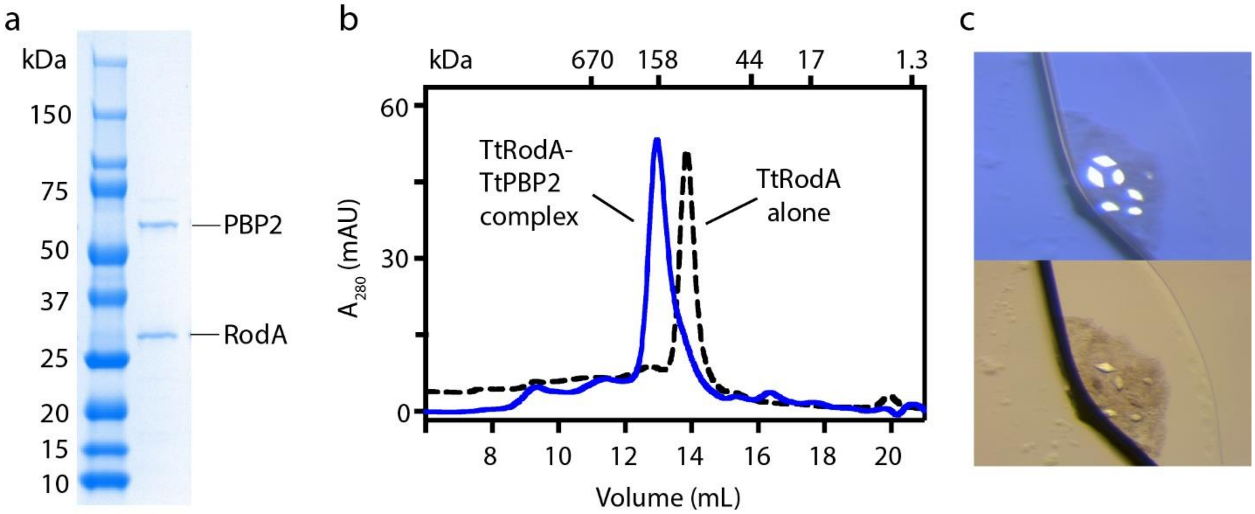
Supplementary Table 1 | Data collection and refinement statistics

	T. thermophilus Wild-type RodA:PBP2 complex[‡]	*T. thermophilus* RodA (D255A):PBP2 complex[‡]
Data collection		
Space group	$P3_221$	$P3_221$
Cell dimensions		
a, b, c (Å)	119.6, 119.6, 267.3	118.44, 118.44 265.80
α, β, γ (°)	90, 90, 120	90, 90, 120
Resolution (Å)	50.0–3.5 (3.6–3.5)[*]	50.0–3.3 (3.4–3.3)[*]
R_{merge}	15.9 (269.0)	24.3 (339.3)
$I / \sigma I$	8.15 (0.62)	5.86 (0.58)
Completeness (%)	99.3 (99.8)	99.8 (99.7)
Redundancy	6.11 (6.11)	6.77 (6.82)
$CC_{1/2}$ (%)	99.9 (33.4)	99.9 (33.0)
Refinement		
Resolution (Å)	50.0–3.50 (3.55–3.50)	50.0–3.30 (3.34–3.30)
No. reflections	50777 (1761)	59327 (1320)
R_{work} / R_{free}	28.7 / 30.5	27.7 / 30.8
No. atoms		
Protein	6843	6864
Ligand/ion	-	24
B-factors		
Protein	175.8	156.9
Ligand/ion	-	168.9
R.m.s. deviations		
Bond lengths (Å)	0.002	0.002
Bond angles (°)	0.494	0.463

