**Supplementary figure legends**

**Suppl. Fig. 1. Transgene insertion, LRRK2 overexpression and parkinsonian pathophysiology in LRRK2 mice.** (A) FISH analysis showing transgene insertion in R1441G(135Cjli/J), R1441G(3IMjff/J), G2019S(340Djmo/J) and G2019S(2AMjff/J) mice. (B) Representative western blots of LRRK2 protein expression in various tissue from adult transgenic mice overexpressing human mutant LRRK2 (indicated as "Tg") and background matched non-transgenic littermates (indicated as "WT"). (C) Number of TH-positive DA neurons in the SNpc from different strains of two years old transgenic mice overexpressing human mutant LRRK2 and their non-transgenic littermates. Data are mean ± SEM, n=3-8 for each genotype. (D) Number of activated Iba-1-positive microglial cells in the SNpc from different strains of two years old transgenic mice overexpressing human mutant LRRK2 and their non-transgenic littermates. Data are mean ± SEM, n=3-5 for each genotype. (E) Number of activated Iba-1-positive microglial cells in the SNpc of FVB/NJ and C57Bl/6 mice in norm and following systemic LPS injection. (F) LRRK2 expression in the tissues used in this study (SN, striatum and PBMCs) from transgenic R1441G and G2019S strains used for the LPS experiments and their non-transgenic littermates. Data are mean ± SEM, n=4-9 for SN and STR, n=3 for PBMCs for each genotype. *$p<0.05$ vs. background matched non-transgenic littermate controls.

**Suppl. Fig. 2. Systemic inflammation causes neuronal loss in the SNpc of mutant G2019S mice.** (A) Number of TH-positive DA neurons in the SNpc of FVB and C57BL/6 mice used as strain-matched WT controls for transgenic R1441G and G2019S strains, respectively. Data are

mean ± SEM, n=5. (B) Number of activated Iba-1-positive microglial cells in the SNpc of FVB and C57BL/6 mice. Data are mean ± SEM, n=5. *p<0.05 vs FVB. (C) Loss of DA neurons in the SNpc of G2019S mice up to 7 months after single systemic LPS injection. Data are mean ± SEM, n=5 for NaCl group, n=4-5 for each post-LPS group. *p<0.05, **p<0.005, ***p<0.001, ****p<0.0001. (D) Representative images of TH-positive DA neurons in the SNpc of WT and G2019S mice after LPS challenge. Note the significant reduction of TH-positive processes in the SN pars reticulata (SNpr) of G2019S mice 2 months to 7 months post-LPS. Sections are matched at the same level of the SNpc (Bregma -3.08 - 3.16 mm) (Paxinos and Franklin, 2004). Scale bar, 100 μm. (E) Representative western blots of LRRK2 protein expression in various tissue from adult transgenic mice overexpressing human WT LRRK2 (WT-OX) (indicated as "OX") and background matched non-transgenic littermates (indicated as "WT"). (F) LRRK2 expression in the SN, striatum and PBMC of WT-OX and background matched non-transgenic littermates (WT). (G) Number of activated Iba-1-positive microglial cells in the SNpc of R1441G, WT-OX and background- and aged-matched WT mice 7 days after systemic LPS injection. Data are mean ± SEM, n=4-5. *p<0.05 vs NaCl control. (H) Number of TH-positive DA neurons in the SNpc of WT-OX and background matched non-transgenic WT mice 7 days after systemic LPS injection. Data are mean ± SEM, n=4-8 for NaCl group, n=3-5 for LPS 7 days group.

**Suppl. Fig. 3. T cells do not infiltrate the inflamed CNS of mutant LRRK2 mice.** (A) No CD3-positive cells were found by IHC in the brain of R1441G mice 24 hours – 7 months post-LPS. Insets show spleen section that was used as a positive control for CD3 staining. (B) Flow cytometric analysis of a single-cell suspension prepared from the whole brain of R1441G mice

also demonstrated the absence of infiltrated T cells in the post-LPS brains. Scale bar in A, 100 μm. Scale bar in A insets, 10 μm (spleen) 20 μm (brain).

