Bronchoalveolar Lavage Proteomics in Patients with Suspected Lung Cancer

Carvalho et. al. 2017

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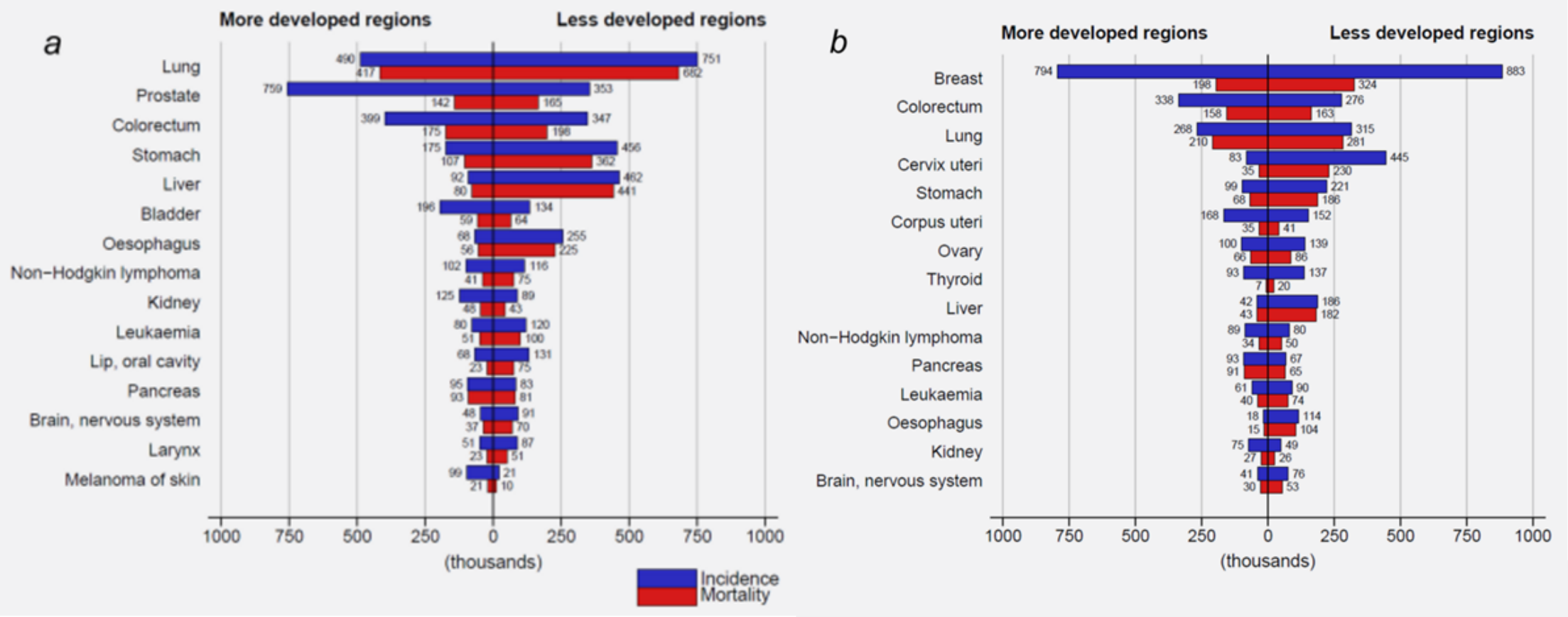
Outline

- Lung cancer and recent developments in non-invasive diagnosis
- Use of omics to identify cancerous from non-cancerous lesions
- Present study: attempt to extend methods to prospective study
- Findings and discussion about future directions
- Potential impact and caveats

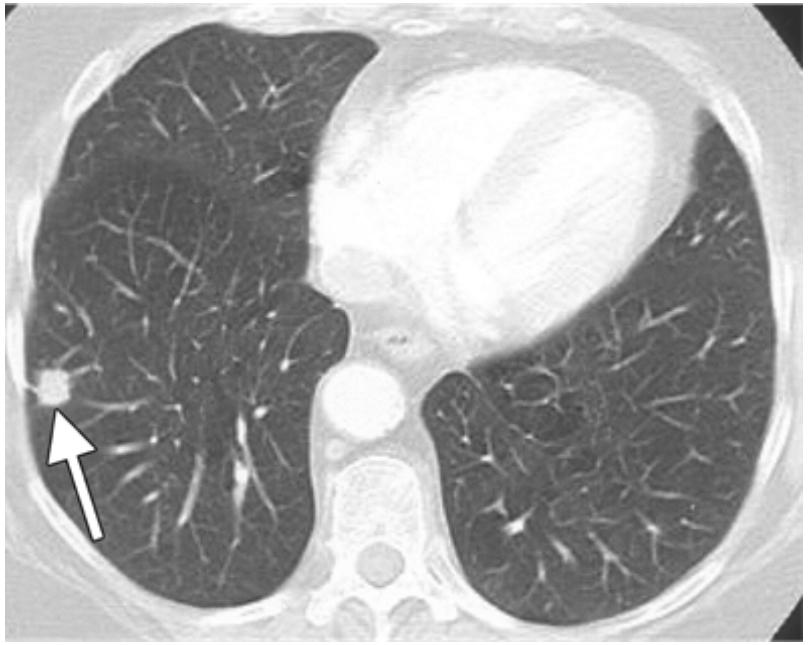
Some lung cancer stats



Ferlay et. al. 2015

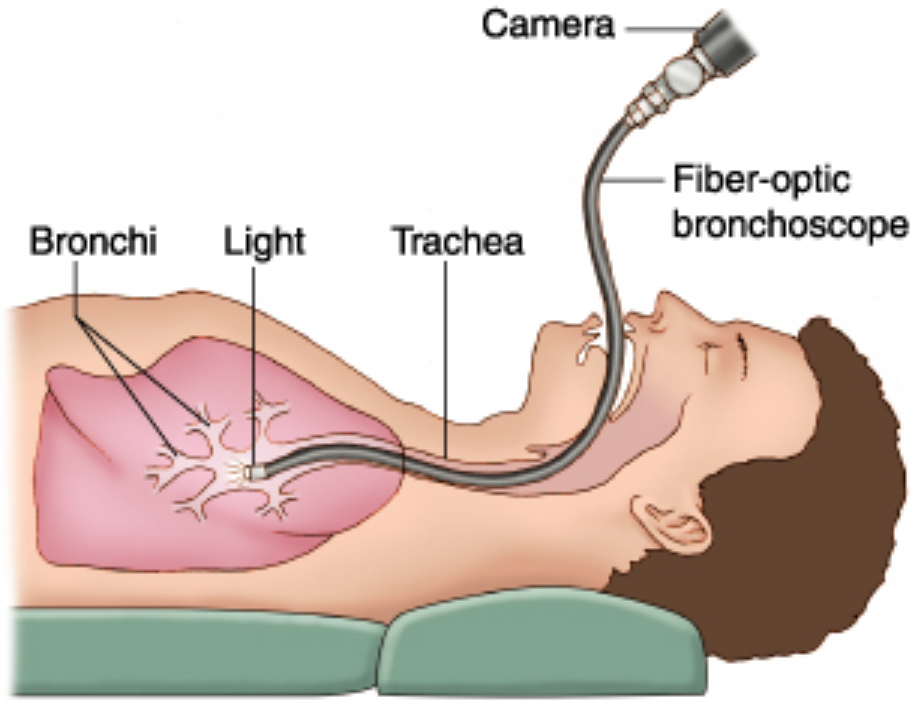
Diagnosis of lung cancer

- Advances in non-surgical diagnosis: transbronchial lung biopsy (TBLB), computed tomography (CT)
- Advances in earlier diagnosis
  - Chest radiography or low-dose CT scan
- Limitations
  - TBLB takes several samples, could be less representative
  - TBLB may miss lesions in peripheral areas of the lung
- Combining these traditional bronchoscopic diagnostics with BAL increases diagnostic rate

