Supporting Information

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SI Materials and Methods

Generation and Maintenance of Macrophage-Specific *Slc39a10*-Knockout Mice and *p53*-Knockout Mice. Gene targeting was performed in embryonic stem cells to delete exon 3 of the endogenous *Slc39a10* locus (Fig. S1), and $Slc39a10^{fl/+}$ offspring were generated. $Slc39a10^{fl/+}$ mice were then backcrossed to the C57BL6/J background for more than eight generations. The resulting $Slc39a10^{fl/+}$ mice were bred with LysM-Cre transgenic mice to generate $Slc39a10^{fl/fl}$;$LysM\text{-}Cre^{+}$ mice, thereby selectively knocking out *Slc39a10* expression in myeloid cells. *p53* floxed mice on the C57BL6/J background were obtained from Kwok-Kin Wong, Dana-Farber Cancer Institute, Harvard Medical School, Boston. All mice were housed under a 12-h/12-h light/dark cycle in a specific pathogen-free facility and were fed a purified AIN-76A diet (Research Diets, Inc.). All animal experiments were approved by the Institutional Animal Care and Use Committee of Zhejiang University.

PCR Genotyping of $Slc39a10^{fl/fl}$ and $p53^{fl/fl}$ Mice. The following primer pair was used to genotype the $Slc39a10^{fl/fl}$ mice: forward: TGTATCAGATCGACAGCAGACTT; reverse: CATGAAGCCAGAACCTGCTA. The following primer pair was used to genotype the $p53^{fl/fl}$ mice: forward: CACAAAAACAGGTTAAACCCAG; reverse: AGCACATAGGAGGCAGAGAC.

LPS-Induced Inflammation Model. LPS (2.5 mg/kg) and D-galactosamine (0.25 g/kg) were injected i.p. to induce acute inflammation. Except where indicated otherwise, all experiments carried out after LPS stimulation were conducted 6 h after LPS injection.

***E. coli* Infection Model and Bacterial Growth Measurements.** Mice were given an i.p. injection of TOP10 strain *E. coli* (42) (5×10^7 cfu), and the survival was plotted for 60 h. Organ/peritoneal cavity cfus were determined by plating dilutions of organ homogenates or peritoneal lavage fluid on LB plates.

Antibodies and Reagents. Anti-p53 (no. 2524), anti-p53-488 (no. 2015), anti-AIF (no. 4642), MitoTracker Red (no. 9082), anti-MLKL (no. 28640), anti-LC3 (no. 4108s), and anti-cleaved caspase-3 (no. 9664) were purchased from Cell Signaling Technology. Anti–caspase-1 p10 (no. sc-514) was purchased from Santa Cruz Biotechnology. Anti–F4/80-FITC, anti–F4/80-PE, anti–CD11b-APC, anti–CD11b-FITC, anti–Ly6C-PEcy7, anti–BrdU-FITC, anti–CD11c-APC, anti–CD206-APC, and mouse IgG1 isotype-FITC were purchased from BD Pharmingen. Anti-F4/80 (no. ab111101) was purchased from Abcam. C11-BODIPY (no. 581/591) was purchased from Invitrogen. PFTα (no. S2929) was purchased from Selleck Chemicals. LPS, D-galactosamine, $ZnCl_2$, TPEN, BrdU, ferrostatin-1, necrostatin-1, Z-VAD-FMK, and 3-MA were purchased from Sigma-Aldrich.

Cytokine Analysis. Serum levels of TNF-α, IL-1β, IL-6, MCP-1, IFN-β, and IFN-γ were measured using ELISA (R&D Systems).

Serum ALT and AST. Serum ALT and AST levels were measured using an alanine aminotransferase reagent kit and an aspartate aminotransferase regent kit, respectively (Shensuoyoufu).

Collection and Culture of PMs and BMDMs. Macrophages were maintained in complete DMEM supplemented with 10% (vol/vol) FBS and penicillin/streptomycin (100 U/mL). To obtain PMs, mice were injected i.p. with 1 mL sterile 4% thioglycollate medium; 60 h later, PMs were harvested. BMDMs were generated by differentiating bone marrow in L929-conditioned DMEM for 7 d, with two medium changes during the 7 d in culture.

Cell-Viability Assay. Cell viability was measured using the Cell Counting Kit-8 viability assay (Sigma-Aldrich).

H&E Staining and Immunohistochemistry. H&E staining and immunohistochemical staining of F4/80 were performed as previously described (43). The images in all H&E and immunohistochemical staining are representative of three or more independent experiments.

