Supplementary Material for

The Psp system of *Mycobacterium tuberculosis* integrates envelope stress sensing and envelope preserving functions

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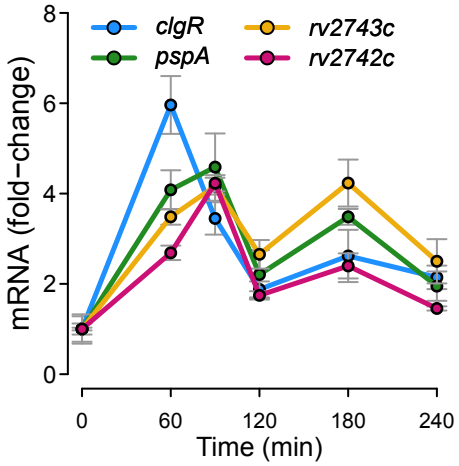

Figure S1. Stress response of the *clgR-pspA-rv2743c-rv2742c* genes.

Mid-log cultures of the wild-type strain were treated with 0.03% SDS, and samples were harvested pre-treatment (time 0) and at multiple times post treatment. Transcript enumeration was performed as described in the legend to Fig. 2C. Data are presented as fold-change relative to time 0.

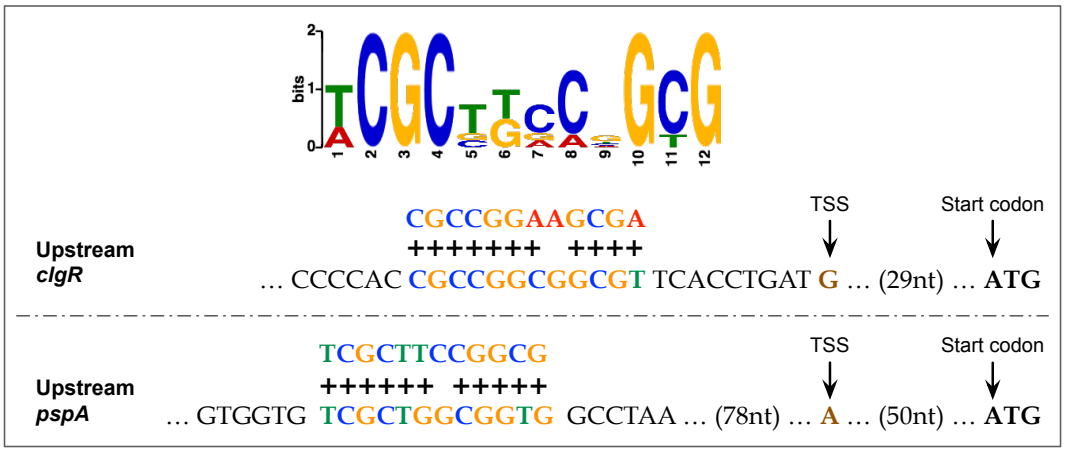

Figure S2. Identification of ClgR-binding sites in the *clgR-pspA* region.

The consensus binding site for ClgR (Estorninho *et al.*, 2010), presented as a sequence logo indicating the relative frequency of each base at each position, was used to search for potential ClgR binding sequences upstream of *clgR* and *pspA*. Shown are sequence matches associated with *p*-values of 1.2E-5 (top row) and of 6.2E-6 (bottom row). Transcription start sites and start codons are shown as in Fig. 2E. It is noted that promoter and predicted ClgR binding sequences in IG1 are maintained in the *clgR* knock-out mutant used in this study.

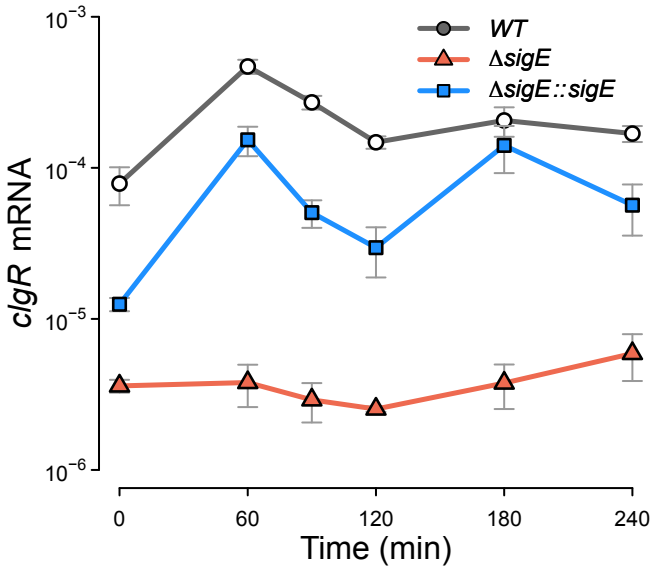

Figure S3. Requirement of *sigE* for *clgR* surface stress response.

