**Characterization of human frataxin missense variants in cancer tissues**

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**SUPPORTING INFORMATION**

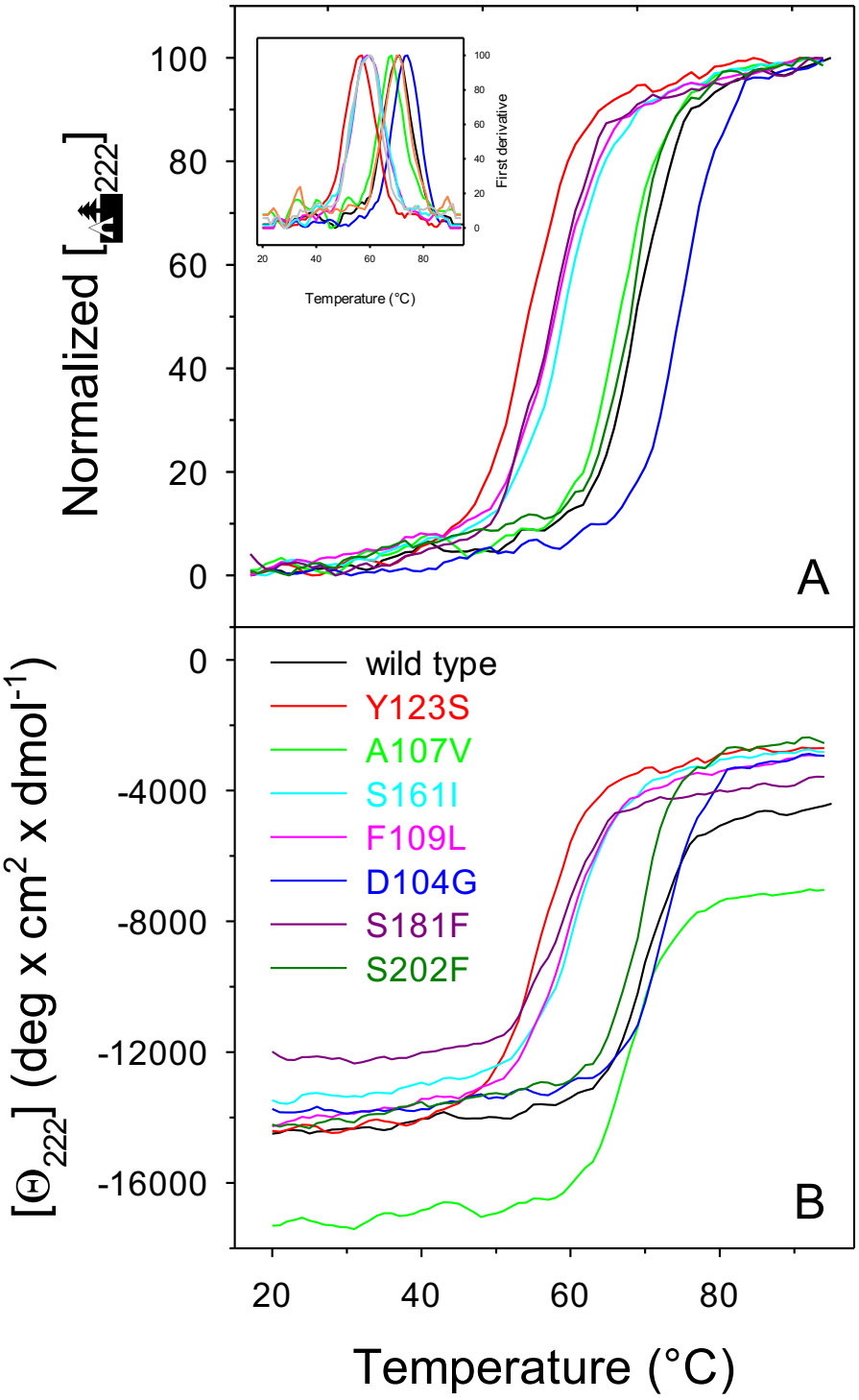



**Figure S1. Thermal unfolding of FXN wild type and variants.** FXN variants and wild type (130 μg/mL) were heated from 20°C to 95°C in 20 mM Tris-HCl, pH 8.0 containing 0.2 M NaCl and 0.2 mM DTT. The molar ellipticity at 222 nm ($[\Theta_{222}]$) was monitored continuously every 0.5 °C. (A) Normalized $[\Theta_{222}]$; the inset shows the first derivative of the same data as in (A). (B) $[\Theta_{222}]$ before normalization.

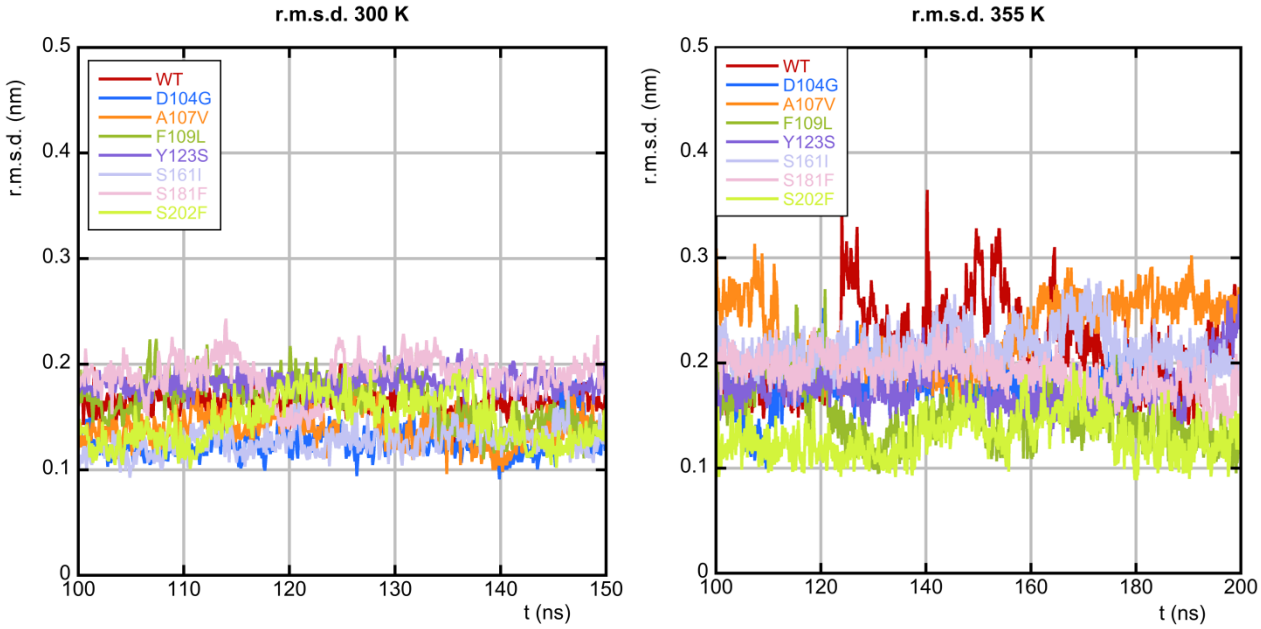



**Figure S2. Root mean square displacement.** Root mean square displacement (r.m.s.d.) computed on the backbone atoms of FXN wild type and variants with respect to the structure at the end of the equilibration procedure.

