

Attenuation of neutrophil driven inflammation and fungal growth by pilocarpine hydrochloride

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Supplementary figures

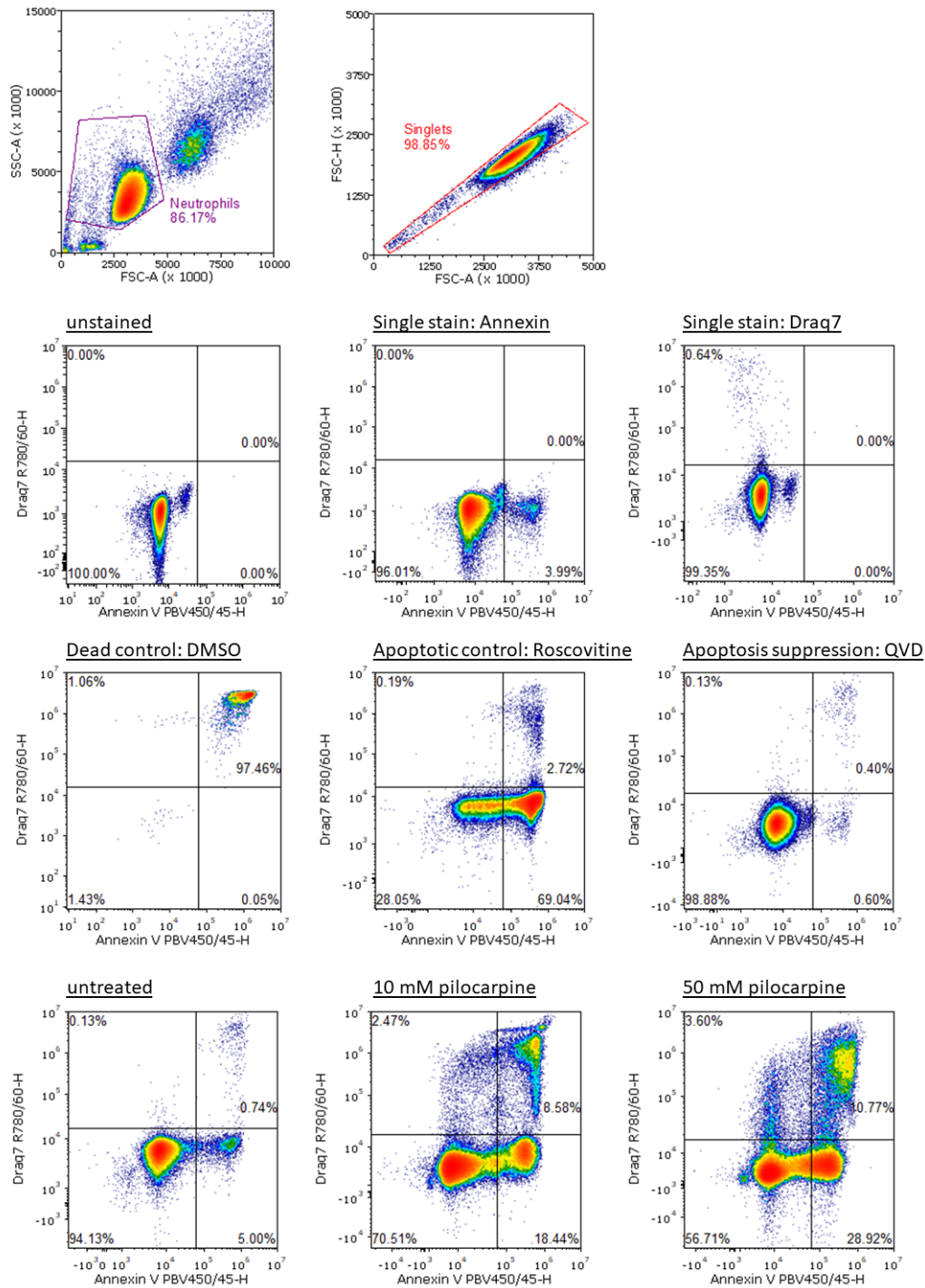


Fig. S1: Gating strategy for analysis of apoptosis and necrosis of human neutrophils by flow cytometry. Debris and doublet cells were excluded from the cells, before analysis for Annexin V and Draq7 staining. Annexin V⁺/Draq7⁻ cells were scored as apoptotic, Draq7⁺ cells as necrotic, and double negative cells as viable. Unstained and single-stained cells were used to adjust the quadrant gate. Examples of dimethyl sulfoxide (DMSO) treated neutrophils for necrosis induction, Roscovitine treated cells as positive control for apoptosis, and pan-caspase inhibitor Q-VD-OPh (QVD) for suppression of both are displayed. Further example plots show untreated, 10 mM and 50 mM pilocarpine treated neutrophils at 6 hours.

