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Lentiviral vectors containing mouse Csf1r control elements direct macrophage-restricted expression in multiple species of birds and mammals

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The development of macrophages requires signaling through the lineage-restricted receptor Csf1r. Macrophage-restricted expression of transgenic reporters based upon Csf1r requires the highly conserved Fms-intronic regulatory element (FIRE). We have created a lentiviral construct containing mouse FIRE and promoter. The lentivirus is capable of directing macrophage-restricted reporter gene expression in mouse, rat, human, pig, cow, sheep, and even chicken. Rat bone marrow cells transduced with the lentivirus were capable of differentiating into macrophages expressing the reporter gene in vitro. Macrophage-restricted expression may be desirable for immunization or immune response modulation, and for gene therapy for lysosomal storage diseases and some immunodeficiencies. The small size of the Csf1r transcription control elements will allow the insertion of large "cargo" for applications in gene therapy and vaccine delivery.

INTRODUCTION
Haemopoietic stem cells (HSC) in the bone marrow (BM) give rise to monocytes in circulation which can then enter tissues to become resident macrophages. Mammalian macrophages are numerous in all tissues, and apart from their role in the innate immune response are required for many aspects of development and homeostasis (reviewed in ref. 1). Macrophages are also involved in the pathogenesis of inflammatory diseases such as atherosclerosis2 and rheumatoid arthritis3 and in the initiation, progression, and metastasis of tumors.4 The active recruitment of monocyte-macrophages in all forms of inflammation makes them an attractive candidate for therapeutic gene delivery. Gene-targeted macrophages could therefore be an effective method to focus protein expression to the site of diseased tissues and nearby blood vessels. Macrophages can cross the blood–brain barrier and therefore could deliver proteins to the brain in the treatment of neurological disorders.5 Macrophage lineage cells function as antigen-presenting cells (APC) for the activation of T lymphocytes.6 Restricted expression in vaccine vectors could provide increased immunogenicity by avoiding expression in non-APC, where the outcome may be tolerogenic.

A feature of macrophages is the high expression of lysosomal enzymes.7 Lineage-restricted gene therapy has been considered in the treatment of lysosomal storage diseases and other phagocyte defects. Lysosomal storage diseases are a large group of metabolic disorders caused by a deficiency in lysosomal enzymes.8 In affected patients, these defects are most obvious in macrophages. Phagocyte function is specifically compromised in chronic granulomatous disease where defects in genes coding for components of the NADPH oxidase enzyme system result in impaired macrophage function.9 A recent study by Brendel et al.10 used a novel myeloid-specific miR223 promoter for chronic granulomatous disease therapy in mice. While expression of p47(PHOX) and gp91(PHOX) was targeted to granulocytes and macrophages, expression was also detected in other cells such as HSC and lymphocytes.

Colonystimulating factor 1 receptor (Csf1r) is a member of the type III protein tyrosine kinase receptor family whose expression in adults is restricted to cells of the macrophage lineage.11 We have previously identified the elements of the Csf1r locus required for lineage-restricted expression in the MacGreen reporter mice.12 The construct contained a 7.2kb region comprising the promoter and first intron, which includes a critical conserved enhancer termed the Fms-intronic regulatory element (FIRE). A construct of this size is clearly not practical for gene therapy. Here, we have extracted sequences of the mouse Csf1r promoter and FIRE to create various lentiviral vectors. These vectors drive expression of several different reporter genes in monocytes and macrophages in a variety of species, including human, rat, pig, cow, sheep, and chicken. Aside from the practical applications for delivery to macrophages, the results indicate that the high sequence conservation of FIRE is reflected in conserved functional activity from birds to mammals.

RESULTS
The Csf1r promoter and FIRE drives EGFP and mCherry expression in RAW264.7 cells

