Supplementary Material

(To be published online but not to be printed)

Supplementary TABLE 1.

List of PCR primers used in this study

Primer name	Primer sequence (5' to 3')
External primers for generating mutations in *def:*	
CR26 (sense)[a]	5' GGAATTCCATATGGCAGTCGTACCC 3'
CR27 (antisense)[a]	5' CCCAAGCTTTTAGTGACCGAACGG 3'
Internal primers for deletion mutations in insertion region of *def*:	
CR23 (antisense)	5' TTAAACGCCCCAGCCATG 3'
CR25 (sense)	5' ACCGCGCAGGTGTGGTCATCAAT 3'
CR24 (antisense)	5' ACCACACCTGCGCGGTCCG 3'
CR88 (sense)	5' GCGGACCGCGCAGTGCTTGAGACCTC 3'
CR87 (antisense)	5' GAGGTCTCAAGCACTGCGCGGTCCG 3'
CR105 (sense)	5' GACCGAGCACGCCGACGC 3'
CR106 (antisense)	5' GCGTCGGCGTGCTCGGTC 3'
Internal primers for Arginine point mutants in insertion region of *def*:	
CR60 (sense)	5' ACCGCC**GC**CCGACG**G** 3'
CR61 (antisense)	5' GCGTCGG**GC**GGCGGTCAT 3'
CR62 (sense)	5' ATGACCGCC**AAG**CGACGC 3'
CR63 (antisense)	5' GCGTCG**CTT**GGCGGTCAT 3'
CR64 (sense)	5' ATGACCGCC**GAT**CGACGC 3'
CR65 (antisense)	5' GCGTCG**ATC**GGCGGTCAT 3'
CR66 (sense)	5' CGC**GCC**CGCGGTGTGG 3'
CR67 (antisense)	5' GCG**GGC**GCGGGCGGT 3'
CR68 (sense)	5' CGC**AAG**CGCGGTGTGGTC 3'
CR78 (antisense)	5' GCG**CTT**GCGGGC**TGT**CATT 3'
CR70 (sense)	5' CGC**GAT**CGCGGTGTGGTC 3'

CR71 (antisense)	5’ GCG**ATC**GCGGGCGGTCAT 3’
CR72 (sense)	5’ CGCCGA**GC**CGGTGTGGTC 3’
CR73 (antisense)	5’ CCACACCG**GC**TCGGCG 3’
CR74 (sense)	5’ CCGCCGA**AAG**GGTGTGGTC 3’
CR75 (antisense)	5’ GACCACACC**CTT**TCGGCGG 3’
CR76 (sense)	5’ CCCGCCGA**GAT**GGTGTGGTC 3’
CR77 (antisense)	5’ GACCACACC**ATC**TCGGCGGG 3’
CK32: (sense)	5’ CAATGACCGCC**AAGAAGAAG**GGTGTG 3’

PTO modified oligodeoxyribonucleotides:

PS-ODN$^{34\text{-}39}$: 5’ GTCCTGGATGAGCGCGGG 3’

PS-ODN$^{104\text{-}111}$: 5’ GCCGGGAACCGACAGACAGCCTTC 3’

PS-ODN$^{74\text{-}83}$: 5’ GATGACCACACCGCGTCGGCGGGCGGTCAT 3’

PS^{b}-ODNF$^{74\text{-}83}$: 5’ GATGACCACACCGCGTCGGCGGGCGGTCAT 3’

PS^{c}-ODNAM$^{76\text{-}85}$: 5’ CGGATTGATGACCACACCGCGTCGGCG 3’

PS-ODN$^{147\text{-}154}$: 5’ ATCAAGGTGCCCGGTTTCGTGCTG 3’

PS-ODN$^{188\text{-}197}$: 5’ TTAGTGACCGAACGGGTCGGGGTCCTCGCC 3’

PS^{d}-ODN$^{S74\text{-}83}$: 5’ GATCACGACGCCGCGGCGGCGTGTGGTCTG 3’

PS^{d}-ODN$^{S188\text{-}197:}$ 5’ TTAGTGCCCGAACGGATCGGGCACCTCACC 3’

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[a]CR26 and CR27 were used as external primers for the amplification of *def* along with mutation. GGAATTCCAT in CR26 and CCCAAGCTT in CR27 do not correspond to genome sequence but has been introduced to incorporate *Nde*I and *Hin*d III sites at 5’ and 3’ ends respectively of the PCR amplified product.