Mid-log cultures of *M. tuberculosis* were treated with 0.03% SDS, aliquots were harvested at time 0 and at multiple times post-treatment, as indicated. The *clgR* induction profile was determined by transcript enumeration in wild-type, *sigE* mutant, and complemented strains and plotted, as described in the legend to Fig. 2C. Mean values (+/- standard error of the mean) from triplicate experiments are shown.

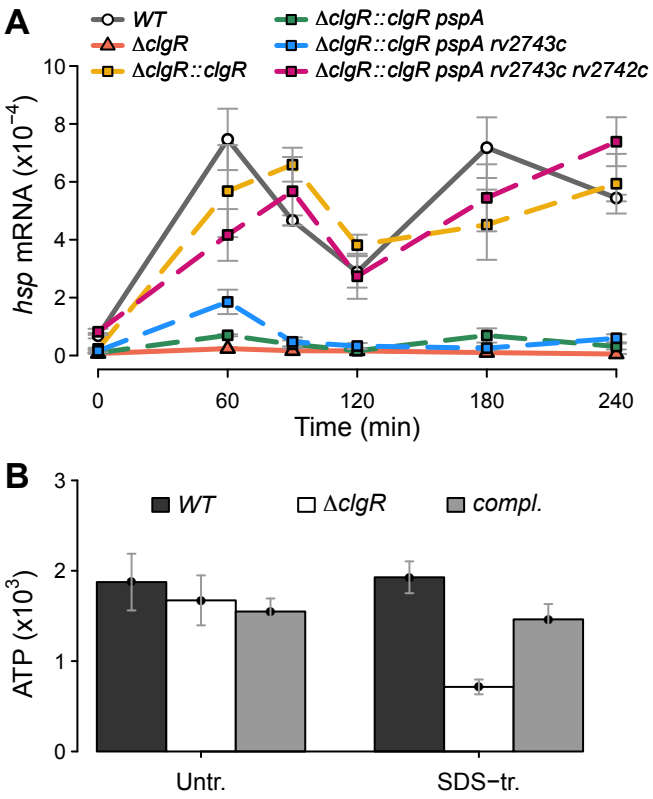

Figure S4. Gene expression and phenotypic effects of *clgR* inactivation.

(**A**) Effects on *hsp* stress response of *clgR* deletion and sequential complementation with *clgR-psp* operon genes. Expression of the sentinel target gene *hsp* was used as readout of ClgR activity (Estorninho *et al.*, 2010). Complementation of the *clgR* deletion was performed sequentially by introducing *clgR* alone, *clgR* plus *pspA*, plus *pspA-rv2743c,* and plus *pspA-rv2743c-rv2742c*. Wild-type, mutant, and complemented strains were subjected to surface stress and *hsp* transcripts were enumerated and transcript copy numbers normalized to 16S rRNA, as described in the legend to Fig. 2C. Normalized data are presented as mean values (+/- standard error of the mean) from triplicate experiments. (**B**) Effect on intracellular ATP levels. Mid-log cultures of wild-type, *clgR* mutant, and *clgR-pspA-rv2743c-rv2742c* complemented (compl.) strains were treated with 0.03% SDS for 24 hrs. Extracts from pre- and post-treatment cultures were then assayed for ATP levels and expressed as ng of ATP per mg of total protein. Data are presented as mean values (+/- standard error of the mean) from triplicate experiments.

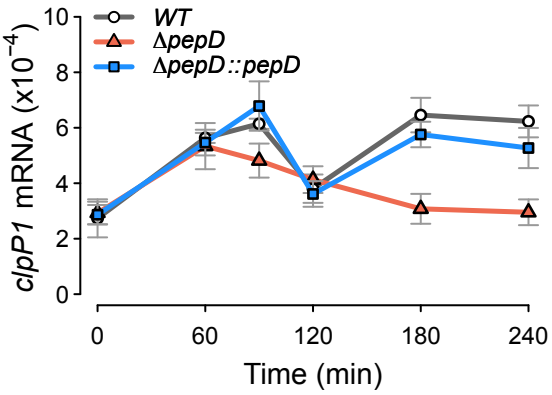

Figure S5. Role of *pepD* in ClgR activity.

ClgR activity was assessed as expression of the sentinel target gene *clpP1* as in Fig. 3. Wild-type, *pepD* mutant, and complemented strains were subjected to surface stress and *clpP1* transcripts were enumerated and plotted as described in the legend to Fig. 2C. Normalized data are presented as mean values (+/- standard error of the mean) from triplicate experiments.

