Supporting Information

An Improved Intracellular Synthetic Lipidation-Induced Plasma Membrane Anchoring System for SNAP-Tag Fusion Proteins

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Supplementary Figures

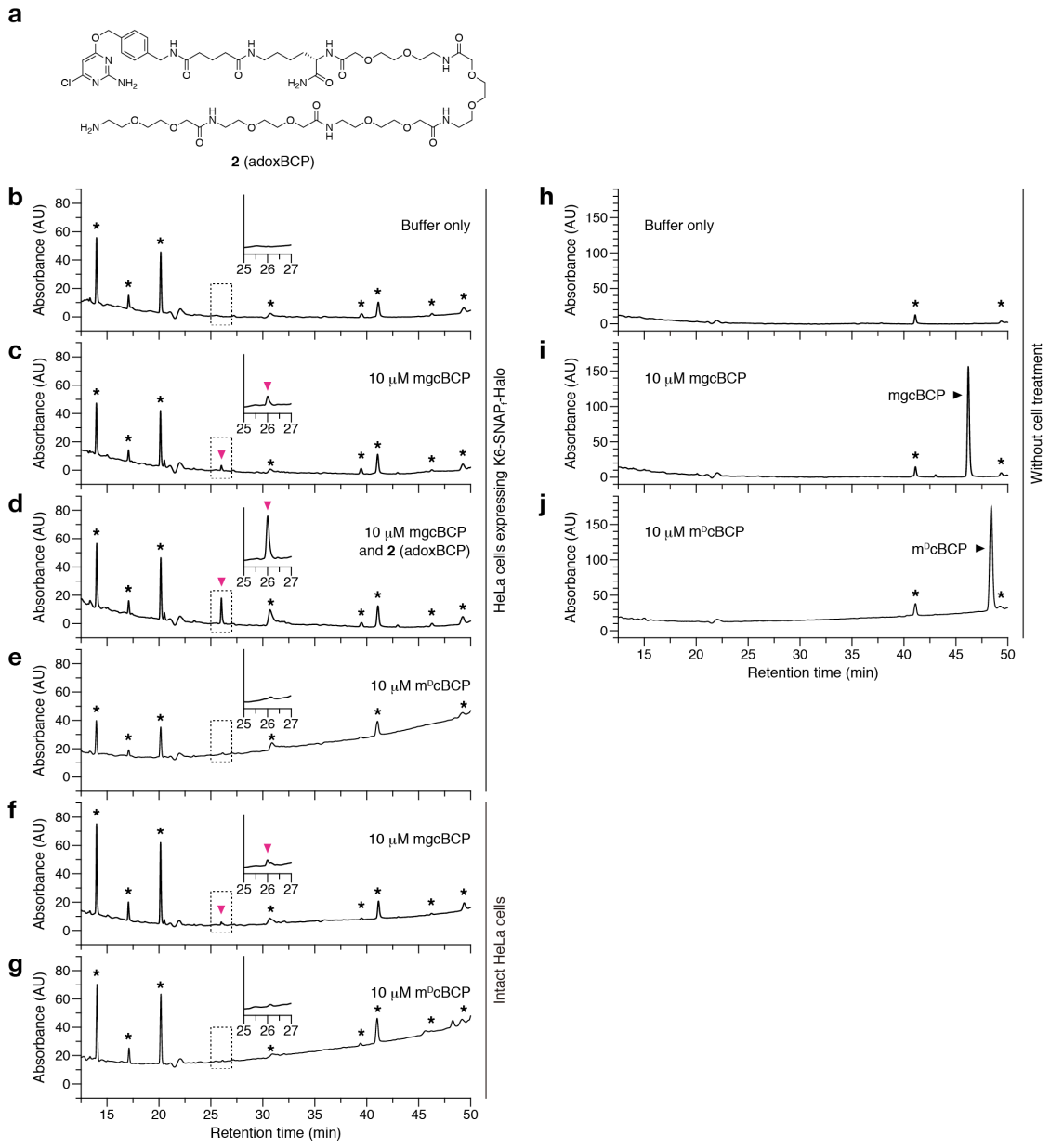

Figure S1. HPLC analysis of mgcBCP cleavage. (**a**) Chemical structure of compound **2** (adoxBCP). (**b**) HPLC of HEPES buffered saline (HBS) (no ligand) collected after incubation with K6-SNAP$_f$-Halo-expressing HeLa cells at 37 °C for 3 h. (**c**) HPLC of mgcBCP (10 μM)-containing HBS collected after incubation with K6-SNAP$_f$-Halo-expressing HeLa cells at 37 °C for 3 h. (**d**) HPLC of a mixture of mgcBCP (10 μM)-containing HBS treated as described in (**c**) and chemically synthesized compound **2**. (**e**) HPLC of m^{D}cBCP (10 μM)-containing HBS treated as described in (**c**). (**f**) HPLC of mgcBCP (10 μM)-containing HBS collected after incubation with

intact HeLa cells at 37 °C for 3 h. (**g**) HPLC of m^{D}cBCP (10 μM)-containing HBS treated as described in (**f**). (**h**) HPLC of HBS alone (no ligand and no cell treatment). (**i**) HPLC of mgcBCP (10 μM)-containing HBS incubated at 37 °C for 3 h (without cells). (**j**) HPLC of m^{D}cBCP (10 μM)-containing HBS incubated at 37 °C for 3 h (without cells). Asterisks indicate background peaks. In **c**, the new peak (around 26 min) indicated by a pink arrowhead was collected and analyzed by high-resolution mass spectrometry. The observed mass was consistent with the theoretical mass of compound **2** (adoxBCP): calculated for $[M+H]^{+}$, 1231.5973; found, 1231.5924. In **d**, the new peak overlapped with the chemically synthesized adoxBCP. These results demonstrate that the amide bond C-terminal to the Cys residue of mgcBCP was cleaved during incubation with cells. No such degradation was observed in the case of m^{D}cBCP [**e** and **g**].

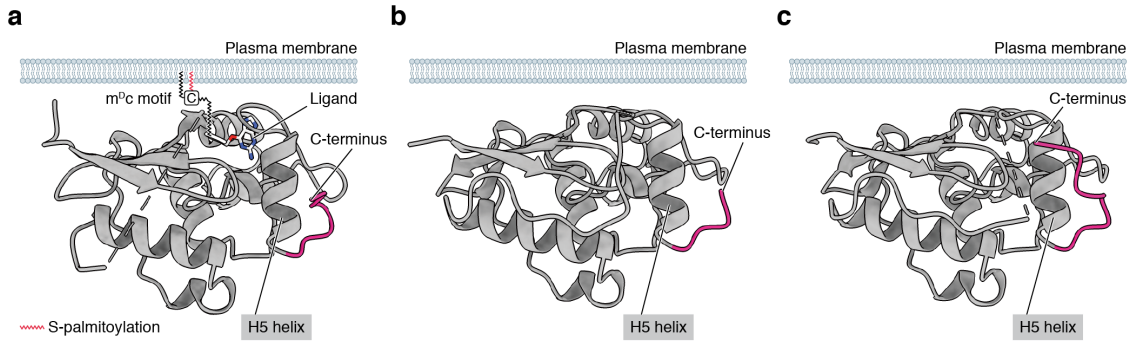

Figure S2. The C-terminus of SNAP-tag points to the PM when it is anchored on the PM by m^{D}cBCP. (**a**) Crystal structure of SNAP-tag bound to its substrate benzylguanine (PDB: 3KZZ[S1]). The structure is depicted in a manner where the SNAP-tag is anchored on the putative PM by m^{D}cBCP. (**b**,**c**) Crystal structures of human O^6-alkylguanine-DNA alkyltransferase [PDB IDs: 1QNT[S2] (**b**) and 1EH6[S3] (**c**)]. The C-terminal polypeptide chain following the H5 helix is shown in magenta. All three crystal structures suggest that the C-terminus of SNAP-tag appears to point toward the PM in this membrane-anchored orientation. These models were displayed with UCSF ChimeraX.[S4]

