Supplementary Information

Extra-mitochondrial mouse frataxin and its implications for mouse models of Friedreich's ataxia

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Supplementary Figures



Supplementary Fig. 1: UHPLC-PRM/MS analysis of the N-terminal peptide from human mature SILAC frataxin isolated by IP using two different antibodies non-covalently bound to protein G magnetic beads. (A) Abcam anti-frataxin rabbit pAb 175402. (B) Abcam anti-frataxin mouse mAb 113691. The recovery determined from mean of the ratios of the major PRM signals for S⁷⁸GTLGHPGSLDETTYER⁷⁹ from human SILAC-mature frataxin (81-210) isolated using rabbit pAb 175402 was 10 % compared with S⁷⁸GTLGHPGSLDETTYER⁷⁹ from human SILAC-mature frataxin (81-210) isolated using mouse mAb 113691. L= [¹³C₆¹⁵N₁]-leucine.



Supplementary Fig. 2: Product ion spectra of N-terminal tryptic peptides from mouse mature frataxin isolated from mouse liver. (A) L⁷⁸GTLDNPSSLDETAYER⁹⁴. (B) G⁷⁹TLDNPSSLDETAYER⁹⁴ (C). L⁸¹DNPSSLDETAYER⁹⁴.



Supplementary Fig. 3: Markers of sub-cellular fractionation of mouse liver tissues (n=2) visualized by western blot with cytoplasmic, mitochondrial, and nuclear fractions indicated. (A) FASN cytosolic protein (200 kDa). (B) Histone H3 and H4 (17 kDa and 11 kDa respectively). (C) VDAC mitochondrial membrane protein (31 kDa) using the stripped blot shown in Panel (A).

SGTLGHPGS 50 LDETTYER ³⁺	(A) RT = 21.2 y ₁₁ + = 100 %	NL: 1.38E5 m/z = 607.2867 → 1274.5948-1274.5998
GTLGHPGS 50 LDETTYER ³⁺ N-1	RT = 21.2 y ₁₁ ⁺ = 0 %	NL: 0 m/z = 578.2761 → 1274.5948-1274.5998
eg 50 DETTYER ³⁺	RT = 21.2 $y_{11}^+ = 0 \%$	NL: 0 m/z = 563.9470 → 1274.5948-1274.5998
LGHPGSL DETTYER ³⁺ 9 0	RT = 20.4 y ₁₁ ⁺ = 0 %	NL: 0 m/z = 525.5864 → 1274.5948-1274.5998
GHPGSLD 50 ETTYER ³⁺	RT = 19.6 y ₈ ⁺ = 0 %	NL: 0 m/z = 487.8917 → 1033.4807-1033.5013
100 50 ETTYER ³⁺ N-5	RT = 19.6 $y_8^+ = 0\%$	NL: 0 m/z = 471.2236 → 1033.4807-1033.5013
100 PGSLDE 50 TTYER ²⁺ N-6	RT = 19.4 y ₈ ⁺ = 0 %	NL: 0 m/z = 637.8023 → 1033.4807-1033.5013
10 15	20 25 30 Time (min)	35 40

Supplementary Fig. 4: UHPLC-PRM/HRMS analysis of potential truncated peptides from human SILAC-frataxin internal standard (25 ng) added to mouse heart tissue. Red arrows denote the retention times of authentic peptide standards. $L = [{}^{13}C_{6}{}^{15}N_{1}]$ -leucine.

ative Abundance	SGTLGHPGS 50 LDETTYER ³⁺	(B) $RT = 21.3$ $y_{11}^+ ratio = 5.5$ <u>98.4</u> %	NL: 7.80E5 m/z = 607.2867 1267.5674-1267.5928
	SGTLGHPG 50 N (Internal stand	RT = 21.3 y ₁₁ +	NL: 1.43E5 m/z = 611.9649 1274.5846-1274.6100
	GTLGHPGS 50 LDETTYER ³⁺ N-1	RT = 20.7 y ₁₁ = 0.1 %	NL: 2.43E3 m/z = 578.2761 → 1267.5776-1267.5826
	100 50 DETTYER ³⁺ N-2	RT = 20.7 y ₁₁ = 1.5 %	NL: 1.22E4 m/z = 563.9470 → 1267.5776-1267.5826
	100 50 DETTYER ³⁺	RT = 20.4 y ₁₁ * = 0 %	NL: 0 <i>m</i> /z = 525.5864 → 1267.5776-1267.5826
Re	100 50 ETTYER ³⁺	RT = 19.6 y ₈ * = 0 %	NL: 0 <i>m</i> / <i>z</i> = 487.8917 1033.4807-1033.5013
100 50	100 50 HPGSLDE 50 TTYER ³⁺ N-5	RT = 19.6 y ₈ * = 0 %	NL: 0 <i>m/z</i> = 468.8845 → 1033.4807-1033.5013
	100 50 N-6	RT = 19.5 y ₈ ⁺ = 0 %	NL: 0 m/z = 636.3672 → 1033.4807-1033.5013
	16 18	20 22 24 Time (min)	26

Supplementary Fig. 5: UHPLC-PRM/MS analysis of potential truncated peptides from human frataxin. Human SILAC-frataxin internal standard (25 ng) added to mouse heart tissue. Red arrows denote the retention times of authentic peptide standards. $L = [{}^{13}C_{6}{}^{15}N_{1}]$ -leucine.

$\frac{100}{50} H^{74}LRNLGTLDNPSSLDE^{2+} MH_2^{2+} = 0 \%$	NL: 0 <i>m/z</i> = 890.9421-890.9475	
$ \begin{array}{c} 100\\ 50\\ 0\\ 0\\ 0\\ 0\\ 0\\ 0\\ 0\\ 0\\ 0\\ 0\\ 0\\ 0\\ 0$	NL: 0 <i>m/z</i> = 822.4129-822.4179	
100 R ⁷⁶ NLGTLDNPSSLDE2+ 50 MH ₂ + = 0%	NL: 0 <i>m/z</i> = 765.8711-765.8757	
	NL: 0 <i>m/z</i> = 687.8207-687.8249	
$\begin{array}{c} & & \\$	NL: 7.90E5 <i>m/z</i> = 630.7994-630.8032	
$\begin{array}{c} 10 \\ \hline 10 \\ \hline 2 \\ \hline 3 \\ \hline 5 \\ 0 \\ \hline 1 \\ \hline 3 \\ \hline$	NL: 5.47E6 <i>m/z</i> = 574.2576-574.2610	
$100 = T^{80}LDNPSSLDE^{2+}$ $50 = MH_2^{2+} = 1.2 \%$ 22.3	NL: 1.19E5 <i>m/z</i> = 545.7470-545.7502	
100 100 101 100 101 101 101 101	NL: 3.08E6 m/z= 495.2232-495.2262:	
20 21 22 23 24 Time (min	25 26 27)	

Supplementary Fig. 6: UHPLC-PRM/MS analysis of potential N-terminal Glu-C peptides from mouse liver frataxin.