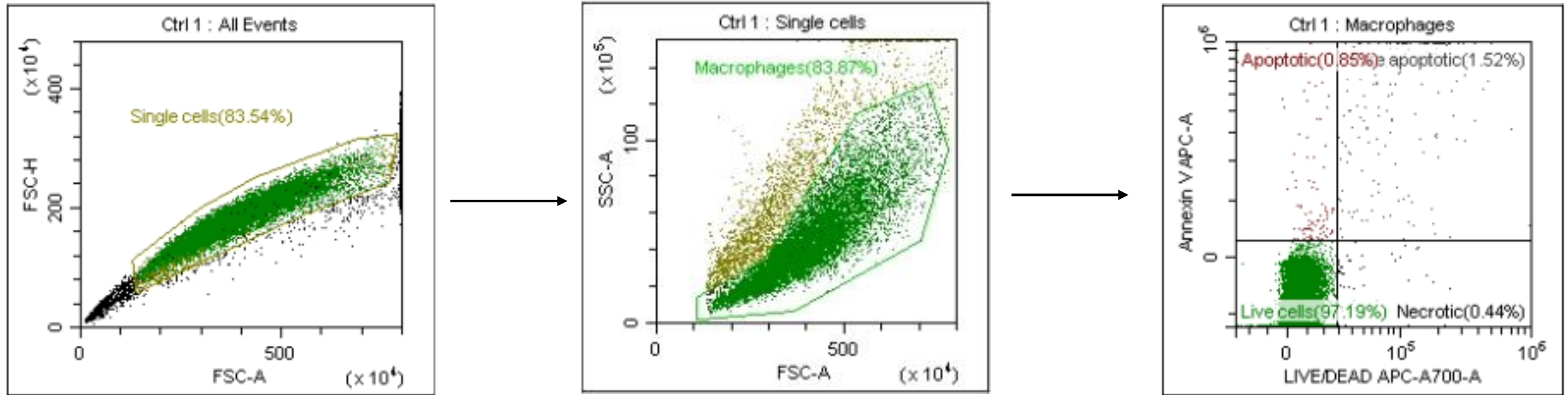


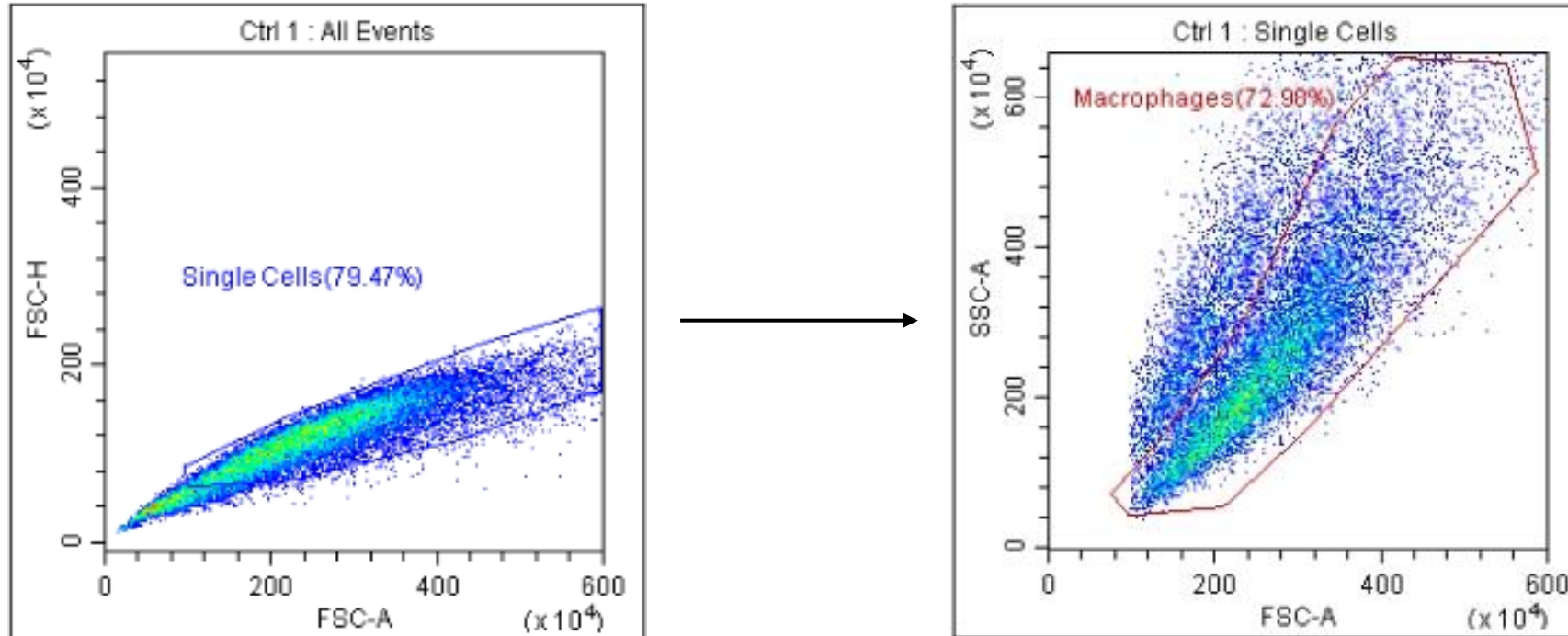
Supplementary table 1. Flow cytometry markers

