Supplementary Information

Physical and functional interaction between nucleoid-associated proteins HU and Lsr2 of *Mycobacterium tuberculosis*: altered DNA-binding and gene regulation

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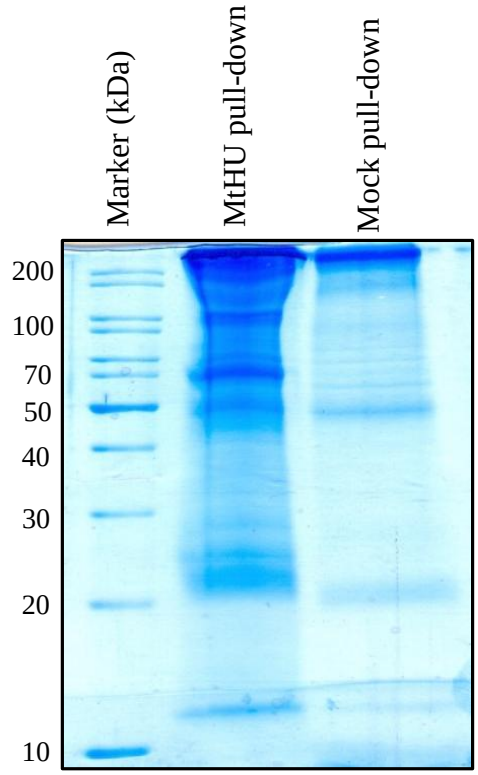
A.



B.

Sample name	Protein found in database	Entry name	Calculated MW	Score	Seq. cov.
H	Nucleoid-associated protein Lsr2 OS=Mycobacterium tuberculosis (strain ATCC 25618 / H37Rv) GN=lsr2 PE=1 SV=1	LSR2_MYCTU	12091	123	13%

