

Supplementary Materials:

# Proteomic Insights into Phycobilisome Degradation, A Selective and Tightly Controlled Process in The Fast-Growing Cyanobacterium *Synechococcus elongatus* UTEX 2973

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| Q1 mass | Q3 mass  | Collision energy | Identity                   |
|---------|----------|------------------|----------------------------|
| 495.247 | 394.208  | 13               | MAHENIFK_light             |
| 495.247 | 583.229  | 17               | MAHENIFK_light             |
| 495.247 | 650.351  | 14               | MAHENIFK_light             |
| 495.247 | 787.41   | 13               | MAHENIFK_light             |
| 495.247 | 858.447  | 14               | MAHENIFK_light             |
| 499.254 | 398.216  | 13               | MAHENIFK_heavy             |
| 499.254 | 583.229  | 17               | MAHENIFK_heavy             |
| 499.254 | 658.365  | 14               | MAHENIFK_heavy             |
| 499.254 | 795.424  | 13               | MAHENIFK_heavy             |
| 499.254 | 866.461  | 14               | MAHENIFK_heavy             |
| 738.883 | 502.298  | 20               | EDLEDLFIEVVR_light         |
| 738.883 | 615.382  | 21               | EDLEDLFIEVVR_light         |
| 738.883 | 875.535  | 24               | EDLEDLFIEVVR_light         |
| 738.883 | 990.562  | 21               | EDLEDLFIEVVR_light         |
| 738.883 | 1119.604 | 20               | EDLEDLFIEVVR_light         |
| 738.883 | 1232.689 | 22               | EDLEDLFIEVVR_light         |
| 743.887 | 512.307  | 20               | EDLEDLFIEVVR_heavy         |
| 743.887 | 625.391  | 21               | EDLEDLFIEVVR_heavy         |
| 743.887 | 885.543  | 24               | EDLEDLFIEVVR_heavy         |
| 743.887 | 1000.57  | 21               | EDLEDLFIEVVR_heavy         |
| 743.887 | 1129.613 | 20               | EDLEDLFIEVVR_heavy         |
| 743.887 | 1242.697 | 22               | EDLEDLFIEVVR_heavy         |
| 778.068 | 696.693  | 5                | MLPPLPDFSLSVEQQFDLQK_light |
| 778.068 | 778.409  | 15               | MLPPLPDFSLSVEQQFDLQK_light |
| 778.068 | 915.461  | 6                | MLPPLPDFSLSVEQQFDLQK_light |
| 778.068 | 1044.536 | 13               | MLPPLPDFSLSVEQQFDLQK_light |
| 778.068 | 1134.579 | 20               | MLPPLPDFSLSVEQQFDLQK_light |
| 778.068 | 1421.727 | 25               | MLPPLPDFSLSVEQQFDLQK_light |
| 780.739 | 699.365  | 5                | MLPPLPDFSLSVEQQFDLQK_heavy |
| 780.739 | 786.424  | 15               | MLPPLPDFSLSVEQQFDLQK_heavy |
| 780.739 | 915.461  | 6                | MLPPLPDFSLSVEQQFDLQK_heavy |
| 780.739 | 1048.543 | 13               | MLPPLPDFSLSVEQQFDLQK_heavy |
| 780.739 | 1142.593 | 20               | MLPPLPDFSLSVEQQFDLQK_heavy |
| 780.739 | 1429.741 | 25               | MLPPLPDFSLSVEQQFDLQK_heavy |

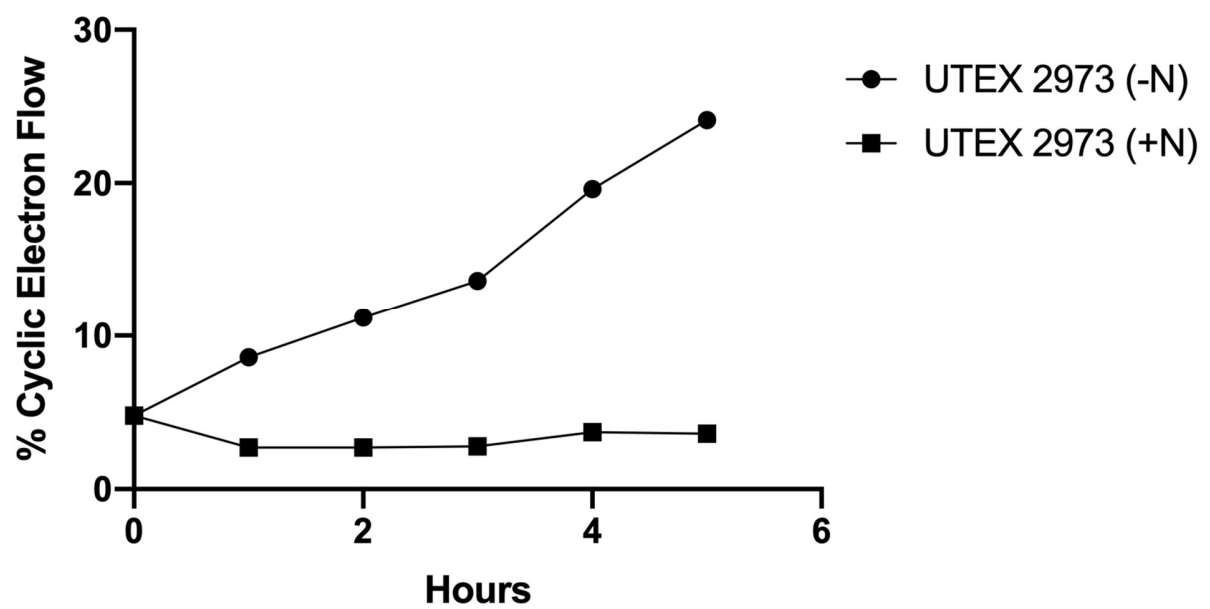
**Table S1.** Transitions used for targeted SRM quantitation of NblA.

|                 | Protein             | protein mass | modification mass | putative identification of modification           |
|-----------------|---------------------|--------------|-------------------|---|
|                 |                     | 17279.9      | 0.0               | unmodified  |
|                 |                     | 17418.0      | 138.1             | degraded PCB ( $\alpha$ )                         |
|                 |                     | 17434.0      | 154.1             | degraded PCB ( $\alpha$ ) + O                     |
|                 |                     | 17596.1      | 316.1             | degraded PCB ( $\alpha\beta$ )                    |
|                 |                     | 17611.1      | 331.2             | degraded PCB ( $\alpha\beta$ ) + O                |
| ApcA            | 2-161:full length   | 17758.2      | 478.2             | degraded PCB ( $\alpha\beta\gamma$ )              |
| M744_01380      | (Met excision)      | 17775.2      | 495.2             | degraded PCB ( $\alpha\beta\gamma$ ) + O          |
|                 |                     | 17792.2      | 512.3             | degraded PCB ( $\alpha\beta\gamma$ ) + 2O         |
|                 |                     | 17865.2      | 585.3             | PCB ( $\alpha\beta\gamma\delta$ )                 |
|                 |                     | 17881.2      | 601.3             | PCB ( $\alpha\beta\gamma\delta$ ) + O             |
|                 |                     | 17897.2      | 617.3             | PCB ( $\alpha\beta\gamma\delta$ ) + 2O            |
|                 |                     | 17914.2      | 634.3             | PCB ( $\alpha\beta\gamma\delta$ ) + 3O            |
|                 |                     | 18049.2      | 769.2             | degraded PCB ( $\alpha\beta\gamma$ ) + GSH -me    |
|                 |                     | 18170.3      | 890.3             | PCB ( $\alpha\beta\gamma\delta$ ) + GSH           |
|                 |                     | 18203.3      | 923.4             | PCB ( $\alpha\beta\gamma\delta$ ) + GSH + 2O      |
|                 |                     | 17534.0      | 152.1             | degraded PCB ( $\alpha$ ) + me                    |
|                 |                     | 17550.0      | 168.1             | degraded PCB ( $\alpha$ ) + me + O                |
|                 |                     | 17698.1      | 316.2             | degraded PCB ( $\alpha\beta$ )                    |
| ApcB            | 1-161:full length   | 17875.2      | 493.2             | degraded PCB ( $\alpha\beta\gamma$ ) + me         |
| M744_01385      |                     | 17981.2      | 599.3             | PCB ( $\alpha\beta\gamma\delta$ ) + me            |
|                 |                     | 17997.2      | 615.3             | PCB ( $\alpha\beta\gamma\delta$ ) + me + O        |
|                 |                     | 18013.2      | 631.4             | PCB ( $\alpha\beta\gamma\delta$ ) + me + 2O       |
|                 |                     | 18318.3      | 936.4             | PCB ( $\alpha\beta\gamma\delta$ ) + me + 2O + GSH |
| ApcC            | 1-67:full length    | 8109.2       | 305.0             | GSH   |
| M744_01390      |                     |              |                   |   |
| ApcD            | 2-163:full length   | 18574.0      | 585.6             | PCB ( $\alpha\beta\gamma\delta$ )                 |
| M744_01830      | (Met excision)      |              |                   |   |
| ApcF            | 1-169:full length   | 19159.9      | 599.4             | PCB ( $\alpha\beta\gamma\delta$ ) + me            |
| M744_05565      |                     |              |                   |   |
|                 |                     | 17284.8      | 138.1             | degraded PCB ( $\alpha$ )                         |
|                 |                     | 17462.8      | 316.2             | degraded PCB ( $\alpha\beta$ )                    |
|                 | 2-163:full length   | 17624.9      | 478.2             | degraded PCB ( $\alpha\beta\gamma$ )              |
|                 | (Met excision)      | 17731.9      | 585.2             | PCB ( $\alpha\beta\gamma\delta$ )                 |
|                 |                     | 17763.9      | 617.3             | PCB ( $\alpha\beta\gamma\delta$ ) + O             |
| CpcA            |                     | 14526.3      | 138.1             | degraded PCB ( $\alpha$ )                         |
| M744_11415      |                     | 14704.4      | 316.2             | degraded PCB ( $\alpha\beta$ )                    |
|                 | 29-163:truncation   | 14866.5      | 478.2             | degraded PCB ( $\alpha\beta\gamma$ )              |
|                 |                     | 14973.5      | 585.3             | PCB ( $\alpha\beta\gamma\delta$ )                 |
|                 |                     | 15005.5      | 617.3             | PCB ( $\alpha\beta\gamma\delta$ ) + O             |
| CpcB            | 98-120: truncation  | 2846.3       | 305.1             | GSH   |
| M744_11420      | 96-120: truncation  | 3080.4       | 305.1             | GSH   |
| (two PCB sites) | 2-31: truncation    | 3236.6       | -18.0             | water loss  |
|                 | 130-159: truncation | 3242.7       | 139.1             | degraded PCB ( $\alpha$ )                         |

