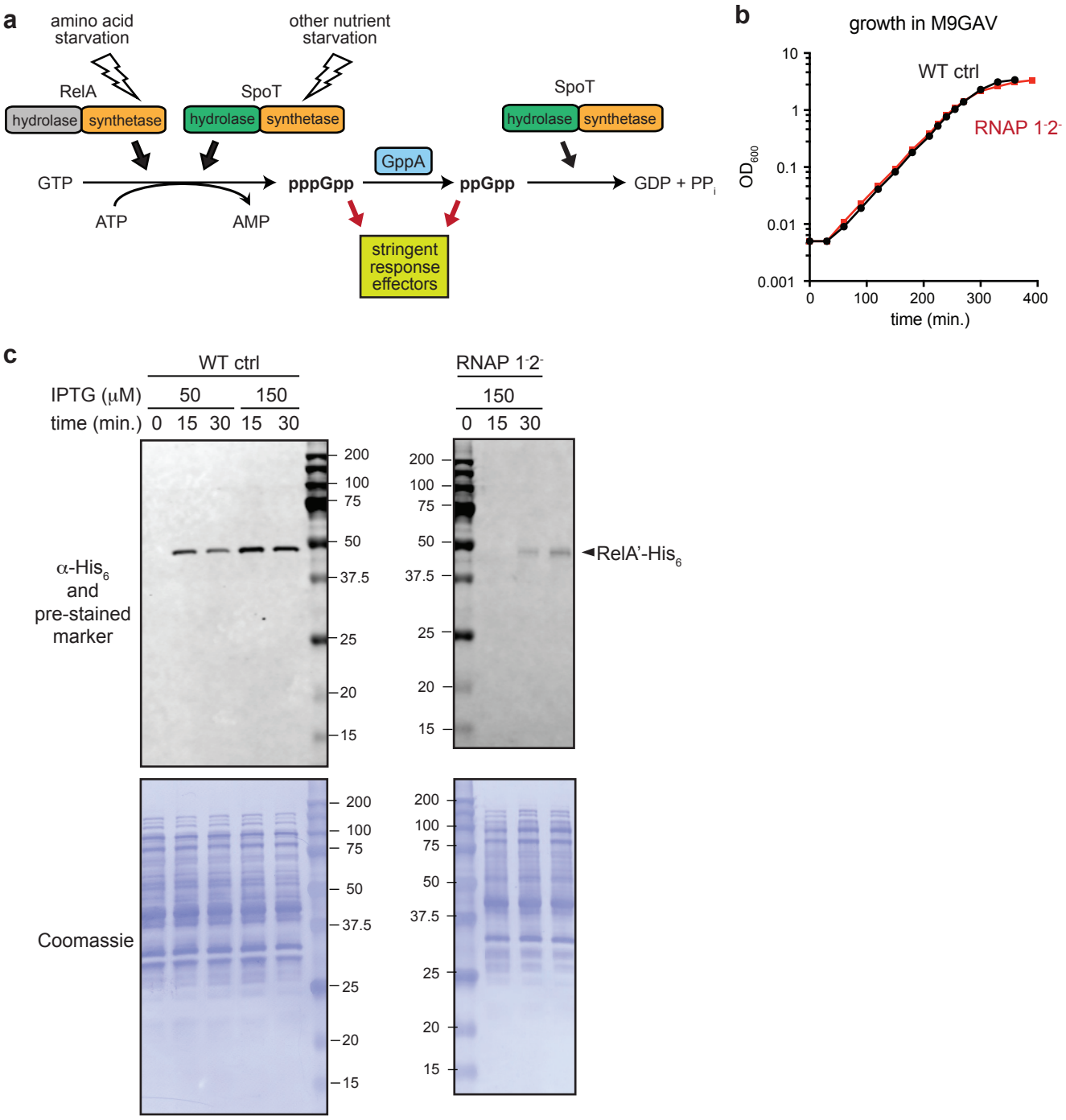
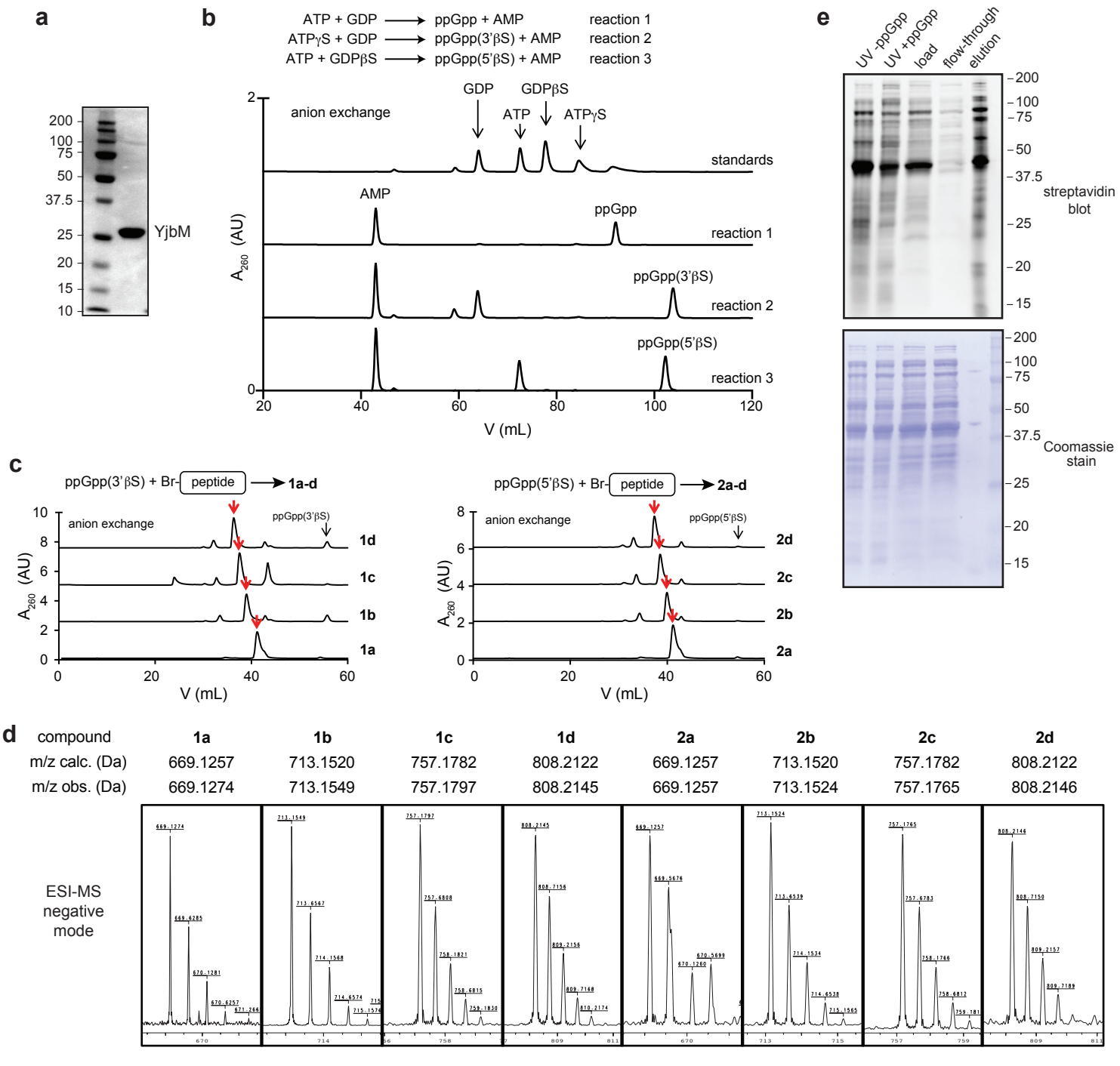


