SUPPLEMENTAL DATA

Structural Insights into the Catalytic Active Site and Activity of Human Nit2/ω-Amidase: Kinetic Assay and Molecular Dynamics Simulation [†]

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Supplemental Materials and Methods

Materials. KGM was synthesized according to the method of Krasnikov et al. (1,2). Succinamate, hydroxylamine HCl, dithiothreitol (DTT), sodium nitroprusside, and 3-phenylpropionitrile (3-PPN) were purchased from Sigma-Aldrich Company. The plasmid pQE70, obtained from the QIAexpress system of QIAGEN, was used for the expression of *hNit2* in bacteria M15[pREP4]. The Ni-NTA column was purchased from GE Healthcare.

Methods
Cloning and expression of hNit2 in the *E. coli* pQE70/pREP4 expression system. The human *Nit2* gene was amplified from pFLAG-Nit2 as described originally (3). The forward primer (N-terminal): CCGGCATGCATATGACCTCTTTCCGC and reverse primer (C-terminal):

GCAGATCTGGGCTTTTTCATCTCCGC were used for polymerase chain reactions (PCR). A *Sph*I restriction site was designed for the N terminal primer and a *Bgl*II restriction site was designed for the C-terminal primer, respectively. The PCR reaction, using proofreading pfx DNA polymerase (Gibco BRL, Inc. USA) for amplification of human *Nit2*, was performed in a Perkin-Elmer apparatus. The steps for PCR were as follows: 3 min of denaturation at 94 °C, 30 cycles for denaturation at 94 °C for 1 min, and 53 °C for 40 s (annealing), followed by polymerization reaction at 72 °C for 1 min. The amplified DNA fragment was digested with *Sph*I and *Bagl*II, and the insert was cloned into pQE70 pre-digested with the same restriction enzymes. The resulting plasmid was designated pQE70-hNit2. The plasmid pQE70-hNit2 was transformed into the M15[pREP4] *E. coli* bacterial strain.

Construction of the pQE70-hNit2 Δ116-128 clone (see Figs. S1-S3).
pQE70-hNit2 plasmid was used as template to construct hNit2 Δ116-128 clone. KAPA HiFi™ HotStart ReadyMix (Kapa Biosystems, KK2601) was used in PCR. Two PCR products 390 bp (PCR1) and 609 bp (PCR2) were obtained by primer F1: CCG G<u>GA ATT C</u>AT TAA AGA GGA GAA ATT; R1: AA<u>G GTA CCC</u> AGA TGG ATC TTT CTA TAC TT; and F2: TAA <u>GGT ACC</u> TCT AAA ACA TTG AGT CCG; R2: TA<u>G CTA GC</u>T TGG ATT CTC ACC AAT AAA, respectively (underlines are restriction sites). Primers are listed in Table S1. PCR conditions were: 95 ℃ for 2 min; 35 cycles of 98 ℃ for 20s, 58 ℃ for 15s, 72 ℃ for 15s; final was 72 ℃ for 1 min. After PCR, the amplified DNA was cleaned up with EasyPure kit (Bioman, PG-300). Restriction reactions were performed at 37 ℃ for 1hr. Vector pQE70, PCR product fragment 1, and PCR product fragment 2 were digested by *EcoR*I/*Nhe*I, *EcoR*I/*Kpn*I, and *KpnI/Nhe*I, respectively. Vector dephosphorylation was performed with Shrimp Alkaline Phosphatase (Promega, M820A) at 37 ℃ for 15 min. PCR fragment 1 and PCR fragment 2 were ligated first to be cloned in pQE70 vector. Vectors and insert DNA were separated in 1 % agarose gel electrophoresis and followed by extraction with buffer. DNA transformation was performed with ECOS™ competent cells (Yeastern Biotech, YE607-J), which were spread in LB/Amp plate evenly and incubated at 37 ℃ for 18hr. hNit2 Δ116-128 clone was selected and plasmid was purified by plasmid mini kit (Geneaid, PD300), and over expressed in *E.coli* M15 (pREP4) PD300. R1-checking primer: GAA ATG TAA TTT TTC CAG GAA CAT CAA TGT C was used for checking the deleted fragment.

Construction and expression of *hNit2* gene with point mutaion. Point mutation of *hNit2* gene was generated with QuikChange II site-directed mutagenesis kit (Stratagene, USA). Primers containing desired mutations are listed in Table S1.

The PfuUltra™ high-fidelity (HF) DNA polymerase was used for mutagenic primer-directed replication of both plasmid strands with the highest fidelity. A supercoiled double-stranded DNA (dsDNA) vector containing *hNit2* sequence is used as template in the mutagenesis reaction. After thermal cycling, the synthesized product was treated with *Dpn* I. The DNA containing each desired point mutation of *hNit2* was then transformed into XL1-Blue supercompetent cells. Each mutated *hNit2* was sub-cloned into pQE-70/M15 expression system for generating each mutated hNit2 protein. The DNA sequence of mutated *hNit2* gene in plasmid pQE70 was checked with ABI model 310 Sequencer.

Purification of wild type hNit2 and all mutant proteins and confirmation of proteins by LC/MS/MS (see Figs. S4-S6). A single colony of M15[pQE70-hNit2] was inoculated into 25 ml of LB broth containing 100 μg/ml of ampicillin and 25 μg/ml of kanamycin, and was incubated overnight at 37°C. hNit2 overexpression was induced with 2 mM of IPTG and maintained for 6 hours. The crude protein obtained from the periplasmic fraction was centrifuged ($14,000 \times g$, 4 °C, 30 min); the supernatant was collected and filtered through a 0.45 μm membrane filter. The filtrate was applied to a column (1 cm × 5 cm) containing Ni-NTA Sepharose gel at 25 °C, which was pre-equilibrated with 10 mM phosphate buffer (pH 7.4) containing 10 mM imidazole. After washing the affinity gel with 20 mM imidazole in 10 mM phosphate buffer (pH 7.4), the His$_6$-tagged hNit2 was eluted with 200-300 mM imidazole in 10 mM phosphate buffer (pH 7.4). hNit2 protein in the eluate was detected by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) analysis using 12% acrylamide gels. Fractions containing the desired protein were pooled and subsequently desalted and concentrated using an Amicon Ultra-15 Centrifugal Filter Unit (Millipore, Massachusetts, USA) with a molecular mass cut-off of 10 kDa. A typical yield of hNit2 was ～3.5 mg.

The purified hNit2 protein was subjected SDS-PAGE analysis using a 12% acrylamide gel and stained with Coomassie Brilliant blue. The gel band corresponding to hNit2 was excised and washed with deionized water, followed by trypsin digestion. Electrospray mass spectrometry (MS) was performed using a Finnigan LTQ Qrbitrap hybrid mass spectrometer for identification of trypsin-digested hNit2 fragments.
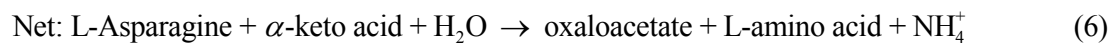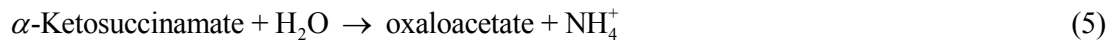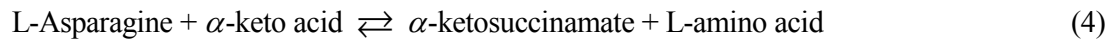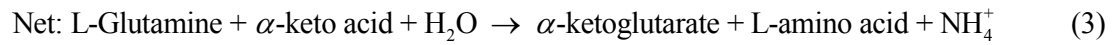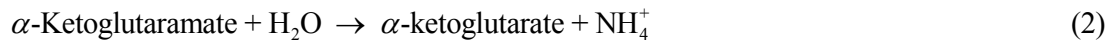
ω-Amidase Assay Method 1 – Hydrolysis of KGM. hNit2-catalyzed hydrolysis of KGM to α-ketoglutarate was determined by measuring α-ketoglutarate as its 2,4-dinitrophenylhydrazone (2,3). The enzymatic assay mixture contained 5 mM KGM, 5 mM DTT, 100 mM Tris-HCl buffer (pH 8.5) and 1 μg of purified enzyme in a final volume of 0.02 ml. After incubation at 37 ℃ for three minutes, the reaction was terminated by addition of 0.01 ml of 5 mM 2,4-dinitrophenylhydrazine in 2 M HCl. After a further incubation for 5 min at 37 ℃, 0.17 ml of NaOH was added, and the absorbance was read at 430 nm within 5 min. The ε_{430nm} value of α-ketoglutarate 2,4-dinitrophenylhydrazone is 16×10^3 $M^{-1}cm^{-1}$. The blank contained complete reaction mixture (but no enzyme) carried through the same procedure.

ω-Amidase Assay Method 2 – Hydroxaminolysis of Succinamate. This method relies on the fact that hydroxylamine competes affectively with water as the attacking nucleophile in several ω-amidase-catalyzed reactions (4,5). The hydroxaminolysis assay was carried out as described by Krasnikov et al. (2). The assay mixture containing 0-120 mM of SM and 1μg of purified enzyme was in 100 mM potassium phosphate buffer, pH 7.2 and was incubated for 3 minutes at 37 ℃. Ferric ion solution was added to form brow succinyl hydroxamate ferric complex. The absorbance of succinyl hydroxamate ferric complex was determined at 535 nm (ε_{535nm} 920 $M^{-1}cm^{-1}$)(2). The blank contained complete reaction mixture (but no enzyme) carried through the same procedure.

ω-Amidase Assay Method 3 – Quantitation of Released Ammonia (Nitrilase Activity). Enzyme preparations were desalted by means of PD-10 columns (Amersham Pharmacia Biotech) to remove ammonium ion present in the bacterial lysates. We chose 3-PPN as a model nitrile and used a sensitive ammonia assay [the Bertholet reaction (6)]. The assay mixture (0.14 ml), containing 10 mM 3-PPN, 10 μg of purified enzyme and 0.05 M potassium phosphate buffer, pH 8.0, was incubated at 37 °C for 30 minutes. The Bertholet ammonia reaction was performed and the absorbance was read at 640 nm by an ELISA reader. The blank consisted of complete reaction mixture plus a boiled enzyme solution. Each experiment was calibrated with NH_4Cl solutions of known concentrations.

The specific activity of purified enzyme was about 25 U/mg protein under standard assay conditions (assay method 1), where one unit (U) of hNit2 enzyme activity is defined as the amount of enzyme required to liberate 1 μmol of α-ketoglutarate per minute from KGM at 37 °C. Protein concentration was measured by the Bradford procedure with bovine serum albumin as a standard. Kinetic parameters were performed by nonlinear least squares regression analysis using the SigmaPlot enzyme kinetic module 1.3 program.

Equation S1: Transamination reactions of glutamine and asparagine coupled to hydrolysis reactions catalyzed by ω-amidase.

L-Glutamine + α-keto acid $\rightleftarrows$ α-ketoglutaramate + L-amino acid (1)

α-Ketoglutaramate + H_2O $\rightarrow$ α-ketoglutarate + NH_4^+ (2)

Net: L-Glutamine + α-keto acid + H_2O $\rightarrow$ α-ketoglutarate + L-amino acid + NH_4^+ (3)

L-Asparagine + α-keto acid $\rightleftarrows$ α-ketosuccinamate + L-amino acid (4)

α-Ketosuccinamate + H_2O $\rightarrow$ oxaloacetate + NH_4^+ (5)

Net: L-Asparagine + α-keto acid + H_2O $\rightarrow$ oxaloacetate + L-amino acid + NH_4^+ (6)

Table S1. Mutagenic oligonucleotides used for the construction of mutant clones.

