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Supplementary Information for

ASK1 signaling regulates phase specific glial interactions during neuroinflammation

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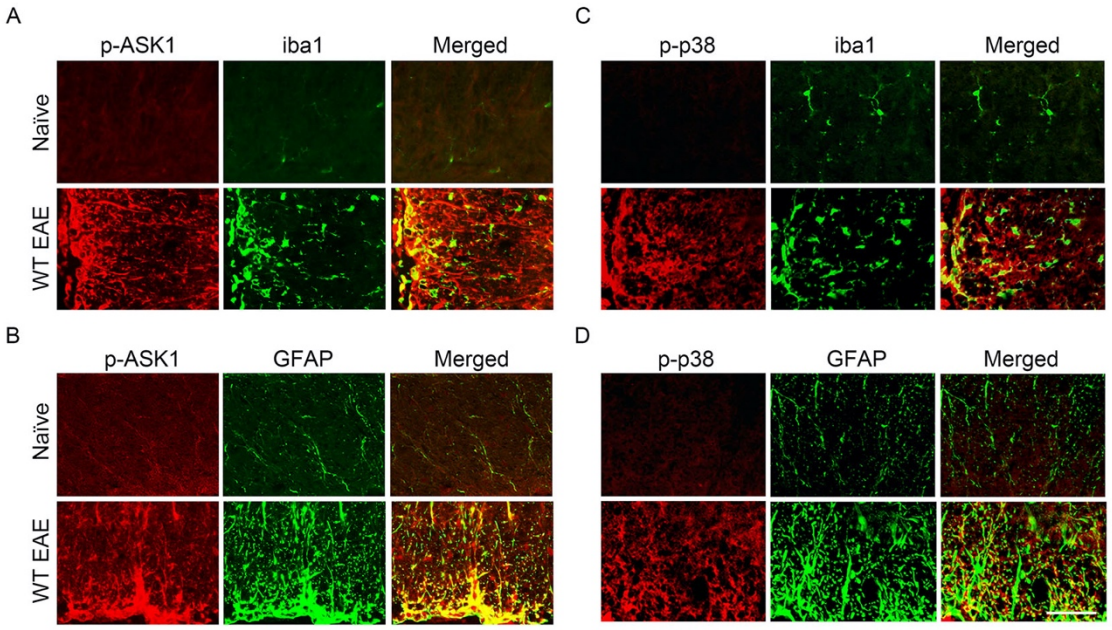

Figure S1. Immunohistochemistry staining of p-ASK1 and p-p38 in the white matter of the spinal cord of EAE mice.

A Immunohistochemical staining with p-ASK1 and iba1 in WT normal and WT EAE mice.

B Immunohistochemical staining with p-ASK1 and GFAP in WT normal and WT EAE mice.

C Immunohistochemical staining with p-p38 and iba1 in WT normal and WT EAE mice.

D Immunohistochemistry staining with p-p38 and GFAP in WT normal and WT EAE mice. Scale bar: 100 μm.

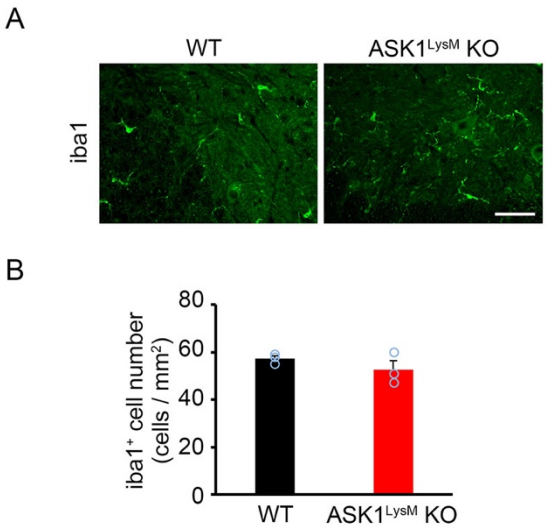

Figure S2. Cell numbers of microglia/macrophages in the spinal cords of naïve WT and ASK1^{LysM} KO mice.

A Immunohistochemistry staining of microglia/macrophage in the white matter of the spinal cords of naïve WT and ASK1^{LysM} KO mice. Scale bar: 50 μm.

B Quantification of iba1-positive cells in (A). *n* = 3 mice per group.

