Supporting Information

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SI Materials and Methods

Generation of CD47 Floxed Mice. For the generation of CD47 floxed ($CD47^{f/f}$) mice, a BAC clone containing C57BL/6J mouse *Cd47* genomic DNA was obtained from BACPAC. The targeting vector was constructed with a 5.5-kb DNA fragment including the upstream region of exon 1 as a 5′ homologous region, a 6.4-kb DNA fragment including the downstream region of exon 1 as a 3′ homologous region, a neomycin resistance cassette flanked by two FRT (Flp recognition target) sites for positive selection of ES cell clones, and a diphtheria toxin A (DTA) chain gene cassette for negative selection (Fig. S7*A*). LoxP sites were also inserted into the upstream sequence of exon 1 and into intron 1 of the targeting vector. The targeting vector was introduced into ES cells by electroporation. Positive ES cell clones were injected into C57BL/6J blastocysts, which then were implanted into pseudopregnant mice. For deletion of the FRT-flanked neomycin resistance cassette, the chimeric offspring were bred with C57BL/6J mice expressing the *Flp* gene (UNITECH), resulting in the generation of mice harboring the CD47 floxed ($CD47^{f}$) allele. CD11c-*Cre* or E2A-*Cre* mice were crossed with $CD47^{f/f}$ mice to yield $CD47^{f/f}$;CD11c-*Cre* ($CD47^{\Delta DC}$) and $CD47^{f/f}$;E2A-*Cre* ($CD47^{-/-}$) offspring for study.

Antibodies and Reagents. An agonistic rat mAb to mouse LTβR (4H8) was the kind gift of C. F. Ware, Sanford Burnham Prebys Medical Discovery Institute, San Diego. A FITC-conjugated mAb to B220 (RA3-6B2), phycoerythrin (PE)-conjugated mAbs to CD4 (RM4-5) and to CD3ε (145-2C11), a peridinin chlorophyll (PerCP)-conjugated mAb to CD8α (53-6.7), and allophycocyanin (APC)-conjugated mAbs to CD11c (HL3) and to CD4 (RM4-5) were obtained from BD Biosciences. An FITC-conjugated mAb to Ki-67 (SolA15), a PE-conjugated mAb to F4/80 (BM8), purified mAbs to CD16/32 (93) and to Pdpn (eBio8.1.1), and biotin-conjugated mAbs to IL-7Rα (A7R34), LTβR (eBio3C8), and Thy1.2 (30-H12) were from eBioscience. An Alexa Fluor 488–conjugated mAb to CD3ε (17A2), PE-conjugated mAbs to CD8α (53-6.7) and to CD31 (MEC13.3), PerCP- and Cy5.5-conjugated mAbs to CD45 (30-F11) and to Ter119 (TER-119), a PE- and Cy7-conjugated mAb to CD11c (N418), an APC-conjugated mAb to Pdpn (8.1.1), APC- and Cy7-conjugated mAbs to B220 (RA3-6B2) and to I-A/I-E (M5/114.15.2), a brilliant violet 421-conjugated mAb to CD11b (M1/70), biotin-conjugated mAbs to CD11b (M1/70) and to TNFR1 (55R-170), a brilliant violet 510-conjugated mAb to CD8α (53-6.7), and a Zombie Aqua Fixable Viability Kit were from BioLegend. PerCP- and Cy5.5-conjugated mAbs to CD3ε (145-2C11), to CD19 (1D3), and to NK1.1 (PK136) were from Tonbo Biosciences. Cy3-conjugated donkey polyclonal antibodies (pAbs) to goat IgG and to hamster IgG and FITC- or Cy3-conjugated streptavidin were from Jackson ImmunoResearch. A mouse mAb to β-actin (AC-40) and propidium iodide were from Sigma-Aldrich. Mouse p55 TNFR (TNFR1)–Fc and LTβR–Fc chimeras, the Mouse TNF-α Quantikine ELISA Kit, and goat pAbs to mouse CCL21, CCL19, or CXCL13 were from R&D Systems. Recombinant mouse TNF-α was obtained from Peprotech. Hoechst 33342 and Rhodamine-conjugated phalloidin were from Invitrogen. A mouse mAb to IκBα (L35A5) and rabbit pAbs to NF-κB2 p100/p52 were from Cell Signaling Technology. Etanercept (Enbrel, p75 TNFR-Ig) was obtained from Takeda Pharmaceuticals. Microbeads conjugated with antibodies to biotin or with mAbs to mouse CD45 or CD11c (N418) were obtained from Miltenyi Biotech.

Cell Preparation and Flow Cytometry. Cell suspensions were prepared from the spleen and LNs as described previously (14), with minor modifications. In brief, tissue was minced and then digested for 20 min at 37 °C in RPMI 1640 (Wako) containing collagenase IV (1 mg/mL; Worthington Biochemical), DNase I (40 μg/mL; Sigma-Aldrich), and 2% FBS. Undigested fibrous material was removed by filtration through a 70-μm nylon mesh, and red blood cells in the filtrate were lysed with Pharm Lyse buffer (BD Biosciences). For preparation of BM cell suspensions, BM cells were isolated from the femur and tibia with the use of a syringe fitted with a 25-gauge needle, the cell preparation was passed through a nylon mesh, and red blood cells in the filtrate were lysed.

For flow cytometric analysis, cells were washed twice with PBS containing 2% FBS and 2 mM EDTA, incubated first with a mAb specific for mouse CD16/32 to prevent nonspecific binding of labeled mAbs to FcγRs and then with specific mAbs, and then were analyzed with the use of a FACSVerse instrument (BD Biosciences) or sorted with the use of a FACSAriaIII instrument (BD Biosciences). All data were analyzed with FlowJo X software (FlowJo; LLC).

