SUPPLEMENTARY INFORMATION

Lack of formylated methionyl-tRNA has pleiotropic effects on *Bacillus subtilis*

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Table S1 Strains and plasmids (the strains are CU1065 background, unless indicated)

Strain	**Genotype**	**Reference**
CU1065	*trpC2 att SPβ*	Lab stock
NCIB 3610	*Marburg undomesticated strain*	(1)
HB21001	*defA:null defB:erm with the deletion of base pair 119A of fmt*	This study
HB21002	*defA:null defB:erm*	This study
HB21003	*defA::erm*	This study
HB21004	*defB::erm*	This study
HB21005	*defA:null defB:erm with 909C deleted, G916T and G917T, the DNA from 813 to 905 deleted.*	This study
HB21006	*fmt::km*	This study
HB21009	*NCIB3610 fmt::km*	This study
HB21011	*fmt:km katA::tet*	This study
HB21012	*fmt::km yfmC::mls*	This study
HB21014	*fmt::km ahpCF::cat*	This study
HB21015	*fmt::km katA::mls ahpCF::cat*	This study
HB21016	*fmt::km* P_{spac} *fmt*	This study
HB21017	*NCIB3610 fmt::km* P_{spac} *fmt*	This study
HB21018	*fmt::km bshC::mls*	This study
HB5612	*yfmC::mls*	(2)
HB14110	*katA::mls*	(3)
HB17882	*katA::mls ahpCF::cat*	(4)
HB17821	*ahpCF::cat*	(4)
HB11212	*bshC::mls*	(5)
pPL82	Expression of gene under $P_{spac(hy)}$ promoter	(6)

Table S2. Oligonucleotides

No.	Name	Sequence
6863	defA-up-fwd	CCGATAGCCAGGATCAAAGAT
6864	defA-do-rev	GGAGGAGGTGTCAGTACTTT
6865	defB-up-fwd	CACCTTTGCGCTAATCGT TG
6866	defB-do-rev	TTGAATACGTTGGGACAGGC
6891	fmt-up-fwd	GACCACTTAGACGGTGTGCT
6892	fmt-do-rev	TCAGCAGCAGGTTGCTGTAT
6893	defB-up-fwd	CGCGGATCCGTTTTATAGCGTCTTTCACGT
6896	defB-do-rev	CGCGAATTCTGTTCCCGAACTGAAAGAAAT
6897	defA-up-fwd	GCGAAGCTTCTCACGTCTTGGAGGGTAA
6900	defA-do-rev	CGCAGATCTCTTAAAACAGGAACTGAAAAATC
7110	fmt-up-rev(km)	CCTATCACCTCAAATGGTTCGCTGCAAATCATCCTTCCATA TCCGCT
7111	fmt-do-fwd(km)	CGAGCGCCTACGAGGAATTTGTATCGACTAGTGTTCGTGA CATCGC
7112	fmt-do-rev	AGCAGCCATTTGGTCAAGCA
7285	fmt-up-f-ppl82	GCGAAGCTTATGAACTAGC GGATATGGAA
7286	fmt-do-r-ppl82	CGCAGATCTTTGTTCTAATTTGATCAGCG

Table S3 Suppressor mutations of *ΔdefA defB::erm*

strains	*fmt*	*foldD*	*glyA*
ΔdefA defB::erm fmt1	119A deleted	no	no
ΔdefA defB::glyA1	no	no	Δ813 to 905, Δ909C, G916T, G917T
*ΔdefA defB::erm (sup3)**	no	no	no

*This suppressed strain did not have a mutation in any of the three sequenced loci.

