SI MATERIAL AND METHODS

Immunohistochemistry. Immunohistochemistry (IHC) was performed as previously described (1, 2). Briefly, mice were perfusion-fixed with 4% PFA (Sigma-Aldrich). Spinal cords were dissected and post-fixed for 30 min in 4% PFA at 4 ^{0}C and cryoprotected in 20% (w/v) sucrose (Sigma-Aldrich) in PBS overnight before freezing in O.C.T. on the surface of dry ice. Twelve-micrometer-thick spinal cord sections were cut using cryostat (Leica CM1900), collected on SuperFrostPlus slides (VWR International), and allowed to dry for 30 min before storing at -80 ^{0}C. Sections were de-stained to reduce NR intensity with a solution of 50% ethanol/ 1% glacial acetic acid (Sigma-Aldrich) for 10 min prior to IHC as previously described (3). For IHC, sections were permeabilized with 1% Triton^{TM} X-100 in TBS for 5 min and incubated in blocking solution (10% donkey serum, 0.25% Triton^{TM} X-100 in TBS) for 1h at room temperature (RT). Primary antibodies were diluted in blocking solution and applied overnight at 4 ^{0}C. Sources and dilutions of primary antibodies were as follows: rabbit anti-Iba1 (1:200, Wako), rabbit anti-Olig2 (1:300, Millipore), mouse anti-GFAP (1:400, Sigma), mouse anti-iNOS (1:100, BD Pharmigen), chicken anti-Arg1 (1:1000, Millipore), and rat anti-CD68 (1;100, BioLegend). Fluorescent dye-conjugated secondary antibodies were obtained from Life Technologies or Jackson Laboratories and used according to the manufacturer's instruction. Images were taken with Zeiss LSM880 confocal microscope (Zeiss, Germany).

RNA extraction, cDNA synthesis, and RT-qPCR. Total RNA was isolated using the TRIzol® Reagent protocol (Life Technologies) and homogenizer (Argos). Purified RNA was reverse transcribed into cDNA using iScript™ gDNA Clear cDNA Synthesis Kit

(BioRad). All quantitative polymerase chain reaction (PCR) primers were purchased from Bio-Rad. SYBR® Green real time quantitative PCR was performed using the SsoAdvanced™ Universal SYBR® Green Supermix (Bio-Rad) and analyzed by the CFX96 Touch™ Real-Time PCR Detection System (Bio-Rad). Results were normalized against peptidylprolyl isomerase A (*Ppia)* and beta-2-microglobulin (*B2m*) and were expressed as mean ± standard error of the mean (SEM). *Ppia* and *B2m* are recommended normalization factors for gene expression studies (4).

Flow cytometry. NR labeled lesion and adjacent unlabeled non-lesion sites from the same spinal cord were dissected at 5 and 10 dpl. The NR labeled and unlabeled tissue samples from 4-5 mice belonging to the same group were combined and mechanically dissociated. For characterization of oligodendroglial populations, we followed cell purification protocol using Accutase (Sigma-Aldrcih) for tissue dissociation and 40 % Percoll (Sigma-Aldrcih) to remove myelin debris as previously described (5). 10^6 cells in 1x PBS was labeled with Zombie NIR™ kit (BioLegend) to assess cell viability. After being washed with Cell Staining Buffer (BioLegend), the cells were incubated with 1:100 antibodies (BioLegend) to the surface markers of macrophages (anti-CD45 PE/Dazzle 594; anti-F4/80 PE/Cy7; and anti-CD11b BV650); dendritic cells (anti-CD45 PE/Dazzle 594; anti-F4/80 PE/Cy7; and anti-CD11c BV510). For oligodendrocyte lineage cells we used markers for OPCs (anti-Pdgfra FITC), later progenitors/early differentiated oligodendrocytes (anti-O4 APC), and mature oligodendrocytes (anti-GALC PE). Oligodendrocyte specific antibodies were conjugated using lightning-link antibody kit according to the manufacturer's protocol (Novus). Cells were analyzed on the flow

cytometer at the Georgetown Lombardi Comprehensive Cancer Center Flow Cytometry and Cell Sorting Shared Resource (FCSR).

