INVENTORY OF SUPPLEMENTAL INFORMATION

viii. Supplementary Table 2: F- and p-values regarding Sholl´s analysis of newborn granule neurons (Related to Figures 2 and 4).

ix. Supplementary Table 3: F- and p-values regarding the density of postsynaptic clusters (Related to Figures 3 and 4).

x. Supplementary Table 4: F- and p-values regarding area of postsynaptic clusters (Related to Figures 3 and 4).

SUPPLEMENTARY EXPERIMENTAL PROCEDURES

Experimental design

Supplementary Figure **S1** provides details of the experimental design. The experiments can be conceptually grouped as follows:

1) Retroviral labeling, and LPS and Ibuprofen treatments (Supplementary Figure **S1A**-**D**). This set of experiments sought to determine whether peripheral *E. coli lipopolysaccharide* (LPS) administration affects the morphology and connectivity of newborn neurons and to analyze the putative reversibility of these alterations by Ibuprofen treatment. For this purpose, we used 88 8-week-old female C57BL/6JRcc mice. Retroviruses were stereotaxically delivered to the hippocampus (timepoint 0), and osmotic pumps chronically delivering LPS (or PBS) were subcutaneously administered at various time points. To analyze morphology and mossy fiber terminals, we used GFP-expressing retroviruses, while for the number and size of postsynaptic clusters we used PSD95-GFP-expressing retroviruses. Four mice per experimental group (treatment and time point) were used. The following groups were analyzed:

    a. 2-week-old newborn neurons (16 mice) (Supplementary Figure **S1A**):
        i. PBS (2 Weeks);
        ii. LPS (2 Weeks);
        iii. PBS + Ibuprofen (2 Weeks)
        iv. LPS + Ibuprofen (2 Weeks).
    b. 4-week-old newborn neurons (32 mice) (Supplementary Figure **S1B**):
        i. PBS (4 Weeks);
        ii. LPS (4 Weeks);
        iii. PBS (2 Weeks) + 2 Weeks (after osmotic pump withdrawal)
        iv. LPS (2 Weeks) + 2 Weeks (after osmotic pump withdrawal)
        v. 2 Weeks (prior to osmotic pump implantation) + PBS (2 Weeks)
        vi. 2 Weeks (prior to osmotic pump implantation) + LPS (2 Weeks)
        vii. PBS + Ibuprofen (4 Weeks)
        viii. LPS + Ibuprofen (4 Weeks)

Since no differences were observed between the groups that received PBS treatments at different time points, all groups were plotted in the graphs together and labeled as PBS (4 Weeks).

c. 8-week-old newborn neurons (40 mice) (Supplementary Figure **S1C**):
    i. PBS (8 Weeks)
    ii. LPS (8 Weeks)
    iii. PBS (2 Weeks) + 6 Weeks (after osmotic pump withdrawal)
    iv. LPS (2 Weeks) + 6 Weeks (after osmotic pump withdrawal)
    v. 2 Weeks (prior to osmotic pump implantation) + PBS (2 Weeks) + 4 Weeks (after osmotic pump withdrawal)
    vi. 2 Weeks (prior to osmotic pump implantation) + LPS (2 Weeks) + 4 Weeks (after osmotic pump withdrawal)
    vii. 6 Weeks (prior to osmotic pump implantation) + PBS (2 Weeks)
    viii. 6 Weeks (prior to osmotic pump implantation) + LPS (2 Weeks)
    ix. PBS + Ibuprofen (8 Weeks)
    x. LPS + Ibuprofen (8 Weeks).

Since no differences were observed between the groups that received PBS treatments at different time points, all groups were plotted in the graphs together and labeled as PBS (8 Weeks).

2) Retroviral labeling, GSK-3β overexpression, and Ibuprofen treatment (16 mice) (Supplementary Figure **S1D**). The GSK-3β-overexpressing (GSK-3-OE) murine model of AD was used in order to determine whether an anti-inflammatory treatment with Ibuprofen ameliorates the cellular alterations previously described (Llorens-Martin et al., 2013). For this aim, 8-week-old GSK-3-OE mice and their wild-type littermates (WT mice) were stereotaxically injected with PSD95-GFP-expressing retroviruses and immediately subjected to an 8-week Ibuprofen treatment, after which the morphology and connectivity of newborn granule neurons were analyzed. The following groups were used: WT, GSK-3-OE, WT + Ibuprofen, and GSK-3-OE + Ibuprofen.

3) LPS and Ibuprofen treatment, behavioral tests, and biochemical analysis (48 mice) (Supplementary Figure **S1E**). The aim of this set of experiments was to determine the effects of a 2-week period of peripheral LPS administration on anxiety-like behavior and behavioral hippocampal-dependent pattern separation. Animals were evaluated after the 2-week treatment and after an additional 2-week recovery period. For this purpose, a group of 48 female mice aged 8 weeks were divided into the following groups:

    a. PBS (2 Weeks)
    b. LPS (2 Weeks)

c. PBS (2 Weeks) + 2 Weeks (after osmotic pump withdrawal)
d. LPS (2 Weeks) + 2 Weeks (after osmotic pump withdrawal)
e. (PBS + Ibuprofen) (2 Weeks);
f. (LPS + Ibuprofen) (2 Weeks).

LPS was administered via osmotic pumps. Eight mice per group were used for this set of experiments. After the animals had been sacrificed, hippocampi were used for biochemical determinations (Cytokine antibody array).

4) GSK-3-OE mice, Ibuprofen treatment, behavioral tests, and biochemical analysis (48 mice) (Supplementary Figure **S1F**). Eight-week-old GSK-3-OE mice and their WT littermates were subjected to a 4-week Ibuprofen treatment, and anxiety-like behavior and hippocampal-dependent pattern separation were evaluated. This duration was chosen because 4 weeks is the minimal time required for the repressor Doxycycline (Llorens-Martin et al., 2013) to switch off the transgene. The following groups were used: WT; GSK-3-OE; WT + Doxycycline; GSK-3-OE + Doxycycline; WT + Ibuprofen; and GSK-3-OE + Ibuprofen. Each group comprised 6 mice. After the mice had been sacrificed, hippocampi were used for biochemical determinations (Cytokine antibody array).

5) LPS and Ibuprofen treatments, and histological analysis (28 mice) (Supplementary Figure **S1E**). Mice were subjected to a 2-week peripheral LPS (or PBS) treatment. A histochemical analysis of the dentate gyrus (DG) was then performed. The following groups were used: PBS (2 Weeks); LPS (2 Weeks); (PBS + Ibuprofen) (2 Weeks); and (LPS + Ibuprofen) (2 Weeks). Each group comprised 7 mice.

6) GSK-3-OE mice, Ibuprofen treatment, and histological analysis (24 mice) (Supplementary Figure **S1F**). Eight-week-old GSK-3-OE mice and their WT littermates were subjected to a 4-week Ibuprofen treatment, after which a histochemical analysis of the DG was performed. The following groups were used: WT; GSK-3-OE; WT + Doxycycline; GSK-3-OE + Doxycycline; WT + Ibuprofen; and GSK-3-OE + Ibuprofen. Each group comprised 6 mice.

7) LPS treatment and electron microscopy (12 mice). In order to study the alterations induced by LPS on the general organization of the DG, as well as the putative alterations on the ultrastructural organization of the subgranular zone (SGZ), mice were subjected to either a 2- or 8-week peripheral LPS (or PBS) treatment. An ultrastructural analysis of the DG was

then performed. The following groups of mice were used: PBS (2 Weeks); LPS (2 Weeks); PBS (8 Weeks); and LPS (8 Weeks). Each group comprised 3 mice.

Stereotaxic surgery

Mice were anesthetized with Isoflouran and placed in a stereotaxic frame equipped with mouse adaptor for anesthesia. The scalp was incised and a skull hole was drilled. Coordinates (mm) relative to bregma in the anteroposterior, mediolateral, and dorsoventral planes were as follows: DG [-2.0, 1.4, 2.2]. 2 µl/DG of virus solution was infused at 0.2 µl/min via a glass micropipette, which was left in place for 5 additional min to ensure diffusion.

Sacrifice

Mice were fully anesthetized with an intraperitoneal pentobarbital injection (EutaLender, 60 mg/kg). Animals used for histology and those injected with retroviruses were transcardially perfused with saline followed by 4% paraformaldehyde in phosphate buffer. Brains were removed and post-fixed overnight in the same fixative. Animals used for biochemical analysis were perfused only with saline. Brains were removed and hippocampi were quickly dissected on ice and frozen in dry ice. Mice used for electron microscopy were perfused with saline followed by 4% paraformaldehyde + 1% glutaraldehyde in phosphate buffer. Brains were post-fixed overnight in the same fixative.

