

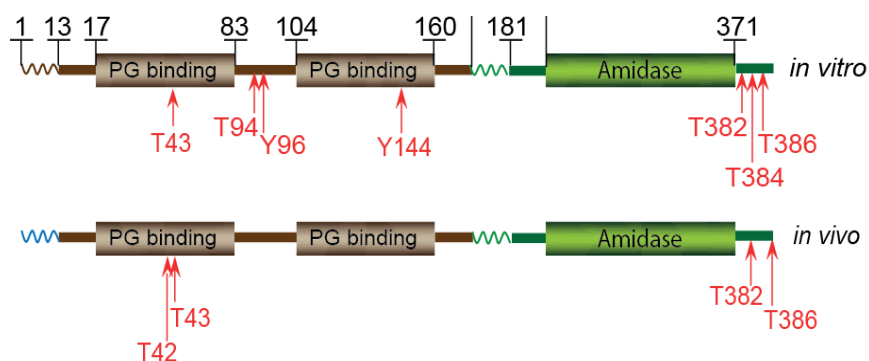
## Supplemental Information

### Two Faces of CwIM, an Essential PknB

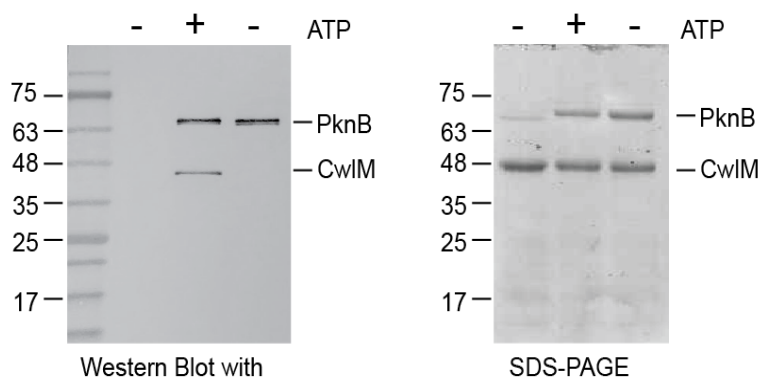
#### Substrate, in *Mycobacterium tuberculosis*

Obolbek Turapov, Francesca Forti, Baleegh Kadhim, Daniela Ghisotti, Jad Sassine, Anna Straatman-Iwanowska, Andrew R. Bottrill, Patrick J. Moynihan, Russell Wallis, Philippe Barthe, Martin Cohen-Gonsaud, Paul Ajuh, Waldemar Vollmer, and Galina V. Mukamolova

## SUPPLEMENTAL FIGURES



**B**



**C**

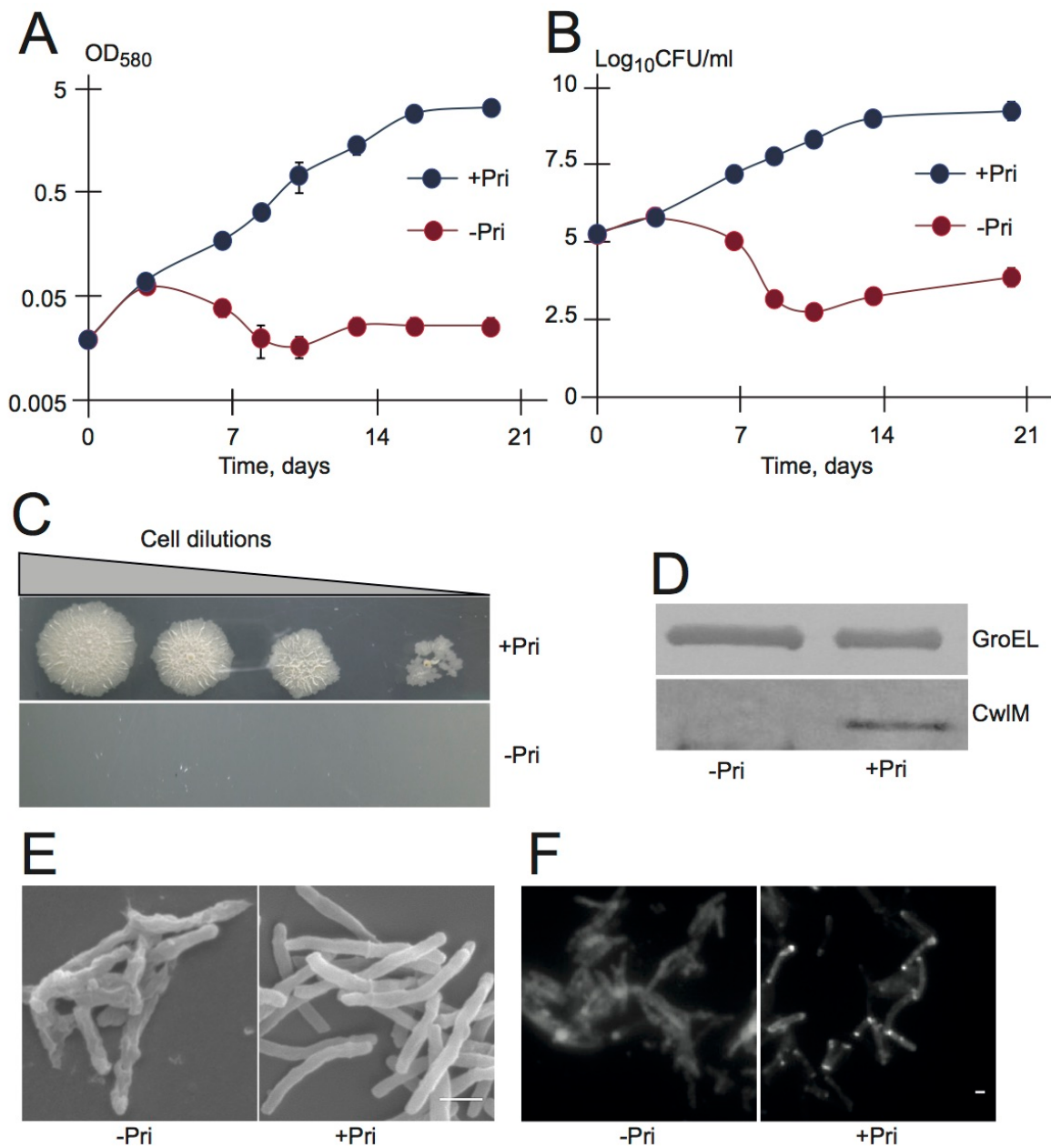
Western Blot with  
Phospho-Threonine  
Antibody

