

Fig. S1 ExoY has no cytotoxic effect on epithelial cells.

(A) cAMP level in A549 cells infected with PAO1 or PAO1 Δ exoY for 0 to 8 h, MOI=2. (B) Analysis of LDH-release-based cell death of A549 cells infected with PAO1 or PAO1 Δ exoY at the indicated times, MOI=10. Data are expressed as mean values \pm s.e.m (A) or mean values \pm s.d. (B) from three technical replicates. ***, $p < 0.001$. Data shown are representative of three independent experiments.

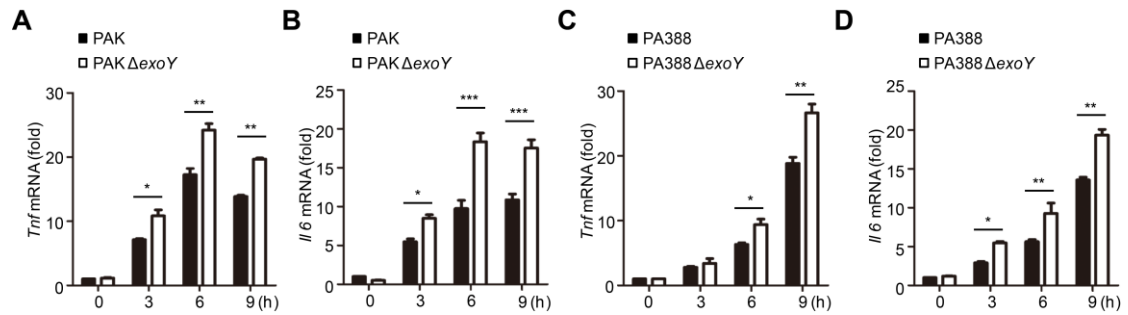


Fig. S2 *ExoY* inhibits cytokine production in epithelial cells.

(A and B) qPCR analysis of *Tnf* or *Il-6* mRNA in A549 cells infected with PAK or PAK Δ *exoY* for 0 to 9 h. (C and D) qPCR analysis of *Tnf* or *Il-6* mRNA in A549 cells infected with PA388 or PA388 Δ *exoY* for 0 to 9 h. MOI=10 in A-D. (A-D) are expressed as mean values \pm s.d. from three technical replicates. *, $p < 0.05$, **, $p < 0.01$, ***, $p < 0.001$. Data shown are representative of three independent experiments.

