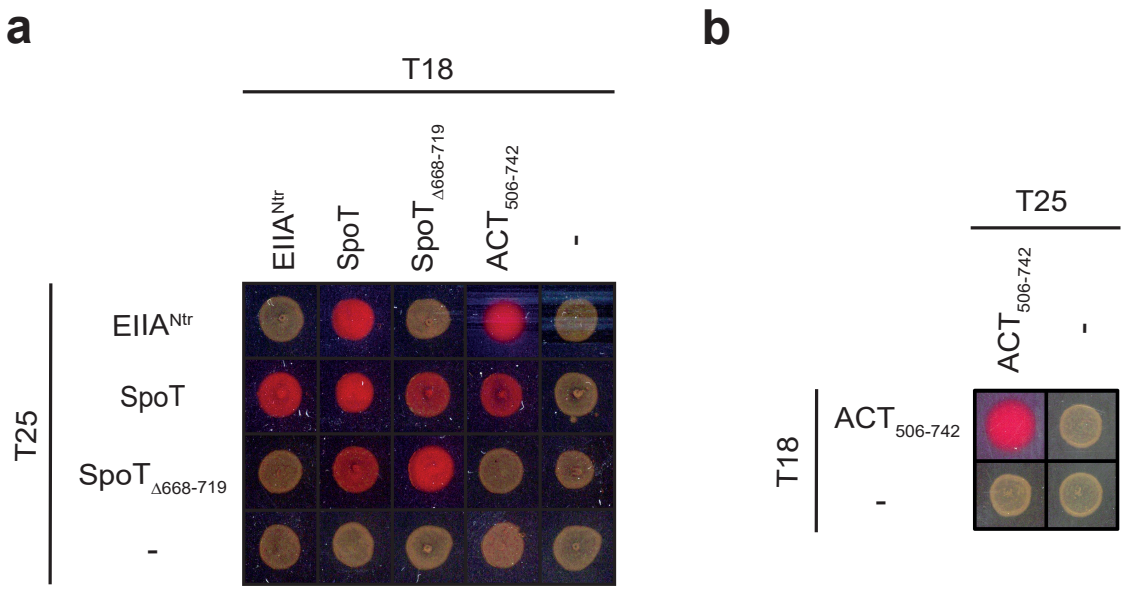
Supplementary Figures

Supplement Figure 1



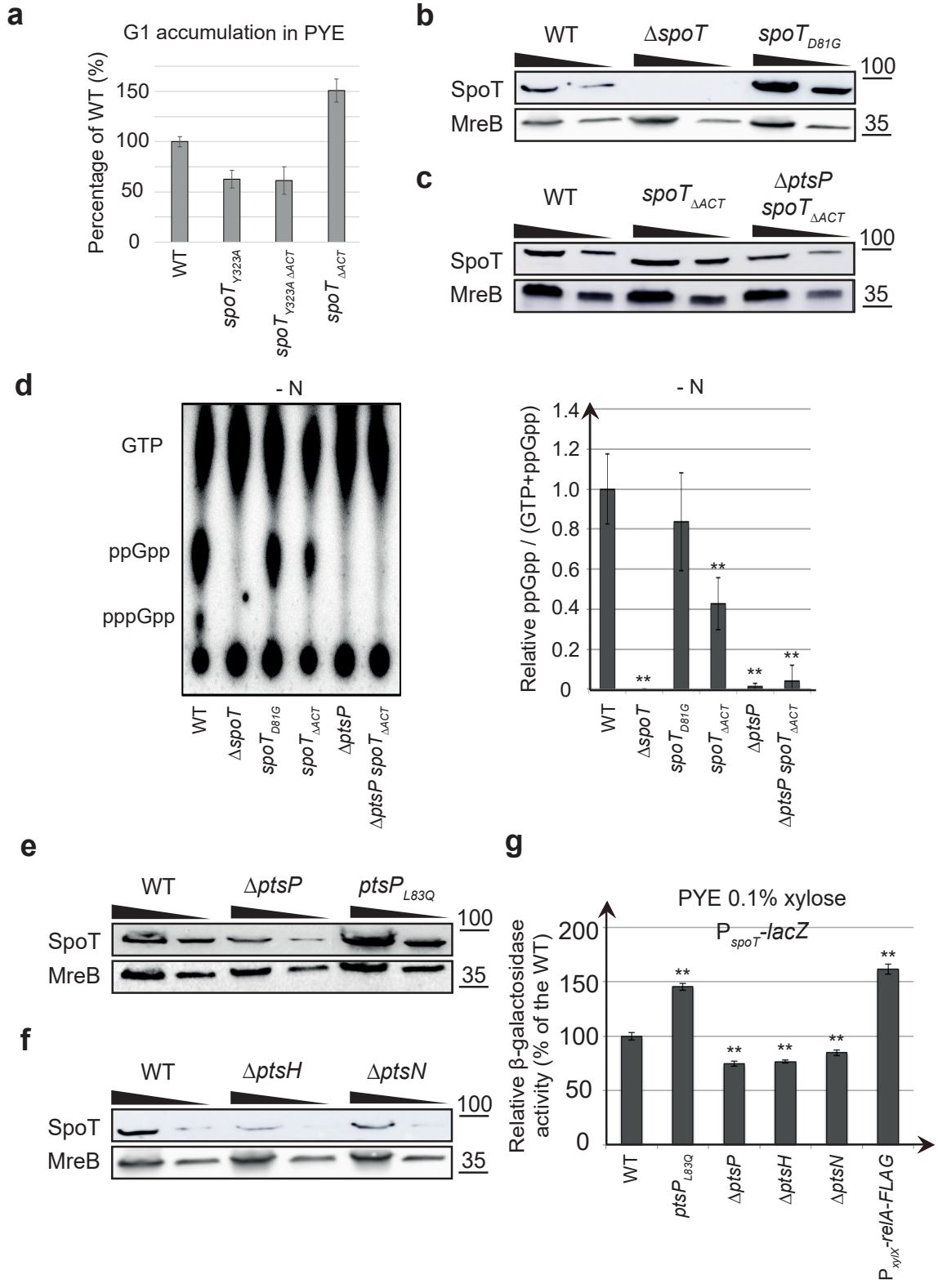
Supplementary Figure 1 The ACT domain is required for the interaction between SpoT and EIIA^{Ntr}~P and interacts with itself in a bacterial two-hybrid assay.

