Gifford et al., Supplemental Figures

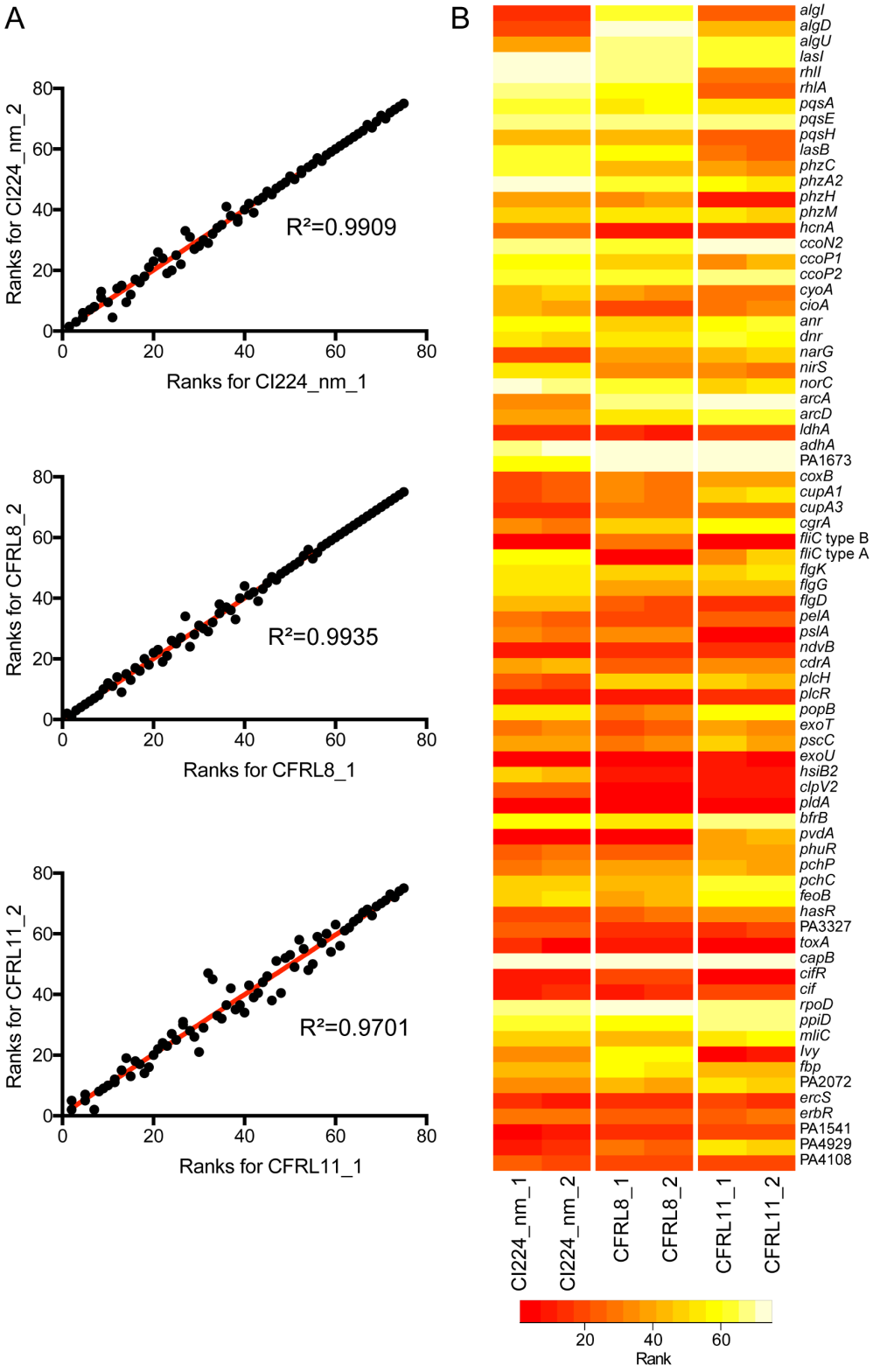

Fig. S1. Analysis of the reproducibility between duplicate samples. A. Correlation plot of rank abundances of raw counts obtained from PAV2 Nanostring analysis of colony biofilms formed by three *P. aerugionsa* clinical strains with different colony phenotypes (two colonies per strain). B. Rank abundance heatmap of the three *P. aeruginosa* clinical strains from the PAV2 Nanostring analysis. The raw data are in Table S6.

