

SUPPLEMENTARY MATERIAL

Mtb	TACCTGCATATCGTCGGGCCCCGACATGGCCGACCAGCCCTAACGGGTGGGATCAACCTGGACAAACACGGGAATTGGTCCGGTTCTCTAAGGGCACGGCTCGAACGCATCCGCAATGACGGG 120
Msmeg	TACCTGCACATCGTCGGGCCCCAGCTGAGCAGGAGCCCTACGGCATGGGGTGAAACCTGGAAACACCGGCTGGGCGCTGGTGAACGGGACGTTGGAGCGCATCCGCCGTGACGGG 120
Mtb	ACCTGGAACACGGTTGATCGCAAGTGGCTGACGGTGTCTGGCCAGCCCCGGCCACCGAGGTATGTGGACTGATGCC-----AAAGCGT-----CAGAGACCGA-- 223
Msmeg	ACCTGGTACACCTCTACCGCAAGTGTGTTGACGGTGTGGGCCCCGGCCCGAACCTCCGTGGGAGGTACGTGACTGATGACTTACCCGACAAACCTGATCTCCAGCGCCGACG 240
Mtb	ACGTTCG-----GGCCCCGGCACCCAACGGCGGAACGCCA-GACCGGGACGT-----CGCGGAGGTTGACGCCCTGAGCACCCAGGGGTGTTCCGCCCGATTTCGGG 324
Msmeg	ACGTTACCTGCACACGGCGCGCAACACCGCGAGGCTGGAAAGACCTGACATGGACTCGGCTGACCATGGGCCCAATGGGACGCAAGGCCGTGACCGGAGTTTCGACG 360
Mtb	ATGAGGACAACTCCGCCATCCGACGCTGGCCGGACACCCGAGCGCAAGACGGGATGGCCACCCACGGCGGGTGCGCCACCGGCTGAGCGGCTGGCGGGCCCTGGTGGAAATCC 444
Msmeg	&ACCCGACGGACATGGCGGACGGT-----GGTEACCGAGGCTACGACCGGTACCCATGGCACGGCGCATTGTCCTCCGATGCGGGCCCTGGCGGGAGATCC 474
Mtb	CGCGGCCGCCGATATCGATCCGCTTGGGGCCCTGATGACCAACCGGTGGTGCCTGGAGTCCACCGGTTCTGCTGGAACTGTGGACGTECCGTCGGCCGGTCCGACTCCGAGACCAAGG 564
Msmeg	CGCGGCCGCCGAAACGGACCCGCTACCGCGTTGATGACCAATCCGGTTGTGGCGAGTCGAAACGGTTCTGCTGGAACCTGGCAAGGCCGTGGACGGTCGACGCCAGACGGCGC 594

Figure S1. Investigation of the translational start of *M. smegmatis pknG*. Alignment of the first 328 and 388 nucleotides of *pknG* from *M. tuberculosis* (Mtb) and *M. smegmatis* (Msmeg), respectively, including the upstream 200 nucleotides. The limits of gene upstream of *pknG*, *glnH*, is indicated. Identical residues are shown in grey. The translational start codon of *M. smegmatis pknG* was annotated on the TIGR Comprehensive Microbial Resource (CMR) home page at a GTG (underlined) further upstream of the kinase domain compared to the other *pknG* homologues (see Figure 1). However, alignment of the *M. smegmatis* and *M. tuberculosis pknG* including their upstream nucleotides revealed not only that the upstream regions are highly conserved as well, but also that an ATG downstream of the annotated start codon in the *M. smegmatis* sequence aligned to the start codon of *M. tuberculosis pknG* (both are framed). Since *M. tuberculosis PknG* with this start codon is an active kinase (Walburger et al., 2004) and rescues *M. bovis BCG ΔpknG* from lysosomal delivery when expressed in *trans* (Houben et al., 2006), we propose this ATG is the most probable start codon of *M. smegmatis pknG*. *M. smegmatis pknG* with this start codon was cloned into pMV361 to obtain pMV-PknG^{smeg}.

