Supplementary Material

1 Supplementary Figures and Tables

1.1 Supplementary Figures

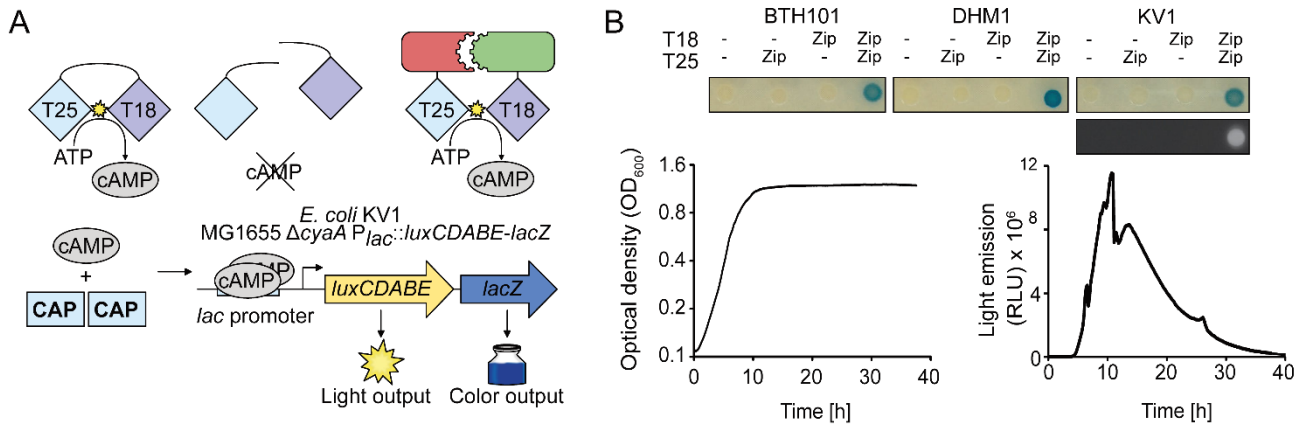

Figure S1. (A) Molecular principle of the Euromedex bacterial two-hybrid system with *E. coli* KV1 (adjusted from the Euromedex KIT Manual): The *Bordetella pertussis* adenylate cyclase CyaA apoprotein catalyzes the formation of cyclic AMP from ATP. Splitting the enzyme into two parts – T18 and T25 – renders CyaA inactive even upon co-expression. Fusing T18 and T25 to interacting proteins brings the fragments into close proximity and thus allows reconstitution of the Apo-CyaA. In *E. coli* KV1, the cyclic AMP dependent lac promoter precedes a translational fusion of the *lux*-operon and *lacZ*, allowing the indirect measurement of protein-protein interactions by light emission and colorimetric detection. **(B)** Proof of principle of *E. coli* KV1 as an *in vivo* reporter for protein-protein interaction using the self-interacting leucine zipper of GCN4. The colorimetric detection in *E. coli* KV1 cells was assessed semi quantitatively based on the formation of blue colonies on LB (Miller)-plates containing 40 µg/mL X-Gal. *E. coli* KV1 light emission was measured in a time course experiment recording relative luminescence (RLU) and optical density at 600 nm (OD600) in time intervals of 10 minutes.