Immunohistochemistry

Sagittal brain sections were obtained on a Leica VT1200S vibratome (50-µm thick sections). For immunohistochemical analysis, series of brain slices were randomly made up of one section from every ninth. Slices were initially pre-incubated in phosphate buffer with 0.5% Triton X-100 and 0.5% bovine serum albumin, and then dual immunohistochemistry was performed as described previously (Llorens-Martin et al., 2013). The following primary antibodies were used: mouse anti-β-Galactosidase (Promega 1:3,000); rabbit anti-GFP (Invitrogen 1:1000); mouse anti-NeuN (Chemicon 1: 1,000); goat anti-Doublecortin (DCX) (Santa Cruz Biotechnologies 1: 500); rabbit anti-Iba1 (Wako 1: 500); and rabbit anti-fractin (BD Biosciences 1: 500). The following secondary Alexa-conjugated antibodies from Molecular Probes were also used at a final concentration of 1:1,000 to detect the primary antibodies: Donkey Alexa 555-conjugated anti-rabbit (GFP, fractin and Iba1); Donkey Alexa 488-conjugated anti-rabbit (GFP); Donkey Alexa 647-conjugated anti-mouse (β-Galactosidase and

NeuN); Donkey Alexa 633-conjugated anti-goat (DCX); and Donkey Alexa 555-conjugated anti-goat (DCX). All sections were counterstained with DAPI (Calbiochem, 1:10,000). The incubation period was 48 h at 4°C for primary antibodies, 24 h at 4°C for secondary antibodies, and 10 min for DAPI.

Volume estimation of the dentate gyrus

To measure the DG volume, we used a semi-automatic Cavalieri system (ImageJ v.1.47, NIH, USA, http://rsbweb.nih.gov/ij/) in 50-µm sections stained with Toluidine blue, as previously described (Llorens-Martin et al., 2013).

Cell counts

The total number of $Iba1^+$, $Fractin^+$, and $NeuN^+$ mature granule neurons was calculated under a LSM710 Zeiss confocal microscope (63x Oil immersion objective) using the physical dissector method adapted for confocal microscopy (Llorens-Martin et al., 2013).

Morphometric analysis

Three series of sections from each animal were used for the immunohistochemical detection of GFP. Sixty randomly selected neurons were reconstructed under a LSM710 Zeiss confocal microscope (25x Oil immersion objective). Confocal stacks of images were obtained (Z-axis interval: 1 µm), and z-projections were analyzed for the determination of total dendritic length and Sholl´s analysis. All cells were traced using the *NeuronJ* plugin for ImageJ software (ImageJ v.1.47, NIH, USA, http://rsbweb.nih.gov/ij/). Sholl´s analysis was performed using the plugin *ShollAnalysis* for ImageJ.

Number and size of PSD95-GFP^+ clusters

PSD95-GFP^+ clusters were examined in each dendritic tree branching order. A minimum of 30 segments belonging to each experimental condition were analyzed for each branching order. Confocal stacks of images were obtained in a LSM710 Zeiss confocal microscope (63x Oil immersion objective, xy dimensions: 67.4 µm) (Z-axis interval: 0.13 µm). Two-channel stack Z-projections were obtained. The dendritic length of each segment was measured (red channel, GFP), and the number and size (area) of PSD-GFP^+ clusters was

analyzed using the semi-automatic *Particle Analyzer* plugin for ImageJ (green channel). A minimum of 1,500 postsynaptic densities (PSDs) belonging to each experimental condition were examined for each branching order in the case of PSD area analysis.

Measurement of mossy fiber terminal area

The area of mossy fiber terminals (MFTs) was measured in the CA3 region. A minimum of 500 terminals per experimental condition were determined. Confocal stacks of images were obtained in a LSM710 Zeiss confocal microscope (63x Oil immersion objective, xy dimensions: 100 µm) (Z-axis interval: 0.13 µm). GFP Z-projections were obtained, and the area of each terminal button was measured manually in ImageJ, as previously described (Zhao et al., 2006).

Electron microscopy

After a post-fixation step, 200-µm sagittal sections were obtained on a Leica VT1200S vibratome. Four sections per mouse were post-fixed in 2% osmium tetroxide (OsO4) for 2 h. They were then rinsed, dehydrated, and embedded in Durcupan (Durcupan, Fluka). Serial semi-thin sections (1.5 µm) were cut with a diamond knife and stained with 1% Toluidine blue. Subsequently, the area of interest was trimmed, and ultrathin sections (0.06 µm) were obtained with a diamond knife. These sections were then stained with lead citrate and examined under a JEM1010 Jeol electron microscope equipped with a 4Kx4K TemCam-F416 Digital camera. In these sections, the number of proliferative clusters (number of clusters/SGZ length) and progenitor cells per cluster (astrocytes/Type B cells + Type D cells) was quantified, as previously described (Sirerol-Piquer et al., 2011). In addition, the ultrastructural organization of the proliferative clusters was examined, and the percentage of precursor cells located outside of the SGZ (in the hilus or more than 2 layers of cells inside the granule layer (GL)) was calculated. In addition, the presence of microglial cells within the clusters was determined and represented as number of microglial cells/cluster.

Human subjects

The use of human brain tissue samples was coordinated by local Brain Bank (Banco de Tejidos CIEN, Madrid), following national laws and international ethical and technical guidelines on the use of human samples for biomedical research purposes. Brain tissue

donation, processing, and use for research were in compliance with published protocols (Best Practices for Repositories (2012)), which included obtaining informed consent for brain tissue donation from living donors, and approval of the whole donation process by an ethical committee.

Behavioral tests

- Anxiety-like behavior: animals were tested in a 5-min single trial of the Elevated Plus Maze (EPM) (Cibertec, Madrid, Spain) paradigm (See Figure **6** for a schematic diagram), in which the mouse is allowed to move freely along the apparatus under a constant intense white light. Animal movement was recorded and subsequently analyzed. Data are presented as the total time spent standing or walking on the open arms (red). The criterion was the head + both forelimbs being placed on an open arm.

- Behavioral pattern separation: as previously described (Bekinschtein et al., 2013), the novel location preference (NLP) paradigm is highly sensitive to variations in hippocampal neurogenesis. Animals were tested in this paradigm in order to analyze neurogenesis-dependent behavioral pattern separation. The test was applied using a squared (45 x 45 cm), constantly illuminated, open-field methacrylate arena. The test was performed on 3 consecutive days, on which animals were subjected to a single 10-min trial (See Figure **6** for a schematic diagram). On the first day, they were placed inside the arena and allowed to explore it for habituation. During the second day (sample phase), two identical objects were placed symmetrically in the central part of the arena. On the third day (test phase), one of the objects (novel-located object) was moved to a peripheral position, while the other remained unaltered. Animal performance was recorded with a video camera and subsequently analyzed. The number of explorations and total time exploring each object were measured. Index memory (time exploring novel-located object/time exploring novel + unaltered object) is shown in the graphs. In addition, the total number of explorations is indicated.

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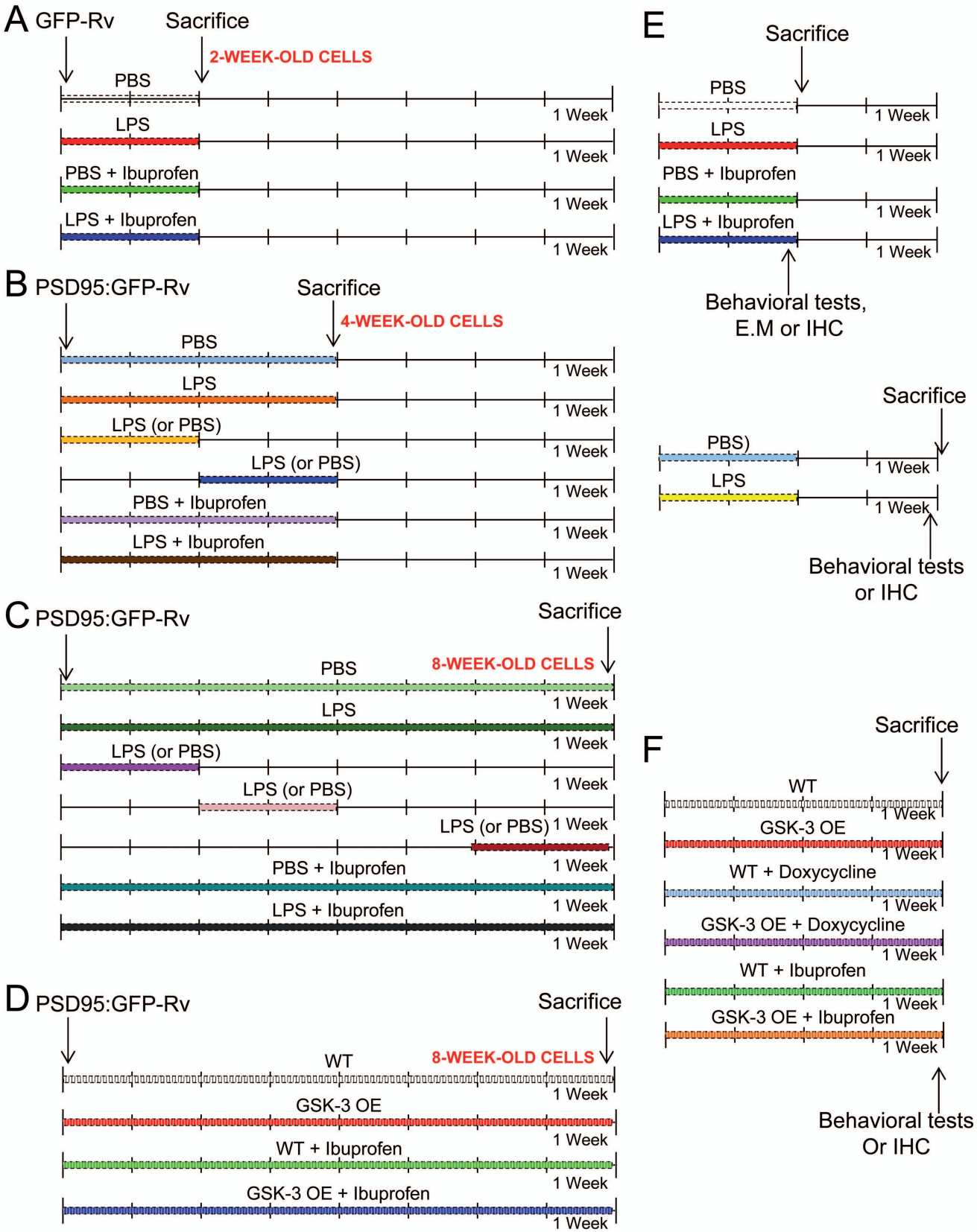
Sirerol-Piquer, M., Gomez-Ramos, P., Hernandez, F., Perez, M., Moran, M.A., Fuster-Matanzo, A., Lucas, J.J., Avila, J., and Garcia-Verdugo, J.M. (2011). GSK3beta overexpression induces neuronal death and a depletion of the neurogenic niches in the dentate gyrus. Hippocampus *21*, 910-922.

