Supporting Information

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SI Materials and Methods

Bacterial Strains, Plasmids, and General Growth Conditions. Strains, plasmids, and primers used in this study are listed in Tables S1–S3. All plasmids were constructed in *E. coli* strain DH5α. Overproduction of recombinant proteins was carried out in *E. coli* strain BL21 (DE3). *E. coli* strains were routinely cultured at 37 °C in LB medium supplemented with ampicillin (100 μg/mL) or kanamycin (35 μg/mL) and 1 mM IPTG when indicated. The *B. subtilis* strains used in this study were derivatives of 168 Marburg strain PB2. All general methods were carried out as described (49). *B. subtilis* strains were routinely grown at 37 °C in LB. Sporulation was carried out by resuspension in Difco sporulation medium (DSM) (50) or in Sterlini–Mandelstam medium (51) as indicated in the text. When appropriate, medium was supplemented with chloramphenicol (5 μg/mL), MLS (1 μg/mL erythromycin and 25 μg/mL lincomycin), kanamycin (10 μg/mL), spectinomycin (100 μg/mL), or tetracycline (10 μg/mL) and IPTG (1 mM). All chemicals were obtained from Sigma-Aldrich unless otherwise specified. Deletions on the *Bacillus* genome were produced by PCR using long, flanking homology regions (52).

Fractionation and Lysis of Mother Cells and Forespores. Strains were grown in CH medium at 37 °C, and sporulation was induced by resuspension in A+B exhaustion medium (51). Five hours after the initiation of sporulation (T5), cells were collected, washed in TE buffer [10 mM Tris·HCl (pH 7.5) and 0.5 mM EDTA] and were resuspended in protoplasting buffer [100 mM Tris·HCl (pH 7.5), 20 mM $MgCl_2$, 0.6 mM KCl, 1 mM NaCl, 0.9 mM Na_2SO_4, 18.7 mM NH_4Cl, and 5% (vol/vol) sucrose]. Lysozyme was added to 1 mg/mL, and samples were incubated for 10 min at 37 °C. Protoplasts then were pelleted, flash frozen in liquid N_2, and lysed by vigorous vortexing in lysis buffer [50 mM Tris·HCl (pH 7.5), 250 mM NaCl, and 1 mM EDTA] with 1 mM PMSF, followed by a 30-min incubation on ice with 3 mM MgCl2 and 10 μg/mL DNase. The resulting supernatant (lysed mother cells) and pellet (forespores) were separated by centrifugation at 13,000 × *g* for 3 min at 4 °C and were treated separately. Mother cell lysate was cleared by a second centrifugation step at 20,000 × *g* for 20 min at 4 °C. The pellet was processed further to isolate forespores from cell debris by three washes with lysis buffer. Forespores then were treated with 4 mg/mL of lysozyme in lysis buffer with 1 mM PMSF, 3 mM MgCl2, and 10 μg/mL DNase for 5 min at 37 °C, followed by 20 min on ice. Lysis was performed by mixing samples with 1 volume of 100 μM Zirconia/Silica beads (BioSpec) and processing three times in a FastPrep homogenizer (MP Biomedicals) at settings of 6.5 m/s, for 45 s each time, with 5 min of ice cooling between cycles. Finally, forespores were incubated with 1% Nonidet P-40 for 30 min at 4 °C and were cleared in two centrifugation steps: (*i*) 1 min at 20,000 × *g* at 4 °C to remove the beads, and (*ii*) 20 min at 20,000 × *g* at 4 °C. To facilitate forespore lysis, the strains used in this experiment contained Δ*cotE* Δ*gerE* mutations that lead to a defective spore coat and therefore are lysed more easily (53). In addition, these strains contained fluorescent reporters that are specifically expressed in mother cell (P_{spoIID}-*yfp*) or forespore (P_{spoIIQ}-*cfp*) compartments to monitor fractionation and lysis by fluorescence microscopy.

