Compounds with potential activity against *Mycobacterium tuberculosis*

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Supplementary Figure S1

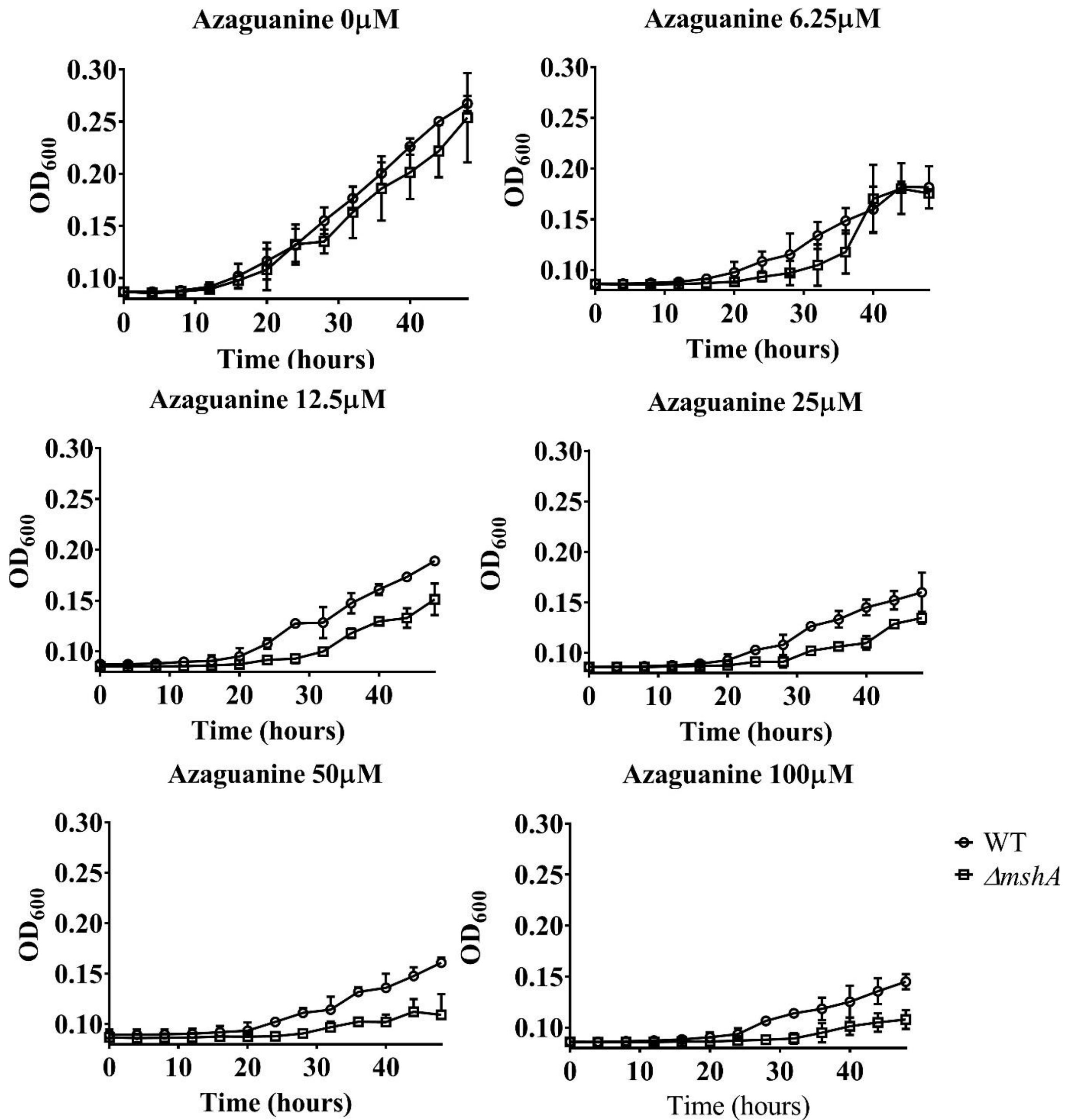


Figure S1. Azaguanine dose response curve of the *M. smegmatis ΔmshA* mutant square relative to the wild type (WT) circle

