Supplementary Information

Validation of biofilm formation on human skin wound models and demonstration of clinically translatable bacteria-specific volatile signatures

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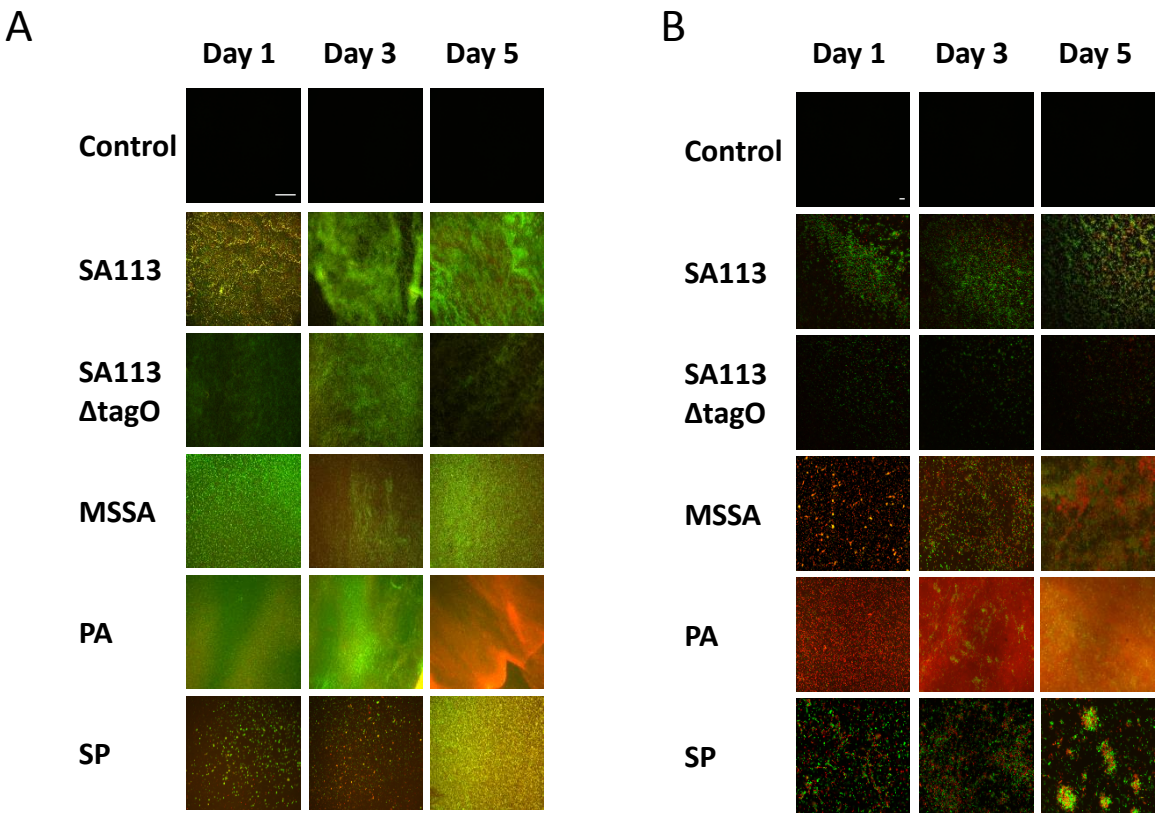

Figure S1. ***In vitro*** **model of biofilm formation**. Aerial view of biofilms on coverslips showing viable bacteria stained green with the nucleic acid stain SYTO® 9 and dead cells and extracellular DNA stained red with the nucleic acid stain propidium iodide (PI). (**A)** Stereo-fluorescence microscopy. Scale bar: 250µm. (**B)** Wide-field fluorescence microscopy. Scale bar: 10µm is equal in all images.

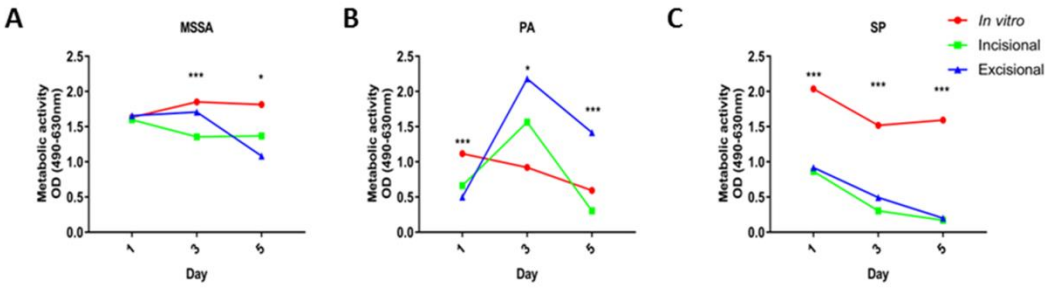

Figure S2. **Inter-model comparisons of metabolic activity of biofilms**. Inter-model comparisons of metabolic activity of MSSA (**A**), PA (**B**) and SP (**C**) biofilms following XTT reduction assay. Mean ± standard error of the mean (n=6), * P < 0.05, ** P < 0.01, *** P < 0.001, as determined by one-way analysis of variances with accompanying Tukey post hoc analyses.