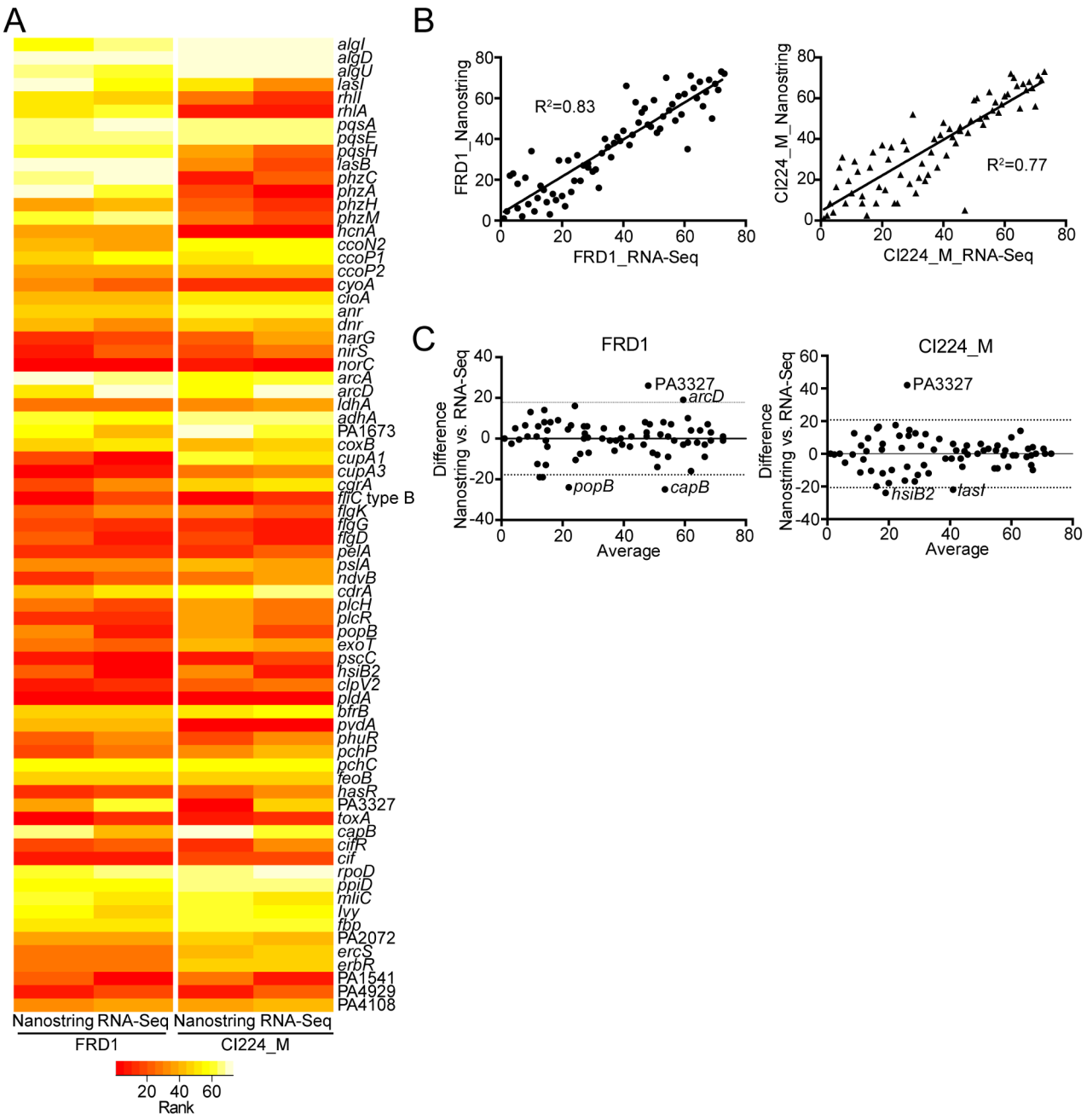

Fig. S2. Analysis of the agreement between Nanostring analysis and RNA-Seq. A. Rank abundance heatmap of the *P. aeruginosa* strains FRD1 and CI224_M from the PAV2 Nanostring analysis (Nanostring) and the RNA-Seq analysis (RNA-Seq). B. Correlation plots of rank abundances of normalized counts obtained from PAV2 Nanostring analysis and the RNA-Seq analysis for the strains FRD1 and CI224_M. C. Bland-Altman plots of rank abundances of normalized counts obtained from PAV2 Nanostring analysis and the RNA-Seq analysis. The dotted lines represent the 95% confidence interval for agreement between the two transcript level measuring methods. The raw data are in Table S4 (RNA-Seq) and Table S5 (Nanostring).

