

SUPPLEMENTARY DATA

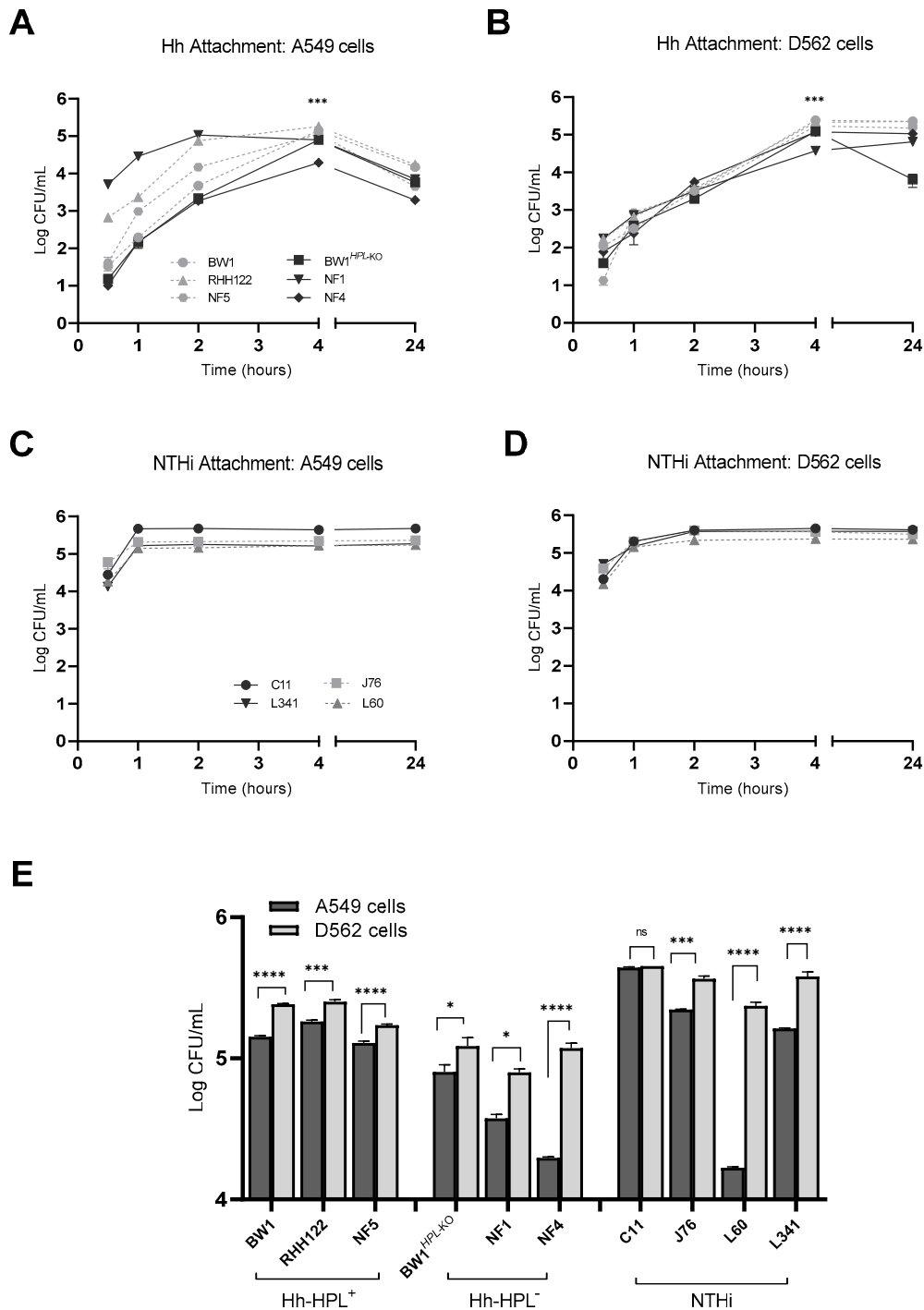


Figure S1] Baseline bacterial attachment to epithelial cells. Colony counts (CFU/mL) of Hh attached to A549 (A), and D562 (B) cell monolayers, showing significant variation between Hh strains. Colony counts (CFU/mL) of NTHi to A549 (C), and D562 (D) during a 24-hour incubation period. A summary of Hh and NTHi attachment to A549 and D562 cell monolayers at maximum attachment time points is shown (E). Error bars represent the \pm SEM or three biological replicates. ns not significant, * $p < 0.05$, *** $p < 0.001$, **** $p < 0.0001$.