Our recent study suggested that the function of FIRE, in situ, is position and orientation dependent,13 but it has previously been reported to have conventional enhancer activity.14 In order to drive expression of enhanced green fluorescent protein (EGFP) specifically in monocytes...
and macrophages, the Csf1r promoter (including start codon) was fused to the coding sequence of EGFP and cloned upstream of FIRE in a lentiviral vector (Figure 1). mCherry constructs (data not shown) were also created as well as one in which expression of EGFP or mCherry was localized to membranes using a myristoylated motif (Figure 1c). The specificity of the lentivirus was initially tested by transduction of the murine cell lines EL4 (T lymphocyte) and RAW264.7 (monocyte/macrophage) with Csf1r:EGFP-FIRE. After 10 days of selection in blasticidin, only RAW264.7 cells expressed EGFP when viewed by confocal microscopy (Figure 2a). Cells transduced with Csf1r:myr:EGFP-FIRE displayed EGFP expression at slightly lower levels via flow cytometry (Figure 2b). However, in these cells, as anticipated, the EGFP was only localized to the cell membrane and also to membranes of intracellular vesicles (Figure 2c). Lentiviral transduction of RAW264.7 cells with the alternative vector containing mCherry also resulted in reporter expression as detected by confocal microscopy (Figure 2c). In summary, with all reporters, the expression was not only restricted to macrophages, but was sufficient to enable ready visualization.

Primary rat macrophages transduced with Csf1r:EGFP-FIRE lentivirus express EGFP

We currently have Csf1r-EGFP-positive transgenic mice, which have been widely used.12 A similar reporter in rat would have great utility, but rat transgenesis is less straightforward and lentiviral delivery would provide an alternative route. The Csf1r:EGFP-FIRE lentivirus was also active in primary cells and in the rat. Rat BM-derived macrophages were transduced with the lentivirus and the majority of transduced cells were EGFP⁺ as determined by fluorescent microscopy (Figure 3a). We therefore wished to determine whether the virus, once integrated into progenitors, would maintain expression during differentiation. Rat BM cells were cultured for 2 days in IL-3, IL-6, and stem cell factor (SCF) to support myeloid progenitors and then transduced with Csf1r:EGFP-FIRE. Two days following transduction, growth factors were replaced with rhCSF1 to allow macrophage development. Prior to addition of rhCSF1, few cells were EGFP⁺ (Figure 3b, i), but as the cells differentiated into macrophages, the number of EGFP⁺ increased. After 7 days, the large majority of cells were EGFP⁺ (Figure 3b, iii) and were confirmed as functional macrophages by their ability to phagocytose fluorescently labeled zymosan particles (Figure 3b, iv). Csf1r:EGFP-FIRE drives EGFP expression exclusively in cells expressing Csf1r

To confirm the specificity of Csf1r:EGFP-FIRE, rat BM cells were transduced with the lentivirus after 2 days in culture and reverse

![Diagram of murine Csf1r:EGFP constructs](https://example.com/diagram1.png)

**Figure 1** Csf1r:EGFP lentiviral constructs. Schematic of murine Csf1r:EGFP constructs. All constructs contained a blasticidin-resistance gene (BSD) driven by the EM7/SV40 promoter. (a) Csf1r:EGFP-FIRE (b) Csf1r:EGFP-sFIRE (c) Csf1r:myr:EGFP-FIRE. cPPT, central polypurine tract; FIRE, Fms-Intronic Regulatory Element; ψ, HIV-1 packaging signal; LTR, long terminal repeat; RRE, rev response elements; sFIRE, short FIRE; myr, myristoylated motif.

![Confocal microscopy images of EL4 and RAW264.7 cells transduced with Csf1r:EGFP-FIRE](https://example.com/images2.png)

**Figure 2** Murine Csf1r elements in a lentivirus drive reporter gene expression in macrophages but not T lymphocytes. (a) Representative confocal microscopy images of EL4 and RAW264.7 cells transduced (n = 3) with Csf1r:EGFP-FIRE. (b) FACS analysis of RAW264.7 cells transduced with Csf1r:EGFP-FIRE or a membrane-bound EGFP (Csf1r:myr:EGFP-FIRE). (c) RAW264.7 cells transduced with (i) Csf1r:myr:mCherry-FIRE and Csf1r:EGFP-FIRE, (ii) Csf1r:myr:EGFP-FIRE, (iii) and Csf1r:mCherry-FIRE. Bar = 20µm or 10µm in C i and iii.
transcriptase PCR performed for Csf1r expression in sorted GFP− and GFP+ populations following selection in blasticidin. Cells were cultured in the presence of IL-6, FLT3-L, thrombopoietin, and SCF to support the growth of progenitors and provide a mixture of haemopoietic lineages for analysis. Three weeks following transduction, approximately one quarter of the cells expressed EGFP (Figure 3c) and only these cells expressed Csf1r as determined by reverse transcriptase PCR (Figure 3d). These results confirm that Csf1r:EGFP-FIRE drives EGFP expression only in haemopoietic cell types expressing Csf1r.

