Supporting Information

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SI Materials and Methods

Reagents. LPS (0111:B4), MG132 were purchased from Sigma-Aldrich. Antibodies to total Akt and Akt phosphorylated at Ser473 or Thr308, and antibodies to phosphorylated ERK1/2, p38, JNK, Gab1, as well as p-Gab1, were from Cell Signaling Technology. Antibodies against $G\alpha_{i1}$ (sc-391), $G\alpha_{i3}$ (sc-262), Gab1 (sc-9049), and Akt1/2(sc-8312), as well as goat antibody against rabbit IgG (IgG) conjugated to horseradish peroxidase (HRP) (sc-2030) were purchased from Santa Cruz Biotechnology. Anti-GIPN was purchased from Abgent. Goat antibody against mouse IgG-HRP (s0368G) was purchased from Beijing Biosynthesis Biotechnology (Bioss). Recombinant mouse IL-4, IFNγ and recombinant human IFN-γ were from Peprotech. D-galactosamine was purchased from TCI.

Western Blot Analysis. Aliquots of 30 μg of protein from each sample (treated as indicated in the legends) were subjected to 12% SDS–polyacrylamide gel electrophoresis (SDS/PAGE) and transferred onto a polyvinylidene difluoride (PVDF) membrane (Millipore). After blocking with 10% instant nonfat dry milk for 30 min, membranes were incubated with specific antibodies overnight at 4 °C followed by incubation with secondary antibodies (HRP-conjugated anti-rabbit or anti-mouse IgG at the appropriate dilutions) for 45 min to 1 h at room temperature. Antibody binding was detected with the enhanced chemiluminescence (ECL) detection system (Pierce).

Immunoprecipitation. Cells treated with the appropriate stimuli were lysed with lysis buffer [200 mM NaCl (pH 7.4), 1% Triton X-100, 10% glycerol, 0.3 mM EDTA, 0.2 mM Na_3VO_4, and protease inhibitor mixture (Roche Diagnostics)]. Aliquots of 500-μg proteins from each sample were precleared by incubation with 35 μL of protein A/G Sepharose beads (Santa Cruz Biotechnology) for 2 h at 4 °C. Precleared samples were incubated with specific antibodies in lysis buffer overnight at 4 °C. To this mixture was added 20 μL of protein A/G beads, and the samples were incubated for 2 h at 4 °C. The beads were washed five times with PBS and once with lysis buffer, boiled, subjected to 12% SDS/PAGE, and transferred onto a PVDF membrane followed by Western blot analysis as described above.

Flow Cytometric Analysis. For in vivo studies, sterile PBS was injected into the peritoneal cavity of treated mice and cells lavaged. Cells collected were washed with PBS and then stained with FITC-F4/80, PE-CD11b (E-Biosciences). Staining was assessed with a Amnis Flowsight (Millipore). Samples were compensated and analyzed by using Amnis IDEAS software.

RNA Quantification. Total RNA was prepared by using TRIzol reagent (Invitrogen) according to the manufacturer's instructions. cDNA was synthesized from 2 μg of total RNA by reverse transcriptase (Takara). Quantitative real-time RT-PCR (QRT-PCR) analysis was performed with LightCycler (Roche Diagnostics) and the SYBR RT-PCR kit (Takara) as described (1). Primers used for quantitative-PCR amplification of mouse GAPDH mRNA were 5′-AGTGGCAAAGTGGAGATT-3′(sense) and 5′-GTGGAGTCATACTGGAACA-3′ (antisense), of mouse IL-6 mRNA were 5′-ACCTGTCTATACCACTTC-3′(sense) and 5′-GCATCATCGTTGTTCATA-3′ (antisense), of mouse TNFα mRNA were 5′-TTCTGTCTACTGAACTTC-3′(sense) and 5′-CCATAGAACTGATGAGAG-3′ (antisense), of mouse IL-12 mRNA 5′-ACATCTGCTGCTCCACAAG-3′(sense) and 5-′GGTGCTTCACACTTCAGGAA-3′(antisense), mouse iNOS mRNA 5′- TTGACGCTCGGAACTGTA-3′(sense) and 5′-ACCTGATGTTGCCATTGTT-3′ (antisense), mouse $G\alpha_{i3}$ mRNA 5′-GTGATTACGACCTTGTTCTG -3′ (sense) and 5′-AACCTGTGTATTCTGGATAAC-3′(antisense). Data were normalized by the level of GAPDH expression in each sample.

