1   **Supplementary information**
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3   **Supplementary Table 1. X-ray data collection, phase and structure refinement**
4   **statistics.**
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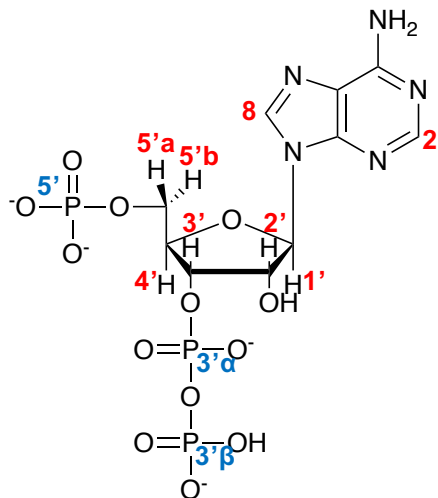|	Tas1$_{tox}$-Tis1 complex (6OX6)	PurF^{EC}-ppApp complex (6OTT)
Data Collection		
Space group	P4$_3$2$_1$2	C222$_1$
Cell dimensions		
a, b, c (Å)	66.4, 66.4, 147.9	115.2, 156.8, 107.5
α, β, γ (°)	90.0, 90.0, 90.0	90.0, 90.0, 90.0
Wavelength (Å)	0.979	0.979
Resolution (Å)	50.00 – 2.17	47.6-2.55
R$_{merge}$[a]	0.083 (0.243)*	0.106 (1.27)
I / σ(*I*)	33.4 (6.2)	9.0 (1.0)
Completeness (%)	98.0 (96.4)	99.8 (100)
Redundancy	9.2 (4.1)	5.8 (6.2)
Refinement		
Resolution (Å)	49.6 – 2.17	47.6 – 2.55
No. reflections		
Total	18200	186253
Unique	17596	32044
Free (%)	5.00	6.23
R$_{work}$ / *R*$_{free}$ (%)[b]	18.5/23.1	22.3/26.1
No. atoms		
Protein	2119	15508
Ligand/ion	28	28
Water	316	0
B-factors (Å^2)		
Protein	40.8	74.6
Ligand/ion	72.0	61
Water	53.7	-
r.m.s deviations		
Bond lengths (Å)	0.006	0.02
Bond angles (°)	0.993	0.547

6   Single crystals were used to collect data for each structure. *values in brackets value refer
7   to highest resolution shells.
8   [a]$R_{merge} = \Sigma_{hkl}\Sigma_j|I_{hkl.j} - \langle I_{hkl}\rangle|/\Sigma_{hkl}\Sigma_jI_{hk.j}$, where $I_{hkl.j}$ and $\langle I_{hkl}\rangle$ are the *j*th and mean measurement
9   of the intensity of reflection *j*.
10   [b]$R = \Sigma|F_p^{obs} - F_p^{calc}|/\Sigma F_p^{obs}$, where F_p^{obs} and F_p^{calc} are the observed and calculated structure
11   factor amplitudes, respectively.
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1 **Supplementary Table 2. ^{1}H and ^{31}P NMR assignments for pApp.** The chemical shifts
2 (in ppm) for pApp. α-proton of the pyrophosphate group was identified from its ddd pattern
3 with a characteristic J-coupling constant with the proximal ^{31}P nuclei at 8.5Hz (0.014 ppm
4 for protons or 0.036ppm for ^{31}P). This proton was assigned to the 3′ location by comparing
5 proton chemical shifts to those observed for adenosine-5′-phosphate (AMP, BMRB
6 database ID: BMSE000837) and a chemically synthesized adenosine-3′, 5′-bisdiphosphate
7 (ppApp) under the same condition[1]. Note that compared to a free hydroxyl group,
8 pyrophosphorylation increases the chemical shift of the α-proton by 0.3-0.4 ppm.
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		Base (^{1}H)		Ribose (^{1}H)					Phosphate (^{31}P)		
	Position	2	8	1′	2′	3′	4′	5′a / 5′b	5′α	3′α	3′β
pApp	Chemical Shift (ppm)	8.15	8.59	6.16	4.88	4.93	4.55	4.02	3.75	-10.00	-5.06
	Integration (multiplicity)	1H (s)	1H (s)	1H (d)	1H (dd)	1H (ddd)	1H (m)	2H (m)	1P (s)	1P (dd)	1P (d)
ppApp	Chemical Shift (ppm)	8.19	8.48	6.12	4.79	4.90	4.54	4.16	-	-	-
	Integration (multiplicity)	1H (s)	1H (s)	1H (d)	1H (dd)	1H (ddd)	1H (m)	2H (m)	-	-	-
AMP	Chemical Shift (ppm)	8.13	8.48	6.09	4.75	4.51	4.38	4.10	-	-	-
	Integration (multiplicity)	1H (s)	1H (s)	1H (d)	1H (dd)	1H (dd)	1H (m)	2H (m)	-	-	-

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28 *   *"-" indicates assignment not clear or data not available.*
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Supplementary Table 3. Absolute nucleotide quantification of *P. aeruginosa* cells undergoing Tas1-mediated intoxication.

