Supplemental Materials

Pseudomonas aeruginosa modulates neutrophil granule exocytosis in an *in vitro* model of airway infection

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Measured			
GM-CSF	granulocyte-macrophage colony-stimulating factor	**IP-10**	interferon gamma-induced protein 10
SCF	stem cell factor	**PDGF-BB**	platelet-derived growth factor B subunits
GRO-α	growth-regulated oncogene-alpha	**VEGF**	vascular endothelial growth factor
MIF	macrophage migration inhibitory factor	**IL2-RA**	interleukin-2 receptor alpha chain
IL-6	interleukin 6	**IL-16**	interleukin 16
TRAIL	TNF-related apoptosis-inducing ligand	**β-NGF**	beta-nerve growth factor
IL-9	interleukin 9	**Eotaxin**	N/A
TNF-β	tumor necrosis factor beta	**CTACK**	cutaneous T cell-attracting chemokine
MIP-1β	macrophage inflammatory protein-1 beta	**IFN-α2**	interferon alpha-2
SDF-1α	stromal cell-derived factor-1 alpha	**IFN-y**	interferon gamma
IL-2	interleukin 2	**LIF**	leukemia inhibitory factor
bFGF	basic fibroblast growth factor	**MIG**	monokine induced by gamma interferon
HGF	hepatocyte growth factor	**RANTES**	regulated on activation, normal T cell expressed and secreted
TNF-α	tumor necrosis factor alpha	**IL-8**	interleukin 8
M-CSF	macrophage colony-stimulating factor	**IL-15**	interleukin 15
IL-18	interleukin 18	**MIP-1 α**	macrophage inflammatory protein-1 alpha
IL-1α	interleukin 1 alpha	**MCP-1**	monocyte chemoattractant protein-1
IL-1β	interleukin 1 beta	**G-CSF**	granulocyte colony stimulating facto
IL-1ra	interleukin 1 receptor antagonist	**SCGF-β**	stem cell growth factor beta

Undetectable			
IL-3	interleukin 3	**IL-12 p40**	interleukin 12 subunit beta
IL-4	interleukin 4	**IL-12 p70**	interleukin 12
IL-5	interleukin 5	**IL-13**	interleukin 13
IL-7	interleukin 7	**IL-17**	interleukin 17
IL-10	interleukin 10	**MCP-3**	monocyte chemotactic protein-3

Supplementary table 1. Cytokine abbreviations

Baseline (Uninfected) pg mL^{-1}																		
	GM-CSF	**SCF***	**GRO-α**	**MIF**	**IL-6**	**TRAIL**	**IL-9**	**TNF-β**	**MIP-1β**	**SDF-1α**	**PDGF-BB**	**VEGF**	**IL2-RA**	**IL-16**	**β-NGF**	**Eotaxin**	**CTACK**	**IFN-α2**	**IFN-γ**
Non-CF	44.3 ± 20.2	110.7 ± 22.4	84062.5 ± 83675.6	12094.8 ± 4082.6	1546.5 ± 743.4	2234.3 ± 952.9	39.0 ± 7.2	4.6 ± 1.1	14.2 ± 2.0	66.7 ± 9.8	37.9 ± 3.7	213.4 ± 48.8	16.6 ± 1.4	54.1 ± 4.2	2.4 ± 0.7	0.4 ± 0.1	3.3 ± 0.9	9.2 ± 4.9	18.8 ± 11.2
CF	120.0 ± 43.1	68.9 ± 14.6	286043.7 ± 19344.2	10771.7 ± 1155.9	537.1 ± 381.6	858.3 ± 367.8	34.6 ± 8.0	4.6 ± 0.4	13.9 ± 4.9	56.4 ± 7.2	34.2 ± 5.2	199.9 ± 13.6	12.3 ± 2.6	50.1 ± 5.4	2.3 ± 0.1	0.5 ± 0.2	2.5 ± 1.6	5.1 ± 1.4	35.9 ± 7.7
	LIF	**IL-2**	**bFGF**	**HGF**	**TNF-α**	**M-CSF***	**IL-18***	**IL-1α**	**IL-1β**	**IL-1ra**	**IP-10**	**MIG**	**RANTES**	**IL-8**	**IL-15**	**MIP-1 α**	**MCP-1**	**G-CSF**	**SCGF-β**
Non-CF	152.8 ± 50.8	3.1 ± 1.3	13.7 ± 2.8	11.3 ± 3.4	30.4 ± 9.6	236.5 ± 94.9	16.4 ± 6.3	20.6 ± 7.7	5.2 ± 0.5	2313.0 ± 2128.4	254.6 ± 121.3	13.6 ± 8.4	17.4 ± 12.3	2316.2 ± 535.2	383.0 ± 16.7	0.5 ± 0.1	136.1 ± 97.2	1757.5 ± 838.6	3433.6 ± 1513.1
CF	80.9 ± 59.4	3.2 ± 1.2	13.1 ± 1.3	12.2 ± 1.6	45.2 ± 30.6	61.8 ± 17.9	6.5 ± 1.6	23.9 ± 9.9	4.4 ± 0.9	4853.9 ± 1999.1	268.2 ± 59.0	20.2 ± 5.9	29.9 ± 10.7	1319.4 ± 396.9	402.0 ± 35.2	2.8 ± 5.2	141.2 ± 242.7	952.0 ± 881.5	2824.1 ± 1494.3

