Electronic supplementary information

Photocleavable Proteins that Undergo Fast and Efficient Dissociation

Xiaocen Lu[a], Yurong Wen‡[b,c], Shuce Zhang‡[a], Wei Zhang[a,d], Yilun Chen[b], Yi Shen[a], M. Joanne Lemieux[b], and Robert E. Campbell*[a,e]

a. Department of Chemistry, University of Alberta, Edmonton, Alberta, T6G 2G2, Canada. E-mail: robert.e.campbell@ualberta.ca

b. Department of Biochemistry, University of Alberta, Edmonton, Alberta, T6G 2H7, Canada.

c. Current address: Talent Highland, The First Affiliated Hospital, Xi'an Jiaotong University, Xi'an, Shaanxi, 710061, China.

d. Current address: Department of Chemistry, Stanford University, Stanford, California, 94305, United States.

e. Department of Chemistry, The University of Tokyo, Tokyo, 113-0033, Japan.

‡ These authors contributed equally to this work.

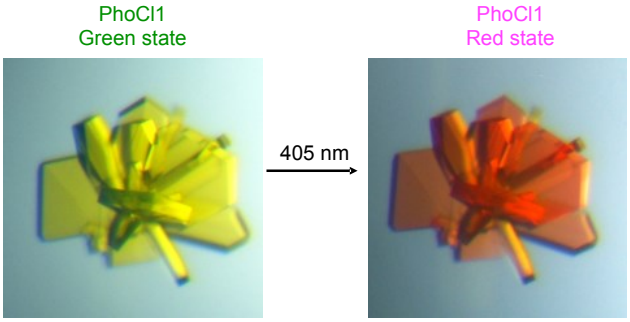

Fig. S1 Visible color change of a crystal of PhoCl1 upon illumination with violet light (15 s light with 405 nm LED array, 3.46 mW/mm^2).

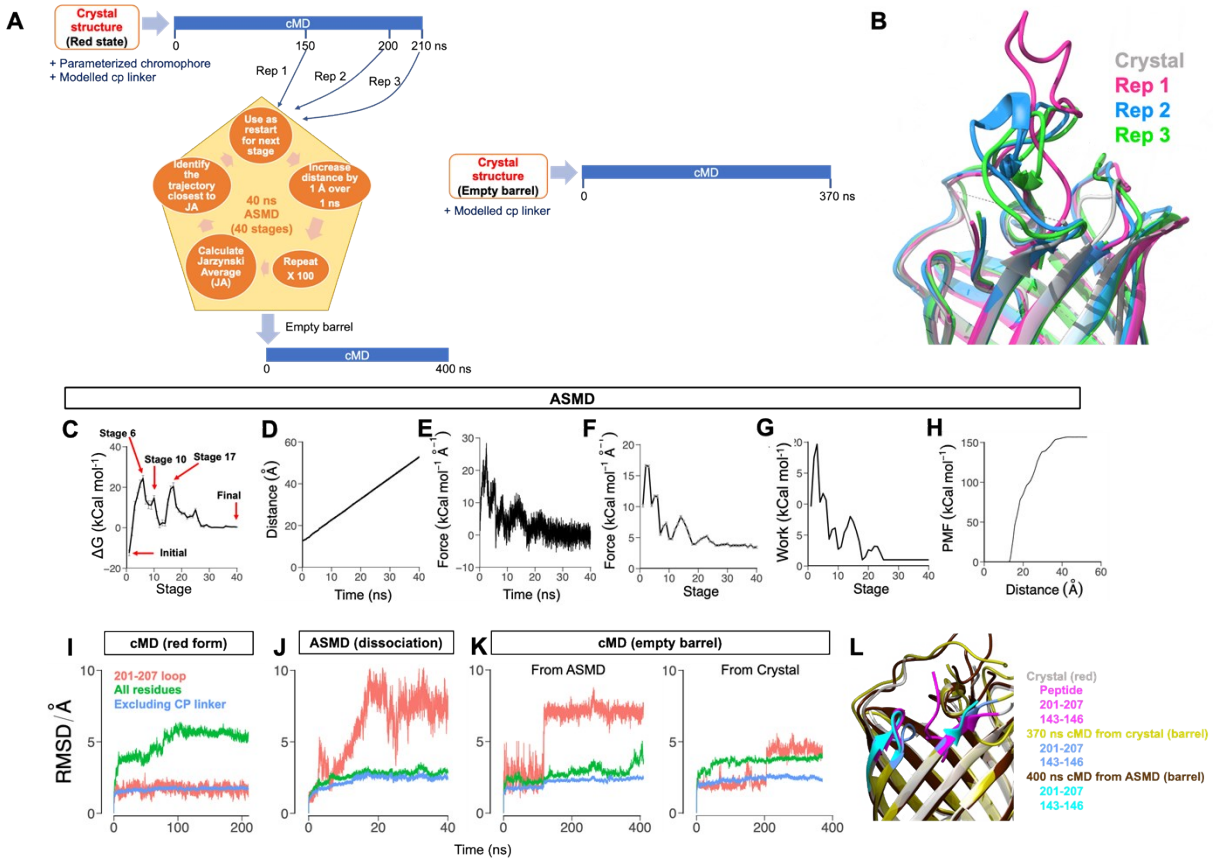

Fig. S2 Additional information on the molecular dynamic simulation of the dissociation process. (A) Schematic of the workflow of molecular dynamic simulations. Rep, replication. (B) Structure alignment of the crystal structure of the PhoCl1 red state and the simulated initial stages of 3 ASMD replications with different conformations of the cp linker. The crystal structure of the PhoCl1 red state is shown in silver, Rep1 is shown in magenta, Rep2 is shown in blue and Rep3 is shown in green. (C) Gibbs free energy of activation over stages from Rep3 with the final stage as reference (ΔG = 0 kCal/mol). Values are means ± SEM (n = 20 snapshots per stage). The stages represented in Fig. 2A,B are indicated by red arrows. (D-H) During the ASMD, the distance between the centres-of-mass of the C-terminal peptide and the N-terminal barrel increased by 40 Å over 40 ns (D) imposed by a harmonic restraint. With a constant speed of movement, the force imposing such distance constraint is changing (E,F). The curve is smoothened by averaging the adjacent 10 snapshots (E) or averaging all snapshots within a stage for clarity (F). The work done by such force in each stage is shown in (G). The cumulative potential of mean force (PMF) throughout the ASMD is shown in (H). The RMSD for all residues (green), all residues other than cp linker (blue), and the 201-207 loop (pink) was plotted

over time for the cMD with the red state crystal structure (I), ASMD Rep 3 (J) and the cMD for empty barrel (K) following ASMD Rep3 (left) and starting from the empty barrel crystal structure. The analysis suggests that most of the movements take place at the cp linker while the rest of the protein remains relatively still in cMD, including the 201-207 loop in the first cMD, after which the cp linker has reached a relatively stable structure. (L) A dissociation-dependent conformational change of the 201-207 loop takes place during ASMD followed by its repositioning during the cMD that follows the ASMD.

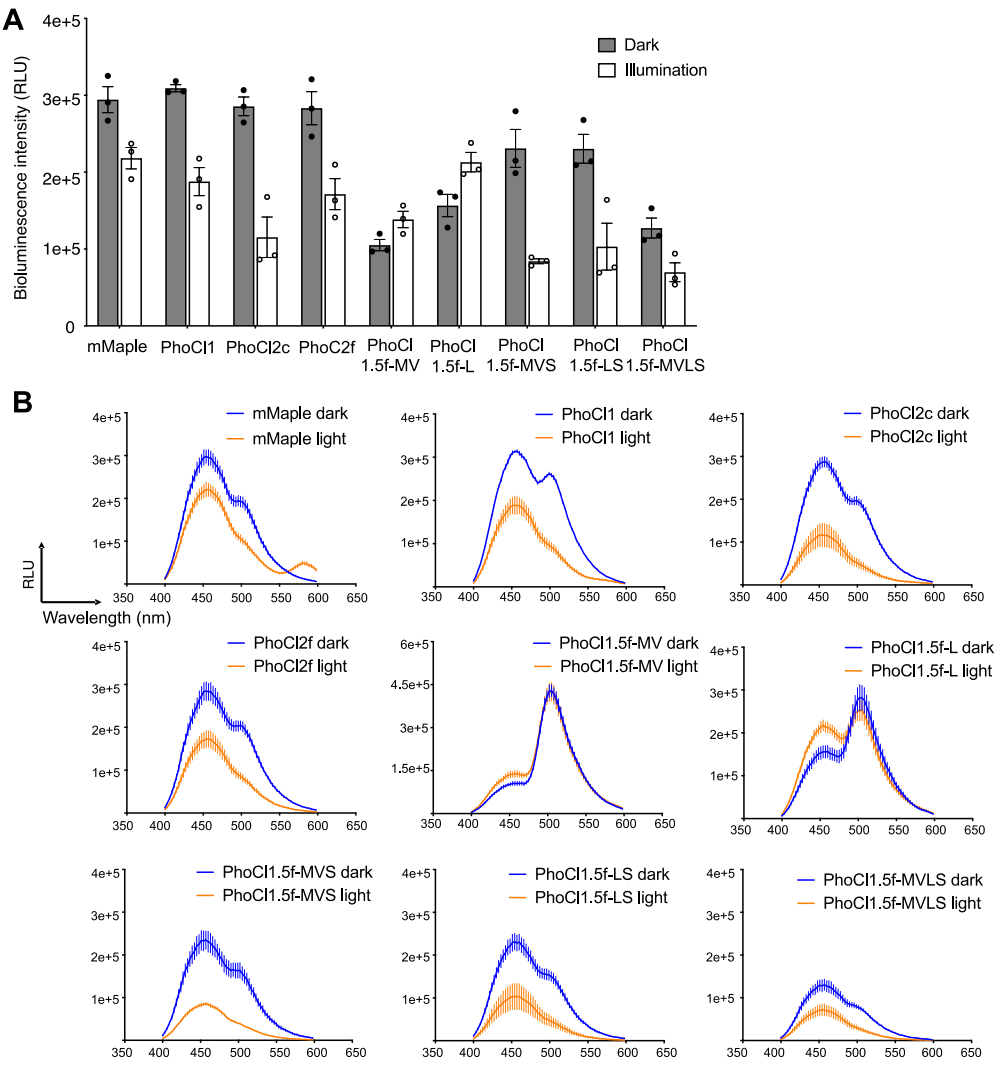

Fig. S3 NanoBiT-based bioluminescence assay of PhoCl dissociation. (A) Summary data of bioluminescence intensity (at 460 nm) from key variants. Purified LgBiT-PhoCl-SmBiT-MBP fusion protein (500 nM; construct represented in Fig. 3A) was used in this assay. At 30 min after illumination (15 s with LED array), the luminescence emission spectra of the protein, both with and without photoconversion, were measured immediately after treatment with the luciferase substrate furimazine (final concentration: 10 µg/mL). Values are means ± SEM (n = 3 independent experiments). (B) Bioluminescence spectra from the NanoBiT-based assay of PhoCl variants with (light) or without (dark) illumination. Measurements performed as described in (A).

