

## **Biofilm in group A streptococcal necrotizing soft tissue infections**

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**Supplemental Table 1. Clinical characteristics of NSTI patients**

| Patient ID | emm-type | Gen. | Age | Comorb.                                     | Septic Shock <sup>#</sup>     | SAPSII | Preoperative<br>(only clinical finding from primary or tertiary hospital)                      | Tissue Severity<br>(1st or 2nd surgical intervention at primary or tertiary hospital)                         | Organ failure                               | Surv. (90 d) | Length of stay <sup>‡</sup> | Surgery <sup>§</sup> | Treatment  |                      |                   |
|------------|----------|------|-----|---|-------------------------------|--------|--|---|---|--------------|-----------------------------|----------------------|--|----------------------|-------------------|
|            |          |      |     |   |                               |        |  |   |   |              |                             |                      | Antibiotics <sup>†</sup>   | HBO <sup>&amp;</sup> | IVIG <sup>†</sup> |
| 2002       | NI       | m    | 63  | none  | Severe sepsis<br>septic shock | 52     | Severe pain<br>Skin bullae<br>Skin bruising<br>Skin anaesthesia<br>Gas visualized on radiology | Skin bullae<br>Skin erythema<br>Fascia greyish<br>Muscle contractions<br>Muscle seeping of blood<br>Frank pus | Mechanical ventilation<br>Inotropic support | Y            | 6/30                        | 4 (5)                | Penicillin<br>Tasobactam<br>Gentamycin<br>Metronidazol<br>Meropenem<br>Ciprofloxacin<br>Clindamycin  | 1/90                 | 3x                |
| 2006       | emm1     | f    | 61  | cardiovascular disease                      | Severe sepsis<br>septic shock | 41     | Severe pain  | Skin erythema<br>Fascia greyish<br>Muscle contractions<br>Muscle seeping of blood                             | Mechanical ventilation<br>Inotropic support | Y            | 7/21                        | 4 (3)                | Meropenem<br>Ciprofloxacin<br>Clindamycin<br>Meropenem<br>Ceftriaxon<br>Ciprofloxacin<br>Clindamycin | 2/180                | 4x                |
| 2015       | emm87    | f    | 69  | malignant disease<br>metastatic carcinoma   | Severe sepsis                 | 43     | N/A  | Skin erythema   | Mechanical ventilation                      | Y            | 8/11*                       | 6 (5)                | Meropenem<br>Ciprofloxacin<br>Clindamycin<br>Meropenem<br>Ciprofloxacin<br>Clindamycin               | 2/180                | 3x                |
| 2017       | emm28    | f    | 37  | none  | Severe sepsis<br>septic shock | 24     | Severe pain  | Skin erythema<br>Muscle seeping of blood  | Mechanical ventilation<br>Inotropic support | Y            | 3/9*                        | 4 (2)                | Meropenem<br>Cefuroxim<br>Clindamycin<br>Meropenem<br>Ciprofloxacin<br>Clindamycin                   | 1/90                 | 2x                |
| 2028       | emm3     | f    | 59  | malignant disease<br>cardiovascular disease | Severe sepsis<br>septic shock | 34     | Palpable gas (crepitus)<br>Gas visualized on radiology   | Skin erythema<br>Fascia greyish<br>Fascia dishwater fluid<br>Muscle contractions<br>Muscle seeping of blood   | Mechanical ventilation<br>Inotropic support | Y            | 8/18                        | 7 (6)                | Piperacillin<br>Meropenem<br>Cefuroxim<br>Ceftriaxon<br>Clindamycin<br>Meropenem<br>Ciprofloxacin    | 1/90                 | 3x                |
| 5004       | emm28    | f    | 51  | none  | Septic shock                  | 70     | Skin purple/black<br>discoloration<br>Skin bruising  | Skin necrosis<br>Fascia greyish and swollen<br>Fascis dishwater fluid   | Mechanical ventilation<br>Inotropic support | Y            | 5/27                        | 5 (3)                | Meropenem<br>Cefotaxim   | 5/350                | 4x                |

|      |      |   |    |   |                               |    |   |  |   |   |       |       |  |   |    |
|------|------|---|----|---|-------------------------------|----|---|--|---|---|-------|-------|--|---|----|
|      |      |   |    |   |                               |    |   | Muscle contractions<br>Muscle seeping of blood   |   |   |       |       | Tobramycin<br>Meropenem<br>Clindamycin   |   |    |
| 5006 | emm1 | m | 37 | none  | none                          | 26 | Skin bruising<br>Gas visualized on radiology  | Skin erythema<br>Frank pus   | none  | Y | 3/14  | 5 (4) | Penicillin<br>Cefotaxim<br>Clindamycin<br>Penicillin<br>Clindamycin                  | 0 | 1x |
| 6013 | emm1 | f | 34 | Retts syndrome<br>chronic wound<br>other skin disease | Severe sepsis<br>septic shock | 29 | oedema<br>rubor                               | Skin erythema<br>Subcutis dissolved<br>Fascia greyish<br>Fingertest positive<br>Fascia dishwater fluid<br>Muscle contractions<br>Muscle seeping of blood   | Mechanical ventilation<br>Inotropic support | Y | 37/41 | 3 (3) | Dicloxacillin<br>Clindamycin<br>Penicillin<br>Meropenem<br>Gentamycin<br>Clindamycin | 0 | 3x |
| 6025 | emm1 | m | 38 | chronic wound<br>psoriasis<br>other skin disease      | Severe sepsis                 | 26 | oedema<br>rubor<br>increased skin temperature | Skin erythema<br>Subcutis dissolved<br>Fascia greyish<br>Facia positive finger test<br>Fascia dishwater fluid<br>Muscle contractions<br>Muscle seeping of blood<br>Muscle brownish discoloration | none  | Y | 0/16  | 6 (6) | 0<br>Penicillin<br>Dicloxacillin<br>Gentamycin                                       | 0 | 0  |

# Sepsis, septic shock or severe septic shock are calculated according to the guidelines laid out in references(1-3).

± Length of hospital stay (ICU/final discharge from hospital)

§ Number of surgical interventions (interval days)

▫ Antibiotic treatment pre ICU at referral hospital/During ICU at INFECT study hospital

& Number of HBO treatment/accumulated minutes of treatment at 284 kPa atm. abs.

