Volatile organic compound signature from co-culture of lung epithelial cell line with *Pseudomonas aeruginosa*

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SUPPLEMENTARY MATERIAL

A549-bacterial optimisation

A multiplicity of infection (MOI) of 50 and 100 were tested on A549 cells seeded in T25 flasks. After cell count by Trypan blue dye exclusion, both MOIs yielded a comparable number of viable cells (Fig S1). A MOI of 100 was subsequently used for the infection experiment.

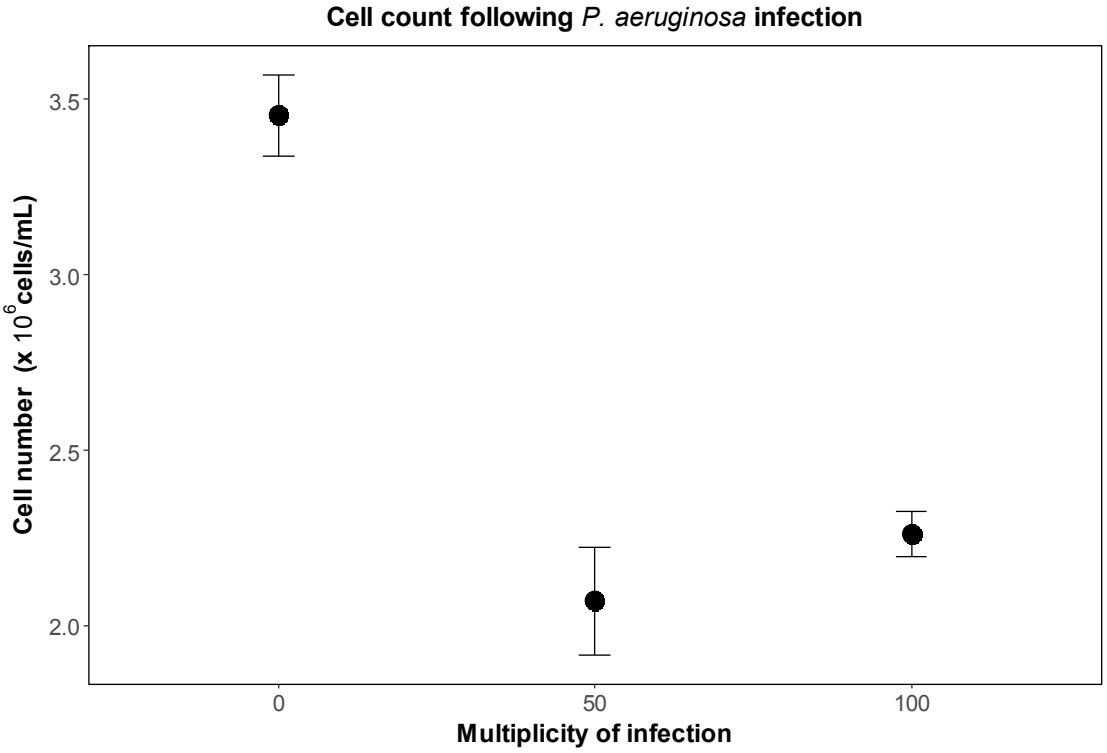


Fig S1. Viable cell count by Trypan blue exclusion after infection of A549 cells in T25 flasks. The averages of three replicates are shown for each condition and the error bars represent standard errors (SE).

Treatment of A549 cells with H_2O_2

A549 cells were treated with varying concentrations of H_2O_2 and cell viability was determined using the alamarBlue™ assay. High fluorescence intensity indicates a large proportion of viable cells. Decreasing fluorescence intensity indicating cell death was observed from 10 mM dose (Fig S2).

