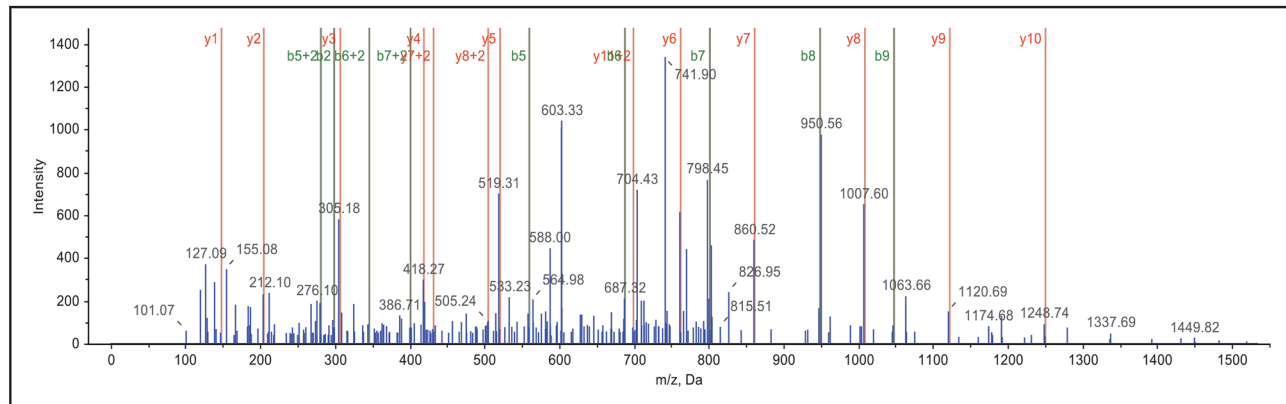


## SUPPLEMENTARY FIGURES AND TABLE

**A**

| Residue | b        | b+2      | y        | y+2      |
|---------|----------|----------|----------|----------|
| L       | 142.0863 | 71.5468  | 1806.99  | 903.9984 |
| R       | 298.1874 | 149.5973 | 1665.911 | 833.4589 |
| G       | 355.2088 | 178.1081 | 1509.809 | 755.4083 |
| G       | 412.2303 | 206.6188 | 1452.788 | 726.8976 |
| M!      | 559.2657 | 280.1365 | 1395.766 | 698.3869 |
| Q       | 687.3243 | 344.1658 | 1248.731 | 624.8692 |
| I       | 800.4083 | 400.7078 | 1120.673 | 560.8399 |
| F       | 947.4767 | 474.242  | 1007.588 | 504.2978 |
| V       | 1046.545 | 523.7762 | 860.52   | 430.7636 |
| K*      | 1288.683 | 644.8452 | 761.4516 | 381.2294 |
| T       | 1389.731 | 695.369  | 519.3137 | 260.1605 |
| L       | 1502.815 | 751.911  | 418.266  | 209.6366 |
| T       | 1603.863 | 802.4349 | 305.1819 | 153.0946 |
| G       | 1660.884 | 830.9456 | 204.1343 | 102.5708 |
| K       | 1788.979 | 894.9931 | 147.1128 | 74.06    |

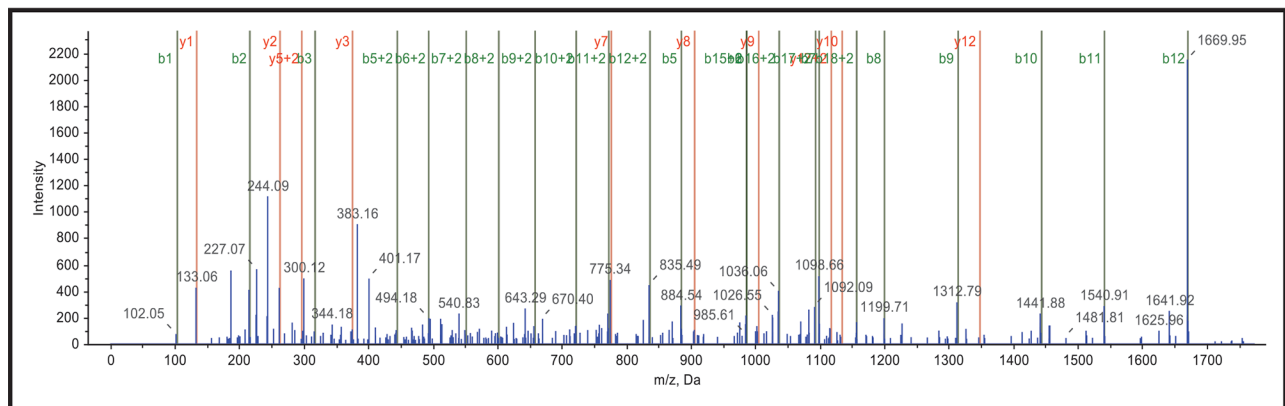


### Ubiquitination at Lys6: LRGGM!QIFVK\*TLGK

**Supplementary Figure S1: Spectrum of peptides obtained after tryptic digest of ubiquitinated species.** After the ubiquitination assay, the mixture is resolved on 12.5% SDS-PAGE gel and the ubiquitinated species were excised, digested with trypsin and analysed by mass spectrometry. Each spectrum indicates the type of isopeptide linkage formed. K\*- lysine residue modified by di-peptide (-GG). K\*\* - lysine residue modified by (-LRGG) peptide due to miscleavage. M! - oxidized methionine.

**B**

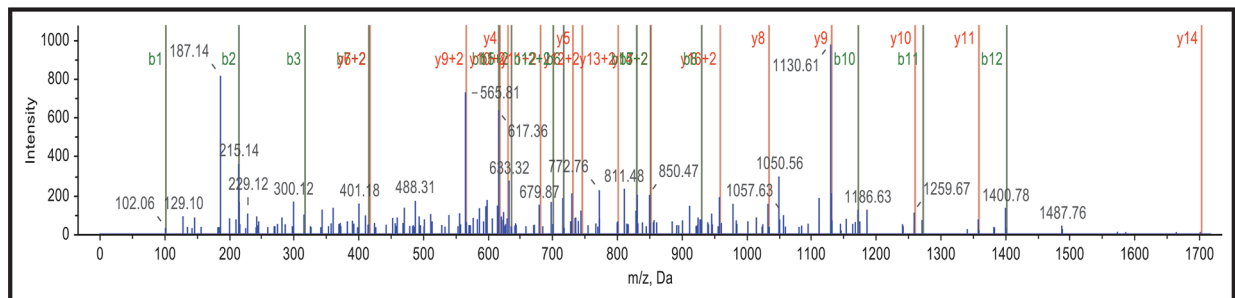
| Residue | b        | b+2      | y        | y+2      |
|---------|----------|----------|----------|----------|
| T       | 102.055  | 51.5311  | 2444.288 | 1222.648 |
| L       | 215.139  | 108.0731 | 2343.24  | 1172.124 |
| T       | 316.1867 | 158.597  | 2230.156 | 1115.582 |
| G       | 373.2082 | 187.1077 | 2129.109 | 1065.058 |
| K**     | 884.5312 | 442.7693 | 2072.087 | 1036.547 |
| T       | 985.5789 | 493.2931 | 1560.764 | 780.8856 |
| I       | 1098.663 | 549.8351 | 1459.716 | 730.3618 |
| T       | 1199.711 | 600.359  | 1346.632 | 673.8197 |
| L       | 1312.795 | 656.901  | 1245.585 | 623.2959 |
| E       | 1441.837 | 721.4223 | 1132.5   | 566.7539 |
| V       | 1540.906 | 770.9565 | 1003.458 | 502.2326 |
| E       | 1669.948 | 835.4778 | 904.3894 | 452.6984 |
| P       | 1767.001 | 884.0042 | 775.3468 | 388.1771 |
| S       | 1854.033 | 927.5202 | 678.2941 | 339.6507 |
| D       | 1969.06  | 985.0337 | 591.262  | 296.1347 |
| T       | 2070.108 | 1035.558 | 476.2351 | 238.6212 |
| I       | 2183.192 | 1092.1   | 375.1874 | 188.0974 |
| E       | 2312.234 | 1156.621 | 262.1034 | 131.5553 |
| N       | 2426.277 | 1213.642 | 133.0608 | 67.034   |



