

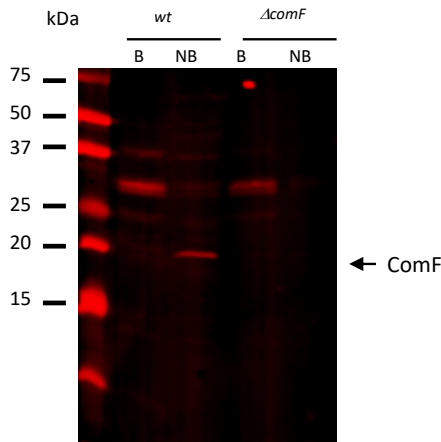
## **Supplementary Information**

### **ComFC mediates transport and handling of single-stranded DNA during natural transformation**

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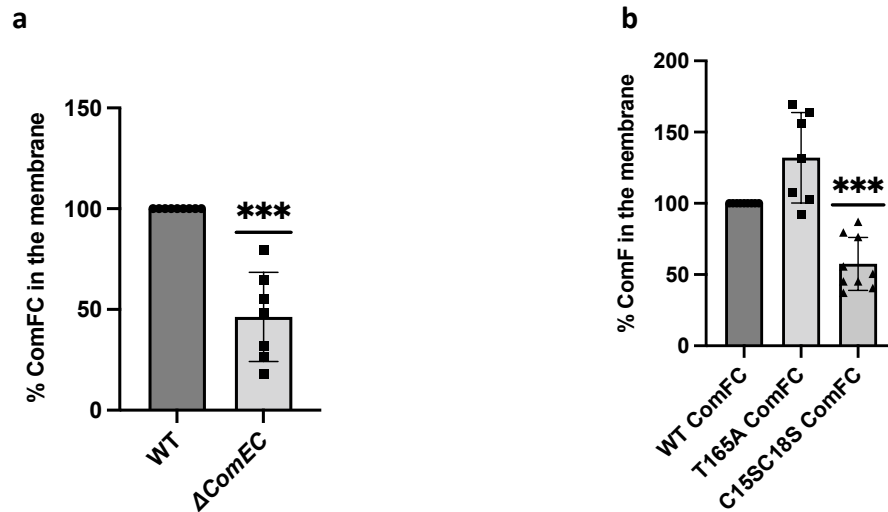
**Other Supplementary Information for this manuscript includes the following:**

Movies S1 to S3



**Supplementary Figure 1.**

**ComF(C) detection by immunoblotting.** Total extracts from wild-type (*wt*) and *comFC* mutant (*comF*) *H. pylori* strains. Samples were either boiled (B) or not boiled (NB) prior gel loading. ComF is specifically detected in the NB condition. Blot is representative of two experiments.



**Supplementary Figure 2.**

**ComFC association with the membrane.** (a) Quantification of Western blots comparing the amount of ComFC in membrane fraction of wild-type and  $\Delta comEC$  strains. Data are from at least three independent fractionations.  $n = 7$  for each genotype (b) Quantification of Western blots comparing the wild-type and mutant ComFC present in the membrane fraction ( $n = 9$  for WT ComFC,  $n = 7$  for T165A ComFC and  $n = 9$  for C155 C18S ComFC). Quantification was carried out by normalising the ComFC band intensity to that of the MotB band to take account of the membrane fraction recovery efficiency. Values for the wild-type strain (panel 2a) and wild-type protein (panel 2b) were set to 100%. Bars correspond to the mean  $\pm$  SD. \*\*\*  $p < 0.001$  with respect to *wt* defined as 100% (One sample t test)

Hpy

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Hpy .....
Sau .....
Efa .....
Spy .....
Spu .....
Bsu .....
Lmo .....
Cje .....
Cpe .....
Tth .....
Vch .....
Hin .....