Supplementary Figure 1. Growth of the RNAP 1-2- strain and its expression of RelA'.
(a) Schematic of enzymatic activities governing (p)ppGpp levels in *E. coli*. RelA and SpoT can each synthesize pppGpp in response to various signals. SpoT also has an active hydrolase domain; the hydrolase domain in RelA is non-functional.
(b) Growth of the WT control and the RNAP 1-2- strains in M9GAV medium. One representative growth curve from three experiments is shown.
(c) Western blot for RelA'-His6 expressed in the WT control and RNAP 1-2- strains for the times indicated with RelA'-His_6 induced with the concentration of IPTG indicated. The corresponding Coomassie stained gel indicating loading is shown below.

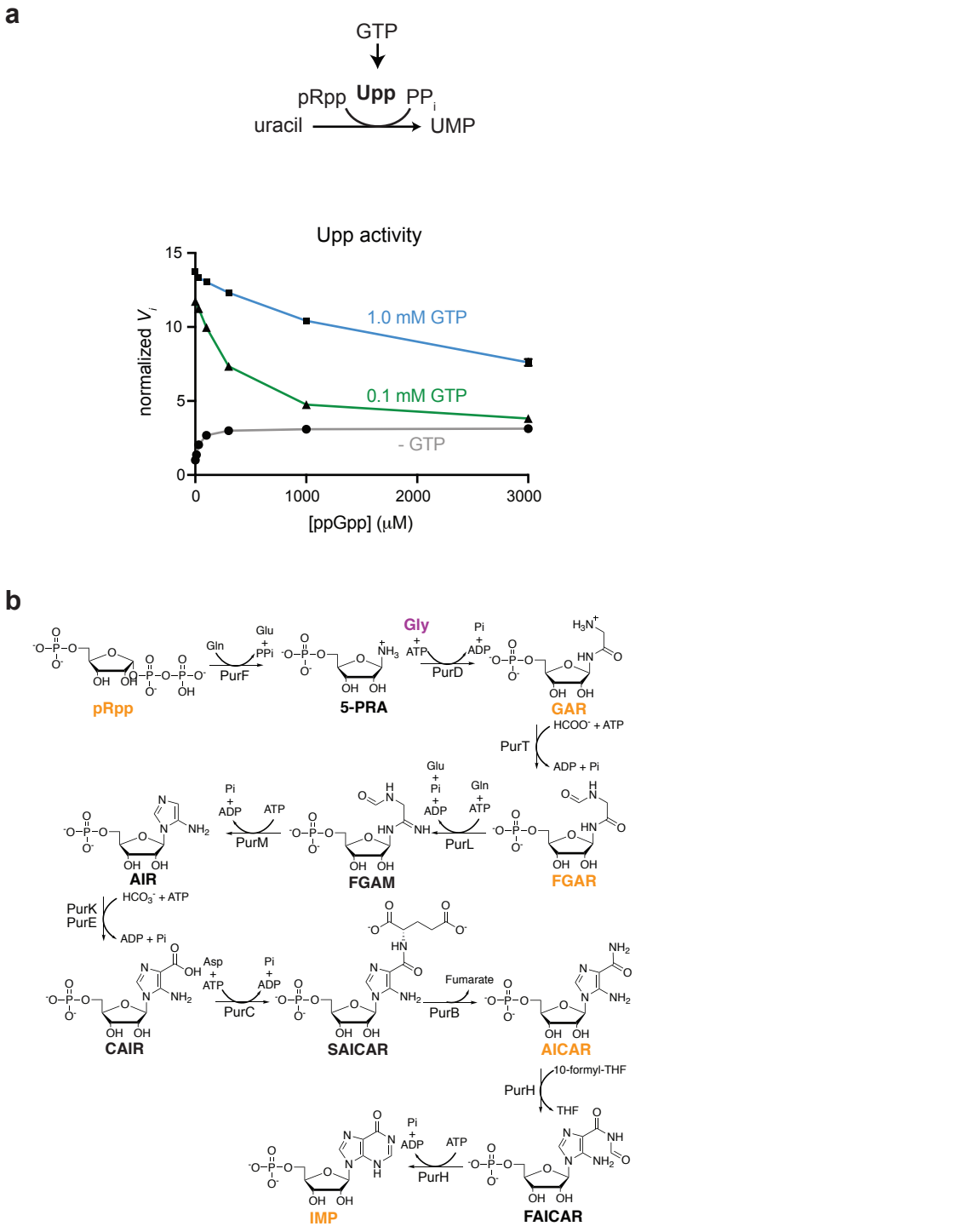


d

compound	1a	1b	1c	1d	2a	2b	2c	2d
m/z calc. (Da)	669.1257	713.1520	757.1782	808.2122	669.1257	713.1520	757.1782	808.2122
m/z obs. (Da)	669.1274	713.1549	757.1797	808.2145	669.1257	713.1524	757.1765	808.2146

