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Supplemental Information

**Cystic Fibrosis Lung Transplant Recipients Have
Suppressed Airway Interferon Responses
during *Pseudomonas* Infection**

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SUPPLEMENTAL TABLES & FIGURES

Table ST1: Longitudinal CLAD-Survival Cohort
(Related to Human Subjects in STAR Methods and Figure 1)

	CF-	CF+	p-value
N (%)	362 (91.4)	34 (8.6)	
Recipient Age, mean (SD)	58.20 (10.23)	36.01 (11.15)	<0.001
Donor Age, mean (SD)	34.31 (14.79)	30.82 (12.25)	0.196
Transplant Indication Category (%)			<0.001
A (Obstructive)	59 (16.6)	0 (0)	
B (Pulm. Vascular)	16 (4.5)	0 (0)	
C (CF)	0 (0)	34 (100)	
D (Restrictive)	281 (78.9)	0 (0)	
Recipient Gender Male (%)	210 (59.0)	17 (53.1)	0.647
Donor Gender Male (%)	217 (61.0)	27 (84.4)	0.015
Recipient Ethnicity (%)			0.015
White	239 (67.1)	29 (90.6)	
Black	28 (7.9)	3 (9.4)	
Hispanic	64 (18.0)	0 (0)	
Other	25 (7.0)	0 (0)	
Donor Ethnicity (%)			0.001
White	164 (46.1)	15 (46.9)	
Black	28 (7.9)	9 (28.1)	
Hispanic	115 (32.3)	7 (21.9)	
Other	49 (13.8)	1 (3.1)	
Transplant Type (%)			0.177
Double-Lung	321 (90.2)	32 (100)	
Single-Lung	27 (7.6)	0 (0)	
Heart-Lung	8 (2.2)	0 (0)	
HLA mismatches, mean (SD)	4.62 (1.16)	4.78 (1.04)	0.45
CMV Status (%)			0.385
Donor +, Recipient -	101 (28.4)	9 (28.1)	
Donor +, Recipient +	138 (38.8)	9 (28.1)	
Other	117 (32.9)	14 (43.8)	
PsA+ cultures, mean (SD)	0.19 (0.65)	1.47 (1.88)	<0.001

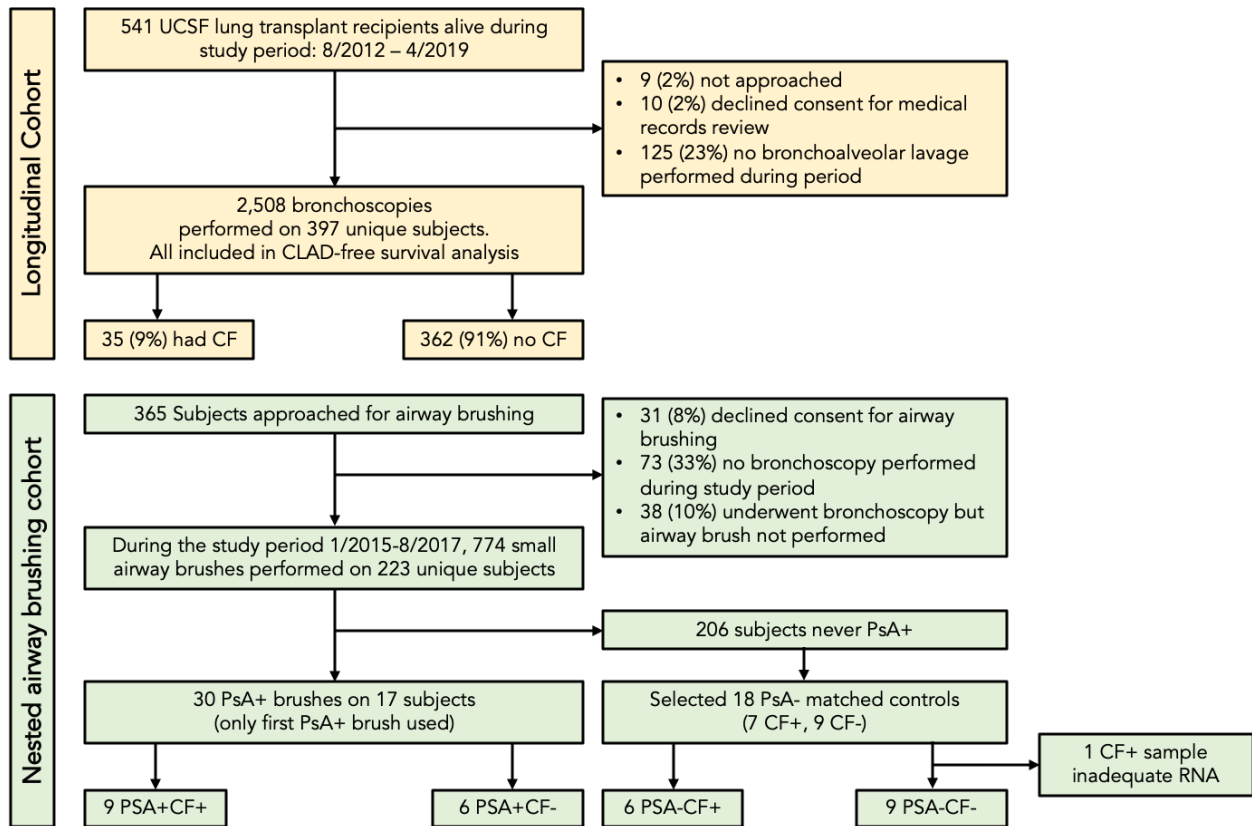
Table ST2: Transcriptome and Metagenome Cohort
(Related to Figure 1 and STAR Methods Human Subjects)

