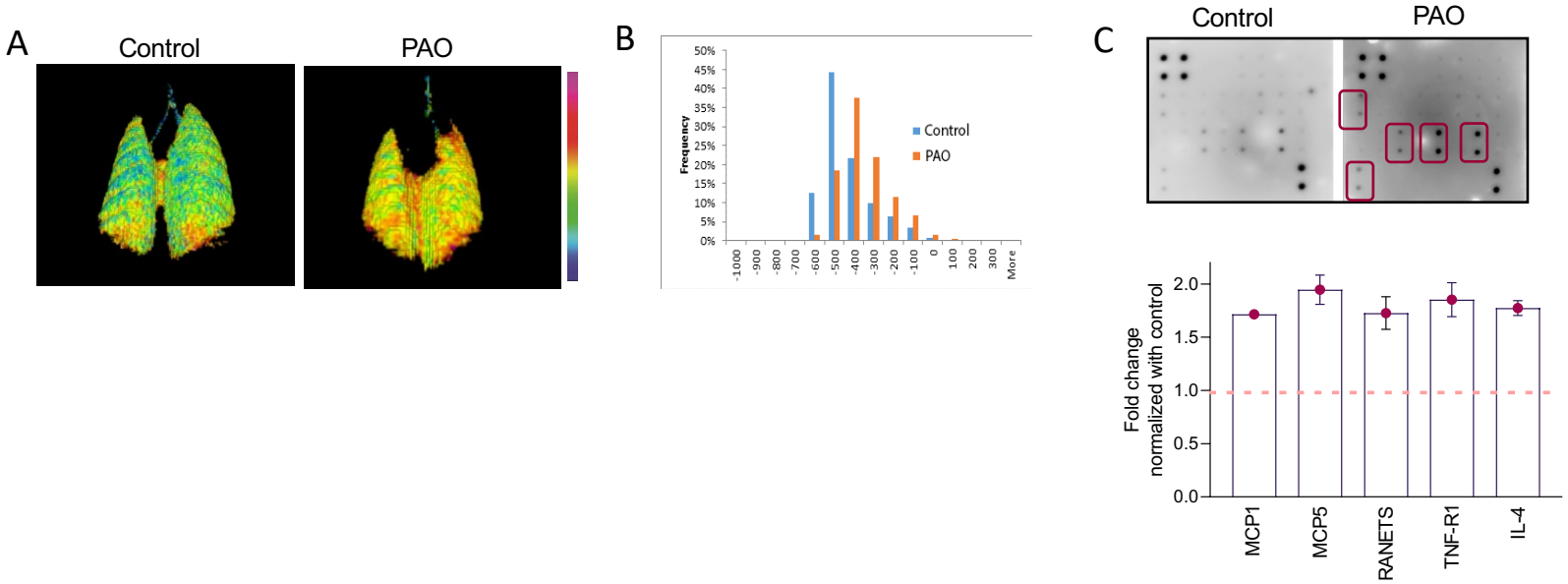
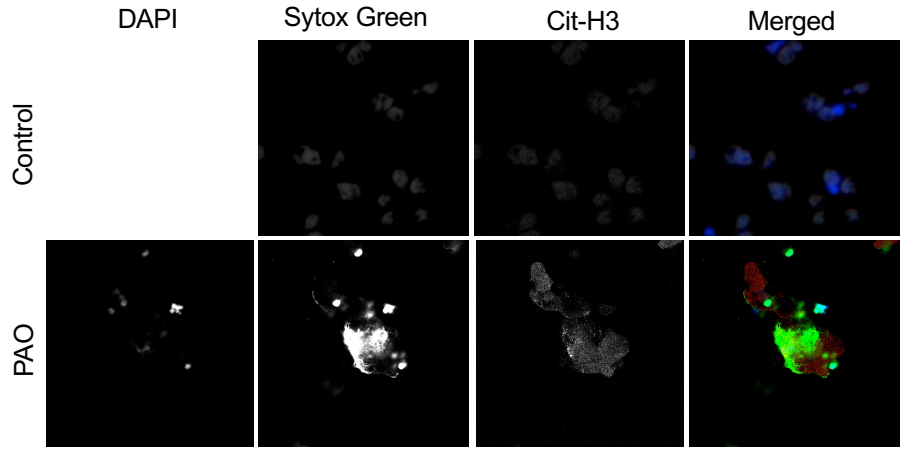
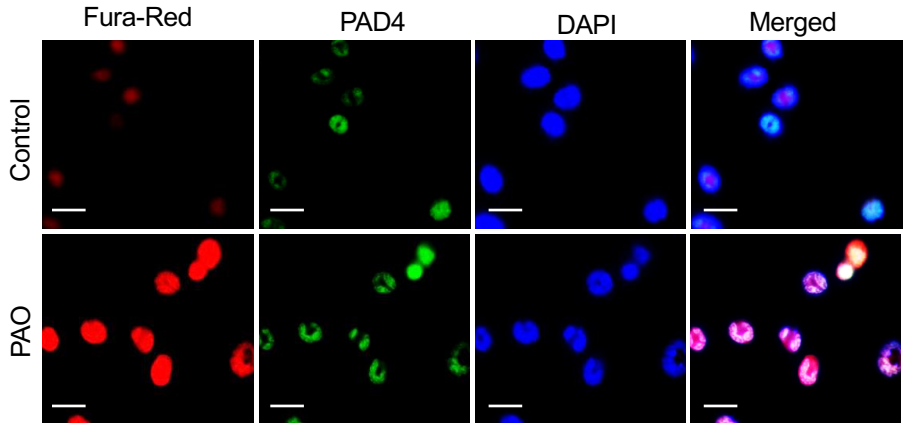