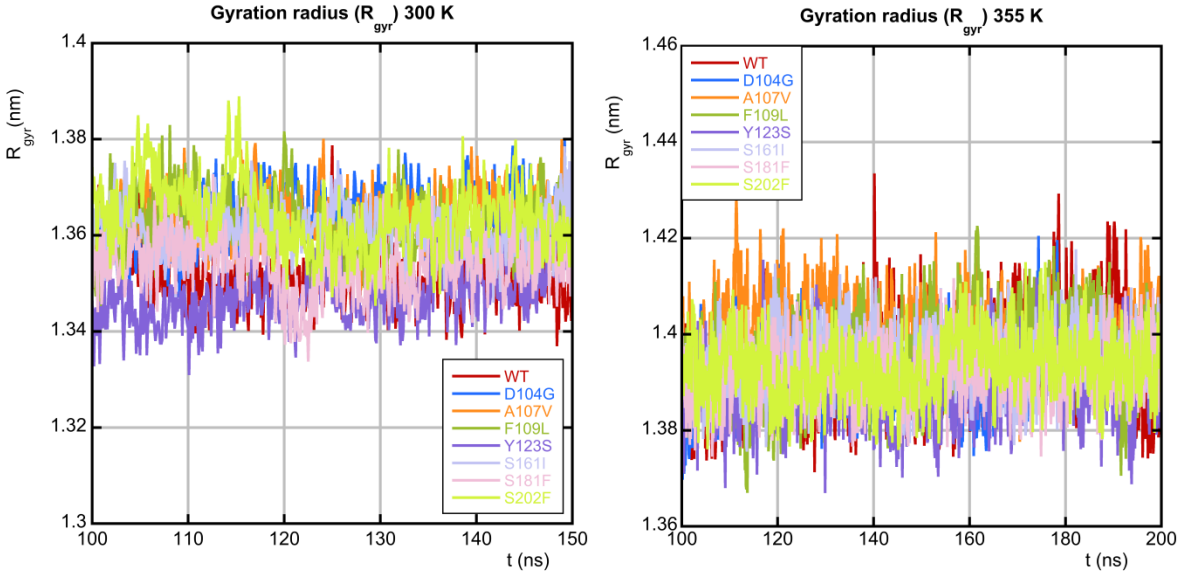



**Figure S3. Radius of gyration.** Radius of gyration ($R_{gyr}$) computed on the backbone atoms of FXN wild type and variants.

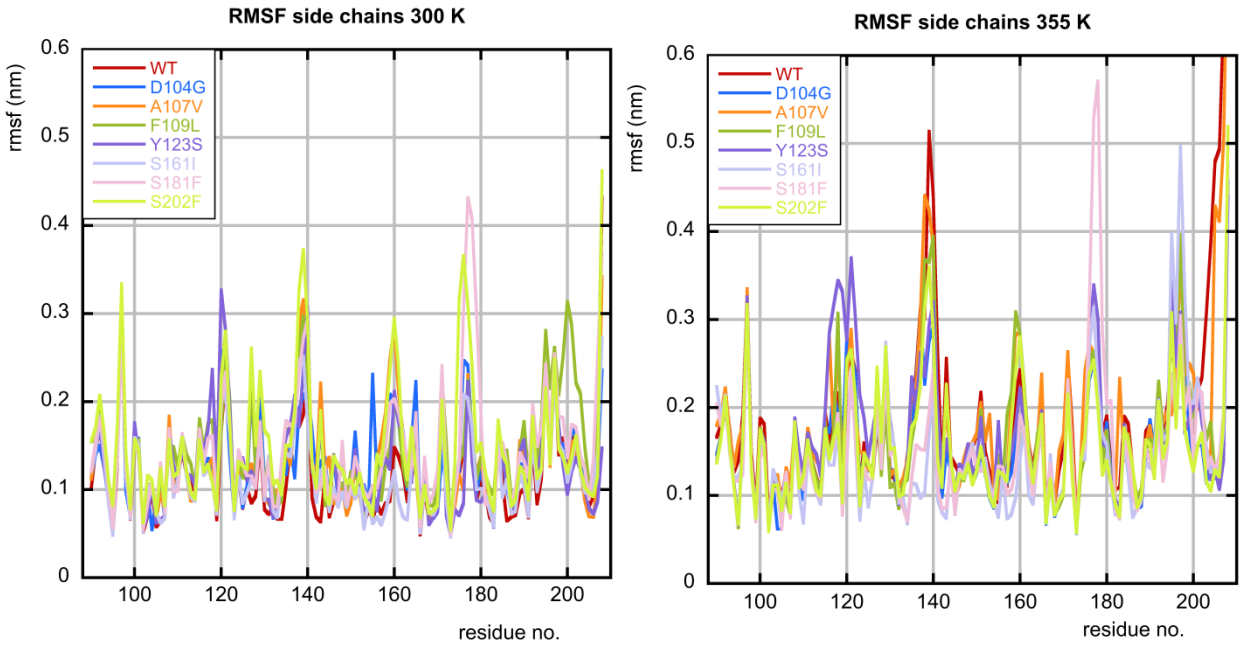



**Figure S4. Root mean square fluctuations.** Root mean square fluctuations (r.m.s.f.) computed for the side chains of FXN wild type and variants. The r.m.s.f. is computed in the last 50 ns (100 ns) of the simulations at 300 K (355 K).

