Supplemental data for

"Structure of Mlc Titration Factor A (MtfA/YeeI) Reveals a Prototypical Zinc Metallopeptidase Related to Anthrax Lethal Factor"

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Table S1. Oligonucleotides and sequences

Oligonucleotides	Sequence (5' to 3')
MtfAkpnEcoRV+	GGGGATATCATTTAAATGGCCCTGG
MtfAkpnKpnI	CCCGGTACCG TGGTGGTGGTGGTGGTGAACAATGCGCCG
H112Akpn+	GATATTGGTCTGGTCGCCAACCAGCGGGTGGTACAGTCG
H112Akpn-	CGACTGTACCACCCGCTGGTTGGCGACCAGACCAATATC
Y205Akpn+	CAGTATTGACGCCGCTGCCGCCACCGATCCCGCAGAG
Y205kpn-	CTCTGCGGGATCGGTGGCGGCAGCGGCGTCAATACTG
E212kpn+	CCACCGATCCCGCAGCGTGCTTTGCTGTCCTCTC
E212kpn-	GAGAGGACAGCAAAGCACGCTGCGGGATCGGTGG
H149,E150,H153Akpn+	CCTTGTGGTGGCTGCAGTGGCGGCTAAGCTTGATACGCGGAAC
H149,E150,H153Akpn-	GTTCCGCGTATCAAGCTTAGCCGCCACTGCAGCCACCACAAGG

+ forward, - reverse

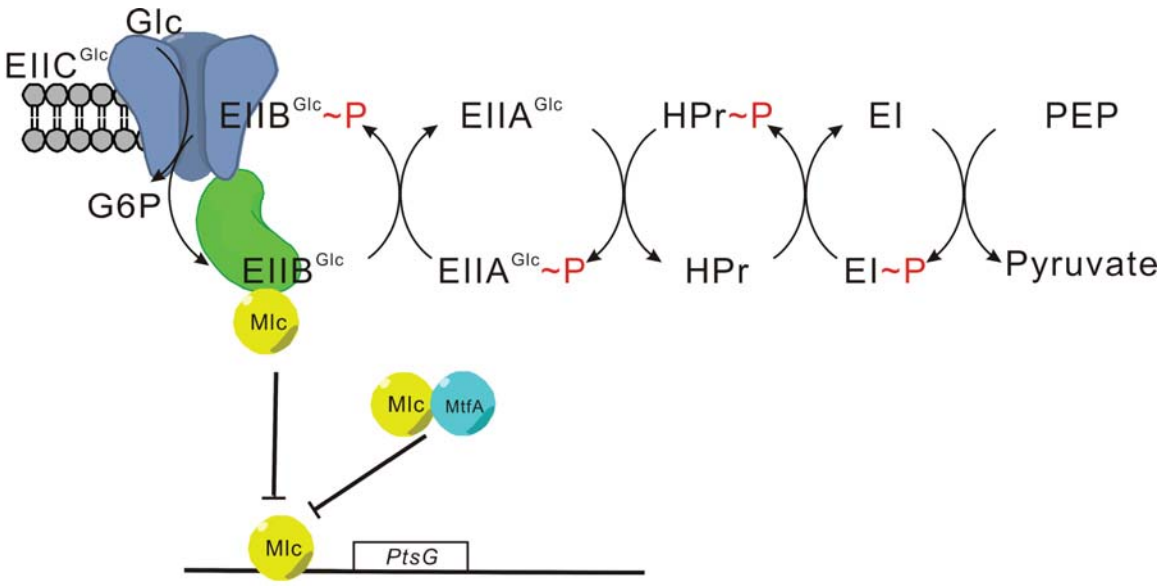


Fig. S1. A schematic drawing of the glucose PTS cascade showing the transfer of phosphorus from phosphoenolpyruvate to the incoming glucose and two possible mechanisms of Mlc inactivation by $EIICB^{Glc}$ and MtfA. $EIIA^{Glc}$ and $EIICB^{Glc}$ become dephosphorylated in the presence of Glc. $EIIB^{Glc}$ (green, unphosphorylated) binds the Mlc repressor, allowing the transcription of the downstream glucose utilization genes. The mechanism of Mlc inactivation by MtfA is currently unknown.

