Supplemental methods

Clinical data: Pulmonary exacerbations were defined as courses of episodic oral or IV antibiotics prescribed by the treating physician for a diagnosis of CF pulmonary exacerbation (1).

Samples were categorized by clinical state (Baseline, Exacerbation, Treatment, Recovery) at the time of sample collection as previously described (2). In brief, Baseline samples were collected during a subject's state of stable health. Exacerbation samples were collected during the time of signs and symptoms of pulmonary exacerbation, but prior to initiation of oral or intravenous (IV) antibiotics for treatment of the exacerbation. Treatment samples were collected while the subject was receiving oral or IV antibiotics for a pulmonary exacerbation. Recovery samples were collected in the 30-day period following completion of oral or IV antibiotics for pulmonary exacerbation treatment. Disease aggressiveness was assigned as mild, moderate, or severe, based on longitudinal lung function values in relation to age, as previously described (3, 4). Body mass index at time of incident NTM infection was defined as acceptable (weight >25th percentile in pediatric patients, BMI >23 or >22 for adult male and female patients, respectively), at risk (weight between 10th and 25th percentile for pediatric patients, BMI of 22-23 or 21-22 for adult male and female patients, respectively), or nutritional failure (weight <10th percentile, BMI <22 or <21 for adult male and female patients, respectively) (5, 6).

DNA extraction and 16S rRNA gene sequencing: Sputum samples were thawed on ice then homogenized with 10% Sputolysin (MilliporeSigma, Burlington, MA, USA). DNA extractions for sputum samples and reagent controls were performed with mechanical

disruption by bead beating followed by incubation with Bacterial Lysis Buffer (Roche Diagnostics Corp., Indianapolis, IN,USA), lysostaphin (MilliporeSigma, Burlington, MA,USA), and lysozyme (MilliporeSigma, Burlington, MA,USA), followed by treatment with proteinase K (Qiagen,Germantown, MD,USA) as previously described (7). DNA were extracted and purified using a MagNA Pure nucleic acid purification platform (Roche Diagnostics Corp., Indianapolis,IN,USA) according to the manufacturer's protocol. Prior studies have demonstrated stability of bacterial DNA in CF sputum stored at -80◦C over a 15 year time period (8).

The V4 region of the bacterial 16S rRNA gene was amplified using touchdown PCR with barcoded dual-index primers. The touchdown PCR cycles consisted of 2 min at 95°C, followed by 20 cycles of 95°C for 20 sec, 60°C (start from 60°C, the annealing temperature decreased 0.3°C each cycle) for 15 sec and 72°C for 5 min, then followed by another 20 cycles of 95°C for 20 sec, 55°C for 15 sec and 72°C for 5 min and held at 72°C for 10 min. The amplicon libraries were then normalized and sequenced on Illumina sequencing platform using a MiSeq Reagent Kit V2 (Illumina, San Diego, CA). The final load concentration was 4-5.5 pM with a 15% PhiX spike to add diversity.

Bacterial 16S rRNA gene ddPCR: The following primers and probe were used: Forward primer, 5'-TCCTACGGGAGGCAGCAGT-3', reverse primer, 5'-GGACTACCAGGGTATCTAATCCTGTT-3', and modified probe, (6-FAM)-5'-CGTATTACCGCGGCTGCTGG-3'-(IABkFQ). Each 25 µL reaction was comprised of 12.5 uL 2X ddPCR Supermix for Probes with no dUTP (Bio-Rad, Hercules, CA), 1.25 µL of each primer and probe (final concentrations of primer and probe per reaction were 900 nM and 250 nM, respectively), 6.75 µL UltraPure water (Invitrogen, Carlsbad, CA),

and 2 µL of template DNA (diluted by a factor of 1:250). Reactions were transferred to the Automated Droplet Generator, followed by gene amplification in a C1000 Touch Thermal Cycler (Bio-Rad, Hercules, CA). Cycling conditions were as follows: 1 cycle at 95°C for 10 minutes, 40 cycles at 94°C for 30 seconds and 58°C for 2 minutes, and 1 cycle at 98°C for 10 minutes, with a ramp rate of 2°C/second per step. DNA quantification was performed with the QX200 Droplet Reader, and data analysis with QuantaSoft Analysis Pro (Bio-Rad, Hercules, CA). All reactions were assayed in duplicate, and those with less than 10,000 droplets per reaction were omitted from analysis. A singular threshold was set for all assays by taking 50% of the difference between the lowest droplet on the positive band and the highest droplet on the negative band and adding that to the amplitude of the highest droplet on the negative band. Output DNA concentration values between replicates were then averaged and multiplied by 250 to correct for the dilution factor, then multiplied by 12.5 to obtain copies of target gene per uL of the starting DNA sample.