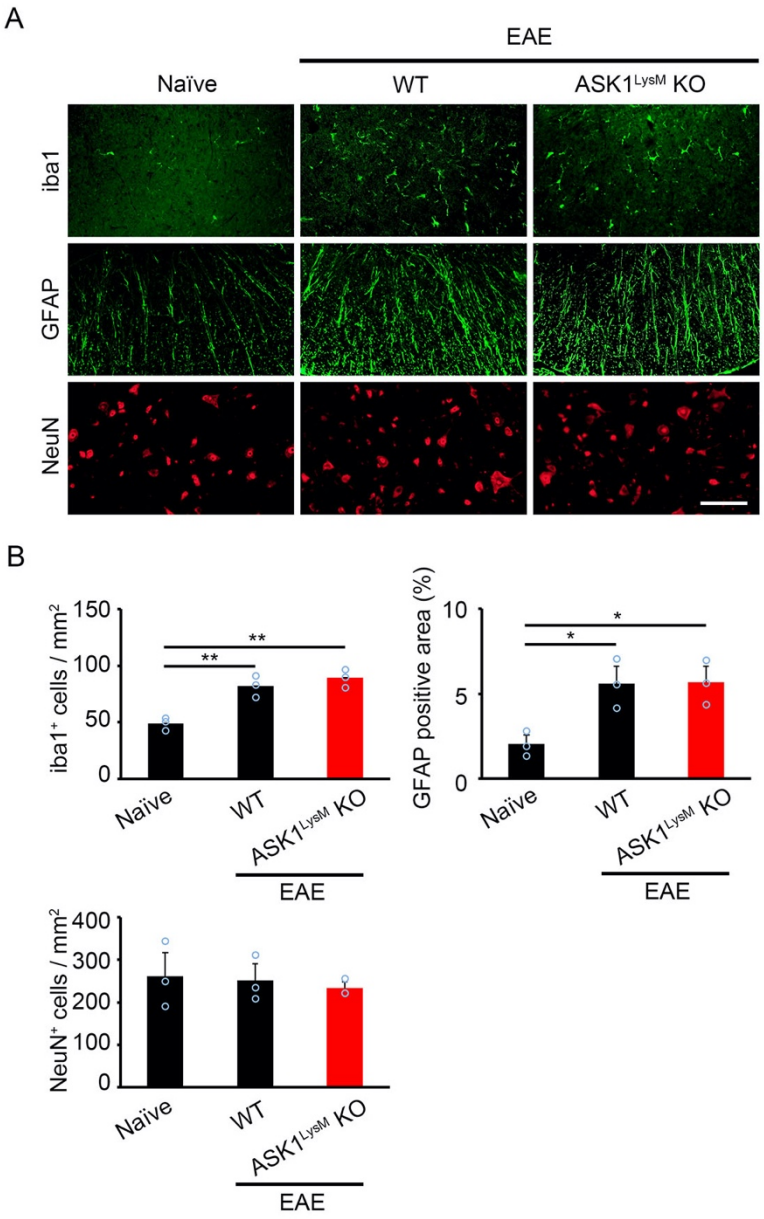

Figure S3. Immunohistochemistry staining of microglia/macrophage, astrocytes and neurons in the spinal cords of EAE mice during the pre-symptomatic phase of EAE.

A Immunohistochemistry staining of iba1 and GFAP in the white matter and NeuN in the gray matter of the spinal cords of WT naïve, WT EAE and ASK1^{LysM} KO EAE mice during the pre-symptomatic phase of EAE. Scale bar: 100 μm.

B Quantification of iba1-, GFAP- and NeuN-immunopositive areas in (A). n = 3 mice per group. $^{**}P < 0.01$; $^{*}P < 0.05$.