SDS-PAGE

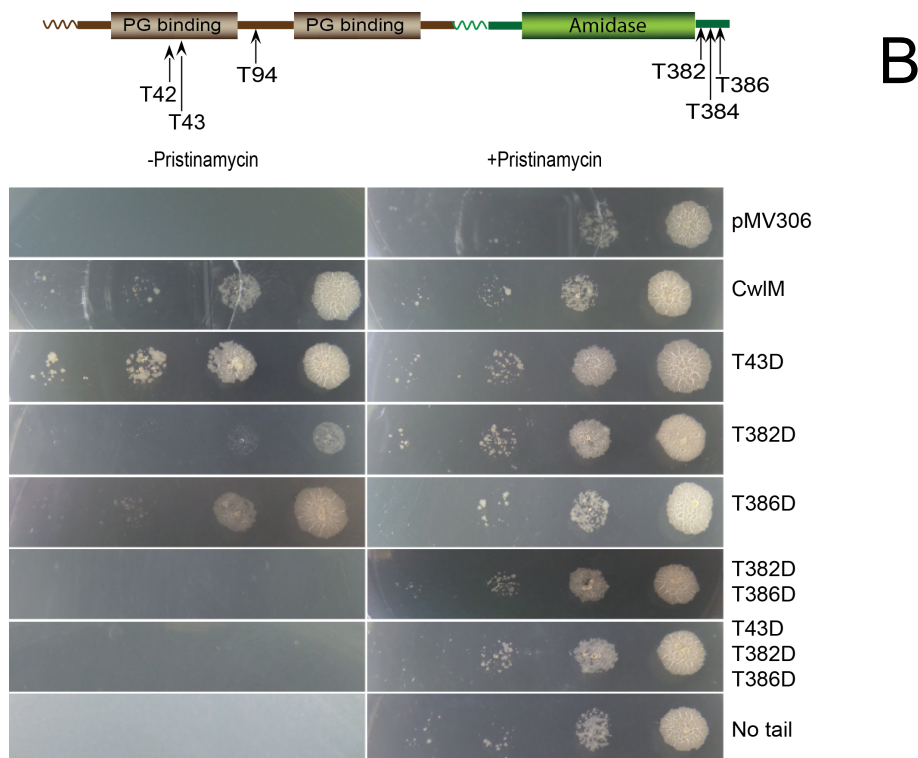
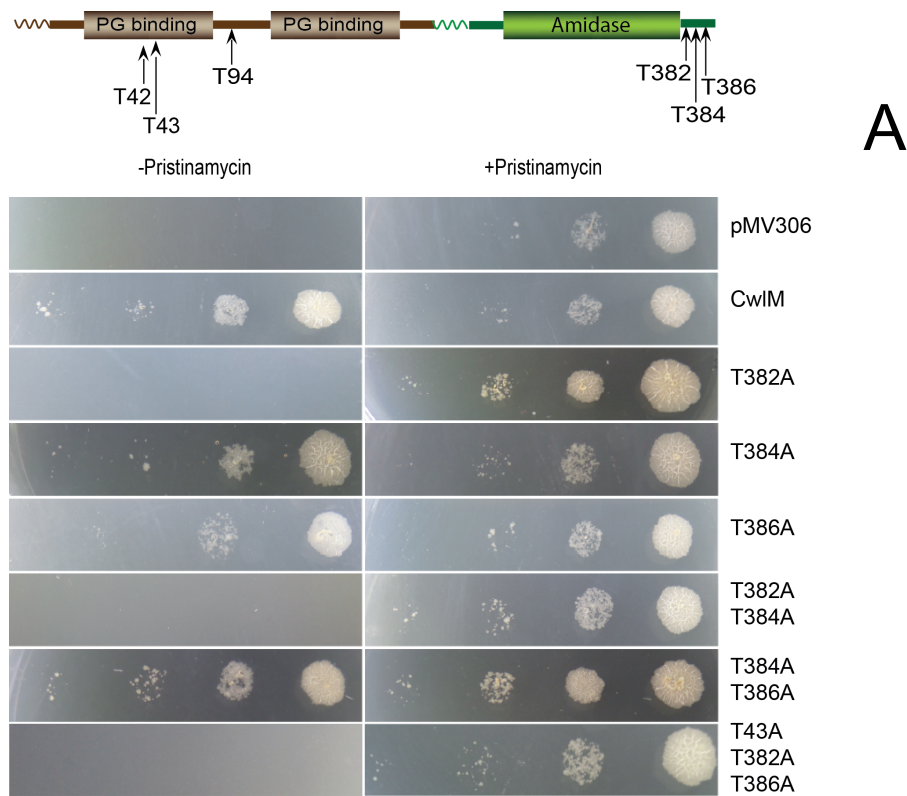
| Peptide                         | Phospho<br>residue | Observed<br>mass (charge) | Actual mass        |
|---------------------------------|--------------------|---------------------------|--------------------|
| AALTALGMLDHQEEDLT <b>T</b> GR   | T43                | 1111.52 (2)               | 2221.03            |
| RLGARTLYHQFGAPLYGDDVATLQAR      | T94                | 938.47 (3)                | 2814.4             |
| RLGARTLYHQFGAPLYGDDVATLQAR      | Y96                | 704.11 (4)                | 2812.4             |
| EYGLAADGICGPETLR                | Y144               | 595.93 (3)                | 1784.78            |
| NDRPT <b>T</b> GTFTFAELLAHELVER | T382               | 862.08 (3)<br>646.82 (4)  | 2583.22<br>2583.22 |
| NDRPT <b>T</b> GTFTFAELLAHELVER | T384               | 646.81 (4)<br>862.09 (4)  | 2583.22<br>2583.22 |
| NDRPT <b>T</b> GTFTFAELLAHELVER | T382<br>T384       | 888.74 (3)                | 2663.2             |
| NDRPT <b>T</b> GTFTFAELLAHELVER | T382<br>T386       | 888.41 (3)                | 2662.2             |
| NDRPTGT <b>T</b> FTFAELLAHELVER | T386               | 861.76 (3)                | 2582.26            |
| NDRPTGT <b>T</b> FTFAELLAHELVER | T384<br>T386       | 888.41 (3)                | 2662.20            |