¶ One dose equals 25 g/24 h

\* Time represents length of stay at tertiary (INFECT study) hospital. Patient discharged to primary hospital. Length of stay at a primary hospital not available.

Abbreviations used: Gen, Gender; Comorb, comorbidities; Surv, survival.

**Supplemental Table 2. Biofilm formation\* of GAS NSTI strains on different surfaces**

| Strain | Polystyrene |     |      |       | Glass    |    |      |       |
|--------|-------------|-----|------|-------|----------|----|------|-------|
|        | uncoated    | FN  | Co I | Co IV | uncoated | FN | Co I | Co IV |
| 5626   | 1           | 1   | 0    | 0     | 1        | 1  | 0    | 0     |
| 8003   | 1           | 1   | 0    | 0     | 0        | 0  | 0    | 0     |
| 8157   | 0/1         | 0/1 | 0    | 0     | 0        | 0  | 0    | 0     |

Abbreviations: FN, fibronectin; Co I, collagen I; Co IV, collagen IV; 0, no biofilm; 1 biofilm.

\*Determined by Nile Red, Wheat germ agglutinin and DAPI positivity.

**Supplemental Table 3. Clinical parameters in patients with or without biofilm during the ICU stay**

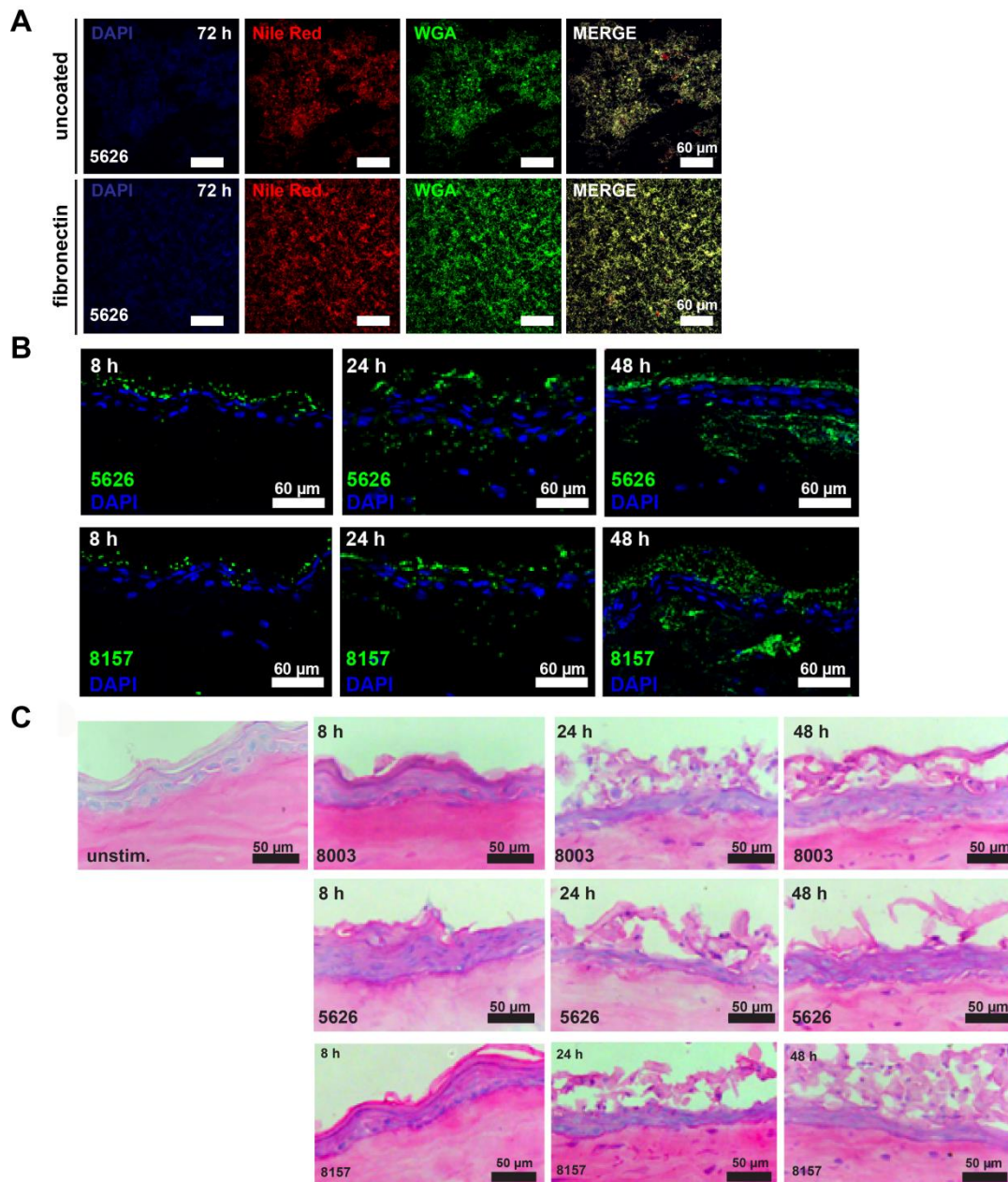
| Parameters*   | Biofilm<br>(n=10) | Non-Biofilm<br>(n=21) | p value |
|---|-------------------|-----------------------|---------|
| ICU stay [d]  | 6                 | 5                     | 0.9155  |
| Hospital stay [d]   | 26                | 21.5                  | 0.7266  |
| No. of surgical interventions within ICU                                | 4                 | 5                     | 0.5980  |
| Period between 1 <sup>st</sup> and last surgery during the ICU stay [d] | 3                 | 5                     | 0.4175  |
| SAPSII <sup>#</sup> (min)   | 37.5              | 38                    | 0.8271  |
| SAPSII <sup>#</sup> (max)   | 42.5              | 40                    | 0.9586  |
| SOFA <sup>#</sup> d0 (min)  | 5.5               | 8                     | 0.2523  |
| SOFA <sup>#</sup> d1 (min)  | 8                 | 6                     | 0.7140  |
| SOFA <sup>#</sup> d2 (min)  | 7.5               | 6                     | 0.6982  |
| SOFA <sup>#</sup> d0 (max)  | 7                 | 9                     | 0.8266  |
| SOFA <sup>#</sup> d1 (max)  | 8                 | 9                     | 0.4447  |
| SOFA <sup>#</sup> d2 (max)  | 7.5               | 6                     | 0.8904  |
| Co-morbidities (n)  | 1                 | 0                     | 0.4701  |

