Supporting Information

Characterization of Two Site-specific Mutations in Human Dihydrolipoamide Dehydrogenase

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Table S1. Primers for the site-directed mutagenesis. The mismatched bases are underlined

Mutations/Primers	Primer Sequences
Pro-325 to Ala	
sense	5'-GATGTAGTTGCTGGTGCAATGCTGGCTCACAAAG-3'
antisense	5'-CTTTGTGAGCCAGCATTGCACCAGCAACTACATC-3'
Trp-366 to Ala	
sense	5'-ACCCTGAAGTTGCTGCAGTTGGCAAATCAG-3'
antisense	5'-TCTGATTTGCCAACTGCAGCAACTTCAGGG-3'

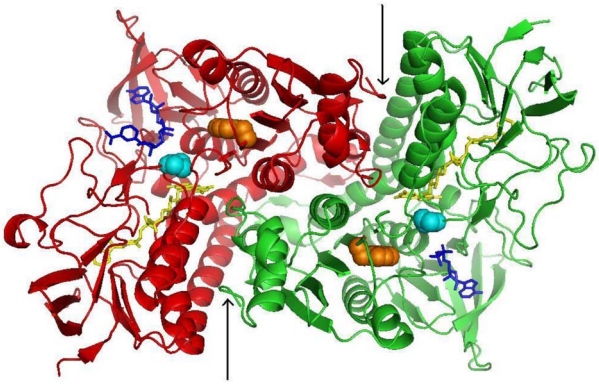

Figure S1. Location of Pro-325 and Trp-366 in human E3. Two subunits of the human E3 homodimeric structure are shown as cartoons, representing secondary structures, in a single color (red and green, respectively). FAD (yellow) and NAD^+ (blue) are shown as sticks whereas Pro-325 (cyan) and Trp-366 (orange) are shown as spheres. The arrows indicate the dihydrolipoamide binding channels. The structure was drawn using the PyMOL program (DeLano Scientific LLC). The PDB ID for the human E3 structure is 1ZMC.

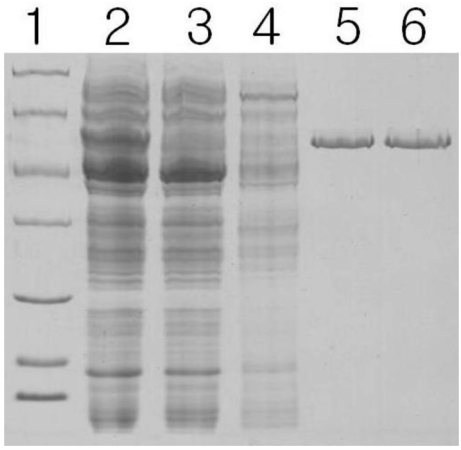

Figure S2. SDS-polyacrylamide gel for purification of the Ala-325 mutant. Lane 1, molecular weight markers (from top to bottom, β-galactosidase 116.3 kDa, bovine serum albumin 66.2 kDa, ovalalbumin 45.0 kDa, lactate dehydrogenase 35.0 kDa, REase Bsp981 25 kDa, β-lactoglobulin 18.4 kDa, lysozyme 14.4 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.

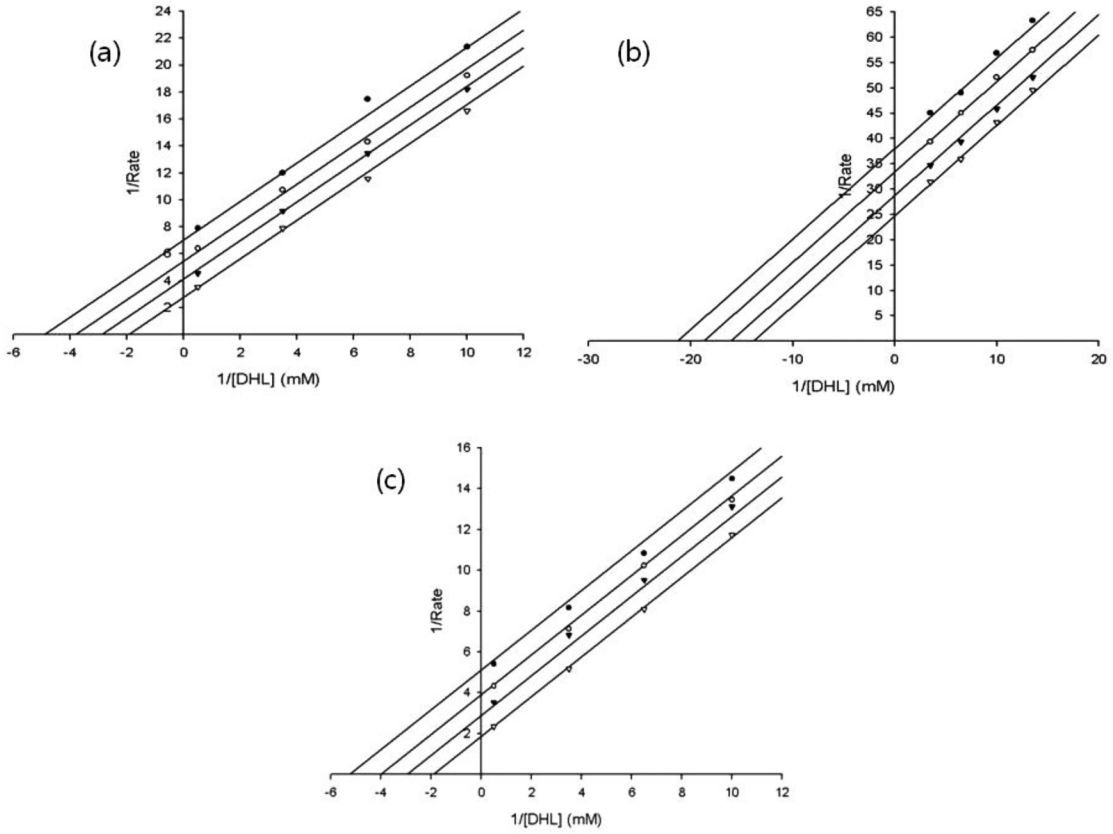

Figure S3. Double reciprocal plots for the normal (a), Ala-325 (b) and Ala-366 mutant (c) human E3s. The E3 activities were determined at 37 °C in a 50 mM potassium phosphate buffer (pH 8.0) containing 1.5 mM EDTA with various concentrations of the substrates, dihydrolipoamide (DHL) and NAD^+. The plots were drawn using the SigmaPlot Enzyme Kinetics Module program. The NAD^+ concentrations from top to bottom were 0.1, 0.154, 0.286 and 2 mM in (a) and (c) whereas those were 0.074, 0.1, 0.154 and 0.286 mM in (b). The DHL concentrations from the right to left were 0.1, 0.154, 0.286 and 2 mM in (a) and (c) whereas those were 0.074, 0.1, 0.154 and 0.286 mM in (b).