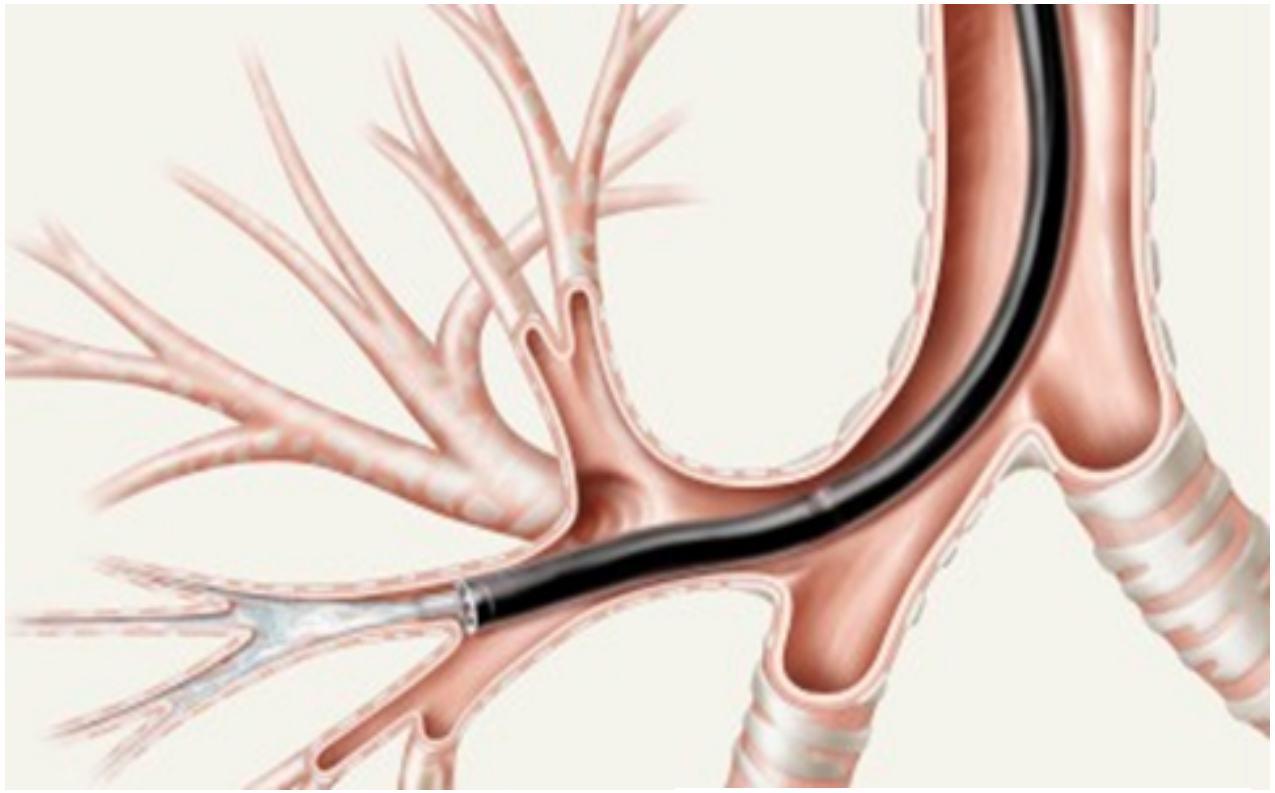


http://rizwan-nurani.com/lung-cancer-diagnosis-.html

What is bronchoalveolar lavage (BAL)?



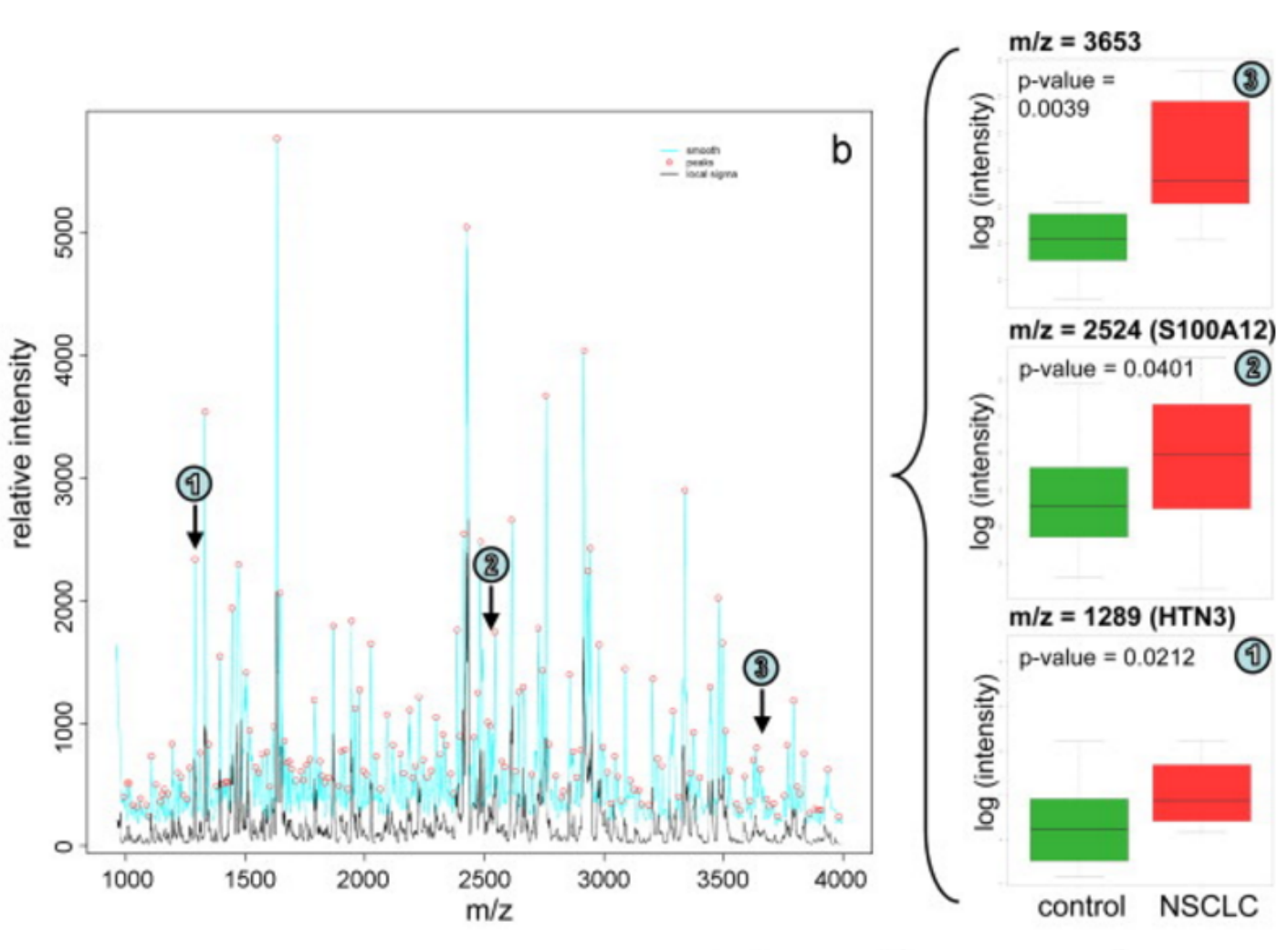
http://www.mdguidelines.com /bronchoscopy



https://www.olympus-europa.com/

Why BAL?

- Less invasive than surgery
- Can get a large amount of information from the samples
- Genomic and proteomic analysis have been established
- Highly specific although less highly sensitive



Oumeraci et. al. 2011

Objectives of this study

- Use BAL to analyze potential cases of lung cancer *prospectively*
- Identify further areas to explore for increased diagnosis rate

