Supporting Information for

Deciphering endogenous SUMO-1 landscape: a novel combinatorial peptide enrichment strategy for global profiling and disease association

Xiaoyu Zhang[#a], Bowen Zhong[#a, e], Yue Sun[a], Dan Liu[a], Xiancheng Zhang[a], Dongdong Wang[a], Cunli Wang[a], Huiling Gao[c], Manli Zhong[c], Haijuan Qin[d], Yang Chen[a], Zhiying Yang[a, b], Yan Li[a], Haijie Wei[a, b], Xindi Yang[a], Yukui Zhang[a], Bo Jiang*[a], Lihua Zhang*[a] and Guangyan Qing*[a]

[a]State Key Laboratory of Medical Proteomics, National Chromatographic R. & A. Center, CAS Key Laboratory of Separation Science for Analytical Chemistry, Dalian Institute of Chemical Physics, Chinese Academy of Sciences, Dalian 116023, P. R. China

[b]University of Chinese Academy of Sciences, Beijing 100039, P. R. China

[c]College of Life and Health Sciences, Northeastern University, Shenyang 110819, P. R. China

[d]Research Centre of Modern Analytical Technology, Tianjin University of Science and Technology, Tianjin 300000, P. R. China

[e]School of Chemistry and Materials Science, University of Science and Technology of China, Hefei 230026, P. R. China

[#]These authors contributed equally to this work.

*Corresponding authors: jiangbo@dicp.ac.cn (B.J.), lihuazhang@dicp.ac.cn (L.Z.), qinggy@dicp.ac.cn. (G.Q.)

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1. Experimental Details

1.1 Materials and Instruments

Materials. Unless specified, all chemicals were analytical-grade reagents obtained from commercial suppliers. Phage display peptide libraries and Escherichia coli ER2738 host cells were obtained from New England Biolabs (Ipswich, MA, USA). Peptides were synthesized by China Peptides Co., Ltd (Suzhou, P. R. China) and Synpeptide Co., Ltd. (Nanjing, P. R. China), purified using high-performance liquid chromatography with a C18 reversed phase column (> 95% purity). Peptides (KLRWTDYAKKRK) labeled with fluorescein amidite (FAM) at the N-terminus were used. Amino bonded silica microspheres (average particle size: 5 μm, average pore diameter: 300 Å; Chromatorex, NH2 SPS300-5) was purchased from Fuji Silysia Chemical Ltd. (Japan). 4-cyano-4-(phenylcarbonothioylthio)pentanoic acid (CPPA, > 97%) was obtained from Macklin Inc. (Shanghai, P. R. China). Glycidyl methacrylate (GMA, 97%), azobisisobutyronitrile (AIBN, 99%), *N,N*′-dicyclohexylcarbodiimide (DCC, 99%), 1- (3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC), *N*-hydroxysuccinimide (NHS, 98%), (3-aminopropyl)triethoxysilane (APTES, 99%), and *N,N*-dimethylformamide (DMF, AR) were purchased from Aladdin (Shanghai, P. R. China). Reagents including poly(ethylene glycol)methylether acrylate (PEGMEA, average M_n 480), bovine serum albumin (BSA), protease inhibitor cocktail, proteasomal inhibitor MG132, urea, dithiothreitol (DTT), iodoacetamide (IAA) and formic acid (FA) were sourced from Sigma-Aldrich (St. Louis, MO, U.S.A.). MEM, DMEM, Fetal Bovine Serum (FBS), and penicillin/streptomycin were purchased from Gibco Thermo fisher scientific Co., Ltd. (China). Trypsin, Asp-N, and Glu-C were procured from Promega (Madison, WI, U.S.A.). Acetonitrile (ACN, HPLC grade) and trifluoroacetic acid (TFA, HPLC grade) were acquired from Merck (Darmstadt, Germany). The Oasis HLB 6 cc Vac Cartridge (200 mg Sorbent per Cartridge, 30 mm) was obtained from Waters (Milford, MA, U.S.A.). Durashell C18 particles and XBP C18 particles (5 mm, 100 Å pore) were sourced from Bona-Agela Technologies (Tianjin, P. R. China). Fused-silica capillaries (150 mm i.d./360 mm o.d.) were purchased from Sino Sumtech (Hebei, P. R. China). Deionized water underwent purification through a Milli-Q

system from Millipore (Milford, MA, U.S.A.).

Instruments. Absorbance was recorded by an ultraviolet spectrophotometer (Multiskan FC, Thermo Fisher Scientific, U. S. A.). Biolayer interferometry (BLI) data were obtained on an Octet K2 System (FortéBio, U. S. A.). Isothermal titration microcalorimetry (ITC) experiments were conducted on a MicroCal PEAQ-ITC (Malvern Panalytical Ltd., UK). ^{1}H and ^{13}C NMR spectra were recorded on a Brüker 400 M Ultra Shield spectrometer. Thermo-gravimetric analysis (TGA) was performed under N2 atmosphere at a heating rate of 20 $K \cdot min^{-1}$; X-ray photoelectron spectroscopy (XPS) spectra of the samples were obtained using an XPS equipped with an Al Ka X-ray source (XSAM800, Kratos Analytical); Scanning electron microscopy (SEM) was carried out on HITACHI FlexSEM 1000. LC-MS/MS samples were analyzed using an Orbitrap Fusion Lumos Tribrid mass spectrometer (Thermo Fisher, Waltham, MA, USA) coupled with an EASY-nLC 1200 (Thermo Fisher, Waltham, MA, USA). MALDI-TOF MS analysis were conducted using the Bruker Ultraflex III MALDI-TOF/TOF MS instrument (Bruker Daltonics, Billerica, MA, U. S. A.). Cell imaging data were obtained on the FV1000MPE two-photon confocal microscope system (Olympus, Japan). The frozen brain samples were sectioned using a cryostat microtome (Leica CM1860, Leica Microsystems, Wetzlar, Germany). MALDI-MSI experiments were performed using a MALDI-TOF/TOF (Ultraflex III, Bruker Daltonics) mass spectrometer equipped with a Smartbeam II 2 kHz laser and operated in positive-ion mode.

1.2 Identification of peptide ligands targeting the C-terminus of SUMO-1 remnant by phage display

For DV12, dodecapeptide (Ph.D.-12) library was used to screen against DV12 through a complete biopanning procedure. Firstly, to minimize nonspecific binding, an initial negative screening with BSA was performed. Briefly, 100 μL of 0.5% BSA solution was incubated overnight at 4°C in 96-well plates, followed by six washes with 0.01% TBST. Subsequently, the plate was exposed to 100 μL of the original phage library (~1×10^{12} plaque-forming units, PFUs). After 30 minutes of

shaking at room temperature, the supernatant was collected and used to infect E. coli ER2738 cells for amplification as per the manufacturer's instructions. Then, four rounds of affinity screening against DV12 were conducted. 100 μL of DV12 (200 μg/mL) was added to a 96-well plate (100 μL/well), followed by overnight incubation at 4°C. The plate was washed three times with 0.01% TBST solution and blocked with 0.05% BSA solution at 4°C for 1 h. After blocking, 100 μL of phage broth (from the previous BSA negative screening step, titer approximately 10^{10} pfu/mL) was added to the plate and incubated for 30 min (25°C, 100 rpm). After a quick wash with TBST, 100 μL of 0.2 M glycine-HCl buffer (pH 2.2) was added and shaken for 10 min (25 °C, 200 rpm). The eluate phages were collected, neutralized with 1 M Tris-HCl (pH 9.1), and stored at –20°C with glycerol for further use. The titer was then measured and expanded to the desired titer for subsequent screening rounds. Besides, in the third and fourth rounds, washing with 0.05% TBST was performed to enhance stringency and improve biopanning affinity.

For QTGG, a loop constrained heptapeptide (Ph.D.-C7C) library was used to screen against QTGG. The biopanning procedures were the same as described above. One round of negative screening with BSA and three rounds of affinity screening against QTGG was performed.

After completing all biopanning procedures, 16 well-isolated phage clones from the last round of DV12 screening, as well as 10 well-isolated phages from the thirds round of QTGG screening, were randomly selected for DNA sequencing.[1] Phage DNA was extracted by using M13 single-stranded DNA extraction kit (BioTeke, China) and DNA sequencing was entrusted to Shanghai Sangon Biotech Corporation (Shanghai, P. R. China).

1.3 Peptide binding affinity assay

1.3.1 Enzyme-linked immunosorbent assay (ELISA)

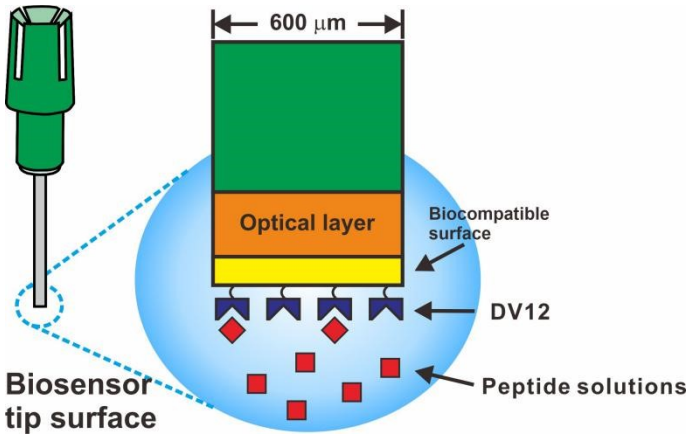
Phage ELISA was first used to evaluate the binding affinity of the identified peptides with DV12 or QTGG.[2] The ELISA plate coated with target (20 μg/mL) was gently shaken overnight at 4°C. Then, 0.05% BSA blocking buffer was added and incubated for 1 h. After washing with 0.05% TBST, phage monoclones which bearing identified peptides were added to each well and

incubated at room temperature for 1 h. Subsequently, the plate was incubated with HRP-conjugated anti-M13 monoclonal antibody (dilution 1:5000) for 1 h, followed by 0.05% TBST. Finally, according to the EL-ABTS (C510031, Sangon Biotech, P. R. China) manufacturer's instructions, the reaction buffer and the stop buffer were added. Absorbance was measured at 405 nm recorded by an ultraviolet spectrophotometer (Multiskan FC, Thermo Fisher Scientific, U. S. A.).

1.3.2 Biolayer interferometry (BLI)

Binding affinity between DV12 and the peptides was measured by biolayer interferometry (BLI) on an Octet K2 System (FortéBio, U. S. A.). Briefly, DV12 was immobilized onto the surface of amine reactive 2nd -generation (AR2G) biosensors through standard EDC-catalyzed amide bond formation to create a covalent bond between the *N*-terminal amine on DV12 and the carboxy-terminated biosensor surface following the manufacturer's instructions. After the activation and immobilization, the excess NHS esters on the biosensor surface were quenched using ethanolamine to minimize non-specific interactions further. A series of peptides solutions with different concentrations were prepared, and DV12 modified-biosensor was exposed to these peptides solutions individually (Scheme 1), each step was followed by dissociation in ultrapure water. Moreover, for binding study between SUMO-1 protein and PEP-4, NTA biosensor was employed to immobilize human SUMO-1 protein (His tag) (13095-H07E, Sino Biological) through the His tag, subsequently, the protein-modified biosensor was exposed to different concentrations of PEP-4 solutions.

The kinetics of association and dissociation were recorded, and the data were analyzed to determine association rate constants (K_{on}), dissociation rate constants (K_{off}), and equilibrium dissociation constants (K_D) using the software provided by FortéBio (Data Analysis 11.0). A reference biosensor was run with an assay buffer blank without peptides for the association and dissociation steps. Reference subtraction was performed before data analysis to subtract out background dissociation, ensuring accurate calculation of kinetic constants.[3]



Scheme 1. Covalent immobilization of DV12 on AR2G biosensors with subsequent analyte binding. After activation, immobilization and quenching, the kinetics of association and dissociation between the immobilized ligand and analyte are measured.

1.3.3 Isothermal titration calorimetry (ITC)

Isothermal titration calorimetry (ITC) analysis was employed to investigate the interaction of QTGG with corresponding peptides. All ITC titrations were performed at 25ºC using a Microcal PEAQ-ITC system (Malvern Panalytical Ltd., UK). QTGG solution (5 mM) was loaded into the syringe and titrated into the calorimetric cell containing different peptide solutions (0.1 mM). The reference cell was filled with Milli-Q water. The titration sequence consisted of a single 0.4 μL injection followed by a series of 2 μL solution, with a time interval of 120 s between injections to ensure that the thermal power returns to the baseline before the next injection. The stirring speed was 750 rpm. The exothermic heat value was analyzed by using Origin7.0 software package (OriginLab, Northampton, MA). Peptides have multiple identical binding sites for QTGG. The obtained association constants could be adequately used for the qualitative analysis and comparison of the binding of QTGG with different peptides.

1.4 Molecular docking

Molecular docking was conducted using AutoDock 4.2.[4] to analyze the interactions between

PEP-4 and DV12 as well as CP-1 with QTGG. Models of the peptides were constructed using the educational version of PyMOL 2.3 software and preprocessed with AutoDock. Semi-flexible docking was performed, and the binding model with the minimum energy was selected based on the scoring function. A maximum of 20 conformations were generated by molecular docking, employing the Lamarckian Genetic Algorithm (LGA) for global search. Subsequently, PyMOL was utilized for in-depth analysis to identify the key residues involved in the interactions between PEP-4 and DV12 or CP-1 with QTGG.