Oligonucleotides	Sequence (5' → 3')
E43A-F	ATAGTTTCTTTGCCGG**GCC**TGCTTTAATTCTCCATATG
E43A-R	CATATGGAGAATTAAAGCA**GGC**CGGCAAAGAAACTAT
K112A-F	CTAGCAAAGTATAGA**GCC**ATCCATCTGTTTGAC
K112A-R	GTCAAACAGATGGAT**GGC**TCTATACTTTGCTAG
C153A-F	GTGGGTCTGGGCATC**GCC**TACGACATGCGGTTTG
C153A-R	CAAACCGCATGTCGTA**GGC**GATGCCCAGACCCAC
△116-128-F1	CCGG<u>GAATTC</u>ATTAAAGAGGAGAAATT (*EcoR*I)
△116-128-R1	AA<u>GGTACC</u>CAGATGGATCTTTCTATACTT (*Kpn*I)
△116-128-F2	TAA<u>GGTACC</u>TCTAAAACATTGAGTCCG (*Kpn*I)
△116-128-R2	TA<u>GCTAGC</u>TTGGATTCTCACCAATAAA (*Nhe*I)

<u>   </u> indicates the codon of mutated amino acid.

<u>   </u> indicates the restriction site.

Table S2. Expressed proteins: amino acid sequences of wild type hNit2, cloned wild type hNit2 and recombinant hNit2 Δ116-128 (The sequence of the deleted fragment is shown in red).

Wild type hNit2 (NP_064587) with 276 amino acids
MTSFRLALIQLQISSIKSDNVTRACSFIREAATQGAKIVSLPECFNSPYGAKYFPEYAEKIPGE
STQKLSEVAKECSIYLIGGSIPEEDAGKLYNTCAVFGPDGTLLAKYRKIHLFDIDVPGKITFQ
ESKTLSPGDSFSTFDTPYCRVGLGICYDMRFAELAQIYAQRGCQLLVYPGAFNLTTGPAHW
ELLQRSRAVDNQVYVATASPARDDKASYVAWGHSTVVNPWGEVLAKAGTEEAIVYSDIDL
KKLAEIRQQIPVFRQKRSDLYAVEMKKP

Recombinant hNit2 (wild type hNit2) with 286 amino acids
MHMTSFRLALIQLQISSIKSDNVTRACSFIREAATQGAKIVSLPECFNSPYGAKYFPEYAEKI
PGESTQKLSEVAKECSIYLIGGSIPEEDAGKLYNTCAVFGPDGTLLAKYRKIHLFDIDVPGKI
TFQESKTLSPGDSFSTFDTPYCRVGLGICYDMRFAELAQIYAQRGCQLLVYPGAFNLTTGPA
HWELLQRSRAVDNQVYVATASPARDDKASYVAWGHSTVVNPWGEVLAKAGTEEAIVYSD
IDLKKLAEIRQQIPVFRQKRSDLYAAEMKKPRSHHHHHH

MH: added for cloning purposes
FDIDVPGKITFQE: the sequence was designed to be deleted in the **Δ**116-128 mutant to remove the lid region of wild type hNit2.
RSHHHHHH: restriction site and x6His

Recombinant hNit2 Δ116-128 with 274 amino acids
MHMTSFRLALIQLQISSIKSDNVTRACSFIREAATQGAKIVSLPECFNSPYGAKYFPEYAEKIP
GESTQKLSEVAKECSIYLIGGSIPEEDAGKLYNTCAVFGPDGTLLAKYRKIHLGTSKTLSPGD
SFSTFDTPYCRVGLGICYDMRFAELAQIYAQRGCQLLVYPGAFNLTTGPAHWELLQRSRAVD
NQVYVATASPARDDKASYVAWGHSTVVNPWGEVLAKAGTEEAIVYSDIDLKKLAEIRQQIP
VFRQKRSDLYAAEMKKPRSHHHHH

MH: added for cloning the recombinant gene
GT: inserted as GGT ACC (*Kpn*I) in F2 primer
RSHHHHHH: restriction site and x6His
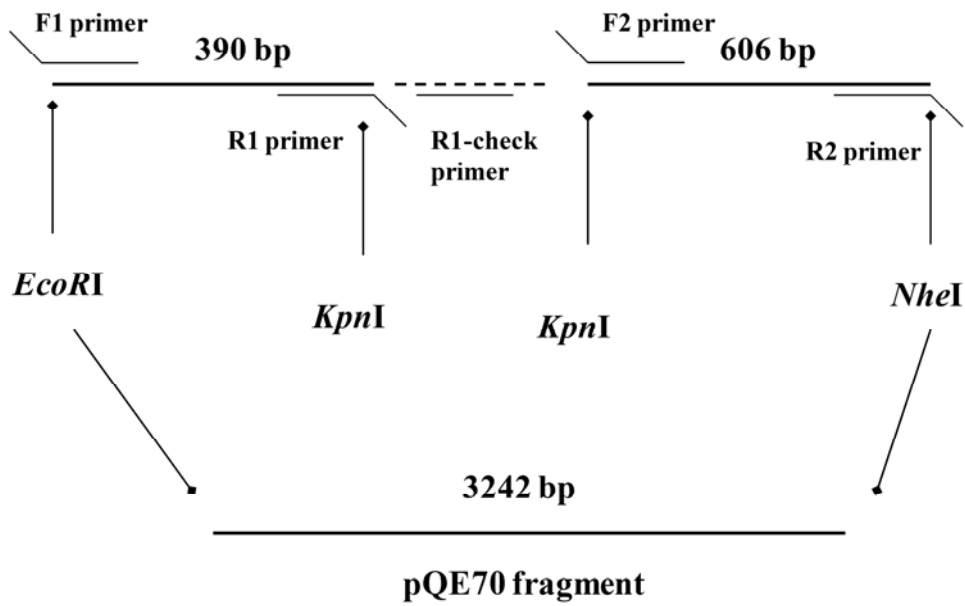
Fig. S1: Illustration of the construction of the pQE70-*hNit2* Δ346-384 clone. (------) indicates the deleted fragment, which was replaced with the Gly-Thr dipeptide. pQE70 is 3426 bp, pQE70-*hNit2* is 4248 bp and pQE70-*hNit2* Δ346-384 is 4215 bp.
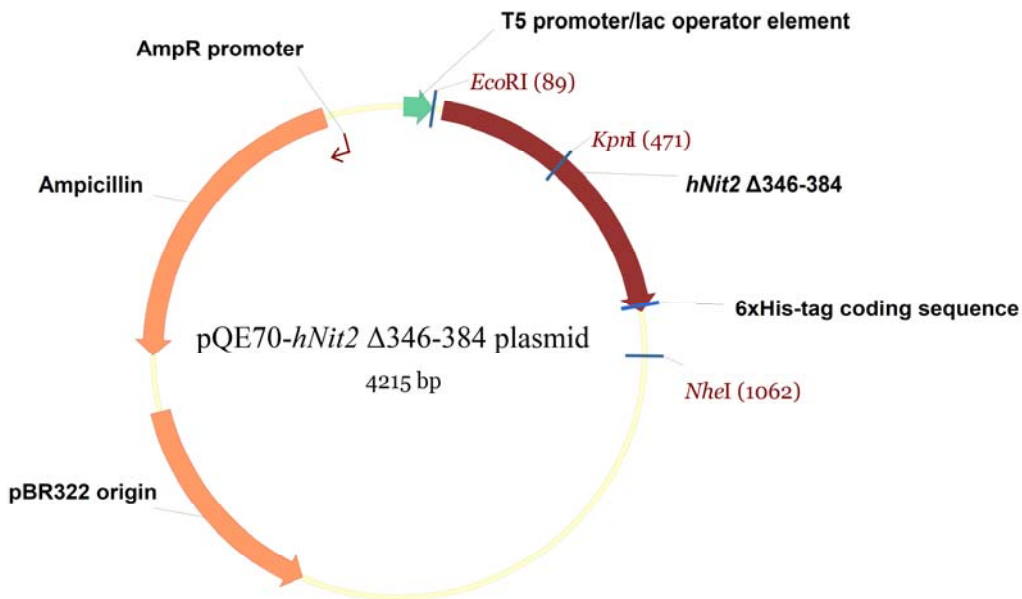
Fig. S2: Map of the pQE70-*hNit2* Δ346-384 plasmid.
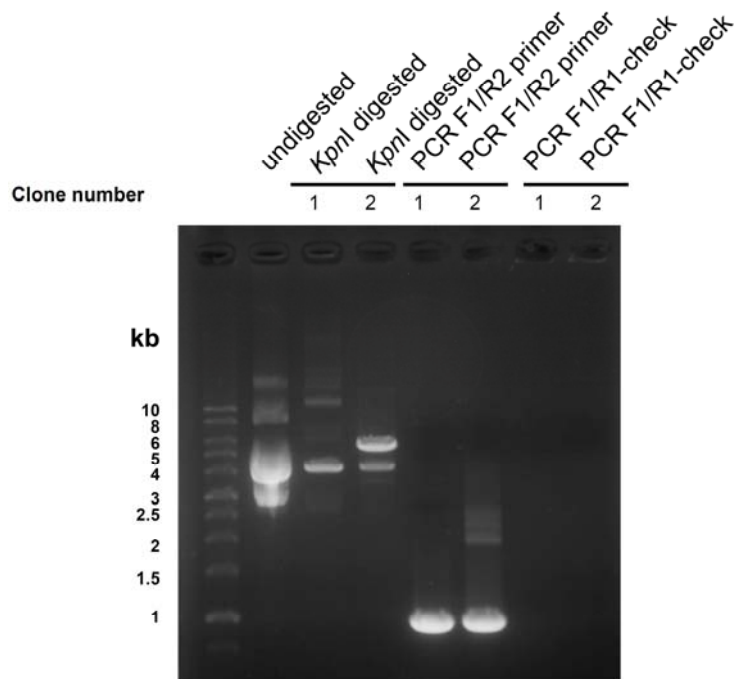
Fig. S3: Electrophoresis of the cloned pQE70-*hNit2* Δ346-384 plasmid.
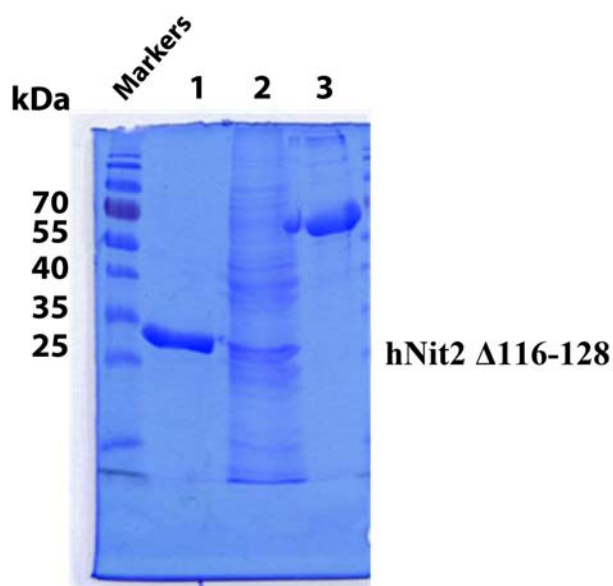
Fig. S4: SDS-PAGE of the purified hNit2 Δ116-128 protein. The method for purification of the hNit2 Δ116-128 mutant was the same as that employed for the wild type enzyme, as described in the Supporting Materials and Methods. Lane 1: 20 μg of hNit2 Δ116-128 protein eluted from a Ni-NTA Sepharose column with 200 mM imidazole in 10 mM phosphate buffer. Lane 2: 30 μg of the periplasmic fraction from M15[pQE70-hNit2 Δ116-128]. Lane 3: 3 μg of bovine serum albumin.
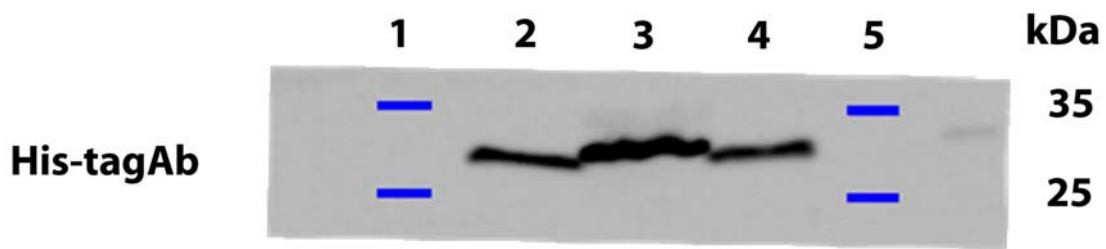
Fig. S5: Western blot analysis of hNit2 Δ116-128. A bacterial periplasmic fraction was prepared by means of a continuous high pressure cell disrupter TS-0.75kW at 40 kpsi. Wild type hNit2-His$_6$ and hNit2 Δ116-128-His$_6$ proteins were purified on a Ni-NTA Sepharose gel column followed by elution with 200 mM of imidazole/20 mM phosphate buffer, pH 7.4. Western blot analysis of hNit2-His$_6$ and hNit2 Δ116-128-His$_6$ proteins was carried out using a 6x His-tag monoclonal antibody. Lane 1: Protein markers. Lane 2: Supernatant of a bacterial extract of hNit2 Δ116-128. Lane 3: Purified hNit2 His$_6$ wild type protein. Lane 4: Purified hNit2 Δ116-128 protein. Lane 5: Protein markers.

