Supporting Information

O'Connor *et al.* 10.1073/pnas.0803386105

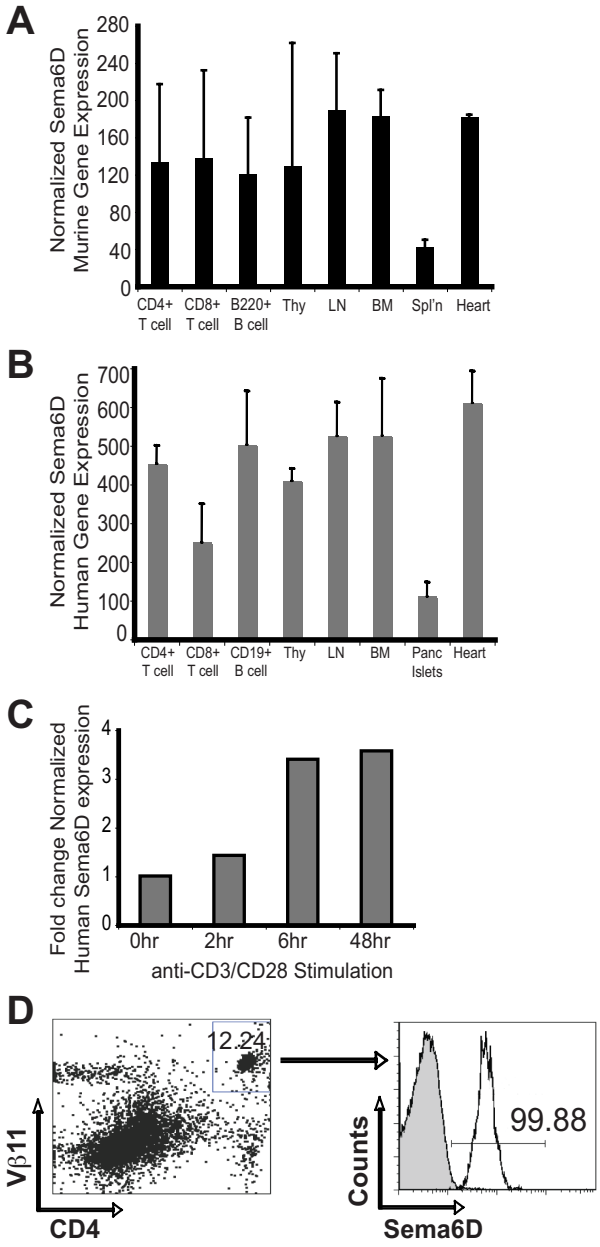

Fig. S1. Semaphorin 6D (Sema6D) expression is enhanced by activation. (*A–C*) Analysis of public database for T cell expression of Sema6D. (*A*) SymAtlas gene array mouse cell and tissue expression of Sema6D mRNA (1, 2). (*B*) SymAtlas gene array human cell and tissue expression of Sema6D mRNA (1, 2). (*A* and *B*) Thy, thymus; LN, lymph node; BM, bone marrow; Spl'n, spleen; Panc Islets, pancreatic islets. (*C*) Public gene array data for Sema6D mRNA expression in anti-CD3- and anti-CD28-stimulated T cells (3). (*D*) MOG-specific 2D2 Vβ11$^+$ TCR Tg CD4$^+$ T cells were stimulated *in vitro* for 4 days for Sema6D expression analysis. Sema6D induction was analyzed by flow cytometry.

1. Su AI, *et al.* (2002) Large-scale analysis of the human and mouse transcriptomes. *Proc Natl Acad Sci USA* 99:4465–4470.
2. Su AI, *et al.* (2004) A gene atlas of the mouse and human protein-encoding transcriptomes. *Proc Natl Acad Sci USA* 101:6062–6067.
3. Lund R, Aittokallio T, Nevalainen O, Lahesmaa R (2003) Identification of novel genes regulated by IL-12, IL-4, or TGF-β during the early polarization of CD4$^+$ lymphocytes. *J Immunol* 171:5328–5336.
4. Eun SY, *et al*. (2006) Cutting edge: Rho activation and actin polarization are dependent on plexin-A1 in dendritic cells. *J Immunol* 177:4271–4275.