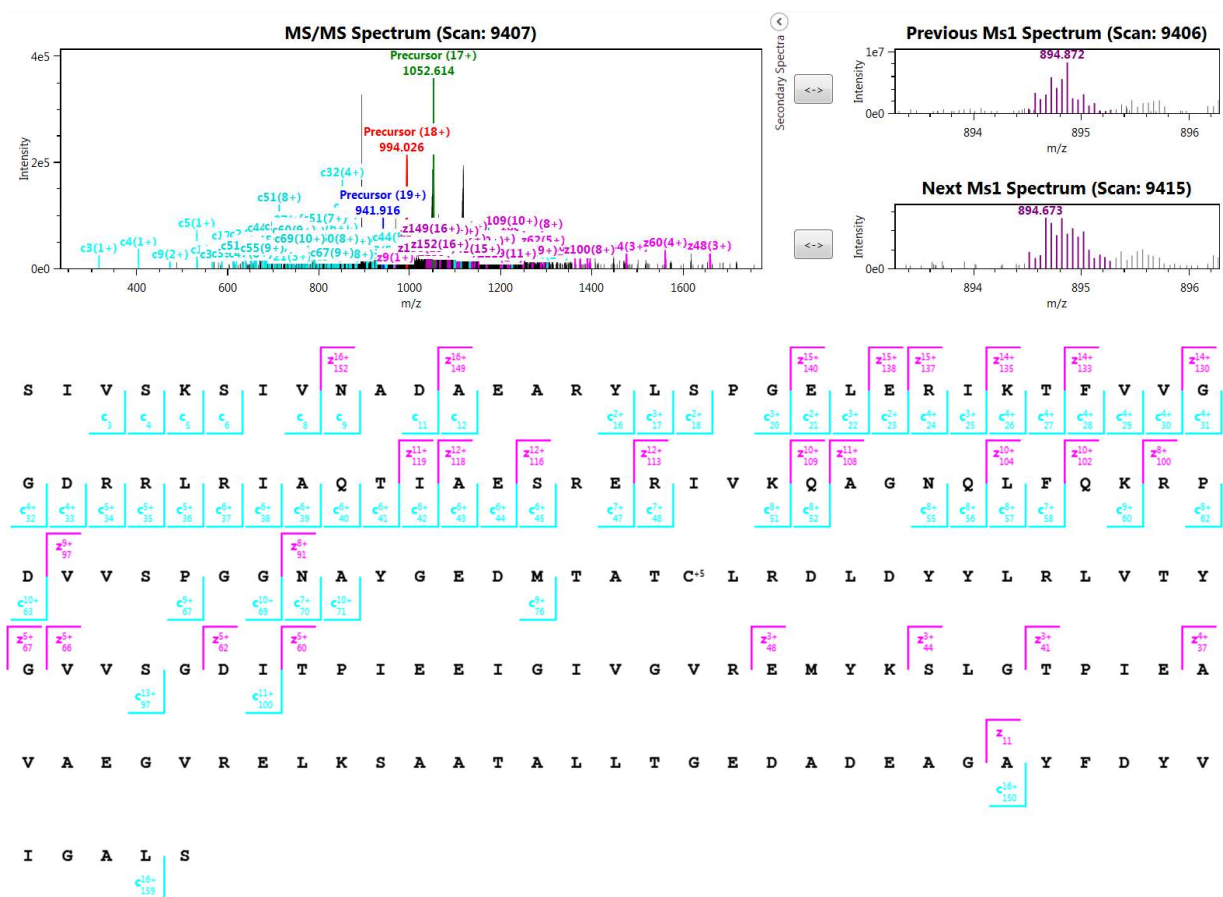
|                    |                     |         |        |  |
|--------------------|---------------------|---------|--------|--|
| CpcD<br>M744_11425 | 2-32: truncation    | 3335.7  | -18.0  | water loss                                     |
|                    | 2-33: truncation    | 3424.8  | 0.0    |  |
|                    |                     | 3477.7  | 52.9   | possible Fe <sup>3+</sup> on Q                 |
|                    | 121-162: truncation | 4293.2  | 139.1  | degraded PCB ( $\alpha$ )                      |
|                    | 2-42: truncation    | 4437.3  | 0.0    |  |
|                    | 12-54: truncation   | 4524.3  | -17.0  | Pyro-glutamic acid                             |
|                    |                     | 4541.4  | 0.0    |  |
|                    | 2-43: truncation    | 4551.3  | 0.0    |  |
|                    | 2-47: truncation    | 4978.6  | 0.0    |  |
|                    | 2-48: truncation    | 5092.6  | 0.0    |  |
|                    | 121-173: truncation | 5387.8  | 139.1  | degraded PCB ( $\alpha$ )                      |
|                    | 2-54: truncation    | 5620.9  | 0.0    |  |
|                    | 124-173: truncation | 5727.9  | 748.4  | unknown  |
|                    |                     | 5850.0  | 870.4  | unknown  |
|                    | 2-73: truncation    | 7494.9  | -4.0   | unknown near C-term                            |
|                    |                     | 7512.9  | 14.0   | methylation                                    |
|                    | 2-120: truncation   | 13495.9 | 599.3  | PCB ( $\alpha\beta\gamma\delta$ ) + me         |
|                    | 2-173: full length  | 19312.9 | 1185.6 | PCB ( $\alpha\beta\gamma\delta$ )*2 + me       |
|                    |                     | 19619.0 | 1491.7 | PCB ( $\alpha\beta\gamma\delta$ )*2 + me + GSH |
|                    | 29-53: truncation   | 2896.5  | 0.0    |  |
|                    | 18-43: truncation   | 3134.6  | 0.0    |  |
|                    | 17-43: truncation   | 3221.6  | 0.0    |  |
|                    | 54-81: truncation   | 3249.8  | 0.0    |  |
|                    | 17-44: truncation   | 3278.6  | 0.0    |  |
|                    | 17-47: truncation   | 3606.8  | 0.0    |  |
|                    | 17-48: truncation   | 3753.9  | 0.0    |  |
|                    | 10-43: truncation   | 3833.9  | 0.0    |  |
|                    | 9-43: truncation    | 3890.9  | 0.0    |  |
|                    | 17-51: truncation   | 4078.1  | 0.0    |  |
|                    | 17-52: truncation   | 4241.1  | 0.0    |  |
|                    | 17-53: truncation   | 4312.2  | 0.0    |  |
|                    | 17-54: truncation   | 4468.3  | 0.0    |  |
|                    | 1-43: truncation    | 4690.4  | 0.0    |  |
|                    |                     | 4706.4  | 16.0   | +O   |
|                    | 17-58: truncation   | 4971.4  | 0.0    |  |
|                    | 1-47: truncation    | 5075.6  | 0.0    |  |
|                    | 1-48: truncation    | 5222.6  | 0.0    |  |
|                    | 17-60: truncation   | 5240.6  | 0.0    |  |
|                    | 25-71: truncation   | 5496.9  | 0.0    |  |
|                    | 23-71: truncation   | 5725.0  | 0.0    |  |
|                    | 22-71: truncation   | 5888.0  | 0.0    |  |
|                    | 17-66: truncation   | 5950.1  | 0.0    |  |