Protein Expression and Purification. The kinase domain of YabT from *B. subtilis* (amino acids 1–277) was expressed as an N-terminal 6xHis-tag fusion and purified as described (54). The kinase domain of PknA from *M. tuberculosis* (amino acids 1–338) was expressed with an N-terminal 6xHis-SUMO tag and purified essentially as described (55), but using Ni^{2+}-NTA affinity purification. PrpC from *B. subtilis* was expressed as a C-terminal Strep-tag II tag recombinant protein and was purified by Strep-Tactin affinity purification (IBA GmbH) in 100 mM Tris·HCl (pH 7.5), 150 mM NaCl, 1 mM EDTA, and 1 mM TCEP supplemented with 2.5 mM desthiobiotin. Purified PrpC then was dialyzed into 20 mM Tris·HCl (pH 7.5), 50 mM NaCl, 10 mM MnCl2, 10% (vol/vol) glycerol, and 1 mM TCEP. EF-Tu from *B. subtilis* and *M. tuberculosis* were expressed with a cleavable N-terminal 6xHis-tag and were purified as described (28). EF-Tu point mutants were generated by site-directed mutagenesis (see Table S3 for primers used).

In Vitro Kinase Assays. Purified kinases (0.5 μM) and substrates (2 μM) were incubated with 0.1 M of unlabeled ATP and 1 μCi of [γ-32P]ATP (Perkin-Elmer) in kinase buffer [50 mM Tris·HCl (pH 7.5), 50 mM KCl, 10 mM $MgCl_2$, 10 mM $MnCl_2$, and 0.5 mM TCEP] for 30 min at 37 °C. Reactions were stopped by adding 5× SDS/PAGE loading buffer and were resolved by SDS/PAGE (12%). Gels were dried, exposed to a Storage Phospho Screen (Molecular Dynamics), and imaged using a Molecular Dynamics Typhoon Trio phosphorimager.

EF-Tu Phosphorylation in Vivo. *B. subtilis* EF-Tu was phosphorylated in *E. coli* by coexpression with a tagless YabT kinase domain from a pETDuet-derived plasmid. Purified EF-Tu was resolved by SDS/PAGE (12%) and stained by Coomassie Brilliant Blue or transferred to an Immobilon-P PVDF membrane and stained with the phosphoprotein dye Pro-Q Diamond (Molecular Probes) following the manufacturer's instructions.

Phosphoenrichment of EF-Tu. All biochemical assays were performed with EF-Tu phosphorylated by YabT in *E. coli* and phosphoenriched using the Pro-Q Diamond phosphoprotein enrichment kit (Molecular Probes) following the manufacturer's instructions.

Dephosphorylation of EF-Tu. EF-Tu phosphorylated by YabT in vivo or in vitro was incubated with PrpC at a 1:3 molar ratio in the kinase buffer for 1 h at 37 °C, unless otherwise indicated. Dephosphorylation was assayed by Pro-Q Diamond following the manufacturer's instructions.

GTP-Binding Assay. The GTP-binding assay used was adapted from ref. 25 with the following modifications: Increasing concentrations of EF-Tu samples (0, 0.125, 0.25, 0.5, 1, 1.5, 2, 3, 5, and 10 μM) were incubated with 7 nM of [α-32P]ATP (Perkin-Elmer) in Tris-Polymix buffer [50 mM Tris·acetate (pH 7.5) at 25 °C, 100 mM KCl, 5 mM NH_4OAc, 0.5 mM $Ca(OAc)_2$, 6 mM b-mercaptoethanol (BME), 5 mM putrescine–HCl, and 1 mM spermidine-free base] with 5 mM $Mg(OAc)_2$ for 10 min at 37 °C. The reactions were spotted on nitrocellulose, which was dried and phosphorimaged as described above. The intensity of the spots was quantified using ImageJ software (56), and the GTP-bound fraction was calculated according to ref. 25.

GTP Hydrolysis Assay. Ribosome-dependent multiple-turnover GTP hydrolysis was performed according to ref. 28, with modifications. EF-Tu samples at 0.8 μM were incubated with 0.4 μM 70S ribosomes in the presence of 1 mM unlabeled GTP and 10 nM of [α-32P]GTP in Tris-polymix buffer with 5 mM $Mg(OAc)_2$ in a final volume of 15 μL. Reactions were carried at 37 °C, and 2-mL samples were taken at 5, 15, and 30 min and quenched in

the same volume of 100-mM EDTA, pH 9.5. Samples then were boiled for 1 min at 95 °C and centrifuged for 5 min at 18,000 × *g*. The supernatant (1.5 μL) was spotted onto a PEI-F cellulose plate (EMD Chemicals), and separation of GTP and GDP was done by TLC using 0.9 M guanidine HCl as solvent. The TLC plates were dried and phosphorimaged as above. Bands were quantified using ImageJ (56). Control reactions in the absence of 70S ribosomes, EF-Tu, or both were carried out for 30 min. The 70S ribosomes used in this and the following assays were purified from *E. coli* as described (28).

