

Supplementary Material

Dysregulation of the CD163-haptoglobin axis in the airways of COPD patients

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Supplementary results

There was a numerical trend for reduced sCD163 levels in COPDS compared to healthy current smokers (S) and COPDE compared to healthy ex-smokers (E) but this did not reach statistical significance ($p=0.3$ and $p=0.2$ respectively; Figures S5 and S6). There was no difference in sCD163 levels when comparing S to E ($p=0.9$; Figure S7).

There were no significant differences in the percentage of CD163⁺ macrophages when comparing COPDS to S, COPDE to E or S to E. ($p=0.3 - 0.9$; Figures S5–S7).

There were no significant differences in the levels of free haptoglobin when comparing COPDS to S, COPDE to E or S to E. ($p=0.2 - 0.9$; Figure S5–S7).

Supplementary discussion

We observed a non-significant reduction in sCD163 levels when comparing COPDS to S and COPDE to E. These sub analyses were limited by small sample sizes. Although not significant, this observation supports the concept of a dysfunctional CD163-haptoglobin axis in COPD. In addition, we didn't observe a difference between S and E, although these subgroup analyses were limited by low sample numbers.

Supplementary figure legends

Supplementary Figure S1. The relationship between sCD163 and clinical variables. The relationship between sCD163 in sputum supernatants and forced expiratory volume in 1 second (FEV1) % predicted in the total population ($n=10$ healthy non-smokers (HNS), $n=10$ healthy smokers (HS) and $n=17$ COPD patients) was examined by Pearson's correlation (A). The relationship between sCD163 levels and smoking pack years history in HS and COPD patients was examined by Pearson's correlation (B).

Supplementary Figure S2. CD163 expression in inhaled corticosteroid users vs non-users. SCD163 levels in sputum supernatants (A) and the percentage of CD163⁺ sputum macrophages (B) were compared between COPD patients who use inhaled corticosteroids (ICS yes) and those who do not (ICS no). Data presented as individual values with mean.

Supplementary Figure S3. Haptoglobin expression in inhaled corticosteroid users vs non-users. Haptoglobin levels in sputum supernatants (A) and the percentage of haptoglobin⁺ sputum macrophages (B) were compared between COPD patients who use inhaled corticosteroids (ICS yes) and those who do not (ICS no). Data presented as individual values with mean.

Supplementary Figure S4. Haptoglobin expression in sputum eosinophils. The percentage of haptoglobin⁺ eosinophils was compared between COPD patients and controls (A), COPD current smokers (COPDS) and COPD ex-smokers (COPDE) (B) and COPD patients who use inhaled

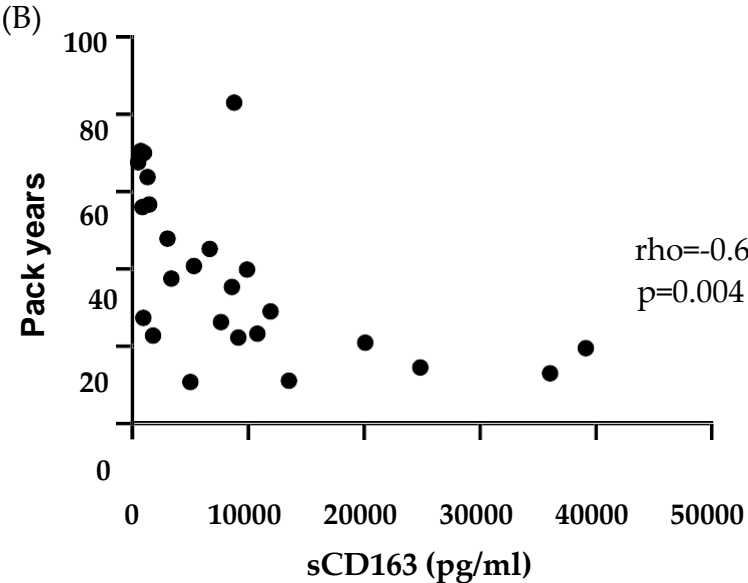
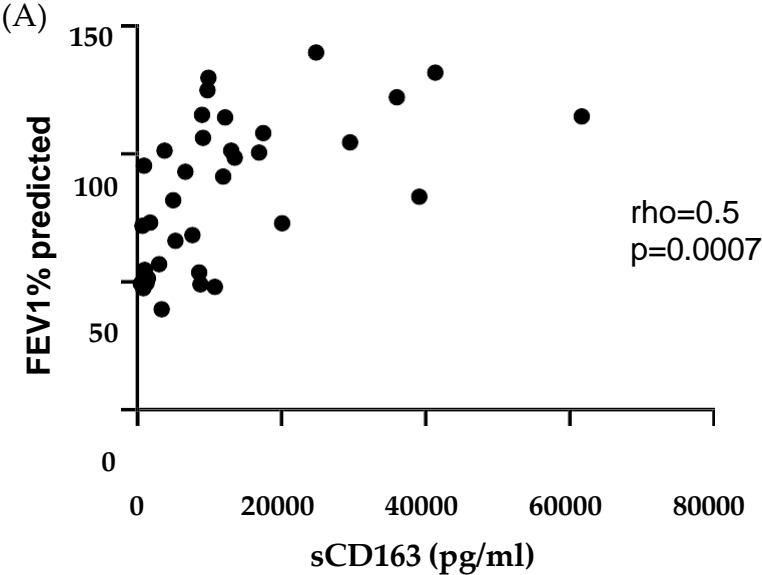
corticosteroids (ICS yes) and those who do not (ICS no) (C). Data presented as individual values with mean.

