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Abbreviations:

CSF1: Colony Stimulating Factor 1, MPS: mononuclear phagocyte system, FIRE: Fms intronic regulatory element, BM: bone marrow.

Abstract

Macrophages are present in large numbers in every tissue in the body where they play critical roles in development and homeostasis. They exhibit remarkable phenotypic and functional diversity, underpinning their adaptation to specialized roles in each tissue niche. CSF1, signaling through the CSF1 Receptor (CSF1R), which is restricted to monocyte-macrophage lineage cells in adults, is a critical growth factor controlling macrophage proliferation, differentiation and many aspects of mature macrophage function. We have generated a macrophage reporter rat, utilizing a construct containing elements of the mouse *Csf1r* promoter and the highly conserved Fms intronic regulatory element (FIRE) to drive mApple fluorescent protein expression. *Csf1r*-mApple was robustly expressed in monocyte-macrophage lineage cells in rat bone marrow, peripheral blood and tissues, with detectable expression in granulocytes and B cells and no evidence of expression in hematopoietic precursors or non-hematopoietic cells. Here, we use the *Csf1r*-mApple transgene to highlight and dissect the abundance and heterogeneity of rat tissue macrophage populations, and to demonstrate parallel increases in blood monocytes and multiple tissue macrophage populations, including bone marrow, liver, spleen and lung, in response to CSF1 treatment *in vivo*. The *Csf1r*-mApple rat is a novel tool enabling analysis of rat macrophages *in situ* by direct imaging and providing an additional phenotypic marker to facilitate exploration of rat tissue macrophage phenotypic and functional heterogeneity.

Introduction

The laboratory rat has been studied extensively in models of cardiovascular, neurological, cancer, diabetes, respiratory and inflammatory diseases.[1, 2] Many of these diseases involve cells of the mononuclear phagocyte system (MPS, monocytes, tissue macrophages) in different states of activation as effectors and are associated with differential genetic susceptibility amongst rat strains (e.g.[3]). Macrophages are a prominent cell population in all major organs and adapt in each site to perform specific functions. In mice, resident tissue macrophages adopt unique transcriptional profiles to perform organ-specific functions in development and homeostasis.[4-7] There are few similar studies in the rat, in part because of the lack of macrophage markers to enable isolation and characterization of tissue macrophages or their localization *in situ.* However, transcriptomic analysis of the brain and spleen of *Csf1r* deficient rats[8] and of the liver of CSF1-treated rats[9] indicated that the macrophage populations of those organs (microglia in brain, marginal zone and red pulp in spleen and Kupffer cells in liver) share many tissue-specific adaptations with mouse.

Analysis of monocyte-macrophage ontogeny, location and function in mice has been driven by the widespread availability of monoclonal antibodies against numerous cell surface proteins, fluorescent reporter transgenes, and conditional and constitutive mutations in the germ line. Amongst many other applications, these tools have enabled the dissection of the differentiation of subsets of peripheral blood monocytes in the mouse. Mammalian peripheral blood monocytes can be separated into subpopulations based upon surface markers; LY6C in mouse and CD14/CD16 in humans (Reviewed in [10]). The subpopulations are also distinguished by their expression of chemokine receptors CCR2 and CX3CR1 in humans, mice and rats.[11] Previous studies of rat monocyte heterogeneity have relied on differential expression of the E-selectin ligand sialophorin

(CD43) and an undefined marker, HIS48, that is shared with granulocytes (expression is somewhat analogous to LY6C in mouse).[12, 13] CD43, which is highly expressed on the non-classical monocyte population in rats,[11] is also highly expressed on LY6C^{lo} and CD16^{hi} non-classical monocytes in mice[14, 15] and humans,[16] respectively. CD43 (*SPN*) mRNA is strongly up-regulated in CD16^{hi} human monocytes relative to CD14^{hi} classical monocytes. Most studies indicate that these populations are a developmental series, with the LY6C^{hi} (mouse) or CD14^{hi} (human) classical monocytes being short-lived immature precursors which may either leave the circulation or differentiate to long-lived progeny.[10, 17]

The available macrophage markers for the rat are limited. There are, for example, no antibodies against rat CD115 (CSF1R), CD64, MERTK, TIMD4 or F4/80 (ADGRE1); each used widely to define subsets of resident tissue macrophages in the mouse. The mRNAs encoding these markers are highly-expressed by rat macrophages[3, 8, 9]. The most widely-used rat macrophage markers in tissues are still CD68 (ED1), CD163 (ED2) and CD169 (ED3).[18] Recently antibodies were generated against rat macrophage C type lectins, MCL1 (CLEC4D) and MINCLE (CLEC4E),[19, 20] but these have not been widely utilized.

The proliferation, differentiation and survival of cells of the MPS are controlled by signals from the macrophage colony-stimulating factor receptor (CSF1R), elicited by binding of its two ligands, CSF1 and IL34[21, 22]. Treatment of mice[23] or pigs[24] with a long-acting form of CSF1, CSF1-Fc, produced an expansion of blood monocyte and tissue macrophage populations. Conversely, a natural mutation of the *Csf1* gene (*Csf1*$^{tl/tl}$)[25] or targeted mutagenesis of the *Csf1r*[8] gene in rats, as in mice[26], is associated with substantial loss of blood monocytes and most tissue macrophage populations. However, the pleiotropic consequences of that loss differ between the rodent

species.[8] Whereas *Csf1r* mutation in mice is lethal prior to weaning on most genetic backgrounds,[27] *Csf1r* mutant rats are viable as adults.[8]

The regulatory elements of the mouse *Csf1r* locus, including a highly-conserved enhancer (the Fms intronic regulatory element (FIRE)) in the first intron, have been used in transgenic mice to drive fluorescent reporter gene expression[28, 29] and cre-recombinase for conditional mutagenesis and lineage tracing[30]. *Csf1r* reporter transgene expression appeared copy-number and position independent and was highly reproducible in multiple independent mouse founders. The *Csf1r* conserved elements also directed monocyte-macrophage reporter gene expression in transgenic chickens[31] and sheep[32]. In mice, granulocytes express *Csf1r* mRNA, but do not express Csf1r protein[28, 33]. Accordingly, both *Csf1r*-EGFP and *Csf1r*-mApple transgenes are expressed in mouse granulocytes, albeit at lower levels than in monocytes. The *CSF1R*-EGFP and *Csf1r*-mApple transgenes are also expressed at low levels in murine B cells, which, like macrophages, express the key transcription factor PU.1. The *Csf1r* locus is epigenetically-silenced during B lymphocyte differentiation[34]; presumably this regulation does not operate fully in a multicopy transgene.

A major advantage of the rat as an experimental model is its much greater size, which permits isolation of large numbers of cells from major organs, experimental surgery including transplantation and dissection of structures such as lymphatics, lymph nodes and peripheral nerves[1]. By contrast to the extensive use of transgenic reporters in mice, there have been few reported in rats, mostly in the context of the neuronal[35-38] and vascular/lymphatic[39, 40] systems. The recent generation of a cre-dependent reporter gene into the rat Rosa26 locus[41] promises rapid advances in applications of lineage tracing in rats, and more broadly the generation of defined mutations in the rat genome using CRISPR-Cas9.

To enable greater utility of the rat as an experimental model for the study of CSF1 biology and tissue monocyte and macrophage populations in rats, including live imaging applications, we generated a *Csf1r* reporter transgene in this species. For this purpose, we chose mApple as a reporter, which was used previously to generate *Csf1r* reporter transgenic mice[28] and chick[31]. mApple has the advantage of resistance to photobleaching[42] and there is relatively little background autofluorescence in this channel. Here we characterize the expression of the *Csf1r*-mApple reporter transgene in adult rats and utilize the reporter to demonstrate the impact of CSF1-Fc treatment on diverse tissue macrophage populations.

Materials and Methods

Generation of transgenic rats and animal maintenance.

Csf1r-mApple transgenic fluorescent reporter rats were generated by pronuclear injection of a *Csf1r*-mApple construct[28] into outbred Sprague-Dawley (SD) embryos under contract with Cyagen Biosciences (Santa Clara, USA). Wild type SD rats were obtained from the Animal Resource Center, Australia. Genotyping was performed by PCR analysis of genomic DNA isolated from ear notches using primers that amplify a 507 bp product within the *Csf1r*-mApple cassette (Forward: CCTACATGTGTGGCTAAGGA, Reverse: CTTGAAGTAGTCGGGGATGT). Rats were bred and maintained in specific pathogen free facilities at the University of Queensland in accordance with the guidelines of The University of Queensland Animal Ethics Unit, under approved protocols. *Csf1r*-mApple^{+} founders were initially mated with wild type SD rats from a separate colony (ARC, Perth, Australia) and then with wild type littermates to avoid mating to homozygosity. Litter sizes averaged 15-17, and there was no evidence of a detrimental impact of the transgene knock-in in juvenile or adult rats.

CSF1-Fc treatment

Male (11 weeks old) and female (8 weeks old) rats were randomized into control and treatment cohorts and injected with saline or CSF1-Fc [23] (1 mg/kg) daily for 4 days in the loose skin over the flank. Animals were euthanized by CO2 inhalation and tissues were harvested on day 5.