**Figure S1. Phosphorylation of CwlM by PknB *in vitro*. Related to Table 1. (A)**

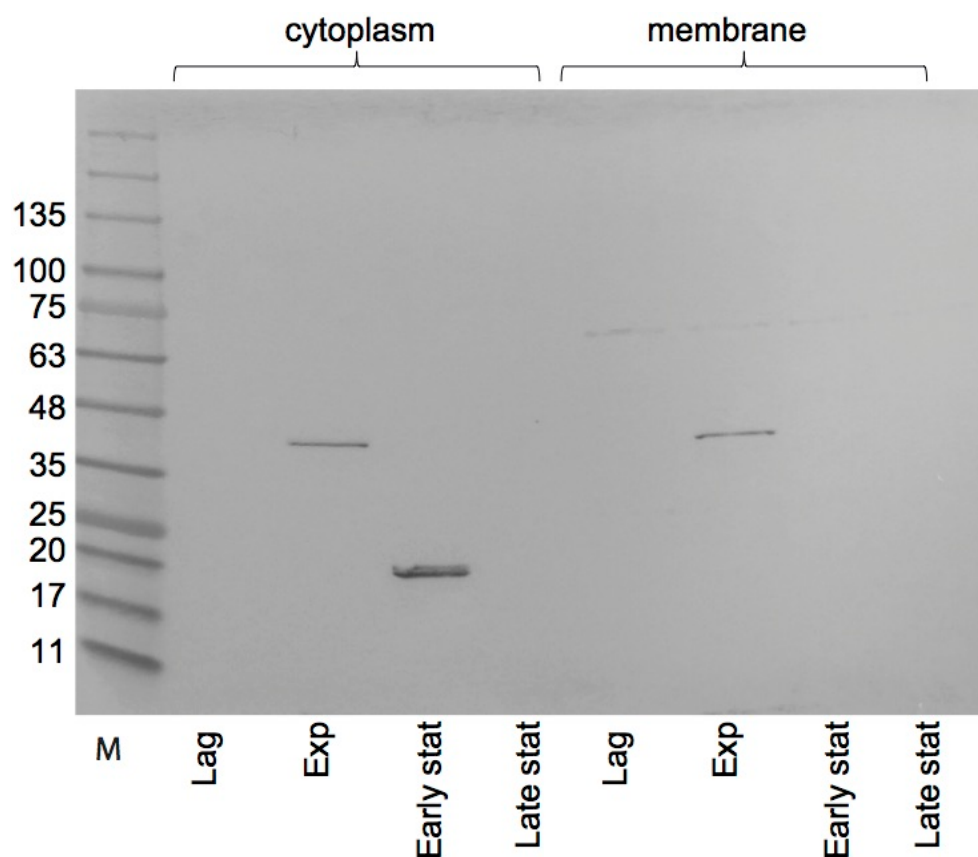
Schematic representation of phosphosites detected *in vitro* (top) and in the phosphoproteomics study (bottom). (B) A recombinant CwlM was phosphorylated by the recombinant enzymatic domain of PknB. Detection of phosphorylated proteins by Western blot analysis using phospho-threonine antibody. (C) Phosphopeptides detected by mass-spectrometry.



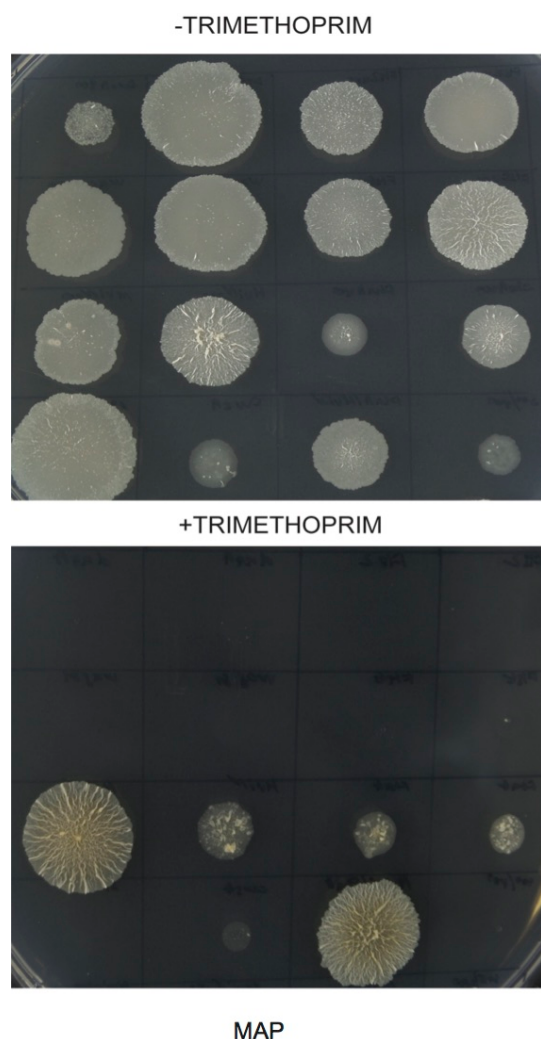
**Figure S2. CwIM is essential for growth of *M. tuberculosis*. Related to Figure 2. See also Figure S3 and Table S3.** A conditional *cwIM* mutant was grown in 7H9 medium with and without pristinamycin (Pri) at 37°C with shaking. Growth was monitored by measurement of OD at 580 nm (A) or by CFU counting on 7H10 agar (B). Data are represented as mean±SEM. (C) Growth of *cwIM*-CM on agar. (D) Detection of CwIM using western blot with anti-CwIM antibody. (E) Scanning electron micrographs of CwIM-producing and CwIM-depleted mycobacteria grown in standard 7H9 medium. (F) Detection of nascent peptidoglycan by Van-BODIPY labelling, data shown for mycobacteria grown in 7H9 medium, similar results were obtained in SMM (not shown for clarity). Scale bars – 1  $\mu$ m.



**Figure S3. Growth of phosphoablative (A) and phosphomimetic (B) *M. tuberculosis* CwIM mutants on 7H10 agar. Related to Figure 2. See also Table S3.** Serially diluted mycobacteria were plated on 7H10 agar with kanamycin and hygromycin +/-pristinamycin and incubated for 4 weeks at 37°C.