Elongation Assay. *B. subtilis* EF-Tu samples were tested in a tripeptide synthesis assay using highly purified *E. coli* components as described (28). Elongation reactions were carried out in three main steps. First, an initiation reaction was started by incubating the initiation factors IF1-3 at 1.6 μM each, 1.5 μM of 70S ribosomes, and 1 mM GTP in Tris-polymix buffer with 3.5 mM $Mg(OAc)_2$, for 10 min at 37 °C. Then, 2.1 μM of T4gp1-20 mRNA (mRNA variant encoding the first 20 amino acids of bacteriophage T4 gene product 32) was added, and the reaction was incubated for 10 min at 37 °C, followed by the addition of 0.5 μM of f-[35S]Met-tRNAfMet and another incubation for 10 min at 37 °C. Second, EF-Tu(GTP)aa-tRNA ternary complexes were prepared by incubating EF-Tu samples at 3.3 μM with 2.5 μM EF-Ts, 800 μM GTP, 2.5 mM phosphoenolpyruvate, and 0.001 U/μL pyruvate kinase in Tris-polymix buffer for 1 min at 37 °C; then 2.5 μM of Lys-$tRNA^{Lys}$ and Phe-$tRNA^{Phe}$ were added, and the mixture was incubated for an additional minute at 37 °C. Third, a mixture of 21 μM EF-G, 1 mM GTP, 3 μM phosphoenolpyruvate, and 0.001 U/μL pyruvate kinase was prepared in Tris-polymix buffer. Then the EF-G mixture and the initiation and ternary complex reactions were combined and incubated at 37°, allowing peptide synthesis to initiate. The final concentrations of components in the peptide synthesis reaction were as follows: 0.5 μM of initiation complexes, 0.25 μM of f-[^{35}S]Met-tRNAfMet, 1.9 μM of mRNA, 1.5 μM of EF-Tu, 1.1 μM EF-Ts, 1.2 μM of each aa-tRNA, and 1.7 μM of EF-G. Samples (0.5 μL) taken at 15 s and 1 and 5 min were quenched with 1 μL of 500 mM KOH, and 0.5 μL of each quenched reaction was spotted onto cellulose TLC plates (EMD). Products were separated using electrophoretic TLC (eTLC) in pyridine acetate buffer (5% pyridine, 20% acetic acid, pH 2.8) for 30 min at 1,200 V. eTLC plates were air-dried and phosphorimaged, and bands were quantified using ImageJ (56) to determine the percentage of f-[^{35}S]Met that is converted into a dipeptide and tripeptide.

Cosedimentation Assays. The EF-Tu ribosome-binding assay used was adapted from ref. 48. EF-Tu samples at 0.05 μM were incubated with 0.025 μM of 70S ribosomes and 1 mM GTP in binding buffer [50 mM Tris·HCl (pH 7.5), 100 mM KCl, 5 mM NH_4Cl_2, 0.5 mM $CaCl_2$, 10 mM $MgCl_2$ and 6 mM BME] in a final volume of 25 μL for 3 min at 37 °C. Reactions were stopped by adding 25 μL of binding buffer (including 1 mM GTP) and by layering the reaction on 50 μL of 10% (vol/vol) sucrose cushions in buffer R [10 mM Tris·HCl (pH 7.2) at 4 °C, 30 mM NH_4Cl, 10 mM $MgCl_2$, 0.5 mM EDTA, and 6 mM BME] with 1 mM GTP. Reactions subsequently were centrifuged at 75,000 rpm for 5 min at 4 °C in a Beckman Optima TLX ultracentrifuge using a Beckman TLA 100 rotor. The supernatant (20 μL collected from the top) and pellet (resuspended in 20 μL) were analyzed by SDS/PAGE (12%) and immunoblotting with a rabbit-raised EF-Tu polyclonal antibody (1:3,000) or a monoclonal antibody against the ribosomal protein S3 (1:3,000; Developmental Studies Hybridoma Bank). A reaction in the absence of ribosomes was done as a control. The relative amounts of EF-Tu in the supernatant and pellet were determined by quantitative densitometry using ImageJ software (56).

Dephosphorylation of EF-Tu prebound to ribosomes was performed by first binding phosphorylated EF-Tu to 70S ribosomes as described above, then splitting the reaction and adding 0.25 μM of PrpC and 10 mM of $MnCl_2$ to one part of the reaction. Both parts of the reaction were incubated further for 30 min at 37 °C. A reaction with unmodified EF-Tu was done as control.

