Supplementary Information

Bactericidal activity of ZnO nanoparticles-anti TB drugs combination towards H37Rv strain and multidrug-resistant isolates of *Mycobacterium tuberculosis* via SufB splicing inhibition

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Figure S1. **MTT assay to determine the cytotoxicity of ZnONPs.** HEK293T cells (human embryonic kidney cell line) were exposed to varied concentrations of ZnONPs for 72 hrs, and the cell viability was determined as explained in the main text. Error bars represent (±1) SEM from (n=3) three independent sets of experiments.

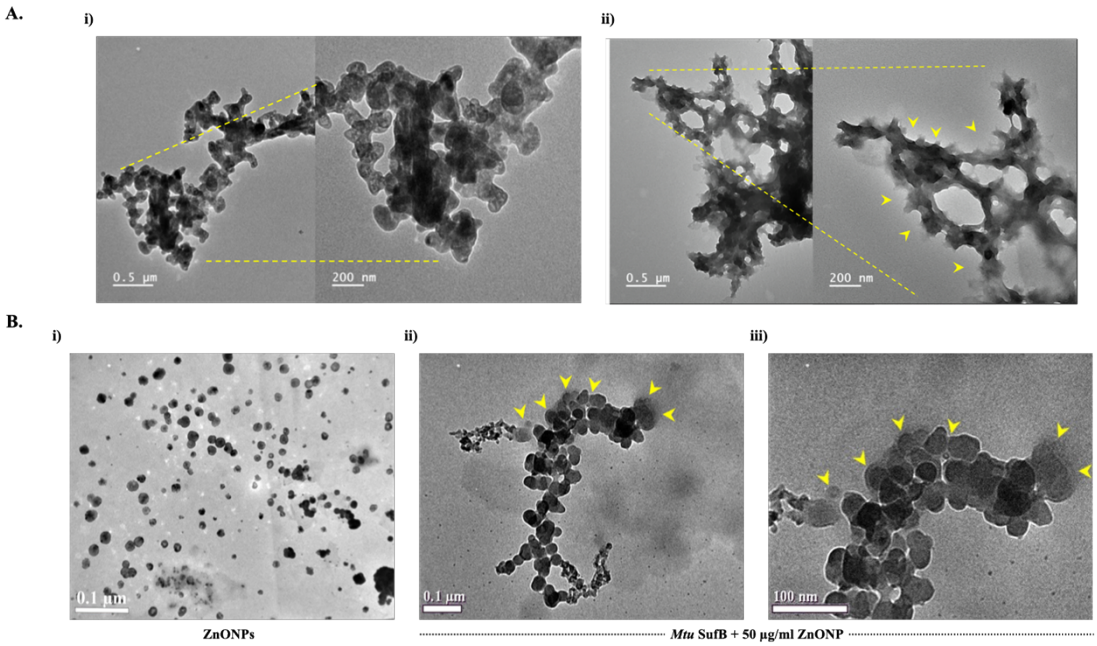

Figure S2. TEM analysis to show ZnONPs interaction with *Mtb* SufB and protein corona formation . (A) Negatively stained TEM images showing (i) ZnONPs, (ii) protein corona formation on the surface of ZnONPs aggregates after interaction with *Mtb* SufB (yellow arrows). **(B)** TEM images

showing (i) discrete ZnONPs, (ii) and (iii) protein corona formation on the surface of ZnONPs aggregates after interaction with *Mtb* SufB (yellow arrows).

Dynamic light scattering (DLS) and zeta potential analysis of ZnONPs-protein complex

Purified *Mtb* SufB precursor protein was refolded in the presence of different concentrations of ZnONPs (26 μg/ml and 50 μg/ml) as explained in section 2.7 for the *in vitro* refolding assay. Following incubation, the treated samples were analyzed via DLS and zeta potential (Malvern Nano – ZS90). To account for potential buffer-nanoparticle interactions, control samples included refolding buffer alone and the buffer with 50 μg/ml of nanoparticles were incubated under the same experimental conditions. The detailed analysis is provided in the Results and Discussion of the main text.

