

Supplemental Figure Legends

Supplemental Figure 1. Number of MS/MS scans, PSMs and unique peptide identifications over a 70-minute LC-MS/MS gradient at MS¹ resolving powers of 15,000, 30,000, 60,000, 120,000, 240,000 and 450,000, at *m/z* 200.

Supplemental Figure 2. Number of MS/MS scans, PSMs and unique peptide identifications over a 70-minute LC-MS/MS gradient at maximum injection times of 30, 35, 45 and 60 msec.

Supplemental Figure 3. Number of MS/MS scans, PSMs and unique peptide identifications over a 70-minute LC-MS/MS gradient at dynamic exclusion settings of 15, 30, 45 and 60 seconds.

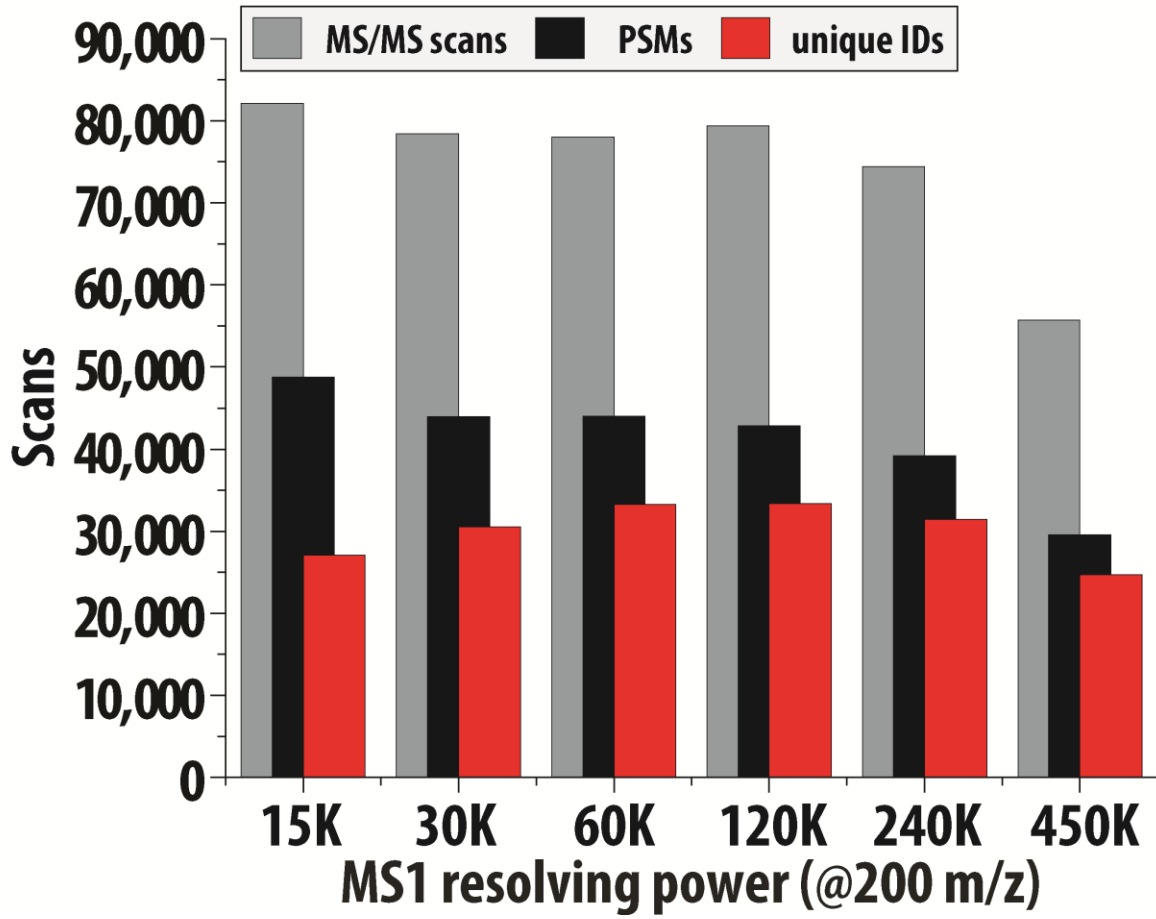
Supplemental Figure 4. Number of MS/MS scans, PSMs and unique peptide identifications over a 70-minute LC-MS/MS gradient using precursor isolation widths of 0.7, 1.0, 1.4, 1.7, 2.0, 2.3 and 2.6 *m/z*.