Supplementary Figure S2

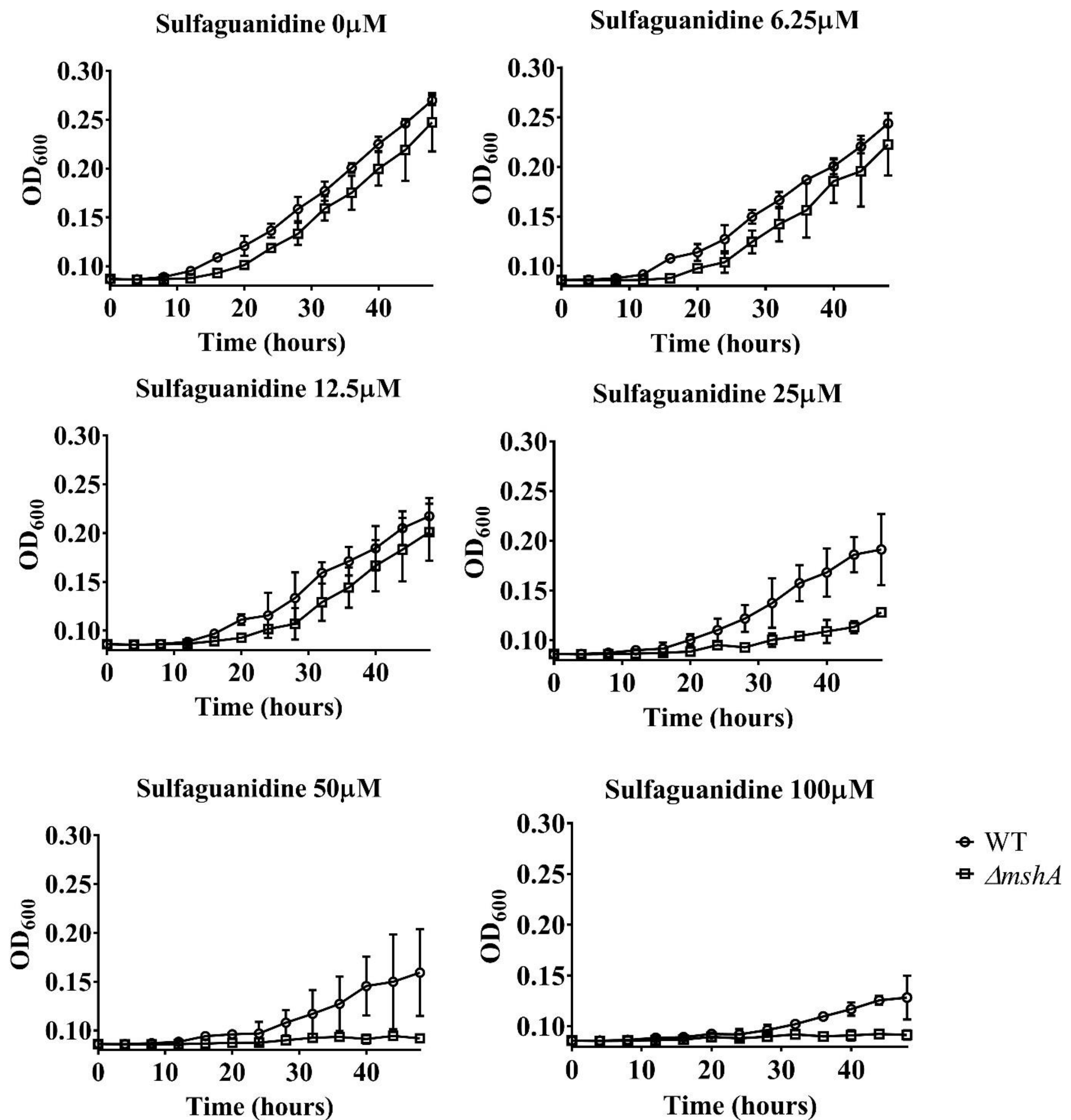


Figure S2. Sulfaguanidine dose response curve of the *M. smegmatis ΔmshA* mutant square relative to the wild type (WT) circle

Supplementary Figure S3

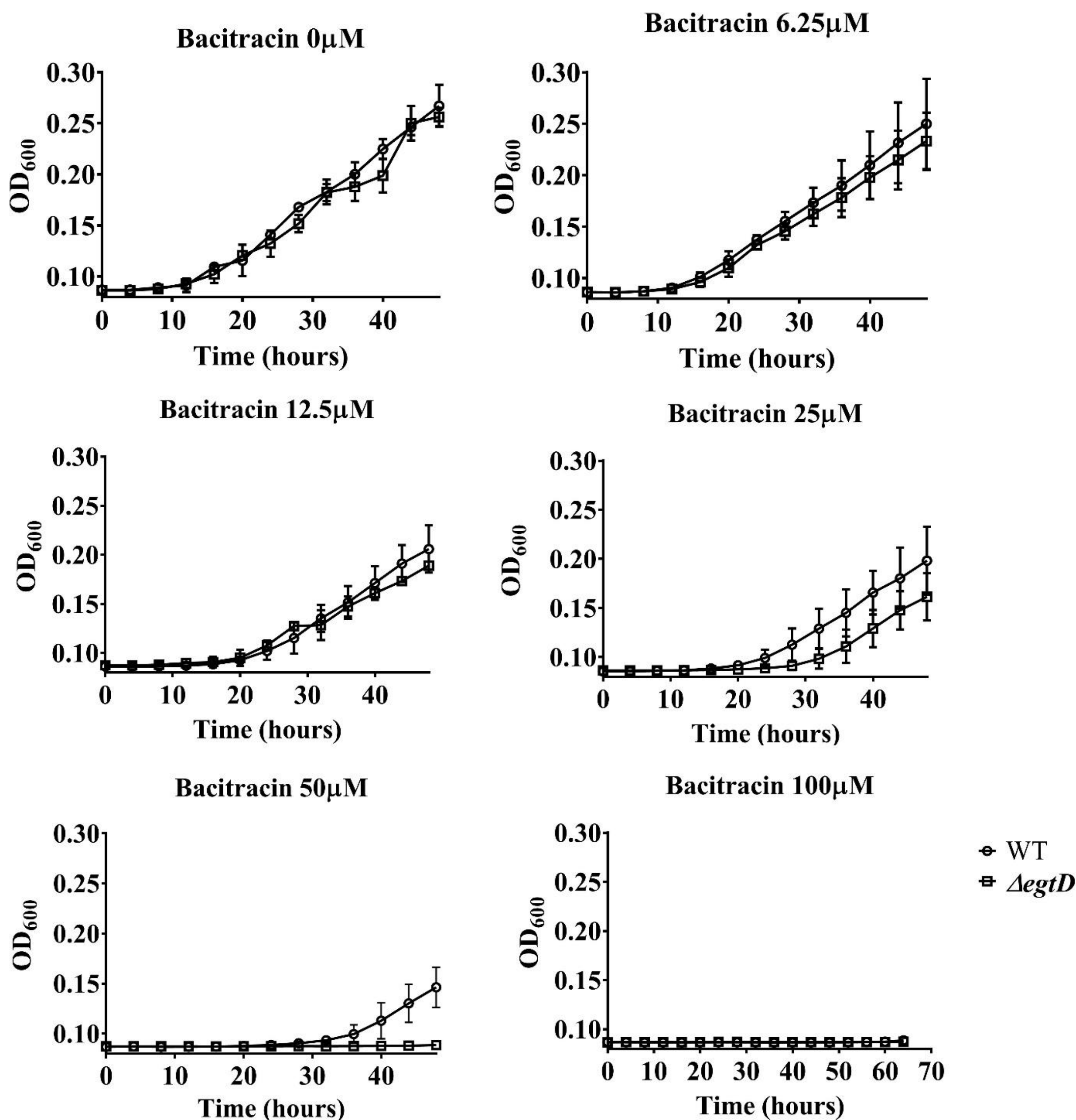


Figure S3. Bacitracin dose response curve of the *M. smegmatis ΔegtD* mutant square relative to the wild type (WT) circle