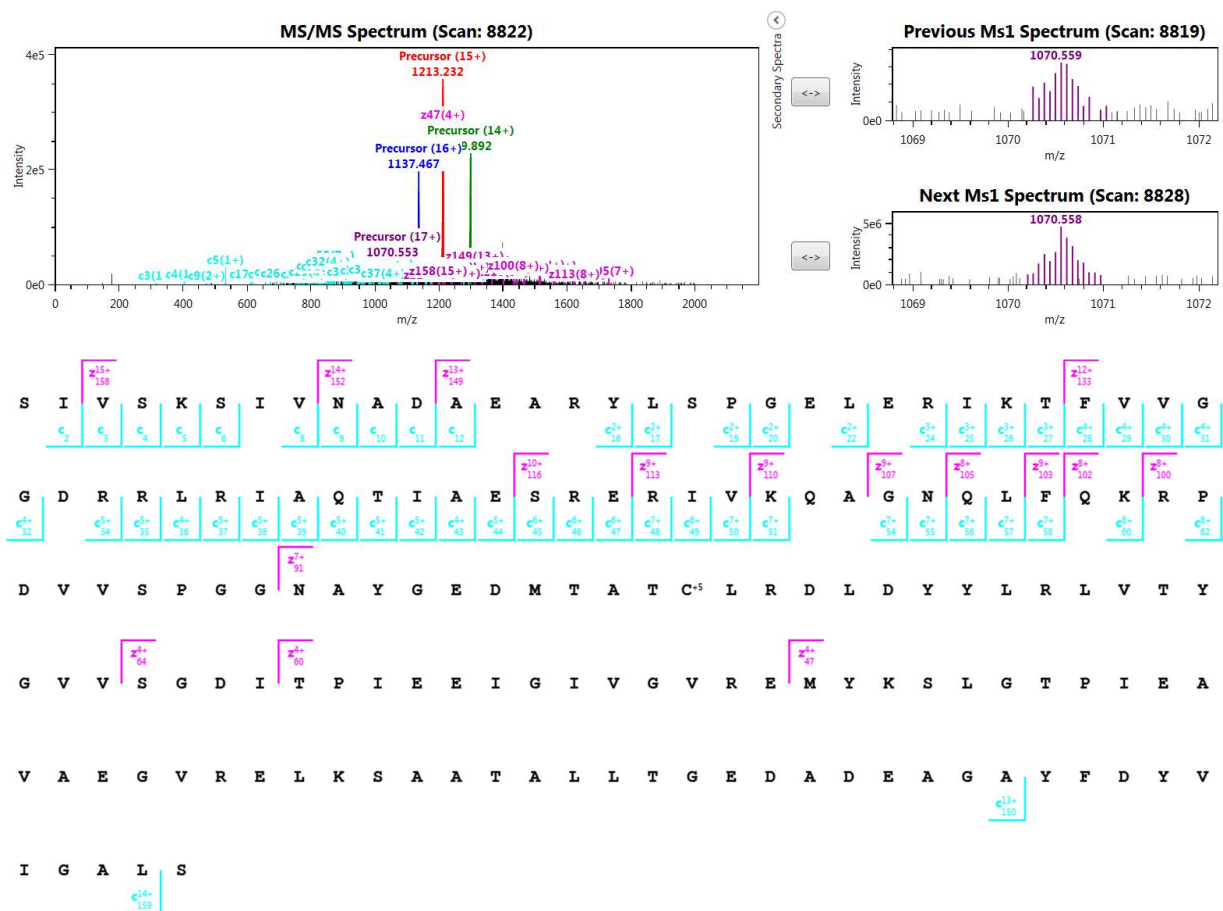
|            |                   |        |       |  |
|------------|-------------------|--------|-------|--|
|            |                   | 5966.1 | 16.0  | +O                                       |
|            | 17-67: truncation | 6021.1 | 0.0   |  |
|            |                   | 6037.1 | 16.0  | +O                                       |
|            | 17-70: truncation | 6389.4 | 0.0   |  |
|            | 17-71: truncation | 6476.4 | 0.0   |  |
|            |                   | 6492.4 | 16.0  | +O                                       |
|            | 22-81: truncation | 6955.6 | 0.0   |  |
|            | 10-71: truncation | 7088.7 | 0.0   |  |
|            | 9-71: truncation  | 7145.7 | 0.0   |  |
|            | 1-66: truncation  | 7418.8 | 0.0   |  |
|            | 1-67: truncation  | 7489.9 | 0.0   |  |
|            |                   | 7543.9 | 0.0   |  |
|            | 17-81: truncation | 7559.9 | 16.0  | +O                                       |
|            |                   | 7596.8 | 52.9  | Fe <sup>3+</sup> (52.92) near C-terminus |
|            | 3-71: truncation  | 7701.0 | 0.0   |  |
|            | 1-70: truncation  | 7858.1 | 0.0   |  |
|            |                   | 7945.2 | 0.0   |  |
|            | 1-71: truncation  | 7961.2 | 16.0  | +O                                       |
|            |                   | 7977.2 | 32.0  | +2O                                      |
|            |                   | 7998.1 | 52.9  | Fe <sup>3+</sup> (52.92)                 |
|            | 10-81: truncation | 8156.3 | 0.0   |  |
|            | 9-81: truncation  | 8213.3 | 0.0   |  |
|            | 1-79: truncation  | 8750.6 | -18.0 | water loss                               |
|            | 3-81: truncation  | 8768.6 | 0.0   |  |
|            | 1-81: full length | 8879.7 | -18.0 | water loss                               |
|            |                   | 9012.7 |       |  |
|            |                   | 9028.7 | 16.0  | +O                                       |
|            |                   | 9044.7 | 32.0  | +2O                                      |
|            |                   | 9065.6 | 52.9  | Fe <sup>3+</sup> (52.92) near C-terminus |
|            |                   | 9111.6 | 98.9  | Sulfate addition                         |
| NbIA       | 1-59: full length | 7046.6 |       |  |
| M744_05740 | 3-59: truncation  | 6802.5 |       |  |

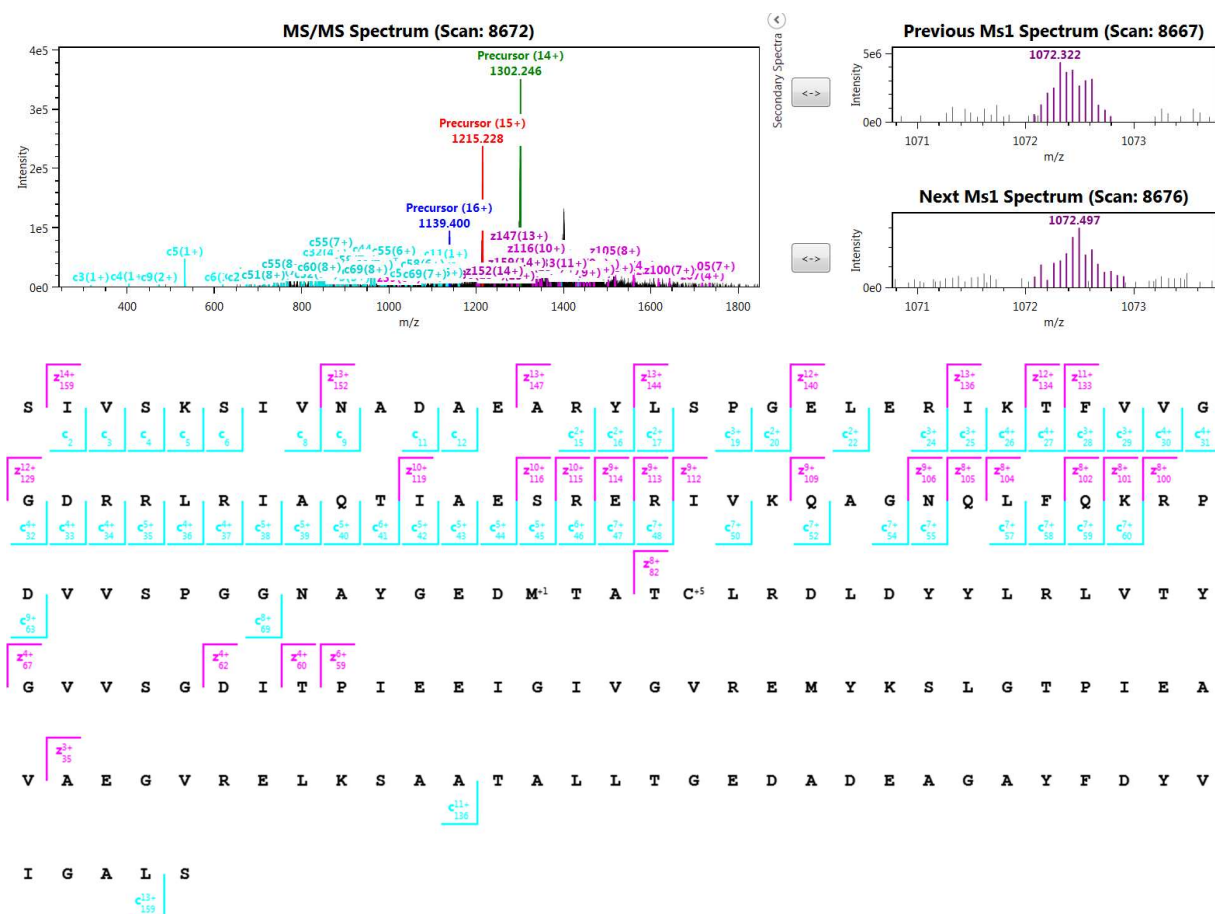
**Table S2.** Identified proteoforms of PBS proteins with top-down proteomics workflow (PCB – phycocyanobilin pigment; O – oxidation; GSH – glutathione; me – methylation). The partially degraded PCBs are annotated with Greek alphabets as discussed in Figure 3.



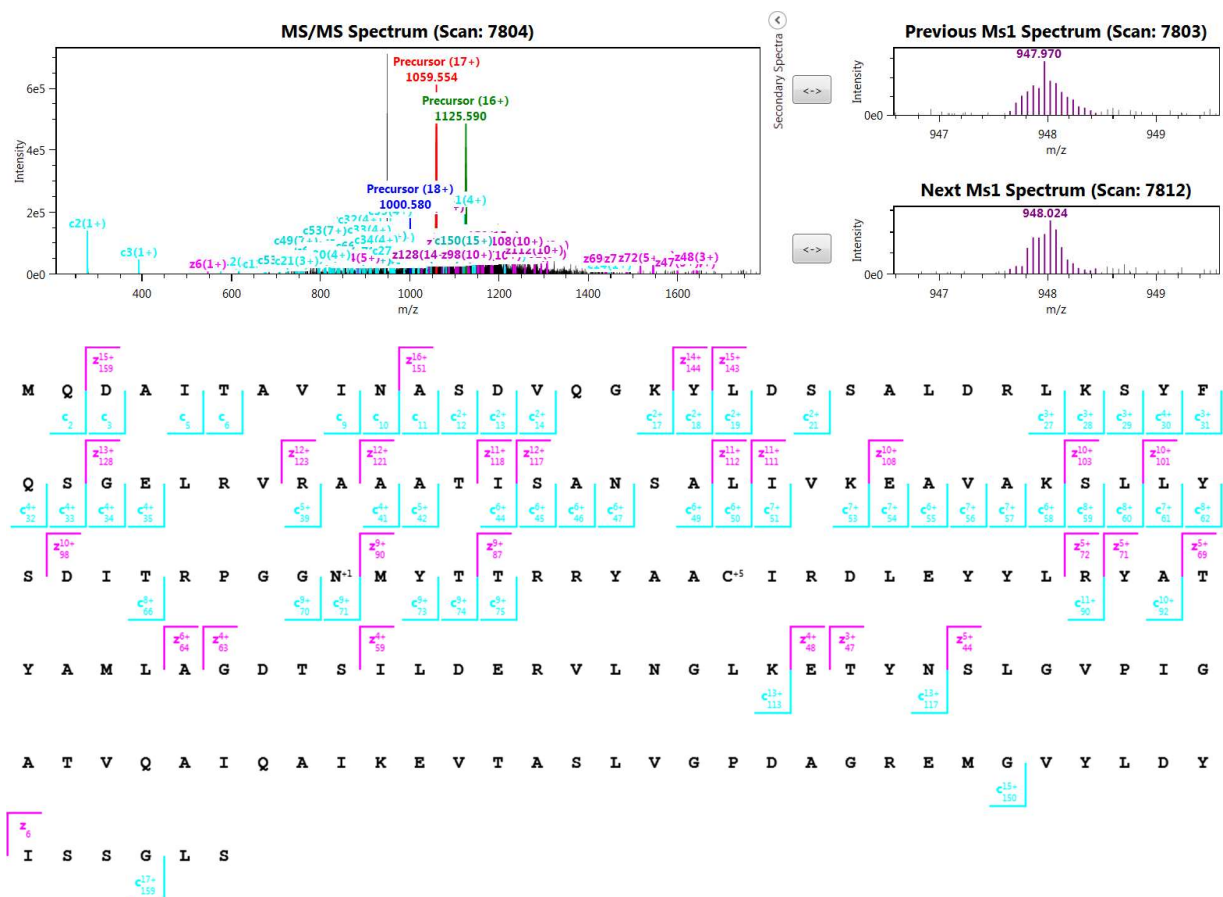
**Figure S1.** Cyclic electron flow in UTEX 2973 under nitrogen depletion (-N) and repletion (+N).