**Suppl. Fig. 4. FACS analysis of a single-cell brain suspension.** (A, B) Representative plots of FACS of cells sorted from NaCl- (control) and LPS-treated brains. Cells were sorted based on Thy1 (Thy1.1-FITC, Thy1.2-FITC), GLAST (GLAST-APC) and O4 (O4-PE) fluorescent intensity. Positive and negative gates were drawn based on the intensity of an unstained control sample and isotype bone marrow control. (C) Viability of sorted cells based on DAPI staining. Data are mean ± SEM, n=3. (D) Validation of sorted cells using cell-type-specific protein expression. Protein extract from the whole brain tissue was used as a positive control. M, Mw marker.

**Suppl. Fig. 5. Analysis of peripheral immune response.** (A, B) Gating strategy for FACS analysis in whole blood from NaCl- (A) and LPS-treated (B) R1441G mice. After eliminating debris/dead cells and doublet cells by forward- and side-scatter, cells were gated based on CD4 (PerCP-Cy5.5), CD8 (PE-Cy7), CD19 (APC-Cy7), CD25 (APC), CD49 (PE), Mac-1 (Alexa700) and Gr-1 (PerCP) fluorescent intensity. Positive and negative gates were drawn based on the intensity of an unstained control sample and isotype bone marrow control, (C) The number of peripheral leukocytes sub-populations in mutant R1441G mice and background matched non-transgenic littermate controls (WT) following systemic LPS injection. The final data are represented as the percentage of each sub-population to the total number of cells analyzed. Data are mean ± SEM; n=20 for NaCl group; n=10-15 for each post-LPS group. *$p<0.05$, **$p<0.005$, ***$p<0.001$, ****$p<0.0001$ vs. NaCl group. (D) LPS-induced activation of pathways related to

B-cells function is exacerbated by mutant LRRK2. GSEA plots and Heat maps show up-regulation of B-cells related genes (GSE12366; GSE22886) in R1441G leukocytes isolated from LPS-treated R1441G mice compared to leukocytes from LPS-treated WT mice. Each bar at the bottom of GSEA panel represents a member gene/protein of the respective pathway. For each GSEA, the nominal p value and normalized enrichment score (NES) are shown.

**Supplementary Table Legend**

**Suppl. Table 1**. Differentially expressed proteins that satisfy $\log_2(FC) \geq 0.37$ and $p \leq 0.01$ in the brain of LPS treated R1441G mice compared to WT controls. Proteins that satisfy cutoff $\log_2(FC) \geq 0.22$ and $p \leq 0.01$ are presented in grey font. Based on the threshold analysis described in (Zhou *et al.*, 2010), we found that 0.37 cutoff results in 4% FDR and 0.22 cutoff results in 6% FDR.

**Supplementary Materials and Methods**

**Animals and LPS administration**

Two, three, 12 and 24 months old FVB/NJ, C57BL/6J, FVB/N-Tg(LRRK2*R1441G)135Cjli/J, FVB/N-Tg(LRRK2)1Cjli/J (WT-OX), B6;C3-Tg(PDGFB-LRRK2*G2019S)340Djmo/J, C57BL/6J-Tg(LRRK2*R1441G)3IMjff/J and C57BL/6J-Tg(LRRK2*G2019S)2AMjff/J mice (all Jackson Laboratory, Bar Harbor, ME) were used in this study. Mice were crossed to either FVB/NJ or C57Bl/6 mice to obtain heterozygous mutant mice. At one month, all littermates were genotyped. Genotype was determined by PCR of tail DNA using a protocol from Jackson Laboratory. In all experiments, age- and background strain-matched littermate mice were used as WT controls for transgenic strains. Specifically, FVB mice were used as controls for R1441G LRRK2 mice while C57BL/6 were used as controls for G2019S LRRK2 mice. Two- and three-

