Supporting Information

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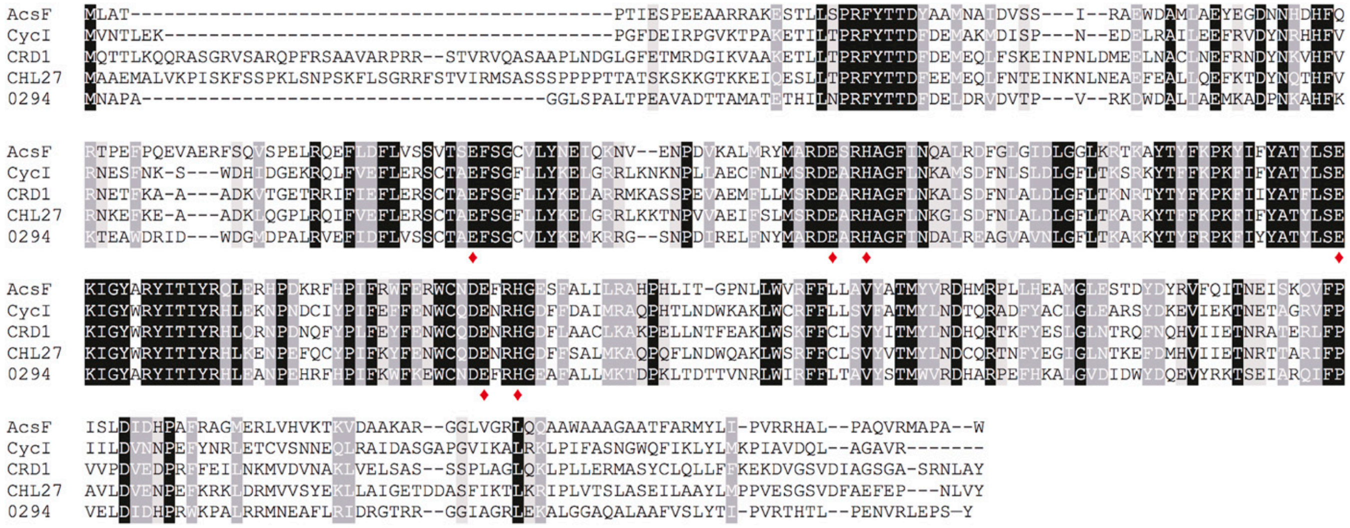

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AcsF   MLAT-------------------------------------------PTIESPEEAARRAKESTLLSPRFYTTDYAAMNAIDVSS---I--RAEWDAMLAEYEGDNNHDHFQ
CycI   MVNTLEK----------------------------------------PGFDEIRPGVKTPAKETILLTPRFYTTDFDEMAKMDISP---N--EDELRAILEEFRVDYNRHHFV
CRD1   MQTTLKQQRASGRVSARQPFRSAAVARPRR--STVRVQASAAPLNDGLGFETMRDGIKVAAKETLLLTPRFYTTDFDEMEQLFSKEINPNLDMEELNACLNEFRNDYNKVHFV
CHL27  MAAEMALVKPISKFSSPKLSNPSKFLSGRRFSTVIRMSASSSPPPPTTATSKSKKGTKKEIQESLLTPRFYTTDFEEMEQLFNTEINKNLNEAEFEALLQEFKTDYNQTHFV
0294   MNAPA------------------------------------GGLSPALTPEAVADTTAMATETHILNPRFYTTDFDELDRVDVTP---V--RKDWDALIAEMKADPNKAHFK

AcsF   RTPEFPQEVAERFSQVSPELRQEFLDFLVSSVTSEFSGCVLYNEIQKNV--ENPDVKALMRYMARDESRHAGFINQAIRDFGLGIDLGGLKRTKAYTYFKPKYIFYATYLSE
CycI   RNESFNK-S---WDHIDGEKRQLFVEFLERSCTAEFSGFLLYKELGRRLKNKNPLLAECFNLMSRDEARHAGFLNKAMSDFNLSLDLGFLTKSRKYTFFKPKFIFYATYLSE
CRD1   RNETFKA-A---ADKVTGETRRIFIEFLERSCTAEFSGFLLYKELARRMKASSPEVAEMFLLMSRDEARHAGFLNKALSDFNLALDLGFLTKNRTYTYFKPKFIIYATFLSE
CHL27  RNKEFKE-A---ADKLQGPLRQIFVEFLERSCTAEFSGFLLYKELGRRLKKTNPVVAEIFSLMSRDEARHAGFLNKGLSDFNLALDLGFLTKARKYTFFKPKFIFYATYLSE
0294   KTEAWDRID---WDGMDPALRVEFIDFLVSSCTAEFSGCVLYKEMKRRG--SNPDIRELFNYMARDEARHAGFINDALREAGVAVNLGFLTKAKKYTYFRPKFIYYATYLSE