In Vitro Translation of CotE-FLAG. The PURExpress system (New England Biolabs) was used according to the manufacturer's instructions to transcribe and translate a C-terminal FLAG-tagged CotE protein. The New England Biolabs control plasmid expressing CotE-FLAG under the T7 promoter was added to the complete reaction mixture (solution A + solution B), and 10-μL reactions were carried out for 5 min at 37 °C in the absence or presence of 0.92 μM of nonphosphorylated or phosphorylated *B. subtilis* EF-Tu. The reactions were stopped by the addition of SDS/PAGE loading buffer, resolved by SDS/PAGE (12%), and analyzed by immunoblotting with a FLAG-tag antibody (1:3,000; Sigma). The amount of CotE-FLAG was determined by quantitative densitometry using ImageJ (56) and is shown relative to the amount of CotE-FLAG synthesized in the absence of *B. subtilis* EF-Tu.

Fractionation and Lysis of Mother Cells and Forespores for Ribosome Isolation. Mother cells and endospores were fractionated essentially as described above except that the lysis buffer was replaced by buffer R and lysis was carried out in the presence of 1× protease and phosphatase inhibitor mixtures (Pierce) and 40 U/mL of RNase inhibitor.

Lysis of Mature Spores for Ribosome Isolation. Strains containing a Δ*cotE* Δ*gerE* double-knockout mutation were sporulated by exhaustion in DSM medium for 24 h at 37 °C (50). Cells then were collected, washed with distilled water, and incubated in decoating buffer (0.1 M NaCl, 0.1 M NaOH, 0.5% SDS, and 0.1 M DTT) for 1 h at 65 °C to remove the remaining layers of the coat. After extensive washes with distilled water, decoated spores were resuspended in buffer R with 1× protease and phosphatase inhibitor mixtures (Pierce) and 40 U/mL of RNase inhibitor and were lysed as described.

Isolation of Intact Ribosomes from *B. subtilis*. Cell lysates from mother cells, endospores, or mature spores were produced as described above. Total cell lysates were quantified by the BCA protein assay kit (Pierce), and normalized samples were loaded in 1.5-mL sucrose cushions [buffer R with 37.7% (vol/vol) sucrose]. Ribosomes were isolated by ultracentrifugation at 85,000 rpm for 2 h at 4 °C in a Beckman Optima TLX ultracentrifuge using a Beckman TLA 100.3 rotor. The supernatant was discarded, and the pellet containing the ribosome complexes was resuspended overnight at 4 °C in 2× SDS/PAGE loading buffer. The EF-Tu levels in the lysate and sucrose pellet were analyzed by SDS/PAGE (12%) and immunoblotting with an EF-Tu polyclonal antibody (1:3,000) or a phospho-Thr antibody (1:1,000; Cell Signaling). The relative amount of EF-Tu present in the ribosomal fraction (pellet) was determined by quantitative densitometry using ImageJ software (56) and was expressed as a fraction of wild-type EF-Tu.

Induction of Protein Translation in Cells Entering Dormancy. Strains harboring a fluorescent *yfp* reporter under the IPTG-inducible $P_{hyper\text{-}spanc}$ promoter were sporulated as described (51). At T4 (4 h after cells initiated sporulation), IPTG was added to the culture to a final concentration of 1 mM to induce *yfp* expression, and 40 min later cells were visualized by fluorescent microscopy. Fluorescence was measured across the spore as schematized in Fig. 6*B*.

***yfp* Expression in Forespores.** Total RNA was extracted from the same samples and in the same conditions used to determine induction of protein translation in cells entering dormancy (see above).

Forespores were isolated as described above and were lysed by shaking with 100 μM Zirconia/Silica beads (BioSpec) in a FastPrep homogenizer (MP Biomedicals) at 6.5 m/s for 40 s. Total RNA was isolated using TRIzol (Invitrogen) and reverse transcribed by SuperScript III (Invitrogen) according to the manufacturer's instructions. Quantitative RT-PCR was performed with SYBR Green (Quanta Biosciences) on a CFX96 (Bio-Rad).

Spontaneous Germination Assay. Strains were sporulated by resuspension in DSM for 48 h at 37 °C (50). Remaining vegetative cells were removed by incubating the spore suspensions at 80 °C for 20 min, followed by three washes with 10 mM Tris·HCl, pH 7.5. Purified spores were stored in the same buffer and were visualized by phase-contrast microscopy after 48 h at room temperature.