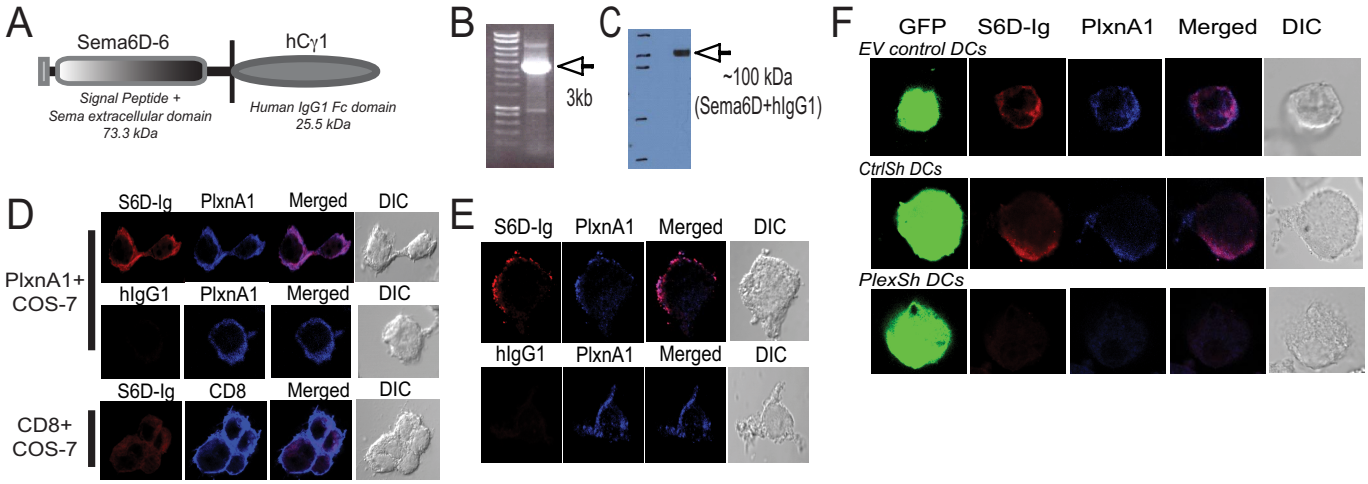

Fig. S2. Detection of Sema6D-Ig fusion protein colocalization with Plxna1 via confocal microscopy. (*A*) The extracellular portion of Sema6D was fused with the Fc portion of human IgG1 in the pcDNA3 backbone. (*B*) A 3-kb piece of cDNA encoding Sema6D isoform 6 (S6D-6) was isolated from mouse brain RNA by RT-PCR. (*C*) Expression of Sema6D-Ig (100 kDa) from drug-selected Chinese hamster ovary stable cell lines was confirmed by Western blotting. (*D*) COS-7 cells were transfected with either a Plxna1- or CD8a-expression plasmid and then incubated with biotin-conjugated Sema6D-Ig fusion protein (S6D-Ig). Biotin-Sema6D-Ig was detected by streptavidin (SA)-conjugated Alexa Fluor 555 (red). Plxna1 was detected by staining with anti-Plxna1 rabbit polyclonal Ab (Abcam) followed by Alexa Fluor 647-conjugated anti-rabbit Ig (blue). CD8 was detected with an anti-CD8a mAb followed by anti-mouse Alexa647. Purple color demonstrates colocalization of biotin-S6D-Ig with Plxna1. (*E*) Plxna1-expressing mouse DCs cultured for 12 days were incubated with biotin-S6D-Ig or control human IgG followed by the staining procedure described in *D*. (*F*) To confirm that Plxna1 expression by DCs was necessary for Sema6D-Ig association, we used a small hairpin RNA (shRNA) to reduce Plxna1 expression as described in ref. 4. DCs transduced with an empty retroviral control (EV), a retrovirus containing a mutated shRNA (CtrlSh), or a retrovirus bearing shRNA for Plxna1 (PlexSh) were used in a localization assay with Sema6D-Ig. The staining protocol is identical to the one described in *D*. Purple color demonstrates that the red signal for biotin-S6D-Ig colocalized with the blue signal for Plxna1. A reduction in Plxna1 expression correlated with a reduced capacity of Sema6D-Ig to associate with DCs.