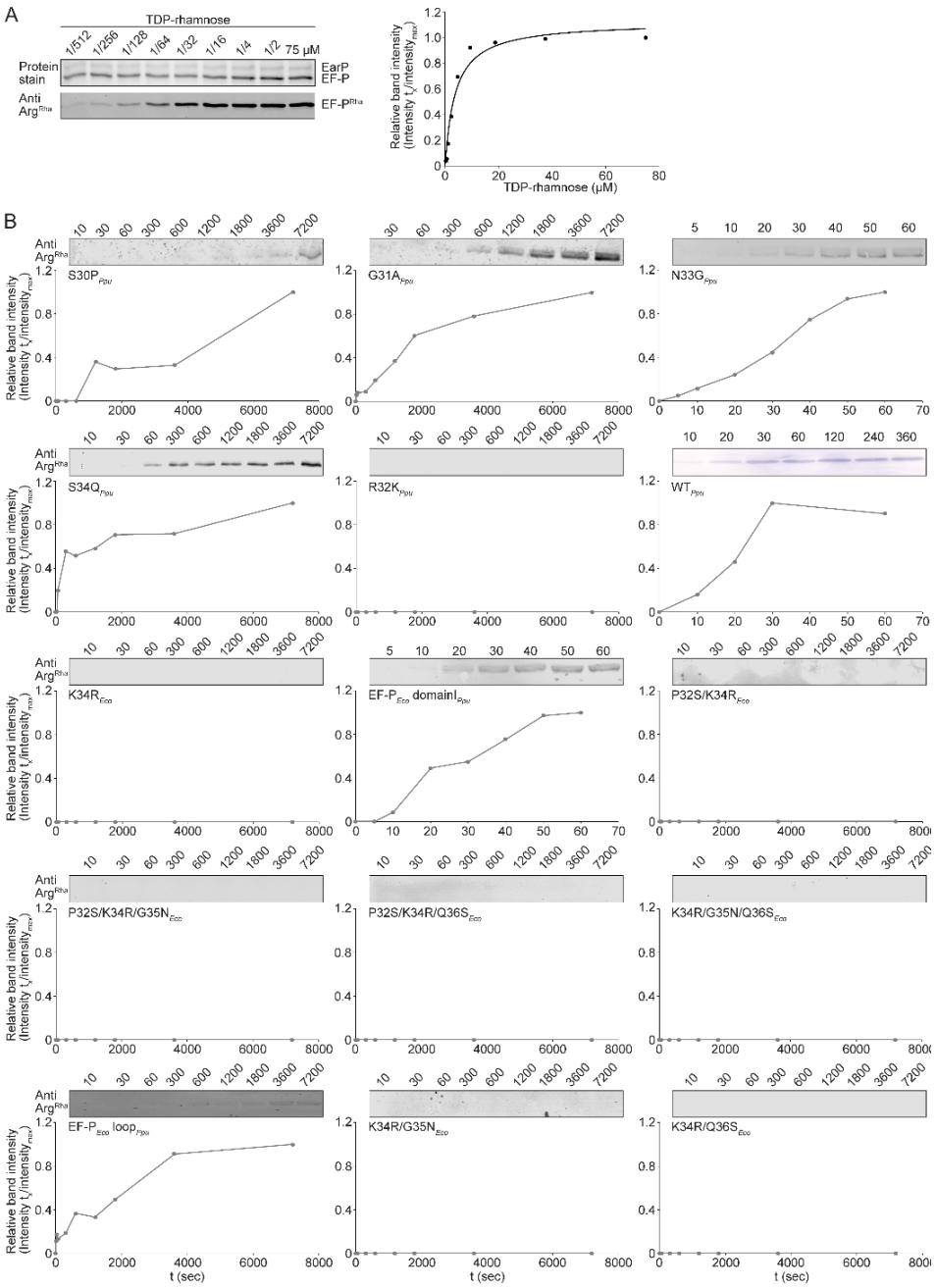

Figure S2. (A) Left: Analysis of EarP$_{Ppu}$ kinetic parameters. 0.5 µg of EF-P$_{Ppu}$ and 0.05 µg of EarP$_{Ppu}$ were subjected to SDS-PAGE after *in vitro* rhamnosylation (see Material and Methods) for 20 seconds at varying TDP-rhamnose concentrations. Proteins were transferred to nitrocellulose membrane by horizontal Western blotting. Rhamnosylated EF-P$_{Ppu}$ was detected using 0.25 µg/ml of Anti-Arg^{Rha}. Right: TDP-rhamnose saturation curve of EarP$_{Ppu}$. Band intensities on nitrocellulose membrane were quantified using ImageJ and relative band intensities were plotted against TDP-rhamnose concentration. **(B)** Timecourse analysis of various EF-P$_{Ppu}$ and EF-P$_{Eco}$ variants. 0.5 µg of EF-P$_{Ppu}$ and 0.05 µg of EarP$_{Ppu}$ were subjected to SDS-PAGE after *in vitro* rhamnosylation at a TDP-rhamnose concentration of 50 µM for varying timespans. Band intensities on nitrocellulose membrane were quantified using ImageJ and relative band intensities were plotted against time.

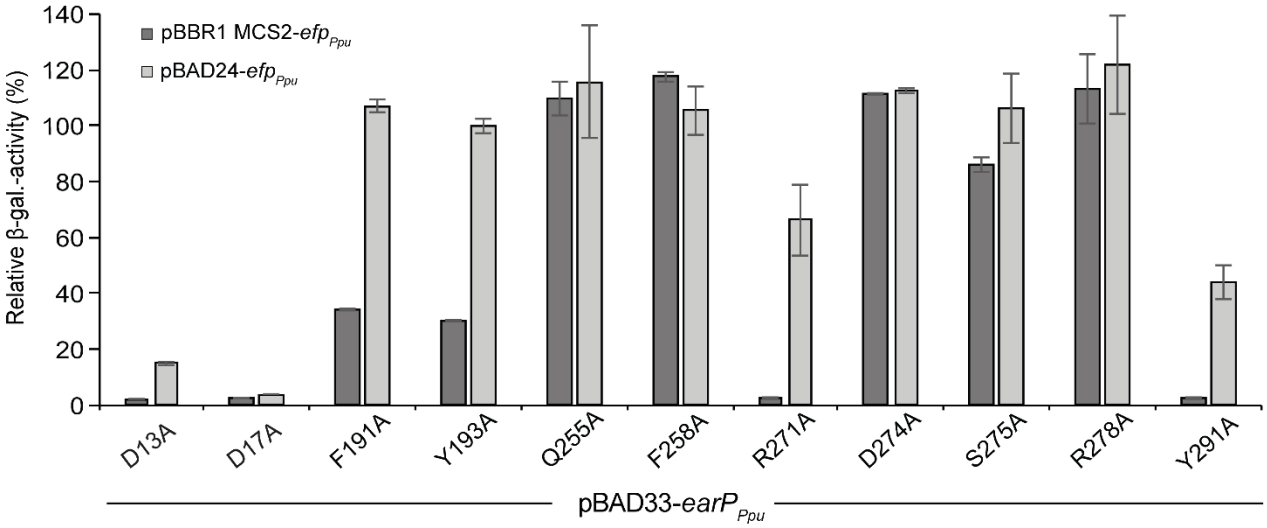

Figure S3. β-galactosidase activity in *E. coli* MG-CR-*efp*-Kan^S upon expression (dark grey) or co-overexpression (light grey) of EF-P$_{Ppu}$ and EarP$_{Ppu}$ and single-amino-acid substitution variants. Cells were incubated under *cadBA*-inducing conditions (LB, pH 5.8) at 30°C o/n.

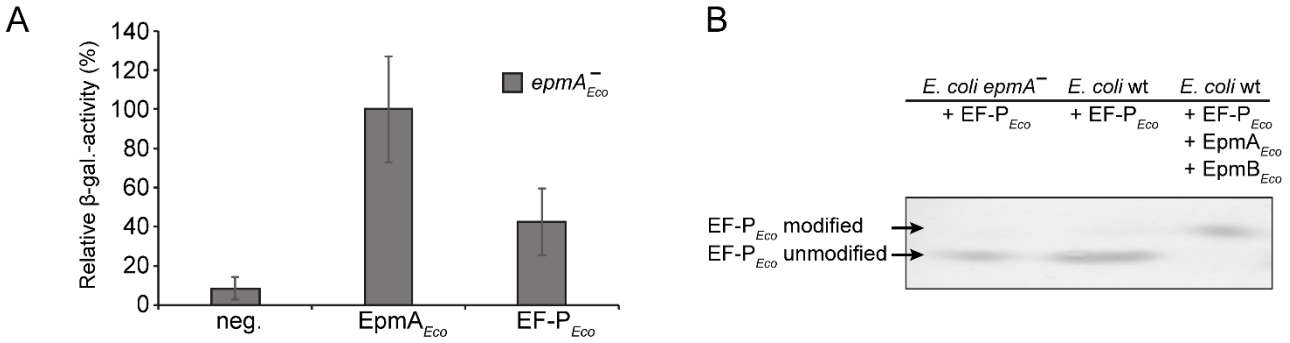

Figure S4. (A) β-galactosidase activity in the Δ*epmA* reporter strain (*E. coli* MG-CL-12-yjeA) harboring either a plasmid borne copy of *epmA* (pBAD33-*epmA*) or *efp* (pBAD24-*efp*$_{Eco}$). **(B)** Isoelectric focusing of *E. coli* EF-P, either overproduced in *E. coli epmA*$^-$ (BW25113-epmA), in which *epmA* was chromosomally deleted, or in *E. coli* wild type cells (BW25113). Furthermore, EF-P was overproduced in combination with its PTM proteins EpmA and EpmB in *E. coli* wild type (BW25113). Production of EF-P was verified by Western blot analysis.