Table S1. Average[a] MIC (µg/ml) of compounds that were validated in *M.tb*

Compound	MIC
Bacitracin (Ba)	~1700 [b]
Sulfaguanidine (Su)	~100 [c]
Azaguanine (Aza)	~120
Fusaric acid (Fu)	~5

[a] mean concentration between the well with complete growth inhibition and the adjacent well with no visual growth inhibition, [b]in culture supplemented with Tween 80, but this average MIC is higher (~6250) in culture supplemented with tyloxapol. [c] When stock is made at 20mg/ml in 50%HCl, however this average MIC is lower (~9) when stock is made at 10mg/ml in 50% HCl

Supplementary Figure S4

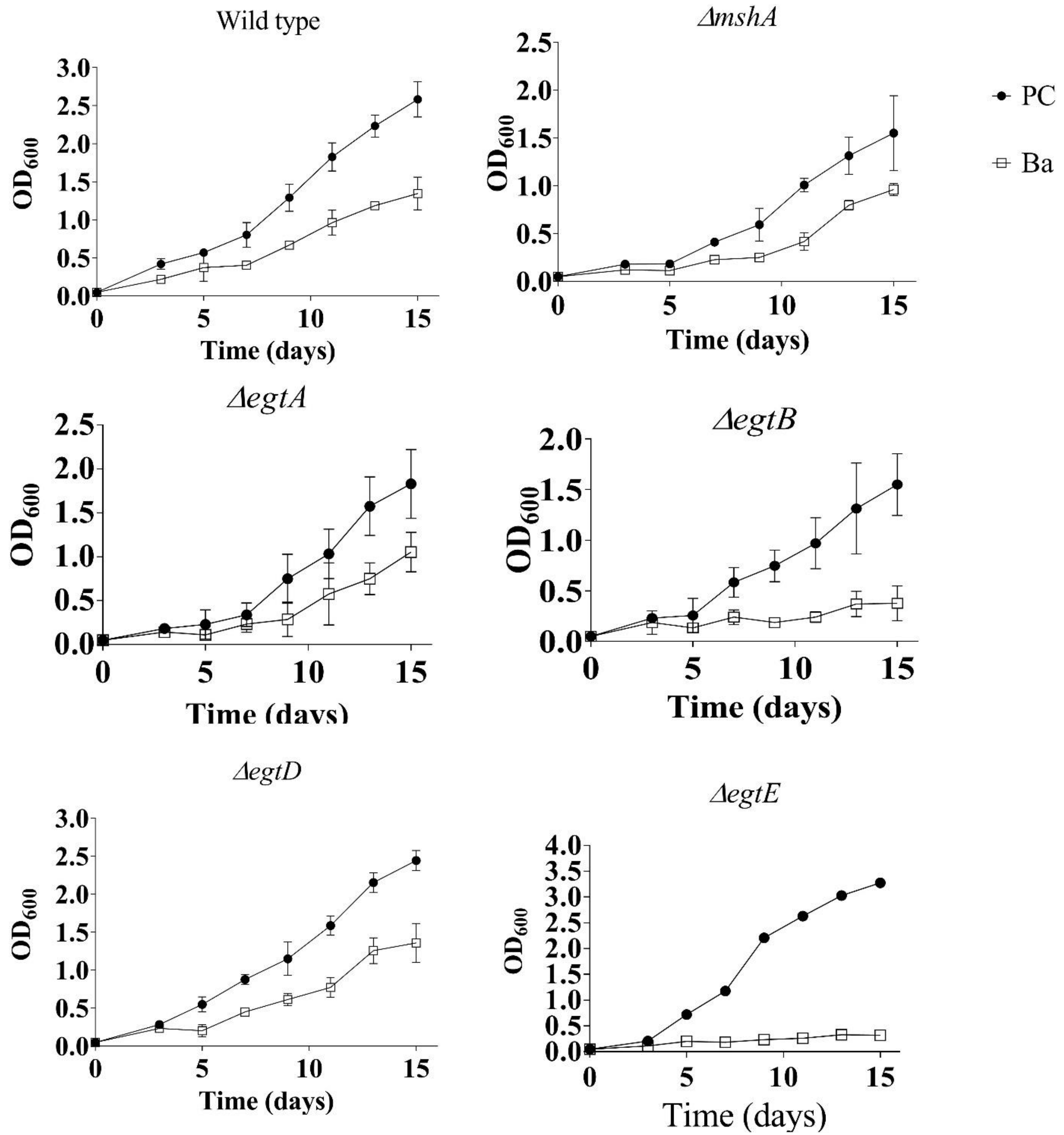


Figure S4. Growth curves of *M. tuberculosis* strains when treated with Bacitracin (Ba) relative to the untreated positive control (PC)