(a) MG1655 *cyaA::frt* (RH785) strains coexpressing T18- fused to *ptsN, spoT*, *spoT*$_{\Delta 668\text{-}719}$ or *ACT*$_{506\text{-}742}$ or alone with T25- fused to *ptsN*, *spoT* or *spoT*$_{\Delta 668\text{-}719}$ or alone were spotted on MacConkey Agar Base plates supplemented with 1% maltose.

(b) MG1655 *cyaA::frt* (RH785) strains coexpressing T18- fused to *ACT*$_{506\text{-}742}$ or alone with T25- fused to *ACT*$_{506\text{-}742}$ or alone were spotted on MacConkey Agar Base plates supplemented with 1% maltose.

Plates were incubated for one day at 30 °C and the red color indicates positive interactions.

Supplement Figure 2



Supplementary Figure 2 The synthetase activity of a *spoT*$_{\Delta ACT}$ strain is still sensitive to PTS^{Ntr}.

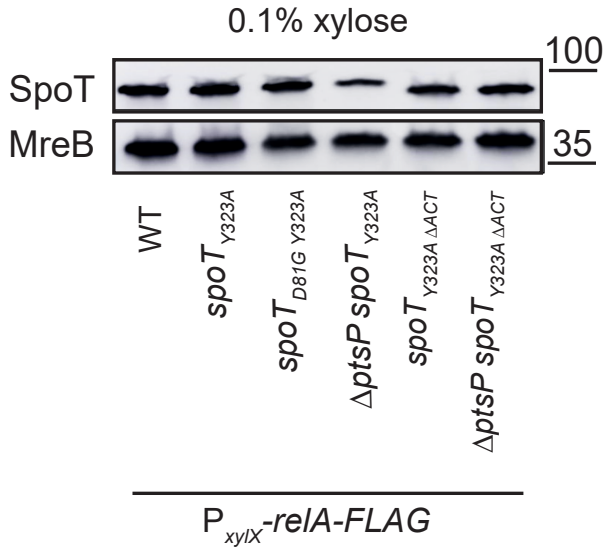
(a) Abolishing the synthetase activity of *spoT*$_{\Delta ACT}$ suppresses the extension of G1/swarmer cell lifetime. DNA content was measured in WT (RH50), *spoT* $_{Y323A}$ (RH1844), *spoT* $_{Y323A\ \Delta ACT}$ (RH2491) and *spoT*$_{\Delta ACT}$ (RH1476) strains grown in complex media (PYE) and normalized to the WT (100%). Error bars = SD, n = 3.

(b-c) Abundance of SpoT increased in strains accumulating (p)ppGpp. Immunoblotting of protein samples extracted from WT (RH50), Δ*spoT* (RH1755), *spoT*$_{D81G}$ (RH1752), *spoT*$_{\Delta ACT}$ (RH1476), Δ*ptsP* *spoT*$_{\Delta ACT}$ (RH1478) strains grown in complex media (PYE) to determine the steady-state levels of native SpoT variants and MreB. Two different volumes (15 μL and 5 μL) of protein lysates were loaded for each strain.

(d) The *spoT*$_{\Delta ACT}$ strain is still able to accumulate (p)ppGpp upon nitrogen starvation by a EI^{Ntr}-dependent way. The intracellular levels of (p)ppGpp were evaluated by TLC after nucleotides extraction from WT (RH50), Δ*spoT* (RH1755), *spoT*$_{D81G}$ (RH1752), *spoT*$_{\Delta ACT}$ (RH1476), Δ*ptsP* (RH1758) and Δ*ptsP* *spoT*$_{\Delta ACT}$ (RH1478) strains grown for 6 hrs in nitrogen-deplete (-N) conditions.