Metabolite		Concentration in Tis1 depleted cells (nmol/OD)				Concentration in Tis1 expressing cells (nmol/OD)		
		time (min)				time (min)		
		0	30	60	120	0	30	60
AMP								
	R1	0.179	0.193	0.238	0.315	0.253	0.323	0.317
	R2	0.151	0.172	0.207	0.236	0.369	0.326	0.342
	R3	0.218	0.213	0.170	0.284	0.365	0.350	0.490
ADP								
	R1	0.547	0.441	0.387	0.330	0.631	0.747	0.652
	R2	0.507	0.407	0.379	0.232	0.700	0.634	0.644
	R3	0.731	0.537	0.340	0.346	0.707	0.681	0.667
ATP								
	R1	2.716	2.091	1.462	0.939	2.648	2.484	2.096
	R2	3.212	2.054	1.299	0.614	2.042	2.281	2.000
	R3	3.191	1.992	1.264	0.916	2.495	2.221	1.641
ppApp								
	R1	0.128	1.020	1.186	1.154	n.d.	n.d.	n.d.
	R2	0.126	1.095	1.073	0.773	n.d.	n.d.	n.d.
	R3	0.154	1.223	1.105	1.226	n.d.	n.d.	n.d.
pppApp								
	R1	0.355	3.588	3.964	3.768	n.d.	n.d.	n.d.
	R2	0.576	3.986	3.763	2.609	n.d.	n.d.	n.d.
	R3	0.588	4.628	3.184	3.909	n.d.	n.d.	n.d.
GMP								
	R1	0.077	0.021	0.032	0.034	0.072	0.093	0.080
	R2	0.048	0.019	0.022	0.003	0.090	0.094	0.068
	R3	0.050	0.074	0.031	0.048	0.098	0.076	0.091
GDP								
	R1	0.132	0.113	0.096	0.102	0.197	0.233	0.184
	R2	0.141	0.115	0.085	0.065	0.203	0.219	0.173
	R3	0.171	0.141	0.082	0.093	0.210	0.226	0.163
GTP								
	R1	0.874	0.748	0.490	0.340	1.095	0.972	0.749
	R2	1.060	0.742	0.410	0.249	0.785	0.907	0.652
	R3	0.928	0.703	0.433	0.335	0.916	0.799	0.526
UTP								
	R1	1.548	1.140	0.719	0.393	2.111	2.235	1.780
	R2	1.780	1.192	0.590	0.284	1.745	2.046	1.779
	R3	1.498	1.134	0.642	0.411	1.870	1.774	1.259
dATP								
	R1	0.147	0.126	0.088	0.063	0.114	0.108	0.082
	R2	0.164	0.120	0.078	0.041	0.093	0.090	0.080

R3	0.163	0.116	0.073	0.057	0.102	0.088	0.065
dCTP							
R1	0.233	0.152	0.100	0.057	0.206	0.189	0.146
R2	0.258	0.159	0.082	0.040	0.167	0.168	0.143
R3	0.224	0.157	0.087	0.057	0.189	0.158	0.108
dTTP							
R1	0.264	0.220	0.168	0.090	0.241	0.228	0.222
R2	0.306	0.224	0.150	0.072	0.202	0.199	0.216
R3	0.297	0.222	0.143	0.106	0.228	0.195	0.178
NAD^+							
R1	0.676	0.896	0.814	0.746	0.572	0.577	0.554
R2	0.675	0.837	0.841	0.628	0.542	0.531	0.562
R3	0.760	0.868	0.787	0.677	0.583	0.543	0.598
$NADP^+$							
R1	0.272	0.359	0.309	0.331	0.241	0.216	0.196
R2	0.292	0.328	0.310	0.264	0.213	0.222	0.210
R3	0.308	0.355	0.310	0.338	0.223	0.199	0.185
UDP-GlcNAc							
R1	0.313	0.215	0.154	0.121	0.364	0.394	0.372
R2	0.353	0.204	0.135	0.117	0.326	0.390	0.354
R3	0.432	0.187	0.140	0.108	0.343	0.404	0.389
IMP							
R1	0.010	0.008	0.007	0.004	0.024	0.024	0.017
R2	0.011	0.010	0.007	n.d.	0.031	0.016	0.014
R3	0.011	0.015	0.008	n.d.	0.028	0.015	0.007
dGTP/pApp							
R1	0.129	0.156	0.130	0.108	0.169	0.096	0.089
R2	0.140	0.159	0.119	0.084	0.105	0.088	0.119
R3	0.105	0.203	0.137	0.132	0.124	0.086	0.108