Isolation and Culture of Splenic Stromal Cells. Splenic SCs were isolated according to a procedure for LN SC isolation (26), with minor modifications. In brief, the spleen was cut into small pieces and digested for 30 min with RPMI 1640 medium containing collagenase P (0.2 mg/mL; Roche), dispase (0.8 mg/mL; Roche), DNase I (100 μg/mL), and 2% FBS. Undigested fibrous material was removed by filtration through a 70-μm nylon mesh, and red blood cells in the filtrate were lysed with Pharm Lyse buffer. The remaining cells were cultured at a density of 5×10^5 cells/cm^2 in αMEM supplemented with 10% FBS and 1% penicillin-streptomycin, nonadherent cells were removed after 24 h, and the remaining attached cells were cultured further in the same medium. After 5 d, the cells were harvested, and $CD45^-$ cells were enriched with the use of anti-CD45 microbeads and an autoMACS Pro Separator (Miltenyi Biotec). The $CD45^-$ cells then were plated at a density of 2.5×10^4 per well in a 24-well plate and cultured for 24 h, the end of which was designated day 0. For coculture of DCs with splenic SCs, $CD11c^+$ cells were isolated from splenocytes with the use of anti-CD11c microbeads and an autoMACS Pro Separator. The purity of the isolated $CD11c^+$ DCs was >95% as determined by flow cytometry. The $CD11c^+$ cells then were cocultured in a 1:1 ratio with isolated splenic $CD45^-$ cells at a density of 2.5×10^4 per well in the presence of RPMI 1640 (Wako) supplemented with 10% FBS and 1% penicillin-streptomycin. For determination of the survival of splenic SCs, the isolated $CD45^-$ cells were cocultured with DCs in RPMI 1640 supplemented with 0.5% FBS and 1% penicillin-streptomycin. For Transwell cultures (pore size, 0.4 μm; Corning), the isolated $CD45^-$ cells were plated in the lower chamber of Transwell plates and then were cultured with DCs placed in the upper chamber. For neutralization of TNFR or LTβR ligands, TNFR1-Fc (0.5 μg/mL) or LTβR-Fc (0.5 μg/mL) chimeras were added to cocultures at day 0.

Determination of BrdU Incorporation in Splenic Stromal Cells. Incorporation of BrdU by splenic SCs was determined as described previously (14). In brief, mice were injected i.p. with 1 mg of BrdU (Sigma) and then were continuously provided with BrdU (0.8 mg/mL) in drinking water that was changed every other day. Six days after BrdU injection, the spleen was isolated and digested as described above for flow cytometry. Control mice that did not

receive BrdU were prepared in parallel. The digested cells were initially stained for surface antigens and then were fixed, permeabilized, stained for BrdU with the use of an FITC BrdU Flow Kit (BD Biosciences), and analyzed by flow cytometry.

Analysis of the Cell-Cycle Profile and Apoptosis in Splenic Stromal Cells. For snapshot cell-cycle analysis in splenic SC subsets, splenocytes were isolated, stained for surface antigens, and then fixed and permeabilized with the use of a Fix/Perm Kit (BD Biosciences). After washing, the cells were stained with FITC-conjugated antibodies to Ki-67 and Hoechst 33342 and then were analyzed by flow cytometry. For determination of Annexin V^+ cells in splenic SC subsets, splenocytes were isolated, stained first for surface antigens and then with FITC-conjugated Annexin V together with Hoechst 33342 in the presence of Annexin V-binding buffer (BioLegend), and finally were analyzed by flow cytometry.

H&E Staining. The spleen was fixed with 10% formalin (Wako), cut into pieces, embedded in paraffin, and sectioned for staining with Mayer's H&E. Images were acquired with a Keyence BZ-X700 microscope. Image analysis was performed by separating the hematoxylin-stained component (the white pulp area) and the eosin-stained component (the total spleen area) with the use of the H&E color deconvolution plugin of ImageJ (NIH).

Immunohistofluorescence Analysis. The spleen was embedded in optimal cutting temperature (O.C.T.) compound (Sakura Fine Technical), immediately frozen in isopentane cooled with liquid nitrogen, and cut into 5- to 7-μm sections with a cryostat (Leica). The sections were dried with a cold airstream for 30 min, fixed with ice-cold acetone (for Thy1.2, CCL21, or CXCL13 staining) or methanol (for Pdpn staining) for 10 min, and then incubated for 1 h at room temperature in blocking solution (PBS containing 5% BSA) before exposure overnight at 4 °C to primary antibodies diluted in blocking solution. They were then washed with PBS, stained for 1 h at room temperature with secondary antibody and DAPI diluted in blocking solution, and imaged with a Keyence BZ-X700 microscope. Images were processed with Adobe Photoshop software. For measurement of the $Thy1.2^+$, $B220^+$, $CCL21^+$, $CXCL13^+$, or $Pdpn^+$ areas in the spleen, cross-sections were made through different segments of the tissue, and images were acquired with a 10× objective magnification. The areas of positively stained regions in each image were determined with the use of ImageJ software and averaged for at least five sections per spleen.

Cell Sorting of cDC Subsets and Monocytes. For cell sorting of cDC subsets, splenocytes isolated from $Sirpa^{f/f}$ or $Sirpa^{\Delta DC}$ mice were incubated with anti-CD11c microbeads, and $CD11c^+$ cells then were collected with the use of an autoMACS Pro Separator. The isolated cells were further stained, and $CD4^+$, CD8, and DN cDC subsets were sorted as lineage (CD3, CD19, NK1.1, Ter119)$^-$ $CD11c^+$I-A/I-$E^+CD4^+CD8^-$, lineage$^-CD11c^+$I-A/I-$E^+CD4^-CD8^+$, and lineage$^-CD11c^+$I-A/I-$E^+CD4^-CD8^-$ cells, respectively, with the use of the FACSAriaIII instrument. For isolation of monocytes, splenocytes isolated from $Sirpa^{f/f}$ or $Sirpa^{\Delta DC}$ mice were incubated with biotinylated anti-CD11b antibody, followed by anti-streptavidin microbeads, and $CD11b^+$ cells were collected by an autoMACS Pro Separator. The $CD11b^+$ cells were further stained, and $Ly6C^{hi}$ monocytes were sorted as lineage (CD3, B220)$^-CD11b^+CD11c^-Ly6C^{hi}Ly6G^-$ cells. The purity of the isolated cells was >98% as determined by flow cytometry.

