S1

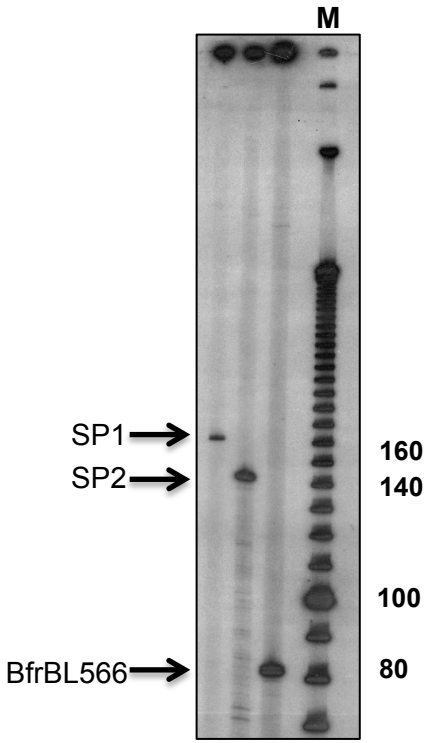

Fig. S1. Primer extension of *bfrB* mRNA. Total RNA was isolated from a *Mtb* culture grown in high iron conditions. The 5' mRNA termini was mapped by extension of γP^{32} labeled reversed primers specific to the *Mtb bfrB mRNA*. A 10 bp ladder (M) was used as a molecular weight standard to determine the length of the primer extension product. The transcriptional start point was verified using primers bfrBSP1, bfrBSP2 and bfrBL566 (Table S1) whose 3' ends were 20 to 60 bp apart. The extension product of each primer is labeled in the gel as lane 1,2 and 3 respectively.