(A)

LC/MS/MS spectral analysis of wild type hNit2

                    "TurboSequest" software for identification

Sample: human Nit2

 A gi|12005906   286 spectra   zTot: 0.0e0
>gi|12005906|gb|AAG44665.1|AF260334_1 CUA002 [Homo sapiens]
MTSFRLALIQ LQISSIKSDN VTRACSFIRE AATQGAKIVS LPECFNSPYG AKYFPEYAEK IPGESTQKLS EVAKECSIYL
IGGSIPEEDA GKLYNTCAVF GPDGTLLAKY RKIHLFDIDV PGKITFQESK TLSPGDSFST FDTPYCRVGL GICYDMRFAE
LAQIYAQRGC QLLVYPGAFN LTTGPAHWEL LQRSRAVDNQ VYVATASPAR DDKASYVAWG HSTVVNPWGE VLAKAGTEEA
IVYSDIDLKK LAEIRQQIPV FRQKRSDLYA VEMKKP

#	zBP	File	z	dM	MH+	Xcorr	dCn	Sp	RSp	Ions	Reference	( )Sequence
66	-----	1747	3	0.6	2487.2	5.27	0.56	1424	1	34/88	gi|12005906 +6	(-)EAATQGAKIVSLPECFNSPYGAK
49	-----	1594	2	1.1	1751.9	5.25	0.59	1469	1	22/30	gi|12005906 +3	(-)AGTEEAIVYSDIDLKK
83	-----	1832	2	-1.0	1625.8	5.20	0.41	1985	1	22/28	gi|12005906 +3	(-)AGTEEAIVYSDIDLK
116	-----	2172	2	1.1	1751.9	5.16	0.59	1514	1	22/30	gi|12005906 +3	(-)AGTEEAIVYSDIDLKK
22	-----	1234	2	-1.1	1563.8	5.12	0.40	2004	1	21/28	gi|12005906 +3	(-)AVDNQVYVATASPAR
103	-----	2019	2	0.0	1624.8	5.12	0.45	1932	1	21/28	gi|12005906 +3	(-)AGTEEAIVYSDIDLK
67	-----	1748	3	1.6	2486.2	5.03	0.53	1571	1	36/88	gi|12005906 +6	(-)EAATQGAKIVSLPECFNSPYGAK
125	-----	2223	3	-2.4	2633.3	4.98	0.42	2124	1	40/92	gi|12005906 +2	(-)DDKASYVAWGHSTVVNPWGEVLAK
45	-----	1578	2	0.1	1752.9	4.94	0.54	1380	1	21/30	gi|12005906 +3	(-)AGTEEAIVYSDIDLKK
72	-----	1764	3	-2.4	2490.2	4.88	0.42	1377	1	35/88	gi|12005906 +6	(-)EAATQGAKIVSLPECFNSPYGAK
178	-----	2597	2	-1.0	1625.8	4.79	0.38	1641	1	21/28	gi|12005906 +3	(-)AGTEEAIVYSDIDLK
188	-----	2750	2	1.1	1751.9	4.76	0.59	1428	1	21/30	gi|12005906 +3	(-)AGTEEAIVYSDIDLKK
124	-----	2221	3	1.4	2334.2	4.65	0.52	1639	1	34/80	gi|12005906 +3	(-)AGTEEAIVYSDIDLKKLAEIR
51	-----	1596	2	-1.8	1889.9	4.61	0.42	1651	1	25/30	gi|12005906 +5	(-)YFPEYAEKIPGESTQK
169	-----	2526	3	0.4	2216.2	4.61	0.44	2329	1	38/72	gi|12005906 +5	(-)KIHLFDIDVPGKITFQESK
76	-----	1781	2	1.2	1886.9	4.49	0.60	1406	1	23/30	gi|12005906 +5	(-)YFPEYAEKIPGESTQK
5	-----	0888	3	1.3	2163.1	4.48	0.43	1901	1	35/76	gi|12005906 +3	(-)SRAVDNQVYVATASPARDDK
208	-----	2869	2	1.0	1623.8	4.46	0.38	1628	1	20/28	gi|12005906 +3	(-)AGTEEAIVYSDIDLK
15	-----	0967	3	-1.9	1807.9	4.43	0.44	1396	1	29/64	gi|12005906 +3	(-)SRAVDNQVYVATASPAR
139	-----	2295	2	0.4	2272.1	4.41	0.62	1308	1	21/40	gi|12005906 +2	(-)ASYVAWGHSTVVNPWGEVLAK
113	-----	2155	2	-0.9	1753.9	4.40	0.45	1158	1	20/30	gi|12005906 +3	(-)AGTEEAIVYSDIDLKK
129	-----	2244	2	0.4	2335.2	4.32	0.53	1020	1	21/40	gi|12005906 +3	(-)AGTEEAIVYSDIDLKKLAEIR
130	-----	2245	2	1.4	2334.2	4.26	0.56	1242	1	22/40	gi|12005906 +3	(-)AGTEEAIVYSDIDLKKLAEIR

