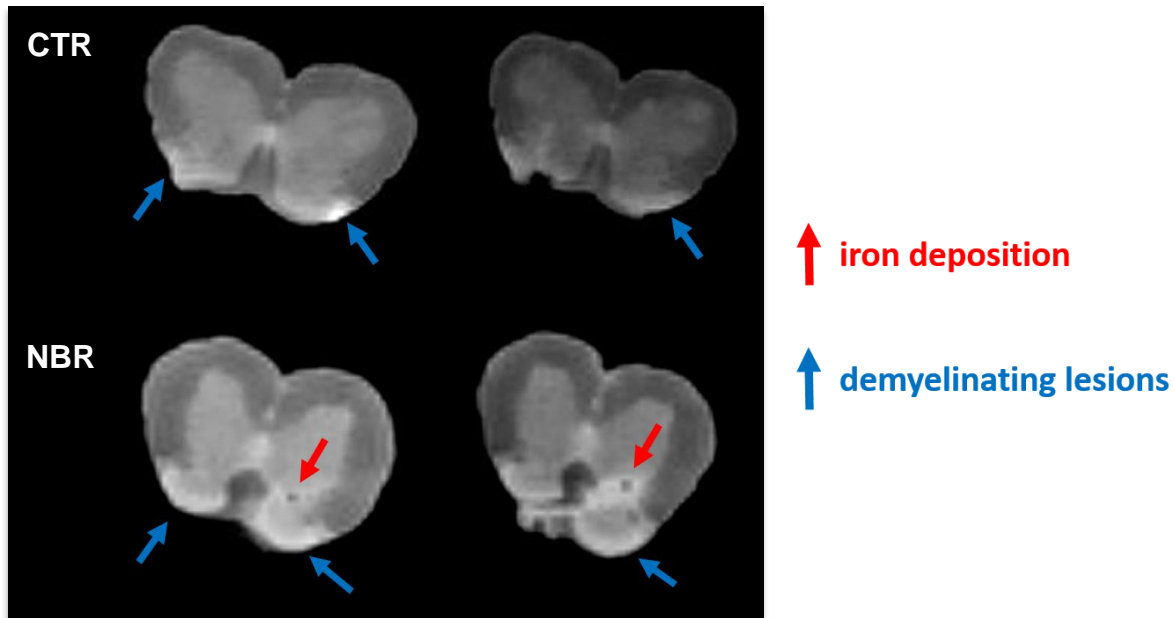


Supplementary Fig.1. Excitation (black line) and emission (red line, λ_{EXC} 480 nm) spectra of Fe₃O₄-DDA-Fluo nanoparticles.

Reliable absorption spectra of the fluorophore cannot be recorded, because of the intrinsic absorption of Fe₃O₄, overlapped to the fluorescein absorption spectrum, as well as to nanoparticle scattering.



Supplementary Fig2. MRI images of a spinal cord isolated from an EAE mouse after the passive transfer of NBR loaded TCL.

The figure shows MRI findings at the thoracic-lumbar region of spinal cord from an EAE mouse (CTR) and from an EAE mouse undergone passive transfer of NBR loaded TCL (NBR). Post mortem axial T2 weighted multi slice multi echo (T2w MSME) MRI images obtained by NMR microscope system at 9.4 T (in-plane resolution = 47 x 63 μm , slice thickness= 0.3 mm) showing hyperintense lesions (blue arrows) and focal hypointense areas (red arrows) suggestive of the NBR presence.

Supplementary Methods

Synthesis of Fluorescein488-NHS.

6-Carboxypentylamido-exanoic fluorescein succinimidyl ester **7** (Fluorescein488-NHS) was synthesized by using a slight modification of the procedure of Wu and Fan (Scheme 1).(1) All chemicals were of analytically pure grade, unless otherwise mentioned. 1,2,4-Benzenetricarboxylic anhydride (CAS 552-30-7) and resorcinol (CAS 108-46-3) were purchased from Acros Organics and were respectively of 97% and 98% analytically grade. N-hydroxysuccinimidyl trifluoroacetate (NHS-TFA) was prepared accordingly to a previously reported procedure.(2) All organic solvents were anhydrous grade.

5(6)-carboxy-fluorescein (1). 1,2,4-Benzenetricarboxylic anhydride (2.056 g, 10.70 mmol) and resorcinol (2.61 g, 23.70 mmol) were dissolved in 99.7% methansulphonic acid (50 ml). The reaction mixture was kept at 50 °C for 4 h and then at 110 °C for 24 h in a nitrogen atmosphere. The solution was cooled at room temperature and slowly poured in and water/ice mixture. A yellow solid precipitated, which was filtered off and washed three times with 0.1 M HCl.