Figure S3. Dynamic light scattering (DLS) and zeta potential analysis of ZnONPs-protein complex. (A) Hydrodynamic diameter distribution. **(B)** Zeta potential profile. Error bars indicate ±1 SD for mean from three independent experiments (n=3).

Table S1. Hydrodynamic diameter (nm) and zeta potential (mV) measurements. Data represent mean (±1 SD) calculated from three independent experiments (n=3).

Samples	Hydrodynamic diameter (nm)	Zeta Potential (mV)
Buffer	144.9 ± 21.3	-5.2 ± 1.7
Buffer + *Mtb* SufB	334.6 ± 115	-2.3 ± 1.5
Buffer + ZnONPs (50 µg/ml)	242.7 ± 2.6	5.1 ± 1.4
Buffer + *Mtb* SufB + ZnONPs (26 µg/ml)	2535 ± 66.5	-4.0 ± 0.6
Buffer + *Mtb* SufB + ZnONPs (50 µg/ml)	2355.3 ± 157.6	-4.0 ± 1.1

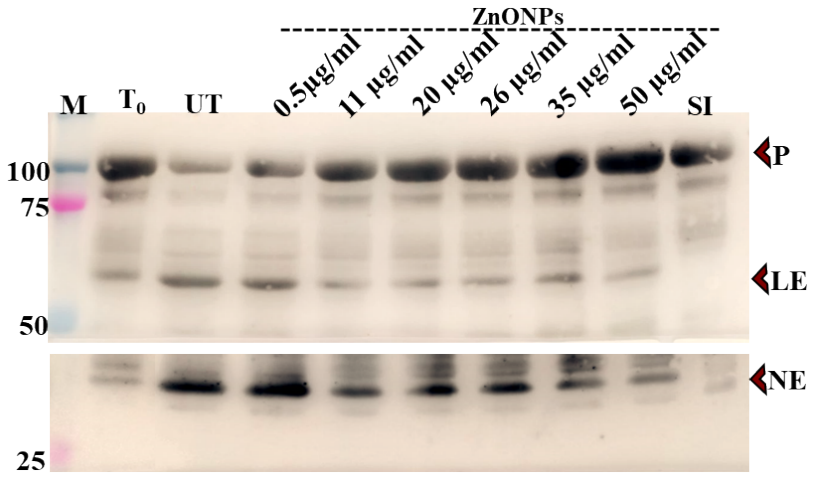

Figure S4. Confirmation of splicing and N-terminal cleavage products of *Mtb* SufB precursor via western blot. *Mtb* SufB precursor protein was refolded *in vitro* in presence varied concentrations of ZnONPs, resolved through 5%-10% gradient SDS PAGE, and the protein bands were detected by using anti-(His) antibody. The details of the methods and the results are mentioned in the main text. P: *Mtb* SufB precursor; LE: Ligated exteins; NE: N-extein; T_0: protein products at time 0, UT: untreated SufB precursor; M: protein molecular weight ladder

Figure S5. Effects of intermediate concentration range of ZnONPs on *Mtb* SufB precursor splicing and N-terminal cleavage reactions. (A) Products from *in vitro* protein refolding experiment were resolved through 4–10% gradient SDS-PAGE. (T_0): splicing and cleavage reactions at 0 h, (UT): untreated protein sample; and Lanes 4-9 show protein products induced by varied concentrations ZnONPs: (i) (2.5 µg/ml -25 µg/ml) (ii) (30 µg/ml to 50 µg/ml), Lane 10 (SI) for (i) and (ii): Splicing inactive SufB double mutant (Cys1Ala/Asn359Ala) is used as a negative control for splicing. **(B)** Western Blot analysis to validate the identity of protein products by using anti-(His) antibody. The details of the methods and the results are mentioned in the main text. P: *Mtb* SufB precursor; LE: Ligated exteins; NE: N-extein; T_0: protein products at time 0, UT: untreated SufB precursor; M: protein molecular weight ladder.

