Electronic Supplementary Material (ESI) for ChemComm.

Supporting Information

Thiirane linkers directed histone H2A diubiquitination suggests plasticity in 53BP1 recognition

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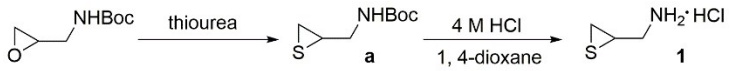
Experimental methods

1. HPLC, mass spectrometry (MS)

Reversed phase HPLC was performed on Shimadzu Prominence HPLC systems (with LC-20AT as the solvent delivery units). For peptide analysis, Reprosil-Pur Basic-C18 (4.6 × 250mm, 5 μm particle size) and C18 (4.6 × 150mm, 5 μm particle size) columns were used at a flow rate of 1.0 mL/min. For peptide purification, Vydac C4 (10 × 250 mm, 5 μm particle size), and Reprosil-Pur Basic-C18 (10 × 250 mm, 5 μm particle size) columns were used at flow rates of 4 mL/min. The UV absorption at 214 nm and 254 nm were monitored for the injections. Water (with 0.1% TFA) and acetonitrile (with 0.08-0.1% TFA) were chosen as the solvents. The gradient of the solvents was optimized for each peptide. Normal ESI mass spectra were measured on LC/MS 2020 (SHIMADZU). High-resolution ESI mass spectra were measured on Agilent 6210 Time of Flight Mass Spectrometer.

2. Preparation of thiiran-2-ylmethanamine hydrochloride



Scheme S1: The Synthesis route for thiiran-2-ylmethanamine hydrochloride, which was modified from the previously reported procedure.[1]

Compound a: N-Boc-oxiran-2-ylmethanamine (0.35 g, 2.0 mmol) was dissolved in ethanol (5.0 ml), and then thiourea (0.16 g, 2.1 mmol) was added. The resulting solution was stirred for 24 h at room temperature. After completion, the solution was concentrated under reduced pressure and purified by column chromatography to give N-Boc-thiiran-2-ylmethanamine (**a**) as a white solid (0.3 g, 80%). ^{1}H NMR (CDCl3, 400 MHz): δ (ppm) = 1.45 (s, 9H), 2.23 (dd, 1H), 2.49 (dd, 1H), 3.13 (m, 1H), 3.23 (dt, 1H), 3.63 (m, 1H), 4.79 (s, 1H). ^{13}C NMR (CDCl3, 400 MHz): δ= 23.3, 28.5, 34.5, 44.9, 79.8, 155.6. m/z (ESI^{+}): observed, 190.08897 Da [M+H]$^{+}$; calculated for C8H15NO2S, 189.08 Da.

Compound 1: Boc-thiiran-2-ylmethanamine (**a**) (0.18 g, 0.95 mmol) was dissolved in 4 M hydrogen chloride/1,4-dioxane (3.0 ml), the resulting solution was stirred for 2 h at room temperature. Then diethyl ether (30.0 ml) was added, and the solution was cooled on ice. The white precipitate was collected by centrifugation (0.1 g, 85%). ^{1}H NMR (D_2O, 400 MHz): δ (ppm)= 2.34 (dd, 1H), 2.62 (dd, 1H), 2.77-2.88 (m, 1H), 3.09 (m, 1H), 3.5(m, 1H). ^{13}C NMR (D_2O, 400 MHz): δ (ppm)= 23.9, 30.2, 44.9. m/z (ESI^{+}): observed, 90.03696 Da [M+H]$^{+}$; calculated for C3H7NS, 89.03 Da.

3. Construction of protein expression plasmids

Ub-77D: 77D (codon: GAC) was added to Ubiquitin gene cloned in pET-22b plasmid vector by site-directed mutagenesis kit (Takara). Amino acid sequence: MQIFVKTLTGKTITLEVEPSDTIENVKAKIQDKEGIPPDQQRL IFAGKQLEDGRTLSDYNIQ KESTLHLVLRLRGGD.

UbK27C-77D: K27C point mutation was introduced to Ub-77D gene cloned in pET-22b plasmid vector by site-directed mutagenesis kit (Takara). Amino acid sequence: MQIFVKTLTGKTITLEVEPSDTIENV**C**AKIQDKEGIP PDQQRLIFAGKQLEDGRTLSDYNIQKESTLHLVLRLRGGD.

UbK48C-77D: K48C point mutation was introduced to Ub-77D gene cloned in pET-22b plasmid vector by site-directed mutagenesis kit (Takara). Amino acid sequence: MQIFVKTLTGKTITLEVEPSDTIENVKAKIQDKEGIPP DQQRLIFAG**C**QLEDGRTLSDYNIQKESTLHLVLRLRGGD.

H2AK13C: K13C point mutation was introduced to Human H2A gene cloned in pET-30a plasmid vector by site-directed mutagenesis kit (Takara). Amino acid sequence: MKDLLSGRGKQGGKARA**C**AKTRSSRAGLQFP VGRVHRLLRKGNYSERVGAGAPVYLAAVLEYLTAEILELAGNAARDNKKTRIIPRHLQLAIRNDEELNKLLGKVT IAQGGVLPNIQAVLLPKKTESHHKAKGK.

H2AK15C: K15C point mutation was introduced to Human H2A gene cloned in pET-30a plasmid vector by site-directed mutagenesis kit (Takara). Amino acid sequence: MKDLLSGRGKQGGKARAKA**C**TRSSRAGLQFP VGRVHRLLRKGNYSERVGAGAPVYLAAVLEYLTAEILELAGNAARDNKKTRIIPRHLQLAIRNDEELNKLLGKVT IAQGGVLPNIQAVLLPKKTESHHKAKGK.

4. Protein expression and purification

4.1 Expression and purification of histones

According to the reported methods,[2] the plasmid of histone was transformed into *E. coli* BL21 (DE3) cells. A single colony was picked and transferred into 5 mL LB with 100 μg/mL ampicillin, which was cultivated at 220 rpm and 37ºC overnight. The resulted LB was then transferred into 1 L LB with 100 μg/mL ampicillin and cultivated for another 6-7 h at 37ºC. When the OD600 reached 0.8, 0.4 mM (final concentration) isopropyl β-D-1-thiogalactopyranoside (IPTG) was added to induce the expression for another 12-16h. Then, the cells were harvested by centrifugation at 4ºC. The precipitation was re-suspended by Lysis buffer (20 mM Tris, 150 mM NaCl, 1 mM DTT, 1 mM PMSF, pH 8.0) and lysed by ultrasonication for 60 min at 4ºC. After centrifugation (12000 rpm at 4ºC) for 30 min, the precipitation was collected and subsequently washed twice each with TW buffer (50 mM Tris, 100 mM NaCl, 1 mM Na-EDTA, 1 mM benzamidine, 5 mM DTT, 1% (v/v) Triton X-100, pH 7.5) and wash buffer (TW buffer without Triton X-100). After centrifugation, the precipitation was collected and resolved by unfolding buffer (6M guanidine hydrochloride, 20 mM Tris, 10 mM DTT, pH 7.5), and the undissolved precipitate was removed by centrifugation. Next, the supernatant was dialysed into ddH_2O containing 0.1% TFA overnight. The precipitation was removed by another centrifugation. After filtration with 0.22 μm filter paper, the resulted supernatant was purified by RP-HPLC and characterized by ESI-MS. Finally, the desired histones were lyophilized and stored at -20 ºC.

