

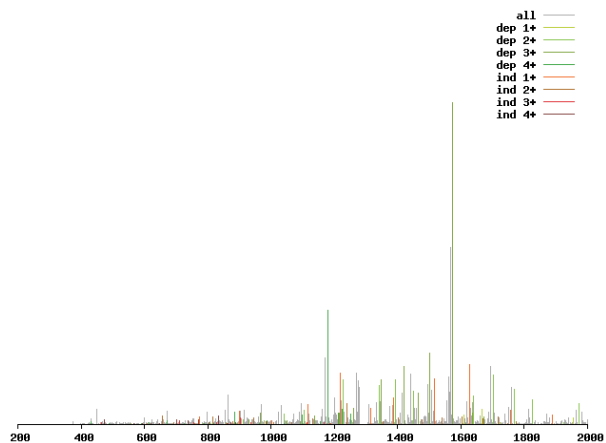
Supplemental Table 1

| NEDD8-UbcH12 Linkage | Peptide Sequence | Predicted <i>m/z</i> NEDD8-modified LysC Peptide | Predicted <i>m/z</i> GG-modified Tryptic Peptide |
|----------------------------------|--|--|--|
| K8 (aa 4-11) | (K ₃)LFSLKQQK ₁₁ (K) | (2+) 1274.28 (3+) 849.86 (4+) 637.64 | (2+) 553.32 (3+) 369.22 (4+) 277.16 |
| K11 (aa 9-12) | (K ₈)QQKK ₁₂ (E) | (2+) 1044.14 (3+) 696.43 (4+) 522.58 | (2+) 323.19 (3+) 215.79 (4+) 162.10 |
| K12 (aa 12-21) | (K ₁₁)KEEESAGGTK ₂₁ (G) | (2+) 1296.23 (3+) 864.49 (4+) 648.62 | (2+) 575.27 (3+) 383.85 (4+) 288.14 |
| K21 (aa 13-25) | (K ₁₂)EEESAGGTKGSSK ₂₅ (K) | (2+) 1411.77 (3+) 941.52 (4+) 706.39 | (2+) 690.81 (3+) 460.88 (4+) 345.91 |
| K25 (aa 22-26) | (K ₂₁)GSSKK ₂₆ (A) | (2+) 1031.63 (3+) 688.09 (4+) 516.32 | (2+) 310.67 (3+) 207.45 (4+) 155.84 |
| K26 (aa 26-36 or aa 26-33) | (K ₂₅)KASAAQLRIQK ₃₆ (D) - LysC or (K ₂₅)KASAAQLR ₃₃ (I) - Trypsin | (2+) 1385.35 (3+) 923.90 (4+) 693.18 | (2+) 479.77 (3+) 320.19 (4+) 240.39 |

Supplemental Figure 1

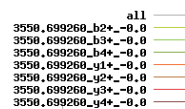
A

| Scan | Charge | relPos | modScore | precursor mass | matched ions | TIC | %TIC | BPI |
|------|--------|--------|----------|----------------|--------------|--------|----------|---------|
| 6001 | 4 | 1 | 1 | 1609.81 | 131 | 172878 | 0.455145 | 10802.8 |



| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 | 25 | 26 | 27 | 28 | 29 | 30 | 31 | 32 | 33 |
|--------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|----|
| dep 1+ | 1609.66 | 1666.71 | 1723.76 | 1824.87 | 1953 | 2081.13 | 2209.26 | 2356.43 | 2455.57 | 2570.66 | 2683.82 | 2784.92 | 2900.01 | 3029.12 | 3144.21 | 3273.33 | 3404.52 | 3533.64 | 3646.8 | 3774.93 | 3846.01 | 3943.12 | 4044.23 | 4159.32 | 4260.42 | 4389.54 | 4503.64 | 4616.8 | 4713.92 | 4842.05 | 4899.1 | 5014.19 | |
| dep 2+ | 805.332 | 833.858 | 862.384 | 912.937 | 977.002 | 1041.07 | 1105.13 | 1178.72 | 1228.29 | 1285.83 | 1342.41 | 1392.96 | 1450.51 | 1515.07 | 1572.61 | 1637.17 | 1702.76 | 1767.32 | 1823.9 | 1887.97 | 1923.51 | 1972.07 | 2022.62 | 2080.16 | 2130.71 | 2195.27 | 2252.32 | 2308.9 | 2357.46 | 2421.53 | 2450.05 | 2507.6 | |
| dep 3+ | 537.224 | 556.242 | 575.259 | 608.961 | 651.671 | 694.381 | 737.091 | 786.15 | 819.194 | 857.557 | 895.277 | 928.979 | 967.342 | 1010.38 | 1048.74 | 1091.78 | 1135.51 | 1178.53 | 1216.27 | 1258.98 | 1282.67 | 1315.05 | 1348.75 | 1387.11 | 1420.81 | 1463.85 | 1501.89 | 1539.61 | 1571.98 | 1614.69 | 1633.7 | 1672.07 | |
| dep 4+ | 403.17 | 417.433 | 431.696 | 456.972 | 489.005 | 521.038 | 553.07 | 589.865 | 614.648 | 643.42 | 671.71 | 696.986 | 725.758 | 758.037 | 786.809 | 819.088 | 851.886 | 884.163 | 912.455 | 944.488 | 962.257 | 986.536 | 1011.81 | 1040.58 | 1065.86 | 1098.14 | 1126.67 | 1154.96 | 1179.24 | 1211.27 | 1225.53 | 1254.3 | |
| ind 1+ | 3551.71 | 3494.66 | 3437.6 | 3336.5 | 3208.37 | 3080.24 | 2952.11 | 2804.93 | 2705.8 | 2590.71 | 2477.55 | 2376.44 | 2261.36 | 2132.24 | 2017.15 | 1888.04 | 1756.84 | 1627.73 | 1514.57 | 1386.44 | 1315.36 | 1218.24 | 1117.14 | 1002.05 | 900.943 | 771.828 | 657.724 | 544.564 | 447.448 | 319.317 | 262.265 | 147.177 | |
| ind 2+ | 1776.36 | 1747.83 | 1719.31 | 1668.75 | 1604.69 | 1540.62 | 1476.56 | 1402.97 | 1353.4 | 1295.86 | 1239.28 | 1188.73 | 1131.18 | 1066.62 | 1009.08 | 944.522 | 878.925 | 814.368 | 757.788 | 693.723 | 658.183 | 609.625 | 559.072 | 501.528 | 450.976 | 386.418 | 329.366 | 272.786 | 224.228 | 160.162 | 131.637 | 74.0922 | |
| ind 3+ | 1184.57 | 1165.56 | 1146.54 | 1112.84 | 1070.13 | 1027.42 | 984.707 | 935.648 | 902.604 | 864.241 | 826.522 | 792.82 | 754.457 | 711.418 | 673.056 | 630.017 | 586.286 | 543.248 | 505.528 | 462.818 | 439.125 | 406.753 | 373.051 | 334.688 | 300.986 | 257.948 | 219.913 | 182.193 | 149.821 | 107.111 | 88.0936 | 49.7308 | |
| ind 4+ | 888.683 | 874.42 | 860.157 | 834.88 | 802.848 | 770.815 | 738.782 | 701.988 | 677.205 | 648.433 | 620.143 | 594.867 | 566.095 | 533.816 | 505.044 | 472.765 | 439.967 | 407.688 | 379.398 | 347.365 | 329.596 | 305.316 | 280.04 | 251.268 | 225.992 | 193.713 | 165.187 | 136.897 | 112.618 | 80.5851 | 66.3222 | 37.55 | |
| | 33 | 32 | 31 | 30 | 29 | 28 | 27 | 26 | 25 | 24 | 23 | 22 | 21 | 20 | 19 | 18 | 17 | 16 | 15 | 14 | 13 | 12 | 11 | 10 | 9 | 8 | 7 | 6 | 5 | 4 | 3 | 2 | 1 |

- o >SUMO2WT
- o EGVK(0)TENNDRINK
- o 1609.81
- o matched ions: 36
- o %TIC: 0.0790599
- o Cons: 0.142857
- o Delta Score: 0
- o target score: **0.933333**

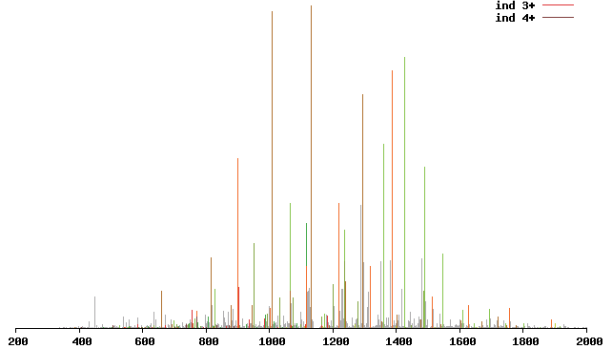


| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 |
|----------------------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|----|
| | E | G | V | K(0) | T | E | N | N | D | H | I | N | L | K |
| 3550.699260_b2+_-0.0 | 65.5656 | 94.0916 | 143.658 | 1983.09 | 2033.65 | 2098.2 | 2155.26 | 2212.31 | 2269.85 | 2338.42 | 2395 | 2452.06 | 2508.63 | |
| 3550.699260_b3+_-0.0 | 44.0464 | 63.0637 | 96.1078 | 1322.4 | 1356.1 | 1399.14 | 1437.17 | 1475.21 | 1513.57 | 1559.28 | 1597 | 1635.04 | 1672.76 | |
| 3550.699260_b4+_-0.0 | 33.2867 | 47.5497 | 72.3328 | 992.051 | 1017.33 | 1049.61 | 1078.13 | 1106.66 | 1135.43 | 1169.72 | 1198.01 | 1226.53 | 1254.82 | |
| 3550.699260_y1+_-0.0 | 5033.34 | 4976.28 | 4877.15 | 1198.28 | 1097.17 | 968.057 | 853.954 | 739.85 | 624.761 | 487.62 | 374.461 | 260.357 | 147.197 | |
| 3550.699260_y2+_-0.0 | 2517.17 | 2488.65 | 2439.08 | 599.643 | 549.09 | 484.533 | 427.481 | 370.429 | 312.884 | 244.314 | 187.734 | 130.682 | 74.1026 | |
| 3550.699260_y3+_-0.0 | 1678.45 | 1659.43 | 1626.39 | 400.098 | 366.396 | 323.358 | 285.323 | 247.288 | 208.926 | 163.212 | 125.492 | 87.4575 | 49.7376 | |
| 3550.699260_y4+_-0.0 | 1259.09 | 1244.83 | 1220.04 | 300.325 | 275.049 | 242.77 | 214.244 | 185.718 | 156.946 | 122.661 | 94.371 | 65.8451 | 37.5552 | |
| | 14 | 13 | 12 | 11 | 10 | 9 | 8 | 7 | 6 | 5 | 4 | 3 | 2 | 1 |

B

| Scan | Charge | relPos | modScore | precursor mass | matched ions | TIC | %TIC | BPI |
|------|--------|--------|----------|----------------|--------------|-------------|----------|--------|
| 4873 | 4 | 1 | 1 | 1053.61 | 108 | 3.05424e+06 | 0.663465 | 120069 |

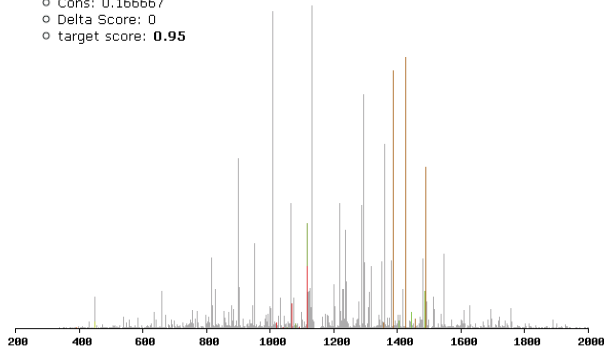
all
dep 1+
dep 2+
dep 3+
dep 4+
ind 1+
ind 2+
ind 3+
ind 4+



| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 | 25 | 26 | 27 | 28 | 29 | 30 | 31 | 32 | 33 |
|--------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|----|
| dep 1+ | 1053.45 | 1110.5 | 1167.56 | 1268.66 | 1396.79 | 1524.92 | 1653.05 | 1800.23 | 1899.36 | 2014.45 | 2127.61 | 2228.72 | 2343.8 | 2472.92 | 2588.01 | 2717.12 | 2848.32 | 2977.43 | 3090.59 | 3218.72 | 3289.8 | 3386.92 | 3488.02 | 3603.11 | 3704.22 | 3833.33 | 3947.44 | 4060.6 | 4157.71 | 4285.84 | 4342.9 | 4457.98 | |
| dep 2+ | 527.23 | 555.756 | 584.282 | 634.835 | 698.9 | 762.966 | 827.031 | 900.619 | 950.186 | 1007.73 | 1064.31 | 1114.86 | 1172.41 | 1236.96 | 1294.51 | 1359.07 | 1424.66 | 1489.22 | 1545.8 | 1609.87 | 1645.4 | 1693.96 | 1744.52 | 1802.06 | 1852.61 | 1917.17 | 1974.22 | 2030.8 | 2079.36 | 2143.43 | 2171.95 | 2229.5 | |
| dep 3+ | 351.823 | 370.84 | 389.857 | 423.559 | 466.269 | 508.98 | 551.69 | 600.749 | 633.793 | 672.156 | 709.876 | 743.577 | 781.94 | 824.979 | 863.342 | 906.38 | 950.111 | 993.149 | 1030.87 | 1073.58 | 1097.27 | 1129.64 | 1163.35 | 1201.71 | 1235.41 | 1278.45 | 1316.48 | 1354.2 | 1386.58 | 1429.29 | 1448.3 | 1486.67 | |
| dep 4+ | 264.119 | 278.382 | 292.645 | 317.921 | 349.954 | 381.987 | 414.019 | 450.814 | 475.597 | 504.369 | 532.659 | 557.935 | 586.707 | 618.986 | 647.758 | 680.037 | 712.835 | 745.114 | 773.404 | 805.437 | 823.206 | 847.485 | 872.762 | 901.534 | 926.81 | 959.089 | 987.615 | 1015.9 | 1040.18 | 1072.22 | 1086.48 | 1115.25 | |
| ind 1+ | 3551.71 | 3494.66 | 3437.6 | 3336.5 | 3208.37 | 3080.24 | 2952.11 | 2804.93 | 2705.8 | 2590.71 | 2477.55 | 2376.44 | 2261.36 | 2132.24 | 2017.15 | 1888.04 | 1756.84 | 1627.73 | 1514.57 | 1386.44 | 1315.36 | 1218.24 | 1117.14 | 1002.05 | 900.943 | 771.828 | 657.724 | 544.564 | 447.448 | 319.317 | 262.265 | 147.177 | |
| ind 2+ | 1776.36 | 1747.83 | 1719.31 | 1668.75 | 1604.69 | 1540.62 | 1476.56 | 1402.97 | 1353.4 | 1295.86 | 1239.28 | 1188.73 | 1131.18 | 1066.62 | 1009.08 | 944.522 | 878.925 | 814.368 | 757.788 | 693.723 | 658.183 | 609.625 | 559.072 | 501.528 | 450.976 | 386.418 | 329.366 | 272.786 | 224.228 | 160.162 | 131.637 | 74.0922 | |
| ind 3+ | 1184.57 | 1165.56 | 1146.54 | 1112.84 | 1070.13 | 1027.42 | 984.707 | 935.648 | 902.604 | 864.241 | 826.522 | 792.82 | 754.457 | 711.418 | 673.056 | 630.017 | 586.286 | 543.248 | 505.528 | 462.818 | 439.125 | 406.753 | 373.051 | 334.688 | 300.986 | 257.948 | 219.913 | 182.193 | 149.821 | 107.111 | 88.0936 | 49.7308 | |
| ind 4+ | 888.683 | 874.42 | 860.157 | 834.88 | 802.848 | 770.815 | 738.782 | 701.988 | 677.205 | 648.433 | 620.143 | 594.867 | 566.095 | 533.816 | 505.044 | 472.765 | 439.967 | 407.688 | 379.398 | 347.365 | 329.596 | 305.316 | 280.04 | 251.268 | 225.992 | 193.713 | 165.187 | 136.897 | 112.618 | 80.5851 | 66.3222 | 37.55 | |
| | 33 | 32 | 31 | 30 | 29 | 28 | 27 | 26 | 25 | 24 | 23 | 22 | 21 | 20 | 19 | 18 | 17 | 16 | 15 | 14 | 13 | 12 | 11 | 10 | 9 | 8 | 7 | 6 | 5 | 4 | 3 | 2 | 1 |

o >SUMO2WT >SUMO3WT
o HTPLSK(O)LMK
o 1053.61
o matched ions: 14
o %TIC: 0.117636
o Cons: 0.166667
o Delta Score: 0
o target score: 0.95