AcsF   KIGYARYITIYRQLERHPDKRFHPIFRWFERWCNDEFRHGESFALILRAHPHLIT-GPNLLWVRFFLLAVYATMYVRDHMRPLHEAMGLESTDYDYRVFQITNEISKQVFP
CycI   KIGYWRYITIYRHLEKNPNDCIYPIFEFFENWCQDENRHGDFFDAIMRAQPHTLNDWKAKLWCRFFLLSVFATMYLNDTQRADFYACLGLEARSYDKEVIEKTNETAGRVFP
CRD1   KIGYWRYITIYRHLQRNPDNQFYPLFEYFENWCQDENRHGDFLAACLKAKPELLNTFEAKLWSKFFCLSVYITMYLNDHQRTKFYESLGLNTRQFNQHVIIETNRATERLFP
CHL27  KIGYWRYITIYRHLKENPEFQCYPIFKYFENWCQDENRHGDFFSALMKAQPQFLNDWQAKLWSRFFCLSVYVTMYLNDCQRTNFYEGIGLNTKEFDMHVIIETNRTTARIFP
0294   KIGYARYITIYRHLEANPEHRFHPIFKWFKEWCNDEFRHGEAFALLMKTDPKLTDTTVNRLWIRFFLTAVYSTMWVRDHARPEFHKALGVDIDWYDQEVYRKTSEIARQIFP

AcsF   ISLDIDHPAFRAGMERLVHVKTKVDAAKAR--GGLVGRLQQAAWAAAGAATFARMYLI-PVRRHAL--PAQVRMAPA--W
CycI   IILDVNNPEFYNRLETCVSNNEQLRAIDASGAPGVIKALRKLPIFASNGWQFIKLYLMKPIAVDQL--AGAVR-------
CRD1   VVPDVEDPRFFEILNKMVDVNAKLVELSAS--SSPLAGLQKLPLLERMASYCLQLLFFKEKDVGSVDIAGSGA-SRNLAY
CHL27  AVLDVENPEFKRKLDRMVVSYEKLLAIGETDDASFIKTLKRIPLVTSLASEILAAYLMPPVESGSVDFAEFEP---NLVY
0294   VELDIDHPRWKPALRRMNEAFLRIDRGTRR--GGIAGRLEKALGGAQALAAFVSLYTI-PVRTHTL--PENVRLEPS—Y
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Fig. S1. Amino acid sequence alignments of known AcsF proteins. Sequences are those from *Rvi. gelatinosus* (AcsF), *Synechocystis* (CycI), *C. reinhardtii* (CRD1), *A. thaliana* (CHL27), and *Rba. sphaeroides* (Rsp_0294; abbreviated as 0294). Conserved, highly similar, and similar residues are highlighted in black, dark gray, and light gray, respectively. The putative diiron center ligands are marked by red diamonds.

