

Cell Host & Microbe, Volume 24

Supplemental Information

**Accelerated Aging and Clearance
of Host Anti-inflammatory Enzymes
by Discrete Pathogens Fuels Sepsis**

Won Ho Yang, Douglas M. Heithoff, Peter V. Aziz, Benjamin Haslund-Gourley, Julia S. Westman, Sonoko Narisawa, Anthony B. Pinkerton, José Luis Millán, Victor Nizet, Michael J. Mahan, and Jamey D. Marth

Figure S1

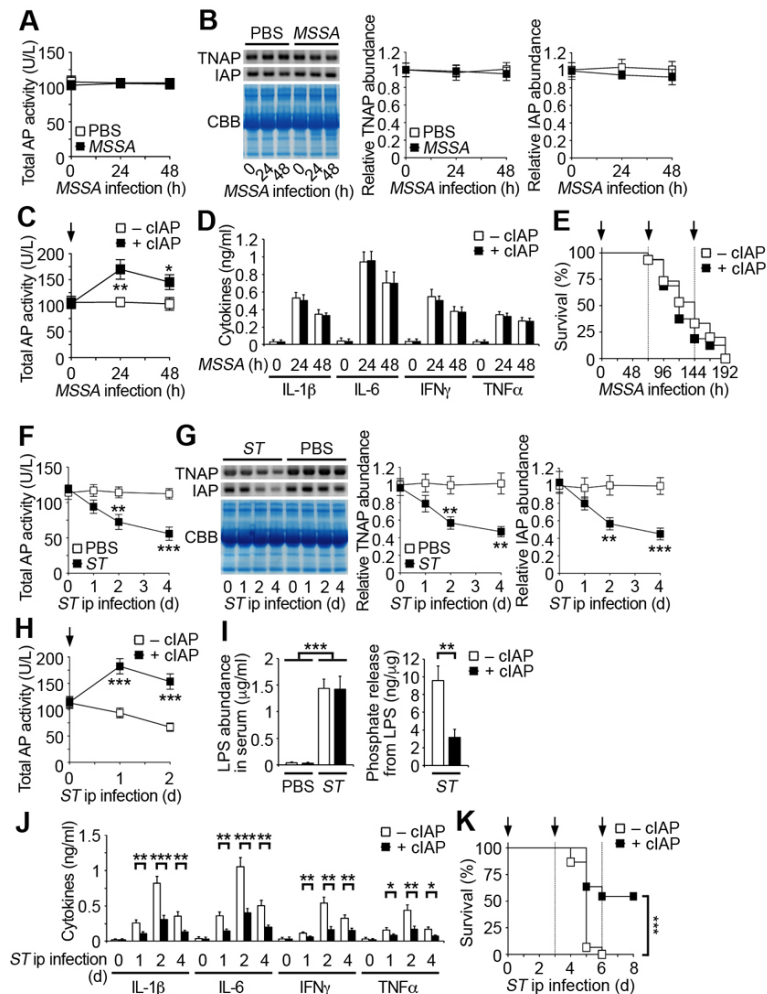


Figure S1. Regulation of AP level during sepsis induced by i.v. infection with *MSSA* or i.p. infection with *ST*. Related to Figure 1.

(A and B) Total AP activity and TNAP and IAP abundance measured in WT mouse serum after intravenous (i.v.) infection with *MSSA* (5×10^8 cfu).

(C) Serum AP activity of WT mice receiving i.v. injections of cIAP (75 U/kg) at indicated times (arrows) following infection with *MSSA*.

(D) Serum inflammatory cytokine expression of WT mice receiving i.v. injections of cIAP following infection with *MSSA*.

(E) Survival of WT mice receiving i.v. injections of cIAP at indicated times (arrows) following infection with *MSSA*.

(F and G) Total AP activity and TNAP and IAP abundance measured in WT mouse serum after i.p. infection with *ST* (10^3 cfu).

(H) Serum AP activity of WT mice receiving i.v. injections of cIAP (75 U/kg) at indicated times (arrows) following i.p. infection with *ST*.

(I) LPS abundance and phosphate amount released from LPS in serum of WT mice 48 h after i.p. infection with *ST* in the presence or absence of cIAP.

(J) Serum inflammatory cytokine expression of WT mice following i.p. infection with *ST* in the presence or absence of cIAP.

