Supporting Information

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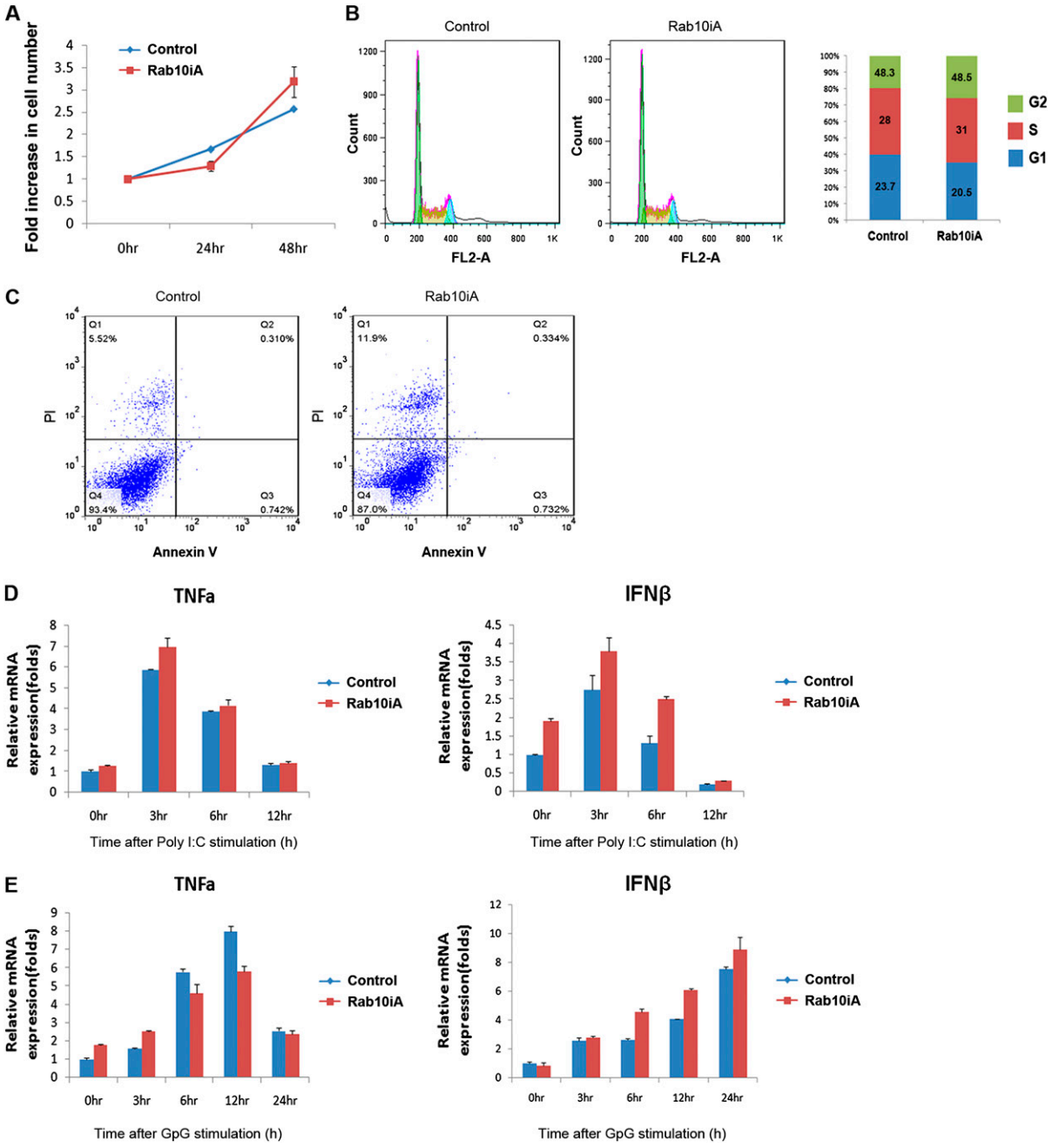