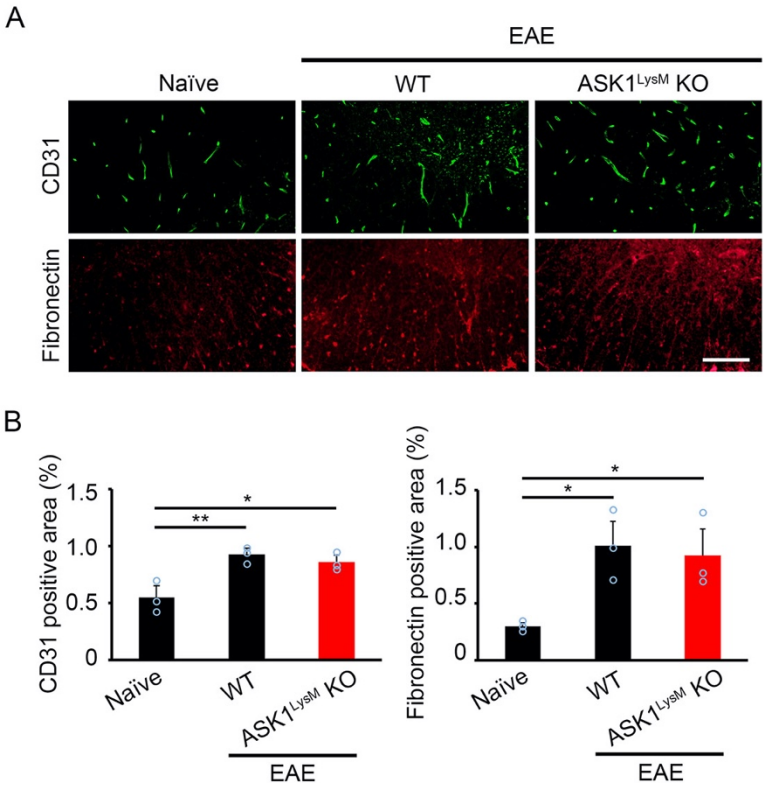

Figure S4. Immunohistochemistry staining of epithelial cells and fibronectin in the spinal cords of EAE mice during the pre-symptomatic phase of EAE.

A Immunohistochemistry staining of epithelial cells and fibronectin in the white matter of the spinal cords of WT naïve, WT EAE and ASK1^{LysM} KO EAE mice during the pre-symptomatic phase of EAE. Scale bar: 100 μm.

B Quantification of CD31- or fibronectin-positive areas in (A). n = 3 mice per group. $^{**}P < 0.01$; $^{*}P < 0.05$.

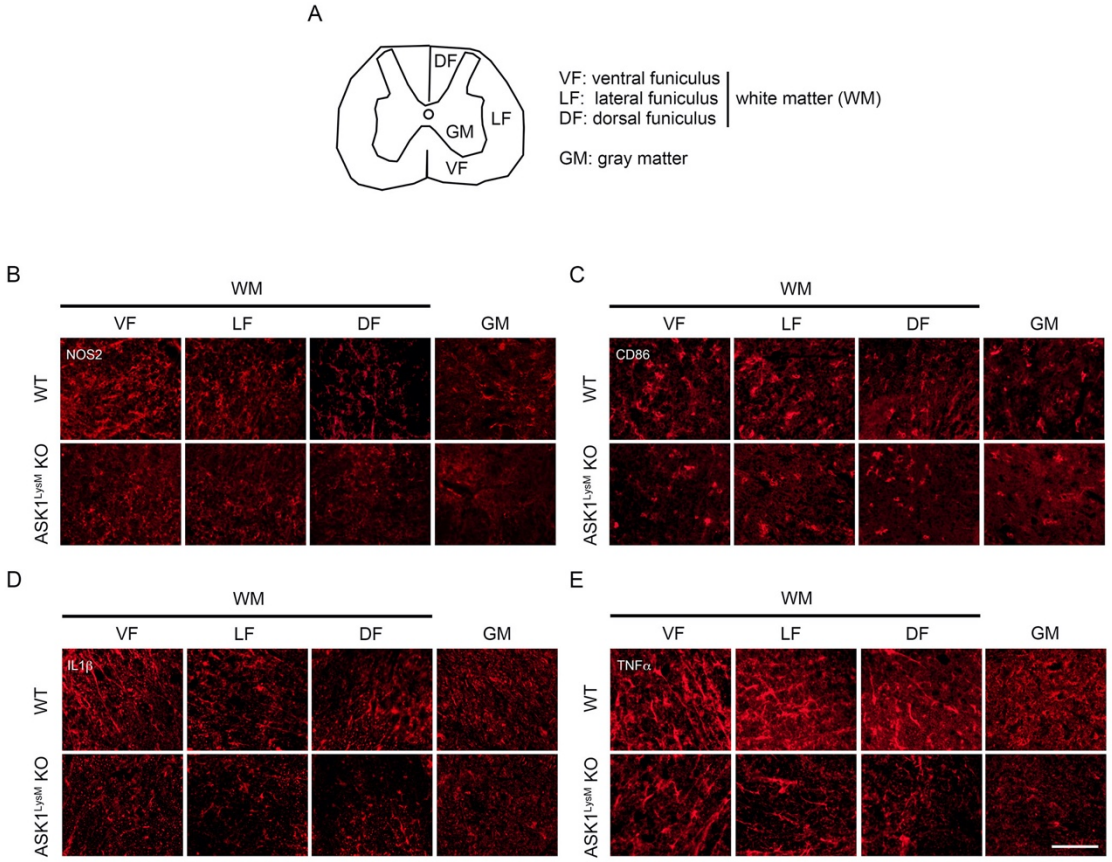

Figure S5. Immunohistochemistry staining of key genes in the spinal cord of WT and ASK1^{LysM} KO EAE mice on d17.