Preparation of cDNA and RT-qPCR Analysis. Total RNA was extracted either from freshly isolated spleen tissue with the use of Sepasol (Nacalai) and an RNeasy Mini Kit (Qiagen) or from freshly isolated cDC subsets or monocytes with the use of an RNeasy Micro Kit (Qiagen). The total RNA was subjected to reverse transcription with the use of a QuantiTect Reverse Transcription Kit (Qiagen) followed by qPCR analysis in 96-well or 384-well plates (Roche) with the use of a QuantiTect SYBR Green PCR Kit (Qiagen) and a LightCycler 480 instrument (Roche). The amplification results were analyzed with the use of LightCycler 480 software (Roche) and were normalized by the corresponding amount of *Gapdh* mRNA. Primer sequences for qPCR (forward and reverse, respectively) were as follows: *Gapdh*, 5′-AGGTCGGTGTGAACGGATTTG-3′ and 5′-TGTAGACCATGTAGTTGAGGTCA-3′; *Ccl21*, 5′-ATCCCGGCAATCCTGTTCTC-3′ and 5′-GGGGCTTTGTTTCCCTGGG-3′; *Ccl19*, 5′-GGGGTGCTAATGATGCGGAA-3′ and 5′-CCTTAGTGTGGTGAACACAACA-3′; *Il7*, 5′-GATAGTAATTGCCCGAATAATGAACCA-3′ and 5′-GTTTGTGTGCCTTGTGATACTGTTAG-3′; *Tnf*, 5′-TGGAACTGGCAGAAGAGGCACT-3′ and 5′-GAGATAGCAAATCGGCTGACGG-3′; *Lta*, 5′-TCCACTCCCTCAGAAGCACT-3′ and 5′-AGAGAAGCCATGTCGGAGAA-3′; *Ltb*, 5′-TGCGGATTCTACACCAGATCC-3′ and 5′-ACTCATCCAAGCGCCTATGA-3′; and *Il1b*, 5′-CAACCAACAAGTGATATTCTCCATG-3′ and 5′-GATCACCACTCTCCAGCTGCA-3′.

Generation of BM Chimeras. Single or mixed BM chimeras were generated as described previously (14), with minor modifications. In brief, recipient C57BL/6J × B6.SJL F1 ($CD45.1^+CD45.2^+$) mice were lethally irradiated (9.5 Gy) and then were injected i.v. with 5×10^6 BM cells obtained from $Sirpa^{f/f}$ ($CD45.2^+$) or $Sirpa^{\Delta DC}$ ($CD45.2^+$) mice or with 5×10^6 mixed BM cells obtained from both B6.SJL ($CD45.1^+$) and $Sirpa^{\Delta DC}$ mice (1:1 ratio). Flow cytometry revealed that the frequency of donor-derived cells was >95% in recipient blood at 6 wk after transplantation. Eight weeks after BM transplantation, splenocytes isolated from the recipient mice were analyzed by flow cytometry or the spleen was subjected to immunohistofluorescence analysis.

Immunoblot Analysis. Cells were homogenized on ice in radioimmunoprecipitation assay (RIPA) buffer [20 mM Tris·HCl (pH 7.5), 150 mM NaCl, 2 mM EDTA, 1% Nonidet P-40, 1% sodium deoxycholate, 0.1% SDS, 50 mM NaF] containing 1 mM sodium vanadate and a protease inhibitor mixture (Nacalai). The lysates were centrifuged at 17,500 × *g* for 15 min at 4 °C, and the resulting supernatants were subjected to SDS/PAGE followed by immunoblot analysis. Immune complexes were detected with a Chemi-Lumi One L Kit (Nacalai) and a LAS4000 image analyzer (Fujifilm). The intensity of bands was determined by densitometric analysis with the use of ImageJ software.

Determination of Soluble LTα in Culture Supernatants. Soluble LTα in culture supernatants was detected by immunoprecipitation and immunoblot analysis. For preparation of CHO-Ras cells that produce mouse LTα, a cDNA encoding the full-length protein (amino acids 1–202) was amplified by PCR with the primers 5′-CCGAATTCGACATGACACTGCTC-3′ (forward) and 5′-GCTCTAGACTACAGTGCAAAGGCTC-3′ (reverse), and the PCR product was subcloned into pcDNA3.1 (Invitrogen). CHO-Ras cells stably express an active form of H-Ras and were kindly provided by S. Shirahata, Kyushu University, Fukuoka, Japan. CHO-Ras cells were transfected with pcDNA3.1-LTα with the use of the Lipofectamine 2000 reagent (Invitrogen) and then were cultured in serum-free medium for 7 d. Culture supernatants were harvested and incubated for 2 h at room temperature with pAbs to mouse LTα (R&D Systems) before the addition of protein G-Sepharose (GE Healthcare). The immunoprecipitated material was isolated and subjected to immunoblot analysis with the same pAbs.

Statistical Analysis. Data are presented as means ± SE and were analyzed by Student's *t* test, ANOVA followed by Tukey's test, or two-way ANOVA followed by Sidak's test with the use of Prism6 software (GraphPad Software). A P value of <0.05 was considered statistically significant.