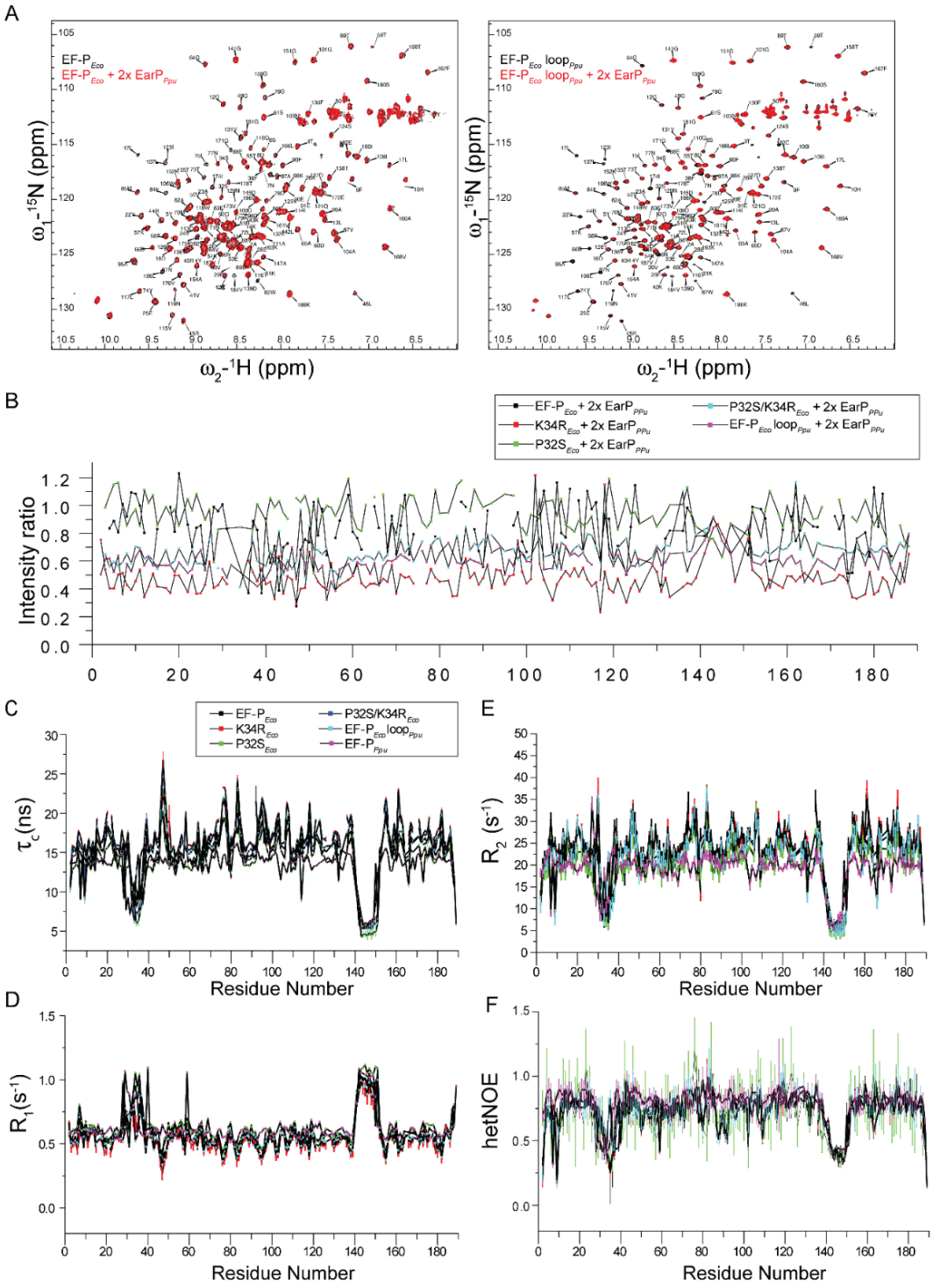

Figure S5. (A) ^{1}H-^{15}N HSQC titrations of EF-P$_{Eco}$ and EF-P$_{Eco}$ loop$_{Ppu}$ with EarP$_{Ppu}$ is shown along with the backbone assignments. Both proteins show a decrease in intensity upon titration with EarP$_{Ppu}$ indicating their interaction with EarP$_{Ppu}$. **(B)** Intensity ratio of all the EF-P$_{Eco}$ mutants on titration with EarP$_{Ppu}$ is shown. **(C)** The correlation time (τ_c) for EF-P$_{Eco}$ and its variants along with **(D)** R1, **(E)** R2 rates and **(F)** hetNOE are shown.

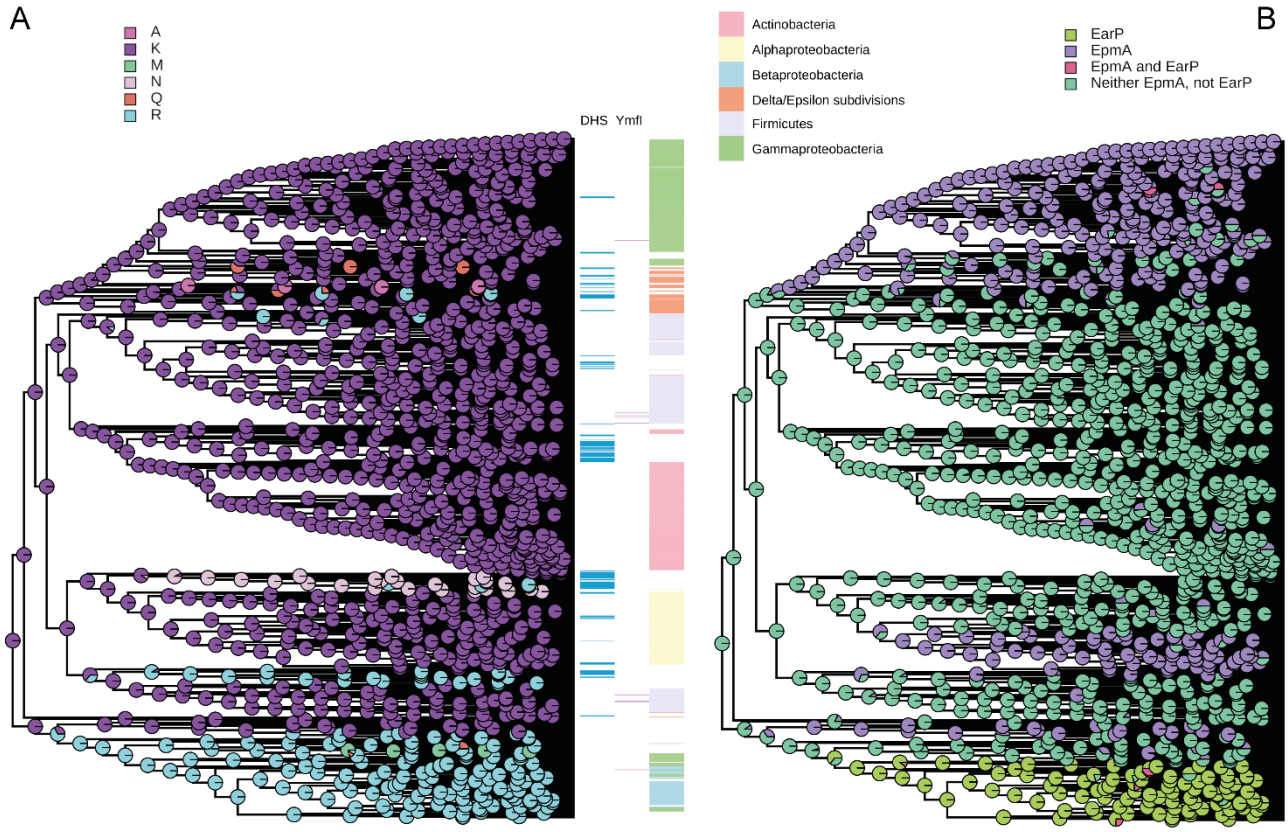

Figure S6. (A) Phylogenetic trees of the EF-P KOW-like N-domain I, with reconstructed state of the 34th position (A, left), presence of known modification systems and taxonomy (A, right colored bars). **(B)** Phylogenetic reconstruction of emergence of EF-P modification systems.

