Electronic supplementary information

Photocleavable Proteins that Undergo Fast and Efficient Dissociation

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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/mm²).



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 ($\Delta G = 0$ kCal/mol). Values are means \pm 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 μ g/mL). Values are means \pm 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 LIGPLOT¹. 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 μ M 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 $5 \times$ increased contrast.



Fig. S10 Red fluorescence intensity localization ratios (cytoplasm to nucleus) of NES-PhoClmCherry before and after photoconversion. Values are means \pm 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-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 μ m.



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

Crystal	PhoCl1 green state	PhoCl1 red state	PhoCl1 empty barrel
Data collection			
Spacegroup	P212121	P1	P21
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-	38.57-2.30 (2.38-	43.11-2.82 (2.92-2.82)
	2.10)	2.30)	
Rmerge	0.066 (0.709)	0.042 (0.533)	0.073 (0.760)
Rmeas	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)
Ι/σ(Ι)	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	25.64	28.86	43.91
$(Å^2)$			
Refinement			
Total Reflections	194926 (31020)	103548 (16288)	44693 (7133)
Unique	57043 (9153)	58440 (9388)	16400 (2688)
Reflections			
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	34.07	35.53	38.70
(Å ²)			
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-Squar	e-Deviation:		
Bond lengths (Å)	0.012	0.004	0.005
Bond Angle (°)	1.50	1.0	0.82

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

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

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

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

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, lle207Ser
PhoCl1.5f-LS	Lys49Gln, Tyr117Cys, Arg145Lys, Asn146Thr, Lys202Leu, lle207Ser
PhoCl1.5f-MVLS	Lys49Gln, Tyr117Cys, Val143Met, Ala144Val, Arg145Lys, Asn146Thr, Lys202Leu, lle207Ser
PhoCl2f	Lys49Gln, Tyr117Cys, Arg145Lys, Asn146Thr, lle207Ser
PhoCl2c	Lys49Gln, Cys99Gly, Tyr117Cys, Arg145Lys, Asn146Thr

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

 Table S4. Relative chromophore formation efficiencies.

Note: The relative chromophore formation efficiency was characterized by the ratio of Abs488/(Abs280* ε 488)*100. Abs, absorbance; ε , extinction coefficients (mM⁻¹cm⁻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.

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

 Table S5. Summary data of bioluminescence assay.

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.

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

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

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 con	struct
F-Xhol-LgBiT	GCAGGCTCGAGGATGGTCTTCACACTCGAAGATTTCGTTGGG
R-LgBiT-linker1-KpnI-PhoCl overlap PCR	GAAGTAGTCAGGGATCACGGTACCAGGTTCTCCGCCGCTAGAACCGCCTCCGCTACTCCC
F-linker1-Kpn1-PhoCl overlap PCR	GGCGGAGAACCTGGTACCGTGATCCCTGACTACTTCAAGCAGAGC
R1-PhoCl-Xbal-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-Kpnl-PhoCl	CGGAGAACCTGGTACCGTGATCCCT
EP-R-Xbal-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-Ncol-PhoCl	ATATACCATGGTGATCCCTGACTACTTCAAGCAGAGCTTC	
R-PhoCl1-linker-Xhol	GTAGCCTCGAGACCTCCACCTCCCCGTGGGTACTTGGTGAACACGC	
pBAD/HisB-PhoCl construct		
F-Xhol-g-PhoCl	CCGAGCTCGAGTGTGATCCCTGACTAC	
R-PhoCl*-KpnI	CATCCGCCAAAACAGCCAAGCTTGGTACCTTACCGTGGGTACTTGGTGAACAC	
pBAD/HisB-PhoCl-MBP construct		
F-Xhol-g-PhoCl	CCGAGCTCGAGTGTGATCCCTGACTAC	
R-PhoCl-linker-Kpnl	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-Nhel-NBid	AGTCAGCTAGCGCCACCATGGATTGTGAGGTCAATAACGG	
R-BamHI-NBid overlap PCR	GGATCCCTCATCGTAGCCTTCCCAC	
F-NBid-BamHI-PhoCl overlap PCR	GTGGGAAGGCTACGATGAGGGATCCGTGATCCCTGACTACTTCAAGC	
R-PhoCl-KpnI-CBid overlap PCR	GATCGGTTTCCATCGGTTTGAAGGGTACCCCGTGGGTACTTGGTGAAC	
F-Kpnl-CBid overlap PCR	GGTACCCTTCAAACCGATGGAAACCGATC	
R-CBid*-Xhol	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-Nhel-NES	CACTGGCTAGCGCCACCATGAACCTGGTGGACCTGCAGAAGAAGCTGGAGG	
F2-NES	GACCTGCAGAAGAAGCTGGAGGAGCTGGAGCTGGACGAGCAGCAGGATCCGCCTCCGGC	
F3-DEVD-mCardinal	CAGGGATCCGCCTCCGGCGATGAGGTGGATGGAGCCGTGAGCAAGGGCGAGGAG	
R1-mCardinal-NLS	CGTTTTTTTTGGTCCGGAGCCCTTGTACAGCTCGTCCATGCC	
R2-NLS-NLS	GGGGTCTACTTTGCGCTTCTTTTTGGGTCAACTTTTCGTTTTTTTT	
R3-NLS*-Xhol	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)	Jequence 93 ATGGTCTTCACACTCGAAGATTTCGTTGGGGACTGGGAACAGACAG
PhoCl2c (PhoCl is highlighted in green and mutations compared to PhoCl1 are highlighted in magenta)	GTGATCCCTGACTACTTCAAGCAGAGCTTCCCCGAGGGCTACAGCTGGGAGCGCAGCATGACCT ACGAGGACGGCGGCATCTGCATCGCACCAACGACATCACAATGGAGGGGGACAGCTTCATCA ACAAGATCCACTTCCAGGGCACGAACTTCCCCCCCAACGGCCCGTGATGCAGAAGAGGACCGT GGGCTGGGAGGCCAGCACCGAAGAAGATGTACGAGCGCGACGGCGTGCTGAAGGGCGACGTGA AGATGAAGCTGCTGCTGAAGGGCGGCGGCCACTATCGCGGCGACTACCGCACCACCTACAAGG TCAAGCAGAAGCCCGTAAAGCTGCCCGACTGCCACTTCGTGGACCACCGCATCGAGATCCTGAG CCACGACAAGGACTACAACAAGGTGAAGCTGTACGAGCACGCGTGGCCAAGGACTTCCACCGA CAGCATGGACGAGCTGCAAAGGTGGAAGCTGTACGAGCACGCGTGGCCAAGGACTTCCACCGA TTACAAGCGTGATCAAGGTGGCAGCGGTGGCATGGTGAGCAAGGGCGAGGAGACCA TTACAAGCGTGATCAAGCCTGACATGAAGAACAAGCTGCGCATGGAGGGCAACGTGAACGGCC ACGCCTTCGTGATCGAGGGCGAGGGCAGGGC
PhoCl2f (PhoCl is highlighted in green and	GTGATCCCTGACTACTTCAAGCAGAGCTTCCCCGAGGGCTACAGCTGGGAGCGCAGCATGACCT ACGAGGACGGCGGCATCTGCATCGCCACCAACGACATCACAATGGAGGGGGACAGCTTCATCA ACAAGATCCACTTCCAGGGCACGAACTTCCCCCCCAACGGCCCCGTGATGCAGAAGAGGACCGT GGGCTGGGAGGCCAGCACCGAGAAGATGTACGAGCGCGACGGCGTGCTGAAGGGCGACGTGA AGATGAAGCTGCTGCTGAAGGGCGGCGGCCACTATCGCTGCGACTACCGCACCACCTACAAGGT CAAGCAGAAGCCCGTAAAGCTGCCCGACTGCCACTTCGTGGACCACCGCATCGAGATCCTGAGC

