Methods for Supplementary Figures:

Electrophoretic mobility shift assays for Fig. S2: A 550-bp of *Mtb ori*C was cloned into the pBluescript II vector and amplified with FITC-labeled primer specific to the *ori*C 5'end and Cy3-primer specific to the 3' end. In a 20 µl final reaction containing MtrA and MtrA~P at indicated concentration, 50 mM Tris-HCl, pH 7.0, 50 mM NaCl, 10 mM $MgCl_2$ and 5 % glycerol, 20 pmoles polydI/dC 20, 1 µg sheared salmon sperm, 1 µg BSA, and 200 fmoles DNA substrate were prepared on ice. Reactions were incubated at 37^0C for 15 min, mixed with sample buffer containing 10% glycerol, resolved on 5% polyacrylamide gels and scanned in a BioRad Molecular Imager Fx and photographed. As control, *ori*C incubated with EnvZ under the same conditions and the samples was resolved and processed as described above.

Electrophoretic mobility shift assays for Fig.s S3 and S4: The DNA sequences on the top strand of these oligonucleotides were as follows: '*fbpB*' 5'-GGG AGG CCA AAT GTC GAT TCG GGC GCA AAG TCG TCT CAT TTC CGT ATC GGT TAC CGC-3', '*oriC* F2(+2) '5'-GCT TCT TCC TCC CAG GTC ACA CCA CGG TCA CAG AGA TTA GCT G-3', 'wt' *ori* F2 5'- GCT TCT TCC TCC CAG GTC ACA CCA GTC ACA GAG ATT GGA TG-3', 'mut F2+2' 5'- GCT TCT TCC TCC CAG CAG TTC CCA CGG TCA CAG AGA TTA GCT G-3'.
MtrA~P was prepared by mixing a 10:1 molar ratio of MtrA:MBP-EnvZ in 20 mM Na phosphate, pH=7.8, 150 mM NaCl, 0.1 mM EDTA, 1 mM DTT, 1.5 mM ATP, 5 mM MgCl2, 10% glycerol and incubating at 37^oC for 30 min. Serial dilutions of this kinase reaction (originally containing 360 picomoles of MtrA protein) in 10 µl were mixed with 8 femtomoles of ^{32}P-dsDNA in 10 µl (20 mM Tris, pH= 7.6; 15mM MgCl2; 1 mM CaCl2; 100 mM KCl, 20 µg/ml poly-dI-dC). These 20 ul binding reactions were held on ice for 10 min before addition of 2 µl (20 mM Tris, pH= 7.6; 20 mM EDTA, 30% glycerol). Finally, reactions were resolved by electrophoresis on 8 % PA (0.5X TBE), dried and exposed to a phosphor-imaging screen. Quantification was done with Quantity One analysis software (BioRad).

Macrophage infections: **PBMCs isolation and culture.** Human peripheral blood mononuclear cells (PBMC) were prepared from whole blood obtained from healthy volunteers, using standard density gradient Ficoll-Paque Plus (Amersham Biosciences, Piscataway, NJ). Monocytes were isolated from PBMCs by adherence. $5x10^6$ PMBCs per well were incubated at 37°C with 5% CO_2 for 90min in RPMI-1640 media containing 2 mM L-glutamine (Invitrogen), 1 mM sodium pyruvate (Invitrogen), 10% fetal bovine serum (Invitrogen) and 100 U/ml penicillin (Sigma) in 24–well tissue culture plates. Nonadherent cells were removed by washing with RPMI-1640 media. The complete medium was replaced and monocytes matured after 4 days to monocytes-derived macrophages (MDMØs). After that, medium was changed and cells were used for infection. Single cell suspensions of *Mtb* strains in RPMI 1640 media were used to infect 4.5 x 10^5 macrophages in triplicate in a 24-well plate at a multiplicity of infection of 1:2–4. After 3 hours of infection, macrophages were lysed in 0.09% SDS and *Mtb* viability was determined to get t0 count. Subsequently, macrophages were also processed 3 and 6 days post-infection in order to determine the *Mtb* viability.

