Efferocytosis induces a novel SLC program to promote glucose uptake and lactate release

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Development and routine tissue homeostasis require a high turnover of apoptotic cells. These cells are removed by professional and non-professional phagocytes via efferocytosis1. How a phagocyte maintains its homeostasis while coordinating corpse uptake, processing ingested materials and secreting anti-inflammatory mediators is incompletely understood1,2. Here, using RNA sequencing to characterize the transcriptional program of phagocytes actively engulfing apoptotic cells, we identify a genetic signature involving 33 members of the solute carrier (SLC) family of membrane transport proteins, in which expression is specifically modulated during efferocytosis, but not during antibody-mediated phagocytosis. We assessed the functional relevance of these SLCs in efferocytic phagocytes and observed a robust induction of an aerobic glycolysis program, initiated by SLC2A1-mediated glucose uptake, with concurrent suppression of the oxidative phosphorylation program. The different steps of phagocytosis5— that is, ‘smell’ (‘find-me’ signals or sensing factors released by apoptotic cells), ‘taste’ (phagocyte–apoptotic cell contact) and ‘ingestion’ (corpsse internalization)—activated distinct and overlapping sets of genes, including several SLC genes, to promote glycolysis. SLC16A1 was upregulated after corpse uptake, increasing the release of lactate, a natural by-product of aerobic glycolysis5. Whereas glycolysis within phagocytes contributed to actin polymerization and the continued uptake of corpses, lactate released via SLC16A1 promoted the establishment of an anti-inflammatory tissue environment. Collectively, these data reveal a SLC program that is activated during efferocytosis, identify a previously unknown reliance on aerobic glycolysis during apoptotic cell uptake and show that glycolytic by-products of efferocytosis can influence surrounding cells.

To identify the pathways potentially involved in efferocytosis, we performed RNA sequencing of LR73 hamster phagocytes engulfing apoptotic human Jurkat cells (to clearly distinguish phagocyte-derived RNA; Fig. 1a, Extended Data Fig. 1). Efferocytic phagocytes displayed changes in multiple transcriptional programs, including decreased expression of pro-inflammatory genes, increased expression of actin rearrangement/cell motility genes and increased expression of anti-inflammatory genes, consistent with previous findings4,5 (Fig. 1a). In addition, we identified gene programs such as upregulation of glycolysis-associated genes and downregulation of genes required for oxidative phosphorylation (OXPHOS), fatty acid oxidation (FAO) and de novo cholesterol synthesis (Fig. 1a, Supplementary Table 1).

We also noted extensive modulation of genes encoding solute carrier (SLC) proteins. SLCs are plasma membrane proteins, localized in the plasma membrane, mitochondrial membrane and other internal membranes, which facilitate transport of molecules including sugars, nucleotides and amino acids across the membrane6–8. Mutations in approximately 100 of the 400 known SLCs are linked to human disease6–8. In LR73 phagocytes, expression levels of 33 SLCs (out of 165 detected) were modified during efferocytosis: 19 were upregulated and 14 were downregulated (Extended Data Fig. 2, Supplementary Table 2).

We categorized the 33 SLCs on the basis of association with physiological processes, experimentally or by homology (Extended Data Fig. 2b), and constructed an integrated network linking each SLC and its assigned functions with other SLCs modified during efferocytosis (Fig. 1b). All but two SLCs (Slc4a6 and Slc4a4) could be clustered into eight categories: carbohydrate metabolism; intracellular pH regulation; membrane stability and volume regulation; nucleoside salvage; vitamin transport; glycolysis; amino acid transport and catabolism; and OXPHOS and FAO. This analysis also reveals coordinated regulation of SLCs linked to particular physiological function(s): multiple SLCs associated with carbohydrate metabolism were upregulated, whereas SLCs associated with OXPHOS and FAO were downregulated (Fig. 1b).

The changes in SLC expression were confirmed by quantitative reverse transcription with PCR (qRT–PCR) using hamster–specific primers (Extended Data Fig. 3a, b). Thus, efferocytosis induces a specific SLC signature that potentially modulates multiple physiological processes.

Mouse peritoneal macrophages displayed similar modulation of SLCs during efferocytosis, suggesting that professional and non-professional phagocytes shared a similar response (Fig. 2a, Extended Data Fig. 3b and Supplementary Table 3). Notably, macrophages ingesting Jurkat cells coated with CD3 antibody (with comparable phagocytosis), did not show changes in the same SLCs, except for Slc29a2 and Slc29a3, which responded in the opposite direction to the efferocytosis response (Fig. 2a). Thus, in addition to the type of engulfed cell, the type of phagocytic receptor used influences the SLC program in phagocytes. Furthermore, when apoptotic Jurkat cells were injected intraperitoneally into mice, efferocytic CD11bhigh/F4/80high macrophages exhibited similar changes in SLC gene expression to those seen in the in vitro efferocytosis (Fig. 2b).

In a time course of efferocytosis, expression of several SLCs was modulated early (0–4 h), whereas others were upregulated later (after 4 h). Even within a group of SLCs linked to a particular function, we observed early and late modulation of expression. These changes were not strict, and mRNA and protein concentrations exhibited continuous variation through the time course (Extended Data Fig. 4).

Distinct stages or phases in efferocytosis have been identified1,2, including (i) smell phase, in which apoptotic cells and phagocytes communicate via soluble mediators; (ii) taste phase, in which ligand–receptor interactions are established between apoptotic cells and phagocytes; and (iii) ingestion phase, in which corpses are internalized and processed. To identify SLCs that are modulated during the different stages of efferocytosis, we performed and compared RNA-seq analyses...
of LR73 cells treated only with apoptotic cell supernatants with those of phagocytosing LR73 cells treated with cytochalasin D, which allows corpse binding but not internalization (Fig. 2c). These data, using SLC induction as a readout, provide further evidence for ongoing communication between apoptotic cells and phagocytes.

