P. aeruginosa rhamnolipids stabilize human rhinovirus 14 virions

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Abstract

Many mammalian viruses encounter bacteria and bacterial molecules over the course of infection. Previous work has shown that the microbial ecology of the gut plays an integral role in poliovirus and coxsackievirus infection, where bacterial glycans can facilitate virus-receptor interactions, enhance viral replication, and stabilize viral particles. However, how airway bacteria alter respiratory viral infection is less understood. Therefore, we investigated whether a panel of airway bacteria affect rhinovirus stability. We found that *Pseudomonas aeruginosa,* an opportunistic airway pathogen, protects human rhinovirus 14 from acid or heat inactivation. Further investigation revealed that *P. aeruginosa* rhamnolipids, glycolipids with surfactant properties, are necessary and sufficient for stabilization of rhinovirus virions. Taken together, this work demonstrates that specific molecules produced by an opportunistic airway pathogen can influence a respiratory virus.

Importance

Bacteria can enhance viral stability and infection for enteric members of the *Picornaviridae* such as poliovirus and coxsackievirus; however, whether bacteria influence respiratory picornaviruses is unknown. In this study, we examined impacts of airway bacteria on rhinovirus, a major etiological agent of the common cold. We found that *P. aeruginosa* protects human rhinovirus 14 from both acid and heat inactivation through rhamnolipids. Overall, this work demonstrates bacterial effects on respiratory virus through specific bacterial molecules.

Introduction

Rhinoviruses are the most common cause of the common cold (1-3). Rhinoviruses are a large and diverse group of enteroviruses that are divided into three species that bind various receptors—ICAM-1, LDLR, or CDHR3—that are found in the airway (2, 3). Although most rhinovirus infections are mild and self-limiting, severe and long-term consequences are possible. Rhinoviruses are the most common viral infection in those with cystic fibrosis and contribute to exacerbations (4-14). Cystic fibrosis disease is the result of ion imbalance at the cell surface (15), leading to aggregation of thick, sticky mucus and chronic colonization of opportunistic bacterial pathogens (16-18).

Previous work from our lab has shown that intestinal bacteria bind related enteroviruses such as poliovirus and coxsackievirus (19-23). Bacteria-virus interactions stabilize these viruses and protect from heat inactivation (19). Further, bacteria promote viral replication *in vivo,* as demonstrated by reduced titers of poliovirus and coxsackievirus in antibiotic-treated animals (22). Similarly, intestinal viruses in other families also benefit from bacteria, including mouse mammary tumor virus, murine norovirus, and certain strains of reovirus (21, 24-28). Although these interactions have been examined for these enteric viruses and related enteroviruses such as poliovirus and coxsackievirus, whether bacteria influence rhinovirus infections is unknown.

To determine if respiratory bacteria stabilize rhinovirus, we incubated human rhinovirus 14 (HRV14) with a panel of respiratory bacteria at an inactivating acidic pH of 5.8 or inactivating heat of 49°C and found that *P. aeruginosa,* a notorious cystic fibrosis pathogen, protects HRV14 from inactivation. Mechanistically, we found that rhamnolipids, biosurfactants produced by *P. aeruginosa,* are necessary and sufficient for

this stabilization. Taken together, these results demonstrate that specific molecules from a ubiquitous bacterium can stabilize HRV14.

Results

P. aeruginosa stabilizes HRV14.

Given that rhinoviruses likely encounter airway bacteria during infection, we questioned whether airway bacteria influence viral infection. In contrast to many other enteroviruses, rhinoviruses are acid sensitive (29). The healthy upper airway has an acidic pH that increases from the nares through the nose and sinuses with pHs of 5.5 to 6.5, respectively (30) . The healthy lower airway has a neutral pH between 7.0-7.5 (31, 32). However, in the presence of inflammation, the respiratory tract pH can decrease. During asthma exacerbations, exhaled breath condensate falls to 5.2 (33). In those with cystic fibrosis, exhaled breath condensate is reduced to 5.8 basally and to 5.3 during exacerbations (34). To examine potential effects of bacteria on HRV14 pH sensitivity, we first incubated 10^5 PFU HRV14 in synthetic nasal media (35) at a pH of 6.8 or 5.8 for one hour before quantifying titer by plaque assay using H1 HeLa cells **(Fig 1AB)**. As expected, we found a >1000-fold reduction in viral titer at pH 5.8 (**Fig 1B**) compared with pH 6.8 (**Fig 1A)**. Next, we repeated the assay in the presence of a panel of airway bacteria. Many of the bacteria used in this screen (e.g. *M. catarrhalis, D. pigrum, S. aureus, S. epidermidis*) are commonly found in the upper airways from the nares to the sinuses (36, 37). However, some of these bacteria are enriched in the upper airways and colonize the lower airways during chronic pulmonary diseases such as cystic fibrosis (e.g. *P. aeruginosa, S. aureus, S. parasanguinis*)(38, 39). Overnight cultures of bacteria (10^6-10^8 CFU (**Table 1**)) were washed and resuspended in media at a pH of either 5.8 or 6.8, 10^5 PFU HRV14 was added, and bacteria and virus were incubated together for one hour at 33°C prior to plaque assay. Bacteria had no effect on rhinovirus titers at a non-inhibitory pH of 6.8 (**Fig 1A**). At a pH of 5.8, HRV14 titers were reduced across all samples, with no bacterial strain significantly protecting HRV14 from acid inactivation (**Fig 1B**), although *P. aeruginosa* strains had increased yields that were not statistically significant in this initial broad screen. We repeated the pH 5.8 stability assay for HRV14 incubated with *P. aeruginosa* PAO1 and found that it significantly increased viral stability by 10-fold (**Fig 1B inset**).

We next determined whether airway bacteria could protect HRV14 from heat inactivation. For these experiments, HRV14 was incubated at 33°C or 49°C for two hours, followed by titer analysis via plaque assay. As expected, HRV14 titers were reduced by >1000-fold after incubation at 49°C (**Fig 1C**). In the presence of airway bacterial strains, only *P. aeruginosa* PAO1 significantly increased HRV14 titers at 49°C (**Fig 1C**). We next examined whether increased HRV14 viability in the presence of *P. aeruginosa* was unique to the strain PAO1 or if other strains of *P. aeruginosa* conferred protection. We compared *P. aeruginosa* strains PAO1, PA14, and FRD1. All three of these strains are typical lab strains of *P. aeruginosa*; however, exopolysaccharide and virulence factor production vary (40-42). We found that the PAO1 strain significantly increased HRV14 recovery after heat exposure, but PA14 and FRD1 strains did not (**Fig 1D**), suggesting that PAO1 stabilizes HRV14 more than other strains of *P. aeruginosa.*

HRV14 does not have increased binding to *P. aeruginosa.*

Our group previously reported that direct binding to bacteria and bacterial glycans stabilizes related picornaviruses such as poliovirus and coxsackievirus (19-22, 43). Therefore, to determine whether *P. aeruginosa* PAO1 has increased binding to HRV14, potentially explaining its virion stabilization phenotype, we quantified binding of purified, ^{35}S-radiolabeled HRV14 to bacterial strains. We incubated our panel of airway bacteria (**Table 1**) with ^{35}S-radiolabeled HRV14 (4,000 CPM/10^6 PFU) at pHs of 5.8 or 6.8 to determine if HRV14 binds relevant airway bacteria. Virus was also incubated with 2.8 µm streptavidin beads to account for nonspecific binding. *E. coli* and *S. aureus* Wichita had significantly increased HRV14 binding compared to the bead control at a pH of 6.8 (**Fig 2A**), although no significant differences in binding were observed at pH 5.8 (**Fig 2B**). Surprisingly, HRV14 did not display enhanced binding to *P. aeruginosa*, suggesting that direct binding may not be a major facet of stabilization against acid inactivation.

Heat-killed *P. aeruginosa* stabilizes HRV14.

To determine if *P. aeruginosa*-mediated protection of HRV14 from acid and heat inactivation was due a heat-sensitive factor or relied upon active *P. aeruginosa* metabolism, HRV14 was incubated with live or heat-killed *P. aeruginosa* at a pH of 6.8 vs. 5.8 (**Fig 3A**) or at 33°C vs. 49°C (**Fig 3B**). Heat-killed *P. aeruginosa* protected HRV14 from acid inactivation, suggesting that a heat stable *P. aeruginosa* factor stabilizes HRV14 (**Fig 3A**).

