# Supporting information

**Materials and Methods**

Agar was purchased from MACKLIN. Yeast extract and tryptone were purchased from OXOID. Isopropyl-β-D-thiogalactoside (IPTG) was purchased from Chemsynlab, and L-arabinose was purchased from MERYER. 2×Super Pfx MasterMix DNA polymerase was obtained from CWBIO. DpnI was obtained from NEB. Gibson assembly master mix and dNTPs were obtained from New England Biolabs. Oligonucleotide primers were purchased from TSINGKE (Supplementary Table S2 lists the oligonucleotides used in this report). Plasmid DNA preparation was carried out with the EndoFree Plasmid Mini Kit (CWBIO). High Affinity Ni-NTA Resin was purchased from GenScript. Protease inhibitor was purchased from Roche diagnostics (cOmplete, EDTA-free Protease Inhibitor Cocktail), and the BCA kit was purchased from Coolaber. Antibiotics were obtained from SolarBio. Yeast RNA was purchased from SolarBio. L-Glutamic acid γ-benzyl ester was purchased from Aladdin. An ATPIO XO-900D sonic disruptor was used. Optical density was measured with Thermo Fisher Scientific NanoDrop™ ONE$^C$ Spectrophotometer. Absorbance and emission spectra were measured on Thermo Scientific Varioskan LUX. Protein mass spectrometry was carried out on Agilent 6545 LC/ESI-QTOF or Waters (Xev0 G2-XS Q-TOF). Fluorescence imaging was carried out on Leica inverted research grade microscope DMi3000B.

## 1. Procedure of PylRS library selection.

The library encoded in the pBK vector (Kan$^R$) was transformed into *E. coli* DH10B cells harboring a positive selection plasmid (pRep-Pyl, Tet$^R$) that encodes both chloramphenicol acetyltransferase (with an Asp112TAG mutation) and tRNA$^{Pyl}$. Over 1.0 X $10^8$ transformants were obtained to cover an expected library size of ~3.0 X $10^7$ members. Positive selection was performed on LB agar plates containing 50 μgmL$^{-1}$ kanamycin, 25 μgmL$^{-1}$ tetracycline, 50 μgmL$^{-1}$ chloramphenicol and 2 mM BnE for 48 h at 37 $^o$C. The pBK plasmid was isolated from surviving colonies and transformed into *E. coli* DH10B cells harboring a negative selection plasmid (pNeg-Pyl, Amp$^R$) that contains tRNA$^{Pyl}$ and a toxic barnase gene with two amber mutations (Gln2TAG and Asp44TAG) under control of the araBAD promoter. The negative selection was carried out on LB agar containing 100 μgmL$^{-1}$ ampicillin, 50 μgmL$^{-1}$ kanamycin and 0.2% L-arabinose for 12 h at 37 $^o$C. The pBK plasmid was isolated from surviving cells, and a second round of positive selection was carried out as described above in the presence 50 μgmL$^{-1}$ chloramphenicol. After 48 h growth at 37 $^o$C, 96 single colonies were picked and re-grown on LB plates with 50 μgmL$^{-1}$ chloramphenicol in the presence or absence of 2 mM BnE. Ten colonies showed obvious growth advantage in the presence of BnE, and the corresponding pBK plasmids were separated and sequenced.

## 2. Procedure of the fluorescence-based assay to evaluate PylRS variants for the ability to incorporate BnE.

*E. coli* DH10B were co-transformed with plasmid pBK-BnERS1-3(Kan$^R$) and pLeiG-PylT-sfGFP(D134TAG) (Cm$^R$). Three single colonies of each were picked and inoculated into 400 μL LB broth, and grown overnight. The saturated culture was diluted 100 folds (4 μL to 400 μL) with LB broth and grown at 37 $^o$C. After 3 h, the culture was supplemented with or without 5 mM BnE and grown for another 15 min before

induction with 1 mM IPTG. The expression was performed for 8 h at 37 °C, and then the cells were pelleted and washed twice with 10 mM pH 7.4 phosphate buffer before fluorescence measurement. Fluorescence was normalized by (fluorescence intensity)/OD600.

### 3. A general procedure for the expression and purification of protein containing BnE.

*E. coli* DH10B was co-transformed with pUltra-PylT-BnERS ($Spec^R$) and pET22b-T5-sfGFP-Y151TAG ($Amp^R$). A single colony was picked and inoculated into 5 mL LB broth, and grown overnight. The saturated culture was diluted 100 folds (250 µL to 25 mL) with LB broth and grown at 37 °C. When the OD reached 0.8, 5 mM BnE was added as powder. The culture was grown for another 15 min before induction with 1 mM IPTG. The induced cells were grown an additional 12 h at 37 °C. The cells were pelleted and lysed with a sonic disruptor in the presence of protease inhibitors, and insoluble protein and cell debris were removed by centrifugation. The protein was purified by $Ni^{2+}$ affinity chromatography, and finally buffer exchanged with an Amicon centrifugal filter. Protein concentration was determined with a NanoDrop™ $ONE^C$ Spectrophotometer (according to the manufacture's protocol). The expression yield was calculated as [purified protein (mg)/ volume of culture (mL)] X1000.

### 4. Procedure for genetic incorporation of BnE in HEK293T cells, and the western blot analysis.

HEK293T cells were maintained in Dulbecco's Modification of Eagle's Medium (DMEM) supplemented with 10% heat inactivated fetal bovine serum (FBS) at 37 °C with 5% $CO_2$. For fluorescence imaging, HEK293T cells were seeded in 24-well plates at 2× $10^5$ cells/well in DMEM supplemented with 10% FBS. Transfection was performed using Lipofectamine™ 3000 Transfection Reagent (Thermo Scientific) according to manufacturer's protocol with pcDNA- EGFP-Y39TAG and pCMV-PylT-BnERS. 5 mM BnE was added 1 h after transfection. Fluorescence microscopic imaging was carried out 24 hours after transfection.

Western blot: HEK293T cells expressing EGFP-Y39TAG with or without BnE were boiled in SDS loading buffer and subjected to SDS-PAGE gel, followed by transferring the proteins from the gel onto PVDF membrane under the condition of 180 mA for 70 min. Then, the membrane was washed by TBST buffer (TBS with 0.1 % tween-20, 10 mL) on the shaker there times with 10 min intervals. After that, the membrane was coated with 5% (w/v) fat-free milk (50 mL) for 1 h at room temperature. The washing step was repeated. The membrane was immunobloted with anti-GFP monoclonal antibody (1:3000, 3mL) at room temperature for 1 hour. After washed, the membrane was treated with secondary antibody (1: 5000, 5 mL) at room temperature for 1 h. Finally, the membrane was washed by TBST buffer on the shaker twice with 20 min intervals. The membrane was subjected to chemiluminescence (ECL) reaction and visualized with Tanon-5200.

