

## SUPPLEMENTAL DATA

for

### Engineering the respiratory complex I to an energy-converting NADPH:ubiquinone oxidoreductase

by

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#### Supplementary Tables:

**Table S1:** Primers for site directed mutagenesis.

**Table S2:** Primers for the insertion of the *nptI-sacRB* cartridge and for amplification of the PCR fragment carrying the point mutation.

**Table S3:** Preparation of the variant Glu183Asp<sup>F</sup>.

**Table S4:** Preparation of the variant Glu183Gln<sup>F</sup>.

**Table S5:** Preparation of the variant Glu183Asn<sup>F</sup>.

**Table S6:** Preparation of the variant Glu183His<sup>F</sup>.

#### Supplementary Figures:

**Figure S1: Sequence alignment of NuoF (numbering according to the *E. coli* subunit) around the nucleotide binding site.** The position of Glu183 is marked in red.

**Figure S2: SDS-Gel of the preparations of complex I from the parental strain (A) and the Glu183His<sup>F</sup> variant (C).** Lane (B) shows the pattern of the marker (PageRuler Protein unstained Ladder, Fermentas). The molecular mass of the marker proteins is indicated. The SDS-Gel of the other preparations looked virtually identical to the ones shown in this figure.

**Figure S3: Detection of superoxide formation.** The spectrum was recorded with the Glu183Asp<sup>F</sup> variant reconstituted in phospholipids in the presence of 100 mM DEPMPO, 100  $\mu$ M decyl-ubiquinone, and 1 mM NADPH. EPR conditions were: microwave frequency: 9.65 GHz; modulation amplitude: 0.1 mT; time constant: 0.164 s; scan rate: 5.4 mT/min.

**Table S1:** Primers for site directed mutagenesis. The mutations introduced are marked in bold. The new restriction site for *VspI* is underlined.

<b>Primer</b>	<b>Sequence</b>
<i>nuoF</i> E183D_fwd	5'-CTGCGGGGAAG <b>AT</b> ACAGC <u>CATTAAT</u> CAACTCCCTGG-3'
<i>nuoF</i> E183D_rev	5'-CCAGGGAGTTG <u>GATTAAT</u> GCTGT <b>AT</b> CTTCCCCGCAG -3'
<i>nuoF</i> E183H_fwd	5'-CTGCGGGGAAC <b>AC</b> ACAGC <u>CATTAAT</u> CAACTCCCTGG-3'
<i>nuoF</i> E183H_rev	5'-CCAGGGAGTTG <u>GATTAAT</u> GCTGT <b>GT</b> GTTCCCCGCAG -3'
<i>nuoF</i> E183Q_fwd	5'-CTGCGGGGAAC <b>AG</b> ACAGC <u>CATTAAT</u> CAACTCCCTGG-3'
<i>nuoF</i> E183Q_rev	5'-CCAGGGAGTTG <u>GATTAAT</u> GCTGT <b>CT</b> GTTCCCCGCAG -3'
<i>nuoF</i> E183N_fwd	5'-CTGCGGGGAAA <b>AC</b> ACAGC <u>CATTAAT</u> CAACTCCCTGG-3'
<i>nuoF</i> E183N_rev	5'-CCAGGGAGTTG <u>GATTAAT</u> GCTGT <b>GTTT</b> CCCCGCAG -3'

**Table S2:** Primers for the insertion of the *nptI-sacRB* cartridge and for amplification of the PCR fragment carrying the point mutation. Regions homologous to *nuoF* are underlined. Regions homologous to the *nptI-sacRB* cartridge are marked bold.

<b>Primer</b>	<b>Sequence</b>
<i>nuoF::nptI-sacRB</i> _fwd	5'- <u>CCGCTGACCTGGCGTCTGCGCGATGACAAACAGCCAGTG</u> <u>TGGCTGGACGGTACCGGATCCGTCGACCTG</u> -3'
<i>nuoF::nptI-sacRB</i> _rev	5'- <u>GCCAGGCTTTAAATTT</u> CAGACCATCACGCATACCACCGGC <u>GTAATCTTCGGAATTCCCCGGGGGATCCG</u> -3'
<i>nuoF2</i> _fwd	5'-AACATTATCCGTACTCCCGAAACG-3'
<i>nuoF</i> _rev	5'-CAGATCAAGGTGCGCTTC-3'

**Table S3:** Isolation of the complex I variant Glu183Asp<sup>F</sup> from 25 g cells (wet weight).

preparation	NADH/ferricyanide-oxidoreductase activity				
	volume [mL]	protein [mg]	total [ $\mu\text{mol}\cdot\text{min}^{-1}$ ]	specific [ $\mu\text{mol}\cdot\text{min}^{-1}\cdot\text{mg}^{-1}$ ]	yield [%]
membranes	17.8	1337	4146	3.1	100
extract	59.5	1021	3469	3.4	82
Fractogel EMD	70	154	2961	19.2	71
ProBond Ni <sup>2+</sup> -IDA	1	2.4	185	77	4