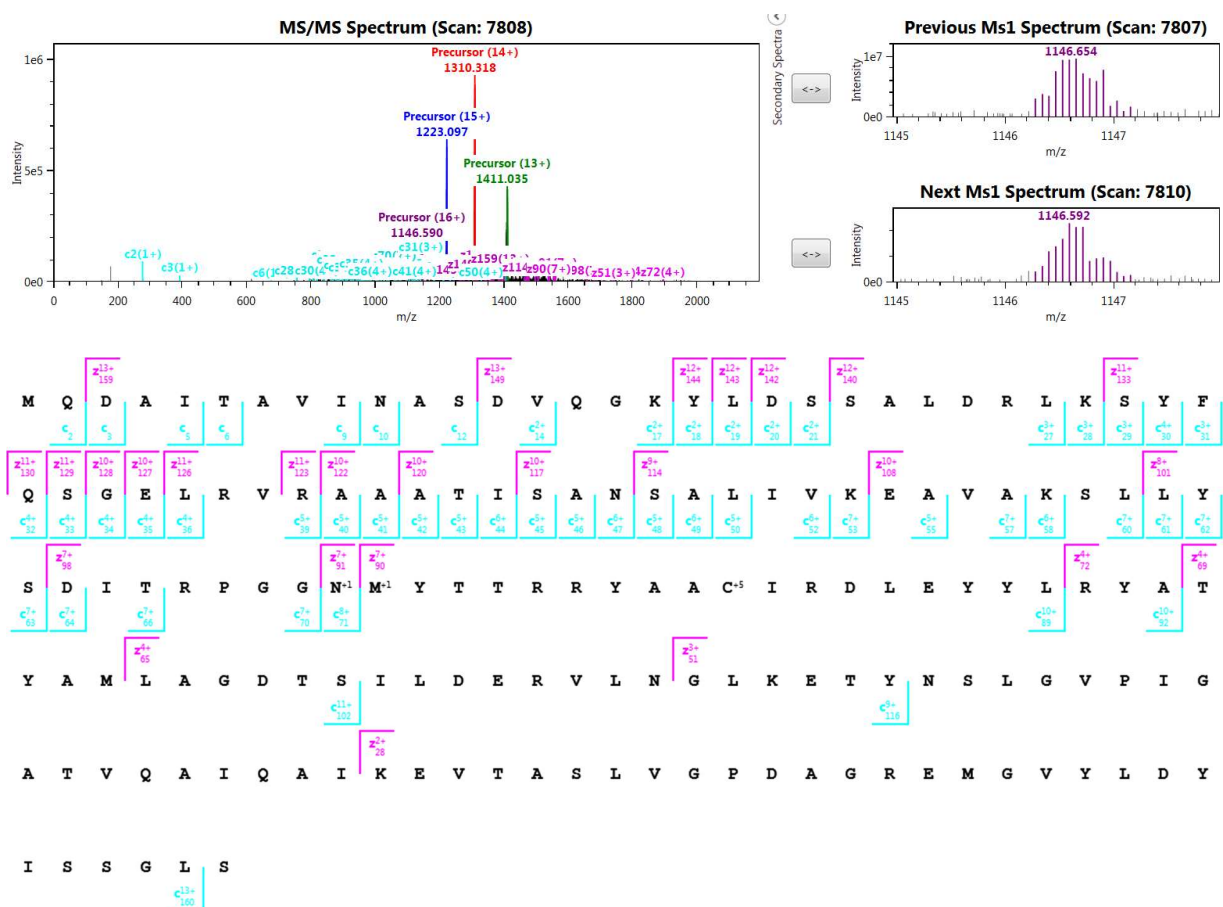




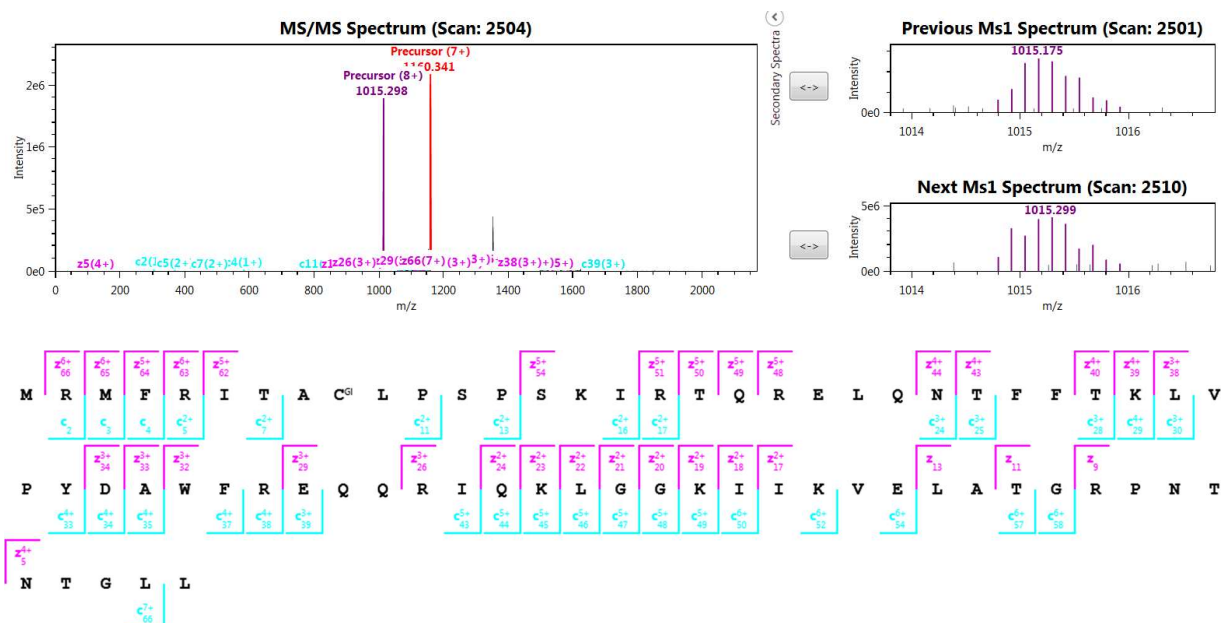
**Figure S4.** Example data for identified ApcA proteoform with modification mass of 923Da, which is tentatively assigned to one PCB and one GSH on Cys81, and two oxidations on Met77 (File: UTEX2973\_topD\_21\_lumos\_29Sep16\_Bane\_16-04-17, scan 8671, charge state +17). Top panel showed the annotated MS/MS spectrum on the left, and the adjacent MS spectra zoomed to the precursor regions. Bottom panel showed the coverage map.



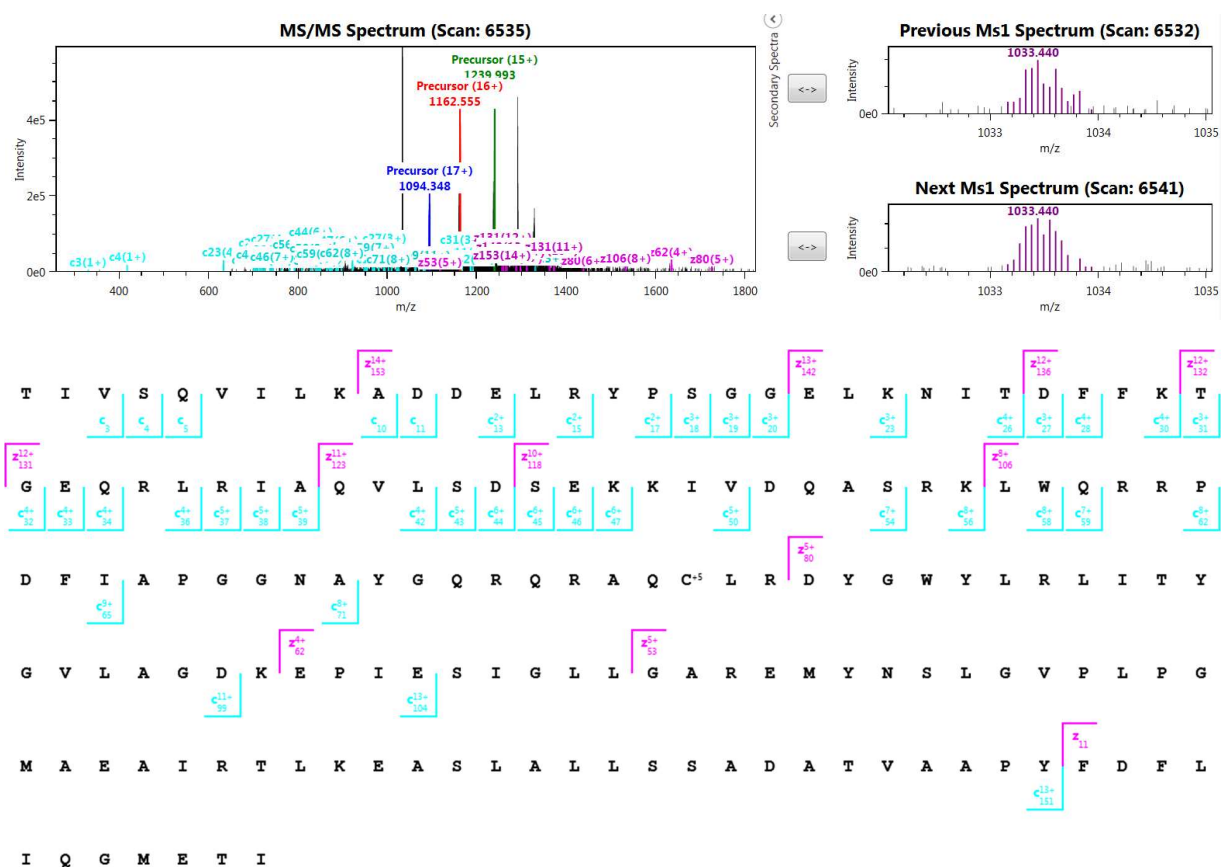
**Figure S5.** Example data for identified ApcB proteoform with one PCB on Cys81, and one methyl on N71 (File: UTEX2973\_topD\_21\_lumos\_29Sep16\_Bane\_16-04-17, scan 7404, charge state +19). Top panel showed the annotated MS/MS spectrum on the left, and the adjacent MS spectra zoomed to the precursor regions. Bottom panel showed the coverage map.



