A group A *Streptococcus* ADP-ribosyltransferase toxin stimulates a protective IL-1β-dependent macrophage immune response

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SUPPLEMENTAL FIGURE LEGENDS

Figure S1. SpyA is highly expressed in a hyperinvasive animal-passaged M1T1 GAS strain. (A) Real-time qPCR of SpyA transcript level in non-animal passaged (non- AP) strains and animal passaged (AP) GAS strains (B) Plasmid based complementation of the ΔspyA mutant leads to high level SpyA mRNA expression Error bar = S.E.M. ***P < 0.001; n = 3, Student's unpaired two-tailed t-test. (C) Western blot illustrating SpyA protein expression in the membrane fraction of WT + pSpyA, ΔspyA and ΔspyA + pSpyA GAS (AP), using rabbit anti-SpyA antibody.

Figure S2. Increased ΔspyA GAS survival in macrophage killing assays. Murine macrophage J774 was infected with GAS M1 (animal passaged) at MOI~15 for total killing (A) and intracellular killing (B) assays. For total killing assays, macrophages were grown in media supplemented in 2% FBS and CFUs were recovered at 30 min intervals as indicated. To assess intracellular killing, cells were infected for 30 min followed by 1 h of gentamicin treatment (100μg/ml) (t=0) and incubated in serum-free media for an additional 1h- 4h before cells were lysed to recover internalized bacteria. (C) Number of bacterial CFU recovered per 100 of BMDM after gentamicin treatment. 5 x 10^5 BMDMs in 24 wells were infected with ~2 x 106 CFU (pooled result from 3 experiments). Error bar = S.E.M; *P < 0.05, **P < 0.01; ***P < 0.001; Student's unpaired two tailed t-test,

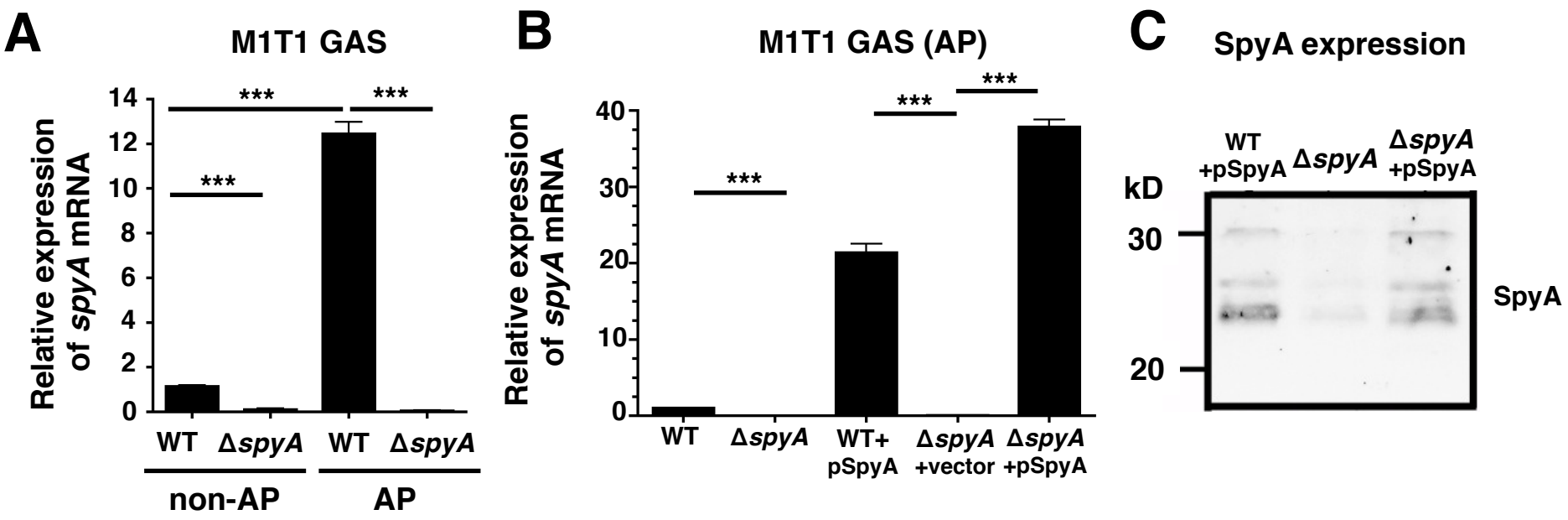
Figure S3. SpyA transcript level in *S. aureus* and J774 murine macrophages. Real-time qPCR was used to assess level of SpyA transcripts in (A) Staphylococcus aureus RN4220 (a laboratory attenuated strain) was transformed with pSpyA or pDCerm (empty vector). RNA of S. aureus was isolated for real-time qPCR to evaluate level of SpyA mRNA and (B) murine macrophages J774 that were transfected with SpyA expressing vectors via JetPEI (Polyplus) (n=6). (C) Relative LDH released by J774 transfected with DsRed(control), DsRed-SpyA or DsRed-SpyA with point mutation at E187A, after 4 h infection (D) Growth curve of S. aureus RN4220 +pDCerm (vector) and S. aureus RN4220 + pSpyA in 37°C stationary culture (n=3). (E) Relative LDH released by BMDM after 4 h infection with *S. aureus* RN4220 expressing control vector, pSpyA or filter-sterile overnight *S. aureus* + pSpyA culture (sup) (n=4). Error bar= S.E.M., ***P < 0.001; ** P < 0.01, * P < 0.05, N.S = not significant Student's unpaired t- test, two-tailed.