**Table S4:** Isolation of the complex I variant Glu183Gln<sup>F</sup> from 22 g cells (wet weight).

preparation	NADH/ferricyanide-oxidoreductase activity				
	volume [mL]	protein [mg]	total [ $\mu\text{mol}\cdot\text{min}^{-1}$ ]	specific [ $\mu\text{mol}\cdot\text{min}^{-1}\cdot\text{mg}^{-1}$ ]	yield [%]
membranes	10,1	714	3414	4.8	100
extract	60	409	3150	7.7	92
Fractogel EMD	35	322	3115	9.7	91
ProBond Ni <sup>2+</sup> -IDA	0.3	2.9	237	81	7

**Table S5:** Isolation of the complex I variant Glu183Asn<sup>F</sup> from 17 g cells (wet weight).

preparation	NADH/ferricyanide-oxidoreductase activity				
	volume [mL]	protein [mg]	total [ $\mu\text{mol}\cdot\text{min}^{-1}$ ]	specific [ $\mu\text{mol}\cdot\text{min}^{-1}\cdot\text{mg}^{-1}$ ]	yield [%]
membranes	6	398	2430	5.4	100
extract	31	548	1717	3.1	71
Fractogel EMD	2.5	121	1433	11.8	59
ProBond Ni <sup>2+</sup> -IDA	0.3	2.0	120	60	5

**Table S6:** Isolation of the complex I variant Glu183His<sup>F</sup> from 20 g cells (wet weight).

preparation	NADH/ferricyanide-oxidoreductase activity				
	volume [mL]	protein [mg]	total [ $\mu\text{mol}\cdot\text{min}^{-1}$ ]	specific [ $\mu\text{mol}\cdot\text{min}^{-1}\cdot\text{mg}^{-1}$ ]	yield [%]
membranes	8,2	613	1542	2.5	100
extract	30	454	1269	2.8	82
Fractogel EMD	21	153	1163	7.6	75
ProBond Ni <sup>2+</sup> -IDA	0.5	4.4	342	78	22

		63	73	80	90	100	110
<i>E. coli</i>	VKDAGLKGRG	GAGFSTGLKW	SLMPKD---E	SMNIRYLLCN	ADEMPEGTYK	DRLLMEQLPH	
<i>T. thermophilus</i>	VKRSGLRGRG	GAGFPTGLKW	SFMPKD---D	GK-QHYLICN	ADESEPGSFK	DRYILEDVPH	
<i>A. aeolicus</i>	VDKSTLRGRG	GAGFPTGKKW	KF&VQN---P	GP--RYFICN	ADESEPGTFK	DRIIERDPH	
<i>P. denitrificans</i>	MKASGLRGRG	GAGFPTGMKW	SFMPKE---S	DGRPSYLVIN	ADESEPATCK	DREIMRHDPH	
<i>N. crassa</i>	VKASGLRGRG	GAGFPSGLKW	SFMNFKDWDK	DDKPRYLVVN	ADEGEPGTC	DREIMRKDPH	
<i>Y. lipolytica</i>	IKKSGLRGRG	GAGFPSGLKW	SFMNPPGWEK	NEGPRYLVVN	ADEGEPGTC	DREIMRKDPH	
<i>B. taurus</i>	VKTSGLRGRG	GAGFPTGLKW	SFMNKP---S	DGRPKYLVVN	ADEGEPGTC	DREIIRHDPH	
<i>H. sapiens</i>	IKTSGLRGRG	GAGFPTGLKW	SFMNKP---S	DGRPKYLVVN	ADEGEPGTC	DREILRHDPH	
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		120	130	140	150	160	170
<i>E. coli</i>	LLVEGMLISA	FALKAYRGI	FLRGEYIEAA	VMLRRAIAEA	TEAGLLGKNI	MGTGDFDFELF	
<i>T. thermophilus</i>	LLIEGMILAG	YAIRATVGI	YVRGEYRRAA	DRLEQAIKEA	RARGYLGNL	FGTDFSFDLH	
<i>A. aeolicus</i>	LLIEGIIISS	YAIGANEAYI	YIRGEYPAGY	YILRDAIEEA	KKKGFGLKNI	LGSGFDLEIY	
<i>P. denitrificans</i>	TLIEGALIAS	FAMGAHAAYI	YIRGEFIRER	EALQAAIDEC	YDAGLLGRNA	AGSGWDFDLY	
<i>N. crassa</i>	KLVEGCLVAG	RAMNATAAYI	YIRGEFIQEA	AILQNAINEA	YADGLIGKNA	CGSGYDFDVY	
<i>Y. lipolytica</i>	KLVEGCLLAG	RAMNATAAYI	YIRGEFYNEA	AVLQTAINEA	YAAGLIGKDA	CGSGYDFDVY	
<i>B. taurus</i>	KLVEGCLVGG	RAMGARAAYI	YIRGEFYNEA	SNLQVAIREA	YEAGLIGKNA	CGSGYDFDVF	
<i>H. sapiens</i>	KLLEGCLVGG	RAMGARAAYI	YIRGEFYNEA	SNLQVAIREA	YEAGLIGKNA	CGSGYDFDVF	
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		180	190	200	210	220	230
<i>E. coli</i>	VHTGAGRYIC	GEETALINSL	EGRANPRSK	PPFPATSGAW	GKPTCVNVE	TLCNVPAILA	
<i>T. thermophilus</i>	VHRGAGAYIC	GEETALMNSL	EGLRANPRLK	PPFPAQSGLW	GKPTTINVE	TLASVVPIME	
<i>A. aeolicus</i>	VARGAGAYIC	GEETALIESL	EKKRGHPRLK	PPYPVQKGLW	GKPTVVMNVE	TIANVFFIIS	
<i>P. denitrificans</i>	LHHGAGAYIC	GEETALLESL	EKKKGMPRMK	PPFPAGAGLY	GCPTTVNVE	SI&VVP&TILR	
<i>N. crassa</i>	LHRGAGAYVC	GEETSLIESL	EKGPKPRLK	PPFPA&VGLF	GCPST&NVE	TV&V&PTICR	
<i>Y. lipolytica</i>	IHRMG&YVC	GEETSLIESL	EKG&GK&PRLK	PPFP&G&VGLF	GRPST&NVE	TV&V&PTILR	
<i>B. taurus</i>	VVRGAGAYIC	GEETALIESI	EKGQ&GK&PRLK	PPFP&D&V&GVF	GCPTT&NVE	TV&V&SPTICR	
<i>H. sapiens</i>	VVRGAGAYIC	GEETALIESI	EKGQ&GK&PRLK	PPFP&D&V&GVF	GCPTT&NVE	TV&V&SPTICR	
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Fig. S1; Morina *et al.*