(*p<0.05 non-CF vs CF uninfected washes, t-test)

Supplementary table 2. Baseline epithelial cytokine concentrations in pg mL^{-1} (mean ± SD)

Compound	Description	Use	FDA Approval	Concentration	Solvent	Reference
Azithromycin	macrolide antibiotic	antimicrobial and anti-inflammatory	Yes	1 μg mL^{-1}	DMSO	1
IL-1Ra	recombinant protein	IL-1α and β antagonist	Yes	2 μg mL^{-1}	ddH_2O + 0.1% BSA	2
Infliximab	monoclonal antibody	TNF-α inhibitor	Yes	50 μg mL^{-1}	ddH_2O	3
LPS-RS	LPS from *R. sphaeroides*	TLR4 antagonist	No	5 μg mL^{-1}	ddH_2O	4
TAK 242	small molecule	TLR4 inhibitor	No	20 nM	DMSO	5
MCC950	small molecule	NLRP3 inhibitor	No	1 μM	DMSO	6
S(+)-ibuprofen	biologically active enantiomer of ibuprofen	COX-1 and 2 inhibitor	Yes	1 mM	DMSO	7

Supplementary table 3. Compounds for anti-GRIM drug screen

1) Ling KM, Hillas J, Lavender MA, Wrobel JP, Musk M, Stick SM, et al. Azithromycin reduces airway inflammation induced by human rhinovirus in lung allograft recipients. Respirology. 2019;24(12):1212-9.

2) Chen G, Sun L, Kato T, Okuda K, Martino MB, Abzhanova A, et al. IL-1beta dominates the promucin secretory cytokine profile in cystic fibrosis. J Clin Invest. 2019;129(10):4433-50.

3) Zhang C, Shu W, Zhou G, Lin J, Chu F, Wu H, et al. Anti-TNF-alpha Therapy Suppresses Proinflammatory Activities of Mucosal Neutrophils in Inflammatory Bowel Disease. Mediators Inflamm. 2018;2018:3021863.

4) Forrest OA, Ingersoll SA, Preininger MK, Laval J, Limoli DH, Brown MR, et al. Frontline Science: Pathological conditioning of human neutrophils recruited to the airway milieu in cystic fibrosis. J Leukoc Biol. 2018;104(4):665-75.

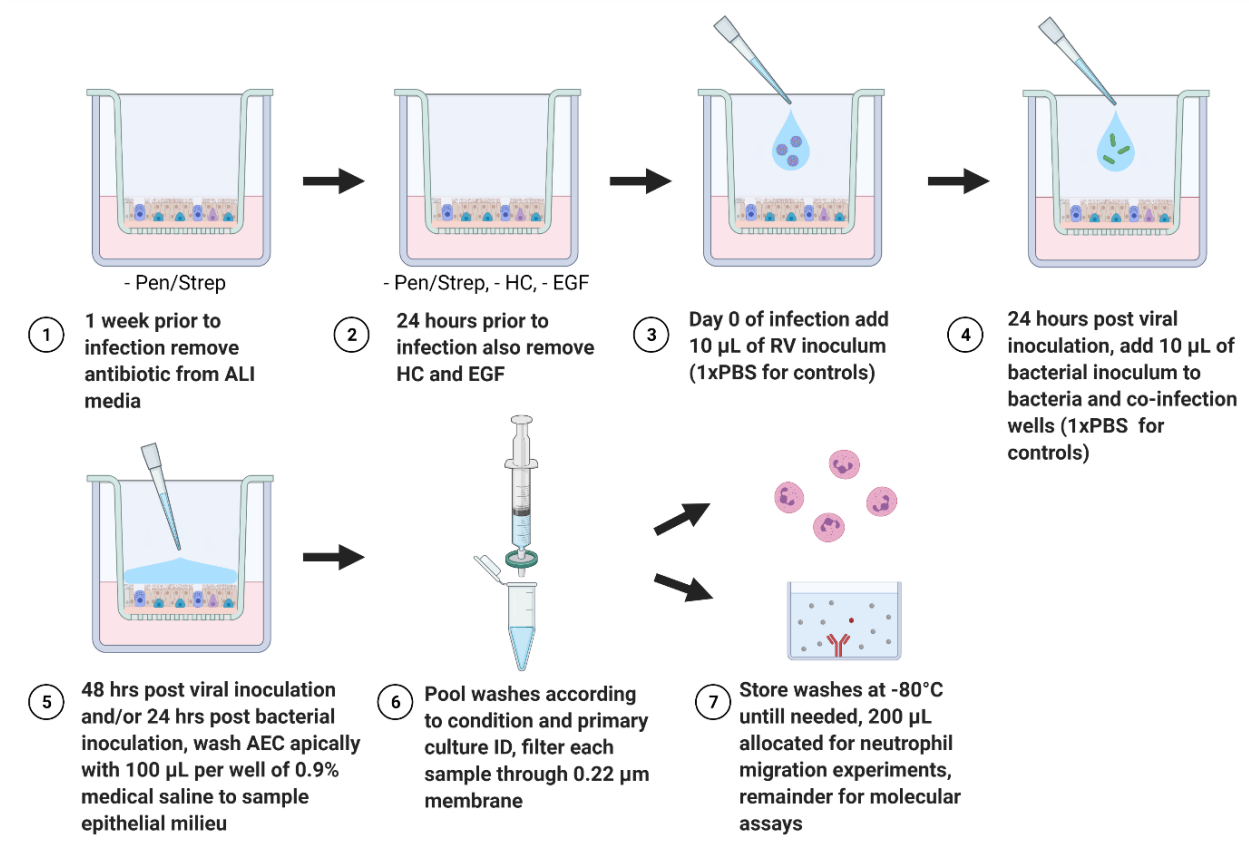
5) He GY, Zhao CH, Wu DG, Cheng H, Sun LA, Zhang DL, et al. S100A8 Promotes Inflammation via Toll-Like Receptor 4 After Experimental Traumatic Brain Injury. Front Neurosci. 2020;14:616559.