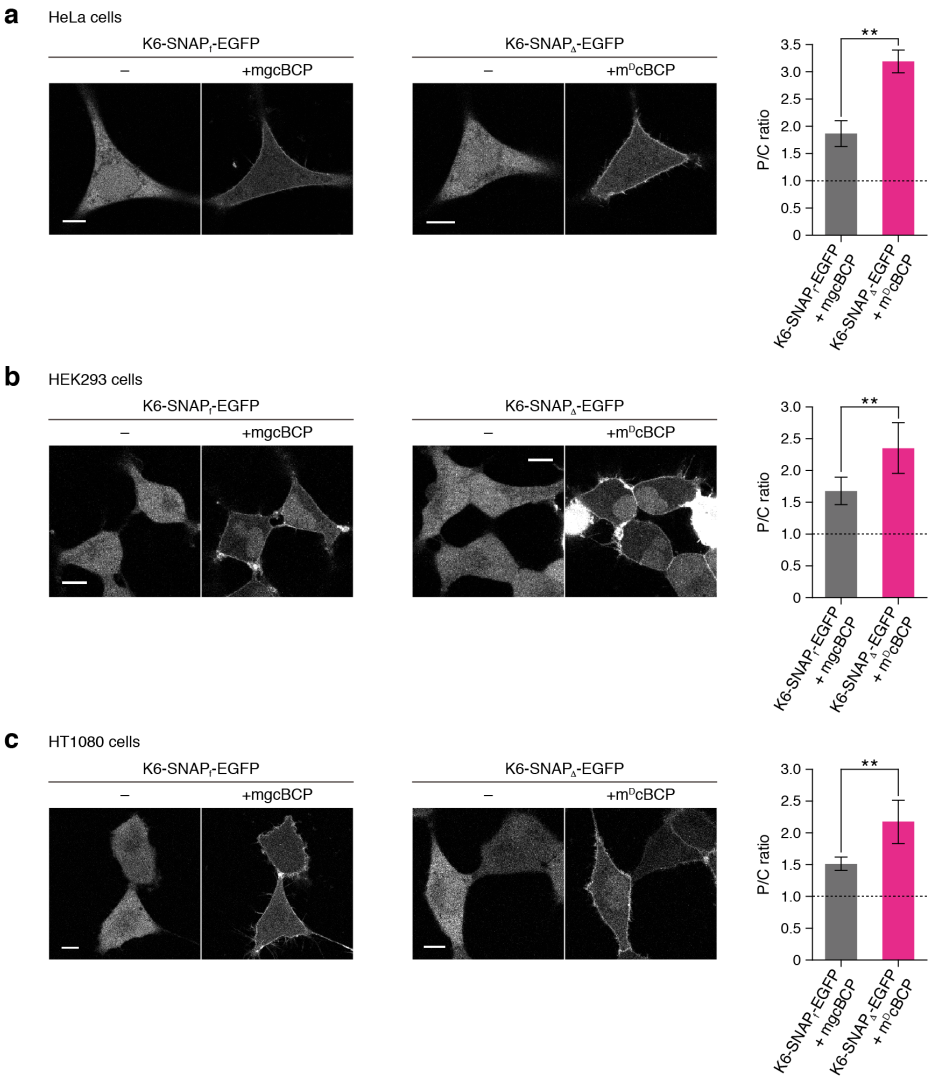

Figure S3. PM recruitment of EGFP fusion protein by the original and second-generation SNAP-tag SLIPT systems. (**a**) HeLa cells. (**b**) HEK293 cells. (**c**) HT1080 cells. (**Left panel**) Confocal fluorescence images of cells expressing K6-SNAP$_f$-EGFP were taken before (left) and 60 min after incubation with mgcBCP (10 μM) (right). Scale bars, 10 μm. (**Center panel**) Confocal fluorescence images of cells expressing K6-SNAP$_\Delta$-EGFP were taken before (left) and 60 min after incubation with m^{D}cBCP (10 μM) (right). (**Right panel**) Quantification of the PM anchoring efficiency. The ratios of the PM to the cytosolic fluorescence intensity (P/C ratios) of the indicated construct were quantified after treatment with the indicated compound (10 μM) for 60 min. Cells with similar expression levels (i.e., fluorescence intensities) were observed and analyzed. Data are presented as the mean ± SD ($n > 6$ cells). The symbols indicate the results of *t* test analysis; n.s.: $p > 0.01$, *$p < 0.01$, **$p < 0.001$.

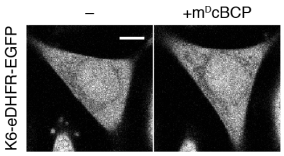

Figure S4. Control experiment using K6-eDHFR-EGFP. Confocal fluorescence images of cells expressing K6-eDHFR-EGFP were taken before (left) and 60 min after incubation with m^{D}cBCP (10 μM) (right). Scale bar, 10 μm.

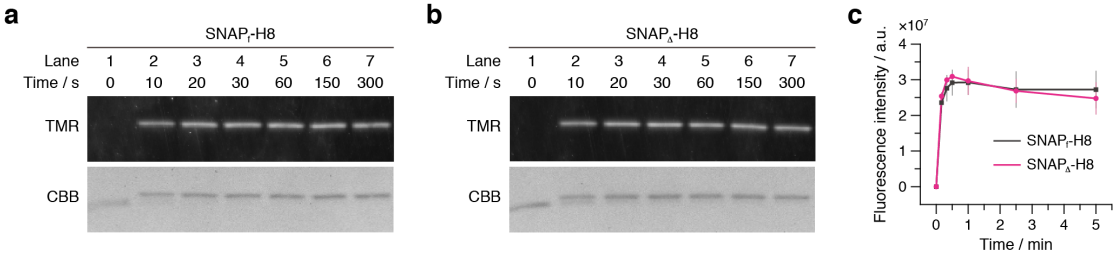

Figure S5. Evaluation of in vitro labeling properties of $SNAP_f$ and $SNAP_\Delta$ proteins. (**a**) Purified His-tagged $SNAP_f$ ($SNAP_f$-H8) (5 μM) was incubated with SNAP-Cell TMR-Star (10 μM) in buffer (pH 7.2) at 25 °C for the indicated time. Samples were subjected to SDS-PAGE and analyzed by in-gel fluorescence imaging (TMR) and Coomassie brilliant blue (CBB) staining. (**b**) Labeling of purified His-tagged $SNAP_\Delta$ ($SNAP_\Delta$-H8) was performed in the same manner as described in (**a**). (**c**) Quantification of the fluorescent labeling. Fluorescence intensities of the bands were plotted as a function of the reaction time. The experiments were carried out in triplicate and data are presented as the mean ± SD.

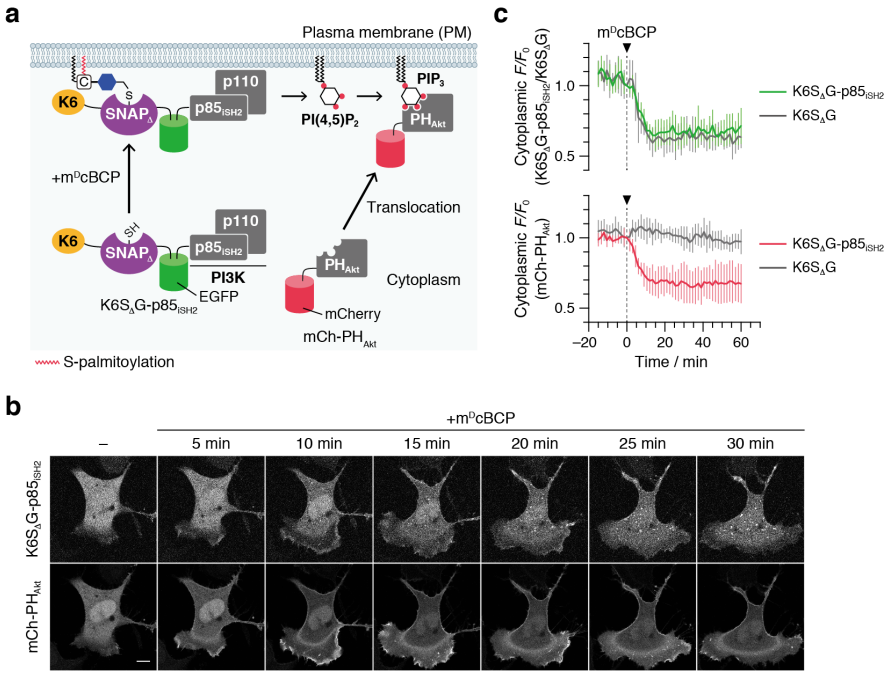

Figure S6. Synthetic PI3K-mediated PI(3,4,5)P_3 production by the second-generation SNAP-tag SLIPT system. (**a**) Schematic illustration of the experimental setup for the PI3K-mediated PI(3,4,5)P_3 production.[S5] (**b**) Time-lapse confocal fluorescence images of HeLa cells coexpressing K6-SNAP$_\Delta$-EGFP-p85$_{iSH2}$ (K6S$_\Delta$G-p85$_{iSH2}$) and mCherry-PH$_{Akt}$[S6] (mCh-PH$_{Akt}$) were taken before and after the addition of 10 μM m^{D}cBCP. Scale bar, 10 μm. (**c**) Time course of K6S$_\Delta$G-p85$_{iSH2}$ recruitment and PI(3,4,5)P_3 production. To evaluate the K6S$_\Delta$G-p85$_{iSH2}$ recruitment (green), normalized fluorescence intensities of K6S$_\Delta$G-p85$_{iSH2}$ in the cytoplasm were plotted as a function of time. To evaluate the PI(3,4,5)P_3 production (red), normalized fluorescence intensities of mCh-PH$_{Akt}$ in the cytoplasm were plotted as a function of time. Gray lines correspond to control experiments using K6S$_\Delta$G lacking the p85$_{iSH2}$ domain. Data are presented as the mean ± SD (n > 4 cells).

