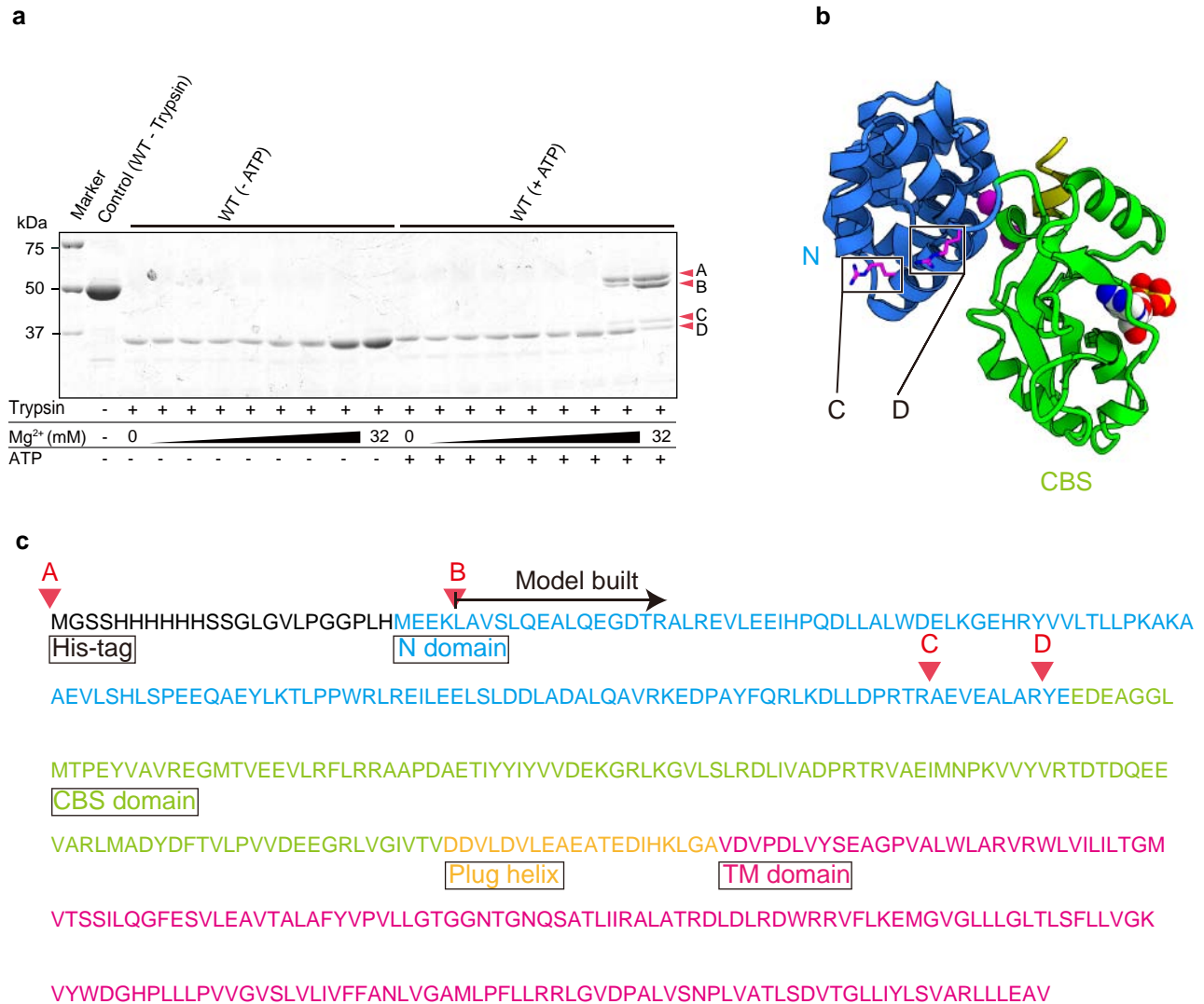


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