

Supplemental materials

Requirement and Synergistic Contribution of PAF acetylhydrolase Sse and Streptolysin S to Inhibition of Neutrophil Recruitment and Systemic Infection by Hypervirulent *emm3* Group A *Streptococcus* in Subcutaneous Infection of Mice

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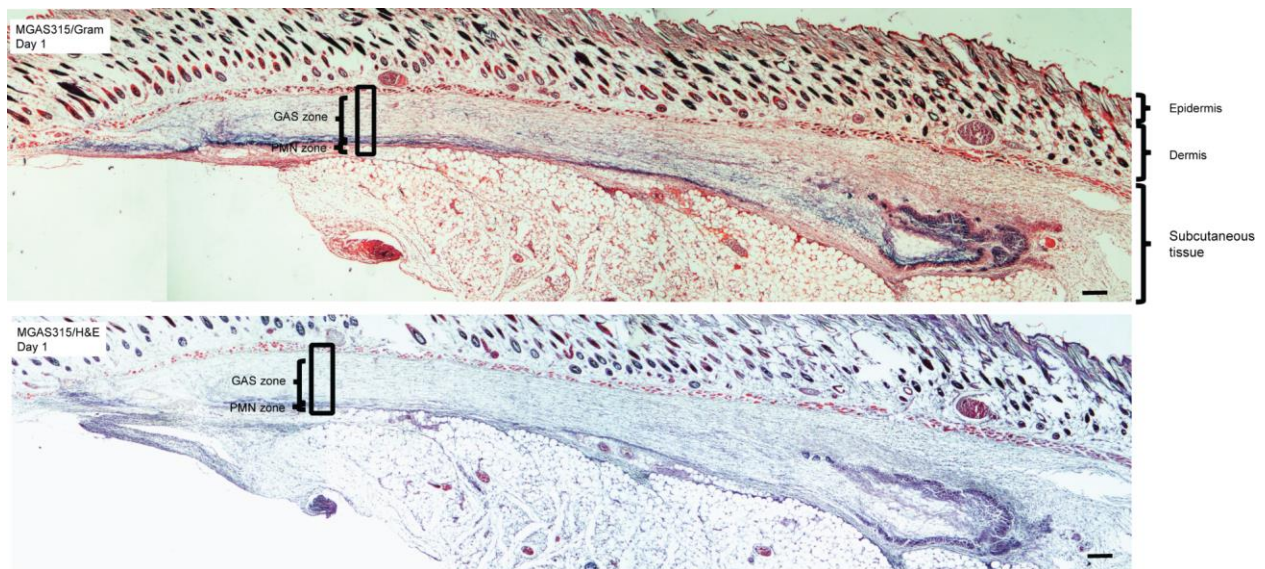


Figure S1. Histological analyses of MGAS315 skin infection sites in mice. Five 6-week old female C57BL/6J mice were subcutaneously inoculated with 1.7×10^8 cfu MGAS315. Skin infection sites were collected on day 1 after inoculation, fixed, and analyzed with Gram and H&E stains, as described in the Materials and Methods section. Presented are representative Gram and H&E stain images taken at 2X magnification with the bar represents 200 μm . The half curly parentheses indicate the bacterial and neutrophil (PMN) zones. The boxes indicate the areas that are shown in Fig. 4 at higher magnification.

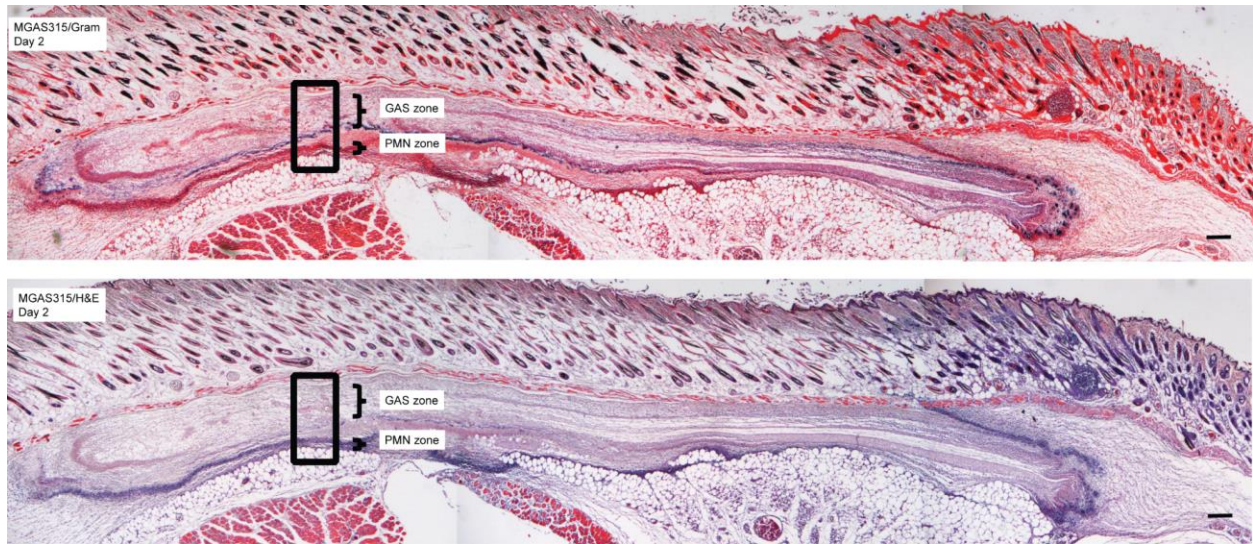


Figure S2. Histological analyses of MGAS315 skin infection sites in mice. Five 6-week old female C57BL/6J mice were subcutaneously inoculated with 1.7×10^8 cfu MGAS315. Skin infection sites were collected on day 2 after inoculation, fixed, and analyzed with Gram and H&E stains, as described in the Materials and Methods section. Presented are representative Gram and H&E stain images taken at 2X magnification with the bar represents 200 μ m. The half curly parentheses indicate the bacterial and PMN zones. The boxes indicate the areas that are shown in Fig. 5 at higher magnification.