Mass spectrometry analysis. NR labeled lesion and unlabeled non-lesion sites were dissected from spinal cords at 5, 10, 20 dpl as described above and kept at -80 ^{0}C before processing for mass spectrometry analysis at the Proteomics & Metabolomics Facility at Georgetown University. Tissue samples were homogenized, centrifuged to remove proteins, dried under a vacuum, and reconstituted in water. The samples were then extracted and subjected to UPLC-MRM-MS analysis. Targeted quantitation of all essential amino acids and biogenic amines, was performed using stable isotope dilution in conjunction with multiple reaction monitoring mass spectrometry. Signal intensities for different amino acids and biogenic amines were ranked, and two independent analyses were performed for each sample. Multiple quality control estimates were performed during the runs, showing <20 % variance and indicating high degree of confidence in the generated results.

Experimental autoimmune encephalomyelitis. C57BL/6 female mice (Charles River) at age 10–12 weeks were acclimatized for 7 days prior to EAE. EAE was induced using the EAE kit (Hooke Laboratories, Cat. No: EK-2110) according to the Hooke Laboratories protocol (http://hookelabs. com/protocols/eaeAI_C57BL6.html). Briefly, mice were immunized by an emulsion of MOG35-55 in complete Freund's adjuvant (CFA) injected subcutaneously at two sites (Day 0), followed by administration of pertussis toxin (PTX) intraperitoneally, first on the day of immunization (Day 0), and then again, the following

day (Day 1). Approximately 115 ng of PTX (Hooke Laboratories, lot#1007) for each of the two PTX administrations were used. The mice were scored blindly and daily from EAE Day 7 until at least EAE Day 30 according to the protocol from Hooke Laboratories. The scoring system used was as follows: 0.0 = no obvious changes in motor function; 0.5 = tip of tail is limp; 1.0 = limp tail; 1.5 = limp tail and hind leg inhibition; 2.0 = limp tail and weakness of hind legs or signs of head tilting; 2.5 = limp tail and dragging of hind legs or strong head tilting; 3.0 = limp tail and complete paralysis of hind legs or limp tail with paralysis of one front and one hind leg; 3.5 = limp tail and complete paralysis of hind legs plus mouse unable to right itself when placed on its side; 4.0 = limp tail, complete hind leg and partial front leg paralysis, mouse is minimally moving but appears alert and feeding; 4.5 = complete hind and partial front leg paralysis, no movement around the cage, mouse is not alert; 5.0 = mouse is found dead due to paralysis or mouse is euthanized due to severe paralysis. The clinical scores and weight of mice were recorded daily until the end of experiment. Mice that had spontaneously recovered from EAE or did not reach a score of 3.0 were not considered in the analysis of the therapeutic study. For each experiment, 8 to 10 mice from each group were analyzed.

Corpus callosum lesion using lysolecithin. Lysolecithin-induced demyelination of corpus collosum was performed as previously described (6, 7) .Briefly, 1 µL of 1% lysolecithin was delivered into the corpus callosum of B6/C57 mice using stereotaxic frame (Narishige, Tokyo, Japan) with the following coordinates: anteroposterior − 1.0 mm from the bregma, mediolateral + 0.5 mm from the midline, dorsoventral − 1.0 mm from the dura. Hamilton syringe (31-gauge, Sigma-Aldrich) connected to the micro injector (IMS-

3, Narishige) was inserted into the corpus callosum, and was kept for 3 min before the injection. The mice were sacrificed at 5 days after the injection.

LPS injection. On P14, C57/B6 mice received an intraperitoneal injection of lipopolysaccharide (LPS, 10 mg/kg, Sigma)**.** Mice were sacrificed 24 h later and processed for immunohistochemistry as described above.

