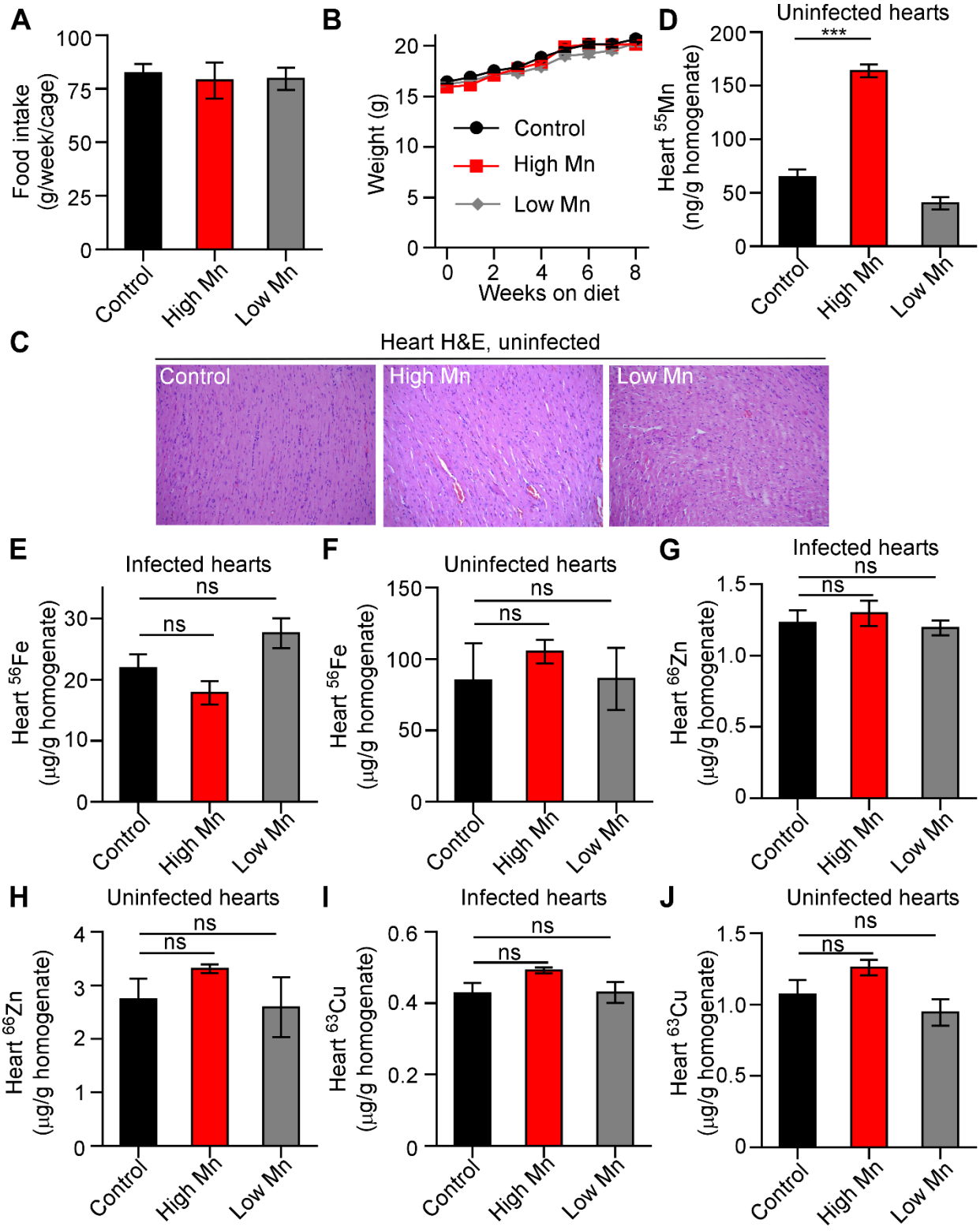
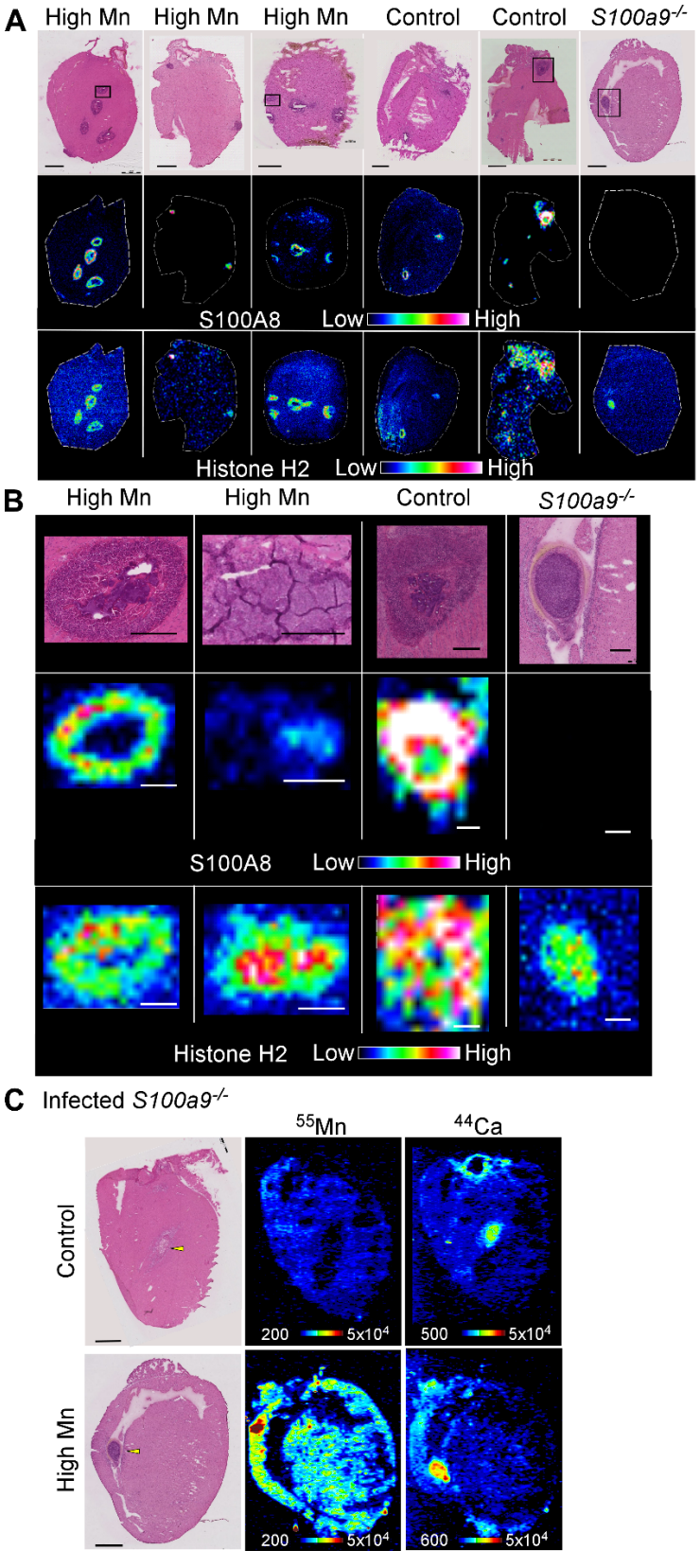


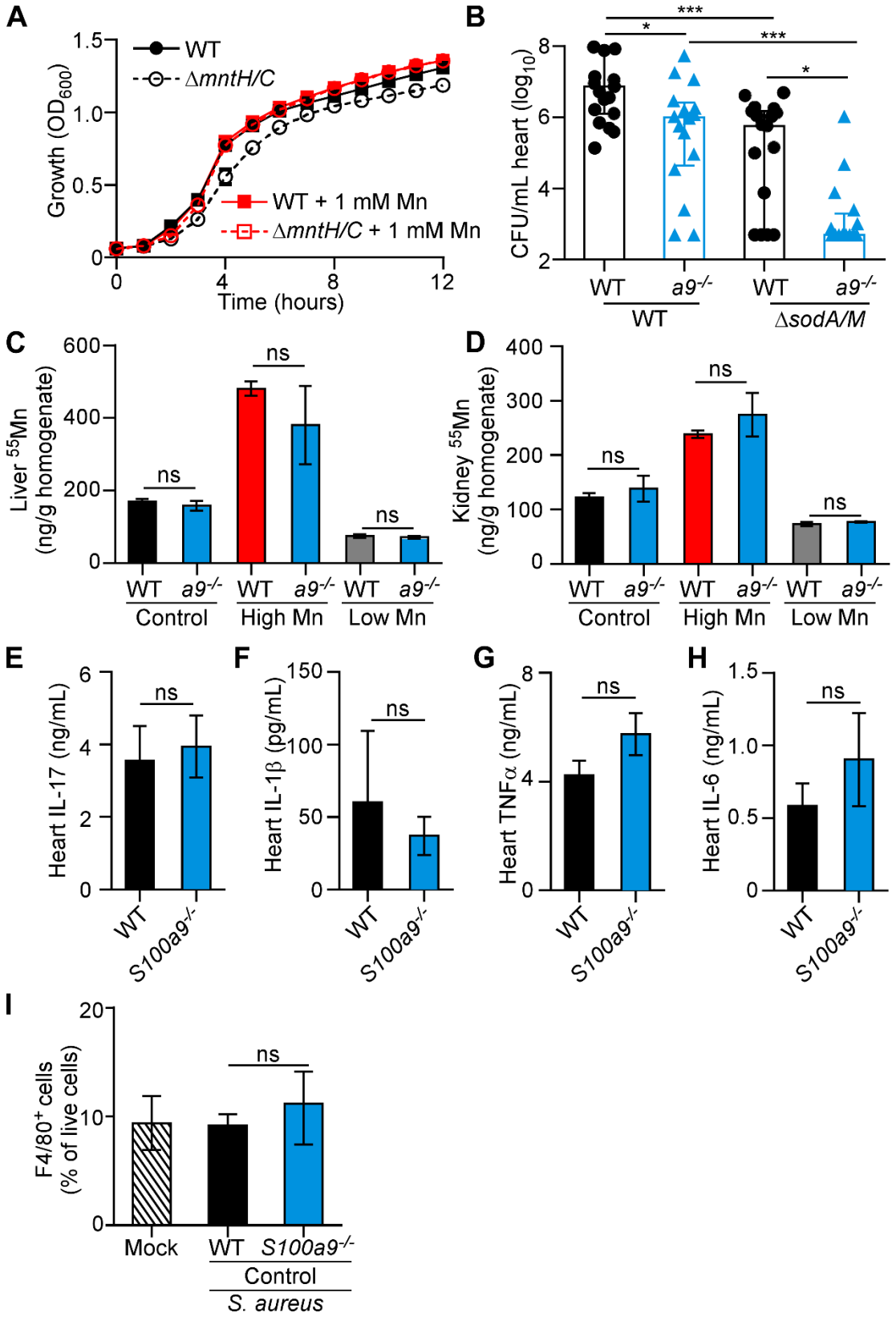
Supplementary Figure 1

Supplementary Figure 1. Impact of dietary Mn on health and tissue metal levels, related to Figure 1. C57BL/6 (WT) mice were fed control, high Mn, or low Mn diet for eight weeks. **(A)** Average food intake (grams consumed/cage/week) over the diet timecourse. $N = 2$ cages per diet with diet weight measured once a week for eight weeks. Bars indicate mean and standard deviation. **(B)** Weight of mice over the diet timecourse. $N = 10$. Bars indicate mean and