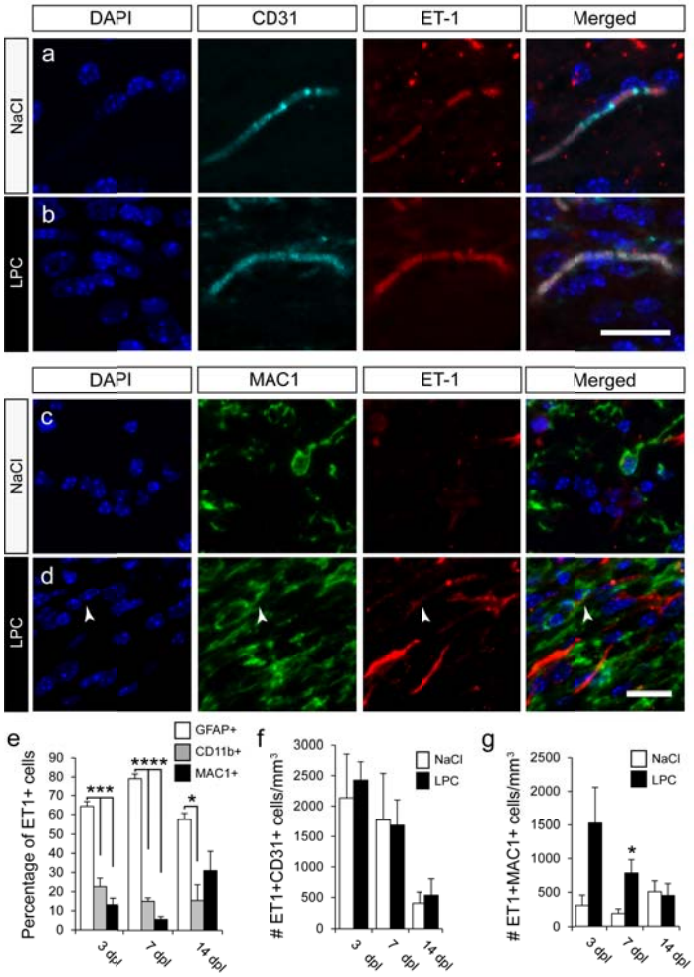
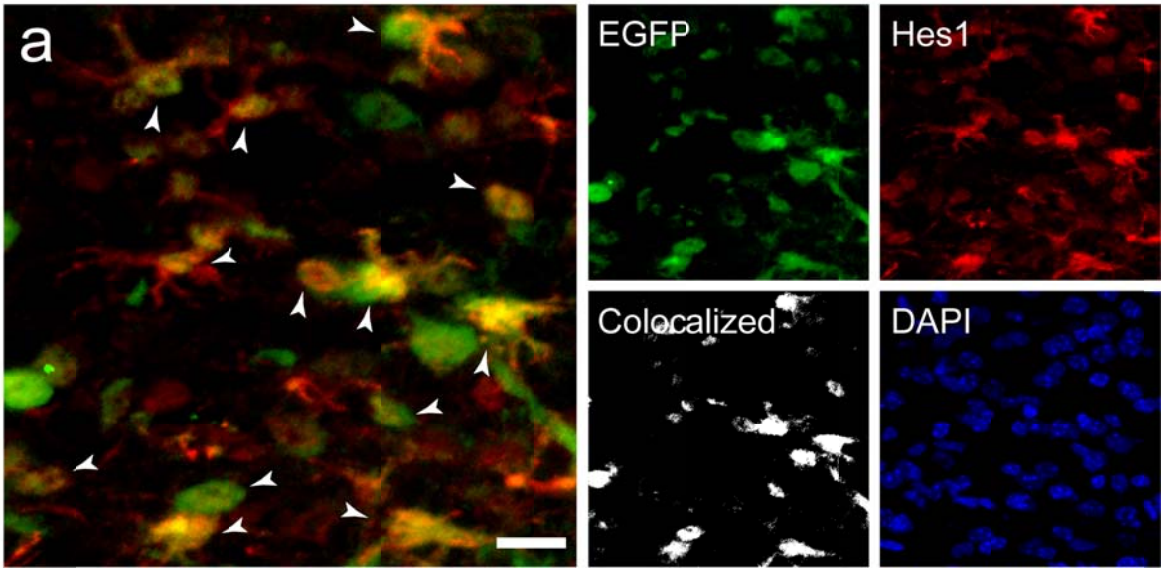
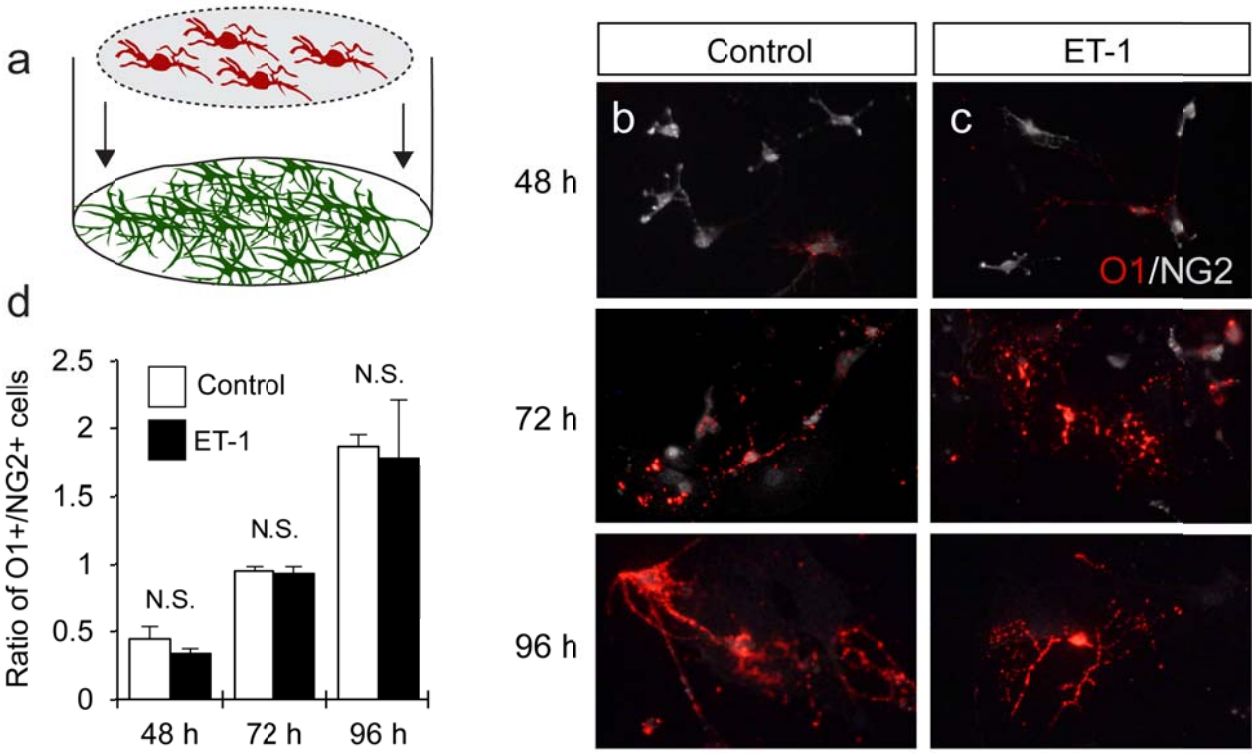