A Schematic structure of the spinal cord.

B-E Immunohistochemical staining of NOS2 (B), CD86 (C), IL1β (D) and TNFα (E) in ventral funiculus (VF), lateral funiculus (LF), dorsal funiculus (DF) of the white matter (WM) and gray matter (GM) in the spinal cords of WT and ASK1^{LysM} KO EAE mice on d17. Scale bar: 110 μm.

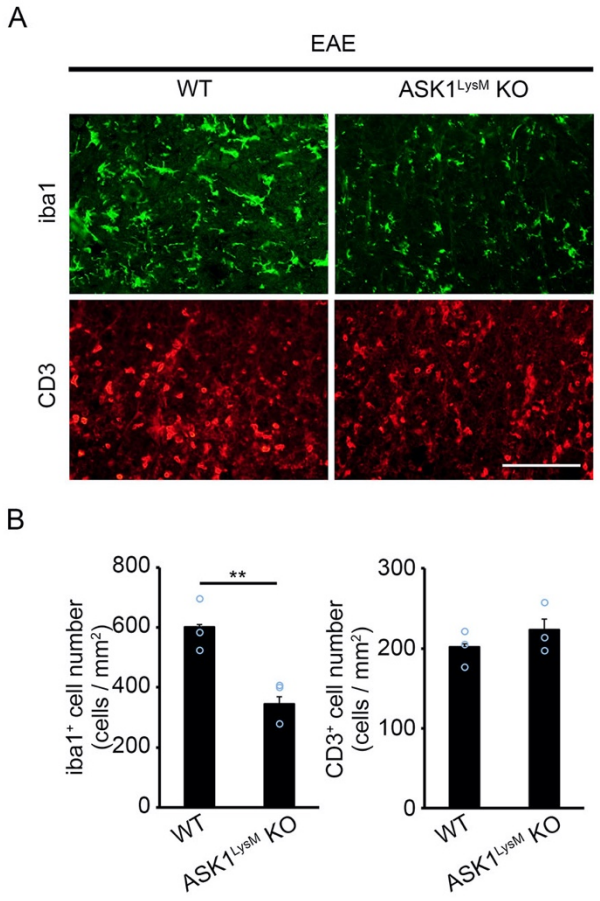

Figure S6. Cell numbers of microglia/macrophages and T cells in the spinal cords of WT and ASK1^{LysM} KO EAE mice on d17.

A Immunohistochemistry staining of microglia/macrophage and T cell in the white matter of the spinal cords of EAE mice on d17. Scale bar: 100 μm.

B Quantification of cell numbers in (A). Values are shown as mean ± SEM. n = 3 mice per group.

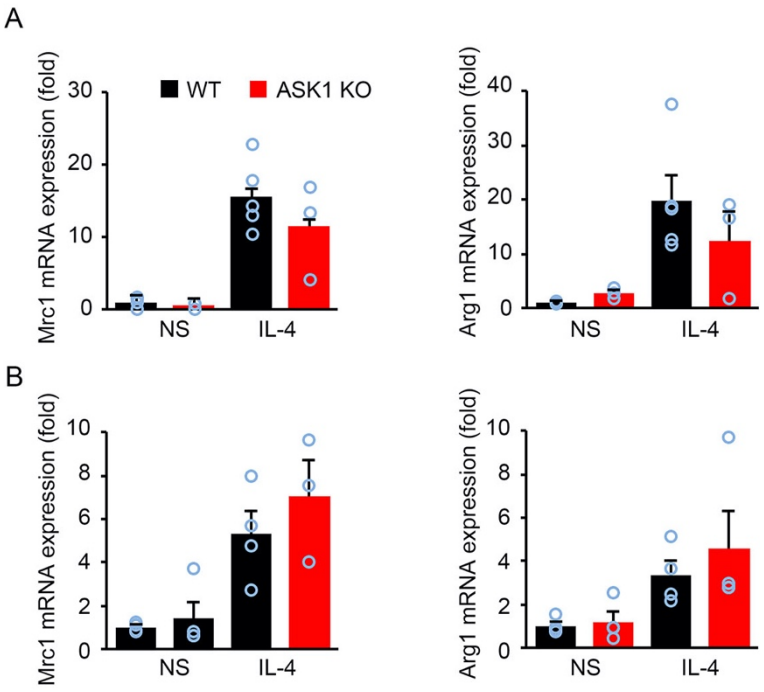

Figure S7. ASK1 deficiency has no effects on the polarization of anti-inflammatory microglia/macrophages.

