Supplementary Information (SI) for Chemical Science.
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1. General remarks on materials

All Fmoc-amino acids, coupling reagents for SPPS, and chemical reagents for NCL were purchased from either Aldrich or GL Biochem, and were used without further purification. All solvents in reagent grade (RCI) or HPLC grade (DUKSAN) were used without purification, with the exception that anhydrous dichloromethane (DCM) was freshly distilled over calcium hydride (CaH_2) before use. Analytical LC-MS was performed on Waters UPLC H-class system equipped with an ACQUITY UPLC photodiode array detector and a Waters SQ Detector 2 mass spectrometer. LC was performed using a Waters ACQUITY BEH C18 column (1.7 μm, 130 Å, 2.1 × 50 mm) at a flow rate of 0.4 mL/min. Preparative HPLC was performed either on a Waters system using a Vydac 218TPTM C18 column (10 μm, 22 × 250 mm) at a flow rate of 10 mL/min or a Vydac 218TPTM C18 column (10 μm, 30 × 250 mm) at a flow rate of 20 mL/min, or on SHIMADZU Essentia LC-16P HPLC system with SPD-16 UV-VIS detector and Welch Materials XB-C18 Ultimate HPLC column (10×250 mm, 5 μm particle). Mobile phases of HPLC used are as follows: Solvent A, 0.1% TFA (v/v) in acetonitrile; Solvent B, 0.1% TFA (v/v) in water.

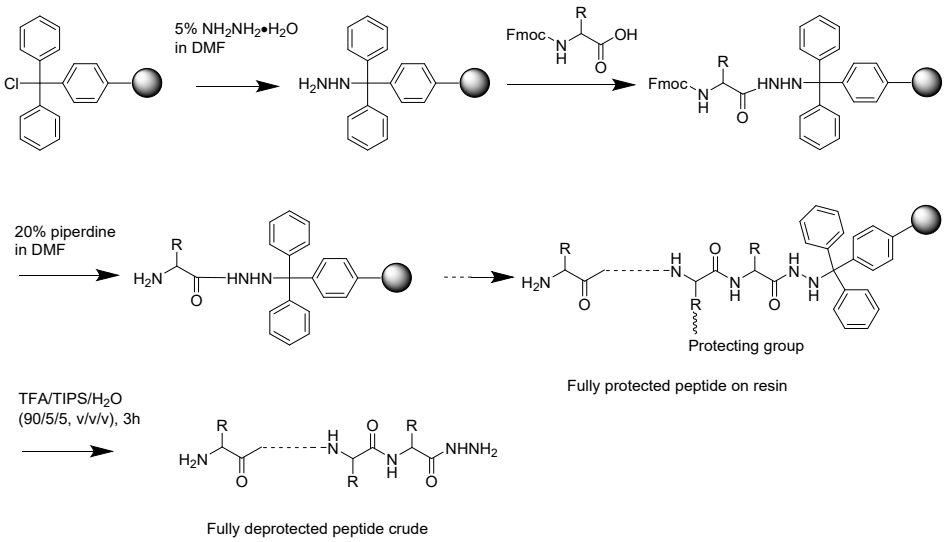
2. General experimental procedures

2.1 Fmoc-based solid-phase peptide synthesis (SPPS) and cleavage

For Fragment 1, 2, and 4 (Figure S1), the peptide hydrazides were prepared by SPPS on hydrazine-modified 2-chlorotrityl chloride resin (GL Biochem, loading capacity: 0.5 mmol/g), following published protocol (Scheme 1).[1] Specifically, the 2-chlorotrityl chloride resin (500 mg, 0.5 mmol/g loading) was placed in a 12 mL polypropylene column with a polyethylene filter at the bottom. The resin was swollen with dry DCM for 20 min. Then it was washed with DCM (8 mL × 3 times) and DMF (8 mL × 3 times). The $NH_2NH_2{\cdot}H_2O$/DMF solution (1/15, v/v, 6 mL) was added to the resin and the mixture was shaken for 1 hour. The resin was then washed by DCM (8 mL × 3 times) and DMF (8 mL × 3 times). Another portion of $NH_2NH_2{\cdot}H_2O$/DMF solution (1/15, v/v, 6 mL) was added, and the mixture was shaken for 30 min. The resin was then washed by DCM (8 mL × 3 times) and DMF (8 mL × 3 times) again. The remaining 2-chlorotrityl chloride resin was quenched by methanol//DMF solution (1/15, v/v, 6 mL) for 20 min. The resin was then washed by DMF (8 mL × 3 times) and ready for iteration of Fmoc-SPPS. Peptide for Fragment 4-S and Fragment 4-***pS*** with an amide end were synthesized on Rink-Amide AM resin (GL Biochem, 0.54mmol/g).

Fmoc amino acids from GL Biochem were employed, which include Fmoc-Ala-COOH, Fmoc-Cys(Trt)-COOH, Fmoc-Asp(O^{t}Bu)-COOH, Fmoc-Glu(O^{t}Bu)-COOH, Fmoc-Phe-COOH, Fmoc-Gly-COOH, Fmoc-His(Trt)-COOH, Fmoc-Ile-COOH, Fmoc-Lys(Boc)-COOH, Fmoc-Leu-COOH, Fmoc-Met-COOH, Fmoc-Asn(Trt)-COOH, Fmoc-Pro-COOH, Fmoc-Gln(Trt)-COOH, Fmoc-Arg(Pbf)-COOH, Fmoc-Ser(^{t}Bu)-COOH, Fmoc-Thr(^{t}Bu)-COOH, Fmoc-Val-COOH, Fmoc-Trp(Boc)-COOH, Fmoc-Tyr(^{t}Bu)-COOH, and Fmoc-His(Boc)-COOH. Fmoc-SPPS was performed following standard procedures.[2] Briefly, peptide on the resin was deblocked with a mixture of piperidine/DMF (20/80, v/v). The resin was washed with DMF, CH_2Cl_2, and DMF (5 mL × 3 times

for each solvent), then mixed with a solution of Fmoc protected amino acid (4.0 equiv. according to the resin capacity), HATU (4.0 equiv.) and DIEA (10 equiv.) in DMF. The coupling proceeded with gentle agitation of the resin at room temperature for 1h. After removing of the coupling solution, the resin was washed with the same procedure mentioned above, then used for the next SPPS cycle.



Scheme 1. Synthesis of peptide hydrazide by SPPS and acidic deprotection, which also releases the peptide from the resin.

After SPPS, the on-resin fully protected peptide, obtained as described in the Fmoc-SPPS section, was subjected to the acidic cleavage cocktail (5-10 mL) of TFA/TIPS/H_2O (90/5/5, v/v/v, TIPS: triisopropylsilane) for 2-3 h. After filtration, the resulting cleavage solution was combined and concentrated to give the crude peptide bearing either a hydrazide group or an amide group at the C-terminus in case the Rink-amide resin was employed.

Table S1. mShc1^{CH1} sections synthesized by Fmoc-SPPS for NCL.

Name	Length (AA)	Sequence	Calculated M.W.	Found
1-YY	30	Biotin-EPPDHQYYNDFPGKEPPLGGVVDMRLREGA-$NHNH_2$	3625.02	3624.76
1-***pY***Y	30	Biotin-EPPDHQ***pY***YNDFPGKEPPLGGVVDMRLREGA-$NHNH_2$	3705.00	3704.72
1-***pYpY***	30	Biotin-EPPDHQ***pYpY***NDFPGKEPPLGGVVDMRLREGA-$NHNH_2$	3784.98	3784.68
2	24	**C**RPTLPSAQMSSHLGATLPIGQHA-$NHNH_2$	2487.89	2488.83
3-Y	35	**C**GDHEVRKQMLPPPP**C(ACM)**PGRELFDDPSYVNIQNLDK-$NHNH_2$	4094.66	4094.40
3-***pY***	35	**C**GDHEVRKQMLPPPP**C(ACM)**PGRELFDDPS***pY***VNIQNLDK-$NHNH_2$	4174.65	4173.98
4-S	18	**C**RQAGGGAGPPNPSLNGS-$CONH_2$	1638.78	1639.45
4-***pS***	18	**C**RQAGGGAGPPNP***pS***LNGS-$CONH_2$	1718.76	1719.41

2.2 A General procedure for native chemical ligation

2.2.1 Native Chemical Ligation (NCL)

The peptide hydrazide was cleanly and rapidly converted to peptide thioester in an epimerization-free manner by an operationally simple $NaNO_2$ activation and thiolysis process with 10 equiv. of $NaNO_2$ followed by 100 equiv. of mercaptophenylacetic acid (MPAA) at 100 mM concentration. The peptide bearing an N-terminal Cys was then directly added for NCL in a one-pot manner without the need to purify the peptide thioester. To monitor the progress of NCL reaction, approximately 10 μL of reaction mixture was taken out and placed into a 1.5 mL Eppendorf tube. Then 200 μL of diethyl ether (20/1, v/v) was added to quench the reaction. The mixture was centrifuged at 4000 rpm for 4 mins. After decanting the ether, the peptide was dissolved in MeCN/H_2O (1/1, v/v) at a concentration of 0.2 mM, filtered by 0.22 μm syringe filter, then analyzed by UPLC. Upon completion of the ligation, equal volume (about 0.4 mL) of 0.1 M TCEP neutral solution[1] was added to the reaction mixture and the solution was agitated at room temperature by magnetic stirring for 20 min.

To remove the excessive MPAA, which coelute with the ligation crude during HPLC, the reaction mixture after ligation was transferred to a 15 mL centrifuge tube with 10 equiv. of diethyl ether (v/v). The mixture was shaken vigorously and then the ether was carefully removed by pipetting. The wash was repeated for another 2 times, which minimized the amount of remaining MPAA in the ligation product after HPLC purification (Figure S1).

2.2.2 Desulfurization (deS)

After the assembly of all 4 Shc1 fragments, all cysteines at the 3 ligation sites on each of the 4 peptides (**1+2+3+4**-YYYS, **1+2+3+4-*pY***YYS, **1+2+3+4-*pYpY***YS, and **1+2+3+4-*pYpYpYpS*)** were converted to alanine by ADD desulfurization with TCEP/$NaBEt_4$ either in a separated step (for YYYS and ***pY***YYS), or performed in one-pot with the 3rd NCL. Reactions were performed following previously published procedure.[3] Briefly, 0.5M TCEP was prepared by dissolving TCEP·HCl in 0.2 M phosphate buffer with 6 M Gn·HCl, then adjusted the pH to 4.0-5.0 using 6 M NaOH (aq.). The peptide was dissolved in the TCEP solution to achieve a final concentration of 1.0 to 10.0 mg/ml. The peptide solution was incubated at room temperature for about 5 minutes to ensure disulfide bond reduction. A solution of $NaBEt_4$ in H_2O (approximately 0.5 to 1.0 g/ml) was prepared in a separate tube, then was carefully added to the peptide solution to achieve a final concentration of 0.1 M. The Eppendorf tube was sealed and shaken vigorously to ensure thorough mixing. The reaction was left for 30 seconds or a few minutes to allow the bubbles generated during the reaction to dissipate. To monitor the reaction progress, aliquots of the reaction solution were collected for UPLC analysis. If necessary, another portion of aqueous $NaBEt_4$ can be added to ensure complete desulfurization. Finally, the remaining reaction mixture was diluted with H_2O/MeCN for subsequent HPLC purification.

2.2.3 Deprotection of Cys302 (deACM)

The S-acetamidomethyl (S-ACM) protection on Cys302 was removed following an established

protocol.[4] Peptide was dissolved in aqueous solution containing 6 M Gn•HCl, 0.2 M Na_2HPO_4, and 40 mM TCEP (pH 7.0) to the concentration of 2 mM, after which 50 equiv. $PdCl_2$ was dissolved in the ligation buffer with 6 M Gn•HCl (0.2 M Na_2HPO_4, pH 7.0) to make the concentration of about 200 mM, and was sonicated for 10 min to ensure complete dissolution of $PdCl_2$. The clear $PdCl_2$ solution was added to the peptide solution to achieve a final concentration of 0.5 mM. The reaction mixture was stirred overnight at 37 °C. Subsequently, 250 equiv. 1 M DTT in the ligation buffer (pH 7.0) was added and the reaction mixture was stirred for 1 h, followed by centrifugation. The supernatant was injected for HPLC purification.

For most NCL, desulfurization, and deACM steps, the reactions were performed multiple times and optimized, and the best yields were reported below (Table S2).

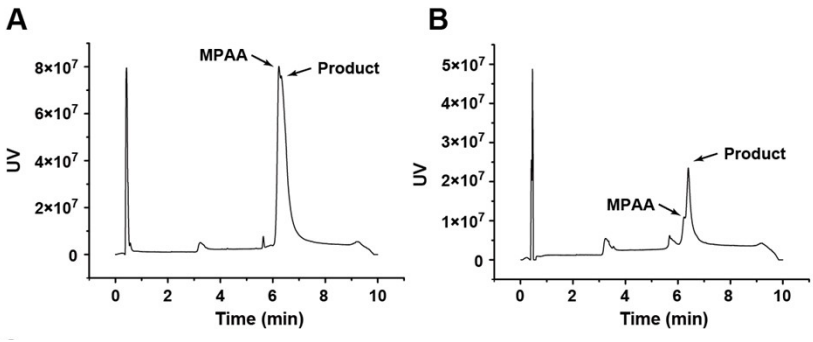


Figure S1. The remove of MPAA contamination. (A-B) HPLC traces of the NCL crude of fragment **1**+**2**+**3**-YYY before (A) or after (B) the ether wash.

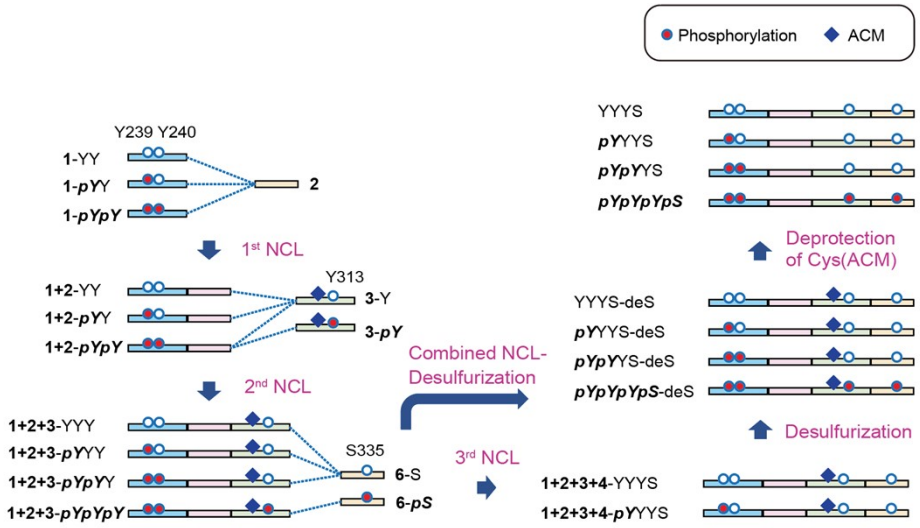


Figure S2. Overview of the assembly of 4 Shc1^{CH1} fragments by three NCLs (dash lines), one desulfurization, and one S-ACM deprotection step in total.

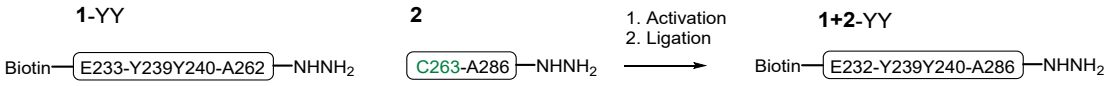
Table S2. Summary of the best yields for each NCL, desulfurization, and ACM removal step. Overall yield for each probe is calculated by multiplying the best yield of each synthetic step. The isolated yield marked by N/A are calculated as 100% as the related step was combined with the next step.

Fragment	1st NCL	2nd NCL	3rd NCL.	deS	deACM	Overall
YYYS	47%	32%	50%	80%	50%	3.0%
pYYYS	46%	56%	42%	81%	40%	3.5%
pYpYYS	60%	48%	N/A	40%	50%	5.8%
pYpYpYpS	See ***pYpY***YS	60%	N/A	54%	40%	7.8%

3. Synthesis of individual peptide

3.1 Synthesis of fragment YYYS

3.1.1 Ligation of Segment 1-YY with Segment 2



Shc1 Segment **1**-YY (7.0 mg, 1.9 μmol) was transferred to a 2 mL Eppendorf tube followed by 0.2 ml of ligation buffer (0.2 M sodium phosphate solution, 6 M Gn·HCl, pH 3.0-3.1).[1] The solution was vortexed and sonicated in an ultrasonic bath to ensure complete dissolvement of the peptide hydrazide. The tube was centrifuged at 7,200g for 1 min at room temperature to recover the solution that sticks to the tube wall, then placed into the -15 °C bath. The solution was gently agitated by magnetic stirring for 15 min. Meanwhile, 4.6 mg (1.8 μmol, 1.0 equiv.) of Shc1 Segment **2** and 6.8 mg of MPAA were dissolved in 0.2 ml of the ligation buffer. After adjusting the pH to 6.5 with 6 M NaOH (about 100 μL), the solution was vortexed and sonicated until everything was dissolved.