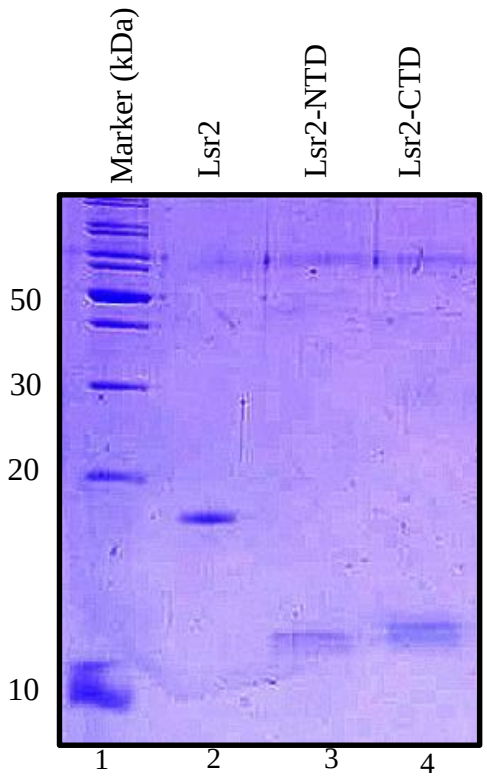
H

1 MAKKVTVTLV DDFDGSGAAD ETVEFGLDGV TYEIDLSTKN ATKLRGDLKQ

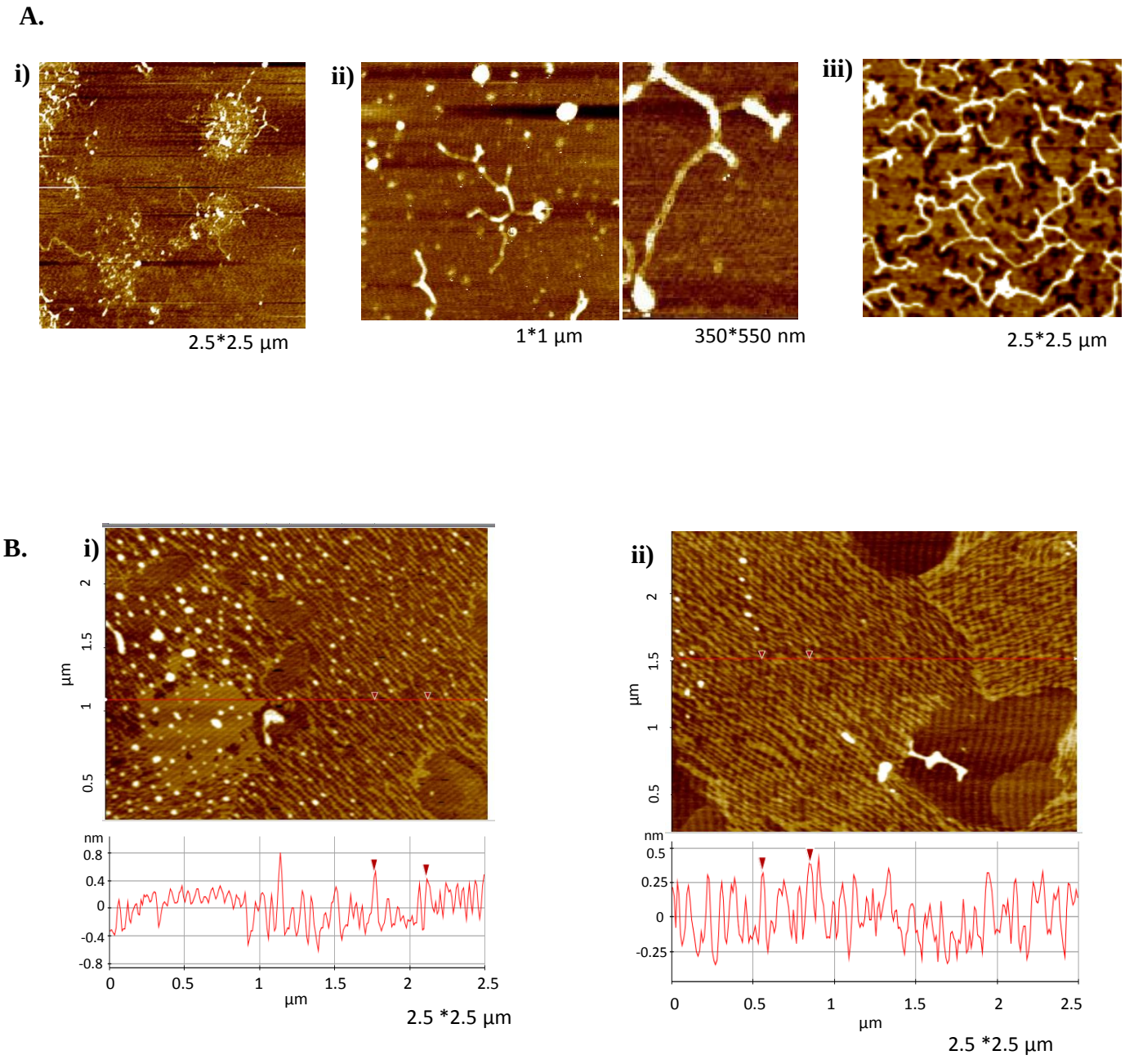
51 WVAAGRRVGG RRRGRSGSGR GRGAIDREQS AAIREWARRN GHNVSTR**GRI**

101 **PADVIDAYHA AT**

Supporting information Figure S1 A. MtHU was immuno-precipitated from *Mtb*H37Ra cell lysate using affinity-purified anti-MtHU antibody. The pool of proteins immuno-precipitated with MtHU as a test (Lane 2), and purified mice IgG as a mock (Lane 3) were electrophoresed in a 12% SDS-polyacrylamide gel and analysed by staining with Coomassie G-250. **B**. Tryptic digests of the pool of precipitated proteins (from *Mtb*H37Ra cultures grown under hypoxia) were analysed by mass spectrometry (LC MS/MS) (shown in tabular form). The unique tryptic digests in the MtHU co-immunoprecipitated fraction, which maps to MtLsr2, has been shown in bold and underlines.