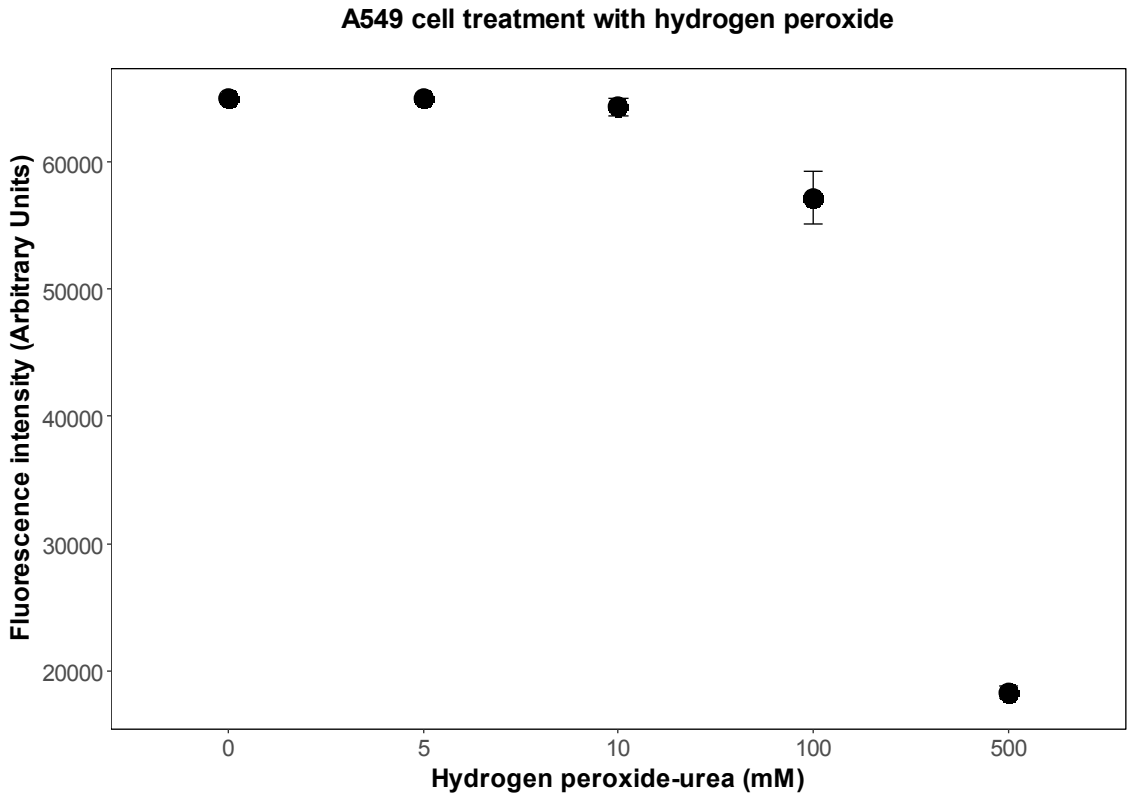


Fig S2. Cell viability of A549 epithelial cell determined by alamarBlue assay after treatment with varying concentrations of H_2O_2. The error bars represent SE of eight repeats.

Viable cell count after hydrogen peroxide treatment

A representative viable cell count is shown following 100 mM H_2O_2 treatment of A549 epithelial cells in glass bottles and counted after headspace collection (Fig S3).