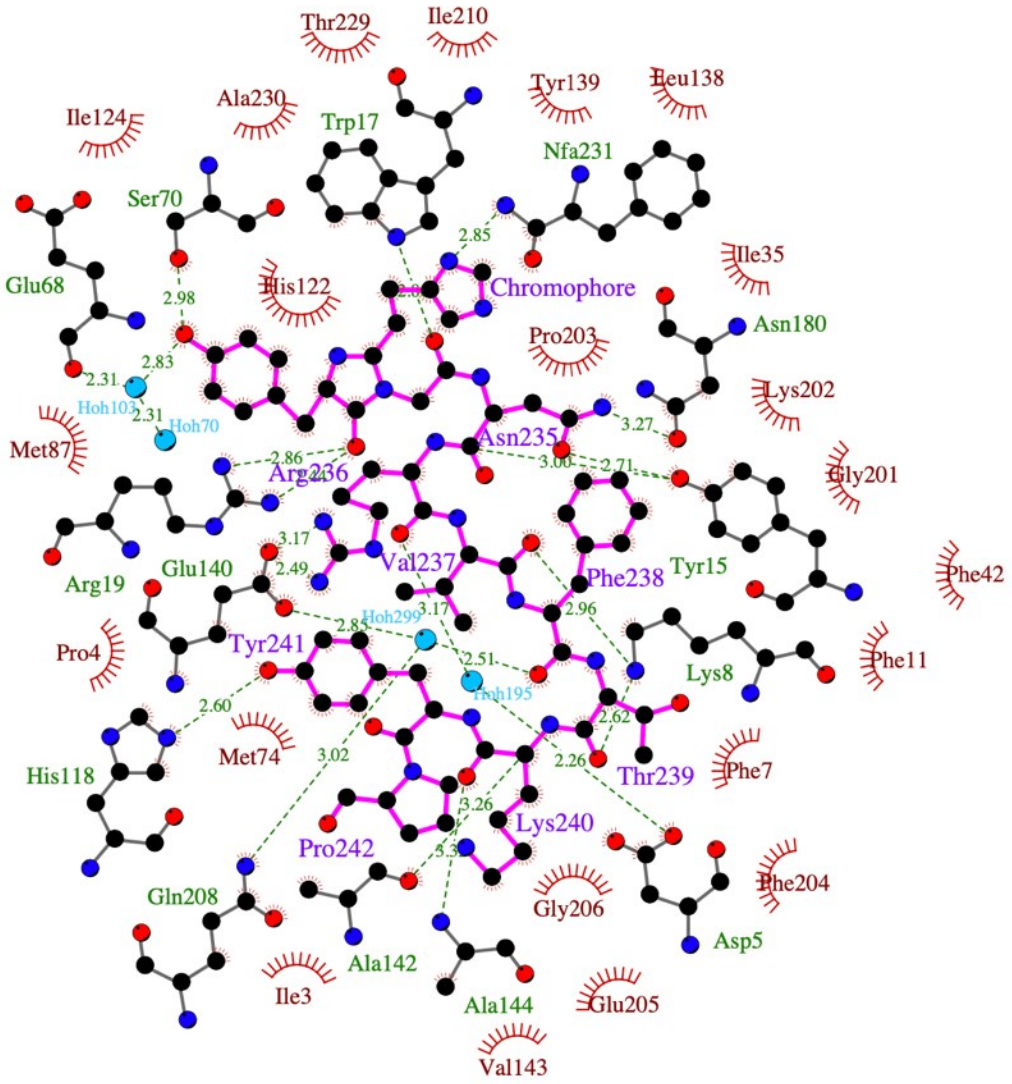

Fig. S4 Schematic diagram of the dissociable peptide and chromophore in the PhoCl1 red state generated by LIGPLOT[1]. The peptide and chromophore are represented with magenta-colored bonds. Hydrogen bonds are represented in green dashed lines and the hydrogen-bonded residues are represented with grey-colored bonds. Hydrophobic contacts are represented as red arcs with radiating lines.

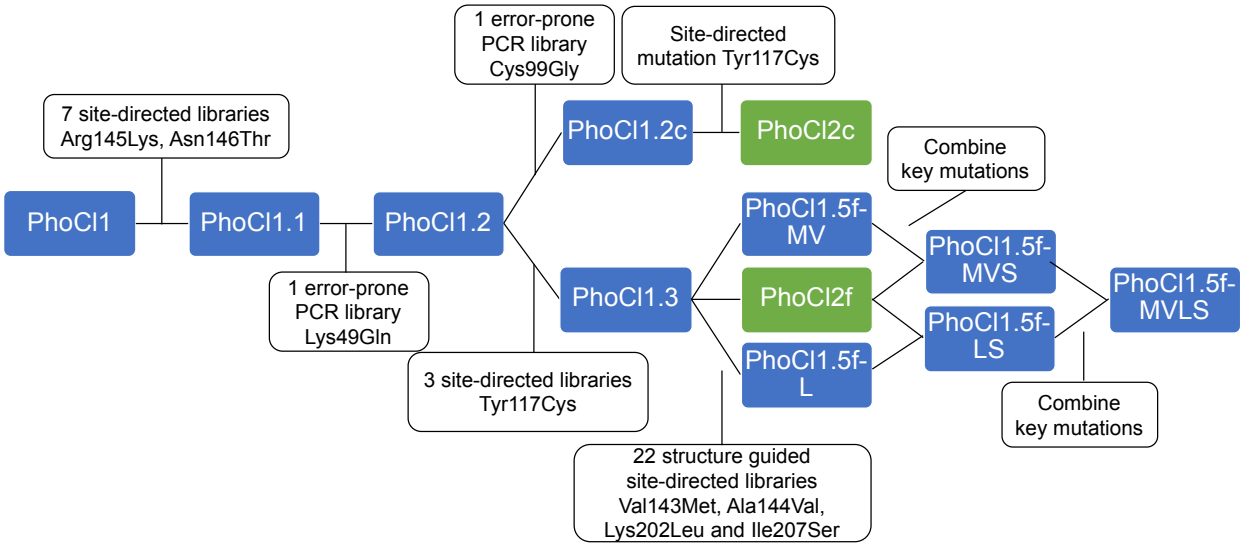

Fig. S5 Flow chart of PhoCl evolution. The PhoCl variants discovered during the screening process are represented in blue rectangles. The final PhoCl2 variants are represented in green rectangles. The library generation method, specific mutations discovered, and other key points, are represented in white rectangles.

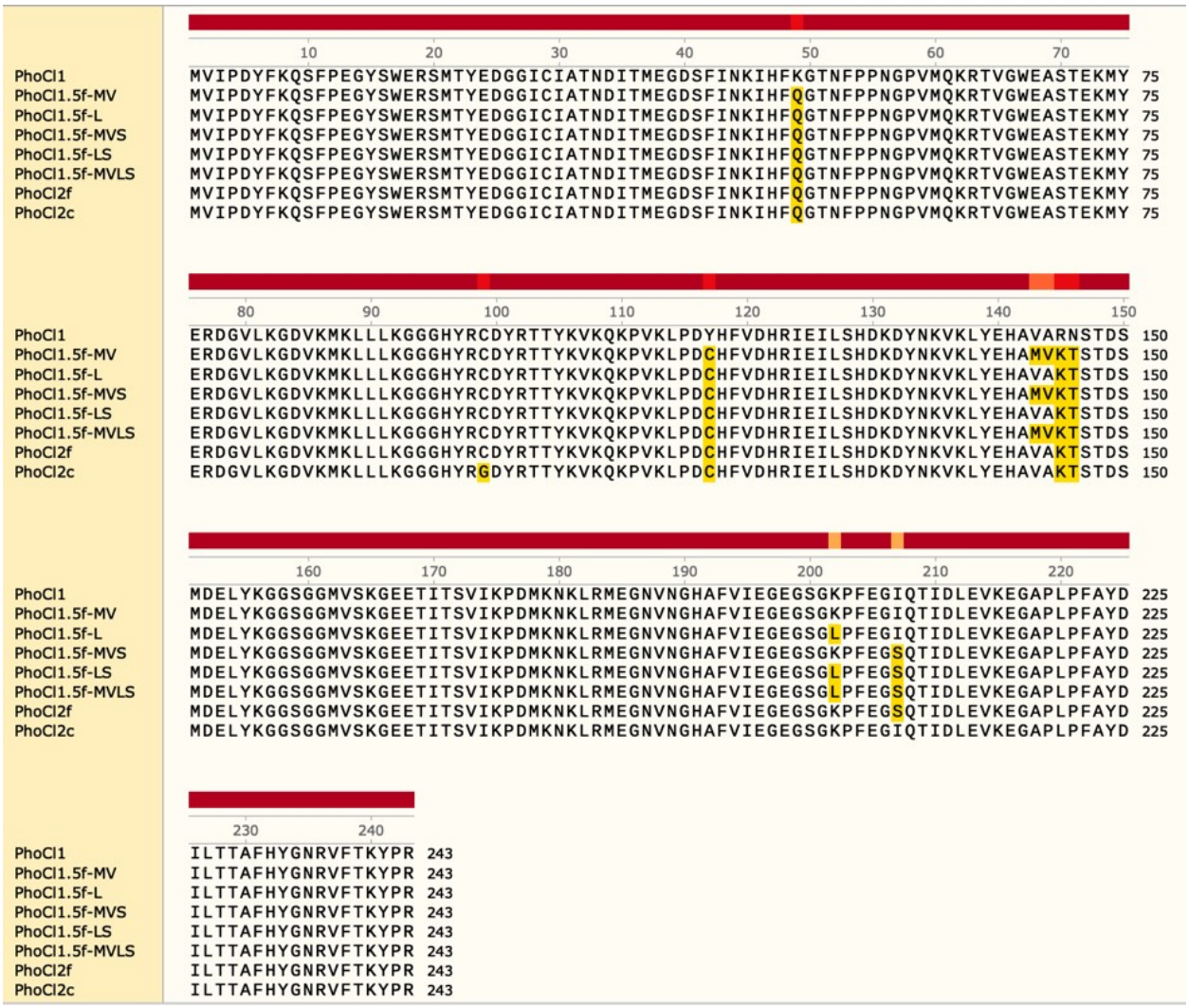

Fig. S6 Sequence alignment of PhoCl variants. Alignment was performed using SnapGene software. The sequence of PhoCl1 was used as the reference sequence and mutations are highlighted in yellow-orange.

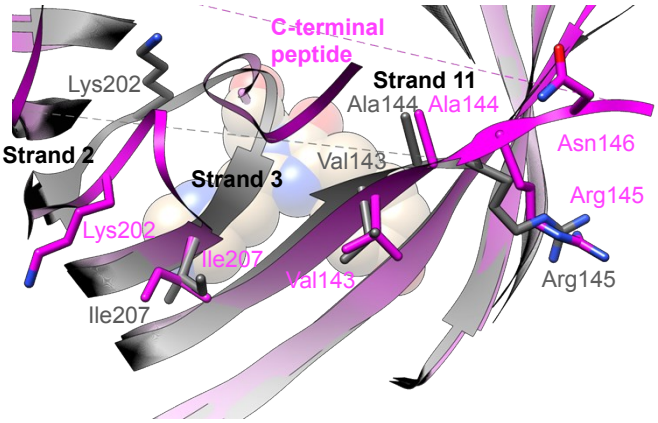

Fig. S7 Key mutations at positions in or near the 201-207 loop that were identified during screening.