Yield: 4.30 g, 11.37 mmoli, 88.2 %

¹H-NMR (DMSO-d₆, 400 MHz) δ 9.96 (s, 1H), 8.18 (s, 0.5 H), 8.08 (d, 0.5 H), 8.01 (d, 0.5 H), 7.90 (d, 0.5 H), 7.43 (s, 0.5 H), 7.18 (d, 0.5 H), 6.48 (s, 2H), 6.40 (d, 2H), 6.34 (d, 2H).

Elemental analysis. Calcd: C%: 66.67 ; H%: 3.73. Found: C%: 55.34; H%: 3.68.

ESI Mass spectrum: m/z = 377

6-carboxyfluorescein-dipivalate diisopropylamine salt (2). Compound **1** (2.0 g, 4.39 mmol) was dissolved in pivalic anhydride (PiV₂O) (8.2 ml, 40.42 mmoli). The resulting solution was refluxed for 2 h under a nitrogen atmosphere. The reaction mixture was then cooled at room temperature and added to 24 ml of a THF/water mixture (1:1, vol:vol). After vigorous stirring (2 h), the solution was added with diethyl ether (30 ml). The organic layer was separated and washed with a phosphate buffer (1.4 M) at pH 7 (4 x 15 ml), 1 M HCl (2 x 15 ml) and brine (3 x 15 ml). The solution was dried over anhydrous MgSO₄ and rotoevaporated to dryness. The resulting yellowish oil was dissolved in anhydrous EtOH and diisopropylamine (DIPA, 48 ml, 10.59 mmol), was slowly added to the solution. After the addition, the resulting solution was cooled at -20 °C for 24 h. A yellowish solid precipitated, which was filtered off and washed with cold EtOH and acetone.

Yield: 800 mg, 1.24 mmol, 28.1%.

¹H-NMR (400 MHz, CDCl₃); δ 8.34 (d, 1H); 8.13 (d, 1H); 7.85 (s, 1H); 7.08 (s, 2H); 6.69-6.30 (m, 4H); 3.36 (m, 2H); 1.42 (d, 12H); 1.35 (s, 18H) ppm.

Elemental analysis. Calcd: C%: 68.61 ; H%: 7.00 N%: 2.16. Found: C%: 62.40; H%: 6.87; N%: 1.88.

ESI Mass spectrum: m/z = 646

Dipivaloyl-6-carboxy-fluorescein (3). Compound **2** (800 mg, 1.24 mmol) was dissolved in CH₂Cl₂ (25 ml). The resulting solution was washed with HCl 1M (4 x 25ml). The organic layer was dried over anhydrous Na₂SO₄ and rotoevaporated to dryness to afford compound **3** as a yellow solid.

Yield: 486 mg, 0.89 mmol, 71.77 %.

¹H-NMR (400 MHz, CDCl₃); δ 9.74 (s, 1H); 8.34 (d, 1H); 8.13 (d, 1H); 7.85 (s, 1H); 7.08 (s, 2H); 6.69-6.30 (m, 4H); 1.35 (s, 18H) ppm.

Elemental analysis. Calcd: C%: 68.12 ; H%: 5.53. Found: C%: 63.87; H%: 4.86.

ESI Mass spectrum: m/z = 545.

Dipivaloyl-6-carboxyfluorescein N-hydroxysuccinimidyl ester (4). Compound **3** (380 mg, 0.70 mmol) was dissolved in anhydrous CH₂Cl₂ (4 ml). A solution of N-hydroxy-succinimidyl-trifluoroacetate (NHS-TFA, 1.29 g, 6.08 mmol) in pyridine (2.5 ml) was then added to the solution of **3**. The reaction mixture was vigorously stirred for 6 h at room temperature. Anhydrous CH₂Cl₂ (10 ml) was added and the resulting solution was washed with HCl 1M (2 x 20 ml) and brine (2 x 10 ml). The organic layer was finally dried over anhydrous Na₂SO₄ and rotoevaporated to dryness to afford a yellow solid.

Yield: 250 mg, 0.39 mmol, 55 %.

¹H-NMR (400 MHz, CDCl₃); δ 8.40 (d, 1H); 8.18 (d, 1H); 7.91 (s, 1H); 7.10 (s, 2H); 6.83 (dd, 2H); 6.78 (d, 2H); 2.89 (s, 4H); 1.36 (s, 18H) ppm.

