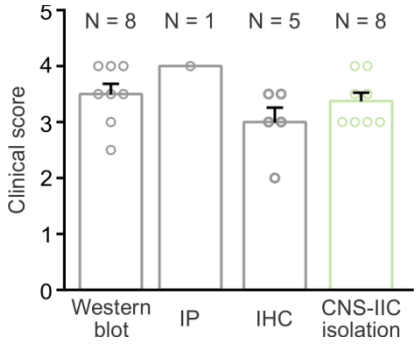
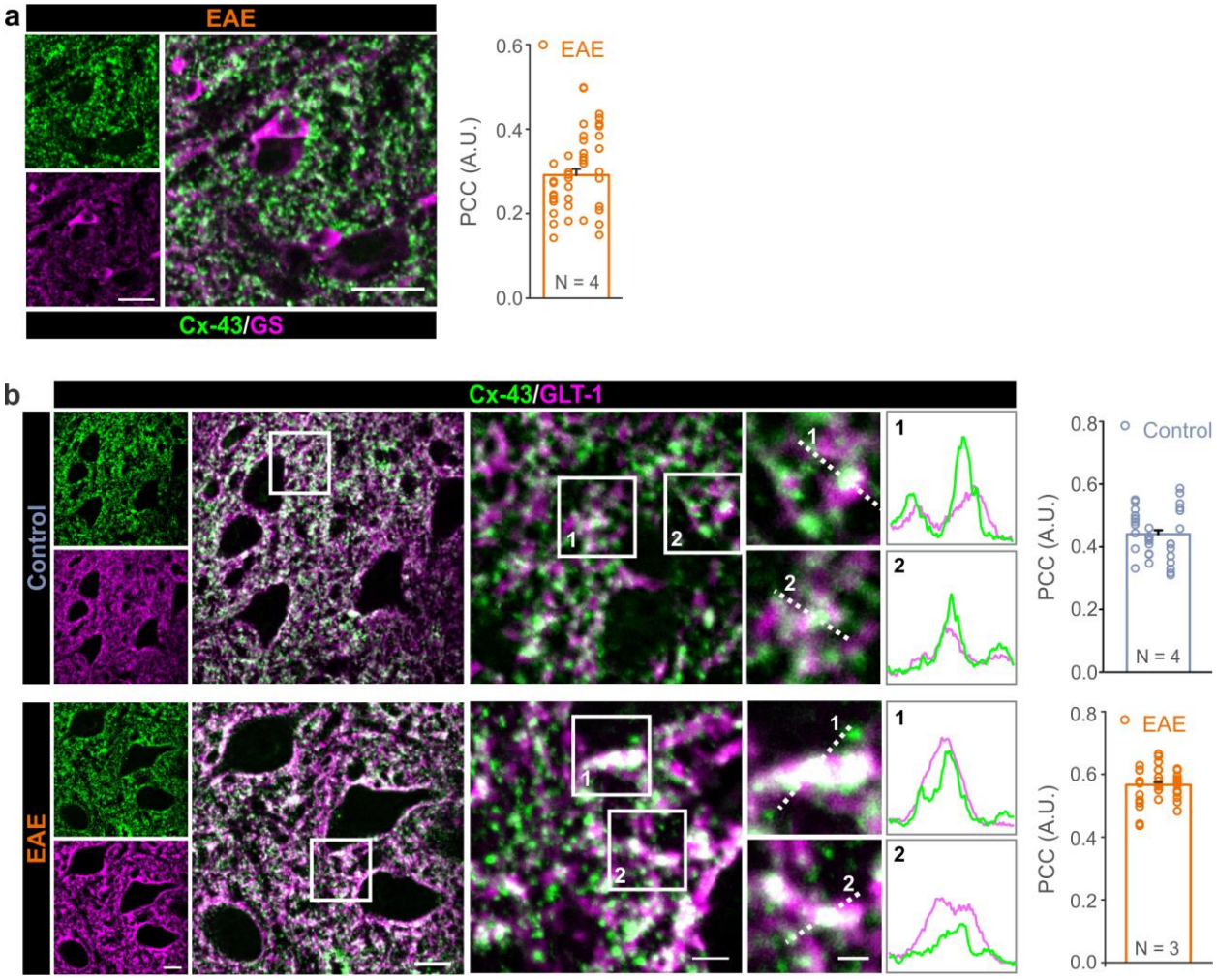
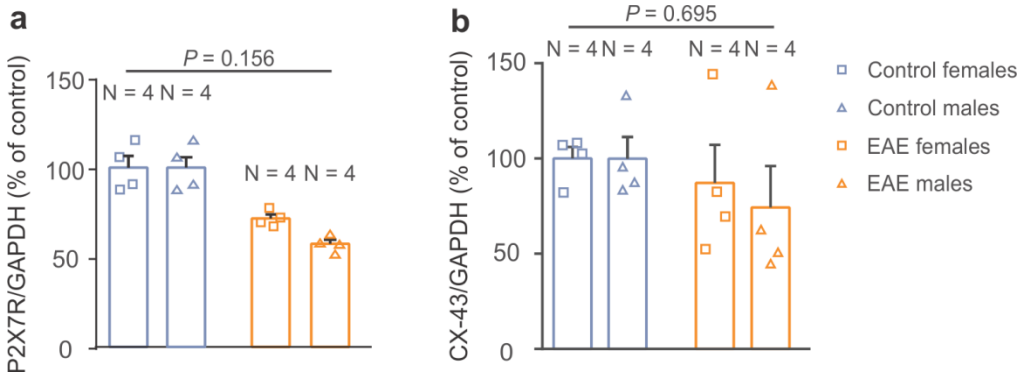
**Supplementary Information**



