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Supplementary Material

Identification of the Protein Target of Myelin-Binding Ligands by Immunohistochemistry and Biochemical Analyses

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Supplementary Figure 1

Supplementary Figure 1. Purity of native MBP used in fluorescence polarization experiments as detected by 15% SDS Polyacrylamide gel. The first lane contains the molecular weight markers, while the next three lanes contain the purified MBP.

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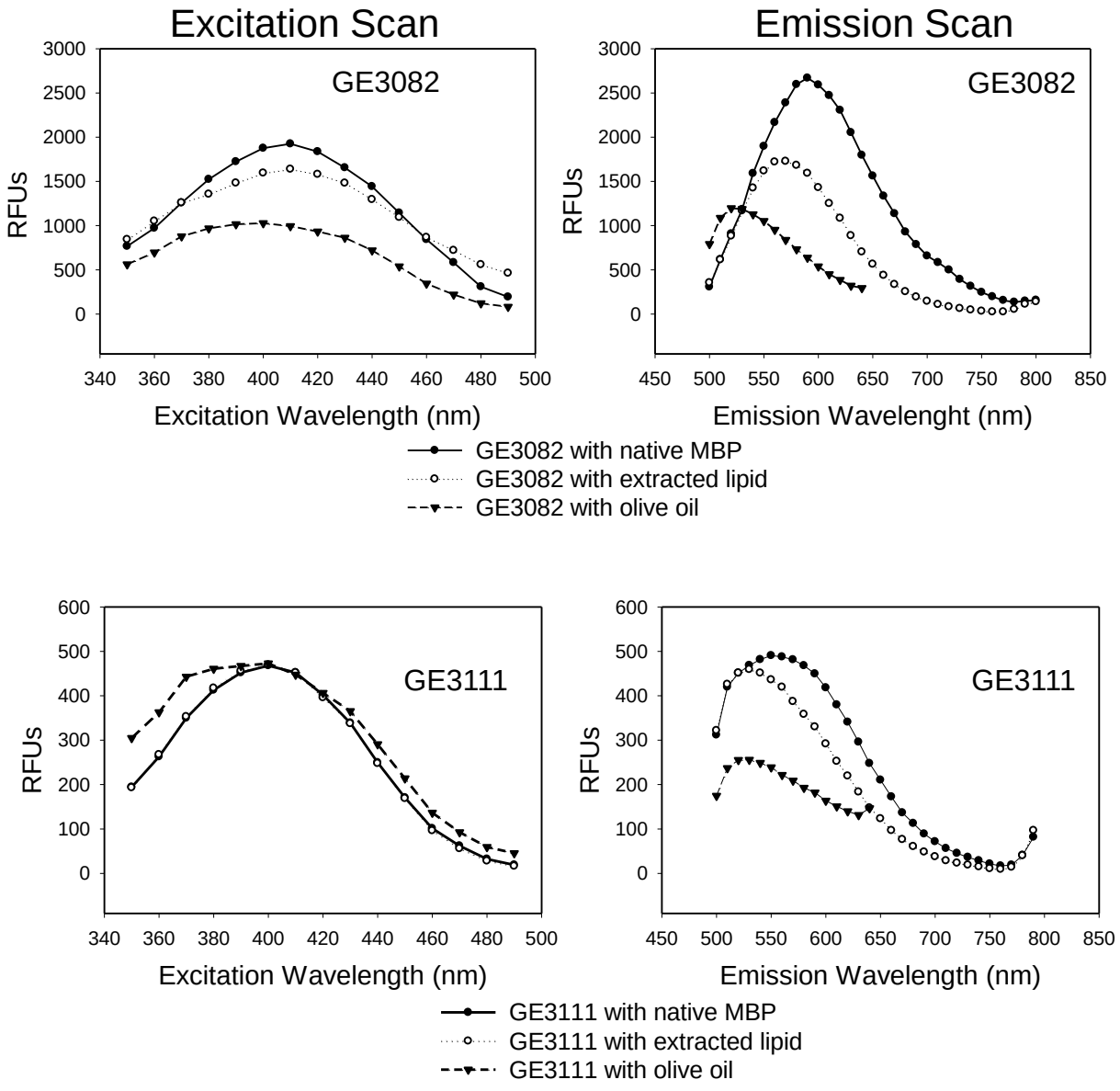
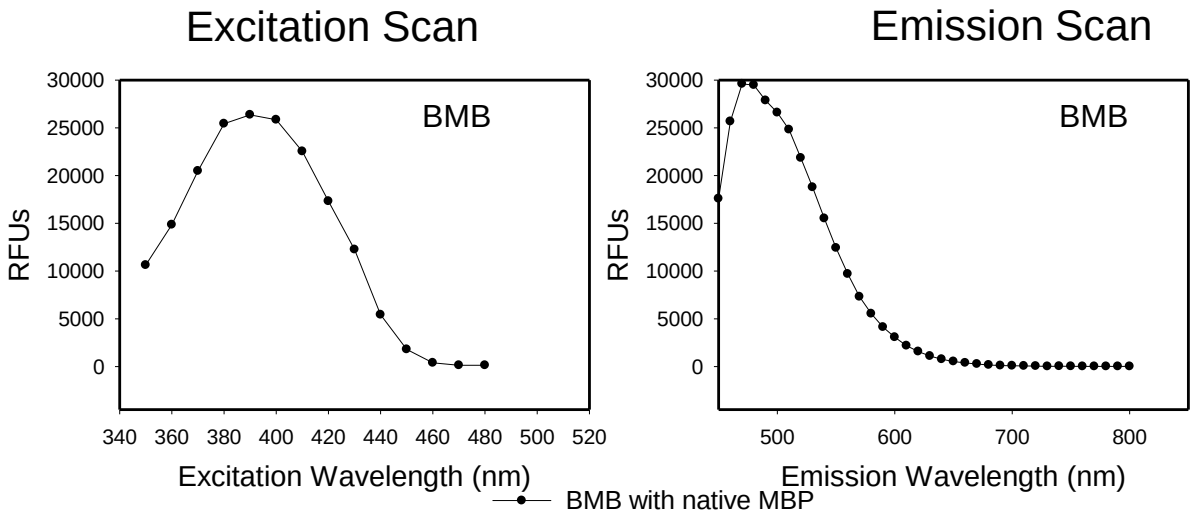
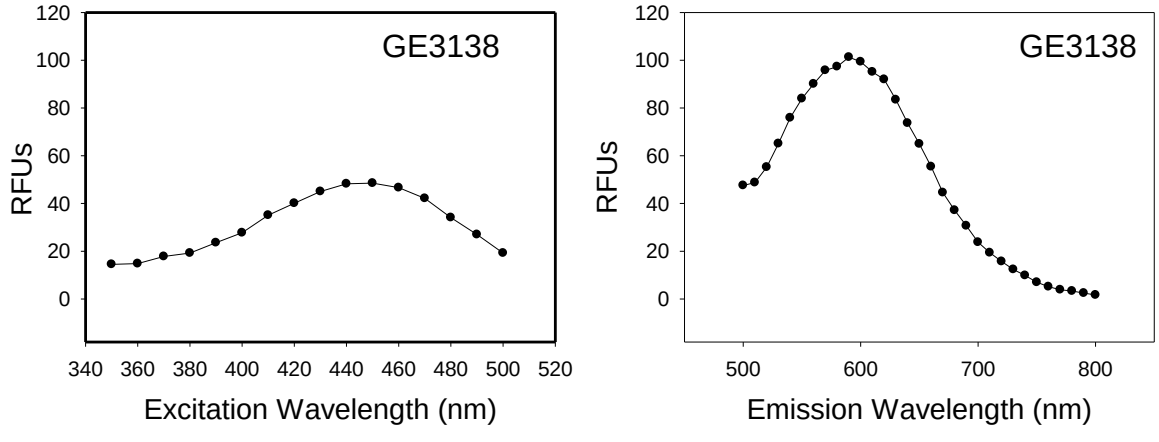
Supplementary Figure 2



