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Supplementary information for "Elastin-derived peptides favor type 2 innate lymphoid cells in COPD"

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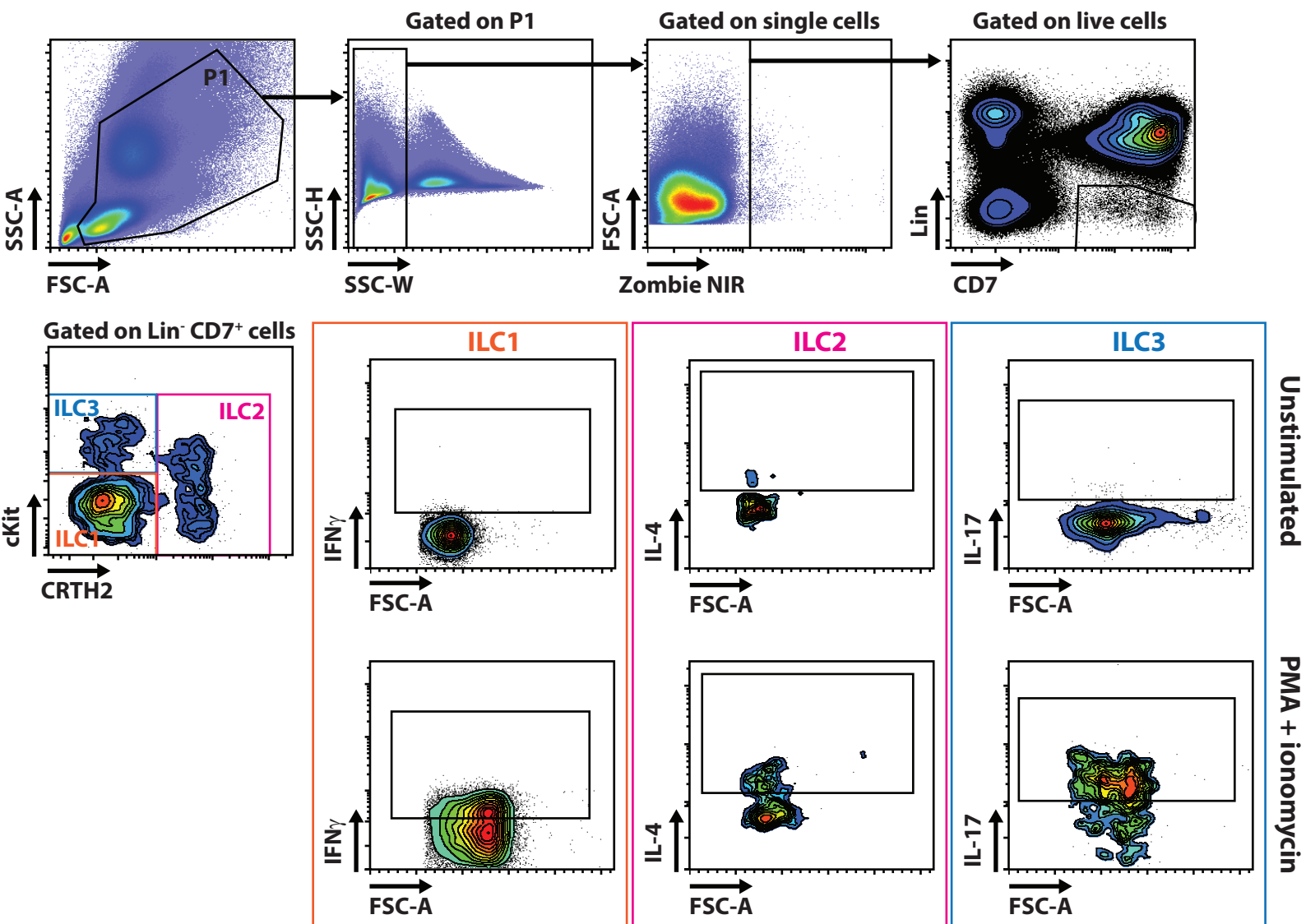
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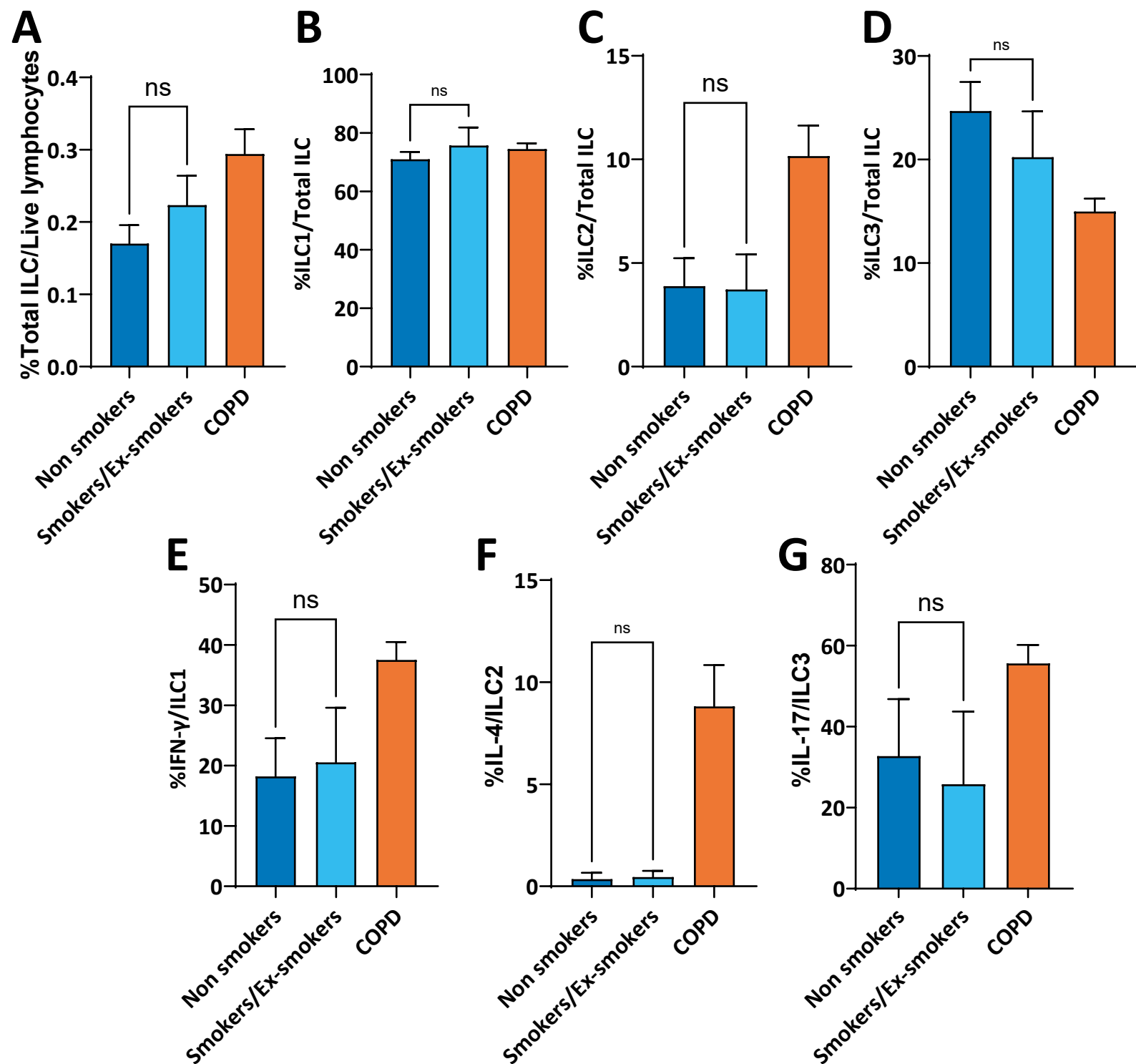