4.2 Expression and purification of Ubiquitin (Ub) mutants

The plasmid containing Ub mutant gene was transformed into *E. coli* BL21 (DE3) cells. The cells were grown in 1 L LB media containing 100 μg/mL ampicillin to OD600 of 0.6-0.8 and induced by a final concentration of 0.4 mM IPTG for 5 h at 37 ºC. After centrifugation at 8000 rpm for 5 min at 4 ºC, cells were resuspended in lysis buffer (50 mM Tris-HCl, 100 mM NaCl, 1 mM DTT, pH 7.5) and lysed by ultra-sonication for 60 min in ice bath. After centrifugation at 12000 rpm for 30 min at 4 ºC, 70% perchloric acid was added to the lysate with 1.0% final volume concentration. The mixture was stirred for 5 min and centrifuged at 12000 rpm for 30 min at 4 ºC. After filtration with 0.22 μm filter paper, the supernatant was dialyzed with 3.5 KDa dialysis bag in dialysis buffer (25 mM Tris-HCl, 100 mM NaCl, pH 7.5) and then concentrated to the desired concentration for the next hydrazinolysis reaction, or the supernatant can be dialyzed to ddH2O (0.1% TFA) followed by direct lyophilization.

4.3 Expression and purification of YUH1

YUH1 was cloned in pET-30a vector containing an N-terminal His tag, and expressed in *E. coli* BL21 (DE3) cells. The cells grew in LB medium containing kanamycin (30 μg/mL) at 37 ºC until OD600 reached to 0.6-0.8, then 0.4 mM (final concentration) IPTG was added to induce the expression at 16 ºC for 14 h. The cells were harvested by centrifugation at 8000 rpm for 5 min and then lysed by sonication in lysis buffer (20 mM Tris-HCl, 300 mM NaCl, pH 7.0). After centrifugation at 12000 rpm for 30 min, supernatant was loaded onto a Ni-NTA column (Histrap 5 mL, GE healthcare). The column was washed with washing buffer (20 mM Tris-HCl, 300 mM NaCl, 30 mM imidazole, pH 7.0) and then eluted with elution buffer (20 mM Tris-HCl, 300 mM NaCl, 250 mM imidazole, pH 7.0). YUH1 was further purified by FPLC with Superdex TM 200 Increase 10/300 GL column.

4.4 Expression and purification of GST-Tudor-UDR

GST-Tudor-UDR was recombinantly expressed in *E. Coli* using the reported protocols.[3] The plasmid (1484-1631) was transformed into *E. coli* BL21 (DE3) cells. A single colony was picked and grew in 10 mL LB containing 100 μg/mL ampicillin at 220 rpm and 37 °C. After 12 hours, the resulted cells were transferred to 1 L of LB containing 100 μg/mL ampicillin, and cultured at 37 °C for another 3 hours. When OD600 reached 0.8, 0.3 mM (final concentration) IPTG was added to induce expression at 18 °C for 18h. The harvested cells were resuspended in Lysis buffer (20 mM Tris, 150 mM NaCl, 1 mM DTT, 1 mM PMSF, pH 7.5) and subsequently lysed by sonication at 4 °C for 30 min. After removal of the precipitation by centrifugation (12000 rpm for 30 minutes), the GST-beads were added to the supernatant and incubated for 2 hours at 4 °C. Then, the beads were filtered and washed with Lysis buffer. Finally, the desired protein was collected by the elution of wash buffer (20 mM Tris, 150 mM NaCl, 30 mM GSH, pH 7.5) and purified by size exclusion chromatography (Superdex 200 10/300 column).

5. Preparation of Ub-$NHNH_2$, 3

For the preparation of segment **3,** Ub-77D (15 mg, 2 μmol) was dissolved in 50 mM Tris-HCl buffer (pH 7.4, 1.0 ml) containing 5% hydrazine hydrate. The mixture was cooled on ice, and then YUH1 was added with final concentration of 1 μM. The reaction mixture was kept in ice bath for 30min. and monitored by analytical RP-HPLC. After completion, the reaction mixture was quenched with TFA, and purified by semi-preparative RP-HPLC.

6. Preparation of segments 2 and 11

For the preparation of segment **2**, UbK27C-77D (17.2 mg, 2 μmol) was dissolved in 0.2 M sodium phosphate buffer (pH 7.5, 2.0 ml) containing 10 mM glyoxylic acid, 5 mM tris(2-carboxyethyl)phosphine hydrochloride, 6 M Gn·HCl, Then thiiran-2-ylmethanamine hydrochloride (2.5 mg, 20 μmol) was added and the pH value of the reaction mixture was adjusted to 8.0. The solution was stirred at 37 °C for 12h. DTT (15.4 mg, 50mM) and O-methylhydoxylamine hydrochloride (33.2 mg, 200mM) were added to the solution, which was then stirred at 37 °C for an additional 24 h. the reaction was dialyzed into ddH2O (0.1% TFA) followed by direct lyophilization. The lyophilized protein (final concentration 8 mg/ml) was dissolved in 50 mM Tris-HCl buffer (pH 7.4, 1.0ml) containing 5% hydrazine hydrate. The mixture was cooled on ice, and then YUH1 was added with final concentration of 1 μM. The reaction mixture was kept in ice bath for 30min. and monitored by analytical RP-HPLC. After completion, the reaction mixture was quenched with TFA, and purified by semi-preparative RP-HPLC.

7. Preparation of segments 4 and 4'

For the preparation of segment **4**, H2AK13C (29.1 mg, 2 μmol) was dissolved in 0.2 M sodium phosphate buffer (pH 7.5, 2.0 ml) containing 10 mM glyoxylic acid, 5 mM tris(2-carboxyethyl)phosphine hydrochloride, 6 M Gn·HCl, Then thiiran-2-ylmethanamine hydrochloride (2.5 mg, 20 μmol) was added and the pH value of reaction mixture was adjusted to 8.0. The solution was stirred at 37 °C for 12h. DTT (15.4 mg, 50mM) and O-methylhydoxylamine hydrochloride (33.2 mg, 200 mM) were added to the solution, which was then stirred at 37 °C for an additional 24 h. The reaction mixture was purified by semi-preparative RP-HPLC. Segment **4'** was obtained in a similar way.

8. Semi-synthesis of di-ubiquitin modified H2A analogues

The strategy for the synthesis of di-ubiquitin modified H2A analogues involves two steps of hydrazide-based native chemical ligation. Here, we take the synthesis of $H2AK13_CdiUb^{K27C}$ as an example. Segment **3** (9.5 mg, 1.1μmol) was dissolved in 0.2 M sodium phosphate buffer (6 M Gn·HCl, pH 3.0,) at a final concentration of 1 mM. The mixture was cooled in ice-salt bath, and 39 μl (7.0 equiv.) solution of 0.2 M $NaNO_2$ was added to the mixture. Reaction was incubated on ice for 30 min with stirring. Then 8.4 mg (50.0 equiv.) MPAA was added to the mixture and the pH value of the resulting solution was adjusted to 5.0. The solution was stirred for 5 min at room temperature. Segment **2** (8.6 mg, 1.0μmol) was then added and reaction solution was adjusted to pH 6.7 with 1.0 M NaOH solution slowly. The reaction was monitored by analytical RP-HPLC. After 12h, the reaction mixture was purified by semi-preparative RP-HPLC. For RP-HPLC analysis and purification, the reaction mixture was reduced by TCEP before injection.

