HHS Public Access

Author manuscript

Cytometry A. Author manuscript; available in PMC 2023 February 15.

Published in final edited form as:

Cytometry A. 2021 December ; 99(12): 1209–1217. doi:10.1002/cyto.a.24469.

A simplified method for separating renal MPCs using SLAMF9

Joseph A. Mikulin,

Briana L. Bates,

Timothy J Wilson*

Miami University, Department of Microbiology, 700 E. High Street, Oxford, OH, USA 45056

Abstract

Mononuclear phagocytes comprise an array of tissue-resident and monocyte-derived cells with important roles in tissue homeostasis and resistance to infection. Their diverse phenotypes make functional characterization within tissues challenging, because multiple surface markers are typically required for subset identification and isolation by cell sorting methods. Analysis of SLAMF9 expression within renal mononuclear phagocyte populations by multi-parametric flow cytometry indicates that SLAMF9 is a specific marker for identification of kidney-resident CD45+ CD11c+ MHC-II+ cells corresponding to prominent tissue-resident MPC populations derived from dendritic cell progenitors in adult mice. High SLAMF9 expression was sufficient to identify and sort these cells from disaggregated tissue using a user-operated cell sorter. The population can be further subdivided according to expression of CD11b and CD14 to identify IRF8^{high} cDC1 cells and cleanly separate the CD11b^{high} F4/80^{low} and CD11b^{int} F4/80^{high} CD11c^{+} MPC subsets. Therefore, SLAMF9 expression allows for the identification and sorting of kidney-resident CD11b^{+} CD11c^{+} CD64^{+} F4/80^{+} CX$_3$CR1^{+} MHC-II^{+} MPCs without the need for complex antibody panels or reporter mice, simplifying isolation of these cells for study ex vivo.

Keywords

mononuclear phagocytes; SLAMF9; dendritic cells; kidney

Introduction

Mononuclear phagocytes (MPCs) are a diverse group of leukocytes important for immune responses and tissue homeostasis. Traditionally divided into macrophages and dendritic cells (DCs), these cells comprise a broad array of subsets with partially overlapping functions which depend on the organ and tissue microenvironment. Among these cell types, conventional dendritic cells (cDCs) are critically important for sensing infection and trafficking antigens from peripheral tissues into lymph nodes for presentation to T

*Correspondence to: Timothy Wilson, wilso265@miamioh.edu, Tel.: +1 (513) 529-1694.

Author Contributions:

J.A.M.: Performed research, analyzed data, wrote manuscript; B.L.B.: performed research; directed research, analyzed data, wrote and edited manuscript.

Conflict of interest disclosure:

T.J.W. has licensed the anti-SLAMF9 (M349) antibody to Cambridge Enterprise Limited for commercial sub-licensing and distribution. The authors have no other competing financial interests to declare.

cells. Depending on the cell type and the stimulus, conventional DCs may induce either inflammatory or tolerogenic functions in T cells, and both their potency and flexibility are of continuing interest (1).

The mononuclear phagocyte system (MPS) of the murine kidney can be both beneficial and deleterious to the delicate organization and functioning of the renal parenchyma, as it is responsible both for tissue repair and debris clearance at steady state (2). Renal MPCs resist simple classification as exclusively macrophage or dendritic cell. Otherwise characteristic macrophage markers, such as F4/80, are expressed by subsets of renal MPCs with high-migratory and T cell-stimulatory activity (3–6), and dendritic cell marker CD11c can be upregulated in cells which phenotypically resemble macrophages during inflammation, or by long-lived renal MPCs at steady state (7–9). Additionally, macrophage-like populations in the murine kidney have historically been categorized by their Ly6C expression, where $Ly6C^{-}$ macrophages are involved in the patrolling and maintaining of renal tissue and $Ly6C^{+}$ macrophages with providing much of the phagocytic capacity and pro-inflammatory cytokine production of the non-parenchymal renal compartment (10–12). Thus, the nomenclature and classification of MPCs in the kidney has been historically understood as a continuum of functionally and phenotypically overlapping groups, rather than discrete populations (3,5,6).

Generally, dendritic cells of the steady state mouse are classified according to their surface phenotype and master transcriptional regulator. The two broad groups of tissue-resident dendritic cells are the Siglec-$H^{+}CD11c^{+}B220^{+}BST\text{-}2^{+}$ E2-2-dependent plasmacytoid dendritic cells (pDCs) (13–18), and the classical dendritic cells (cDCs), consisting of the $CD11b^{-}CD8\alpha^{+}$ BATF3-dependent lymphoid cDC1, the $CD11b^{-}CD103^{+}$ IRF-8-dependent myeloid cDC1 (19,20), and the $CD11b^{+}CD172\alpha^{+}$ IRF4-dependent cDC2 subsets (20–23). These subsets of DCs perform unique functions within the immune cell compartments of both lymphoid and non-lymphoid tissues. Plasmacytoid DCs are potent producers of type I interferons upon viral infection or antigenic challenge (16). Conventional DC1 cells, which develop from Siglec-$H^{-}Ly6c^{-}$ pre-DCs, play a disproportionately large role in cross-presenting antigen to and activating $CD8^{+}$ cytotoxic T cells in draining lymph nodes, skewing the immune response toward ILC1 and T_H1 cell activation and polarization (24–26). Similarly, cDC2 cells appear to be capable activators of ILC3 and T_H17-mediated immunity against extracellular bacterial challenges, as well as of T_H2 and ILC2-mediated anti-parasitic responses (27–29).

The macrophage and dendritic cell compartment of the steady state murine kidney is comprised of a heterogeneous blend of resident and monocyte-derived MPCs which fulfill these outlined niches throughout the tissue. Phenotypically and functionally distinct groups of these cells can be determined on the basis of their expression of integrins CD11b and CD11c (3,30). However, because of the substantial functional and phenotypic overlap within these subsets, additional strategies have been employed to further delineate MPC subsets, including transcriptional and fate-mapping analyses (31–33). Because few markers are expressed exclusively by any cell group (3,31,32), isolating any specific group of MPCs for *ex vivo* and *in vitro* analysis by fluorescence-activated cell sorting (FACS) is complicated by the fact that several surface markers must be used, and this antibody staining has the

potential to cause unintended effects on cell activation. In this study, we identify SLAMF9 as a specific marker for resident $CD45^+CD11c^+MHC\text{-}II^+$ MPCs in resting kidney aligning with populations previously identified as classical dendritic cells or $F4/80^{high}$ $CD64^+$ MPCs descended from the common DC progenitor (31,32). We outline a simplified gating strategy using only SLAMF9 as a marker to separate the above populations from other renal MPCs, and show the need for only two additional surface markers to cleanly distinguish $SLAMF9^+$ cDCs and $F4/80^{high}$ MPCs from each other.