	CACGACAAGGACTACAACAAGGTGAAGCTGTACGAGCACGCCGTGGCCAAGACTTCCACCGAC
	AGCATGGACGAGCTGTACAAGGGTGGCAGCGGTGGCATGGTGAGCAAGGGCGAGGAGACCAT
	TACAAGCGTGATCAAGCCTGACATGAAGAACAAGCTGCGCATGGAGGGCAACGTGAACGGCCA
	CGCCTTCGTGATCGAGGGCGAGGGCAGCGGCAAGCCCTTCGAGGGCTCTCAGACGATTGATT
	GGAGGTGAAGGAGGGCGCCCCGCTGCCCTTCGCCTACGACATCCTGACCACCGCCTTCCACTAC
	GGCAACCGCGTGTTCACCAAGTACCCACGG
	GTGATCCCTGACTACTTCAAGCAGAGCTTCCCCGAGGGCTACAGCTGGGAGCGCAGCATGACCT
	ACGAGGACGGCGGCATCTGCATCGCCACCAACGACATCACAATGGAGGGGGGACAGCTTCATCA
	ACAAGATCCACTTCCAGGGCACGAACTTCCCCCCCAACGGCCCCGTGATGCAGAAGAGGACCGT
	GGGCTGGGAGGCCAGCACCGAGAAGATGTACGAGCGCGACGGCGTGCTGAAGGGCGACGTGA
PhoCl1.5f-MV	AGATGAAGCTGCTGCTGAAGGGCGGCGGCCACTATCGCTGCGACTACCGCACCACCTACAAGGT
(PhoCl is highlighted in green and	CAAGCAGAAGCCCGTAAAGCTGCCCGACTGCCACTTCGTGGACCACCGCATCGAGATCCTGAGC
mutations compared to PhoCl1 are	CACGACAAGGACTACAACAAGGTGAAGCTGTACGAGCACGCCATGGTTAAGACTTCCACCGACA
highlighted in magenta)	GCATGGACGAGCTGTACAAGGGTGGCAGCGGTGGCATGGTGAGCAAGGGCGAGGAGACCATT
	ACAAGCGTGATCAAGCCTGACATGAAGAACAAGCTGCGCATGGAGGGCAACGTGAACGGCCAC
	GCCTTCGTGATCGAGGGCGAGGGCAGCGGCAAGCCCTTCGAGGGCATCCAGACGATTGATT
	GAGGTGAAGGAGGGCGCCCCGCTGCCCTTCGCCTACGACATCCTGACCACCGCCTTCCACTACG
	GCAACCGCGTGTTCACCAAGTACCCACGG
	GTGATCCCTGACTACTTCAAGCAGAGCTTCCCCCGAGGGCTACAGCTGGGAGCGCAGCATGACCT
	ACGAGGACGGCGGCATCTGCATCGCCACCAACGACATCACAATGGAGGGGGGACAGCTTCATCA
	ACAAGATCCACTTCCAGGGCACGAACTTCCCCCCCAACGGCCCCGTGATGCAGAAGAGGACCGT
	GGGCTGGGAGGCCAGCACCGAGAAGATGTACGAGCGCGACGGCGTGCTGAAGGGCGACGTGA
PhoCl1.5f-L	AGATGAAGCTGCTGCTGAAGGGCGGCGGCCACTATCGCTGCGACTACCGCACCACCTACAAGGT
(PhoCl is highlighted in green and	CAAGCAGAAGCCCGTAAAGCTGCCCGACTGCCACTTCGTGGACCACCGCATCGAGATCCTGAGC
mutations compared to PhoCl1 are	CACGACAAGGACTACAACAAGGTGAAGCTGTACGAGCACGCCGTGGCCAAGACTTCCACCGAC
highlighted in magenta)	AGCATGGACGAGCTGTACAAGGGTGGCAGCGGTGGCATGGTGAGCAAGGGCGAGGAGACCAT
	TACAAGCGTGATCAAGCCTGACATGAAGAACAAGCTGCGCATGGAGGGCAACGTGAACGGCCA
	CGCCTTCGTGATCGAGGGCGAGGGCAGCGGGCTTCCCTTCGAGGGCATCCAGACGATTGATT
	GAGGTGAAGGAGGGCGCCCCGCTGCCCTTCGCCTACGACATCCTGACCACCGCCTTCCACTACG
	GCAACCGCGTGTTCACCAAGTACCCACGG
	GTGATCCCTGACTACTTCAAGCAGAGCTTCCCCCGAGGGCTACAGCTGGGAGCGCAGCATGACCT
	ACGAGGACGGCGGCATCTGCATCGCCACCAACGACATCACAATGGAGGGGGGACAGCTTCATCA
	ACAAGATCCACTTCCAGGGCACGAACTTCCCCCCCAACGGCCCCGTGATGCAGAAGAGGACCGT
	GGGCTGGGAGGCCAGCACCGAGAAGATGTACGAGCGCGACGGCGTGCTGAAGGGCGACGTGA
PhoCl1.5f-MVL	AGATGAAGCTGCTGCTGAAGGGCGGCGGCCACTATCGCTGCGACTACCGCACCACCTACAAGGT
(PhoCl is highlighted in green and	CAAGCAGAAGCCCGTAAAGCTGCCCGACTGCCACTTCGTGGACCACCGCATCGAGATCCTGAGC
mutations compared to PhoCl1 are	CACGACAAGGACTACAACAAGGTGAAGCTGTACGAGCACGCCATGGTTAAGACTTCCACCGACA
highlighted in magenta)	GCATGGACGAGCTGTACAAGGGTGGCAGCGGTGGCATGGTGAGCAAGGGCGAGGAGACCATT
	ACAAGCGTGATCAAGCCTGACATGAAGAACAAGCTGCGCATGGAGGGCAACGTGAACGGCCAC
	GCCTTCGTGATCGAGGGCGAGGGGCAGCGGGCTTCCCTTCGAGGGCATCCAGACGATTGATT
	GAGGTGAAGGAGGGCGCCCCGCTGCCCTTCGCCTACGACATCCTGACCACCGCCTTCCACTACG
	GCAACCGCGTGTTCACCAAGTACCCACGG
	GTGATCCCTGACTACTTCAAGCAGAGCTTCCCCGAGGGCTACAGCTGGGAGCGCAGCATGACCT
	ACGAGGACGGCGGCATCTGCATCGCCACCAACGACATCACAATGGAGGGGGGACAGCTTCATCA
	ACAAGATCCACTTCCAGGGCACGAACTTCCCCCCCAACGGCCCCGTGATGCAGAAGAGGACCGT
	GGGCTGGGAGGCCAGCACCGAGAAGATGTACGAGCGCGACGGCGTGCTGAAGGGCGACGTGA
PhoCl1.5f-MVS	AGATGAAGCTGCTGCTGAAGGGCGGCGGCCACTATCGCTGCGACTACCGCACCACCTACAAGGT
(PhoCl is highlighted in green and	CAAGCAGAAGCCCGTAAAGCTGCCCGACTGCCACTTCGTGGACCACCGCATCGAGATCCTGAGC
mutations compared to PhoCl1 are	CACGACAAGGACTACAACAAGGTGAAGCTGTACGAGCACGCCATGGTTAAGACTTCCACCGACA
highlighted in magenta)	GCATGGACGAGCTGTACAAGGGTGGCAGCGGTGGCATGGTGAGCAAGGGCGAGGAGACCATT
	ACAAGCGTGATCAAGCCTGACATGAAGAACAAGCTGCGCATGGAGGGCAACGTGAACGGCCAC
	GCCTTCGTGATCGAGGGCGAGGGCAGCGGCAAGCCCTTCGAGGGCTCTCAGACGATTGATT
	GAGGTGAAGGAGGGCGCCCCGCTGCCCTTCGCCTACGACATCCTGACCACCGCCTTCCACTACG
	GCAACCGCGTGTTCACCAAGTACCCACGG
	GTGATCCCTGACTACTTCAAGCAGAGCTTCCCCCGAGGGCTACAGCTGGGAGCGCAGCATGACCT
	ACGAGGACGGCGGCATCTGCATCGCCACCAACGACATCACAATGGAGGGGGACAGCTTCATCA
	ACAAGATCCACTTCCAGGGCACGAACTTCCCCCCCAACGGCCCCGTGATGCAGAAGAGGACCGT
	GGGCTGGGAGGCCAGCACCGAGAAGATGTACGAGCGCGACGGCGTGCTGAAGGGCGACGTGA
PhoCl1.5f-LS	AGATGAAGCTGCTGCTGAAGGGCGGCGGCCACTATCGCTGCGACTACCGCACCACCTACAAGGT
(PhoCl is highlighted in green and	CAAGCAGAAGCCCGTAAAGCTGCCCGACTGCCACTTCGTGGACCACCGCATCGAGATCCTGAGC
mutations compared to PhoCl1 are	CACGACAAGGACTACAACAAGGTGAAGCTGTACGAGCACGCCGTGGCCAAGACTTCCACCGAC
highlighted in magenta)	AGCATGGACGAGCTGTACAAGGGTGGCAGCGGTGGCATGGTGAGCAAGGGCGAGGAGACCAT

