# Supplementary Information

# Two-component carnitine monooxygenase from *Escherichia coli*: Functional characterization, Inhibition and mutagenesis of the molecular interface

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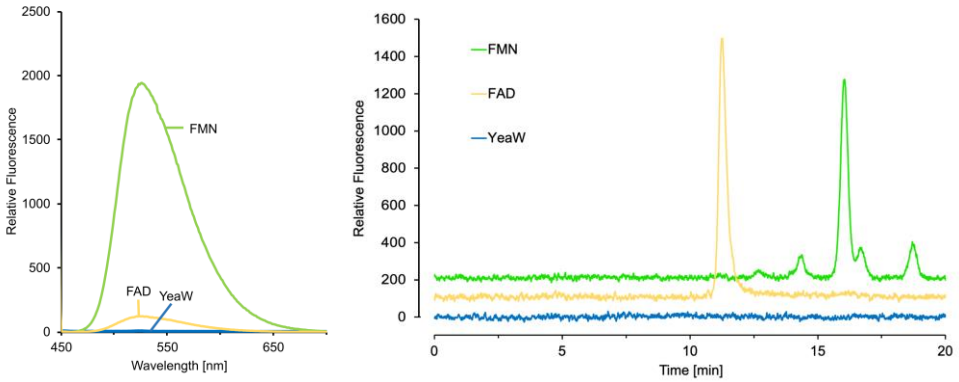



**Figure S1. Fluorescence and HPLC analyses of YeaW.** *left*, fluorescence measurement of a purified YeaW sample (370 nm excitation). Equimolar samples of an FMN or FAD standard were analyzed accordingly. *right*, a purified sample of YeaW (~50 µM) in a volume of 300 µl was incubated at 99 °C for 10 min. The resulting supernatant after centrifugation was chromatographed on a Reprosil 100 C18 column using excitation/emission wavelengths of 370/526 nm (*blue*). Authentic samples of FAD (*yellow*) or FMN (*green*) were analyzed accordingly.

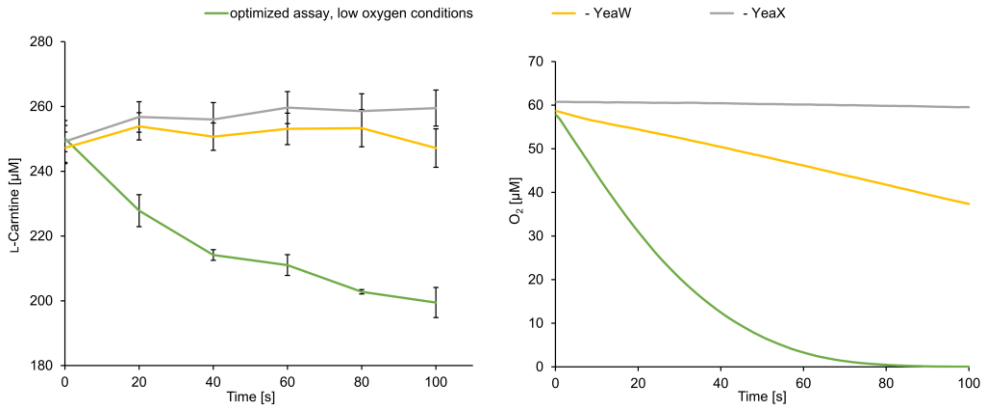
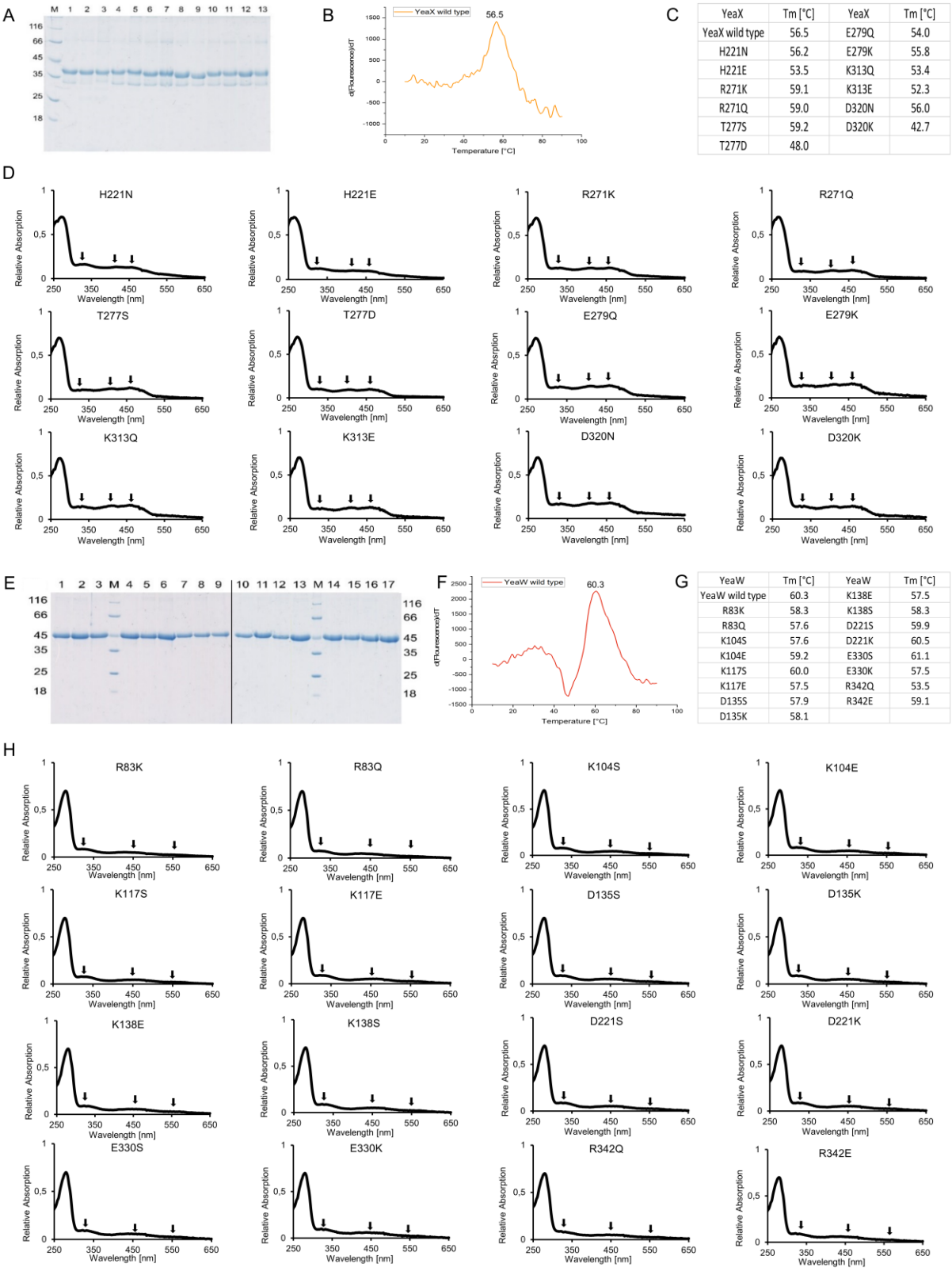