ESI-MS negative mode

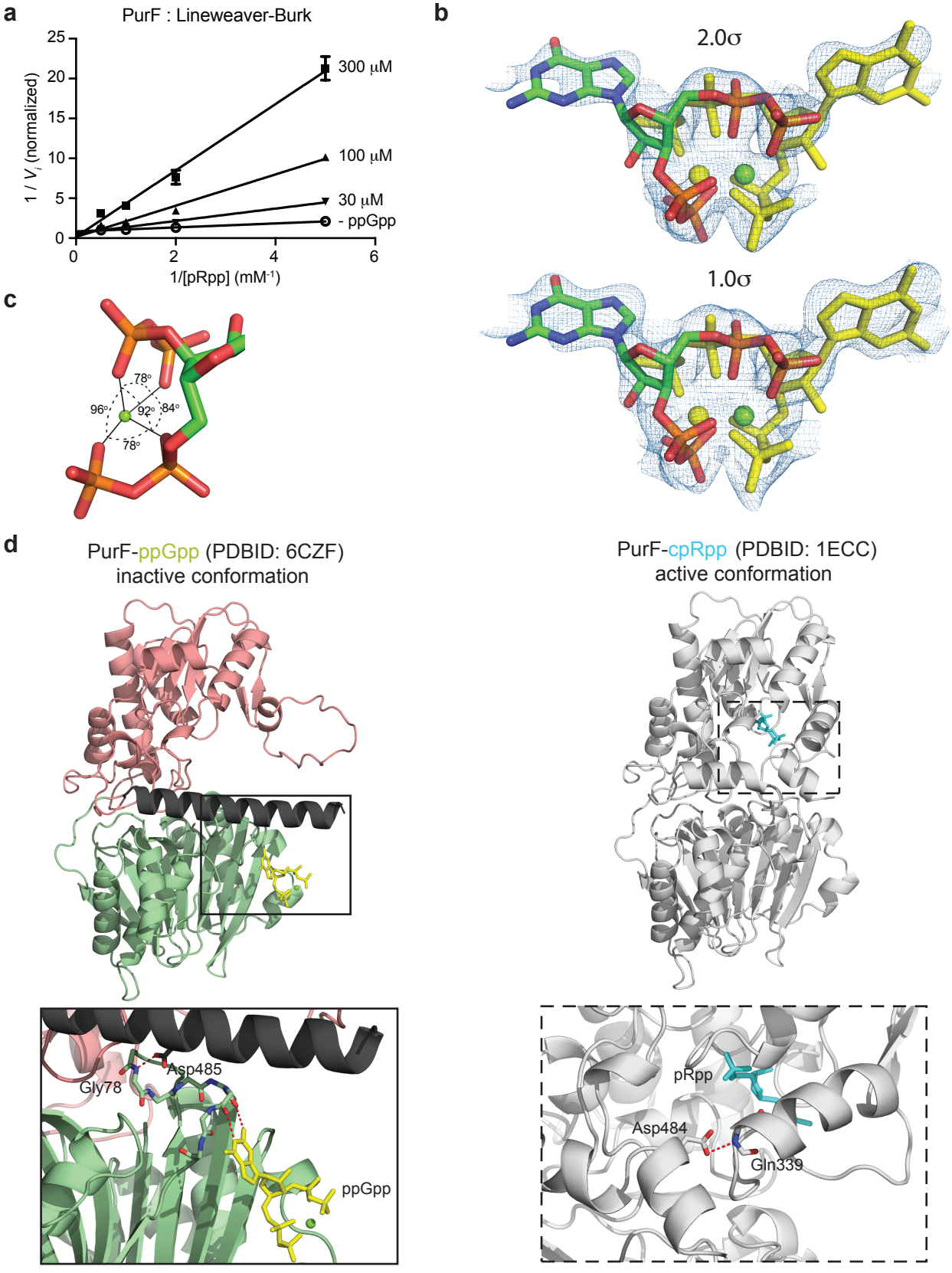
Supplementary Figure 2. Synthesis of crosslinkable ppGpp derivatives.
(a) Coomassie stained gel indicating the purity of YjbM enzyme used to synthesize ppGpp, ppGpp(3'γS), and ppGpp(5'γS).
(b) Anion-exchange chromatography traces for reactions producing ppGpp, ppGpp(3'γS), and ppGpp(5'γS). The top trace was from a mixture of starting materials.
(c) Anion-exchange chromatography traces for conjugation reactions producing **1a-d** (left) and **2a-d** (right). Red arrows indicate desired compounds listed on the far right and shown in Fig. 2a.
(d) High-resolution mass spectra for the 8 crosslinkable ppGpp compounds **1a-d** and **2a-d**. The calculated and observed m/z in Da are listed above each spectrum.
(e) Streptavidin blot (top) and Coomassie brilliant blue staining (bottom) for post-crosslinking lysates (left two lanes) and fractions from streptavidin pulldown (right three lanes).



