Supporting Information

Proteomic Analysis of Plasma-derived Extracellular Vesicles in Smokers and Patients with Chronic Obstructive Pulmonary Disease

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Supplementary Figure Legends:

Figure S1. Representative sample of plasma-derived EVs isolated by ExoSpin method was split into two to compare before and after depletion of IgG. Lane 1: Protein molecular weight marker; Lane 2: EV lysate before IgG depletion; Lane 3: EV lysates passed through slurry of Protein G sepharose fast flow prior to running the gel.

Figure S2. Hierarchical clustering analyses of plasma-derived EV peptide and PSMs counts. (A) Intensity heatmap of peptide counts identified by LC-MS/MS proteomics. (B) Intensity heatmap of PSM counts identified by LC-MS/MS proteomics.

Figure S3. Hierarchical clustering analyses of plasma-derived EVs based of peptide counts. Intensity heatmap of PSM counts for significant proteins identified by LC-MS/MS proteomics among (A) Non-smokers vs. Smokers. (B) Non-smokers vs. COPD and (C) Smokers vs. COPD groups.

Figure S4. STRING protein-protein interaction (PPI) network using the proteins identified in this study vs. Top 100 EV markers from plasma proteome database (PPD). (A) String PPI network connectivity of human plasma-derived EV protein cluster compared between non-smokers vs. smokers based on peptides. Network contains 29 edges (vs. 1 expected edges); clustering coefficient 0.53; enrichment p-value < 0.001. Confidence score threshold was set at 0.4 (medium) for the analysis. (B) String PPI network connectivity of human plasma-derived EV protein cluster compared between smokers vs. COPD based on peptides. Network contains 49 edges (vs. 4 expected edges); clustering coefficient 0.47; enrichment p-value < 0.001. Confidence score threshold was set at 0.4 (medium) for the analysis.

Figure S5. Full membrane used for slot-blot analysis of plasma-derived EVs surface markers. Equal volume of lysed EV proteins were first loaded into nitrocellulose membranes using slot blot apparatus before probing for EV surface markers (proteins) such as TSG101, Rab-5b and Alix using target specific antibodies.