Efferocytosis is an energy-intensive process, as it requires energy for dynamic actin rearrangements to engulf corpses that are often nearly as large as the phagocyte. We focused on SLC2A1 (also known as GLUT1), a glucose transporter that facilitates uptake of glucose from the extracellular medium, as SLC2A1 was strongly upregulated in LR73 cells and macrophages early during efferocytosis (Fig. 2, Extended Data Fig. 4b). First, overexpressing SLC2A1 in LR73 cells increased efferocytosis (Fig. 3a). Second, treatment with STF-31, an inhibitor of SLC2A1, reduced efferocytosis in wild-type and SLC2A1-overexpressing LR73 cells (Fig. 3a). Third, small interfering RNA (siRNA) knockdown of Slc2a1 reduced corpse uptake (Fig. 3b); this effect was rescued by co-transfection with siRNA-resistant Slc2a1 (Fig. 3b). Fourth, CRISPR-Cas9 deletion of Slc2a1 reduced efferocytosis in LR73 cells (Fig. 3c). Fifth, Slc2a1-deficient bone marrow–derived macrophages (BMDMs) displayed decreased efferocytosis (Fig. 3d), but retained normal antibody-mediated phagocytosis (which does not modulate Slc2a1 expression) compared to untreated cells (Extended Data Fig. 5a). Deletion efficiencies for Slc2a1 are shown in Extended Data Fig. 5b–d.

In vivo administration of STF-31 before intraperitoneal injection of apoptotic Jurkat cells decreased efferocytosis by peritoneal macrophages (Fig. 3e), but not phagocytosis of CD3–antibody-coated Jurkat cells (Extended Data Fig. 5e). Moreover, STF-31 did not further reduce efferocytosis in Slc2a1–deficient LR73 cells or BMDMs (Extended Data Fig. 5f), indicating that the effects were specific to SLC2A1. We also tested corpse clearance in the thymus after induction of apoptosis by dexamethasone injection, with or without co-injection of STF-31. There was a modest increase in unclearly or secondarily necrotic thymocytes in response to STF-31 alone, and a significant increase in necrotic thymocytes in response to treatment with both dexamethasone and STF-31 (Fig. 3f, Extended Data Fig. 5g).

To complement the pharmacological approach, we used two genetic approaches. First, using zebrafish expressing GFP in macrophages (Tg(mpeg1:GFP)), we targeted the Slc2a1 orthologue via morpholino oligonucleotide injection. In control morpholino-treated embryos, GFP+ macrophages exhibited numerous phagocytic puncta (containing and/or associated with neutral red), whereas slc2a1a morphant embryos had fewer such associations (Fig. 3g). Quantifying z-stack images, and focusing on macrophages in the trunk region, slc2a1a morphants displayed fewer neutral red+ macrophages and less neutral-red staining per macrophage (Fig. 3g). This also suggested an evolutionarily conserved role for SLC2A1. Second, we tested the Slc2a1 requirement in a mouse model of atherosclerosis, in which defective apoptotic cell clearance can manifest as increased necrotic cores within plaques. Bone marrow transplantation was performed with cells from myeloid-targeted LysM–Cre Slc2a1+/− mice (myeloid differentiation was not affected) into atherosclerosis-prone Ldlr−/− mice. After Western diet feeding (12 weeks), there was a significant increase of the necrotic core area in the aortic roots of mice deficient in Slc2a1 in the myeloid lineage (Fig. 3h). The number of TUNEL-positive nuclei (late apoptotic cells) also significantly increased within necrotic cores, implicating defective corpse clearance (Fig. 3h). Taken together, these results show that SLC2A1 contributes to engulfment of apoptotic cells both in vitro and in vivo.

As SLC2A1 is a glucose transporter, we investigated whether glucose uptake is important for efferocytosis. Switching LR73 cells to glucose-free medium at the initiation of efferocytosis reduced uptake of apoptotic cells (Fig. 4a); conversely, adding exogenous glucose increased uptake of apoptotic cells (Fig. 4a), a phenotype that was attenuated by silencing Slc2a1 (Extended Data Fig. 6a). We also measured glucose uptake directly using the non-metabolizable glucose analogue 2-deoxyglucose (2-DG); 2-DG uptake increased approximately threefold during efferocytosis (Fig. 4b). Further, phagocytes pre-treated with 2-DG showed decreased efferocytosis (Extended Data Fig. 6b). Thus, SLC2A1–mediated glucose uptake is an important step in efferocytosis.

Seahorse analysis of LR73 phagocytes (Fig. 4c) or BMDMs (Extended Data Fig. 6c) showed increased aerobic glycolysis and decreased OXPHOS in engulfing phagocytes (Fig. 4c). Analysis of the RNA-seq data from engulfing phagocytes (Fig. 1a) revealed upregulation of multiple glycolysis genes (Fig. 4d, Extended Data Fig. 6d), with concurrent downregulation of OXPHOS and FAO genes. siRNA-mediated knockdown of PDK1 (Fig. 4e) or PDK4 (Fig. 4f), which promote aerobic glycolysis, or treatment with the pan-PDK inhibitor dichloroacetate (Fig. 4g), resulted in decreased efferocytosis. Notably, when we compared BMDMs treated with dichloroacetate to block aerobic glycolysis with BMDMs treated with rotenone and antimycin A1 to block OXPHOS, we found that PDK inhibition reduced efferocytosis but not antibody-mediated phagocytosis, whereas OXPHOS inhibition reduced antibody-mediated phagocytosis, but not efferocytosis (Extended Data Fig. 7a).

Phosphorylation of SLC2A1 by SGK1 increases the abundance of Slc2a1 mRNA at the plasma membrane during efferocytosis (Fig. 4d, Extended Data Fig. 6d), and targeting SGK1
The isolated CD11b high fraction by qRT–PCR using mouse-specific CD11b low F4/80 low macrophages, and engulfing peritoneal macrophages. SLC expression is shown (left). CypHer5E fluorescence in macrophages after 2 h (bottom left). Quantification of SLC expression in the isolated CD11b high fraction by qRT–PCR using mouse-specific primers (right). In b, right panel, the grey bar for Slc29a2 signifies that there was no increase in expression of this SLC in the presence of apoptotic cells, relative to the control condition. Data represent two replicates with 6 mice per group per experiment. c, Specific SLC signature during different stages of efferocytosis. RNA-seq was performed using mRNA from LR73 necrotic bodies (Fig. 2). Phagocytes frequently ingest multiple apoptotic corpses sequentially. Phagocytic SLC2A1 function was required for uptake of the first corpse; as for continued uptake of further corpses, we treated phagocytes with inhibitors for SLC2A1 or SGK1 at different times during efferocytosis. SLC2A1 function was required for uptake of the first corpse as well as for continued uptake of additional corpses (Extended Data Fig. 7d).