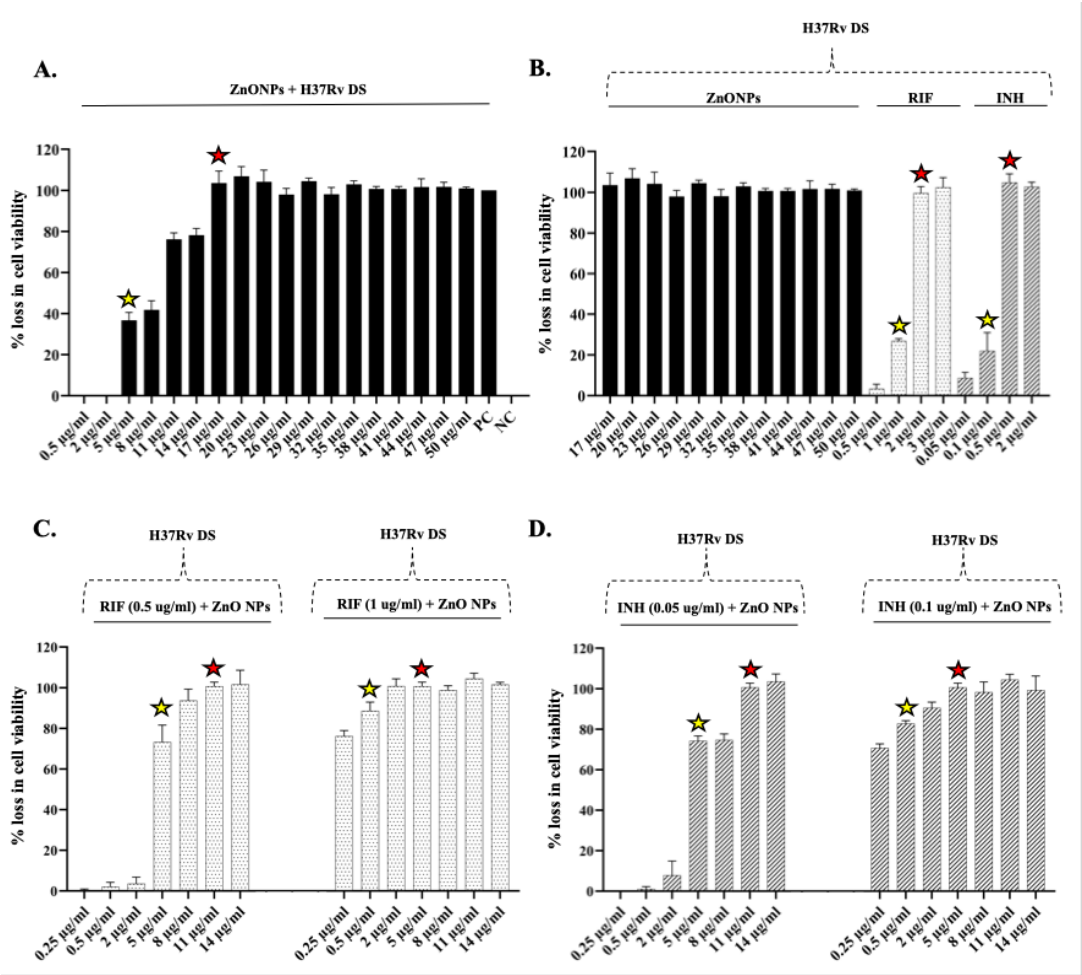

Figure S6. Graphical representation of spectral quantification data for Alamar blue assay (Figure 3 of main text) showing the minimum inhibitory concentrations (MIC) of ZnONPs towards H37Rv *Mtb* cells. Mycobacterial cells (10^6 CFU) were incubated in a 96 well plate, in presence of varied treatment conditions for 14 days, followed by addition of Alamar Blue reagent. Further incubation at 37ºC for 24 hours, led to colour change in the wells (Figure 3**)**. At this stage, absorbance was recorded at 570 nm (OD_{570}) using a UV-Vis spectrophotometer. The percentage loss in cell viability was calculated using a standard formula, and the results were represented graphically as shown here. **(A)** Percentage loss of cell viability in presence of varied concentrations (0.5 to 50 µg/ml) of ZnONPs. MIC of ZnONPs was identified as 5 µg/ml, whereas complete loss of cell viability was seen at 17 µg/ml and higher concentration of ZnONPs. **(B)** Comparison of the activity of first line anti-TB drugs; INH & RIF and varied concentration of ZnONPs. All the cells reached 100% loss of cell viability at a concentration of 17 µg/ml for the NPs, 2 µg/ml for RIF, 0.5 µg/ml for INH, and at respective higher concentrations. Synergistic activity of ZnONPs and anti-TB drugs; **(C)** RIF & **(D)** INH. A combination of RIF (0.5 µg/ml)+ ZnONPs (11 µg/ml) & RIF

(1 µg/ml)+ ZnONPs (5 µg/ml) showcased complete loss of cell viability. Likewise, INH (0.05 µg/ml) + ZnONPs (11 µg/ml) & INH (0.1 µg/ml) + ZnONPs (5 µg/ml) combinations resulted in complete loss of cell viability. Error bars represent ± 1 SEM from mean, where experiments have been realized in triplicates. Red stars- complete loss of viability & Yellow stars- partial loss of viable cells at a lower concentration of ZnONPs/Anti-TB drugs. DS: Drug sensitive H37Rv *Mtb*, RIF: Rifampicin, INH: Isoniazid.