To oxidize the peptide hydrazide to the corresponding azide, 20 μl of 0.5 M $NaNO_2$ was pipetted into the **1**-YY solution, which was then gently agitated for 15 min at -15 °C in the ice/salt bath. Segment **2** and MPAA solution was subsequently added, which allowed simultaneous generation of peptide **1** thioester and its consumption by reacting with Segment **2**. The reaction tube was removed from the ice bath and warmed up to room temperature. The pH of the ligation reaction was adjusted to 6.8–7.0 with 6 M NaOH. The reaction tube was placed on a shaker for 4 h at room temperature. Upon completion, the reaction was quenched by TCEP solution. The reaction mixture was washed by diethyl ether according to the procedure mentioned above in the 2.3 section. The solution was then filtered by 0.45 μm syringe filter and injected for preparative HPLC. After lyophilization, 5.2 mg (0.84 μmol) of purified Segment **1**+**2**-YY were obtained (47% yield, Figure S3).

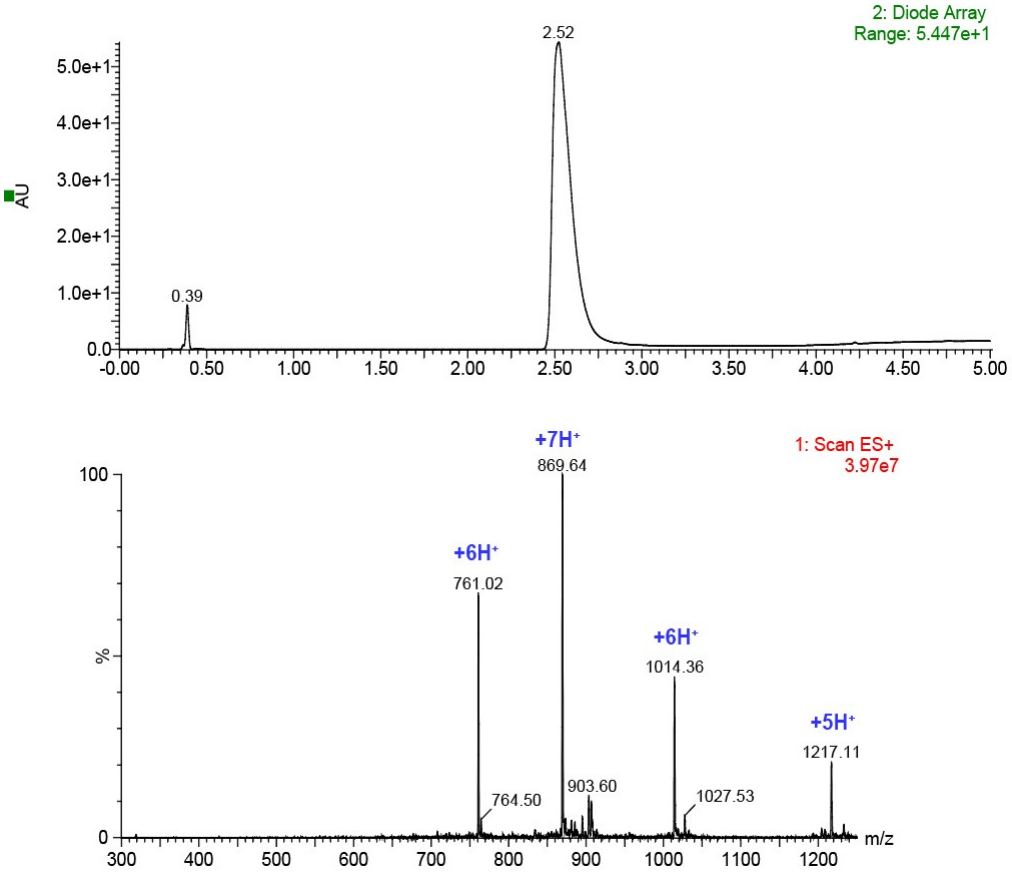
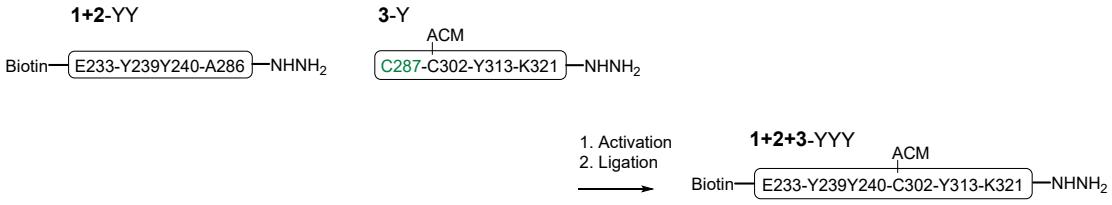


Figure S3. LC-MS of the HPLC purified Segment **1**+**2**-YY with a gradient of 5-95% MeCN/H_2O with 0.1% TFA over 5 min at a flow rate of 0.4 mL/min. The theoretical molecular weight of **1**+**2**-YY ($C_{265}H_{410}N_{78}O_{79}S_4$) is 6080.9020. Calcd. m/z for $[M+5H]^{5+}$ is 1217.19, found 1217.11, $[M+6H]^{6+}$ m/z = 1014.49, found 1014.36, $[M+7H]^{7+}$ m/z = 869.71, found 869.64, $[M+8H]^{8+}$ m/z = 761.12, found 761.02.

3.1.2 Ligation of Segment 1+2-YY with Segment 3-Y



The Segment **1**+**2**-YY (5.0 mg, 0.80 μmol, 1.0 equiv.) was ligated to the Segment **3**-Y (4.2 mg, 1.1 μmol) according to the procedure above, yielding 2.6 mg (0.26 μmol) of purified Segment **1**+**2**+**3**-YYY (32% yield, Figure S4).

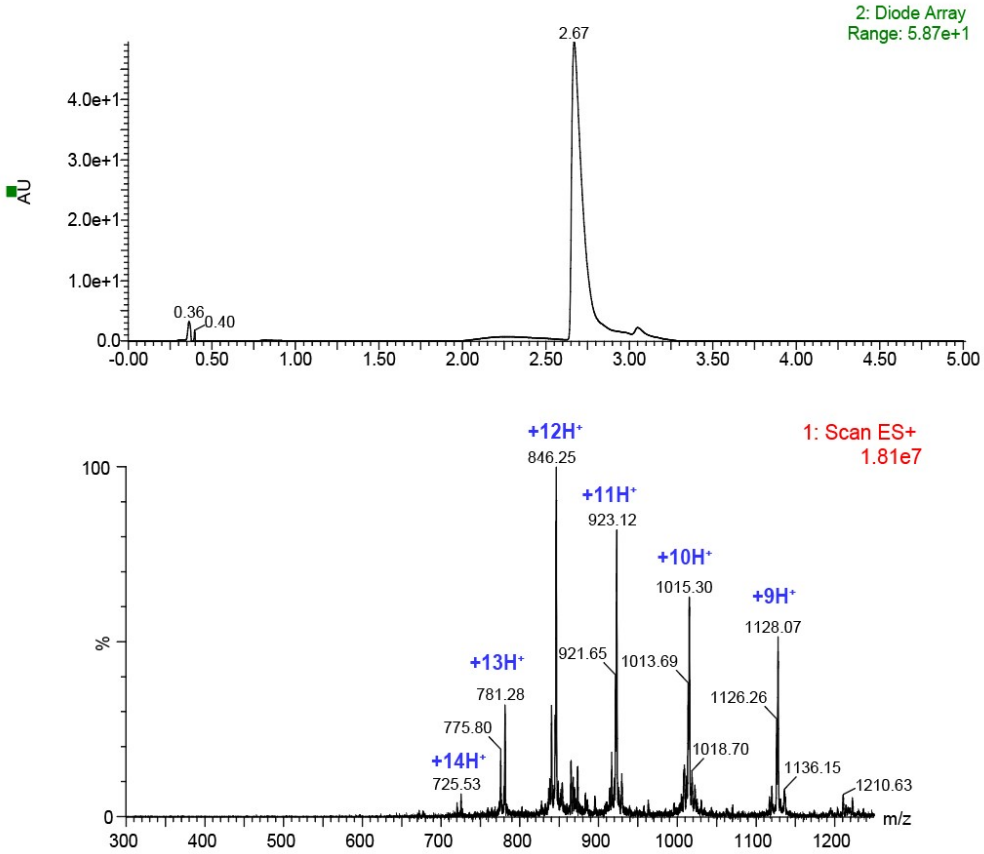


Figure S4. LC-MS of the purified **1**+**2**+**3**-YYY with a gradient of 5-95% MeCN/H_2O with 0.1% TFA over 5 min at a flow rate of 0.4 mL/min. The theoretical molecular weight of **1**+**2**+**3**-YYY ($C_{442}H_{684}N_{128}O_{133}S_7$) is 10143.517. ESI-MS calcd. for $[M+9H]^{9+}$ m/z = 1128.07, found 1128.07, $[M+10H]^{10+}$ m/z = 1015.36, found 1015.30, $[M+11H]^{11+}$ m/z = 923.14, found 923.12, $[M+12H]^{12+}$ m/z = 846.30, found 846.25, $[M+13H]^{13+}$ m/z = 781.28, found 781.28.

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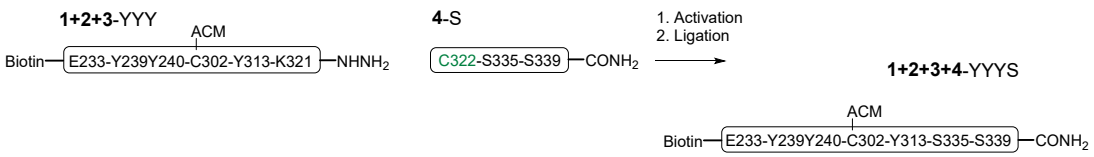
3.1.3 Ligation of Segment 1+2+3-YYY with Segment 4-S



The Segment **1**+**2**+**3**-YYY (2.0 mg, 0.20 µmol) was ligated to the Segment **4**-S (0.80 mg, 0.49 µmol) according to the procedure above, yielding 1.2 mg (0.10 µmol) of purified **1**+**2**+**3**+**4**-YYY (50% yield, Figure S5).

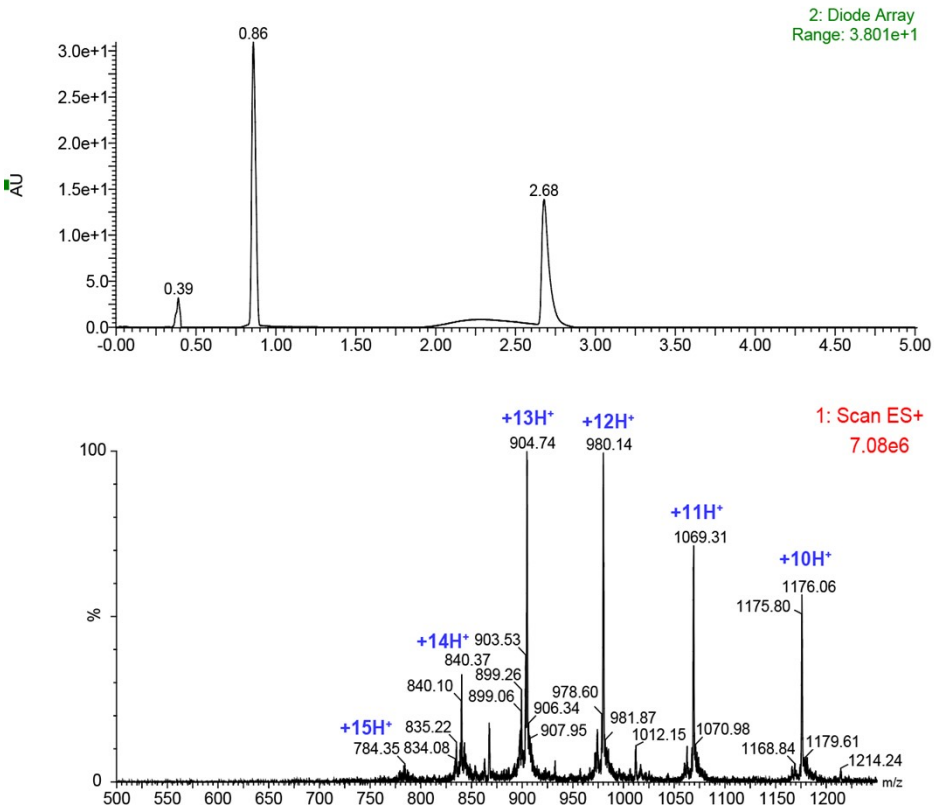
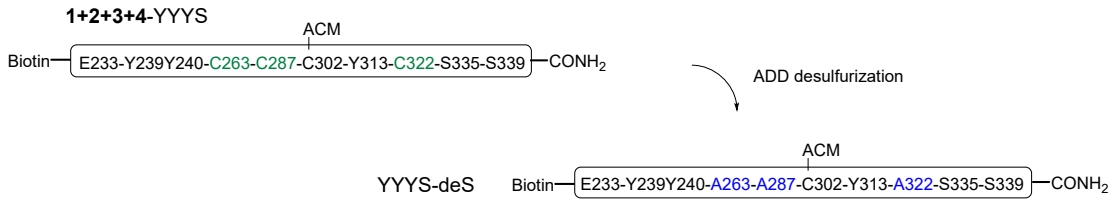


Figure S5. LC-MS of the HPLC purified **1**+**2**+**3**+**4**-YYYS with a gradient of 5-95% MeCN/H_2O with 0.1% TFA over 8 min at a flow rate of 0.4 mL/min. The theoretical molecular weight of **1**+**2**+**3**+**4**-YYYS ($C_{507}H_{786}N_{150}O_{157}S_8$) is 11751.238. ESI-MS calcd. for $[M+10H]^{10+}$ m/z = 1176.13, found 1176.06, $[M+11H]^{11+}$ m/z = 1069.30, found 1069.31, $[M+12H]^{12+}$ m/z = 980.28, found 980.14, $[M+13H]^{13+}$ m/z = 904.95, found 904.74, $[M+14H]^{14+}$ m/z = 840.38, found 840.37.

3.1.4 Desulfurization of 1+2+3+4-YYYS to YYYS-deS



The **1**+**2**+**3**+**4**-YYYS (1.8 mg, 0.15 µmol) was desulfurized according to the procedure above, yielding 1.4 mg (0.12 µmol) of purified YYYS-deS (80% yield, Figure S6).

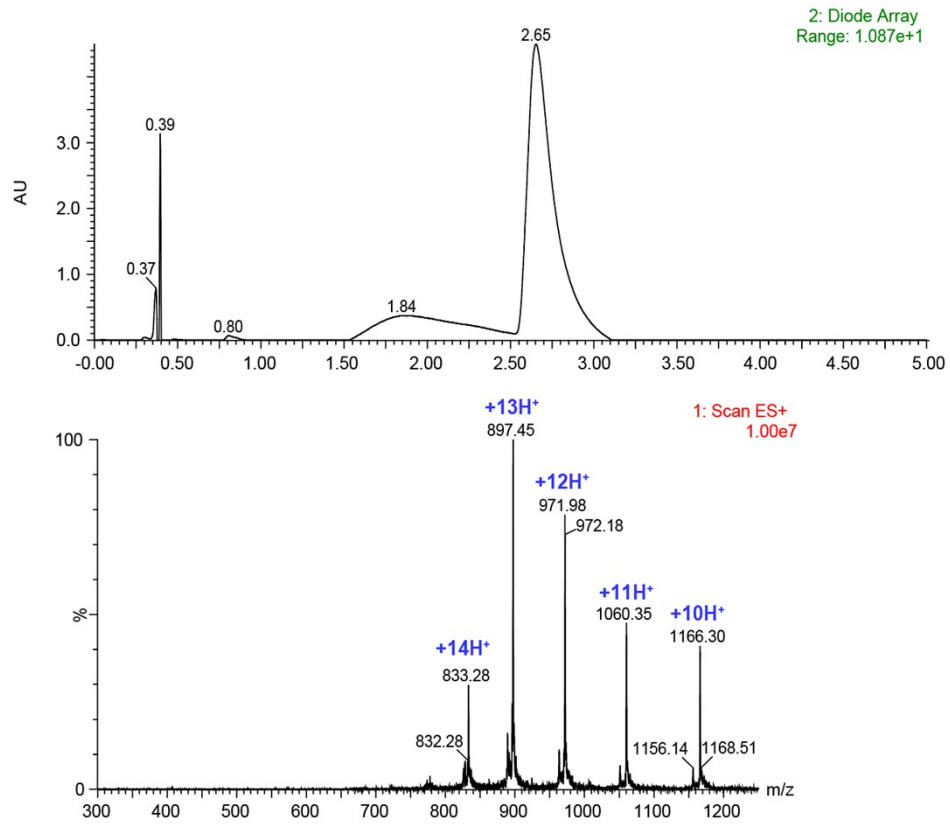
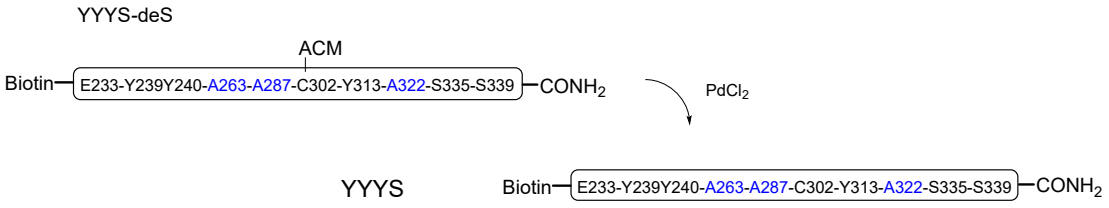


Figure S6. LC-MS of the HPLC purified YYYS-deS with a gradient of 5-95% MeCN/H_2O with 0.1% TFA over 5 min at a flow rate of 0.4 mL/min. The theoretical molecular weight of YYYS-deS ($C_{507}H_{786}N_{150}O_{157}S_5$) is 11655.058. ESI-MS calcd. for $[M+10H]^{10+}$ m/z = 1166.51, found 1166.30, $[M+11H]^{11+}$ m/z = 1060.56, found 1060.36, $[M+12H]^{12+}$ m/z = 972.26, found 972.18, $[M+13H]^{13+}$ m/z = 897.55, found 897.45, $[M+14H]^{14+}$ m/z = 833.51, found 833.28.