## 5. Herceptin Fab Expression and Purification.

Plasmid pBAD-Herceptin Fab (wild type, or SUMO-Fab-E1TAG) was transformed into *E. coli* DH10B cell along with pUltra-BnERS. Cells were cultured in 40 mL LB media, supplemented with 100 μg/mL ampicillin and 50 μg/mL spectinomycin. BnE was added to a final concentration of 5 mM when the $OD_{600}$ reached 0.6, and protein expression was induced at an $OD_{600}$ ~1 by addition of 0.2% arabinose and 1 mM isopropyl β-D-1-thiogalactopyranoside (IPTG). Cells were cultured at 37°C for 10 hours. The cell pellet obtained after centrifugation was frozen at – 80°C for 30 min, and then thawed at room temperature. The cell pellet was re-suspended with 10% of culture volume periplasmic lysis buffer (20% sucrose, 30 mM Tris, 1 mM EDTA aqueous solution at pH 7.8; with 0.2 mg/mL lysozyme added before use) and incubated in a 37°C shaker for 1 hour. Cell debris was removed by centrifugation, and the supernatant was applied to a 1 mL protein G MagBeads (GenScript) column. The column was washed with 50 mL protein G washing buffer (50 mM
NaOAc, pH = 5.2), and the protein was eluted with 5 mL protein G elution buffer (100 mM Glycine, pH 2.8) followed by an immediate buffer exchange with PBS using centrifugal filtration with a 10 kD cutoff.

## 6. Experimental condition for the use of recombinant Ulp1 to expose BnE at protein N-terminus.

Ulp1 was recombinantly expressed in *E. coli* DH10B, and purified through the C-terminal His-Tag. Specifically, 10 nM purified Ulp1 was added to 5 μM SUMO-sfGFP-S2BnE (or SUMO-sfGFP-S2E) in a buffer containing 25 mM Tris, 150 mM NaCl, 2 mM DTT, 0.5 mM EDTA at pH 8, and then incubated at 30°C for 2.5 h. The product was buffer exchanged with phosphate-buffered saline (137 mM NaCl, 2.7 mM KCl, 10 mM $Na_2HPO_4$, 2 mM $KH_2PO_4$, pH 7.4) before further analysis.

## 7. Labeling reaction of pGlu-His dipeptide on sfGFP with arylboronic acid reagent.

7.2 μM pGlu-His-sfGFP (or pGlu-sfGFP) and 100 mM (2-nitrophenyl)boronic acid was incubated in 10 mM pH 9.5 N,N'-diethylpiperazine (NEP) buffer containing 200 μM $Ni(OAc)_2$ at 37°C for 30 min. The product was buffer exchanged with phosphate-buffered saline (137 mM NaCl, 2.7 mM KCl, 10mM $Na_2HPO_4$, 2 mM $KH_2PO_4$, pH 7.4) before further analysis.

## 8. Assay used to evaluate barnase activity.

Barnase-E73BnE alkaline hydrolysis: Barnase-E73BnE was incubated in 100 mM pH 11 Tris buffer at 37°C for 7 h.

Barnase activity was originally analyzed based on a yeast RNA based assay.[1] To 2 mg/mL yeast RNA in aqueous solution (3% ammonium hydroxide) was added with 0.28 μM barnase-E73BnE or the hydrolyzed product, then incubated at 37°C and monitored by absorbance at 298 nm for a period of 30 min.

Barnase activity was calculated based on a reported protocol.[2] To 2 mg/mL yeast RNA solution(125mM Tris-HCl, pH 8.5) was added properly diluted hydrolyzed barnase-E73BnE, the final concentration of the enzyme is 0.4nM, 0.8nM, 6.4nM, 12.8nM. The mixture was incubated at 37°C for 15 minutes and then the reaction was stopped by the addition of equal reaction volume 6% $HClO_4$. After 15 minutes at 0°C, the precipitate was centrifuged out, a quarter of the supernatant was diluted with water and monitored the absorbance at 260nm by Thermo Scientific Varioskan LUX at 28°C. In the barnase activity calculation, an increase in $A_{260}$ of 1.0 under the experimental condition was defined as 100 units of enzyme activity.

**9. Condition used to treat sfGFP-Y151BnE with $NH_2NH_2$ and $NH_2OH$.**

$NH_2NH_2$ or $NH_2OH$ was added to 7.8 μM sfGFP-Y151BnE in pH 7.4 PBS Buffer at a final concentration of 4% v/v, and incubated at 37°C for 1 h. The product was buffer exchanged with PBS Buffer (4X reaction volume each time, five times) before further analysis.

**10. Procedures used to express and purify recombinant H3.**

*E. coli* BL21(DE3) was co-transformed with pUltra-PylT-BnERS ($Spec^R$) and pET22b-T5-H3-Q56TAG ($Amp^R$). A single colony was picked and inoculated into 5 mL LB broth, and grown overnight. The saturated culture was diluted 100 folds (250 μL to 25 mL) with LB broth and grown at 37 °C. When the OD reached 0.8, 8 mM BnE was added. The culture was grown for another 15 min before induction with 1 mM IPTG. The induced cells were grown an additional 10 h at 37 °C. The cells were pelleted and lysed with a sonic disruptor. After centrifugation, the liquid part was discarded, and the solid part was dissolved in pH 8 100 mM $Na_2HPO_4$, 6 M Gn•HCl buffer. Insoluble cell debris was removed by centrifugation. The protein was purified by $Ni^{2+}$ affinity chromatography (Washing Buffer: 100 mM $Na_2HPO_4$, 6 M Gn•HCl, 10 mM imidazole, pH=8; Elution Buffer: 100 mM $Na_2HPO_4$, 6 M Gn•HCl, 250 mM imidazole, pH=8). Protein concentration was determined by BCA assay kit (according to the manufacture's protocol).

**11. Procedure for protein labeling with Fl-Cys.**

sfGFP harboring acyl hydrazide at Y151 (14 μM) in 20 mM, pH 3 phosphate buffer was added with 1 mM $NaNO_2$, and incubated for 20 min in ice−salt bath. Then, 2 mM Fl-cys and 1 mM 4-mercaptophenylacetic acid (MPAA,) was added, and pH was carefully adjusted to 7.0 with 5.0 M NaOH. The reaction mixture was incubated at 25 °C for 4 h. The product was buffer exchanged with PBS before further analysis.

**12. Monitoring the fluorescence change of HA variants of sfGFP in the presence of metal cation.**

To 0.9 μM sfGFP variants with one, two and three HA respectively in 10 mM pH 7.4 Tris-HCl buffer was added with 0 - 30 μM $Fe^{3+}$ or $Cu^{2+}$, and then incubated at 25 °C for 15 min. The fluorescence change was measured on Thermo Scientific Varioskan LUX. The excitation was at 485 nm, and the emission was at 510 nm.