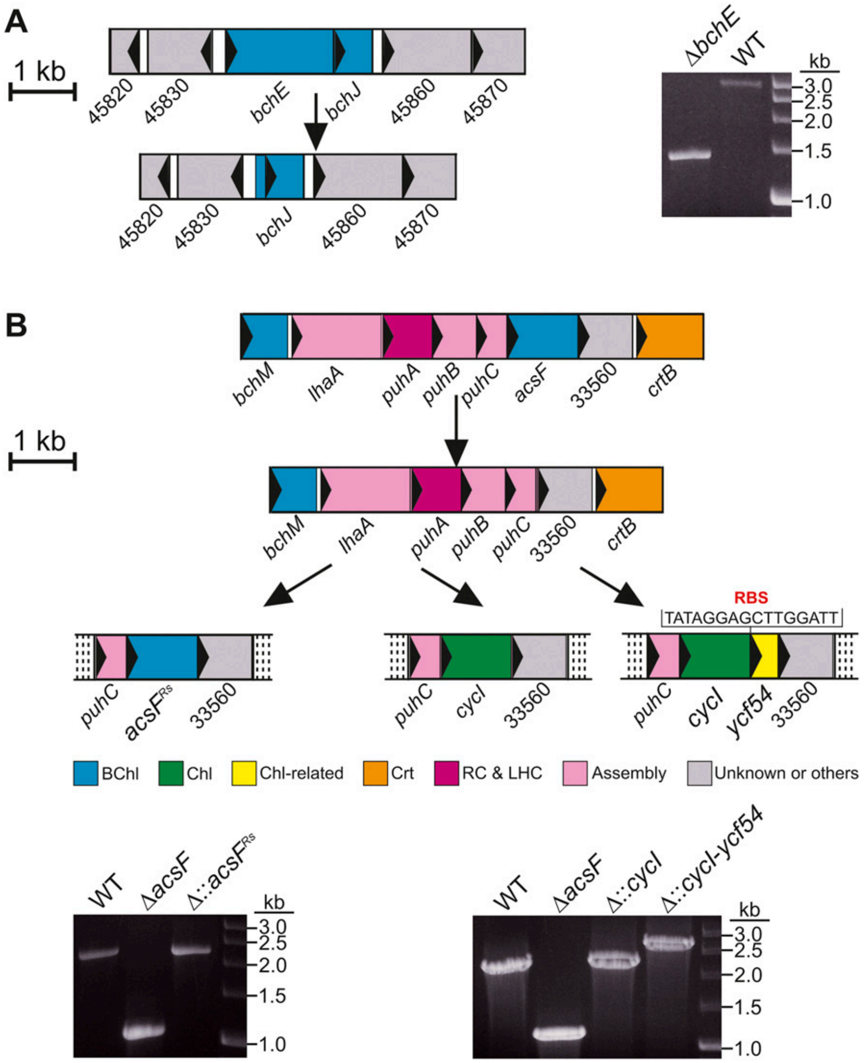

Fig. S2. Genetic knockouts and replacements in *Rvi. gelatinosus.* (*A*) Depiction of the deletion of *bchE* (*Left*), confirmed by colony PCR (*Right*). (*B*) Depiction of deletion of *acsF*, and subsequent integration of foreign genes at the *acsF* locus, under control of the native promoter (*Upper*), confirmed by colony PCR (*Lower*). The regions subjected to genetic manipulation are depicted in proportion to the scale bar. ORFs are represented as colored filled rectangles, within which the arrow indicates the direction of transcription. Crt, carotenoid biosynthesis; RC&LHC, reaction center and light-harvesting complexes.

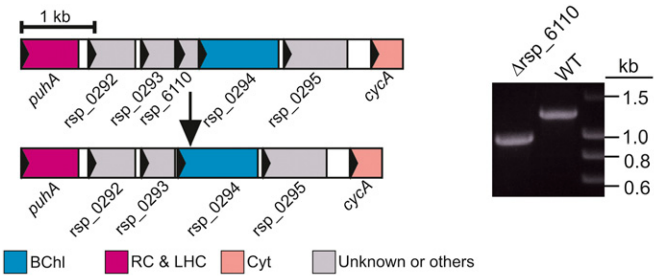

Fig. S3. Deletion of rsp_6110 in *Rba. sphaeroides.* Diagram depicting deletion of rsp_6110 (*Left*), and confirmation by colony PCR (*Right*).

