Supporting Information

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SI Materials and Methods

For isolation of peritoneal macrophages, mice were injected intraperitoneally (IP) with 1.5 mL of 3% Brewer's thioglycollate broth. Six days later, primary macrophages were collected from euthanized animals by peritoneal lavage using 10 mL of ice ice-cold RPMI supplemented with 2% FBS, 1 unit/mL heparin and penicillin/streptomycin. Cells were washed using lavage media without heparin and plated in macrophage culture media of DMEM supplemented with 5% FBS, penicillin/streptomycin, Glutamax, and 15 mM Hepes (pH 8.0) and incubated at 37 °C at 5% CO_2 for 2 h. Cultures were washed three times with PBS to remove nonadherent cells and left in culture media overnight (1). Treatments were initiated the next day. RAW264.7 cells, representing a mouse macrophage cell line, were obtained from ATCC and maintained in DMEM supplemented with 10% FBS, penicillin/streptomycin, Glutamax, and 1 mM sodium pyruvate. Early passages of RAW264.7 cells were transduced with adenoviruses using cholesterol and centrifuged for 2 h following the protocol outlined (2). After overnight incubation to allow viral expression, macrophages were stimulated with LPS and IFNγ.

For isolation of primary immune cells, total spleen cells were recovered from C57BL/6NCr mice ranging in age from 6 wk to 18 mo, purchased from the Animal Production Area of the National Cancer Institute. Spleens were mechanically disrupted before lysis of red blood cells using ACK lysis (NH_4Cl 8,024 mg/L, $KHCO_3$ 1,001 mg/L, $EDTA.Na_2{\cdot}2H_2O$ 3.722 mg/L; Quality Biological). Cells were washed two times in PBS (Lonza) and blocked for 30 min on ice in PBS containing 1% heat-inactivated FCS (HyClone), 1% human AB serum, and 1% goat serum (Sigma). Blocked cells were stained with anti-CD45, anti-CD3ε, antiCD11b, and anti-CD19 for 1 h before being washed with PBS and subjected to flow-activated cell sorting using a BD FACS AriaII. Cell sorting was performed by the Cancer and Inflammation Program Fluorescence-Activated Cell Sorter Core staff. Cells were sorted into CD45+CD3ε+ (T-cells), CD45+CD19+ (B-cells), and CD45+CD11b+ (myeloid+NK cell) subsets. Neutrophils were isolated from bone marrow recovered from the tibias and femurs of the same mice from which spleen cells were isolated using the Percoll gradient protocol reported earlier (3). Mature neutrophils collect between the 62 and 81% Percoll layers. Purified cells were washed once in PBS before lysis in TRIzol and subsequent RNA purification.

1. Fortier AH, Falk LA (2001) Isolation of murine macrophages. *Curr Protoc Immunol* Chapter 14:Unit 14.1.
2. Zhang X, Edwards JP, Mosser DM (2009) The expression of exogenous genes in macrophages: Obstacles and opportunities. *Methods Mol Biol* 531:123–143.
3. Lowell CA, Fumagalli L, Berton G (1996) Deficiency of Src family kinases p59/61hck and p58c-fgr results in defective adhesion-dependent neutrophil functions. *J Cell Biol* 133: 895–910.

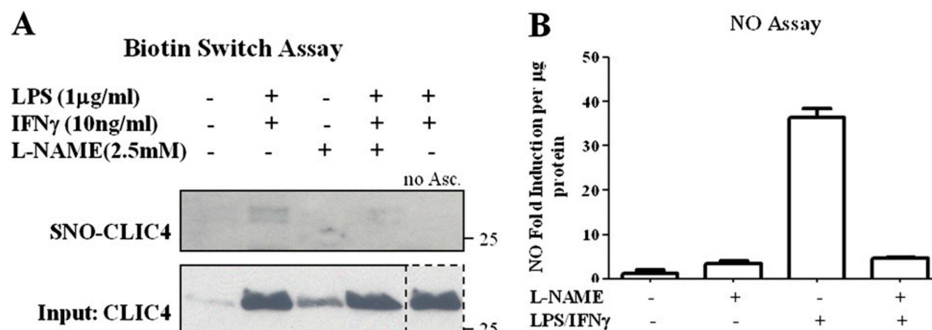

Fig. S1. S-nitrosylation of CLIC4 is dependent on NOS activity in primary macrophages. (*A* and *B*) Peritoneal macrophages were pretreated with (N^G-Nitro-L-arginine-methyl ester hydrogen chloride) L-NAME (2.5 mM) for 1 h, where indicated, before treatment with LPS and IFNγ (1 μg/mL and 10 ng/mL, respectively) for 24 h. (*A*) Lysates were used to perform biotin switch assays to detect S-nitrosylation. Ten percent of lysates were used as input controls and immunoblotted for CLIC4. (*B*) Media from control and treated cells was assayed for nitrite + nitrate levels using a fluorescent assay. Bars represent the mean ± SEM.

Fig. S2. CLIC4 knockout macrophages exhibit lower levels of TNFα, IFNβ, and IL-6 but not of IP-10 mRNA after stimulation with LPS (100 ng/mL). Mouse peritoneal macrophages from CLIC4 knockout and wild-type mice were treated with LPS (100 ng/mL) for the indicated times. Lysates were used for real-time PCR analysis. Bars represent the mean ± SEM of three replicates. Statistical significance was determined using a two-tailed unpaired Student's *t* test and is indicated.

Fig. S3. CLIC4 knockout macrophages show similar viability compared with wild-type cells. Wild-type and CLIC4 knockout animals were injected with thioglycollate and 6 d later injected with LPS (12.5 μg/g) for 24 h. Animals were injected with pHrodo-labeled *Escherichia coli* for 1 h before peritoneal macrophages were collected by lavage and stained for APC-F4/80 and with DAPI. F4/80-stained cells from each animal were analyzed for DAPI fluorescence using flow cytometry. Statistical significance was determined using a two-tailed unpaired Student's *t* test. Results are not significant ($P = 0.085$). Each dot represents results from a single animal.