**Ubiquitination at Lys 11: TLTGK\*\*TITLEVEPSDTIEN**

C

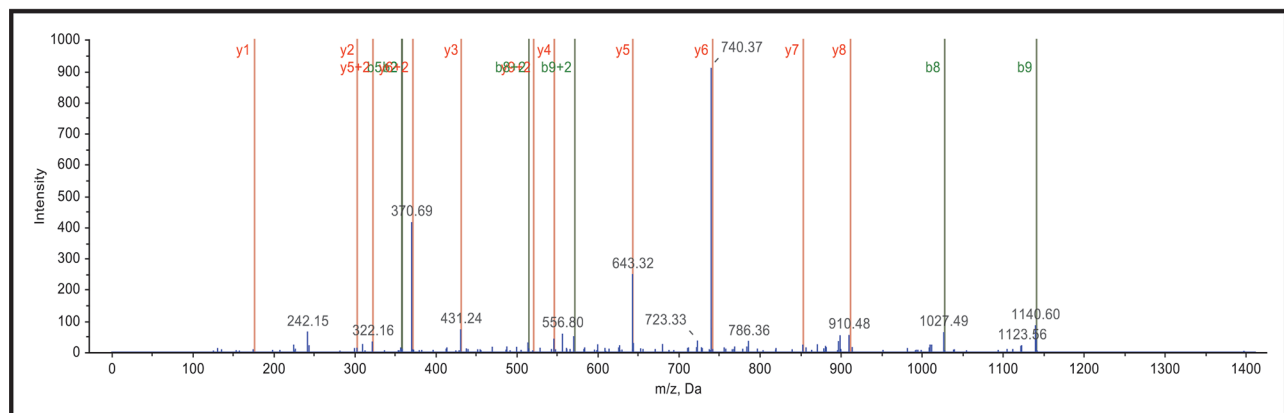
| Residue | b        | b+2      | y        | y+2      |
|---------|----------|----------|----------|----------|
| T       | 102.055  | 51.5311  | 2530.325 | 1265.666 |
| L       | 215.139  | 108.0731 | 2429.277 | 1215.142 |
| T       | 316.1867 | 158.597  | 2316.193 | 1158.6   |
| G       | 373.2082 | 187.1077 | 2215.145 | 1108.076 |
| K*      | 615.3461 | 308.1767 | 2158.124 | 1079.566 |
| T       | 716.3937 | 358.7005 | 1915.986 | 958.4966 |
| I       | 829.4778 | 415.2425 | 1814.938 | 907.9727 |
| T       | 930.5255 | 465.7664 | 1701.854 | 851.4307 |
| L       | 1043.61  | 522.3084 | 1600.807 | 800.9069 |
| E       | 1172.652 | 586.8297 | 1487.722 | 744.3648 |
| V       | 1271.721 | 636.3639 | 1358.68  | 679.8435 |
| E       | 1400.763 | 700.8852 | 1259.611 | 630.3093 |
| P       | 1497.816 | 749.4116 | 1130.569 | 565.788  |
| S       | 1584.848 | 792.9276 | 1033.516 | 517.2617 |
| D       | 1699.875 | 850.4411 | 946.484  | 473.7456 |
| T       | 1800.923 | 900.9649 | 831.4571 | 416.2322 |
| I       | 1914.007 | 957.5069 | 730.4094 | 365.7083 |
| E       | 2057.065 | 1029.036 | 617.3253 | 309.1663 |
| N       | 2171.108 | 1086.058 | 474.2671 | 237.6372 |
| V       | 2270.176 | 1135.592 | 360.2241 | 180.6157 |
| K*      | 2512.314 | 1256.661 | 261.1557 | 131.0815 |



**Ubiquitination at Lys11 and Lys27: TLTKG\*TITLEVEPSDTI-ENNK\***

## D

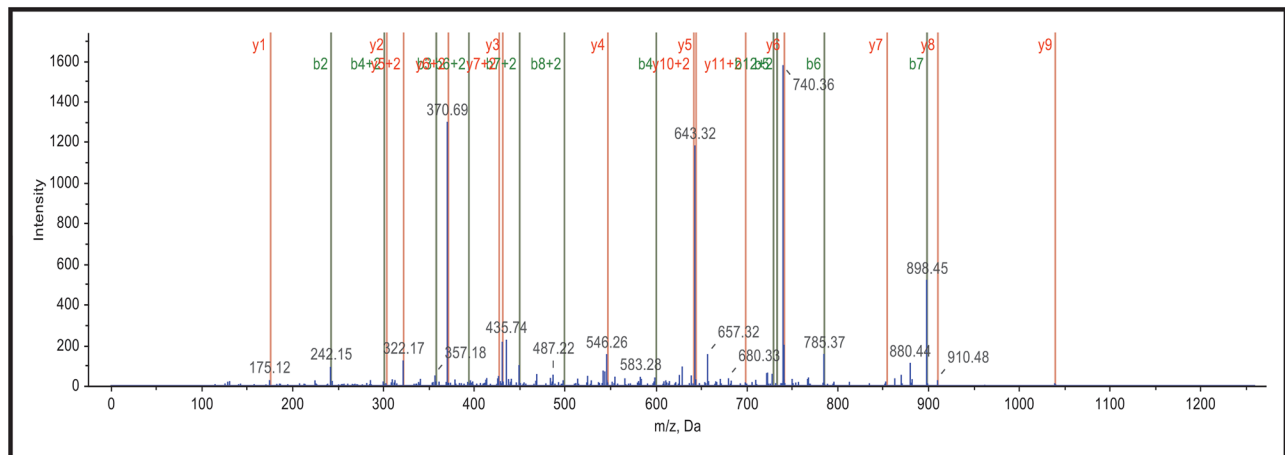
| Residue | b        | b+2      | y        | y+2      |
|---------|----------|----------|----------|----------|
| A       | 115.0502 | 58.0287  | 1879.962 | 940.4847 |
| K*      | 357.1881 | 179.0977 | 1765.919 | 883.4632 |
| I       | 470.2722 | 235.6397 | 1523.781 | 762.3943 |
| Q       | 598.3307 | 299.669  | 1410.697 | 705.8522 |
| D       | 713.3577 | 357.1825 | 1282.639 | 641.8229 |
| K       | 841.4526 | 421.23   | 1167.612 | 584.3095 |
| E       | 970.4952 | 485.7513 | 1039.517 | 520.262  |
| G       | 1027.517 | 514.262  | 910.4741 | 455.7407 |
| I       | 1140.601 | 570.804  | 853.4526 | 427.23   |
| P       | 1237.654 | 619.3304 | 740.3686 | 370.6879 |
| P       | 1334.706 | 667.8568 | 643.3158 | 322.1615 |
| D       | 1449.733 | 725.3703 | 546.2631 | 273.6352 |
| Q       | 1577.792 | 789.3995 | 431.2361 | 216.1217 |
| Q       | 1705.85  | 853.4288 | 303.1775 | 152.0924 |
| R       | 1861.952 | 931.4794 | 175.119  | 88.0631  |