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Table S1. List of primers and probe used for cloning of the constructs analyzed in Figure 8 and for the (q)RT-PCR reactions in Figure 7 and 8.

Primer name	Sequence (5' to 3')
AclI-BCG0451c.fw	GTGCTAAGAATAACGTTAGTTCGTTCGGTAG CCGAA
BCG-Pk-EcoRV.rev	CCTCAAGCGGATCGATATCG
AclI-MSMEG_0788.fw	GTGCTAAGAATAACGTTAGAGCCGTAAGCATCG AAAC
Smeg-Pk-XmnI.rev	CAGTTCCAGCAGAACCGTTTC
Smeg-Pk_BCG-UTR.rev	GGTGAAGTCATCAGTCCACATACCTCGG
BCG-Pk_Smeg-UTR.rev	GCTTG GCCATCAGTCGACGTACCTCG
BCG-UTR_Smeg-Pk.fw	TATGTGGACTGATGACTTCACCCGAGAACCC
Smeg-UTR_BCG-Pk.fw	ACGTCGACTGATGGCAAAGCGTCAGAGAC
RT-PknG.fw	GCCACCGACATCTACACCGT
RT-PknG.rev	GGTGTGCCACCAGCAG
16S-RNA.fw	ACGAACAAACGCGACAAACC
16S-RNA.rev	CCAGCAGCCGCGGTAA
qRT-BCG.fw	CGGCCAGTACGAGGTCAAAG
qRT-BCG.rev	GCGGTCGAGAGCGAGGTAG
qRT-smeg.fw	CGACCAGTACGAGATCAAGGGT
qRT-smeg.rev	TCTTGTGAAACGCCAGGTACA
PknG-probe	6FAM-CCGCCGTGCGCGAT-MGBNFQ

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Table S2. Sources and growth conditions of the various mycobacterial species analyzed for PknG expression in Figure 9.

Mycobacterial species	Source	Medium	Temperature
<i>M. abscessus</i>	ATCC 19977	mod. LJ*	35°C
<i>M. africanum</i>	Swiss NCM ***	mod. LJ	35°C
<i>M. avium</i> subsp. <i>avium</i>	MAC101	mod. LJ	35°C
<i>M. boemicum</i>	Clinical isolate	mod. LJ	35°C
<i>M. bovis</i>	ATCC 19210	mod. LJ	35°C
<i>M. bovis</i> biovar. <i>BCG</i>	ATCC 35734	mod. LJ	35°C
<i>M. bovis</i> subsp. <i>caprae</i>	Clinical isolate	mod. LJ	35°C
<i>M. chelonae</i>	DSM 43804	mod. LJ	35°C
<i>M. fortuitum</i>	ATCC 49403	mod. LJ	35°C
<i>M. gordoniæ</i>	Pasteur 14021.001	mod. LJ	35°C
<i>M. haemophilum</i>	ATCC 29548	MB 7H10**	28°C
<i>M. intracellulare</i>	Clinical isolate	mod. LJ	35°C
<i>M. kansasii</i>	NCTC 10268	mod. LJ	35°C
<i>M. lentiflavum</i>	Clinical isolate	mod. LJ	35°C
<i>M. malmoense</i>	NCTC 11298	mod. LJ	35°C
<i>M. marinum</i>	ATCC 927	mod. LJ	28°C
<i>M. scrofulaceum</i>	Pasteur 14022.0031	mod. LJ	35°C
<i>M. simiae</i>	Clinical isolate	mod. LJ	35°C
<i>M. smegmatis</i>	Pasteur 14133.0001	mod. LJ	35°C
<i>M. terrae</i>	Clinical isolate	mod. LJ	35°C
<i>M. tuberculosis</i> H37Rv	Pasteur 14001.0001	mod. LJ	35°C
<i>M. xenopi</i>	Clinical isolate	mod. LJ	35°C

* modified Löwenstein-Jensen

** Middlebrook with 10% OADC-Enrichment supplement with 0.4% Ferrum-Ammonium-Citrat

***Swiss NCM: Swiss National Centre for Mycobacteria, Zurich.