Tissue collection for imaging and disaggregation for flow cytometry analysis

Peripheral blood (100 ul) was routinely collected into EDTA tubes by cardiac puncture following euthanasia. Blood was subjected to hematology analysis (Mindray BC-5000) followed by red blood cell lysis for 2 minutes in ACK lysis buffer (150 mM NH4Cl, 10 mM KHCO3, 0.1 mM EDTA, pH 7.4) and resuspended in flow cytometry (FC) buffer (PBS/2 % FBS) for staining. In experiments designed to maximize recovery of the marginal pool of monocytes, rats were perfused through the left ventricle with 500 ml of ice-cold PBS-EDTA as described by Scriba et al.[43] The resulting perfusate was collected and centrifuged. The mononuclear cells were isolated from the resulting cell pellet by Ficoll density gradient centrifugation. In some experiments the peritoneal and pleural cavities were sequentially lavaged using 100 ml and 50 ml PBS, respectively. Bronchoalveolar lavage was performed by inserting a plastic cannula into the trachea and inflating the lungs three times with 10 ml of $Ca2^{+}/Mg^{+}$ free PBS using a syringe. Following lavage procedures tissues of interest were removed and dissected for disaggregation and imaging. To remove the blood for some imaging experiments, euthanized animals were flushed with approximately 50 ml PBS via a syringe inserted into the left ventricle. Tissues for imaging were stored in PBS on ice and imaged within 2 h. Tissues for disaggregation, including liver, spleen and lung, were finely chopped in digestion solution containing 1 mg/ml Collagenase IV (Worthington) and 20 ug/ml DNAse1 (Roche) and placed on ice until further processing (~1 g tissue/10 ml). Tissues in digestion solution were placed on a rocking platform at 37°C for 45 minutes prior to mashing through a 70 µm filter (Falcon). For all tissues other than the liver the cell pellet was collected by centrifugation at 400 g for 5 min and resuspended in FC buffer. Percoll density gradient centrifugation was used to isolate the liver mononuclear cell fraction, which was then subjected to red blood cell lysis, washed and resuspended in FC buffer, as previously described[44]. For each tissue the cell count was estimated using a hematology analyzer

(estimated as white blood cell count) and 1 X 10^6 cells were stained for flow cytometry analysis.

Flow cytometry

Cell preparations were stained for 45 min on ice in FC buffer containing unlabeled CD32 (BD Bioscience), to block Fc receptor binding, with antibody cocktails comprising combinations of CD3-FitC, CD161-AF647, HIS48-FitC, CD11b/c-BV570, CD45R-BV785 (BD Biosciences), CD172-AF405 (Novus), CD4-APC-Cy7, CD43 AF647 (Biolegend). For intracellular staining (CD68-AF647, BD Biosciences), following surface staining cells were fixed in 4 % paraformaldehyde for 10 min at room temperature, washed and stained with antibody in FC buffer containing 0.1 % saponin. Cells were washed twice following staining and resuspended in FC buffer containing 7AAD (Life Technologies) for acquisition using a Cytoflex flow cytometer (Becton Dickinson). Relevant single-color controls were used for compensation and unstained and fluorescence-minus-one controls were used to confirm gating strategies. Flow cytometry data were analysed using FlowJo 10 (Tree Star). Live single cells were identified for phenotypic analysis by excluding doublets (FSC-A > FSC-H), 7AAD+ dead cells and debris. Absolute cell counts were calculated by multiplying the frequency of the cell type of interest by the total mononuclear cell yield per gram of disaggregated tissue.

Confocal microscopy and immunohistochemistry.

Direct imaging of tissues was performed using a Nikon/Spectral Spinning Disc Confocal microscope (X-1 Yokogawa spinning disc with Borealis modification). Ki67 staining was performed on paraformaldehyde-fixed paraffin-embedded liver tissue with heat-induced

epitope retrieval in 10 mM sodium citrate buffer pH 6.0, rabbit anti-Ki67 (Abcam ab16667) and DAKO Envision anti-rabbit HRP detection reagents.

Statistics

Statistical tests were performed using GraphPad Prism 7. Comparisons between control and treated groups were performed using the unpaired student's t-test with Welch's correction for unequal variance where population variance significantly differed (F test).

Results

Transgenic *Csf1r*-mApple rats on the outbred Sprague-Dawley (SD) background were generated by pronuclear injection using the same *Csf1r*-mApple cassette used previously to generate *Csf1r*-mApple mice[28]. The mouse Csf1r regulatory elements in this construct, which include the highly conserved FIRE sequence[45] are the same as used in the original mouse *Csf1r*-EGFP transgene[29]. One founder line that showed germ line transmission and expression of the reporter in blood cells was chosen for further detailed characterization.

Expression of Csf1r-mApple *in peripheral blood leukocytes*

Rat monocytes were reported to be uniformly positive for the myeloid markers CD172A (SIRPα), but varied in expression of CD43, HIS48, CD11c, CD4 and MHCII.[12] The *Csf1r*-mApple transgene provides an additional myeloid marker. $CD172A^{+}$ myeloid cells in peripheral blood from *Csf1r*-mApple rats exhibited a spectrum of mApple expression (Figure 1A). Consistent with expression in mice with the same transgene[28], $CD172A^{+}$/$mApple^{+}$ myeloid cells included $mApple^{Int}SSC^{Hi}$ granulocytes and SSC^{Low} monocytes (Figure 1B). The monocyte population could be further segregated based on CD43 and HIS48 expression (Figure 1C). As previously reported, $CD43^{Low}$

monocytes expressed high levels of the granulocyte marker HIS48 whereas CD43^{Hi} cells were HIS48^{Low} (Figure 1C). There was also a small population of CD43^{Low}/HIS48^{-} cells (<5% of monocytes) (Figure 1C), that may represent immature myeloid cells. As previously reported CD43^{Hi}/HIS48^{Low} 'non-classical' monocytes selectively expressed CD4,[11] whereas median CD11b/c expression was higher in CD43^{Low}/HIS48^{Hi} 'classical' monocytes (Figure 1D). The rat monocyte subpopulations were not clearly delineated consistent with evidence, as in humans and mice, that they represent a differentiation series.[11] There was a spectrum of mApple expression in both the HIS48^{Low}/CD43^{Hi} and HIS48^{Hi}/CD43^{Low} monocyte populations (Figure 1E). Amongst the non-myeloid (CD172A^{-}) population approximately 90% of CD45R^{+} (B220^{+}) peripheral blood B cells expressed detectable mApple (Figure 1A,F), similar to the profile observed in *Csf1r*-mApple mice.[28]

CD43^{Hi}/HIS48^{Low} non-classical monocytes were present in approximately 4-fold excess compared to CD43^{Low}/HIS48^{Hi} classical monocytes (Figure 1C). Previous studies have reported an excess of CD43^{Hi}:CD43^{low} monocytes in peripheral blood ranging from approximately 2-fold to 8-fold [11] [12]. Different gating strategies or rat genetic backgrounds may have contributed to this discrepancy. The latter study also employed whole body perfusion with PBS-EDTA in an attempt to include the marginal pool of monocytes but did not compare the perfusate to more conventional sampling of the bulk flow.[11] Given the nature of surface markers including selectin ligands, and the proposed function of non-classical monocytes in monitoring the vessel wall, one possibility was that the CD43^{hi} cells are selectively marginated. We compared the profiles of cells obtained from bulk flow with those obtained by flushing the circulation with PBS-EDTA. The PBS-EDTA eluate contained abundant leukocytes, but there was no selective enrichment in monocyte subsets or other mononuclear cells (data not shown).

Expression of Csf1r-mApple *in the bone marrow.*

Approximately 50% of bone marrow (BM) cells expressed mApple, of which ~50% co-expressed CD172A (Figure 2A). The large majority of mApple^{Hi}/CD172A^{-} non-myeloid cells expressed the B cell marker CD45R (B220) as in peripheral blood (Figure 2B). There was no detectable expression of mApple in CD3^{+} T cells or CD161^{+} NK cells (data not shown). Bone marrow (BM) myeloid CD172A^{+}/mApple^{+} cells comprised a minor (10%) population of SSC^{Hi} cells (putative granulocytes) and 2 SSC$^{Low/Int}$ populations (Figure 2C). CD172A^{+}/mApple^{+}/SSC$^{Low/Int}$ monocytes/macrophages fell into 2 sub-populations distinguished by reciprocal high expression of CD43 and HIS48 (~70:30 ratio) (Figure 2D). CD43^{Hi} cells expressed CD11b/c and CD4, whereas HIS48^{Hi} BM cells were largely negative for both markers (Figure 2E). On cytospin analysis of sorted cells, the SSC^{Int}/CD43^{Hi} BM cells had irregular nuclei and abundant cytoplasm consistent with identity as resident marrow macrophages, whereas the SSC^{Low}/HIS48^{Hi} BM cells resembled monocytes, with round nuclei and relatively scant cytoplasm (not shown). CD68, an endosomal protein commonly-employed as a macrophage marker in rats, was most highly expressed in SSC^{Hi} granulocytes, followed by SSC^{Int}/HIS48^{Low} myeloid cells, again consistent with identity as resident macrophages, but was poorly expressed in SSC^{Low} monocyte-like cells (Figure 2F). CD68 was not routinely included in phenotyping panels because fixation and permeabilization for intracellular staining compromised staining of several antibodies of interest. Overall, our data show that *Csf1r*-mApple transgene expression in the bone marrow is restricted to cells that express myeloid (CD172A, CD11b/c, HIS48, CD68) or B cell (CD45R) markers. Accordingly, we infer that the transgene is not expressed in lineage-negative cells, consistent with previous studies of the regulation of mouse *Csfr1r* transcription during lineage commitment.[46]

Csf1r-mApple *transgene expression in rat serous cavities and tissues.*

Mouse serous cavities contain two populations of macrophage lineage cells distinguished by surface markers. A population of small F4/80^{low} short-lived cells turns over continuously from monocytes but can also contribute to replacement of the long-lived resident population.[47] Equivalent populations have been suggested in humans[48], but have not been identified in rats. We isolated cells from the peritoneal and pleural cavities to investigate the expression of myeloid markers on *Csf1r*-mApple^{+} cells and to seek equivalent heterogeneity. Approximately 70% of peritoneal and pleural exudate cells expressed mApple, 95% of which co-expressed CD172A (Figure 3A,D). In contrast to the uniform high reporter expression in mouse peritoneal macrophages[28], there was a large spectrum of *Csf1r*-mApple expression in rat peritoneal and pleural cells. 10-15% of CD172A^{+}/mApple^{+} cells were also SSC^{Hi} (Figure 3B,E) and resembled granulocytes on cytospins (not shown). The majority (>90%) of SSC^{Low} resident macrophages homogeneously expressed CD4 and CD11b/c (Figure 3C,F), but very low or no HIS48 and CD43 (not shown). Minor populations of CD11b/c$^{Low/Neg.}$ (5-10%) and CD11b/c^{+}/CD4^{-} were also identified (Figure 3C,F). In both the peritoneal and pleural cavities, mApple^{+}/CD172A^{+}/SSC^{Hi} granulocytes, but not SSC^{Low} monocyte-macrophages, expressed CD68 (Figure 3G,H and data not shown). Consistent with published data[49], CD163, another commonly used rat tissue macrophage marker, which is not expressed on the surface of blood monocytes[49], labelled approximately 50% of peritoneal mApple^{+}/CD172A^{+}/SSC^{Low} cells but did not define an obvious subpopulation (Figure 3G,H). Overall, the marker profile suggests that the peritoneal macrophages represent a spectrum of maturation. Consistent with that view, cytospins of flow cytometry-sorted peritoneal populations revealed a broad spectrum of morphologies from monocyte-like to mature macrophage (Figure 3I). We also assessed expression of the transgene in bronchoalveolar lavage cells. Like macrophages of serous cavities, lavage cells expressed very high levels of mApple, were highly autofluorescent and expressed CD172A, CD4 and CD43, but not HIS48 and CD11b/c (Figure 3J and data not shown).

