Supplementary Information

Title: **Identification of the thioredoxin partner of VKOR in mycobacterial disulfide bond formation**

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Supplementary Table 1. DsbA homologs from *M. tuberculosis* H37Rv

Gene in *M. tuberculosis*	Annotation[#]	Essential*	TM[o]	SP[o]	Length (amino acids)	Homolog in *M. smegmatis*
Rv0526	Possible Thioredoxin Protein (Thiol-Disulfide Interchange Protein) Lipoprotein	Y	0	Y	216	MSMEG_0971
Rv0816c **(ThiX)**	Probable Thioredoxin	N	0	Y	140	MSMEG_5786
Rv1677 **(DsbF)**	Probable Conserved Lipoprotein	N	0	Y	182	MSMEG_3543
Rv2878c **(DsbE)**	Soluble Secreted Antigen Mpt53 Precursor	N	0	Y	173	MSMEG_3543
Rv2969c **(DsbA)**	Conserved Membrane Protein	Y	Y	0	255	MSMEG_2410

[#] Gene annotation was obtained from TubercuList (1).

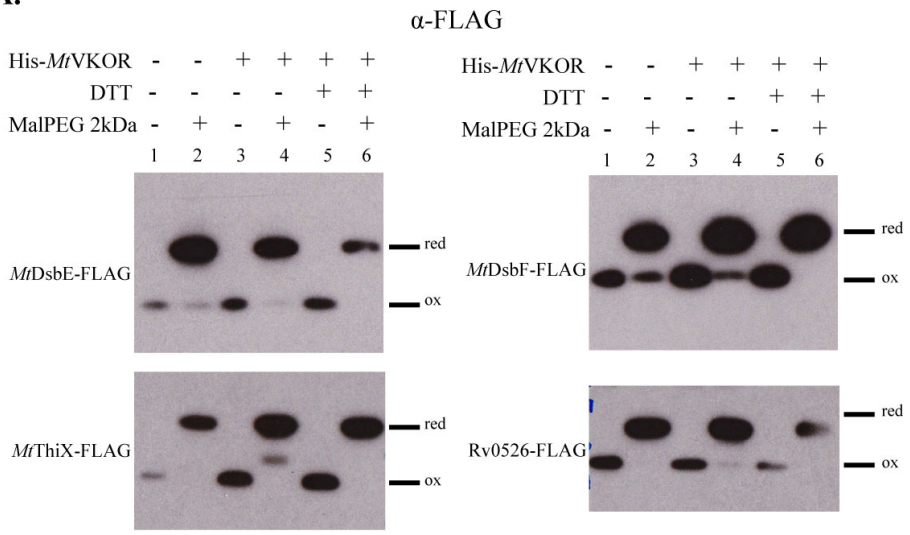
* Essentiality was determined by a Himar1-base transposon mutagenesis study (2).

[o] The transmembrane sequences and signal peptides were predicted by Phobius (3).

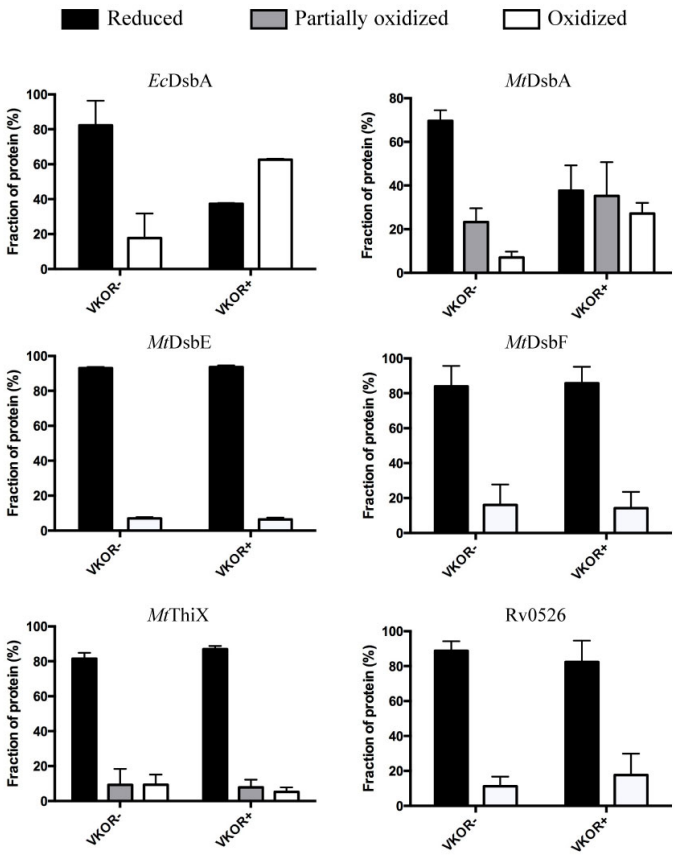
N: No; Y: Yes; and 0: no transmembrane segment.

