Supplementary Information for

Influence of the N-terminal segment and the PHY-tongue element on light-regulation in bacteriophytochromes.

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Figures S1 to S4

Table S1

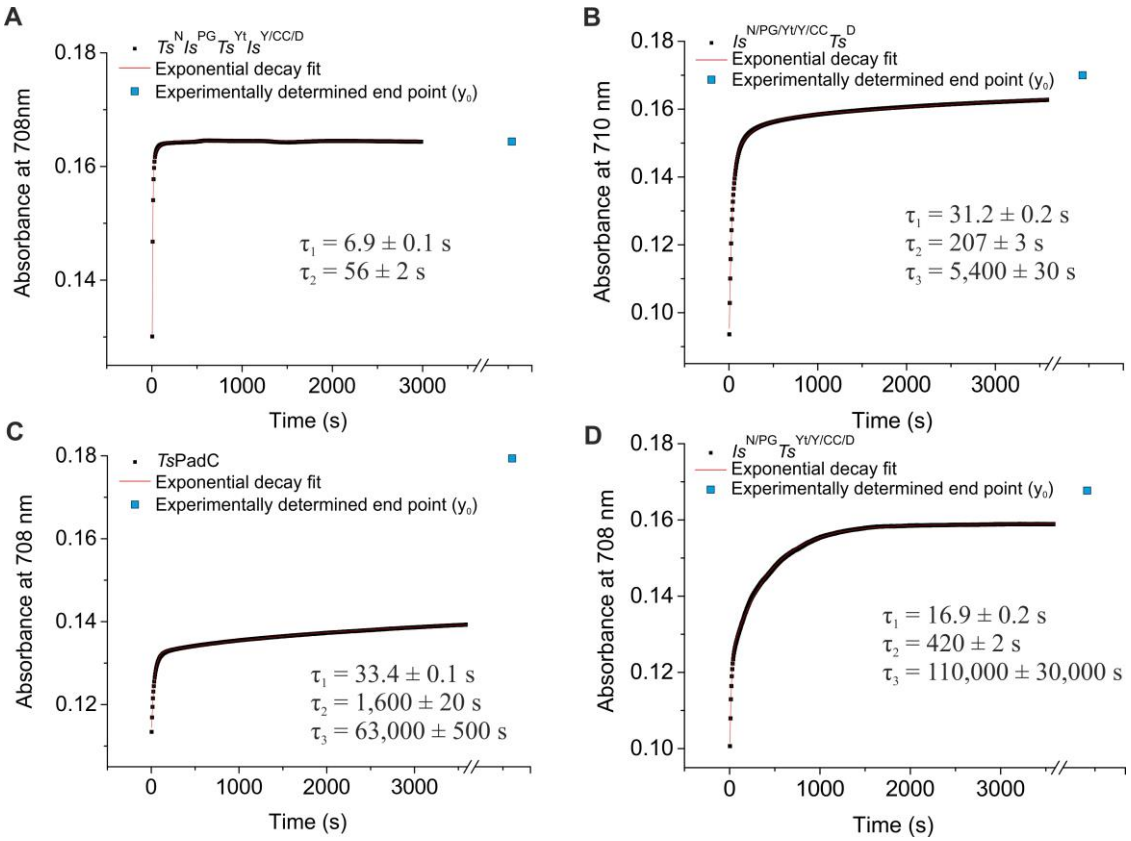

Figure S1. Exponential fitting of representative thermal recoveries. Panels **(A)** to **(D)** represent dark state recovery kinetics of representative constructs featuring very fast to very slow thermal recoveries. Time traces correspond to the maximum absorbance of the Q-band in Pr after 1 min red light illumination for a protein sample of 2 µM equilibrated at 20 °C. Absorbance values were recorded every 5 sec and fitted using the sum of two or three exponential decay functions with the additional restraint of a fixed end point (y_0) extracted from the previously measured dark state spectrum (More details in the *Experimental Procedures*).

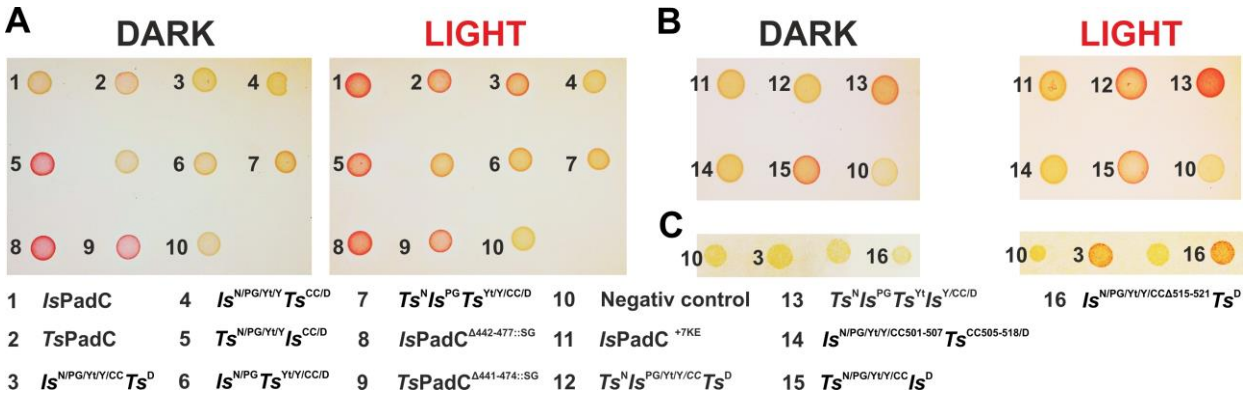

Figure S2. *In vivo* screening of DGC activity. From (**A**) to (**C**) are six different screening plates from where individual colonies have been extracted for Fig. 1 to facilitate the comparison of constructs. Colonies without number associated are constructs which are not discussed further in this study.

