Cigarette smoke-induced reduction of C1q promotes emphysema

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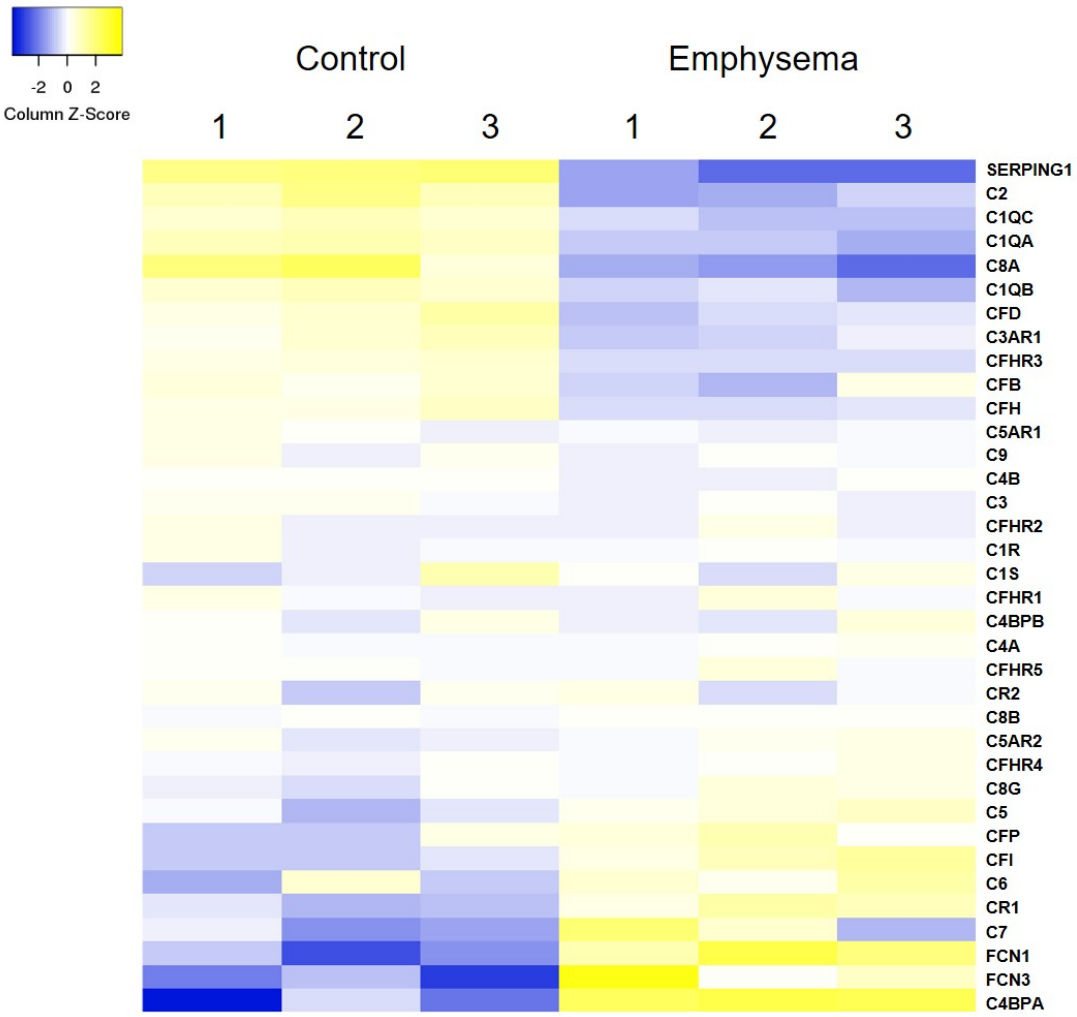
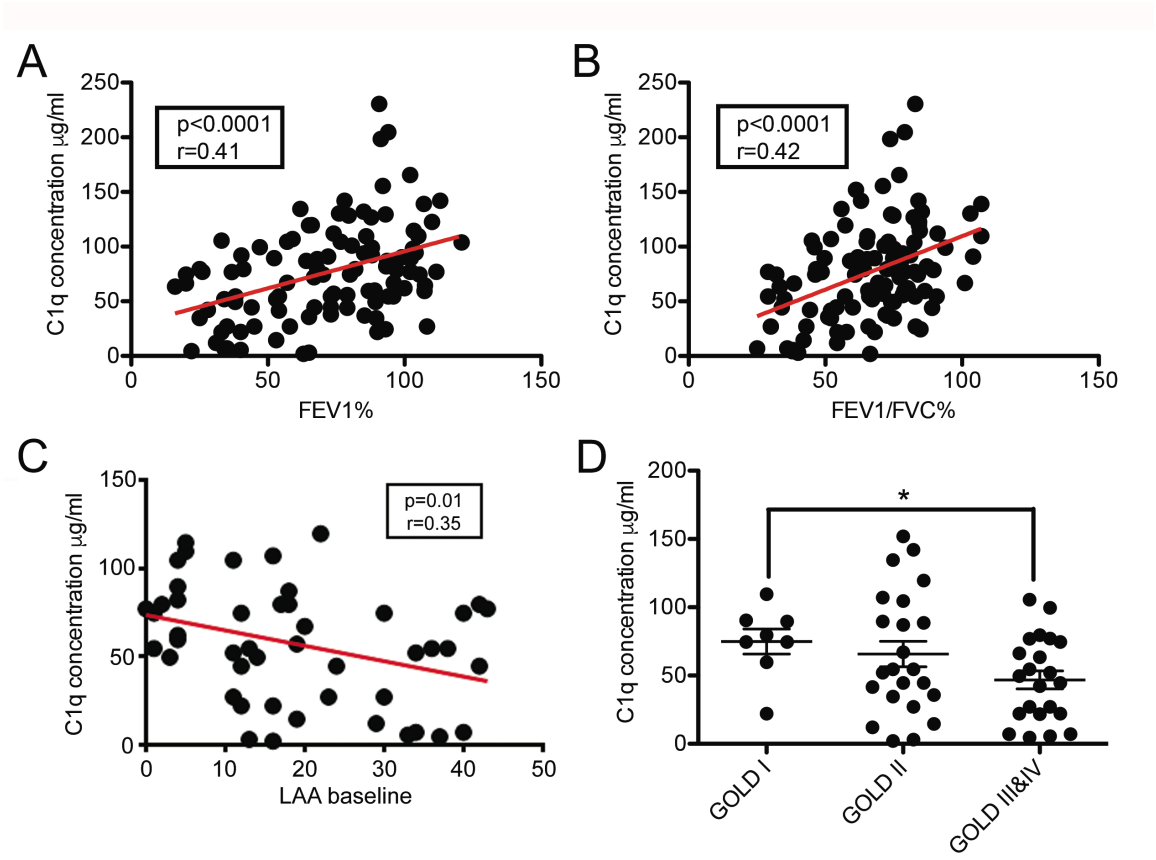
Supplementary Figures 1-8

