Supporting Information for

"Creating Red Light-Switchable Protein Dimerization Systems as Genetically Encoded Actuators with High Specificity"

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SUPPLEMENTARY FIGURES

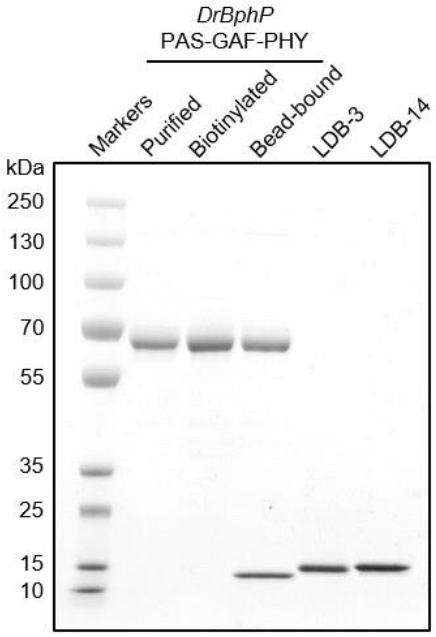

Figure S1. SDS-PAGE analysis of purified *Dr*BphP and nanobodies. Proteins were purified by nickel affinity and SEC chromatography. To examine *in vitro* biotinylation efficiency by BirA, the biotinylated protein was bound to streptavidin beads (Dynabeads M-280 Streptavidin, Thermo Fisher Scientific) and the bound protein (lane 4) was compared with the input protein (lane 3). The lower band in the lane 4 was streptavidin released from the beads when boiling the sample in an SDS loading buffer.

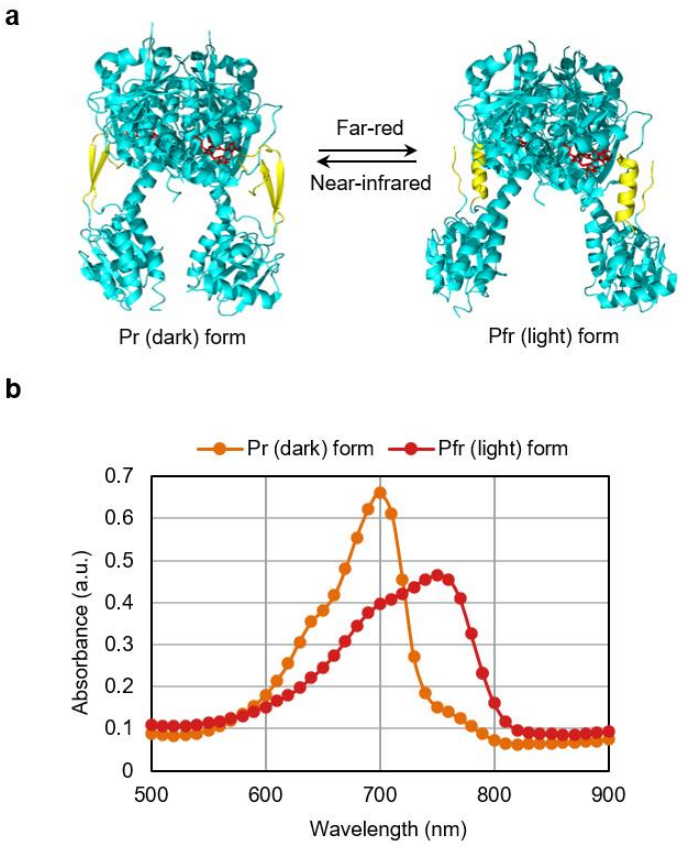

Figure S2. Structures and spectra of the dark and light forms of *Dr*BphP. (a) Structures of *Dr*BphP dark and light forms previously reported[1] showing the biliverdin chromophore (red sticks) bound to a tri-domain photosensory module (cyan cartoon) and conformational changes of a tongue motif (yellow) interacting with the biliverdin binding pocket. (b) Absorption spectra of the dark and light states of the biotinylated *Dr*BphP after the 775-nm (0.3 mW/cm^2, 10 min) and 654-nm (0.5 mW/cm^2, 2 min) illuminations, respectively.

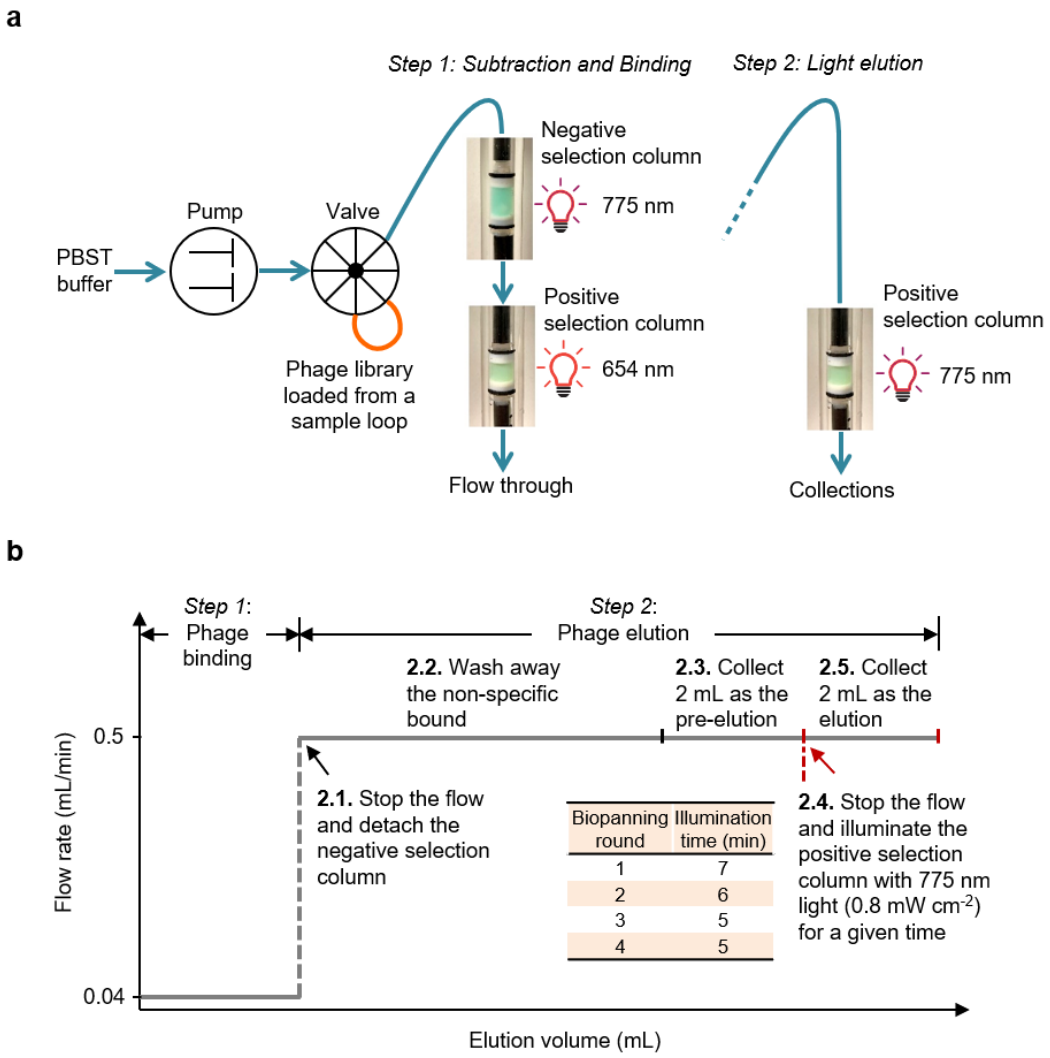

Figure S3. Column chromatography-based phage display selection. (a) Two-step biopanning FPLC setup. (b) Flow rate and illumination time setup. In the Step 1, 2 mL phage-displayed nanobodies were loaded to two connected transparent glass columns (HR 5/5, GE Healthcare) packed with 0.4 and 0.2 mL streptavidin agarose resin (Pierce). Before divided into the two columns, the resin was incubated with 1.2 mL 20 μM biotinylated *Dr*BphP in the dark for 30 min. Next, *Dr*BphP in the first (negative selection) and second (positive selection) columns were converted to the dark and light forms by the 775-nm (0.3 mW/cm^2, 10 min) and 654-nm (0.5 mW/cm^2, 2 min) illumination, respectively. After the phage injection, the flow rate was set to be 0.04 mL/min and then decreased to 0 when the UV 280 nm baseline was stable (i.e., non-bound phages were washed out). In the Step 2, the first column was removed, and phages were eluted from the second column by the 775-nm (0.8 mW/cm^2) illumination for a given time. A pre-elution fraction was collected as a control for the phage count comparison with a light elution fraction to estimate the ratio of phages specifically eluted by the light to those non-specifically eluted (refer to Table S2).

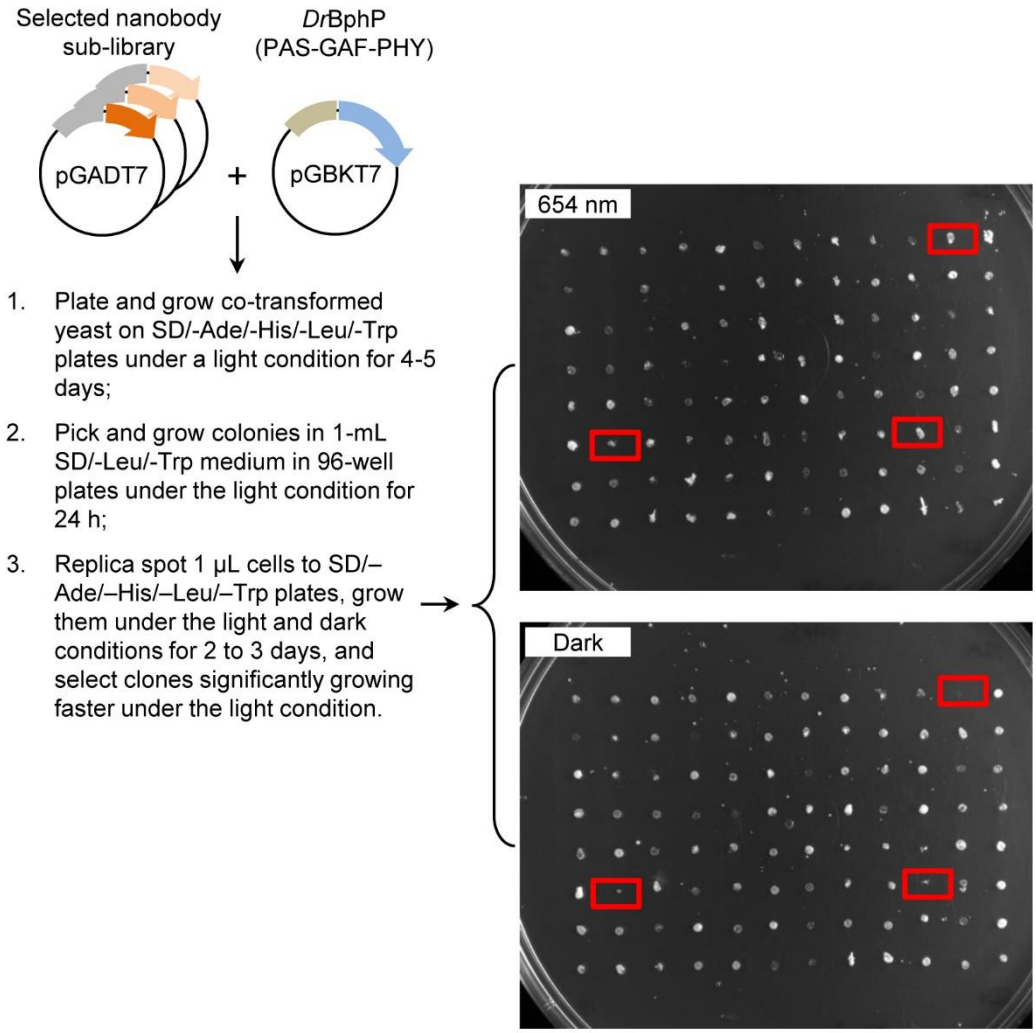

Figure S4. Yeast two-hybrid screening. Phage display-enriched nanobodies (orange), as preys, were subcloned to pGADT7 encoding a GAL4 AD domain (grey). *Dr*BphP (blue), as a bait, was inserted to pGBKT7 encoding a GAL4 DNA-binding domain (green). The right panel shows a representative result of two replica spotted plates incubated in the dark or under the 654-nm illumination.

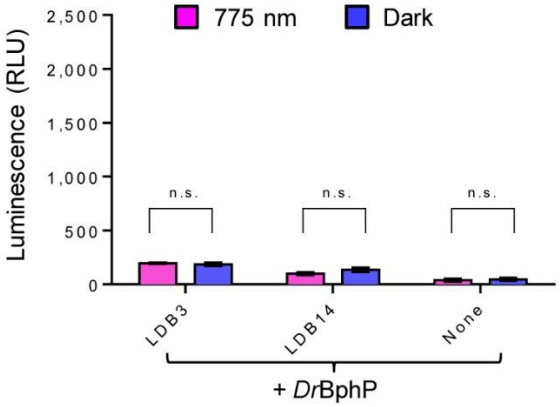

Figure S5. Comparison of basal luciferase expression of LDB-3 and LDB-14 LID systems under 775 nm illumination and in the dark. HEK293T cells were transiently co-transfected with the bait, prey and GAL4UAS-luciferase reporter plasmids (~0.25 μg each) in a 0.5 mL culture. Cells were cultured under the 775 nm illumination (0.2 mW/cm^2) or in the dark for 24 hours before measuring luciferase levels. None, the negative control transfected with only the bait and the luciferase reporter plasmids. Data represent mean values of 3 measurements; error bars, the standard error of the mean.

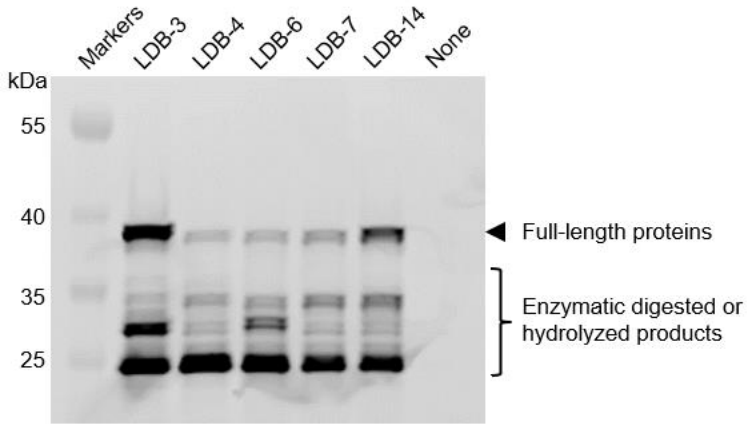

Figure S6. In-gel detection of fluorescently labelled nanobodies expressed in HEK293T cells. Cells were transiently transfected with plasmids encoding SNAP-tagged nanobody fusions. Proteins in supernatants of sonication-lysed cells were specifically labeled with SNAP-Surface 649 and analyzed by SDS-PAGE and fluorescence imaging with an Odyssey CLx imaging system. Degraded proteins might also be caused by protein hydrolysis during sample boiling (10 min at 95˚C) in the SDS sample loading buffer.