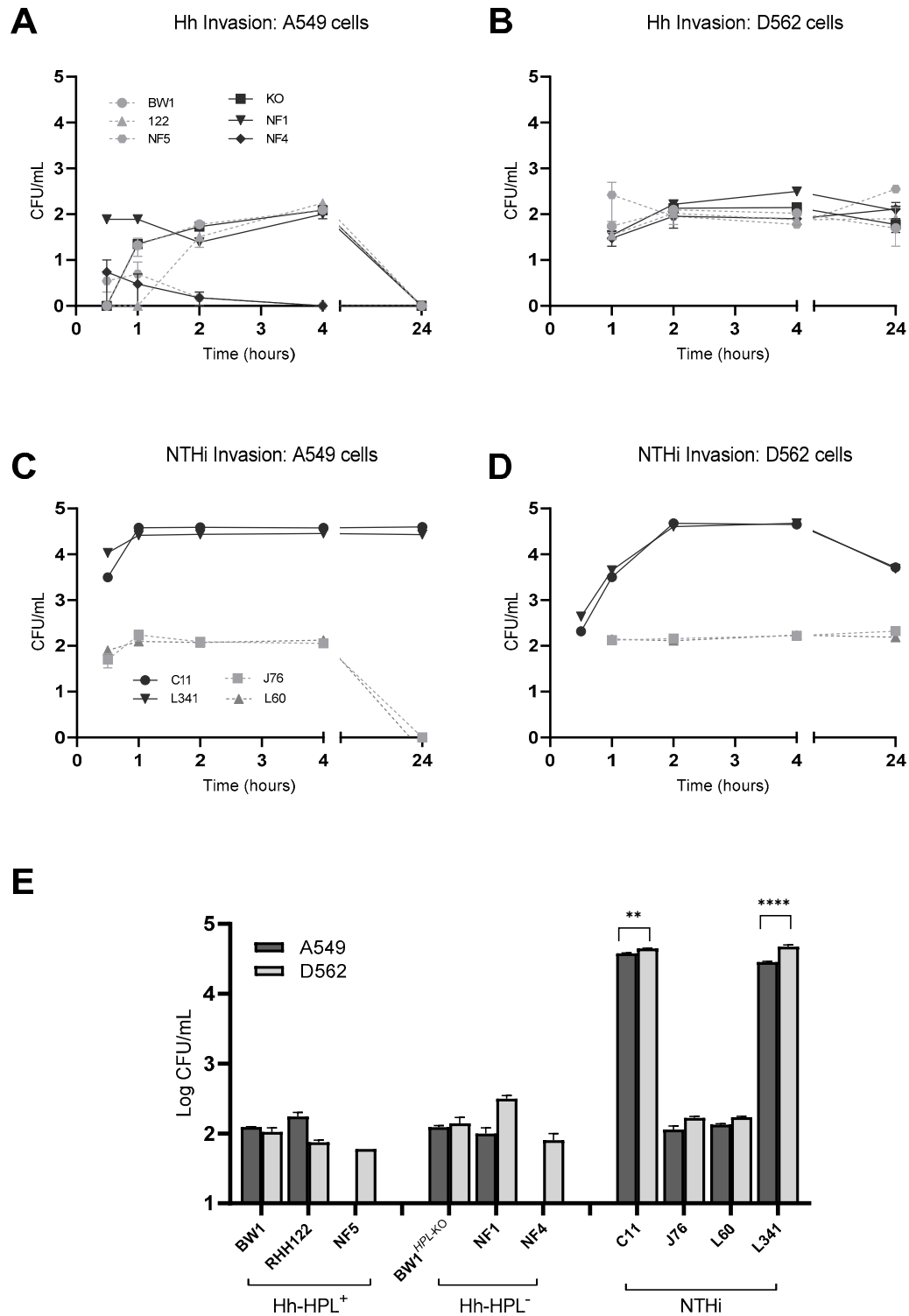


Figure S2| Baseline bacterial invasion of epithelial cells. Colony counts (CFU/mL) of internalised Hh in A549 (A), and D562 (B) cell monolayers, and NTHi in A549 (C), and D562 (D) during a 24-hour incubation period. A summary of Hh and NTHi invasion of A549 and D562 cell monolayers at maximum invasion time points is shown (E). Error bars represent the \pm SEM or three biological replicates. ^{ns} not significant, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.