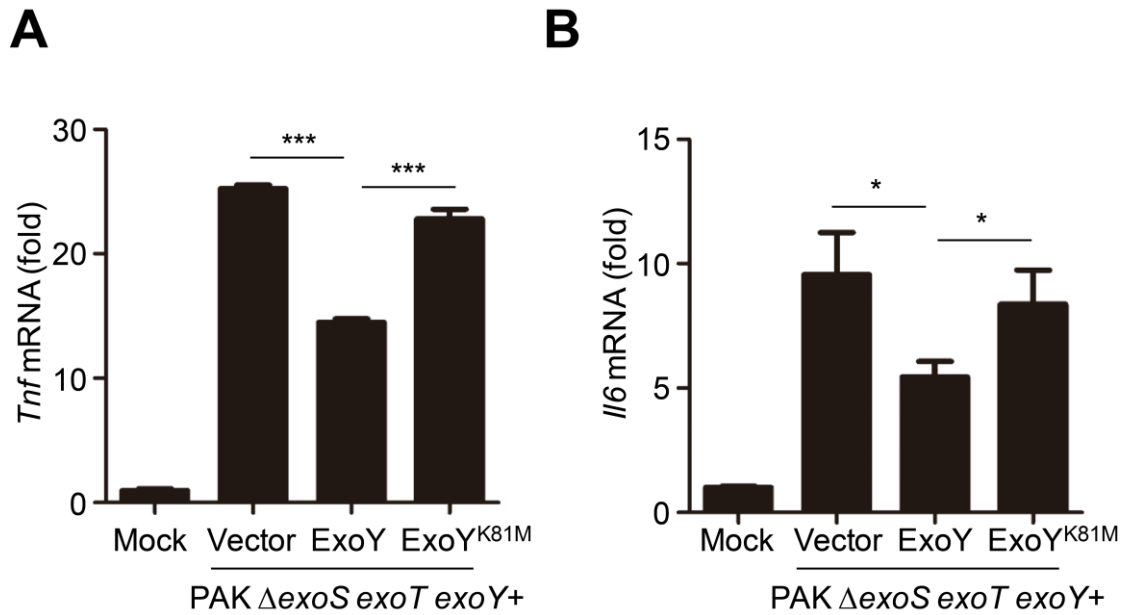


Fig. S3 The inhibitory effect of ExoY on cytokine production is dependent on nucleotidyl cyclase activity.

(A and B) qPCR analysis of *Tnf* or *Il6* mRNA in A549 cells infected with PAK Δ exoS exoT exoY(pUCP19), PAK Δ exoS exoT exoY(pUCPexoY) or PAK Δ exoS exoT exoY(pUCPexoY^{K81M}) for 6 h. MOI=10 in A and B. (A and B) are expressed as mean values \pm s.d. from three technical replicates. *, $p < 0.05$, ***, $p < 0.001$. Data shown are representative of two independent experiments.