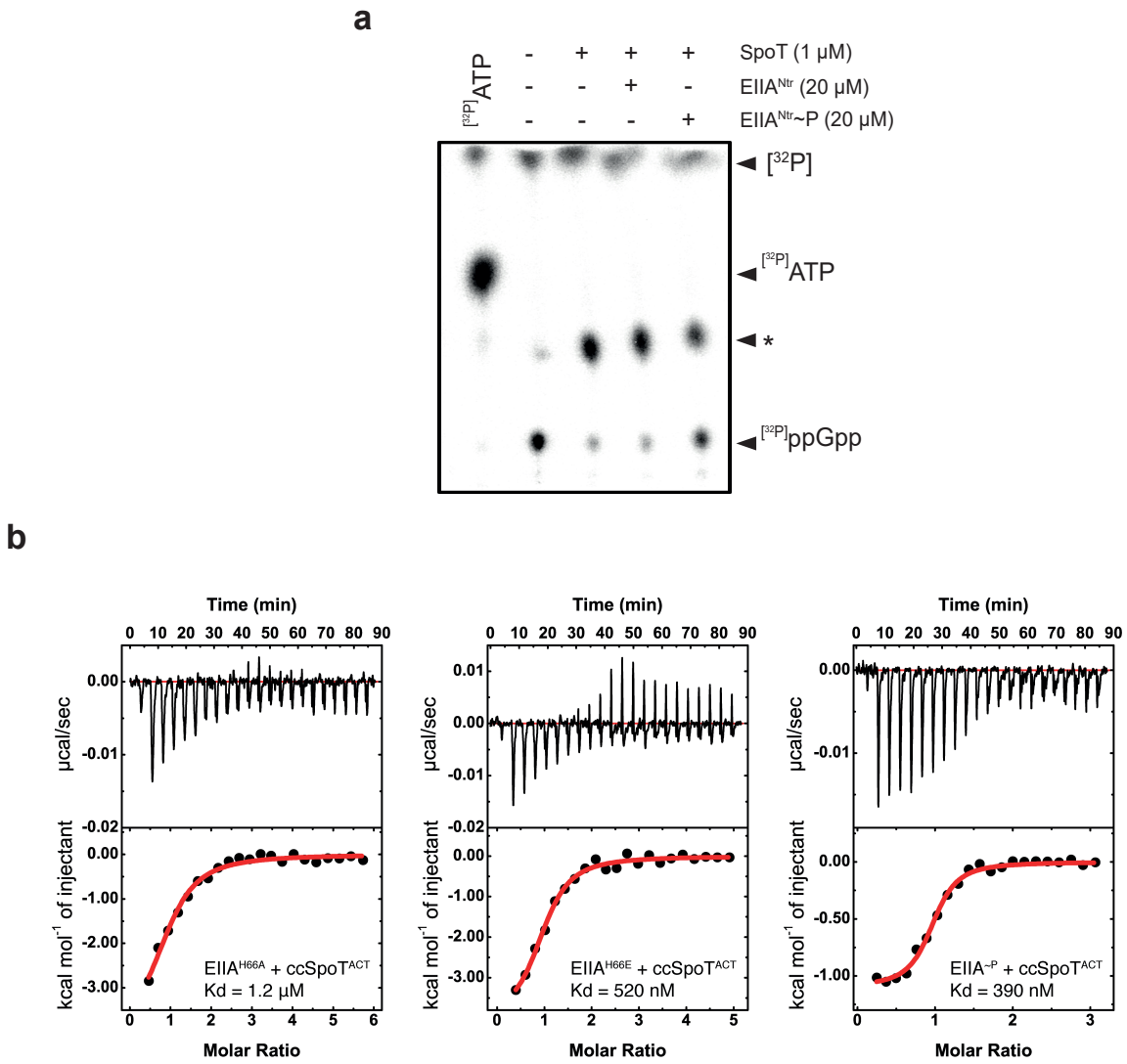
(e-g) Positive feedback loop of (p)ppGpp on SpoT abundance and *spoT* promoter activity. (e-f) Immunoblotting of protein samples extracted from WT (RH50), Δ*ptsP* (RH1758), *ptsP*$_{L83Q}$ (RH1748), Δ*ptsH* (RH1621) and Δ*ptsN* (RH1819) strains grown in complex media (PYE) to determine the steady-state levels of native SpoT and MreB. Two different volumes (15 μL and 5 μL) of protein lysates were loaded for each strain. (g) β-galactosidase assays were performed on WT (RH50), Δ*ptsP* (RH1758), *ptsP*$_{L83Q}$ (RH1748), Δ*ptsH* (RH1621), Δ*ptsN* (RH1819) and P$_{xylX}$*::relA-FLAG* strains harbouring a P$_{spoT}$*::lacZ* fusion, grown for 6 hrs in complex media (PYE) supplemented with 0.1% of xylose and normalized to the WT (100%) Error bars = SD, n = 3.

Supplement Figure 3



Supplementary Figure 3 The steady state levels of SpoT variants are similar in strains harbouring P*xylX::relA-FLAG*. Immunoblotting of protein samples extracted from WT (RH50), *spoT*$_{Y323A}$ (RH1844), *spoT*$_{D81G\ Y323A}$ (RH2193), Δ*ptsP spoT*$_{Y323A}$ (RH2196) *spoT* $_{Y323A\ \Delta ACT}$ (RH1586), and Δ*ptsP spoT* $_{Y323A\ \Delta ACT}$ (RH2492) strains grown for 6 hrs in PYE medium supplemented with 0.1% of xylose to determine the steady-state levels of native SpoT variants and MreB.