Materials and methods

Mice

C57BL/6N mice were obtained from Taconic. *Slamf9*$^{-/-}$ mice (C57BL/6N-$Slamf9^{tm1b(EUCOMM)Wtsi}$/Wtsi) were obtained from the Wellcome Trust Sanger Institute. Genotyping of mice was performed by PCR as previously described (34). All animal procedures were performed in accordance with protocols approved by the Miami University Institutional Animal Care and Use Committee.

Preparation of single-cell suspension and staining of renal leukocytes

Healthy mice were euthanized via CO_2 asphyxiation. Kidneys were excised and cut into fine pieces with scissors in 7mL Bijou containers (Greiner Bio-One) containing 3 mL HBSS w/ Ca^{2+} and Mg^{2+} and 1mg/mL STEMxyme 1 (Worthington Biochemical), 25U/mL Benzonase (Sigma), and 10mM HEPES pH 7.4 (ThermoFisher). Kidneys were then gently rocked for 30 min at room temperature. After incubation, the homogenates were passed through 70 μM mesh filters and then the homogenates were transferred to 50 mL conical tubes and centrifuged twice at 50×G for 1 min to pellet debris, recovering the supernatant each time. The homogenates were washed twice with FACS buffer (PBS with 2% BCS and 1mM EDTA) and then over-laid onto 10 mL of Histopaque 1119 (Sigma) in 50 mL conical tubes. The use of 50 mL tubes instead of 15 mL tubes for density gradient centrifugation was a critical parameter for consistent separation of leukocytes from other cells. Cells were then centrifuged at 900×G for 30 minutes at room temperature with the rotor brake deactivated. Cells at the gradient interface were then extracted and washed in 50 mL FACS buffer.

Flow cytometry

Isolated renal leukocytes were stained with antibodies diluted to 2 μg/mL in FACS buffer targeting the following surface proteins: CCR2 (REA538), CCR5 (REA354), CD8α (53-6.7), CD11b (M1/70), CD11c (HL3 and N418), CD14 (SA14-2), CD19 (6D5), CD26 (H194-112), CD45 (30-F11), CD64 (X54-5/7.1), CD172α (P84), CX_3CR1 (SA011F11), EP-CAM (G8.8), F4/80 (BM8), IRF4 (3E4), IRF8 (V3GYWCH), Ly6c (AL-21), MHC-II (M5/114.15.2), NK1.1 (PK136), and XCR-1 (ZET). SLAMF9 staining was performed using biotinylated mouse monoclonal antibody clone M349 previously described (34). PE, APC, or BV785-conjugated streptavidin was used to label M349. Fluorophore conjugated staining reagents were purchased from BD Biosciences, Biolegend, and Miltenyi Biotec. Non-specific staining by Fc receptors was blocked using CD16.2 (9E9 - Biolegend), and CD16/32 (2.4G2- BioXCell). Cell viability was assessed using Zombie Aqua dye from BioLegend. Intracellular flow cytometry for transcription factors was performed using

the eBioscience Foxp3 / Transcription Factor Staining Buffer Set (ThermoFisher). Data acquisition was performed using LSR-II (BD Biosciences) and Attune NxT (ThermoFisher) flow cytometers. All gating strategies for surface and intracellular stains incorporated single cell discrimination using FSC-A and FSC-H along with a viability dye. All reported markers were assessed in at least three independent kidney preparations. Each kidney preparation comprises two kidneys from a single mouse. Compensation matrices were determined using BD CompBeads incubated with the relevant antibody conjugate. Analysis was performed in FlowJo v10. Primary data used in flow cytometry analysis may be obtained by contacting the corresponding author.

Cell sorting

Isolated renal leukocytes were stained with Zombie Aqua (Biolegend) and anti-SLAMF9 (M349) as described above. SLAMF9-expressing kidney leukocytes were then sorted using a FACSMelody cell sorter (BD Biosciences) using HEPES buffered saline (Leinco Technologies) as sheath fluid at a speed of 7000-8000 events/second. Post-sort analysis was done using FlowJo.

In vitro stimulation of sorted cells and quantitative RT-PCR

Sorted cells were washed in DMEM and then plated in a TC-treated v-bottom 96 well plate with complete DMEM (10% FCS, 1x Sodium pyruvate, non-essential amino acids, Glutamax, pen/strep). Phosphate-buffered saline (PBS), Dulbecco's Modified Eagle Medium (DMEM), Hanks balanced salt solution (HBSS) and bovine calf serum (BCS) were purchased from Sigma (St. Louis, MO). Gibco 100X Sodium pyruvate, Glutamax, MEM non-essential amino acids, penicillin/streptomycin solutions were purchased from ThermoFisher, as was 0.5M EDTA. Fetal bovine serum (FBS) and 1M HEPES were purchased from GE Lifesciences. The cells were stimulated with 1ug/mL of LPS for 24 hours. After 24 hours cells were lysed and RNA was isolated from sorted cells using PureLink RNA Micro Scale Kit (Invitrogen).

First strand cDNA synthesis was performed using the Accuris qMax cDNA Synthesis Kit (AE Bios, Cincinnati, OH) according to manufacturer protocols. Quantitative PCR was performed on a Rotor-Gene Q (Qiagen) using qMax Probe No ROX qPCR mix (Accuris). Probes for qPCR analysis were obtained from Integrated DNA Technologies (Coralville, IA). Relative gene expression was measured in triplicate using probes reactive to the following transcripts: *Il12b* (Mm.PT.58.12409997), and *Actb* (Mm.PT.39a.22214843.g). ΔC_t values for *Il12b* were calculated by subtraction from beta-actin. Statistical analyses were performed using Graphpad Prism software.

Results

SLAMF9 was previously shown to be expressed by multiple populations of antigen-presenting cells in lymphoid tissues, liver, peritoneal cavity, and tumors, including populations of conventional DCs, plasmacytoid DCs, and macrophages (34–36). To determine which cells express SLAMF9 in the kidney, we performed multi-parametric flow cytometry on enzyme-disaggregated kidneys. $CD45^+SSC^{low}SLAMF9^+$ live single cells

from mouse kidneys were analyzed for the presence and abundance of various cell surface markers associated with mononuclear phagocytes. Compared to all $CD45^{+}SSC^{low}$ cells, the $SLAMF9^{+}$ cells within this gate express high levels of MHC-II and CX_3CR1, as well as CD11c, F4/80, CD64, CD172α, and low levels of XCR1 and IRF4. They are also heterogeneous in expression of CD11b, CD14, and IRF8 (Figure 1). This phenotype was reminiscent of mononuclear phagocyte populations MPC1 and MPC3 described by Kawakami et al. as possessing the migratory and T-cell stimulatory functions of classical dendritic cells (3).

