Supplemental information

Metatranscriptomics to characterize respiratory virome, microbiome, and host response directly from clinical samples

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Figure S1: Plot showing the RNA integrity, Related to Figure 1 and STAR Methods. 

a) Plot showing the RNA integrity number (RIN) for RNA extracted from the nasal swab samples. The median RIN is shown as a red line.

b) Rarefaction analysis. Microbiome reads from five samples were sub-sampled five times at 1 million reads intervals, and their average number of species identified are plotted.
Figure S2: RSV-A and RSV-B Phylogenetic trees and Pairwise comparison of RSV genomes, Related to Figure 2.

The Maximum likelihood (ML) method was used to build a phylogenetic tree for complete RSVA and RSVB genomes. a) Phylogenetic tree for 28 complete RSVA genomes. b) Phylogenetic tree for complete RSVB genomes. The genomes recovered from healthy control samples are labelled in green, RSV-ARI mild samples are labelled in orange and RSV-ARI severe samples are labelled in red. c) Pairwise comparison of RSVA genomes showing the number of nucleotide differences between them. The number of nucleotide differences is shown in each box, which is colored red (high) to blue (low).
Figure S3: Species-level comparison of the nasal microbiome, Related to Figure 3 and Figure 4.
Species-level comparison of the nasal microbiome, after excluding the subjects reported taking antibiotics in one month prior to the sample collection. A color-coded bar plot shows the average bacterial species (measured using mOTUs) distribution in the RSV-ARI and healthy control groups. Only the top 20 most abundant species are shown here.
**Figure S4:** The relative abundance of nasal bacteria profiled using the metatranscriptomics method, Related to Figure 4.

a) A color-coded bar plot shows the relative abundance of nasal bacteria profiled using the metatranscriptomics method. The samples are portioned into HC and RSV-ARI groups. The RSVARI samples were portioned into RSV mild and RSV severe groups. Only the top 20 most abundant species are shown here. b) similar to (a), the relative abundance of the nasal bacteria was profiled using 16S rRNA marker gene sequencing. c-d) Correlation of nasal bacterial profiles obtained by 16S rRNA marker gene sequencing and metatranscriptomics methods. Normalized genus counts for the most abundant genera identified by 16S and metatranscriptomics methods were used for linear regression analysis. The samples are partitioned into HC (c) and RSV-ARI (d) groups. The regression coefficient shows a positive correlation between the bacterial profiles obtained from 16S and metatranscriptomics methods.
Figure S5: Active metabolic pathways of nasal bacteria, Related to Figure 4 and Discussion.

Active metabolic pathways of nasal bacteria. The figures show the nasal bacterial species contributions to functional pathways that were significantly different (q-value < 0.05) between HC and RSV-ARI groups. For each identified enzyme class or pathway, HUMAnN2 calculates species contribution. The bar plots show total stratified abundance of species linked with functional attributes, and each species abundance is shown in a different color. In the y-axis, contributing samples are colored based on the groups.
Figure S6: Upregulated genes in Interferon-alpha/beta and MyD88 deficiency pathways, Related to Figure 5 and Discussion.

Upregulated genes in Interferon-alpha/beta and MyD88 deficiency pathways. a) Plot showing Interferon alpha/beta signaling (R-HSA-909733) genes that are significantly up-regulated in the RSV-ARI group. On the x-axis is displayed the q-value for the up-regulated genes with q-values < 0.05 are shown. On the y-axis is displayed the log2 fold change for those genes. The size of the circles represents "base mean" which is the mean of normalized counts. b) Similar to (a), MyD88 deficiency (TLR2/4) (R-HSA-5602498) pathway genes that are significantly up-regulated in the RSVARI group are shown.