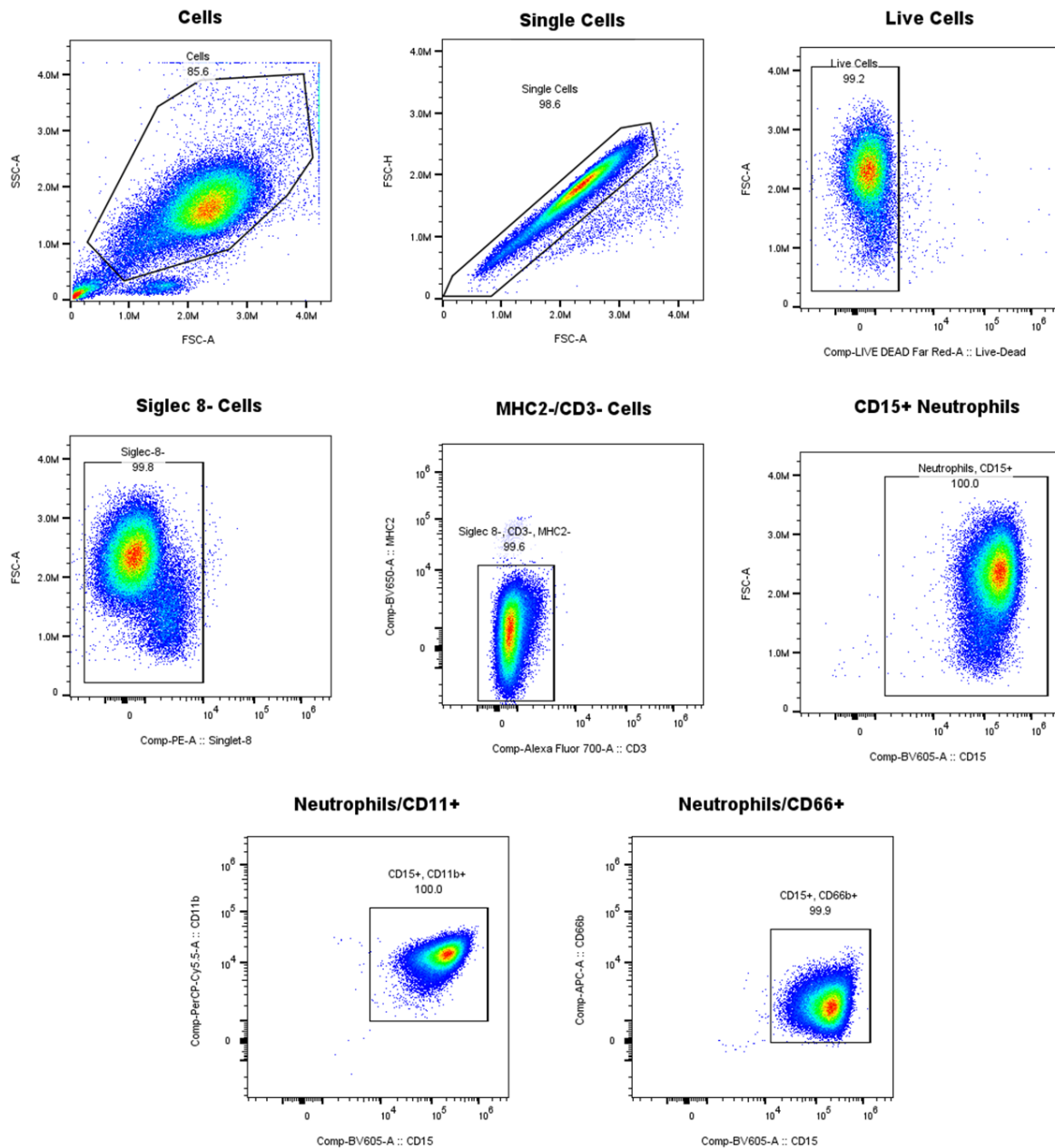


Fig. S2: Gating strategy for neutrophil activation marker analysis by flow cytometry. Debris and doublets were excluded prior to gating on live cells (live/dead far red dye⁻). From this, Siglec⁺ (eosinophils and mast cells) and MHC2/CD3⁺ (lymphocytes and monocytes) cells were excluded, and only CD15⁺ neutrophils included. Activation