To determine whether SLAMF9+ cells comprise a group of kidney resident classical type-2 dendritic cells, multi-parametric flow cytometry was again used to examine their surface phenotype. Using the paradigm of dividing MPCs according to CD11b and CD11c expression allows the identification populations of mononuclear phagocytes with differing functional characteristics. Those with the greatest phagocytic ability are found among $CD11b^{high}CD11c^{low/-}$ MPC2 cells and the greatest antigen-presenting and T cell polarizing activity found among the $CD11c^{int/high}$ MPC1, MPC3, and MPC4 subsets (3). Since circulating B cells and NK cells were previously found not to express SLAMF9 (34), these subsets were removed by negative gating to limit Fc receptor-based staining artifacts. The relatively rare subset previously identified as “MPC5” was not implicated as a potential source of $SLAMF9^{+}$ cells in Figure 1 and so was not separated from T cells in our gating strategy, leaving them simply bound within gate 5 (Figure 2). However, MPC groups 1-4 were gated as described by Kawakami et al., with MPC populations designated according to expression of CD11b and CD11c (Figure 2A). Examining these populations for SLAMF9 expression reveals that MPC1 ($CD11b^{high}CD11c^{int}$) and MPC3 ($CD11b^{int}CD11c^{int}$) are predominantly $SLAMF9^{+}$, with MPC3 as the only one demonstrating homogeneous expression of SLAMF9 (Figure 2A). Back-gating on $SLAMF9^{+}$ cells and comparison with $SLAMF9^{neg}$ cells indicates that $SLAMF9^{+}$ cell populations are centered within the MPC1 and MPC3 gates, with some spill-over into other gated areas (Figure 2B). Importantly, examination of peripheral blood has found no counterpart for MPC3 in the blood and minimal potential contribution of blood-borne cells to the gated MPC1 population (Figure 2C), suggesting that $CD11b^{+}CD11c^{+}SLAMF9^{+}$ cells represent a tissue-resident population and make up a substantial fraction of myeloid cells within the resting murine kidney. Notably, $SLAMF9^{+}$ cells in the kidney are definitively negative for Ly6C, further supporting the notion that circulating populations, particularly inflammatory monocytes, do not contribute to the $CD11b^{+}$ $CD11c^{+}$ $SLAMF9^{+}$ groups.

Since SLAMF9 is able to mark the MPC1 and MPC3 populations in the kidney, we hypothesized that it would be sufficient as a marker to sort these functionally similar populations. To sort SLAMF9-expressing renal MPCs, a single cell suspension of renal cells was stained with anti-SLAMF9 monoclonal antibody M349 and the viability dye, Zombie Aqua (Figure 3). The living cells were then gated for singlets based on FSC-A and FSC-H, excluding any cell debris or aggregates. Performing these gates in the opposite order can also yield equivalent results. Finally, SLAMF9+ cells were clearly identified on a SSC-A x SLAMF9 plot for sorting. This gating strategy allows for the identification of a clear SLAMF9 expressing population without the need for antibodies to multiple markers. Post-sort analysis of these gated cells indicates a purity of approximately 88%, confirming

that the proposed sorting strategy is stringent enough to effectively enrich SLAMF9+ MPCs from among the renal leukocytes and stromal debris (Figure 3A).

To verify that sorted SLAMF9^{+} renal cells retain biological function after sorting, we stimulated sorted cells with 1 μg/mL LPS for 16 hours at 37°C. The transcripts encoding IL-12p40 and beta-actin were analyzed using qRT-PCR. Transcripts for *Il-12b*, which was not detected in the plated, unstimulated cells, were expressed only with LPS stimulation, indicating the recovery of viable and functional cells (Figure 3B). Among the critical parameters for successful isolation of SLAMF9+ MPCs were the effective generation of a single-cell suspension using mechanical and enzymatic disaggregation, together with effective enrichment of leukocytes using the Histopaque 1119 gradient. Data on cellular yield are shown in Table 1.

Since SLAMF9^{+} MPCs exhibit some degree of heterogeneity in expression of markers CD11b, CD14, F4/80, and IRF8 (Figure 1), a deeper examination of the presence of SLAMF9^{+} subsets was undertaken. Using the surface markers CD11b and CD14, it was found that SLAMF9^{+} MPCs could be cleanly separated into at least three distinct subsets: CD11b^{low} CD14^{neg}, CD11b^{int} CD14^{+}, and CD11b^{high} CD14^{low}. The subset with intermediate expression of CD11b and highest expression of CD14 made up the bulk of the population, with around 80% of SLAMF9^{+} cells falling within that gate. The proportion of cells within each subset was consistent whether or not pre-gating with CD45 and lineage markers was performed, indicating that SLAMF9 is sufficient for enrichment of these MPC populations (Figure 3C).

Since all cells expressing SLAMF9 in the kidney also constitutively express CD11c and MHC-II (Figure 1), ontological paradigms and markers of dendritic cells were employed to categorize these cells (31,32). SLAMF9^{+} renal MPCs were gated according to CD11b and CD14 as before and their expression of MPC markers was examined by surface and intracellular staining (Figure 4). The CD11b^{low} CD14^{neg} subset is reliably the fewest in number of the three populations, typically comprising about 6% of SLAMF9^{+} MPCs, which are themselves fewer than half of the CD11b-expressing cells found in a non-perfused kidney (Figure 4B). They show high expression of IRF8, are negative for F4/80 and CD64, and are positive for CD26, indicating that they are cDC1 cells (Figure 4A). The dominant population of SLAMF9^{+} MPCs, (CD11b^{int} CD14^{+}) was found to have the highest expression of CD64 and F4/80, intermediate expression of IRF8, and no expression of CD26. These cells correspond to a population that has been differentially categorized as macrophages (31) or F4/80^{high} ‘macrophage-like’ MPCs derived from the common DC progenitor (CDP) in adult mice (32). It is also notable that these cells express the highest amount of SLAMF9 and are smallest in size by FSC, and so without the use of other surface markers, they can be enriched above 80% by using a more stringent gate for high SLAMF9 expression and lower FSC. Reducing the SLAMF9 gating stringency to include SLAMF9^{low} cells favors increased numbers of CD11b^{low} and CD11b^{high} subsets within the SLAMF9^{+} gate (Figure 4). The subset of SLAMF9^{+} cells that is CD11b^{high} has intermediate expression of F4/80, is positive for the cDC marker CD26 (31), and has the highest expression of IRF4 (Figure 4A). It has heterogeneous expression of CD64 that segregates into two overlapping CD64^{neg} and CD64^{+} populations (Figure 4B) corresponding to cells previously identified

respectively as cDC2 and CD11b^{high} 'cDC2-like' CDP progeny (31,32). The *bona fide* renal macrophage population that is CD11b^{high} CD64^{+} F4/80^{int} CD11c^{neg} is found within the SLAMF9^{neg} gate (Figure 4B).