Supplementary Figure 1



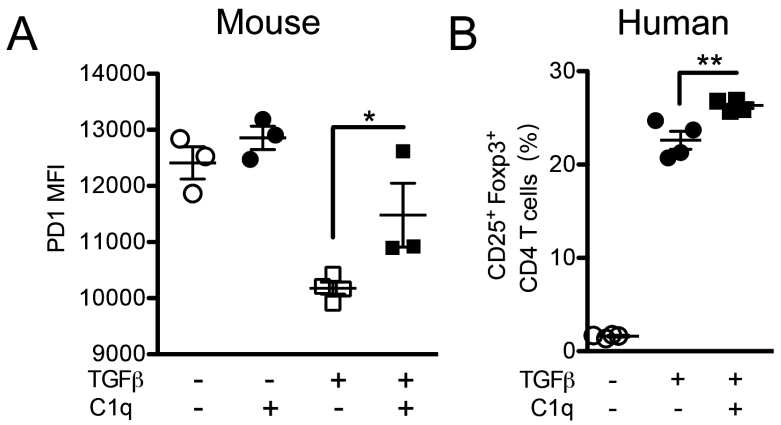
The heatmap of differentially regulated complement pathway genes. CD1a^{+} APCs isolated from control and emphysema patients were used to generate the heatmap from GSE26296 microarray dataset (N=6; 3 emphysema and 3 healthy controls). Briefly, lung CD1a^{+} APCs were isolated and RNA samples were collected for the analysis. Genes that fall into the complement cascade (Reactome Id: R-HSA-166658.2) are shown in the complied heatmap.

