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**Supplementary Material to:**

**Induced ectopic expression of HigB toxin in Mycobacterium tuberculosis results in growth inhibition, reduced abundance of a subset of mRNAs and cleavage of tmRNA**

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**Table S1.** RNA sequencing analysis.

**Table S2.** Oligonucleotides used in this study.

**Fig. S1.** Quantitative RT-PCR analysis of higB expression in ΔTAC strains.

**Fig. S2.** HigB expression with varying metal ion concentrations.
Table S1. RNA sequencing analysis of M. tuberculosis H37Rv overexpressing HigB and the empty vector control strain. Number of reads mapped and RPKM (reads per kilobase per million) values are indicated for each of the annotated H37Rv genes. (Essentiality is taken from Sassett et al., 2003).

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**Table S2.** Oligonucleotides used in this study.

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Fig. S1. Quantitative RT-PCR analysis of higB expression in *M. tuberculosis* ΔTAC strains.

*M. tuberculosis* ΔTAC strains carrying vector control ("Vector") or the HigB expression plasmid ("HigB") were grown to mid-exponential phase and treated with 300 ng mL⁻¹ ATc. RNA was isolated 24 h after addition of ATc. Quantitative RT-PCR analysis was carried out relative to sigA mRNA. All results are the mean values and standard deviation of three independent biological replicates. A significant difference (as determined by Student’s t-test) between strains is marked by **** for p<0.0001.
Figure S2. HigB expression in strains grown in media with varying metal ion concentrations. Strains carrying vector control (“Vector”) or the HigB expression plasmid (“HigB”) were grown to OD ~ 0.7 and inducer was added. A. Growth in Dubos medium. Upon addition of ATc, medium was also supplemented with 300 µM ferric ammonium citrate and 30 µM zinc sulphate heptahydrate (“+ zinc/iron”) to increase the concentration of iron and zinc in the medium. B. Growth in Sauton’s medium. Upon addition of ATc, liquid cultures were supplemented with 0.5 µM (“+ 0.5 TPEN”) or 1 µM (“+ 1.0 TPEN”) of the zinc chelator TPEN (Cayman Chemical) to deplete the amount of zinc in the medium.