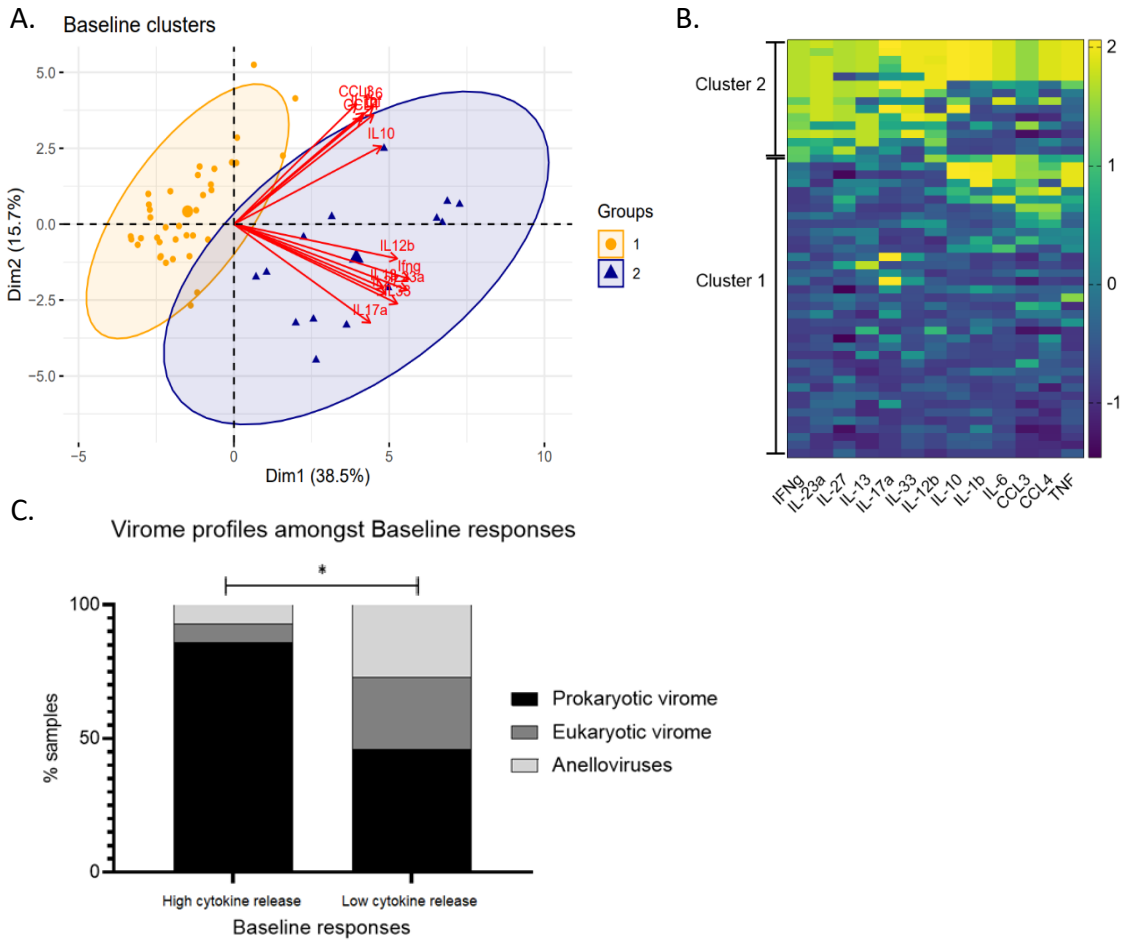

Figure 1. In preschool age children, respiratory virome profiles are associated with spontaneous cytokine release signatures from PBMCs. (A) Study subjects (n=51) were clustered in two groups based on their spontaneous cytokine release at baseline. Cytokines contributing to the clustering are represented by arrows (clockwise: CCL3, IL-6, IL-1b, CCL4, TNF, IL-10, IL-12b, IFN-ɣ, IL-23a, IL-13, IL-27, IL-33 and IL-17a). (B) A quantitative response pattern differentiates baseline spontaneous release clusters. Each line represents a subject and each column a cytokine. The color scale represents level of cytokine release. Cluster 2 subjects show higher overall values, in comparison to Cluster 1 subjects. (C) High spontaneous cytokine release from PBMC is associated with increased prokaryotic- and decreased eukaryotic- and anellovirus-dominated virome types. Cluster 1 (low spontaneous cytokine release) subjects (n=37) were divided between the virus profile groups (prokaryotic (PVPG) 46%, eukaryotic (EVPG) 27%, anellovirus (AVPG) 27%). In contrast, subjects with high spontaneous cytokine release (n=14) included mostly PVPG (86%) and only 7% of EVPG and AVPG. (p=0.018)