	CF+PsA+	CF+PsA-	CF-PsA-	CF-PsA+	p-value
N	9	6	9	6	
Recipient Age, mean (SD)	32.2 (10.8)	39.3 (8.2)	59.6 (8.1)	59.5 (9.6)	<0.001
Donor Age, mean (SD)	27.3 (10.0)	32.8 (9.1)	37.2 (16.7)	37.5 (15.3)	0.72
Transplant Indication (%)					<0.001
A (Obstructive)	0 (0)	0 (0)	2 (22.2)	1 (16.7)	
B (Pulm. Vascular)	0 (0)	0 (0)	0 (0)	0 (0)	
C (CF)	9 (100)	6 (100)	0 (0)	0 (0)	
D (Restrictive)	0 (0)	0 (0)	7 (77.8)	5 (83.3)	
Recipient Gender Male (%)	4 (44.4)	2 (33.3)	6 (66.7)	5 (83.3)	0.556
Donor Gender Male (%)	7 (77.8)	6 (100)	5 (55.6)	5 (83.3)	0.643
Recipient Ethnicity (%)					0.561
White	7 (77.8)	6 (100)	5 (55.6)	3 (50.0)	
Black	2 (22.2)	0 (0)	2 (22.2)	2 (33.3)	
Hispanic	0 (0)	0 (0)	1 (11.1)	1 (16.7)	
Other	0 (0)	0 (0)	1 (11.1)	0 (0)	
Donor Ethnicity (%)					0.296
White	3 (33.3)	4 (66.7)	2 (22.2)	2 (33.3)	
Black	2 (22.2)	2 (33.3)	1 (11.1)	1 (16.7)	
Hispanic	4 (44.4)	0 (0)	4 (44.4)	1 (16.7)	
Other	0 (0)	0 (0)	2 (22.2)	2 (33.3)	
Transplant Type (%)					
Double-Lung	9 (100)	6 (100)	9 (100)	6 (100)	
Single-Lung	0 (0)	0 (0)	0 (0)	0 (0)	
Heart-Lung	0 (0)	0 (0)	0 (0)	0 (0)	
HLA mismatches, mean (SD)	4.9 (1.1)	4.3 (0.8)	4.8 (1.1)	4.3 (1.4)	0.674
CMV Status (%)					0.948
Donor +, Recipient -	3 (33.3)	2 (33.3)	3 (33.3)	2 (33.3)	
Donor +, Recipient +	2 (22.2)	2 (33.3)	3 (33.3)	3 (50.0)	
Other	4 (44.4)	2 (33.3)	3 (33.3)	1 (16.7)	
Years post-transplant, mean (SD)	1.56 (2.59)	1.84 (2.50)	1.23 (1.80)	2.71 (1.78)	0.636
Bronchoscopy data					
Surveillance	6 (66.7)	3 (50.0)	8 (88.9)	1 (16.7)	0.041
Acute Symptoms	1 (11.1)	2 (33.3)	0 (0)	2 (33.3)	0.221
Fever, cough, or dyspnea	1 (11.1)	2 (33.3)	3 (33.3)	3 (50.0)	0.432
Follow up for rejection or infection	2 (22.2)	0 (0)	2 (22.2)	4 (66.7)	0.064
Treated for infection	4 (44.4)	2 (33.3)	3 (33.3)	6(100.0)	0.051
Mucoid PsA	7 (78.8)			0 (0)	0.015
PsA pre transplant (Yes/No/Unknown)	6/1/2			0/2/4	0.036
Mucoid concordant PsA (Yes/No/NA)	5/1/3			0/0/6	0.036
Acute cellular rejection, A-grade >0, (%)	1 (13)	1 (17)	2 (22)	0 (0)	0.768
Lymphocytic bronchiolitis, B-grade >0 (%)	0 (0)	0 (0)	1 (11)	0 (0)	0.557