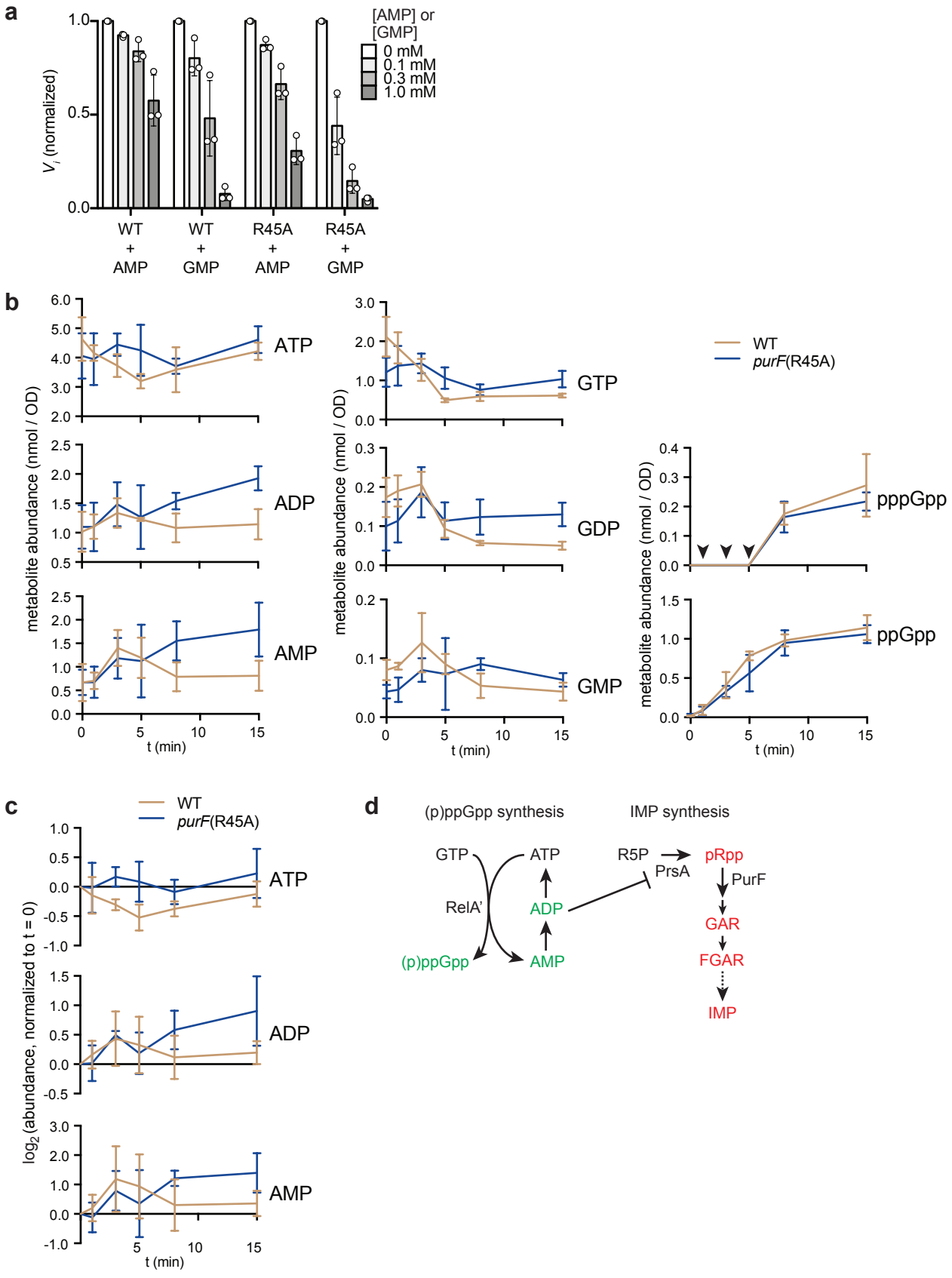
Supplementary Figure 3. Regulation of Upp by ppGpp and summary of de novo purine nucleotide synthesis pathway.

(a) Upp produces UMP from pRpp and uracil, and is activated by GTP (top). Upp activity in the presence of ppGpp and GTP at the concentrations indicated (bottom). Data points represent mean of two kinetic experiments.

(b) Metabolic pathway for *de novo* synthesis of IMP, the precursor to ATP and GTP, from pRpp. Metabolites listed in orange were reliably detected by metabolomics (see Fig. 4c, 5e). Glycine, which was used for isotopic labeling of purines, is highlighted in purple.



Supplementary Figure 4. Structural changes induced by ppGpp binding to PurF.
(a) Lineweaver-Burk analysis showing that ppGpp is a competitive PurF inhibitor with respect to pRpp. Points represent mean of two independent experiments with bars indicating range.
(b) Fo–Fc omit electron density of ppGpp (blue mesh) contoured at 2σ (left) and 1σ (right), each overlaid with stick model of ppGpp-Mg in two orientations, one colored by element and the other in yellow. Also see Online Methods for details of modeling.
(c) Configuration of ppGpp coordinating a Mg^{2+} ion in the PurF-ppGpp structure. All O-Mg-O bond angles displayed should be 90° in an ideal octahedral coordination.
(d) Zoom-in views of key contacts involving the C-terminal helix of PurF in the ppGpp-bound conformation (left) and the cpRpp-bound conformation (right).



Supplementary Figure 5. Additional characterizations of PurF(R45A).
(a) Activity of WT PurF or PurF(R45A) in the presence of indicated concentrations of AMP or GMP. Initial velocities of each reaction were normalized to that of WT PurF without ligand. Error bars indicate S.D., n=3.
(b-c) Time courses of net abundance changes of all adenosine and guanosine nucleotides (b) and fold changes of adenosine nucleotides (c) in WT and *purF(R45A)* strains grown in M9GAV after inducing pRelA' with 40 μM IPTG. Error bars indicate S.D, n=3. Arrowheads in (b) indicate not detectable.
(d) Scheme showing the PurF-independent effects of ppGpp on the *de novo* synthesis of IMP. Metabolites increased (green) or decreased (red) early following RelA' induction are highlighted.

Supplementary Dataset. SILAC mass spectrometry results
For each protein, the H / L ratio, number of total peptides, and number of unique peptides is shown for each of three capture-ID experiments. Entries shaded grey are proteins predicted to be secreted. Entries shaded orange were purified and studied with ITC (see Supplementary Table 2)

Supplementary Table 1. Previously characterized ppGpp-binding proteins in *E. coli*