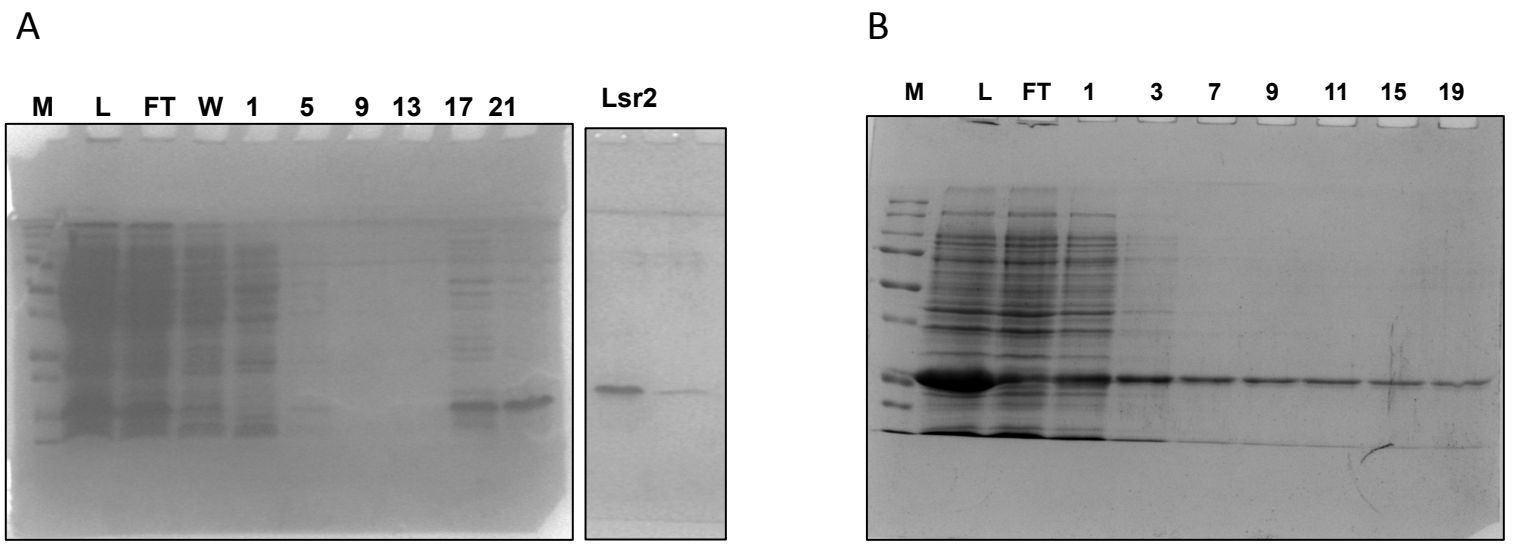

Fig. S2. Protein purification: Lsr2 and IdeR were expressed and purified as described in Materials and Methods A. Lsr2 purification. B. IdeR purification. In A and B: M= molecular weight markers, L= lysate from the over expression strain that was loaded on the His-Trap column, FT= flow though, unbound protein, W= wash with 50 mM imidazole. A. 1-21, eluted fractions. Fractions 17-21 enriched in Lsr2 were pooled dialyzed and after affinity purification using Talon matrix pure Lsr2 was obtained (lanes labeled as Lsr2). In B. 1 to 19, eluted fractions from His-trap column. Fractions 9-19 containing pure IdeR were combined and concentrated.

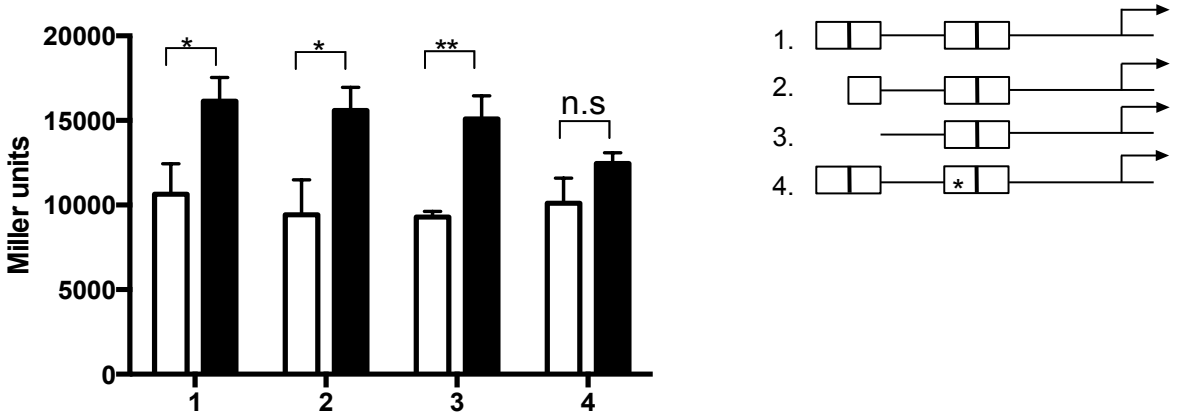

Fig. S3. The intact or modified DNA sequence upstream of *bfrB* containing the four iron boxes (IB1 to IB4) were fused to *lacZ*. The fusions were individually introduced into *Isr2* strain and the effect of the modifications on iron induction of *PbfrB* was determined by measuring β-galactosidase activity in cells grown in high iron medium comparing to those from low iron condition. 1. Intact P*bfrB* upstream sequence. 2. Deletion of IB1. 3. Deletion of IB1 and IB2. 4. Mutated IB3 sequence* (AGCCTT changed to CTATGC). The background activity of strains transformed with the vector alone was subtracted from the total activity. The values represent means ± SD from biological triplicates. *, $p<0.05$, **, $p<0.005$ and n.s not significant.

Table-S1 Sequence of oligonucleotides used in the study:

Primer	Sequence
IdeRFp	5’-GCAAGCATATGAACGAGTTGGTTG-3’
IdeRRp	5’-CCAGAAGCTTGGGTAGGTGC GGGTTAGC-3’
bfrBSP1	5'-CGACCGCTTGGCTGTA-3'
bfrBSP2	5'-AATGCTTCGCCAACTG-3'
bfrBL566	5'-TTGTGCCGCTGTGAA-3'
bfrB339	5’-AGGGCGACAAGAGTAA-3’
bfrB523	5’-ACGCGTGGAATTTTGTGT-3’
bfrBFp	5’-CGGATTCAGCGAGTTCGC-3’
bfrBRp	5’-TTGTGCCGCTGTGAATTC-3’
lsr2 Fp	5’-GACAATTGCGGATCCCGGTCTGAGTGCGTAGTACCT-3’
lsr2 Rp	5’-TTCTGCAGCTGGATCCGTTGATGATGGCGTTGACAG-3’
*mbtb*Fp	5’-GGCATCGGAGAGCACGGTGT-3’
*mbtb*Rp	5’-CTGTGGTCAACCCTGGTCCG-3’