Supporting Information

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SI Materials and Methods

Histology. For histological analysis, mice were killed, immediately perfused with PBS (pH 7.5) and organs were fixed in 4% formaldehyde. Five-micrometer-thick sections were stained with Luxol Fast Blue to visualize spinal cord demyelination. Images were acquired using a Leica DMRA microscope and processed using Adobe Photoshop (Adobe Systems). For immunofluorescence analysis, mice were killed and immediately perfused with PBS, followed by PBS/4% paraformaldehyde (PFA; pH 7.5). Organs were further fixed in PBS/4% PFA for at least 12 h and embedded in 4% low melting agarose (USB). Twenty-micrometer sections were cut using a vibratome (Leica VT 1200S). Free-flowing sections were permeabilized in 1× PBS, 1% Triton × 100 (Sigma), and 1% FCS and blocked with 1× PBS, 0.1% Triton, 10% FCS, and 0.5 μg/mL Fc block. Sections were further incubated with biotin-conjugated anti-CD4 (BioLegend), followed by Alexa Fluor 549-conjugated streptavidin 1:1,000 (Jackson ImmunoResearch), anti-B220 Alexa Fluor 647 (BioLegend), and purified IgG anti-GFP/YFP (Clontech), followed by an anti-rabbit IgG Alexa Fluor 488 (Jackson ImmunoResearch). Nuclei were stained with DAPI (Sigma), and sections were mounted with fluorescence mounting solution (Dako). Images were acquired using Zeiss LSM710 microscope and processed using ZEN software (Zeiss) and Adobe Photoshop.

Brain Cell Isolation and Flow Cytometry. Brains were mechanically disrupted, and the leukocytes were further enriched using a 70–30% Percoll gradient (GE Healthcare) and centrifugation for 25 min at 800 × *g*. Analysis of MHV-specific $CD8^+$ T-cell responses was performed using phycoerythrin (PE)-conjugated MHV S598/$H\text{-}2K^b$ tetramers (Sanquin Reagents). For surface staining, the following mAbs were used: PE-labeled anti-CD138 (BD Biosciences), PerCP-labeled anti-CD45 (Biolegend), APC-labeled anti-CD8, anti-CD4, and anti-CXCR3 (Biolegend), and APC-Alexa Fluor 780 anti-B220 (eBioscience). 7-Amino-actinomycin D (Calbiochem) was used to discriminate dead cells in flow cytometric analysis. For peptide-specific cytokine production, 10^6 splenocytes were restimulated with S598 or M133 peptides in the presence of brefeldin A (5 μg/mL) for 5 h at 37 °C. Cells were stimulated with phorbolmyristateacetate (50 ng/mL) and ionomycin (500 ng/mL; both purchased from Sigma) as positive control or left untreated as a negative control. For intracellular staining, restimulated cells were surface-stained and fixed with cytofix-cytoperm (BD Biosciences) for 20 min. Fixed cells were incubated at 4 °C for 40 min with permeabilization buffer (2% FCS/0.5% saponin/PBS) containing anti-IFNγ mAb (BD Biosciences). Samples were analyzed by flow cytometry using a FACSCanto (Becton Dickinson), and data were analyzed using FlowJo software (Tree Star). Both M133 (TVYVRPIIE-DYHTLT) and S598 (RCQIFANI) peptides were purchased from Neosystems.