Study participants

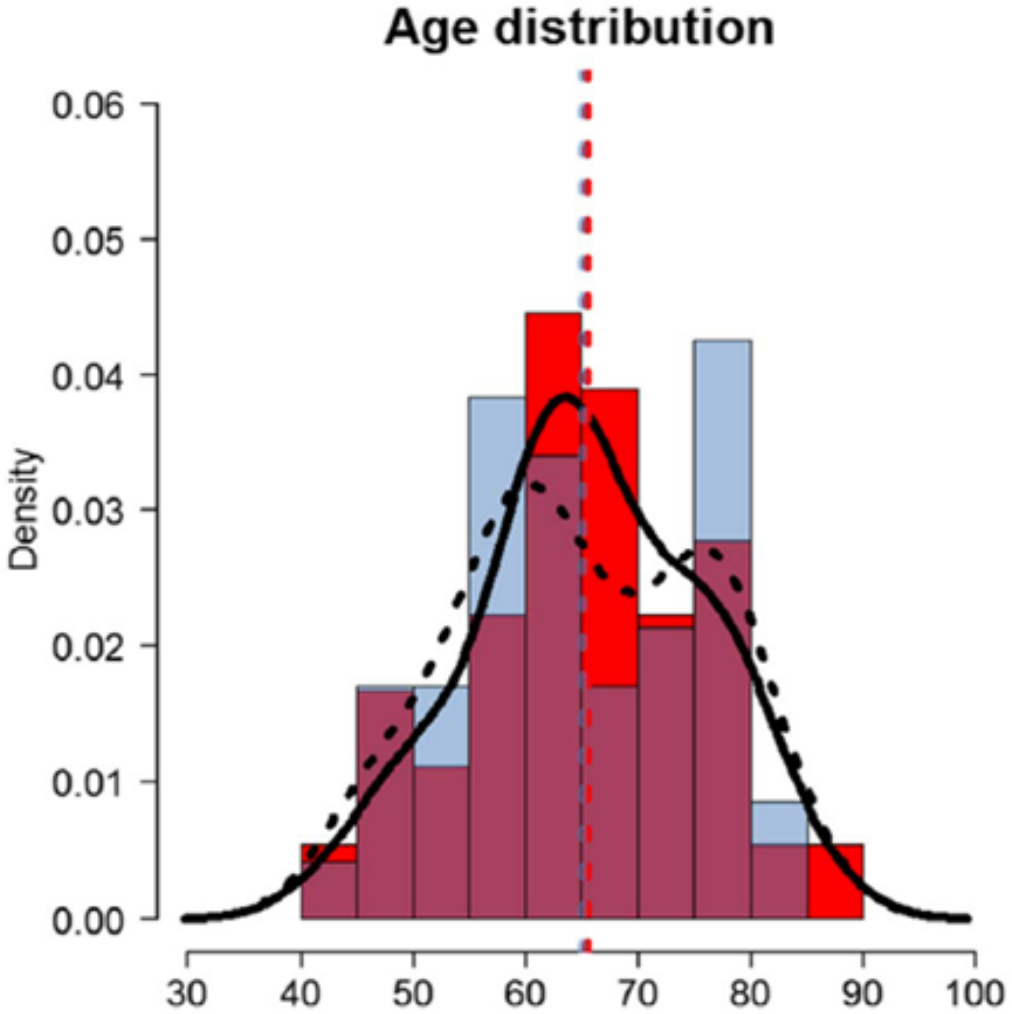
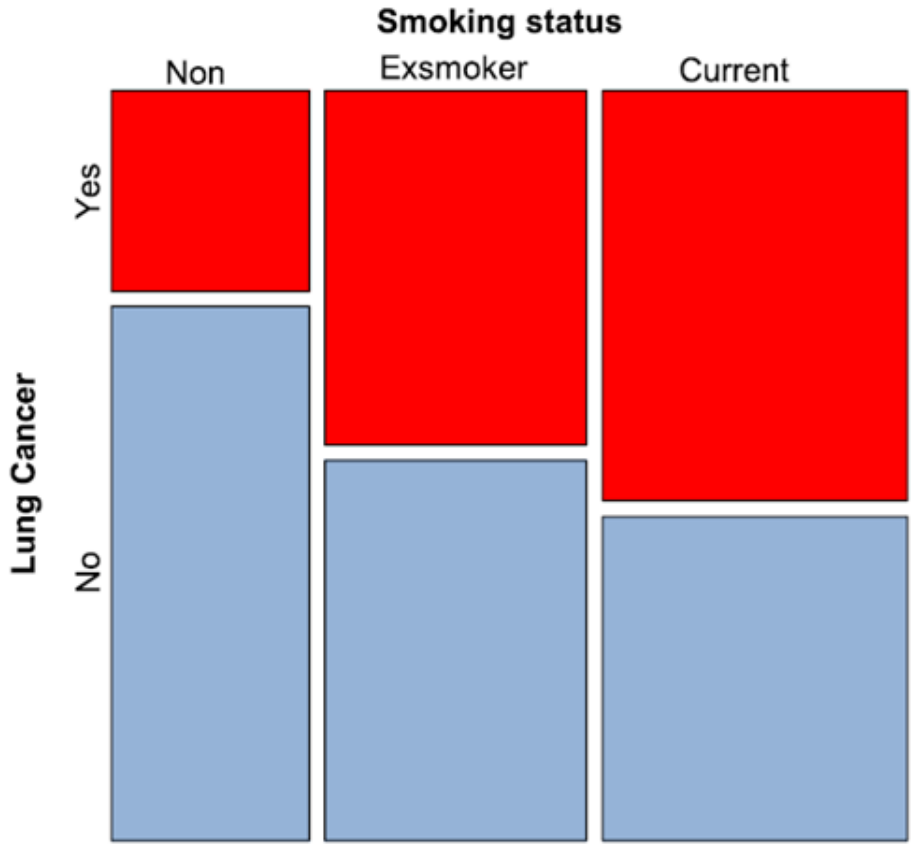
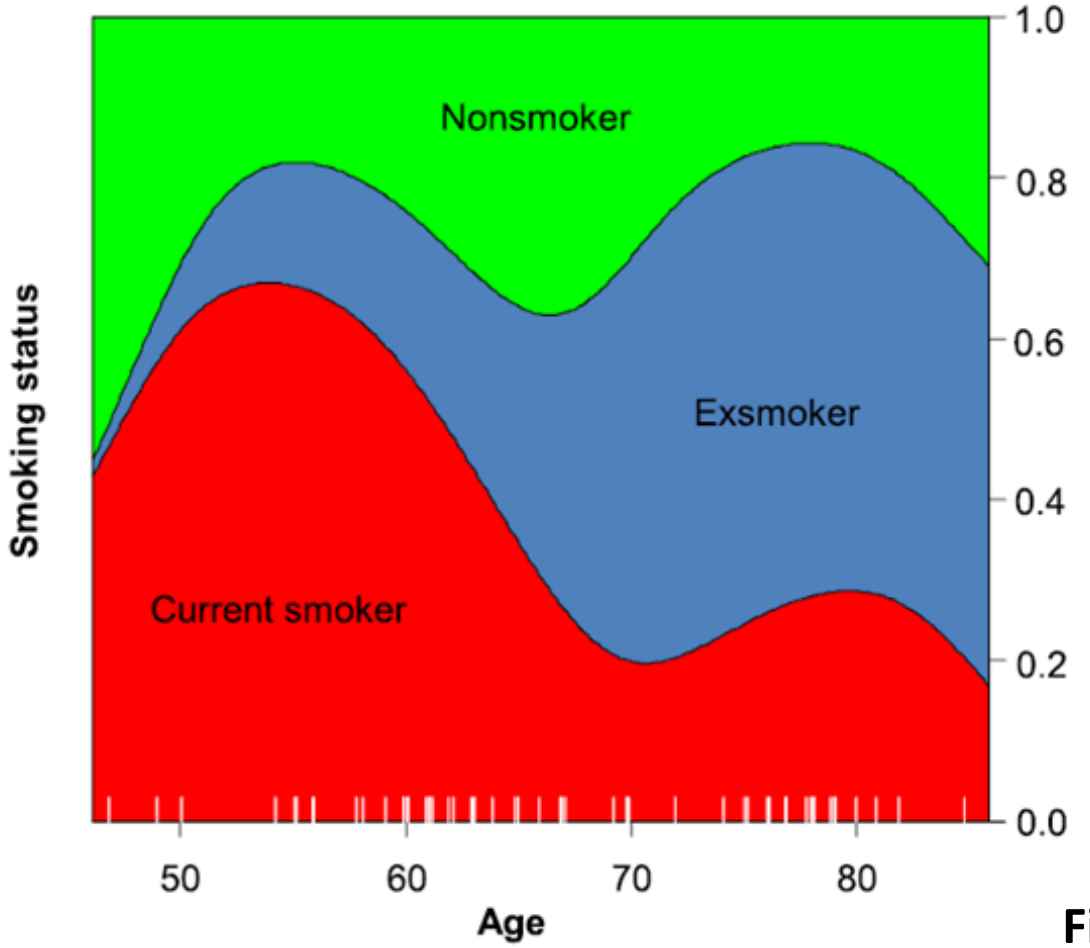