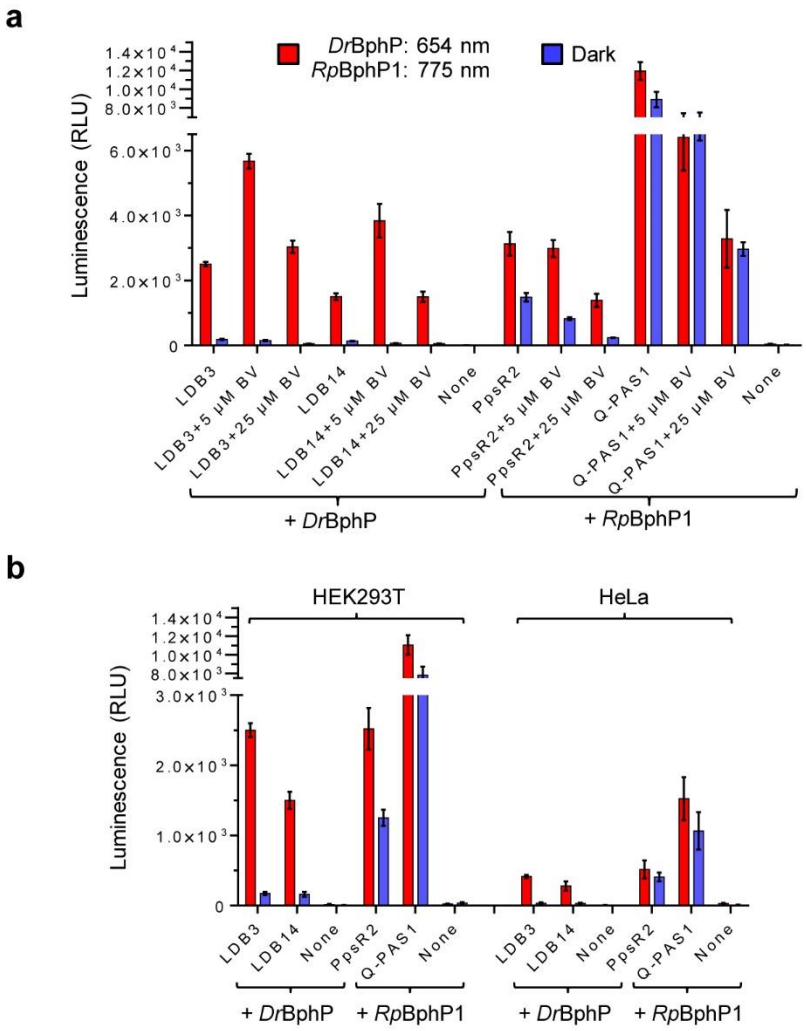

Figure S7. Specificity comparison of *Dr*BphP (or nanobody) and *Rp*BphP1-based LIDs in mammalian cells. The specificity was compared with (a) extra supply of biliverdin and (b) decreased cellular levels of LID proteins. Either HEK293T or HeLa cells were transiently co-transfected with the bait, prey and GAL4UAS-luciferase reporter plasmids (~0.25 μg each) in a 0.5 mL culture. Cells were maintained under the illumination either at 654-nm (0.2 mW/cm^2) or 775-nm (0.2 mW/cm^2), or in the dark for 24 hours before measuring luciferase levels. Luminescence intensities of HEK293T and HeLa cells were normalized by cell counts. BV, biliverdin; None, the negative control transfected with only the bait and the luciferase reporter plasmids. Data represent mean values of 3 measurements; error bars, the standard error of the mean.

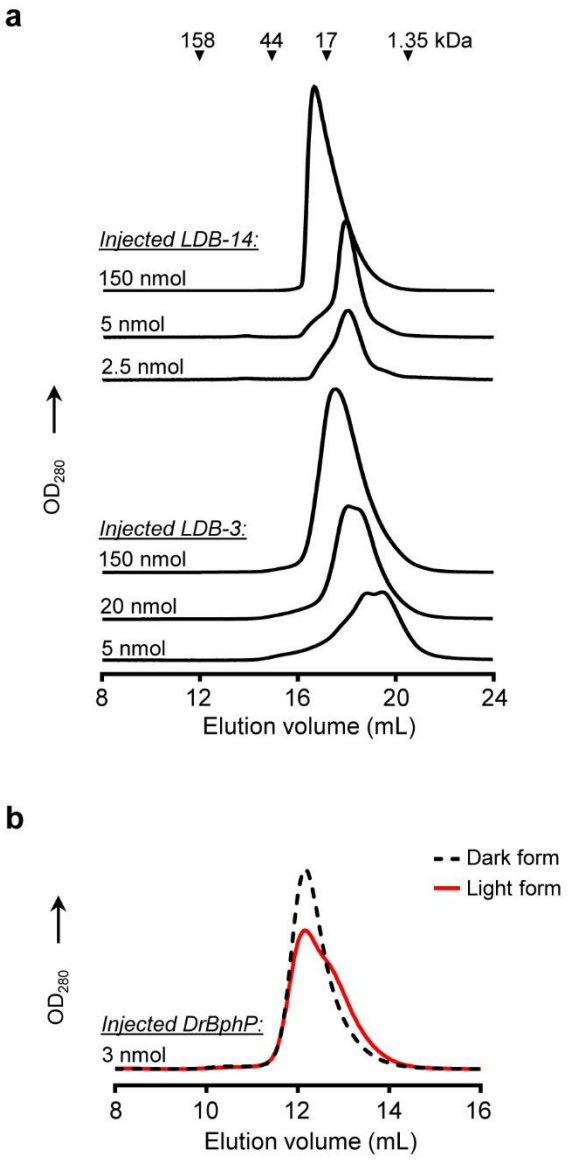

Figure S8. Analytical SEC analyses of (a) nanobodies at different concentrations and (b) *Dr*BphP in the light and dark forms. Proteins were loaded to a Superdex 200 Increase 10/300 GL column pre-equilibrated with 1× PBS and eluted at a flow rate of 0.75 mL/min at 4˚C.

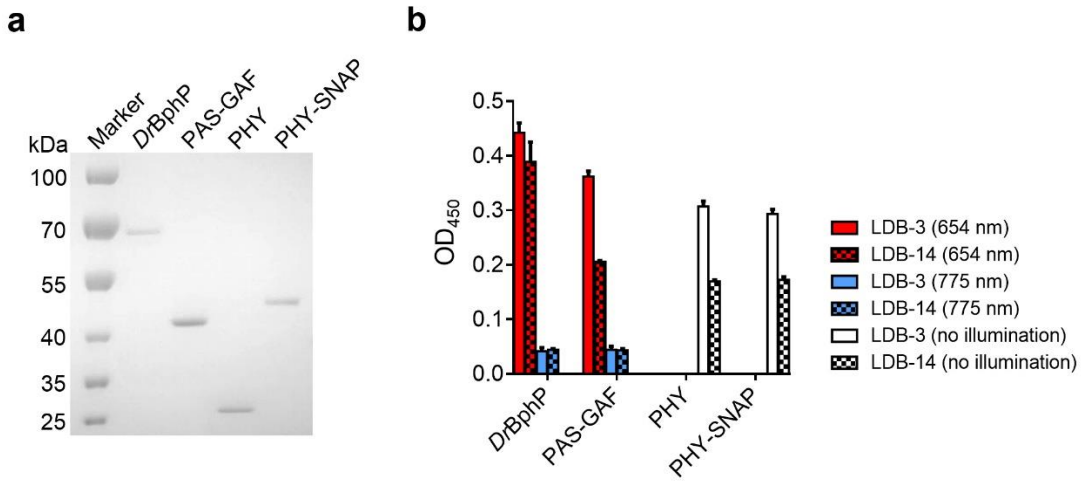

Figure S9. Analysis of nanobody binding sites on *Dr*BphP. (a) SDS-PAGE analysis of biotinylated, truncated *Dr*BphP proteins. The tridomain *Dr*BphP (PAS-GAF-PHY), didomain (PAS-GAF), and monodomain (PHY) proteins bearing a C-terminal AviTag and HisTag were *E. coli* expressed, purified and biotinylated as targets for ELISA. Due to relatively low expression solubility, PHY was also prepared as a SNAP tag fusion. (b) Detection of nanobody binding to truncated *Dr*BphP proteins by ELISA. Phage-displayed nanobodies were bound to *Dr*BphP and PAS-GAF in microtiter plates, which were illuminated with 654-nm (0.3 mW/cm^2) or 775-nm (0.2 mW/cm^2) lights during binding and wash steps. PHY and PHY-SNAP were assayed under normal lighting condition. Data represent mean values of 6 measurements; error bars, standard deviation.

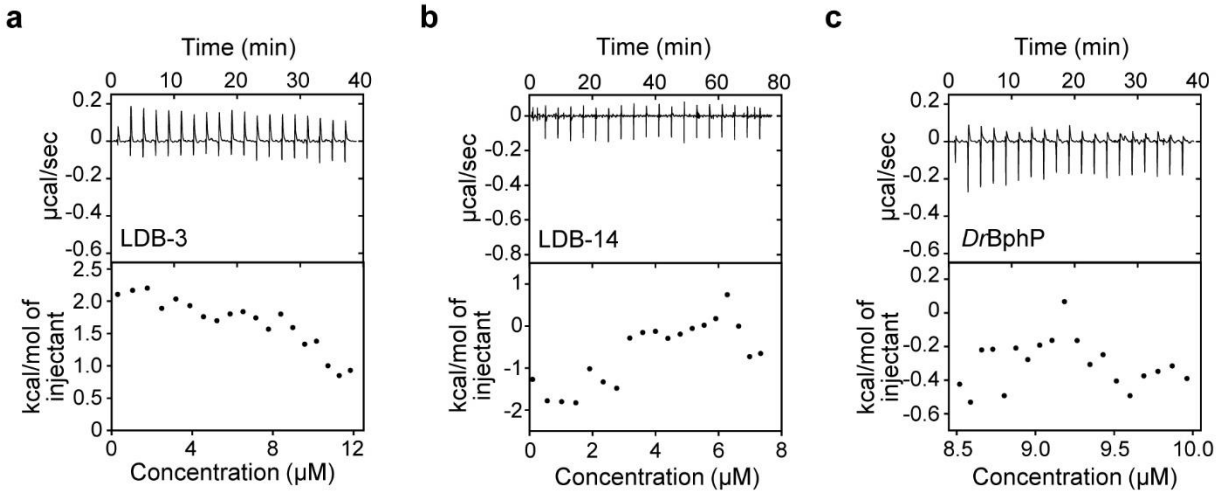

Figure S10. ITC analysis of the titration of (a) 80 μM LDB-3 or (b) 50 μM LDB-14 into 1× PBS buffer, and (c) the titration of 1× PBS buffer into 10 μM *Dr*BphP. The raw data (top) and the integration of heats (bottom) for each titration are shown.

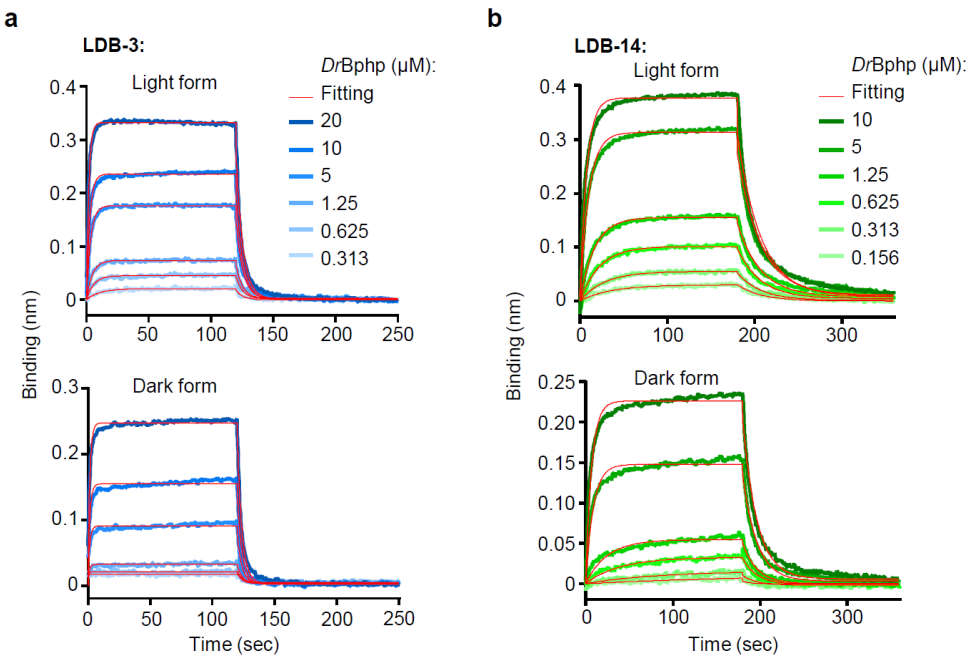

Figure S11. BLI analysis of LDB-3 and LDB-14 binding kinetics. BLI sensorgrams show *Dr*BphP binding to LDB-3 (a) and LDB-14 (b). Nanobodies were immobilized on Streptavidin biosensors and interacted with *Dr*BphP after the 654-nm (light form) or 775-nm (dark form) illumination. Data were fitted using a global 1:1 model. *Note:* the dark-form *Dr*BphP was likely converted to the light form by the white light applied to BLI biosensors.

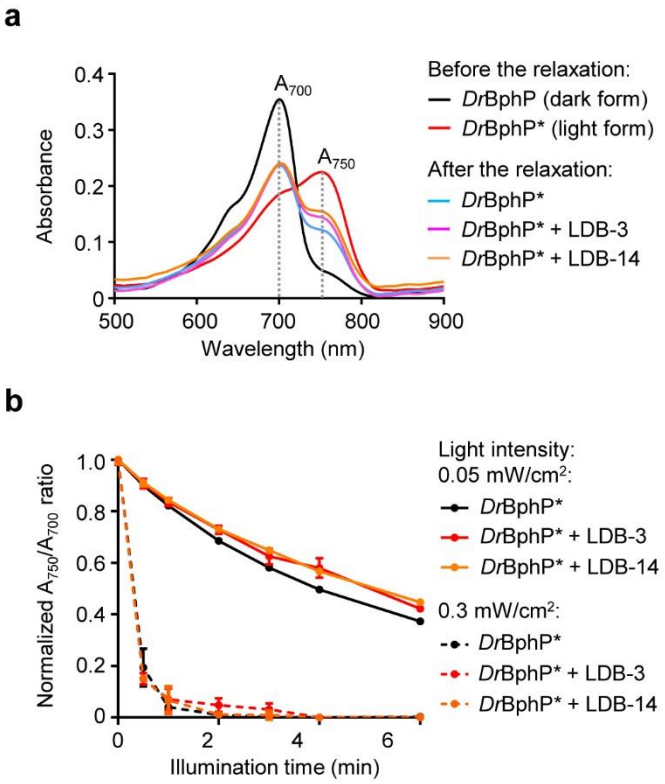

Figure S12. Inhibition of *Dr*BphP photoconversion to the dark form by the nanobody binding. (a) Representative absorption spectra of the photoconverted light and dark forms and after photoconversion by 775-nm illumination with or without LDB-3 or LDB-14 binding. (b) Time-course analysis of photoconversion rates of unbound and nanobody-bound light-form *Dr*BphP by the 775-nm illumination. 400 µl 5 µM (final concentration) light-form *Dr*BphP (after the 654-nm illumination at 0.5 mW/cm^2 for 2 min) was incubated with 5 µM (final concentration) LDB-3 or LDB-14 for 10 min before the photoconversion.

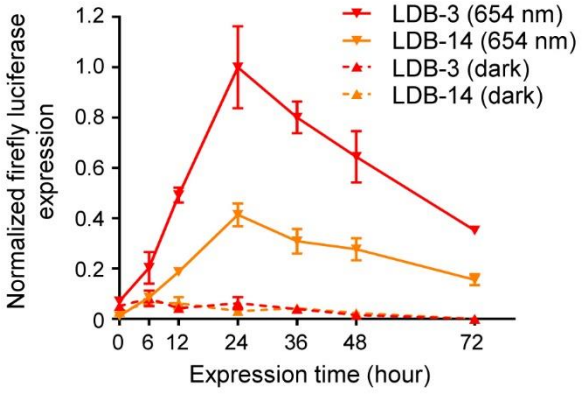

Figure S13. Time-course analysis of red light-induced luciferase expression. HEK293T cells were co-transfected with the bait, prey, and GAL4UAS-luciferase reporter plasmids (~0.25 μg each) in a 0.5 mL culture. Transfected cells were incubated under the 654-nm (0.2 mW/cm^2) illumination or in the dark. Data represent mean values of 3 measurements; error bars, standard deviation.