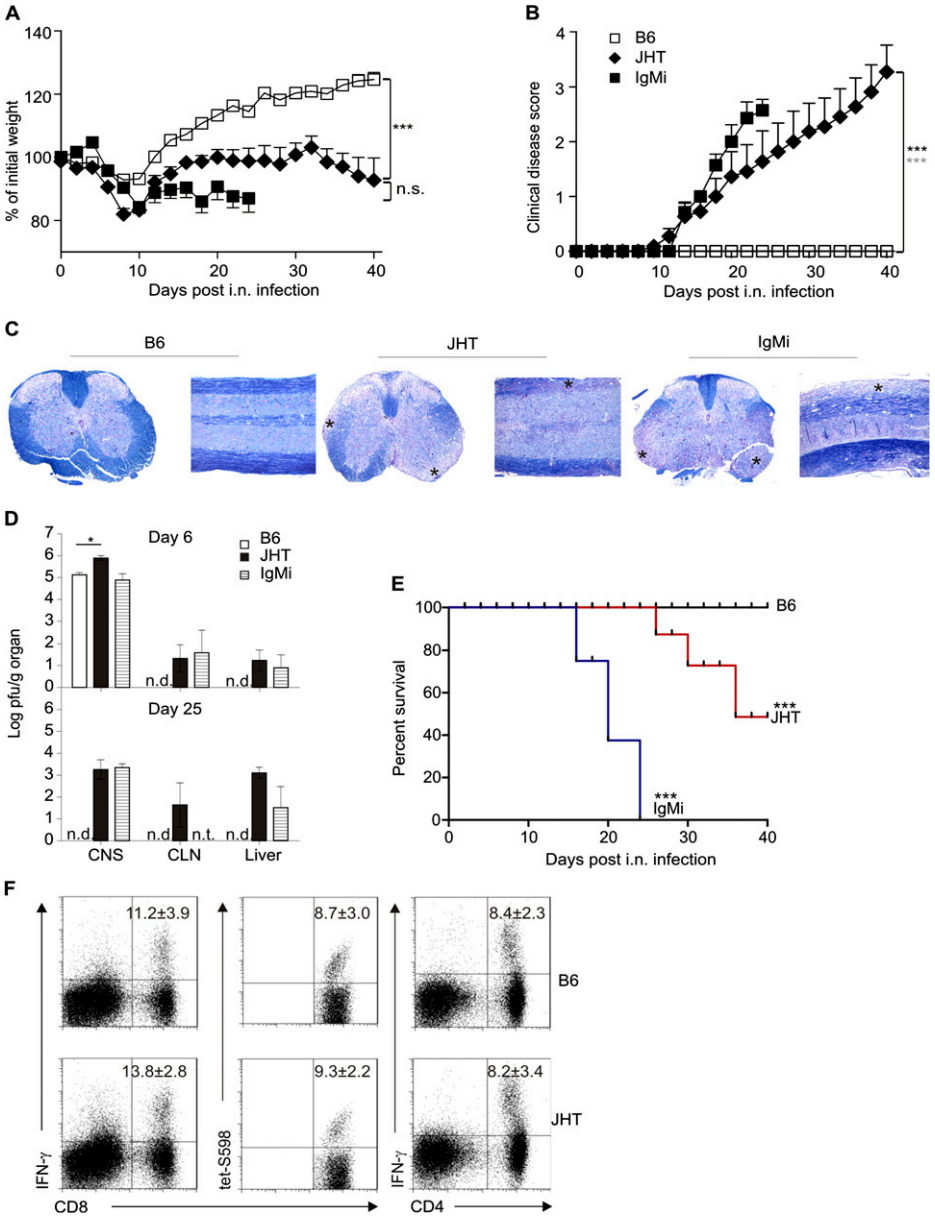

Fig. S1. Antibodies protect against MHV-induced demyelinating disease. B6, JHT, or IgMi mice were intranasally (i.n.) infected with 5×10^4 pfu of MHV A59. (*A*) Weight loss and (*B*) development of clinical symptoms were recorded during the indicated time points after infection. Values in *A* indicate mean percentage of the initial weight ± SEM (n = 8–10 mice per group). Data in *B* indicate clinical scores (n = 8–10 mice per group). (*C*) Spinal cord sections from i.n. infected mice were analyzed on day 40 (JHT and B6 mice) or day 25 (IgMi mice) using Luxol fast blue staining. Asterisks indicate areas of extensive myelin loss. (*D*) Viral titers in CNS, cervical lymph nodes (CLN), spleen, and liver were determined at days 6 and 25 after infection. Data indicate means of log-transformed values ± SEM (n = 5 mice per group). n.d., not detectable; n.t., not tested. (*E*) Survival was recorded during the indicated time period. Mice that showed a sustained loss of weight of >20% without recovery and/or reached level 4 of the clinical score were killed. (*F*) Tetramer-binding and IFN-γ production by CNS-infiltrating T cells was evaluated by flow cytometry on day 8 after infection. Values in the upper right quadrant indicate peptide-specific responses in the respective T-cell population (mean ± SEM, n = 3 mice per group, data are from one representative of three independent experiments). Statistical analyses in *A* and *B* were performed using one-way ANOVA with Tukey's postanalysis; in *D*, Student's t test was used; and in *E* the Mantel Cox test and χ^2 test were used. $^*P < 0.05$; $^{***}P < 0.001$.