**13. Procedure for modifying H3 variants with site-specific Gln methylation.**

To 12 μM H3 variant harboring BnE at Q5, Q56 and Q94 respectively in 6 M Gn•HCl PBS Buffer (137mM NaCl, 2.7mM KCl, 10mM $Na_2HPO_4$, 2mM $KH_2PO_4$, pH 7.4) was added with $NH_2NH_2$ at a final concentration of 20% (v/v), and incubated for 1.5 h at 37 °C. After that, the solution was dialyzed with 1 L PB buffer (20 mM $Na_2HPO_4$, 6M Gn•HCl, pH 3) for four times to remove the excessive $NH_2NH_2$. After concentrated the product with a Millipore Ultrafiltration centrifuge tube (3KD cut-off), the reaction mixture was added with 200 mM acetyl acetone, and then incubated for 1.5 h at 37 °C. Lastly, methylamine in DMSO (2 M stock) was added, and the final concentration was 1 M and the pH was carefully adjusted to 9 with 5.0 M NaOH. The reaction mixture (50% DMSO) was incubated at 37 °C for 5 h, and then was dialyzed with PBS buffer (137mM NaCl, 2.7mM KCl, 10mM $Na_2HPO_4$, 2mM $KH_2PO_4$, pH 7.4) before further analysis.

**14. Plasmid construction and protein sequence in this work.**

Genes encoding barnase, Ulp1, H3, 14-3-3γ and TrxA were inserted into pET22b-T5 vector through Gibson Assembly. Site Directed Mutagenesis was performed with 2×Super Pfx MasterMix DNA polymerase. BnERS/PylT and Ulp1 co-expression vector (pUltra-PylT-BnERS-Ulp1) was constructed by inserting Ulp1 gene after the BnERS expression cassette in pUltra vector.[3] SUMO and Fab co-expression vector (pBAD-SUMO-Fab-E1TAG) was constructed by inserting SUMO gene before the Heavy chain in pBAD vector. All the primers are listed in table S2.

**Barnase sequence:**

MAQVINTFDGVADYLQTYHKLPDNYITKSEAQALGWVASKGNLADVAPGKSIGGDIFSNREGKLPGKSGRTWR**E*** ADI
NYTSGFRNSDRILYSSDWLIYKTTDHYQTFTKIRGSHHHHHH

**H3 sequence:**

MARTK**Q*** TARKSTGGKAPRKQLATKAARKSAPATGGVKKPHRYRPGTVALREIRRYQ*KSTELLIRKLPFQRLVREIAQDF
KTDLRFQSSAVMAL**Q*** EACEAYLVGLFEDTNLCAIHAKRVTIMPKDIQLARRIRGERAHHHHHH

**14-3-3γ sequence:**

**V*** DREQLVQKARLAEQAERYDDMAAAMKNVTELNEPLSNEERNLLSVAYKNVVGARRSSWRVISSIEQKTSADGNEKKI
EMVRAYREKIEKELEAVCQDVLSLLDNYLIKNCSETQYESKVFYLKMKGDYYRYLAEVATGEKRATVVESSEKAYSEAHEIS
KEHMQPTHPIRLGLALNYSVFYYEIQNAPEQACHLAKTAFDDAIAELDTLNEDSYKDSTLIMQLLRDNLTLWTSDQQDDD
GGEGNNLEHHHHHH

**TrxA sequence:**

**V*** DKIIHLTDDSFDTDVLKADGAILVDFWAEWCGPCKMIAPILDEIADEYQGKLTVAKLNIDQNPGTAPKYGIRGIPTLLLF
KNGEVAATKVGALSKGQLKEFLDANLAHHHHHH

**15. Method for ESI-QTOF mass spectrometry**

Purified protein samples in PBS were subjected to liquid chromatography-electrospray ionization-quadrupole Time-of-Flight (LC-ESI-QTOF) mass spectrometry. The sample was injected into an LC column (Agilent ZORBAX 300SB-C8, 2.1 X 150 mm) and run through by gradient elution at a column temperature of 70 $^{o}$C. The mobile phases consisted of $H_2O$ containing 0.1% formic acid (buffer A) and acetonitrile containing 0.1% formic acid (buffer B). The gradient conditions were as follows: buffer A, 95% in 0-2 min, 95-40% in 2-10 min, 40-20% in 10-11 min, 20-10% in 11-12 min, 10% in 12-14 min, 10-95% in 14-14.5 min, 95% in 14.5-16.5 min. The mobile-phase flow rate was 0.5 ml/min. Protein mass deconvolution from LC-ESI-QTOF mass spectrometry data was conducted by using Agilent Qualitative Analysis software (Agilent Technologies Inc.)

**16. Method for enzymatic digestion and MS/MS analysis of semi-synthesized H3 variants.**

Sample preparation:

Proteins were dissolved in 100 μL $NH_4HCO_3$ buffer (50 mM, pH 8.2) and digested by GluC for 20 h at 37 $^{o}$ C with a 1:25 (w/w) enzyme/substrate ratio. The digest was desalted by using C18 tip and dried for MS analysis.

MS analysis:

The digestions were dissolved with 20 μL of 0.1% FA, and then separated by nano-LC and analyzed by online mass spectrometry. The experiments were performed on a Q-Extractive mass spectrometer, equipped with an Ultimate 3000 system. The peptide sample was separated on the analytical column (reverse capillary analytical C18, 75 μm × 15 cm) with a linear gradient. The elution method was carried out as follows: 0–2% mobile phase B (80% ACN/0.1% FA) for 10 min, 2–45% mobile phase B for 35 min, 45–90% mobile phase B for 10 min, 90% mobile phase B for 5 min, and finally, equilibration with mobile phase A for 10 min (A, 0.1% FA). The Q-Exactive mass spectrometer was operated in data dependent MS/MS acquisition mode. Survey full-scan MS spectra (m/z 350–1800) were acquired with a mass resolution of 70 K. The 10 most intense parent ions were fragmented by higher-energy collisional dissociation (HCD) with normalized collision energy (NCE) of 30%. The MS/MS acquisitions were performed in an Orbitrap with a resolution of 175 00 (m/z 200). Dynamic exclusion was set as 30 s. System control and data collection were carried out by Xcalibur software.

Data analysis:

The MS raw data files were exported using the pFind (version 3.1) for all database searches, and the database was H3-1. The parameters were as follows: precursor mass tolerance, ±20 ppm; fragment mass tolerance, ±20 ppm; two missed cleavages were allowed by GluC digestion with the cleavage site at the C-terminal of Glu. False discovery rates (FDR) were controlled lower than 1% at both the peptide level. Methyl on glutamine (Gln, Q) was set as variable modifications.

**Reference:**


[1] G. Schreiber, C. Frisch, A. R. Fersht, *J. Mol. Biol.* **1997**, *270*, 111-122.

[2] G. W. Rushizky, A. E. Greco, R. W. Hartley, Jr., H. A. Sober, *Biochemistry* **1963**, *2*, 787-793.

[3] A. Chatterjee, S. B. Sun, J. L. Furman, H. Xiao, P. G. Schultz, *Biochemistry* **2013**, *52*, 1828-1837.

Figure S1. Fluorescence analysis of BnERS1-3 for their abilities to encode BnE. Error bars represent s.d. of three independent biological replicates.