standard deviation. **(C)** Hematoxylin and eosin stained heart sections from uninfected mice fed control, high Mn, or low Mn diet for eight weeks. No histopathologic differences between groups were noted by a pathologist. Representative images are shown. These findings were replicated in three separate experiments. (**D-J**) Metals were assayed in heart homogenates by ICP-MS in uninfected mice or mice infected with *S. aureus* after 6 weeks on custom-synthesized diets. **(D)** Mn concentrations in uninfected hearts. **(E)** Fe levels in infected hearts. **(F)** Fe levels in uninfected hearts. **(G)** Zn levels in infected hearts. **(H)** Zn levels in uninfected hearts. **(I)** Copper (Cu) levels in infected hearts. **(J)** Cu levels in uninfected hearts. (**D-J**) $N = 3$. Bars depict mean and SEM. *** $P < 0.001$, ns = not significant by ANOVA with Dunnett's multiple comparisons test.



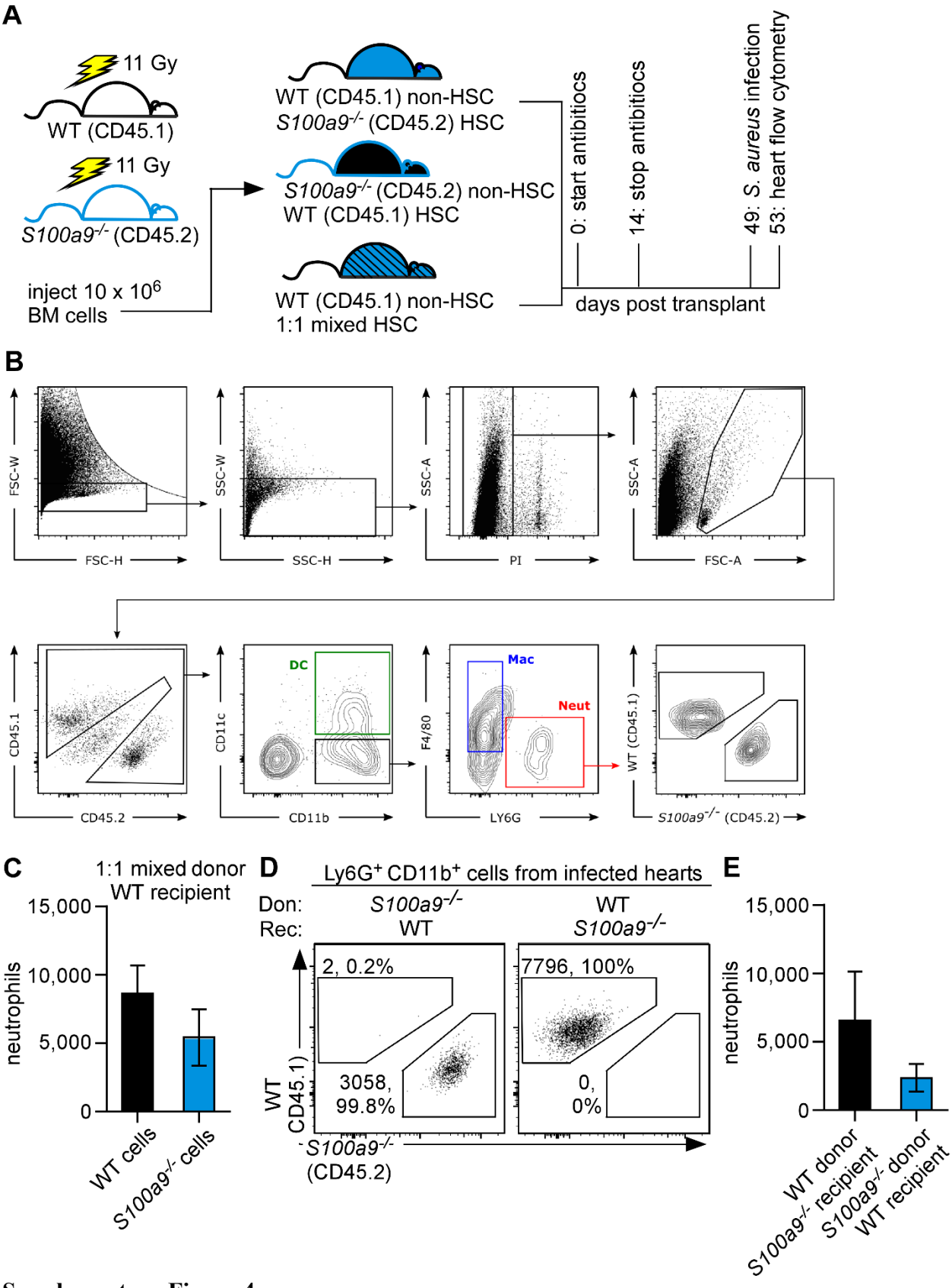
Supplementary Figure 2

Supplementary Figure 2. Imaging of WT and *S100a9*$^{-/-}$ hearts, related to Figures 3 & 6. (**A-B**) MALDI-IMS imaging of S100A8 and histone H2 in hearts from WT mice that were fed high Mn or control diet and infected with *S. aureus* for 4 days. One infected *S100a9*-/- heart is included as a control. H&E staining is shown for comparison. **(A)** Scale bars are 1 mm. (**B**) High power view of area inside the outlined box. Scale bars are 200 μm. Images were acquired at separate times; thus, the heat maps intensities are relative and a given color cannot be directly compared across images. **(C)** *S100a9*$^{-/-}$ mice were fed high Mn or control diet and infected with *S. aureus* WT for 4 days. Relative concentrations of Mn and Ca in infected heart sections were assessed by LA-ICP-MS. H&E stained serial sections are shown to the left. Scale bar is 1 mm.