(K) Survival of WT mice receiving i.v. injections of cIAP at indicated times (arrows) following i.p. infection with *ST*.

(A) n = 10 per condition. (B–D) n = 6 per condition. (E) n = 15-16 per condition. (F–I) n = 6 per condition. (J) n = 8 per condition. (K) n = 11-15 per condition. Data are presented as means \pm SEM from two independent experiments.

Figure S2

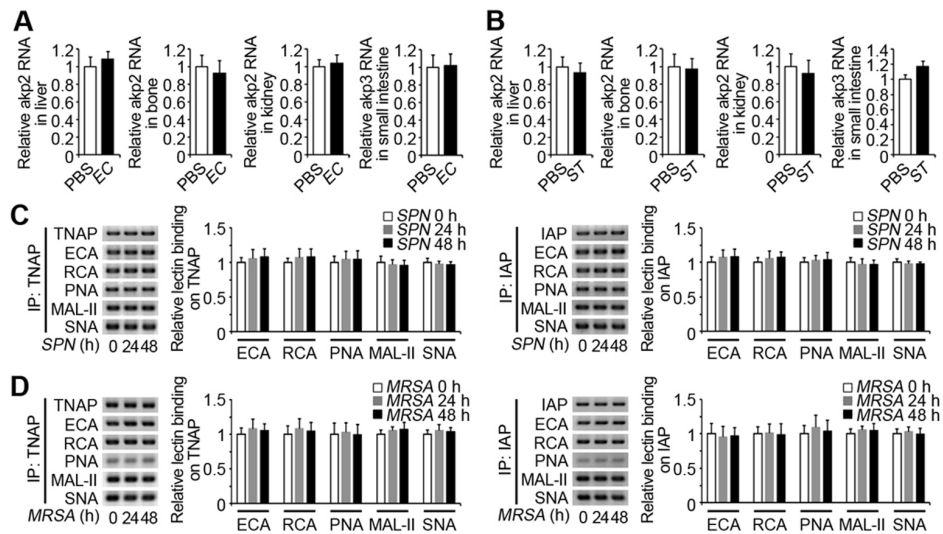


Figure S2. mRNA expression and glycosylation of TNAP and IAP during sepsis caused by different microbial pathogens. Related to Figure 1 and Figure 2.

(A and B) Relative TNAP and IAP mRNA expression among liver, bone, kidney and small intestine of *EC*-infected WT mice (12 h) or *ST*-infected WT mice (day 5) measured by qRT-PCR. TNAP is encoded by the *akp2* gene and IAP is encoded by the *akp3* gene.

(C and D) Lectin blotting analyses are presented from identical amounts of TNAP and IAP isolated from WT mouse serum after i.p. infection with *SPN* (10^4 cfu) or i.v. infection with *MRSA* (10^8 cfu).

(A–D) $n = 6$ per condition. Data are presented as means \pm SEM from two independent experiments.