Immune Cell Classification. The numbers of monocytes, neutrophils, and lymphocytes in the blood were analyzed using a Sysmex KX-21N Automated Hematology Analyzer (Sysmex America, Inc.).

Zn Uptake Measurements Using FluoZin-3 AM. BMDMs were incubated for 1 h in PBS containing 1 μM FluoZin-3 AM (Molecular Probes; Life Technologies) and then were washed once with PBS. Fluorescence intensity (494 mm excitation/516 nm emission) was measured at 1-min intervals for 20 min using a microplate reader (SpectraMax; Molecular Devices). Zn (10 μM) was added at the 4-min time point, and TPEN (10 μM) was added at the 15-min time point.

ICP-MS Analysis. The protein concentration in each sample was measured, and each sample was diluted to 2 mg/mL protein. Next, HNO_3 was used to digest 200 μL cells, followed by ICP-MS. All measured values were corrected by subtracting the value measured with medium (HNO_3). Digested samples were measured using a 7700× ICP-MS device (Agilent Technologies) equipped with an ASX-520 autosampler (Agilent Technologies). For each element analyzed, a five-point calibration curve, including a blank, was established with the highest standard being 0.2 ppm.

FACS Analysis. Cells were suspended in PBS and stained with the appropriate antibodies. Approximately 10^4 cells were collected using a Cytomics FC 500 MCL flow cytometer (Beckman Coulter, Inc.) and were analyzed using FlowJo software (TreeStar, Inc.).

Real-Time qRT-PCR. Total RNA was isolated from cells or tissues using TRIzol (Invitrogen) and reverse-transcribed into cDNA using the PrimeScript RT kit (Takara). The sequences of the primers used are provided in Table S2. Real-time PCR was performed using the two-step qRT-PCR method (Bio-Rad). The level of each amplified target gene was normalized to the respective *Hprt* mRNA level.

Western Blot Analysis. Samples were prepared and subjected to Western blot analysis as described previously (43).

Apoptosis Analysis. Apoptosis was measured using the FITC Annexin-V Apoptosis Detection Kit with PI (Sony Biotechnology, Inc.) in accordance with the manufacturer's instructions.

Phagocytosis Assay and Macrophage-Killing Assays. For the phagocytosis assay, thioglycollate-stimulated peritoneal macrophages were incubated with Alexa Fluor 488-conjugated heat-killed *E. coli* (Molecular Probes) for 1 h. Extracellular fluorescence was then quenched by adding 100 μL of Trypan Blue. The cells then were washed in cold PBS and examined using flow cytometry (FACS analysis). For the killing assay, thioglycollate-stimulated peritoneal macrophages were incubated with TOP10 strain *E. coli*

for 20 min. The macrophages were then washed with PBS (to remove the bacteria), incubated in 100 μg/mL gentamycin in DMEM for 20 min (to kill extracellular bacteria), and then incubated for an additional 120 min in 25 μg/mL gentamycin in DMEM. The cfu values were then measured from macrophage lysates.

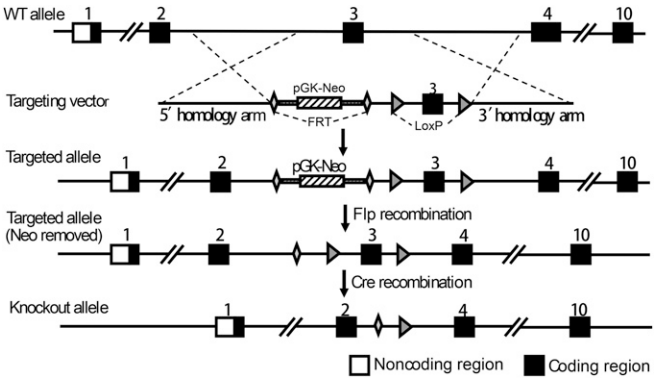

Fig. S1. Targeting strategy for generating macrophage-specific *Slc39a10*-knockout mice. The targeting vector was designed to excise exon 3 of *Slc39a10* by inserting a LoxP sequence and *Neo* cassette into the region between exons 2 and 4. Crossing *Slc39a10*$^{fl/fl}$ mice with the LysM-Cre mouse deletes the *Neo* cassette and produces *Slc39a10*$^{fl/fl}$;*LysM-Cre*$^{+}$ offspring.

