Table S1: List of bacterial strains, plasmid vectors and primers used in this study.

***Francisella* Strains**	**Genotype**	**Source**
F. tularensis LVS	Wild type strain	ATCC
Δ*tolC* mutant	*Deletion mutant of F. tularensis* LVS *tolC* gene	(Gil *et al.*, 2006)
acrB mutant	*F. tularensis* LVS, *acrB ::Tn5*, Kan^r	This Study
secA mutant	*F. tularensis* LVS, *secA ::EZ::Tn*, Kan^r	(Su *et al.*, 2007)
secE mutant	*F. tularensis* LVS, *secB ::EZ::Tn*, Kan^r	(Su *et al.*, 2007)
Δ*KatG* mutant	*Deletion mutant of F. tularensis* LVS catalase gene	(Lindgren *et al.*, 2007)
Δ*KatG* + pΔS*KatG*	Δ*KatG mutant transcomplemented with* pΔS*KatG lacking putative KatG signal sequence*	This Study
emrA1 mutant	*F. tularensis* LVS, *FTL_0687 ::Tn5*, Kan^r	This Study
emrA1 transcomplement (*emrA1* + p*emrA1*)	*F. tularensis* LVS, *FTL-0687 ::Tn5*, (pMP822+*FTL_0687*),Kan^r, Hygro^r	This Study
SodB mutant	*Point mutant of F. tularensis LVS Fe-SOD gene*	(Bakshi *et al.,* 2006)
SodBC mutant	*Point mutant of F. tularensis LVS Fe-SOD gene and deletion mutant of Cu-ZnSOD gene*	(Melillo *et al.,* 2009)
***E. coli* Strains**		
DH5α	F– Φ80*lacZ*ΔM15 Δ(*lacZ*YA-*arg*F) U169 *rec*A1 *end*A1 *hsd*R17 (rK–, mK+) *pho*A *sup*E44 λ– *thi*-1 *gyr*A96 *rel*A1	Invitrogen
ME006	DH5α with pMP822+*FTL_0687* ,Hygro^r	This Study
Plasmids		
pMOD3	EZ::Tn5,Kan^r	Epicentre
pMP822	*E. coli-Francisella* shuttle vector ,Hygro^r	(LoVullo *et al.*, 2006)
pMM001	pMP822 + FTL_0687,Hygro^r	This Study
pΔS*KatG*	pMP822 + Δ*SKatG lacking putative KatG signal sequence*, Hygro^r	This Study

Primers	**Sequence**	**Purpose**
Sqfp	5'-GCCAACGACTACGCACTAGCCAAC-3'	Forward primer for sequencing transposon insertion sites
Sqrp	5'-GAGCCAATATGCGAGAACACCCGAGAA-3'	Reverse primer for sequencing transposon insertion sites
MP003	5'- AGGGATCCATGTCAGAAGAAAAATTACAAC-3'	Forward primer for cloning *FTL_0687* gene at *BamHI* site of pMP822
MP004	5'-CGCTGCAGCTATAATGTATCTACTTTTAC-3'	Reverse primer for cloning *FTL_0687* gene at *PstI* site of pMP822
MP015	5'-AAGCCAAGTTAGTGCTGCATATTT-3'	Forward primer for qRT-PCR of *FTL_0686*
MP016	5'-AAGCCAAGTTAGTGCTGCATATTT-3'	Reverse primer for qRT-PCR of *FTL_0686*

MP019	5’-TCCTAATCCCTGAATAGCCGTTGT-3’	Forward primer for qRT-PCR of *FTL_0688*
MP020	5’-AGGTGTTGCCGCTATTATTGGTGC-3’	Reverse primer for qRT-PCR of *FTL_0688*
MP029	5’-TCGCAGGTTTAGCGAGCTGTTCTA-3’	Forward primer for qRT-PCR internal control
MP030	5’-ACAGCAGCAGCTTGCTCAGTAGTA-3’	Reverse primer for qRT-PCR internal control
MP105	5’- CAAGAAATCAACAAACCACCTATC-3’	Forward primer for RT-PCR of *FTL_0688-0687* intergenic region (Primer E)
MP106	5’-CAAATTGGTTGTTGTGGATATAGA-3’	Reverse primer for RT-PCR of *FTL_0688-0687* intergenic region (Primer F)
MP107	5’-AGCCAGGGCAAAAAGTTGAA-3’	Forward primer for RT-PCR of *FTL_0687-0686* intergenic region (Primer C)
MP 108	5’-TTTATCTCAGCATTAGCTTGTAGG-3’	Reverse primer for RT-PCR of *FTL_0687-0686* intergenic region (Primer D)
MP111	5’-GTTACATCTGCTATGGTTTCAACA-3’	Forward primer for RT-PCR of *FTL_0688-0689* intergenic region (Primer A)
MP112	5’-GAGCCATTAGGGGTACTAACATGA-3’	Reverse primer for RT-PCR of *FTL_0688-0689* intergenic region (Primer B)