1.5 NMR titration experiments

Two-dimension NMR spectra were used to study the interaction between PEP-4 and DV12, as well as CP-1 with QTGG at 25°C with DMSO-d_6 as solvent. ^{1}H, ^{13}C, ^{13}C–^{1}H (heteronuclear singular quantum correlation) HSQC, and ^{1}H–^{1}H (correlation spectroscopy) COZY NMR experiments were performed to validate the chemical shift attribution of each H proton and C atom of the peptides. Subsequently, equimolar concentrations (20 mM) of PEP-4 and DV12, or CP-1 and QTGG, were mixed and tested under the same conditions. The chemical shift variation were recorded and analyzed in order to discover possible binding sites between the peptide and its respective target.[5]

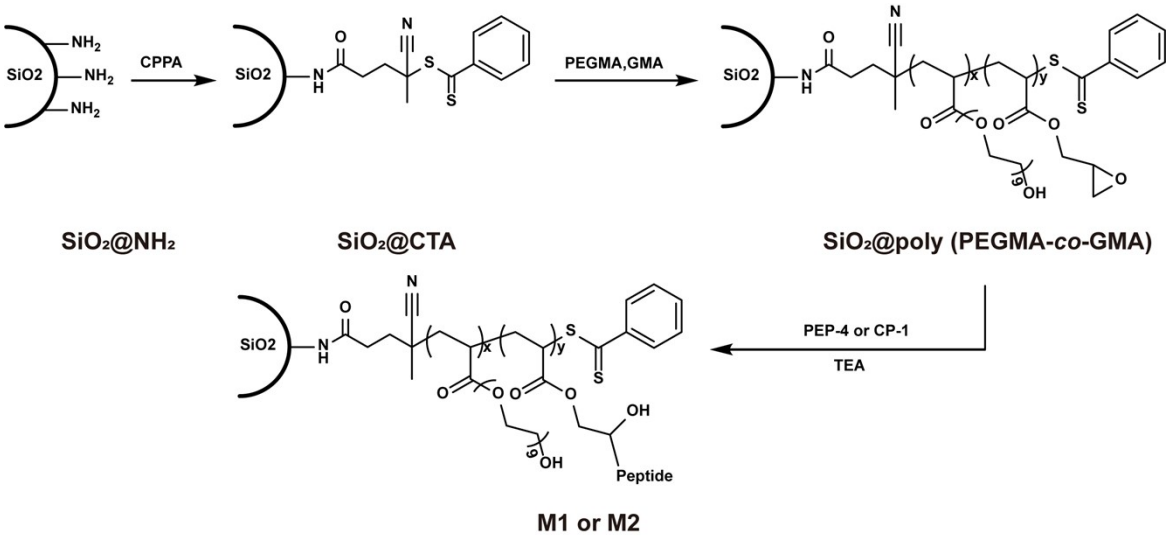
1.6 Preparation and characterization of M1 and M2 enrichment materials

First, through surface-initiated reversible addition−fragmentation chain transfer (SI-RAFT) polymerization, poly(PEGMEA-*co*-GMA) was grafted on a porous silica microsphere substrate. PEP-4 or CP-1 modification through the ring-opening reaction with the epoxy groups of GMA to establish stable covalent linkages (Scheme 2).[6]

Firstly, a RAFT chain transfer agent was introduced onto the surface of the silica microspheres: CPPA (100 mg, 0.35 mmol), DCC (333 mg, 1.61 mmol), and NHS (192 mg, 1.67 mmol) were dissolved in 20 mL of DMF. The solution was stirred at 30 °C for 2 h. Then, 300 mg SiO_2@NH_2 (average particle size: 5 μm, average pore diameter: 300 Å) suspended in 15 mL of DMF was

added, and the suspension was stirred at 30 °C for 48 h, yielding RAFT chain transfer agent-modified silica microspheres (SiO_2@CTA). SiO_2@CTA was collected by centrifugation at 8000 rpm for 10 min, washed with DMF, CH_3OH, and ultrapure water several times, and dried via vacuum freeze-drying.

Following this, GMA (355 mg, 2.5 mmol), PEGMEA (1.2 g, 2.5 mmol), and AIBN (0.41 mg, 2.5 μmol) were dissolved in 50 mL of DMF. To this solution, 50 mg of SiO_2@CTA was added, and the mixture was stirred at 65 °C for 24 h after three freeze−pump−thaw cycles, resulting in poly(PEGMEA-*co*-GMA) grafted silica microspheres, denoted as SiO_2@poly(PEGMEA-*co*-GMA). Multiple reactions could be performed in parallel.



Scheme 2. Preparation procedure of **M1** and **M2**.

The SiO_2@poly(PEGMEA-*co*-GMA) was collected by centrifugation at 8000 rpm for 10 min, washed with DMF and ultrapure water several times, and dried via vacuum freeze-drying. Finally, 100 mg of SiO_2@poly(PEGMEA-*co*-GMA) was suspended in 10 mL of PEP-4 or CP-1 peptide solution (2 mg/mL in ultrapure water), and 10 μL triethylamine (TEA) was added. TEA served as a catalyst to promote the open-ring reaction between the GMA and the peptide. The suspension was stirred at 45 °C for 48 h to graft PEP-4 or CP-1 onto the surface of the silica microspheres, referred to as **M1** and **M2**, respectively. Following this, **M1** or **M2** was collected by centrifugation

at 8000 rpm for 10 min, washed with ultrapure water several times, and dried via vacuum freeze-drying.

Thermo-gravimetric analysis (TGA) was performed under N_2 atmosphere at a heating rate of 20 $K \cdot min^{-1}$; X-ray photoelectron spectroscopy (XPS) spectra of the samples were obtained using an XPS equipped with an Al Ka X-ray source (XSAM800, Kratos Analytical); Solid ^{13}C NMR spectra were recorded on a Brüker AVABCE NEO 400 M spectrometer; Scanning electron microscopy (SEM) was carried out on HITACHI FlexSEM 1000. Water contact angle (WCA) analysis was used to assess wettability changes after each reaction step. The RAFT chain transfer agent of was introduced on the silicon wafer instead of silica microspheres. Then the WCAs of the surfaces after each reaction step were recorded.

1.7 Enrichment ability of M1 and M2 toward SUMO-1 modified peptides

To assess the performance of the material in enriching SUMO-1 modified peptides, we synthesized two standard SUMOylated peptide: P1 (LLVHMGLL**K**(GGTQEQYVEIVD)SE) and P2 (LLVHMGLL**K**(GGTQ)SE). Using 1 mg **M1** material to enrich 2 μg P1. The enrichment process was conducted overnight. The material underwent a single wash with water, followed by stepwise elution using 10%, 20%, 30%, and 80% ACN, respectively. The resulting peptides were then dried using a SpeedVac. For the enrichment using **M2** material, the same enrichment steps were carried out accordingly.

1.8 MALDI-TOF MS analysis

1 μL of the collected peptides and 1 μL CHCA (7 mg/mL, in 60% ACN, 0.2% TFA) were successively dry-dropped onto a MALDI plate. All experiments were conducted using the Bruker Ultraflex III MALDI-TOF/TOF MS instrument (Bruker Daltonics, Billerica, MA, U. S. A.). Spectra were acquired in a pulsed smart beam laser positive ionization mode with reflector detection.

1.9 Cell culture

HeLa cells were purchased from American Type Culture Collection (ATCC) and cultured in MEM supplemented with 10% FBS, 100 units/mL penicillin, and 100 mg/mL streptomycin at 37°C under 5% CO_2. For MG132-stimulated samples, cells were subjected to proteasome inhibition by adding 10 mM MG132 for 8 hours. SHSY5Y cells were obtained from Shanghai (China) and cultured in DMEM supplemented with 10% FBS and 1% penicillin/streptomycin in 5% CO_2 at 37°C.

1.10 Lysis of cells

Following the treatment, the growth medium was discarded, and cells underwent an immediate rinse with ice-cold PBS. Subsequently, cells were gently scraped at 4 °C, pelleted by centrifugation at 350 g for 5 minutes, and washed again with ice-cold PBS before a second round of centrifugation. The collected cells were lysed in a solution containing 8 M urea, 1% (v/v) protease inhibitor cocktail, 50 mM NH_4HCO_3 (pH 8.0), and sonicated on ice for 120 seconds (5 seconds pulse on time, 5 seconds pulse off time). Following the sonication, the cellular debris was eliminated through centrifugation at 16,000 g at 4 °C for 10 minutes. The protein concentration was determined using BCA protein assay kits from Bio-Rad (Hercules, CA, U. S. A.).

1.11 Mice

All animal procedures were conducted in accordance with the Guidelines for Care and Use of Laboratory Animals of Northeastern University and approved by the Animal Ethics Committee of College of Life and Health Sciences of Northeastern University. Tau P301S transgenic mice [B6C3-Tg (PrnpMAPT*P301S) PS19 Vle/J, AD model] were originally purchased from the Jackson laboratory (ME, U. S. A.) and C57BL/6 wild type mice were obtained from Beijing HuaFuKang Bioscience Co., Ltd. (Beijing, P. R. China). When the mice grew to 9 months old, all mice were euthanized and the whole brain was promptly excised and snap-frozen in liquid nitrogen. The samples were then transferred to −80°C and stored until further processing.

1.12 Extraction of proteins from mouse brain tissues

Mouse brain tissue samples obtained from Tau P301S transgenic mice and C57BL/6 wild-type mice were separately lysed in a buffer that consisted of 8 M urea, 1% (v/v) protease inhibitor cocktail, 50 mM NH_4HCO_3 (pH 8.0) for 10 minutes. The preliminary lysates were sonicated on ice for 240 seconds (5 seconds pulse on time, 5 seconds pulse off time). Following the sonication, the lysates were centrifuged at 16,000 g for 10 minutes at 4 °C, and the supernatants were reserved. The protein concentration in each sample was determined using BCA protein assay kits.

1.13 Protein lysate digestion

The samples preparation protocol is the same for cell line and tissue samples. In brief, the extracted protein samples (40 mg, recommended initial protein amount) were reduced with 20 mM DTT at 56 °C for 30 minutes. Subsequently, the reduced cysteine residues were alkylated with 40 mM IAA for 30 minutes at room temperature in darkness. Following this, the urea buffer was diluted to 1 M by adding 50 mM NH_4HCO_3, and the tryptic digestion was conducted with a trypsin/protein ratio (m/m) of 1:40 at 37 °C for 12 hours.

1.14 Second-stage digestion and SUMOylated peptide enrichment

The samples preparation protocol is the same for cell line and tissue samples. In brief, the extracted protein samples (40 mg) were reduced with 20 mM DTT at 56 °C for 30 minutes. Subsequently, the reduced cysteine residues were alkylated with 40 mM IAA for 30 minutes at room temperature in darkness. Following this, the urea buffer was diluted to 1 M by adding 50 mM NH_4HCO_3, and the tryptic digestion was conducted with a trypsin/protein ratio (m/m) of 1:40 at 37 °C for 12 hours. For **M1** enrichment method, tryptic digested peptides were purified utilizing Oasis HLB cartridges following the manufacturer's guidelines, employing 200 mg of sorbent per 10 mg of digested protein. The obtained peptides were subsequently dried using a SpeedVac. The lyophilized peptides were reconstituted in 4 mL ultrapure water, and an enrichment process was carried out with **M1** at a ratio of 1:1 (material/peptide, m/m), and incubated overnight.

Subsequently, the material underwent a single wash with water. Following this, the material was eluted using 80% ACN, and the obtained peptides were then dried using a SpeedVac. Then, the lyophilized peptides were once again reconstituted with 200 µL, 50 mM NH_4HCO_3 (pH 8.0). Glu-C enzyme was added to the peptides with a Glu-C /peptide ratio (m/m) of 1:40, and the mixture was incubated at 37 °C for 12 hours. Finally, the peptides were acidified with formic acid (FA), and the peptides were subsequently dried using a SpeedVac.

For **M2** enrichment method, Glu-C enzyme was added to the tryptic digested peptides at an enzyme ratio of 40:1, and the mixture was incubated at 37 °C for 12 hours. The digested peptides were purified utilizing Oasis HLB cartridges, and the obtained peptides were subsequently dried using a SpeedVac. The lyophilized peptides were reconstituted in 4 mL ultrapure water, and an enrichment process was conducted using **M2** at a ratio of 1:1 (material/peptide, m/m) and incubated overnight. Subsequently, the material underwent a single wash with water. SUMOylated peptides were eluted using 80% ACN, and the eluted peptides were then dried using a SpeedVac.

For the two-step sequential enrichment method, the first tryptic digestion was conducted with a trypsin/protein ratio (m/m) of 1:40 at 37 °C for 12 hours. The tryptic digested peptides were purified utilizing Oasis HLB cartridges and the obtained peptides were subsequently dried using a SpeedVac. The lyophilized peptides were reconstituted in 4 mL ultrapure water, and an enrichment process was carried out with **M1** at a ratio of 1:1 (material/peptide, m/m), and incubated overnight. Subsequently, the material underwent a single wash with water. Following this, the material was eluted using 80% ACN, and the obtained peptides were then dried using a SpeedVac. Then, the lyophilized peptides were once again reconstituted with 200 µL, 50 mM NH_4HCO_3 (pH 8.0). The peptides underwent trypsin digestion with an enzyme ratio of 40:1, and the mixture was incubated at 37 °C for 8 hours. Subsequently, Glu-C enzyme was added to the tryptic-digested peptides with a Glu-C/peptide ratio (m/m) of 1:40, and the mixture underwent a subsequent incubation at 37 °C for 12 hours. Then, the digested peptides were purified utilizing Oasis HLB cartridges and dried using a SpeedVac. Following this, the lyophilized peptides were reconstituted in 4 mL ultrapure, and an enrichment process was conducted using **M2** at a ratio of

1:1 (material/peptide, m/m) and incubated overnight, after which the material underwent a single wash with water. SUMOylated peptides were eluted using 80% ACN, and the eluted peptides were then dried using a SpeedVac.

1.15 High-pH fractionation

Following the enrichment, the collected SUMOylated peptides were reconstituted in a 10 mM ammonium bicarbonate buffer (pH 10.0) and then subjected to small-scale reversed-phase (sRP) chromatography using a custom-made C18 column. The peptides were fractionated into twelve fractions by stepwise increasing ACN from 6 to 50% under basic conditions (pH 10.0). These fractions were subsequently combined into six or nine samples, vacuum-dried, and stored at –80 °C until later use for liquid chromatography tandem mass spectrometry (LC-MS/MS) analysis.