3.1.5 Removal of ACM protection of YYYS-deS to produce fragment YYYS



The desulfurized YYYS-deS (0.80 mg, 0.069 μmol) with ACM-protected Cys302 was deprotected by $PdCl_2$ according to the procedure above, yielding 0.40 mg (0.034 μmol) of purified fragment YYYS (50% yield, Figure S7).

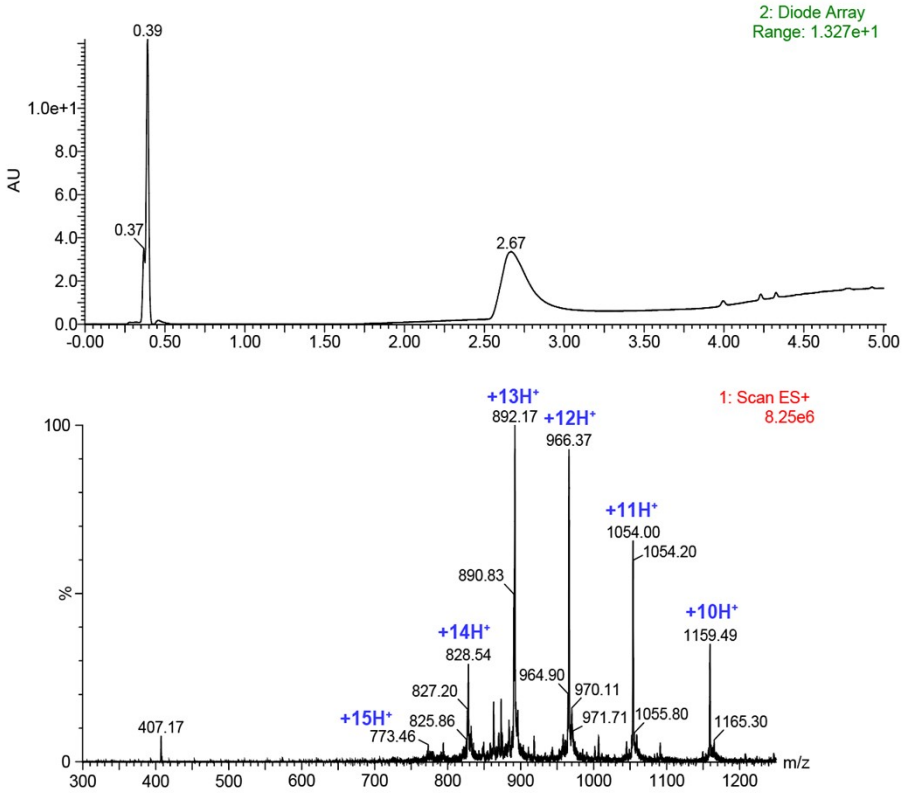
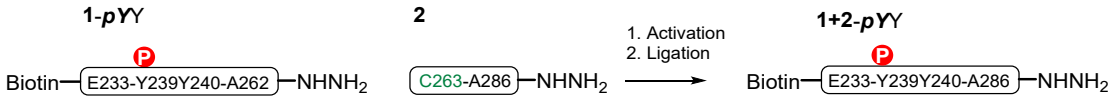


Figure S7. LC-MS of the HPLC purified fragment YYYS with a gradient of 5-95% MeCN/H_2O with 0.1% TFA over 5 min at a flow rate of 0.4 mL/min. The theoretical molecular weight of fragment YYYS ($C_{504}H_{781}N_{149}O_{156}S_5$) is 11583.979. ESI-MS calcd. for $[M+10H]^{10+}$ m/z = 1159.40, found 1159.49, $[M+11H]^{11+}$ m/z = 1054.09, found 1054.00, $[M+12H]^{12+}$ m/z = 966.34, found 966.37, $[M+13H]^{13+}$ m/z = 892.08, found 892.17, $[M+14H]^{14+}$ m/z = 828.43, found 828.54.

3.2 Synthesis of fragment *pY*YYS

3.2.1 Ligation of Segment 1-*pY*Y with Segment 2



Segment **1**-***pY***Y (5.0 mg, 1.3 μmol) was ligated to Segment **2** (2.6 mg, 1.0 μmol) according to the procedure above, yielding 2.9 mg (0.46 μmol) of purified Segment **1+2**-***pY***Y (46% yield, Figure S8).

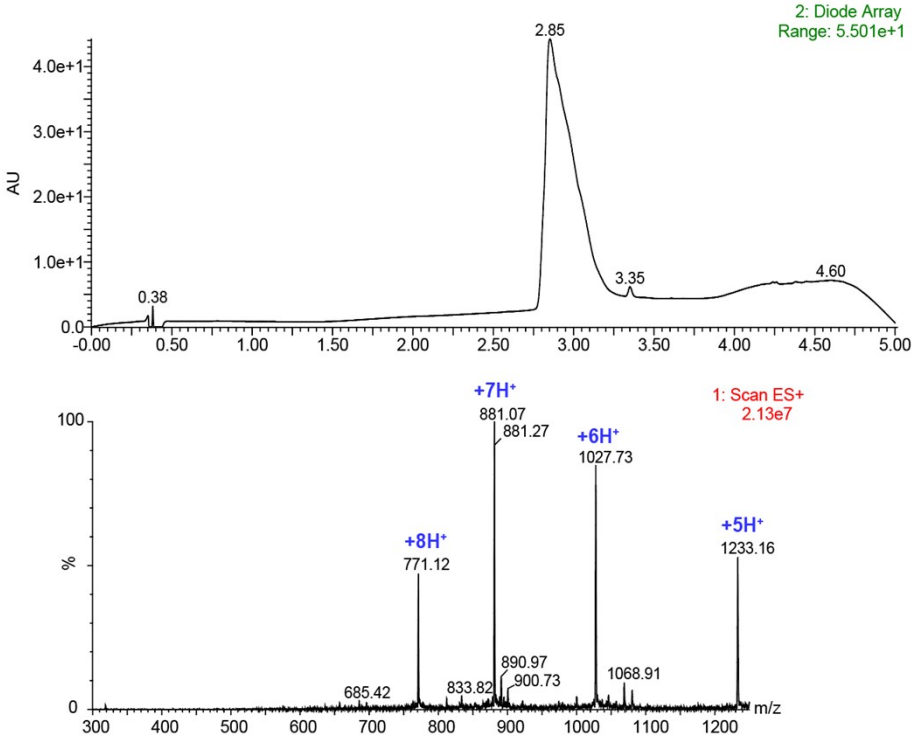
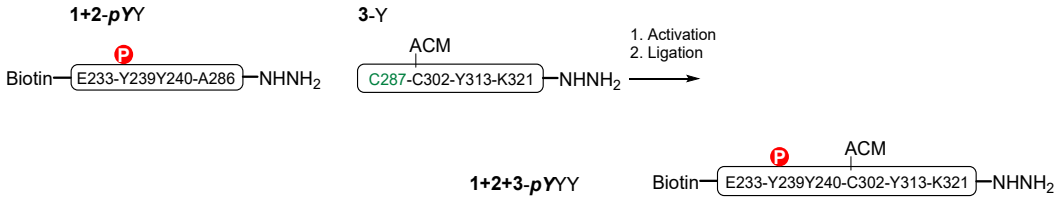


Figure S8. LC-MS of the HPLC purified Segment **1+2-*pY*Y** with a gradient of 5-95% MeCN/H_2O with 0.1% TFA over 5 min at a flow rate of 0.4 mL/min. The theoretical molecular weight of **1+2-*pY*Y** ($C_{265}H_{411}N_{78}O_{82}PS_4$) is 6160.8808. ESI-MS calcd. for $[M+5H]^{5+}$ m/z = 1233.18, found 1217.16, $[M+6H]^{6+}$ m/z = 1027.82, found 1027.73, $[M+7H]^{7+}$ m/z = 881.13, found 881.07, $[M+8H]^{8+}$ m/z = 771.12, found 771.12.

3.2.2 Ligation of Segment 1+2-*pY*Y with Segment 3-Y



The Segment **1+2-*pY*Y** (2.0 mg, 0.32 μmol) was ligated to Segment **3**-Y (2.5 mg, 0.65 μmol) according to the procedure above, yielding 1.8 mg (0.18 μmol) of purified Segment **1+2+3-*pY*YY** (56% yield, Figure S9).

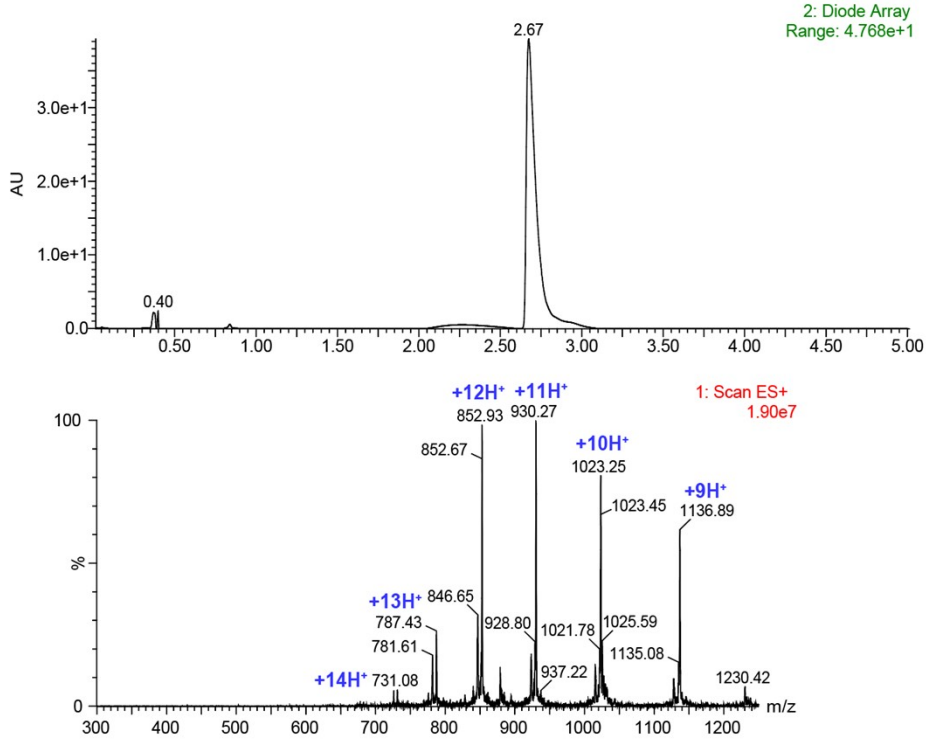
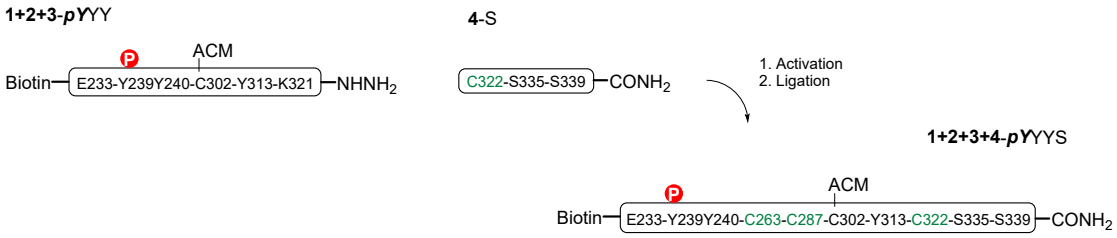


Figure S9. LC-MS of the HPLC purified Segment **1**+**2**+**3**-***pY***YY with a gradient of 5-95% MeCN/H_2O with 0.1% TFA over 5 min at a flow rate of 0.4 mL/min. The theoretical molecular weight of **1**+**2**+**3**-***pY***YY ($C_{442}H_{685}N_{128}O_{136}PS_7$) is 10223.4958. ESI-MS calcd. for $[M+9H]^{9+}$ m/z = 1136.95, found 1136.89, $[M+10H]^{10+}$ m/z = 1023.35, found 1023.25, $[M+11H]^{11+}$ m/z = 930.42, found 930.27, $[M+12H]^{12+}$ m/z = 852.96, found 852.93, $[M+13H]^{13+}$ m/z = 787.43, found 787.43.

3.2.3 Ligation of Segment 1+2+3-*pY*YY with Segment 4-S



The Segment **1**+**2**+**3**-***pY***YY (1.0 mg, 0.10 µmol) was ligated to Segment **4**-S (0.5 mg, 0.31 µmol) according to the procedure above, yielding 0.5 mg (42 nmol) of purified **1**+**2**+**3**+**4**-***pY***YYS (42% yield, Figure S10).

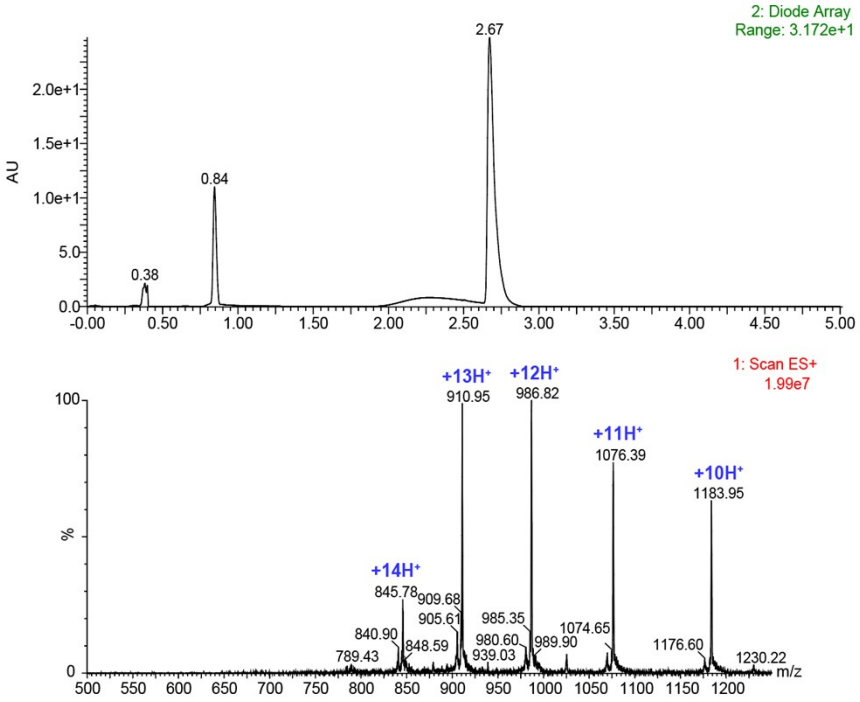
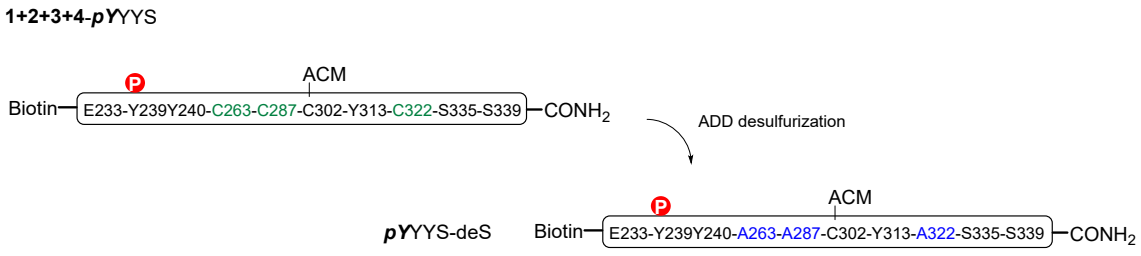


Figure S10. LC-MS of the HPLC purified **1+2+3+4-*pY*YYS** with a gradient of 5-95% MeCN/H_2O with 0.1% TFA over 5 min at a flow rate of 0.4 mL/min. The theoretical molecular weight of **1+2+3+4-*pY*YYS** ($C_{507}H_{787}N_{150}O_{160}PS_8$) is 11831.2168. ESI-MS calcd. for $[M+10H]^{10+}$ m/z = 1184.13, found 1183.95, $[M+11H]^{11+}$ m/z = 1076.57, found 1076.39, $[M+12H]^{12+}$ m/z = 986.94, found 986.82, $[M+13H]^{13+}$ m/z = 911.10, found 910.95, $[M+14H]^{14+}$ m/z = 846.09, found 845.78.

3.2.4 Desulfurization of 7-*pY*YYS to *pY*YYS-deS



The **1+2+3+4-*pY*YYS** (0.5 mg, 42 nmol) was desulfurized according to the procedure above, yielding 0.4 mg (34 nmol) of ***pY*YYS-deS** (81% yield, Figure S11).