Given that heat-killed *P. aeruginosa* was sufficient to protect HRV14 from acid inactivation, and our past work demonstrated that heat stable bacterial lipopolysaccharide (LPS) stabilizes picornaviruses, we hypothesized that LPS stabilizes HRV14. As an external glycan moiety on Gram-negative bacterial surfaces, LPS is a common factor that rhinovirus is likely to encounter. Previous work from our lab demonstrated that poliovirus binds LPS and that binding to LPS stabilizes poliovirus, Aichivirus, and coxsackievirus (19, 22). Conversely, LPS destabilizes enveloped influenza virions as well as alphavirus and flavivirus virions (44, 45). To assess LPS effects, HRV14 was incubated with LPS isolated from *E. coli* or *P. aeruginosa* at a pH of 5.8 vs. 6.8 for one hour (**Fig 3C**) or at 33°C vs. 49°C for two hours (**Fig 3D**) followed by plaque assay. Surprisingly, LPS did not protect HRV14 from acid or heat, suggesting that some other *P. aeruginosa* factor is responsible for stabilization.

Rhamnolipids stabilize HRV14.

We next hypothesized that other heat-stable, high abundance *P. aeruginosa* molecules stabilize HRV14. Like LPS, rhamnolipids are glycolipids that are produced by *P. aeruginosa* at high concentrations (46, 47). Rhamnolipids are important for biofilm formation and architecture, motility, and protection from phagocytosis (48-51). Rhamnolipids are synthesized by the enzymes RhlA, RhlB, and RhlC (**Fig 4A**)(52). RhlA catalyzes the conversion of B-hydroxyacyl-ACP into the fatty acid dimer 3-(3-hydroxyalkanoyloxy)alkanoates (HAA)(53, 54). RhlB is a rhamnosyltransferase that catalyzes a reaction between HAA and dTDP-L-rhamnose to produce mono-rhamnolipids (55). RhlC acts as a second rhamnosyltransferase that catalyzes the conversion of mono-rhamnolipids and dTDP-L-rhamnose to di-rhamnolipids (56).

To examine the potential impact of *P. aeruginosa* rhamnolipids on HRV14 stabilization, we used strains with transposon insertions within rhamnolipid synthesis genes to test for necessity, and addition of purified rhamnolipids to test for sufficiency. First, we obtained *rhlA, rhlB,* and *rhlC* mutants in the *P. aeruginosa* PAO1 background (57) and incubated them with HRV14 at a pH of 5.8 vs. 6.8 for one hour (**Fig 4B**) or at 33°C vs. 49°C for two hours **(Fig 4C)** followed by plaque assay. All mutants in the rhamnolipid synthesis pathway failed to protect HRV14 from acid inactivation (**Fig 4B**). The *rhlC* mutant partially restored protection of HRV14 from heat inactivation, suggesting that the production of mono-rhamnolipids are somewhat protective against heat (**Fig 4C**). Next, we tested whether purified rhamnolipids could stabilize HRV14 in the absence of bacteria. HRV14 was incubated with various concentrations of rhamnolipids at a pH of 5.8 vs. 6.8 for one hour (**Fig 5A**) or at 33°C vs. 49°C for two hours **(Fig 5B)** followed by plaque assay. Rhamnolipids protected HRV14 from both acid and heat inactivation at a concentration of 0.5 mg/mL. Overall, data in Figures 4 and 5 indicate that rhamnolipids are necessary and sufficient for stabilization of HRV14 by *P. aeruginosa*.

To confirm that rhamnolipids stabilize HRV14 using an assay independent from viral viability assays, we performed a cell-free Particle Stability Thermal Release assay (PaSTRy)(58). Through this assay, virion RNA release is measured over a temperature gradient using SYBR green II dye to define the exact temperature of virion inactivation. RNA release was measured for HRV14 in the presence or absence of *P. aeruginosa* LPS (as a negative control) or rhamnolipids (**Fig 6**). Untreated HRV14 released RNA at 48.7°C. As expected from our plaque-based assays, LPS had no effect on the temperature at which HRV14 RNA release occurred. However, rhamnolipids shifted HRV14 RNA release temperatures by ~1°C at 0.05 and 0.1mg/mL concentrations and by ~3°C at 1mg/mL concentration. Taken together, these results demonstrate that rhamnolipids stabilize HRV14.

Discussion

Rhinoviruses are important respiratory pathogens, but potential impacts of bacteria on rhinovirus infection are largely unknown. Here, we screened a panel of respiratory bacteria and found that *P. aeruginosa*, an opportunistic pathogen that establishes chronic infections in those with chronic airway diseases, protects HRV14 from acid and heat inactivation. Investigation of *P. aeruginosa* strains deficient for rhamnolipid production and addition of exogenous rhamnolipids revealed that rhamnolipids were necessary and sufficient for HRV14 stabilization.

Rhamnolipids are glycolipids that are important for *P. aeruginosa* physiology and infection. *P. aeruginosa* produces copious amounts of rhamnolipids, with wildtype *P. aeruginosa* PAO1 producing as much as 39 g/L (46). Critically, rhamnolipids are present in sputum samples from people with cystic fibrosis that are colonized with *P. aeruginosa* (59, 60). Rhamnolipids help shape biofilm architecture (49), mediate *P. aeruginosa* dispersal (61-63), enhance *P. aeruginosa* motility (62), decrease phagocytosis (50, 51), and damage cell membranes (51, 64). Rhamnolipids inhibit the colonization and disperse a wide array of other bacteria (63, 65-70). Additionally, rhamnolipids inactivate enveloped viruses such as herpesviruses, coronaviruses, and respiratory syncytial virus via envelope disruption (71-75).

Less is known about interactions between rhamnolipids and nonenveloped viruses, such as rhinoviruses and other picornaviruses. Rhamnolipids have no effect on poliovirus stability (71), but *in silico* modeling of HRV14 suggested that rhamnolipids interact with the canyon region of the capsid, where rhinoviruses bind their receptors (76). This interaction may be responsible for the stabilization phenotype herein, but further studies are required to fully delineate the role of rhamnolipids during rhinovirus infection. Beyond viral stability, exposure to biosurfactants, such as rhamnolipids, can increase pathogenesis of other picornaviruses, such as encephalomyocarditis virus (EMCV)(77-79). The pesticides dichloro-diphenyl-trichloroethane (DDT) and fenitrothion are associated with clusters of Reye's Syndrome, a rare condition involving liver pathology and brain swelling that often accompanies viral infection. These surfactants increase EMCV uncoating in treated cells (78). Additionally, these compounds reduce interferon responses, contributing to increased morbidity and mortality in mice (78).

Taken together, we found that rhamnolipids, glycolipids produced by the opportunistic pathogen *P. aeruginosa,* increases stability of HRV14. This interaction may be clinically relevant as many people with cystic fibrosis are chronically colonized by *P. aeruginosa* and rhinoviruses are a common cause of exacerbation events. Future studies are necessary to determine the role rhamnolipids and other compounds play over the course of rhinovirus infection.

Methods

Cells and viruses.

HeLa H1 cells were propagated in DMEM supplemented with 10% calf bovine serum and 1% antibiotics. Cells were grown at 37°C with 5% CO_2. HRV14 was propagated from an infectious clone (gift of William Jackson) and infections were performed at 33°C 5% CO_2.

Bacterial strains, culture conditions, and reagents.

P. aeruginosa PAO1, FRD1, PA14 and the PAO1 isogenic mutants*, rhlA, rhlB, rhlC*, *S. aureus, S. epidermidis, E. coli,* and *K. pneumoniae* were maintained on lysogeny broth (LB) agar and grown on LB at 37°C with shaking at 250 rpm. *M. catarrhalis* was grown in brain heart infusion media at 37°C with shaking at 250 rpm. *D. pigrum* and *S. parasanguinis* were grown in Todd Hewitt broth/agar at 37°C with 5% CO_2. Synthetic nasal media was prepared as described in Krismer et al 2014 (35).

Table 1. Bacterial strains.