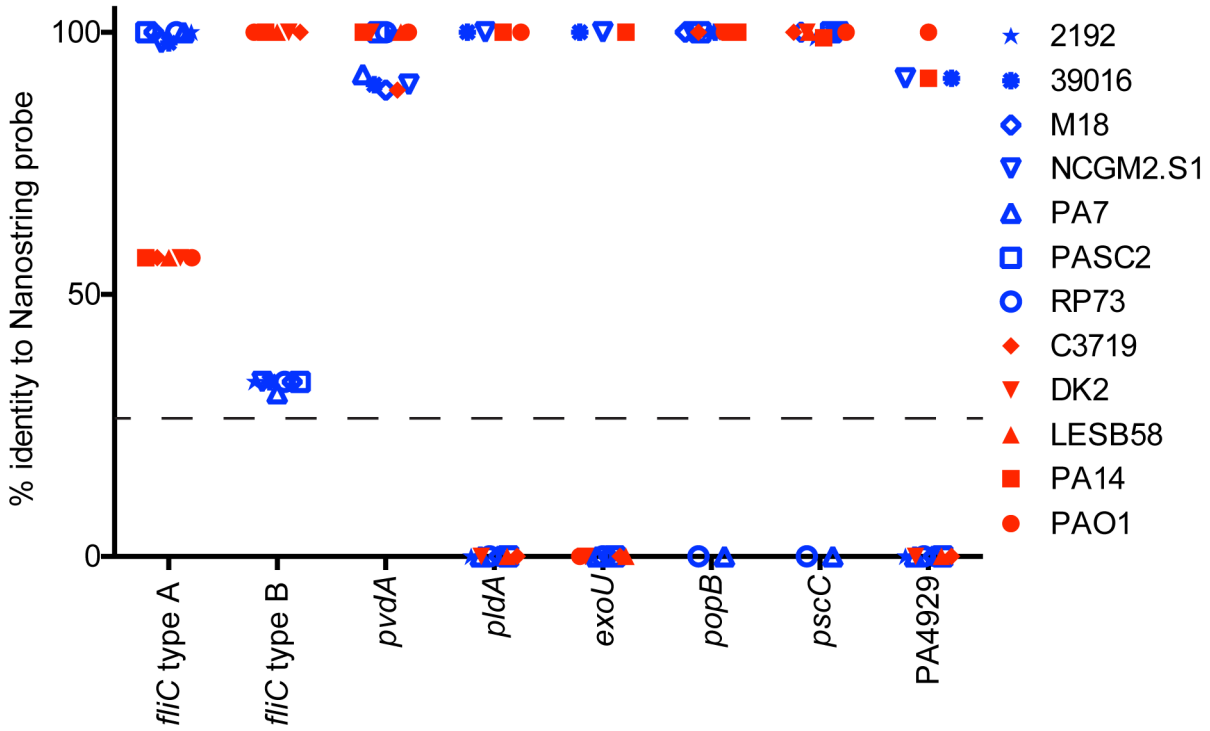

Fig. S3. Genomic sequence identities for genes that vary across strains. Genes with variation in the regions complementary to the Nanostring probe sequence are shown. Each point reflects the percent identity to the PAO1 reference sequence or absence of a detectable homolog. The *P. aeruginosa* strains used in the comparison are shown in the legend. Only genes with at least one instance of a match with less than <90% identity are shown.