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\* \* Zn finger \* \*

Hpy

TTT 1 10 α1 00000 20

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Hpy .....MR.....LTCLKLS.FKPL.....LNCLN
Sau .....MNNCLSCGAKLYENITTYNLF...KKPNRLDRCKENWDNIKLDIKARRCSRCLK
Efa .....MDCKNCASPIRLETTLKMLLRFKKWARPSLQHCQAKFQKIPMIGT...CFGCSR
Spy .....MILLCGQISQTPISITIEIFLR.RISSPIQQCQKSFQKIGK.SV...CATCCA
Spu .....MKLLCGQTMKTVLTFSSLLLR.NDDSCLSDCDSTFERIGE.EN...CPNCKM
Bsu MNKTESHLLCSQLLKTSVITWKIL.LIK.QFTPTICTRCTAR.....
Lmo .....MTNCLLCFQPVQRQSASWEIS.WLF.EPLPICNLCLAGFEKLTG.LL...CKNCSK
Cje .....MRINCGAFA.LLCF.....DLCGL
Cpe .....VDYLFQCFLEAI.....YPTEEKYVNTG.EKII...CDKCKG
Tth .....ALLGHA.....CPGGGLDLPAL...GRCRA
Vch APPLTPECHLCRLALDT.....NSPFGVSAQAWLEHGYSR...CARCGL
Hin MNFFNFRCHRCRNLHI.....AKNGLCSGQKQIKSFPY...GHGA

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Hood domain

Hpy

β1 30 β2 40 β3 50 α2 50 η1 60 η2 60

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Hpy DL.PLSLKVRL.L.....EGVSVYSFYAYS...EIEELIKSKYTIIGSRILP
Sau HLNQDEAYCLDCK...FLSAHF.NLMEQLYCFQYDGLMKEMIHQYKFLKDYIYLCCLLA
Efa ..VSQERYCFDCK...RWQLLYPEYSFHNAALFHYDEGMQSWMERYKFGDYRLRMCFN
Spy ..NSDIIACRDCL...KWEN.KGVNVNHRSLCYNAAMKAYFSQYKFGDYLRKVF
Spu ..TELSTKQDCQ...FWCK.EGVGVSHRAIFTYNQAMKOFFSRYKFGDYLRKVF
Bsu .....F...EHSQSITALYQYNEAMKDYLHQFQKFLQDVALLAKVFR
Lmo .....SRT...HFLDSNKSIYRYNDFAKSYMKKKFGDYIYGAIFK
Cje ELSEPSLNVKRLD.....NFKVYSFYKYH...EIQHLLHSRHYFYGYFYVYK
Cpe KIQAQKET...L.IIEECE.MLICSYYSFIV.K...DLILRLKYKGFHAGEILV
Tth GLEAF.....STGE...MVYLGHYAR.V.G...PLVRALKYGRREALARALA
Vch PTLTPIDQCGQCLGQPPPWRK...LMCVGDYRFPL.S...DAVHQLKYQRQFVQAQRLA
Hin ELQYYAQHCNGCLKQEPSWDK...MVIIGHYVEPL.S...VLIHRFKYQNFVWDRILA

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PPI loop

Hpy

α3 80 β4 90 TT 100 α4 110 TT 120

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Hpy LLSQKAGAEFAKILQEKGLNTPYGIATDDRIK.SFYSHSAAQLKGFQGNLRKPYGRTR
Sau HLIEIPQT...S.....YDYIVPISSPAHDLSRTFNPEAVLK...AKGIRFDKILK
Efa EEINFYFOQ.QS.....ADYIIPVPLSEKRMQERGFNOVIGLLE...AADVPYSPFLI
Spy VFLADVITKYYK...GYIPVVPVSPGCFRERGFNOVSAILLE...AANVSYLSLFE
Spu SFLSEELKK.YK.....EYQFVVPVSPDRYANRGNQVEGLVE...AAGFEYLDLLE
Bsu QELYTRFNK..E.....KAMIVPIMHPLKQERTFSHTNELLT...AANIPIYQLLE
Lmo KELSTFLSE..K.....KEKIVPVPVSETRKLEKRGFNQTTAILK...QSGILYEELLA
Cje MLAKLSFAKFKDFDPP...RMTIVVIALDDKVEDMLYSHSAILARYLKTFRVFNVLK
Cpe MLEEKIKESNLD...VDFIITYVPVAKDSIRKKEFNQCEYLSKELGKRLGIKSIETLK
Tth EPLARAVAARGW...R.LQGVTAVPTLPHRLALRGYNPPEVLARALAGALGVYRPVLF
Vch KLATQINEP.....APLLCSVPLHWQRRLRGNQSDLRLARELANVLNIEYDHLF
Hin RLYLAVRDAKRTHLKQPEAIPVPLYHFRQWRBRYNQADLSRQLSRWLDIPNLNNIV