PhoCl1.5F.MVIS GGGTGGGAGGGGCAGGGGCAGGGGCAGCGGGCTTCGCAGGCAG		TACAAGCGTGATCAAGCCTGACATGAAGAACAAGCTGCGCATGGAGGGCAACGTGAACGGCCA
EAGGTGAAGGAGGCCCCCCGTCCCTTCGCCTACGGACGCCCGCC		CGCCTTCGTGATCGAGGGCGAGGGCAGCGGGCTTCCCTTCGAGGGCTCTCAGACGATTGATT
CCAACCCCTGTTCACCAAGTACCCACGG CGACCCCCGCGCGCCCCCCGCGGCCCCCCGCGGCCCCGCGCGC		GAGGTGAAGGAGGGCGCCCGCTGCCCTTCGCCTACGACATCCTGACCACCGCCTTCCACTACG
PhoCI1.51-MVLS GGGCTGGGGAGCCACCTCGCAGGCACCAGCGAGGGGGCAGCTCGAGAGGGGGGACGTCGACAGGGGGGAGCGTCGCAGCAGGGGGGGCAGCTCGCGGGGGGGG		GCAACCGCGTGTTCACCAAGTACCCACGG
PhoCI1.51-MVL5 ACGAGGACGECGCATCTGCATCGCCACCGACACCACATCACAT		GTGATCCCTGACTACTTCAAGCAGAGCTTCCCCCGAGGGCTACAGCTGGGAGCGCAGCATGACCT
ACAAGATCCACTTCCAGGGCACGAACTTCCCCCCCAAGGCCCCCTGTCGAAGAGGGGCAGCGGA PhoCI1.5F.WUS GGGCGGGGACGCCCCCCCCCCCCCCCCCCCCCCCCCCC		ACGAGGACGGCGGCATCTGCATCGCCACCAACGACATCACAATGGAGGGGGGACAGCTTCATCA
PhoCLI_SI-MVLS GGGCTGGGAGGCCACCACCAGAGAGTTATGGCTGGAGCTGCGACCACCGCACCAGCTAAGG (PhoCl is highlighted in green and mutations compared to PhoCl1 are highlighted in magenta) GGGCTGGGAGGCCGTAAGGCTGCGGCACCACGCGGCGGCACCTGGAGGCCACCGGAGGGCACCGGAGGGCACGGGAGGCACGGAGGGCACCGGAGGGCACGGAGGGCACGGAGGGCACGGAGGGCACGGAGGGCACGGAGGGCACGGAGGGCACGGAGGGCACGGAGGGCACGGAGGGCACGGAGGGCACGGAGGCACGGAGGCACGGAGGGCACGGAGGGCACGGAGGGCACGGAGGGCACGGAGGGAGGCCCCGCACGGAGGGCACGGGAGGCAGGGAGGCACGGAGGGCACGGAGGGCACGGAGGGCACGGAGGGCACGGAGGGCACGGAGGGCACGGAGGGCACGGAGGGCACGGAGGGCACGGAGGGCACGGAGGGCACGGAGGGCACGGAGGGAGGCAGGAGG		ACAAGATCCACTTCCAGGGCACGAACTTCCCCCCCAACGGCCCCGTGATGCAGAAGAGGACCGT
PhoCl.5:FMVLS AGATCANGCTGCTGCTGCTGAAGGGCGGCGGCCACTATCGCTGCCGCACCGCACCTGACAGGC (PhoCl is highlighted in pragenta) CAAGCAAGGCCGTAAAGCTGCCGCGAGGGCGCCACTGGTGGACGCGCGCAGGGGAAGCCCATGGAGGGGAAGCCCACGGGCAGGGGAAGCCCATGGAGGGAAGCCCATGGAGGGAAGCCCATGGAGGGAG		GGGCTGGGAGGCCAGCACCGAGAAGATGTACGAGCGCGACGGCGTGCTGAAGGGCGACGTGA
(iPbc1i is highlighted in green and mutations compared to Phc01i are highlighted in magenta) CAGGACAAGGACTACAACAAGGTGGACACCTTGGTGGACCACGGCAAGGTGAAGACGACA CAGGACAAGGACTACAACAAGGTGGACACGGTGGACACGGGACAGTGAAGAGACACAC CACGACAAGGACTACAAGGGCGAGGGACGCACGGTGAACACAGGGCAAGGTGAACGCACG CCTTCGTGATCCAAGCCGGCGCGCCCCTCCCACCACGCACG	PhoCl1.5f-MVLS	AGATGAAGCTGCTGCTGAAGGGCGGCGGCCACTATCGCTGCGACTACCGCACCACCTACAAGGT
nutation: compared to PhoCl are highlighted in magenta) CACGACAAGGACTACAACAAGGTGAACCAGCGCGTGGCACGCAGGGGGCGAGGGGAGGCAGGGCAAGGCGAGGCAAGGGCAAGGGCAAGGGCAAGGCCACGCCACGCCCCCC	(PhoCl is highlighted in green and	CAAGCAGAAGCCCGTAAAGCTGCCCGACTGCCACTTCGTGGACCACCGCATCGAGATCCTGAGC
highlighted in magenta) GCATGGACGAGCTGTACAAGGGCGGCGGCGGCAGCAGCAGGAGCAGGGCGAGCGGAGCCATT ACAAGGCGTGATCAAGGCTGACAAGGAGAAAGCTGGCACAGGGAAGGGGAACGGGAACCGGAAGCGGAACGGGAACGCGGACCCGCCG	mutations compared to PhoCl1 are	CACGACAAGGACTACAACAAGGTGAAGCTGTACGAGCACGCCATGGTTAAGACTTCCACCGACA
ACARGEGTCATCAAGCCTGACATGAAGAGCAAGCGGGGCACGGGGGGGG	highlighted in magenta)	GCATGGACGAGCTGTACAAGGGTGGCAGCGGTGGCATGGTGAGCAAGGGCGAGGAGACCATT
GCCTTCGTATCGAGGGCAGGGGCAGCGGGCTTCCTCGAGGGCTTCAGACAATTGGTTGAGGGGGGGG	5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5	ACAAGCGTGATCAAGCCTGACATGAAGAACAAGCTGCGCATGGAGGGCAACGTGAACGGCCAC
NBid-PhoCl1-CBid AGGTGAAGGAGGCCCCCGTTCGACTAGGCGACATCCTGACCACCGCCTTCCACTACGG CAACGGCGTGTCACCTTCAAGCAGGAGCTTCCCCCAAGGAGCTACAGCTGGGGGGGG		GCCTTCGTGATCGAGGGCGAGGGGCAGCGGGCTTCCCTTCGAGGGCTCTCAGACGATTGATT
CAACCCCCGTGTTCACCAAGTACCCCAGGG ATGGTGATCCCTGACTACTTCAAGGCAGAGGCTCCCCGAGGGCAGGCA		AGGTGAAGGAGGGCGCCCCGCTGCCCTTCGCCTACGACATCCTGACCACCGCCTTCCACTACGG
NBid-PhoCl1-CBid ATGGTATCCCTGACTACTCAAGGAGAGCCCGCAACGACGAGGACGAGCATCGA CCTACGAGGACGCGGCATCTGACGAGAACTCTCCCCCCAACGAGCATCGACGAGGACGCCCTACAA CCAACAAGATCCACCCTCACAAGGACCCCCCCCCAAGAAGCACCCCCCCC		CAACCGCGTGTTCACCAAGTACCCACGG
PhoC11-6His CCTACGAGGACGCCGCATCTGCATCGCCACCAACGACATTGCAGAAGAGGGGGACAGCTTCAT CAACAAGATCCACTTCAAGGGCACGGAACTTCCCCCCCGATGACGGCCGCGCGCTGCTGAAGAGAGGCCACCTCACACT CCAACAAGGCCCTAAAGGGCCACGGACGCCCCCGTGCTGCAAGAGGGCGACGC PhoC11-6His GGTCAAGCAGAGCCGCACCTGCACACACGCACCGCCCGCGCGCCCCCCCC		ATGGTGATCCCTGACTACTTCAAGCAGAGCTTCCCCGAGGGCTACAGCTGGGAGCGCAGCATGA
PhoCl1-6His CAACAAGATCCACTTCAAGGGCACGAACTTCCCCCCAACGGCCCCGCAGGCGCCACGATGCACGACGCGG PhoCl1-6His GGTCAAGGCAGGCCCGGCAGCCCCGACGCACGCACGCACG		CCTACGAGGACGGCGGCATCTGCATCGCCACCAACGACATCACAATGGAGGGGGACAGCTTCAT
PhoCl1-6His GTGGGCTGGCAGGCCCGGCGGCGGCGCGCACCTACCACCTCCACA PhoCl1-6His GGTCAGCCAGAGACCCGTAAGGGCGGCGCCACCTATCCCTTCGCGACTACCGACCCGCACCTCACA (PhoCl is highlighted in yellow) GGTCAGCCAGGCGGCGCCCGCATACCACCTCGCGCGCACCCCGCACCTCACAC (PhoCl is highlighted in yellow) GGTCAGCCAGCGTGGCACAGGCGCGGCGCGCGCAGGCAGG		CAACAAGATCCACTTCAAGGGCACGAACTTCCCCCCCAACGGCCCCGTGATGCAGAAGAGGACC