Figure 1

Study participants





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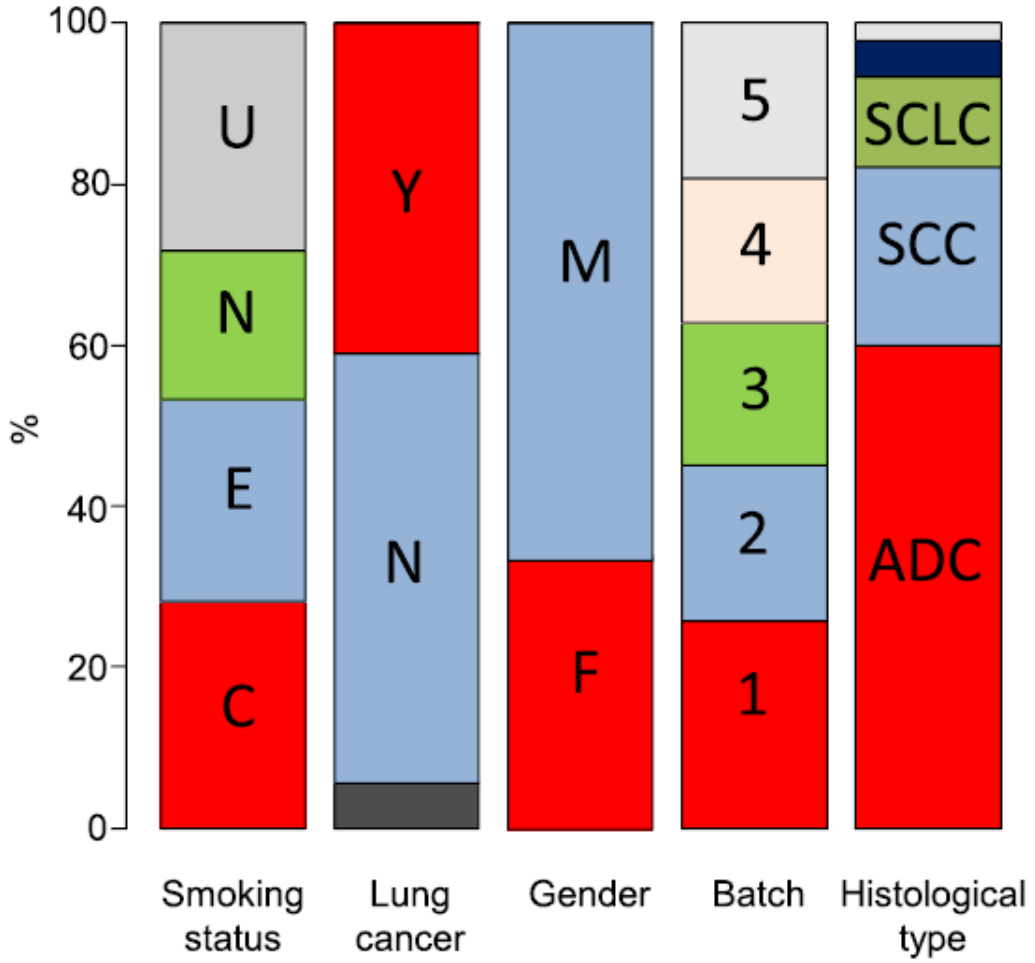
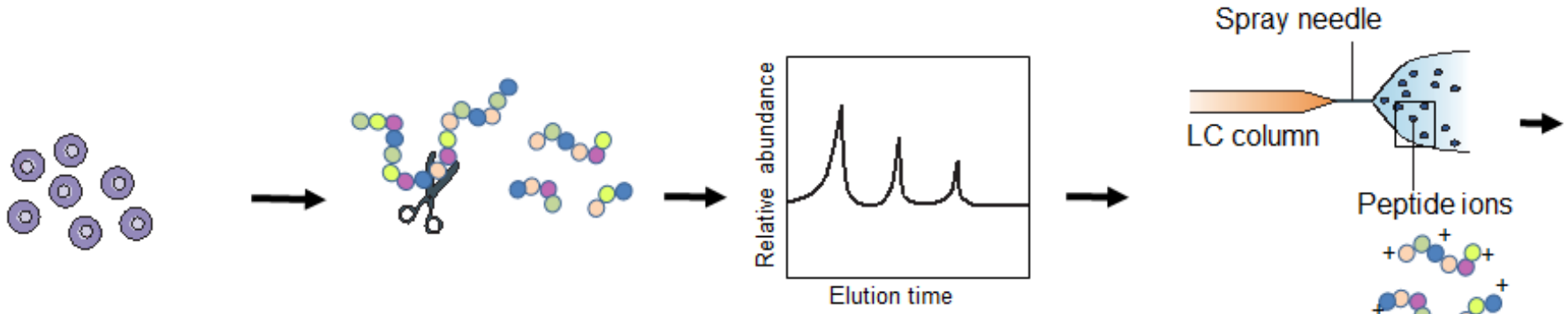
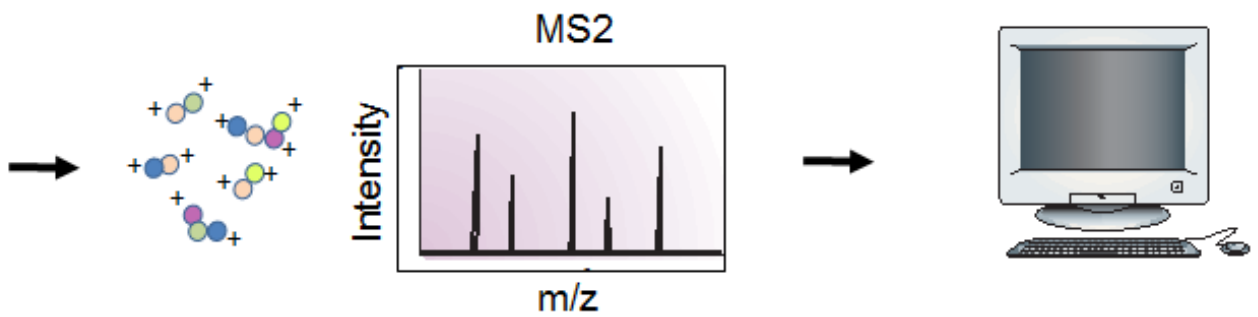


Figure 1

Liquid Chromatography Tandem Mass Spectrometry



1. Protein isolation and purification
2. Trypsin digest
3. Peptide separation by liquid chromatography (LC)
4. Peptide ionization