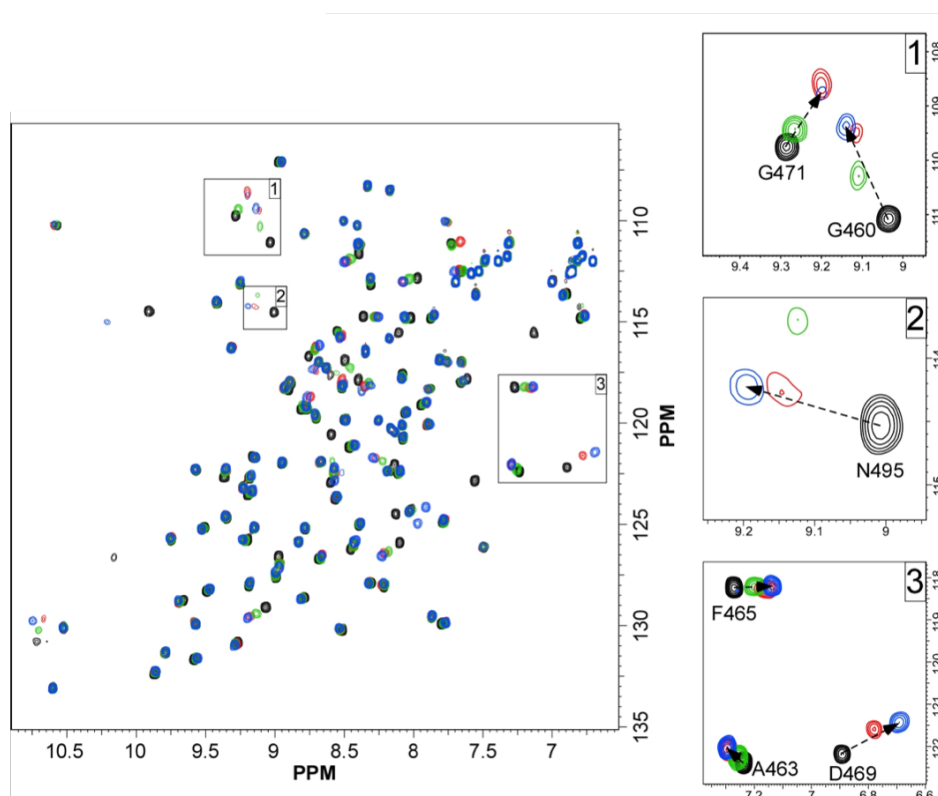


**Figure S4. Detection of CwIM in cytoplasm and membrane fractions of *M. smegmatis* during different growth stages. Related to Figure 3.** Bacteria were grown inoculated in 7H9 medium and incubated for 1 hour (lag-phase, Lag), grown to OD 0.8 (exponential phase, Exp), 3.6 (early stationary phase, Early stat) and 7.4 (late stationary phase, Late stat) at 37°C with shaking. Fractionation and Western blot analysis using the anti-CwIM antibody were done as described in Methods.

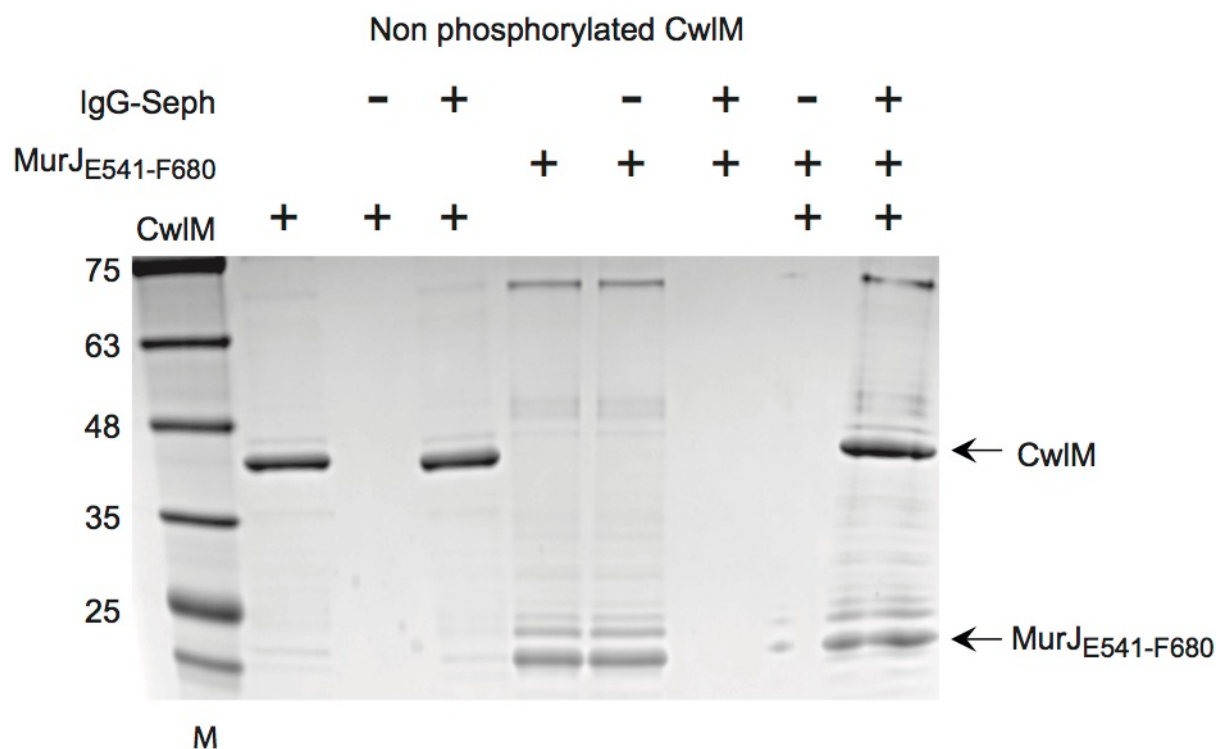


|                                               |                                               |                                               |                                            |
|-----------------------------------------------|-----------------------------------------------|-----------------------------------------------|--------------------------------------------|
| DnaA <sub>400</sub><br>CwIM <sub>300</sub>    | DnaA <sub>200</sub><br>CwIM <sub>100</sub>    | FtsE <sub>400</sub><br>CwIM <sub>300</sub>    | FtsE <sub>200</sub><br>CwIM <sub>100</sub> |
| Wag31 <sub>400</sub><br>CwIM <sub>300</sub>   | Wag31 <sub>200</sub><br>CwIM <sub>100</sub>   | FtsZ <sub>400</sub><br>CwIM <sub>300</sub>    | FtsZ <sub>200</sub><br>CwIM <sub>100</sub> |
| MurJ <sub>1cd400</sub><br>CwIM <sub>300</sub> | MurJ <sub>1cd200</sub><br>CwIM <sub>100</sub> | FhaA <sub>400</sub><br>CwIM <sub>300</sub>    | FhaA <sub>200</sub><br>CwIM <sub>100</sub> |
| pUAB200<br>CwIM <sub>100</sub>                | CwsA <sub>400</sub><br>CwIM <sub>300</sub>    | MurJ <sub>1cd100</sub><br>FhaA <sub>200</sub> | pUAB300<br>pUAB400                         |

**Figure S5. Detection interactions of CwIM with other proteins by a mycobacterial protein fragment complementation assay. Related to Figure 5. See also Table S4.** 300/400 is a negative empty plasmid control (pUAB300+pUAB400); CwIM/400 – control (pUAB300::cwlM+pUAB400); MurJ<sub>1cd</sub>/FhaA – positive control (pUAB100:: *murJN*<sub>1cd</sub> +pUAB200::fhaA). Growth in the presence of trimethoprim indicates interactions between proteins fused to dihydrofolate-reductase domains. MAP shows the position of strains used in the assays (strains are detailed in Table S2).