A lentivirus containing a smaller region of intronic DNA results in EGFP expression but at lower levels in RAW264.7 cells. The plasmid encoding Csf1r:EGFP-FIRE consists of 6.5 kb sequence between the two lentiviral long terminal repeats (LTR). To increase the potential “cargo” capacity available for expression of genes of interest, we created a smaller vector (Csf1r:EGFP-sFIRE), which reduced the region surrounding FIRE by around 1.2 kb, so that there was only 5.3 kb between LTRs. The truncated construct retained EGFP expression, albeit at a slightly lower level compared to Csf1r:EGFP-FIRE (Figure 3e).

Murine Csf1r:EGFP-FIRE drives expression of EGFP in macrophages from multiple species

The FIRE element is highly conserved from birds to humans. We therefore examined whether murine Csf1r:EGFP-FIRE could direct the expression of EGFP in macrophages of other species. Figure 4 clearly shows EGFP expression in BM-derived macrophages from pig (Figure 4a), monocyte-derived macrophages from cow (Figure 4b) and human (Figure 4c) as well as a chicken macrophage cell line (HD11, Figure 4d), and sheep alveolar macrophages (Figure 4e).

DISCUSSION

Differentiation of BM progenitors into macrophages requires macrophage colony-stimulating factor (Csf1), which signals via its receptor Csf1r.15,16 Expression of Csf1r mRNA in mice is restricted to cells of the myeloid lineage. The only other expression occurs in placental trophoblasts during development, where it is driven by a distinct promoter.17 MacGreen mice were created previously by placing EGFP expression under the control of the Csf1r proximal promoter and FIRE located in the first intron.12 These mice have consistent expression of EGFP in the same locations as the endogenous gene and...
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provide a valuable tool for those interested in macrophage biology. Lentiviral gene delivery to embryos results in higher percentages of transgenic animals when compared to standard pronuclear injection techniques. The region of Csf1r used to create the MacGreen mouse (7.2 kb) was considered too large to incorporate into a lentiviral vector. Including EGFP, the sequence between the two LTRs would be 10.5 kb. HIV-based lentiviral vectors generally contain a maximum sequence of 9 kb between the two LTRs as this is the size of the native HIV genome. Insertion of larger sequences is possible but typically results in lower titers of virus. To enable inclusion of cargo, we created a vector in which a 0.5 kb region of the murine Csf1r promoter was placed 5’ to the EGFP coding sequence with a distal portion of the Csf1r second intron containing FIRE (1.8 kb) placed 3’ to the EGFP stop codon. We also created a smaller vector, in which the region surrounding FIRE was further reduced. This decreased expression somewhat, possibly due to the exclusion of a second known DNase hypersensitive site upstream of FIRE. Both vectors enabled expression of the EGFP reporter. We also tested a membrane-targeted reporter.

Metachromatic leukodystrophy patients are deficient in arylsulfatase A, a causing build-up of the enzyme’s substrate in various cell types including microglia and macrophages of the peripheral nervous system. Biffi et al. reported promising results in a phase 1/2 clinical trial involving three patients where HSC were transduced with a lentivirus overexpressing arylsulfatase A from a ubiquitous promoter. Gene delivery driven by such promoters could have undesirable effects in nontarget cell types, and current lentivirus-based technologies aim to deliver proteins only to target cells. As macrophages have the potential to deliver therapeutic proteins to many target tissues, they make an attractive target cell for gene therapy. Several macrophage promoters such as CD68 and the scavenger receptor-A have been studied. Their limitations are reviewed in ref. 27. He et al. generated macrophage-specific synthetic promoters by random ligation of myeloid/macrophage cis elements and reported that these promoters were 100-fold more effective at delivering green fluorescent protein (GFP) to macrophage cell lines than the native Csf1r promoter. However, their Csf1r constructs did not include the intronic region containing FIRE, which is essential for EGFP expression in the MacGreen mice, so the baseline for comparison was very low. The promoter of CD68 has been suggested as the best currently available macrophage-specific promoter, but CD68 reporter mice do not express GFP in the majority of cells expressing F4/80, which is a well-documented marker of macrophages. For myeloid-restricted gene expression, the Csf1r promoter/enhancer appears superior in terms of efficacy, specificity, and reproducibility. The production of transgenic sheep using the Csf1r-based lentivirus is currently under way, and initial analysis of peripheral blood mononuclear cell in these animals shows EGFP expression is restricted to CD14+ monocytes (unpublished data). We have shown that macrophages from chicken, cow, human, and pig as well as sheep alveolar macrophages can also be transduced with the vector, and express EGFP, so the mouse sequence provides a generic vehicle that could be tested in both veterinary, preclinical, and human clinical applications.