Strain	Characteristics	Overnight CFU/mL	Reference/Source
P. aeruginosa PAO1	Wound, lab isolate	10^8	(57, 80)
PAO1 *rhlA*	Transposon insertion in *rhlA*	10^8	(57)
PAO1 *rhlB*	Transposon insertion in *rhlB*	10^8	(57)
PAO1 *rhlC*	Transposon insertion in *rhlC*	10^8	(57)
P. aeruginosa FRD1	Cystic fibrosis, lab isolate	10^8	(81)

P. aeruginosa PA14	Wildtype, lab isolate	10^8	(82)
M. catarrhalis	Wildtype	10^6	(83)
E. coli K12	Lab isolate	10^8	(21)
K. pneumoniae NCTC 9633	Lab isolate	10^8	(84)
D. pigrum	Wildtype	10^6	(85)
S. aureus USA200	MRSA	10^8	(86)
S. aureus Wichita	Wound, lab isolate	10^8	(87)
S. epidermidis	Wildtype	10^8	(88)
S. parasanguinis FW213	Wildtype	10^8	(89)

Quantifying effects of bacteria on viral stability

For acid sensitivity assays, overnight cultures of bacteria (**Table 1**, 10^6 or 10^8 CFU, depending on the strain) were centrifuged, washed in synthetic nasal media (SNM) at a pH of either 5.8 or 6.8, centrifuged and resuspended in (SNM) at a pH of either 5.8 or 6.8. HRV14 (10^5 PFU) was added, and the virus and bacteria of interest were incubated at 33°C with 5% CO_2 for one hour. For heat sensitivity assays, overnight cultures were centrifuged, washed with PBS, centrifuged and resuspended in PBS. HRV14 was added as above, and the mixture was incubated at either 33°C or 49°C for two hours. After each incubation, samples were centrifuged and PFU in the supernatants were quantified via plaque assay as described (19). Briefly, samples were diluted in PBS supplemented with 100 µg/mL $CaCl_2$ and 100 µg/mL $MgCl_2$ and allowed to attach to cells for 30 minutes at 33ºC with 5% CO_2. Agar overlays containing DMEM with 10% calf bovine serum and 1% antibiotics was added and removed 48 hours after infection. PFU were enumerated following crystal violet staining of monolayers.

Quantifying viral binding to bacterial cells

^{35}S-radiolabeled HRV14 was generated as previously described (20). Briefly, infected cells were pulsed with ^{35}S-amino acids to label progeny virions, cell-associated virions were collected, and purified using Capto Core 700 beads (Cytivia) according to the manufacturer's instructions. Briefly, rhinovirus was mixed end-over-end at 4°C with capto core beads for 45-minute increments three times. The slurry was centrifuged and virus from the supernatant was assessed for purity by SDS-PAGE. For binding assays, ~4000 counts per minute (CPM)(10^6 PFU) HRV14 was added to overnight bacterial cultures or streptavidin beads (Invitrogen, Dynabeads) resuspended in SNM pH 5.8 or 6.8. Incubation proceeded for one hour at 33ºC with 5% CO_2 and the mixture was centrifuged and washed to remove unbound virus. The pellet was resuspended in Budget-Solve complete counting cocktail (Research Products International) and CPM was determined by scintillation counting.

Quantifying effects of lipopolysaccharide and rhamnolipids on viral stability

Live or heat-killed *P. aeruginosa* PAO1 was incubated with HRV14 as above. PAO1 was heat-killed by incubating at 95ºC for 10 minutes. LPS (at 1 mg/mL) from *E. coli* (O111:B4, Sigma) or *P. aeruginosa* (PA-10, Sigma) was resuspended in SNM pH 5.8 or PBS and incubated at 33ºC or 49ºC and quantified via plaque assay as above.

Exogenous rhamnolipids from *P. aeruginosa* (Sigma) were added and incubated via the same scheme.

Particle Stability Thermal Release assay (PaSTRy)

Capto-core (Cytiva) purified and Amicon filter-concentrated (Sigma) HRV14 (~10^5 PFU) was combined with rhamnolipids, SYBR green II (10x final concentration, Invitrogen), and buffer (10mM HEPES at pH 8, 200mM NaCl). The 50 uL reactions were heated from 25ºC to 95ºC on a 1% gradient in an ABI 7500 real-time thermocycler (Applied Biosystems) with fluorescent monitoring.

Data analysis

All statistical analyses were performed using GraphPad Prism version 10.4.2 for macOS. Normality was assessed via the Shapiro-Wilk test. Further analyses were performed where indicated.

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Figure Legends

Figure 1. *P. aeruginosa* stabilizes HRV14.
A/B/C) HRV14 (10^5 PFU) was incubated for one hour at a pH of 6.8 (A) or 5.8 (B) or for two hours at 33°C or 49°C (C) in the presence or absence of a panel of airway bacteria (10^6-10^8 CFU). Samples were centrifuged and PFU were quantified from the supernatant by plaque assay. D) HRV14 was incubated in the presence or absence of *P. aeruginosa* PAO1, PA14, or FRD1 at 33°C or 49°C for two hours prior to plaque assay. n=6, 3 biological replicates with 2 technical replicates. **, p<0.01, ***, p<0.001, ****, p<0.0001 (A-D Kruskal-Wallis, Dunnett's post hoc test, B insert unpaired t test).

Figure 2. HRV14 does not have enhanced binding to *P. aeruginosa*.
A/B) ^{35}S-radiolabeled HRV14 (~4,000 CPM/ 10^6 PFU) was incubated in the presence or absence of streptavidin beads (2.8 µm) or 10^6-10^8 CFU bacteria in media at a pH of 6.8 (A) or 5.8 (B) at 33°C for one hour. Samples were centrifuged and washed to remove unbound virus. Bound virus was quantified via scintillation counting and normalized to input. n=3. A) ns, p>0.05 (Kruskal-Wallis, Dunnett's post hoc test). B) *, p<0.05, **, p<0.01 (one-way ANOVA, Dunnett's post hoc test).

Figure 3. Heat-killed *P. aeruginosa* stabilizes HRV14.
A/B) HRV14 (10^5 PFU) was incubated in the presence or absence of 10^8 CFU live or heat-killed (HK) *P. aeruginosa* PAO1 at a pH of 5.8 or 6.8 at 33°C for one hour (A) or

33°C or 49°C for two hours (B) prior to plaque assay. C/D) HRV14 was incubated in the presence or absence of 1 mg/ml LPS from *E. coli* or *P. aeruginosa* at a pH of 5.8 or 6.8 for one hour (C) or 33°C or 49°C for two hours (D) prior to plaque assay. n=6, 3 biological replicates with 2 technical replicates. *, p<0.05, ****, p<0.0001 (A/C/D, Kruskal-Wallis, Dunnett's post hoc test, B, one way ANOVA, Dunnett's post hoc test).

Figure 4. Insertion mutation of rhamnolipid synthesis genes ablates HRV14 stabilization.

A) *P. aeruginosa* rhamnolipid synthesis pathway. B/C) HRV14 (10^5 PFU) was incubated in the presence or absence of 10^8 CFU PAO1, *rhlA, rhlB,* or *rhlC* transposon insertion mutants at a pH of 5.8 or 6.8 at 33°C for one hour (B) or 33°C or 49°C for two hours (C) prior to plaque assay. n=6-8, 3-4 biological replicates with 2 technical replicates. *, p<0.05, ** p<0.01, ****, p<0.0001 (Kruskal-Wallis, Dunnett's post hoc test).

Figure 5. Rhamnolipids stabilize HRV14.

A/B) HRV14 (10^5 PFU) was incubated in the presence or absence of various concentrations of rhamnolipids at a pH of 5.8 or 6.8 at 33°C for one hour (A) or 33°C or 49°C for two hours (B) prior to plaque assay. n=6, 3 biological replicates with 2 technical replicates. *, p<0.05, ** p<0.01, ***, p<0.001 ****, p<0.0001(Kruskal-Wallis, Dunnett's post hoc test).

Figure 6. Rhamnolipids enhance HRV14 thermostability.

HRV14 thermostability profile using a cell-free Particle Stability Thermal Release assay (PaSTRy). HRV14 (10^5 PFU) was added to SYBR green II with or without LPS or rhamnolipids. Samples were heated from 25°C to 95°C on a 1% stepwise gradient with fluorescence monitoring. n=6, 3 biological replicates with 2 technical replicates. **, p<0.01, ****, p<0.0001 (one-way ANOVA, Dunnett's post hoc test).

1. Arruda E, Pitkäranta A, Witek TJ, Doyle CA, Hayden FG. Frequency and natural history of rhinovirus infections in adults during autumn. Journal of Clinical Microbiology. 1997;35(11):2864-8.
2. Palmenberg AC, Gern JE. Classification and Evolution of Human Rhinoviruses. In: Jans DA, Ghildyal R, editors. Rhinoviruses: Methods and Protocols. New York, NY: Springer New York; 2015. p. 1-10.
3. Palmenberg AC, Spiro D, Kuzmickas R, Wang S, Djikeng A, Rathe JA, et al. Sequencing and Analyses of All Known Human Rhinovirus Genomes Reveal Structure and Evolution. Science. 2009;324(5923):55-9.
4. Goffard A, Lambert V, Salleron J, Herwegh S, Engelmann I, Pinel C, et al. Virus and cystic fibrosis: Rhinoviruses are associated with exacerbations in adult patients. Journal of Clinical Virology. 2014;60(2):147-53.