Supplementary Figure S5

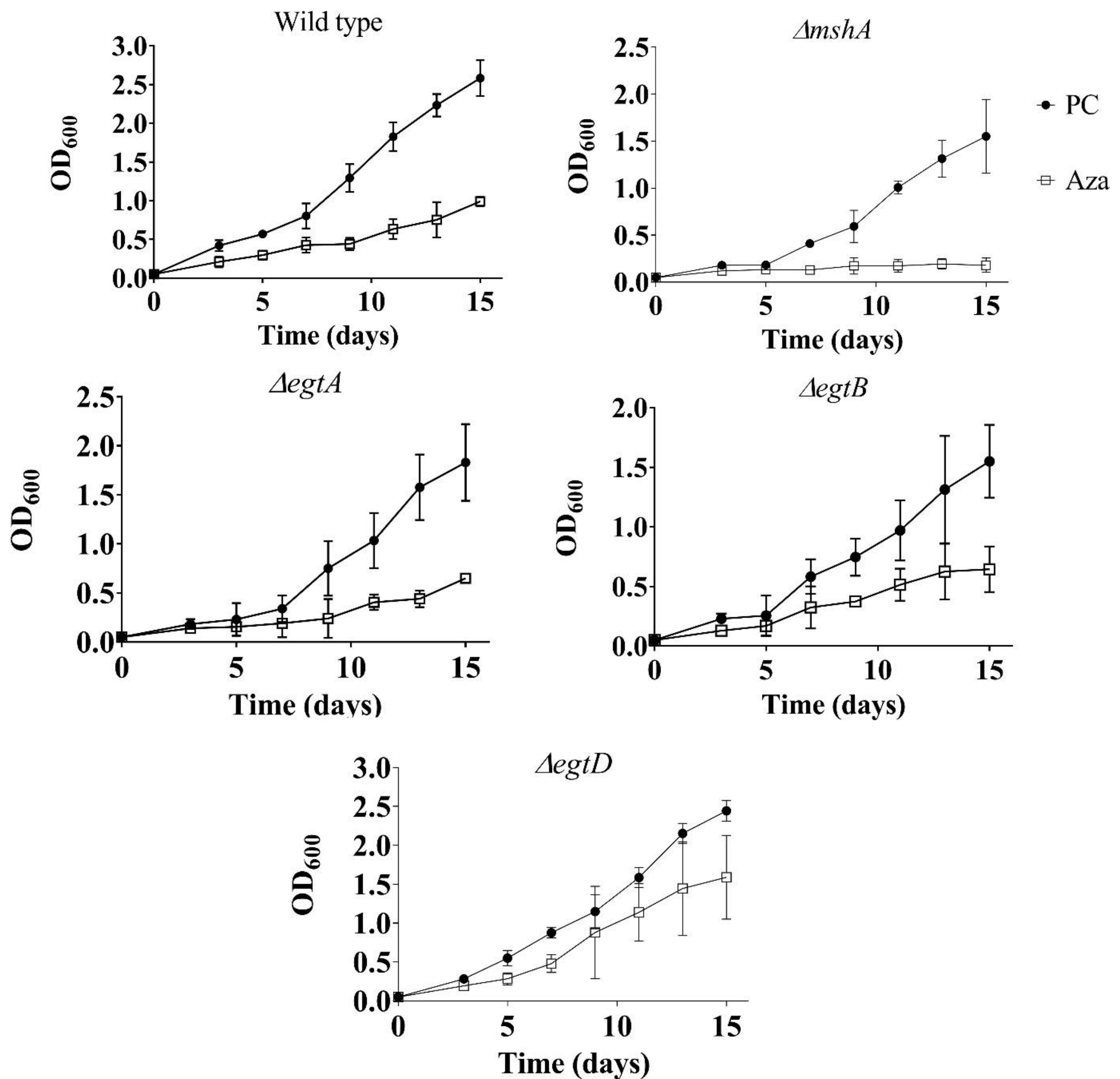


Figure S5. Growth cures of *M. tuberculosis* mutants when treated with Azaguanine (Aza) at average MIC relative to the untreated positive control (PC)

Supplementary Figure. S6

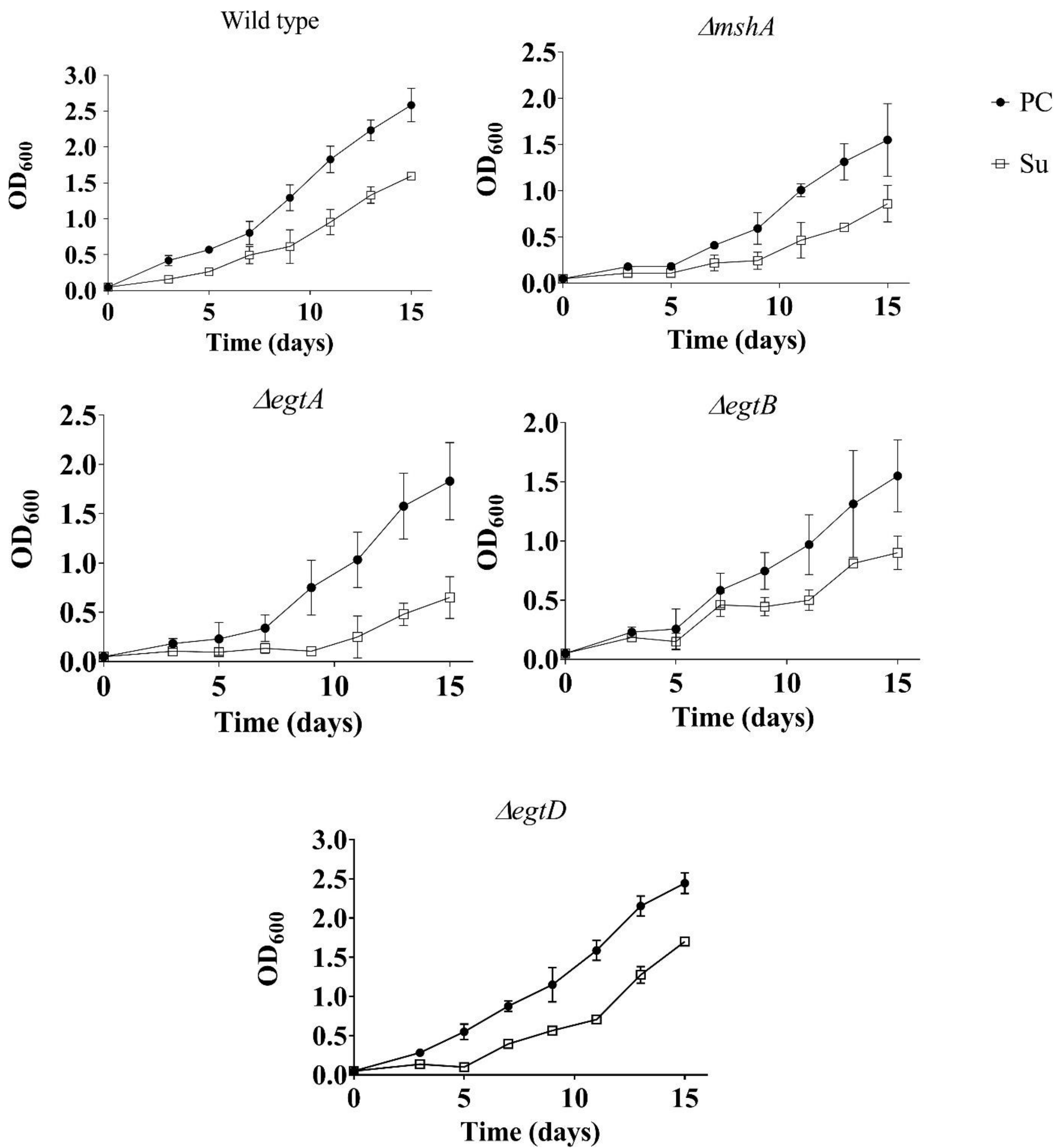


Figure S6. Growth curves of *M.tb* mutants when treated with Sulfaguanidine (Su) at average MIC relative to the untreated positive control (PC)

Supplementary Figure S7.