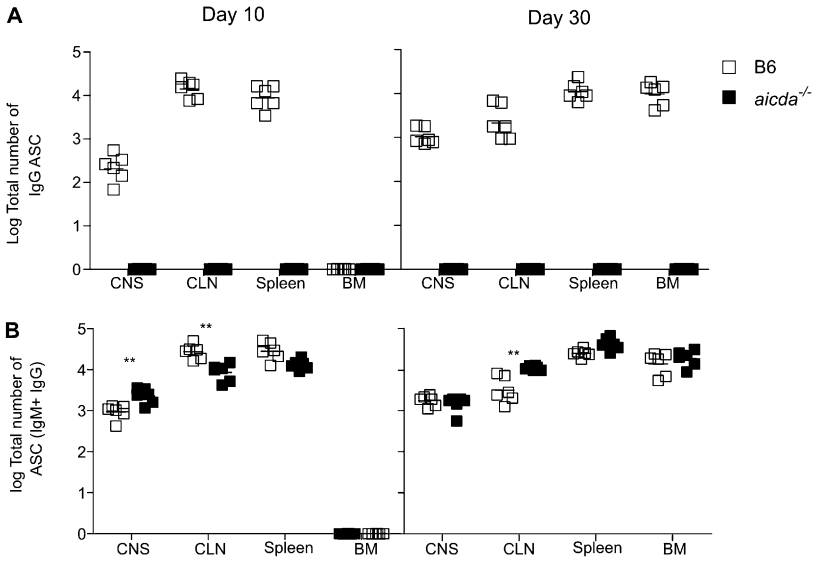

Fig. S2. Induction of antibody-secreting cells (ASCs) in B6 and *aicda*$^{-/-}$ mice. (*A*) IgG-specific ASCs and (*B*) IgM- and IgG-specific ACSs were enumerated in CNS, cervical lymph node (CLN), spleen (SPL), and bone marrow (BM) at days 10 and 30 after infection. The statistical analyses in *B* were performed using Student's *t* test. $^{**}P < 0.01$.

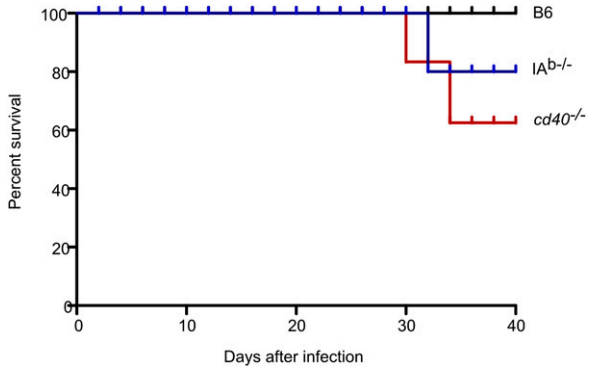

Fig. S3. Survival of cd40$^{-/-}$ and IA$^{b-/-}$ mice after MHV A59 intranasal infection. Survival was recorded during the indicated period. Mice that showed a sustained loss of weight (<20%) without recovery and/or reached level 4 of the clinical score were killed. $n = 8$–10 mice per group. Mantel Cox test and χ^2 test were used and did not reveal statistically significant differences.