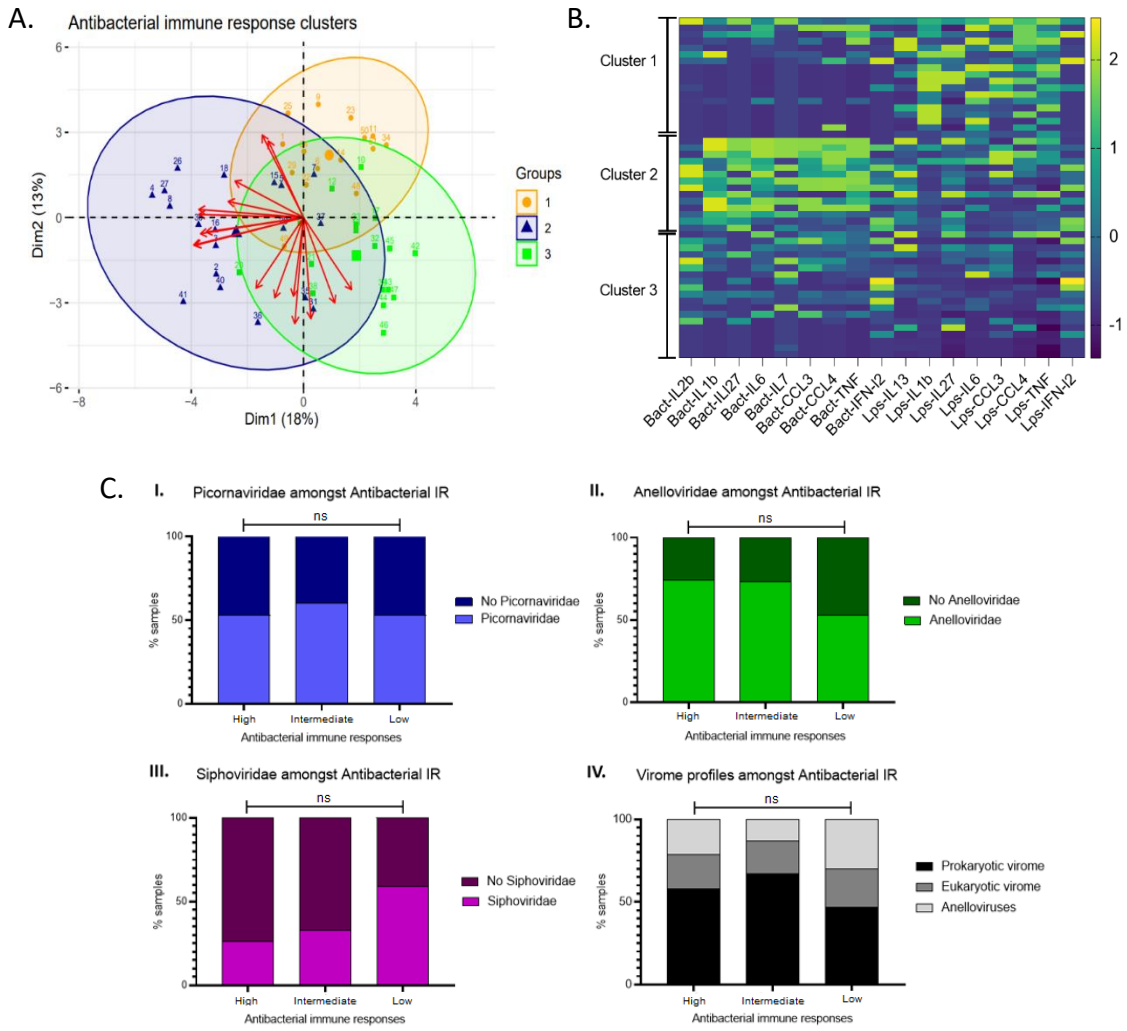

Figure 2. Bacterial immune signatures have minimal associations with respiratory viral signatures. (A) Bacterial stimuli trigger high, medium and low inflammatory responses. Subjects (n=51) were clustered based on their cytokine induction profile following stimulation with LPS and Bacterial DNA (Bact). Cytokines contributing to the clustering are represented by arrows (counterclockwise: LPS-IFN-λ2, Bact-IFN-λ2, Bact-IL12b, Bact-IL7, Bact-CCL3, Bact-CCL4, Bact-IL27, Bact-IL1β, Bact-IL6, Bact-TNF, LPS-IL13, LPS-IL27, LPS-CCL3, LPS-CCL4, LPS-TNF, LPS-IL6, LPS-IL1β). (B) Antibacterial immune response clusters.
Each line represents a subject and each column a cytokine (induced by either LPS or Bacterial DNA (Bact)). The color scale represents level of induction. Cluster 2 subjects show higher overall induction values, while Cluster 1 includes low responders. Cluster 3 represents an intermediate response. (C) Innate immune responses against bacterial stimuli are mostly independent from the virome composition. The presence of Picornaviridae (I) and Anelloviridae (II) is equally distributed among cytokine response clusters. A gradient in regard to the presence of Siphoviridae (III), did not reach statistical significance in this analysis, but was significant in multivariate regression (see Suppl. Table 5).