markers CD11b and CD66b were then quantified by their geometric mean.

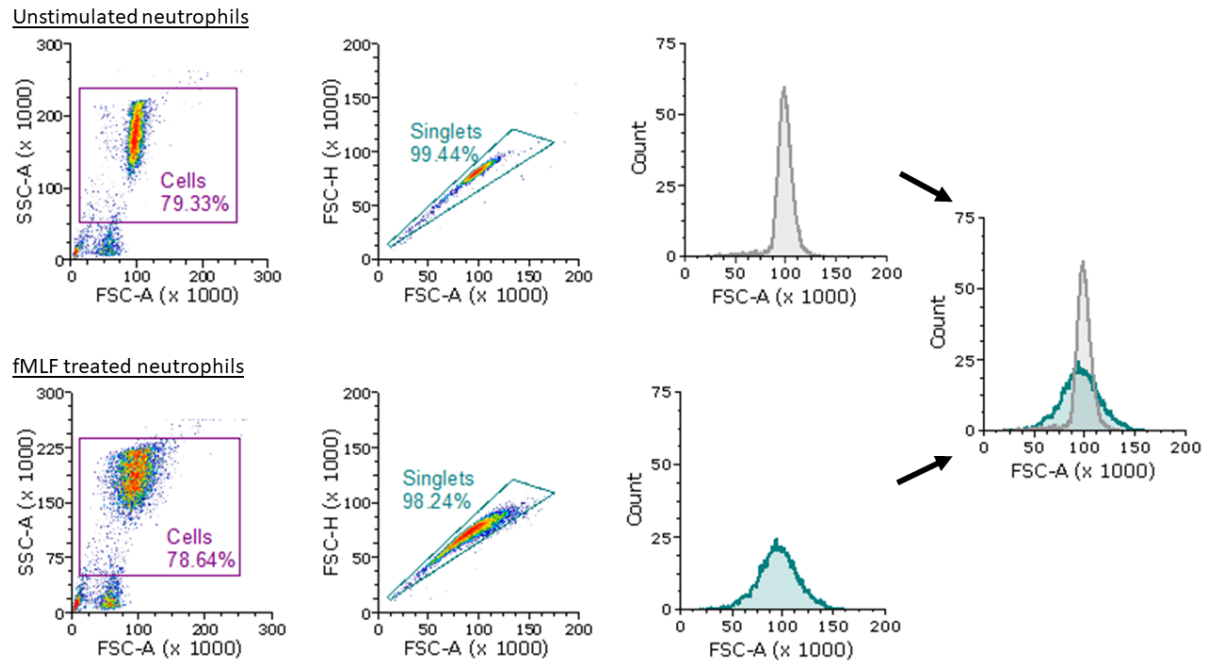


Fig. S3: Gating strategy for neutrophil shape change by FCS-A. Debris and doublets were excluded before analysis of FCS-A on unstained samples. Examples displayed show gating of unstimulated versus N-formylmethionine-leucyl-phenylalanine (fMLF)-treated neutrophils.