1.2 Supplementary Tables

Table S1. Strains used in this study

Strain	Genotype	Reference
DH5αλ*pir*	*recA1 endA1 gyrA96 thi-1 hsdR17 supE44 relA1* Δ*lacZYA-argF* U169 ϕ80d*lacZ*ΔM15 λpir	(Macinga et al., 1995)
LMG194	F$^-$ Δ*lacX74 galE galK thi rpsL* Δ*phoA* (PvuII) Δ*ara714 leu*::Tn*10*	(Guzman et al., 1995)
BL21(DE3)	F$^-$ *ompT gal dcm lon hsdS$_B$*(rB$^-$ mB$^-$) λ(DE3)	(Studier and Moffatt, 1986)
DHM1	F$^-$ *cya-854 recA1 endA1 gyrA96* (*NalR*) *thi1 hsdR17 spoT1 rfbD1 glnV44*(*AS*)	(Karimova et al., 2005)
BTH101	F$^-$ *cya-99 araD139 galE15 galK16 rpsL1* (*StrR) hsdR2 mcrA1 mcrB1* additional *relA1* mutation reported by (Battesti and Bouveret, 2012)	Euromedex
JW4107	Δ*(araD-araB)567*, Δ*lacZ4787*(::rrnB-3), *λ$^-$*, *rph-1*, Δ*(rhaD-rhaB)568*, Δ*efp-772::kan*, *hsdR514*	(Baba et al., 2006)
KV1	MG1655 *rpsL150* Δ*cyaA* P$_{lac}$::*luxCDABE-lacZ*	This study
LF1	MG1655 *rpsL150 P$_{lac}$::rpsL-neo-kan::lacZ$^{\Delta 1-100}$ bp;Kan^R Strp^S*	(Fried et al., 2012)
MG-CR-*efp*	MG1655 Δ*lacZ*::*tet rpsL*150 *efp*::*npt* Δ*cadBA* P$_{cadBA}$::*lacZ*	(Lassak et al., 2015)
MG-CR-*efp*-KanS	MG1655 Δ*lacZ*::*tet rpsL*150 Δ*efp* Δ*cadBA* P$_{cadBA}$::*lacZ*	This study
MG-CR-*efp-epmA*-KanR	MG1655 Δ*lacZ*::*tet rpsL*150 Δ*efp* Δ*cadBA epmA*::*npt* P$_{cadBA}$::*lacZ*	This study
MG-CL-12-*yjeA*	MG1655 Δ*lacZ*::*tet rpsL*150 *yjeA*::*npt* Δ*cadBA cadBA*::*lacZ*	(Ude, 2013)
BW25113	Δ(*araD-araB*)*567*, Δ*lacZ4787*(::rrnB-3), λ-, rph-1, Δ(*rhaD-rhaB*)*568*, *hsdR514*	(Datsenko and Wanner, 2000)
BW25113-*epmA*	Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, rph-1, Δ(rhaD-rhaB)568, hsdR514, ΔepmA	This study
JW4116	F$^-$, *Δ(araD-araB)567*, *ΔlacZ4787*(::rrnB-3), *λ$^-$*, *rph-1*, *Δ(rhaD-rhaB)568*, *ΔpoxA782::kan*, *hsdR514*	(Baba et al., 2006)

Kan^R: kanamycin resistance, Srep^S: streptomycin sensitive

Table S2. Plasmids used in this study

Plasmid	Features	Reference
Plasmids for strain construction		
pRED/ET® Amp	λ-RED recombinase in pBAD24; Amp^R	GeneBridges, Germany
FRT-PGK-gb2-neo-FRT template DNA	PCR-template (plasmid DNA) for generating a FRT-flanked PGK-gb2-neo cassette, Kan^R	GeneBridges, Germany
709-FLPe, amp	Plasmid for removal of FRT flanked resistance cassette, Supplier ID: A106	GeneBridges, Germany
pBAD/HisA-Lux	Contains the *luxCDABE* operon from *Photorhabdus luminescens*	(Volkwein et al., 2017)
Plasmids for mutational analysis of the *E. coli* loop and for overproduction		
pBAD24	Amp^R-cassette, pBBR322 origin, *araC* coding sequence, *ara* operator	(Guzman et al., 1995)
pBAD24-efp_{Eco}	C-terminal His_6-tagged *E. coli efp* amplified from pBAD33-$efp_{E.c.}$-His6 (Lassak et al., 2015) using P1/P2	this study
pBAD24-efp_{Eco} P32G	C-terminal His_6-tagged *E. coli efp* substitution variant P32G. Overlap PCR fragment was amplified from pBAD24-efp_{Eco} using P1/P16 and P2/P15	this study
pBAD24-efp_{Eco} P32S	C-terminal His_6-tagged *E. coli efp* substitution variant P32S. Overlap PCR fragment was amplified from pBAD24-efp_{Eco} using P1/P18 and P2/P17	this study
pBAD24-efp_{Eco} G33A	C-terminal His_6-tagged *E. coli efp* substitution variant G33A. Overlap PCR fragment was amplified from pBAD24-efp_{Eco} using P1/P20 and P2/P19	this study
pBAD24-efp_{Eco} G33S	C-terminal His_6-tagged *E. coli efp* substitution variant G33S. Overlap PCR fragment was amplified from pBAD24-efp_{Eco} using P1/P22 and P2/P21	this study
pBAD24-efp_{Eco} K34A	C-terminal His_6-tagged *E. coli efp* substitution variant K34A. Amplified from pBAD33-$efp_{E.c.}$-His6-K34A (Lassak et al., 2015) using P1/P2	this study
pBAD24-efp_{Eco} K34M	C-terminal His_6-tagged *E. coli efp* substitution variant K34M. Overlap PCR fragment was amplified from pBAD24-efpEco using P1/P24 and P2/P23	this study