Supplementary Figure S5. Comparison between healthy current smokers (S) and COPD current smokers (COPDS). SCD163 levels (A) the percentage of CD163⁺ sputum macrophages (B) and free haptoglobin levels (C) were compared between S (n=3) and COPDS (n=8). Data presented as individual values with median.

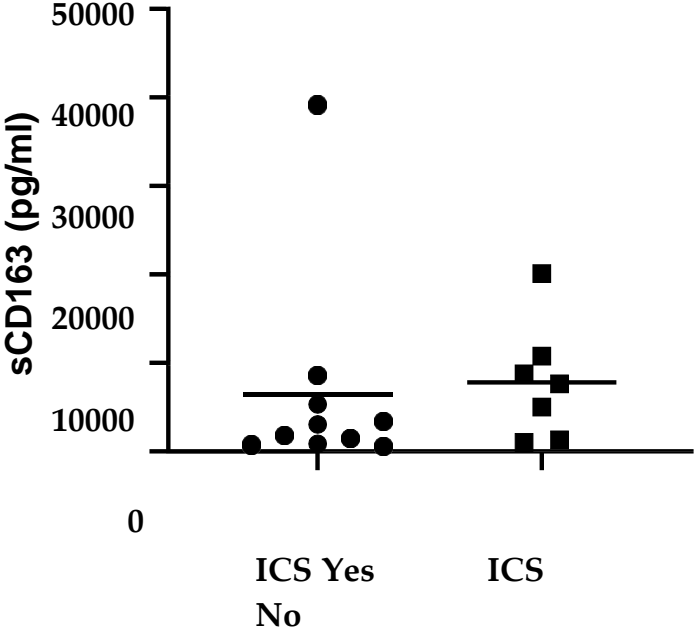
Supplementary Figure S6. Comparison between healthy ex-smokers (E) and COPD ex- smokers (COPDE). SCD163 levels (A) the percentage of CD163⁺ sputum macrophages (B) and free haptoglobin levels (C) were compared between E (n=7) and COPDS (n=9). Data presented as individual values with median.

Supplementary Figure S7. Comparison between healthy current smokers (S) and healthy ex- smokers (E). SCD163 levels (A) the percentage of CD163⁺ sputum macrophages (B) and free haptoglobin levels (C) were compared between S (n=3) and E (n=7). Data presented as individual values with median.

