# ATP-dependent ligases and AEP primases affect the profile and frequency of mutations in mycobacteria under oxidative stress.

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Supplementary Figures and Tables:

Tables:

1. Table S1 – Bacterial strains
2. Table S2 – Plasmids and primers
3. Table S3 –Description of strains sequenced by NGS (see separate data set, Excel)
4. Table S4 - Summary of NGS analysis (see separate data set, Excel)
5. Table S5 – Mutations detected in *M. smegmatis* cells growing in the presence of CHP

Figures:

1. Figure S1 – Southern blot analysis of the Δ*ku-ligD-ligC1-ligC2-prim* mutant
2. Figure S2 – Phenotypic analysis of *M. smegmatis* strains in the presence of various DNA damaging assaults
3. Figure S3 – Southern blot analysis of sucrose-resistant mutants

**Table S1 – Bacterial strains**

| Name/genotype | **STRAINS** | Reference |
|---|---|---|
| Top10F' | *Escherichia coli* strain | Invitrogen |
| $mc^2155$ | *M. smegmatis* - wild type | Laboratory stock |
| Δ*ku* | *M. smegmatis* defective in the synthesis of Ku | Korycka-Machala et al., 2007; Pitcher et al., 2007 |
| Δ*ligD* | *M. smegmatis* defective in the synthesis of LigD | Korycka-Machala et al., 2007; Pitcher et al., 2007 |
| Δ*ku-ligD* | *M. smegmatis* defective in the synthesis of Ku and LigD | Korycka-Machala et al., 2007; Pitcher et al., 2007 |
| Δ*ligC$_1$-ligC$_2$-primC* | *M. smegmatis* defective in the synthesis of $LigC_1$, $LigC_2$ and PrimC | Plocinski et al., 2017 |
| Δ *ku-ligD-ligC$_1$-ligC$_2$-primC* | *M. smegmatis* defective in the synthesis of Ku, LigD, $LigC_1$, $LigC_2$ and PrimC | This study |

**Table S2 - Plasmids and primers**

| **Plasmids used for this study** | **CLONING VECTORS** | |
|---|---|---|
| pJET 1.2/blunt | Blunt cloning vector, AmpR | Thermo Scientific |
| p2NIL | Recombination vector, nonreplicating in mycobacteria, $Kan^R$ | Parish& Stoker, 2000 |
| pGoal17 | Source of PacI cassette and *sacB,* $Amp^R$ | Parish& Stoker, 2000 |
| pMV306K | Mycobacterial integrative vector, $Kan^R$ | Med-Immune Inc |
| pMV306Km-sacB | Integrative plasmid for selection of sucrose resistant ($sacB^R$) colonies | This study |
| **Primers used in this study** | | |
| Name of primer | Sequence (5'>3') | Application |
| Msmeg_ligC1,ligC2, Prim2 probe-F | TCACTGCTTGCGGTTGCCCTGC | Southern blotting - hybridization probe |
| Msmeg_ligC1,ligC2, Prim2 - probe-R | GGCGAGCGGTTGTTCATCG | |
| sacBNotI-F | CGCGGCCGCTCGAGTGACCACAACGACGC | *sacB* cloning into pMV306Km |
| sacB XbaI-R | CTCTAGATCGGCATTTTCTTTTGCGTTT | |

**Table S3 – Description of strains sequenced by NGS (see separate data set, Excel)**

**Table S4 – Summary of NGS analysis (see separate data set, Excel)**

**Table S5 - Mutations detected in *M. smegmatis* cells growing in the presence of CHP.**

| | ***M. smegmatis strains*** | | |
|---|---|---|---|
| | **wild-type** | **CCP** | **KuDCCP** |
| **substitution** | **number of positions affected** | | |
| C→T | 0 | 0 | 1 |
| G→T | 37 | 32 | 26 |
| G→C | 29 | 27 | 34 |
| C→G | 48 | 25 | 43 |
| A→C | 54 | 45 | 56 |
| T→G | 67 | 58 | 57 |
| A→T | 23 | 10 | 16 |
| A→G | 5 | 1 | 5 |
| C→A | 48 | 28 | 35 |
| T→A | 24 | 17 | 17 |
| T→C | 4 | 2 | 0 |
| G→A | 0 | 0 | 0 |
| total | 339 | 245 | 290 |

The culture of wild-type *M. smegmatis* as well as mutants Δ*ku-ligD-ligC1-ligC2-primC* (KuDCCP) and Δ*ligC1-ligC2-primC* (CCP) growing in the presence of CHP were sequenced using NGS technology and analyzed concerning the presence of mutations. The most abundant changes are highlighted by gray background.