1 *NADH and NADPH were not detected due to instability during extraction procedure and
2 storage conditions for extracts.
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Supplementary Table 4. Genotypes of *P. aeruginosa* and *E. coli* strains used in this study.

Organism	Genotype	Description	Reference
P. aeruginosa PAO1	wild-type		[2]
	ΔPA4856	*retS* deletion strain	This study
	ΔPA4856 attB::*lacZ*	*retS* deletion strain, constitutive *lacZ* expression strain, Tet[R]	This study
	ΔPA4856 ΔPA0093	*retS tse6* deletion strain	This study
P. aeruginosa PA14	wild-type		[3]
	ΔPA14_		
	ΔPA14_52570	*rsmA* deletion strain	This study
	ΔPA14_52570 ΔPA14_68450	*rsmA rsmF* deletion strain	This study
	ΔPA14_52570 ΔPA14_68450 attB::*lacZ*	*rsmA rsmF* deletion strain, constitutive *lacZ* expression strain, Tet[R]	This study
	ΔPA14_52570 ΔPA14_68450 ΔPA14_01100	*rsmA rsmF clpV1* deletion strain	This study
	ΔPA14_52570 ΔPA14_68450 ΔPA14_01140	*rsmA rsmF tas1* deletion strain	This study
	ΔPA14_52570 ΔPA14_68450 ΔPA14_01120	*rsmA rsmF tsi6* deletion strain	This study
	ΔPA14_52570 ΔPA14_68450 ΔPA14_01140 ΔPA14_01130 attB::*lacZ*	*rsmA rsmF tas1 tis1* deletion strain	This study
	ΔPA14_52570 ΔPA14_68450 ΔPA14_01140 ΔPA14_01130 ΔPA14_01120 attB::*lacZ*	*rsmA rsmF tas1 tis1 tsi6* deletion strain	This study
	ΔPA14_52570 ΔPA14_68450 ΔPA14_01150	*rsmA rsmF eagT6* deletion strain	This study
	ΔPA14_52570 ΔPA14_68450 ΔPA14_01110	*rsmA rsmF vgrG1* deletion strain	This study
	ΔPA14_52570 ΔPA14_68450 ΔPA14_01160	*rsmA rsmF vgrG2* deletion strain	This study
	ΔPA14_52570 ΔPA14_68450 ΔPA14_29390	*rsmA rsmF vgrG4* deletion strain	This study
	ΔPA14_64230	*retS* deletion strain	This study

	ΔPA14_64230 ΔPA14_57520	*retS sspB* deletion strain	This study
	ΔPA14_64230 ΔPA14_57520 PA14_01130-DAS+4	*retS sspB* deletion strain expressing Tis1 with a C-terminal DAS+4 tag	This study
	ΔPA14_52570 ΔPA14_68450 PA14_01140-VSV-G	*rsmA rsmF* deletion strain expressing Tas1 with a C-terminal VSV-G tag	This study
	ΔPA14_52570 ΔPA14_68450 PA14_01140-E382A	*rsmA rsmF* deletion strain expressing Tas1[E382A]	This study
E. coli MG1655	wild-type		[4]
E. coli SM10 λpir	Km[R], *thi-1 thr leu tonA lac Y supE recA*::RP4-2-Tc::Mu, pir	Conjugation strain	BioMedal LifeScience
E. coli XL-1 Blue	*recA1 endA1 gyrA96 thi-1 hsdR17 supE44 relA1 lac* [F′ *proAB lacI*q Z∆*M15* Tn*10* (Tet[R])]	Cloning strain	Agilent
E. coli BL21 (DE3) CodonPlus	F[-] *ompT gal dcm lon hsdS*$_B$(r$_B$[-] m$_B$[-]) λ(DE3) pLysS(Cm[R])	Protein expression strain	Novagen

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Supplementary Table 5. Plasmids used for allelic exchange, protein expression and toxicity assays.

