalytic subunit, alpha isoform	CAGTCGGACG GGACGAGC	AGCCGGTGAC TTGGTGGAAA
Mm.222178	NM_011101	*Prkca*	Protein kinase C, alpha	GGGACCTGAC ACTGACGACC	TCCGCAGAGG CTAGGGACAT
Mm.7980	NM_011102	*Prkcc*	Protein kinase C, gamma	ACAATGTACC GGTGGCCGAT	TGGCACCAAA GAAGCATCGC

Mm.381172	NM_011160	*Prkg1*	Protein kinase, cGMP-dependent, type I	CGCTCGGTGATCCGGC	GCCTCCTTTATGAGATCCTTCGAC
Mm.328431	NM_007393	*Actb*	Actin, beta	TGTCGAGTCGCGTCCACC	GATGGAGGGGAATACAGCCCG
Mm.163	NM_009735	*B2m*	Beta-2 microglobulin	GGTCGCTTCAGTCGTCAGCA	GAGGCGGGTGGAACTGTGTT
Mm.343110	NM_008084	*Gapdh*	Glyceraldehyde-3-phosphate dehydrogenase	CTGGATAAGCAGGGCGGGA	CCGTTCACACCGACCTTCAC
Mm.3317	NM_010368	*Gusb*	Glucuronidase, beta	CAAGAAGCAGCCCTTCGGGA	TCAGCCTCAAAGGGGAGGTG
Mm.2180	NM_008302	*Hsp90ab1*	Heat shock protein 90 alpha (cytosolic), class B member 5	GGATGACATCACGCAGGAGGA	CGGGGAATGAAGAGCAATGCC
Mm.3037	NM_007399	*Adam10*	A disintegrin and metallopeptidase domain 10	GGGCTGGGAGGTCAGTATGG	AGGGAAGTGTCCCTCTTCATTCG
Mm.259733	NM_009622	*Adcy1*	Adenylate cyclase 1	GCTGTTCTCATGCACGCTGG	GAAGTGCTGTGCGACGTGAG
Mm.1425	NM_009623	*Adcy8*	Adenylate cyclase 8	AGACCTATCTGGCCCGCAAC	AGTACTCTGGGTAGGAGCAGA
Mm.6645	NM_009652	*Akt1*	Thymoma viral proto-oncogene 1	TAGGCCCAGTCGCCCG	AGACAGGGGTAACCCAGGGA
Mm.25405	NM_018790	*Arc*	Activity regulated cytoskeletal-associated protein	ACACCAGGTCTCAAGGCT	CTTCAGGAGAAGAGAGGATGGT
Mm.1442	NM_007540	*Bdnf*	Brain derived neutrophic factor	AGCCGCAAAGAAGTTCCA	CTTGTCCGTGGACGTTTACT
Mm.131530	NM_177407	*Camk2a*	Calcium/calmodulin-dependent protein kinase II alpha	TTCCCAGCCTAAAGCCTCGC	CTGACCAGCCAGCACCTTCA
Mm.235182	NM_178597	*Camk2g*	Calcium/calmodulin-dependent protein kinase II gamma	AGGGTGCCATCCTCACAACC	CTTGGGCTGGGCTTACGAGA
Mm.257437	NM_007664	*Cdh2*	Cadherin 2	ATGTGCACGAAGGACAGCCC	CAGGAACTTTGCCTGCTCTGC

Mm.439656	NM_009883	*Cebpb*	CCAAT/enhancer binding protein (C/EBP), beta	CCGCCTTATAAACCTCCCGC	AGGCAGTCGGGCTCGTAGTA
Mm.347407	NM_007679	*Cebpd*	CCAAT/enhancer binding protein (C/EBP), delta	AACCCGCGGCCTTCTACGAG	TGTAGGCGCTGAAGTCGATGG
Mm.7992	NM_007726	*Cnr1*	Cannabinoid receptor 1 (brain)	TTGTAGCAGAGAGCCAGCCC	AGGTGGTATCTGCAAGGCCG
Mm.453295	NM_133828	*Creb1*	CAMP responsive element binding protein 1	AAACTCCAGCGAGATCCGGG	TGGCTGGGCTTGAACTGTCA
Mm.5244	NM_013498	*Crem*	CAMP responsive element modulator	AAGAAGCAACTCGCAAGCGG	TTCTTCCTGCGACACTCCCG
Mm.27256	NM_007864	*Dlg4*	Discs, large homolog 4 (Drosophila)	AGATGAAGACACGCCCCCTC	CTGCAACTCATATCCTGGGGCT
Mm.181959	NM_007913	*Egr1*	Early growth response 1	CAATCCTCAAGGGGAGCCGA	TGATGGGAGGCAACCGAGTC
Mm.290421	NM_010118	*Egr2*	Early growth response 2	AGGCCGTAGACAAAATCCCAG	AGGGGTCTCTTCTCTCCAGTCA
Mm.103737	NM_018781	*Egr3*	Early growth response 3	TCAACCTCTTCTCCGGCAGC	TCAACCTCTTCTCCGGCAGC
Mm.44137	NM_020596	*Egr4*	Early growth response 4	CTCCACCTGAGCGACTTCTCC	TGCTCAAAGCCCAGCTCAAGA
Mm.250981	NM_010142	*Ephb2*	Eph receptor B2	TATGGGCGCTACAGTGGCAA	CGCTCAAACCCCCGTCTGTTA
Mm.246513	NM_010234	*Fos*	FBJ osteosarcoma oncogene	GAAGACCGTGTCAGGAGGCA	CTCCTCCGATTCCGGCACTT
Mm.254629	NM_010305	*Gnai1*	Guanine nucleotide binding protein (G protein), alpha inhibiting 1	GAGCCCCCTCACGATATGCT	ATCTGTGGCGCACGTGAAGT
Mm.4920	NM_008165	*Gria1*	Glutamate receptor, ionotropic,	TTGCAACACCCAAGGGGTCC	GCACTCGCCCTTGTCGTACC