Figure S1

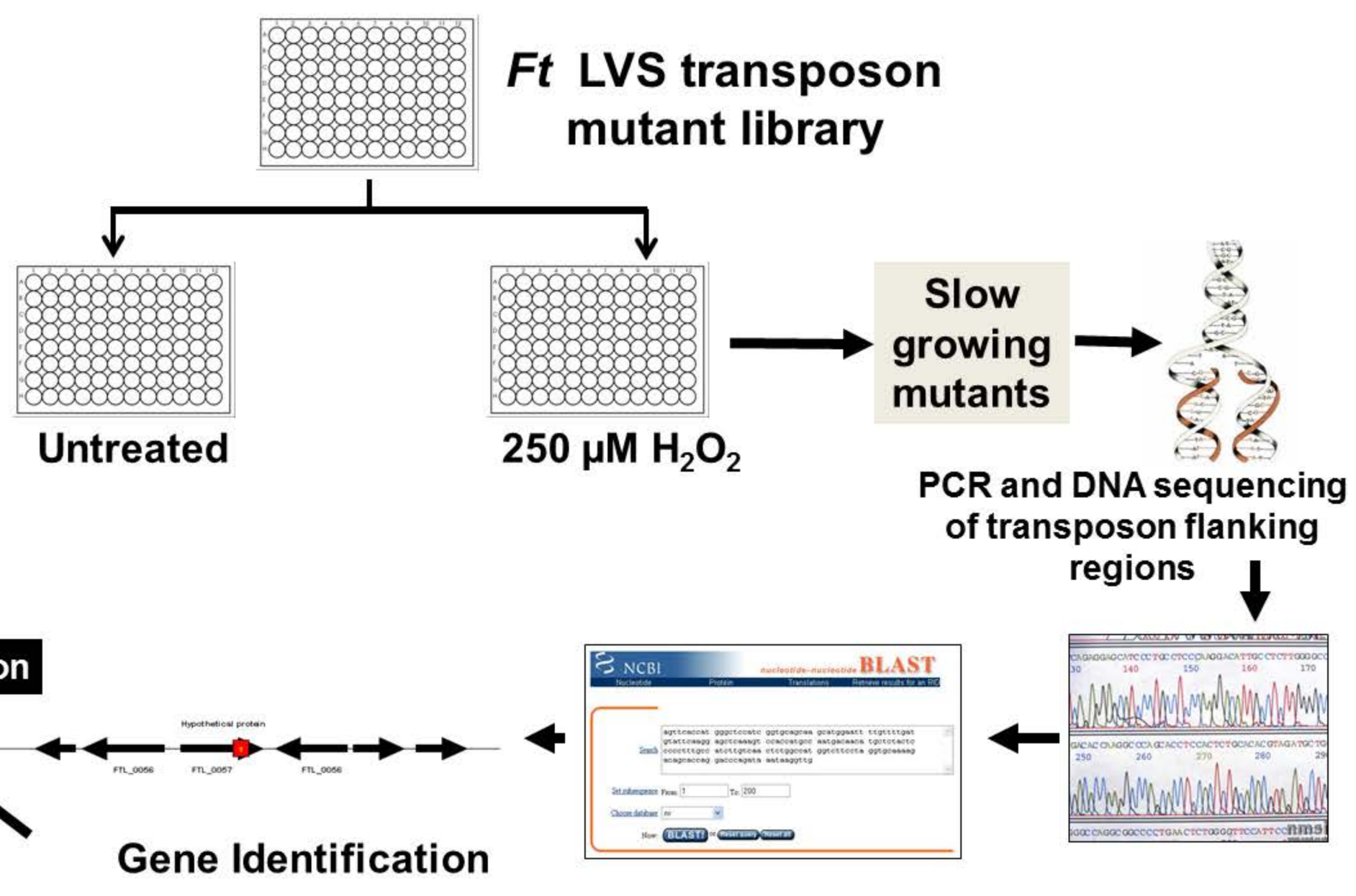

Fig. S1: Scheme for screening of transposon mutants of *F. tularensis* LVS for the identification of H_2O_2 sensitive mutants.