protein	function / annotation	ID'd here?	characterization	reference
SpeC	ornithine decarboxylase	Y	inhibition and binding (ITC) *in vitro*	1
Gpt	xanthine-guanine phosphoribosyltransferase	Y	inhibition *in vitro*	2
GdhA	glutamate dehydrogenase	Y	binding (microdialysis) *in vitro*	3
Upp	uracil phosphoribosyltransferase	Y	inhibition *in vitro* activation *in vitro* with low GTP	4 5
Hpt	hypoxanthine phosphoribosyltransferase	Y	inhibition *in vitro*	2
PurA	adenylosuccinate synthetase	Y	inhibition of partially purified protein *in vitro*	6
EF-G	elongation factor G	Y	binding (ITC) *in vitro* inhibition of translation *in vitro*	7 8
Der	ribosome-associated GTPase	Y	binding *in vitro*	9
IF-2	translation initiation factor 2, GTPase	Y	binding (ITC) *in vitro* inhibition *in vitro*	7 10
EF-Tu	translation elongation factor Tu	Y	binding (ITC) *in vitro* inhibition of translation *in vitro*	7 17
BipA	ribosome biogenesis GTPase	Y	binding (ITC) *in vitro* + structure	11
Ppx	exopolyphosphatase	Y	inhibition *in vitro*, polyP accumulation *in vivo*	12
GuaB	inosine-5'-monophosphate dehydrogenase	Y	inhibition *in vitro*	6
ObgE	GTPase, putative ribosome disassembly factor	Y	inhibition *in vitro*	13
HisG	ATP phosphoribosyltransferase	N	inhibition *in vitro*	14
LdcC	lysine decarboxylase 2	N	inhibition *in vitro*	1
LdcI	lysine decarboxylase 1	N	inhibition *in vitro* + structure	15
DksA	RNA polymerase-binding transcription factor	Y	inhibition *in vitro* RNAP $1^{-}2^{-}$ strain defective in nutritional shifts	16 17
RpoC	RNA polymerase subunit β'	N	structure	18
RpoZ	RNA polymerase subunit ω	N	RNAP $1^{-}2^{-}$ strain defective in nutritional shifts	17
SpoT	bifunctional ppGpp synthase/hydrolase	Y	overexpression reduces ppGpp *in vivo*	19
DnaG	DNA primase	N	inhibited *in vitro*	20
YgdH	nucleotide 5'-monophosphate nucleosidase	N	DRaCALA	21
RF-3	peptide chain release factor 3, GTPase	Y	DRaCALA	21
Era	ribosome biogenesis GTPase	Y	DRaCALA	21
EF-4	translation elongation factor 4	Y	DRaCALA	21
RsgA	ribosome biogenesis GTPase	Y	DRaCALA	21
HypB	hydrogenase isozymes nickel incorp. protein	N	DRaCALA	21

Supplementary Table 2. Summary of exposed (p)ppGpp termini in crystal structures

PDBID	resolution (Å)	(p)ppGpp	species	protein	solvent-exposed terminal P_i	reference
2J4R	2.7	ppGpp	*A. aeolicus*	GppA	5'	22
1LNZ	2.6	ppGpp	*B. subtilis*	Obg	3' and 5'	23
3VR1	2.8	ppGpp	*D. vulgaris*	RF3	3'	24
4ZCM	3.3	ppGpp	*E. coli*	BipA	3'	11
5A9Y	4.0	ppGpp	*E. coli*	BipA	3'	25
3N75	2.0	ppGpp	*E. coli*	LdcI	3' and 5'	15
4JK1	3.9	ppGpp	*E. coli*	RNAP	5'	26
4JKR	4.2	ppGpp	*E. coli*	RNAP	3' and 5'	18
5VSW	4.3	ppGpp	*E. coli*	RNAP-DksA	3' and 5' (chain E) 3' and 5' (chain J) 3' (chain M)	26
5U51	3.0	ppGpp	*F. tularensis*	MglA-SspA	3' and 5'	27
5VOG	1.5	ppGpp	*N. gonorrhoeae*	Hpt	3'	N/A
4EDT	2.0	ppGpp	*S. aureus*	DnaG	3' and 5'	28
5DED	2.9	pppGpp	*B. subtilis*	YjbM	3' and 5'	29
4JK2	4.2	pppGpp	*E. coli*	RNAP	5'	18
4EDV	2.0	pppGpp	*S. aureus*	DnaG	3' and 5'	28
4QRH	1.6	pppGpp	*S. aureus*	Gmk	5'	30

Supplementary Table 3. Binding of ppGpp to purified metabolic enzymes

Protein	Function / Annotation	#subunits	# binding sites	K_D (μM)	% activity + 1 mM ppGpp
Gsk	inosine-guanosine kinase	2	2^	0.4 ± 0.07 14 ± 2	9 ± 1
SpeC	ornithine decarboxylase	2	1.14 ± 0.004	1.6 ± 0.05	1.6 ± 0.2
PurF	glutamine amidophosphoribosyltransferase	4	2.13 ± 0.005	1.6	8 ± 2
GdhA	glutamate dehydrogenase	6	2^	2.8 ± 0.4 29 ± 2	119
Gpt	xanthine-guanine phosphoribosyltransferase	2	1.02 ± 0.002	3.2 ± 0.05	13 ± 5
Hpt	hypoxanthine phosphoribosyltransferase	2	0.97 ± 0.03	32 ± 4	4 ± 1
Upp	uracil phosphoribosyltransferase	2	2.98 ± 0.04	47 ± 3	76 ± 1
PurA	adenylosuccinate synthetase	2	2.02 ± 0.03	61 ± 4	75 ± 2
GpmA	phosphoglycerate mutase	2	2^	52 ± 3	147
Cmk	cytidylate kinase	1	1^	79 ± 1	111
FolC	dihydrofolate synthase	1	1^	130 ± 3	N.T.
Icd	isocitrate dehydrogenase	2	1^	132 ± 5	96 ± 1
Gnd	6-phosphogluconate dehydrogenase	2	1^	175 ± 3	114 ± 1
Pgk	phosphoglycerate kinase	1	1^	493 ± 30	85
Mpl	UDP-MurNAc--L-Ala-γ-D-Glu-meso-DAP ligase	1	1^	752 ± 46	N.T.
PurC	SAICAR synthase	3	N.D.	N.D.	N.T.
PurB	adenylosuccinate lyase	4	N.D.	N.D.	N.T.
GuaB	inosine 5'-monophosphate dehydrogenase	4	N.D.	N.D.	106 ± 5

red = new hits
^number of sites was fixed rather than inferred from fitted ITC data
ITC errors are fitting errors from one binding isotherm.
Activity errors are range of two replicates.
N.D. = no detectable heat release by ITC
N.T. = not tried

Supplementary Table 4. Data collection and refinement statistics for the PurF-ppGpp structure

	PDB: 6CZF
Data collection	
Space group	$P2_12_12_1$
Cell dimensions	
a, *b*, *c* (Å)	107.29, 115.34, 156.07
α, β, γ (°)	90, 90, 90
Resolution (Å)	48.6-1.95 (1.98-1.95)
R_{sym}	0.087 (1.49)
$I / \sigma(I)$	10.9 (0.70)
Completeness (%)	99.9 (99.0)
Redundancy	6.4 (5.8)
Refinement	
Resolution (Å)	1.95
No. reflections	140,744
R_{work} / R_{free}	0.2163 (0.371) / 0.2409 (0.431)
No. atoms	
Protein	15577
Ligand/ion	148*
Water	380
B-factors	
Protein	47.68
Ligand/ion	36.01*
Water	44.27
R.m.s. deviations	
Bond lengths (Å)	0.006
Bond angles (°)	0.993