Figure S4. Caspase-1 and GAS co-localization. Representative image illustrates co- localization of caspase-1 active cells (FMK-YVAD-FMK, green) with GAS (M1, red) after 2 h intracellular killing. DAPI stains cell and bacterial nuclei. Scale bar= 20 μm.

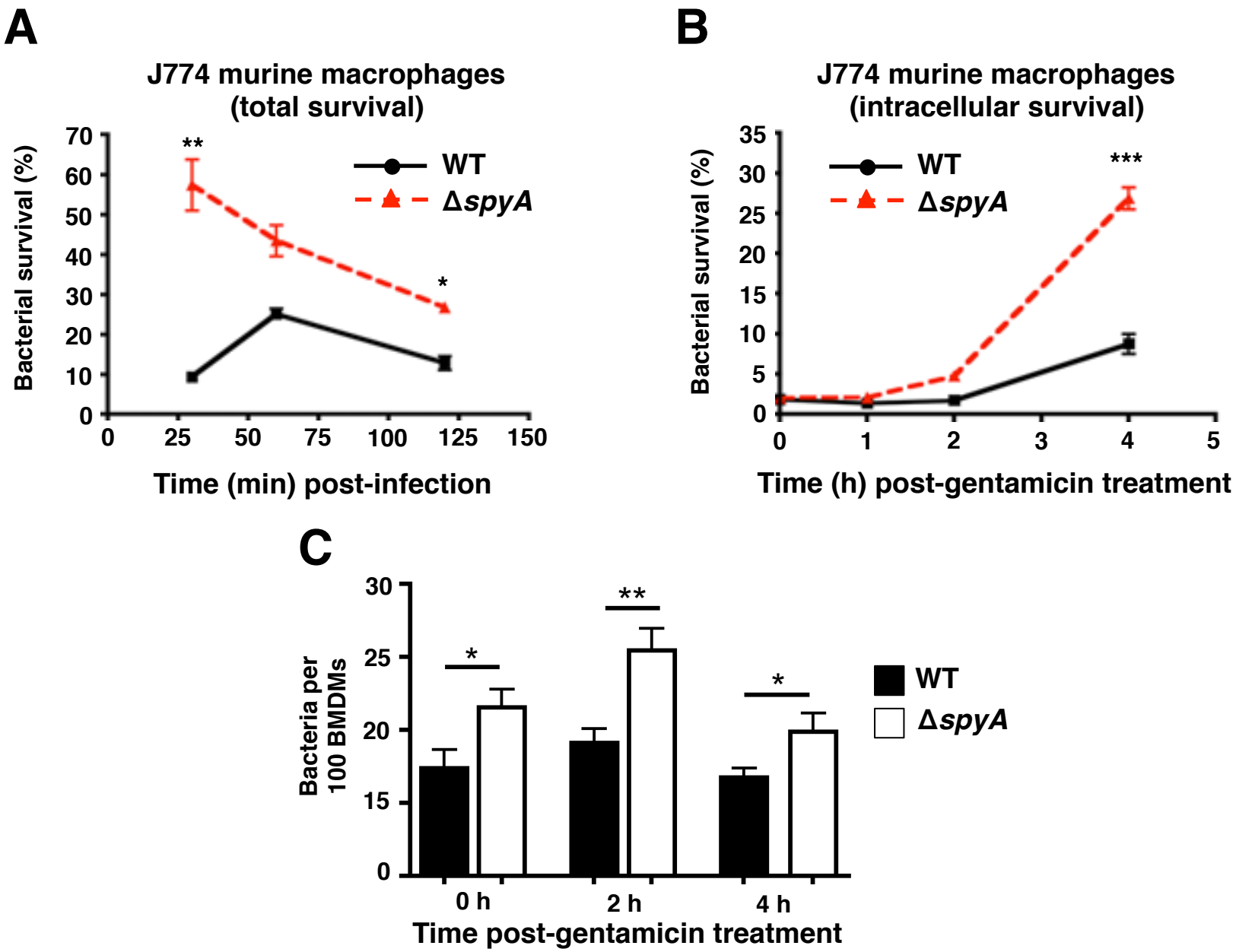
Figure S5. Bacterial recovery from GAS infected mice at early time points. CD1 mice were infected with 107 CFU of GAS (AP) WT or ΔspyA (n=7-8) for 6, 12 and 24 h. Higher CFUs were recovered from spleens (A) and blood (B) of ΔspyA-infected animals 12 h and 24 h post-infection, respectively. Error bar = S.E.M; *$P < 0.05$; Student's unpaired t-test.