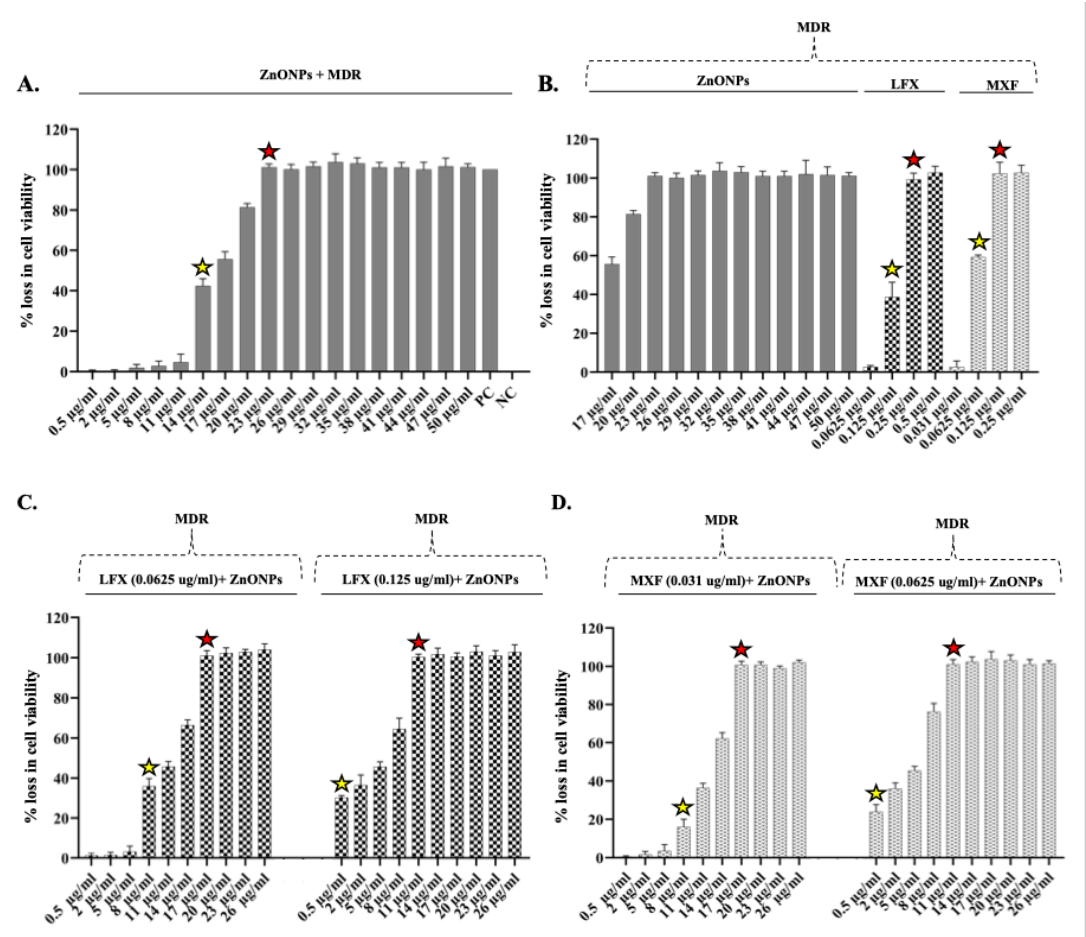

Figure S7. Graphical representation of spectral quantification data for Alamar blue assay showing the minimum inhibitory concentrations (MIC) of ZnONPs towards MDR *Mtb* cells. Mycobacterial cells (10^6 CFU) were incubated in a 96 well plate, in presence of varied treatment conditions for 14 days, followed by addition of Alamar Blue reagent. Further incubation at 37ºC for 24 hours, led to colour change in the wells. At this stage, absorbance was recorded at OD_{570nm} using a UV-Vis spectrophotometer. The percentage loss in cell viability was calculated using a standard formula, and the results were represented graphically as shown here. **(A)** Percentage loss of cell viability in presence of varied concentrations (0.5 to 50 µg/ml) of ZnONPs. MIC of ZnONPs was identified as 14 µg/ml, whereas complete loss of viable cell population was seen at 23 µg/ml of

ZnONPs. **(B)** Comparison of the activity of anti-TB drugs; Levofloxacin (LFX) & Moxifloxacin (MXF) and varied concentration of ZnONPs. All the cells reached 100% loss of cell viability at a concentration of 23 µg/ml for the ZnONPs and in presence of LFX (0.25 µg/ml), MXF (0.125 µg/ml) and at higher concentrations. Synergistic activity of ZnONPs and anti-TB drugs; **(C)** LFX & **(D)** MXF. A combination of LFX (0.0625 µg/ml) + ZnONPs (17 µg/ml) & LFX (0.125 µg/ml) + ZnONPs (11 µg/ml) yielded complete loss of cell viability. Likewise, MXF (0.031 µg/ml) + ZnONPs (17 µg/ml) & MXF (0.0625 µg/ml) + ZnONPs (11 µg/ml) combinations resulted in 100% loss of viability. Error bars represent ± 1 SEM from mean, where experiments have been realized in triplicates. Red stars- complete loss of viability & Yellow stars- partial loss of viable cells at a lower concentration of ZnONPs/Anti-TB drugs. MDR: Multidrug-resistant, LFX: Levofloxacin, MXF: Moxifloxacin