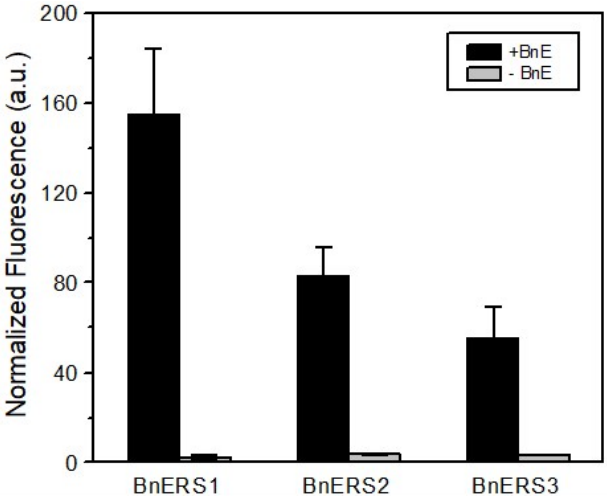


Figure S2. Comparing the incorporation efficiency of BnE to BocK in *E. coli* DH10B. pUltra-BnERS and pUltra-PylRS-Y349F was used to encode two ncAAs respectively. Error bars represent s.d. of three independent biological replicates.

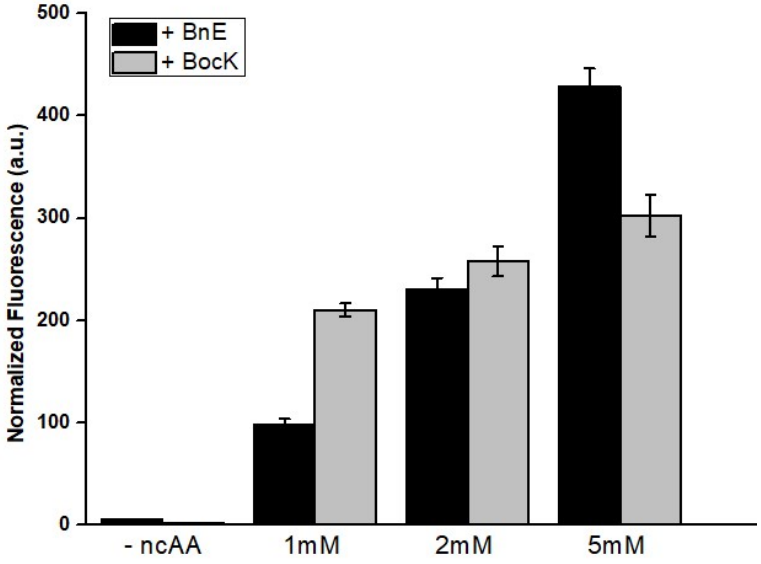


Figure S3. Recombinant expression of EGFP-Y39BnE in HEK293T cells. A) BnE-dependent fluorescence enhancement. B) Western blot analysis of EGFP-Y39BnE expressed in HEK293T cells.

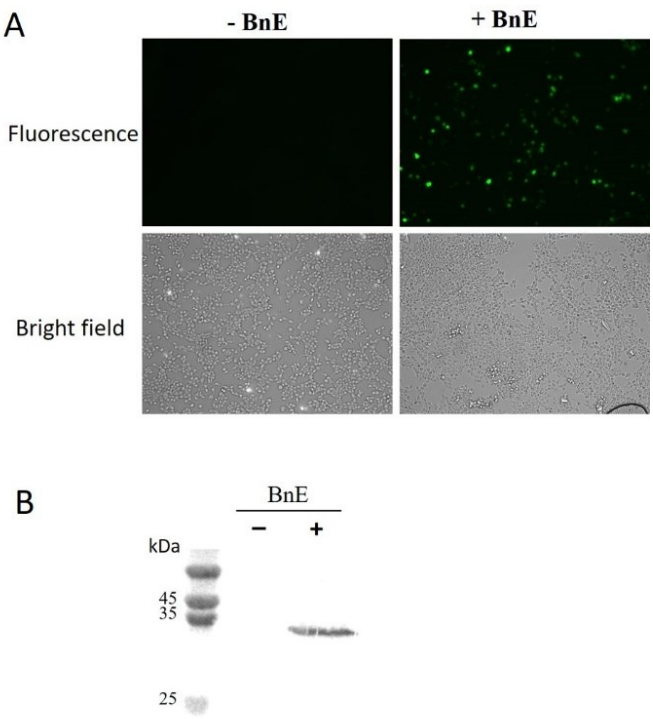


Figure S4. Mass spectrum of purified sfGFP-S2BnE revealed a failed removal of the first Met.

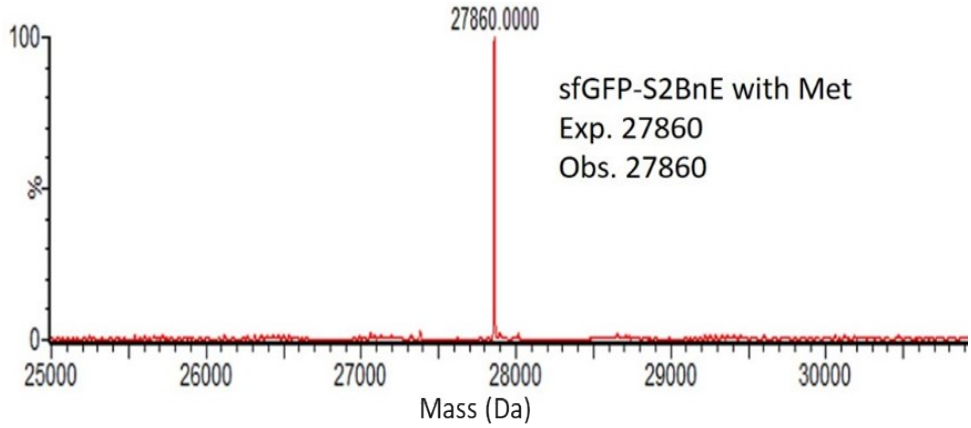


Figure S5. Mass spectra of purified SUMO-sfGFP-S2E before (A) and after (B) treatment with Ulp1.

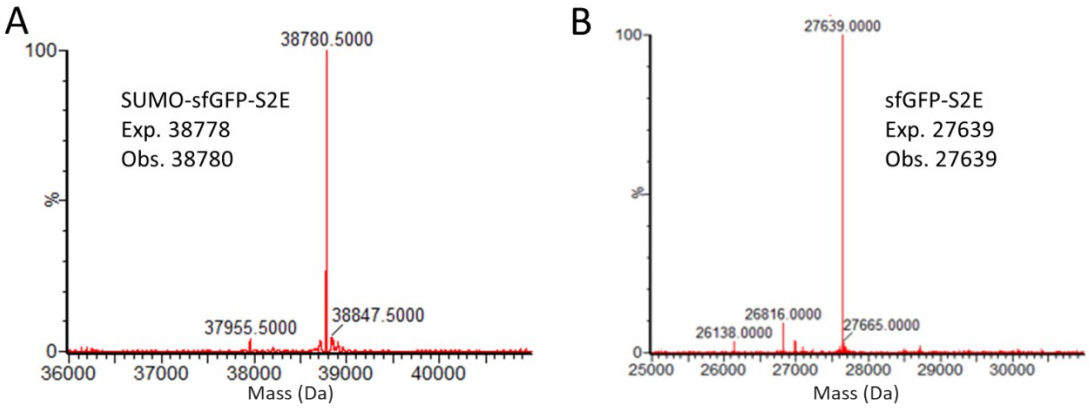


Figure S6. Co-expression of SUMO-sfGFP-S2Q with Ulp1 didn't afford obvious pGlu formation. The mass of pGlu-sfGFP is expected at 27621 Da.