Discussion

This study demonstrates that SLAMF9, a member of the CD2/SLAM receptor family, is selectively expressed on populations of renal CD11c^{+} MHC-II^{+} leukocytes corresponding to cell populations derived from the common DC progenitor in adult mice (32). While the surface phenotype of renal CD11b^{int} F4/80^{high} SLAMF9^{+} MPCs could support classification as either DCs or macrophages, cells expressing the same surface markers as renal F4/80^{high} SLAMF9^{+} MPCs have been shown to migrate to the draining lymph nodes and present antigen to T cells following systemic LPS challenge, indicating the presence of classical DC function *in vivo* (3). This ability to migrate to lymph nodes and activate T cells, coupled with their high frequency within the myeloid compartment of resting kidneys, makes CD11b^{int}F4/80^{high} renal MPCs an interesting and consequential target of investigation.

The role of renal mononuclear phagocytes in renal injury is an active field of investigation. Specifically, the renal MPS is the fulcrum upon which ischemia-reperfusion injury (IRI) is either exacerbated or resolved; these cells are responsible for the production of pro-inflammatory cytokines and chemokines, presentation of self-peptides to T cells, fibrosis, and ultimately the clearance of debris and immunoregulation that are all associated with acute and late phases of IRI (37–41). Additionally, CD11b^{+} DCs are involved in the initiation and modulation of autoimmune and infectious nephritis (2), and so functional characterization of these cells is important for understanding the pathogenesis of multiple diseases. While we have not yet examined the utility of using SLAMF9 as a marker for DC isolation under these disease states, it may provide a simpler alternative for cellular identification and isolation in studies of renal homeostasis and disease. Furthermore, where studies of cDC1 and cDC2 cells are complicated by the higher abundance of F4/80^{high} and CD11c^{neg} MPCs, SLAMF9 staining can be used to clearly define these populations during analysis and sorting.

In summary, we have identified a method of enriching for tissue-resident dendritic cells from mouse kidney by fluorescence-activated cell sorting that requires only a single antibody to highly enrich for CD45^{+} lymphoid lineage^{neg} CD11c^{+} MHC-II^{+} populations. This finding will facilitate functional analysis of these cells where genetic reporters are unavailable or where other antibody combinations might interfere with the functional activity of the cells in downstream assays.

Acknowledgements

The authors thank the Wellcome Trust Sanger Institute Mouse Genetics Project (Sanger MGP) and its funders for providing the mutant mouse line (C57BL/6N-Slamf9/Wtsi:[tm1b(EUCOMM)Wtsi]), and INFRAFRONTIER/EMMA (www.infrafrontier.eu) (42,43). Funding information may be found at www.sanger.ac.uk/mouseportal and associated primary phenotypic information at www.mousephenotype.org. Instrumentation support was provided by the Center for Bioinformatics and Functional Genomics at Miami University

Funding Information:

Funding for this work was provided by: NIAID R15-AI138184 and NSF MRI-1726645

References:

1. Eisenbarth SC. Dendritic cell subsets in T cell programming: location dictates function. Nat Rev Immunol 2019;19:89–103. [PubMed: 30464294]
2. Rogers NM, Ferenbach DA, Isenberg JS, Thomson AW, Hughes J. Dendritic cells and macrophages in the kidney: a spectrum of good and evil. Nat Rev Nephrol 2014;10:625–43. [PubMed: 25266210]
3. Kawakami T, Lichtnekert J, Thompson LJ, Karna P, Bouabe H, Hohl TM, Heinecke JW, Ziegler SF, Nelson PJ, Duffield JS. Resident renal mononuclear phagocytes comprise five discrete populations with distinct phenotypes and functions. J Immunol 2013;191:3358–72. [PubMed: 23956422]
4. Miller JC, Brown BD, Shay T, Gautier EL, Jojic V, Cohain A, Pandey G, Leboeuf M, Elpek KG, Helft J and others. Deciphering the transcriptional network of the dendritic cell lineage. Nat Immunol 2012;13:888–99. [PubMed: 22797772]
5. Nelson PJ, Rees AJ, Griffin MD, Hughes J, Kurts C, Duffield J. The renal mononuclear phagocytic system. J Am Soc Nephrol 2012;23:194–203. [PubMed: 22135312]
6. Gottschalk C, Kurts C. The Debate about Dendritic Cells and Macrophages in the Kidney. Front Immunol 2015;6:435. [PubMed: 26388867]
7. Ferenbach D, Hughes J. Macrophages and dendritic cells: what is the difference? Kidney Int 2008;74:5–7. [PubMed: 18560360]
8. Hume DA. Plenary perspective: the complexity of constitutive and inducible gene expression in mononuclear phagocytes. J Leukoc Biol 2012;92:433–44. [PubMed: 22773680]
9. Puranik AS, Leaf IA, Jensen MA, Hedayat AF, Saad A, Kim KW, Saadalla AM, Woollard JR, Kashyap S, Textor SC and others. Kidney-resident macrophages promote a proangiogenic environment in the normal and chronically ischemic mouse kidney. Sci Rep 2018;8:13948. [PubMed: 30224726]
10. Davies LC, Rosas M, Jenkins SJ, Liao CT, Scurr MJ, Brombacher F, Fraser DJ, Allen JE, Jones SA, Taylor PR. Distinct bone marrow-derived and tissue-resident macrophage lineages proliferate at key stages during inflammation. Nat Commun 2013;4:1886. [PubMed: 23695680]
11. Geissmann F, Manz MG, Jung S, Sieweke MH, Merad M, Ley K. Development of monocytes, macrophages, and dendritic cells. Science 2010;327:656–61. [PubMed: 20133564]
12. Schulz C, Gomez Perdiguero E, Chorro L, Szabo-Rogers H, Cagnard N, Kierdorf K, Prinz M, Wu B, Jacobsen SE, Pollard JW and others. A lineage of myeloid cells independent of Myb and hematopoietic stem cells. Science 2012;336:86–90. [PubMed: 22442384]
13. Blasius AL, Cella M, Maldonado J, Takai T, Colonna M. Siglec-H is an IPC-specific receptor that modulates type I IFN secretion through DAP12. Blood 2006;107:2474–6. [PubMed: 16293595]
14. Blasius AL, Giurisato E, Cella M, Schreiber RD, Shaw AS, Colonna M. Bone marrow stromal cell antigen 2 is a specific marker of type I IFN-producing cells in the naive mouse, but a promiscuous cell surface antigen following IFN stimulation. J Immunol 2006;177:3260–5. [PubMed: 16920966]
15. Cisse B, Caton ML, Lehner M, Maeda T, Scheu S, Locksley R, Holmberg D, Zweier C, den Hollander NS, Kant SG and others. Transcription factor E2-2 is an essential and specific regulator of plasmacytoid dendritic cell development. Cell 2008;135:37–48. [PubMed: 18854153]
16. Murphy TL, Grajales-Reyes GE, Wu X, Tussiwand R, Briseno CG, Iwata A, Kretzer NM, Durai V, Murphy KM. Transcriptional Control of Dendritic Cell Development. Annu Rev Immunol 2016;34:93–119. [PubMed: 26735697]
17. Swiecki M, Colonna M. The multifaceted biology of plasmacytoid dendritic cells. Nat Rev Immunol 2015;15:471–85. [PubMed: 26160613]
18. Zhang J, Raper A, Sugita N, Hingorani R, Salio M, Palmowski MJ, Cerundolo V, Crocker PR. Characterization of Siglec-H as a novel endocytic receptor expressed on murine plasmacytoid dendritic cell precursors. Blood 2006;107:3600–8. [PubMed: 16397130]