**Ubiquitination at Lys29: AK\*IQDKEAGIPPDQQR**

## E

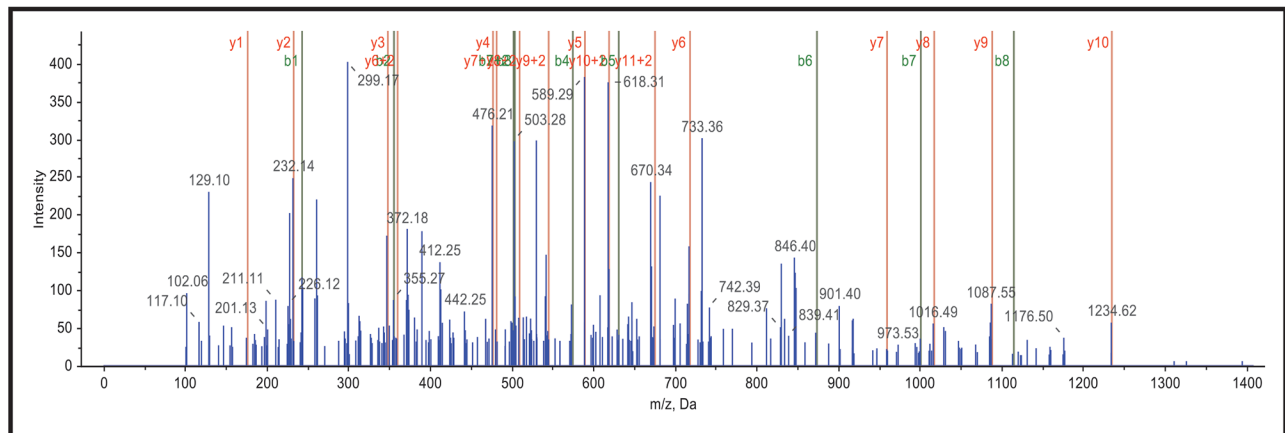
| Residue | b        | b+2      | y        | y+2      |
|---------|----------|----------|----------|----------|
| I       | 114.0913 | 57.5493  | 1637.824 | 819.4157 |
| Q       | 242.1499 | 121.5786 | 1524.74  | 762.8737 |
| D       | 357.1769 | 179.0921 | 1396.682 | 698.8444 |
| K*      | 599.3148 | 300.161  | 1281.655 | 641.3309 |
| E       | 728.3573 | 364.6823 | 1039.517 | 520.262  |
| G       | 785.3788 | 393.193  | 910.4741 | 455.7407 |
| I       | 898.4629 | 449.7351 | 853.4526 | 427.23   |
| P       | 995.5156 | 498.2615 | 740.3686 | 370.6879 |
| P       | 1092.568 | 546.7878 | 643.3158 | 322.1615 |
| D       | 1207.595 | 604.3013 | 546.2631 | 273.6352 |
| Q       | 1335.654 | 668.3306 | 431.2361 | 216.1217 |
| Q       | 1463.713 | 732.3599 | 303.1775 | 152.0924 |
| R       | 1619.814 | 810.4104 | 175.119  | 88.0631  |



**Ubiquitination at Lys33: IQDK\*EGIPPDQQR**

**F**

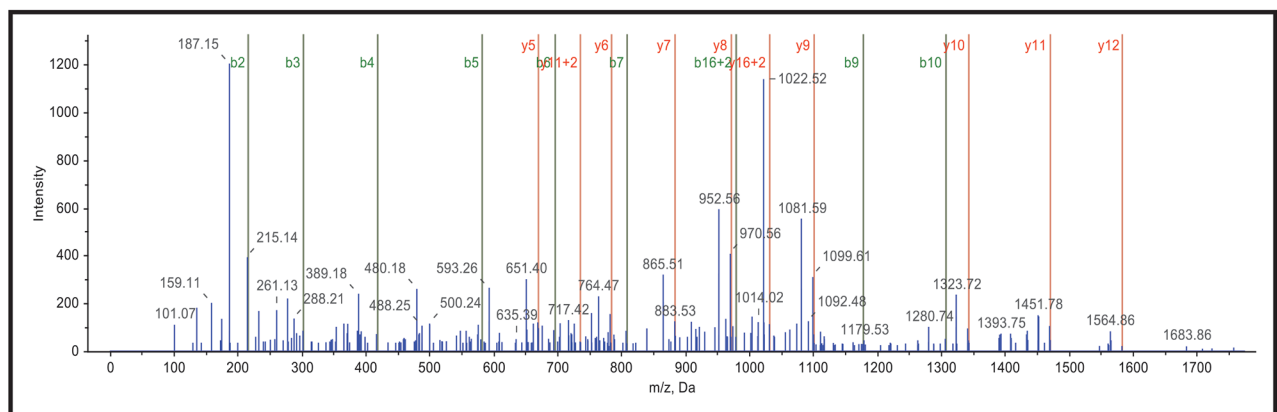
| Residue | b        | b+2      | y        | y+2      |
|---------|----------|----------|----------|----------|
| L       | 242.1863 | 121.5968 | 1588.881 | 794.9439 |
| I       | 355.2704 | 178.1388 | 1347.702 | 674.3544 |
| F       | 502.3388 | 251.673  | 1234.618 | 617.8124 |
| A       | 573.3759 | 287.1916 | 1087.549 | 544.2782 |
| G       | 630.3974 | 315.7023 | 1016.512 | 508.7596 |
| K*      | 872.5352 | 436.7713 | 959.4905 | 480.2489 |
| Q       | 1000.594 | 500.8006 | 717.3526 | 359.1799 |
| L       | 1113.678 | 557.3426 | 589.294  | 295.1506 |
| E       | 1242.721 | 621.8639 | 476.21   | 238.6086 |
| D       | 1357.747 | 679.3774 | 347.1674 | 174.0873 |
| G       | 1414.769 | 707.8881 | 232.1404 | 116.5738 |
| R       | 1570.87  | 785.9386 | 175.119  | 88.0631  |



**Ubiquitination at Lys48: LIFAGK\*QLEDGR**

G

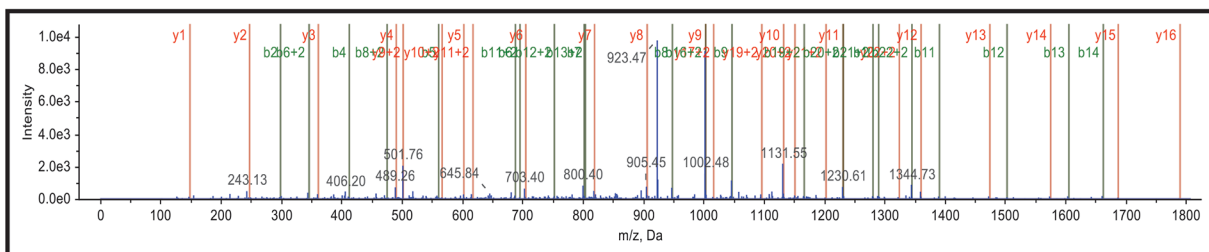
| Residue | b        | b+2      | y        | y+2      |
|---------|----------|----------|----------|----------|
| T       | 102.055  | 51.5311  | 2276.188 | 1138.598 |
| L       | 215.139  | 108.0731 | 2175.14  | 1088.074 |
| S       | 302.171  | 151.5892 | 2062.056 | 1031.532 |
| D       | 417.198  | 209.1026 | 1975.024 | 988.0158 |
| Y       | 580.2613 | 290.6343 | 1859.997 | 930.5023 |
| N       | 694.3042 | 347.6558 | 1696.934 | 848.9707 |
| I       | 807.3883 | 404.1978 | 1582.891 | 791.9492 |
| Q       | 935.4469 | 468.2271 | 1469.807 | 735.4072 |
| K*      | 1177.585 | 589.296  | 1341.749 | 671.3779 |
| E       | 1306.627 | 653.8173 | 1099.611 | 550.3089 |
| S       | 1393.659 | 697.3333 | 970.568  | 485.7876 |
| T       | 1494.707 | 747.8572 | 883.536  | 442.2716 |
| L       | 1607.791 | 804.3992 | 782.4883 | 391.7478 |
| H       | 1744.85  | 872.9287 | 669.4042 | 335.2058 |
| L       | 1857.934 | 929.4707 | 532.3453 | 266.6763 |
| V       | 1957.003 | 979.0049 | 419.2613 | 210.1343 |
| L       | 2070.087 | 1035.547 | 320.1928 | 160.6001 |
| R       | 2258.178 | 1129.592 | 207.1088 | 104.058  |