Figure S6. Distribution of ClgR-PspA-Rv2743c-Rv2742c proteins in ClgR-positive Actinobacteria.

Significant matches and similarity scores were obtained as described in Methods. The heat map represents the similarity score per target species or sub-species (or the highest score in the case of multiple matches), expressed as percent of protein sequence similarity for the longest contiguous match. Representative species of Actinobacteria (taxid: 201174) were ordered on the basis of phylogenetic similarity ((Wattam *et al.*, 2014) and http://www.patricbrc.org). For each comparison, the length of the bar is proportional to the similarity score, in % (full bar = 100% homology). *Mycobacterium spp.* (shown in greater detail in Fig. 9) are presented in a shaded box. Proteins annotated as PspA in ~ 40 species of Actinobacteria, including ~10 in *Streptomyces spp*., scored below cut-off in the present analysis and do not appear in the figure. With increasing evolutionary distance, ClgR sequence conservation specifically mapped to the XRE-type, helix-turn-helix DNA binding domain (SM00530, PF01381) of the protein.

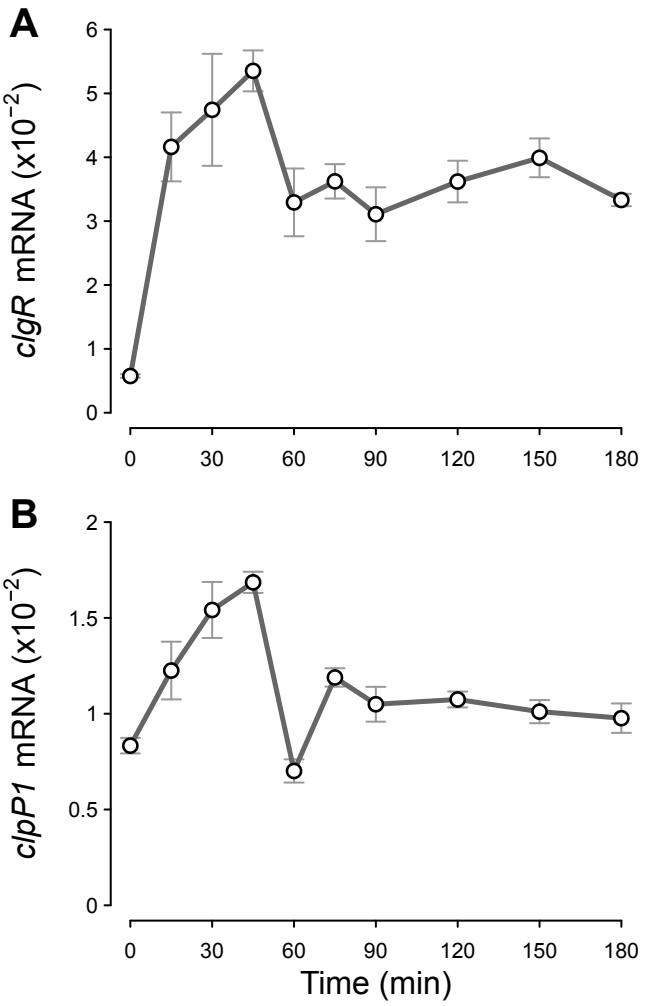

Figure S7. Stress response of *clgR* (*MSMEG 2694*) and *clpP1* (*MSMEG 4673*) in *M. smegmatis*.

Mid-log cultures of *M. smegmatis* were treated with a bacteriostatic concentration of SDS (0.02%), and culture aliquots were harvested pre-treatment (time 0) and at multiple times post-treatment, as indicated. Transcripts were enumerated and plotted as described in the legend to Fig. 2C.