6) Coll RC, Robertson AA, Chae JJ, Higgins SC, Munoz-Planillo R, Inserra MC, et al. A small-molecule inhibitor of the NLRP3 inflammasome for the treatment of inflammatory diseases. Nat Med. 2015;21(3):248-55.

7) Hanna N, Graboski S, Laskin DL, Weinberger B. Effects of ibuprofen and hypoxia on neutrophil apoptosis in neonates. Biol Neonate. 2004;86(4):235-9.

Primer	Sequence	Annealing Temp (°C)	Amplicon Size (bp)
Rhinovirus 5'UTR Fwd	CCTCCGGCCCCTGAAT	60	316
Rhinovirus 5'UTR Rev	AAACACGGACACCCAAAGTAGT	60	
P. aeruginosa 16S rRNA Fwd	GGAGAAAGTGGGGGATCTTC	60	117
P. aeruginosa 16S rRNA Rev	CCGGTGCTTATTCTGTTGGT	60	

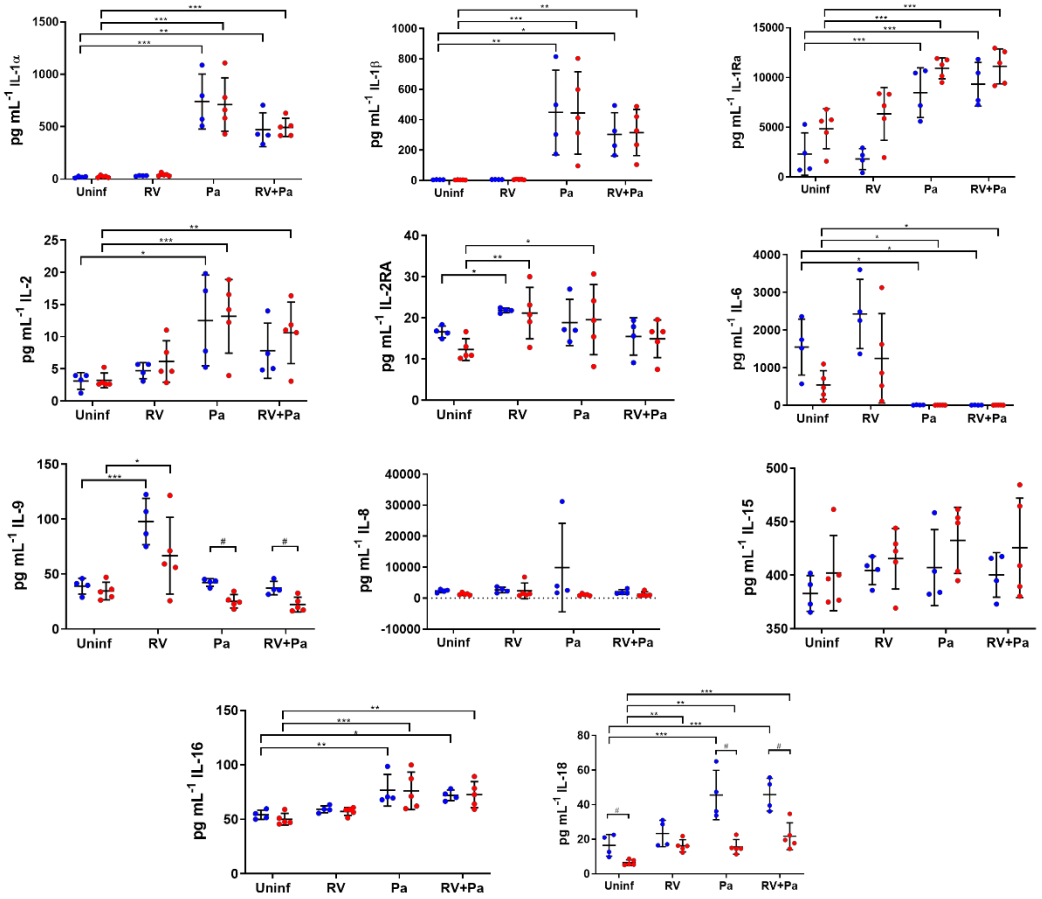
Supplementary table 4. Primers for RV and Pa quantification