**Figure S6. Superimposition of  $[^1\text{H}-^{15}\text{N}]$  HSQC spectra of Rv0020c FHA domain alone and in presence of 3 different phospho-peptides. Related to Figure 5.** Superimpositions of  $[^1\text{H}-^{15}\text{N}]$  HSQC spectra of uniformly labelled samples Rv0020c FHA domain (50  $\mu\text{M}$ ) alone (black) and in presence of 50  $\mu\text{M}$  of T382 phospho-peptide (red) or T386 phospho-peptide (green) or the double T382 and T386 phospho-peptide (blue). Three areas of the HSQC spectrum are expanded to the right. In each zoom, peaks are assigned and an arrow indicates the displacement of the peak when titrating with the double T382 and T386 phospho-peptide. The displacement observed for the double phosphor-peptide corresponds to the displacement observed for the T382 phospho-peptide.



**Figure S7. CwIM interacts with an E541-F680 linker of MurJ<sub>ICD</sub>. Related to Figure 4.**

Recombinant proteins were immunoprecipitated and analysed by SDS-PAGE. M- protein markers; “+” – reagent added; “-” designates flow-through fractions.

## SUPPLEMENTAL TABLES

**Table S2. Strains generated in the study. Related to Figure 1-4.**

| Strain name                                                     | Strain description                         | Plasmid                                                | Comments                                               |
|-----------------------------------------------------------------|--------------------------------------------|--------------------------------------------------------|--------------------------------------------------------|
| <b><i>Mycobacterium tuberculosis</i> H37Rv strains</b>          |                                            |                                                        |                                                        |
| <i>pknB</i> -CM                                                 | PknB conditional mutant                    | pAZI9479:: <i>pkn</i>                                  | PknB depletion                                         |
| <i>cwIM</i> -CM                                                 | CwIM conditional mutant                    | pAZI9479:: <i>cwIM</i>                                 | CwIM depletion                                         |
| <i>cwIM</i> -CM <sub>pMV</sub>                                  | Empty plasmid control                      | pMV306                                                 | <i>cwIM</i> -CM complementation                        |
| <i>cwIM</i> -CM <sub>WT</sub>                                   | CM-CwIM wild type <i>cwIM</i> complemented | pMV306:: <i>cwIM</i>                                   | <i>cwIM</i> -CM complementation                        |
| <i>cwIM</i> -CM <sub>T42A</sub>                                 | T42A CwIM variant                          | pMV306:: <i>cwIMT42A</i>                               | <i>cwIM</i> -CM complementation                        |
| <i>cwIM</i> -CM <sub>T43A</sub>                                 | T43A CwIM variant                          | pMV306:: <i>cwIMT43A</i>                               | <i>cwIM</i> -CM complementation                        |
| <i>cwIM</i> -CM <sub>T42A+T43A</sub>                            | T42A+T43A CwIM variant                     | pMV306:: <i>cwIMT42A</i> + <i>T43A</i>                 | <i>cwIM</i> -CM complementation                        |
| <i>cwIM</i> -CM <sub>T94A</sub>                                 | T94A CwIM variant                          | pMV306:: <i>cwIMT94A</i>                               | <i>cwIM</i> -CM complementation                        |
| <i>cwIM</i> -CM <sub>T382A</sub>                                | T382A CwIM variant                         | pMV306:: <i>cwIMT382A</i>                              | <i>cwIM</i> -CM complementation                        |
| <i>cwIM</i> -CM <sub>T384A</sub>                                | T384A CwIM variant                         | pMV306:: <i>cwIMT384A</i>                              | <i>cwIM</i> -CM complementation                        |
| <i>cwIM</i> -CM <sub>T386A</sub>                                | T386A CwIM variant                         | pMV306:: <i>cwIMT386A</i>                              | <i>cwIM</i> -CM complementation                        |
| <i>cwIM</i> -CM <sub>T382A+T384A</sub>                          | T382A +T384A CwIM variant                  | pMV306:: <i>cwIMT382A</i> + <i>384A</i>                | <i>cwIM</i> -CM complementation                        |
| <i>cwIM</i> -CM <sub>T382A+T384A+T386A</sub>                    | T382A +T384A+T386A CwIM variant            | pMV306:: <i>cwIMT382A</i> + <i>384A</i> + <i>T386A</i> | <i>cwIM</i> -CM complementation                        |
| <i>cwIM</i> -CM <sub>T43A+T382A+T386A</sub>                     | T43A+T382A +T384A CwIM variant             | pMV306:: <i>cwIMT43A</i> + <i>T382A</i> + <i>386A</i>  | <i>cwIM</i> -CM complementation                        |
| <i>cwIM</i> -CM <sub>T384A+T386A</sub>                          | T384A +T386A CwIM variant                  | pMV306:: <i>cwIMT384A</i> + <i>T386A</i>               | <i>cwIM</i> -CM complementation                        |
| <i>cwIM</i> -CM <sub>T43D</sub>                                 | T43D CwIM variant                          | pMV306:: <i>cwIMT43D</i>                               | <i>cwIM</i> -CM complementation                        |
| <i>cwIM</i> -CM <sub>T382D</sub>                                | T382D CwIM variant                         | pMV306:: <i>cwIMT382D</i>                              | <i>cwIM</i> -CM complementation                        |
| <i>cwIM</i> -CM <sub>T386D</sub>                                | T386D CwIM variant                         | pMV306:: <i>cwIMT386D</i>                              | <i>cwIM</i> -CM complementation                        |
| <i>cwIM</i> -CM <sub>T382D+T386D</sub>                          | T382D+T386D CwIM variant                   | pMV306:: <i>cwIMT382D</i> + <i>T386D</i>               | <i>cwIM</i> -CM complementation                        |
| <i>cwIM</i> -CM <sub>T43D+T382D+T386D</sub>                     | T43D+T382D+T386D CwIM variant              | pMV306:: <i>cwIMT43D</i> + <i>T382D</i> + <i>T386D</i> | <i>cwIM</i> -CM complementation                        |
| <i>cwIM</i> -CM <sub>R2</sub>                                   | Truncated CwIM version (M1- K370)          | pMV306:: <i>cwIMR2</i>                                 | <i>cwIM</i> -CM complementation                        |
| <b><i>Mycobacterium smegmatis</i> mc<sup>2</sup>155 strains</b> |                                            |                                                        |                                                        |
| pUAB300+<br>pUAB400                                             | Empty plasmid control                      | pUAB300<br>pUAB400                                     | Mycobacterial protein fragment complementation (M-PFC) |