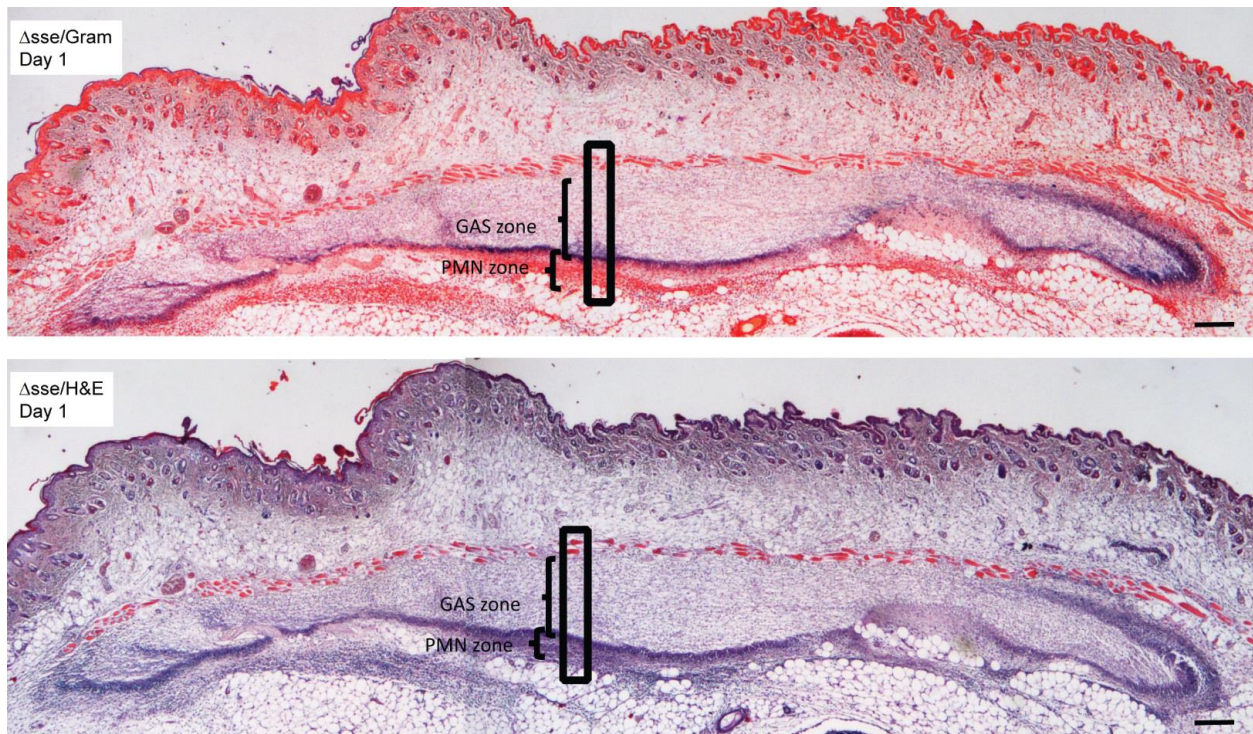


Figure S3. Histological analyses of MGAS315 Δ sse skin infection sites in mice. Five 6-week old female C57BL/6J mice were subcutaneously inoculated with 2.0×10^8 cfu Δ sse. Skin infection sites were collected on day 1 after inoculation, fixed, and analyzed with Gram and H&E stains, as described in the Materials and Methods section. Presented are representative Gram and H&E stain images taken at 2X magnification with the bar represents 200 μ m. The half curly parentheses indicate the bacterial and neutrophil (PMN) zones. The boxes indicate the areas that are shown in Fig. 4 at higher magnification.

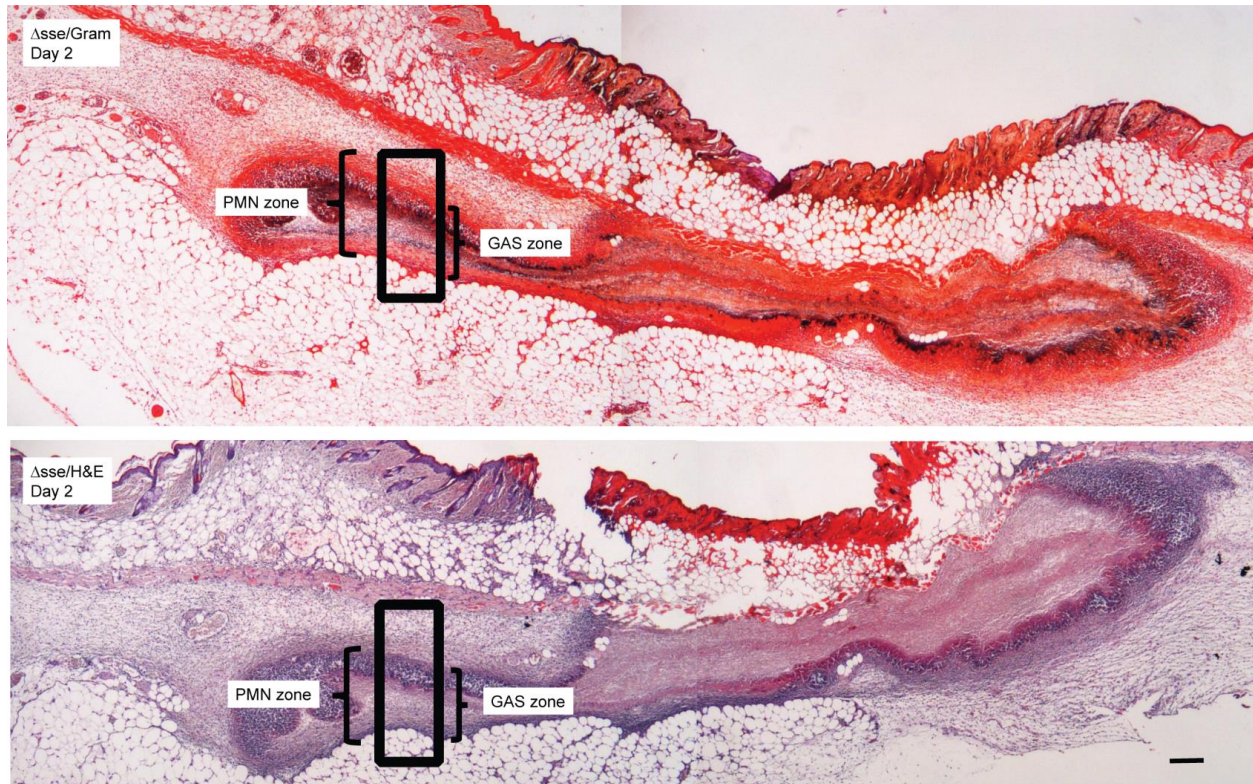


Figure S4. Histological analyses of MGAS315 Δ *sse* skin infection sites in mice. Five 6-week old female C57BL/6J mice were subcutaneously inoculated with 2.0×10^8 cfu Δ *sse*. Skin infection sites were collected on day 2 after inoculation, fixed, and analyzed with Gram and H&E stains, as described in the Materials and Methods section. Presented are representative Gram and H&E stain images taken at 2X magnification with the bar represents 200 μ m. The half curly parentheses indicate the bacterial and neutrophil (PMN) zones. The boxes indicate the areas that are shown in Fig. 5 at higher magnification.