Fig. S1. Rab10 knockdown does not affect the viability of macrophages and cytokine production induced by Poly I:C and CpG. (*A*) For measuring the proliferation of the cells, Rab10 knockdown RAW264.7 cells and cells transfected with empty vector were cultured in complete cell culture medium; cell numbers were counted after different time period as indicated. (*B*) Typical flow cytometric analysis of the cell cycle: RAW264.7 cells stably transfected with Rab10iA or vector plasmid were stained with propidium iodide (PI) and analyzed using flow cytometry; respective percentages of G1, S, and G2 phase cells were analyzed by Flowjo software. (*C*) Apoptosis analysis: Rab10 knockdown RAW264.7 cells and control cells were stained with Annexin V and PI and assayed by flow cytometry. (*D* and *E*) RAW264.7 cells stably transfected with Rab10iA or vector plasmid were stimulated with 10 μg/mL poly I:C (*D*) or 0.3 μM CpG (*E*) for the indicated time periods. Relative mRNA expression of TNF-α and IFN-β was measured by quantitative PCR.

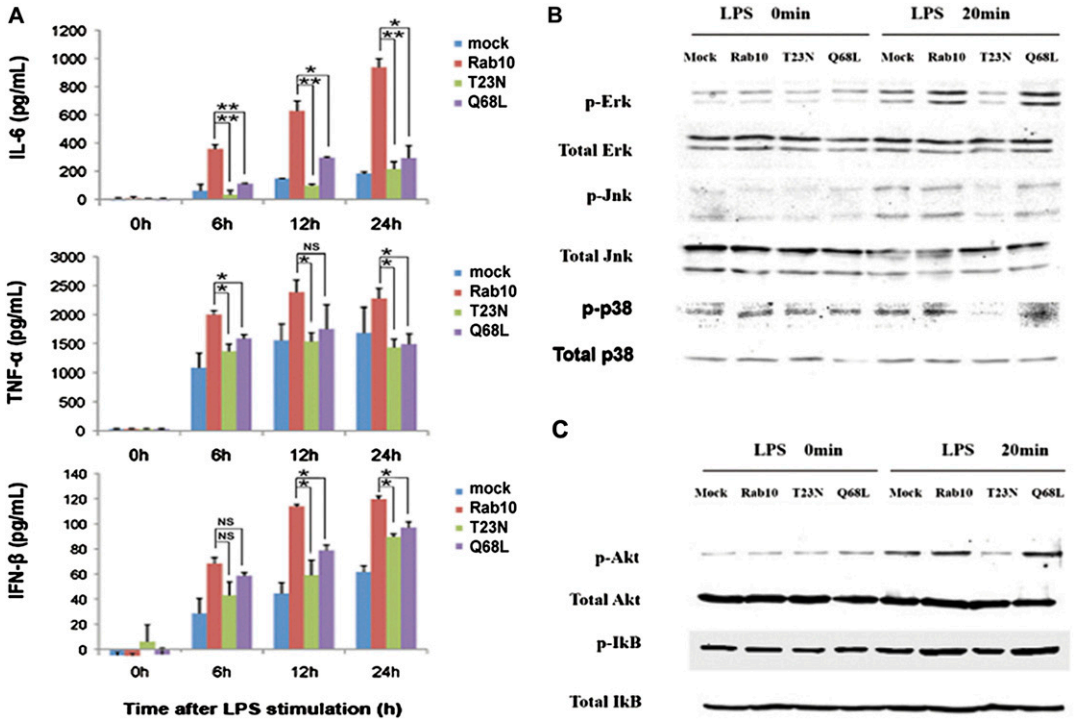

Fig. S2. Overexpression of Rab10T23N served as dominant-negative form of Rab10 in LPS-initiated signaling pathways in macrophages. Western blotting assay of MAPK (*B*), Akt, and NF-κB (*C*) activation after LPS treatment in RAW264.7 cells. Cell lysates of Rab10, Rab10T23N, or Rab10Q68L transiently overexpressed RAW264.7 cells were prepared and blotted with the indicated Abs. Total Erk, total Jnk, total P38, total Akt, and total IκB were probed as quantitative controls. (*A*) Enzyme-linked immunoassay of IL-6, TNF-α, and IFN-β production by Rab10, Rab10T23N, or Rab10Q68L overexpressed RAW264.7 cells after LPS treatment as indicated was performed as described in Fig. 2*B*. Similar results were obtained in three independent experiments. $*P < 0.05$, $**P < 0.01$.