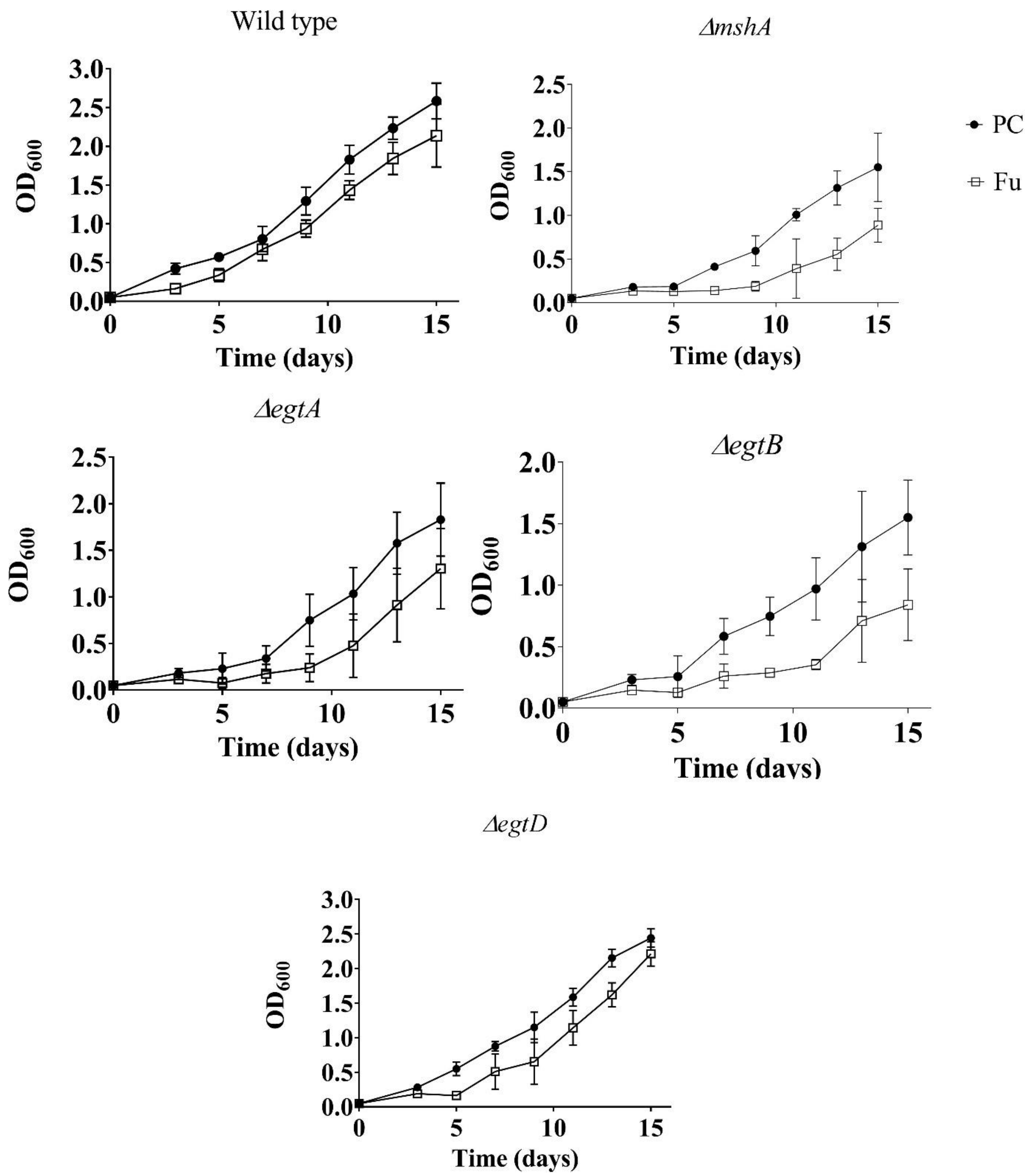


Figure S7. Growth curves of *M.tb* strains when treated with Fusaric acid (Fu) relative to the untreated positive control (PC)

Table S2. Level of intracellular (IM) and extracellular (EM) MSH in Area/105 CFUs

	IM1	IM2	EM1	EM2
M,tb (CDC1551)	67,7	102,9	3,7	10,6
Δ*egtA* (CDC1551)	92,4	294,2	64,0	220
Δ*egtB* (CDC1551)	102,02	152,1	0	0
Δ*egtC* (CDC1551)	55,4	100,1	23,4	52,3
Δ*egtD* (CDC1551)	121,6	188,2	59	87,2
Δ*egtE* (CDC1551)	40,1	42,3	<1	<1
Δ*mshA*(CDC1551)	<1	<1	<1	<1
Δ*egtAc* (CDC1551)	42,1	106,7	53	115,7
Δ*egtBc* (CDC1551)	184,7	137,8	5,4	3
Δ*egtCc* (CDC1551)	133,3	17,9	63,2	9,9
Δ*egtDc* (CDC1551)	55,9	65,9	31,6	29
Δ*mshAc*(CDC1551)	146,2	84,8	72	62,9

Table S3. Level of intracellular (IE) and extracellular (EE) ERG in pg/105 CFUs

	IE1	IE2	EE1	EE2
M,tb (CDC1551)	189	115,25	80,5	68,7
Δ*egtA* (CDC1551)	4,4	1,95	8,5	1,6
Δ*egtB* (CDC1551)	<1	<1	<1	<1
Δ*egtC* (CDC1551)	13,7	21	3,15	1,06
Δ*egtD* (CDC1551)	<1	<1	<1	<1
Δ*egtE* (CDC1551)	62,4	89,52	14	13
Δ*mshA* (CDC1551)	234,3	207,83	190,8	87,8
Δ*egtA*$_c$ (CDC1551)	528,3	346,16	343,1	288,8
Δ*egtB*$_c$(CDC1551)	3,45	2,81	<1	<1
Δ*egtC*$_c$(CDC1551)	180,5	122,2	29,14	48
Δ*egtD*$_c$ (CDC1551)	329,55	162,2	61,9	46,62
Δ*mshA*$_c$(CDC1551)	120,4	112,2	104,84	40,64
ΔegtE*$_{op}$ (CDC1551)	118,86	103,46	17,08	17,5