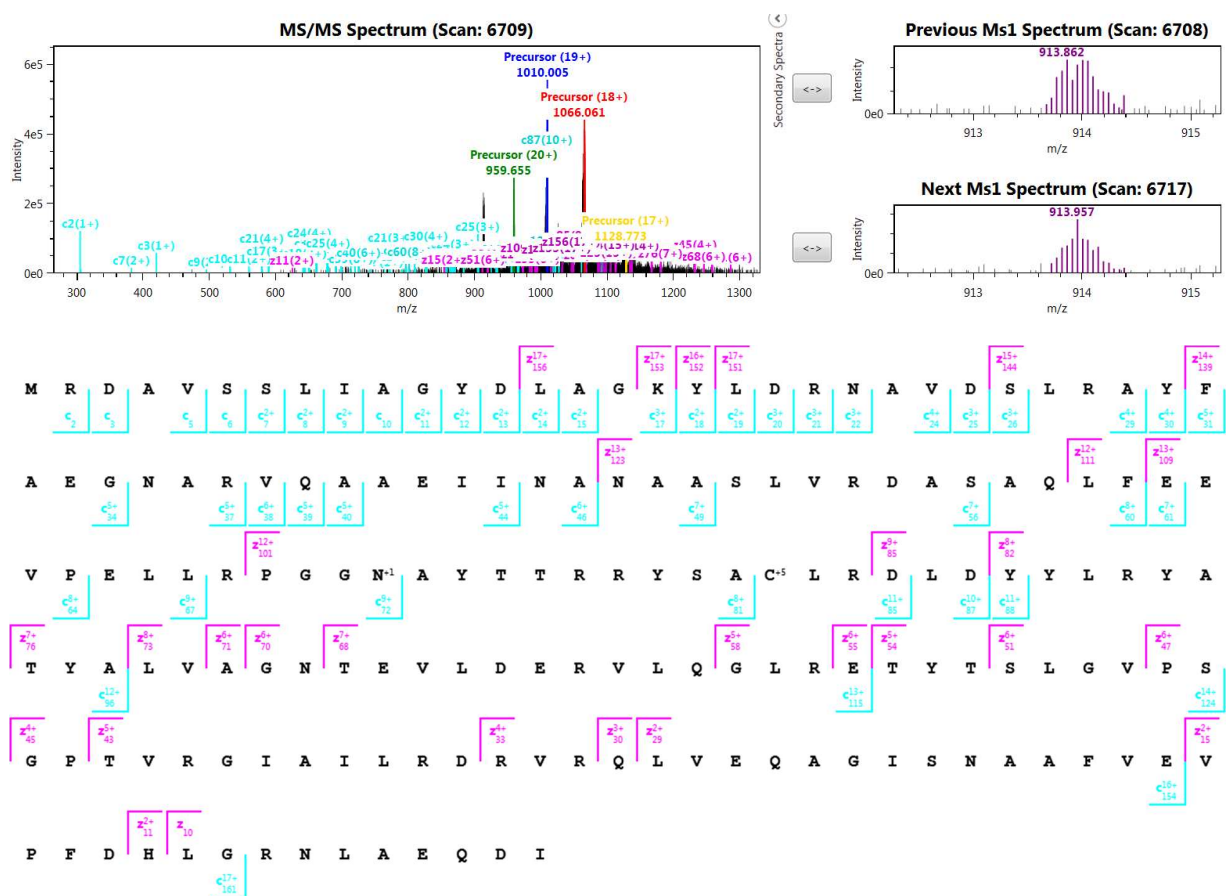
**Figure S6.** Example data for identified ApcB proteoform with modification mass of 936Da, which is tentatively assigned to one PCB and one GSH on Cys81, one methyl on Asn71, and two oxidation on Met72, (File: UTEX2973\_topD\_25\_lumos\_29Sep16\_Bane\_16-04-17, scan 7808, charge state +16). One of the two oxidations could potentially be assigned to Met96 without significantly reducing the sequence coverage. Similar to the ApcA species assigned to carry modifications with both GSH and PCB, the chemistry forming this ApcB species is unclear because only one Cys is in the sequence. Top panel showed the annotated MS/MS spectrum on the left, and the adjacent MS spectra zoomed to the precursor regions. Bottom panel showed the coverage map.



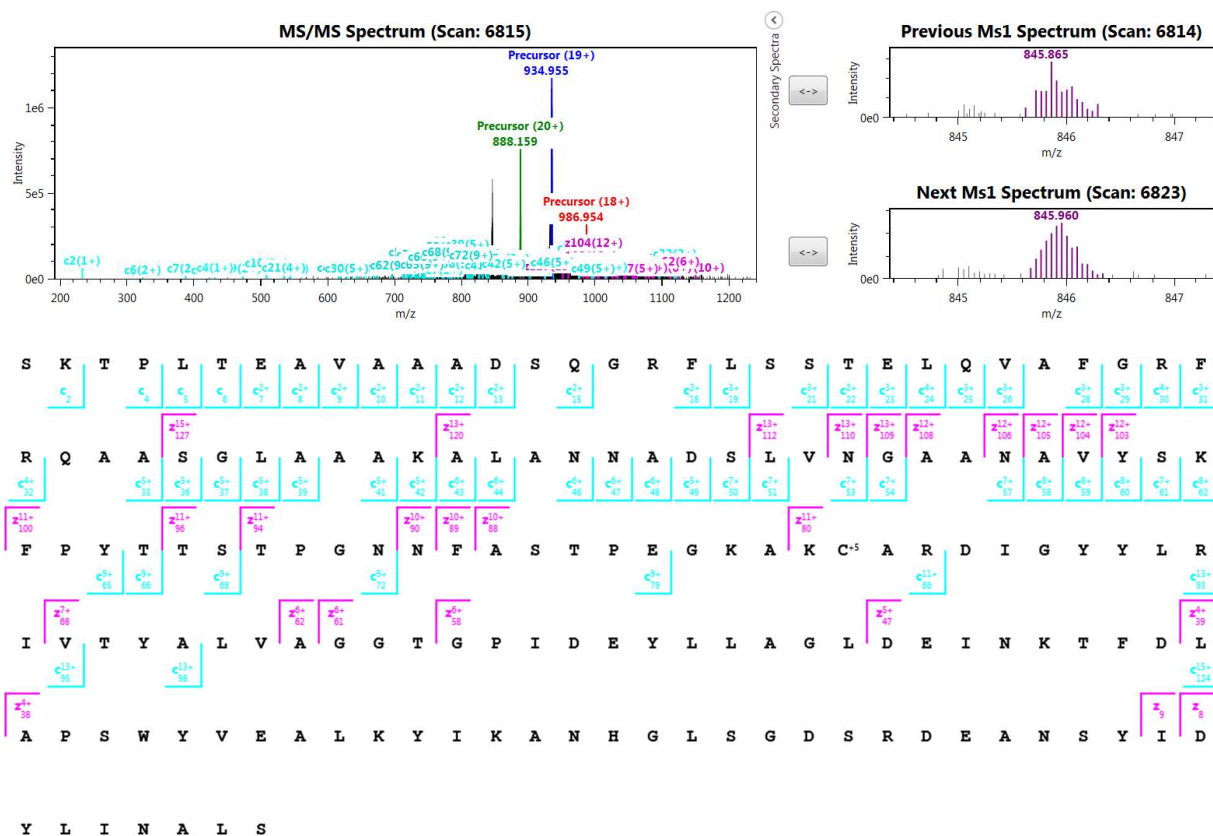
**Figure S7.** Example data for identified ApcC proteoform with glutathione on Cys9 (File: UTEX2973\_topD\_25\_lumos\_29Sep16\_Bane\_16-04-17, scan 2504, charge state +8). All detected, high confidence proteoforms of ApcC had the glutathione modification. Top panel showed the annotated MS/MS spectrum on the left, and the adjacent MS spectra zoomed to the precursor regions. Bottom panel showed the coverage map.