Interfering RNAs. $G\alpha_{i1}$- and $G\alpha_{i3}$-specific RNAi duplexes were purchased from Santa Cruz Biotechnology. Twenty microliters of $G\alpha_{i1}$-specific (sc-41751) and $G\alpha_{i3}$-specific (sc-29325) RNAi duplexes (10 μM, diluted in siRNA Dilution Buffer from Santa Cruz Biotechnology) were mixed with liposome 2000 (Invitrogen). The complex were added to the well containing 2 mL of medium with a final siRNA concentration of 100 nM. BMDMs (1×10^6) were seeded into each well of 6-well plates and incubated overnight, and then transfected with siRNA duplexes. After 24 h, the supernatant was removed and fresh medium was added. The cells were cultured for another 24 h before further experiments. We also use shRNA for $G\alpha_{i1}$ (sc-41751-SH) and $G\alpha_{i3}$ (sc-29325-SH) in macrophage phenotype research.

Gab1-specific siRNA: 5′-AGGAGACAAACAAGUCGAATT-3′ (sense)
/5′-UUCGACUUGUUUGUCUCCUTT -3′ (antisense);
Gab1 nonsense control sequence: 5′-UUCUCCGAACGUGUCACGUTT-3′ (sense)
/5′- ACGUGACACGUUCGGAGAATT -3′ (antisense).

Cytokine Assays. Production of the cytokines IL-6, TNF-α, IFNβ, and IL-12 were measured in the serum or supernatants by ELISA by using kits from R&D Systems.

Nitric Oxide Detection. Cells plated at 1.5×10^5cells per well in 24-well culture dishes were incubated overnight before stimulation. After the cells were treated with 100 ng/mL LPS for 24 h, the culture medium was collected for analysis by using the Griess Reagent kit (Bytime). Nitrite concentrations were determined by the measurement of the optical density at 570 nm.

Determination of Arginase Activity. Arginase activity was measured in cell lysates as described (1). Briefly, cells were lysed with 100 μL of 0.1% Triton X-100. After 30 min on a shaker, 100 μL of 25 mM Tris·HCl was added. To 100 μL of this lysate, 10 μL of 10 mM $MnCl_2$ was added, and the enzyme was activated by heating for 10 min at 56 °C. Arginine hydrolysis was conducted by incubating the lysate with 100 μL of 0.5 M L-arginine (pH 9.7) at 37 °C for 15–120 min. The reaction was stopped with 900 μL of H_2SO_4 (96%)/H_3PO_4 (85%)/H_2O (1/3/7, vol/vol/vol). The urea concentration was measured at 540 nm after addition of 40 μL of α-isonitrosopropiophenone (dissolved in 100% ethanol) followed by heating at 95 °C for 30 min. One unit of enzyme activity is defined as the amount of enzyme that catalyzes the formation of 1 μmol of urea per min.

Microscopy. BMDMs were cultured as 5×10^5 cells per well in 6-well plates on sterile coverslips overnight and stimulated with LPS (100 ng/mL) for indicated times. Then cells were fixed with 4% paraformaldehyde for 15 min at room temperature, permeabilized with 0.1% Triton X-100, and incubated with $G\alpha_{i1}$, $G\alpha_{i3}$ antibody and EEA1 overnight. Binding was detected by using Alexa Fluor 488-labeled goat anti-rabbit IgG antibody or Alexa Fluor 647-labeled goat anti-mouse IgG antibody (Molecular Probes). Fixed cells imaging were performed by using a Leica TCS SPE.

1. Munder M, et al. (1999) Th1/Th2-regulated expression of arginase isoforms in murine macrophages and dendritic cells. *J Immunol* 163(7):3771–3777.