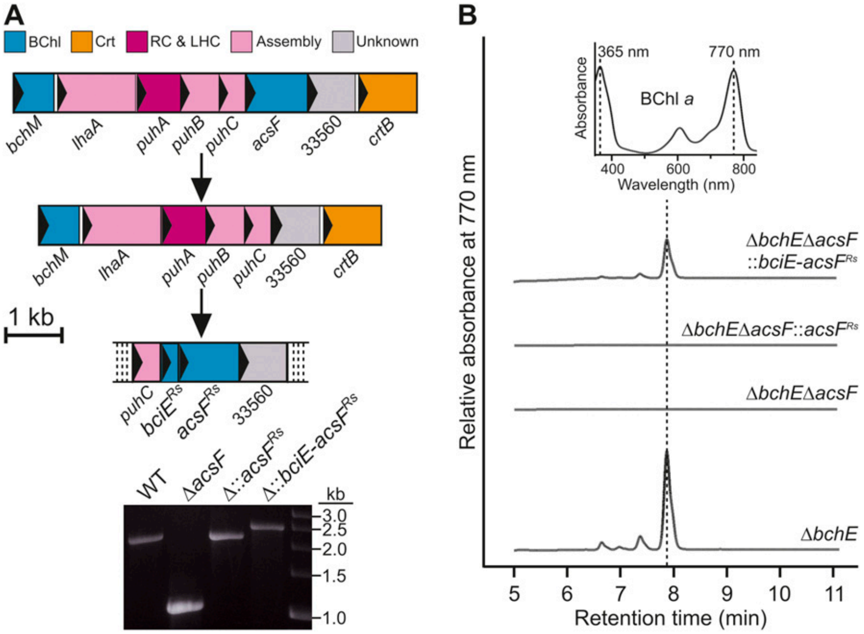

Fig. S4. Construction and phenotypic analysis of *Rvi. gelatinosus* mutant expressing *bciE* and *acsF* from *Rba. sphaeroides.* (*A*) Diagram depicting integration of *bciE* and *acsF* from *Rba. sphaeroides* in place of the native *acsF* in *Rvi. gelatinosus* (*Upper*), and confirmation by colony PCR (*Lower*). (*B*) HPLC analysis of pigments extracted from *Rvi. gelatinosus* strains, extracted from the same number of cells of each strain except for the Δ*bchE* strain, which had a much greater BChl *a* content compared with the other strains. (*Inset*) Retention times and Soret/Q_y maxima of peaks were used to identify BChl *a*.klj.

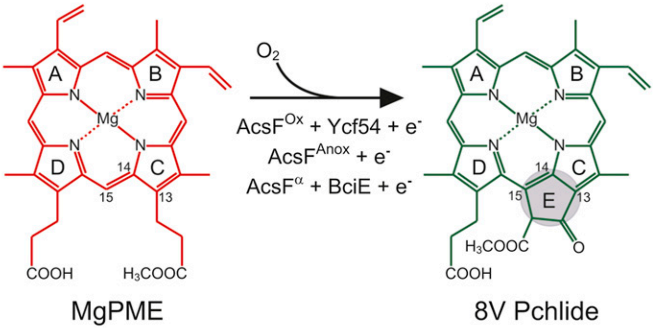

Fig. S5. Current status of known components of the oxygen-dependent cyclase. AcsF^{α}, $\text{AcsF}^{\text{Anox}}$, and AcsF^{Ox} represent AcsF proteins from Alphaproteobacteria, anoxygenic phototrophs other than the Alphaproteobacteria, and oxygenic phototrophs, respectively. e^{-} denotes the electron donor to the diiron center of AcsF.

Table S1. Distribution of *acsF* and *bchE* genes among sequenced phototrophic Proteobacteria, along with the presence of orthologs of rsp_6110