Csf1r-mApple *expression in rat tissues.*

To assess the expression of *Csf1r*-mApple in tissue macrophage populations *in situ*, we imaged a variety of fresh unfixed tissues from adult male and female reporter rats using a spinning disc confocal microscope. Representative images are shown in Figure 4. The abundance, distribution and stellate morphology of cells expressing detectable *Csf1r*-mApple in a diverse array of tissues mirrors previous analysis of mouse *Csf1r* transgenes[28, 29] and, in most cases, the location of mouse macrophages defined by the F4/80 antigen.[50] Consistent with the expression of the transgene in blood and bone marrow B cells *Csf1r*-mApple was apparently abundant in structures that resemble lymphoid follicles in the spleen, lymph nodes and Peyer's patches (Figure 4). One striking finding made possible with the transgene is the imaging of macrophages in skeletal, cardiac and smooth muscle of the diaphragm and muscularis externa of the gut (Figure 4). Macrophages of the diaphragm are implicated in control of lymphangiogenesis[51, 52] and those of muscularis externa in the regulation of gut motility in the mouse[53]. In each location, macrophages are abundant and spread in the plane of the muscle fibres. There have been few published studies of skeletal muscle macrophages in the mouse[54], but the expression of the transgene is consistent with much older studies of the localization of ED2 (CD163) and ED3 (CD169) in rat skeletal muscle[55].

In mice, the *Csf1r*-EGFP transgene provides a sensitive marker for imaging microglia[56]. For reasons that are not clear, mApple was readily detected by flow cytometry in $CD45^{Low}CD11b^{+}$ microglia in *Csf1r*-mApple mice[28] but the reporter gene was difficult to detect in whole mount brain preparations. This was also the case in *Csf1r*-mApple rats. Microglia were readily detectable in the brain meninges (Figure 4) but only faintly labelled in whole mounts of brain regions, although

Csf1r-mApple^{+} myeloid cells (CD11b/c^{+}, ~20 % of brain cells) were readily detected in disaggregated brain of *Csf1r*-mApple rats (data not shown). We conclude that there is some form of quenching of the mApple signal in nervous tissue.

The effect of CSF1-Fc administration on circulating monocytes and tissue macrophage populations.

The main purpose of the generation and characterization of the *Csf1r*-mApple transgene was to provide a marker for studies of macrophage biology in the rat. In particular, our focus is on potential therapeutic applications of CSF1 and the role of CSF1R signalling in development.[8] In neonatal rats, the administration of a CSF1-Fc fusion protein expanded the macrophage populations of the liver and other tissues but did not increase the blood monocyte count.[9] However, much earlier studies demonstrated increased peripheral blood monocytes in CSF1-treated adult rats.[57] We therefore decided to investigate the effect of CSF1-Fc in adult rats using the *Csf1r*-mApple transgene as a marker. This study also serves to validate the CSF1-dependence of mApple^{+} tissue macrophage populations identified with the transgene by direct imaging and by flow cytometry analysis of disaggregated tissues. Using the same protocol as employed previously in mice,[23] in separate experiments we injected cohorts of adult male and female *Csf1r*-mApple rats with pig CSF1-Fc (1 mg/kg) on each of 4 days, sacrificed them on the 5th day and harvested cell populations and tissues for analysis.

Consistent with the findings in mice and pigs, CSF1-Fc treatment produced a marked increase in the size of the liver and spleen in both male and female rats (Figure 5A,B). Liver growth was associated with an increase in the number of Ki67^{+} hepatocytes as well as non-parenchymal cells (Figure 5C). In the blood we confirmed an ~4-fold increase in total leukocytes, and a 4-fold increase in monocytes (Figure 5D-E), but no change in granulocytes or other hematological parameters (not shown).

Consistent with these findings, flow cytometry analysis revealed an ~3-fold increase in the *Csf1r*-mApple^{+} mononuclear cell population in peripheral blood (Figure 5F). There was no apparent change in the relative proportions of HIS48^{Hi} and CD43^{Hi} monocytes, but the minor population of HIS48^{-}/CD43^{Low} monocytes almost completely disappeared upon CSF1-Fc treatment (p=0.04, data not shown). The monocytosis initiated in response to CSF1-Fc treatment was associated with an increased prevalence of CD172A^{+}/*Csf1r*-mApple^{+} cells in the BM, which was attributable to an increase in the SSC^{Low}/HIS48^{Hi} cells shown previously to resemble monocytes (Figure 2, Figure 6A-C). In the peritoneum CSF1-Fc treatment modestly increased the total yield of exudate cells (approximately 1.5-fold, p=0.06), without changing the proportions of cells positive for CD172A.

The combination of the transgenic reporter and whole mount imaging enabled a global overview of the impact of CSF1-Fc treatment. An increase in both the size of the macrophages and their abundance was visibly obvious in images of fresh tissue examined using the spinning disc confocal. Figure 7 shows representative images of the lung, liver, diaphragm, mesentery, mesenteric lymph node, Peyer's Patches, colon and isolated lymphoid follicles with colonic patches from control and CSF1-Fc-treated *Csf1r*-mApple rats. In the liver, the obvious massive increase in *Csf1r*-mApple^{+} cells appeared to involve smaller, less stellate monocyte-like cells. Consistent with findings in the intestinal muscularis externa of CSF1-Fc treated mice,[28] individual *Csf1r*-mApple expressing macrophages in the diaphragm, where they can be readily imaged, were spread in the plane of the muscle and appeared larger and less ramified (Figure 7). The mesentery similarly provides a location where adipose tissue macrophages can be readily imaged. *Csf1r*-mApple^{+} cells were distributed between adipocytes in control rats, but in CSF1-Fc treated mice they appeared larger and surrounded each adipocyte (Figure 7).

To confirm the substantial expansion of the tissue macrophage populations observed with direct imaging we disaggregated selected tissues and analyzed cell surface marker expression. In saline treated control rats approximately 45% of spleen cells expressed *Csf1r*-mApple, 80-90% of which co-expressed CD45R and lacked expression of CD172A, CD11b/c, CD43 and HIS48, consistent with expression by B cells (Figure 8A and data not shown). In contrast to other tissues in which $CD172A^{-}/CD45R^{+}$ cells exhibited intermediate mApple expression, the spleen contained an $mApple^{Hi}$ population (Figure 8A). CSF1-Fc treatment increased splenic white blood cells (WBC) approximately 2-fold, including an increase in the proportion and number of $mApple^{+}/CD172A^{+}$ myeloid cells ($p=0.005$ and $p=0.028$, respectively), but no significant change in the $mApple^{+}/CD172A^{-}$ compartment (Figure 8B). The increase in the myeloid compartment was driven by an increase in SSC^{Low} monocyte-macrophages, including $CD43^{Low}$ cells with variable CD4 expression and a $CD43^{Hi}/CD4^{Hi}$ sub-population (Figure 8C). Both $CD172A^{+}$ monocyte-macrophage populations were $CD11b/c^{+}$ and the majority were $HIS48^{-}$ (data not shown). Spleen $mApple^{+}/CD172A^{+}$ myeloid cells also included a population of relatively granular cells (Figure 8A), which were uniformly $HIS48^{+}/CD43^{+}$ and not affected by CSF1-Fc administration.

In the liver of saline-treated control rats, approximately 40% of non-parenchymal cells expressed mApple, of which 60% expressed CD172A (Figure 8D). CSF1-Fc treatment increased the yield of mononuclear cells after liver disaggregation >5-fold ($p=0.007$) and dramatically increased the proportion and number of $CD172A^{+}/mApple^{+}$ cells (Figure 8E), in particular $CD172A^{+}/SSC^{Low}$ monocyte-macrophages with intermediate mApple expression. In control animals, 3 populations of liver monocyte-macrophages with distinct phenotypes were identified: $CD43^{-}/CD4^{-}/mApple^{Int}$,

$CD4^+/CD43^-/mApple^{Int}$ and $CD4^+/CD43^+/mApple^{Hi}$ (Figure 8D). CSF1-Fc treatment led to increases in all 3 sub-populations, especially $CD4^+CD43^-$ cells (Figure 8F).

Whole mount imaging of the lung suggested that there was a substantial increase in the number of *Csf1r*-$mApple^+$ cells in this organ in response to CSF1-Fc treatment (Figure 7). The lung has not previously been examined in detail, but in mouse we showed that injection of labelled CSF1-Fc accesses interstitial but not bronchoalveolar macrophages.[28] Consistent with the imaging data, CSF1-Fc treatment induced a significant increase in $mApple^+/CD172A^+$ cells that were released by enzymic disaggregation of the lung (Figure 8G,H). As in other tissues, in both control and CSF1-Fc treated lungs, these cells varied in granularity. The SSC^{low} monocyte-macrophage population could be subdivided based upon CD43 and CD4 expression, with $CD43^{Hi}/CD4^+$ cells comprising 70% of the total (Figure 8G). CSF1-Fc increased the SSC^{low} population without changing the relative proportion of $CD43^{Hi}$ cells (Figure 8I). The SSC^{Hi} and SSC^{Int} granulocytic cells had a similar surface marker profiles; $CD43^+/HIS48^+/CD4^-/CD11b/c^+$ (data not shown). Neither $CD172A^+/SSC^{Hi}/SSC^{Int}$ nor *Csf1r*-$mApple^+/CD172^-/CD45R^+$ B cells were affected by CSF1-Fc administration (Figure 8H,I).