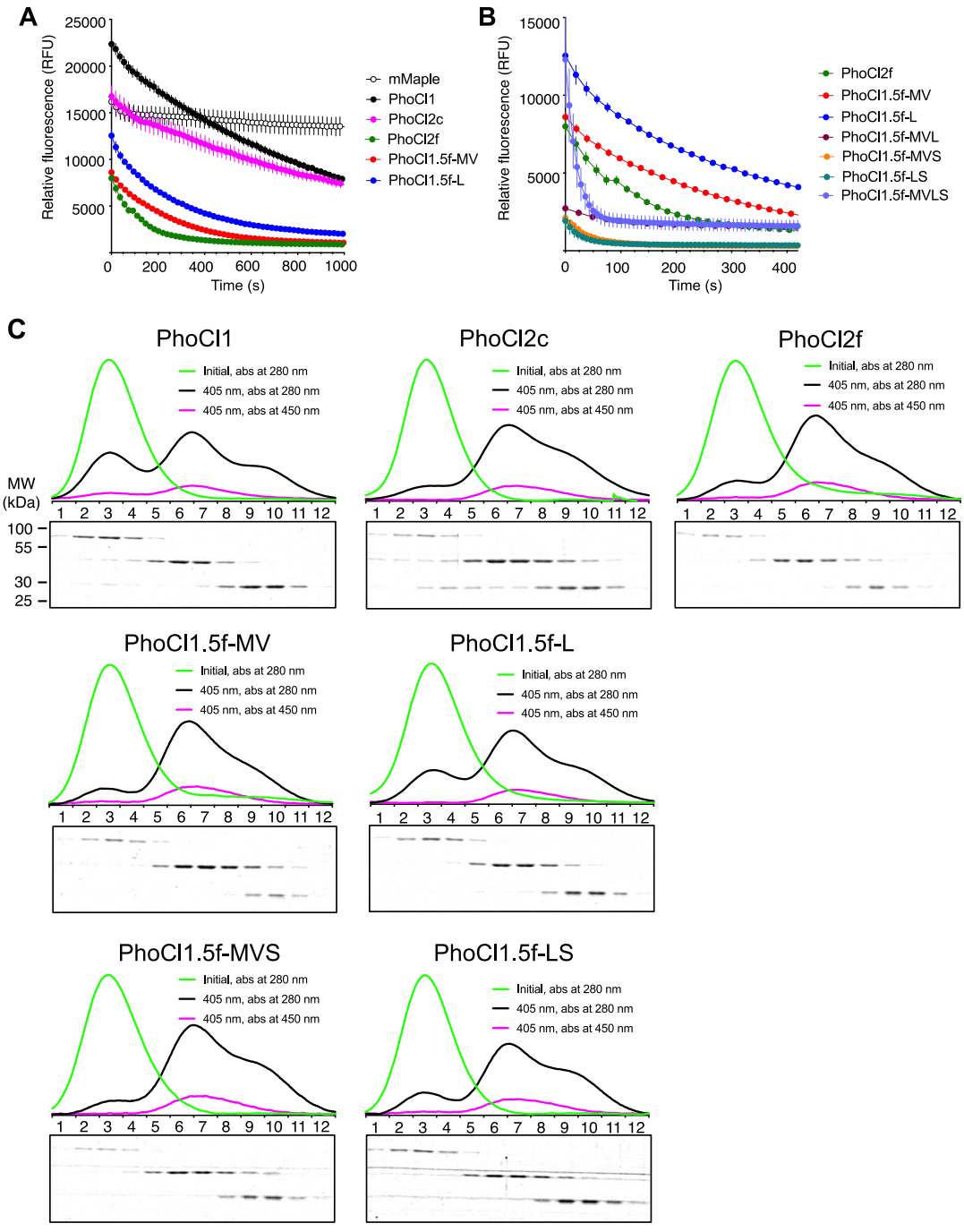

Fig. S8. Additional data on dissociation kinetics and dissociation efficiencies of PhoCl variants. (A,B) Loss of red fluorescence after photoconversion without normalization. This is the same data shown in Fig. 3B (panel A) and Fig. 3C (panel B). RFU, relative fluorescence units. Each protein was at a concentration of 500 nM, except for PhoCl1.5f-MVLS which was at 5 μM due to its poor protein expression and dim fluorescence. (C) GFC and SDS-PAGE analysis of PhoCl-

MBP fusions. SDS-PAGE analysis of GFC fractions for partially photoconverted fusion protein is labeled according to fraction numbers (12 × 1.5 mL, 43.5 - 61.5 mL elution volume).

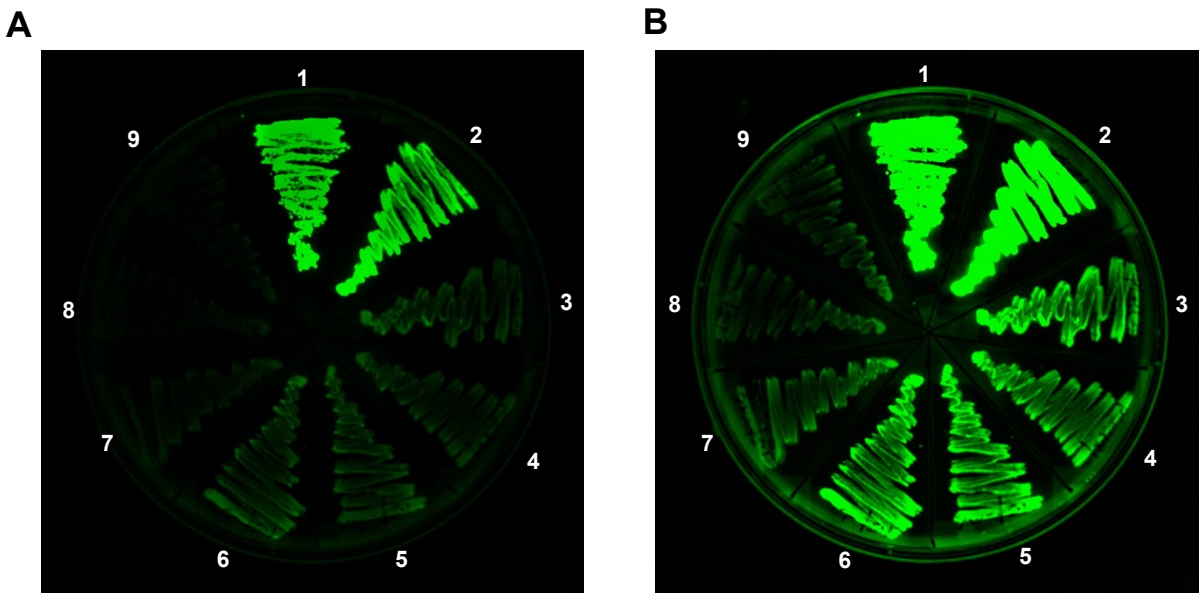

Fig. S9 Green fluorescence image of *E. coli* expressing PhoCl variants on agar media in a Petri dish. 1: mMaple, 2: PhoCl1, 3: PhoCl2c, 4: PhoCl2f, 5: PhoCl1.5f-MV, 6: PhoCl1.5f-L, 7: PhoCl1.5f-MVS, 8: PhoCl1.5f-LS, 9: PhoCl1.5f-MVLS. (B) is the same image as (A) with 5× increased contrast.

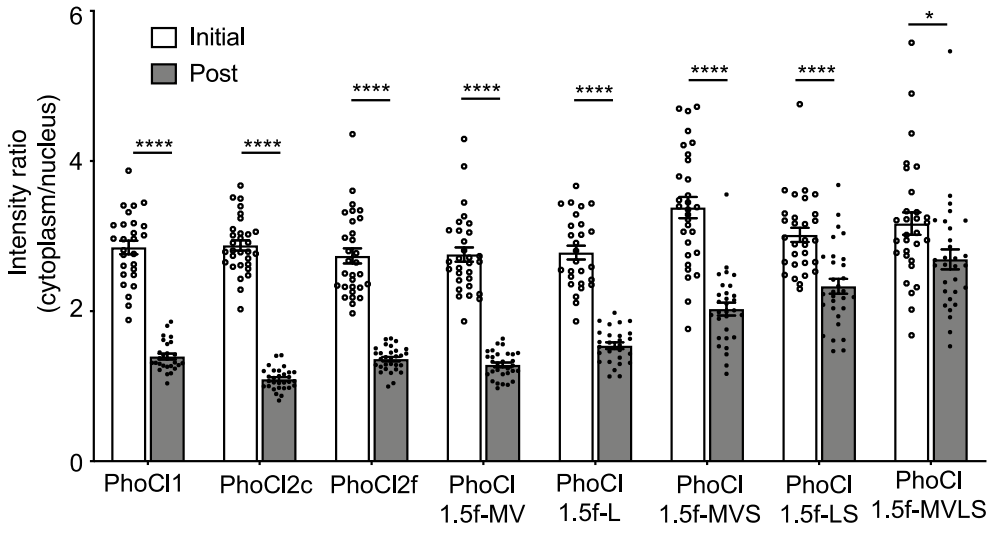

Fig. S10 Red fluorescence intensity localization ratios (cytoplasm to nucleus) of NES-PhoCl-mCherry before and after photoconversion. Values are means ± SEM. The data is from the experiments shown in Fig. 4. PhoCl1: ****P < 0.000001, t (52) = 14.85, n = 27 cells from 3 cultures; PhoCl2c: ****P < 0.000001, t (58) = 24.41, n = 30 cells from 3 cultures; PhoCl2f: ****P < 0.000001, t (58) = 13.09, n = 30 cells from 3 cultures; PhoCl1.5f-MV: ****P < 0.000001, t (58) = 14.47, n = 30 cells from 3 cultures; PhoCl1.5f-L: ****P < 0.000001, t (52) = 12.03, n = 27 cells from 3 cultures; PhoCl1.5f-MVS: ****P < 0.000001, t (58) = 8.187, n = 30 cells from 3 cultures; PhoCl1.5f-LS: ****P = 0.000006, t (58) = 4.997, n = 30 cells from 3 cultures; PhoCl1.5f-MVLS: *P = 0.020512, t (58) = 2.382, n = 30 cells from 3 cultures. Multiple t tests were used to determine significant differences between group means.

