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Supporting Information

Characterization of Two Site-Specifically Modified Human Dihydrolipoamide Dehydrogenase Mutants (Pro-282 to Ala and Pro-298 to Ala)

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Site-directed mutagenesis was carried out using a mutagenesis kit according to the manufacturer's protocol. Two mutagenic primer pairs were used for the mutations, as shown in Table S1. PCR was carried out using the human E3 expression vector, pPROEX-1:E3, as a template in a programmable PCR machine. The entire DNA sequence of the human E3 coding region was sequenced to confirm the integrity of the DNA sequences other than the anticipated mutations. The mutant was expressed in *E. coli* by IPTG induction (1 mM). Purification of the mutants was performed using a nickel affinity column. The purification steps were followed by SDS-polyacrylamide gel electrophoresis, as shown in Figure S2. The gel revealed the mutants to be highly purified.

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                                282                 298
sp|P09622|DLDH_HUMAN    DVLLVCIGRRPFTKNLGLEELGIELDPRGRIPVNTRF
sp|P09623|DLDH_PIG      DVLLVCIGRRPFTQNLGLEELGIELDPRGRIPVNTRT
sp|P09624|DLDH_YEAST    EVLLVAVGRRPYIAGLGAEKIGLEVDKRGRLVIDDQF
sp|P0A9P0|DLDH_ECOLI    DAVLVAIGRVPNGKNLDAGKAGVEVDDRGFRVDKQL
sp|P14218|DLDH_PSEFL    DKLIVAVGRRPVTTDLLAADSGVTLDERGFYVDDHC
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                               βF2       α7
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Figure S1. Sequence alignment of the Pro-282 and Pro-298 regions of human E3 with the corresponding regions of the E3s from various sources (from top to bottom; human, pigs, yeast, *Escherichia coli* and *Pseudomonas fluorescens*). Pro-282, Pro-298 and the corresponding residues are underlined. Alignment analysis was performed using the MAFFT program at the ExPASy Proteomics Server (Swiss Institute of Bioinformatics).

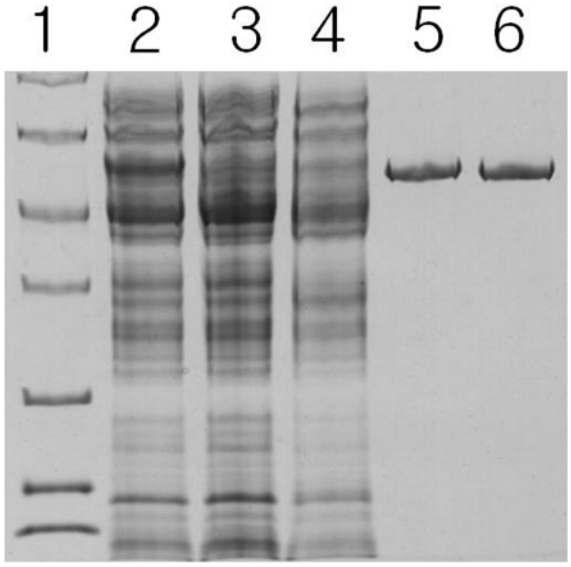

Figure S2. SDS-PAGE gel for the purification of the Ala-298 mutant. Lane 1, molecular weight markers (from top to bottom, β-galactosidase 116.3 kDa, bovine serum albumin 66.2 kDa, ovalalbumin 45.0 kDa, lactate dehydrogenase 35.0 kDa, REase Bsp981 25 kDa, β-lactoglobulin 18.4 kDa, lysozyme 14.4 kDa); lane 2, supernatant; lane 3, flow-through; lane 4, Binding buffer containing 50 mM imidazole; lane 5, Binding buffer containing 250 mM imidazole; lane 6, previously purified recombinant human E3 as a control.