Table ST4: DNA Methylation Cohort
(Related to Figure 3 and STAR Methods Human Subjects)

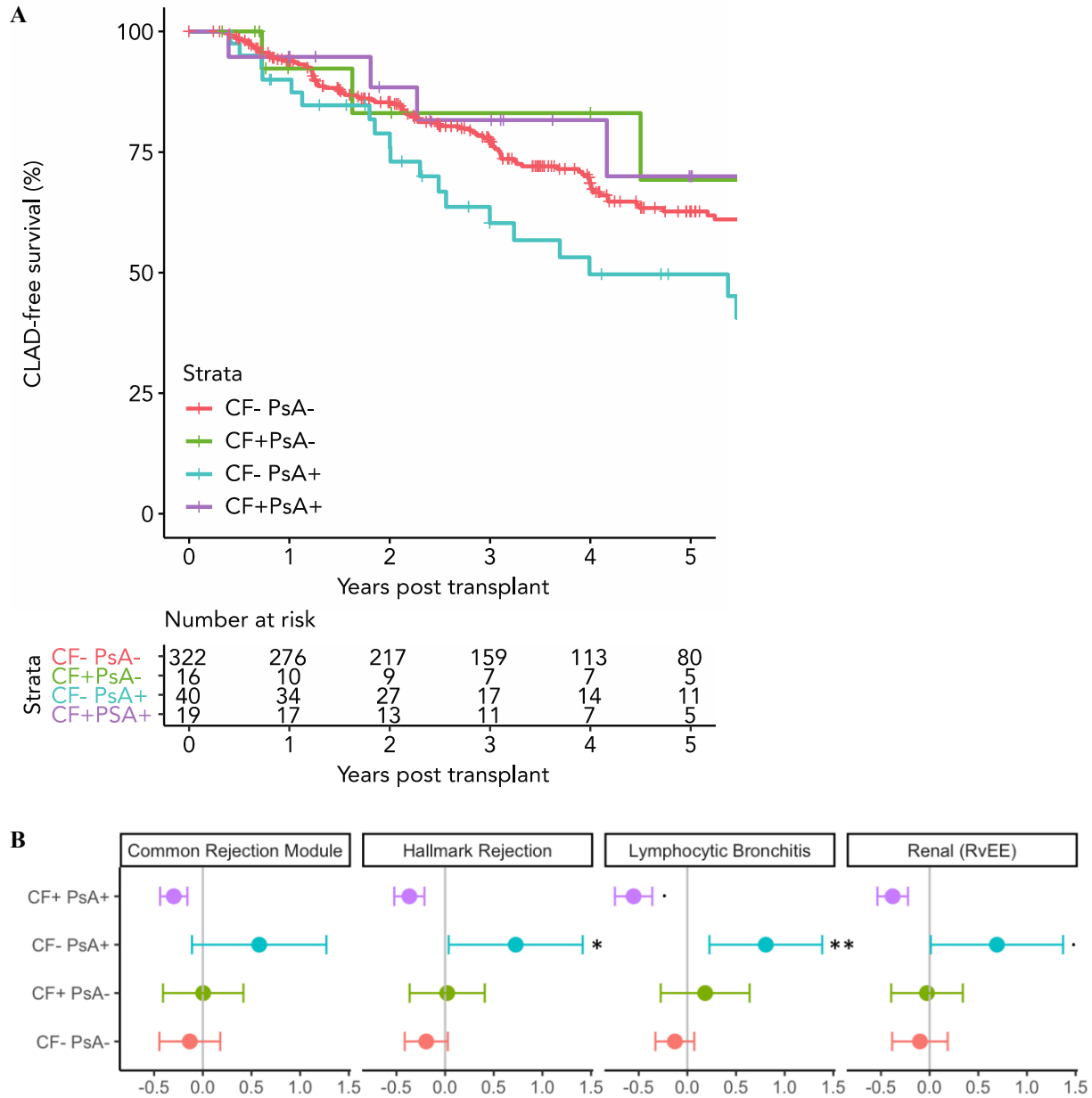
	CF	Restrictive	p-value
N	5	12	
Recipient Age, mean (SD)	25.0 (2.7)	61.2 (9.1)	<0.001
Donor Age, mean (SD)	31.4 (11.0)	37.04 (17.4)	0.723
Transplant Indication (%)			<0.001
C (CF)	5 (100)	0 (0)	
D (Restrictive)	0 (0)	12 (100)	
Recipient Gender Male (%)	1 (20.0)	8 (66.7)	0.182
Donor Gender Male (%)	4 (80.0)	9 (75.0)	0.478
Recipient Ethnicity (%)			0.632
White	4 (80.0)	10 (83.3)	
Black	1 (20.0)	0 (0)	
Hispanic	0 (0)	2 (16.7)	
Other	0 (0)	0 (0)	
Donor Ethnicity (%)			0.812
White	4 (80.0)	6 (50.0)	
Black	0 (0)	1 (8.3)	
Hispanic	1 (20.0)	3 (25.0)	
Other	0 (0)	2 (16.7)	
Transplant Type (%)			0.78
Double-Lung	5 (100)	12 (100)	
Single-Lung	0 (0)	0 (0)	
Heart-Lung	0 (0)	0 (0)	
HLA mismatches, mean (SD)	4.60 (0.89)	4.45 (1.21)	0.872
CMV Status (%)			0.492
Donor +, Recipient -	0 (0)	5 (41.7)	
Donor +, Recipient +	3 (60.0)	3 (25.0)	
Other	2 (40.0)	4 (33.3)	
<i>P. aeruginosa</i> positive	4 (80.0)	2 (16.7)	0.028