Plasmid	Relevant features	Reference
pEXG2	Allelic replacement vector containing *sacB*, Gm^R	[5]
pPSV38-CV	Expression vector with *lacI*, *lacUV5* promoter, C-terminal VSV-G tag, Gm^R	[6]
pPSV39-CV	Expression vector with *lacI*, *lacUV5* promoter, C-terminal VSV-G tag, Gm^R	[7]
pSCrhaB2-CV	Expression vector with *PrhaB*, Tmp^R	[8]
pETDuet-1	Co-expression vector with *lacI*, T7 promoter, N-terminal His$_6$ tag in MCS-1, Amp^R	Novagen
pET28b	Expression vector with *lacI*, T7 promoter, C-terminal His$_6$ tag, Amp^R	Novagen
pCfa	Expression vector with *lacI*, T7 promoter, C-terminal Cfa-His$_6$ intein tag, Amp^R	[9]
pKSV45-Amp^R	Expression vector with *tetR* and *ptet* promoter, replaced *kan^R* with *amp^R*	[10]
pALS13	Expression vector for (p)ppGpp synthetase fragment of RelA 1-455 (RelA′)	[11]
pSW196	MiniCTX1 plasmid, Tet^R	[12]
pEXG2::ΔPA0905	*rsmA* deletion construct for PAO1	This study
pEXG2::ΔPA5182	*rsmF* deletion construct for PAO1	This study
pEXG2::ΔPA0093	*tse6* deletion construct	This study
pEXG2::ΔPA0090	*clpV1* deletion construct for PAO1	This study
pEXG2::ΔPA14_52570	*rsmA* deletion construct for PA14	This study
pEXG2::ΔPA14_68450	*rsmF* deletion construct for PA14	This study
pEXG2::ΔPA14_01100	*clpV1* deletion construct for PA14	This study
pEXG2::ΔPA14_01140	*tas1* deletion construct	This study
pEXG2::ΔPA14_01120	*tsi6* deletion construct	This study
pEXG2::ΔPA14_01140-01130	*tas1 tis1* deletion construct	This study
pEXG2::ΔPA14_01140-01120	*tas1 tis1 tsi6* deletion construct	This study
pEXG2::ΔPA14_01150	*eagT6* deletion construct	This study
pEXG2::ΔPA14_01110	*vgrG1* deletion construct	This study
pEXG2::ΔPA14_01160	*vgrG2* deletion construct	This study
pEXG2::ΔPA14_29390	*vgrG4* deletion construct	This study