\*Median values for each group are presented.

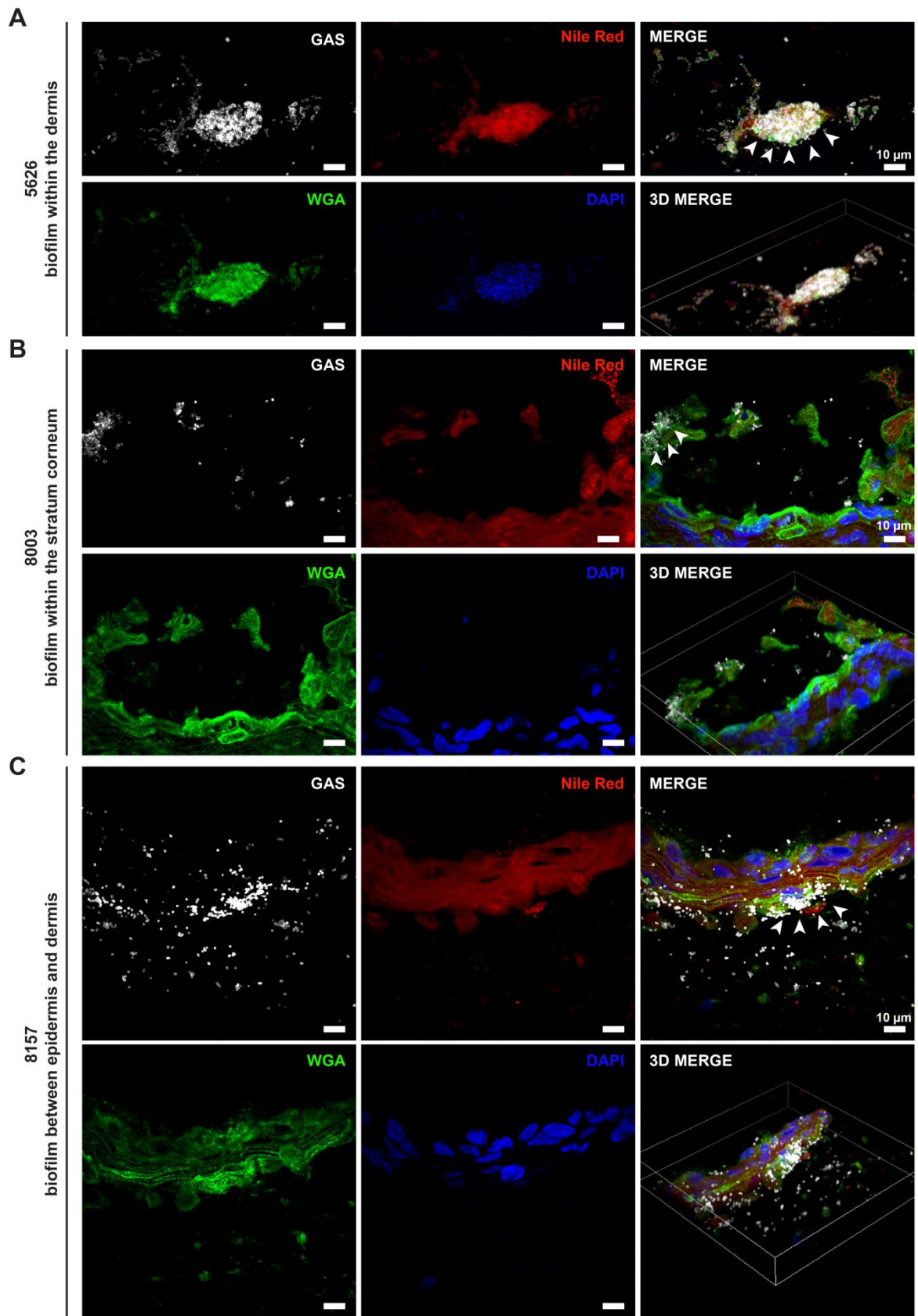
# SOFA or SAPSII min/max, missing values are replaced by minimum or maximum values, respectively.