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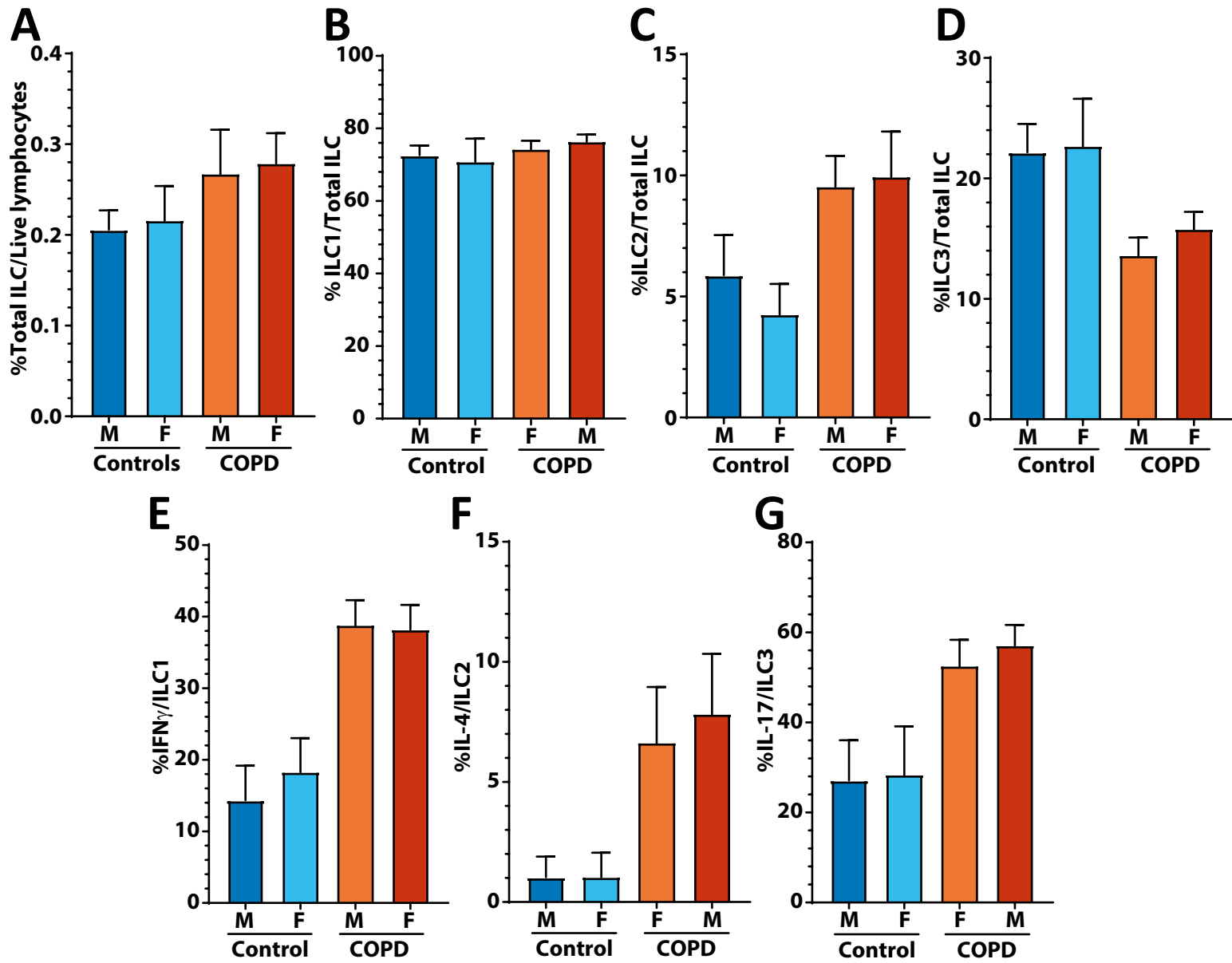


Supplementary figure S1: Gating strategy used for cytometry analysis.