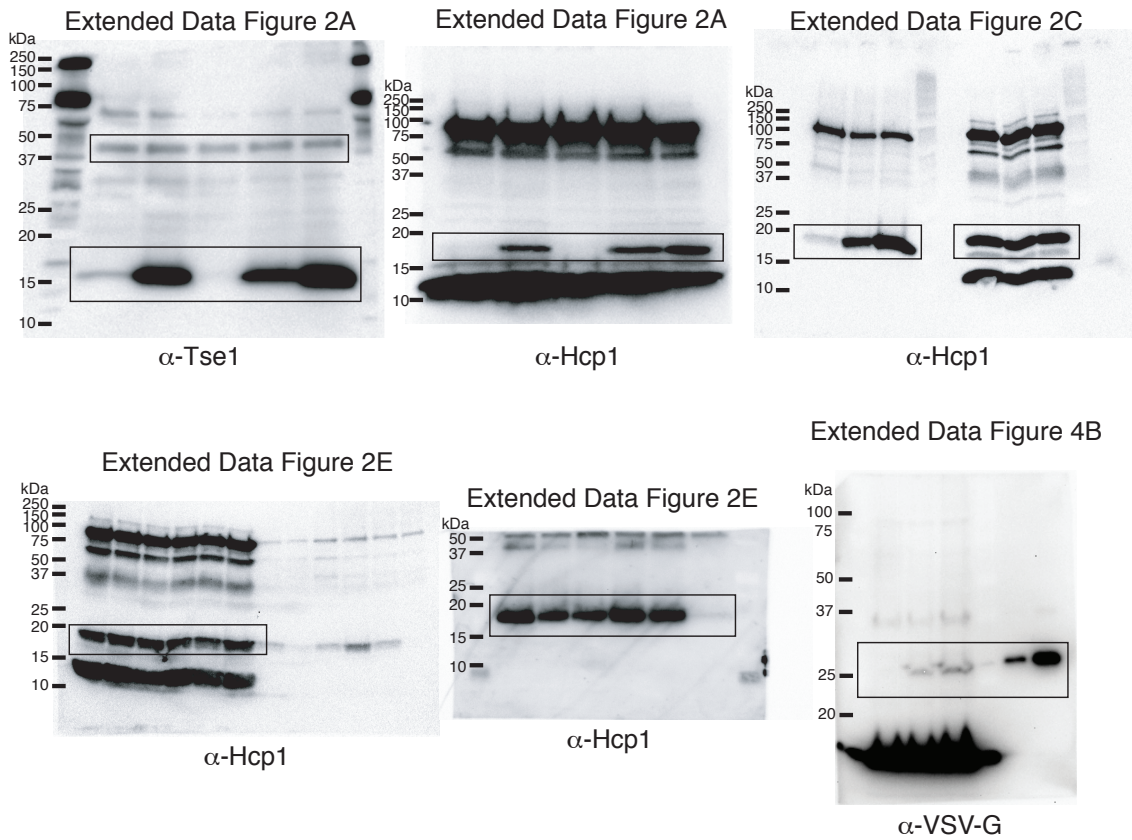|		
pEXG2::ΔPA14_64230	*retS* deletion construct	This study
pEXG2::ΔPA14_57520	*sspB* deletion construct	This study
pEXG2::PA14_01130_DAS+4	For generating strains encoding Tis1 fused to a C-terminal DAS+4 tag (AANDENYSENYADAS)	This study
pEXG2::PA14_01140-VSV-G	For generating strains encoding Tas1 fused to a C-terminal VSV-G epitope tag (YTDIEMNRLGK)	This study
pEXG2::PA14_01140_E382A	For generating strains encoding Tas1^{E382A}	This study
pPSV39-CV::PA14_01130	Expression vector for *tas1*	This study
pPSV39-CV::PA14_01120	Expression vector for *tsi6*	This study
pPSV39-CV::PA14_57520	Expression vector for *sspB*	This study
pPSV39-CV::PA5338	Expression vector for *spoT*	This study
pSCrhaB2-CV::PA14_01140_251-CT	Expression vector for the C-terminal toxin domain of Tas1 (Tas1$_{tox}$)	This study
pSCrhaB2-CV::PA14_01140_251-CT_K305A	Expression vector for Tas1$_{tox}$^{K305A}	This study
pSCrhaB2-CV::PA14_01140_251-CT_K313A	Expression vector for Tas1$_{tox}$^{K313A}	This study
pSCrhaB2-CV::PA14_01140_251-CT_K326A	Expression vector for Tas1$_{tox}$^{K326A}	This study
pSCrhaB2-CV::PA14_01140_251-CT_D327A	Expression vector for Tas1$_{tox}$^{D327A}	This study
pSCrhaB2-CV::PA14_01140_251-CT_R330A	Expression vector for Tas1$_{tox}$^{R330A}	This study
pSCrhaB2-CV::PA14_01140_251-CT_E382A	Expression vector for Tas1$_{tox}$^{E382A}	This study
pSCrhaB2-CV::PA14_01140_251-CT_E382D	Expression vector for Tas1$_{tox}$^{E382D}	This study
A1-2	Expression vector for Tas1$_{tox}$ with N-terminal His$_6$ and C-terminal VSV-G epitope	This study
pETDuet-1::His$_6$_PA14_01140_251-CT :: PA14_01130	Co-expression vector for Tas1$_{tox}$ with N-terminal His$_6$ and Tis1	This study
pETDuet-1::His$_6$_PA14_01140_251-CT_VSV-G:: PA14_01130	Co-expression vector for Tas1$_{tox}$ with N-terminal His$_6$ and C-terminal VSV-G and Tis1	This study
pETDuet-1::PA5338_1-387_His$_6$	Expression vector for the SpoT$_{1-387}$ fragment with C-terminal His$_6$	This study
pET28b:: D8B36_07150	Expression vector for PurF^{EC}	9
pET28b:: D8B36_21350	Expression vector for PurD^{EC}	9
pCfa:: D8B36_07150	Expression vector for PurF^{EC}	9
pCfa:: D8B36_07150_R62A	Expression vector for PurF$^{EC\ R62A}$	9

| pCfa:: PA14_23290 | Expression vector for PurF[PA] | This study |
| pSW196:: *lacZ* | *lacZ* in miniCTX1 plasmid | [13] |

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1 **Supplementary Figure 1. Source gels.** Uncropped blots for Extended Data figures.

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Extended Data Figure 2A

α-Tse1

Extended Data Figure 2A

α-Hcp1

Extended Data Figure 2C

α-Hcp1

Extended Data Figure 2E

α-Hcp1

Extended Data Figure 2E

α-Hcp1

Extended Data Figure 4B

α-VSV-G

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