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Flexible loop

PRPP loop

Hpy

β6 130 TT 140 α5 150 β7 160 β8 170 α6 170

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Hpy ..ANNITISYAGKSLKFRANNPRDFTFK.GD...ENLDYFLDDTITIGTTLKRALKYLK
Sau ..M.SNRPKQSHLTKKERLADENFFIIDTEL..DLNGKEILLVDDTYTIGTLIHRAGCKLY
Efa RK.EENVPQSKTKRERMQLQPPFAIQKENQKLLKNCVILVDDTYTIGRTLHFHAAAVIN
Spy ..K.LDNTHSSSRKRELLVEKSYRLLKVS..NIP.DKILLVDDTYTIGSTIIALRKOIA
Spu ..K.REERASSSKRSERLGTTELPFFIKSGV..TIP.KKILLVDDTYTIGATINRKKLLE
Bsu ..K.TITEAGSKRREQLRAAPLFRLEKGA..NVEHKDYLLVDDTYTIGTTLQHAADVLM
Lmo ..K.KHTEKQSKTKRERLASEQAFYFSGKE..ILKSTEVILVDDTYTIGSTLNLAAQILK
Cje ..K.AQNSVKYSKSLERQKHKRNYKLL.KT...IHEPVILVDDTYTIGSSLLAKKVE
Cpe KK.NNVKEKSLKREERKNVQNAFKLRYKN.LEGKSILLVDDTYTIGSTLAKVRELK
Tth RRRY.TPQPTRGFKERLLPGDLFGARVR...VEGAWLLVDDTYTIGATFLRARGALL
Vch ARRRATPHQQGLSKAQRHNLRDFAVNLNHP...NQTHVAIVDDTYTIGSTIRHCDLL
Hin KRVKHTHTQRLGSAKDRQNLKNAFSLAVLKNFPPYHRVALVDDTYTIGSTLNEISKLIR

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Hpy

β9 180 190

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Hpy I.LNKARFAIALCSADE.....
Sau AKNIRKFKVFAFAR.....
Efa DCYPKSLNFTLAR.....
Spy KVANSDIKSLSIAR.....
Spu EAGAKDKVTFSLVR.....
Bsu QAGAKNVQYFTLIEG.....
Lmo EAGVHKVSALTIFR.....
Cje ENKISVLFALVLADAKV.....
Cpe KIKDKIKIFLLTIAKSNL.....
Tth EAGAAAY..VYGFALVARDPFGALGPYR
Vch DVGVQSIIDYICICRTEPEPKDSHG..
Hin KLVGEEITQVWGLARA.....

