**Supplemental Information**

 The external PASTA domain of the essential serine/threonine protein kinase PknB regulates mycobacterial growth

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**Table S5.** List of proteins differently present in the membrane fractions of TMP and MIND (submitted as a separate excel file). Peptides from the TMP and MIND samples were labelled with different tandem mass tags. During MS/MS fragmentation each tag generates a reporter-ion with a different m/z value. The peaks areas of the reporter-ion can thus be compared to give a relative quantitative value. The data were normalized separately within each acquisition run. Intensities for each peptide identification were normalized within the assigned protein. The reference channel (MIND) was normalized to produce a 1:1 fold change. All normalization calculations were performed using medians to multiplicatively normalize data. The values shown thus correspond to the log2 fold change for the TMP sample normalized against the MIND (reference) sample.

**Figure S1.**

**Figure S2.**

**Figure S3.**

**Figure S1**. Growth of *M. smegmatis* MIND and TMP strains in Sauton’s medium supplemented with hygromycin and tetracycline after *in vitro* passage. Mycobacteria were grown in Sauton’s medium to stationary phase and used for inoculation of microtitre plates as described in Materials and Methods.



**Figure S2.** Superimposition of [1H, 15N] HSQC spectra from PknB\_PASTA in 25 mM sodium acetate pH 4.6 in the presence (black) or absence (blue) of 25 mM MgCl2, recorded at 37°C.

**Figure S3.** Analytical gel filtration chromatograms of PknB\_PASTA (50 µl at 50 µM) with (blue) or without (red) 25 mM MgSO4. Both profile are almost identical indicating that the protein is not subject to any major conformational changes or oligomerization in presence of a large excess of MgSO4.

**Table S1.** Plasmids and strains generated for over-expression studies

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Plasmid Name**  | **Strain Name** | **Insert Size (bp)** | **Primers** | **Description** |
| pMind-*pknB1* | PknB1 | 1881 | PknBF1 & PknBR1 | Full length gene |
| pMind *pknB5* | PknB5 | 1062 | PknBF1 & PknBR5-TM | ΔPASTA 1-4 |
| pMind *pknB7*  | TMP | 888 | PknBF2 & PknBR1 | TM-PASTA1-4 |
| pMind *pknB9* | PknB9 | 72 | PknBF2 & PknBR5-TM | TM only |
| pMind *pknB10* | PknB10 | 276 | PknBF2 & PknBR4-P | TM-PASTA1 |
| pMind *pknB11* | PknB11 | 480 | PknBF2 & PknBR3-2P | TM-PASTA1-2 |
| pMind *pknB12*  | PknB12 | 684 | PknBF2 & PknBR3-3P | TM-PASTA1-3 |
| pMind *pknB13* | PknB13 | 819 | PknBF3 & PknBR1 | PASTA 1-4 |
| pMind *pknB14* | PknB14 | 879 | PknBF3 &MycHisR | PASTA 1-4-MycHis-tag |
| pMind *pknB15* | TMPH | 951 | PknBF1 & PknBHR2 | TM-PASTA1-4- Myc-His-tag |
| pMind | MIND | N/A | N/A | Empty plasmid control |

**Table S2.** Primers used in the study

|  |  |  |  |
| --- | --- | --- | --- |
| **N** | **Primer** | **Sequence 5’-3’** | **Description** |
| 1. | PknBF1 | gat*GGATCC*atgaccaccccttcccacctgtcc | Cloning of *pknB* in pMind plasmid |
| 2. | PknBF2 | Gac*GGATCC*atgcgttgggttgcggtggtc | Cloning of *pknB8* in pMind plasmid |
| 3. | PknBF3 | Gca*GGATCC*atgggc ggcatcacccgcgacgttcaa | Amplification pknB10 |
| 4. | PknBR1 | cgg*ACTAGT*ctactg gcc gaa cct cag cgt gat | Cloning of *pknB* in pMind plasmid |
| 5. | PknBR4-P | ttg*ACTAGT*ctatcc ggt gga cac gtt gac tgt | Cloning of *pknB* in pMind plasmid |
| 6. | PknBR5-TM | ggt*ACTAGT*ctagcc gaa cgt gtt gat ggc gat | Cloning of *pknB* in pMind plasmid |
| 7. | PknBHR2 | cgg*ACTAGT*cta*ACG CGT* ctg gcc gaa cct cag cgt  | Cloning of *pknB* in pMind plasmid |
| 8. | Myc-HisF | Gca *ACG CGT* gaa caa aaa ctc atc tca | Amplification of 6XHis-Myc tag |
| 9. | Myc-HisR  | Gcg *ACT AGT* Taa tct gta tca ggc gaa  | Amplification of 6XHis-Myc tag |
| 10. | MindF2 | tgagtcatagttgcactttatcat | Sequencing of pMind constructs |
| 11. | MindR3 | TCCGAATCAATACGGTCGAGA | Sequencing of pMind constructs |
| 12. | PknBR2-3P | cgg*ACTAGT* ctactcttggacacctgtagttc  | Cloning of *pknB12* in in pMind plasmid |
| 13. | PknBR3-2P | cgg*ACTAGT*cta gccaacgatgatgat | Cloning of *pknB11* in in pMind plasmid |
| 14. | RT-PknBF1 | TCAGAACGGAATCATCCACCGTGA | qRT-PCR |
| 15. | RT-PknBR1 | GCGATGCCGAAATCCATCACCTTT | qRT-PCR |
| 16. | RT-PknBF2 | AGAACCTCAACGTCTACGGCTTCA | qRT-PCR |
| 17. | RT-PknBR2 | ATGACGAATTGGTTGCCCTTGGAC | qRT-PCR |