5 (7.0 mg, 0.4 μmol) was dissolved in 0.3 mL acidified ligation buffer (aqueous solution of 6 M Gn·HCl and 0.2 M Na_2HPO_4, acidified to pH 3.0 by HCl). The mixture was cooled in an ice-salt bath, and 20 μL solution of 0.2 M $NaNO_2$ (in acidified ligation buffer) was added to the mixture. Reaction was kept in ice-salt bath with stirring for 30 min. Then 3.5 mg MPAA in 0.1 ml neutral ligation buffer (6 M Gn·HCl and 0.2 M Na_2HPO_4) was added, and the pH value of the resulting solution was adjusted to 5.0 at room temperature. Then segment **4** (6.0 mg, 0.4 μmol) was added. Subsequently the resulting solution was adjusted to pH 6.7 and the ligation was kept at room temperature. After 12 h, reaction mixture was analysed and then purified by HPLC to give $H2AK13_CdiUb^{K27C}$ in 30% isolated yield (3.8 mg). For HPLC analysis, aliquots from reaction mixture were reduced by TCEP before injection. The reaction mixture was also treated by TCEP before HPLC purification.

9. Preparation of the wild-type modified histones

Lys20 dimethylated histone H4 (H4K20me2) was prepared by total chemical synthesis according to our previously reported method.[4] Generally, the sequence was divided into three segments and two alanine residues (Ala38 and Ala76) were substituted by cysteines to facilitate ligations of peptide hydrazides in a N-to-C sequential strategy. The desired H4K20me2 was finally obtained after desulfurization.

Both the wild-type Lys13-monoubiquitinated H2A (H2AK13monoUb) and Lys15-diubiquitinated H2A ($H2AK15diUb^{WT}$) were also prepared by total chemical synthesis as same as our reported methods.[4] Any synthetic details can be found in the reference 4.

10. Nucleosome core particles reconstruction

The nucleosome core particles (NCPs) containing di-ubiquitinated H2A analogues were reconstituted according to standard procedures.[5] Firstly, the four histones (molar ratio 1:1:1:1) were respectively dissolved in unfolding buffer at a concentration of 2 mg/ml. After incubation for 30 min, the histones were mixed and the total histone concentration was adjusted to 1 mg/ml with unfolding buffer. Then, the mixture was dialyzed against refolding

buffer (2M NaCl, 10 mM Tris. HCl, 1 mM 2-mercaptoethanol, pH 7.5) for 12 hours at 4 °C with three times. After 36 hours, the mixture was concentrated to less than 1 mL and then purified by size-exclusion chromatography (Superdex 200 increase column). The purified octamers confirmed by SDS-PAGE were stored at -80ºC or immediately used to assemble NCPs with 601 DNA. To constitute NCPs, the octamers and DNA were mixed in a high salt buffer (2 M NaCl, 10 mM Tris-HCl, pH 7.5, 1 mM EDTA and 1 mM DTT) and dialyzed against 400 ml high salt buffer as a starting buffer. The TE buffer (10 mM Tris-HCl, pH 7.5, 1 mM EDTA and 1 mM DTT) was slowly dropped into the high salt buffer at a flow rate of 0.6 mL/min with a peristaltic pump until the concentration of NaCl reached about 0.1M. Then, the mixture was further dialyzed against 1 L of TE buffer overnight to produce the desired nucleosomes.

11. Pull-down assays

The GST pull-down assays with NCPs were conducted as the reported methods.[3] 10 μg of the GST-Tudor-UDR (1484-1631) immobilized on GST-beads was incubated with 6 μg of NCP complex in pull down buffer (50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 1 mM DTT, 0.05% NP-40 (v/v), 0.1% (w/v) BSA) for 2 hours at 4 °C. Then, the mixture was carefully washed with 0.8 mL of pull-down buffer for four times and followed by adding of 2 x Laemmli SDS-PAGE loading buffer. The resulted samples were further analysed by SDS-PAGE with coomassie brilliant blue and immunoblotting with anti-H2A specific antibody. In the pull-down assays of LANA peptide competition, the protocols were almost the same except the incubation of GST-53BP1 with NCPs was performed in the presence of the LANA peptide or its mutant.

12. Immunoblotting

The SDS-PAGE separated samples were transferred to Nitrocellulose Blotting membrane (NC, GE Healthcare) in Transfer buffer (25 mM Tris base, 192 mM Glycine, 20% ethanol) for 80 min under 300 mA at 4 °C. TBS (20 mM Tris base, 137 mM NaCl, pH 7.6) containing 5% (w / v) skim milk powder was used to block the membrane for 2 h at room temperature. The rabbit anti-H2A primary antibody (ab18255, Abcam) diluted to 2000-fold with TBS (20 mM Tris base, 137 mM NaCl, pH 7.6) containing 3% (w/v) BSA was then added to the NC membrane. After incubation with oscillating for 2h at room temperature, the NC membrane was washed by TBS for four times. Then, HRP goat anti-rabbit IgG secondary antibody (Beyotime, Shanghai, China) diluted to 2000-fold by TBS containing 3% (w /v) BSA was added to the NC membrane with incubation at room temperature. After 2 hours, the NC membrane was further washed with TBS for four times and subsequently treated by the freshly prepared HRP chemiluminescent reagent (highly sensitive ECL luminescent reagent, Sangon), which was finally exposed and analyzed by the ChemiDocTM Touch Imaging system (Bio-rad).

References

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4. J.-B. L, Y.-K. Q, Q.-Q. He, H.-S. Ai, S.-L. Liu, J.-X. Wang, J.-S. Zheng, L. Liu and C. Tian, *Cell Res*, 2018, **28**, 257-260.
5. P. T. Lowary and J. Widom, *J. Mol. Biol.* **1998**, 276, 19-42.