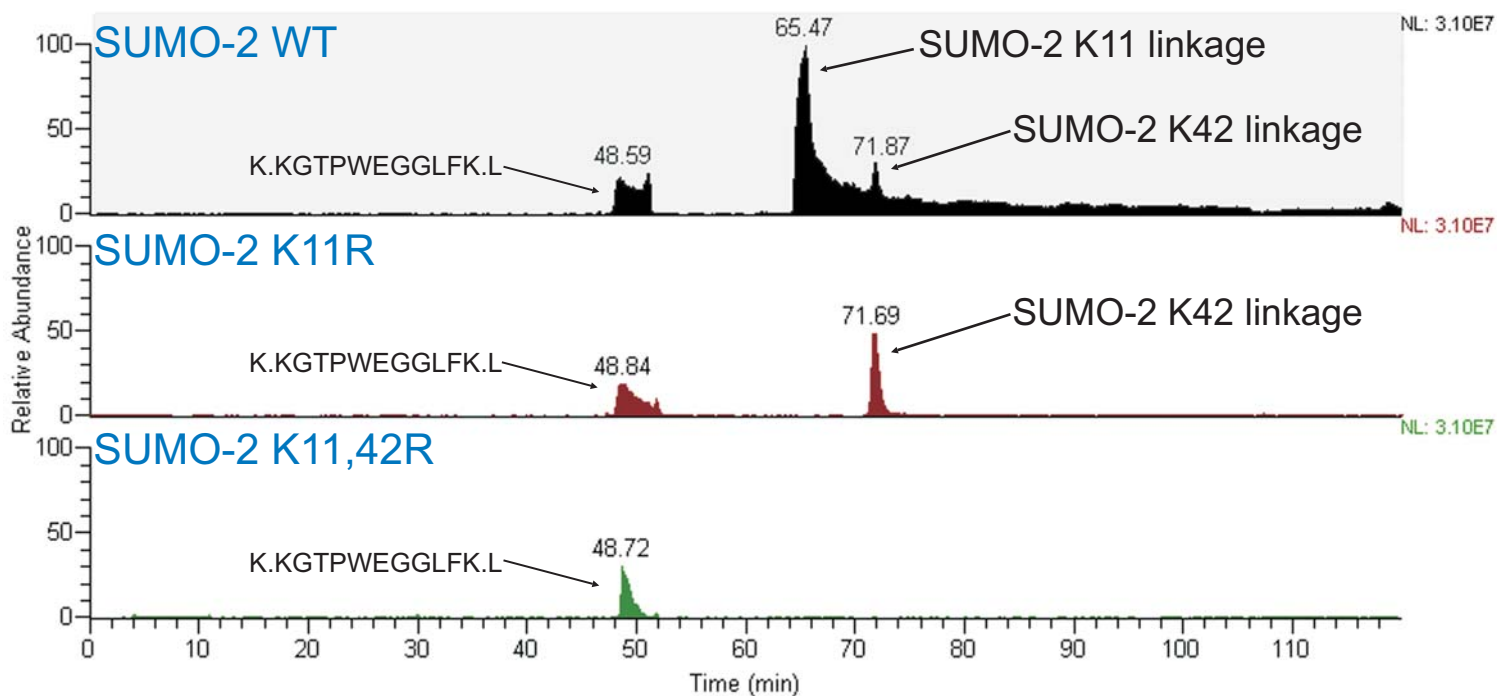
all
3550.699260_b1+_-0.0
3550.699260_b3+_-0.0
3550.699260_b4+_-0.0
3550.699260_y1+_-0.0
3550.699260_y3+_-0.0
3550.699260_y4+_-0.0



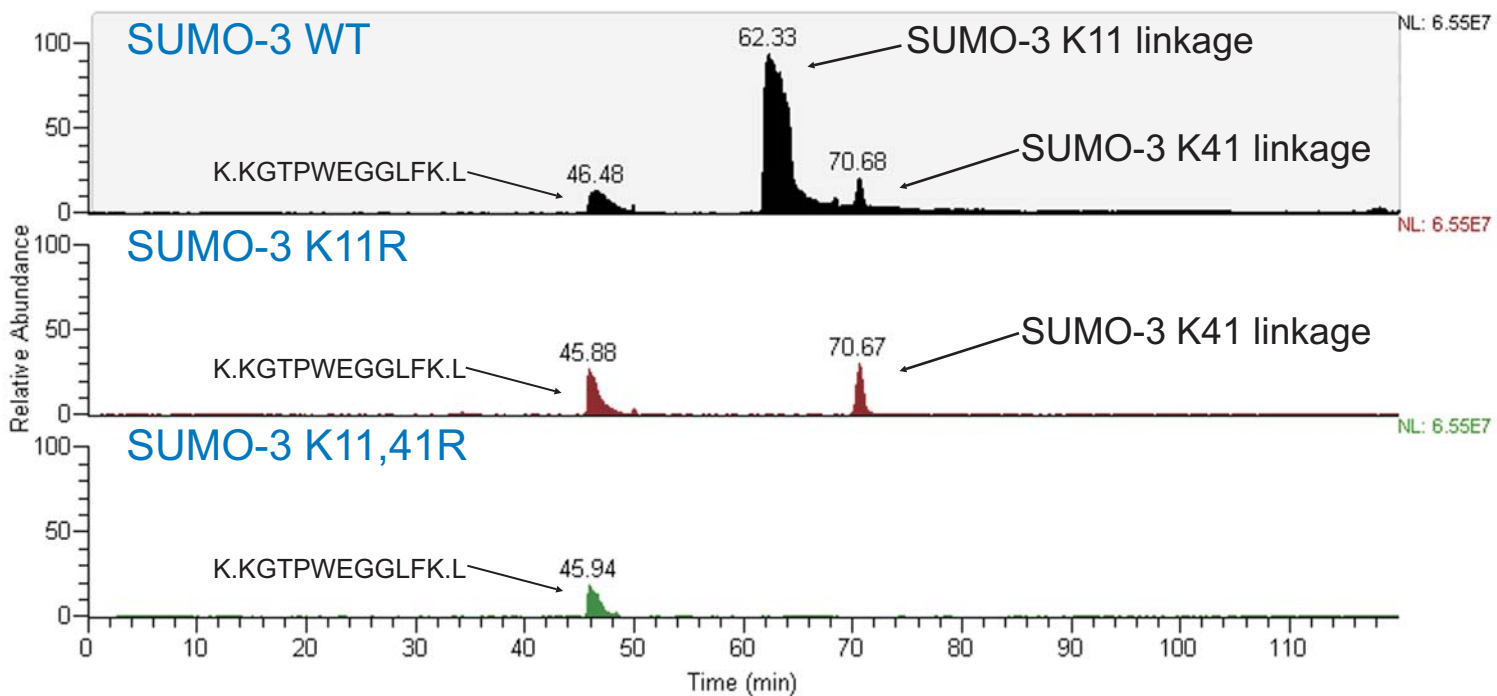
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
|----------------------|---------|---------|---------|---------|---------|---------|---------|---------|---------|
| | H | T | P | L | S | K(O) | L | M | K |
| 3550.699260_b1+_-0.0 | 138.149 | 239.254 | 336.371 | 449.53 | 536.608 | 4215.48 | 4328.64 | 4459.83 | |
| 3550.699260_b3+_-0.0 | 46.7216 | 80.4232 | 112.795 | 150.515 | 179.541 | 1405.83 | 1443.55 | 1487.28 | |
| 3550.699260_b4+_-0.0 | 35.2931 | 60.5694 | 84.8486 | 113.138 | 134.908 | 1054.63 | 1082.92 | 1115.71 | |
| 3550.699260_y1+_-0.0 | | 4468.88 | 4367.78 | 4270.66 | 4157.5 | 4070.42 | 391.549 | 278.39 | 147.197 |
| 3550.699260_y3+_-0.0 | | 1490.3 | 1456.6 | 1424.23 | 1386.51 | 1357.48 | 131.188 | 93.4685 | 49.7376 |
| 3550.699260_y4+_-0.0 | | 1117.98 | 1092.7 | 1068.42 | 1040.13 | 1018.36 | 98.6432 | 70.3533 | 37.5552 |
| | 9 | 8 | 7 | 6 | 5 | 4 | 3 | 2 | 1 |

Supplemental Figure 2

A



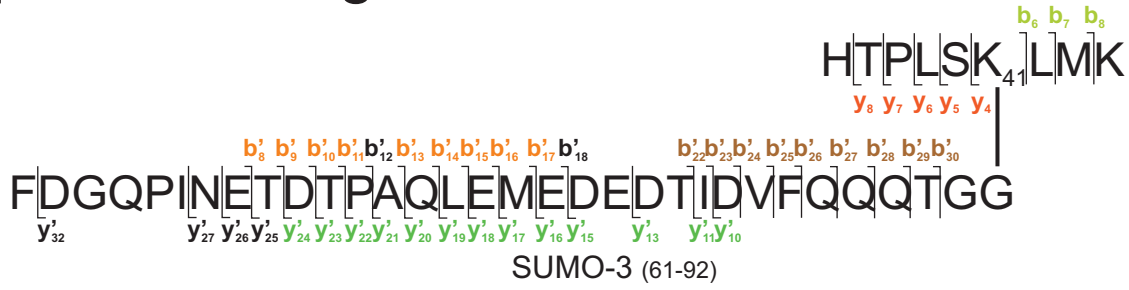
B



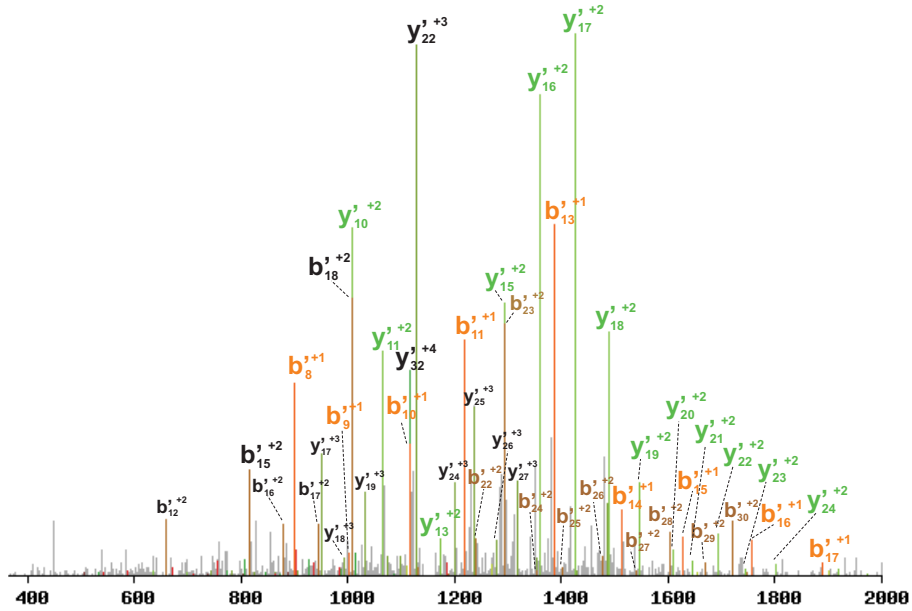
Supplemental Figure 3

SUMO-3 (36-44)