Discussion

The 7.2kb mouse *Csf1r* promoter cassette used to generate the *Csf1r*-mApple transgenic rat line produced consistent position and copy number-independent expression of reporter genes in multiple independent mouse lines[28, 29, 58] and was also used to generate *Csf1r*-cre recombinase transgenes for lineage trace studies[30]. The expression of the reporter gene in this construct depends upon an enhancer, the Fms Intronic Regulatory Element (FIRE)[29] that is highly-conserved in vertebrates[45]. A FIRE-like sequence, in the same relative location in the chicken CSF1R locus, is

also required for expression of reporter genes in macrophages in chicken transgenic lines[31] and a lentivirus containing mouse FIRE was shown previously to able to direct myeloid-specific expression in rat, human, pig, cow, sheep and even chicken[59]. FIRE is 96 % identical between mouse and rat and there only 5 transversions none of which affects the documented binding sites for transcription factors[60]. The 7.2kb mouse Csf1r promoter used to generate the reporter rat line can be aligned across its whole length with the rat Csf1r locus with approximately 85 % conservation. Most differences are due to repeat insertions and expansion. All the regulatory elements in the proximal promoter (reviewed in[60]) are also conserved between mouse and rat. The widespread expression of Csf1r-mApple in macrophage lineage cells in the reporter rat supports the view that the regulation is conserved. Notwithstanding that view, we recently demonstrated that germ-line deletion of FIRE in mice leads to selective loss of CSF1R expression in monocytes and only a subset of tissue macrophages[61]. There are likely to be regulatory elements immediately upstream of the proximal promoter region[58] and within the upstream Pdgfrb gene[60] that contribute to Csf1r regulation. In a detailed study of the role of CSF1R in brain development, Erblich et al[62] found that *Csf1r*-EGFP expression accurately reflected the presence of *Csf1r* mRNA exclusively in microglia. Several studies have suggested that CSF1R can be expressed in neurons and in some epithelia. One such study claimed that CSF1R was expressed in Paneth cells in the intestinal crypt, but this was recently demonstrated not to be the case and *Csf1r*-EGFP expression in the lamina propria accurately reflected the expression of *Csf1r* mRNA exclusively in macrophages including those closely-associated with the crypts[63]. In rat intestine there was a similar close apposition of *Csf1r*-mApple^{+} cells with the crypts, and the transgene was not expressed by epithelial cells. We have similarly seen no evidence for transgene expression in neurons or in any other location in tissues inconsistent with restriction to hematopoietic cells. The analysis of disaggregated tissues by

flow cytometry strongly supports the restriction of transgene expression in tissues to monocyte/macrophage lineage cells, and generally low expression in granulocytes and B cells.

The *Csf1r*-mApple reporter provides an additional marker for blood monocytes. As previously reported in studies in inbred PVG[11] and outbred SD rats[12], the $CD43^{Hi}$ "non-classical monocyte population was dominant in our SD *Csf1r*-mApple rats (4-fold compared to $CD43^{Low}$ "classical" monocytes). As in a previous study, rat monocytes were also distinguished by expression of CD4 and the granulocyte marker HIS48[12]. CSF1-Fc treatment significantly increased peripheral blood monocyte count, as has been shown in other species. In mice, the relative abundance of the monocyte populations varies in different reports and may be strain and method dependent. On an outbred genetic background, the $CD43^{+}$/$Ly6C^{Low}$ non-classical monocytes comprised around 60% of total monocytes[14]. However, in C57BL/6 mice $Ly6C^{Hi}$ monocytes are clearly in the substantial majority[28, 64]. In humans the non-classical $CD16^{+}$ monocytes constitute a minor population (~10%), but this depends upon the gating strategy and changes with disease status[65-67]. The delineation of a definitive "non-classical" monocyte is also difficult in other species[32, 68]. The original identification of $CD43^{Hi}$ monocytes in rats reported a similar relative abundance to mice[69], but subsequent studies indicated an apparent excess of $CD43^{Hi}$ non-classical cells[11, 12, 70]. The identification of an excess of $CD43^{Hi}$ non-classical monocytes in the rat depends obviously upon the definition of monocytes. The *Csf1r*-mApple transgene provides an additional informative marker.

The abundance and regular distribution of macrophages within rat tissues is remarkably consistent. Monocyte-macrophage homeostasis has been attributed to a combination of regulated growth factor availability and either mutual repulsion or competition for spatially-defined niches within

tissues[17, 21, 71]. In previous studies, we showed that labelled CSF1-Fc injected into the circulation can access all tissue macrophage populations other than brain microglia and bronchoalveolar macrophages in mice[28] and that short-term CSF1-Fc administration increases the abundance of multiple tissue macrophage populations, in part due to resident macrophage proliferation[9, 23, 72, 73]. CSF1-Fc treatment also increased liver and splenic macrophage content in neonatal rats[9]. At this age, resident macrophage proliferation appeared to be the dominant mechanism since there was no impact on monocyte production. By contrast, in adult rats, CSF1-Fc treatment promoted a significant increase in circulating blood monocytes, and a massive expansion of tissue macrophages in every organ investigated. Changes in size and morphology were also evident. The lack of CSF1-Fc-induced monocytosis thus appears specific to neonates and adult rats respond to CSF1-Fc in a similar manner to mice and pigs[23, 24, 73]. Although CSF1 has been shown to promote monocyte maturation in mice and humans[74], we did not observe any difference in the relative proportions of monocyte subsets upon CSF1-Fc treatment. CSF1-Fc treatment of adult rats also promoted hepatomegaly (associated with hepatocyte and non-parenchymal cell proliferation) and splenomegaly, as observed in neonatal rats as well as mice and pigs[9, 23, 73]. The impacts of CSF1-Fc treatment in mice are age- and gender-dependent (unpublished data). In this study, we observed a greater impact of CSF1-Fc treatment on liver size in 8-week old female compared to 11-week old male rats. The underlying mechanism is not known, however CSF1-dependent liver macrophages may interact with other pathways controlling sex-specific liver gene expression, organism growth and other functions.

Tissue macrophage heterogeneity has been extensively studied in mice (e.g. www.Immgen.org), and some phenotypic and/or functional characteristics have been translated to human macrophages and informed our understanding of macrophage heterogeneity and plasticity in

health and disease. Although rats are considered to be superior to mice for modelling many aspects of human physiology, including cardiovascular disease and cognition[1], there is a paucity of studies on rat tissue macrophages, in part due to the limited range of antibodies available. Here we use *Csf1r*-mApple to highlight and begin to dissect the remarkable macrophage heterogeneity in a diverse range of rat tissues. In addition to their differential expression on circulating monocytes, CD43 and HIS48 have been reported as markers of monocyte-macrophage subsets in rat bone marrow, liver, lung and spleen[12]. We used *Csf1r*-mApple and CD172A to identify myeloid lineage cells in multiple tissues and investigated co-expression of CD11b/c and the monocyte-subset-specific markers CD43 and HIS48. We identified $CD43^{Hi}$ and $HIS48^{Hi}$ bone marrow populations that resembled macrophages and monocytes, respectively, and demonstrated a >3-fold increase in the $HIS48^{Hi}$ monocyte population in response to CSF1-Fc treatment. CSF1-Fc treatment also produced a dramatic increase in splenic monocyte-macrophages, including both $CD43^{Hi}$ and $CD43^{Low}$ sub-populations, the majority of which lacked HIS48 expression. The largest impact of CSF1-Fc treatment was seen in the liver, with significant increases in 3 monocyte-macrophage populations distinguished by differential CD43 and CD4 expression. CSF1-Fc treatment also increased monocyte-macrophage content in the lung, with parallel increases in $CD43^{Hi}$ and $CD43^{Low}$ sub-populations. Although the available markers do not definitively distinguish monocytes, monocyte-derived macrophages and embryonic-derived macrophages, these data suggest CSF1-Fc acts on multiple resident macrophage subsets and potentially promotes monocyte infiltration, consistent with data in mice, in which CSF1-Fc treatment promoted both monocyte infiltration and resident macrophage proliferation in the liver[73]. [75] Taken together, we show that CSF1-Fc treatment specifically expanded *Csf1r*-mApple/$CD17A^{+}$ populations in every tissue examined, without impacting *Csf1r*-$mApple^{+}$ granulocytes and B cells, consistent with their identification as monocyte-macrophage lineage cells and highlighting the systemic impact of CSF1-Fc. Although HIS48 robustly labelled

granulocytes and has previously been reported as a marker of tissue macrophage subsets[12], we did not find this marker to be a universally applicable monocyte-macrophage marker. On the other hand, CD43 and CD4 reliably distinguished macrophage sub-populations in multiple tissues. Further detailed investigations will be required to understand the ontogeny and function of the rat tissue macrophage sub-populations identified.

The propensity of immune cell reporter genes to be downregulated by inflammatory stimuli is a potential limitation to their use. For example, a human CD68 promoter-driven GFP transgenic macrophage reporter mouse was shown to be superior to Cxcr3-GFP for studies of inflammation[75]. *Csf1r*-mApple expression in response to inflammatory stimuli has not yet been tested, however reporter downregulation has not previously been observed in *Csf1r* reporter mice in inflammatory settings, and there were no identifiable effects of CSF1-Fc treatment upon mApple expression in *Csf1r*-mApple rats.

Circulating CSF1 is heavily buffered by macrophage-mediated clearance in the liver and spleen[21]. Nevertheless, increased circulating CSF1 has been measured in many different inflammatory and malignant disease states[76]. In obese patients, the level varied by an order of magnitude and was weakly correlated with insulin resistance[77]. Another study noted elevated circulating CSF1 in response to exercise and training[78]. Our key conclusion is that CSF1 is not saturating for any tissue macrophage populations. The results indicate that any increase in circulating CSF1 would drive increased macrophage numbers in every organ system. In effect, the mononuclear phagocyte system functions as a single regulated unit. The pleiotropic impacts need to be considered in any therapeutic application of either CSF1 or CSF1 antagonists.

