

Supplementary data for:

Probing the mechanistic role of the long α -helix in subunit L of respiratory Complex I from *Escherichia coli* by site-directed mutagenesis

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Table S1
Oligonucleotide primers used for cloning and mutagenesis of *E.coli* *nuoL* gene

Primer	Sequence ^a
<i>for PCR</i>	
UpLF	ATGGCAGGTATGGCAATCGACG
UpLXhoR	GGTAA <u>CTCGAG</u> CATGTTTCATCCG
DnLXhoF	<u>GCTCGAG</u> GGGCATATAAGAACGTAG
DnLR	ACAGCGGCAGGGAGAAACG
UpLNheR	GGTAAAGGCTAGCATGTTTCATCCG
LnNheF	ATTCCGCT <u>AGC</u> TTATCTCTGCTTCC
<i>for mutagenesis^b</i>	
Y590St	TGCTGTTAAGTGAGAACGGCTAA <u>ACTGCGCTGGTAT</u>
D542N	GGTGGTACAACGCCTGGGGATTTA <u>ACTGGCTGTATG</u>
D542R	GGTACAACGCCTGGGGATTT <u>CGCTGGCTGTATGACAA</u>
K551Q	ATGACAAAGTGTTTCGTCC <u>CAGCCGTTCCCTGGGTATT</u>
K551E	ATGACAAAGTGTTTCGTCC <u>GAGCCGTTCCCTGGGTATT</u>
P552A	CAAAGTGTTTCGTCAAGG <u>CGTTCCCTGGGTATTGC</u>
P552Q	CAAAGTGTTTCGTCAAGC <u>AGTTCCCTGGGTATTGCCT</u>
P552C	TATGACAAAGTGTTTCGTCAAGT <u>GCTTCCCTGGGTATTGCCTGGTTG</u>
G532ins ⁶	GCCGTCTGCTGGGC <u>GATGGTGATGGTGACCCTACCTGGTGGTACAAC</u>
G532ins ⁷	CCGTCTGCTGGGC <u>CCAGATGGTGATGGTGACCCTACCTGGTGGTACAAC</u>
G532sub ⁷	CAACAGTGCGCCGGCCGTCTGCTGGGC <u>CCAGATGGCGACGGCGATCCG</u> GGATTT GACTGGCTGTATGACAAAGTG
P552ins ⁶	GTGTTTCGTCAAGCCGATGGTGATGGTGACCATTCCCTGGGTATTGCC
P552ins ⁷	GTGTTTCGTCAAGCCGATGGTGATGGTGACCCTTTCCCTGGGTATTGCC
P564ins ⁶	TGCTGAAACGCGATCCGGATGGTGATGGTGACCCTGA <u>ACTCAATGATGAAC</u>
P564sub ⁷	CCTGGGTATTGCCTGGTTGCTGAAACGCGATCCG <u>GGTGACGGTGATGGTGACCCA</u> CCGGCTGTCTTTCCCGCTTTGCAGGTAAAGGTC
P572ins ⁶	CAATGATGAACATCCCGGATGGTGATGGTGACCAGCTGTCCTTTCCCGCT

^a New restriction sites are underlined, altered nucleotides are in bold, and nucleotide sequence in insertions/substitutions is shaded

^b Sequence represents sense primer, antisense primer had reverse complementary sequence

Fig.S2. Growth curves of NuoL mutants with insertion/substitution in AH in minimal malate medium.

GR70N, wild type, black squares; GRL3 (Δ nucL), red diamonds; G542 ins^7 , wine, solid line and cycles; G542 ins^6 , wine, dashed line, open cycles; G542 sub^7 , wine, dotted line, open cycles with cross, P552 ins^6 , green, solid line and triangles; P552 ins^7 , green, dashed lane, open triangles; P564 ins^6 , blue, solid line and diamonds; P654 sub^7 , blue, dashed line, open diamonds; P572 ins^6 , dark yellow stars.

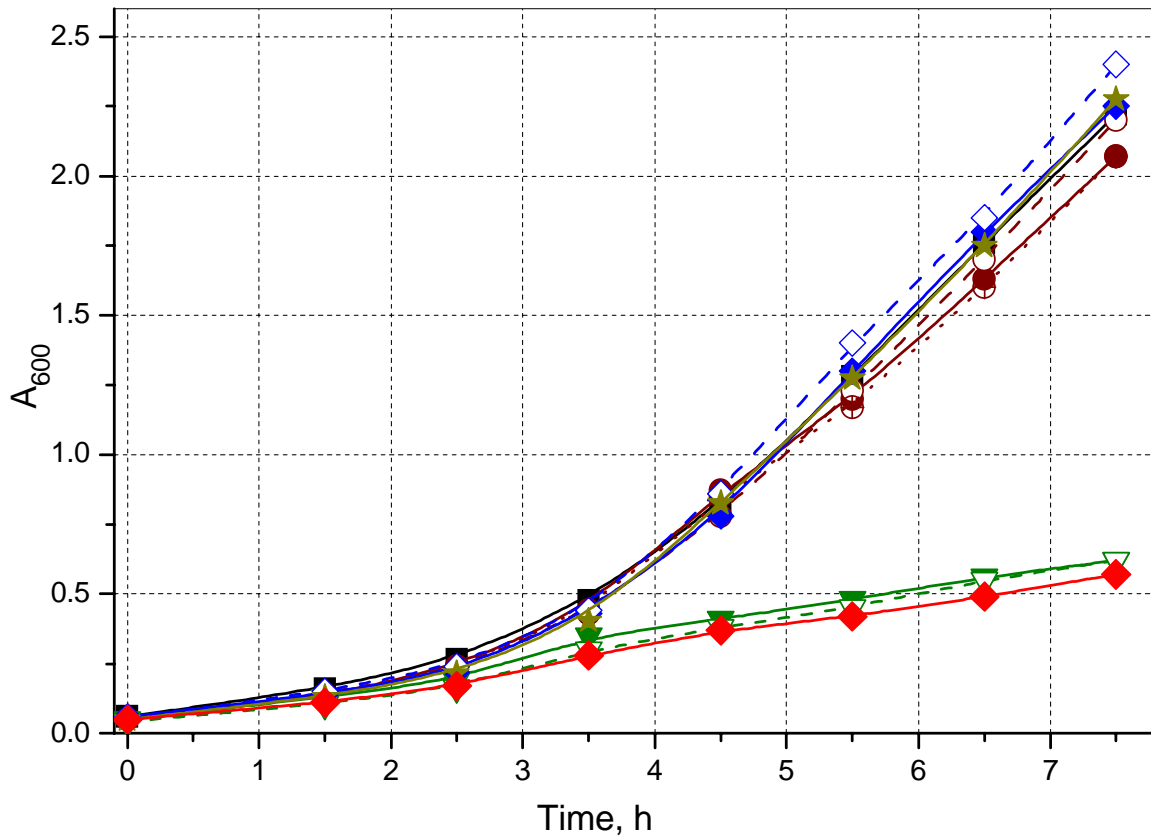


Fig.S3. SDS-PAGE of purified *E. coli* Complex I.

Lane 1, molecular mass markers (kDa). Lane 2, P552ins⁶ variant. Lane 3, wild type. The assignment of the observed bands to the individual subunits of Complex I is given on the right.

