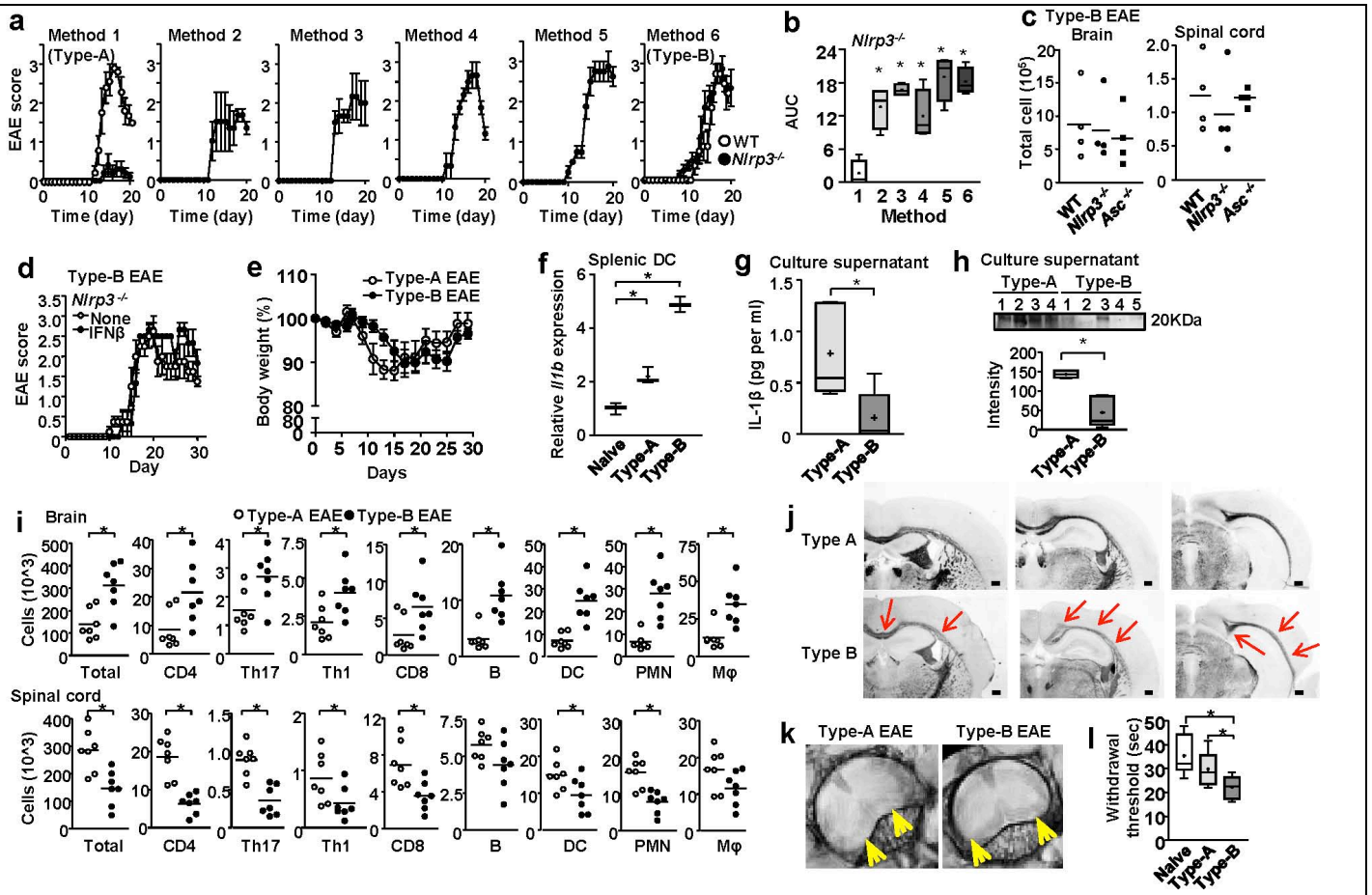
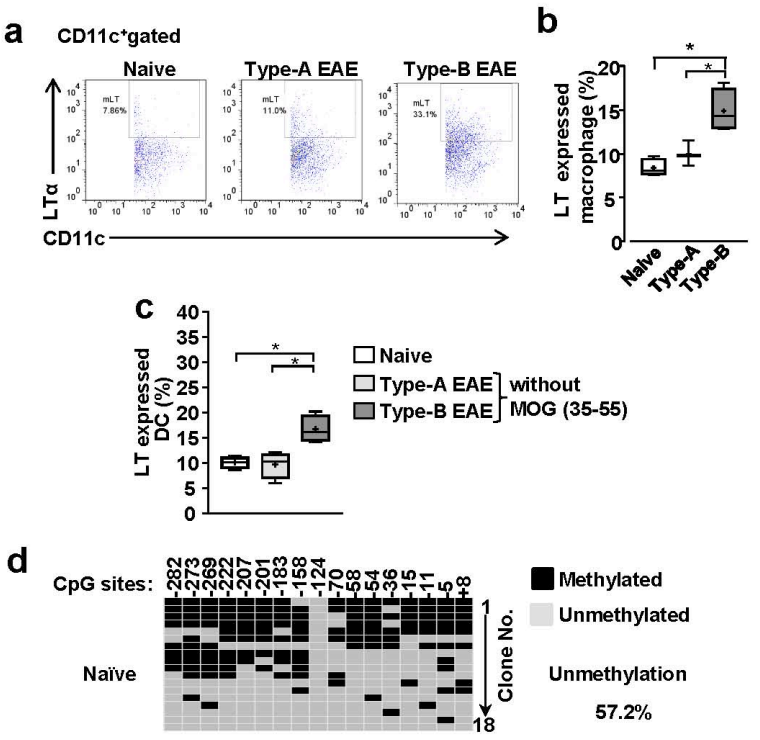


