**Fig. 1S** Gating strategy of CNS immune cells in cuprizone model by multi-dimensional flow cytometry. Live leukocytes were gated to CD45lo and CD45hi cells. CD45hi cells were gated to CNS-infiltrating myeloid cells (CD45hiCD11b+) and lymphocytes (CD45hiCD3+). Infiltrating myeloid cells (CD45hiCD11b+) were further gated to monocytes (CD45hiCD11b+LysC+) and granulocytes (CD45hiCD11b+ Ly6G+).

**Fig. 2S** Characterization of glial cell pathology in S1P1-GFP signaling mice following cuprizone exposure. (**a**) Representative images of oligodendrocytes (Olig2+) in the CNS of S1P1-GFP signaling mice during demyelination (0-6 weeks) and remyelination (8 week) in the cuprizone model. The MCC is shown in the square box. In the upper right corner magnification of oligodendrocytes in the S1P1 signaling mice depicted. (**b**) Degree of astrocytosis (GFAP+) during demyelination (0-6 weeks) and remyelination (8 weeks). Arrowheads represent MCC, SVZ and CTX in the brain. (**c**) Macrophage/microglia activation (CD68+) following exposure to cuprizone diet. Arrowheads indicate MCC, SVZ, LCC and CP. (**d**) Graph representing the number of Olig2+ cells in 0.1 mm2 of MCC upon cuprizone induced demyelination (0-6 weeks), and remyelination (8 weeks), (n=3 mice/groups). Values shown are expressed as mean ± SEM. Unpaired t-test was used to determine the statistical significance between two groups, \**p*<0.05. Scale bars: A, 50 and 20 µm, B and C 400 µm. MCC; Medial corpus callosum, LCC; Lateral corpus callosum, CP; caudoputamen, CTX; Cortex, SVZ; Subventricular zone, GFAP; Glial fibrillary acidic protein.

**Fig. 3S** GFP expression in the corpus callosum of GFP control and S1P1-GFP signaling mice. (**a-b**) GFP expression in the LCC of S1P1-GFP signaling and GFP reporter mice at 0, 2, 4, 6, and 8 weeks of cuprizone diet. (**c-d**) GFP expression in the MCC of S1P1-GFP signaling and GFP control mice at 0, 2, 4, 6, and 8 weeks of cuprizone diet. scale bars, 50 µm. (**e**) The graph represents the number of GFP+ cells in 0.1 mm2 of the MCC in GFP reporter and S1P1-GFP signaling mice at 0, 2, 4, 6, and 8 weeks of cuprizone diet. Values shown are expressed as mean ± SEM. LCC; Lateral corpus callosum, MCC; Medial corpus callosum.

**Fig. 4S** S1P1 signaling in oligodendrocytes progenitor cells depicted by GFP expression during remyelination.GFP expression in NG2+Olig2+ OPCs in the SVZ of S1P1-GFP signaling mice at 8 weeks following exposure to cuprizone. White arrowheads indicate S1P1 signaling in NG2+Olig2+ cells. Magenta arrowheads indicate Olig2+NG2+ cells. The image on the right shows a higher magnification of GFP expression in Olig2+NG2+ cells. Scale bars, 20 µm.

**Fig. 5S** S1P1 signaling in oligodendrocytes, neural stem cells and myeloid cells.The graphs represent GFP expression in myeloid cells, oligodendrocytes and neural stem cells to the total GFP+ cells in 0.1 mm2 of theCC in GFP reporter and S1P1-GFP signaling mice (n=3 mice/group).

**Fig. 6S** Flow cytometry analysis of S1P1 signaling via GFP expression in cerebral immune cells upon cuprizone induced demyelination.Number of CNS infiltrating immune cells at 0, 2, 4 and 6 weeks of cuprizone diet in GFP reporter and S1P1-GFP signaling mice. (**a**)Number of CNS infiltrating immune cells (CD45hi), (**b**) myeloid cells (CD45hi CD11b+), (**c**) lymphocytes (CD45hiCD3+) and (**d**) granulocytes (CD45hiLysG+) during demyelination (0-6 weeks) in S1P1-GFP signaling and GFP control mice. (**e**) The number of GFP+ cells in infiltrating immune cells, (**f**) myeloid cells (CD11b+CD45hi), (**g**) lymphocytes (CD3+CD45hi) and (**h**) granulocytes (LysG+CD45hi) in GFP reporter and S1P1-GFP signaling mice. The number of (**i**) GFP+CD45lo and (**J**) GFP+CD45loCD11b+ (resident microglia) in GFP reporter and signaling mice. Values shown are expressed as mean ± SEM. Unpair t-test was used to analyze significant difference between two groups. \**p*<0.05, \*\*p<0.01, \*\*\*P<0.001, and \*\*\*\*P<0.0001.

**Fig. 7S** Flow cytometry analysis of S1P1 signaling in the splenic immune cells upon cuprizone induced demyelination.S1P1 signaling via GFP expression in myeloid cells and lymphocytes during demyelination in the cuprizone model (0, 2, 4 and 6 weeks)**.** (**a**) Number of GFP expressing leukocytes (GFP+CD45+), (**b**) myeloid (CD11b+) and (**c**) lymphocytes (CD3+) in the spleens of S1P1-GFP signaling and GFP reporter mice at different time points of cuprizone diet. (**d**) Number of myeloid (CD11b+) cells and (**e**) lymphocytes (CD3+) in the spleens of GFP reporter and S1P1-GFP signaling mice. Values shown are expressed as mean ± SEM. Unpair t-test was used to analyze significant difference between two groups, \**p*<0.05 and \*\**p*<0.01.

**Fig. 8S** Flow cytometry analysis of S1P1 signaling via GFP expression in splenic myeloid cells of EAE mice. Gating of GFP expression in myeloid cells (CD45+CD11b+) isolated from the spleen of mice at day 8 post immunization**.** GFP+CD11b+ cell percentage is shown for naïve wild type, naïve GFP reporter, EAE GFP reporter and EAE S1P1-GFP signaling mice. EAE; Experimental autoimmune encephalomyelitis

**Fig. 9S** Flow cytometry analysis of S1P1 signaling via GFP expression in splenic lymphocytes of EAE mice.Gating of GFP expression in lymphocytes isolated from the spleen of

mice at day 8 post immunization. (**a**) Gating represents GFPexpression in T cells (CD3+) and (**b**) B cells (CD19+) from naïve wild type, naïve GFP reporter, EAE GFP control and EAE S1P1-GFP

signaling mice.