Note: In addition to PM translocation of K6S$_\Delta$G-p85$_{iSH2}$, dispersed fluorescent puncta were observed in the EGFP channel after the addition of m^{D}cBCP (**b**). The reason for this phenomenon is unclear, but we hypothesize that it may be due to unexpected partial aggregation of K6S$_\Delta$G-p85$_{iSH2}$, which was induced by binding to m^{D}cBCP. Such puncta formation was seen only for this p85$_{iSH2}$ domain among the tested proteins.

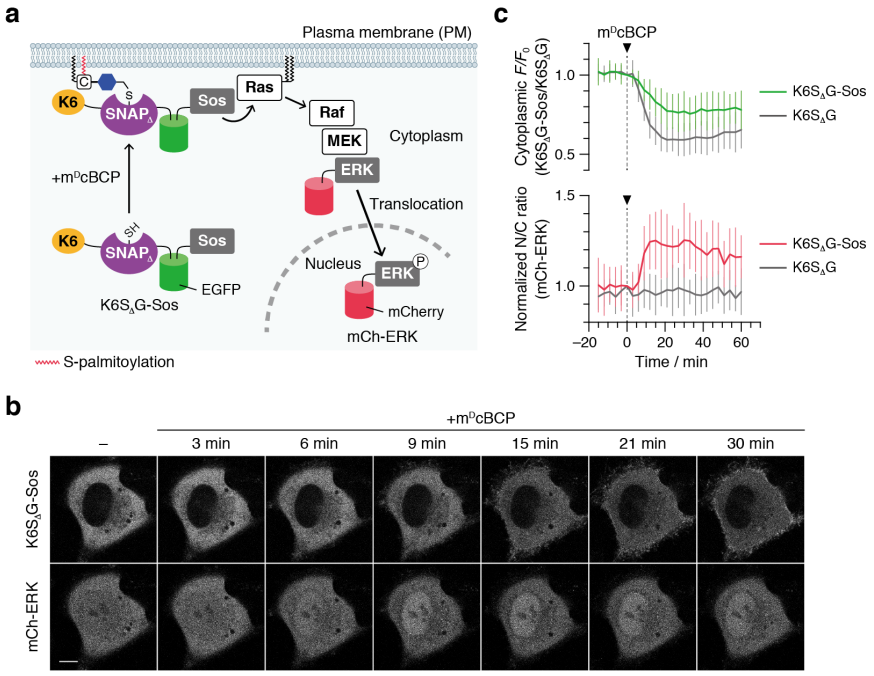

Figure S7. Synthetic Sos-mediated Ras/ERK activation by the second-generation SNAP-tag SLIPT system. (**a**) Schematic illustration of the experimental setup for the Sos-mediated Ras/ERK activation.[S7] (**b**) Time-lapse confocal fluorescence images of HeLa cells coexpressing K6-SNAP$_{\Delta}$-EGFP-Sos (K6S$_{\Delta}$G-Sos) and mCherry-ERK[S8] (mCh-ERK) were taken before and after the addition of 10 μM m^{D}cBCP. Scale bar, 10 μm. (**c**) Time course of K6S$_{\Delta}$G-Sos recruitment and ERK activation. To evaluate the K6S$_{\Delta}$G-Sos recruitment (green), normalized fluorescence intensities of K6S$_{\Delta}$G-Sos in the cytoplasm were plotted as a function of time. To evaluate the ERK activity (red), normalized ratios of the nuclear to cytoplasm fluorescence intensity (N/C ratios) of mCh-ERK were plotted as a function of time. Gray lines correspond to control experiments using K6S$_{\Delta}$G lacking the Sos. Data are presented as the mean ± SD (n > 10 cells).

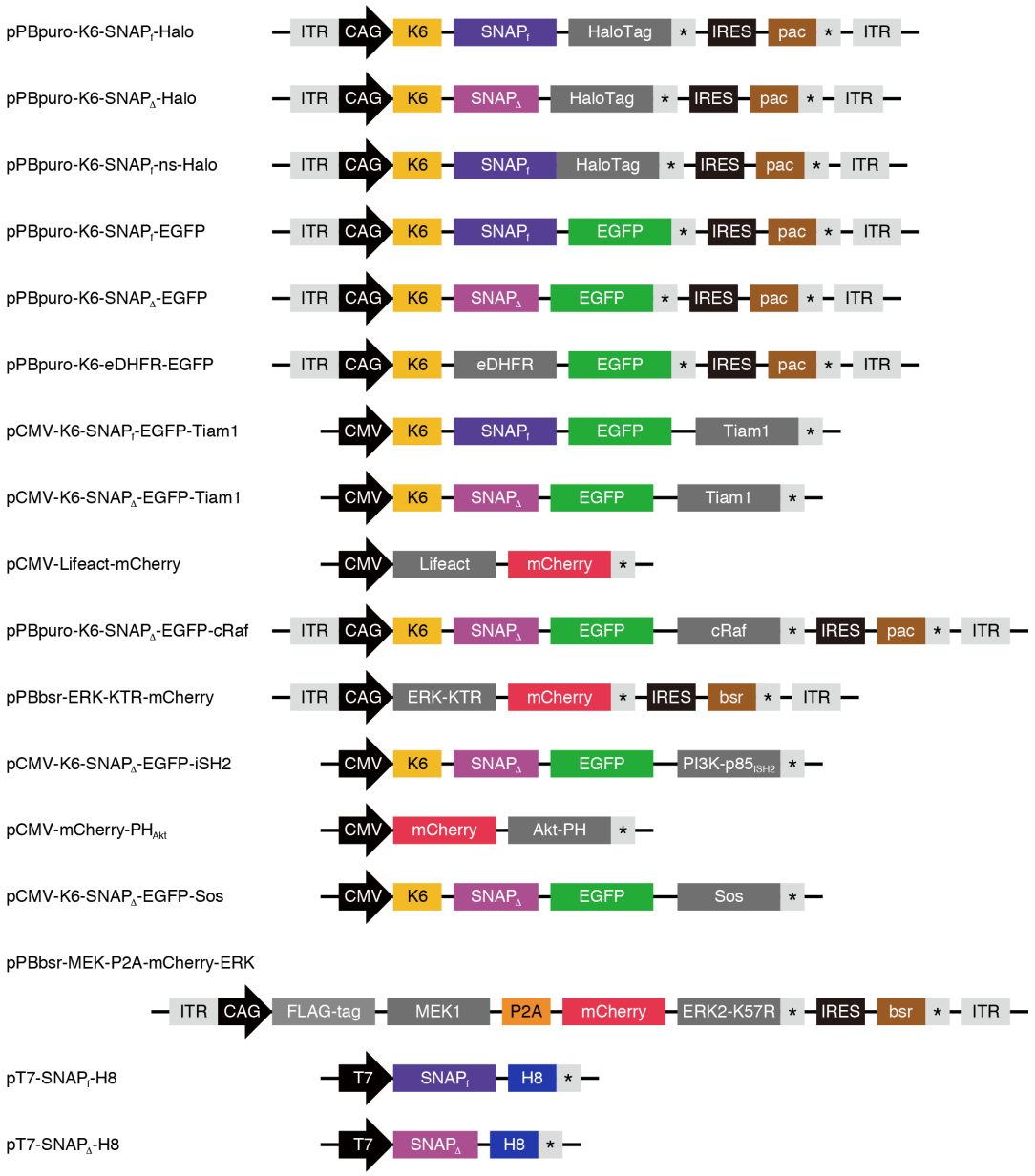

Figure S8. Schematic illustration of the domain structures of constructs used in this study.