Figure S2

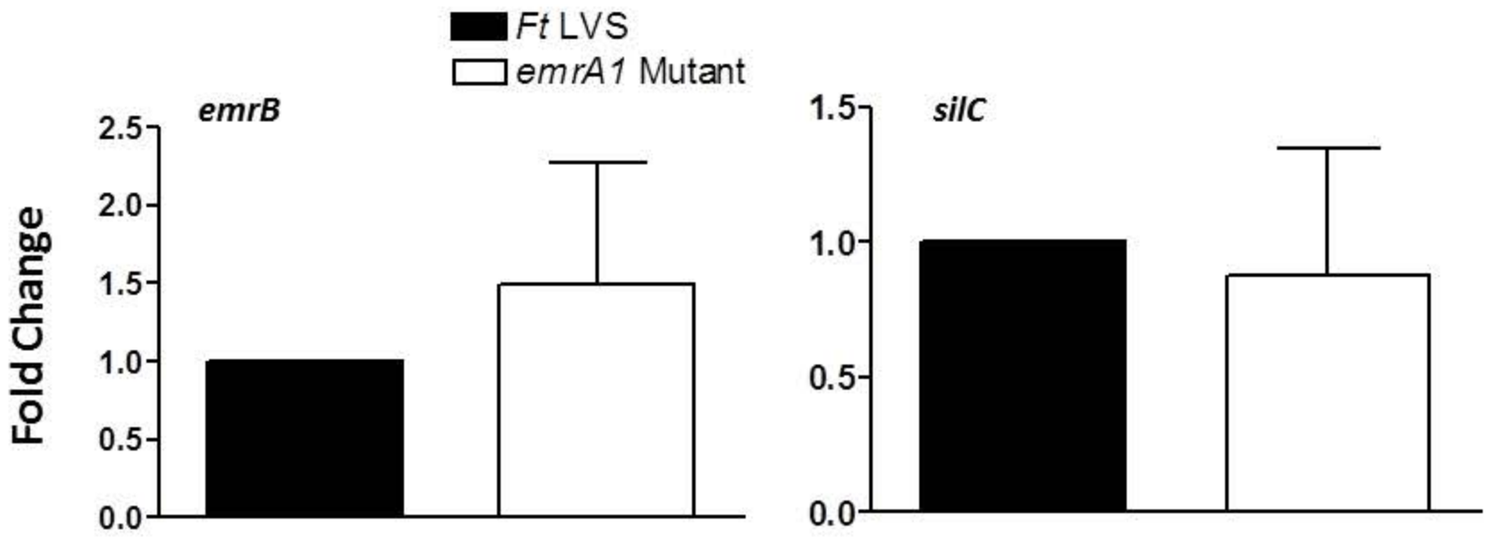

Fig. S2: Quantitative RT-PCR analysis of genes upstream (*silC)* and downstream *(emrB)* of the *emrA1* gene.