Legends for Supplementary Figures:

Figure S1: Viability of *Mtb mtr*A merodiploid strains in PBMC: Viability after 3h, 3 and 6 days post infection was determined and data are presented as Log CFU. Black bars indicates Rv-78 *Mtb*(MtrA+) whereas grey bar – Rv-129 *Mtb*(D53N MtrA+) and white bar – Rv-19 wild-type.

Figure S2: Polyacrylamide gel analysis of *ori*C-MtrA complexes: The concentrations of MtrA and MtrA~P used are marked. EnvZ blank refers to the *ori*C incubated with phosphorylated EnvZ and processed as described above. *ori*C blank refers to *ori*C in the absence of MtrA and EnvZ processed as described above.

Figure S3: MtrA~P forms a second complex (c2) with ***fbpB*** that requires threshold MtrA~P concentrations. (A) Rapid lose ***fbpB*** binding upon gradual dilution of MtrA~P. Lane 1 reaction omitted MtrA and the lane 2 reaction contained 360 pmoles of MtrA but omitted ATP from the kinase reaction. The upper most bands in lane 2 are non-specific since they are suppressed by extra poly-dI-dC competitor (not shown) and by MtrA~P (+ATP, lane 3). For reactions in lanes 3-15, gradual 9/10-fold serial dilutions of the complete kinase reaction (containing 360, 324, 292, 263, … down to 91 picomoles of MtrA protein) in 10 µl were mixed with 8 femtomoles of ^{32}P-***fbpB*** dsDNA in 10 µl. These 20 ul binding reactions were resolved by electrophoresis on 8 % PA (0.5X TBE), dried and exposed to a phosphor-imaging screen. These experiments clearly show that a concentration threshold is required for binding. Unfortunately, much of the complexes dissociate during electrophoresis and their measurements are unreliable. (B) MtrA~P (ATP-specific) protein-DNA complexes driven by extra high DNA target concentrations. Otherwise identical reactions used 360 picomoles of MtrA protein and 50 picomoles of the specified unlabeled dsDNA. The poly-dI-dC was omitted and the gels were stained with ethidium bromide. The *fbpB* DNA with 4 MtraA direct repeats (Fig. 2B), forms two complexes (c1 and c2, lane 2), while the *oriC* F2(+2) DNA with 2 MtrA direct repeats (Fig. 4) forms only one complex (lane 4).

Figure S4: Effect of spacing on MtrA affinity for its DNA binding motifs. EMSA was performed with serial three-fold dilutions of MtrA~P and ^{32}P-labelled, dsDNA oligonucletides modeled after the *oriC* F2 region. Shown in (A) are two identical experiments for each of three related probes: a 'wt' *ori* F2 sequence (left), a '+2' spacing variant ('*ori* F2+2'; middle), and an analogous spacing variant with additional bp changes that change the left-most GTCACA motif ('mut F2+2'; right). Labeled DNA species having reduced mobility compared to the free probe are found in at least two distinct complexes. Additional experiments (not shown) reveal that the top-most (slowest-migrating) complex is non-specific, as it fails to form in the presence of (1ng/uL) poly dIdC. (B) Histograms plotting the fraction of total bound probe with decreasing protein concentration. Bars in the histogram are split to reflect the contribution of each type of MtrA:DNA complex to the total bound DNA in each lane. The fraction of specifically-bound probe (excluding signals from non-specific complexes and from remaining 'free' probe) can be plotted against the MtrA dilution series to determine the concentration required for 'half-maximal' binding. Data from the '*ori* F2+2' probe can be reliably fit to a logarithmic curve (R^2 = 0.96; not shown), allowing determination of the dissociation constant for the binding reaction (Kd = 4.6 µ*M*). In contrast, neither of the 'wt *ori* F2', or the 'mut F2+2' probes become more

than 50% bound, even with undiluted MtrA~P. Thus, we can only put an upper limit on dissociation constants for these sequences, such that Kd > 20 μ*M*.