CypHer5E fluorescence in macrophages (Cypher5E+) (bottom left). Quantification of SLC expression in the isolated CD11b high fraction by qRT–PCR using mouse-specific primers (right). In b, right panel, the grey bar for Slc29a2 signifies that there was no increase in expression of this SLC in the presence of apoptotic cells, relative to the control condition. Data represent two replicates with 6 mice per group per experiment. c, Specific SLC signature during different stages of efferocytosis. RNA-seq was performed using mRNA from LR73 cells treated for 4 h with supernatants of apoptotic cells, or LR73 cells treated with cytochalasin D and incubated with apoptotic cells. SLC genes altered by supernatant alone (smell), and cytochalasin D-sensitive SLCs (ingestion) were used to identify SLCs responding to ligand–receptor interactions (taste) (red, upregulated; blue, downregulated). For clarity, SLCs in more than one stage are not shown. In all figures, *P < 0.05, **P < 0.01, ***P < 0.001. with siRNA or the SGK1 inhibitor GSK650394 decreased uptake of apoptotic cells (Fig. 4h, Extended Data Fig. 7b). Further, efferocytic BMDMs from transgenic mice expressing an extracellular Myc tag on SLC2A1 exhibited increased cell-surface expression of Myc–SLC2A1; this effect was also increased by GSK650394 (Extended Data Fig. 7c).

Phagocytes frequently ingest multiple apoptotic corpses sequentially. To address whether SLC2A1 is required for the uptake of the first corpse, or for continued uptake of further corpses, we treated phagocytes with inhibitors for SLC2A1 or SGK1 at different times during efferocytosis. SLC2A1 function was required for uptake of the first corpse as well as for continued uptake of additional corpses (Extended Data Fig. 7d).

Corpse internalization requires substantial actin polymerization, which has been linked to aerobic glycolysis during cell migration. Increased actin polymerization in efferocytic phagocytes (indicated by phalloidin staining) was inhibited by either STF-31 or 2-DG (Fig. 4i). Inhibiting PDK1, which favours aerobic glycolysis, also reduced F-actin formation (Fig. 4i). Thus, SLC2A1-mediated glucose uptake and glucose utilization in aerobic glycolysis contribute to actin polymerization during efferocytosis.

Distinct steps of SLC2A1-dependent aerobic glycolysis in phagocytes were regulated by the smell, taste and ingestion phases of efferocytosis (Fig. 5a–c). Apoptotic supernatant was sufficient to increase Sgk1 expression, but not that of Slc2a1 (Fig. 5a). ATP (a known find-me signal) also increased Sgk1 expression (Extended Data Fig. 8a). Similarly,
binding of apoptotic targets or phosphatidylycerine (PtdSer) liposomes to phagocytes (without internalization) was sufficient to induce Slc2a1 (Fig. 5b). Masking PtdSer on targets (using BA11-DSPE, the PtdSer-binding domains of BA11) reduced upregulation of Slc2a1 (but not that of Sgk1) during efferocytosis (Extended Data Fig. 8b). Therefore, Sgk1—triggered by factors released from apoptotic cells—prepares the phagocytes by increasing abundance of endogenous Slc2a1 on the plasma membrane, and PtdSer-dependent interactions increase new transcription and expression of Slc2a1.

Following corpse internalization in phagocytes, there was increased expression of Slc16a1, a plasma membrane proton-driven monocarboxylate transporter of lactate and pyruvate (Figs. 1b, 2a–c, 5c, Extended Data Fig. 8b). siRNA-mediated knockdown of Slc16a1 in LR73 cells reduced efferocytosis in vitro (Extended Data Fig. 8c), and SR13800—a bioactive inhibitor of Slc16a1—reduced apoptotic cell uptake by peritoneal macrophages in vivo (Extended Data Fig. 8d). Supernatants of engulfing LR73 cells contained threefold-higher lactate concentration (approximately 5 mM) compared to supernatants of phagocytes without engulfing cells (approximately 1.5 mM) (Fig. 5d). siRNA knockdown of Slc16a1 reduced lactate concentration in the supernatants to approximately 2 mM, with concomitant accumulation in phagocytes (Fig. 5d), indicating that Slc16a1 contributes to lactate release from engulfing phagocytes.

Lactate released from solid tumours could act on naive macrophages to induce M2 macrophage-like polarization 1. To test whether factors released via Slc16a1 during efferocytosis might also promote anti-inflammatory skewing of naive macrophages, we tested the effect of supernatants of engulfing LR73 phagocytes on BMDMs (Fig. 5e). These supernatants induced upregulation of anti-inflammatory macrophage genes such as Tgfb1 and Il10 as well as anti-inflammatory or M2-like markers, including Vegfa, Mgl1, Mgl2 and CD206 (also known as Mrcl).
whereas pro-inflammatory markers (Tnf and Il6) were not affected. Slc16a1 knockdown attenuated this effect in engulfing LR73 cells (Fig. 5f, Extended Data Fig. 8e, f). Therefore, aerobic glycolysis induced during efferocytosis affects efferocytosis at two levels: regulation of corpse uptake through regulation of actin polymerization (involving SLC2A1); and modulation of expression of anti-inflammatory genes in neighbouring cells (via SLC16A1).