Supplemental Figure 1. ET-1 expression in microglia and endothelial cells is low compared to astrocytes following demyelination, Related to Figure 1. Confocal image of NaCl ***(a)*** and LPC ***(b)*** lesions at 7 dpl, co-immunolabeled with anti-CD31 and anti-ET-1. Confocal image of NaCl ***(c)*** and LPC ***(d)*** lesions at 3 dpl, co-immunolabeled with anti-MAC1 and anti-ET-1. Low-level ET-1 expression in microglia is marked by the white arrow. ET-1^+ astrocytic processes can be seen between microglia. ***(e)*** Plot of the relative percentage of labeled ET-1^+ cells from 3-14 dpl. At 3 and 7 dpl when ET-1 expression was elevated in the lesion, ET-1^+ astrocytes comprised 64% and 79% of ET-1^+ cells, respectively. When ET-1 levels were low at 14 dpl, the percentage of ET-1^+ astrocytes dropped slightly to 57%. ***(f)*** Quantification of the number of ET-1^+CD31^+ cells from 3-14 dpl. Little or no change was seen between NaCl and LPC-lesions at any time point. ***(g)*** Quantification of the number of ET-1^+MAC1^+ cells from 3-14 dpl. (For e-g: N=3-4, *p<0.05, ***p<0.001, ****p<0.0001, ANOVA Bonferroni post hoc, mean +/- SEM). Scale bar=20μm for all images.