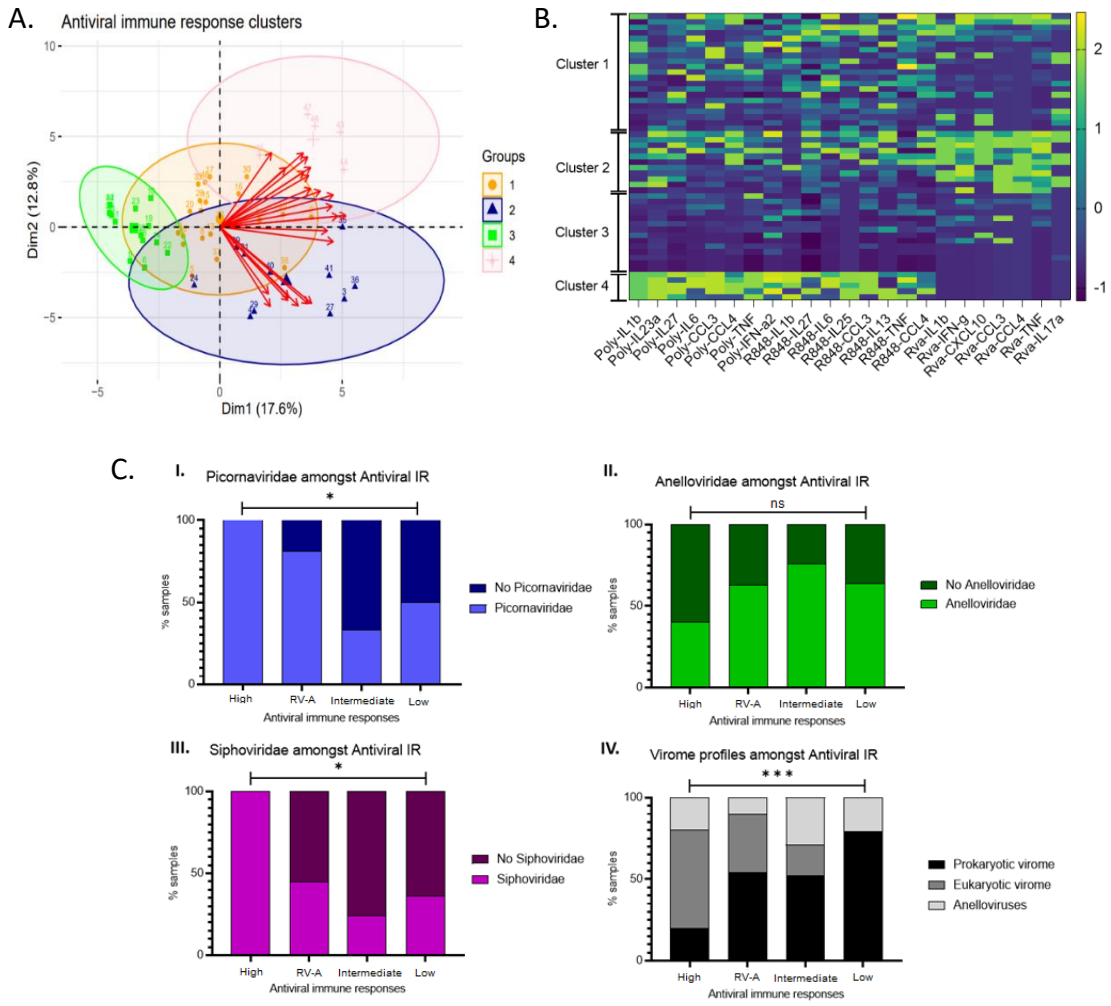

Figure 3. Viral immune signatures correlate to the presence of picornaviruses and bacteriophages in the respiratory virome. (A) Viral stimuli trigger four distinct types of responses. Subjects (n=51) were clustered based on their cytokine induction profile following stimulation with R848, polyIC and RV-A. Cytokines contributing to the clustering are represented by arrows (clockwise: R848-IL1b, R848-TNF, Poly-TNF, Poly-IL1b, Poly-IL6, R848-IL6, Poly-CCL3, Poly-IFN-α2, R848-CCL3, R848-IL25, Poly-IL23a, Poly-IL27, Poly-CCL4, R848-CCL4, R848-IL27, RVA-TNF, RVA-CCL4, RVA-IFN-ɣ, RVA-IL6, RVA-IL13, RVA-CXCL10, RVA-IL17a, RVA-CCL3). (B) Antiviral immune response clusters. Each line represents a subject and each column a cytokine (induced by either R848, polyIC, or RV-A). The color scale represents level of induction. Cluster 3 subjects show low induction, in contrast to Clusters 2 and 4 which include high responders to RV-A or polyIC/R848 respectively. Cluster 1 represents an intermediate response. (C) Correlation of antiviral immune responses with virus families and virome patterns. While Anelloviridae (II) are equally distributed among antiviral response groups, Picornaviridae (I), as well as Siphoviridae (III) are more prevalent in subjects with strong antiviral responses. Subjects with low antiviral responses have significantly more prokaryotic and less eukaryotic viromes (IV).