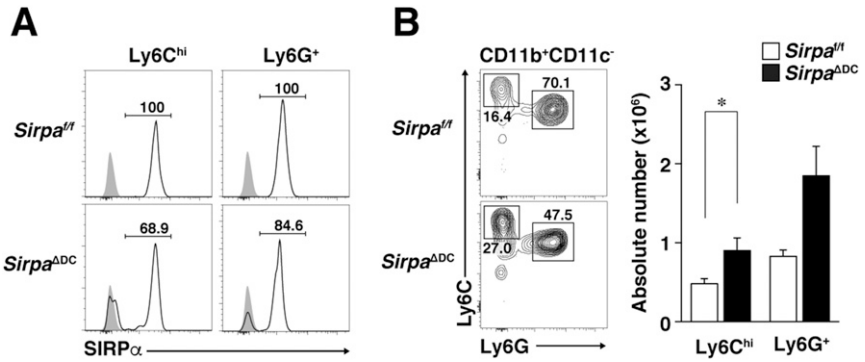

Fig. S1. Characterization of Ly6C^{hi} monocytes or Ly6G^{+} neutrophils in the spleens of *Sirpa*$^{f/f}$ and *Sirpa*$^{\Delta DC}$ mice. (*A*) Expression of SIRPα (open traces) or isotype control (solid traces) on monocytes and Ly6G neutrophils among lineage (CD3ε^{+}B220^{-}) CD11b^{+}CD11c^{-} splenocytes from *Sirpa*$^{\Delta DC}$ mice. Data are representative of three independent experiments. (*B*) Absolute number of Ly6C^{hi} monocytes and Ly6G^{+} granulocytes in the spleens of *Sirpa*$^{f/f}$ or *Sirpa*$^{\Delta DC}$ mice. Data are pooled from three independent experiments and are expressed as the mean ± SE for six mice per group. *$P < 0.01$ (Student's *t* test).

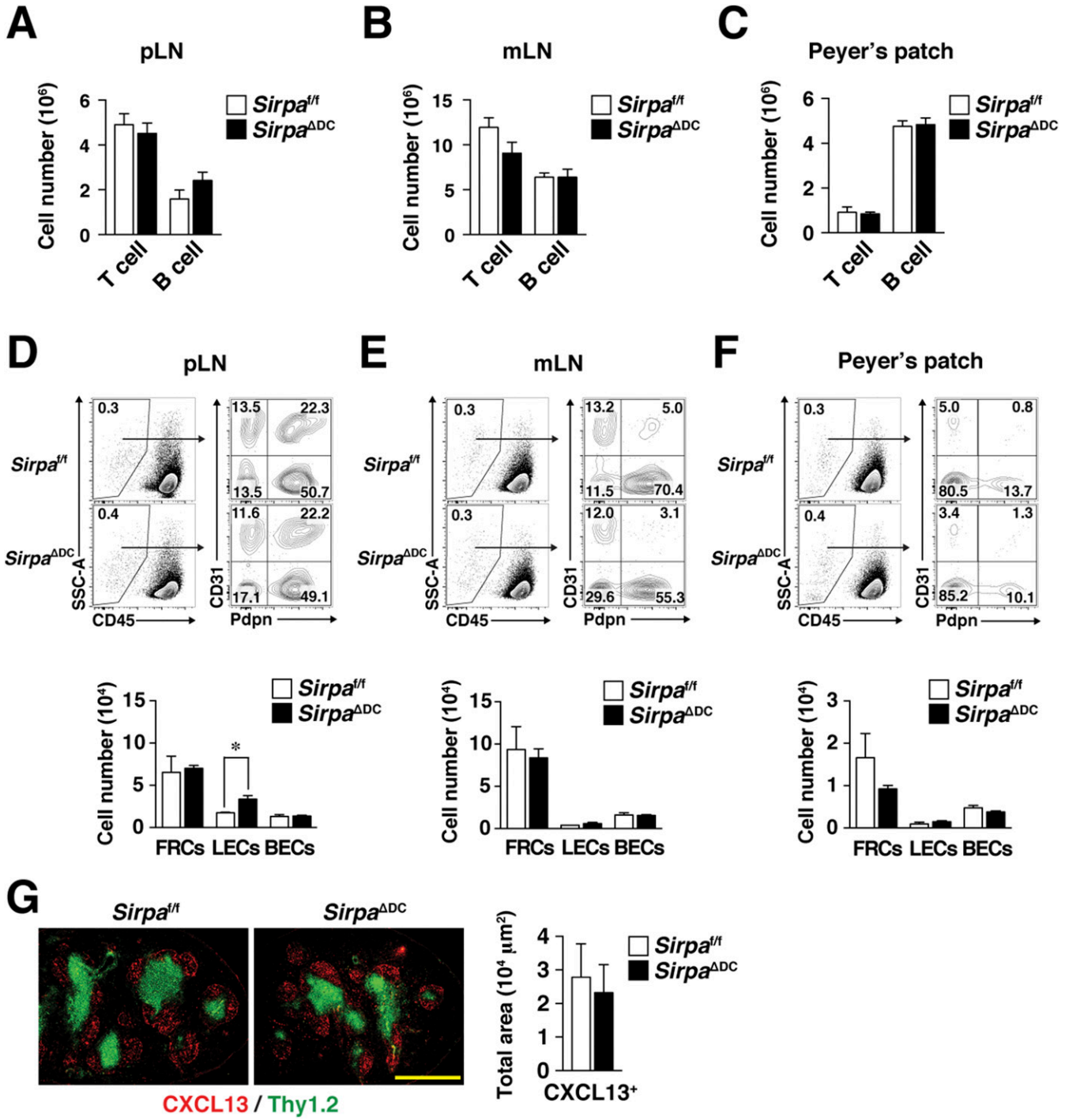

Fig. S2. Characterization of T and B cells and SC subsets in SLOs of *Sirpa*$^{f/f}$ and *Sirpa*$^{\Delta DC}$ mice. (*A*–*C*) Absolute numbers of T cells (CD3ε$^+$B220$^-$) and B cells (CD3ε$^-$B220$^+$) in peripheral LNs (pLNs) (*A*), mesenteric LNs (mLNs) (*B*), and Peyer's patch (*C*) of *Sirpa*$^{f/f}$ or *Sirpa*$^{\Delta DC}$ mice. Data are pooled from three independent experiments and are expressed as the means ± SE for three mice per group. (*D*–*F*) SC subsets isolated from peripheral LNs (*D*), mesenteric LNs (*E*), and Peyer's patch (*F*) of *Sirpa*$^{f/f}$ or *Sirpa*$^{\Delta DC}$ mice were analyzed by flow cytometry. Representative plots for FRCs (CD31$^-$Pdpn$^+$), lymphatic endothelial cells (LECs; CD31$^+$Pdpn$^+$), and BECs (CD31$^+$Pdpn$^-$) among CD45$^-$ cells (*Upper*) and the absolute numbers of these cells (*Lower*) are shown. Data are pooled from two independent experiments and are expressed as the means ± SE for four mice per group. *$P < 0.05$ (Student's *t* test). (*G*, *Left*) Frozen sections of spleens from *Sirpa*$^{f/f}$ or *Sirpa*$^{\Delta DC}$ mice were stained with antibodies to CXCL13 (red) and to Thy1.2 (green). (Scale bar, 500 μm.) (*Right*) The CXCL13$^+$ area in each image was measured with the use of ImageJ software. Data are expressed as the means ± SE for five mice per group.