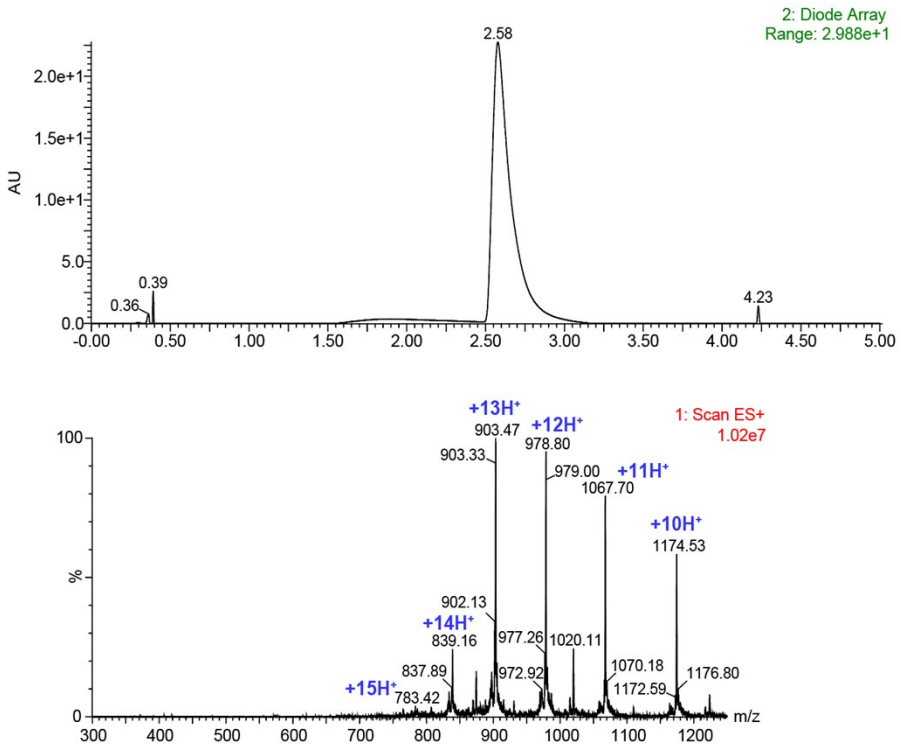
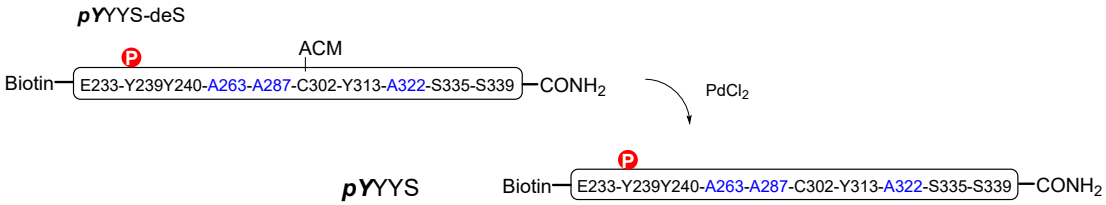


Figure S11. LC-MS of the HPLC purified ***pY***YYS-deS with a gradient of 5-95% MeCN/H_2O with 0.1% TFA over 5 min at a flow rate of 0.4 mL/min. The theoretical molecular weight of ***pY***YYS-deS ($C_{507}H_{787}N_{150}O_{160}PS_5$) is 11735.0368. ESI-MS calcd. for $[M+10H]^{10+}$ m/z = 1174.51, found 1174.53, $[M+11H]^{11+}$ m/z = 1067.83, found 1067.70, $[M+12H]^{12+}$ m/z = 978.93, found 978.80, $[M+13H]^{13+}$ m/z = 903.70, found 903.47, $[M+14H]^{14+}$ m/z = 839.22, found 839.16.

3.2.5 Removal of ACM protection of *pY*YYS-deS to produce fragment *pY*YYS



The desulfurized ***pY***YYS-deS (1.5 mg, 0.13 μmol) with ACM-protected Cys302 was deprotected by $PdCl_2$ according to the procedure above, yielding 0.60 mg (0.051 μmol) of purified fragment ***pY***YYS (40% yield, Figure S12).

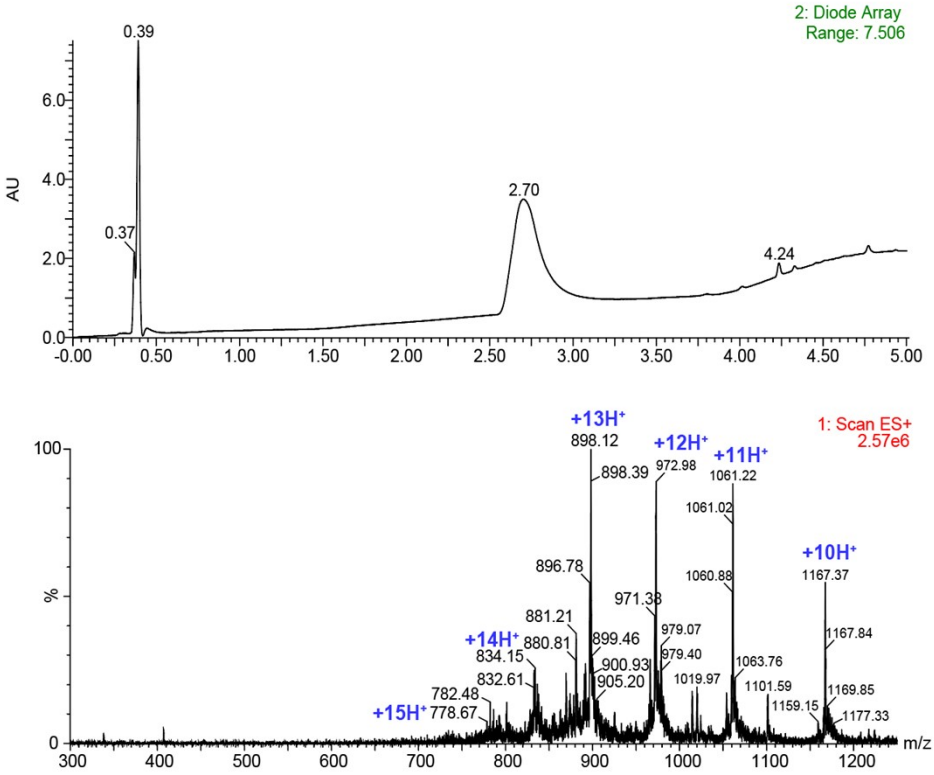
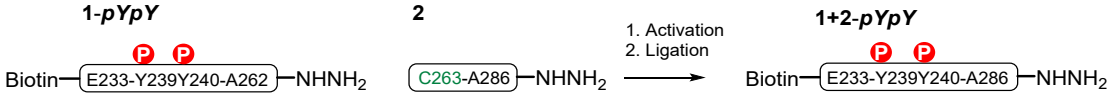


Figure S12. LC-MS of the HPLC purified fragment ***pY***YYS with a gradient of 5-95% MeCN/H_2O with 0.1% TFA over 5 min at a flow rate of 0.4 mL/min. The theoretical molecular weight of fragment ***pY***YYS ($C_{504}H_{782}N_{149}O_{159}PS_5$) is 11663.9578. ESI-MS calcd. for $[M+10H]^{10+}$ m/z = 1167.40, found 1167.37, $[M+11H]^{11+}$ m/z = 1061.36, found 1061.22, $[M+12H]^{12+}$ m/z = 973.00, found 972.98, $[M+13H]^{13+}$ m/z = 898.23, found 898.12, $[M+14H]^{14+}$ m/z = 834.15, found 834.15.

3.3 Synthesis of fragment *pYpY*YS

3.3.1 Ligation of Segment 1-*pYpY* with Segment 2



Segment **1-*pYpY*** (3.0 mg, 0.77 μmol) was ligated to Segment **2** (2.0 mg, 0.80 μmol) according to the procedure above, yielding 2.9 mg (0.46 μmol) of purified Segment **1+2-*pYpY*** (60% yield, Figure S13).

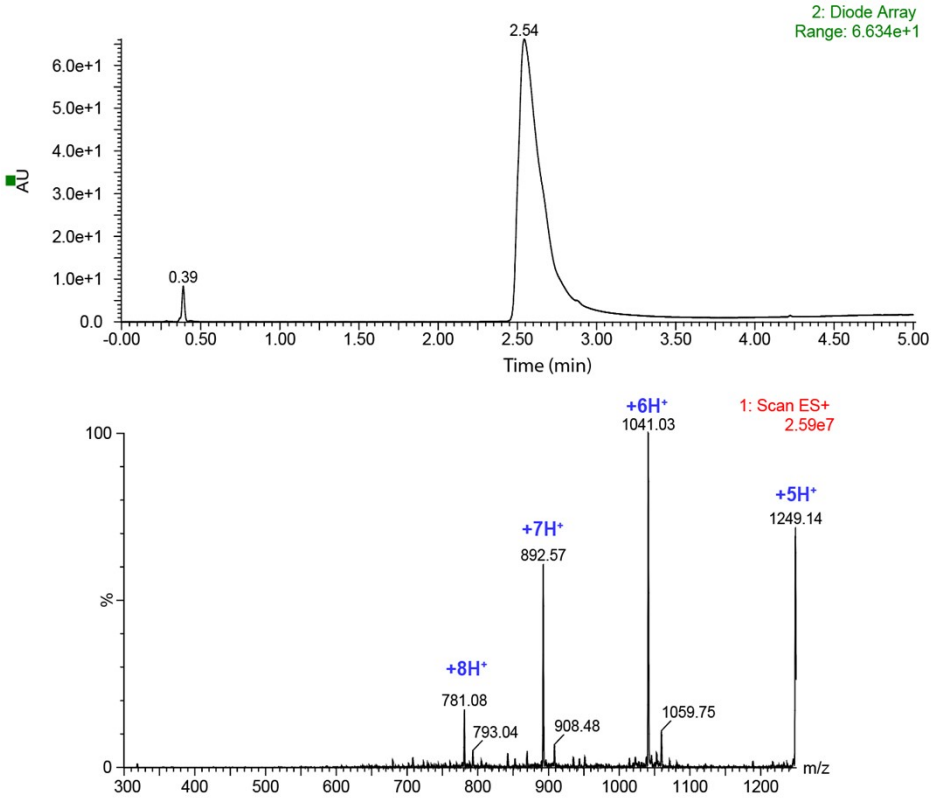
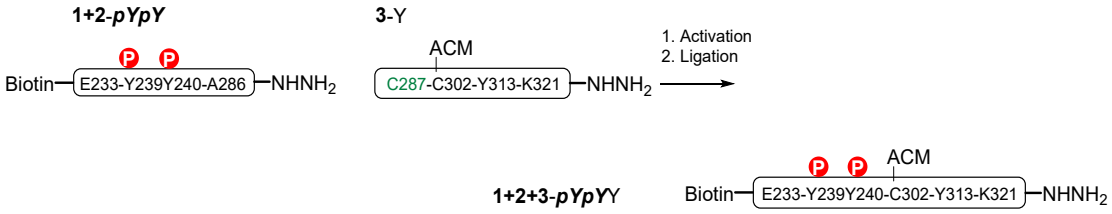


Figure S13. LC-MS of the HPLC purified Segment **1**+**2**-***pYpY*** with a gradient of 5-95% MeCN/H_2O with 0.1% TFA over 5 min at a flow rate of 0.4 mL/min. The theoretical molecular weight of **1**+**2**-***pYpY*** ($C_{265}H_{412}N_{78}O_{85}P_2S_4$) is 6240.8595. ESI-MS calcd. for $[M+5H]^{5+}$ m/z = 1249.18, found 1249.14, $[M+6H]^{6+}$ m/z = 1041.15, found 1041.03, $[M+7H]^{7+}$ m/z = 892.56, found 892.57, $[M+8H]^{8+}$ m/z = 781.12, found 781.08.

3.3.2 Ligation of Segment 1+2-*pYpY* with Segment 3-Y



The Segment **1**+**2**-***pYpY*** (1.0 mg, 0.16 μmol) was ligated to the Segment **3**-Y (0.8 mg, 0.20 μmol) according to the procedure above, yielding 0.8 mg (77 nmol) of purified Segment **1**+**2**+**3**-***pYpY***Y (48% yield, Figure S14).

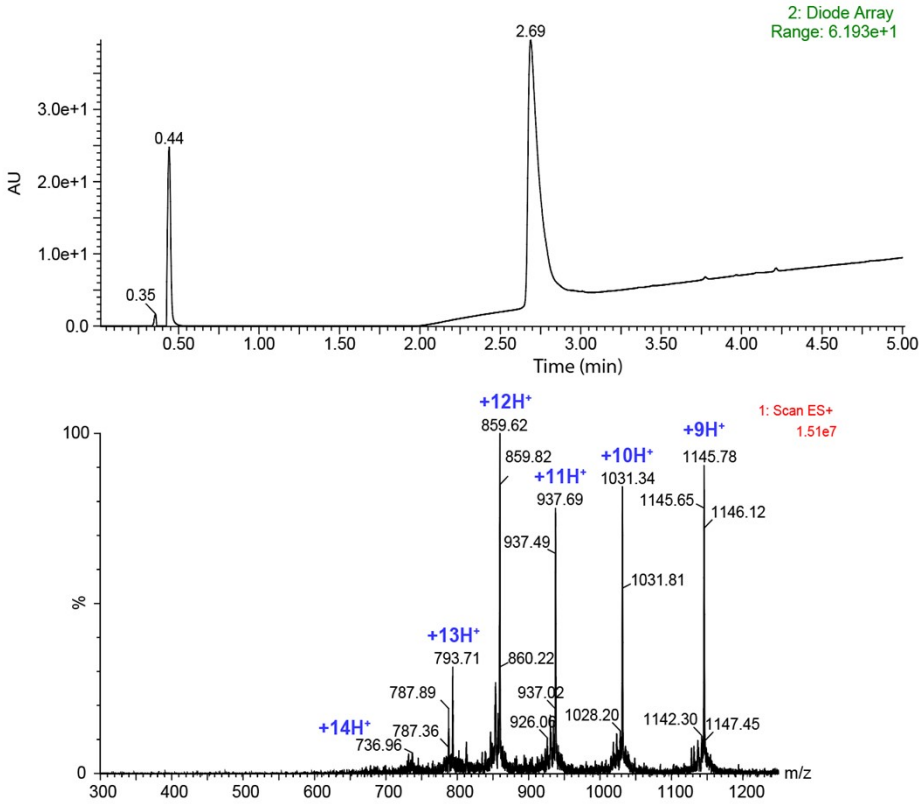
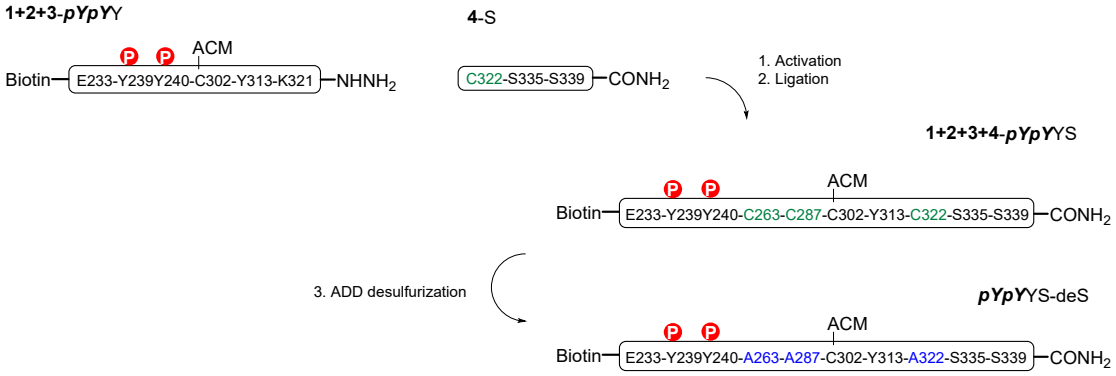


Figure S14. LC-MS of the HPLC purified Segment **1+2+3-*pYpY*Y** with a gradient of 5-95% MeCN/H_2O with 0.1% TFA over 5 min at a flow rate of 0.4 mL/min. The theoretical molecular weight of Segment **1+2+3-*pYpY*Y** ($C_{442}H_{686}N_{128}O_{139}P_2S_7$) is 10303.4745. ESI-MS calcd. for $[M+9H]^{9+}$ m/z = 1145.84, found 1145.78, $[M+10H]^{10+}$ m/z = 1031.35, found 1031.34, $[M+11H]^{11+}$ m/z = 937.69, found 937.69, $[M+12H]^{12+}$ m/z = 859.63, found 859.62, $[M+13H]^{13+}$ m/z = 793.58, found 793.71.

3.3.3 Ligation of Segment 1+2+3-*pYpY*Y with Segment 4-S, followed by desulfurization



The Segment **1+2+3-*pYpY*Y** (6.0 mg, 0.58 μmol, 1.0 equiv.) was ligated to Segment **4**-S (3 mg, 0.6 μmol, 1.0 equiv.) according to the procedure above., After extraction of the MPAA, the solution

further diluted to around 600 μM by 0.2 M phosphate buffer containing 0.5M TCEP, and acidified by adding HCl solution to pH 4.5-5.0. Subsequently, the ligated crude peptide was subjected to ADD desulfurization in one pot by proportionally adding aqueous $NaBEt_4$ (15~30 mg in 30~60 μL H_2O) and stirred for a few minutes to allow the completion of the reaction. The crude was purified by RP-HPLC (20-40% MeCN/H_2O with 0.1% TFA over 60 min), and 1.7 mg (0.23 μmol) ***pYpY***YS-deS was obtained as a white powder after lyophilization (40% yield over NCL and desulfurization, Figure S15).