Table S4. Thiols in mutants during the stationarry phase using ERG standard (pg/10^5CFUs)

	IE1	IE2	EE1	EE2	IM1	IM2	EM1	EM2
CDC1551	111	296	33	97	12	40	<1	<1
ΔmshA	430	385	160	233	<1	<1	<1	<1
ΔegtA	11	12	<1	<1	212	109	7	3
ΔegtB	<1	<1	<1	<1	118	60	<1	<1
ΔegtD	<1	<1	<1	<1	37	90	1.4	2

Supplementary Figure S8.

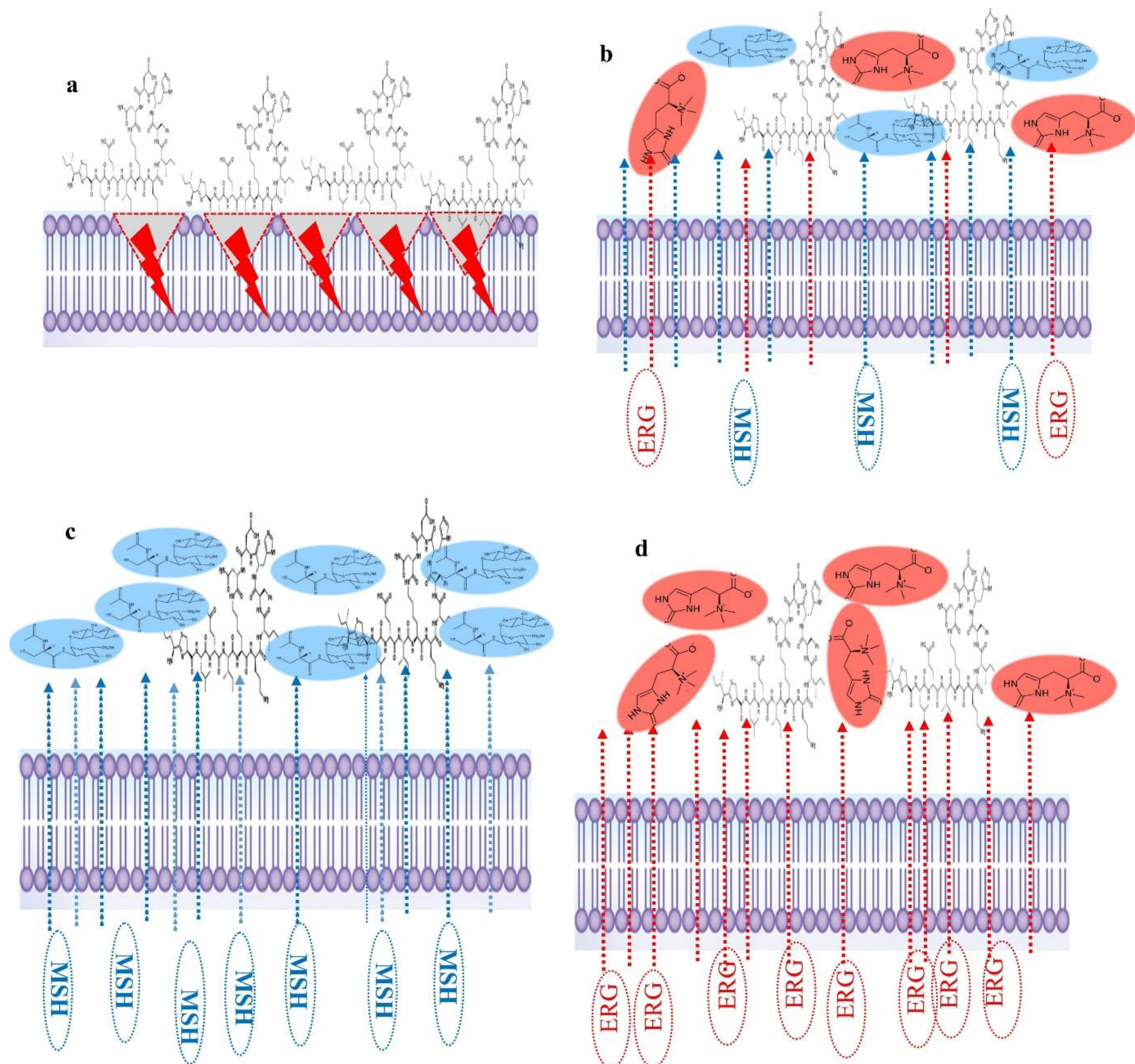
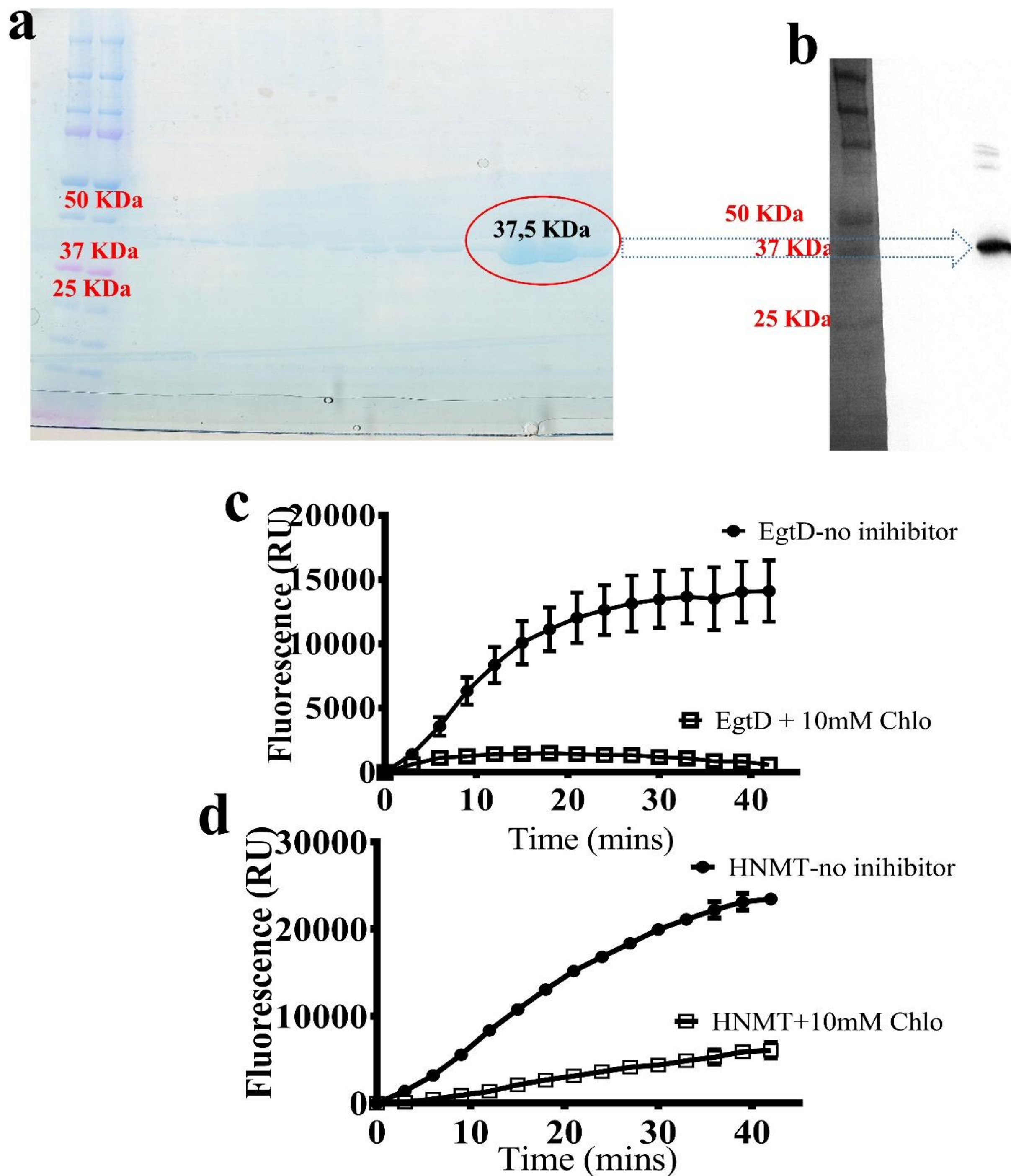