			AMPA1 (alpha 1)		
Mm.220224	NM_013540	*Gria2*	Glutamate receptor, ionotropic, AMPA2 (alpha 2)	ACGACAAAGGAGAGTGCGGC	AAACCAAGGCCCCCGACAAG
Mm.327681	NM_016886	*Gria3*	Glutamate receptor, ionotropic, AMPA2 (alpha 3)	GCTCCTGCCACCAACACTCA	CATTGCATGCTTCCGGTGGG
Mm.209263	NM_019691	*Gria4*	Glutamate receptor, ionotropic, AMPA2 (alpha 4)	AAACTCAGTGAGGCAGGCGT	TTGCTCAGGCTCAAGGCACT
Mm.278672	NM_008169	*Grin1*	Glutamate receptor, ionotropic, NMDA1 (zeta 1)	GCGTGAGTCCAAGGCAGAGA	CACACGTACCCAGAGCCAGT
Mm.2953	NM_008170	*Grin2a*	Glutamate receptor, ionotropic, NMDA2A (epsilon 1)	ACCAGAGGGGCGTAGAGGAT	GTACCCGCTCCCAATGGTCA
Mm.436649	NM_008171	*Grin2b*	Glutamate receptor, ionotropic, NMDA2A (epsilon 2)	ACCCTGCCTCTTGGGTTTCTC	CTCCATAGAGCCAAGCCCCG
Mm.39090	NM_010350	*Grin2c*	Glutamate receptor, ionotropic, NMDA2A (epsilon 3)	CTACCCGTACTGGTCCCGC	CTTTGCCGGGTTCCTTGCAG
Mm.322594	NM_008172	*Grin2d*	Glutamate receptor, ionotropic, NMDA2A (epsilon 4)	CAGCGGCACTTGCATCAGAG	TGCAGCATCTCTTCTCCGGG
Mm.196692	NM_133442	*Grip1*	Glutamate receptor interacting protein 1	AGCAGGAGAGAGAGGAATTCAAGG	CCCACATCCAGCTGGTCACT

Mm.391904	NM_016976	*Grm1*	Glutamate receptor, metabotropic 1	GCTGCAGTCC TCTGACCTGA	TCCGCTTCTTC CTGCTGGTC
Mm.410822	NM_001160 353	*Grm2*	Glutamate receptor, metabotropic 2	TGGCGGCTCC TACAGTGATG	GCACGGTGCG AGCAAAGTAA
Mm.318966	NM_181850	*Grm3*	Glutamate receptor, metabotropic 3	AGGAGTCATT GGCGGTTCGT	GTCGCTGAGTT TGGCACTGG
Mm.358940	NM_001013 385	*Grm4*	Glutamate receptor, metabotropic 4	TACACCACCT GCATCGTCTG G	TGGCGGGCTG AATTCCACG
Mm.235018	NM_001081 414	*Grm5*	Glutamate receptor, metabotropic 5	GCAAGTCATC ATCCGCTGCC	GACTGCTGTCT GGTTGGGGT
Mm.240881	NM_177328	*Grm7*	Glutamate receptor, metabotropic 7	AAACCCAGTG ACAGGCCCA A	GCCAGGCTGG GTGACAGAAT
Mm.320732	NM_008174	*Grm8*	Glutamate receptor, metabotropic 8	AATCTGAACT TGCTCGGCCA	TTTCCAACAGA TCCTCCGTTCA
Mm.37533	NM_152134	*Homer1*	Homer homolog 1 (Drosophila)	TATGGCAGGG GTGGTAGCAA A	TAACTGCATGC TTGCTGGTGG
Mm.268521	NM_010512	*Igf1*	Insulin-like growth factor 1	TGCTCTAACA TCTCCCATCT CTC	GCGCCAGGTA GAAGAGGTGT
Mm.8042	NM_008380	*Inhba*	Inhibin beta-A	GGGGGAGAA CGGGTATGTG G	ACACTTCTGCA CGCTCCACT
Mm.275071	NM_010591	*Jun*	Jun oncogene	GCTACCGGCC AGCAACTTTC	CAGTCTCGGTG GCAGCCTTA
Mm.1167	NM_008416	*Junb*	Jun-B oncogene	CATCCACGGC CAACATGCTC	AACGTTTGCAA CTGCTGCGT

SUPPLEMENTAL REFERENCES

1. Roy A, Jana M, Corbett GT, Ramaswamy S, Kordower JH, Gonzalez FJ, and Pahan K. Regulation of cyclic AMP response element binding and hippocampal plasticity-related genes by peroxisome proliferator-activated receptor alpha. *Cell Rep.* 2013;4(4):724-37.

2. Roy A, Modi KK, Khasnavis S, Ghosh S, Watson R, and Pahan K. Enhancement of morphological plasticity in hippocampal neurons by a physically modified saline via phosphatidylinositol-3 kinase. *PLoS One.* 2014;9(7):e101883.

3. Jana M, Jana A, Pal U, and Pahan K. A simplified method for isolating highly purified neurons, oligodendrocytes, astrocytes, and microglia from the same human fetal brain tissue. *Neurochem Res.* 2007;32(12):2015-22.