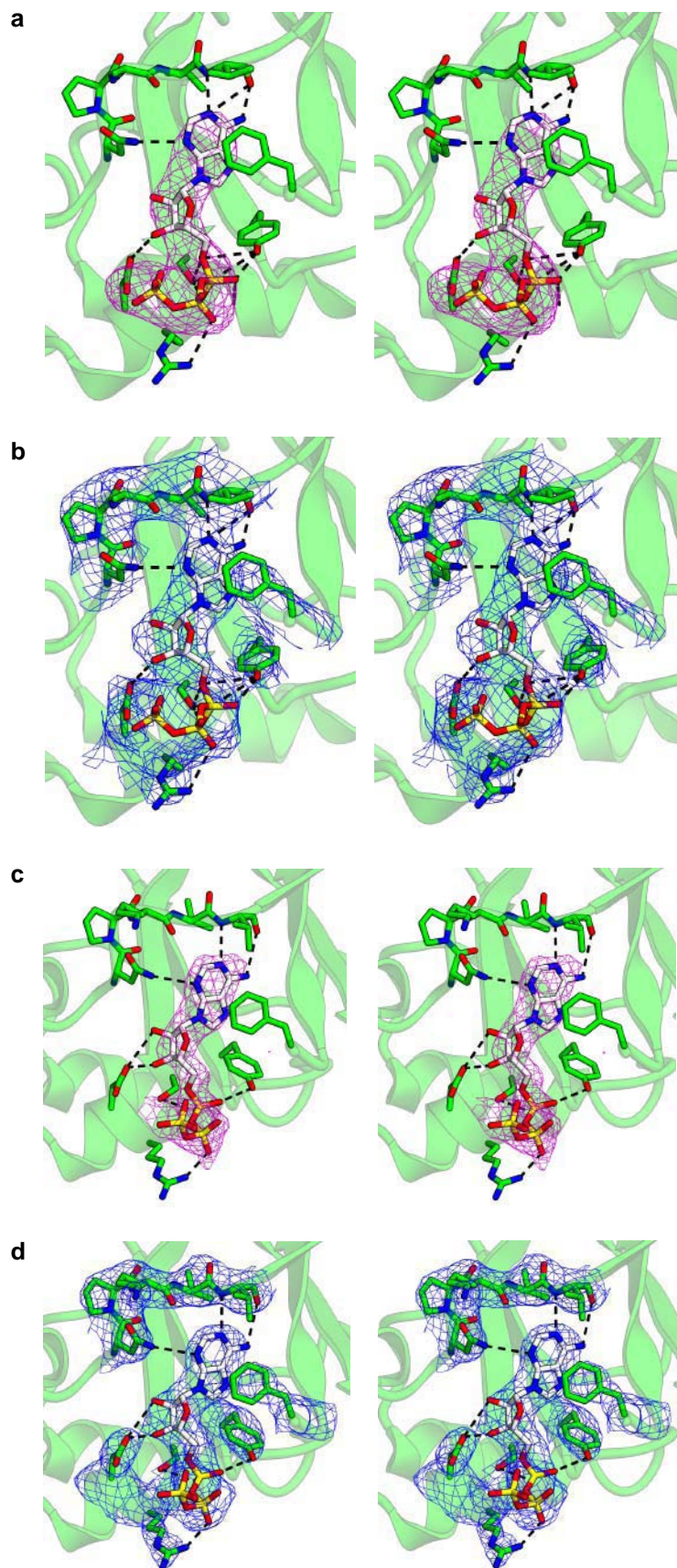
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