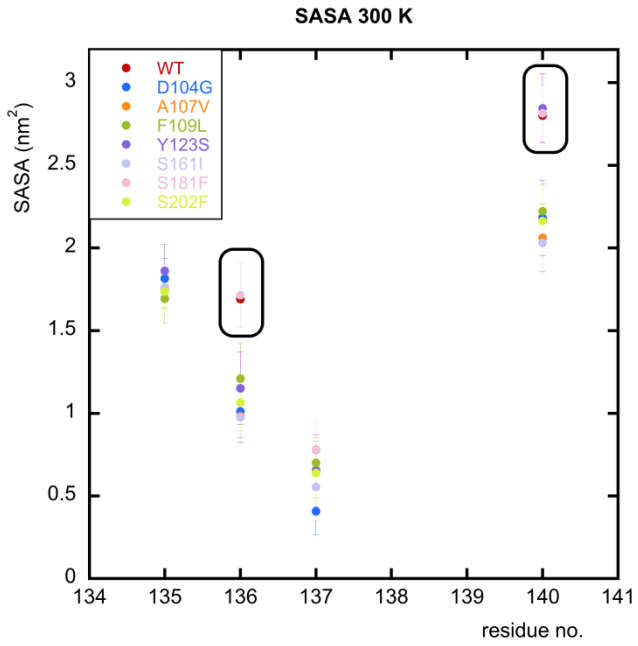
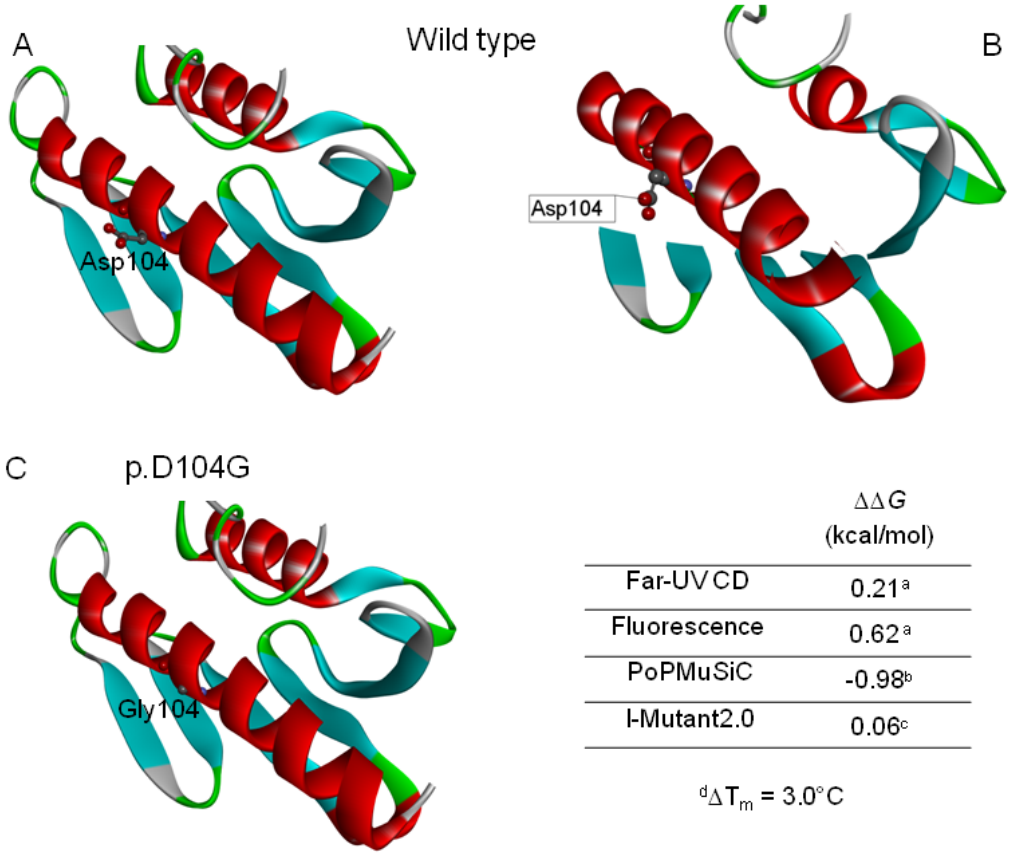



**Figure S5. Solvent accessible surface area.** Solvent accessible surface area (SASA) values for residues 135, 136, 137 and 140 (involved in the interaction between FXN and IscS) for FXN wild type and variants computed in the last 50 ns of the simulation at 300 K.




| | ΔΔG (kcal/mol) |
|---|---|
| Far-UV CD | 0.21[a] |
| Fluorescence | 0.62[a] |
| PoPMuSiC | -0.98[b] |
| I-Mutant2.0 | 0.06[c] |

[d]$\Delta T_m$ = 3.0°C

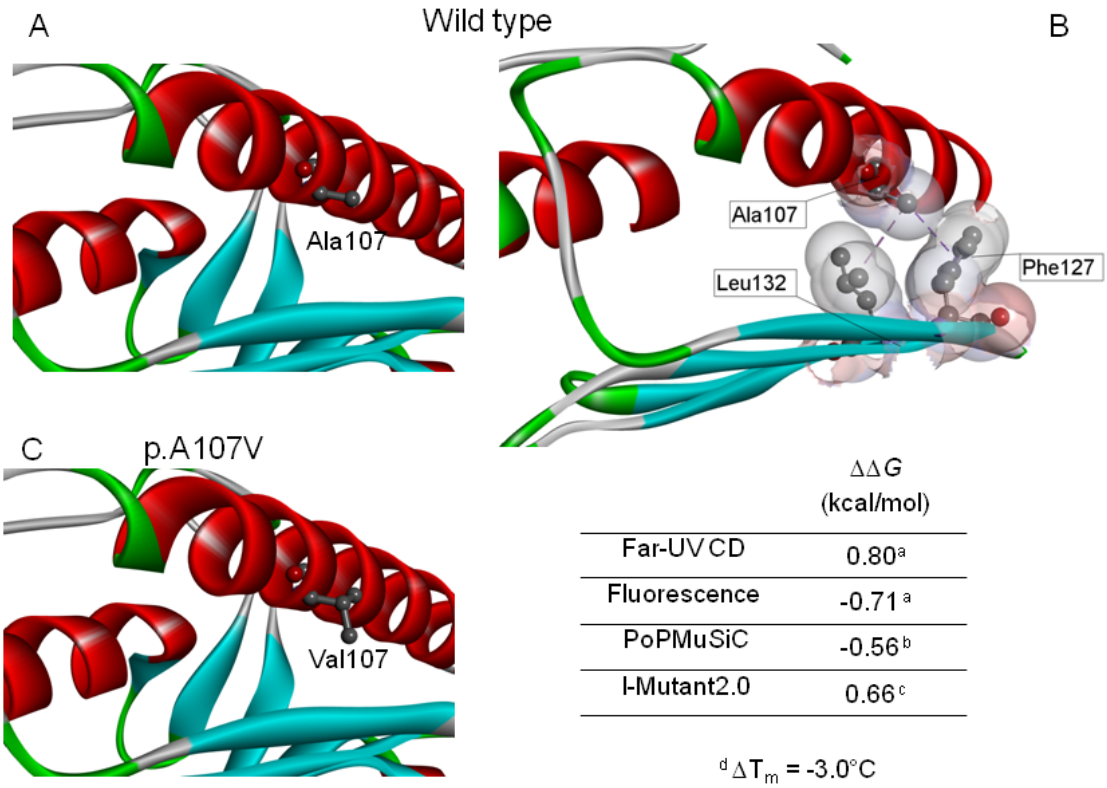
**Figure S6. FXN wild type and variant p.D104G.** (A) Location of residue Asp104 on FXN wild type structure (PDB: 1EKG) shown as a ribbon diagram. The residue Asp104 is depicted in ball and stick. (B) The local environment of residue Asp104, depicted in scaled ball and stick, does not show specific interactions with other residues. The figure was generated using BIOVIA Discovery Studio Visualizer 2016 (DS Visualizer 2016). (C) Replacement of the native residue Asp104 with the mutated residue Gly104, depicted in ball and stick, on the wild type structure using the "mutate protein" option of BIOVIA Discovery Studio Visualizer 2016.

[a]$\Delta\Delta G$ was calculated as $\Delta\Delta G = \Delta G^{H_2O}_{variant} - \Delta G^{H_2O}_{wild\ type}$; $\Delta G^{H_2O}_{variant}$ and $\Delta G^{H_2O}_{wild\ type}$ values for urea-induced unfolding equilibrium data were obtained from Eq (2) monitoring the far-UV CD and the fluorescence changes and are the same as those reported in Table 1 of the main manuscript.