Supplementary Fig. 1: Single exposure of PAO on the skin results in acute lung injury. (**A**) 3D virtual composition of lung micro-computed tomography (CT) slice images of individual mice, from controls and PAO exposed group. The red-to-blue color scale (more density to less density) demonstrated changes in lung density. (**B**) micro-CT image signal histogram of the volume of lung tissue over the full range of tissue density for a single whole lung from a control mouse (blue bars), and PAO mouse (orange bars) (n=5-7 for A-B). (**C**) Cytokine protein array (Raybio® C series AAM-CYT-1-4) for acute inflammation in whole lung lysate of control and PAO groups. The selected cytokine levels are represented in the graph after normalization with the control group (n=3).

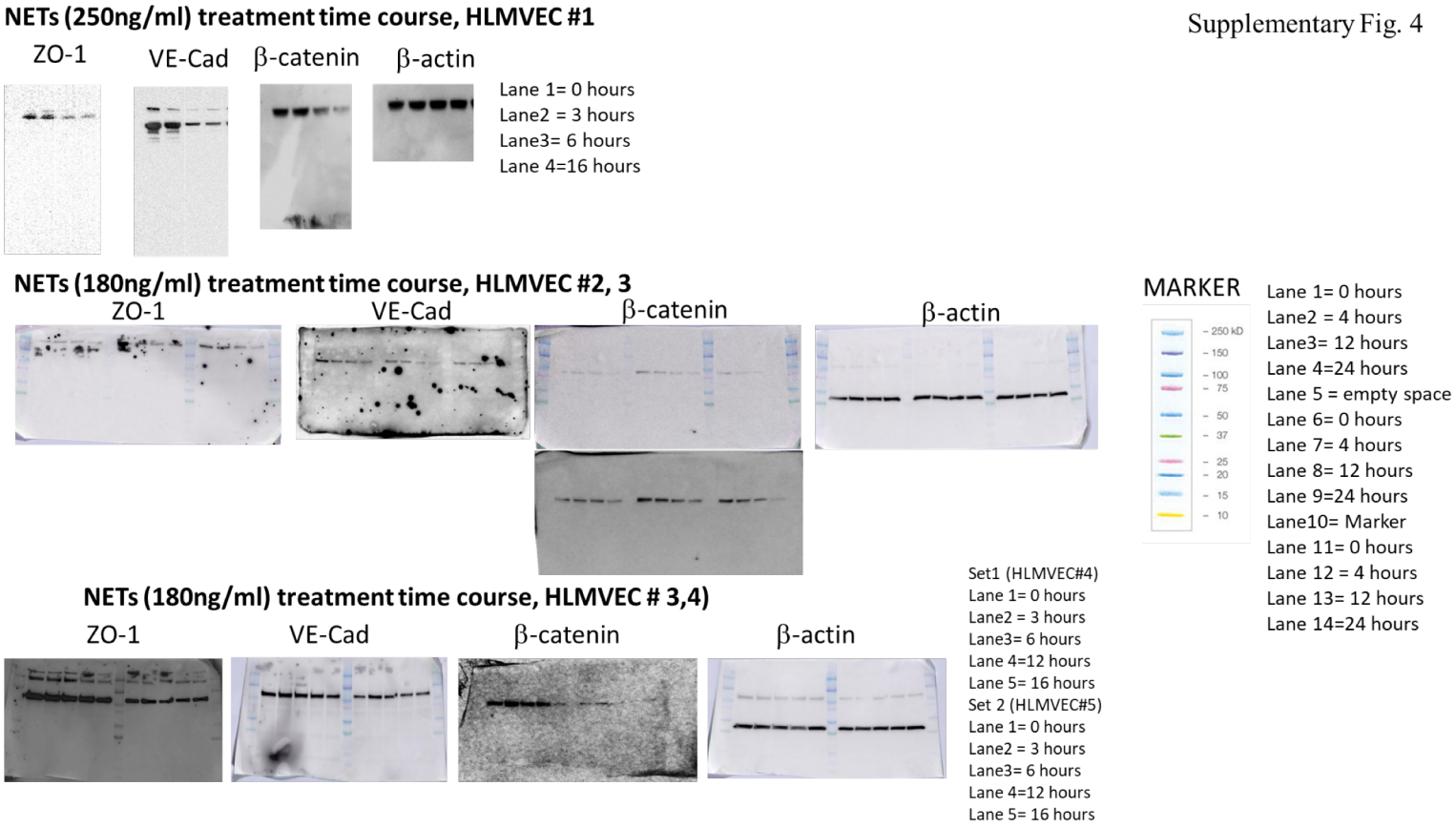


Supplementary Fig. 2: PAO treatment induces release of Cit-H3 positive NETs in human neutrophils. Neutrophils were treated with 250nM PAO for 4 hours. The un-permeablized neutrophils were stained for Sytox green (ds DNA) and Cit-H3 (tagged with Alexa-594).

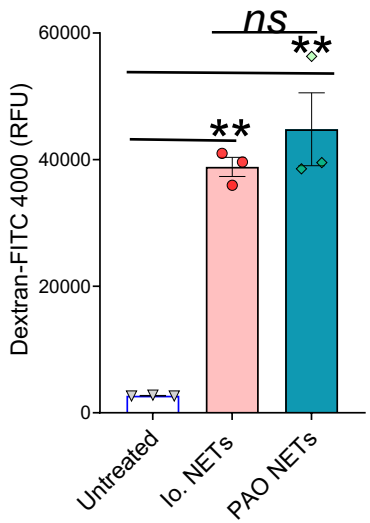


Supplementary Fig. 3: PAO treatment increases calcium and PAD4 levels in neutrophils. Human peripheral blood neutrophils were loaded with FURA-red for the detection of intracellular calcium levels (FURA-red) in controls and PAO treated group. The live neutrophils were stained for anti-PAD4-Alexa 488.

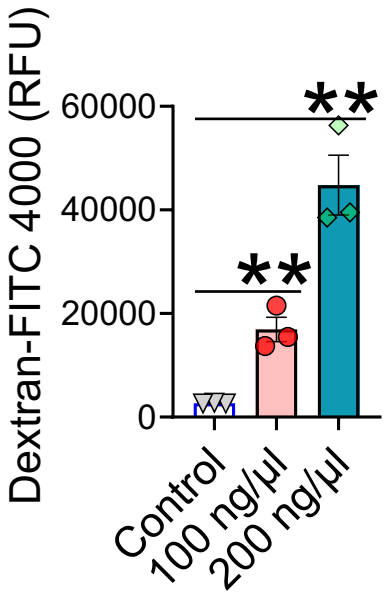