PhoCl1-6His GAAGATGAAGCTGCTGCTGCAAGGGCGGCGCCACTATCGGCACCACCACCACCACCACCACCACACACA		GTGGGCTGGGAGGCCAGCACCGAGAAGATGTACGAGCGCGACGGCGTGCTGAAGGGCGACGT
PhoCl1-6His (PhoCl is highlighted in green and His tag is highlighted in yellow) GGTCAAGCAGAAGCCCGTAAAGCTGCCCGCATCCAGAGCACCGCGATCGAGAACTCCTAC GCACGCATGCACAGGAGCACTACAAACAAGGTGCAAGCGGTGCAGGGGCGAGGGCAAGCG CACTACAAGCAGCAGCGCGCGCGCTGCTCACAGGCGCAGGGCAAGCGGCAAGCGGCAAGCGG CACGCCTTCGTGAATCCAAGGGCGAGGGCAAGCGCCACGCGCAAGCCCTCCAACCGCCCCCCACCCA		GAAGATGAAGCTGCTGCTGAAGGGCGGCGGCCACTATCGCTGCGACTACCGCACCACCTACAA
(PhoCl is highlighted in green and His tag is highlighted in yellow) AGCCACGACAAGGACTACAACAAGGTGAAGCTGTACGAGACGCGCGTGGCCCGCAAGCTGACAGGAGAGAGA	PhoCl1-6His	GGTCAAGCAGAAGCCCGTAAAGCTGCCCGACTACCACTTCGTGGACCACCGCATCGAGATCCTG
tag is highlighted in yellow) GACAGCATGGACGAGCGTGTACAAGGGTGGCAGCGGTGGCATGGTGGCAGGGCAAGGGCAAGGGCAACGGGAACGGCAACGGGAAGGCACCGGCATGGACTGGAACGG CATTCCAAGCCTTCGTAGTCAGGGCCAGGGCAAGCGGCAAGCGCACCGGCACCGCACCCACCA	(PhoCl is highlighted in green and His	AGCCACGACAAGGACTACAACAAGGTGAAGCTGTACGAGCACGCCGTGGCCCGCAACTCCACC
CATTACAAGCGTGATCAAGCCTGACATGAAGAACAAGCTGCGCATGGAGGGCAACGTGAACGG CCACGCCTTCGTGATCGAGGGCGAGGCAAGCCCTTCGAGGGCATCCAGACGCATTGA TTTGGAGGTGAAGGAGGGCGCCCGCGCCCCCGCGCCTCCGACGCCAGCCCAGCCCACCCA	tag is highlighted in vellow)	GACAGCATGGACGAGCTGTACAAGGGTGGCAGCGGTGGCATGGTGAGCAAGGGCGAGGAGAC
NBid-PhoCl1-CBid CCACGCCTTCGTGATCGAGGGCAGGGCAGGGCAGGGCATCCAAGACCCCTCGACTAACGAACTGACGGCAGGGGGGGG		CATTACAAGCGTGATCAAGCCTGACATGAAGAACAAGCTGCGCATGGAGGGCAACGTGAACGG
TTIGGAGGTGAAGGAGGGCGCCCCGGTGCCCTTCGCACACGACACCGCCCTTCCACT ACGGCAACCGCGTGTTCACCAAGTACCCACGGGGAGGTGAAGGTCTAGACACCCACC		CCACGCCTTCGTGATCGAGGGCGAGGGCAGCGGCAAGCCCTTCGAGGGCATCCAGACGATTGA
ACGGCAACCGCGGTGTTCACCAAGGACCACGGGGAGGTGGAGGTCCGAGCACCACCACCACCACCACCACCACCACCACCACCA		TTTGGAGGTGAAGGAGGGCGCCCCGCTGCCCTTCGCCTACGACATCCTGACCACCGCCTTCCACT
ACCACTGA ATGGATTGTGAGGTCAATAACGGTTCATCTCTCCGAGACGAATGCATAACGAACTTGCTCGTTTT CGGCTTTCTGCAATCCGCGCACGACGACAGAGAGACGAGAACTTGACGACCTCGTGATCACCGACATGAAC TCCCCAGTACTCGCCCCCACAGGGGAGAGGCTACGATGAGGACCTGACGCATGAGCGCGGCGGCGCCCTCGA GCAGAGCTTCCCCCGAGGGCTACAGCTGGGAGGGGCAGCGTCACACAACGAGCCCCTCAAGGGC CATCGCCACCCACCGACCACCACCGCGCGCGGGGGCAGCAGCCTCACCAACAGACCCACTCAAGGGC ACGAACGTTCCCCCCAACGGCCCGCGGGGCAGCAGCCCCACCACACAAGACCCACCTCAAGGGC ACGAACGTTCCCCCCACGGCCCGCGGCGCGCGCGGCAGCGAGCG		ACGGCAACCGCGTGTTCACCAAGTACCCACGGGGGGGGGG
ATGGATTGTGAGGTCAATAACGGTTCATCTCTCCGAGACGATAGCATAACGAACTTGCTCGTTTT CGCGTTTCTGCAATCCTGCCGCGCGATAATTCTTTCAGAAGAGAACTTGACGCTCTTGGACATGAAC TCCCAGTACTCGCCCACAGTGGGAAGGCTACGATGAGGGGGACCGCGTGTGACGATGACGCCGGCGCATCTG CCCAGGCTTCCCCCCAACGGCCACAGCTGGGAGGGCACGCTCACGACGAGGAGGCCACGCCCGGAGAGAGGCGAGCGCGGCG		ACCACTGA
CGGCTTTCTGCAATCCTGCAGCGATAATTCTTTCAGAAGAGAACTTGACGCTCTTGGACATGAAC TCCCAGTACTCGCTCCCAAGTGGGAGGGCACGATCGATGACGCTTGGACCATGAGC CGCAGAGCTTCCCCGAGGGCTACAGCTGGGAGCGCAGCATCGATCCTGATCCACTTCAAGGGC CAGAACTTCCCCCCAACGGACAATGAAGGGGGACAGCATCAAGAGAGAG		ATGGATTGTGAGGTCAATAACGGTTCATCTCTCCGAGACGAATGCATAACGAACTTGCTCGTTTT
NBid-PhoCl1-CBid TCCCAGTACTCGCTCCACAGTGGGAAGGCTACGACTGAGCGCAGGAGCGCCAGCGCGCATCTG CAGAGCTTCCCCCCACGGGGCTACAGCTGGGAAGGCTCACACAGAGAGCGCCAGGCGCAGCGC CAGCAGACTTCCCCCCACAGGGGGGGGACGCTGCACAAGCGGCGGCGGCGGCGGCAGCGCAGAGACGCCGGCAGCGCGAGAGCGCAGCA		CGGCTTTCTGCAATCCTGCAGCGATAATTCTTTCAGAAGAGAACTTGACGCTCTTGGACATGAAC
Bid-PhoCl1-CBid GCAGAGCTTCCCCCGAGGGCTACAGCTGGGAGCGCAGCATGACCTACGAGGACGGCGGCATCTG CATCGCCACCAACGACACCCACCGCCGGCGTGCTGAAGAGGGGCGACGGCGGGGGGGG		TCCCAGTACTCGCTCCACAGTGGGAAGGCTACGATGAGGGATCCGTGATCCCTGACTACTTCAA
NBid-PhoCl1-CBid CATCGCCACCAACGACATCACAATGGAGGGGGACAGCTTCATCAACAAGATCCACTTCAAGGGC NBid-PhoCl1-CBid GGGCGGCCGCCCCCCAACGGCCGCGCGCCCCCGCACCACC		GCAGAGCTTCCCCGAGGGCTACAGCTGGGAGCGCAGCATGACCTACGAGGACGGCGGCATCTG
ACGAACTTCCCCCCCCAACGGCCCCGTGATGCAGAAGAGGACCGTGGGAGGCCAGCACC GAGAAGATGTACGAGCGCGAGCGCGGCGGCGACGGCACGTGAAGAGCGGAGGCGACGTGCTGAA GGCCGGCGGCGCCACTATCGCTGCGACTACCGCACCACCACCAAGGACGAAGGCCGTAAA GCCCCCCCACACCCCCTCGGCCCCGCACGCCCCGACGCCACGCCACGACGAGAGCCCGTAAA GCCCCCCCCACTACCACTTCGTGGACCACCGCCGCGACGCCACGCCAGGAGAGCCAAGGACTACAAC AAGGGTGGAAGCTGTACGAGCACGCCGTGGCCCGCAACTCCCACAGCATGGAAGAGCGGACGAGGCGTGTAC AAGGGTGGCAGCGGCGGCAGCGGCGGCAGGGCAAGGGCGAGGAGACCATTACAAGCGTGAAGCG (NBid is highlighted in yellow, PhoCl is highlighted in green and CBid is highlighted in blue) CGAGGGCAGCGGCAGCGGCGCAGCGGCGGCGCGGCAGGGCAACGGCAAGCGCGCGCCGC		CATCGCCACCAACGACATCACAATGGAGGGGGGACAGCTTCATCAACAAGATCCACTTCAAGGGC