A, B qPCR analysis of Mrc1 and Arg1 in IL-4-stimulated WT and ASK1 KO microglia (A) and macrophages (B). Bone marrow derived macrophages (BMDM) were generated from WT and ASK1 KO mice. Experiments were carried out in a 96 well plate format with 3-5 wells used for each culture condition. Experiments were repeated three times and representative results were shown. Values are shown as mean ± SEM.

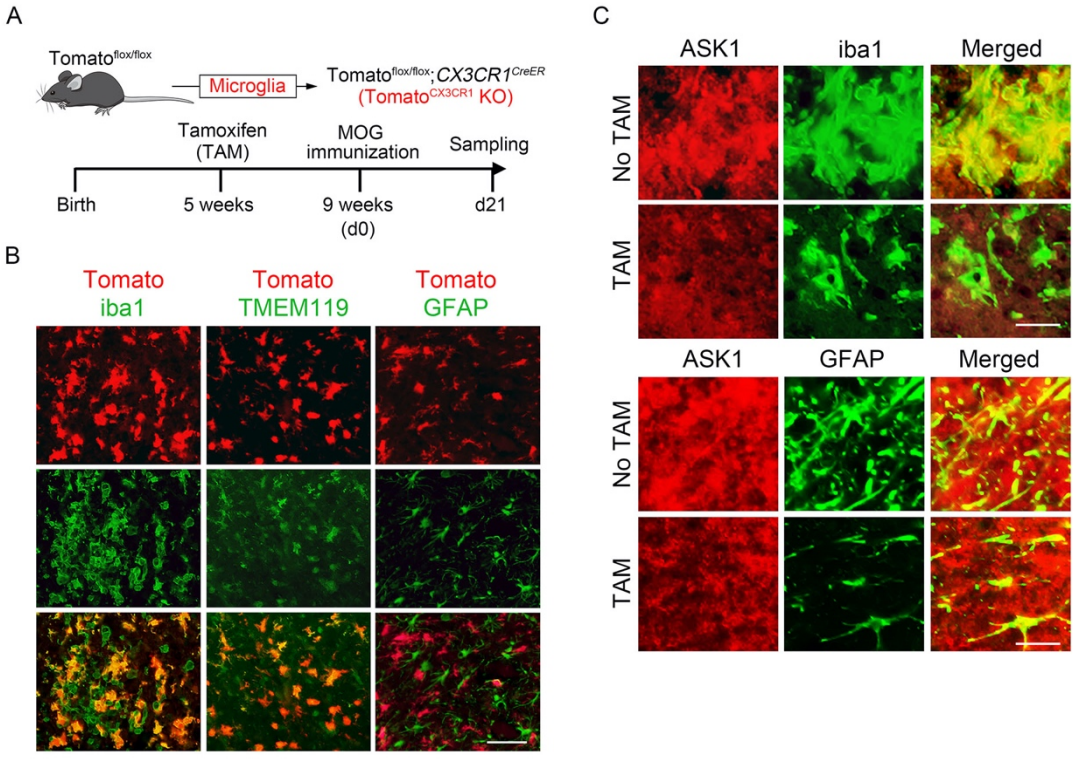

Figure S8. Confirmation of microglia-specific ASK1 deficiency in ASK1^{CX3CR1} KO mice.

A Schematic diagram illustrating the breeding strategy of Tomato^{CX3CR1} transgenic (Tg) mice and experimental design for assessing Cre mediated Tomato expression in microglia.

B Immunohistochemistry staining of iba1, TMEM119 and GFAP in the white matter of the spinal cords of Tomato^{CX3CR1} Tg EAE mice pre-administrated with TAM. Scale bar: 50 μm.

C Immunohistochemical staining of ASK1 and iba1 or GFAP in the white matter of the spinal cords of ASK1^{CX3CR1} KO EAE mice pre-administrated with or without TAM on d30. Scale bar: 20 μm.