Zhao, C., Teng, E.M., Summers, R.G., Jr., Ming, G.L., and Gage, F.H. (2006). Distinct morphological stages of dentate granule neuron maturation in the adult mouse hippocampus. J Neurosci *26*, 3-11.

SUPPLEMENTARY FIGURES

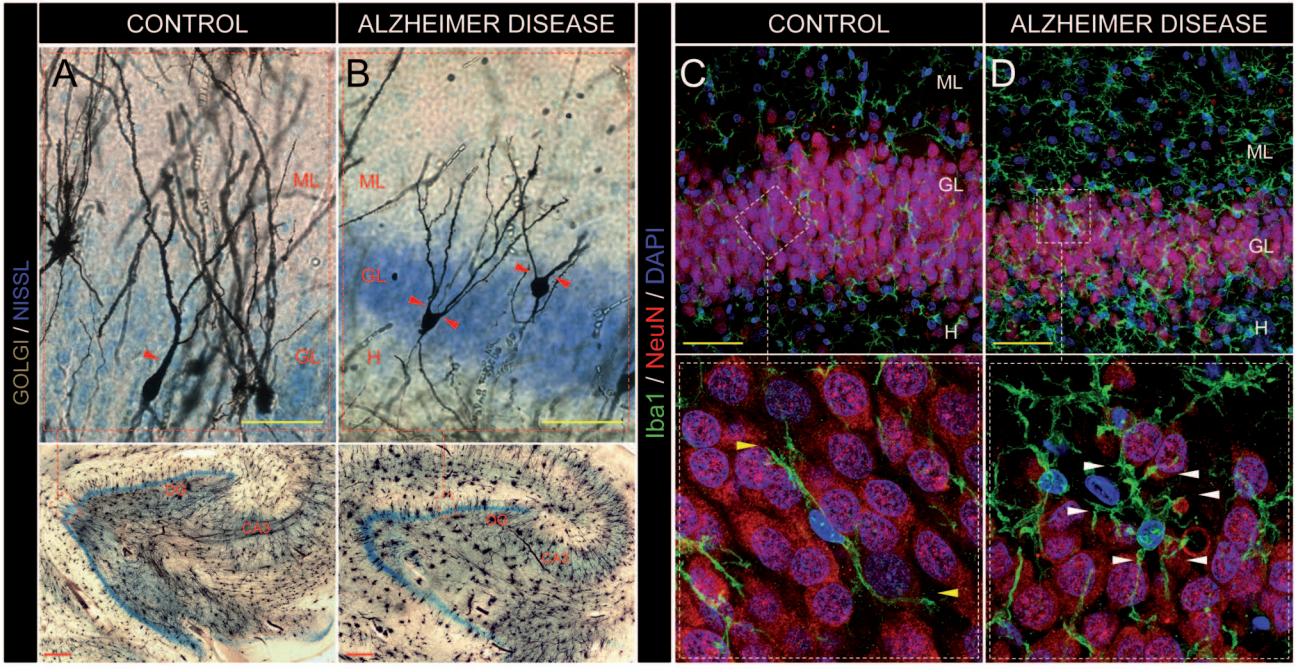
SUPPLEMENTARY FIGURE S1

Experimental design. A-C: Retroviral labeling + LPS treatment experiments. The aim of this set of experiments was to determine how peripheral *E. coli lypopolysacharide* (LPS) administration affects the morphology and connectivity of newborn neurons. GFP- or PSD95:GFP-expressing retroviruses were delivered into the hippocampus, and LPS was administered during different periods of time, following the experimental design shown in the figure. Mice were sacrificed 2, 4, or 8 weeks after retroviral injections, in order to analyze newborn neuron morphology at different cell ages, or after different treatments or recovery periods. **D**: Retroviral labeling + GSK-3β overexpression + Ibuprofen treatment. This murine model of Alzheimer disease was used to determine whether an anti-inflammatory treatment with Ibuprofen ameliorates the cellular alterations previously described [17]. For this aim, 8-week-old GSK-3-OE mice and their WT littermates were stereotaxically injected with PSD95-GFP-expressing retroviruses and immediately subjected to an 8-week Ibuprofen treatment. The morphology and connectivity of newborn granule neurons were then analyzed. **E-F**: Histological analysis + Electron microscopy + Behavioral tests + Biochemical quantifications. **E**: Mice were subjected to a 2-week peripheral LPS (or PBS) treatment in the presence or absence of Ibuprofen, and the respective *post-mortem* determinations were performed. F: 8-week-old GSK-3-OE mice and their WT littermates were subjected to a 4-week Ibuprofen treatment, after which the aforementioned measurements were taken.


A GFP-Rv
Sacrifice
2-WEEK-OLD CELLS
PBS
LPS
PBS + Ibuprofen
LPS + Ibuprofen
1 Week
B PSD95:GFP-Rv
Sacrifice
4-WEEK-OLD CELLS
PBS
LPS
LPS (or PBS)
LPS (or PBS)
PBS + Ibuprofen
LPS + Ibuprofen
1 Week
C PSD95:GFP-Rv
Sacrifice
8-WEEK-OLD CELLS
PBS
LPS
LPS (or PBS)
LPS (or PBS)
LPS (or PBS)
PBS + Ibuprofen
LPS + Ibuprofen
1 Week
D PSD95:GFP-Rv
Sacrifice
8-WEEK-OLD CELLS
WT
GSK-3 OE
WT + Ibuprofen
GSK-3 OE + Ibuprofen
1 Week
E
Sacrifice
PBS
LPS
PBS + Ibuprofen
LPS + Ibuprofen
1 Week
Behavioral tests,
E.M or IHC
Sacrifice
PBS)
LPS
1 Week
Behavioral tests
or IHC
F
Sacrifice
WT
GSK-3 OE
WT + Doxycycline
GSK-3 OE + Doxycycline
WT + Ibuprofen
GSK-3 OE + Ibuprofen
1 Week
Behavioral tests
Or IHC