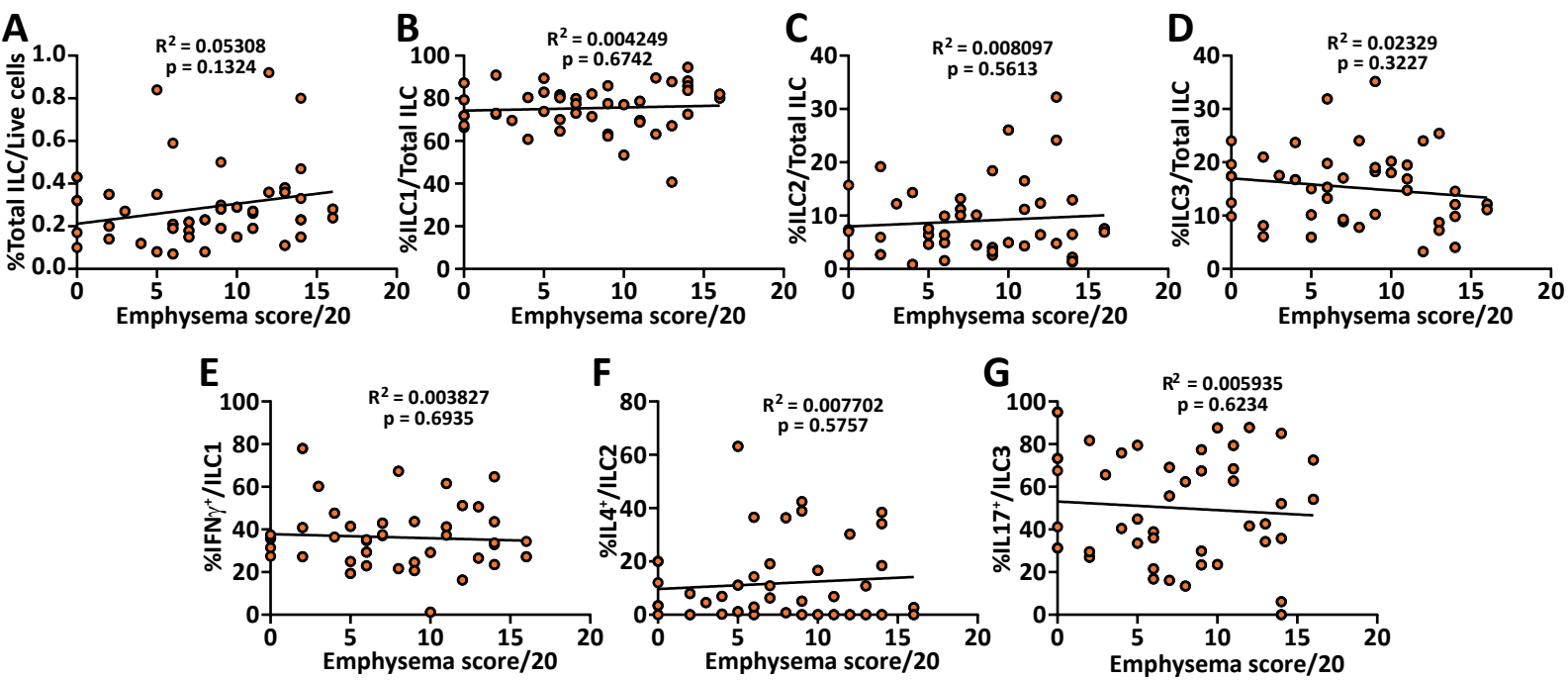
The size and complexity of peripheral blood mononuclear cells were used to select leukocytes. After exclusion of doublets and dead cells, innate lymphoid cells were defined as Lin⁻ and CD7⁺. Lineage (CD3/14/16/19/20/56) allowing us to eliminate T and B lymphocytes, natural killer cells and myeloid cells. Among ILC, the expression of the CD117 and CD294 markers was used to determine the 3 sub-populations: ILC1 (CD117⁻ and CD294⁺), 2 (CD117^{+/+} and CD294⁺) and 3 (CD117⁺ and CD294⁻). The expression of their associated secreting cytokines were also analysed. i.e., IFN- γ for ILC1, IL-4 for ILC2 and IL-17 for ILC3. Gates for cytokines set on unstimulated cells.