Highest-resolution shell is shown in parentheses.
* half occupancy atoms: B-factor weighed by occupancy

Supplementary Table 5. DNA oligos

Primer name	Sequence
G23-1 K46:Kan FP	GCC GGC ATC ATC ACC ATA GAT GCC AAT AAC TGC TTC CGT TTG CGT AAA GAA CTT CAA GAT CCC CTC ACG CTG
G23-1 K46:Kan RP	CGC TGC ATA TGG CGA GCT TCA AAT ACA TCG CTC ACC AGC CCG TTC GCT TCA GAG CGC TTT TGA AGC TGG GG
G23-1 R45A Rec	GAT GCC AAT AAC TGC TTC CGT TTG GCT AAA GCG AAC GGG CTG GTG AGC GAT GTA TTT G
RelA D275G FP	GAT GAG CTG TTT GGT GTG CGT GCG
RelA D275G RP	CGC ACG CAC ACC AAA CAG CTC ATC
pTac EcoRI RP	CTG TTT CCT GTG TGA AAT TGT TAT CC
pTac HindIII FP	AAG CTT GGC TGT TTT GGC GGA TG
pET30 Sumo RP	ACC ACC AAT CTG TTC TCT GTG AGC
pET30 EcoRI FP	GAA TTC GAG CTC CGT CGA CAA G
pCfa NdeI RP	CAC GAA GAT CTG CAT ATG TAT ATC TCC TTC
pCfa Cys FP	TGC CTG TCT TAC GAC ACA GAG
G1-2 5 NdeI	CCT GGT GCC GCG CGG CAG CCA TAT GGA TGA CAA ACA ATG GGA GCG
G1-2 3' XhoI	GTG GTG GTG GTG GTG GTG CTC GAG CTA TTG TTG CTC GCT TCC TTT TTT CTT TC
G9-1 5' NdeI	CCT GGT GCC GCG CGG CAG TCA TAT GTC CAA GCA ACA GAT CGG C
G9-1 3' XhoI	GTG GTG GTG GTG GTG GTG CTC GAG TTA ATC CAG CCA TTC GGT ATG GAA C
G10-1 5' NdeI	CCT GGT GCC GCG CGG CAG CCA TAT GGA TCA GAC ATA TTC TCT GGA GTC
G10-1 3' XhoI	GTG GTG GTG GTG GTG GTG CTC GAG TTA AAT CAC ACC CTG CGC CAG
G13-1 5' NdeI	CCT GGT GCC GCG TGG TAG CCA TAT GGG GCA GGG TTT TCC AC
G13-1 3' XhoI	GTG GTG GTG GTG GTG GTG CTC GAG TTA CTT CAA CAC ATA ACC GTA CAA CC
G15-1 5' NdeI	CCT GGT GCC GCG CGG CAG CCA TAT GAT TAT CAA ACG CAC TCC TCA AG
G15-1 3' XhoI	GTG GTG GTG GTG GTG GTG CTC GAG CCG ATT CTG AAA CTT ACT TGC CAC
G19-1 5' NdeI	CCT GGT GCC GCG CGG CAG CCA TAT GAG CGA AAA ATA CAT CGT CAC C
G19-1 3' XhoI	GTG GTG GTG GTG GTG GTG CTC GAG TTA GCG ACC GGA GAT TGG C
G20-1 5' NdeI	CCT GGT GCC GCG CGG CAG CCA TAT GAA ACA TAC TGT AGA AGT AAT GAT CCC C
G20-1 3' XhoI	GTG GTG GTG GTG GTG GTG CTC GAG TTA CTC GTC CAG CAG AAT CAC TTT G
G21-1 5' NdeI	CCT GGT GCC GCG CGG CAG CCA TAT GAA GAT CGT GGA AGT CAA ACA CC
G21-1 3' XhoI	GTG GTG GTG GTG GTG GTG CTC GAG TTA TTT CGT ACC AAA GAT TTT GTC ACC G
G23-1 5' NcoI	GTT TAA CTT TAA GAA GGA GAT ATA CCA TGT GCG GTA TTG TCG GTA TCG
G23-1 3' XhoI	GTG GTG GTG GTG GTG GTG CTC GAA AAG GCA TCA TCC TTC GTT ATG C
G23-1 5' NdeI Cfa	CTT TAA GAA GGA GAT ATA CAT ATG TGC GGT ATT GTC GGT ATC G
G23-1 3' Cfa	CTC TGT GTC GTA AGA CAG GCA TCC TTC GTT ATG CAT TTC GAG ATT TTC
G24-1 5' NdeI	CCT GGT GCC GCG CGG CAG CCA TAT GAA ATT TCC CGG TAA ACG TAA ATC C
G24-1 3' XhoI	GTG GTG GTG GTG GTG GTG CTC GAG TTA ACG ATC CCA GTA AGA CTC TTC CAG
G25-1 5' Sumo	CAC AGA GAA CAG ATC GGT GGT GCT ACC AAT GCA AAA CCC GTC
G25-1 3' EcoRI	CTT GTC GAC GGA GCT CGA ATT CGA ATT ATT CCG TGA TTA AAG TCC CTT CTT TTT C
G26-1 5' NdeI	CCT GGT GCC GCG CGG CAG CCA TAT GAC TGC AAT TGC CCC GGT TAT TAC
G26-1 3' XhoI	GTG GTG GTG GTG GTG GTG CTC GAG TAC TGC AAA TTC GGT CGC TTA TGC
G27-1 5' NcoI	GTT TAA CTT TAA GAA GGA GAT ATA CCA TG GGT AAC AAC GTC GTC GTA CTG G
G27-1 3' XhoI	GTG GTG GTG GTG GTG GTG CTC GAG TAC CAG AAT TAC GCG TCG AAC G
G28-1 5' NdeI	CCT GGT GCC GCG TGG TAG CCA TAT GGA ATT ATC CTC ACT GAC CGC
G28-1 3' XhoI	GTG GTG GTG GTG GTG GTG CTC GAG TTT ATT TCA GCT CAT CAA CCA TCG TG
G29-1 5' NdeI	CCT GGT GCC GCG CGG CAG CCA TAT GCA AAA GCA AGC TGA GTT GTA TCG
G29-1 3' XhoI	GTG GTG GTG GTG GTG GTG CTC GAG AAC AGA AAA ATC AGT CCA GCT GTA CAC
G30-1 5' Sumo	CAC AGA GAA CAG ATT GGT GGT ATG CTA AGA ATC GCT AAA GAA GCT CTG
G30-1 3' EcoRI	CTT GTC GAC GGA GCT CGA ATT CTC AGG AGC CCA GAC GGT AG
G43-1 5' NdeI	CCT GGT GCC GCG CGG CAG TCA TAT GGA AAG TAA AGT AGT TGT TCC GGC
G43-1 3' XhoI	GTG GTG GTG GTG GTG GTG CTC GAG TTA CAT GTT TTC GAT GAT CGC GTC AC
G45-1 5' NdeI	CCT GGT GCC GCG CGG CAG CCA TAT GTC TGT AAT TAA GAT GAC CGA TCT GG
G45-1 3' XhoI	GTG GTG GTG GTG GTG GTG CTC GAG TGA TTT TTT ACT TCT TAG CGC GCT C