months old mice were injected i.p. with saline (0.9% sterile NaCl) or lipopolysaccharide (LPS, Escherichia coli serotype O111:B4; 5 mg/kg; 500,000 endotoxin units/mg) (Sigma-Aldrich, MO, USA). Immediately after LPS injection, mice were placed on warming pads in order to prevent hypothermia and reduce mortality (Romanovsky *et al.*, 1997; Paul *et al.*, 1999). For long-term experiments, cumulative lethality was monitored 4 days following injections and never exceeded 10% for the LPS-treated groups. All experimental procedures were performed under pathogen-free conditions. All animals were housed within the vivarium at St. Jude Children's Research Hospital or Thomas Jefferson University (TJU) and maintained on a 12:12 h light/dark cycle with *ad libitum* food and water. All of the experimental procedures in the animals were performed in accordance with the NIH Guide for the Care and Use of Laboratory Animals and all protocols, were approved by the St Jude Children's Research Hospital (protocol 270) or TJU (Protocol 1892) IACUCs. Experiments were carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for animal experiments.

**Fluorescent in situ hybridization**

Lungs were harvested from transgenic mice to determine where each LRRK2 transgene inserted into the genome. For BAC FVB/N-Tg(LRRK2*R1441G)135Cjli/J, BAC C57BL/6J-Tg(LRRK2*R1441G)3IMjff/J and BAC C57BL/6J-Tg(LRRK2*G2019S)2AMjff/J mice, purified LRRK2 (RP11-115F18) BAC DNA was labeled with a red-dUTP (Alexa Fluor 594, Molecular Probes) by nick translation. The labeled transgene probe was combined with sheared mouse cot DNA and hybridized to metaphase and interphase nuclei derived from the transgenic mouse lung fibroblast culture in a solution containing 50% formamide, 10% dextran sulfate, and 2X SSC. After confirming transgene signal, an initial chromosomal localization was assigned

based on known banding patterns. For B6;C3-Tg(PDGFB-LRRK2*G2019S)340Djmo/J mice, an agar stab containing the LRRK2 wt plasmid was submitted and DNA was made from this stab by the CSR. The purified LRRK2 wt plasmid DNA was labeled with a red-dUTP (Alexa Fluor 594, Molecular Probes) by nick translation and first hybridized to normal human chromosomes in order to confirm localization to chromosome 12q12. The labeled transgene probe was then combined with sheared mouse cot DNA and hybridized to metaphase and interphase nuclei derived from the transgenic mouse lung fibroblast culture in a solution containing 50% formamide, 10% dextran sulfate, and 2X SSC. Confirmation was then made by co-localization with known chromosomal probes for mChr1 (Pax3 / RP23-260F1), mChr 2 (LHX3[RP23-297I16] /2A3) or mChr 3 (RP24-380G11).

**Microglia isolation**

Mice were deeply anesthetized with an overdose of Avertin and perfused with ice-cold 1xPBS (pH 7.4). The exsanguinated brain was quickly removed and placed in ice-cold HBSS (Gibco, Life Technologies NY, USA) supplemented with 0.5% glucose (Sigma-Aldrich, St. Louis, MO, USA) and 15 mM HEPES (Gibco, Life Technologies NY, USA). Tissue was minced with a razor blade in HBSS containing 0.25% trypsin (Gibco, Life Technologies NY, USA) and 10 U/mL DNAse (Life Technologies, NY, USA). Next, the tissue was incubated for 20 min in a 37C water bath. The enzymatic reaction was quenched by the addition of equal volume of 20% heat-inactivated FBS (Gibco, Life Technologies NY, USA) and 100 ug/mL trypsin inhibitor (Sigma-Aldrich, MO, USA). Then homogenate was filtered through a 40 uM cell strainer (BD Biosciences Discovery Labware, MA, USA) and centrifuged at 5000g for 10 min at 4C. Supernatant was aspirated and the pellet was re-suspended in ice-cold 70% isotonic Percoll (GE

Healthcare Bio-Sciences, PA, USA). Then 30% ice-cold isotonic Percoll was layered on top of the 70% layer and then sterile PBS layered on top of the 30% Percoll layer (Frank *et al.*, 2006; Moussaud and Draheim, 2010; Lee and Tansey, 2013). The density gradient was centrifuged at 1200g in a swinging bucket rotor for 30 min at 4C. Following centrifugation, the thick top layer phase contained all CNS elements was discarded while the lower layer at the interface between the 70 and 30% Percoll phases (which contained microglia) was aspirated. Cells were washed 3 times by adding 1 mL PBS and centrifuged at 3000g for 5 min at 4C to pellet the cells. The purity and the phenotype of microglia were verified by RT-qPCR and Western Blot.