**Ubiquitination of Lys63: TLSDYNIQK\*ESTLHLVLR**

H

| Residue | b        | b+2      | y        | y+2      |
|---------|----------|----------|----------|----------|
| L       | 142.0863 | 71.5468  | 3689.942 | 1845.475 |
| R       | 298.1874 | 149.5973 | 3548.863 | 1774.935 |
| G       | 355.2088 | 178.1081 | 3392.762 | 1696.885 |
| G       | 412.2303 | 206.6188 | 3335.74  | 1668.374 |
| M!      | 559.2657 | 280.1365 | 3278.719 | 1639.863 |
| Q       | 687.3243 | 344.1658 | 3131.683 | 1566.345 |
| I       | 800.4083 | 400.7078 | 3003.625 | 1502.316 |
| F       | 947.4767 | 474.242  | 2890.541 | 1445.774 |
| V       | 1046.545 | 523.7762 | 2743.472 | 1372.24  |
| K*      | 1288.683 | 644.8452 | 2644.404 | 1322.706 |
| T       | 1389.731 | 695.369  | 2402.266 | 1201.637 |
| L       | 1502.815 | 751.911  | 2301.218 | 1151.113 |
| T       | 1603.863 | 802.4349 | 2188.134 | 1094.571 |
| G       | 1660.884 | 830.9456 | 2087.087 | 1044.047 |
| K*      | 1903.022 | 952.0146 | 2030.065 | 1015.536 |
| T       | 2004.07  | 1002.538 | 1787.927 | 894.4673 |
| I       | 2117.154 | 1059.08  | 1686.88  | 843.9434 |
| T       | 2218.201 | 1109.604 | 1573.796 | 787.4014 |
| L       | 2331.285 | 1166.146 | 1472.748 | 736.8776 |
| E       | 2460.328 | 1230.668 | 1359.664 | 680.3355 |
| V       | 2559.396 | 1280.202 | 1230.621 | 615.8142 |
| E       | 2688.439 | 1344.723 | 1131.553 | 566.28   |
| P       | 2785.492 | 1393.25  | 1002.51  | 501.7587 |
| S       | 2872.524 | 1436.766 | 905.4575 | 453.2324 |
| D       | 2987.551 | 1494.279 | 818.4254 | 409.7164 |
| T       | 3088.598 | 1544.803 | 703.3985 | 352.2029 |
| I       | 3201.682 | 1601.345 | 602.3508 | 301.679  |
| E       | 3330.725 | 1665.866 | 489.2667 | 245.137  |
| N       | 3444.768 | 1722.888 | 360.2241 | 180.6157 |
| V       | 3543.836 | 1772.422 | 246.1812 | 123.5942 |
| K       | 3671.931 | 1836.469 | 147.1128 | 74.06    |