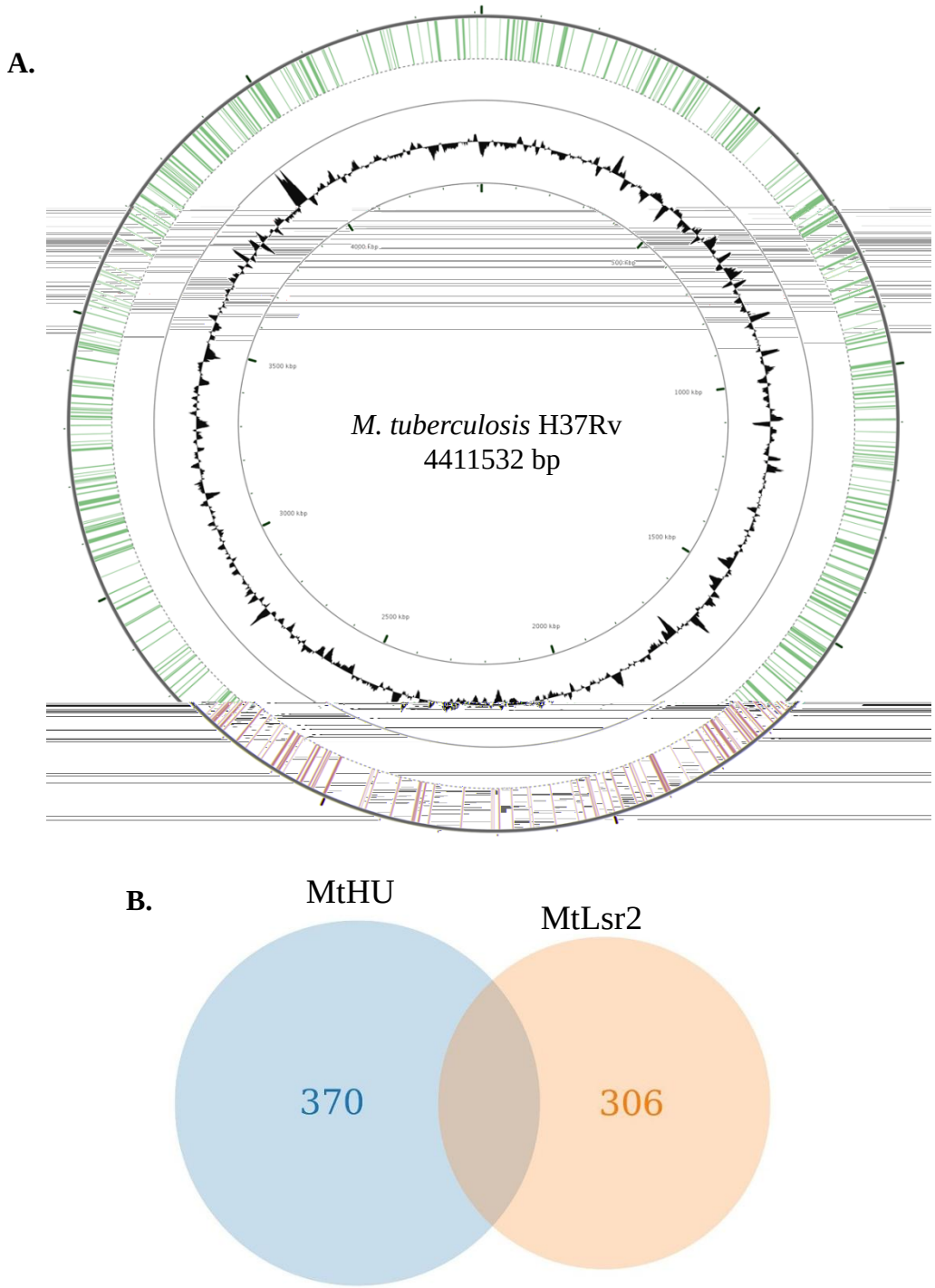


Supporting information Figure S2. Purification profile of MtLsr2, MtLsr2-NTD, and MtLsr2-CTD. 15% SDS-polyacrylamide gel showing hexa-histidine tagged proteins MtLsr2 (lane 2), MtLsr2-NTD (lane 3), MtLsr2-CTD (lane 4), purified to near homogeneity.