Figure S3

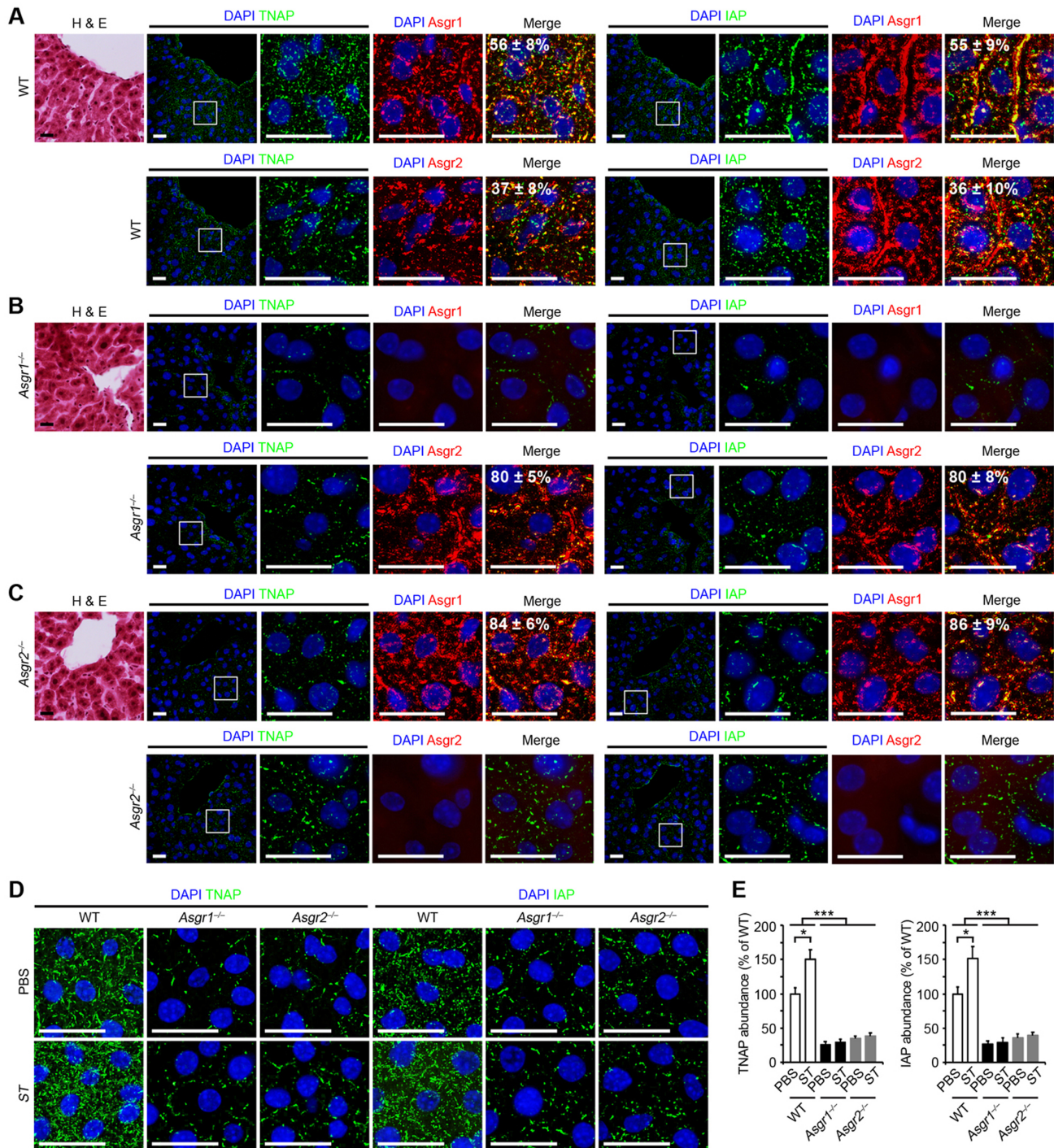


Figure S3. In-situ abundance and co-localization of TNAP and IAP with the AMR. Related to Figure 2.

(A–C) Serial liver sections from WT, *Asgr1*^{-/-}, and *Asgr2*^{-/-} mice stained with hematoxylin and eosin (H & E) or by fluorescence using antibodies specific for either TNAP or IAP. DNA is stained with DAPI. All scale bars indicate 20 μm. The percentage of TNAP or IAP in the liver

co-localized with Asgr1 or Asgr2 is indicated and representative of ten fields of view obtained from three mice of each genotype.

(D) Liver sections from *ST*-infected WT, *Asgr1*^{-/-}, and *Asgr2*^{-/-} mice (day 8) stained by fluorescence using antibodies specific for either TNAP or IAP. DNA is stained with DAPI.

(E) TNAP and IAP abundance in the liver sections of WT, *Asgr1*^{-/-} and *Asgr2*^{-/-} mice were quantified by immunohistochemistry using fluorescent signals obtained from ten fields of view among three mice of each genotype. Data are presented as means ± SEM from two independent experiments.