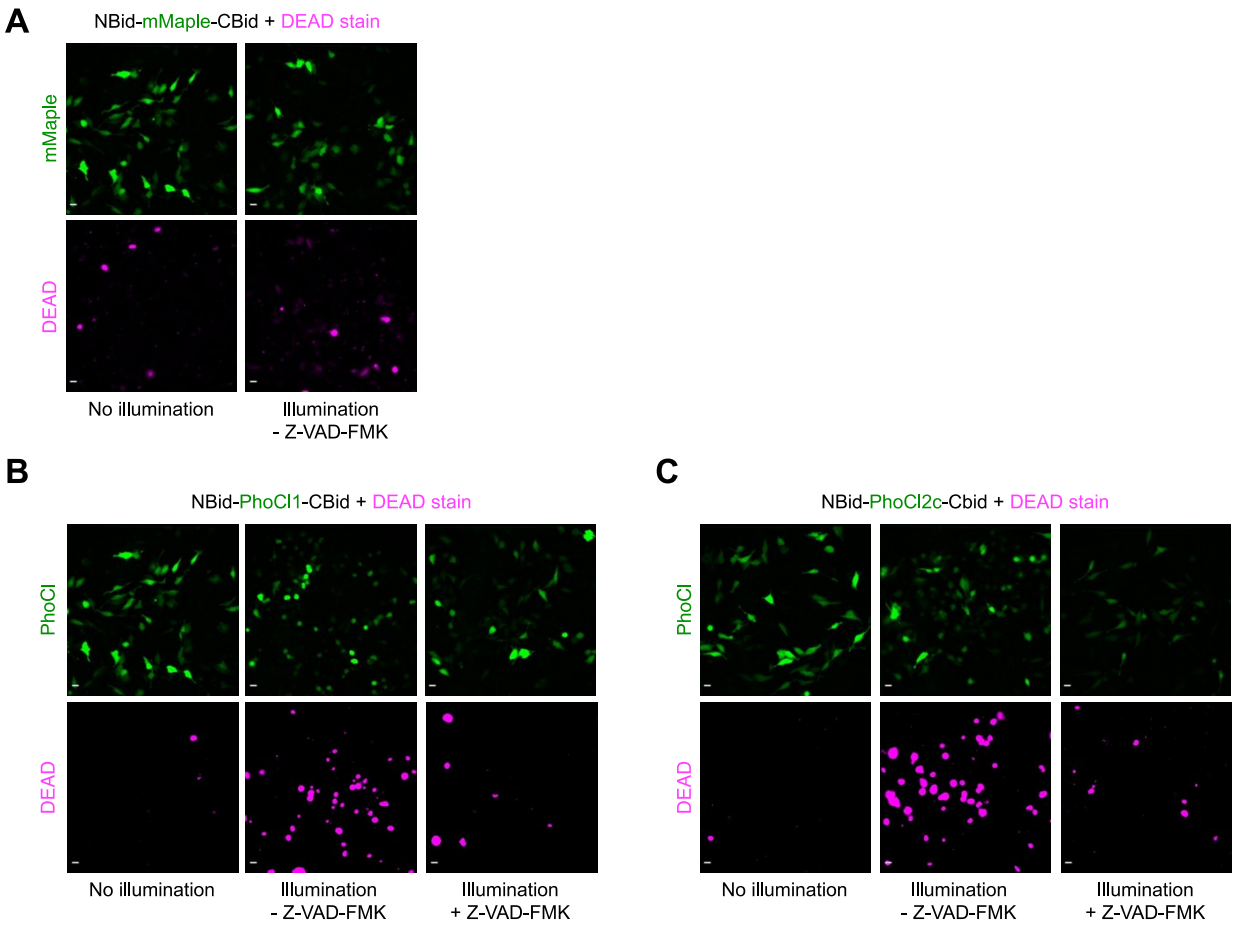

Fig. S11 Demonstration of PhoCl-dependent induction of apoptosis using DEAD cell viability assay. (A-C) Representative cell images of HeLa cells expressing mMaple or PhoCl inserted Bid after DEAD stain. Scale bar, 20 μm.

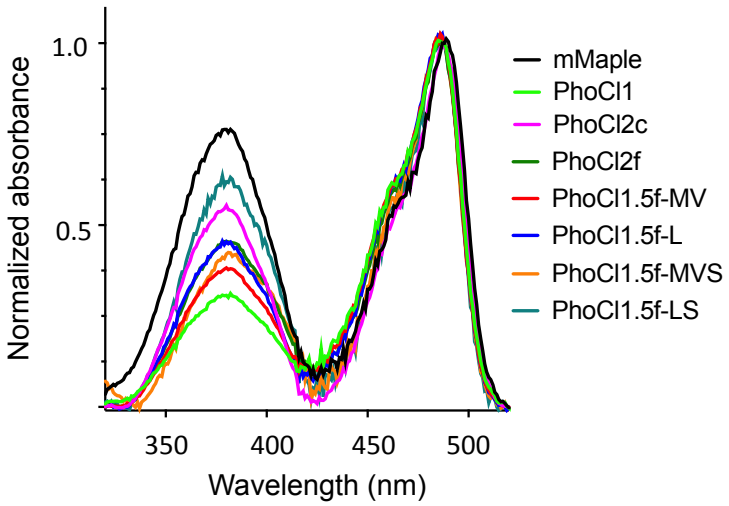

Fig. S12 Absorbance spectra of mMaple and PhoCl variants. Compared to PhoCl1, increased extinction coefficients at 405 nm were observed for the improved variants.

Table S1. X-ray data collection and refinement statistics.

Crystal	PhoCl1 green state	PhoCl1 red state	PhoCl1 empty barrel
Data collection			
Spacegroup	$P2_12_12_1$	P1	$P2_1$
a, b, c (Å)	60.36, 112.76, 144.85	38.93, 72.49, 126.55	46.4, 119.6, 65.3
α, β, γ (°)	90.0, 90.0, 90	92.5, 97.3, 92.5	90, 107.7, 90
Resolution (Å)	42.88-2.10 (2.18-2.10)	38.57-2.30 (2.38-2.30)	43.11-2.82 (2.92-2.82)
*R*merge	0.066 (0.709)	0.042 (0.533)	0.073 (0.760)
*R*meas	0.078 (0.835)	0.059 (0.734)	0.089 (0.936)
Multiplicity	3.4 (3.5)	1.8 (1.7)	2.7 (2.7)
CC(1/2)	0.997 (0.885)	0.998 (0.855)	0.997 (0.811)
CC*	0.999 (0.969)	1 (0.96)	0.999 (0.946)
I/σ(I)	9.73 (1.72)	9.60 (1.20)	8.54 (1.28)
Completeness (%)	96.8 (97.7)	95.9 (94.9)	92.9 (94.5)
Wilson B-factor (Å^2)	25.64	28.86	43.91
Refinement			
Total Reflections	194926 (31020)	103548 (16288)	44693 (7133)
Unique Reflections	57043 (9153)	58440 (9388)	16400 (2688)
R_{Work}/R_{Free}	0.1824/0.2162	0.2186/0.2638	0.2587/0.3029
Number of atoms:			
Protein	6715	10075	5031
Ligands	100	148	-
Water	410	488	-
Average B-factor (Å^2)	34.07	35.53	38.70
Protein ADP (Å^2)	34.35	35.59	38.71
Ligands (Å^2)	22.13	29.24	-
Water	32.51	31.77	-
Ramachandran plot:			
Favored/Allowed (%)	97.70/2.20	94.0/5.4	91.0/8.7
Root-Mean-Square-Deviation:			
Bond lengths (Å)	0.012	0.004	0.005
Bond Angle (°)	1.50	1.0	0.82

Note: Statistics for the highest resolution shell are shown in parentheses.

Table S2. Summary of the interactions of the PhoCl1 dissociable peptide and chromophore in the red state.

Residues	**Hydrogen Bonding**	**Hydrophobic Interaction (2.90 Å – 3.90 Å)**
Chromophore	Trp17 (2.86), Arg19 (2.86, 2.44), Ser70 (2.98), Nfa231 (2.85)	Met87, His122, Ile124, Leu138, Tyr139, Glu140, Gln208, Ile210, Thr229, Ala230, Arg236
Asn235	Tyr15 (2.71), Asn180 (3.27), Val237 (3.30)	Ile35
Arg236	Tyr15 (3.00), Glu140 (3.17, 2.49), Tyr239 (2.92,2.68)	Phe7, Met74, His122, Ala142, Iey233, Phe238
Val237	Lys8 (2.96), Asn235 (3.30)	Phe11, Phe42, Pro203, Thr239
Phe238	NA	Ala142, Asn180, Gly201, Lys202, Pro203, Gly206, Gln208, Arg235
Thr239	Lys8 (2.62), Arg236 (2.68, 2.92), Tyr241 (3.12)	Pro203, Gly206, Val237
Lys240	Ala142 (3.26), Ala144 (3.32)	Val143, Phe204, Glu205, Gly206
Tyr241	His118 (2.60), Thr239 (3.12)	Ile3, Asp5, Pro4
Pro242	NA	NA

Note: The residues were randomised by site-directed mutagenesis to generate libraries are: Ile3, Asp5, Phe7, Lys8, Phe11, Gly14, Tyr15, Ile35, Met37, Glu38, Gly39, Asp40, Phe42, Arg77, Asp78, Gly79, Val80, Met87, Tyr117, His118, Val120, His122, Leu138, Tyr139, Val143, Ala144, Arg145, Asn146, Met162, Asp177, Met178, Gly201, Lys202, Pro203, Phe204, Glu205, Gly206, Ile207, Gln208, Ile210, Thr229, Ala230, Thr239 and Lys240.

Table S3. Summary of mutations in PhoCl2 variants.

Variants	Mutations
PhoCl1.5f-MV	Lys49Gln, Tyr117Cys, Val143Met, Ala144Val, Arg145Lys, Asn146Thr
PhoCl1.5f-L	Lys49Gln, Tyr117Cys, Arg145Lys, Asn146Thr, Lys202Leu
PhoCl1.5f-MVS	Lys49Gln, Tyr117Cys, Val143Met, Ala144Val, Arg145Lys, Asn146Thr, Ile207Ser
PhoCl1.5f-LS	Lys49Gln, Tyr117Cys, Arg145Lys, Asn146Thr, Lys202Leu, Ile207Ser
PhoCl1.5f-MVLS	Lys49Gln, Tyr117Cys, Val143Met, Ala144Val, Arg145Lys, Asn146Thr, Lys202Leu, Ile207Ser
PhoCl2f	Lys49Gln, Tyr117Cys, Arg145Lys, Asn146Thr, Ile207Ser
PhoCl2c	Lys49Gln, Cys99Gly, Tyr117Cys, Arg145Lys, Asn146Thr

Table S4. Relative chromophore formation efficiencies.

Protein	Abs488/(Abs280* ε488) %	Relative chromophore formation efficiency
PhoCl1	1.16	1
PhoCl2c	1.29	1.11
PhoCl2f	1.24	1.07
PhoCl1.5f-MV	1.33	1.15
PhoCl1.5f-L	1.13	0.98
PhoCl1.5f-MVS	2.64	2.24
PhoCl1.5f-LS	1.36	1.17

Note: The relative chromophore formation efficiency was characterized by the ratio of Abs488/(Abs280*ε488)*100. Abs, absorbance; ε, extinction coefficients ($mM^{-1}cm^{-1}$). We assume that folded proteins that do not form the chromophore (absorbance at 280 nm but not at 488 nm) would co-elute with the folded proteins that do form the chromophore (absorbance at both 280 nm and 488 nm). The Abs488 and Abs280 were measured from the GFC analysis of the peak area of PhoCl-MBP fusion (43.5 - 61.5 mL elution volume) without photoconversion. Relative chromophore formation efficiencies were determined be normalizing the ratios to that of PhoCl1.

Table S5. Summary data of bioluminescence assay.