The major limitation of the *Csf1r*-mApple rat is that there are two populations of cells that express mApple that are clearly not macrophages. In mice, both Csf1r transgenes and *Csf1r* mRNA are expressed by granulocytes[33] but they do not express Csf1r protein unless starved of growth factors *in vitro.* They also do not bind labelled CSF1-Fc *in vivo*[28]. In several tissues, notably bone marrow, peritoneum and liver, we also identified *Csf1r*-mApple^{-}/CD172A^{+} myeloid populations that exhibited high granularity and expressed the granulocyte marker HIS48. Circulating B lymphocytes expressed *Csf1r*-mApple in rats, as they do in mice[28]. We have extended that observation to demonstrate the expression in B cells in multiple organs, including spleen and lymph node. As noted above, the Csf1r promoter is active in B cells[34], but there is little to no detectable *Csf1r* mRNA in purified mouse B cells in any state of activation (www.biogps.org; www.immgen.org). It may be that elements outside the promoter construct used, as well as chromatin architecture, serve to restrict B cell *Csf1r* expression.

In summary, we report the generation of the first macrophage reporter transgene in rats. A separate rat macrophage reporter line utilising the human CD68 promoter[75] driving EGFP has also recently been generated (Kevin J Wollard, personal communication), which will be valuable for comparative studies. *Csf1r*-mApple is robustly expressed in monocyte-macrophage lineage cells. We have demonstrated the utility of *Csf1r*-mApple for direct imaging and flow-cytometric identification of rat tissue macrophage populations and for monitoring impacts of modifiers of the mononuclear phagocyte system, exemplified by CSF1-Fc. The mApple reporter will enable two-colour imaging of cell-cell interactions with more commonly-applied EGFP reporters and facilitate more detailed investigations into rat monocyte and tissue macrophage subsets, which are currently limited by the available markers.

Authorship. KMI, MC, MFC, AS, CP, SK and DAH collected and analysed data. GMD generated the transgene construct. KMI and DAH wrote the manuscript.

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Figure Legends:

Figure 1. *Csf1r*-mApple expression in rat peripheral blood leukocytes. Peripheral blood (PB) was collected from *Csf1r*-mApple rats and analyzed by flow cytometry. (A) *Csf1r*-mApple expression in $CD172A^+$ and $CD172A^-$ PB leukoyctes. *Csf1r*-$mApple^+$/$CD172A^+$ cells comprised SSC^{Hi} granulocytes and SSC^{Low} monocytes (B), which could be further divided into $HIS48^{Hi}$/$CD43^{Low}$ (red), $HIS48^{Low}$/$CD43^{Hi}$ (green) and $HIS48^-$/$CD43^{Low}$ (blue) subpopulations (C) exhibiting differential CD4 and CD11b/c expression (D) and a spectrum of mApple expression (E). (F) CD45R expression on $CD172A^-$ PB leukocytes.

Figure 2. *Csf1r*-mApple expression in rat bone marrow. Bone marrow (BM) was collected from *Csf1r*-mApple rats and analyszed by flow cytometry. (A) *Csf1r*-mApple expression in $CD172A^+$ and $CD172A^-$ BM cells. (B) CD45R expression on $CD172A^-$ BM cells. *Csf1r*-$mApple^+$/$CD172A^+$ cells comprised SSC^{Hi} granulocytes, SSC^{Int} and SSC^{Low} monocyte/macrophages (C). CD43 and HIS48 (D) and CD11b/c and CD4 (E) expression on

Csf1r-mApple^{+}/CD172A^{+}/SSC^{Int} and mApple^{+}/CD172A^{+}/SSC^{Low} BM cells. CD68 expression on *Csf1r*-mApple^{+}/CD172A^{+} BM cells (E, population cell counts normalized to mode).

Figure 3. *Csf1r*-mApple expression in rat peritoneal and pleural cavity and alveolar cells. Peritoneal, pleural and bronchoalveolar cells were collected from *Csf1r*-mApple rats by lavage and analyzed by flow cytometry. *Csf1r*-mApple expression in CD172A^{+} and CD172A^{-} peritoneal (PT, A) and pleural (PL, D) cells. PT and PL *Csf1r*-mApple^{+}/CD172A^{+} cells comprised SSC^{Hi} granulocytes and SSC^{Low} monocyte/macrophages (arrow) (B,E). SSC^{Low} monocyte/macrophages exhibited differential CD11b/c expression (C,F). CD163 and CD68 expression on peritoneal monocyte-macrophages (G) and granulocytes (H). Cytospin analysis of *Csf1r*-mApple^{+}/CD172A^{+}/SSC^{Low} peritoneal monocyte-macrophages (I, arrow and arrow head indicate macrophage and monocyte morphology, respectively. 20X magnification). *Csf1r*-mApple expression in CD172A^{+} bronchoalveolar lavage (BAL) cells (J).

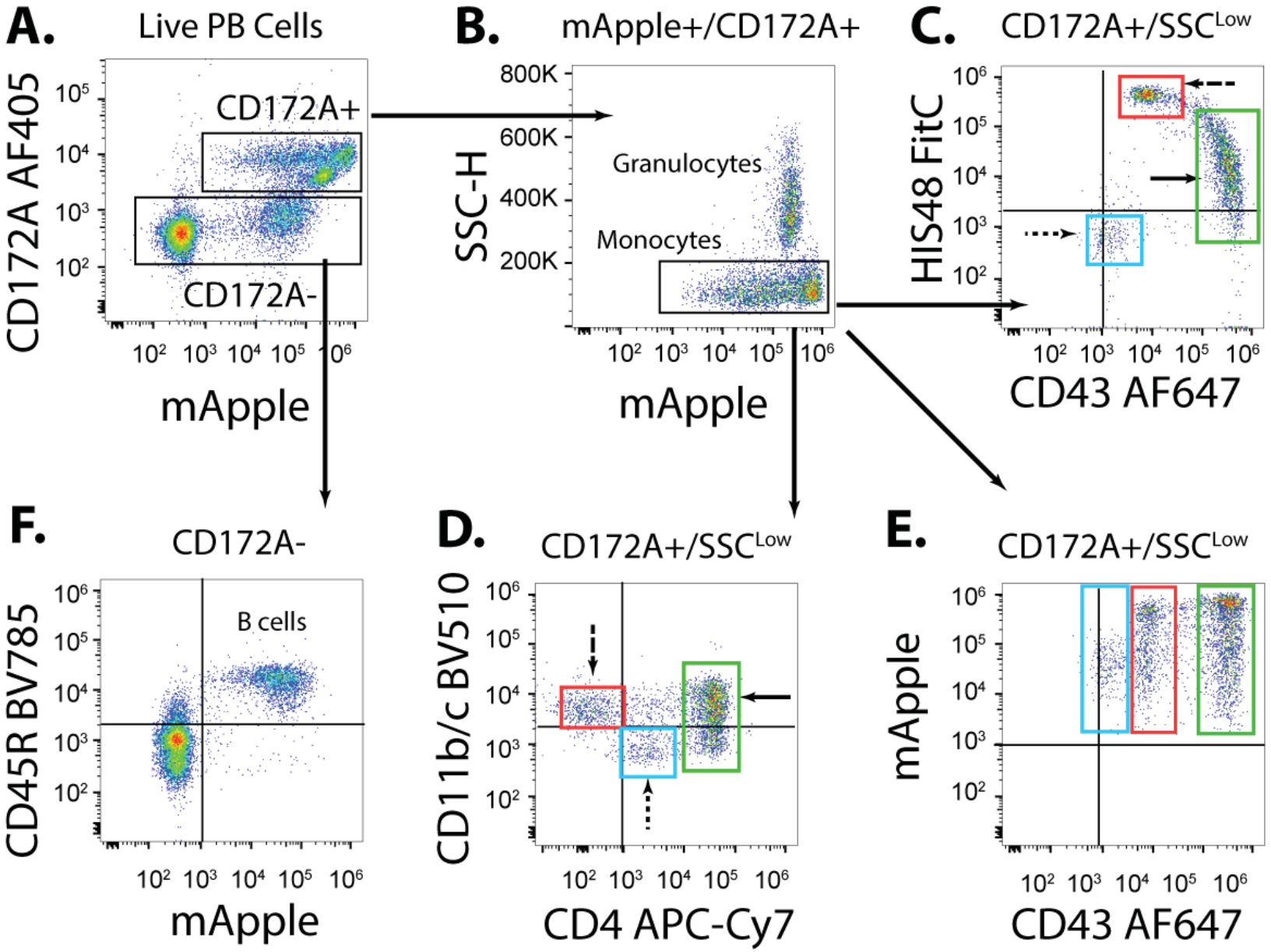
Figure 4. Localization of the Csf1r-mApple^{+} cells in rat tissues. Whole-mount imaging of freshly isolated meninges, optic nerve, liver, kidney, lung, cardiac muscle, diaphragm, muscularis externa of the gut, skeletal muscle, skin epidermis, brown adipose tissue (BAT), white adipose tissue (WAT), pancreas (including islet), spleen, mesenteric lymph node (MLN), inguinal lymph node (ILN), Peyer's patch, and colon from *Csf1r*-mApple transgenic rats. Scale bars represent 50μM not including Peyer's patch and colon, which is 100μM.