1.16 LC-MS/MS analysis

Samples were analyzed using an Orbitrap Fusion Lumos Tribrid mass spectrometer (Thermo Fisher, Waltham, MA, USA) coupled with an EASY-nLC 1200 (Thermo Fisher, Waltham, MA, USA). Fractions were loaded onto and separated by a C18 capillary analytical column (150 mm i.d. x 15 cm), packed in-house with XBP C18 silica particles. The mobile phase for solvent A consisted of H_2O with 2% ACN and 0.1% FA, and solvent B comprised ACN with 20% H_2O and 0.1% FA. The column elution employed various gradients, tailored to the distinct properties of peptides in high-pH fractions. The effective gradient times were set at 65, 75, and 90 minutes, with values of the increasing solvent B ranging from 5% to 45%. Following the effective gradient time, a continuous increase to 95% B over 2 minutes occurred, maintained for 8 minutes at a constant flow rate of 600 nL/min. The spray voltage was set at 2.1 kV, and the ion transfer tube temperature was maintained at 320°C. Full MS scans were conducted in the orbitrap analyzer at a mass resolution of 60,000, with an AGC target value of 4.0E5 and a maximum injection time of 50 ms. The scan ranges spanned from 350 to 1500 m/z. The most intense precursor ions were selected and isolated within a 1.6 m/z window, then fragmented by higher-energy collisional

dissociation (HCD) with a normalized collision energy of 30. Precursor ions with digests ranging from +2 to +7 were chosen for MS/MS analysis, and dynamic exclusion was set to 20 s. MS/MS spectra were acquired in the orbitrap analyzer at an enhanced scan rate, with an AGC target value of 5.0E4 and a maximum injection time of 30 ms.

1.17 Database searching

The MaxQuant software (ver. 2.0.3.0)[7] was employed to process all raw data files. Default settings were utilized, with specific exceptions and crucial configurations highlighted below. The raw files underwent searching against the human protein database retrieved from UniProt.[8] The human database was obtained on 23 August 2021 and the mouse database was acquired on 23 June 2023. For enzyme digestion, both Trypsin and Glu-C were selected, allowing a maximum of 5 missed cleavages. The main search peptide mass tolerance for precursor ions was established at 4.5 ppm, and the MS/MS-FTMS mass tolerance was set to 20 ppm. To ensure data reliability, the required false discovery rate (FDR) was set at 1% and applied to both the peptide and protein levels. Fixed modifications included carbamidomethyl of cysteines, while variable modifications comprised methionine oxidation and protein N-terminal acetylation. Additional variable modifications related to SUMO-1 modifications were defined as follows. SUMO1-Glu-C-QTGG: H21O6C13N5, monoisotopic mass 343.1492, neutral loss b2-QT, diagnostic mass remnants [b2-QT, b3-QTG]. SUMO1-pyro-QTGG: H18O6C13N4, monoisotopic mass 326.1226, neutral loss b2-pyro-QT, diagnostic mass remnants [b2-pyro-QT, b3-pyro-QTG]. SUMO1-VYQEQTGG: H54O14C37N10, monoisotopic mass 862.3821, neutral loss b6-VYQEQT, diagnostic mass remnants [b2-VY, b3-VYQ, b4-VYQE, b5-VYQEQ, b6-VYQEQT, b7-VYQEQTG]. To ensure the reliability of identified SUMO-1 sites, stringent filtering criteria were applied, requiring an Andromeda score of at least 40, an Andromeda delta score of at least 6, a localization score of at least 40, and a localization probability of at least 90%.

1.18 Bioinformatics and statistical analysis

Sequence motif analysis was conducted using WebLogo, utilizing all high-confidence peptides containing SUMO-1 modification sites. A set of 15 amino acid sequences was selected for input into the analysis. Enrichment analysis of Gene Ontology (GO) terms, including cellular component (CC), molecular function (MF), and biological process (BP), along with the Kyoto Encyclopedia of Genes and Genomes (KEGG) for proteins modified by SUMO-1, was executed using the Metascape. The screening for protein kinases and transcription factors within SUMO-1 modified proteins involved consulting the Human Kinome database and the TRRUST database.

1.19 Co-Immunoprecipitation (Co-IP)

Ulk2 IP was conducted using the Pierce™ Classic Magnetic IP/Co-IP Kit following the manufacturer's instructions (88804, Thermo Scientific). In brief, brain tissue total protein lysates from Tau P301S were incubated with anti-Ulk2 antibody (NBP3-12362, Novus Biologicals) for 2 hours at room temperature and precipitated with protein A/G magnetic beads. The eluate was electrophoretically separated on a SDS polyacrylamide gel. Proteins were subsequently transferred to PVDF membranes, followed by standard immunoblot analyses. In summary, after PVDF membrane incubation with a 5% skim milk block, blots were probed with antibodies against the following targets: SUMO-1 (67559-1-Ig, Proteintech) and Ulk2. Membranes were then incubated with HRP-conjugated secondary antibodies, and bands were detected using chemiluminescence reagent. Notes: All steps were performed on ice or at 4°C to maintain immunoprecipitation stability.

For PEP-4 material enrichment, brain tissue total protein lysates from Tau P301S were incubated with **M1** overnight before the Co-IP experiment. After a quick wash with TBST, 100 μL of 0.2 M glycine-HCl buffer (pH 2.2) was added for elution and neutralized with 1 M Tris-HCl (pH 9.1). The eluted proteins were then subjected to the aforementioned Co-IP steps.

1.20 Immunofluorescence

SHSY5Y cells were fixed in 4% formaldehyde for 10 minutes at room temperature, permeabilized

in PBS supplemented with 0.5% Triton X-100 for 10 minutes and then incubated with 1% BSA and 22.52 mg • mL^{-1} glycine in PBST (PBS + 0.1% Tween 20) for 30 min to block the non-specific binding. Immunostaining was performed for two hours at room temperature using FAM-labelled PEP-4 or anti-SUMO-1 antibody (ab32058, Abcam). Cells incubated with anti-SUMO-1 antibody were washed 3 times with PBS and incubated with goat anti-rabbit IgG Alexa 555 (A32732, Thermo Fisher Scientific) for one hour at room temperature. Cells were incubated with 1 μg • mL^{-1} DAPI staining for 5 minutes. Fluorescent signals were detected at room temperature using a laser confocal microscopy (Olympus, Japan).

1.21 MALDI mass spectrometry imaging (MSI)

The frozen brain samples were sectioned using a cryostat microtome (Leica CM1860, Leica Microsystems, Wetzlar, Germany). The sagittal brain sections were prepared with reference to Allen Brain Reference Atlases from http://atlas.brain-map.org/. The brain sections were sliced to a thickness of 10 μm at −20℃. Tissue sections were thaw-mounted onto indium tin oxide-coated (ITO) glass slides (Bruker Daltonics, Bremen, Germany) and stored at −80°C until analysis. Before PEP-4 incubation, serial parallel tissue sections were rinsed six times using the following protocol: 70% ethanol, 100% ethanol, Carnoy's fluid (60% ethanol, 30% chloroform, and 10% acetic acid), 100% ethanol, 0.2% TFA and 100% ethanol. All rinse steps were carried out for 30 s except for the step with Carnoy's solution, which was for 2 min. Following washing, ITO slides were dried in a desiccator for 20 min. Subsequently, the slides were incubated with PEP-4 (1 μg/mL, in PBS) at -4°C overnight. Afterward, the slides underwent two washes with PBS and one wash with ultrapure water, each lasting 10 minutes. Finally, three milliliters of CHCA (7 mg/mL, in 60% ACN, 0.2% TFA) was coated automatically on the brain tissue section using an automatic sprayer (ImagePrep 2.0, Bruker Daltonics). The matrix was sprayed over 30 layers (60s drying time between each layer).

MALDI-MSI experiments were performed using a MALDI-TOF/TOF (Ultraflex III, Bruker Daltonics) mass spectrometer equipped with a Smartbeam II 2 kHz laser and operated in positive-

ion mode. The laser power was optimized at the start of each run and then held constant during the MALDI–MSI experiment. The laser focus was set to large for 80 μm raster width analysis. At each sampling position, 300 shots were used to acquire data for the m/z 1000–2000 range. The methods used were externally calibrated using Peptide Calibration Standard Ⅱ (Bruker Daltonics). MALDI–MSI data were visualized using FlexImaging (Bruker Daltonics, v.4.0) and spectra was normalized against the total ion current (TIC) of all data points.

2. Supporting Figures

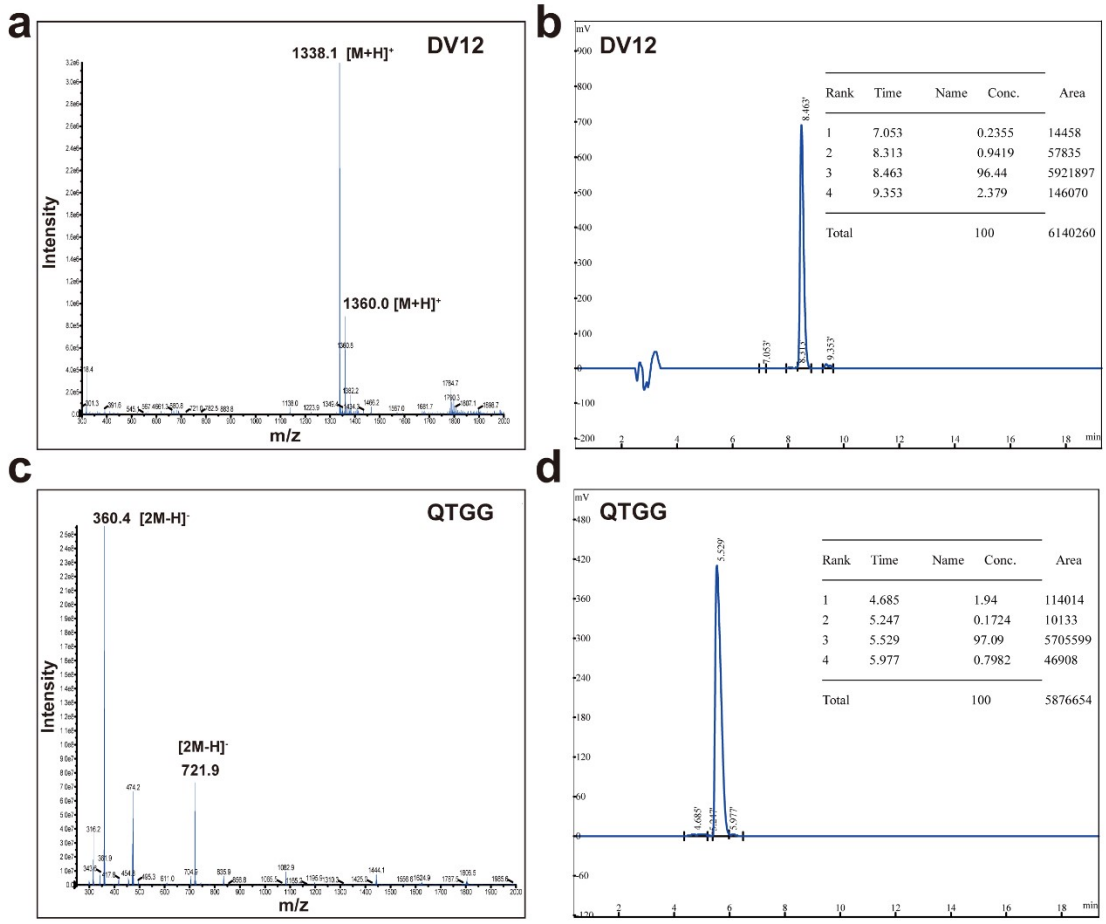


Figure S1. Mass spectra (a, c) and high-performance liquid chromatography (b, d) of DV12 and QTGG (c, d), illustrating the high purities of these peptides.

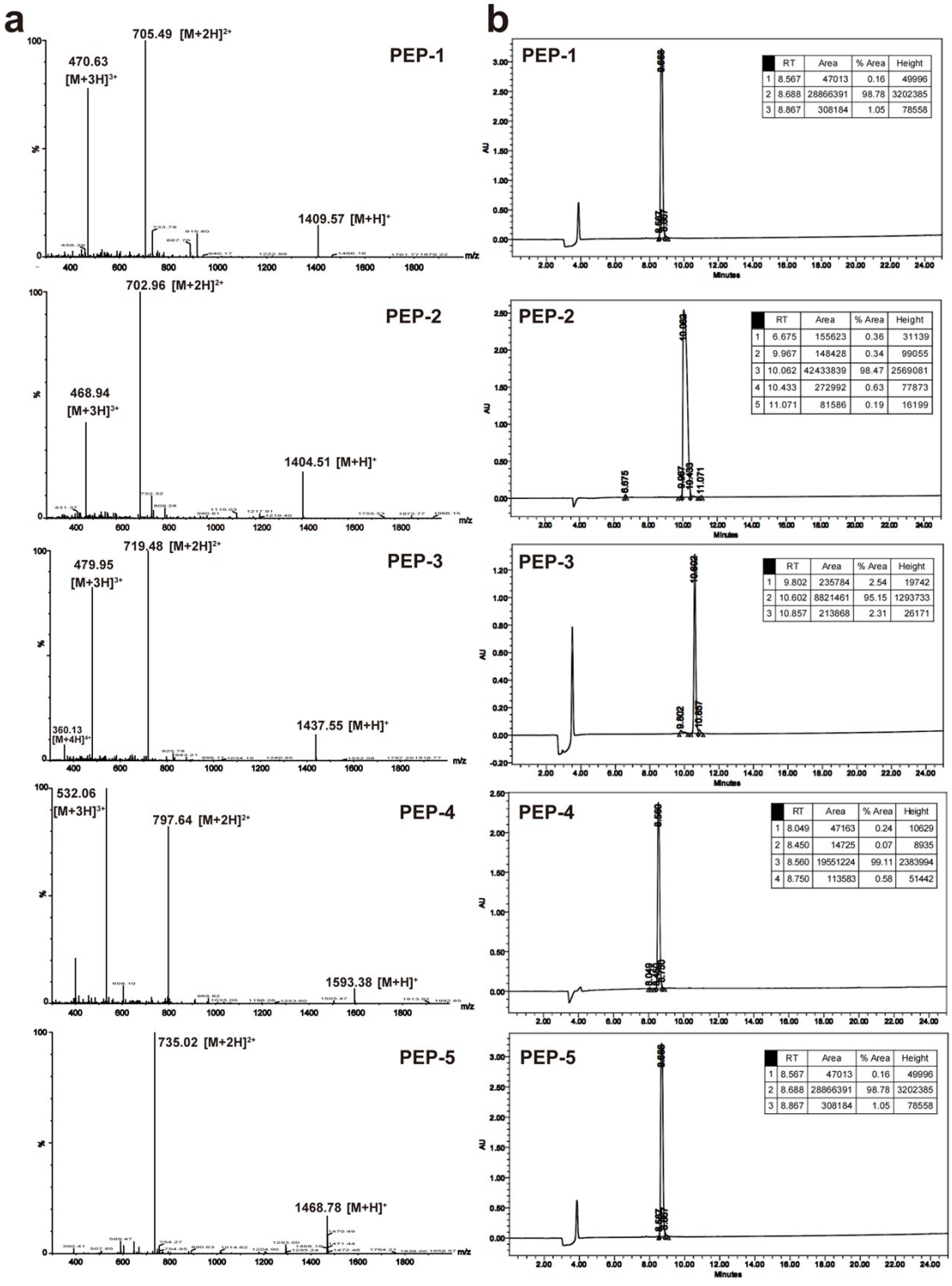


Figure S2. Mass spectra (a) and high-performance liquid chromatography (b) of the peptides PEP 1–5, illustrating the high purities of these peptides obtained from peptide synthesis and subsequent purification.