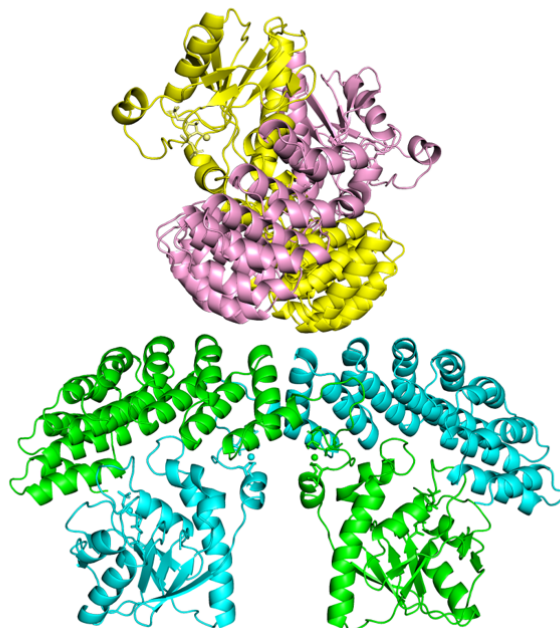
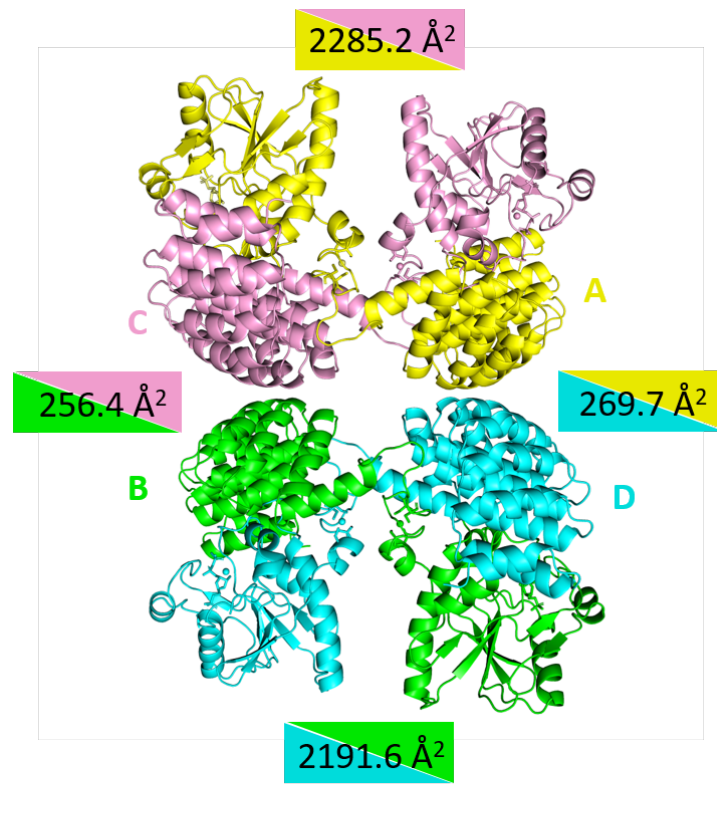
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### Supplementary Figure 3.

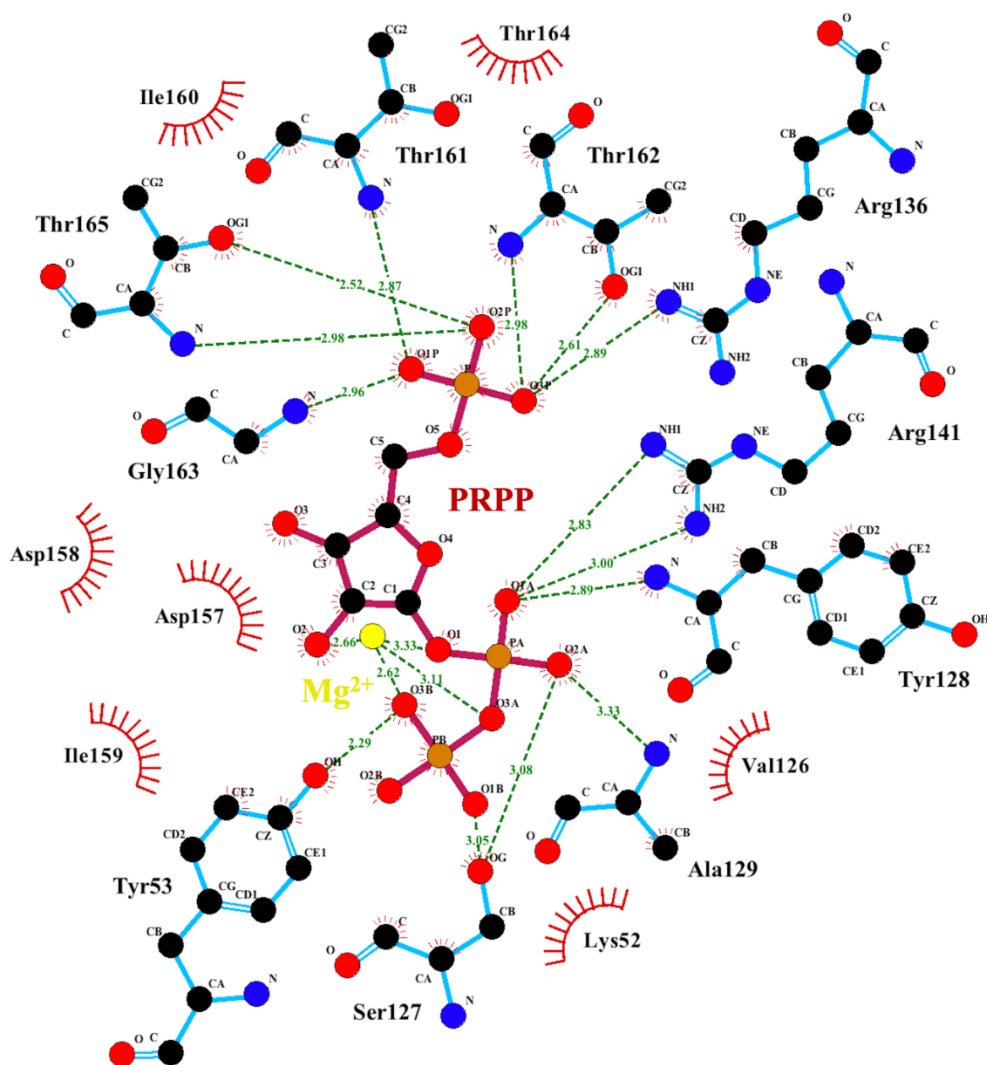
#### Sequence alignment of the ComFC proteins from gram(-) and gram(+) bacteria

