Supporting Information

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SI Materials and Methods

Chemokine (C-C Motif) Receptor Type 7 Expression. Bone marrow-derived dendritic cells (BMDCs) were induced from bone marrow cells of WT mice and analyzed by flow cytometry after staining with an anti-mouse chemokine (C-C motif) receptor type 7 antibody (clone 4B12; BD Biosciences) coupled to peridinin chlorophyll protein-Cy5.5 (Fig. S1).

Chemotaxis. As shown in Fig. S1, unstimulated or LPS-stimulated (100 ng/mL for 24 h) $CD31^{+/+}$ and $CD31^{-/-}$ BMDCs were labeled with carboxyfluorescein succinimidyl ester (CFSE) and allowed to migrate (5×10^5 BMDCs per well) for 4 h at 37 °C on transwell assay plates (ChemoTx Disposable Chemotaxis System, no. 106-5; Neuroprobe) in which chemokine (C-C) motif ligand 21 (1 μg/mL) was present in the lower chamber. The number of CFSE-labeled dendritic cells in the lower chamber was then evaluated by flow cytometry with a BD LSRII analyzer (BD Biosciences) equipped with a high-throughput screening reader. The data were analyzed with BD Diva software (BD Biosciences).

Quantification. To quantify the effect of the CD31 peptide on the internalization of surface CD31 molecules upon LPS stimulation, spleen single-cell suspensions from C57BL/6 mice ($n = 3$, 10^6 cells per tube) were stimulated with 1 μg/mL LPS (for 30 min at 37 °C) in the presence of the CD31 peptide (final concentration of 50 μg/mL) or the vehicle (Fig. S2). At the end of the experiment, cells were transferred on ice and processed for cytometric analysis as described in *Materials and Methods*. The antibodies used for these experiments were phycoerythrin–anti-mouse CD31 (clone MEC13.3; BD Biosciences), allophycocyanin–anti-mouse CD11c (clone HL3; BD Biosciences), and BV421–anti-mouse IA^b (clone M5/114.15.2; Biolegend). In the indicated experiments, intracellular CD31 molecules were detected after fixation [4% (wt/vol) paraformaldehyde for 10 min at 4 °C] and permeabilization (PBS/0.5% Triton X-100 for 10 min), using the antibodies diluted in the permeabilization buffer. Cytometric analysis was performed on a BD LSRII analyzer, as indicated in *Materials and Methods*.