Supplementary Figure 1. *In vivo* redox states of four mycobacterial thioredoxin candidates.

A.



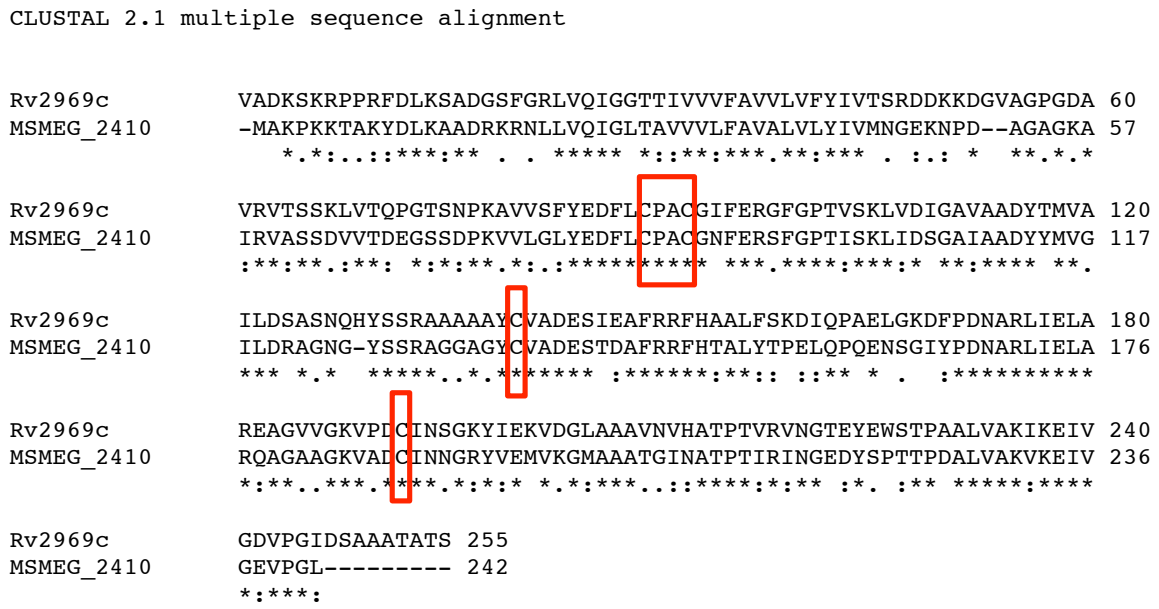
B.



A) *In vivo* oxidation states of four thioredoxin proteins from *M. tuberculosis* with or without *Mt*VKOR in *E. coli*. The four *M. tuberculosis* thioredoxins were cloned into pBAD33 vector and expressed in truncated form (deletion of its original signal peptide or trans-membrane sequence) using an *E. coli* TorT signal sequence in Δ*dsbAdsbB* strain. His-*Mt*VKOR was expressed from pTrc99a plasmid. Cells were grown in M63 media

with antibiotics and 0.2% arabinose to induce the expression of the FLAG-tagged thioredoxin candidates. 1 mM IPTG was used to induce the expression of His-*Mt*VKOR. Proteins were TCA precipitated and then alkylated with Mal-PEG2k. Thioredoxins were detected with anti-FLAG antibody. The sample without Mal-PEG2k treatment shows the position of the oxidized protein, which is the same as that of the reduced protein with no alkylating agent present. The sample with Mal-PEG2k refers to the position of the protein with reduced cysteines that are alkylated with Mal-PEG2k, which adds to the molecular weight of the protein. Red, reduced; ox, oxidized. Blots show representative results of two independent experiments. B) Quantification of reduced, oxidized and partially oxidized forms of the five mycobacterial thioredoxin candidates and *Ec*DsbA in the presence or absence of *Mt*VKOR when expressed in *E. coli*. The fraction of reduced vs oxidized states of each protein were calculated from blots of at least two independent experiments using Image J. Data represent average ± SEM.