|                                                 |                                                      |                                                              |                                          |
|-------------------------------------------------|------------------------------------------------------|--------------------------------------------------------------|------------------------------------------|
| CwIM <sub>100</sub> /<br>DnaA <sub>200</sub>    | Interaction strain                                   | pUAB100:: <i>cwI</i><br>pUAB200:: <i>dnaA</i>                | M-PFC                                    |
| CwIM <sub>300</sub> /<br>DnaA <sub>400</sub>    | Interaction strain                                   | pUAB300:: <i>cwIM</i><br>pUAB400:: <i>dnaA</i>               | M-PFC                                    |
| CwIM <sub>100</sub> /<br>FtsE <sub>200</sub>    | Interaction strain                                   | pUAB100:: <i>cwIM</i><br>pUAB200:: <i>ftsEA</i>              | M-PFC                                    |
| CwIM <sub>300</sub> /<br>FtsE <sub>400</sub>    | Interaction strain                                   | pUAB300:: <i>cwIM</i><br>pUAB400:: <i>ftsE</i>               | M-PFC                                    |
| CwIM <sub>100</sub> /<br>FtsZ <sub>200</sub>    | Interaction strain                                   | pUAB100:: <i>cwIM</i><br>pUAB200:: <i>ftsZ</i>               | M-PFC                                    |
| CwIM <sub>300</sub> /<br>FtsZ <sub>400</sub>    | Interaction strain                                   | pUAB300:: <i>cwIM</i><br>pUAB400:: <i>ftsZ</i>               | M-PFC                                    |
| CwIM <sub>100</sub> /<br>Wag31 <sub>200</sub>   | Interaction strain                                   | pUAB100:: <i>cwIM</i><br>pUAB200:: <i>wag31</i>              | M-PFC                                    |
| CwIM <sub>300</sub> /<br>Wag31 <sub>400</sub>   | Interaction strain                                   | pUAB300:: <i>cwIM</i><br>pUAB400:: <i>wag31</i>              | M-PFC                                    |
| CwIM <sub>100</sub> /<br>FhaA <sub>200</sub>    | Interaction strain                                   | pUAB100:: <i>cwIM</i><br>pUAB200:: <i>fhaA</i>               | M-PFC                                    |
| CwIM <sub>300</sub> /<br>FhaA <sub>400</sub>    | Interaction strain                                   | pUAB300:: <i>cwIM</i><br>pUAB400:: <i>fhaA</i>               | M-PFC                                    |
| CwIM <sub>100</sub> /<br>MurJ <sub>icd200</sub> | Interaction strain                                   | pUAB100:: <i>cwIM</i><br>pUAB200:: <i>murJ<sub>icd</sub></i> | M-PFC                                    |
| CwIM <sub>300</sub> /<br>MurJ <sub>icd400</sub> | Interaction strain                                   | pUAB300:: <i>cwIM</i><br>pUAB400:: <i>murJ<sub>icd</sub></i> | M-PFC                                    |
| CwIM <sub>300</sub> /<br>CwsA <sub>400</sub>    | Interaction strain                                   | pUAB300:: <i>cwIM</i><br>pUAB400:: <i>cwsA</i>               | M-PFC                                    |
| MurJ <sub>icd100</sub> /<br>FhaA <sub>200</sub> | Interaction strain                                   | pUAB100:: <i>murJ<sub>icd</sub></i><br>pUAB200:: <i>fhaA</i> | M-PFC                                    |
| CwIM <sub>100/200</sub>                         | Interaction strain                                   | pUAB100:: <i>cwIM</i><br>pUAB200                             | M-PFC                                    |
| CwIM <sub>300/ 400</sub>                        | Interaction strain                                   | pUAB300:: <i>cwIM</i><br>pUAB400                             | M-PFC                                    |
| MurJ <sub>icd200/ 100</sub>                     | Interaction strain                                   | pUAB100<br>pUAB200:: <i>murJ<sub>icd</sub></i>               | M-PFC                                    |
| MurJ <sub>icd400/300</sub>                      | Interaction strain                                   | pUAB300<br>pUAB400:: <i>murJ<sub>icd</sub></i>               | M-PFC                                    |
| C41(DE3) pET<br>CwIM                            | 6xHis-CwIM expression<br>strain                      | pET15bTEV:: <i>cwIM</i>                                      | M-PFC                                    |
| C41 (DE3)<br>pET MurJ <sub>icd</sub>            | 6xHis-MurJ <sub>icd</sub><br>expression strain       | pET15bTEV:: <i>murJ<sub>icd</sub></i>                        | Recombinant<br>MurJ <sub>icd</sub>       |
| C41 (DE3)pET<br>MurJ <sub>E541-F680</sub>       | 6xHis-MurJ <sub>E541-F680</sub><br>expression strain | pET15bTEV:: <i>murJ<sub>E541-F680</sub></i>                  | Recombinant<br>MurJ <sub>E541-F680</sub> |
| C41 (DE3)<br>pGEX Wag31                         | GST-Wag31 expression<br>strain                       | pGEX:: <i>wag31</i>                                          | Recombinant<br>Wag31                     |
| C41 (DE3)<br>pGEX FhaA                          | GST-FhaA expression<br>strain                        | pGEX:: <i>fhaA</i>                                           | Recombinant<br>FhaA                      |
| BL21 (DE3)<br>pGEX PknB                         | GST-PknB kinase<br>domain expression<br>strain       | pGEX:: <i>pknB<br/>kinase</i>                                | Recombinant<br>PknB kinase<br>domain     |