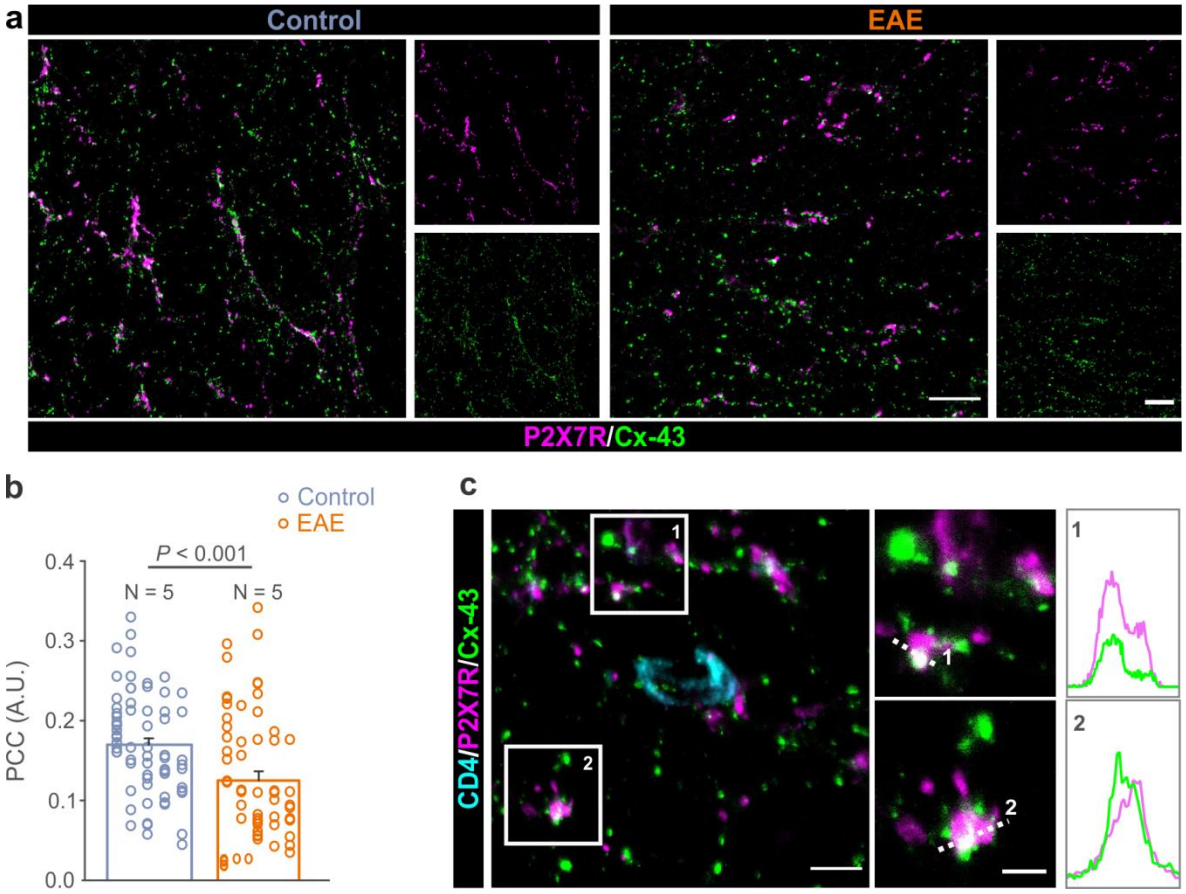

**Supplementary Figure S1. Clinical scores of EAE animals used in experiments.** Graph shows average clinical scores of EAE animals used in Western blot, immunoprecipitation (IP), immunohistochemistry (IHC), and CNS-IIC isolation experiments. CNS-IIC isolation bar comprises $Ca^{2+}$ imaging and ATP assay experiments. Data are presented as mean ± SEM. Dots represent individual animals. N is the number of animals.