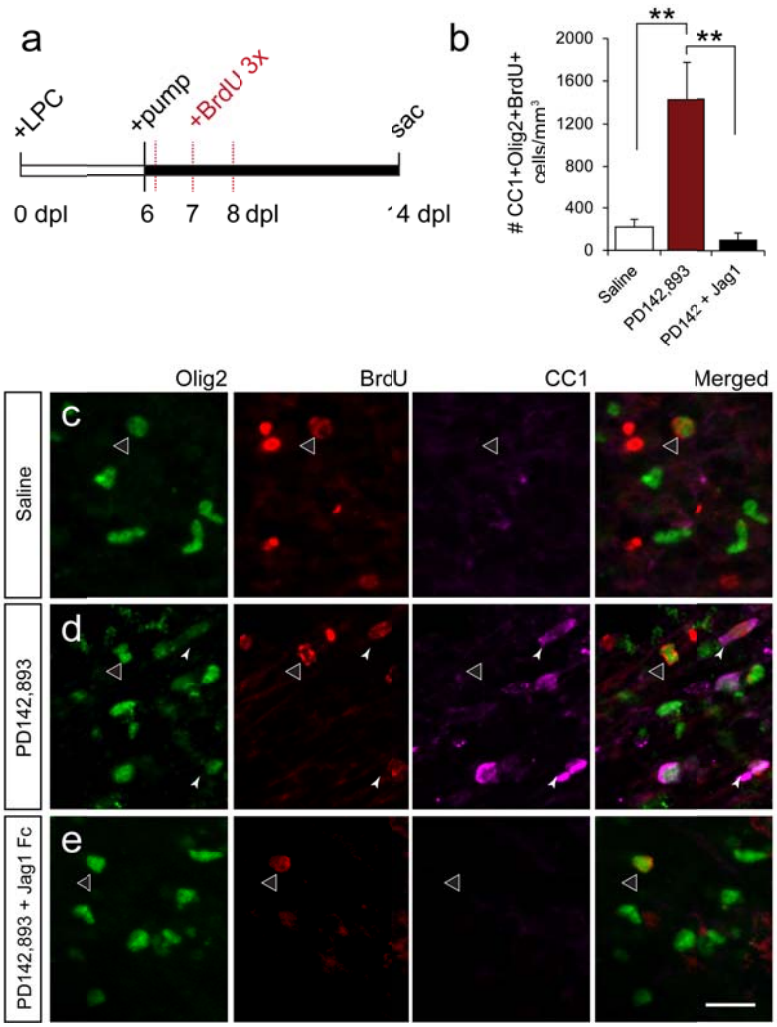


Supplemental Figure 2. Hes1 expression is found in TNR^{+} cells in LPC lesions, Related to Figure 4.