Supplementary Figure 2



Reduced C1q in emphysema and correlation with lung function (**A**) The linear regression was used to determine the correlation between plasma C1q concentration and airway obstruction as measured by forced expiratory volume in 1 sec (FEV1) % (N=108), (**B**) ratio of FEV1 over forced vital capacity (FVC) (N=108), (**C**) % emphysema as measured by low attenuation area (%LAA) determined by chest CT quantification (N=48). (**D**) Concentration of plasma C1q in patients categorized by the GOLD stages (N=8 in GOLD I; N=23 in GOLD II; N=22 in GOLD III&IV). P values were determined by the Mann–Whitney nonparametric test, *P<0.05.

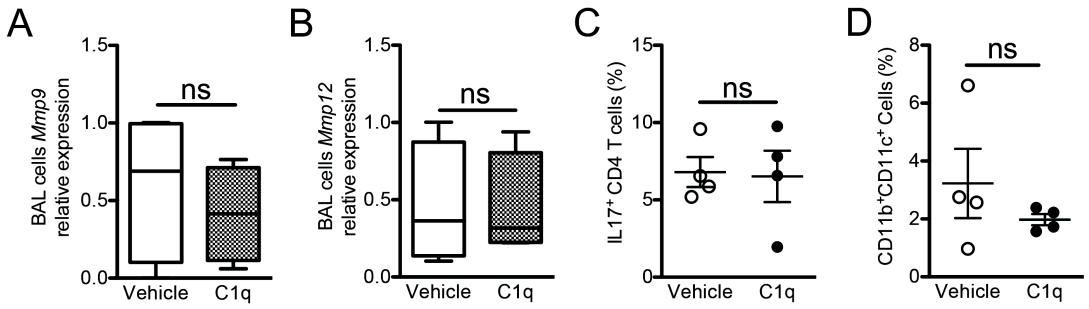
Supplementary Figure 3



The effect of C1q in CD4$^+$ T cell differentiation in vitro

(**A**) Murine splenic CD4$^+$ T cells were differentiated under Treg condition (TGFβ, anti-IL-4, anti-IFN-γ) for three days. Mean fluorescent intensity of PD1 was measured in CD25$^+$Foxp3$^+$ cells (N=3 or 4). Results are represented as mean±s.e.m, representative of two independent experiments. P values were determined by one-way ANOVA test with a Bonferroni's multiple comparisons. (**B**) Naïve human CD4$^+$ T cells were isolated from PBMC and cultured under Treg condition for 10 days. Population of Treg in the culture was identified by flow cytometry staining of CD25 and Foxp3 (N=4 in each group). Results are represented as mean±s.e.m, representative of three independent experiments. P values were determined by the Mann–Whitney nonparametric test, *P<0.05, **P<0.01.