Marker	Fluorophore	Function	Clone	Manufacturer
CD80	Alexa Fluor® 488	M1	2D10	Biolegend
HLA-DR	PE-Cy7	M1	G46-6/L243	BD Biosciences
CD40	BV510	M1	5C3	BD Biosciences
PDL1	BV421	M1	MIH1	BD Biosciences
CD86	BV650	M1	FUN-1	BD Biosciences
MerTK	PE	M2	590H11G1E3	Biolegend
CD16	PerCP/Cyanine5.5	M2	3G8	Biolegend
CD163	PE-CF594	M2	GHI/61	BD Biosciences
CD206	APC/Fire™ 750	M2	15-2	Biolegend
LIVE/DEAD	Scarlet	Necrosis		Invitrogen
Annexin V	APC	Apoptosis		BD Biosciences



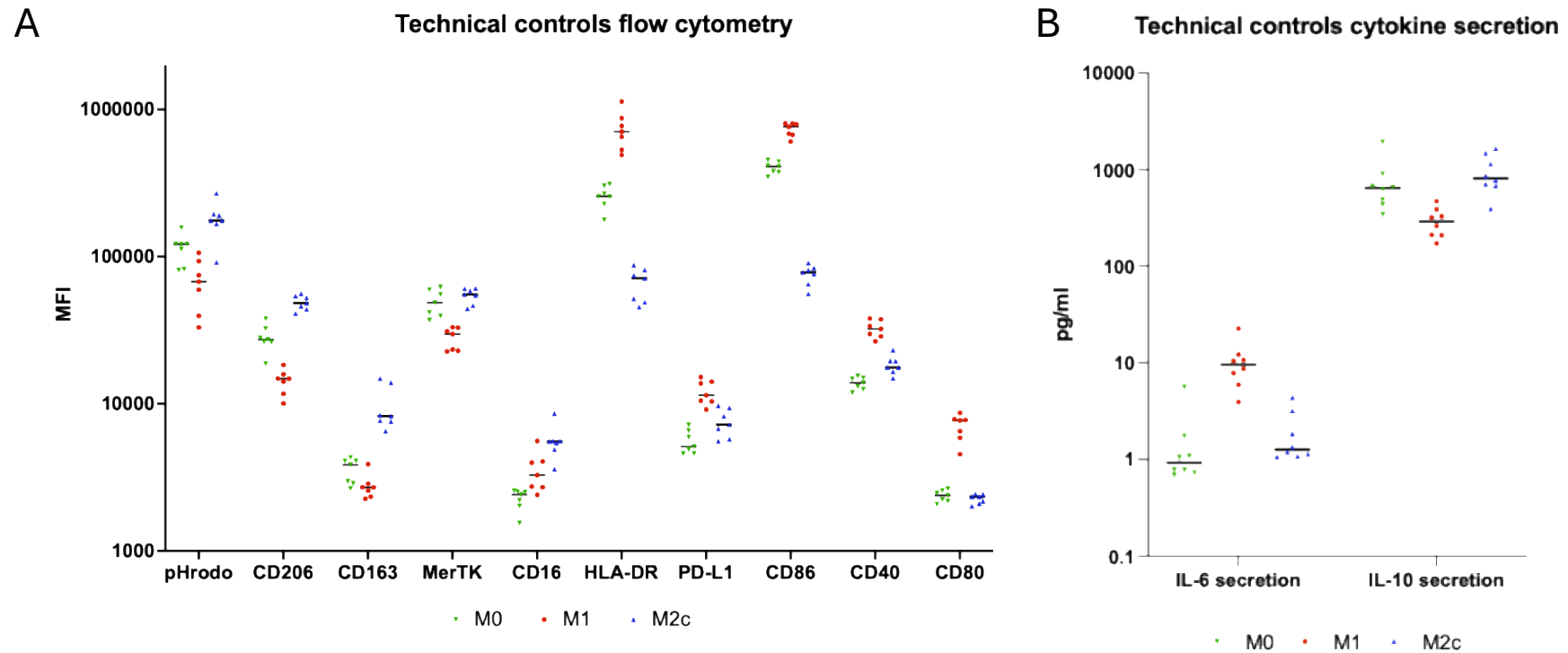
Supplementary figure 1. Gating strategy surface markers