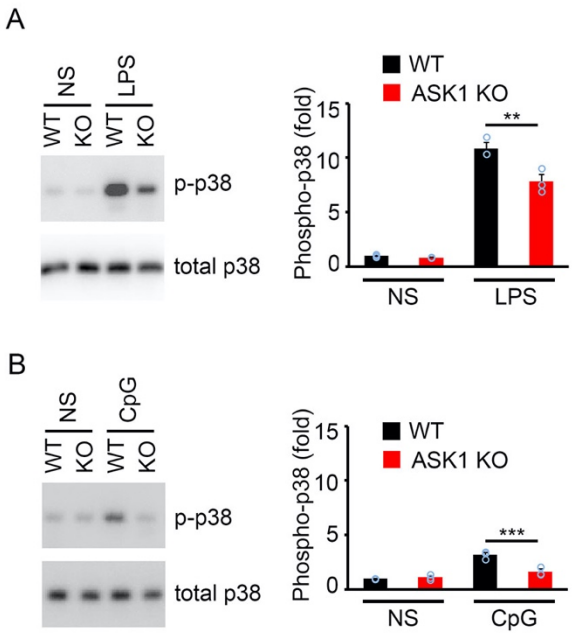

Figure S9. ASK1 is required for TLR ligand-induced p38 activation in microglia.

A Effect of ASK1 on LPS induced p38 activation. Microglia derived from WT and ASK1 KO mice were stimulated with LPS (1 ng) for 30 min, followed by immunoblot analysis of total and phosphorylated p38 (p-p38) in cell lysates. Experiments were carried out in a 96 well plate format with 3 wells used for each culture condition.

B Effect of ASK1 on CpG induced p38 activation. Microglia derived from WT and ASK1 KO mice were stimulated with CpG (10 μM) for 30 min, followed by immunoblot analysis of total and phosphorylated p38 in cell lysates. NS: non-stimulated. Experiments were carried out in a 96 well plate format with 3 wells used for each culture condition.

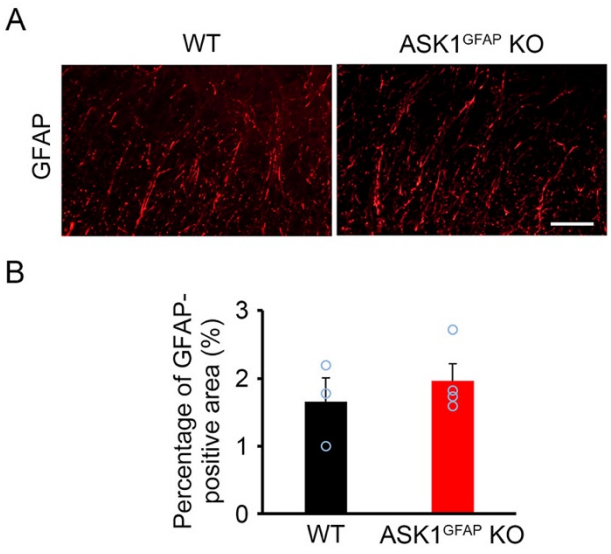

Figure S10. Immunohistochemistry staining of astrocytes in the spinal cord of naïve WT and ASK1^{GFAP} KO mice.

A Immunohistochemistry staining of GFAP in the white matter of the spinal cords of naïve WT and ASK1^{GFAP} KO mice. Scale bar: 50 μm.

B Quantification of GFAP-positive area in (A). $n = 3$ and $n = 4$ mice for naïve WT and ASK1^{GFAP} KO group, respectively.