19. Edelson BT, Kc W, Juang R, Kohyama M, Benoit LA, Klekotka PA, Moon C, Albring JC, Ise W, Michael DG and others. Peripheral CD103+ dendritic cells form a unified subset developmentally related to CD8alpha+ conventional dendritic cells. J Exp Med 2010;207:823–36. [PubMed: 20351058]
20. Segura E, Amigorena S. Inflammatory dendritic cells in mice and humans. Trends Immunol 2013;34:440–5. [PubMed: 23831267]
21. Balan S, Saxena M, Bhardwaj N. Dendritic cell subsets and locations. Int Rev Cell Mol Biol 2019;348:1–68. [PubMed: 31810551]
22. Durai V, Murphy KM. Functions of Murine Dendritic Cells. Immunity 2016;45:719–736. [PubMed: 27760337]
23. Guilliams M, Ginhoux F, Jakubzick C, Naik SH, Onai N, Schraml BU, Segura E, Tussiwand R, Yona S. Dendritic cells, monocytes and macrophages: a unified nomenclature based on ontogeny. Nat Rev Immunol 2014;14:571–8. [PubMed: 25033907]
24. Broz ML, Binnewies M, Boldajipour B, Nelson AE, Pollack JL, Erle DJ, Barczak A, Rosenblum MD, Daud A, Barber DL and others. Dissecting the Tumor Myeloid Compartment Reveals Rare Activating Antigen-Presenting Cells Critical for T Cell Immunity. Cancer Cell 2014;26:938.
25. Mashayekhi M, Sandau MM, Dunay IR, Frickel EM, Khan A, Goldszmid RS, Sher A, Ploegh HL, Murphy TL, Sibley LD and others. CD8alpha(+) dendritic cells are the critical source of interleukin-12 that controls acute infection by Toxoplasma gondii tachyzoites. Immunity 2011;35:249–59. [PubMed: 21867928]
26. Schlitzer A, Sivakamasundari V, Chen J, Sumatoh HR, Schreuder J, Lum J, Malleret B, Zhang S, Larbi A, Zolezzi F and others. Identification of cDC1- and cDC2-committed DC progenitors reveals early lineage priming at the common DC progenitor stage in the bone marrow. Nat Immunol 2015;16:718–28. [PubMed: 26054720]
27. Lewis KL, Caton ML, Bogunovic M, Greter M, Grajkowska LT, Ng D, Klinakis A, Charo IF, Jung S, Gommerman JL and others. Notch2 receptor signaling controls functional differentiation of dendritic cells in the spleen and intestine. Immunity 2011;35:780–91. [PubMed: 22018469]
28. Satpathy AT, Briseno CG, Lee JS, Ng D, Manieri NA, Kc W, Wu X, Thomas SR, Lee WL, Turkoz M and others. Notch2-dependent classical dendritic cells orchestrate intestinal immunity to attaching-and-effacing bacterial pathogens. Nat Immunol 2013;14:937–48. [PubMed: 23913046]
29. Tussiwand R, Everts B, Grajales-Reyes GE, Kretzer NM, Iwata A, Bagaitkar J, Wu X, Wong R, Anderson DA, Murphy TL and others. Klf4 expression in conventional dendritic cells is required for T helper 2 cell responses. Immunity 2015;42:916–28. [PubMed: 25992862]
30. Merad M, Sathe P, Helft J, Miller J, Mortha A. The dendritic cell lineage: ontogeny and function of dendritic cells and their subsets in the steady state and the inflamed setting. Annu Rev Immunol 2013;31:563–604. [PubMed: 23516985]
31. Guilliams M, Dutertre CA, Scott CL, McGovern N, Sichien D, Chakarov S, Van Gassen S, Chen J, Poidinger M, De Prijck S and others. Unsupervised High-Dimensional Analysis Aligns Dendritic Cells across Tissues and Species. Immunity 2016;45:669–684. [PubMed: 27637149]
32. Salei N, Rambichler S, Salvermoser J, Papaioannou NE, Schuchert R, Pakalniskyte D, Li N, Marschner JA, Lichtnekert J, Stremmel C and others. The Kidney Contains Ontogenetically Distinct Dendritic Cell and Macrophage Subtypes throughout Development That Differ in Their Inflammatory Properties. J Am Soc Nephrol 2020;31:257–278. [PubMed: 31932472]
33. Schraml BU, van Blijswijk J, Zelenay S, Whitney PG, Filby A, Acton SE, Rogers NC, Moncaut N, Carvajal JJ, Reis e Sousa C. Genetic tracing via DNGR-1 expression history defines dendritic cells as a hematopoietic lineage. Cell 2013;154:843–58. [PubMed: 23953115]
34. Wilson TJ, Clare S, Mikulin J, Johnson CM, Harcourt K, Lyons PA, Dougan G, Smith KGC. Signalling lymphocyte activation molecule family member 9 is found on select subsets of antigen-presenting cells and promotes resistance to Salmonella infection. Immunology 2020;159:393–403. [PubMed: 31880316]
35. Dollt C, Michel J, Kloss L, Melchers S, Schledzewski K, Becker K, Sauer A, Krewer A, Koll F, Schmieder A. The novel immunoglobulin super family receptor SLAMF9 identified in TAM of murine and human melanoma influences pro-inflammatory cytokine secretion and migration. Cell Death Dis 2018;9:939. [PubMed: 30232321]

