Supplemental Figure 1. Validation of ASC-Citrine system in tissue imaging. (A) Representative images of ASC speck formation detected with ASC-Citrine and ASC antibody signals. Live spleen tissue culture slices from naïve WT and ASC-Citrine mice were used with NLRP3 inflammasome stimulation. Scale bar is 50 µm. (B) Quantification of ASC specks in the iLNs and cLNs of ASC-Citrine mice during EAE. Each datapoint represents a value of an average value from two cross-sections of LNs (25 µm thickness) from one mouse. One-way ANOVA, \( p = 0.0021 \) (iLN), \( p = 0.3235 \) (cLN), with Dunnett’s multiple comparisons test. (C and D) Comparison of ASC speck images and numbers in SC between Type-A and Type-B EAE. Representative images (C) and quantification (D) of ASC specks in the SC of ASC-Citrine mice at 30-dpi for Type A (n=5) and Type B (n=8) EAE. Each datapoint represents a value from one mouse. Mann-Whitney test was used (D). Scale bar is 200 µm. (B, D) ns; not significant (\( p > 0.05 \)), *\( p < 0.05 \), **\( p < 0.01 \). Error bars denote mean ± SEM (B, D).
Supplemental Figure 2. Validation of Bone Marrow Chimeras. (A) BM chimera were created by transferring WT BM cells to irradiated WT or Pycard\textsuperscript{-/-} recipients (n=7 for each group). Reconstitution efficiency of BM chimeras determined by flow cytometry, quantified as % of total CD45\textsuperscript{+} cells in peripheral blood for congenic markers of CD45.1 (donor) or CD45.2 (recipient). Each datapoint represents a value from one mouse. Mann-Whitney test used. (B and C) BM chimera were created by transferring ASC-Citrine BM cells irradiated WT recipients (ASC-Citrine \textrightarrow WT, n=6) and vice versa (WT \textrightarrow ASC-Citrine mice, n=8). Reconstitution efficiency (B) and EAE disease score (C) of indicated BM chimera. Each datapoint represents a value from one mouse (B). Each datapoint denotes mean EAE score per group, and Mann-Whitney test of total AUC was used for statistical evaluation (C). (D) Representative images of SC from WT recipients reconstituted with ASC-Citrine BM cells at indicated time points during EAE. No apparent ASC specks were observed. Scale bar is 500 μm. (E) Representative image of ALDH1L1 counterstaining of astrocytes in ASC-Citrine mice at 30-dpi EAE. Scale bar is 10 μm. (F) Representative images of ASC specks and strings counter-stained with antibodies against NG2 (for OPCs) and MBP (for mature oligodendrocytes) in SC from naïve versus 30-dpi EAE ASC-Citrine mice. Scale bar is 20 μm. (G) Quantification of ASC specks in OPCs and mature oligodendrocytes of SC from naïve versus 30-dpi EAE ASC-Citrine mice. Each datapoint represents a value from one mouse. Two-way repeated measures (RM) ANOVA was used (main effect of cell type: ns, p<0.7807). (H) Percentages of ASC specks detected in OPC or mature oligodendrocytes out of total ASC specks per section. L5 spinal cords at 30-dpi EAE were used for the analysis. (I) Percentage of ChAT\textsuperscript{+} and ChAT\textsuperscript{-} VH neurons containing ASC specks in SC from naïve vs. 30-dpi EAE ASC-Citrine mice. Each datapoint represents a value from one mouse (n=5). Two-way RM ANOVA was used (main effect of cell type: p<0.001) with Sidak’s multiple comparisons test post hoc. ns; not significant (p>0.05), **p<0.01. Error bars denote mean ± SEM (A, B, G-I).
