SUPPLEMENTAL INFORMATION

TABLE 1

ΔsarA	*Staphylococcus aureus, Streptococcus dysgalactiae, Pseudomonas aeruginosa, E. coli, non-haemolytic rough, Staphylococcus hyicus ss hyicus, Proteus vulgaris, Enterococcus faecalis, Lysinibacillus sp., Bacillus sp.*
USA 300	*Staphylococcus aureus, Staphylococcus hyicus ss hyicus, Enterococcus faecalis, Klebsiella oxytoca, E. coli, non-haemolytic rough, Proteus vulgaris, Proteus mirabilis*
ΔrexB	*Staphylococcus aureus, Streptococcus dysgalactiae, Pseudomonas aeruginosa, Enterobacter cloacae, Klebsiella oxytoca, Staphylococcus hyicus ss chromogenes, Pseudomonas aeruginosa*

SUPPLEMENTAL METHODS

Characterization of Polymicrobial Wound Infection

Characterization of polymicrobial wound infection was done as previously described with minor modifications[1]. The minced wound tissue samples were inoculated on TSA II (Becton Dickinson, NJ) with 5% sheep blood agar and on MacConkey agar (Becton Dickinson, NJ) and incubated at 37^{o}C aerobically as well as on TSA II with 5% sheep blood agar with a nurse Staphylococcus aureus streak and Chocolate II (Becton Dickinson, NJ) with hemoglobin, IsoVitalex agar and incubated at 37^{o}C in a 10% CO2 incubator. Growth was checked daily for three days. Bacterial colonies were set on MALDI-TOF™ (Bruker, MA) for identification.

Laser Capture Microdissection

Laser capture microdissection of the granulation tissue of the engineered human scaffolds was done as previously described[2].

Preparation of Human Engineered Skin

Human engineered skin were prepared as described previously[3]

SUPPLEMENTAL FIGURE LEGENDS

Figure S1: Bacterial biofilm of Δ*sarA*, USA300 & Δ*rexB* were developed on polycarbonate membrane. **A,** Digital photographs of the biofilm by the mutants and wild type strains of bacteria on polycarbonate membrane discs (left) along with its inverted images (right). Scale = 10mm. **B,** representative confocal Z stack images of the biofilms of Δ*sarA*, USA300 & Δ*rexB* developed on polycarbonate membrane indicating the thickness of biofilm. The red arrow indicates the horizontal plane and the blue arrow indicates the vertical plane. **C,** bar diagram representing the intensity of the *in vitro* biofilms as calculated from the digital inverted photos. Data are mean±SEM (n=4), *p<0.05 compared to *ΔsarA*. **D,** bar diagram indicates the average thickness of the biofilms developed on PCM membrane, as quantified from confocal images. *p<0.05 compared to *ΔsarA.* **E,** the representative growth curve of the wild type and the mutant strains of *S.aureus.*

Fig S3. Hyper-biofilm infection by S. *aureus USA::ΔrexB* in murine wounds severely compromises tensile strength of the repaired skin. Two 8 x 16-mm full-thickness excisional wounds were made on the dorsal skin of C57BL/6 mice. Each of the two wounds was topically infected with isogenic strains of S. *aureus* USA300, USA300::*rexB* (*ΔrexB)* or USA300::*sarA (ΔsarA).* **A,** miR-143 expression in the healed skin measured on d5 post-wound closure (d21). **B,** Granulation tissue collagen content was determined in the repaired skin measured on d5 post-wound closure (d21) using hydroxyproline assay. **C-E***,* Collagen 1 protein and mRNA expression in the repaired skin measured on d5 post-wound closure (d21). **F-G,** MMP2 expression in the repaired skin measured on d5 post-wound closure (d21). **H,** Tensile strength of the healed wounds was measured in the repaired skin measured on d5 post-wound closure (d21). Data are mean±SEM (n=3-7), **p*<0.05 compared to *ΔsarA*. Scale bar = 30μm.

Figure S5. Bar diagram represents the bacterial collagenase activity measured on day 35 from the porcine burn wounds inoculated with Δ*sarA*, USA300 & Δ*rexB*. Data are mean±SEM (n=6).