a

pPBpuro-K6-SNAP$_f$-Halo

>Amino acid sequence

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b

pPBpuro-K6-SNAP$_{\Delta}$-Halo

>Amino acid sequence

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pPBpuro-K6-SNAP$_f$-ns-Halo

>Amino acid sequence

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pPBpuro-K6-SNAP$_f$-EGFP

>Amino acid sequence

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>DNA sequence

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pPBpuro-K6-SNAP$_{\Delta}$-EGFP

>Amino acid sequence

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>DNA sequence

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pPBpuro-K6-eDHFR-EGFP

>Amino acid sequence

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>DNA sequence

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>Amino acid sequence

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>DNA sequence

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h

pPBpuro-K6-SNAP$_{\Delta}$-EGFP-Tiam1

>Amino acid sequence

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>DNA sequence

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i

pCMV-Lifeact-mCherry

>Amino acid sequence
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>DNA sequence
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j

pPBpuro-K6-SNAP$_{\Delta}$-EGFP-cRaf

>Amino acid sequence

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>DNA sequence

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k

pPBbsr-ERK-KTR-mCherry

>Amino acid sequence

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>DNA sequence

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I

pCMV-K6-SNAP$_{\Delta}$-EGFP-iSH2

>Amino acid sequence

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>DNA sequence

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m

pCMV-mCherry-PH_{Akt}

>Amino acid sequence

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>DNA sequence

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A

n

pCMV-K6-SNAP$_{\Delta}$-EGFP-Sos

>Amino acid sequence

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>DNA sequence

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o

pPBbsr-MEK-P2A-mCherry-ERK

>Amino acid sequence

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>DNA sequence

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p

pT7-SNAP$_f$-H8

>Amino acid sequence
MDKDCEMKRTTLDSPLGKLELSGCEQGLHRIIFLGKGTSAADAVEVPAPAAVLGGPEPLMQ
ATAWLNAYFHQPEAIEEFPVPALHHPVFQQESFTRQVLWKLLKVVKFGEVISYSHLAALAG
NPAATAAVKTALSGNPVPILIPCHRVVQGDLDVGGYEGGLAVKEWLLAHEGHRLGKPGLGG
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>DNA sequence
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q

pT7-SNAP$_\Delta$-H8

>Amino acid sequence
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ATAWLNAYFHQPEAIEEFPVPALHHPVFQQESFTRQVLWKLLKVVKFGEVISYSHLAALAG
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>DNA sequence
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Figure S9. DNA and amino acid sequences of constructs used in this study. (**a**) pPBpuro-K6-SNAP$_f$-Halo: orange, K6-tag; blue, SNAP$_f$; gray box, HaloTag; yellow marker, an internal ribosomal entry site from Encephalomyocarditis virus (EMCV IRES)[S9]; brown, puromycin *N*-acetyltransferase (pac). (**b**) pPBpuro-K6-SNAP$_\Delta$-Halo: orange, K6-tag; purple, SNAP$_\Delta$; gray box, HaloTag; yellow marker, EMCV IRES; brown, pac. (**c**) pPBpuro-K6-SNAP$_f$-ns-Halo: orange, K6-tag; blue, SNAP$_f$; gray box, HaloTag; yellow marker, EMCV IRES; brown, pac. (**d**) pPBpuro-

K6-SNAP$_f$-EGFP: orange, K6-tag; blue, SNAP$_f$; green, EGFP; yellow marker, EMCV IRES; brown, pac. (**e**) pPBpuro-K6-SNAP$_\Delta$-EGFP: orange, K6-tag; purple, SNAP$_\Delta$; green, EGFP; yellow marker, EMCV IRES; brown, pac. (**f**) pPBpuro-K6-eDHFR-EGFP: orange, K6-tag; gray, eDHFR; green, EGFP; yellow marker, EMCV IRES; brown, pac. (**g**) pCMV-K6-SNAP$_f$-EGFP-Tiam1: orange, K6-tag; blue, SNAP$_f$; green, EGFP; gray box, a DH-PH domain (residues 1012–1591) from human Tiam1. (**h**) pCMV-K6-SNAP$_\Delta$-EGFP-Tiam1: orange, K6-tag; purple, SNAP$_\Delta$; green, EGFP; gray box, a DH-PH domain (residues 1012–1591) from human Tiam1. (**i**) pCMV-Lifeact-mCherry: gray box, Lifeact; red, mCherry. (**j**) pPBpuro-K6-SNAP$_\Delta$-EGFP-cRaf: orange, K6-tag; purple, SNAP$_\Delta$; green, EGFP; gray box, cRaf; yellow marker, EMCV IRES; brown, pac. (**k**) pPBbsr-ERK-KTR-mCherry: orange, K6-tag; gray box, ERK-KTR; red, mCherry; yellow marker, EMCV IRES; brown, blasticidin *S*-deaminase (bsr). (**l**) pPBpuro-K6-SNAP$_\Delta$-EGFP-p85$_{iSH2}$: orange, K6-tag; purple, SNAP$_\Delta$; green, EGFP; gray box, an iSH2 domain (residues 617–724) from human PI3K (p85α); yellow marker, EMCV IRES; brown, pac. (**m**) pCMV-mCherry-PH$_{Akt}$: red, mCherry; grey box, a PH domain (residues 1–148) from human Akt1. (**n**) pCMV-K6-SNAP$_\Delta$-EGFP-Sos: orange, K6-tag; purple, SNAP$_\Delta$; green, EGFP; gray box, Sos. (**o**) pPBbsr-MEK-P2A-mCherry-ERK: grey, FLAG-tag; purple, MEK1 from *Xenopus laevis*; black box, 2A self-cleaving peptide (P2A)[S10]; red, mCherry; blue, ERK2(K57R) from *Xenopus laevis*; yellow marker, EMCV IRES; brown, bsr. (**p**) pT7-SNAP$_f$-H8: blue, SNAP$_f$; blue box, polyhistidine tag. (**q**) pT7-SNAP$_\Delta$-H8: purple, SNAP$_\Delta$; blue box, polyhistidine tag.

Supplementary Methods: Chemical Synthesis

General materials and methods

All chemical reagents and solvents were purchased from commercial suppliers (Watanabe Chemical Industries, Tokyo Chemical Industry, and Kanto Chemical) and used without further purification. Reverse-phase HPLC was performed on a Hitachi LaChrom Elite system with UV detection at 220 nm using a YMC-Pack ODS-A column (10 × 250 mm or 20 × 250 mm). ^{1}H NMR spectra were recorded on a Bruker AVANCE III HD400SJ (400 MHz) spectrometer. ^{1}H NMR chemical shifts were referenced to tetramethylsilane (0 ppm). High-resolution mass spectra were measured on a Thermo Scientific Extractive Plus Orbitrap mass spectrometer.

mgcBCP[S6] and compound **3** (BCP-COOH)[S11] were synthesized as described previously.