Supplemental Figure 3. Validation of EAE mice with cell type-specific ASC-Citrine expression. (A) Flow cytometry histograms showing tamoxifen-mediated expression of ASC-Citrine reporter expression in microglia and splenic monocytes from Cx3cr1<sup>CreERT2</sup>;Asc-Citrine<sup>LSL</sup> mice with or without tamoxifen (TAM) treatment. (B) Flow cytometry gating strategy for identifying microglia. (C) EAE disease score of Cx3cr1<sup>CreERT2</sup>;Asc-Citrine<sup>LSL</sup> (n=5) vs. Cx3cr1<sup>CreERT2</sup>;Asc-Citrine<sup>LSL</sup> (n=5) mice. Both groups were treated with TAM. (D and E) EAE disease score of Asc-Citrine<sup>LSL</sup> (n=10) vs. Gfap<sup>Cre</sup>;Asc-Citrine<sup>LSL</sup> (n=13) (D) and Asc-Citrine<sup>LSL</sup> (n=13) vs. Syn1<sup>Cre</sup>;Asc-Citrine<sup>LSL</sup> (n=10) (E). Mann-Whitney test of total AUC of disease score was used for statistical analysis (C, D, E). ns; not significant (p>0.05). Each datapoint denotes mean EAE score per group with an error bar of mean ± SEM (C-E).
Supplemental Figure 4. Expression of inflammasome components and cell death markers in astrocytes during EAE. (A) Gene-set enrichment analysis of inflammasome-associated genes in bulk SC lysates and astrocytes (with astrocyte-specific Ribotag-HA enriched RNA) in naïve and 30-dpi EAE mice. Data represented as raw transcript counts derived from publicly available data (GEO Accession #: GSE100329). (B-G) Representative images (B-D) and quantification (E-F) of caspase-1 (B, E), IL-1β (C, F), and GSDMD (D, G) expression in spleen and SC astrocytes from naïve versus 30-dpi EAE ASC-Citrine mice. Scale bar is 20 μm. Each datapoint represents a value from one mouse. Individual astrocytes were identified using the Imaris software and the mean intensity per cell was quantified for caspase-1 (E), IL-1β (F) and GSDMD (G). Mann-Whitney test was used. (E-G) ns; not significant (p>0.05). Error bars denote mean ± SEM (E-G).
Supplemental Figure 5. Expression of inflammasome components in primary cortical astrocyte cell line (A and B) WB quantitative evaluation of culture supernatant samples of mature caspase-1 (A) and IL-1β (B). (C-F) WB quantitative evaluation of cell lysate samples of pro-caspase-1 (C), pro-IL-1β (D), GSDMD-FL (E), and GSDMD-NT (F). In (A-F), Cells in group 1 were unstimulated. Cells in group 2 were treated with Ultrapure LPS alone. Cells in groups 3 and 4 were pre-treated with Ultrapure LPS, and were further stimulated with nigericin and poly(dA:dT)/liposome to activate the NLRP3 and AIM2 inflammasomes, respectively. (G) Quantification of active caspase-3 (CC3) in spinal cord astrocytes comparing astrocytes with or without ASC specks in GfapCre;Asc-CitrineLSL (n=6) mice at 30-dpi EAE. Each datapoint represents a value from one mouse. Individual astrocytes were identified using the Imaris software and were quantified by CC3 puncta staining. Mann-Whitney test was used. **p<0.01. Error bars denote mean ± SEM.
Fig. S6. Validation of EAE phenotype of Nlrp3−/−;ASC-Citrine mice and immune phenotype of Aim2−/− mice with EAE. (A) EAE disease score of ASC-Citrine (n=7) vs. Nlrp3−/−;ASC-Citrine (n=8) mice with Type B-EAE. Mann-Whitney test of total AUC for disease score was used. Each datapoint denotes mean EAE score per group with an error bar of mean ± SEM. (B–D) Leukocyte counts in SC (B), iLN (C) and spleen (D) at 16-dpi EAE in WT vs. Aim2−/− mice induced with Type-A EAE. Each datapoint represents a value from one mouse. Error bars denote mean ± SEM. Two-way RM ANOVA was used with Sidak’s multiple comparisons test post hoc. (E) Representative image of GFAP staining in SC from WT versus Aim2−/− mice at 30-dpi of EAE. Scale bar is 200 μm. ns; not significant (p>0.05) (A–D).