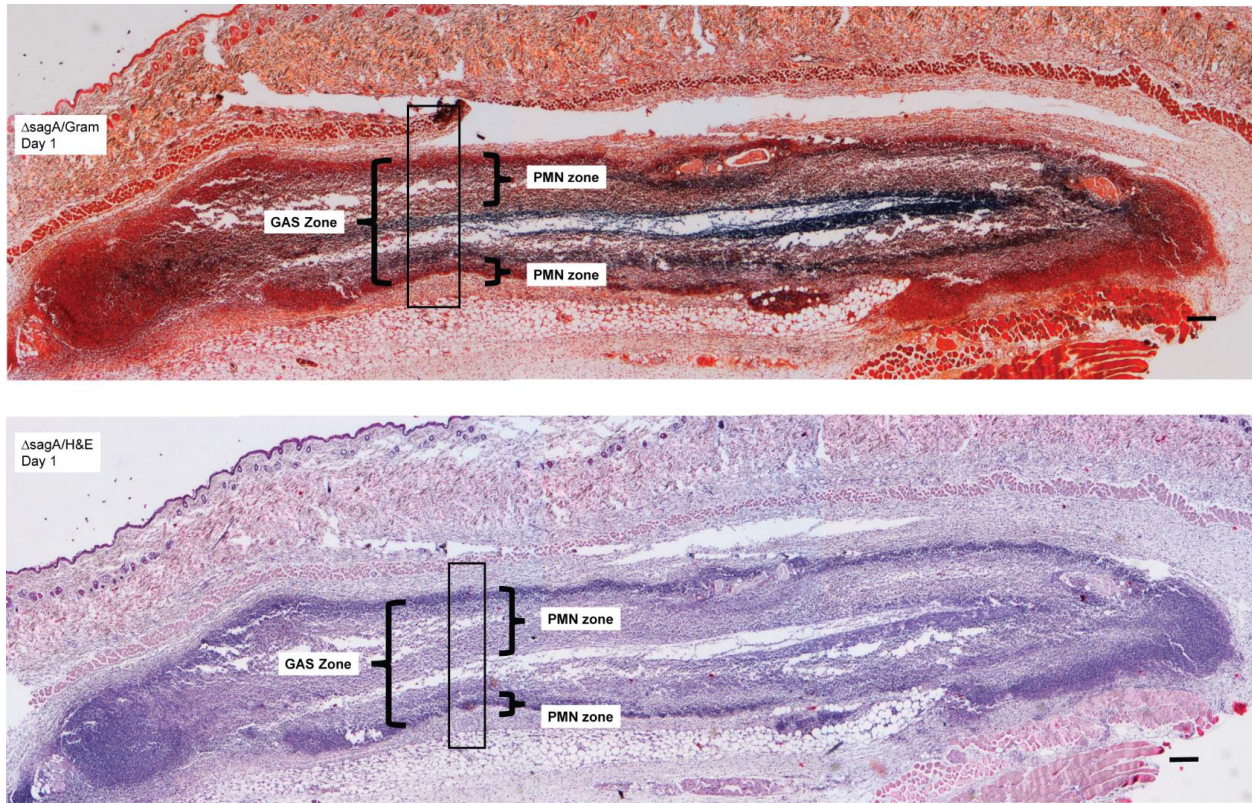


Figure S5. Histological analyses of MGAS315 *ΔsagA* skin infection sites in mice. Five 6-week old female C57BL/6J mice were subcutaneously inoculated with 1.9×10^8 cfu *ΔsagA*. Skin infection sites were collected on day 1 after inoculation, fixed, and analyzed with Gram and H&E stains, as described in the Materials and Methods section. Presented are representative Gram and H&E stain images taken at 2X magnification with the bar represents 200 μ m. The half curly parentheses indicate the bacterial and neutrophil (PMN) zones. The boxes indicate the areas that are shown in Fig. 4 at higher magnification.