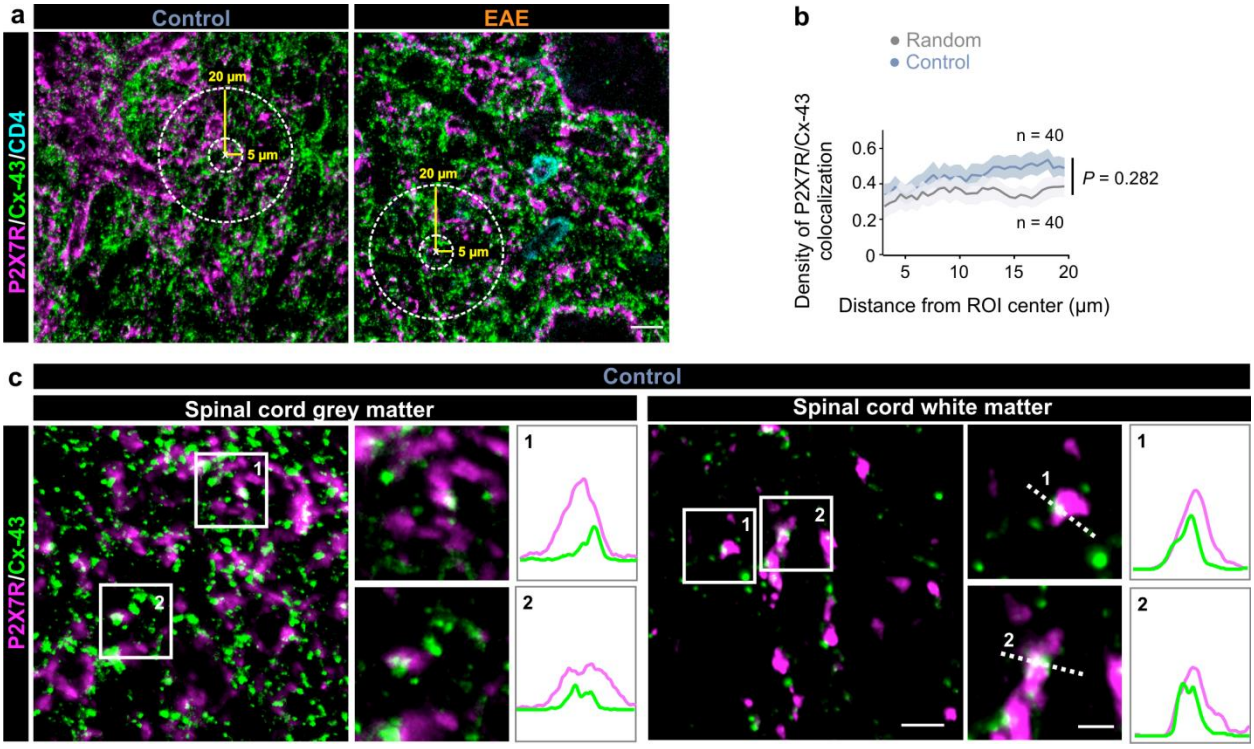

**Supplementary Figure S2. Connexin-43 colocalizes with astrocytic markers glutamine synthetase and glutamate transporter-1. (a)** Left: Confocal images of the lumbar spinal cord grey matter immunostained for connexin-43 (Cx-43, green) and glutamine synthetase (GS, magenta) in EAE. Right: Graph showing Pearson's correlation coefficient (PCC) of colocalization between Cx-43 and GS. **(b)** Confocal images of the lumbar spinal cord grey matter immunostained for Cx-43 (green) and glutamate transporter-1 (GLT-1, magenta) in Control and EAE. White rectangle corresponds to the enlarged region shown on the right, scale bar 5 µm. Numbered (1, 2) rectangles correspond to the regions presented on the right side, scale bar 2 µm. Profile intensity plots of Cx-43 and GLT-1 fluorescent signals are measured along each white dotted line. Graphs show PCC of colocalization between Cx-43 and GLT-1 in Control and EAE. N indicates number of analyzed animals. Each vertical dot plot corresponds to the data points obtained from the individual animal. Data are presented as mean ± SEM.




**Supplementary Figure S3. P2X7R and connexin-43 expression in EAE females and males.** (**a, b**) Graphs showing comparisons of P2X7R (**in a**) and connexin-43 (Cx-43, **in b**) expression in the lumbar spinal cord of female and male rats in control and at the peak of EAE (two-way ANOVA, $P = 0.156$ for P2X7R and $P = 0.695$ for Cx-43). Data are shown as mean ± SEM. Triangles represent data obtained for individual females, squares for individual males. Number of animals is displayed on the graph.