Significance testing: Cross-sectional features from the samples closest in time to the incident NTM infection included relative abundances of OTUs (OTUs with an average relative abundance ≥ 0.01%), alpha diversity measures (chao richness, inverse Simpson index, and nonparametric Shannon diversity), total bacterial load, and percent predicted forced expiratory volume in one second ($ppFEV_1$). To test the within-subject dynamics over time leading up to incident NTM infection, the longitudinal changes of microbiome features (relative abundances of OTUs, alpha diversity measures, and total bacterial load) from all samples and data points available over the study period was

measured with linear regression (scipy stats' linregress) (9), and these slopes were then included as longitudinal features in the significance testing.

ARIMA and network analyses: OTU-based features were filtered for each ARIMA model to include only OTUs with an average relative abundance greater than 1%, thus slightly different OTUs were considered in each ARIMA model. To meet the ARIMA method requirement of evenly spaced time points, all features were interpolated at 0.5 year intervals for each patient within the range of time for which real data was available (i.e. no extrapolation). As the interpolation required normalization of the sample counts, the offset term used by in the cited ARIMA paper was not used. A range of possible parameter values for λ and α were tested with 'cv.glmnet' for parameterization. AIC and pseudo-R^2 were used to rank and determine the best fit models for each OTU. To evaluate the magnitude of the effect of each feature (OTU relative abundance or ppFEV$_1$) on the outcomes, NTM pulmonary disease (primary outcome), and persistent infection (secondary outcome), the outcome variables were forced into their respective models. To identify OTU-OTU interactions that differed between the outcome groups, separate within-cohort ARIMA analyses were performed. The resulting coefficients for OTUs in each ARIMA model were scaled by their mean relative abundance for direct comparison and visualization. For the network analyses, OTUs were grouped at the genus level for vertices, and vertex clustering was calculated by optimizing modularity (with igraph's cluster_optimal function) after running *simplify* to reduce multiple edges between vertices to single edges.

1. Flume PA, Mogayzel PJ, Robinson KA, Goss CH, Rosenblatt RL, Kuhn RJ, Marshall BC, Bujan J, Downs A, Finder J, Goss C, Gutierrez H, Hazle L, Lester M, Quittell L, Sabadosa K, Vender RL, White TB, Willey-Courand DB, Saldanha I, Oyegunle M, Shankar MB, Mckoy N, Sengupta S, Odelola OA, Waybright S. Cystic fibrosis pulmonary guidelines: Treatment of pulmonary exacerbations. *Am J Respir Crit Care Med* 2009;doi:10.1164/rccm.200812-1845PP.

2. Zhao J, Schloss PD, Kalikin LM, Carmody LA, Foster BK, Petrosino JF, Cavalcoli JD, VanDevanter DR, Murray S, Li JZ, Young VB, LiPuma JJ. Decade-long bacterial community dynamics in cystic fibrosis airways. *Proc Natl Acad Sci U S A* 2012;109:5809–5814.

3. Konstan MW, Wagener JS, Vandevanter DR. Characterizing aggressiveness and predicting future progression of CF lung disease. *J Cyst Fibros* 2009;8:S15-19.

4. Schluchter MD, Konstan MW, Drumm ML, Yankaskas JR, Knowles MR. Classifying severity of cystic fibrosis lung disease using longitudinal pulmonary function data. *Am J Respir Crit Care Med* 2006;174:780–786.

5. Stallings VA, Stark LJ, Robinson KA, Feranchak AP, Quinton H. Evidence-Based Practice Recommendations for Nutrition-Related Management of Children and Adults with Cystic Fibrosis and Pancreatic Insufficiency: Results of a Systematic Review. *J Am Diet Assoc* 2008;108:832–839.

6. Turck D, Braegger CP, Colombo C, Declercq D, Morton A, Pancheva R, Robberecht E, Stern M, Strandvik B, Wolfe S, Schneider SM, Wilschanski M. ESPEN-ESPGHAN-ECFS guidelines on nutrition care for infants, children, and

adults with cystic fibrosis. *Clin Nutr* 2016;35:557–77.

7. Caverly LJ, Carmody LA, Haig S-J, Kotlarz N, Kalikin LM, Raskin L, LiPuma JJ. Culture-independent identification of nontuberculous mycobacteria in cystic fibrosis respiratory samples. *PLoS One* 2016;11:e0153876.

8. Acosta N, Whelan FJ, Somayaji R, Poonja A, Surette MG, Rabin HR, Parkins MD. The Evolving Cystic Fibrosis Microbiome: A Comparative Cohort Study Spanning 16 Years. doi:10.1513/AnnalsATS.201609-668OC.

9. Virtanen P, Gommers R, Oliphant TE, Haberland M, Reddy T, Cournapeau D, Burovski E, Peterson P, Weckesser W, Bright J, van der Walt SJ, Brett M, Wilson J, Millman KJ, Mayorov N, Nelson ARJ, Jones E, Kern R, Larson E, Carey C, Polat İ, Feng Y, Moore EW, VanderPlas J, Laxalde D, Perktold J, Cimrman R, Henriksen I, Quintero EA, *et al.* SciPy 1.0--Fundamental Algorithms for Scientific Computing in Python. 2019;at <http://arxiv.org/abs/1907.10121>.