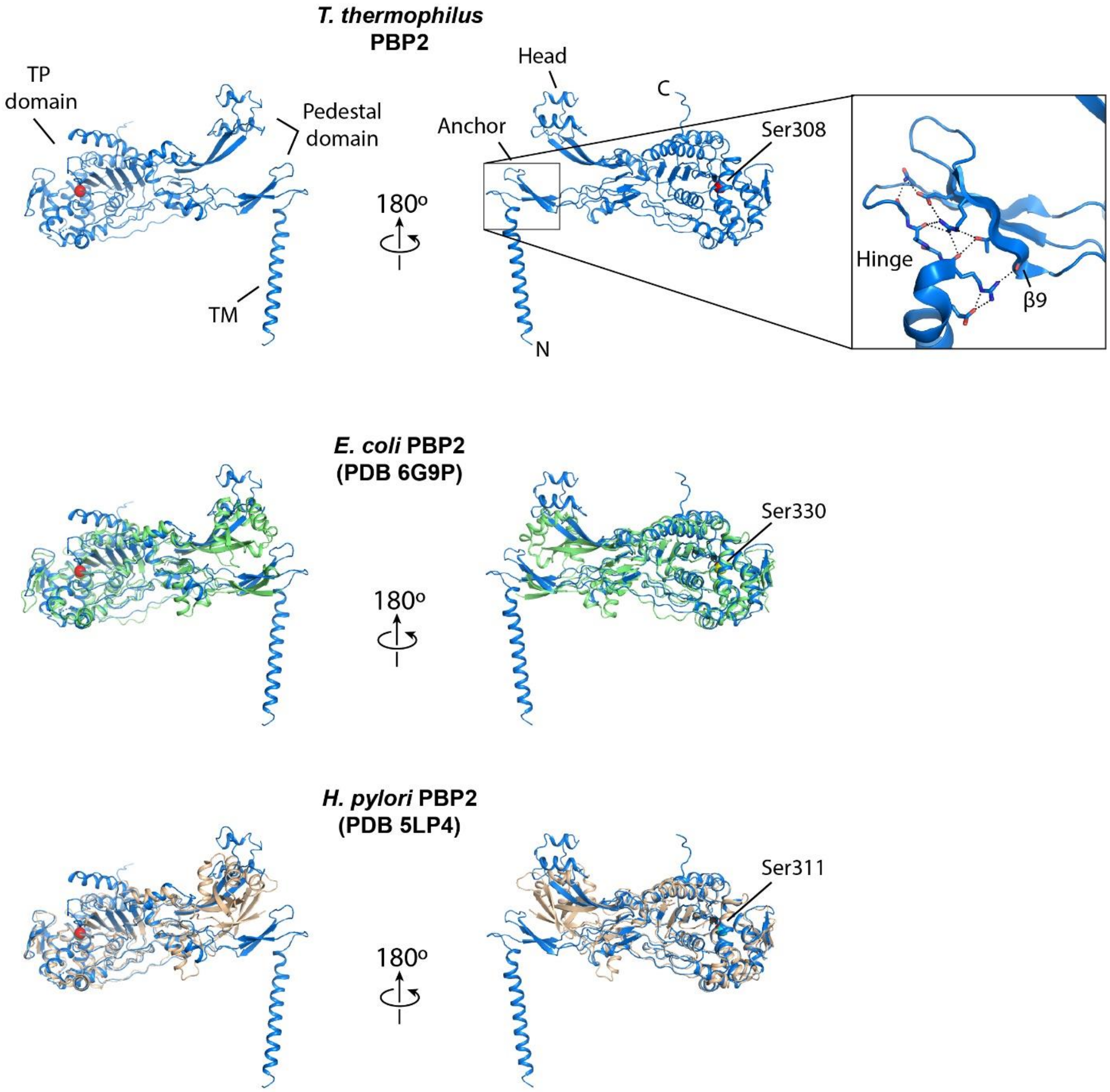
‡ Data shown are derived from 2 and 5 crystals for the wild-type protein and 5 crystals for the D255A variant. *Values in parentheses are for highest-resolution shell.