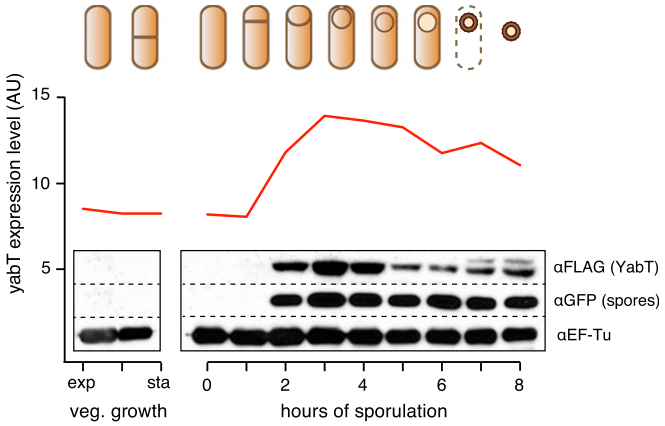

Fig. S1. YabT expression during sporulation. The transcriptional level of *yabT* during the *B. subtilis* cell cycle, including vegetative growth and sporulation, is shown in red (12). To determine YabT protein levels, samples of a *B. subtilis* strain expressing FLAG-tagged YabT were collected at exponential and stationary phases in vegetative growth or at different time points after the induction of sporulation and were analyzed by SDS/PAGE and immunoblotting using a FLAG antibody (1:1,000; Sigma). The levels of CFP (expressed from a sporulation-specific promoter) and EF-Tu also were determined as loading controls using GFP and EF-Tu antibodies (1:3,000).