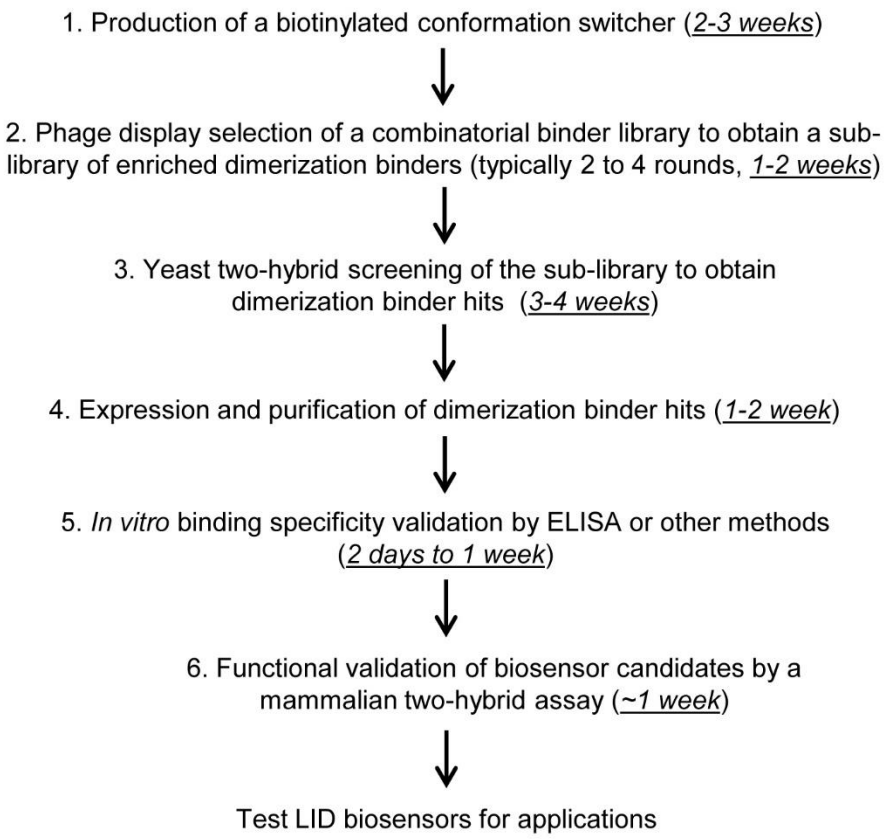

Figure S14. Flowchart and timeline of COMBINES-LID.

SUPPLEMENTARY TABLES

Table S1. Summary of photoswitchable proteins or domains that can potentially be used as conformational switchers in LID systems.

Photo-switchable proteins (or domains)	Example(s)	Chromophore(s)	Excitation λ (nm)	Reversion λ (nm)	Oligomeric state		Natural light induced binder(s)	Reference (s)
					Dark form	Light form		
UV receptors	UVR8	Trp	~300	Dark	Dimer	Monomer	COP1	2-4
Cyanobacteriochromes	CcaS	PCB	~535	~672/dark	Monomer	Monomer	CcaR	5-6
	cPAC	PCB	~410	~520/dark	Dimer	Dimer	Unknown	7
	UirS	PCB	~400	~530/dark	Monomer	Monomer	UirR	8-9
Sensors of blue-light using FAD (BLUF) domains	PixD	FAD or FMN	~450	Dark	Decamer	Dimer	PixE	10-12
	bPAC	FAD or FMN	~450	Dark	Dimer	Dimer	Unknown	13-15
LOV domains	AsLOV2	FMN	~450	Dark	Monomer	Monomer	Unknown	16-18
	YtvA	FAD, FMN, or riboflavin	~450	Dark	Dimer	Dimer	Unknown	19-21
	VVD	FAD or FMN	~450	Dark	Monomer	Dimer	VVD	22-24
	FKF1	FMN	~450	Dark	Monomer	Dimer	GI	25-27
	EL222	FMN	~450	Dark	Monomer	Dimer	Unknown	28-29
Cryptochromes	CRY2	FAD	~450	Dark	Monomer	Monomer	CIB1	30-31
Fluorescent protein domains	Dronpa145K/N	p-HBI	~400	~500/dark	Monomer	Dimer	Unknown	32-33
	PYP	p-coumaric acid	~450	Dark	Monomer	Monomer	Unknown	34-35
Opsins	BeCyclOp	Retinal	~530	Dark	Dimer	Dimer	Unknown	36
Cobalamin binding domains (CBDs)	TtCBD	AdoCbl, MetCbl, or CNCbl	~545	Dark	Tetramer	Monomer	Unknown	37
	MxCBD	AdoCbl, MetCbl, or CNCbl	~545	Dark	Tetramer	Monomer	Unknown	37
Phytochromes	*Rp*BphP1	BV	~740	~636/dark	Dimer	Dimer	PpsR2	38-39
	*Dr*BphP	BV	~655	~780/dark	Dimer	Dimer	Unknown	1, 40-41
	Cph1	PCB or PΦB	~657	~731/dark	Dimer	Dimer	Unknown	42-44
	PhyB	PCB	~660	~740/dark	Monomer	Monomer	PIF3/PIF6	45-47

Table S2. Enrichment of phage titers following each round of biopanning for the dimerization binder selection.

Round	Input count	Pre-elution count*	775 nm light elution count**
1	~1×10^{14}	~5.4×10^{6}	~2.0×10^{6}
2	~1×10^{13}	~1.2×10^{5}	~2.3×10^{5}
3	~1×10^{13}	~1.8×10^{5}	~7.3×10^{5}
4	~1×10^{13}	~1.5×10^{7}	~1.4×10^{8}

Note: After phage binding, the positive selection column was washed with ~30 mL 0.05% PBST buffer. 2 mL pre-elution fraction (*****) was collected at 0.5 mL/min immediately before the 775 nm-illumination at 0 mL/min. 2 mL elution fraction (******) was collected at 0.5 mL/min immediately after the illumination. Phage titers of the collected fractions were measured to determine the enrichment of clones specifically eluted by the light for each selection round.

Table S3. CDR sequences of light-induced dimerization binders (LDBs) characterized in the work.

Nanobody	CDR1	CDR2	CDR3
LDB-3	FTWDHYI	ENGDAWN	IGFDVPSGRSWQGSHFWM
LDB-4	DTSYLYS	WWWNLTQ	WSIYFPPGNDYNGYH
LDB-6	FFSNWSD	FWADGTE	WYGPVNGFYMFD
LDB-7	STSDFES	SWFTNPP	HRSIWYHPT
LDB-14	TTSRWES	WQNNSVP	AQHNFLGHR

Table S4. ITC-derived thermodynamic parameters for LDB-3 and LDB-14 binding to the *Dr*BphP light form.

	n (stoichiometry)	K_D^{app} (μM)	ΔH (kJ/mol)	ΔG (kJ/mol)	-TΔS (kJ/mol)
LDB-3	0.605	1.01	-37.0	-34.2	2.78
LDB-14	0.556	0.47	-112.8	-36.1	76.7

Note: The binding of the nanobodies to the dark form was too weak to be determined by ITC (Figure 5).

Table S5. Kinetic parameters of selected dimerization binders binding to the light form by Bio-layer interferometry.

	Molar ratio	K_D^{app} (10^{-6}M)	k_{on}^{app} (10^4 M^{-1} s^{-1})	k_{off}^{app} (10^{-2} s^{-1})
LDB-3	1:1	7.7	2.4	18.5
LDB-14	1:1	2.4	1.56	3.74

Note: Although the nanobody binding to the dark form was detectable (Figure S11), the binding data are not reliable because the white light applied to BLI biosensors can convert *Dr*BphP to the light form.

Table S6. Synthetic oligos used for plasmid construction in this study.

	Vector backbone	Name	Sequences (5' to 3')	Note
*Dr*BphP-Avi-His	pBAD	*Dr*BphP-Avi-His F	CTTTAAGAAGGAGATAT*GGATCC*ATGAGTCGTGACCCTTTGCCAT	*Bam*HI or *Eco*RI sites are underlined.
		*Dr*BphP-Avi-His R	TGGTGATGGTGATGATG*GAATTC*TTAGTGATGGTGGTGATGATG	
*Dr*BphP-His	pBAD	*Dr*BphP-His F	CTTTAAGAAGGAGATAT*GGATCC*ATGAGTCGTGACCCTTTGCCA	*Bam*HI or *Eco*RI sites are underlined.
		*Dr*BphP-His R	TGGTGATGGTGATGATG*GAATTC*TAATGCGCCAGTAAGAGTGTC	
Nanobody-His	pADL-23c	Nanobody-His F	GGATTGTTATTACTCGCG*GCCCAGCCGGC*CATGGCAGAAGTTCAGCTGCAGGCAAGCGG	*Bgl*I sites are underlined.
		Nanobody-His R	TGATGGTGGTGATGGTGTTG*GCCTCCCGGGC*TGCTGCTAACGGTAACCTGGGTGC	
Nanobody-Avi-His	pADL-23c	Nanobody-Avi-His F	TATTACTCGCG*GCCCAGCCGGC*CATGGCAGAAGTTCAGCTGCAGGCAAGC	*Bgl*I sites are underlined.
		Nanobody-Avi-His R	GGTGGATAAGCTTTG*GCCTCCCGGGC*TGCTGCTAACGGTAACCTGGGTGC	
*Dr*BphP-Yeast	pGBKT7	*Dr*BphP-Yeast F	GAGGAGGACCTG*CATATG*GGAGGCGGTTCCGGTGGCGG	*Nde*I or *Bam*HI sites are underlined.
		*Dr*BphP-Yeast R	CTGCAGGTCGAC*GGATCC*CTAGCTGCTAACGGTAACCTGGG	
Nanobody-Yeast	pGADT7	Nanobody-Yeast F	GATTACGCT*CATATG*GGAGGCGGTTCCGGTGGCGGTTCTGAAGTTCAGCTGCAGGCAAGC	*Nde*I or *Bam*HI sites are underlined.
		Nanobody-Yeast R	CTCGAGCTCGAT*GGATCC*CTAGCTGCTAACGGTAACCTGGGT	
*Dr*BphP-Mammalian	pBobi	*Dr*BphP-Mammalian F	GTTGCCACCATG*GGATCC*ATGAAGCTACTGTCTTCTATC	*Bam*HI or *Xho*I sites are underlined.
		*Dr*BphP-Mammalian R	GGAACCACCACC*CTCGAG*TAATGCGCCAGTAAGAGTGTC	
Nanobody-Mammalian	pBobi	3*NLS F	GTTGCCACCATG*GGATCC*CCCAAGAAGAAGCGCAAGGT	*Bam*HI or *Xho*I sites are underlined.
		3*NLS R	TGCCTGCAGCTGAACTTCTCCGCTGCCACCAGACCCTC	
		Nanobody F	GAAGTTCAGCTGCAGGCAAGC	
		Nanobody R	ACTGCCACCGCCGCCGCTGCTGCTAACGGTAACCTGGGT	
		p65 F	AGCGGCGGCGGTGGCAGTCAGTACCTGCCAGATACAGAC	
		p65 R	GGAACCACCACC*CTCGAG*GGAGCTGATCTGACTCAGCAG	
LDB-14-Mammalian	pcDNA3	3*NLS F	AAGCTGGCTAGTT*AAGCTT*ATGCCCAAGAAGAAGCGCAAGGTG	*Hin*dIII or *Xho*I sites are underlined.
		3*NLS R	TCCGCTGCCACCAGACCCTC	
		Nanobody F	GTTGAAGCATCTGGATCCGGAGGCGGTTCCGGTGGCGG	
		Nanobody R	GCCACTTCCTCCGGTACCGCTGCTAACGGTAACCTGGGT	

		p65 F	GGGTCTGGTGGCAGCGGACAGTACCTGCCAGATACAGACGAT	
		p65 R1 (first round PCR)	TCCACTGCCGCCAGAGCTGCCACTTCCTCCGGAGCTGATCTGACTCAGCAG	
		p65 R2 (second round PCR)	CGGGCCCTCTAGA*CTCGAG*CTACTGAATTCTCCACTGCCGCCAGAGCTGC	
*Rp*BphP1-Mammalian	pcDNA3	GAL4 BD F	ACCCAAGCTGGCTAGTT*AAGCTT*ATGAAGCTACTGTCTTCTATCG	*Hin*dIII or *Xho*I sites are underlined.
		GAL4 BD R	CATATGCAGGTCCTCCTCTGA	
		*Rp*BphP1-F	GAGGAGGACCTGCATATGGTGGCAGGTCATGCCTCTGGC	
		*Rp*BphP1-R	GGGCCCTCTAGA*CTCGAG*CTACTTCTTGTTCTGCGAGCCATT	
PpsR2-Mammalian	pcDNA3	PpsR2 F	GTTGAAGCATCT*GGATCC*GTGGCGTCAAAGTCCGTTCAT	*Bam*HI or *Kpn*I sites are underlined.
		PpsR2 R	GCCACTTCCTCC*GGTACC*ATCCTCTGCGTCGTCTGAG	
Q-PAS1-Mammalian	pcDNA3	Q-PAS1 F	GTTGAAGCATCT*GGATCC*GGCAAGAACATGCAGGCGGT	*Bam*HI or *Kpn*I sites are underlined.
		Q-PAS1 R	GCCACTTCCTCC*GGTACC*GTCGTCGATCGCGGGAGTCG	
Nanobody-SNAP	pBobi	Nanobody F	ACTGAGCTCCTTAAGGTTGCCACCATG*GGATCC*GAAGTTCAGCTGCAGGCAAGC	*Bam*HI or *Xho*I sites are underlined.
		Nanobody R	ACTGCCACCGCCGCCGTTAACGCTGCTAACGGTAACCTGGGT	
		SNAP F	AACGGCGGCGGTGGCAGTGACAAAGACTGCGAAATGAAGCG	
		SNAP R	TCAGCTTCTGCTCACCGGAACCACCACC*CTCGAG*ACCCAGCCCAGGCTTGCCCA	
PAS-GAF-Avi-His	pBAD	PAS-GAF-Avi-His F	AAGCTTGGTGGCGGTAGC	Constructed by Q5 Site-Directed Mutagenesis Kit (New England Biolabs, E0554S) based on *Dr*BphP-Avi-His
		PAS-GAF-Avi-His R	TTCCTTGACTTGCACCTGAAG	
PHY-Avi-His	pBAD	PHY-Avi-His F	GCCGCGGACGTTGCTGCA	
		PHY-Avi-His R	CATGGATCCATATCTCCTTCTTAAAGTTAAACAAAAGGGG	
PHY-SNAP-Avi-His	pLGSA	PHY-SNAP-Avi-His F	ACTTTAAGAAGGAGATATA*CATATG*GCCGCGGACGTTGCTGCATTCC	*Nde*I or *Bam*HI sites are underlined.
		PHY-SNAP-Avi-His R	TCATTTCGCAGTCTTTGTC*GGATCC*TAATGCGCCAGTAAGAGTGTCG	
*Dr*BphP-AcGFP-CAAX	pBobi	*Dr*BphP F	GTTGCCACCATG*GGATCC*ATGAGTCGTGACCCTTTGCCAT	*Bam*HI or *Xho*I sites are underlined.
		*Dr*BphP R	CCGCCACCACCGCTCGAGCCAACTAATGCGCCAGTAAGAGTGTCG	
		AcGFP-CAAX F	GGCTCGAGCGGTGGTGGCGGGAGCGGAGGTATGGTGAGCAAGGGCGCCGA	
		AcGFP-CAAX R	GGAACCACCACC*CTCGAG*TCACATAATGACACACTTGGTTTTGCTTTTCTTCTTTTTTTTCTTCTTGTACAGCTCATCCATGCC	
LDB-3-mCherry	pBobi	LDB-3 F	GTTGCCACCATG*GGATCC*GAAGTTCAGCTGCAGGCAAGC	

		LDB-3 R	CCGCCACCACCGCTCGAGCCAACGCTGCTAACGGTAACCTGGGT	*Bam*HI or *Xho*I sites are underlined.
		mCherry F	GGCTCGAGCGGTGGTGGCGGGAGCGGAGGTATGGTGAGCAAGGGCGAGGAG	
		mCherry R	GGAACCACCACC*CTCGAG*TCAGTTTCCGGACTTGTACAGCTCG	

Table S7. Protein coding sequences (CDSs) and noncommercial vector used in this work.