**Table S3**.Growth of *M. smegmatis* strains over-expressing *pkn*B variants

|  |  |  |
| --- | --- | --- |
| Sauton’s medium | Lysogeny broth | Middlebrook 7H9 medium |
| Strain | Maximum growthrate (h-1) | Lag-phase (h) | OD600nm | Maximum growthrate (h-1) | Lag-phase (h) | OD600nm | Maximum growthrate (h-1) | Lag-phase (h) | OD600nm |
| Mind | 0.12±0.010 | 22±2.2 | 1.46±0.10 | 0.28±0.015 | 21±2.2 | 1.14±0.15 | 0.12±0.010 | 16±1.7 | 0.75±0.12 |
| PknB1 | 0.11±0.010 | 52±4.0 | 1.10±0.12 | 0.09±0.002 | 55±4.4 | 0.98±0.16 | 0.06±0.005 | 32±2.7 | 0.54±0.15 |
| PknB5 | 0.12±0.010 | 46±4.0 | 1.50±0.15 | 0.11±0.008 | 47±4.2 | 1.04±0.18 | 0.09±0.020 | 21±2.0 | 0.61±0.15 |
| TMP | 0.11±0.010 | 50±2.0 | 1.60±0.16 | 0.24±0.020 | 29±2.0 | 1.05±0.10 | 0.10±0.030 | 17±1.5 | 0.73±0.11 |

Experiments were done in the Bioscreen Growth Analyser as described above. Presented data are mean ± SD from three independent experiments. The apparent lag phase was calculated as a time period from the inoculation of the culture until the OD (600 nm) was 0.1.

**Table S4.** Muropeptides and sugars tested in TMP growth assays.

|  |  |  |  |
| --- | --- | --- | --- |
| Substance | Source | Concentration | Effect |
| D-glucose | Sigma | Up to 5 mM | No effect |
| N-acetylglucosamine (GlcNAc) | Sigma | Up to 5 mM | No effect |
| N-acetylmuramic acid (MurNAc) | Sigma | Up to 5 mM | No effect |
| GlcNAc-MurNAc disaccharide | Sigma | Up to 100 μM | No effect |
| MurNAc--dipeptide | Sigma | Up to 100 μM | No effect |
| MurNAc-pentapeptide  | Synthesised in laboratory | Up to 75 μM | No effect |
| GlcNAc-MurNAc--pentapeptide | Synthesised in laboratory | Up to 75 μM | No effect |
| GlcNAc-1,6 anhydroMurNAc- pentapeptide | Synthesised in laboratory | Up to 75 μM | No effect |
| Tetrasaccharide- pentapeptide | Synthesised in laboratory | Up to 75 μM | No effect |
| Sonicated *Mtb* PG | Isolated in laboratory | Up to 1 mg/ml | 20% reduction of lag-phase at 0.5 mg/ml |
| *E. coli* PG  | Isolated in laboratory  | Up to 1 mg/ml | 20% reduction of lag-phase at 0.5 mg/ml |
| Lysozyme-digested *E. coli* PG | Isolated in laboratory | Up to 1 mg/ml | 20% reduction of lag-phase at 0.5 mg/ml |
| Mutanolysin-digested *E. coli* PG | Isolated in laboratory | Up to 1 mg/ml | 20% reduction of lag-phase at 0.5 mg/ml |
| MltA-digested *E. coli* PG | Isolated in laboratory | Up to 0.2 mg/ml | No effect |
| RpfB-digested *E. coli* PG | Isolated in laboratory | Up to 1 mg/ml | 20% reduction of lag-phase at 0.5 mg/ml |
| Sonicated *M. smegmatis* PG  | Isolated in laboratory | Up to 1 mg/ml | 20% reduction of lag-phase at 0.5 mg/ml |
| RpfB-digested *M. smegmatis* PG  | Isolated in laboratory | Up to 1 mg/ml | 20% reduction of lag-phase at 0.5 mg/ml |
| Culture supernatant (Sauton’s medium) | Prepared in laboratory | Up to 5xfold concentrated  | Complete elimination of inhibition  |
| Culture supernatant (7H9 supplemented medium) | Prepared in laboratory | Up to 5xfold concentrated  | Complete elimination of inhibition |

PG – peptidoglycan