4. Roy A, Jana M, Kundu M, Corbett GT, Rangaswamy SB, Mishra RK, Luan CH, Gonzalez FJ, and Pahan K. HMG-CoA Reductase Inhibitors Bind to PPARalpha to Upregulate Neurotrophin Expression in the Brain and Improve Memory in Mice. *Cell Metab.* 2015;22(2):253-65.

5. Corbett GT, Roy A, and Pahan K. Sodium phenylbutyrate enhances astrocytic neurotrophin synthesis via protein kinase C (PKC)-mediated activation of cAMP-response element-binding protein (CREB): implications for Alzheimer disease therapy. *J Biol Chem.* 2013;288(12):8299-312.

6. Patel D, Roy A, Kundu M, Jana M, Luan CH, Gonzalez FJ, and Pahan K. Aspirin binds to PPARα to stimulate hippocampal plasticity and protect memory. *Proc Natl Acad Sci U S A.* 2018;115(31):E7408-E17.

7. Khasnavis S, Roy A, Ghosh S, Watson R, and Pahan K. Protection of dopaminergic neurons in a mouse model of Parkinson's disease by a physically-modified saline containing charge-stabilized nanobubbles. *J Neuroimmune Pharmacol.* 2013;9(2):218-32.

8. Ghosh A, Roy A, Liu X, Kordower JH, Mufson EJ, Hartley DM, Ghosh S, Mosley RL, Gendelman HE, and Pahan K. Selective inhibition of NF-kappaB activation prevents dopaminergic neuronal loss in a mouse model of Parkinson's disease. *Proc Natl Acad Sci U S A.* 2007;104(47):18754-9.

9. Mansuy IM, Mayford M, Jacob B, Kandel ER, and Bach ME. Restricted and regulated overexpression reveals calcineurin as a key component in the transition from short-term to long-term memory. *Cell.* 1998;92(1):39-49.

10. Galeano P, Martino Adami PV, Do Carmo S, Blanco E, Rotondaro C, Capani F, Castano EM, Cuello AC, and Morelli L. Longitudinal analysis of the behavioral phenotype in a novel

transgenic rat model of early stages of Alzheimer's disease. *Front Behav Neurosci.* 2014;8(321.

11. Chen Y, Liang Z, Blanchard J, Dai CL, Sun S, Lee MH, Grundke-Iqbal I, Iqbal K, Liu F, and Gong CX. A non-transgenic mouse model (icv-STZ mouse) of Alzheimer's disease: similarities to and differences from the transgenic model (3xTg-AD mouse). *Mol Neurobiol.* 2013;47(2):711-25.

LEGENDS TO SUPPLEMENTAL FIGURES

Figure S1. Monitoring Hex in hippocampus with the help of EI-GCMS method. Representative plots are for relative abundance of hex in the hippocampi of **(A)** NTG, **(B)** Veh-fed 5xFAD and **(C)** Hex-fed 5xFAD mice. Analysis was done in ISQ7000 single quadrupole mass spectrometer and quantified at Chromeleon 7 chromatography studio. (*Inset*) Magnified views of enclosed squares of **(a)** NTG, **(b)** Veh-fed 5XFAD and **(c)** Hex-fed 5xFAD mice were displayed next to the uncut image.

Figure S2. Unmerged images of merged immunolabeled images in Fig 4C. **(A)** Corresponding unmerged images of hippocampal sections derived from mice across all groups, shown in upper panel of *Fig. 4C* that are double-labeled with BDNF (red) and MAP2 (green). **(B)** Corresponding unmerged images of hippocampal sections derived from mice across all groups, shown lower panel of *Fig. 4C* that are double-labeled with PSD95 (red) and MAP2 (green). The hippocampal sections used for double-labeling are 40 micron thick. Nuclei were counterstained with DAPI (blue). Scale bars 50 µm.

Figure S3. Generation of 5xFAD$^{Ppar\alpha\text{-}\Delta hippo}$ mice. Age-matched *Ppar*$\alpha^{\Delta Hippo}$ mice were crossed with 5xFAD mice to generate littermates heterozygous for both *Ppar*$\alpha^{\Delta Hippo}$ allele and 5xFAD transgenes (*APP* and *PSEN1*). These mice were then backcrossed with homozygous *Ppar*$\alpha^{\Delta Hippo}$ mice to generate ~25% 5xFAD$^{Ppar\alpha\text{-}\Delta Hippo}$ mice. Homozygous 5xFAD$^{Ppar\alpha\text{-}\Delta Hippo}$ mice were further bred up to F4 generation. The genomic DNA isolated from tails of littermates born to breeding pairs made from F4 generation were then analyzed by qualitative RT-PCR using primers against APP/PSEN transgenes, Camk2a^{Cre} alleles and *Ppar*α^{flox} alleles **(Table S2)**.

Figure S4. Hexadecanamide improves short-term memory in 5xFAD mice via hippocampal PPARα. 6 to 7 months-old 5xFAD, 5xFAD$^{Ppar\alpha\text{-null}}$ mice and 5xFAD$^{Ppar\alpha\text{-}\Delta hippo}$ mice (*n=6/group)* were orally treated with Hex (5mg/kg) for 30 days orally. Following Hex treatment, the recognition memory was evaluated on an open field apparatus using the *Noldus software*. **(A)** representative heat maps and track plots of mice movement during novel object recognition test (NORT), generated by *Noldus software*. **(B)** Preferential index for novel object among mice across all groups. Results are represented as mean ± SEM. One-way ANOVA [NORT preferential index: $F_{(6, 35)} = 4.7986$, $p = 0.01$], followed by *Bonferroni's* multiple comparisons test was used to assess the significance of the mean; $^{*}p < 0.05$ vs Veh-fed 5xFAD; $^{**}p < 0.01$ vs Veh-fed 5xFAD; $^{ns}p > 0.05$ vs Veh-fed 5xFAD; ns – not significant.