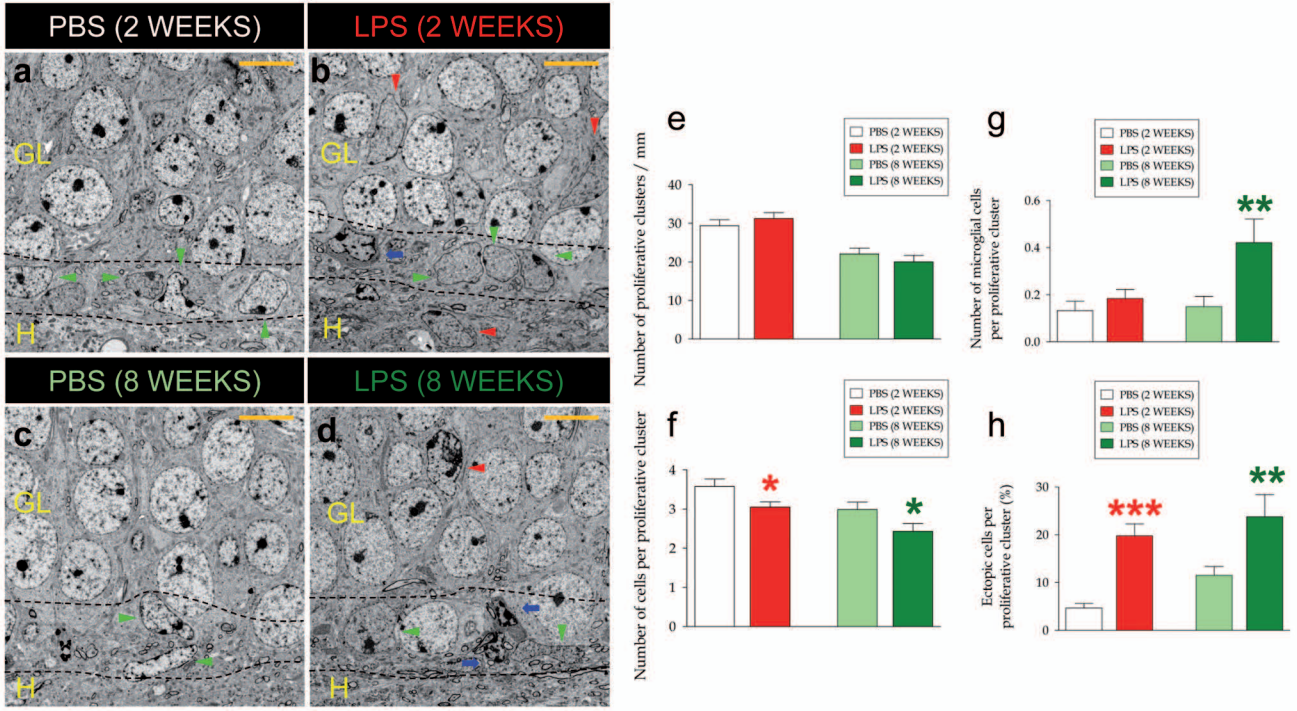
SUPPLEMENTARY FIGURE S2

Human Alzheimer disease (AD) hippocampus showing altered granule neuron morphology and increased number of Iba1$^+$ cells. A-B: Representative images showing Golgi-stained human dentate gyrus counterstained with Toluidine blue (Nissl staining) of a control (**A**) and AD patient (**B**) and their respective high magnification. Most of the cells display several apical primary dendrites in the AD patient (red triangles), in contrast to the single apical primary dendrite observed in the control patient. **C-D**: Representative images of microglial Iba1 staining in the human hippocampus of a control (**C**) and AD (**D**) patient and their respective high magnification, showing an increased number of Iba1$^+$ cells in the AD hippocampus as compared to that of the control patient. Note the abundance of thick and short branches, and amoeboid nuclear shape in the case of AD microglial cells, in contrast to the thin and sparse branching of control patient microglia. ML: Molecular layer. GL: Granule layer. H: Hilus. Yellow scale bar: 50 µm. Red scale bar: 100 µm. Red triangles: Primary apical dendrites of granule neuron. White triangles: Thick and short branches of AD microglial cells. Yellow triangles: thin and long branches in the case of control microglial cells.


CONTROL
ALZHEIMER DISEASE
CONTROL
ALZHEIMER DISEASE
A
B
C
D
ML
GL
H
DG
CA3
GOLGI / NISSL
Iba1 / NeuN / DAPI

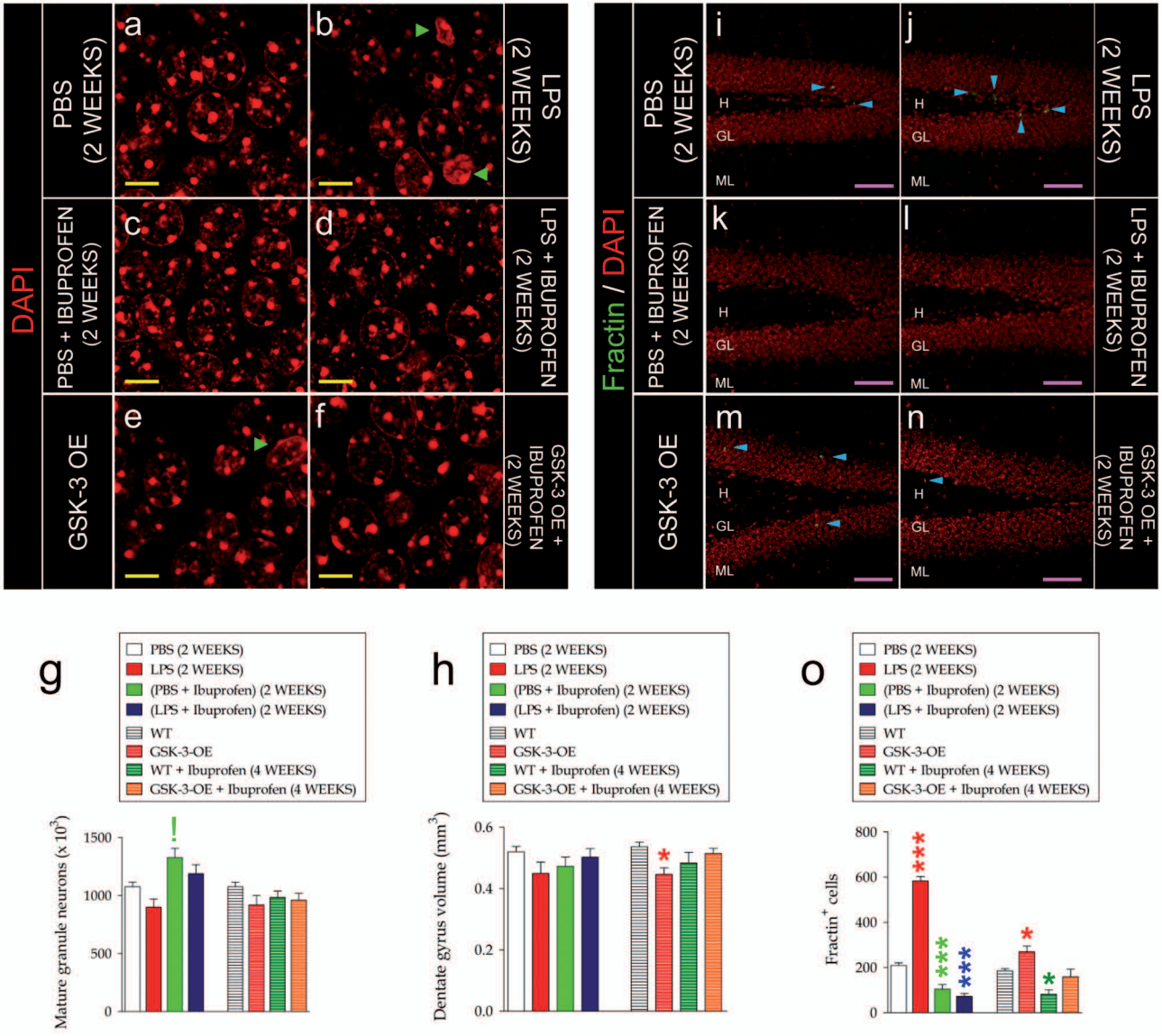
SUPPLEMENTARY FIGURE S3

LPS altered subgranular zone (SGZ) ultrastructure. In order to analyze the ultrastructural organization of the SGZ after LPS treatment, ultrathin sections were examined under an electron microscope. **A**-**D**: Representative images showing ultrathin sections of hippocampal SGZ belonging to different experimental conditions. **E**: Number of proliferative clusters. No changes in the number of proliferative clusters were observed after LPS treatment. **F**: Number of progenitor cells per proliferative cluster. The number of precursor cells per cluster showed a significant reduction after LPS treatment. **G**: Number of microglial cells within the proliferative cluster. LPS increased the presence of microglial cells in the proliferative clusters. **H**: Percentage of ectopic cells (located either in the hilus or more than 2 layers of cells inside the granule layer). LPS produced a drastic disorganization of the cluster, as reflected by the higher percentage of precursor cells located outside the SGZ. GL: Granule layer. H: Hilus. Yellow scale bar: 10 µm. Green triangles: Progenitor cells located within the SGZ (discontinuous black line). Red triangles: Ectopic progenitor cells. Blue Arrows: Microglial cells. (* $0.01 < p < 0.05$) (** $0.001 < p < 0.01$) (*** $p < 0.001$).