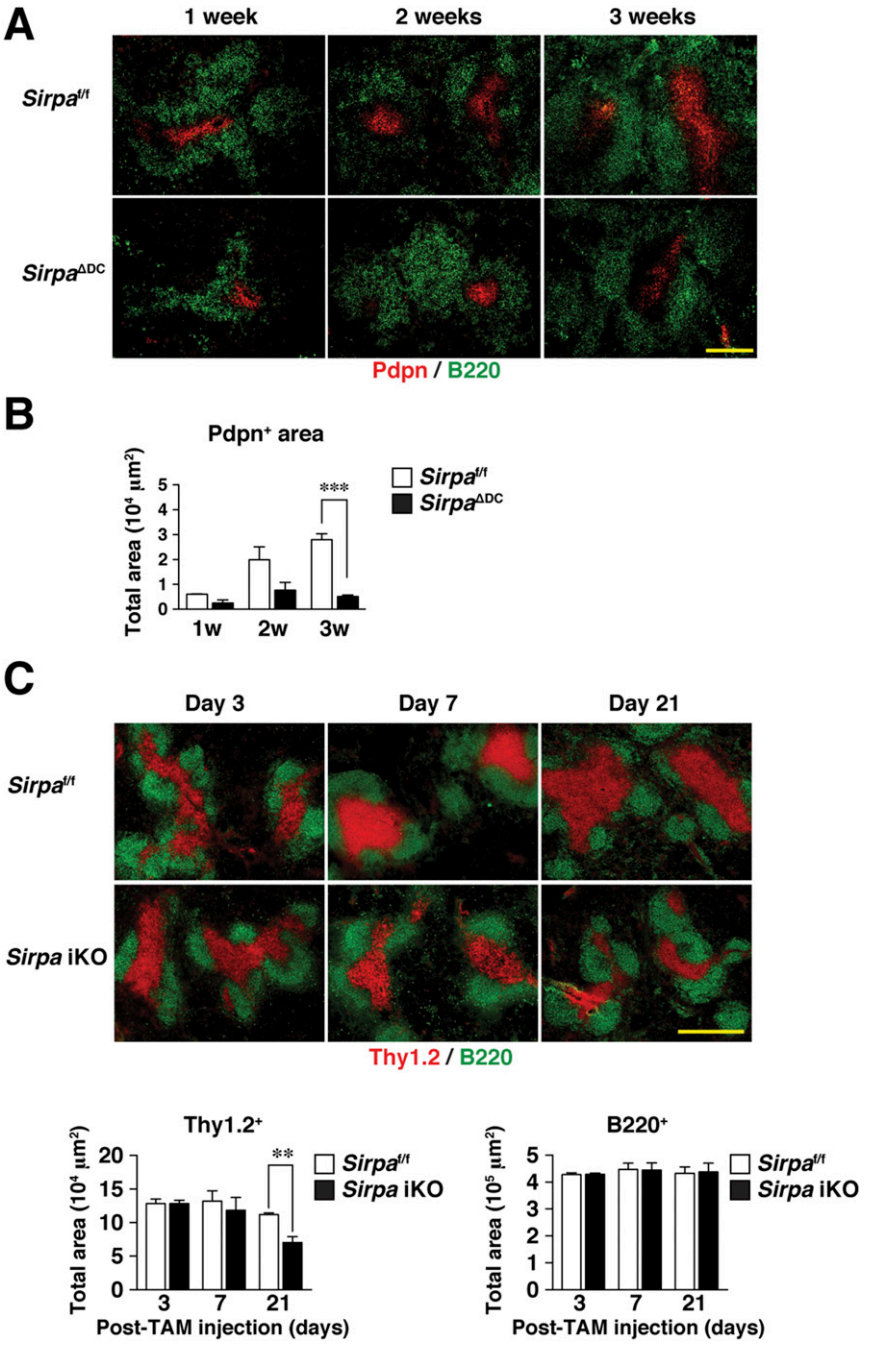

Fig. S3. The Pdpn$^+$ area in the spleens of *Sirpa*$^{f/f}$ and *Sirpa*$^{\Delta DC}$ mice at 1–3 wk of age and the T and B cell areas in the spleens of *Sirpa*-iKO mice. (*A*) Frozen sections of spleens from *Sirpa*$^{f/f}$ and *Sirpa*$^{\Delta DC}$ mice at 1, 2, and 3 wk of age were stained for Pdpn (red) and for B220 (green). (Scale bar, 200 μm.) (*B*) The Pdpn$^+$ area in images similar to those in *A* was measured with the use of ImageJ software. Data are expressed as means ± SE for three mice per group. ***$P < 0.001$ (Student's *t* test). (*C*, *Upper*) Frozen sections of spleens from *Sirpa*$^{f/f}$ and *Sirpa* iKO mice at 3, 7, and 21 d after the last TAM injection were stained with antibodies to Thy1.2 (red) and to B220 (green). (Scale bar, 500 μm.) (*Lower*) The Thy1.2$^+$ and B220$^+$ areas in each image were measured with the use of ImageJ software. Data are expressed as the means ± SE for three mice per group. **$P < 0.01$ (Student's *t* test).

