Supplemental Methods

**Transposon mutant screen.** We used an ordered 10,100 transposon mutant library of Mtb H37Rv made with the mariner based transposon MycoMarT7 as described (1). The library was stored at -80 °C in 15% glycerol. To remove glycerol, each mutant was passaged twice through 7H9 medium containing no carbon source. Subsequently, each transposon mutant was exposed to a medium designed to prevent replication without reducing the number of colony forming units: modified Sauton’s based medium containing KH₂PO₄ (0.5 g/L), MgSO₄.7H₂O (0.5 g/L), ZnSO₄ (1 mg/L), ferric ammonium citrate (50 mg/L), supplemented with either butyrate or isobutyrate (0.05%), NaNO₂ (0.5 mM), NH₄Cl (0.1%), BSA (0.5%), NaCl (0.085%), and tyloxapol (0.02%). The pH was adjusted to 5.5 and the medium filter sterilized. Strains were exposed for 2 weeks at 1% O₂, 5% CO₂ using an oxygen controlled chamber (Biospherix). After exposure, ~5% of the bacteria were transferred to permissive growth conditions in complete 7H9 medium at 21% O₂, 5% CO₂ for 12 to 16 days at which point bacterial
turbidity was measured at 580 nm. Those mutants that failed to recover were selected for further analysis and assessed for viability in repeat experiments by colony forming units (CFU). Early-mid log phase Mtb was washed once in the non-replication medium containing butyrate and centrifuged at 120 g to remove clumps. The single cell suspension (SCS) was adjusted to an OD of 0.05 and incubated for 1 week at 1% O₂ and 5% CO₂. Prior to exposure, an aliquot was serially diluted in 7H9 and plated for CFU on 7H11 agar, representing ‘input’ of each strain. After the one week exposure, strains were serially diluted and plated on 7H11 agar. CFUs were counted after 3 weeks of incubation at 37 ºC.

To identify to which stress(es) which each mutant was susceptible, butyrate was replaced with glycerol (0.05%), NaNO₂ was omitted from the medium and the foregoing experiment was repeated with 0, 0.5 mM or 1.5 mM NaNO₂ with 1% or 21% O₂.

To identify the site of transposon insertion, clones were grown to OD 1.0 and DNA isolated. The DNA was digested overnight with BamHI, an enzyme that cuts frequently in the Mtb genome but does not digest within the Himar transposon. BamHI digested DNA was purified by sequential phenol:chloroform and chloroform extractions and self ligated using high concentration T4 DNA ligase (NEB). Ligated DNA was transformed into *E.coli* pir-11 containing a helper plasmid. Transformants were selected on kanamycin LB agar plates and plasmids were sequenced.

**Gel mobility shift.** UV-irradiated plasmid DNA (25 nM) was incubated with the protein(s) under study for 10 min at 37 ºC in reaction mixtures (15 µL) with buffer containing 25 mM HEPES (pH 7.5), 10 mM MgCl₂, 2 mM ATP and 1 mM DTT.
Glutaraldehyde was then added to a final concentration of 0.2%, incubation continued for 10 min to cross-link protein-DNA complexes, and Tris-HCl (pH 8.0) added to a final concentration of 120 mM to quench the cross-linking reaction. Products were resolved by electrophoresis on 1% agarose gels at 4 °C. Gels were stained with ethidium bromide, images acquired with a Gel Doc (BioRad Laboratories) documentation system.

**Sensitivity of Msm strains to UV.** In order to characterize Msm mutant strains for sensitivity to UV, single cell suspensions (25 µl, OD580 = 0.04) were dispensed into 96-well plates (Corning, #3595). Cells were exposed to varying fluxes of UV. 75 µl of 7H9 medium was added before overnight culture with shaking. Bacterial growth was then measured by Alamar Blue reduction and CFU determination of 10-fold serial dilutions on 7H10-agar plates.