Supplement Figure SF1



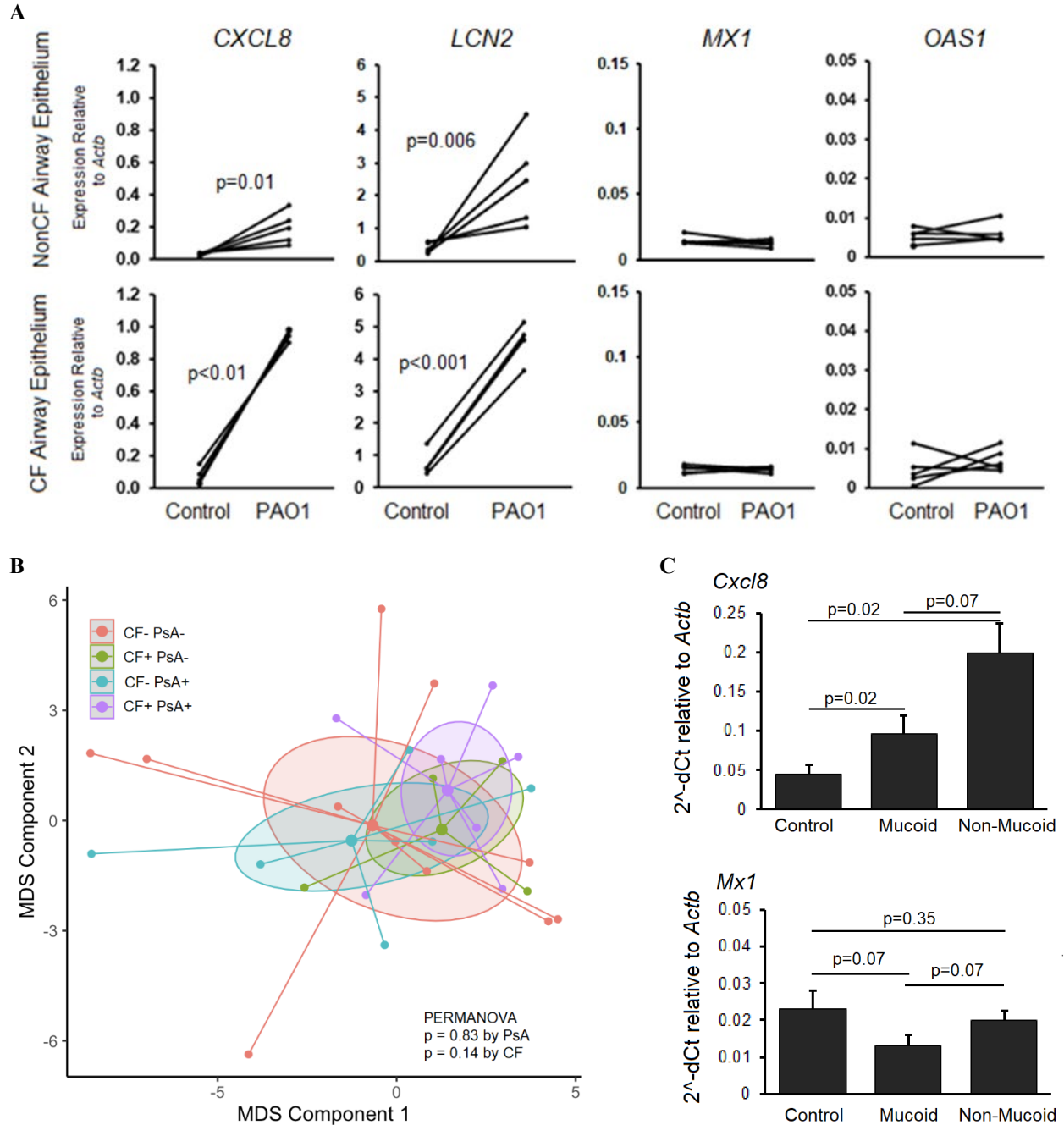
Supplement Figure SF1. CONSORT flow diagram of subject enrollment and allocation. The longitudinal cohort is shown in yellow and airway brushing cohort in green. Airway brushing was performed on consecutive consenting recipients at least 6 weeks from transplant at the provider’s discretion. Brushing was performed in 81% of eligible surveillance bronchoscopies, and 56% of cases with acute symptoms. Related to Human Subjects the STAR Methods.