Fig S6. Hyper-biofilm infection by S. *aureus USA::ΔrexB* in human engineered skin compromises miR-143 expression and attenuates Collagen 1 protein. Human engineered skin was subjected to treatment with conditioned media obtained from the 3 isogenic mutant strains of *Staphylococcus aureus* ∆*sarA*, USA300 & ∆*rexB* for 72h **A,** miR-143 expression in the laser-captured dermal component of the human engineered skin was measured using RT-PCR. **B,** Collagen content of the human engineered skin was determined using hydroxyproline assay. **C-D,** Collagen 1 protein expression, Data are mean±SEM (n=3-6). *p<0.05 compared to *ΔsarA*. Scale bar = 30µm. **D-E***,* MMP2 protein expression. Data are mean±SEM (n=3-6). *p<0.05 compared to *ΔsarA*. Scale bar = 30µm.

REFERENCES

1. Ganesh K, Das A, Dixith S, et al. Electric field based dressing disrupts mixed-species bacterial biofilm infection and restores functional wound healing *Annals of Surgery* 2017; in press.
2. Shapiro JP, Biswas S, Merchant AS, et al. A quantitative proteomic workflow for characterization of frozen clinical biopsies: laser capture microdissection coupled with label-free mass spectrometry. *J Proteomics* 2012; 77:433-40.
3. Ebersole GC, Anderson PM, Powell HM. Epidermal differentiation governs engineered skin biomechanics. *J Biomech* 2010; 43(16):3183-90.

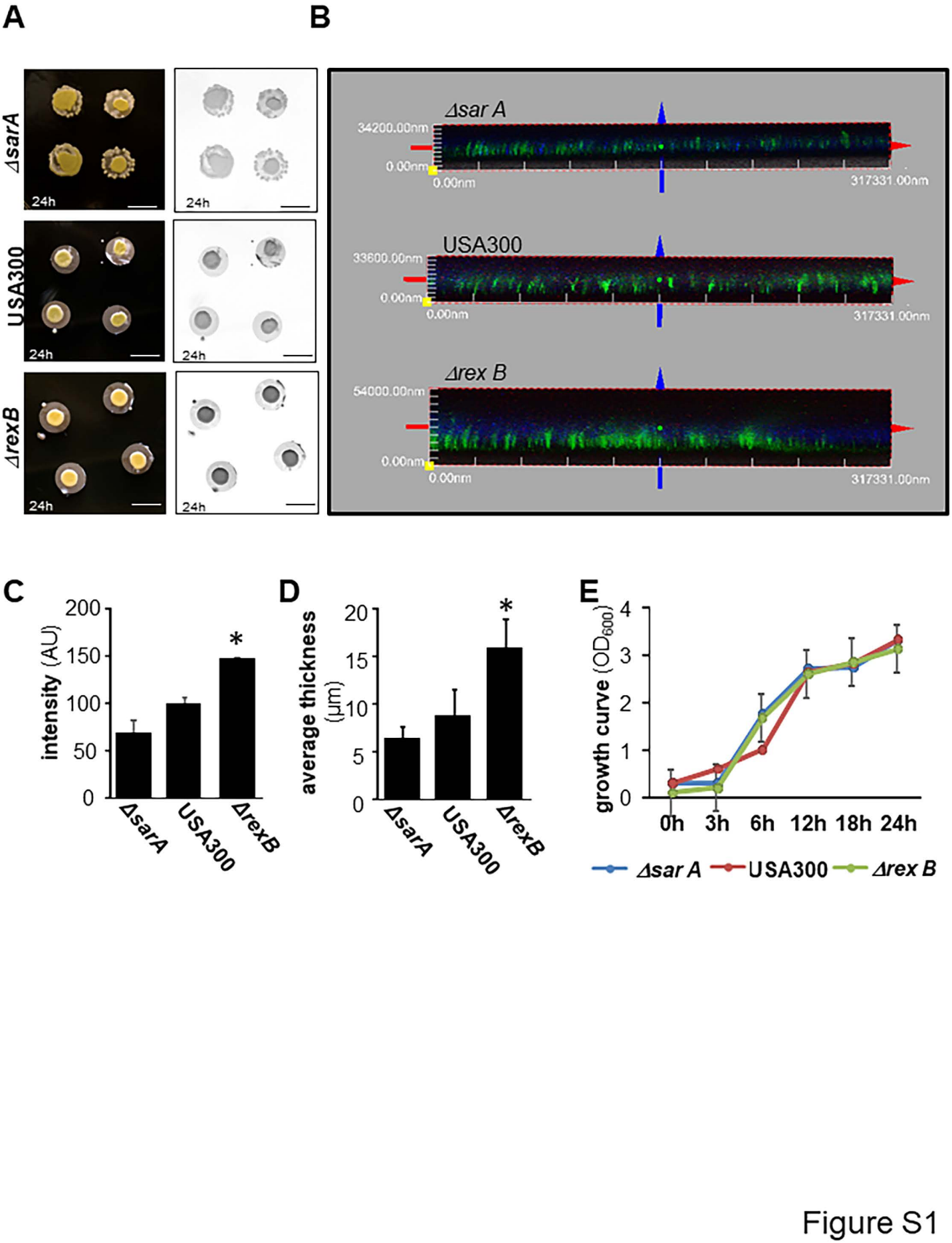
A
B
ΔsarA
USA300
ΔrexB
24h
Δsar A
34200.00nm
0.00nm
0.00nm
317331.00nm
USA300
33600.00nm
0.00nm
0.00nm
317331.00nm
Δrex B
54000.00nm
0.00nm
0.00nm
317331.00nm
C
intensity (AU)
200
150
100
50
0
*
ΔsarA
USA300
ΔrexB
D
average thickness (μm)
20
15
10
5
0
*
ΔsarA
USA300
ΔrexB
E
growth curve (OD600)
4
3
2
1
0
0h
3h
6h
12h
18h
24h
Δsar A
USA300
Δrex B

