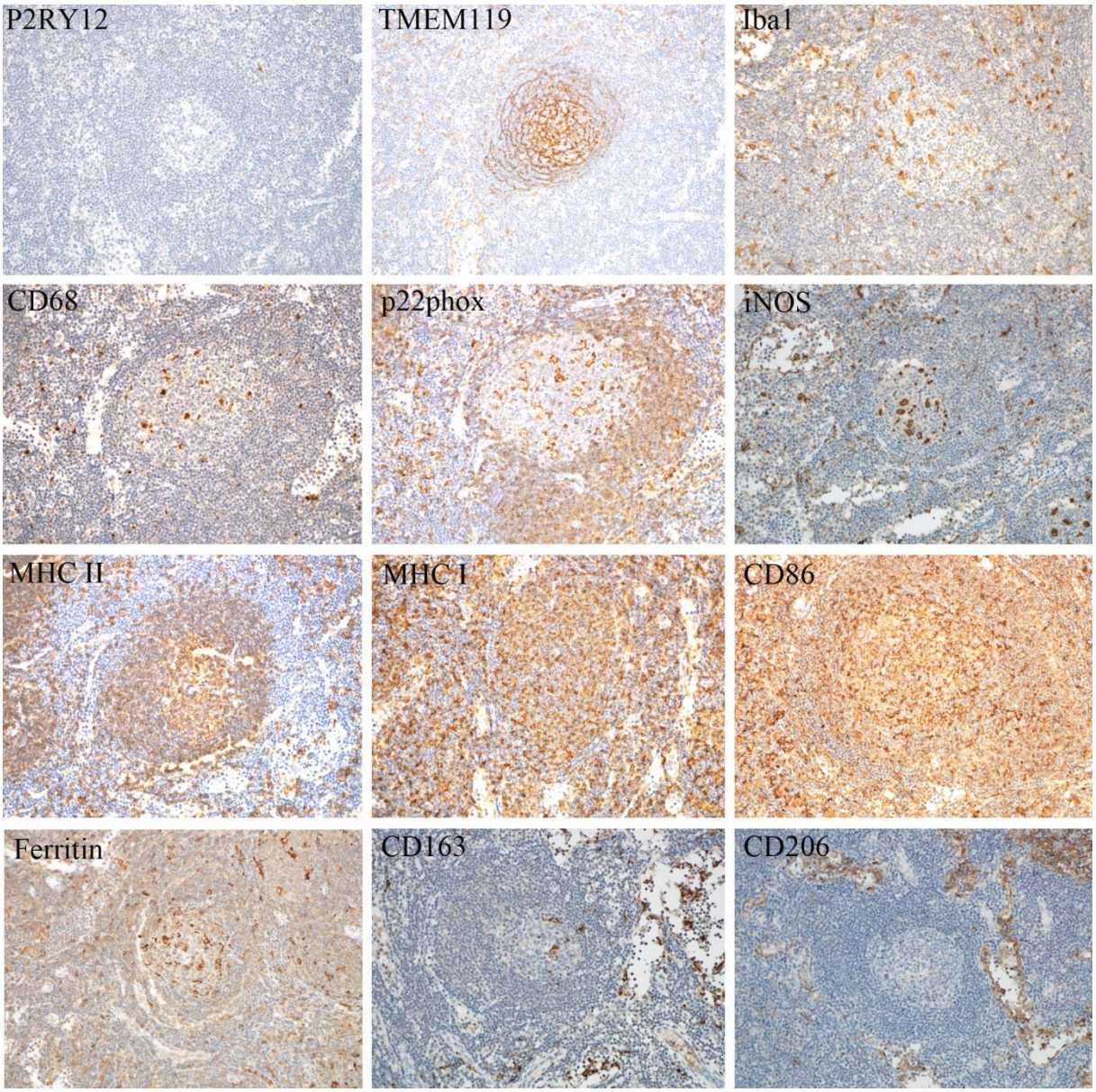
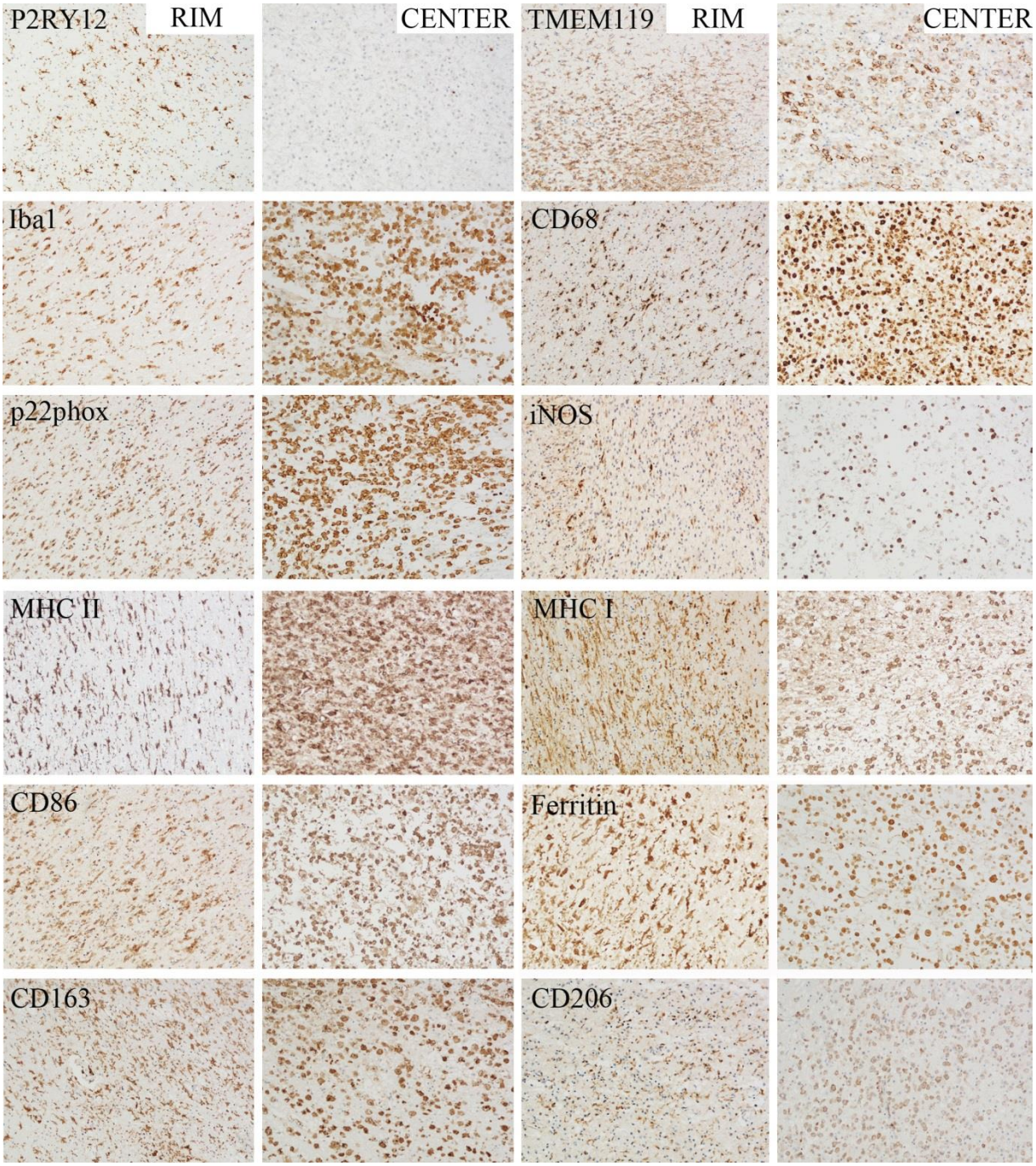
Supplementary Figure 1: Macrophage microglia marker expression in lymph node