Figure S5. Global and hippocampal deletion of PPARα does not influence locomotor function in hexadecanamide-treated 5xFAD mice. 6 to 7 months-old 5xFAD and 5xFAD$^{Ppar\alpha\text{-null}}$ transgenic mice (*n=6/group)* were orally treated with Hex (5 mg/kg body wt.) for 30 days orally. Following Hex treatment, the locomotor activity was evaluated on an open field apparatus using the *Noldus software*. **(A)** representative heat maps of locomotor activity of mice across all groups during open field test (OFT), generated by *Noldus software*. **(B-C)** Corresponding OFT parameter analyses; **(B)** total distance moved and **(C)** Velocity. Results are represented as mean ± SEM. One-way ANOVA [OFT total distance moved: $F_{(6, 35)} = 1.3101$, $p = 0.279$ and OFT velocity $F_{(6, 35)} = 1.3164$, $p = 0.276$], followed by *Bonferroni's* multiple comparisons test was used to assess the significance of the mean; $^{ns}p > 0.05$ vs Veh-fed 5xFAD; ns – not significant.

Figure S6. Uncropped western blot images. **(A)** Corresponding uncropped image of western blot shown in Fig. 1C. **(B)** Corresponding uncropped image of western blot shown in Fig. 1F. **(C)** Corresponding uncropped image of western blot shown in Fig. 1I. **(D-F)** Corresponding uncropped images of western blots for BDNF **(D)**, βactin **(E)**, PSD95 **(E)**, GluR1 **(F)** and NR2A **(G)**, shown in Fig. 2C. **(H-I)** Corresponding uncropped images of western blots for BDNF **(H)**, PSD95 **(I)** and βactin **(I)**, shown in Fig. 5A. Red dotted box represents the cropped area for a particular protein depicted to the right of the images. The protein standards are depicted to the left of the images.

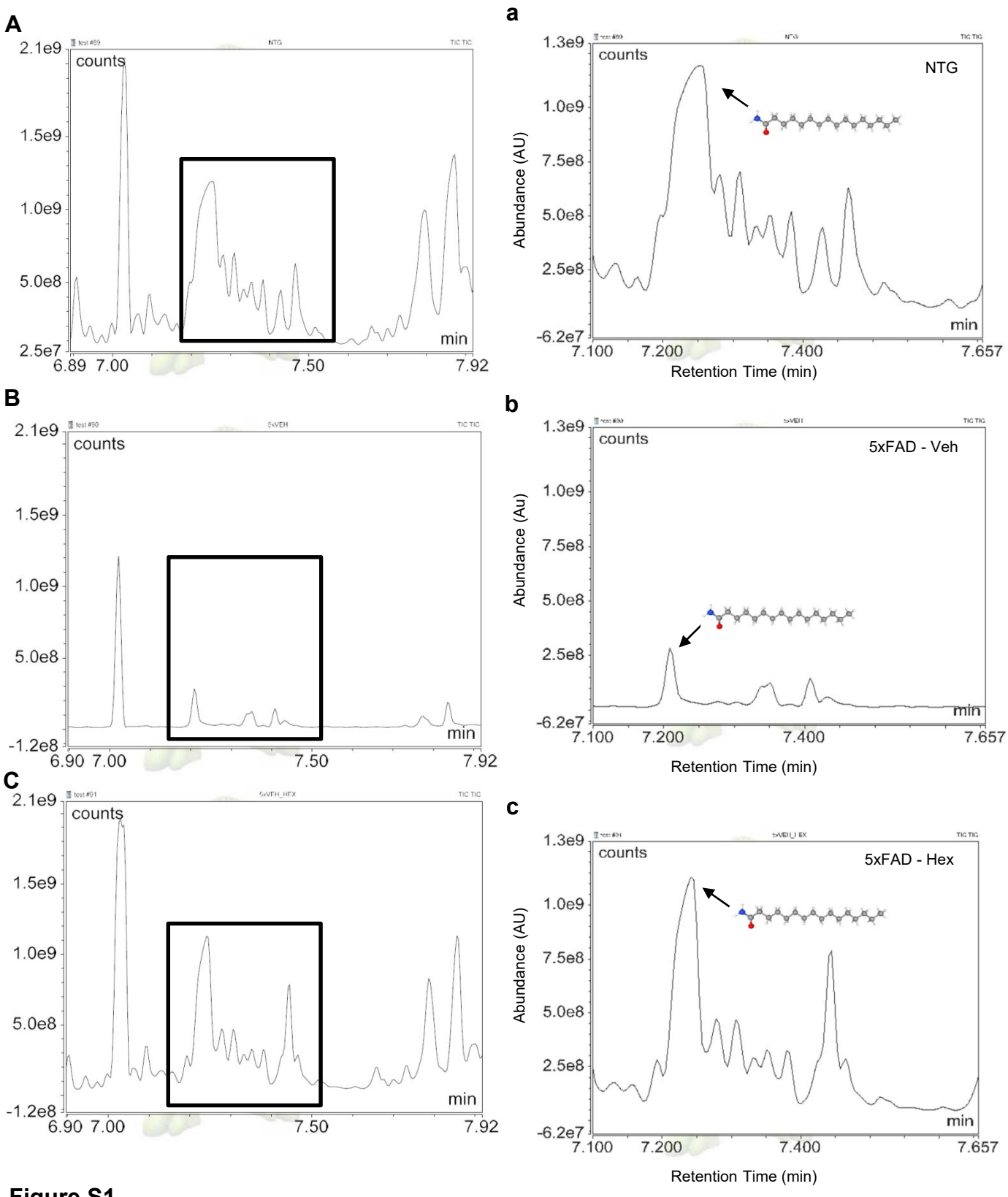

A
a
counts
NTG
min
Abundance (AU)
Retention Time (min)
B
b
5xFAD - Veh
C
c
5xFAD - Hex

Figure S1.