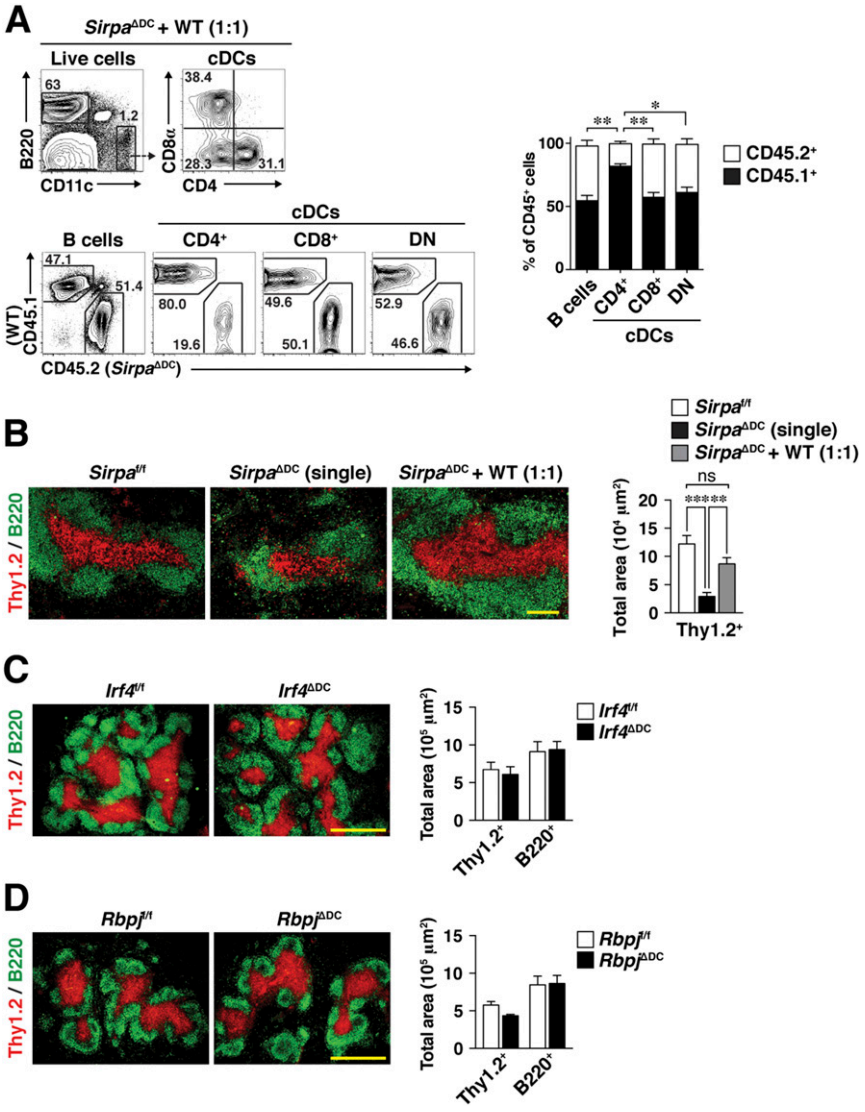

Fig. S4. Characterization of *Sirpa*$^{\Delta DC}$ BM chimeric mice and *Irf4*$^{\Delta DC}$ and *Rbpj*$^{\Delta DC}$ mice. (*A*) Lethally irradiated WT (CD45.1$^+$CD45.2$^+$) mice were reconstituted with an equal mixture of BM cells from *Sirpa*$^{\Delta DC}$ (CD45.2$^+$) and WT (CD45.1$^+$) mice for the generation of *Sirpa*$^{\Delta DC}$ + WT (1:1) BM chimeras. Eight weeks after cell transplantation, the spleen was isolated from the chimeras for analysis. (*Upper Left*) Representative flow cytometric profiles for cDCs (CD11c^{high}B220$^-$) and B cells (CD11c$^-$B220$^+$) and for CD8$^+$, CD4$^+$, and DN cDCs. (*Lower Left*) Representative flow cytometric profiles for *Sirpa*$^{\Delta DC}$-derived CD45.1$^-$CD45.2$^+$ or WT-derived CD45.1$^+$CD45.2$^-$ cells among B cells or among CD8$^+$, CD4$^+$, and DN cDC subsets. (*Right*) The percentages of CD45.1$^+$CD45.2$^-$ (CD45.1$^+$) or CD45.1$^-$CD45.2$^+$ (CD45.2$^+$) cells among total CD45$^+$ cells for B cells and for the CD4$^+$, CD8$^+$, and DN cDC subsets. Data are pooled from three independent experiments and are expressed as the means ± SE for four mice per group. *$P < 0.05$, **$P < 0.01$ (two-way ANOVA and Sidak's test). (*B*, *Left*) Frozen sections of the spleens from the indicated BM chimeras were stained with antibodies to Thy1.2 (red) and to B220 (green). (Scale bar, 200 μm.) The Thy1.2$^+$ area in each image was measured with the use of ImageJ software. (*Right*) Data are pooled from three independent experiments and are expressed as the means ± SE for four mice per group. **$P < 0.01$, ***$P < 0.001$; ns, not significant (one-way ANOVA and Tukey's test). (*C*, *Left* and *D*, *Left*) Frozen sections of the spleens from *Irf4*$^{f/f}$ and *Irf4*$^{\Delta DC}$ mice (*C*) or from *Rbpj*$^{f/f}$ and *Rbpj*$^{\Delta DC}$ mice (*D*) were stained with antibodies to Thy1.2 (red) and to B220 (green). (Scale bars, 1 mm.) (C, *Right* and *D*, *Right*) The Thy1.2$^+$ and B220$^+$ areas in each image were measured. Data are pooled from three independent experiments and are expressed as the means ± SE for three (*C*) or four (*D*) mice per group.

