

DISCRIMINATIVE PROTEIN MARKERS OF SECOND-HAND SMOKE EXPOSURE IDENTIFIED BY SHOTGUN PROTEOMICS

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Background:

- Second-hand smoke (SHS) is a major indoor environmental pollutant, generated mainly from cigarette combustion.
- SHS is a complex mixture of toxic chemicals that poses serious health risks to non-smokers¹.
- SHS chronic exposure has been linked to tobacco-related diseases, yet the molecular mechanisms of SHS-induced pathogenesis remain unclear.
- Identifying biomarkers of response and risk is essential for advancing public health protection.

Objectives:

We conducted a proteomic study in healthy non-smokers restaurant employees occupationally exposed to SHS, aiming to uncover differentially abundant proteins that best discriminated SHS exposed individuals. The objective was to discover molecular signatures that could serve as early indicators of risk and/or response to SHS exposure.

Methods:

- Protein extracts from nasal samples and plasma from healthy, non-smoking restaurant workers (exposed, n=21; not exposed, n=19) were analyzed by mass spectrometry-based proteomics through a shotgun approach (see Fig.1).
- Independent Samples Mann-Whitney U Test was applied for group comparisons to address the differences between the protein's abundances ($\alpha=0.05$).
- Proteomic data was integrated in to molecular pathways by functional enrichment term analysis.
- A generalized linear model with binomial distribution was employed to identify the differentially abundant proteins (nasal epithelium and plasma), that best discriminated SHS exposed *versus* not exposed individuals. A model was fitted for each individual protein. A 2nd model was fitted using the top 3 proteins ($p<0.05$ and $AUC>0.72$). ROC curves were used to evaluate each model performance.

Results:

- In the nasal epithelium, SHS exposure was associated with modulation of proteins involved in HIF1 α -regulated glycolytic pathways and xenobiotic metabolism (see Fig.2).
- In plasma, SHS exposure was related to systemic inflammation and thrombosis (see Fig.3).
- Altogether the logistic regression identified the top 3 proteins: *Histidine-rich glycoprotein* (HRG), *Vitamin D-binding protein* (GC), and *Leucine-rich alpha-2-glycoprotein* (LRG1) - as significant discriminators of SHS exposure (Fig 4).