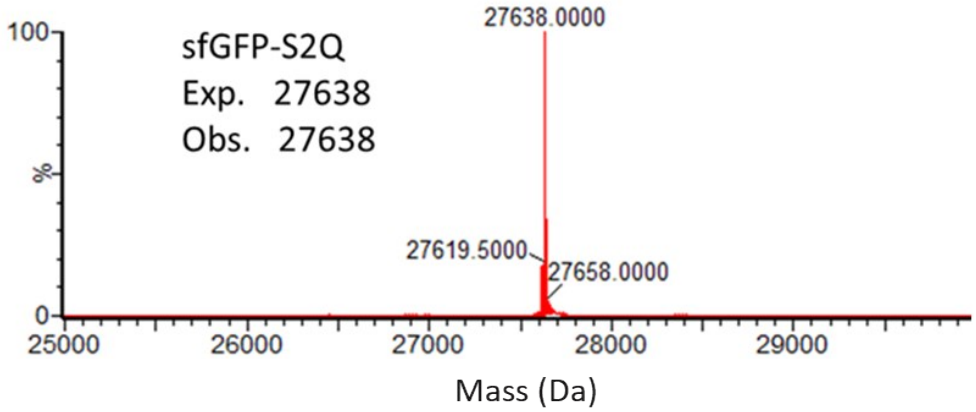


Figure S7. SDS-PAGE (A) and ESI-QTOF mass spectrometry (B and C) of purified pGlu-14-3-3γ and pGlu-TrxA.

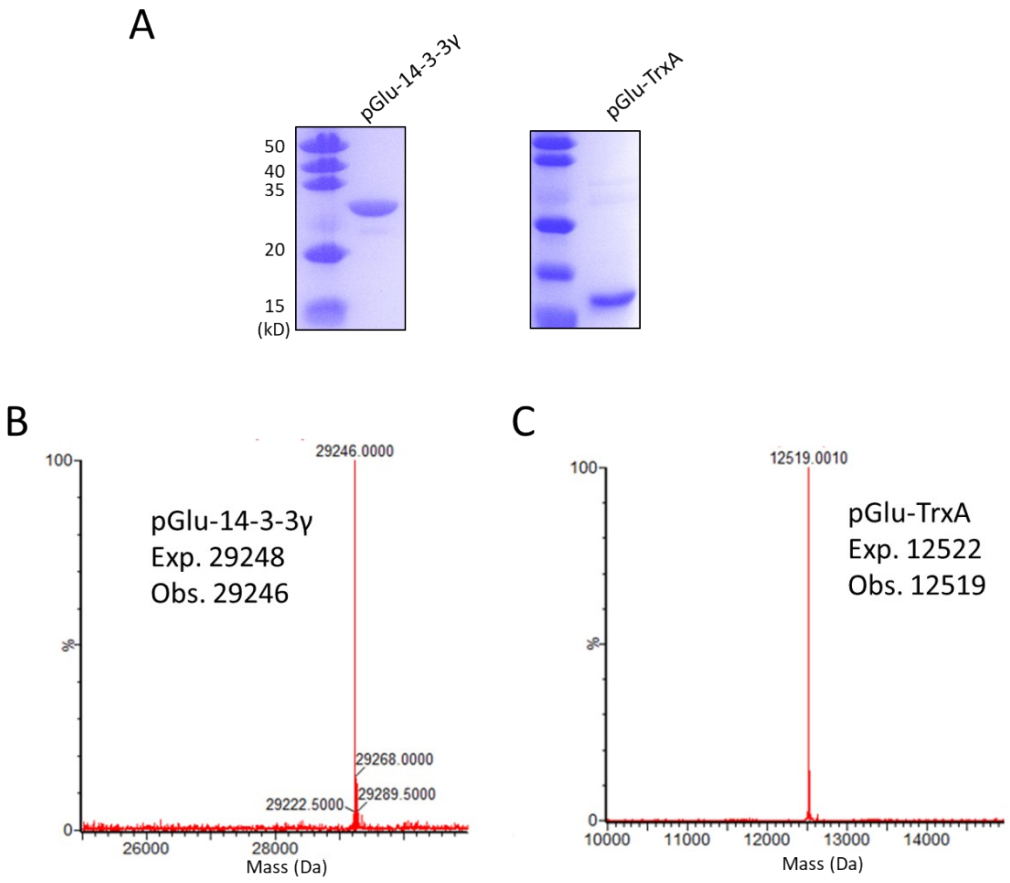


Figure S8. SDS-PAGE (A) and ESI-QTOF mass spectrometry (B) of the purified Herceptin Fab WT and the pGlu variant.

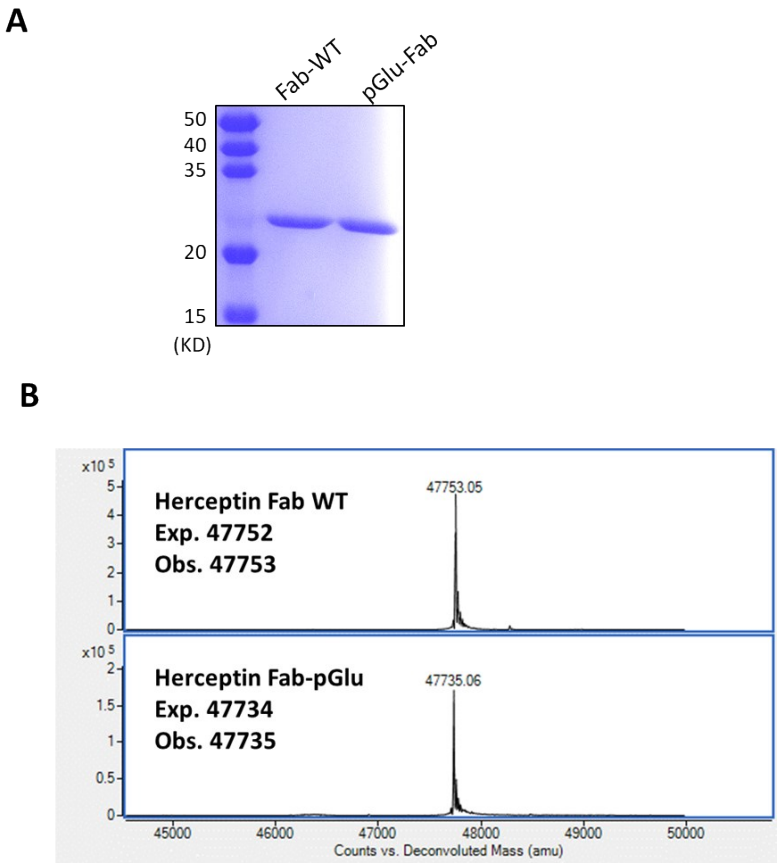


Figure S9. A) pGlu-His dipeptide at the N-terminus of sfGFP was used in labeling experiment with (2-nitrophenyl)boronic acid. B) Mass spectrum of purified pGlu-His-sfGFP. C) Mass spectrum of labeling product. D) Without the adjacent His, pGlu-sfGFP could not be labeled.