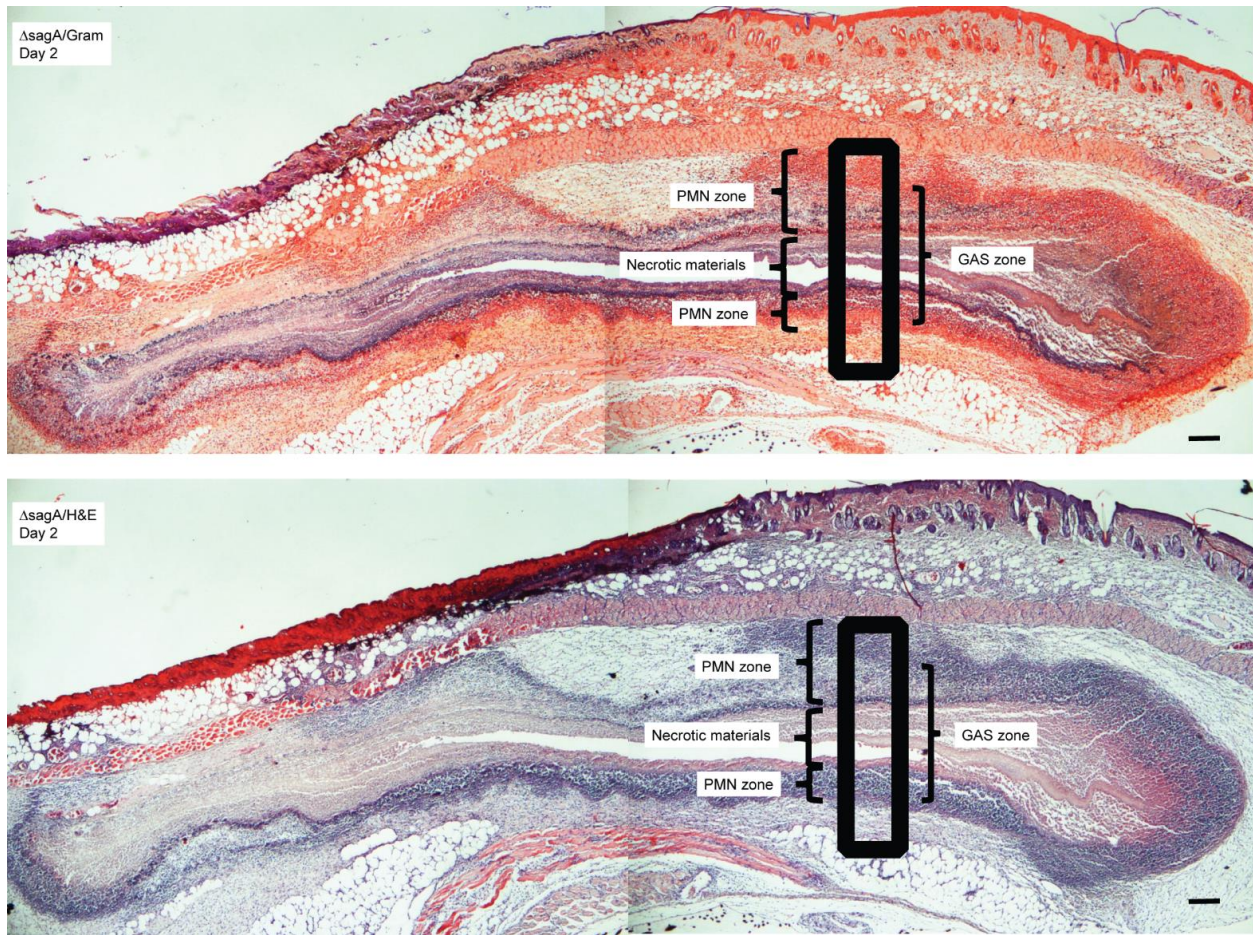


Figure S6. Histological analyses of MGAS315 *ΔsagA* skin infection sites in mice. Five 6-week old female C57BL/6J mice were subcutaneously inoculated with 1.9×10^8 cfu *ΔsagA*. Skin infection sites were collected on day 2 after inoculation, fixed, and analyzed with Gram and H&E stains, as described in the Materials and Methods section. Presented are representative Gram and H&E stain images taken at 2X magnification with the bar represents 200 μm . The half curly parentheses indicate the bacterial and neutrophil (PMN) zones. The boxes indicate the areas that are shown in Fig. 5 at higher magnification.

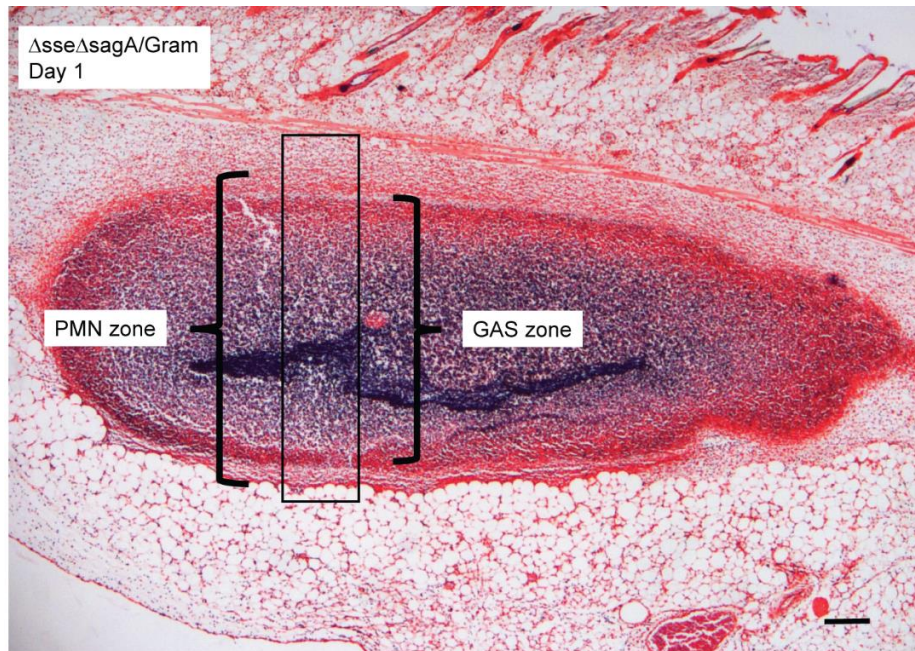


Figure S7. Histological analyses of MGAS315 *ΔsseΔsagA* skin infection sites in mice. Five 6-week old female C57BL/6J mice were subcutaneously inoculated with 2.0×10^8 cfu *ΔsseΔsagA*. Skin infection sites were collected on day 1 after inoculation, fixed, and analyzed with Gram and H&E stains, as described in the Materials and Methods section. Presented are representative Gram and H&E stain images taken at 2X magnification with the bar represents 200 μm . The half curly parentheses indicate the bacterial and neutrophil (PMN) zones. The boxes indicate the areas that are shown in Fig. 4 at higher magnification.