Table S1 | Specific Hh treatment load (CFU/mL) for competitive colonisation experiments, standardised for differences in attachment capacity.

Hh strain	Treatment load for A549 cells (CFU/mL)	Treatment load for D562 cells (CFU/mL)
BW1	1.50×10^5	2.50×10^5
BW1 ^{HPL-KO}	3.00×10^5	5.00×10^5
RHH122	1.50×10^5	2.50×10^5
NF1	3.00×10^6	1.25×10^6
NF4	1.50×10^5	5.00×10^5
NF5	1.50×10^5	5.00×10^5

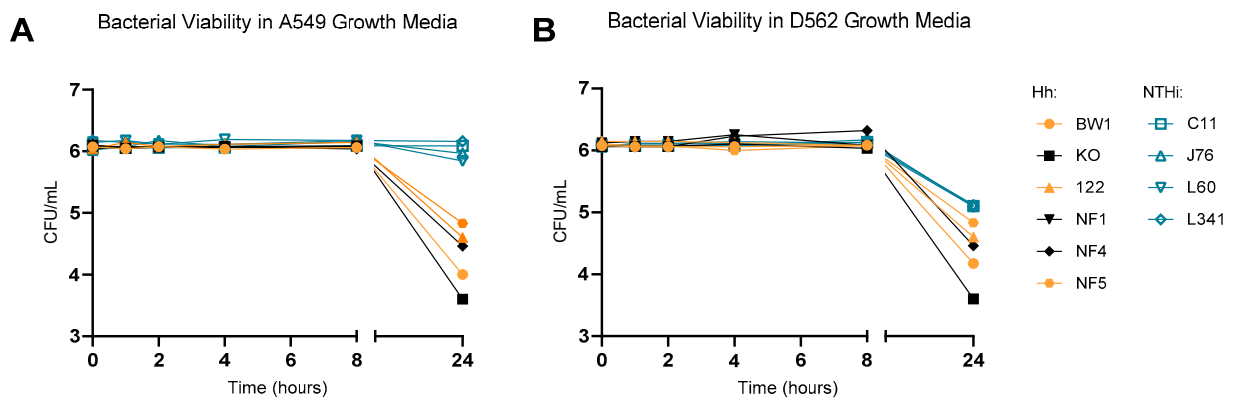


Figure S3 | Bacterial viability in cell culture media. Colony counts (CFU/mL) Hh and NTHi strains grown in RPMI (A549 growth media) **(A)**, and EMEM (D562 growth media) **(B)** over 24 hours. Error bars represent the \pm SEM of two biological replicates.