This remarkable cross-species reactivity highlights the widespread implications that this vector could have in current gene therapy technologies. If the Csf1r:EGFP-FIRE lentivirus is to be used as a gene therapy vehicle, it would be advantageous to further decrease the size of the vector in order for sequences larger than EGFP to be inserted. The smaller vector we created apparently reduced expression and the titer of the two lentiviruses was similar, indicating the larger vector was not inhibiting virus production (data not shown). We may be able to optimize expression further by creating a hybrid or synthetic combination of FIRE (which is only around 300 bp) and the upstream element. What is clearly the case is that the enhancer can be located conveniently downstream of the gene of interest or reporter gene, so further optimization will be straightforward. The safety concerns of using lentiviral vectors in gene therapy technologies are well documented. There is the risk of insertional mutagenesis following viral transduction and current gene therapy research focuses on the use of integrase-deficient packaging plasmids. If Csf1r:EGFP-FIRE were to be used in clinical settings, it would be quite simple to mutate (D64V) the viral integrase, which decreases integration up to 104-fold.

Aside from gene therapy, lentiviral vectors have also been considered as vaccines to drive efficient antigen expression and presentation in APC both in vitro and in vivo, leading to the activation of cellular immunity and humoral immune responses. The injection of a lentivirus containing ovalbumin driven by a ubiquitous promoter protected mice from the development of OVA-expressing tumor cells. However, in some cases, ubiquitous expression of a potential antigen in non-APC could potentially drive peripheral tolerance, rather than effective immunization (e.g., ref. 36). The Csf1r lentivirus addresses this question by...
driving expression in professional APC. Classical Steinman–Cohn dendritic cells as well as macrophages express Csf1r-EGFP in the MacGreen mouse.37 As Csf1r:EGFP-sFIRE is only 5.3 kb between LTRs, there is the possibility to insert larger antigens for which previous vaccination approaches have proved difficult. One such example is the blood-stage of malaria. There is currently no vaccine following lentiviral transduction, the concentration is listed in Table 2.

Cell culture
All cells were maintained at 37 °C in a humidified 5% CO 2 incubator and cultured as per Table 2. All media contained 1 mmol/l GlutaMAX (Life Technologies, Paisley, UK) except for rat BM, which contained 2 mmol/l. If cells were selected with blasticidin following lentiviral transduction, the concentration was 8 (D-17) µg/ml.

Lentivirus production and titration
High-titer lentivirus was prepared by transfection of HEK293T cells with a Csf1r construct as well as psPax2 (encoding gag/pol/rev proteins) and pLP/vesicular stomatitis virus G (for the expression of vesicular stomatitis virus G glycoprotein). Prior to transfection, HEK293T cells were plated at 105 cells/cm2 and cotransfection of the three plasmids was performed with Fugene HD (Promega, Southampton, UK) according to manufacturer’s instructions. The following day medium was replaced with serum-free culture media. Supernatant was harvested 48 hours post-transfection and filtered through 0.45 µm filters prior to concentration in Centricon Plus-70 columns (Millipore, Watford, UK). Once the supernatant was concentrated to 105 transducing units/ml, it was then ultracentrifuged for 2 hours at 69K x g at 4°C. Viral pellets were resuspended in 100 mmol/l NaCl, 20 mmol/l Tris pH 7.3, 10 mg/ml sucrose, and 10 mg/ml mannitol and stored at —80 °C until use. The viral titer (typically between 8.5 × 108 and 2 × 109 transducing units/ml) was assayed by end-point dilution on the D-17 cell line. For some experiments, unconcentrated lentivirus (108 transducing units/ml) was used.