Figure S1

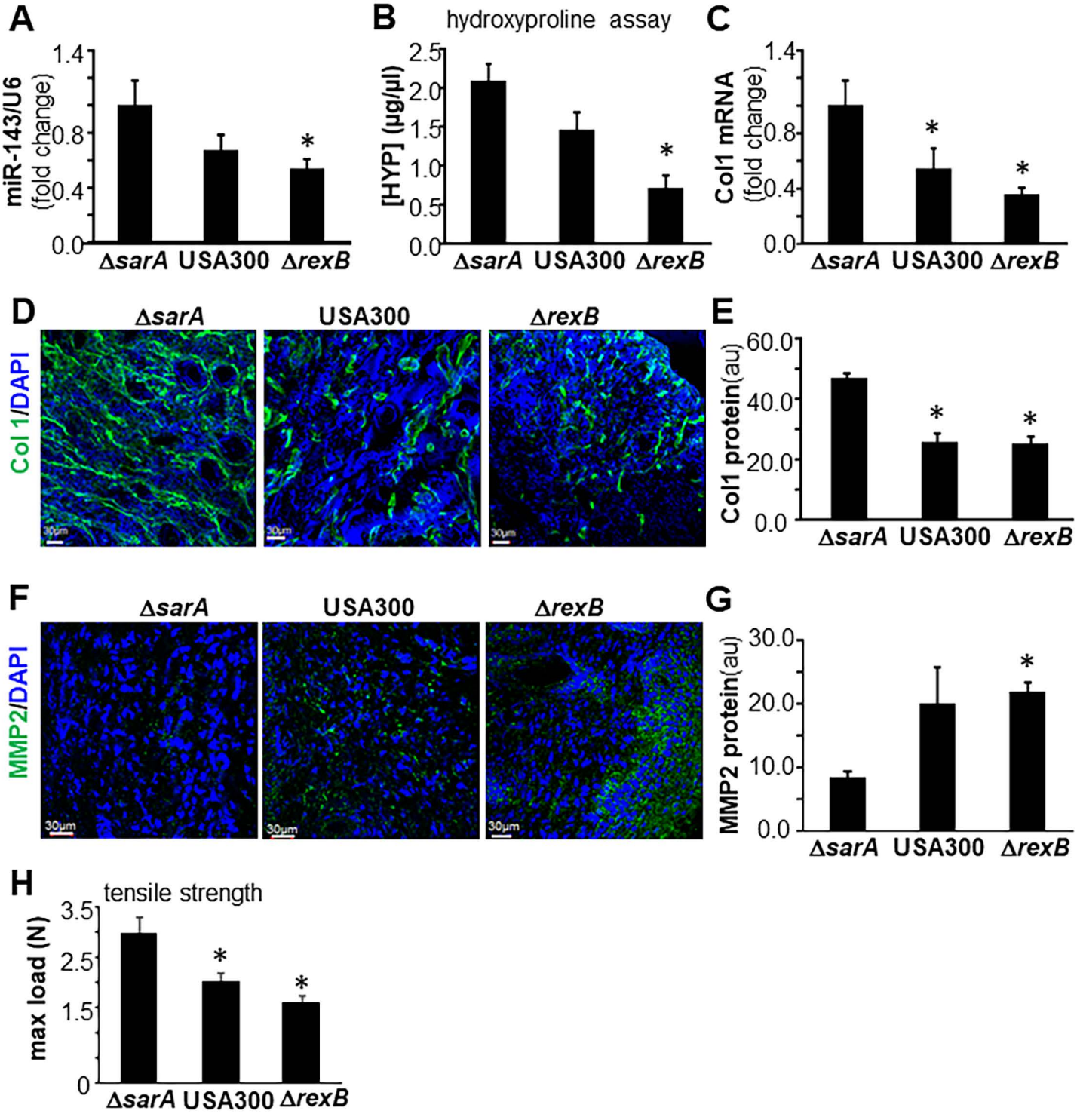
A
miR-143/U6 (fold change)
1.4
0.8
0.4
0.0
*
ΔsarA USA300 ΔrexB
B
hydroxyproline assay
[HYP] (μg/μl)
2.5
2.0
1.5
1.0
0.5
0.0
*
ΔsarA USA300 ΔrexB
C
Col1 mRNA (fold change)
1.4
0.8
0.4
0.0
* *
ΔsarA USA300 ΔrexB
D
ΔsarA USA300 ΔrexB
Col 1/DAPI
30μm
E
Col1 protein(au)
60.0
40.0
20.0
0.0
* *
ΔsarA USA300 ΔrexB
F
ΔsarA USA300 ΔrexB
MMP2/DAPI
30μm
G
MMP2 protein(au)
30.0
20.0
10.0
0.0
*
ΔsarA USA300 ΔrexB
H
tensile strength
max load (N)
3.5
2.5
1.5
0
* *
ΔsarA USA300 ΔrexB