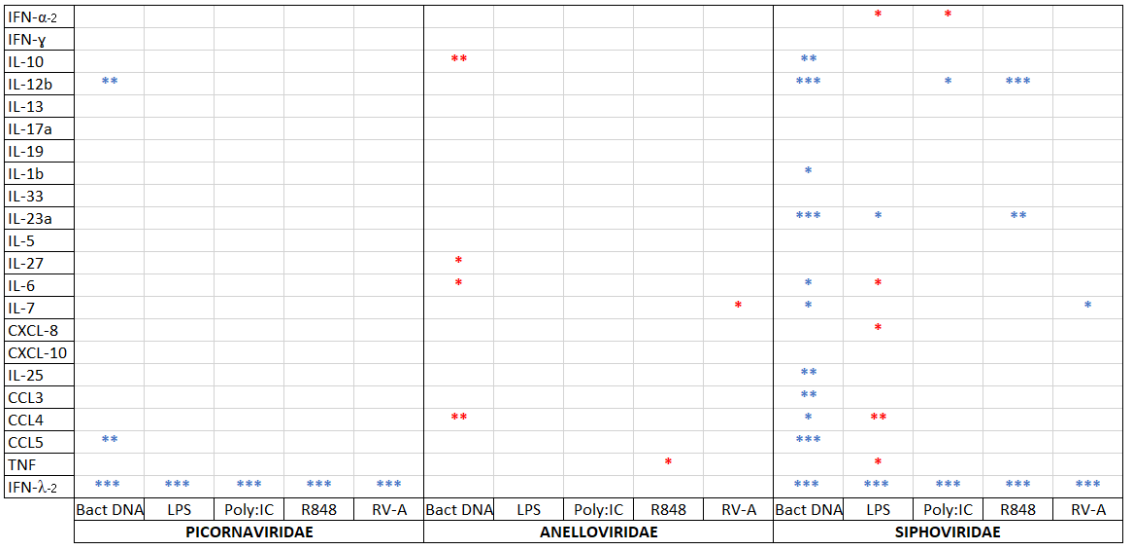

Figure 4. Cytokine changes in the presence versus absence of Picornaviridae, Anelloviridae and Siphoviridae following bacterial and viral stimuli. The p-value is represented by asterisks: $p<0.05$ *, $p<0.01$ **, $p<0.001$ ***. Red asterisks show a significant increase of the corresponding cytokine in presence of the viral family, blue asterisks a decrease. Antibacterial responses: bacterial DNA, LPS; antiviral responses: Poly:IC, R848, RV-A