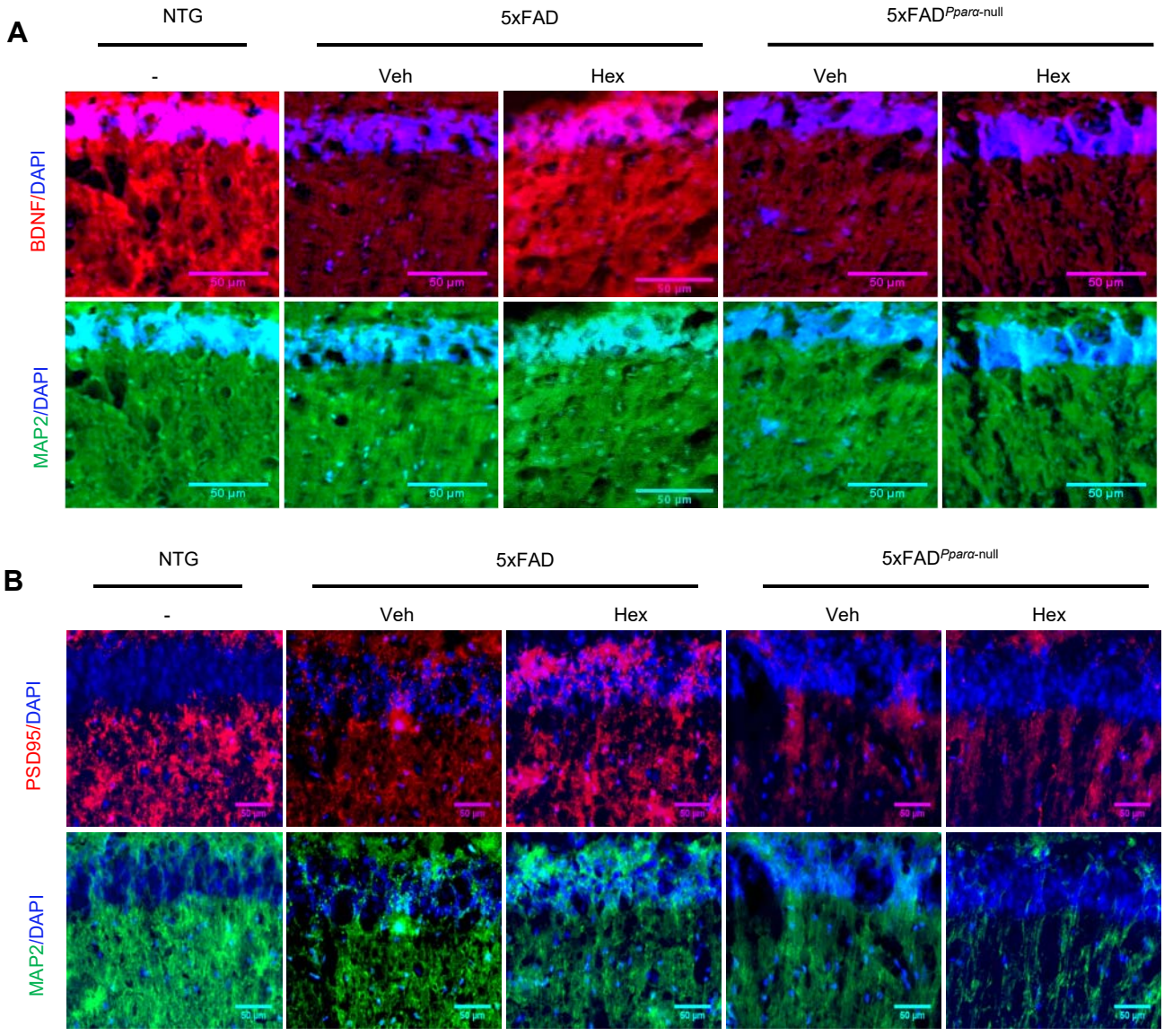

A
NTG
5xFAD
5xFAD Pparα-null
-
Veh
Hex
Veh
Hex
BDNF/DAPI
MAP2/DAPI
50 µm
B
NTG
5xFAD
5xFAD Pparα-null
-
Veh
Hex
Veh
Hex
PSD95/DAPI
MAP2/DAPI
50 µm