[b]$\Delta\Delta G$ was computed from PoPMuSiC as $\Delta\Delta G = \Delta G_{variant} - \Delta G_{wild\ type}$

[c]$\Delta\Delta G$ was computed from I-Mutant2.0 as $\Delta\Delta G = \Delta G_{variant} - \Delta G_{wild\ type}$

[d]$\Delta T_m$ was calculated as $\Delta T_m = T_{m\ variant} - T_{m\ wild\ type}$ ; $T_{m\ variant}$ and $T_{m\ wild\ type}$ are the same as those reported in Table 1 of the main manuscript




| | ΔΔG (kcal/mol) |
|---|---|
| Far-UV CD | 0.80[a] |
| Fluorescence | -0.71[a] |
| PoPMuSiC | -0.56[b] |
| I-Mutant2.0 | 0.66[c] |

[d] $\Delta T_m$ = -3.0°C

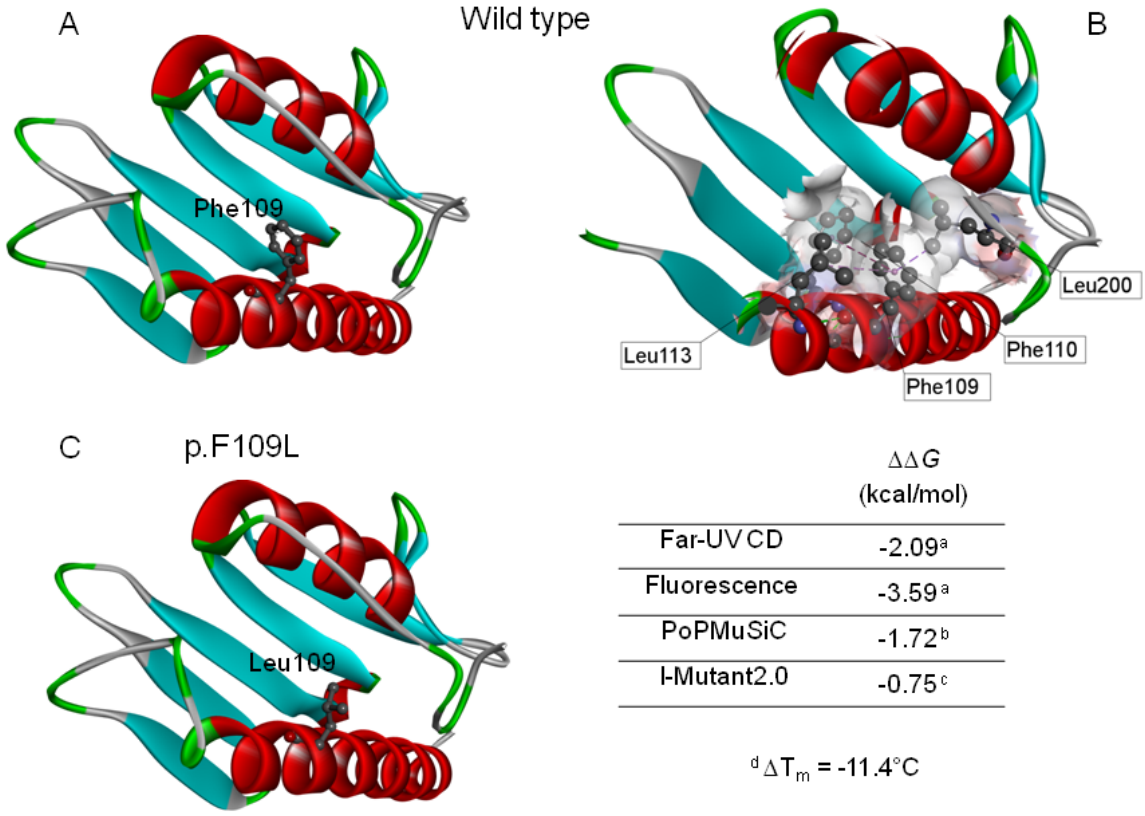
**Figure S7. FXN wild type and variant p.A107V.** (A) Location of residue Ala107 on FXN wild type structure (PDB: 1EKG) shown as a ribbon diagram. The residue Ala107 is depicted in ball and stick. (B) Local environment of residue Ala107 involved in hydrophobic interactions (dashed purple lines) with Phe127 and Leu132, shown as van der Waals spheres. The residues are depicted in scaled ball and stick. The figure was generated using BIOVIA Discovery Studio Visualizer 2016 (DS Visualizer 2016). (C) Replacement of the native residue Ala107 with the mutated residue Val107, depicted in ball and stick, on the wild type structure using the "mutate protein" option of BIOVIA Discovery Studio Visualizer 2016.

[a] $\Delta\Delta G$ was calculated as $\Delta\Delta G = \Delta G^{H_2O}_{variant} - \Delta G^{H_2O}_{wild\ type}$; $\Delta G^{H_2O}_{variant}$ and $\Delta G^{H_2O}_{wild\ type}$ values for urea-induced unfolding equilibrium data were obtained from Eq (2) monitoring the far-UV CD and the fluorescence changes and are the same as those reported in Table 1 of the main manuscript.

[b] $\Delta\Delta G$ was computed from PoPMuSiC as $\Delta\Delta G = \Delta G_{variant} - \Delta G_{wild\ type}$

[c] $\Delta\Delta G$ was computed from I-Mutant2.0 as $\Delta\Delta G = \Delta G_{variant} - \Delta G_{wild\ type}$

[d] $\Delta T_m$ was calculated as $\Delta T_m = T_{m\ variant} - T_{m\ wild\ type}$ ; $T_{m\ variant}$ and $T_{m\ wild\ type}$ are the same as those reported in Table 1 of the main manuscript.




| | ΔΔG (kcal/mol) |
|---|---|
| Far-UV CD | -2.09[a] |
| Fluorescence | -3.59[a] |
| PoPMuSiC | -1.72[b] |
| I-Mutant2.0 | -0.75[c] |