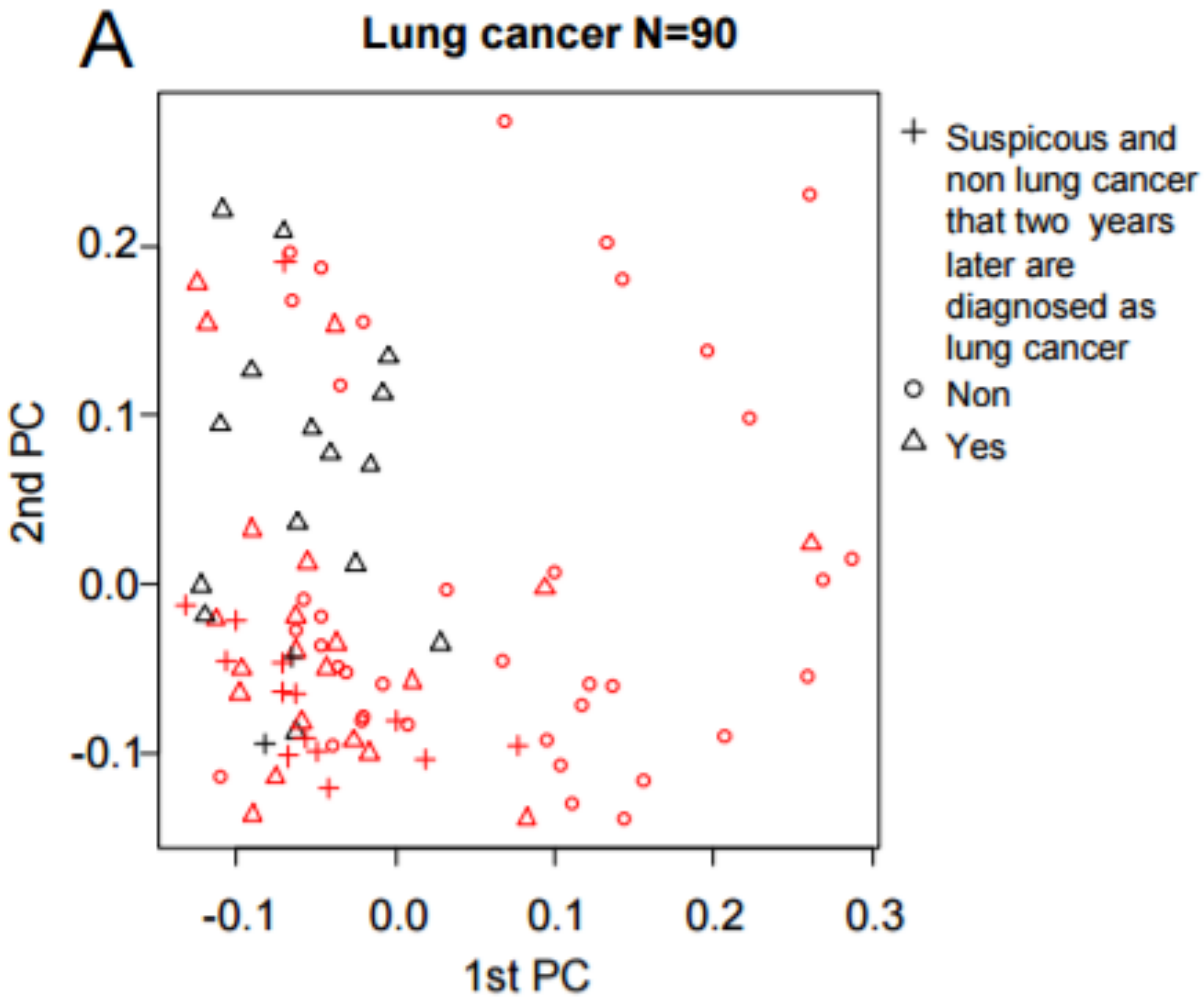


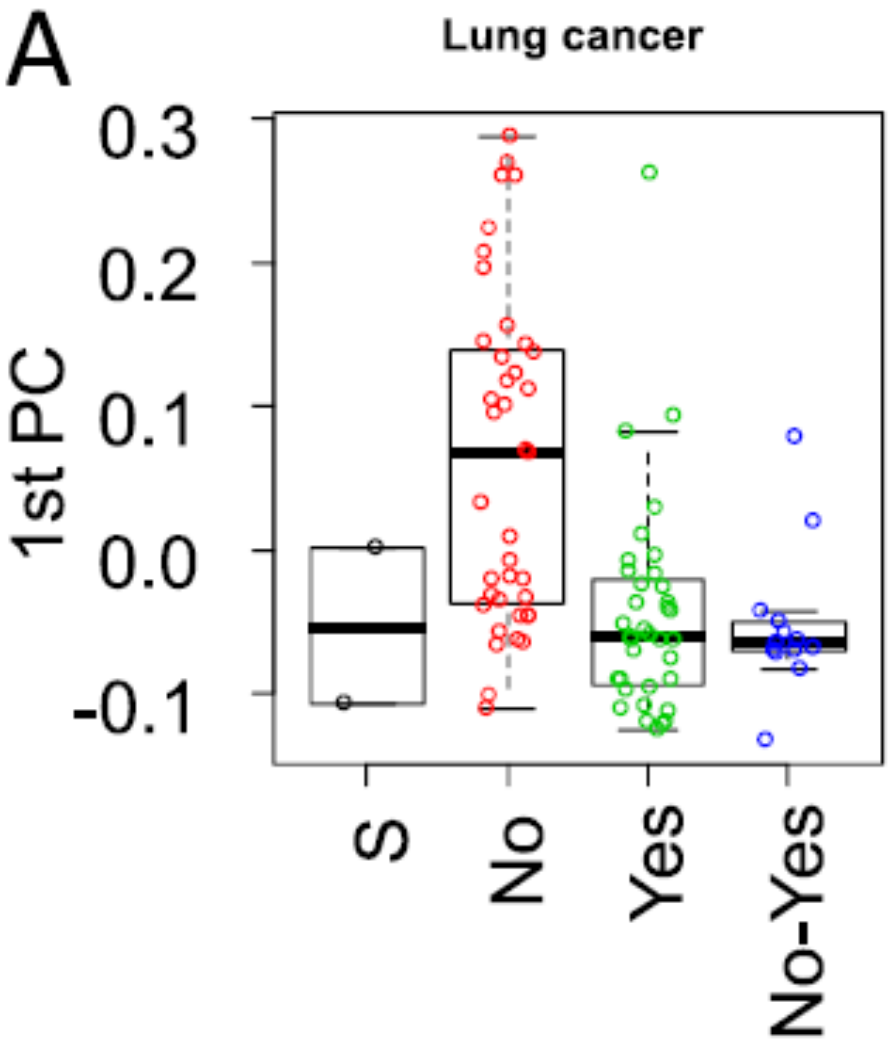
5. Peptide ion fragmentation and detection by mass spectrometer (MS2 scan)
6. Data analysis
   - compare MS2 scans to database

Liquid Chromatography Tandem Mass Spectrometry

- Protein isolated from bronchoalveolar lavage fluid
- UPLC Column separation
- Tandem MS
  - Data dependent acquisition
  - Top 6 hits per scan ionized
  - 60 second dynamic exclusion
- Database matching – MaxQuant and VEMS
- Peptide/protein quantification
  - Spectral counts
  - Integration-based intensity values

Figure 2: PCA Analysis





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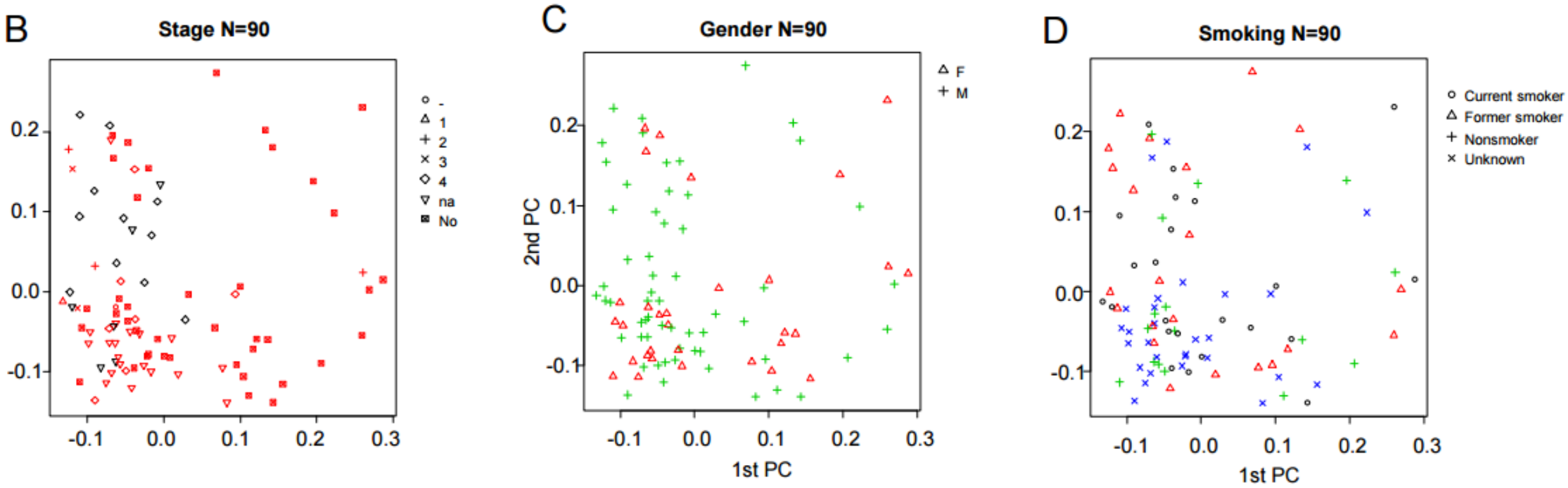

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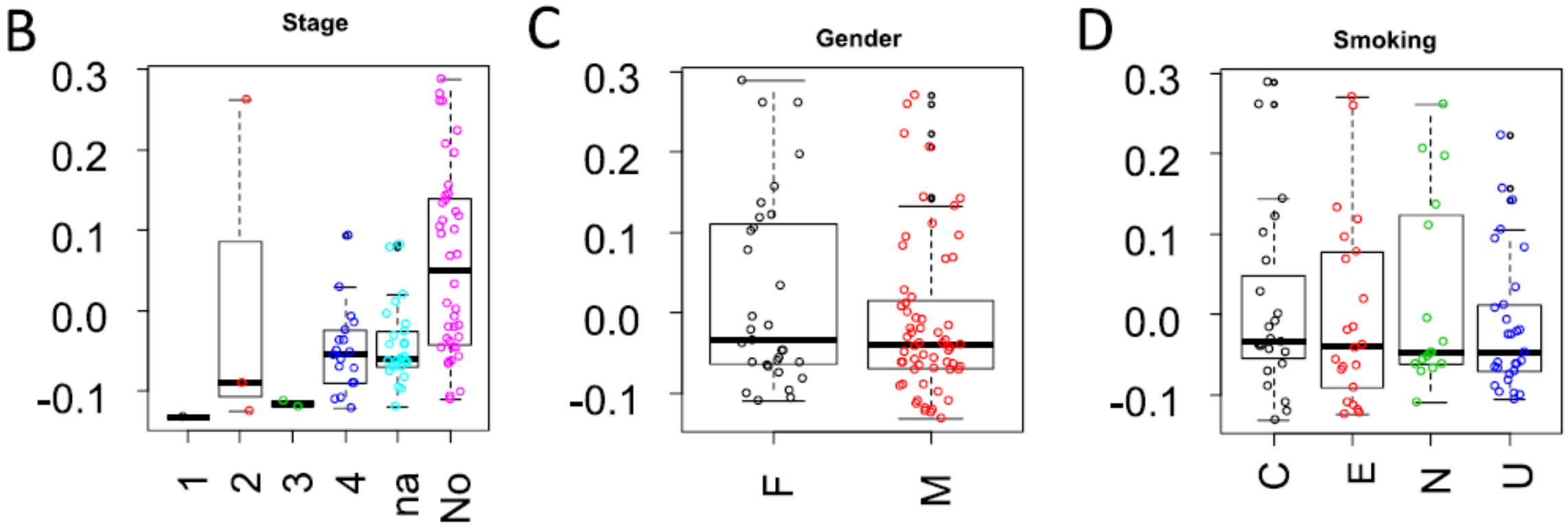