Figure S6. Controls for caspase-1 detection assays. BMDMs from WT, Casp-1-/- or Nlrp3 -/- C57BL/6 mice were infected with GAS for 2 h at MOI~25 (A) Western blot illustrating level of pro-caspase1 protein in WT and Casp-1-/- BMDM whole cell lysates. (B) FAM YVAD-FMK staining identifies active Casp-1 in WT BMDM but not in Caspase-1-/- and Nlrp3-/- BMDMs infected with GAS (20x magnification). Images are representations of more than 5 random fields of views of views per sample (n=3).

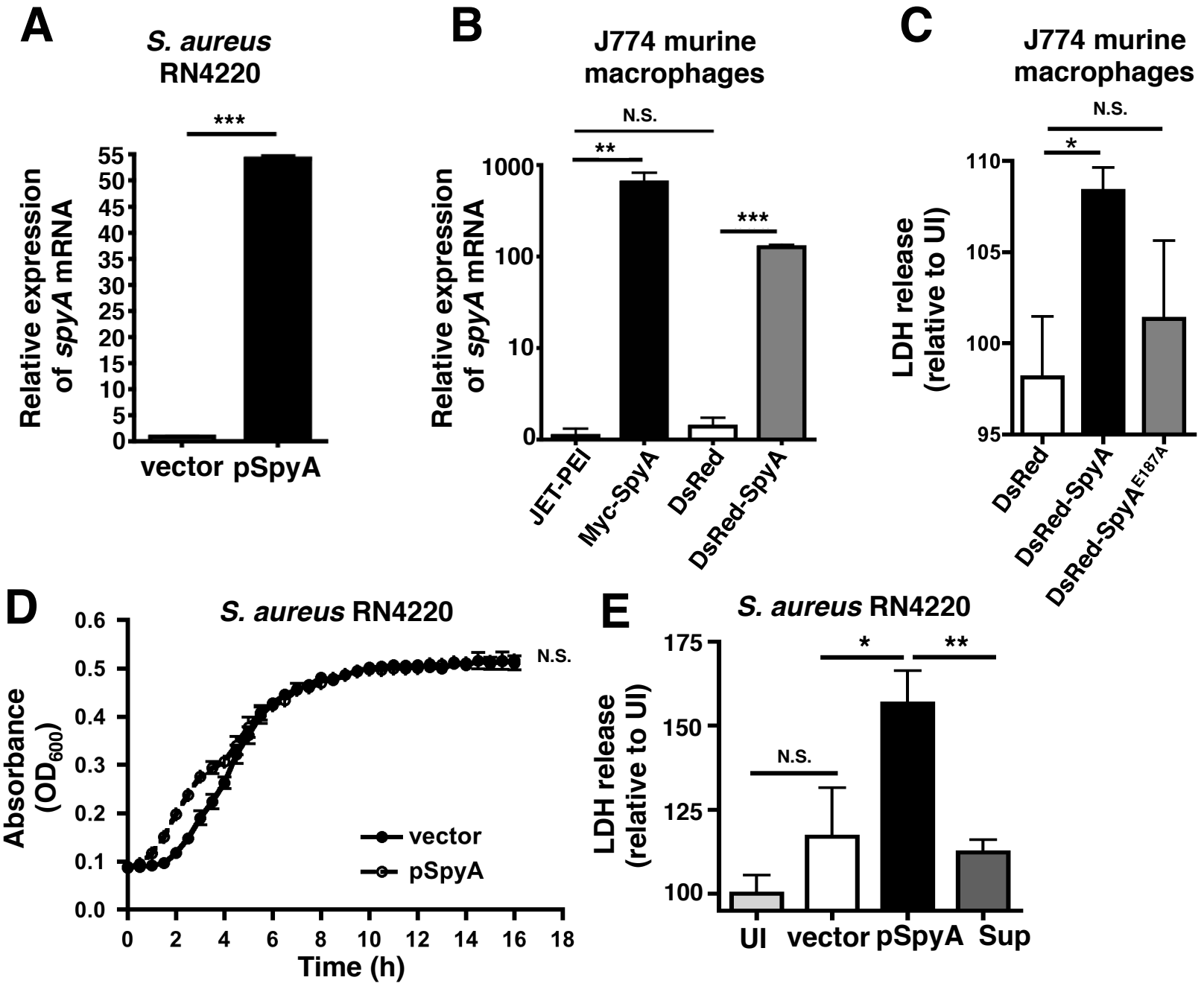