G46-1 5’ NdeI	CCT GGT GCC GCG CGG CAG TCA TAT GGC TGT AAC TAA GCT GGT TCT G
G46-1 3’ XhoI	GTG GTG GTG GTG GTG GTG CTC GAG AAT GAC GTT TAC TTC GCT TTA CCC
G53-1 5’ NdeI	CCT GGT GCC GCG CGG CAG CCA TAT GCG CAT TCA TAT TTT AGG AAT TTG TG
G53-1 3’ XhoI	GTG GTG GTG GTG GTG GTG CTC GAG AAT TAC TGC GCG GCT TCC G
G55-1 5’ NdeI	CCT GGT GCC GCG TGG CAG TCA TAT GAA AGT ATT AGT GAT TGG TAA CGG C
G55-1 3’ XhoI	GTG GTG GTG GTG GTG GTG CTC GAG TTA GTT CTG CTC GCG TTC G
G61-1 5’ NdeI	CCT GGT GCC GCG CGG CAG TCA TAT GGC TAT TGA ACG TAC TTT TTC CAT C
G61-1 3’ XhoI	GTG GTG GTG GTG GTG GTG CTC GAG AAA TTA TTA ACG GGT GCG CGG G
G62-15’ NdeI	CCT GGT TCC GCG CGG CAG TCA TAT GGC TCA AGG CAC GCT TTA TAT TG
G62-1 3’ XhoI	GTG GTG GTG GTG GTG GTG CTC GAG TTC AGT CTG CCA ACA ATT TGC TG
G23-1 R45A FP	CGT TTG GCT AAA GCG AAC GGG CTG GTG
G23-1 R45A RP	CCG TTC GCT TTA GCC AAA CGG AAG CAG TTA TTG G
G23-1 N48A FP	CGT TTG CGT AAA GCG GCC GGG CTG GTG AG
G23-1 N48A RP	CTC ACC AGC CCG GCC GCT TTA CGC AAA CG
G23-1 R58A FP	GCG ATG TAT TTG AAG CCG CCC ATA TGC AGC GTT TG
G23-1 R58A RP	CAA ACG CTG CAT ATG GGC TGC TTC AAA TAC ATC GC
G23-1 H59A FP	GTA TTT GAA GCT CGC GCT ATG CAG CGT TTG CAG
G23-1 H59A RP	CTG CAA ACG CTG CAT AGC ACG AGC TTC AAA TAC
G23-1 R62A FP	CCA TAT GCA GGC TTT GCA GGG CAA TAT GGG C
G23-1 R62A RP	CCC TGC AAA GCC TGC ATA TGG CGA GCT TCA AAT AC

Supplementary Table 6. Protein expression vectors

Vector backbone	Selection marker	Relevant features	Preparation
pET-nt	*kan*^{R}	No affinity tag	NcoI/XhoI double digestion of pET28b (Novagen)
pET28b	*kan*^{R}	N-His**$_6$** tag, thrombin cleavage site	NdeI/XhoI double digestion of pET28b (Novagen)
pSumo	*kan*^{R}	N-His$_6$-Sumo tag	Amplify pET30-His$_6$-Sumo-CfaN with primers "pET30 Sumo RP" and "pET30 EcoRI FP"
pCfa	Carb^R	C-Cfa-His$_6$ tag	Amplify pTXB1-Ub-Cfa with primers "pCfa NdeI RP" and "pCfa Cys FP"

Supplementary Table 7. Construction of protein expression plamids

Plasmid name	Vector backbone	Insert information			
		Protein	Species	Primers for insert amplification	
pG1-2	pET28b	YjbM	*B. subtilis*	G1-2 5 NdeI	G1-2 3' XhoI
pG9-1	pET28b	Gnd	*E. coli*	G9-1 5' NdeI	G9-1 3' XhoI
pG10-1	pET28b	GdhA	*E. coli*	G10-1 5' NdeI	G10-1 3' XhoI
pG13-1	pET28b	SpeC	*E. coli*	G13-1 5' NdeI	G13-1 3' XhoI
pG15-1	pET28b	FolC	*E. coli*	G15-1 5' NdeI	G15-1 3' XhoI
pG19-1	pET28b	Gpt	*E. coli*	G19-1 5' NdeI	G19-1 3' XhoI
pG20-1	pET28b	Hpt	*E. coli*	G20-1 5' NdeI	G20-1 3' XhoI
pG21-1	pET28b	Upp	*E. coli*	G21-1 5' NdeI	G21-1 3' XhoI
pG23-1Cfa	pCfa	PurF	*E. coli*	G23-1 5' NcoI	G23-1 3' XhoI
pG23-1nt	pET-nt	PurF	*E. coli*	G23-1 5' NdeI Cfa	G23-1 3' Cfa
pG24-1	pET28b	Gsk	*E. coli*	G24-1 5' NdeI	G24-1 3' XhoI
pG25-1Sumo	pSumo	PyrH	*E. coli*	G25-1 5' Sumo	G25-1 3' EcoRI
pG26-1	pET28b	Cmk	*E. coli*	G26-1 5' NdeI	G26-1 3' XhoI
pG27-1nt	pET-nt	PurA	*E. coli*	G27-1 5' NcoI	G27-1 3' XhoI
pG28-1	pET28b	PurB	*E. coli*	G28-1 5' NdeI	G28-1 3' XhoI
pG29-1	pET28b	PurC	*E. coli*	G29-1 5' NdeI	G29-1 3' XhoI
pG30-1Sumo	pSumo	GuaB	*E. coli*	G30-1 5' Sumo	G30-1 3' EcoRI
pG43-1	pET28b	Idh	*E. coli*	G43-1 5' NdeI	G43-1 3' XhoI
pG45-1	pET28b	Pgk	*E. coli*	G45-1 5' NdeI	G45-1 3' XhoI
pG46-1	pET28b	GpmA	*E. coli*	G46-1 5' NdeI	G46-1 3' XhoI
pG53-1	pET28b	Mpl	*E. coli*	G53-1 5' NdeI	G53-1 3' XhoI
pG55-1	pET28b	PurD	*E. coli*	G55-1 5' NdeI	G55-1 3' XhoI
pG61-1	pET28b	Ndk	*E. coli*	G61-15' NdeI	G61-1 3' XhoI
pG62-1	pET28b	Gmk	*E. coli*	G62-15' NdeI	G62-1 3' XhoI