**Figure S1 - Southern blot analysis of the Δ*ku-ligD-ligC1-ligC2-prim* mutant**

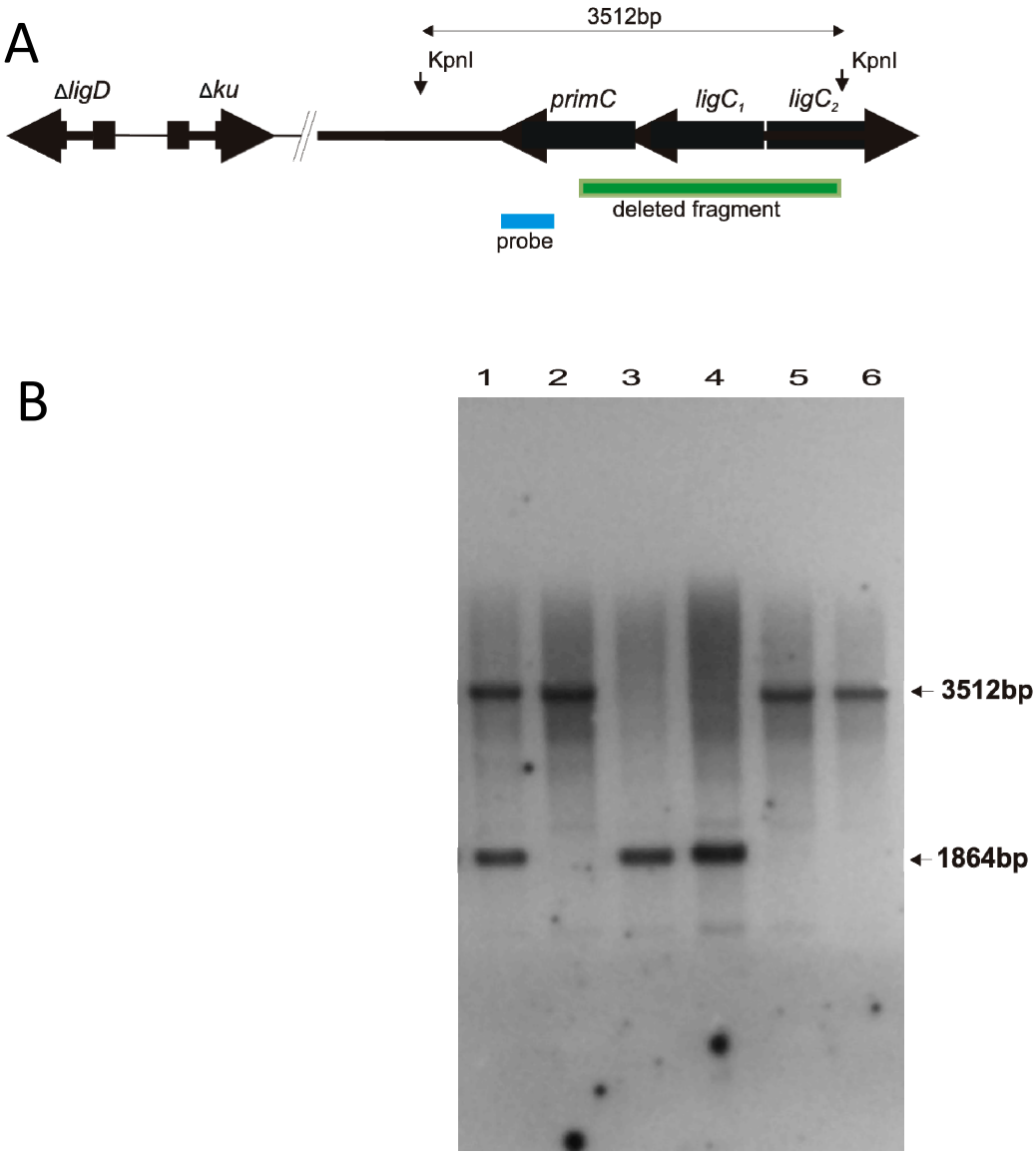


**Fig. S1.** Confirmation of mutant construction by Southern blotting analysis. (**A**) Genomic DNA fragment containing *ligC₁* (1056 bp), ligC₂ (1065 bp) and *primC* (1116 bp) as well as mutated genes Δ*ku* and Δ*ligD* (genetic background of the strain for mutagenesis) are shown. KpnI restriction sites, internal deletion fragment and genetic probe are indicated. **(B)** Southern blot autoradiogram shows the presence of the intact genes (*ligC₁*-ligC₂-*primC*) in the wild type strain (**lane 2;** 3512 bp), both intact and mutated genes in the single crossover mutant (**lane 1;** 3512 and 1864 bp), mutated genes in the double crossover mutants (**lanes 3-4;** 1864 bp), and wild-type genes in the double crossover strains reversed to the wild-type genotype (**lanes 5-6;** 3512 bp).