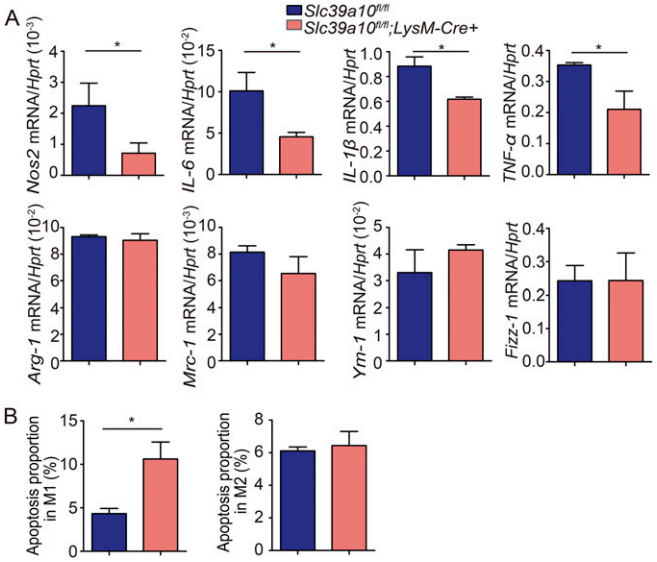

Fig. S2. The biomarker levels and the apoptosis status of M1 and M2 macrophages in *Slc39a10*$^{fl/fl}$;*LysM-Cre*$^{+}$ and control mice. (*A*) The respective markers for M1 (Nos2, Il-6, Il-1β, and Tnf-α) and M2 (Arg-1, Mrc-1, Ym-1, and Fizz-1) macrophages were measured in the PMs of mice following LPS stimulation ($n = 3$ mice per group). (*B*) The percentages of apoptotic M1 macrophages (CD11C^{+}F4/80^{+}CD11b^{+}) and M2 macrophages (CD206^{+}F4/80^{+}CD11b^{+}) in LPS-stimulated *Slc39a10*$^{fl/fl}$;*LysM-Cre*$^{+}$ and control mice ($n = 4$ mice per group). Student's *t* test was used to compare two groups. *$P < 0.05$.

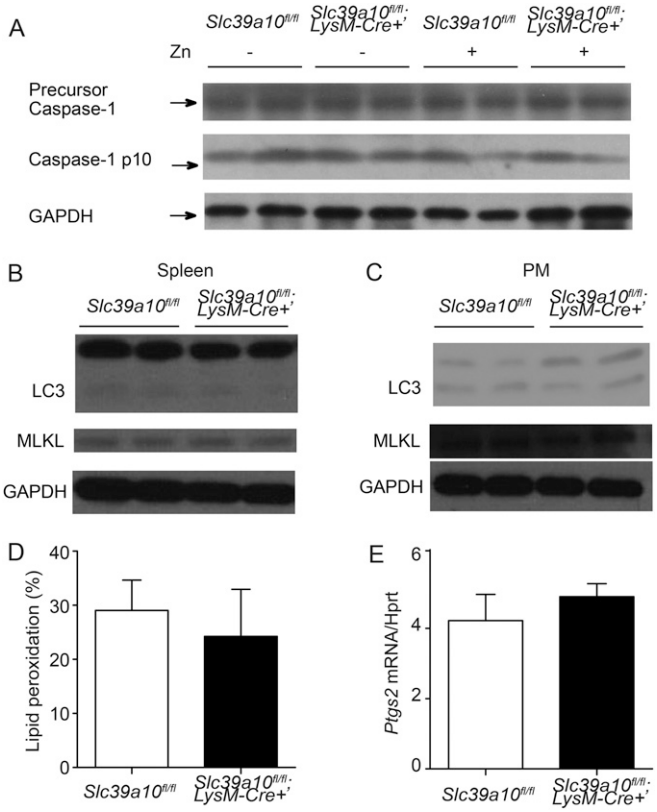

Fig. S3. The expression of markers of pyroptosis, autophagy, and ferroptosis are similar in *Slc39a10*$^{fl/fl}$;*LysM-Cre*$^{+}$ and control mice following LPS stimulation. (*A*) PMs were isolated from *Slc39a10*$^{fl/fl}$;*LysM-Cre*$^{+}$ and control mice 6 h after LPS stimulation with and without Zn (100 μM), and the protein levels of caspase-1 (a marker of pyroptosis) were measured using Western blot analysis. Data are representative of two or more independent experiments. (*B* and *C*) The protein levels of LC3 (a marker of autophagic activity) and MLKL (a marker of necroptosis) were measured in the spleen and PMs of *Slc39a10*$^{fl/fl}$;*LysM-Cre*$^{+}$ and control mice 6 h after LPS stimulation. Data are representative of two or more independent experiments. (*D*) Lipid peroxidation was measured using flow cytometry in PMs isolated from *Slc39a10*$^{fl/fl}$;*LysM-Cre*$^{+}$ and control mice ($n = 3$ mice per group). (*E*) The mRNA levels of *Ptgs2* (a marker of ferroptosis) were measured in PMs isolated from *Slc39a10*$^{fl/fl}$;*LysM-Cre*$^{+}$ and control mice 6 h after LPS stimulation ($n = 3$ mice per group). Student's *t* test was used to compare two groups.