Supplementary Table 8. Plasmids

Plasmid name	Relevant Genotype	Source
pKD46	P_{araB}-*gam-bet-exo*, *repA101*(ts)	31
pKD4	FRT:*kan*^{R}:FRT	31
pALS13	P_{Tac}-RelA(1-455)	32
R1-1His	P_{Tac}-RelA(1-455)-His_8	This study
pET28b		Novagen
pET30-His_6-Sumo-CfaN	P_{T7}-His_6-Sumo-CfaN	33
pTXB1-Ub-Cfa	P_{T7}-Ubiquitin-Cfa-His_6	33
G1-2	P_{T7}-His_6-YjbM^{Bst}	This study
G9-1	P_{T7}-His_6-Gnd^{Eco}	This study
G10-1	P_{T7}-His_6-GdhA^{Eco}	This study
G13-1	P_{T7}-His_6-SpeC^{Eco}	This study
G15-1	P_{T7}-His_6-FolC^{Eco}	This study
G19-1	P_{T7}-His_6-Gpt^{Eco}	This study
G20-1	P_{T7}-His_6-Hpt^{Eco}	This study
G21-1	P_{T7}-His_6-Upp^{Eco}	This study
G23-1Cfa	P_{T7}-PurF^{Eco}-Cfa-His_6	This study
G23-1nt	P_{T7}-PurF^{Eco}	This study
G24-1	P_{T7}-His_6-Gsk^{Eco}	This study
G25-1Sumo	P_{T7}-His_6-Sumo-PyrH^{Eco}	This study
G26-1	P_{T7}-His_6-Cmk^{Eco}	This study
G27-1nt	P_{T7}-PurA^{Eco}	This study
G28-1	P_{T7}-His_6-PurB^{Eco}	This study
G29-1	P_{T7}-His_6-PurC^{Eco}	This study
G30-1Sumo	P_{T7}-His_6-Sumo-GuaB^{Eco}	This study
G43-1	P_{T7}-His_6-Idh^{Eco}	This study
G45-1	P_{T7}-His_6-Pgk^{Eco}	This study
G46-1	P_{T7}-His_6-GpmA^{Eco}	This study
G53-1	P_{T7}-His_6-Mpl^{Eco}	This study
G55-1	P_{T7}-His_6-PurD^{Eco}	This study
G61-1	P_{T7}-His_6-Ndk^{Eco}	This study
G62-1	P_{T7}-His_6-Gmk^{Eco}	This study

Supplementary Table 9: Strains

Strain #	Background	plasmid	Source
ML006	MG1655		
ML2907	MG1655 *rpoZ* (WT)-*kanR* , *rpoC* (WT)-tet*AR*		17
ML2908	MG1655 *rpoZ*Δ2-5-*kanR, rpoC* R362A R417A K615A N680A K681A-*tetAR*		17
ML2909	F-, *glnX44*(AS), *λ*$^{-}$, *cysJ43*, *argA21*, *lysA22*, *rpsL104*, *malT1*(λ^{R}), *xyl-7*, *mtlA2*, *thiE1*		34
ML2912	MG1655 *purF* R46A		This study
ML2914	ML2907	pR1-1His	This study
ML2915	ML2907	pR1-1His(D275G)	This study
ML2916	ML2908	pR1-1His	This study
ML2917	ML2908	pR1-1His(D275G)	This study
ML2920	ML006	pR1-1His	This study
ML2921	ML006	pR1-1His(D275G)	This study
ML2924	ML2912	pR1-1His	This study
ML2926	BL21(DE3)	pG1-2	This study
ML2927	BL21(DE3)	pG9-1	This study
ML2928	BL21(DE3)	pG10-1	This study
ML2929	BL21(DE3)	pG13-1	This study
ML2930	BL21(DE3)	pG15-1	This study
ML2931	BL21(DE3)	pG19-1	This study
ML2932	BL21(DE3)	pG20-1	This study
ML2933	BL21(DE3)	pG21-1	This study
ML2934	BL21(DE3)	pG23-1Cfa	This study
ML2935	BL21(DE3)	pG23-1nt	This study
ML2940	BL21(DE3)	pG24-1	This study
ML2941	BL21(DE3)	pG25-1Sumo	This study
ML2942	BL21(DE3)	pG26-1	This study
ML2943	BL21(DE3)	pG27-1nt	This study
ML2944	BL21(DE3)	pG28-1	This study
ML2945	BL21(DE3)	pG29-1	This study
ML2946	BL21(DE3)	pG30-1Sumo	This study
ML2947	BL21(DE3)	pG43-1	This study
ML2948	BL21(DE3)	pG45-1	This study
ML2949	BL21(DE3)	pG46-1	This study
ML2950	BL21(DE3)	pG53-1	This study
ML2951	BL21(DE3)	pG55-1	This study
ML2952	BL21(DE3)	pG61-1	This study
ML2953	BL21(DE3)	pG62-1	This study

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