Figure S5: Conservation of *oriC* DNA sequences at MtrA footprint F2. The *oriC* DNA sequences were obtained through the integrated microbial genomes (IMG) system, http://img.jgi.doe.gov (45). The abbreviations correspond to the following species: M tub, *Mycobacterium tuberculosis* H37Rv, M lep, *Mycobacterium leprae* TN, M avi, *Mycobacterium avium* subsp. Paratuberculosis, M smeg, *Mycobacterium smegmatis* MC2 155, M flav, *Mycobacterium flavescens* PYR-GCK, M MCS, *Mycobacterium sp.* MCS, M abs, *Mycobacterium abscessus*. The corresponding *dnaA* 3' to 5' *dnaN* intergenic DNA sequences were analyzed by multi-way DNA alignment (CLUSTAL W (1.7), scoring matrix: mismatch = 9, open gap = 1, extended gap = 9, Clone Manager Software version 7). The most prominent DNA sequence alignments occur at the *dnaA* 3' region shown here. The output file was manually annotated only to add colors and to underline the GTCACA direct repeat motifs identified by the present studies. The AT-rich (DNA unwinding region) and the DnaA boxes, also identified in *Mycobacterium tuberculosis,* are shown.

Figure S6: Southern analysis of *Mtb* merodiploid strains: Genomic DNA preparations of *Mtb* merodiploid strains (Rv19-219; Rv78-219, Rv129-219) along with WT and Rv-219 genomic DNA were digested with *Bam*HI and *Eco*RI enzymes, transferred onto Nytran nitrocellulose membranes and blots were probed with ^{32}P- *ori*C. Arrowhead marked with '1' represents DNA band corresponding to 3-kb chromosomal *ori*C, whereas '2' and '3' represent the bands corresponding to 5.6-kb and 4. 2-kb plasmid DNA, respectively. Digestion of pMQ219 plasmid with *Bam*H-*Eco*RI produces 4. 2-kb fragment, if replicating extrachromosomally, whereas ~ 5. 5-kb if integrating at the region of homology. The pMQ219 plasmid could not be recovered from Rv19, Rv-78 and Rv-129 cells, although could be recovered from Rv WT. The pZErO2.1 plasmid and the integration proficient plasmid used for altering MtrA levels share ~880-bp sequence region of homology. Thus, the appearance of higher size band (5. 5-kb) is indicative of recombination events between these two plasmids in the region of homology. It remains to be tested if integrations at the chromosomal *ori*C region can be detected by characterizing several independent transformants. Stars (*) represent bands corresponding to DNA due to rearrangements/ deletions in some fraction of cells. More detailed studies are required to define the nature of these rearrangements, if any.

Figure S7 : Alignement of the 175 bp up-stream region of the *fbp*B gene : The up-stream 175 bp regions of *Mtb, M. ulcerans, M. leprae* and *M. avium* have been compared. For clarity MtrA footprint (from Fig. 3) alongwith direct repeats are marked. Note the alignement shows significant sequence conservation under the footprinted region in all these mycobacterial speceis. The conserved region includes plausible -35 and -10 sites with perfect 18 bp spacing, a characteristic of mycobacterial promoters (46).

Figure S8: qRT-PCR analysis of *fbp*A RNA expression. RNA samples from intracellular and broth grown cultures of wild type, Rv-78 *Mtb*(MtrA+) and Rv-129 *Mtb*(D53N MtrA) were used to analyze the expression of *fbp*A essentially as described for *fbp*B under Figure 5.