Protein	Emission (460 nm)	BRET ratio (dark)	BRET ratio (light)
mMaple	-26%	0.63	0.44
PhoCl1	-39%	0.82	0.47
PhoCl2c	-60%	0.66	0.40
PhoCl2f	-39%	0.69	0.45
PhoCl1.5f-MV	+32%	4.08	3.07
PhoCl1.5f-L	+36%	1.80	1.18
PhoCl1.5f-MVS	-64%	0.68	0.43
PhoCl1.5f-LS	-55%	0.63	0.41
PhoCl1.5f-MVLS	-45%	0.58	0.38

Note: The BRET ratio (acceptor/donor ratio) is the ratio of the intensity of the PhoCl emission peak (505 nm) to the intensity of the NanoBiT emission peak (460 nm). All data is the mean from triplicate measurements for each variant.

Table S6. Summary data of optogenetic manipulation of protein translocation assay with PhoCl variants.

Protein	Photoconversion Half Time (s)	Dissociation Half Time (s)
PhoCl1	75.5	241
PhoCl2c	62.7	114
PhoCl2f	78.1	135
PhoCl1.5f-MV	83.6	160
PhoCl1.5f-L	69.6	155

Plateau followed by one phase decay fit was used in both analyses. For fit of photoconversion, *R*-squared values range from 0.9900 to 0.9951. For fit of dissociation, *R*-squared values range from 0.7006 to 0.9040.

Table S7. Primers used in this study.

Primers	Sequence 5’-3’
pBAD/HisB-LgBiT-PhoCl-SmBiT-MBP construct	
F-XhoI-LgBiT	GCAGGCTCGAGGATGGTCTTCACACTCGAAGATTTCGTTGGG
R-LgBiT-linker1-KpnI-PhoCl overlap PCR	GAAGTAGTCAGGGATCACGGTACCAGGTTCTCCGCCGCTAGAACCGCCTCCGCTACTCCC
F-linker1-Kpn1-PhoCl overlap PCR	GGCGGAGAACCTGGTACCGTGATCCCTGACTACTTCAAGCAGAGC
R1-PhoCl-XbaI-linker2-SmBiT	CACGCCGCCACTGCTTCCGCCACCGGACGAACCTCTAGACCGTGGGTACTTGGTGAACAC
R2-linker2-SmBiT-EcoRI	CAGCTGAATTCCAGGATCTCTTCAAAAAGTCTGTATCCAGTCACGCCGCCACTGCTT
F-EcoRI-linker3-MBP	GTACGGAATTCGGGGGTGGAGGTTCAAAAATCGAAGAAGGTAAACTGGTAATCTGGAT
R-MBP*-HindIII	CAGCCAAGCTTTTAAGTCTGCGCGTCTTTCAGGG
Error-prone PCR for PhoCl libraries	
EP-F-KpnI-PhoCl	CGGAGAACCTGGTACCGTGATCCCT
EP-R-XbaI-PhoCl	CGGACGAACCTCTAGACCGTGGGTA
QuickChange primers for PhoCl libraries	
I3-antisense	TCTGCTTGAAGTAGTCAGGMNNCACGGTACCAGGTTCTCCG
D5-sense	GAACCTGGTACCGTGATCCCTNNKTACTTCAAGCAGAGCTTCCCC
F7-antisense	CCTCGGGGAAGCTCTGCTTMNNGTAGTCAGGGATCACGGTA
K8-antisense	GCCCTCGGGGAAGCTCTGMNNGAAGTAGTCAGGGATCAC
F11-antisense	CCAGCTGTAGCCCTCGGGMNNGCTCTGCTTGAAGTAGTC
G14 Y15-antisense	AGGTCATGCTGCGCTCCCAGCTMNNMNNCTCGGGGAAGCTCTGCTTGAAG
I35-antisense	CTGTCCCCCTCCATTGTMNNGTCGTTGGTGGCGATGC
M37 E38-antisense	TGATGAAGCTGTCCCCMNNMNNTGTGATGTCGTTGGTGGCGATGCAGATGCCG
G39 D40-antisense	CTGGAAGTGGATCTTGTTGATGAAGCTMNNMNNCTCCATTGTGATGTCGTTGGTGGCGAT
F42-antisense	CTTGAAGTGGATCTGGTTGATMNNGCTGTCCCCCTCCATTGTGAT
R77 D78-antisense	TCACGTCGCCCTTCAGCACGCCMNNMNNCTCGTACATCTTCTCGGTGCTG
G79 V80-antisense	CTTCATCTTCACGTCGCCCTTCAGMNNMNNGTCGCGCTCGTACATCTTCTCGGT
M87-sense	GCCCTTCAGCAGCAGCTTMNNCTTCACGTCGCCCTTCAG
Y117-antisense	CGGTGGTCCACGAAGTGMNNGTCGGGCAGCTTTACGG
Y117 H118-antisense	TCTCGATGCGGTGGTCCACGAAMNNMNNGTCGGGCAGCTTTACGGGCTTC
V120-antisense	TCTCGATGCGGTGGTCMNNGAAGTGGCAGTCGGGC
H122-antisense	GCTCAGGATCTCGATGCGMNNGTCCACGAAGTGGCAGTC
L138 Y139-antisense	GGAAGTCTTGGCCACGGCGTGCTCMNNMNNCTTCACCTTGTTGTAGTCCTTGTC
V143 A144-antisense	GTCCATGCTGTCGGTGGAAGTCTTMNNMNNGGCGTGCTCGTACAGCTTCACCTT
R145 N146-antisense	CTCGTCCATGCTGTCGGTGGAMNNMNNGGCCACGGCGTGCTCGTACAG
M162-sense	GGTGGCAGCGGTGGCNNKGTGAGCAAGGGCGAG
D177 M178-sense	GAGACCATTACAAGCGTGATCAAGCCTNNKNNKAAGAACAAGCTGCGCATGGAGGGCAAC
G201 K202-antisense	CGTCTGGATGCCCTCGAAGGGMNNMNNGCTGCCCTCGCCCTCGATCAC
P203 F204-antisense	ATCAATCGTCTGGATGCCCTCMNNMNNCTTGCCGCTGCCCTCGCCCTC
F204-antisense	CGTCTGGATGCCCTCMNNGGGCTTGCCGCTGCC
E205 G206-antisense	ACCTCCAAATCAATCGTCTGGATMNNMNNGAAGGGCTTGCCGCTGCCCTCGC
I207-antisense	CTCCAAATCAATCGTCTGMNNGCCCTCGAAGGGCTTGCC
I207 Q208-antisense	TCCTTCACCTCCAAATCAATCGTMNNMNNGCCCTCGAAGGGCTTGCCGCTGC
I210-antisense	CCTCCTTCACCTCCAAATCMNNCGTCTGGATGCCCTCGAAG
T229 A230-antisense	AACACGCGGTTGCCGTAGTGGAAMNNMNNGGTCAGGATGTCGTAGGCGAAGG
T239-antisense	CATTATCCCGTGGGTACTTMNNGAACACGCGGTTGCCGTAG
K240-antisense	ACCTCTAGACCGTGGGTAMNNGGTGAACACGCGGTTGCC

QuickChange primers for the combinations of point mutations	
K202L-antisense	GATGCCCTCGAAGGGAAGCCCGCTGCCCTCGCCC
I207S-antisense	CTCCAAATCAATCGTCTGAGAGCCCTCGAAGGGCTTGCC
K202L I207S-antisense	CCTCCAAATCAATCGTCTGAGAGCCCTCGAAGGGAAGCCC
pET-28a-PhoCl-6His construct	
F-NcoI-PhoCl	ATATACCATGGTGATCCCTGACTACTTCAAGCAGAGCTTC
R-PhoCl1-linker-XhoI	GTAGCCTCGAGACCTCCACCTCCCCGTGGGTACTTGGTGAACACGC
pBAD/HisB-PhoCl construct	
F-XhoI-g-PhoCl	CCGAGCTCGAGTGTGATCCCTGACTAC
R-PhoCl*-KpnI	CATCCGCCAAAACAGCCAAGCTTGGTACCTTACCGTGGGTACTTGGTGAACAC
pBAD/HisB-PhoCl-MBP construct	
F-XhoI-g-PhoCl	CCGAGCTCGAGTGTGATCCCTGACTAC
R-PhoCl-linker-KpnI	TTCATGGTACCTCCACCTCCCCGTGGGTACTTGGTGAACAC
pcDNA-NES-PhoCl-mCherry construct	
quickchange-NES-HindIII-antisense	AGTAGTCAGGGATCACGCTAAGCTTGCCTCCCTGCTGCTCGTCC
F-HindIII-PhoCl	CTTAGAAGCTTAGCGTGATCCCTGACTACTTCAAG
R-PhoCl-KpnI	CCTCCGGTACCCCGTGGGTACTTGGTGAACACG
pcDNA-NBid-PhoCl-CBid construct	
F-NheI-NBid	AGTCAGCTAGCGCCACCATGGATTGTGAGGTCAATAACGG
R-BamHI-NBid overlap PCR	GGATCCCTCATCGTAGCCTTCCCAC
F-NBid-BamHI-PhoCl overlap PCR	GTGGGAAGGCTACGATGAGGGATCCGTGATCCCTGACTACTTCAAGC
R-PhoCl-KpnI-CBid overlap PCR	GATCGGTTTCCATCGGTTTGAAGGGTACCCCGTGGGTACTTGGTGAAC
F-KpnI-CBid overlap PCR	GGTACCCTTCAAACCGATGGAAACCGATC
R-CBid*-XhoI	TGACTCTCGAGTTACTTGTTCAACCTGAGCAACCACG
F-BamHI-PhoCl	GTCAAGGATCCGTGATCCCTGACTACTTCAAGCAGAG
R-PhoCl-KpnI	CCTCCGGTACCCCGTGGGTACTTGGTGAACACG
pcDNA-NBid-mMaple-CBid construct	
F-BamHI-mMaple	AGTAGGAATCCGTGAGCAAGGGCGAGGAGACCA
R-mMaple-KpnI	GCACTGGTACCCTTGTACAGCTCGTCCATGCTG
pcDNA-NES-DEVD-mCardinal-NLS construct	
F1-NheI-NES	CACTGGCTAGCGCCACCATGAACCTGGTGGACCTGCAGAAGAAGCTGGAGG
F2-NES	GACCTGCAGAAGAAGCTGGAGGAGCTGGAGCTGGACGAGCAGCAGGGATCCGCCTCCGGC
F3-DEVD-mCardinal	CAGGGATCCGCCTCCGGCGATGAGGTGGATGGAGCCGTGAGCAAGGGCGAGGAG
R1-mCardinal-NLS	CGTTTTTTTTTTGGTCCGGAGCCCTTGTACAGCTCGTCCATGCC
R2-NLS-NLS	GGGGTCTACTTTGCGCTTCTTTTTTGGGTCAACTTTTCGTTTTTTTTTTGGTCCGGAGCC
R3-NLS*-XhoI	ACTGACTCGAGTTAGGTACCTACCTTGCGTTTTTTCTTGGGGTCTACTTTGCGCTTC

Table S8. Nucleotide sequences of gene expression constructs.