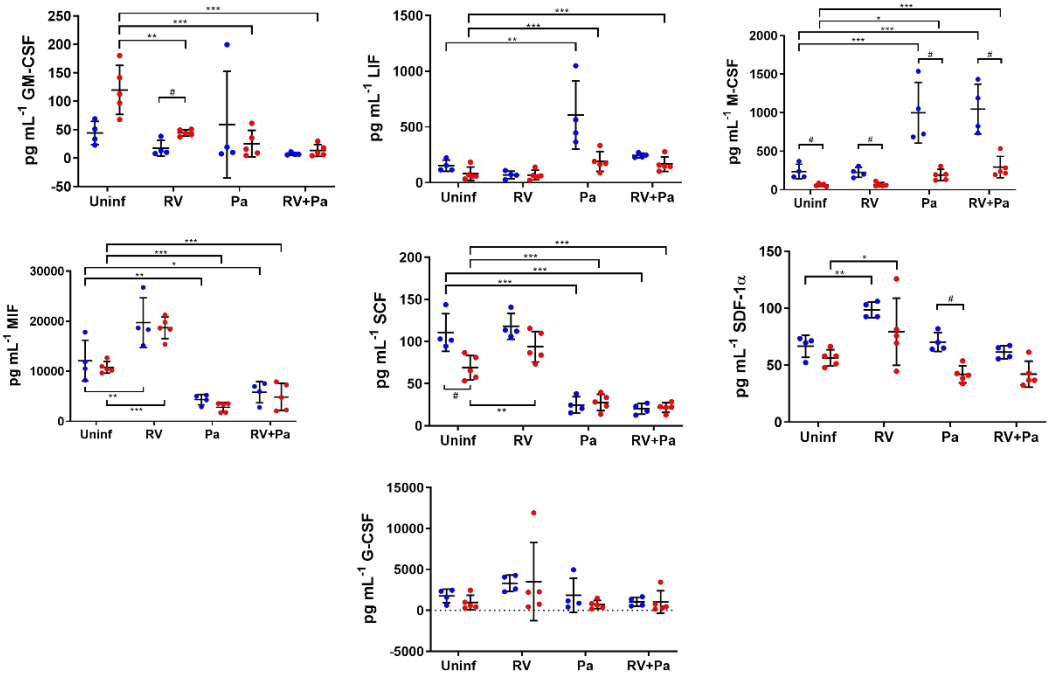
Supplementary figure 1. Timeline of *in vitro* AEC infections (Created with BioRender.com)

(AEC) airway epithelial cell, (ALI) air liquid interface, (Pen/Strep) penicillin/streptomycin, (HC) hydrocortisone, (EGF) epidermal growth factor, (PBS) Phosphate Buffered Saline

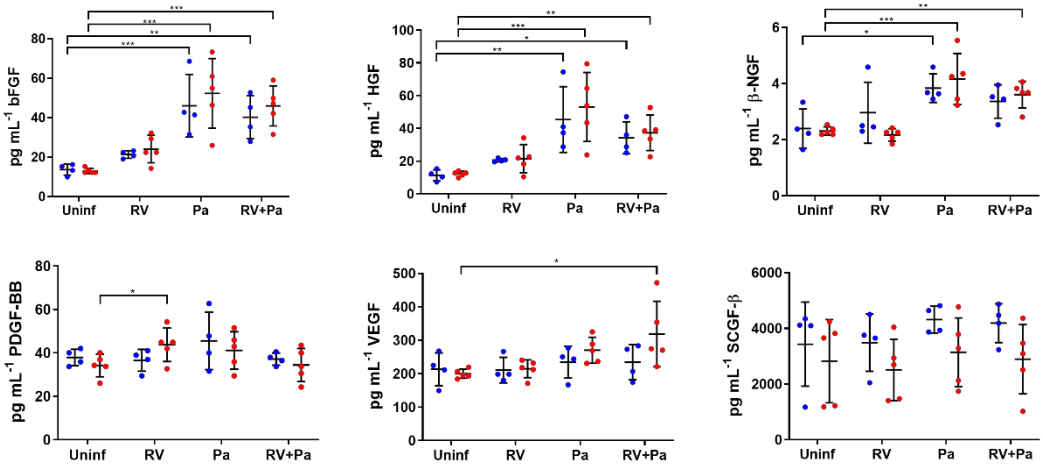
(a)