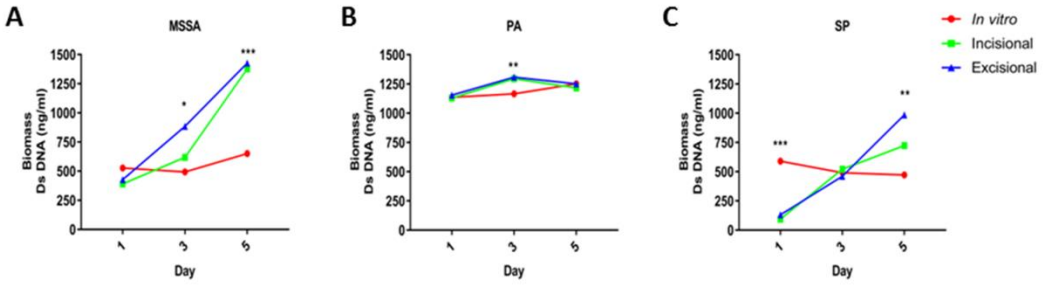

Figure S3. **Inter-model comparisons of biomass of biofilms**. Inter-model comparisons of biomass of MSSA (**A**), PA (**B**) and SP (**C**) biofilms following Quant-iT PicoGreen dsDNA reagent assay. Mean ± standard error of the mean (n=6), * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, as determined by one-way analysis of variances with accompanying Tukey post hoc analyses.

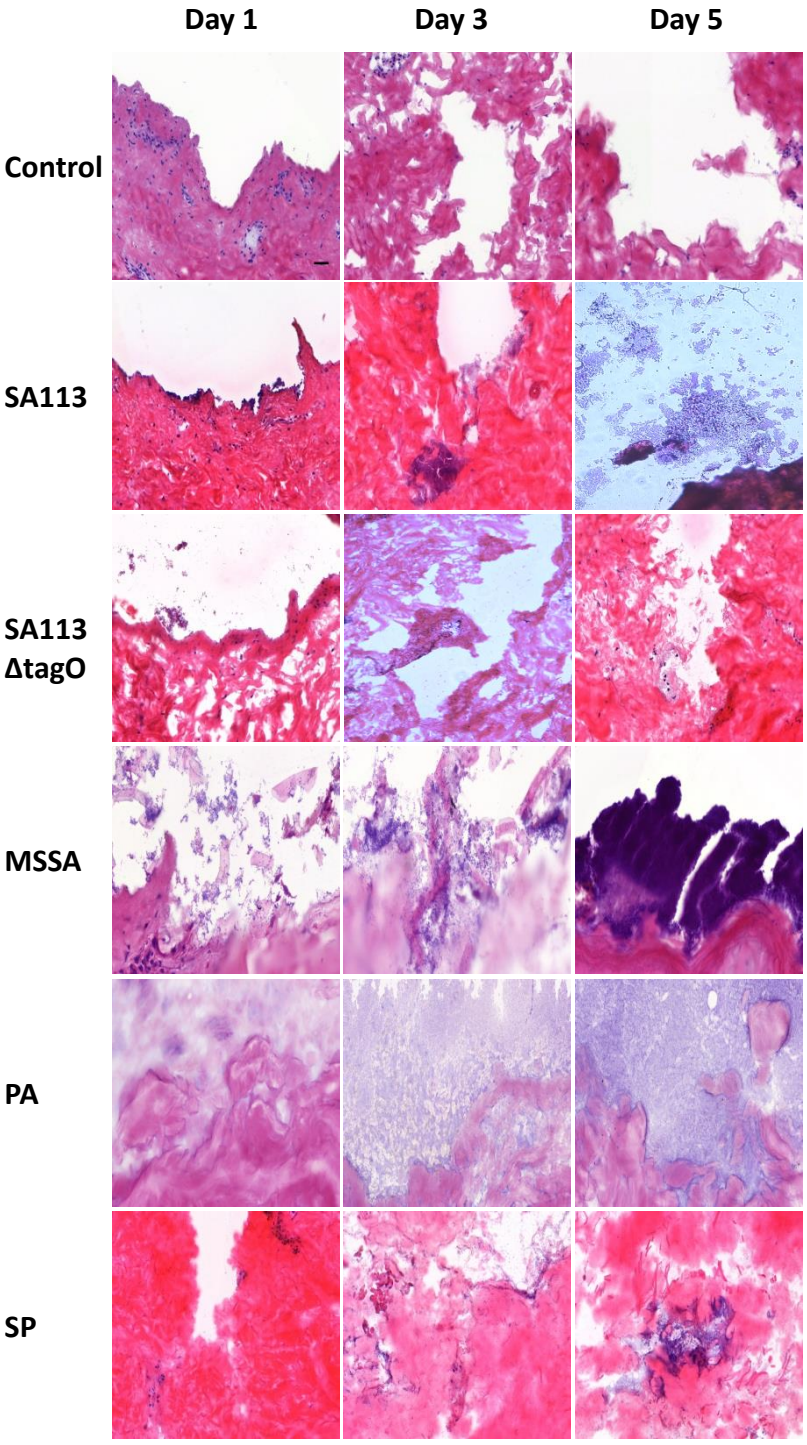

Figure S4. **Haematoxylin and eosin stained microscopy of biofilms of *ex vivo* incisional wound model.** Wound tissue is stained pink and biofilm material blue or purple. Scale bar: 20 µm is equal in all images.