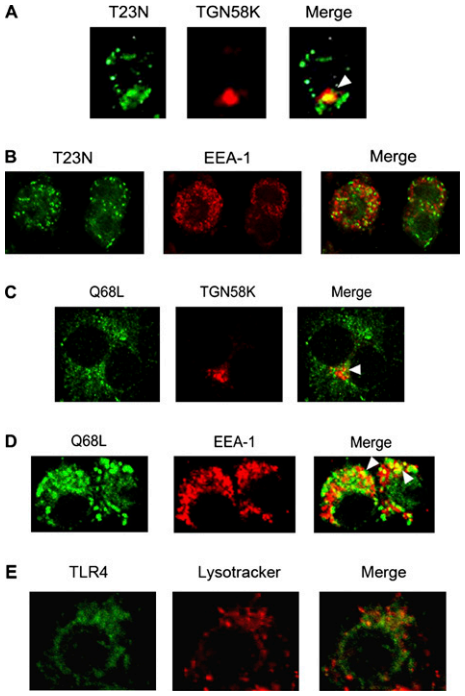

Fig. S3. Rab10T23N is mainly localized to Golgi, whereas Rab10Q68L is mainly localized to early endosomes. (*A* and *B*) Confocal analysis of Rab10T23N subcellular localization. RAW264.7 cells were transiently transfected with GFP-Rab10T23N plasmid; 48 h later, cells were labeled with primary Ab against TGN58K (*A*) and EEA-1 (*B*). (*C* and *D*) Confocal analysis of Rab10Q68L subcellular localization. RAW264.7 cells were transiently transfected with GFP-Rab10Q68L plasmid; 48 h later, cells were labeled with first Ab against TGN58K (*C*) and EEA-1 (*D*). Confocal analysis of TLR4 subcellular location: RAW264.7 cells were transiently transfected with TLR4-HA plasmid and incubated for 48 h. Cells were labeled with LysoTracker Red followed by immunostaining with anti-HA Ab plus secondary antibody before images analysis using confocal microscopy.