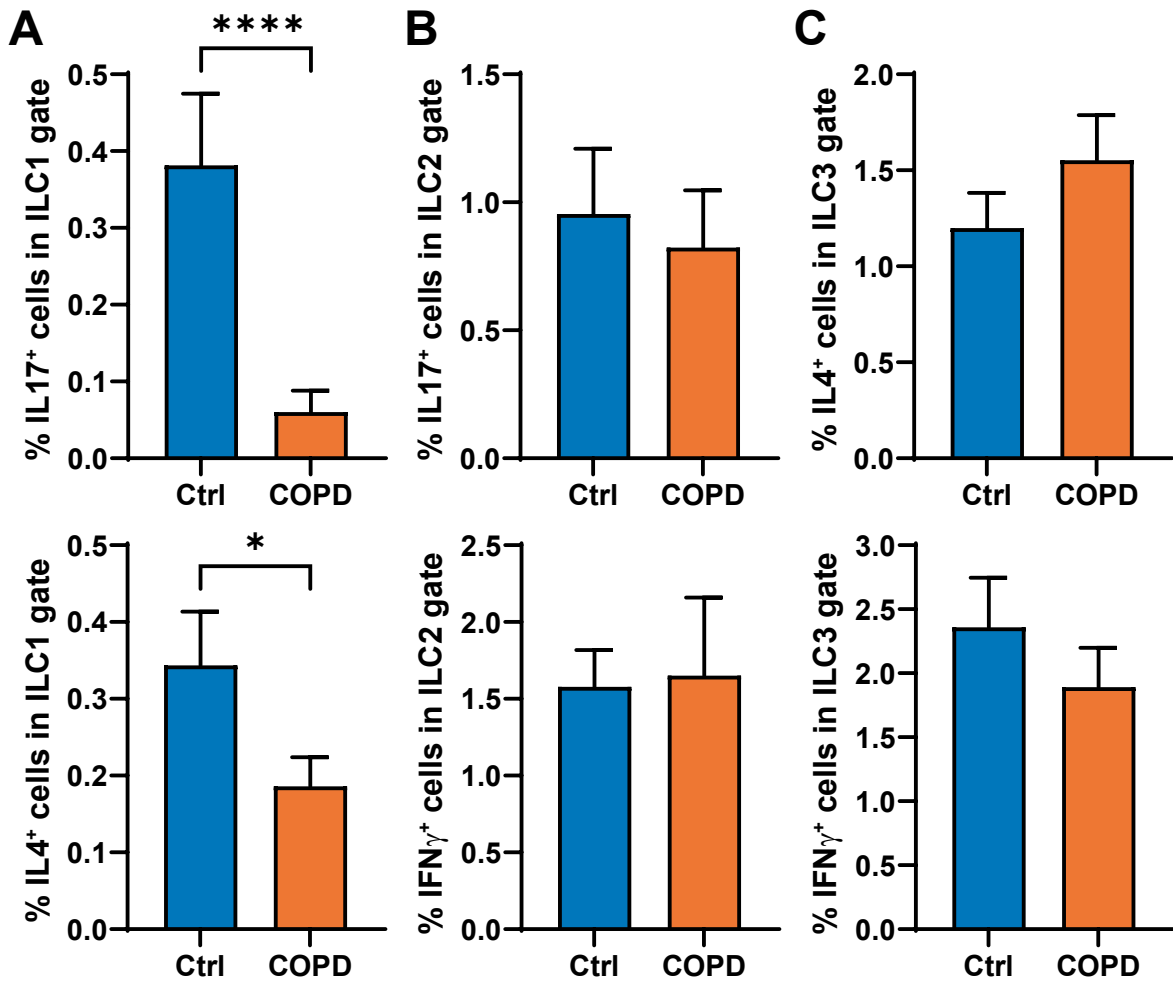
Supplementary figure S2: Comparison of (A) Total ILC proportion, (B) ILC1 proportion, (C) ILC2 proportion, (D) ILC3 proportion, (E) Proportion of IFN- γ secreting-ILC1, (F) Proportion of IL-4 secreting-ILC2, (G) Proportion of IL-17 secreting-ILC3 between nonsmokers (n=12), ex-smokers and current smokers' controls (=13) and COPD patients. Data are presented as mean \pm SEM. Differences between two groups were evaluated by using the Wilcoxon-Mann-Whitney test unless otherwise specified. P values <0.05 were considered significant.