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134 ----- 2288    3   0.4 2272.2 4.25 0.39 1624   1  35/80  gi|12005906 +2  (-)ASYVAWGHSTVVNPWGEVLAK
  4 ----- 0887    3   0.3 2164.1 4.24 0.46 1803   1  35/76  gi|12005906 +3  (-)SRAVDNQVYVATASPARDDK
241 ----- 3316    2   1.1 1751.9 4.24 0.57 1319   1  20/30  gi|12005906 +3  (-)AGTEEAIVYSDIDLKK
185 ----- 2733    3   0.6 2630.3 4.21 0.27 1015   2  33/92  gi|12005906 +2  (-)DDKASYVAWGHSTVVNPWGEVLAK
123 ----- 2220    3   0.4 2335.2 4.18 0.42 1105   1  31/80  gi|12005906 +3  (-)AGTEEAIVYSDIDLKKLAEIR
 13 ----- 0955    2   1.1 1804.9 4.16 0.45 1039   1  22/32  gi|12005906 +3  (-)SRAVDNQVYVATASPAR
184 ----- 2733    2  -0.9 1753.9 4.13 0.48  870   1  17/30  gi|12005906 +3  (-)AGTEEAIVYSDIDLKK
136 ----- 2291    2   1.0 1623.8 4.13 0.43 1784   1  21/28  gi|12005906 +3  (-)AGTEEAIVYSDIDLK
150 ----- 2342    2   0.3 1999.9 4.12 0.50  490   1  17/32  gi|12005906 +4  (-)TLSPGDSFSTFDTPYCR
170 ----- 2527    3   1.4 2215.2 4.12 0.37 1699   1  32/72  gi|12005906 +5  (-)KIHLFDIDVPGKITFQESK
272 ----- 3735    2  -1.0 1625.8 4.12 0.42 1446   1  20/28  gi|12005906 +3  (-)AGTEEAIVYSDIDLK
 18 ----- 1039    2   0.9 1561.8 4.11 0.44 1664   1  20/28  gi|12005906 +3  (-)AVDNQVYVATASPAR
151 ----- 2343    3   0.6 2487.2 4.09 0.45 1007   1  30/88  gi|12005906 +6  (-)EAATQGAKIVSLPECFNSPYGAK
 38 ----- 1477    3   0.3 2164.1 4.09 0.47 1522   1  33/76  gi|12005906 +3  (-)SRAVDNQVYVATASPARDDK
128 ----- 2241    3  -0.6 2217.2 4.08 0.47 1404   1  31/72  gi|12005906 +5  (-)KIHLFDIDVPGKITFQESK
265 ----- 3633    3   1.4 2215.2 4.07 0.37 1516   1  31/72  gi|12005906 +5  (-)KIHLFDIDVPGKITFQESK
143 ----- 2306    3  -2.6 2275.2 4.04 0.18 1532   1  35/80  gi|12005906 +2  (-)ASYVAWGHSTVVNPWGEVLAK
 41 ----- 1509    2   0.2 1887.9 4.03 0.53  976   1  20/30  gi|12005906 +5  (-)YFPEYAEKIPGESTQK
190 ----- 2764    2  -0.1 1562.8 4.00 0.42 1283   1  18/28  gi|12005906 +3  (-)AVDNQVYVATASPAR
230 ----- 3087    3   0.4 2216.2 3.99 0.41 1578   1  32/72  gi|12005906 +5  (-)KIHLFDIDVPGKITFQESK
  7 ----- 0906    3  -2.7 2167.1 3.98 0.25 1717   1  34/76  gi|12005906 +3  (-)SRAVDNQVYVATASPARDDK
163 ----- 2462    2  -1.1 1563.8 3.97 0.54 1214   1  18/28  gi|12005906 +3  (-)AVDNQVYVATASPAR
140 ----- 2296    2   1.4 2271.1 3.96 0.60 1151   1  20/40  gi|12005906 +2  (-)ASYVAWGHSTVVNPWGEVLAK
 50 ----- 1595    2  -1.9 1754.9 3.96 0.20 1380   1  21/30  gi|12005906 +3  (-)AGTEEAIVYSDIDLKK
162 ----- 2461    3   1.6 2486.2 3.91 0.52 1107   1  30/88  gi|12005906 +6  (-)EAATQGAKIVSLPECFNSPYGAK
 87 ----- 1883    2  -1.1 1563.8 3.88 0.28 1613   1  20/28  gi|12005906 +3  (-)AVDNQVYVATASPAR
159 ----- 2411    2  -2.0 1626.8 3.83 0.07 1767   1  21/28  gi|12005906 +3  (-)AGTEEAIVYSDIDLK
235 ----- 3170    2  -1.0 1625.8 3.78 0.37 1647   1  21/28  gi|12005906 +3  (-)AGTEEAIVYSDIDLK
135 ----- 2289    3   1.4 2271.1 3.78 0.27 1377   2  33/80  gi|12005906 +2  (-)ASYVAWGHSTVVNPWGEVLAK
147 ----- 2326    3  -1.7 2090.1 3.76 0.42 1657   1  36/68  gi|12005906 +5  (-)IHLFDIDVPGKITFQESK
156 ----- 2363    2   1.2 1886.9 3.76 0.52  900   1  19/30  gi|12005906 +5  (-)YFPEYAEKIPGESTQK
267 ----- 3651    3  -1.6 2218.2 3.69 0.36 1853   1  35/72  gi|12005906 +5  (-)KIHLFDIDVPGKITFQESK
 61 ----- 1713    2   0.2 1730.9 3.66 0.51  519   1  19/28  gi|12005906 +6  (-)IVSLPECFNSPYGAK
205 ----- 2854    3  -1.6 2274.2 3.65 0.15 1842   2  34/80  gi|12005906 +2  (-)ASYVAWGHSTVVNPWGEVLAK
191 ----- 2765    2   0.9 1561.8 3.63 0.28 1062   1  15/28  gi|12005906 +3  (-)AVDNQVYVATASPAR
243 ----- 3332    2   0.9 1561.8 3.62 0.29 1482   1  18/28  gi|12005906 +3  (-)AVDNQVYVATASPAR
105 ----- 2032    3   0.9 1381.8 3.60 0.36 1912   1  25/44  gi|12005906 +5  (-)KIHLFDIDVPGK
240 ----- 3299    3   0.6 2630.3 3.60 0.41 1059   2  32/92  gi|12005906 +2  (-)DDKASYVAWGHSTVVNPWGEVLAK
 32 ----- 1390    3   0.1 1920.9 3.59 0.40  495   1  31/68  gi|12005906 +3  (-)AVDNQVYVATASPARDDK
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 94 ----- 1934     2  1.0 1557.8 3.58 0.16  535   1 19/26 gi|12005906 +5 (-)ACSFIREAATQGAK
 29 ----- 1357     2 -1.0 1559.8 3.55 0.18  261  20 14/26 gi|12005906 +5 (-)ACSFIREAATQGAK
203 ----- 2852     3  1.4 2271.1 3.50 0.40 1559   2 33/80 gi|12005906 +2 (-)ASYVAWGHSTVVNPWGEVLAK
106 ----- 2071     2  0.9 1381.8 3.49 0.43  887   1 16/22 gi|12005906 +5 (-)KIHLFDIDVPGK
200 ----- 2814     3  1.1 1751.9 3.48 0.49  607   4 28/60 gi|12005906 +3 (-)AGTEEAIVYSDIDLKK
 63 ----- 1726     3 -1.1 1471.9 3.46 0.32 1369   1 27/44 gi|12005906 +3 (-)LAEIRQQIPVFR
115 ----- 2169     3 -1.9 1754.9 3.46 0.49 1072   1 30/60 gi|12005906 +3 (-)AGTEEAIVYSDIDLKK
144 ----- 2308     2  0.2 1730.9 3.44 0.56  519   1 19/28 gi|12005906 +6 (-)IVSLPECFNSPYGAK
 62 ----- 1714     2  1.2 1729.9 3.43 0.60  578   1 20/28 gi|12005906 +6 (-)IVSLPECFNSPYGAK
250 ----- 3432     2  1.0 1623.8 3.41 0.46 1424   1 20/28 gi|12005906 +3 (-)AGTEEAIVYSDIDLK
 12 ----- 0951     3  1.1 1804.9 3.41 0.42 1586   1 29/64 gi|12005906 +3 (-)SRAVDNQVYVATASPAR
 23 ----- 1251     2 -1.2 1213.6 3.40 0.37  896   1 16/18 gi|12005906 +3 (-)RSDLYAVEMK
175 ----- 2580     2  1.3 1998.9 3.39 0.44  618   1 18/32 gi|12005906 +4 (-)TLSPGDSFSTFDTPYCR
181 ----- 2665     2 -0.8 1888.9 3.37 0.48  861   1 19/30 gi|12005906 +5 (-)YFPEYAEKIPGESTQK
114 ----- 2168     3  1.1 1751.9 3.37 0.53  869   1 30/60 gi|12005906 +3 (-)AGTEEAIVYSDIDLKK
 53 ----- 1612     2  0.9 1561.8 3.37 0.34  865   1 19/28 gi|12005906 +3 (-)AVDNQVYVATASPAR
 88 ----- 1884     3  3.2 2484.7 3.36 0.52 1195   1 31/88 gi|12005906 +6 (-)EAATQGAKIVSLPECFNSPYGAK
234 ----- 3154     2  1.3 1998.9 3.36 0.52  687   1 20/32 gi|12005906 +4 (-)TLSPGDSFSTFDTPYCR
 79 ----- 1798     3  1.6 2514.3 3.30 0.51  648   1 33/84 gi|12005906 +5 (-)YFPEYAEKIPGESTQKLSEVAK
 98 ----- 1952     2 -1.0 1559.8 3.28 0.21  431   1 18/26 gi|12005906 +5 (-)ACSFIREAATQGAK
233 ----- 3153     2  0.3 1999.9 3.28 0.51  505   1 17/32 gi|12005906 +4 (-)TLSPGDSFSTFDTPYCR
268 ----- 3684     2  1.8 1623.0 3.26 0.48 1444   1 19/28 gi|12005906 +3 (-)AGTEEAIVYSDIDLK
 28 ----- 1356     2  1.0 1557.8 3.25 0.08  408   2 17/26 gi|12005906 +5 (-)ACSFIREAATQGAK
118 ----- 2174     3  1.8 2757.3 3.25 0.50 1159   1 39/88 gi|12005906 +5 (-)IVSLPECFNSPYGAKYFPEYAEK
142 ----- 2305     3  1.3 2087.1 3.25 0.37 1605   1 35/68 gi|12005906 +5 (-)IHLFDIDVPGKITFQESK
187 ----- 2746     3 -0.9 1753.9 3.24 0.47  687   1 29/60 gi|12005906 +3 (-)AGTEEAIVYSDIDLKK
245 ----- 3344     3  0.4 2335.3 3.22 0.36  873   1 28/80 gi|12005906 +3 (-)AGTEEAIVYSDIDLKKLAEIR
 59 ----- 1694     2 -1.3 1057.5 3.22 0.44 1126   1 14/16 gi|12005906 +3 (-)SDLYAVEMK
109 ----- 2091     2 -0.8 1888.9 3.21 0.42  874   1 19/30 gi|12005906 +5 (-)YFPEYAEKIPGESTQK
248 ----- 3397     3 -0.9 1753.9 3.19 0.48  461   9 25/60 gi|12005906 +3 (-)AGTEEAIVYSDIDLKK
281 ----- 3916     3  1.4 2334.2 3.17 0.38  995   4 29/80 gi|12005906 +3 (-)AGTEEAIVYSDIDLKKLAEIR
209 ----- 2870     3  0.3 2088.1 3.15 0.49 1024   1 32/68 gi|12005906 +5 (-)IHLFDIDVPGKITFQESK
239 ----- 3299     2 -0.9 1753.9 3.15 0.43  716   1 16/30 gi|12005906 +3 (-)AGTEEAIVYSDIDLKK
 47 ----- 1590     3  1.1 1751.9 3.13 0.47  568   3 27/60 gi|12005906 +3 (-)AGTEEAIVYSDIDLKK
216 ----- 2936     2  1.2 1886.9 3.11 0.53  841   1 19/30 gi|12005906 +5 (-)YFPEYAEKIPGESTQK
168 ----- 2512     2  0.0 1558.8 3.11 0.04  391   7 16/26 gi|12005906 +5 (-)ACSFIREAATQGAK
217 ----- 2938     3 -1.4 2489.2 3.09 0.28 1759   1 36/88 gi|12005906 +6 (-)EAATQGAKIVSLPECFNSPYGAK
126 ----- 2231     2  0.6 2630.3 3.09 0.54  776   1 19/46 gi|12005906 +2 (-)DDKASYVAWGHSTVVNPWGEVLAK
102 ----- 2008     2  2.5 1997.7 3.09 0.50  633   1 19/32 gi|12005906 +4 (-)TLSPGDSFSTFDTPYCR
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 48 ----- 1591    3 -2.0 1754.9 3.08 0.38  703   1 32/60 gi|12005906 +3 (-)AGTEEAIVYSDIDLKK
120 ----- 2189    2  0.9 1561.8 3.07 0.21 1090   1 16/28 gi|12005906 +3 (-)AVDNQVYVATASPAR
161 ----- 2424    3  1.0 1598.0 3.07 0.43  525   9 24/48 gi|12005906 +3 (-)KLAEIRQQIPVFR
104 ----- 2021    3  2.5 2612.4 3.05 0.40  732   2 32/92 gi|12005906 +5 (-)LSEVAKECSIYLIGGSIPEEDAGK
194 ----- 2785    3  0.4 2335.3 3.04 0.35  691   1 30/80 gi|12005906 +3 (-)AGTEEAIVYSDIDLKKLAEIR
 26 ----- 1352    2  0.7 1055.5 3.01 0.46 1171   1 14/16 gi|12005906 +3 (-)SDLYAVEMK
 43 ----- 1574    3  0.1 1805.9 3.00 0.31 1632   1 31/64 gi|12005906 +3 (-)SRAVDNQVYVATASPAR
278 ----- 3882    2  1.1 1751.9 2.98 0.52  776   1 18/30 gi|12005906 +3 (-)AGTEEAIVYSDIDLKK
 19 ----- 1183    2  0.8 1211.6 2.97 0.41  906   1 16/18 gi|12005906 +3 (-)RSDLYAVEMK