Figure S1

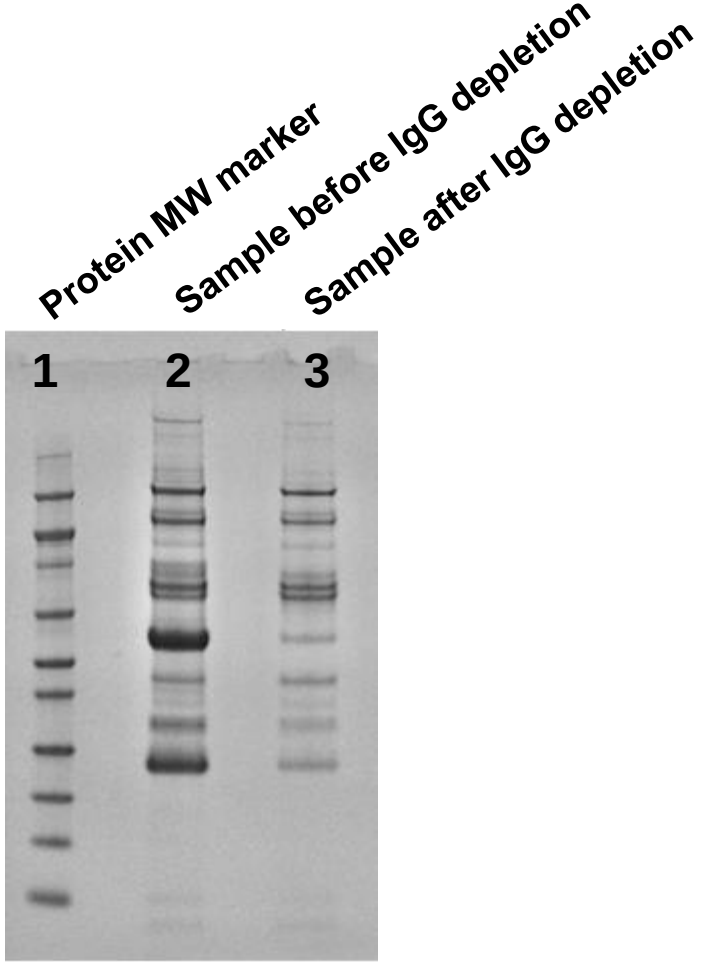

Protein MW marker
Sample before IgG depletion
Sample after IgG depletion
1
2
3

Figure S2

Hierarchical cluster analysis of peptides and PSMs identified by LC-MS/MS