Figure S2

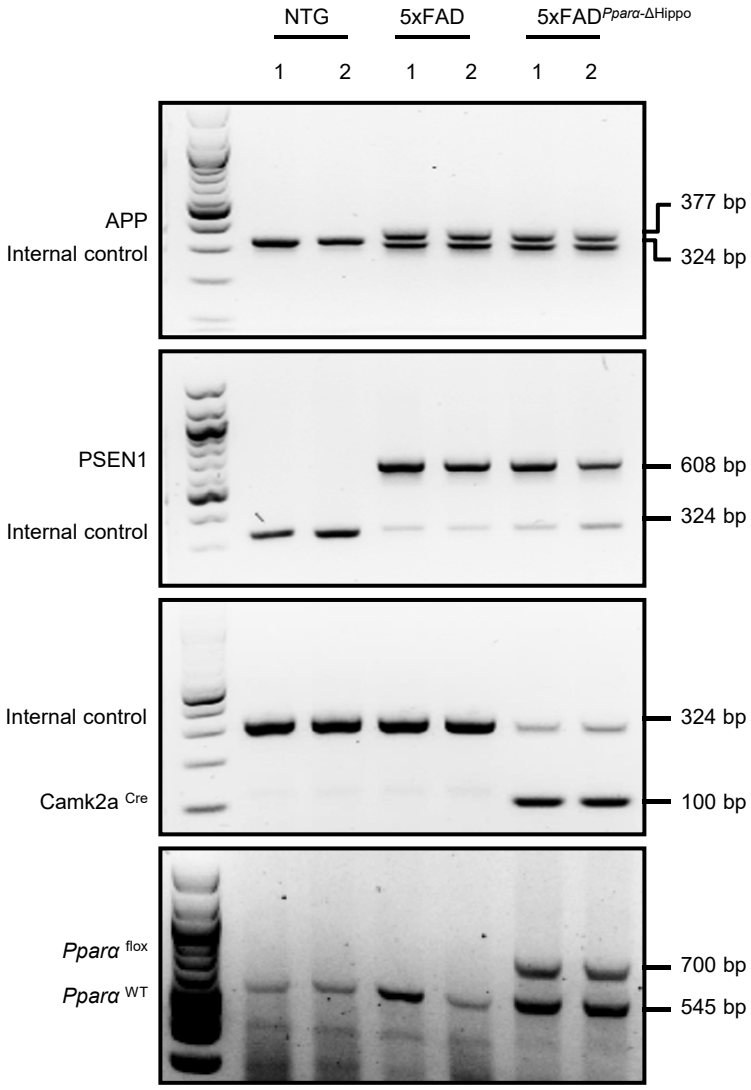
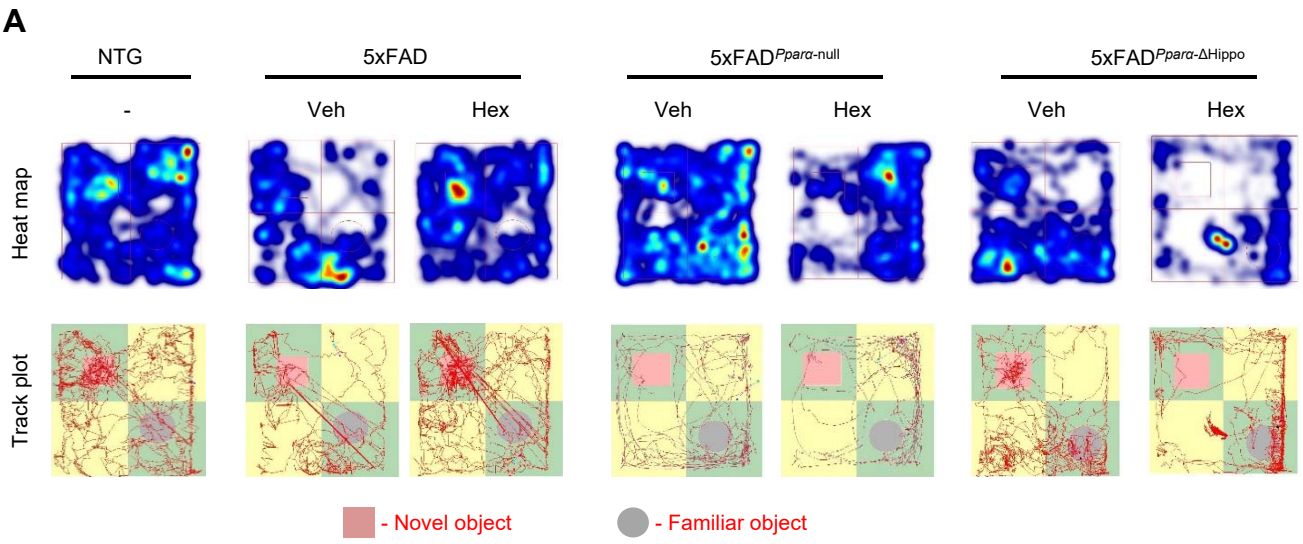

NTG
5xFAD
5xFAD Pparα-ΔHippo
1 2 1 2 1 2
APP
Internal control
377 bp
324 bp
PSEN1
Internal control
608 bp
324 bp
Internal control
Camk2a Cre
324 bp
100 bp
Pparα flox
Pparα WT
700 bp
545 bp

Figure S3.


A
NTG
5xFAD
5xFAD Ppara-null
5xFAD Ppara-ΔHippo
-
Veh
Hex
Veh
Hex
Veh
Hex
Heat map
Track plot
- Novel object
- Familiar object