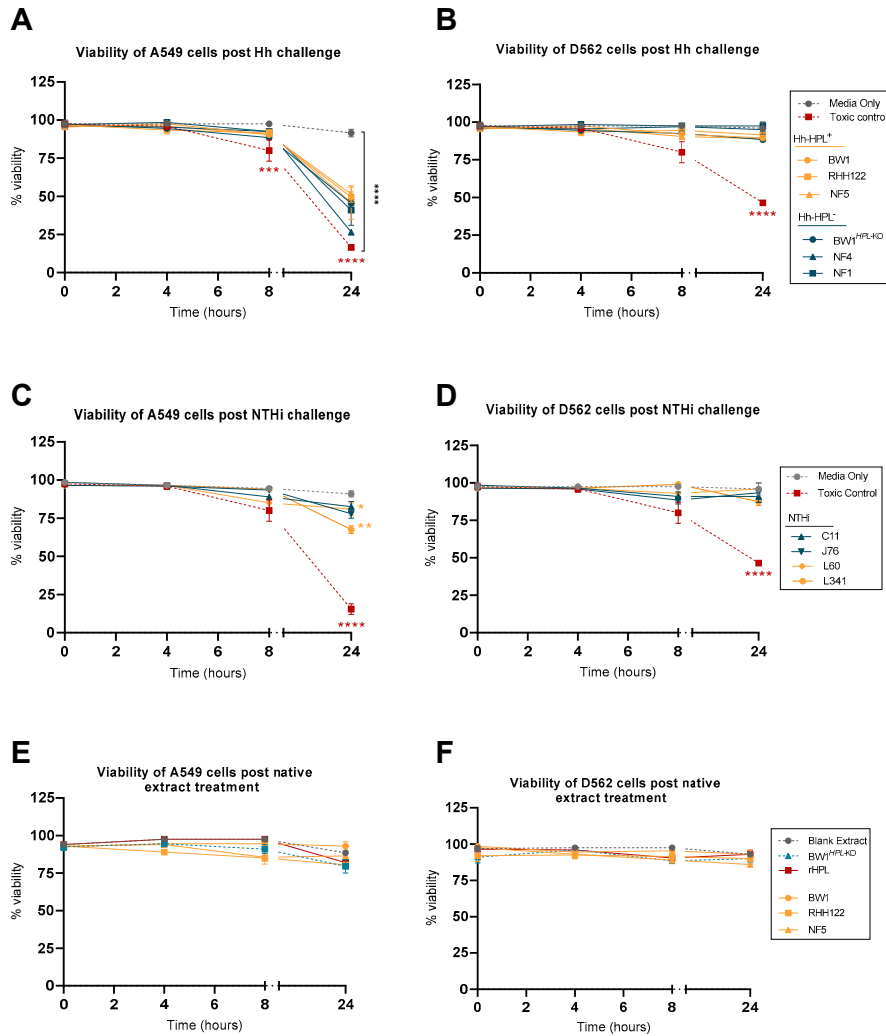


Figure S4 | Viability of respiratory epithelium post bacterial challenge. Effect of 24-hour Hh challenge on A549 (A), and D562 (B) cell viability, compared to media alone. Effect of 24-hour NTHi challenge on A549 (C), and D562 (D) cell viability, compared to media alone. Effect of 24-hour HPL treatment on A549 (E), and D562 (F) cell viability, compared to media alone. Error bars represent the \pm SEM of three biological replicates. * $p < 0.05$, ** $p < 0.005$, **** $p < 0.0001$.