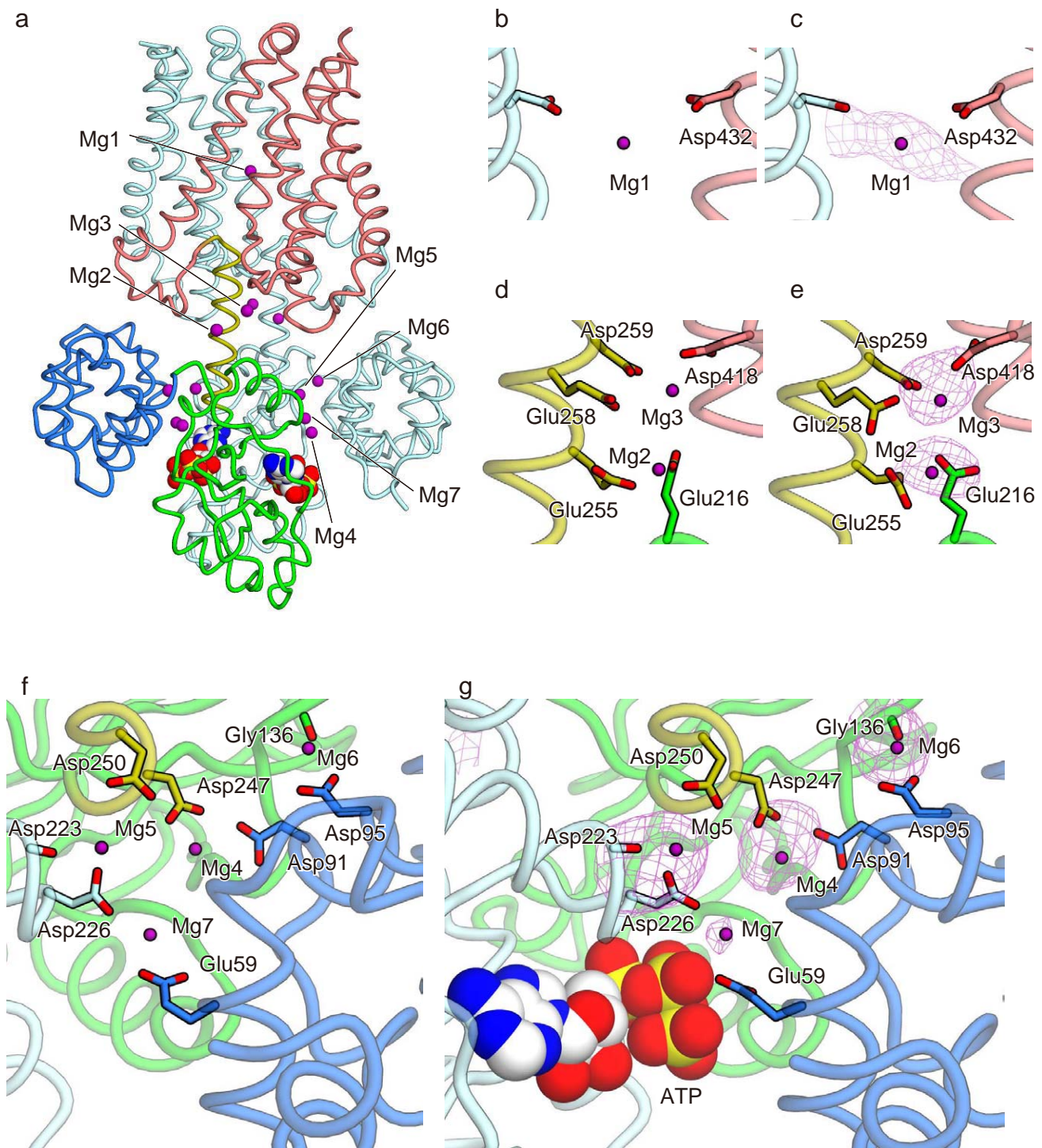
### Supplementary Figure 1 | Cleavage sites of limited proteolysis.