Supplement Figure 4

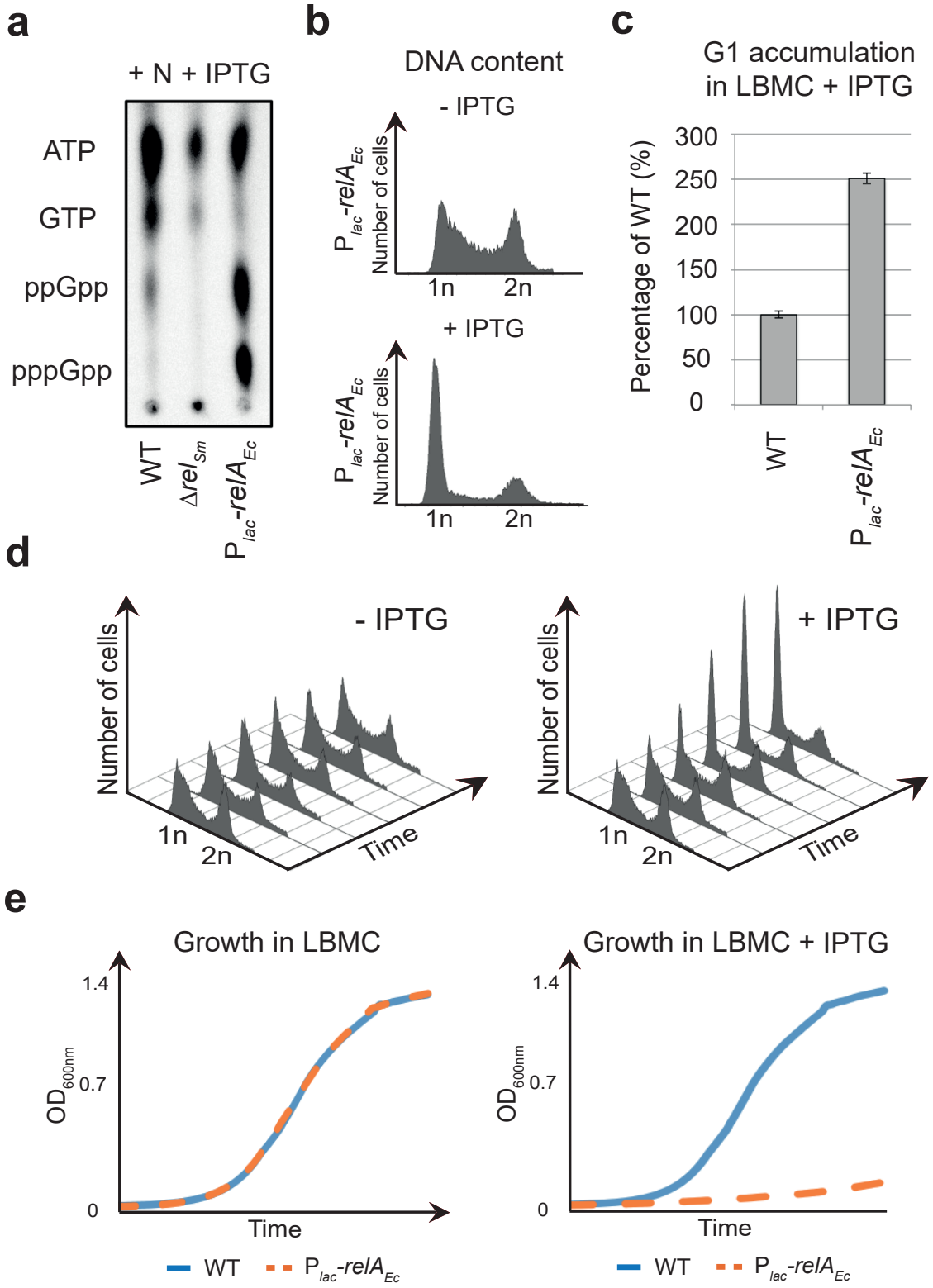


Supplementary Figure 4 EIIA^{Ntr}~P inhibits the hydrolase activity of SpoT *in vitro* and binds ACT.

(a) Phosphorylated EIIA^{Ntr} protects ppGpp from hydrolysis by SpoT. Radiolabelled $^{[32P]}$ppGpp incubated with 1 μM of SpoT in the presence of 20 μM of repurified EIIA^{Ntr} or EIIA^{Ntr}~P was separated by TLC. The star "*" indicates a $^{[32P]}$ppGpp intermediate degradation. $^{[32P]}$ATP and $^{[32P]}$ppGpp were used as references.

(b) Phosphorylation of EIIA^{Ntr} enhanced interaction with ACT. The Kd between the ACT domain of SpoT (ccSpoT^{ACT}) and the phospho-dead (EIIA$_{H66A}$), the phosphomimetic (EIIA$_{H66A}$) or the phosphorylated (EIIA$^{\sim P}$) variant of EIIA^{Ntr} were calculated by Isothermal Titration Calorimetry (ITC).

Supplement Figure 5



Supplementary Figure 5 *Sinorhizobium meliloti* accumulates G1 cells upon (p)ppGpp accumulation.

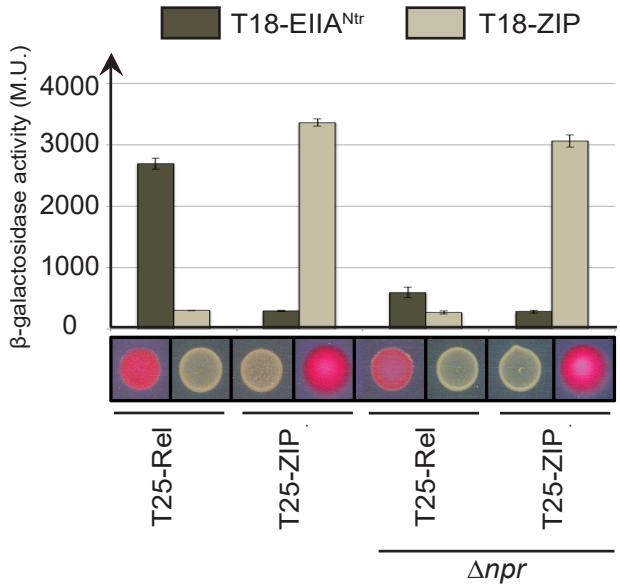
(a) The intracellular levels of (p)ppGpp were evaluated by TLC after nucleotides extraction from WT (RH2000), Δ*rel* (RH2327) and P_{lac}-$relA_{Ec}$ strains grown in nitrogen-replete (+N) conditions supplemented with 1 mM IPTG.