Supplementary figures

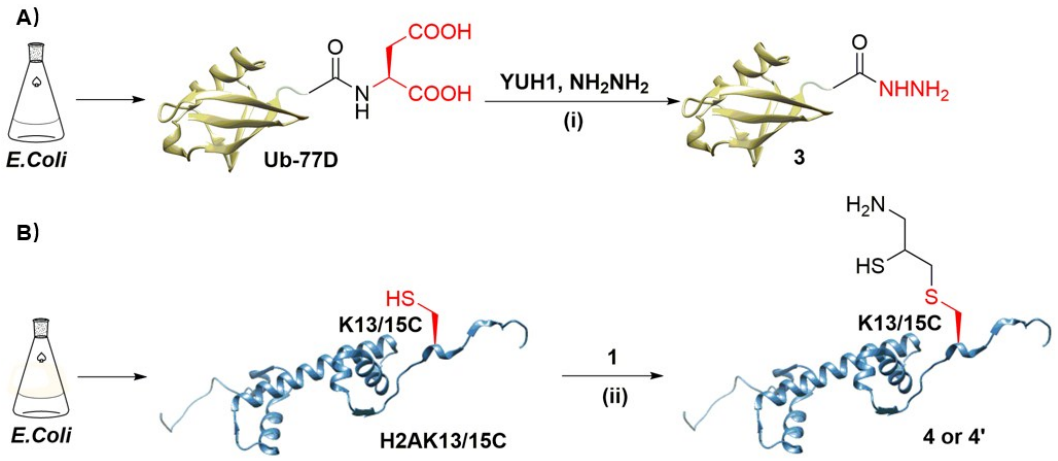

Figure. S1 Scheme for the synthesis of segments 3 and 4 (or 4'). (A) Segment 3 was obtained through the hydrazine treatment of Ub-77 in the presence of C-terminal hydrolase YUH1. (i) Ub-77D, YUH1, 5% NH_2NH_2, 50 mM Tris buffer, pH 7.4, 0°C, 1h. (B) The histone mutants H2AK13C and H2AK15C were respectively modified by thiirane **1** in the presence of glyoxylic acid, followed by the treatment of CH_3ONH_2, producing **4** or **4'**. (ii) H2AK13C or H2AK15C, **1**, CHOCOOH, 0.2 M phosphate buffer, 5 mM TCEP, 6 M Gn·HCl, pH 8.0, 37 °C, 12h; then CH_3ONH_2, DTT, 37 °C, 24h.

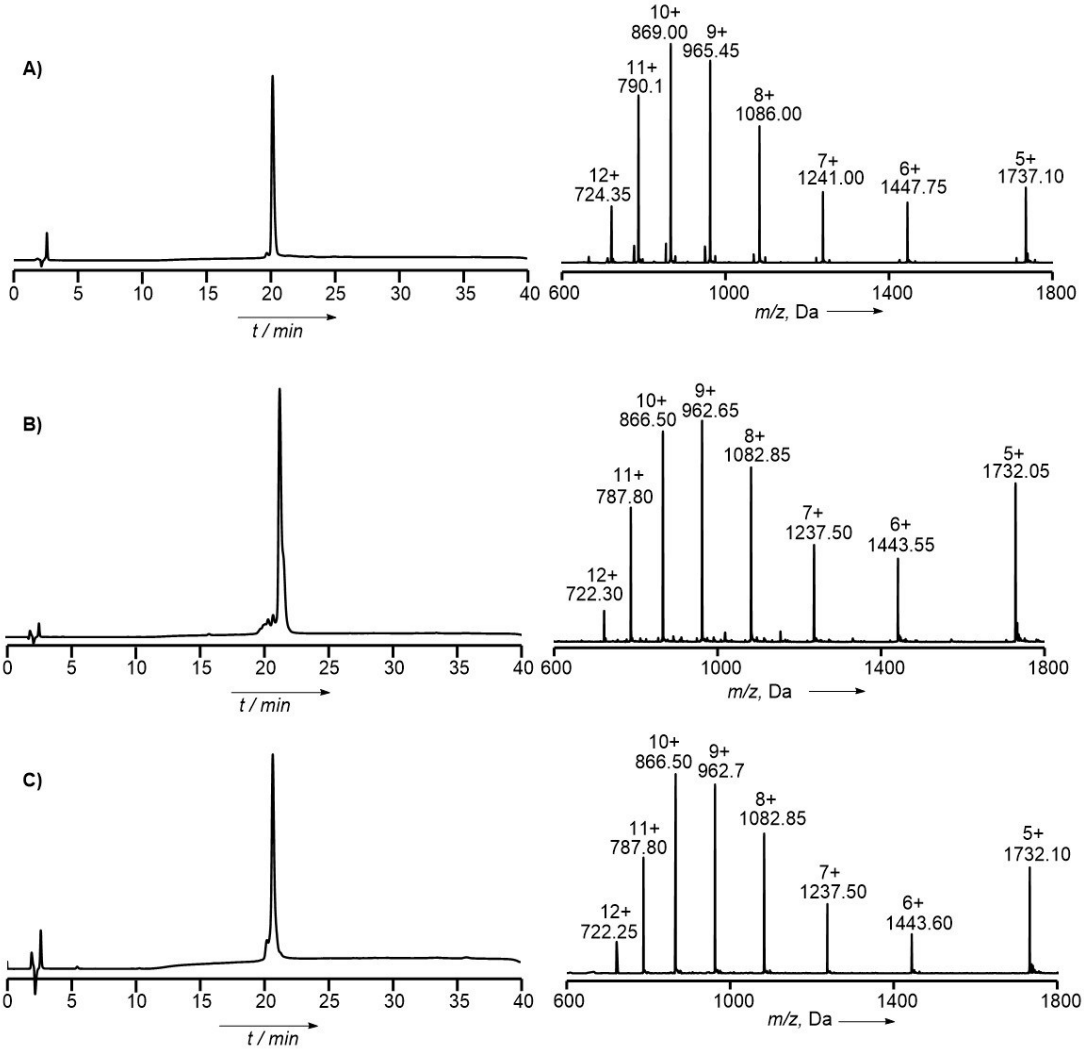

Figure. S2 Characterization of the recombinant ubiquitin mutants. (A) The RP-HPLC (λ=214 nm) and ESI-MS (average isotope) analysis of Ub-77D, observed: 8680.2Da; cacld: 8680.9 Da. (B) The RP-HPLC (λ=214 nm) and ESI-MS (average isotope) analysis of UBK27C-77D, observed: 8654.8 Da; cacld: 8654.9 Da. (C) The RP-HPLC (λ=214 nm) and ESI-MS (average isotope) analysis of UbK48C-77D, observed: 8655.3 Da; cacld: 8654.9 Da.