[d] $\Delta T_m$ = -11.4°C

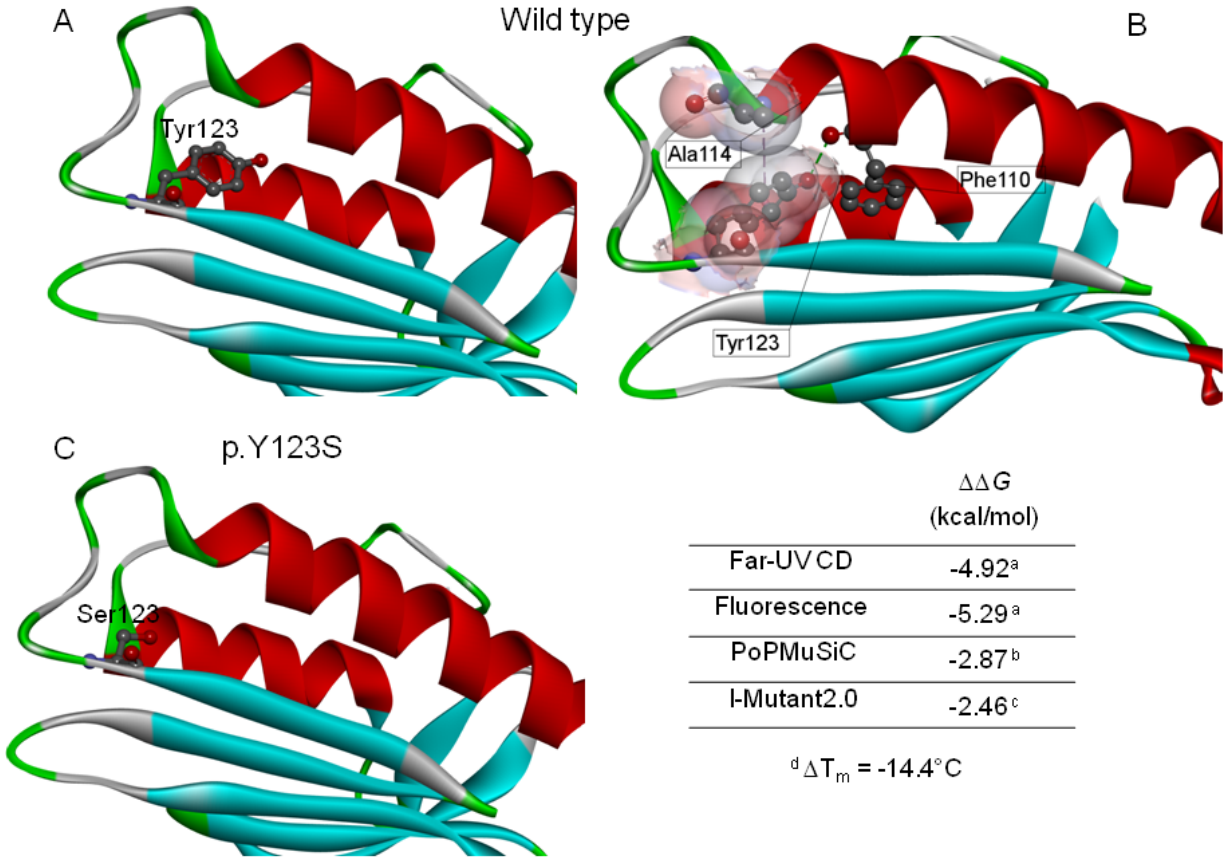
**Figure S8. FXN wild type and variant p.F109L.** (A) Location of residue Phe109 on FXN wild type structure (PDB: 1EKG) shown as a ribbon diagram. The residue F109 is depicted in ball and stick. (B) Local environment of residue Phe109 involved in a network of hydrophobic interactions (dashed purple lines) with Phe110, Leu113 and Leu200. The residues are depicted in scaled ball and stick. Interactions are shown as van der Waals spheres. The figure was generated using BIOVIA Discovery Studio Visualizer 2016 (DS Visualizer 2016). (C) Replacement of the native residue Phe109, depicted in ball and stick. with the mutated residue Leu109 on the wild type structure using the "mutate protein" option of BIOVIA Discovery Studio Visualizer 2016.

[a] $\Delta\Delta G$ was calculated as $\Delta\Delta G = \Delta G^{H_2O}_{variant} - \Delta G^{H_2O}_{wild\ type}$; $\Delta G^{H_2O}_{variant}$ and $\Delta G^{H_2O}_{wild\ type}$ values for urea-induced unfolding equilibrium data were obtained from Eq (2) monitoring the far-UV CD and the fluorescence changes and are the same as those reported in Table 1 of the main manuscript.

[b] $\Delta\Delta G$ was computed from PoPMuSiC as $\Delta\Delta G = \Delta G_{variant} - \Delta G_{wild\ type}$

[c] $\Delta\Delta G$ was computed from I-Mutant2.0 as $\Delta\Delta G = \Delta G_{variant} - \Delta G_{wild\ type}$

[d] $\Delta T_m$ was calculated as $\Delta T_m = T_{m\ variant} - T_{m\ wild\ type}$ ; $T_{m\ variant}$ and $T_{m\ wild\ type}$ are the same as those reported in Table 1 of the main manuscript




| | ΔΔG (kcal/mol) |
|---|---|
| Far-UV CD | -4.92[a] |
| Fluorescence | -5.29[a] |
| PoPMuSiC | -2.87[b] |
| I-Mutant2.0 | -2.46[c] |

[d] $\Delta T_m$ = -14.4°C

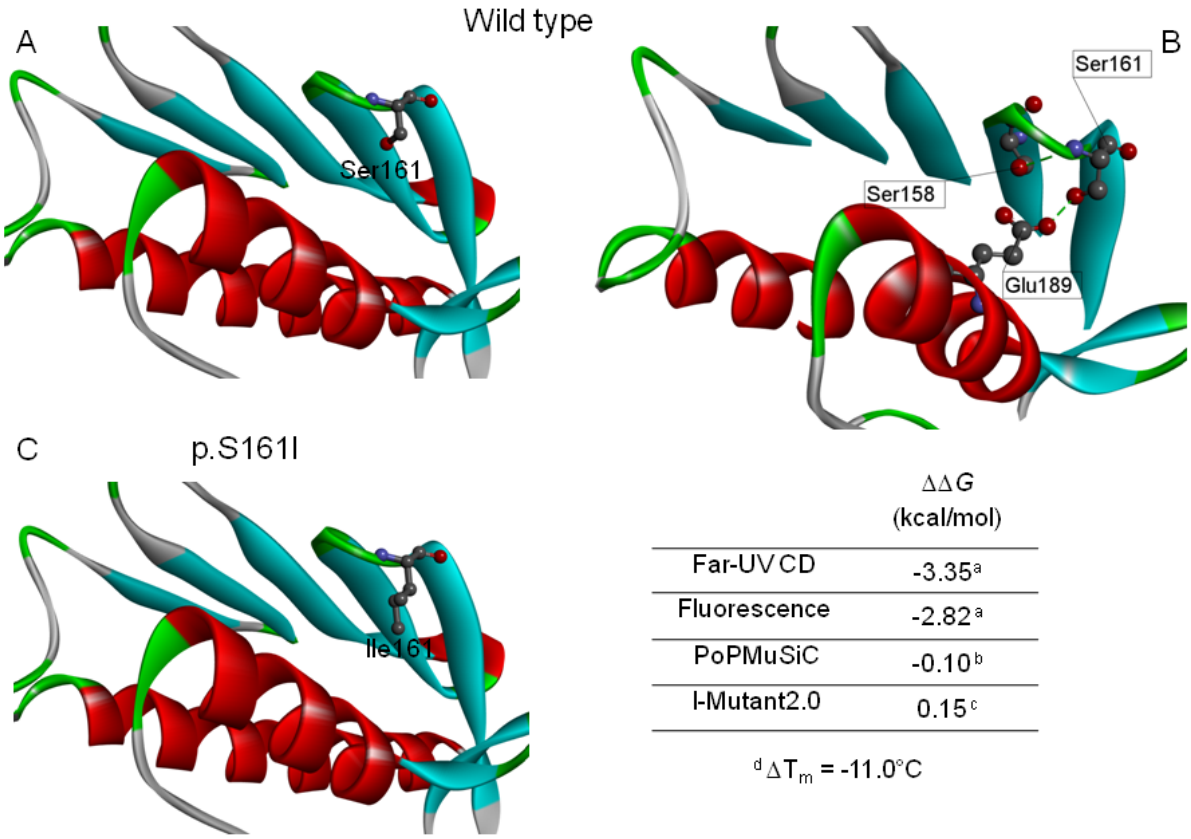
**Figure S9. FXN wild type and variant p.Y123S.** (A) Location of residue Tyr123 on FXN wild type structure (PDB: 1EKG) shown as a ribbon diagram. The residue Tyr123 is depicted in ball and stick. (B) Local environment of residue Tyr123, depicted in scaled ball and stick, involved in a hydrophobic interactions (dashed purple lines) with Ala114, shown as van der Waals spheres, and hydrogen bonded to Phe110 (dashed green line). The residues are depicted in scaled ball and stick.The figure was generated using BIOVIA Discovery Studio Visualizer 2016 (DS Visualizer 2016). (C) Replacement of the native residue Tyr123 with the mutated residue Ser123, depicted in ball and stick on the wild type structure using the "mutate protein" option of BIOVIA Discovery Studio Visualizer 2016.