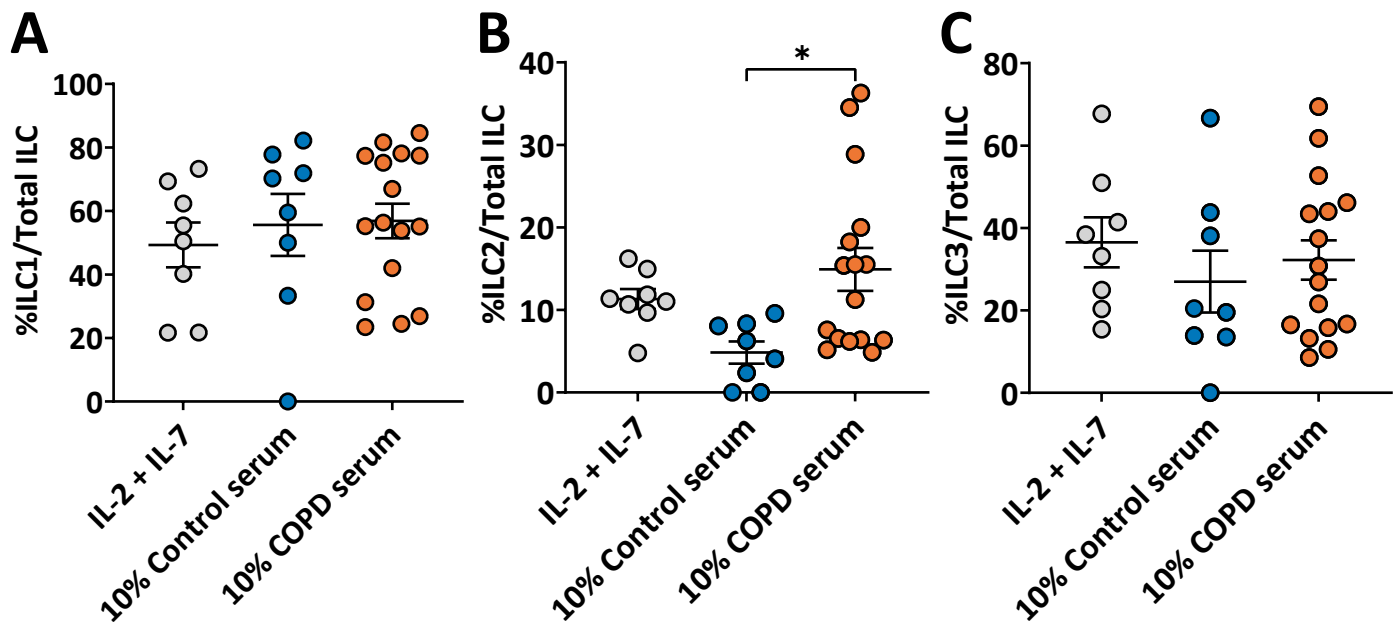
Supplementary figure S3: Comparison of (A) Total ILC proportion, (B) ILC1 proportion, (C) ILC2 proportion, (D) ILC3 proportion, (E) Proportion of IFN- γ secreting-ILC1, (F) Proportion of IL-4 secreting-ILC2, (G) Proportion of IL-17 secreting-ILC3 between male (M) (n=18) and female (F) (n=17) controls and male (n=21) and female (n=25) COPD patients. Data are presented as mean \pm SEM. Differences between two groups were evaluated by using the Wilcoxon-Mann-Whitney test unless otherwise specified. P values <0.05 were considered significant.