The multialignment was generated by Clustalw2 (32). The figure was generated using ESPRIPT (33). The secondary structure elements of ComFC are in black on the top of the multialignment. Grey and green bars respectively localize the Hood domain and the three PRTase-loops. The 4 cysteine residues of the Zn-finger are indicated by orange stars. Hpy: *Helicobacter pylori*, Sau: *Streptococcus aureus*, Efa: *Enterococcus faecalis*, Spy: *Streptococcus pyrogenis*, Spn: *Streptococcus pneumoniae*, Bsu: *Bacillus subtilis*, Lmo: *Listeria monocytogenes*, Cje: *Campylobacter jejuni*, Cpe: *Clostridium perfringens*, Tth: *Thermus thermophilus*, Vch: *Vibrio cholerae*, Hin: *Haemophilus influenzae*.

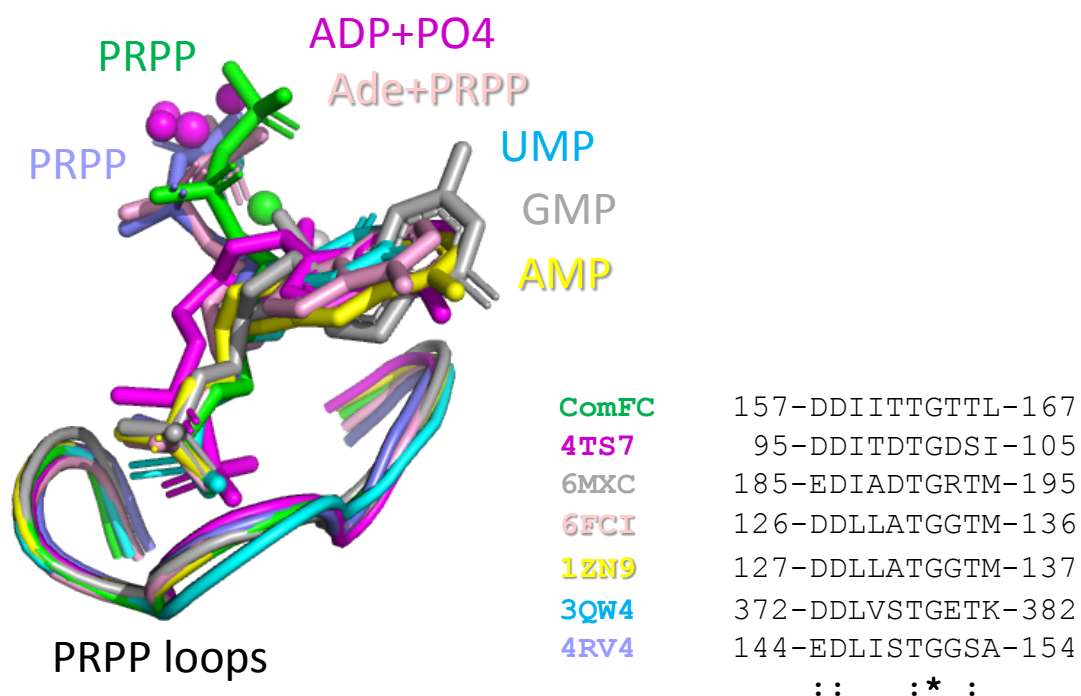
Supplementary Figure 4.