 56 ----- 1626    3 -2.0 1729.0 2.97 0.24  610   7 23/52 gi|12005906 +3 (-)LAEIRQQIPVFRQK
131 ----- 2246    2  1.6 2629.3 2.96 0.56  543   1 16/46 gi|12005906 +2 (-)DDKASYVAWGHSTVVNPWGEVLAK
226 ----- 3071    3 -2.6 2219.2 2.96 0.25 1697   1 34/72 gi|12005906 +5 (-)KIHLFDIDVPGKITFQESK
224 ----- 3038    3  1.6 2486.2 2.94 0.33  690   8 28/88 gi|12005906 +6 (-)EAATQGAKIVSLPECFNSPYGAK
145 ----- 2309    2  1.2 1729.9 2.93 0.54  452   1 17/28 gi|12005906 +6 (-)IVSLPECFNSPYGAK
110 ----- 2100    2  0.8 1253.7 2.91 0.49  810   1 14/20 gi|12005906 +5 (-)IHLFDIDVPGK
282 ----- 3917    3 -2.0 2337.7 2.90 0.34 1150   1 29/80 gi|12005906 +3 (-)AGTEEAIVYSDIDLKKLAEIR
111 ----- 2117    3  0.9 1469.9 2.89 0.34 1227   3 25/44 gi|12005906 +3 (-)LAEIRQQIPVFR
 93 ----- 1932    2  0.7 1055.5 2.88 0.39 1161   1 14/16 gi|12005906 +3 (-)SDLYAVEMK
279 ----- 3899    2  0.9 1561.8 2.88 0.51  952   1 16/28 gi|12005906 +3 (-)AVDNQVYVATASPAR
210 ----- 2883    3  1.3 2087.1 2.84 0.37  897   2 28/68 gi|12005906 +5 (-)IHLFDIDVPGKITFQESK
 44 ----- 1575    3  1.1 1804.9 2.83 0.23 1002  16 28/64 gi|12005906 +3 (-)SRAVDNQVYVATASPAR
 42 ----- 1513    2  0.1 1920.9 2.81 0.40 1052   1 18/34 gi|12005906 +3 (-)AVDNQVYVATASPARDDK
223 ----- 3037    3  0.6 2487.2 2.81 0.30  695   6 25/88 gi|12005906 +6 (-)EAATQGAKIVSLPECFNSPYGAK
 77 ----- 1783    3  0.6 2515.3 2.81 0.30  590   1 29/84 gi|12005906 +5 (-)YFPEYAEKIPGESTQKLSEVAK
155 ----- 2360    2 -1.7 2001.9 2.78 0.35  489   1 18/32 gi|12005906 +4 (-)TLSPGDSFSTFDTPYCR
146 ----- 2312    2 -1.6 2274.2 2.78 0.37 1131   1 19/40 gi|12005906 +2 (-)ASYVAWGHSTVVNPWGEVLAK
 54 ----- 1621    1 -0.9 1753.9 2.78 0.44 1034   1 18/30 gi|12005906 +3 (-)AGTEEAIVYSDIDLKK
149 ----- 2338    3 -2.1 1472.9 2.74 0.26 1290   2 25/44 gi|12005906 +3 (-)LAEIRQQIPVFR
252 ----- 3443    3  1.3 2087.1 2.72 0.51 1334   1 34/68 gi|12005906 +5 (-)IHLFDIDVPGKITFQESK
160 ----- 2423    3  0.0 1599.0 2.72 0.26  425  11 22/48 gi|12005906 +3 (-)KLAEIRQQIPVFR
260 ----- 3548    3 -0.1 2891.5 2.72 0.41  653   1 29/96 gi|12005906 +3 (-)GCQLLVYPGAFNLTTGPAHWELLQR
 92 ----- 1931    2  0.9 1469.9 2.71 0.25  403   4 12/22 gi|12005906 +3 (-)LAEIRQQIPVFR
251 ----- 3434    3 -1.7 2090.1 2.71 0.44 1277   1 32/68 gi|12005906 +5 (-)IHLFDIDVPGKITFQESK
 60 ----- 1711    2 -0.1 1470.9 2.70 0.26  493   1 14/22 gi|12005906 +3 (-)LAEIRQQIPVFR
176 ----- 2581    2  0.8 1309.7 2.70 0.23  472   9 12/20 gi|12005906 +4 (-)FAELAQIYAQR
201 ----- 2822    3 -0.1 2891.5 2.70 0.26  786   1 30/96 gi|12005906 +3 (-)GCQLLVYPGAFNLTTGPAHWELLQR
192 ----- 2784    3 -0.5 2336.2 2.69 0.18  440   5 25/80 gi|12005906 +3 (-)AGTEEAIVYSDIDLKKLAEIR
167 ----- 2510    2  0.7 1055.5 2.69 0.40  971   1 13/16 gi|12005906 +3 (-)SDLYAVEMK
247 ----- 3348    3  1.4 2334.2 2.68 0.32 1006   1 30/80 gi|12005906 +3 (-)AGTEEAIVYSDIDLKKLAEIR
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183 ----- 2695     3  0.9 1469.9 2.67 0.36 1539    1  26/44 gi|12005906 +3 (-)LAEIRQQIPVFR
 33 ----- 1391     3  1.1 1919.9 2.66 0.48  430    3  27/68 gi|12005906 +3 (-)AVDNQVYVATASPARDDK
 39 ----- 1505     3  0.2 1887.9 2.65 0.25  453   27  23/60 gi|12005906 +5 (-)YFPEYAEKIPGESTQK
 86 ----- 1851     3 -2.5 2518.3 2.65 0.39  422    1  28/84 gi|12005906 +5 (-)YFPEYAEKIPGESTQKLSEVAK
261 ----- 3549     3  0.9 2890.5 2.64 0.22  577    9  27/96 gi|12005906 +3 (-)GCQLLVYPGAFNLTTGPAHWELLQR
257 ----- 3512     3  1.1 1751.9 2.63 0.45  751    4  30/60 gi|12005906 +3 (-)AGTEEAIVYSDIDLKK
213 ----- 2906     2 -0.7 2000.9 2.63 0.42  495    1  16/32 gi|12005906 +4 (-)TLSPGDSFSTFDTPYCR
228 ----- 3083     2 -0.1 1470.9 2.61 0.14  586    1  15/22 gi|12005906 +3 (-)LAEIRQQIPVFR
193 ----- 2785     2  1.6 1557.2 2.61 0.15  351   19  15/26 gi|12005906 +5 (-)ACSFIREAATQGAK
242 ----- 3329     3 -2.0 1754.9 2.60 0.33  398  111  24/60 gi|12005906 +3 (-)AGTEEAIVYSDIDLKK
 71 ----- 1762     2  0.8 1211.6 2.59 0.29  826    3  15/18 gi|12005906 +3 (-)RSDLYAVEMK
157 ----- 2393     3 -0.5 2516.3 2.59 0.24  700    1  31/84 gi|12005906 +5 (-)YFPEYAEKIPGESTQKLSEVAK
180 ----- 2661     3 -1.8 1889.9 2.59 0.32 1102    2  27/60 gi|12005906 +5 (-)YFPEYAEKIPGESTQK
 16 ----- 0971     2 -1.9 1807.9 2.58 0.18 1133    1  23/32 gi|12005906 +3 (-)SRAVDNQVYVATASPAR
 55 ----- 1624     3  1.1 1726.0 2.57 0.44  651    2  24/52 gi|12005906 +3 (-)LAEIRQQIPVFRQK
 17 ----- 1022     2 -2.1 1564.8 2.57 0.16 1830    1  21/28 gi|12005906 +3 (-)AVDNQVYVATASPAR
177 ----- 2582     3 -2.4 2490.2 2.56 0.06 1350    1  33/88 gi|12005906 +6 (-)EAATQGAKIVSLPECFNSPYGAK
 70 ----- 1761     2 -2.1 1472.9 2.55 0.06  520    2  14/22 gi|12005906 +3 (-)LAEIRQQIPVFR
117 ----- 2173     3  0.8 2758.3 2.55 0.41  788    1  35/88 gi|12005906 +5 (-)IVSLPECFNSPYGAKYFPEYAEK
227 ----- 3072     3  2.5 2214.1 2.51 0.31 1306    1  29/72 gi|12005906 +5 (-)KIHLFDIDVPGKITFQESK
211 ----- 2886     2  0.2 1730.9 2.48 0.44  351    1  15/28 gi|12005906 +6 (-)IVSLPECFNSPYGAK
  3 ----- 0833     2 -1.4  803.4 2.48 0.25  214    3   9/10 gi|12005906 +7 (-)ACSFIR
158 ----- 2394     3  0.3 2614.7 2.47 0.06  835    2  28/92 gi|12005906 +5 (-)LSEVAKECSIYLIGGSIPEEDAGK
132 ----- 2270     3 -1.8 1384.4 2.46 0.30  423    3  22/44 gi|12005906 +5 (-)KIHLFDIDVPGK
 35 ----- 1425     2  0.9 1467.8 2.46 0.23  349   23  13/22 gi|12005906 +3 (-)QKRSDLYAVEMK
 84 ----- 1836     2  1.3 1985.9 2.44 0.42  820    1  17/34 gi|12005906 +5 (-)ECSIYLIGGSIPEEDAGK
 82 ----- 1830     3  1.0 1598.0 2.44 0.13  645    2  24/48 gi|12005906 +3 (-)KLAEIRQQIPVFR
212 ----- 2887     2  1.2 1729.9 2.43 0.42  353    2  15/28 gi|12005906 +6 (-)IVSLPECFNSPYGAK
 90 ----- 1917     3  2.6 2513.2 2.43 0.12  356    7  26/84 gi|12005906 +5 (-)YFPEYAEKIPGESTQKLSEVAK
 37 ----- 1460     2  0.9 1486.8 2.42 0.35  473    1  16/26 gi|12005906 +6 (-)IPGESTQKLSEVAK
246 ----- 3345     2  0.0 1558.8 2.39 0.21  245   83  13/26 gi|12005906 +5 (-)ACSFIREAATQGAK
229 ----- 3084     2  0.9 1469.9 2.39 0.22  365   17  12/22 gi|12005906 +3 (-)LAEIRQQIPVFR
166 ----- 2509     2  0.9 1469.9 2.39 0.11  387    6  13/22 gi|12005906 +3 (-)LAEIRQQIPVFR
231 ----- 3101     2  0.7 1055.5 2.39 0.37  884    1  13/16 gi|12005906 +3 (-)SDLYAVEMK
256 ----- 3502     2  1.2 1886.9 2.37 0.48  722    1  15/30 gi|12005906 +5 (-)YFPEYAEKIPGESTQK
198 ----- 2799     2 -1.0 1213.4 2.37 0.34  566    7  13/18 gi|12005906 +3 (-)RSDLYAVEMK
127 ----- 2238     3  0.0 1727.0 2.36 0.09  553   30  22/52 gi|12005906 +3 (-)LAEIRQQIPVFRQK
 85 ----- 1849     2  1.0 1597.9 2.35 0.31  383   12  12/24 gi|12005906 +3 (-)KLAEIRQQIPVFR
285 ----- 3968     2 -1.1 1563.8 2.34 0.38  410   14  13/28 gi|12005906 +3 (-)AVDNQVYVATASPAR
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270 ----- 3718    2  0.3 1999.9 2.34 0.47  312    1  15/32 gi|12005906 +4  (-)TLSPGDSFSTFDTPYCR
 64 ----- 1730    2 -1.8 1732.9 2.34 0.11  504    1  18/28 gi|12005906 +6  (-)IVSLPECFNSPYGAK
 97 ----- 1949    3  1.0 1557.8 2.33 0.29  435    1  26/52 gi|12005906 +5  (-)ACSFIREAATQGAK
 81 ----- 1829    3  0.0 1599.0 2.32 0.17  520    4  24/48 gi|12005906 +3  (-)KLAEIRQQIPVFR
 69 ----- 1755    2 -1.4 2489.2 2.31 0.14  394    2  16/44 gi|12005906 +6  (-)EAATQGAKIVSLPECFNSPYGAK
195 ----- 2786    2  2.3 1556.5 2.30 0.13  282   56  13/26 gi|12005906 +5  (-)ACSFIREAATQGAK
153 ----- 2356    2  0.8 1211.6 2.30 0.35  841    1  15/18 gi|12005906 +3  (-)RSDLYAVEMK
179 ----- 2611    3  0.9 1381.8 2.29 0.30 1210    1  26/44 gi|12005906 +5  (-)KIHLFDIDVPGK
 31 ----- 1370    3  1.0 1557.8 2.29 0.33  465    2  27/52 gi|12005906 +5  (-)ACSFIREAATQGAK
262 ----- 3552    3  1.9 2889.5 2.25 0.12  696   14  27/96 gi|12005906 +3  (-)GCQLLVYPGAFNLTTGPAHWELLQR
101 ----- 1987    2 -1.3 1311.8 2.25 0.04  862    3  13/20 gi|12005906 +4  (-)FAELAQIYAQR
263 ----- 3599    3  0.6 2487.2 2.24 0.11  937    4  28/88 gi|12005906 +6  (-)EAATQGAKIVSLPECFNSPYGAK
244 ----- 3333    2 -1.1 1563.8 2.24 0.32  615    4  14/28 gi|12005906 +3  (-)AVDNQVYVATASPAR
204 ----- 2853    3 -0.8 1888.9 2.24 0.17  643   11  25/60 gi|12005906 +5  (-)YFPEYAEKIPGESTQK
199 ----- 2801    3 -2.4 2633.3 2.23 0.00 1747    1  35/92 gi|12005906 +2  (-)DDKASYVAWGHSTVVNPWGEVLAK
277 ----- 3865    3  0.6 2630.3 2.22 0.08  687   83  25/92 gi|12005906 +2  (-)DDKASYVAWGHSTVVNPWGEVLAK
286 ----- 3982    3  0.4 2272.2 2.20 0.29  781   28  26/80 gi|12005906 +2  (-)ASYVAWGHSTVVNPWGEVLAK
249 ----- 3416    3  0.4 2272.2 2.20 0.24 1222    3  30/80 gi|12005906 +2  (-)ASYVAWGHSTVVNPWGEVLAK
 73 ----- 1766    3  0.3 1999.9 2.19 0.18  607    7  25/64 gi|12005906 +4  (-)TLSPGDSFSTFDTPYCR