NBid-PhoCl1-CBidGAGAAGATGTACGAGCGCGACGGCGACGGCGACGTGAAGGTGAAGATGAAGCTGCTGCTGAAA GGGCGGCGCCACTATCGCTGCGACTACCGCACCCGCACAGGCAAGGCAGGAGAGCCGTAAA GCTGCCCGACTACCACCTTCGTGGACCACCGCACCGCAC		ACGAACTTCCCCCCCAACGGCCCCGTGATGCAGAAGAGGACCGTGGGCTGGGAGGCCAGCACC
NBid-PhoCl1-CBid GGGCGGCGGCCACTATCGCTGCGACTACCGCACCACCTACAAGGTCAAGCAGAAGCCCGTAAA GCTGCCCGACTACCACTTCGTGGACCACCGCGCGACACGACGACGACAAGGACTACAAC AAGGTGAAGCTGTACAAGCGGCGACGCCGGCGACGACGCACGACAGGACACAGGACCACGACAAGGACTACAAC (NBid is highlighted in yellow, PhoCl is highlighted in green and CBid is highlighted in blue) CGAGGGCAGCGGTGGCAGGGCAGGGGCAAGGGCAACGGCAACGGCACACGCCTCGTGAACGAGGCG CCCCGCGTGCCCTTCGCCTACGACGCACGGCAAGGGCAACGGCAACGGCAACGGCAACGGGCAACGGGCAACGGGCAACGGGCAACGGGCAACGGCGCAACGGCGCACCGCGCTTCCACACAGGCGCACCGCGCTTCACACAAGGAAGG		GAGAAGATGTACGAGCGCGACGGCGTGCTGAAGGGCGACGTGAAGATGAAGCTGCTGCTGAA
NBid-PhoCl1-CBid GCTGCCCGACTACCACTTCGTGGACCACCGCATCGAGATCCTGAGCCACGACAAGGACTACAAC AAGGTGAAGCTGTACGAGCAGCGCGTGGCCCGCAACTCCACCGCACGACAAGGACTACAACC AAGGTGAAGCTGTACGAGCAGCGCGAGGAGAGCCATTGAAGGCGAGGCGACGACGGCAAGCGCTACGACGGC highlighted in yellow, PhoCl is highlighted in green and CBid is highlighted in blue) TGACATGAAGAACAAGCTGCGCATGGGAGGGCGAACGGCAACGGCACGCCCTCGGAGGAGGCGACGCGCAGCGGCAAGCGCTTCGAGGGCAACCGGCCACGCCTTCGGAGGGGAGCGACGGCAAGCGCGTGGCACGCGCTTCGACGACGGCTACCGACGGCGACGGCAAGCGCGGCGACGGCGAGGGCGACCCGCCCTCCCACGGGCACCGGCCTTGGAGGGGGGGG		GGGCGGCGGCCACTATCGCTGCGACTACCGCACCACCTACAAGGTCAAGCAGAAGCCCGTAAA
NBid-PhoCl1-CBidAAGGTGAAGCTGTACGAGCACGCCGTGGCCCGCAACTCCACCGACAGCAGGAGAGCTGTAC(NBid is highlighted in yellow, PhoCl is highlighted in green and CBid is highlighted in blue)AAGGGTGGCAGCGGTGGCATGGTGAGCAAGGGCAACGTGAACGGCCACGCCTTCGTGATCGAGGG CGAGGGCAGCGGCAAGCCGCTTCGACGACGGCAACGGCCACGCCTTCGAAGGAGGGCG CCCCGCTGCCCTTCGCCACGACGCCTTCCAACGGCAACCGGCAACGGCGAGGGGCAA CCCCGCTGCCCTTCGACGAGGTCAGGAGGCAACCGACTGCTCCACTACGGCAACCGGCGTTCCACA AGTACCCACGGGGTACCCTTCCAACGGAGGAAACCGACTGCTCCACTAGGGCAAACGGACATAATAGGCAATATGGAAGAGAGAG		GCTGCCCGACTACCACTTCGTGGACCACCGCATCGAGATCCTGAGCCACGACAAGGACTACAAC
(NBid is highlighted in yellow, PhoCl is highlighted in green and CBid is highlighted in blue)AAGGGTGGCAGCGGTGGCATGGTGAGCAAGGGCGAGGAGACCATTACAAGCGTGATCAAGGC CGAGGGCAGCGGCAAGCGCGCATCGAGGGGCAACGTGAACGGCCACGCCTTCGTGATCGAGGGG CGAGGGCAGCGGCAAGCCCTTCGACGACGCCTTCCACACGGCCACGCCCTTCGAGGGGAAGCGGCG CCCCGCTGCCCTCGCCACGACGCCTCCACGACGCGCTCCACTACGGCAACCGCGGTTCACCA AGTACCCACGGGGTACCCTTCAAACCGATGGAAACCGATCATCACGGCAACCGCGGTTCACCA AGTACCCACGGGGTACCCTTCAACGGAGGACCATAATACGCAATATACGCAATATAGGCAACGGCCTGCAACGGCC GGTGATAGCAAGGAGGAGGACGACATAATACGCAATATACGCAATATAGCGCAACGCCTGCAACGGCT GGTGATAGCAAGGAGGAGGACGACATAATACGCAATATACGCAATATGGACACCCTTGCACACGGCC GGTGATAGCAAGGAGGAGAGAGAGAAGAAGAACAATGGTCGGCGACCGGCTTGACAACGCTTCCCA AGCCTTCCAAGGAGGAGAGAAGAAGAAAAACAATGGCTGGC	NBid-PhoCl1-CBid	AAGGTGAAGCTGTACGAGCACGCCGTGGCCCGCAACTCCACCGACAGCATGGACGAGCTGTAC
highlighted in green and CBid is highlighted in blue)TGACATGAAGAACAAGCTGCGCATGGAGGGCAACGTGAACGGCCACGCCTTCGTGATCGAGGG CGAGGGCAGCGGCAGCGGCAAGCCTTCGAGGGCAACGGCCACGGCCTTCCACGACGACGGCCAAGGAGGGGCG CCCCGCTGCCCTTCGCCTACGACATCCTGACCACGACGACTGCACGGCGAACGGCGGCGAAT TGAAGCAGATAGTAGCAGGAGGACGCGCCCCCCAGGCGACCGCGCTTCAACAGGTTGGGCCGCGACA AGTACCCACGGGGTGACAGGAGGACATAATACGCAATATAGCTCAACGGACTGGCAACGGCGAAG GGTGATAGCATGGACGGCACGCGCTCTATCCCCCCAGGTTTGGTTAACGGATTGGCCCGCACAGGTCC GGTGATAGCATGGAAGGAGAGAAGAAGATAGAAATCGAGAACCGGCCGCGCTTGAACAGCTTCTCA AGCCTATCCCAGAGAGTGAAGAAGAAGAAGAAAAACAATGCTGGCGCACCGCGCTTGAACAGCTTCTTCA AGCCTATCCCAGAGAGTGAAGAAGAAGAAGAAAAAAAAAA	(NBid is highlighted in yellow, PhoCl is	AAGGGTGGCAGCGGTGGCATGGTGAGCAAGGGCGAGGAGACCATTACAAGCGTGATCAAGCC
highlighted in blue) CGAGGGCAGCGGCAAGCCCTTCGAGGGCATCCAGACGATTGATT	highlighted in green and CBid is	TGACATGAAGAACAAGCTGCGCATGGAGGGCAACGTGAACGGCCACGCCTTCGTGATCGAGGG
CCCCGCTGCCCTTCGCCTACGACATCCTGACCACCGCCTTCCACTACGGCAACCGCGTGTTCACCA AGTACCCACGGGGTACCCTTCAAACCGATGGAAACCGATCATCTCATTCAAGGTTGGGGCGAAT TGAAGCAGGATAGTGAGAGTCAGGAGGACATAATACGCAATCTGCGACACCTTGCACAGGTC GGTGATAGCATGGACCGCTCTATCCCCCCAGGTTTGGTTAACGGATTGGCCCTGCAACTGCGAA ACACTTCAAGGAGTGAAGAAGATAGAAATCGAGACCTGGCGACCGCGCTTGAACAGCTTCTCA AGCCTATCCCAGAGATATGGAGAAGAAAAAACAATGCTCGTGCTGCACTGCTGCTGGCAAAG AAAGTAGCCTCTAACACACACCATCCCTCTTGAGAGACGTCTTCCATACCACGGTAAATTTCATAAAC CAGAACCTTAGGACGATATGGCGAAGGACAAAAACAATGCTCGTGCTGGCACTGCTGCTGCCACTG GCCCCTCAATGCGTGGTGCTCAGGTTGACAAGGTATGACTGAGTTTCATCAACCCGT GCCCCTCAATGCGTGGTGCTCAGGTTCATCTCTCCCGAGACGAATGCATAACGAACTTGCTCGTTTT CGGGCTTTCTGCAATCGCGCAGCGCAAGGCTACGAATGCATAACGAACTTGCTCGTTTT CGGCTTTCTGCAATCCGCACGCGCAAGGCTACGATGAAGAGAACTTGAACGAAC	highlighted in blue)	CGAGGGCAGCGGCAAGCCCTTCGAGGGCATCCAGACGATTGATT