a. Two views of the crystal structure of the 4 molecules of the  $\alpha$ Rep-HpComFC fusion, forming 2 domain-swapped dimers in the asymmetric unit. The two protein fusions of the first dimer are in green and blue (colored as in Fig. 5), the two others are in pink and yellow. The PDBePISA server calculates the interface areas.



- b. **ComFC binds the PRPP** (in red sticks) and  $Mg^{2+}$  ion (in yellow circle) through hydrogen bonds (green dotted lines, involved residues in cyan sticks) and hydrophobic forces (red half circles and dashes). The PRPP and the flexible loops are involved. The 9 residues of the PRPP loop are the characteristic motif of the PRTase family. 5 residues of the flexible loop can close the active site pocket to sequester the PRPP. Two other residues (K52 and Y53) located between helices  $\alpha 2$  and  $\alpha 3$  are also involved in the binding of the PRPP.



**c. Structural alignment of the PRPP loops of various PRTases co-crystallised with ligands and Mg ions.**

PDB ID 4TS7 : Adenine Phosphoribosyltransferase from *Sulfolobus solfataricus*

PDB ID 6MXC : Hypoxanthine-guanine phosphoribosyltransferase from *Trypanosoma brucei brucei*

PDB ID 6FCI : Adenine Phosphoribosyltransferase from Human

PDB ID 1ZN9 : Adenine Phosphoribosyltransferase from Human

PDB ID 3QW4 : UMP synthase from *Leishmania donovani*

PDB ID 4RV4 : Orotate phosphoribosyltransferase from *Bacillus anthracis*

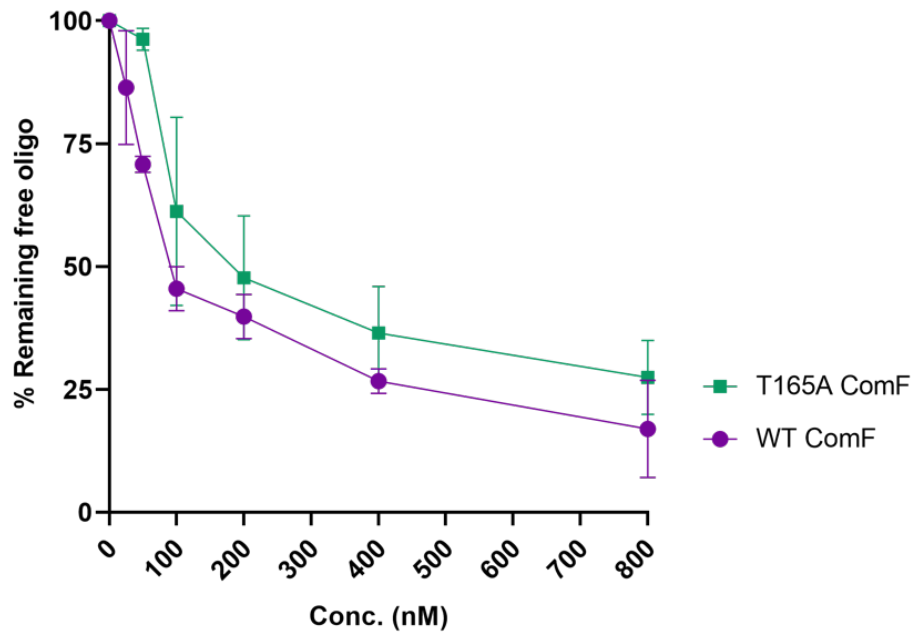
PRPP : phosphoribosylpyrophosphate

UMP : uridine-5'-monophosphate

GMP : guanosine-5'-monophosphate

AMP : adenine-5'-monophosphate

Ade : Adenine



**Supplementary Figure 5.**

**Comparison of DNA binding affinities of WT ComFC with ComFC T165A.** No significant difference was observed between the binding of WT and T165A ComF proteins to ssDNA. Quantification of three independent electrophoretic mobility shift assays was performed using Image Studio software. Data corresponds to the Mean +/- SD.