Supplementary Figure 1

EAE induced by several immunization methods and phenotypes on cell migration, demyelination and nociceptive sensitivity in mice with Type-A and Type-B EAE.

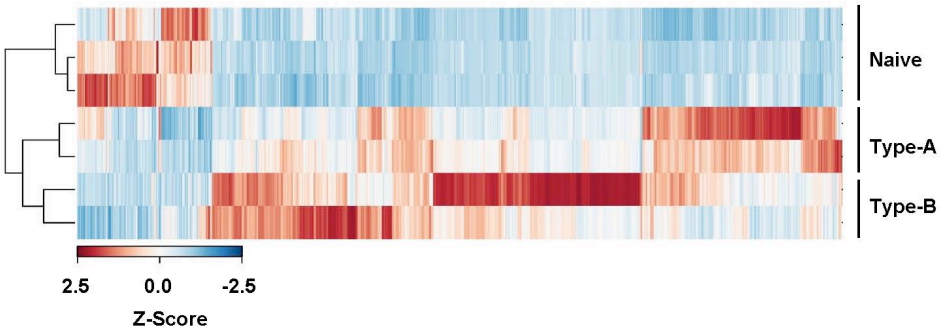
(a) EAE severity in *Nlrp3*$^{-/-}$ mice was evaluated. EAE was induced with 6 different methods, as indicated in Online Methods. **(b)** AUC between 0 and 20-dpi. Method 1 (n=4), Method 2-6 (n=5). $P_{(Method\ 1\ vs\ 2)}$=0.0006, $t(7)$=5.98, $P_{(Method\ 1\ vs\ 3)}$ <0.0001, $t(7)$=12.54, $P_{(Method\ 1\ vs\ 4)}$=0.0027, $t(7)$=4.54, $P_{(Method\ 1\ vs\ 5)}$ <0.0001, $t(7)$=8.15, $P_{(Method\ 1\ vs\ 6)}$ <0.0001, $t(7)$=10.63. **(c)** Numbers of total immune cells were evaluated in the brains and spinal cords (S.C.) of mice with Type-B EAE at 17-dpi. n=4. Brain: $P_{(WT\ vs\ Nlrp3\text{-}/\text{-})}$=0.8159, $t(6)$=0.2433, $P_{(WT\ vs\ Asc\text{-}/\text{-})}$=0.5685, $t(6)$=0.6031. Spinal cord: $P_{(WT\ vs\ Nlrp3\text{-}/\text{-})}$=0.5226, $t(6)$=0.6787, $P_{(WT\ vs\ Asc\text{-}/\text{-})}$=0.8984, $t(6)$=0.1332. **(d)** IFNβ treatment on *Nlrp3*$^{-/-}$ mice with Type-B EAE induced with Method 6. IFNβ ($3x10^4$ unit/mouse) were *i.p.* injected every other day from day 0 to 8 as previously performed. **(e)** Time course on body weight change in Type-A and Type-B EAE. **(f)** *Il1b* mRNA levels in splenic DC from naïve, Type-A, and Type-B EAE mice at 9 dpi. $P_{(Naive\ vs\ Type\text{-}A)}$=0.0059, $t(4)$=5.344, $P_{(Naive\ vs\ Type\text{-}B)}$<0.0001, $t(4)$=18.55, $P_{(Type\text{-}A\ vs\ Type\text{-}B)}$=0.0004, $t(4)$=10.83. **(g,h)** Levels of extracellular IL-1β (f) and p20 caspase-1 (g) in 24h splenocyte culture