Figure S4

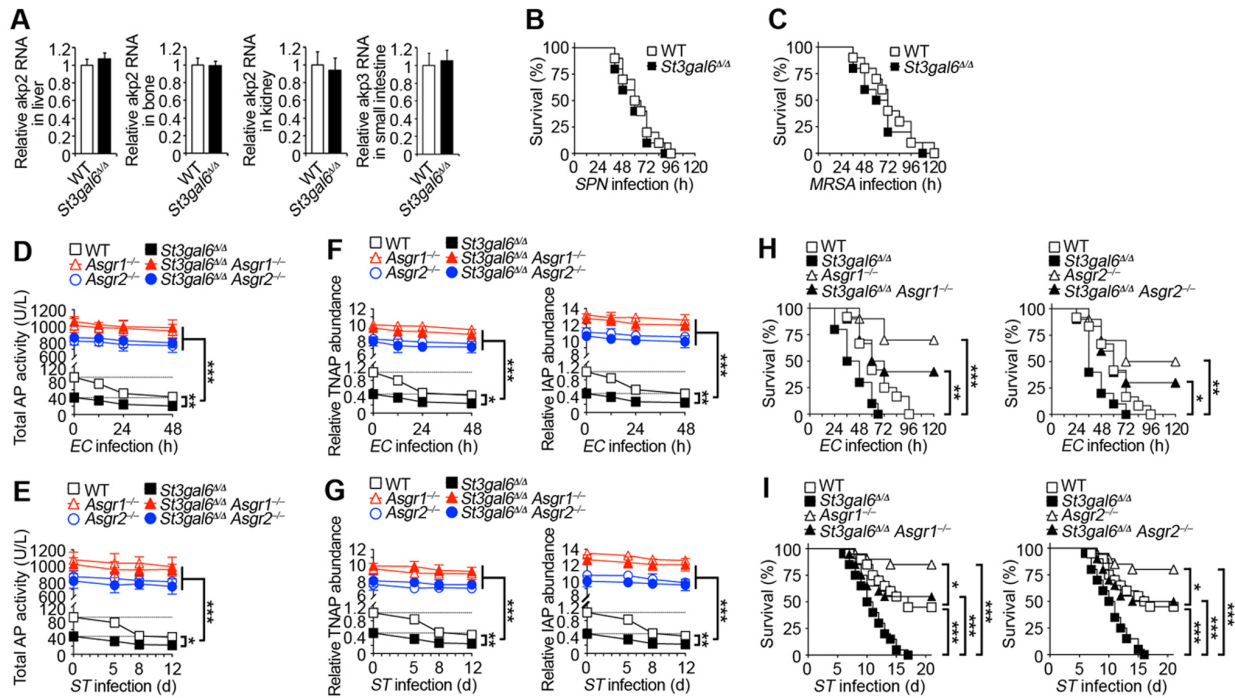


Figure S4. ST3Gal6 and AMR deficiency during sepsis caused by different microbial pathogens. Related to Figure 4.

(A) Relative TNAP and IAP mRNA expression among liver, bone, kidney and small intestine of ST3Gal6-deficient mice at 8-10 weeks old measured by qRT-PCR.

(B and C) Survival of ST3Gal6-deficient mice following i.p. infection with *SPN* (10^4 cfu), or i.v. infection with *MRSA* (10^8 cfu).

(D–G) Total AP activity and TNAP and IAP abundance measured in the serum of indicated genotypes after i.p. infection with *EC* (10^7 cfu) or oral infection with *ST* (10^7 cfu).

(H and I) Survival of indicated genotypes following i.p. infection with *EC* (10^7 cfu) or oral infection with *ST* (5×10^5 cfu).

(A) $n = 8$ per condition. (B and C) $n = 10-12$ per condition. (D–G) $n = 6$ per condition. (H) $n = 10-12$ per condition. (I) $n = 20$ per condition. Data are presented as means \pm SEM from two independent experiments.