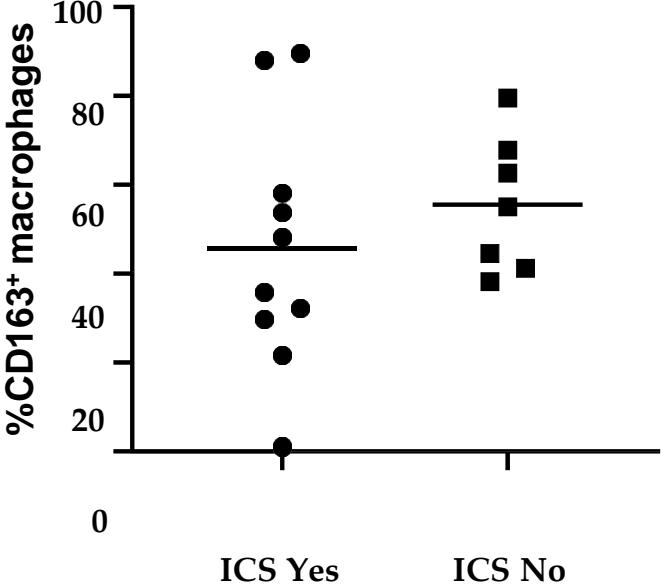
Supplementary Figure S1



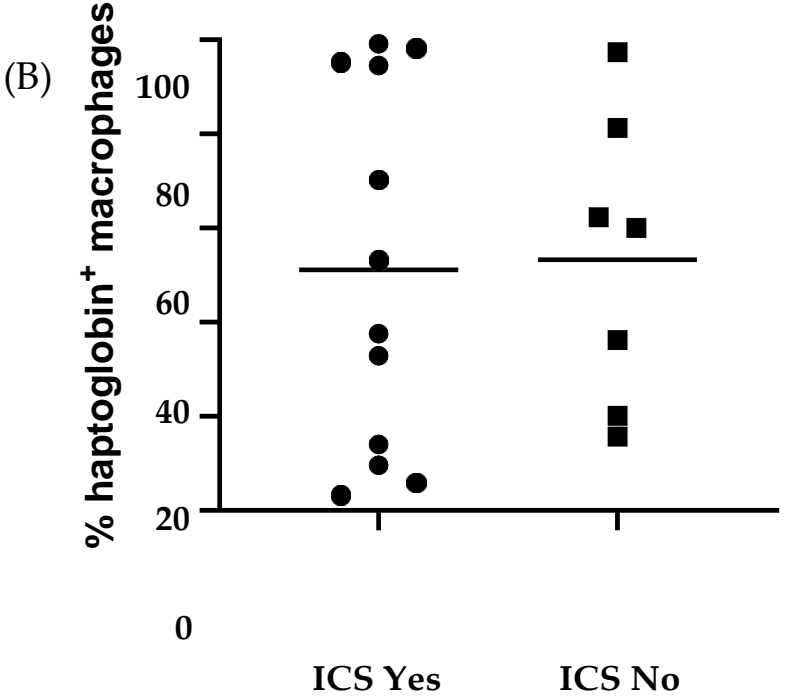
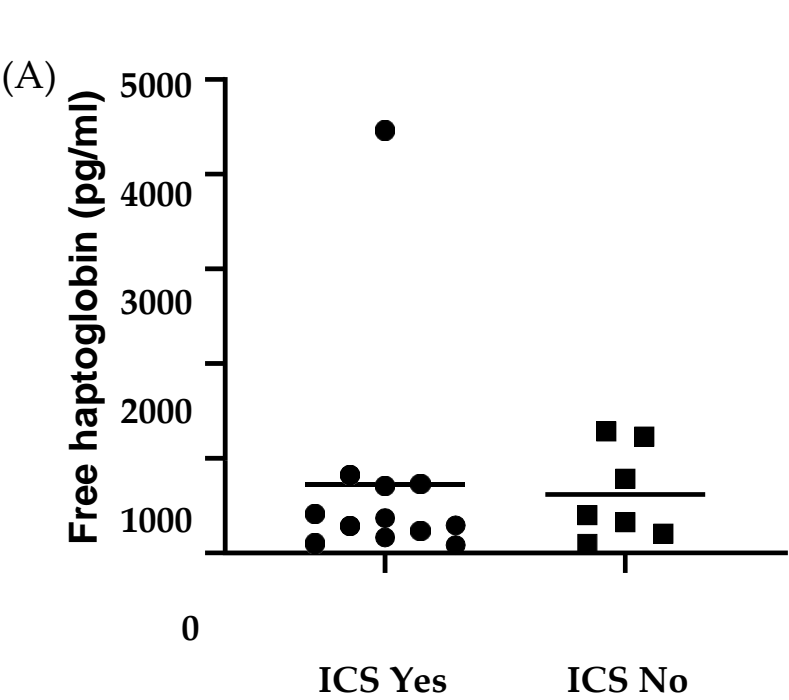
(A)