Figure S3

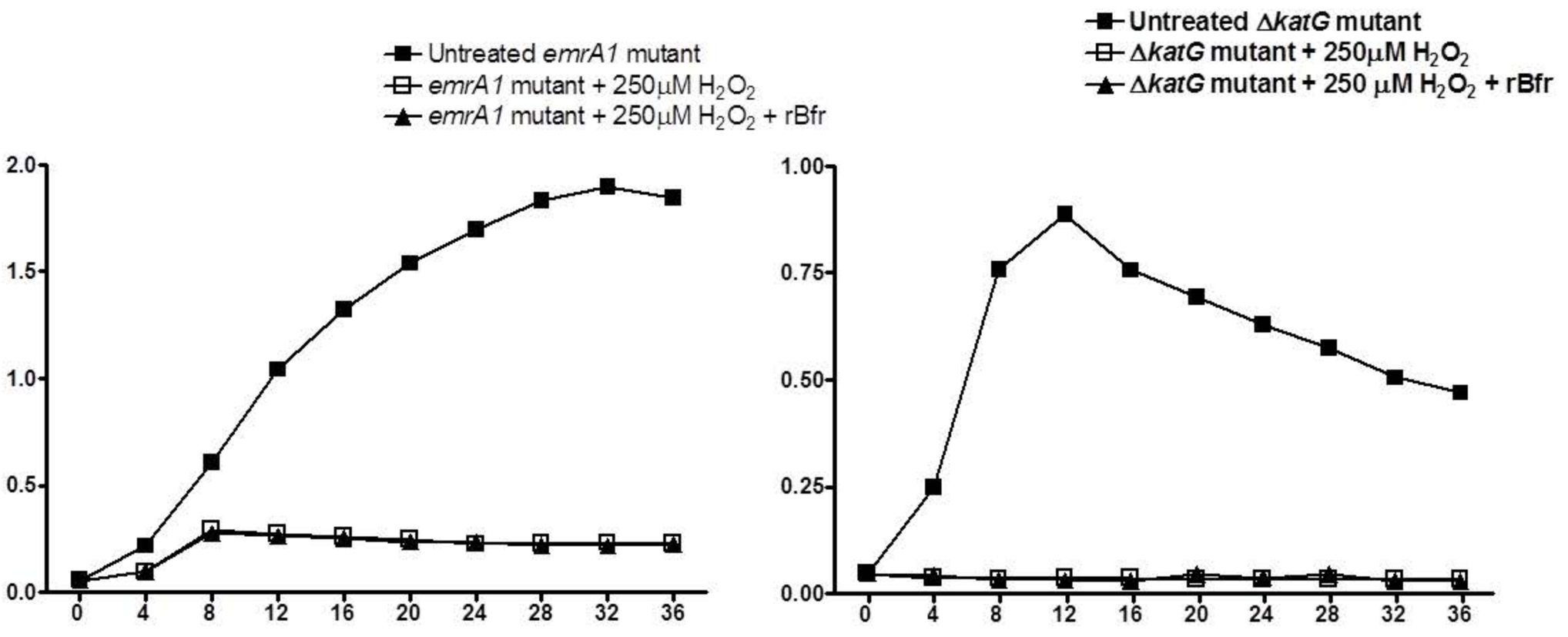

Fig. S3: Growth curves of *F. tularensis* (*Ft*) LVS and the *emrA1* mutant (B) and Δ*katG* mutant (C) in the absence or presence of 250μM of H_2O_2 and with or without the addition of recombinant bacteroferritin (rBfr). The cultures were grown for 24 hrs and OD_{600} readings were recorded every 4 hrs