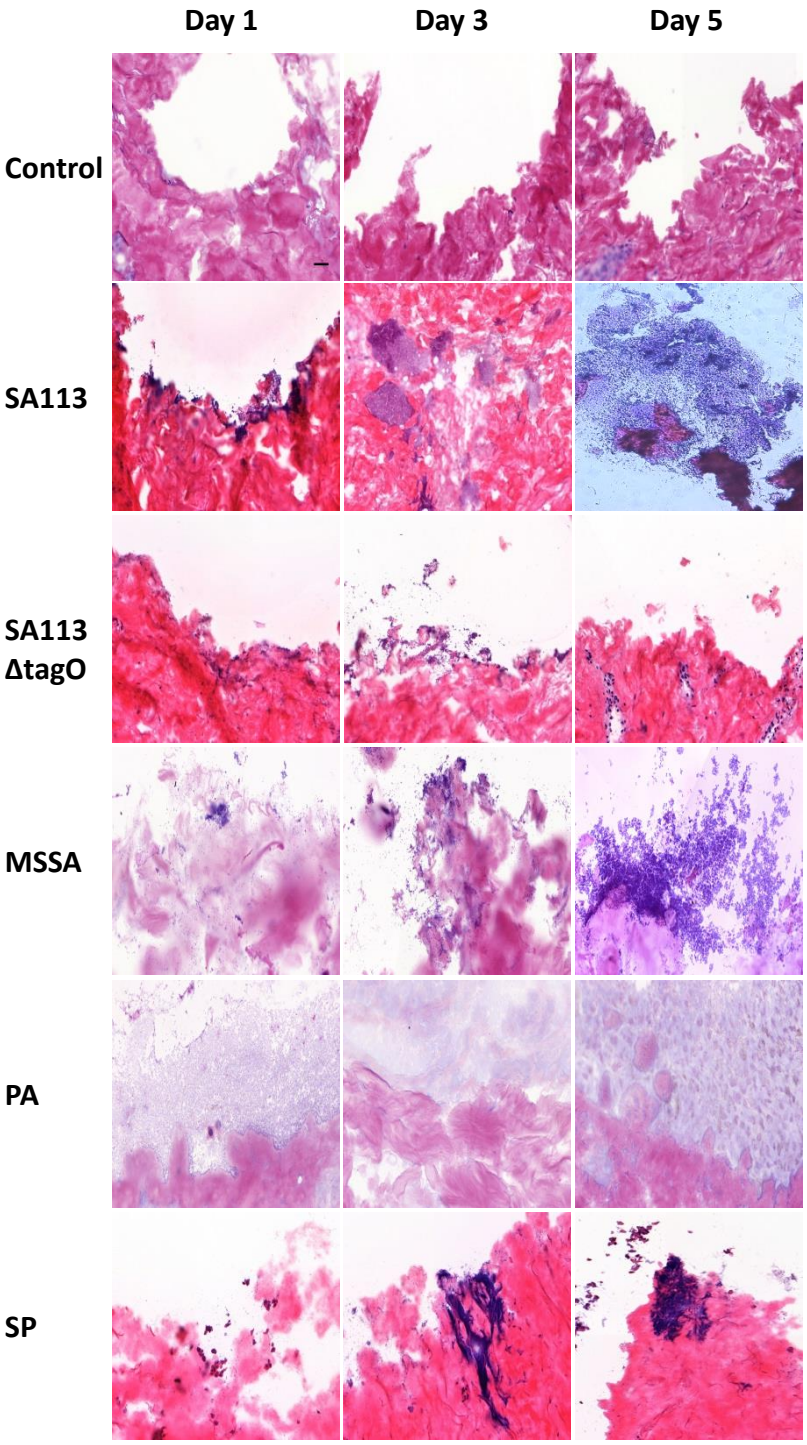

Figure S5. Haematoxylin and eosin stained microscopy of biofilms of *ex vivo* excisional wound model. Wound tissue is stained pink and biofilm material blue or purple. Scale bar: 20 µm is equal in all images.