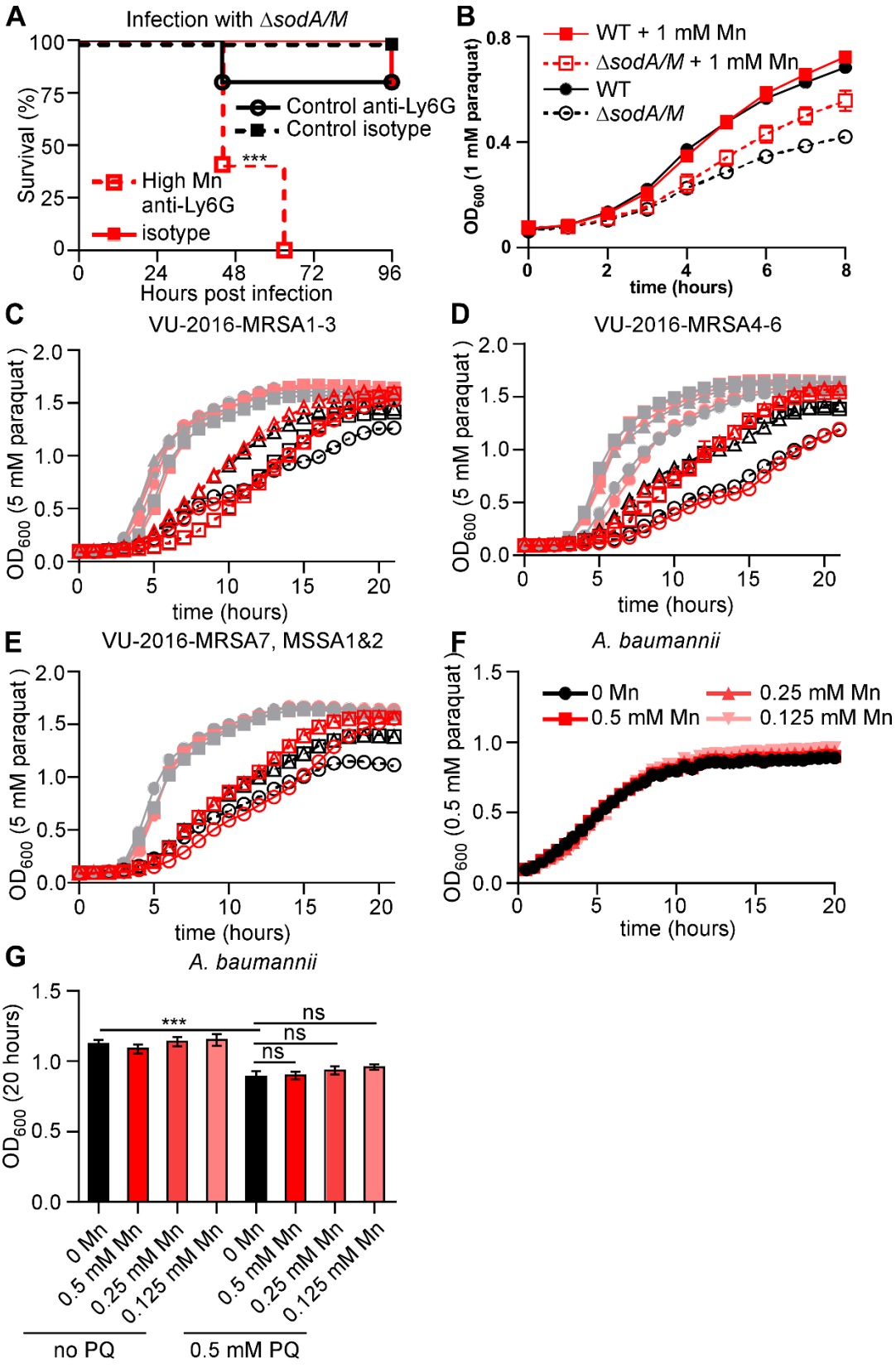
Supplementary Figure 3

Supplementary Figure 3. Metal levels and inflammation in *S100a9*$^{-/-}$ mice, related to Figures 4 & 5. (A) Growth of *S. aureus* WT or Δ*mntH/C* with or without the addition of 1 mM $MnCl_2$. Growth was measured by OD_{600} over time. Data are combined from three independent experiments and depict the mean and S.E.M. (**B**) Heart bacterial burdens from WT and *S100a9*$^{-/-}$ mice provided normal chow and infected for 4 days with *S. aureus* WT or Δ*sodA/M*. $N = 14$. *, $P < 0.05$; ***, $P < 0.001$ by one-way ANOVA with Tukey's multiple comparisons test. (**C-D**) Mn concentrations in infected homogenates were measured by inductively-coupled plasma mass spectrometry (ICP-MS). Organs were harvested from WT or *S100a9*$^{-/-}$ (*a9*$^{-/-}$) mice fed high Mn, control, or low Mn diet and infected with *S. aureus* for 4 days. (**C**) Liver. (**D**) Kidney. $N = 3$. Bars depict mean and SEM. ns = not significant by ANOVA with Sidak's multiple comparisons test. (**E-K**) WT and *S100a9*$^{-/-}$ mice were infected with *S. aureus,* hearts were harvested 24 hours post-infection, and the indicated cytokines were measured in supernatants harvested from heart homogenates: (**E**) IL-17; (**F**) IL-1β; (**G**) TNFα; (**H**) IL-6. $N = 9$ (*S100a9*$^{-/-}$), 10 (WT). (**I**) Flow cytometry quantification of F4/80+ cells from hearts harvested from WT and *S100a9*$^{-/-}$ mice infected with *S. aureus* for four days. Previous gate, live cells. $N = 2$ (mock), 3 (*S100a9*$^{-/-}$), 3 (WT). (**E-H**) Bars depict mean and SEM. (**I**) Bars and error are median and interquartile range. ns = not significant by Student's *t*-test.