pBAD24-*efp*$_{Eco}$ K34N	C-terminal His$_6$-tagged *E. coli efp* substitution variant K34N. Overlap PCR fragment was amplified from pBAD24-*efp*$_{Eco}$ using P1/P26 and P2/P25	this study
pBAD24-*efp*$_{Eco}$ K34Q	C-terminal His$_6$-tagged *E. coli efp* substitution variant K34Q. Overlap PCR fragment was amplified from pBAD24-*efp*$_{Eco}$ using P1/P28 and P2/P27	this study
pBAD24-*efp*$_{Eco}$ K34R	C-terminal His$_6$-tagged *E. coli efp* substitution variant K34R. Amplified from pBAD33-*efp*$_{E.c.}$-His6-K34R (Lassak et al., 2015) using P1/P2	this study
pBAD24-*efp*$_{Eco}$ G35N	C-terminal His$_6$-tagged *E. coli efp* substitution variant G35N. Overlap PCR fragment was amplified from pBAD24-*efp*$_{Eco}$ using P1/P30 and P2/P29	this study
pBAD24-*efp*$_{Eco}$ Q36S	C-terminal His$_6$-tagged *E. coli efp* substitution variant Q36S. Overlap PCR fragment was amplified from pBAD24-*efp*$_{Eco}$ using P1/P32 and P2/P31	this study
Plasmids for mutational analysis of the *P. putida* loop and for overproduction		
pBAD24-*efp*$_{Ppu}$	C-terminal His$_6$-tagged *P. putida efp* amplified from *Pseudomonas putida* KT2440 using P1/P2	this study
pBAD24-*efp*$_{Ppu}$ K29A	C-terminal His$_6$-tagged *efp P. putida* substitution variant K29A. Overlap PCR fragment was amplified from pBAD24-*efp*$_{Ppu}$ using P1/P36 and P2/P35	this study
pBAD24-*efp*$_{Ppu}$ K29R	C-terminal His$_6$-tagged *P. putida efp* substitution variant K29R. Overlap PCR fragment was amplified from pBAD24-*efp*$_{Ppu}$ using P1/P37 and P2/P35	this study
pBAD24-*efp*$_{Ppu}$ S30A	C-terminal His$_6$-tagged *P. putida efp* substitution variant S30A. Overlap PCR fragment was amplified from pBAD24-*efp*$_{Ppu}$ using P1/P38 and P2/P35	this study
pBAD24-*efp*$_{Ppu}$ S30G	C-terminal His$_6$-tagged *P. putida efp* substitution variant S30G. Overlap PCR fragment was amplified from pBAD24-*efp*$_{Ppu}$ using P1/P39 and P2/P35	this study
pBAD24-*efp*$_{Ppu}$ S30P	C-terminal His$_6$-tagged *P. putida efp* substitution variant S30P. Overlap PCR fragment was amplified from pBAD24-*efp*$_{Ppu}$ using P1/P40 and P2/P35	this study

pBAD24-*efp*$_{Ppu}$ G31A	C-terminal His_6-tagged *P. putida efp* substitution variant G31A. Overlap PCR fragment was amplified from pBAD24-*efp*$_{Ppu}$ using P1/P41 and P2/P35	this study
pBAD24-*efp*$_{Ppu}$ G31S	C-terminal His_6-tagged *P. putida efp* substitution variant G31S. Overlap PCR fragment was amplified from pBAD24-*efp*$_{Ppu}$ using P1/P42 and P2/P35	this study
pBAD24-*efp*$_{Ppu}$ R32K	C-terminal His_6-tagged *P. putida efp* substitution variant R32K. Overlap PCR fragment was amplified from pBAD24-*efp*$_{Ppu}$ using P1/P44 and P2/P43	this study
pBAD24-*efp*$_{Ppu}$ N33D	C-terminal His_6-tagged *P. putida efp* substitution variant N33D. Overlap PCR fragment was amplified from pBAD24-*efp*$_{Ppu}$ using P1/P46 and P2/P45	this study
pBAD24-*efp*$_{Ppu}$ N33G	C-terminal His_6-tagged *P. putida efp* substitution variant N33G. Overlap PCR fragment was amplified from pBAD24-*efp*$_{Ppu}$ using P1/P47 and P2/P45	this study
pBAD24-*efp*$_{Ppu}$ S34A	C-terminal His_6-tagged *P. putida efp* substitution variant S34A. Overlap PCR fragment was amplified from pBAD24-*efp*$_{Ppu}$ using P1/P48 and P2/P45	this study
pBAD24-*efp*$_{Ppu}$ S34Q	C-terminal His_6-tagged *P. putida efp* substitution variant S34Q. Overlap PCR fragment was amplified from pBAD24-*efp*$_{Ppu}$ using P1/P49 and P2/P45	this study
pBAD24-*efp*$_{Ppu}$ A35S	C-terminal His_6-tagged *P. putida efp* substitution variant A35S. Overlap PCR fragment was amplified from pBAD24-*efp*$_{Ppu}$ using P1/P50 and P2/P45	this study
pBAD33	Cm^R-cassette, p15A origin, *araC* coding sequence, *ara* operator	(Guzman et al., 1995)
pBAD33 PP1857-His6	C-terminal His6-Tag *earP* version from *P. putida* KT2440	(Krafczyk et al., 2017)

Plasmids for cross modification/actication and overproduction		
pBBRMCS2	pBBR origin of replication, *oriT*, KanR	(Kovach et al., 1995)
pBAD24-efp_{Eco} domainI-efp_{Ppu}	C-terminal His_6-tagged *E. coli efp* where the first 65 amino acids (domainI) were substituted by the first 65 amino acids from *P. putida efp*. Overlap PCR fragment was amplified from pBAD24-efp_{Ppu} using P1/P52 and pBAD24-efp_{Eco} P2/P51	this study
pBAD24-efp_{Eco} loop-efp_{Ppu}	C-terminal His_6-tagged *E. coli efp* P32S K34R G35N Q36S multiple amino acid substitution variant, corresponding to EF-P of *E. coli* carrying the acceptor loop of EF-P from *P. putida.* Overlap PCR fragment was amplified from pBAD24-efp_{Eco} using P1/P54 and P2/P53	this study
pBAD24-efp_{Eco} P32S K34R	C-terminal His_6-tagged *E. coli efp* substitution variant P32S K34R. Overlap PCR fragment was amplified from pBAD24-efp_{Eco} K34R using P1/P56 and P2/P55	this study
pBAD24-efp_{Eco} K34R G35N	C-terminal His_6-tagged *E. coli efp* substitution variant K34R G35N. Overlap PCR fragment was amplified from pBAD24-efp_{Eco} K34R using P1/P58 and P2/P57	this study
pBAD24-efp_{Eco} K34R Q36S	C-terminal His_6-tagged *E. coli efp* substitution variant K34R Q36S. Overlap PCR fragment was amplified from pBAD24-efp_{Eco} K34R using P1/P60 and P2/P59	this study
pBAD24-efp_{Eco} P32S K34R G35N	C-terminal His_6-tagged *E. coli efp* substitution variant P32S K34R G35N. Overlap PCR fragment was amplified from pBAD24-efp_{Eco} P32S K34R using P1/P62 and P2/P61	this study
pBAD24 -efp_{Eco} P32S K34R Q36S	C-terminal His_6-tagged *E. coli efp* substitution variant P32S K34R Q36S. Overlap PCR fragment was amplified from pBAD24-efp_{Eco} P32S K34R using P1/P64 and P2/P63	this study
pBAD24-efp_{Eco} K34R G35N Q36S	C-terminal His_6-tagged *E. coli efp* substitution variant K34R G35N Q36S. Overlap PCR fragment was amplified from pBAD24-efp_{Eco} K34R using P1/P66 and P2/P65	this study