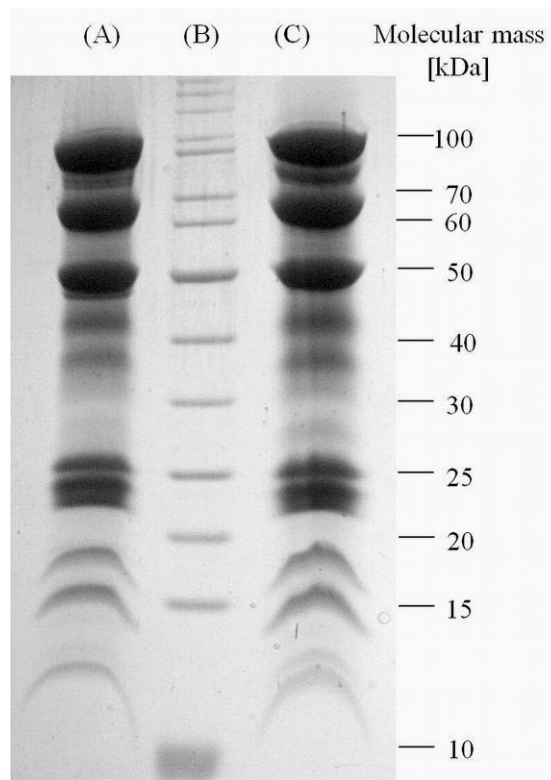


Fig. S2; Morina *et al.*

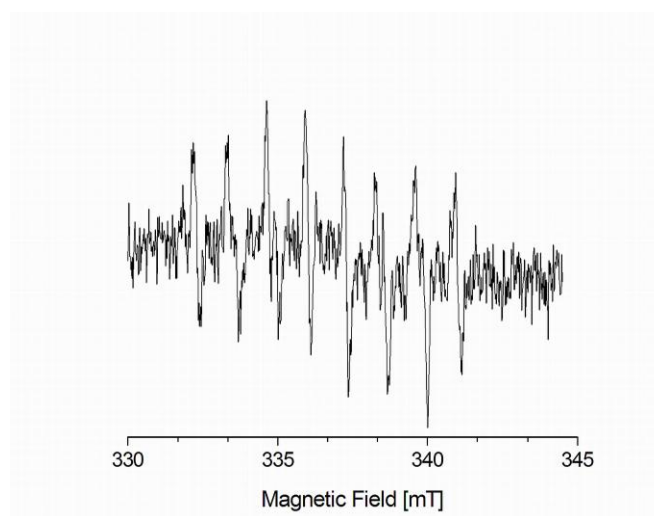


Fig. S3; Morina *et al.*