5. Meyer VMC, Siqueira MM, Costa P, Caetano BC, Oliveira Lopes JC, Folescu TW, et al. Clinical impact of respiratory virus in pulmonary exacerbations of children with Cystic Fibrosis. PLoS One. 2020;15(10):e0240452.
6. Ling KM, Garratt LW, Gill EE, Lee AHY, Agudelo-Romero P, Sutanto EN, et al. Rhinovirus Infection Drives Complex Host Airway Molecular Responses in Children With Cystic Fibrosis. Front Immunol. 2020;11:1327.
7. Banjar H, Chaballout M, Karkour K, Al-Ghamdi H, Al-Mogarri I, Al-Haider S, et al. The prevalence of viral infections in children with cystic fibrosis in a tertiary care center in Saudi Arabia. Int J Pediatr Adolesc Med. 2020;7(2):83-7.
8. O Loughlin DW, Coughlan S, De Gascun CF, McNally P, Cox DW. The role of rhinovirus infections in young children with cystic fibrosis. Journal of Clinical Virology. 2020;129:104478.
9. Schögler A, Stokes AB, Casaulta C, Regamey N, Edwards MR, Johnston SL, et al. Interferon response of the cystic fibrosis bronchial epithelium to major and minor group rhinovirus infection. J Cyst Fibros. 2016;15(3):332-9.
10. Thornton CS, Caverly LJ, Kalikin LM, Carmody LA, McClellan S, LeBar W, et al. Prevalence and Clinical Impact of Respiratory Viral Infections from the STOP2 Study of Cystic Fibrosis Pulmonary Exacerbations. Ann Am Thorac Soc. 2024;21(4):595-603.
11. Eymery M, Morfin F, Doleans-Jordheim A, Perceval M, Ohlmann C, Mainguy C, et al. Viral respiratory tract infections in young children with cystic fibrosis: a prospective full-year seasonal study. Virol J. 2019;16(1):111.
12. Smyth AR, Smyth RL, Tong CY, Hart CA, Heaf DP. Effect of respiratory virus infections including rhinovirus on clinical status in cystic fibrosis. Archives of Disease in Childhood. 1995;73(2):117.
13. Kieninger E, Singer F, Tapparel C, Alves MP, Latzin P, Tan H-L, et al. High Rhinovirus Burden in Lower Airways of Children With Cystic Fibrosis. Chest. 2013;143(3):782-90.
14. Dijkema JS, van Ewijk BE, Wilbrink B, Wolfs TFW, Kimpen JLL, van der Ent CK. Frequency and Duration of Rhinovirus Infections in Children With Cystic Fibrosis and Healthy Controls: A Longitudinal Cohort Study. The Pediatric Infectious Disease Journal. 2016;35(4).
15. Quinton PM. Chloride impermeability in cystic fibrosis. Nature. 1983;301(5899):421-2.
16. Rubin BK. Mucus structure and properties in cystic fibrosis. Paediatric Respiratory Reviews. 2007;8(1):4-7.
17. Parkins MD, Floto RA. Emerging bacterial pathogens and changing concepts of bacterial pathogenesis in cystic fibrosis. Journal of Cystic Fibrosis. 2015;14(3):293-304.
18. Surette MG. The Cystic Fibrosis Lung Microbiome. Annals of the American Thoracic Society. 2014;11(Supplement 1):S61-S5.
19. Aguilera ER, Nguyen Y, Sasaki J, Pfeiffer JK. Bacterial Stabilization of a Panel of Picornaviruses. mSphere. 2019;4(2):10.1128/msphere.00183-19.
20. Erickson AK, Jesudhasan PR, Mayer MJ, Narbad A, Winter SE, Pfeiffer JK. Bacteria Facilitate Enteric Virus Co-infection of Mammalian Cells and Promote Genetic Recombination. Cell Host & Microbe. 2018;23(1):77-88.e5.

21. Kuss SK, Best GT, Etheredge CA, Pruijssers AJ, Frierson JM, Hooper LV, et al. Intestinal microbiota promote enteric virus replication and systemic pathogenesis. Science. 2011;334(6053):249-52.
22. Robinson CM, Jesudhasan PR, Pfeiffer JK. Bacterial lipopolysaccharide binding enhances virion stability and promotes environmental fitness of an enteric virus. Cell host & microbe. 2014;15(1):36-46.
23. Robinson Christopher M, Woods Acevedo Mikal A, McCune Broc T, Pfeiffer Julie K. Related Enteric Viruses Have Different Requirements for Host Microbiota in Mice. Journal of Virology. 2019;93(23):10.1128/jvi.01339-19.
24. Baldridge MT, Nice TJ, McCune BT, Yokoyama CC, Kambal A, Wheadon M, et al. Commensal microbes and interferon-λ determine persistence of enteric murine norovirus infection. Science. 2015;347(6219):266-9.
25. Kane M, Case LK, Kopaskie K, Kozlova A, MacDearmid C, Chervonsky AV, et al. Successful transmission of a retrovirus depends on the commensal microbiota. Science. 2011;334(6053):245-9.
26. Jones MK, Watanabe M, Zhu S, Graves CL, Keyes LR, Grau KR, et al. Enteric bacteria promote human and mouse norovirus infection of B cells. Science. 2014;346(6210):755-9.
27. Erickson AK, Sutherland DM, Welsh OL, Maples RW, Dermody TS, Pfeiffer JK. A single mutation in an enteric virus alters tropism and sensitivity to microbiota. Proc Natl Acad Sci U S A. 2025;122(16):e2500612122.
28. Berger AK, Yi H, Kearns DB, Mainou BA. Bacteria and bacterial envelope components enhance mammalian reovirus thermostability. PLoS Pathogens. 2017;13(12):e1006768.
29. Hughes JH, Thomas DC, Hamparian VV. Acid Lability of Rhinovirus Type 14: Effect of pH, Time, and Temperature. Proceedings of the Society for Experimental Biology and Medicine. 1973;144(2):555-60.
30. McShane D, Davies JC, Davies MG, Bush A, Geddes DM, Alton EWFW. Airway surface pH in subjects with cystic fibrosis. European Respiratory Journal.21(1):37-42.
31. Rehman T, Welsh MJ. Inflammation as a Regulator of the Airway Surface Liquid pH in Cystic Fibrosis. Cells. 2023;12(8):1104.
32. Zajac M, Dreano E, Edwards A, Planelles G, Sermet-Gaudelus I. Airway Surface Liquid pH Regulation in Airway Epithelium Current Understandings and Gaps in Knowledge. International Journal of Molecular Sciences. 2021;22(7):3384.
33. HUNT JF, FANG K, MALIK R, SNYDER A, MALHOTRA N, PLATTS-MILLS TAE, et al. Endogenous Airway Acidification. American Journal of Respiratory and Critical Care Medicine. 2000;161(3):694-9.
34. Ojoo JC, Mulrennan SA, Kastelik JA, Morice AH, Redington AE. Exhaled breath condensate pH and exhaled nitric oxide in allergic asthma and in cystic fibrosis. Thorax. 2005;60(1):22.
35. Krismer B, Liebeke M, Janek D, Nega M, Rautenberg M, Hornig G, et al. Nutrient Limitation Governs Staphylococcus aureus Metabolism and Niche Adaptation in the Human Nose. PLOS Pathogens. 2014;10(1):e1003862.
36. Boeck ID, Wittouck S, Martens K, Claes J, Jorissen M, Steelant B, et al. Anterior Nares Diversity and Pathobionts Represent Sinus Microbiome in Chronic Rhinosinusitis. mSphere. 2019;4(6):10.1128/msphere.00532-19.