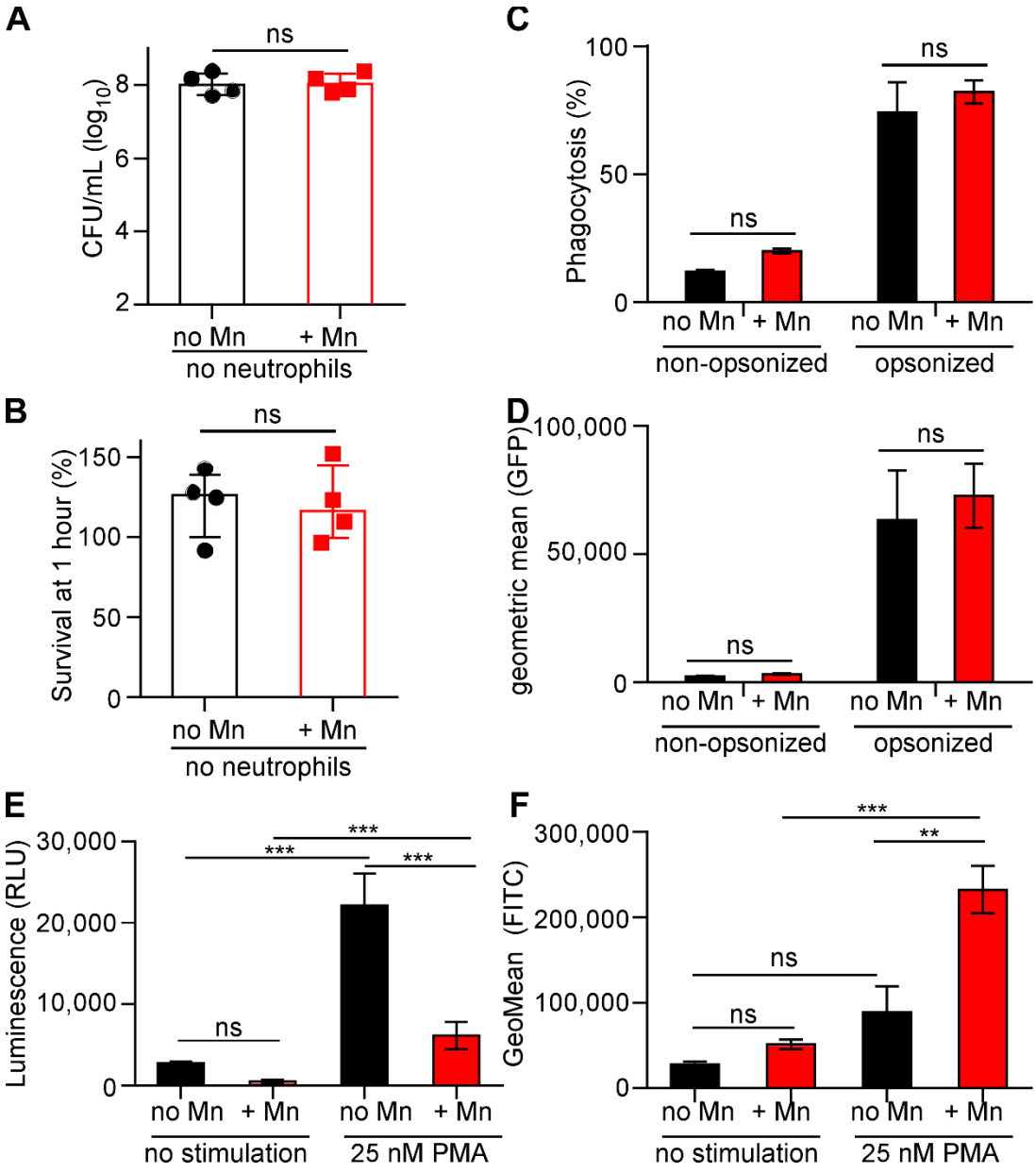
Supplementary Figure 4

Supplementary Figure 4. Bone marrow transplant model and gating strategy for studying neutrophil recruitment, related to Figure 5. (**A**) Design of bone marrow transplant experiments. (**B**) Gating strategy for distinguishing desired immune cells in heart homogenates. FSC = forward scatter; SSC = side scatter; PI = propidium iodide (live/dead marker). Lineages were distinguished as follows: CD45 for myeloid lineage cells, CD11b and CD11c for dendritic cells (DC), CD11b and F4/80 for macrophages (Mac), and CD11b Ly6G for neutrophils (neut). When appropriate, bone marrow lineages were delineated by CD45.1 or CD45.2. (**C**) Total neutrophil counts (Ly6G+CD11b+) for WT cells (CD45.1) and *S100a9*$^{-/-}$ cells (CD45.2) following BM transplantation of a 1:1 mix of WT congenic BM (CD45.1) and *S100a9*$^{-/-}$ BM (CD45.2) into WT mice. Previous gate, CD45+ cells. $N = 3$. (**D-E**) Neutrophils (Ly6G^{+}CD11b^{+}) were quantified following reciprocal bone marrow transplantation (BMT) between WT congenic mice (CD45.1) and *S100a9*$^{-/-}$ mice (CD45.2). Mice were infected with *S. aureus* intravenously and hearts were harvested four days post infection. Previous gate, CD45+ cells. (**D**) Representative flow plot. (**E**) Quantification of total neutrophils in hearts. Data are representative of $N = 2$ (*S100a9*$^{-/-}$ donor) and $N = 4$ (WT donor) from a single experiment. This experiment was repeated twice.