The results presented here provide several key insights. Although the SLC family is the second largest among membrane proteins (after G-protein–coupled receptors), there is much less knowledge of SLC function in specific physiological contexts. This work describes coordinated regulation of select SLCs during efferocytosis (Fig. 5g). The small, touch and ingestion phases of efferocytosis induce distinct and overlapping sets of SLC genes with functional consequences; these sets of genes are distinct from those that are activated during anti-body-mediated phagocytosis. Efferocytosis induces a metabolic gene program promoting glucose uptake and subsequent glycolysis, with concurrent downregulation of genes linked to OXPHOS and FAO. Although efferocytic macrophages are more M2-like, and M2-like macrophages are reported to be OXPHOS-dependent, our study of the first few hours of efferocytosis does not rule out a role for OXPHOS at later times. Although glycolysis is linked to inflammation, effec-

trocytic phagocytes can influence non-engulfing naive macrophages in the tissue microenvironment towards anti-inflammatory polarization by SLC16A1-mediated lactate release, as well as other factors released during efferocytosis such as TGFβ and IL-10.

**Online content**

Any methods, additional references, Nature Research reporting summaries, source data, statements of data availability and associated accession codes are available at https://doi.org/10.1038/s41586-018-0735-5.

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**Author contributions**

S.M. and J.S.A.P. designed and performed most experiments, with input from K.S.R., M.H.R., C.B.M., V.S., N.O.G., J.C.R., Y.Z., S.K., L.Z., and L.M. performed and/or assisted with specific experiments. S.M., J.S.A.P. and K.S.R. wrote the manuscript with input from co-authors.

**Competing interests**

The authors declare no competing interests.

**Additional information**

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METHODS

The investigators were not blinded to allocation during experiments and outcome assessment.

In vitro engulfment assay. For induction of apoptosis22, human Jurkat T cells resuspended in RPMI with 1% BSA were treated with 150 µM caffeine and 30 µM 2-deoxyglucose (FBS) + 10% fetal bovine serum (FBS) + 1% penicillin–streptomycin–glutamine. Medium was replenished every 2–3 days and cells were harvested according to the manufacturer’s instruction (Invitrogen). When primary macrophages were used as phagocytes, there was an inherent difference in the absolute percentage uptake of corpses between experiments performed on different days. Therefore, phagocytic index was used to better compile data from multiple experiments. All cell lines (except LR73 cells) were obtained from ATCC. LR73 cells were obtained from C. Stanners23. Cells were routinely tested for mycoplasma contamination and all cell lines tested negative.

RNA sequencing. LR73 cells were co-cultured with apoptotic Jurkat cells for 2 h, unbound Jurkat cells were removed by washing with PBS and the phagocytes were rested in culture medium for an additional period of time. Cells were dissociated from the plate with trypsin and the phagocytes were assessed by a flow cytometry-based assay or analysed for RNA or protein24. Phalloidin staining was conducted according to the manufacturer’s instruction (Invitrogen). When primary macrophages were used as phagocytes, there was an inherent difference in the absolute percentage uptake of corpses between experiments performed on different days. Therefore, phagocytic index was used to better compile data from multiple experiments. All cell lines (except LR73 cells) were obtained from ATCC. LR73 cells were obtained from C. Stanners23. Cells were routinely tested for mycoplasma contamination and all cell lines tested negative.

Quantitative gene expression analysis. Total RNA was extracted from cells using the RNeasy Mini Kit (Qiagen) + dexamethasone (Sigma) with manufacturer’s instructions. For staining, BMDMs were stained with F4/80 APC-eFluor 780 (eBioscience, Cat #: 25-0082-82) and F4/80 APC-eFluor 780 (eBioscience, Cat: #47-4801-80), and the uptake of the injected Cy5phTh5R + Ic1b1b-F4/80+mph cells was assessed by flow cytometry. For sequencing or other types of analysis of serum (FBS). The collected cells were stained with CD11b PE-Cy7 (eBioscience, C-1171) before use in the engulfment assays. Chinese hamster LR73 cells or mouse macrophages were seeded in a 24-well plate and incubated with targets at a 1:10 phagocytosetarget ratio for the indicated times. Targets were then washed with PBS. Where indicated, phagocytes were rested in culture medium for an additional period of time. Cells were dissociated from the plate with trypsin and the phagocytes were assessed by a flow cytometry-based assay or analysed for RNA or protein24. Phalloidin staining was conducted according to the manufacturer’s instruction (Invitrogen). When primary macrophages were used as phagocytes, there was an inherent difference in the absolute percentage uptake of corpses between experiments performed on different days. Therefore, phagocytic index was used to better compile data from multiple experiments. All cell lines (except LR73 cells) were obtained from ATCC. LR73 cells were obtained from C. Stanners23. Cells were routinely tested for mycoplasma contamination and all cell lines tested negative.

Network analysis. The network was analysed using analytical software Gephi v.0.9.1 (https://gephi.org/). Standard algorithms for network analysis were used, including: edge betweenness, betweenness centrality, degree centrality, eccentricity, closeness centrality, and other parameters. For each gene, we calculated the degree centrality, which is the number of direct connections (edges) of a node. The nodes with the highest degree centrality were considered the most important nodes in the network.

In vitro bone marrow transplantation and analysis of necrotic core areas in atherosclerosis. At six weeks of age, Ldlr−/− mice were exposed to two doses of X-ray irradiation (500 cGy × 2, 4 h apart; X-RAD), and were then transplanted with bone marrows isolated from Slc2a1a5/5 or Lsd1−/−/Slc2a1a5/5 donor mice. Control animals were transplanted with HNR buffer only—these mice died within 10–11 days of lethal irradiation. Following bone marrow transplantation, chimeric Ldlr−/− mice were transferred to sterile cages with ad libitum access to sterile mouse chow and sterile water and were maintained on chow diet for 4 weeks before challenge with Western Diet (Harlan Teklad TD88137, 42% of Cal from milk fat with 0.15% added cholesterol) for 12 weeks. Following mice dissection, hearts were isolated for formalin-fixed paraffin-embedded sections. The sections were stained with Masson’s trichrome for quantification of necrotic core areas and normalized to total quantified area. Necrotic core size was measured using Aperio ePathology software (Aperio), TUNEL staining was conducted according to the manufacturer’s instructions (Promega).