Constructs	Sequence 5'-3'
LgBiT-PhoCl1-SmBiT-MBP (LgBiT is highlighted in yellow, PhoCl1 is highlighted in green, SmBiT is highlighted in cyan and MBP is highlighted in grey)	ATGGTCTTCACACTCGAAGATTTCGTTGGGGACTGGGAACAGACAGCCGCGTACAACCTGGACC AAGTCCTTGAACAGGGAGGTGTGTCCAGTTTGCTGCAGAATCTCGCGGTGTCCGTAACTCCGAT CCAAAGGATTGTCCGCAGCGGTGAAAATGCGCTGAAGATCGACATCCATGTCATCATCCCGTAT GAAGGTCTGAGCGCGGACCAAATGGCACAGATCGAAGAAGTGTTTAAGGTGGTGTACCCTGTG GATGATCATCACTTTAAGGTGATCCTGCCGTATGGCACACTGGTAATCGACGGGGTTACGCCGA ACATGCTGAACTATTTCGGACGGCCGTATGAAGGCATCGCCGTGTTCGACGGCAAAAAGATCAC TGTAACAGGGACCCTGTGGAACGGCAACAAAATTATCGACGAGCGCCTGATCACCCCCGACGGC TCCATGCTGTTCCGAGTAACCATCAACAGCGGGAGTAGCGGAGGCGGTTCTAGCGGCGGAGAA CCTGGTACCGTGATCCCTGACTACTTCAAGCAGAGCTTCCCCGAGGGCTACAGCTGGGAGCGCA GCATGACCTACGAGGACGGCGGCATCTGCATCGCCACCAACGACATCACAATGGAGGGGGACA GCTTCATCAACAAGATCCACTTCAAGGGCACGAACTTCCCCCCCAACGGCCCCGTGATGCAGAA GAGGACCGTGGGCTGGGAGGCCAGCACCGAGAAGATGTACGAGCGCGACGGCGTGCTGAAGG GCGACGTGAAGATGAAGCTGCTGCTGAAGGGCGGCGGCCACTATCGCTGCGACTACCGCACCA CCTACAAGGTCAAGCAGAAGCCCGTAAAGCTGCCCGACTACCACTTCGTGGACCACCGCATCGA GATCCTGAGCCACGACAAGGACTACAACAAGGTGAAGCTGTACGAGCACGCCGTGGCCCGCAA CTCCACCGACAGCATGGACGAGCTGTACAAGGGTGGCAGCGGTGGCATGGTGAGCAAGGGCG AGGAGACCATTACAAGCGTGATCAAGCCTGACATGAAGAACAAGCTGCGCATGGAGGGCAACG TGAACGGCCACGCCTTCGTGATCGAGGGCGAGGGCAGCGGCAAGCCCTTCGAGGGCATCCAGA CGATTGATTTGGAGGTGAAGGAGGGCGCCCCGCTGCCCTTCGCCTACGACATCCTGACCACCGC CTTCCACTACGGCAACCGCGTGTTCACCAAGTACCCACGGTCTAGAGGTTCGTCCGGTGGCGGA AGCAGTGGCGGCGTGACTGGATACAGACTTTTTGAAGAGATCCTGGAATTCGGGGGTGGAGGT TCAAAAATCGAAGAAGGTAAACTGGTAATCTGGATTAACGGCGATAAAGGCTATAACGGTCTCG CTGAAGTCGGTAAGAAATTCGAGAAAGATACCGGAATTAAAGTCACCGTTGAGCATCCGGATAA ACTGGAAGAGAAATTCCCACAGGTTGCGGCAACTGGCGATGGCCCTGACATTATCTTCTGGGCA CACGACCGCTTTGGTGGCTACGCTCAATCTGGCCTGTTGGCTGAAATCACCCCGGACAAAGCGTT CCAGGACAAGCTGTATCCGTTTACCTGGGATGCCGTACGTTACAACGGCAAGCTGATTGCTTACC CGATCGCTGTTGAAGCGTTATCGCTGATTTATAACAAAGATCTGCTGCCGAACCCGCCAAAAACC TGGGAAGAGATCCCGGCGCTGGATAAAGAACTGAAAGCGAAAGGTAAGAGCGCGCTGATGTTC AACCTGCAAGAACCGTACTTCACCTGGCCGCTGATTGCTGCTGACGGGGGTTATGCGTTCAAGT ATGAAAACGGCAAGTACGACATTAAAGACGTGGGCGTGGATAACGCTGGCGCGAAAGCGGGTC TGACCTTCCTGGTTGACCTGATTAAAAACAAACACATGAATGCAGACACCGATTACTCCATCGCA GAAGCTGCCTTTAATAAAGGCGAAACAGCGATGACCATCAACGGCCCGTGGGCATGGTCCAACA TCGACACCAGCAAAGTGAATTATGGTGTAACGGTACTGCCGACCTTCAAGGGTCAACCATCCAA ACCGTTCGTTGGCGTGCTGAGCGCAGGTATTAACGCCGCCAGTCCGAACAAAGAGCTGGCAAAA GAGTTCCTCGAAAACTATCTGCTGACTGATGAAGGTCTGGAAGCGGTTAATAAAGACAAACCGC TGGGTGCCGTAGCGCTGAAGTCTTACGAGGAAGAGTTGGTGAAAGATCCGCGTATTGCCGCCA CTATGGAAAACGCCCAGAAAGGTGAAATCATGCCGAACATCCCGCAGATGTCCGCTTTCTGGTA TGCCGTGCGTACTGCGGTGATCAACGCCGCCAGCGGTCGTCAGACTGTCGATGAAGCCCTGAAA GACGCGCAGACTTAA
PhoCl2c (PhoCl is highlighted in green and mutations compared to PhoCl1 are highlighted in magenta)	GTGATCCCTGACTACTTCAAGCAGAGCTTCCCCGAGGGCTACAGCTGGGAGCGCAGCATGACCT ACGAGGACGGCGGCATCTGCATCGCCACCAACGACATCACAATGGAGGGGGACAGCTTCATCA ACAAGATCCACTTCCAGGGCACGAACTTCCCCCCCAACGGCCCCGTGATGCAGAAGAGGACCGT GGGCTGGGAGGCCAGCACCGAGAAGATGTACGAGCGCGACGGCGTGCTGAAGGGCGACGTGA AGATGAAGCTGCTGCTGAAGGGCGGCGGCCACTATCGCGGCGACTACCGCACCACCTACAAGG TCAAGCAGAAGCCCGTAAAGCTGCCCGACTGCCACTTCGTGGACCACCGCATCGAGATCCTGAG CCACGACAAGGACTACAACAAGGTGAAGCTGTACGAGCACGCCGTGGCCAAGACTTCCACCGA CAGCATGGACGAGCTGTACAAGGGTGGCAGCGGTGGCATGGTGAGCAAGGGCGAGGAGACCA TTACAAGCGTGATCAAGCCTGACATGAAGAACAAGCTGCGCATGGAGGGCAACGTGAACGGCC ACGCCTTCGTGATCGAGGGCGAGGGCAGCGGCAAGCCCTTCGAGGGCATCCAGACGATTGATT TGGAGGTGAAGGAGGGCGCCCCGCTGCCCTTCGCCTACGACATCCTGACCACCGCCTTCCACTA CGGCAACCGCGTGTTCACCAAGTACCCACGG
PhoCl2f (PhoCl is highlighted in green and mutations compared to PhoCl1 are	GTGATCCCTGACTACTTCAAGCAGAGCTTCCCCGAGGGCTACAGCTGGGAGCGCAGCATGACCT ACGAGGACGGCGGCATCTGCATCGCCACCAACGACATCACAATGGAGGGGGACAGCTTCATCA ACAAGATCCACTTCCAGGGCACGAACTTCCCCCCCAACGGCCCCGTGATGCAGAAGAGGACCGT GGGCTGGGAGGCCAGCACCGAGAAGATGTACGAGCGCGACGGCGTGCTGAAGGGCGACGTGA AGATGAAGCTGCTGCTGAAGGGCGGCGGCCACTATCGCTGCGACTACCGCACCACCTACAAGGT CAAGCAGAAGCCCGTAAAGCTGCCCGACTGCCACTTCGTGGACCACCGCATCGAGATCCTGAGC