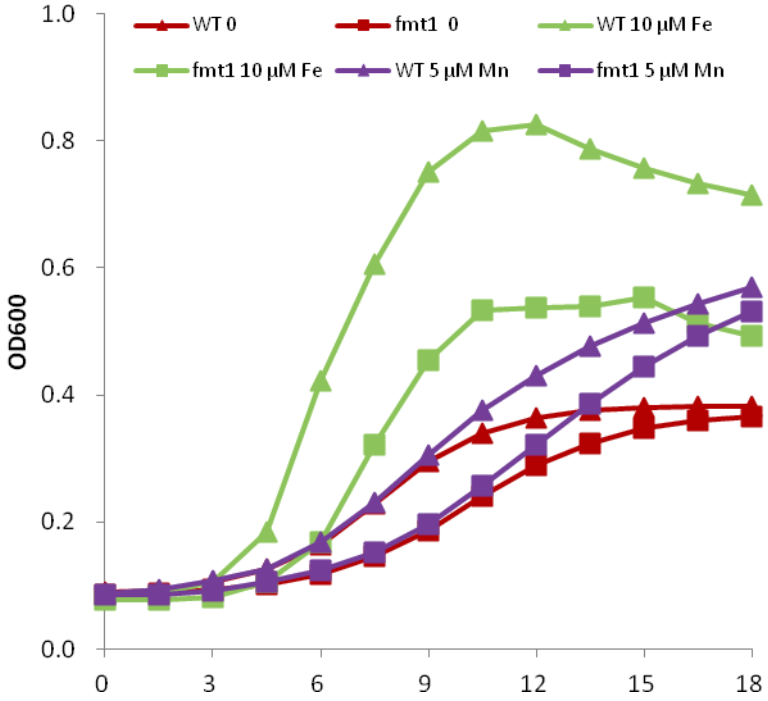

FIG S1 Growth curves of the WT and *ΔdefA defB::erm fmt1* (fmt1, HB21001) mutant in MM plus different metal (OD_{600} at the time indicated in hours). The WT in MM (red triangle), in MM plus 10 µM Fe (green triangle), in MM plus 5µM Mn (purple triangle). The *fmt1* mutant in MM (red square), in MM plus 10 µM Fe (green square) and MM plus 5µM Mn (purple square).

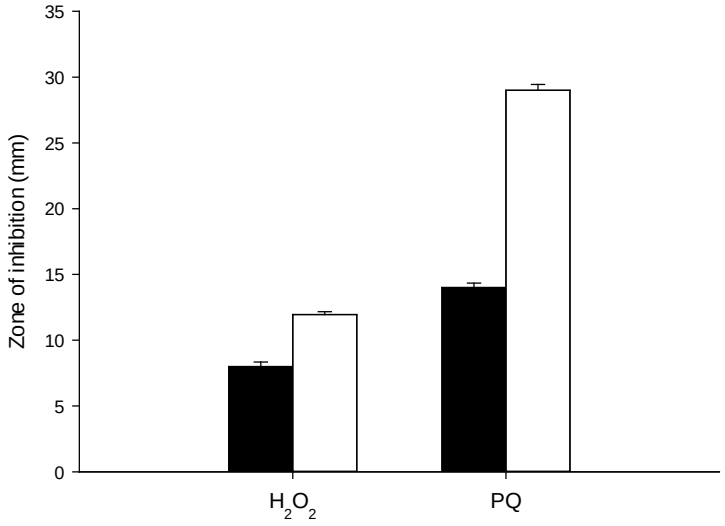

FIG S2 an *ΔdefA defB::erm fmt1* (HB21001) mutant has elevated sensitivity to hydrogen peroxide and PQ. Sensitivity of WT (black column) and *ΔdefA defB::erm fmt1* (white column), to hydrogen peroxide stress as monitored using a disk diffusion assay. The results are expressed as the diameter of the inhibition zone (mm) minus the diameter of the filter paper disk (6.5 mm). The disks were spotted with 3 µl of 0.8M H_2O_2 or 5 µl of 0.5 M PQ. The mean ± SE from at least three biological replicates are reported.

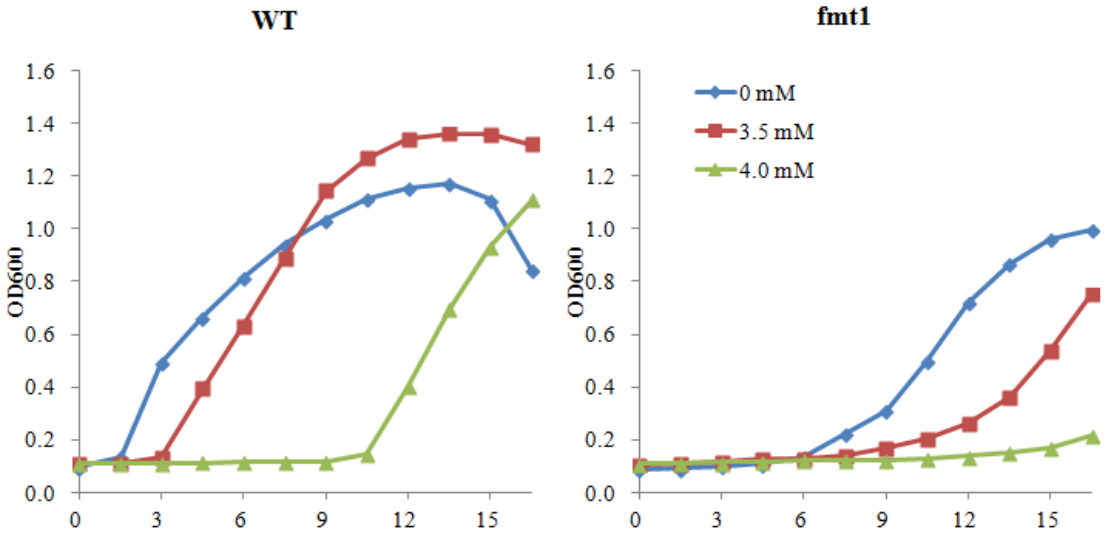

FIG S3 The *ΔdefA defB::erm fmt1* mutant is sensitive to iron intoxication. Growth of WT (CU1065) and *ΔdefA defB::erm fmt1*(HB21001) under iron intoxication condition by monitoring cell growth in liquid culture (OD_{600} vs. hours). Iron concentration dependence of growth inhibition for the strains in LBC medium amended with various concentrations of $FeSO_4$ (added from a 100 mM stock prepared in 0.1 N HCl). Growth inhibition is apparent with 3.5 mM Fe(II) (red square) and 4 mM Fe(II) (green trangle). Growth curves are an average of four cultures monitored in parallel (technical replicates), and the results are representative of experiments performed at least three times.