**Table S1.****Table S1.**

Approximate melting temperature (°C) of wild-type ComFC and ComFC-T165A with or without added ligand

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<b>Ligand</b>	<b>Wild-type ComFC</b>	<b>ComFC-T165A</b>
AMP (0)	45.7	42.1
AMP (5mM)	55.1	43.7
AMP (10 mM)	55.0	45.2
ADP (0)	45.0	41.2
ADP (5mM)	51.4	44.6
ADP (10 mM)	51.6	42.9
ATP (0)	46.7	41.4
ATP (5mM)	45.0	41.2
ATP (10 mM)	45.8	41.5
Ribose-5-Phosphate (0)	44.9	41.3
Ribose-5-Phosphate (5 mM)	48.2	42.0
Ribose-5-Phosphate (10 mM)	48.2	41.8

**Table S2.****Strains used in this study**

<b>Strain</b>	<b>Genotype</b>	<b>Source</b>
LR1	26695	
LR133	26695 <i>strep</i> <sup>R</sup>	Lab. collection
LR293, LR294	26695 <i>recA::Cm</i>	Lab. collection
LR827, LR828	26695 <i>dprA::Cm</i>	Lab. collection
LR768, LR769	26695 <i>comB2::Cm</i>	Lab. collection
LR776, LR777	26695 <i>comEC::Km</i>	Lab. collection
LR887	26695 <i>pUreA-GFPmut2-Km</i>	Lab. collection
LR901, LR902	26695 <i>pUreA-GFPmut2-Km comEC::Cm</i>	Lab. collection
LR982	26695 <i>pUreA-GFPmut2-Km hp1473::Cm</i>	This work
LR762	26695 <i>hp1473::Cm</i>	This work
LR965	26695 <i>hp1473::Cm rdxA ::hp1473-Km</i>	This work
LR1038	26695 <i>hp1473::Cm rdxA ::hp1473-FLAG-Km</i>	This work
LR1000	26695 <i>hp1473::Cm rdxA ::hp1473-T165A-Km</i>	This work
LR1051	26695 <i>hp1473::Cm rdxA ::hp1473-C15SC18S-FLAG-Km</i>	This work
LR1209	26695 <i>pUreA-hp1473 flag-Cm hp1473::Apra</i>	This work
LR1211	26695 <i>pUreA-hp1473 flag-Cm hp1473::Apra</i>	This work
LR1213	26695 <i>pUreA-hp1473 flag-Cm hp1473::Apra</i>	This work