supernatants (n=4 for Type-A, n=5 for Type-B). P=0.0261, $t(6)$=2.724 (g). P=0.0020, $t(6)$=4.794 (h). **(i)** Infiltrated cell numbers in the brain and spinal cord (n=7). Brain: $P_{(Total)}$=0.0028, $t(12)$=3.747, $P_{(CD4)}$=0.018, $t(12)$=2.737, $P_{(Th17)}$=0.0167, $t(12)$=2.78, $P_{(Th1)}$=0.0165, $t(12)$=2.784, $P_{(CD8)}$=0.0329, $t(12)$=2.41, $P_{(B)}$=0.0013, $t(12)$=4.165, $P_{(DC)}$=0.0003, $t(12)$=5.008, $P_{(PMN)}$=0.0004, $t(12)$=4.912, $P_{(Mac)}$=0.0027, $t(12)$=3.759. Spinal cord: $P_{(Total)}$=0.0035, $t(12)$=3.621, $P_{(CD4)}$=0.0002, $t(12)$=5.372, $P_{(Th17)}$=0.0003, $t(12)$=4.997, $P_{(Th1)}$=0.00465, $t(12)$=2.221, $P_{(CD8)}$=0.0104, $t(12)$=3.032, $P_{(B)}$=0.0918, $t(12)$=1.833, $P_{(DC)}$=0.0429, $t(12)$=2.263, $P_{(PMN)}$=0.0011, $t(12)$=4.29, $P_{(Mac)}$=0.089, $t(12)$=1.849. **(j)** Representative LFB-stained images of brains at 17-dpi. Red arrows indicate reduced LFB intensity, *i.e.,* reduced myelin. Scale bars, 200 μm. **(k)** T2 FLAIR MRI analysis of spinal cords obtained from mice at 18-dpi. Yellow arrows indicate areas of potential myelin loss. **(l)** Thermal sensitivity evaluated by a hot-plate test in 9-dpi mice, which did not show any EAE symptoms and motor dysfunction. n=8. $P_{(Naive\ vs\ Type\text{-}A)}$=0.1524, $t(14)$=1.514, $P_{(Naive\ vs\ Type\text{-}B)}$=0.0013, $t(14)$=4.026, $P_{(Type\text{-}A\ vs\ Type\text{-}B)}$=0.0217, $t(14)$=2.582. *; p<0.05. All statistical analyses in this figure were performed by two-tailed unpaired Student's t-test. All the experimental data and images are representatives from at least 2 similar experiments for each.



Supplementary Figure 2

mLT expression on DC.