Supplemental Figure SF2



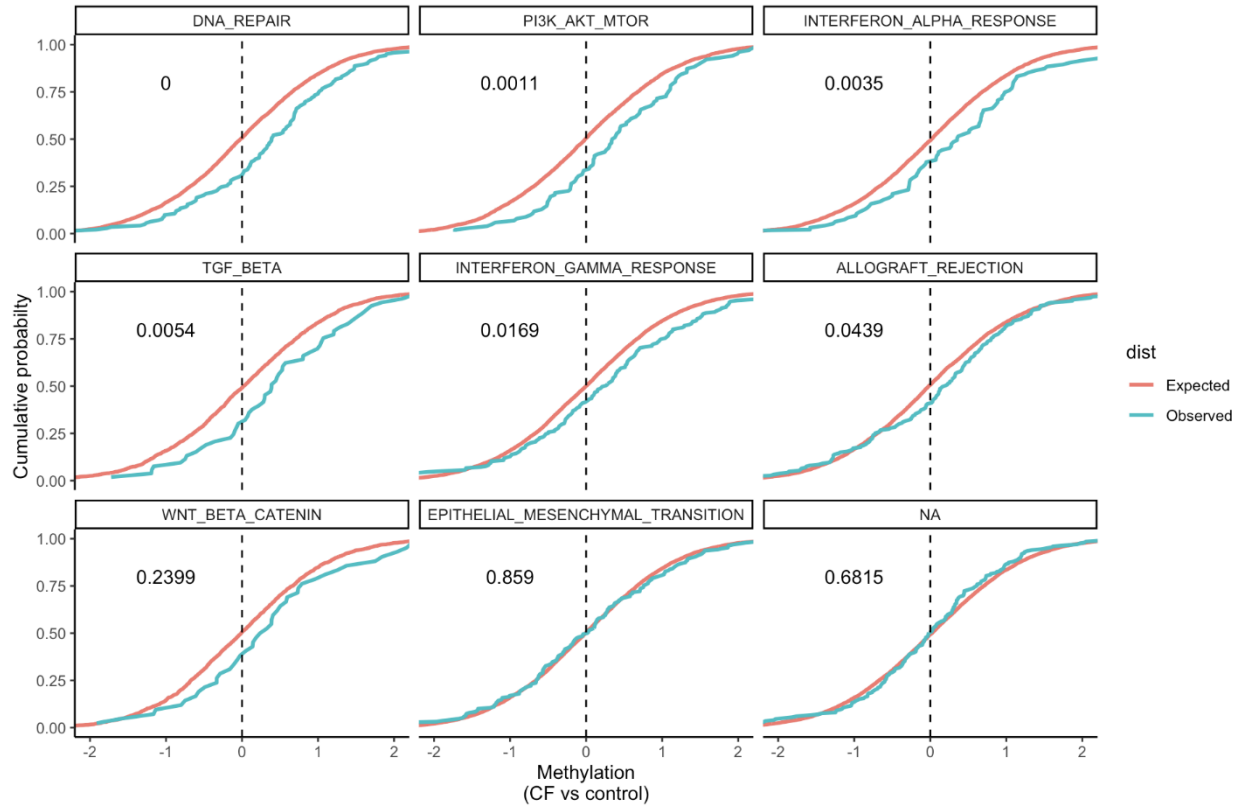
Supplemental Figure SF2. (A) Kaplan-Meier analysis of CLAD-free survival stratified by CF and PsA status. Shown is time to CLAD or death for the 397 subjects stratified by CF as the indication for lung transplantation and the presence of PsA detected in bronchoalveolar lavage fluid at any time post-transplantation. Statistics are not calculated for this model because of the time-dependent nature of PsA status. **(B) RNA-Seq gene signatures for rejection in airway brushes from subjects stratified by CF and PsA status.** Z-scores for mean and standard error of the sum of normalized counts for genes in select Broad Hallmark airway epithelial cell gene sets are shown for each group (General Linear Model; • p<0.1, * p<0.05, ** p<0.01). These figures are related to Figure 1. (CF-PsA- n=9, CF+PsA- n=6, CF-PsA+ n=6, CF+PsA+ n=9).

Supplement Figure SF3



Supplemental Figure SF3. (A) Native AEC response to PAO1, heat-killed laboratory strain of *P. aeruginosa*. PAO1 significantly induced transcription of *CXCL8* and *LCN2* in CF and non-CF AEC. The difference between control and PAO1 in *CXCL8* ($P < 0.0001$) and *LCN2* ($P = 0.02$) induction was greater in the