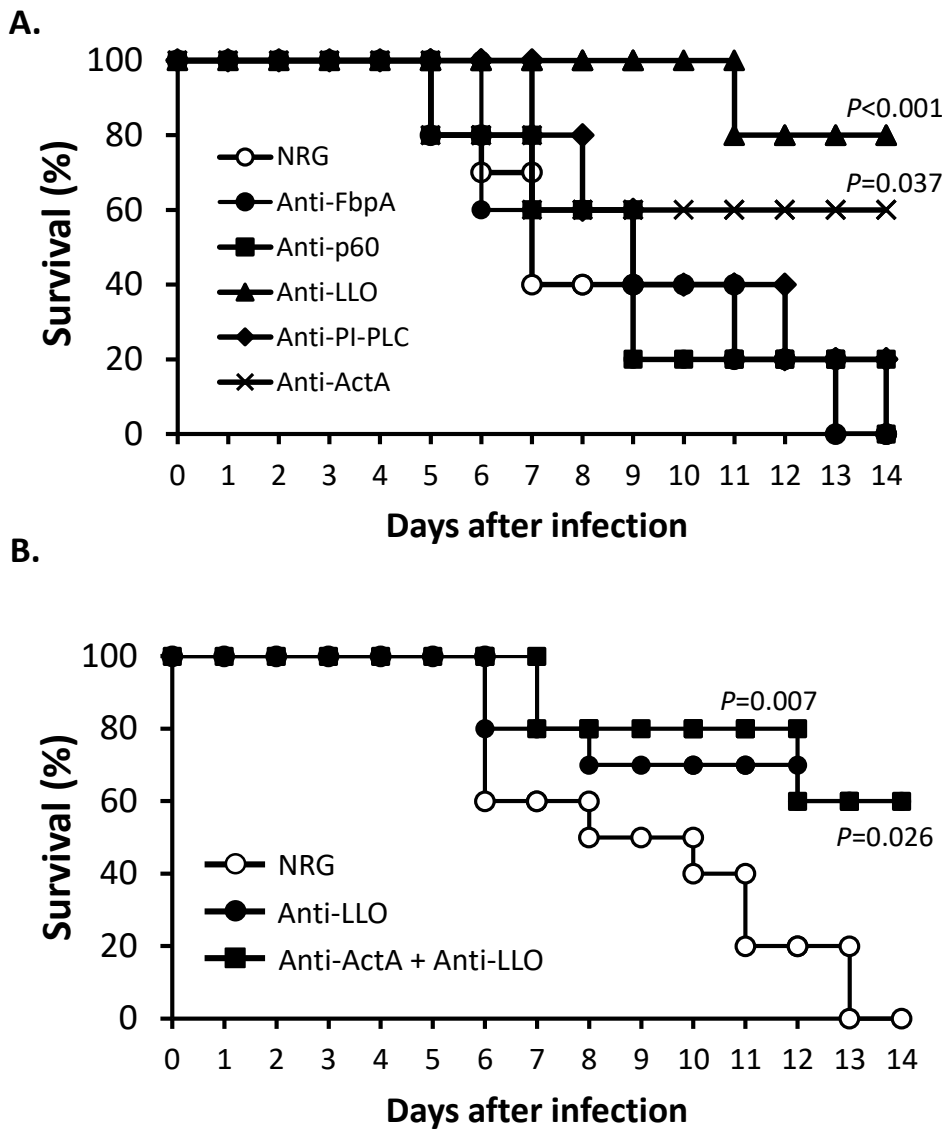


**Passive immunization with anti-ActA and anti-listeriolysin O antibodies protects against *Listeria monocytogenes* infection in mice**