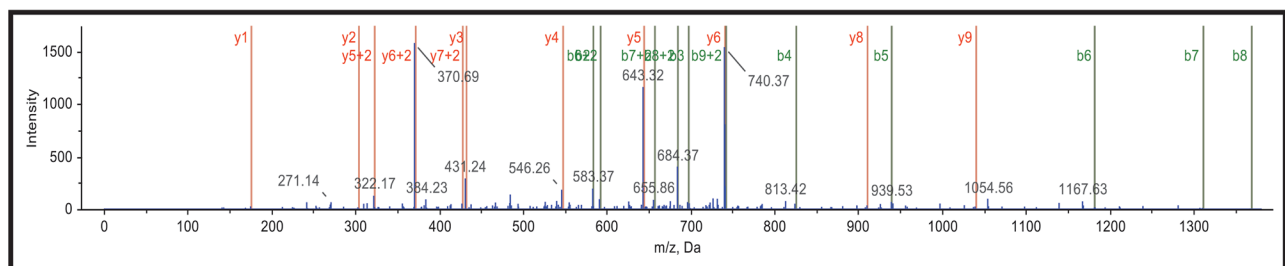


**Ubiquitination at Lys6 and Lys11: LRGGM!QIFVK\*TLTGK\*-TITLEVEPSDTIENVK**

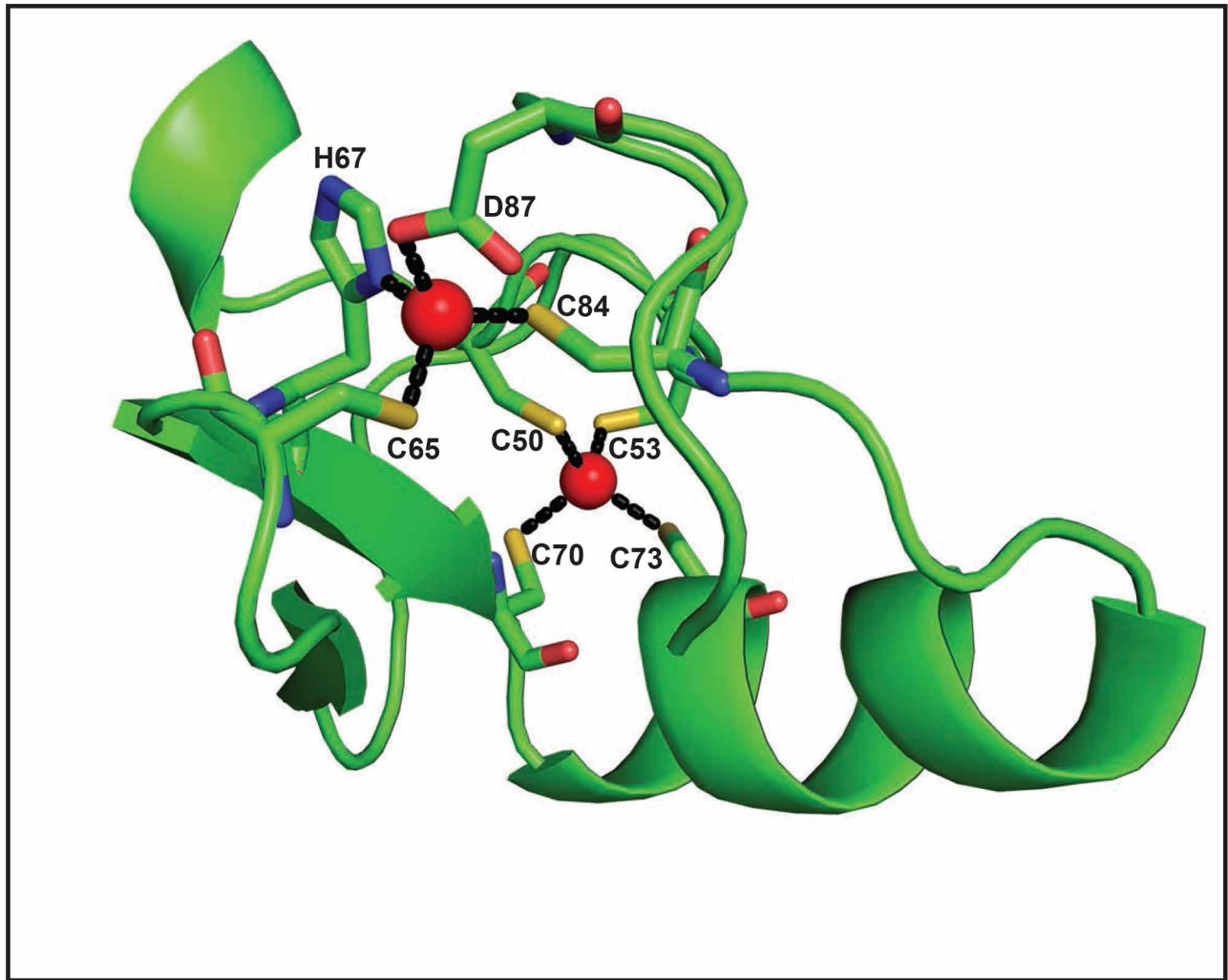


## I

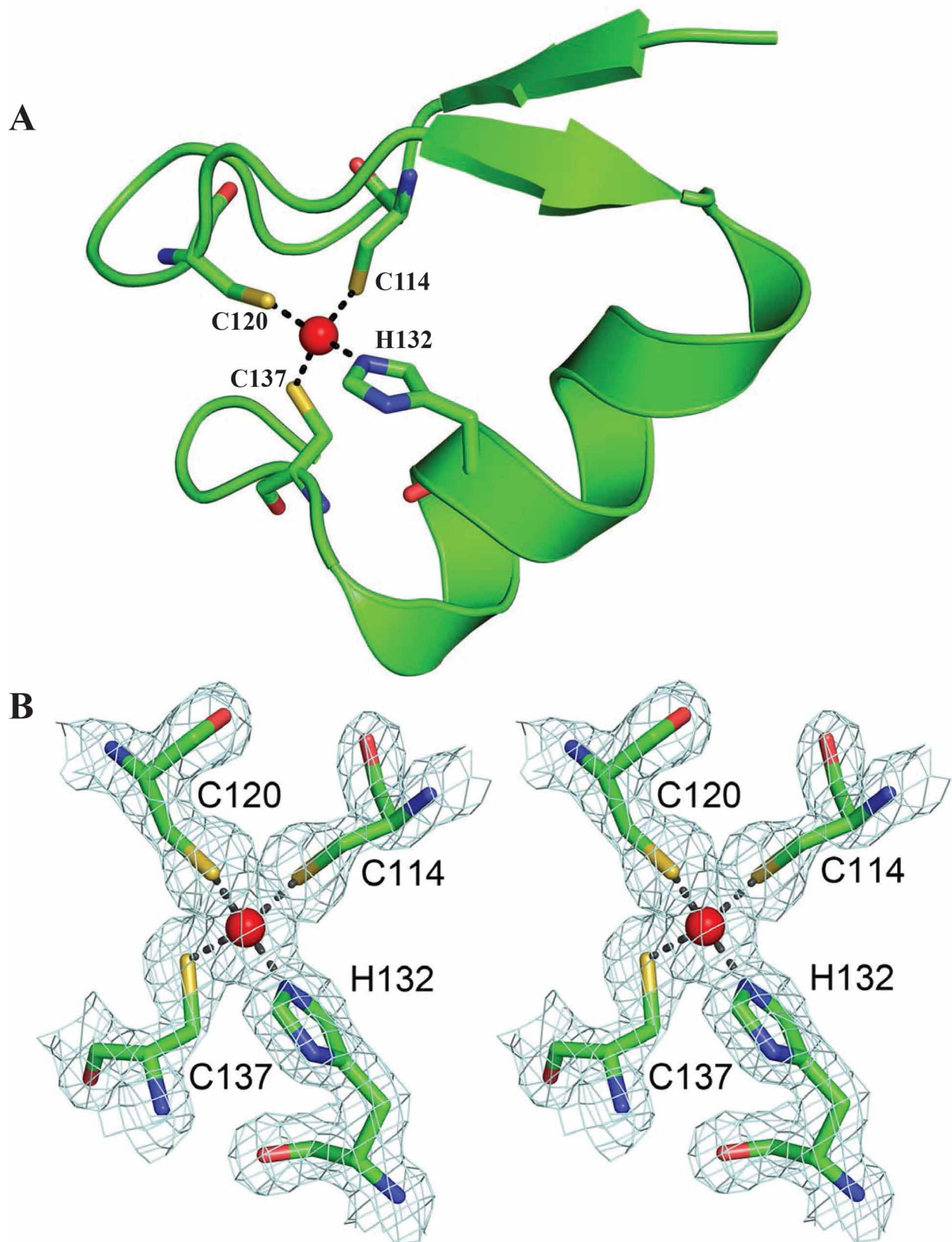
| Residue | b        | b+2      | y        | y+2      |
|---------|----------|----------|----------|----------|
| A       | 72.0444  | 36.5258  | 2220.184 | 1110.596 |
| K**     | 583.3675 | 292.1874 | 2149.147 | 1075.077 |
| I       | 696.4515 | 348.7294 | 1637.824 | 819.4157 |
| Q       | 824.5101 | 412.7587 | 1524.74  | 762.8737 |
| D       | 939.537  | 470.2722 | 1396.682 | 698.8444 |
| K*      | 1181.675 | 591.3411 | 1281.655 | 641.3309 |
| E       | 1310.718 | 655.8624 | 1039.517 | 520.262  |
| G       | 1367.739 | 684.3731 | 910.4741 | 455.7407 |
| I       | 1480.823 | 740.9152 | 853.4526 | 427.23   |
| P       | 1577.876 | 789.4415 | 740.3686 | 370.6879 |
| P       | 1674.929 | 837.9679 | 643.3158 | 322.1615 |
| D       | 1789.956 | 895.4814 | 546.2631 | 273.6352 |
| Q       | 1918.014 | 959.5107 | 431.2361 | 216.1217 |
| Q       | 2046.073 | 1023.54  | 303.1775 | 152.0924 |
| R       | 2202.174 | 1101.591 | 175.119  | 88.0631  |