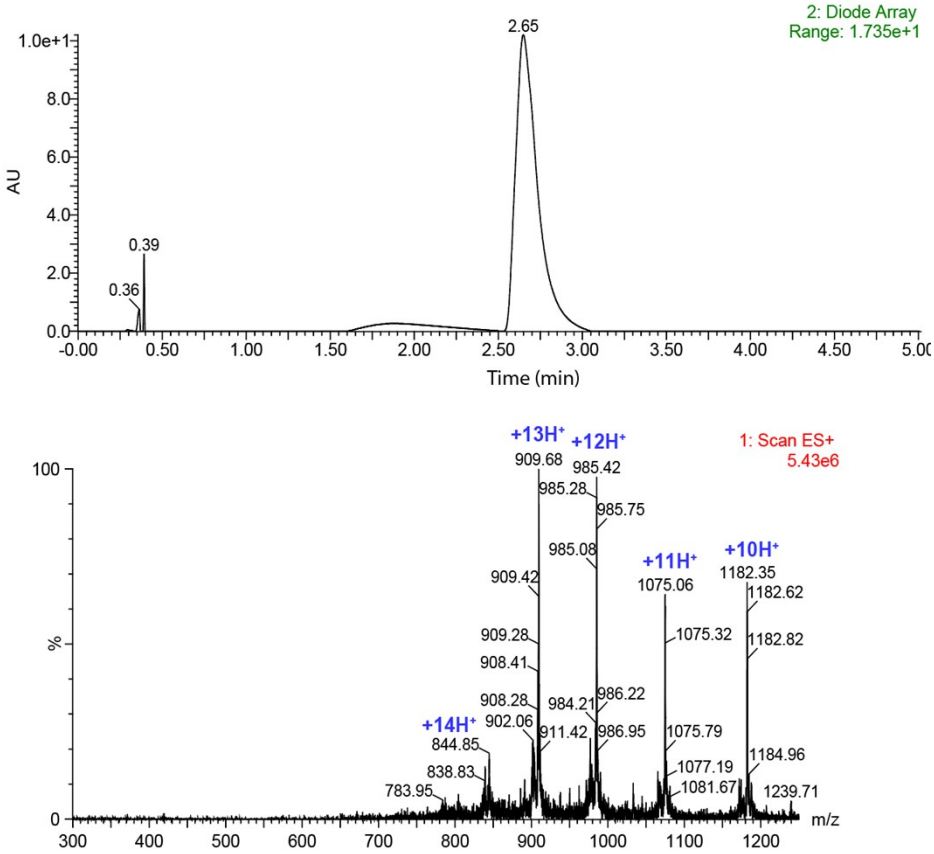
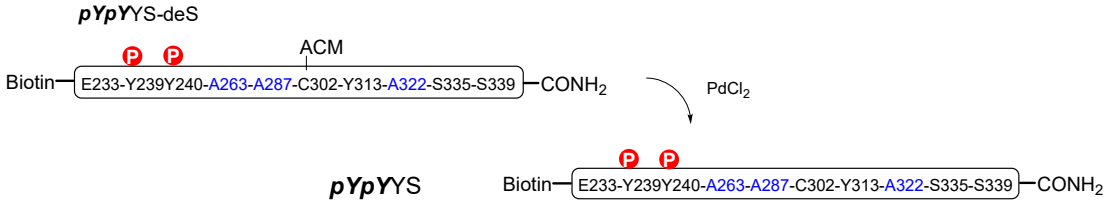


Figure S15. LC-MS of the HPLC purified ***pYpY***YS-deS with a gradient of 5-95% MeCN/H_2O with 0.1% TFA over 5 min at a flow rate of 0.4 mL/min. The theoretical molecular weight of ***pYpY***YS-deS ($C_{507}H_{788}N_{150}O_{163}P_2S_5$) is 11815.0155. ESI-MS calcd. for $[M+10H]^{10+}$ m/z = 1182.51, found 1182.35, $[M+11H]^{11+}$ m/z = 1075.10, found 1075.06, $[M+12H]^{12+}$ m/z = 985.59, found 985.42, $[M+13H]^{13+}$ m/z = 909.85, found 909.68, $[M+14H]^{14+}$ m/z = 844.94, found 844.85.

3.3.4 Removal of ACM protection of *pYpY*YS-deS to produce fragment *pYpY*YS



The desulfurized ***pYpY***YS-deS (2.5 mg, 0.21 μmol) with ACM-protected Cys302 was deprotected by $PdCl_2$ according to the procedure above, yielding 1.20 mg (0.11 μmol) of purified fragment ***pY***YYS (50% yield, Figure S16).

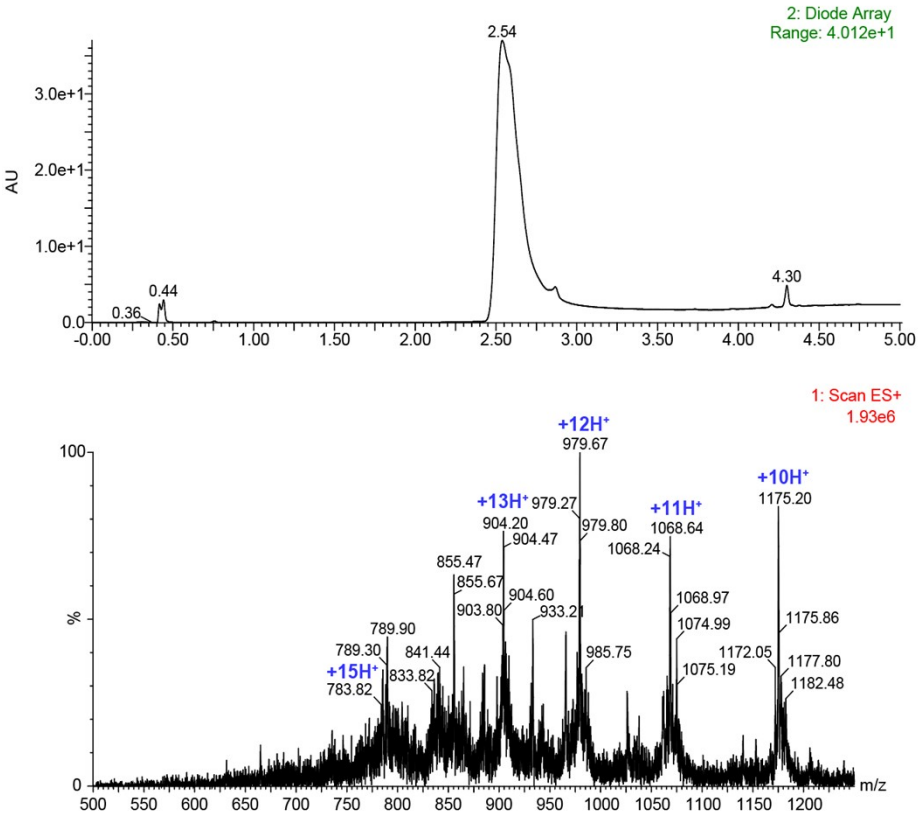
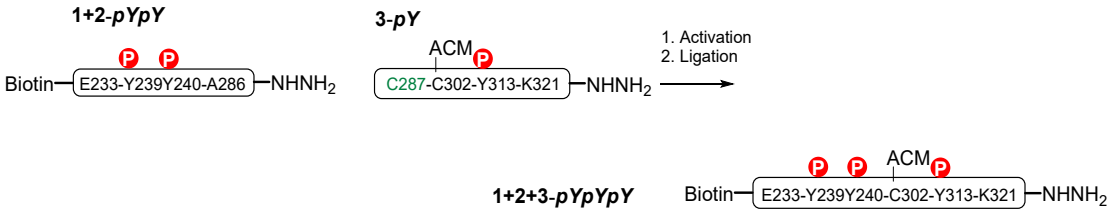


Figure S16. LC-MS of the HPLC purified fragment ***pYpY***YS with a gradient of 5-95% MeCN/H_2O with 0.1% TFA over 5 min at a flow rate of 0.4 mL/min. The theoretical molecular weight of fragment ***pYpY***YS ($C_{504}H_{783}N_{149}O_{162}P_2S_5$) is 11743.9365. ESI-MS calcd. for $[M+10H]^{10+}$ m/z = 1175.40, found 1175.20, $[M+11H]^{11+}$ m/z = 1068.64, found 1068.64, $[M+12H]^{12+}$ m/z = 979.67, found 979.67, $[M+13H]^{13+}$ m/z = 904.38, found 904.20, $[M+14H]^{14+}$ m/z = 839.86, found 841.44, $[M+15H]^{15+}$ m/z = 783.94, found 783.82.

3.4 Synthesis of fragment *pYpYpYpS*

3.4.1 Ligation of Segment 1+2-*pYpY* with Segment 3-*pY*



The Segment **1+2-*pYpY*** (1.0 mg, 0.16 µmol, 1.0 equiv., see section 3.3.1) was ligated to Segment **3-*pY*** (0.80 mg, 0.20 µmol) according to the procedure above, yielding 1.0 mg (96 nmol) of purified Segment **1+2+3-*pYpYpY*** (60% yield, Figure S17).

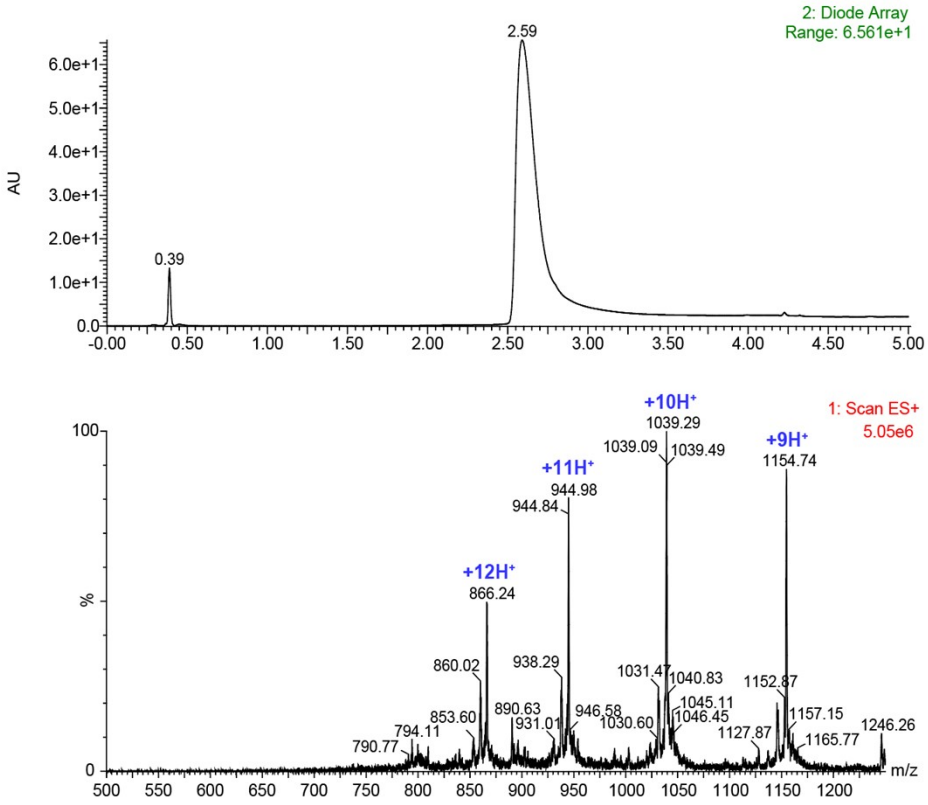
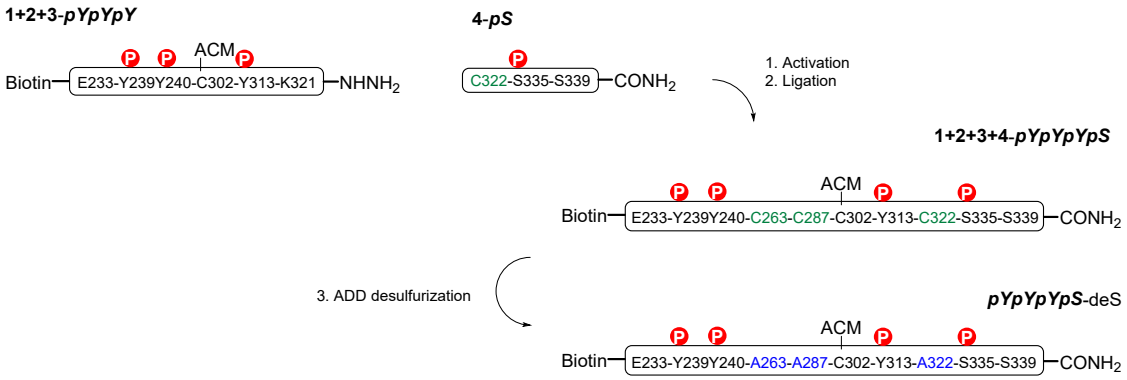


Figure S17. LC-MS of the HPLC purified Segment **1+2+3-*pYpYpY*** with a gradient of 5-95% MeCN/H_2O with 0.1% TFA over 5 min at a flow rate of 0.4 mL/min. The theoretical molecular weight of **1+2+3-*pYpYpY*** ($C_{442}H_{687}N_{128}O_{142}P_3S_7$) is 10383.4533. ESI-MS calcd. for $[M+9H]^{9+}$ m/z = 1154.72, found 1154.74, $[M+10H]^{10+}$ m/z = 1039.35, found 1039.29, $[M+11H]^{11+}$ m/z = 944.96, found 944.98, $[M+12H]^{12+}$ m/z = 866.30, found 866.24.

3.4.2 Ligation of Segment 1+2+3-*pYpYpY* with Segment 4-*pS*, followed by desulfurization



The Segment **1+2+3-*pYpYpY*** (3.0 mg, 0.29 μmol, 1.0 equiv.) was ligated to Segment **4-*pS*** (1.5 mg, 0.87 μmol), followed by desulfurization according to the procedure above, yielding 1.9 mg (0.16 μmol) of purified ***pYpYpYpS***-deS (54% yield, Figure S18).

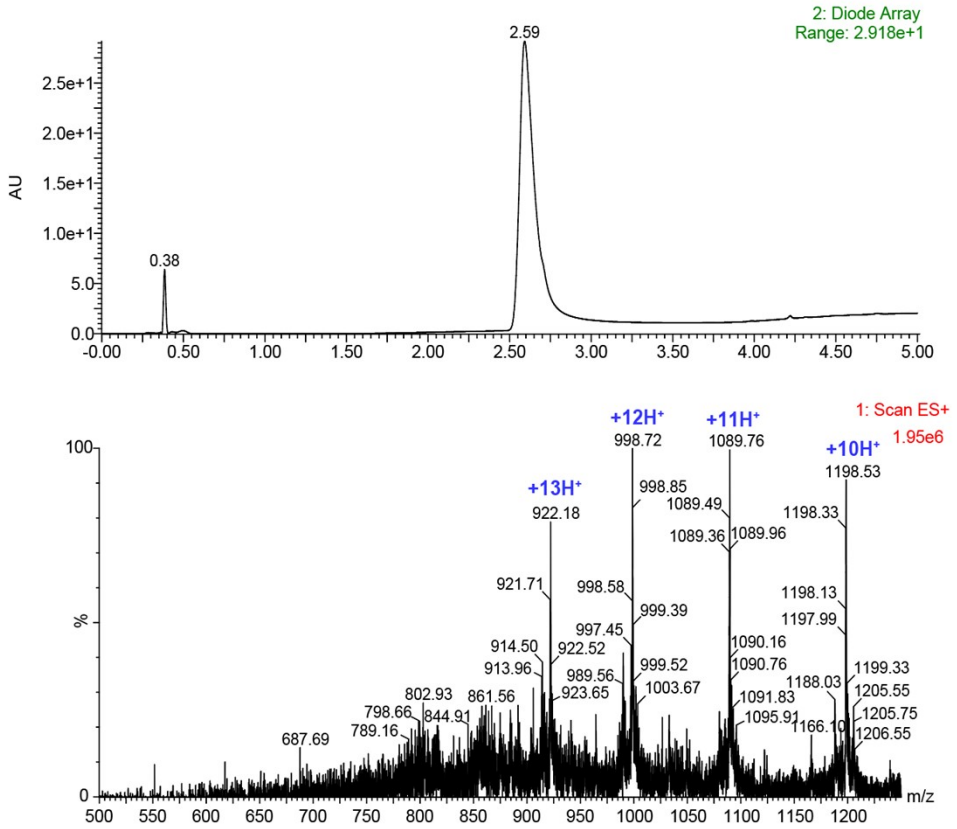
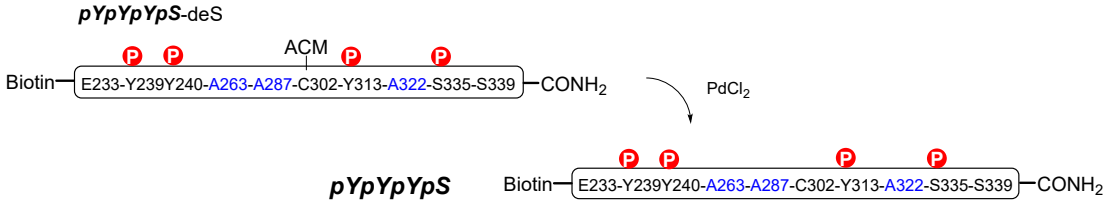


Figure S18. LC-MS of the HPLC purified ***pYpYpYpS***-deS with a gradient of 5-95% MeCN/H_2O with 0.1% TFA over 5 min at a flow rate of 0.4 mL/min. The theoretical molecular weight of ***pYpYpYpS***-deS ($C_{507}H_{790}N_{150}O_{169}P_4S_5$) is 11974.973. ESI-MS calcd. for $[M+10H]^{10+}$ m/z = 1198.51, found 1198.53, $[M+11H]^{11+}$ m/z = 1089.64, found 1089.76, $[M+12H]^{12+}$ m/z = 998.92, found 998.72, $[M+13H]^{13+}$ m/z = 922.16, found 922.18.

3.4.3 Removal of ACM protection of *pYpYpYpS*-deS to produce fragment *pYpYpYpS*



The desulfurized ***pYpYpYpS***-deS (0.90 mg, 75 nmol) with ACM-protected Cys302 was deprotected by $PdCl_2$ according to the procedure above, yielding 0.36 mg (30 nmol) of purified fragment ***pY***YYS (40% yield, Figure S19).