Figure S7: Hierarchical Clustering

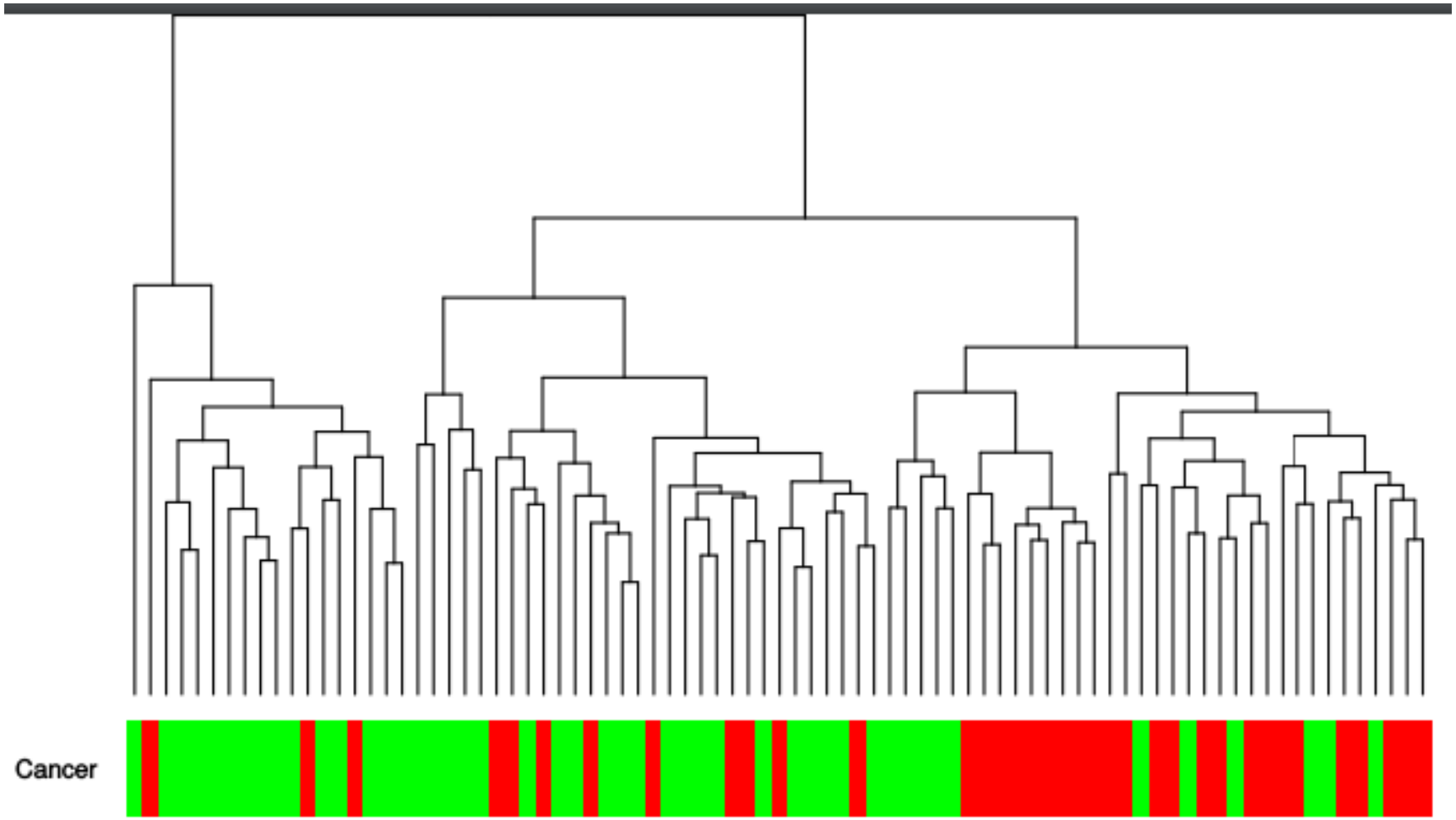


Figure 3: Boxplot of iBAQ expression values for the nine most significant regulated proteins

Method: LC-MS, Label-free quantification (iBAQ)

- Two search engines: MaxQuant and VEMS
- Filtering of proteins
  1. Benjamini & Hochberg method to decrease false positives ($p<0.05$)
  2. > two-fold regulation
  3. Same direction of regulation in both MaxQuant and VEMS

Takeaways:

- 133 significantly regulated proteins identified
- Top 9 shown here (all are upregulated in lung cancer)
- Potential markers for lung cancer

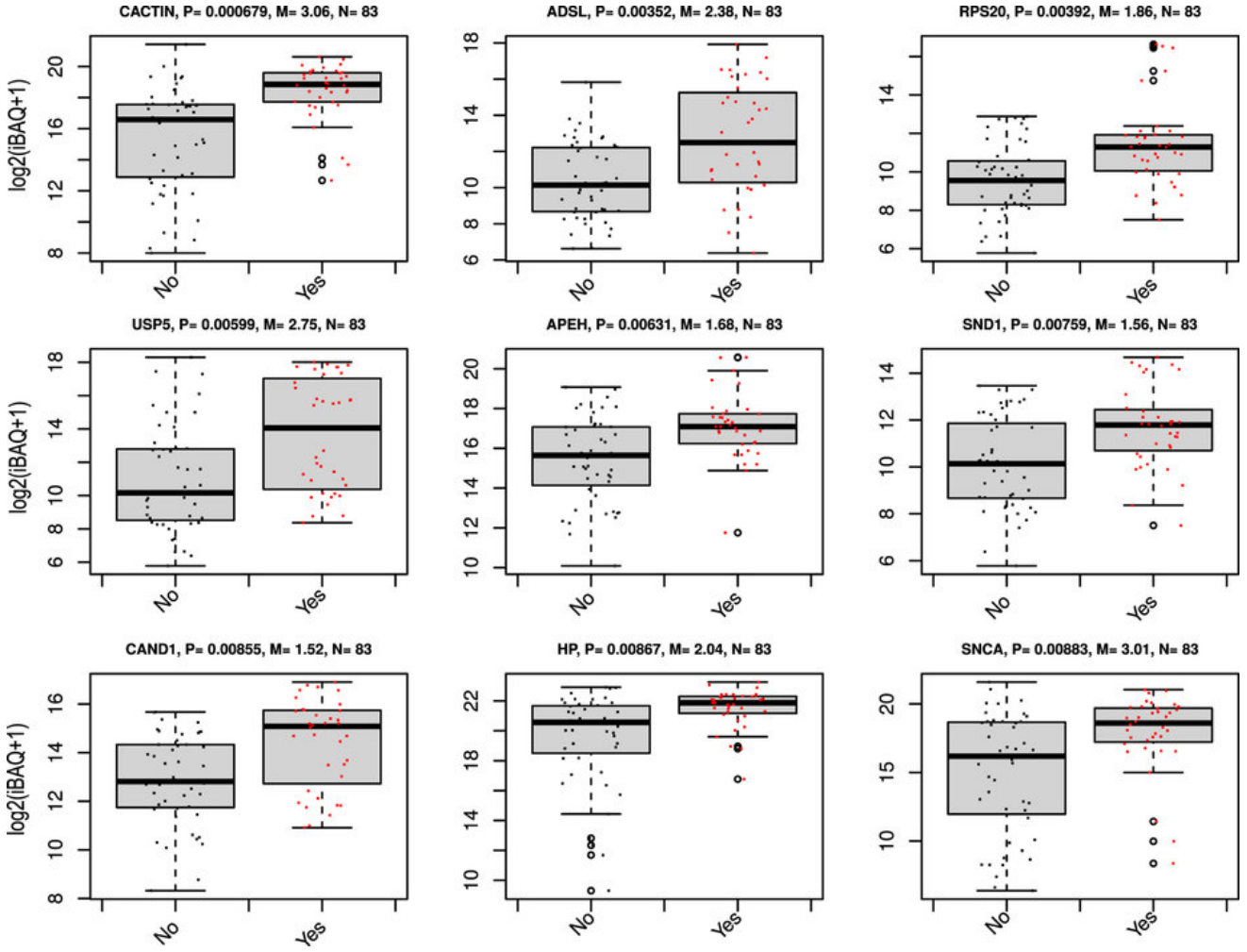
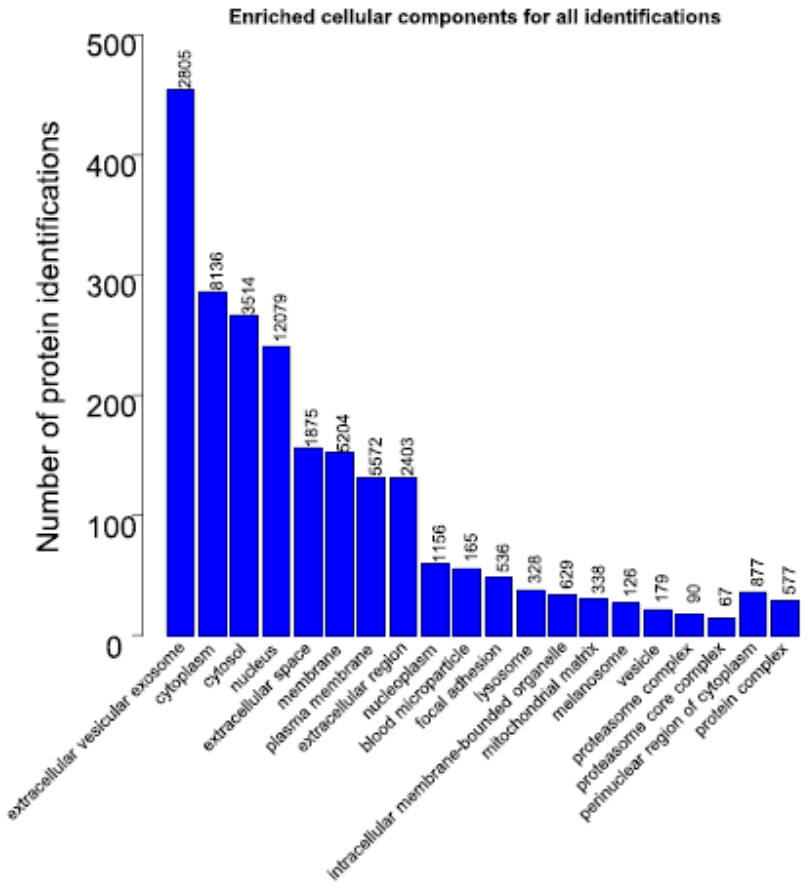
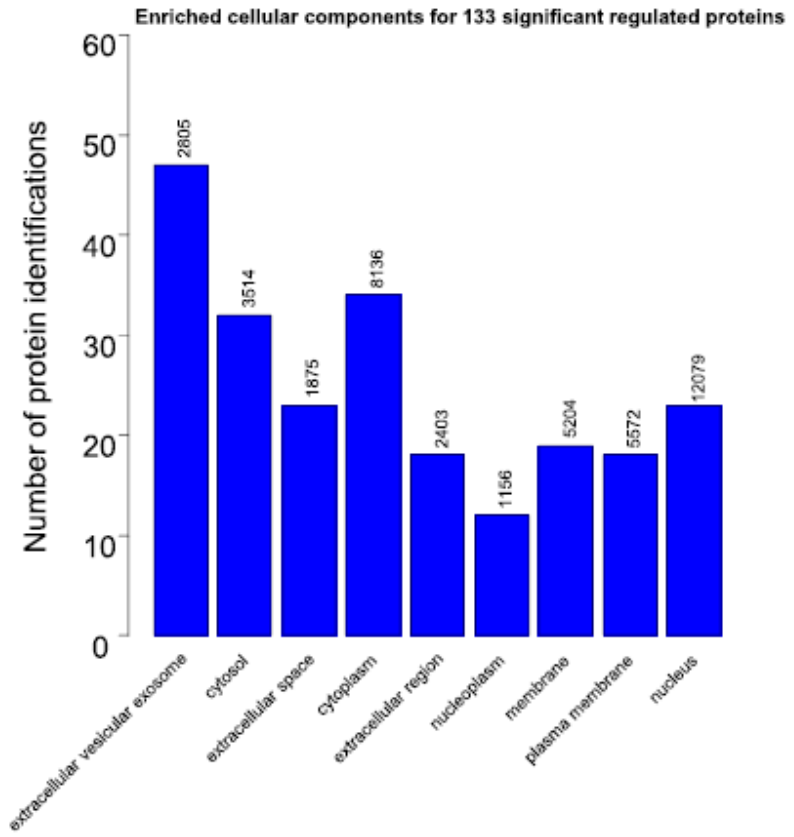