			Presence/absence of:		
Organism	**GenBank no.**	**Group**	**6110**	***acsF***	***bchE***
Acidiphilium multivorum AIU301	AP012035	α1, AAP	●	●	○
Phaeospirillum molischianum DSM 120	ZP_09875400	α1, PNB	X	X	○
Rhodospirillum centenum SW	CP000613	α1, PNB	●	●	○
Rhodospirillum rubrum ATCC 11170	CP000230	α1, PNB	X	X	○
Rhodospirillum photometricum DSM 122	YP_005416037	α1, PNB	X	X	○
Ahrensia sp. strain R2A130	NZ_AEEB01000017	α2, AAP	●	●	X
Agrobacterium albertimagni AOL15	ALJF00000000	α2, AAP	●	●	○
Hoeflea phototrophica DFL43	NZ_ABIA02000022	α2, AAP	●	●	X
Labrenzia alexandrii DFL11	NZ_EQ973123.1	α2, AAP	●	●	○
Methylobacterium sp. strain 4-46	CP000943	α2, AAP	●	●	○
Methylobacterium radiotolerans	CP001001	α2, AAP	●	●	X
Methylobacterium populi BJ001	YP_001927978	α2, AAP	●	●	X
Methylobacterium extorquens AM1	YP_002966142	α2, AAP	●	●	X
Methylocella silvestris BL2	CP001280	α2, AAP	●	●	X
Bradyrhizobium sp. strain BTAi1	CP000494	α2, PNB	●	●	●
Bradyrhizobium sp. strain ORS278	CU234118	α2, PNB	●	●	●
Rhodomicrobium vannielii ATCC 17100	NC_014664	α2, PNB	X	X	○
Rhodopseudomonas palustris	Multiple	α2, PNB	●	●	○
Dinoroseobacter shibae DFL12	CP000830	α3, AAP	●	●	○
Jannaschia sp. strain CCS1	CP000264	α3, AAP	●	●	X
Loktanella vestfoldensis SKA53	AAMS01000000	α3, AAP	●	●	X
Roseobacter denitrificans Och 114	CP000362	α3, AAP	●	●	○
Roseobacter litoralis Och 149	ABIG00000000	α3, AAP	●	●	○
Roseobacter sp. strain AzwK-3b	ABCR00000000	α3, AAP	●	●	●
Roseobacter sp. strain CCS2	AAYB00000000	α3, AAP	●	●	X
Roseovarius sp. strain TM1035	ABCL00000000	α3, AAP	●	●	○
Roseovarius sp. strain 217	AAMV00000000	α3, AAP	●	●	○
Rhodobacter capsulatus SB 1003	NC_014034	α3, PNB	X	X	●
Rhodobacter sphaeroides	Multiple	α3, PNB	●	●	●
Rhodobacter sp. strain SW2	ZP_05842911	α3, PNB	●	●	●
Erythrobacter sp. strain NAP1	AAMW00000000	α4, AAP	●	●	X
Citromicrobium bathyomarinum JL354	ZP_06861151	α4, AAP	●	●	○
Sphingomonas spp.	Multiple	α4, AAP	●	●	X
Brevundimonas subvibrioides ATCC 15264	CP002102	α4, AAP	●	●	X
Rubrivivax gelatinosus IL-114	NC_017075	β, PNB	X	●	○
Rubrivivax benzoatilyticus JA2	NZ_AEWG01000000	β, PNB	X	●	○
Methyloversatilis universalis FAM5	ZP_08506871	β, PNB	X	●	X
Limnohabitans sp. strain Rim28	ALKN00000000	β, PNB	X	●	X
Limnohabitans sp. strain Rim47	ALKO00000000	β, PNB	X	●	X
Allochromatium vinosum DSM 180	NC_013851	γ, PSB	X	X	○
Ectothiorhodospira sp. strain PHS-1	NZ_AGBG01000002	γ, PSB	X	X	○
Halorhodospira halophila SL1	CP000544	γ, PSB	X	X	●
Marichromatium purpuratum 984	NZ_AFWU01000001	γ, PSB	X	X	○
Thiocapsa marina 5811	NZ_AFWV01000003	γ, PSB	X	X	●○
Thiocystis violascens DSM198	AGFC00000000	γ, PSB	X	X	○
Thioflavicoccus mobilis 8321	NC_019940	γ, PSB	X	X	○
Thiorhodococcus drewsii AZ1	NZ_AFWT01000007	γ, PSB	X	X	○
Thiorhodospira sibirica ATCC 700588	AGFD01000016	γ, PSB	X	X	○
Congregibacter litoralis KT71	AAOA01000014	γ, AAP	X	●	●
gamma proteobacterium NOR5-3	ZP_05125815	γ, AAP	X	●	●
Luminiphilus syltensis NOR51-B	NZ_DS999411	γ, AAP	X	●	X
marine gamma proteobacterium HTCC2080	NZ_DS999405	γ, AAP	X	●	X
gamma proteobacterium HIMB55	ZP_09691978	γ, AAP	X	●	X

Modified from (26). ●, gene present in PGC; ○, gene present outside PGC; **X**, gene absent. The red box indicates an identical pattern of presence/absence of orthologs of rsp_6110 and *acsF* among Alphaproteobacteria. AAP, aerobic anoxygenic phototroph; PNB, purple nonsulfur bacterium; PSB, purple sulfur bacterium.