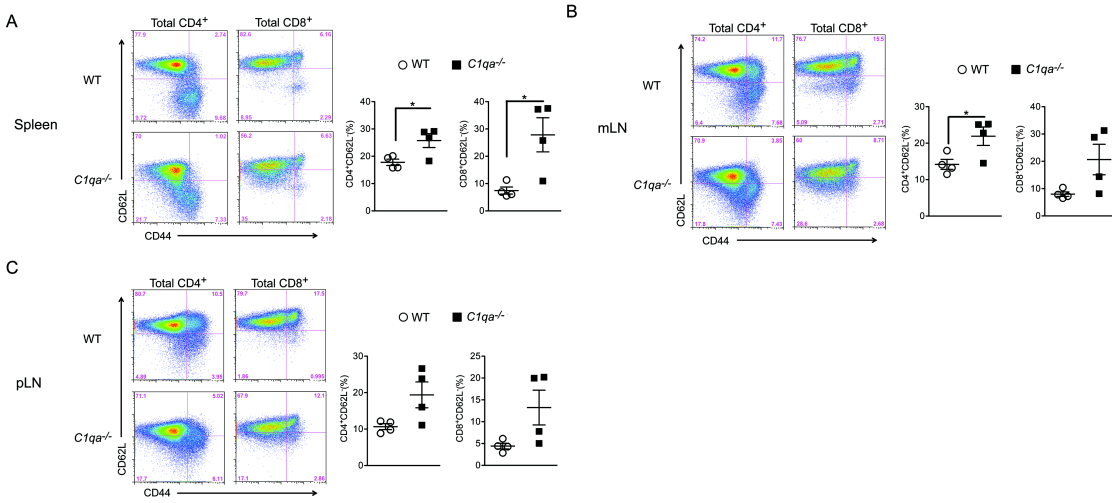
Supplementary Figure 4



Intranasal C1q does not cause lung inflammation

8-week-old WT naïve mice were treated with intranasal C1q (20ug, twice per week) for three weeks. Expression of *Mmp9* (**A**) and *Mmp12* (**A**) in BAL cells was measured by quantitative reverse transcription PCR (qPCR) (normalized to 18S); N=4 in each group. Box: median and interquartile range; whiskers: min to max range, representative of two independent experiments. (**C**) Cumulative flow cytometry analysis showed the population of $IL17^+$ $CD4^+$ T cells in the lungs. N=4 in each group. Results are represented as mean±s.e.m, representative of two independent experiments. (**D**) Cumulative flow cytometry analysis showed the population of $CD11b^+$ $CD11c^+$ dendritic cells in the lungs. N=4 in each group. Results are represented as mean±s.e.m, representative of two independent experiments. P values were determined the student t-test.

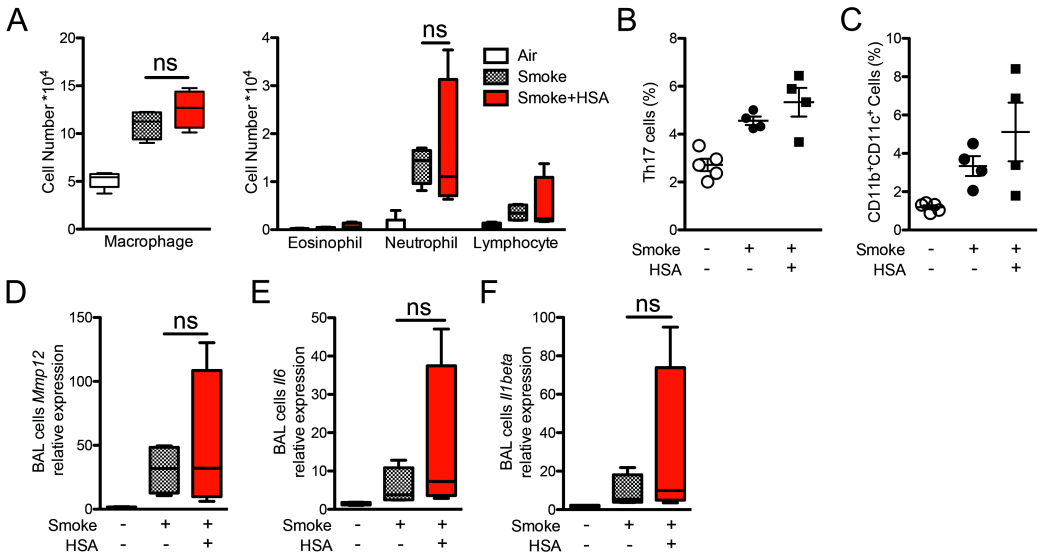
Supplementary Figure 5



Increased T cell activation in peripheral lymphoid organs in 5-month-old C1qa$^{-/-}$ mice