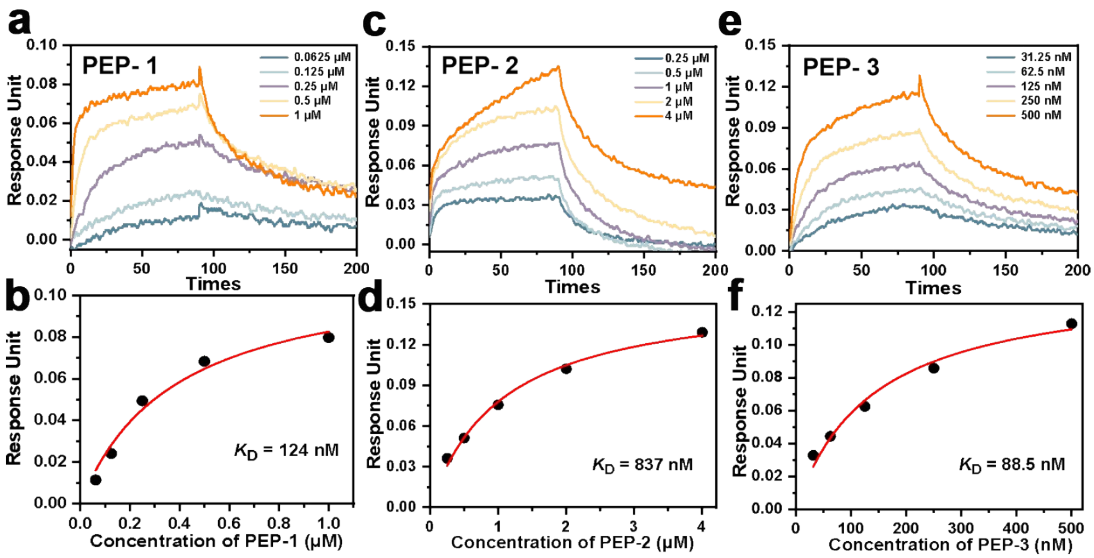


Figure S3. Association and dissociation kinetics (a, c, e) and binding affinity (b, d, f) of DV12 with (a, b) PEP-1, PEP-2 (c, d) and PEP-3 (e, f).

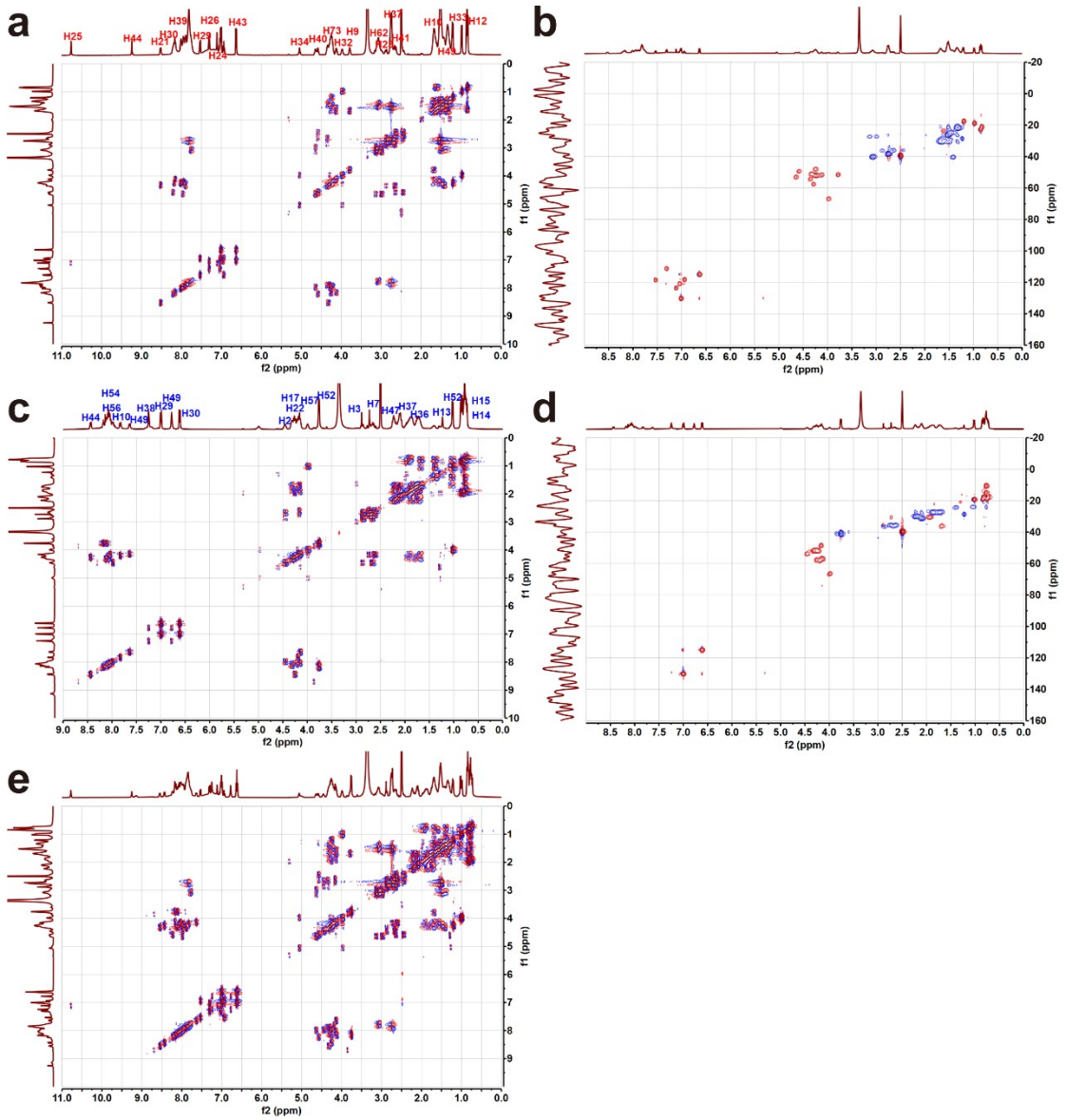


Figure S4. ^{1}H– ^{1}H correlation NMR spectrum of PEP-1 (20 mM, a), DV12 (20 mM, c) and PEP-1 with DV12 mixed at a molar ratio of 1:1 (e), ^{13}C– ^{1}H correlation NMR spectrum of PEP-1 (20 mM, b) and DV12 (20 mM, d) in DMSO-*d*6 at 25°C.

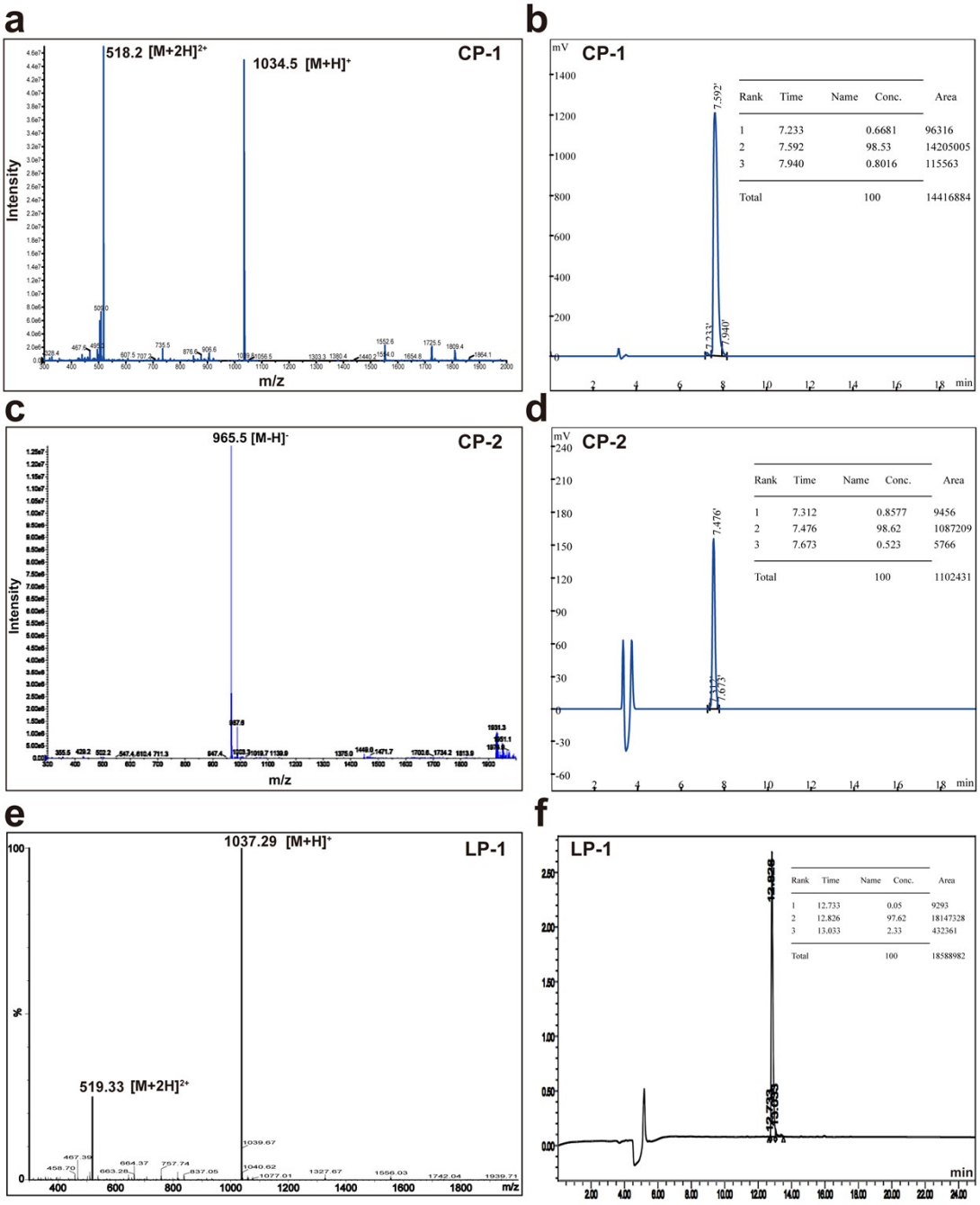


Figure S5. Mass spectra (a, c, e) and high-performance liquid chromatography (b, d, f) of peptides CP-1 (a, b), CP-2 (c, d), and LP-1 (e, f).

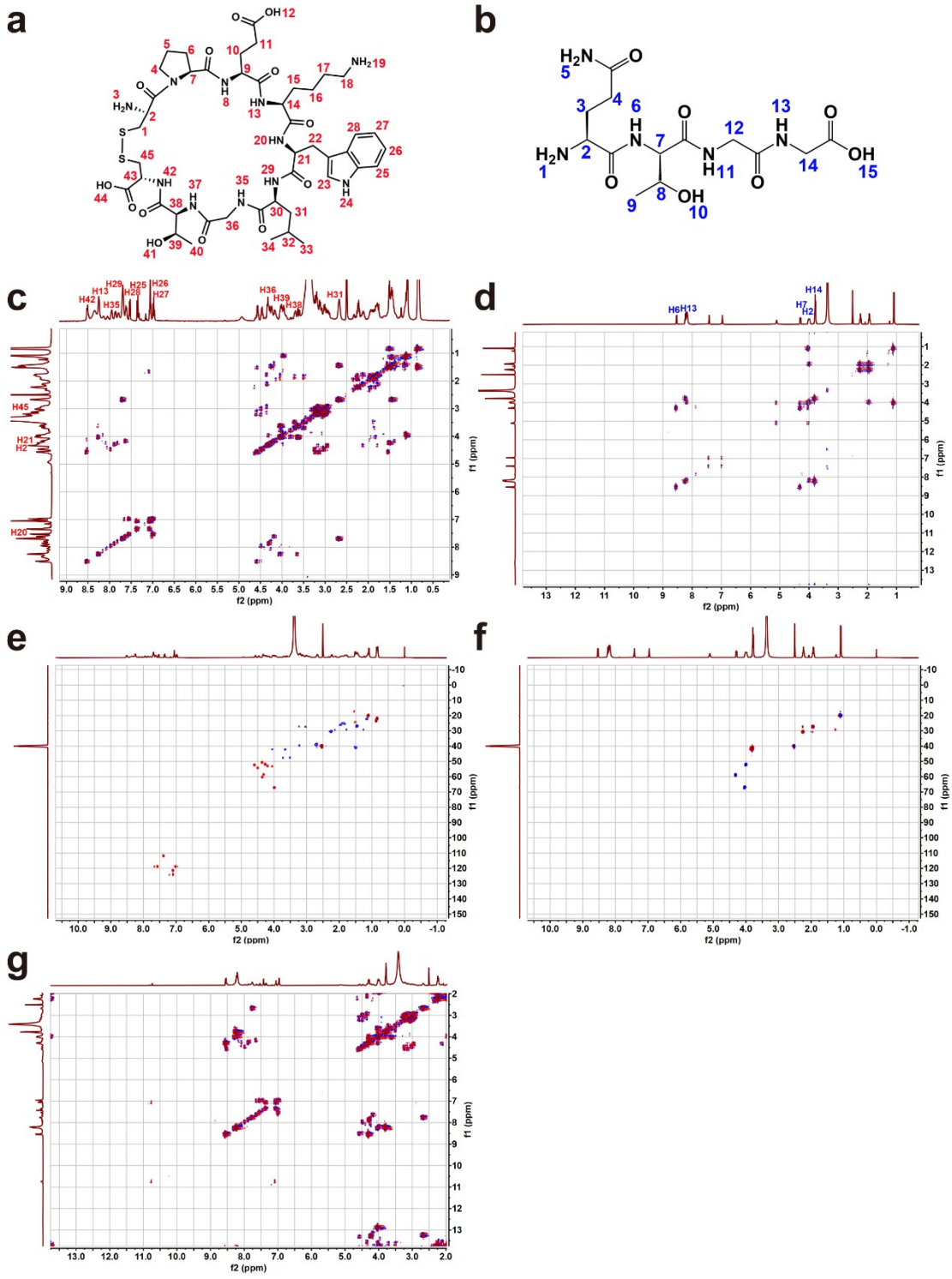


Figure S6. (a, b) Chemical structure of CP-1 (a) and QTGG (b); (c, d, g) ^{1}H– ^{1}H correlation NMR spectrum of CP-1 (20 mM, c), QTGG (20 mM, d) and CP-1 with QTGG mixed at a molar ratio of 1:1 (g); (e, f) ^{13}C–^{1}H correlation NMR spectrum of CP-1 (20 mM, e) and QTGG (20 mM, f) in DMSO-*d*6 at 25°C.