**Table S3. Effect of threonine mutations on growth of *M. tuberculosis* in various media.**

**Related to Figures 2 and S3.**

| Strain                                | HK 7H10 agar | HKP 7H10 agar  | HK 7H9 liquid | HKP 7H9 liquid | SMM | SMP |
|---------------------------------------|--------------|----------------|---------------|----------------|-----|-----|
| CM-CwIM <sub>pMV</sub>                | -            | +++            | -             | +++            | -   | ++  |
| CM-CwIM <sub>WT</sub>                 | +++          | +++            | +++           | +++            | +++ | +++ |
| CM-CwIM <sub>T43A</sub>               | +++          | +++            | +++           | +++            | +++ | +++ |
| CM-CwIM <sub>T382A</sub>              | -            | +++            | -             | +++            | ++  | +++ |
| CM-CwIM <sub>T386A</sub>              | ++           | +++            | ++            | +++            | +++ |     |
| CM-CwIM <sub>T382A+T386A</sub>        | No colonies  | No colonies    | N/A           | N/A            | N/A | N/A |
| CM-CwIM <sub>T382A+T386A+T43A</sub>   | -            | +++            | -             | +++            | ++  | +++ |
| CM-CwIM <sub>T42A</sub>               | +++          | +++            | +++           | +++            | +++ | +++ |
| CM-CwIM <sub>T42A+T43A</sub>          | +++          | +++            | ++            | +++            | +++ | +++ |
| CM-CwIM <sub>T94A</sub>               | +++          | +++            | +++           | +++            | +++ | +++ |
| CM-CwIM <sub>T384A</sub>              | +++          | +++            | +++           | +++            | +++ | +++ |
| CM-CwIM <sub>T382A+T384A</sub>        | -            | +++            | -             | +++            | ++  | +++ |
| CM-CwIM <sub>T384A+T386A</sub>        | +++          | +++            | +++           | +++            | +++ | +++ |
| CM-CwIM <sub>T382A+T384A+T386A</sub>  | -            | Small colonies | N/A           | N/A            | N/A | N/A |
| CM-CwIM <sub>T43D</sub>               | +++          | +++            | +++           | +++            | +++ | +++ |
| CM-CwIM <sub>T382D</sub>              | ++           | +++            | ++            | +++            | ++  | +++ |
| CM-CwIM <sub>T386D</sub>              | +++          | +++            | +++           | +++            | +++ | +++ |
| CM-CwIM <sub>T382D+T386D</sub>        | -            | +++            | -             | +++            | -   | +++ |
| CM-CwIM <sub>T382D + T386D+T43D</sub> | -            | +++            | -             | +++            | ++  | -   |
| CM-CwIM <sub>T42D+T43D</sub>          | +++          | +++            | +++           | +++            | +++ | +++ |
| CM-CwIM <sub>T382D+T384D</sub>        | -            | +++            | -             | +++            | N/D | N/D |
| CM-CwIM <sub>R2</sub>                 | -            | ++             | -             | ++             | -   | ++  |

N/A – not applicable; N/D – not determined; “+++” - good growth compared with WT Mtb strain; “++” or “+” growth with defect; - no growth; “HK” with addition of hygromycin and kanamycin; “HKP” with addition of hygromycin, kanamycin and pristinamycin; SMM – sucrose magnesium medium.

**Table S4. Identification of potential CwIM partners by immuno-precipitation and protein fragment complementation assays. Related to Figure 4.**

| Protein | Gene           | Function                                          | Fraction  | M-PFC        |
|---------|----------------|---------------------------------------------------|-----------|--------------|
| DnaA    | <i>Rv0001</i>  | Chromosomal replication initiator protein         | Cytoplasm | N            |
| CwsA    | <i>Rv0008c</i> | Membrane protein involved in division             | Membrane  | Y            |
| FhaA    | <i>Rv0020c</i> | Conserved protein with FhaA                       | Cytoplasm | Y            |
| MurA    | <i>Rv1315</i>  | UDP-N-acetylglucosamine 1-carboxyvinyltransferase | Cytoplasm | Not assessed |
| Wag31   | <i>Rv2145c</i> | DivIVA family protein                             | Cytoplasm | N            |
| FtsZ    | <i>Rv2150c</i> | Cell division protein                             | Cytoplasm | N            |
| FtsE    | <i>Rv3102c</i> | Putative cell division ATP-binding protein        | Membrane  | N            |
| MviN    | <i>Rv3910</i>  | Potential Lipid II flippase MurJ                  | Membrane  | Y            |

N – no growth on trimethoprim plates; Y- interaction.

**Table S5. Primers used in the study. Related to Figures 2-4, S2-S5.**

| PRIMER       | SEQUENCE 5'-3'                            | DESCRIPTION                     |
|--------------|-------------------------------------------|---------------------------------|
| CMRv3915F    | ACTGCCATGGGCCCCGAGTCCGCGCGAA              | <i>cw/M</i> -CM generation      |
| CMRv3915R    | ACGTGCATGCTCATGCGTCGGACGGACTACG           | <i>cw/M</i> -CM generation      |
| FG3106       | CGTTGGCGGTGGCGGCAC                        | <i>cw/M</i> -CM confirmation    |
| FG2224       | CCGTACACCGTACAAGGAG                       | <i>cw/M</i> -CM confirmation    |
| Rv3915pEF    | ACACATATGCCGAGTCCGCGCCGCGAAGA             | Recombinant CwIM                |
| RV3915PETR1  | ACAGGATCCTTAAGAACCGCCGAGTCTACCCG          | Recombinant CwIM                |
| 3915PMV306F2 | ACTGGTACC AGCCGGTGAAACGAATCGTT            | <i>cw/M</i> -CM complementation |
| 3915PMV306R1 | TACAAGCTTTAAGAACCGCCGAGTCTACC             | <i>cw/M</i> -CM complementation |
| 3915PMV306R3 | TACAAGCTTTCAAACGGCTGTATCTGTTA             | <i>cw/M</i> -CM complementation |
| 3915T43AF    | CAGGAAGAAGACCTGACGGCGGGCCGTAACGTGCGCCTT   | CwIM SDM                        |
| 3915T43AR    | AAGGGCGACGTTACGGCCCCGCCGTCAGGTCTTCTTCCTG  | CwIM SDM                        |
| 3915T43DF    | CAGGAAGAAGACCTGACGGATGGCCGTAACGTGCGCCTT   | CwIM SDM                        |
| 3915T43DR    | AAGGGCGACGTTACGGCCATCCGTCAGGTCTTCTTCCTG   | CwIM SDM                        |
| 3915T382AF   | GCAAGAACGATCGGCCCCGCTGGCACATTCACTTTCGC    | CwIM SDM                        |
| 3915T382AR   | GCGAAAGTGAATGTGCCAGCGGGCCGATCGTTCTTGC     | CwIM SDM                        |
| 3915T382DF   | GGCAAGAACGATCGGCCCCGACGGCACATTCACTTTCGCC  | CwIM SDM                        |
| 3915T382DR   | GGCGAAAGTGAATGTGCCGTGCGGGCCGATCGTTCTTGCC  | CwIM SDM                        |
| 3915T386AF   | CGGCCCACCGGCACATTCGCTTTCGCCGAGTTGCTGGCC   | CwIM SDM                        |
| 3915T386AR   | GGCCAGCAACTCGGCGAAAGCGAATGTGCCGGTGGGCCC   | CwIM SDM                        |
| 3915T386DF   | CGGCCCACCGGCACATTCGATTTGCCCGAGTTGCTGGCC   | CwIM SDM                        |
| 3915T386DR   | GGCCAGCAACTCGGCGAAATCGAATGTGCCGGTGGGCCC   | CwIM SDM                        |
| 3915T382-6AF | GCAAGAACGATCGGCCCCGCTGGCACATTCGCTTTCG     | CwIM SDM                        |
| 3915T382-6AR | GCGAAAGCGAATGTGCCAGCGGGCCGATCGTTCTTGC     | CwIM SDM                        |
| 3915T382-6DF | CGGCCCCGACGGGCACATTCGATTTGCCCGAGTTGCTGGCC | CwIM SDM                        |
| 3915T382-6DR | GGCCAGCAACTCGGCGAAATCGAATGTGCCGTGCGGGCCG  | CwIM SDM                        |
| 3915T384AF2  | ACGATCGGCCCCACCGGCGCATTCACTTTCGCCGAGTT    | CwIM SDM                        |
| 3915T384AR2  | AACTCGGCGAAAGTGAATGCGCCGGTGGGCCGATCGT     | CwIM SDM                        |
| 2AtailF      | AACGATCGGCCCCGCTGGCGCATTCACTTTCGCCGAGTTG  | CwIM SDM                        |
| 2AtailR      | CAACTCGGCGAAAGTGAATGCGCCAGCGGGCCGATCGTT   | CwIM SDM                        |
| 2AendF       | ACGATCGGCCCCACCGGCGCATTCGCTTTCGCCGAGTT    | CwIM SDM                        |
| 2AendR       | AACTCGGCGAAAGCGAATGCGCCGGTGGGCCGATCGT     | CwIM SDM                        |
| 3AtailF      | AACGATCGGCCCCGCTGGCGCATTCGCTTTCGCCGAGTTG  | CwIM SDM                        |
| 3AtailR      | CAACTCGGCGAAAGCGAATGCGCCAGCGGGCCGATCGTT   | CwIM SDM                        |
| T42AF        | CATCAGGAAGAAGACCTGGCGACGGGCCGTAACGTGCGCC  | CwIM SDM                        |
| T42AR        | GGCGACGTTACGGCCCCGTGCGCAGGTCTTCTTCCTGATG  | CwIM SDM                        |
| T42-43AF     | CATCAGGAAGAAGACCTGGCGGGCGGGCCGTAACGTGCGCC | CwIM SDM                        |
| T42-43AR     | GGCGACGTTACGGCCCCGCCGCGCAGGTCTTCTTCCTGATG | CwIM SDM                        |
| T94AF        | TACCGGCTCGGGGCCCGCGCGCTGTACCACCAATTCGGC   | CwIM SDM                        |
| T94AR        | GCCGAATTGGTGGTACAGCGCGCGGGCCCCGAGCCGGTA   | CwIM SDM                        |
| 2DtailF      | AACGATCGGCCCCGACGGCGACTTCACTTTCGCCGAGTTG  | CwIM SDM                        |
| 2DtailR      | CAACTCGGCGAAAGTGAAGTCGCCGTGCGGGCCGATCGTT  | CwIM SDM                        |
| T42DF        | CATCAGGAAGAAGACCTGGACACGGGGCCGTAACGTGCGCC | CwIM SDM                        |
| T42DR        | GGCGACGTTACGGCCCCGTGTCCAGGTCTTCTTCCTGATG  | CwIM SDM                        |
| UAB1003915F  | ATAGGATCCATGCCGAGTCCGCGCCGCGAA            | M-PFC                           |
| UAB1003915R  | CACATCGATAGAACCGCCGAGTCTACCCGC            | M-PFC                           |