274 ----- 3800    3  1.3 1998.9 2.18 0.40  428    9  25/64 gi|12005906 +4  (-)TLSPGDSFSTFDTPYCR
218 ----- 2949    3  2.0 1750.9 2.18 0.18  547   20  26/60 gi|12005906 +3  (-)AGTEEAIVYSDIDLKK
222 ----- 3001    2  0.8 1211.6 2.18 0.27  906    1  15/18 gi|12005906 +3  (-)RSDLYAVEMK
138 ----- 2293    2 -1.1 1471.9 2.17 0.01  305   18  11/22 gi|12005906 +3  (-)LAEIRQQIPVFR
174 ----- 2565    3  1.1 1919.9 2.17 0.25  385   18  25/68 gi|12005906 +3  (-)AVDNQVYVATASPARDDK
196 ----- 2786    3  1.4 2334.2 2.16 0.11  552    2  25/80 gi|12005906 +3  (-)AGTEEAIVYSDIDLKKLAEIR
164 ----- 2495    3  0.6 2515.3 2.14 0.26  762    2  27/84 gi|12005906 +5  (-)YFPEYAEKIPGESTQKLSEVAK
121 ----- 2203    3  0.1 1805.9 2.13 0.15  613  179  24/64 gi|12005906 +3  (-)SRAVDNQVYVATASPAR
148 ----- 2331    2 -0.6 2217.2 2.11 0.19  853    1  19/36 gi|12005906 +5  (-)KIHLFDIDVPGKITFQESK
 14 ----- 0958    2 -2.9 1923.9 2.08 0.02  787    1  17/34 gi|12005906 +3  (-)AVDNQVYVATASPARDDK
 96 ----- 1948    3  0.0 1558.8 2.06 0.25  308   13  23/52 gi|12005906 +5  (-)ACSFIREAATQGAK
108 ----- 2085    3  0.8 1253.7 2.05 0.15  605   25  19/40 gi|12005906 +5  (-)IHLFDIDVPGK
253 ----- 3447    2  1.2 1729.9 2.05 0.37  322    3  13/28 gi|12005906 +6  (-)IVSLPECFNSPYGAK
 40 ----- 1506    3  1.2 1886.9 2.05 0.17  353   92  20/60 gi|12005906 +5  (-)YFPEYAEKIPGESTQK
202 ----- 2836    3  1.8 2757.3 2.03 0.23  486    3  25/88 gi|12005906 +5  (-)IVSLPECFNSPYGAKYFPEYAEK
 21 ----- 1200    3  0.8 1211.6 2.03 0.16  745    6  19/36 gi|12005906 +3  (-)RSDLYAVEMK
258 ----- 3531    3 -1.8 1889.9 2.02 0.08  589    5  24/60 gi|12005906 +5  (-)YFPEYAEKIPGESTQK
264 ----- 3618    3  1.6 2486.2 2.02 0.29 1165    1  32/88 gi|12005906 +6  (-)EAATQGAKIVSLPECFNSPYGAK
122 ----- 2220    2  1.6 1557.2 2.02 0.17  221  172  12/26 gi|12005906 +5  (-)ACSFIREAATQGAK
  2 ----- 0816    2  0.6  801.4 2.01 0.33  234    6   9/10 gi|12005906 +7  (-)ACSFIR
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276 ----- 3865     2 -0.9 1753.9 2.00 0.25  373  35  12/30 gi|12005906 +3 (-)AGTEEAIVYSDIDLKK
112 ----- 2138     2  2.5 1468.3 2.00 0.30  325  29  12/22 gi|12005906 +3 (-)LAEIRQQIPVFR
152 ----- 2355     3 -1.7 2001.9 1.99 0.20  339   2  25/64 gi|12005906 +4 (-)TLSPGDSFSTFDTPYCR
225 ----- 3070     3 -0.4 2516.3 1.99 0.23  527  10  26/84 gi|12005906 +5 (-)YFPEYAEKIPGESTQKLSEVAK
255 ----- 3500     2  0.2 1887.9 1.97 0.29  452   3  15/30 gi|12005906 +5 (-)YFPEYAEKIPGESTQK
271 ----- 3719     2  1.3 1998.9 1.96 0.27  325   6  13/32 gi|12005906 +4 (-)TLSPGDSFSTFDTPYCR
 74 ----- 1769     2 -0.7 2000.9 1.96 0.28  391   2  13/32 gi|12005906 +4 (-)TLSPGDSFSTFDTPYCR
197 ----- 2797     3  0.2 1730.9 1.96 0.25  196 177  19/56 gi|12005906 +6 (-)IVSLPECFNSPYGAK
 36 ----- 1456     2 -1.4  803.4 1.95 0.09  208  15   9/10 gi|12005906 +7 (-)ACSFIR
 46 ----- 1587     1  0.1 1752.9 1.94 0.40  233   8  12/30 gi|12005906 +3 (-)AGTEEAIVYSDIDLKK
 10 ----- 0926     2  0.2 1920.9 1.93 0.33  482   2  14/34 gi|12005906 +3 (-)AVDNQVYVATASPARDDK
 89 ----- 1905     2  1.1 1919.9 1.93 0.28  251  28  12/34 gi|12005906 +3 (-)AVDNQVYVATASPARDDK
 99 ----- 1981     2  0.6  801.4 1.91 0.03  231  15   9/10 gi|12005906 +7 (-)ACSFIR
100 ----- 1985     3  1.1 1919.9 1.91 0.08  262 173  21/68 gi|12005906 +3 (-)AVDNQVYVATASPARDDK
 80 ----- 1806     2  1.6 2514.3 1.91 0.34  223  15  14/42 gi|12005906 +5 (-)YFPEYAEKIPGESTQKLSEVAK
280 ----- 3900     2  1.7 1469.1 1.90 0.25  234 255   9/22 gi|12005906 +3 (-)LAEIRQQIPVFR
 58 ----- 1629     2  1.1 1726.0 1.89 0.19  186  25  12/26 gi|12005906 +3 (-)LAEIRQQIPVFRQK
266 ----- 3650     2  0.9 1469.9 1.87 0.06  573   2  13/22 gi|12005906 +3 (-)LAEIRQQIPVFR
236 ----- 3232     3  1.3 1998.9 1.85 0.29  433   5  26/64 gi|12005906 +4 (-)TLSPGDSFSTFDTPYCR
221 ----- 2999     3  1.0 1597.9 1.82 0.21  419   6  21/48 gi|12005906 +3 (-)KLAEIRQQIPVFR
273 ----- 3769     3 -0.4 2516.3 1.82 0.02  546   7  29/84 gi|12005906 +5 (-)YFPEYAEKIPGESTQKLSEVAK
284 ----- 3967     3  0.8 2758.3 1.81 0.04  509   1  25/88 gi|12005906 +5 (-)IVSLPECFNSPYGAKYFPEYAEK
207 ----- 2866     3  1.2 1886.9 1.80 0.12  493  74  22/60 gi|12005906 +5 (-)YFPEYAEKIPGESTQK
 75 ----- 1777     3  1.3 1998.9 1.78 0.17  394  33  22/64 gi|12005906 +4 (-)TLSPGDSFSTFDTPYCR
232 ----- 3134     2  0.6  801.4 1.78 0.03  231 251   8/10 gi|12005906 +7 (-)ACSFIR
189 ----- 2751     3 -0.2 2759.3 1.78 0.01  316  35  25/88 gi|12005906 +5 (-)IVSLPECFNSPYGAKYFPEYAEK
141 ----- 2304     3 -1.8 1732.9 1.77 0.23  329   3  23/56 gi|12005906 +6 (-)IVSLPECFNSPYGAK
133 ----- 2287     3  1.2 1886.9 1.77 0.13  428  59  22/60 gi|12005906 +5 (-)YFPEYAEKIPGESTQK
171 ----- 2542     3  1.0 1557.8 1.76 0.12  201 497  16/52 gi|12005906 +5 (-)ACSFIREAATQGAK
 25 ----- 1318     2 -1.3 1048.5 1.75 0.08  219  23  10/14 gi|12005906 +5 (-)YFPEYAEK
  8 ----- 0922     3 -1.9 1922.9 1.75 0.01  288  52  25/68 gi|12005906 +3 (-)AVDNQVYVATASPARDDK
137 ----- 2292     3  2.1 1886.0 1.73 0.15  452  89  22/60 gi|12005906 +5 (-)YFPEYAEKIPGESTQK
172 ----- 2551     2  0.4 2216.2 1.73 0.31  618   1  16/36 gi|12005906 +5 (-)KIHLFDIDVPGKITFQESK
 24 ----- 1278     2  2.3 1918.8 1.72 0.38  223  38  12/34 gi|12005906 +3 (-)AVDNQVYVATASPARDDK
275 ----- 3862     2  0.7 1055.5 1.72 0.38  428  58  11/16 gi|12005906 +3 (-)SDLYAVEMK
107 ----- 2083     3  0.2 1887.9 1.71 0.21  727   4  27/60 gi|12005906 +5 (-)YFPEYAEKIPGESTQK
 34 ----- 1403     2  0.6  801.4 1.70 0.19  225   6   9/10 gi|12005906 +7 (-)ACSFIR
173 ----- 2559     2  0.6  801.4 1.64 0.07  269  24   9/10 gi|12005906 +7 (-)ACSFIR
 57 ----- 1628     2  0.1 1727.0 1.64 0.17  116 135  12/26 gi|12005906 +3 (-)LAEIRQQIPVFRQK
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269 ----- 3705      3 -2.3 2893.7 1.63 0.05  295 217  22/96  gi|12005906 +3  (-)GCQLLVYPGAFNLTTGPAHWELLQR
214 ----- 2923      2 -2.3 2002.5 1.63 0.17  363   4  15/32  gi|12005906 +4  (-)TLSPGDSFSTFDTPYCR
206 ----- 2857      2 -3.3 2003.5 1.59 0.01  364   4  14/32  gi|12005906 +4  (-)TLSPGDSFSTFDTPYCR
154 ----- 2359      3  1.3 1998.9 1.59 0.18  501   1  28/64  gi|12005906 +4  (-)TLSPGDSFSTFDTPYCR
220 ----- 2990      3  0.9 2890.5 1.57 0.04  229 220  23/96  gi|12005906 +3  (-)GCQLLVYPGAFNLTTGPAHWELLQR
215 ----- 2932      3  0.3 1999.9 1.57 0.10  355   2  24/64  gi|12005906 +4  (-)TLSPGDSFSTFDTPYCR
 68 ----- 1751      2  1.3 1998.9 1.57 0.20  207  59  11/32  gi|12005906 +4  (-)TLSPGDSFSTFDTPYCR
 20 ----- 1184      2  0.7 1046.5 1.55 0.31  189  51   8/14  gi|12005906 +5  (-)YFPEYAEK
237 ----- 3241      2 -0.8 1888.9 1.48 0.03  421   2  13/30  gi|12005906 +5  (-)YFPEYAEKIPGESTQK
  9 ----- 0925      2 -0.8 1921.9 1.47 0.02  377   8  13/34  gi|12005906 +3  (-)AVDNQVYVATASPARDDK
186 ----- 2739      2  1.1 1919.9 1.45 0.24  463   5  14/34  gi|12005906 +3  (-)AVDNQVYVATASPARDDK
 65 ----- 1738      2  1.6 2486.2 1.44 0.29  109  15   8/44  gi|12005906 +6  (-)EAATQGAKIVSLPECFNSPYGAK
259 ----- 3533      3  2.1 1886.0 1.37 0.02  353 259  20/60  gi|12005906 +5  (-)YFPEYAEKIPGESTQK
283 ----- 3955      2  2.2 1998.0 1.37 0.25  140  52  11/32  gi|12005906 +4  (-)TLSPGDSFSTFDTPYCR
254 ----- 3468      2 -1.7 2001.9 1.36 0.01  236  22  11/32  gi|12005906 +4  (-)TLSPGDSFSTFDTPYCR
  6 ----- 0904      1  0.6  801.4 1.34 0.25  135  51   7/10  gi|12005906 +7  (-)ACSFIR
 30 ----- 1369      2 -1.5  889.5 1.30 0.02  296   9  11/12  gi|12005906 +3  (-)QQIPVFR
 95 ----- 1947      2 -1.2  889.2 1.28 0.00  203  10  10/12  gi|12005906 +3  (-)QQIPVFR
182 ----- 2678      2  0.5  887.5 1.23 0.18  263   3  11/12  gi|12005906 +3  (-)QQIPVFR
 11 ----- 0927      2  1.1 1919.9 1.21 0.18  400   1  14/34  gi|12005906 +3  (-)AVDNQVYVATASPARDDK
 27 ----- 1353      2  0.5  887.5 1.19 0.11  187  10  10/12  gi|12005906 +3  (-)QQIPVFR
165 ----- 2508      2 -0.5  888.5 1.17 0.08  133 207   8/12  gi|12005906 +3  (-)QQIPVFR
238 ----- 3249      2  0.5  887.5 1.16 0.03   81 282   5/12  gi|12005906 +3  (-)QQIPVFR
 52 ----- 1604      1  1.1 1751.9 1.13 0.18   56 150   7/30  gi|12005906 +3  (-)AGTEEAIVYSDIDLKK
 91 ----- 1930      2  0.5  887.5 1.07 0.17  196  21  10/12  gi|12005906 +3  (-)QQIPVFR
119 ----- 2180      2  0.8 2758.3 1.03 0.00   92   8   8/44  gi|12005906 +5  (-)IVSLPECFNSPYGAKYFPEYAEK
  1 ----- 0550      2  0.5  852.4 0.94 0.06  146  72   7/12  gi|12005906 +5  (-)ITFQESK
219 ----- 2956      2  1.4 2271.1 0.86 0.05  116 349   8/40  gi|12005906 +2  (-)ASYVAWGHSTVVNPWGEVLAK
 78 ----- 1789      2  0.6 2515.3 0.83 0.12   84  24   8/42  gi|12005906 +5  (-)YFPEYAEKIPGESTQKLSEVAK
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(B)