[a] $\Delta\Delta G$ was calculated as $\Delta\Delta G = \Delta G^{H_2O}_{variant} - \Delta G^{H_2O}_{wild\ type}$; $\Delta G^{H_2O}_{variant}$ and $\Delta G^{H_2O}_{wild\ type}$ values for urea-induced unfolding equilibrium data were obtained from Eq (2) monitoring the far-UV CD and the fluorescence changes and are the same as those reported in Table 1 of the main manuscript.

[b] $\Delta\Delta G$ was computed from PoPMuSiC as $\Delta\Delta G = \Delta G_{variant} - \Delta G_{wild\ type}$

[c] $\Delta\Delta G$ was computed from I-Mutant2.0 as $\Delta\Delta G = \Delta G_{variant} - \Delta G_{wild\ type}$

[d] $\Delta T_m$ was calculated as $\Delta T_m = T_{m\ variant} - T_{m\ wild\ type}$ ; $T_{m\ variant}$ and $T_{m\ wild\ type}$ are the same as those reported in Table 1 of the main manuscript




| | ΔΔG (kcal/mol) |
|---|---|
| Far-UV CD | -3.35[a] |
| Fluorescence | -2.82[a] |
| PoPMuSiC | -0.10[b] |
| I-Mutant2.0 | 0.15[c] |

[d] $\Delta T_m$ = -11.0°C

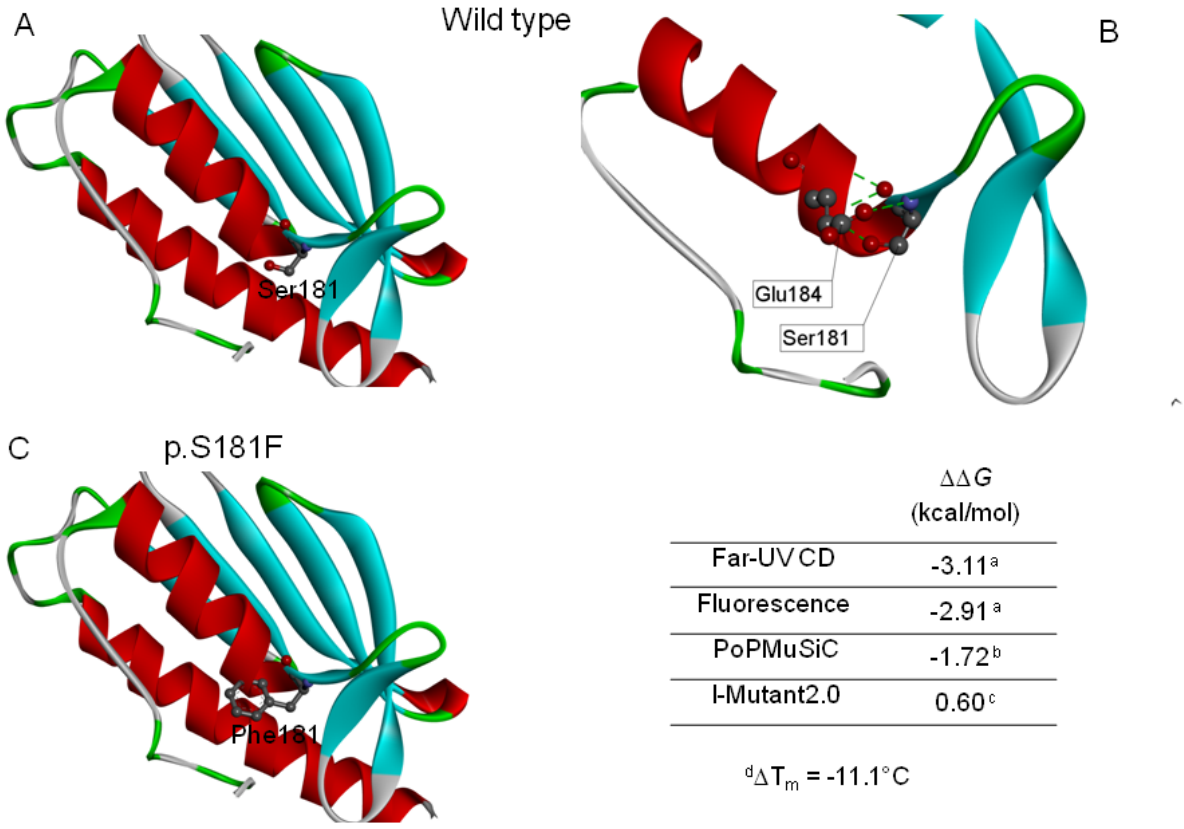
**Figure S10. FXN wild type and variant p.S161I.** (A) Location of residue Ser161 on FXN wild type structure (PDB: 1EKG) shown as a ribbon diagram. The residue Ser161 is depicted in ball and stick. (B) The local environment of residue Ser161 involved in a hydrogen bond with Glu189 and Ser158 (dashed green lines). The residues are depicted in scaled ball and stick. The figure was generated using BIOVIA Discovery Studio Visualizer 2016 (DS Visualizer 2016). (C) Replacement of the native residue Ser161 with the mutated residue Ile161, depicted in ball and stick, on the wild type structure using the "mutate protein" option of BIOVIA Discovery Studio Visualizer 2016.

[a] $\Delta\Delta G$ was calculated as $\Delta\Delta G = \Delta G^{H_2O}_{variant} - \Delta G^{H_2O}_{wild\ type}$; $\Delta G^{H_2O}_{variant}$ and $\Delta G^{H_2O}_{wild\ type}$ values for urea-induced unfolding equilibrium data were obtained from Eq (2) monitoring the far-UV CD and the fluorescence changes and are the same as those reported in Table 1 of the main manuscript.

[b] $\Delta\Delta G$ was computed from PoPMuSiC as $\Delta\Delta G = \Delta G_{variant} - \Delta G_{wild\ type}$

[c] $\Delta\Delta G$ was computed from I-Mutant2.0 as $\Delta\Delta G = \Delta G_{variant} - \Delta G_{wild\ type}$

[d] $\Delta T_m$ was calculated as $\Delta T_m = T_{m\ variant} - T_{m\ wild\ type}$ ; $T_{m\ variant}$ and $T_{m\ wild\ type}$ are the same as those reported in Table 1 of the main manuscript




| | ΔΔG (kcal/mol) |
|---|---|
| Far-UV CD | -3.11[a] |
| Fluorescence | -2.91[a] |
| PoPMuSiC | -1.72[b] |
| I-Mutant2.0 | 0.60[c] |