|               |                                        |                                            |
|---------------|----------------------------------------|--------------------------------------------|
| UAB200Wag31F  | ACACAATTGATGCCGCTTACACCTGCCGAC         | M-PFC                                      |
| UAB200Wag31R  | ACATCGATGTTTTTGGCCCGTTGAATTGA          | M-PFC                                      |
| UAB200FhaAF   | GACACAATTGATGGGTAGCCAGAAAAGGCT         | M-PFC                                      |
| UAB200FhaAR   | AACGTCGACGTGCATGCGGACGATGATCTC         | M-PFC                                      |
| UAB200FtsEF   | ACACAATTGATGATCACCCCTGGACCATGTC        | M-PFC                                      |
| UAB200FtsER   | GAC ATCGAT GCGATCCATCCCGTAGACGCC       | M-PFC                                      |
| UAB200FtsZF   | ACACAATTGATGACCCCCCGCACAACTAC          | M-PFC                                      |
| UAB200FtsZR   | GAC ATCGATGCGGGCGCATGAAGGGCGGCAC       | M-PFC                                      |
| UAB200DnaAF   | CACAATTGTTGACCGATGACCCCGGTTCA          | M-PFC                                      |
| UAB200DnaAR   | GAC ATCGATGCGCTTGGAGCGCTGACGGAT        | M-PFC                                      |
| UAB400FhaAF   | ACAGAATTCGATGGGTAGCCAGAAAAGGCT         | M-PFC                                      |
| UAB400-FhaAR  | CACAAGCTTTCAGTGCATGCGGACGATGAT         | M-PFC                                      |
| UAB200 MviNF1 | ACACAATTGGCCGTGCGAGCCCGAATCAGGA        | M-PFC                                      |
| UAB200MviNR1  | AACATCGATGTTGCGACGGCGGGGTTGA           | M-PFC                                      |
| UAB400MviNR1  | GACATCGATCTAGTTGCGACGGCGGGGTT          | M-PFC                                      |
| UAB100MviNF2  | CACGTTAACATG GCCGTGCGAGCCCGAATCA       | M-PFC                                      |
| pETMviNF      | CACCATATGGCCGTGCGAGCCCGAATCAGGA        | Recombinant<br>MurJ <sub>icd</sub>         |
| pETMviNR      | GAC GCT AGCCTAGTTGCGACGGCGGGGTA        | Recombinant<br>MurJ <sub>icd</sub>         |
| FhaALEICF     | TACTTCCAATCCATGGGTAGCCAGAAAAGGCTGGT    | Recombinant<br>FhaA                        |
| FhaALEICR     | TATCCACCTTTACTGTCACTGATGCGGACGATGATCT  | Recombinant<br>FhaA                        |
| Wag31LeicF    | TACTTCCAATCCATGCCGCTTACACCTGCCGACGT    | Recombinant<br>Wag31                       |
| Wag31LeicR    | TATCCACCTTTACTGTCACTAGTTTTTGGCCCGGTTGA | Recombinant<br>Wag31                       |
| RTPknBF1      | TCAGAACGGAATCATCCACCGTGA               | qRT-PCR                                    |
| RTPknBR1      | GCGATGCCGAAATCCATCACCTTT               | qRT-PCR                                    |
| PMV306F       | TGGTATCTTTATAGTCCTGTC                  | pMV306-specific<br>primer                  |
| pMV306R2      | TAGTTAACTACGTGCACATCGA                 | pMV306-specific<br>primer                  |
| MviNpETR3     | CATGGATCCCTAGAACGCAATCGGCCACG          | Recombinant<br>MurJ <sub>E541-F680</sub>   |
| MviNpETF2     | TATCATATGGAGGCGCGGGCGGCGCTGGAT         | Recombinant<br>MurJ <sub>E541-F680</sub> I |