(a) Protease protection of MgtE by  $Mg^{2+}$  and nucleotides, from Figure 1b. Arrows with letters indicate the four limited proteolysis products. (b) Ribbon representation of the MgtE cytosolic domain in complex with ATP. The coloring scheme is the same as that in Figure 2. ATP and  $Mg^{2+}$  ions are shown as CPK models. The arginine residues followed by the cleavage sites C and D are shown as stick models. (c) The amino acid sequences of MgtE and the cleavage sites are shown. Red arrows with letters indicate the cleavage sites.



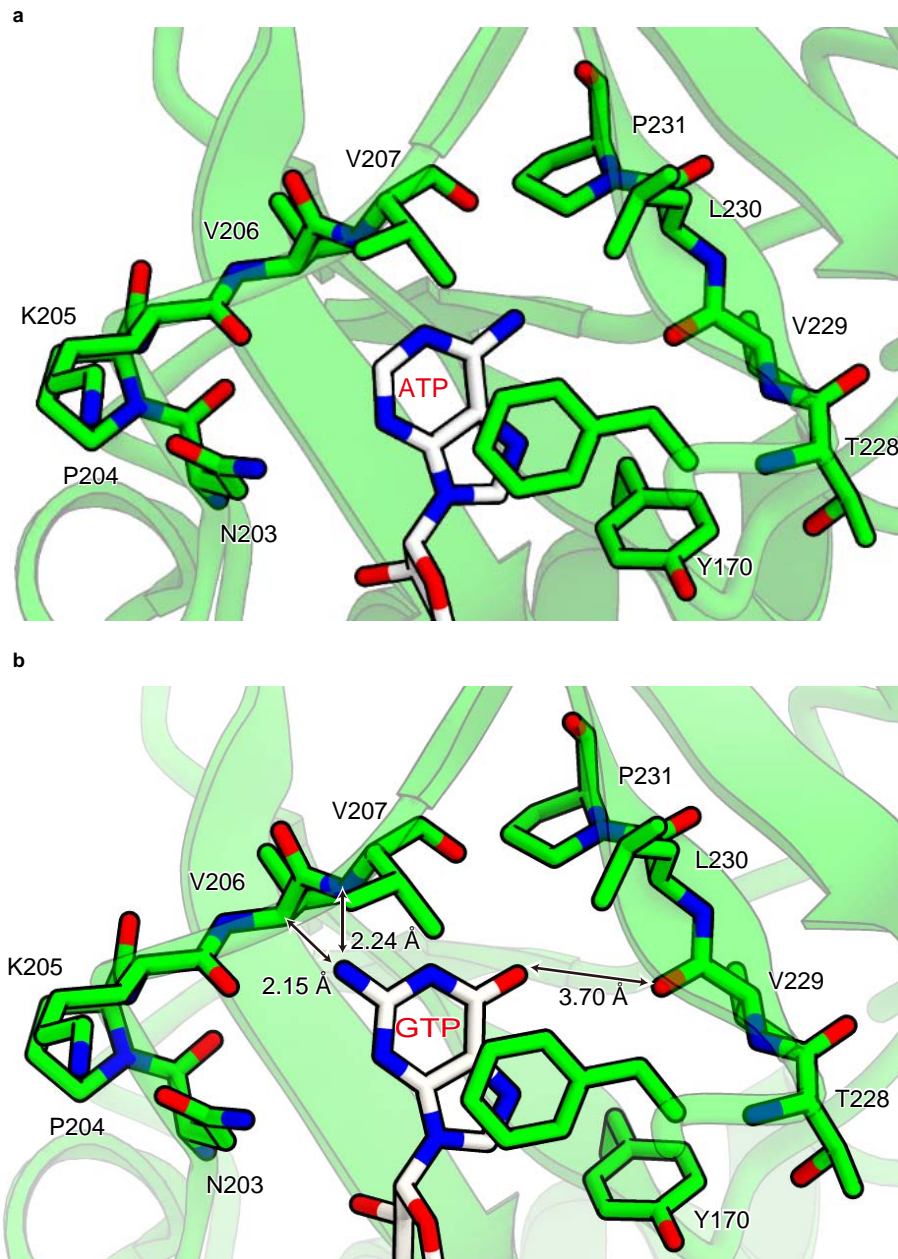
**Supplementary Figure 2 | Electron density for ATP.**