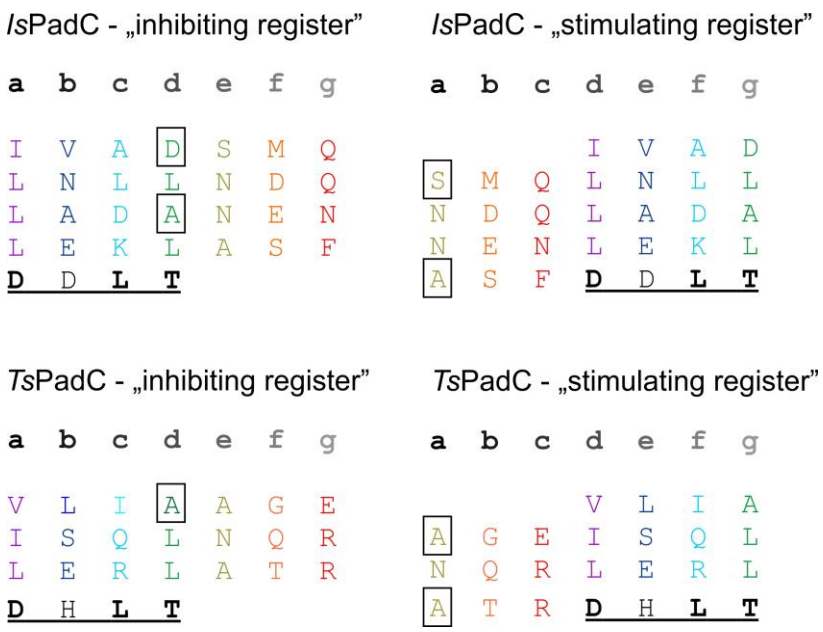

Figure S3. *Is*PadC and *Ts*PadC coiled-coil linker registers. Schematic representation of the inhibiting and stimulating coiled-coil register of *Is*PadC and *Ts*PadC. Destabilizing residues are boxed.

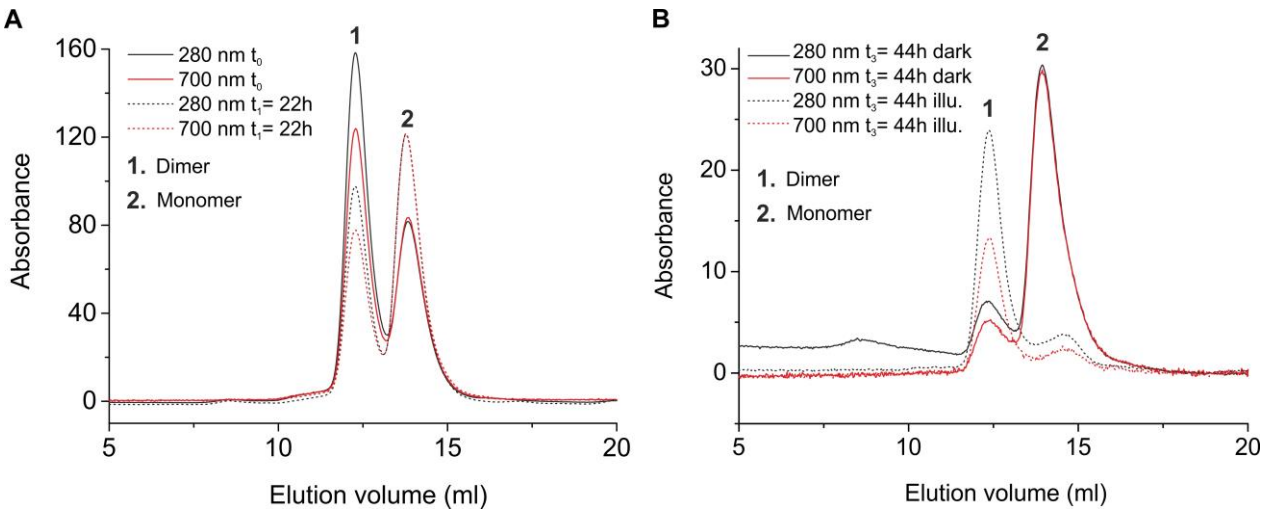

Figure S4. ***Ts*$^{N/PG/Yt/Y}$*Is*$^{CC/D}$ monomerization upon dark state recovery. (A)** Gel filtration analysis of the time dependent monomerization of *Ts*$^{N/PG/Yt/Y}$*Is*$^{CC/D}$ during Pr-state recovery using a Superdex200 10/300. **(B)** After 44h in the dark a sample of *Ts*$^{N/PG/Yt/Y}$*Is*$^{CC/D}$ was illuminated for 1 min with red light and analyzed again via gel filtration under constant illumination. Redimerization of the full-length protein supports the reversibility of the monomer/dimer equilibrium of this construct.