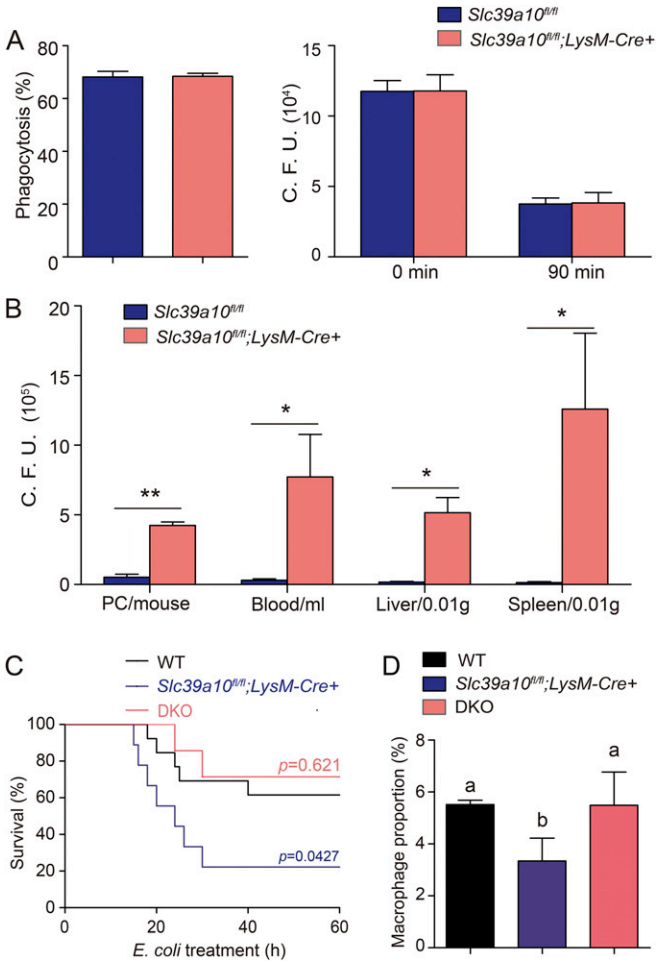

Fig. S4. The phagocytosis capacity, bacterial burden, and survival rates of *E. coli*-infection experiments. (*A*) Phagocytosis (*Left*) and *E. coli*-killing capacity (*Right*) were measured in PMs isolated from *Slc39a10*$^{fl/fl}$;*LysM-Cre*$^{+}$ and *Slc39a10*$^{fl/fl}$ mice (n = 3 mice per group). (*B*) The number of bacteria present in the peritoneal cavity, blood, liver, and spleen was measured 12 h after an *E. coli* injection in *Slc39a10*$^{fl/fl}$;*LysM-Cre*$^{+}$ and *Slc39a10*$^{fl/fl}$ mice (n = 5 mice per group). (*C*) Kaplan–Meier survival curve of wild-type (n = 13), *Slc39a10*$^{fl/fl}$;*LysM-Cre*$^{+}$ (n = 9), and DKO (n = 7) mice following *E. coli* infection. The survival rates of the *Slc39a10*$^{fl/fl}$;*LysM-Cre*$^{+}$ and DKO groups were each compared with the wild-type group, with significance indicated above their respective survival curves. (*D*) Percentages of macrophages in the peritoneal cavity of wild-type, *Slc39a10*$^{fl/fl}$;*LysM-Cre*$^{+}$, and DKO mice following *E. coli* infection, measured using flow cytometry. *A*, *Left* and *B* were analyzed by *t* test; *A*, *Right* and *C*, *Right* were analyzed by ANOVA; *C*, *Left* was analyzed by log-rank test. *P < 0.05; **P < 0.01. Groups labeled without a common letter were significantly different (P < 0.05).