**High-throughput whole-cell screen for DNA repair inhibitors.** Sets of ten 384-well plates (Perkin Elmer) were filled with 20 µl per well of 2× 7H9 medium without detergent using a Multidrop Combi nL Reagent Dispenser (Thermo Scientific). Compounds were added using a Perkin-Elmer Janus Mini Workstation to produce 5 sets of duplicate plates. *M. smegmatis ΔuvrB::uvrB* Mtb was grown to log phase in 7H9 medium. A single cell suspension in water was diluted to OD_{580} = 0.015. The suspension (150 mL) was placed in a sterile Pyrex lasagna pan, 50 mL removed to serve as non-irradiated controls (-UV) and the remainder exposed to 200 µWatts/min for 20 sec (40 J/m²) with shaking. Preliminary experiments demonstrated that this UV exposure markedly extended the growth lag but still allowed logarithmic growth to be detected the
next day. Thereafter the bacteria were kept in the dark. Twenty µl of the UV exposed cells (+UV) were plated per well in one set of replicate screening plates and 20 µl of the -UV cell into the other set. The final concentration of compounds was 19 µM. Serial dilutions of the two bacterial suspensions were plated as standard curves on each plate in wells with no compounds. The plates were incubated in humidified air at 37°C for 18-20 hours. Alamar Blue (Invitrogen) was added to 10% final concentration. After 1.5 hours the -UV plates were brought to RT for 15 min and fluorescence recorded on a Perkin-Elmer Envision plate reader at excitation 535 nm and emission 590 nm. About 6 hours later, when fluorescence in the +UV plates also reached the linear range of the standard curve, it was similarly recorded. The fluorescence value from Alamar Blue reduction in each well with inhibitor was converted to cell number with respect to the standard curve. The data on each plate were standardized by subtracting from the value for each well, the value for the mean of all compound-containing wells on the plate and dividing the remainder by 1 SD of the values on the plate, creating an inhibitor-associated delta. The inhibitor-associated delta for wells exposed to UV was subtracted from the corresponding value for wells containing the same compound but not exposed to UV, creating a set of “net values”. We considered an inhibitor a candidate-active compound if its “net value” was greater than 3 SD from the mean of “net values” for the inhibitors on the same plate. Candidate-actives were cherry picked and re-tested.

Reference:
1. Darwin KH, Ehrt S, Gutierrez-Ramos JC, Weich N, & Nathan CF (2003) The proteasome of *Mycobacterium tuberculosis* is required for resistance to nitric oxide. *Science* 302(5652):1963-1966.
Supplemental Figure Legends

Figure S1. Sensitivity of transposon mutants in *uvrA* and *uvrB* genes to acidified nitrite. Single cell suspensions were prepared and adjusted to an OD = 0.05. An aliquot was removed, serially diluted and plated on 7H11 agar. This represented the ‘input’ CFU. Either 0 mM, 0.5 mM or 1.5mM NaNO₂ at pH 4.5 was added to each suspension. Cells were incubated at 1% O₂ for one week, serially diluted and plated for CFU on 7H11 agar. CFUs were counted after 3 weeks of incubation at 37 °C. Data are means ± SD from 3 independent experiments.

Figure S2. Incision activity of Mtb UvrABC on UV-damaged plasmid substrate; dependence on concentration of Uvr proteins. (A) UV-irradiated plasmid (25 nM) was incubated with increasing concentrations of UvrA, 300 nM UvrB and 150 nM UvrC (gel lanes “a”); with 100 nM UvrA and increasing concentrations of UvrB and 150 nM UvrC (gel lanes “b”); or with 100 nM UvrA, 300 nM UvrB and increasing concentrations of UvrC (gel lanes “c”). (B) Panel A results are plotted. UvrA data are from 2 independent experiments; UvrB data are means ± SD from 3 independent experiments; UvrC data are from 2 independent experiments. Results in 8 additional independent experiments at 150 nM UvrC were 74 ± 6 (mean ± SD).

Figure S3. Dependence of UV-damaged plasmid incision by UvrABC on physiological ATP concentration. (A) UV-irradiated supercoiled plasmid (25 nM) was incubated with 100 nM UvrA, 300 nM UvrB and 150 nM UvrC in the presence of
increasing concentrations of ATP. (B) The graph shows the means ± SD of triplicates in 1 experiment representative of 2 independent experiments.

**Figure S4. Dependence of UV-damaged plasmid incision by UvrABC on physiological pH.** UV-irradiated supercoiled plasmid (25 nM) was incubated with 100 nM UvrA, 300 nM UvrB and 150 nM UvrC at the indicated pH. The graph (lower panel) shows the means ± SD of triplicates in 1 experiment.