(b-c) DNA content (b) and G1 proportion (c) were measured in the same strains grown in complex media (LBMC) with or without 1 mM IPTG for 5 hrs. G1 proportions were normalized to the WT (100%). Error bars = SD, n = 3.

(d) Kinetic of G1 accumulation in cells harbouring P_{lac}-$relA_{Ec}$ with or without 1 mM IPTG. The same experiment than in (b) was performed by withdrawing samples every hour for 5 hrs.

(e) Growth of WT with (orange) or without (blue) P_{lac}-$relA_{Ec}$ in presence or absence of 1 mM IPTG was measured for 24 hrs.

Supplement Figure 6



Supplementary Figure 6 The phosphorylated form of *Rhodobacter sphaeroides* $EIIA^{Ntr}$ interacts with Rel in a bacterial two-hybrid assay.

MG1655 *cyaA::frt* (RH785) or (b) MG1655 *cyaA::frt* Δ*npr* (RH2122) strains coexpressing T18- fused to $ptsN^{Rs}$ or *ZIP* with T25- fused to rel^{Rs} or *ZIP* were spotted on MacConkey Agar Base plates supplemented with 1% maltose. Plates were incubated for one day at 30 °C. The red color indicates positive interactions. β-galactosidase assays were performed on the same strains. Error bars = SD, n = 3

Supplementary Tables

Supplementary Table 1 Oligonucleotides used in this study.

Inserted restriction sites are indicated in capital letter

Name	Sequence
897	GGATCCcgctgtcgaaagcttcgagg
899	gcgcgagatccatgaagagc
923	cgcctaagcggaacaacggg
926	ggggcagctgatcgtgttcg
927	gacgttccacagcaggatgg
933	cctggtcgaggaacatctcg
978	tcCATATGacgggcatggcttcaaga
1159	cctaagtaactaaCATATGtcatggtctggcgccgtcggtggattatcgtcggc
1174	GAATTCacgcagtcaggtcgtgaaatgc
1175	cctaagtaactaaTCTAGAgttgtcgtctgtggcccccgtcgctg
1176	ggatccccgggtaGAATTCctgaaggagagggtcctcg
1178	ggatccccgggtaGAATTCtaccgctcctcgtcgaaccgg
1194	cctaagtaactaaGAATTCgcttttgtcagacggcgacg
1204	tcGGTACCgaagctgaaggacgtgtcgc
1281	tcAAGCTTacatttcggaagacggctcg
1282	tcGAATTCcggacctgcggaaaggtctc
1283	tcGAATTCcagctcctggtcgatttcgc
1284	tcGGATCCtgctcgcggcgtatctctcg
1285	gcctatgccaaggatgaagc
1286	tcGAATTCcagctcctggtcgatttcgc
1287	tcAAGCTTagactgcatcgacaaggtcg
1288	tcGAATTCctgcatggcgtagacatagg
1289	tcGAATTCcagctcaaggaattgccgag
1290	tcGGATCCaaagaccttgccggccagg

1291 ccgccgcatctcttgagtcg

1292 ctgaattcgcgcacggaatg

1369 cctaagtaactaaGCTAGCttgtcgtctgtggcccccgtcgctg

1370 cctaagtaactaaTCTAGAggaagagttcctgcgtctggg

1435 ggatccccgggtaGAATTCtacatgccttccaggtcgagcc

1439 cctaagtaactaaTCTAGAgatgcgccaatacgagctcg

1440 ggatccccgggtaGAATTCgaacaggcgccctattcg

1441 cctaagtaactaaTCTAGAtaaaagattggcattggcaggc

1442 ggatccccgggtaGAATTCatgctcttccaaccggctcc

1553 cgtaagtaactaaGGTACCgaaggttccgaaccgcttcg

1554 ggatccccgggtaAAGCTTcaacgcgcccgattcggtcc

1564 ggatccccgggtaGAATTCctagcgctcgaggatgcgatagccg

1608 cctaagtaactaaTCTAGAcatggaactatccaaacttctgatgccagg

1609 ggatccccgggtaGAATTCaggatgtgatgaagaacgggc

1610 cctaagtaactaaTCTAGAcatgatcgacgtcgaagacctgatcgcc

1611 ggatccccgggtaGAATTCgccgcttgaagaccacttcc

1676 cctaagtaactaaGCTAGccacaccatcgactgtccgc

1818 cctaagtaactaaGCTAGCgagatggtcagctccagcgagc

Supplementary Table 2 Plasmids used in this study.

Name	description	Reference
	pNPTS138	M. R. Alley, Imperial College London (UK), unpublished
	pKT25	[1]
	pUT18C	[1]
	pKT25-*zip*	[1]
	pUT18C-*zip*	[1]
	pCP20	[2]
	pBAD33	[3]
	pXTCYC-4-*relA-FLAG*	[4]
	pSRKKm	[5]
	p*lacZ*290-P*ctrA*	[6]
pHR639	pNPTS138-Δ*ptsP*	[7]
pHR689	pUT18C-*spoT*	[7]
pHR693	pKT25-*ptsN*	[7]
pHR704	pUT18C-*ptsN*	[7]
pHR797	pET-28a-*spoT*	This study
pHR819	pET-28a-*ptsH*	This study
pHR820	pET-28a-*ptsN*	This study
pHR821	pET-28a-$ptsN_{H66E}$	This study
pHR833	pET-28a-$ptsN_{H66A}$	This study
pHR839	pET-28a-$spoT_{D81G}$	This study
pHR896	pSRKKm-*relA-FLAG*	This study
pHR897	pNPTS138-Δ$ptsP^{Sm}$	This study
pHR898	pNPTS138-Δrel^{Sm}	This study
pHR918	pKT25-$ACT_{506-742}$	This study
pHR930	pET28a-$spoT_{\Delta 668-719}$	This study