Purpose	Name	CDS or vector sequence	Subcloning note
E. coli expression	*Dr*BphP-Avi-His	ATGAGTCGTGACCCTTTGCCATTCTTTCCTCCTCTTTATCTGGGTGGACCCGAGAT TACAACAGAAAACTGCGAACGCGAACCAATTCACATCCCGGGATCTATTCAACCAC ACGGTGCATTGCTGACGGCAGACGGACATTCCGGAGAGGTTTTACAGATGTCGCTT AACGCAGCAACGTTTCTGGGACAAGAGCCTACGGTTTTGCGCGGCCAGACGTTAGC GGCTCTGTTGCCAGAGCAATGGCCGGCCTTACAGGCGGCATTGCCTCCAGGGTGCC CCGATGCATTGCAATACCGCGCGACACTGGATTGGCCGGCGGCAGGACATCTTTCT CTGACAGTCCACCGCGTGGGCGAGCTGTTGATCCTGGAGTTTGAACCTACGGAGGC CTGGGACTCGACTGGCCCGCACGCGTTACGCAATGCGATGTTCGCTCTTGAATCAG CGCCAAACTTGCGCGCGTTAGCTGAAGTGGCCACACAAACCGTACGCGAGCTTACA GGCTTTGACCGCGTGATGTTATACAAATTCGCACCCGATGCGACAGGCGAGGTAAT CGCCGAAGCCCGCCGCGAGGGGTTGCATGCCTTTCTTGGCCATCGTTTTCCGGCCT CAGATATTCCCGCCCAAGCGCGCGCCCTTTACACTCGCCATCTGCTTCGTTTGACT GCGGACACGCGCGCGGCGGCCGTTCCCTTAGACCCAGTACTTAATCCTCAGACTAA CGCTCCTACCCCCTTAGGGGGGGCAGTGCTGCGTGCGACGTCGCCTATGCACATGC AGTACCTTCGCAATATGGGCGTCGGCTCCTCTTTAAGTGTATCAGTGGTAGTTGGG GGGCAGTTATGGGGTCTGATTGCGTGCCATCATCAGACCCCCTATGTTTTGCCACC AGACCTTCGTACTACTCTTGAATACTTGGGGCGTTTATTAAGCCTTCAGGTGCAAG TCAAGGAAGCCGCGGACGTTGCTGCATTCCGTCAGTCACTTCGCGAACACCATGCG CGCGTCGCCTTAGCGGCAGCGCATTCCCTGTCGCCGCACGATACTCTTTCCGACCC TGCACTTGATCTTCTGGGTCTGATGCGTGCTGGGGGCTTAATCCTGCGTTTTGAAG GTCGTTGGCAGACGTTAGGAGAAGTCCCGCCCGCTCCCGCAGTCGATGCACTGCTT GCATGGCTTGAAACCCAACCAGGGGCGCTTGTTCAGACTGATGCATTGGGGCAGTT GTGGCCGGCGGGGGCTGATTTGGCTCCCTCAGCCGCGGGTCTGCTTGCCATTTCAG TAGGGGAGGGATGGAGTGAGTGCTTGGTTTGGTTACGTCCCGAACTGCGCCTTGAG GTTGCGTGGGGTGGAGCAACTCCAGACCAGGCCAAGGACGACCTGGGCCCTCGTCA CAGTTTCGATACTTACTTAGAAGAGAAGCGTGGGTATGCAGAACCCTGGCATCCCG GAGAGATTGAGGAAGCTCAGGATTTGCGCGACACTCTTACTGGCGCATTAAAGCTT GGTGGCGGTAGCGAGAATTTGTATTTTCAGGGTGGCGGTGGCAGTAGCTTATCCAC CCCGCCGACCCCGAGCACTCCTCCTACCGGTCTGAACGACATCTTCGAGGCTCAGA AAATCGAATGGCACGAACATCATCACCACCATCAC	The CDS was inserted into pBAD (Addgene #80341) using *BamH*I/*Eco*RI restriction sites.
	*Dr*BphP-His	(*Dr*BphP)-GAATTCCATCATCACCATCACCAT	The sequence of *Dr*BphP is the same as above. The CDS was inserted into pBAD (Addgene #80341) using *BamH*I/*Eco*RI restriction sites.
	LDB-3-His	GAAGTTCAGCTGCAGGCAAGCGGTGGTGGTTTTGTTCAGCCTGGTGGTAGCCTGCG TCTGAGCTGTGCAGCCAGCGGTTTTACCTGGGATCATTACATCATGGGCTGGTTTC GCCAGGCACCGGGTAAAGAACGTGAATTTGTTAGCGCAATCAGCGAAAATGGTGAT GCATGGAATTATTATGCCGATAGCGTGAAAGGTCGCTTTACCATTAGCCGTGATAA TAGCAAAAATACCGTTTACCTGCAGATGAATAGTCTGCGTGCAGAAGATACCGCAA CCTATTATTGTGCAATCGGTTTTGATGTTCCATCTGGTCGTTCTTGGCAGGGTTCT CATTTTTGGATGTATTGGGGTCAGGGCACCCAGGTTACCGTTAGCAGCAGCCCGGG AGGCCAACACCATCACCACCATCAT	The CDS was inserted into pADL-23c (Antibody Design Labs) using a *Bgl*I restriction site.
	LDB-3-Avi-His	(LDB-3)- AGCCCGGGAGGCCAAAGCTTATCCACCCCGAGTGTAGATCTCGGTGGTCGCCGTAT CATTGGTCTGAACGACATCTTCGAGGCTCAGAAAATCGAATGGCACGAACATCATC ACCACCATCACTCT	The sequence of LDB-3 is the same as above. The CDS was inserted into pADL-23c using a *Bgl*I restriction site.
	LDB-14-His	GAAGTTCAGCTGCAGGCAAGCGGTGGTGGTTTTGTTCAGCCTGGTGGTAGCCTGCG TCTGAGCTGTGCAGCCAGCGGTACCACCTCTCGTTGGGAATCTATGGGCTGGTTTC GCCAGGCACCGGGTAAAGAACGTGAATTTGTTAGCGCAATCAGCTGGCAGAATAAT TCTGTTCCATATTATGCCGATAGCGTGAAAGGTCGCTTTACCATTAGCCGTGATAA TAGCAAAAATACCGTTTACCTGCAGATGAATAGTCTGCGTGCAGAAGATACCGCAA CCTATTATTGTGCAGCACAGCATAACTTTCTGGGTCATCGTTATTGGGGTCAGGGC ACCCAGGTTACCGTTAGCAGCAGCCCGGGAGGCCAACACCATCACCACCATCAT	The CDS was inserted into pADL-23c using a *Bgl*I restriction site.
	LDB-14-Avi-His	(LDB-14)- AGCCCGGGAGGCCAAAGCTTATCCACCCCGAGTGTAGATCTCGGTGGTCGCCGTAT CATTGGTCTGAACGACATCTTCGAGGCTCAGAAAATCGAATGGCACGAACATCATC ACCACCATCACTCT	The sequence of LDB-14 is the same as above. The CDS was inserted into pADL-23c using a *Bgl*I restriction site.

	PAS-GAF-Avi-His	ATGAGTCGTGACCCTTTGCCATTCTTTCCTCCTCTTTATCTGGGTGGACCCGAGAT TACAACAGAAAACTGCGAACGCGAACCAATTCACATCCCGGGATCTATTCAACCAC ACGGTGCATTGCTGACGGCAGACGGACATTCCGGAGAGGTTTTACAGATGTCGCTT AACGCAGCAACGTTTCTGGGACAAGAGCCTACGGTTTTGCGCGGCCAGACGTTAGC GGCTCTGTTGCCAGAGCAATGGCCGGCCTTACAGGCGGCATTGCCTCCAGGGTGCC CCGATGCATTGCAATACCGCGCGACACTGGATTGGCCGGCGGCAGGACATCTTTCT CTGACAGTCCACCGCGTGGGCGAGCTGTTGATCCTGGAGTTTGAACCTACGGAGGC CTGGGACTCGACTGGCCCGCACGCGTTACGCAATGCGATGTTCGCTCTTGAATCAG CGCCAAACTTGCGCGCGTTAGCTGAAGTGGCCACACAAACCGTACGCGAGCTTACA GGCTTTGACCGCGTGATGTTATACAAATTCGCACCCGATGCGACAGGCGAGGTAAT CGCCGAAGCCCGCCGCGAGGGGTTGCATGCCTTTCTTGGCCATCGTTTTCCGGCCT CAGATATTCCCGCCCAAGCGCGCGCCCTTTACACTCGCCATCTGCTTCGTTTGACT GCGGACACGCGCGCGGCGGCCGTTCCCTTAGACCCAGTACTTAATCCTCAGACTAA CGCTCCTACCCCCTTAGGGGGGGCAGTGCTGCGTGCGACGTCGCCTATGCACATGC AGTACCTTCGCAATATGGGCGTCGGCTCCTCTTTAAGTGTATCAGTGGTAGTTGGG GGGCAGTTATGGGGTCTGATTGCGTGCCATCATCAGACCCCCTATGTTTTGCCACC AGACCTTCGTACTACTCTTGAATACTTGGGGCGTTTATTAAGCCTTCAGGTGCAAG TCAAGGAAAAGCTTGGTGGCGGTAGCGAGAATTTGTATTTTCAGGGTGGCGGTGGC AGTAGCTTATCCACCCCGCCGACCCCGAGCACTCCTCCTACCGGTCTGAACGACAT CTTCGAGGCTCAGAAAATCGAATGGCACGAACATCATCACCACCATCAC	PAS-GAF-Avi-His was constructed by Q5 Site-Directed Mutagenesis Kit (New England Biolabs, E0554S) based on *Dr*BphP-Avi-His.
	PHY-Avi-His	ATGGCCGCGGACGTTGCTGCATTCCGTCAGTCACTTCGCGAACACCATGCGCGCGT CGCCTTAGCGGCAGCGCATTCCCTGTCGCCGCACGATACTCTTTCCGACCCTGCAC TTGATCTTCTGGGTCTGATGCGTGCTGGGGGCTTAATCCTGCGTTTTGAAGGTCGT TGGCAGACGTTAGGAGAAGTCCCGCCCGCTCCCGCAGTCGATGCACTGCTTGCATG GCTTGAAACCCAACCAGGGGCGCTTGTTCAGACTGATGCATTGGGGCAGTTGTGGC CGGCGGGGGCTGATTTGGCTCCCTCAGCCGCGGGTCTGCTTGCCATTTCAGTAGGG GAGGGATGGAGTGAGTGCTTGGTTTGGTTACGTCCCGAACTGCGCCTTGAGGTTGC GTGGGGTGGAGCAACTCCAGACCAGGCCAAGGACGACCTGGGCCCTCGTCACAGTT TCGATACTTACTTAGAAGAGAAGCGTGGGTATGCAGAACCCTGGCATCCCGGAGAG ATTGAGGAAGCTCAGGATTTGCGCGACACTCTTACTGGCGCATTAAAGCTTGGTGG CGGTAGCGAGAATTTGTATTTTCAGGGTGGCGGTGGCAGTAGCTTATCCACCCCGC CGACCCCGAGCACTCCTCCTACCGGTCTGAACGACATCTTCGAGGCTCAGAAAATC GAATGGCACGAACATCATCACCACCATCAC	PHY-Avi-His was constructed by Q5 Site-Directed Mutagenesis Kit (New England Biolabs, E0554S) based on *Dr*BphP-Avi-His.
	PHY-SNAP-Avi-His	(PHY)- GGATCCGACAAAGACTGCGAAATGAAGCGCACCACCCTGGATAGCCCTCTGGGCAA GCTGGAACTGTCTGGGTGCGAACAGGGCCTGCACCGTATCATCTTCCTGGGCAAAG GAACATCTGCCGCCGACGCCGTGGAAGTGCCTGCCCCAGCCGCCGTGCTGGGCGGA CCAGAGCCACTGATGCAGGCCACCGCCTGGCTCAACGCCTACTTTCACCAGCCTGA GGCCATCGAGGAGTTCCCTGTGCCAGCCCTGCACCACCCAGTGTTCCAGCAGGAGA GCTTTACCCGCCAGGTGCTGTGGAAACTGCTGAAAGTGGTGAAGTTCGGAGAGGTC ATCAGCTACAGCCACCTGGCCGCCCTGGCCGGCAATCCCGCCGCCACCGCCGCCGT GAAAACCGCCCTGAGCGGAAATCCCGTGCCCATTCTGATCCCCTGCCACCGGGTGG TGCAGGGCGACCTGGACGTGGGGGGCTACGAGGGCGGGCTCGCCGTGAAAGAGTGG CTGCTGGCCCACGAGGGCCACAGACTGGGCAAGCCTGGGCTGGGTCCGGGAGGCCA AAGCTTATCCACCCCGCCGACCCCGAGCACTCCTCCTACCGGTCTGAACGACATCT TCGAGGCTCAGAAAATCGAATGGCACGAACTCGAGCACCACCACCACCACCACCAC	The sequence of PHY is the same as above. The CDS was inserted into pLGSA vector using *Nde*I/*BamH*I restriction sites.
Yeast two-hybrid	GAL4-*Dr*BphP	ATGAAGCTACTGTCTTCTATCGAACAAGCATGCGATATTTGCCGACTTAAAAAGCT CAAGTGCTCCAAAGAAAAACCGAAGTGCGCCAAGTGTCTGAAGAACAACTGGGAGT GTCGCTACTCTCCCAAAACCAAAAGGTCTCCGCTGACTAGGGCACATCTGACAGAA GTGGAATCAAGGCTAGAAAGACTGGAACAGCTATTTCTACTGATTTTTCCTCGAGA AGACCTTGACATGATTTTGAAAATGGATTCTTTACAGGATATAAAAGCATTGTTAA CAGGATTATTTGTACAAGATAATGTGAATAAAGATGCCGTCACAGATAGATTGGCT TCAGTGGAGACTGATATGCCTCTAACATTGAGACAGCATAGAATAAGTGCGACATC ATCATCGGAAGAGAGTAGTAACAAAGGTCAAAGACAGTTGACTGTATCGCCGGAAT TTGTAATACGACTCACTATAGGGCGAGCCGCCATCATGGAGGAGCAGAAGCTGATC TCAGAGGAGGACCTGCAT-(*Dr*BphP)	The sequence of *Dr*BphP is the same as above. The CDS was inserted into pGBKT7 vector (Clontech) using *Nde*I/*BamH*I restriction sites.
	AD-LDB-3	ATGGATAAAGCGGAATTAATTCCCGAGCCTCCAAAAAAGAAGAGAAAGGTCGAATT GGGTACCGCCGCCAATTTTAATCAAAGTGGGAATATTGCTGATAGCTCATTGTCCT TCACTTTCACTAACAGTAGCAACGGTCCGAACCTCATAACAACTCAAACAAATTCT CAAGCGCTTTCACAACCAATTGCCTCCTCTAACGTTCATGATAACTTCATGAATAA TGAAATCACGGCTAGTAAAATTGATGATGGTAATAATTCAAAACCACTGTCACCTG GTTGGACGGACCAAACTGCGTATAACGCGTTTGGAATCACTACAGGGATGTTTAAT ACCACTACAATGGATGATGTATATAACTATCTATTCGATGATGAAGATACCCCACC AAACCCAAAAAAAGAGATCTTTAATACGACTCACTATAGGGCGAGCGCCGCCATGG AGTACCCATACGACGTACCAGATTACGCTCATATGGGAGGCGGTTCCGGTGGCGGT TCT-(LDB-3)	The sequence of LDB-3 is the same as above. The CDS was inserted into pGADT7 (Clontech) using *Nde*I/*BamH*I restriction sites.
	AD-LDB-4	(AD)- ATCTTTAATACGACTCACTATAGGGCGAGCGCCGCCATGGAGTACCCATACGACGT ACCAGATTACGCTCATATGGGAGGCGGTTCCGGTGGCGGTTCTGAAGTTCAGCTGC AGGCAAGCGGTGGTGGTTTTGTTCAGCCTGGTGGTAGCCTGCGTCTGAGCTGTGCA GCCAGCGGTGATACCTCTTACCTGTACTCTATGGGCTGGTTTCGCCAGGCACCGGG	The CDS was inserted into pGADT7 using *Nde*I/*BamH*I restriction sites. The