pg mL-1 IL-1α
pg mL-1 IL-1β
pg mL-1 IL-1Ra
pg mL-1 IL-2
pg mL-1 IL-2RA
pg mL-1 IL-6
pg mL-1 IL-9
pg mL-1 IL-8
pg mL-1 IL-15
pg mL-1 IL-16
pg mL-1 IL-18
Uninf
RV
Pa
RV+Pa

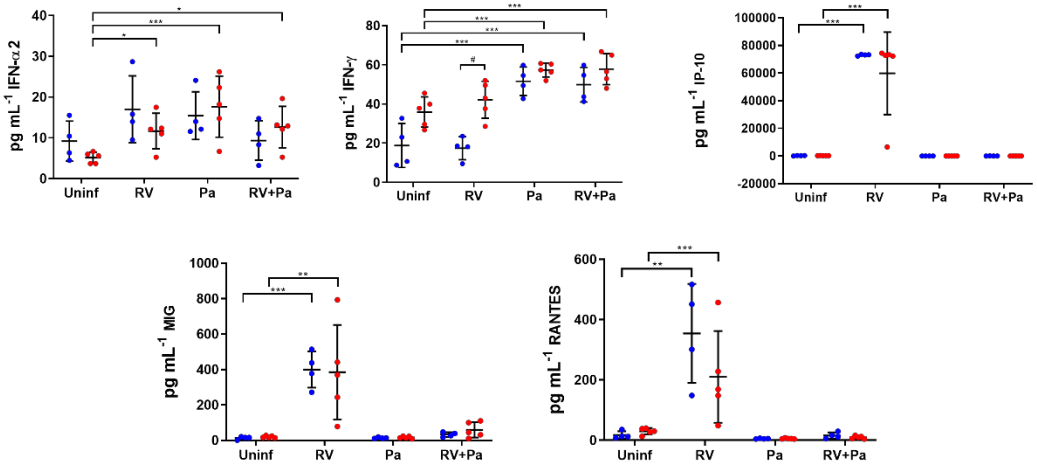
(b)


pg mL-1 GM-CSF
pg mL-1 LIF
pg mL-1 M-CSF
pg mL-1 MIF
pg mL-1 SCF
pg mL-1 SDF-1α
pg mL-1 G-CSF
Uninf
RV
Pa
RV+Pa

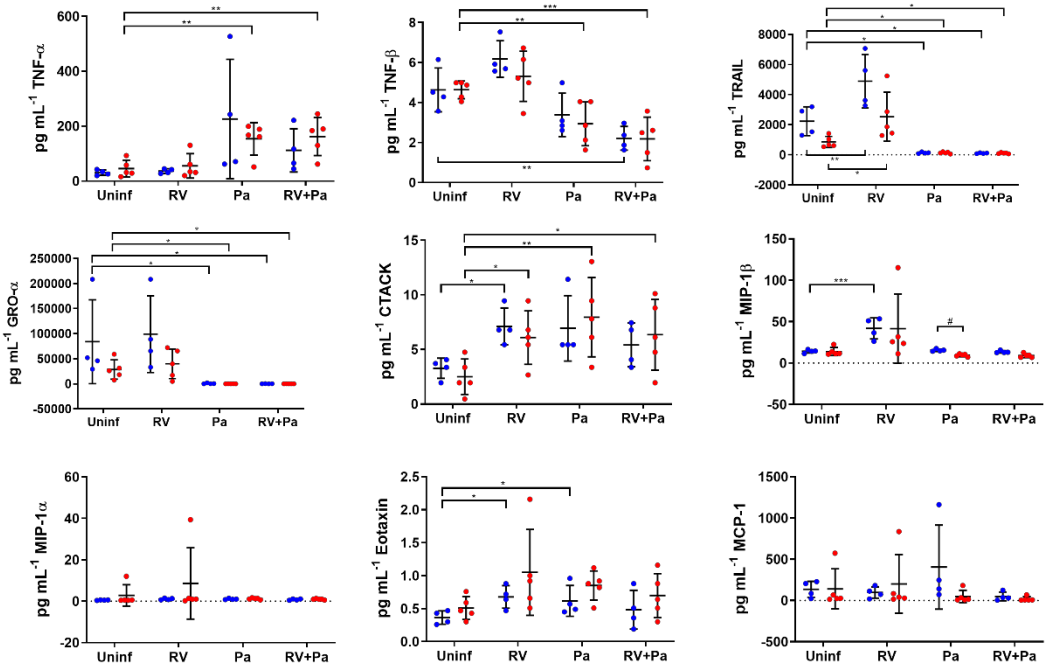
(c)