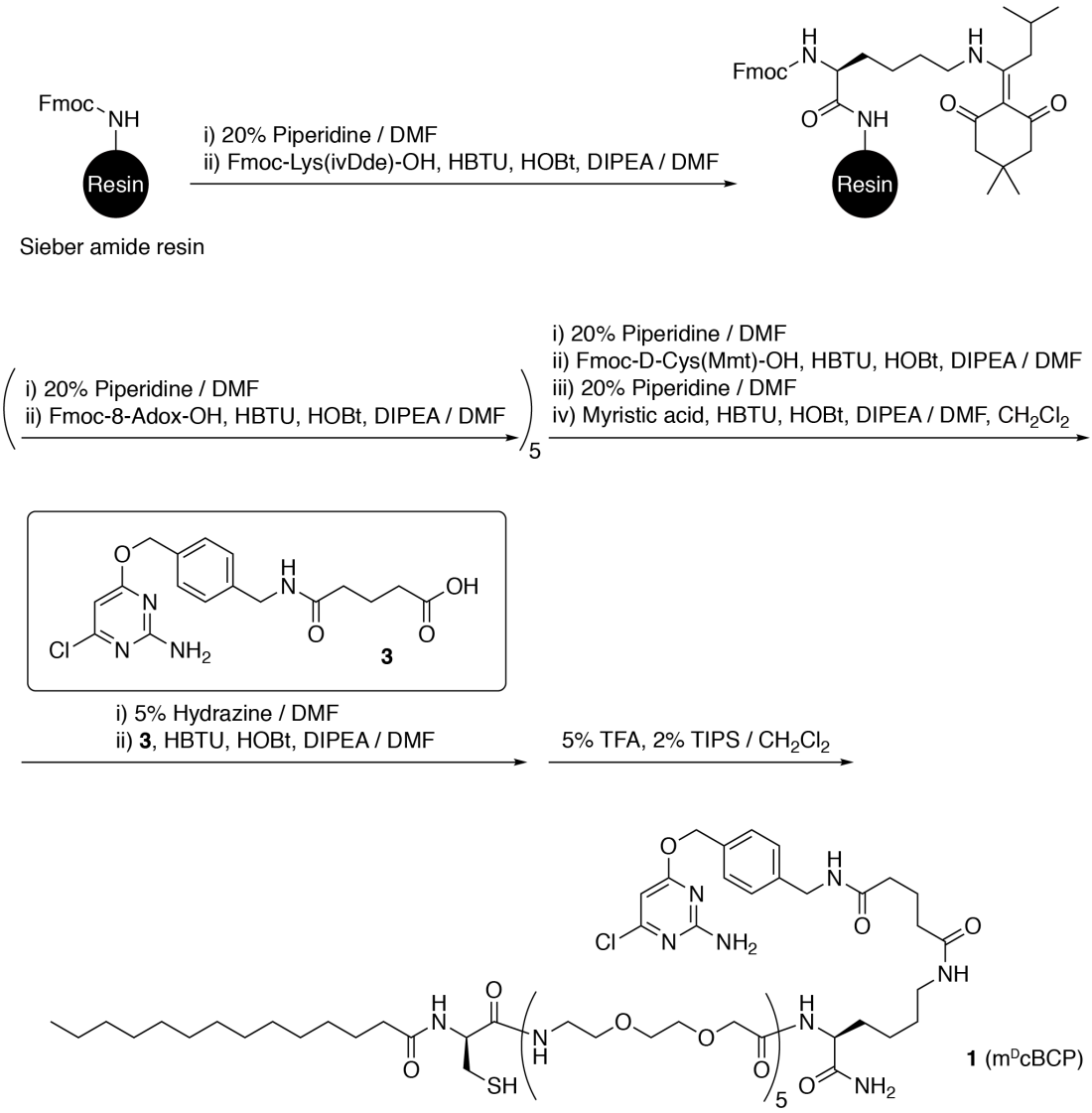
Reagent abbreviations

Boc_2O: di-*tert*-butyl dicarbonate
DIPEA: *N*,*N*-diisopropylethylamine
DMF: *N*,*N*-dimethylformamide
Fmoc-Adox-OH: Fmoc-8-amino-3,6-dioxaoctanoic acid
HBTU: *O*-(benzotriazole-1-yl)-*N*,*N*,*N*',*N*'-tetramethyluronium hexafluorophosphate
HOBt: 1-hydroxybenzotriazole (monohydrate)
TFA: trifluoroacetic acid
TIPS: triisopropylsilane
TMS: tetramethylsilane

General methods for solid-phase synthesis

Compounds **1** (m^{D}cBCP) and **2** (adoxBCP) were synthesized manually on Sieber amide resin by standard Fmoc-based solid-phase peptide synthesis protocols. Fmoc deprotection was performed with 20% piperidine in DMF at room temperature for 30 min. Amino acid coupling reactions were performed at room temperature with a mixture of Fmoc-protected amino acid (3.1 eq.), HBTU (3.0 eq.), HOBt (3.0 eq.), and DIPEA (6.0 eq.) in DMF. All Fmoc deprotection and coupling steps were monitored by the Kaiser test.[S12] Unless otherwise stated, all washing procedures were performed with DMF.

Synthesis of Compound 1



Scheme S1. Synthetic route of **1** (m^DcBCP)

Compound **1** (m^DcBCP) was synthesized manually on Sieber amide resin (0.69 mmol/g) (58 mg, 40 μmol). First, Fmoc-Lys(ivDde)-OH, Fmoc-Adox-OH (×5), and Fmoc-D-Cys(Mmt)-OH were coupled to the resin. The N-terminus was then myristoylated using a mixture of myristic acid (3.1 eq.), HBTU (3.0 eq.), HOBt (3.0 eq.), and DIPEA (6.0 eq.) in DMF/CH_2Cl_2 (1/1). After washing the resin with DMF, the ivDde group was deprotected by treatment with DMF containing 5% hydrazine monohydrate for 30 min (one time). The resin was then washed with DMF. **3** (BCP-COOH) was coupled to the side chain of the lysine with a mixture of **3** (3.1 eq.), HBTU (3.0 eq.),

HOBt (3.0 eq.), and DIPEA (6.0 eq.) in DMF. After washing with DMF, MeOH, and CH_2Cl_2, the resin was dried *in vacuo*. Deprotection and cleavage from the resin was performed with CH_2Cl_2 containing 5% TFA and 2% TIPS. The crude product was precipitated by Et_2O and purified by reversed-phase HPLC using a semi-preparative C18 column (a linear gradient of MeCN containing 0.1% TFA and 0.1% aqueous TFA) to afford **1** (m^DcBCP) as a white solid [15.7 mg, 23.6% (as a mono-TFA salt)].

Compound **1** (m^DcBCP)

^{1}H NMR (400 MHz, CD_3OD): δ 7.39 (d, J = 8.1 Hz, 2H), 7.29 (d, J = 7.9 Hz, 2H), 6.10 (s, 1H), 5.33 (s, 2H), 4.50–4.39 (m, 2H), 4.38–4.33 (m, 2H), 4.05–3.95 (m, 10H), 3.72–3.67 (m, 20H), 3.62–3.53 (m, 10H), 3.49–3.36 (m, 10H), 3.21–3.10 (m, 2H), 2.90–2.71 (m, 2H), 2.26 (t, J = 7.4 Hz, 4H), 2.21 (t, J = 7.4 Hz, 2H), 1.95–1.67 (m, 4H), 1.66–1.57 (m, 2H), 1.56–1.47 (m, 2H), 1.41–1.36 (m, 2H), 1.28 (s, 20H), 0.89 (t, J = 6.8 Hz, 3H).
HRMS (ESI): calculated for $[M+H]^+$, 1544.8047; found, 1544.8002.