**Supplemental Table 4. Primers used in this study**

| <b>Gene</b>     | <b>Primer</b>  | <b>Sequence (5'-3')</b> |
|-----------------|----------------|-------------------------|
| <i>ihk</i>      | ihk_g-for      | ACTGCTCTATCCTTCCTTGC    |
|                 | ihk_g-rev      | TTGCTAGCATCTGTTACGGG    |
| <i>irr</i>      | irr_g-for      | TAGACAAGTGGCAGACAACG    |
|                 | irr_g-rev      | CGTCATCTAATGCCGTTAGC    |
| <i>mga</i>      | mga_g-for      | CCATCAACATTGCGGTTTGC    |
|                 | mga_g-rev      | GACGCTTGAGTGTTGAAAGG    |
| <i>nra/rofA</i> | nra/rofA_g-for | GAACAAGATTGTCCGGTGAGG   |
|                 | nra/rofA_g-rev | TAAGGTGGGTGGA ACTATGG   |
| <i>speB</i>     | speB_for       | TGCTAACCCAGTATTTGCCG    |
|                 | speB_rev       | ATATCTTCTGCGCTTCGTGC    |
| <i>gyrA</i>     | gyrA_g-for     | TGCCATGAGTGTCATTGTGG    |
|                 | gyrA_g-rev     | ACATCACCCGTAATACGAGC    |



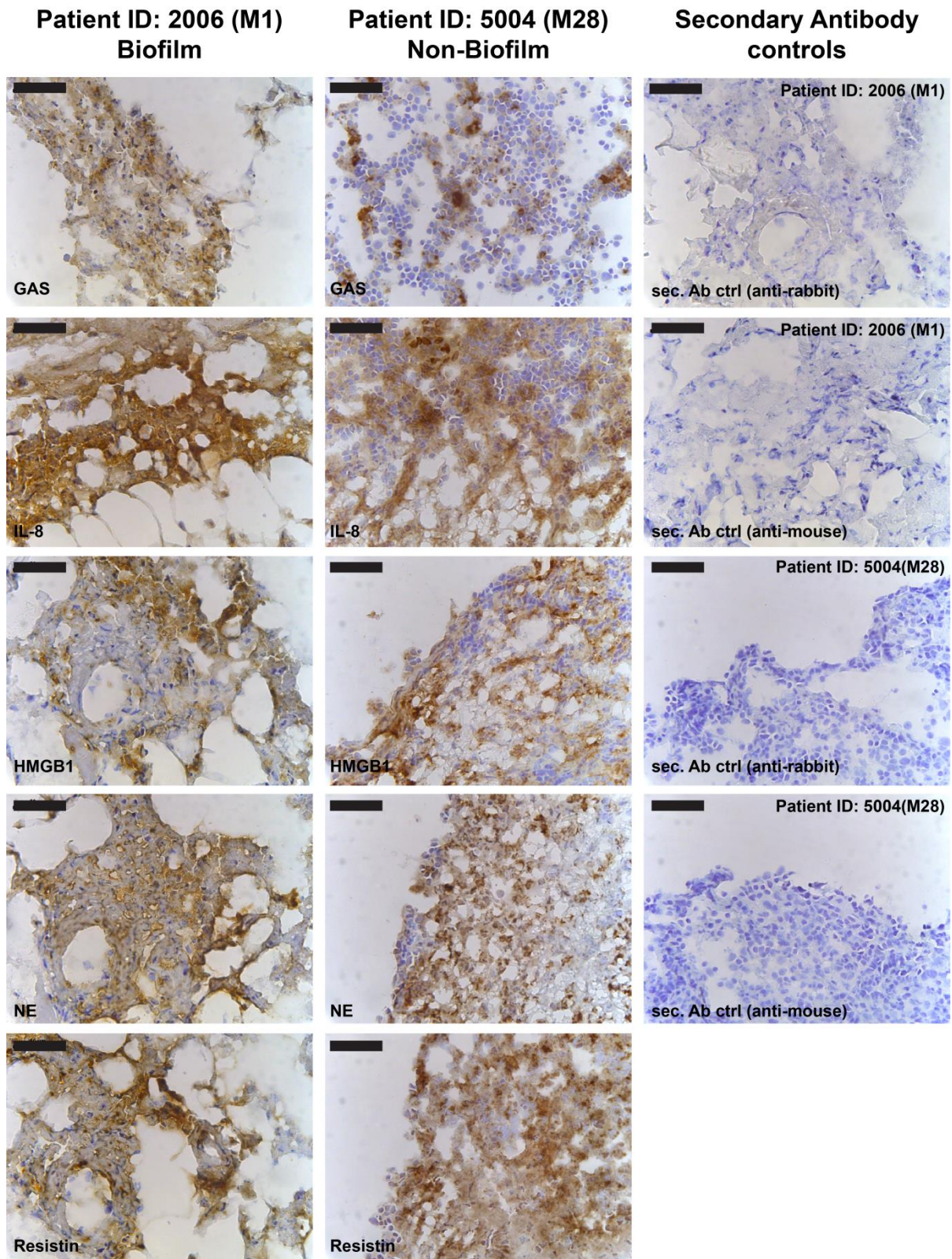
**Supplemental Figure 1. Biofilm and infection phenotype of clinical NSTI strains.** (A) Biofilm formation of 5626 strain on uncoated and fibronectin coated glass surfaces. Biofilm was detected through staining of Nile Red, wheat germ agglutinin (WGA), and DAPI. No staining was evident for strains 8003 and 8157. (B) Immuno-fluorescence analysis of GAS infected tissue 8, 24, and 48 h post infection with indicated strains. (C) Histological analysis of the skin model tissue after infection. Representative micrographs with indicated strains are shown.