Supplemental Figure 5. Number of MS/MS scans, PSMs and unique peptide identifications over a 70-minute LC-MS/MS gradient using MS¹ AGC targets of 8e4, 1e5, 2e5 and 5e5.

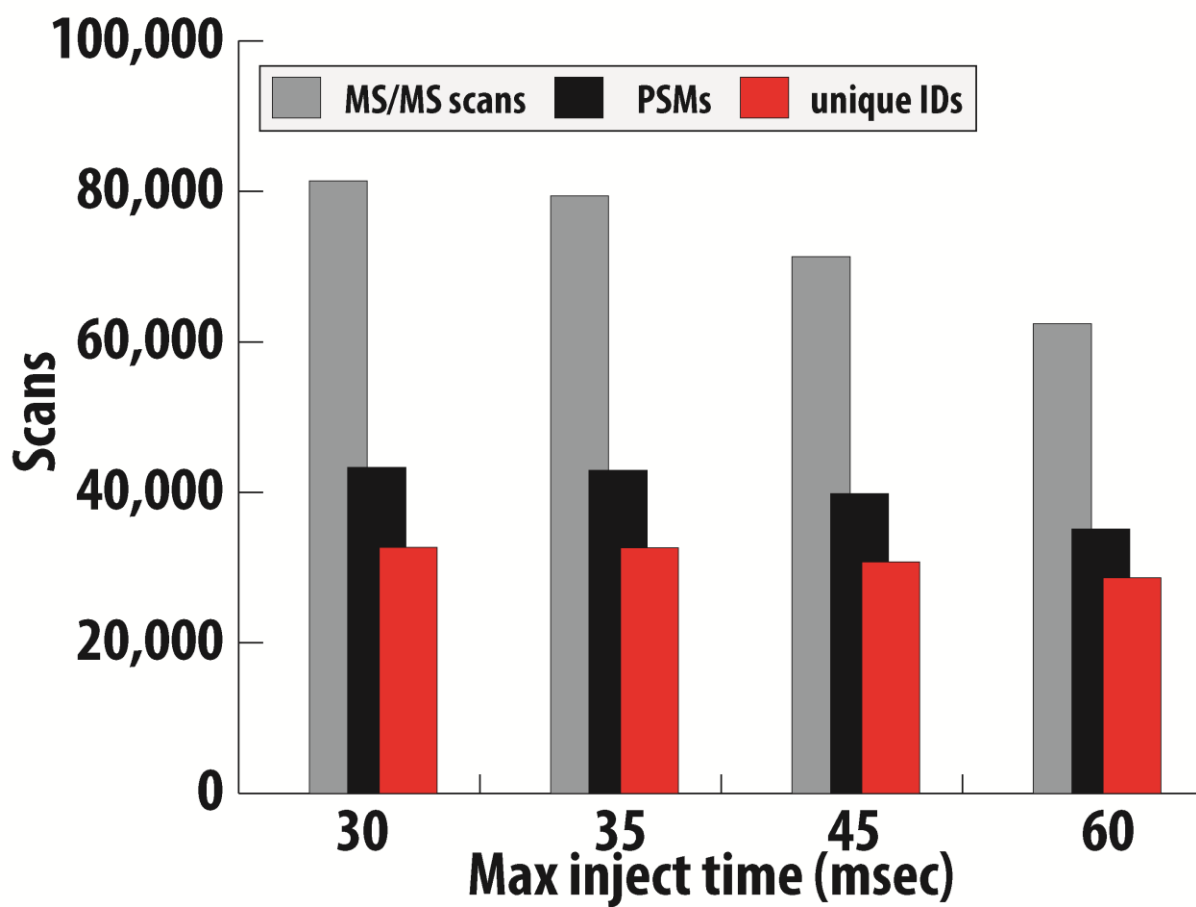
Supplemental Figure 6. Number of MS/MS scans, PSMs and unique peptide identifications over a 70-minute LC-MS/MS gradient using MS² AGC targets of 3e3, 5e3, 7e3 and 1e4, all at an MS² max inject time of 35 msec.

