SUPPORTING INFORMATION

The veterinary anti-parasitic selamectin is a novel inhibitor of the *Mycobacterium tuberculosis* DprE1 enzyme

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Table S1. Oligonucleotides used for DprE1 mutagenesis

Name	Sequence
DprE1 C394A For	CGATCCCGGGCTGGAACGTGGCCGTGGACTTCCCGATCAAGG
DprE1 C394A Rev	CCTTGATCGGGAAGTCCACGGCCACGTTCCAGCCCGGGATCG
DprE1 C394G For	CGATCCCGGGCTGGAACGTGGGCGTGGACTTCCCGATCAAG
DprE1 C394G Rev	CTTGATCGGGAAGTCCACGCCCACGTTCCAGCCCGGGATCG
DprE1 C394S For	GATCCCGGGCTGGAACGTGTCCGTGGACTTCCCGATCAAGG
DprE1 C394S Rev	CCTTGATCGGGAAGTCCACGGACACGTTCCAGCCCGGGATC
DprE1 L282F For	CGCCGCAACTGCTCACGTTTCCGGACATCT
DprE1 L282F Rev	AGATGTCCGGAAACGTGAGCAGTTGCGGCG
DprE1 L282V For	CGCCGCAACTGCTCACGGTGCCGGACATCT
DprE1 L282V Rev	AGATGTCCGGCACCGTGAGCAGTTGCGGCG

Table S2. Oligonucleotides used for *M. smegmatis* recombineering. Point mutations introduced with mutagenic oligos are highlighted

Name	Sequence
rpsL+	GCGACCTTCCGGAGCGCCGAGTTCGGCTTCCTCGGAGTGGTGGTGT AAACGCGCGTGCACA
L282F	AGCTGCAGAAGGATCCACTGAAATTCGATGCGCCGCAACTGCTCA CGTTTCCGGACATCTTCCCGAACGGCCTGGCCAACAAGTTCACGTT CATGCCGAT
L282V	AGCTGCAGAAGGATCCACTGAAATTCGATGCGCCGCAACTGCTCA CGGTTCCGGACATCTTCCCGAACGGCCTGGCCAACAAGTTCACGTT CATGCCGA
dprE1-seq-F	GTGAGCCTGGACCAGTTGATGAAAGC
dprE1-seq-R	TACAGCCTGCCACCGAACTCC

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Mab      MARASGLCHRPGDCDSHQFHRPARGDLPNPLSRYAQLPMTTPKSELPLTPRALTGFGRTA    60
Mav      ----------------------------------------MSSTDPLITPARLTGFGRTA    20
Msm      ------------------------------MGAV--PSLTMSTTEFPTTTKRLMGWGRTA    28
Mtb      ----------------------------------------MLSVGATTTATRLTGWGRTA    20
Mbo      ----------------------------------------MLSVGATTTATRLTGWGRTA    20
Mka      ----------------------------------------MSSNASSTTPTRLTGWGRTA    20
                                                      .     *   * *:****

Mab      PTVAHVLSTPDVDVIAEAVRQVADASSHSPAHLRRGIVARGLGRSYGDHACNGGGIVVDM    120
Mav      PSVAQVLRTRDPEVIAKAVARVADSGH----SKGRGVIARGLGRSYGDNAQNGGGLVIDM    76
Msm      PTVASVLSTSDPEVIVRAVTRAAEE-------GGRGVIARGLGRSYGDNAQNGGGLVIDM    81
Mtb      PSVANVLRTPDAEMIVKAVARVAESG------GGRGAIARGLGRSYGDNAQNGGGLVIDM    74
Mbo      PSVANVLRTPDAEMIVKAVARVAESG------GGRGAIARGLGRSYGDNAQNGGGLVIDM    74
Mka      PSVADVLRTPDPEVIAKAVARAAES-------GARGVIARGLGRSYGDNAQNGGGLVIDM    73
         *:** ** * * ::*..** :.*:          ** :**********:* ****:*:**

Mab      TPLKRVHSISAETAVADVDAGVSLDQLMKAALPFGLWVPVLPGTRQVTVGGAIGSDIHGK    180
Mav      TGLNRIHSISADTRLVDVDAGVSLDQLMKAALPFGLWVPVLPGTRQVTVGGAIACDIHGK    136
Msm      PALNRIHSIDSGTRLVDVDAGVSLDQLMKAALPHGLWVPVLPGTRQVTVGGAIGCDIHGK    141
Mtb      TPLNTIHSIDADTKLVDIDAGVNLDQLMKAALPFGLWVPVLPGTRQVTVGGAIACDIHGK    134
Mbo      TPLNTIHSIDADTKLVDIDAGVNLDQLMKAALPFGLWVPVLPGTRQVTVGGAIACDIHGK    134
Mka      SGLNNIHSISADTKLADVDAGVNLDQLMKAALPFGLWVPVLPGTRQVTVGGAIACDIHGK    133
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Mab      NHHSAGSFGNHVLSMDLLMADGEVHTITPDGP---ASELFWATVGGNGLTGIVVRARIAM    237
Mav      NHHSAGSFGNHVRSMELLMADGTVRTITPDGPDASDAELFWATVGGNGLTGIVLRATIAM    196
Msm      NHHSAGSFGNHVRSMELLTANGEVRHLTPAGP---DSDLFWATVGGNGLTGIILRATIEM    198
Mtb      NHHSAGSFGNHVRSMDLLTADGEIRHLTPTGE---DAELFWATVGGNGLTGIIMRATIEM    191
Mbo      NHHSAGSFGNHVRSMDLLTADGEIRHLTPTGE---DAELFWATVGGNGLTGIIMRATIEM    191
Mka      NHHSAGSFGNHVRSMDLLLANGEVRRLSPDGD---EAELFWATVGGNGLTGIILRATIEM    190
         ************ **:** *:* :: ::* *     ::***************::** * *