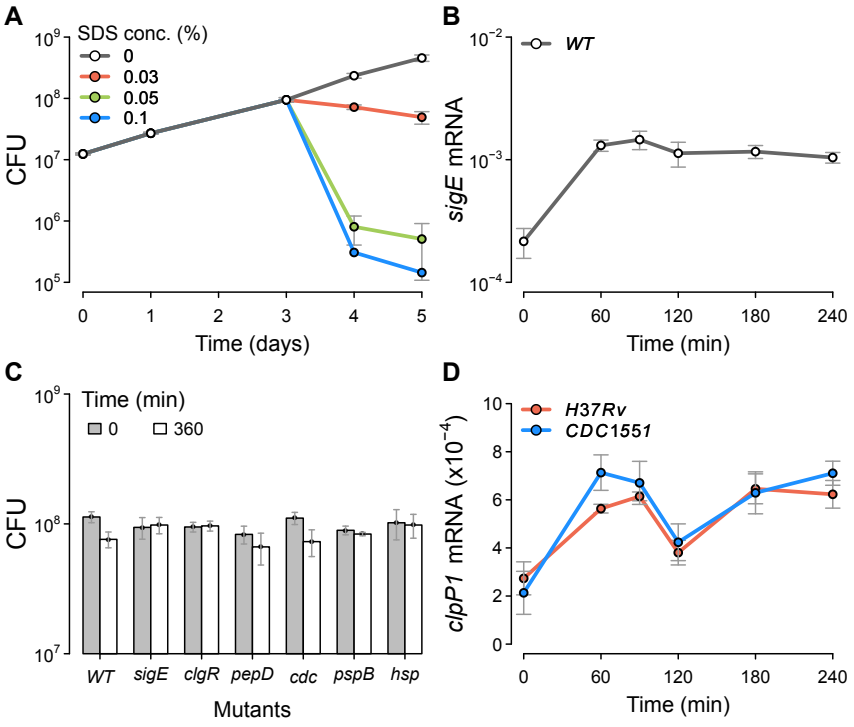

Figure S8. Stress conditions used in this study.

(**A**) Determination of bacteriostatic SDS concentration. An *M. tuberculosis* culture was grown to exponential phase and divided into multiple culture tubes that were treated with increasing concentrations of SDS, as indicated. Culture aliquots were harvested at various times (as indicated), diluted, and plated for CFU enumeration. (**B**) Effect of surface stress on *sigE* expression. Wild-type strain was subjected to treatment with 0.03% SDS, and *sigE* transcripts were enumerated, as described in the legend to Fig. 2C. Mean values (+/- standard error of the mean) from triplicate experiments are shown. (**C**) Susceptibility of various mutants to bacteriostatic SDS concentration. Mid-log cultures of wild-type and mutant strains were subjected to surface stress for 6 hrs. Culture aliquots were collected before (time 0) and after treatment (360 min), diluted, and plated for CFU enumeration. Mean values (+/- standard error of the mean) from triplicate experiments are shown. (**D**) Surface stress response of *clpP1* in two *M. tuberculosis* strains. Mid-log cultures of the reference strain *M. tuberculosis* H_{37}Rv and the clinical strain *M. tuberculosis* CDC1551 were treated with 0.03% SDS and *clpP1* transcripts were enumerated as described in the legend to Fig. 2C. Mean values (+/- standard error of the mean) from triplicate experiments are shown.

Table S1. Primers used in the study

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Primers for construction of complementation fragments		
Genes	**Primer pairs(5'-3')**	**Mutations**
clgR	Fwd: ATATAGGTACCATCGGATTGTCGATGCTC Rev: ATATTCTAGATTAGGCCACCGCCAGCGACACCA	
clgR-pspA	Fwd: ATATAGGTACCATCGGATTGTCGATGCTC Rev: ATATTCTAGACTACTGACCGTAGGGCTGCTC	
clgR-pspA-rv2743c	Fwd: ATATAGGTACCATCGGATTGTCGATGCTC Rev: ATAATTCTAGACTAACGCCGGGGCAATCC	
clgR-pspA-rv2743c-rv2742c	Fwd: ATATAGGTACCATCGGATTGTCGATGCTC Rev: ATCATAAGCTTGCTGCTGGAGGGCCAGAA	
ΔclgR-ΔpspA-rv2743c-rv2742c	Fwd: ATATAGGTACCATCGGATTGTCGATGCTC Rev: ATAATTCTAGACAGCGCCATGAGGTACTT	895bp
	Fwd: ATAATTCTAGAACTTCTGGCGGGGCTATT Rev: ATCATAAGCTTGCTGCTGGAGGGCCAGAA	1848bp (Δ*pspA*)
	Fwd: GGAGCGCGGATGTAATCGCAGGGTCGG Rev: CCGACCCTGCGATTACATCCGCGCTCC	stop TAA (Δ*clgR*)
P_{clgR}::*lacZ*	Fwd: TATAGATCTATCGGATTGTCGATGCTCGGCGAC Rev: TATGGTACCGCGCAGCACGTCACCAACGACCTC	
IG1::*lacZ*	Fwd: ATATAGATCTGGCGCCACCATTGACGCCAGCAC Rev: ATATGGTACCCGCCATGAGGTACTTCCAGGCTTT	