Elemental analysis. Calcd: C%: 65.31 ; N%: 2.18; H%: 5.17. Found: C%: 58.65; N%: 1.93; H%: 4.91.

ESI Mass spectrum: m/z = 643.

Dipivaloyl-6-(5-carboxypentylaminocarbonyl) fluorescein (5). Compound **4** (250 mg, 0.40 mmol) were dissolved in anhydrous CH₂Cl₂ (5 ml). To the resulting solution was added a solution of 6-amino-hexanoic acid (70 mg, 0.53 mmol) in anhydrous CH₂Cl₂ (6 ml). The reaction mixture was stirred for 2 h at room temperature. The solution was rotoevaporated to dryness to afford a yellow solid.

Yield: 219 mg, 0.33 mmol, 83 %.

¹H-NMR (400 MHz, DMSO-d₆); δ 9.68 (s, 1H); 8.45 (d, 1H); 8.33 (d, 1H); 8.16 (s, 1H); 7.30 (d, 2H); 7.05 (d, 2H); 6.93 (dd, 2H); 3.20 (t, 2H); 2.88 (m, 2H); 2.18 (t, 2H); 1.48 (m, 4H); 1.33 (s, 18H) ppm.

Elemental analysis. Calcd: C%: 67.36 ; N%: 2.12; H%: 6.26. Found: C%: 59.63; N%: 1.87; H%: 3.98.

6-(5-carboxypentylaminocarbonyl) fluorescein (6).

An aqueous solution of 28% NH₃ (1 ml) was added to solution of **5** (204.5 mg, 0.31 mmol) in anhydrous CH₂Cl₂ (5 ml). The resulting mixture was stirred for 2 h. Water (10 ml) was added and the two layers were separated. The pH of the aqueous phase was adjusted to 2 by addition of 10% HCl, to induce the precipitation of a yellow solid. After cooling in ice, the resulting yellow precipitate was filtered off, washed with cold water and dried in desiccator.

Yield: 72.7 mg, 0.15 mmol, 48 %.

¹H-NMR (400 MHz, CDCl₃); δ 9.94 (s, 1H), 8.40 (d, 1H); 8.18 (d, 1H); 7.91 (s, 1H); 7.10 (s, 2H); 6.83 (d, 2H); 6.78 (d, 2H); 3.29 (t, 2H); 2.23 (t, 2H); 1.53 (m, 4H); 1.34 (m, 2H) ppm.

Elemental analysis. Calcd: C%: 66.25 ; N%: 2.86; H%: 4.74. Found: C%: 56.55; N%: 2.49; H%: 3.69.

ESI Mass spectrum: m/z = 488.

6-(fluorescein-6-carboxamido)-hexanoic acid succinimidyl ester (7). Compound **7** (15 mg, 0.031 mmol) was suspended

in anhydrous CH₂Cl₂ and added of a solution of N-hydroxy-succinimidyl-trifluoroacetate (NHS-TFA, 62.7 mg, 0.30 mmol) in anhydrous pyridine (0.2 ml). The resulting yellow solution was vigorously stirred for 6 h at room temperature. Anhydrous CH₂Cl₂ (3 ml) was added and the resulting solution was washed with HCl 1M (2 x 3 ml) and brine (2 x 3 ml). The organic layer was dried over anhydrous Na₂SO₄ and rotoevaporated to dryness to afford a yellow solid.

Yield: 17.3 mg, 0.029 mmol, 94 %.