Supplementary Figure 2. Comparison of *M. tuberculosis* and *M. smegmatis* DsbA proteins.



ClustalW2 protein sequence alignment of *M. tuberculosis* DsbA (top) and *M. smegmatis* DsbA (bottom). Catalytic or structural cysteines are highlighted in red.

Protein Sequences of the five thioredoxin candidates.
Cysteines are highlighted in yellow. Underlined amino acids indicate the portion of the protein that was removed to fuse to *Ec*TorT signal sequence.

0526 >gi|2113988|emb|CAB08993.1| POSSIBLE THIOREDOXIN PROTEIN (THIOL-DISULFIDE INTERCHANGE PROTEIN) [Mycobacterium tuberculosis H37Rv]
MQSRATRRSGALTMRRLVIAAAVSALLLTGCSGRDAVAQGGTFEFVSPGGKTDI
FYDPPASRGRPGPLSGPELADPARSVSLDDFPGQVVVVNVWGQWCGPCRAEVS
QLQRVYDATRGAGVSFLGIDVRDNNRQAPQDFINDRHVTYPSIYDPAMRTLIAF
GGKYPTSVIPSTLVLDRQHRVAAVFLRELLAADLQPVVERVAEEEPSGRAPVGA
Q

0816 >gi|2916874|emb|CAA17622.1| PROBABLE THIOREDOXIN THIX [Mycobacterium tuberculosis H37Rv]
MTTMIVASVATGALATIARWLLTRRSVILREVGPETTPAAPARTAELGLSGAGPT
VVHFRAPGCAPCDRVRRGVGDVCADLGDVAHIEVDLDSNPQAARRFSVLSLPTT
LIFDVDGRQRYRTSGVPKAADLRSALKPLLA

1677 >gi|2916975|emb|CAA17607.1| PROBABLE CONSERVED LIPOPROTEIN DSBF [Mycobacterium tuberculosis H37Rv]
MTHSRLIGALTVVAIIVTACGSQPKSQPAVAPTGDAAAATQVPAGQTVPAQLQFS
AKTLDGHDFHGESLLGKPAVLWFWAPWCPTCQGEAPVVGQVAASHPEVTFVG
VAGLDQVPAMQEFVNKYPVKTFTQLADTDGSVW

2878 >gi|1403398|emb|CAA98354.1| SOLUBLE SECRETED ANTIGEN MPT53 PRECURSOR [Mycobacterium tuberculosis H37Rv]
MSLRLVSPIKAFADGIVAVAIAVVLMFGLANTPRAVAADERLQFTATTLSGAPFD
GASLQGKPAVLWFWTPWCPFCNAEAPSLSQVAAANPAVTFVGIATRADVGAMQ
SFVSKYNLNFTNLNDADGVIWARYNVPWQPAFVFYRADGTSTFVNNPTAAMSQ
DELSGRVAALTS

2969 >M. tuberculosis H37Rv|Rv2969c|Rv2969c
VADKSKRPPRFDLKSADGSFGRLVQIGGTTIVVVFAVVLVFYIVTSRDDKKDGV
AGPGDAVRVTSSKLVTQPGTSNPKAVVSFYEDFLCPACGIFERGFGPTVSKLVDI
GAVAADYTMVAILDSASNQHYSSRAAAAAYCVADESIEAFRRFHAALFSKDIQP
AELGKDFPDNARLIELAREAGVVGKVPDCINSGKYIEKVDGLAAAVNVHATPTV
RVNGTEYEWSTPAALVAKIKEIVGDVPGIDSAAATATS

References

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3. Käll L, Krogh A, Sonnhammer ELL. 2004. A combined transmembrane topology and signal peptide prediction method. J Mol Biol 338:1027–1036.