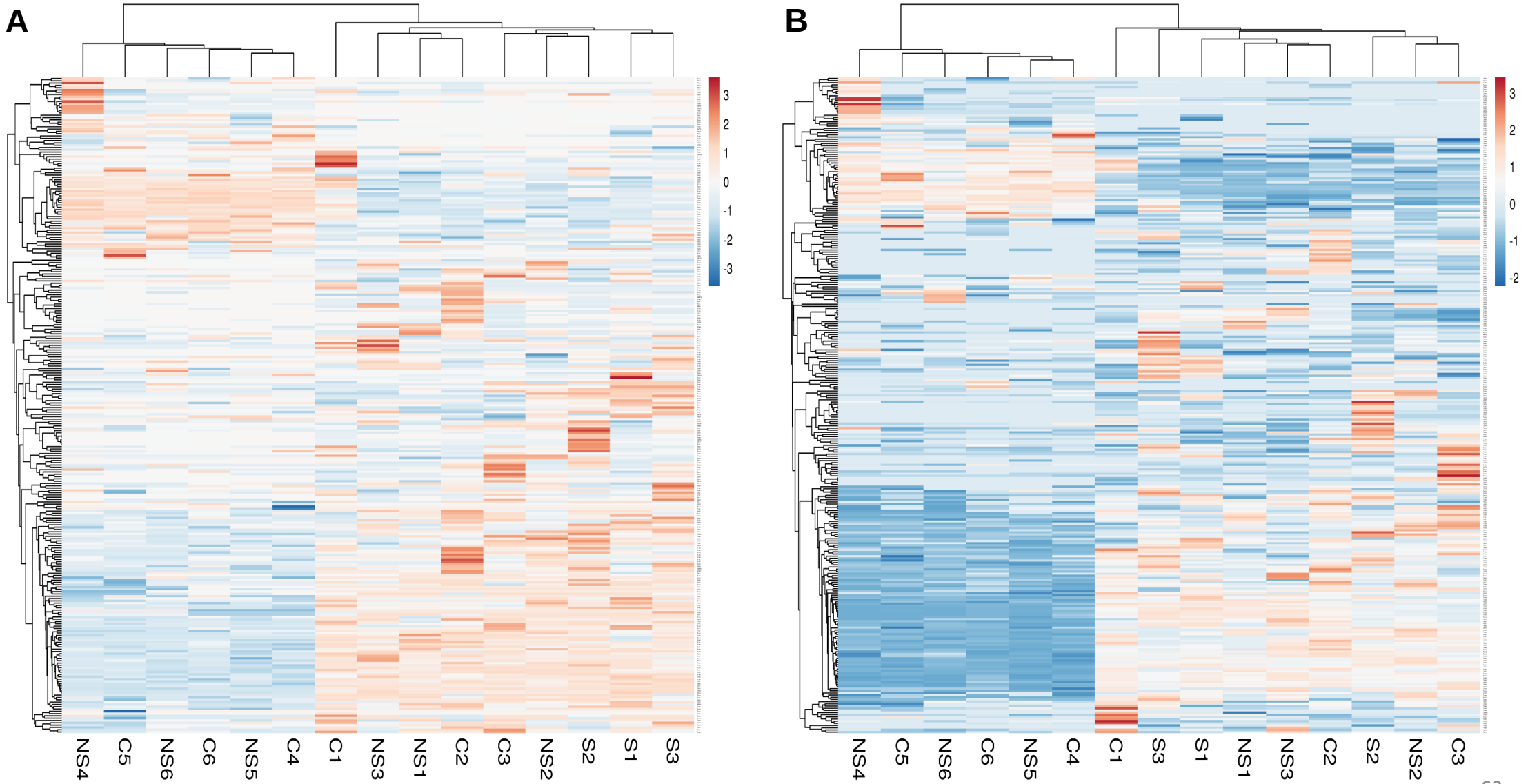


Figure S3

Hierarchical cluster analysis of significant peptides identified by LC-MS/MS

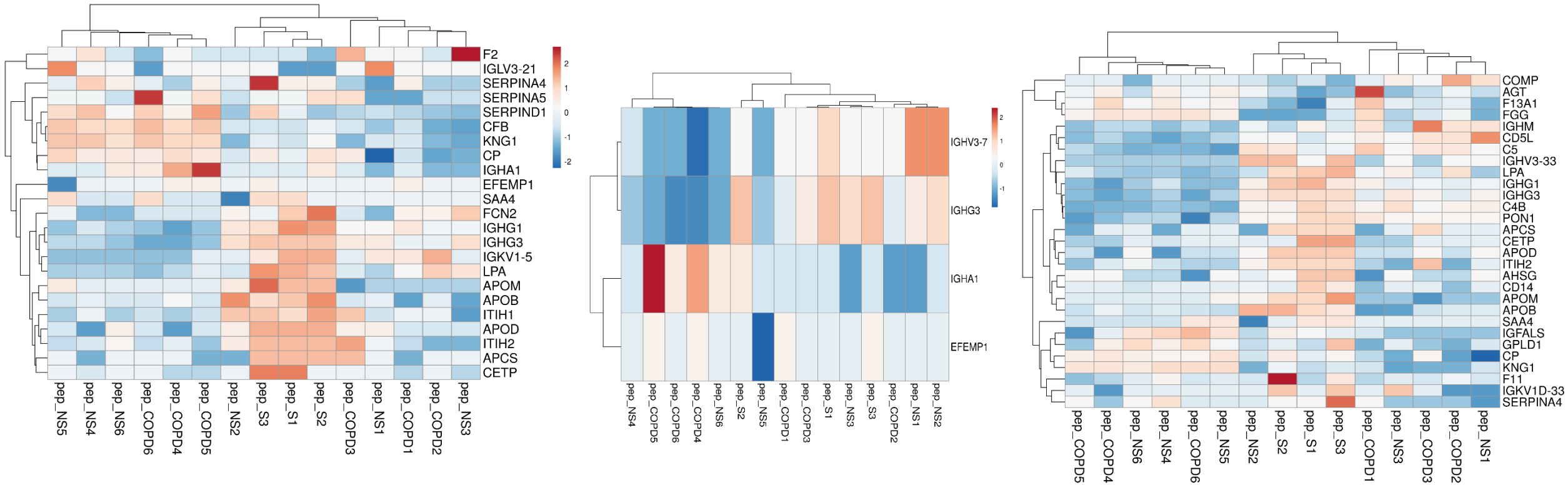
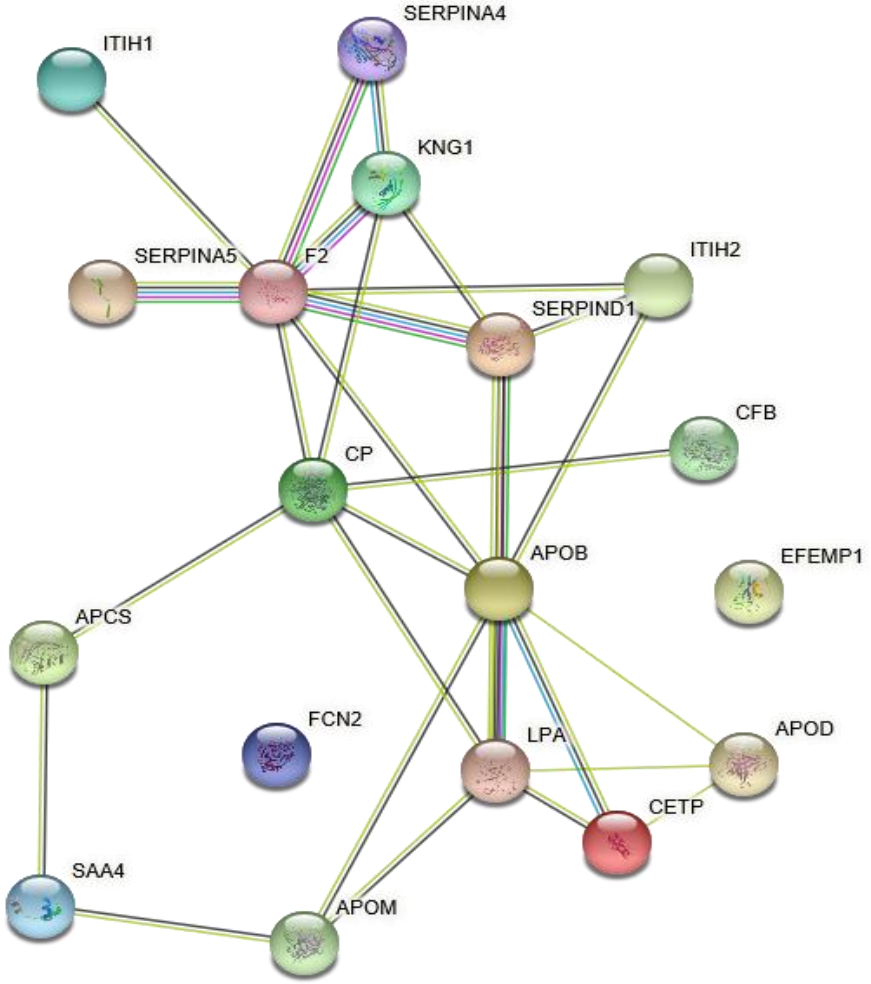