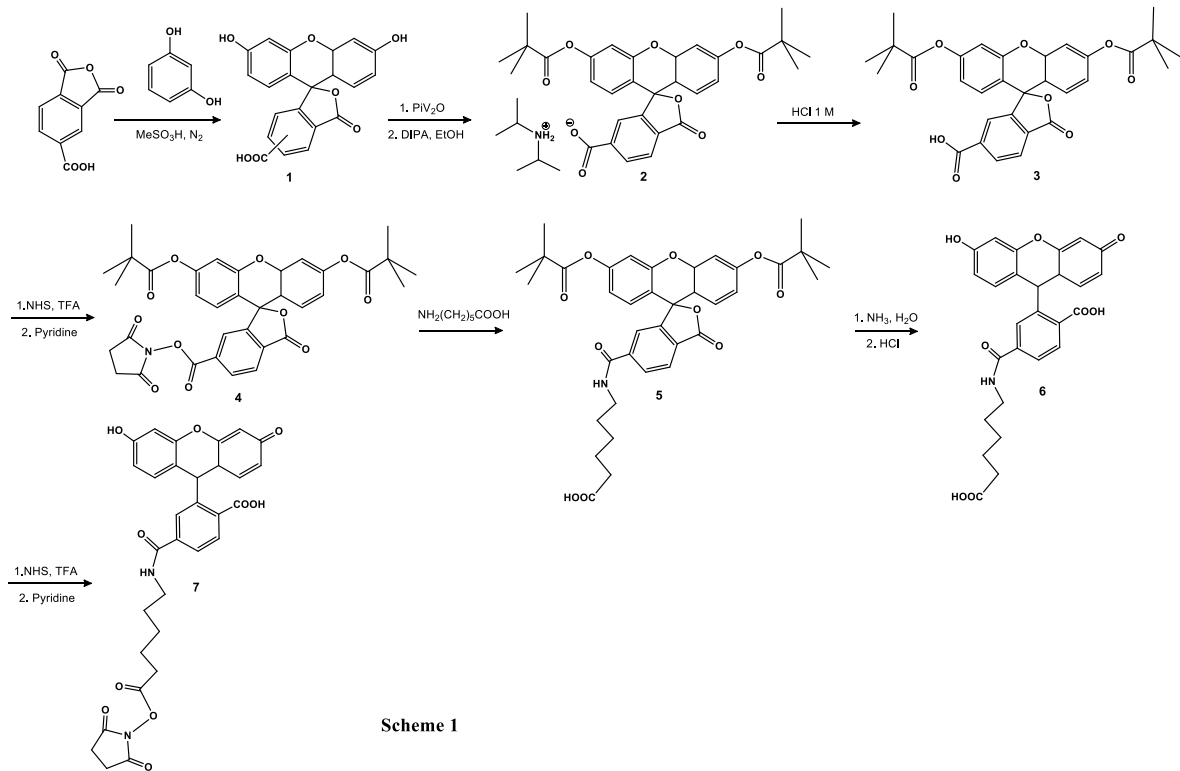
¹H-NMR (400 MHz, CDCl₃); δ 9.95 (s, 1H), 8.40 (d, 1H); 8.17 (d, 1H); 7.91 (s, 1H); 7.10 (s, 2H); 6.83 (d, 2H); 6.77 (d, 2H); 3.31 (t, 2H); 2.28 (t, 2H); 1.61 (m, 2H); 1.44 (m, 4H); 2.69 (t, 4H) ppm.

Elemental analysis. Calcd: C%: 63.48 ; N%: 4.78; H%: 4.47. Found: C%: 56.12; N%: 3.98; H%: 3.79.

ESI Mass spectrum: m/z = 585.

1) Wu XL, Fan WT. Synthesis Spectroscopic Properties, and Cell Imaging of Novel Chlorinated Fluorescent Proteins-labeling Probe, *Journal of Life Sciences and Technologies* 2013 Dec; 1(4): 210-215.

2) Thomas DA, Sohn CH, Gao J, and Beauchamp JL. Hydrogen Bonding Constrains Free Radical Reaction Dynamics at Serine and Threonine Residues in Peptides, *J. Phys. Chem. A*, 2014, 118, 8380–8392.



Post mortem MRI

EAE mice were perfused with PFA 4%, then spinal cord and brain were explanted and embedded in perfluoropolyether lubricant (Fomblin PFPE, Solvay) in glass tubes. Imaging was performed in a 9.4 T Avance II 400WB MRI scanner (Bruker Biospin, Germany) equipped with a great 40 Gradient Amplifiers, Micro2.5 Gradient and MicWB40 10 mm 1H Resonator. We used data acquired from a high-resolution axial T2 weighted multi slice multi echo (T2w MSME; nominal in-plane resolution = 47 x 63 μ m, field of view 12x12 mm, slice thickness 0.3 mm, number of echoes 12, number of slices 40, repetition time 3500 milliseconds, echo time 4.77 milliseconds, number of averages 32, acquisition time 5 hours and 58 minutes). Data were analyzed using MIPAV software (NIH, Bethesda, MD, USA). Images were screened for the presence of focal areas of signal hypointensity compatible with paramagnetic NBR loaded cells infiltration. The presence of areas of hyperintense signal abnormality reflecting inflammatory demyelination was also assessed. To achieve a correlation between MRI and pathology, visually matched histopathological sections were analysed for the presence of NBR loaded cells in areas corresponding to MRI signal abnormalities.