		TAAAGAACGTGAATTTGTTAGCGCAATCAGCTGGTGGTGGAATCTGACTCAGTATT ATGCCGATAGCGTGAAAGGTCGCTTTACCATTAGCCGTGATAATAGCAAAAATACC GTTTACCTGCAGATGAATAGTCTGCGTGCAGAAGATACCGCAACCTATTATTGTGC ATGGTCTATCTACTTTCCACCAGGTAACGATTACAACGGTTACCATTATTGGGGTC AGGGCACCCAGGTTACCGTTAGCAGC	sequences of AD and LDB-14 are the same as above.
	AD-LDB-6	(AD)- ATCTTTAATACGACTCACTATAGGGCGAGCGCCGCCATGGAGTACCCATACGACGT ACCAGATTACGCTCATATGGGAGGCGGTTCCGGTGGCGGTTCTGAAGTTCAGCTGC AGGCAAGCGGTGGTGGTTTTGTTCAGCCTGGTGGTAGCCTGCGTCTGAGCTGTGCA GCCAGCGGTTTTTTTTCTAACTGGTCTGATATGGGCTGGTTTCGCCAGGCACCGGG TAAAGAACGTGAATTTGTTAGCGCAATCAGCTTTTGGGCAGATGGTACTGAATATT ATGCCGATAGCGTGAAAGGTCGCTTTACCATTAGCCGTGATAATAGCAAAAATACC GTTTACCTGCAGATGAATAGTCTGCGTGCAGAAGATACCGCAACCTATTATTGTGC ATGGTACGGTCCAGTTAACGGTTTTTACATGTTTGATTATTGGGGTCAGGGCACCC AGGTTACCGTTAGCAGC	
	AD-LDB-7	(AD)- ATCTTTAATACGACTCACTATAGGGCGAGCGCCGCCATGGAGTACCCATACGACGT ACCAGATTACGCTCATATGGGAGGCGGTTCCGGTGGCGGTTCTGAAGTTCAGCTGC AGGCAAGCGGTGGTGGTTTTGTTCAGCCTGGTGGTAGCCTGCGTCTGAGCTGTGCA GCCAGCGGTTCTACCTCTGATTTTGAATCTATGGGCTGGTTTCGCCAGGCACCGGG TAAAGAACGTGAATTTGTTAGCGCAATCAGCTCTTGGTTTACTAATCCACCATATT ATGCCGATAGCGTGAAAGGTCGCTTTACCATTAGCCGTGATAATAGCAAAAATACC GTTTACCTGCAGATGAATAGTCTGCGTGCAGAAGATACCGCAACCTATTATTGTGC ACATCGTTCTATCTGGTACCATCCAACCTATTGGGGTCAGGGCACCCAGGTTACCG TTAGCAGC	
	AD-LDB-14	(AD)- ATCTTTAATACGACTCACTATAGGGCGAGCGCCGCCATGGAGTACCCATACGACGT ACCAGATTACGCTCATATGGGAGGCGGTTCCGGTGGCGGTTCT-(LDB-14)	
Mammalian two-hybrid	GAL4-*Dr*BphP	GAL4-*Dr*BphP	The sequence of GAL4-*Dr*BphP is the same as above. The CDS was inserted into pBobi (see below for the sequence) using *BamH*I/*Xho*I restriction sites.
	NLS-LDB-3-p65	ATGGGATCCCCCAAGAAGAAGCGCAAGGTGGAAGCTAGCGCTTCCCCGAAGAAAAA GCGGAAAGTCGAGGCCTCCGCATCTCCAAAAAAAAAAAAGCAAGGTTGAAGCATCTG GATCCGGTACCGGAGGAAGTGGCAGCTCTGGCGGCAGTGGAGGGTCTGGTGGCAGC GGA-(LDB-3)- AGCGGCGGCGGTGGCAGTCAGTACCTGCCAGATACAGACGATCGTCACCGGATTGA GGAGAAACGTAAAAGGACATATGAGACCTTCAAGAGCATCATGAAGAAGAGTCCTT TCAGCGGACCCACCGACCCCCGGCCTCCACCTCGACGCATTGCTGTGCCTTCCCGC AGCTCAGCTTCTGTCCCCAAGCCAGCACCCCAGCCCTATCCCTTTACGTCATCCCT GAGCACCATCAACTATGATGAGTTTCCCACCATGGTGTTTCCTTCTGGGCAGATCA GCCAGGCCTCGGCCTTGGCCCCGGCCCCTCCCCAAGTCCTGCCCCAGGCTCCAGCC CCTGCCCCTGCTCCAGCCATGGTATCAGCTCTGGCCCAGGCCCCAGCCCCTGTCCC AGTCCTAGCCCCAGGCCCTCCTCAGGCTGTGGCCCCACCTGCCCCCAAGCCCACCC AGGCTGGGGAAGGAACGCTGTCAGAGGCCCTGCTGCAGCTGCAGTTTGATGATGAA GACCTGGGGGCCTTGCTTGGCAACAGCACAGACCCAGCTGTGTTCACAGACCTGGC ATCCGTCGACAACTCCGAGTTTCAGCAGCTGCTGAACCAGGGCATACCTGTGGCCC CCCACACAACTGAGCCCATGCTGATGGAGTACCCTGAGGCTATAACTCGCCTAGTG ACAGGGGCCCAGAGGCCCCCCGACCCAGCTCCTGCTCCACTGGGGGCCCCGGGGCT CCCCAATGGCCTCCTTTCAGGAGATGAAGACTTCTCCTCCATTGCGGACATGGACT TCTCAGCCCTGCTGAGTCAGATCAGCTCC	The CDS was inserted into pBobi vector using *BamH*I/*Xho*I restriction sites. The sequence of LDB-3 is the same as above.
	NLS-LDB-4-p65	(NLS)- GGATCCGGTACCGGAGGAAGTGGCAGCTCTGGCGGCAGTGGAGGGTCTGGTGGCAG CGGA-(LDB-4)-AGCGGCGGCGGTGGCAGT-(p65)	The sequences of NLS, nanobody and p65 are the same as above. The CDS was inserted into pBobi using *BamH*I/*Xho*I restriction sites.
	NLS-LDB-6-p65	(NLS)- GGATCCGGTACCGGAGGAAGTGGCAGCTCTGGCGGCAGTGGAGGGTCTGGTGGCAG CGGA-(LDB-6)-AGCGGCGGCGGTGGCAGT-(p65)	
	NLS-LDB-7-p65	(NLS)- GGATCCGGTACCGGAGGAAGTGGCAGCTCTGGCGGCAGTGGAGGGTCTGGTGGCAG CGGA-(LDB-7)-AGCGGCGGCGGTGGCAGT-(p65)	
	NLS-LDB-14-p65	ATGCCCAAGAAGAAGCGCAAGGTGGAAGCTAGCGCTTCCCCGAAGAAAAAGCGGAA AGTCGAGGCCTCCGCATCTCCAAAAAAAAAAAAGCAAGGTTGAAGCATCTGGATCCG GAGGCGGTTCCGGTGGCGGTTCT-(LDB-14)- GGTACCGGAGGAAGTGGCAGCTCTGGCGGCAGTGGAGGGTCTGGTGGCAGCGGA- (p65)-GGAGGAAGTGGCAGCTCTGGCGGCAGTGGA	The CDS was inserted into pcDNA3 (Invitrogen) using *Hind*III/*Xho*I restriction sites. The sequences of LDB-14 and p65

			are the same as above.
	GAL4-*Rp*BphP1	(GAL4)- CCGGAATTTGTAATACGACTCACTATAGGGCGAGCCGCCATCATGGAGGAGCAGAA GCTGATCTCAGAGGAGGACCTGCATGTGGCAGGTCATGCCTCTGGCAGCCCCGCAT TCGGGACCGCCGATCTTTCGAATTGCGAACGTGAAGAGATCCACCTCGCCGGCTCG ATCCAGCCGCATGGCGCGCTTCTGGTCGTCAGCGAGCCGGATCATCGCATCATCCA GGCCAGCGCCAACGCCGCGGAATTTCTGAATCTCGGAAGCGTGCTCGGCGTTCCGC TCGCCGAGATCGACGGCGATCTGTTGATCAAGATCCTGCCGCATCTCGATCCCACC GCCGAAGGCATGCCGGTCGCGGTGCGCTGCCGGATCGGCAATCCCTCCACGGAGTA CGACGGTCTGATGCATCGGCCTCCGGAAGGCGGGCTGATCATCGAGCTCGAACGTG CCGGCCCGCCGATCGATCTGTCCGGCACGCTGGCGCCGGCGCTGGAGCGGATCCGC ACGGCGGGCTCGCTGCGCGCGCTGTGCGATGACACCGCGCTGCTGTTTCAGCAGTG CACCGGCTACGACCGGGTGATGGTGTATCGCTTCGACGAGCAGGGCCACGGCGAAG TGTTCTCCGAGCGCCACGTGCCCGGGCTCGAATCCTATTTCGGCAACCGCTATCCG TCGTCGGACATTCCGCAGATGGCGCGGCGGCTGTACGAGCGGCAGCGCGTCCGCGT GCTGGTCGACGTCAGCTATCAGCCGGTGCCGCTGGAGCCGCGGCTGTCGCCGCTGA CCGGGCGCGATCTCGACATGTCGGGCTGCTTCCTGCGCTCGATGTCGCCGATCCAT CTGCAGTACCTGAAGAACATGGGCGTGCGCGCCACCCTGGTGGTGTCGCTGGTGGT CGGCGGCAAGCTGTGGGGCCTGGTTGCCTGTCACCATTATCTGCCGCGCTTCATCC ATTTCGAGCTGCGGGCGATCTGCGAACTGCTCGCCGAAGCGATCGCGACGCGGATC ACCGCGCTTGAGAGCTTCGCGCAGAGCCAGTCGGAGCTGTTCGTGCAGCGGCTCGA ACAGCGCATGATCGAAGCGATCACCCGTGAAGGCGATTGGCGCGCAGCGATTTTCG ACACCAGCCAATCGATCCTGCAGCCGCTGCACGCCGACGGTTGCGCGCTGGTGTAC GAAGACCAGATCAGGACCATCGGTGACGTACCTTCCACGCAGGATGTTCGCGAGAT CGCCGGGTGGCTCGATCGCCAGCCACGTGCGGCGGTGACCTCGACCGCGTCGCTCG GTCTCGACGTGCCGGAGCTCGCGCATCTGACGCGGATGGCGAGCGGCGTGGTCGCG GCGCCGATTTCGGATCATCGCGGCGAGTTTCTGATGTGGTTCCGCCCCGAGCGCGT CCACACCGTTACCTGGGGCGGCGATCCGAAGAAGCCGTTCACGATGGGCGATACAC CGGCGGATCTGTCGCCGCGGCGCTCCTTCGCCAAATGGCATCAGGTTGTCGAAGGC ACGTCCGATCCGTGGACGGCCGCCGATCTCGCCGCGGCTCGCACCATCGGTCAGAC CGTCGCCGACATCGTGCTGCAATTCCGCGCGGTGCGGACACTGATCGCCCGCGAAC AGTACGAACAGTTTTCGTCCCAGGTGCACGCTTCGATGCAGCCGGTGCTGATCACC GACGCCGAAGGCCGCATCCTGCTGATGAACGACTCGTTCCGCGACATGTTGCCGGC GGGGTCGCCATCCGCCGTCCATCTCGACGATCTCGCCGGGTTCTTCGTCGAATCGA ACGATTTCCTGCGCAACGTCGCCGAACTGATCGATCACGGCCGCGGGTGGCGCGGC GAAGTTCTGCTGCGCGGCGCAGGTAATCGCCCGTTGCCGCTGGCAGTGCGCGCCGA TCCGGTGACGCGCACGGAGGACCAGTCGCTCGGCTTCGTGCTGATCTTCAGCGACG CTACCGATCGTCGCACCGCAGATGCCGCACGCACGCGTTTCCAGGAAGGCATTCTT GCCAGCGCACGTCCCGGCGTGCGGCTCGACTCCAAGTCCGACCTCTTGCACGAGAA GCTGCTGTCCGCGCTGGTCGAGAACGCGCAGCTTGCCGCATTGGAAATTACTTACG GCGTCGAGACCGGACGCATCGCCGAGCTGCTCGAAGGCGTTCGCCAGTCGATGCTG CGCACCGCCGAAGTGCTCGGCCATCTGGTGCAGCACGCGGCGCGCACGGCCGGCAG CGACAGCTCGAGCAATGGCTCGCAGAACAAGAAG	The CDS was subcloned into pcDNA3 using *Hind*III/*Xho*I restriction sites. The sequence of GAL4 is the same as above.
	NLS-PpsR2-p65	ATGCCCAAGAAGAAGCGCAAGGTGGAAGCTAGCGCTTCCCCGAAGAAAAAGCGGAA AGTCGAGGCCTCCGCATCTCCAAAAAAAAAAAGCAAGGTTGAAGCATCTGGATCCG GAGGCGGTTCCGGTGGCGGTTCTGTGGCGTCAAAGTCCGTTCATGCCGACATCACC CTTCTGCTCGATATGGAGGGTGTGATTCGCGAAGCCACCCTGTCTCCGACGATGGC GGCCGAGAGCGTGGACGGTTGGCTGGGGCGTCGCTGGAGCGACATCGCCGGCGCCG AAGGCGGCGACAAGGTTCGCCGCATGGTCGAAGACGCCCGCCGCAGCGGCATCTCG GCTTTCCGCCAGATCAATCAGCCTTTCCCGAGCGGCGTCGAAATCCCGATCGAATT CACCACGATGCTGCTGGGCGACCGCACCGGCATGATCGCGGTCGGCAAGAACATGC AGGCGGTCACCGAGCTGCATTCCCGGCTGATCGCTGCGCAGCAGGCGATGGAGCGC GACTATTGGCGGTTGCGTGAATTGGAGACTCGCTACCGCCTGGTGTTCGACGCTGC CGCCGATGCGGTGATGATCGTCTCCGCCGGCGACATGCGCATCGTCGAAGCCAACC GGGCGGCGGTGAATGCGATCAGCCGCGTCGAGCGCGGCAATGACGACCTTGCGGGG CGTGATTTCCTCGCCGAAGTGGCGGCTGCCGATCGCGATGCGGTGCGCGACATGCT GGCCCAGGTGCGTCAGCGCGGCACCGCACTCAGCGTCCTCGTTCATCTCGGCCGTT ACGACCGCGCCTGGATGCTGCGCGGTTCGCTGATGTCGTCCGAGCGTCGTCAGGTT TTCCTGCTGCACTTCACCCCGGTGACCACGACTCCCGCGATCGACGACGTCGACGA TGATGCCGTGCTGCGCGGGCTGATCGATCGCATTCCCGACGGGTTCGTCGCACTGG ATTCGGAAGGCGTCGTTCGTCACGCCAACCAGGCGTTTCTCGATCTGGTCCAGATC GGCTCCAAGCCTGCGGCGGTCGGACGATCGCTGGGCGTCTGGATGGGTCGTCCGGG CGCCGATCTGTCCAGCTTGCTGACGCTGCTGCGGCGCTACAAGACGGTGCGGCTGT TCCAAACGACGATCCGCGGCGAGCTCGGCACCGAGACTGAAGTCGAGGTCTCGGCC GTCGACGGCGAGGACGACCAATACATCGGCGTTCTGATGCGCAATGTCGCGCGACG CCTCGACGCTGCGGACGACCACGATGCCTTGCGTCAGGCGCTCGGCCCGATCAGCA AGCAGCTCGGGCGATCCTCGCTGCGCAAGCTGGTGAAGAACGCCGTGAGCATTGTC GAGCAGCACTACGTGAAGGAAGCGCTGTTGCGATCCAAGGGCAATCGCACGGCAAC TGCCGAACTGCTCGGATTGAGCCGGCAGAGCCTTTATGCAAAACTCAACTCCTACG GCTTCGACGACAAAGGTGTCGTTGCTTCTGCTGCCGACGGTGCAGAGGGCGCCTCA	The CDSs were inserted into pcDNA3 using *Hind*III/*Xho*I restriction sites. The sequence of p65 is the same as above.