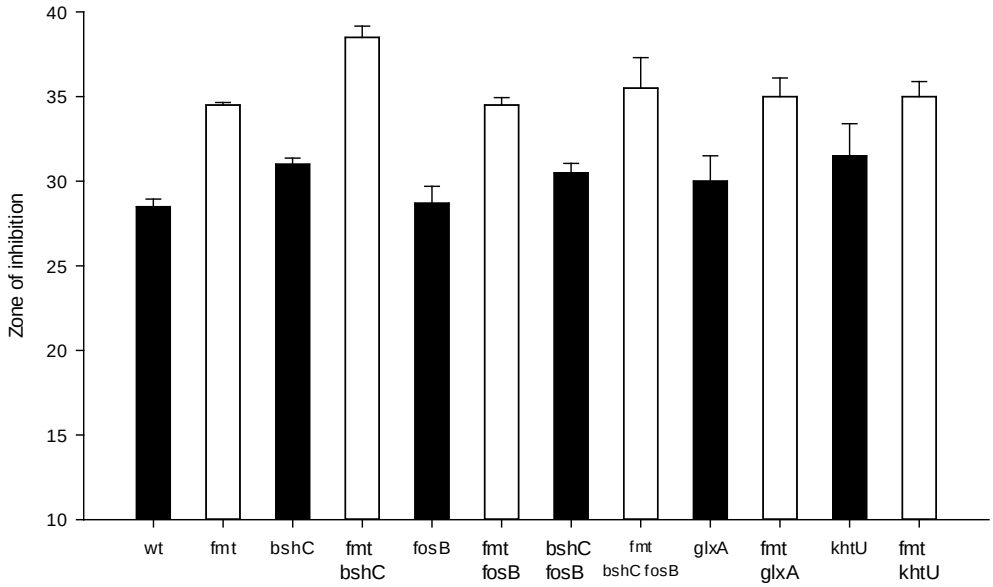

FIG S4 Disk diffusion assay of WT (WT; CU1065; black bars) and mutant strains on Methylglyoxal (MG, 22 μmol). The results are expressed as the diameter of the inhibition zone (mm) minus the diameter of the filter paper disk (6.5 mm). The mean ± SE from at least three biological replicates are reported.

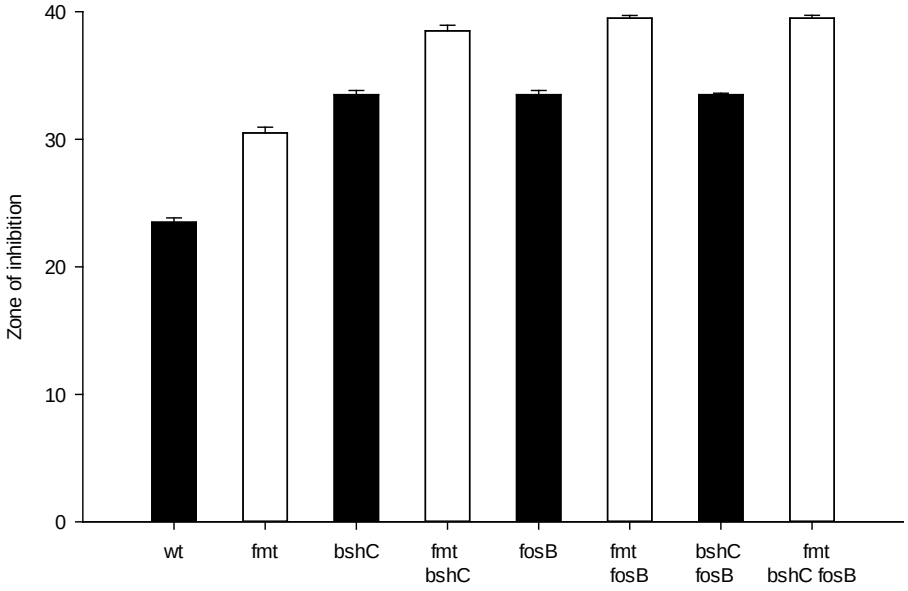

FIG S5 Disk diffusion assay of WT (WT; CU1065; black bars) and mutant strains on fosfomycin (250 µg). The results are expressed as the diameter of the inhibition zone (mm) minus the diameter of the filter paper disk (6.5 mm). The mean ± SE from at least three biological replicates are reported.

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