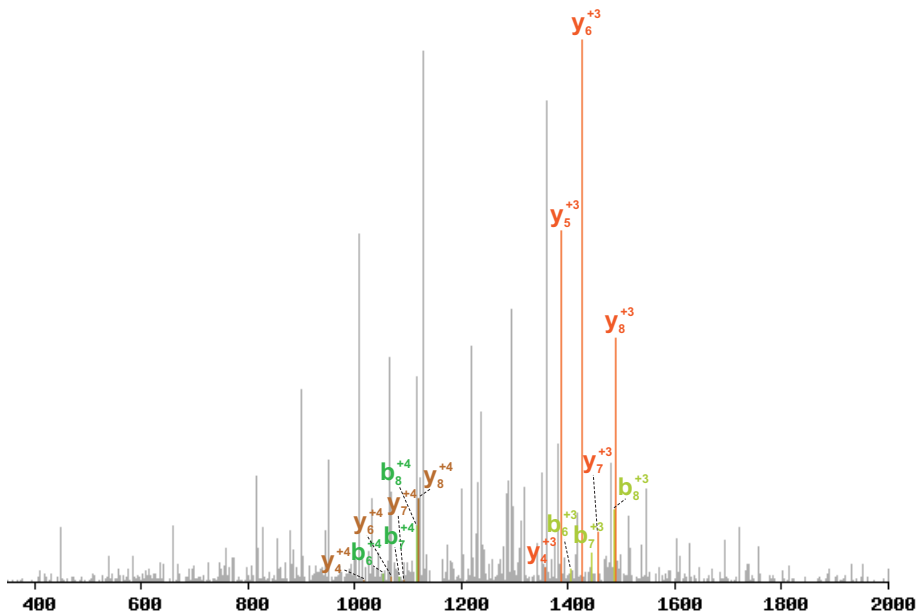
A



B Modification



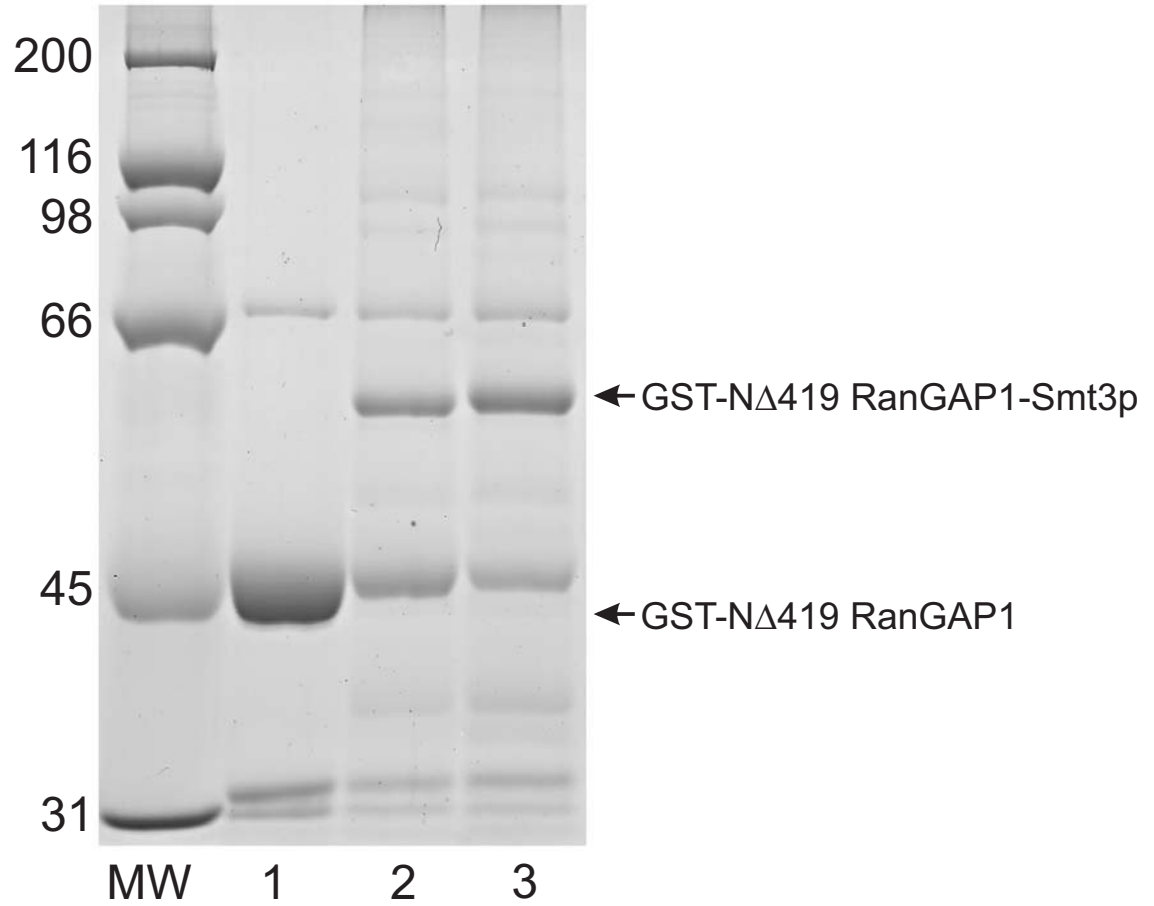
C Target



Supplemental Figure 4

A

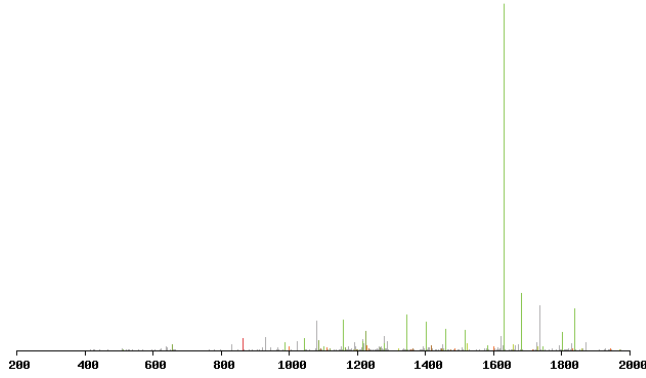
| | | | |
|----------------------------|---|---|---|
| E1,E2 and ATP | - | + | + |
| Smt3p WT | - | + | - |
| Smt3p R93A | - | - | + |
| GST-N Δ 419 RanGAP1 | + | + | + |



B

| Scan | Charge | relPos | modScore | precursor mass | matched ions | TIC | %TIC | BPI |
|------|--------|--------|----------|----------------|--------------|--------|----------|---------|
| 3989 | 3 | 1 | 1 | 964.527 | 70 | 190259 | 0.461746 | 39681.3 |

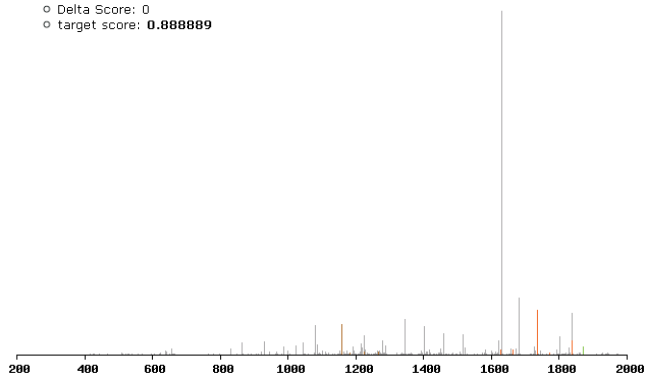
all
dep 1+
dep 2+
dep 3+
ind 1+
ind 2+
ind 3+



| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 | 25 | 26 | 27 | 28 |
|--------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|----|
| dep 1+ | 964.762 | 1021.81 | 1078.86 | 1192.02 | 1320.15 | 1449.26 | 1520.34 | 1657.48 | 1728.56 | 1857.67 | 1970.83 | 2083.99 | 2199.08 | 2313.18 | 2428.27 | 2557.38 | 2688.58 | 2803.67 | 2916.83 | 3031.92 | 3161.03 | 3258.15 | 3359.25 | 3487.38 | 3602.47 | 3673.55 | 3801.68 | |
| dep 2+ | 482.885 | 511.41 | 539.935 | 596.515 | 660.58 | 725.135 | 780.675 | 829.245 | 864.785 | 929.34 | 985.92 | 1042.5 | 1100.04 | 1157.09 | 1214.64 | 1279.19 | 1344.79 | 1402.34 | 1458.92 | 1516.46 | 1581.02 | 1629.58 | 1680.13 | 1744.19 | 1801.74 | 1837.28 | 1901.34 | |
| dep 3+ | 322.259 | 341.276 | 360.292 | 398.012 | 440.722 | 483.759 | 507.452 | 553.166 | 576.859 | 619.896 | 657.616 | 695.336 | 733.699 | 771.732 | 810.096 | 853.132 | 896.866 | 935.229 | 972.949 | 1011.31 | 1054.35 | 1086.72 | 1120.42 | 1163.13 | 1201.5 | 1225.19 | 1267.9 | |
| ind 1+ | 2950.08 | 2893.03 | 2835.98 | 2722.82 | 2594.69 | 2465.58 | 2394.5 | 2257.36 | 2186.28 | 2057.17 | 1944.01 | 1830.85 | 1715.76 | 1601.66 | 1486.57 | 1357.46 | 1226.26 | 1111.17 | 998.01 | 882.92 | 753.81 | 656.69 | 555.59 | 427.46 | 312.37 | 241.29 | 113.16 | |
| ind 2+ | 1475.54 | 1447.02 | 1418.49 | 1361.91 | 1297.85 | 1233.29 | 1197.75 | 1129.18 | 1093.64 | 1029.09 | 972.509 | 915.929 | 858.384 | 801.334 | 743.789 | 679.234 | 613.634 | 556.089 | 499.509 | 441.964 | 377.409 | 328.849 | 278.299 | 214.234 | 156.689 | 121.149 | 57.0839 | |
| ind 3+ | 984.032 | 965.015 | 945.999 | 908.279 | 865.569 | 822.532 | 798.839 | 753.125 | 729.432 | 686.395 | 648.675 | 610.955 | 572.392 | 534.559 | 496.195 | 453.159 | 409.425 | 371.062 | 333.342 | 294.979 | 251.942 | 219.569 | 185.869 | 143.159 | 104.795 | 81.1019 | 38.3919 | |
| | 28 | 27 | 26 | 25 | 24 | 23 | 22 | 21 | 20 | 19 | 18 | 17 | 16 | 15 | 14 | 13 | 12 | 11 | 10 | 9 | 8 | 7 | 6 | 5 | 4 | 3 | 2 | 1 |

- o >Smt3
- o LMEAFK(0)R
- o 964.521
- o matched ions: 11
- o %TIC: 0.0657265
- o Cons: 0.25
- o Delta Score: 0
- o target score: **0.888889**

all
2949.072060_b1+_-0.0
2949.072060_b2+_-0.0
2949.072060_y2+_-0.0
2949.072060_y3+_-0.0

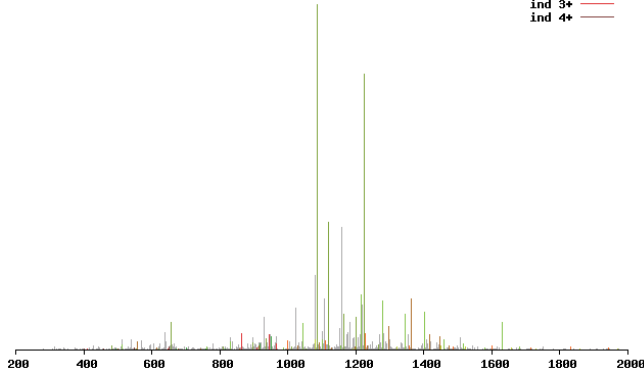


| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
|----------------------|---------|---------|---------|---------|---------|---------|---------|---------|
| | L | M | E | A | F | A | K(0) | R |
| 2949.072060_b1+_-0.0 | 114.167 | 245.36 | 374.475 | 445.554 | 592.731 | 663.81 | 3741.06 | |
| 2949.072060_b2+_-0.0 | 57.5876 | 123.184 | 187.742 | 223.281 | 296.869 | 332.409 | 1871.03 | |
| 2949.072060_y2+_-0.0 | | 1901.55 | 1835.96 | 1771.4 | 1735.86 | 1662.27 | 1626.73 | 88.1093 |
| 2949.072060_y3+_-0.0 | | 1268.04 | 1224.31 | 1181.27 | 1157.58 | 1108.52 | 1084.82 | 59.0754 |
| | 8 | 7 | 6 | 5 | 4 | 3 | 2 | 1 |

C

| Scan | Charge | relPos | modScore | precursor mass | matched ions | TIC | %TIC | BPI |
|------|--------|--------|----------|----------------|--------------|--------|---------|---------|
| 4035 | 4 | 1 | 1 | 964.522 | 97 | 928687 | 0.36897 | 81150.6 |

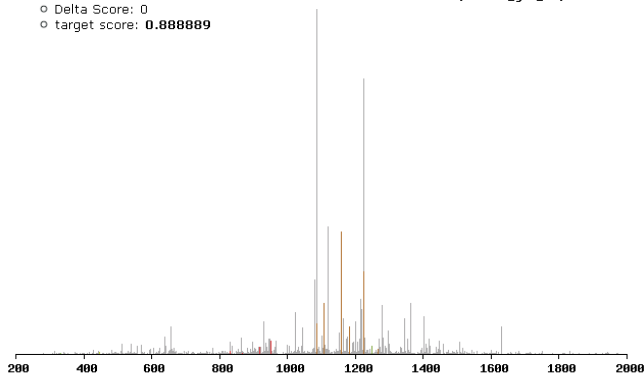
all
dep 1+
dep 2+
dep 3+
dep 4+
ind 1+
ind 2+
ind 3+
ind 4+



| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 | 25 | 26 | 27 | 28 |
|--------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|----|
| dep 1+ | 964.737 | 1021.81 | 1078.86 | 1192.02 | 1320.15 | 1449.26 | 1520.34 | 1657.48 | 1728.56 | 1857.67 | 1970.83 | 2083.99 | 2199.08 | 2313.18 | 2428.27 | 2557.38 | 2688.58 | 2803.67 | 2916.83 | 3031.92 | 3161.03 | 3258.15 | 3359.25 | 3487.38 | 3602.47 | 3673.55 | 3801.68 | |
| dep 2+ | 482.882 | 511.407 | 539.932 | 596.512 | 660.577 | 725.132 | 760.672 | 829.242 | 864.782 | 929.337 | 985.917 | 1042.5 | 1100.04 | 1157.09 | 1214.64 | 1279.19 | 1344.79 | 1402.34 | 1458.92 | 1516.46 | 1581.02 | 1629.58 | 1680.13 | 1744.19 | 1801.74 | 1837.28 | 1901.34 | |
| dep 3+ | 322.257 | 341.274 | 360.291 | 398.011 | 440.721 | 483.757 | 507.451 | 553.164 | 576.857 | 619.894 | 657.614 | 695.334 | 733.697 | 771.731 | 810.094 | 853.131 | 896.864 | 935.227 | 972.947 | 1011.31 | 1054.35 | 1086.72 | 1120.42 | 1163.13 | 1201.49 | 1225.19 | 1267.9 | |
| dep 4+ | 241.945 | 256.207 | 270.47 | 298.76 | 330.792 | 363.07 | 380.84 | 415.125 | 432.895 | 465.172 | 493.462 | 521.752 | 550.525 | 579.05 | 607.822 | 640.1 | 672.9 | 701.672 | 729.962 | 758.735 | 791.012 | 815.292 | 840.567 | 872.6 | 901.372 | 919.142 | 951.175 | |
| ind 1+ | 2950.08 | 2893.03 | 2835.98 | 2722.82 | 2594.69 | 2465.58 | 2394.5 | 2257.36 | 2186.28 | 2057.17 | 1944.01 | 1830.85 | 1715.76 | 1601.66 | 1486.57 | 1357.46 | 1226.26 | 1111.17 | 998.01 | 882.92 | 753.81 | 656.69 | 555.59 | 427.46 | 312.37 | 241.29 | 113.16 | |
| ind 2+ | 1475.54 | 1447.02 | 1418.49 | 1361.91 | 1297.85 | 1233.29 | 1197.75 | 1129.18 | 1093.64 | 1029.09 | 972.509 | 915.929 | 858.384 | 801.334 | 743.789 | 679.234 | 613.634 | 556.089 | 499.509 | 441.964 | 377.409 | 328.349 | 278.299 | 214.234 | 156.689 | 121.149 | 57.0839 | |
| ind 3+ | 984.032 | 965.015 | 945.999 | 908.279 | 865.569 | 822.532 | 798.839 | 753.125 | 729.432 | 686.395 | 648.675 | 610.955 | 572.592 | 534.559 | 496.195 | 453.159 | 409.425 | 371.062 | 333.342 | 294.979 | 251.942 | 219.569 | 185.869 | 143.159 | 104.795 | 81.1019 | 38.3919 | |
| ind 4+ | 738.276 | 724.013 | 709.751 | 681.461 | 649.428 | 617.151 | 599.381 | 565.096 | 547.326 | 515.048 | 486.758 | 458.468 | 429.696 | 401.171 | 372.398 | 340.121 | 307.321 | 278.548 | 250.258 | 221.486 | 189.208 | 164.928 | 139.653 | 107.621 | 78.8484 | 61.0784 | 29.0459 | |
| | 28 | 27 | 26 | 25 | 24 | 23 | 22 | 21 | 20 | 19 | 18 | 17 | 16 | 15 | 14 | 13 | 12 | 11 | 10 | 9 | 8 | 7 | 6 | 5 | 4 | 3 | 2 | 1 |

o >Smt3
o LMEAFK(D)R
o 964.521
o matched ions: 15
o %TIC: 0.088307
o Cons: 0.166667
o Delta Score: 0
o target score: **0.888889**

all
2949.072060_b1+_-0.0
2949.072060_b2+_-0.0
2949.072060_b3+_-0.0
2949.072060_y2+_-0.0
2949.072060_y3+_-0.0
2949.072060_y4+_-0.0