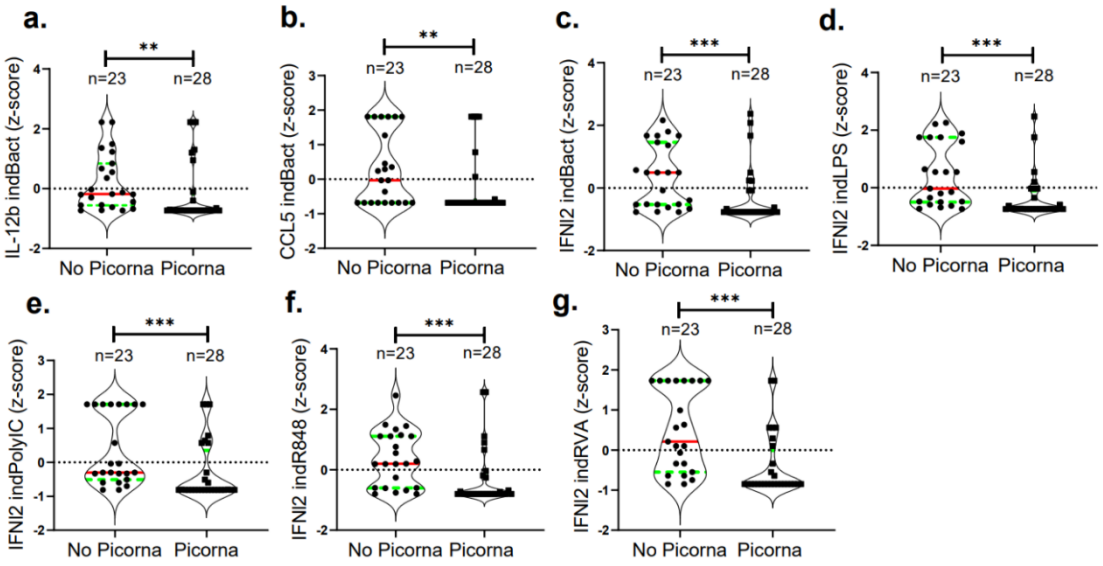

Figure 5. In children with Picornaviridae in their nasopharynx, reduced IFN responses, to both viral and bacterial stimuli are observed. (a) Bacterially-induced IL-12b (p:0.004), (b) Bacterially-induced CCL-5 (p:0.019), (c) Bacterially-induced IFN-λ-2 (p:0.001), (d) LPS-induced IFN-λ-2 (p:0), (e) PolyIC-induced IFN-λ-2 (p:0.001), (f) R848-induced IFN-λ-2 (p:0), (g) RVA-induced IFN-λ-2 (p:0).