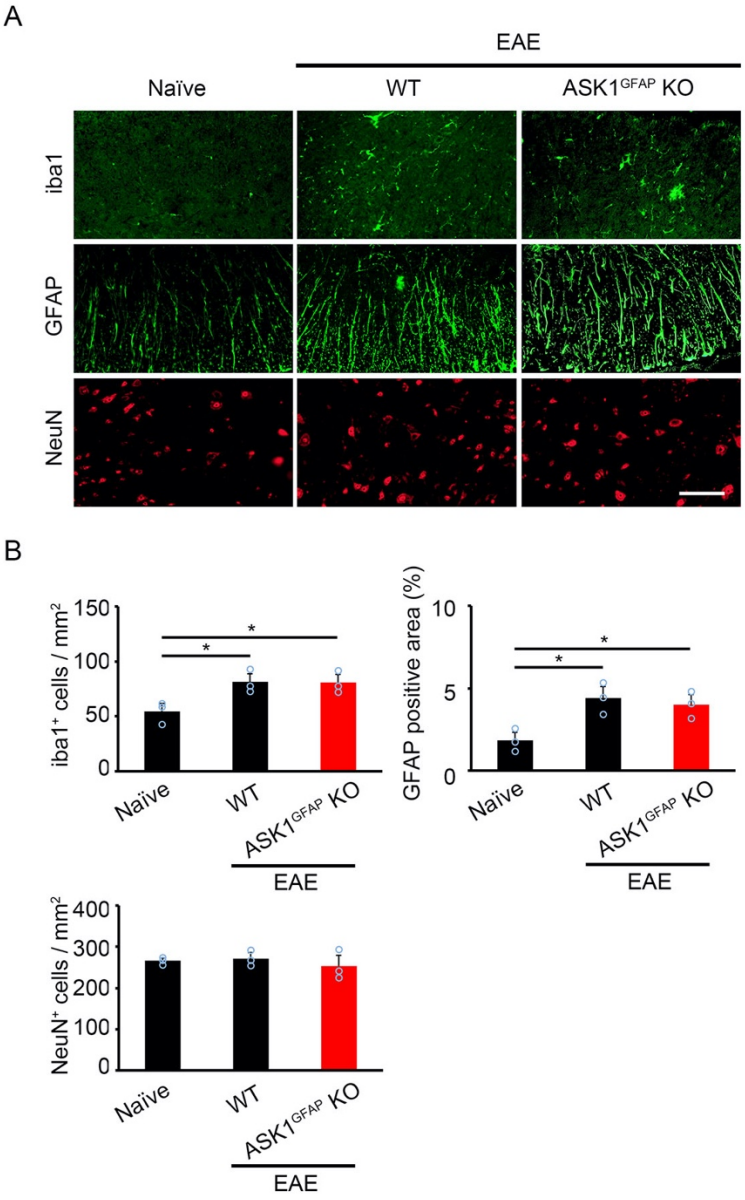

Figure S11. Immunohistochemistry staining of microglia/macrophage, astrocytes and neuron in the spinal cords of EAE mice during the pre-symptomatic phase of EAE.

A Immunohistochemistry staining of iba1 and GFAP in the white matter and NeuN in the gray matter of the spinal cords of WT naïve, WT EAE and ASK1^{GFAP} KO EAE mice during the pre-symptomatic phase of EAE. Scale bar: 100 μm.

B Quantification of iba1-, GFAP- and NeuN-immunopositive areas in (A). n = 3 mice per group. *$P < 0.05$.

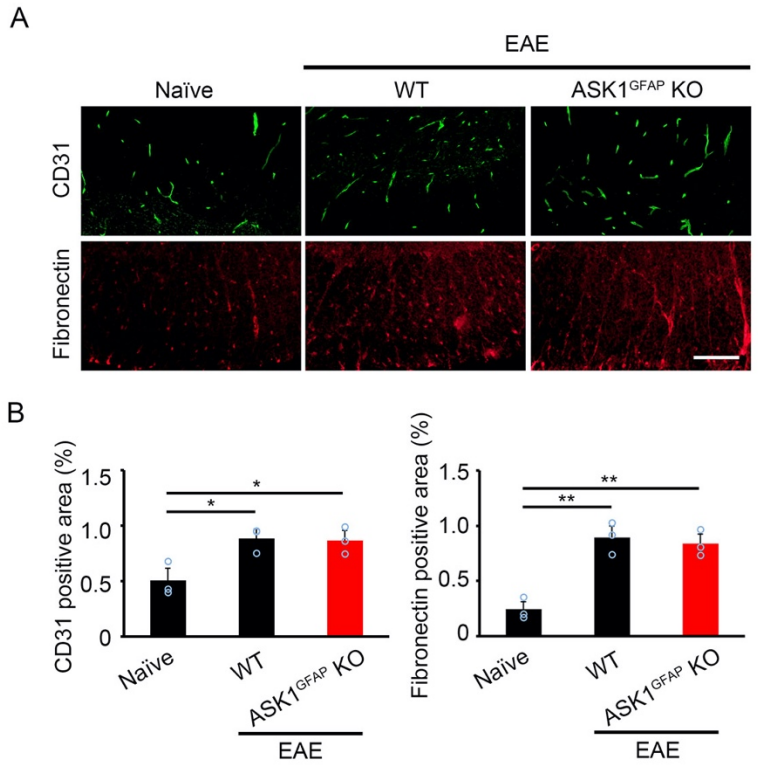

Figure S12. Immunohistochemistry staining of epithelial cells and fibronectin in the spinal cords of EAE mice during the pre-symptomatic phase of EAE.

A Immunohistochemistry staining of epithelial cells and fibronectin in the white matter of the spinal cords of WT naïve, WT EAE and ASK1^{GFAP} KO EAE mice during the pre-symptomatic phase of EAE. Scale bar: 100 μm.

B Quantification of CD31- or fibronectin-positive areas in (A). n = 3 mice per group. $^{**}P < 0.01$; $^{*}P < 0.05$.