Supplementary Note 1

The Ph.D.-C7C library's randomized segment is flanked by a pair of cysteine residues. During phage assembly, these cysteine residues undergo oxidation, forming a disulfide linkage that presents the displayed peptides to the target in loop configurations. After three rounds of biopanning, the titer of the binding phages reached a plateau, where the enrichment of the binding phages toward QTGG was observed (Table S3). Ten phage clones were randomly selected for sequencing and analyzed, leading to 7 newly displayed peptide sequences (Main Figure 3a). Sequences of clone #1 and #2 were repeated two and three times, respectively. The binding affinities of the above phage monoclones which bear identified peptides toward QTGG were then evaluated by ELISA. Among the tested binders, #1 and #6 exhibited the highest affinity toward QTGG (Main Figure 3b). The two candidate peptides mentioned above, CPEKWLGTC (named CP-1) and CMTPNPTTC (named CP-2), were subsequently synthesized via solid-phase peptide synthesis, resulting in cystine-linked cyclic heptapeptides. Furthermore, a linear peptide corresponding to the open-loop form of CP-1 (named LP-1) was also synthesized for use as a control. The synthesized peptides were characterized through HPLC and MS (Figure S5).

The synthetic peptides bound to QTGG with K_D values of 71.8 nM, 1.8 μM, and 122.8 nM revealed high affinity, as measured by isothermal calorimetry (ITC) (Main Figure 3c-f). Initially, we used BLI for all peptide interaction measurements. However, during the assessment of interactions involving smaller molecules, BLI could not provide reliable K_D values due to the minor variations in the responding signal. The operation manual suggests that BLI is generally more suitable for studying interactions between macromolecules. In contrast, ITC is better suited for investigating interactions involving small molecules. Therefore, we employed ITC to measure the K_D values between QTGG and the peptides. ITC results showed that CP-1 exhibited the highest binding affinity to QTGG (K_D: 71.78 nM), while LP-1, corresponding linear form of CP-1, displayed weaker binding affinity (K_D: 122.8 nM), underscoring the essential role of the rigid cyclic structure in molecular recognition. Remarkable heat releases presented by the ITC titration

curves (Upper panels of Main Figure 3d-f) indicated that their complexation was driven by entropy increase, corresponding to spontaneous thermodynamics processes. Subsequently, we employed 2D NMR techniques and MD simulations to investigate the interaction model between CP-1 and QTGG. 2D ^{1}H–^{1}H total correlation NMR spectra of CP-1 before and after interaction with an equimolar amount of QTGG (Figure S6) provided a comprehensive depiction of the intermolecular interactions between them. Specifically, as shown in Main Figure 3g and 3h, significant changes in the chemical shift variations were observed for H8 (N-H of glutamic acid), H13 (N-H of lysine;), H20 (N-H of trptophan), H29 (N-H of leucine), H35 (N-H of glycine), and H45 (C-H of cysteine) in CP-1, as well as H2 (C-H of glutamine;), H6, H7 (N-H, C-H of threonine), and H13, H14 (N-H, C-H of glycine) in QTGG. Furthermore, MD simulations show that the cyclic peptide effectively encircled QTGG, forming a close complex that established 15 sets of binding interactions (Main Figure 3f). Notably, the glutamine residue of QTGG formed hydrogen bonds with multiple amino acids in CP-1. These findings confirmed the robust binding between CP-1 and QTGG.

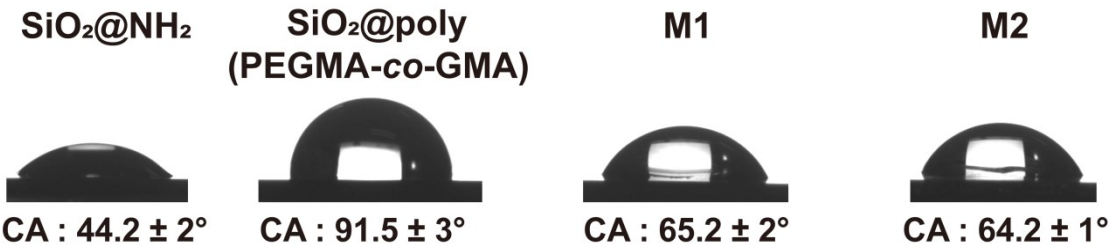


Figure S7. Water droplet profiles and the corresponding water contact angles (WCA) on amine, PEGMA-*co*-GMA, PEGMA-*co*-PEP-1, and PEGMA-*co*-CP-1-modified silica wafer surfaces. Remarkable changes in the WCA indicated the successful modifications on the surface. The test temperature was 25 ^{o}C.

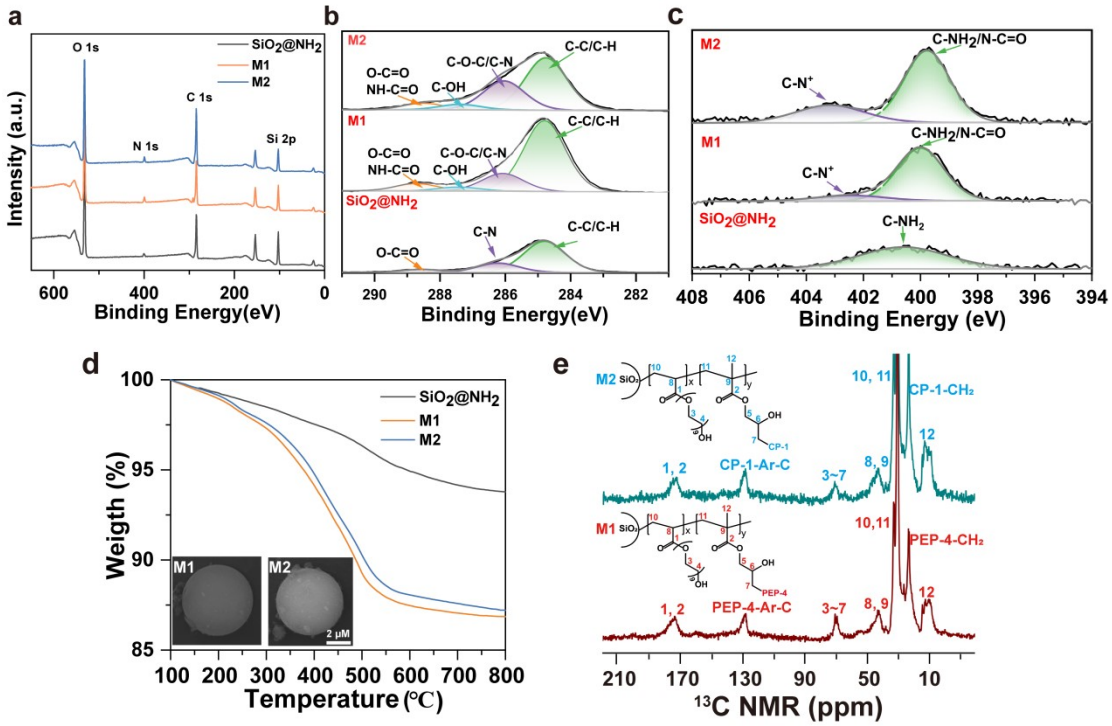


Figure S8. Characterization of functional polymer incorporating peptide ligands. Comparison of XPS full spectra (a), C_{1s} spectrum (b), N_{1s} spectrum (c) and TGA curves (inset: SEM images) (d) of SiO_2@NH_2, the prepared enrichment materials **M1** and **M2**. Solid ^{13}C NMR spectra of **M1** and **M2** (e).

Supplementary Note 2

First, wettability was assessed using water contact angle (WCA) analysis, changes in wettability after each reaction step (Figure S7) corresponded to the successful progression of material synthesis. The static WCA value of the poly(PEGMA-*co*-GMA) grafted silicon surface was 91.5 ± 3°, which decreased to 65.2 ± 2° and 64.2 ± 1° after modification with PEP-4 and CP-1, respectively, owing to the hydrophilic nature of the peptides. Elemental composition of the materials was further characterized by X-ray photoelectron spectroscopy (XPS) spectra (Figure. S8a-c and Table S5). The binding energies of C (284.6 eV), N (400.5 eV), O (532.5 eV), and Si (152.5 and 102.5 eV) speaks were observed, respectively. In addition, the relative atomic content of nitrogen (N) of SiO_2-NH_2 was 1.9%, after the polymer grafting, this value increased to 3.6%

and 4.2% for **M1** and **M2**, respectively. Subsequently, the spectrum of C_{1s} for **M1** and **M2** was deconvoluted into multiple peaks. New peaks assigned to C−OH (287.4 eV) were observed (Figure S8b), which indicated the success of the ring-opening reaction owing to the peptide grafting. In addition, the deconvolution of the N_{1s} spectra revealed a new peak at 403.2 eV (**M1**) or 402.3 eV (**M2**) (Figure. S8c), corresponding to the N located in the indole ring of tryptophan. Meanwhile, the signals attributed to amide-N or NH_2 (at 400.5 eV or 399.8 eV) of **M1** and **M2** increased remarkably compared to that of SiO_2@NH_2. These evident variations in the XPS spectra demonstrated that the PEP-4 and CP-1 had been successfully immobilized on the copolymer-modified silica gel. The contents of poly(PEGMEA-*co*-PEP-4) and poly(PEGMEA-*co*-CP-1) on the silica microspheres were respectively calculated as 6.92% and 6.57% according to thermogravimetric analysis (TGA) results (Figure S8d). Scanning electron microscopy (SEM) images revealed that **M1** and **M2** effectively maintained the spherical morphology of the silica microspheres. Solid ^{13}C NMR analysis further validated this presumption. Each NMR signal peak could be well assigned according to the structures of the copolymers, as shown in Figure S8e. Particularly, the clear peak at 127.6 ppm could be attributed to the carbon atoms of indole group in tryptophan, which only belonged to the PEP-4 or CP-1. This illustrated the high grafting ratios of the peptide, which was favorable for the SUMO-1 peptide enrichment.

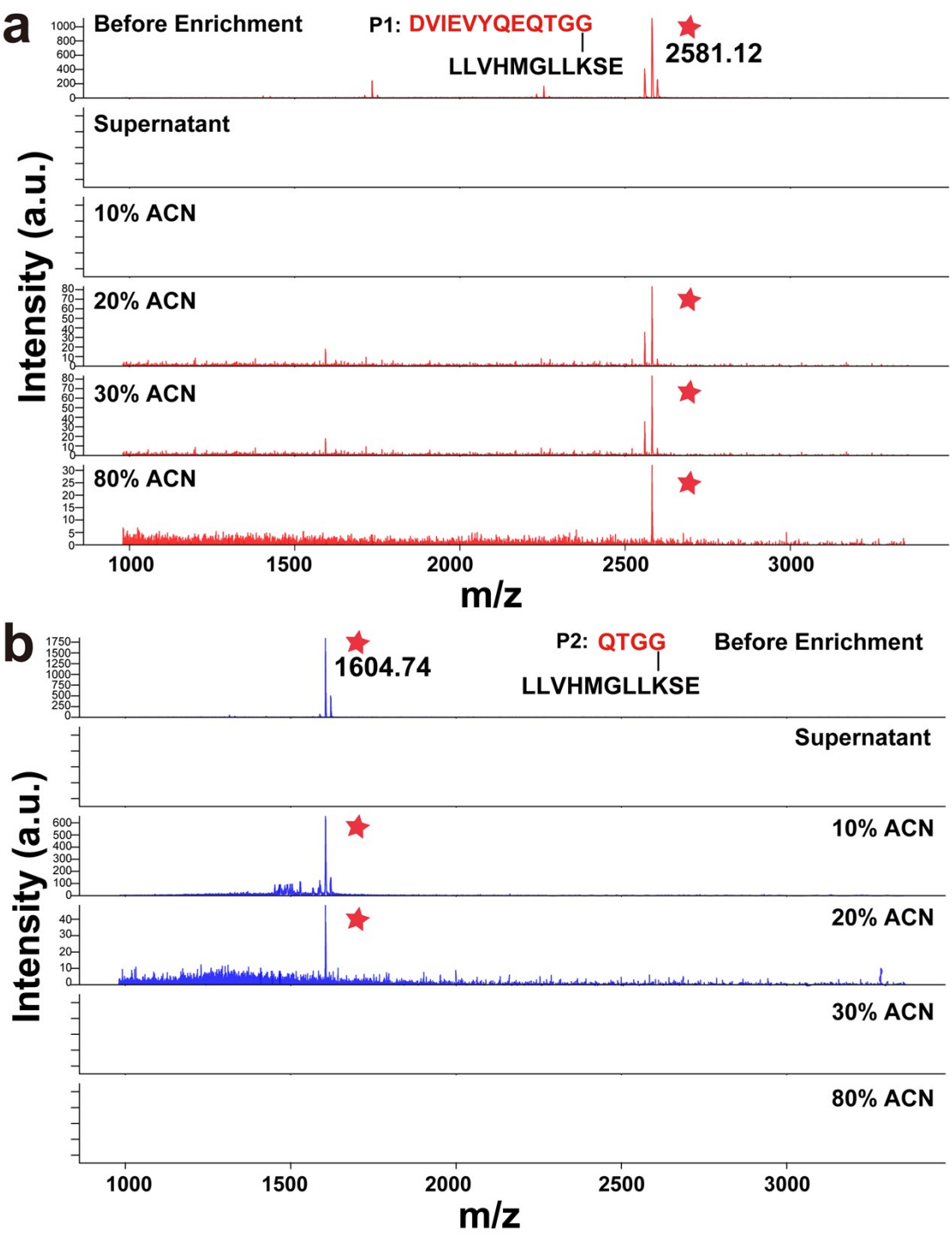


Figure S9. MALDI-TOF/TOF spectra of P1 or P2 before and after enrichment using **M1** (a) or **M2** (b), eluted with ACN/H_2O mixtures with different proportions.