36. Sever L, Radomir L, Stirm K, Wiener A, Schottlender N, Lewinsky H, Barak AF, Friedlander G, Ben-Dor S, Becker-Herman S and others. SLAMF9 regulates pDC homeostasis and function in health and disease. Proc Natl Acad Sci U S A 2019;116:16489–16496. [PubMed: 31346085]

37. Dai H, Thomson AW, Rogers NM. Dendritic Cells as Sensors, Mediators, and Regulators of Ischemic Injury. Front Immunol 2019;10:2418. [PubMed: 31681306]

38. Dong X, Swaminathan S, Bachman LA, Croatt AJ, Nath KA, Griffin MD. Resident dendritic cells are the predominant TNF-secreting cell in early renal ischemia-reperfusion injury. Kidney Int 2007;71:619–28. [PubMed: 17311071]

39. Han HI, Skvarca LB, Espiritu EB, Davidson AJ, Hukriede NA. The role of macrophages during acute kidney injury: destruction and repair. Pediatr Nephrol 2019;34:561–569. [PubMed: 29383444]

40. Snelgrove SL, Lo C, Hall P, Lo CY, Alikhan MA, Coates PT, Holdsworth SR, Hickey MJ, Kitching AR. Activated Renal Dendritic Cells Cross Present Intrarenal Antigens After Ischemia-Reperfusion Injury. Transplantation 2017;101:1013–1024. [PubMed: 27495751]

41. Xu L, Sharkey D, Cantley LG. Tubular GM-CSF Promotes Late MCP-1/CCR2-Mediated Fibrosis and Inflammation after Ischemia/Reperfusion Injury. J Am Soc Nephrol 2019;30:1825–1840. [PubMed: 31315923]

42. Bradley A, Anastassiadis K, Ayadi A, Battey JF, Bell C, Birling MC, Bottomley J, Brown SD, Burger A, Bult CJ and others. The mammalian gene function resource: the International Knockout Mouse Consortium. Mamm Genome 2012;23:580–6. [PubMed: 22968824]

43. Skarnes WC, Rosen B, West AP, Koutsourakis M, Bushell W, Iyer V, Mujica AO, Thomas M, Harrow J, Cox T and others. A conditional knockout resource for the genome-wide study of mouse gene function. Nature 2011;474:337–42. [PubMed: 21677750]

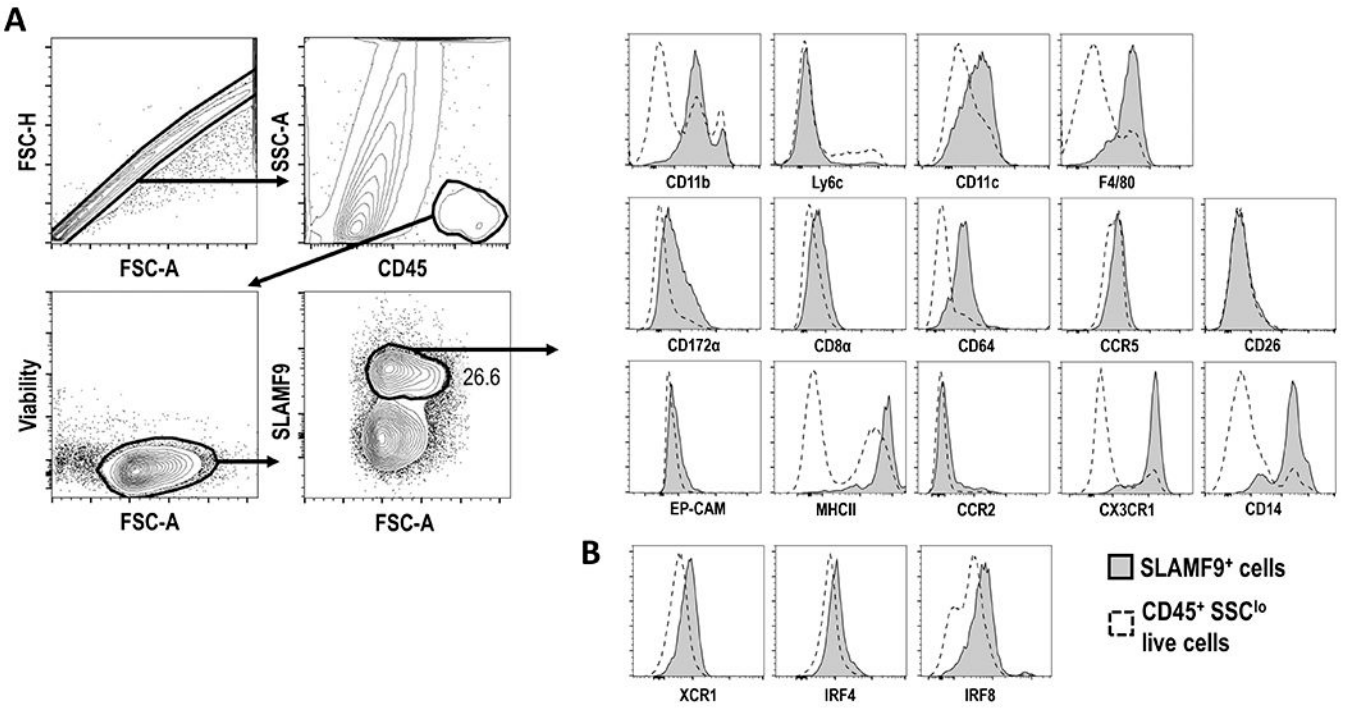

Figure 1. Surface phenotype of SLAMF9+ cells in mouse kidney.

Kidneys from heathy C57BL/6 mice were mechanically and enzymatically disaggregated and stained for the indicated markers by (A) surface or (B) intracellular staining. Cells were gated as indicated on the left with live, $CD45^+$ leukocytes identified among single cells within the kidney suspension. $SLAMF9^+$ cells (shaded histogram) were compared with all cells in their parent gate (dashed line) to determine their phenotype using the indicated markers.