pHR931	pNPTS138-Δ*ACT*	This study
pHR935	pET28a-*spoT*$_{633\text{-}742}$	This study
pHR939	pUT18C-*ACT*$_{506\text{-}742}$	This study
pHR946	pNPTS138-*spoT*$_{\Delta 668\text{-}719}$	This study
pHR989	pET28a-*spoT*$_{1\text{-}373}$	This study
pHR998	pKT25-*rel*^{Sm}	This study
pHR999	pKT25-*rel*$^{Sm}{}_{\Delta ACT}$	This study
pHR1000	pKT25-*rel*^{Rs}	This study
pHR1001	pUT18C-*ptsN*^{Sm}	This study
pHR1002	pUT18C-*ptsN*^{Rs}	This study
pHR1025	p*lacZ*290-P*spoT*	This study
pHR1027	pET-28a-*ptsP*$_{161\text{-}754}$	This study
pHR1046	pKT25-*spoT*$_{\Delta 668\text{-}719}$	This study
pHR1047	pUT18C-*spoT*$_{\Delta 668\text{-}719}$	This study

Supplementary Table 3 Strains used in this study.

Name	Description and relevant genotype	Reference
RH10	S17-1 ((F- λ- *13 endA thi pro recA hsdr2* (r-m+) RP4-2-Tet::Mu-Km ::Tn7)	[8]
RH319	MT607(*pro*-82 *thi*-I *hsdR17* (r-m+) *supE44 recA56*)	[9]
RH603	BL21 (DE3)	Novagen
RH783	Top10 (*ϕ80lacZΔM15 araD139* Δ(*ara-leu*)7697 *galE15 galK16* Δ (*lac*)X74 *rpsL*(StrR) *nupG recA1 endA1 mcrA* Δ (*mrr-hsdRMS-mcrBC*)	Life Technologies
RH785	MG1655 *cya::frt*	[10]
RH2122	MG1655 *cya::frt* Δ*npr*	[7]
RH2292	NM522 pQE-30-*ptsI_Bs*	[11]
RH50	*C. crescentus* NA1000	[12]
RH1476	NA1000 $spoT_{\Delta ACT}$	This study
RH1478	NA1000 Δ*ptsP* $spoT_{\Delta ACT}$	This study
RH1586	NA1000 $spoT_{Y323A\ \Delta ACT}$	This study
RH1621	NA1000 Δ*ptsH*	[7]
RH1748	NA1000 $ptsP_{L83Q}$	[7]
RH1752	NA1000 $spoT_{D81G}$	[7]
RH1755	NA1000 Δ*spoT*	[7]
RH1758	NA1000 Δ*ptsP*	[7]
RH1819	NA1000 Δ*ptsN*	[7]
RH1844	NA1000 $spoT_{Y323A}$	[7]
RH1882	NA1000 Δ*spoT*	[13]
RH2017	NA1000 $ptsN_{H66E}$	[7]
RH2018	NA1000 $ptsN_{H66A}$	[7]
RH2193	NA1000 $spoT_{D81G\ Y323A}$	[7]
RH2196	NA1000 Δ*ptsP* $spoT_{Y323A}$	[7]

RH2142	NA1000 Δ*spoT*$_{\Delta ACT}$	[13]
RH2447	NA1000 Δ*ptsP spoT*$_{\Delta 668\text{-}719}$	This study
RH2491	NA1000 Δ*ptsP spoT*$_{\Delta ACT}$	This study
RH2492	NA1000 Δ*ptsP spoT*$_{Y323A\ \Delta ACT}$	This study
RH2000	*S. meliloti* Rm1021	[14]
RH2326	Sm1021 Δ*ptsP*	This study
RH2327	Sm1021 Δ*rel*	This study
RH2278	*R. sphaeroides* 2.4.1	[15]

Supplementary Methods

Plasmids construction

pHR797 (pET-28a-*spoT*)

C. crescentus CCNA_01622 was amplified from NA1000 gDNA by PCR with primers 1369 and 1176, digested with *Nhe* I and *Eco* RI and ligated into the pET-28a vector cut with the same restriction enzymes.

pHR819 (pET-28a-*ptsH*)

C. crescentus CCNA_00241 was amplified from NA1000 gDNA by PCR with primers 978 and 1178, digested with *Nde* I and *Eco* RI and ligated into the pET-28a vector cut with the same restriction enzymes.

pHR820 (pET-28a-*ptsN*)

C. crescentus CCNA_00241 was amplified from NA1000 gDNA by PCR with primers 1159 and 1174, digested with *Nde* I and *Eco* RI and ligated into the pET-28a vector cut with the same restriction enzymes.

pHR821 (pET-28a-$ptsN_{H66E}$)

C. crescentus CCNA_00241 was amplified from NA1000 $ptsN_{H66E}$ (RH2017) gDNA by PCR with primers 1159 and 1174, digested with *Nde* I and *Eco* RI and ligated into the pET-28a vector cut with the same restriction enzymes.

pHR833 (pET-28a-$ptsN_{H66A}$)

C. crescentus CCNA_00241 was amplified from NA1000 $ptsN_{H66A}$ (RH2018) gDNA by PCR with primers 1159 and 1174, digested with *Nde* I and *Eco* RI and ligated into the pET-28a vector cut with the same restriction enzymes.