Supplementary Fig. 4



Supplementary Fig. 4: PAO treatment increases permeability of HLMVEC monolayers. HLMVECs were treated with NETs (180-250ng/ml) for indicated hours. Immunoblots of ZO-1, β-catenin, and VE-cadherin demonstrated decreased levels over the period of time upon PAO treatment. β-actin used as loading control.



Supplementary Fig. 5: FITC Dextran permeability assay: The HLMVECs monolayers were treated with equal concentrations of NETs obtained from neutrophils treated with Ionomycin (5 μM) for 30 minutes, and PAO (250 nM) for 4 hours. Neutrophils were obtained from 3 individuals. *ns*=not significant,**$P<0.01$, 1-way ANOVA followed by Tukey's multiple comparisons test



Supplementary Fig. 6: FITC Dextran permeability assay: The HLMVECs monolayers were treated with different concentrations of NETs for 24 hours. Neutrophils were obtained from 3 individuals. $^{**}P<0.01$, 1-way ANOVA followed by Tukey's multiple comparisons test

Supplementary Table 1

ARDS patients

Case ID	Age	Sex	Race	ARDS	Pneumonia
1	70	Male	White	+	-
2	25	Male	Black	+	-
3	38	Male	White	+	-
4	59	Male	White	+	-
5	35	Male	Black	+	-
6	31	Male	White	+	-
7	27	Male	Hispanic	+	-
8	53	Female	White	+	-
9	48	Male	White	+	-

Healthy donors

Case ID	Age	Sex	Race
1	26	Female	Black
2	35	Female	White
3	23	Female	White
4	41	Female	White
5	43	Male	White
6	30	Male	White
7	27	Male	White
8	24	Male	Hispanic
9	48	Female	Black
10	51	Male	White

Supplementary table 1: ARDS patients demographics