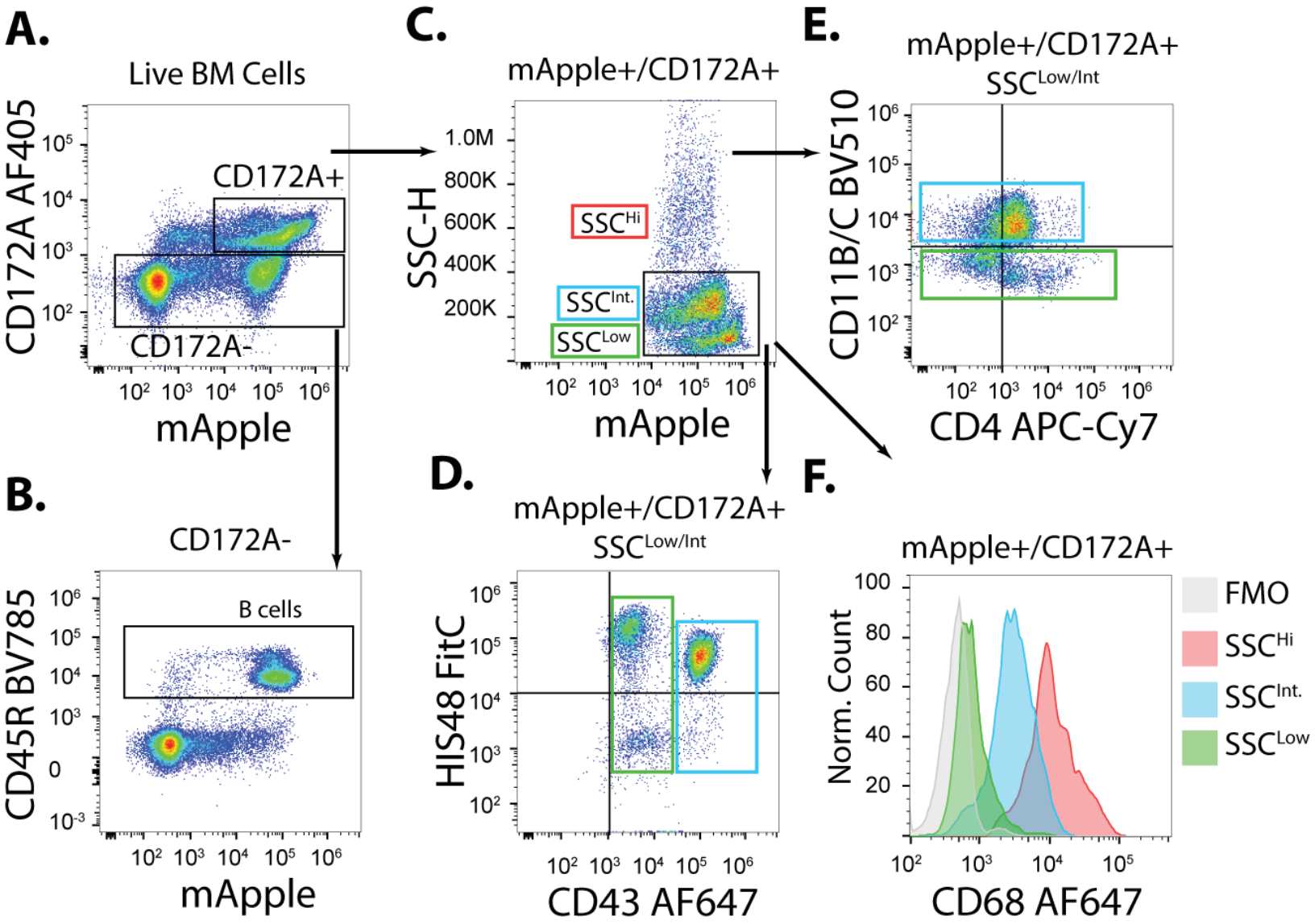
Figure 5. CSF1-Fc administration increases blood monocyte count and promotes hepatomegaly and splenomegaly. *Csf1r*-mApple rats were administered 1 mg/kg CSF1-Fc daily for 4 days prior to sacrifice on day 5. Liver (A) and spleen (B) weight/body weight ratio, liver Ki67 expression (C, D, 20X magnification), and peripheral white blood cell (WBC) count (E) and monocyte proportion (F) in PBS compared to CSF1-Fc-treated rats.

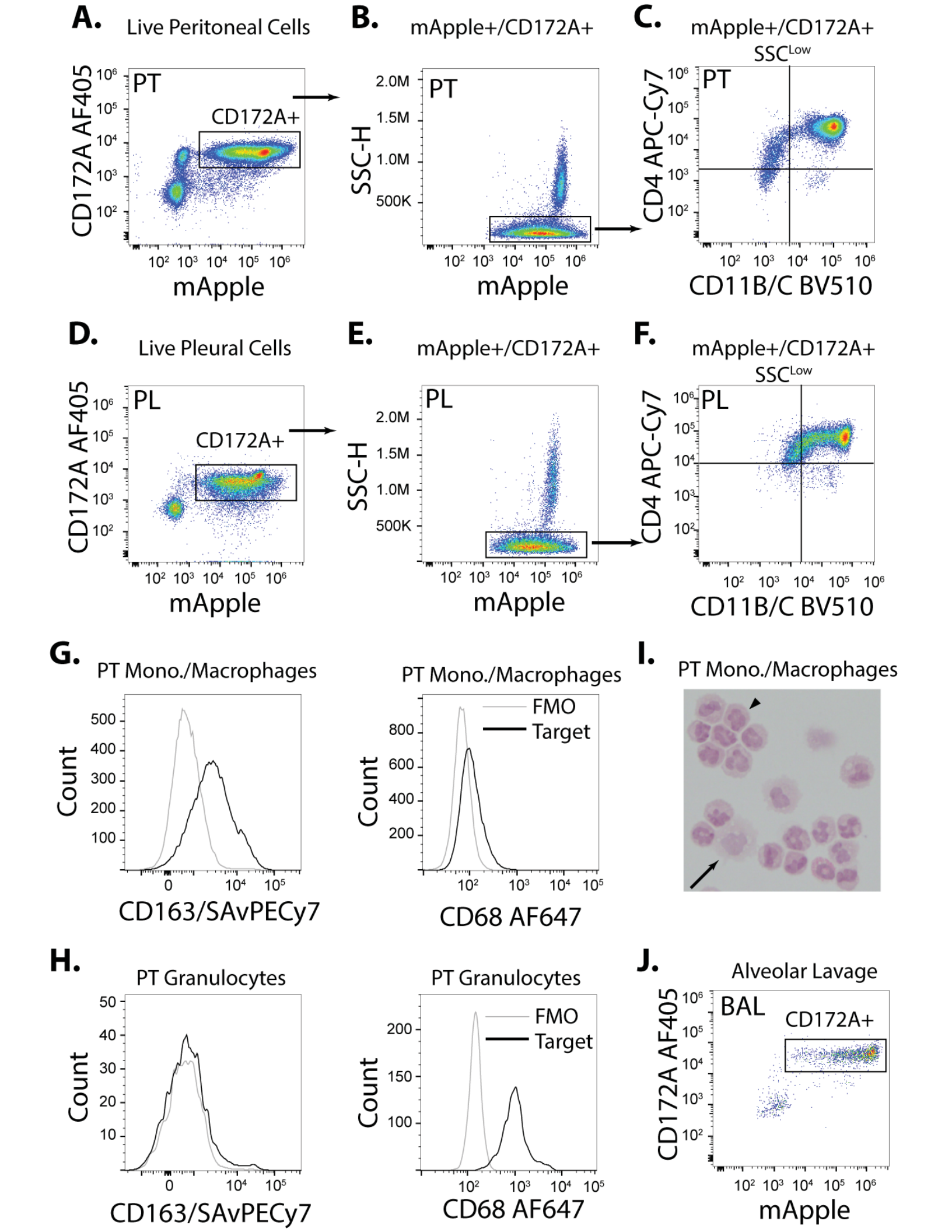
Figure 6. Impact of CSF1-Fc administration on bone marrow leukocytes. Bone marrow (BM) cells were harvested from *Csf1r*-mApple rats administered 1 mg/kg CSF1-Fc daily for 4 days prior to sacrifice on day 5. The impact of CSF1-Fc treatment on *Csf1r*-mApple $CD172A^{+}$ and $CD172A^{-}$ BM myeloid cells (A) and $CD172A^{+}/SSC^{Int}$ (solid arrow) and $CD172A^{+}/SSC^{Low}$ (dashed arrow) BM cells in control and CSF1-Fc-treated rats (B-C).

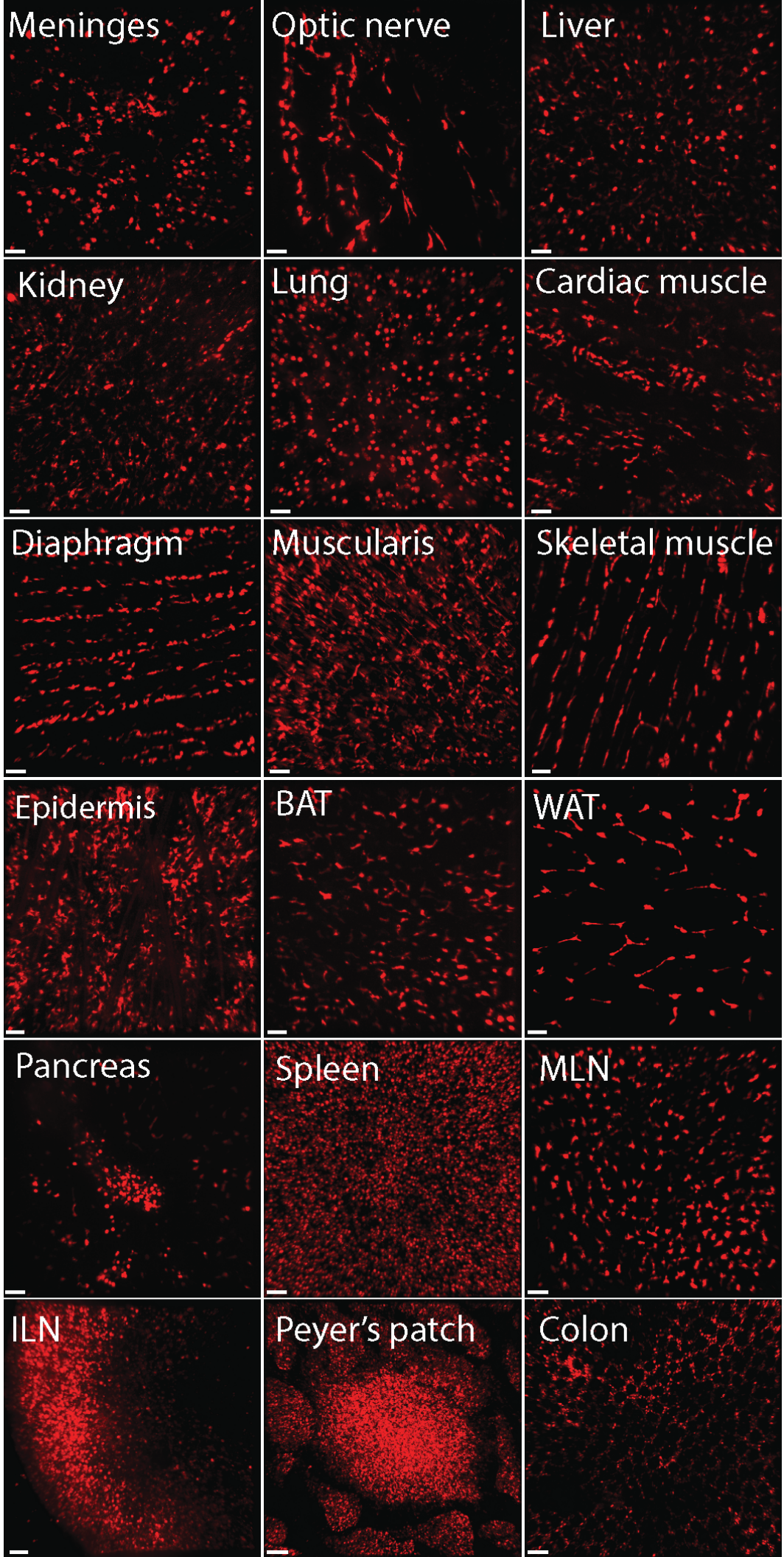
Figure 7: Localization of Csf1r-mApple+ cells in tissues of CSF1-Fc-treated rats. Whole mount images of freshly isolated lung, liver, diaphragm, mesentery, mesenteric lymph node (MLN), Peyer's patches (PP) and their surrounding villi (V), colon (including isolated lymphoid follicles (ILF)) and colonic patches (CP) from saline- and CSF1-Fc-treated (below) *Csf1r*-mApple rats. Scale bars represent 50 µM in lung, liver, diaphragm, mesentery and MLN. Scale bars represent 100 µM in Peyer's patches, colon and colonic patches.