Healthy control 1 used as an example. Duplet exclusion was attained by FSC-H/FSC-A, gating Single cells. Macrophages were gated by analyzing Single cells by SSC-A/FSC-A. Apoptotic, late apoptotic and necrotic cells within the Macrophages population were excluded by gating away cells with higher levels of Annexin V and/or LIVE/DEAD. The remaining macrophages were deemed Live cells. Surface markers were measured in MFI (Median Fluorescent Intensity) in the Live cells group.



Supplementary figure 2. Gating strategy phagocytosis

Healthy control 1 used as an example. Duplet exclusion was attained by FSC-H/FSC-A, gating Single cells. Macrophages were gated by analyzing Single cells by SSC-A/FSC-A. MFI (Median Fluorescent Intensity) of pHrodo was measured in the Macrophages group.



Supplementary figure 3. Technical controls

Seven separate experiments were performed to stimulate macrophages with sputum samples from PCD patients and healthy controls. The figure shows technical controls M0, M1 and M2c from all seven occasions. (A) Flow cytometry analysis of surface marker expression after polarization. (B) Measurements of cytokine secretion from polarized macrophages.

Supplementary table 2. Pathogens in regular sputum culture	PCD	
	Children (n=10)	Adults (n=17)
<i>Haemophilus influenzae</i> n	4	8
<i>Pseudomonas aeruginosa</i> n	0	5
<i>Staphylococcus aureus</i> n	2	1
<i>Moraxella catarrhalis</i> n	1	2
<i>Klebsiella pneumoniae</i> n	1	0
<i>Streptococcus equi</i> n	1	0
<i>Klebsiella oxytoca</i> group n	0	1
<i>Enterobacter cloacae</i> complex n	0	1
<i>Aspergillus species</i> n	1	0
<i>Aspergillus niger</i> complex n	0	1
<i>Aspergillus fumigatus</i> n	0	2
<i>Exophiala dermatitidis</i> n	0	1
No relevant pathogens n	4	2
No culture n	0	2

Supplementary table 3. Sputum characteristics comparing sputum samples with positive and negative sputum culture	Positive sputum culture		Negative sputum culture		Mann-Whitney (p)	Spearman's correlation to 16S DNA	
	Median	Range	Median	Range		p	r (95 % confidence interval)
16S DNA (ng/μl)	1.473	0.10-3.16	9.665	0.19-52.44	0.036		
IL-1β (pg/ml)	670.7	104.8-3840	7668	29.73-59127	0.026	0.0001	0.78 (0.57-0.90)
IL-6 (pg/ml)	430.8	62.6-20000	9.921	0.128-1381	0.0069	0.026	-0.47 (-0.72 - -0.093)

MFI = Median Fluorescent Intensity

Supplementary table 4. Baseline clinical characteristic comparing children to adults with PCD

	Controls (n=7)	PCD (n=27)		Mann-Whitney (p)
		Children (n=10)	Adults (n=17)	
Median age, years (range)	46 (29-62)	10.8 (7.6-17.7)	29.6 (18.7-60.0)	
Female n (%)	5 (71)	5 (50)	9 (53)	
FEV1pp median (range)		90 (75-116)	60 (19-99)	0.0004
Presence of bronchiectasis n (%) ^a		6 (60)	16 (94)	0.047
Genetically confirmed diagnosis n (%)		10 (100)	11 (65)	0.057
Clinical diagnosis or unknown n (%)		0 (0)	6 (35)	0.057
Ongoing exacerbation n (%)		1 (10)	3 (18)	0.99
Positive sputum culture n (%)		6 (60)	13 (87)	0.18
- <i>Haemophilus influenzae</i> n (%)		4 (40)	8 (53)	
- <i>Pseudomonas aeruginosa</i> n (%)		0 (0)	5 (33)	
- <i>Staphylococcus aureus</i> n (%)		2 (20)	1 (7)	
Inhaled cortisone treatment n (%)		8 (80)	7 (41)	0.11
Presence of antibiotic treatment n (%)		3 (30)	7 (41)	0.69