Figure 4: GO enrichment analysis of all identified proteins

A



B



Method: Gene Ontology analysis and binning of the significantly regulated proteins in their respective cellular components

Takeaway: Most of the proteins were from the extracellular vesicular exosome, and this enrichment was compared to the relative enrichment estimates, p values and number of identified proteins.

Figure S19

- Further characterization of the GO proteins

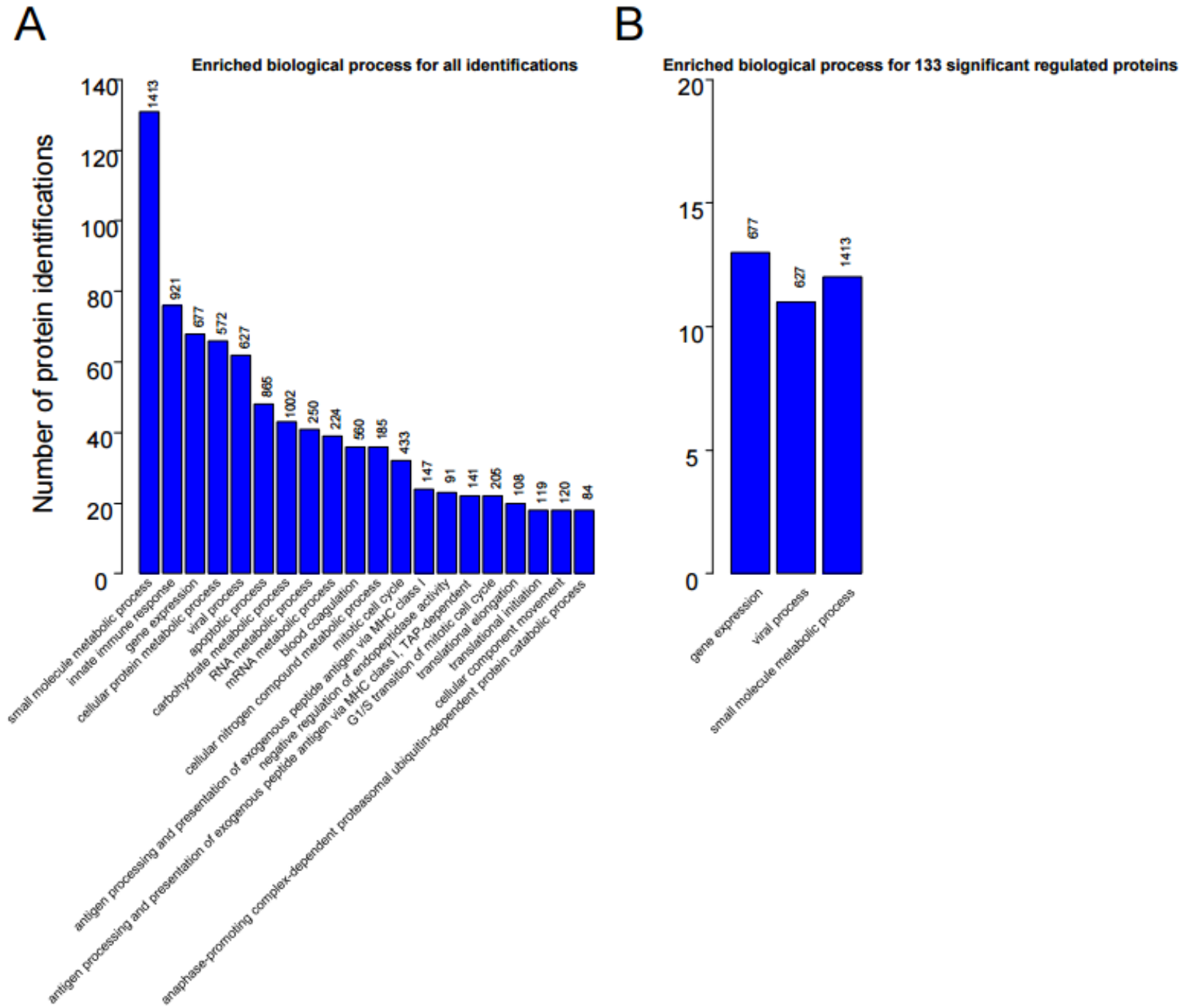


Figure 5: Overview of potential lung cancer biomarkers up- or down- regulated

Method: Heat map that compared biomarkers identified in this study to those in literature

Takeaways:

- Lack of consistency between tissue, BAL, BAL cells, and plasma
- Metabolic pathways most significant among differentially regulated genes
    - Possibility of fluorescently tagging metabolic enzyme inhibitors and using them for diagnostic purposes
- Other important KEGG pathways: spliceosome, focal adhesion, Epstein-Barr Virus infection