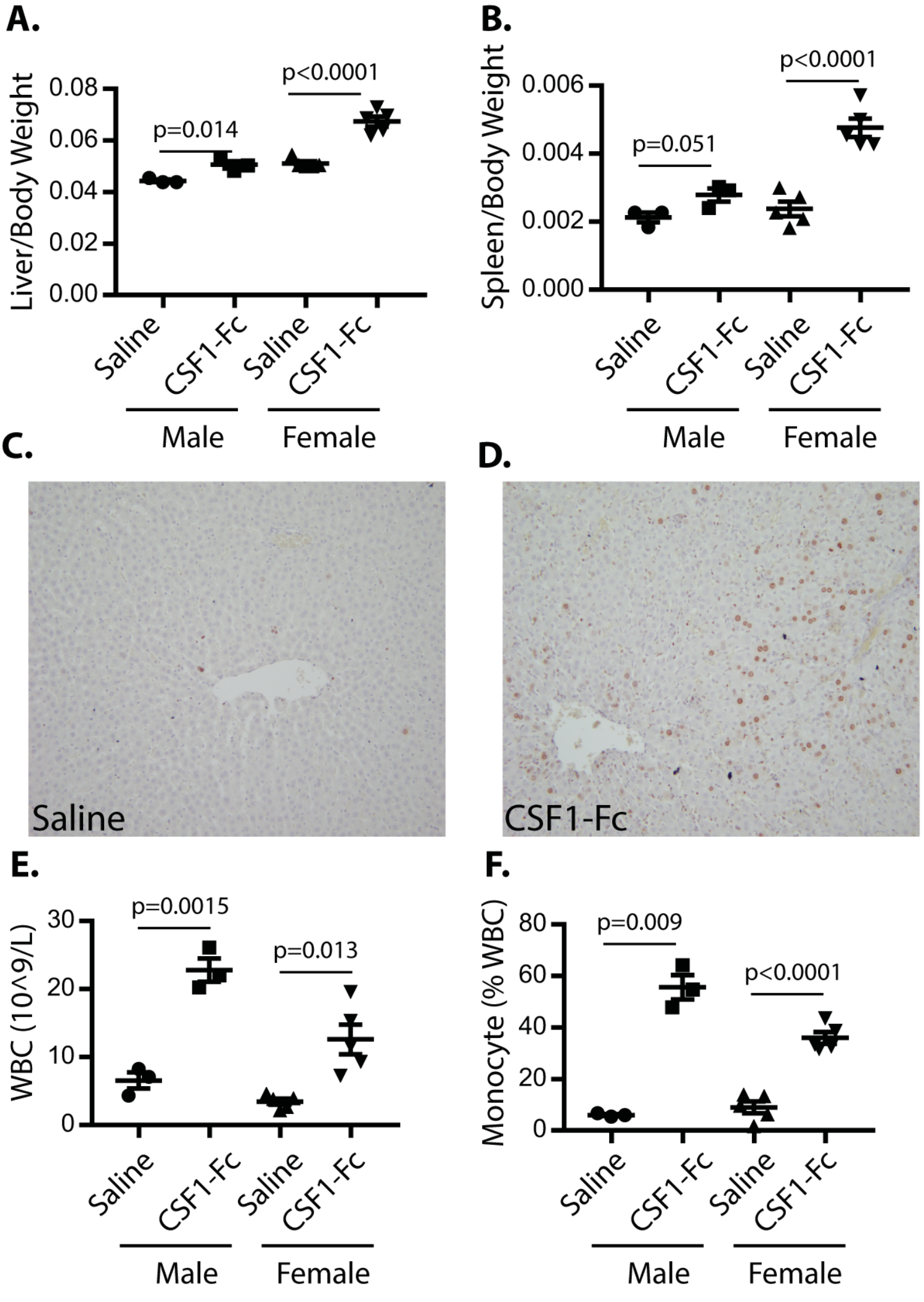
Figure 8. *Csf1r*-mApple expression in rat spleen, liver and lung leukocyte populations and the impact of CSF1-Fc administration. Spleens, livers and lungs were harvested from *Csf1r*-mApple rats administered 1 mg/kg CSF1-Fc daily for 4 days prior to sacrifice on day 5. *Csf1r*-mApple expression in $CD172A^{+}$ and $CD172A^{-}$ splenocytes (A) and their abundance (B) in control and CSF1-Fc-treated rats. *Csf1r*-$mApple^{+}/CD172A^{+}/SSC^{Low}$ spleen cells comprised $CD43^{Low}$ and $CD43^{Hi}$ sub-populations (A,C). *Csf1r*-mApple expression in $CD172A^{+}$ and $CD172A^{-}$ liver leukocytes (D) and their abundance (E) in control and CSF1-Fc-treated rats. *Csf1r*-$mApple^{+}/CD172A^{+}/SSC^{Low}$ liver cells comprised $CD43^{-}/CD4^{-}$, $CD43^{-}/CD4^{+}$ and $CD43^{+}/CD4^{+}$ sub-populations (D,F). *Csf1r*-mApple expression in $CD172A^{+}$ and $CD172A^{-}$ lung leukocytes (G) and their abundance (H) in control and CSF1-Fc-treated rats. The impact of CSF1-Fc treatment on $CD172A^{+}/SSC^{Hi}$ and $CD172A^{+}/SSC^{Low}$ subpopulations (C).

