

Fig. S1. Glutamate is required for growth of the *B. subtilis* $\Delta rfaA$ mutant. **a**, Comparison of the relative fitness of the $\Delta rfaA$ and the $\Delta gltA$ mutants in *B. subtilis*. Data used to compare the two strains was obtained from ¹⁸. **b**, Growth assay of *B. subtilis* wild type and $\Delta rfaA$ mutant. Bacterial growth of the *B. subtilis* wild type strain 168 and the $\Delta rfaA$ mutant GP1468 has been assessed on minimal medium in absence or in presence of glutamate (E), glutamine (Q) and aspartate (D), respectively. Additionally the growth has been analyzed by drop dilution assays displayed below.

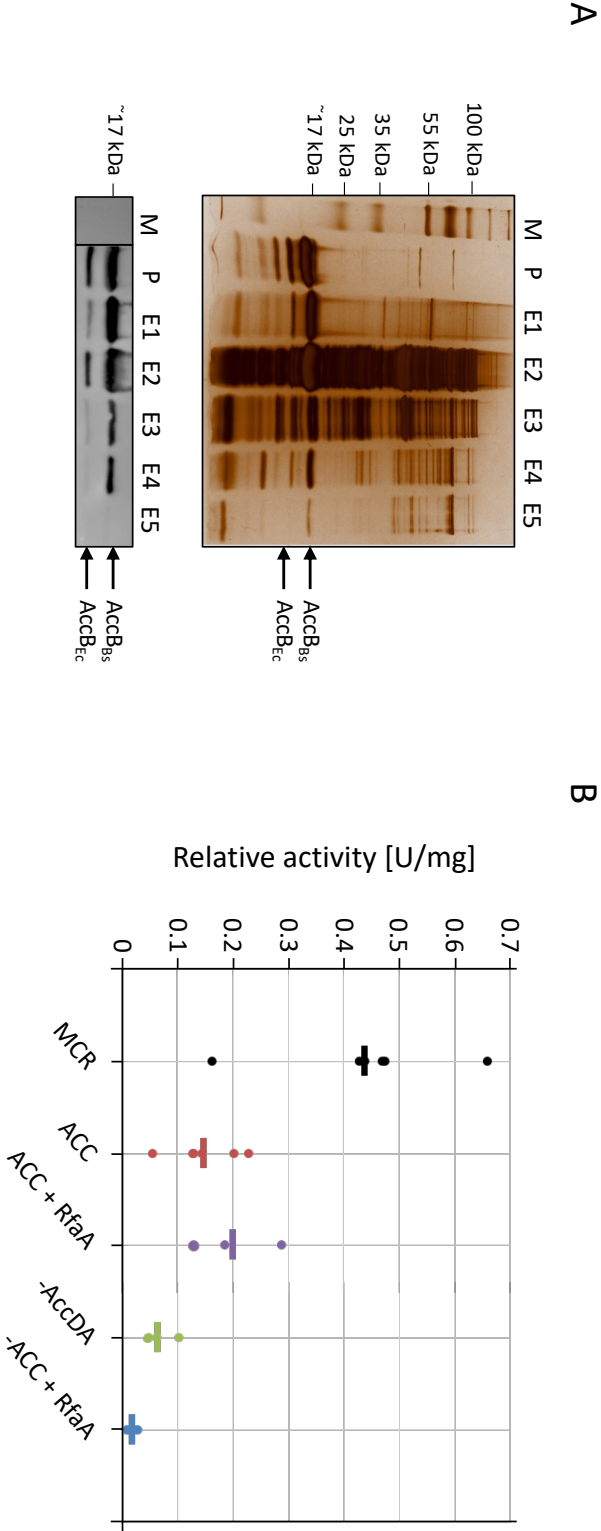


Fig. S2. RfaA does not affect ACCase activity. **a**, Confirmation of the biotinylation of AccB. The purified AccB protein (P) was used for *in vitro* biotinylation and purification via a heparin column. The presence of AccB was confirmed by SDS PAGE of the elution fractions (E1-E5) and subsequent silver staining. The biotinylation state has been analyzed by Western blot analysis using streptavidin-HRP. **b**, Activity of the ACCase complex was assayed by a coupled enzyme assay. The production of malonyl-CoA by the ACCase was coupled to the NADPH-dependent reaction of the malonyl-CoA reductase (MCR). The oxidation of NADPH was monitored using a multi-well PlateReader. Dots represent single measurements and bars indicate the mean value of four replicates.