Mab      TRTETAYFIADGVATRDLDETIAVHQDGTEDNYTYSSAWFDLINPPPKLGRAAVSRGSLA    297
Mav      TPTETAYFIADGVATKDLDETVAVHLDGSEADYTYSSAWFDLISPPPKLGRAAVSRGSLA    256
Msm      TPTETAYFIADGDVTGSLDETIAFHSDGSEANYTYSSAWFDAISKPPKLGRAAISRGSLA    258
Mtb      TPTSTAYFIADGDVTASLDETIALHSDGSEARYTYSSAWFDAISAPPKLGRAAVSRGRLA    251
Mbo      TPTSTAYFIADGDVTASLDETIALHSDGSEARYTYSSAWFDAISAPPKLGRAAVSRGRLA    251
Mka      TPTETAYFIADGDVTATLDETIALHSDGSEADYTYSSAWFDAISAPPKLGRAAISRGSLA    250
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Mab       KLDQLPPKLAKDPLKFSAPQLPGLPDLFPVNFMIKPSLMAIGEAFYRMSGNYQGKIVNLT    357
Mav       RLDQLPKKLAKNPLKFDAPQLLTVPDVFPVSAMNKLSFMAIGEVYYRLGGTYTGKVMNLS    316
Msm       KLDQLPSKLQKDPLKFDAPQLLTLPDIFPNGLANKFTFMPIGELWYRKSGTYRNKVQNLT    318
Mtb       TVEQLPAKLRSEPLKFDAPQLLTLPDVFPNGLANKYTFGPIGELWYRKSGTYRGKVQNLT    311
Mbo       TVEQLPAKLRSEPLKFDAPQLLTLPDVFPNGLANKYTFGPIGELWYRKSGTYRGKVQNLT    311
Mka       RLEQLPTKLQRNPLKFDAPQLLTFPDVFPNGLANKYTFGPIGELWYRKSGTYRGKIQNLT    310
           ::*** **  :****.****  .**:** .   * ::  *** :** .*.* .*: **:

Mab       QFYHMLDITTGWQYAYGPAGFAQHQFLVPPDALEEFKGIIRWIQTQGQYSALNVFKLFGP    417
Mav       QFYHMLDLVSGWNNAYGPRGFAQHQFLVPPDAMDEFKAIIRWIQTRGHYSALNVFKLFGP    376
Msm       QFYHPLDMFGEWNRAYGSAGFLQYQFVVPTEAVEEFKSIIVDIQRSGHYSFLNVFKLFGP    378
Mtb       QFYHPLDMFGEWNRAYGPAGFLQYQFVIPTEAVDEFKKIIGVIQASGHYSFLNVFKLFGP    371
Mbo       QFYHPLDMFGEWNRAYGPAGFLQYQFVIPTEAVDEFKKIIGVIQASGHYSFLNVFKLFGP    371
Mka       QFYHPLDMFGEWNRAYGPAGFLQYQFVIPTEAVDEFKKIIRDIQASGHYSFLNVFKLFGP    370
          **** **:   *: ***  ** *:**::* :*::*** **  **  *:** *********

Mab       GNKAPLSFPMKGWNVAMDFPNKPGVNEFLNELDNRAMEFGGRVYTAKDSRVSAEKFHRMY    477
Mav       GNRAPLSFPMAGWNVAMDFPNKPGVNEFLNELDRRVLQFGGRVYTAKDSRTNAETFHAMY    436
Msm       GNQAPLSFPIPGWNVCVDFPIKAGLHEFVTELDRRVLEFGGRLYTAKDSRTTAETFHAMY    438
Mtb       RNQAPLSFPIPGWNICVDFPIKDGLGKFVSELDRRVLEFGGRLYTAKDSRTTAETFHAMY    431
Mbo       RNQAPLSFPIPGWNICVDFPIKDGLGKFVSELDRRVLEFGGRLYTAKDSRTTAETFHAMY    431
Mka       GNRAPLSFPIPGWNICVDFPIKAGLNEFVSELDRRVLEFGGRLYTAKDSRTTAETFHAMY    430
           *:******: ***:.:*** * *: :*:.***.*.::****:*******..**.** **