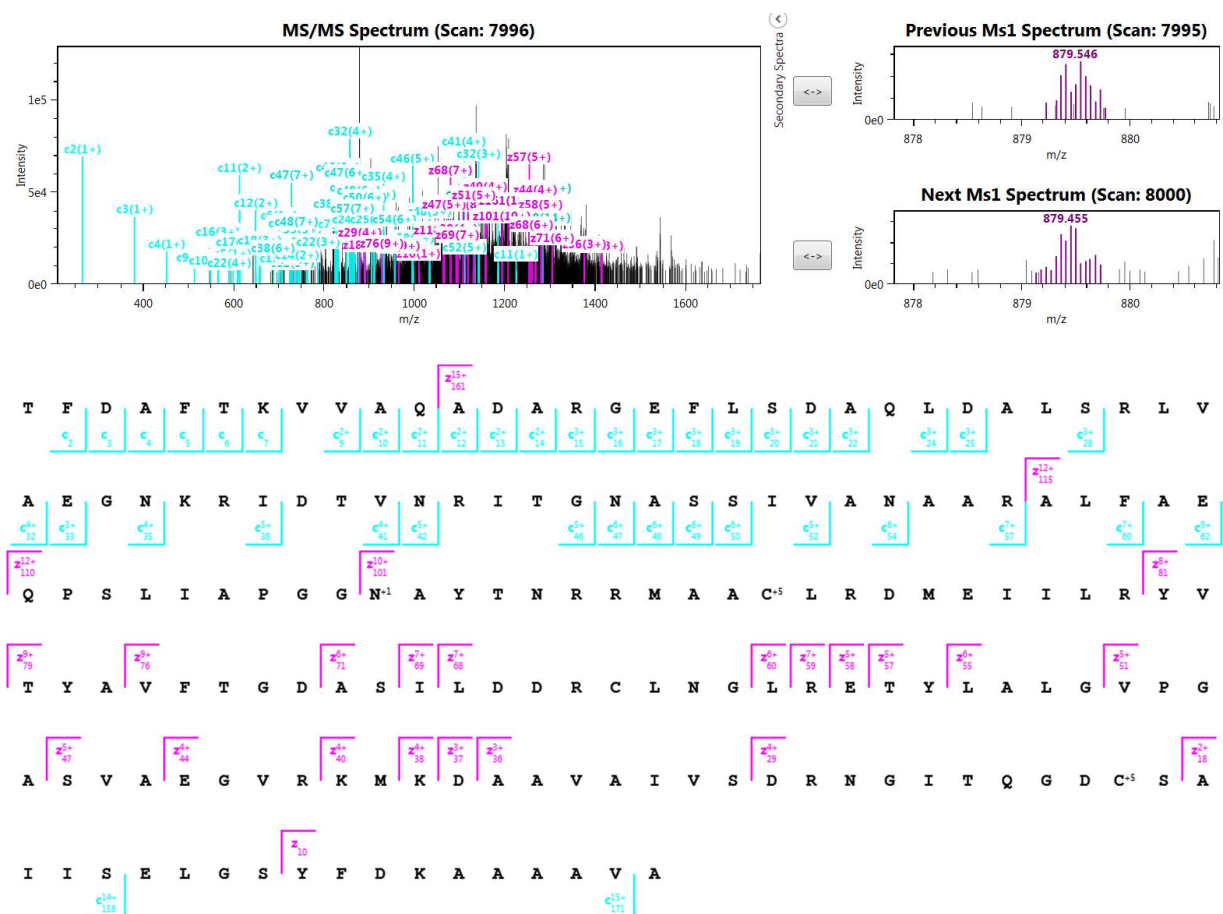
**Figure S8.** Example data for identified ApcD proteoform with one PCB on Cys81 (File: UTEX2973\_topD\_21\_lumos\_29Sep16\_Bane\_16-04-17, scan 6535, charge state +18). Top panel showed the annotated MS/MS spectrum on the left, and the adjacent MS spectra zoomed to the precursor regions. Bottom panel showed the coverage map.



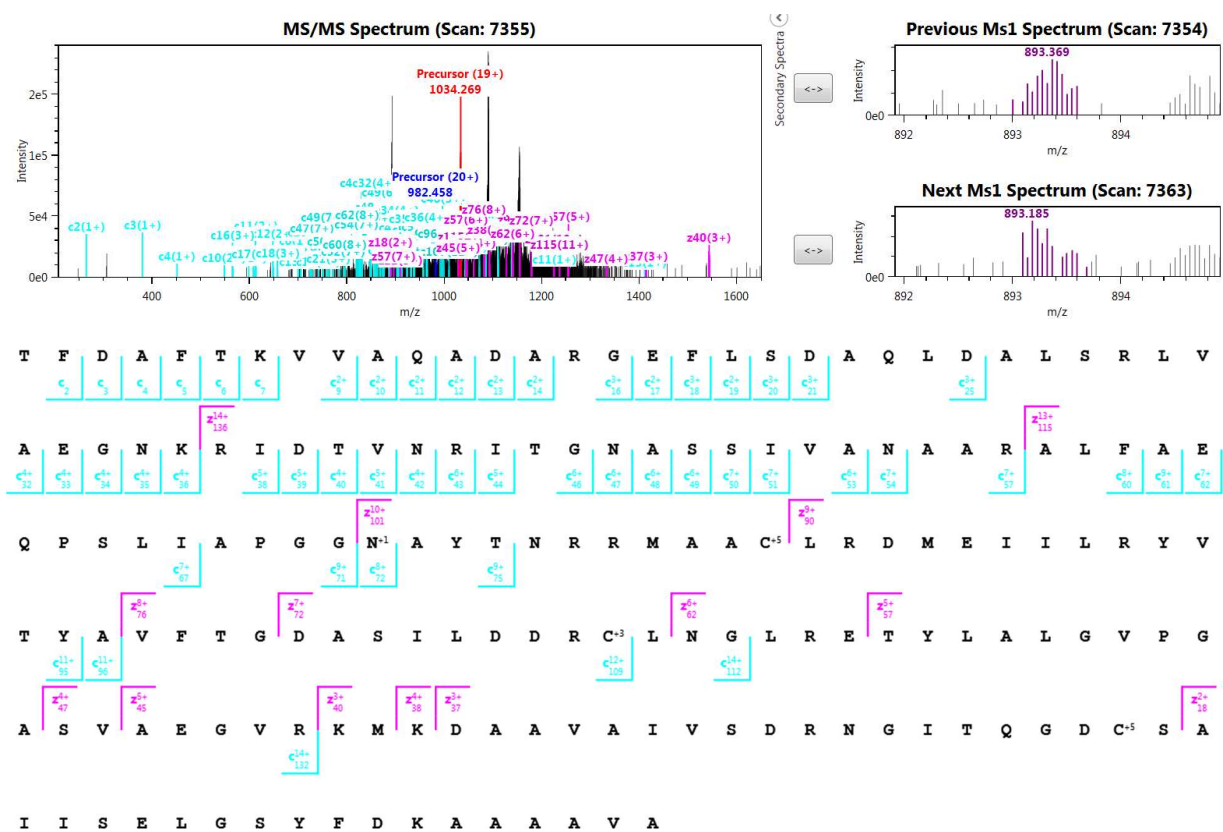
**Figure S9.** Example data for identified ApcF proteoform with one PCB on Cys82, and one methyl on N72 (File: UTEX2973\_topD\_21\_lumos\_29Sep16\_Bane\_16-04-17, scan 6709, charge state +21). Top panel showed the annotated MS/MS spectrum on the left, and the adjacent MS spectra zoomed to the precursor regions. Bottom panel showed the coverage map.



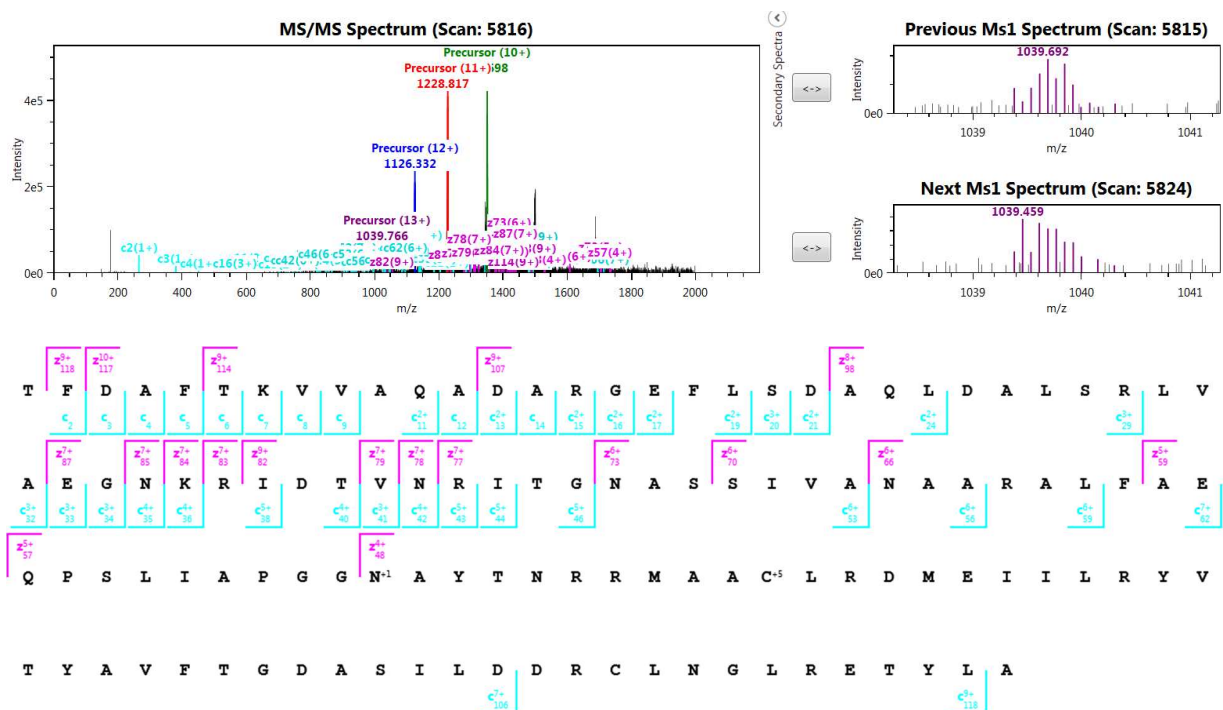
**Figure S10.** Example data for identified CpcA proteoform with one PCB on Cys85 (File: UTEX2973\_topD\_14\_lumos\_29Sep16\_Bane\_16-04-17, scan 6815, charge state +21). Top panel showed the annotated MS/MS spectrum on the left, and the adjacent MS spectra zoomed to the precursor regions. Bottom panel showed the coverage map.