Supplementary Table 2 | Plasmids and *E. coli* strains used in this study

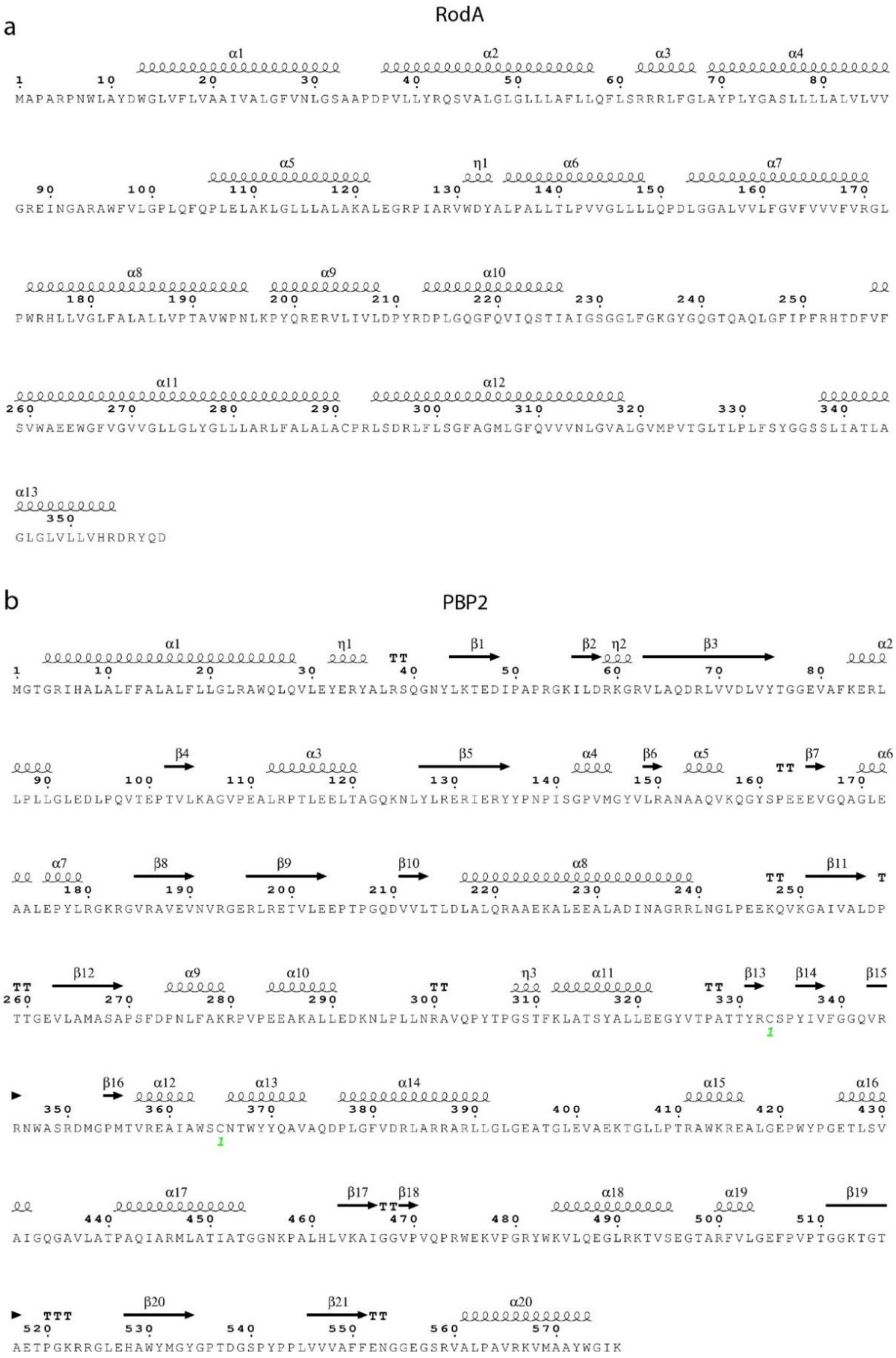
Plasmid	Description/Genotype	origin	Source/ Reference
pAM174	*p_{BAD}-His6-Ulp1(403–621)*	p15A	[1]
pMS211	*p_{T7}-His6-SUMO-Flag-3C-TtRodA*	ColE1	[13]
pMS235	*p_{T7}-TtPBP2-3C-PrtC*	ColA	this study
pMS239	*p_{T7}-TtPBP2-3C-PrtC; p_{T7}-His6-SUMO-Flag-3C-TtRodA*	ColA	this study
pMS244	*p_{T7}-TtPBP2-3C-PrtC; p_{T7}-His6-SUMO-Flag-3C-TtRodA(D255A)*	ColA	this study
pMS292	*p_{T7}-TtPBP2(L43R)-3C-PrtC; p_{T7}-His6-SUMO-Flag-3C-TtRodA*	ColA	this study
pMS293	*p_{T7}-TtPBP2(A186R)-3C-PrtC; p_{T7}-His6-SUMO-Flag-3C-TtRodA*	ColA	this study
pMS294	*p_{T7}-TtPBP2(R149-GlySer-E162)-3C-PrtC; p_{T7}-His6-SUMO-Flag-3C-TtRodA*	ColA	this study
pMW1010	*p_{T7}-His6-EfPBPX(T32-P429)*	ColE1	[24]
pRY47	*cat* $\mathrm{P_{lac}}$::empty	ColE1	[20]
pHC857	*cat* $\mathrm{P_{lac}}$::*pbpA-rodA*	ColE1	[20]
pPR148	*cat* $\mathrm{P_{lac}}$::*pbpA(A147C)-rodA*	ColE1	this study
pPR151	*cat* $\mathrm{P_{lac}}$::*pbpA(R237C)-rodA*	ColE1	this study
pPR152	*cat* $\mathrm{P_{lac}}$::*pbpA(A147C/R237C)-rodA*	ColE1	this study
pPR190	*cat* $\mathrm{P_{lac}}$::*pbpA(L61R/A147C/R237C)-rodA*	ColE1	this study
FB38	*rph1 ilvG rfb-50 ΔlacIZYA::frt mrdAB::aph*		[37]
TB28	*rph1 ilvG rfb-50 ΔlacIZYA::frt*		[40]



Supplementary Figure 1 | Lipid cubic phase crystallography of *T. thermophilus* RodA-PBP2 complex. a, Coomassie-stained gel of purified ^{Tt}RodA and ^{Tt}PBP2. **b,** Analytical SEC trace of ^{Tt}RodA-PBP2 complex. For comparison, the SEC trace of ^{Tt}RodA alone is shown. Estimated molecular weights are shown at the top based on the elution profiles of a set of standards. The molecular weight is consistent with expected size of the detergent-solubilized (DDM) proteins. Results in a and b are representative of more than 3 experiments. **c,** Representative crystals of TtRodA-PBP2 complex.



Supplementary Figure 2 | *T. thermophilus* PBP2 is structurally similar to PBP2 of *E. coli* and *H. pylori*. *T. thermophilus* PBP2 is shown as ribbons colored blue and aligned to the previously determined structures of PBP2 from *E. coli* (green), *H. pylori* (tan). The catalytic serine of each homolog is shown as a red sphere. The inset in the top panel highlights the network of hydrogen bonds and salt bridges formed between the hinge region and the anchor subdomain in *T. thermophilus* PBP2.



Supplementary Figure 3 | The secondary structure features of *T. thermophilus* **a**, RodA and **b**, PBP2 are annotated above the amino-acid sequence of each.