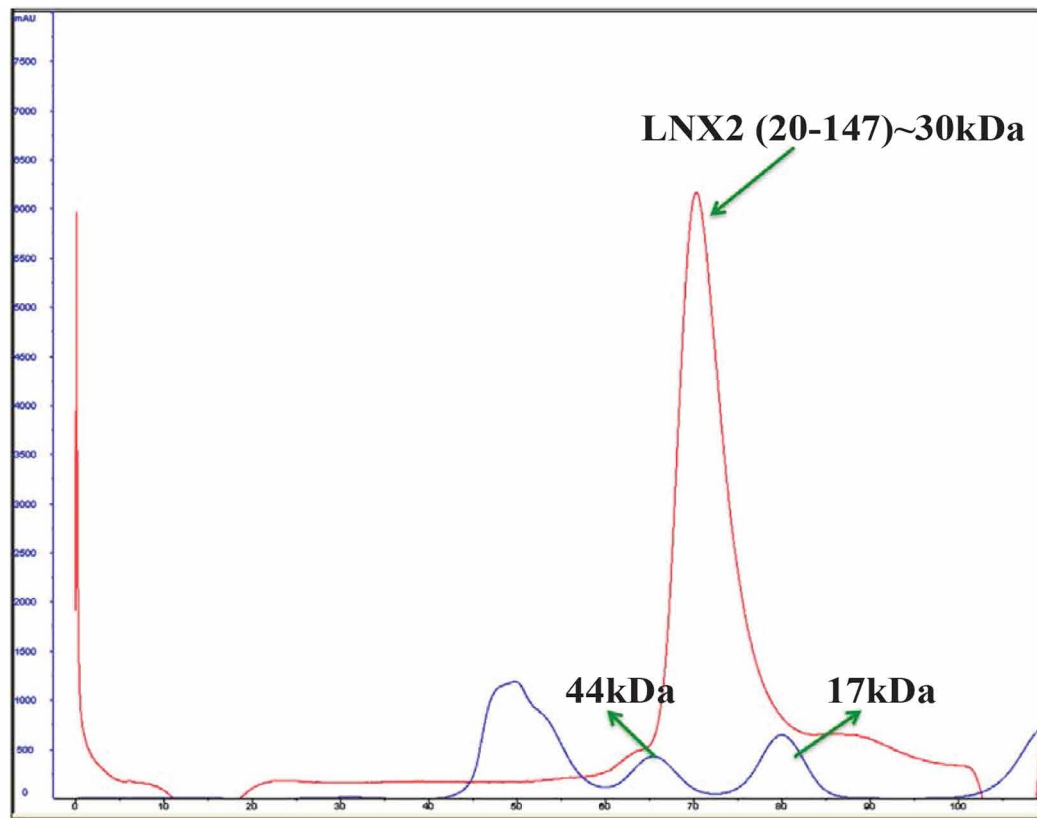
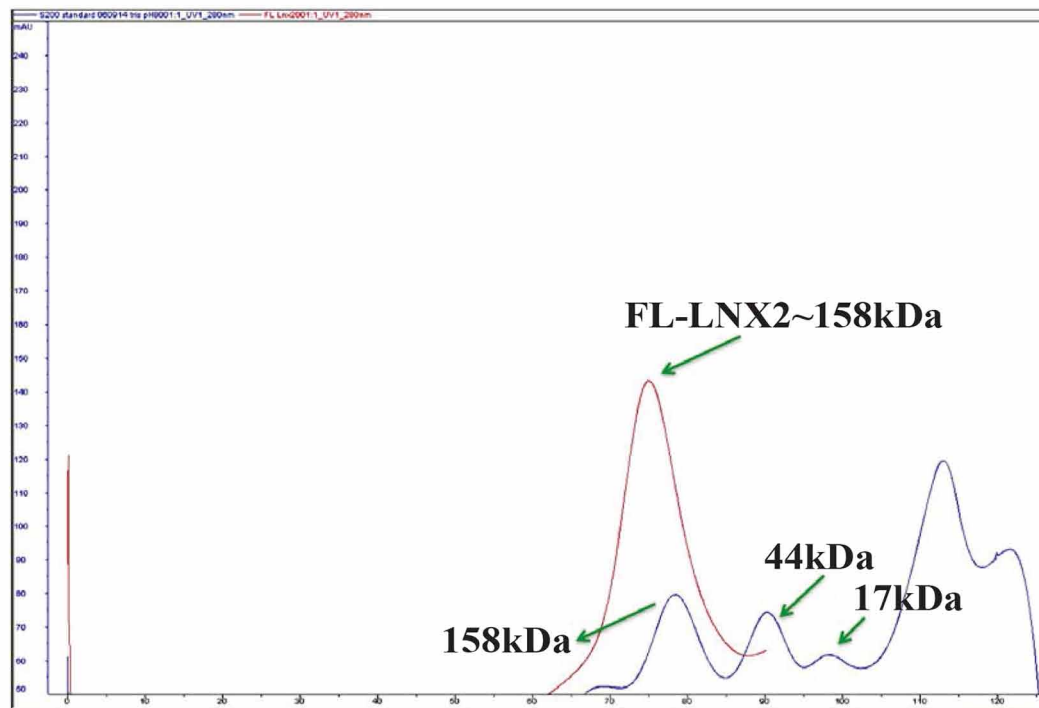
**Ubiquitination at Lys29 and Lys33: AK\*\*IQDK\*EGIPPDQQR**



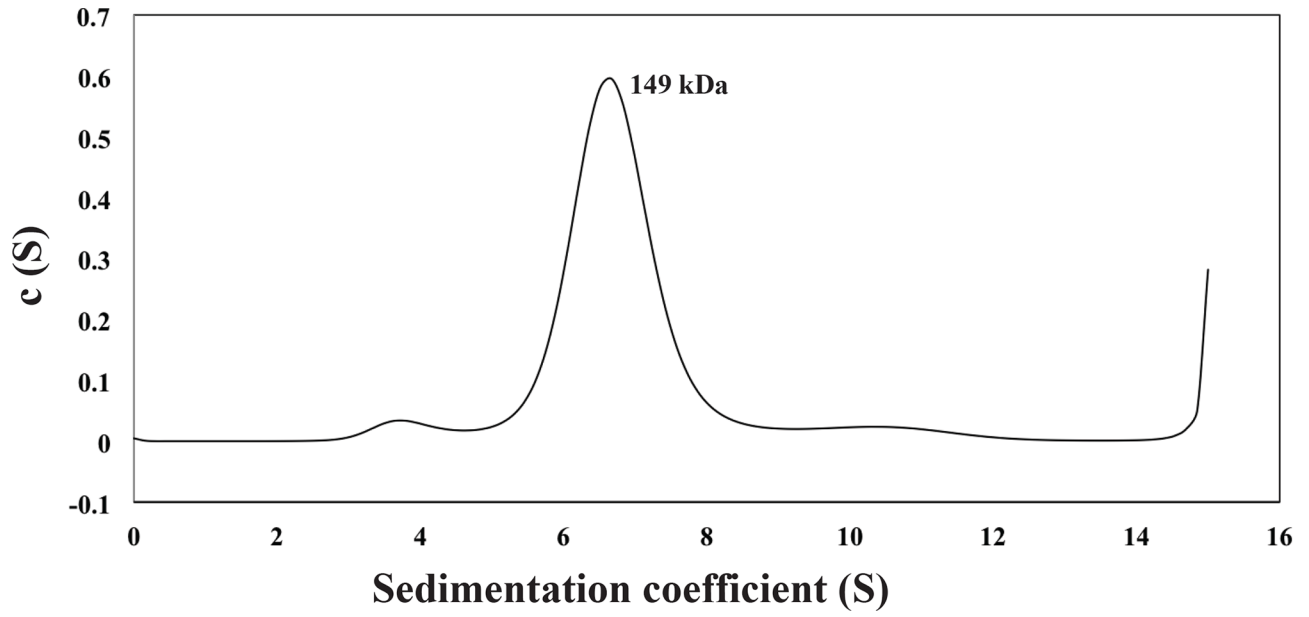
**Supplementary Figure S2: Structure of RING.** The structure of the RING domain of LNX2 with zinc coordination residues and zinc ions (red spheres) for one of the monomers. Unlike the Cys4HisCys3 “cross-brace” formation, one of the cysteine residues is replaced by an aspartic acid residue (Asp87) as shown here.