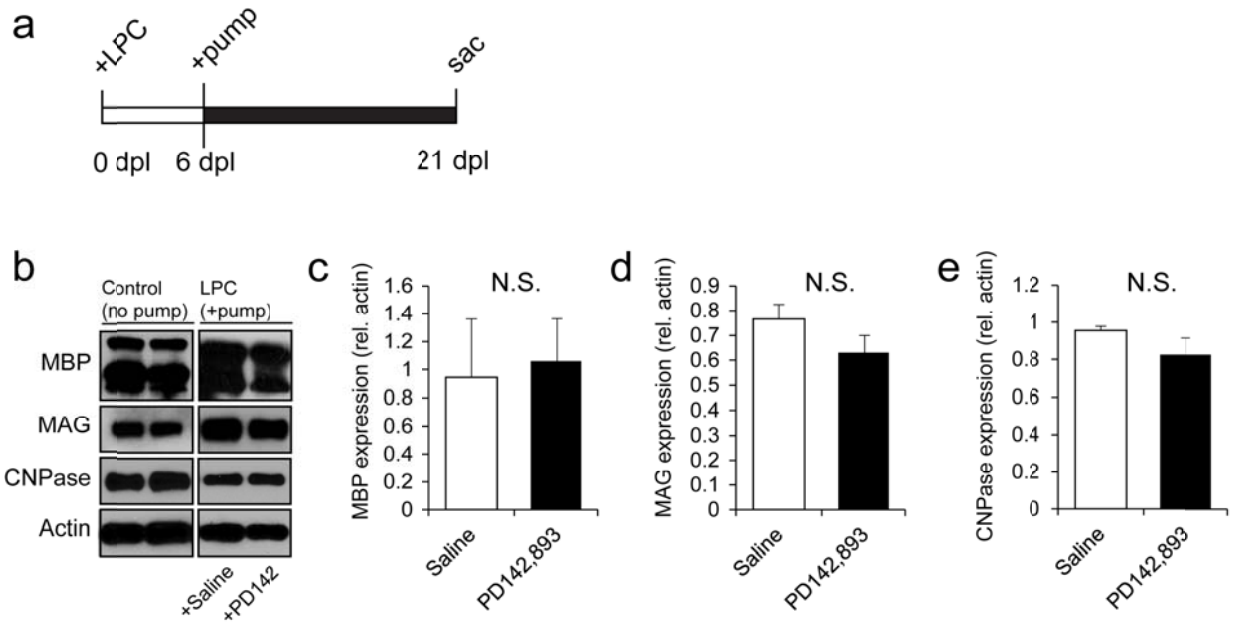
In order to validate Notch activation in TNR-EGFP^{+} cells, tissue was stained with an anti-Hes1 antibody. Hes1 is a direct downstream target of the CSL/CBF1/RPGJ transcriptional regulators which EGFP expression reports in the TNR mouse. ***(a)*** Confocal image of a TNR^{+} LPC lesion at 5 dpl co-immunolabeled with anti-Hes1. The majority of EGFP^{+} cells were also Hes1^{+}. Solid white arrows indicate EGFP^{+}Hes1^{+} cells. Scale bar=15μm.



Supplemental Figure 3. ET-1 pretreated astrocytes require cell-cell contact to alter OPC differentiation, Related to Figure 6. ***(a)*** To account for any soluble factors released into the culture media following ET-1 pretreatment, and because Notch signaling requires cell-cell contact, OPCs were plated on glass coverslips and placed into the same culture media of control or ET-1 pretreated astrocytes. Flourescent images of control ***(b)*** and ET-1 ***(c)*** pretreated cultures co-immunolabeled with anti-O1 and anti-NG2. Cells were left for 48, 72, or 96 h to differentiate. ***(d)*** Quantification of the $O1^+$ to $NG2^+$ ratio for control and ET-1 pretreated samples. No changes were found between groups at any time point. (N=3 independent cultures with multiple replicates per sample, N.S.=not significant, unpaired t-test, mean +/- SEM).