1. Psachoulia K*, et al.* (2016) IL4I1 augments CNS remyelination and axonal protection by modulating T cell driven inflammation. *Brain* 139(Pt 12):3121-3136.
2. Chamberlain KA, Chapey KS, Nanescu SE, & Huang JK (2017) Creatine Enhances Mitochondrial-Mediated Oligodendrocyte Survival After Demyelinating Injury. *J Neurosci* 37(6):1479-1492.
3. Repetto G, del Peso A, & Zurita JL (2008) Neutral red uptake assay for the estimation of cell viability/cytotoxicity. *Nat Protoc* 3(7):1125-1131.
4. Gong ZK*, et al.* (2014) Identification and validation of suitable reference genes for RT-qPCR analysis in mouse testis development. *Mol Genet Genomics* 289(6):1157-1169.
5. Robinson AP, Rodgers JM, Goings GE, & Miller SD (2014) Characterization of oligodendroglial populations in mouse demyelinating disease using flow cytometry: clues for MS pathogenesis. *PLoS One* 9(9):e107649.
6. Yamazaki R, Baba H, & Yamaguchi Y (2018) Unconventional Myosin ID is Involved in Remyelination After Cuprizone-Induced Demyelination. *Neurochem Res* 43(1):195-204.
7. Sahel A*, et al.* (2015) Alteration of synaptic connectivity of oligodendrocyte precursor cells following demyelination. *Front Cell Neurosci* 9:77.