Figure S4

STIRNG: network analysis based significant peptides identified by LC-MS/MS

A Non-smokers vs. Smokers



B Smokers vs. COPD

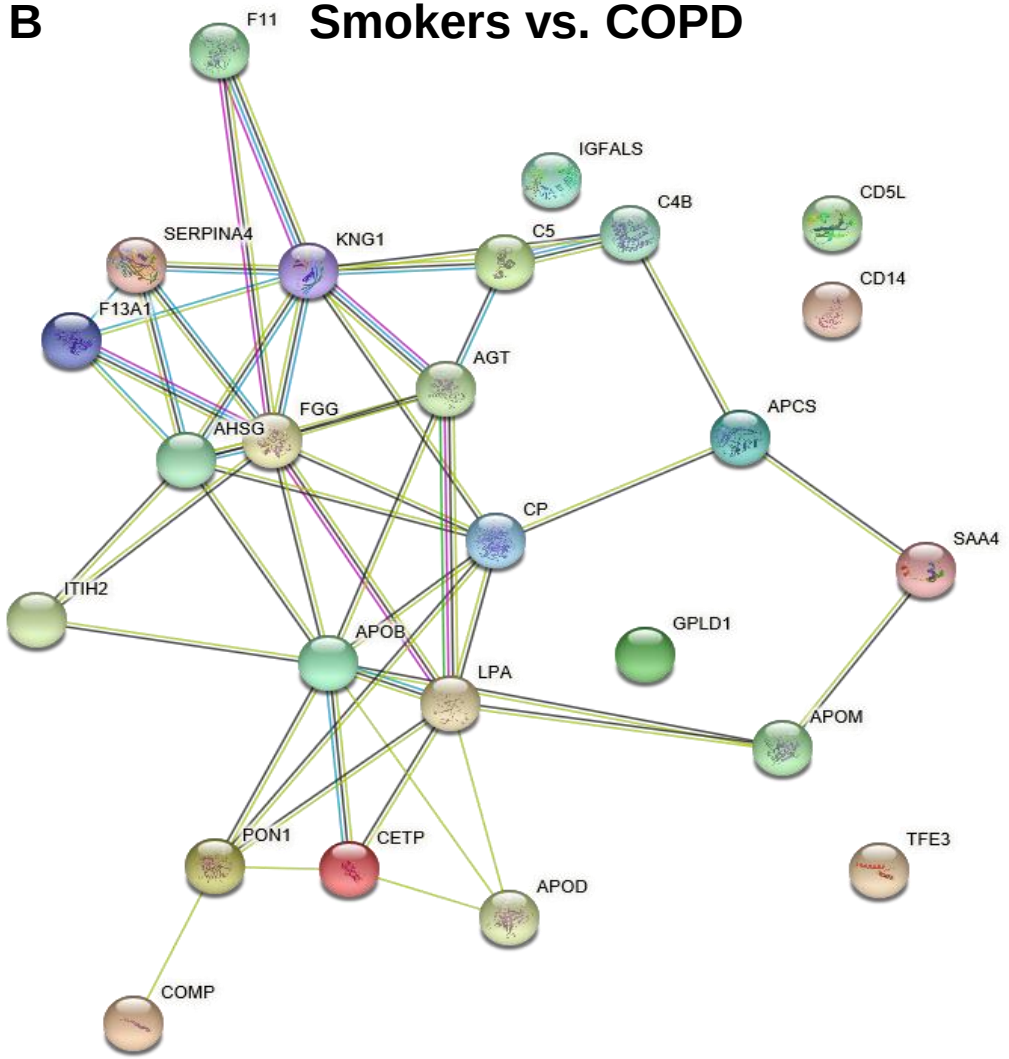


Figure S5