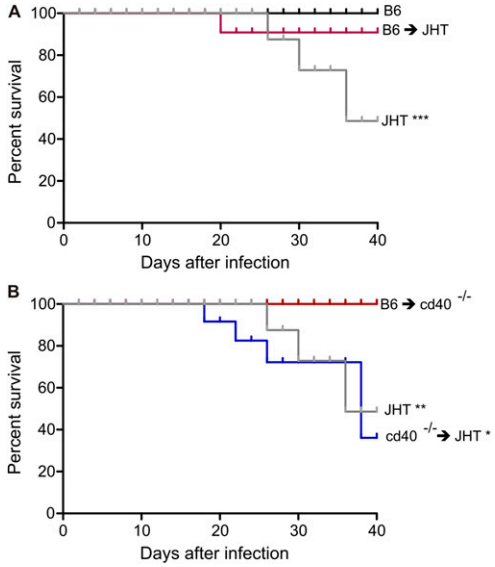

Fig. S4. CD40 expression on B cells is required to protect against demyelinating disease. Sorted B cells (1.5×10^7) from B6 or CD40-deficient donors were transferred into JHT or CD40-deficient recipient mice on days 3 and 1 before MHV infection. B6 and JHT mice served as controls. (*A* and *B*) Survival rates were determined during the indicated time period after infection in the indicated strains or B cell-reconstituted recipients (n = 8 mice per group) mice after MHV A59 intranasal infection. Mice that showed a sustained loss of weight (>20%) without recovery and/or reached level 4 of the clinical score were killed. Mantel Cox test and χ^2 test were used. *$P < 0.05$; **$P < 0.01$; ***$P < 0.01$.

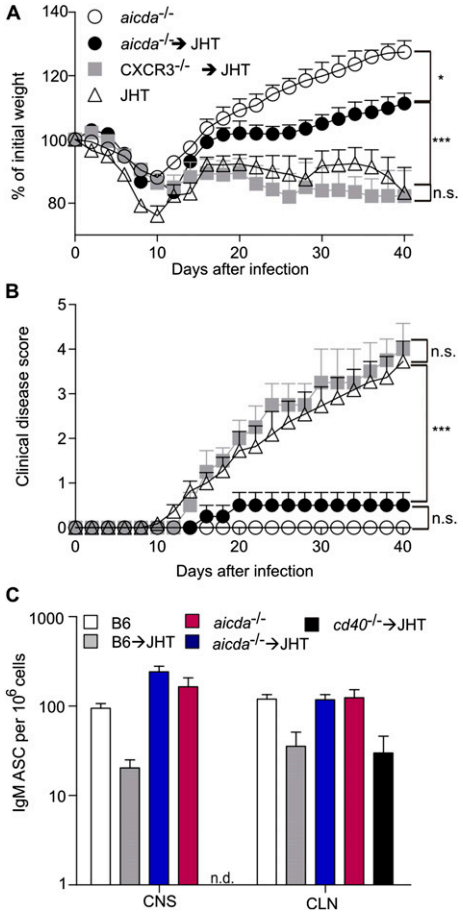

Fig. S5. Activation-induced cytidine deaminase (AID)-deficient but not CXCR3-deficient B cells protect against MHV-induced demyelinating disease. Sorted B cells (1.5×10^7) from *aicda*$^{-/-}$ or *cxcr3*$^{-/-}$ donors were transferred into JHT recipient mice on days 3 and 1 before MHV infection. *aicda*$^{-/-}$ and JHT mice served as controls. (*A* and *B*) Weight loss and development of clinical symptoms were recorded during the indicated time period after infection in the indicated strains or B cell-reconstituted recipients (n = 4 mice per group). (*C*) IgM-specific antibody secreting cells were enumerated in CNS and CLN on day 10 after infection. Values indicate mean numbers of IgM-secreting cells per 10^6 cells ± SEM from 4 mice per group. n.d., not detectable. Statistical analyses in *A* and *B* were performed using one-way ANOVA with Tukey's postanalysis. *$P < 0.05$; **$P < 0.01$, ***$P < 0.001$. n.s., not significant.