		GACGACGCAGAGGATGGTACCGGAGGAAGTGGCAGCTCTGGCGGCAGTGGAGGGTC TGGTGGCAGCGGA-(p65)-GGAGGAAGTGGCAGCTCTGGCGGCAGTGGA	
	NLS-Q-PAS1-p65	ATGCCCAAGAAGAAGCGCAAGGTGGAAGCTAGCGCTTCCCCGAAGAAAAAGCGGAA AGTCGAGGCCTCCGCATCTCCAAAAAAAAAAAGCAAGGTTGAAGCATCTGGATCCG GAGGCGGTTCCGGTGGCGGTTCTGGCAAGAACATGCAGGCGGTCACCGAGCTGCAT TCCCGGCTGATCGCTGCGCAGCAGGCGATGGAGCGCGACTATTGGCGGTTGCGTGA ATTGGAGACTCGCTACCGCCTGGTGTTCGACGCTGCCGCCGATGCGGTGATGATCG TCTCCGCCGGCGACATGCGCATCGTCGAAGCCAACCGGGCGGCGGTGAATGCGATC AGCCGCGTCGAGCGCGGCAATGACGACCTTGCGGGGCGTGATTTCCTCGCCGAAGT GGCGGCTGCCGATCGCGATGCGGTGCGCGACATGCTGGCCCAGGTGCGTCAGCGCG GCACCGCACTCAGCGTCCTCGTTCATCTCGGCCGTTACGACCGCGCCTGGATGCTG CGCGGTTCGCTGATGTCGTCCGAGCGTCGTCAGGTTTTCCTGCTGCACTTCACCCC GGTGACCACGACTCCCGCGATCGACGACGGTACCGGAGGAAGTGGCAGCTCTGGCG GCAGTGGAGGGTCTGGTGGCAGCGGA-(p65)- GGAGGAAGTGGCAGCTCTGGCGGCAGTGGA	
Detection of nanobody expression in mammalian cells	LDB-3-SNAP	ATGGGATCC-(LDB-3)- GTTAACGGCGGCGGTGGCAGTGACAAAGACTGCGAAATGAAGCGCACCACCCTGGA TAGCCCTCTGGGCAAGCTGGAACTGTCTGGGTGCGAACAGGGCCTGCACCGTATCA TCTTCCTGGGCAAAGGAACATCTGCCGCCGACGCCGTGGAAGTGCCTGCCCCAGCC GCCGTGCTGGGCGGACCAGAGCCACTGATGCAGGCCACCGCCTGGCTCAACGCCTA CTTTCACCAGCCTGAGGCCATCGAGGAGTTCCCTGTGCCAGCCCTGCACCACCCAG TGTTCCAGCAGGAGAGCTTTACCCGCCAGGTGCTGTGGAAACTGCTGAAAGTGGTG AAGTTCGGAGAGGTCATCAGCTACAGCCACCTGGCCGCCCTGGCCGGCAATCCCGC CGCCACCGCCGCCGTGAAAACCGCCCTGAGCGGAAATCCCGTGCCCATTCTGATCC CCTGCCACCGGGTGGTGCAGGGCGACCTGGACGTGGGGGGCTACGAGGGCGGGCTC GCCGTGAAAGAGTGGCTGCTGGCCCACGAGGGCCACAGACTGGGCAAGCCTGGGCT GGGT	The CDS was inserted into pBobi vector using *BamH*I/*Xho*I restriction sites. The sequence of LDB-3 is the same as above.
	LDB-4-SNAP	ATGGGATCC-(LDB-4)-GTTAACGGCGGCGGTGGCAGT-(SNAP)	The CDSs were inserted into pBobi using *BamH*I/*Xho*I restriction sites. The sequences of nanobodies and SNAP are the same as above.
	LDB-6-SNAP	ATGGGATCC-(LDB-6)-GTTAACGGCGGCGGTGGCAGT-(SNAP)	
	LDB-7-SNAP	ATGGGATCC-(LDB-7)-GTTAACGGCGGCGGTGGCAGT-(SNAP)	
	LDB-14-SNAP	ATGGGATCC-(LDB-14)-GTTAACGGCGGCGGTGGCAGT-(SNAP)	
Colocalization in mammalian cells	*Dr*BphP-AcGFP-CAAX	ATGGGATCC-(*Dr*BphP)- GTTGGCTCGAGCGGTGGTGGCGGGAGCGGAGGTATGGTGAGCAAGGGCGCCGAGCT GTTCACCGGCATCGTGCCCATCCTGATCGAGCTGAATGGCGATGTGAATGGCCACA AGTTCAGCGTGAGCGGCGAGGGCGAGGGCGATGCCACCTACGGCAAGCTGACCCTG AAGTTCATCTGCACCACCGGCAAGCTGCCTGTGCCCTGGCCCACCCTGGTGACCAC CCTGAGCTACGGCGTGCAGTGCTTCTCACGCTACCCCGATCACATGAAGCAGCACG ACTTCTTCAAGAGCGCCATGCCTGAGGGCTACATCCAGGAGCGCACCATCTTCTTC GAGGATGACGGCAACTACAAGTCGCGCGCCGAGGTGAAGTTCGAGGGCGATACCCT GGTGAATCGCATCGAGCTGACCGGCACCGATTTCAAGGAGGATGGCAACATCCTGG GCAATAAGATGGAGTACAACTACAACGCCCACAATGTGTACATCATGACCGACAAG GCCAAGAATGGCATCAAGGTGAACTTCAAGATCCGCCACAACATCGAGGATGGCAG CGTGCAGCTGGCCGACCACTACCAGCAGAATACCCCCATCGGCGATGGCCCTGTGC TGCTGCCCGATAACCACTACCTGTCCACCCAGAGCGCCCTGTCCAAGGACCCCAAC GAGAAGCGCGATCACATGATCTACTTCGGCTTCGTGACCGCCGCCGCCATCACCCA CGGCATGGATGAGCTGTACAAGAAGAAAAAAAAgAAGAAAAGCAAAACCAAGTGTG TCATTATG	The CDSs were inserted into pBobi using *BamH*I/*Xho*I restriction sites. The sequence of *Dr*BphP is the same as above.
	LDB-3-mCherry	ATGGGATCC-(LDB-3)- GTTGGCTCGAGCGGTGGTGGCGGGAGCGGAGGTATGGTGAGCAAGGGCGAGGAGGA TAACATGGCCATCATCAAGGAGTTCATGCGCTTCAAGGTGCACATGGAGGGCTCCG TGAACGGCCACGAGTTCGAGATCGAGGGCGAGGGCGAGGGCCGCCCCTACGAGGGC ACCCAGACCGCCAAGCTGAAGGTGACCAAGGGTGGCCCCCTGCCCTTCGCCTGGGA CATCCTGTCCCCTCAGTTCATGTACGGCTCCAAGGCCTACGTGAAGCACCCCGCCG ACATCCCCGACTACTTGAAGCTGTCCTTCCCCGAGGGCTTCAAGTGGGAGCGCGTG ATGAACTTCGAGGACGGCGGCGTGGTGACCGTGACCCAGGACTCCTCCCTGCAGGA CGGCGAGTTCATCTACAAGGTGAAGCTGCGCGGCACCAACTTCCCCTCCGACGGCC CCGTAATGCAGAAGAAGACCATGGGCTGGGAGGCCTCCTCCGAGCGGATGTACCCC GAGGACGGCGCCCTGAAGGGCGAGATCAAGCAGAGGCTGAAGCTGAAGGACGGCGG CCACTACGACGCTGAGGTCAAGACCACCTACAAGGCCAAGAAGCCCGTGCAGCTGC CCGGCGCCTACAACGTCAACATCAAGTTGGACATCACCTCCCACAACGAGGACTAC ACCATCGTGGAACAGTACGAACGCGCCGAGGGCCGCCACTCCACCGGCGGCATGGA CGAGCTGTACAAGTCCGGAAAC	The CDSs were inserted into pBobi using *BamH*I/*Xho*I restriction sites. The sequence of LDB-3 is the same as above.
	pBobi vector	TGACGGATCGGGAGATCTCCCGATCCCCTATGGTCGACTCTCAGTACAATCTGCTC TGATGCCGCATAGTTAAGCCAGTATCTGCTCCCTGCTTGTGTGTTGGAGGTCGCTG AGTAGTGCGCGAGCAAAATTTAAGCTACAACAAGGCAAGGCTTGACCGACAATTGC	

		ATGAAGAATCTGCTTAGGGTTAGGCGTTTTGCGCTGCTTCGCGATGTACGGGCCAG ATATACGCGTTGACATTGATTATTGACTAGTTATTAATAGTAATCAATTACGGGGT CATTAGTTCATAGCCCATATATGGAGTTCCGCGTTACATAACTTACGGTAAATGGC CCGCCTGGCTGACCGCCCAACGACCCCCGCCCATTGACGTCAATAATGACGTATGT TCCCATAGTAACGCCAATAGGGACTTTCCATTGACGTCAATGGGTGGACTATTTAC GGTAAACTGCCCACTTGGCAGTACATCAAGTGTATCATATGCCAAGTACGCCCCCT ATTGACGTCAATGACGGTAAATGGCCCGCCTGGCATTATGCCCAGTACATGACCTT ATGGGACTTTCCTACTTGGCAGTACATCTACGTATTAGTCATCGCTATTACCATGG TGATGCGGTTTTGGCAGTACATCAATGGGCGTGGATAGCGGTTTGACTCACGGGGA TTTCCAAGTCTCCACCCCATTGACGTCAATGGGAGTTTGTTTTGGCACCAAAATCA ACGGGACTTTCCAAAATGTCGTAACAACTCCGCCCCATTGACGCAAATGGGCGGTA GGCGTGTACGGTGGGAGGTCTATATAAGCAGCGCGTTTTGCCTGTACTGGGTCTCT CTGGTTAGACCAGATCTGAGCCTGGGAGCTCTCTGGCTAACTAGGGAACCCACTGC TTAAGCCTCAATAAAGCTTGCCTTGAGTGCTTCAAGTAGTGTGTGCCCGTCTGTTG TGTGACTCTGGTAACTAGAGATCCCTCAGACCCTTTTAGTCAGTGTGGAAAATCTC TAGCAGTGGCGCCCGAACAGGGACCTGAAAGCGAAAGGGAAACCAGAGCTCTCTCG ACGCAGGACTCGGCTTGCTGAAGCGCGCACGGCAAGAGGCGAGGGGCGGCGACTGG TGAGTACGCCAAAAATTTTGACTAGCGGAGGCTAGAAGGAGAGAGATGGGTGCGAG AGCGTCAGTATTAAGCGGGGGAGAATTAGATCGCGATGGGAAAAAATTCGGTTAAG GCCAGGGGGAAAGAAAAAATATAAATTAAAACATATAGTATGGGCAAGCAGGGAGC TAGAACGATTCGCAGTTAATCCTGGCCTGTTAGAAACATCAGAAGGCTGTAGACAA ATACTGGGACAGCTACAACCATCCCTTCAGACAGGATCAGAAGAACTTAGATCATT ATATAATACAGTAGCAACCCTCTATTGTGTGCATCAAAGGATAGAGATAAAAGACA CCAAGGAAGCTTTAGACAAGATAGAGGAAGAGCAAAACAAAAGTAAGACCACCGCA CAGCAAGCGGCCGCTGATCTTCAGACTTGGAGGAGGAGATATGAGGGACAATTGGA GAAGTGAATTATATAAATATAAAGTAGTAAAAATTGAACCATTAGGAGTAGCACCC ACCAAGGCAAAGAGAAGAGTGGTGCAGAGAGAAAAAAGAGCAGTGGGAATAGGAGC TTTGTTCCTTGGGTTCTTGGGAGCAGCAGGAAGCACTATGGGCGCAGCCTCAATGA CGCTGACGGTACAGGCCAGACAATTATTGTCTGGTATAGTGCAGCAGCAGAACAAT TTGCTGAGGGCTATTGAGGCGCAACAGCATCTGTTGCAACTCACAGTCTGGGGCAT CAAGCAGCTCCAAGCAAGAATCCTAGCTGTGGAAAGATACCTAAAGGATCAACAGC TCCTAGGGATTTGGGGTTGCTCTGGAAAACTCATTTGCACCACTGCTGTGCCTTGG AATGCTAGTTGGAGTAATAAATCTCTGGAACAGATCTGGAATCACACGACCTGGAT GGAGTGGGACAGAGAAATTAACAATTACACAAGCTTAATACACTCCTTAATTGAAG AATCGCAAAACCAGCAAGAAAAGAATGAACAAGAATTATTGGAATTAGATAAATGG GCAAGTTTGTGGAATTGGTTTAACATAACAAATTGGCTGTGGTATATAAAATTATT CATAATGATAGTAGGAGGCTTGGTAGGTTTAAGAATAGTTTTTGCTGTACTTTCTA TAGTGAATAGAGTTAGGCAGGGATATTCACCATTATCGTTTCAGACCCACCTCCCA ATCCCGAGGGGACCCGACAGGCCCGAAGGAATAGAAGAAGAAGGTGGAGAGAGAGA CAGAGACAGATCCATTCGATTAGTGAACGGATCAACTTTTAAAAGAAAAGGGGGGA TTGGGGGGTACAGTGCAGGGGAAAGAATAGTAGACATAATAGCAACAGACATACAA ACTAAAGAATTACAAAAACAAATTACAAAAATTCAAAATTTTATCGATAAGCTTGG GAGTTCCGCGTTACATAACTTACGGTAAATGGCCCGCCTGGCTGACCGCCCAACGA CCCCCGCCCATTGACGTCAATAATGACGTATGTTCCCATAGTAACGCCAATAGGGA CTTTCCATTGACGTCAATGGGTGGAGTATTTACGGTAAACTGCCCACTTGGCAGTA CATCAAGTGTATCATATGCCAAGTACGCCCCCTATTGACGTCAATGACGGTAAATG GCCCGCCTGGCATTATGCCCAGTACATGACCTTATGGGACTTTCCTACTTGGCAGT ACATCTACGTATTAGTCATCGCTATTACCATGGTGATGCGGTTTTGGCAGTACATC AATGGGCGTGGATAGCGGTTTGACTCACGGGGATTTCCAAGTCTCCACCCCATTGA CGTCAATGGGAGTTTGTTTTGGCACCAAAATCAACGGGACTTTCCAAAATGTCGTA ACAACTCCGCCCCATTGACGCAAATGGGCGGTAGGCGTGTACGGTGGGAGGTCTAT ATAAGCAGAGCTCGTTTAGTGAACCGTCAGATCGCCTGGAGACGCCATCCACGCTG TTTTGACCTCCATAGAAGACACCGACTGAGCTCCTTAAGGTTGCCACCATGGGATC CCTCGAGGGTGGTGGTTCCGGTGAGCAGAAGCTGATCTCAGAGGAGGACCTGTGAT CGAGCCATGGAAGCTTGATATCTAACTGACTGAACCGGTGGTACCGAGGAATTAAT TCGCTGTCTGCGAGGGCCAGCTGTTGGGGTGAGTACTCCCTCTCAAAAGCGGGCAT GACTTCTGCGCTAAGATTGTCAGTTTCCAAAAACGAGGAGGATTTGATATTCACCT GGCCCGCGGTGATGCCTTTGAGGGTGGCCGCGTCCATCTGGTCAGAAAAGACAATC TTTTTGTTGTCAAGCTTGAGGTGTGGCAGGCTTGAGATCTGGCCATACACTTGAGT GACAATGACATCCACTTTGCCTTTCTCTCCACAGGTGTCCACTCCCAGGTCCAACT GCAGGTCGAGCATGCATCTAGGGCGGCCAATTCCGCCGATCCTGGAAAAACATGGA GCAATCACAAGTAGCAATACAGCAGCTACCAATGCTGATTGTGCCTGGCTAGAAGC ACAAGAGGAGGAGGAGGTGGGTTTTCCAGTCACACCTCAGACAATCAACCTCTGGA TTACAAAATTTGTGAAAGATTGACTGGTATTCTTAACTATGTTGCTCCTTTTACGC TATGTGGATACGCTGCTTTAATGCCTTTGTATCATGCTATTGCTTCCCGTATGGCT TTCATTTTCTCCTCCTTGTATAAATCCTGGTTGCTGTCTCTTTATGAGGAGTTGTG GCCCGTTGTCAGGCAACGTGGCGTGGTGTGCACTGTGTTTGCTGACGCAACCCCCA CTGGTTGGGGCATTGCCACCACCTGTCAGCTCCTTTCCGGGACTTTCGCTTTCCCC CTCCCTATTGCCACGGCGGAACTCATCGCCGCCTGCCTTGCCCGCTGCTGGACAGG GGCTCGGCTGTTGGGCACTGACAATTCCGTGGTGTTGTCGGGGAAGCTGACGTCCT TTCCATGGCTGCTCGCCTGTGTTGCCACCTGGATTCTGCGCGGGACGTCCTTCTGC	