AGTACCCACGGGGTACCCTTCAAACCGATGGAAACCGATCATCTCATTCAAGGTTGGGGCGAAT TGAAGCAGATAGTGAGAGTGAGAGAGCAGGAGACATAATACGCAATATAGCTCGACACCTTGCACAGGTC GGTGATAGCATGGACCGCTCTATCCCCCCAGGTTTGGTTAACGGATTGGCCCTGCAACTGCGAA ACACTTCAAGGAGTGAAGAAGATAGAAATCGAGACCTGGCGACCGCGCTTGAACAGCTTCTTCA AGCCTATCCCAGAGATATGGAGAAGGAAAAAACAATGCTCGTGCTCGCACTGCTGCGCAAAG AAAGTAGCCTCTAACACACCATCCCTCTTGAGAGACGTCTTCCATACCACGGTAAATTTCATAAAC CAGAACCTTAGGACGTATGTGCGGAGTCTTGCTCGCAATGGTATGACTGAGTTTCATAAAC CAGAACCTTAGGACGTATGTGCGGAGTCTTGCTCGCAATGGTATGACTGAGTTTCATAAAC CAGAACCTTAGGACGTATGTGCGGAGTCTTGCTCGCAATGGTATGACTGAGTTTCAAACCGT GCCCCTCAATGCGTGGTTGCCAGGAGTCTTGCTCGCAATGCATGACGAACTTGCTCGTTTT CGGCTTTCTGCAATCGCGCAACGGCTACGATAGAACTTGACGCTCTTGGACATGAAC TCCCCAGTACTCGCCTCCACAGTGGGAGGCTACGATGAGGAACTTGACGCTCTGGACATGAAC TCCCCAGTACTCGCCCCCCCCCCCCCCCCCCCCCCCCCC		
TGAAGCAGATAGTGAGAGGTCAGGAGGACATAATACGCAATATAGCTCGACACCTTGCACAGGTC GGTGATAGCATGGACCGCTCTATCCCCCCAGGTTTGGTTAACGGATTGGCCCTGCAACTGCGAA ACACTTCAAGGAGTGAAGAAGATAGAAATCGAGACCTGGCGACCGCGCTTGAACAGCTTCTTCA AGCCTATCCCAGAGATATGGAGAAGGAAAAAACAATGCTCGTGCTCGCACTGCTGGCAAAG AAAGTAGCCTCTAACACACCATCCCTCTTGAGAGACGTCTTCCATACCACGGTAAATTTCATAAAC CAGAACCTTAGGACGTATGTGCGGAGTCTTGCTCGCAATGGTATGACTGAGTTTCATAAAC CAGAACCTTAGGACGTATGTGCGGAGTCTTGCTCGCAATGGTATGACTGAGTTTCATAAAC CCCCCCAATGCGTGGTTGCTCAGGTTGAACAAGTAA ATGGATTGTGAGGTCAATAACGGTTCATCTCTCCGAGACGAATGCATAACGAACTTGCTCGTTTT CGGCTTTCTGCAATCGCGCAACGGCAAGGCTACGATGAACTTGACGCTCTTGGACATGAAC TCCCAGTACTCGCCCACAGTGGGAGGCCACCGTTACGAGGAGCCCCGCTGCCCTTGGACATGAACTGCGC ATGGAATGCGCGAGGGAGACCATTATGAGCGTGATCAAGCCTGACATGAAGATCAAGCTGCGC GTGAAGGGCAACGTGAACGGCCACGCCCTCGTGATCAAGCCTGACATGAAGATCAAGCTGCGC ATGGAAGGGCAACGTGAACGGCCACGCCCTCGTGATCAAGCCTGACATGAAGATCAAGCTGCC GAGGGCATCCAAGCGAACGAGACCATTATGAGCGTGAACGAGGCCACGCCAAGCCCTTC GAGGGCATCCAAGCGTAACGGCCACGCCACGCCTTCGTGATCGAGGGCGACGCCAAGCCCTTC GAGGGCATCCCAGCGCTTCCCACAGGAGGAGGCCCCCCCTTCGCCTTCCCCCTCC		AGTACCCACGGGGTACCCTTCAAACCGATGGAAACCGATCATCTCATTCAAGGTTGGGGCGAAT
GGTGATAGCATGGACCGCTCTATCCCCCCAGGTTTGGTTAACGGATTGGCCCTGCAACTGCGAA ACACTTCAAGGAGTGAAGAAGATAGAAATCGAGACCTGGCGACCGCGCTTGAACAGCTTCTTCA AGCCTATCCCAGAGATATGGAGAAGAAGAAAAACAATGCTCGTGCTCGCACTGCTGCTGGCAAAG AAAGTAGCCTCTAACACACCATCCCTCTTGAGAGACGTCTTCCATACCACGGTAAATTTCATAAAC CAGAACCTTAGGACGTATGTGCGGAGTCTTGCTCGCAATGGTATGACTGAGTTTCTTCAAACCG CAGAACCTTAGGACGTATGTGCGGAGTCTTGCTCGCAATGGTATGACTGAGTTTCTTCAACCCGT GCCCCTCAATGCGTGGTTGCTCAGGTTGAACAAGTAA ATGGATTGTGAGGTCAATAACGGTTCATCTCTCCGAGACGAATGCATAACGAACTTGCTCGTTTT CGGCTTTCTGCAATCCTGCAGCGATAATTCTTTCAGAAGAAACTTGACGCTCTTGGACATGAAC TCCCAGTACTCGCTCCACAGTGGGAGACCATTATGAGCGTGAACATGAAGATCAAGCTGGCC GTGAAGGGCAACGGCAACGGCAACGGCCAAGCCCTTC GAGGGCAACCGTGAACGGCCACGCCTTCGTGATCGAGGGCGAGGGCAAGCGGCAAGCCCTTC GAGGGCATCCAAGCGGAACAGGCCACGCCTTCGTGATCGAGGGCGAGGGCAAGCCGCCAAGCCCTTC GAGGGCATCCAAGCGTGAACGGCCACGCCATGAAGAGAGGGCGCCCGCGCTGCCCTTCGCCTACGAC		TGAAGCAGATAGTGAGAGTCAGGAGGACATAATACGCAATATAGCTCGACACCTTGCACAGGTC
ACACTTCAAGGAGTGAAGAAGATAGAAATCGAGACCTGGCGACCGCGCTTGAACAGCTTCTTCA AGCCTATCCCAGAGATATGGAGAAGGAAGAAAAACAATGCTCGTGCTGCACGCGCGAAAG AAAGTAGCCTCTAACACACCATCCCTCTTGAGAGACGTCTTCCATACCACGGTAAATTTCATAAAC CAGAACCTTAGGACGTATGTGCGGAGTCTTGCTCGCAATGGTATGACTGAGTTTCTTCAACCCGT GCCCCTCAATGCGTGGTTGCTCAGGTTGAACAAGTAA ATGGATTGTGAGGTCAATAACGGTTCATCTCTCCGAGACGAATGCATAACGAACTTGCTCGTTTT CGGCTTTCTGCAATCCTGCAGCGATAATTCTTTCAGAAGAACTTGACGCTCTTGGACATGAAC TCCCAGTACTCGCTCCACAGTGGGAAGGCTACGATGAGGGACCC GTGAGGCAACGGCGAGGAGCACTTATGAGCGTGATCAAGCCTGACATGAAGCTGACG ATGGAGGGCAACGTGAACGGCCACGCCTTCGTGACCAGGGCGAGGGCAGCCGCCAAGCCCTTC GAGGGCATCCAGCGGCAGGACACTTGGAGGGGAGG		GGTGATAGCATGGACCGCTCTATCCCCCCAGGTTTGGTTAACGGATTGGCCCTGCAACTGCGAA
AGCCTATCCCAGAGATATGGAGAAGGAAAAAACAATGCTCGTGCTCGCACTGCTGCTGGCAAAG AAAGTAGCCTCTAACACACCATCCCTCTTGAGAGACGTCTTCCATACCACGGTAAATTTCATAAAC CAGAACCTTAGGACGTATGTGCGGAGTCTTGCTCGCAATGGTATGACTGAGTTTCTTCAAACCGT GCCCCTCAATGCGTGGTTGCTCAGGTTGAACAAGTAA ATGGATTGTGAGGTCAATAACGGTTCATCTCTCCCGAGACGAATGCATAACGAACTTGCTCGTTTT CGGCTTTCTGCAATCCTGCAGCGATAATTCTTTCAGAAGAAGATGCATAACGAACTTGGCCGTGTT CCCAGTACTCGCACCGCGAGAGGCTACGATGAGGGAACCTGGACGTCAGACATGAAC TCCCAGTACTCGCTCCACAGTGGGAAGGCTACGATGAAGAGCCTGACATGAAGATCAAGCTGGC GTGAGGCAACGGCGAGGAGCCATTATGAGCGTGATCAAGCCTGACATGAAGATCAAGCTGCGC ATGGAAGGGCAACGTGAACGGCCACGCCTTCGTGATCGAGGGCGAGGGCAGCGGCAAGCCCTTC GAGGGCATCCAAGCGCGCAGGAGAGGTTGAGGGGGCGCCCCGCTGCCCTTCGCCTACGAC		ACACTTCAAGGAGTGAAGAAGATAGAAATCGAGACCTGGCGACCGCGCTTGAACAGCTTCTTCA