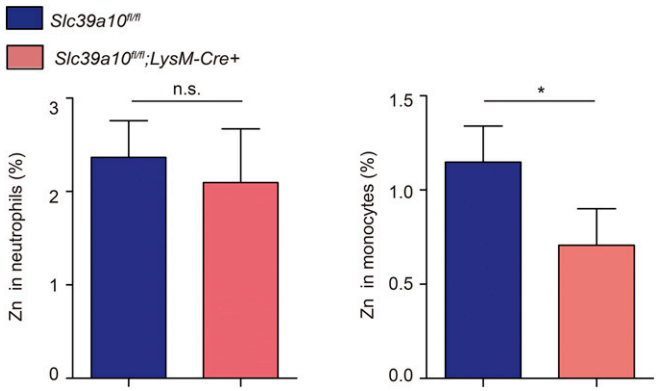

Fig. S5. Zn level in the neutrophils and monocytes of *Slc39a10*$^{fl/fl}$;*LysM-Cre*$^{+}$ and control mice. The percentages of monocytes (CD11b^{+}; Ly6C^{+}) and neutrophils (CD11b^{+}; Ly6G^{+}) positively stained with FluoZin-3 in the peritoneal cavity of LPS-stimulated *Slc39a10*$^{fl/fl}$;*LysM-Cre*$^{+}$ and DKO mice (n = 3 mice per group). Student's *t* test was used to compare two groups. *P < 0.05; n.s., not significant.

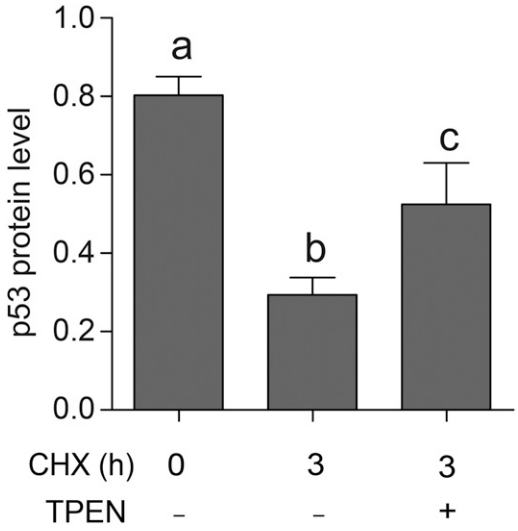

Fig. S6. Quantification of p53 protein levels in wild-type macrophages following TPEN treatment. The p53 protein levels were quantified in the PMs of wild-type mice before and after 3 h of CHX treatment in the presence or absence of TPEN. ($n = 3$ mice per group). The one-way ANOVA with Tukey's post hoc test was used to compare multiple groups. Groups labeled without a common letter were significantly different ($P < 0.05$).

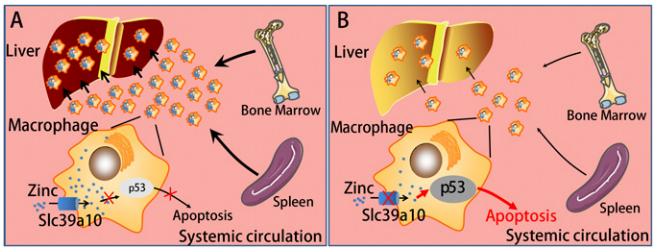

Fig. S7. Proposed model to explain the role of Slc39a10 in macrophages in response to inflammatory stimuli. (*A*) Following a robust inflammatory stimulus in wild-type mice, sufficient numbers of inflammatory macrophages are generated and infiltrate the liver, resulting in hyperactivation of the inflammatory response and liver damage. (*B*) In contrast, macrophages that lack Slc39a10 have impaired Zn influx, which leads to the stabilization of p53 and the subsequent increase in apoptosis following LPS stimulation. As a result of reduced inflammatory macrophages, the animal develops endotoxin resistance and reduced liver damage, thereby improving survival.