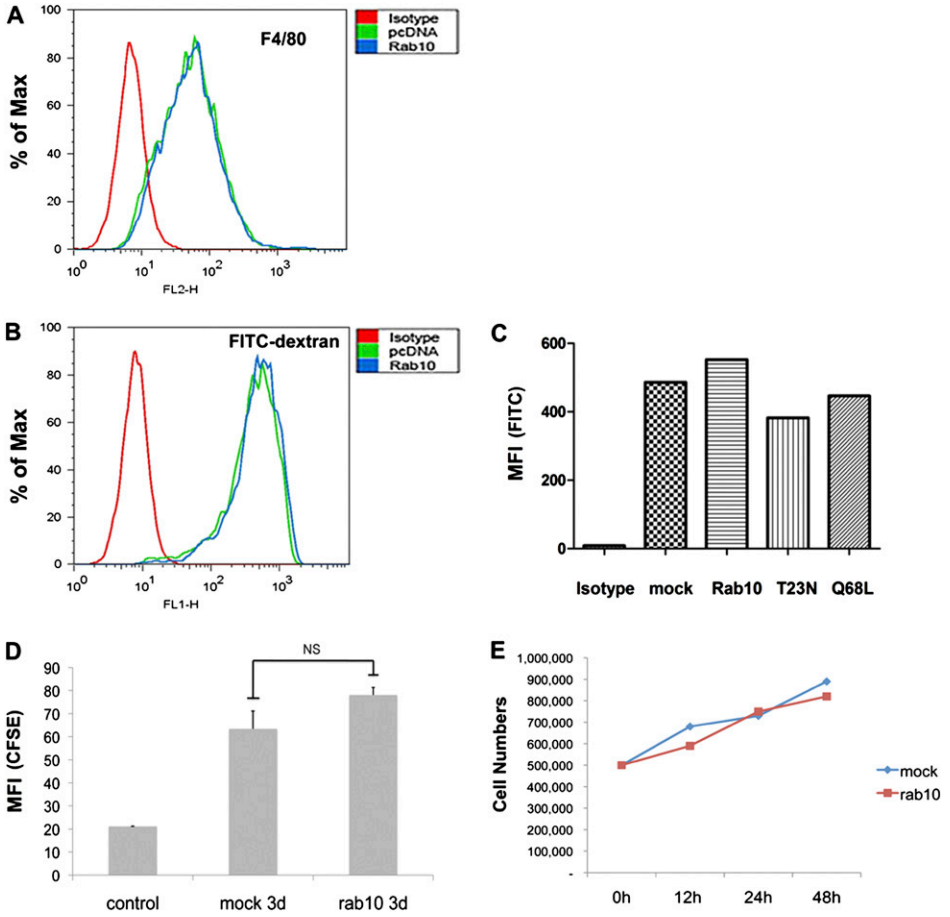

Fig. S4. Overexpression of Rab10 does not affect F4/80 surface expression, phagocytosis ability, antigen-presentation ability, and cell proliferation in macrophages. (*A*). FACS assay of F4/80 expression on plasma membrane of Rab10 overexpressed RAW264.7 cells. (*B*) Measurement of phagocytosis (FITC-dextran uptake), RAW264.7 cells overexpressing Rab10 were incubated with prewarmed medium containing 1 mg/mL FITC-dextran for 10 min at 37 °C. After washing three times with chilled medium, internalized FITC-dextran was measured by FACS. The MFI of internalized FITC-dextran in Rab10, Rab10T23N, or Rab10Q68L overexpressing RAW264.7 cells is also shown (*C*). For measuring antigen-presentation ability of Rab10 overexpressed macrophages, BMDMs were transfected with Rab10 or vector plasmid; 48 h later, BMDMs were plated with 20 U/mL IFN-γ at 3–5 × 10^4 cells/well in 96-well plate and incubated 48 h before use. Cells were antigen loaded overnight with OVA protein added from a 100 mg/mL stock. Cells were rinsed three times with medium and CFSE-labeled $CD4^+$ T cells from OT-2 mice (1–3 × 10^5 cells/well) were added. After 72-h incubation, FACS assay of CFSE-labeled $CD4^+$ T cells was performed (*D*). For measuring the proliferation of Rab10 overexpressed macrophages, RAW264.7 cells overexpressed Rab10 or vector plasmid were performed cell count of different time period as indicated (*E*).