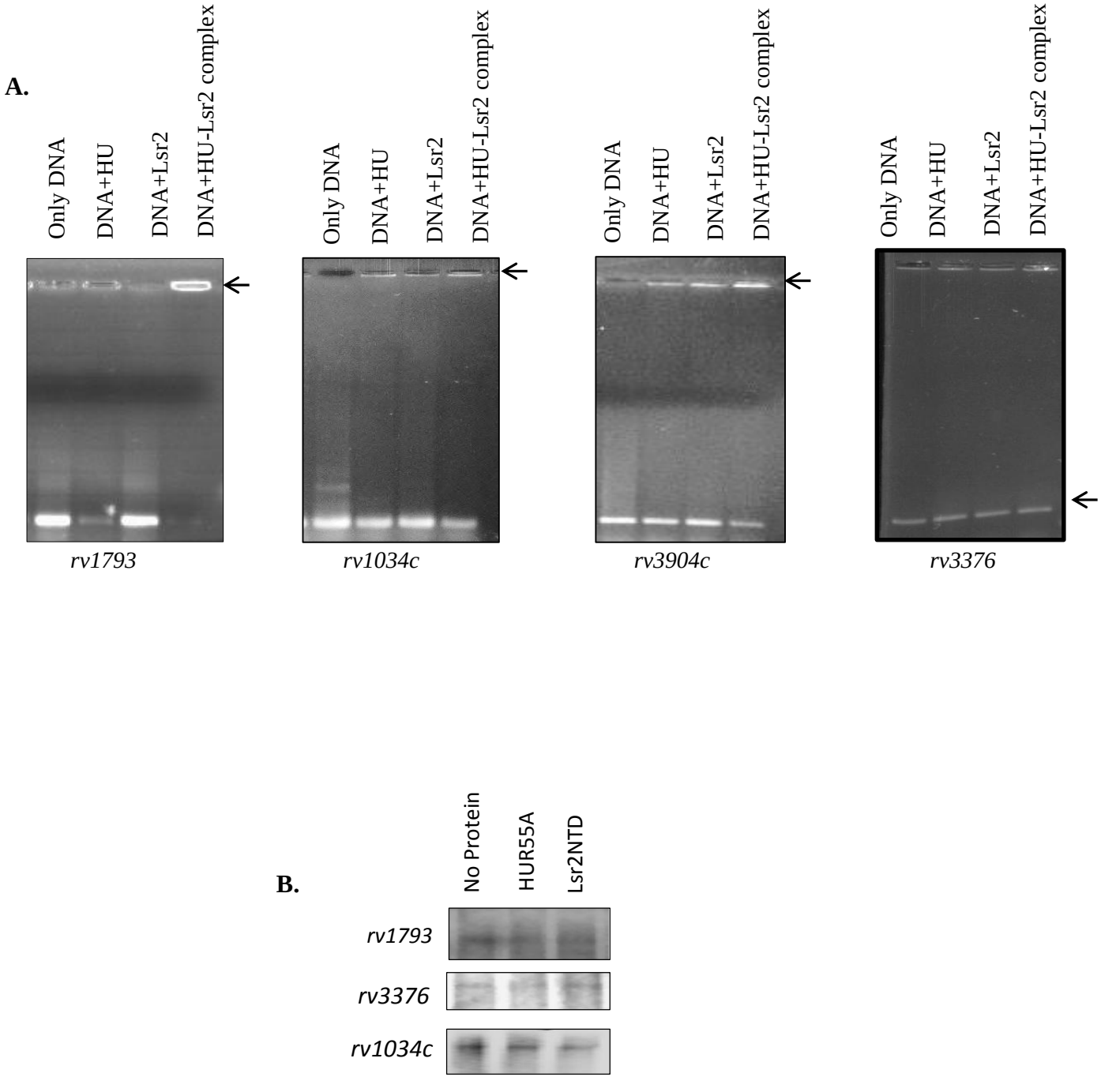


Supporting information Figure S3. A. AFM images of linear pUC18 DNA with i) MtHU ii)MtLsr2 iii) MtHU-Lsr2. (Dimensions of each image are provided along with the AFM pictures)

B. i)MtHUR55A and ii) MtLsr2-NTD, showing their inability to form DNA-protein complexes. The height scan (along the red lines) are show height of DNA (0.4 nm-0.5 nm) marked by red pointers.



Supporting information Figure S4. ChIP-Seq analysis of MtHU. To identify binding sites of MtHU in *Mtb*, ChIP-Seq was carried out following the protocol described in (Ahmed *et al*., 2017). **A.** CG browser view of MtHU occupancy across the *Mtb* genome. The outer circle with green bars indicate the MtHU binding events on the circular genome. The inner circle with the black peaks represent the GC-content. **B**. Venn-diagram showing the number of MtHU and MtLsr2 binding sites across the *Mtb* genome. The intersection of the Venn-diagram represents 61 genomic regions bound by both MtHU and MtLsr2 (see Table S2 and S3 for a listing of overlapping sites).



Supporting information Figure S5. A. EMSA showing the binding of MtHU, MtLsr2 and the MtHU-Lsr2 complex with the upstream elements of *rv1793*, *rv1034c*, *rv3904c*, and *rv3376*. **B**. *In vitro* transcription assays carried out with MtHUR55A and MtLsr2-NTD as controls.

Oligonucleotide	Sequence
Lsr2FP	5’ATGCATATGGCGAAGAAAGTAACCG3’
Lsr2RP	5’TCATCTAGACTCGAGGGTCGCCGCGTGGTA3’
Lsr2NTDFP	5’TATAGCTAGCGCGAAGAAAGTAACCGTCACC3’
Lsr2NTDRP	5’TATACTCGAGTCAGCCCGCCGCCACCC3’
Lsr2CTDFP	5’TATAGCTAGCCGTCGCGTCGGTGGGCGC3
Lsr2CTDRP	5’TATACTCGAGTCAGGTCGCCGCGTGGTATG3’
30mer TS	5’TCAACTCTGTATAAAAAACACCCCGCGAAAC3’
30mer BS	5’GTTTCGCGGGGTGTTTTTTATACAGAGTTGA3’
1793FP	5’GATGAATCAGGCGTTTCGC3’
1793RP	5’CCTGCTCGTAGATCACCTG3’
1034FP	5’GTCAGATTGTGGCGCAGACC3’
1034RP	5’CTTCGAGACCGCCGATCC3’
3904FP	5’TGGGCATTGTTAGGTTGCGG 3’
3904RP	5’ CCAAGGACTCAATCTCGGCG3’
3376FP	5’TCCACGACGTGGACATCCTG3’
3376RP	5’ATGGTAGGCCCGTGATTCGC3’

Supporting information Table S1. Oligonucleotides used in the study