[d]$\Delta T_m$ = -11.1°C

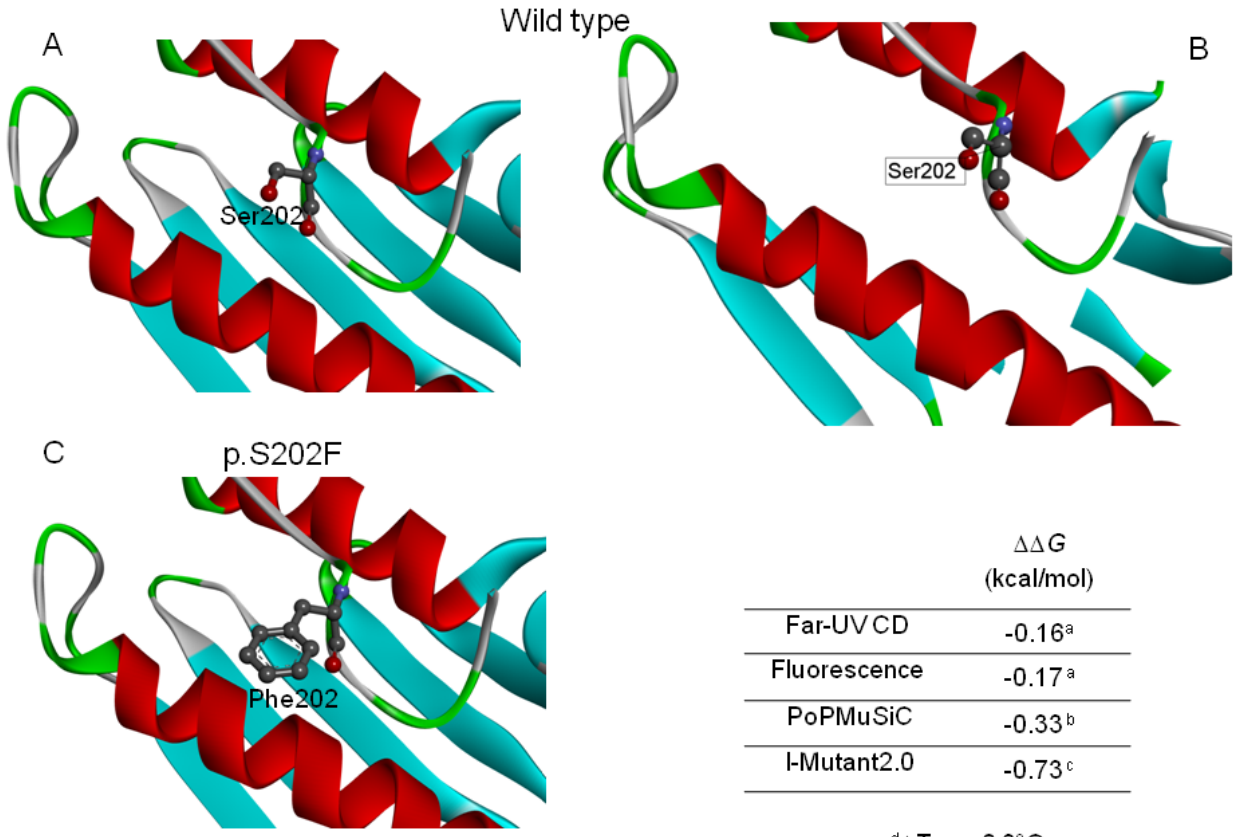
**Figure S11. FXN wild type and variant p.SI81F.** (A) Location of residue Ser181 on FXN wild type structure (PDB: 1EKG) shown as a ribbon diagram. The residue Ser181 is depicted in ball and stick. (B) The local environment of residue Ser181 involved in a network of hydrogen bond with Glu184. The residues are depicted in scaled ball and stick. The figure was generated using BIOVIA Discovery Studio Visualizer 2016 (DS Visualizer 2016). (C) Replacement of the native residue Ser181 with the mutated residue Phe181, depicted in ball and stick, on the wild type structure using the "mutate protein" option of BIOVIA Discovery Studio Visualizer 2016.

[a]$\Delta\Delta G$ was calculated as $\Delta\Delta G = \Delta G^{H_2O}_{variant} - \Delta G^{H_2O}_{wild\ type}$; $\Delta G^{H_2O}_{variant}$ and $\Delta G^{H_2O}_{wild\ type}$ values for urea-induced unfolding equilibrium data were obtained from Eq (2) monitoring the far-UV CD and the fluorescence changes and are the same as those reported in Table 1 of the main manuscript.

[b]$\Delta\Delta G$ was computed from PoPMuSiC as $\Delta\Delta G = \Delta G_{variant} - \Delta G_{wild\ type}$

[c]$\Delta\Delta G$ was computed from I-Mutant2.0 as $\Delta\Delta G = \Delta G_{variant} - \Delta G_{wild\ type}$

[d]$\Delta T_m$ was calculated as $\Delta T_m = T_{m\ variant} - T_{m\ wild\ type}$ ; $T_{m\ variant}$ and $T_{m\ wild\ type}$ are the same as those reported in Table 1 of the main manuscript.




| | ΔΔG (kcal/mol) |
|---|---|
| Far-UV CD | -0.16[a] |
| Fluorescence | -0.17[a] |
| PoPMuSiC | -0.33[b] |
| I-Mutant2.0 | -0.73[c] |

[d]$\Delta T_m$ = -0.3°C

**Figure S12. FXN wild type and variant p.S202F.** (A) Location of residue Ser202 on FXN wild type structure (PDB: 1EKG) shown as a ribbon diagram. The residue Ser202 is depicted in ball and stick. (B) The local environment of residue Ser202, depicted in scaled ball and stick, does not show specific interactions with other residues. The figure was generated using BIOVIA Discovery Studio Visualizer 2016 (DS Visualizer 2016). (C) Replacement of the native residue Ser181 with the mutated residue Phe202, depicted in ball and stick, on the wild type structure using the "mutate protein" option of BIOVIA Discovery Studio Visualizer 2016.

[a]$\Delta\Delta G$ was calculated as $\Delta\Delta G = \Delta G^{H_2O}_{variant} - \Delta G^{H_2O}_{wild\ type}$; $\Delta G^{H_2O}_{variant}$ and $\Delta G^{H_2O}_{wild\ type}$ values for urea-induced unfolding equilibrium data were obtained from Eq (2) monitoring the far-UV CD and the fluorescence changes and are the same as those reported in Table 1 of the main manuscript.

[b]$\Delta\Delta G$ was computed from PoPMuSiC as $\Delta\Delta G = \Delta G_{variant} - \Delta G_{wild\ type}$

[c]$\Delta\Delta G$ was computed from I-Mutant2.0 as $\Delta\Delta G = \Delta G_{variant} - \Delta G_{wild\ type}$

[d]$\Delta T_m$ was calculated as $\Delta T_m = T_{m\ variant} - T_{m\ wild\ type}$; $T_{m\ variant}$ and $T_{m\ wild\ type}$ are the same as those reported in Table 1 of the main manuscript.