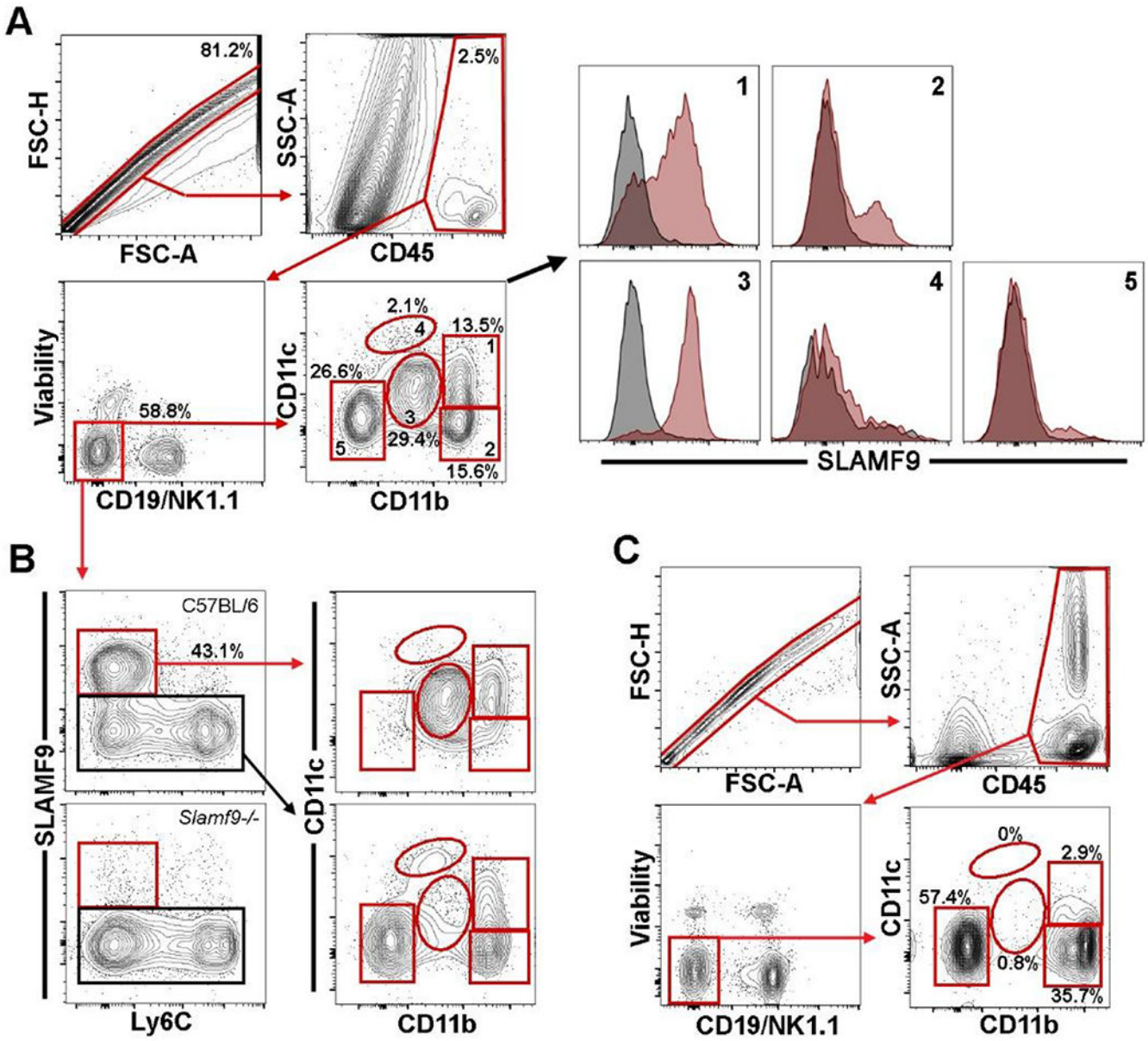

Figure 2. Examination of SLAMF9 expression among tissue-resident and blood MPC populations.

A) Gating strategy to identify SLAMF9 positive cells among mononuclear phagocyte populations in mouse kidney. Gates numbered 1-4 correspond to previously characterized MPC populations. Histograms represent staining for SLAMF9 among these numbered populations in C57BL/6 (red) or *Slamf9*−/− (gray) mice. B) Back-gating on SLAMF9 $CD45^{+}$ $CD19^{neg}$ $NK1.1^{neg}$ $SLAMF9^{+}$ cells to identify which populations are the source of $SLAMF9^{+}$ cells in (A). C) Staining of peripheral blood using the same gating strategy as (A) to examine which populations in kidney may be derived from blood. The viability dye used to exclude dead cells in all cases was Zombie Aqua.