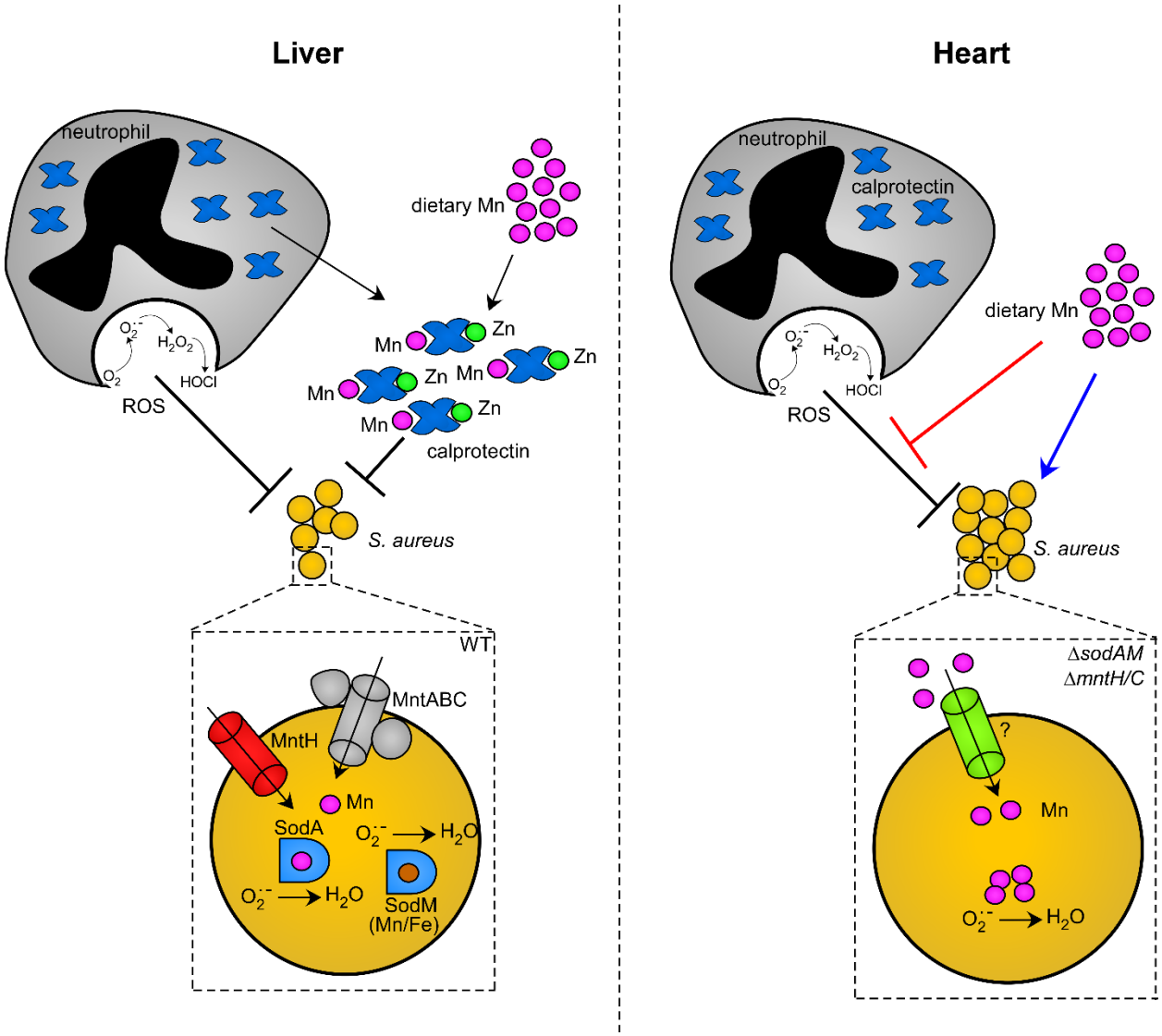
Supplementary Figure 5

Supplementary Figure 5. The impact of Mn on growth in superoxide and virulence, related to Figure 7. (**A**) Mice fed control diet or high Mn diet were treated with anti-Ly6G to deplete neutrophils or treated with an isotype control and infected with *S. aureus* Δ*sodA/M*. Survival was monitored over four days. $N = 5$ per group. ***, $P < 0.001$ by log-rank test. (**B**) Growth of *S. aureus* WT or Δ*sodA/M* in 1 mM paraquat with or without the addition of 1 mM $MnCl_2$. Growth was measured by OD_{600} over time. Data are combined from three independent experiments and depict the mean and S.E.M. (**C-E**). Growth of individual *S. aureus* clinical isolates as measured by OD_{600}. Closed gray symbols indicate TSB alone and closed pink symbols are TSB + 1 mM $MnCl_2$. Open symbols with dashed lines indicate treatment with 5 mM paraquat alone (black outline) or in combination with 1 mM $MnCl_2$ (red outline). Symbols represent the mean and error is shown as standard deviation. A single representative experiment of three is shown. (**C**) Square indicates isolate VU-2016-MRSA-1, triangle indicates isolate VU-2016-MRSA-2, circle indicates isolate VU-2016-MRSA-3. (**D**) Square indicates isolate VU-2016-MRSA-4, triangle indicates isolate VU-2016-MRSA-5, circle indicates isolate VU-2016-MRSA-6. (**D**) Square indicates isolate VU-2016-MRSA-7, triangle indicates isolate VU-2016-MSSA-1, circle indicates isolate VU-2016-MSSA-2. (**F, G**) Growth of *A. baumannii* 500 μM paraquat. $MnCl_2$ was supplemented at 500 μM, 250 μM, or 125 μM. Data are combined from three independent experiments and depict the mean and S.E.M. (**F**) Growth was measured over 20 hours. (**G**) OD_{600} at 20 hours. ***, $P < 0.001$; ns, not significant by one way ANOVA with Sidak's multiple comparisons test.



Supplementary Figure 6

Supplementary Figure 6. Impact of Mn on neutrophil ROS production and phagocytosis, related to Figure 7. (A-B) *S. aureus* growth with or without 1 mM $MnCl_2$ during the neutrophil killing assay. Bacterial growth in the absence of neutrophils is shown as colony forming units (**A**) or survival compared to the zero time point (**B**). $N = 4$ biological replicates. Bars depict median and interquartile