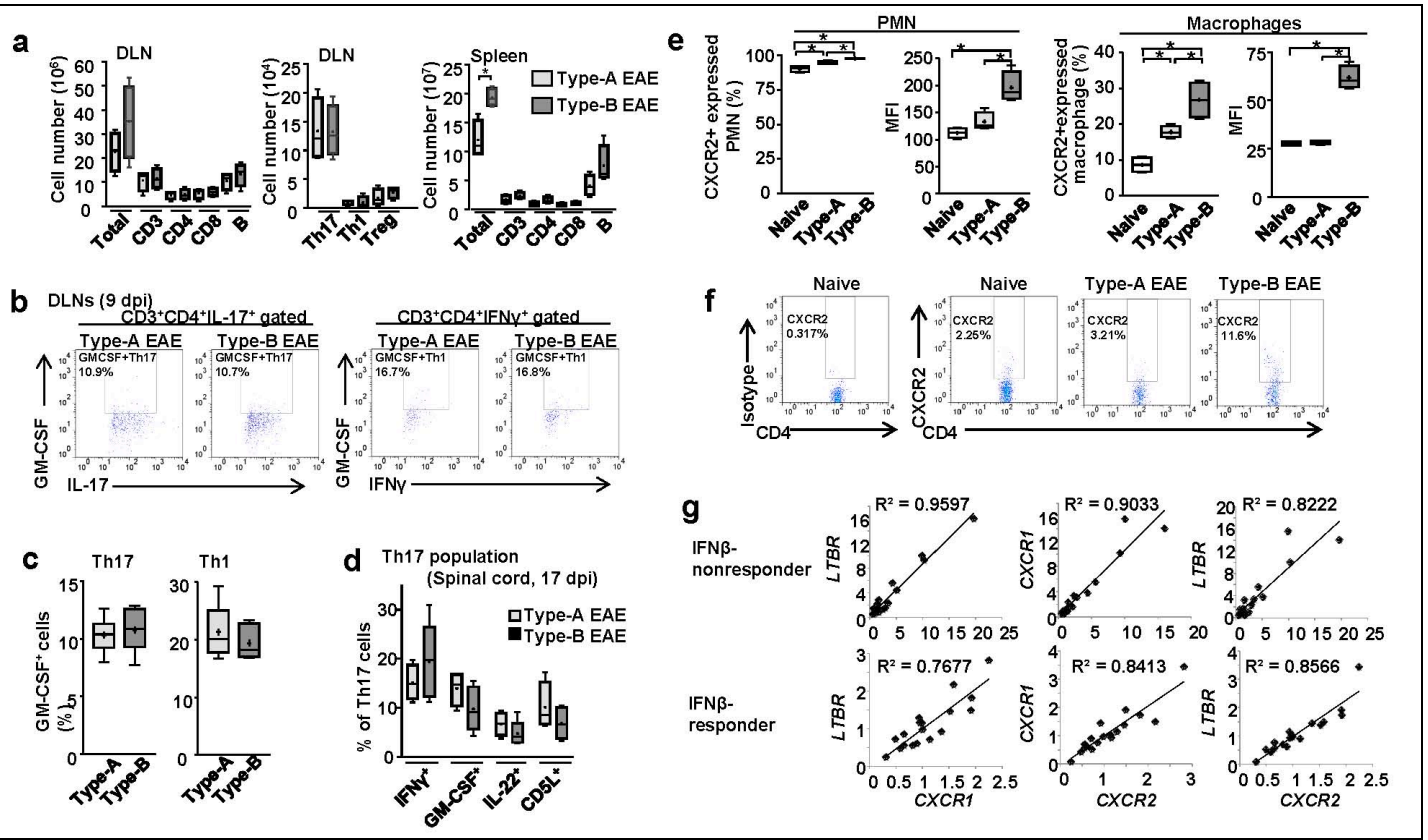
(a) Flow charts showing mLT expression in DC ($CD11c^+$ gated) obtained from naïve mice, Type-A EAE mice, and Type-B EAE mice at 9-dpi. **(b)** Percentages of $LT\alpha^+$ (*i.e.,* mLT^+) macrophages, determined by flow cytometry, in DLNs of naïve mice or mice with EAE at 9-dpi. Naïve (*n*=4), Type-A (n=3), Type-B (n=4). $P_{(Naive\ vs\ Type\text{-}A)}$=0.1340, $t(5)$=1.7887, $P_{(Naive\ vs\ Type\text{-}B)}$=0.0024, $t(6)$=45.021, $P_{(Type\text{-}A\ vs\ Type\text{-}B)}$=0.0268, $t(5)$=3.101, by two-tailed unpaired Student's *t*-test. **(c)** Evaluating mLT expression on DCs from mice received CFA injection alone without MOG. One group received 200 μg *Mtb* in CFA (*Mtb* dosage for Type-A EAE), and another group had 400 μg *Mtb* in CFA twice (*Mtb* dosage for Type-B EAE). (n=6) $P_{(Naive\ vs\ Type\text{-}A)}$=00.7939, $t(6)$=0.2732, $P_{(Naive\ vs\ Type\text{-}B)}$=0.0040, $t(6)$=4.519, $P_{(Type\text{-}A\ vs\ Type\text{-}B)}$=0.0095, $t(6)$=3.748, by two-tailed unpaired Student's *t*-test. **(d)** Methylation analysis was carried out by bisulfite conversion on the *Lta* promoter in DCs from naïve mice. Methylated and unmethylated CpG were shown with black and gray boxes, respectively



