

Supplementary Materials for:

**Investigating neoplastic progression of ulcerative colitis  
with label-free comparative proteomics**

**Spectral Count Comparison**

Spectral counting is a popular approach to label-free quantitation of peptides across samples. In deciding to base our analysis on a comparison of peptide intensity values, we briefly evaluated a very basic spectral counting approach to this problem. The comparison described here is not intended as an indictment of spectral counting approaches (modern incarnations of which are much more sophisticated than the method described here); rather, it demonstrates the relationship between those approaches and the current ion intensity-based approach.

For less-abundant proteins, the spectral counting approach is at the mercy of particulars of data acquisition at the time the peptides in question eluted. As others have noted (see references cited in manuscript) ratios for less-abundant proteins are both less reliable and less precise than ratios for more-abundant proteins.

In this basic spectral-counting analysis, we calculated a crude sum of the spectral counts from all sample runs of a single experimental group (PHGD or NP) and divided that sum by the equivalent sum for the NP group to calculate the protein ratio.

The scatter plots on the next page demonstrate the relationship between the intensity-based protein ratios discussed on the paper, and ratios for the same proteins based on spectral counting, both represented by their natural logs. In both of the comparisons described here (PHGD:NP and PNEG:NP) there is a moderate correlation between the ratios calculated by these two methods. That correlation becomes much higher, however, when spectral counting-based ratios that are based on four or fewer spectra (black dots) are removed from the comparison. Correlations below are based on least-squares linear regression and are not adjusted for outliers.

The manuscript discusses two proteins in particular, CPS1 and TRAP1. The ratios for those proteins derived from ion intensity and from spectral count are provided here. “Infinite” indicates that the count in group NP is 0.

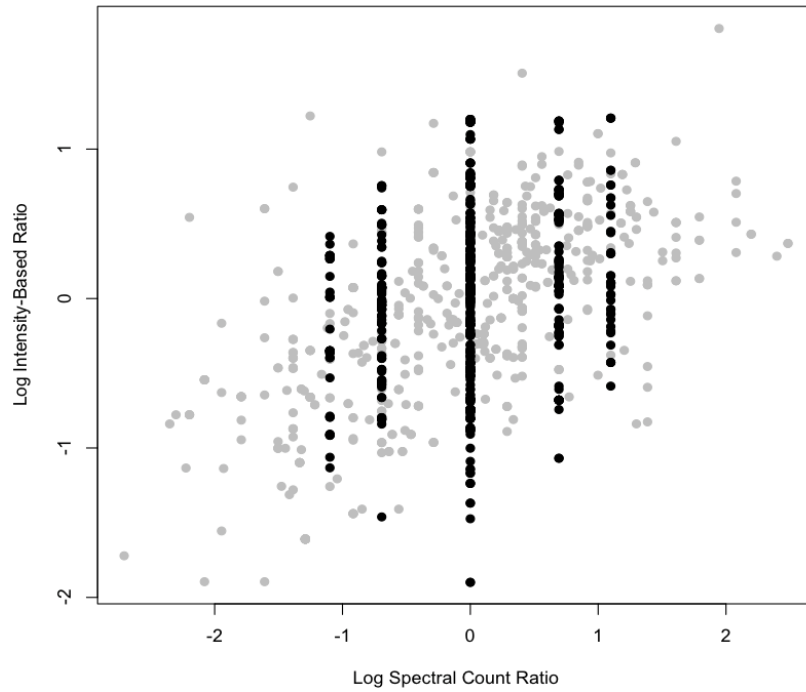
<b>Protein</b>	<b>Ion Intensity Ratio PHGD:NP</b>	<b>Spectral Count Ratio PHGD:NP</b>	<b>Ion Intensity Ratio PNEG:NP</b>	<b>Spectral Count Ratio PNEG:NP</b>
CPS1	3.80	Infinite	3.10	Infinite
TRAP1	1.74	1.09	2.41	1.95

## Log Ratio Comparison: Intensity vs. Spectral Count

### Comparison 1: PHGD:NP

Correlation of log-ratios with spectral counts  $\leq 4$  (black):  $r=0.46$ .

Correlation of log-ratios with spectral counts  $>4$  (gray):  $r=0.61$



### Comparison 1: PHGD:NP

Correlation of log-ratios with spectral counts  $\leq 4$  (black):  $r=0.36$ .

Correlation of log-ratios with spectral counts  $>4$  (gray):  $r=0.49$