**Single brain cell suspension preparation and FACS**

Mouse adult brain tissue was dissociated for FACS sorting following the modified protocol of Brewer (Brewer and Torricelli, 2007). Briefly, mice were perfused with ice-cold 1X PBS, and the brain was quickly removed in 4 mL ice-cold DMEM with Glutamax (Gibco, Life Technologies NY, USA) containing 15 mM HEPES (Gibco, Life Technologies NY, USA) and 1xB27 Supplement (Thermo Fisher Scientific, MA, USA). Tissue was minced with a razor blade and incubated in pre-warmed DMEM containing 15 mM HEPES (Gibco, Life Technologies NY, USA), 1xB27 Supplement (Thermo Fisher Scientific, MA, USA), 0.25% trypsin (Thermo Fisher Scientific, MA, USA) and 10 U/mL DNAse (Life Technologies, NY, USA) for 30 min in a 37C water bath. Then the tissue was transferred to the 15 mL tube containing 2 mL DMEM with 20% heat-inactivated FBS (Gibco, Life Technologies NY, USA) and 100 ug/mL trypsin inhibitor (Sigma-Aldrich, MO, USA). To dissociate cells, the brain tissue was triturated approximately 10 times with a large diameter fire-polished Pasteur pipet. The collected homogenate was filtered through a 70 uM cell strainer (BD Biosciences Discovery Labware, MA, USA). Cells were

pelleted by centrifugation at 1600g for five minutes and re-suspended in PBS, and then incubated with conjugated antibodies against CD11b (BV605), Thy 1.1 (FITC), Thy 1.2 (FITC), F4/80 (PE-Cy7), CD3 (APC-Cy7) (all from BD Biosciences), O4 (PE) and GLAST (APC) (both from Miltenyi Biotec) and DAPI (Molecular Probes) for thirty minutes in the dark at 4°C. Samples were sorted for $Thy^{+}/CD3^{-}$ neurons, $O4^{+}$ oligodendrocytes, $GLAST^{+}$ astrocytes (Schwarz *et al.*, 2013; Sharma *et al.*, 2015) using a FACSAria cell sorter (BD Biosciences). Cell debris and dead cells were excluded from the analysis based on scatter signals and DAPI fluorescence. The viability of sorted cell populations was always at least 95%. A small portion of the cells was incubated without antibodies to gate cells according to their light scattering characteristics, and another small portion of the cells stained with isotype-matched control reagents were used to set the maximum threshold for non-specific binding. Bone marrow cells were also used as an additional isotype control. After sorting, cells were washed by adding 1 mL PBS and centrifuged at 3000g for five minutes at $4^{o}C$ with full brake to pellet cells. The purity of sorted cells was verified by Western Blot.

**Peripheral blood mononuclear cells separation**

Blood was collected from the heart of deeply anesthetized mice into 10% EDTA-treated vacuum tubes, mixed 1:1 with sterile PBS and separated in lymphocytes separation medium (Corning, NY USA). Samples were centrifuged for thirty minutes at 400g at RT and then the leukocytes “white” layer was carefully aspirated and placed into sterile tubes. Cells were washed two times with three volume of sterile PBS, centrifuged for 10 min at 200g at RT, and then stored at −80C until assayed. For TMT-based proteomics experiments, leukocytes for each genotype and treatment were pooled from three-six mice.

**Flow cytometry and FACS**

For flow cytometric analysis, blood was collected from the heart of deeply anesthetized mice into 10% EDTA-treated vacuum tubes. After RBCs lysis with ammonium chloride solution, PBMCs were stained with antibodies against CD4 (PerCP-Cy5.5), CD8 (PE-Cy7), CD19 (APC-Cy7), CD25 (APC), CD49 (PE), Mac-1 (Alexa700), Gr-1 (PerCP) (all from BD Biosciences), B220 (eFluor605; eBiosciences), and DAPI (Molecular Probes) for 30 min at $4^{o}$C. Data were acquired using a FACSCalibur flow cytometer (BD Biosciences) and analyzed with CellQuest Pro software. The final data are represented as the percentage of each subpopulation to the total number of cells analyzed.