Figure S1

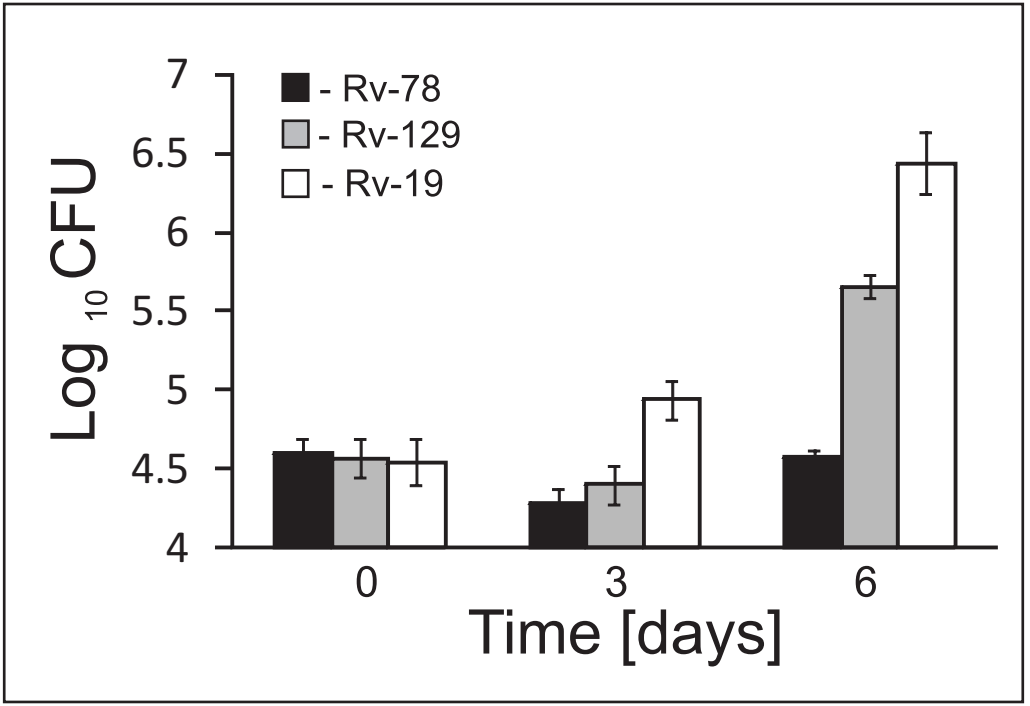


Figure S2

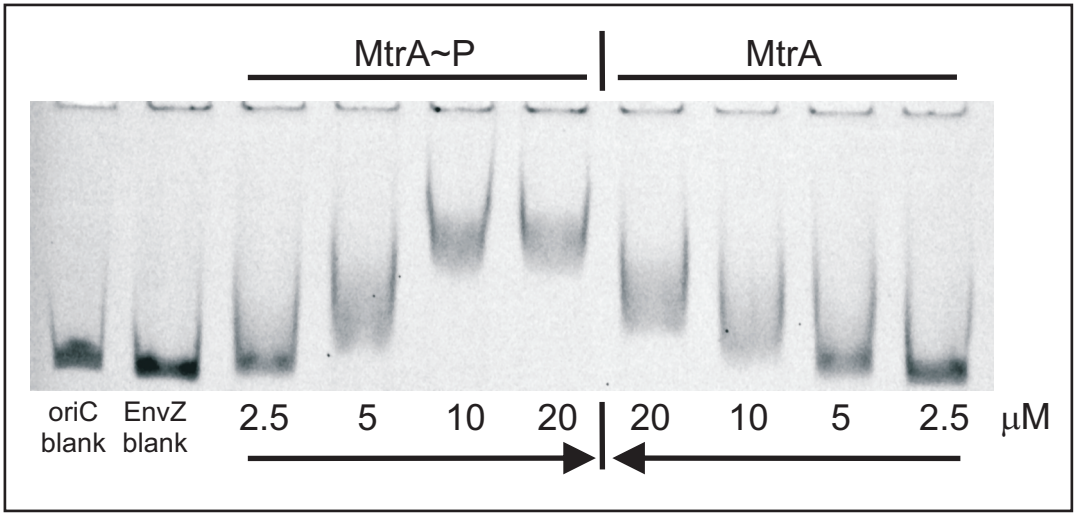


Figure S3

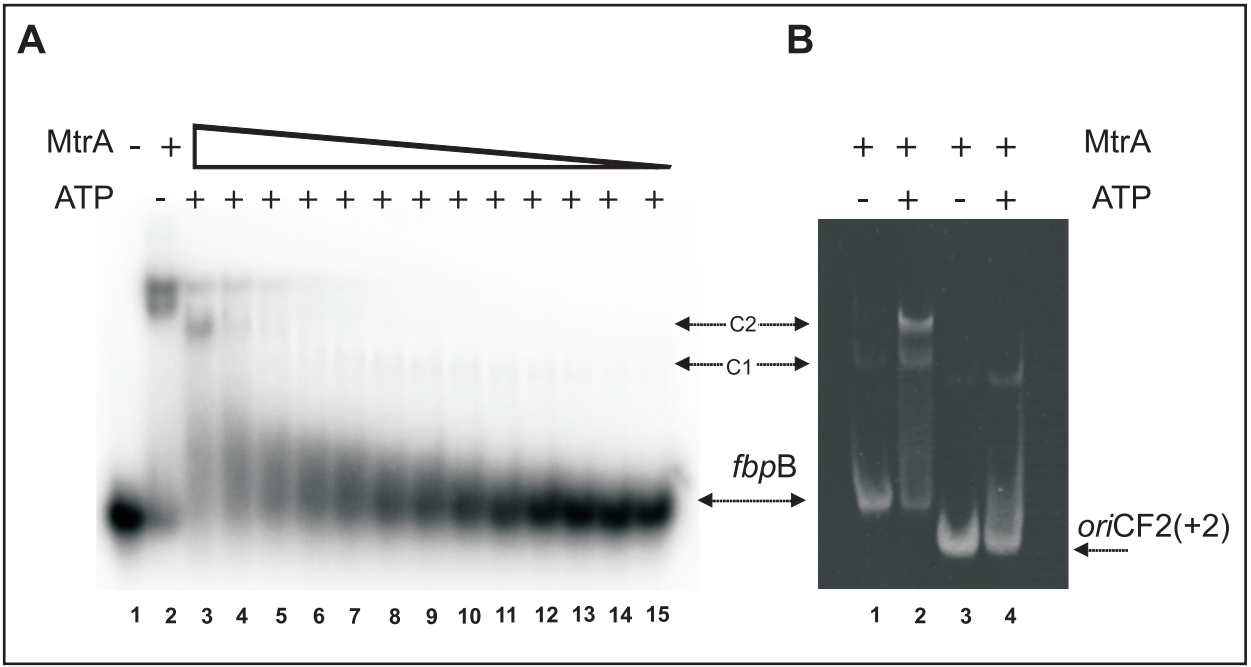

A
MtrA - +
ATP - + + + + + + + + + + + +
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
B
+ + + + MtrA
- + - + ATP
C2
C1
fbpB
oriCF2(+2)
1 2 3 4