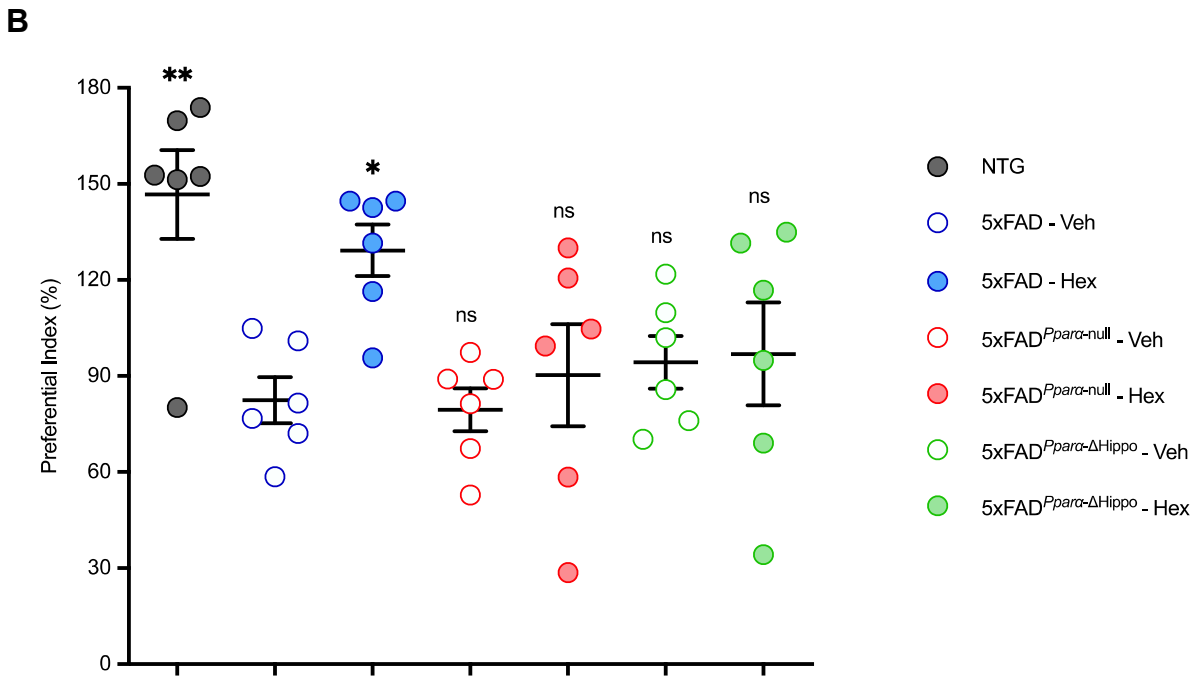

B
Preferential Index (%)
180
150
120
90
60
30
0
**
*
ns
ns
ns
NTG
5xFAD - Veh
5xFAD - Hex
5xFAD Ppara-null - Veh
5xFAD Ppara-null - Hex
5xFAD Ppara-ΔHippo - Veh
5xFAD Ppara-ΔHippo - Hex

Figure S4.

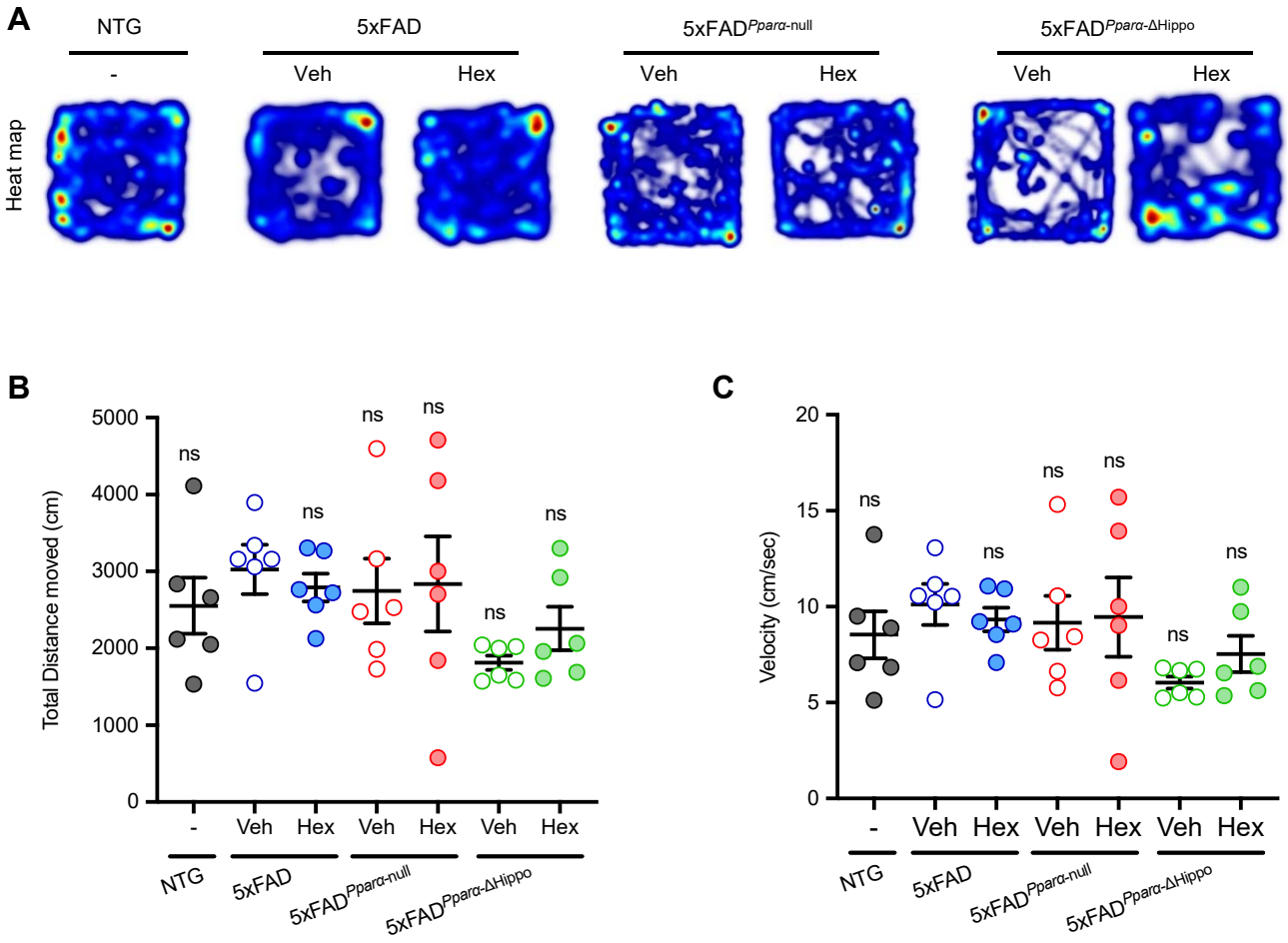

A
NTG
5xFAD
5xFAD Pparα-null
5xFAD Pparα-ΔHippo
-
Veh
Hex
Heat map
B
Total Distance moved (cm)
5000
4000
3000
2000
1000
0
ns
NTG
5xFAD
5xFAD Pparα-null
5xFAD Pparα-ΔHippo
C
Velocity (cm/sec)
20
15
10
5
0

Figure S5.