**Table S3.**  
**Oligonucleotides used in this study**

Name	Sequence (5'-3')	Description
1473 F	ATGCGCTGTTAACCTGTTTG	hp1473 forward
1473 R	TCATTCATCCGCGCTGCAAAG	hp1473 reverse
1473 inverse R	CGGGGTACC GATTTTCACAACTCTGCACC	--
1473 inverse F	CGCGGATCC GGCGTTAAGGGCTAATAATGC	--
Op3	GTAATTTTTCTATGCCTTGTTTTCTTATTCTCCTAGTTAGTCA GGTACC	HP1473 954
Op4	CTTTGCAGCGCGGATGAATGAATGGCTAAAATGAGAATATCAC C	HP1473 KanR
Op13	GCCCTAAAAGAAGCCCTAAAATACCTTAAAAC	TA fw
Op14	GTTTTAAGGTATTTTAGGGCTTCTTTTAGGGCGGTGCCGGTGG TG	TA rev
Op302	GCTTTCT TTT AAG CCT CTT TCC CCA AAT TCC TTGAACGATTTGCCCTTAAGCTTAAAGG	C15SC18S F
Op303	CCTTTAAGCTTAAGGGCAAATCGTTCAAGGAATTTGGGGAAAG AGGCTTAAAAGAAAGC	C15SC18S R
Op247	GAGGGGTTTGTACTAGGGTTTATACGACTACCCTAGAAAGCC TAACTCGGCTTTAAGAAAGGTTGCCAAAGTTC	Streptomycin resistant (75-mer) ssDNA for electroporation
Op611	CTTTAAGAATAGGAGAATAAGGAATTCATGCGCTGTTAACCT GTTTGAAGC	For PureA-ATG/ComFC
Op612	TTACTTATCGTCGTATCCTTGAATCTTCATCCGCGCTGCAA GCGCG	Rev ComFC-FLAG*taa
Op613	GCTTCAAACAGGTTAAACAGCGCATGAATTCCTTATTCTCCTAT TCTTAAAG	Rev PureA- GAATTCATG/ComFC
Op614	GATTACAAGGATGACGACGATAAGTAAAGCGGCCGCGACTCT AGATCATAATCAGCC	For FLAG* - linker
Op853	GAGAATATTGTAGGAGATCTTCTAGAAAAGATAAAGAGGGCTT AAAACAGCGCTTAAGCC	For Eco47- HP1473
Op854	CCGGATGGCTCGAGTTTTTCAGCAAGATTCAGGGCGGTTACCC CCTAAACC	Rev HP1473 dw-Eco47
Op855	GGCTTAAGCGCTGTTTTAAGCCCTCTTATCTTTCTAGAAGATC TCCTACAATATTCTC	Rev Eco47- HP1473up
Op856	GGTTTAGGGGGTAACCGCCCTGAATCTTGCTGAAAACTCGAG CCATCCGG	For HP1473dw -Eco47
Op857	TCGCCGCTTTTATAAAATGCGCTGTTAACCTGTTTGGTACCCG GGTGACTAACT	For HP1473up /20nt HP1473-Apra
Op858	TTAAAAAATAAAATTATAACTCATTTCATCCGCGCTGCAAAGGAT CCCCGTGTCATTATT	Rev Apra- 20nt HP1473+HP1473dw

Op859	AATAATGACACGGGGATCCTTTGCAGCGCGGATGAATGAGTT ATAATTTTATTTTTTAA	For Apra- 20nt HP1473+Hp1473dw
Op860	AGTTAGTCACCCGGGTACCAAACAGGTAAACAGCGCATTTTA TAAAAGCGGCGA	Rev HP1473up /20nt Hp1473-Apr
XV2	TGGGTGAACCTGCAGGTGGGCAAAGATGTCCTAGCAATGTAA TCGTCAAGCTTTATGCCGTT	62 mer ssDNA5-Cy5 labelled
cXV2	AACGGCATAAAGCTTGACGATTACATTGCTAGGACATCTTTGC CCACCTGCAGGTTCAACCA	62 mer ssDNA Complementary to XV2

**Table S4.**  
**Plasmids used in this study**

<b>Name</b>	<b>Description</b>	<b>Source</b>
p978	pJet1.2- <i>hp1473::Cm</i>	This work
P1175	pJet1.2- <i>RdxA:: Km</i>	Lab collection
p1176	pJet1.2- <i>RdxA:: Prom-hp1473-Km</i>	This work
p1204	pJet1.2- <i>RdxA:: Prom-hp1473-T165A-Km</i>	This work
P1284	pJet1.2- <i>RdxA:: Prom-hp1473-FLAG-Km</i>	This work
P1310	pJet1.2- <i>RdxA:: Prom-hp1473-C15SC18S-FLAG-Km</i>	This work
P1410	pET21- <i>His6-TEV- hp1473</i>	This work
P1412	pET21- <i>His6-TEV- hp1473-T165A</i>	This work
P1088	pJet1.2-PromUreA-Cm	Lab collection
P1672	pJet1.2-PromUreA- <i>hp1473-Flag-Cm</i>	This work
P1674	pJet1.2-PromUreA- <i>hp1473-T165A-Flag-Cm</i>	This work
P1676	pJet1.2-PromUreA- <i>hp1473-C15S C18SFlag-Cm</i>	This work
P1699	pJET1.2- <i>hp1473::Apra</i>	This work