37. Lemon KP. Human nasal microbiota. Current Biology. 2020;30(19):R1118-R9.
38. Filkins LM, Hampton TH, Gifford AH, Gross MJ, Hogan DA, Sogin ML, et al. Prevalence of Streptococci and Increased Polymicrobial Diversity Associated with Cystic Fibrosis Patient Stability. Journal of Bacteriology. 2012;194(17):4709-17.
39. Cuthbertson L, Walker AW, Oliver AE, Rogers GB, Rivett DW, Hampton TH, et al. Lung function and microbiota diversity in cystic fibrosis. Microbiome. 2020;8(1):45.
40. Wozniak DJ, Wyckoff TJO, Starkey M, Keyser R, Azadi P, O'Toole GA, et al. Alginate is not a significant component of the extracellular polysaccharide matrix of PA14 and PAO1 Pseudomonas aeruginosa biofilms. Proceedings of the National Academy of Sciences. 2003;100(13):7907-12.
41. Malhotra S, Limoli Dominique H, English Anthony E, Parsek Matthew R, Wozniak Daniel J. Mixed Communities of Mucoid and Nonmucoid Pseudomonas aeruginosa Exhibit Enhanced Resistance to Host Antimicrobials. mBio. 2018;9(2):10.1128/mbio.00275-18.
42. Grace A, Sahu R, Owen DR, Dennis VA. Pseudomonas aeruginosa reference strains PAO1 and PA14: A genomic, phenotypic, and therapeutic review. Frontiers in Microbiology. 2022;Volume 13 - 2022.
43. Erickson AK, Jesudhasan PR, Mayer MJ, Narbad A, Winter SE, Pfeiffer JK. Bacteria facilitate enteric virus co-infection of mammalian cells and promote genetic recombination. Cell host & microbe. 2018;23(1):77-88. e5.
44. Bandoro C, Runstadler JA. Bacterial Lipopolysaccharide Destabilizes Influenza Viruses. mSphere. 2017;2(5).
45. Langendries L, Jacobs S, Abdelnabi R, Verwimp S, Kaptein S, Baatsen P, et al. Perturbation of Alphavirus and Flavivirus Infectivity by Components of the Bacterial Cell Wall. J Virol. 2022;96(6):e0006022.
46. Müller MM, Hörmann B, Syldatk C, Hausmann R. Pseudomonas aeruginosa PAO1 as a model for rhamnolipid production in bioreactor systems. Applied Microbiology and Biotechnology. 2010;87(1):167-74.
47. Chayabutra C, Wu J, Ju L-K. Rhamnolipid production by Pseudomonas aeruginosa under denitrification: Effects of limiting nutrients and carbon substrates. Biotechnology and Bioengineering. 2001;72(1):25-33.
48. Caiazza NC, Shanks RMQ, O'Toole GA. Rhamnolipids Modulate Swarming Motility Patterns of Pseudomonas aeruginosa. Journal of Bacteriology. 2005;187(21):7351-61.
49. Davey ME, Caiazza NC, O'Toole GA. Rhamnolipid Surfactant Production Affects Biofilm Architecture in Pseudomonas aeruginosa PAO1. Journal of Bacteriology. 2003;185(3):1027-36.
50. McClure CD, Schiller NL. Inhibition of Macrophage Phagocytosis by Pseudomonas aeruginosa Rhamnolipids In Vitro and In Vivo. Current Microbiology. 1996;33(2):109-17.
51. McClure CD, Schiller NL. Effects of Pseudomonas aeruginosa rhamnolipids on human monocyte-derived macrophages. J Leukoc Biol. 1992;51(2):97-102.
52. Soberón-Chávez G, Lépine F, Déziel E. Production of rhamnolipids by Pseudomonas aeruginosa. Applied Microbiology and Biotechnology. 2005;68(6):718-25.
53. Déziel E, Lépine F, Milot S, Villemur R. rhlA is required for the production of a novel biosurfactant promoting swarming motility in Pseudomonas aeruginosa: 3-(3-

hydroxyalkanoyloxy)alkanoic acids (HAAs), the precursors of rhamnolipids. Microbiology. 2003;149(8):2005-13.

54. Cabrera-Valladares N, Richardson A-P, Olvera C, Treviño LG, Déziel E, Lépine F, et al. Monorhamnolipids and 3-(3-hydroxyalkanoyloxy)alkanoic acids (HAAs) production using Escherichia coli as a heterologous host. Applied Microbiology and Biotechnology. 2006;73(1):187-94.

55. Ochsner UA, Fiechter A, Reiser J. Isolation, characterization, and expression in Escherichia coli of the Pseudomonas aeruginosa rhlAB genes encoding a rhamnosyltransferase involved in rhamnolipid biosurfactant synthesis. Journal of Biological Chemistry. 1994;269(31):19787-95.

56. Rahim R, Ochsner UA, Olvera C, Graninger M, Messner P, Lam JS, et al. Cloning and functional characterization of the Pseudomonas aeruginosa rhlC gene that encodes rhamnosyltransferase 2, an enzyme responsible for di-rhamnolipid biosynthesis. Molecular Microbiology. 2001;40(3):708-18.

57. Jacobs MA, Alwood A, Thaipisuttikul I, Spencer D, Haugen E, Ernst S, et al. Comprehensive transposon mutant library of Pseudomonas aeruginosa. Proceedings of the National Academy of Sciences. 2003;100(24):14339-44.

58. Walter TS, Ren J, Tuthill TJ, Rowlands DJ, Stuart DI, Fry EE. A plate-based high-throughput assay for virus stability and vaccine formulation. Journal of Virological Methods. 2012;185(1):166-70.

59. Kownatzki R, Tümmler B, Döring G. RHAMNOLIPID OF PSEUDOMONAS AERUGINOSA IN SPUTUM OF CYSTIC FIBROSIS PATIENTS. The Lancet. 1987;329(8540):1026-7.

60. Quinn RA, Phelan VV, Whiteson KL, Garg N, Bailey BA, Lim YW, et al. Microbial, host and xenobiotic diversity in the cystic fibrosis sputum metabolome. The ISME Journal. 2015;10(6):1483-98.

61. Schooling SR, Charaf UK, Allison DG, Gilbert P. A role for rhamnolipid in biofilm dispersion. Biofilms. 2004;1(2):91-9.

62. Boles BR, Thoendel M, Singh PK. Rhamnolipids mediate detachment of Pseudomonas aeruginosa from biofilms. Molecular Microbiology. 2005;57(5):1210-23.

63. Wood TL, Gong T, Zhu L, Miller J, Miller DS, Yin B, et al. Rhamnolipids from Pseudomonas aeruginosa disperse the biofilms of sulfate-reducing bacteria. npj Biofilms and Microbiomes. 2018;4(1):22.

64. Xu Q, Kang D, Meyer MD, Pennington CL, Gopal C, Schertzer JW, et al. Cytotoxic rhamnolipid micelles drive acute virulence in Pseudomonas aeruginosa. Infection and Immunity. 2024;92(3):e00407-23.

65. Bru J-L, Kasallis SJ, Chang R, Zhuo Q, Nguyen J, Pham P, et al. The great divide: rhamnolipids mediate separation between P. aeruginosa and S. aureus. Frontiers in Cellular and Infection Microbiology. 2023;13.

66. Bernier SP, Hum C, Li X, O'Toole GA, Magarvey NA, Surette MG. Pseudomonas aeruginosa-Derived Rhamnolipids and Other Detergents Modulate Colony Morphotype and Motility in the Burkholderia cepacia Complex. Journal of Bacteriology. 2017;199(13):10.1128/jb.00171-17.

67. Dusane DH, Nancharaiah YV, Zinjarde SS, Venugopalan VP. Rhamnolipid mediated disruption of marine Bacillus pumilus biofilms. Colloids and Surfaces B: Biointerfaces. 2010;81(1):242-8.

68. Yamasaki R, Kawano A, Yoshioka Y, Ariyoshi W. Rhamnolipids and surfactin inhibit the growth or formation of oral bacterial biofilm. BMC Microbiology. 2020;20(1):358.
69. e Silva SS, Carvalho JWP, Aires CP, Nitschke M. Disruption of Staphylococcus aureus biofilms using rhamnolipid biosurfactants. Journal of Dairy Science. 2017;100(10):7864-73.
70. Bhattacharjee A, Nusca TD, Hochbaum AI. Rhamnolipids Mediate an Interspecies Biofilm Dispersal Signaling Pathway. ACS Chemical Biology. 2016;11(11):3068-76.
71. Cerqueira dos Santos S, Araújo Torquato C, de Alexandria Santos D, Orsato A, Leite K, Serpeloni JM, et al. Production and characterization of rhamnolipids by Pseudomonas aeruginosa isolated in the Amazon region, and potential antiviral, antitumor, and antimicrobial activity. Scientific Reports. 2024;14(1):4629.
72. Giugliano R, Buonocore C, Zannella C, Chianese A, Palma Esposito F, Tedesco P, et al. Antiviral Activity of the Rhamnolipids Mixture from the Antarctic Bacterium Pseudomonas gessardii M15 against Herpes Simplex Viruses and Coronaviruses. Pharmaceutics. 2021;13(12):2121.
73. Jin L, Black W, Sawyer T. Application of Environment-Friendly Rhamnolipids against Transmission of Enveloped Viruses Like SARS-CoV2. Viruses. 2021;13(2):322.
74. Remichkova M, Galabova D, Roeva I, Karpenko E, Shulga A, Galabov AS. Anti-herpesvirus activities of Pseudomonas sp. S-17 rhamnolipid and its complex with alginate. Z Naturforsch C J Biosci. 2008;63(1-2):75-81.
75. Ali AM, Hill HJ, Elkhouly GE, Bakkar MR, Raya NR, Stamataki Z, et al. Rhamnolipid Nano-Micelles Inhibit SARS-CoV-2 Infection and Have No Dermal or Eye Toxic Effects in Rabbits. Antibiotics [Internet]. 2022; 11(11).
76. Touabi L, Ismail NSM, Bakkar MR, McLean GR, Abo-zeid Y. Antiviral Activity of Rhamnolipids Nano-Micelles Against Rhinoviruses—In Silico Docking, Molecular Dynamic Analysis and In-Vitro Studies. Current Issues in Molecular Biology. 2025;47(5):333.
77. Crocker JF, Ozere RL, Safe SH, Digout SC, Rozee KR, Hutzinger O. Lethal interaction of ubiquitous insecticide carriers with virus. Science. 1976;192(4246):1351-3.
78. Lee SH, Laltoo M, Crocker JF, Rozee KR. Emulsifiers that enhance susceptibility to virus infection: increased virus penetration and reduced interferon response. Appl Environ Microbiol. 1980;40(4):787-93.
79. Rozee KR, Lee SH, Crocker JF, Safe SH. Enhanced virus replication in mammalian cells exposed to commercial emulsifiers. Appl Environ Microbiol. 1978;35(2):297-300.
80. Stover CK, Pham XQ, Erwin AL, Mizoguchi SD, Warrener P, Hickey MJ, et al. Complete genome sequence of Pseudomonas aeruginosa PAO1, an opportunistic pathogen. Nature. 2000;406(6799):959-64.
81. Ohman DE, Chakrabarty AM. Genetic mapping of chromosomal determinants for the production of the exopolysaccharide alginate in a Pseudomonas aeruginosa cystic fibrosis isolate. Infection and Immunity. 1981;33(1):142-8.
82. Rahme LG, Stevens EJ, Wolfort SF, Shao J, Tompkins RG, Ausubel FM. Common Virulence Factors for Bacterial Pathogenicity in Plants and Animals. Science. 1995;268(5219):1899-902.