Supplemental Figure S1



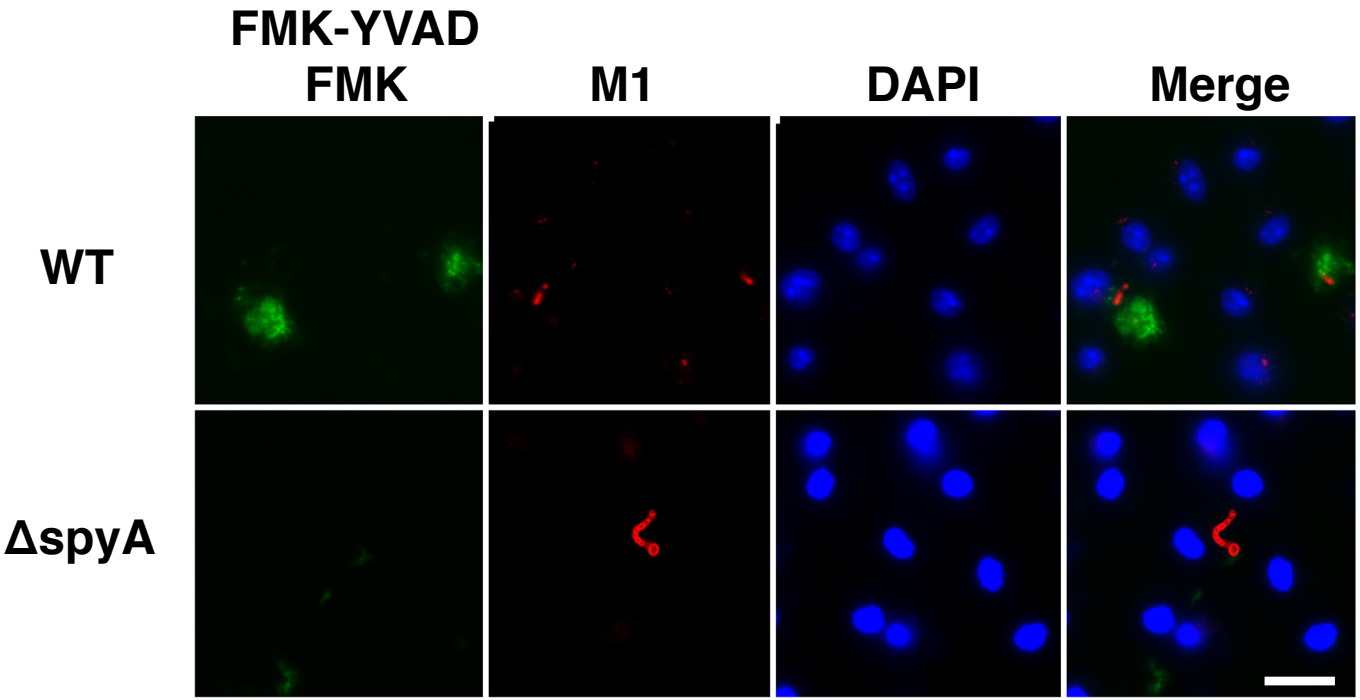
Supplemental Figure S2



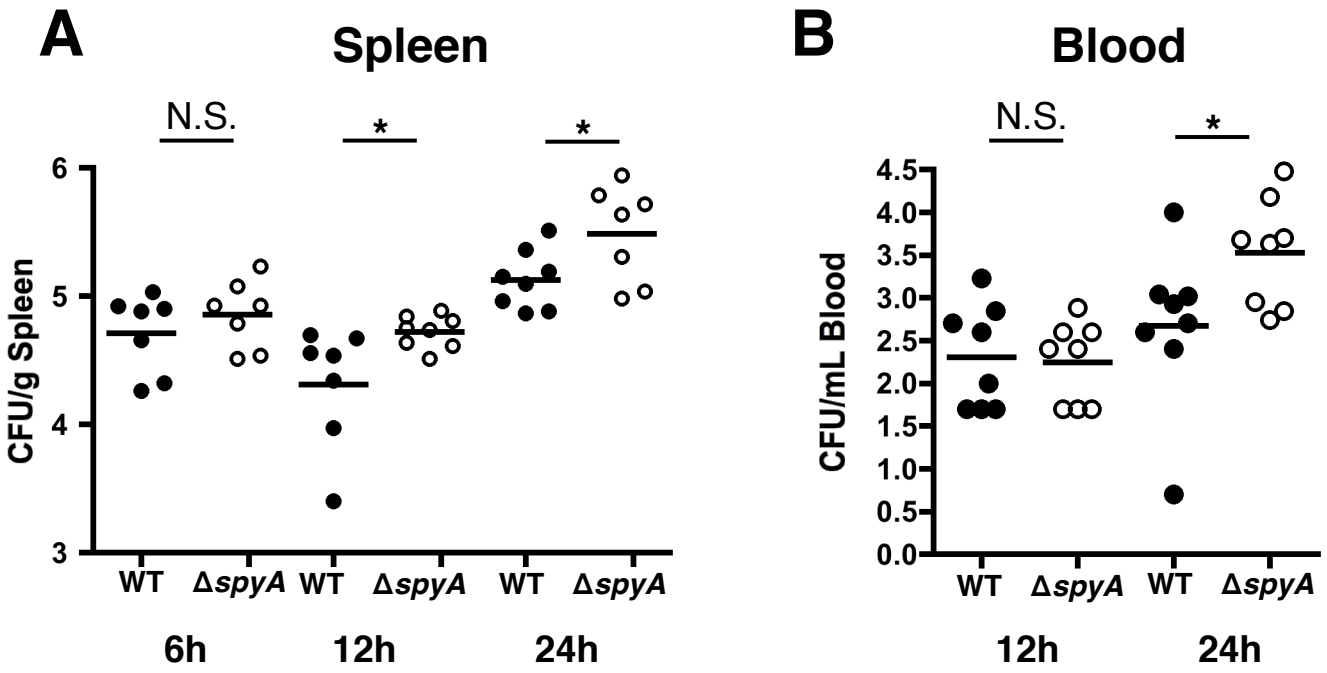
Supplemental Figure S3



Supplemental Figure S4



Supplemental Figure S5



Supplemental Figure S6