pBAD33-*earP*$_{So}$	*earP* from *Shewanella oneidensis* MR-1 amplified from pBAD24-*earP*$_{S.o.}$-His$_6$ (Lassak et al., 2015) using P1/P67	this study
Plasmids for XPPX assay		
p3LC-TL30-APP	p3LC-TL30 + sequence encoding Ala-Pro-Pro	(Peil et al., 2013)
p3LC-TL30-PPD	p3LC-TL30 + sequence encoding Pro-Pro-Asp	(Peil et al., 2013)
p3LC-TL30-PPP	p3LC-TL30 + sequence encoding Pro-Pro-Pro	(Ude et al., 2013)
p3LC-TL30-DPP	p3LC-TL30 + sequence encoding Asp-Pro-Pro	(Peil et al., 2013)
p3LC-TL30-PPG	p3LC-TL30 + sequence encoding Pro-Pro-Gly	(Peil et al., 2013)
p3LC-TL30-PPN	p3LC-TL30 + sequence encoding Pro-Pro-Asn	(Peil et al., 2013)
Plasmids used in the reporter strain MG-CL-12-yjeA		
pBAD33-*epmA*	*epmA* from *E. coli*, amplified using P72/P75	this study
Plasmids for protein overproduction (NMR)		
pET SUMO	pBR322 origin, *lacI*, T7 *lac* promoter, N-terminal His$_6$ tag, SUMO coding sequence, Kan^R, Supplier ID: K300-01	Invitrogen
pET SUMO-*efp*$_{Eco}$	C-terminal genetic fusion of *efp* from *E. coli* to His$_6$-SUMO-tag. Amplified from pBAD24-*efp*$_{Eco}$ using P68/P69	this study
pET SUMO-*efp*$_{Ppu}$	C-terminal genetic fusion of *efp* from *P. putida* KT2440 to His$_6$-SUMO-tag	(Krafczyk et al., 2017)
pET SUMO-*efp*$_{Eco}$ P32S	C-terminal genetic fusion of *efp* from *E. coli* substitution variant P32S to His$_6$-SUMO-tag. Overlap PCR fragment was amplified from pET SUMO-*efp*$_{Eco}$ using P5/P18 and P6/P17	this study
pET SUMO-*efp*$_{Eco}$ K34R	C-terminal genetic fusion of *efp* from *E. coli* substitution variant K34R to His$_6$-SUMO-tag. Overlap PCR fragment was amplified from pET SUMO-*efp*$_{Eco}$ using P5/P71 and P6/P70	this study
pET SUMO-*efp*$_{Eco}$ P32S K34R	C-terminal genetic fusion of *efp* from *E. coli* substitution variant P32S K34R to His$_6$-SUMO-tag. Overlap PCR fragment was amplified from pET SUMO-*efp*$_{Eco}$ using P5/P56 and P6/P55	this study
pET SUMO-*efp*$_{Eco}$ loop$_{Ppu}$	C-terminal genetic fusion of *efp* from *E. coli* substitution variant P32S K34R G35N Q36S to His$_6$-SUMO-tag. Overlap PCR fragment was amplified from pET SUMO-*efp*$_{Eco}$ using P5/P54 and P6/P53	this study

Plasmids for bacterial two-hybrid (BTH)		
pUT18-*zip*	N-terminal genetic fusion of the leucine zipper from GCN4 to the T18 fragment of CyaA	Euromedex
pKT25-*zip*	C-terminal genetic fusion of the leucine zipper from GCN4 to the T25 fragment of CyaA	Euromedex
pUT18C-PP1858	C-terminal genetic fusion of *efp* from *Pseudomonas putida t*o the T18 fragment of CyaA	(Krafczyk et al., 2017)
pKT25-PP1857	C-terminal genetic fusion of *earP* from *Pseudomonas putida* to the T25 fragment of CyaA	(Krafczyk et al., 2017)
pUT18C-efp_{Eco}	C-terminal genetic fusion of *efp* from *Escherichia coli* to the T18 fragment of CyaA. PCR fragment was amplified from pBAD24-efp_{Eco} using P80/P81	this study
pUT18C-efp_{Eco} K34R	C-terminal genetic fusion of *efp* from *Escherichia coli* to the T18 fragment of CyaA. K34R single amino acid exchange variant. PCR fragment was amplified from pBAD24-efp_{Eco} K34R using P80/P81	this study
pUT18C-efp_{Eco} P32S K34R	C-terminal genetic fusion of *efp* from *Escherichia coli* to the T18 fragment of CyaA. P32S K34R double amino acid exchange variant. PCR fragment was amplified from pBAD24-efp_{Eco} P32S K34R using P80/P81	this study
pUT18C-efp_{Eco} $loop_{Ppu}$	C-terminal genetic fusion of *efp* from *E. coli* to the T18 fragment of CyaA. P32S K34R G35N Q36S multiple amino acid exchange variant, corresponding to EF-P of *E. coli* carrying the acceptor loop of EF-P from *P. putida.* PCR fragment was amplified from pBAD24-efp_{Eco} $loop_{Ppu}$ using P80/P81	this study
Plasmids for isoelectric focusing		
pBAD33-*efp*-His_6	C-terminal His_6-Tag *efp* version from *E. coli* into pBAD33	(Lassak et al., 2015)
pBAD33-*efp*-His_6-*epmAB*	C-terminal His_6-tagged *efp* and *epmAB from E. coli.* Overlap PCR fragment was amplified using P73/P76, P72/P75 and P74/P77	this study