Supplemental Figure 7. Increase in peptide identifications with more vigorous bead milling conditions. Conditions are listed as time of constant milling (min), frequency of shaking (Hz), and number of repeats. The (*) indicates that lysate was not cleared.

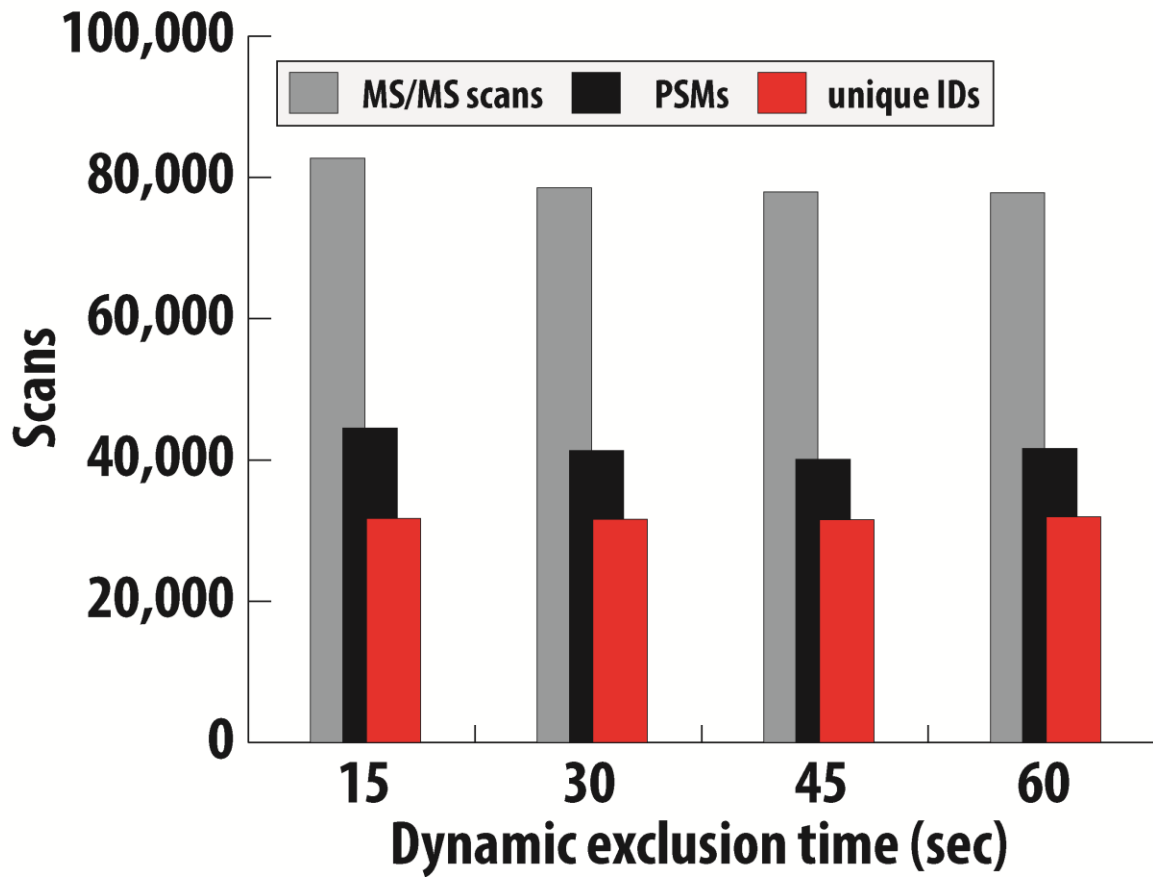
Supplemental Figure 8. A. Distribution of intensities of peptide precursors in the survey scan prior to the MS² event, in which the peptide was identified, with and without the addition of DMSO. B. Total ion current area with and without the addition of DMSO.