Primers Primers and molecular beacons for real time measurements	
Genes	**Primer pairs(5'-3') and Molecular beacon (MB)**
lacZ	Fwd: TTGTTGCCATTGCTACAGGCATCG Rev: TGTAACTCGCCTTGATCGTTGCGA MB: ACCCGGGGTGTCACGCTCGTCGTTTGGCCGGGT
clgR	Fwd: TGAGCCTCGGGTATCTGTCGG Rev: AAGGCGCTCTTGACGCGCCAT MB: ACGGGGCGAGCTGCTCAGTGCGATTTGTACCCCGT
IG1	Fwd: TGTCGCTGGCGGTGGCCTAA Rev: CTTCCAGGCTTTAACGAACGGATT MB: GCGGGGGGGTCGACAAGATGCGCGCCATGGGTGCCCCGC
pspA	Fwd: CAAGGTGCAGATTCAACAGGCCAT Rev: TCTCCAATTGACGCTGGTTACCGA MB: GGCCCCCACCCACCAAGCGCTGACTCAACAGGGGCC
IG2	Fwd: ACTTCTGGCGGGGCTATT Rev: CACGCCGCGCTGCAGCAA MB: GGCGCAGTAGTTGGGCAGACGGTTCGGCATGCGCC
rv2743C	Fwd: GTTCTTCTCGCTGTTGGGTGTCAT Rev: TTGATGGTCGGCACCAGATACGA MB: ACGGGGGAGGTGGTCTCGATGGAGCGGGCCCCGT
IG3	Fwd: GGATTGCCCCGGCGTTAG Rev: CTGCGCGTGCACAATCTT MB: CGCGCTTCGATATACGCCCGGTGAATGTGAGCGCG
rv2742c	Fwd: GGACGCCGAAGTGGTAATG Rev: AACGAACGCACCGAAAC MB: ACCCGCCCGTTGTACCTCGAAAATGCCGCGGGT
clpP1	Fwd: AAAGAAATGTTCCGGCTCAACGCC Rev: CGGGTGATGATGTGATCGACGAAA MB: GGGCCCGACCGCTGGTTCACCGCCGCCGAAGGGCCC
hsp	Fwd: ACCGCTGGCTACGTGACTTCTT Rev: TCAATGCCGGGCAGTTCCAAA MB: ACCCCGTCAAGGATGGCGACGACGCGGGGT
mprA	Fwd: CGCACCAAGCCCGAGGAT Rev: CACGGTTGACTTCGCGGGTTA MB: CGCGCTGCCGCCGAGTCGATGGCCATGAGCGCG
sigB	Fwd: ACAAGCCGGGTTGACAGCGATCT Rev: TGCGCTTGGCCAGTTCGACTTC MB: CGCACGCTCAAAGCCCCGCGGCGGACGTGCG
sigE	Fwd: ACGACTTGCCAACTTATTGCAG Rev: GGATGAGACATGCTGGTCGGA MB: CGCACGATATCACGACCATCACGACCTTGCGTGCG
16S rRNA	Fwd: ATGACGGCCTTCGGGTTGTAA Rev: CGGCTGCTGGCACGTAGTTT MB: CCCCGCCGACGAAGGTCCGGGTTCTCGCGGGG
clgR (MSMEG 2694)	Fwd: AGTCTGGGCTACCTGTCC Rev: CACGCGCGAAAGTGGTA MB: GCGGCATCGAGCGAGCTTCTCAGCGCCGC
clpP1 (MSMEG 4673)	Fwd: GGACATCCACCTGTACATCAA Rev: GAGCAGGAACTCACGCATC MB: ACGCCGGCGATCATCGACACCATGGTGCTCGCGGCGT

Primers for protein expression	
Genes	**Primer pairs(5'-3')**
clgR-Myc	Fwd: CATGCCATGGCGGCTTTGGTGCGTGA Rev: AATAAGCTTTCACAGATCCTCTTCTGAGATGAGTTTTTGTTCGGCCACC GCCAGCGACA
His-pspA	Fwd: AAATTAGATCTTATGGCCAATCCGTTGGTTAA Rev: AAATTGAATTCCTACTGACCGTAGGGCTGCT
rv2743c-Stag	Fwd: AAATTAGATCTTATGGCAGTGAAAGCGGGTCA Rev: AAATTGGTACCACGCCGGGGCAATCCGCCGA
His-rv2742c	Fwd: ATATGGATCCAGTGCTCGTCGACGAGCTCGGGGTC Rev: TATATAAAGCTTTCAGCCACCGGGCGTGCGGCGGCC
rv2742c-Stag	Fwd: ATATAGATCTTAGTGCTCGTCGACGAGCTCGGGGTC Rev: TATATAGGTACCGCCACCGGGCGTGCGGCGGCC

Underlined are the restriction enzyme sites used for cloning.