PBS (2 WEEKS)
LPS (2 WEEKS)
PBS (8 WEEKS)
LPS (8 WEEKS)
a
b
c
d
GL
H
e
f
g
h
PBS (2 WEEKS)
LPS (2 WEEKS)
PBS (8 WEEKS)
LPS (8 WEEKS)
Number of proliferative clusters / mm
Number of cells per proliferative cluster
Number of microglial cells per proliferative cluster
Ectopic cells per proliferative cluster (%)
*
**
***

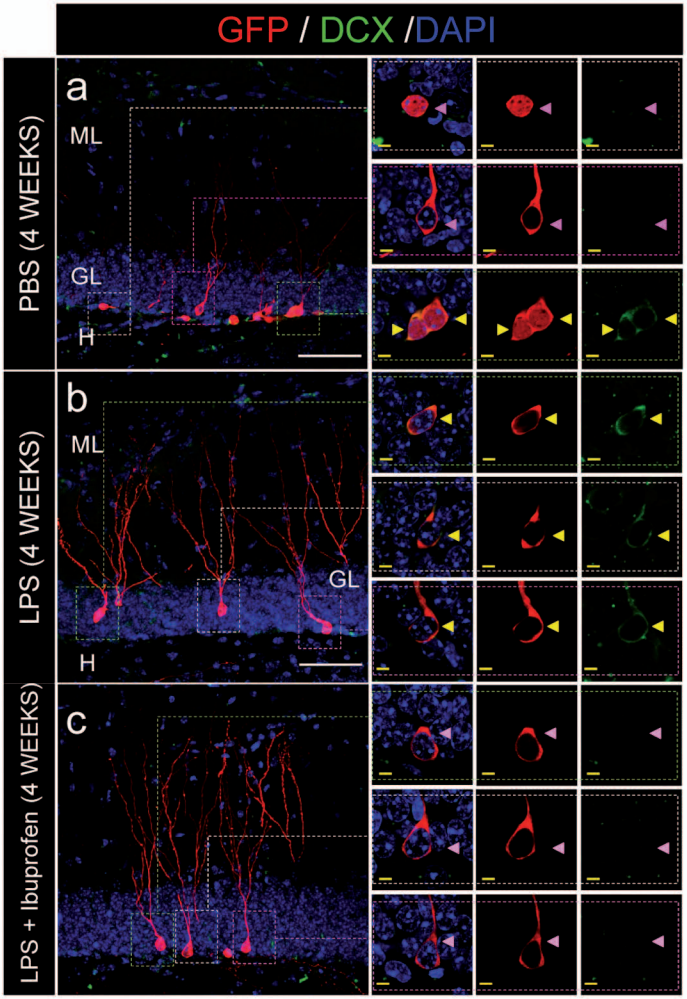
SUPPLEMENTARY FIGURE S4

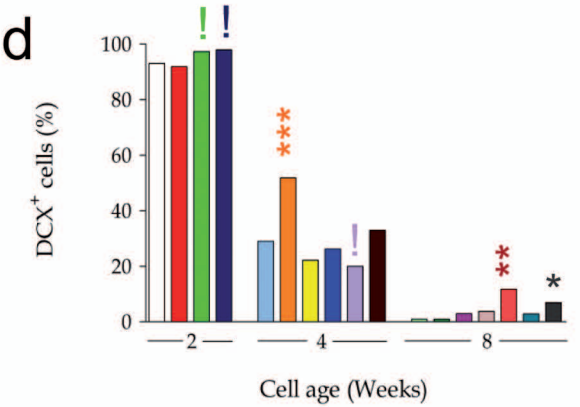
LPS administration and GSK-3-β overexpression produced apoptosis in the dentate gyrus. A-**F**: Representative images of mature granule neuron stained with DAPI. Note that both LPS administration and GSK-3β overexpression increased the presence of apoptotic and pyknotic nuclei (green triangles). **G**: Quantification of the total number of mature granule neurons. **H**: Determination of dentate gyrus volume. No significant changes in the total number of mature granule neurons (**G**) or the dentate gyrus volume (**H**) were observed after any of the treatments, although GSK-3 OE mice tended to show a decrease in the number of mature granule neurons and in dentate gyrus volume. **I**-**N**: Representative images of $Fractin^{+}$ apoptotic cells belonging to the different experimental conditions. **O**: Quantification of $Fractin^{+}$ cells. LPS administration for 2 weeks significantly increased the number of apoptotic $Fractin^{+}$ cells, a phenomenon also observed in GSK-3-OE mice. Both increases were fully normalized after Ibuprofen treatment, although this drug produced an additional decrease in apoptosis in LPS-treated mice; however, this decrease was not observed in GSK-3-OE mice. ML: Molecular layer. GL: Granule layer. H: Hilus. Yellow scale bar: 10 µm. Pink scale bar: 100 µm. Blue triangles: Apoptotic $Fractin^{+}$ cells. (* $0.01 < p < 0.05$) (*** $p < 0.001$) ($^{!}$ $0.05 < p < 0.1$).

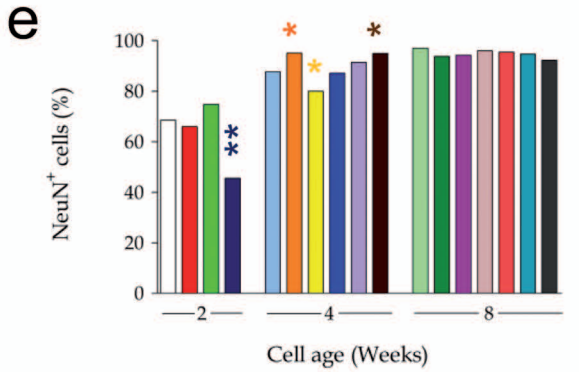

DAPI
PBS (2 WEEKS)
LPS (2 WEEKS)
PBS + IBUPROFEN (2 WEEKS)
LPS + IBUPROFEN (2 WEEKS)
GSK-3 OE
GSK-3 OE + IBUPROFEN (2 WEEKS)
a
b
c
d
e
f
Fractin / DAPI
H
GL
ML
i
j
k
l
m
n
g
h
o
PBS (2 WEEKS)
LPS (2 WEEKS)
(PBS + Ibuprofen) (2 WEEKS)
(LPS + Ibuprofen) (2 WEEKS)
WT
GSK-3-OE
WT + Ibuprofen (4 WEEKS)
GSK-3-OE + Ibuprofen (4 WEEKS)
Mature granule neurons (x 10^3)
Dentate gyrus volume (mm^3)
Fractin+ cells

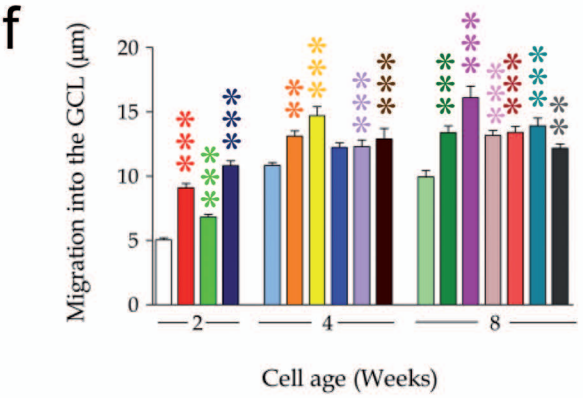
SUPPLEMENTARY FIGURE S5

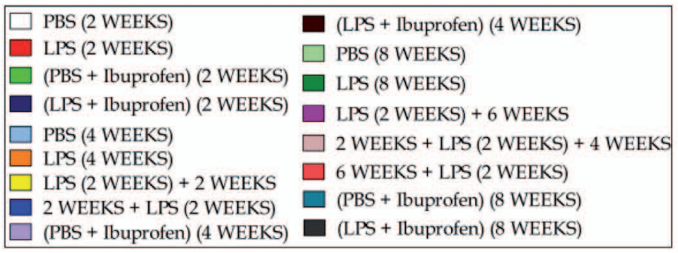
LPS altered newborn neuron maturation. A-C: Representative pictures showing 4-week-old retrovirus-labeled neurons belonging to different experimental conditions. **D**: Percentage of retrovirus-labeled cells of various ages that expressed the transient neuroblast marker Doublecortin (DCX). As shown, LPS administration produced a transient increase in the percentage of 4-week-old neurons that were DCX^+. This phenomenon was reversed after Ibuprofen treatment. **E**: Percentage of retrovirus-labeled cells of different ages that expressed the mature neuron marker NeuN. As can be observed, only slight variations in the percentage of $NeuN^+$ cells were observed in 2- and 4-week-old neurons, thereby suggesting the occurrence of changes in maturation timing without affecting the final maturation or neuronal lineage commitment processes. **F**: Cell migration into the Granule cell layer (GL). As shown, LPS administration, as well as Ibuprofen treatment, significantly increased newborn neuron migration into the GL. ML: Molecular layer. GL: Granule layer. H: Hilus. White scale bar: 50 µm. Yellow scale bar: 10 µm. Yellow triangles: DCX^+ cells. Pink triangles: DCX^- cells. (* $0.01 < p < 0.05$) (** $0.001 < p < 0.01$) (*** $p < 0.001$) (! $0.05 < p < 0.1$).