**Figure S5. UvrA and DNA damage-dependence of UvrB loading on UV damaged DNA.** (A) UV-irradiated plasmid DNA (25 nM) (upper panel) or non-irradiated plasmid DNA (25 nM) (lower panel) was incubated with increasing concentrations of UvrA (0, 20, 50, 100, 250, 500 or 1000 nM) or UvrB (0, 20, 50, 100, 250, 500 or 1000 nM) in buffer containing 25 mM Hepes 7.5, 10 mM MgCl$_2$, 5 mM ATP, 1 mM DTT. Bound and free plasmids are as indicated. This is representative of three independent experiments. (B) 100 nM Mtb UvrA in the presence of UvrB (0, 20, 50, 100, 250, 500 or 1000 nM) in the same buffer as in A with UV-irradiated plasmid DNA (25 nM) (upper panel) or non-irradiated plasmid DNA (25 nM) (lower panel). (C) Panel A and B results are plotted to represent relative bound DNA fraction to total DNA used vs protein concentration of the Uvr proteins; solid shapes refer to UV-irradiated plasmid and empty shapes refer to non-irradiated plasmid. This is representative of two independent experiments.

**Figure S6. Complementation of uvrB deletion.** The *uvrB* deletion mutant of *M. smegmatis* was complemented with the integrative plasmid pMV306-Mtb or a non-
integrative plasmid. (A) Colonies grown on 7H10-agar plates containing 50 µg/ml hygromycin and 20 µg/ml kanamycin were restreaked on 7H10-agar plates, exposed to UV of 30 J/m², and cultured for 4 days. All 10 complemented colonies (1 ~ 10) grew like wild type Msm, while colonies (v1 ~ v4) transformed with the non-integrating plasmid were not restored to normal UV resistance. (B) One complemented strain and one transformed, non-complemented strain along with wild type and mutant strains were tested for growth by the Alamar Blue assay after varying doses of UV. Data show mean ± S. D. from three independent experiments.

**Figure S7. Raw fluorescence data for the two 384 well plates illustrated in Fig. 3.** Columns 1-22 were prefilled with the same 352 test compounds on each plate and then filled with *M. smegmatis* that was or was not exposed to UV. The UV irradiated wells showed less variance because the reading was taken earlier in logarithmic growth than for the non-irradiated cells. The two compounds that were chosen as screen active compounds are in columns 5 (□) and 16 (X).

**Figure S8. Structures of ATBC related compounds.** These four compounds were tested for inhibition of plasmid incision by Mtb UvrABC; no inhibition was observed at 50 µM.

**Figure S9. Structures, IC50s, and DNA intercalation assay results for compounds that exhibited inhibition of NER activity in vitro.** (A) These nine compounds were confirmed hits for NER activity in vitro at 50 µM. (B) Table lists the approximate IC50
after analysis with compound concentrations of 0-50 µM for NER activity as well as after testing for DNA intercalation.

**Fig. S10. Effect of ATBC on incision of a fluoresceinated plasmid by *Bacillus caldotenax* UvrA and UvrB and *Thermatoga maritima* UvrC.** (A) Schematic of the 5’-end-labeled (with *P-32*) fluorescein adducted 50-bp duplex used as a substrate. Top strand: F_{26}50 (fluorescein at position 26), Bottom strand: NDB. (B) Incision of the 5’-end-labeled substrate (F_{26,50}/NDB) in presence of 5% DMSO (control) and ATBC (200 µM) diluted in DMSO. Assay contained 20 nM UvrA, 100 nM UvrB and 50 nM UvrC and proceeded for 30 min at 37 °C before it was stopped with 200 mM EDTA. Incision products were analyzed on a 10% denaturing polyacrylamide gel. (C) Quantitation of the incision gel. Data reported as mean ± S.D. (n=3). *** Student t-test: p-value= 0.008)

**Fig S11. ATBC has no effect on UvrB-deficient *M. smegmatis* at a UV dose low enough to allow its recovery.** Serial dilutions of single cell suspensions of *M. smegmatis* strains from (2.5×10^4 to 2.5 cfu) were spotted on 7H10 agar plates in the absence (“No cpd”) or presence of ATBC or its inactive congener compound 2 at 25 µM. Three plates were exposed to UV at 10 J/m².
Supplemental Figures:

Fig. S1
Fig. S3

A. 

B. 

% Product

ATP (M)
Fig. S7

Raw data from typical (-UV) plate

Raw data from typical (+UV) plate
Fig. S8
A.

Fig. S9

B.

| Compound Name | 7  | 8  | 9  | 10 | 11 | 12 | 13 | 14 | 15 |
|---------------|----|----|----|----|----|----|----|----|----|
| IC 50 (µM)    | 6.0| 12.0| 30.0| 12.0| 12.0| 12.0| 5.0| 30.0| 6.0|
| DNA Intercalation | Yes | No | No | Yes | No | Yes | Yes | Yes | Yes |