	CACGACAAGGACTACAACAAGGTGAAGCTGTACGAGCACGCCGTGGCCAAGACTTCCACCGAC AGCATGGACGAGCTGTACAAGGGTGGCAGCGGTGGCATGGTGAGCAAGGGCGAGGAGACCAT TACAAGCGTGATCAAGCCTGACATGAAGAACAAGCTGCGCATGGAGGGCAACGTGAACGGCCA CGCCTTCGTGATCGAGGGCGAGGGCAGCGGCAAGCCCTTCGAGGGCTCTCAGACGATTGATTT GGAGGTGAAGGAGGGCGCCCCGCTGCCCTTCGCCTACGACATCCTGACCACCGCCTTCCACTAC GGCAACCGCGTGTTCACCAAGTACCCACGG
PhoCl1.5f-MV (PhoCl is highlighted in green and mutations compared to PhoCl1 are highlighted in magenta)	GTGATCCCTGACTACTTCAAGCAGAGCTTCCCCGAGGGCTACAGCTGGGAGCGCAGCATGACCT ACGAGGACGGCGGCATCTGCATCGCCACCAACGACATCACAATGGAGGGGGACAGCTTCATCA ACAAGATCCACTTCCAGGGCACGAACTTCCCCCCCAACGGCCCCGTGATGCAGAAGAGGACCGT GGGCTGGGAGGCCAGCACCGAGAAGATGTACGAGCGCGACGGCGTGCTGAAGGGCGACGTGA AGATGAAGCTGCTGCTGAAGGGCGGCGGCCACTATCGCTGCGACTACCGCACCACCTACAAGGT CAAGCAGAAGCCCGTAAAGCTGCCCGACTGCCACTTCGTGGACCACCGCATCGAGATCCTGAGC CACGACAAGGACTACAACAAGGTGAAGCTGTACGAGCACGCCATGGTTAAGACTTCCACCGACA GCATGGACGAGCTGTACAAGGGTGGCAGCGGTGGCATGGTGAGCAAGGGCGAGGAGACCATT ACAAGCGTGATCAAGCCTGACATGAAGAACAAGCTGCGCATGGAGGGCAACGTGAACGGCCAC GCCTTCGTGATCGAGGGCGAGGGCAGCGGCAAGCCCTTCGAGGGCATCCAGACGATTGATTTG GAGGTGAAGGAGGGCGCCCCGCTGCCCTTCGCCTACGACATCCTGACCACCGCCTTCCACTACG GCAACCGCGTGTTCACCAAGTACCCACGG
PhoCl1.5f-L (PhoCl is highlighted in green and mutations compared to PhoCl1 are highlighted in magenta)	GTGATCCCTGACTACTTCAAGCAGAGCTTCCCCGAGGGCTACAGCTGGGAGCGCAGCATGACCT ACGAGGACGGCGGCATCTGCATCGCCACCAACGACATCACAATGGAGGGGGACAGCTTCATCA ACAAGATCCACTTCCAGGGCACGAACTTCCCCCCCAACGGCCCCGTGATGCAGAAGAGGACCGT GGGCTGGGAGGCCAGCACCGAGAAGATGTACGAGCGCGACGGCGTGCTGAAGGGCGACGTGA AGATGAAGCTGCTGCTGAAGGGCGGCGGCCACTATCGCTGCGACTACCGCACCACCTACAAGGT CAAGCAGAAGCCCGTAAAGCTGCCCGACTGCCACTTCGTGGACCACCGCATCGAGATCCTGAGC CACGACAAGGACTACAACAAGGTGAAGCTGTACGAGCACGCCGTGGCCAAGACTTCCACCGAC AGCATGGACGAGCTGTACAAGGGTGGCAGCGGTGGCATGGTGAGCAAGGGCGAGGAGACCAT TACAAGCGTGATCAAGCCTGACATGAAGAACAAGCTGCGCATGGAGGGCAACGTGAACGGCCA CGCCTTCGTGATCGAGGGCGAGGGCAGCGGGCTTCCCTTCGAGGGCATCCAGACGATTGATTTG GAGGTGAAGGAGGGCGCCCCGCTGCCCTTCGCCTACGACATCCTGACCACCGCCTTCCACTACG GCAACCGCGTGTTCACCAAGTACCCACGG
PhoCl1.5f-MVL (PhoCl is highlighted in green and mutations compared to PhoCl1 are highlighted in magenta)	GTGATCCCTGACTACTTCAAGCAGAGCTTCCCCGAGGGCTACAGCTGGGAGCGCAGCATGACCT ACGAGGACGGCGGCATCTGCATCGCCACCAACGACATCACAATGGAGGGGGACAGCTTCATCA ACAAGATCCACTTCCAGGGCACGAACTTCCCCCCCAACGGCCCCGTGATGCAGAAGAGGACCGT GGGCTGGGAGGCCAGCACCGAGAAGATGTACGAGCGCGACGGCGTGCTGAAGGGCGACGTGA AGATGAAGCTGCTGCTGAAGGGCGGCGGCCACTATCGCTGCGACTACCGCACCACCTACAAGGT CAAGCAGAAGCCCGTAAAGCTGCCCGACTGCCACTTCGTGGACCACCGCATCGAGATCCTGAGC CACGACAAGGACTACAACAAGGTGAAGCTGTACGAGCACGCCATGGTTAAGACTTCCACCGACA GCATGGACGAGCTGTACAAGGGTGGCAGCGGTGGCATGGTGAGCAAGGGCGAGGAGACCATT ACAAGCGTGATCAAGCCTGACATGAAGAACAAGCTGCGCATGGAGGGCAACGTGAACGGCCAC GCCTTCGTGATCGAGGGCGAGGGCAGCGGGCTTCCCTTCGAGGGCATCCAGACGATTGATTTG GAGGTGAAGGAGGGCGCCCCGCTGCCCTTCGCCTACGACATCCTGACCACCGCCTTCCACTACG GCAACCGCGTGTTCACCAAGTACCCACGG
PhoCl1.5f-MVS (PhoCl is highlighted in green and mutations compared to PhoCl1 are highlighted in magenta)	GTGATCCCTGACTACTTCAAGCAGAGCTTCCCCGAGGGCTACAGCTGGGAGCGCAGCATGACCT ACGAGGACGGCGGCATCTGCATCGCCACCAACGACATCACAATGGAGGGGGACAGCTTCATCA ACAAGATCCACTTCCAGGGCACGAACTTCCCCCCCAACGGCCCCGTGATGCAGAAGAGGACCGT GGGCTGGGAGGCCAGCACCGAGAAGATGTACGAGCGCGACGGCGTGCTGAAGGGCGACGTGA AGATGAAGCTGCTGCTGAAGGGCGGCGGCCACTATCGCTGCGACTACCGCACCACCTACAAGGT CAAGCAGAAGCCCGTAAAGCTGCCCGACTGCCACTTCGTGGACCACCGCATCGAGATCCTGAGC CACGACAAGGACTACAACAAGGTGAAGCTGTACGAGCACGCCATGGTTAAGACTTCCACCGACA GCATGGACGAGCTGTACAAGGGTGGCAGCGGTGGCATGGTGAGCAAGGGCGAGGAGACCATT ACAAGCGTGATCAAGCCTGACATGAAGAACAAGCTGCGCATGGAGGGCAACGTGAACGGCCAC GCCTTCGTGATCGAGGGCGAGGGCAGCGGCAAGCCCTTCGAGGGCTCTCAGACGATTGATTTG GAGGTGAAGGAGGGCGCCCCGCTGCCCTTCGCCTACGACATCCTGACCACCGCCTTCCACTACG GCAACCGCGTGTTCACCAAGTACCCACGG
PhoCl1.5f-LS (PhoCl is highlighted in green and mutations compared to PhoCl1 are highlighted in magenta)	GTGATCCCTGACTACTTCAAGCAGAGCTTCCCCGAGGGCTACAGCTGGGAGCGCAGCATGACCT ACGAGGACGGCGGCATCTGCATCGCCACCAACGACATCACAATGGAGGGGGACAGCTTCATCA ACAAGATCCACTTCCAGGGCACGAACTTCCCCCCCAACGGCCCCGTGATGCAGAAGAGGACCGT GGGCTGGGAGGCCAGCACCGAGAAGATGTACGAGCGCGACGGCGTGCTGAAGGGCGACGTGA AGATGAAGCTGCTGCTGAAGGGCGGCGGCCACTATCGCTGCGACTACCGCACCACCTACAAGGT CAAGCAGAAGCCCGTAAAGCTGCCCGACTGCCACTTCGTGGACCACCGCATCGAGATCCTGAGC CACGACAAGGACTACAACAAGGTGAAGCTGTACGAGCACGCCGTGGCCAAGACTTCCACCGAC AGCATGGACGAGCTGTACAAGGGTGGCAGCGGTGGCATGGTGAGCAAGGGCGAGGAGACCAT

	TACAAGCGTGATCAAGCCTGACATGAAGAACAAGCTGCGCATGGAGGGCAACGTGAACGGCCA CGCCTTCGTGATCGAGGGCGAGGGCAGCGGGCTTCCCTTCGAGGGCTCTCAGACGATTGATTTG GAGGTGAAGGAGGGCGCCCCGCTGCCCTTCGCCTACGACATCCTGACCACCGCCTTCCACTACG GCAACCGCGTGTTCACCAAGTACCCACGG
PhoCl1.5f-MVLS (PhoCl is highlighted in green and mutations compared to PhoCl1 are highlighted in magenta)	GTGATCCCTGACTACTTCAAGCAGAGCTTCCCCGAGGGCTACAGCTGGGAGCGCAGCATGACCT ACGAGGACGGCGGCATCTGCATCGCCACCAACGACATCACAATGGAGGGGGACAGCTTCATCA ACAAGATCCACTTCCAGGGCACGAACTTCCCCCCCAACGGCCCCGTGATGCAGAAGAGGACCGT GGGCTGGGAGGCCAGCACCGAGAAGATGTACGAGCGCGACGGCGTGCTGAAGGGCGACGTGA AGATGAAGCTGCTGCTGAAGGGCGGCGGCCACTATCGCTGCGACTACCGCACCACCTACAAGGT CAAGCAGAAGCCCGTAAAGCTGCCCGACTGCCACTTCGTGGACCACCGCATCGAGATCCTGAGC CACGACAAGGACTACAACAAGGTGAAGCTGTACGAGCACGCCATGGTTAAGACTTCCACCGACA GCATGGACGAGCTGTACAAGGGTGGCAGCGGTGGCATGGTGAGCAAGGGCGAGGAGACCATT ACAAGCGTGATCAAGCCTGACATGAAGAACAAGCTGCGCATGGAGGGCAACGTGAACGGCCAC GCCTTCGTGATCGAGGGCGAGGGCAGCGGGCTTCCCTTCGAGGGCTCTCAGACGATTGATTTGG AGGTGAAGGAGGGCGCCCCGCTGCCCTTCGCCTACGACATCCTGACCACCGCCTTCCACTACGG CAACCGCGTGTTCACCAAGTACCCACGG
PhoCl1-6His (PhoCl is highlighted in green and His tag is highlighted in yellow)	ATGGTGATCCCTGACTACTTCAAGCAGAGCTTCCCCGAGGGCTACAGCTGGGAGCGCAGCATGA CCTACGAGGACGGCGGCATCTGCATCGCCACCAACGACATCACAATGGAGGGGGACAGCTTCAT CAACAAGATCCACTTCAAGGGCACGAACTTCCCCCCCAACGGCCCCGTGATGCAGAAGAGGACC GTGGGCTGGGAGGCCAGCACCGAGAAGATGTACGAGCGCGACGGCGTGCTGAAGGGCGACGT GAAGATGAAGCTGCTGCTGAAGGGCGGCGGCCACTATCGCTGCGACTACCGCACCACCTACAA GGTCAAGCAGAAGCCCGTAAAGCTGCCCGACTACCACTTCGTGGACCACCGCATCGAGATCCTG AGCCACGACAAGGACTACAACAAGGTGAAGCTGTACGAGCACGCCGTGGCCCGCAACTCCACC GACAGCATGGACGAGCTGTACAAGGGTGGCAGCGGTGGCATGGTGAGCAAGGGCGAGGAGAC CATTACAAGCGTGATCAAGCCTGACATGAAGAACAAGCTGCGCATGGAGGGCAACGTGAACGG CCACGCCTTCGTGATCGAGGGCGAGGGCAGCGGCAAGCCCTTCGAGGGCATCCAGACGATTGA TTTGGAGGTGAAGGAGGGCGCCCCGCTGCCCTTCGCCTACGACATCCTGACCACCGCCTTCCACT ACGGCAACCGCGTGTTCACCAAGTACCCACGGGGAGGTGGAGGTCTCGAGCACCACCACCACC ACCACTGA
NBid-PhoCl1-CBid (NBid is highlighted in yellow, PhoCl is highlighted in green and CBid is highlighted in blue)	ATGGATTGTGAGGTCAATAACGGTTCATCTCTCCGAGACGAATGCATAACGAACTTGCTCGTTTT CGGCTTTCTGCAATCCTGCAGCGATAATTCTTTCAGAAGAGAACTTGACGCTCTTGGACATGAAC TCCCAGTACTCGCTCCACAGTGGGAAGGCTACGATGAGGGATCCGTGATCCCTGACTACTTCAA GCAGAGCTTCCCCGAGGGCTACAGCTGGGAGCGCAGCATGACCTACGAGGACGGCGGCATCTG CATCGCCACCAACGACATCACAATGGAGGGGGACAGCTTCATCAACAAGATCCACTTCAAGGGC ACGAACTTCCCCCCCAACGGCCCCGTGATGCAGAAGAGGACCGTGGGCTGGGAGGCCAGCACC GAGAAGATGTACGAGCGCGACGGCGTGCTGAAGGGCGACGTGAAGATGAAGCTGCTGCTGAA GGGCGGCGGCCACTATCGCTGCGACTACCGCACCACCTACAAGGTCAAGCAGAAGCCCGTAAA GCTGCCCGACTACCACTTCGTGGACCACCGCATCGAGATCCTGAGCCACGACAAGGACTACAAC AAGGTGAAGCTGTACGAGCACGCCGTGGCCCGCAACTCCACCGACAGCATGGACGAGCTGTAC AAGGGTGGCAGCGGTGGCATGGTGAGCAAGGGCGAGGAGACCATTACAAGCGTGATCAAGCC TGACATGAAGAACAAGCTGCGCATGGAGGGCAACGTGAACGGCCACGCCTTCGTGATCGAGGG CGAGGGCAGCGGCAAGCCCTTCGAGGGCATCCAGACGATTGATTTGGAGGTGAAGGAGGGCG CCCCGCTGCCCTTCGCCTACGACATCCTGACCACCGCCTTCCACTACGGCAACCGCGTGTTCACCA AGTACCCACGGGGTACCCTTCAAACCGATGGAAACCGATCATCTCATTCAAGGTTGGGGCGAAT TGAAGCAGATAGTGAGAGTCAGGAGGACATAATACGCAATATAGCTCGACACCTTGCACAGGTC GGTGATAGCATGGACCGCTCTATCCCCCCAGGTTTGGTTAACGGATTGGCCCTGCAACTGCGAA ACACTTCAAGGAGTGAAGAAGATAGAAATCGAGACCTGGCGACCGCGCTTGAACAGCTTCTTCA AGCCTATCCCAGAGATATGGAGAAGGAAAAAACAATGCTCGTGCTCGCACTGCTGCTGGCAAAG AAAGTAGCCTCTAACACACCATCCCTCTTGAGAGACGTCTTCCATACCACGGTAAATTTCATAAAC CAGAACCTTAGGACGTATGTGCGGAGTCTTGCTCGCAATGGTATGACTGAGTTTCTTCAACCCGT GCCCCTCAATGCGTGGTTGCTCAGGTTGAACAAGTAA
NBid-mMaple-CBid (NBid is highlighted in yellow, mMaple	ATGGATTGTGAGGTCAATAACGGTTCATCTCTCCGAGACGAATGCATAACGAACTTGCTCGTTTT CGGCTTTCTGCAATCCTGCAGCGATAATTCTTTCAGAAGAGAACTTGACGCTCTTGGACATGAAC TCCCAGTACTCGCTCCACAGTGGGAAGGCTACGATGAGGGATCC GTGAGCAAGGGCGAGGAGACCATTATGAGCGTGATCAAGCCTGACATGAAGATCAAGCTGCGC ATGGAGGGCAACGTGAACGGCCACGCCTTCGTGATCGAGGGCGAGGGCAGCGGCAAGCCCTTC GAGGGCATCCAGACGATTGATTTGGAGGTGAAGGAGGGCGCCCCGCTGCCCTTCGCCTACGAC ATCCTGACCACCGCCTTCCACTACGGCAACCGCGTGTTCACCAAGTACCCCGAGGACATCCCTGA CTACTTCAAGCAGAGCTTCCCCGAGGGCTACAGCTGGGAGCGCAGCATGACCTACGAGGACGG CGGCATCTGCATCGCCACCAACGACATCACAATGGAGGAGGACAGCTTCATCAACAAGATCCAC TTCAAGGGCACGAACTTCCCCCCCAACGGCCCCGTGATGCAGAAGAGGACCGTGGGCTGGGAG GTCAGCACCGAGAAGATGTACGTGCGCGACGGCGTGCTGAAGGGCGACGTGAAGATGAAGCT