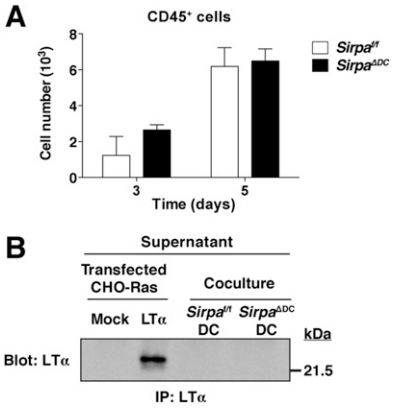

Fig. S5. The proliferation of CD45$^+$ cells and determination of soluble LTα in culture supernatants after coculture of splenic SCs with *Sirpa*$^{f/f}$ or *Sirpa*$^{\Delta DC}$ DCs. (*A*) Splenic CD45$^-$ SCs (at day 0) were cultured for 3 d with Sirpa$^{f/f}$ or Sirpa$^{\Delta DC}$ DCs (CD45$^+$); then the cells were harvested to determine the CD45$^+$ cell yield. Data are expressed as the means ± SE of triplicate determinations and are representative of three independent experiments. (*B*) Culture supernatants of mock-transfected CHO-Ras cells, of CHO-Ras cells transfected with an expression vector for mouse LTα, or of CD45$^-$ splenic SCs cocultured for 5 d with *Sirpa*$^{f/f}$ or *Sirpa*$^{\Delta DC}$ DCs were subjected to immunoprecipitation (IP) with antibodies to LTα. The resulting precipitates were subjected to immunoblot analysis with the same antibodies. Data are representative of two independent experiments.

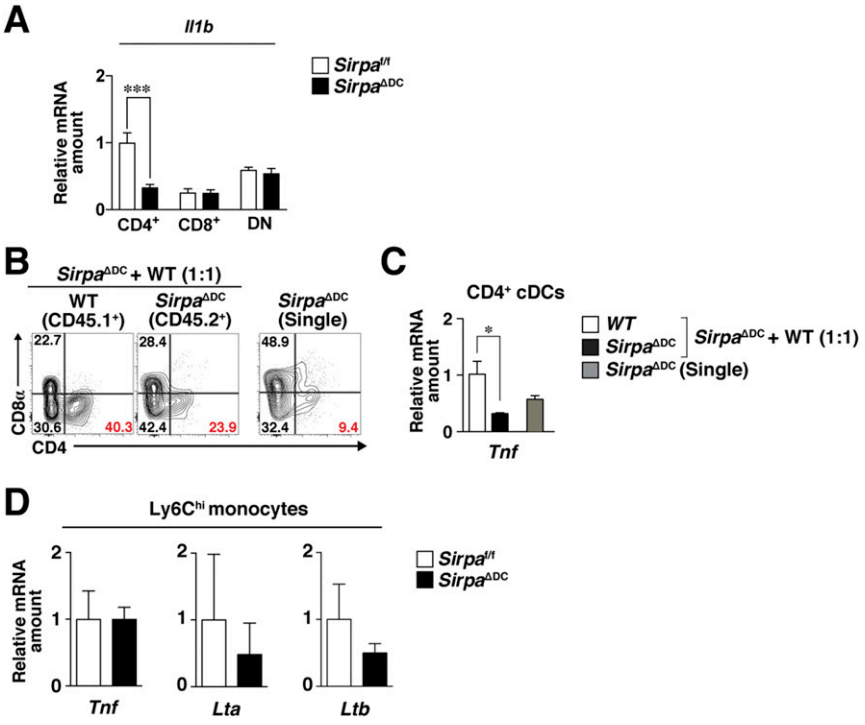

Fig. S6. Cytokine expression of cDC subsets or monocytes in the spleens of *Sirpa*$^{f/f}$ or *Sirpa*$^{\Delta DC}$ mice or mixed BM chimera mice. (*A*) Relative abundance of *Il1b* mRNA in CD4$^+$, CD8$^+$, and DN cDCs sorted from the spleens of *Sirpa*$^{f/f}$ or *Sirpa*$^{\Delta DC}$ mice. The amount of each mRNA was normalized by that of *Gapdh* mRNA. Data are expressed as the means ± SE for six mice per group examined in three independent experiments. ***$P < 0.001$ (Student's *t* test). (*B*) Lethally irradiated WT (CD45.1$^+$CD45.2$^+$) mice were reconstituted with an equal mixture of BM cells from *Sirpa*$^{\Delta DC}$ (CD45.2$^+$) and WT (CD45.1$^+$) mice or with BM cells from *Sirpa*$^{\Delta DC}$ (CD45.2$^+$) mice to generate *Sirpa*$^{\Delta DC}$ + WT (1:1) or *Sirpa*$^{\Delta DC}$(single) BM chimeras, respectively. Six to seven weeks after transplantation, CD4$^+$ cDCs from derived from each donor were sorted. Representative flow cytometric profiles for CD8$^+$, CD4$^+$, and DN cDCs are shown. (*C*) The relative abundance of *Tnf* mRNA in CD4$^+$ cDCs sorted from the spleens of *Sirpa*$^{\Delta DC}$ + WT (1:1) or *Sirpa*$^{\Delta DC}$(single) BM chimeras. The amount of each mRNA was normalized by that of *Gapdh* mRNA. Data are expressed as the means ± SE for three mice per group examined in three independent experiments. *$P < 0.01$ (one-way ANOVA with Tukey's test). (*D*) The relative abundance of *Tnf*, *Lta*, and *Ltb* mRNAs in Ly6C^{hi} monocytes sorted from the spleens of *Sirpa*$^{f/f}$ or *Sirpa*$^{\Delta DC}$ mice. The amount of each mRNA was normalized by that of *Gapdh* mRNA. Data are expressed as the means ± SE for three mice per group examined in three independent experiments.