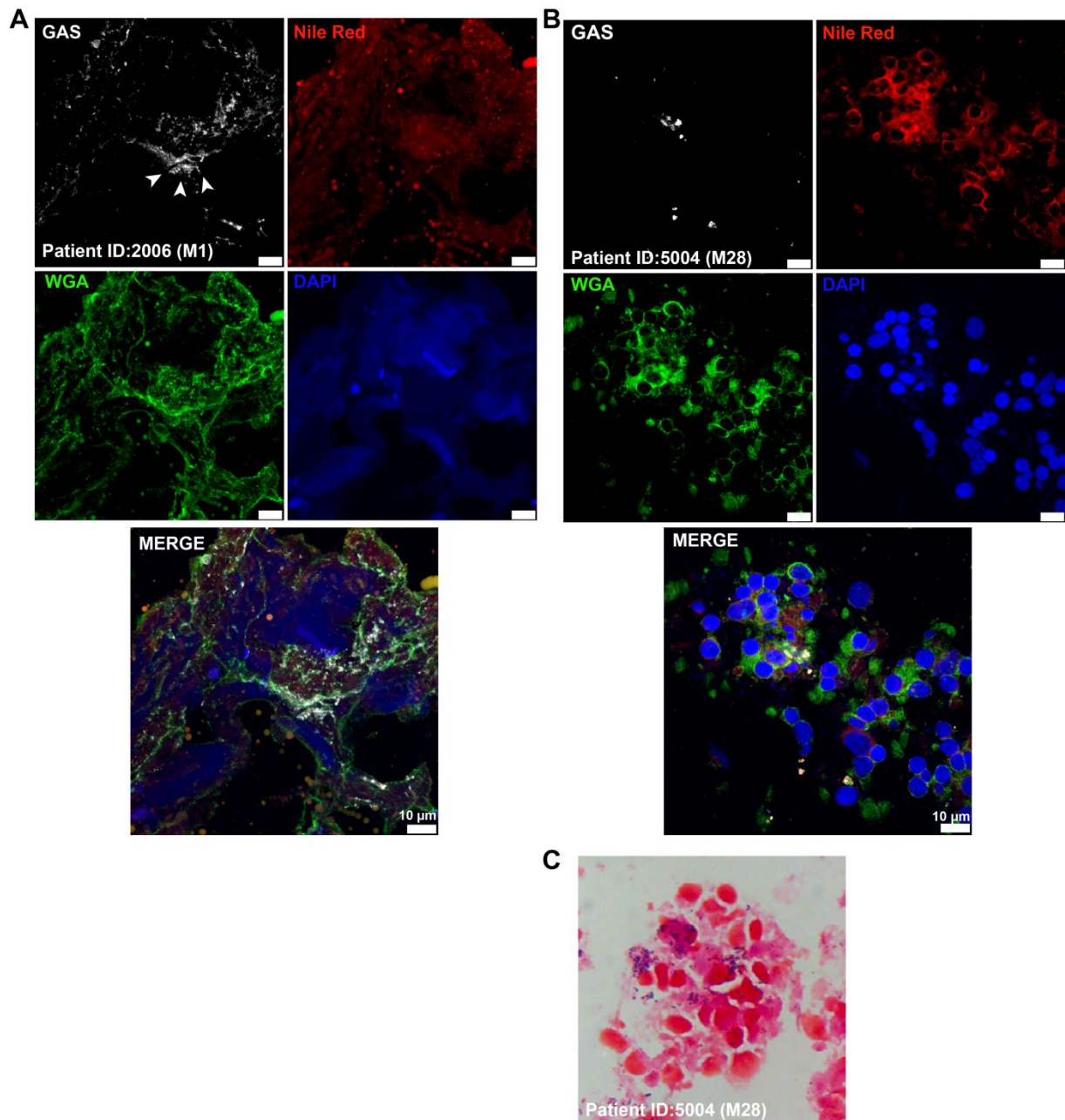
**Supplemental Figure 2. GAS biofilm in skin tissue models.** Identification of bacteria and biofilm structures in tissues infected with 5626 (A), 8003 (B), and 8517 (C) visualized by



immuno-staining with GAS specific antibody, WGA, Nile red, and DAPI. Arrows are indicating biofilm.