Supplementary Figure 3

RNA-seq analysis.

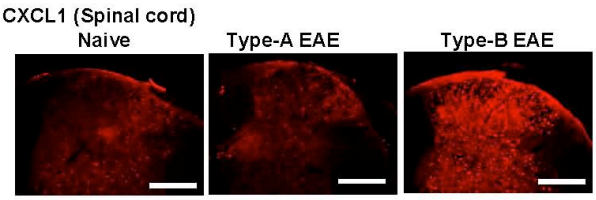
Heatmap for differentially expressed genes in $CD4^+$ T cells isolated from naïve, Type-A EAE mice, and Type-B EAE mice at 9-dpi. Genes and samples have been clustered using correlation distance with complete linkage. A gene is listed on the heatmap if it had a p-value <= 0.05 and a $\log_2$FC >1 or < -1.



Supplementary Figure 4

Comparison of IFNβ-responders and non-responders in mouse EAE and human RRMS.

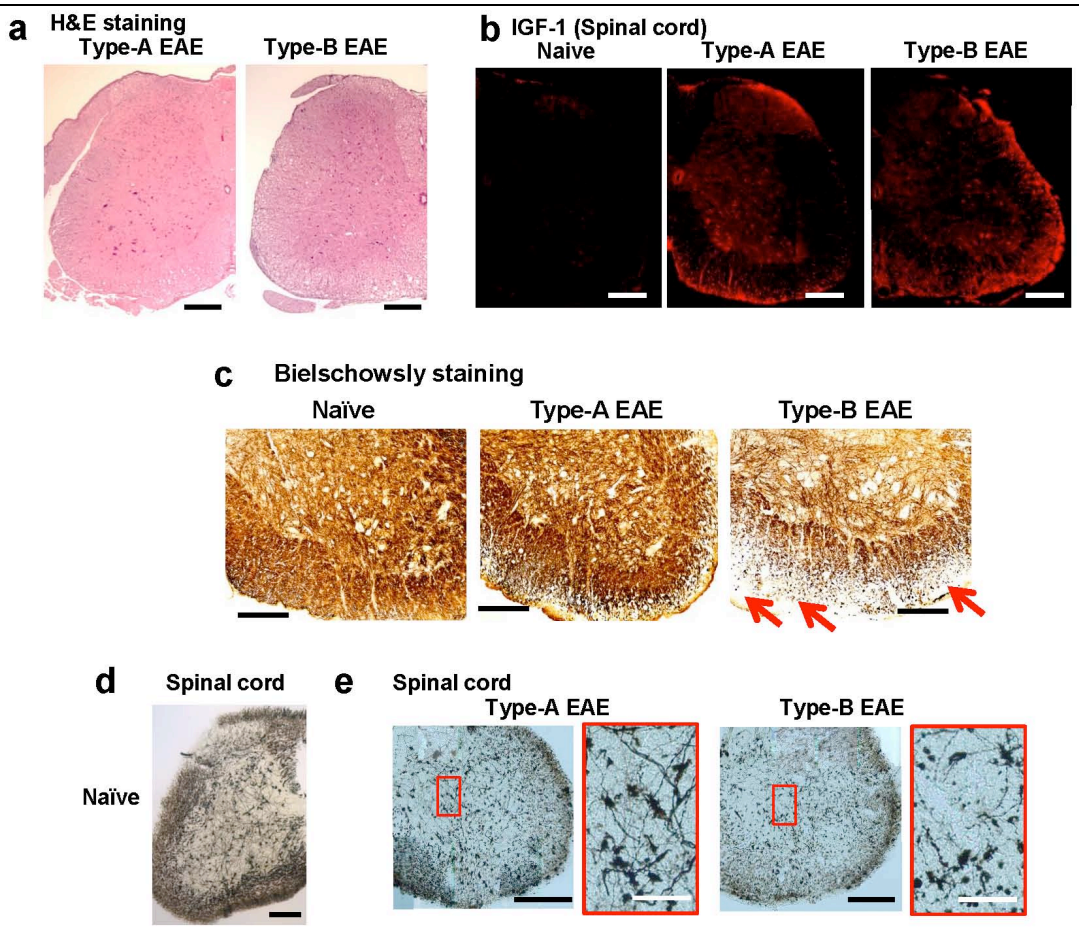
(a) Numbers of indicated cell types obtained from DLNs and spleen on 17-dpi were evaluated between Type-A and Type-B EAE. n=4. DLNs: $P_{(Total)}$=0.2073, $t(6)$=1.413, $P_{(CD3)}$=0.8466, $t(6)$=0.202, $P_{(CD4)}$=0.9285, $t(6)$=0.09353, $P_{(CD8)}$=0.7413, $t(6)$=0.3459, $P_{(B)}$=0.4141, $t(6)$=0.8773, $P_{(Th17)}$=0.9724, $t(6)$=0.03605, $P_{(Th1)}$=0.9017, $t(6)$=0.1289, $P_{(Treg)}$=0.3556, $t(6)$=1.001. Spleen: $P_{(Total)}$=0.0072, $t(6)$=3.99, $P_{(CD3)}$=0.2537, $t(6)$=1.262, $P_{(CD4)}$=0.1781, $t(6)$=1.525, $P_{(CD8)}$=0.3999, $t(6)$=0.906, $P_{(B)}$=0.1371, $t(6)$=1.715. **(b, c)** Representative flow charts (b) and proportion (c) for GM-CSF-producing Th1 and Th17 cells from Type-A and Type-B EAE at 9-dpi. n=6. Th17: P=0.6568, $t(10)$=0.4579, Th1: P=0.3954, $t(10)$=0.8879. **(d)** Proportion for IFNγ-, GM-CSF-, IL-22-producing and CD5L-expressing Th17 cells in spinal cords from Type-A and Type-B EAE mice at 17-dpi. Type-A (n=4), Type-B (n=5). $P_{(IFNγ)}$=0.3493, $t(7)$=1.0037, $P_{(GM\text{-}CSF)}$=0.1760, $t(7)$=1.505, $P_{(Il\text{-}22)}$=0.2859, $t(7)$=1.155, $P_{(CD5L)}$=0.2641, $t(7)$=1.214. **(e)** Proportions and MFI of CXCR2$^+$ neutrophils and macrophages obtained from naïve mice and mice with either Type-A or Type-B EAE at 9-dpi. n=4. PMN %: $P_{(Naive\ vs\ Type\text{-}A)}$=0.0102, $t(6)$=3.695, $P_{(Naive\ vs\ Type\text{-}B)}$=0.0004, $t(6)$= 7.001, $P_{(Type\text{-}A\ vs\ Type\text{-}B)}$=0.0022, $t(6)$=5.131. PMN MFI: $P_{(Naive\ vs\ Type\text{-}A)}$=0.0777, $t(6)$=2.125, $P_{(Naive\ vs\ Type\text{-}B)}$=0.0014, $t(6)$= 5.588, $P_{(Type\text{-}A\ vs\ Type\text{-}B)}$=0.0090, $t(6)$=3.81. Macrophage %: $P_{(Naive\ vs\ Type\text{-}A)}$=0.0005, $t(6)$=6.774, $P_{(Naive\ vs\ Type\text{-}B)}$=0.0006, $t(6)$= 6.48, $P_{(Type\text{-}A\ vs\ Type\text{-}B)}$=0.0161, $t(6)$=3.314. Macrophage MFI: $P_{(Naive\ vs\ Type\text{-}A)}$=0.33, $t(6)$=1.06, $P_{(Naive\ vs\ Type\text{-}B)}$=0.0001, $t(6)$= 11.11, $P_{(Type\text{-}A\ vs\ Type\text{-}B)}$=0.0001, $t(6)$=10.89. **(f)** Flow cytometry results showing CXCR2 protein expression on the CD4$^+$ T surface in each group (naïve mice, Type-A, or Type-B EAE mice at 9-dpi). **(g)** Comparison of relative gene expression levels between *Ltbr* and *Cxcr1*, *Cxcr1* and *Cxcr2*, *Ltbr* and *Cxcr2*, normalized to *Vcam1* expression. Total PBMCs from IFNβ-responder and non-responder RRMS patients were compared. All statistical analyses in this figure were performed by two-tailed unpaired Student's t-test. All the experimental data sets, except for (g), are representatives from at least 2 similar experiments for each.