Supplemental Figure 4. PD142,893 increases the number of newly generated OLs following demyelination, Related to Figure 7. ***(a)*** Drugs were infused using mini-osmotic pumps following LPC-induced demyelination, from 6-14 dpl. BrdU was injected once a day from 6-8 dpl to label proliferating OPCs. ***(b)*** Quantification of the number of $BrdU^+Olig2^+CC1^+$ cells at 14 dpl. A significant increase in triple-labeled cells was found in PD142,893-infused samples versus control and PD142,893+Jagged1Fc samples. (N=4, **p<0.01, ANOVA Bonferroni post hoc, mean +/- SEM). Confocal images of saline- ***(c)***, PD142,893- ***(d)***, and PD142,893+Jagged1Fc-infused ***(e)*** samples co-immunolabeled with anti-Olig2, anti-BrdU, and anti-CC1 antibodies. Solid white arrows indicate $BrdU^+Olig2^+CC1^+$ cells, hollow white arrows indicate $BrdU^+Olig2^+$ cells.



Supplemental Figure 5. Increases in myelin protein levels persist until 21 dpl in PD142,893-infused mice, Related to Figure 8. ***(a)*** Mini-osmotic pumps were used to infuse drugs into LPC lesions from 6-21 dpl. ***(b)*** Western blot analysis of myelin protein expression in micro-dissected tissue from control non-demyelinated tissue (left 2 lanes), and from LPC-injected lesions at 21 dpl infused with saline- (third lane) or PD142,893 (fourth lane). No changes in MBP ***(c)***, MAG ***(d)***, and CNPase ***(e)*** were observed between groups. (N=3, N.S=not significant, unpaired t-test, mean +/- SEM).

Supplemental Experimental Procedures

Immunohistochemistry

Mice were anesthetized by flurane (Ohmeda PPD) inhalation and perfused intracardially with 1X PBS, followed by 4% paraformaldehyde. Brains were removed and postfixed as previously described; immunocytochemical procedures and confocal microscopy analysis (Zeiss LSM 510 NLO confocal microscope) were as previously described (Aguirre et al., 2007). Antigen retrieval was performed for Jagged1 immunostaining by boiling sections in 20 mM Citrate Buffer for 10 min, followed by two washes in PBS for 10 min each, followed by normal blocking and staining procedures. ET-1 immunostaining was performed on frozen sections mounted to charged glass slides.

Analysis: Multi-stack images were collected on a Zeiss confocal microscope. Demyelinated lesions were confirmed by a decrease or absence of CC1 and MBP immunoreactivity, and disruption of the white matter cytoarchitecture. For saline-injected tissue, the injection site was located based on identification of the needle track, and injection site on the skull surface. Images were analyzed using ImageJ software for the parameter outlined for each experiment. At least 3 images were taken per slice in each hemisphere, 3-5 slices per brain, and 4 brains per condition. For analysis of differentiation during remyelination, cellular analysis of signaling during remyelination, and analysis of Notch activation/inhibition using mini-osmotic pumps, images were taken at the edges and core of the demyelination lesion. For TNR cellular analysis, images were taken both within the lesion, and approximately 200-300 μm beyond the lesion border, where Notch activation was seen in a few mature $CC1^+$ OLs. For cell density calculations, the number of cells per parameter was divided by the total volume of the z-stacked image (length x width x stacked depth) to give cells/μm^3. Measurements were then multiplied by 10^9 to give cells/mm^3. Colocalization analysis was performed using the colocalization plug-in on ImageJ. The colocalization plug-in analyzes pixel overlap between channels stack-by-stack to ensure no false overlap occurs between points. The resulting images (in white) represent

overlapping points that meets a minimum intensity threshold. Images were minimally adjusted for clarity and size using Adobe Photoshop software.

Lysolecithin injection and tissue microdissection

Bilateral Injections for Tissue Microdissection: Mice were deeply anaesthetized using 100 mg/kg Ketamine and 10mg/kg Xylazine. Lysolecithin (1%, 2 μL) was injected bi-laterally into the external capsule of 8-12 wk old C57bl/6n mice at the same coordinates described above. Bi-lateral lesions were created to increase tissue yield for protein analysis. In control mice, 0.9% NaCl was injected bilaterally. Only littermates were used for comparison purposes. Brains were removed after 3, 7, 14, and 30 dpl and 400μm coronal sections were taken on a vibratome and collected in D1 media (HBSS, 1M HEPES, Sugar Solution (6% Glucose, 15% Sucrose), 1% Penn/Strep). The lesion sites were identified and micro-dissected based on location beneath the needle injection site and disruption of white matter cytoarchitecture. Saline-injected control tissue was located beneath the needle injection site.