pg mL-1 bFGF
pg mL-1 HGF
pg mL-1 β-NGF
pg mL-1 PDGF-BB
pg mL-1 VEGF
pg mL-1 SCGF-β
Uninf
RV
Pa
RV+Pa

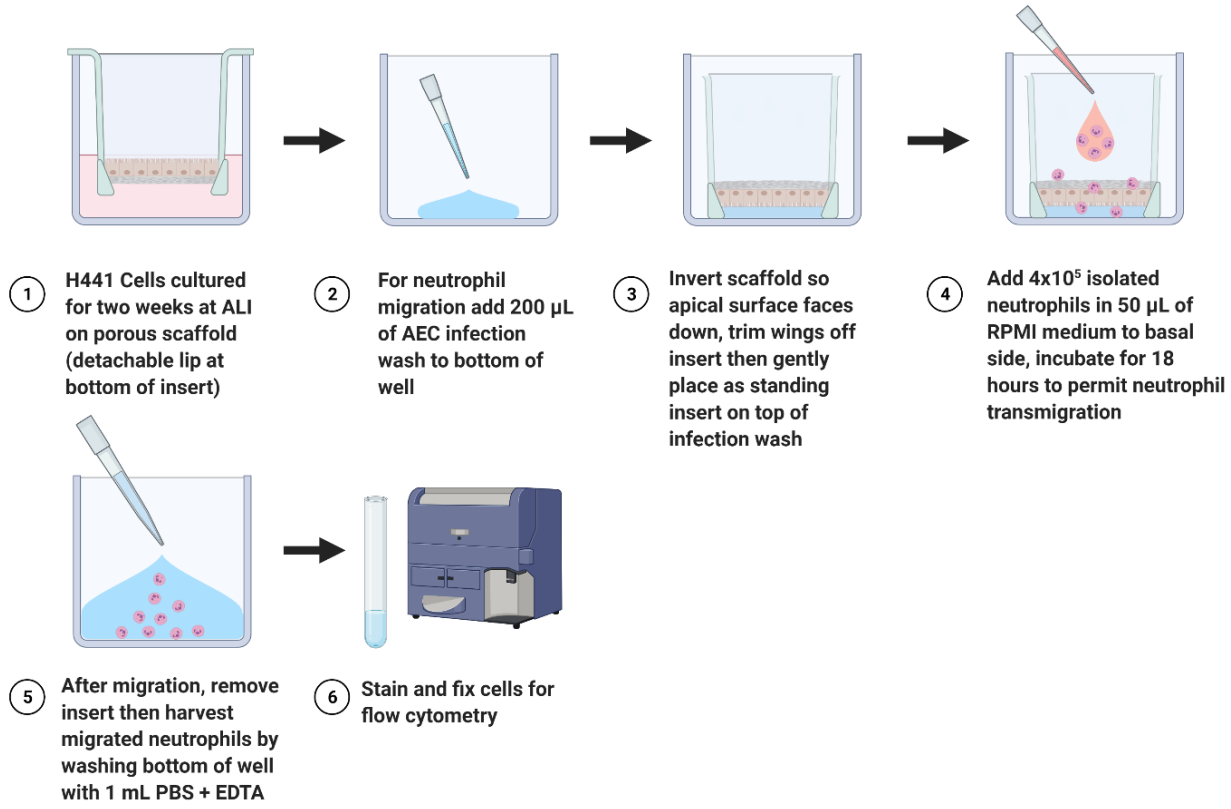
(d)


pg mL-1 IFN-α2
pg mL-1 IFN-γ
pg mL-1 IP-10
pg mL-1 MIG
pg mL-1 RANTES
Uninf
RV
Pa
RV+Pa

(e)