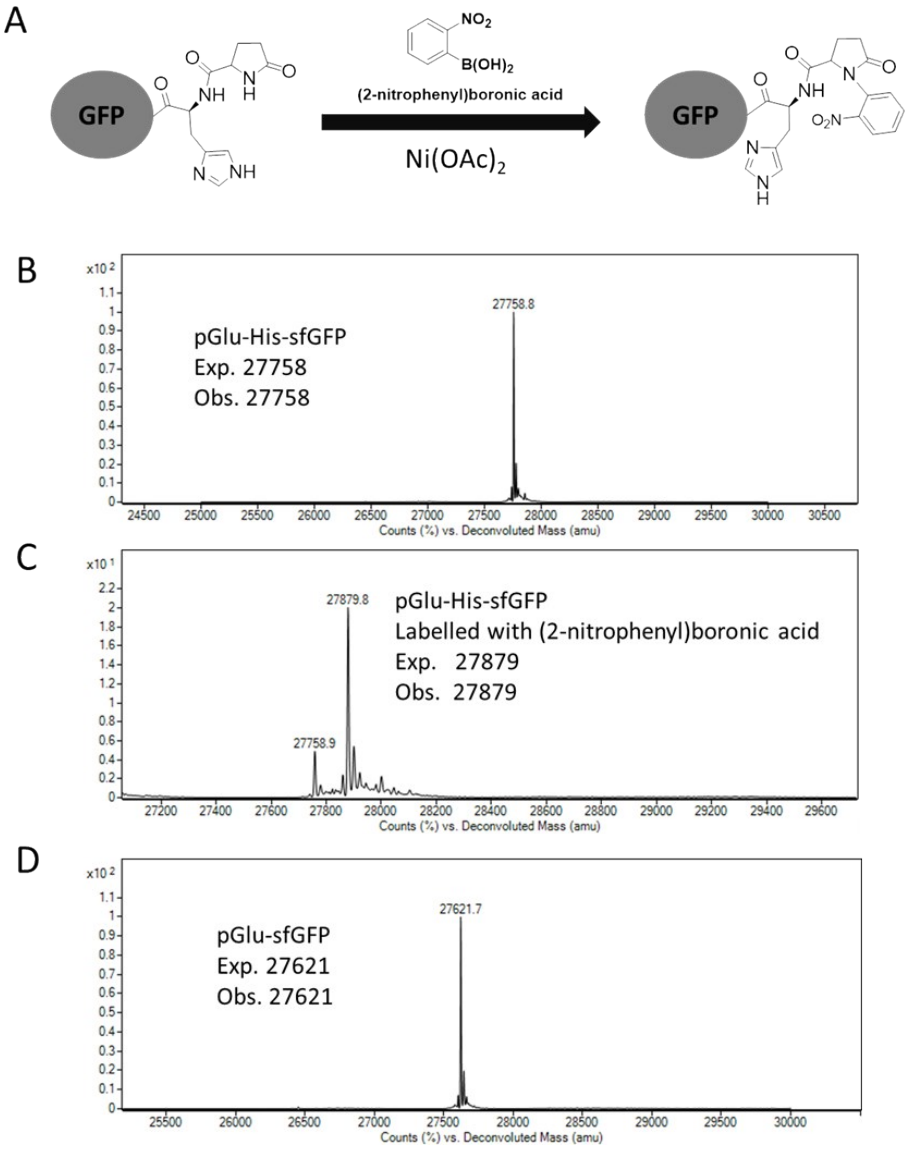


Figure S10. Growth curve of *E. coli* DH10B harboring pUltra-PylT-BnERS and pET22b-T5-barnase-E73TAG in LB media at 37 $^{o}$C.

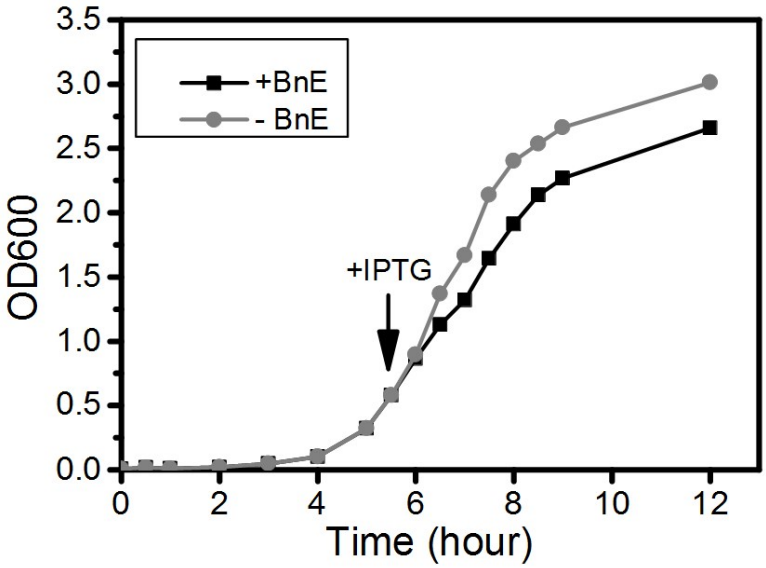


Figure S11. SDS-PAGE analysis of sfGFP variants with one, two and three BnE.

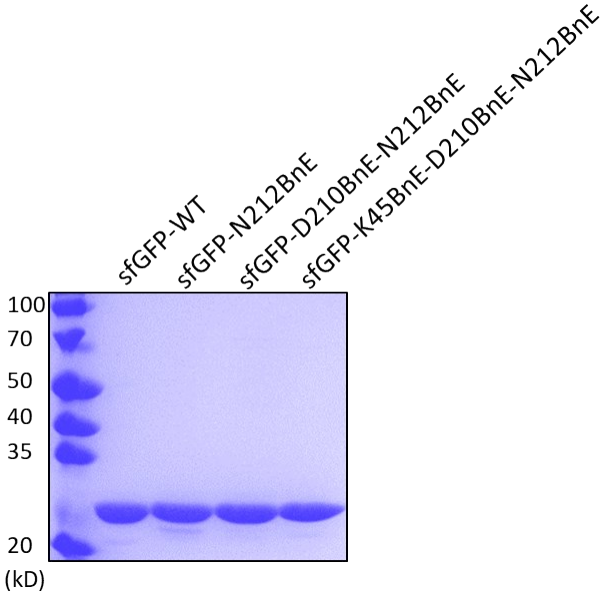


Figure S12. The fluorescence change of sfGFP variants after incubation with different concentration $Fe^{3+}$. Error bars represent s.d. of three independent biological replicates.