In vivo phagocytosis analysis in zebrafish. To inhibit the expression of slc2a1a, antisense morpholinos targeting the translational start site of the zebrafish slc2a1a (5′-GGCCCATCATACGTGACTGTGACACC-3′) were synthesized (Gene Tools). A control morpholino (5′-GGCCCATCATACGTGACTGTGACACC-3′) was used as anegetic control. Morpholino (2.5 ng) was microinjected into Tg(mpeg1:GFP) embryos at the one-cell stage. Embryos were treated with phenylthiooleic acid (0.004%) in egg water at 24 hpf to reduce pigmentation as per standard protocols. 8–10 h later, imaging (embryos were imaged soaked in 2.5 µg/ml neutral red in egg water. At 50 hpf, embryos were anaesthetized with 3-amino-benzoic acid ester (Tricaine), immersed in 0.8% low-melting-point agarose and mounted laterally in glass-bottomed 35-mm Petri dishes for confocal imaging. Three identical z-stack images were taken for each embryo covering hemi-segments of somites 6 to 20. More than 100 macrophages were counted per group for the evaluation of GFP and neutral red colocalization.

CRISPR–Cas9 deletion or siRNA knockdown of SLCA. Stable, individual clones of Cas9–GFP expressing LR73 cells were generated by lentilCas9–EGFP plasmid lenti viral transduction and a protocol adapted from ref. 26, followed by single-cell cloning of GFP-expressing cells and Cas9 expression verification. Slc2a1a was deleted from LR73 cells using two independent Cas9–GFP LR73 cell clones and using lentilGuide-Puro sgRNA plasmid with two unique guides for Slc2a1a. LentilCas9–EGFP was a gift from P. Sharp and F. Zhang (Addgene plasmid # 63952) and lentilGuide-Puro was a gift from E. Zhang (Addgene plasmid # 52963).

Guide RNAs targeting Slc2a1a were generated using the following oligonucleotide pairs. Guide 1: 5′-CAGCGGTTCCTGCGGATCATCCG-3′, 5′-CTTAA GAAAGCCCTTAGTAGGGACA-5′; guide 2: 5′-CACCGTCGGCC GCGTGGGCG-3′, 5′-CGGATTCCACGGGCGTGGGCG-3′. For siRNA and plasmid transduction experiments, LR73 cells were treated with Lipofectamine 2000 (Thermo Fisher) with specific siRNAs, according to the manufacturer’s instructions, 2 days before the engulfment assay. GLUT1–eGFP/pDNA-DEST47 was a gift from W. Frommer (Addgene plasmid #18729)27. siRNAs targeting hamster mRNAs were customized by GE Healthcare Pharmaco. Primers for siRNA against Slc2a1a: 5′-CCAGGAAGUGGUGCAGAAGAAUU-3′. Two siRNAs against Slc2a1a: 5′-CUUCAUGGUGUCGAGAAUU-3′, 5′-CU GCAGAAGAGAGCAAGAAUU-3′. Two siRNAs against Sdc1a: 5′-CGA CAAGAGUUGGGCUUAAU-3′, 5′-GGCAAAAGGUGGAAAGAAU-3′. Two siRNAs against Pdk1: 5′-UCACAGAGAAGGAUAAGAAUU-3′, 5′-CAUCAAGGGUGGAAAAGAAU-3′, 5′-CAUCAAGGGUGGAAAAGAAU-3′, siRNA against Slc16a1: 5′-AGAA CAGGAAGUGGUAAGAAUU-3′.

Macrophage isolation and analysis. To obtain BMDMs, femurs from control mice, mice crossing foxed alleles of Slc2a1a, or Glut1–Myc knock–in mice were removed and flushed with 3 ml sterile PBS containing 5% FBS26,27. The cell suspension was centrifuged, treated with red blood cell lysis buffer, washed and then plated onto sterile Petri dishes in DMEM containing 10% 1:29 medium, 10% FBS and 1% penicillin–streptomycin–glutamine. Medium was replenished every 2–3 days and differentiated cells were used at day 6 post-collection. To delete Slc2a1a, macrophage cultures were treated with TAT-Cre (EMD Millipore), according to the manufacturer’s instructions. For staining, BMDMs were stained with F4/80 APC-eFluor 780 (eBioscience, Cat: #47-4801-80) and subsequently stained with Myc PerCP antibody (Novus Biologicals, 9E10 Cat: #NB600-302PCP) or fixed and permeabilized using Foxp3/Transcription Factor Staining Buffer Set (eBioscience), and intracellular staining was performed using CD206 PE (Biologicent, Cat: #141706). Resident peritoneal macrophages were obtained by flushing the peritoneal cavity of mice with 1 ml cold FBS, thinning the peritoneum down, resuspended in X-VIVO 10 (Lonza), and plated at a concentration of 5 × 105 cells per well. Floating cells were removed the next day, and remaining peritoneal macrophages were used 2 days after isolation.
Glucose uptake assay. LR73 cells were incubated with apoptotic Jurkat cells for 2 h, washed 3 times with PBS and incubated with 10 mM 2-DG, a glucose analogue, in glucose-free medium for 30 min. Following incubation, cells were washed 3 times with PBS and lysed with Extraction Buffer (Sigma Cat#: MAK083). Lysate was frozen–thawed in dry ice/ethanol, and then heated at 85 °C for 40 min. Lysate was then cooled on ice for 5 min and then neutralized by Neutralization Buffer (Sigma Cat#: MAK083). Samples were spun down at 13,000g to remove insoluble fraction and then diluted tenfold by adding Assay Buffer (Sigma Cat#: MAK083). Using the lysate, glucose uptake was measured using Glucose Uptake Colorimetric Assay Kit (Sigma). 2-DG is taken up by cells and phosphorylated by hexokinase to 2-DG6P. 2-DG6P cannot be further metabolized and accumulates in cells, directly proportional to the glucose uptake by cells. 2-DG uptake is determined by a coupled enzymatic assay in which the 2-DG6P is oxidized, resulting in the generation of NADPH, which is then determined by a recycling amplification reaction in which the NADPH is used by glutathione reductase in a coupled enzymatic reaction that produces glutathione. Glutathione reacts with DTNB to produce TNB, which was detected at 412 nm as per the manufacturer’s recommendations.