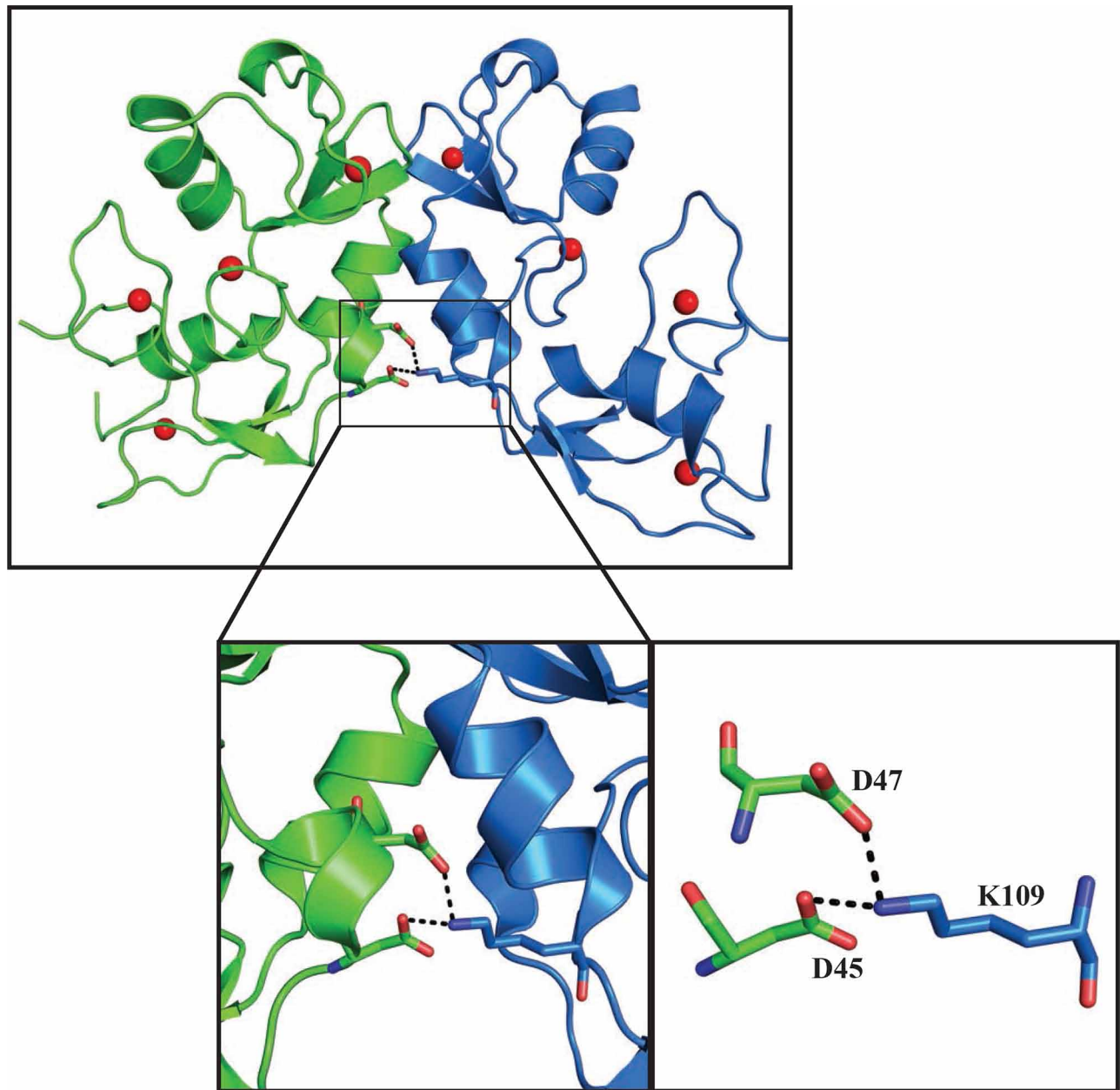
**Supplementary Figure S3: C-terminal Zn finger motif in Zn-RING-Zn domain.** A. Structure of the C-Terminal Zn finger motif in Zn-RING-Zn domain with zinc coordination residues and Zinc ion (red sphere). B. Stereo view of the zinc coordination residues and zinc ion in (A) with the final 2Fo-Fc electron density map contoured at 1.6  $\sigma$ .

**A****B**

**Supplementary Figure S4: Gel filtration profiles of Zn-RING-ZN domain and FL-LNX2 respectively.** Zn-RING-ZN domain and FL-LNX2 elute as a single peak at an elution volume corresponding to molecular mass of approximately 30 kDa **A.** and 158 kDa **B.** respectively. The molecular mass standard is shown in blue.

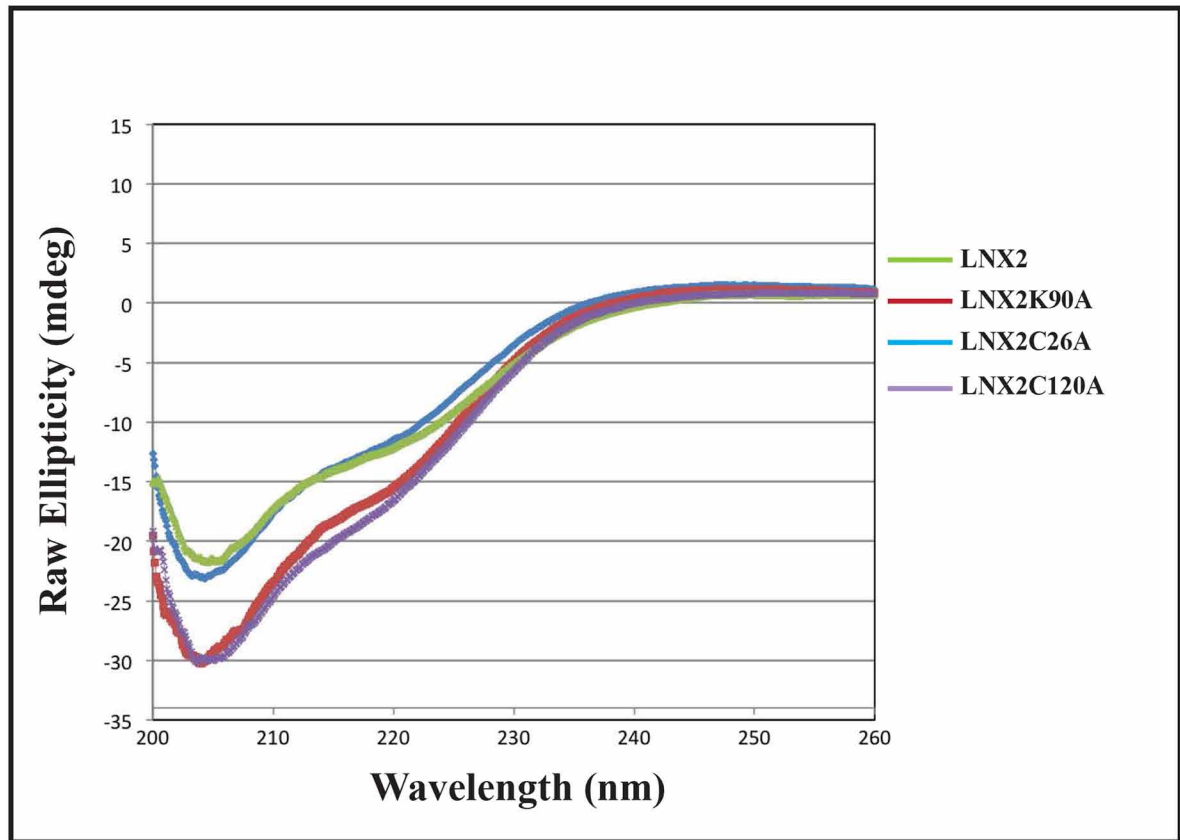


**Supplementary Figure S5: Analytical Centrifugation of FL-LNX2.** AUC analysis of FL-LNX2 showed the presence of dimers in the solution.

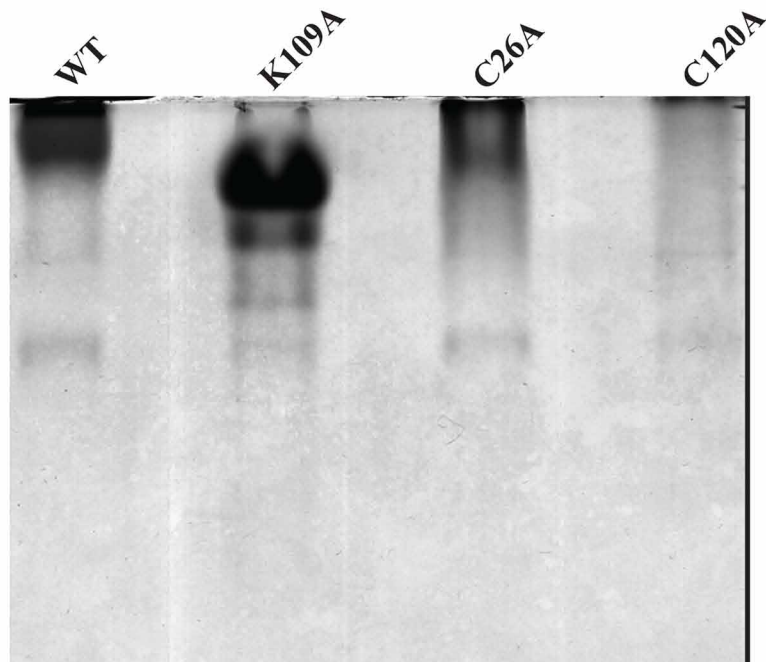


**Supplementary Figure S6: Residues responsible for the dimerization of Zn-RING-Zn domain.** Lys109 of one chain makes hydrogen bond contact with Asp45 and Asp47 of other chain.