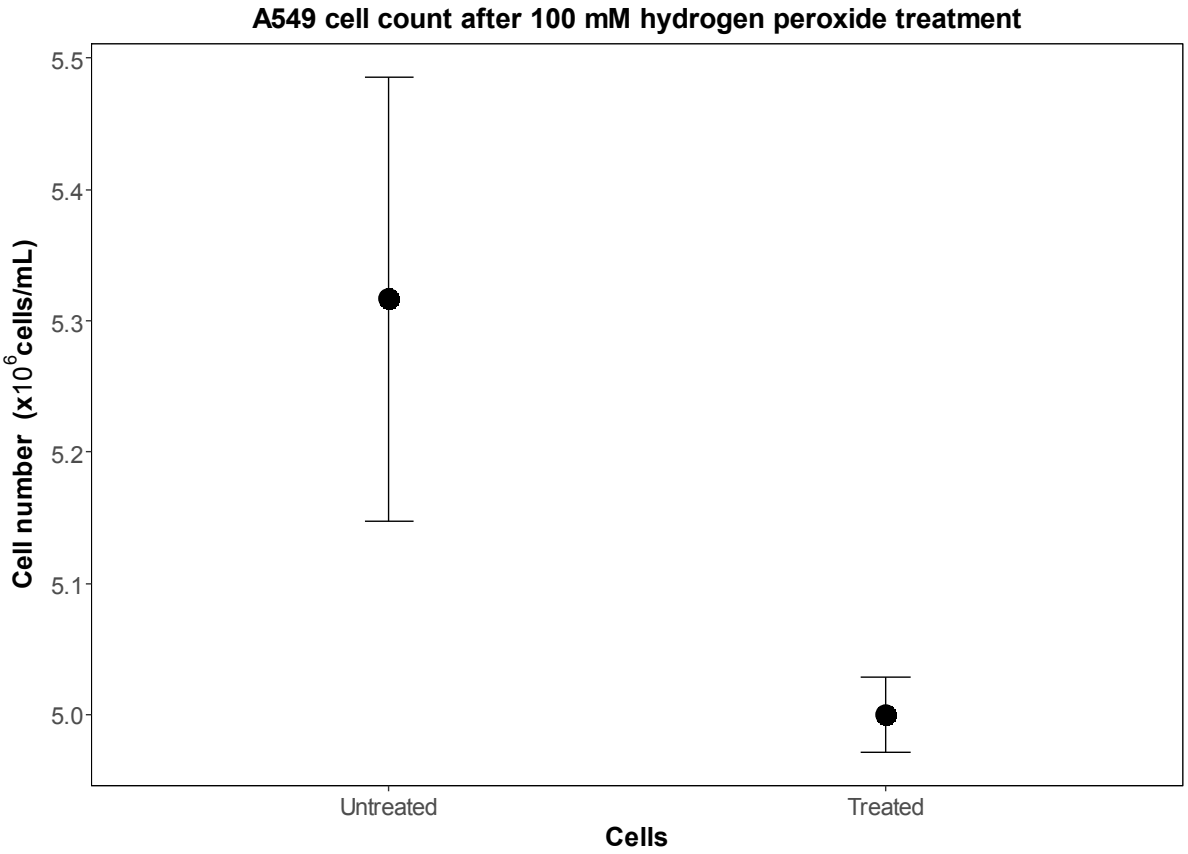
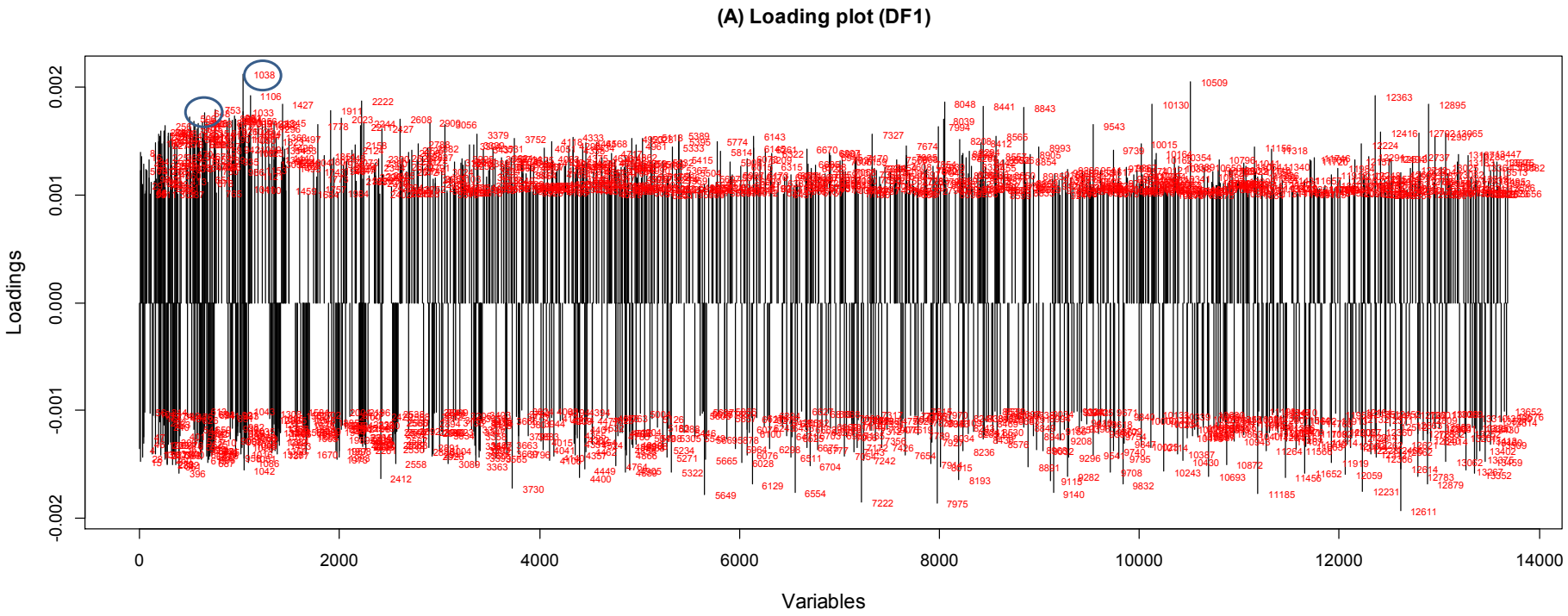


Fig S3. Viable cell count by Trypan blue exclusion after treatment of A549 cell in glass bottles with 100 mM H_2O_2. The averages of three replicates are shown for each condition and the error bars represent SE.