Table 1. Primers for the site-directed mutagenesis. The mismatched bases are underlined

Mutations/Primers	Primer Sequences
Pro-282 → Ala	
sense	5'-GCATTGGCCGACGAGCCTTTACTAAGAATTTGGC-3'
antisense	5'-GCCAAATTCTTAGTAAAGGCTCGTCGGCCAATGC-3'
Pro-298 → Ala	
sense	5'-GGAATTGAACTAGATGCCAGAGGTAGAATTCC-3'
antisense	5'-GGAATTCTACCTCTGGCATCTAGTTCAATTCC-3'

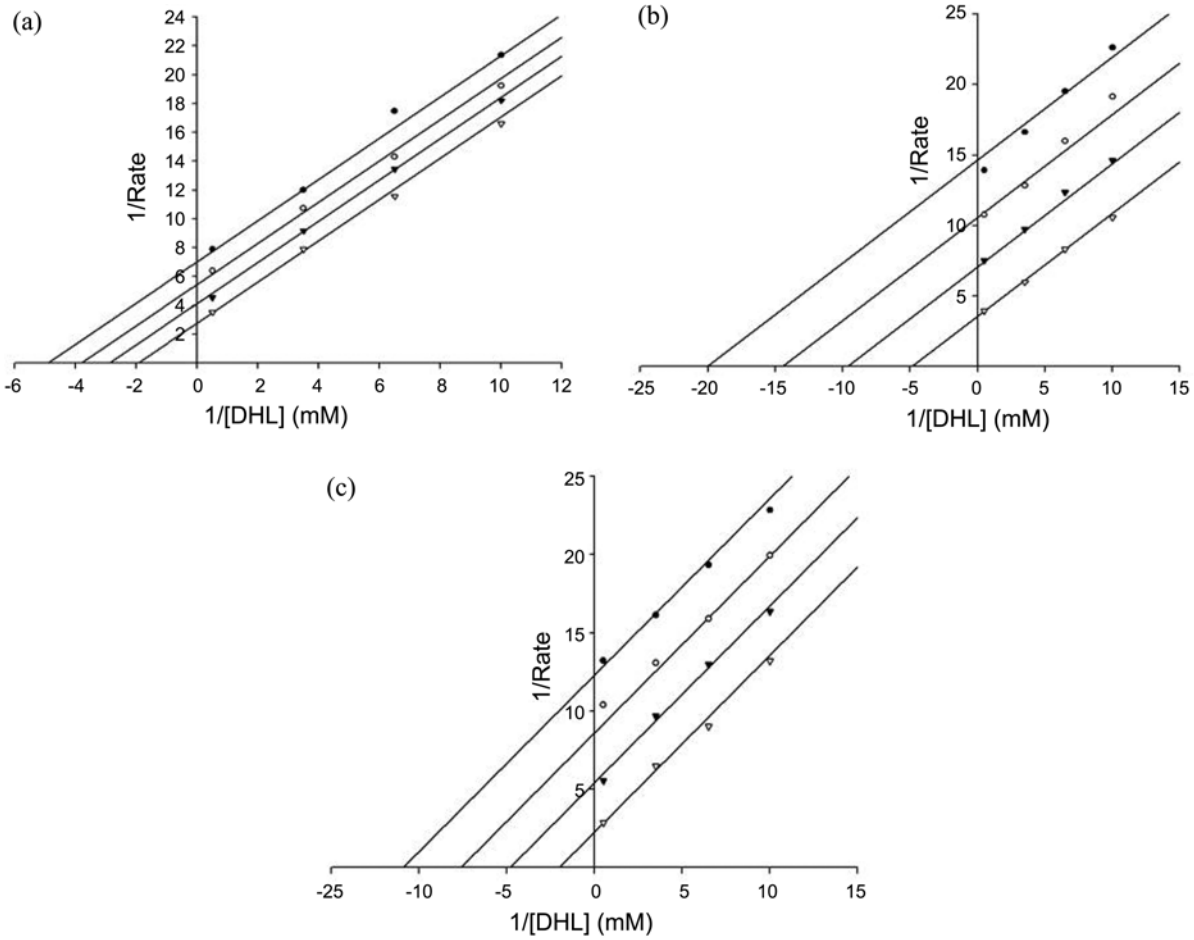

Figure S3. Double reciprocal plots for the normal (A), Ala-282 (B) and Ala-298 mutant (C) human E3s. E3 activities were determined at 37 °C in a 50 mM potassium phosphate buffer (pH 8.0) containing 1.5 mM EDTA with variable concentrations of the substrates, dihydrolipoamide (DHL) and NAD^+. Plots were drawn with the SigmaPlot Enzyme Kinetics Module program. The NAD^+ concentrations from top to bottom are 0.1, 0.154, 0.286 and 2 mM. The DHL concentrations from right to left are 0.1, 0.154, 0.286 and 2 mM.