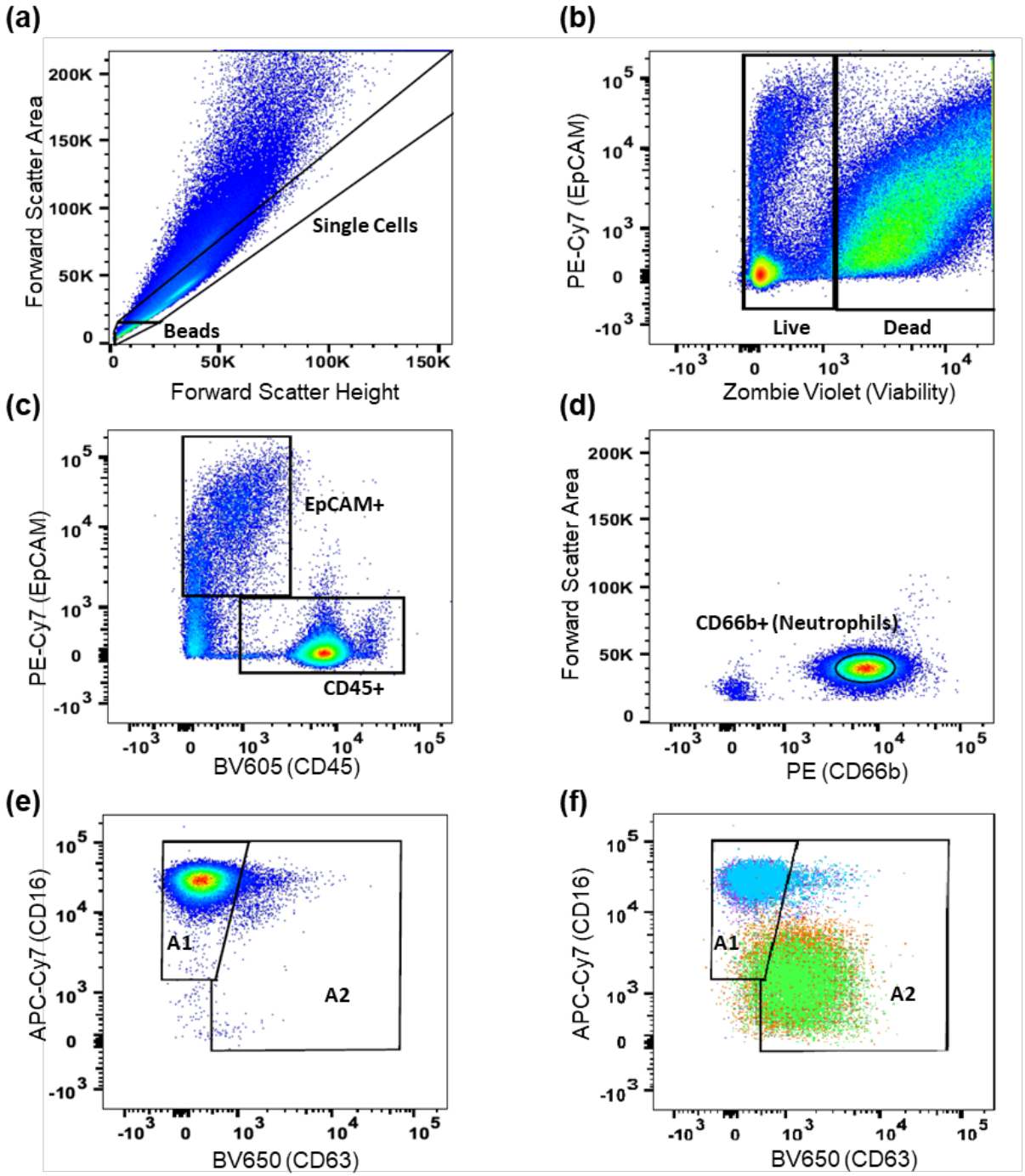

pg mL-1 TNF-α
pg mL-1 TNF-β
pg mL-1 TRAIL
pg mL-1 GRO-α
pg mL-1 CTACK
pg mL-1 MIP-1β
pg mL-1 MIP-1α
pg mL-1 Eotaxin
pg mL-1 MCP-1
Uninf
RV
Pa
RV+Pa

Supplementary figure 2. Cytokine concentrations in apical washes of infected and control ALI cultures. Types of cytokines include **(a)** interleukins, **(b)** various stimulatory and inhibitory factors, **(c)** growth factors, **(d)** cytokines associated with antiviral responses, and **(e)** TNF associated cytokines, activators of monocytes, macrophages, and eosinophils, as well as other inflammatory modulators. Results from non-CF cultures are shown in blue, and CF cultures in red. Each point is indicative of results from a single epithelial cell donor (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ compared to uninfected controls, RM one-way ANOVA; $^{\#}P < 0.05$ non-CF vs CF, *t*-test).