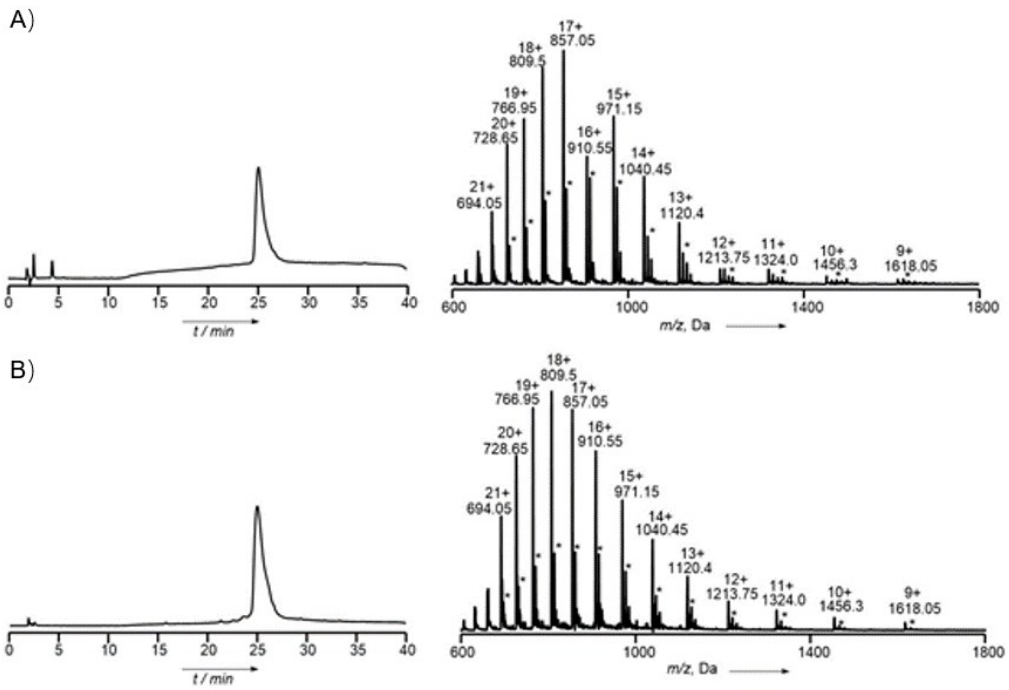

Figure. S3 Characterization of the recombinant histones. (A) The RP-HPLC (λ=214 nm) and ESI-MS (average isotope) analysis of H2AK13C, observed: 14553.4 Da; cacld: 14552.0 Da. (B) The RP-HPLC (λ=214 nm) and ESI-MS (average isotope) analysis of H2AK15C, observed: 14553.4 Da; cacld: 14552.0 Da.

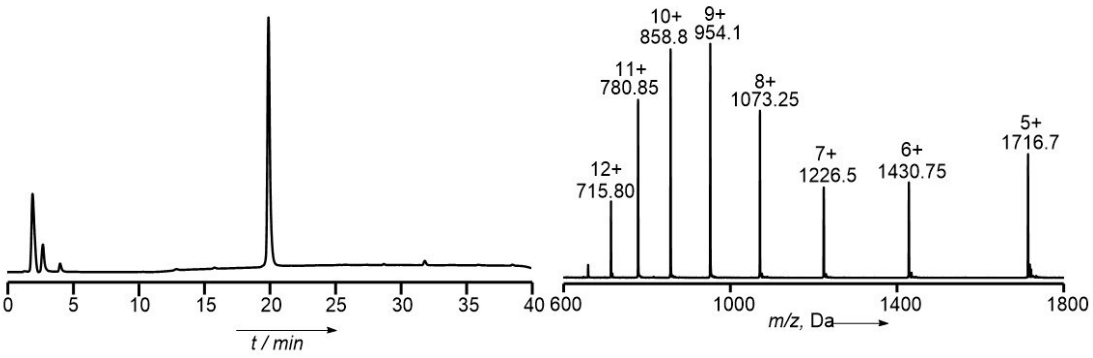

Figure. S4 Characterization of segment 3. The RP-HPLC (λ=214 nm) and ESI-MS (average isotope) analysis of **3**, observed: 8578.2 Da; cacld: 8578.8 Da.

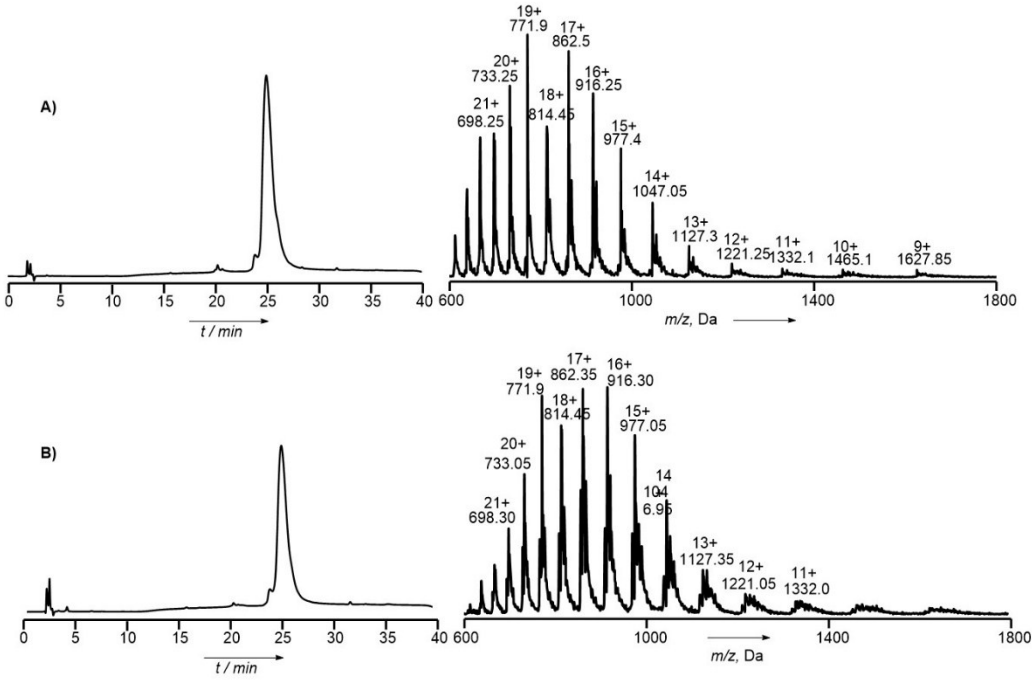

Figure. S5 Characterization of segments 4 and 4'. (A) The RP-HPLC (λ=214 nm) and ESI-MS (average isotope) analysis of **4**, observed: 14643.7 Da; cacld: 14641.2 Da. (B) The RP-HPLC (λ=214 nm) and ESI-MS (average isotope) analysis of **4'**, observed: 14642.2 Da; cacld: 14641.2 Da.

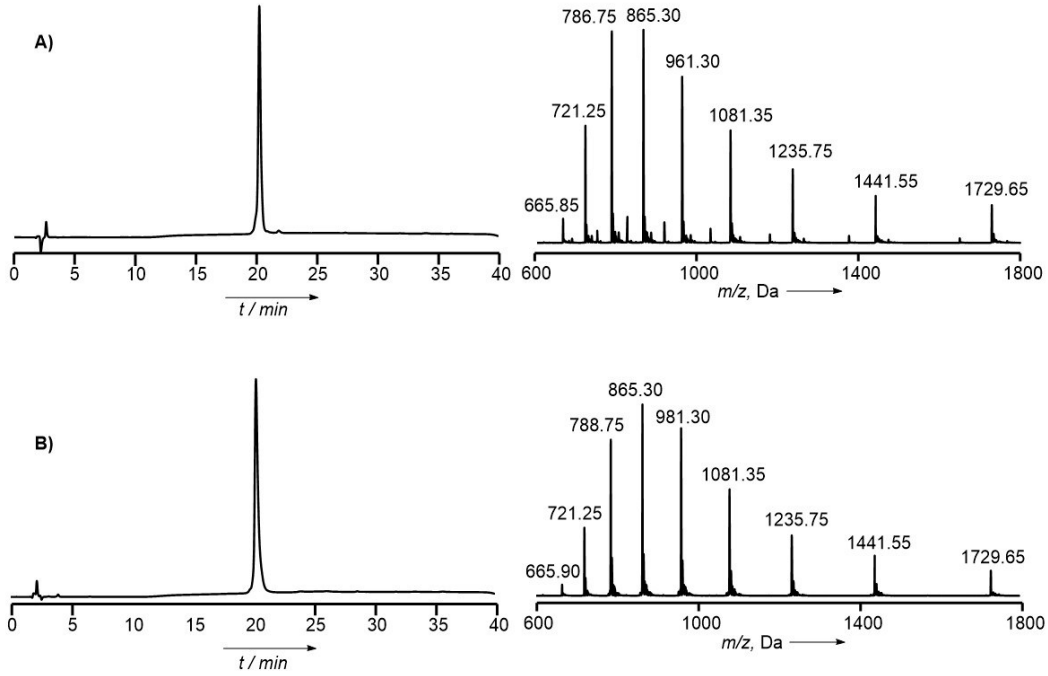

Figure. S6 Characterization of segments 2 and 11. (A) The RP-HPLC (λ=214 nm) and ESI-MS (average isotope) analysis of **2**, observed: 8643.3 Da; cacld: 8643.3 Da. (B) The RP-HPLC (λ=214 nm) and ESI-MS (average isotope) analysis of **11**, observed: 8642.8 Da; cacld: 8643.3 Da.