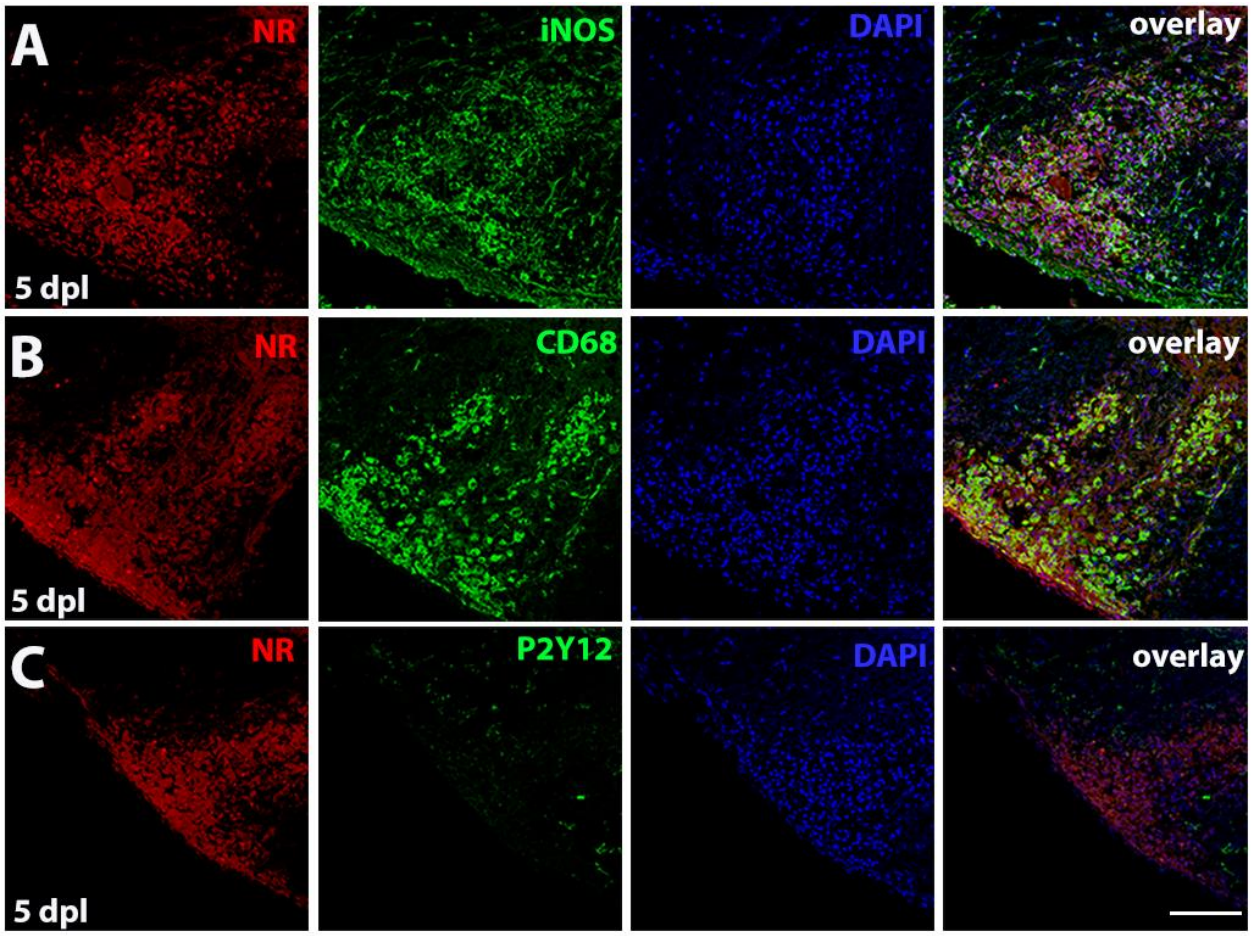

Figure S1. NR co-localizes with activated macrophages/microglia and phagocytotic monocytes but not resting microglia. Representative confocal images showing NR fluorescence (red) and immunostaining (green) for proinflammatory macrophages labeled by iNOS (*A*), phagocytic monocytes labeled by CD68 (*B*), and resting microglia marked by P2Y12 (*C*). Scale bar, 100 µm.