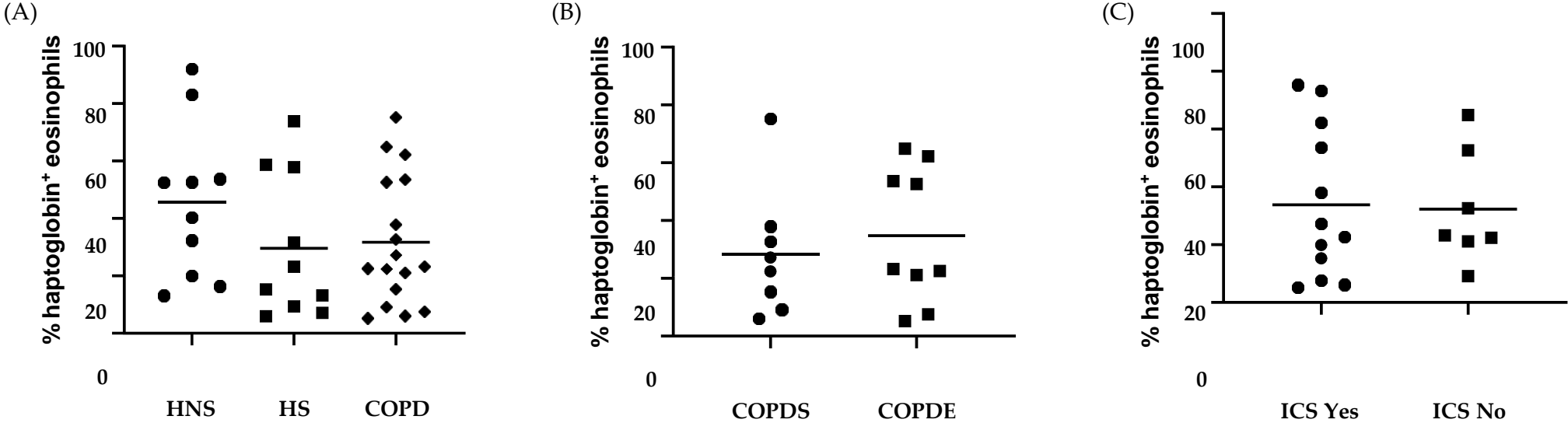
(B)