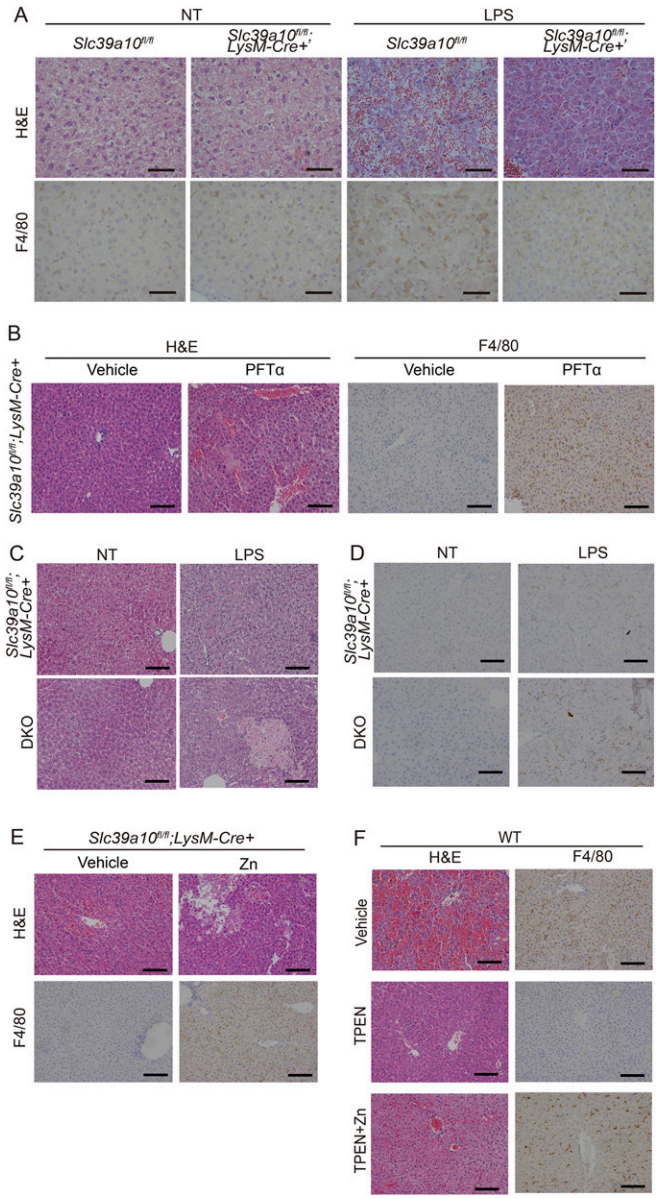

Fig. S8. High-resolution images of histology sections. (*A*) Liver H&E staining and immunohistochemical staining for F4/80 in *Slc39a10*$^{fl/fl}$;*LysM-Cre*$^{+}$ and control mice either with or without LPS stimulation. (*B*) Liver H&E and immunohistochemical staining for F4/80 in PFTα-treated *Slc39a10*$^{fl/fl}$;*LysM-Cre*$^{+}$ mice with or without LPS stimulation. (*C* and *D*) Liver H&E staining (*C*) and immunohistochemical staining (*D*) for F4/80 in *Slc39a10*$^{fl/fl}$;*LysM-Cre*$^{+}$ and DKO mice either with or without LPS stimulation. (*E*) Liver H&E and immunostaining for F4/80 in LPS-stimulated *Slc39a10*$^{fl/fl}$;*LysM-Cre*$^{+}$ mice treated with or without Zn supplementation. (*F*) Liver H&E and immunostaining for F4/80 in wild-type mice treated with vehicle or TPEN (10 mg/kg) with or without Zn (10 mg/kg). NT, no treatment. (Scale bars: 50 μm in *A* and 100 μm in *B*–*F*.)

Table S1. Gene-expression analyses of human and mouse SLC39A and SLC30A family members shown in Fig. 1