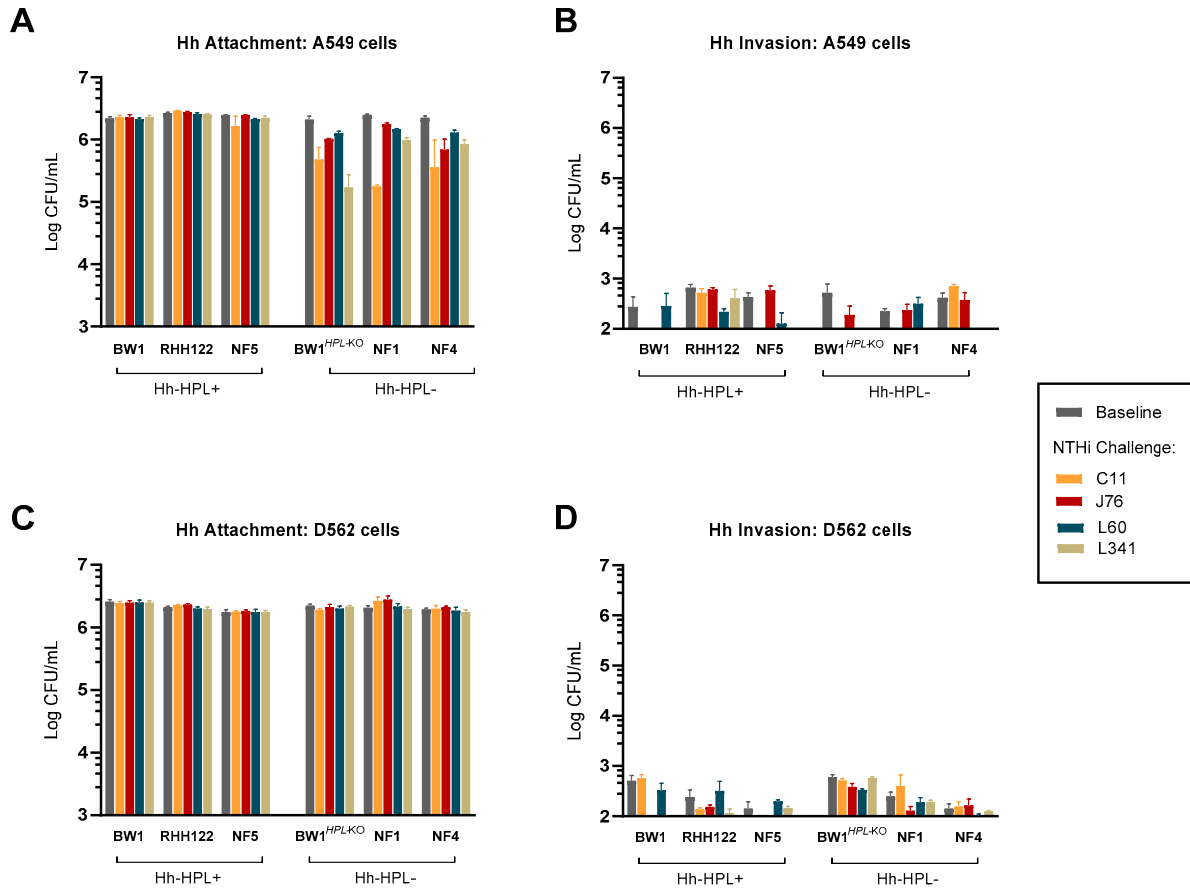


Figure S5 | Hh attachment and invasion of A549 and D562 cells post NTHi challenge. Colony counts (CFU/mL) of Hh attached to A549 (**A**) and D562 (**B**) cell monolayers post NTHi challenge. Colony counts (CFU/mL) of internalised Hh after exposure to A549 (**C**) and D562 (**D**) cell monolayers post NTHi challenge. Error bars represent the \pm SEM of three biological replicates, measured triplicate. * $p < 0.05$, **** $p < 0.0001$.

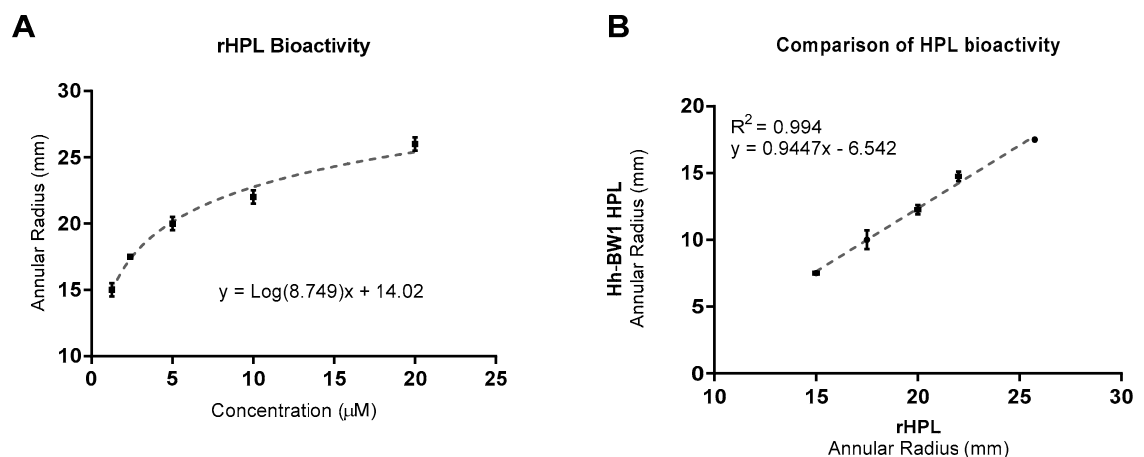


Figure S6 | Bioactivity of recombinant and native HPL. Annular radius of inhibitory zones produced by serial 2-fold dilutions of rHPL on agar containing indicator strain NTHi-252 (**A**). Linear regression comparing the relationship between concentration and anti-NTHi inhibitory activity of rHPL and nHPL from Hh-BW1 (**B**).