Supplementary Figure 5

CXCL1 expression in spinal cords. .

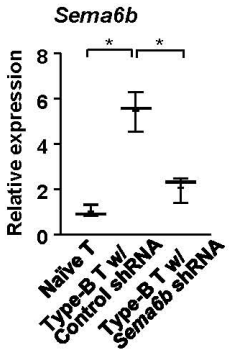
Shown are representative images of typical CXCL1 staining in spinal cords from naïve mice and mice with either Type-A or Type-B EAE at 9-dpi. Scale bars, 200 μm. Images are representatives from 3 similar experiments.



Supplementary Figure 6

.Histology of spinal cords in mice with Type-A or Type-B EAE.

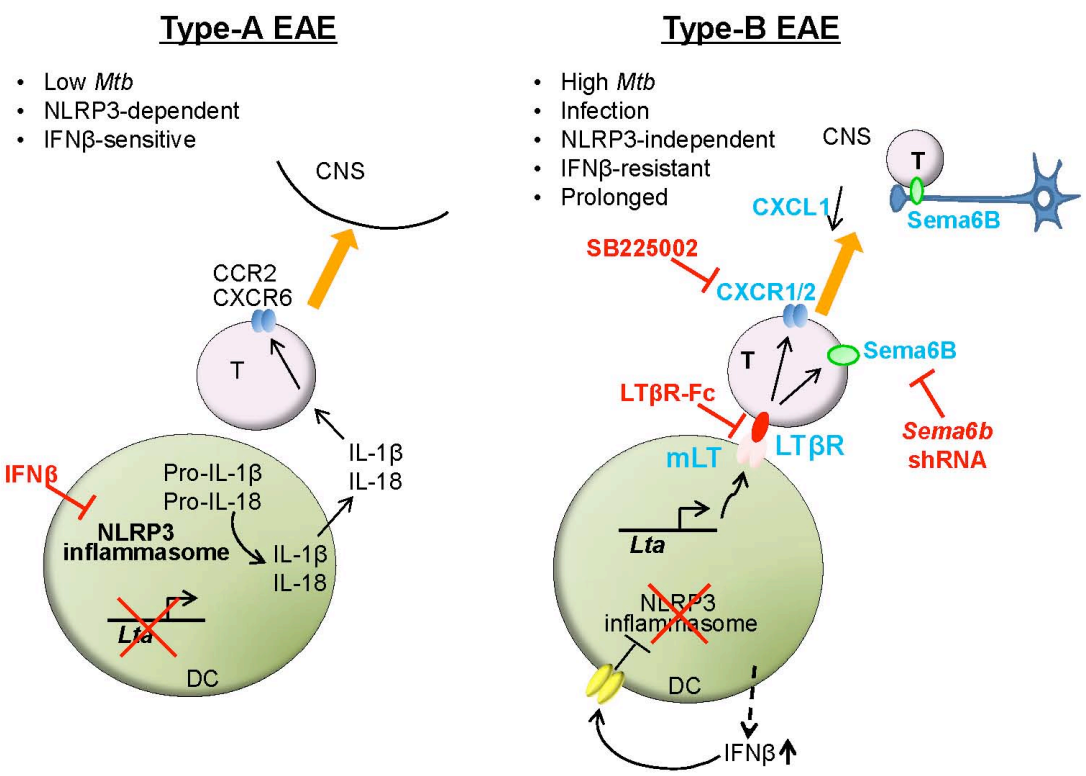
(a-e) Shown are representative images of typical staining from multiple mice. **(a)** H/E staining in the spinal cord of Type-A and Type-B EAE mice at 70-dpi. **(b)** IGF-1 staining in spinal cord of naïve mice and mice with either Type-A or Type-B EAE at 22-dpi. **(c)** Bielschowsky neuron staining in spinal cords of naïve mice (e), and Type-A and Type-B EAE at 30-dpi. Red arrows indicate area showing reduced staining intensity in the spinal cord. **(d,e**) Golgi's silver staining in spinal cord of naïve mice (d), and Type-A and Type-B at 22-dpi (e). All scale bars in this figure except magnified figure in (e), 200 μm. All scale bars in magnified figure in (e), 50 μm. All images are representatives from 3 similar experiments.



Supplementary Figure 7