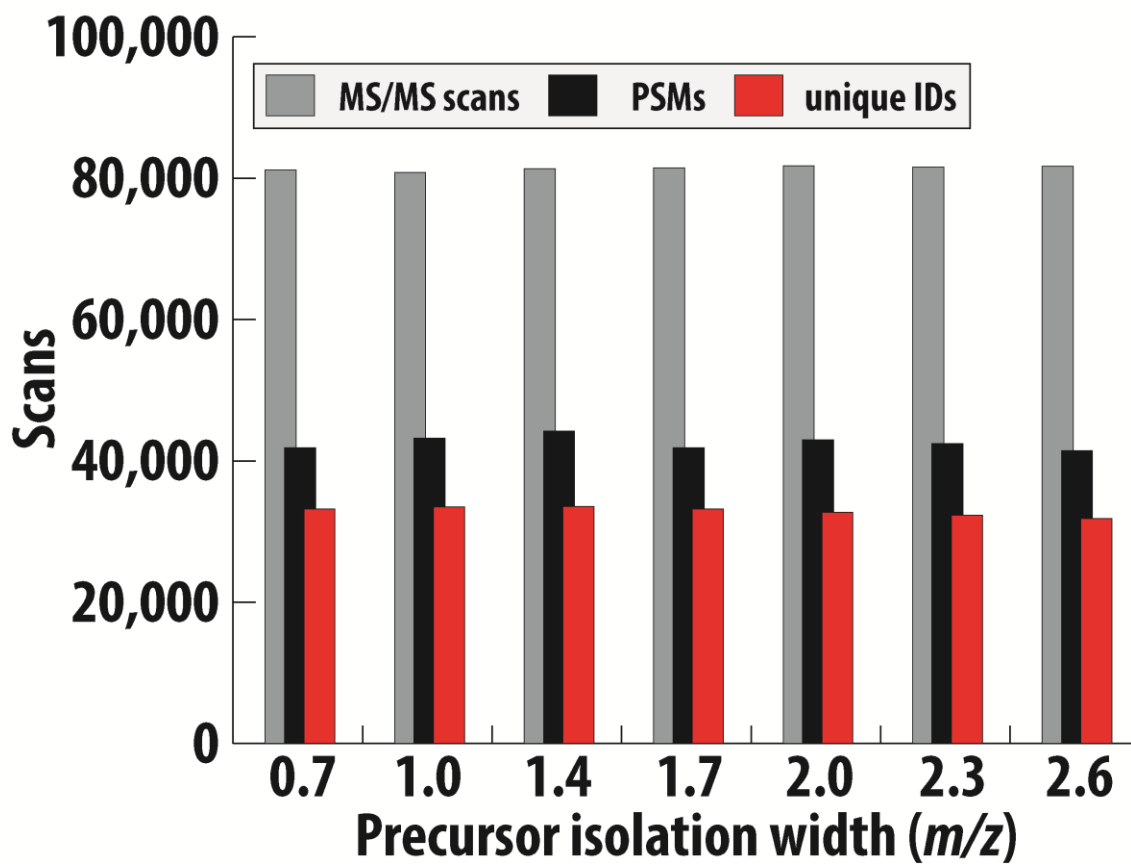
Supplemental Figure 1



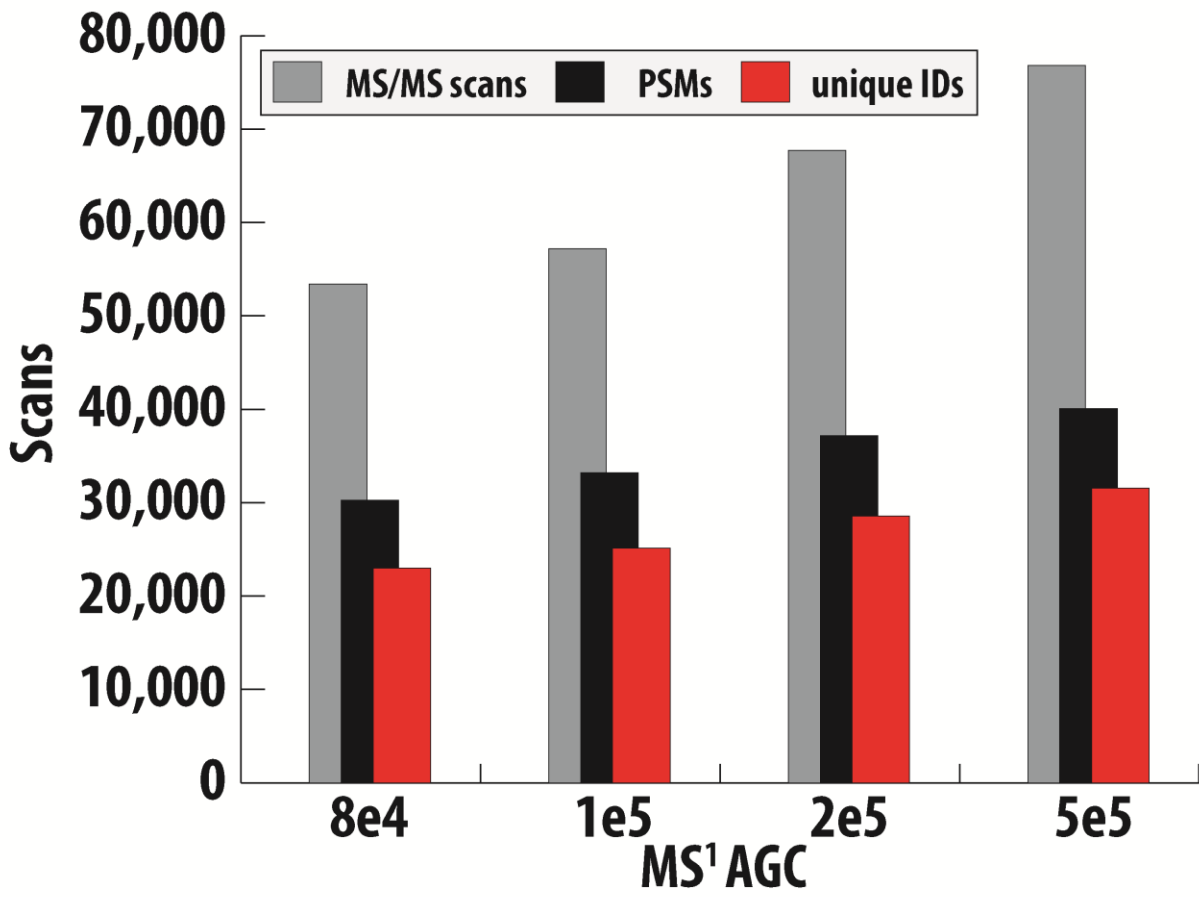