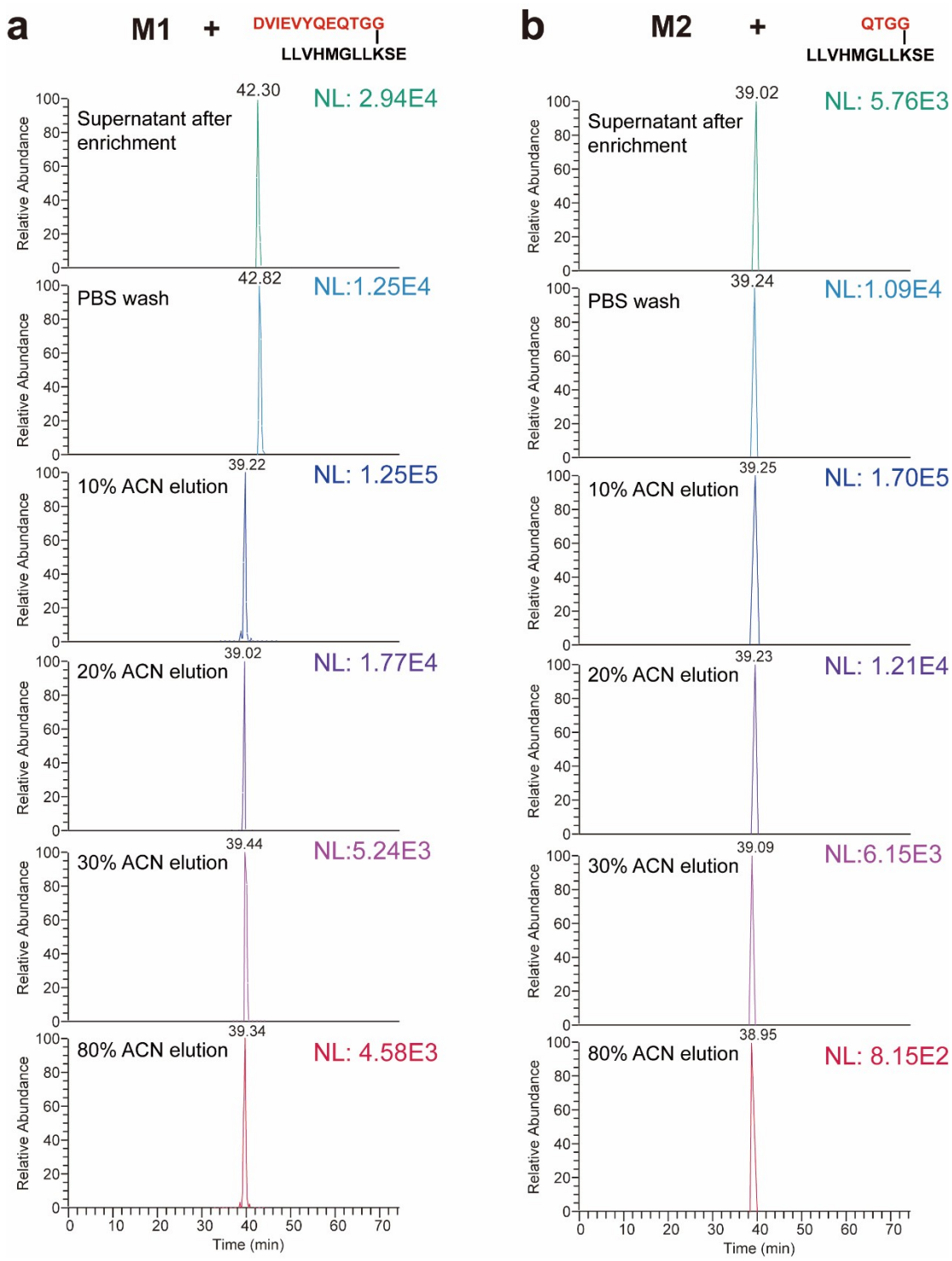


Figure S10. Extracted ion chromatogram of different SUMOylated RanGAP1 peptide remnants (supernatant after enrichment, PBS wash, and ACN gradient elution) after enrichment with **M1** (a) and **M2** (b), respectively.

Supplementary Note 3

Based on the sophisticated design of materials, we proposed a peptide-level enrichment strategy, akin to immunoprecipitation but independent of antibodies, to enhance the identification of endogenous SUMO-1 modified sites. To accomplish this, we synthesized two standard peptides derived from SUMOylated RanGAP1, considering that SUMO-1 site is located at the K524 of RanGAP1, one of the most abundant SUMO-1 modified proteins in mammalian cells. The peptides P1 and P2 (sequences are shown in insets of Figure S9a and 9b), differed in the length of SUMO-1 remnant, were used for evaluating the enrichment performance of **M1** and **M2**, respectively. Subsequently, we performed enrichment analysis using a dispersive solid-phase extraction method with **M1** and **M2**, respectively, and each eluent was subjected to MALDI-TOF/TOF analysis. As depicted in Figure S9a, after **M1** enrichment and subsequent washing with 10% or 20% acetonitrile (ACN)-H_2O mixtures, no peak of P1 was detected in the supernatant and eluents. When the ACN ratio was increased to 30%, P1 peak began to emerge. This indicated the strong binding between the **M1** and P1. Slightly different from **M1**, after **M2** enrichment, P2 was gradually eluted across a gradient range of ACN (10%–20%) (Figure S9b), which also indicated the strong binding between the **M2** and P2. Moreover, the mixtures of standard peptide and tryptic HeLa (1:10, m/m) were used to evaluate the enrichment performance. Different components (i.e., supernatant after enrichment, PBS wash, and ACN gradient elution) were separately collected during the material enrichment process and subjected to LC-MS analysis. As illustrated in Figure S10, there was a significant increase in peak intensity within the peptides chromatogram during the gradient elution, which was similar to that of the standard peptides results. Accordingly, the above results demonstrated the excellent enrichment ability of the materials. In following enriching experiments, water was applied as more moderate loading and washing buffer for reducing loss of absorbed peptides, and 80% ACN was applied as elution buffer for thorough collection of peptides. ACN was preferred over an acidic solution for elution due to its ability to efficiently disrupt peptide-material interactions while preserving peptide integrity. Acidic conditions could risk peptide denaturation, especially for sensitive peptides, which ACN helps to avoid.

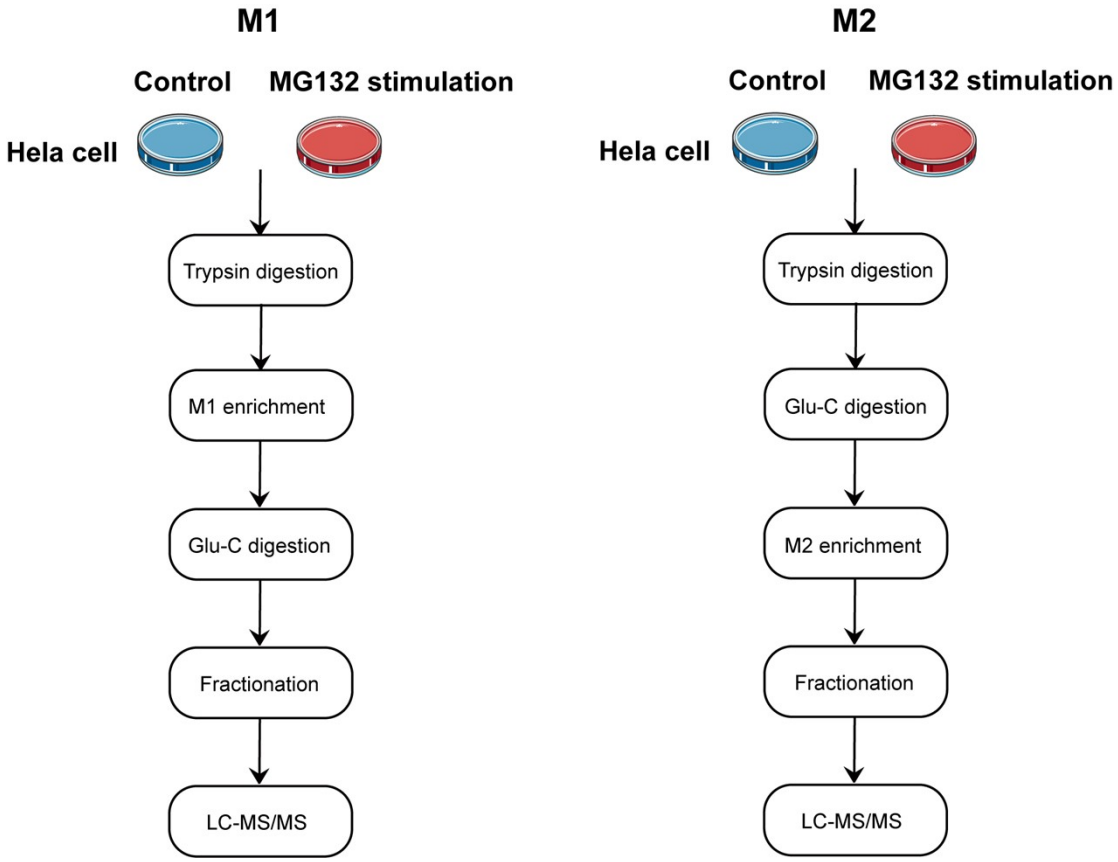


Figure S11. Workflows of the enrichment strategies using **M1** and **M2**, respectively.

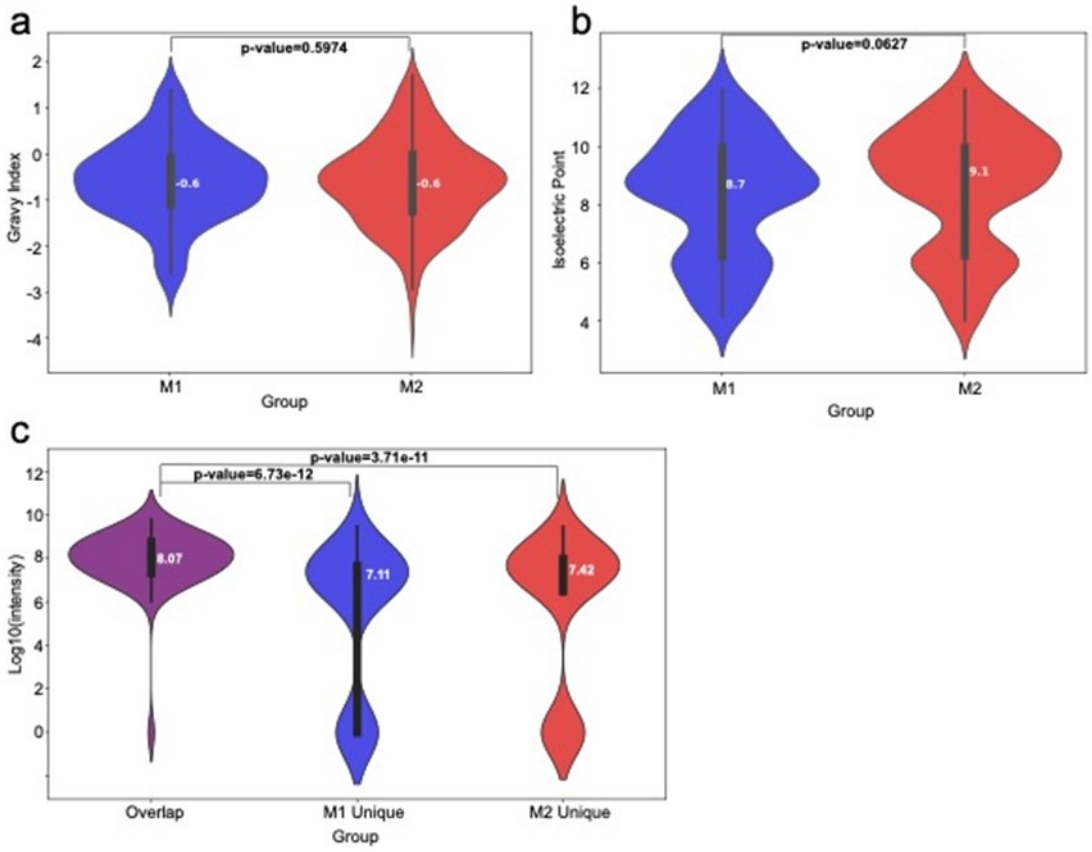


Figure S12. Comparing SUMO-1 sites between **M1**-dependent and **M2**-dependent method. (a) Hydrophobicity (gravy index) distribution of SUMO-1 modified peptides enriched by **M1** and **M2** materials; (b) Isoelectric Point (pI) distribution of SUMO-1 modified peptides enriched by **M1** and **M2** materials; (c) Intensity comparison of SUMO-1 modified sites identified by **M1** and **M2** materials.