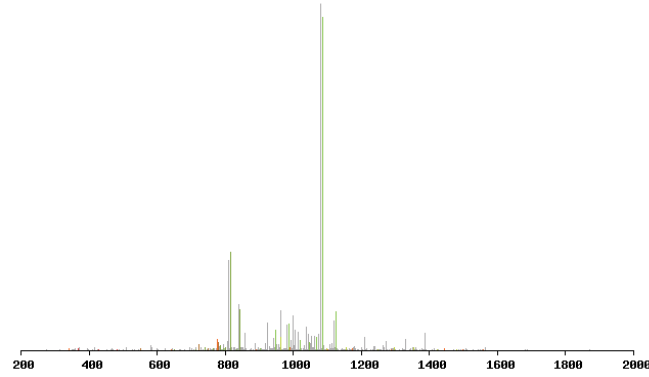
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
|----------------------|---------|---------|---------|---------|---------|---------|---------|---------|
| | L | M | E | A | F | A | K(O) | R |
| 2949.072060_b1+_-0.0 | 114.167 | 245.36 | 374.475 | 445.554 | 592.731 | 663.81 | 3741.06 | |
| 2949.072060_b2+_-0.0 | 57.5876 | 123.184 | 187.742 | 223.281 | 296.869 | 332.409 | 1871.03 | |
| 2949.072060_b3+_-0.0 | 38.7277 | 82.4585 | 125.497 | 149.19 | 198.249 | 221.942 | 1247.69 | |
| 2949.072060_y2+_-0.0 | | 1901.55 | 1835.96 | 1771.4 | 1735.86 | 1662.27 | 1626.73 | 88.1093 |
| 2949.072060_y3+_-0.0 | | 1268.04 | 1224.31 | 1181.27 | 1157.58 | 1108.52 | 1084.82 | 59.0754 |
| 2949.072060_y4+_-0.0 | | 951.281 | 918.482 | 886.204 | 868.434 | 831.64 | 813.87 | 44.5585 |
| | 8 | 7 | 6 | 5 | 4 | 3 | 2 | 1 |

Supplemental Figure 5

A

| Scan | Charge | relPos | modScore | precursor mass | matched ions | TIC | %TIC | BPI |
|------|--------|--------|----------|----------------|--------------|--------|----------|---------|
| 8215 | 4 | 1 | 0.971888 | 1926.01 | 51 | 254378 | 0.287173 | 36952.9 |

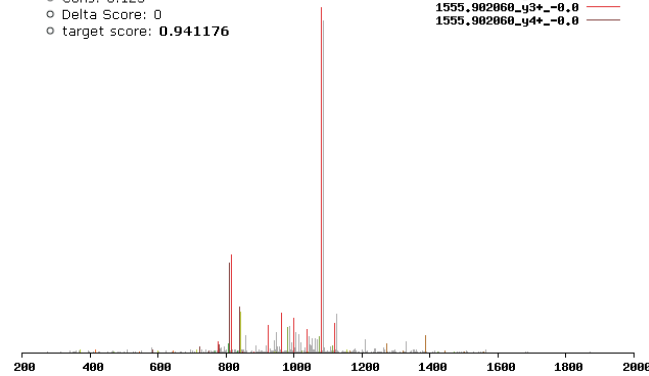
all
dep 2+
dep 3+
dep 4+
ind 1+
ind 2+
ind 3+



| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | |
|--------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|
| dep 2+ | 964.04 | 992.565 | 1021.09 | 1099.18 | 1155.76 | 1191.3 | 1247.88 | 1297.45 | 1354.03 | 1422.6 | 1479.18 | 1528.74 | 1572.28 | 1600.81 | 1629.33 | 1685.91 | | |
| dep 3+ | 643.029 | 662.046 | 681.062 | 733.126 | 770.846 | 794.539 | 832.259 | 865.302 | 903.022 | 948.736 | 986.456 | 1019.5 | 1048.53 | 1067.54 | 1086.56 | 1124.28 | | |
| dep 4+ | 482.524 | 496.786 | 511.049 | 550.096 | 578.386 | 596.156 | 624.446 | 649.229 | 677.519 | 711.804 | 740.094 | 764.876 | 786.646 | 800.909 | 815.171 | 843.461 | | |
| ind 1+ | | 1556.91 | 1499.86 | 1442.81 | 1286.62 | 1173.46 | 1102.38 | 989.22 | 890.09 | 776.93 | 639.79 | 526.63 | 427.5 | 340.42 | 283.37 | 226.32 | 113.16 | |
| ind 2+ | | 778.959 | 750.434 | 721.909 | 643.814 | 587.234 | 551.694 | 495.114 | 445.549 | 388.969 | 320.399 | 263.819 | 214.254 | 170.714 | 142.189 | 113.664 | 57.0839 | |
| ind 3+ | | | 519.642 | 500.625 | 481.609 | 429.545 | 391.825 | 368.132 | 330.412 | 297.369 | 259.649 | 213.935 | 176.215 | 143.172 | 114.145 | 95.1286 | 76.1119 | 38.3919 |

- o >Q15843|HUMAN Nedd8
- o EIEIDIEPTDK(0)VERIK
- o 1926.04
- o matched ions: 48
- o %TIC: 0.388307
- o Cons: 0.125
- o Delta Score: 0
- o target score: **0.941176**

all
1555.982060_b1+_-0.0
1555.982060_b2+_-0.0
1555.982060_b3+_-0.0
1555.982060_b4+_-0.0
1555.982060_y1+_-0.0
1555.982060_y2+_-0.0
1555.982060_y3+_-0.0
1555.982060_y4+_-0.0

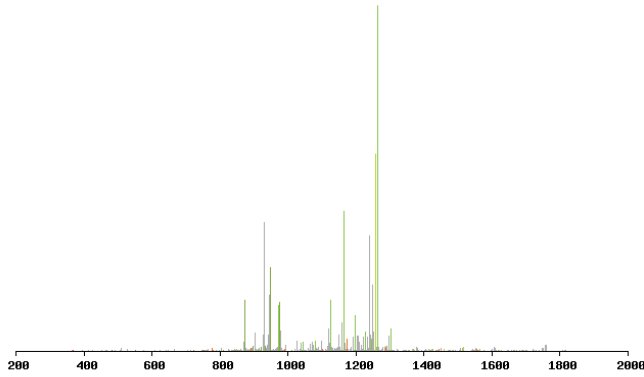


| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | |
|----------------------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|
| | E | I | E | I | D | I | E | P | T | D | K(0) | V | E | R | I | K | |
| 1555.902060_b1+_-0.0 | 130.123 | 243.283 | 372.398 | 485.558 | 600.646 | 713.806 | 842.921 | 940.038 | 1041.14 | 1156.23 | 2840.31 | 2939.44 | 3068.56 | 3224.74 | 3337.9 | | |
| 1555.902060_b2+_-0.0 | 65.5656 | 122.145 | 186.703 | 243.283 | 300.827 | 357.407 | 421.965 | 470.523 | 521.075 | 578.62 | 1420.66 | 1470.22 | 1534.78 | 1612.88 | 1669.46 | | |
| 1555.902060_b3+_-0.0 | 44.0464 | 81.7662 | 124.805 | 162.524 | 200.887 | 238.607 | 281.646 | 314.018 | 347.72 | 386.082 | 947.441 | 980.485 | 1023.52 | 1075.59 | 1113.31 | | |
| 1555.902060_b4+_-0.0 | 33.2867 | 61.5766 | 93.8555 | 122.145 | 150.917 | 179.207 | 211.486 | 235.765 | 261.042 | 289.814 | 710.833 | 735.616 | 767.895 | 806.942 | 835.232 | | |
| 1555.902060_y1+_-0.0 | | 3354.98 | 3241.82 | 3112.7 | 2999.54 | 2884.45 | 2771.29 | 2642.18 | 2545.06 | 2443.96 | 2328.87 | 644.792 | 545.66 | 416.544 | 260.357 | 147.197 | |
| 1555.902060_y2+_-0.0 | | 1677.99 | 1621.41 | 1556.85 | 1500.28 | 1442.73 | 1386.15 | 1321.59 | 1273.03 | 1222.48 | 1164.94 | 322.9 | 273.334 | 208.776 | 130.682 | 74.1026 | |
| 1555.902060_y3+_-0.0 | | | 1119 | 1081.28 | 1038.24 | 1000.52 | 962.156 | 924.437 | 881.398 | 849.026 | 815.324 | 776.961 | 215.603 | 182.558 | 139.52 | 87.4575 | 49.7376 |
| 1555.902060_y4+_-0.0 | | | 839.5 | 811.21 | 778.931 | 750.641 | 721.869 | 693.579 | 661.301 | 637.021 | 611.745 | 582.973 | 161.954 | 137.171 | 104.892 | 65.8451 | 37.5552 |

B

| Scan | Charge | relPos | modScore | precursor mass | matched ions | TIC | %TIC | BPI |
|------|--------|--------|----------|----------------|--------------|--------|----------|--------|
| 8331 | 4 | 1 | 0.977332 | 2458.24 | 48 | 961903 | 0.418254 | 114176 |

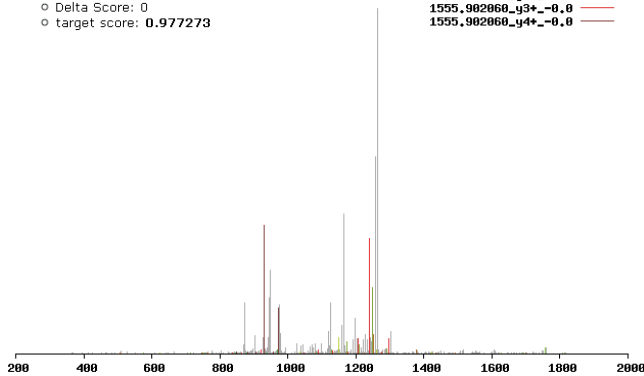
all
dep 2+
dep 3+
dep 4+
ind 1+
ind 2+
ind 3+



| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 |
|--------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|
| dep 2+ | 1230.15 | 1258.68 | 1287.2 | 1365.3 | 1421.88 | 1457.42 | 1514 | 1563.56 | 1620.14 | 1688.71 | 1745.29 | 1794.86 | 1838.4 | 1866.92 | 1895.45 | 1952.03 | |
| dep 3+ | 820.437 | 839.454 | 858.47 | 910.534 | 948.254 | 971.947 | 1009.67 | 1042.71 | 1080.43 | 1126.14 | 1163.86 | 1196.91 | 1225.93 | 1244.95 | 1263.97 | 1301.69 | |
| dep 4+ | 615.58 | 629.842 | 644.105 | 683.152 | 711.442 | 729.212 | 757.502 | 782.285 | 810.575 | 844.86 | 873.15 | 897.932 | 919.702 | 933.965 | 948.227 | 976.517 | |
| ind 1+ | | 1556.91 | 1499.86 | 1442.81 | 1286.62 | 1173.46 | 1102.38 | 989.22 | 890.09 | 776.93 | 639.79 | 526.63 | 427.5 | 340.42 | 283.37 | 226.32 | 113.16 |
| ind 2+ | | 778.959 | 750.434 | 721.909 | 643.814 | 587.234 | 551.694 | 495.114 | 445.549 | 388.969 | 320.399 | 263.819 | 214.254 | 170.714 | 142.189 | 113.664 | 57.0839 |
| ind 3+ | | 519.642 | 500.625 | 481.609 | 429.545 | 391.825 | 368.132 | 330.412 | 297.369 | 259.649 | 213.935 | 176.215 | 143.172 | 114.145 | 95.1286 | 76.1119 | 38.3919 |

- o >Q15843|HUMAN Nedd8 >Q15843|HUMAN Nedd8
- o EGIPPPQQRLIYSGK(0)QMNDEK
- o 2458.23
- o matched ions: 65
- o %TIC: 0.17934
- o Cons: 0.125
- o Delta Score: 0
- o target score: 0.977273

all
1555.902060_b1+_-0.0
1555.902060_b2+_-0.0
1555.902060_b3+_-0.0
1555.902060_b4+_-0.0
1555.902060_y1+_-0.0
1555.902060_y2+_-0.0
1555.902060_y3+_-0.0
1555.902060_y4+_-0.0

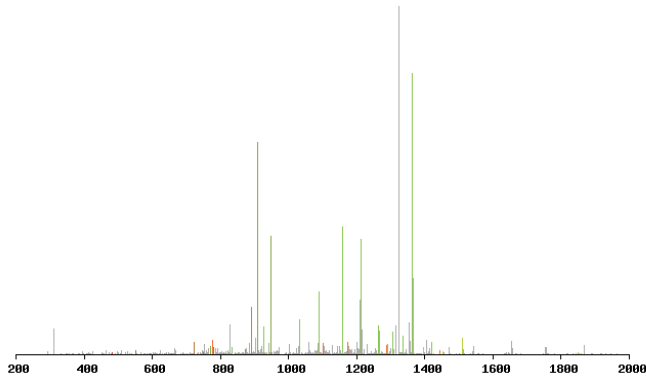


| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 |
|----------------------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|
| 1555.902060_b1+_-0.0 | 130.123 | 187.175 | 300.335 | 397.451 | 494.568 | 622.699 | 750.83 | 878.96 | 1035.15 | 1148.31 | 1261.47 | 1424.64 | 1511.72 | 1568.77 | 3252.85 | 3380.98 | 3512.17 | 3626.28 | 3741.36 | 3870.48 | |
| 1555.902060_b2+_-0.0 | 65.5656 | 94.0916 | 150.671 | 199.23 | 247.788 | 311.853 | 375.919 | 439.984 | 518.078 | 574.658 | 631.237 | 712.825 | 756.364 | 784.89 | 1626.93 | 1690.99 | 1756.59 | 1813.64 | 1871.19 | 1935.74 | |
| 1555.902060_b3+_-0.0 | 44.0464 | 63.0637 | 100.783 | 133.156 | 165.528 | 208.238 | 250.948 | 293.659 | 345.721 | 383.441 | 421.161 | 475.553 | 504.579 | 523.596 | 1084.95 | 1127.67 | 1171.4 | 1209.43 | 1247.79 | 1290.83 | |
| 1555.902060_b4+_-0.0 | 33.2867 | 47.5497 | 75.8396 | 100.119 | 124.398 | 156.431 | 188.463 | 220.496 | 259.543 | 287.833 | 316.123 | 356.917 | 378.686 | 392.949 | 813.968 | 846.001 | 878.799 | 907.325 | 936.097 | 968.376 | |
| 1555.902060_y1+_-0.0 | | 3887.55 | 3830.5 | 3717.34 | 3620.23 | 3523.11 | 3394.98 | 3266.85 | 3138.72 | 2982.53 | 2869.37 | 2756.21 | 2593.03 | 2505.96 | 2448.9 | 764.828 | 636.698 | 505.505 | 391.401 | 276.313 | 147.197 |
| 1555.902060_y2+_-0.0 | | 1944.28 | 1915.75 | 1859.18 | 1810.62 | 1762.06 | 1697.99 | 1633.93 | 1569.86 | 1491.77 | 1435.19 | 1378.61 | 1297.02 | 1253.48 | 1224.96 | 382.918 | 318.853 | 253.257 | 196.205 | 138.66 | 74.1026 |
| 1555.902060_y3+_-0.0 | | 1296.52 | 1277.51 | 1239.79 | 1207.41 | 1175.04 | 1132.33 | 1089.62 | 1046.91 | 994.848 | 957.128 | 919.409 | 865.017 | 835.991 | 816.973 | 255.615 | 212.904 | 169.174 | 131.139 | 92.7761 | 49.7376 |
| 1555.902060_y4+_-0.0 | | 972.644 | 958.381 | 930.091 | 905.812 | 881.533 | 849.5 | 817.468 | 785.435 | 746.388 | 718.098 | 689.809 | 649.015 | 627.245 | 612.982 | 191.963 | 159.93 | 127.132 | 98.6062 | 69.8341 | 37.5552 |

C

| Scan | Charge | relPos | modScore | precursor mass | matched ions | TIC | %TIC | BPI |
|------|--------|--------|----------|----------------|--------------|--------|----------|---------|
| 8954 | 3 | 1 | 0.979072 | 1395.6 | 52 | 330173 | 0.386181 | 32915.6 |