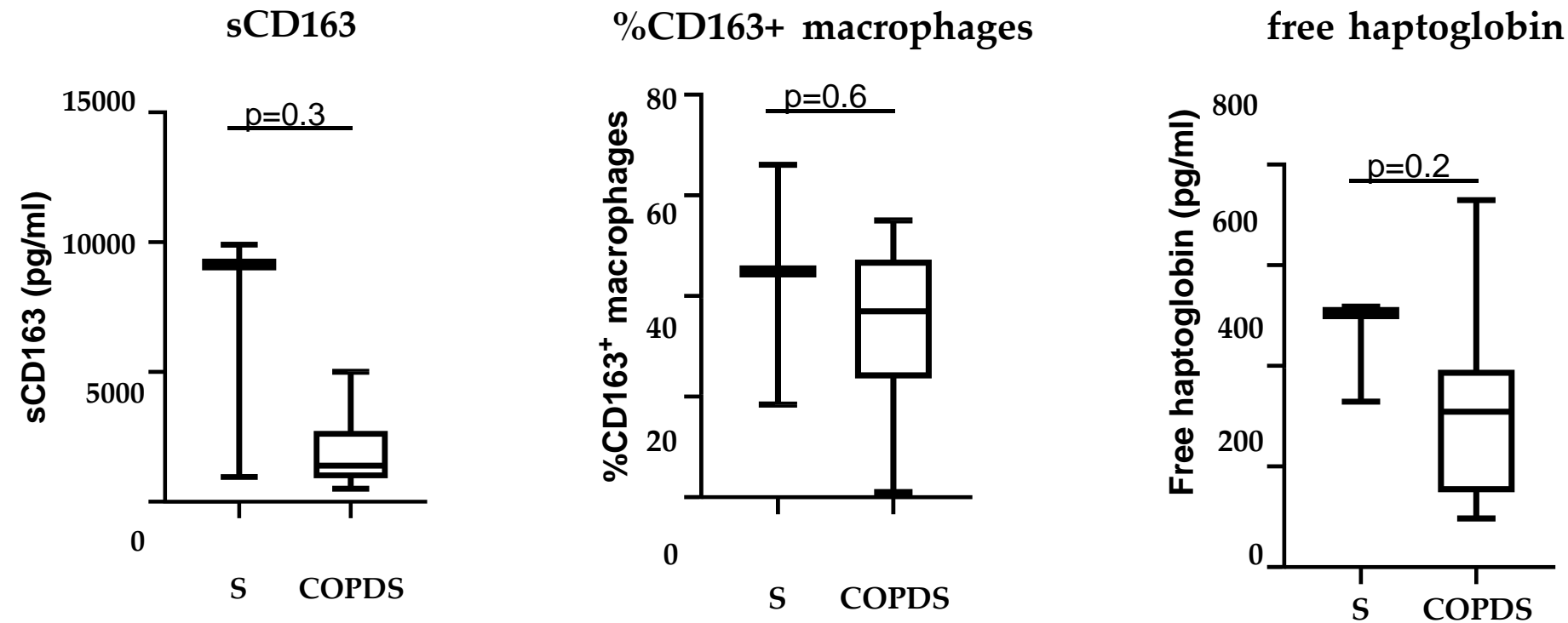
Supplementary Figure S3



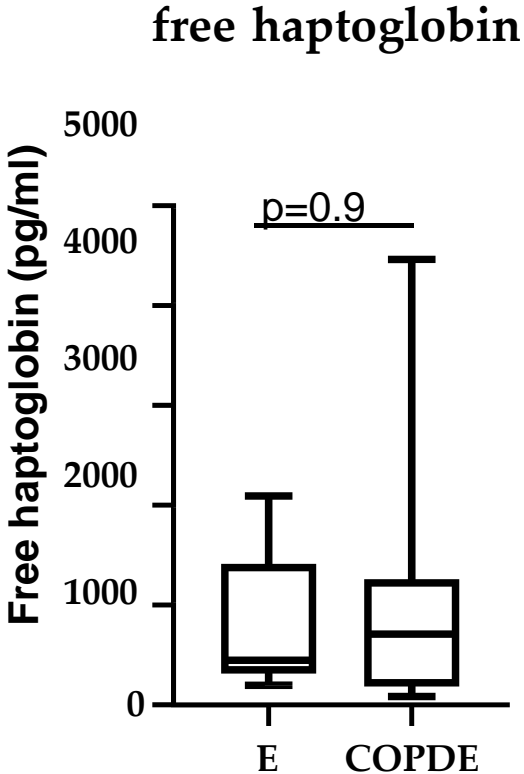
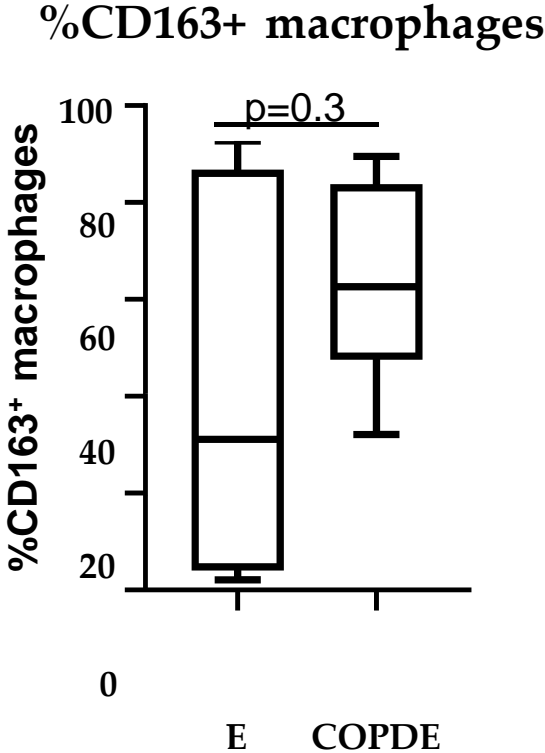
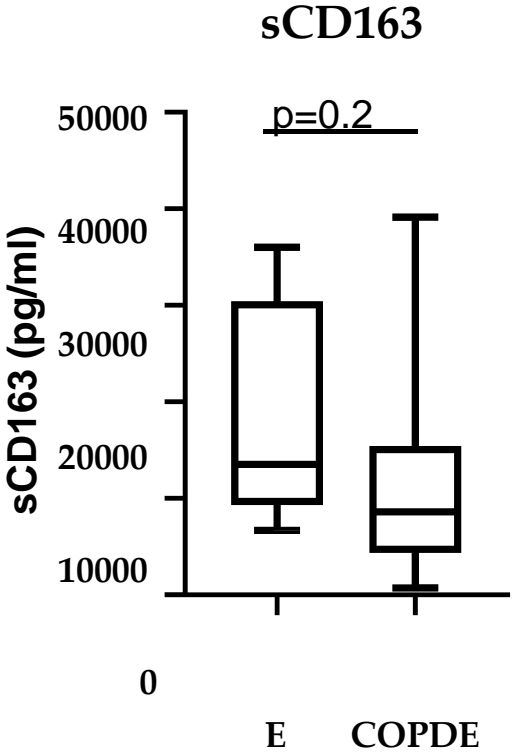
Supplementary Figure S4



Supplementary Figure S5



Supplementary Figure S6



Supplementary Figure S7