Total cells from spleen (**A**), mesenteric lymph node (**B**) and peripheral lymph nodes (**C**) were isolated from 5-month-old WT or *C1qa*$^{-/-}$ mice (N=4 in each group). Representative of flow cytometry analysis and summarized population of CD4^{+}CD62L^{-} and CD8^{+}CD62L^{-} cells were shown. Results are represented as mean±s.e.m, representative of two independent experiments. P values were determined the student t-test, *P<0.05.

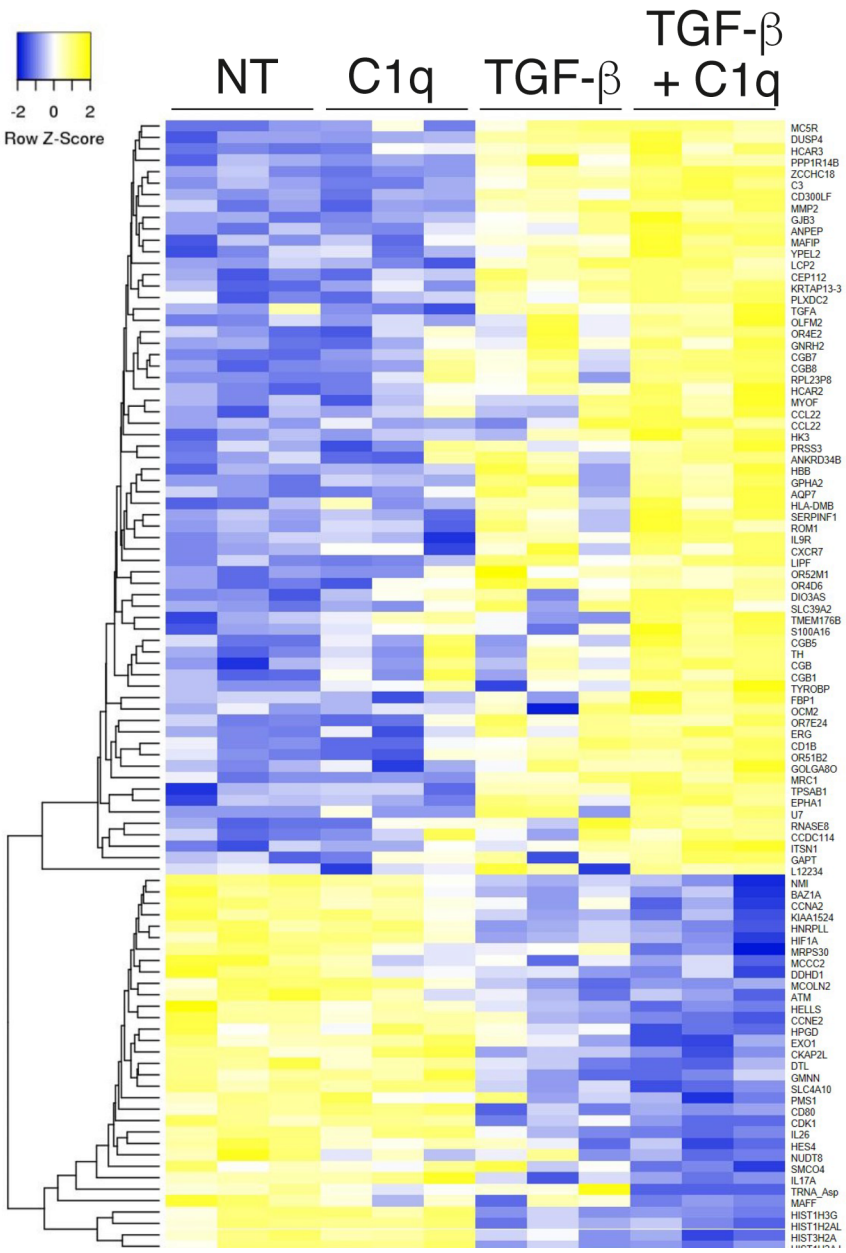
Supplementary Figure 6



Human serum albumin (HSA) has no effect on murine emphysema

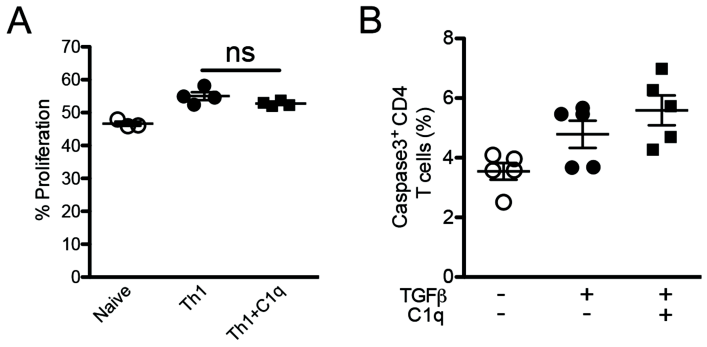
Wild type (WT) mice were exposed to cigarette smoke or air for 6 month. Intranasal HSA was given to smoke-exposed mice (20ug, twice per week) for a total of 6 weeks prior to the sacrifice at 6 months. (**A**) Bronchoalveolar Lavage (BAL) fluid analyses showing macrophages (Mac), neutrophils (Neu), eosinophil (Eos) and lymphocytes (Lymph). N=5 in Air group; N=4 in Smoke and Smoke+HSA. Box: median and interquartile range; whiskers: min to max range, representative of two independent experiments. (**B-C**) Cumulative intracellular cytokine staining of Th17 cells and $CD11b^{+}CD11c^{+}$ dendritic cells. N=5 in Air group; N=4 in Smoke and Smoke+HSA. Results are represented as mean±s.e.m, representative of two independent experiments. (**D-F**) Expression of *Mmp12, Il6 and Il1β* mRNA in BAL cells were measured by quantitative reverse transcription PCR (qPCR) (normalized to 18S N=5 in Air group; N=4 in Smoke and Smoke+HSA. Results are represented as mean±s.e.m, representative of two independent experiments. P values were determined by a one-way ANOVA test with a Bonferroni's multiple comparisons.

