Table S1. Primers used in this study

Primer	Sequence (5'-3')
Nox2F	GACCGTAATAGCAACATGAG
Nox2R	CTTGAATCGCTAAACTGAAC
noxE_NcoI_F	CATGCCATGGGAAAAATCGTAGTTATCG NcoI
noxE_XbaI_R	TGCTCTAGATCCTCCTAACTTTCTATACG XbaI
noxE_XbaI_6H_R	Stop codon CTAGTCTAGATTAGTGATGGTGATGGTGATGTTTGGCATTCAAAGCTGCAAC XbaI 6-His Tag
noxE_XhoI_R	CCGCTCGAGTTTGGCATTCAAAGCTGCAAC XhoI
T7terRev	GCTAGTTATTGCTCAGCGG
T7promRev	TAATACGACTCACTATAGGG
pNZ8048_F	GTTAGATACAATGATTTCG
pNZ8048_R	AAATTTACATTAGTCTCGG
noxE_A303T_F	CGCTCTTGCCTCAAAC**A**CTGTTCGGTCAGGAATTG
noxE_A303T_R	CAATTCCTGACCGAACAG**T**GTTTGAGGCAAGAGCG
noxE_A144T_F	GACGCAGAATTT**A**CCAAAGAAAAAGTAAAGCGTATCGC
noxE_A144T_R	GCGATACGCTTTACTTTTTCTTTGG**T**AAATTCTGCGTC
noxE_K384N_F	CGTATCGTATATGAGACAAA**T**AGTCGCAGAATTATTGGAGC
noxE_K384N_R	GCTCCAATAATTCTGCGACT**A**TTTGTCTCATATACGATACG
noxE_A303G_F	CGCTCTTGCCTCAAACG**G**TGTTCGGTCAGGAATTG
noxE_A303G_R	CAATTCCTGACCGAACA**C**CGTTTGAGGCAAGAGCG
noxE_A300T_F	ACTTATATCGCTCTT**A**CCTCAAACGCTGTTCGG
noxE_A300T_R	CCGAACAGCGTTTGAGG**T**AAGAGCGATATAAGT
noxE_L299T_F	TTTACTTATATCGCT**AC**TGCCTCAAACGCTGTTC
noxE_L299T_R	GAACAGCGTTTGAGGCA**GT**AGCGATATAAGTAAA
noxE_N302S_F	ATCGCTCTTGCCTCAA**G**CGCTGTTCGGTCAGG
noxE_N302S_R	CCTGACCGAACAGCG**C**TTGAGGCAAGAGCGAT
noxE_G307A_F	AACGCTGTTCGGTCAG**C**AATTGTCGCAGGACAC
noxE_G307A_R	GTGTCCTGCGACAATT**G**CTGACCGAACAGCGTT
noxE_G307S_F	AACGCTGTTCGGTCA**A**GC**A**TTGTCGCAGGACAC
noxE_G307S_R	GTGTCCTGCGACAA**T**GC**T**TGACCGAACAGCGTT
noxE_T303A_F	CGCTCTTGCCTCAAAC**G**CTGTTCGGTCAGGAATTG
noxE_T303A_R	CAATTCCTGACCGAACAG**C**GTTTGAGGCAAGAGCG

Fig. S1. SDS-PAGE of the soluble fractions (SF) of *E*. *coli* Rosetta overexpressing WT NoxE (1), no protein (2), NoxE A303T (3), NoxE A144T (4) and NoxE K384N (5). The arrow indicates NoxE.

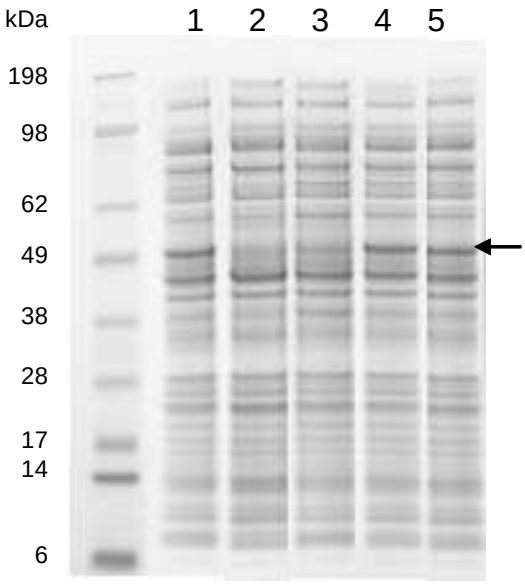

Figure S2. SDS-PAGE of purified TIL46 NoxE (WT) and the derived variants. Molecular weight (kDa) standards were seeblue® plus2 from Invitrogen; 1ug of protein was loaded in each lane; before loading purified proteins were heated at 95℃ for 5 min in the presence of 5% β-mercaptoethanol and 2% SDS in the sample buffer; the percentage of purity estimated by image analysis with SameSpot v2.0 software is indicated in parenthesis

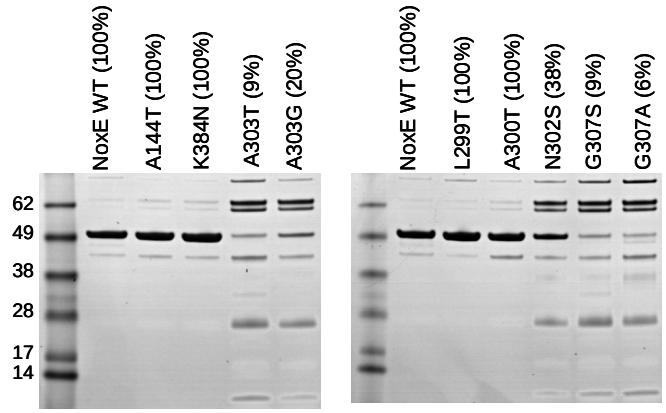

Figure S3 : Particle size distribution of purified recombinant NoxE solutions determined by dynamic light scattering. Recombinant His-tagged NoxE of *L. lactis* TIL46 and the derived variants were at a final concentration of 5 µM in 20 mM MOPS buffer pH 7.