Supplementary Figure 2. Excitation and emission spectra of 10 μM GE3082 (top) and 10 μM GE3111 (bottom) in the presence of native MBP, extracted lipid and olive oil. 10 μM fluorophore was mixed with 1.6 μM native MBP or 1.6 μM extracted lipid or 50% olive in the FP binding buffer buffer (0.25% CHAPS in 20 mM Tris, pH 7.5). The reagents were incubated for 10 min at room temperature in a 96-well plate after which the spectra were recorded using the fluorescence mode of Spectra Max M5 (Molecular Devices).

Bajaj et al. Supplementary Figure 3





Supplementary Figure 3. Excitation and emission spectra of 10 μM BMB and 10 μM GE3138 in the presence of native MBP. 10 μM fluorophore was mixed with 1.6 μM native MBP in the FP binding buffer. The reagents were incubated for 10 min at room temperature in a 96-well plate after which the spectra were recorded using the fluorescence mode of Spectra Max M5 (Molecular Devices).

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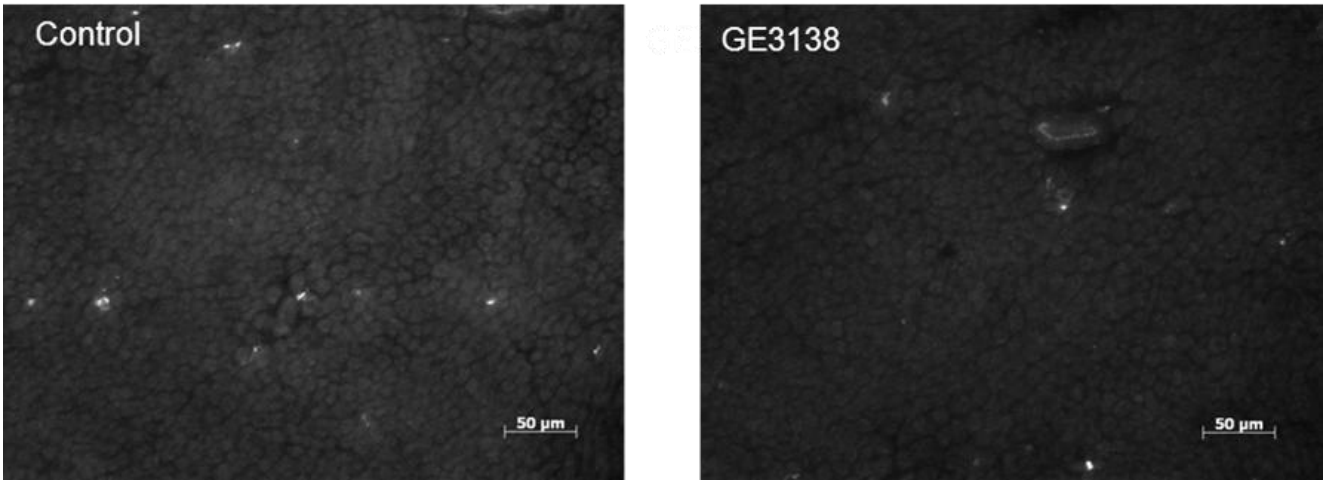
Supplementary Figure 4



Supplementary Figure 4. Ex vivo staining of rat sciatic nerve section with GE3138 and a control nerve section that underwent the same experimental procedure but was not exposed to any fluorophores. GE3138 (with a final concentration of 10 μM) was added onto the tissue in a buffer containing 10% Cremophor EL and 65% rat serum in PBS. The slides were incubated for 1 h in a dark, humid chamber after which they were washed with PBS (3 x 5 min), cover-slipped, and imaged using a custom filter cube (excitation filter: 460 nm with 60 nm band pass, emission filter: 630 nm with 92 nm band pass). A buffer only control (no GE3138) was also performed using exactly the same procedure to determine autofluorescence under the same settings.