Seahorse analysis. LR73 cells or BMDMs were seeded into a Seahorse 24-well tissue culture plate (Agilent Technologies). The cells adhered overnight before treatment. For assessing respiratory capacity, cells were subjected to a mitochondrial stress test. In brief, at the beginning of the assay, the medium was changed to DMEM containing pyruvate (Thermo Fisher Cat#:12800017, pH = 7.35 at 37 °C) and cells were allowed to equilibrate for 30 min. OCR was measured using a Seahorse XF24 Flux Analyzer (Agilent Technologies). After three basal OCR measurements, the drugs of interest were injected into the plate and OCR was measured using four-minute measurement periods. Compounds to modulate cellular respiration (1 µM oligomycin (Sigma-Aldrich); 2 µM BAM15 (Cayman Chemical Company); 1 µM antimycin A and 100 nM rotenone (Sigma-Aldrich)) were injected after every three measurements. Basal respiration was calculated by subtracting the average of the first three measurements by the average of the post-antimycin A and rotenone measurements. Maximum respiratory capacity was calculated by subtracting the average of the post-BAM15 measurements by the post-antimycin A and rotenone measurements. The reserve capacity was calculated by subtracting the average of the basal measurements from the average of the post-BAM15 measurements.

For assessing glycolytic capacity, the cells were subjected to a glycolytic stress test. In brief, ECAR—a measurement of lactate export—was measured using a Seahorse XF24 Flux Analyzer. Cells were seeded into a Seahorse 24-well tissue culture plate. At the beginning of the assay, the medium was changed to unbuffered, glucose-free DMEM (Sigma-Aldrich Cat# D5030, pH 7.35 at 37 °C), supplemented with 143 mM NaCl and 2 mM glutamine. After three basal ECAR measurements, the drugs of interest were injected into the plate and ECAR was measured using 3-min measurement periods. Compounds to modulate glycolysis (20 mM glucose; 1 µM oligomycin; 80 mM 2-DG) (Sigma) were injected after every three measurements. Basal glycolysis was calculated by subtracting the average of the post-2-DG measurements from the average of the post-glucose measurements. Maximum glycolytic capacity was calculated by subtracting the average of the post-2-DG measurements from the average of the post-oligomycin measurements. The glycolytic reserve capacity was calculated by subtracting the average of the post-oligomycin measurements from the average of the post-glucose measurements.

Liposome construction. Liposomes were prepared by dissolving the lipids (phosphatidylethanolamine, dioleoyl phosphatidylcholine, cholesterol and the lipid DiD dye) in chloroform, evaporating chloroform under flow of argon gas in a glass vial and subjecting the lipid layer to overnight lyophilization to remove traces of organic solvent. Normal saline was then added for hydration, and after vortexing was done to prepare multimellar vesicles. Particle size was verified by dynamic light scattering using Nicomp 370.

Determination of lactate concentration. The lactate concentration was measured using a Lactate Assay Kit (Sigma) according to the manufacturer’s instructions. The mean values ± s.d. of the lactate concentration in the medium and cells were calculated for each condition.

Immunoblotting. LR73 cells were seeded in a 100-mm dish at a concentration of 2 million cells per dish. Apoptotic Jurkat cells were added as indicated. Cells were lysed in RIPA buffer and immunoblotted with SLC2A1 (Abcam #ab652), SLC16A1 (LSBio LS-C353287), SLC2A2 (Cell Signaling Technology #14581) and total Erk2 (Santa Cruz Biotechnology, sc-154-G) antibodies in Can Get Signal solution (TOYOBO Cat# NKB-101) followed by chemiluminescence detection. Specific bands were quantified using Adobe Photoshop CS6.

Research animals. Power of 80% was used to determine the number of mice needed to achieve a two-sided 5% significance level to detect a twofold change for each set of in vivo studies. Allocation of mice was random in all in vivo experiments. Mice were taken from littermates. Animal breeding and experiments were performed in a specific pathogen-free animal facility using protocols approved by the University of Virginia Animal Studies Committee. Ethical guidelines determined by the Institutional Animal Care and Use Committee were followed in all experiments performed in this manuscript.

Statistical analysis. Statistical significance was determined using GraphPad Prism 7, using unpaired Student’s two-tailed t-test, one-way ANOVA or two-way ANOVA, according to test requirements. Grubbs’ outlier test was used to determine outliers, which were excluded from final analysis. *P < 0.05, **P < 0.01, ***P < 0.001 were considered significant. Number of replicates and repeats of individual experiments and statistical tests used are shown in Supplementary Table 4.

Code availability. All code used in this manuscript can be accessed from the Github repository https://github.com/perrysj/Perry-R.

Reporting Summary. Further information on research design is available in the Nature Research Reporting Summary linked to this paper.

Data availability
RNA sequencing data presented in this study have been deposited in the NCBI GEO repository under the accession number GSE119273.
Extended Data Fig. 1 | RNA preparation for RNA-seq experiments.
a. Representative fluorescence-activated cell sorting plots of engulfment assays with LR73 hamster phagocytes (left) and annexin V/7-AAD staining of apoptotic human Jurkat cells (right) in conditions matching experiments performed for RNA-seq (2 h with apoptotic cells followed by 2 h rest in the absence of apoptotic cells). b. Principal component analysis on hamster-genome-aligned RNA-seq data as a quality control statistic.
Extended Data Fig. 2 | Regulation of SLC expression during efferocytosis. a, SLC genes are differentially regulated during efferocytosis. Left, plot of the 165 SLC genes detected by RNA-seq of efferocytic LR73 cells, highlighting the 19 significantly upregulated (red) and 14 downregulated (blue) SLC genes that were altered during efferocytosis. The 132 SLC genes that were not altered are located on the midline (black). Right, the current genetic classifications of these 33 SLC genes that are altered during engulfment are shown. b, Efferocytosis-associated SLCs and their properties. Current genetic classification and/or functional linkages of the 33 SLCs modulated during apoptotic cell engulfment. The significantly upregulated and downregulated SLCs and the substrates they are known to transport grouped by predicted general function are shown, as are the known monogenic diseases and single nucleotide polymorphism (SNP) or disease phenotype to which the specific SLCs have been linked.
Extended Data Fig. 3 | qRT–PCR confirmation of the RNA-seq data.