pHR839 (pET-28a-$spoT_{D81G}$)

C. crescentus CCNA_01622 was amplified from NA1000 $spoT_{D81G}$ (RH1752) gDNA by PCR with primers 1369 and 1176, digested with *Nde* I and *Eco* RI and ligated into the pET-28a vector cut with the same restriction enzymes.

pHR896 (pSRKKm-*relA-FLAG*)

relA-FLAG was cut off from pXTCYC-4-*relA-FLAG* with *Nde* I and *Nhe* I and ligated into the pSRKKm vector cut with the same restriction enzymes.

pHR897 (pNPTS138-Δ$ptsP^{Sm}$)

Upstream and downstream regions of *S. meliloti smc02437* were amplified from RM1021 gDNA by PCR respectively with primers 1281/1282 and 1283/1284,

digested respectively with *Hind* III/*Eco* RI and *Eco* RI/ *Bam* HI; and ligated into the pNPTS138 vector cut with *Hind* III and *Bam* HI.

pHR898 (pNPTS138-Δrel^{Sm})

Upstream and downstream regions of *S. meliloti smc02659* were amplified from RM1021 gDNA by PCR respectively with primers 1287/1289 and 1289/1290, digested respectively with *Hind* III/*Eco* RI and *Eco* RI/ *Bam* HI; and ligated into the pNPTS138 vector cut with *Hind* III and *Bam* HI.

pHR918 (pKT25-$ACT_{506\text{-}742}$)

C. crescentus $ACT_{506\text{-}742}$ was amplified from NA1000 gDNA by PCR with primers 1370 and 1176, digested with *Xba* I and *Eco* RI and ligated into the pKT25 vector cut with the same restriction enzymes.

pHR930 (pET-28a-$spoT_{\Delta 668\text{-}719}$)

C. crescentus $spoT_{\Delta 668\text{-}719}$ was amplified from NA1000 $spoT_{\Delta 668\text{-}719}$ (RH2447) gDNA by PCR with primers 1369 and 1176, digested with *Nhe* I and *Eco* RI and ligated into the pET-28a vector cut with the same restriction enzymes.

pHR931 (pNPTS138-Δ*ACT*)

C. crescentus $spoT_{\Delta ACT}$ was amplified from NA1000 *HA-*$spoT_{\Delta ACT}$ (RH2142) gDNA by PCR with primers 1370 and 899 and ligated into the pNPTS138 vector cut with the *Eco* RV.

pHR935 (pET-28a-$spoT_{633\text{-}742}$)

C. crescentus $spoT_{633\text{-}742}$ (encoding the ACT domain of SpoT) was amplified from NA1000 (RH50) gDNA by PCR with primers 1676 and 1176, digested with *Nhe* I and *Eco* RI and ligated into the pET-28a vector cut with the same restriction enzymes.

pHR939 (pUT18c-$ACT_{506\text{-}742}$)

C. crescentus $ACT_{506\text{-}742}$ was amplified from NA1000 gDNA by PCR with primers 1370 and 1176, digested with *Xba* I and *Eco* RI and ligated into the pUT18c vector cut with the same restriction enzymes.

pHR946 (pNPTS138-$spoT_{\Delta 668\text{-}719}$)

DNA fragment of *C. crescentus CCNA_01622* encompassing deletion of the ACT domain from aa 668 to 719 was synthesized as a gBlock (IDT) and cloned into pNPTS138 cut with *Eco* RV.

pHR989 (pET-28a-*spoT*$_{1\text{-}373}$)

C. crescentus spoT$_{1\text{-}373}$ was amplified from NA1000 (RH50) gDNA by PCR with primers 1369 and 1435, digested with *Nhe* I and *Eco* RI and ligated into the pET-28a vector cut with the same restriction enzymes.

pHR998 (pKT25-*rel*^{Sm})

S. meliloti smc02659 was amplified from RM1021 gDNA by PCR with primers 1439 and 1440, digested with *Xba* I and *Eco* RI and ligated into the pKT25 vector cut with the same restriction enzymes.

pHR999 (pKT25-*rel*$^{Sm}{}_{\Delta ACT}$)

S. meliloti rel$^{Sm}{}_{\Delta ACT}$ was amplified from RM1021 *rel*$_{\Delta ACT}$ (RH2327) gDNA by PCR with primers 1439 and 1564, digested with *Xba* I and *Eco* RI and ligated into the pKT25 vector cut with the same restriction enzymes.

pHR1000 (pKT25-*rel*^{Rs})

R. sphaeroides RSP1670 was amplified from *R. sphaeroides* 2.4.1 gDNA by PCR with primers 1610 and 1611, digested with *Xba* I and *Eco* RI and ligated into the pKT25 vector cut with the same restriction enzymes.

pHR1001 (pUT18c-*ptsN*^{Sm})

S. meliloti smc01141 was amplified from RM1021 gDNA by PCR with primers 1441 and 1442, digested with *Xba* I and *Eco* RI and ligated into the pUT18c vector cut with the same restriction enzymes.

pHR1002 (pUT18c-*ptsN*^{Rs})

R. sphaeroides RSP1158 was amplified from *R. sphaeroides* 2.4.1 gDNA by PCR with primers 1608 and 1609, digested with *Xba* I and *Eco* RI and ligated into the pUT18c vector cut with the same restriction enzymes.

pHR1025 (p*lacZ*290-P*spoT*)

C. crescentus P*spoT* was amplified from NA1000 gDNA by PCR with primers 1553 and 1554, digested with *Kpn* I and *Hind* III and ligated into the p*lacZ*290-P*ctrA* vector cut with the same restriction enzymes.