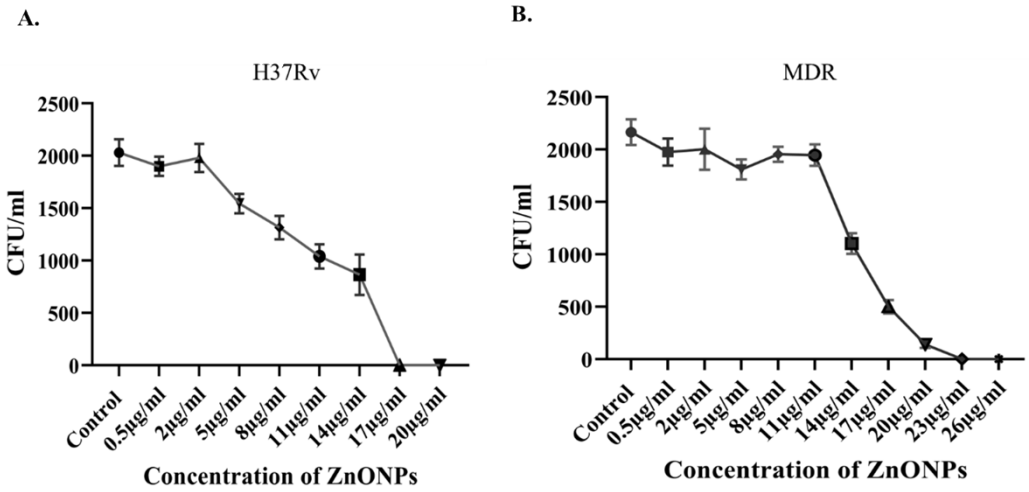

Figure S8. Determination of Minimum bactericidal concentration (MBC) for ZnONPs via Spread plate method. A. MBC of ZnO nanoparticles (ZnONPs) against H37Rv *Mycobacterium tuberculosis* (*Mtb*). **B.** MBC of ZnO nanoparticles (ZnONPs) towards multidrug-resistant (MDR) *Mtb isolates*. The details of the methods and the results are mentioned in the main text. Error bars represent (±1) SEM from (n=3) three independent sets of experiments.

Alamar blue assay to show the effects of ZnONPs on the growth and viability of *Mycobacterium smegmatis (M. sm)*

Mycobacterium smegmatis (*M. sm*) SufB precursor protein lacks an intein sequence in its polypeptide chain (1). Hence *M. sm* was used as a negative control to evaluate ZnONPs effect on its growth and viability, when intein splicing event is missing. All the cells exhibited pink colour in presence of ZnONPs, suggesting no effect of ZnONPs on *M. sm* viability due to lack of regulatory influence on SufB precursor splicing and cleavage reactions.

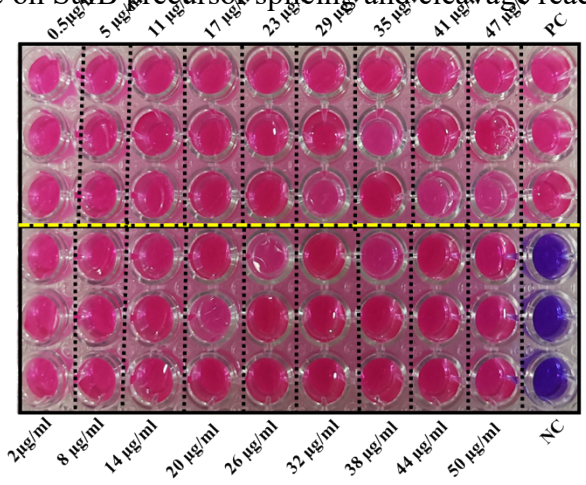

Figure S9. Alamar blue assay to show the effects of ZnONPs on the growth and viability of *Mycobacterium smegmatis (M. sm)*. 10^6 CFU of bacteria were incubated with different concentrations of ZnONPs (0.5 µg/ml to 50 ug/ml). After 24h of incubation growth and viability of mycobacterial cells were analyzed by a colour change. The details of the methods and the results are mentioned in the main text. *M. sm* cells remained viable (pink) in presence of varied concentrations of ZnONPs. NC- Negative control (*M. sm* cells without ZnONPs), PC – positive control (*M. sm* cells + RIF 3 µg/ml)

Reference

1. Perler FB. InBase: the Intein Database. *Nucleic Acids Research*. 2002;30(1): 383–384. https://doi.org/10.1093/NAR/30.1.383.