Figure S4

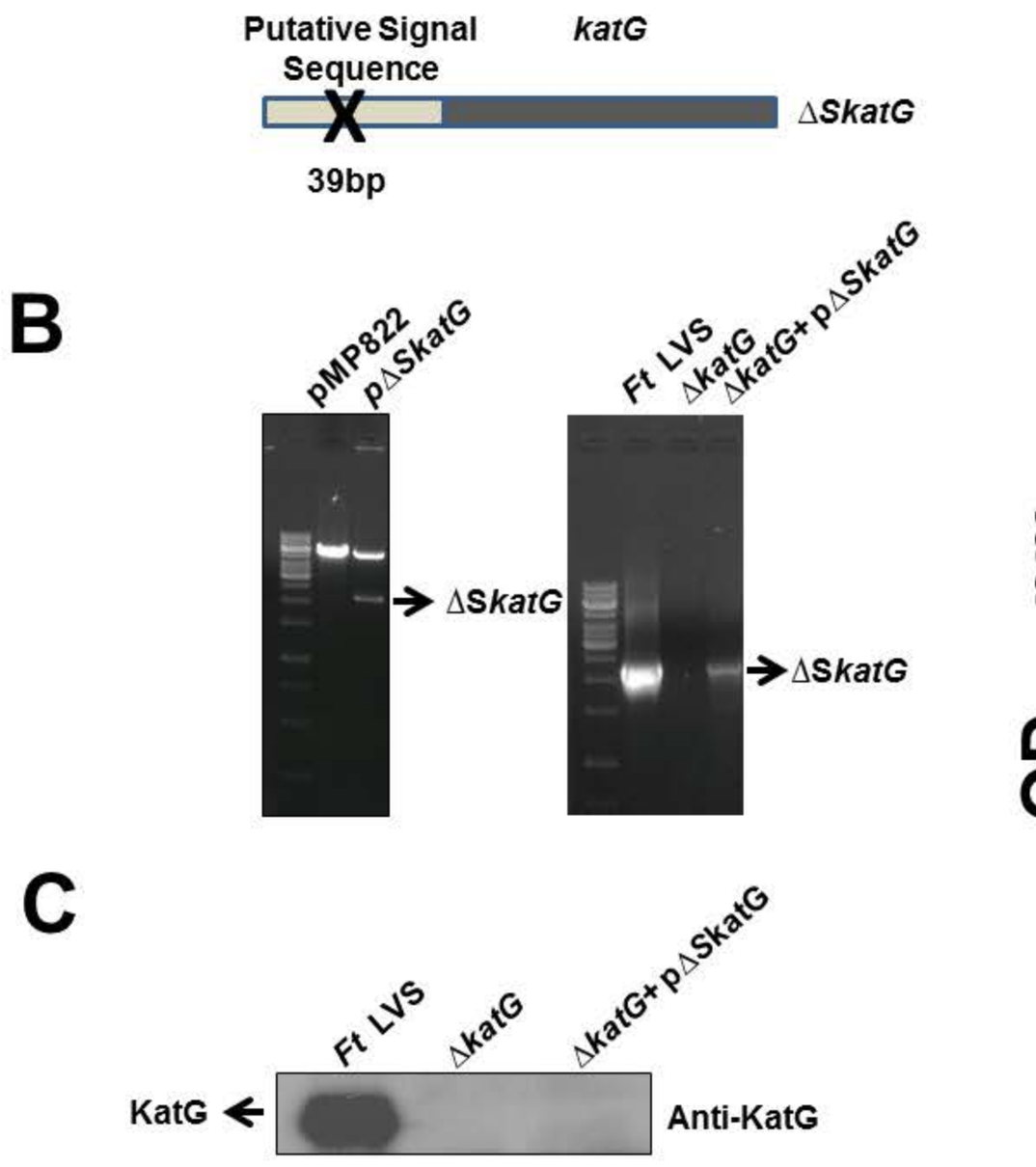

Fig. S4: Signal sequence is required for the expression of KatG in *F. tularensis* LVS. (A) Schematic representation of the strategy used for generation of *katG* transcomplement strain. The *katG* gene was amplified using LVS genomic DNA as a template employing primers KatG *PstI* F (GCATGCCTGCAGATGCTACTAGCTTCTAGCAATGC) and KatG *XhoI* R (GCATGCCTCGAGTTATTGTTGAACATCAAATCTGCC). The first 39 nucleotides of the katG gene at 5' end which spans the putative signal sequence were excluded. The PCR product was digested with *Pst*I and *Xho*I and ligated into similarly digested pMP822 plasmid.

(B) Confirmation of *katG* transcomplement strain. Left gel represents the release of *katG* insert after restriction enzyme digestion of pΔ*SkatG* with *Pst*I and *Xho*I. The pMP822 plasmid was used as vector control. Right gel represents PCR of LVS parent strain, Δ*katG* and Δ*katG* + pΔ*SkatG* strains. Primers are specific for a region within *katG* gene to confirm the presence of katG.

(C) Western blot analysis of ΔSkatG transcomplement strain. Cell lysates were prepared from LVS parent strain, Δ*katG* and Δ*katG* + pΔ*SkatG* strains. The blot was probed with anti-KatG antibody to indentify KatG protein.

(D) Growth curve of Δ*katG* and Δ*katG* + pΔ*SkatG* strains in presence or absence of H_2O_2. The graph represents A_{600} measurements of Δ*katG* and Δ*katG* + pΔ*SkatG* strains following 16 hours of treatment with 250 μM of H_2O_2.