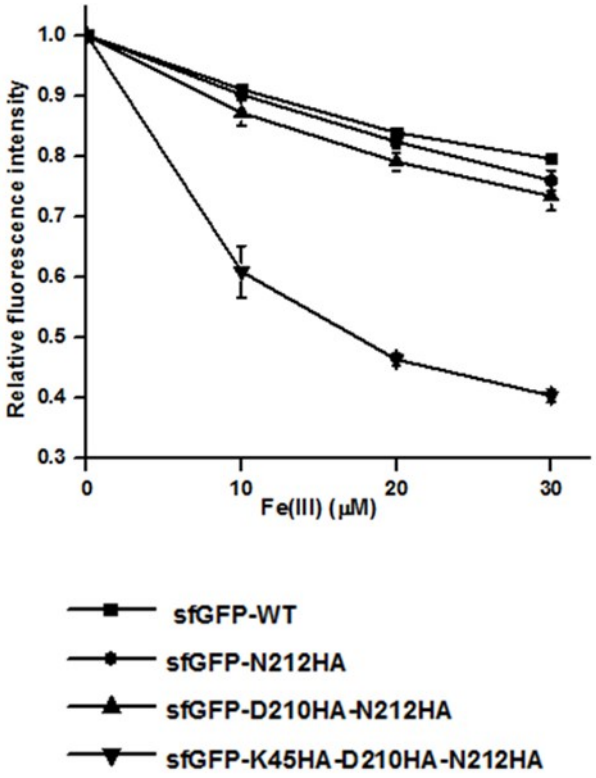
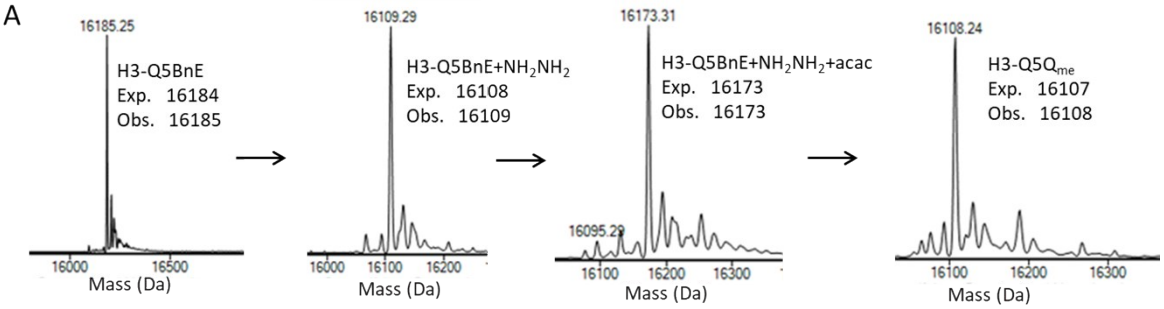


Figure S13. Generating methylated Gln at Q5 on H3. A) ESI-QTOF analysis of H3-Q5 variants in the process of preparing H3-Q5$Q_{me}$. B) SDS-PAGE analysis of H3 wt and H3-Q5$Q_{me}$.



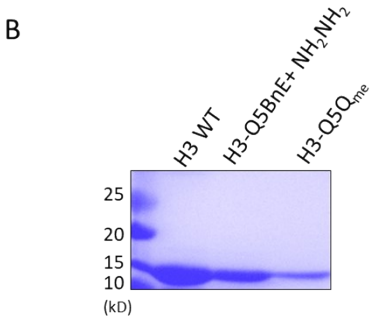



Figure S14. Generating methylated Gln at Q94 on H3. A) ESI-QTOF analysis of H3-Q94 variants in the process of preparing H3-Q94$Q_{me}$. B) SDS-PAGE analysis of H3 wt and H3-Q94$Q_{me}$. C) The MS/MS analysis of H3-Q94$Q_{me}$.

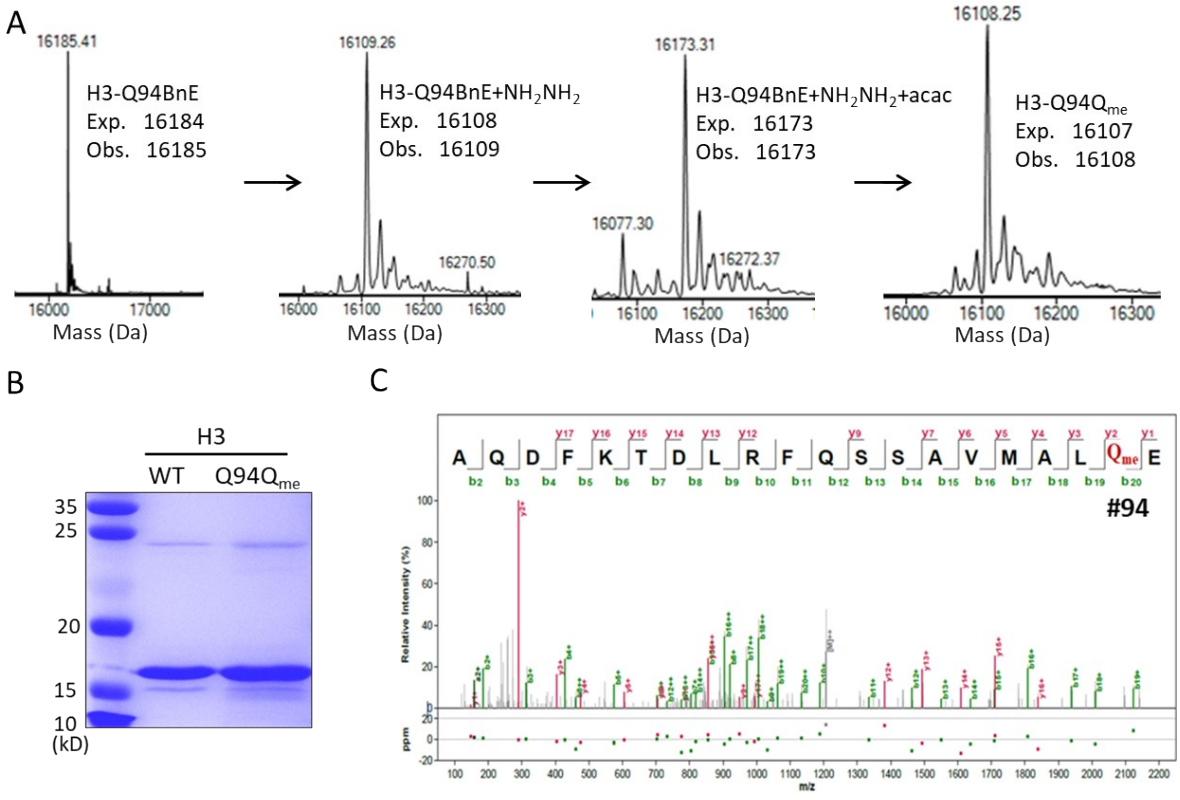


Table S1. DNA sequencing results for the evolution of PylRS to encode BnE. Mutation sites are highlighted in bold.