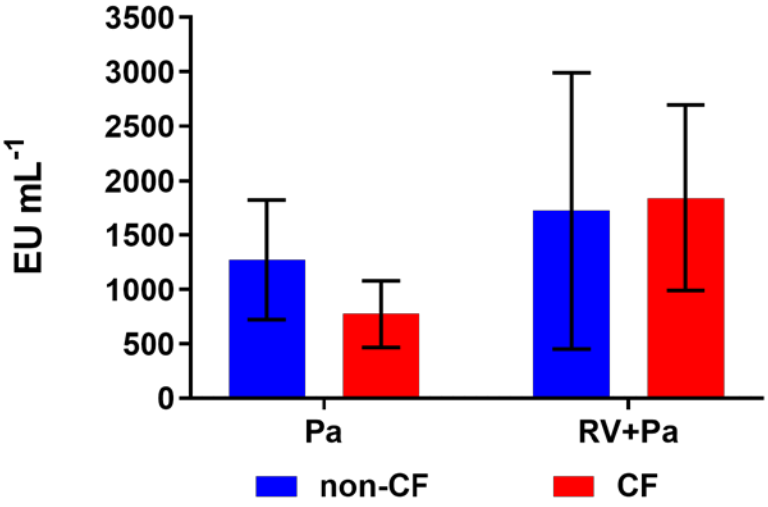


Supplementary figure 3. Neutrophil Transmigration Assay (Created with BioRender.com)

(ALI) air liquid interface, (AEC) airway epithelial cell

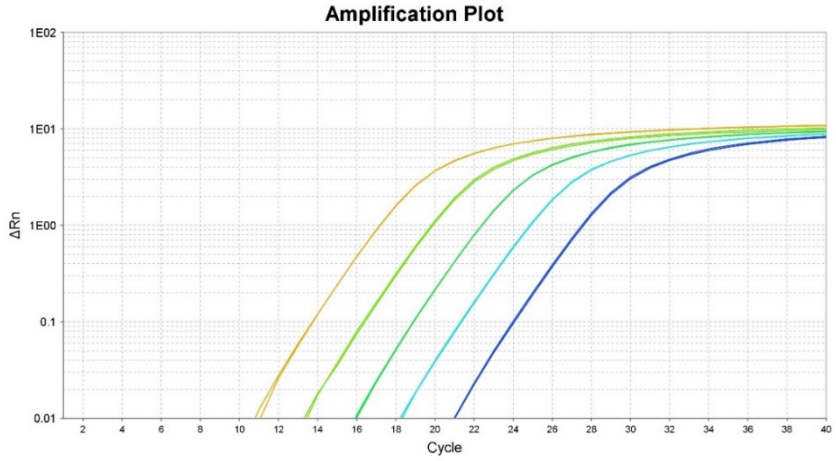


Supplementary figure 4. Gating strategy for neutrophil phenotyping. Flow gating strategy starting with **(a)** singlet gate (forward scatter area vs height), **(b)** viability gate (EpCAM vs Zombie Violet viability), **(c)** CD45+ cells (EpCAM vs CD45) and **(d)** CD66b+ neutrophils (forward scatter area vs CD66b). **(e)** A1 vs. A2 (GRIM) populations according to CD16/CD63 expression. **(f)** Example of shifts in neutrophil populations according to type of apical wash used for migration, uninfected (purple), RV (cyan), *Pa* (orange), and RV+*Pa* co-infected (green).

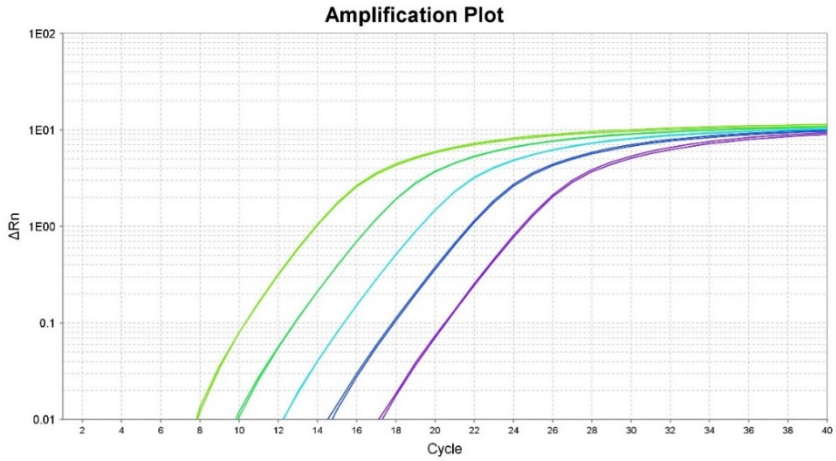


Supplementary figure 5. Endotoxin units per mL (EU mL^{-1}) of lipopolysaccharide (LPS) in ALI washes from *Pa* infected cultures

(a)



(b)



Supplementary figure 6. qPCR Amplification pots. **(a)** Standard curve for rhinovirus 5'UTR qPCR in a 1 in 5 dilution series, beginning with amplification corresponding to approximately $2x10^6$ PFU. **(b)** Standard curve for *P. aeruginosa* 16srRNA qPCR in a 1 in 5 dilution series, beginning with amplification corresponding to approximately $4x10^8$ CFU.