Sample information	Family member													
Fig. 1*A**	*SLC39A1*	*SLC39A3*	*SLC39A4*	*SLC39A6*	*SLC39A7*	*SL C39A8*	*SLC39A9*	*SLC39A10*	*SLC39A11*	*SLC39A13*	*SLC39A14*			
Nonsurvivors														
Mean	78.984457	26.465905	10.421327	1.9997217	36.160134	6.3210106	10.464486	1.5465558	7.8046074	27.964722	0.7995294			
SD	37.063843	14.265818	6.2337999	2.0375986	17.251867	9.5617923	7.0149453	1.1456287	4.9710112	17.730824	0.7326807			
n	33	33	33	33	33	33	33	33	33	33	33			
Survivors														
Mean	55.981771	22.191199	6.567035	1.6611954	32.988384	5.2851864	6.7720811	0.802346	5.4573534	17.746266	0.7467987			
SD	32.58557	15.59787	4.6225486	1.4824262	17.46554	9.302352	5.1529709	0.6105101	3.9749141	12.134944	0.8917611			
n	97	97	97	97	97	97	97	97	97	97	97			
Fold change	0.7087695	0.8384825	0.6301534	0.8307133	0.912286	0.83613	0.647149	0.5187954	0.6992477	0.6345948	0.9340479			
P value	0.0009028	0.1691631	0.0003335	0.3131375	0.3674587	0.589399	0.0016446	2.963E-05	0.0072109	0.0003784	0.7634621			
Fig. 1*B**	*SLC30A1*	*SLC30A4*	*SLC30A5*	*SLC30A6*	*SLC30A7*	*SLC30A8*	*SLC30A9*							
Nonsurvivors														
Mean	1.860168	0.2581352	4.2372546	5.6756504	7.4166256	0.4339532	2.7848397							
SD	1.686792	0.4919569	3.2149119	3.4329673	5.0363187	0.4603985	2.200874							
n	33	33	33	33	33	33	33							
Survivors														
Mean	1.2208611	0.2678322	3.5836658	5.9409183	7.140459	0.5711299	2.2738787							
SD	1.137736	0.3180686	2.2829215	4.4804779	5.9679599	0.707706	2.37292							
n	97	97	97	97	97	97	97							
Fold change	0.6563176	1.0375658	0.8457518	1.0467379	0.9627638	1.3161095	0.8165205							
P value	0.016999	0.8980901	0.208433	0.759088	0.8137424	0.3078593	0.2842843							
Fig. 1*C*	*Slc39a1*	*Slc39a2*	*Slc39a3*	*Slc39a4*	*Slc39a5*	*Slc39a6*	*Slc39a7*	*Slc39a8*	*Slc39a9*	*Slc39a10*	*Slc39a11*	*Slc39a12*	*Slc39a13*	*Slc39a14*
Wild type														
Mean	0.1265819	0.0001785	0.0106262	0.0284126	0.0001295	0.0603443	0.1509926	0.0035189	0.0283295	0.0256666	0.0128381	0.0001253	0.0074268	0.0072784
SD	0.0758748	4.452E-05	0.0017073	0.0063955	2.483E-05	0.0327989	0.0915371	0.0028563	0.0211901	0.0077181	0.0030515	9.761E-05	0.0037184	0.0006077
n	3	3	3	3	3	3	3	3	3	3	3	3	3	3
Wild type + LPS														
Mean	0.2081873	0.0001519	0.0082971	0.0562594	0.0002267	0.0772167	0.262937	0.0014562	0.0283759	0.0105322	0.0081853	0.0001592	0.0173401	0.0286509
SD	0.033383	5.147E-05	0.0013523	0.016654	7.375E-05	0.0403415	0.0802445	0.000164	0.0050438	0.0027674	0.0019321	0.0001164	0.0171217	0.0067575
n	3	3	3	3	3	3	3	3	3	3	3	3	3	3
Fold change	1.645	0.851	0.781	1.980	1.751	1.280	1.741	0.414	1.002	0.410	0.638	1.271	2.335	3.936
P value	0.163	0.635	0.138	0.054	0.219	0.604	0.186	0.280	0.997	0.039	0.120	0.719	0.383	0.005
Fig. 1*D*	*Slc30a1*	*Slc30a2*	*Slc30a3*	*Slc30a4*	*Slc30a5*	*Slc30a6*	*Slc30a7*	*Slc30a8*	*Slc30a9*	*Slc30a10*				
Wild type														
Mean	0.0058904	0.0055395	0.0016455	0.0020284	0.1296186	0.0332242	0.0840123	1.353E-05	0.0399326	0.0004966				
SD	0.0030814	0.0058272	0.002319	0.0006059	0.0627691	0.0160805	0.039409	7.365E-06	0.0119471	0.0004741				
n	3	3	3	3	3	3	3	3	3	3				
Wild type + LPS														
Mean	0.008504	0.0087277	0.0032737	0.0012159	0.0990576	0.0852689	0.1266293	1.508E-05	0.0466413	0.000174				
SD	0.0008385	0.0064025	0.0030759	0.0001304	0.0069768	0.0197384	0.0260438	7.63E-06	0.0006958	9.066E-05				
n	3	3	3	3	3	3	3	3	3	3				
Fold change	1.444	2.307	1.990	0.599	0.764	2.566	1.507	1.114	1.168	0.350				
P value	0.229	0.355	0.408	0.086	0.449	0.024	0.193	0.856	0.387	0.311				

A and *B*: mRNA levels of *SLC39A* (*A*) and *SLC30A* (*B*) genes were measured in both sepsis nonsurvivors ($n = 28$ patients) and survivors ($n = 78$ patients). *C* and *D*: mRNA levels of *Slc39* (*C*) and *Slc30* (*D*) genes were measured in wild-type mouse BMDMs stimulated with or without LPS ($n = 3$ mice per group).

