Supplemental Tables and Figures

Table S1. **List of primers**

Chromosomal sequences are in upper cases and restriction sites are underlined.

Primers	Sequence 5'- 3'	Purpose
Ebm732	GCGGATATATGCCGTGTTGTTAAAAAAAGTCTTGAGTCCAGGGTGtccatggaaaagagaag	IacP_TAP
Ebm734	ATAACAATTAATCTTATTCAATTGTTGTCAAGCGAGAGAAAAATAcatatgaatatcctccttag	tag
Ebm735	gaagaattcATGGCTTGCACATGCAGCGTT	*iacP*_TAP
Ebm736	ATCATCATATATTGCAGCCATAC	cloning
Ebm798	GATCTTTACGCTGACACATTGGATTTAATTG	$IacP_{S38T}$
Ebm799	CAATTAAATCCAATGTGTCAGCGTAAAGATC	mutagenesis
Ebm674	tctagaattcATGAATATGGATATTGAAGCAAGAGTC	*iacP* cloning
Ebm675	ttgctcgagCTACACCCTGGACTCAAGAC	(2H, pKO3)
Ebm773	gaagaattcATGGCGATTCTCGGCCTGGGA	*acpS* cloning
Ebm774	ctcctcgagCTAACTTTCCAGAATGACCGT	(2H, pP_{TET})
Ebm775	gaagaattcATGCTGACATCTCATTTTCCC	*entD* cloning
Ebm776	ctcctcgagTTATCGGGGTATTGCGCTAAG	(2H, pP_{TET})
Ebm777	gaagaattcATGTACCAGGTCGTTCTGGGA	*acpT* cloning
Ebm778	ctcctcgagTCATAGCGCTTTCGTTATCGT	(2H, pP_{TET})
Ebm779	gaagaattcATGGCGACTCACTTTGCCAGA	*yieE* cloning
Ebm780	ctcctcgagTTATGAACGATTAAGCGTATA	(2H, pP_{TET})
Ebm904	GGCGGTGGCTGAAATGAAACGTTTGATGCTGGAAGCGCGCGGCTAAtcattggctggcaccaagcag	P_{BAD}*acps*
Ebm905	GCGGGCAATCTCTACAATATCCGTTCCCAGGCCGAGAATCGCCATcgtttcactccatccaaaaaaacggg	
Ebm133	gaccggatccAAATCACTGGCGCGCGAAG	pKO3*iacP*
Ebm959	CGAGTTTTGATAGGAAATTTAAGAGTATGAATATGGATATTGAAGCAAGAG	
Ebm960	CTCTTGCTTCAATATCCATATTCATACTCTTAAATTTCCTATCAAAACTCG	

Table S2. **List of plasmids**

Name	Lab code	Description	Reference
pKD46	pEB267	Amp^R, repA101ts & oriR101	(17)
pJL72	pEB793	Amp^R, Kan^R	(18)
pKT25link	pEB354	Kan^R, p15A ori, P*lac*, T25	(20)
pT25_ACP	pEB375		(24)
pT25_ACP_{S36T}	pEB885		(24)
pT25_IacP	pJV4		This work
pT25_AcpS	pJV9		This work
pT25_EntD	pJV10		This work
pT25_AcpT	pJV11		This work
pT25_YieE	pJV12		This work
pT25_$IacP_{S38T}$	pJV17		This work
pUT18Clink	pEB355	Amp^R, colE1 ori, P*lac*, T18	(20)
pT18_ACP	pEB379		(20)
pT18_ACP_{S36T}	pEB612		(20)
pT18_IacP	pJV1		This work
pT18_AcpS	pJV13		This work
pT18_EntD	pJV14		This work
pT18_AcpT	pJV15		This work
pT18_YieE	pJV16		This work
pT18_$IacP_{S38T}$	pJV18		This work
pUC18_IacP_TAP	pJV21	Amp^R, pMB1 ori, MCS *lacZ*a	This work
pUC18_$IacP_{S38T}$_TAP	pJV22		This work
pP_{TET}	pEB1242	Amp^R, colE1 ori, P_{TET}, 6His	pASK-IBA37plus, IBA
pP_{TET}*acpS*	pJV37		This work
pP_{TET}*entD*	pJV38		This work
pP_{TET}*acpT*	pJV39		This work
pP_{TET}*yieE*	pJV40		This work
pKO3	pEB232	Cm^R, repA(ts) ori, M13 ori, *sacB*	(22)
pKO3*acpP*	pEB1334		(30)
pKO3*iacP*	pEB1453		This work

Figure Legends

Fig. S1: Post-translational modifications occurring on the acyl carrier protein ACP. Components are indicated on top and designations of ACP forms are indicated at the bottom.

Fig. S2: Purified IacP_CBP and IacP$_{S38T}$_CBP recovered using the TAP method, ran on SDS-PAGE 15% and stained with Coomassie Blue.