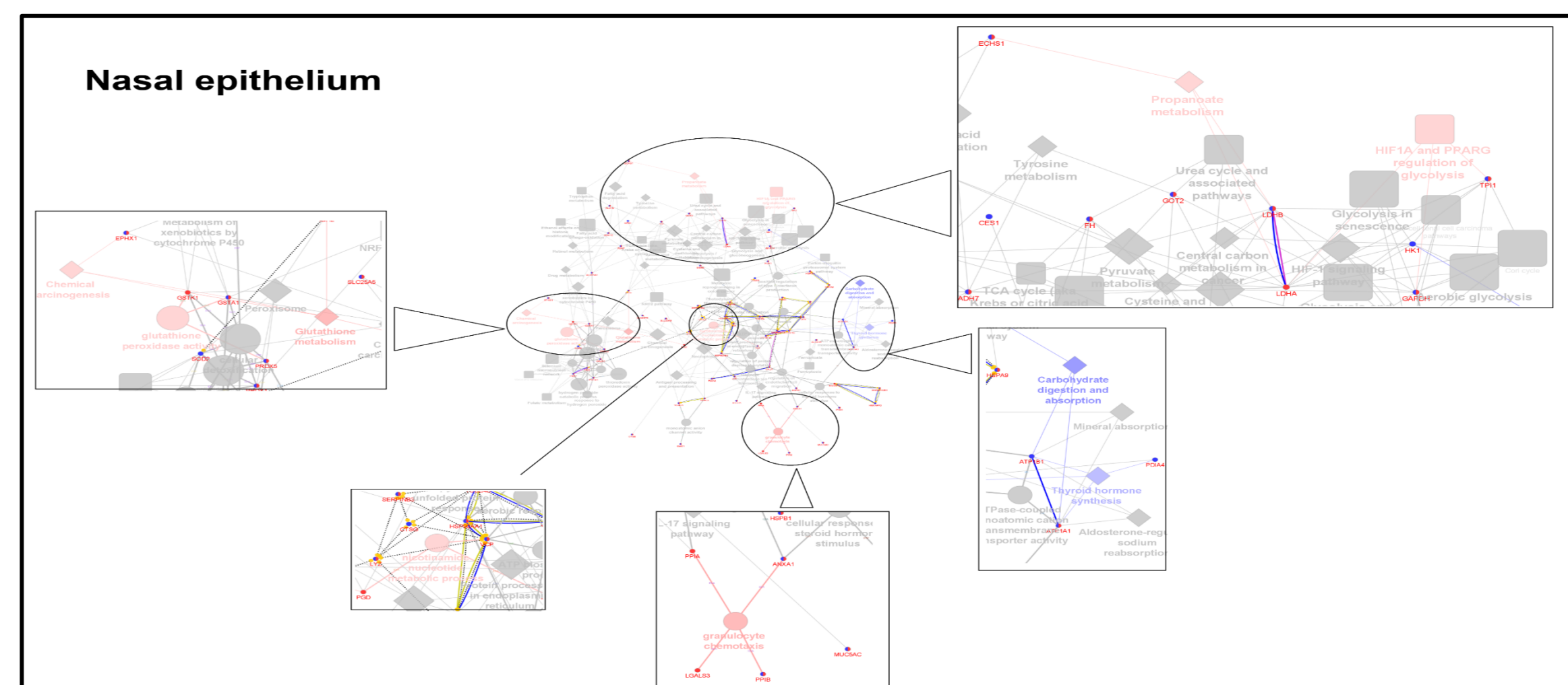
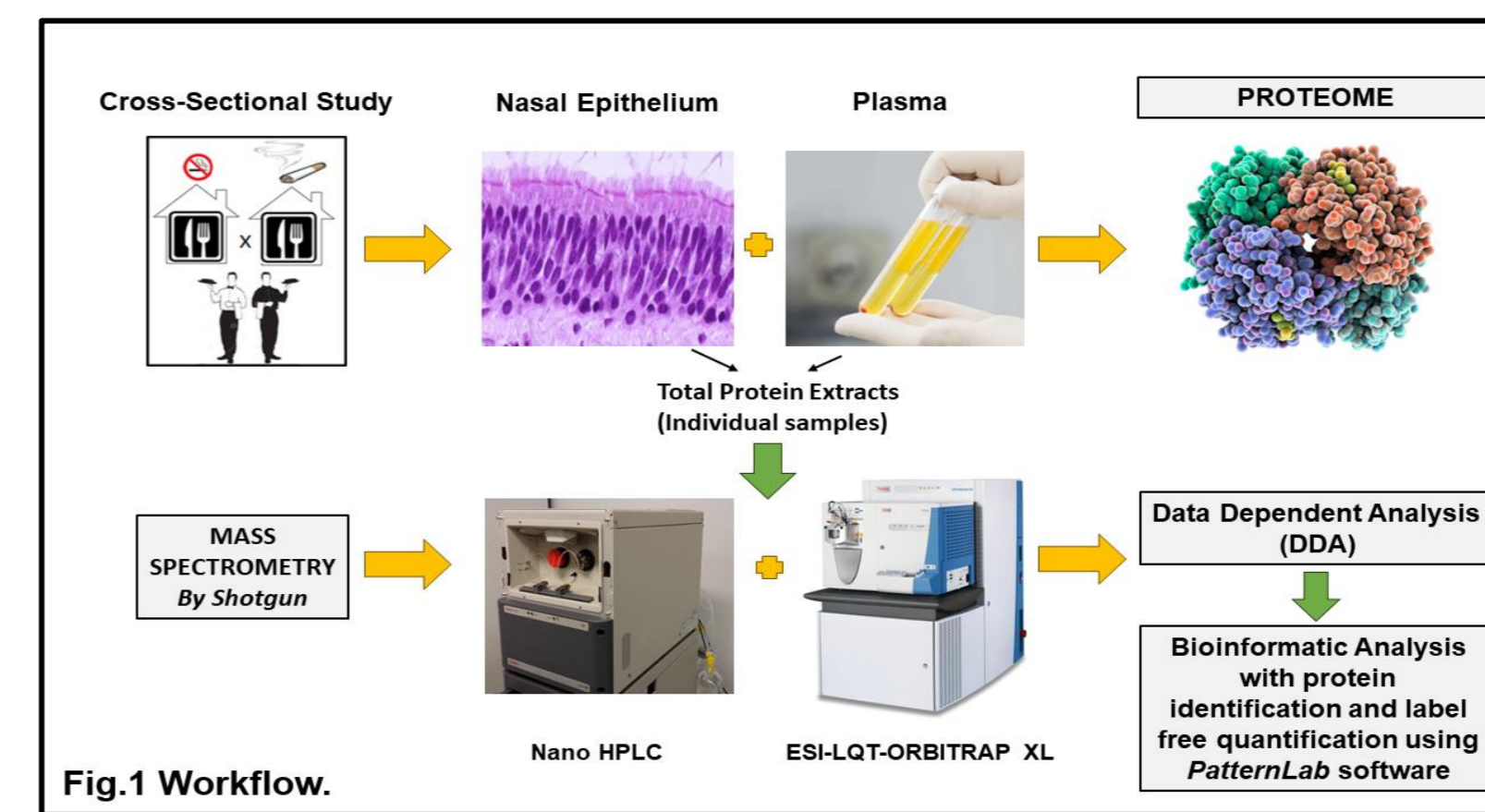