Mab       PRVDEWIATRRKADPHGVFASDMARRLELL    507
Mav       PRIDEWIAVRRKVDPTGVFASDMARRLELL    466
Msm       PRIDEWIRIRRSVDPDGVFASDMARRLQLL    468
Mtb       PRVDEWISVRRKVDPLRVFASDMARRLELL    461
Mbo       PRVDEWISVRRKVDPLRVFASDMARRLELL    461
Mka       PRIDEWIAVRRKVDPLRVFASDMARRLELL    460
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Figure S1. Sequence alignment of mycobacterial DprE1. Residues predicted to be relevant for selamectin binding to DprE1 are highlighted in yellow (Leu275 is shown in red) *Mab: Mycobacterium abscessus*; *Mav*: *Mycobacterium avium*; *Msm*: *Mycobacterium smegmatis*; *Mtb*: *Mycobacterium tuberculosis*; *Mbo*: *M. bovis*; *Mka*: *M. kansasii*

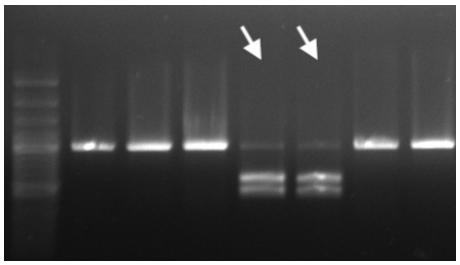

Figure S2. Screening of *M. smegmatis* recombinants for point mutations. Following transformation with the mixture of mutagenic oligonucleotides targeting both *dprE1* and *rpsL*, colonies isolated on streptomycin-containing were screened for the presence of point mutations in *dprE1*. A 944 bp fragment of *dprE1* was amplified by PCR, and then digested with *Kpn*2I. Wild-type *M. smegmatis* shows the undigested 959 bp product, while mixed populations (white arrows) show the undigested fragment and the two digestion products (525 and 419 bp). Left lane: Gene Ruler 100 bp Plus Ladder (ThermoFisher Scientific).

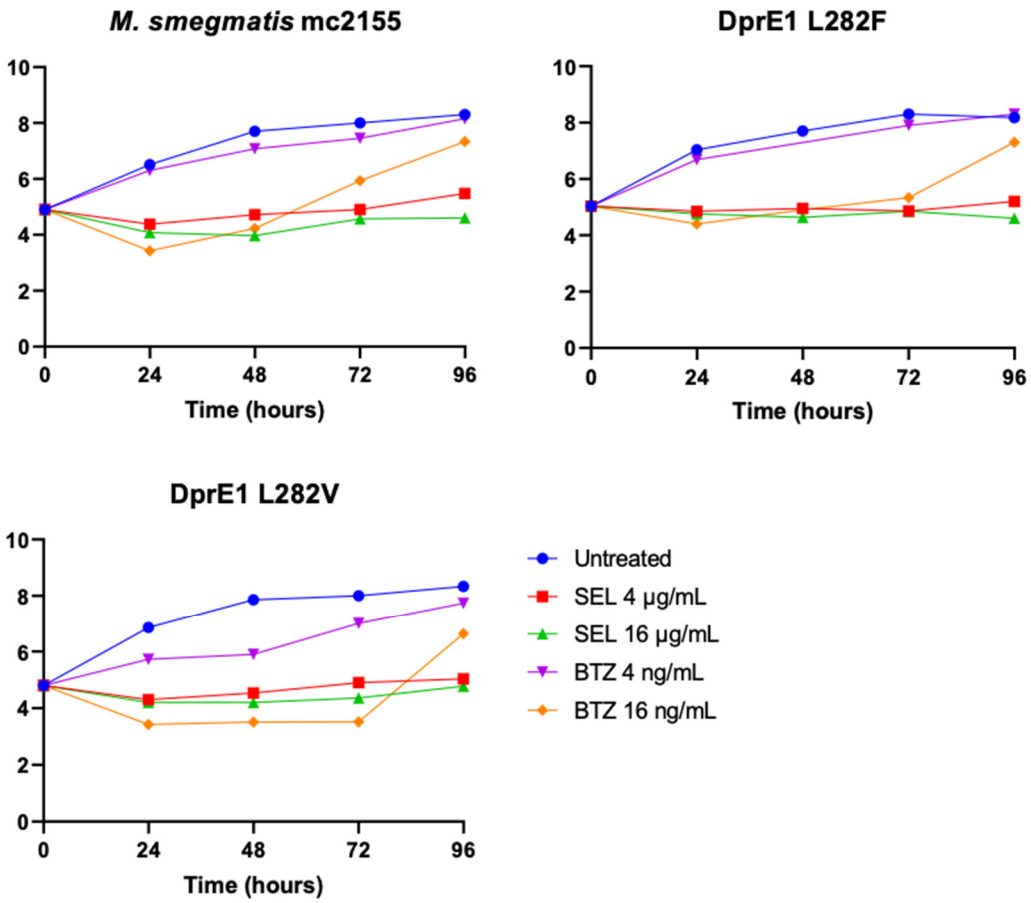

Figure S3. Time-kill kinetics of *M. smegmatis* DprE1 point mutants.