GFP / DCX /DAPI
PBS (4 WEEKS)
LPS (4 WEEKS)
LPS + Ibuprofen (4 WEEKS)
a
b
c
ML
GL
H


d
DCX+ cells (%)
Cell age (Weeks)
2
4
8

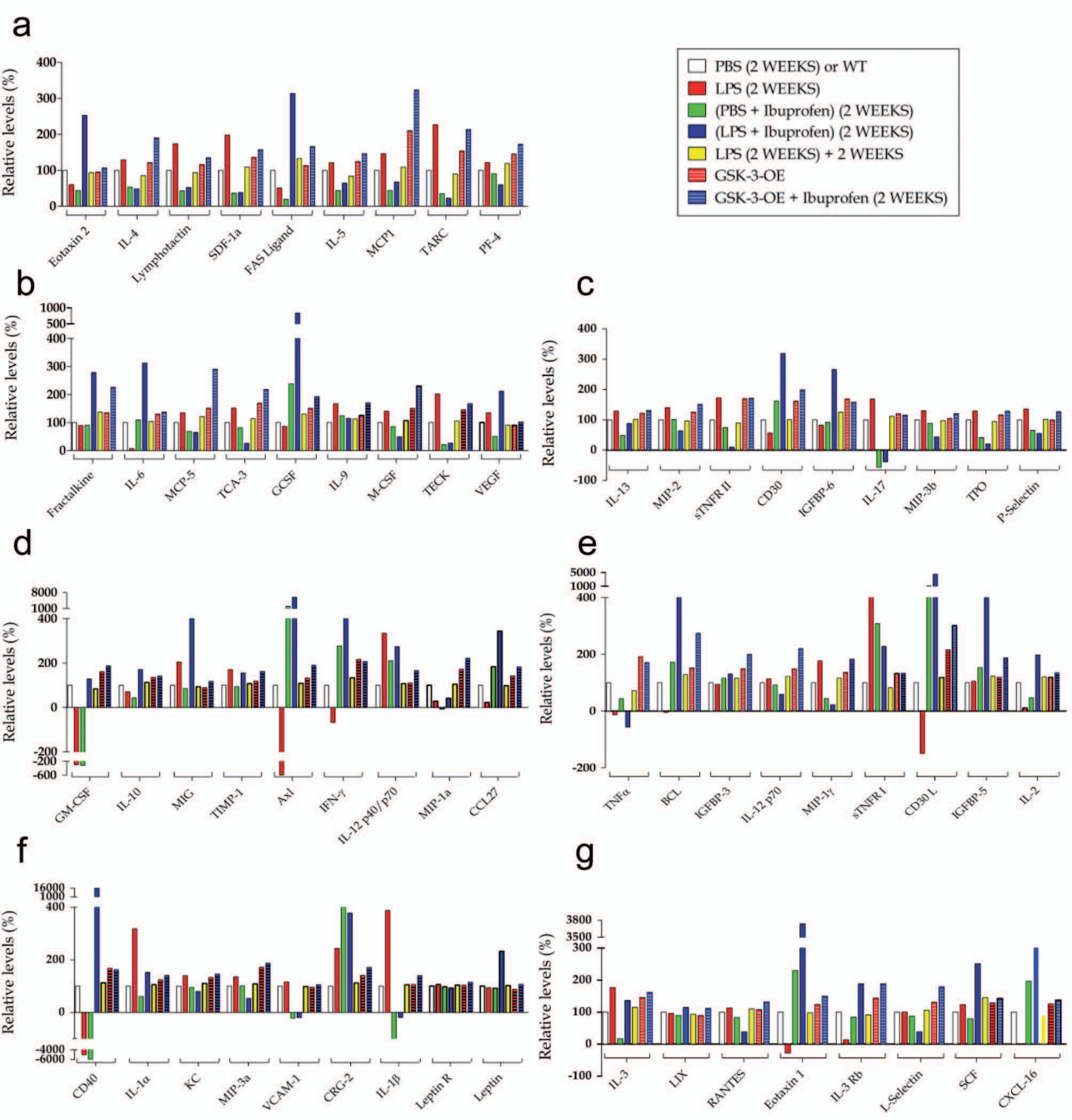

e
NeuN+ cells (%)
Cell age (Weeks)
2
4
8


f
Migration into the GCL (µm)
Cell age (Weeks)
2
4
8


PBS (2 WEEKS)
LPS (2 WEEKS)
(PBS + Ibuprofen) (2 WEEKS)
(LPS + Ibuprofen) (2 WEEKS)
PBS (4 WEEKS)
LPS (4 WEEKS)
LPS (2 WEEKS) + 2 WEEKS
2 WEEKS + LPS (2 WEEKS)
(PBS + Ibuprofen) (4 WEEKS)
(LPS + Ibuprofen) (4 WEEKS)
PBS (8 WEEKS)
LPS (8 WEEKS)
LPS (2 WEEKS) + 6 WEEKS
2 WEEKS + LPS (2 WEEKS) + 4 WEEKS
6 WEEKS + LPS (2 WEEKS)
(PBS + Ibuprofen) (8 WEEKS)
(LPS + Ibuprofen) (8 WEEKS)

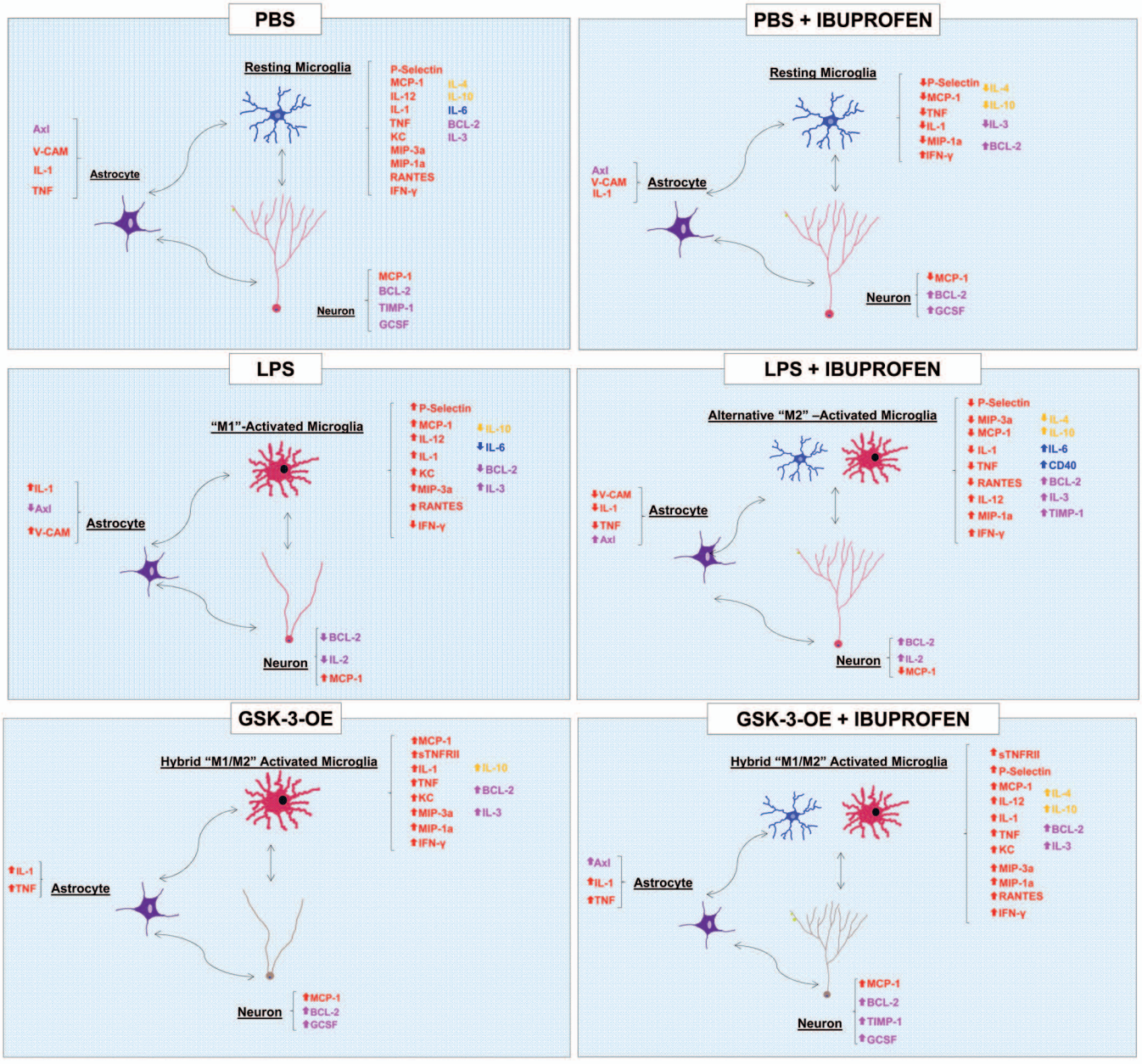
SUPPLEMENTARY FIGURE S6

Peripheral LPS administration and neuronal GSK-3-β overexpression produced different microglial activation phenotypes. A-G: Cytokine antibody array quantification. In general terms, LPS increased the levels of the most important pro-inflammatory cytokines (IL-1, TNF-α, RANTES, KC, among others), thus supporting the abundant literature reporting an M1 classical activation phenotype after LPS treatment. GSK-3-OE mice showed an increase not only in similar pro-inflammatory cytokines, but also in numerous pro-proliferative and pro-survival factors (BCL-2, IL-10, IGFI-BP-3), thereby suggesting the existence of a hybrid microglial activation phenotype in these mice. Ibuprofen produced a complete shift to the M2 neuroprotective phenotype in LPS-treated mice. However, no obvious changes in the microglial phenotype were observed in GSK-3-OE mice treated with Ibuprofen. Two weeks after LPS withdrawal, most of the cytokines reached normal levels. A more detailed description of the expression patterns of these cytokines is shown in Supplementary Figure **S7**. ML: Molecular layer. GL: Granule layer. H: Hilus. Pink scale bar: 100 μm. Blue scale bar: 50 μm. Red asterisks: activated microglial cells. White asterisks: resting microglial cells. (* $0.01 < p < 0.05$) (** $0.001 < p < 0.01$) (*** $p < 0.001$) ($^{!}$ $0.05 < p < 0.1$).