Supplementary Note 4

By comparing the hydrophobicity (Figure S12a) and isoelectric points (Figure S12b) of peptides enriched by **M1** and **M2** materials, we found no significant differences in the physicochemical properties of SUMO-1 sites between the two materials. However, when comparing the abundance of SUMO-1 sites identified by both materials, we observed that the abundance of SUMO-1 sites common to both materials was higher than that of sites enriched by each material individually. Thus, we speculated that randomness in detection caused by the low abundance of SUMO-1 modification is the primary reason for the small overlap in the results identified by the two

materials. Enrichment of peptides by the materials primarily depends on the target segment of SUMO-1, with no significant preference for the peptide sequence connected to the other end.

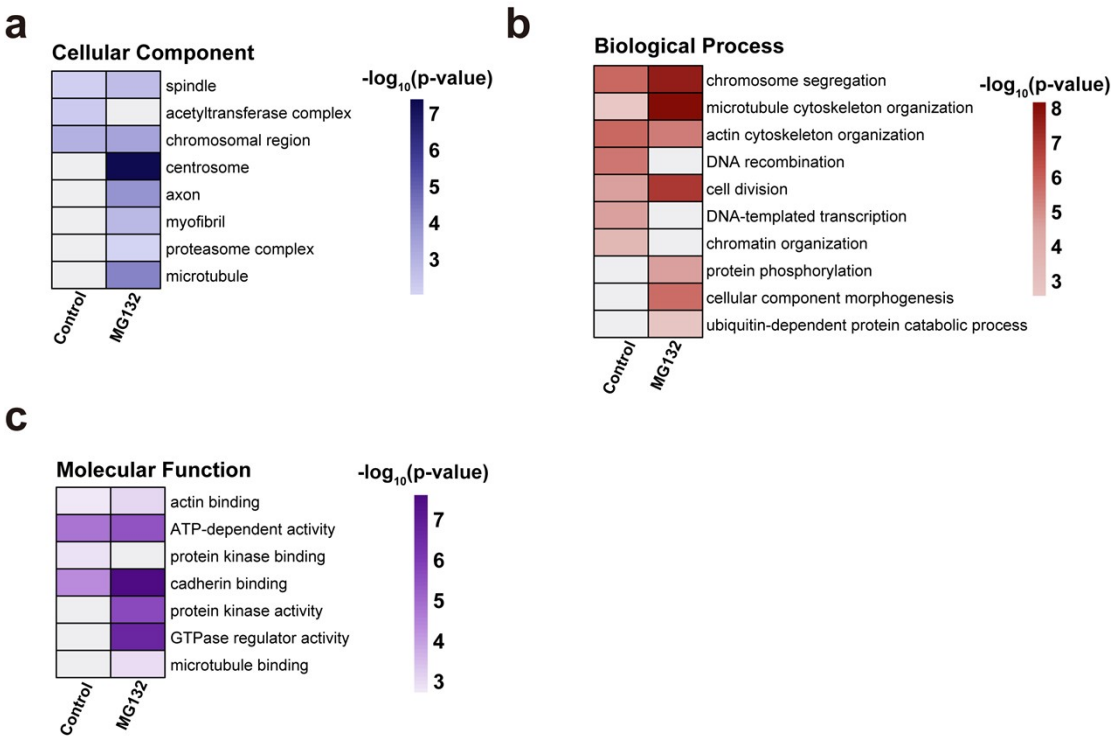


Figure S13. Gene Ontology (GO) enrichment analysis (a, cellular component, b, biological process, and c, molecular function) of merged SUMO-1 modified proteins between the group of no treatment and MG132 stimulation identified by both materials.

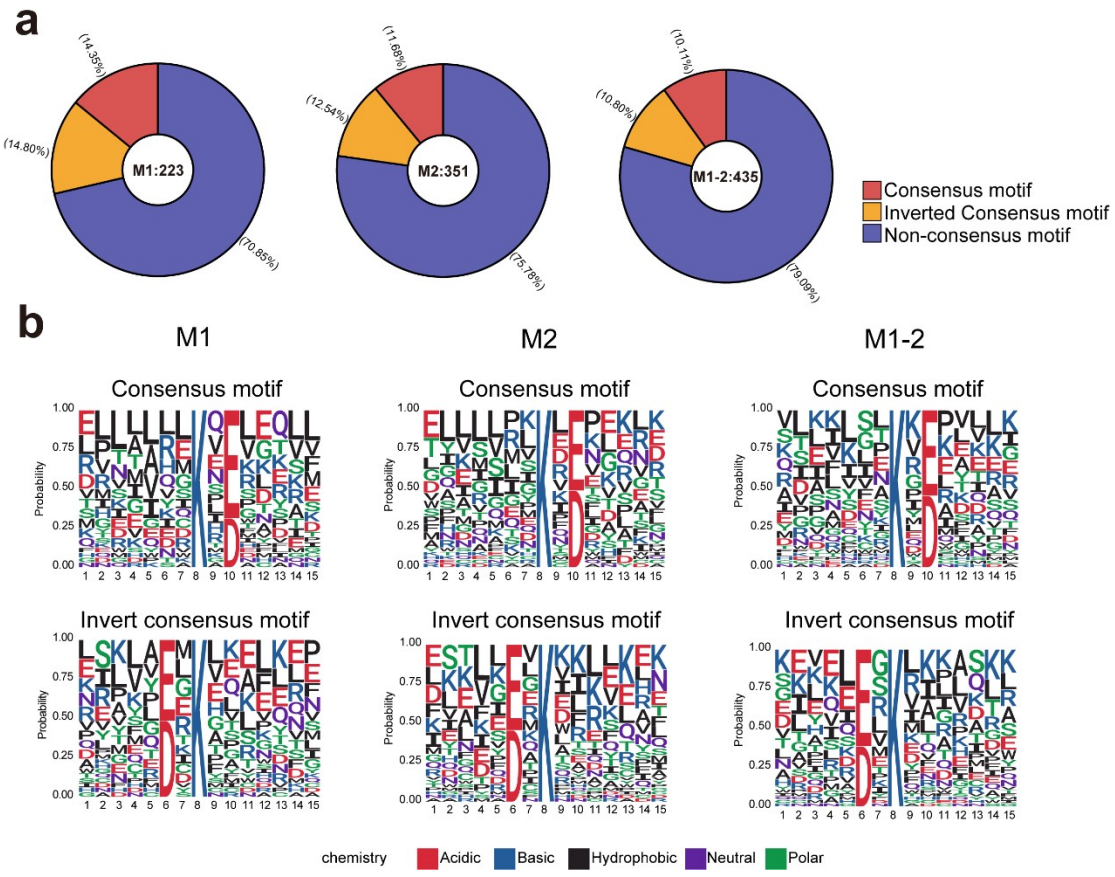


Figure S14. Consensus motif analysis of SUMOylation sites identified by **M1**, **M2**, or the combination of **M1** and **M2**. (a) Distribution of identified SUMO-1 modified sites based on sequence motif; (b) SUMO-1 sites occurring on consensus motif (KxE) and inverted consensus motif (D/ExK), separately.

Supplementary Note 5

We generated a dataset of tryptic peptides from the human proteome (2,296,383 peptides, missed cleavages = 1) and randomly divided it into three equal subsets. For each subset, we calculated the percentage of sequences containing the consensus motif (KxD/E) and the inverted consensus motif. These values were compared to our experimental results using a two-tailed T-test (As shown in Table S6 and Table S7). The p-values for the consensus motif and inverted consensus motif were 0.0016 and 0.016, respectively. These results indicate that the percentages of both the consensus motif and the inverted consensus motif in the identified SUMO-1 modified sites are statistically significantly higher compared to the human proteome.

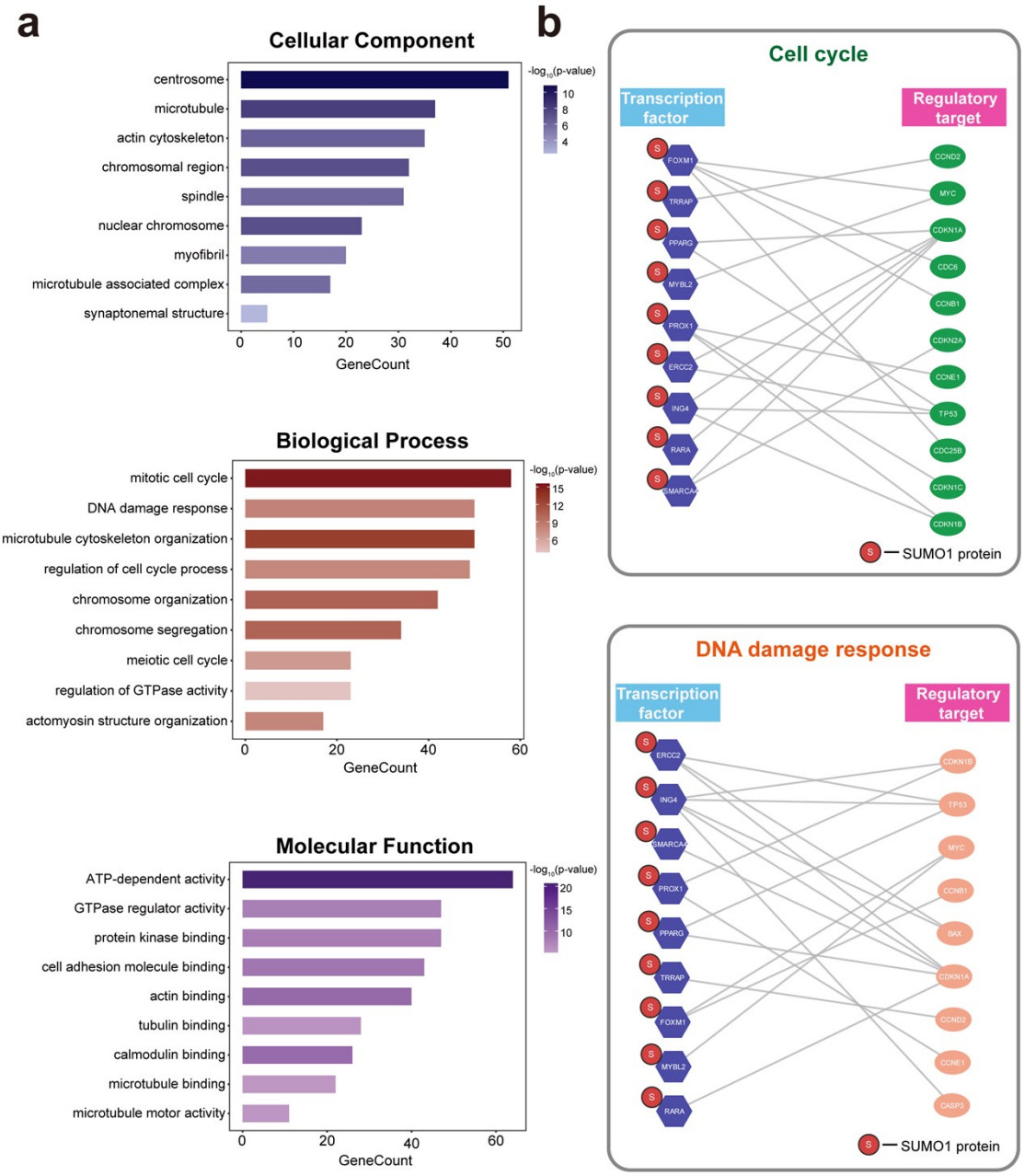


Figure S15. Bioinformatic analysis of identified SUMO-1 modified proteins in HeLa cells. (a) GO analysis of the identified SUMO-modified proteins, including the cellular components, biological processes, and molecular functions. (b) SUMO-1 modified transcription factors and their regulatory target proteins in cell cycle and DNA damage response pathway.

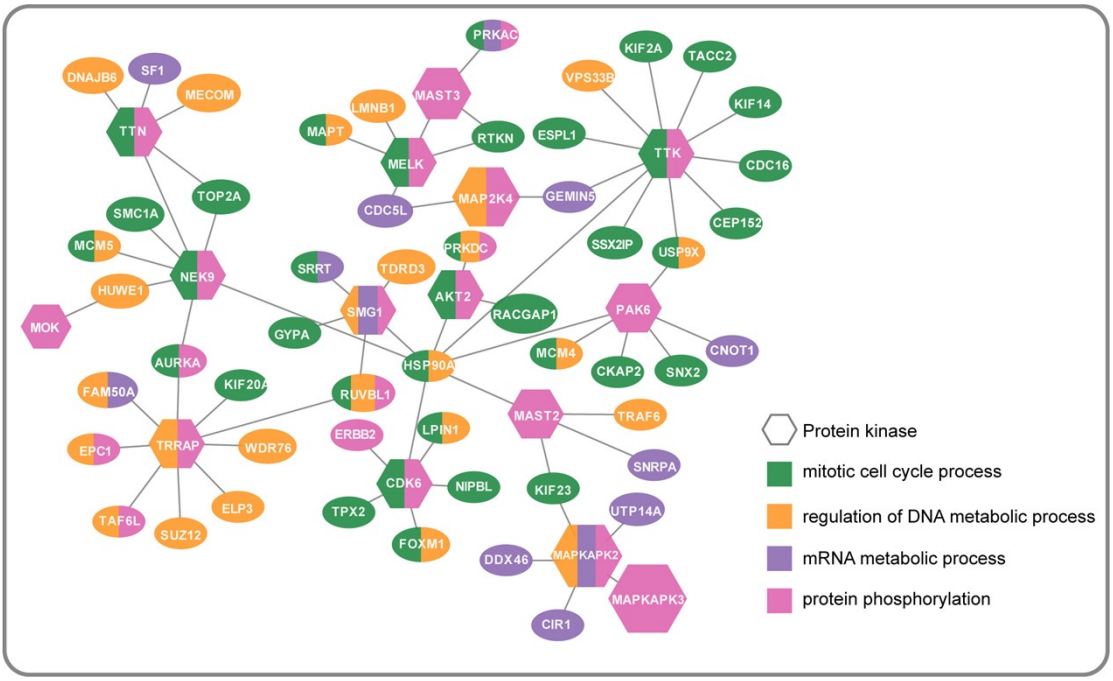


Figure S16. Protein-protein interaction network comprises SUMO-1 modified protein kinases and their interacting proteins.