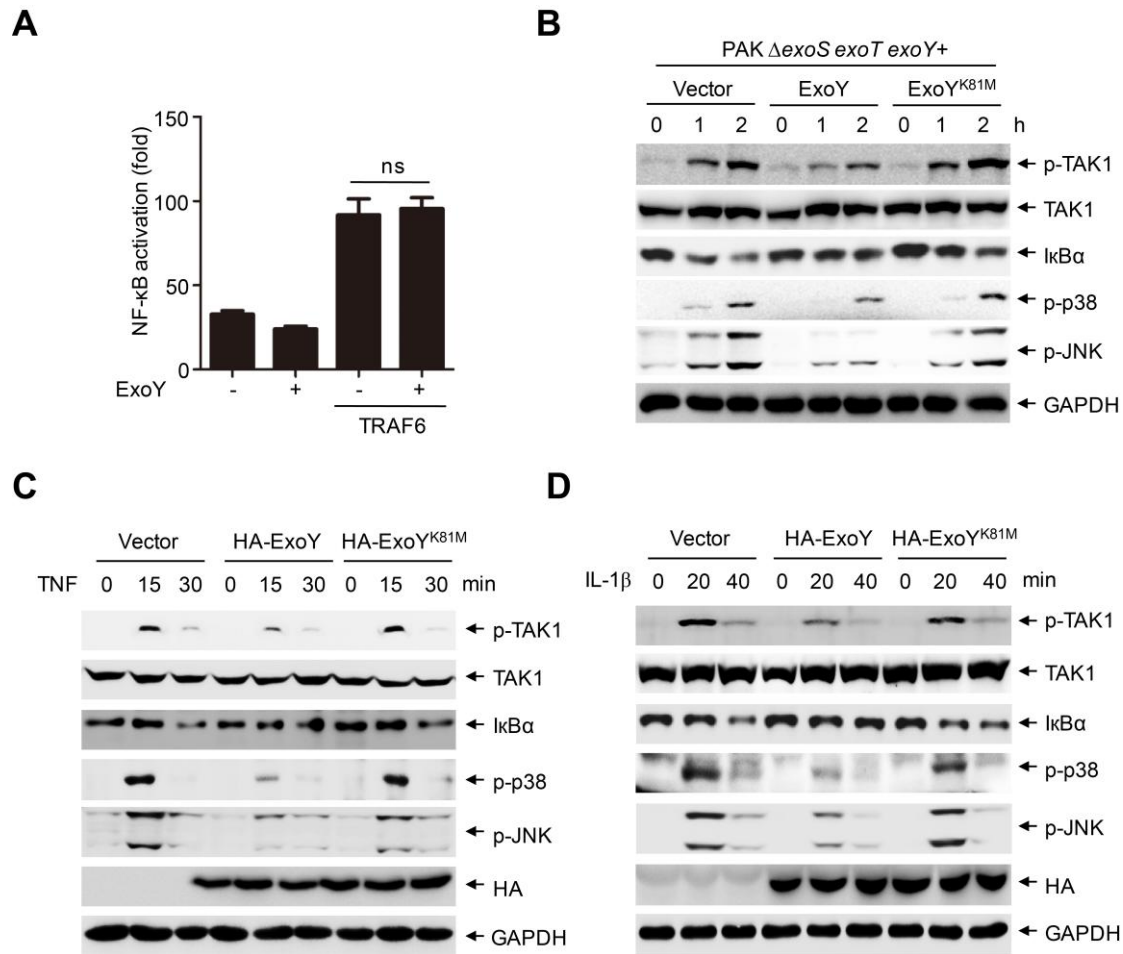


Fig. S4 ExoY suppresses the activation of TAK1.

(A) Luciferase assay of NF- κ B reporter activation in HEK293T cells transfected with various plasmids. (B) IB of lysates from A549 cells infected with PAK Δ exoS exoT exoY(pUCP19), PAK Δ exoS exoT exoY(pUCPexoY) or PAK Δ exoS exoT exoY (pUCPexoY^{K81M}) at the indicated times, MOI=10. (C) IB of lysates from HEK293T cells transfected with various plasmids, and stimulated with TNF (10ng/ml) at the indicated times. (D) IB of lysates from A549 cells transfected with various plasmids, and stimulated with IL-1 β (10 ng/ml) at the indicated times. A is expressed as mean values \pm s.d. from three technical replicates. ns, non-significant. Data shown are representative of three independent experiments (A) or two independent experiments (B-D).

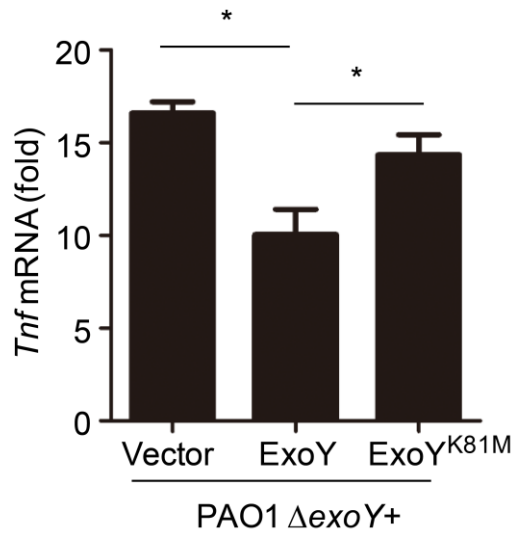
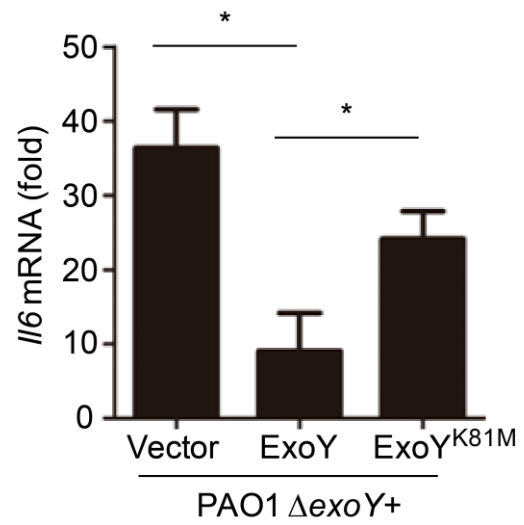
A**B**

Fig. S5 The inhibitory effect of ExoY on cytokine production is dependent on nucleotidyl cyclase activity in mice.

(**A** and **B**) qPCR analysis of *Tnf* or *Il6* mRNA in lung tissues from mice infected with 1×10^6 CFU of PAO1 Δ *exoY*(pUCP19), PAO1 Δ *exoY*(pUCP*exoY*) or PAO1 Δ *exoY*(pUCP*exoY*^{K81M}) for 2 days. n=5 per group. (**A** and **B**) are expressed as mean values \pm s.d. from five mice per group. *, $p < 0.05$. Data shown are representative of at least three independent experiments.