Figure S4

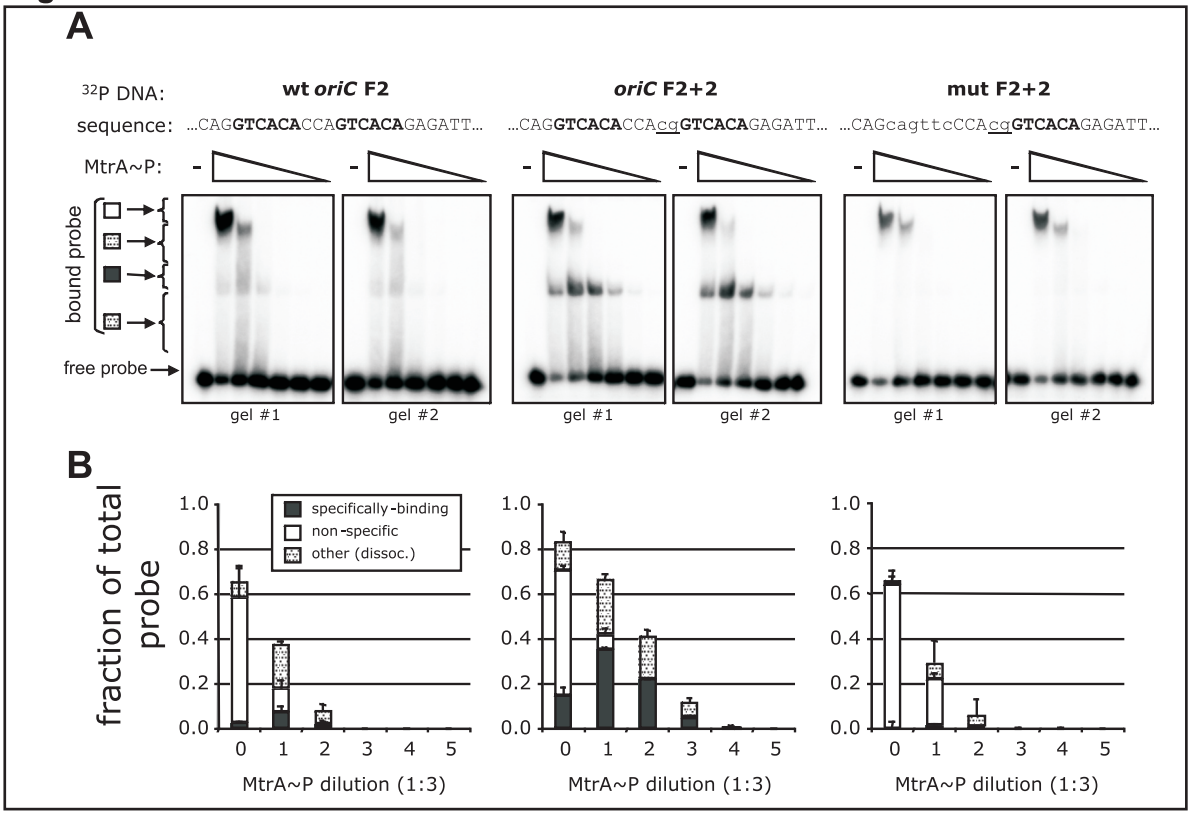

A
32P DNA:
wt oriC F2
oriC F2+2
mut F2+2
sequence:
…CAGGTCACACCAGTCACAGAGATT…
…CAGGTCACACCAcgGTCACAGAGATT…
…CAGcagttcCCAcgGTCACAGAGATT…
MtrA~P:
bound probe
free probe
gel #1
gel #2
gel #1
gel #2
gel #1
gel #2
B
fraction of total probe
specifically-binding
non-specific
other (dissoc.)
1.0
0.8
0.6
0.4
0.2
0.0
0 1 2 3 4 5
MtrA~P dilution (1:3)
MtrA~P dilution (1:3)
MtrA~P dilution (1:3)