intervals (**C-D**) Phagocytosis by human neutrophils of Newman *pOS1-*P^{SarA}*-sodRBS-sGFP* pre-incubated in buffer only (non-opsonized) or in 20% fresh human serum (opsonized). Data are presented as (**C**) percent of GFP-positive neutrophils and (**D**) the geometric mean fluorescence of neutrophils. $N = 4$ donors. (**E-F**) Primary human neutrophil production of reactive oxygen species (ROS) in the presence or absence of 1 mM $MnCl_2$. Production of ROS was stimulated by a 10 minute stimulation with 25 mM phorbol-12-myristate-13-acetate (PMA). (**E**) Detection of neutrophil-derived ROS by luminol, measured by luminescence, expressed as relative light units (RLU). $N = 4$ donors. (**F**) Detection of intracellular ROS by flow cytometry. $N = 4$ donors. (**C-F**) Bars depict mean and SEM. **, $P < 0.01$; ***, $P < 0.001$; ns, not significant by *t*-test (**A, B**) or ANOVA with Tukey's multiple comparisons test (**C-F**).



Supplementary Figure 7

Supplementary Figure 7. Model for dietary Mn enhancing infection of the heart, related to Figure 7. Left, model for infection of the liver. Neutrophils release calprotectin into the staphylococcal abscess (Corbin et al., 2008), binding excess dietary Mn to prevent *S. aureus* acquisition. ROS production by neutrophils effectively limits bacterial growth (Kehl-Fie et al., 2011). Left inset, in the liver *S. aureus* must express high affinity Mn importers to compete with calprotectin for Mn (Kehl-Fie et al., 2013). The superoxide dismutase enzymes SodA and SodM are important for detoxifying ROS in the liver (Kehl-Fie et al., 2013; Garcia et al., 2017). Right, model for infection of the heart. Calprotectin is not released from neutrophils into the abscess center. In a cell-intrinsic manner, calprotectin promotes neutrophil accumulation. Excess Mn from the diet, unbound by calprotectin, is bioavailable to *S. aureus* and enhances growth. Mn also reacts with ROS to decrease neutrophil killing. Right inset, *S. aureus* Δ*mntH/C* has enhanced virulence in the setting of high bioavailable Mn because Mn import through unidentified low-affinity Mn importers is sufficient to support *S. aureus* growth. Abundant intracellular Mn detoxifies superoxide in *S. aureus* Δ*sodA/M* to improve bacterial survival under ROS stress.