(a-b) Electron density maps for ATP in the full-length MgtE structure. The omit *Fo-Fc* maps (contoured at  $3.0\sigma$ , green) (a) and the  $2\text{ }Fo-Fc$  maps (contoured at  $1.0\sigma$ , blue) (b) are shown. (c-d) Electron density maps for ATP in the cytosolic domain structure. The omit *Fo-Fc* maps (contoured at  $3.0\sigma$ , green) (c) and the  $2\text{ }Fo-Fc$  maps (contoured at  $1.0\sigma$ , blue) (d) are shown.



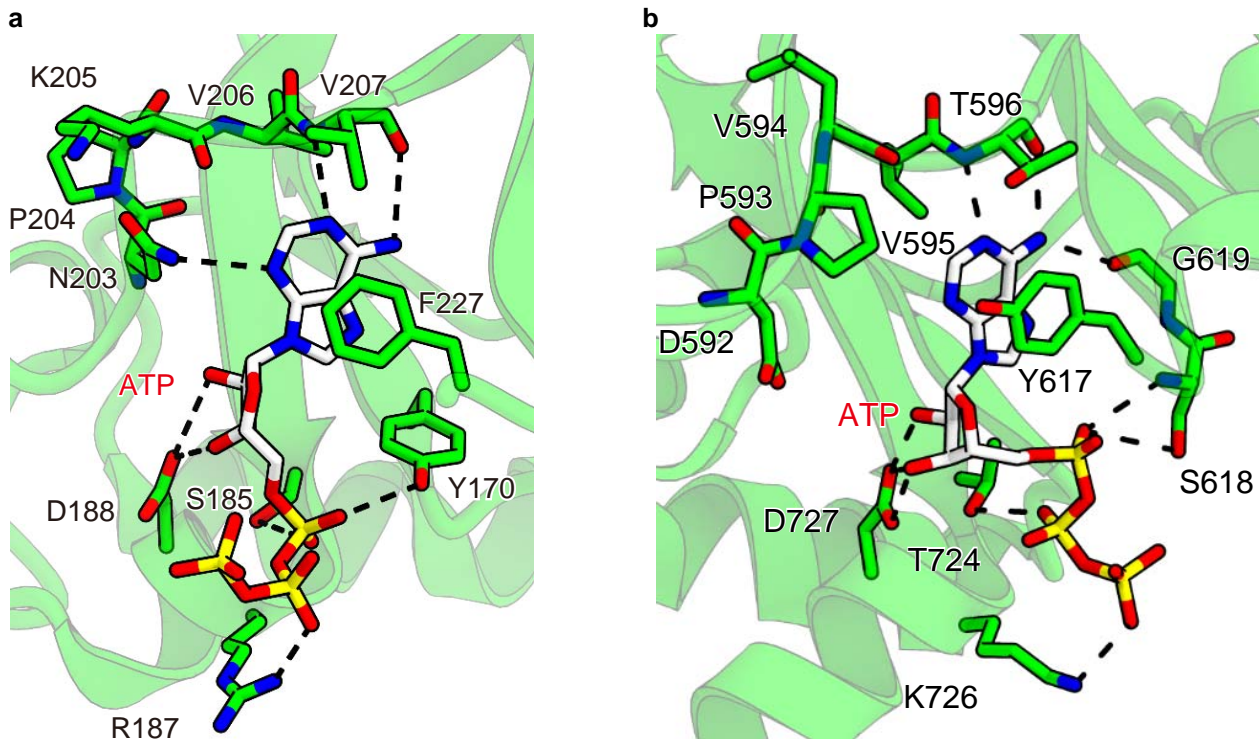
**Supplementary Figure 3 | Comparison of the Mg<sup>2+</sup> binding site of the full-length MgtE-ATP complex with the previously determined structure.**