A

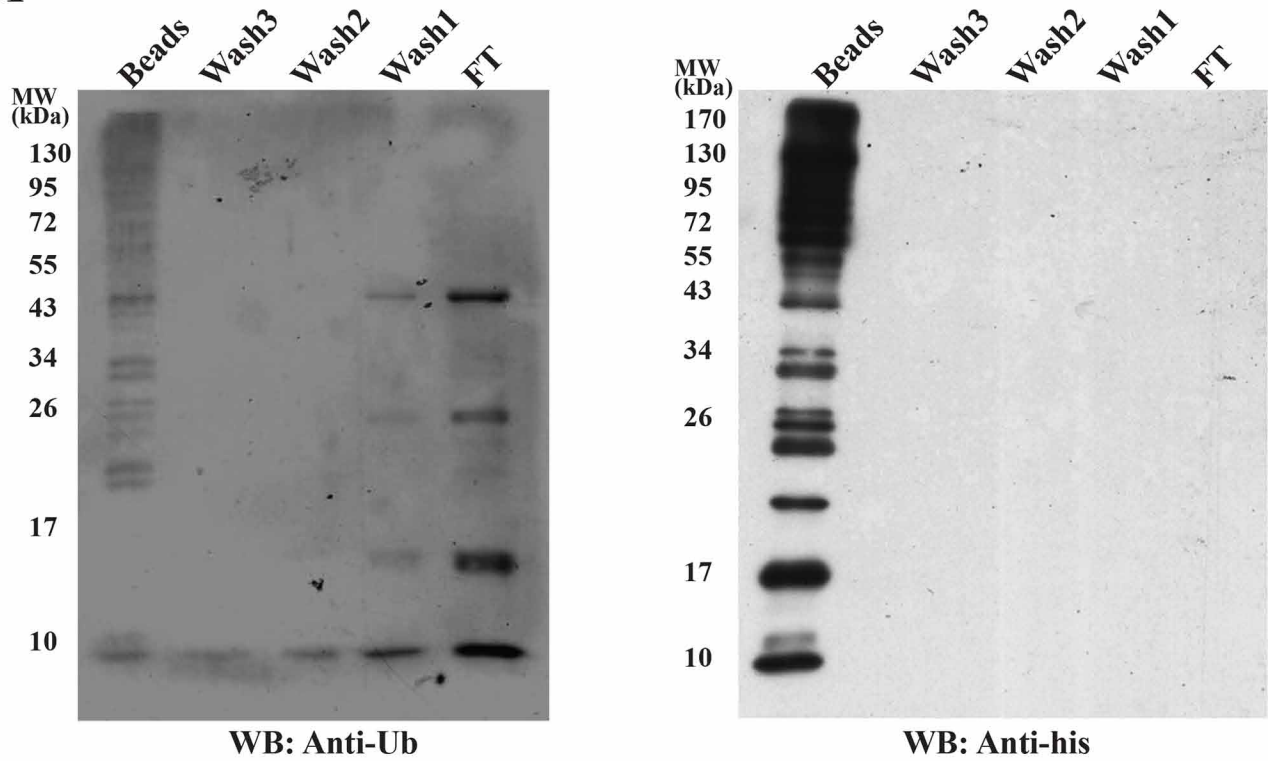


B

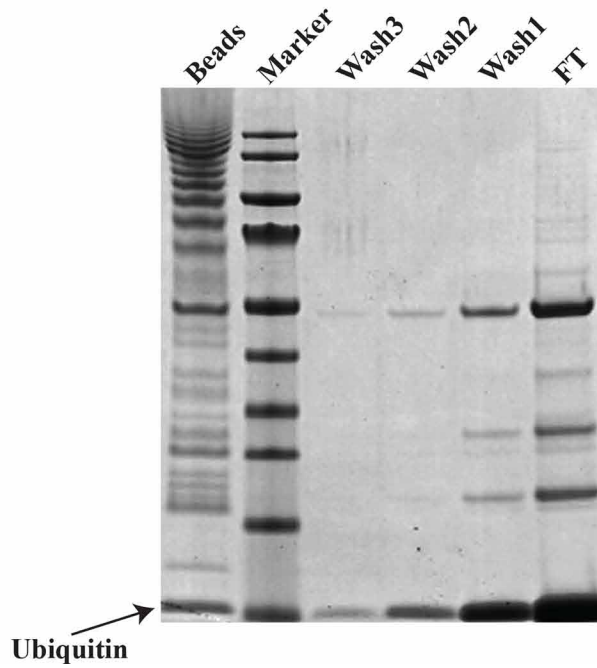


**Supplementary Figure S7: Secondary structure of dimer and Zn finger motifs disruptive mutants of Zn-RING-Zn domain respectively.** A. Circular dichroism analysis shows that the dimeric and two zinc finger disruptive mutants of Zn-RING-Zn domain respectively maintained a well-defined secondary structure, comparable to that of the wild type. B. Native gel analysis of the mutants used in (A) shows the change in oligomerization state of K109A mutant compared to wild type.

**A**



**B**



Supplementary Figure S8: Full blots of the partial western blots shown in the main text. Figure 8B. **B.** SDS-PAGE result for the corresponding blot used in A.



## Supplementary Table S1: Crystallographic data and refinement statistics

| Zinc-SAD  |                                   |
|---|-----------------------------------|
| <b>DATA COLLECTION</b>  |                                   |
| Space group   | P 1 21 1                          |
| Cell Dimensions<br>a, b, c (Å)<br>$\alpha, \beta, \gamma$ (°) | 46.97 69.39 52.25<br>90 115.41 90 |
| Wavelength (Å)  | 1.282                             |
| Resolution range (Å)  | 24.24 – 1.86 (1.93 – 1.86)        |
| Total reflections   | 179976                            |
| Unique reflections  | 25098 (2367)                      |
| R-sym <sup>a</sup>  | 0.1 (0.307)                       |
| Multiplicity  | 7.2 (6.3)                         |
| Completeness (%)  | 98.92 (93.78)                     |
| Mean I/sigma(I)   | 9.91 (5.01)                       |
| <b>REFINEMENT</b>   |                                   |
| Wilson B-factor   | 17.84                             |
| R-factor <sup>b</sup>   | 0.190 (0.222)                     |
| R-free <sup>c</sup>   | 0.232 (0.272)                     |
| Number of atoms   | 2260                              |
| macromolecules  | 1997                              |
| ligands   | 20                                |
| water   | 243                               |
| Protein residues  | 244                               |
| RMS(bonds)  | 0.007                             |
| RMS(angles)   | 1.10                              |
| Ramachandran favored (%)                                      | 99                                |
| Ramachandran outliers (%)                                     | 0                                 |
| Clashscore  | 3.01                              |
| Average B-factor  | 22.60                             |
| macromolecules  | 22.10                             |
| solvent   | 26.50                             |

Statistics for the highest-resolution shell are shown in parentheses.

<sup>a</sup>Rsym =  $\sum |I_i - \langle I \rangle| / \sum I_i$ , where  $I$  is the intensity of the  $i$ -th measurement, and  $\langle I \rangle$  is the mean intensity for that reflection.

<sup>b</sup>Rwork =  $\sum ||F_{obs}| - |F_{calc}|| / \sum |F_{obs}|$ , where  $F_{calc}$  and  $F_{obs}$  are the calculated and observed structure factor amplitudes, respectively.

<sup>c</sup>Rfree = as for Rwork, but was calculated using 10% of data excluded from refinement.