		TACGTCCCTTCGGCCCTCAATCCAGCGGACCTTCCTTCCCGCGGCCTGCTGCCGGC TCTGCGGGCCTCTTCCGCGTCTTCGCCTTCGCCCTCAGACGAGTCGGATCTCCCTTT GGGCCGCCTCCCCGCCTGGAATTCGAGCTCGGTACCTTTAAGACCAATGACTTACA AGGCAGCTGTAGATCTTAGCCACTTTTTAAAAGAAAAGGGGGGACTGGAAGGGCTA ATTCACTCCCAAAGAAGACAAGATATCCTTGATCTGTGGATCTACCACACACAAGG CTACTTCCCTGATTGACAGAACTACACACCAGGGCCAGGGGTCAGATATCCACTGA CCTTTGGATGGTGCTACAAGCTAGTACCAGTTGAGCCAGATAAGATAGAAGAGGCC AATAAAGGAGAGAACACCAGCTTGTTACACCCTGTGAGCCTGCATGGGATGGATGA CCCGGAGAGAGAAGTGTTAGAGTGGAGGTTTGACAGCCGCCTAGCATTTCATCACG TGGCCCGAGAGCTGCATCCGGACTGTACTGGGTCTCTCTGGTTAGACCAGATCTGA GCCTGGGAGCTCTCTGGCTAACTAGGGAACCCACTGCTTAAGCCTCAATAAAGCTT GCCTTGAGTGCTTCAAGTAGTGTGTGCCCGTCTGTTGTGTGACTCTGGTAACTAGA GATCCCTCAGACCCTTTTAGTCAGTGTGGAAAATCTCTAGCAGGGCCGCTTTAAAC CCGCTGATCAGCCTCGACTGTGCCTTCTAGTTGCCAGCCATCTGTTGTTTGCCCCT CCCCCGTGCCTTCCTTGACCCTGGAAGGTGCCACTCCCACTGTCCTTTCCTAATAA AATGAGGAAATTGCATCGCATTGTCTGAGTAGGTGTCATTCTATTCTGGGGGGTGG GGTGGGGCAGGACAGCAAGGGGGAGGATTGGGAAGACAATAGCAGGCATGCTGGGG ATGCGGTGGGCTCTATGGCTTCTGAGGCGGAAAGAACCAGCTGGGGCTCTAGGGGG TATCCCCACGCGCCCTGTAGCGGCGCATTAAGCGCGGCGGGTGTGGTGGTTACGCG CAGCGTGACCGCTACACTTGCCAGCGCCCTAGCGCCCGCTCCTTTCGCTTTCTTCC CTTCCTTTCTCGCCACGTTCGCCGGCTTTCCCCGTCAAGCTCTAAATCGGGGCATC CCTTTAGGGTTCCGATTTAGTGCTTTACGGCACCTCGACCCCAAAAAACTTGATTA GGGTGATGGTTCACGTAGTGGGCCATCGCCCTGATAGACGGTTTTTCGCCCTTTGA CGTTGGAGTCCACGTTCTTTAATAGTGGACTCTTGTTCCAAACTGGAACAACACTC AACCCTATCTCGGTCTATTCTTTTGATTTATAAGGGATTTTGGGGATTTCGGCCTA TTGGTTAAAAAATGAGCTGATTTAACAAAAATTTAACGCGAATTAATTCTGTGGAA TGTGTGTCAGTTAGGGTGTGGAAAGTCCCCAGGCTCCCCAGGCAGGCAGAAGTATG CAAAGCATGCATCTCAATTAGTCAGCAACCAGGTGTGGAAAGTCCCCAGGCTCCCC AGCAGGCAGAAGTATGCAAAGCATGCATCTCAATTAGTCAGCAACCATAGTCCCGC CCCTAACTCCGCCCATCCCGCCCCTAACTCCGCCCAGTTCCGCCCATTCTCCGCCC CATGGCTGACTAATTTTTTTTATTTATGCAGAGGCCGAGGCCGCCTCTGCCTCTGA GCTATTCCAGAAGTAGTGAGGAGGCTTTTTTGGAGGCCTAGGCTTTTGCAAAAAGC TCCCGGGAGCTTGTATATCCATTTTCGGATCTGATCAGCACGTGTTGACAATTAAT CATCGGCATAGTATATCGGCATAGTATAATACGACAAGGTGAGGAACTAAACCATG GCCAAGTTGACCAGTGCCGTTCCGGTGCTCACCGCGCGCGACGTCGCCGGAGCGGT CGAGTTCTGGACCGACCGGCTCGGGTTCTCCCGGGACTTCGTGGAGGACGACTTCG CCGGTGTGGTCCGGGACGACGTGACCCTGTTCATCAGCGCGGTCCAGGACCAGGTG GTGCCGGACAACACCCTGGCCTGGGTGTGGGTGCGCGGCCTGGACGAGCTGTACGC CGAGTGGTCGGAGGTCGTGTCCACGAACTTCCGGGACGCCTCCGGGCCGGCCATGA CCGAGATCGGCGAGCAGCCGTGGGGGCGGGAGTTCGCCCTGCGCGACCCGGCCGGC AACTGCGTGCACTTCGTGGCCGAGGAGCAGGACTGACACGTGCTACGAGATTTCGA TTCCACCGCCGCCTTCTATGAAAGGTTGGGCTTCGGAATCGTTTTCCGGGACGCCG GCTGGATGATCCTCCAGCGCGGGGATCTCATGCTGGAGTTCTTCGCCCACCCCAAC TTGTTTATTGCAGCTTATAATGGTTACAAATAAAGCAATAGCATCACAAATTTCAC AAATAAAGCATTTTTTTCACTGCATTCTAGTTGTGGTTTGTCCAAACTCATCAATG TATCTTATCATGTCTGTATACCGTCGACCTCTAGCTAGAGCTTGGCGTAATCATGG TCATAGCTGTTTCCTGTGTGAAATTGTTATCCGCTCACAATTCCACACAACATACG AGCCGGAAGCATAAAGTGTAAAGCCTGGGGTGCCTAATGAGTGAGCTAACTCACAT TAATTGCGTTGCGCTCACTGCCCGCTTTCCAGTCGGGAAACCTGTCGTGCCAGCTG CATTAATGAATCGGCCAACGCGCGGGGAGAGGCGGTTTGCGTATTGGGCGCTCTTC CGCTTCCTCGCTCACTGACTCGCTGCGCTCGGTCGTTCGGCTGCGGCGAGCGGTAT CAGCTCACTCAAAGGCGGTAATACGGTTATCCACAGAATCAGGGGATAACGCAGGA AAGAACATGTGAGCAAAAGGCCAGCAAAAGGCCAGGAACCGTAAAAAGGCCGCGTT GCTGGCGTTTTTCCATAGGCTCCGCCCCCCTGACGAGCATCACAAAAATCGACGCT CAAGTCAGAGGTGGCGAAACCCGACAGGACTATAAAGATACCAGGCGTTTCCCCCT GGAAGCTCCCTCGTGCGCTCTCCTGTTCCGACCCTGCCGCTTACCGGATACCTGTC CGCCTTTCTCCCTTCGGGAAGCGTGGCGCTTTCTCAATGCTCACGCTGTAGGTATC TCAGTTCGGTGTAGGTCGTTCGCTCCAAGCTGGGCTGTGTGCACGAACCCCCCGTT CAGCCCGACCGCTGCGCCTTATCCGGTAACTATCGTCTTGAGTCCAACCCGGTAAG ACACGACTTATCGCCACTGGCAGCAGCCACTGGTAACAGGATTAGCAGAGCGAGGT ATGTAGGCGGTGCTACAGAGTTCTTGAAGTGGTGGCCTAACTACGGCTACACTAGA AGGACAGTATTTGGTATCTGCGCTCTGCTGAAGCCAGTTACCTTCGGAAAAAGAGT TGGTAGCTCTTGATCCGGCAAACAAACCACCGCTGGTAGCGGTGGTTTTTTTGTTT GCAAGCAGCAGATTACGCGCAGAAAAAAAGGATCTCAAGAAGATCCTTTGATCTTT TCTACGGGGTCTGACGCTCAGTGGAACGAAAACTCACGTTAAGGGATTTTGGTCAT GAGATTATCAAAAAGGATCTTCACCTAGATCCTTTTAAATTAAAAATGAAGTTTTA AATCAATCTAAAGTATATATGAGTAAACTTGGTCTGACAGTTACCAATGCTTAATC AGTGAGGCACCTATCTCAGCGATCTGTCTATTTCGTTCATCCATAGTTGCCTGACT CCCCGTCGTGTAGATAACTACGATACGGGAGGGCTTACCATCTGGCCCCAGTGCTG CAATGATACCGCGAGACCCACGCTCACCGGCTCCAGATTTATCAGCAATAAACCAG CCAGCCGGAAGGGCCGAGCGCAGAAGTGGTCCTGCAACTTTATCCGCCTCCATCCA	

		GTCTATTAATTGTTGCCGGGAAGCTAGAGTAAGTAGTTCGCCAGTTAATAGTTTGC GCAACGTTGTTGCCATTGCTACAGGCATCGTGGTGTCACGCTCGTCGTTTGGTATG GCTTCATTCAGCTCCGGTTCCCAACGATCAAGGCGAGTTACATGATCCCCCATGTT GTGCAAAAAAGCGGTTAGCTCCTTCGGTCCTCCGATCGTTGTCAGAAGTAAGTTGG CCGCAGTGTTATCACTCATGGTTATGGCAGCACTGCATAATTCTCTTACTGTCATG CCATCCGTAAGATGCTTTTCTGTGACTGGTGAGTACTCAACCAAGTCATTCTGAGA ATAGTGTATGCGGCGACCGAGTTGCTCTTGCCCGGCGTCAATACGGGATAATACCG CGCCACATAGCAGAACTTTAAAAGTGCTCATCATTGGAAAACGTTCTTCGGGGCGA AAACTCTCAAGGATCTTACCGCTGTTGAGATCCAGTTCGATGTAACCCACTCGTGC ACCCAACTGATCTTCAGCATCTTTTACTTTCACCAGCGTTTCTGGGTGAGCAAAAA CAGGAAGGCAAAATGCCGCAAAAAAGGGAATAAGGGCGACACGGAAATGTTGAATA CTCATACTCTTCCTTTTTCAATATTATTGAAGCATTTATCAGGGTTATTGTCTCAT GAGCGGATACATATTTGAATGTATTTAGAAAAATAAACAAATAGGGGTTCCGCGCA CATTTCCCCGAAAAGTGCCACCTGACGTC	
	pLGSA vector	AGATCTCGATCCCGCGAAATTAATACGACTCACTATAGGGGAATTGTGAGCGGATA ACAATTCCCCTCTAGAAATAATTTTGTTTAACTTTAAGAAGGAGATATACATATGG GCTCGAGCGGCGGATCCGACAAAGACTGCGAAATGAAGCGCACCACCCTGGATAGC CCTCTGGGCAAGCTGGAACTGTCTGGGTGCGAACAGGGCCTGCACCGTATCATCTT CCTGGGCAAAGGAACATCTGCCGCCGACGCCGTGGAAGTGCCTGCCCCAGCCGCCG TGCTGGGCGGACCAGAGCCACTGATGCAGGCCACCGCCTGGCTCAACGCCTACTTT CACCAGCCTGAGGCCATCGAGGAGTTCCCTGTGCCAGCCCTGCACCACCCAGTGTT CCAGCAGGAGAGCTTTACCCGCCAGGTGCTGTGGAAACTGCTGAAAGTGGTGAAGT TCGGAGAGGTCATCAGCTACAGCCACCTGGCCGCCCTGGCCGGCAATCCCGCCGCC ACCGCCGCCGTGAAAACCGCCCTGAGCGGAAATCCCGTGCCCATTCTGATCCCCTG CCACCGGGTGGTGCAGGGCGACCTGGACGTGGGGGGCTACGAGGGCGGGCTCGCCG TGAAAGAGTGGCTGCTGGCCCACGAGGGCCACAGACTGGGCAAGCCTGGGCTGGGT CCGGGAGGCCAAAGCTTATCCACCCCGCCGACCCCGAGCACTCCTCCTACCGGTCT GAACGACATCTTCGAGGCTCAGAAAATCGAATGGCACGAACTCGAGCACCACCACC ACCACCACTGAGATCCGGCTGCTAACAAAGCCCGAAAGGAAGCTGAGTTGGCTGCT GCCACCGCTGAGCAATAACTAGCATAACCCCTTGGGGCCTCTAAACGGGTCTTGAG GGGTTTTTTGCTGAAAGGAGGAACTATATCCGGATTGGCGAATGGGACGCGCCCTG TAGCGGCGCATTAAGCGCGGCGGGTGTGGTGGTTACGCGCAGCGTGACCGCTACAC TTGCCAGCGCCCTAGCGCCCGCTCCTTTCGCTTTCTTCCCTTCCTTTCTCGCCACG TTCGCCGGCTTTCCCCGTCAAGCTCTAAATCGGGGGCTCCCTTTAGGGTTCCGATT TAGTGCTTTACGGCACCTCGACCCCAAAAAACTTGATTAGGGTGATGGTTCACGTA GTGGGCCATCGCCCTGATAGACGGTTTTTCGCCCTTTGACGTTGGAGTCCACGTTC TTTAATAGTGGACTCTTGTTCCAAACTGGAACAACACTCAACCCTATCTCGGTCTA TTCTTTTGATTTATAAGGGATTTTGCCGATTTCGGCCTATTGGTTAAAAAATGAGC TGATTTAACAAAAATTTAACGCGAATTTTAACAAAATATTAACGTTTACAATTTCA GGTGGCACTTTTCGGGGAAATGTGCGCGGAACCCCTATTTGTTTATTTTTCTAAAT ACATTCAAATATGTATCCGCTCATGAATTAATTCTTAGAAAAACTCATCGAGCATC AAATGAAACTGCAATTTATTCATATCAGGATTATCAATACCATATTTTTGAAAAAG CCGTTTCTGTAATGAAGGAGAAAACTCACCGAGGCAGTTCCATAGGATGGCAAGAT CCTGGTATCGGTCTGCGATTCCGACTCGTCCAACATCAATACAACCTATTAATTTC CCCTCGTCAAAAATAAGGTTATCAAGTGAGAAATCACCATGAGTGACGACTGAATC CGGTGAGAATGGCAAAAGTTTATGCATTTCTTTCCAGACTTGTTCAACAGGCCAGC CATTACGCTCGTCATCAAAATCACTCGCATCAACCAAACCGTTATTCATTCGTGAT TGCGCCTGAGCGAGACGAAATACGCGATCGCTGTTAAAAGGACAATTACAAACAGG AATCGAATGCAACCGGCGCAGGAACACTGCCAGCGCATCAACAATATTTTCACCTG AATCAGGATATTCTTCTAATACCTGGAATGCTGTTTTCCCGGGGATCGCAGTGGTG AGTAACCATGCATCATCAGGAGTACGGATAAAATGCTTGATGGTCGGAAGAGGCAT AAATTCCGTCAGCCAGTTTAGTCTGACCATCTCATCTGTAACATCATTGGCAACGC TACCTTTGCCATGTTTCAGAAACAACTCTGGCGCATCGGGCTTCCCATACAATCGA TAGATTGTCGCACCTGATTGCCCGACATTATCGCGAGCCCATTTATACCCATATAA ATCAGCATCCATGTTGGAATTTAATCGCGGCCTAGAGCAAGACGTTTCCCGTTGAA TATGGCTCATAACACCCCTTGTATTACTGTTTATGTAAGCAGACAGTTTTATTGTT CATGACCAAAATCCCTTAACGTGAGTTTTCGTTCCACTGAGCGTCAGACCCCGTAG AAAAGATCAAAGGATCTTCTTGAGATCCTTTTTTTCTGCGCGTAATCTGCTGCTTG CAAACAAAAAAACCACCGCTACCAGCGGTGGTTTGTTTGCCGGATCAAGAGCTACC AACTCTTTTTCCGAAGGTAACTGGCTTCAGCAGAGCGCAGATACCAAATACTGTCC TTCTAGTGTAGCCGTAGTTAGGCCACCACTTCAAGAACTCTGTAGCACCGCCTACA TACCTCGCTCTGCTAATCCTGTTACCAGTGGCTGCTGCCAGTGGCGATAAGTCGTG TCTTACCGGGTTGGACTCAAGACGATAGTTACCGGATAAGGCGCAGCGGTCGGGCT GAACGGGGGGTTCGTGCACACAGCCCAGCTTGGAGCGAACGACCTACACCGAACTG AGATACCTACAGCGTGAGCTATGAGAAAGCGCCACGCTTCCCGAAGGGAGAAAGGC GGACAGGTATCCGGTAAGCGGCAGGGTCGGAACAGGAGAGCGCACGAGGGAGCTTC CAGGGGGAAACGCCTGGTATCTTTATAGTCCTGTCGGGTTTCGCCACCTCTGACTT GAGCGTCGATTTTTGTGATGCTCGTCAGGGGGGCGGAGCCTATGGAAAAACGCCAG CAACGCGGCCTTTTTACGGTTCCTGGCCTTTTGCTGGCCTTTTGCTCACATGTTCT TTCCTGCGTTATCCCCTGATTCTGTGGATAACCGTATTACCGCCTTTGAGTGAGCT GATACCGCTCGCCGCAGCCGAACGACCGAGCGCAGCGAGTCAGTGAGCGAGGAAGC	