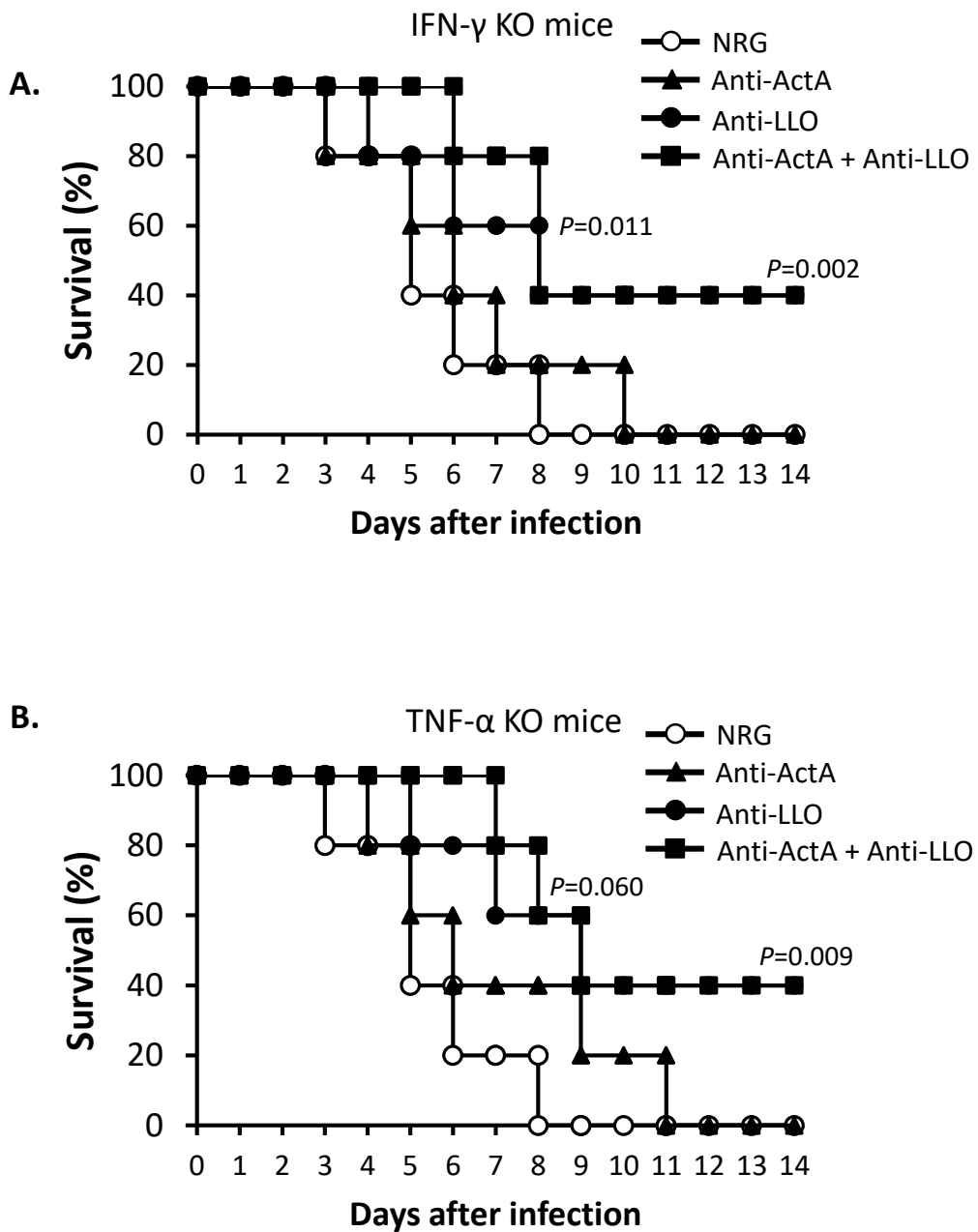
Krisana Asano<sup>1</sup>, Hiroshi Sashinami<sup>1</sup>, Arihiro Osanai<sup>1</sup>, Shouhei Hirose<sup>1</sup>, Hisaya K. Ono<sup>2</sup>, Kouji Natira<sup>3</sup>, Dong-Liang Hu<sup>2</sup>, Akio Nakane<sup>1,\*</sup>

Fig. S1 (Nakane)