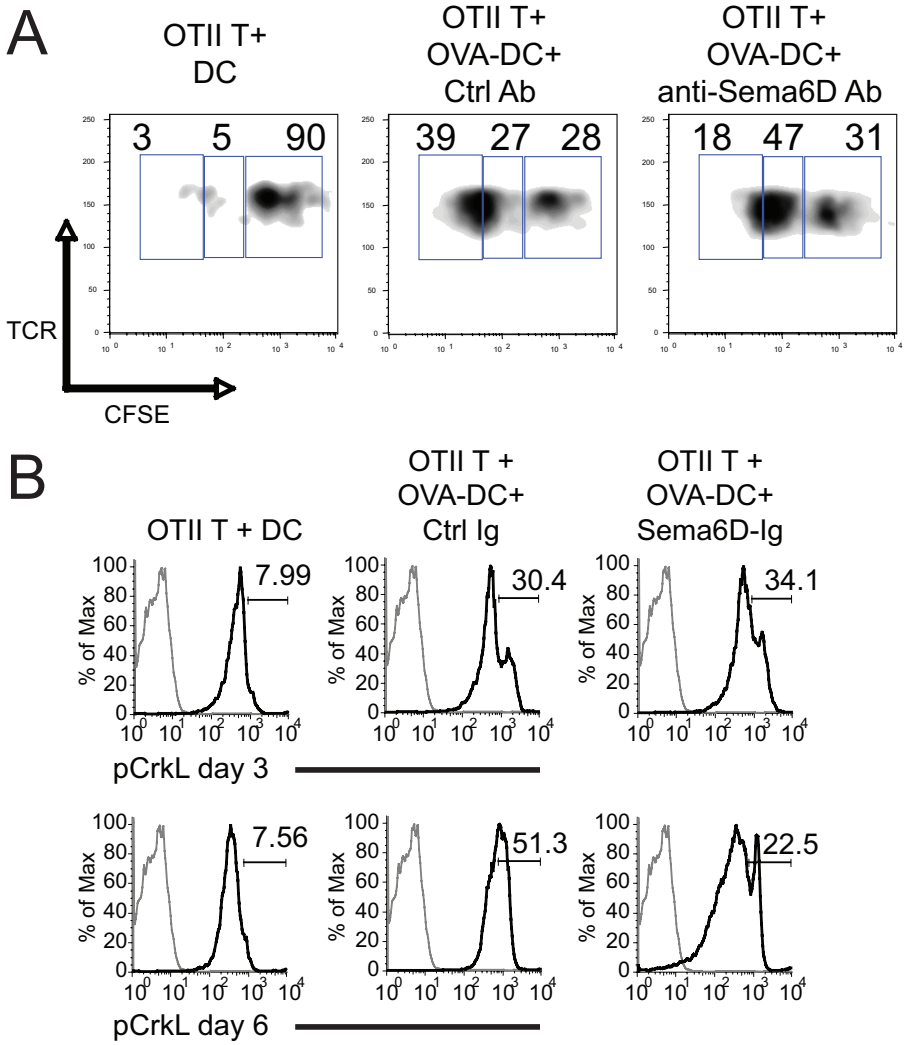

Fig. S3. Blockade of Sema6D inhibits a late phase of T cell activation. (*A*) Delayed targeting of Sema6D inhibits T cell proliferation at a late phase of activation. DCs that were loaded with whole OVA protein (OVA-DC) or unloaded (DC) were cocultured with purified OTII T cells *in vitro*. Before culture initiation, OTII T cells were labeled with CFSE. The dilution of CFSE was determined via flow cytometry for TCR^+ cells gated from $V\beta5^+$ and $CD4^+$ cells at day 6. OVA-stimulated cultures were also treated with an anti-Sema6D monoclonal antibody (R&D Systems) or a control antibody on day 4. The percentage of TCR^+ cells with a $CFSE^{high}$, $CFSE^{int}$, or $CFSE^{low}$ phenotype is indicated for each coculture group. (*B*) PhosphoFlow analysis of CrkL phosphorylation (pCrkL) in T cells cocultured with DCs at days 3 and 6. DCs that were loaded with whole OVA protein (OVA-DC) or unloaded (DC) were cocultured with purified OTII T cells *in vitro*. OVA-stimulated cultures were also treated with either a Sema6D-Ig fusion protein or a control Ig. To reduce nonspecific autofluoresence impacts on pCrkL detection, TCR^+ cells of similar cell size (FSC) were analyzed for each treatment group. The detection of pCrkL was determined in reference to T cells cocultured with unloaded DCs.