Fig. S3: Phosphopantetheinyl transferase protein sequence alignment. The multiple sequence alignment has been produced using ClustalW and displayed using Jalview. The consensus sequences of the PPTase enzyme family defined by Lambalot and collaborators are framed (11) and the alignment has been manually corrected in the N-terminal part to optimize their alignment.

Fig. S4: Growth phenotypes related to AcpS depletion and complementation

A. Growth phenotypes of the IacP_TAP (JV48) and P$_{BAD}$*acpS* IacP_TAP (JV68) strains onto agar LB plates containing 0.2% arabinose (inducing condition of PBAD), 0.2% glucose (repressing condition of P$_{BAD}$) or agar LB without any sugar addition (non-inducing condition of P$_{BAD}$). **B.** Growth phenotypes of P$_{BAD}$*acpS* IacP_TAP harboring the different PPTases genes cloned into pP$_{TET}$, onto agar LB plates containing 0.2% arabinose (inducing condition of PBAD) or agar LB (non-inducing condition of P$_{BAD}$).

Fig. S5 Complementation of *acpP* mutant strains

A. The *E. coli* ACP temperature-sensitive strain (ACP^{ts}) was transformed with the indicated plasmids used for bacterial 2-hybrid and carrying *acpP* or *iacP* coding sequences fused to the T18 fragment of adenylate cyclase. Transformants were spread on LB plates containing ampicillin and incubated at 30°C or 42°C for 3 days. Only strains able to complement the ACP temperature-sensitive phenotype could grow at 42°C. **B**. Using phage P1, the Δ*acpP*::Kan^{R} allele was transduced into *E. coli* MG1655 bearing the empty plasmid pKO3 or pKO3 carrying *acpP* or *iacP* under the control of the *acpP* promoter. Transductants were spread on LB plates containing kanamycin and grown at 30°C. Only strains able to

32 complement the absence of ACP could integrate the Δ*acpP*::Kan^{R} deletion and grow on
33 kanamycin selective medium.

