Table S1. Primers used in this study.

Primer Name	**Primer Utility**	**Sequence(s)**
Kanseq_Rev	Transposon insertion locus determination	5’-GCATCGCCTTCTATCGCCTTC-3’
*fadD2*_For	*fadD2* disruption/deletion complementation	5’-TTTTAAGCTTATGCCTAACCTCACTGATCT-3’
*fadD2*_Rev		5’- TTTTGCTAGCCTACAGCTTGCGTAGCTCAC-3’
*fadD2*_LL	*fadD2* deletion	5’-TTTTTTTTCACAAAGTGTTGGCGTTCTGGTTGAAGTC-3’
*fadD2*_LR		5’-TTTTTTTTCACTTCGTGGGGTCGCAAATTACCATGAG-3’
*fadD2*_RL		5’-TTTTTTTTCCATAGATTGGTCACCGCATCGGTGCCAAAG-3’
*fadD2*_RR		5’-TTTTTTTTCCATCTTTTGGCGCGTTCGGAACCGACAATC-3’
*fadD2*_DelCheck_5’	*fadD2* deletion confirmation	5’-GGCCAACCGCGTCATCCATC-3’
*fadD2*_DelCheck_3’		5’-GGTGGGTGCTGCCATATCTG-3’
*fadD2*_Pur_For	*fadD2* overexpression and protein purification	5’-AAAAGCTAGCATGCCTAACCTCACTGATCT-3’
*fadD2*_Pur_Rev		5’-AAAAAAGCTTCTACAGCTTGCGTAGCTCAC-3’

Table S2. Conservation of the FadD2 amino acid sequence among members of the *M. tuberculosis* complex and other mycobacteria.

Species/Strain	% Identity*	Positives	Gaps	Notable Differences in Sequence*
M. bovis BCG Pasteur	100%	560/560	0/560	—
M. bovis	100%	560/560	0/560	—
M. tuberculosis H37Rv	99%	559/560	0/560	E112D
M. africanum	99%	559/560	0/560	E112D
M. avium	84%	512/560	1/560	—
M. marinum	84%	510/560	6/560	—
M. leprae	82%	505/560	4/560	—
M. smegmatis	77%	488/560	4/560	—

*Relative to the *M. bovis* BCG Pasteur FadD2 amino acid sequence.

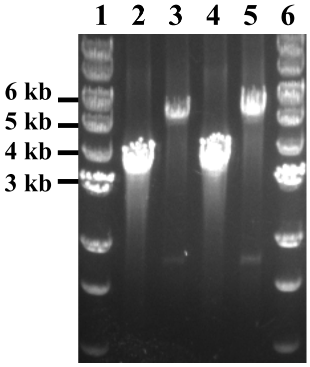

Figure S1. Confirmation of *fadD2* deletion in *M. tuberculosis* H37Rv and in *M. bovis* BCG by PCR. The *fadD2* locus was amplified using wild-type *M. tuberculosis* H37Rv (lane 2), *M. tuberculosis* H37Rv *ΔfadD2* (lane 3), *M. bovis* BCG (lane 4), and *M. bovis* BCG *ΔfadD2* (lane 5) genomic DNA as templates and using primers (*fadD2*_DelCheck_5' and *fadD2*_DelCheck_3' in Table S1) specific to the chromosomal regions adjacent to the flanking regions included in the deletion construct to direct allelic exchange. Lanes 1 and 6 contain NEB 1 Kb ladder. The wild type *fadD2* product is 3.6-kb and *ΔfadD2* product is 5.5-kb due to allelic replacement with the *hyg-sacB* cassette.