Plasmids for EarP mutant test		
pBBR1-MCS2 NP_SO_PP1858-His6	C-terminal His6-Tag *efp* from *Pseudomonas putida* under control of the *Shewanella oneidensis* native *efp* promoter in pBBR1-MCS2	(Krafczyk et al., 2017)
pBAD33 PP1857 D13A-His6	C-terminal His_6-Tag *earP* from *Pseudomonas putida* in pBAD33. Mutant version; Asp13 exchanged to Ala	(Krafczyk et al., 2017)
pBAD33 PP1857 D17A-His6	C-terminal His_6-Tag *earP* from *Pseudomonas putida* in pBAD33. Mutant version; Asp17 exchanged to Ala	(Krafczyk et al., 2017)
pBAD33 PP1857 F191A-His6	C-terminal His_6-Tag *earP* from *Pseudomonas putida* in pBAD33. Mutant version; Phe191 exchanged to Ala	(Krafczyk et al., 2017)
pBAD33 PP1857 Y193A-His6	C-terminal His_6-Tag *earP* from *Pseudomonas putida* in pBAD33. Mutant version; Tyr193 exchanged to Ala	(Krafczyk et al., 2017)
pBAD33 PP1857 Q255A-His6	C-terminal His_6-Tag *earP* from *Pseudomonas putida* in pBAD33. Mutant version; Gln255 exchanged to Ala	(Krafczyk et al., 2017)
pBAD33 PP1857 F258A-His6	C-terminal His_6-Tag *earP* from *Pseudomonas putida* in pBAD33. Mutant version; Phe258 exchanged to Ala	(Krafczyk et al., 2017)
pBAD33 PP1857 R271A-His6	C-terminal His_6-Tag *earP* from *Pseudomonas putida* in pBAD33. Mutant version; Arg271 exchanged to Ala	(Krafczyk et al., 2017)
pBAD33 PP1857 D274A-His6	C-terminal His_6-Tag *earP* from *Pseudomonas putida* in pBAD33. Mutant version; Asp274 exchanged to Ala	(Krafczyk et al., 2017)
pBAD33 PP1857 S275A-His6	C-terminal His_6-Tag *earP* from *Pseudomonas putida* in pBAD33. Mutant version; Ser275 exchanged to Ala	(Krafczyk et al., 2017)
pBAD33 PP1857 R278A-His6	C-terminal His_6-Tag *earP* from *Pseudomonas putida* in pBAD33. Mutant version; Arg278 exchanged to Ala	(Krafczyk et al., 2017)
pBAD33 PP1857 Y291A-His6	C-terminal His_6-Tag *earP* from *Pseudomonas putida* in pBAD33. Mutant version; Tyr291 exchanged to Ala	(Krafczyk et al., 2017)

Amp^R, Cm^R, Kan^R: ampicillin, chloramphenicol, kanamycin resistance.

Table S3. Primers used in this study

Identifier	Oligonucleotide	Sequence (5' - 3')	Restriction site	Reference
Primers for sequencing and cloning				
P1	Seq33 fw	GGC GTC CAC ACT TTG CTA TGC		(Lassak et al., 2010)
P2	pBAD HisA rev	CAG TTC CCT ACT CTC GCA TG		(Lassak et al., 2010)
P3	*epmA* chk fw	TAG GTA CAA CAG TAT AGT CTG ATG GAT AA		this study
P4	*epmA* chk rev	TGA GGC ATG AAA CCA TCC TTC ATT TC		this study
P5	T7 Prom	TAA TAC GAC TCA CTA TAG G		
P6	T7 Term	TAT GCT AGT TAT TGC TCA G		
Primers for strain construction of KV1				
P7	*lacI*-583-fw	GTC TGC GTC TGG CTG GCT GGC ATA		(Fried et al., 2012)
P8	*luxC*-OL-rev	TAG TGC CCA TAG CTG TTT CCT GTG TGA AAT TGT TAT CC		this study
P9	*luxC*-OL-fw	GGA AAC AGC TAT GGG CAC TAA AAA AAT TTC ATT CAT TAT TAA CGG		this study
P10	*luxE*-OL-sRBS-*lacZ*-rev	AAT GTA CCT CCT TAC TTT ATT TAT TGT ATT TGT TTA GCT ATC AAA CGC TTC GGT TAA GCT C		this study
P11	OL-sRBS-*lacZ*-fw	ACA AAT ACA ATA AAT AAA GTA AGG AGG TAC ATT ATG ACC ATG ATT ACG GAT TCA CTG GCC G		this study
P12	*lacZ* 500bp anti	CGA CTG TCC TGG CCG TAA CCG ACC		(Fried et al., 2012)
P13	delta *cyaA* fw	GTT GGC GGA ATC ACA GTC ATG ACG GGT AGC AAA TCA GGC GAT ACG TCT TGA ATT AAC CCT CAC TAA AGG GCG		this study

P14	delta *cyaA* rev	TCC GCT AAG ATT GCA TGC CGG ATA AGC CTC GCT TTC CGG CAC GTT CAT CAT AAT ACG ACT CAC TAT AGG GCT C		this study
Primers *E. coli* loop mutation and overproduction constructs				
P15	*efp Eco* P32G fw	GTA AAA GGC GGT AAA GGC CAG G		this study
P16	*efp Eco* P32G rev	CCT GGC CTT TAC CGC CTT TTA C		this study
P17	*efp Eco* P32S fw	CGT AAA ATC GGG TAA AGG CCA GG		this study
P18	*efp Eco* P32S rev	CCT GGC CTT TAC CCG ATT TTA CG		this study
P19	*efp Eco* G33A fw	TAA AAC CGG CGA AAG GCC AGG		this study
P20	*efp Eco* G33A rev	CCT GGC CTT TCG CCG GTT TTA		this study
P21	*efp Eco* G33S fw	GTA AAA CCG TCG AAA GGC CAG G		this study
P22	*efp Eco* G33S rev	CCT GGC CTT TCG ACG GTT TTA C		this study
P23	*efp Eco* K34M fw	GTA AAA CCG GGT ATG GGC CAG GCA TTT		this study
P24	*efp Eco* K34M rev	AAA TGC CTG GCC CAT ACC CGG TTT TAC		this study
P25	*efp Eco* K34N fw	GTA AAA CCG GGT AAC GGC CAG GCA TTT		this study
P26	*efp Eco* K34N rev	AAA TGC CTG GCC GTT ACC CGG TTT TAC		this study
P27	*efp Eco* K34Q fw	GTA AAA CCG GGT CAG GGC CAG GCA TTT		this study
P28	*efp Eco* K34Q rev	AAA TGC CTG GCC CTG ACC CGG TTT TAC		this study
P29	*efp Eco* G35N fw	CCG GGT AAA AAC CAG GCA TTT GC		this study
P30	*efp Eco* G35N rev	GCA AAT GCC TGG TTT TTA CCC GG		this study
P31	*efp Eco* Q36S fw	GGG TAA AGG CAG CGC ATT TGC		this study