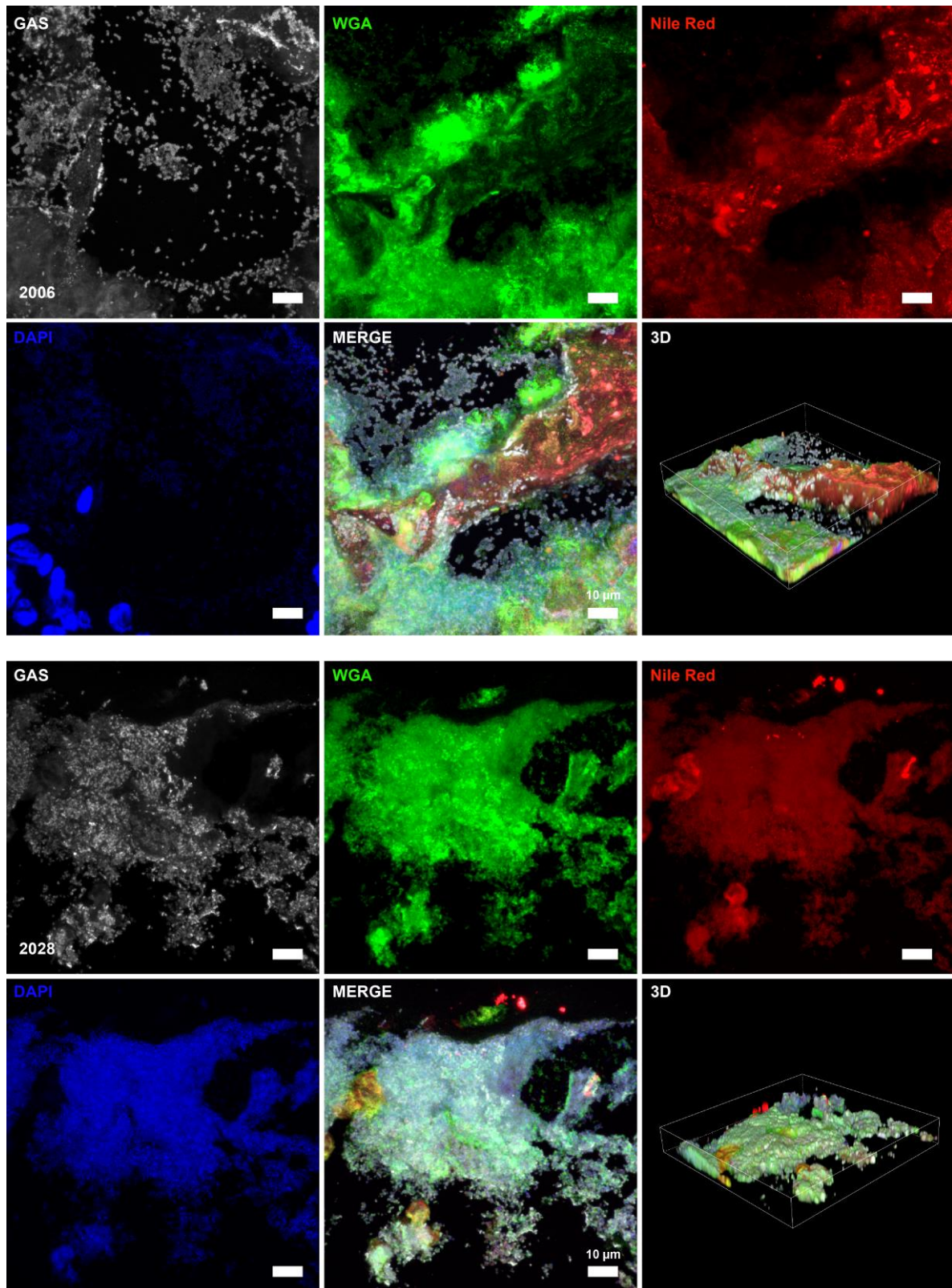


**Supplemental Figure 3. Inflammation and phagocytic infiltration at the local site of infection.** Representative immunohistochemically stained tissue biopsies from biofilm and non-biofilm associated patients. (GAS, group A streptococcus; HMGB1, High-mobility group protein B1; NE, neutrophil elastase).



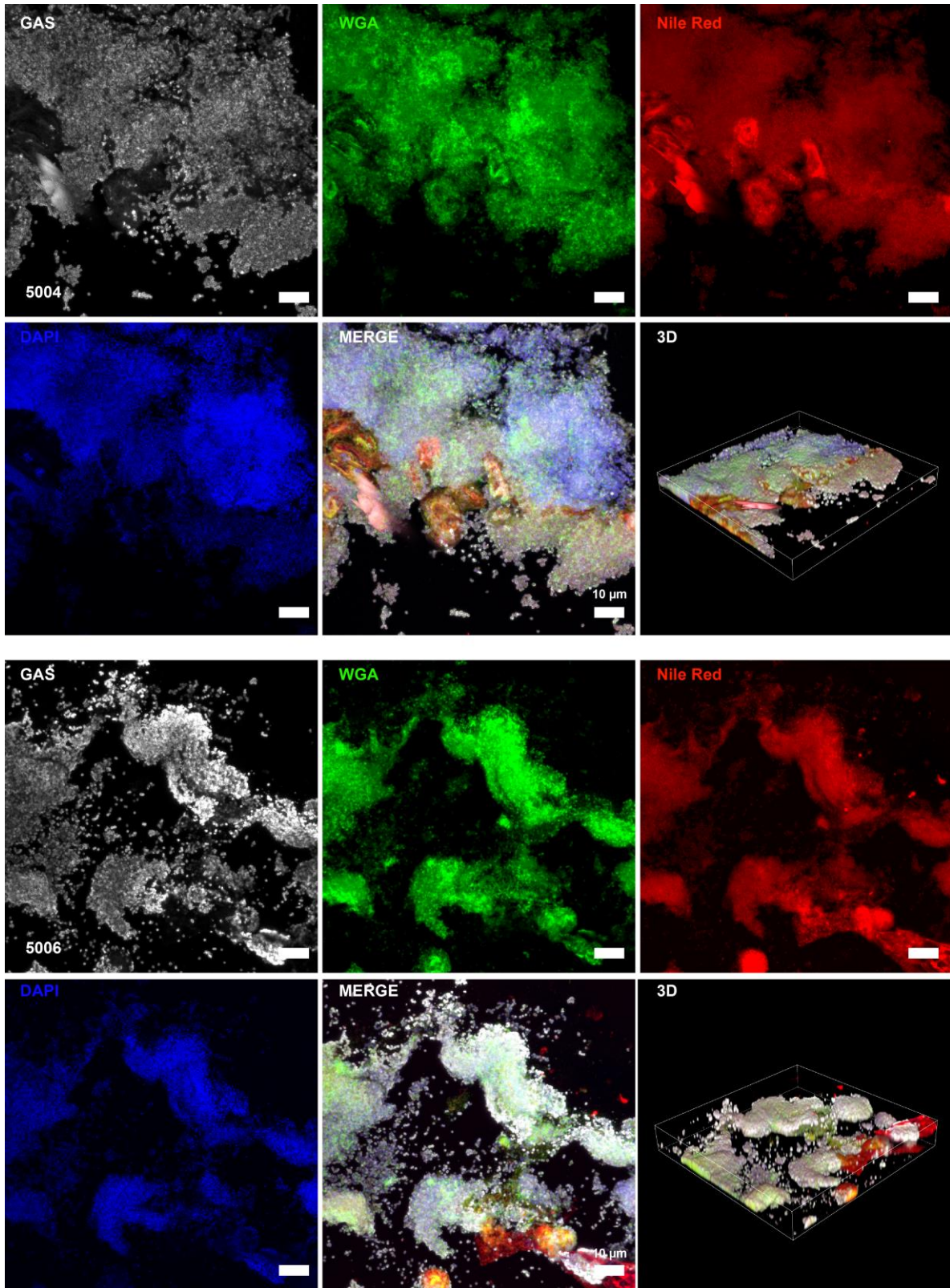
**Supplemental Figure 4. Biofilm in GAS NSTI patients.** Identification of bacteria and biofilm structures in biofilm-associated (A) and non-biofilm (B) patient biopsies visualized by immuno-staining with GAS specific antibody, WGA, Nile red, and DAPI. Arrows are indicating biofilm structures. (C) Gram-stained tissue section from indicated patient; magnification 63x.