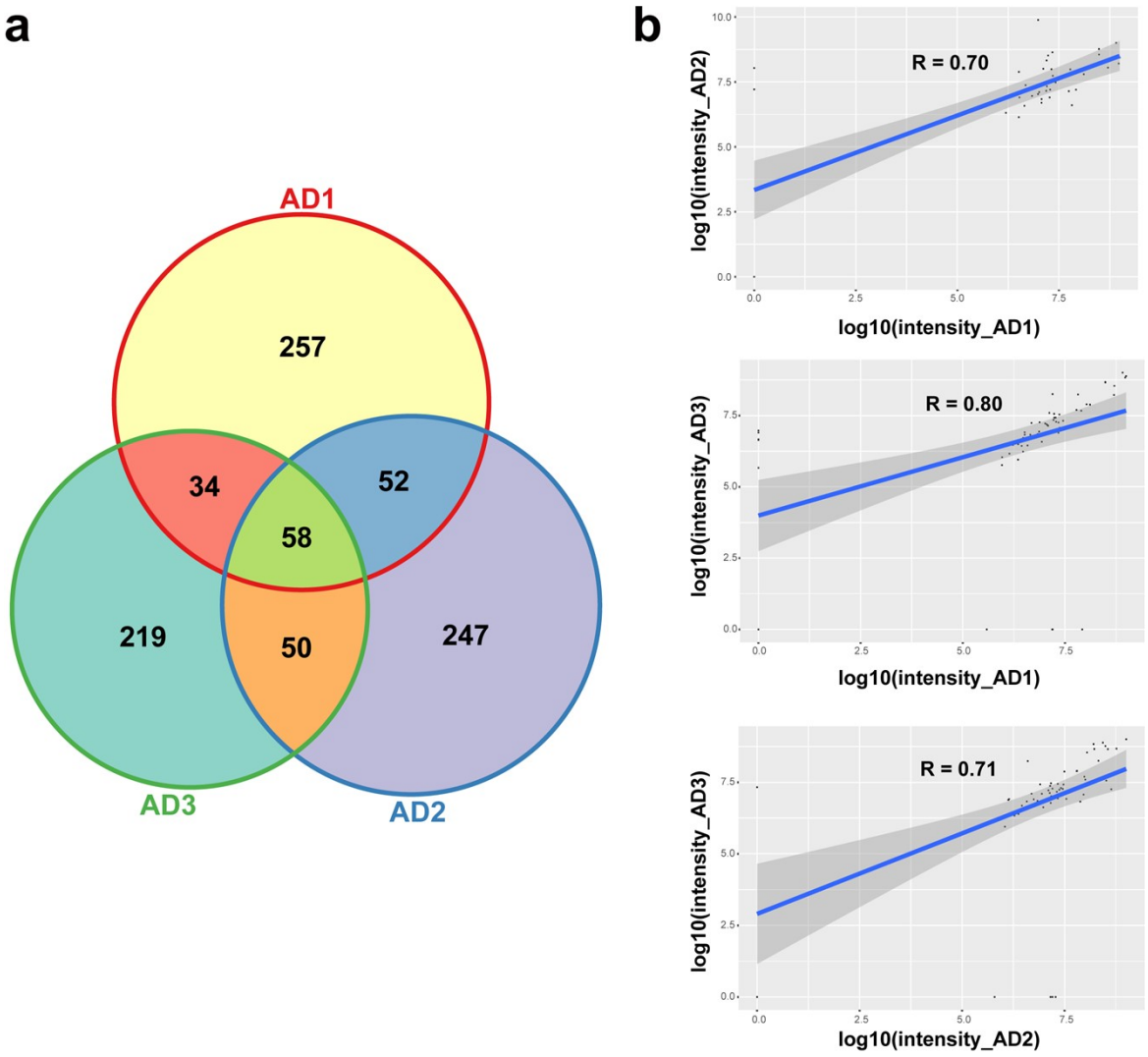


Figure S17. Reproducibility assessment of biological triplicates in AD mouse brain tissue experiments. (a) Overlap of identified SUMO-1 modification sites. (b) Correlation of quantitative intensities for SUMO-1 modified sites.

3. Supporting Tables

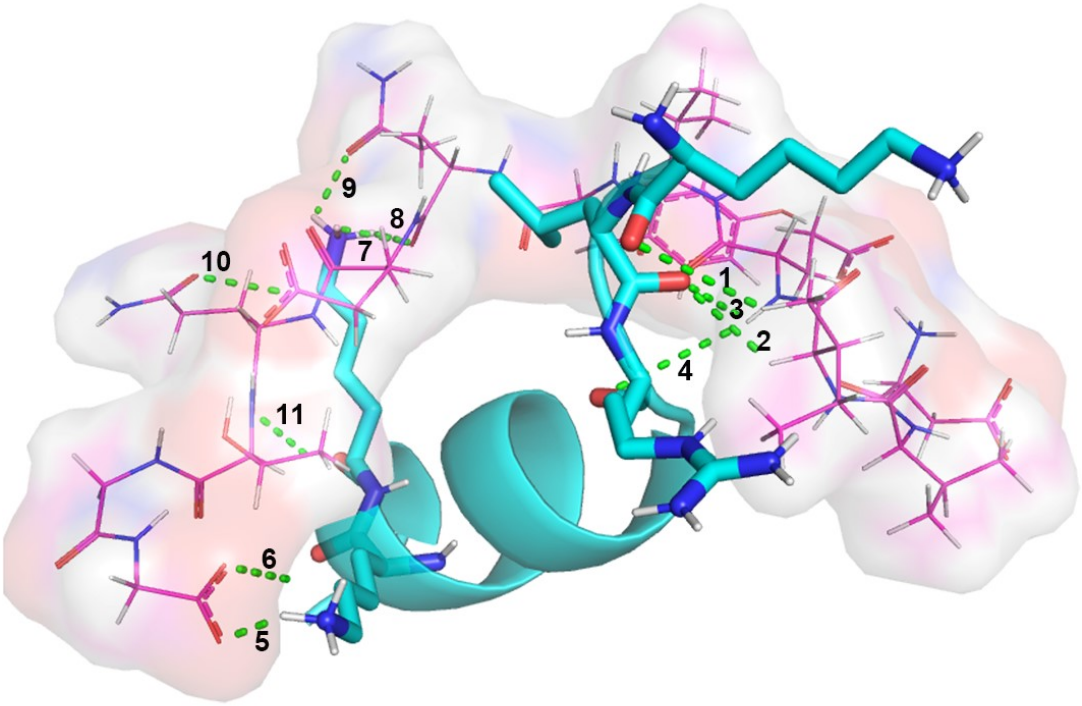
3.1 Table S1. The phage titer results at each stage of each biopanning procedure targeting DV12.

Biopanning steps		Phage Inputs 2.0×10^{12}			
		NO.	Recovered phage titer/ pfu mL^{-1}	Amplified for next round/ pfu mL^{-1}	Recov.*
Ph.D.-12 library	Negative screening against BSA	1st	3.7×10^{10}	4.3×10^{11}	–
	Positive screening against DV12	1st	6.7×10^{6}	3.2×10^{12}	3.1×10^{-4}
		2nd	2.2×10^{6}	1.0×10^{11}	1.4×10^{-5}
		3rd	1.5×10^{6}	1.6×10^{11}	3.0×10^{-4}
		4th	2.6×10^{6}	–	**3.3×10^{-4}**

*Recovery = output/input = virions of supernatant/virions of the appropriate dilution titer of amplified phage from last round

3.2 Table S2. Binding interactions between PEP-4 and DV12.

No.	Bonding	Distance(Å)
1	$O_{K1}\cdots H_{I3}$	3.74
2	$O_{L2}\cdots H_{I3}$	3.06
3	$O_{L2}\cdots H_{E4}$	2.93
4	$O_{R3}\cdots H_{E4}$	4.31
5	$O_{K9}\cdots H_{G12}$	1.90
6	$O_{K9}\cdots H_{G12}$	3.20
7	$H_{K10}\cdots O_{E8}$	2.33
8	$H_{K10}\cdots O_{E8}$	2.59
9	$H_{K10}\cdots O_{E8}$	2.17
10	$H_{K10}\cdots O_{Q9}$	2.62
11	$H_{K10}\cdots O_{T10}$	2.95



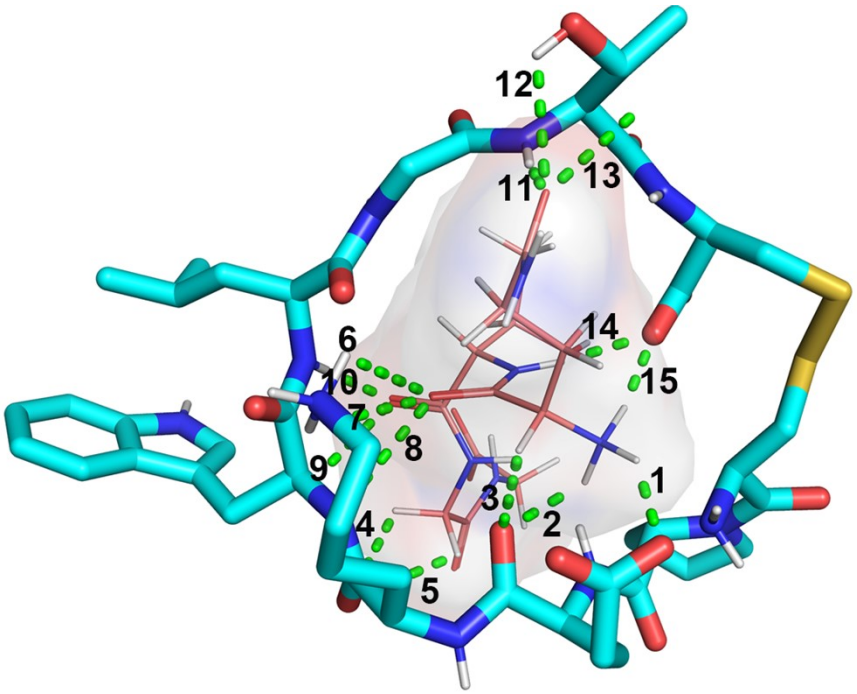
3.3 Table S3. The phage titer results at each stage of each biopanning procedure targeting QTGG.

Biopanning steps		Phage Inputs 2.0×10^{12}			
		NO.	Recovered phage titer/ pfu mL^{-1}	Amplified for next round/ pfu mL^{-1}	Recov.*
Ph.D.-C7C library	Negative screening against BSA	1st	5.3×10^{10}	-	-
	Positive screening against QTGG	1st	5.3×10^{5}	3.5×10^{12}	2.0×10^{-5}
		2nd	5.9×10^{6}	4.6×10^{12}	3.4×10^{-5}
		3rd	1.4×10^{7}	-	**6.1×10^{-5}**

*Recovery = output/input = virions of supernatant/virions of the appropriate dilution titer of amplified phage from last round

3.4 Table S4. Binding interactions between CP-1 and QTGG.

No.	Bonding	Distance(Å)
1	$O_{E3}\cdots H_{Q1}$	1.91
2	$O_{E3}\cdots H_{Q1}$	2.00
3	$O_{E3}\cdots H_{Q1}$	2.60
4	$O_{K4}\cdots H_{G3}$	2.75
5	$O_{K4}\cdots H_{G3}$	2.73
6	$H_{K4}\cdots O_{Q1}$	3.03
7	$H_{K4}\cdots O_{Q1}$	3.05
8	$H_{K4}\cdots O_{Q1}$	2.55
9	$H_{W5}\cdots O_{T2}$	2.38
10	$H_{L6}\cdots O_{T2}$	1.98
11	$H_{T8}\cdots O_{Q1}$	1.95
12	$H_{T8}\cdots O_{Q1}$	2.82
13	$H_{T8}\cdots O_{Q1}$	2.95
14	$O_{C9}\cdots H_{T2}$	2.08
15	$O_{C9}\cdots H_{Q1}$	1.80



3.5 Table S5. Elemental compositions on the surfaces of the samples determined by XPS analysis.

Samples	Relative atomic content (%)			
	C	*O*	*N*	*Si*
SiO_2@NH_2	32.1	44.4	1.9	21.6
M1	44.1	35.6	3.6	16.7
M2	33.7	42.1	4.2	20.0

3.6 Table S6. Consensus motif statistics.

Group	Total Peptides	KxD/E Motif Count	Ratio
M1-dependent enrichment	223	32	14.35%
M2-dependent enrichment	351	41	11.68%
Combinatorial peptide enrichment strategy	436	44	10.11%
Human tryptic peptides 1	765461	20872	2.72%
Human tryptic peptides 2	765461	20863	2.72%
Human tryptic peptides 3	765461	20689	2.70%

3.7 Table S7. Invert consensus motif statistics.

Group	Total Peptides	D/ExK Motif Count	Ratio
M1-dependent enrichment	223	33	14.80%
M2-dependent enrichment	351	44	12.54%
Combinatorial peptide enrichment strategy	436	47	10.80%
Human tryptic peptides1	765461	61917	8.09%
Human tryptic peptides2	765461	61731	8.06%
Human tryptic peptides3	765461	62146	8.12%

3.8 Table S8. Comparison of experimental conditions applied in our work with previous research.

Research	Exogenous/ Endogenous	MS Instrument	LC Gradient	Enrichment Method	Initial Protein Amount	SUMO-1 sites (number)
Proc Natl Acad Sci U S A. 2014;111(34): 12432-12437	Exogenous	LTQ Orbitrap Velos	120 min	His pulldown assays followed by Immunocapture of diglycine-modified peptides	Not specified	295
Mol Cell Proteomics. 2017;16(5):717-727	Endogenous	Q-Exactive Plus/Q-Exactive HF	47 samples, 85 min	Anti-pan-SUMO-1 antibody enrichment	40 mg	132
Anal Chim Acta. 2021;1154:338324	Endogenous	Orbitrap Fusion Lumos	12 fractions, 65 min	SAX chromatography enrichment	40 mg	171
Our work	Endogenous	Orbitrap Fusion Lumos	6 fractions, 90 min	Combinatorial peptide enrichment strategy with specific peptide ligands	40 mg	1312

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