a. qRT–PCR of mRNA of specific SLCs during efferocytosis. Indicated SLC genes were tested for mRNA expression levels during engulfment assays performed similarly to those in Fig. 1a. Data are representative of at least two independent experiments with 3–4 replicates per condition.

b. The table presents the cycle numbers for each species-specific qRT–PCR primer. None of these primers produced signals when tested against human Jurkat cell mRNA (target) alone.
Extended Data Fig. 4 | See next page for caption.
**Extended Data Fig. 4 | Dynamic expression of SLCs during efferocytosis.**

*a*, Schematic of the experiment and time points when RNA from phagocytes was assessed for specific SLC gene expression. Apoptotic Jurkat cells were added to LR73 cells and co-cultured for 2 h. Unbound and floating apoptotic cells were then washed away, and the LR73 cells were cultured in fresh medium for the indicated times. The time scale bar reflects total time of experiment, such that the 4-h time point reflects 2 h with apoptotic cells plus 2 h subsequent incubation (to match the timeframe used in our RNA-seq experiment). Total RNA was subsequently isolated and qRT–PCR was performed for specific SLC genes. Flow cytometry plots indicate that fluorescent signals from the internalized corpses are significantly degraded by the 8 h time point. *b*, Expression of SLC genes is regulated over the time course of efferocytosis. Relative expression of mRNAs for specific SLC genes belonging to different functional classes over the time course of engulfment is shown. Data are representative of three biological replicates. *c*, Immunoblotting for some of the SLCs modified during efferocytosis. Indicated SLCs were probed at various time points after addition of apoptotic cells. Relative intensities of specific bands, normalized to ERK2, are shown below representative blots. *d*, Immunoblotting for the some of the SLCs in LR73 phagocytes and apoptotic Jurkat cells.
Extended Data Fig. 5 | See next page for caption.
Extended Data Fig. 5 | The role of SLC2A1 in efferocytosis. a, Slc2a1

BMDMs were treated with or without TAT-Cre to delete Slc2a1. The cells were then incubated with IgG-coated Jurkat cells and engulfment was assessed by CypHer5E signal within BMDMs. The uptake by control BMDMs (not treated with TAT-Cre, and denoted wild type (WT)) was set to 1. b, siRNA targeting of Slc2a1 downregulates SLC2A1 protein expression. Representative western blots from siRNA knockdown of Slc2a1 versus scrambled siRNA in LR73 cells are shown. LR73 cells expressing siRNA-resistant SLC2A1 are also shown. c, Slc2a1 deletion efficiency in Cas9 LR73 cells. Slc2a1 guide was introduced into Cas9–EGFP+ LR73 cell clones. The efficiency of Slc2a1 deletion was quantified using qRT–PCR. d, Introduction of TAT-Cre into Slc2a1fl/fl BMDMs efficiently knocks down SLC2A1 protein expression. Slc2a1fl/fl bone marrow cells were treated with recombinant TAT-Cre during macrophage differentiation after isolation from the bone marrow. e, STF-31 did not affect antibody-mediated phagocytosis by peritoneal macrophages. C57BL/6 mice were intraperitoneally injected with 10 mg kg⁻¹ of either STF-31 in X-VIVO medium 1 h before injection of IgG-coated Jurkat cells. CypHer5E-labelled Jurkat cells were injected intraperitoneally along with the drug. Mice were euthanized 1 h later, peritoneal cells were collected, and apoptotic cell engulfment by CD11b⁺F4/80high macrophages was analysed by fluorescence-activated cell sorting. f, Slc2a1-deficient LR73 cells or BMDMs were treated with STF-31, and the engulfment assay was conducted using CypHer5E-labelled apoptotic Jurkat cells. CypHer5E⁺ phagocytic cells after 2 h of incubation were identified by flow cytometry. n.s., not significant. Data are representative of at least two independent experiments with 3–4 replicates per condition. g, The SLC2A1 inhibitor STF-31 does not increase the number of thymocytes stained with 7-aminoactinomycin D (7AAD⁺) in vitro. Isolated thymocytes were incubated with dexamethasone (10 µM) with or without STF-31 (2 mM). Four hours later, the cell death of the thymocytes was indicated by annexin7⁻7AAD⁺. Data are representative of two independent experiments.
Extended Data Fig. 6 | The role of glycolytic genes in efferocytosis.

a, The effect of physiological (1 mg ml\(^{-1}\)) or high (5 mg ml\(^{-1}\)) glucose on apoptotic cell engulfment (2 h) in control and Slc2a1-siRNA-treated LR73 cells. Note that the enhanced engulfment due to higher glucose concentration is lost in siRNA-treated conditions. Data are representative of at least three independent experiments with 3–4 replicates per condition.

b, Apoptotic cell engulfment by LR73 cells in the presence of the glucose analogue 2-DG (10 mM). Data are representative of two independent experiments with 2–3 replicates per condition.

c, BMDMs undergo glycolytic flux during apoptotic cell clearance. Glycolytic flux and OXPHOS were measured during engulfment assays using Seahorse XF to assess ECAR (left) and OCR (right), respectively. Data are mean ± s.d. for ECAR and OCR over the course of standard glycolytic flux and cellular respiration tests. Data are representative of four replicates per condition.

d, Genes within the glycolytic pathway that are significantly upregulated during apoptotic cell clearance. A schematic of the glycolytic pathway and subsequent steps is shown, with enzymes that are significantly upregulated (determined via RNA-seq) indicated in red.

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**Extended Data Fig. 7 | Testing SGK1 and glycolysis in efferocytosis.**

**a.** Differential metabolic requirements of macrophages for efferocytosis versus antibody-mediated phagocytosis. BMDMs were co-cultured with apoptotic or antibody-coated Jurkat cells. Mitochondrial respiration was inhibited by addition of the mitochondrial complex I inhibitor rotenone (200 nM), the mitochondrial complex III inhibitor antimycin A1 (1 µM), or both (R + A). Aerobic glycolysis was inhibited by the addition of the pan-PDK inhibitor dichloroacetate (1 mM). Data are representative of three independent experiments.