P32	*efp Eco* Q36S rev	GCA AAT GCG CTG CCT TTA CCC		this study
Primers *P. putida* loop mutation and overproduction constructs				
P33	NheI-NRBS-PP_1858-fw	GCA CTA GCT AGC CGC GGC CTC GAT TTT TAT AAA TCC	*Nhe*I	this study
P34	XbaI-PP_1858-GS-His6-rev	CGT CTA GAT TAG TGA TGG TGA TGG TGA TGC GAG CCC TTC TTG GAG CGG CCT TTG AA	*Xba*I	this study
P35	*efp Ppu* 29 30 31 OL fw	CGT AAC AGC GCG ATC ATG AAG ACC		this study
P36	*efp Ppu* K29A OL rev	CAT GAT CGC GCT GTT ACG GCC CGA CGC GGT GAA CTC AGC		this study
P37	*efp Ppu* K29R OL rev	CAT GAT CGC GCT GTT ACG GCC CGA GCG GGT GAA CTC AGC		this study
P38	*efp Ppu* S30A OL rev	CAT GAT CGC GCT GTT ACG GCC CGC CTT GGT GAA CTC AGC		this study
P39	*efp Ppu* S30G OL rev	CAT GAT CGC GCT GTT ACG GCC GCC CTT GGT GAA CTC AGC		this study
P40	*efp Ppu* S30P OL rev	CAT GAT CGC GCT GTT ACG GCC CGG CTT GGT GAA CTC AGC		this study
P41	*efp Ppu* G31A OL rev	CAT GAT CGC GCT GTT ACG CGC CGA CTT GGT GAA CTC		this study
P42	*efp Ppu* G31S OL rev	CAT GAT CGC GCT GTT ACG GCT CGA CTT GGT GAA CTC		this study
P43	*efp Ppu* R32K fw	ACC AAG TCG GGC AAG AAC AGC GCG ATC		this study
P44	*efp Ppu* R32K rev	GAT CGC GCT GTT CTT GCC CGA CTT GGT		this study
P45	*efp Ppu* 33 34 35 OL fw	ATC ATG AAG ACC AAG CTG AAG AAC CTG		this study
P46	*efp Ppu* N33D OL rev	CTT CAG CTT GGT CTT CAT GAT CGC GCT ATC ACG GCC CGA CTT		this study

P47	*efp Ppu* N33G OL rev	CTT CAG CTT GGT CTT CAT GAT CGC GCT GCC ACG GCC CGA CTT		this study
P48	*efp Ppu* S34A OL rev	CTT CAG CTT GGT CTT CAT GAT CGC CGC GTT ACG GCC CGA		this study
P49	*efp Ppu* S34Q OL rev	CTT CAG CTT GGT CTT CAT GAT CGC CTG GTT ACG GCC CGA		this study
P50	*efp Ppu* A35S OL rev	CTT CAG CTT GGT CTT CAT GAT GCT GCT GTT ACG GCC		this study
Primers cross modification/activation and overproduction				
P51	*efp Eco* domainI Ppu OL fw	AAG CTG GAC GAC GTG ATC CTG GAT ATG AAC CTG ACT TAC CTG		this study
P52	*efp Eco* domainI Ppu OL rev	CAG GTA AGT CAG GTT CAT ATC CAG GAT CAC GTC GTC CAG CTT		this study
P53	*efp Eco* loop *Ppu* OL fw	AAG TCG GGC CGT AAC AGC GCG TTT GCT CGC GTT AAA CTG CGT		this study
P54	*efp Eco* loop *Ppu* OL rev	CGC GCT GTT ACG GCC CGA CTT TAC GAA TTC ACT CGC TTC AAC		this study
P55	*efp Eco* P32S K34R OL fw	GAA TTC GTA AAA AGC GGT CGC GGC CAG GCA TTT		this study
P56	EF-P *Eco* P32S K34R OL rev	AAA TGC CTG GCC GCG ACC GCT TTT TAC GAA TTC		this study
P57	*efp Eco* K34R G35N OL fw	CCG GGT CGC AAC CAG GCA TTT GCT CG		this study
P58	*efp Eco* K34R G35N OL rev	CGA GCA AAT GCC TGG TTG CGA CCC GG		this study
P59	*efp Eco* K34R Q36S OL fw	CGC GGC AGC GCA TTT GCT CGC GTT A		this study
P60	*efp Eco* K34R Q36S OL rev	TAA CGC GAG CAA ATG CGC TGC CGC G		this study
P61	*efp Eco* P32S K34R G35N OL fw	AGC GGT CGC AAC CAG GCA TTT GCT CG		this study

P62	*efp Eco* P32S K34R G35N OL rev	CGA GCA AAT GCC TGG TTG CGA CCG CT		this study
P63	*efp Eco* P32S K34R Q36S OL fw	AGC GGT CGC GGC AGC GCA TTT GCT CG		this study
P64	*efp Eco* P32S K34R Q36S OL rev	CGA GCA AAT GCG CTG CCG CGA CCG CT		this study
P65	*efp Eco* K34R G35N Q36S OL fw	GGG TCG CAA CAG CGC ATT TG		this study
P66	*efp Eco* K34R G35N Q36S OL rev	CAA ATG CGC TGT TGC GAC CC		this study
P67	*earP So* rev	GCG GTA CCC GAT TTT CTA TTT CAG CGC AGC AT	*Kpn*I	this study
Primers pET SUMO constructs				
P68	*efp Eco*-SUMO-fw	ATG GCA ACG TAC TAT AGC AAC GAT TTT		this study
P69	*efp Eco*-SUMO-rev	TTA GTG ATG GTG ATG GTG ATG GCT		this study
P70	*efp Eco* K34R OL fw	GTA AAA CCG GGT CGC GGC CAG GCA TTT		this study
P71	*efp Eco* K34R OL rev	AAA TGC CTG GCC GCG ACC CGG TTT TAC		this study
Primers isoelectric focusing and pBAD33-*epmA*				
P72	*efp-yjeA*-OL-XbaI-fw	CGT GAA GTA ATC TAG ATT GTC AAA AAC TGG AGA TTT AAC TAT GAG C	*Xba*I	this study
P73	*efp-yjeA*-OL-XbaI-rev	TTT TTG ACA ATC TAG ATT ACT TCA CGC GAG AGA CGT ATT CA	*Xba*I	this study
P74	*yjeA-yjeK*-OL-PstI-fw	GCA TAA CTG CAG GGT AGC TAA GCC ACA AAA TGG CG	*Pst*I	this study
P75	*yjeA-yjeK*-OL-PstI-rev	GCT ACC CTG CAG TTA TGC CCG GTC AAC GCT AAA G	*Pst*I	this study
P76	SacI-RBS-*efp*-fw	GCG ATG AGC TCA ATT AAC AAA TTT CAG AGG GCC TTA TGG	*Sac*I	this study

P77	SphI-*yjeK*-rev	GCA TCG CAT GCT TAC TGC TGG CGT AGC TGG AG	*Sph*I	this study
Primer for strain construction of MG-CR-efp-epmA-KanR				
P78	*epmA* fw	CAC CGC TGT TTG ATT CCT GCG T		this study
P79	*epmA* rev	GCT ACA GAA TGG CGC TTA T CA CG		this study
Primer for for bacterial two-hybrid (BTH)				
P80	XbaI-*efp-Eco* FW	GTA TCG TCT AGA GGC AAC GTA CTA TAG CAA CGA TTT TCG TG	*Xba*I	this study
P81	XmaI-*efp-Eco* Rev	GTA TCG CCC GGG ACT TCA CGC GAG AGA CGT ATT CAC C	*Xma*I	this study

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