Figure S3

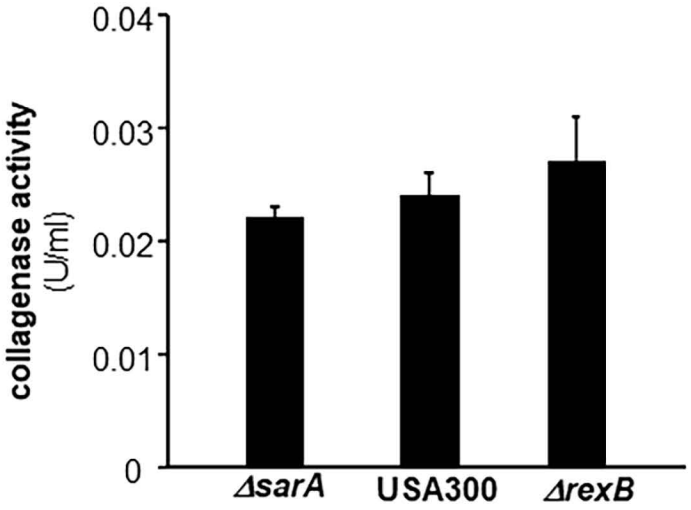
collagenase activity
(U/ml)
0.04
0.03
0.02
0.01
0
ΔsarA
USA300
ΔrexB