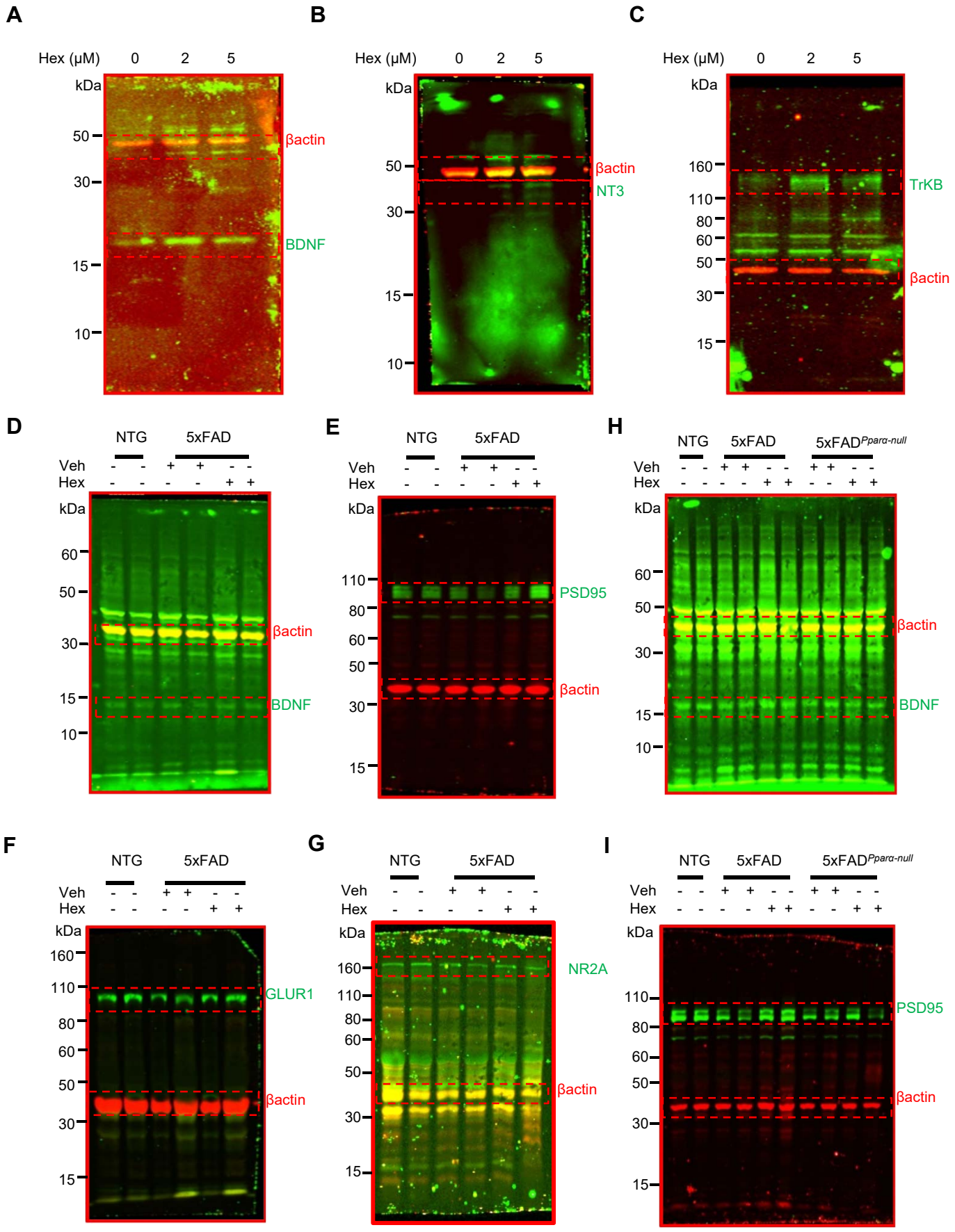

A
Hex (μM) 0 2 5
kDa
50
30
15
10
βactin
BDNF
B
Hex (μM) 0 2 5
kDa
50
30
15
10
βactin
NT3
C
Hex (μM) 0 2 5
kDa
160
110
80
60
50
30
15
TrKB
βactin
D
NTG
5xFAD
Veh - - + + - -
Hex - - - - + +
kDa
60
50
30
15
10
βactin
BDNF
E
NTG
5xFAD
Veh - - + + - -
Hex - - - - + +
kDa
110
80
60
50
30
15
PSD95
βactin
H
NTG
5xFAD
5xFAD Ppara-null
Veh - - + + - - + + - -
Hex - - - - + + - - + +
kDa
60
50
30
15
10
βactin
BDNF
F
NTG
5xFAD
Veh - - + + - -
Hex - - - - + +
kDa
160
110
80
60
50
30
15
GLUR1
βactin
G
NTG
5xFAD
Veh - - + + - -
Hex - - - - + +
kDa
160
110
80
60
50
30
15
NR2A
βactin
I
NTG
5xFAD
5xFAD Ppara-null
Veh - - + + - - + + - -
Hex - - - - + + - - + +
kDa
110
80
60
50
30
15
PSD95
βactin

Figure S6.