FEV1pp = Forced expiratory volume in one second in percent of predicted

^a Diagnosed by chest CT (n=20) or chest tomosynthesis (n=7)

Supplementary table 5. Surface markers comparing macrophages stimulated with healthy sputum vs PCD sputum	Healthy controls (n=7)		PCD patients (n=27)		Mann-Whitney (p)
	Median	Range	Median	Range	
CD163 (MFI)	4155	2675-9049	11882	2388-104460	0.024
CD16 (MFI)	5901	2613-18251	23677	2596-52764	0.0032
CD206 (MFI)	19648	14519-42278	38153	11662-62815	0.044
MerTK (MFI)	43973	36812-54657	47664	19579-105296	0.43
CD80 (MFI)	8568	3844-12765	4672	2303-8777	0.035
CD40 (MFI)	23657	16268-40279	18138	12177-27425	0.013
HLA-DR (MFI)	78185	62066-241678	166092	42567-723030	0.11
PD-L1 (MFI)	8492	6399-13580	11462	4010-24652	0.12
CD86 (MFI)	521516	190659-873272	343892	102085-805254	0.50

MFI = Median Fluorescent Intensity

Supplementary table 6. Correlation of sputum cytokines to macrophage functions	IL-1 β (pg/ml)			IL-6(pg/ml)			16S DNA		
	r	95 % confidence interval	p	r	95 % confidence interval	p	r	95 % confidence interval	p
Phagocytosis (MFI pHrodo)	0.73	0.52 - 0.86	<0.0001	-0.32	-0.60 - 0.033	0.067	0.47	0.14 - 0.70	0.0054
IL-1 β secretion (pg/ml)	-0.42	-0.67 - -0.083	0.01	0.24	-0.12 - 0.54	0.17	-0.16	-0.48 - 0.19	0.35
IL-6 secretion (pg/ml)	-0.29	-0.58 - 0.069	0.10	0.16	-0.20 - 0.48	0.38	-0.062	-0.40 - 0.29	0.73
IL-10 secretion (pg/ml)	0.06	-0.30 - 0.40	0.75	-0.11	-0.44 - 0.25	0.55	0.11	-0.24 - 0.44	0.52

MFI = Median Fluorescent Intensity

Supplementary table 7. Effects of treatment regimens and age on macrophage polarization		CD163 (MFI)	CD16 (MFI)	CD206 (MFI)	CD80 (MFI)	CD40 (MFI)	Phagocytosis (MFI pHrodo)	IL-1 β secretion (pg/ml)	IL-6 secretion (pg/ml)
No inhaled corticosteroids (n=8)	Median	11616	24876	31044	4636	17344	203626	0.75	9.175
	Range	2898-36589	6016-44380	11662-44038	2303-8777	12177-23612	97752-300876	0.75-2.70	1.05-23.87
Inhaled corticosteroids (=19)	Median	12886	23290	46606	4672	18243	161361	0.75	12.02
	Range	2388-104460	2596-52764	23043-62815	2423-7681	13183-27425	42506-297341	0.75-8.271	1.68-101.3
	Mann-Whitney (p)	0.94	0.9	0.025	0.83	0.28	0.49	0.83	0.22
No Azithromycin (n=21)	Median	12886	22794	29265	4699	18138	177244	0.75	10.38
	Range	2388-104460	2596-52764	11662-62815	2303-8777	12177-23612	42506-300876	0.75-8.27	1.05-101.3
Azithromycin (n=6)	Median	10233	26175	50164	4638	18624	220168	0.75	14
	Range	3487-24638	3858-48296	16501-54828	2680-6198	14691-27425	79291-275671	0.75-2.33	2.11-60.67
	Mann-Whitney (p)	0.75	0.41	0.16	0.75	0.48	0.29	0.58	0.89
Children (n=10)	Median	12118	23431	44288	5761	18960	139466	2.369	30.5
	Range	2959-87319	4283-44380	16501-58483	2971-7175	15986-27425	42506-300876	0.75-8.271	4.779-101.3
Adults (n=17)	Median	11882	23677	36259	4573	17971	196837	0.75	5.504
	Range	2388-104460	2596-52764	11662-62815	2303-8777	12177-25340	79291-297341	0.75-2.579	1.053-60.67
	Mann-Whitney (p)	0.68	0.79	0.57	0.36	0.22	0.19	0.015	0.0004

MFI = Median Fluorescent Intensity