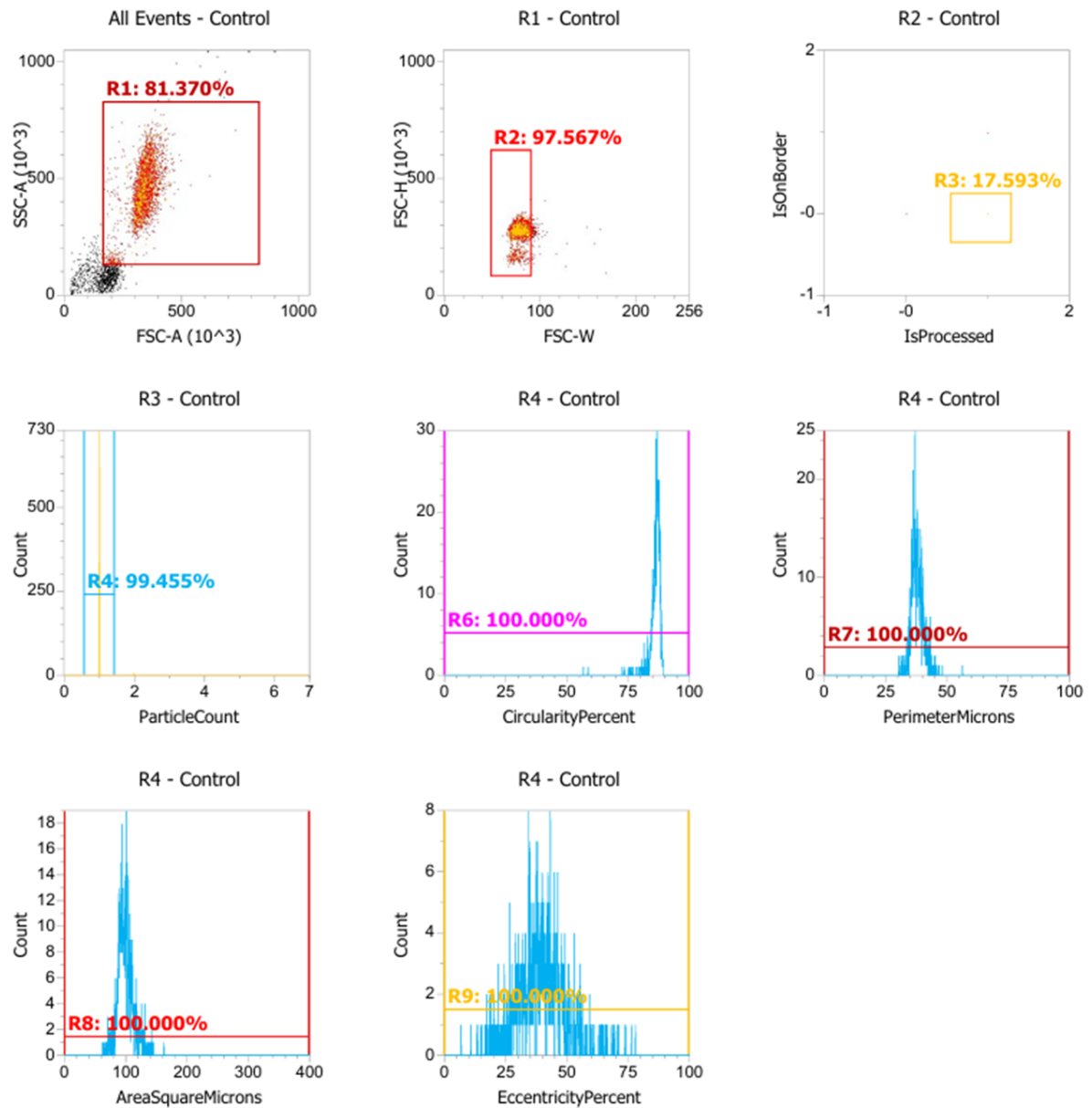


Fig. S4: Gating strategy for neutrophil shape change by imaging flow cytometry. Debris and doublets (FSC-W vs. FSC-H) were excluded and processed cells not located on the border were quantified. From single particles, the software recognition of neutrophils was adjusted. Then, circularity (%), perimeter (μm), area (μm^2), and eccentricity (%) values were obtained.