a
b
c
d
e
f
g
PBS (2 WEEKS) or WT
LPS (2 WEEKS)
(PBS + Ibuprofen) (2 WEEKS)
(LPS + Ibuprofen) (2 WEEKS)
LPS (2 WEEKS) + 2 WEEKS
GSK-3-OE
GSK-3-OE + Ibuprofen (2 WEEKS)
Relative levels (%)
Eotaxin 2
IL-4
Lymphotactin
SDF-1a
FAS Ligand
IL-5
MCP1
TARC
PF-4
Fractalkine
IL-6
MCP-5
TCA-3
GCSF
IL-9
M-CSF
TECK
VEGF
IL-13
MIP-2
sTNFR II
CD30
IGFBP-6
IL-17
MIP-3b
TPO
P-Selectin
GM-CSF
IL-10
MIG
TIMP-1
Axl
IFN-γ
IL-12 p40/p70
MIP-1a
CCL27
TNFα
BCL
IGFBP-3
IL-12 p70
MIP-1γ
sTNFR I
CD30 L
IGFBP-5
IL-2
CD40
IL-1α
KC
MIP-3a
VCAM-1
CRG-2
IL-1β
Leptin R
Leptin
IL-3
LIX
RANTES
Eotaxin 1
IL-3 Rb
L-Selectin
SCF
CXCL-16

SUPPLEMENTARY FIGURE S7

Proposed schematic model. Peripheral LPS treatment caused an increase in the expression of pro-inflammatory cytokines, including IL-1α/β, IFN-γ, RANTES, KC and MCP-1, among others. These mediators have been demonstrated to be crucial in eosinophil, basophil, and neutrophil recruitment by the endothelium and other components of the vascular system. In addition, pro-survival (such as Bcl-2 and Axl) and anti-inflammatory (IL-10 or IL-6) cytokines were also down-regulated. These data suggest a classical "M1" activation phenotype expressed by LPS-stimulated microglia. In contrast, GSK-3β overexpression increased the levels of several pro-inflammatory (IL-1α/β, TNF-α, IFN-γ, KC) but also pro-proliferative (IL-3R, Bcl-2, GCSF, M-CSF) and anti-inflammatory (IL-4, IL-10) cytokines, suggesting a hybrid activation phenotype. Importantly, Ibuprofen treatment promoted a clear shift to a "M2" alternative neuroprotective phenotype in LPS- and in PBS-treated mice, as shown by the increased levels of pro-survival molecules (BCL-2, Axl, G-CSF) and drastically reduced levels of pro-inflammatory factors (IL-1α/β, TNF-α, RANTES and MCP-1 among others). However, Ibuprofen did not cause such a clear shift in GSK-3-OE mice, as the levels of most of the pro-inflammatory cytokines remained increased. Nevertheless, the levels of pro-proliferative factors were also increased after Ibuprofen treatment, thus accounting for the neuroprotective effects exerted by this drug in this AD model. However, this finding indicates that the microglia/vascular/endothelial system of GSK-3-OE mice seems to be less "flexible" and sensitive to the neuroprotective effects of Ibuprofen.


PBS
Resting Microglia
P-Selectin
MCP-1
IL-12
IL-1
TNF
KC
MIP-3a
MIP-1a
RANTES
IFN-γ
IL-4
IL-10
IL-6
BCL-2
IL-3
Axl
V-CAM
IL-1
TNF
Astrocyte
Neuron
MCP-1
BCL-2
TIMP-1
GCSF
PBS + IBUPROFEN
Resting Microglia
↓P-Selectin
↓MCP-1
↓TNF
↓IL-1
↓MIP-1a
↑IFN-γ
↓IL-4
↓IL-10
↓IL-3
↑BCL-2
Axl
V-CAM
IL-1
Astrocyte
Neuron
↓MCP-1
↑BCL-2
↑GCSF
LPS
"M1"-Activated Microglia
↑P-Selectin
↑MCP-1
↑IL-12
↑IL-1
↑KC
↑MIP-3a
↑RANTES
↓IFN-γ
↓IL-10
↓IL-6
↓BCL-2
↑IL-3
↑IL-1
↓Axl
↑V-CAM
Astrocyte
Neuron
↓BCL-2
↓IL-2
↑MCP-1
LPS + IBUPROFEN
Alternative "M2" –Activated Microglia
↓P-Selectin
↓MIP-3a
↓MCP-1
↓IL-1
↓TNF
↓RANTES
↑IL-12
↑MIP-1a
↑IFN-γ
↓IL-4
↑IL-10
↑IL-6
↑CD40
↑BCL-2
↑IL-3
↑TIMP-1
↓V-CAM
↓IL-1
↓TNF
↑Axl
Astrocyte
Neuron
↑BCL-2
↑IL-2
↓MCP-1
GSK-3-OE
Hybrid "M1/M2" Activated Microglia
↑MCP-1
↑sTNFRII
↑IL-1
↑TNF
↑KC
↑MIP-3a
↑MIP-1a
↑IFN-γ
↑IL-10
↑BCL-2
↑IL-3
↑IL-1
↑TNF
Astrocyte
Neuron
↑MCP-1
↑BCL-2
↑GCSF
GSK-3-OE + IBUPROFEN
Hybrid "M1/M2" Activated Microglia
↑sTNFRII
↑P-Selectin
↑MCP-1
↑IL-12
↑IL-1
↑TNF
↑KC
↑MIP-3a
↑MIP-1a
↑RANTES
↑IFN-γ
↑IL-4
↑IL-10
↑BCL-2
↑IL-3
↑Axl
↑IL-1
↑TNF
Astrocyte
Neuron
↑MCP-1
↑BCL-2
↑TIMP-1
↑GCSF

LEGENDS TO SUPPLEMENTARY TABLES

Supplementary Table T1: Morphometric analysis of retrovirus-labeled cells. Statistic F-, χ^2- and p-values for individual comparisons (Total dendritic length, Primary apical dendrite length, Migration into the granule layer (GL), Percentage of cells with more than one primary apical dendrite).

Supplementary Table T2: Sholl´s analysis. F- and p-values for individual comparisons.

Supplementary Table T3: Density of postsynaptic clusters. F- and p-values for individual comparisons.

Supplementary Table T4: Area of postsynaptic clusters. F- and p-values for individual comparisons.

Supplementary Table 1: Morphometrical analysis of retrovirally-labeled cells.

		Total dendritic length length (µm)		Primary apical dendrite length (µm)		Migration into the GL (µm)		Cells with more than one primary apical dendrite (%)	
		$F_{(df,n)}$	p-value	$F_{(df,n)}$	p-value	$F_{(df,n)}$	p-value	$X^2_{(df,n)}$	Pearson
2 Weeks	**ANOVA (Global)**	$F_{(3,204)} = 30.84$	< 0.001	$F_{(3,276)} = 34.931$	< 0.001	$F_{(3,320)} = 78.693$	< 0.001	$X^2_{(3,340)} = 94.22$	< 0.001
	LPS (2 Weeks)		< 0.001		0.007		< 0.001		< 0.001
	PBS + Ibuprofen (2 Weeks)		0.117		< 0.001		< 0.001		0.562
	LPS + Ibuprofen (2 Weeks)		0.533		< 0.001		< 0.001		0.622
4 Weeks	**ANOVA (Global)**	$F_{(5,360)} = 8.075$	< 0.001	$F_{(5,614)} = 18.843$	< 0.001	$F_{(5,681)} = 12.93$	< 0.001	$X^2_{(5,1307)} = 52.3$	< 0.001
	LPS (4 Weeks)		0.001		< 0.001		0.001		< 0.001
	LPS (2 Weeks) + 2 Weeks		0.035		< 0.001		< 0.001		0.316
	2 Weeks + LPS (2 Weeks)		0.042		< 0.001		0.125		< 0.001
	PBS + Ibuprofen (4 Weeks)		0.801		0.374		< 0.001		0.996
	LPS + Ibuprofen (4 Weeks)		0.637		0.099		0.024		0.992
8 Weeks	**ANOVA (Global)**	$F_{(6,303)} = 20.601$	0.001	$F_{(6,541)} = 21.179$	< 0.001	$F_{(6,549)} = 9.55$	< 0.001	$X^2_{(6,579)} = 33.47$	< 0.001
	LPS (8 Weeks)		0.001		< 0.001		< 0.001		0.002
	LPS (2 Weeks) + 6 Weeks		0.021		< 0.001		< 0.001		0.383
	2 Weeks + LPS (2 Weeks) + 4 Weeks		0.955		0.003		< 0.001		0.572
	2 Weeks + LPS (2 Weeks)		0.662		< 0.001		< 0.001		0.589
	PBS + Ibuprofen (8 Weeks)		0.110		0.997		< 0.001		0.223
	LPS + Ibuprofen (8 Weeks)		< 0.001		0.994		0.002		0.404
GSK-3 OE	**ANOVA (Global)**	$F_{(3,180)} = 6.983$	< 0.001	$F_{(3,303)} = 49.415$	< 0.001	$F_{(3,294)} = 26.99$	< 0.001	$X^2_{(3,355)} = 34.34$	< 0.001
	GSK-3 OE		0.997		< 0.001		< 0.001		< 0.001
	WT + Ibuprofen (8 Weeks)		< 0.001		< 0.001		< 0.001		0.680
	GSK-3 OE + Ibuprofen (8 Weeks)		0.014		0.312		< 0.001		0.291