For FACS, blood was pooled from fifteen mice into 10% EDTA-treated vacuum tubes, and then the RBC-depleted PBMCs were incubated with anti-CD4 (APC), anti-CD8 (PerCP-Cy5.5), anti-CD11b (Alexa 700), anti-CD14 (PE), anti-CD19 (APC-Cy7) (all antibodies from BD Biosciences) and DAPI (Molecular Probes) for thirty minutes at 4°C, followed by washing. Sorting of defined subpopulations from PBMCs was performed using a FACSAria cell sorter (BD Biosciences). Gates were set according to unstained samples and isotype controls using bone marrow and sample.

**Cytokines assay**

For cytokines assay, blood was collected into EDTA-treated tubes. Serum was separated by centrifugation and stored at -20C until assay. Brain tissues (SN, striatum) were mechanically homogenized in Bioplex cell lysis buffer containing factors 1 and 2 (Bio-Rad, CA, USA) and centrifuged at 4500×g. The total protein concentration of each sample was determined using the BCA assay (Pierce, IL, USA), with bovine serum albumin as a standard, according to the

manufacturer's protocol. Individual vials of cytokines/chemokines beads were sonicated, vortexed and then mixed with Assay Buffer (Milliplex Map Kit, MCYTOMAG-70K, Millipore, MA, USA). Working standards ranging in concentration from 3.2 to 2000 pg/ml were made by diluting the stock concentration (10.000 pg/ml) in Assay Buffer. Brain and serum samples were added in equal volumes (25 ul) into the wells (96-well plate) containing 25 ul of Assay Buffer and 25 ul of cytokines mixed beads and plate was incubated overnight at 4C. Then plate was washed and incubated following: (i) with detection antibodies for one hour at RT, (ii) Streptavidin-Phycoerythrin for 30 min at RT, (iii) shealth fluid for 5 min. The plates were run on Luminex 200™ according to manufactures recommended procedures and the data analyzed using BioPlex Manager 4.1 software. All samples were run in triplicates. Data were expressed as pg/mg total protein. Since no statistically significant differences were seen in the cytokine expression in the brain of 3 mo old NaCl mice (used as a control for 4 h, 24 h, 7 d post-LPS groups) compared to 5 mo old NaCl mice (used as a control for 2 mo post-LPS), the data from NaCl-injected mice were combined together and used as one NaCl control. Final data in LPS-treated groups are expressed as the percentage of NaCl control.

**Western Blot**

Tissue samples were homogenized in law-salt buffer (Biskup *et al.*, 2007), containing complete protease and phosphatase inhibitors (Roche NJ, USA). The homogenate was incubated on ice for thirty minutes, and centrifuged for twenty minutes at 14000g, 4°C. The total protein concentration was determined using the BCA assay (Pierce, IL, USA), with BSA as a standard, according to the manufacturer's protocol. The protein extracts (30-60 μg) were separated by 4-20% or 12.5% SDS-polyacrylamide gel electrophoresis, and electrotransferred onto a PVDF

membrane. For immunoblotting, membranes were blocked with 5% non-fat dry milk or BSA in TBS-T buffer for 1 hour at RT, incubated with primary antibody overnight at 4C in TBS-T, 5% w/v nonfat dry milk or BSA. The following antibody were used in this study: rabbit anti-LRRK2 (1:1000; clone 41-2, Abcam by MJFF, MA, USA), rabbit anti-pS935 LRRK2 (1:1000; Abcam by MJFF, MA, USA), rabbit anti-CD11b (1:1000; Abcam, MA, USA), rabbit anti-NeuN (1:1000; Abcam, MA, USA), mouse anti-GFAP (1:1000; Sigma-Aldrich, MO, USA), rabbit anti-MAP2 (1:500; Cell Signaling, MA, USA), mouse anti-GFAP (1:500; Cell Signaling, MA, USA), rabbit anti-MBP (1:500; Cell Signaling, MA, USA), rabbit or mouse anti-β-actin (1:2000; Abcam, MA, USA). Labeled proteins were visualized using enhanced chemiluminescence (Amersham Biosciences, UK). Optical density analysis was performed using the Odyssey Image Studio Software (Li-Cor Biotechnology Lincoln NE, USA). Target proteins expression was normalized to an internal β-actin control. Final data were represented as a percentage of the target protein/β-actin ratio in the experimental and control samples (0.9% NaCl) taken as 100%.