Figure S5

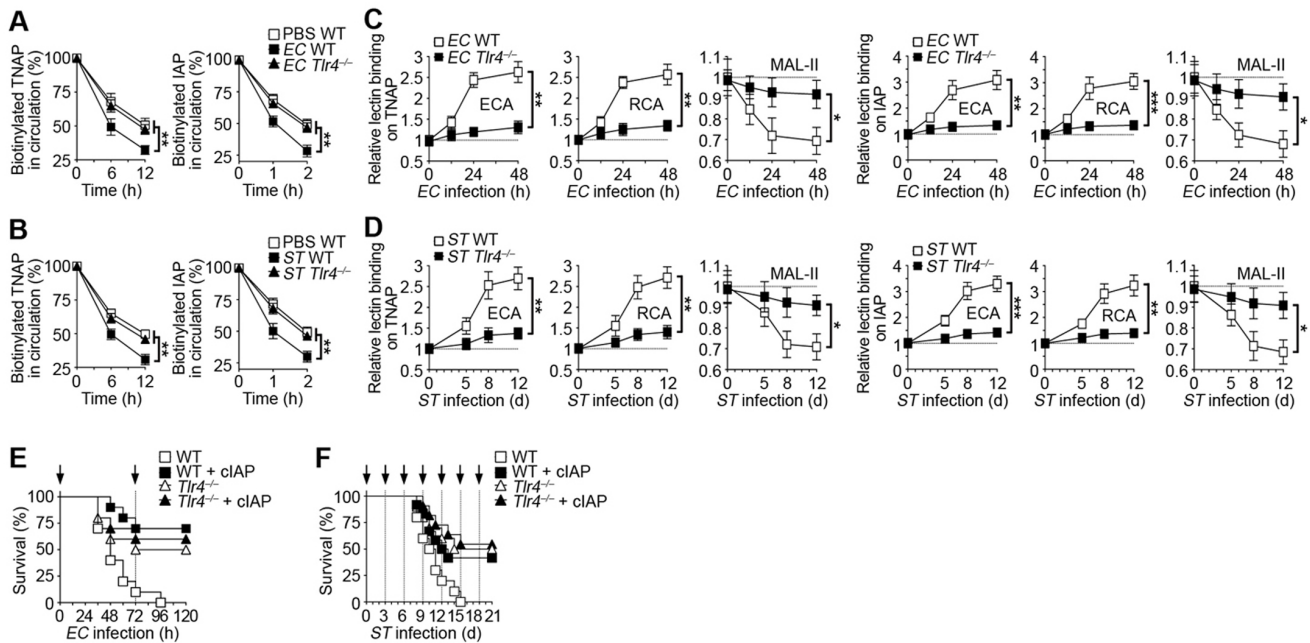


Figure S5. Half-life and lectin binding analyses of TNAP and IAP in TLR4-deficient mice during EC- and ST-induced sepsis. Related to Figure 5.

(A and B) Half-life analyses of TNAP and IAP glycoproteins in circulation of TLR4-deficient mouse serum 24 h after infection with *EC* (10^7 cfu) or day 8 after infection with *ST* (10^7 cfu) following biotinylation.

(C and D) Lectin binding on TNAP and IAP proteins isolated from TLR4-deficient mouse serum after infection with *EC* or *ST*.

(E and F) Survival of TLR4-deficient mice receiving i.v. injections of cIAP (75 U/kg) at indicated times (arrows) following i.p. infection with *EC* or oral infection with *ST*.

(A and B) $n = 8$ per condition. (C and D) $n = 6$ per condition. (E and F) $n = 10-12$ per condition. Data are presented as means \pm SEM from two independent experiments.