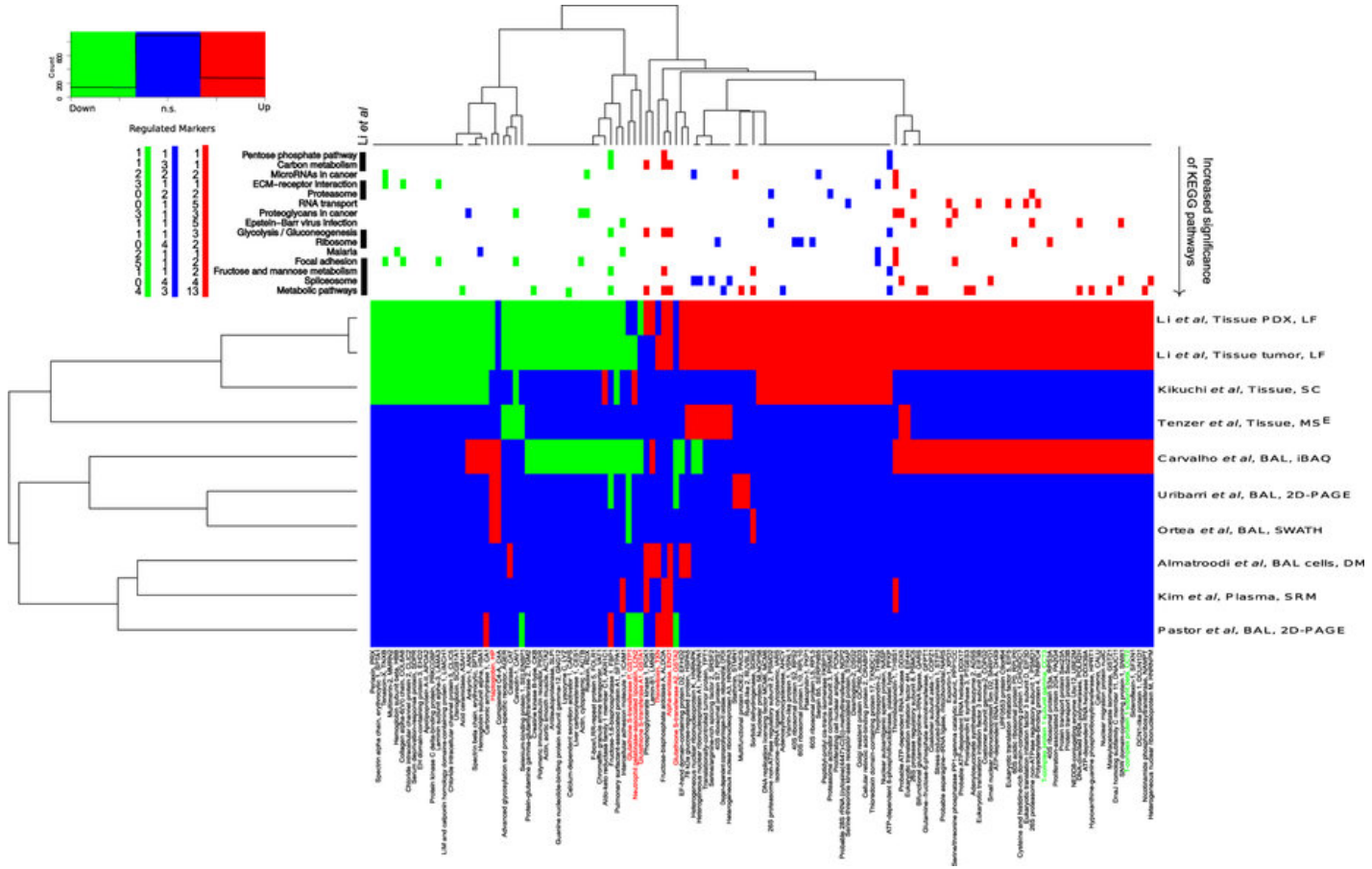
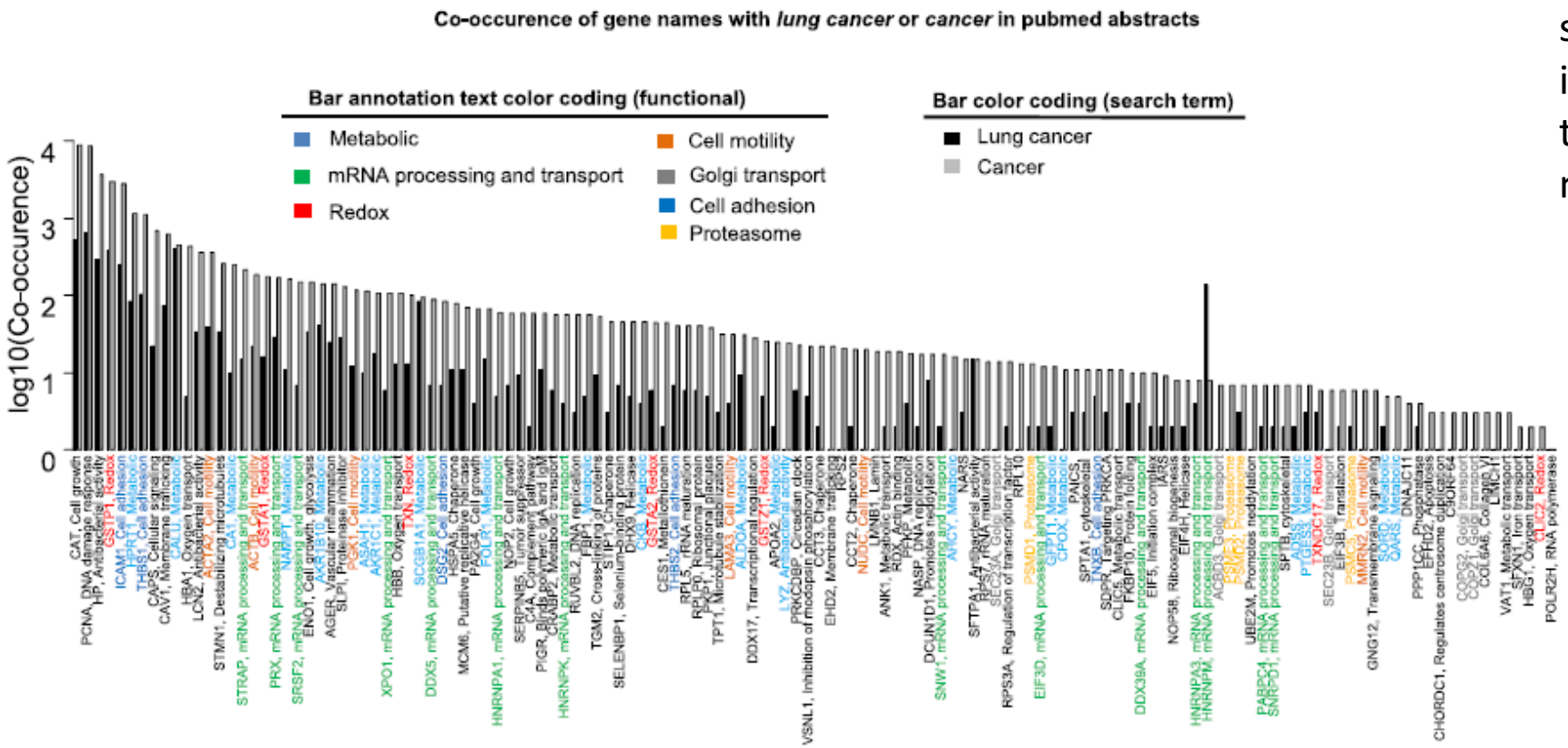


Figure 6



Takeaways: Cancer and Lung cancer have certain proteomes and certain significantly regulated proteins identified from lung cancer tissue from this study have been shown to be novel.

Strengths/Weaknesses

- Strengths:
- Heterogenous clinical population
- Moderate discriminatory ability for cases vs controls
- Potential to augment current diagnostic tools

- Weaknesses:
- Validating against difficult clinical diagnosis
- No classification models used
- Not diagnostic alone

Conclusions

- Demonstrate potential for biomarker diagnosis of lung cancer
  - Although they fall short of true diagnostic
- Some overlap is seen between their results and past studies
  - Mostly in upregulated protein
- Identification of specific cell compartments with differences between tumor and normal point to ways to refine the diagnosis rate

References

- Ferlay, J. *et al.* Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. *International journal of cancer* **136**, E359–386, doi: 10.1002/ijc.29210 (2015).
- Oumeraci, T. *et al.* Bronchoalveolar lavage fluid of lung cancer patients: mapping the uncharted waters using proteomics technology. *Lung Cancer* **72**, 136–138, doi: 10.1016/j.lungcan.2011.01.015 (2011).
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