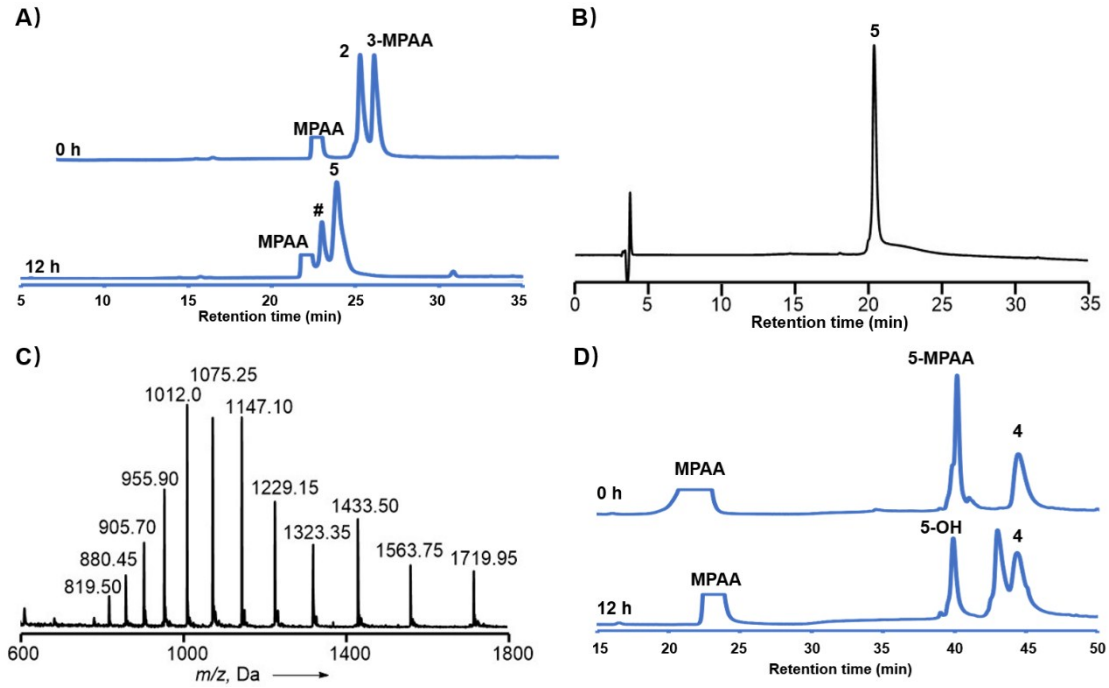

Figure. S7 Synthesis of H2AK13$_C$diUb^{K27C} (A) The HPLC traces (λ=214 nm) of the ligation between **2** and **3**, isolated yield: 55%. (B) The RP-HPLC analysis of purified **5**. (C) The ESI-MS (average isotope) analysis of **5**, observed: 17192.9 Da; cacld: 17190.1 Da. (D) The HPLC traces (λ=214 nm) of the ligation between **5** and **4**, isolated yield: 30%.

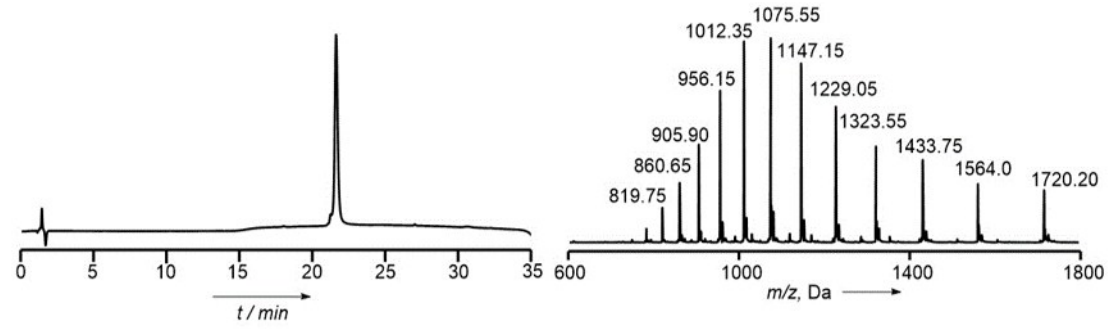

Figure. S8 Characterization of diUb^{K48C}. The RP-HPLC (λ=214 nm) and ESI-MS (average isotope) analysis of **diUb^{K48C}**, observed: 17192.9 Da; cacld: 17190.1 Da.

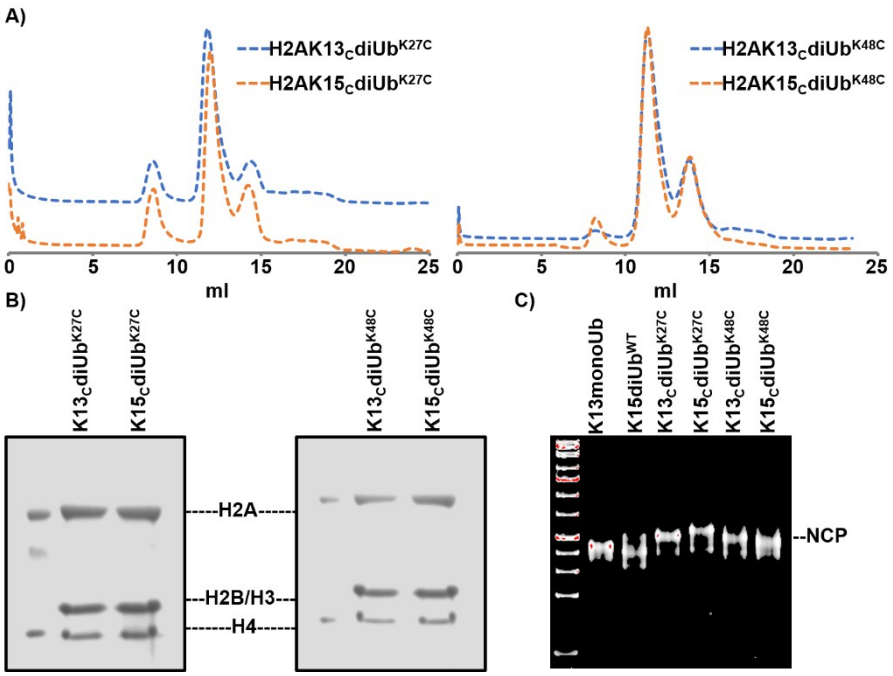

Figure. S9 Reconstitution of di-ubiquitinated H2A-containing octamers and NCPs. (A) Size-exclusion chromatography of octamers containing certain di-ubiquitinated H2A. (B) Purified octamers were analyzed by SDS-PAGE analysis and stained with Coomassie Brilliant Blue. (C) Native gel analysis of reconstituted NCPs.