Table S2. Strains and plasmids described in this study

Strain/plasmid	Genotype/characteristics	Source
E. coli		
JM109	Cloning strain for plasmid constructs	Promega
S17-1	Conjugation strain for pK18*mobsacB* constructs	(48)
Rvi. gelatinosus		
WT	IL144	S. Nagashima[*]
Δ*bchE*	Unmarked deletion mutant of *bchE* in WT	This study
Δ*bchE*Δ*acsF*	Unmarked deletion mutant of *acsF* in Δ*bchE*	This study
Δ*bchE*Δ*acsF*::$acsF^{Rs}$	$acsF^{Rs}$ replacement of *acsF* in Δ*bchE*	This study
Δ*bchE*Δ*acsF*::*bciE*-$acsF^{Rs}$	*acsF* replaced with rsp_6110-$acsF^{Rs}$ in Δ*bchE*	This study
Δ*bchE*Δ*acsF*::*cycI*	*cycI* replacement of *acsF* in Δ*bchE*	This study
Δ*bchE*Δ*acsF*::*cycI-ycf54*	*cycI-ycf54* replacement of *acsF* in Δ*bchE*	This study
Synechocystis		
WT	sp. PCC6803	R. Sobotka[†]
$acsF^{Rg+}$	$acsF^{Rg}$ and Km^R replacement of *psbAII* in WT	This study
$acsF^{Rg+}$ Δ*cycI*	Cm^R replacement of *cycI* in $acsF^{Rg+}$	This study
$acsF^{Rg+}$ Δ*cycI*Δ*ycf54*	Zeo^R replacement of central portion of *ycf54* in $acsF^{Rg+}$Δ*cycI*	This study
Δ*ycf54*	Zeo^R replacement of central portion of *ycf54* in WT	(22)
Rba. sphaeroides		
WT	2.4.1	S. Kaplan[‡]
Δ*bchE*Δ*ccoP*	Unmarked deletion mutant of *bchE* and *ccoP* in WT	(15)
Δ*bchE*Δ*ccoP*Δ*acsF*	Unmarked deletion mutant of *acsF* in Δ*bchE*Δ*ccoP*	(15)
Δ*bchE*Δ*ccoP*Δ6110	Unmarked deletion mutant of rsp_6110 in Δ*bchE*Δ*ccoP*	This study
Plasmids		
pK18*mobsacB*	Allelic exchange vector, Km^R	J. Armitage[§]
pK18Δ$bchE^{Rg}$	Upstream-*NdeI*-downstream of $bchE^{Rg}$ cloned into *Bam*HI/*Hin*dIII sites of pK18*mobsacB*	This study
pK18Δ$acsF^{Rg}$	Upstream-*NdeI*-downstream of $acsF^{Rg}$ cloned into *Bam*HI/*Hin*dIII sites of pK18*mobsacB*	This study
pK18Δ6110	Upstream-downstream of rsp_6110 cloned into *XbaI*/*Hin*dIII sites of pK18*mobsacB*	This study
pK18[$acsF^{Rs}$]	$acsF^{Rs}$ cloned into the *NdeI* site of pK18Δ$acsF^{Rg}$	This study
pK18[6110-$acsF^{Rs}$]	rsp_6110-$acsF^{Rs}$ cloned into the *NdeI* site of pK18Δ$acsF^{Rg}$	This study
pK18[*cycI*]	*cycI* cloned into the *NdeI* site of pK18Δ$acsF^{Rg}$	This study
pK18[*cycI-ycf54*]	*cycI-ycf54* cloned into the *NdeI* site of pK18Δ$acsF^{Rg}$	This study
pPD-FLAG	Cloning site, Km^R, flanked by *psbAII* upstream and downstream regions, Amp^R	(21)
pPD[$acsF^{Rg}$]	$acsF^{Rg}$ cloned into *NdeI*/*BglI*I sites of pPD-FLAG	This study
pBBRBB-$Ppuf_{843\text{–}1200}$	Expression vector carrying the 843–1,200 region of *puf* promoter of *Rba. sphaeroides*, Km^R	(27)
pBB[6110]	rsp_6110 cloned into the *BglII*/*NotI* sites of pBBRBB-$Ppuf_{843\text{–}1200}$	This study

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[§] Department of Biochemistry, University of Oxford, Oxford, United Kingdom.