(a) Side view of the overall structure of the full-length MgtE-ATP complex with bound Mg<sup>2+</sup>. (b-d) Close-up views of the respective Mg<sup>2+</sup> [Mg1-7] binding sites in the previously determined structure of full-length MgtE. (e)-(g) Close-up views of the respective Mg<sup>2+</sup> [Mg1-7] binding sites in the full-length MgtE-ATP complex, with *F<sub>o</sub>-F<sub>c</sub>* omit maps contoured at 2.5σ, calculated with the full-length MgtE-ATP complex excluding Mg<sup>2+</sup>.



**Supplementary Figure 4 | Nucleotide base specificity.**

Stick representation of the ATP binding site in the MgtE cytosolic domain structure in the complex with ATP (a) and with a superimposed model of GTP (b). Distances between the N2 atom of guanine and the C $\alpha$  atom of Val206, the N2 atom of guanine and the N atom of Val207, and the O6 atom of guanine and the O atom of Val229 are shown.



**Supplementary Figure 5 | Structural comparison of CBS domains.**

Structural comparison of the CBS domains of (a) MgtE and (b) human CLC-5 (PDB ID: 2J9L). The ATP binding motif (especially Arg187, Asp188, and Phe227 of MgtE) is well conserved between both domains. The Arg187, Asp188 and Phe227 of MgtE correspond to the Lys726, Asp727 and Tyr617 of human CLC-5.

		1	10	20
T.therm	.....	MEEKLAVS	EQE.ALQEG	...DTRALQEV
T.potens JR	ME.....	F...VKSVPNDKEQILAAIKS.Q	INTH...	REVDLKKM
D.alkalitolerans	.....	MALFNGTFDA	IVL.NLSKG	...NKEKLR
E.faecalis	.....	MNEGQEMEEQFAL	LE.TLKNQ	...QMNFR
S.aureus	MS.....	MNTDEKERVQEELYDETL	LQD.YLEND	...DIDQFR
L.xylanilyticus	.....	MIEERKEKDDVQFDEAR	LRE.MLEAH	...EINAF
E.saphenumc	.....	MEDILTHEGEADERREK	FQA.FIDER	...NFR
Spirochaeta sp. JC230	.....	MSNLREK	KE.YLHLM	...DQDD
M.rufus	.....	MYQ TENREIKA	LEA.ALAEM	...DAFR
M.hydrothermalis	.....	MRTLRENLFEL	LRT.ALEQG	...DIPK
V.cholerae	.....	MAEQI.EFDQAHLLTQE	ITE.ALDNG	...RFVH

N domain

	30	40	50	60	70	80				
T.therm	LEE	VHPQDL	LALWDE	LEGEHR	RYVV	LTLPKAKAAE	VLSHSP	EQE..AEY	LKTP	PPWRLR
T.potens JR	LEE	LHPADIA	AEIIES	LAEKQ	RVS	VFLLSSEKAA	AVLYELNS	E..IVAP	LMESL	DKSIAA
D.alkalitolerans	FDE	IHPYDQA	QILMEL	LSAEHR	RLADFL	TDEEMAE	IIQEL	SIQQ..KL	IIDE	LGIERY
E.faecalis	FLA	LHIYE	QGFYQS	LDEKDR	QHLYNY	LSPKELAD	DMFDV	IEED	NENMKD	YLAE
S.aureus	FLA	LHTYE	QSEYFED	TDEN	RQKIF	PQYLSPEE	VANFFDQ	LDID	DDDEYEL	LFDKM
L.xylanilyticus	FLE	LHPYDQA	TFYEK	VEPDI	RKII	YSFLSPAEMAD	IFEAE	IEDD	DEYTA	LAEM
E.saphenumc	LST	LNVVDI	ANLIDG	LDPQT	GV	ALYRMLPKDVA	AEVFAK	FDPE	QQ..EAI	IYAS
Spirochaeta sp. JC230	LNE	LNSTE	LELWHD	MNDEE	ASEF	LLLSEKRV	VEFLTH	LPEGDQ	..EW	LLLS
M.rufus	LEE	LYPAQIL	LEHWPK	LSAEHR	RVV	LTLPSPDAA	EVFSL	EEAEQ	..AE	LLEAL
M.hydrothermalis	AQT	LHPREIL	DRWAE	LPEYR	YV	LTLPDPDAA	IFAN	LEAADQ	..AE	FLET
V.cholerae	LQD	MEPE	DI	AHLLEAS	PRKAR	EV	LWQLT	DPE	DYGE	ILDE