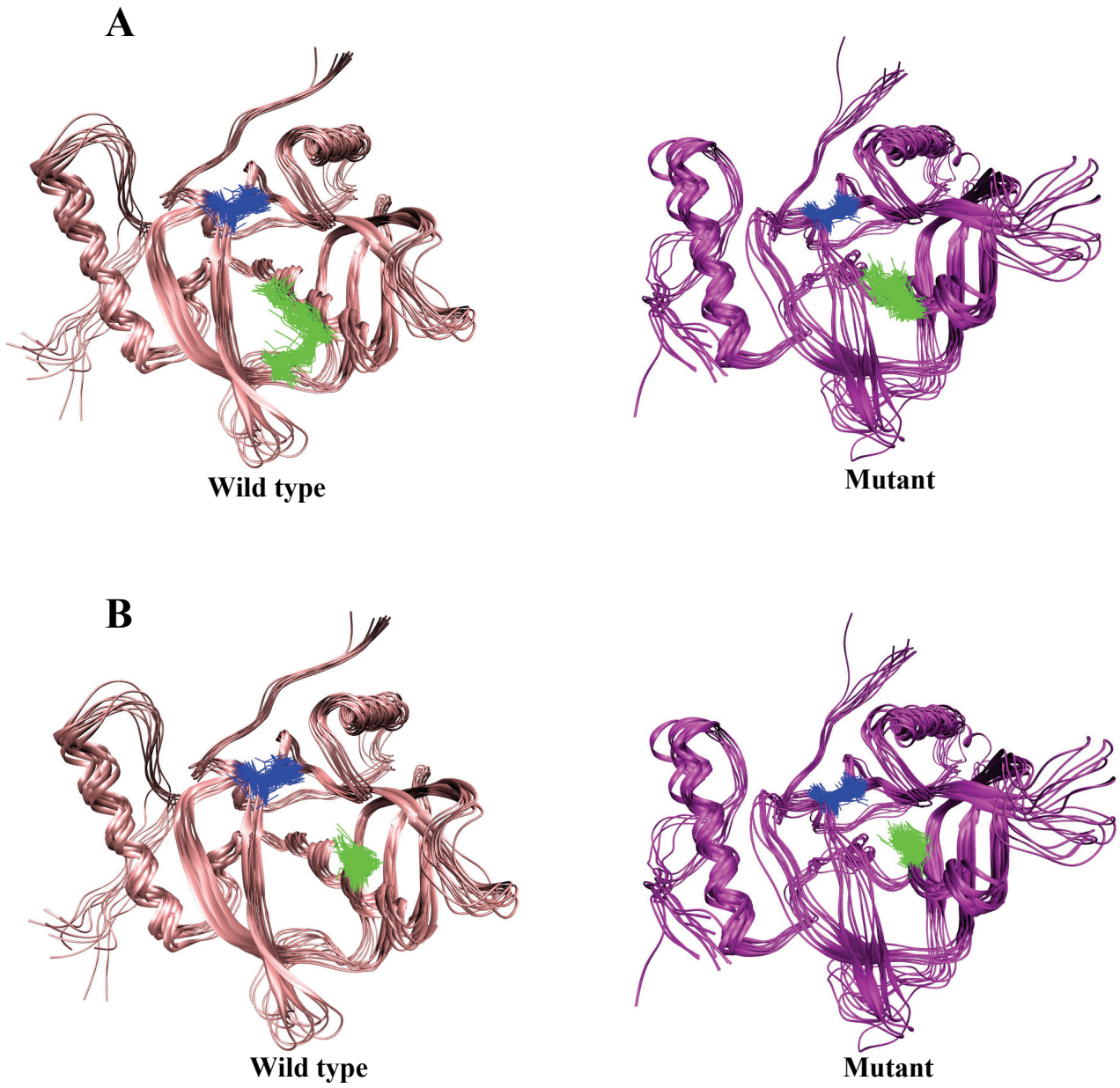
[b]First base at 5’-end was PTO modified and last base at 3’-end was conjugated with flourescein.

[c]All bases were PTO modified.

[d]Antisense oligos designed based on *M. smegmatis def* sequence.

Supplementary Fig. 1.

Multiple sequence alignment of peptide deformylase from different bacteria



Supplementary Fig. 2.

Superimposed snapshots of wild-type and mutant mPDFs showing the dynamics of structural regions. *A*, Movement of side chain L107 (blue) with respect to residue R144 (green). In wild type R144 side chain changes conformation after 4ns and stays in this conformation till the end, whereas in mutant only one conformation is present. *B*, Movement of side chain L107 (blue) with respect to residue M145 (green).