Supplementary Table 2: Sholl´s analysis

		Branching (0 – 50 µm)		Branching (50 – 100 µm)		Branching (100 – 150 µm)		Branching (150 – 200 µm)		Branching (150 – 200 µm)	
		$F_{(df,n)}$	p-value	$F_{(df,n)}$	p-value	$F_{(df,n)}$	p-value	$F_{(df,n)}$	p-value	$F_{(df,n)}$	p-value
2 Weeks	**ANOVA (Global)**	$F_{(3,204)} = 16.246$	< 0.001	$F_{(3,219)} = 39.06$	< 0.001	$F_{(3,208)} = 27.6$	< 0.001	$F_{(3,215)} = 14.3$	< 0.001	--	--
	LPS (2 Weeks)		< 0.001		< 0.001		< 0.001		< 0.001		--
	PBS + Ibuprofen (2 Weeks)		0.06		0.034		< 0.001		< 0.001		--
	LPS + Ibuprofen (2 Weeks)		0.098		0.002		< 0.001		< 0.001		--
4 Weeks	**ANOVA (Global)**	$F_{(5,362)} = 9.314$	< 0.001	$F_{(5,360)} = 4.452$	0.001	$F_{(5,363)} = 4.12$	0.001	$F_{(5,365)} = 8.43$	< 0.001	$F_{(5,353)} = 9.65$	< 0.001
	LPS (4 Weeks)		< 0.001		0.072		0.004		< 0.001		< 0.001
	LPS (2 Weeks) + 2 Weeks		0.114		0.997		1.000		0.115		< 0.001
	2 Weeks + LPS (2 Weeks)		0.001		0.003		0.632		0.005		< 0.001
	PBS + Ibuprofen (4 Weeks)		0.305		0.247		0.693		0.998		0.203
	LPS + Ibuprofen (4 Weeks)		0.260		0.084		1.000		0.999		0.660
8 Weeks	**ANOVA (Global)**	$F_{(6,300)} = 15.084$	< 0.001	$F_{(6,308)} = 6.769$	< 0.001	$F_{(6,307)} = 7.20$	< 0.001	$F_{(6,301)} = 26.1$	< 0.001	$F_{(6,285)} = 34.1$	< 0.001
	LPS (8 Weeks)		< 0.001		0.028		0.011		< 0.001		< 0.001
	LPS (2 Weeks) + 6 Weeks		0.002		0.021		0.995		< 0.001		< 0.001
	2 Weeks + LPS (2 Weeks) + 4 Weeks		< 0.001		< 0.001		0.410		0.983		0.003
	2 Weeks + LPS (2 Weeks)		0.152		0.525		1.000		0.381		< 0.001
	PBS + Ibuprofen (8 Weeks)		0.700		0.964		0.469		0.076		0.08
	LPS + Ibuprofen (8 Weeks)		0.992		0.993		0.031		0.126		< 0.001
GSK-3 OE	**ANOVA (Global)**	$F_{(3,184)} = 49.361$	< 0.001	$F_{(3,186)} = 10.493$	< 0.001	$F_{(3,190)} = 6.07$	0.001	$F_{(3,187)} = 13.1$	< 0.001	$F_{(3,168)} = 37.9$	< 0.001
	GSK-3 OE		< 0.001		0.002		0.083		0.017		< 0.001
	WT + Ibuprofen (8 Weeks)		0.024		0.028		0.097		0.01		< 0.001
	GSK-3 OE + Ibuprofen (8 Weeks)		0.219		0.257		0.039		0.034		< 0.001

Supplementary Table 3: Density of Postsynaptic Clusters

		2nd branching order		3rd branching order		4th branching order		5th branching order	
		F (df,n)	p-value	F (df,n)	p-value	F (df,n)	p-value	F (df,n)	p-value
4 Weeks	**ANOVA (Global)**	$F_{(5,163)} = 2.075$	0.071	$F_{(5,174)} = 5.39$	< 0.001	$F_{(5,168)} = 2.84$	0.017	$F_{(5,155)} = 2.576$	0.029
	LPS (4 Weeks)		0.790		0.045		0.042		0.885
	LPS (2 Weeks) + 2 Weeks		0.850		< 0.001		0.654		0.999
	2 Weeks + LPS (2 Weeks)		0.817		0.309		0.096		1.000
	PBS + Ibuprofen (4 Weeks)		0.202		0.054		0.843		0.938
	LPS + Ibuprofen (4 Weeks)		0.946		0.664		1.000		0.082
8 Weeks	**ANOVA (Global)**	$F_{(6,169)} = 4.787$	< 0.001	$F_{(6,179)} = 4.7$	< 0.001	$F_{(6,175)} = 2.55$	0.022	$F_{(6,175)} = 1.471$	0.191
	LPS (8 Weeks)		0.491		0.039		0.029		0.835
	LPS (2 Weeks) + 6 Weeks		0.459		0.005		1.000		1.000
	2 Weeks + LPS (2 Weeks) + 4 Weeks		0.685		0.218		0.997		1.000
	2 Weeks + LPS (2 Weeks)		0.887		1.000		0.89		0.99
	PBS + Ibuprofen (8 Weeks)		0.529		0.089		0.059		0.307
	LPS + Ibuprofen (8 Weeks)		0.691		0.49		0.68		0.848
GSK-3 OE	**ANOVA (Global)**	$F_{(3,102)} = 6.479$	< 0.001	$F_{(3,135)} = 12.3$	< 0.001	$F_{(3,99)} = 16.2$	< 0.001	$F_{(3,113)} = 21.68$	< 0.001
	GSK-3 OE		0.045		0.024		0.019		0.815
	WT + Ibuprofen (8 Weeks)		0.186		0.155		0.091		0.195
	GSK-3 OE + Ibuprofen (8 Weeks)		0.017		< 0.001		< 0.001		< 0.001

Supplementary Table 4: Area of postsynaptic clusters

		2nd branching order		3rd branching order		4th branching order		5th branching order	
		F (df,n)	p-value	F (df,n)	p-value	F (df,n)	p-value	F (df,n)	p-value
4 Weeks	**ANOVA (Global)**	$F_{(5,2063)} = 5.877$	< 0.001	$F_{(5,5484)} = 3.3$	0.005	$F_{(5,6407)} = 3.5$	0.004	$F_{(5,8000)} = 3.695$	0.002
	LPS (4 Weeks)		1.000		0.982		0.948		0.152
	LPS (2 Weeks) + 2 Weeks		0.999		0.989		0.808		0.064
	2 Weeks + LPS (2 Weeks)		0.742		0.96		0.909		1.000
	PBS + Ibuprofen (4 Weeks)		< 0.001		0.782		1.000		0.141
	LPS + Ibuprofen (4 Weeks)		0.922		0.04		0.01		0.024
8 Weeks	**ANOVA (Global)**	$F_{(3,3847)} = 8.196$	< 0.001	$F_{(6,6382)} = 2.7$	0.015	$F_{(6,6718)} = 9.7$	< 0.001	$F_{(6,11471)} = 12.8$	< 0.001
	LPS (8 Weeks)		0.758		0.925		0.364		0.003
	LPS (2 Weeks) + 6 Weeks		0.104		0.921		< 0.001		< 0.001
	2 Weeks + LPS (2 Weeks) + 4 Weeks		0.036		0.957		< 0.001		< 0.001
	2 Weeks + LPS (2 Weeks)		0.039		0.188		< 0.001		< 0.001
	PBS + Ibuprofen (8 Weeks)		0.875		0.999		< 0.001		< 0.001
	LPS + Ibuprofen (8 Weeks)		0.997		0.89		0.027		0.024
GSK-3 OE	**ANOVA (Global)**	$F_{(3,1858)} = 19.872$	< 0.001	$F_{(3,4626)} = 5.6$	0.001	$F_{(3,5231)} = 27$	< 0.001	$F_{(3,7105)} = 37.01$	< 0.001
	GSK-3 OE		0.135		0.091		0.497		0.036
	WT + Ibuprofen (8 Weeks)		0.039		0.146		< 0.001		0.305
	GSK-3 OE + Ibuprofen (8 Weeks)		< 0.001		0.004		< 0.001		< 0.001