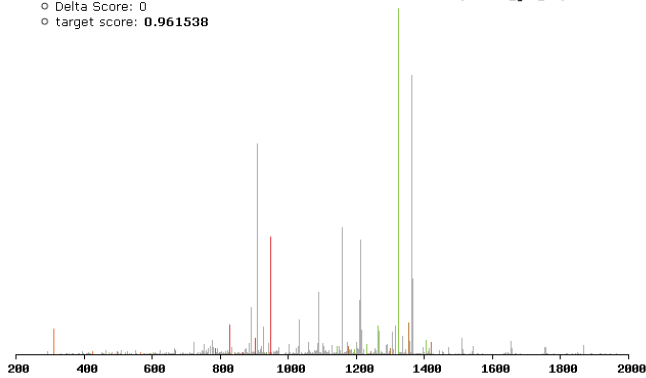
all
dep 1+
dep 2+
dep 3+
ind 1+
ind 2+
ind 3+



| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | |
|--------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|
| dep 1+ | 1396.66 | 1453.71 | 1510.76 | 1666.95 | 1780.11 | 1851.19 | 1964.35 | 2063.48 | 2176.64 | 2313.78 | 2426.94 | 2526.07 | 2613.15 | 2670.2 | 2727.25 | 2840.41 | | |
| dep 2+ | 698.834 | 727.359 | 755.884 | 833.979 | 890.559 | 926.099 | 982.679 | 1032.24 | 1088.82 | 1157.39 | 1213.97 | 1263.54 | 1307.08 | 1335.6 | 1364.13 | 1420.71 | | |
| dep 3+ | 466.225 | 485.242 | 504.259 | 556.322 | 594.042 | 617.735 | 655.455 | 688.499 | 726.219 | 771.932 | 809.652 | 842.695 | 871.722 | 890.739 | 909.755 | 947.475 | | |
| ind 1+ | | 1556.91 | 1499.86 | 1442.81 | 1286.62 | 1173.46 | 1102.38 | 989.22 | 890.09 | 776.93 | 639.79 | 526.63 | 427.5 | 340.42 | 283.37 | 226.32 | 113.16 | |
| ind 2+ | | 778.959 | 750.434 | 721.909 | 643.814 | 587.234 | 551.694 | 495.114 | 445.549 | 388.969 | 320.399 | 263.819 | 214.254 | 170.714 | 142.189 | 113.664 | 57.0839 | |
| ind 3+ | | | 519.642 | 500.625 | 481.609 | 429.545 | 391.825 | 368.132 | 330.412 | 297.369 | 259.649 | 213.935 | 176.215 | 143.172 | 114.145 | 95.1286 | 76.1119 | 38.3919 |
| | 17 | 16 | 15 | 14 | 13 | 12 | 11 | 10 | 9 | 8 | 7 | 6 | 5 | 4 | 3 | 2 | 1 | |

- o >Q15843|HUMAN Nedd8 >Q15843|HUMAN Nedd8
- o Q(-17.026500)MNDEK(0)TAADYK
- o 1395.6
- o matched ions: 27
- o %TIC: 0.198457
- o Cons: 0.166667
- o Delta Score: 0
- o target score: **0.961538**

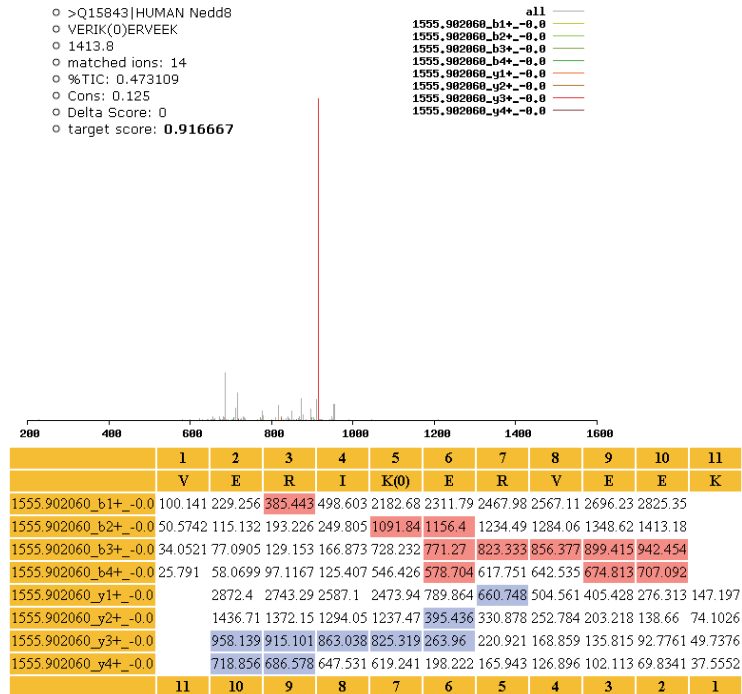
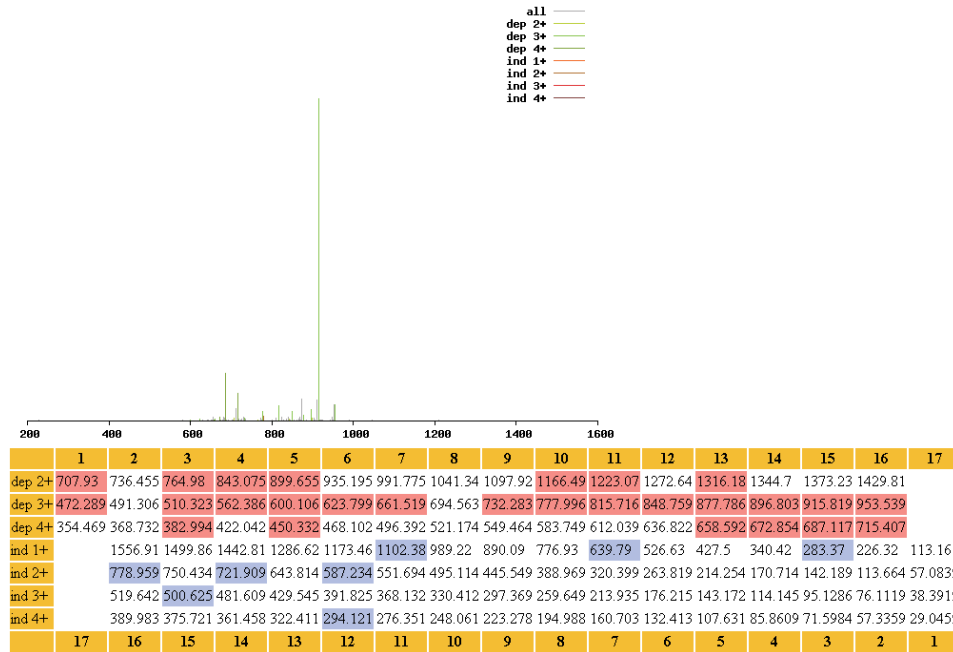
all
1555.902060_b1+_-0.0
1555.902060_b2+_-0.0
1555.902060_b3+_-0.0
1555.902060_y1+_-0.0
1555.902060_y2+_-0.0
1555.902060_y3+_-0.0



| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|----------------------|---------------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|
| | Q(-17.026500) | M | N | D | E | K(0) | T | A | A | D | Y | K |
| 1555.902060_b1+_-0.0 | 112.112 | 243.305 | 357.409 | 472.497 | 601.613 | 2285.69 | 2386.79 | 2457.87 | 2528.95 | 2644.04 | 2807.22 | |
| 1555.902060_b2+_-0.0 | 56.56 | 122.156 | 179.208 | 236.752 | 301.31 | 1143.35 | 1193.9 | 1229.44 | 1264.98 | 1322.52 | 1404.11 | |
| 1555.902060_b3+_-0.0 | 38.0426 | 81.7735 | 119.808 | 158.171 | 201.209 | 762.568 | 796.27 | 819.963 | 843.656 | 882.019 | 936.411 | |
| 1555.902060_y1+_-0.0 | | 2842.3 | 2711.11 | 2597 | 2481.92 | 2352.8 | 668.725 | 567.619 | 496.541 | 425.462 | 310.373 | 147.197 |
| 1555.902060_y2+_-0.0 | | 1421.65 | 1356.06 | 1299.01 | 1241.46 | 1176.9 | 334.866 | 284.314 | 248.774 | 213.235 | 155.691 | 74.1026 |
| 1555.902060_y3+_-0.0 | | 948.106 | 904.375 | 866.34 | 827.977 | 784.939 | 223.58 | 189.878 | 166.185 | 142.493 | 104.13 | 49.7376 |
| | 12 | 11 | 10 | 9 | 8 | 7 | 6 | 5 | 4 | 3 | 2 | 1 |

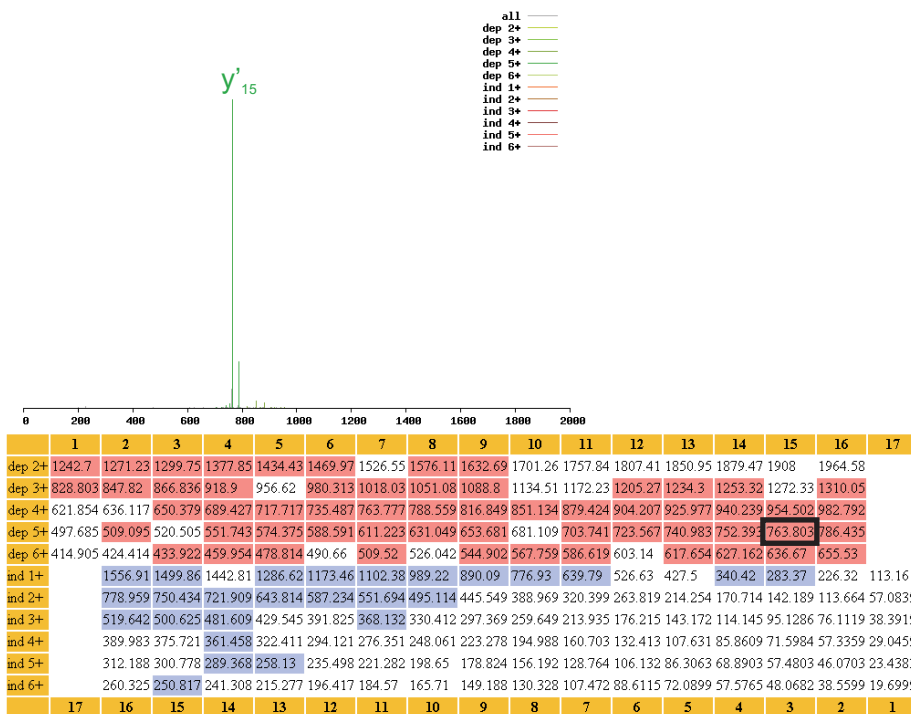
D

| Scan | Charge | relPos | modScore | precursor mass | matched ions | TIC | %TIC | BPI |
|------|--------|--------|----------|----------------|--------------|--------|----------|---------|
| 6881 | 4 | 1 | 1 | 1413.79 | 31 | 186201 | 0.682315 | 83563.5 |

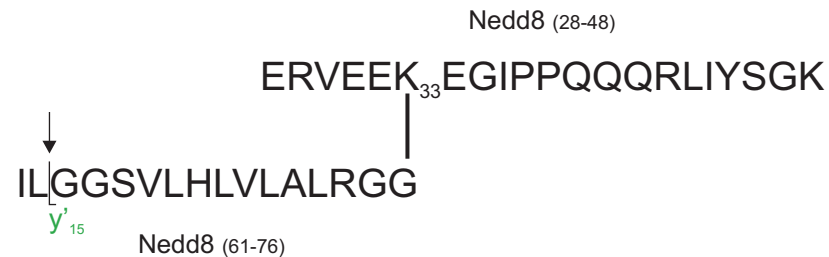


E

| Scan | Charge | relPos | modScore | precursor mass | matched ions | TIC | %TIC | BPI |
|------|--------|--------|----------|----------------|--------------|--------|---------|--------|
| 7887 | 6 | 1 | 1 | 2483.33 | 56 | 614857 | 1.39409 | 385186 |

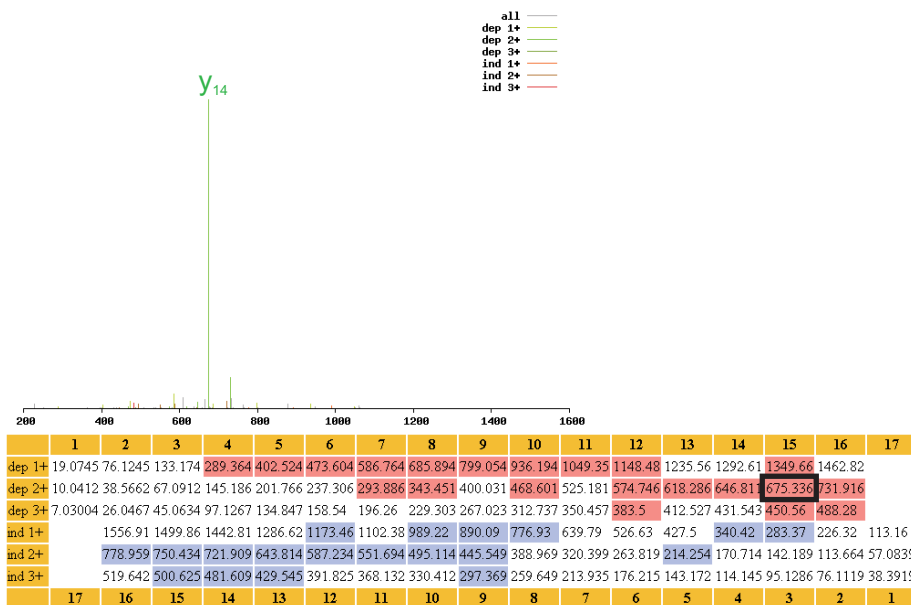


NEDD8 K33 linkage:



F

| Scan | Charge | relPos | modScore | precursor mass | matched ions | TIC | %TIC | BPI |
|-------|--------|--------|----------|----------------|--------------|--------|----------|--------|
| 13273 | 3 | 1 | 0.983017 | 18.0163 | 29 | 341325 | 0.761792 | 193355 |

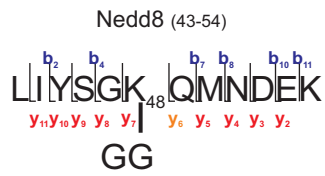


Unconjugated NEDD8 tail:

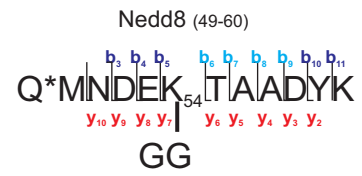


Supplemental Figure 6

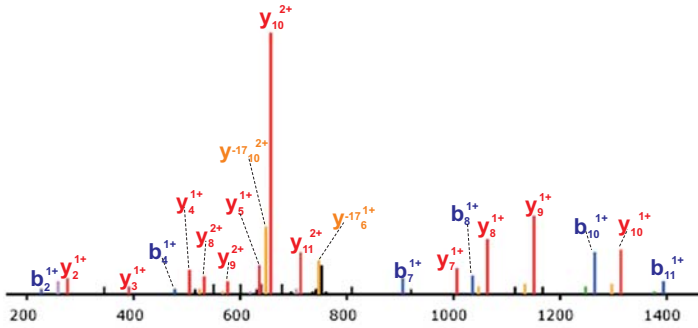
A



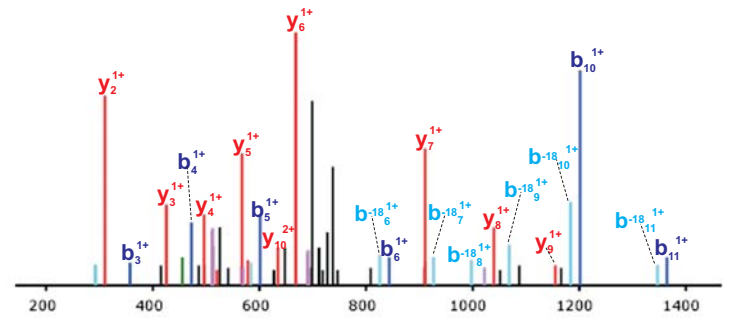
C



B



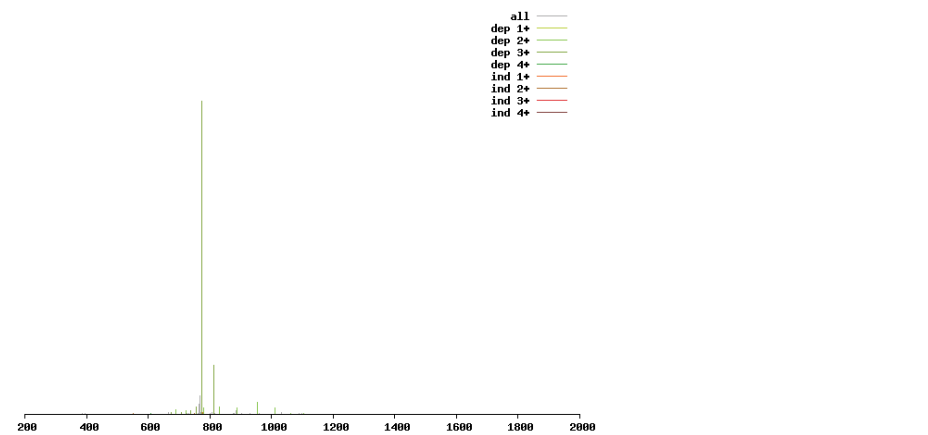
D



Supplemental Figure 7

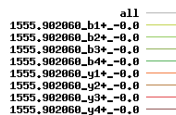
A

| Scan | Charge | relPos | modScore | precursor mass | matched ions | TIC | %TIC | BPI |
|------|--------|--------|----------|----------------|--------------|-------------|----------|-------------|
| 9208 | 4 | 1 | 0.993548 | 990.591 | 57 | 3.41349e+06 | 0.772226 | 1.95188e+06 |



| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | |
|--------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|
| dep 1+ | 991.649 | 1048.7 | 1105.75 | 1261.94 | 1375.1 | 1446.18 | 1559.34 | 1658.47 | 1771.63 | 1908.77 | 2021.93 | 2121.06 | 2208.14 | 2265.19 | 2322.24 | 2435.4 | | |
| dep 2+ | 496.328 | 524.853 | 553.378 | 631.473 | 688.053 | 723.593 | 780.173 | 829.738 | 886.318 | 954.888 | 1011.47 | 1061.03 | 1104.57 | 1133.1 | 1161.62 | 1218.2 | | |
| dep 3+ | 331.222 | 350.238 | 369.255 | 421.318 | 459.038 | 482.732 | 520.452 | 553.495 | 591.215 | 636.928 | 674.648 | 707.692 | 736.718 | 755.735 | 774.752 | 812.472 | | |
| dep 4+ | 248.668 | 262.931 | 277.193 | 316.241 | 344.531 | 362.301 | 390.591 | 415.373 | 443.663 | 477.948 | 506.238 | 531.021 | 552.791 | 567.053 | 581.316 | 609.606 | | |
| ind 1+ | | 1556.91 | 1499.86 | 1442.81 | 1286.62 | 1173.46 | 1102.38 | 989.22 | 890.09 | 776.93 | 639.79 | 526.63 | 427.5 | 340.42 | 283.37 | 226.32 | 113.16 | |
| ind 2+ | | 778.959 | 750.434 | 721.909 | 643.814 | 587.234 | 551.694 | 495.114 | 445.549 | 388.969 | 320.399 | 263.819 | 214.254 | 170.714 | 142.189 | 113.664 | 57.0839 | |
| ind 3+ | | | 519.642 | 500.625 | 481.609 | 429.545 | 391.825 | 368.132 | 330.412 | 297.369 | 259.649 | 213.935 | 176.215 | 143.172 | 114.145 | 95.1286 | 76.1119 | 38.3919 |
| ind 4+ | | | 389.983 | 375.721 | 361.458 | 322.411 | 294.121 | 276.351 | 248.061 | 223.278 | 194.988 | 160.703 | 132.413 | 107.631 | 85.8609 | 71.5984 | 57.3359 | 29.0459 |

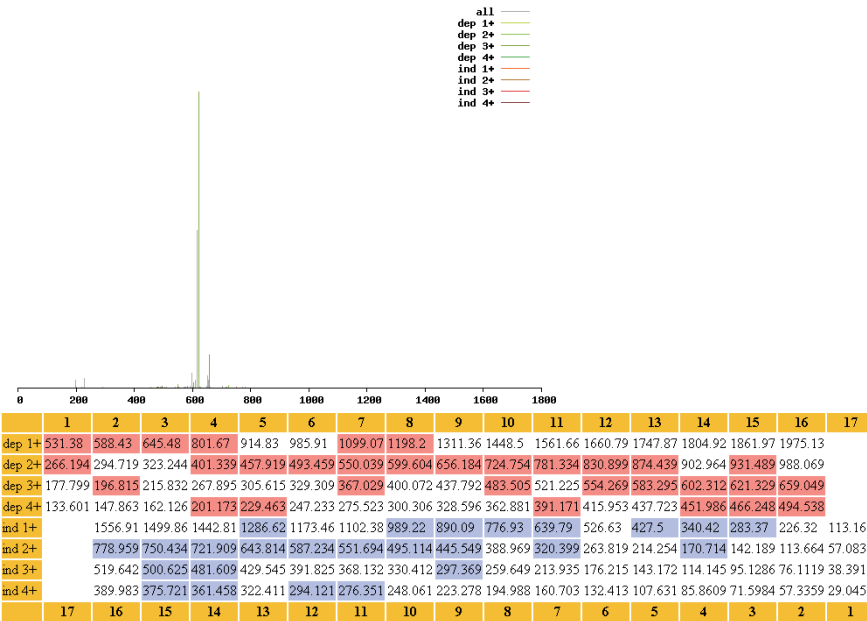
- >P61081|UbcH12 E2M Nedd8 E2
- LFSLK(0)QQK
- 990.591
- matched ions: 13
- %TIC: 0.114867
- Cons: 0.125
- Delta Score: 0
- target score: **0.888889**



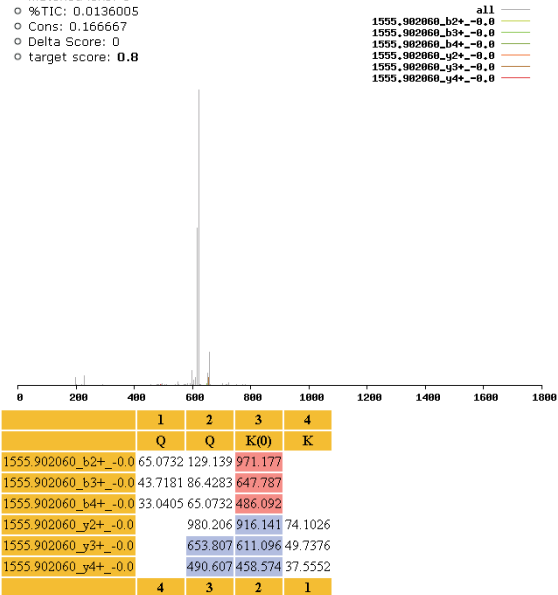
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
|----------------------|---------|---------|---------|---------|---------|---------|---------|---------|
| | L | F | S | L | K(0) | Q | Q | K |
| 1555.902060_b1+_-0.0 | 114.167 | 261.344 | 348.422 | 461.582 | 2145.66 | 2273.79 | 2401.92 | |
| 1555.902060_b2+_-0.0 | 57.5876 | 131.176 | 174.715 | 231.295 | 1073.33 | 1137.4 | 1201.46 | |
| 1555.902060_b3+_-0.0 | 38.7277 | 87.7865 | 116.813 | 154.532 | 715.891 | 758.601 | 801.312 | |
| 1555.902060_b4+_-0.0 | 29.2977 | 66.0919 | 87.8614 | 116.151 | 537.17 | 569.203 | 601.236 | |
| 1555.902060_y1+_-0.0 | | 2434.95 | 2287.77 | 2200.69 | 2087.53 | 403.459 | 275.328 | 147.197 |
| 1555.902060_y2+_-0.0 | | 1217.98 | 1144.39 | 1100.85 | 1044.27 | 202.233 | 138.168 | 74.1026 |
| 1555.902060_y3+_-0.0 | | 812.322 | 763.263 | 734.237 | 696.517 | 135.158 | 92.4479 | 49.7376 |
| 1555.902060_y4+_-0.0 | | 609.493 | 572.699 | 550.929 | 522.64 | 101.621 | 69.5879 | 37.5552 |

B

| Scan | Charge | relPos | modScore | precursor mass | matched ions | TIC | %TIC | BPI |
|------|--------|--------|----------|----------------|--------------|--------|----------|--------|
| 7981 | 4 | 1 | 0.972805 | 530.322 | 38 | 632102 | 0.546491 | 293220 |

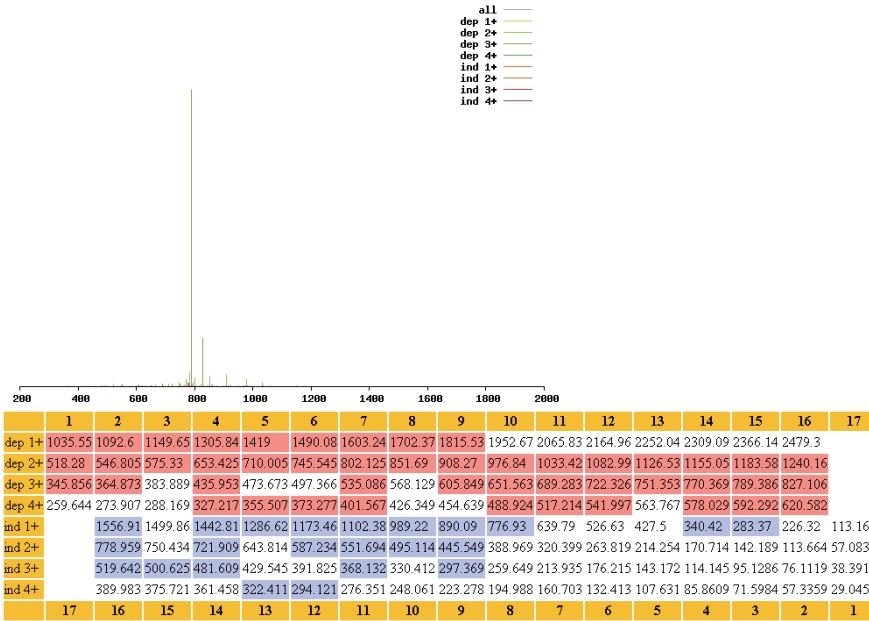


- >P61081|UbcH12 E2M Nedd8 E2
- QK(0)K
- 530.322
- matched ions: 6
- %TIC: 0.0136005
- Cons: 0.166667
- Delta Score: 0
- target score: 0.8

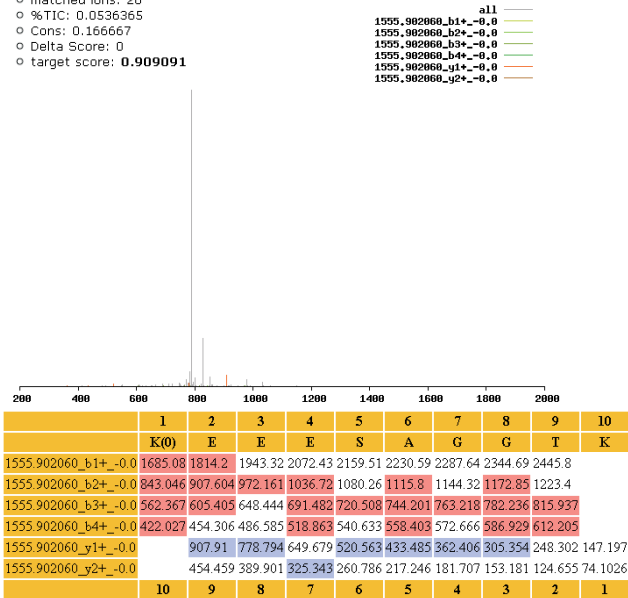


C

| Scan | Charge | relPos | modScore | precursor mass | matched ions | TIC | %TIC | BPI |
|------|--------|--------|----------|----------------|--------------|--------|----------|--------|
| 7952 | 4 | 1 | 0.981308 | 1034.49 | 56 | 562157 | 0.719293 | 295007 |

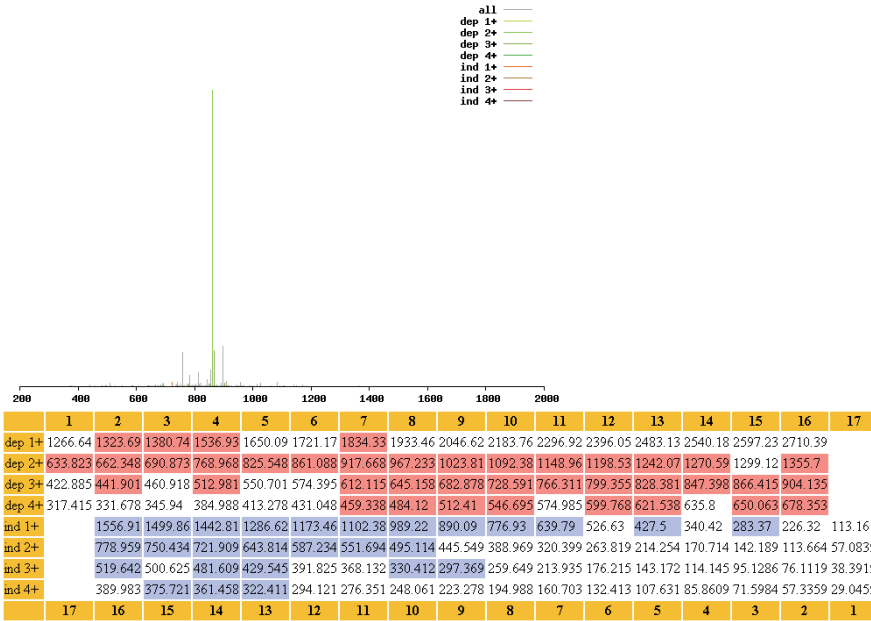


- o >P61081|UbcH12 E2M Nedd8 E2
- o K(O)EEESAGGTK
- o 1034.49
- o matched ions: 20
- o %TIC: 0.0536365
- o Cons: 0.166667
- o Delta Score: 0
- o target score: **0.909091**

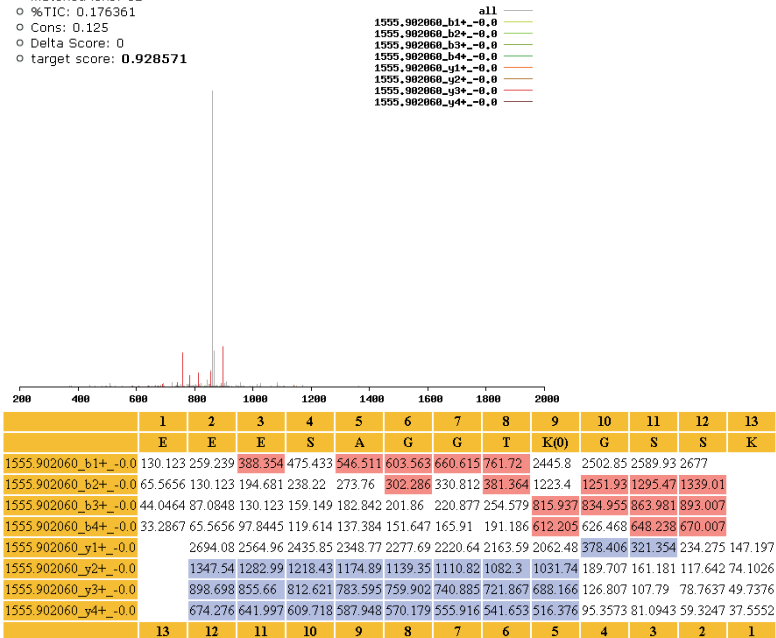


D

| Scan | Charge | relPos | modScore | precursor mass | matched ions | TIC | %TIC | BPI |
|------|--------|--------|----------|----------------|--------------|--------|---------|--------|
| 8029 | 4 | 1 | 0.922825 | 1265.58 | 37 | 449434 | 0.47149 | 176930 |