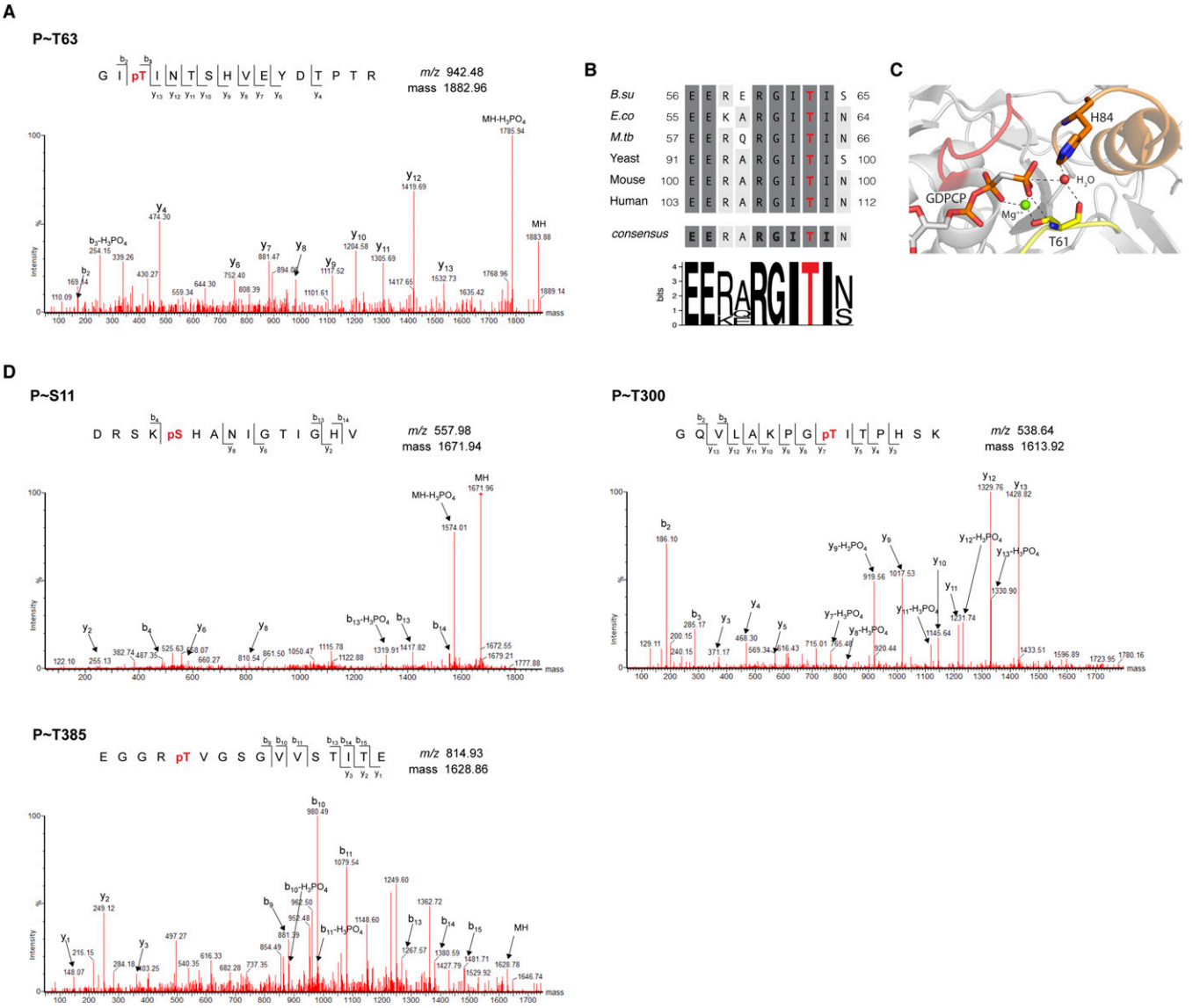

Fig. S2. EF-Tu phosphorylation sites. (*A*) Representative MS/MS spectra of the phosphopeptide obtained after trypsin digestion of wild-type *B. subtilis* EF-Tu. The phosphorylation site was unambiguously assigned to Thr-63 (represented as a red-labeled pT). (*B*) Amino acid sequence alignment of the switch I region in EF-Tu for *B. subtilis* (B.su; P33166), *E. coli* (E.co; NP_417798), *M. tuberculosis* (M.tb; P0A558), and mitochondrial EF-Tu for *Saccharomyces cerevisiae* (Yeast; P02992), *Mus musculus* (Mouse; NP_766333), and *Homo sapiens* (Human; NP_003312). Dark and light shading represent identities and similarities, respectively. The absolutely conserved residues are shown in bold in the consensus sequence. The Thr residue in the GITI motif of the switch I region that is the primary site of phosphorylation in *B. subtilis* EF-Tu is shown in red. The sequence logo was generated using GENIO/logo (www.biogenio.com/logo). (*C*) GTP-binding domain of *E. coli* EF-Tu (Protein Data Bank ID code 2XQD). The structure shows the interactions between the GTP analog GDPCP and EF-Tu p-loop (red), Thr-61 in the switch I region (yellow), and the catalytic His-84 residue in the switch II region (orange). The Mg^{2+} ion and the catalytic water molecule are shown as green and red spheres, respectively. (*D*) Representative MS/MS spectra of phosphopeptides from the *B. subtilis* EF-Tu T63A mutant showing phosphorylation at Ser-11, Thr-300, and Thr-385. Phosphopeptides were obtained after digestion with endoprotease Asp-N (Ser-11 peptide) or trypsin (remaining peptides). The red-labeled pS and pT represent phospho-Ser and phospho-Thr, respectively.

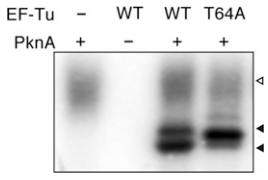

Fig. S3. Phosphorylation of *M. tuberculosis* EF-Tu. Purified wild-type *M. tuberculosis* EF-Tu or the T64A point mutant (T64A) incubated with the Ser/Thr kinase PknA in the presence of [γ-^{32}P]ATP. Phosphorylation was assayed by phosphorimaging. Bands containing EF-Tu and kinase are indicated by closed and open arrowheads, respectively.