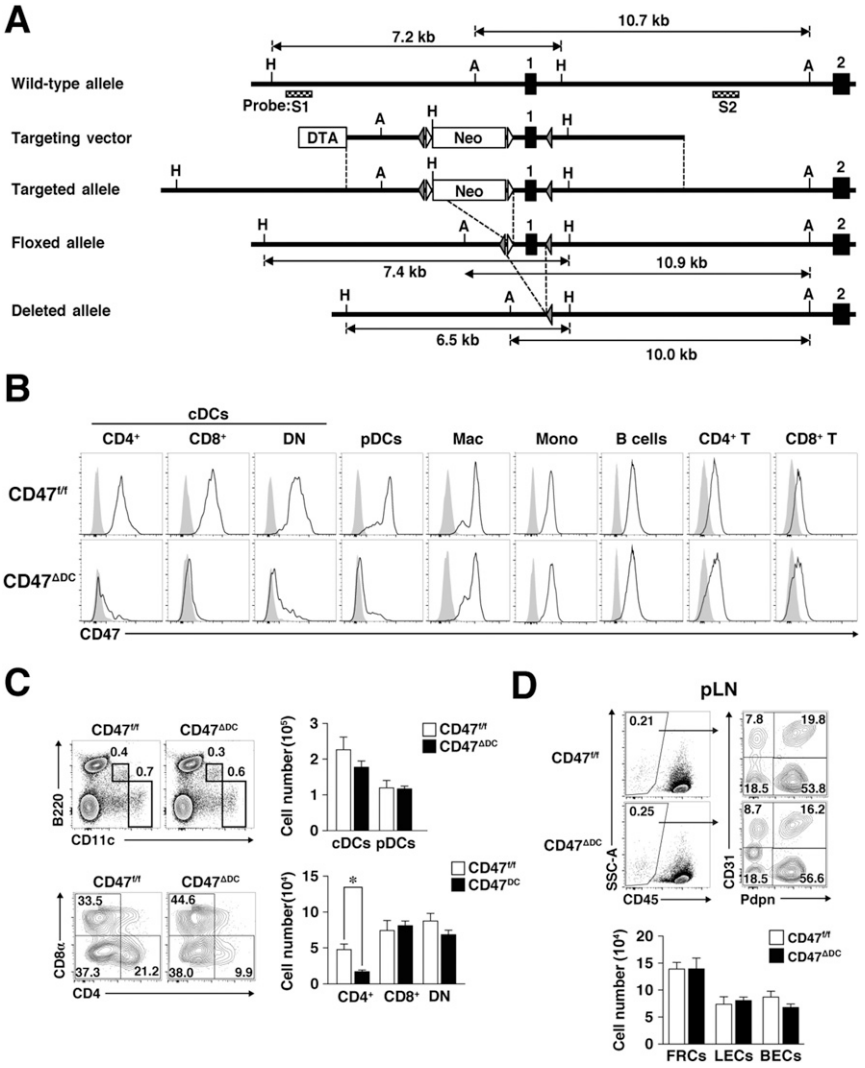

Fig. S7. Generation of DC-specific CD47 conditional-knockout mice. (*A*) Schematic representation of the mouse CD47 gene (WT allele) and the targeting vector, targeted allele, floxed allele, and deleted allele. Open and shaded triangles indicate FRT and loxP sites, respectively. Hatched bars indicate the positions of the 5′ probe (S1) and 3′ probe (S2) for Southern blot analysis to confirm the homologous recombination or deletion events. A, AflII; DTA, diphtheria toxin A cassette; H, HindIII; Neo, neomycin resistance cassette; 1, exon1; 2, exon2. (*B*) Splenocytes from $CD47^{f/f}$ or $CD47^{\Delta DC}$ mice were stained with antibodies to CD47 (open traces) or isotype control antibodies (filled traces) and with antibodies to CD4, CD8α, CD11c, and B220 (cDC subsets, pDCs, B cells, and T cells), with antibodies to CD11b [macrophages (Mac)] and F4/80 [monocytes (Mono)], and with Aqua (for detection of dead cells). The expression of CD47 on cDCs ($CD11c^{high}B220^{-}$), $CD4^{+}$ cDCs ($CD4^{+}CD8\alpha^{-}$ cDCs), DN cDCs ($CD4^{-}CD8\alpha^{-}$ cDCs), $CD8^{+}$ cDCs ($CD4^{-}CD8\alpha^{+}$ cDCs), pDCs ($CD11c^{int}B220^{+}$), B cells ($CD11c^{-}B220^{+}$), $CD4^{+}$ T cells ($CD11c^{-}B220^{-}CD4^{+}CD8\alpha^{-}$), $CD8^{+}$ T cells ($CD11c^{-}B220^{-}CD4^{-}CD8\alpha^{+}$), macrophages ($CD11c^{-}B220^{-}CD11b^{+}F4/80^{+}$), and monocytes ($CD11c^{-}B220^{-}CD11b^{+}Ly6C^{hi}$) among $Aqua^{-}$ cells was analyzed by flow cytometry. (*C*) Representative flow cytometric plots for cDCs ($CD11c^{high}B220^{-}$), pDCs ($CD11c^{int}B220^{+}$), and $CD4^{+}$, DN, and $CD8^{+}$ cDCs (*Left*) and absolute numbers of these cells (*Right*) isolated from peripheral LNs of $CD47^{f/f}$ or $CD47^{\Delta DC}$ mice. (*D*, *Upper*) Representative flow cytometric plots for SCs (FRCs, LECs, and BECs) among $CD45^{-}$ cells isolated from peripheral LNs of $CD47^{f/f}$ or $CD47^{\Delta DC}$ mice. (*Lower*) Absolute numbers of these cells. Data in *B* are representative of three independent experiments, and those in *C* and *D* are pooled from two independent experiments and are expressed as the means ± SE for four mice per group. $*P < 0.05$ (Student's *t* test).

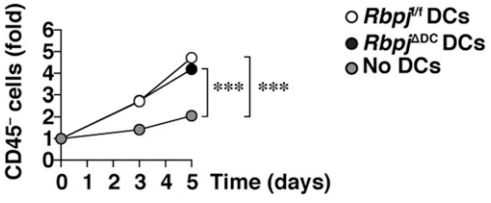

Fig. S8. Splenic DCs of $Rbpj^{\Delta DC}$ mice support the proliferation of splenic SCs. Splenic $CD45^{-}$ SCs isolated from C57BL/6J mice (Fig. 5) were cultured for 5 d with or without $CD11c^{+}$ DCs isolated from the spleens of $Rbpj^{f/f}$ or $Rbpj^{\Delta DC}$ mice; then the $CD45^{-}$ cell yield was determined by flow cytometry. Data are expressed as the means ± SE of triplicate determinations and are representative of two independent experiments. $***P < 0.001$ (two-way ANOVA with Sidak's test).