Table S1. Overview of oligonucleotides and buffers. Related to figure 1. (A) Oligonucleotides used in this study. **(B)** Buffers used for purification and storage of the different *Is*PadC variants.

Panel **A** - oligonucleotides		
Construct	**Oligonucleotide (5'-3')**	**Template**
Ts$^{N/PG/Yt/Y}$*Is*$^{CC/D}$	fw: atctggcaagcgatctggccattgtggcagatagcatg rv: tgctcgagtgcggccgcttactggctacaaacctgattac	*Is*PadC
	fw: taagcggccgcactcgag rv: ggccagatcgcttgccag	*Ts*PadC
Is$^{N/PG/Yt/Y/CC}$*Ts*^{D}	fw: taagcggccgcactcgag rv: atcaaagctggccagtttttcc	*Is*PadC
	fw: tggaaaaactggccagctttgatcatctgaccggtctg rv: tgctcgagtgcggccgcttaatctgcgctaacttcaac	*Ts*PadC
Is$^{N/PG/Yt/Y}$*Ts*$^{CC/D}$	fw: taagcggccgcactcgag rv: cagcagatcacgtgcaatatcac	*Is*PadC
	fw: atattgcacgtgatctgctggttctgattgcagccggt rv: tgctcgagtgcggccgcttaatctgcgctaacttcaacg	*Ts*PadC
Is$^{N/PG/Yt/Y/CC501-507}$*Ts*$^{CC505-518/D}$	fw: attgtggcagatagcatgcagattagccagctgaatcagc rv: ctgcatgctatctgccacaatcagcagatcacgtgc	*Is*$^{N/PG/Yt/Y}$*Ts*$^{CC/D}$
Is$^{N/PG}$*Ts*$^{Yt/Y/CC/D}$	fw: taagcggccgcactcgag rv: atgaatcagccacagacgttct	*Is*PadC
	fw: cgtctgtggctgattcatgcacgtgatgatgcagttctgat rv: tgctcgagtgcggccgcttaatctgcgctaacttcaacg	*Ts*PadC
Ts^{N}*Is*^{PG}*Ts*$^{Yt/Y/CC/D}$	fw: tggttgcagcactggaagcatgtgaacgt gaaccgattcatattccgaatgcaattcagc rv: cttccagtgctgcaaccagttccggttcatccat ggcgccctgaaaataaagattctcagtagtg	*Is*$^{N/PG}$*Ts*$^{Yt/Y/CC/D}$
IsPadC^{PSM}	fw: gcacgtgatctgctg taagcggccgcactcg rv: cagcagatcacgtgc aatatcacgtgctgcatacagctg	*Is*PadC
Is$^{N/PG/Yt/Y/CCΔ515-521}$*Ts*^{D}	fw: aatgatcagctggaaaaactggccagctttgatcatctgaccggtctgtg rv: ctgatcattcagcagattcagc	*Is*$^{N/PG/Yt/Y/CC}$*Ts*^{D}
Ts$^{N/PG/Yt/Y/CC}$*Is*^{D}	fw: tggaacgtctggcaacccgtgatgatctgaccggtatc rv: tgctcgagtgcggccgcttactggctacaaacctgattac	*Is*PadC
	fw:taagcggccgcactcgag rv: acgggttgccagacgttc	*Ts*PadC
Ts^{N}*Is*$^{PG/Yt/Y/CC}$*Ts*^{D}	fw: tggttgcagcactggaagcatgtgaacgtgaaccgattcatattccgaatgcaattcagc rv: cttccagtgctgcaaccagttccggttcatccatggcgccctgaaaataaagattctcagtagtg	*Is*$^{N/PG/Yt/Y/CC}$*Ts*^{D}
Ts^{N}*Is*^{PG}*Ts*^{Yt}*Is*$^{Y/CC/D}$	fw: agcggtaaaagccagc rv: ctgtgcaacacgaaacag	*Ts*^{N}*Is*$^{PG/Yt/Y/CC}$*Ts*^{D}
	fw: ctgtttcgtgttgcacaggcagaaacccgtatttgggcag rv: gctggcttttaccgctaacggtttctttccagcttgcaaag	*Ts*PadC

Panel **B** – buffer systems	
Use	**Buffer composition**
Storage buffer	10 mM HEPES pH 7, 0.5 M NaCl, 2 mM $MgCl_2$
Lysis buffer	50 mM HEPES pH 7, 0.5 M NaCl, 2 mM $MgCl_2$, 10 mM imidazole
Dialysis buffer	50 mM HEPES pH 7, 0.5 M NaCl, 2 mM $MgCl_2$, 1 mM DTE
HPLC buffer	50 mM HEPES pH 7, 0.5 M NaCl, 50 mM $MgCl_2$