**RT-qPCR**

Microglia, leukocytes and brain samples were processed to obtain RNA in accordance with the protocol outlined in PureLink® RNA Mini Kit or ARCTURUS® PicoPure® RNA isolation Kit (Thermo Fisher Scientific, MA, USA). The RNA samples were treated with DNase (Thermo Fisher Scientific, MA, USA) and total RNA was quantified using a NanoDrop 8000 (Thermo Fisher Scientific, MA, USA). Isolated RNA was converted to cDNA using a High Capacity RNA to cDNA kit (Applied Biosystems, CA, USA) according to manufacturer instructions. This cDNA was subsequently used for RT-qPCR analysis using specific validated primers (Taqman® assays) obtained from Life Technologies. Reaction was performed using

TaqMan Gene Expression Master Mix or TaqMan® Fast Advanced Master Mix in a 7300 Real Time PCR System or 7500 Fast Real Time PCR System (Applied Biosystems, USA), respectively. β-actin gene was used as the normalizing/housekeeping gene. The alterations in gene expression have been expressed as $2^{-\Delta\Delta Ct}$ denoting fold-change in mRNA levels for each gene.

**Quantitative analysis of proteome by 10-plex TMT-LC/LC-MS/MS**

Brain samples or leukocytes were lysed in fresh lysis buffer (50 mM HEPES, pH 8.5, 8 M urea, 0.5% sodium deoxycholate). The protein concentration of the lysate was quantified by the BCA protein assay (Thermo Fisher Scientific MA, USA) and confirmed on a short SDS gel followed by Coomassie staining with titrated BSA as a standard (Xu *et al.*, 2009). Proteins (0.1 mg) from each sample were digested with Lys-C (Wako, 1:100 w/w) at RT for 2 hours, diluted four times with 50 mM HEPES, pH 8.5, and further digested with trypsin (Promega, 1:50 w/w) overnight at RT. The reaction was quenched with 1% trifluoroacetic acid followed by peptide desalting with Sep-Pak C18 cartridge (Waters), and dried by speedvac. Each sample was then resuspended in 50 mM HEPES, pH 8.5, and labeled with 10-plex TMT reagents following the manufacturer's instruction. Finally, the TMT labeled samples were equally mixed, desalted again and dried. Next, the pooled TMT labeled peptides were solubilized in 70 µl of buffer A (10 mM ammonium formate, pH 8) and separated on two connected XBridge C18 columns (3.5 µm particle size, 4.6 mm x 25 cm, Waters) into eighty fractions with a three hour gradient from 15% to 35% buffer B (95% acetonitrile, 10 mM ammonium formate, pH 8, flow rate of 0.4 ml/min) and every other fractions were dried for whole proteome analysis.

The analysis was performed using our optimized platform (Wang *et al.*, 2015). Brain samples were performed on an Orbitrap Fusion mass spectrometer and PBMCs samples were performed on a Q Exactive™ HF hybrid quadrupole-Orbitrap mass spectrometer (Thermo Fisher Scientific MA, USA). For whole proteome analysis, the dried peptides were reconstituted in 5% formic acid, loaded on a reverse phase column (75 µm x 30 cm, 1.9 µm C18 resin (Dr. Maisch GmbH, Germany). Peptides were eluted by 10-36% buffer B gradient (buffer A: 0.2% formic acid, 5% DMSO; buffer B: buffer A plus 65% acetonitrile, flow rate of 0.28 µl/min). Mass spectrometer was operated in data-dependent mode with a survey scan in Orbitrap (60,000 resolution, 2 x 105 AGC target and 50 ms maximal ion time). During data dependent acquisition, Orbitrap survey spectra were scheduled for execution at least every three sec, with the embedded control system determining the number of MS/MS (60,000 resolution, 1 x 105 AGC target, 150 ms maximal ion time, HCD, 38 normalized collision energy, 1 m/z isolation window, and 20 s dynamic exclusion) acquisitions executed during this period.