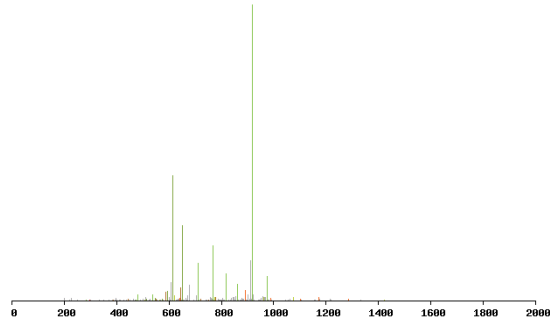
- o >P61081|UbcH12 E2M Nedd8 E2
- o EEE5AGGK(0)GSSK
- o 1265.58
- o matched ions: 32
- o %TIC: 0.176361
- o Cons: 0.125
- o Delta Score: 0
- o target score: **0.928571**



E

| Scan | Charge | relPos | modScore | precursor mass | matched ions | TIC | %TIC | BPI |
|------|--------|--------|----------|----------------|--------------|--------|----------|--------|
| 7965 | 3 | 1 | 1 | 505.292 | 58 | 584666 | 0.696372 | 148405 |

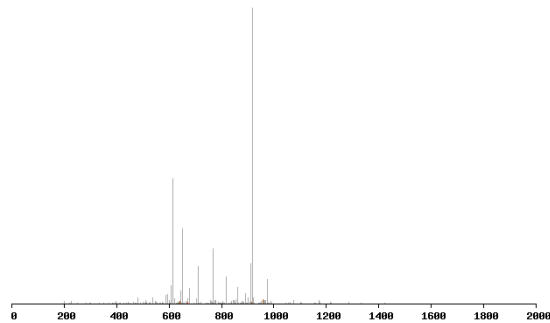
all
dep 1+
dep 2+
dep 3+
ind 1+
ind 2+
ind 3+



| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 |
|--------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|
| dep 1+ | 506.35 | 563.4 | 620.45 | 776.64 | 889.8 | 960.88 | 1074.04 | 1173.17 | 1286.33 | 1423.47 | 1536.63 | 1635.76 | 1722.84 | 1779.89 | 1836.94 | 1950.1 | |
| dep 2+ | 253.679 | 282.204 | 310.729 | 388.824 | 445.404 | 480.944 | 537.524 | 587.089 | 643.669 | 712.239 | 768.819 | 818.384 | 861.924 | 890.449 | 918.974 | 975.554 | |
| dep 3+ | 169.455 | 188.472 | 207.489 | 259.552 | 297.272 | 320.965 | 358.685 | 391.729 | 429.449 | 475.162 | 512.882 | 545.925 | 574.952 | 593.969 | 612.985 | 650.705 | |
| ind 1+ | | 1556.91 | 1499.86 | 1442.81 | 1286.62 | 1173.46 | 1102.38 | 989.22 | 890.09 | 776.93 | 639.79 | 526.63 | 427.5 | 340.42 | 283.37 | 226.32 | 113.16 |
| ind 2+ | | 778.959 | 750.434 | 721.909 | 643.814 | 587.234 | 551.694 | 495.114 | 445.549 | 388.969 | 320.399 | 263.819 | 214.254 | 170.714 | 142.189 | 113.664 | 57.0839 |
| ind 3+ | | 519.642 | 500.625 | 481.609 | 429.545 | 391.825 | 368.132 | 330.412 | 297.369 | 259.649 | 213.935 | 176.215 | 143.172 | 114.145 | 95.1286 | 76.1119 | 38.3919 |

- o >P61081|UbcH12 E2M Nedd8 E2
- o GSSK(0)K
- o 505.291
- o matched ions: 3
- o %TIC: 0.00238028
- o Cons: 0.2
- o Delta Score: 0
- o target score: 0

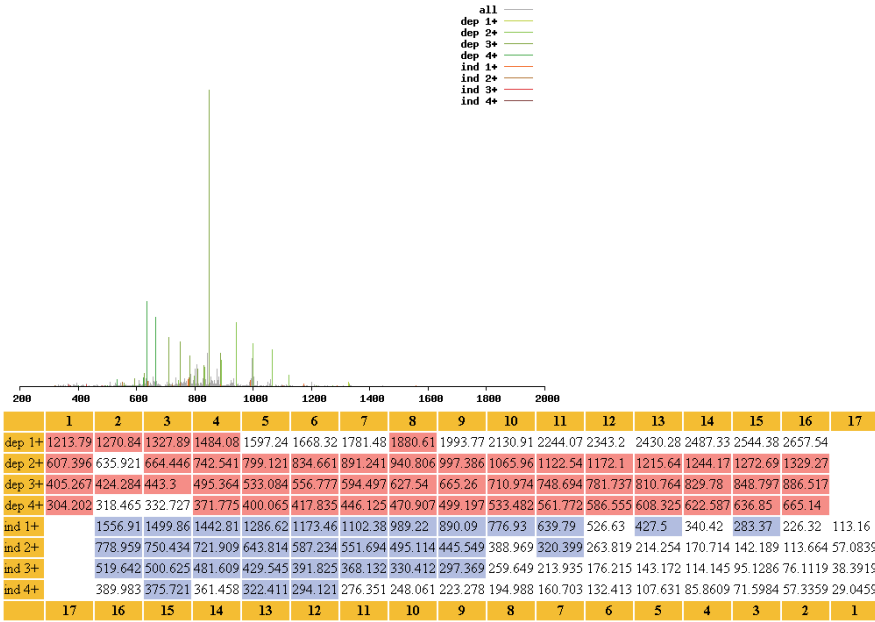
all
1555.902060_b2+_-0.0
1555.902060_b3+_-0.0
1555.902060_y1+_-0.0
1555.902060_y2+_-0.0
1555.902060_y3+_-0.0



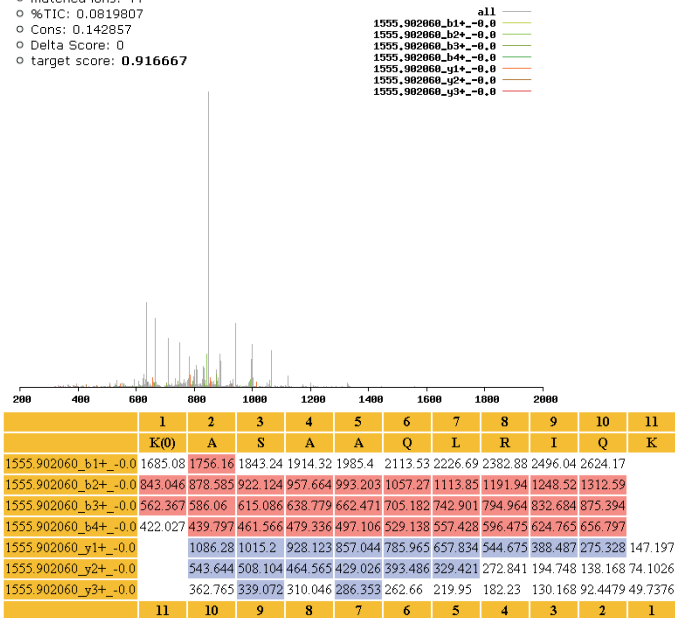
| | 1 | 2 | 3 | 4 | 5 |
|----------------------|---------|---------|---------|---------|---------|
| | G | S | S | K(0) | K |
| 1555.902060_b2+_-0.0 | 29.5338 | 73.0729 | 116.612 | 958.65 | |
| 1555.902060_b3+_-0.0 | 20.0252 | 49.0512 | 78.0773 | 639.436 | |
| 1555.902060_y1+_-0.0 | | 2005.43 | 1918.35 | 1831.27 | 147.197 |
| 1555.902060_y2+_-0.0 | | 1003.22 | 959.68 | 916.141 | 74.1026 |
| 1555.902060_y3+_-0.0 | | 669.149 | 640.122 | 611.096 | 49.7376 |

F

| Scan | Charge | relPos | modScore | precursor mass | matched ions | TIC | %TIC | BPI |
|------|--------|--------|----------|----------------|--------------|--------|----------|---------|
| 7534 | 4 | 1 | 1 | 1212.73 | 75 | 432025 | 0.507157 | 61270.5 |



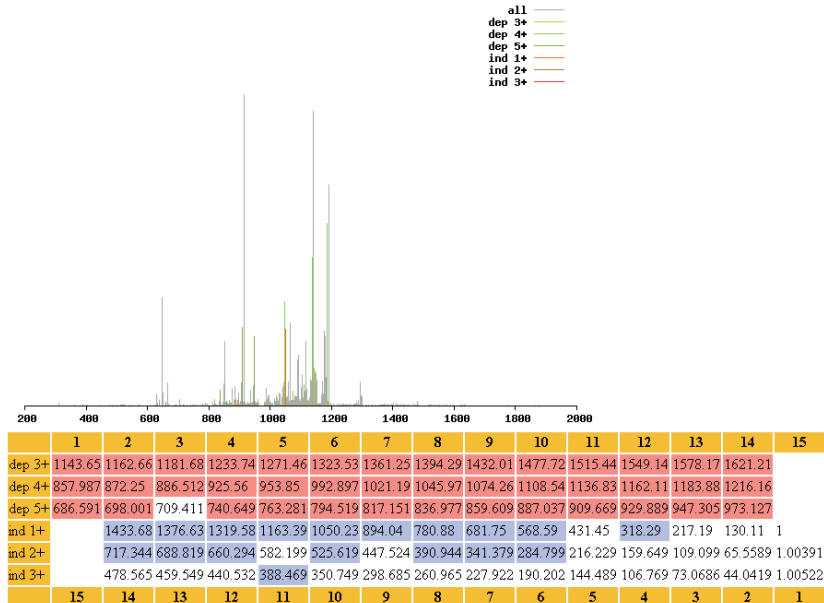
- o >P61081|UbcH12 E2M Nedd8 E2
- o K(O)ASAAQLRIQK
- o 1212.73
- o matched ions: 44
- o %TIC: 0.0819807
- o Cons: 0.142857
- o Delta Score: 0
- o target score: **0.916667**



Supplemental Figure 8

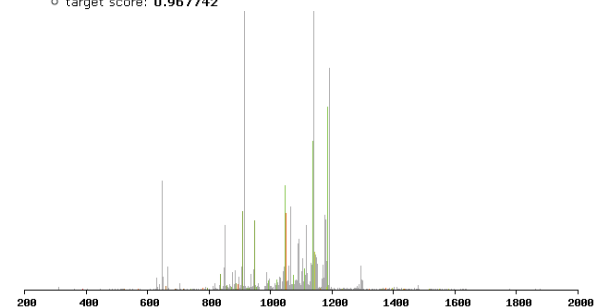
A

| Scan | Charge | relPos | modScore | precursor mass | matched ions | TIC | %TIC | BPI |
|------|--------|--------|----------|----------------|--------------|--------|----------|---------|
| 6451 | 5 | 1 | 0.914141 | 3428.76 | 40 | 943305 | 0.193573 | 70218.2 |



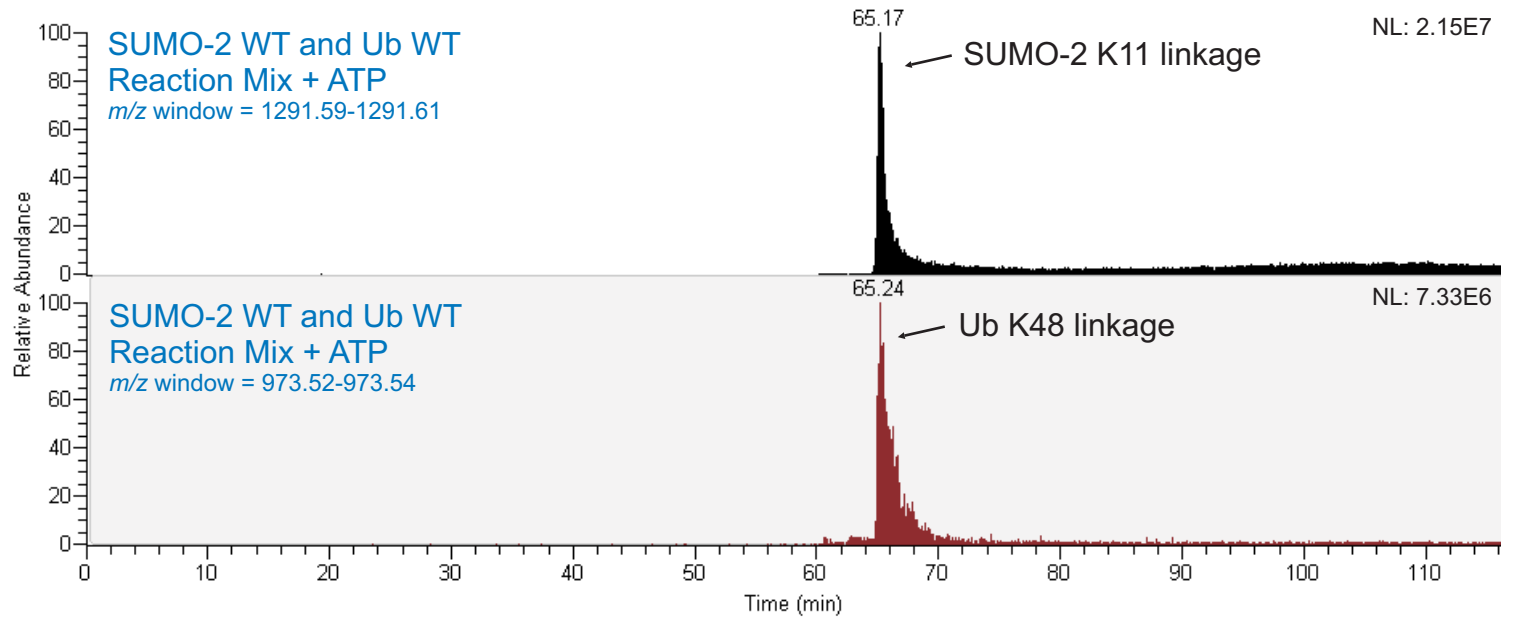
- >P62988|HUMAN Ubiquitin
- EGIPDQQLIFAGK(Q)QLEDGRTLSDYNIQK
- 3428.77
- matched ions: 100
- %TIC: 0.508359
- Cons: 0.1
- Delta Score: 0
- target score: 0.967742