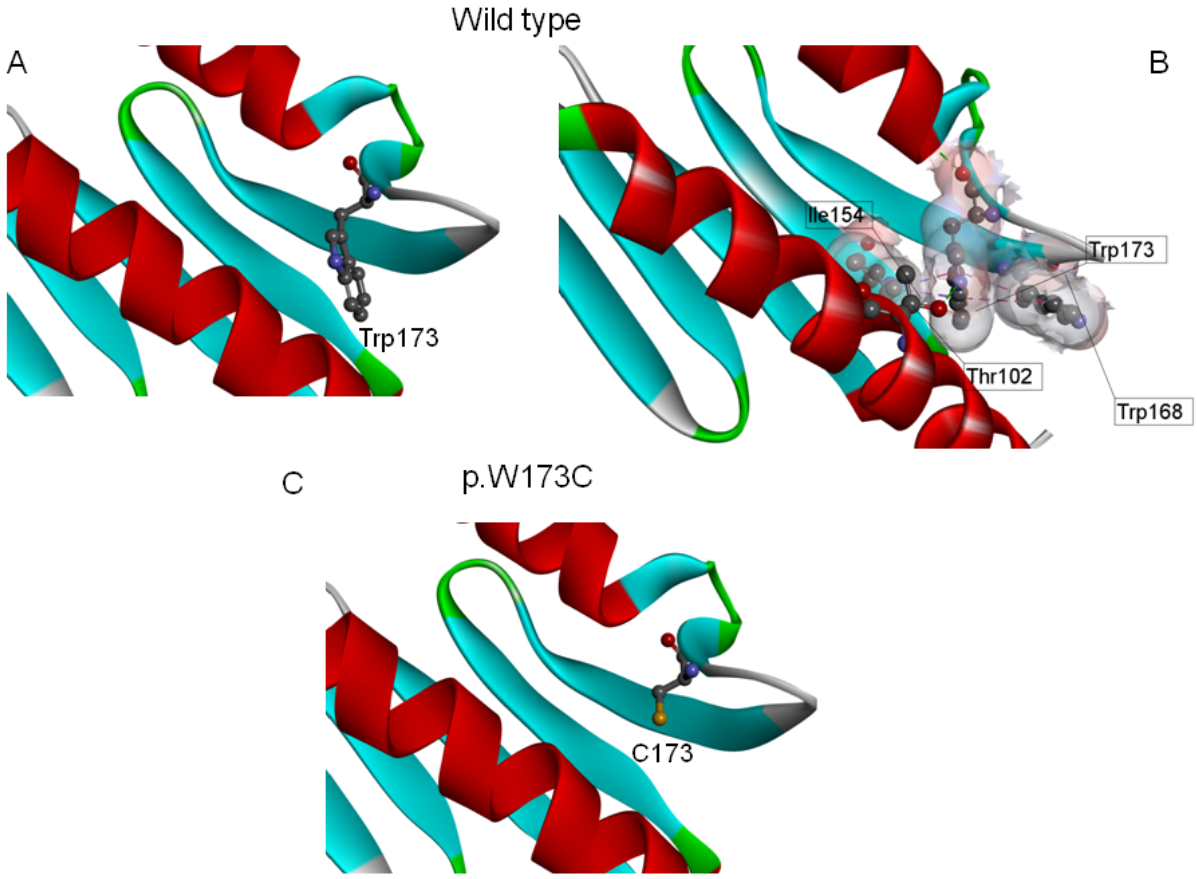



**Figure S13. FXN wild type and variant p.W173C.** (A) Location of residue Trp173 on FXN wild type structure (PDB: 1EKG) shown as a ribbon diagram. The residue Trp173 is depicted in ball and stick. (B) Local environment of residue Trp173, depicted in scaled ball and stick, involved in a network of hydrophobic interactions (dashed purple lines) with Ile154 and Trp168, shown as van der Waals spheres, and hydrogen bonded to Thr102. The figure was generated using BIOVIA Discovery Studio Visualizer 2016 (DS Visualizer 2016). (C) Replacement of the native residue Trp173 with the mutated residue Cys173, depicted in ball and stick, on the wild type structure using the "mutate protein" option of BIOVIA Discovery Studio Visualizer 2016.

[a] $\Delta\Delta G$ was calculated as $\Delta\Delta G = \Delta G^{H_2O}_{variant} - \Delta G^{H_2O}_{wild\ type}$; $\Delta G^{H_2O}_{variant}$ and $\Delta G^{H_2O}_{wild\ type}$ values for urea-induced unfolding equilibrium data were obtained from Eq (2) monitoring the far-UV CD and the fluorescence changes and are the same as those reported in Table 1 of the main manuscript.

[b] $\Delta\Delta G$ was computed from PoPMuSiC as $\Delta\Delta G = \Delta G_{variant} - \Delta G_{wild\ type}$

[c] $\Delta\Delta G$ was computed from I-Mutant2.0 as $\Delta\Delta G = \Delta G_{variant} - \Delta G_{wild\ type}$

[d] $\Delta T_m$ was calculated as $\Delta T_m = T_{m\ variant} - T_{m\ wild\ type}$ ; $T_{m\ variant}$ and $T_{m\ wild\ type}$ are the same as those reported in Table 1 of the main manuscript.

**Table S1. List of oligonucleotides used for site-directed mutagenesis (protein seq. ref. NP-_000135.2)**

| | |
|---|---|
| p.F109L FW | AGCCTTGCTGAGCTCTTTGAGGATCTT |
| p.F109L REV | AAGATCCTCAAAGAGCTCAGCAAGGCT |
| p.Y123S FW | ACCTTTGAGGACTCCGATGTGTCCTTT |
| p.Y123S REV | AAAGGACACATCGGAGTCCTCAAAGGT |
| p.S161I FW | GTTCACCTAGCATTGGCCCAAAAC |
| p.S161I REV | GTTTTGGGCCAATGCTAGGTGAAC |
| p.S181F FW | GATGGCGTATTCTTGCATGAACTG |
| p.S181F REV | CAGTTCATGCAAGAATACGCCATC |
| p.S202F FW | TGGATCTGAGCTTTCTCGCGTATT |
| p.S202F REV | AATACGCGAGAAAGCTCAGATCCA |

**Table S2. Difference in free energy changes of FXN missense variants measured by far-UV CD and fluorescence spectroscopy and computed by PoPMuSiC and I-Mutant2.0.**

| Protein Variant | [a]$\Delta\Delta G$ (kcal/mol) (far-UV CD ) | [a]$\Delta\Delta G$ (kcal/mol) (Fluorescence) | [b]$\Delta\Delta$G (kcal/mol) (PoPMuSiC) | [c]$\Delta\Delta$G (kcal/mol) (I-Mutant2.0) |
|---|---|---|---|---|
| p.D104G | 0.21 | 0.62 | -0.98 | 0.06 |
| p.A107V | 0.80 | -0.71 | -0.56 | 0.66 |
| p.F109L | -2.09 | -3.59 | -1.72 | -0.75 |
| p.Y123S | -4.92 | -5.29 | -2.87 | -2.46 |
| p.S161I | -3.35 | -2.82 | -0.10 | 0.15 |
| p.S181F | -3.11 | -2.91 | -1.72 | 0.60 |
| p.S202F | -0.16 | -0.17 | -0.33 | -0.73 |

[a] $\Delta\Delta G$ was calculated as $\Delta\Delta G = \Delta G^{H_2O}_{variant} - \Delta G^{H_2O}_{wild\ type}$; $\Delta G^{H_2O}$ was obtained from the analysis of the far-UV CD and fluorescence equilibrium unfolding transitions and calculated according to Eq (2), as reported in the main manuscript. The standard error was 5-10%. [b,c] $\Delta\Delta G$ was computed from PoPMuSiC and I-Mutant2.0 and reported as $\Delta\Delta G = \Delta G_{variant} - \Delta G_{wild\ type}$.

A positive $\Delta\Delta G$ value indicates that the mutation is stabilizing, a negative one indicates that the mutation is destabilizing.