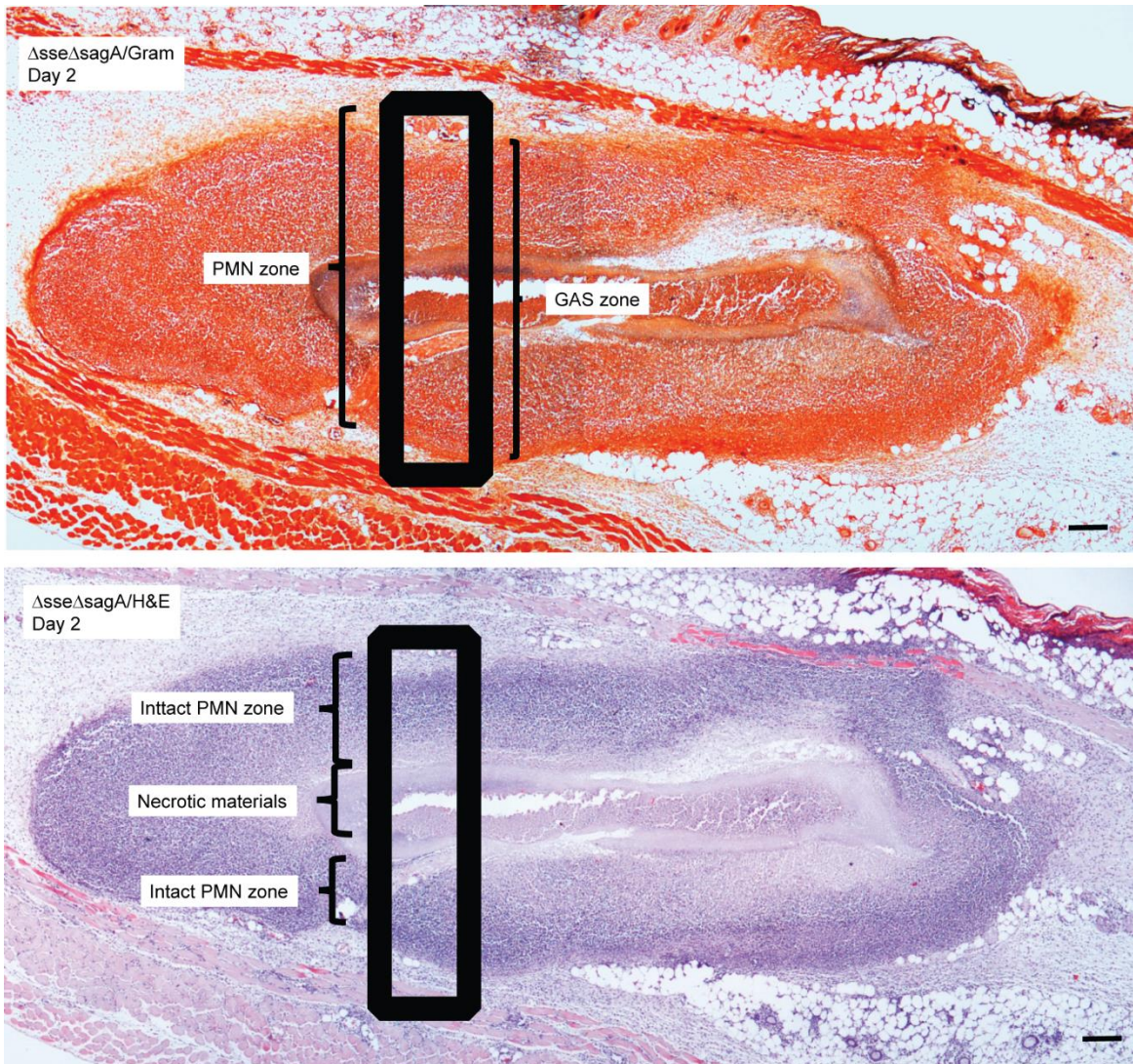


Figure S8. Histological analyses of MGAS315 $\Delta sse\Delta sagA$ skin infection sites in mice. Five 6-week old female C57BL/6J mice were subcutaneously inoculated with 2.0×10^8 cfu $\Delta sse\Delta sagA$. Skin infection sites were collected on day 2 after inoculation, fixed, and analyzed with Gram and H&E stains, as described in the Materials and Methods section. Presented are representative Gram and H&E stain images taken at 2X magnification with the bar represents 200 μm . The half curly parentheses indicate the bacterial and neutrophil (PMN) zones. The boxes indicate the areas that are shown in Fig. 5 at higher magnification.