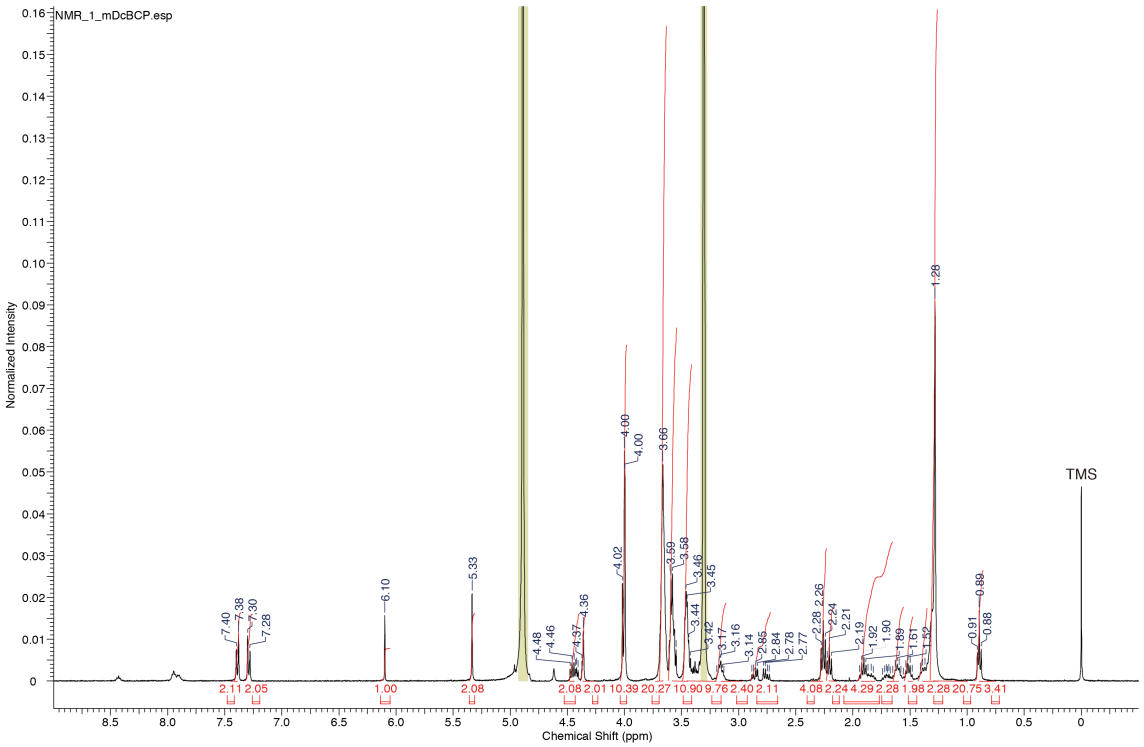
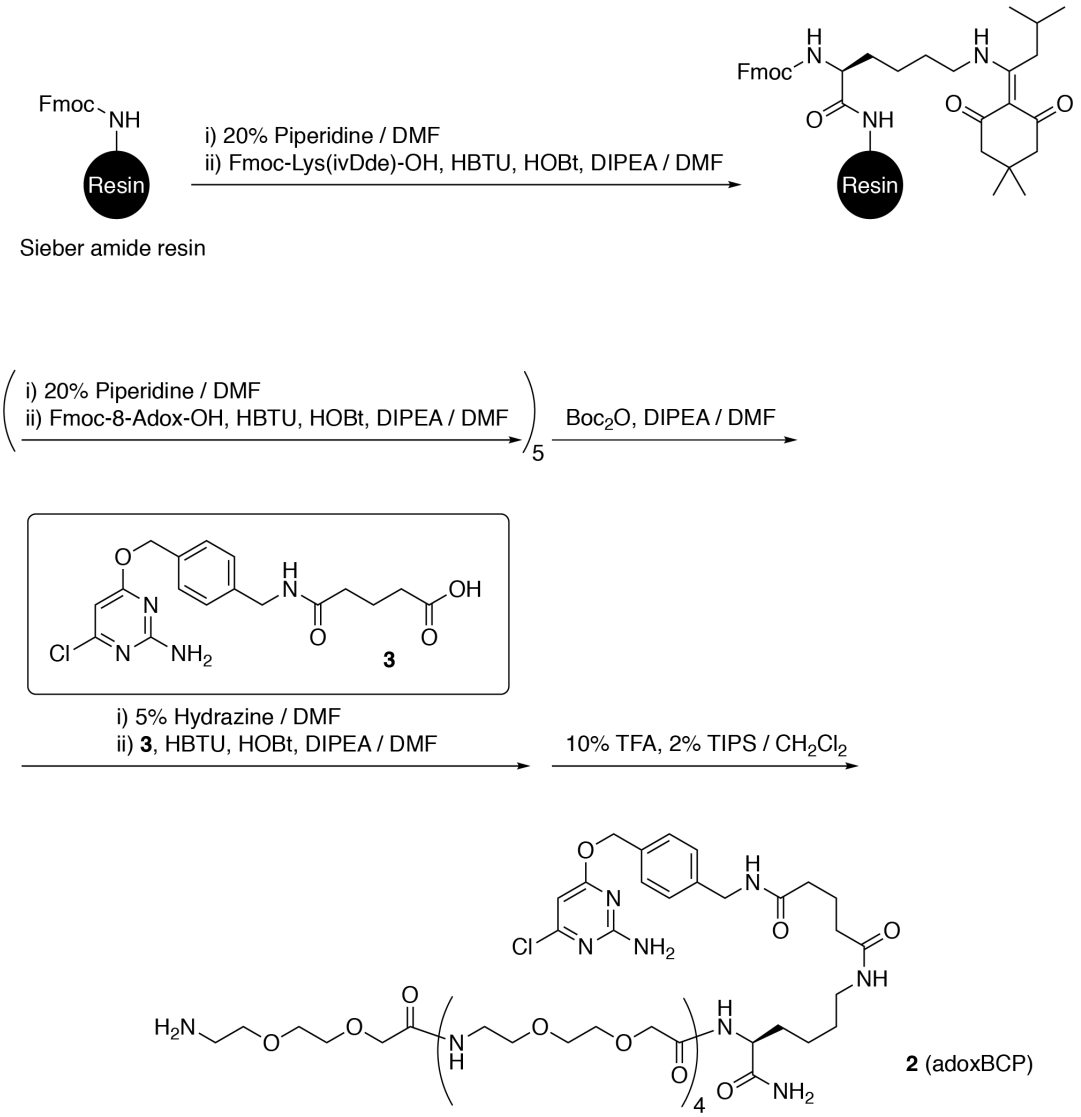


Figure S10. ^{1}H NMR spectrum of **1** (m^DcBCP) in CD_3OD

Synthesis of Compound 2



Scheme S2. Synthetic route of **2** (adoxBCP)

Compound **2** (adoxBCP) was synthesized on Sieber amide resin (0.69 mmol/g) (29 mg, 20 μmol). First, Fmoc-Lys(ivDde)-OH and Fmoc-Adox-OH (×5) were coupled to the resin. After Fmoc deprotection, the N-terminus was Boc-protected using a mixture of Boc_2O (3.1 eq.) and DIPEA (6.0 eq.) in DMF. After washing the resin with DMF, the ivDde group was deprotected by treatment with DMF containing 5% hydrazine monohydrate for 30 min (one time). The resin was then washed with DMF. **3** was coupled to the side chain of the lysine with a mixture of **3** (3.1 eq.), HBTU (3.0 eq.), HOBt (3.0 eq.), and DIPEA (6.0 eq.) in DMF. After washing with DMF, MeOH, and CH_2Cl_2, the

resin was dried *in vacuo*. Deprotection and cleavage from the resin was performed with CH_2Cl_2 containing 10% TFA and 2% TIPS. The crude product was precipitated by Et_2O and purified by reversed-phase HPLC using a semi-preparative C18 column (a linear gradient of MeCN containing 0.1% TFA and 0.1% aqueous TFA) to afford **2** (adoxBCP) as a colorless oil [10.5 mg, 35.9% (as a di-TFA salt)].

Compound **2** (adoxBCP)

^{1}H NMR (400 MHz, CD_3OD): δ 7.38 (d, J = 8.1 Hz, 2H), 7.28 (d, J = 8.1 Hz, 2H), 6.10 (s, 1H), 5.33 (s, 2H), 4.46–4.39 (m, 1H), 4.36 (s, 2H), 4.00 (m, 10H), 3.72–3.63 (m, 22H), 3.61–3.55 (m, 8H), 3.48–3.42 (m, 8H), 3.18–3.11 (m, 4H), 2.26 (t, J = 7.5 Hz, 2H), 2.20 (t, J = 7.5 Hz, 2H), 1.90 (quin, J = 7.5 Hz, 2H), 1.57–1.46 (m, 2H), 1.45–1.24 (m, 4H)
HRMS (ESI): calculated for $[M+H]^+$, 1231.5972; found, 1231.5924.