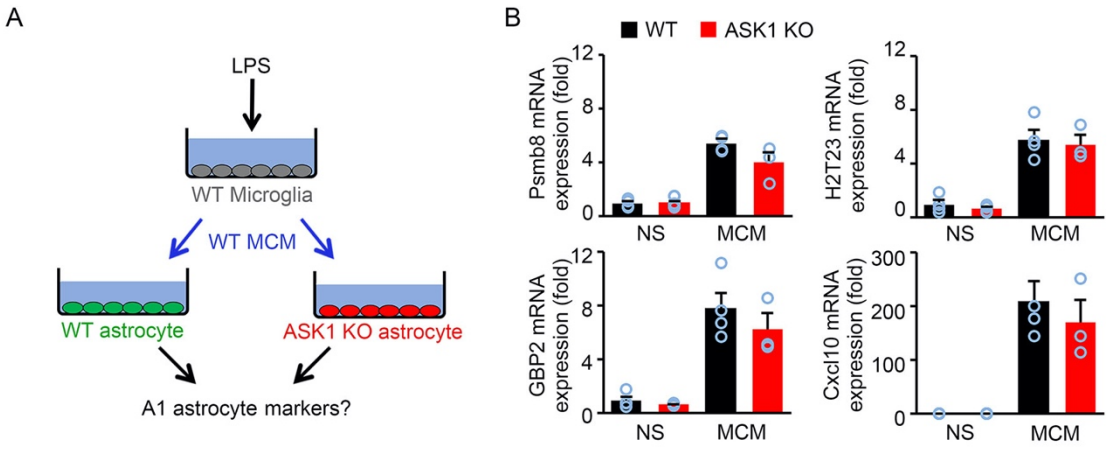

Figure S13. ASK1 deficiency in astrocytes has no effects on the induction of A1 astrocytes.

A Schematic representation of the experimental procedure for A1 astrocyte induction from LPS-stimulated WT microglia.

B A1 astrocyte marker expression (Psmb8, H2T23, and GBP2) in WT and ASK1 KO astrocytes stimulated with WT microglia conditioned medium (MCM) for 6 h. The expression of Cxcl10, one of the pan-reactive astrocyte markers, was also quantified. Experiments were carried out in a 96 well plate format with 3-4 wells used for each culture condition. Experiments were repeated three times and representative results were shown.

Table S1. Sequences of PCR primers used in this study

Genes	Sequence (5'-3')
Arg1	F: CAGAAGAATGGAAGAGTCAG R: CAGATATGCAGGGAGTCACC
C1q	F: TCTCAGCCATTCGGCAGAAC R: ACAGACAAAGGTCCCACTTG
CCL2	F: CCCCACTCACCTGCTGCTACT R: GGCATCACAGTCCGAGTCACA
CD86	F: TTGTGTGTGTTCTGGAAACGGAG R: AACTTAGAGGCTGTGTTGCTGGG
CSF1	F: GACCCTCGAGTCAACAGAGC R: TGTCAGTCTCTGCCTGGATG
Cxcl10	F: AAATCATCCCTGCGAGCCTATC R: GGAGCCCTTTTAGACCTTTTTTGG
Fibronectin	F: AATGGAAAAGGGGAATGGAC R: CTCGGTTGTCCTTCTTGCTC
GAPDH	F: TGCACCACCAACTGCTTAG R: GGATGCAGGGATGATGTTC
GBP2	F: TTGGCCCAGATAGAGAACT R: GACCTCAATGGCCTCACTCTCA
H2T23	F: ACCAACAGAGGGCATACCTG R: GGTCTCCACAAGCTCCATGT
HSPG2	F: AGTGTCTCCCCACCAATGAG R: GTCTCCTGGGCATCAATGTT
IL1α	F: CTCTAGAGCTCCATGCTACAGAC R: AGACAGCTTTAAGGACGGGA
IL1β	F: GGGCCTCAAAGGAAAGAATC R: TACCAGTTGGGGAACTCTGC
LCN2	F: ATGTCACCTCCATCCTGGTC R: ACAGCTCCTTGGTTCTTCCA
Mrc1	F: GGGCAGTGAAAGCTTATGGA R: CCTGTCAGGTATGTTTGCTCA
NOS2	F: CGTGAAGAAAACCCCTTGTG R: CAGCTTGTCCAGGGATTCTG
Psmb8	F: TGATGCTGCAGTACCGGGGGATGG R: TAGCTCTGCGGCCAAGGTCGTAGG
TNFα	F: CGTCAGCCGATTTGCTATCT R: CGGACTCCGCAAAGTCTAAG