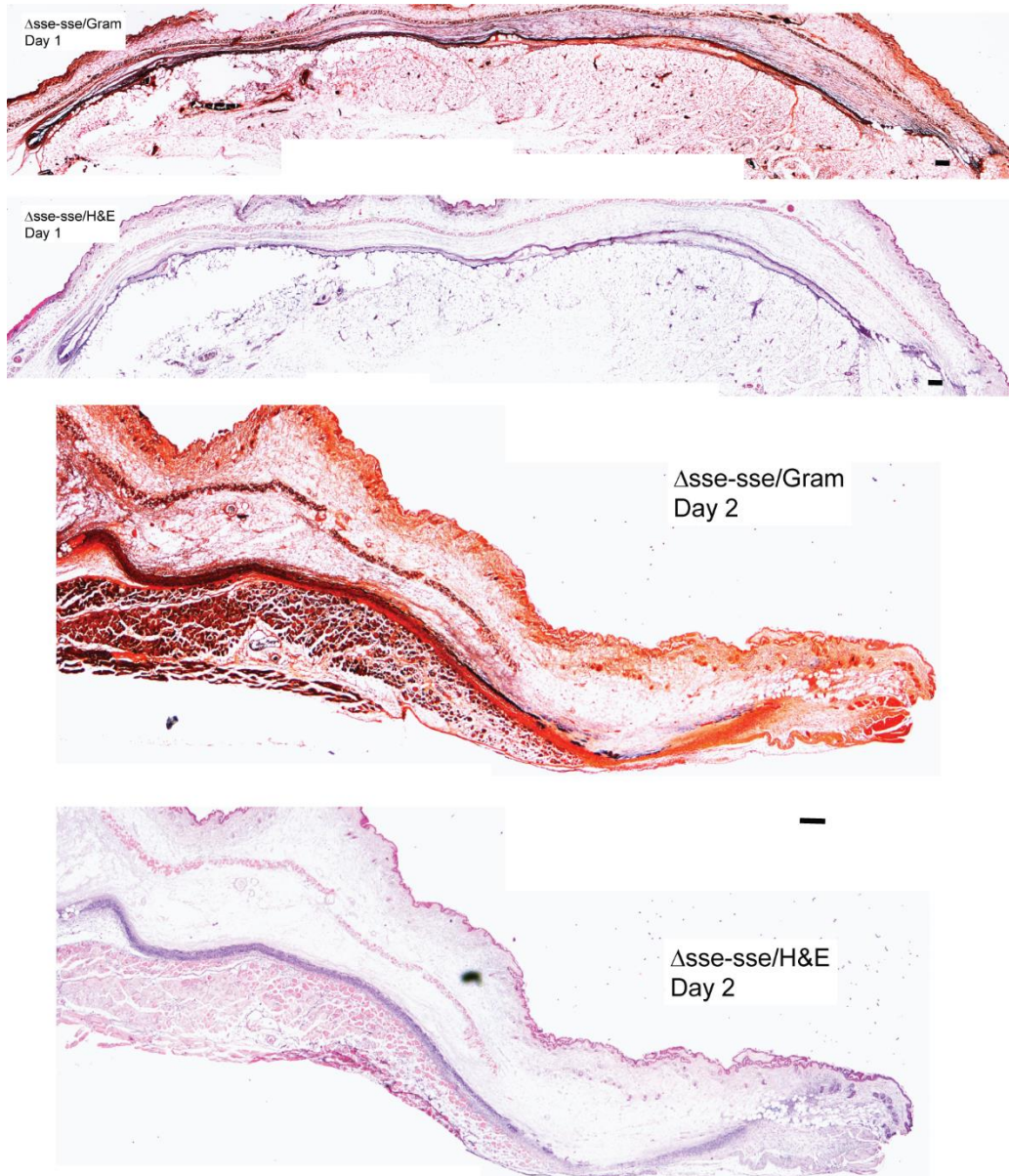


Figure S9. Histological analyses of $\Delta sse-sse$ skin infection sites in mice. Five 6-week old female C57BL/6J mice were subcutaneously inoculated with 1.8×10^8 cfu $\Delta sse-sse$. Two and three mice were sacrificed on days 1 and 2 after inoculation, respectively, to collect skin infection sites. The skin infection sites were fixed and analyzed with Gram and H&E stains, as described in the Materials and Methods section. Presented are representative Gram and H&E stain images on days 1 and 2 after inoculation. The bar represents 200 μ m.

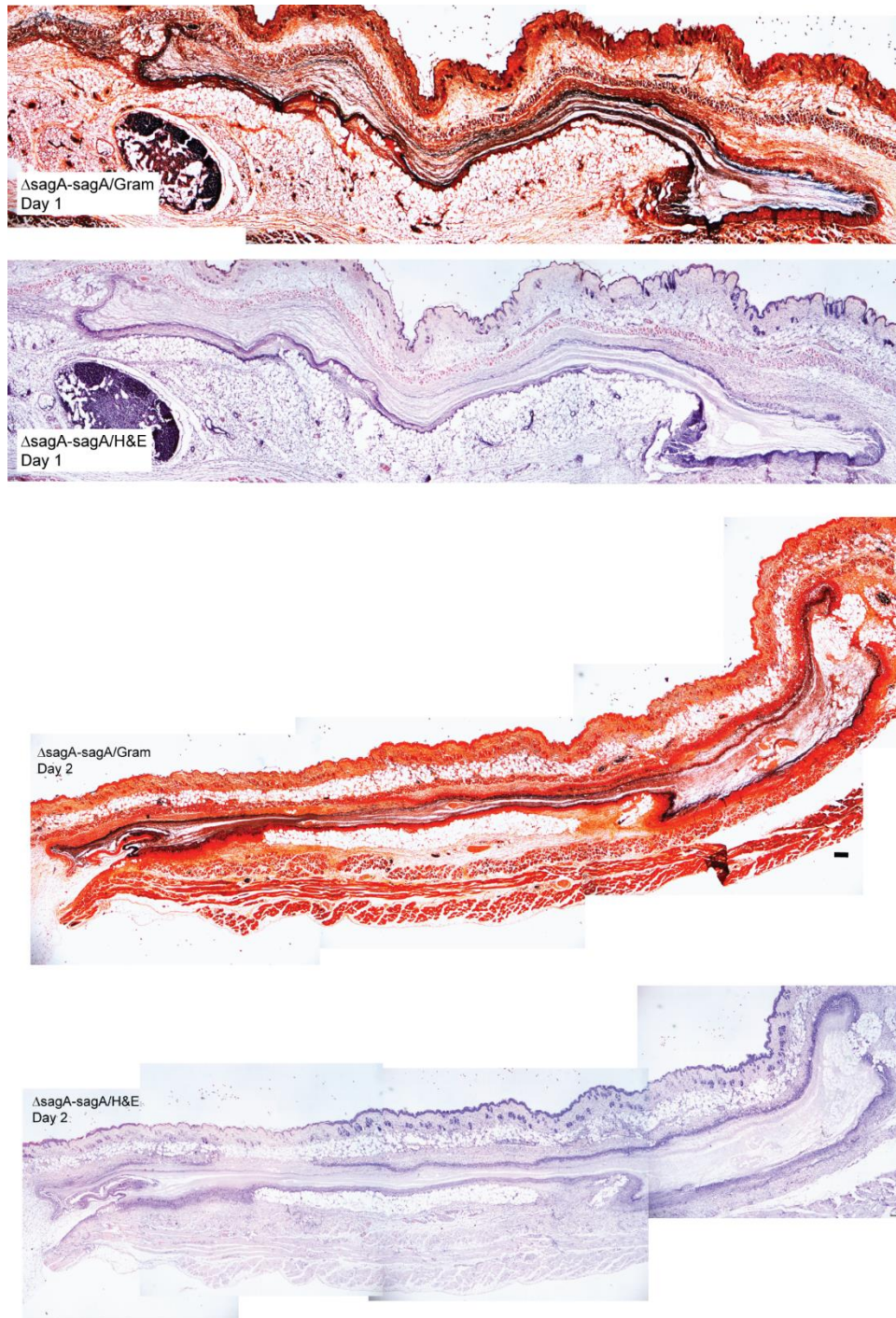


Figure S10. Histological analyses of $\Delta sagA-sagA$ skin infection sites in mice. Five 6-week old female C57BL/6J mice were subcutaneously inoculated with 1.4×10^8 cfu $\Delta sagA-sagA$. Two and three mice were sacrificed on days 1 and 2 after inoculation, respectively, to collect skin infection sites. The skin infection sites were fixed and analyzed with Gram and H&E stains, as described in the Materials and Methods section. Presented are representative Gram and H&E stain images on days 1 and 2 after inoculation. The bar represents 200 μm .