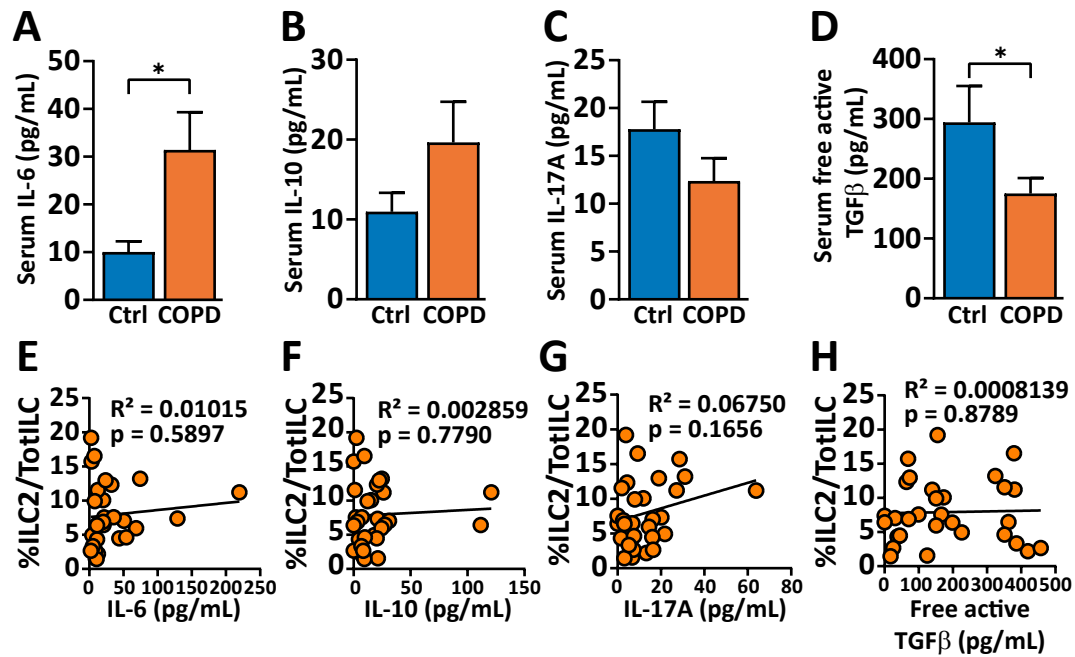
Supplementary figure S4: Pearson correlation between emphysema score out of 20 and proportion of total ILC (A), ILC1 (B), ILC2 (C), ILC3 (D) and ILC1, ILC2 and ILC3 subpopulations secreting IFN- γ , IL-4 and IL-17 respectively (E-G) in COPD patients. Pearson coefficient was used for correlation analysis of COPD patients (n=46). P values <0.05 were considered significant.



Supplementary figure 5: Proportion of expression of cytokines related to another ILC lineage. (A) Proportion of IL-17 (top) and IL-4 (bottom) producing ILC1. (B) Proportion of IL-17 (top) and IFN γ (bottom) producing ILC2. (C) Proportion of IL-4 (top) and IFN γ (bottom) producing ILC3. ILC isolated from the blood of COPD patients (n=46) and controls (n=25) were used for phenotypic and functional characterization by flow cytometry. Data are presented as mean \pm SEM. Differences between two groups were evaluated by using the Wilcoxon-Mann-Whitney test unless otherwise specified. P values <0.05 were considered significant.



Supplementary figure S6: (A) Enriched proportion of ILC1. (B) Enriched proportion of ILC2. (C) Enriched proportion of ILC3. Enriched ILC isolated from the blood of controls (n=2-8) were cultured for 5 days in the presence of either IL-2 and IL-7 (n=8), 10% control serum (n=8) or 10% COPD serum isolated from patients (n=27) before being analyzed by flow cytometry. Data are presented as Mean±SEM. Significance between three groups was calculated using one-way ANOVA with Tukey's multiple comparison test. P values <0.05 were considered significant.



Supplementary figure 7: Quantification of pro- and anti-inflammatory cytokines. (A-D) Quantification of (A) IL-6, (B) IL-10, (C) IL-17A and (D) free active TGFβ in the *sera* from COPD patients (n=32) and controls (n=13). (E-H) Pearson correlation between ILC2 proportion and IL-6 (D), IL-10 (E), IL-17A (F) and free active TGFβ (H) concentration in COPD patients. Cytokines quantification was performed using LEGENDplex. Data are presented as Mean±SEM. Difference between two groups were evaluated by using the Wilcoxon-Mann-Whitney test. Pearson coefficient was used for correlation analysis. P values <0.05 were considered significant.