LC/MS/MS spectral analysis of hNit2 Δ116-128 mutant

Proteomics Research Center at National Yang-Ming University

The deletion fragment of peptide is not matched in the sequence, as shown in blue character in the second line of sequence.

>**sp|q9nqr4|nit2**_HUMAN Nitrilase homolog 2 OS=Homo sapiens GN=NIT2 PE=1 SV=1

MTSFR**LALIQ LQISSIKSDN VTRACSFIRE** AATQGAK**IVS LPECFNSPYG AKYFPEYAEK IPGESTQKLS EVAKECSIYL**

IGGSIPEEDA GKLYNTCAVF GPDGTLLAKY RKIHL FDIDV PGKITFQ ESK **TLSPGDSFST FDTPYCRVGL GICYDMRFAE**

LAQIYAQRGC QLLVYPGAFN LTTGPAHWEL LQRSRAVDNQ VYVATASPAR DDKASYVAWG HSTVVNPWGE VLAKAGTEEA

IVYSDIDLKK LAEIRQQIPV FRQK**RSDLYA VEMK**KP

Mass (mono): 30588.6 **Identifier:** sp|q9nqr4|nit2_ **Database:** C:/Xcalibur/database//SPHuman_081030.fasta

Protein Coverage: 238/276 = **86.2%** by amino acid count, 26213.2/30588.6 = **85.7%** by mass
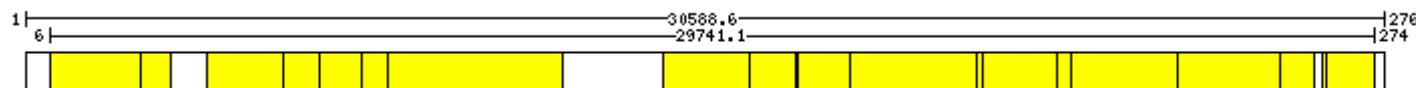
<>Nitrilase homolog 2 OS=Homo sapiens GN=NIT2 PE=1 SV=1

336	-----	00724	3	0.0	2629.3	**6.86 0.53 3438**	1	48/92	sp|q9nqr4|nit2_	(R)DDKASYVAWGHSTVVNPWGEVLAK
362	-----	00765	3	0.0	3854.9	**6.78 0.59 2083**	1	44/136	sp|q9nqr4|nit2_	(K)ECSIYLIGGSIPEEDAGKLYNTCAVFGPDGTLLAK
348	-----	00742	3	0.0	2629.3	**6.61 0.57 2761**	1	47/92	sp|q9nqr4|nit2_	(R)DDKASYVAWGHSTVVNPWGEVLAK
349	-----	00744	2	0.0	2271.2	**6.29 0.66 2477**	1	26/40	sp|q9nqr4|nit2_	(K)ASYVAWGHSTVVNPWGEVLAK
314	-----	00683	3	0.0	4172.1	**6.25 0.64 1997**	1	45/152	sp|q9nqr4|nit2_	(R)AVDNQVYVATASPARDDKASYVAWGHSTVVNPWGEVLAK
352	-----	00748	3	0.0	3854.9	**6.24 0.57 1942**	1	46/136	sp|q9nqr4|nit2_	(K)ECSIYLIGGSIPEEDAGKLYNTCAVFGPDGTLLAK
266	-----	00599	2	0.0	1986.0	**5.97 0.63 2257**	1	28/34	sp|q9nqr4|nit2_	(K)ECSIYLIGGSIPEEDAGK
108	-----	00314	3	0.0	2163.1	**5.96 0.44 2358**	1	42/76	sp|q9nqr4|nit2_	(R)SRAVDNQVYVATASPARDDK
264	-----	00596	2	0.0	1986.0	**5.90 0.61 1881**	1	25/34	sp|q9nqr4|nit2_	(K)ECSIYLIGGSIPEEDAGK
285	-----	00630	2	0.0	1888.0	**5.85 0.65 1936**	1	27/32	sp|q9nqr4|nit2_	(K)LYNTCAVFGPDGTLLAK
275	-----	00615	2	0.0	1888.0	**5.79 0.63 1766**	1	26/32	sp|q9nqr4|nit2_	(K)LYNTCAVFGPDGTLLAK
215	-----	00515	2	0.0	1751.9	**5.77 0.59 2232**	1	24/30	sp|q9nqr4|nit2_	(K)AGTEEAIVYSDIDLKK
360	-----	00762	4	0.0	3854.9	**5.73 0.60 2435**	1	62/204	sp|q9nqr4|nit2_	(K)ECSIYLIGGSIPEEDAGKLYNTCAVFGPDGTLLAK