**Figure S2 – Phenotypic analysis of *M. smegmatis* strains in the presence of various DNA damaging assaults**

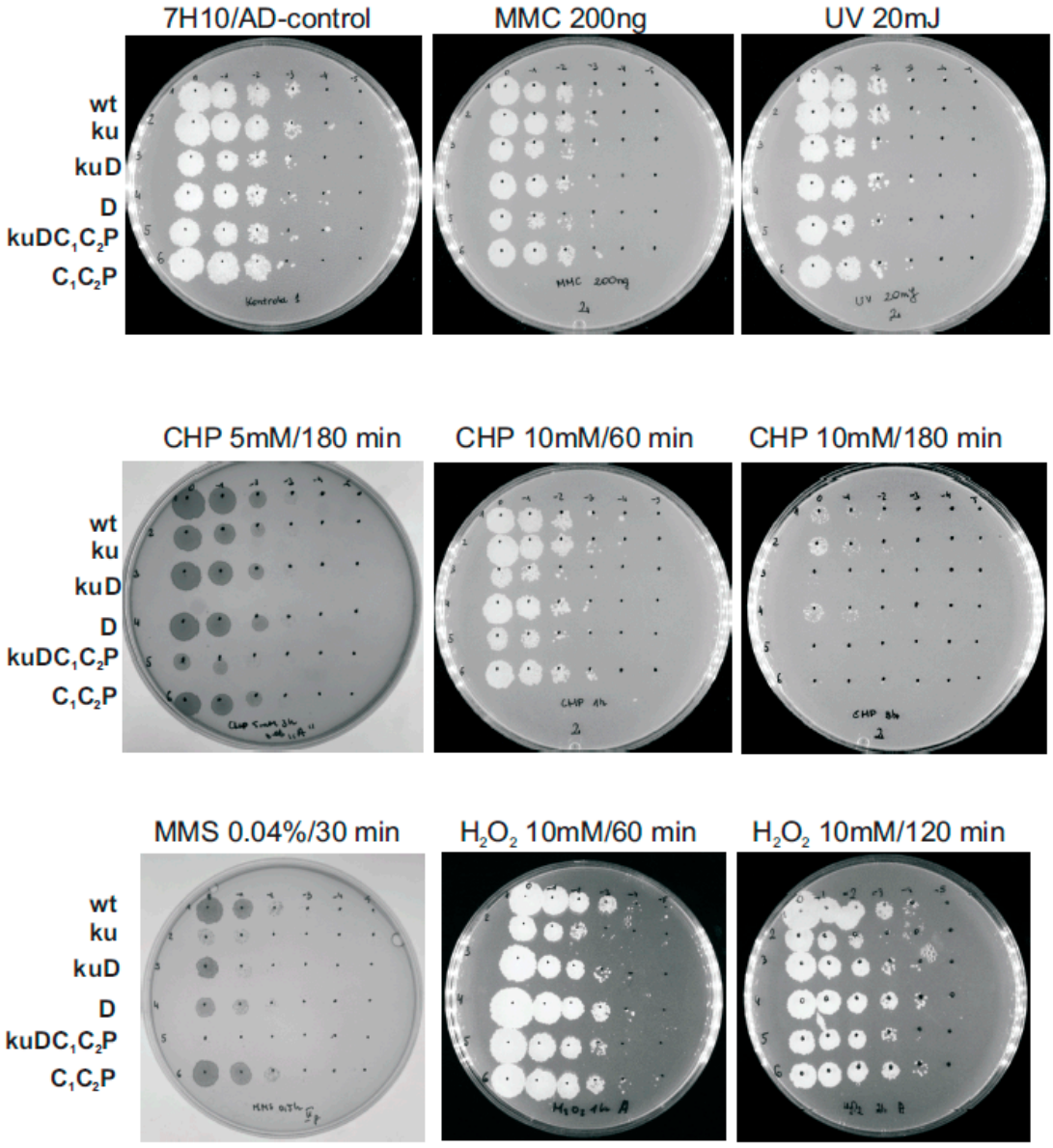


**Fig. S2.** The survival of *M. smegmatis* wild type (wt) and mutant cells: Δ*ku* (ku); Δ*ku-ligD* (kuD); Δ*ligD* (D); Δ*ku-ligD ligC1-ligC2-PolC* (kuDC1 C2P) and Δ*ligC1-ligC2-PolC* (C1C2P) in the absence of DNA damage (7H10/AD control) and after treatment with MMC, UV, CHP, MMS and $H_2O_2$. For qualitative analysis, the 5µl of indicated dilutions of cells ($10^0$-$10^{-5}$) were plated and after 3 days of incubation the plates were photographed using the gel documentation system.