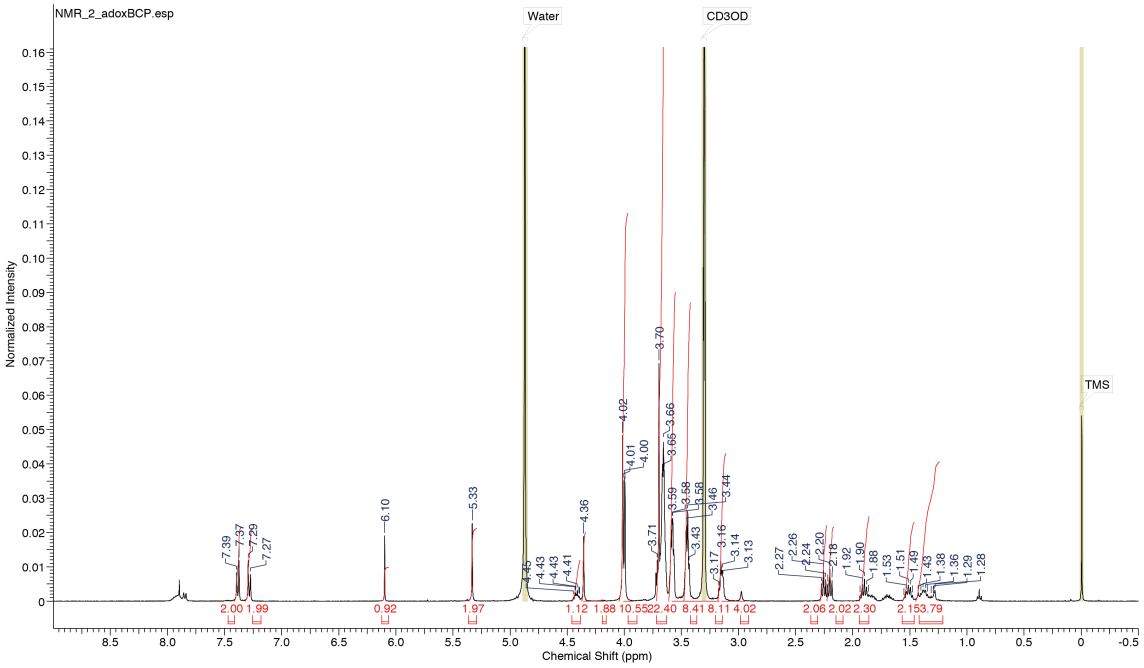


Figure S11. ^{1}H NMR spectrum of **2** (adoxBCP) in CD_3OD

Supplementary Methods: Molecular and Cell Biology Experiments

Plasmid construction

All of the cDNA and amino acid sequences of the constructs used in this study are listed in **Figures S8** and **S9**. We used pPB-CAG.EBNXN (provided by Dr. Allan Bradley, Wellcome Trust Sanger Institute),[S13] pEGFP-C1 (Clontech), pmCherry-N1 (Clontech), and pmCherry-C1 (Clontech) as vector backbones. We also used pPBbsr[S6,S14] (blasticidin *S* resistance) and pPBpuro[S6] (puromycin resistance), as *piggyBac* donor vectors for the establishment of stable cell lines. We used pET-41a(+) (Novagen) as a vector for recombinant protein production. All expression plasmids were generated using standard cloning procedures.

Cell culture and transfection

HeLa and HEK293 cells were obtained from the Cell Resource Center for Biomedical Research, Institute of Development, Aging and Cancer, Tohoku University. HT1080 cells were a gift from Dr. Michiyuki Matsuda (Kyoto University). Cells were cultured in DMEM (Wako) supplemented with 10% heat-inactivated FBS (Biowest), penicillin (100 U/mL), and streptomycin (100 μg/mL) at 37 °C under a humidified 5% CO_2 atmosphere. For transient expression experiments, cells were transfected using Lipofectamine LTX (Invitrogen) or 293fectin (Invitrogen) in accordance with the manufacturer's protocol.

Establishment of stable cell lines

A *piggyBac* transposon system[S13] was employed to establish HeLa cell lines stably expressing the indicated constructs. HeLa cells were cotransfected with a *piggyBac* donor vector (pPBpuro) encoding a desired protein(s) and pCMV-mPBase encoding the *piggyBac* transposase[S13,S15] (provided by Dr. Allan Bradley, Wellcome Trust Sanger Institute) using 293fectin (Invitrogen). Cells were selected with 2 μg/mL puromycin for at least 10 days. Bulk populations of selected cells were used.

Live cell imaging

Fluorescence imaging was performed with an IX83/FV3000 confocal laser-scanning microscope (Olympus) equipped with a PlanApo N 60×/1.42 NA oil objective (Olympus), a Z drift compensator system (IX3-ZDC2, Olympus), and a stage top incubator (Tokai

Hit). Lasers used for excitation were as follows: 488 nm for EGFP, 561 nm for mCherry. Time-lapse live cell imaging was performed at 37 °C. Fluorescence images were analyzed using the Fiji distribution of ImageJ.[S16] The cell outline was traced using the QuimP software.[S17]

SLIPT assays

To conduct the SLIPT assay, HeLa cells stably expressing K6-SNAP$_f$-Halo (established using pPBpuro-K6-SNAP$_f$-Halo), K6-SNAP$_\Delta$-Halo (pPBpuro-K6-SNAP$_\Delta$-Halo), K6-SNAP$_f$-ns-Halo (pPBpuro-K6-SNAP$_f$-ns-Halo), K6-SNAP$_f$-EGFP (pPBpuro-K6-SNAP$_f$-EGFP), or K6-SNAP$_\Delta$-EGFP (pPBpuro-K6-SNAP$_\Delta$-EGFP) were plated at 1.0×10^5 cells in 35 mm glass-bottomed dishes (Iwaki Glass) coated with collagen type I-C (Nitta gelatin). The cells were then cultured for 24 h at 37 °C in 5% CO_2. For cells expressing HaloTag fusion proteins, HaloTag was labeled as described below before the SLIPT assay. The medium was changed to serum-free DMEM supplemented with penicillin (100 U/mL) and streptomycin (100 μg/mL) [DMEM(–)], and the cells were observed by time-lapse imaging before and after addition of the indicated compounds (10 μM) dissolved in DMSO (final DMSO concentration <0.1% v/v).

HaloTag labeling

Cells expressing HaloTag fusion proteins were stained as follows. After 24 h incubation of the cells at 37 °C in 5% CO_2, the cells were washed with DMEM(–) and incubated with 50 nM HaloTag® TMR ligand (Promega) in DMEM(–) for 15 min at 37 °C in 5% CO_2. The cells were then washed with DMEM(–) and incubated in (dye-free) DMEM(–) for 30 min at 37 °C in 5% CO_2. The cells were washed with DMEM(–) once again and used for the SLIPT assay.

HPLC analysis of mgcBCP degradation

Cellular degradation of mgcBCP was analyzed as previously reported.[S10] Intact or K6-SNAP$_f$-Halo-expressing HeLa cells were plated at 4.0×10^5 cells in 60 mm plastic dishes (TPP) and cultured for 24 h at 37 °C. The cells were washed with HEPES buffered saline (HBS) (25 mM HEPES, 119 mM NaCl, 5 mM KCl, 2 mM $MgCl_2$, 2 mM $CaCl_2$, 30 mM glucose, pH 7.4) (2 mL×2) and the medium was changed to 1 mL of HBS containing mgcBCP or m^{D}cBCP (10 μM). After incubation for 3 h at 37 °C, the buffer solution was

collected and filtered with a membrane filter (Millipore, Millex-LH, 0.45 μm). A total of 900 μL of the filtrate was analyzed by reversed-phase HPLC using a C18 column (YMC-Pack ODS-A column, 10×250 mm) with a linear gradient of MeCN containing 0.1% TFA (A) and 0.1% aqueous TFA (B): A/B = 0/100 to 100/0 for 60 min. The HPLC profiles were monitored at 220 nm. The new product shown in **Figure S1** was characterized by high-resolution mass spectrometry (Thermo Scientific Extractive Plus Orbitrap mass spectrometer). For control experiments, mgcBCP or m^{D}cBCP (10 μM) dissolved in HBS was incubated for 3 h at 37 °C and analyzed by reversed-phase HPLC as described above.

Expression and purification of His-tagged SNAP-tag proteins

A His-tagged recombinant $SNAP_f$ protein ($SNAP_f$-H8) was expressed and purified as follows. The pET-41a(+) vector encoding the protein (pT7-$SNAP_f$-H8) was transformed into *E. coli* strain BL21(DE3), and the cells were first cultured in 5 mL of LB broth containing 20 μg/mL kanamycin. The culture was transferred to 400 mL of Terrific Broth (TB) containing 20 μg/mL kanamycin and cells were grown at 37 °C until the optical density at 660 nm reached 0.6. Isopropyl-β-D-thiogalactopyranoside was then added to a final concentration of 100 μM to induce protein expression. The cells were further cultured at 16 °C for 42 h. The cells were collected by centrifugation and lysed by sonication. The lysate was cleared by centrifugation and purified by a HisTrap FF column (GE Healthcare) in accordance with the manufacturer's protocol. The purified protein was dialyzed and stored in 50 mM HEPES, 100 mM NaCl, 1 mM dithiothreitol (DTT), pH 7.2 at 4 °C. A His-tagged recombinant $SNAP_Δ$ ($SNAP_Δ$-H8) protein was obtained in the same manner. The concentration of the proteins was determined by UV spectroscopy using the molar extinction coefficient at 280 nm of 21,345 $M^{-1}cm^{-1}$.[S18]

In vitro SNAP-tag labeling

SNAP-tag labeling experiments were performed by incubating purified $SNAP_Δ$-H8 (5 μM) with a fluorescent SNAP-tag substrate SNAP-Cell TMR-Star (New England Biolabs) (10 μM) in a reaction buffer (50 mM HEPES, 100 mM NaCl, 1 mM DTT, pH 7.2) at 25 °C. Aliquots were taken at defined times, mixed with 2× SDS-PAGE sample buffer [62.5 mM Tris-HCl (pH 6.8), 2% SDS, 5% 2-mercaptoethanol, 25% glycerol, 0.01% bromophenol blue] containing 100 μM of the free BCP ligand (**3**, the structure is shown on p. S44), and boiled at 95 °C for 5 min. The samples were applied to 12% SDS-

PAGE and the labeled SNAP-tag proteins were detected by *in-gel* florescence imaging using a ChemiDoc MP system (Bio-Rad Laboratories). After fluorescence detection, the gel was stained with CBB. Fluorescence band intensities were quantified using the Image Lab software (Bio-Rad Laboratories).