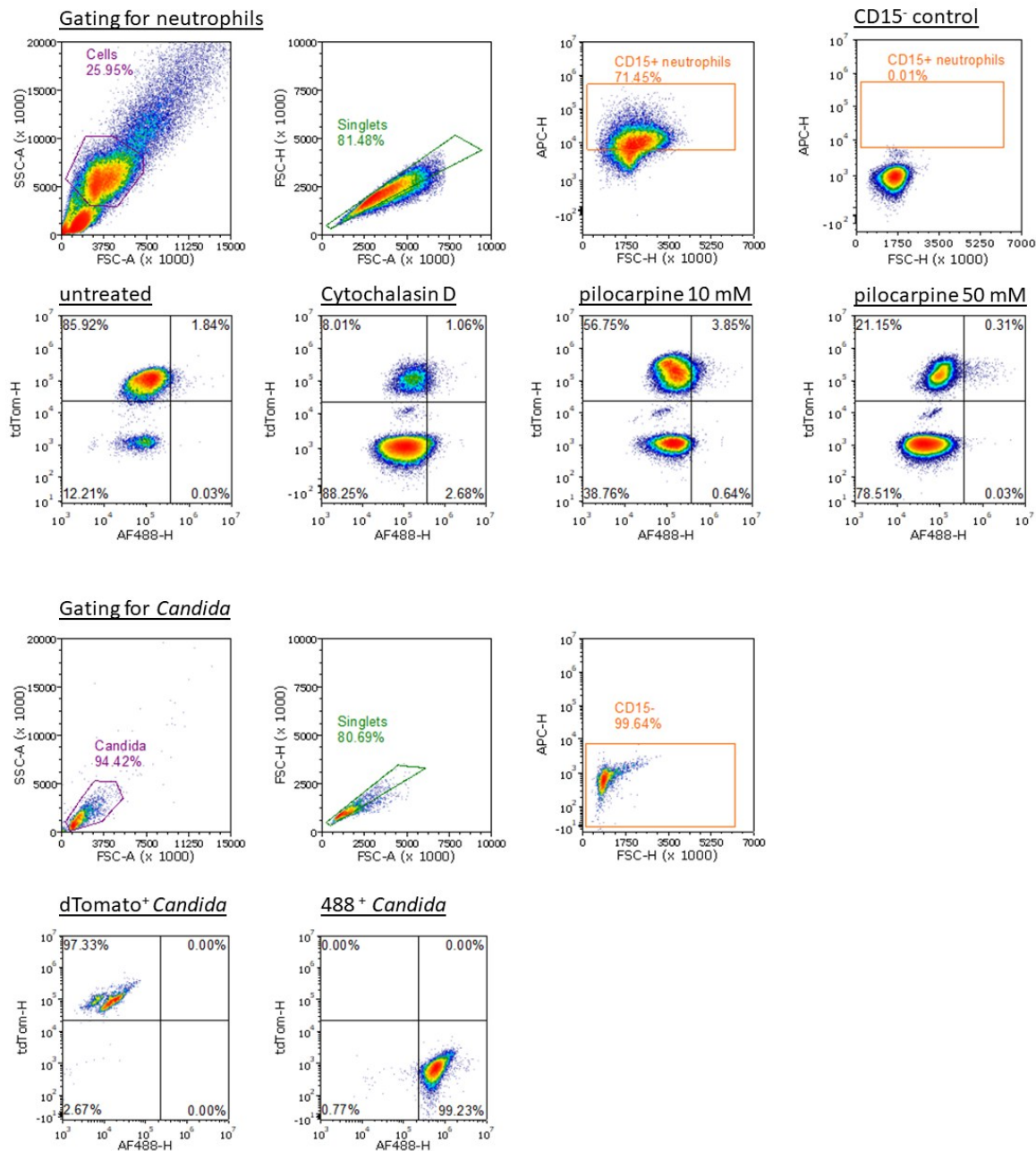


Fig. S5: Gating strategy for flow cytometry analysis of phagocytosis of *C. albicans* yeast by human neutrophils. Debris and doublet cells were excluded prior to gating on CD15⁺ neutrophils to exclude *C. albicans* yeast. dTomato⁺/488⁻ neutrophils were scored as positive for phagocytosis, while dTomato⁺/488⁺ neutrophils were considered to have adherent but not phagocytosed yeast and were therefore excluded. Double negative neutrophils did not phagocytose *C. albicans* yeast. Pre-incubation of neutrophils with cytochalasin D (CytD) at 10 μ g/mL served as control for inhibition of phagocytosis. Example plots display untreated, CytD treated, and pilocarpine treated samples. The gating for *C. albicans* excluded doublets and only included CD15⁻ cells. Example plots of single stained samples (dTomato positive yeast versus dTomato negative yeast stained with anti human IgG-488 antibody after opsonisation by human autologous serum) are shown.