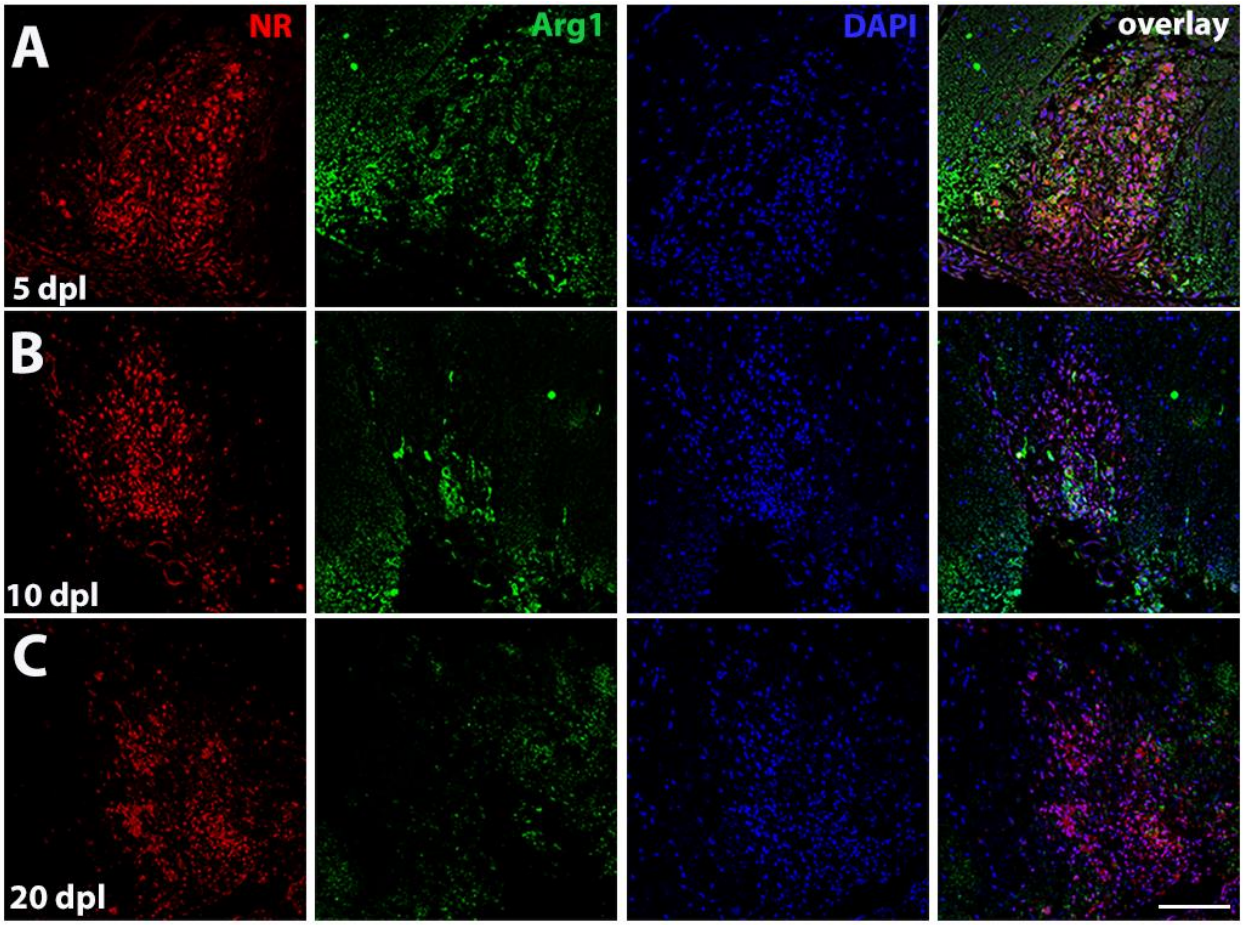

Figure S2. NR labels alternatively activated macrophages. Representative confocal images showing NR fluorescence (red) co-localized with Arg1 (green), a marker of alternatively activated macrophages in a lesion, detected by enhanced DAPI staining (blue) at 5 dpl (*A*), 10 dpl (*B*), and 20 dpl (*C*) (n=3 animals per time point examined). Scale bar, 100 µm.