Multiple sclerosis tissue samples and immunohistochemistry

Frozen post-mortem multiple sclerosis and control brain samples were obtained from the UK Multiple Sclerosis tissue Bank (R. Reynolds, Imperial College). Two randomly chosen multiple sclerosis cases (See table, Supplemental Experimental Procedures) were studied, including secondary progressive and one primary progressive. For these multiple sclerosis cases, the mean age was 44 years (range: 37-51) and disease duration of 7 years. The death-tissue preservation delay varied between 7 and 11 h. Histological assessment of the lesions was performed using Luxol fast blue/Cresyl violet and Oil-red-O histological staining. The expression of ET-1 was studied in MS lesions with active borders and chronic silent core normal appearing.

Tissue sections (12 μm) were hydrated in PBS and microwaved in unmasking solution, according to the manufacturer's protocol (Vector). Briefly, sections were pre-incubated in blocking buffer (10% normal goat serum, 0.1% Triton-X 100 in PBS for 1 h and incubated overnight with primary antibodies at 4 °C. After overnight incubation, slides were extensively washed in PBS/0.1% Triton X-100 and incubated with appropriate secondary antibodies. The following primary antibodies were used: anti-ET1 (1:100, Abbiotec), anti-GFAP (1:100, Millipore), anti-Olig1 (1:100, R&D system) and mouse monoclonal anti-MHCII (1:100, Dako).

Clinical data of subject used in this study

Multiple Sclerosis

Case	Sex	Age (years)	PMD (h)	Disease duration (years)	Cause of death	Course
MS 94	F	42	11	6	MS, bronchpneumonia	PP
MS 100	M	46	7	8	Pneumonia	SP
Control						
C 19	F	90	15		Old age (as per death certificate)	
C 20	F	84	24		Congestive cardiac failure, ischaemic	

Post Mortem Disease (PMD), Secondary Progressive (SP), Primary Progressive (PP)

Electron microscopy

Mice were prepared for standard electron microscopic analysis as previous described (Marcus et al., 2006). Mice were perfused in Millonig's buffer containing 4% paraformaldehyde and 2.5% glutaraldehyde. A brain matrix was used to isolate the regions of interest, which were post fixed in 1% osmium tetroxide, dehydrated and embedded in PolyBed resin. Thick (1 um) and thin sections were stained with toluidine blue and a combination of uranyl acetate and lead citrate, respectively. A minimum of 10 electron micrographs taken at 5,000X were captured per mouse using a JEOL JEM 1230 TEM equipped with a Gatan 4K X 4K Ultrascan digital camera.

Cell cultures

Barrier co-cultures: Astrocytes monolayers were grown in 6-well plates as described above. Astrocytes were pre-treated with vehicle or ET-1 for 48 h, and then the media was replaced with untreated N1 (without added ET-1 or growth factors). OP cultures were plated on poly-L-lysine treated glass coverslips and placed in the same wells as the pre-treated astrocytes to prevent cell-cell contact The OPCs were left for 48, 72 or 96 h to differentiate, and then the coverslips were removed and analyzed by immunocytochemistry.

Immunocytochemistry

Coverslips were then washed twice in ice-cold PBS, and then incubated in 4% PFA for 5 minutes at R/T. Coverslips were washed twice in PBS and then incubated in cell blocking solution (5% NGS, 0.3% triton X-100 in PBS) for 1 h at R/T. Primary antibodies were then diluted in cell blocking solution and incubated for 2 h at R/T. Coverslips were then washed 3 times in PBS. Secondary antibodies were all diluted at a concentration of 1:500 in cell blocking solution and coverslips were incubated for 1 h at R/T. Coverslips were then washed 3 times in PBS, and mounted on charged glass slides using MOWIOL mounting medium.