**Database searching and TMT-based protein quantification from the JUMP software suite**

The MS/MS raw files were converted into mzXML format and searched by our recently developed JUMP algorithm against a composite target/decoy database (Peng *et al.*, 2003) to estimate FDR. The target protein database was downloaded from the Uniprot mouse database (52,490 protein entries) and the decoy protein database was generated by reversing all target protein sequences. Spectra were searched with ± 10 ppm for precursor ion and product ion mass tolerance, fully tryptic restriction, static mass shift for TMT-tagged N-terminus and lysine (+229.16293), dynamic modification for the oxidation (+15.99492) of methionine, two maximal missed cleavages, three maximal modification sites, and the assignment of a, b, and y ions.

Putative peptide spectra matches were first filtered by MS mass accuracy (± 4 standard deviations, ~2 ppm, which was determined by all empirical good matches of doubly charged peptides with JUMP Jscore of at least 30). These good matches were also used for global mass recalibration prior to the filtering. The survived matches were grouped by precursor ion charge state and further filtered by Jscore and dJn values. The cutoff values for these values were adjusted until a protein FDR lower than 1% was achieved. If one peptide was matched to multiple proteins, the peptide was represented by the protein with the highest peptide-spectrum matches (PSM) according to the rule of parsimony.

The analysis was performed in the following steps as previously reported with modifications (Mertz *et al.*, 2015): (i) exacting TMT reporter ion intensities of each PSM; (ii) correcting the raw intensities based on isotopic distribution of each labeling reagent (e.g. TMT126 generates 91.8%, 7.9% and 0.3% of 126, 127, 128 *m/z* ions, respectively; (iii) excluding PSMs of very low intensities (e.g. minimum intensity of 1,000 and median intensity of 5,000); (iv) removing sample loading bias by normalization with the trimmed median intensity of all PSMs; (v) calculating the mean-centered intensities across samples (e.g. relative intensities between each sample and the mean), which has the merits as follows: (1) each scan is fairly treated regardless of its intensity level to avoid bias toward highly or lowly abundant PSMs; (2) outliers can be readily detected by statistical tests (e.g. Dixon's Q-test or generalized extreme Studentized deviate test); (vi) summarizing protein relative intensities by averaging related PSMs; (vii) finally deriving protein absolute intensities by multiplying the relative intensities by the grand-mean of three most highly abundant PSMs. Technical effects were removed via mean-centering (Sims *et al.*, 2008).

Differential expression analysis was performed using LIMMA (Linear Models for Microarray and RNA-seq Data) (Ritchie *et al.*, 2015) with a p value cutoff of 0.01 or 0.05 along with fold change (FC) cutoff ≥ 0.37. The 0.37 $\log_2$(FC) cutoff was derived based on a null experiment compared to the mutant vs control experiment (Zhou *et al.*, 2010). Gene set enrichment analysis (GSEA) was performed on Gene Ontology, Hallmark and Immune related gene sets by permuting gene sets over 1000 times and a $\log_2$(FC)ranking metric.

**Immunohistochemistry**

Mice were intracardially perfused with 1X phosphate-buffered saline (PBS), pH 7.4. followed by 3% paraformaldehyde in PBS, pH 7.4. Each brain was dissected out of the skull following perfusion and post-fixed overnight in fresh fixative. Brains were then dehydrated through a graded series of ethanols, defatted in mixed xylenes and embedded in paraffin (Paraplast-Xtra™, Fisher Scientific, MA, USA), and then blocked and cut in the coronal plane and serially-sectioned at 10 µm. Every section from the rostral hippocampus to the anterior aspects of the cerebellar-midbrain junction was saved and mounted, five sections per slide, onto Superfrost-Plus slides (Fisher Scientific, MA, USA). Every other slide (first series) was double labeled for TH (1:250; mouse monoclonal, Sigma-Aldrich, MO, USA) and ionized calcium-binding adapter molecule 1 (Iba-1) (1:200; rabbit polyclonal, Wako, VA, USA) as described previously (Jang *et al.*, 2012). The double labeling was carried out using a two-color DAB protocol. In some experiments after TH and Iba-1 counting coverslips were dehydrated through a graded series of ethanol, defatted in mixed xylenes and then slides were counterstained with the Nissl stain. A second series was labeled for T-cells (CD3, 1:300; Santa Cruz Biotechnology, TX, USA) and then counterstained with a Nissl stain.