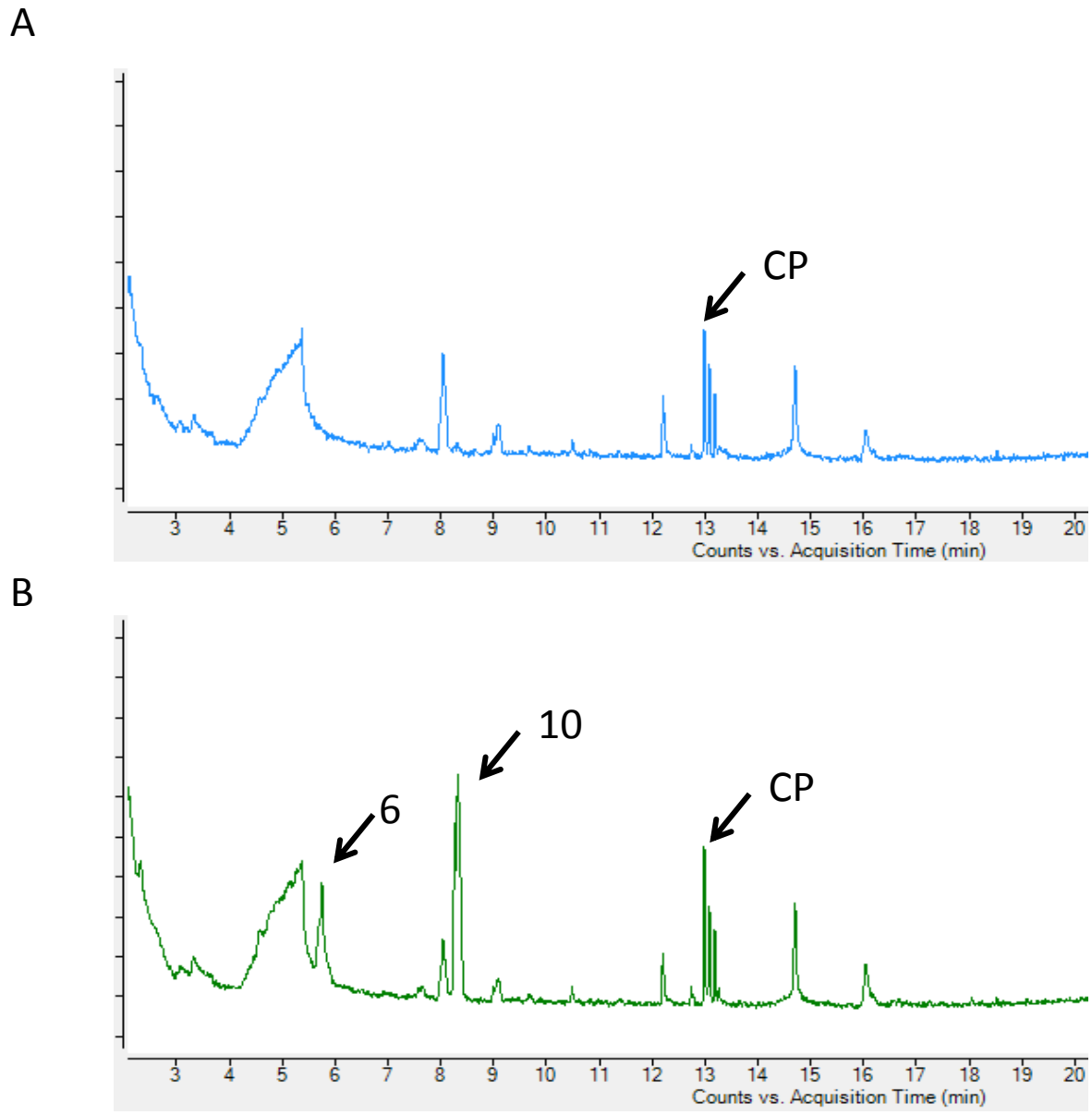

Figure S6. Representative chromatograms at day 1 of (A) *ex vivo* excisional cutaneous wound explant control and (B) *ex vivo* excisional cutaneous wound explant inoculated with MSSA. 6 - 2-methyl-1-propanol; 10 - 3-methyl-1-butanol; CP – common peak. Snapshots of chromatograms up to 20 min after which there were no further peaks. Non-labelled peaks were shared between inoculated and control samples.

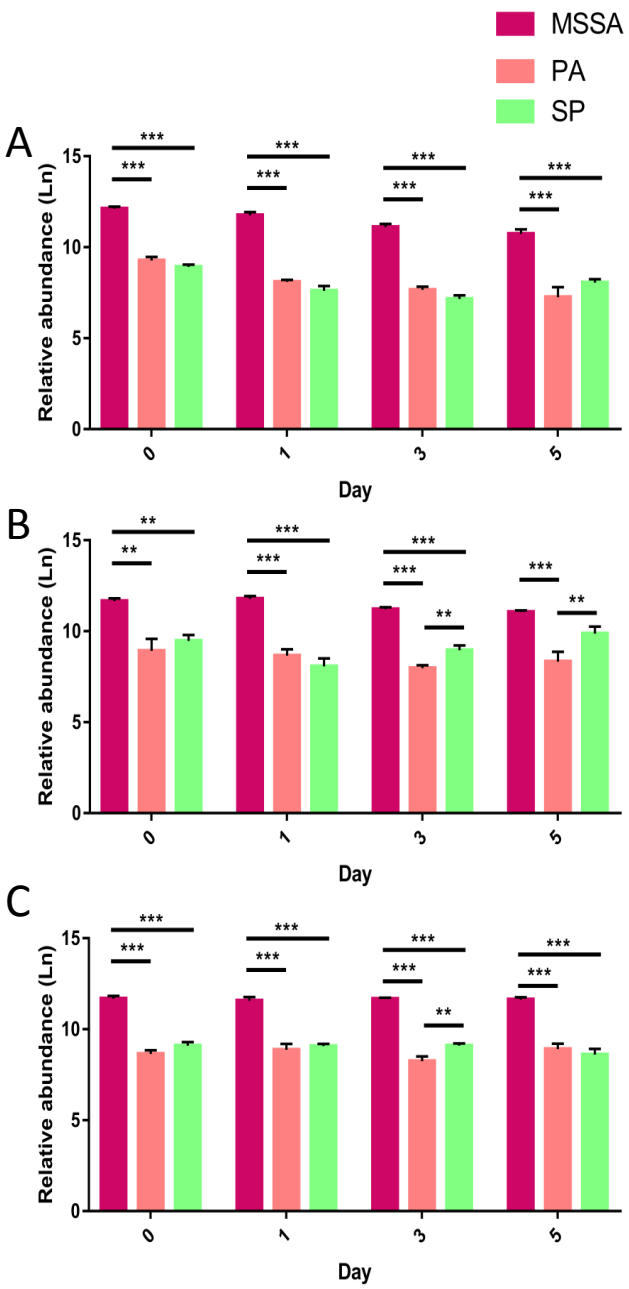

Figure S7. **The presence of 2-methyl-1-propanol among bacterial species and in different wound models. (A)** *in vitro*; (**B)** *ex vivo* incisional and (**C)** *ex vivo* excisional wound model. Relative abundance of 2-methyl-1-propanol on days 0, 1, 3 and 5 produced by MSSA (pink), PA (peach) and SP (green) biofilms. Mean ± standard error of the mean (n=6), * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, as determined by one-way analysis of variances with accompanying Tukey post hoc analyses.