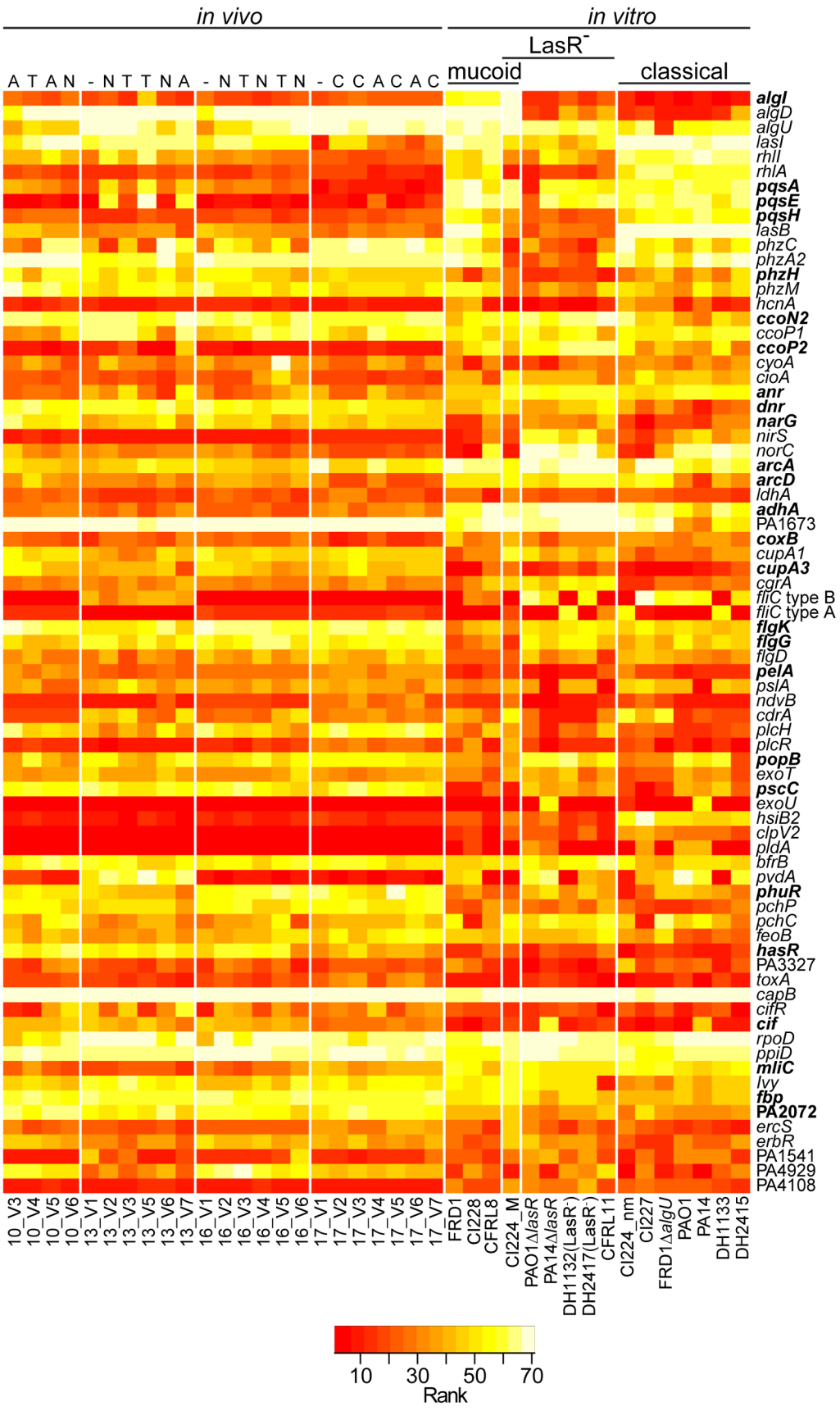
in vivo
in vitro
LasR-
mucoid
classical
A T A N - N T T N A - N T N T N - C C A C A C
algI
algD
algU
lasI
rhlI
rhlA
pqsA
pqsE
pqsH
lasB
phzC
phzA2
phzH
phzM
hcnA
ccoN2
ccoP1
ccoP2
cyoA
cioA
anr
dnr
narG
nirS
norC
arcA
arcD
ldhA
adhA
PA1673
coxB
cupA1
cupA3
cgrA
fliC type B
fliC type A
flgK
flgG
flgD
pelA
pslA
ndvB
cdrA
plcH
plcR
popB
exoT
pscC
exoU
hsiB2
clpV2
pldA
bfrB
pvdA
phuR
pchP
pchC
feoB
hasR
PA3327
toxA
capB
cifR
cif
rpoD
ppiD
mliC
lvy
fbp
PA2072
ercS
erbR
PA1541
PA4929
PA4108
10_V3
10_V4
10_V5
10_V6
13_V1
13_V2
13_V3
13_V5
13_V6
13_V7
16_V1
16_V2
16_V3
16_V4
16_V5
16_V6
17_V1
17_V2
17_V3
17_V4
17_V5
17_V6
17_V7
FRD1
CI228
CFRL8
CI224_M
PAO1ΔlasR
PA14ΔlasR
DH1132(LasR-)
DH2417(LasR-)
CFRL11
CI224_nm
CI227
FRD1ΔalgU
PAO1
PA14
DH1133
DH2415
10
30
50
70
Rank