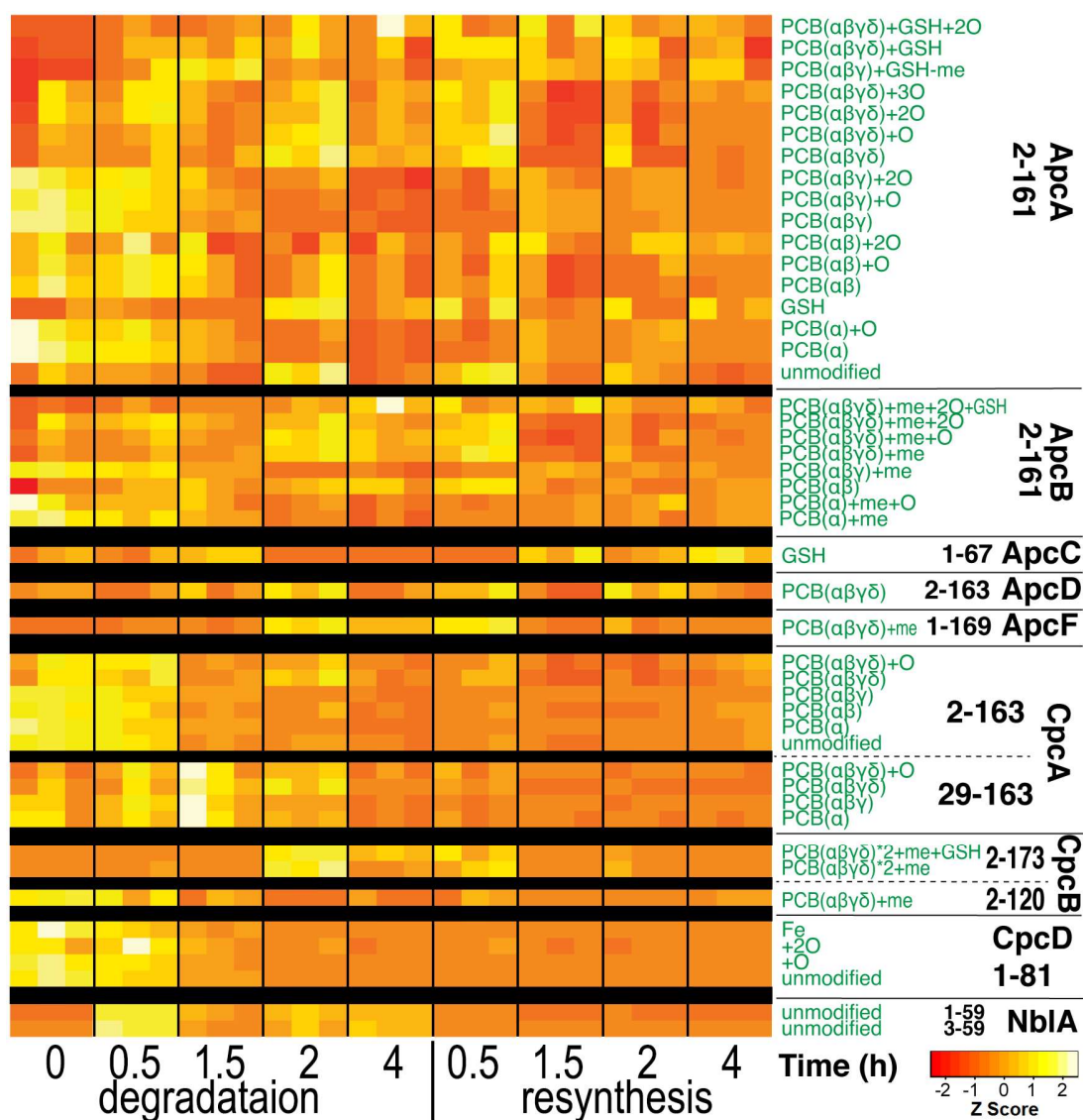
**Figure S11.** Example data for identified CpcB proteoform with methyl on N73, two PCBs on Cys83 and Cys154 (File: UTEX2973\_topD\_19\_lumos\_29Sep16\_Bane\_16-04-17, scan 7996, charge state +22). Modification sites were assigned based on the coverage and the preknowledge of the modifications. Top panel showed the annotated MS/MS spectrum on the left, and the adjacent MS spectra zoomed to the precursor regions. Bottom panel showed the coverage map.



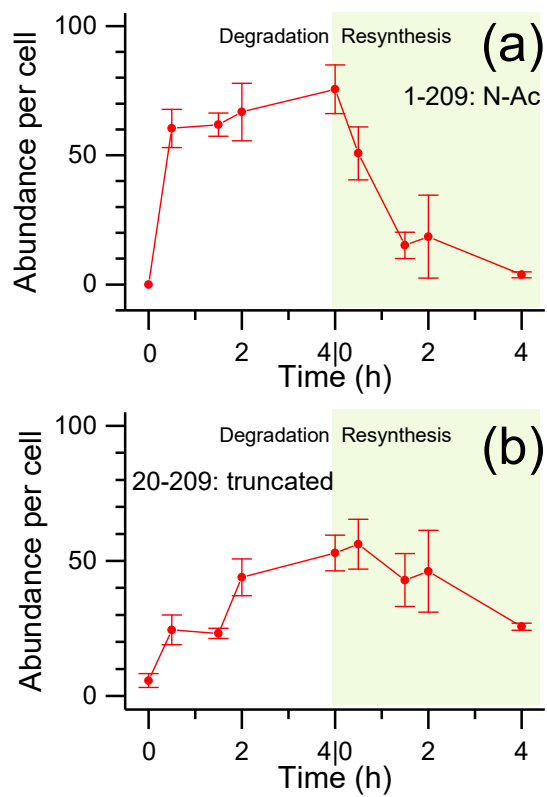
**Figure S12.** Example data for identified CpcB proteoform with methyl on N73, two PCBs on Cys83 and Cys154, and glutathione on Cys110 (File: UTEX2973\_topD\_18\_lumos\_29Sep16\_Bane\_16-04-17, scan 7355, charge state +22). Although the coverage is not sufficient to confidently assign all modification sites, we did detect CpcB fragments with PCB on Cys154, methyl on N73, and glutathione on Cys110. Data on these degraded CpcB fragments supported the assignment of modifications on the full-length CpcB. Top panel showed the annotated MS/MS spectrum on the left, and the adjacent MS spectra zoomed to the precursor regions. Bottom panel showed the coverage map.



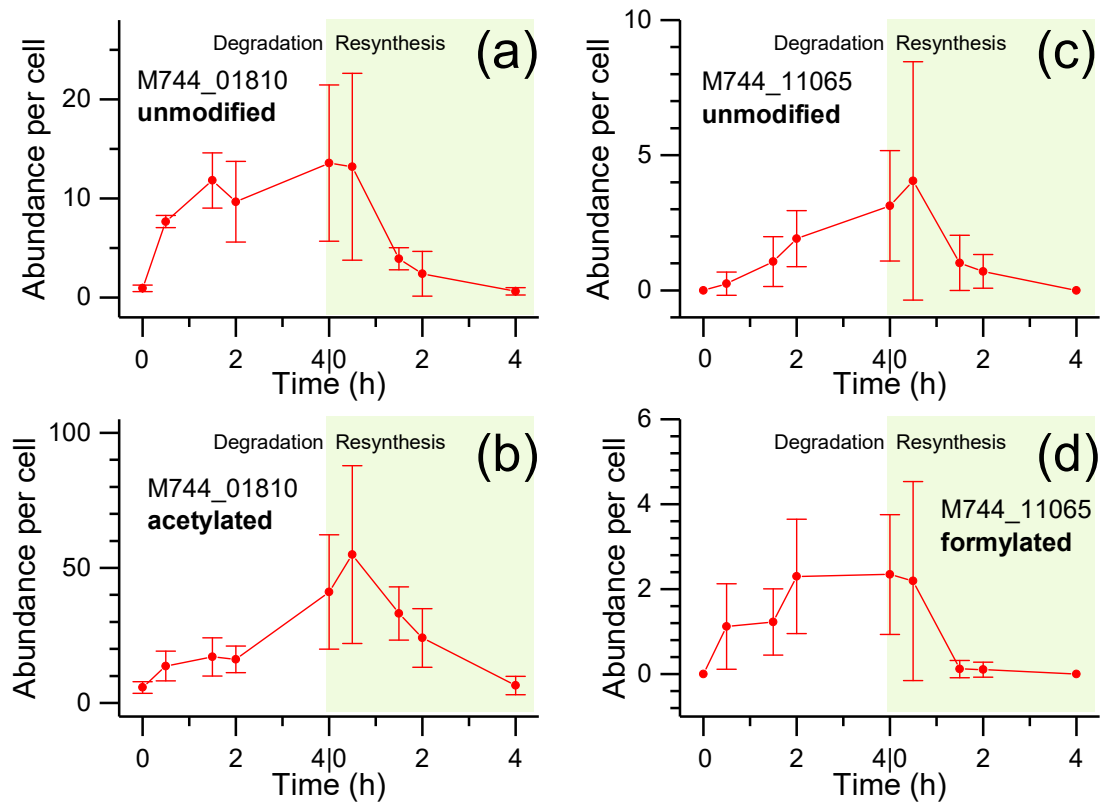
**Figure S13.** Example data for the truncated CpcB proteoform (residue 2-120) with methyl on N73, one PCs on Cys83 (File: UTEX2973\_topD\_05\_lumos\_29Sep16\_Bane\_16-04-17, scan 5816, charge state +13). The sites of modifications were assigned based on the knowledge of the protein due to the limited coverage near the C-terminus of the proteins. Top panel showed the annotated MS/MS spectrum on the left, and the adjacent MS spectra zoomed to the precursor regions. Bottom panel showed the coverage map.