all
dep 3+
dep 4+
dep 5+
ind 1+
ind 2+
ind 3+

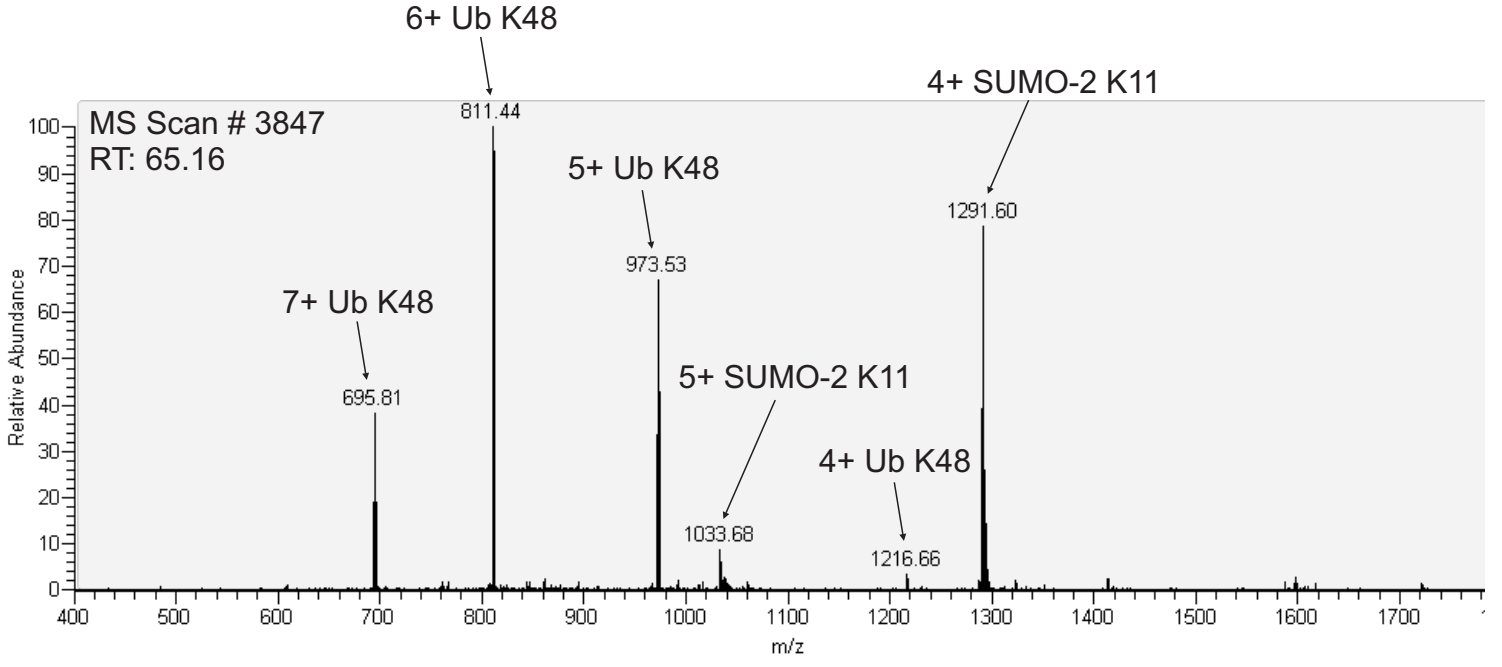


| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 | 25 | 26 | 27 | 28 | 29 | 30 | | |
|----------------------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|--|--|
| | E | G | I | P | P | D | Q | Q | R | L | I | F | A | G | K(Q) | Q | L | E | D | G | R | T | L | S | D | Y | N | I | Q | K | | |
| 1432.672060_b1+_-0.0 | 130.123 | 187.175 | 300.335 | 397.451 | 494.568 | 609.657 | 737.787 | 865.918 | 1022.11 | 1135.27 | 1248.42 | 1395.6 | 1466.68 | 1523.73 | 3084.58 | 3212.71 | 3325.87 | 3454.98 | 3570.07 | 3627.12 | 3783.31 | 3884.42 | 3997.58 | 4084.65 | 4199.74 | 4362.92 | 4477.02 | 4590.18 | 4718.31 | | | |
| 1432.672060_b2+_-0.0 | 65.5656 | 94.0916 | 150.671 | 199.23 | 247.788 | 305.332 | 369.398 | 433.463 | 511.557 | 568.136 | 624.716 | 698.304 | 733.844 | 762.37 | 1542.79 | 1606.86 | 1663.44 | 1728 | 1785.54 | 1814.07 | 1892.16 | 1942.71 | 1999.29 | 2042.83 | 2100.38 | 2181.96 | 2239.02 | 2295.59 | 2359.66 | | | |
| 1432.672060_b3+_-0.0 | 44.0464 | 63.0637 | 100.783 | 133.156 | 165.528 | 203.891 | 246.601 | 289.311 | 341.374 | 379.094 | 416.813 | 465.872 | 489.565 | 508.582 | 1028.86 | 1071.57 | 1109.29 | 1152.33 | 1190.7 | 1209.71 | 1261.78 | 1295.48 | 1333.2 | 1362.22 | 1400.59 | 1454.98 | 1493.01 | 1530.73 | 1573.44 | | | |
| 1432.672060_b4+_-0.0 | 33.2867 | 47.5497 | 75.8396 | 100.119 | 124.398 | 153.17 | 185.203 | 217.235 | 256.282 | 284.572 | 312.862 | 349.656 | 367.426 | 381.689 | 771.9 | 803.933 | 832.223 | 864.502 | 893.274 | 907.537 | 946.584 | 971.86 | 1000.15 | 1021.92 | 1050.69 | 1091.49 | 1120.01 | 1148.3 | 1180.33 | | | |
| 1432.672060_b5+_-0.0 | 26.8309 | 38.2413 | 60.8732 | 80.2966 | 99.7199 | 122.738 | 148.364 | 173.99 | 205.227 | 227.859 | 250.491 | 279.926 | 294.142 | 305.553 | 617.722 | 643.348 | 665.98 | 691.803 | 714.821 | 726.231 | 757.469 | 777.69 | 800.321 | 817.737 | 840.755 | 873.39 | 896.211 | 918.843 | 944.469 | | | |
| 1432.672060_y1+_-0.0 | | 4735.39 | 4678.33 | 4565.18 | 4468.06 | 4370.94 | 4255.85 | 4127.72 | 3999.59 | 3843.4 | 3730.25 | 3617.09 | 3469.91 | 3398.83 | 3341.78 | 1780.93 | 1652.8 | 1539.64 | 1410.53 | 1295.44 | 1238.39 | 1082.2 | 981.094 | 867.934 | 780.856 | 665.767 | 502.591 | 388.487 | 275.328 | 147.197 | | |
| 1432.672060_y2+_-0.0 | | 2368.2 | 2339.67 | 2283.09 | 2234.53 | 2185.97 | 2128.43 | 2064.37 | 2000.3 | 1922.21 | 1865.63 | 1809.05 | 1735.46 | 1699.92 | 1671.39 | 890.97 | 826.905 | 770.325 | 705.767 | 648.223 | 619.697 | 541.603 | 491.051 | 434.471 | 390.932 | 333.388 | 251.8 | 194.748 | 138.168 | 74.1026 | | |
| 1432.672060_y3+_-0.0 | | 1579.13 | 1560.12 | 1522.4 | 1490.02 | 1457.65 | 1419.29 | 1376.58 | 1333.87 | 1281.81 | 1244.09 | 1206.37 | 1157.31 | 1133.62 | 1114.6 | 594.316 | 551.606 | 513.886 | 470.847 | 432.485 | 413.467 | 361.405 | 327.703 | 289.983 | 260.957 | 222.594 | 168.202 | 130.168 | 92.4479 | 49.7376 | | |
| 1432.672060_y4+_-0.0 | | 1184.6 | 1170.34 | 1142.05 | 1117.77 | 1093.49 | 1064.72 | 1032.69 | 1000.65 | 961.607 | 933.317 | 905.027 | 868.233 | 850.463 | 836.2 | 445.989 | 413.956 | 385.666 | 353.388 | 324.615 | 310.352 | 271.306 | 246.029 | 217.739 | 195.97 | 167.198 | 126.404 | 97.8777 | 69.5879 | 37.5552 | | |
| 1432.672060_y5+_-0.0 | | 947.884 | 936.473 | 913.841 | 894.418 | 874.995 | 851.977 | 826.351 | 800.725 | 769.487 | 746.855 | 724.223 | 694.788 | 680.572 | 669.162 | 356.993 | 331.367 | 308.735 | 282.912 | 259.894 | 248.483 | 217.246 | 197.025 | 174.393 | 156.977 | 133.96 | 101.325 | 78.5038 | 55.8719 | 30.2457 | | |

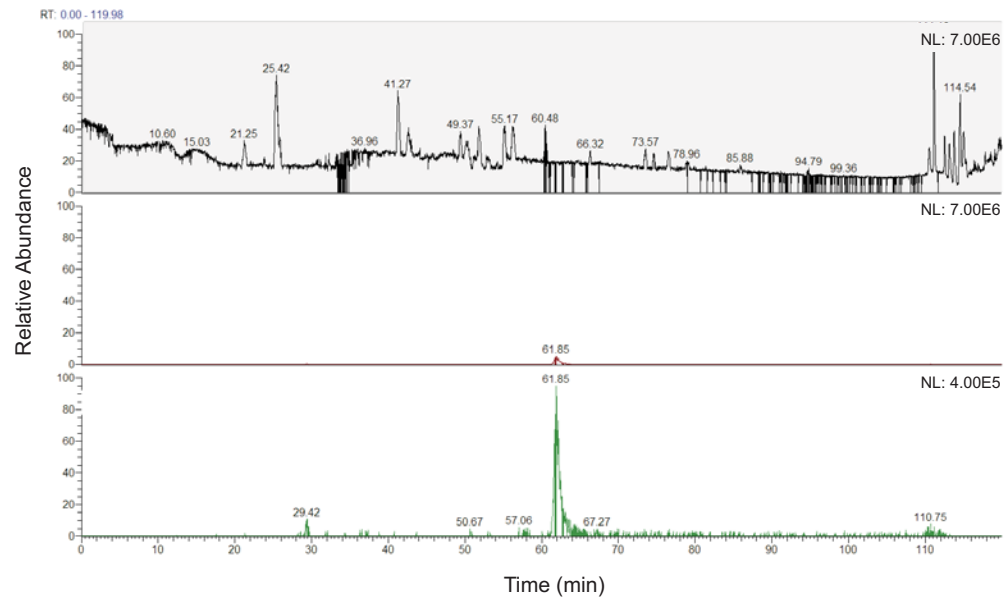
B



C

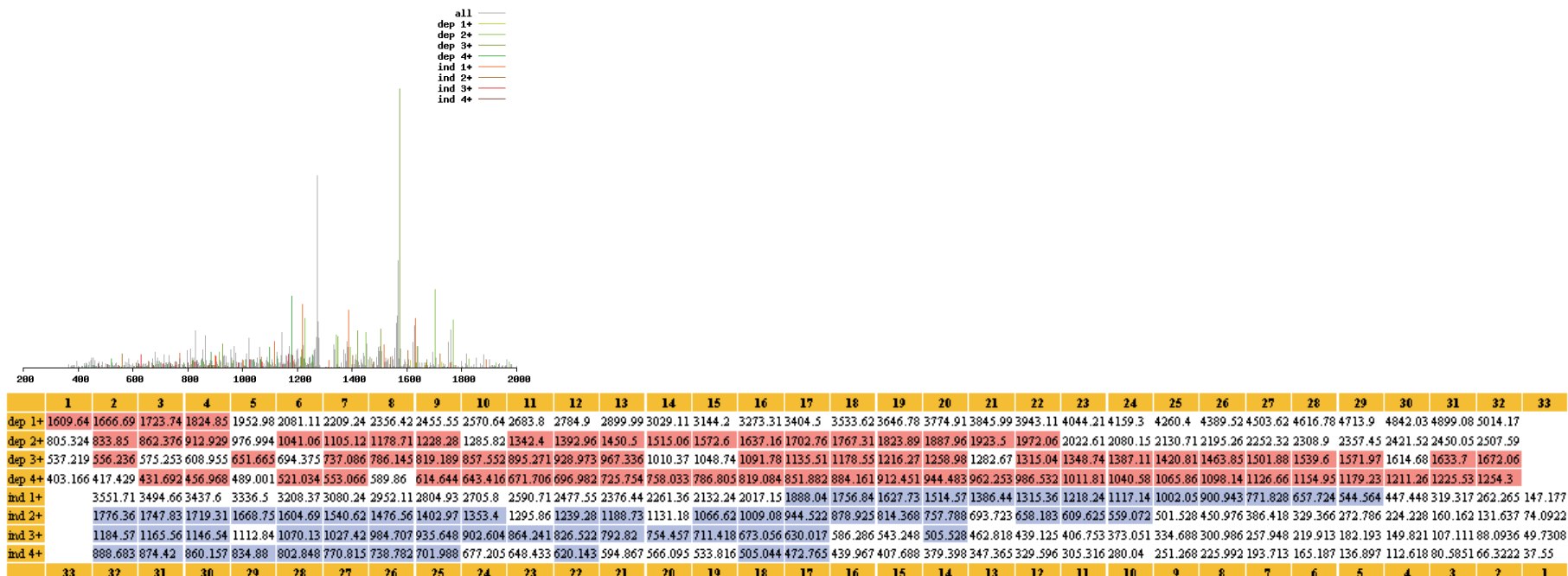


Supplemental Figure 9

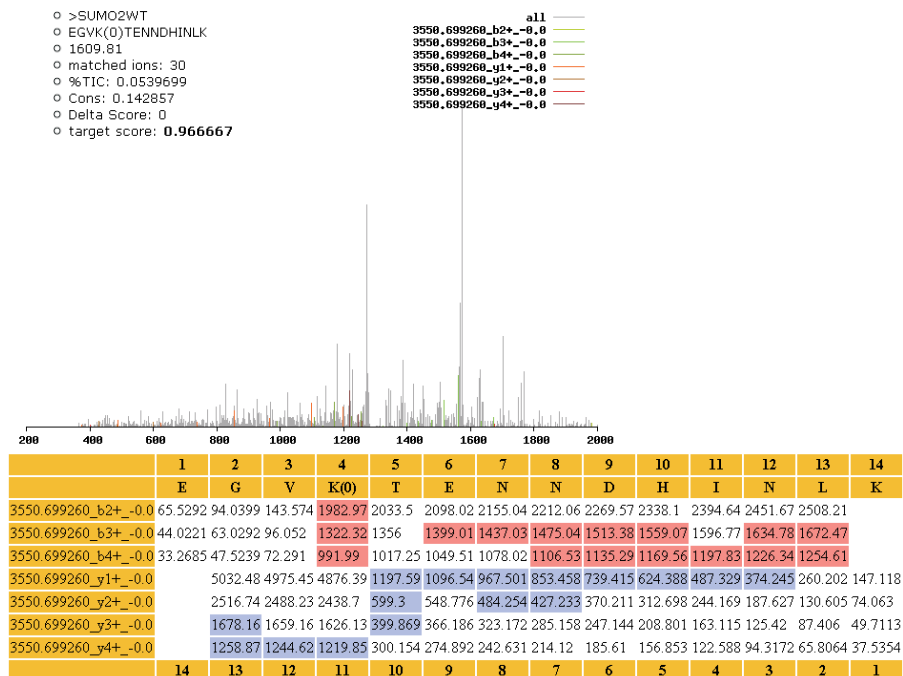


Supplemental Figure 10

| Scan | Charge | relPos | modScore | precursor mass | matched ions | TIC | %TIC | BPI |
|------|--------|--------|----------|----------------|--------------|---------|----------|---------|
| 2354 | 4 | 1 | 0.882353 | 1609.79 | 132 | 48020.8 | 0.379819 | 2348.23 |



- >SUMO2WT
- EGVK(O)TENNNDHINLK
- 1609.81
- matched ions: 30
- %TIC: 0.0539699
- Cons: 0.142857
- Delta Score: 0
- target score: **0.966667**



Supplemental Figure 11