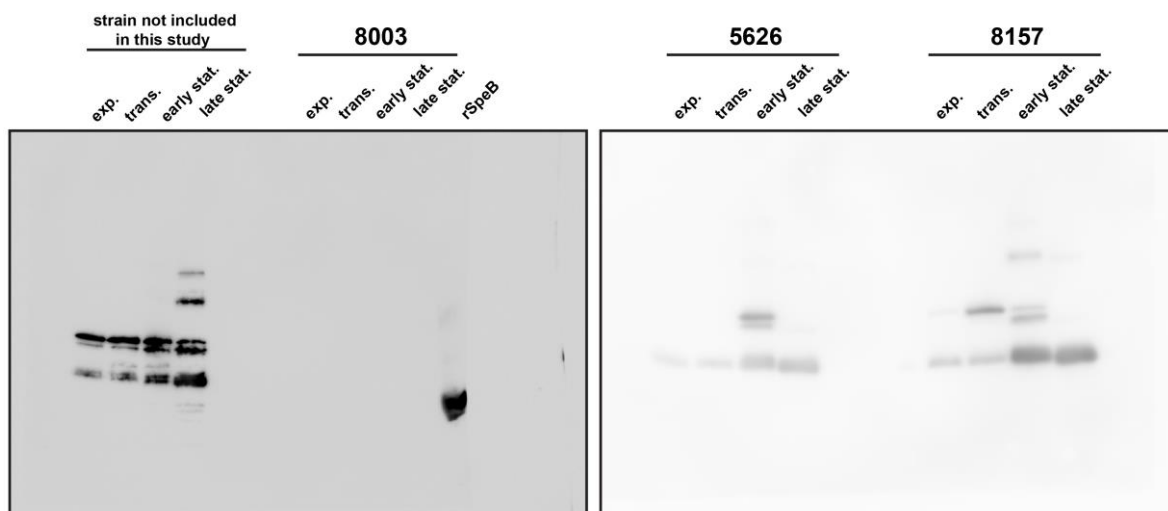


**Supplemental Figure 5. GAS biofilm in skin tissue models.** Identification of bacteria and biofilm structures in tissues infected with clinical GAS strains 2006 and 2028 visualized by immuno-staining with GAS specific antibody, WGA, Nile red, and DAPI; magnifications 100x.