Supplemental Figure 2



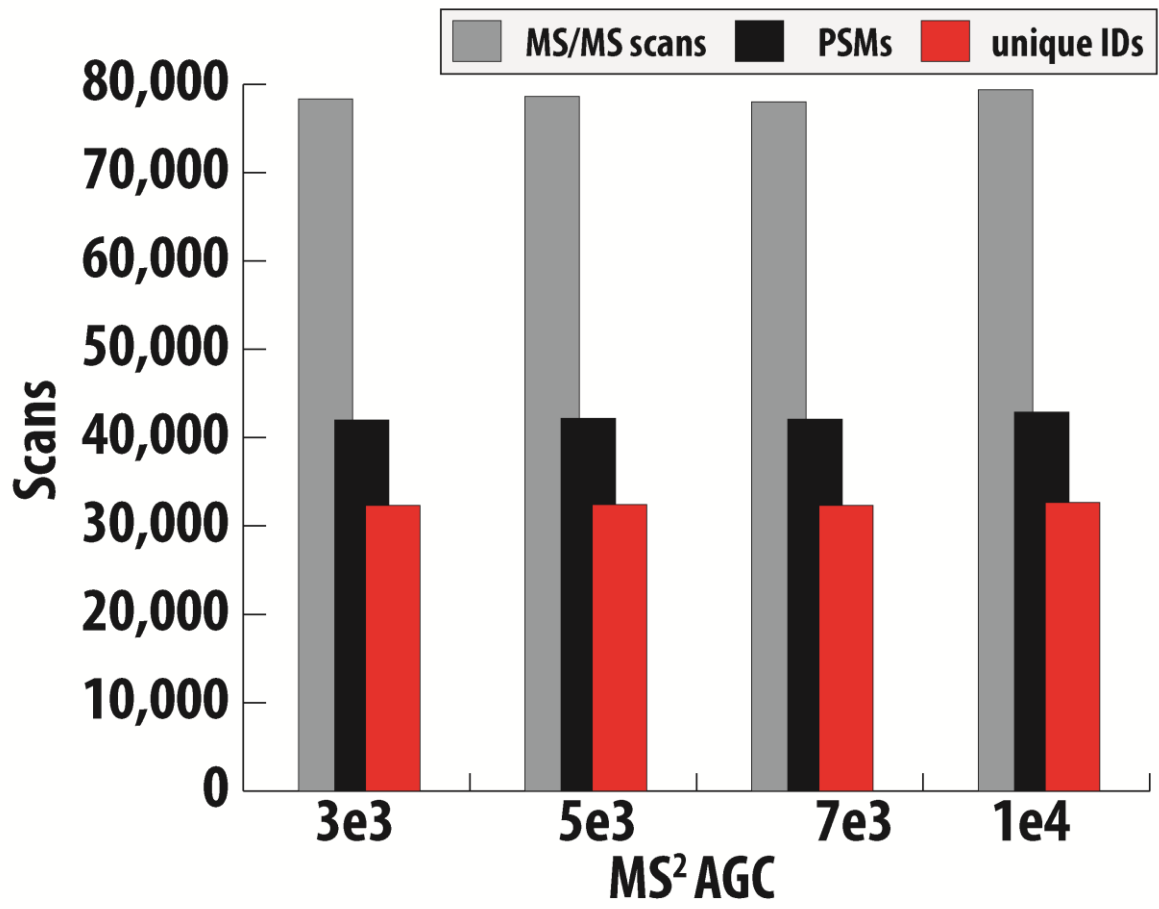
Supplemental Figure 3



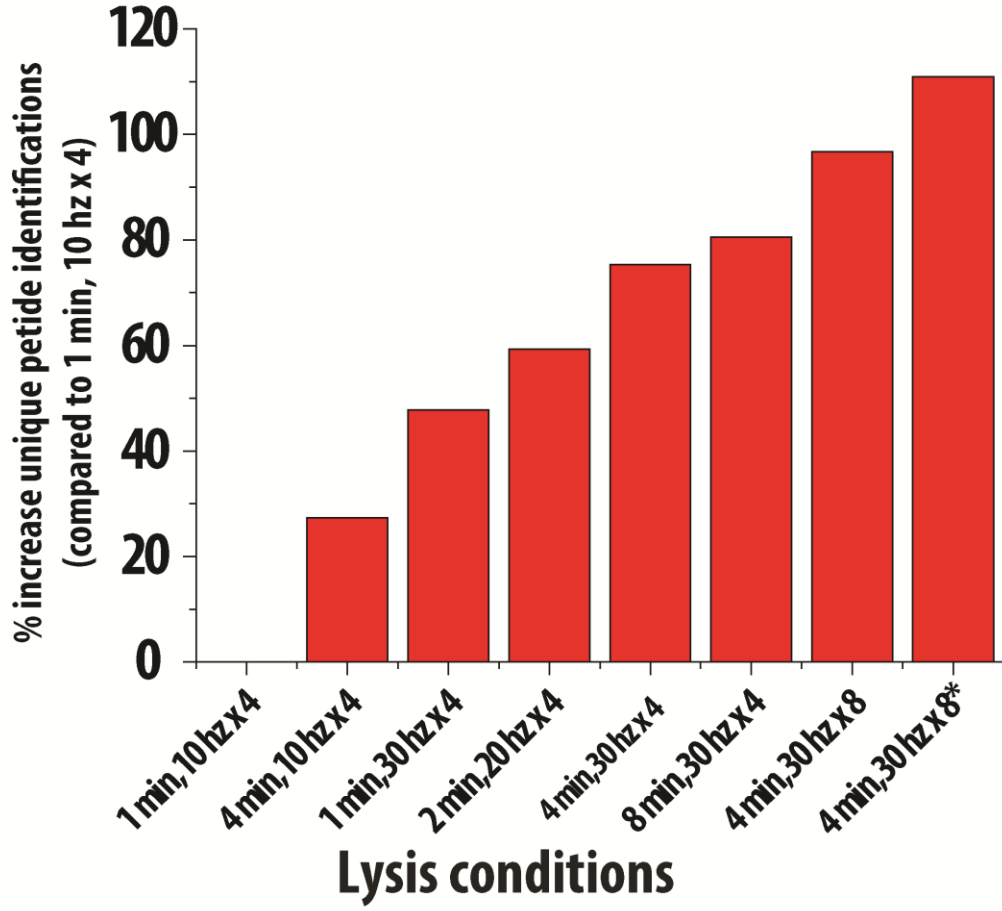
