SUPPLEMENTAL METHODS

***In vitro* stimulation.** Peritoneal macrophages were cultured on a 24-well plate (1×10^5 cells/well) and treated with PBS, LPS (0.5 μg/mL, Sigma L8643) or *A. baumannii* EVs (100 ng/mL in total protein concentration), with or without polymyxin B (20 mg/mL), in the medium containing 0.5% fetal bovine serum for 12 h. The cell-free conditioned medium was collected and stored at -80°C until the cytokine concentrations were determined.

Cytokine measurement. The concentrations of TNF-α were measured in conditioned media by DuoSet ELISA Development kit (R&D Systems, Minneapolis, MN, USA), according to the manufacturer's instructions.

SUPPLEMENTAL FIGURE LEGEND

FIG S1. The roles of LPS in *A. baumannii* EV-mediated release of TNF-α from macrophages. Peritoneal macrophages from wild-type mice were stimulated with PBS, LPS (0.5 μg/mL), or *A. baumannii* EVs (100 ng/mL in total protein concentration), with or without polymyxin B (PMB; 20 mg/mL). At 12 h after stimulation, the concentrations of TNF-α in conditioned media were determined. ***, $P < 0.001$.

Supplemental Figure 1