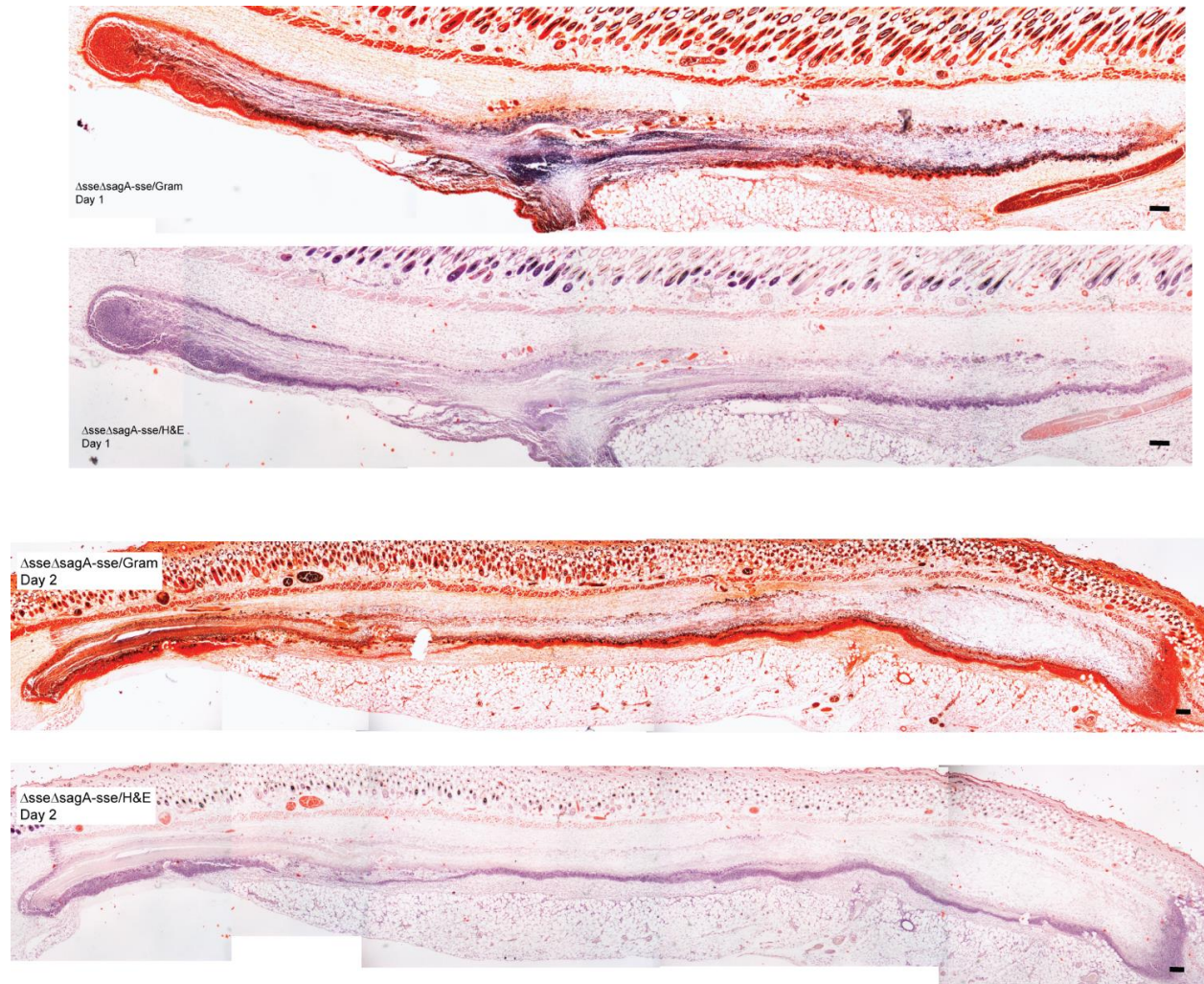


Figure S11. Histological analyses of $\Delta sse\Delta sagA-sse$ skin infection sites in mice. Five 8-week old female C57BL/6J mice were subcutaneously inoculated with 1.6×10^8 cfu $\Delta sse\Delta sagA-sse$. Two and three mice were sacrificed on days 1 and 2 after inoculation, respectively, to collect skin infection sites. The skin infection sites were fixed and analyzed with Gram and H&E stains, as described in the Materials and Methods section. Presented are representative Gram and H&E stain images on days 1 and 2 after inoculation. The bar represents 200 μ m.