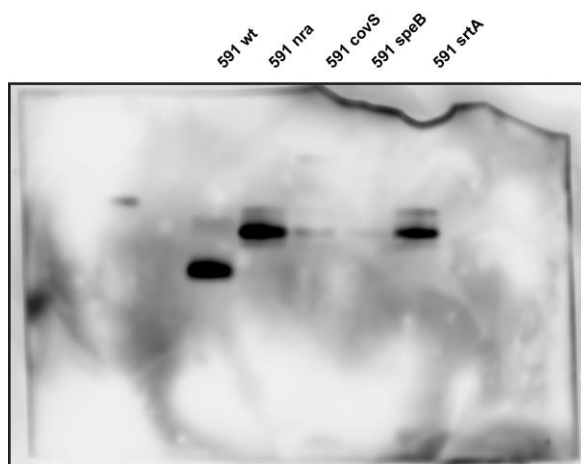




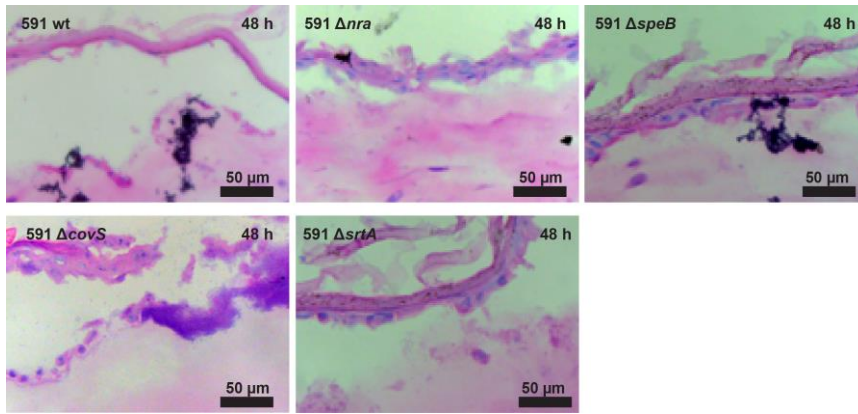
**Supplemental Figure 6. GAS biofilm in skin tissue models.** Identification of bacteria and biofilm structures in tissues infected with clinical GAS strains 5004 and 5006 visualized by immuno-staining with GAS specific antibody, WGA, Nile red, and DAPI; magnifications 100x.



exp., exponential; trans., transition; stat., stationary; rSpeB, recombinant SpeB

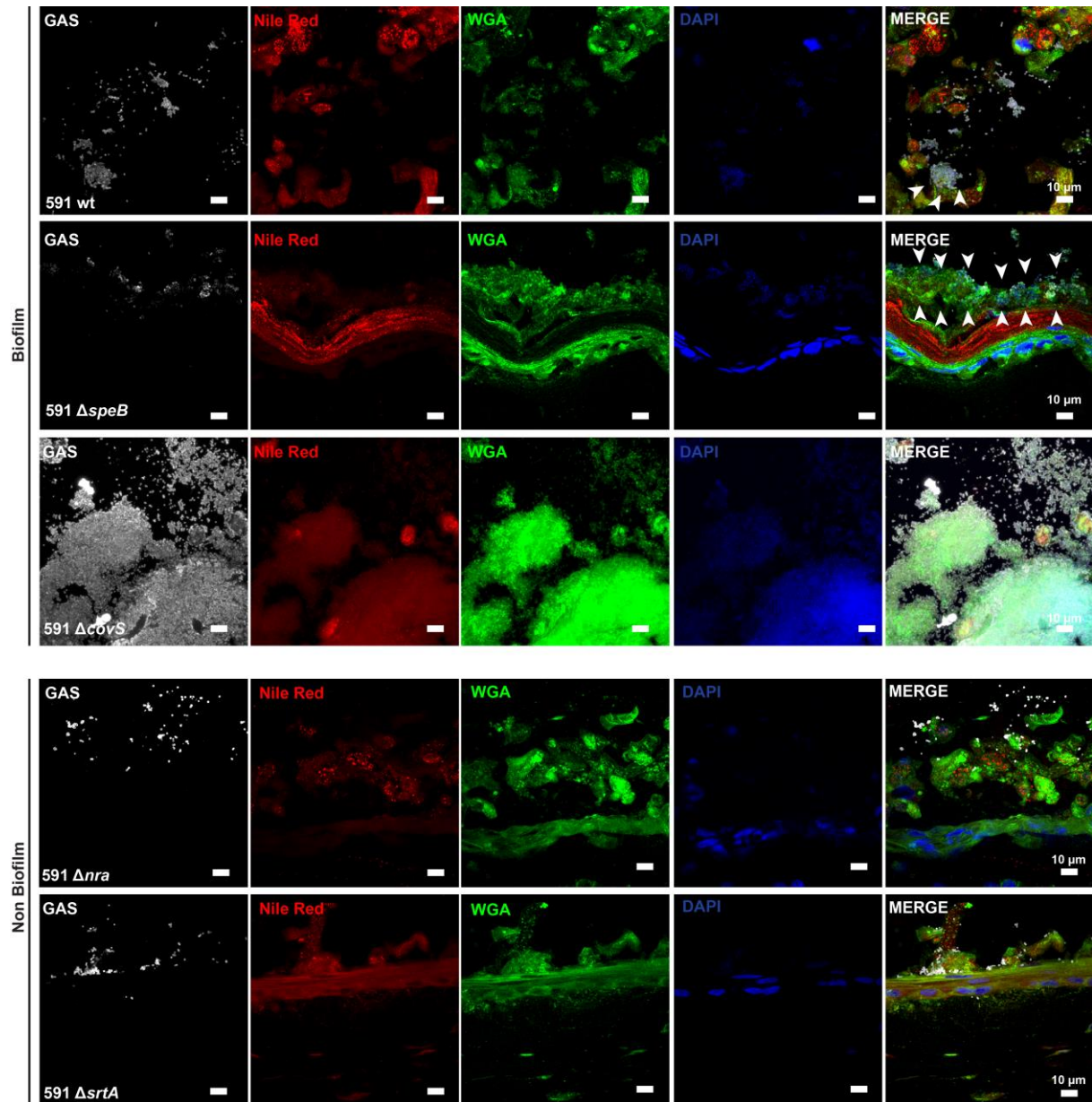


**Supplemental Figure 7. SpeB expression during different growth stages.** Original SpeB-Blots (cropped versions are shown in Figure 5 and Figure 7).



**Supplemental Figure 8. Tissue injury after 48 h of infection.** Representative haematoxylin/eosin stained tissue sections of the skin model infected with indicated bacteria at indicated time points.





**Supplemental Figure 9. Nra and SrtA mediated biofilm formation.** Representative immunofluorescence images of biofilm or bacterial single staining after 48 h of skin model infections. GAS specific antibody, wheat germ agglutinin visualizing carbohydrates, and Nile red visualizing lipids were used.



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