Figure 5

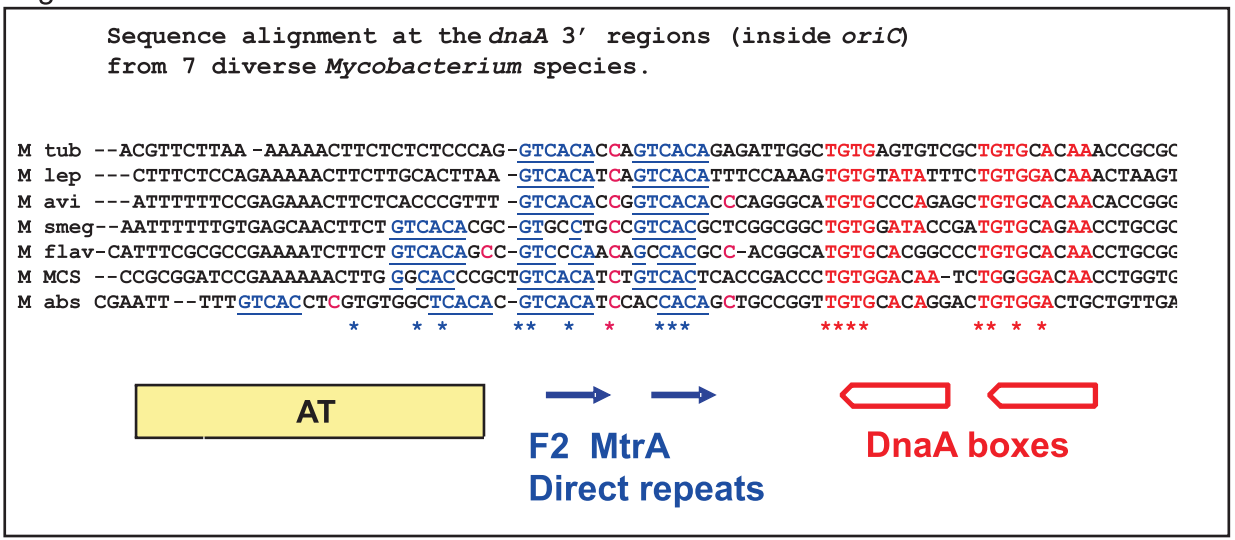

Sequence alignment at the dnaA 3′ regions (inside oriC)
from 7 diverse Mycobacterium species.
M tub --ACGTTCTTAA-AAAAACTTCTCTCTCCCAG-GTCACACCAGTCACAGAGATTGGCTGTGAGTGTCGCTGTGCACAAACCGCGC
M lep ---CTTTCTCCAGAAAAACTTCTTGCACTTAA-GTCACATCAGTCACATTTCCAAAGTGTGTATATTTCTGTGGACAAACTAAGT
M avi ---ATTTTTTCCGAGAAACTTCTCACCCGTTT-GTCACACCGGTCACACCCAGGGCATGTGCCCAGAGCTGTGCACAACACCGGG