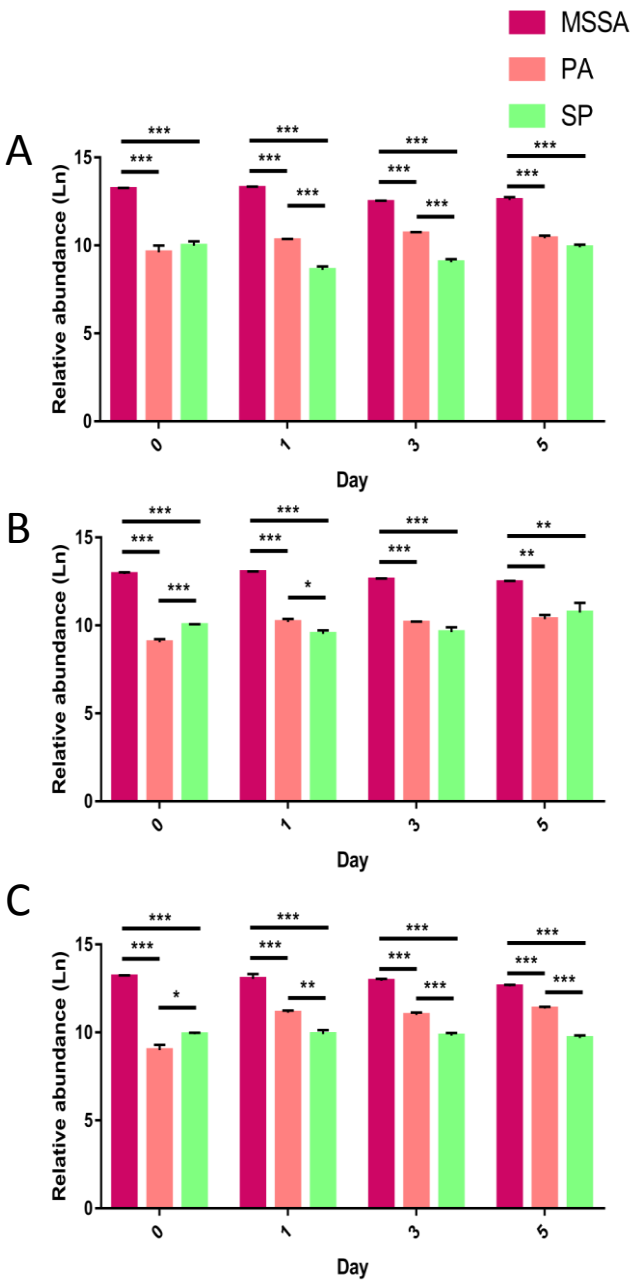

Figure S8. **The presence of 3-methyl-1-butanol among bacterial species and in different wound models. (A)** *in vitro*; (**B)** *ex vivo* incisional; and (**C)** *ex vivo* excisional wound model. Relative abundance of 3-methyl-1-butanol on days 0, 1, 3 and 5 produced by MSSA (pink), PA (peach) and SP (green) biofilms. Mean ± standard error of the mean (n=6). * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, as determined by one-way analysis of variances with accompanying Tukey post hoc analyses.