*The patients' data were reanalyzed from Gene Expression Omnibus dataset GSE63042.

Table S2. Primers used for qRT-PCR analysis

Gene name	Forward primer	Reverse primer
Il-6	TGTATGAACAACGATGATGCACTT	ACTCTGGCTTTGTCTTTCTTGTTATCT
Tnf-α	AGTGACAAGCCTGTAGCCC	GAGGTTGACTTTCTCCTGGTAT
Il-1β	CTGGTACATCAGCACCTCAC	AGAAACAGTCCAGCCCATAC
Ifn-γ	AAAGAGATAATCTGGCTCTGC	GCTCTGAGACAATGAACGCT
Ifn-β	CCACAGCCCTCTCGATCAACTATAAGGAGG	AGCTCTTCAACTGGAGAGCAGTTGAGG
Mt-1	GCGTCACCACG ACTTCAAC	GTCACATCAGGCACAGCAC
Ptgs2	CTGCGCCTTTTCAAGGATGG	GGGGATACACCTCTCCACCA
Hprt	TTTCCCTGGTTAAGCAGTA	TGGCCTGTATCCAACACTTCGAGA
Nos2	TCACCTTCGAGGGCAGCCGA	TCCGTGGCAAAGCGAGCCAG
Arg-1	CTCCAAGCCAAAGTCCTTAGAG	AGGAGCTGTCATTAGGGACATC
Mrc-1	CTCTGTTCAGCTATTGGACGC	CGGAATTTCTGGGATTCAGCTTC
Ym-1	CAGGTCTGGCAATTCTTCTGAA	GTCTTGCTCATGTGTGTAAGTGA
Fizz-1	CCAATCCAGCTAACTATCCCTCC	ACCCAGTAGCAGTCATCCCA
Slc39a1	ACTACCTGGCTGCCATAGA	TGAACTCTTGCAGTGGGAAC
Slc39a2	CTGGAGGGAATTGAGTCAGAAA	AAGCAGCATCACGAGAAGAA
Slc39a3	CCTGCAGTGAGGGACAAG	GGTAGTCGGTGCTGATGTG
Slc39a4	GGACCAGCTCAGTCAAACA	GACCGAACACAGCACAGA
Slc39a5	TGCTAGAGAACACACTAGGACT	CAGGGTTTGGTTCTCCAAGAT
Slc39a6	CGTACTCACACTGATCAAGCA	TGCTTCTTGCTCTCCACATC
Slc39a7	GACATGGACACTCCCACAG	GCGACAATCCCACTGAGAA
Slc39a8	TCTAAGAAAGCACAACGCAAAG	AGGAGAGAGGCCAGATTGATA
Slc39a9	TCATTCCTTTGGCTGTTAATTTCTC	GCAGTTCCACAGAGAAGACC
Slc39a10	ACTCTGGTTCCTGAAGATAAGAC	GCAGACTAATGACGGTGATAGA
Slc39a11	CGGAGAGTGAACTTTCCATCC	CAGTAGCTGCCACCTTCTTC
Slc39a12	AGTACTTTGGCACTTCCAGTAG	CAGATTCCCTCTGCAGAATCTTA
Slc39a13	GAAGATGTTCCTCAACAGCAAG	CAGACAGTGGCCTCCATT
Slc39a14	TTTCCCAGCCCAAGGAAG	CAAAGAGGTCTCCAGAGCTAAA
Slc30a1	CCAACACCAGCAATTCCAAC	CTTGTACATCCACTGGGTCATC
Slc30a2	CTGTCTGTCCACATAGCCATT	CTAGCCACCTTCAGCACAG
Slc30a3	TTACTTACCACGTTGCCTCTG	CAGCCAGGACAGCTTCAG
Slc30a4	CGCATCATATGGGACACAGTAG	CTACATTCAAATGGCTTGGTACAC
Slc30a5	GCCATGATCACGGTCACA	CTGCCAACACATGGAGAAATAC
Slc30a6	ACCCTTGGATTTGGCTCATT	CCATTTGTTCGTTCGCATCTC
Slc30a7	CCAGGTGGATCTTAAGCCAAA	CTGTACATAAAGCTGTCTCACTCC
Slc30a8	CACTGACTGTGAACCAAGTGA	TCCTGTCCGCACAGACT
Slc30a9	CTCTAGGAGCTGAAGTAGACAGA	TGCCGAACTTCAGGGTTTC
Slc30a10	GGTCAACATGGAAGAGCTGA	CACTTCATGCACACTGCTAATC