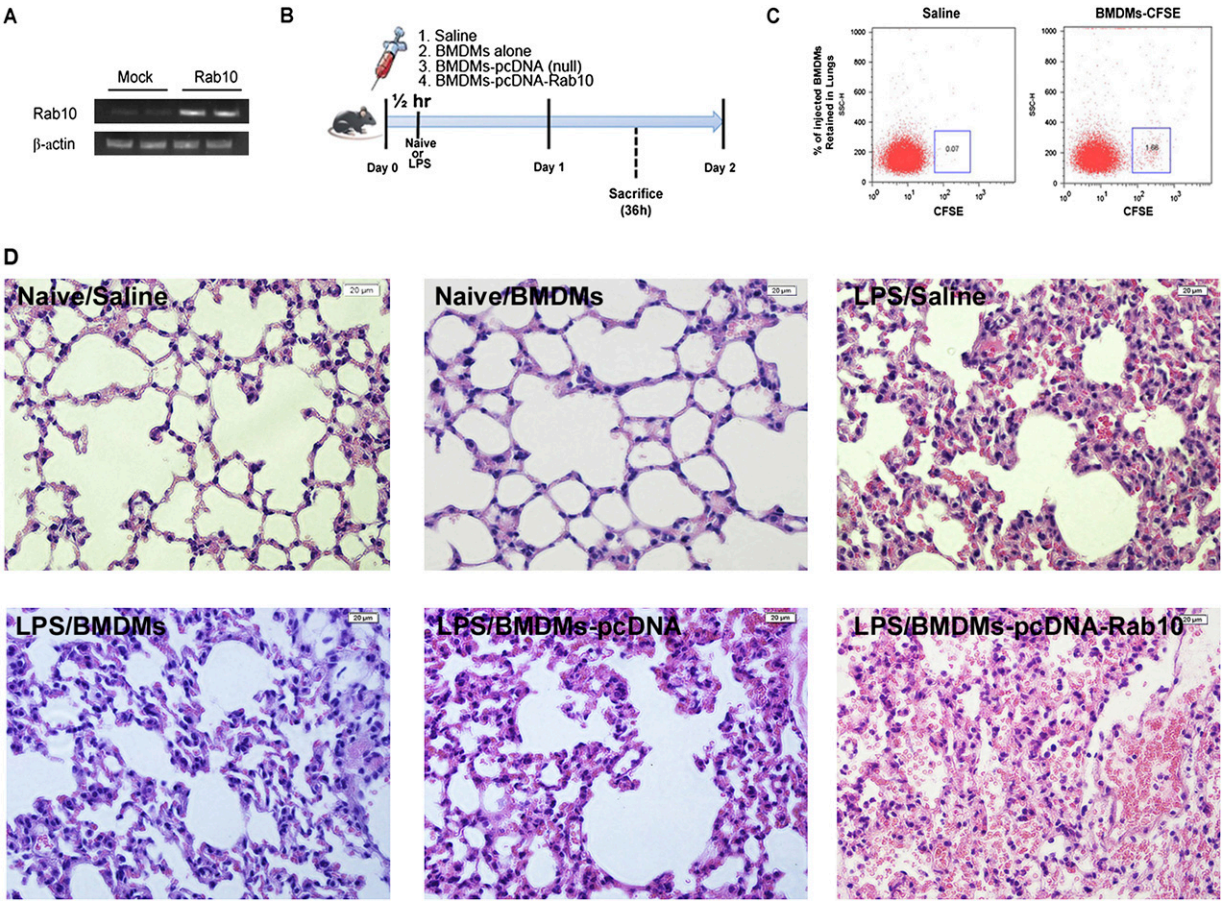

Fig. S5. Adoptive transfer of BMDMs-Rab10 aggravated LPS-induced lung injury in mice by histological analysis. (*A*) BMDMs were transiently transfected with plasmids encoding Rab10 or mock vector. The efficiency of transfection was evaluated by RT-PCR. (*B*) Female mice at 6–10 wk were divided into six groups (n = 6, 5, 7, 5, 7, 6, respectively). Saline, BMDMs, BMDMs transfected with pcDNA3.1, or pcDNA3.1-mRab10 plasmid (2×10^6 cells, 200 μL total volume each) were infused via a jugular venous canula 30 min before LPS challenge. Mice were then anesthetized and endotracheally intubated with 1.5 mg/mL LPS or saline. Mice were killed after 36 h to evaluate pulmonary inflammation. (*C*) To ensure the infiltration of injected macrophages to the lung, carboxyfluorescein diacetate succinimidyl ester (CFSE) was used to label BMDMs before injection into animals. Lung lobes were enzyme-digested into single cells before CFSE-labeled BMDMs were counted by flow cytometry. *(D)* Lung tissues fixed in 4% paraformaldehyde, embedded in paraffin, were cut into 5-μm thick sections before staining with H&E (Beyotime), followed by microscopy analysis with an Olympus TH4-200 microscope using a 40× objective. Lung pathology was evaluated blindly by a pathologist according to four criteria: alveolar congestion, hemorrhage, infiltration or aggregation of neutrophils in airspaces or vessel walls, and thickness of alveolar wall/hyaline membrane formation. Representative images of H&E stained lung sections from six experimental groups are shown. Lungs were fixed with 4% paraformaldehyde, embedded in paraffin, and then cut into 5-μm-thick sections before staining. Photomicrographs were obtained with an Olympus TH4-200 microscope with a 40× objective. (Scale bar = 20 μm.)