Figure S5

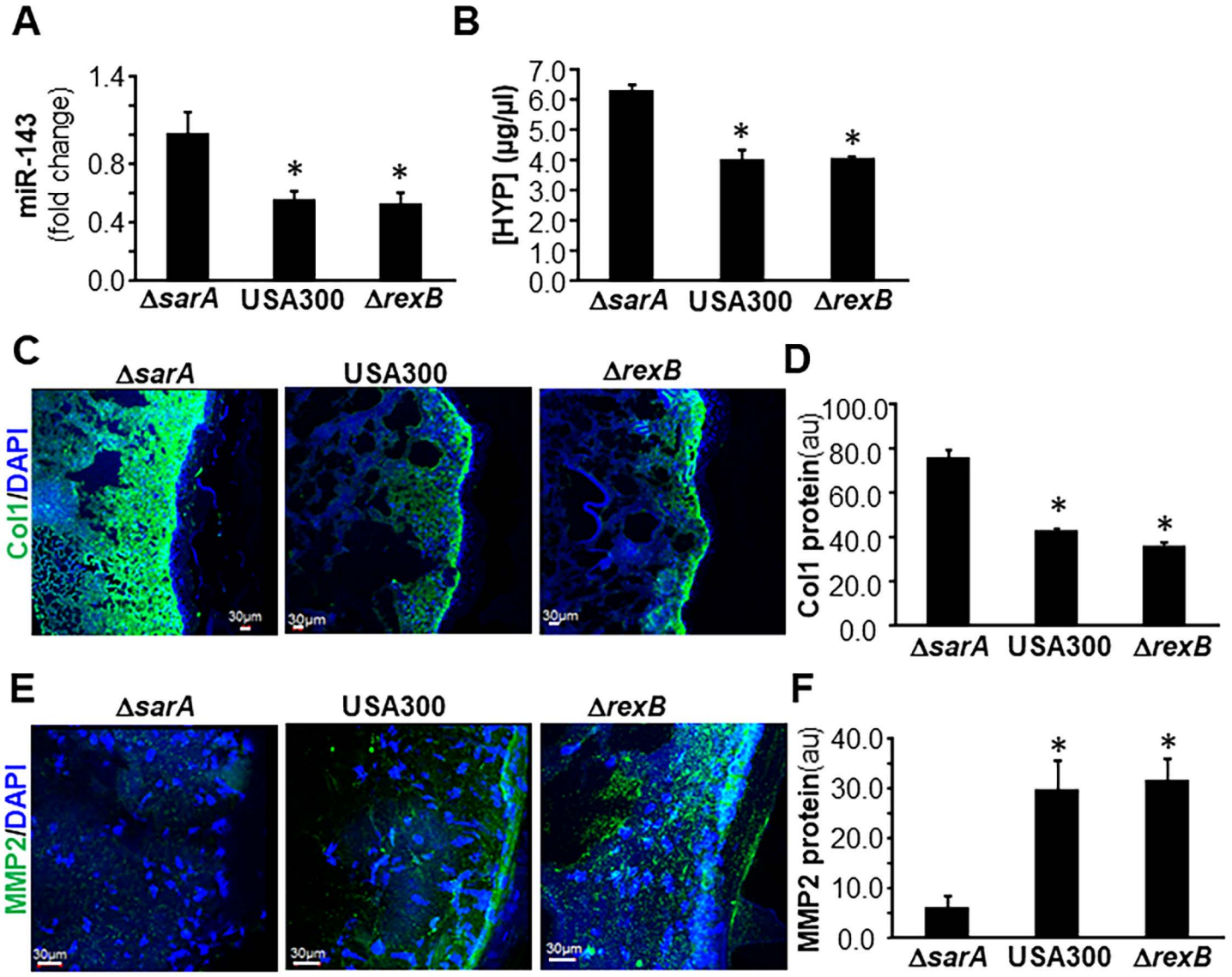
A
miR-143
(fold change)
1.4
0.8
0.4
0.0
*
*
ΔsarA
USA300
ΔrexB
B
[HYP] (μg/μl)
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
*
*
ΔsarA
USA300
ΔrexB
C
ΔsarA
USA300
ΔrexB
Col1/DAPI
30μm
30μm
30μm
D
Col1 protein(au)
100.0
80.0
60.0
40.0
20.0
0.0
*
*
ΔsarA
USA300
ΔrexB
E
ΔsarA
USA300
ΔrexB
MMP2/DAPI
30μm
30μm
30μm
F
MMP2 protein(au)
40.0
30.0
20.0
10.0
0.0
*
*
ΔsarA
USA300
ΔrexB

Figure S6