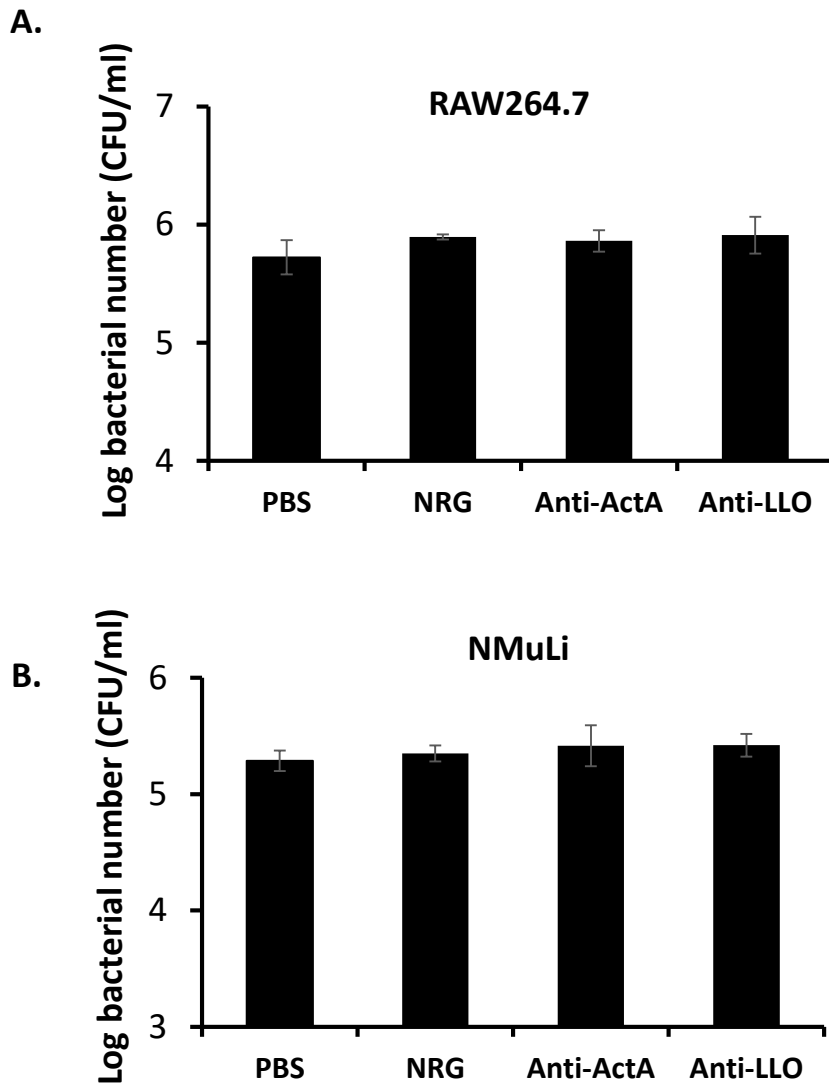
**Figure S1. Effect of listerial antigen-specific antibodies on protection of mice against listerial infection.** (A) Mice were administered intravenously with the antibody or NRG 1 mg/mouse. Mice were infected intravenously with  $1 \times 10^6$  CFU *L. monocytogenes* 24 h later. (B) Mice were infected intravenously with  $1 \times 10^6$  CFU *L. monocytogenes* for 6 h prior to intravenous administration with the antibody or NRG 1 mg/mouse. Survival was observed for 14 days (n=10). *P*-values calculated by log rank test are indicated.

Fig. S2 (Nakane)



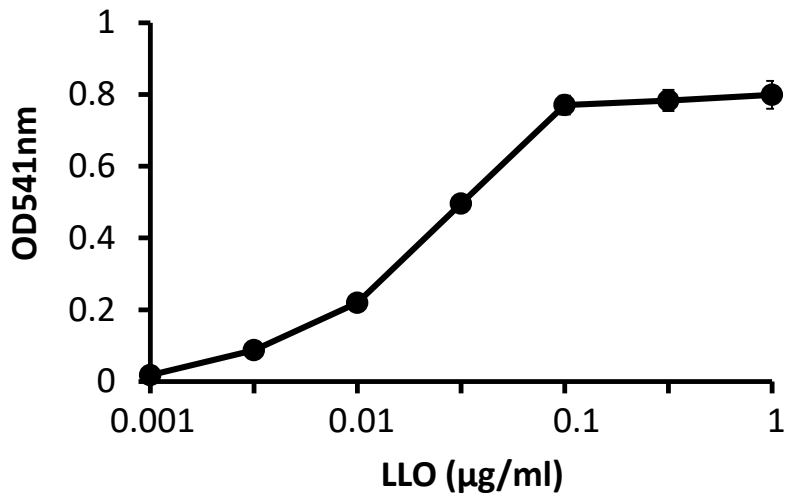
**Figure S2. Passive immunization of anti-ActA and anti-LLO antibodies partially protects mice from listerial infection in IFN- $\gamma^{-/-}$  and TNF- $\alpha^{-/-}$  mice.** (A) IFN- $\gamma^{-/-}$  and (B) TNF- $\alpha^{-/-}$  mice were administered intravenously with the antibody or NRG 1 mg/mouse. Mice were infected intravenously with  $1 \times 10^5$  CFU *L. monocytogenes* 24 h later. Survival was observed for 14 days (n=10). *P*-values calculated by log rank test are indicated.

**Fig. S3 (Nakane)**



**Figure S3. Antibodies did not promote bacterial adhesion into mouse macrophages and murine hepatocytes.** (A) RAW264.7 and (B) NMuLi cells were plated on 24-well plates at  $1 \times 10^6$  and  $5 \times 10^5$  cell/well, respectively. The cells were treated with 100  $\mu$ g/ml antibody, NRG or PBS and infected simultaneously with *L. monocytogenes*. RAW264.7 and NMuLi cells were infected with *L. monocytogenes* at MOI 10 and MOI 100, respectively. At 15 min of incubation, the non-adhesive bacterial cells were washed with PBS and the adhesive bacterial cells were enumerated on TSA plates (mean  $\pm$  S.D., n=6).

**Fig. S4 (Nakane)**



**Figure S4. Hemolytic activity of rLLO.** rLLO was diluted in PBS and incubated with sheep RBC at 37°C for 45 min. Then, lysis of RBC was determined at absorbance 541 nm (mean  $\pm$  S.D., n=6).