CF as compared to non-CF AEC. Neither *MX1* nor *OAS1* were induced by PAO1. Expression is $2^{-\Delta\text{Ct}}$ relative to *ACTB*. Related to Figure 2. (n=5 control, n=5 PAO1 paired analysis). **(B and C) PsA Characteristics in CF and Lung Transplantation. (B)** MDS plot showing comparisons of Beta-Diversity measure by CF and PsA status (CF-PsA- n=9, CF+PsA- n=6, CF-PsA+ n=6, CF+PsA+ n=9). **(C)** Control media, clinical mucooid PsA, or clinical non-mucooid PsA used to stimulate normal ALI AEC for 24hr before RT-PCR measurement of relative *CXCL8* and *MX1* expression referenced to *ACTB* ($2^{-\Delta\text{Ct}}$). Mucoidity of PsA did not result in differential responses *in vitro* (n=5 control, n=5 mucooid, n=5 non-mucooid paired analysis). Related to Figure 4.

Supplement Figure SF4



Supplemental Figure SF4. Kolmogorov-Smirnoff plot for expected DNA methylation distribution. Promoters of several curated gene sets and a random sample (NA) of genes showed a distribution that matched the expected distribution while other pathways were found to be hypermethylated in CF subjects. A random sampling of genes showed no difference in promoter DNA methylation between CF (n=5) and non-CF (n=12) subjects. Related to Figure 3.