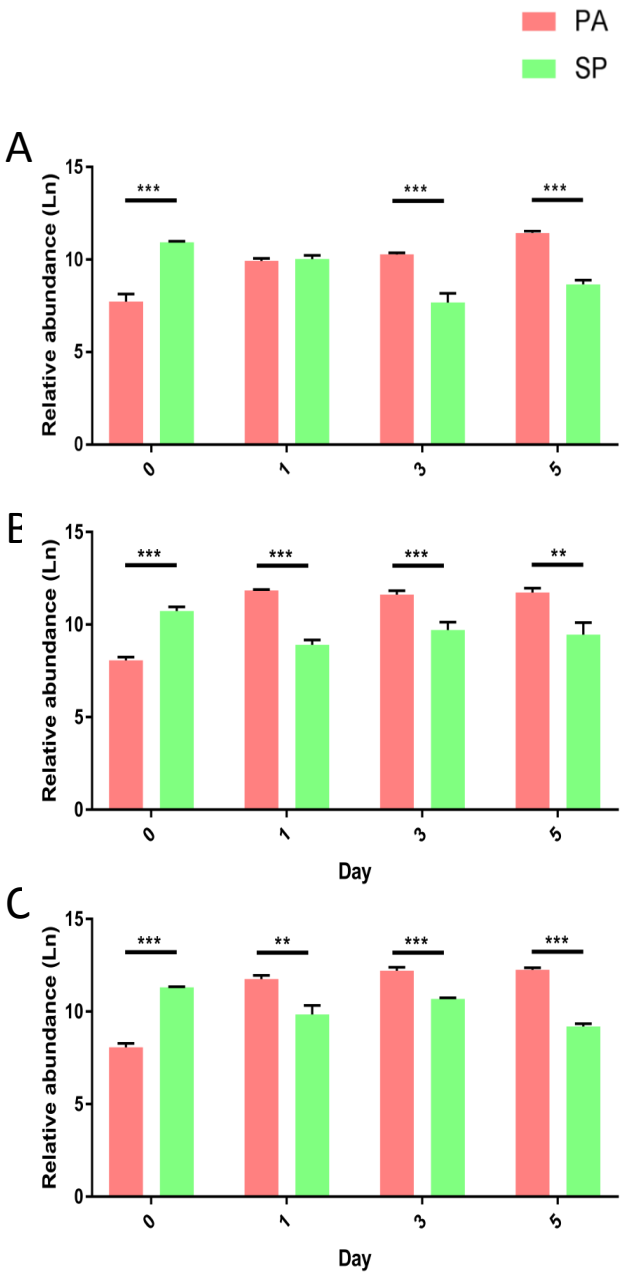

Figure S9. **The presence of 1-undecene among bacterial species and in different wound models (except MSSA). (A)** *in vitro*; (**B)** *ex vivo* incisional; and (**C)** *ex vivo* excisional wound model. Relative abundance of 1-undecene on days 0, 1, 3 and 5 produced by PA (peach) and SP (green) biofilms. Mean ± standard error of the mean (n=6), * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, as determined by one-way analysis of variances with accompanying Tukey post hoc analyses.

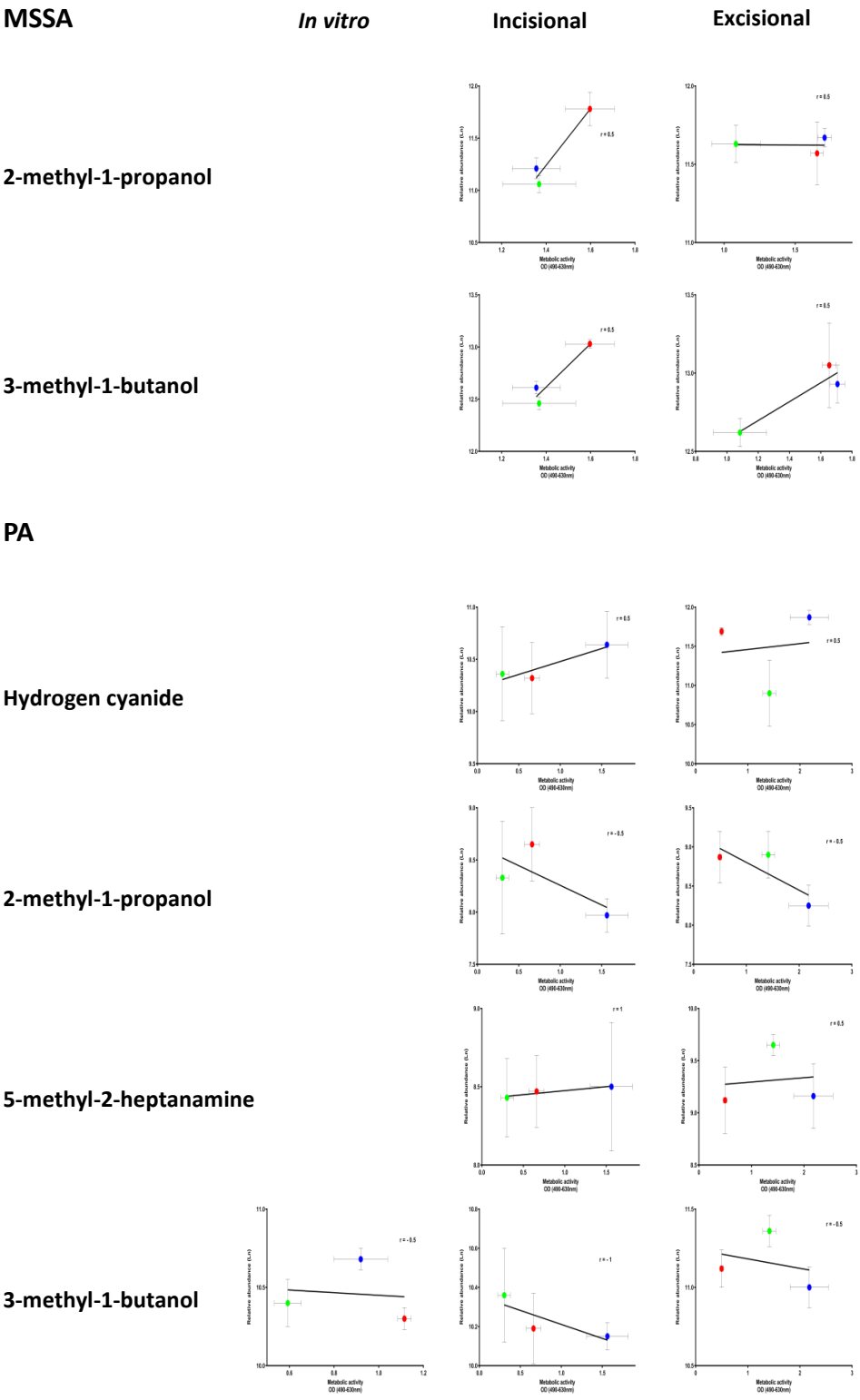

Figure S10. Correlations between biofilm metabolic activity and abundance of VOCs across models on day 1 (red), 3 (blue) and 5 (green). Mean ± SEM (n=6).