Figure S8. Suggested mechanistic Effect of Bacitracin on different strains. **(a)** Bacitracin (Ba) is a high molecular weight cyclic peptides which hardly penetrates the thick lipid layer of mycobacterial membrane. Strains that have a compromised membrane lipid composition such as the *ΔegtE* mutant of CDC551. In addition, strains that cannot produce extracellular thiols such as the *ΔegtB* mutant of *M. tuberculosis* and the *ΔegtD* mutant of *M. smegmatis* are also sensitive. **(b)** The wild-type strain of CDC1551 can produce extracellular ERG and MSH under stress conditions to counteract Ba in order to minimize the toxic effect of Ba**.** **(c)** The ERG-deficient *ΔegtA* and *ΔegtD* mutants of *M. tuberculosis* CDC1551 can produce extracellular MSH that counteracts Ba **(d)** The MSH-deficient *ΔmshA M. tuberculosis* and *M. smegmatis* mutants produce a high level of extracellular ERG that counteracts Ba

Supplementary Figure S9



Supplementary Figure S9. Investigation of the ability of Chloroquine to inhibit the activity of EgtD. (a) SDS-PAGE of purified Hist-tagged EgtD. (b) Western blotting analysis of purified His-tagged EgtD. **(c)** Investigation of the effect of chloriquine (Chlo) on the activity of EgtD which is proportionnal to the fluorescence intensity**. (d)** Investigation of Chlo effect on the activity of the human histamine methyltransfrease (HNMT).

Supplementary Method.

Cloning, expression of EgtD and the human histamine methyltransferase (HNMT)

Mycobacterium tuberculosis egtD gene (*Rv3701*) was amplified (AAGTTCTGTTCCAGGGGCCCATGAGAGTGTCGGTTGCC and CGCCATTAACCTGATGTTCTGGGGAATATAA) and cloned into PETM 14 expression vector using the Gibson assembly method [1] to generate a full length EgtD N-terminal His_6-tag. The integrity of the inserted gene was confirmed by sequencing and the resulting PETM 14-EgtD plasmid was used to transform the *E.coli* expression strain BL21. A positive colony was grown in 3 ml LB liquid culture containing 50 µg/ml kanamycin overnight. Then the overnight culture was diluted 100 X and further cultured to an OD_{600} of ~0.6. Then this culture was induced with 0.2 mM IPTG at 18 ºC overnight. Cells were harvested at 4000 rpm (4 ºC), frozen for 30 mins and re-suspended in the ice cold nickel column loading buffer (12.5 mM Tris-HCl, pH 8, 2.5 mM imidazole, 1.63% glycerol, 125 mM NaCl) containing 1% Triton and 2mM DTT. Cells were subsequently lysed using the French Press. Cell lysate was centrifuged at 40 000 rpm (4ºC) for 30 mins. The supernatant was loaded on a Ni-NTA resin column that was previously equilibrated with the loading buffer. The elution buffer consisted of 6.25 mM Tris-HCl, 125 mM Imidazole, 1.63% glycerol and 75 mM NaCl. Fractions containing EgtD were collected, checked for purity by SDS PAGE and Western Blot (using an anti-histidine antibody), flash frozen in liquid nitrogen and stored at -80ºC.