Figure S6

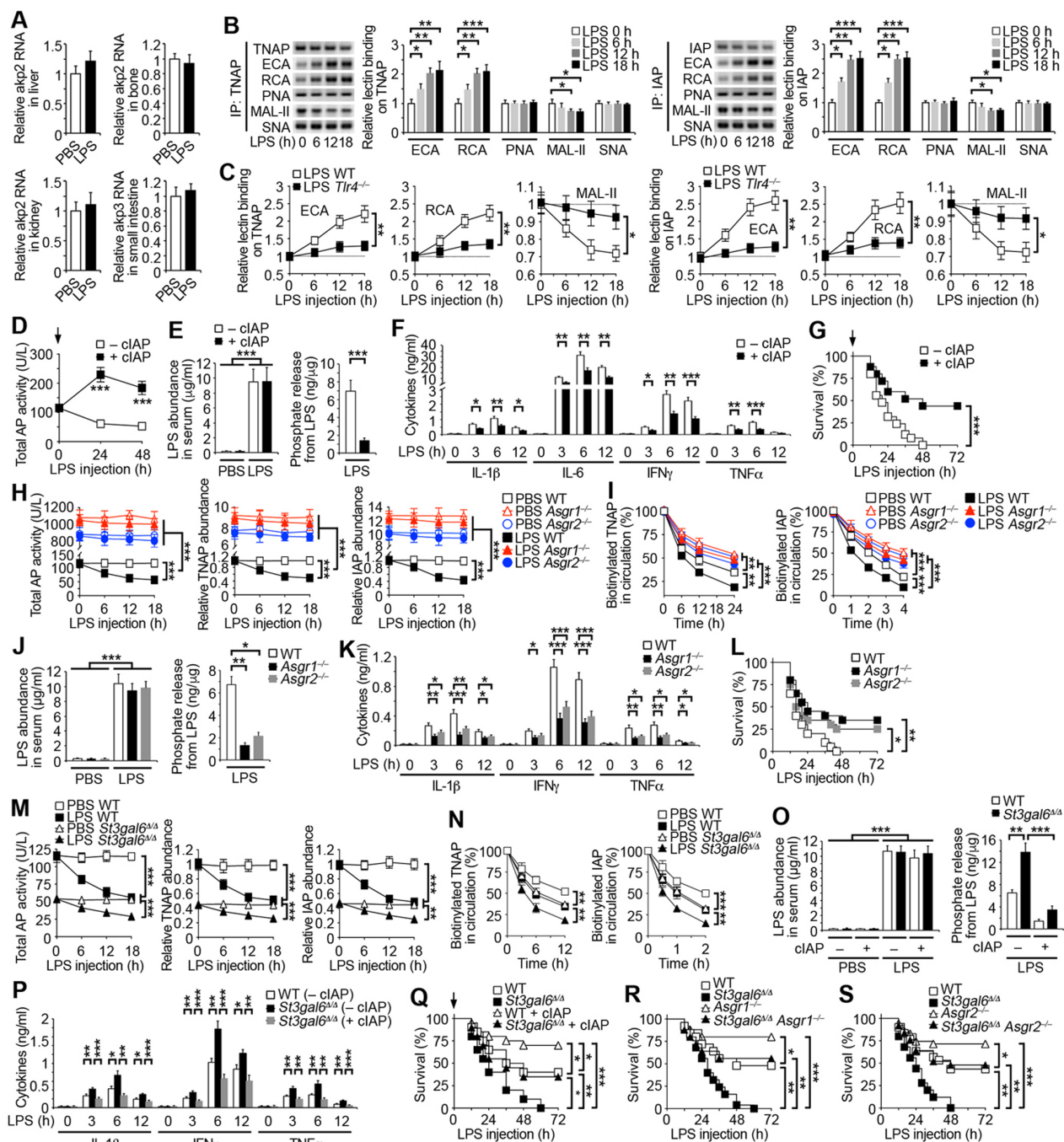


Figure S6. Roles of host TLR4, AMR, and ST3Gal6 in regulating AP levels and determining survival during LPS-induced toxicity. Related to Figure 5.

(A) Relative TNAP and IAP mRNA expression among liver, bone, kidney and small intestine of LPS-injected WT mice (6 h) measured by qRT-PCR.

- (B) Lectin blotting analyses are presented from identical amounts of TNAP and IAP isolated from WT mouse serum after i.p. injection with LPS (40 mg/kg).
- (C) Lectin binding analyses of TNAP and IAP isolated from TLR4-deficient mouse serum after i.p. injection with LPS (40 mg/kg).
- (D) Serum AP activity of WT mice receiving i.v. injections of cIAP (75 U/kg) at indicated times (arrows) following i.p. injection with LPS (40 mg/kg).
- (E) LPS abundance and phosphate amount released from LPS in serum of WT mice 12 h after LPS injection (40 mg/kg) in the presence or absence of cIAP.
- (F) Serum inflammatory cytokine expression of WT mice following i.p. injection with LPS (40 mg/kg) in the presence or absence of cIAP.
- (G) Survival of WT mice following i.v. injections of cIAP at indicated times (arrows) and i.p. injection with LPS (40 mg/kg).
- (H) Total AP activity and TNAP and IAP abundance in AMR-deficient mouse serum after i.p. injection with LPS (40 mg/kg).
- (I) Half-life analyses of TNAP and IAP glycoproteins in circulation of AMR-deficient mouse serum 12 h after i.p. injection with LPS (40 mg/kg) following biotinylation.
- (J) LPS abundance and phosphate amount released from LPS in serum of AMR-deficient mice 12 h after LPS injection (40 mg/kg).
- (K) Serum inflammatory cytokine expression in AMR-deficient mouse serum following i.p. injection with LPS (40 mg/kg).
- (L) Survival of AMR-deficient mice following i.p. injection with LPS (40 mg/kg).
- (M) Total AP activity and TNAP and IAP abundance in ST3Gal6-deficient mouse serum after i.p. injection with LPS (40 mg/kg).
- (N) Half-life analyses of TNAP and IAP glycoproteins in circulation of ST3Gal6-deficient mouse serum 12 h after i.p. injection with LPS (40 mg/kg) following biotinylation.
- (O) LPS abundance and phosphate amount released from LPS in serum of ST3Gal6-deficient mice 12 h after LPS injection (40 mg/kg).
- (P) Serum inflammatory cytokine expression in ST3Gal6-deficient mouse serum following i.p. injection with LPS (40 mg/kg).
- (Q) Survival of ST3Gal6-deficient mice after i.p. injection with LPS (20 mg/kg) in the absence or presence of cIAP (75 U/kg; arrow).
- (R and S) Survival of ST3Gal6 and AMR double-deficient mice after i.p. injection with LPS (20 mg/kg).
- (A–E, H, M) n = 6 per condition. (F, I–K, N–P) n = 8 per condition. (G, L, Q–S) n = 20–25 per condition. Data are presented as means \pm SEM from two independent experiments.

