SUPPLEMENTAL INFORMATION

Supplemental Data include seven figures, one table, Supplemental Material and Methods and six movies.

ONLINE METHODS

Mice

Plxna1$^{-/-}$ [20] and *Sema3A*$^{-/-}$ [31] mice were previously characterized. *Nrp1*$^{sema-}$ knock-in mice were kindly provided by D.D. Ginty and A. L. Kolodkin [32]. OT-II Tg mice were kindly provided by W. R. Heath [26]. *Sema6C*$^{-/-}$ and *Sema6D*$^{-/-}$ mice were newly generated as described in the Supplemental Experimental Procedures. All experimental procedures were performed following the guidelines by the Research Institute for Microbial Diseases, Osaka University.

Adoptive transfer experiments

BMDCs were labeled with 5 μM CFSE in PBS for 10 min at room temperature and then extensively washed with PBS. One million DCs in PBS were injected subcutaneously into the hind footpads of recipient mice. The popliteal LNs were collected at 24 and 48 h post

injection and then treated with 1 mg/ml collagenase D for 30 min at 37°C. The cells were counted and analyzed by flow cytometry. The percentage of migrated DCs corresponds to the ratio of fluorescent DCs among the total LN cells [49].

Transwell experiments

Uncoated transwells (pore size, 5.0 μm; CORNING) were placed in 24-well plates filled with 0.6 ml of 0.1% BSA in RPMI containing CCL19, CCL21 or CXCL12. A solution of 1 $x10^5$ DCs in 0.1 ml was added to the upper well of the transwell and incubated at 37°C for 3 h. The cells in the lower chamber were detached with 5 mM PBS-EDTA for 5 min and counted by Guava. For *in vitro* transmigration assays, fibronectin (10 mg/ml), type I collagen (3.0 mg/ml) or lymphatic endothelial cells were layered on the membrane of the upper chamber. Briefly, 2 $x10^4$ SVEC4-10 or HMVEC-dLy cells were seeded on the upper or lower surface of the transwell insert that was coated with 2 μg/ml fibronectin. After two days of culture, the integrity of the confluent layers was assessed by phalloidin staining. The transmigration assays were performed for 6 h as previously described [50]. Myosin II and ROCK were inhibited by treating DCs with 50 μM blebbistatin or 30 μM Y-27632, respectively, for 30 min at 37°C as previously described [11].

***In vivo* transmigration of DCs**

To observe DCs exiting the periphery, contact hypersensitivity was induced by applying oxazolone to the abdomen as previously described [7]. Briefly, six days after sensitization, oxazolone was applied to the ear. Eight hours after challenge, 10^6 CFSE-labeled BMDCs were dermally injected. After 24 h, the animals were sacrificed, and the ear tissues were fixed in paraformaldehyde. Whole-mount staining was performed using a biotinylated anti-Lyve-1 antibody plus streptavidin-Cy3 to detect the lymphatics. Images were obtained by confocal Z-stack imaging, and the number of cells retained in the periphery was determined.

Two-dimensional migration assays in 3D collagen matrices

Bone marrow-derived DCs that had been treated with LPS (500 ng/ml) for 12 h were suspended in type I collagen (3 mg/ml) (BD Biosciences) containing 2% FCS with either a control IgG (5 μg/ml) or Sema3A-Fc (5 μg/ml) and then placed on one side of the Zigmond chamber to cover the stage with gel. The cells were incubated at 37°C for 30 min to polymerize the matrix, and then RPMI containing 0.5% BSA with CCL21 (5 μg/ml) was

added to the other chamber. After a 20-min incubation, DC locomotion was examined at 1-min intervals by a confocal time-lapse video-microscope as described above.

Statistical analysis

Two-tailed Student's t-test and one-way ANOVAs were performed after the data were confirmed to fulfill the criteria. Otherwise, Mann-Whitney's U-test and Kruskal-Wallis test were performed. If the ANOVA or Kruskal-Wallis test was significant, Tukey's test or Scheffe's F test was used as a post hoc test. Analyses were performed with Statcel2.

References for Online Methods

49. Cavanagh, L.L. *et al.* Activation of bone marrow-resident memory T cells by circulating, antigen-bearing dendritic cells. *Nat. Immunol.* **6**, 1029-37 (2005).

50. Ledgerwood, L.G. *et al*. The sphingosine 1-phosphate receptor 1 causes tissue retention by inhibiting the entry of peripheral tissue T lymphocytes into afferent lymphatics. *Nat. Immunol.* **9**, 42-53 (2008).