M smeg--AATTTTTTGTGAGCAACTTCTGTCACACGC-GTGCCTGCCGTCACGCTCGGCGGCTGTGGATACCGATGTGCAGAACCTGCGC
M flav-CATTTCGCGCCGAAAATCTTCTGTCACAGCC-GTCCCAACAGCCACGCC-ACGGCATGTGCACGGCCCTGTGCACAACCTGCGG
M MCS --CCGCGGATCCGAAAAAACTTGGGCACCCGCTGTCACATCTGTCACTCACCGACCCTGTGGACAA-TCTGGGGACAACCTGGTG
M abs CGAATT--TTTGTCACCTCGTGTGGCTCACAC-GTCACATCCACCACAGCTGCCGGTTGTGCACAGGACTGTGGACTGCTGTTGA
AT
F2 MtrA
Direct repeats
DnaA boxes

Figure S6

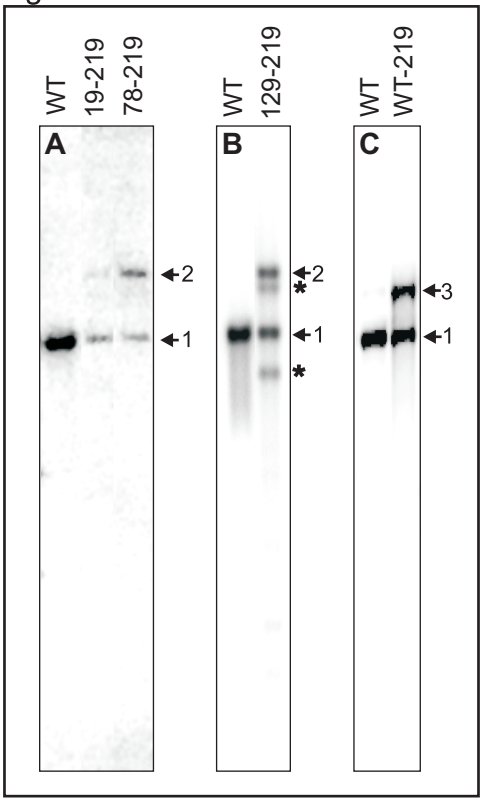
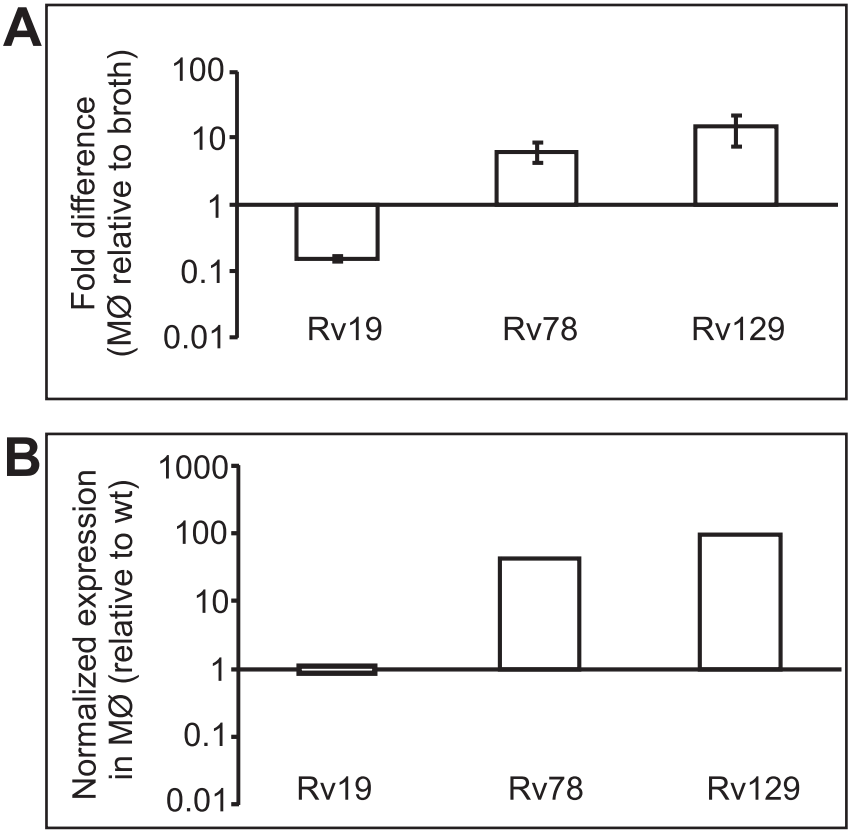