	GCTGCTGAAGGGCGGCAGCCACTATCGCTGCGACTTCCGCACCACCTACAAGGTCAAGCAGAAG GCCGTAAAGCTGCCCGACTACCACTTCGTGGACCACCGCATCGAGATCCTGAGCCACGACAAGG ACTACAACAAGGTGAAGCTGTACGAGCACGCCGTGGCCCGCAACTCCACCGACAGCATGGACG AGCTGTACAAGGGTACCCTTCAAACCGATGGAAACCGATCATCTCATTCAAGGTTGGGGCGAAT TGAAGCAGATAGTGAGAGTCAGGAGGACATAATACGCAATATAGCTCGACACCTTGCACAGGTC GGTGATAGCATGGACCGCTCTATCCCCCCAGGTTTGGTTAACGGATTGGCCCTGCAACTGCGAA ACACTTCAAGGAGTGAAGAAGATAGAAATCGAGACCTGGCGACCGCGCTTGAACAGCTTCTTCA AGCCTATCCCAGAGATATGGAGAAGGAAAAAACAATGCTCGTGCTCGCACTGCTGCTGGCAAAG AAAGTAGCCTCTAACACACCATCCCTCTTGAGAGACGTCTTCCATACCACGGTAAATTTCATAAAC CAGAACCTTAGGACGTATGTGCGGAGTCTTGCTCGCAATGGTATGACTGAGTTTCTTCAACCCGT GCCCCTCAATGCGTGGTTGCTCAGGTTGAACAAGTAA
NBid-PhoCl2c-CBid (NBid is highlighted in yellow, PhoCl2c is highlighted in green and CBid is highlighted in blue)	ATGGATTGTGAGGTCAATAACGGTTCATCTCTCCGAGACGAATGCATAACGAACTTGCTCGTTTT CGGCTTTCTGCAATCCTGCAGCGATAATTCTTTCAGAAGAGAACTTGACGCTCTTGGACATGAAC TCCCAGTACTCGCTCCACAGTGGGAAGGCTACGATGAGGGATCCGTGATCCCTGACTACTTCAA GCAGAGCTTCCCCGAGGGCTACAGCTGGGAGCGCAGCATGACCTACGAGGACGGCGGCATCTG CATCGCCACCAACGACATCACAATGGAGGGGGACAGCTTCATCAACAAGATCCACTTCCAGGGC ACGAACTTCCCCCCCAACGGCCCCGTGATGCAGAAGAGGACCGTGGGCTGGGAGGCCAGCACC GAGAAGATGTACGAGCGCGACGGCGTGCTGAAGGGCGACGTGAAGATGAAGCTGCTGCTGAA GGGCGGCGGCCACTATCGCGGCGACTACCGCACCACCTACAAGGTCAAGCAGAAGCCCGTAAA GCTGCCCGACTGCCACTTCGTGGACCACCGCATCGAGATCCTGAGCCACGACAAGGACTACAAC AAGGTGAAGCTGTACGAGCACGCCGTGGCCAAGACTTCCACCGACAGCATGGACGAGCTGTAC AAGGGTGGCAGCGGTGGCATGGTGAGCAAGGGCGAGGAGACCATTACAAGCGTGATCAAGCC TGACATGAAGAACAAGCTGCGCATGGAGGGCAACGTGAACGGCCACGCCTTCGTGATCGAGGG CGAGGGCAGCGGCAAGCCCTTCGAGGGCATCCAGACGATTGATTTGGAGGTGAAGGAGGGCG CCCCGCTGCCCTTCGCCTACGACATCCTGACCACCGCCTTCCACTACGGCAACCGCGTGTTCACCA AGTACCCACGGGGTACCCTTCAAACCGATGGAAACCGATCATCTCATTCAAGGTTGGGGCGAAT TGAAGCAGATAGTGAGAGTCAGGAGGACATAATACGCAATATAGCTCGACACCTTGCACAGGTC GGTGATAGCATGGACCGCTCTATCCCCCCAGGTTTGGTTAACGGATTGGCCCTGCAACTGCGAA ACACTTCAAGGAGTGAAGAAGATAGAAATCGAGACCTGGCGACCGCGCTTGAACAGCTTCTTCA AGCCTATCCCAGAGATATGGAGAAGGAAAAAACAATGCTCGTGCTCGCACTGCTGCTGGCAAAG AAAGTAGCCTCTAACACACCATCCCTCTTGAGAGACGTCTTCCATACCACGGTAAATTTCATAAAC CAGAACCTTAGGACGTATGTGCGGAGTCTTGCTCGCAATGGTATGACTGAGTTTCTTCAACCCGT GCCCCTCAATGCGTGGTTGCTCAGGTTGAACAAGTAA
NES-DEVD-mCardinal-NLS (NES is highlighted in yellow, DEVD is highlighted in green, mCardinal is highlighted in magenta and 3x NLS is highlighted in cyan)	ATGAACCTGGTGGACCTGCAGAAGAAGCTGGAGGAGCTGGAGCTGGACGAGCAGCAGGGATC CGCCTCCGGCGATGAGGTGGATGGAGCCGTGAGCAAGGGCGAGGAGCTGATCAAGGAGAACA TGCACATGAAGCTGTACATGGAAGGCACCGTGAACAACCACCACTTCAAGTGCACCACCGAAGG GGAGGGCAAGCCCTACGAGGGCACCCAGACCCAGAGGATTAAGGTGGTGGAGGGAGGCCCCC TGCCGTTCGCATTCGACATCCTGGCCACCTGCTTTATGTACGGGAGCAAGACCTTCATCAACCAC ACCCAGGGCATCCCCGATTTCTTTAAGCAGTCCTTCCCTGAGGGCTTCACATGGGAGAGAGTCAC CACATACGAAGACGGGGGCGTGCTTACCGTTACCCAGGACACCAGCCTCCAGGACGGCTGCTTG ATCTACAACGTCAAGCTCAGAGGGGTGAACTTCCCATCCAACGGCCCTGTGATGCAGAAGAAAA CACTCGGCTGGGAGGCCACCACCGAGACCCTGTACCCCGCTGACGGCGGCCTGGAAGGCAGAT GCGACATGGCCCTGAAGCTCGTGGGCGGGGGCCACCTGCACTGCAACCTGAAGACCACATACA GATCCAAGAAACCCGCTAAGAACCTCAAGATGCCCGGCGTCTACTTTGTGGACCGCAGACTGGA AGAATCAAGGAGGCCGACAATGAGACCTACGTCGAGCAGCACGAGGTGGCTGTGGCCAGATA CTGCGACCTCCCTAGCAAACTGGGGCACAAACTTAATGGCATGGACGAGCTGTACAAGGGCTCC GGACCAAAAAAAAAACGAAAAGTTGACCCAAAAAAGAAGCGCAAAGTAGACCCCAAGAAAAAA CGCAAGGTAGGTACCTAA

Movie S1. Molecular dynamic simulations on dissociation process of PhoCl1. Rep, replication. PhoCl1 is shown in grey, dissociated peptide fragment is shown as magenta for the peptide portion and cyan sticks for the chromophore. Residues within and near the 201-207 loop are highlighted in green. The schematic of the workflow of molecular dynamic simulations has been described in Fig. S2A.

Movie S2. Additional unconstrained cMD. The ASMD empty barrel structure and the empty barrel crystal structure are shown in grey, residues within the 201-207 loop are highlighted in red. The schematic of the workflow of molecular dynamic simulations has been described in Fig. S2A.

Movie S3. Optogenetic manipulation of cell apoptosis with caspase-3 reporter. Transient transfected HeLa cells co-expressing NBid-mMaple-CBid or NBid-PhoCl-CBid with caspase-3 reporter. Cells were illuminated with 10 s violet light pulses (395/40 nm, 2 mW/mm^2) every 15 s for 6 mins, then imaged 2 hours after photoconversion.

References:

1 A. C. Wallace, R. A. Laskowski and J. M. Thornton, *Protein Eng.*, 1995, **8**, 127–134.

2 N. C. Shaner, P. A. Steinbach and R. Y. Tsien, *Nat. Methods*, 2005, **2**, 905–909.