**Supplementary Figure S4. P2X7 receptor colocalization with connexin-43 in the spinal cord white matter in EAE. (a)** Representative confocal images of the spinal cord white matter immunostained for P2X7R (magenta) and connexin-43 (Cx-43, green) in control and EAE. Scale bars 20 µm. **(b)** Graph showing Pearson correlation coefficient (PCC) between Cx-43 and P2X7R in the white matter in control and EAE (Mann-Whitney Rank Sum Test, $P < 0.001$). N indicates number of animals. Each vertical dot plot corresponds to the data points obtained from the individual animal. Data are presented as mean ±SEM. **(c)** Confocal images of Cx-43 (green) and P2X7R (magenta) fluorescent signals in the close vicinity of infiltrated $CD4^+$ T cells (cyan) in the white matter of the spinal cord of EAE rats. Scale bar 5 µm. Numbered (1, 2) rectangles correspond to the regions presented on the right side. Scale bar 2 µm. Profile intensity plots of Cx-43 and P2X7R fluorescent signals are measured along each white dotted line.




**Supplementary Figure S5. P2X7 receptor colocalization with connexin-43 in the spinal cord of control healthy animal. (a)** Confocal images of P2X7R (magenta), Cx43 (green) and $CD4^+$ T cell (cyan) immunofluorescent labeling in Control and EAE. Depicted regions of interests (ROI) are used for analysis of P2X7R, Cx-43 signal intensity and colocalization in the random region of interest (ROIs). Yellow and dashed white lines mark 5 µm and 20 µm radial distances measured from the center of ROIs. Scale bar 10 µm. (**b**) Graph showing density of P2X7R/Cx-43 colocalization in Control (pale blue) and random (grey) ROIs (two-way ANOVA, P = 0.282). Data are presented as mean ± SEM; n is the number of analyzed ROIs. (**c**) Confocal images of Cx-43 and P2X7R fluorescent signals in the white and the grey matter of the spinal cord of Control rats. Scale bar 5 µm. Numbered (1,2) white rectangles correspond to the regions presented on the right side. Scale bars 2 µm. Profile intensity plots of Cx-43 and P2X7R fluorescent signals are measured along each white dotted line.