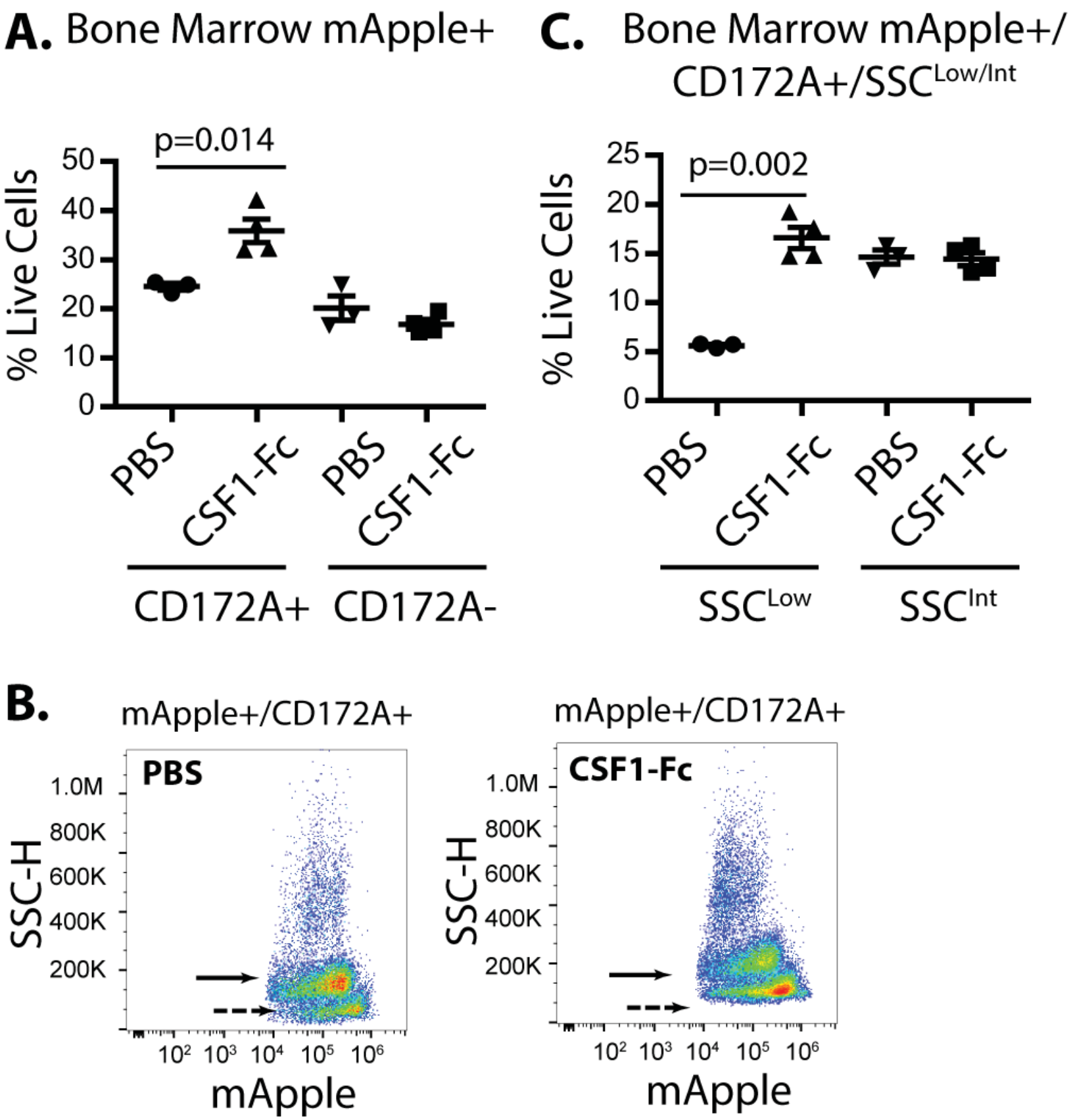

A.
Live PB Cells
CD172A+
CD172A-
CD172A AF405
mApple
B.
mApple+/CD172A+
Granulocytes
Monocytes
SSC-H
mApple
C.
CD172A+/SSC^Low
HIS48 FitC
CD43 AF647
F.
CD172A-
B cells
CD45R BV785
mApple
D.
CD172A+/SSC^Low
CD11b/c BV510
CD4 APC-Cy7
E.
CD172A+/SSC^Low
mApple
CD43 AF647


A.
Live BM Cells
CD172A AF405
CD172A+
CD172A-
mApple
B.
CD172A-
CD45R BV785
B cells
mApple
C.
mApple+/CD172A+
SSC-H
SSC^Hi
SSC^Int.
SSC^Low
mApple
D.
mApple+/CD172A+
SSC^Low/Int
HIS48 FitC
CD43 AF647
E.
mApple+/CD172A+
SSC^Low/Int
CD11B/C BV510
CD4 APC-Cy7
F.
mApple+/CD172A+
Norm. Count
CD68 AF647
FMO
SSC^Hi
SSC^Int.
SSC^Low

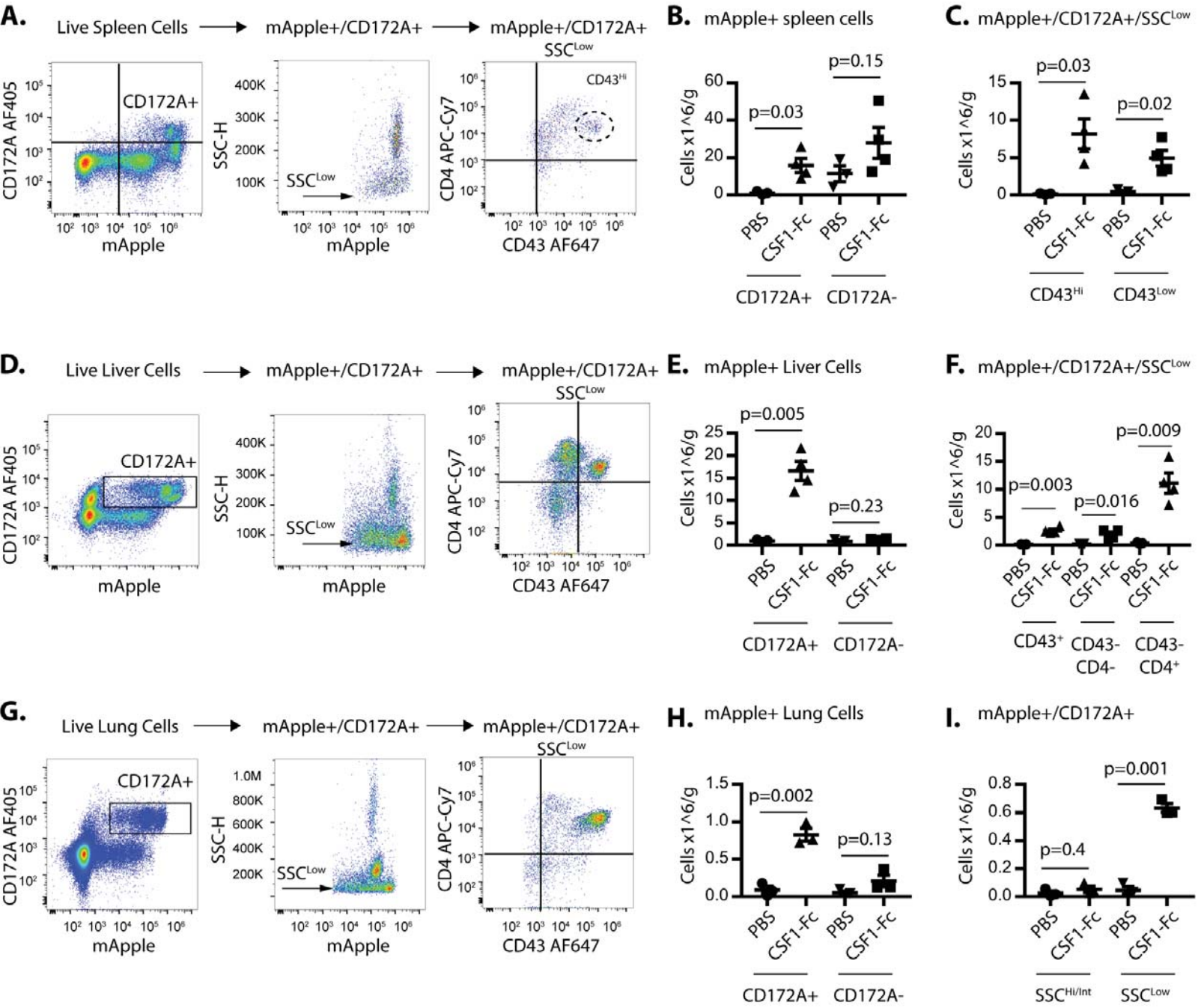

A.
Live Peritoneal Cells
PT
CD172A+
CD172A AF405
mApple
B.
mApple+/CD172A+
PT
SSC-H
mApple
C.
mApple+/CD172A+
SSC^Low
PT
CD4 APC-Cy7
CD11B/C BV510
D.
Live Pleural Cells
PL
CD172A+
CD172A AF405
mApple
E.
mApple+/CD172A+
PL
SSC-H
mApple
F.
mApple+/CD172A+
SSC^Low
PL
CD4 APC-Cy7
CD11B/C BV510
G.
PT Mono./Macrophages
Count
CD163/SAvPECy7
PT Mono./Macrophages
FMO
Target
Count
CD68 AF647
I.
PT Mono./Macrophages
H.
PT Granulocytes
Count
CD163/SAvPECy7
PT Granulocytes
FMO
Target
Count
CD68 AF647
J.
Alveolar Lavage
BAL
CD172A+
CD172A AF405
mApple


Meninges
Optic nerve
Liver
Kidney
Lung
Cardiac muscle
Diaphragm
Muscularis
Skeletal muscle
Epidermis
BAT
WAT
Pancreas
Spleen
MLN
ILN
Peyer's patch
Colon


A.
Liver/Body Weight
0.08
0.06
0.04
0.02
0.00
p=0.014
p<0.0001
Saline
CSF1-Fc
Saline
CSF1-Fc
Male
Female
B.
Spleen/Body Weight
0.006
0.004
0.002
0.000
p=0.051
p<0.0001
Saline
CSF1-Fc
Saline
CSF1-Fc
Male
Female
C.
Saline
D.
CSF1-Fc
E.
WBC (10^9/L)
30
20
10
0
p=0.0015
p=0.013
Saline
CSF1-Fc
Saline
CSF1-Fc
Male
Female
F.
Monocyte (% WBC)
80
60
40
20
0
p=0.009
p<0.0001
Saline
CSF1-Fc
Saline
CSF1-Fc
Male
Female


A. Bone Marrow mApple+
p=0.014
% Live Cells
50
40
30
20
10
0
PBS
CSF1-Fc
PBS
CSF1-Fc
CD172A+
CD172A-
C. Bone Marrow mApple+/
CD172A+/SSC^Low/Int
p=0.002
% Live Cells
25
20
15
10
5
0
PBS
CSF1-Fc
PBS
CSF1-Fc
SSC^Low
SSC^Int
B.
mApple+/CD172A+
PBS
SSC-H
1.0M
800K
600K
400K
200K
10^2 10^3 10^4 10^5 10^6
mApple
mApple+/CD172A+
CSF1-Fc
SSC-H
1.0M
800K
600K
400K
200K
10^2 10^3 10^4 10^5 10^6
mApple

Saline
CSF1-Fc
Saline
CSF1-Fc
Lung
Liver
Diaphragm
Mesentery
MLN
Peyer's Patches
Colon
Colonic Patch
V
PP
ILF
CP


A.
Live Spleen Cells
mApple+/CD172A+
mApple+/CD172A+ SSC^Low
CD172A+
CD172A AF405
mApple
SSC-H
SSC^Low
CD43^Hi
CD4 APC-Cy7
CD43 AF647
B.
mApple+ spleen cells
Cells x1^6/g
p=0.03
p=0.15
PBS
CSF1-Fc
CD172A+
CD172A-
C.
mApple+/CD172A+/SSC^Low
p=0.03
p=0.02
CD43^Hi
CD43^Low
D.
Live Liver Cells
mApple+/CD172A+
mApple+/CD172A+ SSC^Low
E.
mApple+ Liver Cells
p=0.005
p=0.23
F.
mApple+/CD172A+/SSC^Low
p=0.003
p=0.016
p=0.009
CD43+
CD43- CD4-
CD43- CD4+
G.
Live Lung Cells
mApple+/CD172A+
mApple+/CD172A+ SSC^Low
H.
mApple+ Lung Cells
p=0.002
p=0.13
I.
mApple+/CD172A+
p=0.001
p=0.4
SSC^Hi/Int
SSC^Low