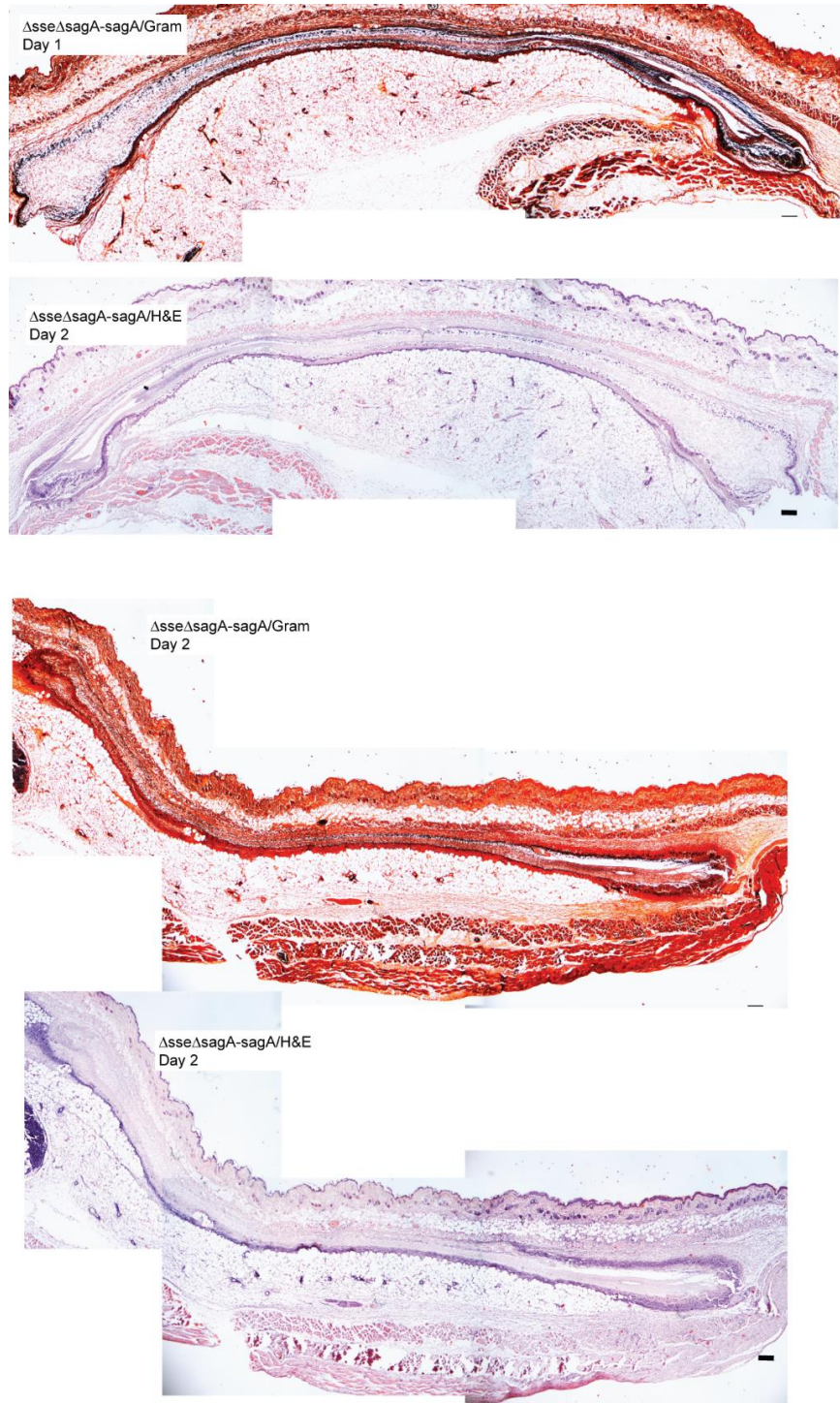


Figure S12. Histological analyses of $\Delta sse\Delta sagA-sagA$ skin infection sites in mice. Five 6-week old female C57BL/6J mice were subcutaneously inoculated with 1.5×10^8 cfu $\Delta sse\Delta sagA-sagA$. Two and three mice were sacrificed on days 1 and 2 after inoculation, respectively, to collect skin infection sites. The skin infection sites were fixed and analyzed with Gram and H&E stains, as described in the Materials and Methods section. Presented are representative Gram and H&E stain images on days 1 and 2 after inoculation. The bar represents 200 μm .

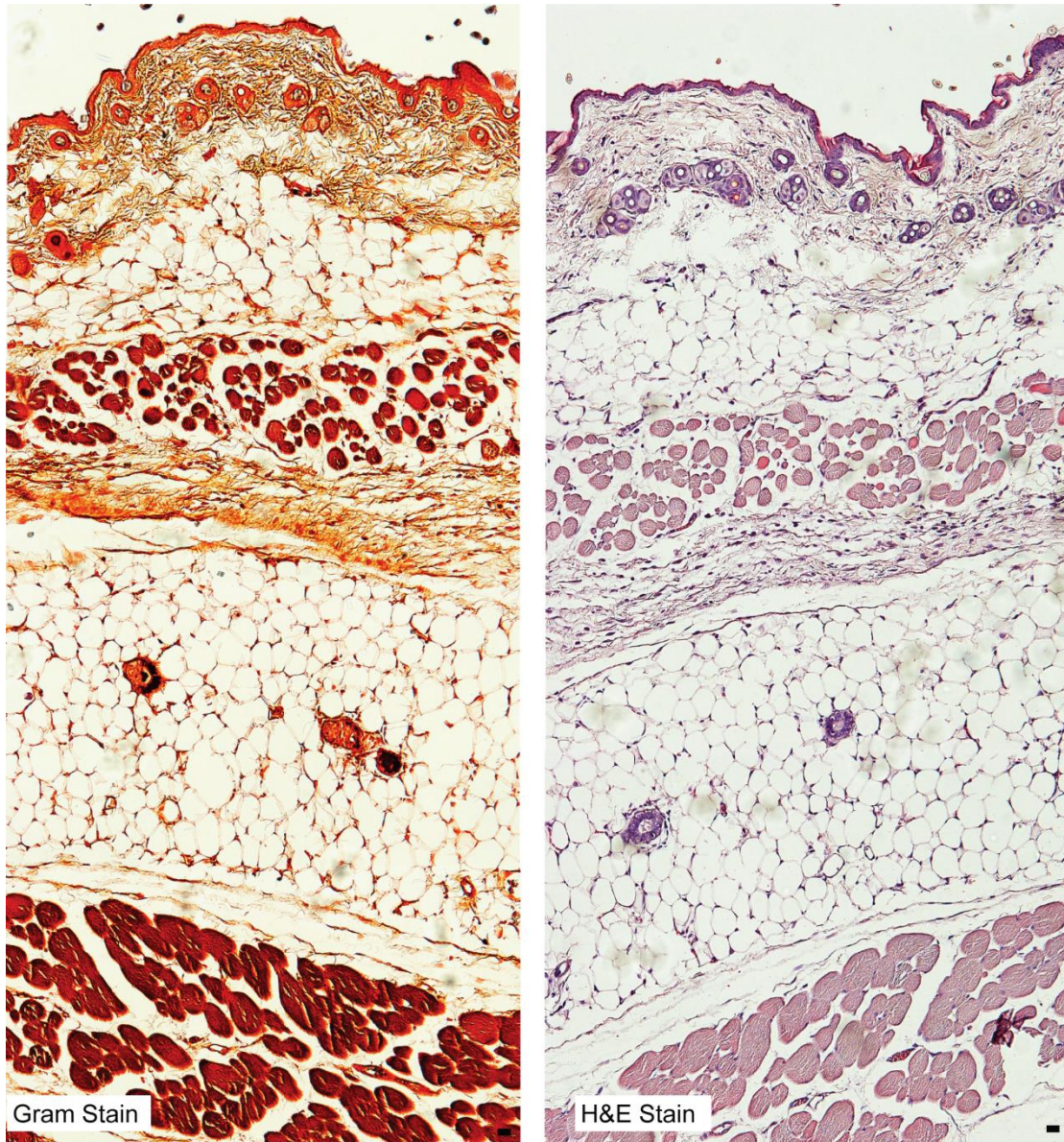


Figure S13. Gram and H&E stain images of the skin of a C57BL/6J mouse. *eof ΔsseΔsagA* skin infection sites in mice. The skin infection sites were fixed and analyzed with Gram and H&E stains, as described in the Materials and Methods section. The bar represents 20 µm.