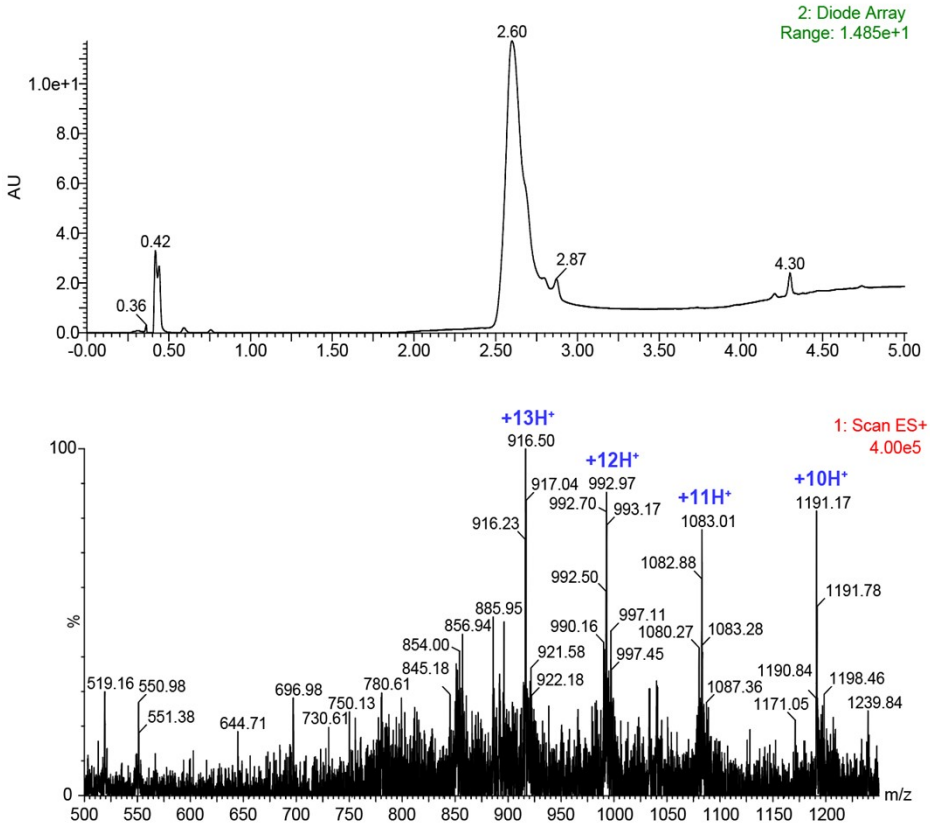


Figure S19. LC-MS of the HPLC purified fragment ***pYpYpYpS*** with a gradient of 5-95% MeCN/H_2O with 0.1% TFA over 5 min at a flow rate of 0.4 mL/min. The theoretical molecular weight of fragment ***pYpYpYpS*** ($C_{504}H_{785}N_{149}O_{168}P_4S_5$) is 11903.894. ESI-MS calcd. for $[M+10H]^{10+}$ m/z = 1191.39, found 1191.17, $[M+11H]^{11+}$ m/z = 1083.18, found 1083.01, $[M+12H]^{12+}$ m/z = 992.99, found 992.97, $[M+13H]^{13+}$ m/z = 916.69, found 916.50, $[M+14H]^{14+}$ m/z = 851.28, found 851.18.

4. Cell line construction and cell culture

Mouse breast cancer cell line 4T1 was gifted from Prof. Ying Sun in Southern University of Science and Technology. The human breast cancer cell lines MDA-231 (MDA-MB-231), MDA-468 (MDA-MB-468) and BT-474 were purchased from American Type Culture Collection (ATCC). The mouse 4T1 and human BT-474 cell lines were cultured in RPMI 1640 Medium (Corning). The MDA-231,

MDA-468, and engineered Hela cell lines were cultured in DMEM medium (Corning). All cells were cultured in mediums supplemented with 10% (v/v) FBS and at 37 °C in 5% CO_2. The human cervical cancer cell line Hela stably transfected with GFP/mShc1 WT/mutants was constructed according to the published protocol.[5] The mouse p52Shc1 with N-terminal FLAG and eGFP tags was cloned into the pCAGGS expression vector（XhoI-2xFlag-EGFP- Shc1 cDNA -EcoRI). And Shc1phosphorylation site mutants were generated by a QuikChange™ Site-Directed Mutagenesis Kit (Stratagene) with the following primers (forward): Y239F: GACCATCAG**TTC**TACAATGAC TTTCCAGGGAAGGAACCCCCTCTTGG; Y240F: GACCATCAGTAC**TTC**AATGACTTT CCAGGGAAGGAACCCCCTCTTGG; Y313F: TCTTCGATGACCCCTCC**TTT**GTCAACATC CAGAAT; 3F (Y313/239/240F): CCCCCTGACCAT CAG**TTCTTC**AATGACTTTCCAGGG, and TCTTCGATGACCCCTCC**TTT**GTCAACATC CAGAAT, with the mutation sites indicated by the bold characters. The plasmids with wild type or mutant p52Shc1 were transfected into Hela cells according to Lipofectamine™ 3000 Reagent protocol (Polyplus 114-75). Hela cells was seeded to be around 80% confluent. Plasmid and reagent were incubated with the plasmid-lipid complex, which was adopted by cells through 2-4 days of incubation. The acquired cells were sorted by fluorescence activated cell sorting cycles (FACS) according to the eGFP signal.

5. Fully integrated spintip-based AP (FISAP)

Cells were harvested after reaching 80% confluence either by centrifugation (for 4T1 and BT-474 cells) or trypsin digestion (for MDA-231, MDA-468, and Hela WT/mutants). Cell lysate was prepared according to the published protocol and was diluted to 1 mg/mL by the lysis buffer [25 mM Tris·HCl, pH7.4, 150 mM NaCl, 1% (v/v) Nonidet P-40, supplemented with home-made protease and phosphatase inhibitors] according to the concentration measured by Pierce Protein Assay Reagent (Thermo).[6] Peptide AP-MS experiments was performed with 1.5 μL Pierce streptavidin agarose beads suspension (Thermo Scientific, 0.75 μL bead volume) loaded with 0.6 nmol biotinated peptide probe. After loading, 100 μL cell lysate was added to the spin tip. After AP, the sample was desalted following the published integrated protocol.[7] For AP-western blot, the AP experiment was scale up with 1.5 μL bead volume. After AP, the bead section was cut off from the pipette tip and directly boiled with the loading buffer for western blot.

6. LC-MS analysis and proteomic data procession for AP-MS

The desalted sample was redissolved in 10 μL 0.1% (v/v) formic acid and 2 μL was injected for proteomic analysis on Thermo Fisher Q Exactive HF-X mass spectrometer coupled with Easy-nLC 1000 nano HPLC system according to published protocol.[8] The MS data was acquired in DDA mode and searched using MaxQuant against either Mus musculus or Homo sapiens proteomes downloaded from UniProt database. Among the tryptic peptides from $Shc1^{CH1}$ fragments, the peptide “EPPLGGVVDMR” has the highest MS/MS count in all our peptide AP-MS experiments (QC-1C). Before statistical analysis by Perseus, the LFQ of all proteins in each sample was normalized according to the intensity of this most abundant tryptic peptide.

7. Shc1 IP-MS

Hela-GFP, Hela-dt-Shc1, Hela-dt-Shc1-Y239F, Hela-dt-Shc1-Y240F, Hela-dt-Shc1-Y313F, Hela-dt-Shc1-Y239240F, Hela-dt-Shc1-Y239313F, Hela-dt-Shc1-Y240313F, Hela-dt-Shc1-3F cell lines were each cultured in one 15 cm dish to 90% confluence (dt: double-tagged). All cells except the EGF- groups were stimulated by murine EGF (PeproTech Cat#: 315-09, source: *E.coli*, 100 ng/mL) for 5 min before quenching by ice-cold PBS buffer, then lysed in 1 mL lysis buffer for each culture dish. The anti-flag M2 beads were treated with 5% BSA in lysis buffer at 4 °C overnight, then washed by the lysis buffer. The treated bead slurry (12 μL, about 50% bead volume) was then mixing with each diluted cell lysate (8 mL) and incubated at 4 °C for 4-6 h. The beads were then collected by centrifugation at 1500 rpm for 3 min, and transferred to a 1.5 mL EP tube, washed by 1 mL lysis buffer 3 times, then 50 mM ammonium bicarbonate buffer twice before on-bead digestion. The samples were desalted with the sample protocol as that in peptide AP-MS and analyzed by Thermo Fisher Q Exactive HF mass spectrometer. For the database search, mouse p52Shc1 (P98083-2) sequence from UniProt was added to the fasta file to ensure the generation of the correct LFQ of the bait protein for normalization.

8. Western blot and proteomic analysis of mouse and human breast cancer cell lines

The western blot analysis of EGFR and HER2 in mouse 4T1, human MDA-231, MDA-468, and BT-474 cell lines were conducted with the loading of 15 μg cell lysate for each lane (Figure S24A). The proteomic sample of MDA-231, MDA-468, and BT-474 were prepared by Bayomics SISPROT SE kit (10 μg cell lysate for each sample), and analyzed by Thermo Fisher Q Exactive HF-X mass spectrometer in DDA mode, and processed with the same settings as that in AP-MS analysis (Figure S24B-C).

9. Structure prediction of the Shc1^{CH1} complexes by AlphaFold3

The AlphaFold3 structure prediction was performed on https://alphafoldserver.com/. The 107 AA sequence of mouse Shc1^{CH1}-***pYpYpYpS***, with 4 PTMs were entered to chelate with the full-length mouse version of Grb2, Pik3r1, and Ubash3b with 1:1 ratio. The canonical sequence of these prey proteins was downloaded from UniProt (uniprot.org) and was entered in full. The prediction result was downloaded from the AlphaFold3 server and further edited by PyMOL (version 3.0.3) to reveal residues forming polar interactions with the phosphate group on pY, which were calculated by PyMOL with the default setting of the distance (Figure 4C, S21). In each case, the prediction with the highest probability ranking among the 5 given structures by AlphaFold3 was shown.

10. Data availability

All associated MS raw data files have been deposited to the ProteomeXchange Consortium via the PRIDE partner repository (https://www.ebi.ac.uk/pride/) with the dataset identifier PXD049113. The reviewer account details are as below.

Username: reviewer_pxd049113@ebi.ac.uk

Password: gptV0Fwe

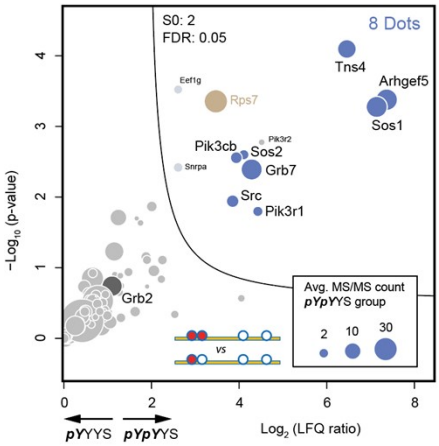


Figure S20. Volcano plot of the AP-MS experiment comparing Shc1^{CH1} fragments ***pYpY***YS with ***pY***YYS.

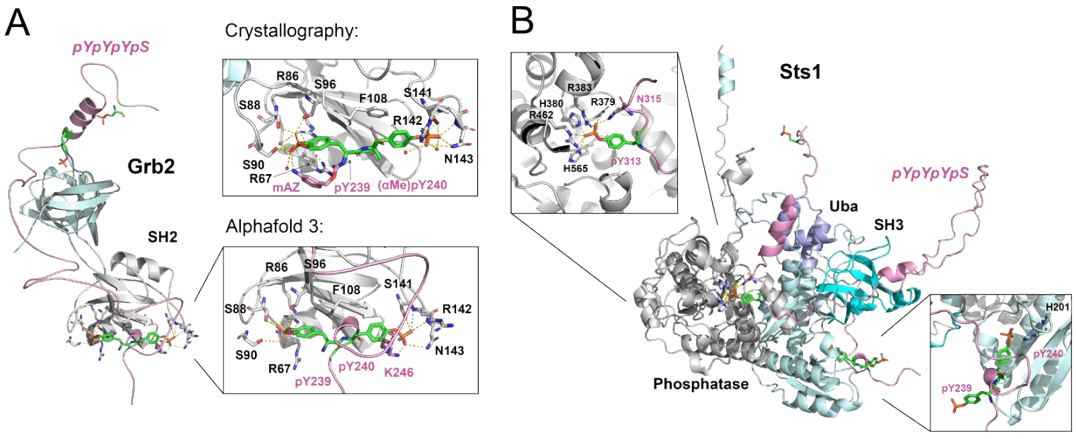


Figure S21. Predicted structures of the pY-dependent protein complexes with Shc1^{CH1}-***pYpYpYpYpS*** by AlphaFold3, with the mouse sequences for all entries. (A) Comparing AlphaFold3 prediction of mouse Grb2-***pYpYpYpYpS*** complex with the crystallography data of human GRB2 SH2 domain in complex with Shc1-peptide mimic mAZ-pY-(αMe)pY-N-NH_2, with mAZ (3-aminobenzoylcarbonyl) as the N-terminal protection, and α-methylation on pY240 (PDB 1JYQ). (B) Predicted complex of mouse Sts1 to ***pYpYpYpYpS***. In all graphics, the Shc1^{CH1} peptide ***pYpYpYpYpS*** were colored in pink, and the interacting domains on the preys to pY239-240 or pY313 were colored in grey.

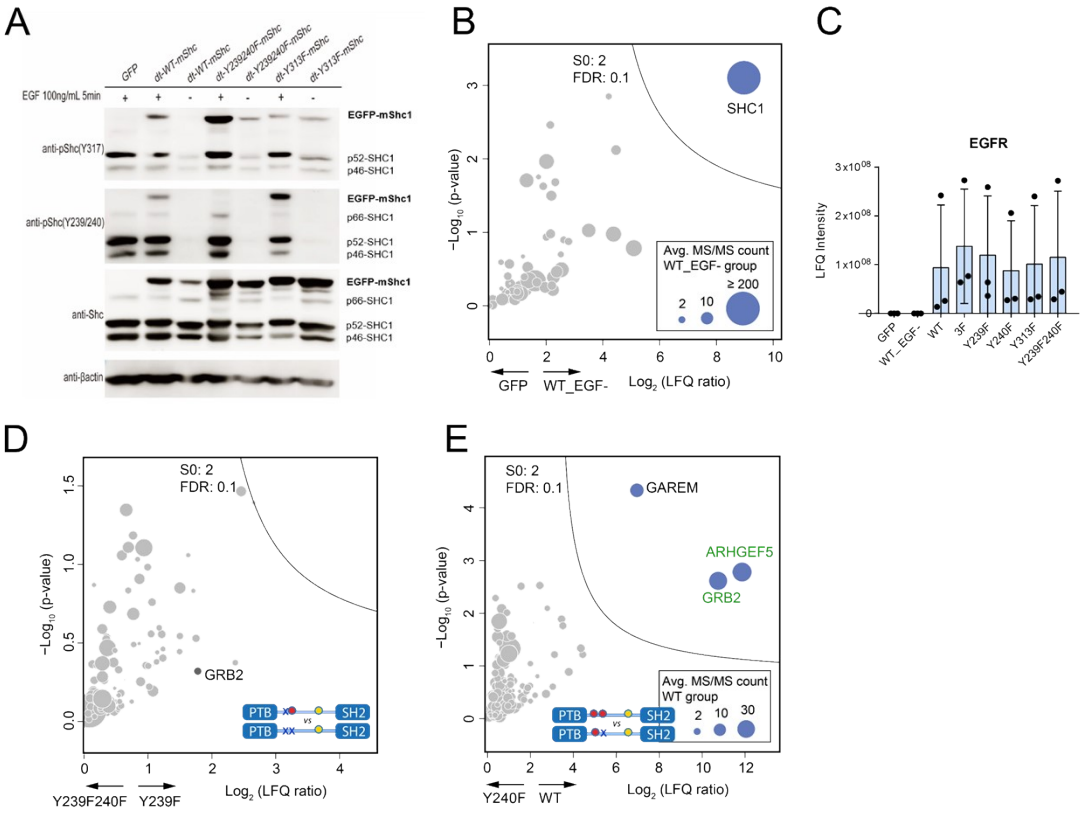


Figure S22. IP-MS in Hela cells stably transformed with a tagged mouse Shc1 for the wild type (WT) or mutated Shc1 to confirm its site-specific interactions. (A) WB of cell lysate for various stable cell lines with mouse Shc1 (mShc1) WT or mutant for the detection of Y239/240 or Y313 (Y317 in human) phosphorylation. Intrinsic human SHC1 isoforms including p46, p52 and p66 were also detected. (B-E) IP-MS validation in Hela cells stably transformed with a tagged mouse Shc1 for the wild type (WT) or mutated Shc1 to confirm its site-specific interactions. (B) Volcano plots of the IP-MS experiments to compare the interactome of unstimulated Shc1 WT (WT_EGF-) with that of the GFP. (C) LFQ intensity of EGFR in the IP-MS experiment. (D-E) Volcano plots of the IP-MS experiments comparing the interactomes between stimulated Shc1 mutants Y239FY240F and Y239F (D), or between Y240F and WT (E).