621 83. Baumann P, Doudoroff M, Stanier RY. Study of the Moraxella group. I. Genus
622 Moraxella and the Neisseria catarrhalis group. J Bacteriol. 1968;95(1):58-73.
623 84. Edwards PR, Fife MA. Studies on the Klebsiella-Aerobacter group of bacteria. J
624 Bacteriol. 1955;70(4):382-90.
625 85. Aguirre M, Morrison D, Cookson BD, Gay FW, Collins MD. Phenotypic and
626 phylogenetic characterization of some Gemella-like organisms from human infections:
627 description of Dolosigranulum pigrum gen. nov., sp. nov. J Appl Bacteriol.
628 1993;75(6):608-12.
629 86. King Jessica M, Kulhankova K, Stach Christopher S, Vu Bao G, Salgado-Pabón
630 W. Phenotypes and Virulence among Staphylococcus aureus USA100, USA200,
631 USA300, USA400, and USA600 Clonal Lineages. mSphere.
632 2016;1(3):10.1128/msphere.00071-16.
633 87. Barry AL, Jones RN, Thornsberry C, Ayers LW, Gerlach EH, Sommers HM.
634 Antibacterial activities of ciprofloxacin, norfloxacin, oxolinic acid, cinoxacin, and nalidixic
635 acid. Antimicrobial Agents and Chemotherapy. 1984;25(5):633-7.
636 88. HUGH R, ELLIS MA. The neotype strain for Staphylococcus epidermidis
637 (Winslow and Winslow 1908) Evans 1916. International Journal of Systematic and
638 Evolutionary Microbiology. 1968;18(3):231-9.
639 89. Facklam RR. Physiological differentiation of viridans streptococci. J Clin
640 Microbiol. 1977;5(2):184-201.
641

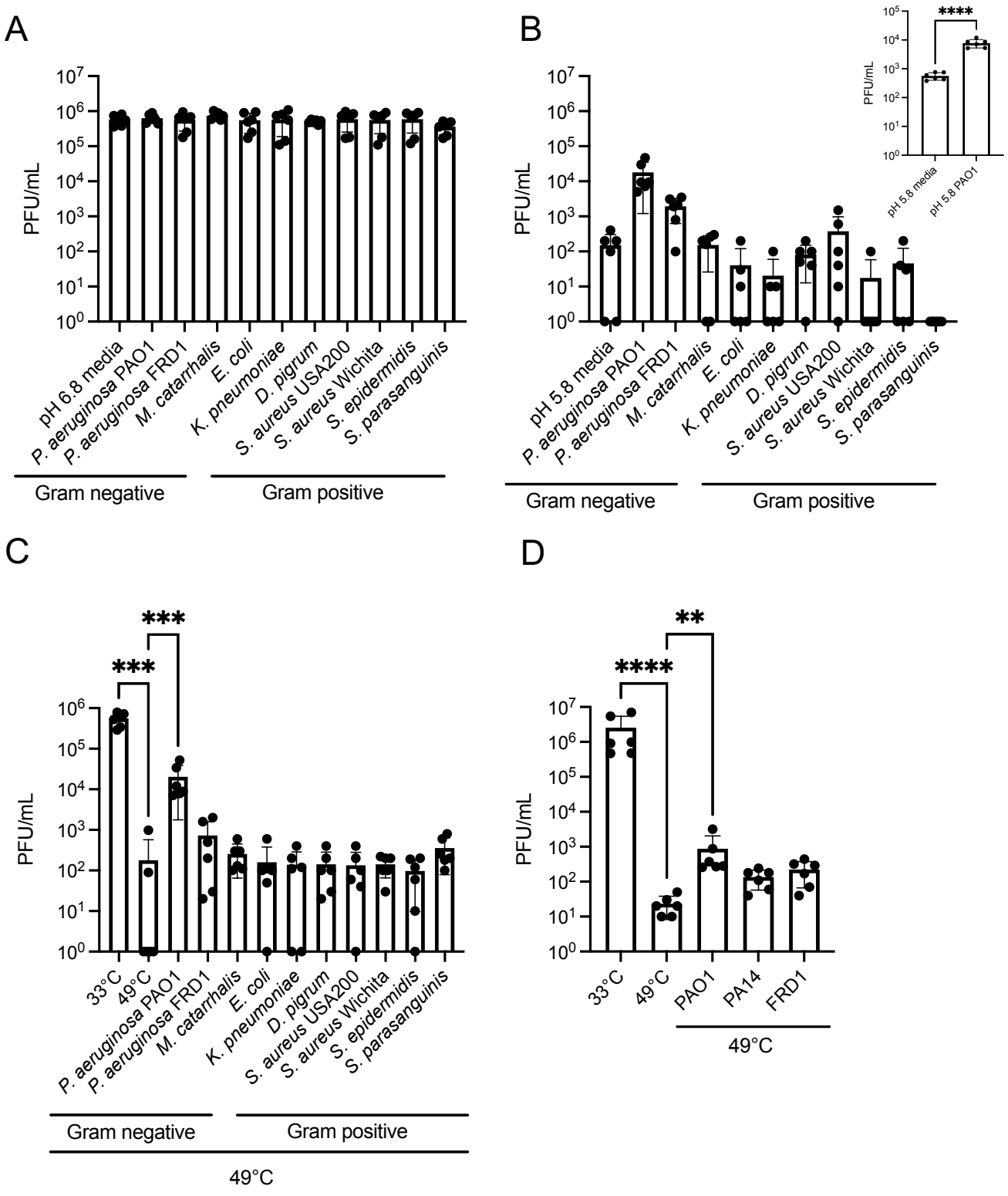

Figure 1. *P. aeruginosa* stabilizes HRV14.
A/B/C) HRV14 (10^5 PFU) was incubated for one hour at a pH of 6.8 (A) or 5.8 (B) or for two hours at 33°C or 49°C (C) in the presence or absence of a panel of airway bacteria (10^6-10^8 CFU). Samples were centrifuged and PFU were quantified from the supernatant by plaque assay. D) HRV14 was incubated in the presence or absence of *P. aeruginosa* PAO1, PA14, or FRD1 at 33°C or 49°C for two hours prior to plaque assay. n=6, 3 biological replicates with 2 technical replicates. **, p<0.01, ***, p<0.001, ****, p<0.0001 (A-D Kruskal-Wallis, Dunnett's post hoc test, B insert unpaired t test).