***Sema6b* shRNA knock-down in T cells.** .

Sema6b expression levels in $CD4^+$ T cells isolated from naïve or Type-B EAE mice at 9-dpi were evaluated by qPCR. *Sema6b* was knocked down by shRNA in the indicated group of $CD4^+$ T cells. Control shRNA (scrambled shRNA sequence) was used for the control group. Values shown are obtained from three independent trials using one mouse per each. n=3. $P_{(Naive\ T\ vs\ Type\text{-}B\ T+Control\ shRNA)}$=0.0011, $t(4)$=8.396, $P_{(Type\text{-}B\ T+Control\ shRNA\ vs\ Type\text{-}B\ T+Sema6b\ shRNA)}$=0.0051, $t(4)$=5.559. *; p<0.05.



Supplementary Figure 8

Schematic diagram of distinct pathology between two EAE subtypes. .

Type-A EAE is NLRP3 inflammasome-dependent and IFNβ-sensitive. Type-B EAE is induced by immunization with higher doses of *Mtb* than Type-A EAE induction, and is NLRP3 inflammasome-independent and IFNβ-resistant. Type-B EAE can also be induced with Type-A EAE induction methods with MHV68 infection or with rLT (rLTα2β1) injection. In Type-B EAE, *Lta* gene expression is epigenetically induced in DCs and the expression of membrane-bound LT (mLT) on DCs are enhanced. mLT stimulate LTβR on $CD4^+$ T cells, resulting in the upregulation of CXCR2 on $CD4^+$ T cells. Blockade of LTβR (with LTβR-Fc) and CXCR2 (with SB225002) selectively inhibits the Type-B EAE progression. mLT is also involved in the induction of Sema6B in T cells. Sema6B causes neural damages, and this may be a reason for the prolonged and minimal remission in Type-B EAE.