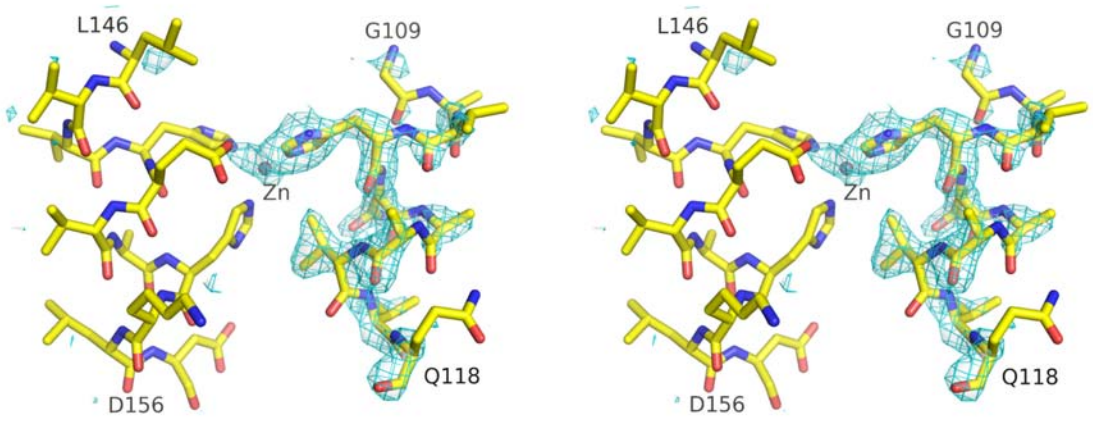
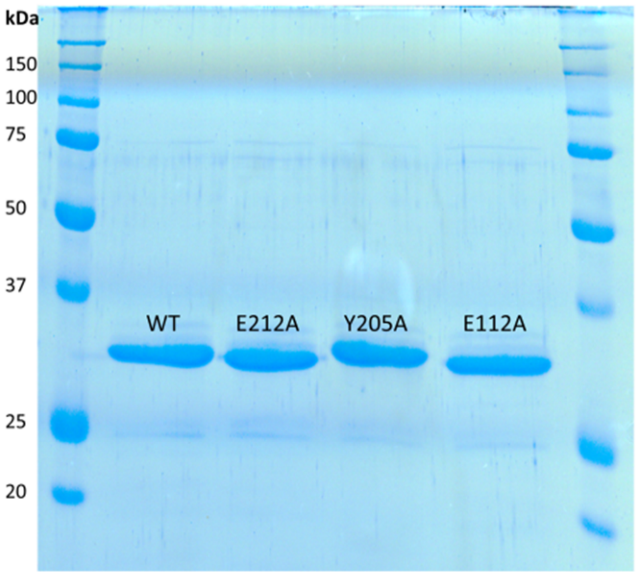
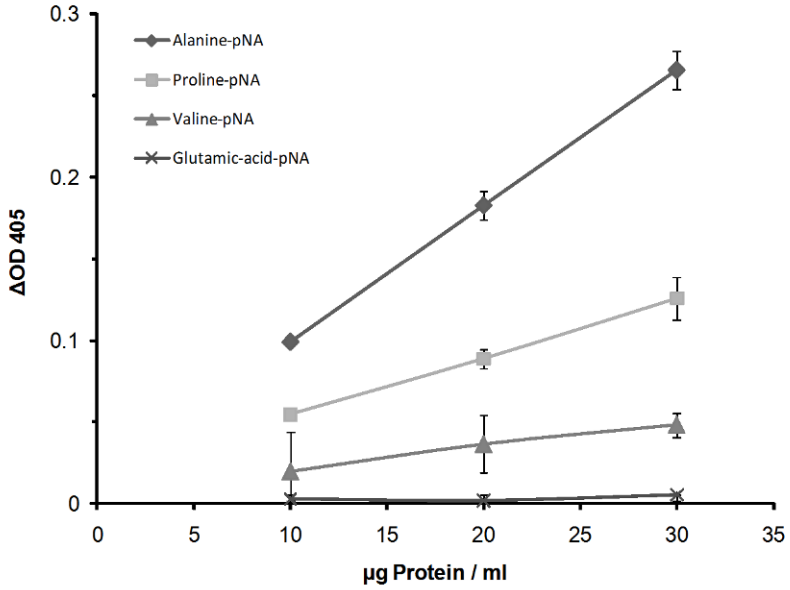


Fig. S2. Stereoview of the simulated annealing Fo-Fc omit map (contoured at 2.5 σ) for the 109-118 region of the MtfA holoenzyme structure. The refined model is shown as sticks and the zinc as a grey sphere.