AAAGTAGCCTCTAACACACCATCCCTCTTGAGAGACGTCTTCCATACCACGGTAAATTTCATAAAC CAGAACCTTAGGACGTATGTGCGGAGTCTTGCTCGCAATGGTATGACTGAGTTTCTTCAAACCGT GCCCCTCAATGCGTGGTTGCTCAGGTTGAACAAGTAA ATGGATTGTGAGGTCAATAACGGTTCATCTCTCCGAGACGAATGCATAACGAACTTGCTCGTTTT CGGCTTTCTGCAATCCTGCAGCGATAATTCTTTCAGAAGAGAACTTGACGTCTTGGACATGAAC TCCCAGTACTCGCTCCACAGTGGGAAGGCTACGATGAGGGATCC GTGAGGCAAGGGCGAGGAGACCATTATGAGCGTGATCAAGCTGACATGAAGATCAAGCTGCGC ATGGAGGGCAACGTGAACGGCCACGCCTTCGTGATCAGGGCGAGGGCAGCGGCAAGCCTTC GAGGGCATCCAGACGACGACGAGTTAGTTGGAGGGGGAGGCGCCCCGCTGCCCTCCGCCTACGAC		AGCCTATCCCAGAGATATGGAGAAGGAAAAAAAAAAGAATGCTCGTGCTCGCACTGCTGGCAAAG
CAGAACCTTAGGACGTATGTGCGGAGTCTTGCTCGCAATGGTATGACTGAGTTTCTTCAACCCGT GCCCCTCAATGCGTGGTTGCTCAGGTTGAACAAGTAA ATGGATTGTGAGGTCAATAACGGTTCATCTCTCCGAGACGAATGCATAACGAACTTGCTCGTTTT CGGCTTTCTGCAATCCTGCAGCGATAATTCTTTCAGAAGAGAACTTGACGTCTTGGACATGAAC TCCCAGTACTCGCTCCACAGTGGGAAGGCTACGATGAGGGATCC GTGAGCAAGGGCGAGGAGACCATTATGAGCGTGATCAAGCTGAACATCAAGCTGCGC ATGGAGGGCAACGTGAACGGCCACGCCTTCGTGATCAAGGCGGCGAGGGCAGCGGCAAGCCCTTC GAGGGCATCCAGACGACGACGAGTGAATTGGAGGGGGCGCCCCCGCTGCCTTCGCCTACGAC		AAAGTAGCCTCTAACACCACCCTCTTGAGAGACGTCTTCCATACCACGGTAAATTTCATAAAC
GCCCCTCAATGCGTGGTTGCTCAGGTTGAACAAGTAA ATGGATTGTGAGGTCAATAACGGTTCATCTCTCCCGAGACGAATGCATAACGAACTTGCTCGTTTT CGGCTTTCTGCAATCCTGCAGCGATAATTCTTTCAGAAGAGAACTTGACGTCTTGGACATGAAC TCCCAGTACTCGCTCCACAGTGGGAAGGCTACGATGAGGGATCC GTGAGCAAGGGCGACGAGGAGCACTATTATGAGCGTGATCAAGCTGACGGAAGGCAGCGCCA ATGGAAGGCCACGGAGAGCCATTATGAGCGTGATCAAGCCTGACAGAGAGCAAGCCTTC GAGGGCAACCGTGAACGGCCACGCCTTCGTGATCGAGGGCGCCCCCGCTGCCCTTCGCCTACGAC ATGCACCCCCCTTCCCACACGCCTTCCCCACGCGCAGGCCCCCCCC		CAGAACCTTAGGACGTATGTGCGGAGTCTTGCTCGCAATGGTATGACTGAGTTTCTTCAACCCGT
ATGGATTGTGAGGTCAATAACGGTTCATCTCTCCGAGACGAATGCATAACGAACTTGCTCGTTTT CGGCTTTCTGCAATCCTGCAGCGATAATTCTTTCAGAAGAGAACTTGACGCTCTTGGACATGAAC TCCCAGTACTCGCTCCACAGTGGGAAGGCTACGATGAGGGATCC GTGAGGAGGGCAAGGGGGGGGAGACCATTATGAGCGTGATCAAGCCTGACATGAAGATCAAGCTGCGC ATGGAGGGCAACGTGAACGGCCACGCCTTCGTGATCGAGGGCGAGGGCGACGGCAAGCCCTTC GAGGGCATCCAGACGATTGACTTAGGAGGGGGGCGCCCCGCTGCCCTCGCCTACGAC		GCCCCTCAATGCGTGGTTGCTCAGGTTGAACAAGTAA
CGGCTTTCTGCAATCCTGCAGCGATAATTCTTTCAGAAGAGAACTTGACGCTCTTGGACATGAAC TCCCAGTACTCGCTCCACAGTGGGAAGGCTACGATGAGGGATCC GTGAGCAAGGGCGAGGAGACCATTATGAGCGTGATCAAGCCTGACATGAAGATCAAGCTGCGC ATGGAGGGCAACGTGAACGGCCACGCCTTCGTGATCGAGGGCGACGGCGAGGGCAAGCCCTTC GAGGGCATCCAGACGATTGATTTGGAGGTGAAGGAGGGCGCCCCGCTGCCCTTCGCCTACGAC		ATGGATTGTGAGGTCAATAACGGTTCATCTCTCCGAGACGAATGCATAACGAACTTGCTCGTTTT
TCCCAGTACTCGCTCCACAGTGGGAAGGCTACGATGAGGGATCC GTGAGCAAGGGCGAGGAGACCATTATGAGCGTGATCAAGCCTGAAGATCAAGCTGCGC ATGGAGGGCAACGTGAACGGCCACGCCTTCGTGATCGAGGGCGAGGGCAGCGGCAAGCCCTTC GAGGGCATCCAGACGATTGGATTTGGAGGTGAAGGAGGGCGCCCCGCTGCCCTCGCCTACGAC		CGGCTTTCTGCAATCCTGCAGCGATAATTCTTTCAGAAGAGAACTTGACGCTCTTGGACATGAAC
GTGAGCAAGGGCGAGGAGACCATTATGAGCGTGATCAAGCCTGACATGAAGATCAAGCTGCGC ATGGAGGGCAACGTGAACGGCCACGCCTTCGTGATCGAGGGCGAGGGCAGCGGCAAGCCCTTC GAGGGCATCCAGACGATTGATTTGGAGGTGAAGGAGGGCGCCCCGCTGCCCTCGCCTACGAC		TCCCAGTACTCGCTCCACAGTGGGAAGGCTACGATGAGGGATCC
ATGGAGGGCAACGTGAACGGCCACGCCTTCGTGATCGAGGGCGAGGGCAGCGGCAAGCCCTTC GAGGGCATCCAGACGATTGATTTGGAGGTGAAGGAGGGCGCCCCGCTGCCCTCGCCTACGAC		GTGAGCAAGGGCGAGGAGACCATTATGAGCGTGATCAAGCCTGACATGAAGATCAAGCTGCGC
GAGGGCATCCAGACGATTGATTTGGAGGTGAAGGAGGCGCCCCGCTGCCCTTCGCCTACGAC		ATGGAGGGCAACGTGAACGGCCACGCCTTCGTGATCGAGGGCGAGGGCAGCGGCAAGCCCTTC
		GAGGGCATCCAGACGATTGATTTGGAGGTGAAGGAGGGCGCCCCGCTGCCCTTCGCCTACGAC
		ATCCTGACCACCGCCTTCCACTACGGCAACCGCGTGTTCACCAAGTACCCCGAGGACATCCCTGA
CTACTTCAAGCAGAGCTTCCCCGAGGGCTACAGCTGGGAGCGCAGCATGACCTACGAGGACGG		CTACTTCAAGCAGAGCTTCCCCGAGGGCTACAGCTGGGAGCGCAGCATGACCTACGAGGACGG
CGGCATCTGCATCGCCACCAACGACATCACAATGGAGGAGGACAGCTTCATCAACAAGATCCAC		CGGCATCTGCATCGCCACCAACGACATCACAATGGAGGAGGACAGCTTCATCAACAAGATCCAC
NBid-mMaple-CBid TTCAAGGGCACGAACTTCCCCCCCAACGGCCCCGTGATGCAGAAGAGGACCGTGGGGCTGGGAG	NBid-mMaple-CBid	TTCAAGGGCACGAACTTCCCCCCCAACGGCCCCGTGATGCAGAAGAGGACCGTGGGGCTGGGAG
(NBid is highlighted in yellow, mMaple GTCAGCACCGAGAAGATGTACGTGCGCGACGGCGTGCTGAAGGGCGACGTGAAGATGAAGCT	(NBid is highlighted in yellow, mMaple	GTCAGCACCGAGAAGATGTACGTGCGCGACGGCGTGCTGAAGGGCGACGTGAAGATGAAGCT