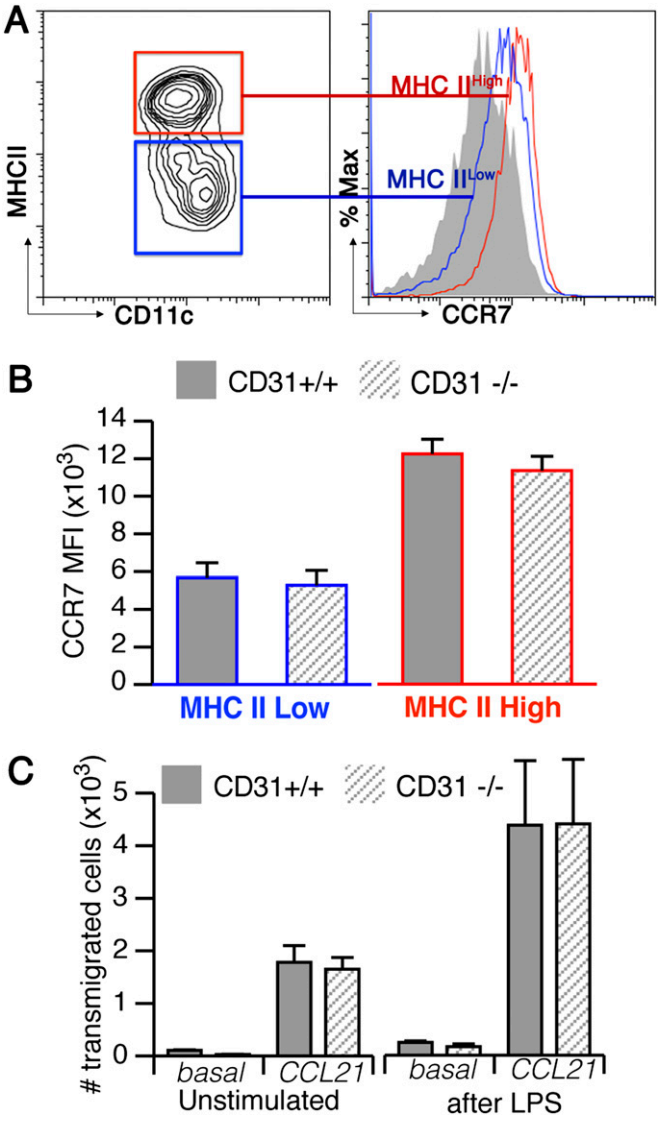

Fig. S1. CD31 expression does not modify BMDC migration against chemokine (C-C) motif ligand 21 (CCL21) in vitro. (*A*) Flow cytometry analysis of surface chemokine (C-C motif) receptor type 7 (CCR7) expression on CD11c^{+}, CD31$^{+/+}$, and CD31$^{-/-}$ BMDCs based on their maturation state: MHC class II (MHCII)^{high} and MHCII^{low}. %Max, percentage of maximum. (*B*) CCR7 expression levels [median fluorescence intensity (MFI)] on CD31$^{+/+}$ and CD31$^{-/-}$ dendritic cells (DCs) were similar, regardless of the maturation state. (*C*) BMDCs from CD31$^{+/+}$ and CD31$^{-/-}$ mice were stimulated or not stimulated with LPS (100 ng/mL) overnight, CFSE-labeled, and allowed to migrate across a transwell chamber, the wells of which contained CCL21 (1 μg/mL). Flow cytometric analysis of the CFSE^{+} cells that migrated to the lower chamber revealed that there were no differences between CD31$^{+/+}$ and CD31$^{-/-}$ mice with respect to the numbers of immature and mature DCs.

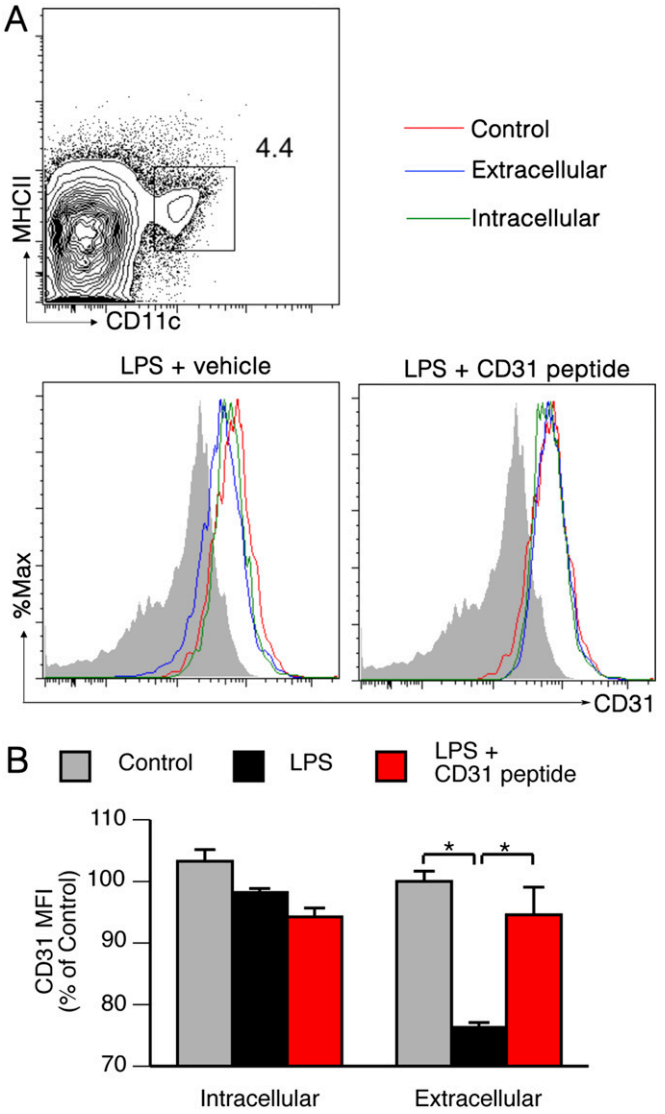

Fig. S2. Effect of the agonist peptide on the internalization of surface CD31 molecules by DCs stimulated with LPS. (*A*) Example of flow cytometry analysis of the surface (extracellular, blue) and internalized (intracellular, green) CD31 molecules in spleen DCs submitted to LPS stimulation with or without the agonist peptide. Control cells were left unstimulated (red). The representative histogram overlays show that the fluorescent intensity of the "Extracellular" (surface) CD31 immunostaining was consistently reduced on DCs ($CD11c^{+}MHCII^{+}$) that were subjected to LPS stimulation compared with control (unstimulated) cells. The CD31 molecules were likely internalized, because the CD31 signal was unchanged when the staining was performed after cell fixation and permeabilization ("Intracellular"). Remarkably, the presence of the CD31 peptide during LPS stimulation prevented the internalization of the surface CD31 molecules. (*B*) Quantitative analysis of the CD31 mean fluorescence intensity in the different conditions, normalized to the control (extracellular staining, unstimulated cells).