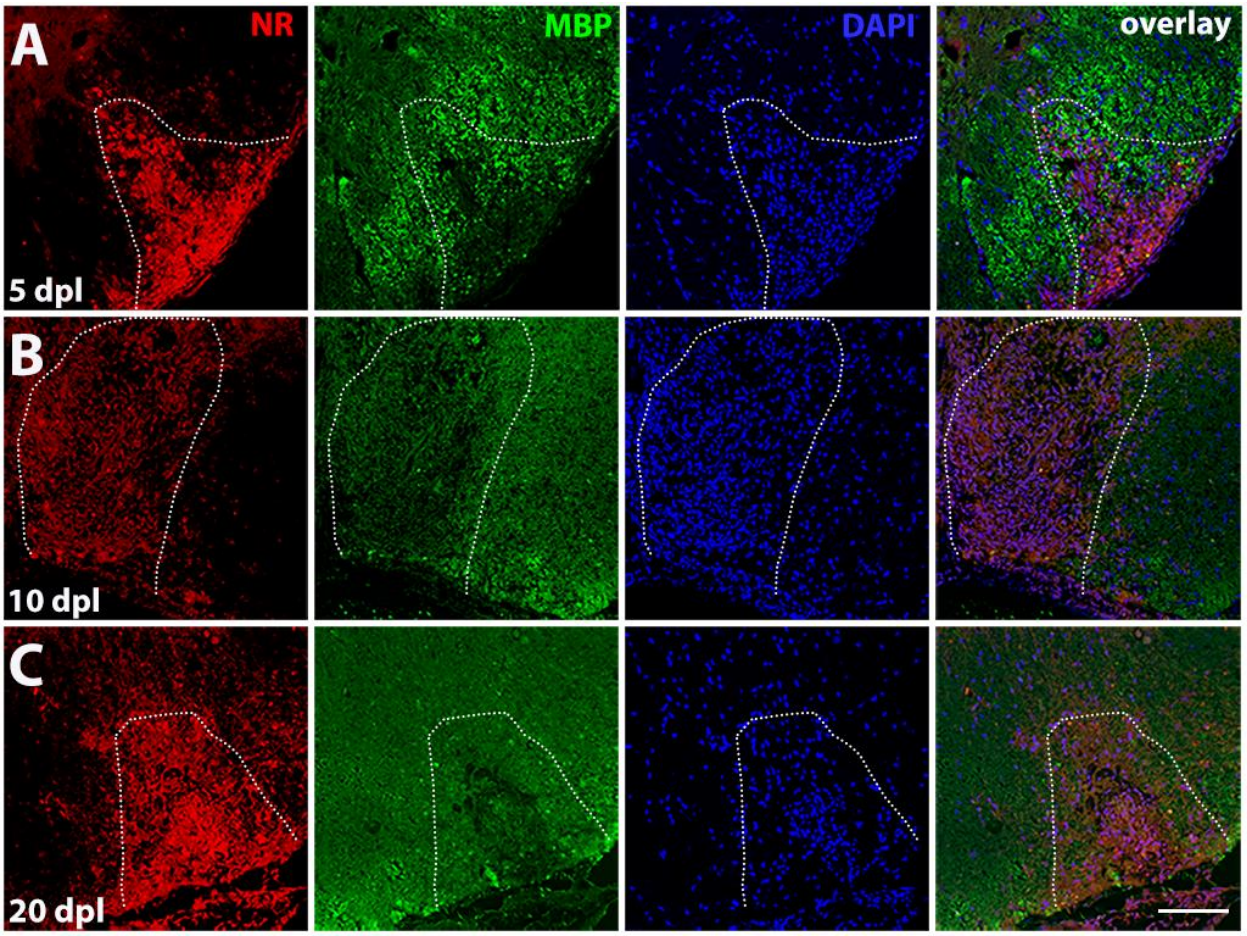

Figure S3. NR labelling covers MBP$^+$ area of the lesion during remyelination. Representative confocal images showing NR fluorescence (red) present in the lesion detected by enhanced DAPI staining (blue) at 5 dpl (*A*), 10 dpl (*B*), and 20 dpl (*C*) (n=3 animals per group). MBP staining (green) shows various stages of remyelination in the lesion labeled by NR. Scale bar, 100 µm.

