Supplementary Materials: Protective Role of Proton-Sensing TDAG8 in Lipopolysaccharide-Induced Acute Lung Injury

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Figure S1. No appreciable effect of TDAG8 deficiency on TLR4 protein expression. The proteins were extracted from lung tissues and alveolar macrophages in WT and TDAG8-deficient mice with a lysis buffer composed of 20 mM Tris–HCl, pH = 8.0, 150 mM NaCl, 1mM EDTA–PBS, 0.1% sodium dodecyl sulfate, 0.5% sodium deoxycholate, 1% Nonidet P-40, and 1% proteinase inhibitor cocktail (Sigma-Aldrich, St. Louis, MO, USA). The lysate was centrifuged at 14,000×g for 20 min. The cell extracts were then subjected to Western blotting analysis using sodium dodecyl sulfate–polyacrylamide gel electrophoresis for detection of TLR4 and β-actin proteins. Protein bands were detected by alkaline phosphatase method. The primary antibodies were obtained from Sigma-Aldrich (St. Louis, MO, USA) for anti-β-actin, and from Santa Cruz Biotechnologies (Dallas, TX, USA) for anti-TLR4. Two different batches of the tissues and cells were used.

Figure S2. CD14 mRNA expression in lungs and alveolar macrophages. The mRNA expression of CD14 was evaluated by a quantitative real-time TaqMan PCR for lung tissues (A) and alveolar macrophages (B) in WT and TDAG8-deficient mice.
Figure S3. Proton-sensing GPCR mRNA expression in various types of cells. The mRNA expressions of proton-sensing GPCRs were evaluated by a quantitative real-time TaqMan PCR. Cell types are as follows: (A) mouse alveolar macrophages (Figure 2C in the present study); (B) mouse peritoneal macrophages (data from Mogi et al. [10]); (C) human neutrophils (data from Murata et al. [11]); (D) h.ASMC (human airway smooth muscle cells, data from Ichimonji et al. [24]); (E) h.BEAS-2B (human bronchial epithelial cells, unpublished results); (F) h.HBE (human bronchial epithelial cells, unpublished results) and (G) m.bEnd.3 (mouse brain endothelial cells, unpublished results). Results are expressed as a ratio relative to GAPDH.