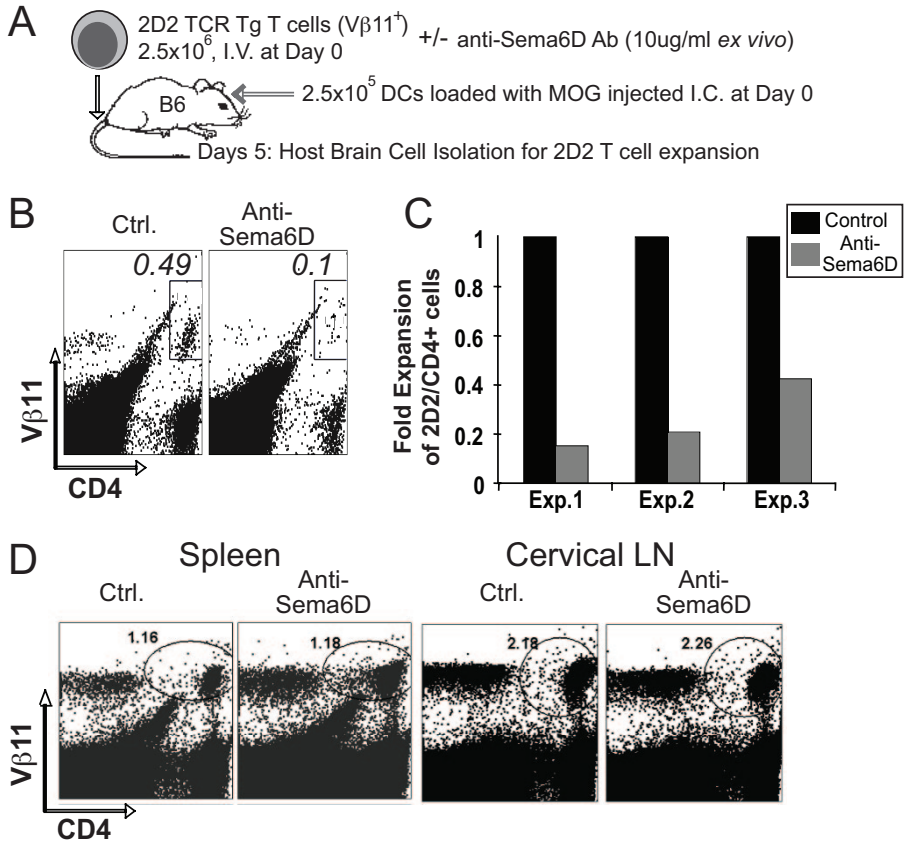

Fig. S4. Disruption of Sema6D with an anti-Sema6D antibody reduces T cell neuroinflammation. (*A*) MOG-specific 2D2 TCR Tg $CD4^+$ T cells were stimulated *in vitro* for 4 days to induce Sema6D expression (Fig. S1*D*). At day 4 of *in vitro* stimulation, T cells were treated with a monoclonal anti-Sema6D Ab (R&D Systems) at a final concentration of 10 μg/ml for 24 h. After treatment, $\approx 2.5 \times 10^6$ T cells were adoptively transferred to recipient mice by i.v. injection. At the same time, $\approx 2.5 \times 10^5$ DCs loaded with MOG antigen were transferred by intracranial (I.C.) injection. Five days after the adoptive transfer, the brain cells of recipient mice were isolated and analyzed by flow cytometry. (*B*) The expansion of 2D2 $CD4^+$ T cells was tracked by expression of the Tg Vβ11 TCR chain. The percentage of $V\beta11^+$ $CD4^+$ T cells in the total isolated brain cell population is indicated. (*C*) The expansion of 2D2 T cells treated with anti-Sema6D Ab was compared for three independent experiments in relation to a control group. (*D*) The percentage of 2D2 Tg T cells in the periphery was unaffected by anti-Sema6D antibody treatment. Flow cytometry detection of $CD4^+$ $V\beta11^+$ T cells in the spleen and lymph node 5 days after adoptive transfer is shown.