PC-DFA loadings plot

The loadings plots from PC-DFA analysis are shown in Fig S4. The variables at the extreme ends of the loading plots were investigated to determine those features that contributed most to the observed separation in the scores plot (Fig 6). Fragments 1038 and 506 belongs to 3-methyl-1-butanol and ethylidenecyclopropane respectively (Fig S4A) and fragment 94 to methyl tert butyl ether (Fig S4B). Fragments 16, 730 and 478 represent tert-butyl ethyl ether. The identity of the other fragments is still unknown.


(A) Loading plot (DF1)
Loadings
Variables
0.002
0.001
0.000
-0.001
-0.002
0
2000
4000
6000
8000
10000
12000
14000

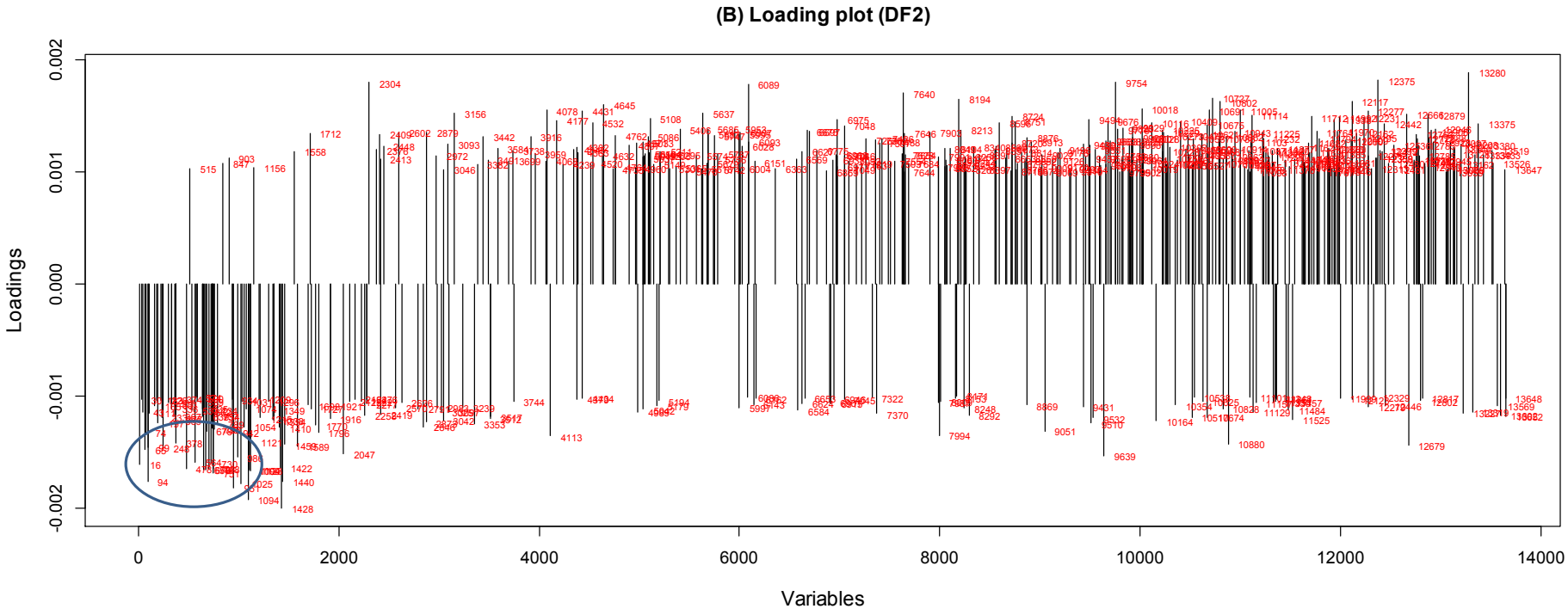


Fig S4. PC-DFA loadings plot for (A) DF1, (B) DF2.