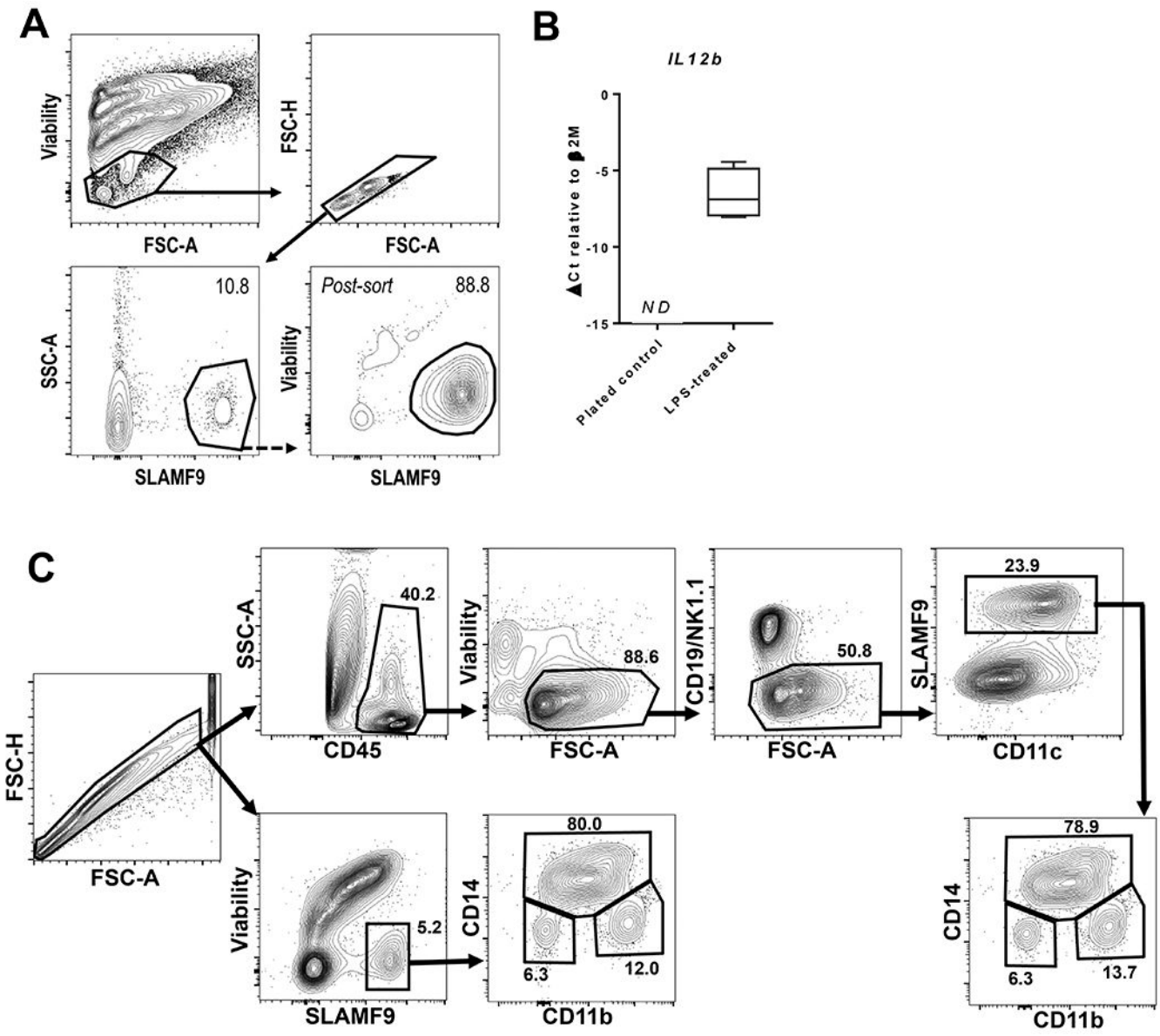

Figure 3. Fluorescence activated cell sorting of renal MPCs using SLAMF9 as a marker.

A) Cell sorting of SLAMF9^{+} cells from disaggregated kidney cell suspensions with post-sort analysis. B) Quantitative RT-PCR measurement of *Il12b* transcript in SLAMF9-expressing MPCs after with and without LPS stimulation. C) Assessment of the equivalence of using SLAMF9 as a primary marker for identification and/or cell enrichment compared with alternative pre-gating strategies using CD45 and lineage markers prior to SLAMF9 gating.

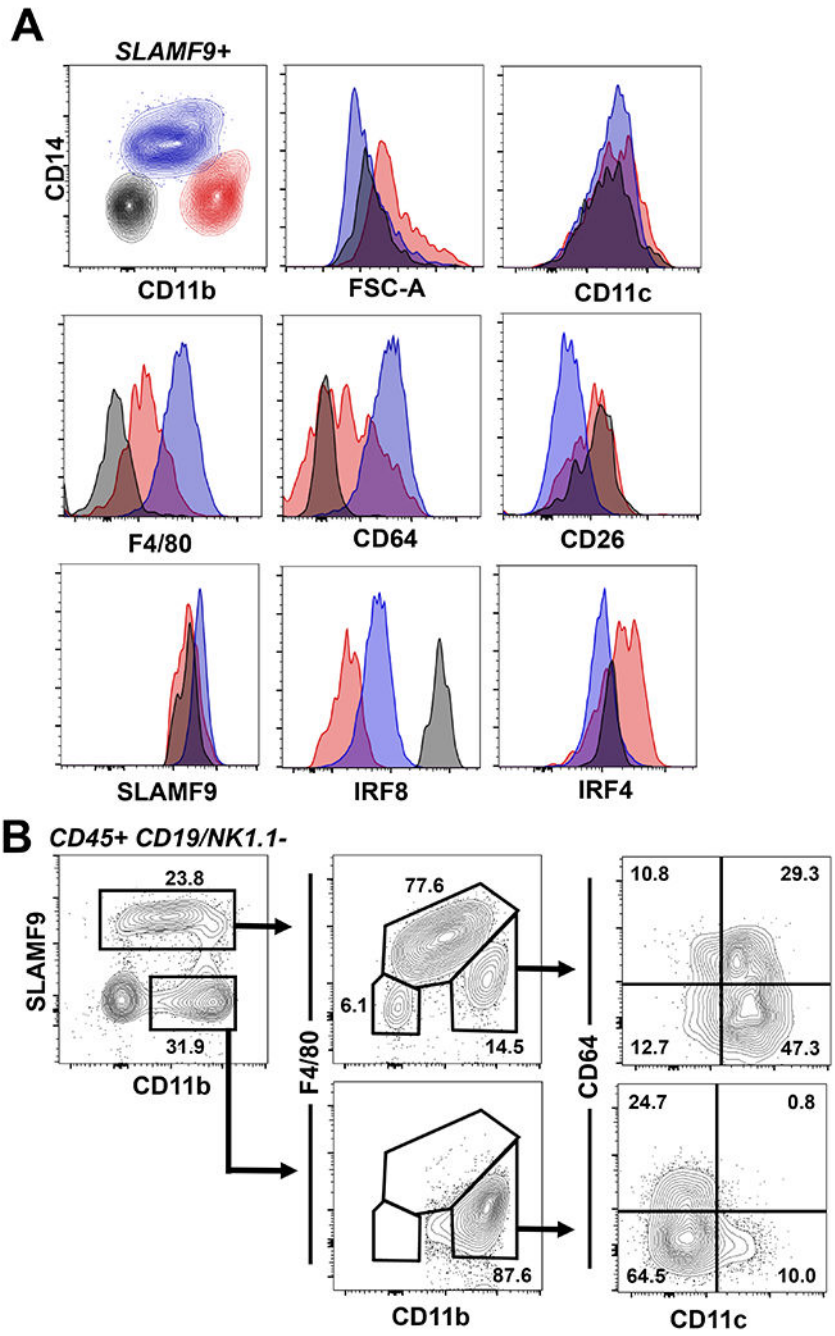

Figure 4. Characterization of renal SLAMF9+ MPC subpopulations.

A) Live, SLAMF9^{+}, single cell populations gated according to CD11b and CD14 expression were examined by surface or intracellular flow cytometry for the indicated markers. Black histograms correspond to CD14^{neg} CD11b^{low} cells; blue indicates CD11b^{int} CD14^{+} cells; red indicates CD11b^{high} CD14^{low} populations. B) Alternative gating strategy for characterization of heterogenous CD11b^{high} populations.

Table 1.

Yield of renal SLAMF9^{+} MPCs.

Summary statistics for yields of SLAMF9^{+} renal cDCs using 6 kidneys per sorting experiment. Summary data indicate the average values and range from 8 different sorting cell sorting experiments on healthy C57BL/6 mice.

	Average (Range)
Events Sorted	11.7×10^{6} ($6.1\text{-}18.0\times10^{6}$)
% SLAMF9+	0.39% (0.15-0.65%)
Total Recovered	43,212 (14,763 – 64,912)
Recovered/Kidney	7,202 (2,461 – 12,178)