A


kDa
150
100
75
50
37
25
20
WT
E212A
Y205A
E112A

B


Alanine-pNA
Proline-pNA
Valine-pNA
Glutamic-acid-pNA
ΔOD 405
0.3
0.2
0.1
0
0
5
10
15
20
25
30
35
µg Protein / ml

C

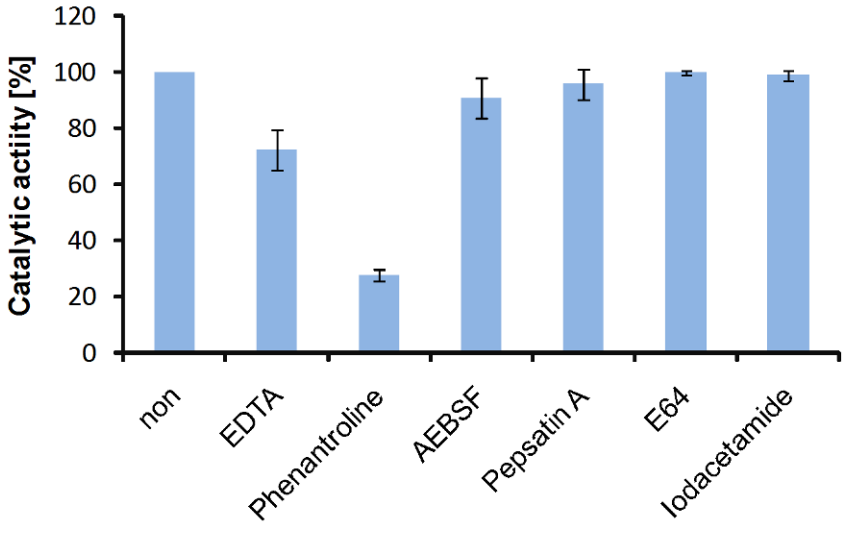

Catalytic actiity [%]
120
100
80
60
40
20
0
non
EDTA
Phenantroline
AEBSF
Pepsatin A
E64
Iodacetamide

Fig. S3. Aminopeptidase activity of MtfA. (**A**) SDS-PAGE analysis of the MtfA wildtype and mutant proteins, with molecular weight ladder indicated on the left. (**B)** Amino peptidase activity of purified MtfA with different L-amino 4-nitroanilide substrates is shown. Activity was monitored by measuring the change of the optical density at 405 nm (ΔOD_{405}) triggered by the release of 4-nitroanilide after cleavage. The highest cleavage rate was obtained with L-alanine 4-nitroanilide as substrate. Lower affinities were detected for other nonpolar amino acid substrates such as L-proline or L-valine fused to 4-nitroanilide. Glutamic-acid 4-nitroanilide with an acidic amino acid and L-arginine 4-nitroanilide with a basic amino acid (not shown) cannot be cleaved by MtfA. (**C)** The catalytic activity of the purified MtfA with respect to cleavage of L-alanine 4-nitroanilide was characterized via competitive inhibition with different substrates. Proteolytic activity was measured in the presence of different inhibitors and is shown relatively to a sample without any inhibitor. Assays were supplemented with the following standard inhibitor concentrations: EDTA, phenantroline, and AEBSF: 1 mM; pepsatin A: 5 μM; E64: 10 μM; iodacetamide: 100 μM. The catalytic activity of MtfA was only significantly inhibited by the metal chelators, EDTA and phenantroline. Inhibitors for serine- (AEBSF), aspartyl- (pepsatin A), or cysteine-proteases (E64, iodacetamide), do not decrease activity. All data are the mean values of three independent experiments.