Table 1 Oligonucleotides used in cloning

| Oligonucleotide | Sequence/s |
|-----------------|------------|
| Mouse Csf1 promoter | 5′ TTACAGTTGGTCCAGAGG |
| Csf1r:EGFP splice overlap | 5′ CACGCTATTCCCTGGAGACTATGTGGAGCAAGGCGC; 3′ AACAGCTCTGTGCTTGACGACGAGGGTGAGAGCC |
| EGFP | 3′ CGGCCGCTTTACTTGT |
| FIRE | 5′ GAGTTGGAAGGGGCTGAT; 3′ AGACCATACTGCTGGCTGTT |
| sFIRE | 5′ GGTTGGAAGGGGCTGAT; 3′ GCCAGCTCTGGCTGCTGTC |
| Csf1r:myrEGFP splice overlap | 5′ CACGCTATTCCCTGGAGACTATGTGGAGCAAGGCGC; 3′ GCCAGCTCTGGCTGCTGTC |
| myrEGFP splice overlap | 5′ GCAGCCAGGGGCACCCGGTCACCCTAGGACAGGGCC; 3′ GCCAGCTCTGGCTGCTGTC |
| mCherry | 3′ TTGATTGCTCTGGAGGCG |
| Csf1r:mCherry splice overlap | 5′ GGTTGGAAGGGGCTGAT; 3′ GCCAGCTCTGGCTGCTGTC |

FIRE, Fms-intronic regulatory element.

Table 2 Cell culture media

| Cells | Media | Polybrene (µg/ml) | Blasticidin (µg/ml) |
|-------|-------|-----------------|-----------------|
| RAW264.7 | DMEM, 10% FCS | 8 | 5 |
| HEK293T,D-17 | DMEM, 10% FCS, 100 U/ml penicillin, 100 µg/ml streptomycin, nonessential amino acids | 8 (D-17) | 15 (D-17) |
| EL4 | DMEM, 10% horse serum | 8 | 10 |
| HD11 | RPMI, 10% FCS, 2% chicken serum | 8 | 7.5 |
| Rat BM, rat BMDM | DMEM, 10% FCS, 100 U/ml penicillin, 100 µg/ml streptomycin | 5 | 8 (Rat BM) |

BMDM, bone marrow–derived macrophages; DMEM, Dulbecco’s modified Eagle medium; RPMI, Roswell Park Memorial Institute medium.

MATERIALS AND METHODS

Constructs
Lentiviral vector plasmid pLent6-R4-R2-VSDEST (Invitrogen, Paisley, UK) was modified by removal of the attR4-attR2 region with EcoRl and self-ligation. The resultant plasmid was cut with Clal/Hpnl and a polymerase chain reaction (PCR) product encoding central polypurine tract inserted. Two genomic DNA regions containing the FIRE were amplified by PCR and cloned into the XbaI site. The sizes of the genomic DNA containing FIRE were 1,798 bp and 537 bp, respectively. The murine Csf1r promoter (mm10, chr18:61,105,950–61,105,950) was amplified via splice overlap PCR so that the ATG of Csf1r initiates translation of EGFP. The construct used to produce MacGreen mice was used as template DNA.12 The Csf1r promoter:EGFP fragment was cloned into the EcoRl site upstream of FIRE. A construct was also created where mCherry replaced EGFP; mCherry or EGFP was also fused to a myristoylated motif (myr) to localize expression to membranes. The vector containing myr:mCherry39 was a generous gift from Mary E. Dickinson (Baylor College of Medicine, Houston, TX). A list of oligonucleotides used in the construction of these vectors can be found in Table 1.

In summary, we have created a lentiviral vector that drives expression of genes of interest in macrophage lineage cells of species from birds through livestock to humans.
Lentiviral transduction
Cells were cultured in 24-well plates or Lab-Tek II 4-well chamber slides and on the morning of transduction, placed in a minimal volume of media containing polybrene and lentivirus (as specified in more detailed protocols below and in Table 2). Cells were incubated at 37 °C for 3–4 hours before the wells were topped up with 1 ml fresh media.

Lentiviral transduction of cell lines
EL4 and RAW264.7 cells were transduced with 9.2 × 10⁶ transducing units of lentivirus (Figure 2a). Unconcentrated virus was used to transduce RAW264.7 cells in Figure 2c.

FACS analysis of RAW264.7 cells
RAW264.7 cells were harvested and placed in PBS containing 2% fetal calf serum (FCS) and 1 µg/ml propidium iodide (Sigma, Dorset, UK) to exclude dead cells. EGFP expression was analyzed on a FACSCalibur (BD, Oxford, UK).