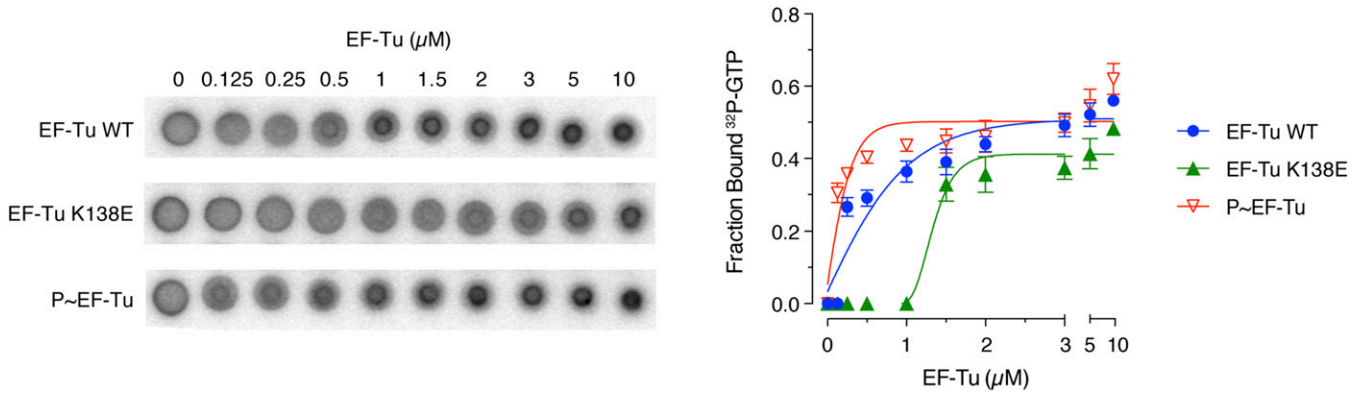

Fig. S4. EF-Tu GTP-binding kinetics. Increasing concentrations of wild-type EF-Tu, K138E GTP-binding–deficient mutant (K138E), and phosphorylated EF-Tu (P~EF-Tu) were incubated with α-[^{32}P]GTP. The reactions were spotted on nitrocellulose, and the diffusion patterns of the radiolabeled nucleotide were visualized by phosphorimaging and are shown in representative autoradiograms. The fraction of bound nucleotide was calculated as described in *Materials and Methods* and is plotted as a function of the EF-Tu concentration. Error bars indicate the SD for at least three spots.

Table S1. Bacterial strains

Strain	Relevant genotype	Description	Source or reference
E. coli strains			
DH5a			Invitrogen
BL21(DE3)			Stratagene
JDE1715	pSFP65 amp	BL21(DE3) expressing PrpC-Strep	This study
JDE1741	pSFP75 amp	BL21(DE3) expressing His-YabT (amino acids 1–277)	This study
JDE1788	pSFP80 amp	BL21(DE3) expressing His-EF-Tu	This study
JDE1824	pSFP80T63A amp	BL21(DE3) expressing His-EF-TuT63A	This study
JDE1840	pSFP83 amp	BL21(DE3) expressing His-EF-TuT63A	This study
JDE1890	pSFP89 amp	BL21(DE3) coexpressing YabT (amino acids 1–277) and His-EF-Tu	This study
JDE2386	pSFP180 amp	BL21(DE3) expressing His-EF-Tu from *M. tuberculosis*	This study
JDE2424	pSFP187 kan	BL21(DE3) expressing His-PknA (amino acids 1–338)	This study
JDE2517	pSFP180T64A amp	BL21(DE3) expressing His-EF-Tu T64A from *M. tuberculosis*	This study
B. subtilis strains			
168	TrpC2	*B. subtilis* wild-type strain	(57)
JDB3485	Δ*cotE*::*tet* Δ*gerE*::*cat*	Coat-deficient mutant	This study
JDB3516	*amyE*::P_{spoIIQ}-*cfp* spec *sacA*::P_{spoIID}-*yfp kan* Δ*cotE*::*tet* Δ*gerE*::*cat*	Reporter strain used in mother cell/endospore fractionation	This study
JDB3557	*amyE*::$P_{hyperspanc}$-*yfp cat*	YFP reporter strain used to assess protein synthesis in sporulating cells	This study
JDB3566	Δ*prkC* Δ*yabT*::*erm* Δ*ybdM*::*spec* *amyE*::$P_{hyperspanc}$-*yfp cat*	YFP reporter in a Δkinase background used to assess protein synthesis in sporulating cells	This study
JDB3564	Δ*yabT*::*erm* *amyE*::$P_{hyperspanc}$-*yfp cat*	Δ*yabT* in JDB3557	This study
JDB3598	Δ*yabT*::*erm* Δ*cotE*::*tet* Δ*gerE*::*cat*	Δ*yabT* in JDB3485	This study
JDB3709	Δ*yabT*::*erm* Δ*cotE*::*tet* Δ*gerE*::*cat* *amyE*::P_{spoIIQ}-*cfp* spec *sacA*::P_{spoIID}-*yfp kan*	Δ*yabT* in JDB3516	This study
JDB3969	Δ*cotE*::*tet* Δ*gerE*::*cat* *amyE*::P_{spoIIQ}-*cfp* spec *yabT*-FLAG *erm*	YabT-FLAG translation fusion expressed from *yabT* native locus	This study
M. tuberculosis strain			
Erdman		*M. tuberculosis* wild-type strain	(58)