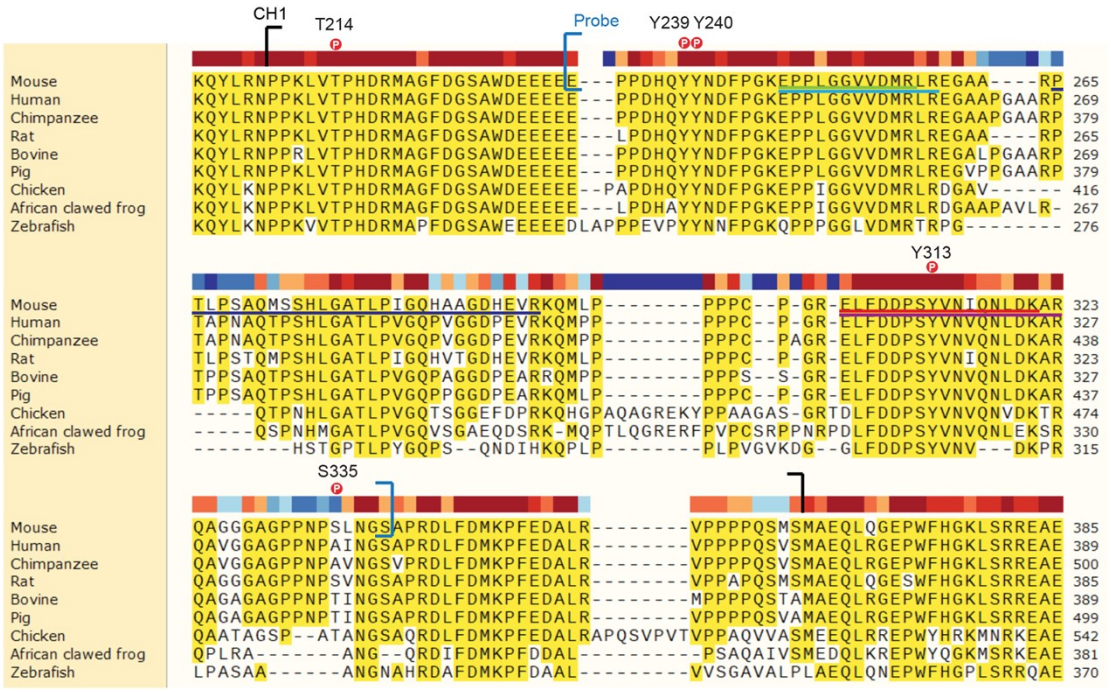


Figure S23. Sequence alignment of Shc1^{CH1} from mouse, human, and other species. Phosphorylation sites of mouse Shc1^{Ch1} were marked by the red dots. The sequence conservation was represented by the yellow blocks. The CH1 region was marked by black lines.[9] The 107 AA section covered by our synthetic fragments was bracketed. The 5 typical tryptic peptides from Shc1^{CH1} fragments identified by MS were underlined with corresponding colors (QC-1C).

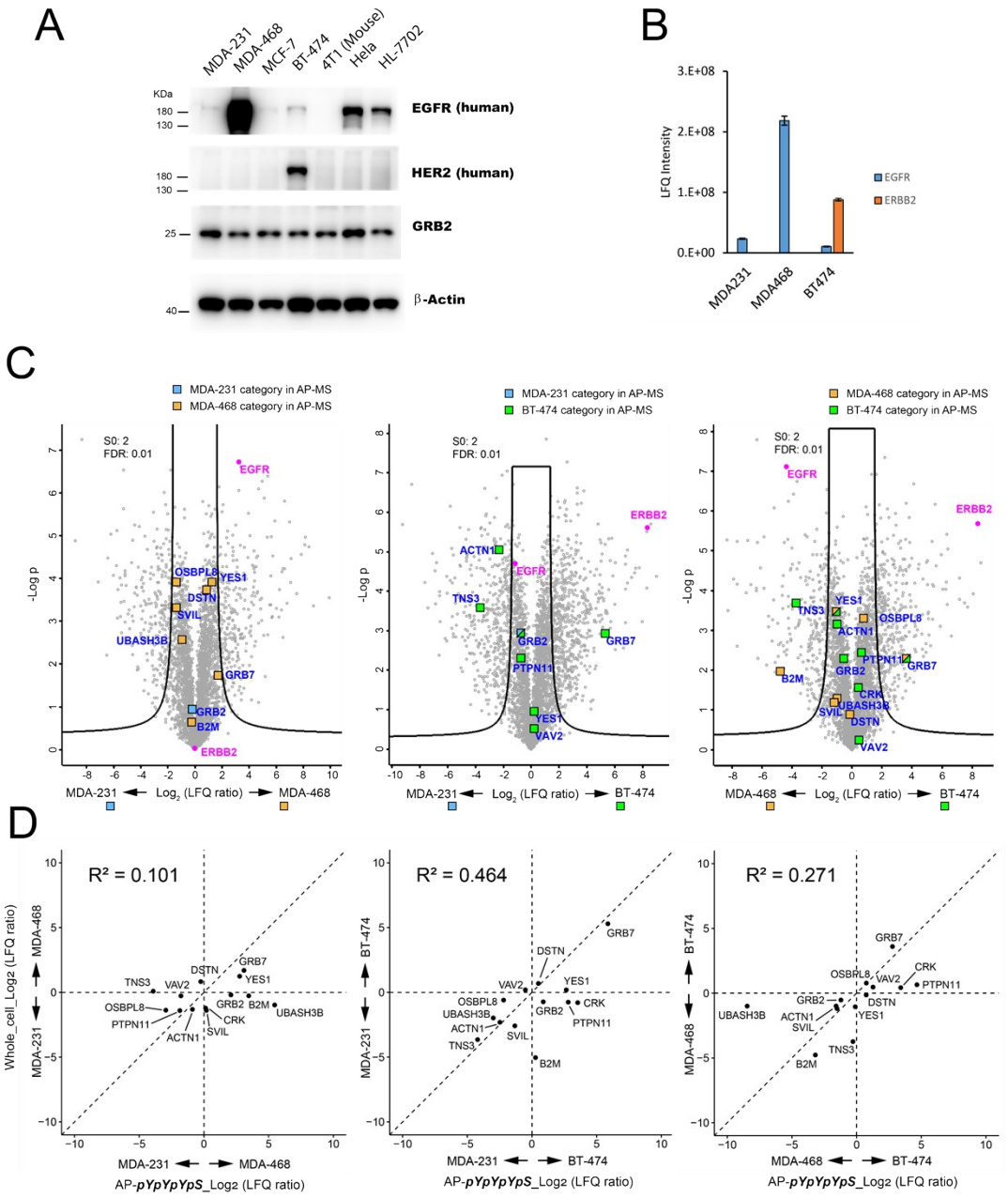


Figure S24. Western blot and proteomic analysis of various human and mouse cell lysate. (A) Western blot analysis of available human and mouse breast cancer cell lines. Antibodies against human variant of the targeting proteins were applied. (B) LFQ of EGFR and HER2 (ERBB2) in 3 human breast cancer cell lines: MDA-231 (EGFR/HER2 -/-), MDA-468 (EGFR/HER2 +/-), and BT-474 (EGFR/HER2 -/+). (C) Volcano plots comparing the proteomes of three human cell lines. EGFR and ERBB2 were highlighted in pink. Interacting proteins identified by our AP-MS analysis in the corresponding cell lines are shown with their categories in the AP-MS, which are indicated by the color of their square (**Figure 5D**). (D) Scatter plots showing the correlation of Log_2 LFQ ratios between Shc1^{CH1}-***pYpYpYpS*** AP-MS and whole cell proteomic analysis for the selected proteins shown above across the three cell lines.

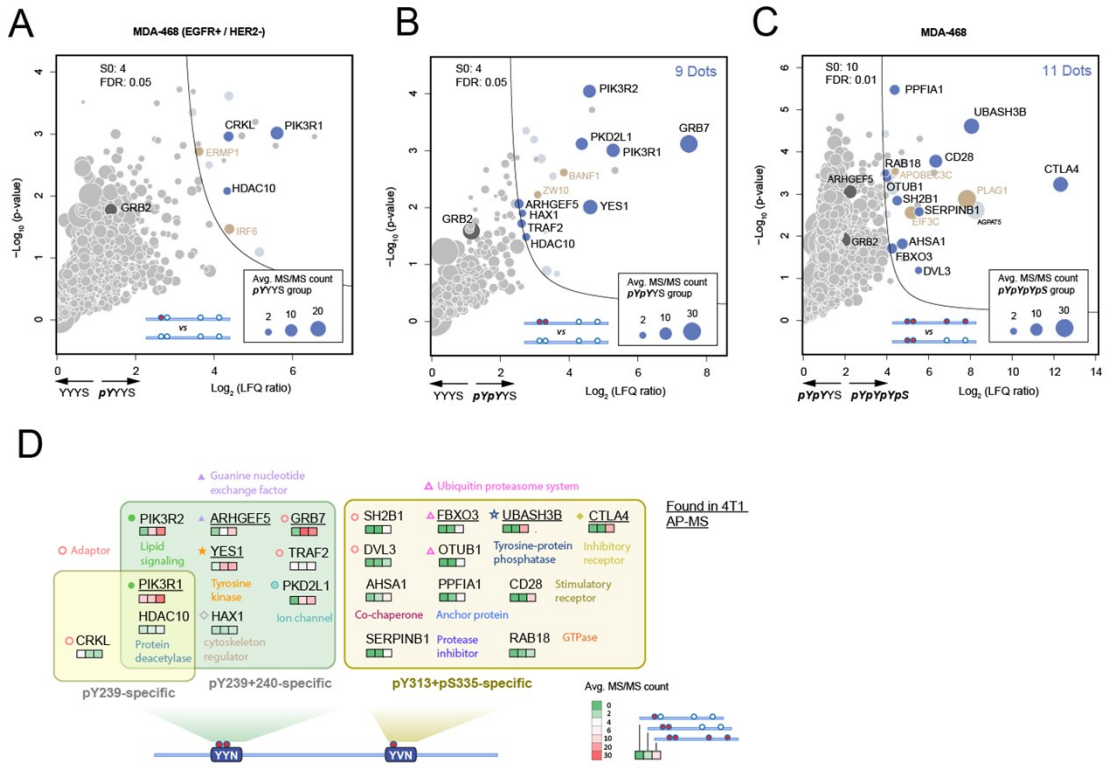
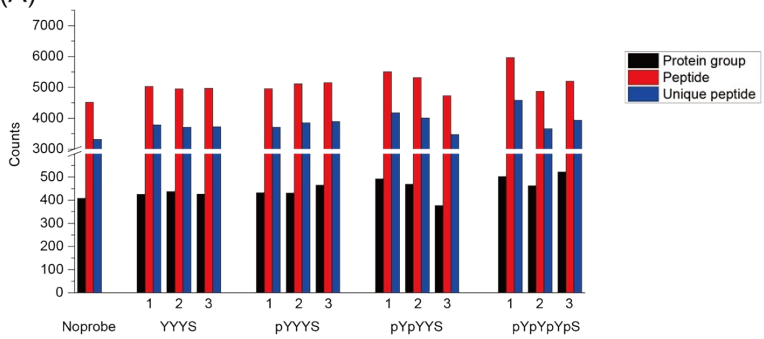


Figure S25. Site-specific interactome of Shc1 in human MDA-468 cell. (**A-D**) Volcano plot of the standard AP-MS comparing different Shc1^{CH1} fragments for the specific interactomes of pY239 (**A**), pY239+pY240 (**B**), and pY313 (**C**).

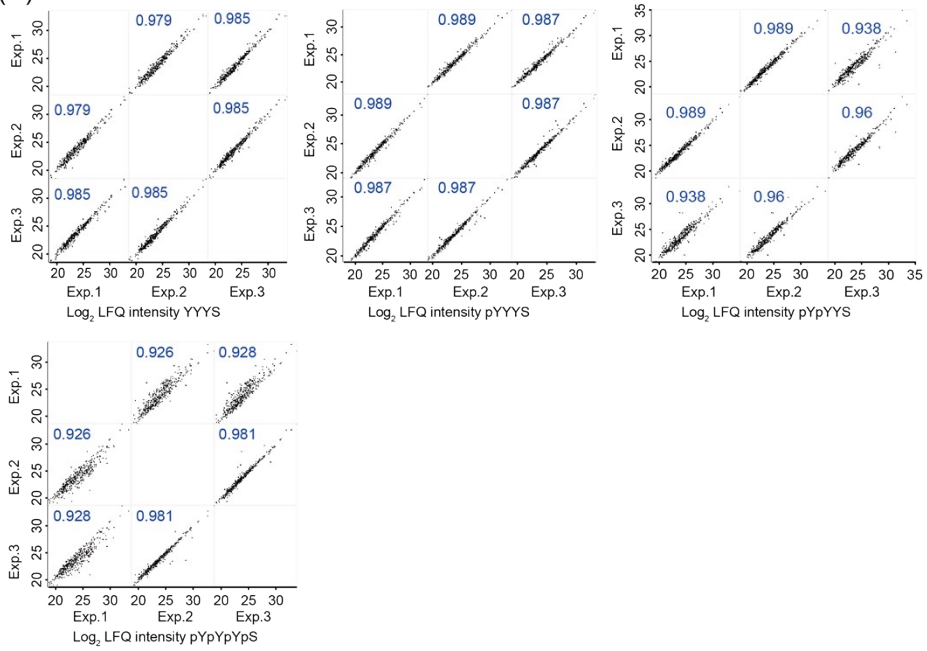
11. QC figures

(A)



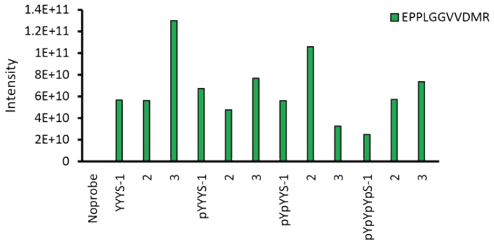
	No probe	YYYS			pYYYS			pYpYYS			pYpYpYpS		
		1	2	3	1	2	3	1	2	3	1	2	3
Protein group	408	425	437	426	433	431	465	492	469	377	502	463	522
Peptide	4519	5031	4949	4968	4958	5117	5155	5504	5313	4731	5962	4875	5198
Unique peptide	3315	3778	3707	3717	3708	3858	3890	4171	4004	3470	4582	3655	3938

(B)

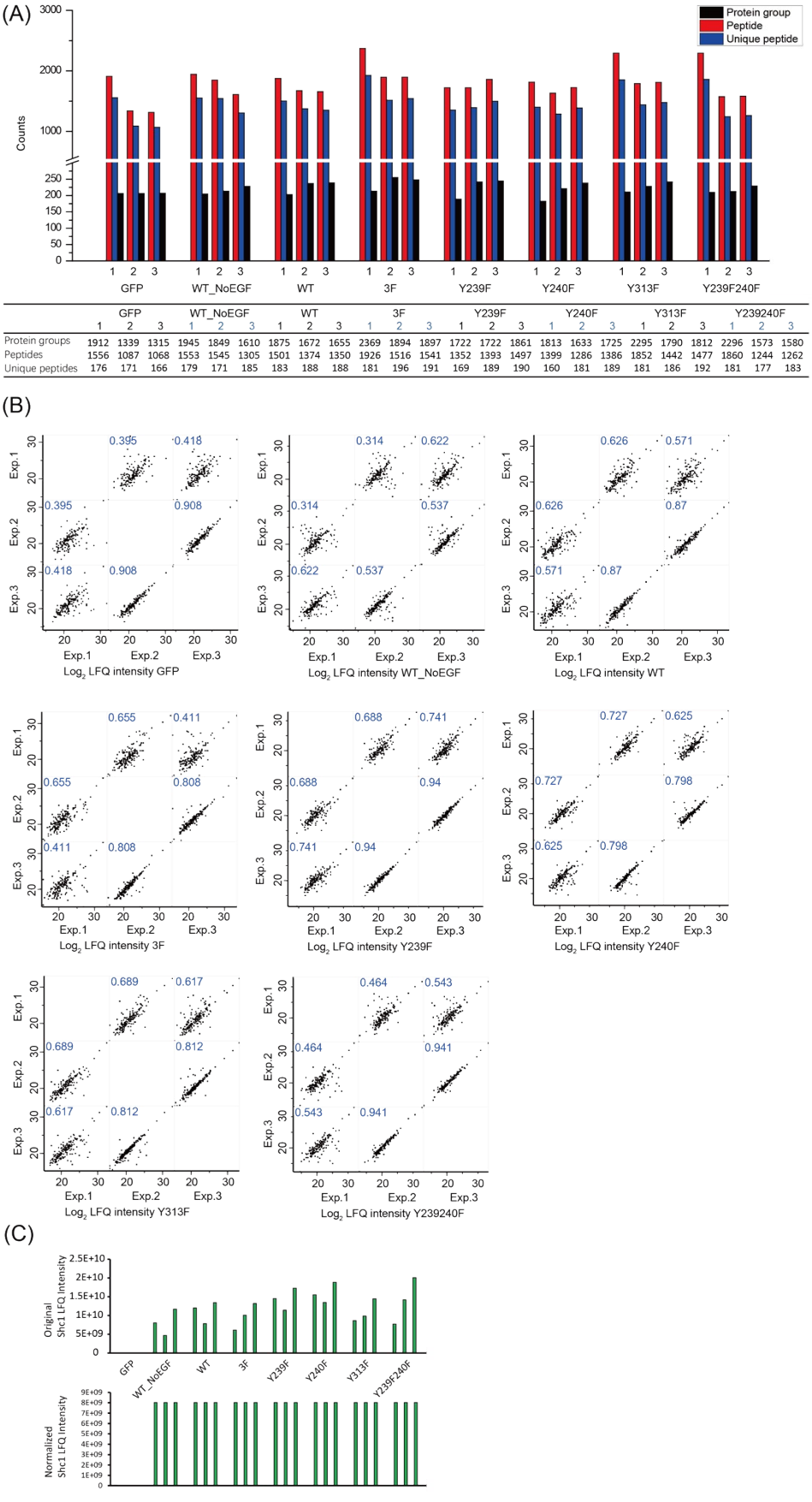


(C)

Sequence	MS/MS Count
EPPLGGVVDMR	790
ELFDDPSY VNIQNLDK	166
EPPLGGVVDMRLR	64
EGAARPTLPSAQMSSHLGATLPIGQHAAGDHEVR	19
PTLPSAQMSSHLGATLPIGQHAAGDHEVR	17
ELFDDPSY VNIQNLDKAR	16
EGAARPTLPSAQMSSHLGATLPIGQHAAGDHEVRK	9
QAGGGAGPPNPSLNGS	8
QMLPPPPCPGR	4
KQMLPPPPCPGR	1
PTLPSAQMSSHLGATLPIGQHAAGDHEVRK	1



QC-1. Quality control of the AP-MS assay of the 4 Shc1^{CH1} fragments for mouse 4T1 cell (**Fig. 2**). (a) Identified peptide, unique peptide, and protein groups for each sample. (b) Correlation of LFQ value within each experimental group. R^2 value was labelled at the upper left corner of each scatter plot. (c) All tryptic peptides from the Shc1^{CH1} fragments. The peptide with the highest MS/MS count was shown in green. MS Intensities of this most abundant peptide fragment was shown on the right.