Figure S5

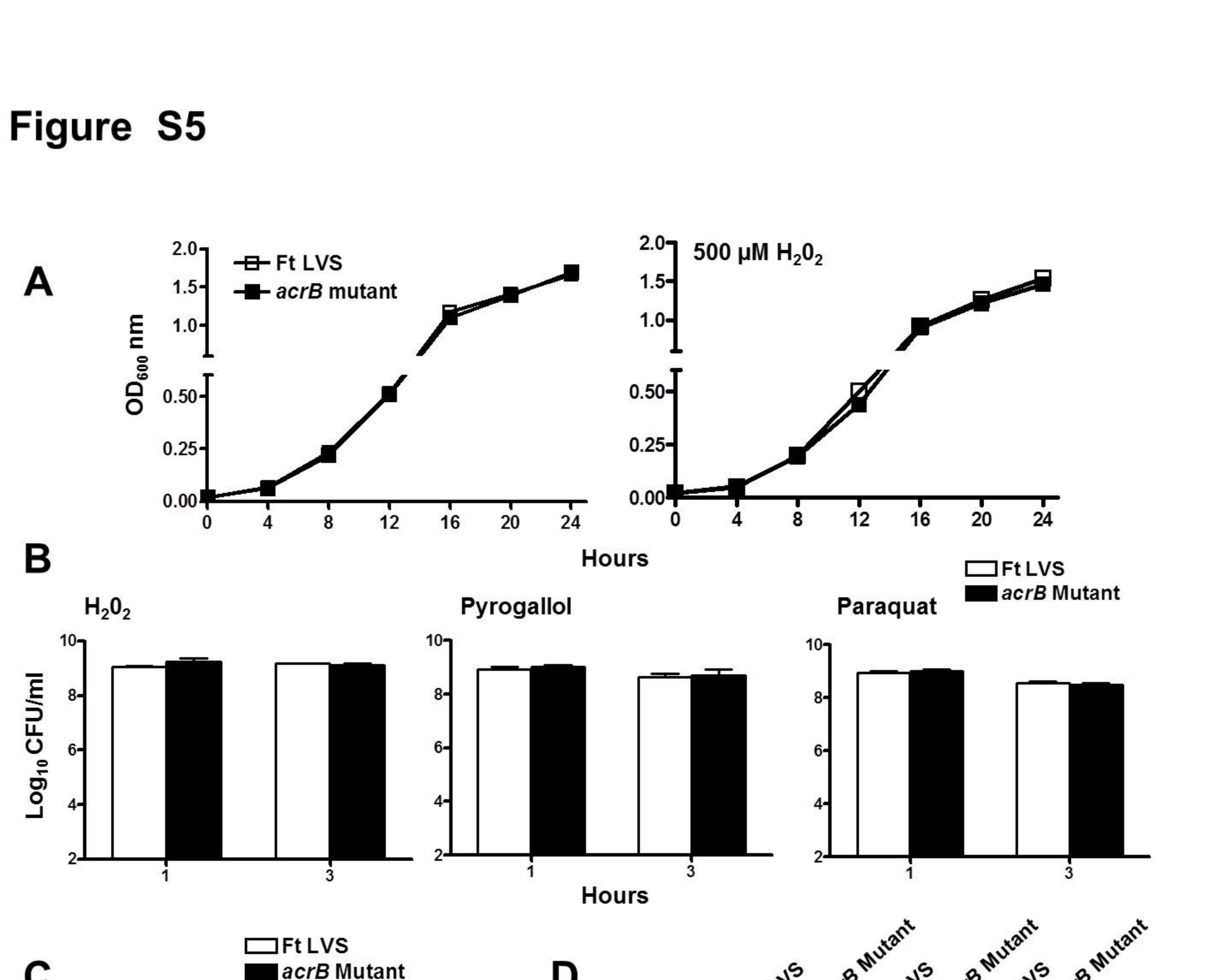
A
Ft LVS
acrB mutant
OD600 nm
2.0
1.5
1.0
0.50
0.25
0.00
0
4
8
12
16
20
24
Hours
500 μM H2O2
B
Ft LVS
acrB Mutant
H2O2
Pyrogallol
Paraquat
Log10 CFU/ml
10
8
6
4
2
1
3
Hours
C
Ft LVS
acrB Mutant
Log10 CFU/ml
8
7
6
5
4
4
24
Hours
D
Ft LVS
acrB Mutant
Ft LVS
acrB Mutant
Ft LVS
acrB Mutant
Bacterial lysate
Supernatant
Bacterial lysate
Supernatant
SodB
KatG
4
8
12
Hours

Fig. S5: Membrane fusion protein AcrB of RND multidrug efflux pump is not required for resistance to oxidants and secretion of antioxidant enzymes SodB and katG.

(A) Growth curves of *F. tularensis* (*Ft*) LVS and the *acrB* mutant in the absence or presence of 500μM of H_2O_2. The cultures were grown for 24 hrs and OD_{600} readings were recorded every 4 hrs.

(B) The bacterial killing assay. The *F. tularensis* LVS or the *acrB* mutant cultures were exposed to 1mM of hydrogen peroxide, 1mM Paraquat or 1 mM Pyrogallol for 1 and 3 hrs (n=3). The cultures were diluted 10-fold and plated on MH-chocolate agar plates for bacterial enumeration. The results are expressed as Log_{10} CFU/ml.

(C) Macrophage invasion assay. Murine BMDMs derived from wild type C57BL/6 were infected with Ft LVS or the *acrB* mutant at 100 MOI. The cells were lysed at 4 and 24 hrs and plated on MH-chocolate agar plates for enumeration of bacterial CFU (n=3 biological replicates).

(D) The culture filtrates or the lysates of the bacterial pellet of Ft LVS or the *acrB* mutant grown in BHI broth were analyzed at the indicated times by western blot analysis using anti-SodB and anti-KatG antibodies.