Fig. 2 Cluster Network Analysis of the pathways and biological processes associated with the proteome of Non-Smokers exposed (NSE) (red dots) versus the proteome of Non-Smokers not exposed (NS) (blue dots). The "diamond" shape represents a pathway from the data base KEGG (23.03.2022); the "circle" shape represents biologic processes from the Gene Ontology (GO) (03.03.2023); the "hexagon" shape represents the pathway from the Reactome data base (03.03.2023); and the "rectangle" shape represents the pathways from the Wikipathways (23.02.2022). The red shapes are NSE specific and the blue shapes are NS specific; the grey colour is common to both clusters of studied proteins. Connection between genes is coloured according with the type of action between the par: "binding" coloured by blue edges, "activation" arrow with green point, inhibition an arrow with red point, "reaction" dotted black edges, "catalysis" by yellow golden edges and "experimental data" by dark pink edges. Proteins are identified by their gene name.

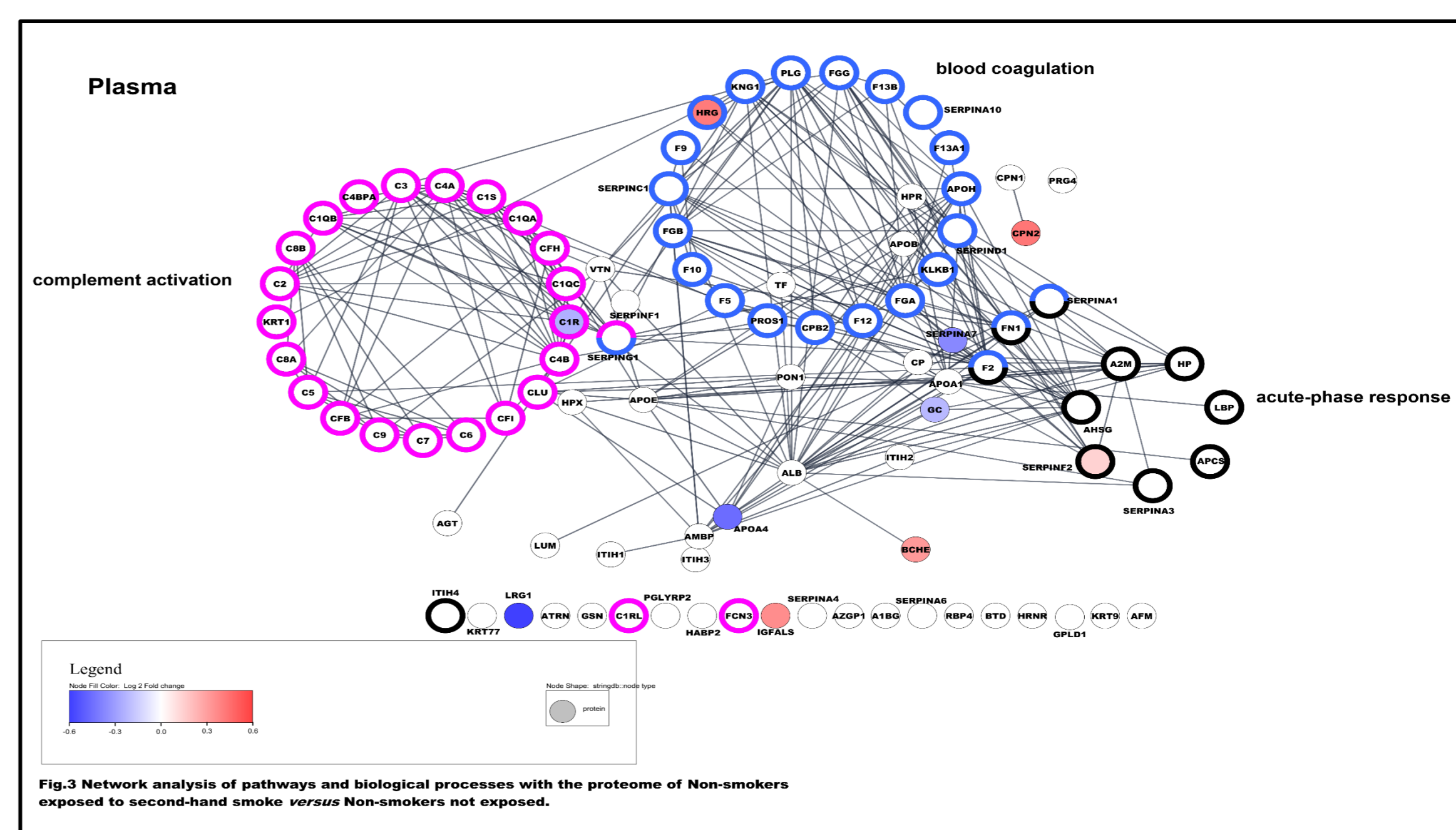
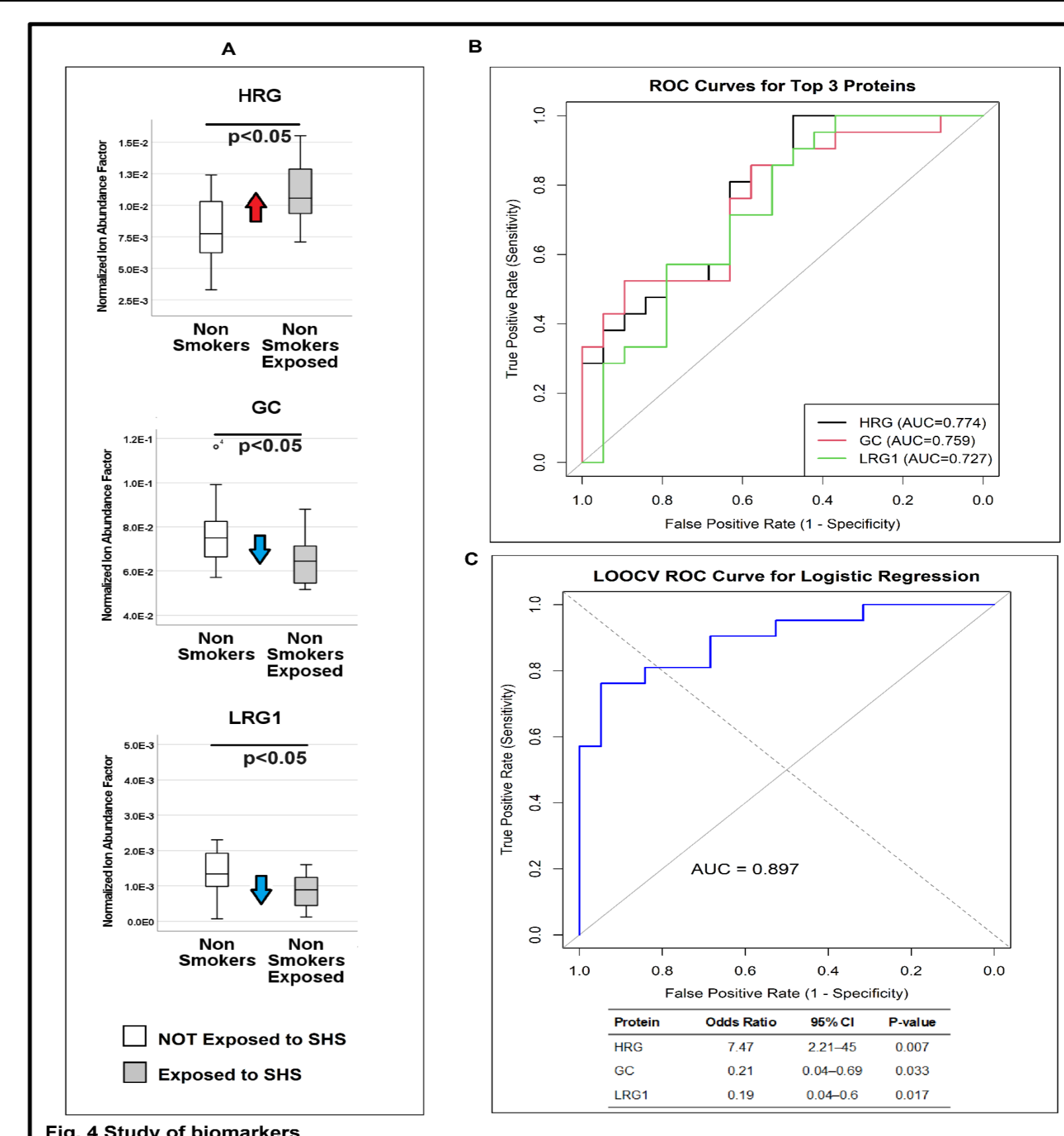


Fig.3 Network analysis of pathways and biological processes with the proteome of Non-smokers exposed to secondhand smoke versus Non-smokers not exposed.



Conclusions:

All the proteins identified in plasma: HRG, GC, and LRG1, are promising response biomarkers for SHS exposure ($AUC=0.90$). HRG and LRG1 are associated with immune response, GC with clearance of F-actin filaments released during cell damage. Their expression profiles may support the development of molecular tools for individual response assessment associated with SHS.

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References:

1. Carreras, G., Lugo, A., Gallus, S., Cortini, B., Fernández, E., López et al. Burden of disease attributable to second-hand smoke exposure: A systematic review. In Preventive Medicine .2019;129:105833. Academic Press Inc. <https://doi.org/10.1016/j.ypmed.2019.105833>