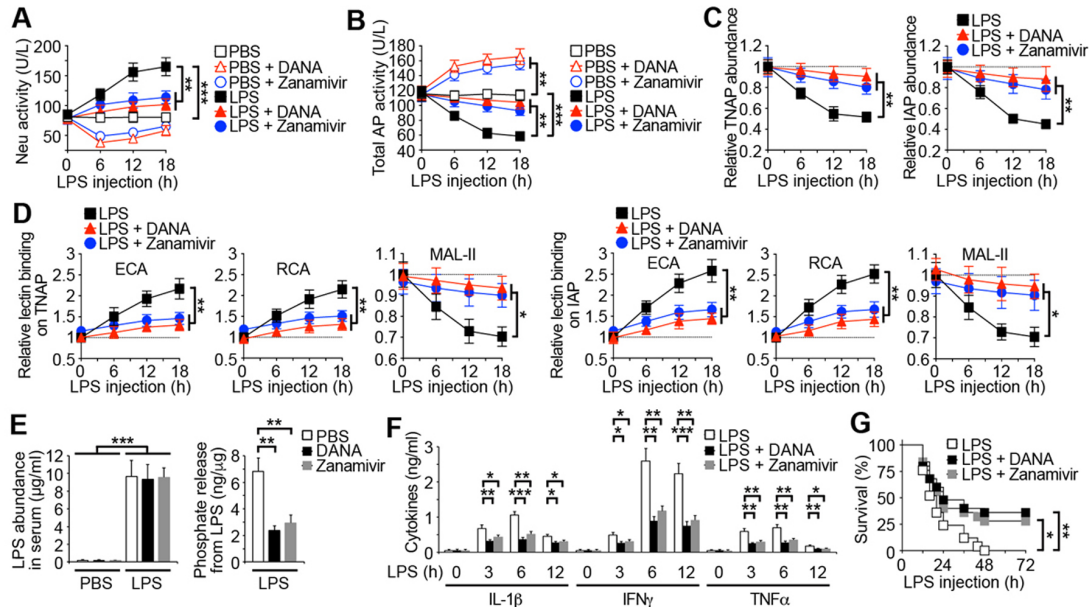


Figure S7. Therapeutic effects of neuraminidase inhibitors on LPS-induced toxicity.

Related to Figure 6.

(A) Total Neu activity measured in WT mouse serum after i.p. injection with LPS (40 mg/kg) in the absence or presence of the broad-spectrum neuraminidase inhibitor DANA (250 mg/kg) or Zanamivir (250 mg/kg) delivered immediately post-infection by i.v. injection.

(B and C) Total AP activity and TNAP and IAP abundance measured in WT mouse serum after i.p. injection with LPS in the presence or absence of DANA or Zanamivir.

(D) Lectin binding analyses of TNAP and IAP in WT mouse serum after i.p. injection with LPS in the presence or absence of DANA or Zanamivir.

(E) LPS abundance and phosphate amount released from LPS in serum of WT mice 12 h after LPS injection in the presence or absence of DANA or Zanamivir.

(F) Serum inflammatory cytokine expression in WT mouse serum following i.p. injection with LPS in the presence or absence of DANA or Zanamivir.

(G) Survival of WT mice (n = 20-25 per condition) after i.p. injection with LPS in the absence or presence of DANA (250 mg/kg, every 24 h) or Zanamivir (250 mg/kg, every 24 h).

(A–D, F) n = 6 per condition. (E) n = 10 per condition. (G) n = 25 per condition. Data are presented as means ± SEM from two independent experiments.