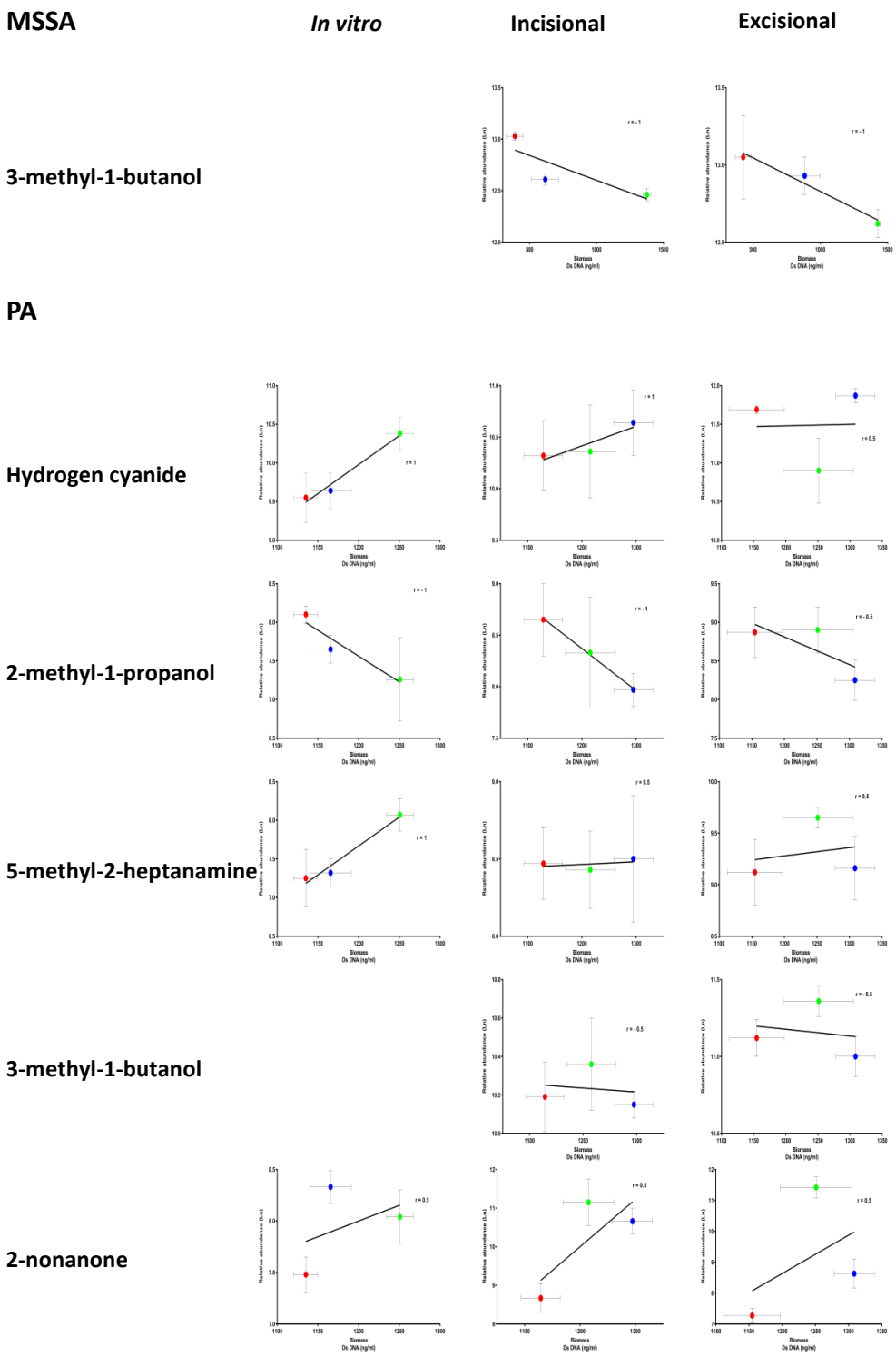

Figure S11. Correlations between biofilm biomass and abundance of VOCs across models on day 1 (red), 3 (blue) and 5 (green). Mean ± SEM (n=6).

Table S1. Proposed chemical compound of the peaks identified by GC-MS based on National Institute of Standards and Technology library spectral matching (spectra of all three species combined).

Peak	Proposed compound	Match	R. Match	RI	Prob (%)
1	3-methylbutanal	818	870	652	69.0
2	ethanol	831	901	427	93.3
3	pentanal	858	881	699	35.2
4	2-butanol	671	724	598	8.3
5	hydrogen cyanide	857	961	300	99.0
6	2-methyl-1-propanol	819	852	625	88.5
7	5-methyl-2-hexanamine	657	827		44.1
8	5-methyl-2-heptanamine	669	715		7.28
9	1-undecene	873	874	1091	7.8
10	3-methyl-1-butanol	912	918	736	67.0
11	2-nonanone	915	917	1092	76.4
12	2-undecanone	914	922	1294	80.7

R. Match – reverse match; RI – retention index; Prob – probability

Table S2. VOCs identified from biofilms of different bacterial species in the *in vitro* model indicating the statistical differences between time point measurements

VOC	D0 vs. D1	D0 vs. D3	D0 vs. D5	D1 vs. D3	D1 vs. D5	D3 vs. D5
MSSA						
3-methylbutanal	> 0.05	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**	> 0.05
pentanal	**0.001**	**< 0.001**	**< 0.001**	**< 0.001**	**<0.001**	> 0.05
2-methyl-1-propanol	> 0.05	**0.006**	**< 0.001**	> 0.05	**0.005**	> 0.05
3-methyl-1-butanol	> 0.05	**< 0.001**	**0.002**	**< 0.001**	**0.001**	> 0.05
PA						
hydrogen cyanide	**< 0.001**	**< 0.001**	**< 0.001**	> 0.05	> 0.05	> 0.05
2-methyl-1-propanol	> 0.05	**0.007**	**0.001**	> 0.05	> 0.05	> 0.05
5-methyl-2-hexanamine	**< 0.001**	**< 0.001**	**< 0.001**	**0.004**	**< 0.001**	**< 0.001**
5-methyl-2-heptanamine	**< 0.001**	**< 0.001**	**< 0.001**	> 0.05	> 0.05	> 0.05
1-undecene	**< 0.001**	**< 0.001**	**< 0.001**	> 0.05	**0.001**	**0.009**
3-methyl-1-butanol	> 0.05	**0.01**	> 0.05	> 0.05	> 0.05	> 0.05
2-nonanone	**< 0.001**	**< 0.001**	**< 0.001**	**0.012**	> 0.05	> 0.05
SP						
ethanol	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**	> 0.05
2-butanol	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**	> 0.05
2-methyl-1-propanol	**0.001**	**< 0.001**	**0.03**	> 0.05	> 0.05	**0.022**
1-undecene	> 0.05	**< 0.001**	**< 0.001**	**< 0.001**	**0.016**	> 0.05
3-methyl-1-butanol	**< 0.001**	**0.013**	> 0.05	> 0.05	**0.001**	**0.025**