N domain

		Mg4	Mg6				Mg6															
	90	100	110	120	130	140																
T.therm	EIL	EE	LSL	DDLADAL	QAV	RRKED	PAYFQR	LKDL	DP	PR	TR	AE	VE	EA	LAR	YE	ED	AG	GM	MT	PE	
T.potens JR	EIM	DEM	ST	DDAADII	GDL	KDE...	DKAK	LLNL	ME	YAD	AED	VQ	EL	MP	Y	PE	ED	AG	GM	MT	PE	
D.alkalitolerans	KI	I	VE	MPS	DDAADLL	GDL	DED...	RLNQ	VL	DL	MG	DKE	EA	DL	RE	LM	Y	PE	ED	AG	GM	MT
E.faecalis	DM	LA	EM	YTD	NAVDLL	NML	DKS...	QKAKY	LS	LL	SSE	EAGE	IK	EL	LY	VE	DE	AG	GM	MT	PE	
S.aureus	HIL	EE	MS	YD	NAVDIL	NE	LTKP...	KVAS	LL	TL	MNK	DDANE	IK	AL	LH	Y	DE	ED	AG	GM	MT	
L.xylanilyticus	EM	LS	MYA	DDAADV	LNE	LDTK...	QRESYL	GM	DE	E	TAE	IN	EL	LS	Y	DE	ED	AG	GM	MT	PE	
E.saphenumc	ELI	ED	MFL	DD	V	DLLE	EM	PSG...	V	SR	I	LE	NT	GH	S	DR	SM	IN	Q	L	K	
Spirochaeta sp. JC230	EIL	MR	LEP	DDLADII	QAI	T	SPE...	V	RES	V	W	NS	L	S	DE	A	K	Q	E	T	R	
M.rufus	EIL	EE	LSL	DDLADTI	NAV	VEE	ENS	PE	AAQ	AL	R	Q	L	D	P	E	T	R	A	E	V	
M.hydrothermalis	ELL	EAL	LD	DD	L	DAL	QAL	Q	E	Q	N	P	Q	L	A	Q	E	L	I	A	S	
V.cholerae	EAT	E	G	M	D	I	DD	V	Y	V	L	R	S	L	P	D	D	...	V	S	R	

N domain

CBS domain

				R187	D188
	150	160	170	180	190
T.therm	YVA	VRE	EGM	TVEE	VL
T.potens JR	YVA	I	REDI	T	VER
D.alkalitolerans	YIV	L	PEDF	T	ADE
E.faecalis	FVS	I	VANQ	T	VRS
S.aureus	YLS	L	KAHT	P	VKE
L.xylanilyticus	YVA	I	PENS	T	VRS
E.saphenumc	YIS	L	KKEM	T	VSS
Spirochaeta sp. JC230	YVA	L	RSNI	T	VQA
M.rufus	YIA	V	DDMR	V	VEE
M.hydrothermalis	FIA	V	RASM	T	VEE
V.cholerae	VIT	I	R	ADV	D