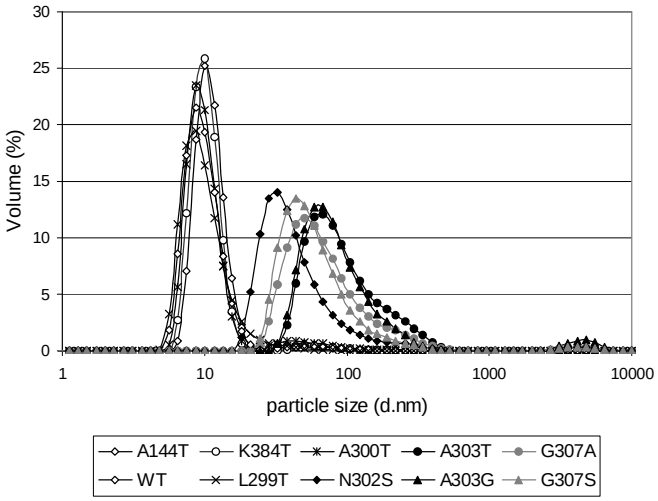

Figure S4: Circular dichroism spectra of the recombinant His-tagged NoxE of *L. lactis* TIL46 and the A303T variant in the far UV region. All measurements were in 20 mM MOPS pH 7.0

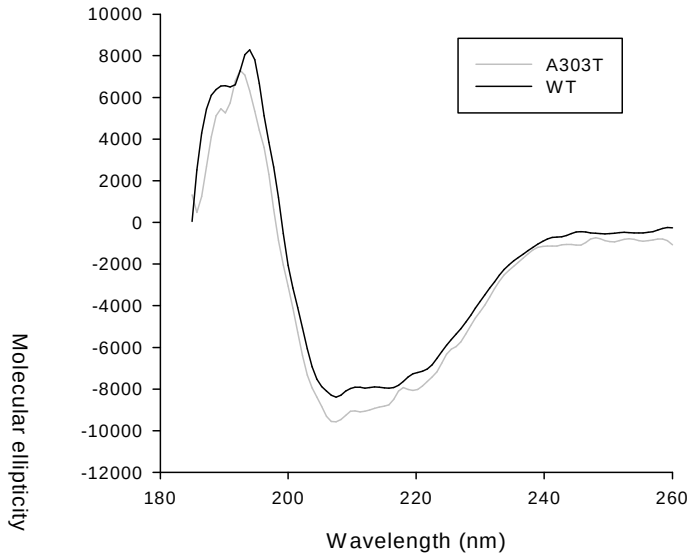

Figure S5: CLUSTAL W sequence alignment of NADH oxidases sharing from 33% to 53.8% of identity with *L*. *lactis* NoxE. L. cremoris, *Lactococcus lactis* subsp. *cremoris* MG1363 (Gene ID: 4796799); L lactis, *Lactococcus lactis* subsp. *lactis* IL1403 (Gene ID: 1114002); S mutans UA159, *Streptococcus mutans* (Gene ID: 1028431); E faecalis, *Enterococcus faecalis* V583 (Gene ID:1200486); Lb san, *Lactobacillus sanfransiscensis* (Prot ID: BAB19268.1); Treponema, *Treponema denticola* (Gene ID: 2741711).

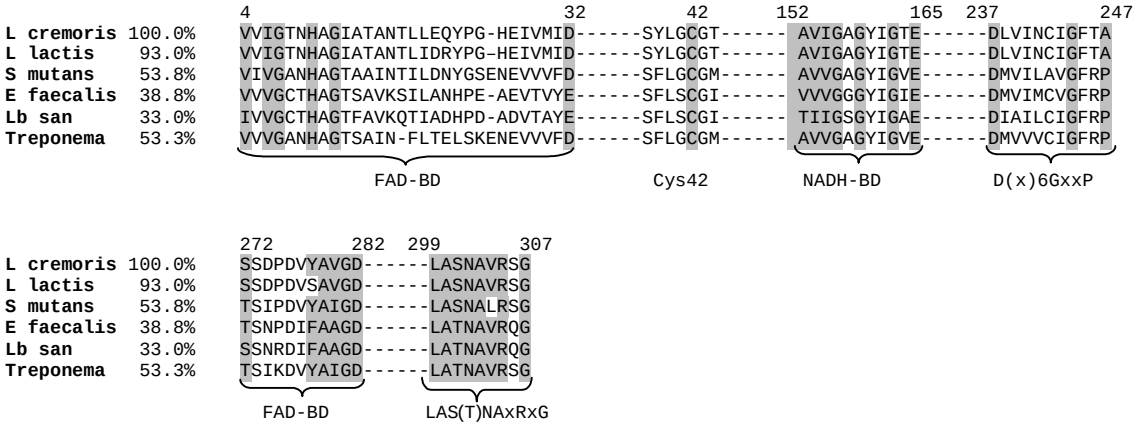

Fig. S6. Ribbon representation of the *Streptococcus pyogenes* NADH oxidase dimer (PDB code 2BC0). Subunit A is in red and subunit B is in blue. The α-helix formed by residues corresponding to the 299-307 sequence of *L. lactis* NoxE (residues 309-317) is illustrated by purple VDW spheres. The FAD, shown in VDW spheres, is colored by atom type.