Supplemental Figure 4



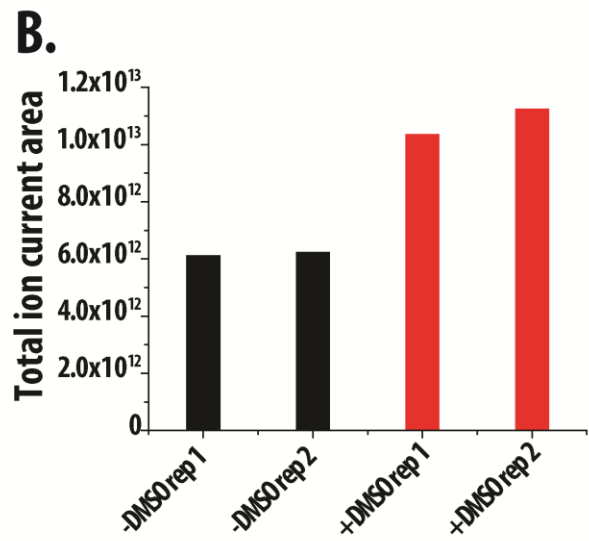
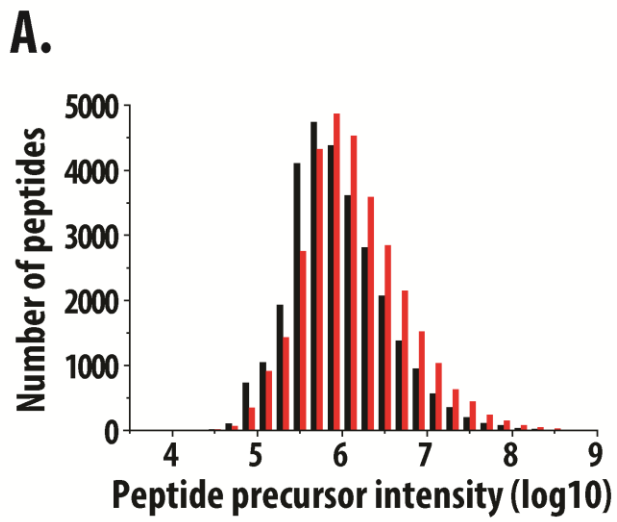
Supplemental Figure 5



Supplemental Figure 6



Supplemental Figure 7



Supplemental Figure 8