Synthetic Tiam1-mediated Rac1 activation and lamellipodia formation

HeLa cells were plated at 0.2×10^5 cells in 35 mm glass-bottomed dishes coated with poly-L-lysine solution (Sigma). The cells were cotransfected with pCMV-Lifeact-mCherry and either pCMV-K6-SNAP$_f$-EGFP-Tiam1 or pCMV-K6-SNAP$_\Delta$-EGFP-Tiam1. Approximately 24 h after transfection, the medium was changed to DMEM(–), followed by serum-starvation for 1 h. The cells were imaged before and after the addition of mgcBCP or m^{D}cBCP (10 μM).

Synthetic cRaf-mediated ERK activation

HeLa cells were plated at 1.0×10^5 cells in 35 mm glass-bottomed dishes coated with collagen type I-C. The cells were cotransfected with pPBpuro-K6-SNAP$_\Delta$-EGFP-cRaf and pPBbsr-ERK-KTR-mCherry. Approximately 24 h after transfection the medium was changed to DMEM(–), followed by serum-starvation for 1 h. The cells were imaged before and after the addition of m^{D}cBCP (10 μM).

Synthetic PI3K-mediated PI(3,4,5)P$_3$ production

HeLa cells were plated at 1.0×10^5 cells in 35 mm glass-bottomed dishes coated with collagen type I-C. The cells were cotransfected with pCMV-K6-SNAP$_\Delta$-EGFP-iSH2 and pCMV-mCherry-PH$_{Akt}$. Approximately 24 h after transfection, the medium was changed to DMEM(–), followed by serum-starvation for 1 h. The cells were imaged before and after the addition of m^{D}cBCP (10 μM).

Synthetic Sos-mediated Ras/ERK activation

HeLa cells were plated at 1.0×10^5 cells in 35 mm glass-bottomed dishes coated with collagen type I-C. The cells were cotransfected with pPBpuro-K6-SNAP$_\Delta$-EGFP-Sos and pPBbsr-MEK-P2A-mCherry-ERK. Approximately 24 h after transfection, the medium was changed to DMEM(–), followed by serum-starvation for 1 h. The cells were imaged before and after the addition of m^{D}cBCP (10 μM).

References

S1. Mollwitz, B., Brunk, E., Schmitt, S., Pojer, F., Bannwarth, M., Schiltz, M., Rothlisberger, U., and Johnsson, K. (2012) Directed evolution of the suicide protein O^6-alkylguanine-DNA alkyltransferase for increased reactivity results in an alkylated protein with exceptional stability. *Biochemistry 51*, 986–994.

S2. Wibley, J. E. A., Pegg, A. E., and Moody, P. C. E. (2000) Crystal structure of the human O^6-alkylguanine-DNA alkyltransferase. *Nucleic Acids Res. 28*, 393–401.

S3. Daniels, D. S., Mol, C. D., Arvai, A. S., Kanugula, S., Pegg, A. E., and Tainer, J. A. (2000) Active and alkylated human AGT structures: a novel zinc site, inhibitor and extrahelical base binding. *EMBO J. 19*, 1719–1730.

S4. Goddard, T. D., Huang, C. C., Meng, E. C., Pettersen, E. F., Couch, G. S., Morris, J. H., and Ferrin, T. E. (2018) UCSF ChimeraX: meeting modern challenges in visualization and analysis. *Protein Sci. 27*, 14–25.

S5. Suh, B.-C., Inoue, T., Meyer, T., and Hille, B. (2006) Rapid chemically induced changes of PtdIns(4,5)P_2 gate KCNQ channels. *Science 314*, 1454–1457.

S6. Nakamura, A., Oki, C., Kato, K., Fujinuma, S., Maryu, G., Kuwata, K., Yoshii, T., Matsuda, M., Aoki, K., and Tsukiji, S. (2020) Engineering orthogonal, plasma membrane-specific SLIPT systems for multiplexed chemical control of signaling pathways in living single cells. *ACS Chem. Biol. 15*, 1004–1015.

S7. Holsinger, L. J., Spencer, D. M., Austin, D. J., Schreiber, S. L., and Crabtree, G. R. (1995) Signal transduction in T lymphocytes using a conditional allele of Sos. *Proc. Natl. Acad. Sci. U. S. A. 92*, 9810–9814.

S8. Nakamura, A., Oki, C., Sawada, S., Yoshii, T., Kuwata, K., Rudd, A. K., Devaraj, N. K., Noma, K., and Tsukiji, S. (2020) Designer palmitoylation motif-based self-localizing ligand for sustained control of protein localization in living cells and *Caenorhabditis elegans*. *ACS Chem. Biol. 15*, 837–843.

S9. Bochkov, Y. A. and Palmenberg, A. C. (2006) Translational efficiency of EMCV IRES in bicistronic vectors is dependent upon IRES sequence and gene location. *BioTechniques 41*, 283–288.

S10. Kim, J. H., Lee, S.-R., Li, L.-H., Park, H.-J., Park, J.-H., Lee, K. Y., Kim, M.-K., Shin, B. A., and Choi, S.-Y. (2011) High cleavage efficiency of a 2A peptide derived from Porcine Teschovirus-1 in human cell lines, zebrafish and mice. *PLoS One 6*,

e18556.

S11. Srikun, D., Albers, A. E., Nam, C. I., Iavarone, A. T., and Chang, C. J. (2010) Organelle-targetable fluorescent probes for imaging hydrogen peroxide in living cells via SNAP-tag protein labeling. *J. Am. Chem. Soc. 132*, 4455–4465.

S12. Kaiser, E., Colescott, R. L., Bossinger, C. D., and Cook, P. I. (1970) Color test for detection of free terminal amino groups in the solid-phase synthesis of peptides. *Anal. Biochem. 34*, 595–598.

S13. Yusa, K., Rad, R., Takeda, J., and Bradley, A. (2009) Generation of transgene-free induced pluripotent mouse stem cells by the *piggyBac* transposon. *Nat. Methods 6*, 363–369.

S14. Komatsu, N., Aoki, K., Yamada, M., Yukinaga, H., Fujita, Y., Kamioka, Y., and Matsuda, M. (2011) Development of an optimized backbone of FRET biosensors for kinases and GTPases. *Mol. Biol. Cell 22*, 4647–4656.

S15. Cadiñanos, J. and Bradley, A. (2007) Generation of an inducible and optimized *piggyBac* transposon system. *Nucleic Acids Res. 35*, e87.

S16. Schindelin, J., Arganda-Carreras, I., Frise, E., Kaynig, V., Longair, M., Pietzsch, T., Preibisch, S., Rueden, C., Saalfeld, S., Schmid, B., Tinevez, J.-Y., White, D. J., Hartenstein, V., Eliceiri, K., Tomancak, P., and Cardona, A. (2012) Fiji: an open-source platform for biological-image analysis. *Nat. Methods 9*, 676–682.

S17. Baniukiewicz, P., Collier, S., and Bretschneider, T. (2018) QuimP: analyzing transmembrane signaling in highly deformable cells. *Bioinformatics 34*, 2695–2697.

S18. Pace, C. N., Vajdos, F., Fee, L., Grimsley, G., Gray, T. (1995) How to measure and predict the molar absorption coefficient of a protein. *Protein Sci. 4*, 2411–2423.