Table S2. Plasmids

Plasmid	Description	Source or reference
pET11a	Cloning vector, ampR	Novagen
pETPhos	Cloning vector, ampR	(59)
pETDuet-1	Cloning vector, ampR	EMD Biosciences
pSMT3	Cloning vector, kanR	(60)
pSFP65	pET11a with *prpC* amplified from TrpC2 genomic DNA with oligos SFP237 and SFP238 (NdeI/BamHI)	This study
pSFP75	pETPhos with *yabT* amplified from TrpC2 genomic DNA with oligos SFP239 and SFP255 (NdeI/BamHI)	This study
pSFP80	pETPhos with *tuf* amplified from TrpC2 genomic DNA with oligos SFP271 and SFP272 (NdeI/BamHI)	This study
pSFP80T63A	pSFP80 with *tuf*T63A generated by site-directed mutagenesis with oligos SFP273 and SFP274	This study
pSFP83	pETDuet-1 with *yabT* amplified from TrpC2 genomic DNA with oligos SFP286 and SFP255 (NcoI/BamHI)	This study
pSFP89	pSFP83 with *tuf* amplified from TrpC2 genomic DNA with oligos SFP311 and SFP296 (BglII/XhoI)	This study
pSFP180	pETPhos with *tuf* amplified from *M. tuberculosis* genomic DNA with oligos SFP494 and SFP495 (NdeI/BamHI)	This study
pSFP187	pSMT3 with *pknA* amplified from *M. tuberculosis* genomic DNA with oligos SFP506 and SFP507 (BamHI/XhoI)	This study
pSFP180T64A	pSFP180 with *tuf*T64A generated by site-directed mutagenesis with oligos SFP527 and SFP528	This study

Restriction sites used are shown in parentheses.

Table S3. Primers

Oligo	Sequence	Characteristics	Origin
SFP237	GGAATTC*CATATG*ttgttaacagccttaaaaacag	*NdeI*; ATG	This study
SFP238	TGA*GGATCC*TTA**TTTTTCGAACTGCGGGTGGCTCCA**AGCGCCgcactgatcttcaccctc	*BamHI*; STOP **Strep-tag**	This study
SFP239	GGAATTC*CATatg*atgaacgacgctttgacgag	*NdeI*	This study
SFP255	GA*AGATCT*TTAaggctgttttctttgtgctgc	*BglII*; STOP	This study
SFP271	GGAATTC*CATatgg*ctaaagaaaaattcg	*NdeI*	This study
SFP272	GTC*GGATcc*atactattactcagtg	*BamHI*	This study
SFP273	cgagcgcggtatcGcaatctctactg	T63A	This study
SFP274	cagtagagattgCgataccgcgctcg	T63A	This study
SFP286	CATG*CCATGGG*Catgatgaacgacgctttgacgag	*NcoI*	This study
SFP296	CTCG*CTCGAG*ccatactattactcagtg	*XhoI*	This study
SFP311	GGA*AGATCT***ACATCATCATCATCATCAT**GGTatggctaaagaaaaattcg	*BglII*; **His-tag**	This study
SFP494	ATTC*CATATG*gtggcgaaggcgaagttcc	*NdeI*	This study
SFP495	CGC*GGATCC*cctacttgatgatcttgg	*BamHI*	This study
SFP506	TGA*GGATCC*atgagcccccgagttggcgt	*BamHI*	This study
SFP507	TAGT*GTCGAC*TTAacgctgaccggacgaaaacgtgc	*SalI*; STOP	This study
SFP527	cagcgcggtatcGccatcaacatcg	T64A	This study
SFP528	cgatgttgatggCgataccgcgctg	T64A	This study
SFP656	ggccctgtccttttaccaga	*yfp* qPCR F	This study
SFP657	atgccatgtgtaatcccagca	*yfp* qPCR R	This study
SFP658	gcgacatggtagacgacgaa	*tuf* qPCR F	This study
SFP659	tcagcgtctccttcaagagc	*tuf* qPCR R	This study

Homology sequences are shown in lowercase. Nonhomolog sequences are shown in uppercase and include restriction sites (in italic), STOP codons (underlined), tags (bold), and point mutations.