P values (one-way analysis of variances with accompanying Tukey post hoc analyses); D0 – planktonic phase; D 1, 3 and 5 – biofilm phase (days)

Table S3. VOCs identified from biofilms of different bacterial species in the *ex vivo* human cutaneous incisional model indicating the statistical differences between time points

VOC	D0 vs. D1	D0 vs. D3	D0 vs. D5	D1 vs. D3	D1 vs. D5	D3 vs. D5
MSSA						
3-methylbutanal	**< 0.001**	**< 0.001**	**< 0.001**	> 0.05	> 0.05	> 0.05
pentanal	**< 0.001**	**< 0.001**	**< 0.001**	> 0.05	> 0.05	> 0.05
2-methyl-1-propanol	> 0.05	> 0.05	**0.015**	**0.021**	**0.003**	> 0.05
3-methyl-1-butanol	> 0.05	**0.019**	**0.001**	**0.001**	**< 0.001**	> 0.05
PA						
hydrogen cyanide	**< 0.001**	**< 0.001**	**< 0.001**	> 0.05	> 0.05	> 0.05
2-methyl-1-propanol	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05
5-methyl-2-hexanamine	**< 0.001**	**< 0.001**	**< 0.001**	> 0.05	> 0.05	> 0.05
5-methyl-2-heptanamine	**< 0.001**	**< 0.001**	**< 0.001**	> 0.05	> 0.05	> 0.05
1-undecene	**< 0.001**	**< 0.001**	**< 0.001**	> 0.05	> 0.05	> 0.05
3-methyl-1-butanol	**0.001**	**0.001**	**< 0.001**	> 0.05	> 0.05	> 0.05
2-nonanone	**< 0.001**	**< 0.001**	**< 0.001**	**0.009**	**0.001**	> 0.05
2-undecanone	**< 0.001**	**< 0.001**	**< 0.001**	> 0.05	> 0.05	> 0.05
SP						
ethanol	**< 0.001**	**< 0.001**	**< 0.001**	> 0.05	> 0.05	> 0.05
2-butanol	**< 0.001**	**< 0.001**	**< 0.001**	> 0.05	> 0.05	> 0.05
2-methyl-1-propanol	> 0.05	> 0.05	> 0.05	> 0.05	**0.01**	> 0.05
1-undecene	**0.03**	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05
3-methyl-1-butanol	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05

P values (one-way analysis of variances with accompanying Tukey post hoc analyses); D0 – planktonic phase; D 1, 3 and 5 – biofilm phase (days)

Table S4. VOCs identified from biofilms of different bacterial species in the *ex vivo* human cutaneous excisional model indicating the statistical differences between time points

VOC	D0 vs. D1	D0 vs. D3	D0 vs. D5	D1 vs. D3	D1 vs. D5	D3 vs. D5
MSSA						
3-methylbutanal	**< 0.001**	**< 0.001**	**< 0.001**	> 0.05	> 0.05	> 0.05
pentanal	**< 0.001**	**< 0.001**	**< 0.001**	> 0.05	> 0.05	> 0.05
2-methyl-1-propanol	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05
3-methyl-1-butanol	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05
PA						
hydrogen cyanide	**< 0.001**	**< 0.001**	**< 0.001**	> 0.05	> 0.05	**0.024**
2-methyl-1-propanol	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05
5-methyl-2-hexanamine	**< 0.001**	**< 0.001**	**< 0.001**	**0.017**	**0.039**	> 0.05
5-methyl-2-heptanamine	**< 0.001**	**< 0.001**	**< 0.001**	> 0.05	> 0.05	> 0.05
1-undecene	**< 0.001**	**< 0.001**	**< 0.001**	> 0.05	> 0.05	> 0.05
3-methyl-1-butanol	**< 0.001**	**< 0.001**	**< 0.001**	> 0.05	> 0.05	> 0.05
2-nonanone	**< 0.001**	**< 0.001**	**< 0.001**	**0.028**	**< 0.001**	**< 0.001**
2-undecanone	**< 0.001**	**< 0.001**	**< 0.001**	> 0.05	> 0.05	> 0.05
SP						
ethanol	**0.001**	> 0.05	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**
2-butanol	**< 0.001**	**0.011**	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**
2-methyl-1-propanol	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05
1-undecene	**0.004**	> 0.05	**< 0.001**	> 0.05	> 0.05	**0.003**
3-methyl-1-butanol	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05

P values (one-way analysis of variances with accompanying Tukey post hoc analyses); D0 – planktonic phase; D 1, 3 and 5 – biofilm phase (days)