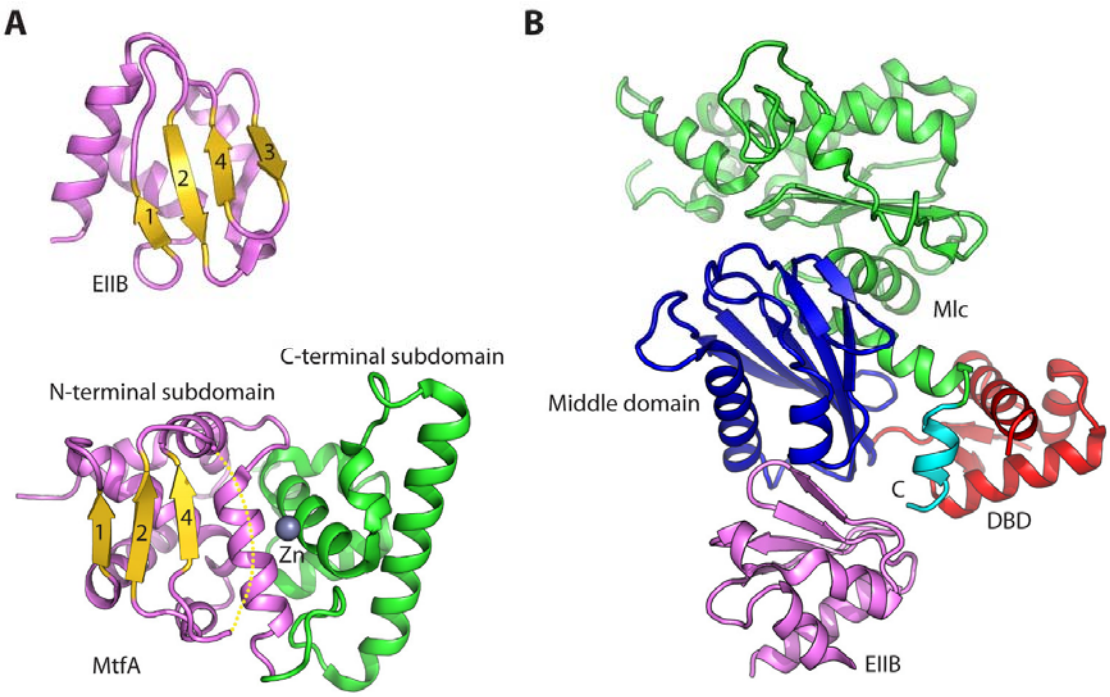


Fig. S4. Structural comparison of EIIB and MtfA. (**A**) Structures of $EIIB^{Glc}$ (top) and MtfA (bottom). (**B**) The Mlc-$EIIB^{Glc}$ complex structure (PDB ID 3bp8). The three Mlc domains are colored in red, blue and green. The C-terminal helix of Mlc is highlighted in cyan. For clarity, only one half of the dimeric complex is shown.

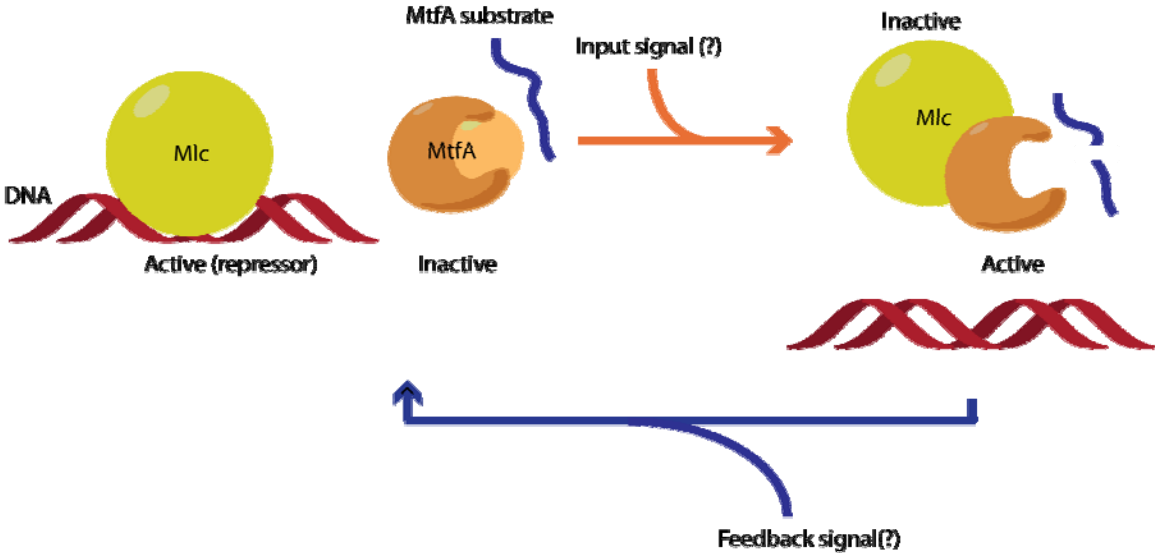


Fig. S5. A hypothetical model of the interplay between MtfA and Mlc. Upon receiving an input signal, such as glucose, Mlc dissociates from DNA and interacts with inactive MtfA. The interaction between Mlc and MtfA impedes the ability of Mlc to bind DNA, but activates the peptidase function of MtfA. The binding of a negative feedback signal to the complex disrupts MtfA-Mlc association.