FIGURE S1

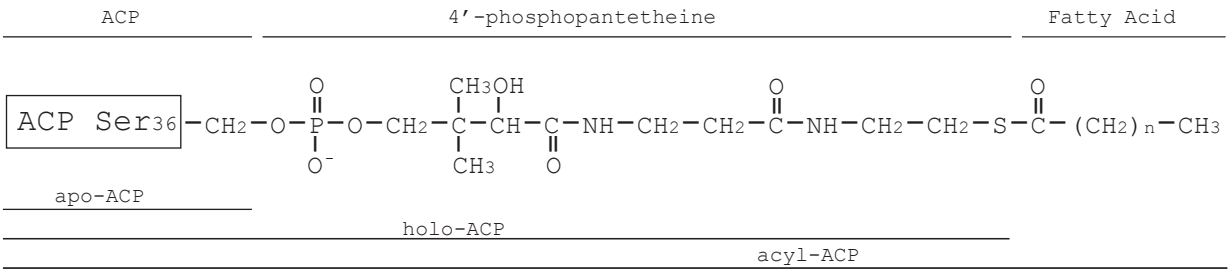

FIGURE S2

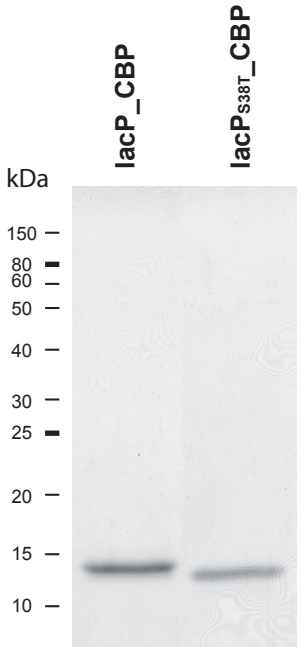

FIGURE S3

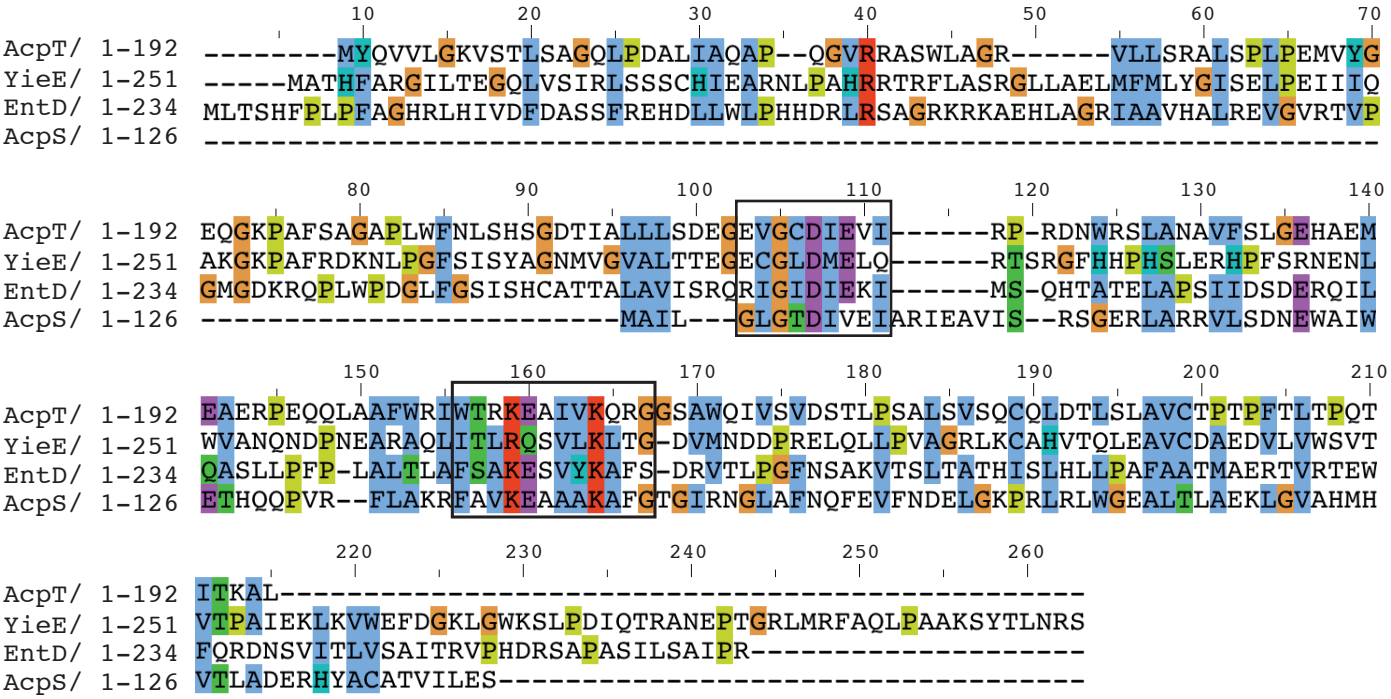

FIGURE S4

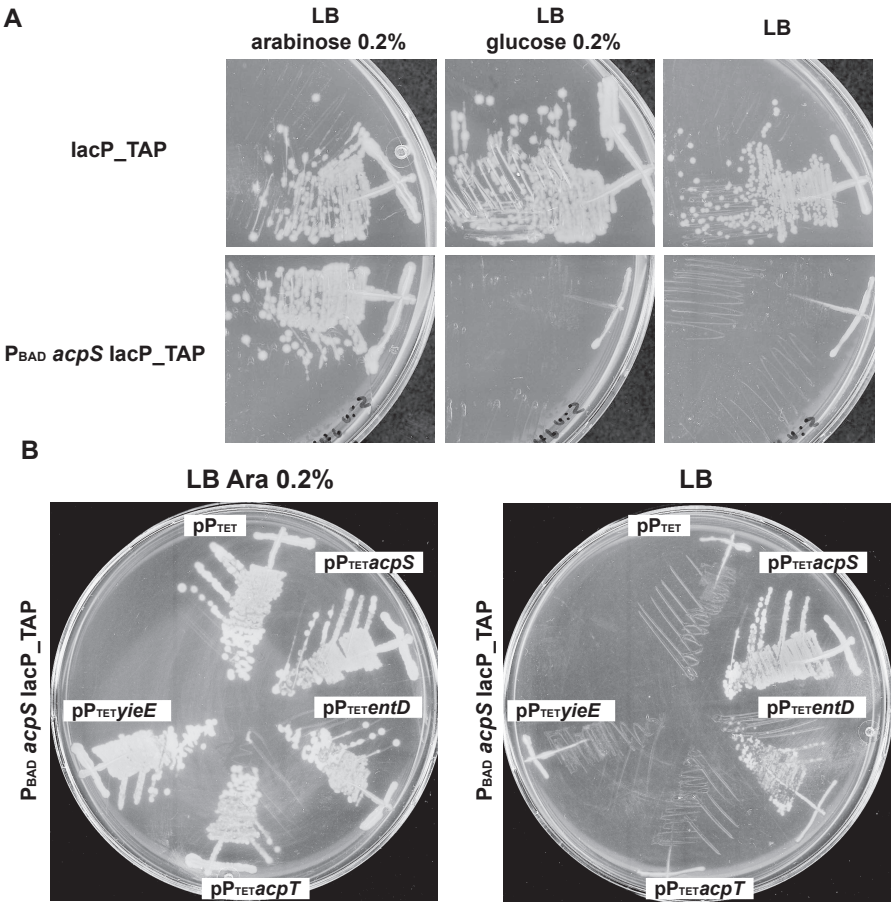

FIGURE S5