Supplementary Figure 2: Macrophage/microglia marker expression in the MS brain



RIM: microglia like cells at the lesion margin

CENTER: macrophage like cells in the active lesion center

Supplementary Figure 3: Double staining for P2RY12 or Iba-1 with TMEM119

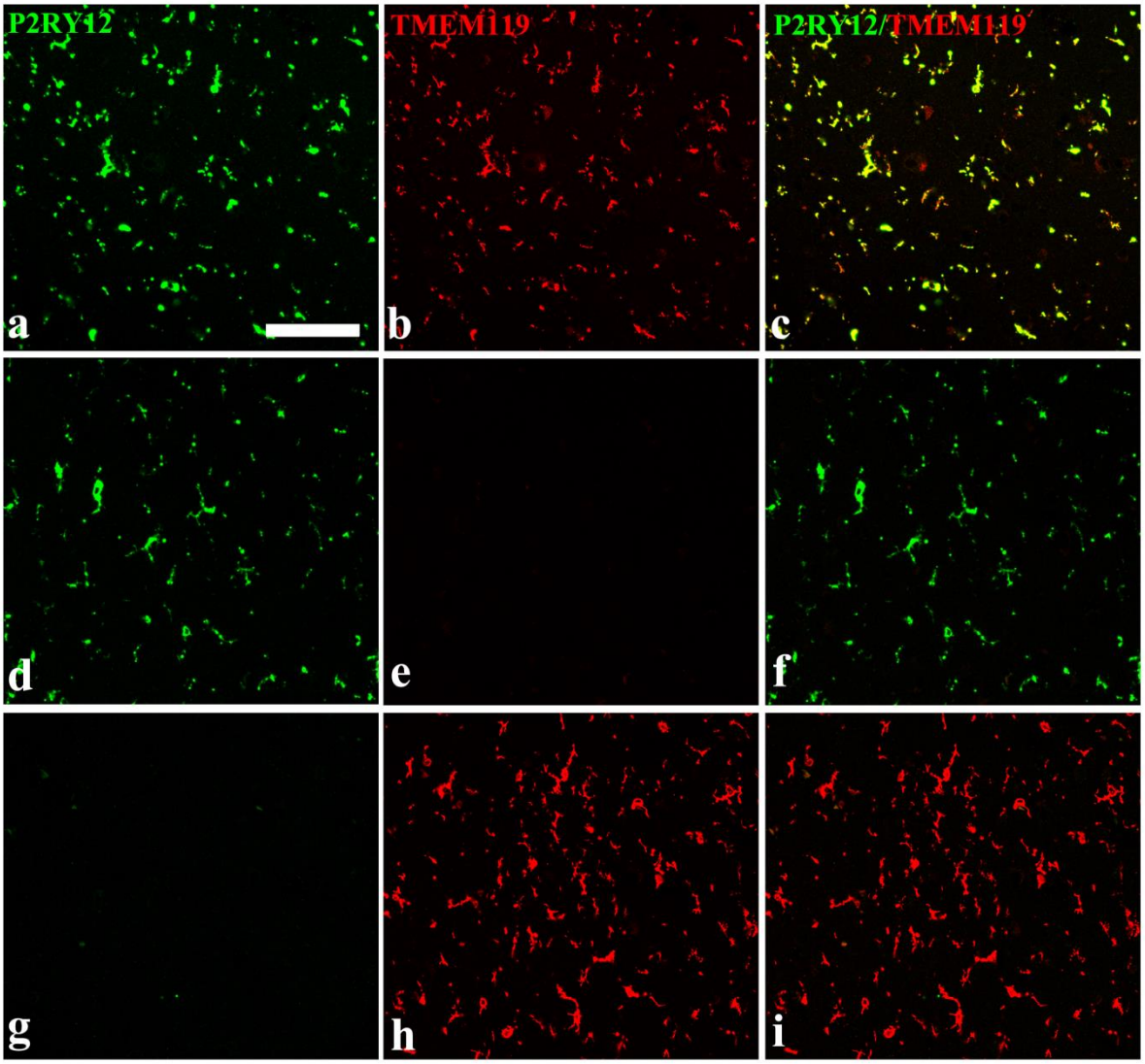


Figure legend:

Double staining for P2RY12 and TMEM119 was performed as described below. Human control brain white matter was stained. The first row (figures a-c) shows the full double staining and the results are documented for the single color channels for the detection of P2RY12 (green) and TMEM 119 (red) and co-localization results in a yellow image in panel c. Note that most of the microglia cells in the normal human white matter express both, P2RY12 and TMEM119, while there are few additional TMEM119 positive microglia, which lack immunoreactivity for P2RY12 (red cells in panel c).

The lower panels show the specificity controls of the immunocytochemical reaction. In Figure d-e the entire reaction was performed, but the primary antibody for TMEM119 was omitted, thus giving selectively a signal for P2RY12 (green). In figure g-I the primary antibody for P2RY12 was omitted, thus showing only a signal for TMEM119 (red).

Method:

Double staining for P2RY12 or Iba1 and TMEM119 was performed according to Bauer and Lassmann (2016), since both primary antibodies come from the same species (rabbit). We used extensive heat-induced epitope retrieval between the subsequent immunohistochemical reactions. After de-paraffination and a primary round of antigen retrieval (Table 2) the sections were incubated with anti-P2RY12 or Iba-1, followed by biotinylated anti-rabbit antibody, by avidin peroxidase and catalyzed signal amplification with biotinylated tyramide. Then another round of antigen retrieval for 30 minutes at pH 9.5 was performed, which abolishes antibody reactivity from the previous round, but leaves the amplified avidin binding sites intact, thus allowing the localized binding Cy2 labeled streptavidin. Then the second immunohistochemistry reaction was applied using TMEM119 antibody as the primary antibody and (goat) anti rabbit Cy3 as a secondary antibody (Table 1). For control serial sections were stained with the identical protocol, but the P2RY12 or the TMEM119 primary antibody were omitted. Fluorescent preparations were examined using a Leica SP2 confocal laser scan microscope (Mannheim, Germany).