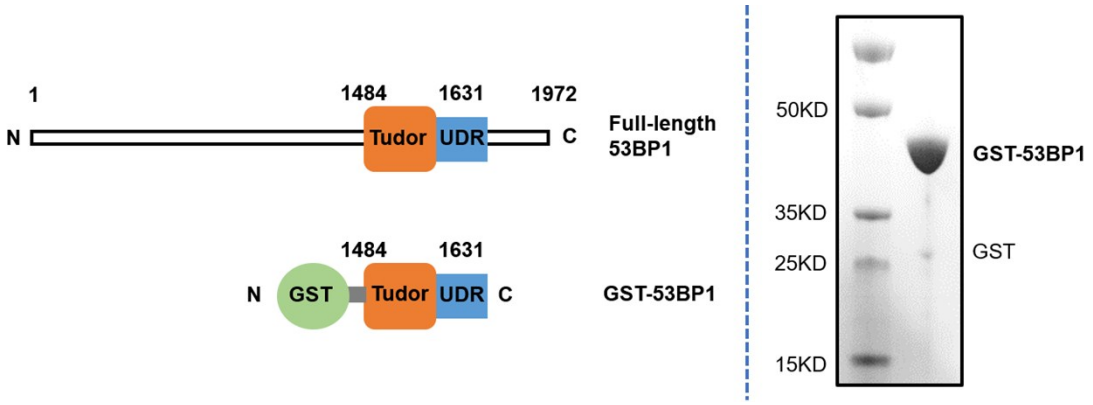

Figure. S10 Expression of GST-53BP1. Left is the schematic representation of full-length 53BP1 and GST-53BP1 construct consisting of Tudor and UDR domains used in this study. Right is the SDS-PAGE analysis of recombinant GST-53BP1.

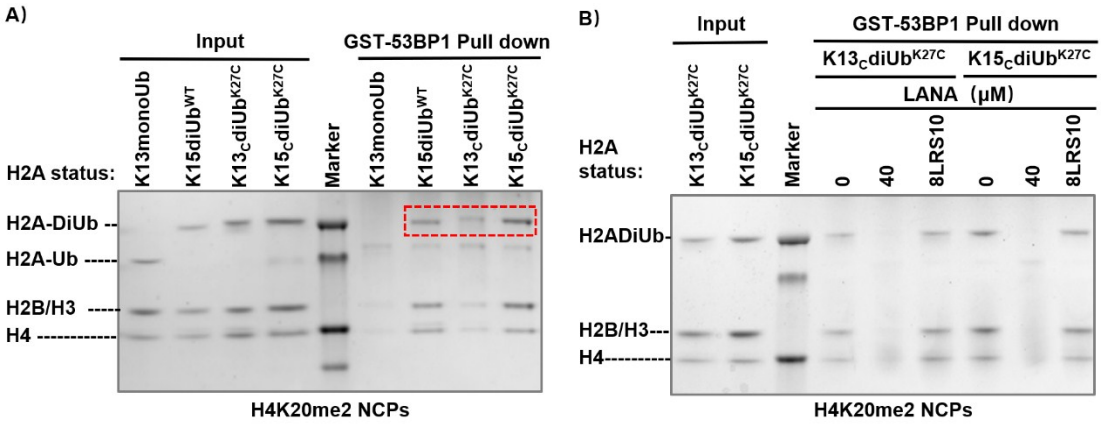

Figure. S11 Pull-down assays of NCPs containing K27-linked di-ubiquitin modified H2As. (A) H2AK13$_C$diUb^{K27C} and H2AK15$_C$diUb^{K27C} were pulled down by GST-53BP1 and stained with Coomassie brilliant blue. H2AK13monoUb and H2AK15diUb^{WT} were used as a negative control and a positive control respectively. (B) Pull-down assays in the presence of LANA peptide (Sequence: MAPPGMRLRSGRSTGAPLTRGSY) or its mutant 8LRS10, stained with Coomassie brilliant blue.

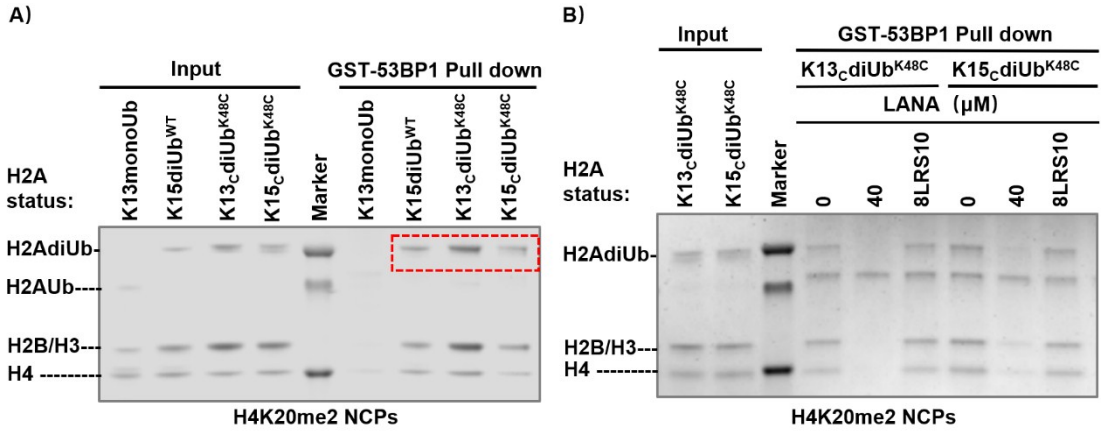

Figure. S12 GST-53BP1 Pull-down assays of NCPs containing K48-linked di-ubiquitin modified H2As. (A) H2AK13$_C$diUb^{K48C} and H2AK15$_C$diUb^{K48C} were pulled down by GST-53BP1 and stained with Coomassie brilliant blue. H2AK13monoUb and H2AK15diUb^{WT} were used as a negative control and a positive control respectively. (B) Pull-down assays in the presence of LANA peptide or its mutant 8LRS10, stained with Coomassie brilliant blue.