For Tmem119 and Iba-1 double-immunostaining, brain and spleens were sectioned on a cryostat (20μm) and thaw mounted onto polyionic coated slides. Slides were blocked for one hour at RT in 10% serum in PBSTx (PBS + 0.5% Triton X) and incubated with Tmem119 (rabbit monoclonal; clone 28-3; provided by Prof. Ben Barres) and Iba-1 (goat polyclonal; Abcam MA, USA) antibodies diluted in 1% serum PBSTx overnight at 4°C. Then sections were washed and incubated with anti-rabbit Alexa-Fluor 488 and anti-goat Alexa Fluor 594 secondary antibodies (Life Technologies, NY, USA) in 1% serum PBSTx for two hours at RT. For CCR2 and Iba-1 double-immunostaining, brain and spleens sections (20μm) were incubated with CCR2 (goat polyclonal; Thermo Fisher Scientific, MA, USA) and Iba-1 (rabbit polyclonal; Wako, VA, USA) antibodies diluted in 1% serum PBSTx overnight at 4°C. Then sections were washed and incubated with anti-goat Alexa Fluor 594 and anti-rabbit Alexa-Fluor 488 secondary antibodies (Life Technologies, NY, USA) in 1% serum PBSTx for two hours at RT. Slides were washed and coverslips mounted with Vectashield with DAPI (Vector Labs). Sections incubated without primary antibody were used as a negative control. Spleen sections were used as a positive and negative control in CCR2 and Tmem119 staining, respectively.

**Microscopy and model-based (2D) cell counting**

The total number of SNpc TH-positive DA neurons and Nissl-positive neurons, as well as Iba-1-positive microglial cells was estimated using model-based stereology (Baquet *et al.*, 2009; Sadasivan *et al.*, 2015). On average, forty sections per SNpc were analyzed. TH-positive or Iba-1-positive cells were counted as present within a section if they exhibited DAB (TH) or VIP (Iba-1) reaction product in the cytoplasm and there was a clear and complete nucleus present. Microglia was deemed as “resting” if it contained a small oval Iba-1-positive cell body that

averaged three microns or less in diameter with long slender processes, and as “activated” when the cell body was slightly increased in size compared to resting microglia and had an irregular shape with shorter and had thickened processes (Graeber and Streit, 2010).

In all histology experiments, three mo old NaCl-injected mice were used as a control for 24 h, 7 d and 2 mo post-LPS time-points, while 1 year old NaCl-injected mice were used as a control for the 7 mo post-LPS time point. However, since our data showed that regardless of genotype there were no differences in the number of TH neurons in the SNpc of NaCl-injected three mo, 1 and 2 year old mice; the data on TH neurons were combined together and used as one NaCl control.

The number of Nissl-stained hippocampal CA1 pyramidal cells was counted in every fifth section starting at Bregma -2.7 through - 3.6 mm (Paxinos and Franklin, 2004). Only neurons with normal visible nuclei were counted. The total number of CA1 neurons in right and left hemispheres was calculated for NaCl and 7 mo post-LPS groups.

Double-stained Iba1- and Tmem119-, and Iba-1- and CCR2-immunostained sections were examined on a fluorescent microscope (Olympus BX51, Olympus Corporation, Tokyo, Japan) under 10x and 20x objectives.

**Statistical analysis**

All data are represented as mean ± SEM. Statistical analysis was performed using the GraphPad Prism® version 4.03 software. Differences between groups were determined by one-way ANOVA. If overall statistical significance was found, post hoc comparisons were performed.

**Supplementary References**


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