Figure S7


M tub 85B 175 CCGCGATAGATCCATACCGCCATACCGTTTGTGAGCCCCCTAAGCACACTTGCTCTGTCC
M ulc 85b 175 GGGAGGTTATTACCGAGAAGTCGGGATCGTGGCGGCTCGCGTAGCACAGGTGCTGTGTCC
M lep 85B 175 -GAACAAGAATCCGCTGGTAGGAACGGCTTCCGTGCGTCGACCAAACGGTTGCGGTATCC
M avi 85B 175 -ATCCCCGCACCCGTCGCCGCCCGGCGCTGCGGGCGGCGAAATTGCGAGGTGAAAACCTG
654321nn987654321nn987654321987654321
<<<<<< <<<<<< <<<<<< <<<<<< Direct repeats
G AC TG GAC TG G C GAC
GGCGGTAACC GATACGGAAATGAGACGACTTTGCGCCCGAATCGACATTTGGCCTCC
MtrA footprint
M tub 85B 175 GCGGCGG -TAACCGATACGGAAATGAGACGACTTTGCGCCCGAATCGACATTTGGCCTCC
M ulc 85b 175 GTGGCGG -TGGCCGATATCCAAATGAAATGAGTTCGCGTCCCGATCGACATTTGCCGTAC
M lep 85B 175 GTGACGGGTAACC GATACAGAACTGAGATCGCTTCGCGTCTCGATCGACATCTGGGCTAT
M avi 85B 175 CGAGACGAAAAGA GATGCGAAACCGAGACTCGTTCGCGTCCCTATCGACATTTGACGGAC
TTGACA -35
M tub 85B 175 ACACACGG TATGTTCTGGCCCGAGCACACGACGACATACAGGACAAAGGGGCACAA
M ulc 85b 175 TCACGCGGTAAGTTCTGCCGGGAGCACACGACGACATACGGACAAGGGGCACAGGT
M lep 85B 175 TCGCAGGGTAAATTCTGCCGAGAGCACCGACAACATACGATAAGGGGTATTAGGTT
M avi 85B 175 TCACACGGTAAATTCCTGTGAGACCACGATCACATGCGACCACATCCGACAAGGGG
fbpB Coding
TA---T +1
-10
TTGCGA-(N=18)-TAnnnT Agarwal and Tyagi 2006 NAR
-35 -10

Figure S8


A
Fold difference
(MØ relative to broth)
100
10
1
0.1
0.01
Rv19
Rv78
Rv129
B
Normalized expression
in MØ (relative to wt)
1000
100
10
1
0.1
0.01
Rv19
Rv78
Rv129