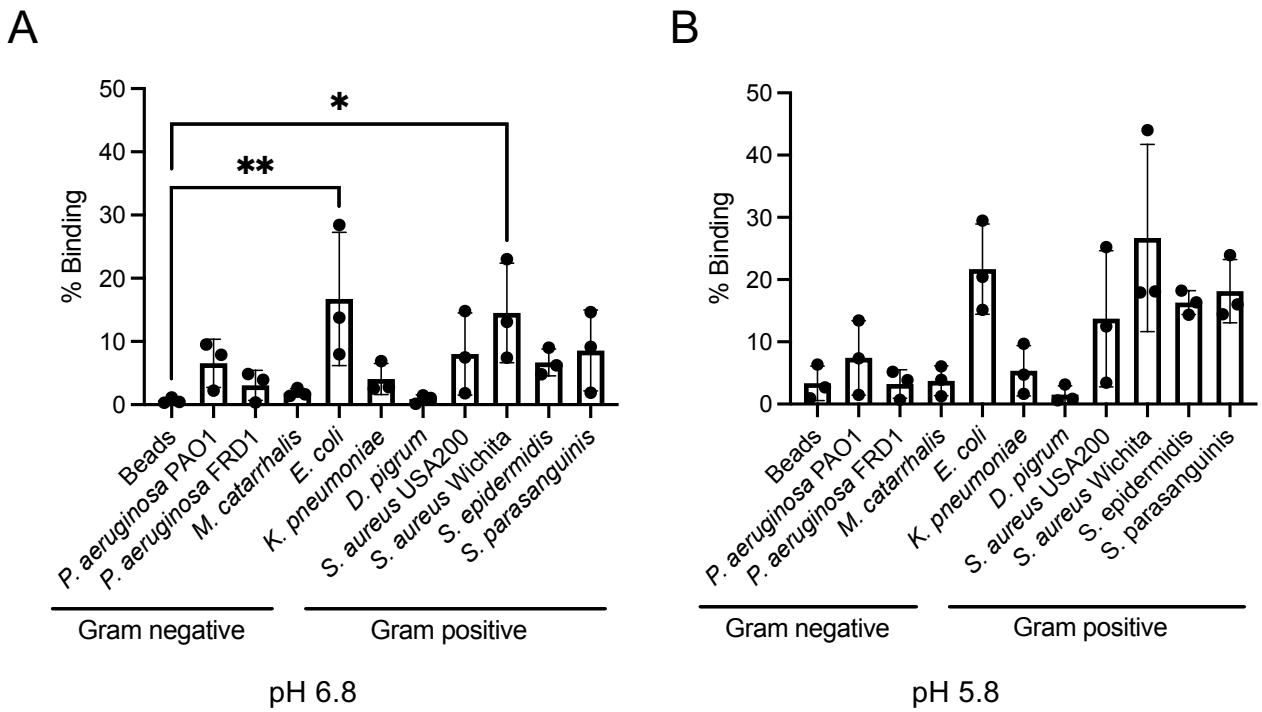

Figure 2. HRV14 does not have enhanced binding to *P. aeruginosa*.
A/B) ^{35}S-radiolabeled HRV14 (~4,000 CPM/ 10^6 PFU) was incubated in the presence or absence of streptavidin beads (2.8 µm) or 10^6-10^8 CFU bacteria in media at a pH of 6.8 (A) or 5.8 (B) at 33°C for one hour. Samples were centrifuged and washed to remove unbound virus. Bound virus was quantified via scintillation counting and normalized to input. n=3. A) ns, $p>0.05$ (Kruskal-Wallis, Dunnett's post hoc test). B) *, $p<0.05$, **, $p<0.01$ (one-way ANOVA, Dunnett's post hoc test).

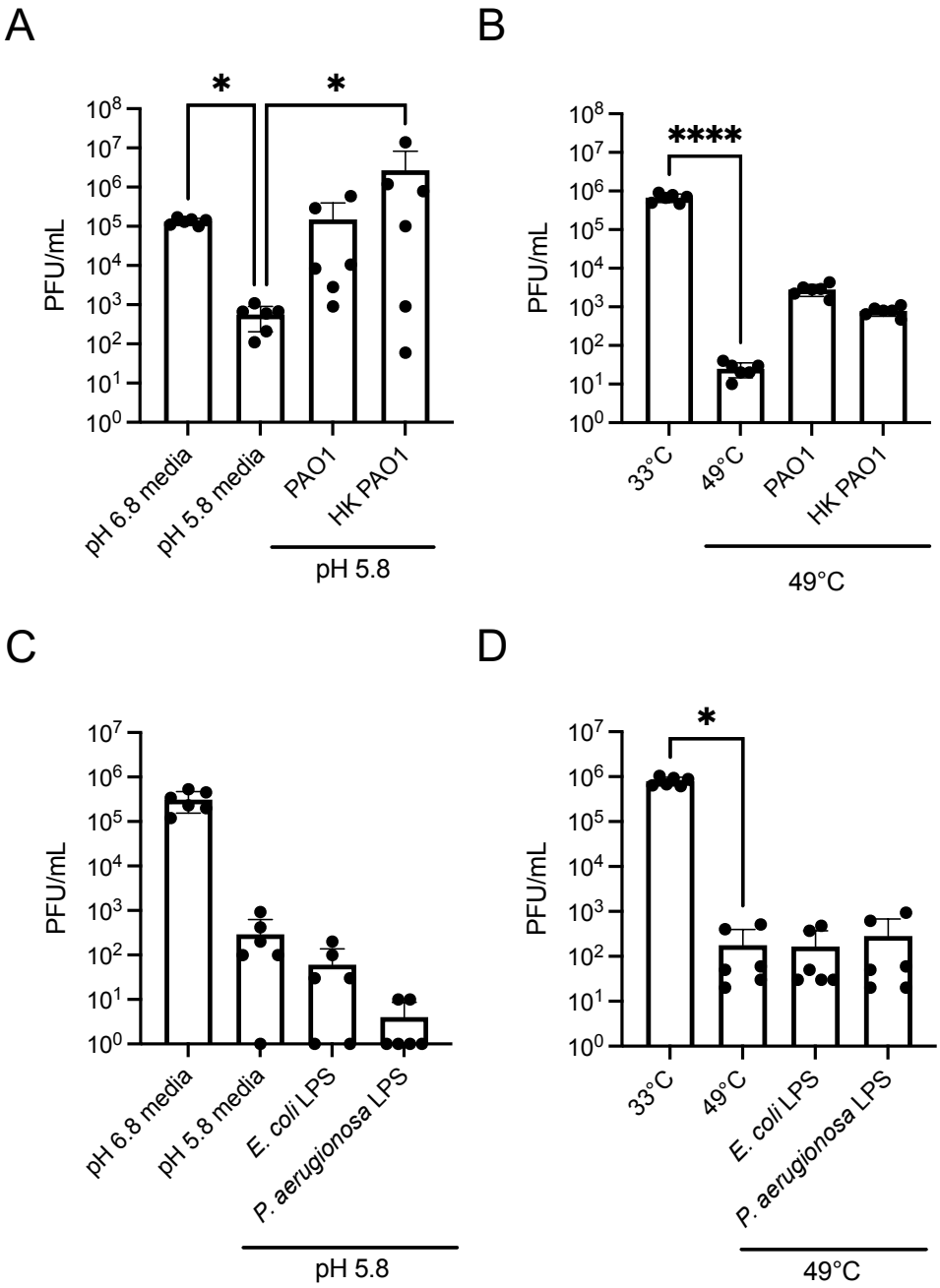

Figure 3. Heat-killed *P. aeruginosa* stabilizes HRV14.
A/B) HRV14 (10^5 PFU) was incubated in the presence or absence of 10^8 CFU live or heat-killed (HK) *P. aeruginosa* PAO1 at a pH of 5.8 or 6.8 at 33°C for one hour (A) or 33°C or 49°C for two hours (B) prior to plaque assay. C/D) HRV14 was incubated in the presence or absence of 1 mg/ml LPS from *E. coli* or *P. aeruginosa* at a pH of 5.8 or 6.8 for one hour (C) or 33°C or 49°C for two hours (D) prior to plaque assay. n=6, 3 biological replicates with 2 technical replicates. *, p<0.05, ****, p<0.0001 (A/C/D, Kruskal-Wallis, Dunnett's post hoc test, B, one way ANOVA, Dunnett's post hoc test).