**Figure S2. Carnitine monooxygenase activity under low oxygen conditions.** The L-carnitine depletion assay and the $O_2$ depletion assay (*green traces*) were performed in an anaerobic chamber using $N_2$ saturated buffers. Mixtures of oxygen-free and air saturated assay components facilitated for the adjustment of a specific oxygen partial pressure of ~5%. A fiber-optic oxygen meter (FireSting, PyroScience) was used to validated the resulting oxygen values. Control reactions in the absence of YeaW (*yellow traces*) or YeaX (*grey traces*) were performed. *left*, L-carnitine depletion assay (prepared in triplicate, details under experimental procedures). *right*, continuous $O_2$ depletion assay. One representative graph from three measurements is shown.




| YeaX | Tm [°C] | YeaX | Tm [°C] |
|---|---|---|---|
| YeaX wild type | 56.5 | E279Q | 54.0 |
| H221N | 56.2 | E279K | 55.8 |
| H221E | 53.5 | K313Q | 53.4 |
| R271K | 59.1 | K313E | 52.3 |
| R271Q | 59.0 | D320N | 56.0 |
| T277S | 59.2 | D320K | 42.7 |
| T277D | 48.0 | | |

| YeaW | Tm [°C] | YeaW | Tm [°C] |
|---|---|---|---|
| YeaW wild type | 60.3 | K138E | 57.5 |
| R83K | 58.3 | K138S | 58.3 |
| R83Q | 57.6 | D221S | 59.9 |
| K104S | 57.6 | D221K | 60.5 |
| K104E | 59.2 | E330S | 61.1 |
| K117S | 60.0 | E330K | 57.5 |
| K117E | 57.5 | R342Q | 53.5 |
| D135S | 57.9 | R342E | 59.1 |
| D135K | 58.1 | | |

**Figure S3. Characterization of YeaX and YeaW proteins. A** and **E**, SDS-PAGE analysis of purified YeaX mutants (*lanes 1-13*: wild type, H221N, H221E, R271K, R271Q, T277S, T277D, E279Q, E279K, K313Q, K313E, D320N and D320K) and of YeaW variants (*lanes 1-17*: wild type, R83K, R83Q, K104S, K104E, K117S, K117E, D135S, D135K, K138S, K138E, D221S, D221K, E330S, E330K, R342E and R342Q; lanes M: molecular mass marker, relative molecular masses (x 1,000) are indicated. **B** and **F**, thermal denaturation experiments (Thermofluor assay) for wild type YeaX and YeaW. **C** and **G**, melting temperature of variant proteins of YeaX and YeaW. **D** and **H**, Normalized UV-visible absorption spectra of purified YeaX and YeaW proteins.