**Figure S3 – Southern blot analysis of sucrose resistant mutants**

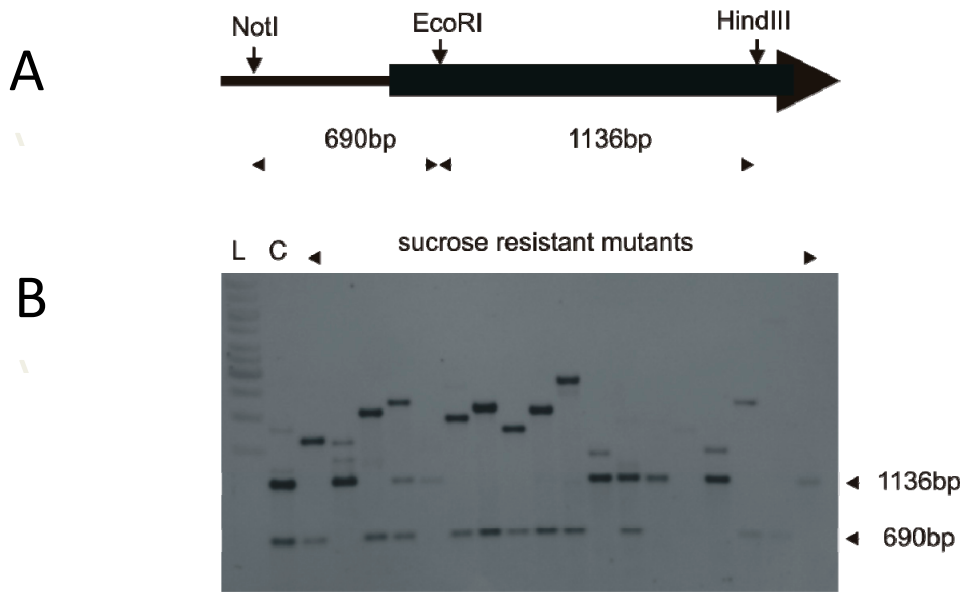


**Fig. S3.** The analysis of *sacB* gene by Southern blotting. (**A**) The black arrow represents *sacB* gene and its promoter introduced into the chromosomal DNA of *M. smegmatis* strain. The restriction sites and DNA fragments generated by digestion of chromosomal DNA by NotI/EcoRI/HindIII are indicated. **(B)** Southern blot autoradiogram shows the presence of the fragments of *sacB* gene and its promoter in the wild-type strain (C; 1136 and 690 bp) and selected sucrose-resistant mutants. L represents 1kb-DNA ladder.