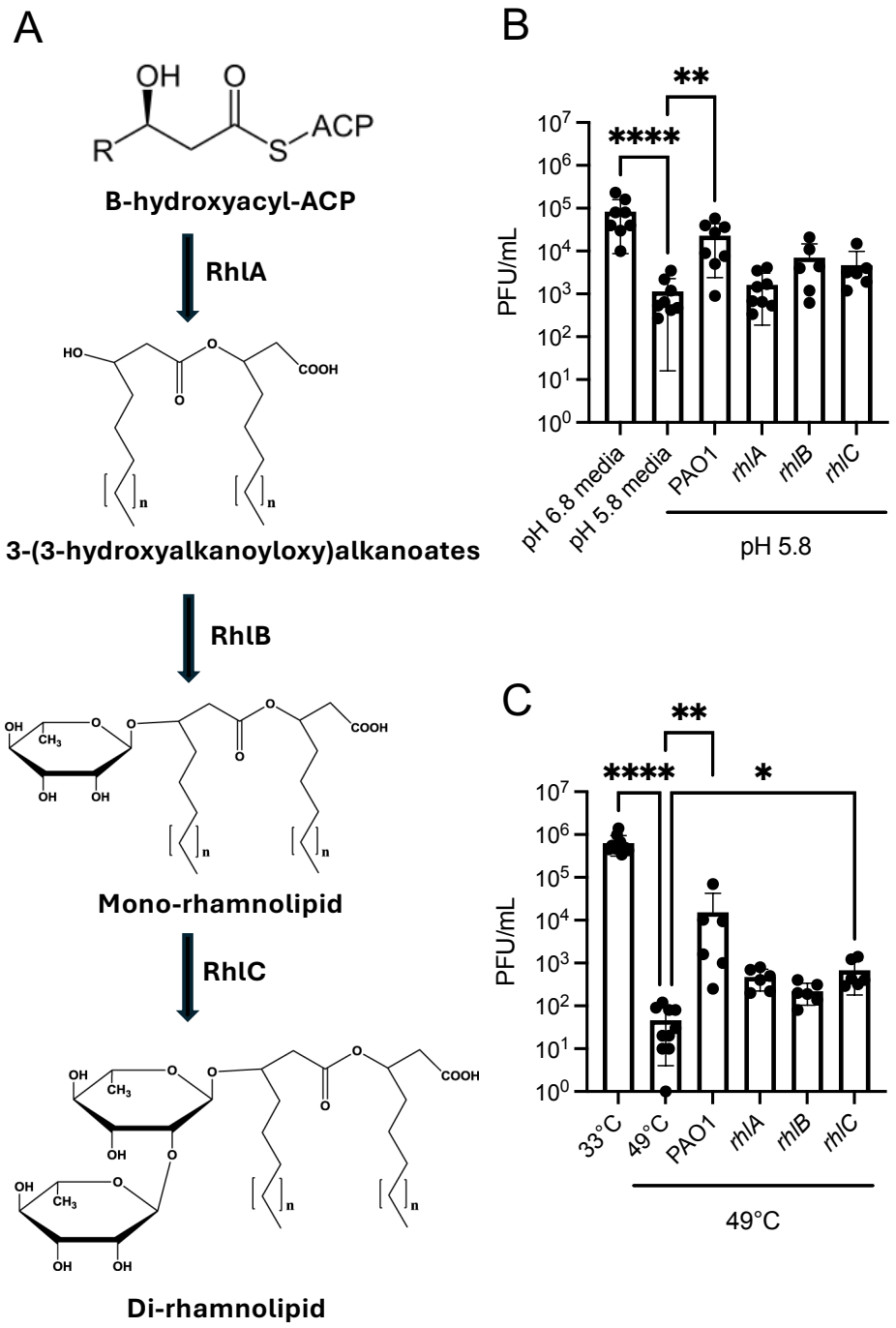

Figure 4. Insertion mutation of rhamnolipid synthesis genes ablates HRV14 stabilization.
A) *P. aeruginosa* rhamnolipid synthesis pathway. B/C) HRV14 (10^5 PFU) was incubated in the presence or absence of 10^8 CFU PAO1, *rhlA, rhlB,* or *rhlC* transposon insertion mutants at a pH of 5.8 or 6.8 at 33°C for one hour (B) or 33°C or 49°C for two hours (C) prior to plaque assay. n=6-8, 3-4 biological replicates with 2 technical replicates. *, $p<0.05$, ** $p<0.01$, ****, $p<0.0001$ (Kruskal-Wallis, Dunnett's post hoc test).

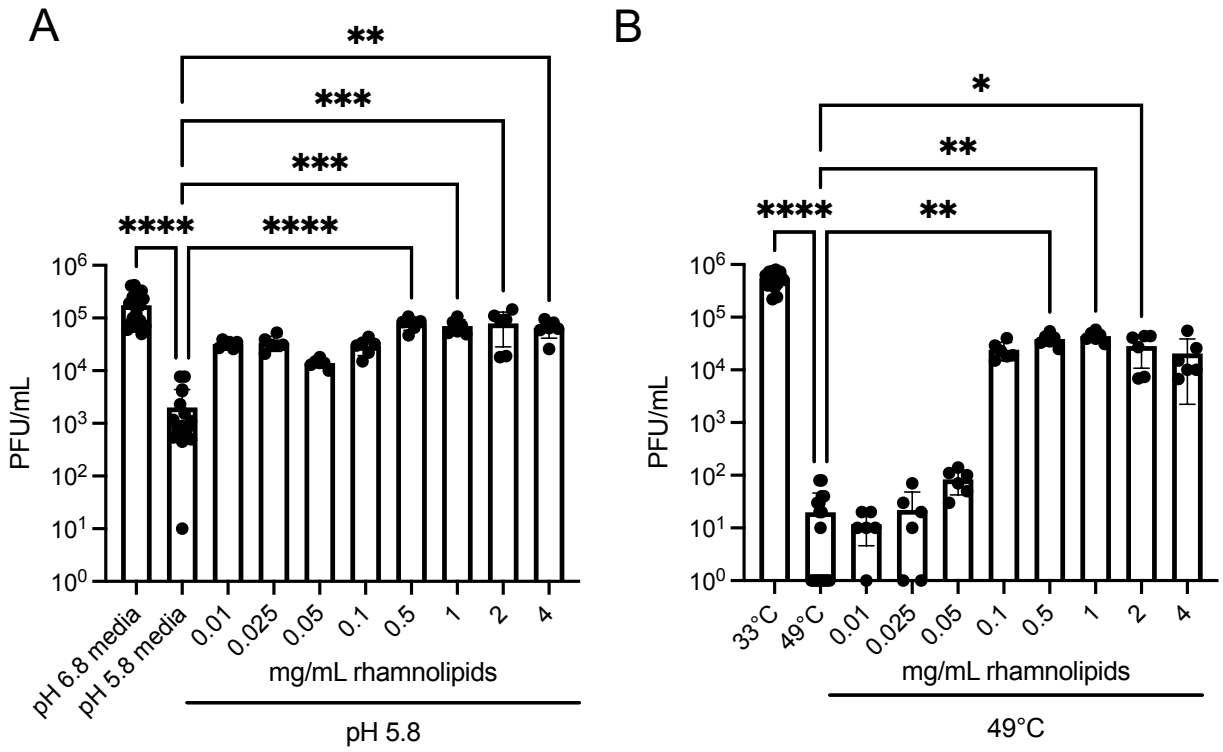

Figure 5. Rhamnolipids stabilize HRV14.
A/B) HRV14 (10^5 PFU) was incubated in the presence or absence of various concentrations of rhamnolipids at a pH of 5.8 or 6.8 at 33°C for one hour (A) or 33°C or 49°C for two hours (B) prior to plaque assay. n=6, 3 biological replicates with 2 technical replicates. *, p<0.05, ** p<0.01, ***, p<0.001 ****, p<0.0001(Kruskal-Wallis, Dunnett's post hoc test).

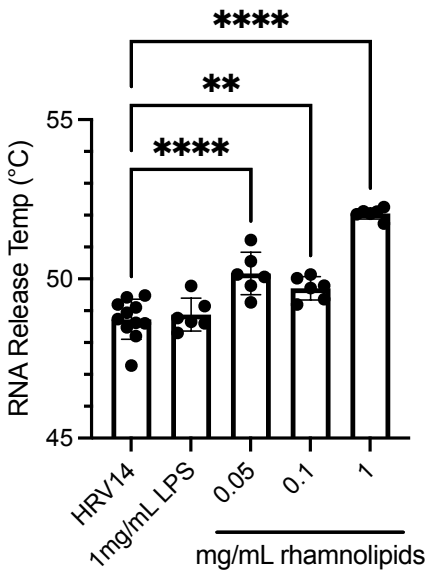

Figure 6. Rhamnolipids enhance HRV14 thermostability.
HRV14 thermostability profile using a cell-free Particle Stability Thermal Release assay (PaSTRy). HRV14 (10^5 PFU) was added to SYBR green II with or without LPS or rhamnolipids. Samples were heated from 25°C to 95°C on a 1% stepwise gradient with fluorescence monitoring. n=6, 3 biological replicates with 2 technical replicates. **, $p<0.01$, ****, $p<0.0001$ (one-way ANOVA, Dunnett's post hoc test).