Microscopy
Cells were imaged via confocal microscopy using either an eC1 (Nikon, Kingston Upon Thames, UK) or LSM710 (Zeiss, Cambridge, UK). Standard light microscopes were also used. For detection of EGFP expression in macrophages from multiple species, GFP gain was initially set using untransduced cells and the same EGFP gain used for all samples.

Lentiviral transduction of rat BM-derived macrophages
All animal care and experimentation was conducted in accordance with guidelines of Roslin Institute and the University of Edinburgh and under Home Office Project License PPL 60/4259. Cryopreserved BM cells from a male Wistar rat were thawed and cultured on bacteriological plates as per Table 2 in media containing 10 U/ml (100 ng/ml) recombinant human CSF1 (rhCSF1), a gift from Chiron, Emeryville, CA. On day 8, 1.9 × 10⁶ cells/well were plated in a 24-well plate and transduced the following day with Csf1rEGFP-FIRE with 4 × 10⁶ transducing units of lentivirus.

Lentiviral transduction of rat BM
BM cells from an 8-week-old male Dark Agouti rat were isolated from femurs by flushing the bones with cell culture media. Red blood cells were removed using lysis buffer (BioLegend, London, UK) according to instructions. Cells were cultured at a concentration of 1 × 10⁶ cells/ml in media (Table 2) containing 10 ng/ml rat IL-3b (Peprotech, London, UK), 100 ng/ml murine stem cell factor, and 50 ng/ml murine IL-6 (Sigma). The following day cells were concentrated back to 1 × 10⁶ cells/ml via centrifugation. On day 2, cells were transduced with 1.2 × 10⁶ transducing units of Csf1rEGFP-FIRE lentivirus. Media was replaced the following day and on day 4, rhCSF1 (10 µg/ml) was added for macrophage differentiation. In experiments where BM was FACs sorted for EGFP expression, cells were harvested as described above and cultured in 15% FCS, penicillin, streptomycin, 50 µmol/l 2-Mercaptoethanol, 2 mmol/l GlutaMAX, 50 ng/ml murine IL-6, 120 ng/ml murine FLT3-L, 50 ng/ml rat thrombopoietin, and 100 ng/ml murine SCF (Sigma). Cells were concentrated to 1 × 10⁶ cells/ml and transduced on day 2 as described above. On day 3, the media was replaced and on day 4 Blastidin (8 µg/ml) was added to select for transduced cells. Cells were cultured for 3 weeks before EGFP positive and negative cells were sorted on a FACSAria III (BD). SYTOX Blue (Invitrogen) was used to exclude dead cells. Following cell sorting, cells were centrifuged and RNA isolated using an RNeasy Mini kit (QIAGEN, Venlo, Netherlands) according to instructions.

Phagocytosis assay
Phagocytosis assays were performed as described on www.macrophages.com. Zymosan A BioParticles labeled with Alexa Fluor 594 (Invitrogen) were used.

cDNA synthesis and PCR
DNA was removed from RNA with Ambion DNA-free and cDNA prepared using SuperScript III reverse transcriptase and random hexamers (Invitrogen). Rat Actb and Csf1r were amplified from EGFP- and EGFP-expressing human tissues with Invitrogen Taq polymerase using the following oligonucleotides. Actb 5′- caaactcttgtagctcct, Actb 3′-ctctagctggttcggga, Csf1r 5′- AGGAAGCTGTGCTGCCCTGT, and Csf1r 3′-CTCCCTGGAATCCCTCACA.

Isolation of primary macrophages from multiple species
Pig BM-derived macrophages, cow, and human monocyte-derived macrophages were derived according to protocols described in ref. 40–42 respectively. Sheep alveolar macrophages were isolated as per ref 43 and cultured in Roswell Park Memorial Institute medium containing 20% sheep serum containing 10 U/ml (100 ng/ml) rhCSF1.

Lentiviral transduction of macrophages from multiple species
All cells were transduced with 2 × 10⁶ transducing units of lentivirus in media containing 8 µg/ml polybrene except for human and pig monocyte-derived macrophages, which were transduced with unconcentrated lentivirus.

CONFLICT OF INTEREST
The authors declared no conflict of interest.

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