Table S3. Primers used in this study

Primer	Sequence (5′-3′)
6110UpF	GCTCTAGAGGAGCTGATCCCGCCCTTCC
6110UpR	GGAGAGCCCTCCGGCCGGCGCGTTCATGGGGGTTCCCTTCTCTTGG
6110DownF	CCAAGAGAAGGGAACCCCCATGAACGCGCCGGCCGGAGGGCTCTCC
6110DownR	GCAAGCTTCCCAGGTTCACCGCCACGCC
6110CheckF	GCCCCGGAGCGACAAGGAC
6110CheckR	GTATTTCTTGGCCTTGGTCAGG
6110F_NdeI	GAGTCTCATATGGGTCTGTTCACGAAACAAGCG
6110F_BglII	GGCAGATCTATGGGTCTGTTCACGAAACAAGCGGAA
6110R_NotI	TCTGCGGCCGCTCACAGCGTCACCTGCTCGGAGAA
0294F_NdeI	CCAGTACATATGTGAACGCGCCGGCCGGAGG
0294R_NdeI	CCAGTACATATGTCAATAGCTCGGCTCCAGTCGG
45840UpF	CTAGGTCAAGTAGGATCCTCATGCCGGCGGCGATCATG
45840UpR	CTAGGTCAAGTACATATGGGAAACGGCTCCTCGCGATTC
45840DownF	CTAGGTCAAGTACATATGCGACGGCTGGGTCACGATGC
45840DownR	CTAGGTCAAGTAAAGCTTTGCCGGTGTAGAAGTCGCACGC
45840CheckF	TAGCCGCCGACCATGCCGA
45840CheckR	GCGGTGCACCAGCACCGTGA
33550UpF	GAGTCTGGATCCCTGCATGAGCGACAACGCGTC
33550UpR	GAGTCTCATATGGAGGGTCTCCGTGGTGTGTCA
33550DownF	GAGTCTCATATGAAGCGAGGACAGGATGCTGAGC
33550DownR	GAGTCTAAGCTTGGAACTCCTCGCTCAGGTTGCG
33550CheckF	GAACGTTTGCCGGACACGGT
33550CheckR	ACGAGGTACTTCAGGTGCTCC
33550F_NdeI	GAGTCTCATATGCTCGCGACCCCGACGATCG
33550R_BamHI	GAGTCTGGATCCTCACCATGCCGGGGCCATG
1214UpF	GCCGATCCGGTTAACCTAGGCA
1214UpR	ATATCCAGTGATTTTTTTCTCCATAGAGTTGTTTAAAATAGTTTCC
1214UpCmF	GGAAACTATTTTAAACAACTCTATGGAGAAAAAAATCACTGGATAT
1214DownCmR	GGTGATCCAGCGGAAGACAACCTTACGCCCCGCCCTGC
1214DownF	GCAGGGCGGGGCGTAAGGTTGTCTTCCGCTGGATCACC
1214DownR	TGGAGTTGTTGGGAGAGTTCGGTC
1214F_NdeI	GGAATTCCATATGGTTAATACCCTCGAAAAGCCCG
1214R_NdeI	GGAATTCCATATGTTAGCGCACAGCTCCAGCCA
1214RBS1780F	GTTGGCTGGAGCTGTGCGCTAATATAGGAGCTTGGATTGTGGAAAGTTGGGCATTGACGA
1214RBS1780R	TCGTCAATGCCCAACTTTCCACAATCCAAGCTCCTATATTAGCGCACAGCTCCAGCCAAC
1780F	GTGGAAAGTTGGGCATTGACG
1780R	CTAATCCAGGGATGCAAGGGG
1780R_NdeI	GAGTCTCATATGCTAATCCAGGGATGCAAGGGG