| MbPylRS (frequence in sequencing) | A267 | Y271 | N311 | C313 | Y349 |
|---|---|---|---|---|---|
| BnERS1 (6/10) | Ala | Tyr | **Ser** | **Ala** | **Phe** |
| BnERS2 (3/10) | Ala | Tyr | **Ser** | **Ser** | **Phe** |
| BnERS3 (1/10) | Ala | Tyr | **Ser** | **Val** | **Trp** |

Table S2. Primers used in this study.

| **oligonucleotide** | **Sequence (5'-3')** |
|---|---|
| BnERS-F | GAATTTACCATGGTTAGCTTCGCCCAAATGGGCAGCGGTTGTACGCGC |
| BnERS-R | GCTGCCCATTTGGGCGAAGCTAACCATGGTAAATTCTTCCAGGTGTTC |
| sfGFP_S2E-F | AAATTACATATGGAAAAAGGAGAAGAACTTTTCACTGGAG |
| sfGFP_S2E-R | CTTCTCCTTTTTCCATATGTAATTTCTCCTCTTTAATGAAT |
| SUMO-sfGFP_S2E-F | CAAATTGGTGGCGAAAAAGGAGAAGAACTTTTCACTGG |
| SUMO-sfGFP_S2E-R | CTTCTCCTTTTTCGCCACCAATTTGCTCACGGTGCGC |
| barnase-E73TAG-F | GAACATGGCGTTAGGCGGATATTAACTATACATCAGG |
| barnase-E73TAG-R | TTAATATCCGCCTAACGCCATGTTCGTCCGCTTTTG |
| pCMV-BnERS-311_313-F | TTTACCATGGTTAGCTTCGCCCAAATGGGCAGCGGTTGTACGCGCG |
| pCMV -BnERS-311_313-R | TGCCCATTTGGGCGAAGCTAACCATGGTAAATTCTTCCAGGTGTTC |
| pCMV -BnERS-349-F | CTTGCATGGTGTTTGGCGATACCCTGGACATCATGCA |
| pCMV -BnERS-349-R | CAGGGTATCGCCAAACACCATGCAAGAATCACCGACG |
| SUMO-F | CAATTTCACACAGAATTCATTAAAGAGGAGAAATTACATATGGGCAGCGATAGCGAGGTG AACCAGG |
| SUMO-R | GACAACTCCAGTGAAAAGTTCTTCTCCTTTCTAGCCACCAATTTGCTCACGGTGCGC |
| pET22b-vector-F | TAGAAAGGAGAAGAACTTTTCACTGGAGTTGTCCC |
| pET22b-vector-R | CATATGTAATTTCTCCTCTTTAATGAATTCTGTGTGAAATTGTTATCCGC |
| barnase-vector-F | GGATCCCACCACCACCACCACCACTAA |
| barnase-vector-R | CATATGTAATTTCTCCTCTTTAATGAATTCTGTGTGAAATTGTTAT |
| barnase-F | CACACAGAATTCATTAAAGAGGAGAAATTACATATGGCACAGGTTATCAACACGTTTGA |
| barnase-R | TAGTGGTGGTGGTGGTGGTGGGATCCTCTGATTTTTGTAAAGGTCTGATAATGGTCCGT |
| pUltra-F | ATTAAATCAGAACGCAGAAGCGGTCTGAT |
| pUltra-R | GAATCTGTATCAGGCTGAAAATCTTCTCTCATCCGCC |
| SUMOprotease-F | atgagagaagattttcagcctgatacagattcattaaggaggagaaattacatATGCACCATCATCACCACCAC GGTGGCAGC |
| SUMOprotease-R | gaccgcttctgcgttctgatttaatTTATTACTTCAGCGCATCGGTCAGAATCAGGTG |
| Vector-F | CACCACCACTAAAAGCTTAATTAGCTGAGCTTG |
| Vector-R | CTAGCCACCAATTTGCTCACGGTGCGCTTC |
| 1433-F | GCGCACCGTGAGCAAATTGGTGGCTAGGATCGCGAACAGCTTGTTCAAAAAGCTCGCC |
| 1433-R | CTCAGCTAATTAAGCTTTTAGTGGTGGTGGTGGTGGTGCTCGAGGT |
| Trx-F | GCACCGTGAGCAAATTGGTGGCTAGGATAAAATTATTCACCTGACTGACGACAGTTTTG |
| Trx-R | CTCAGCTAATTAAGCTTTTAGTGGTGGTGGTGGTGGTGCGCCAGGTTAGCGTCGAGGAA |
| BnE-His-sfGFP-F | AATTGGTGGCTAGCACAAAGGAGAAGAACTTTTCACTGGA |
| BnE-His-sfGFP-R | TCTCCTTTGTGCTAGCCACCAATTTGCTCACGGTGCGC |
| H3-insert-F | aggagaaattacatATGGCGCGTACCAAACAGACCG |
| H3-insrt-R | GTGGTGGTGGTGGTGCGCACGTTCGCCACGGATACGA |
| H3-Vector-F | TGGCGAACGTGCGCACCACCACCACCACCACTAAaagc |

| | |
|---|---|
| H3-Vector-R | GTTTGGTACGCGCCATatgtaatttctcctctttaatgaattctgtgtgaaattgttat |
| H3-Q5TAG-F | GCGTACCAAATAGACCGCGCGTAAAAGCACCGGTGGCA |
| H3-Q5TAG-R | TACGCGCGGTCTATTTGGTACGCGCCATatgtaatttctcct |
| H3-Q56TAG-F | TCCGTCGTTACTAGAAAAGCACCGAACTGCTGATCCGT |
| H3-Q56TAG-R | TTCGGTGCTTTTCTAGTAACGACGGATTTCACGCAGCGC |
| H3-Q94TAG-F | TTATGGCGCTGTAGGAAGCGTGCGAAGCGTACCTGGTT |
| H3-Q94TAG-R | CGCACGCTTCCTACAGCGCCATAACCGCGCTGCTCTG |
| SUMO-S2Q-F | AATTGGTGGCCAGAAAGGAGAAGAACTTTTCACTGGAGTTGTCC |
| SUMO-S2Q-R | AGTTCTTCTCCTTTCTGGCCACCAATTTGCTCACGGTGCGC |
| 210-212-F | TCTGTCCTTTCGAAATAGCCCTAGGAAAAGCGTGACCACATG |
| 210-212-R | TTTCCTAGGGCTATTTCGAAAGGACAGATTGTGTCGACAG |
| K45TAG-F | ACTCACCCTTTAGTTTATTTGCACTACTGGAAAACTACCTGTTCC |
| K45TAG-R | AGTAGTGCAAATAAACTAAAGGGTGAGTTTTCCGTTTGTAGCATC |
| SUMO-Fab-vector-F | gtgcagctggtggagtctggag |
| SUMO-Fab-vector-R | agcgtacgcgtttgtagcaatagaaaaaa |
| SUMO-Fab-insert-F | tattgctacaaacgcgtacgctATGGGCAGCGATAGCGAGGTGA |
| SUMO-Fab-insert-R | ctccagactccaccagctgcacCTAGCCACCAATTTGCTCACGGTGC |