Supplementary table S1. Strains used in this study.

Name	Genotype	Source or Reference
168	<i>trpC2</i>	Laboratory collection
GP807	<i>trpC2 ΔgltAB::tet</i>	21
GP1468	<i>trpC2 ΔrfaA::ermC</i>	13
GP3784	<i>trpC2 ΔclpE::neo</i>	This work

Supplementary table S2. Plasmids used in this study.

Plasmid	Relevant characteristics	Source or Reference
p25-N	N-term. fusion of the target protein to the T25 fragment of adenylate cyclase	Karimova
pBP202	pUT18 with <i>clpC</i>	This work
pBP203	pUT18C with <i>clpC</i>	This work
pBP204	pKT25 with <i>clpC</i>	This work
pBP205	p25-N with <i>clpC</i>	This work
pBP206	pUT18 with <i>clpX</i>	This work
pBP207	pUT18C with <i>clpX</i>	This work
pBP208	p25-N with <i>clpX</i>	This work
pBP209	pKT25 with <i>clpX</i>	This work
pGEX-6P-1	Expression of N-term. GST-tagged proteins in <i>E. coli</i>	Cytiva, USA
pGP172	Expression of N-term. Strep-tagged proteins in <i>E. coli</i>	49
pGP382	Expression of C-term. Strep-tagged proteins in <i>B. subtilis</i>	51

pGP574	Expression of C-term. Strep-tagged proteins in <i>E. coli</i>	50
pGP706	Expression of N-term. His-tagged CcpC	56
pGP1025	Expression of N-term. Strep-tagged AccC	This work
pGP1027	Expression of C-term. Strep-tagged AccB	This work
pGP1329	Expression of N-term. Strep-tagged RfaA	This work
pPG1470	pUT18 with <i>rfaA</i>	This work
pPG1471	pUT18C with <i>rfaA</i>	This work
pPG1472	p25-N with <i>rfaA</i>	This work
pPG1473	pKT25 with <i>rfaA</i>	This work
pGP1723	Expression of C-term. Strep tagged AccA in <i>B. subtilis</i>	This work
pGP1724	Expression of C-term. Strep tagged AccD in <i>B. subtilis</i>	This work
pGP1736	pUT18 with <i>accB</i>	This work
pGP1737	pUT18C with <i>accB</i>	This work
pGP1738	p25-N with <i>accB</i>	This work
pGP1739	pKT25 with <i>accB</i>	This work
pGP1740	pUT18 with <i>accC</i>	This work
pGP1741	pUT18C with <i>accC</i>	This work
pGP1742	p25-N with <i>accC</i>	This work
pGP1743	pKT25 with <i>accC</i>	This work
pGP2157	pUT18 with <i>clpE</i>	This work
pGP2158	pUT18-C with <i>clpE</i>	This work
pGP2159	p25-N with <i>clpE</i>	This work
pGP2160	pKT25 with <i>clpE</i>	This work
pGP2649	Expression of N-term. His-tagged AccB	This work
pGP3644	pGEX-6P-1 with <i>rfaA</i>	This work

pGP3646	Expression of N-term. His-tagged AccC	This work
pGP3650	Expression of N-term. Strep-tagged RnpM	55
pKT25	C-term. fusion of the target protein to the T25 fragment of adenylate cyclase	53
pKT25-zip	C-term. fusion of the leucine zipper to the T25 fragment of adenylate cyclase (control)	53
pTrc99A-mcr	Expression of N-term. Strep-tagged MCR from <i>Chloroflexus</i> <i>aurantiacus</i>	54
pUT18	N-term. fusion of the target protein to the T18 fragment of adenylate cyclase	53
pUT18-C	C-term. fusion of the target protein to the T18 fragment of adenylate cyclase	53
pUT18-zip	N-term. fusion of the leucine zipper to the T25 fragment of adenylate cyclase (control)	53
pWH844	Expression of N-term. His-tagged proteins in <i>E. coli</i>	52