		GGAAGAGCGCCTGATGCGGTATTTTCTCCTTACGCATCTGTGCGGTATTTCACACC GCATATATGGTGCACTCTCAGTACAATCTGCTCTGATGCCGCATAGTTAAGCCAGT ATACACTCCGCTATCGCTACGTGACTGGGTCATGGCTGCGCCCCGACACCCGCCAA CACCCGCTGACGCGCCCTGACGGGCTTGTCTGCTCCCGGCATCCGCTTACAGACAA GCTGTGACCGTCTCCGGGAGCTGCATGTGTCAGAGGTTTTCACCGTCATCACCGAA ACGCGCGAGGCAGCTGCGGTAAAGCTCATCAGCGTGGTCGTGAAGCGATTCACAGA TGTCTGCCTGTTCATCCGCGTCCAGCTCGTTGAGTTTCTCCAGAAGCGTTAATGTC TGGCTTCTGATAAAGCGGGCCATGTTAAGGGCGGTTTTTTCCTGTTTGGTCACTGA TGCCTCCGTGTAAGGGGGATTTCTGTTCATGGGGGTAATGATACCGATGAAACGAG AGAGGATGCTCACGATACGGGTTACTGATGATGAACATGCCCGGTTACTGGAACGT TGTGAGGGTAAACAACTGGCGGTATGGATGCGGCGGGACCAGAGAAAAATCACTCA GGGTCAATGCCAGCGCTTCGTTAATACAGATGTAGGTGTTCCACAGGGTAGCCAGC AGCATCCTGCGATGCAGATCCGGAACATAATGGTGCAGGGCGCTGACTTCCGCGTT TCCAGACTTTACGAAACACGGAAACCGAAGACCATTCATGTTGTTGCTCAGGTCGC AGACGTTTTGCAGCAGCAGTCGCTTCACGTTCGCTCGCGTATCGGTGATTCATTCT GCTAACCAGTAAGGCAACCCCGCCAGCCTAGCCGGGTCCTCAACGACAGGAGCACG ATCATGCGCACCCGTGGGGCCGCCATGCCGGCGATAATGGCCTGCTTCTCGCCGAA ACGTTTGGTGGCGGGACCAGTGACGAAGGCTTGAGCGAGGGCGTGCAAGATTCCGA ATACCGCAAGCGACAGGCCGATCATCGTCGCGCTCCAGCGAAAGCGGTCCTCGCCG AAAATGACCCAGAGCGCTGCCGGCACCTGTCCTACGAGTTGCATGATAAAGAAGAC AGTCATAAGTGCGGCGACGATAGTCATGCCCCGCGCCCACCGGAAGGAGCTGACTG GGTTGAAGGCTCTCAAGGGCATCGGTCGAGATCCCGGTGCCTAATGAGTGAGCTAA CTTACATTAATTGCGTTGCGCTCACTGCCCGCTTTCCAGTCGGGAAACCTGTCGTG CCAGCTGCATTAATGAATCGGCCAACGCGCGGGGAGAGGCGGTTTGCGTATTGGGC GCCAGGGTGGTTTTTCTTTTCACCAGTGAGACGGGCAACAGCTGATTGCCCTTCAC CGCCTGGCCCTGAGAGAGTTGCAGCAAGCGGTCCACGCTGGTTTGCCCCAGCAGGC GAAAATCCTGTTTGATGGTGGTTAACGGCGGGATATAACATGAGCTGTCTTCGGTA TCGTCGTATCCCACTACCGAGATATCCGCACCAACGCGCAGCCCGGACTCGGTAAT GGCGCGCATTGCGCCCAGCGCCATCTGATCGTTGGCAACCAGCATCGCAGTGGGAA CGATGCCCTCATTCAGCATTTGCATGGTTTGTTGAAAACCGGACATGGCACTCCAG TCGCCTTCCCGTTCCGCTATCGGCTGAATTTGATTGCGAGTGAGATATTTATGCCA GCCAGCCAGACGCAGACGCGCCGAGACAGAACTTAATGGGCCCGCTAACAGCGCGA TTTGCTGGTGACCCAATGCGACCAGATGCTCCACGCCCAGTCGCGTACCGTCTTCA TGGGAGAAAATAATACTGTTGATGGGTGTCTGGTCAGAGACATCAAGAAATAACGC CGGAACATTAGTGCAGGCAGCTTCCACAGCAATGGCATCCTGGTCATCCAGCGGAT AGTTAATGATCAGCCCACTGACGCGTTGCGCGAGAAGATTGTGCACCGCCGCTTTA CAGGCTTCGACGCCGCTTCGTTCTACCATCGACACCACCACGCTGGCACCCAGTTG ATCGGCGCGAGATTTAATCGCCGCGACAATTTGCGACGGCGCGTGCAGGGCCAGAC TGGAGGTGGCAACGCCAATCAGCAACGACTGTTTGCCCGCCAGTTGTTGTGCCACG CGGTTGGGAATGTAATTCAGCTCCGCCATCGCCGCTTCCACTTTTTCCCGCGTTTT CGCAGAAACGTGGCTGGCCTGGTTCACCACGCGGGAAACGGTCTGATAAGAGACAC CGGCATACTCTGCGACATCGTATAACGTTACTGGTTTCACATTCACCACCCTGAAT TGACTCTCTTCCGGGCGCTATCATGCCATACCGCGAAAGGTTTTGCGCCATTCGAT GGTGTCCGGGATCTCGACGCTCTCCCTTATGCGACTCCTGCATTAGGAAGCAGCCC AGTAGTAGGTTGAGGCCGTTGAGCACCGCCGCCGCAAGGAATGGTGCATGCAAGGA GATGGCGCCCAACAGTCCCCCGGCCACGGGGCCTGCCACCATACCCACGCCGAAAC AAGCGCTCATGAGCCCGAAGTGGCGAGCCCGATCTTCCCCATCGGTGATGTCGGCG ATATAGGCGCCAGCAACCGCACCTGTGGCGCCGGTGATGCCGGCCACGATGCGTCC GGCGTAGAGGATCG	

SUPPLEMENTARY NOTE

Thermodynamic modeling of competitive hetero- and homo-dimerization

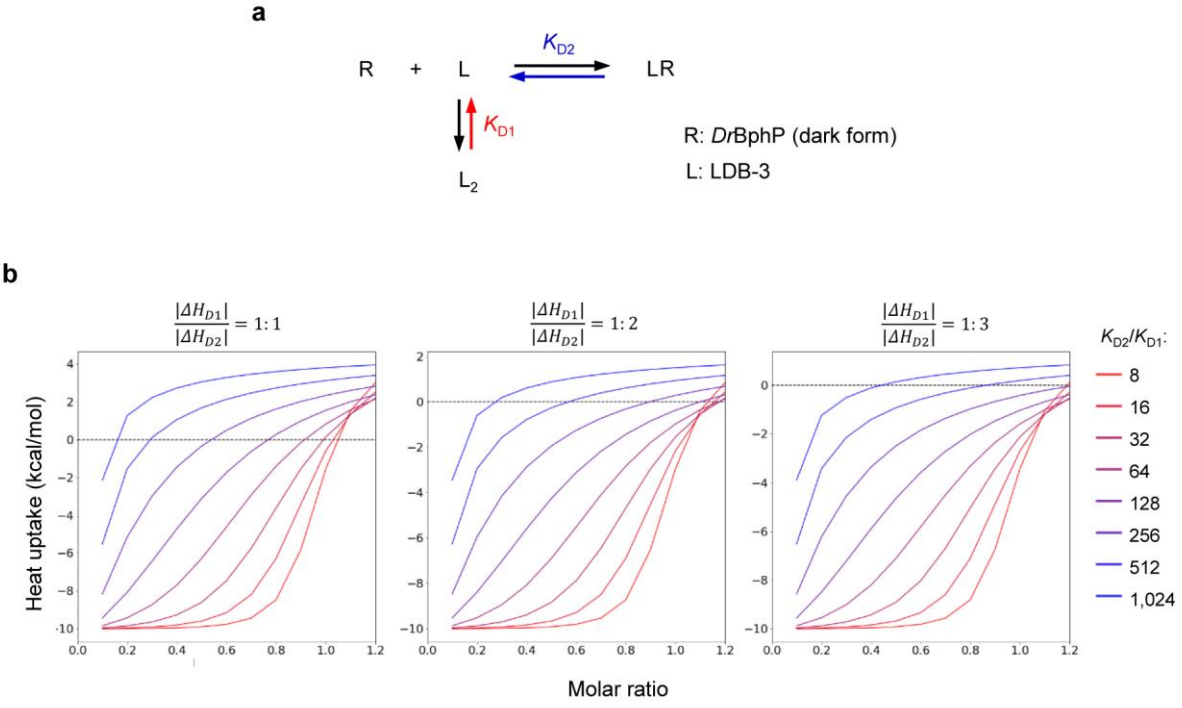

Figure S15. (a) Two-state equilibrium model used in the thermodynamic simulation. (**b**) Thermographs showing the integration of heat transfer in simulated titration experiments.

A simplified thermodynamic model was used to understand the observed transition of heat transfer from heat release to absorption when titrating LDB-3 to the dark-form DrBphP (Fig. 5a). We assume that the dark-form *Dr*BphP–LDB-3 binding and LDB-3 dimerization are competitive (Fig. S12a): $2L \leftrightarrow L_2$, and $L + R \leftrightarrow LR$, where L represents the monomeric LDB-3 and R the dark-form *Dr*BphP. The dissociation constants are $K_{\mathrm{D1}} = \frac{[L]^2}{[L_2]} = \frac{1}{K_{a1}}$ and $K_{\mathrm{D2}} = \frac{[L][R]}{[LR]} = \frac{1}{K_{a2}}$, where [L], [$L_2$], [R], and [LR] are equilibrium concentrations, and K_{a1} and K_{a2} are association constants. The relationships of these concentrations are $[L_T] = [L] + [LR] + 2[L_2]$, and $[R_T] = [R] + [LR]$, where [L_T] represents the initial total concentration of LDB-3 and [R_T] represents the initial total concentration of *Dr*BphP. So, the equilibrium dissociation constants can also be expressed as $K_{D1} = \frac{([L_T]-2[L_2]-[LR])^2}{[L_2]}$, and $K_{D2} = \frac{([L_T]-2[L_2]-[LR])([R_T]-[LR])}{[LR]}$. [$L_2$] and [LR] could be determined if [K_{D1}], [K_{D2}], [L_T] and [R_T] are known.

The equilibrium dissociation constant is associated with the Gibbs energy of dissociation, ΔG_D, and can be expressed in terms of the enthalpy(ΔH_D) and entropy (ΔS_D) changes in the process: $\Delta G_D = -RT \ln K_D = \Delta H_D - T\Delta S_D$. During the ITC assay, we assume that the whole heat transfer is the sum of

ΔH_{D1} and ΔH_{D2} which could be calculated by concentration changes of each component using above equations. To simulate a titration process, the dissociation of the LDB-3 homodimer was set to be *endothermic* ($\Delta H_{D1} > 0$) while the formation of the LDB-3-*Dr*BphP complex was *exothermic* ($\Delta H_{D2} < 0$), which is consistent with our experimental results (Figs. 5a and S7). To calculate heat transfer of the whole system, K_{D2}/K_{D1} was set as a variable, and $\frac{|\Delta H_{D1}|}{|\Delta H_{D2}|}$ was set to be 1:1, 1:2, or 1:3. Thermographs were generated to show the integration of heat transfer in an titration experiment. Below is the command used to run the simulation:

```
import os
import os.path
import sys
from scipy.optimize import fsolve
from matplotlib import pyplot as plt

n_point = 13 # number of drops
L2_lst = [] # the concentration of L2 after each drop
LR_lst = [] # the concentration of LR after each drop
R_lst = [] # the concentration of R after each drop
L_lst = [] # the concentration of L after each drop
for i in range(0, n_point):
      ka1 = 1e5 #ka1
      ka2 = 200 #ka2
      R0 = 1 #Rt
      if i == 0:
            L0 = R0 *  1e-6 / 10 #Lt, avoid 0 in calculation
      else:
            L0 = R0 *  i / 10 # Lt
      results  =  solve([Eq(L2-ka1  *  (L0-2*L2-LR)*(L0-2*L2-LR),  0),  Eq(LR-
ka2*(R0-LR)*(L0-2*L2-LR),0)], [L2, LF]) # solve equations
      L2_lst.append(results[0][0].as_real_imag()[0])
      LR_lst.append(results[0][1].as_real_imag()[0])
      R_lst.append(R0-results[0][1].as_real_imag()[0])
      L_lst.append((L0-results[0][1].as_real_imag()[0]-
2*results[0][0].as_real_imag()[0])/2)

delta_L2_lst = [] #the change of concentration of L2 between two drops
delta_LR_lst = [] #the change of concentration of LR between two drops
delta_R_lst = [] #the change of concentration of R between two drops
delta_L_lst = [] #the change of concentration of L between two drops

for i in range(1, len(L2_lst)):
      delta_L2_lst.append(L2_lst[i]-L2_lst[i-1])
      delta_LR_lst.append(LR_lst[i]-LR_lst[i-1])
      delta_R_lst.append(R_lst[i]-RF_lst[i-1])
      delta_L_lst.append(L_lst[i]-L_lst[i-1])

dLR = -100 #the heat change of L+R->LR
dL2 = 50 # the heat change of L+L->L2
x_lst = list(range(1, n_point)) #molar ratio
for i in range(len(x_lst)):
      x_lst[i] = float(x_lst[i])/10
H_lst = [] #data change
```

```
for i in range(len(delta_L2_lst)):
      H_lst.append(dLR * delta_LR_lst[i] + dL2 * delta_L2_lst[i])

# generate plots
plt.figure(figsize=(10,10))
plt.plot(x_lst, H_lst)
plt.xlabel("Molar Ratio")
plt.ylabel("Heat uptake")
```

The simulation result showed that the clear transition from the heat release to absorption was found when $K_{D2} >> K_{D1}$ (e.g., $K_{D2}/K_{D1} > 100$). The LDB-3 dimer is expected to a relatively weak complex because, in the SEC experiment, a large percentage of the dimer was dissociated at the low-µM concentrations (Figure S8a). Based on our simulation and observed data (Fig. 5a), the dark-form *Dr*BphP–LDB-3 complex (K_{D2}) should be much weaker than the LDB-3 dimer (K_{D1}), 3. It should be noted that this simplified model did not consider *Dr*BphP dimerization and possible binding cooperativity in higher-order complexes. The fitting of the dark form binding data was found to be difficult due to the complexity of protein-protein interactions, so we did not calculate the K_D^{app}. Nevertheless, the ITC experimental data and the modeling supports the low dark activity of LDB-3 observed in other assays.

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