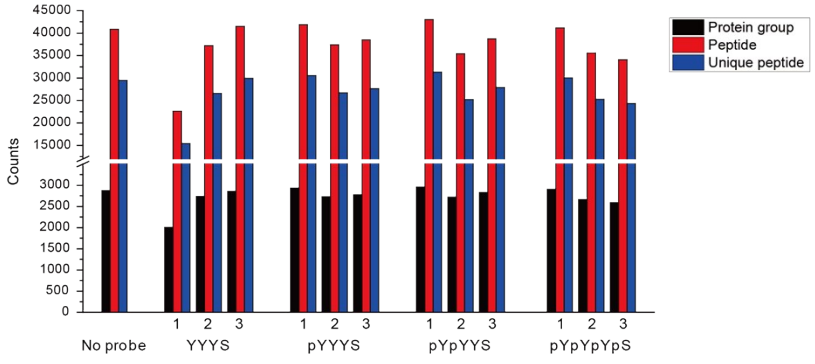


	GFP			WT_NoEGF			WT			3F			Y239F			Y240F			Y313F			Y239240F		
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
Protein groups	1912	1339	1315	1945	1849	1610	1875	1672	1655	2369	1894	1897	1722	1722	1861	1813	1633	1725	2295	1790	1812	2296	1573	1580
Peptides	1556	1087	1068	1553	1545	1305	1501	1374	1350	1926	1516	1541	1352	1393	1497	1399	1286	1386	1852	1442	1477	1860	1244	1262
Unique peptides	176	171	166	179	171	185	183	188	188	181	196	191	169	189	190	160	181	189	181	186	192	181	177	183

QC-2. Quality control of the Shc1 IP-MS assay in Hela cells stably expressing mouse p52Shc1

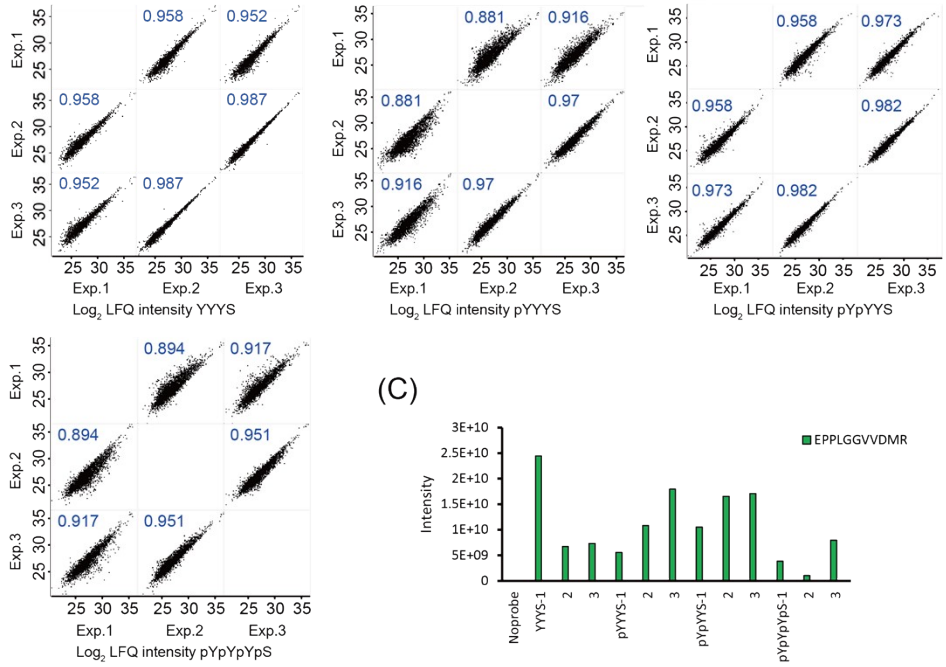
wild-type or mutants (**Figure 4D-F, S22B-E**). (a) Identified peptide, unique peptide, and protein groups for each sample. (b) Correlation of LFQ value within each experimental group. R^2 value was labelled at the upper left corner of each scatter plot. (c) MS Intensities of Shc1 before and after normalization.

(A)



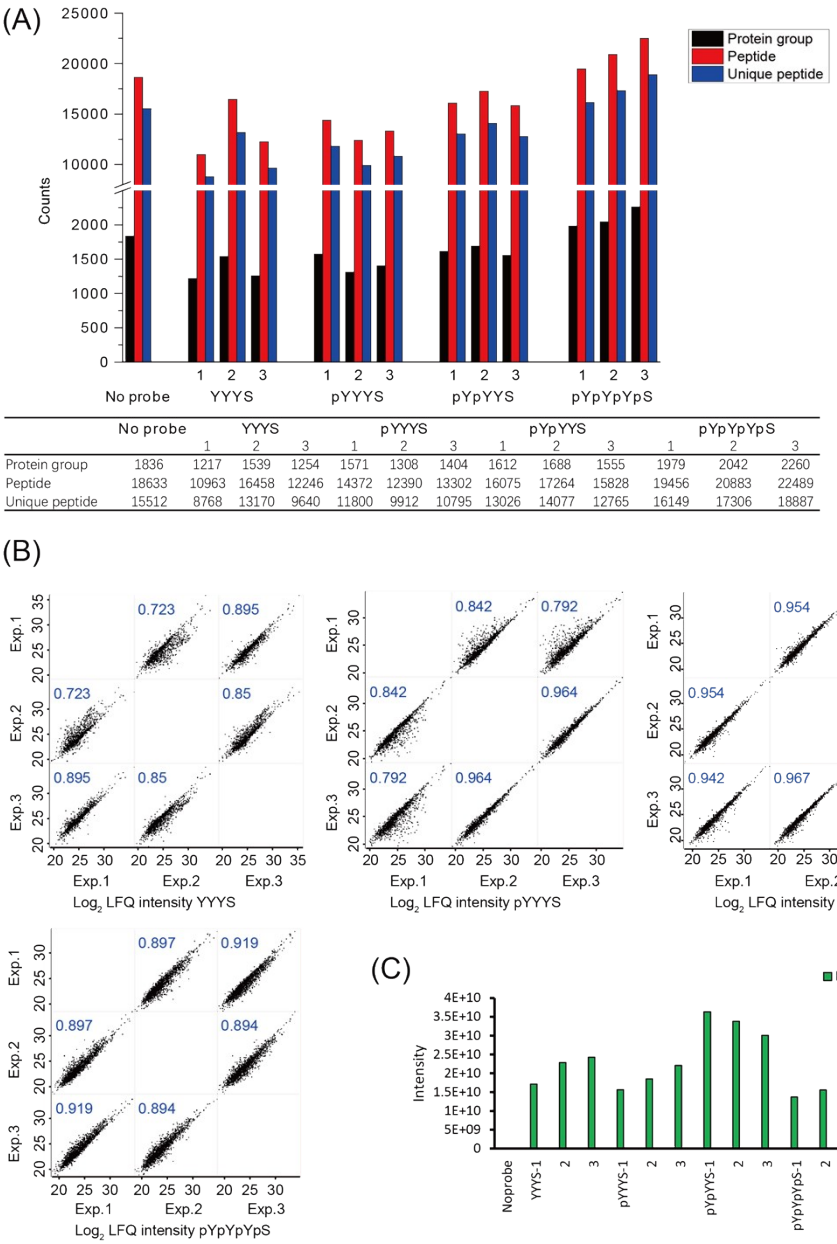
	No probe	YYYS			pYYYS			pYpYYS			pYpYpYpS		
		1	2	3	1	2	3	1	2	3	1	2	3
Protein groups	2872	2008	2736	2856	2926	2728	2774	2956	2716	2827	2900	2661	2589
Peptides	40844	22619	37185	41479	41865	37383	38483	43018	35371	38705	41164	35525	34064
Unique peptides	29488	15398	26554	29933	30524	26666	27620	31268	25198	27890	30010	25231	24288

(B)



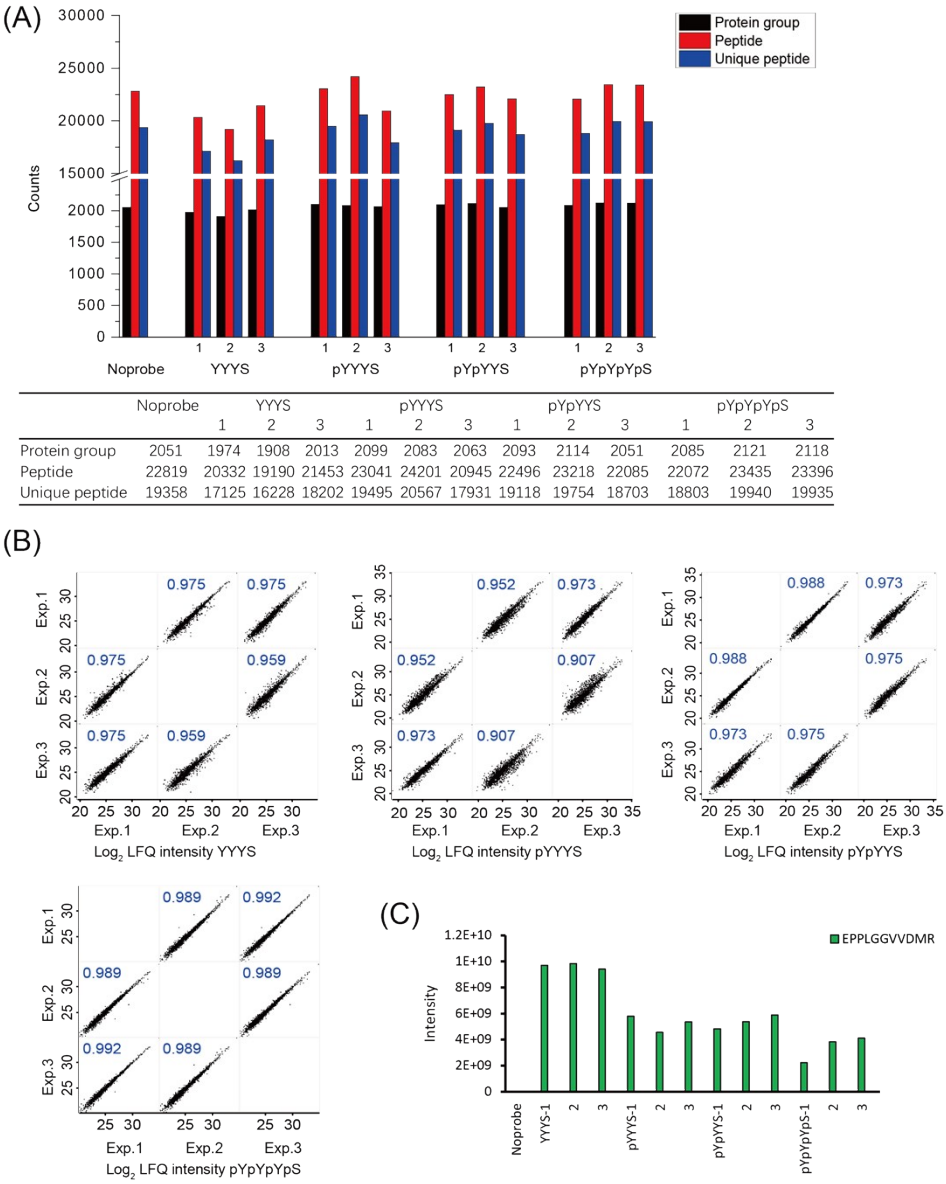
(C)

QC-3. Quality control of the AP-MS assay of the 4 $Shc1^{CH1}$ fragments for human MDA-231 cell (**Figure 5**). (a) Identified peptide, unique peptide, and protein groups for each sample. (b) Correlation of LFQ value within each experimental group. R^2 value was labelled at the upper left corner of each scatter plot. (c) Of all tryptic peptides from the $Shc1^{CH1}$ fragments, the MS Intensities of the peptide with the highest MS/MS count were calculated.



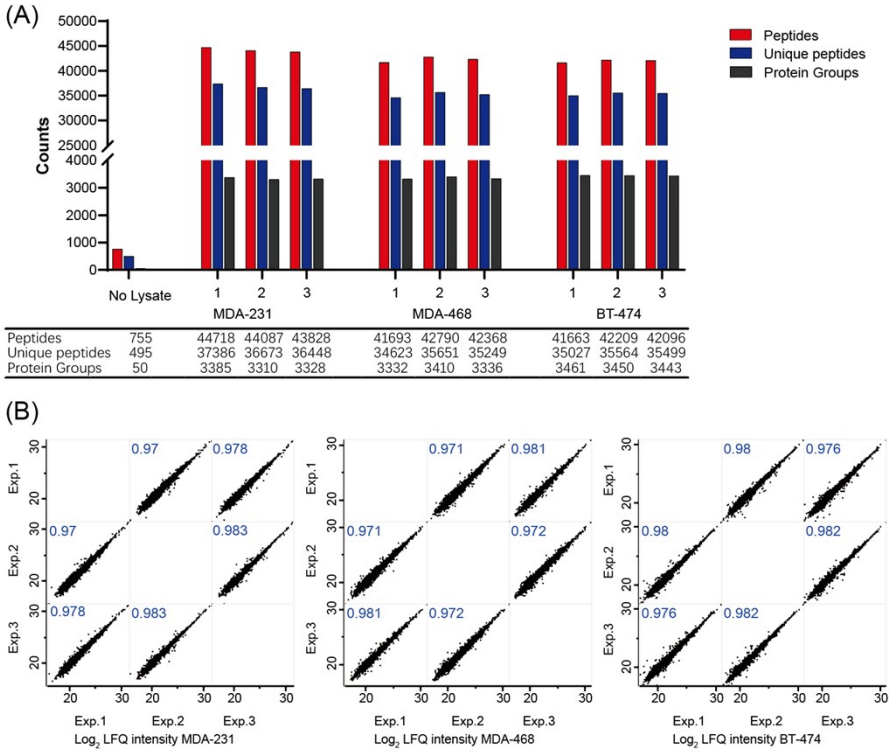
	No probe	YYYS			pYYYS			pYpYYS			pYpYpYpS		
		1	2	3	1	2	3	1	2	3	1	2	3
Protein group	1836	1217	1539	1254	1571	1308	1404	1612	1688	1555	1979	2042	2260
Peptide	18633	10963	16458	12246	14372	12390	13302	16075	17264	15828	19456	20883	22489
Unique peptide	15512	8768	13170	9640	11800	9912	10795	13026	14077	12765	16149	17306	18887

QC-4. Quality control of the AP-MS assay of the 4 Shc1^{CH1} fragments for human MDA-468 cell (**Figure 5**). (a) Identified peptide, unique peptide, and protein groups for each sample. (b) Correlation of LFQ value within each experimental group. R^2 value was labelled at the upper left corner of each scatter plot. (c) Of all tryptic peptides from the Shc1^{CH1} fragments, the MS Intensities of the peptide with the highest MS/MS count were calculated.



	Noprobe	YYYS			pYYYS			pYpYYS			pYpYpYpS		
		1	2	3	1	2	3	1	2	3	1	2	3
Protein group	2051	1974	1908	2013	2099	2083	2063	2093	2114	2051	2085	2121	2118
Peptide	22819	20332	19190	21453	23041	24201	20945	22496	23218	22085	22072	23435	23396
Unique peptide	19358	17125	16228	18202	19495	20567	17931	19118	19754	18703	18803	19940	19935

QC-5. Quality control of the AP-MS assay of the 4 Shc1^{CH1} fragments for human BT-474 cell (**Figure 5**). (a) Identified peptide, unique peptide, and protein groups for each sample. (b) Correlation of LFQ value within each experimental group. R^2 value was labelled at the upper left corner of each scatter plot. (c) Of all tryptic peptides from the Shc1^{CH1} fragments, the MS Intensities of the peptide with the highest MS/MS count were calculated.



		MDA-231			MDA-468			BT-474		
Peptides	755	44718	44087	43828	41693	42790	42368	41663	42209	42096
Unique peptides	495	37386	36673	36448	34623	35651	35249	35027	35564	35499
Protein Groups	50	3385	3310	3328	3332	3410	3336	3461	3450	3443

QC-5. Quality control of the whole cell proteomic assay of the 3 human breast cancer cell lines: MDA-231, MDA-468, and BT-474 (**Figure S24C**). (a) Identified peptide, unique peptide, and protein groups for each sample. (b) Correlation of LFQ value within each experimental group. R^2 value was labelled at the upper left corner of each scatter plot.

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