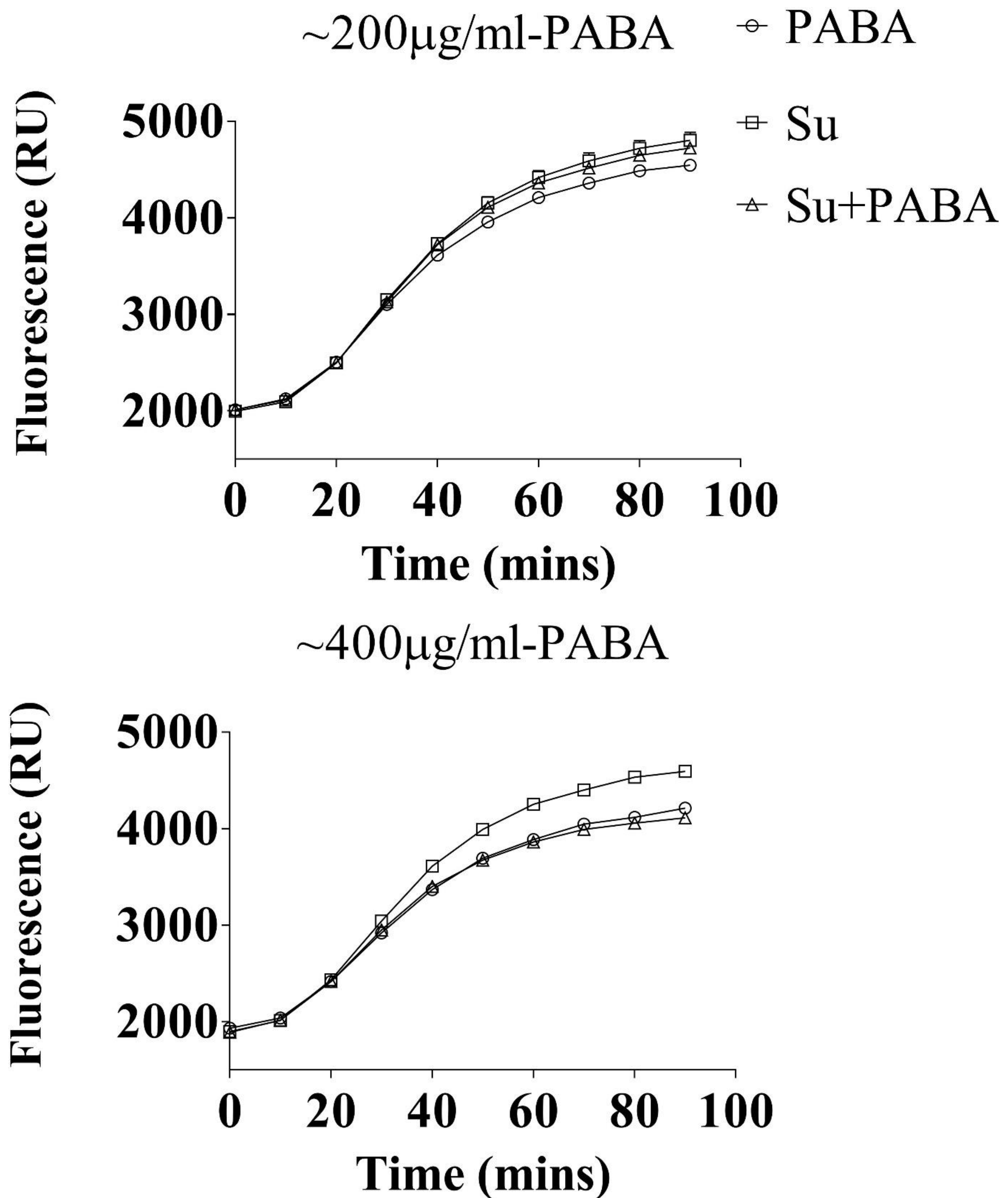
The human histamine methyltransferase (HNMT) from PDONR in the MGC premier human ORFeome v 8.1 library was amplified with the following primers (AAGTTCTCTTCCAGGGGCCCGCATCTTCCATGAGGAGCTTG and CCCCAGAACATCAGGTTAATGGCGTTATGCCTCAATCACTATGAAACTCAG and cloned by the Gibson assembly method to the PETM 33 expression vector to obtain a full length HNMT C-terminal PreScission site-GST-linker-His_6 tag. Following the same expression and

purification process described for EgtD above, HNMT was expressed and purified. The fractions containing HNMT were collected, and the re-suspension buffer of HNMT was exchanged to the GST purification loading buffer (PBS pH 7.3) using the PD-10 desalting column (GE Healthcare Life Sciences) and the GST tag was digested overnight with PreScission protease enzyme (GE Healthcare Life Sciences) at 4ºC. Then HNMT was further purified with the GST affinity column (GE Healthcare Life Sciences), where the unbound sample (that is the flow through), was collected and checked for purity by SDS-PAGE and the sample buffer was subsequently exchanged to the buffer in which EgtD was stored.

Enzymatic assays

This was achieved with the SAMfluoro™: SAM Methyltransferase Assay kit (G-Biosciences 9800 Page Avenue St. Louis, MO 63132-1429 U.S A). The optimized concentrations were 167 µM S-adenosylmethionine (SAM), 87 µM histidine for EgtD or 87 µM histamine for HNMT, 42 nM of the investigated enzyme. The optimized reaction total volume was 11.5 µl that is, 1.5 µl of enzyme and inhibitor added to 10 µl of the assay buffer containing SAM and the specific enzyme substrate. The optimized concentration of chloroquine that elicited an inhibition on both the activity of EgtD and HNMT was 10 mM.

Supplementary Figure S10



Supplementary Figure S10. Investigation of the ability of para-aminobenzoic acid (PABA) to countereact the effect of sulfaguanidine (Su). A wild type *M. tuberculosis* culture grown without catalse of an OD_{600} ~0.2 was treated with either ~100µg/ml Su and/or PABA and the generation of oxidative stress was measured over time at 37°C (Ex~488nm, Em~520nm) when samples were stained with a final concentration of 10µM FEHFC. There was no significant difference when 200µg/ml of PABA was used (top panel). However, 400µg/ml of PABA (botom panel) was able to aleviate the level of oxidative stress generated by Su. Data derive from 5 to 6 replicates represented as means and standard errors of the means.

References

[1] D. G. Gibson, L. Young, R.-Y. Chuang, J. C. Venter, C. A. Hutchison, and H. O. Smith, "Enzymatic assembly of DNA molecules up to several hundred kilobases," *Nat. Methods*, vol. 6, no. 5, pp. 343–345, May 2009.