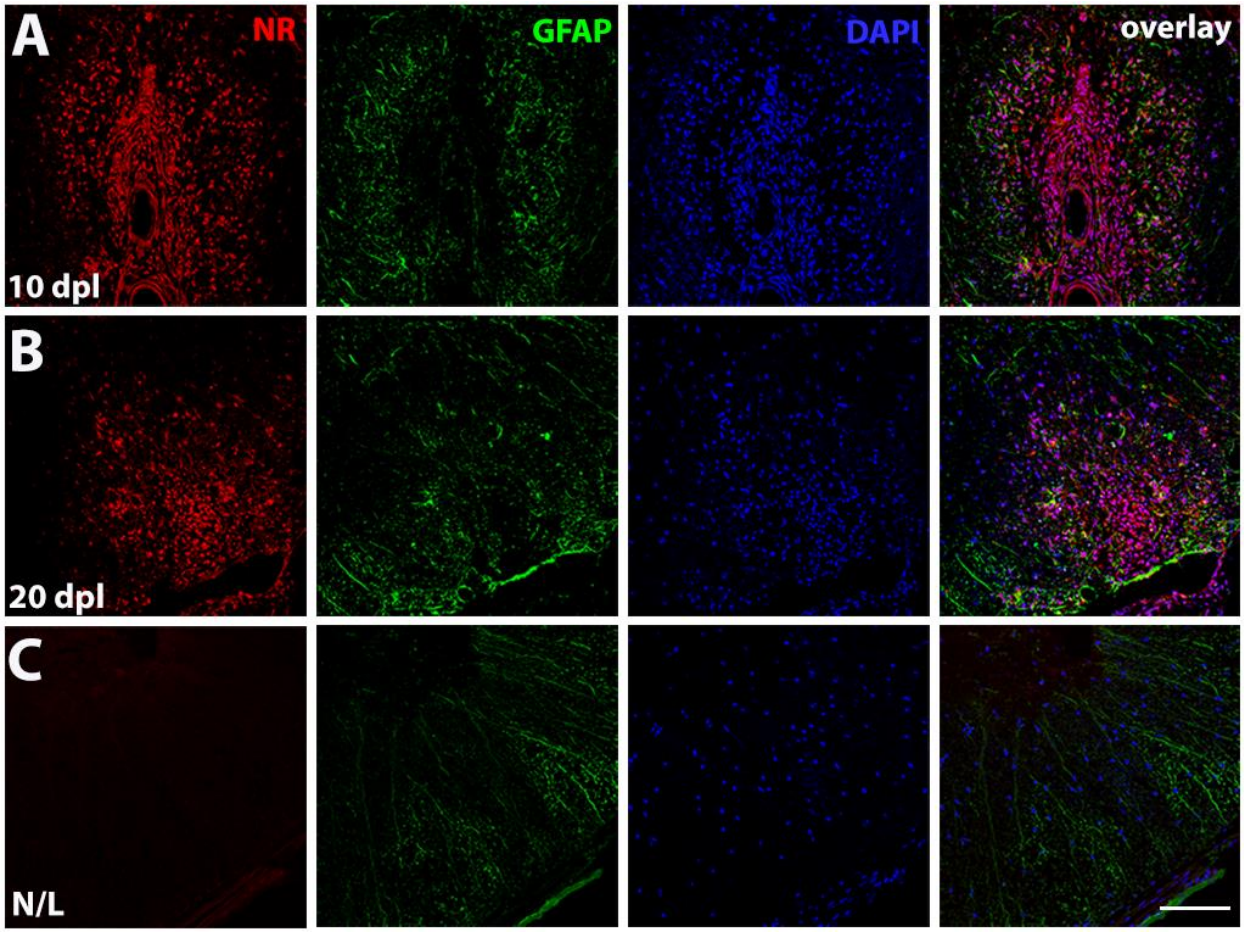

Figure S4. Reactive astrocytes are not present in the lesion labeled by NR at later stages of remyelination. Representative confocal images showing NR fluorescence (red) co-localized with Arg1 (green), a marker of alternatively activated macrophages in a lesion, detected by enhanced DAPI staining (blue) at 5 dpl (*A*), 10 dpl (*B*), and 20 dpl (*C*) (n=3 animals per time point examined). Scale bar, 100 µm.

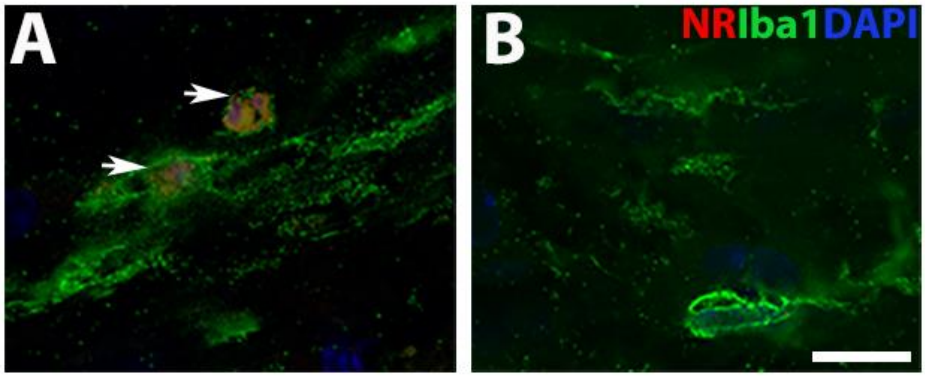

Figure S5. NR labels macrophages/microglia in corpus callosum of LPS mice at P14. Representative confocal images showing NR fluorescence (red) co-localized activated macrophages/microglia (arrows) labeled by Iba1 (green) in corpus callosum of P14 mice 24 h after LPS injection. Scale bar, 10 μm.