	GCTGCTGAAGGGCGGCAGCCACTATCGCTGCGACTTCCGCACCACCTACAAGGTCAAGCAGAAG
	GCCGTAAAGCTGCCCGACTACCACTTCGTGGACCACCGCATCGAGATCCTGAGCCACGACAAGG
	ACTACAACAAGGTGAAGCTGTACGAGCACGCCGTGGCCCGCAACTCCACCGACAGCATGGACG
	AGCTGTACAAGGGTACCCTTCAAACCGATGGAAACCGATCATCTCATTCAAGGTTGGGGCGAAT
	TGAAGCAGATAGTGAGAGTCAGGAGGACATAATACGCAATATAGCTCGACACCTTGCACAGGTC
	GGTGATAGCATGGACCGCTCTATCCCCCCAGGTTTGGTTAACGGATTGGCCCTGCAACTGCGAA
	GCAGAGCTTCCCCGAGGGCTACAGCTGGGAGCGCAGCATGACCTACGAGGACGGCGGCATCTG
	CATCGCCACCAACGACATCACAATGGAGGGGGGACAGCTTCATCAACAAGATCCACTTCCAGGGC
	ACGAACTTCCCCCCCAACGGCCCCGTGATGCAGAAGAGGACCGTGGGGCTGGGAGGCCAGCACC
	GAGAAGATGTACGAGCGCGACGGCGTGCTGAAGGGCGACGTGAAGATGAAGCTGCTGCTGAA
	GGGCGGCGGCCACTATCGCGGCGACTACCGCACCACCTACAAGGTCAAGCAGAAGCCCGTAAA
	GCTGCCCGACTGCCACTTCGTGGACCACCGCATCGAGATCCTGAGCCACGACAAGGACTACAAC
NBid-PhoCl2c-CBid	AAGGTGAAGCTGTACGAGCACGCCGTGGCCAAGACTTCCACCGACAGCATGGACGAGCTGTAC
(NBid is highlighted in yellow, PhoCl2c	AAGGGTGGCAGCGGTGGCATGGTGAGCAAGGGCGAGGAGACCATTACAAGCGTGATCAAGCC
is highlighted in green and CBid is	TGACATGAAGAACAAGCTGCGCATGGAGGGCAACGTGAACGGCCACGCCTTCGTGATCGAGGG
highlighted in blue)	CGAGGGCAGCGGCAAGCCCTTCGAGGGCATCCAGACGATTGATT
	CCCCGCTGCCCTTCGCCTACGACATCCTGACCACCGCCTTCCACTACGGCAACCGCGTGTTCACCA
	AGTACCCACGGGGTACCCTTCAAACCGATGGAAACCGATCATCTCATTCAAGGTTGGGGCGAAT
	TGAAGCAGATAGTGAGAGTCAGGAGGACATAATACGCAATATAGCTCGACACCTTGCACAGGTC
	GGTGATAGCATGGACCGCTCTATCCCCCCAGGTTTGGTTAACGGATTGGCCCTGCAACTGCGAA
	ACACTTCAAGGAGTGAAGAAGATAGAAATCGAGACCTGGCGACCGCGCTTGAACAGCTTCTTCA
	AGCCTATCCCAGAGATATGGAGAAGGAAAAAAAAAACAATGCTCGTGCTCGCACTGCTGGCAAAG
	AAAGTAGCCTCTAACACCATCCCTCTTGAGAGACGTCTTCCATACCACGGTAAATTTCATAAAC
	CAGAACCTTAGGACGTATGTGCGGAGTCTTGCTCGCAATGGTATGACTGAGTTTCTTCAACCCGT
	GCCCCTCAATGCGTGGTTGCTCAGGTTGAACAAGTAA
	ATGAACCTGGTGGACCTGCAGAAGAAGCTGGAGGAGCTGGAGCTGGACGAGCAGCAGGGATC
NES-DEVD-mCardinal-NLS	CGCCTCCGGCGATGAGGTGGATGGAGCCGTGAGCAAGGGCGAGGAGCTGATCAAGGAGAACA
	TGCACATGAAGCTGTACATGGAAGGCACCGTGAACAACCACCACTTCAAGTGCACCACCGAAGG
	GGAGGGCAAGCCCTACGAGGGCACCCAGACCCAGAGGATTAAGGTGGTGGAGGGAG
	TGCCGTTCGCATTCGACATCCTGGCCACCTGCTTTATGTACGGGAGCAAGACCTTCATCAACCAC
	ACCCAGGGCATCCCCGATTTCTTTAAGCAGTCCTTCCCTGAGGGCTTCACATGGGAGAGAGTCAC
(NES is highlighted in vellow, DEVD is	CACATACGAAGACGGGGGGGGGGGGGGTGCTTACCGTTACCCAGGACAGCCAGC
highlighted in green, mCardinal is	ATCTACAACGTCAAGGTCAGAGGGGTGAACTTCCCATCCAACGGCCCTGTGATGCAGAAGAAAA
highlighted in magenta and 3x NI S is	
highlighted in cyan)	GCGACATGGCCCTGAAGCTCGTGGGCGGGGGGGCCACCTGCACCTGAAGACCACATACA

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/mm²) 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.