Analysis: 20x images were taken on an Olympus BX61 fluorescent microscope. At least 5 images were taken per coverslip per condition, with at least 3 independent cultures per condition. The ratio of $NG2^+$ to $O1^+$ cells was determined by co-staining NG2 and O1 on the same coverslip and counting the number of positive cells per field for each label. The number of $NG2^+EGFP^+$ cells was determined by counting the number of co-labeled cells per field.

Western blot

Tissue samples were micro-dissected and homogenized in RIPA lysis buffer with protease inhibitor cocktail (Santa Cruz Biotechnology) using a 20-guage needle. Samples were then put on a shaker at 4^oC for 30 min to promote lysis, and then spun down for 10 min at 12,000rpm. Homogenized protein samples in RIPA lysis buffer were quantified using a Bradford protein

assay and 7.5 μg of each protein was loaded per well into a 10-well 4-20% iGel (Nusep). Samples were separated by size in SDS-page running buffer (KD-Medical), and transferred onto a PVDF membrane in a solution of Tris-Glycine transfer buffer (KD-Medical), 20% methanol, and 0.1% SDS. Membranes were then washed briefly in TBS-T (1x TBS, 0.1% Tween-20), and transferred to blocking buffer (2% BSA, 5% milk, 1x TBS-T) for 1 h at RT. Primary antibodies were diluted in carrier solution (2% BSA, 1x TBS-T) and blots were incubated at 4^{o}C O/N on a shaker. Blots were then washed three times for 5 min each in TBS-T at R/T. Secondary HRP-conjugated antibodies (Santa Cruz) were all diluted 1:10,000 in carrier solution, and blots were incubated for 1 h at R/T on a shaker. Membranes were washed three times in TBS-T, and then incubated for 1 min in ECL (Thermo Scientific). Autoradiography film (Denville Scientific) was then used to capture the signal from each blot and were developed in an automated film developer. Band size and density measurements from each sample were collected using ImageJ. Values were normalized to β-actin loading controls, and reported as a proportion of actin expression.

RT-PCR

RNA was isolated from NaCl and LPC-injected lesions at 3 dpl using Trizol (Invitrogen) (Gadea et al., 2009). Specific primers were obtained from Integrated DNA Technologies. Sequences were as follows: ET-1, sense, 5_-GGTTGGAGGCCATCAGCAACAGCA-3_, and antisense, 5_-AAGGACGCTGGTCCTCTGCCAGT-3_; ET-2, sense, 5_-TGCGTTTTCGTCGTTGCTCCTGC-3_, and antisense, 5_-ATGGCAGAAGGTGGCACAGGCAGA-3_; ET-3, sense, 5_-CTGCCTGCACAGCCTGGAAATGCT-3_, and antisense, 5_-ACTTTCTGGAACTGGCCCCGAGGA-3_; GAPDH, sense, 5_-GGGCTCTCTGCTCCTCCCTGTTCT-3_, and antisense, 5_-TGACTGTGCCGTTGAACTTGCCGT-3_. Genes were amplified by 1 min denaturation at 94°C,

30 sec, annealing at 55°C, and 1 min extension at 72°C for 35 cycles. PCR products were resolved by 1.2% agarose gel electrophoresis and visualized under UV light.

BrdU injections

For fate mapping experiments, BrdU (5mg/mL in PBS, 10 μL/gram body weight) was injected once a day intraperitoneally into mice following installation of osmotic pumps at 6, 7, and 8 dpl. Mice were then left until 14 dpl for immunohistochemical analysis.

Tamoxifen injections

Tamoxifen (Sigma, 90 mg/mL) was dissolved in 100% ethanol, and then diluted in autoclaved sunflower oil (Sigma) to a final concentration of 10 mg/mL. *hGFAP*$^{CreER^{T+}}$*;ET-1*$^{flox/flox}$ mice were injected with 75 mg/kg of Tamoxifen, once per day from 1 to 3 dpl. A vehicle solution of ethanol and sunflower oil was injected into control animals.