Supplementary Figure 7



RNA-Seq and pathway analysis of C1q treated CD4+ T cells. Human $CD4^+$ T cells were isolated from PBMC and cultured with vehicle (no treatment; NT), C1q, TGFβ with or without C1q for 48 hours. Total RNA was isolated from the cells and subjected to RNA-Seq using standard protocols. Unsupervised Heatmap clustering of 125 genes differentially expressed transcripts in C1q + TGF-β (with $p<0.01$ and 1.4-fold change) compared to NT, C1q, TGF-β groups. Row Z-Score shown in the upper left area.

Supplementary Figure 8



Impact of C1q on T cell proliferation and apoptosis

(**A**) Murine splenic $CD4^+$ T cells were labeled with CFSE and differentiated under Th1 condition (IL-2, IL-12 (p70), anti-IL-4) for three days with or without 20ug/ml C1q. Proliferation was quantified using CFSE dilution (N=3 in Naïve; N=4 in Th1 and Th1+C1q). Results are represented as mean±s.e.m, representative of three independent experiments. (**B**) Murine splenic $CD4^+$ T cells were differentiated under Treg condition (TGFβ, anti-IL-4, anti-IFNγ) for three days. Expression of caspase3 in $CD4^+$ T cells was measured by flow cytometry (N=5); results are represented as mean±s.e.m, representative of three independent experiments. P values were determined by a one-way ANOVA test with a Bonferroni's multiple comparisons.