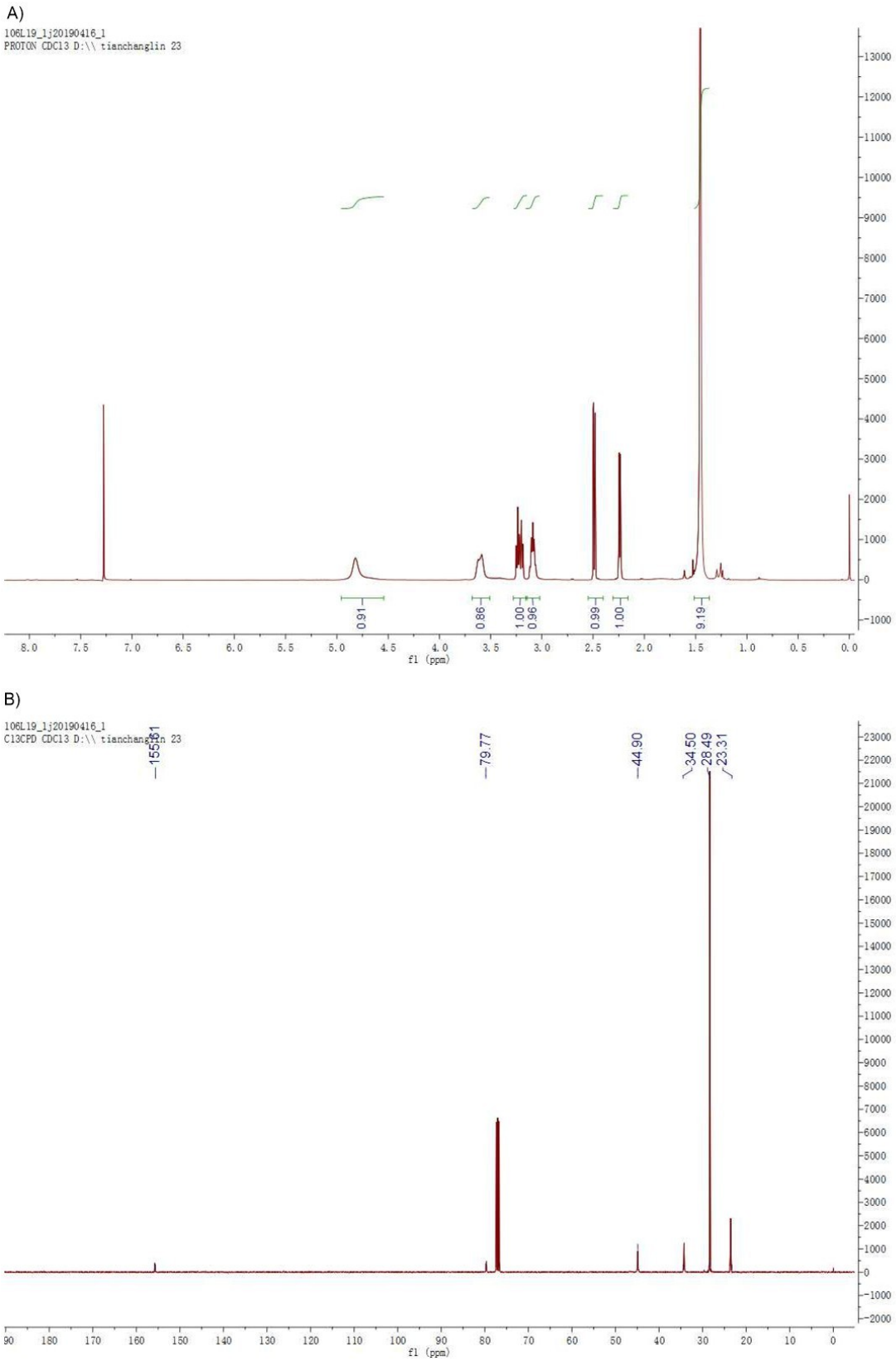

Figure S13. A) The ^{1}H NMR spectra of **a**. B) ^{13}C NMR spectrum of **a**.

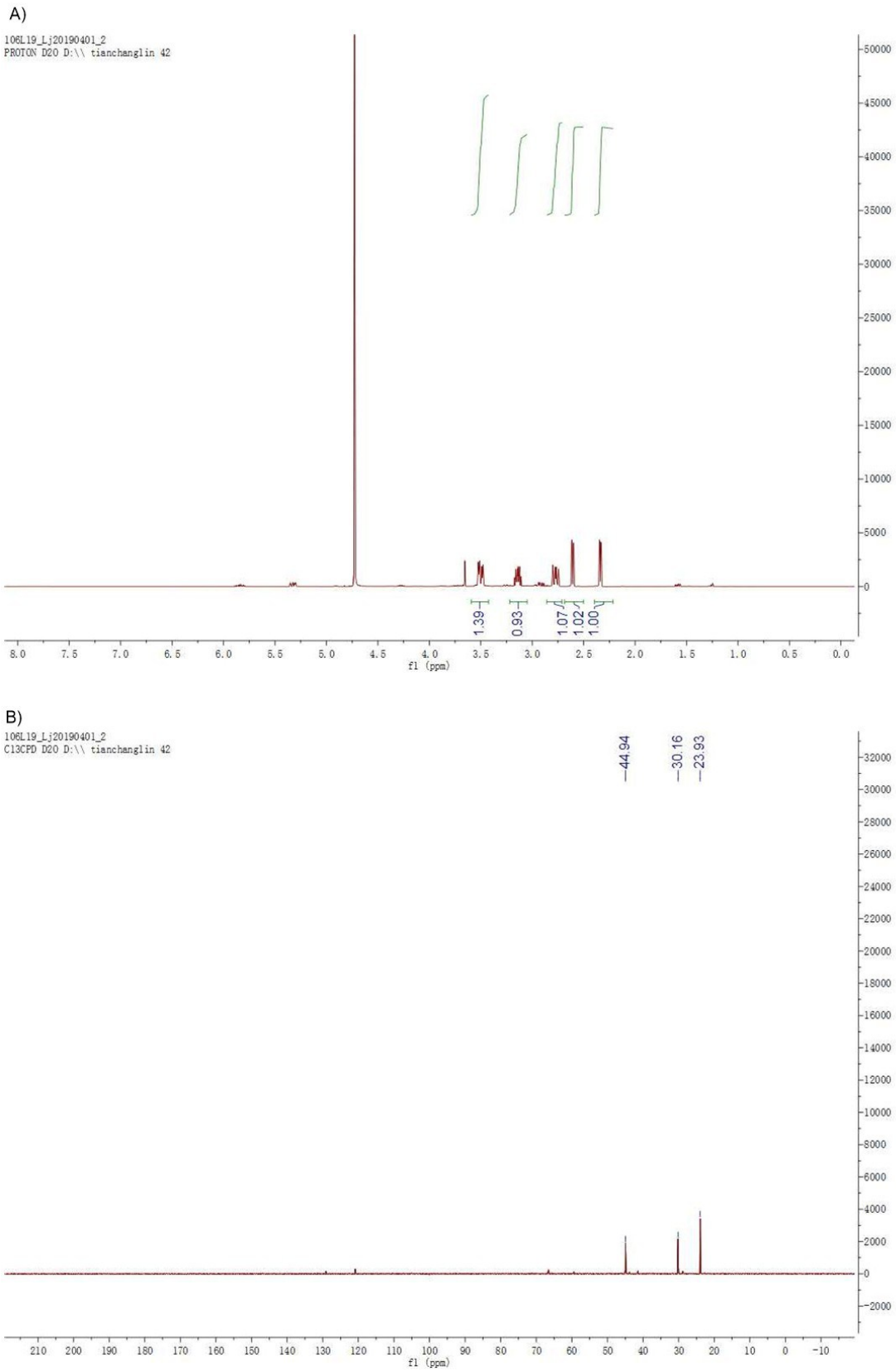

Figure S14. A) The ^{1}H NMR spectra of **1**. B) ^{13}C NMR spectrum of **1**.