pHR1027 (pET-28a-*ptsP*$_{161\text{-}754}$)

C. crescentus ptsP$_{161\text{-}754}$ (encoding EI$^{Ntr}{}_{\Delta GAF}$) was amplified from NA1000 (RH50) gDNA by PCR with primers 1818 and 1194, digested with *Nhe* I and *Eco* RI and ligated into the pET-28a vector cut with the same restriction enzymes.

pHR1046 (pKT25-*spoT*$_{\Delta 668\text{-}719}$)

C. crescentus spoT$_{\Delta 668\text{-}719}$ was amplified from NA1000 *spoT*$_{\Delta 668\text{-}719}$ (RH2447) gDNA by PCR with primers 1175 and 1176, digested with *Xba* I and *Eco* RI and ligated into the pKT25 vector cut with the same restriction enzymes.

pHR1047 (pUT18c-*spoT*$_{\Delta 668\text{-}719}$)

C. crescentus spoT$_{\Delta 668\text{-}719}$ was amplified from NA1000 *spoT*$_{\Delta 668\text{-}719}$ (RH2447) gDNA by PCR with primers 1175 and 1176, digested with *Xba* I and *Eco* RI and ligated into the pUT18c vector cut with the same restriction enzymes.

Description of Strains

RH1476 (NA1000 *spoT*$_{\Delta ACT}$)

Triparental mating between NA1000, RH319 (MT607-pRK600) and RH2378 (Top10-pNPTS138 *spoT*$_{\Delta ACT}$) was selected on PYE Nal Kan, streaked on PYE Kan, cultivated o/n in PYE and plated on PYE 3% Suc. Suc^R candidates were streaked on PYE Kan and PYE. Kan^S colonies were screened by PCR with primers 1204/897.

RH1478 (NA1000 Δ*ptsP spoT*$_{\Delta ACT}$)

Triparental mating between RH1758 (NA1000 Δ*ptsP*), RH319 (MT607-pRK600) and RH2378 (Top10-pNPTS138 *spoT*$_{\Delta ACT}$) was selected on PYE Nal Kan, streaked on PYE Kan, cultivated o/n in PYE and plated on PYE 3% Suc. Suc^R candidates were streaked on PYE Kan and PYE. Kan^S colonies were screened by PCR with primers 1204/897.

RH1586 (NA1000 *spoT*$_{Y323A\ \Delta ACT}$)

Triparental mating between RH1844 (NA1000 *spoT*$_{Y323A}$), RH319 (MT607-pRK600) and RH2378 (Top10-pNPTS138-*spoT*$_{\Delta ACT}$) was selected on PYE Nal Kan, streaked on PYE Kan, cultivated o/n in PYE and plated on PYE 3% Suc. Suc^R candidates were streaked on PYE Kan and PYE. Kan^S colonies were screened by PCR with primers 923/933 and 1175/1176.

RH2447 (NA1000 Δ*ptsP spoT*$_{\Delta 668\text{-}719}$)

Triparental mating between RH1758 (NA1000 Δ*ptsP*), RH319 (MT607-pRK600) and RH2403 (Top10-pNPTS138 *spoT*$_{\Delta 668\text{-}719}$) was selected on PYE Nal Kan, streaked on PYE Kan, cultivated o/n in PYE and plated on PYE 3% Suc. Suc^R candidates were

streaked on PYE Kan and PYE. Kan^S colonies were screened by PCR with primers 1204/897.

RH2491 (NA1000 Δ*ptsP HA-spoT*$_{\Delta ACT}$)

Triparental mating between RH2142 (NA1000 *HA-spoT*$_{\Delta ACT}$), RH319 (MT607-pRK600) and RH1757 (Top10-pNPTS138-Δ*ptsP*) was selected on PYE Nal Kan, streaked on PYE Kan, cultivated o/n in PYE and plated on PYE 3% Suc. Suc^R candidates were streaked on PYE Kan and PYE. Kan^S colonies were screened by PCR with primers 926/927.

RH2492 (NA1000 Δ*ptsP HA-spoT*$_{Y323A\ \Delta ACT}$)

Triparental mating between RH2491 (NA1000 Δ*ptsP HA-spoT*$_{Y323A\ \Delta ACT}$), RH319 (MT607-pRK600) and RH1817 (Top10-pNPTS138-*spoT*$_{Y323A}$) was selected on PYE Nal Kan, streaked on PYE Kan, cultivated o/n in PYE and plated on PYE 3% Suc. Suc^R candidates were streaked on PYE Kan and PYE. Kan^S colonies were screened by PCR with primers 923/933 and 1175/1176.

RH2326 (RM1021 Δ*ptsP*)

Triparental mating between RH2000 (RM1021), RH319 (MT607-pRK600) and RH2323 (Top10-pNPTS138-Δ*smc02437*) was selected on PYE Nal Kan, streaked on PYE Kan, cultivated o/n in PYE and plated on PYE 3% Suc. Suc^R candidates were streaked on PYE Kan and PYE. Kan^S colonies were screened by PCR with primers 1285/1286.

RH2327 (RM1021 Δ*rel*)

Triparental mating between RH2000 (RM1021), RH319 (MT607-pRK600) and RH2323 (Top10-pNPTS138-Δ*smc02659*) was selected on PYE Nal Kan, streaked on PYE Kan, cultivated o/n in PYE and plated on PYE 3% Suc. Suc^R candidates were streaked on PYE Kan and PYE. Kan^S colonies were screened by PCR with primers 1291/1292.

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