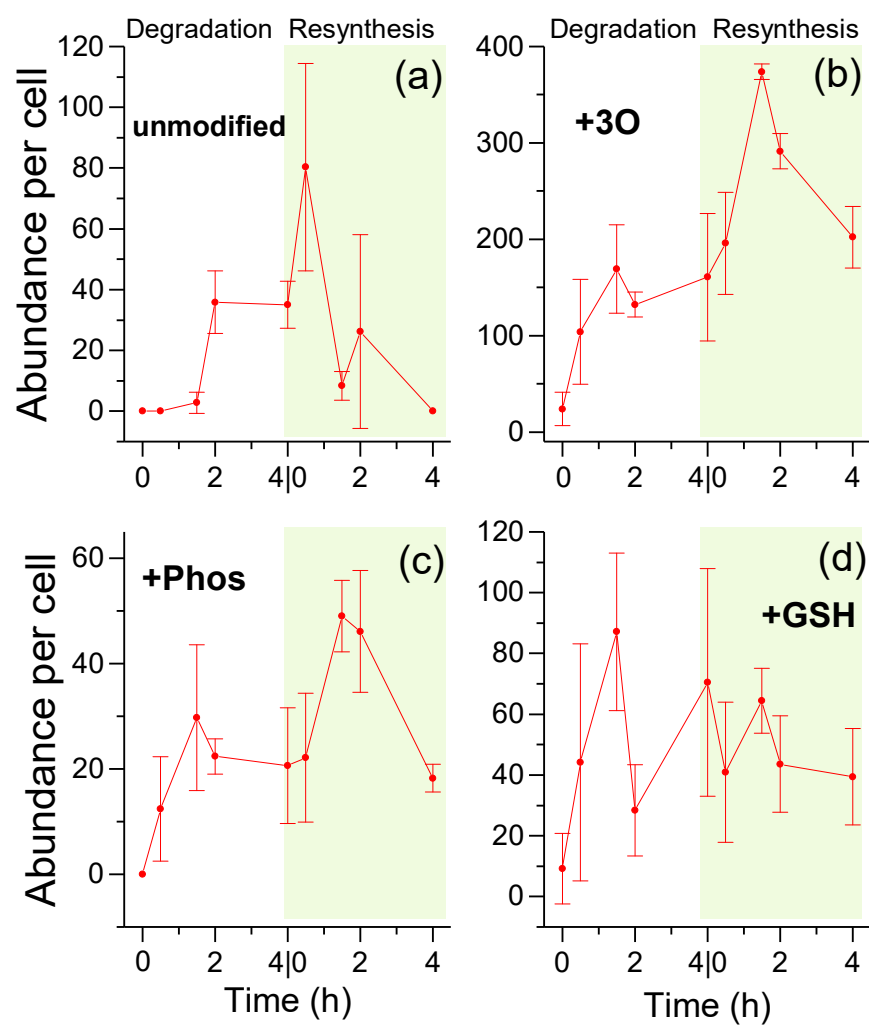
**Figure S14.** Heatmap for abundance changes of proteoforms including all replicates. Three replicates for each time points are grouped and separated from other time points by vertical black lines. In general the replicates are consistent with each other.



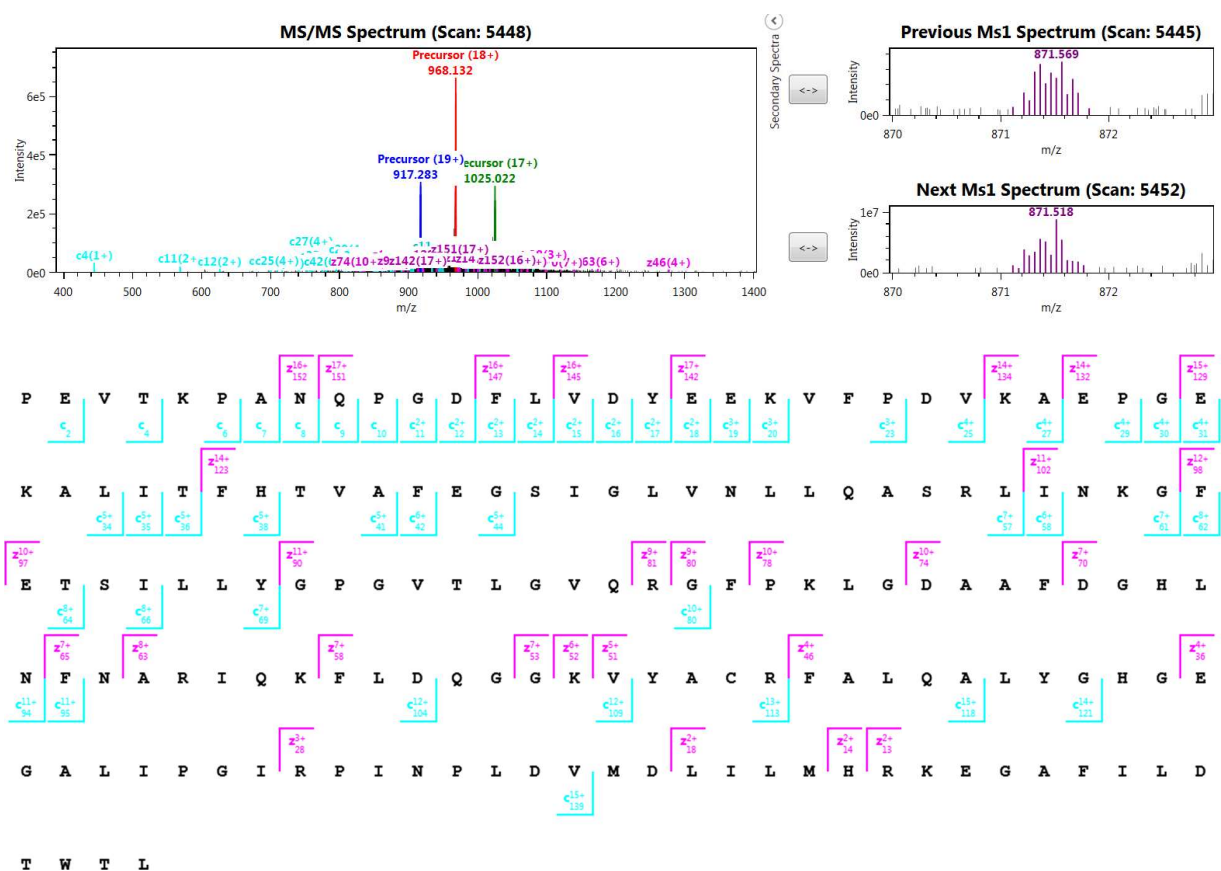
**Figure S15.** Abundance change across time points for M744\_01170 chemotaxis protein CheY (a) full length with N-terminal acetylation, and (b) N-terminally truncated form (residue 20-209).



**Figure S16.** Abundance change across time points for two highlight inducible proteins (a-b) M744\_01810, and (c-d) M744\_11065.

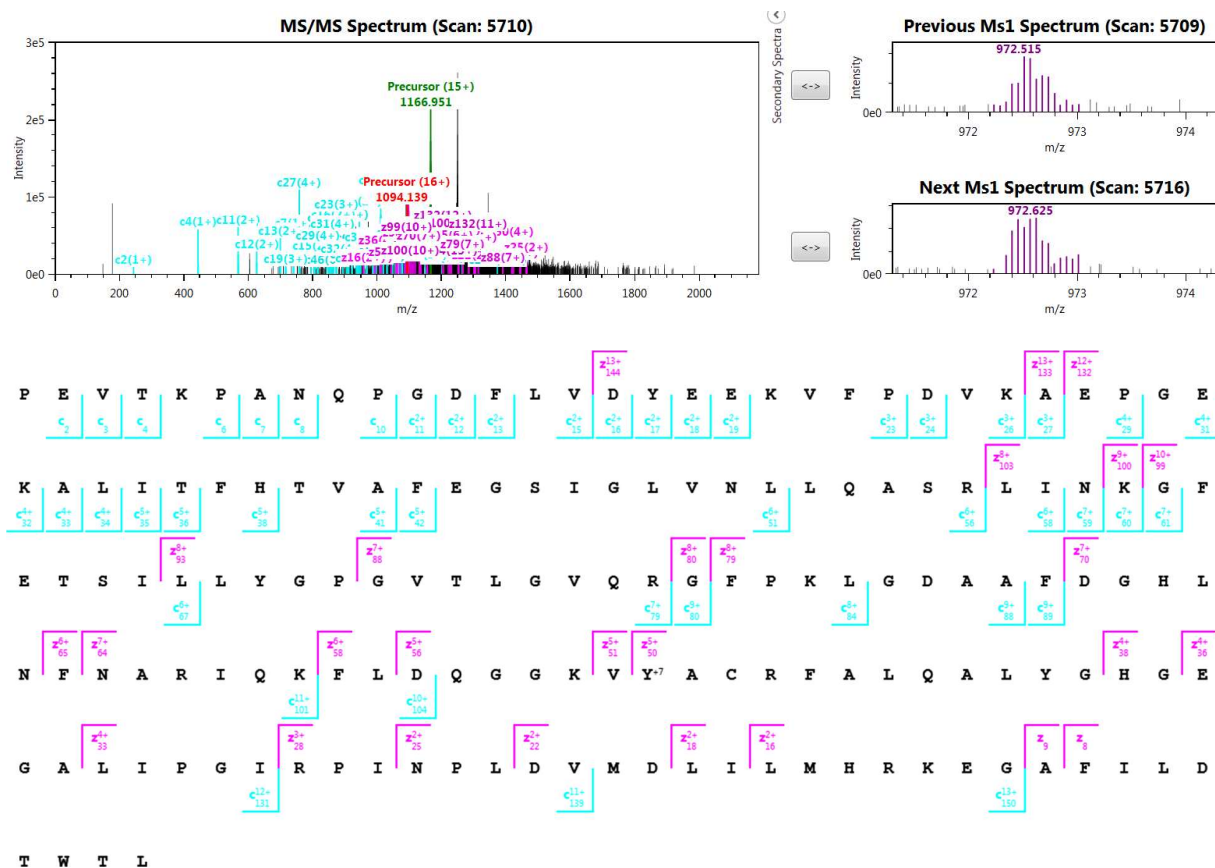


**Figure S17.** Abundance change across time points for the major proteoforms of M744\_12535. This protein blasted to nitrogen starvation response protein.



**Figure S18.** Example data for the nitrogen starvation response protein M744\_12535 without modification (File: UTEX2973\_topD\_29\_lumos\_29Sep16\_Bane\_16-04-17, scan 5448, charge state +20). Top panel showed the annotated MS/MS spectrum on the left, and the adjacent MS spectra zoomed to the precursor regions. Bottom panel showed the coverage map.





**Figure S20.** Example data for the nitrogen starvation response protein M744\_12535 with 79.96 Da modification on Tyr111 (File: UTEX2973\_topD\_26\_lumos\_29Sep16\_Bane\_16-04-17, scan 5710, charge state +18). The PTM is likely to be phosphorylation (+79.966), but it may be assigned as sulphation (+79.957 Da) as well. Top panel showed the annotated MS/MS spectrum on the left, and the adjacent MS spectra zoomed to the precursor regions. Bottom panel showed the coverage map.