CBS domain

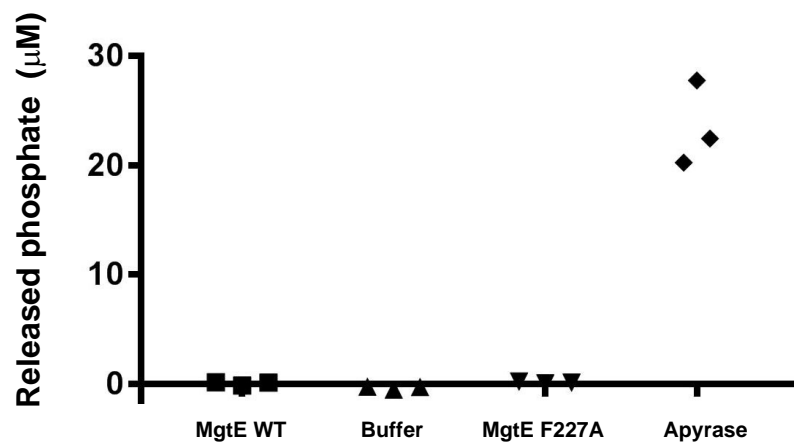
		Mg5	F227
	200	210	220
T.therm	ADP	.....	RTR
T.potens JR	AKS	.....	SAT
D.alkalitolerans	ASP	.....	ETK
E.faecalis	NDD	.....	DTL
S.aureus	AEN	.....	DAY
L.xylanilyticus	ADE	.....	DTL
E.saphenumc	ADD	.....	SKR
Spirochaeta sp. JC230	SND	.....	NDR
M.rufus	ADP	.....	KTK
M.hydrothermalis	ADP	.....	RTR
V.cholerae	TQP	.....	DVP

CBS domain

Supplementary Figure 6 | Amino acid sequence alignment of the cytosolic domain of TtMgtE with its bacterial orthologues.

The amino acid sequence alignment of TtMgtE with its orthologs was constructed with Clustal Omega<sup>1</sup> and ESPript 3.0<sup>2</sup>. Abbreviations of organisms are as follows: *T. therm.*, *Thermus thermophilus* (Gene ID 3168925); *T. potens JR*, *Thermincola potens JR* (Gene ID 9149225); *D. alkalitolerans*, *Desulfitibacter alkalitolerans* (Gene ID 974208913); *E. faecalis*, *Enterococcus faecalis* (Gene ID 514904541); *S. aureus*, *Staphylococcus aureus* (Gene ID 581835250); *L. xylanilyticus*, *Lysinibacillus xylanilyticus* (Gene ID 926258026); *E. saphenum*, *Eubacterium saphenum* (Gene ID 492421711); *Spirochaeta sp. JC230* (Gene ID 739695846); *M. rufus*, *Meiothermus rufus* (Gene ID 738318087); *M. hydrothermalis*, *Marinithermus hydrothermalis* (Gene ID 503469645); *V. cholerae*, *Vibrio cholerae* (Gene ID 903058383). Strictly conserved and similar amino acid residues are highlighted with a red box and by a red letter, respectively. The amino acid residues forming Mg<sup>2+</sup> binding sites and the amino acid residues involved in ATP binding are labeled on the top of the alignment. The protein domains of TtMgtE are shown below its sequence. The side chains of Asp91, Asp92, Asp95, Asn99 and Asp226, and the main chain atoms of Gly136 and Ala223 form Mg<sup>2+</sup> binding sites.





**Supplementary Figure 7 | ATP hydrolysis assay of MgtE.**

The ATP hydrolysis assay of MgtE. All assays were performed in triplicate. Individual measurements are plotted.

## Supplementary References

1. Sievers, F. *et al.* Fast, scalable generation of high-quality protein multiple sequence alignments using Clustal Omega. *Mol. Syst. Biol.* **7**, 539 (2011).
2. Robert, X. & Gouet, P. Deciphering key features in protein structures with the new ENDscript server. *Nucleic Acids Res.* **42**, W320–4 (2014).