Fig. S4. Analysis of the *P. aeruginosa* transcripts that are significantly different between *in vivo* and *P. aeruginosa* mucoid strains grown *in vitro*. The heat map shows the rank abundances for all 75 PAV2 transcripts in all samples analyzed. The in vivo samples (RNA extracted from sputum) and in vitro samples are compared. For the in vitro samples, the strain phenotype (classical, mucoid, or LasR-deficient) is shown. For the in vivo samples, the subject number and treatment code (A = aztreonam, C = colistimethate, T = tobramycin and N = no treatment) is shown. The sample and strain names are along the bottom. For the in vivo samples, the first number indicates the subject (10, 13, 16, and 17) and the number following the "V" indicates the visit number within the six month enrollment period. Letters above each *in vivo* sample column denotes the type of inhaled antibiotic used at the time of sample collection (A = aztreonam, C = colistimethate, T = tobramycin and N = no treatment). Rank abundance analysis indicating levels of transcripts (most abundant in yellow and least abundant in red). The genes highlighted in bold are statistically different between the in vivo samples and the mucoid strains grown in vitro (FDR corrected and corrected for repeated measures, p-value $P<0.05$).

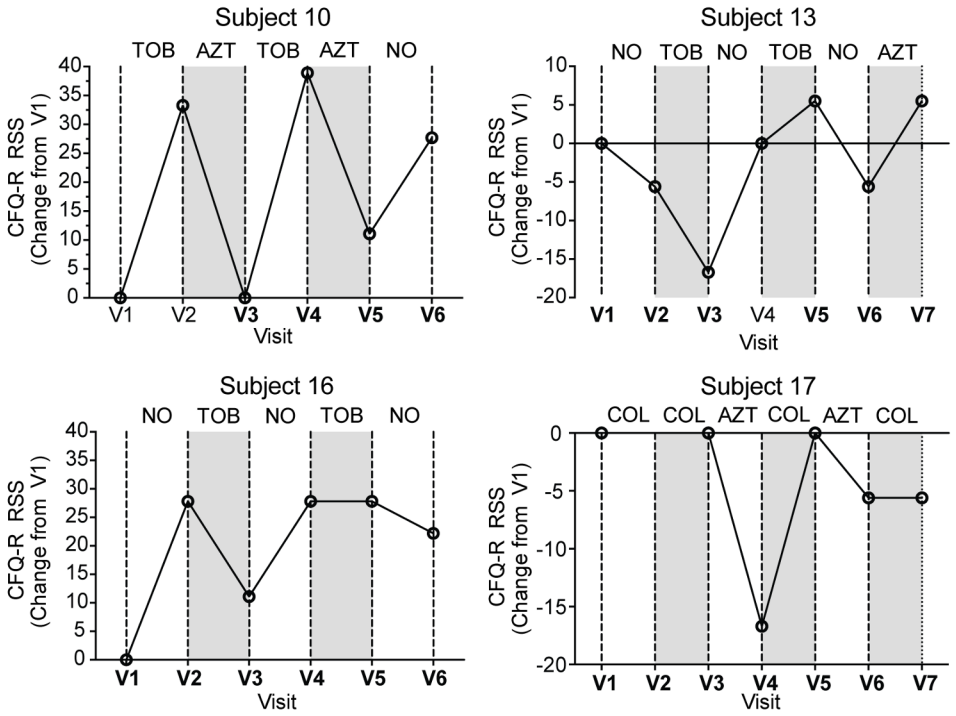

Fig. S5. **Changes in the respiratory symptom score (RSS) of the CFQ-R relative to the first visit for each CF subject.** Each plot shows the RSS for a single subject over the course of the six month study. The numbers show the difference relative to the score obtained during the first visit. The type of inhaled antibiotic used the month prior to sample collection is shown (AZT = aztreonam, COL = colistimethate, TOB = tobramycin and NO = no treatment). The visits highlighted in bold were used for *P. aeruginosa* gene expression analysis by Nanostring (PAV2). Samples not used in the analysis were either not collected due to the inability to produce sputum or to the recovery of only minimal or poor quality RNA.