**b.** *In vitro* efferocytosis with SGK1 inhibitor

**c.** Cell surface SLC2A1 expression

**d.** SLC2A1 is required for continued uptake of corpses

SGK1 is required for continued uptake of corpses

with or without the SGK1 inhibitor GSK650394 (5 µM) for 2 h, unbound apoptotic cells were washed away and the cell-surface expression of SLC2A1 was measured by flow cytometry after staining for surface Myc tag. Data are representative of at least two independent experiments.

Continued uptake of apoptotic thymocytes was determined by the MFI (indicative of corpse-derived signal per phagocyte) of LR73 phagocytes over a time course of engulfment. SLC2A1 or SGK1 inhibitors were added at the beginning of engulfment (left of each pair of graphs) or 1 h post-apoptotic cell addition (right of each pair of graphs). Data are representative of at least three independent experiments with 3–4 replicates per condition.
Extended Data Fig. 8 | See next page for caption.
Extended Data Fig. 8 | Testing SLC16A1 in efferocytosis. a, qRT–PCR determination of Sgk1 expression in phagocytes treated with ATP. LR73 cells were treated with indicated amounts of ATP for 4 h. Expression of Sgk1 was determined by qRT–PCR using hamster-specific primers. Data are representative of at least two independent experiments with 3–4 replicates per condition. b, qRT–PCR determination of SLC2A1, SLC16A1 and Sgk1 expression in phagocytes after addition of the PtdSer-masking peptide (GST–TSR) during efferocytosis. Apoptotic cells (AC) were added with or without TSR peptide (10 ng µl⁻¹) for 4 h. Expression of indicated genes was determined by qRT–PCR using hamster-specific primers. Data are representative of at least two independent experiments with 3–4 replicates per condition. c, SLC16A1 inhibition blocks efferocytosis in vitro. LR73 cells were treated with SLC16A1 siRNA and uptake of CypHer5E-labelled apoptotic Jurkat cells was assessed. d, SLC16A1 inhibitor SR13800 dampens efferocytosis by peritoneal macrophages. C57BL/6 mice were injected intraperitoneally with SR13800 (10 mg kg⁻¹) in X-VIVO medium 1 h before injection of apoptotic cells. CypHer5E-labelled apoptotic Jurkat cells were injected intraperitoneally. After 1 h, apoptotic cell engulfment by CD11b⁺ F4/80high peritoneal macrophages was analysed by flow cytometry. Data are representative of two independent experiments with at least six mice in each group per experiment. e, f, Supernatants were prepared from LR73 cells, treated with control or SLC16A1 siRNA, that were engulfing apoptotic cells. The supernatants were added to BMDMs and incubated for 12 h. e, Expression of inflammatory markers was determined by qRT–PCR. f, After 24 h of incubation, expression of CD206 and F4/80 was determined by flow cytometry. Data are representative of two independent experiments with 2–3 replicates per condition.
Reporting Summary

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For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes

Estimates of effect sizes (e.g. Cohen's d, Pearson's r), indicating how they were calculated

Clearly defined error bars

State explicitly what error bars represent (e.g. SD, SE, CI)

Our web collection on statistics for biologists may be useful.

Software and code

Policy information about availability of computer code

Data collection

- SteoOne Software v2.3, BD FACSDiva V8.0

Data analysis

- GraphPad Prism v.6 and v.7, SPSS v.22, Photoshop CS6, R v3.3.2, Gephi v.0.8.2 beta, FlowJo v.8 Mac and v.10 PC.

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Data Availability

RNA sequencing data presented in this study have been deposited in NCBI GEO repository under the accession #GSE119273.
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Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

| Sample size | No statistical tests were used to predetermine sample size. For in vivo experiments, sample sizes were determined based on the numbers required to achieve statistical significance using non-parametric statistics. |
| Data exclusions | Statistical tests for outliers are routinely performed, Grubbs’ test for outliers was used for excluding outliers. |
| Replication | Consistent results obtained from at least two or three biological replicates with more than two technical replicates per experiment were used in the manuscript. A significant number of the experiments used 3-4 biological replicates and 3-4 technical replicates. |
| Randomization | Allocation of mice was random in all in vivo experiments, taken from littermates. |
| Blinding | The investigators were not blinded to allocation during the experiments and outcome assessment. All experiments required known injections of substances, including apoptotic cells, inhibitors, etc. Therefore, it was not possible to blind the investigator for such experiments. |

Reporting for specific materials, systems and methods

| Materials & experimental systems |
| Involved in the study |
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| Unique biological materials |
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| Palaeontology |
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| Methods |
| Involved in the study |
| n/a |
| ChIP-seq |
| Flow cytometry |
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Flow Cytometry

Plots

Confirm that:
- The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).
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Methodology

Sample preparation

Reported under Methods, in "In vivo engulfment assay and quantitative RT-PCR", "In vivo engulfment assay and quantitative RT-PCR", "In vivo thymus efferocytosis assay" and "Macrophages isolation and analysis". Peritoneal exudates were obtained via cold PBS flush. Thymocytes were obtained by gentle mechanical disruption. Bone marrow cells for BMDMs was obtained by flushing tibia and fibia using serum-complete media. All samples were filtered prior to staining. Samples were kept on ice during staining and collection. All fluorescent antibodies were aliquotted in a sterile hood with minimal light exposure. Staining of samples were protected from light throughout.
Data were collected on a FACS Canto I (Becton Dickinson).

Data were collected analyzed with FlowJo v8 and v10 (Treestar, Inc).

Purity of isolated samples was obtained by antibody stain and FACS. Sample purity was greater than 95% in all experiments.

Standard lymphocyte gates were applied, following by doublet exclusion using FSC-HxW and SSC-HxW. In our studies, macrophages were gated using a combination of CD11b and F4/80. For in vitro experiments, all phagocytes were labeled with GFP or CFSE, whereas all apoptotic cells were labeled with a reporter dye such as CypherSE or TAMRA. Singlets were similarly gated for in vitro experiments prior to analysis of GFP+/CFSE+, CypherSE+/TAMRA+ cells.

Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.