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308 ----- 00675 4 0.0 4172.1 5.66 0.56 2099 1 67/228 sp|q9nqr4|nit2_ (R)AVDNQVYVATASPARDDKASYVAWGHSTVVNPWGEVLAK
107 ----- 00312 3 0.0 2163.1 5.62 0.42 2285 1 42/76 sp|q9nqr4|nit2_ (R)SRAVDNQVYVATASPARDDK
356 ----- 00757 4 0.0 3854.9 5.50 0.52 3048 1 65/204 sp|q9nqr4|nit2_ (K)ECSIYLIGGSIPEEDAGKLYNTCAVFGPDGTLLAK
642 ----- 01339 4 0.0 4172.1 5.49 0.50 2366 1 67/228 sp|q9nqr4|nit2_ (R)AVDNQVYVATASPARDDKASYVAWGHSTVVNPWGEVLAK
311 ----- 00679 4 0.0 4172.1 5.48 0.59 2515 1 67/228 sp|q9nqr4|nit2_ (R)AVDNQVYVATASPARDDKASYVAWGHSTVVNPWGEVLAK
437 ----- 00903 2 1.0 1886.9 5.47 0.60 1617 1 25/32 sp|q9nqr4|nit2_ (K)LYNTCAVFGPDGTLLAK
655 ----- 01372 2 0.0 1751.9 5.38 0.59 1961 1 24/30 sp|q9nqr4|nit2_ (K)AGTEEAIVYSDIDLKK
321 ----- 00696 2 0.0 1999.2 5.37 0.55 2318 1 25/34 sp|q9nqr4|nit2_ (R)LALIQLQISSIKSDNVTR
225 ----- 00531 2 0.0 1751.9 5.37 0.59 1847 1 24/30 sp|q9nqr4|nit2_ (K)AGTEEAIVYSDIDLKK
436 ----- 00901 2 1.0 1886.9 5.30 0.58 1664 1 25/32 sp|q9nqr4|nit2_ (K)LYNTCAVFGPDGTLLAK
389 ----- 00813 2 0.0 2889.5 5.28 0.73 1686 1 27/48 sp|q9nqr4|nit2_ (R)GCQLLVYPGAFNLTTGPAHWELLQR
661 ----- 01384 2 0.0 1751.9 5.27 0.59 1814 1 23/30 sp|q9nqr4|nit2_ (K)AGTEEAIVYSDIDLKK
387 ----- 00809 3 0.0 2889.5 5.26 0.57 1594 1 45/96 sp|q9nqr4|nit2_ (R)GCQLLVYPGAFNLTTGPAHWELLQR
320 ----- 00694 2 0.0 1999.2 5.23 0.58 2020 1 24/34 sp|q9nqr4|nit2_ (R)LALIQLQISSIKSDNVTR
124 ----- 00345 3 -1.0 2164.1 5.18 0.47 2121 1 40/76 sp|q9nqr4|nit2_ (R)SRAVDNQVYVATASPARDDK
653 ----- 01365 2 0.0 2629.3 5.18 0.65 1240 1 21/46 sp|q9nqr4|nit2_ (R)DDKASYVAWGHSTVVNPWGEVLAK
342 ----- 00732 2 0.0 2629.3 5.17 0.59 1488 1 25/46 sp|q9nqr4|nit2_ (R)DDKASYVAWGHSTVVNPWGEVLAK
595 ----- 01223 3 0.0 4172.1 5.15 0.46 1803 1 42/152 sp|q9nqr4|nit2_ (R)AVDNQVYVATASPARDDKASYVAWGHSTVVNPWGEVLAK
260 ----- 00591 3 0.0 1986.0 5.15 0.44 908 3 33/68 sp|q9nqr4|nit2_ (K)ECSIYLIGGSIPEEDAGK
185 ----- 00453 2 0.0 1561.8 5.11 0.62 1878 1 21/28 sp|q9nqr4|nit2_ (R)AVDNQVYVATASPAR
390 ----- 00818 4 0.0 2889.5 5.10 0.62 2053 1 54/144 sp|q9nqr4|nit2_ (R)GCQLLVYPGAFNLTTGPAHWELLQR
393 ----- 00823 3 0.0 2889.5 5.10 0.60 1147 1 40/96 sp|q9nqr4|nit2_ (R)GCQLLVYPGAFNLTTGPAHWELLQR
134 ----- 00361 2 0.0 1804.9 5.06 0.58 1646 1 27/32 sp|q9nqr4|nit2_ (R)SRAVDNQVYVATASPAR
496 ----- 01009 3 0.0 3854.9 5.06 0.61 1879 1 44/136 sp|q9nqr4|nit2_ (K)ECSIYLIGGSIPEEDAGKLYNTCAVFGPDGTLLAK
268 ----- 00603 2 0.0 2613.3 5.04 0.64 1307 1 25/46 sp|q9nqr4|nit2_ (K)LSEVAKECSIYLIGGSIPEEDAGK
533 ----- 01089 2 1.0 2270.2 5.00 0.62 1419 1 24/40 sp|q9nqr4|nit2_ (K)ASYVAWGHSTVVNPWGEVLAK
339 ----- 00728 2 0.0 2629.3 4.99 0.67 1452 1 25/46 sp|q9nqr4|nit2_ (R)DDKASYVAWGHSTVVNPWGEVLAK
221 ----- 00523 2 0.0 2514.3 4.98 0.57 1113 1 26/42 sp|q9nqr4|nit2_ (K)YFPEYAEKIPGESTQKLSEVAK
151 ----- 00392 2 0.0 1804.9 4.88 0.50 1632 1 27/32 sp|q9nqr4|nit2_ (R)SRAVDNQVYVATASPAR
280 ----- 00624 3 0.0 1888.0 4.81 0.42 2053 1 35/64 sp|q9nqr4|nit2_ (K)LYNTCAVFGPDGTLLAK
155 ----- 00402 2 0.0 1561.8 4.80 0.67 1989 1 21/28 sp|q9nqr4|nit2_ (R)AVDNQVYVATASPAR
200 ----- 00486 2 0.0 1886.9 4.69 0.46 1474 1 25/30 sp|q9nqr4|nit2_ (K)YFPEYAEKIPGESTQK
157 ----- 00405 2 0.0 1561.8 4.68 0.64 1659 1 21/28 sp|q9nqr4|nit2_ (R)AVDNQVYVATASPAR
346 ----- 00740 3 0.0 2271.2 4.68 0.47 2402 1 39/80 sp|q9nqr4|nit2_ (K)ASYVAWGHSTVVNPWGEVLAK
283 ----- 00628 2 0.0 1623.8 4.66 0.45 2044 1 22/28 sp|q9nqr4|nit2_ (K)AGTEEAIVYSDIDLK
376 ----- 00789 2 0.0 1326.8 4.65 0.54 1859 1 19/22 sp|q9nqr4|nit2_ (R)LALIQLQISSIK
343 ----- 00736 3 0.0 2271.2 4.62 0.49 2479 1 40/80 sp|q9nqr4|nit2_ (K)ASYVAWGHSTVVNPWGEVLAK
659 ----- 01377 2 0.0 1986.0 4.59 0.55 1402 1 23/34 sp|q9nqr4|nit2_ (K)ECSIYLIGGSIPEEDAGK
555 ----- 01133 2 0.0 1985.9 4.58 0.54 1297 1 22/34 sp|q9nqr4|nit2_ (K)ECSIYLIGGSIPEEDAGK
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123 ----- 00344 2 0.0 1486.8 4.48 0.49 979 1 21/26 sp|q9nqr4|nit2_ (K)IPGESTQKLSEVAK
548 ----- 01120 2 0.0 2629.3 4.46 0.67 976 1 20/46 sp|q9nqr4|nit2_ (R)DDKASYVAWGHSTVVNPWGEVLAK
253 ----- 00579 2 0.0 1998.9 4.42 0.59 698 1 19/32 sp|q9nqr4|nit2_ (K)TLSPGDSFSTFDTPYCR
543 ----- 01113 2 0.0 1623.8 4.41 0.47 2143 1 22/28 sp|q9nqr4|nit2_ (K)AGTEEAIVYSDIDLK
263 ----- 00595 3 0.0 1986.0 4.36 0.44 1074 1 35/68 sp|q9nqr4|nit2_ (K)ECSIYLIGGSIPEEDAGK
292 ----- 00645 2 0.0 1623.8 4.36 0.47 2039 1 22/28 sp|q9nqr4|nit2_ (K)AGTEEAIVYSDIDLK
638 ----- 01334 2 0.0 1623.8 4.34 0.44 2148 1 22/28 sp|q9nqr4|nit2_ (K)AGTEEAIVYSDIDLK
447 ----- 00921 4 0.0 4172.1 4.29 0.50 2643 1 66/228 sp|q9nqr4|nit2_ (R)AVDNQVYVATASPARDDKASYVAWGHSTVVNPWGEVLAK
554 ----- 01132 2 0.0 1998.9 4.23 0.35 706 1 19/32 sp|q9nqr4|nit2_ (K)TLSPGDSFSTFDTPYCR
633 ----- 01323 2 0.0 1623.8 4.23 0.46 2277 1 23/28 sp|q9nqr4|nit2_ (K)AGTEEAIVYSDIDLK
552 ----- 01128 2 0.0 1623.8 4.22 0.42 1879 1 21/28 sp|q9nqr4|nit2_ (K)AGTEEAIVYSDIDLK
249 ----- 00569 2 0.0 1998.9 4.18 0.60 984 1 22/32 sp|q9nqr4|nit2_ (K)TLSPGDSFSTFDTPYCR
641 ----- 01338 2 0.0 1998.9 4.16 0.30 746 1 19/32 sp|q9nqr4|nit2_ (K)TLSPGDSFSTFDTPYCR
272 ----- 00611 2 0.0 1309.7 4.15 0.36 1989 1 18/20 sp|q9nqr4|nit2_ (R)FAELAQIYAQR
353 ----- 00750 2 0.0 3854.9 4.14 0.64 298 1 21/68 sp|q9nqr4|nit2_ (K)ECSIYLIGGSIPEEDAGKLYNTCAVFGPDGTLLAK
350 ----- 00745 2 -1.0 2000.1 4.12 0.34 2501 1 25/34 sp|q9nqr4|nit2_ (R)LALIQLQISSIKSDNVTR
284 ----- 00629 3 0.0 1888.0 4.11 0.42 1760 1 33/64 sp|q9nqr4|nit2_ (K)LYNTCAVFGPDGTLLAK
645 ----- 01346 2 0.0 1561.8 4.10 0.66 1465 1 21/28 sp|q9nqr4|nit2_ (R)AVDNQVYVATASPAR
443 ----- 00916 2 0.0 1623.8 4.07 0.45 2018 1 22/28 sp|q9nqr4|nit2_ (K)AGTEEAIVYSDIDLK
133 ----- 00360 2 0.0 1919.9 4.07 0.57 1049 1 20/34 sp|q9nqr4|nit2_ (R)AVDNQVYVATASPARDDK
382 ----- 00801 2 0.0 1326.8 4.05 0.46 1980 1 19/22 sp|q9nqr4|nit2_ (R)LALIQLQISSIK
430 ----- 00887 2 0.0 1623.8 4.02 0.42 1725 1 20/28 sp|q9nqr4|nit2_ (K)AGTEEAIVYSDIDLK
405 ----- 00843 2 0.0 1998.9 3.96 0.15 782 2 19/32 sp|q9nqr4|nit2_ (K)TLSPGDSFSTFDTPYCR
639 ----- 01335 2 0.0 1561.8 3.95 0.62 1546 1 21/28 sp|q9nqr4|nit2_ (R)AVDNQVYVATASPAR
634 ----- 01325 2 0.0 1888.0 3.93 0.10 1726 1 24/32 sp|q9nqr4|nit2_ (K)LYNTCAVFGPDGTLLAK
364 ----- 00770 3 0.0 1999.2 3.91 0.49 1289 1 32/68 sp|q9nqr4|nit2_ (R)LALIQLQISSIKSDNVTR
530 ----- 01085 3 0.0 2629.3 3.88 0.36 1354 1 37/92 sp|q9nqr4|nit2_ (R)DDKASYVAWGHSTVVNPWGEVLAK
318 ----- 00692 3 0.0 1999.2 3.87 0.58 1358 1 33/68 sp|q9nqr4|nit2_ (R)LALIQLQISSIKSDNVTR
494 ----- 01005 2 0.0 1326.8 3.85 0.53 1984 1 19/22 sp|q9nqr4|nit2_ (R)LALIQLQISSIK
640 ----- 01336 2 0.0 1998.9 3.82 0.26 792 1 19/32 sp|q9nqr4|nit2_ (K)TLSPGDSFSTFDTPYCR
491 ----- 01001 2 0.0 1326.8 3.80 0.53 1989 1 19/22 sp|q9nqr4|nit2_ (R)LALIQLQISSIK
418 ----- 00867 2 0.0 1309.7 3.80 0.32 2058 1 18/20 sp|q9nqr4|nit2_ (R)FAELAQIYAQR
281 ----- 00625 2 0.0 1309.7 3.80 0.38 2073 1 18/20 sp|q9nqr4|nit2_ (R)FAELAQIYAQR
458 ----- 00943 2 0.0 1561.8 3.79 0.63 1304 1 20/28 sp|q9nqr4|nit2_ (R)AVDNQVYVATASPAR
561 ----- 01147 2 0.0 1998.9 3.77 0.32 768 1 19/32 sp|q9nqr4|nit2_ (K)TLSPGDSFSTFDTPYCR
135 ----- 00363 2 0.0 1919.9 3.76 0.52 1112 1 19/34 sp|q9nqr4|nit2_ (R)AVDNQVYVATASPARDDK
582 ----- 01195 2 0.0 1326.8 3.75 0.53 2061 1 19/22 sp|q9nqr4|nit2_ (R)LALIQLQISSIK
392 ----- 00822 4 0.0 2889.5 3.75 0.50 1492 1 53/144 sp|q9nqr4|nit2_ (R)GCQLLVYPGAFNLTTGPAHWELLQR
553 ----- 01129 2 0.0 1561.8 3.73 0.68 1564 1 21/28 sp|q9nqr4|nit2_ (R)AVDNQVYVATASPAR
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325 ----- 00704 3 0.0 1999.2 3.71 0.52 1388 1 33/68 sp|q9nqr4|nit2_ (R)LALIQLQISSIKSDNVTR
420 ----- 00870 2 0.0 1309.7 3.68 0.36 2006 1 18/20 sp|q9nqr4|nit2_ (R)FAELAQIYAQR
461 ----- 00947 2 0.0 1998.9 3.65 0.09 601 1 18/32 sp|q9nqr4|nit2_ (K)TLSPGDSFSTFDTPYCR
646 ----- 01348 2 0.0 1729.9 3.61 0.59 577 1 20/28 sp|q9nqr4|nit2_ (K)IVSLPECFNSPYGAK
565 ----- 01157 2 0.0 1729.9 3.61 0.58 497 1 18/28 sp|q9nqr4|nit2_ (K)IVSLPECFNSPYGAK
244 ----- 00563 2 0.0 1729.9 3.61 0.57 595 1 20/28 sp|q9nqr4|nit2_ (K)IVSLPECFNSPYGAK
606 ----- 01251 2 0.0 1309.7 3.60 0.35 1728 1 17/20 sp|q9nqr4|nit2_ (R)FAELAQIYAQR
665 ----- 01393 2 0.0 1326.8 3.59 0.52 1634 1 18/22 sp|q9nqr4|nit2_ (R)LALIQLQISSIK
576 ----- 01183 2 -0.1 1309.8 3.59 0.34 1753 1 17/20 sp|q9nqr4|nit2_ (R)FAELAQIYAQR
573 ----- 01179 2 -0.1 1309.8 3.58 0.25 1958 1 18/20 sp|q9nqr4|nit2_ (R)FAELAQIYAQR
291 ----- 00644 2 -1.0 1310.7 3.57 0.36 2100 1 18/20 sp|q9nqr4|nit2_ (R)FAELAQIYAQR
662 ----- 01385 2 0.0 1326.8 3.56 0.54 1889 1 19/22 sp|q9nqr4|nit2_ (R)LALIQLQISSIK
584 ----- 01202 2 0.0 1326.8 3.56 0.55 1924 1 19/22 sp|q9nqr4|nit2_ (R)LALIQLQISSIK
231 ----- 00539 2 -1.0 2515.3 3.49 0.42 1038 1 25/42 sp|q9nqr4|nit2_ (K)YFPEYAEKIPGESTQKLSEVAK
455 ----- 00935 2 0.0 1998.9 3.49 0.07 505 1 18/32 sp|q9nqr4|nit2_ (K)TLSPGDSFSTFDTPYCR
651 ----- 01359 2 0.0 1729.9 3.45 0.62 481 1 18/28 sp|q9nqr4|nit2_ (K)IVSLPECFNSPYGAK
594 ----- 01220 4 0.0 2889.5 3.42 0.34 1282 2 48/144 sp|q9nqr4|nit2_ (R)GCQLLVYPGAFNLTTGPAHWELLQR
335 ----- 00722 4 0.0 2334.3 3.41 0.44 1505 1 45/120 sp|q9nqr4|nit2_ (K)AGTEEAIVYSDIDLKKLAEIR
130 ----- 00356 3 0.0 1804.9 3.40 0.46 1414 1 29/64 sp|q9nqr4|nit2_ (R)SRAVDNQVYVATASPAR
560 ----- 01144 2 0.0 1729.9 3.37 0.59 516 1 18/28 sp|q9nqr4|nit2_ (K)IVSLPECFNSPYGAK
608 ----- 01254 2 0.0 1309.7 3.36 0.36 2056 1 18/20 sp|q9nqr4|nit2_ (R)FAELAQIYAQR
559 ----- 01143 2 0.0 1561.8 3.36 0.53 570 3 14/28 sp|q9nqr4|nit2_ (R)AVDNQVYVATASPAR
462 ----- 00948 2 0.0 1729.9 3.31 0.56 526 1 19/28 sp|q9nqr4|nit2_ (K)IVSLPECFNSPYGAK
519 ----- 01061 3 1.0 2270.2 3.30 0.25 725 1 35/80 sp|q9nqr4|nit2_ (K)ASYVAWGHSTVVNPWGEVLAK
111 ----- 00318 2 0.0 2163.1 3.29 0.34 322 2 21/38 sp|q9nqr4|nit2_ (R)SRAVDNQVYVATASPARDDK
500 ----- 01017 4 0.0 2889.5 3.29 0.46 1259 3 45/144 sp|q9nqr4|nit2_ (R)GCQLLVYPGAFNLTTGPAHWELLQR
453 ----- 00932 2 0.0 1561.8 3.22 0.58 908 1 18/28 sp|q9nqr4|nit2_ (R)AVDNQVYVATASPAR
499 ----- 01015 4 0.0 2889.5 3.22 0.43 1176 4 47/144 sp|q9nqr4|nit2_ (R)GCQLLVYPGAFNLTTGPAHWELLQR
628 ----- 01303 2 1.0 1918.9 3.21 0.47 1084 1 20/34 sp|q9nqr4|nit2_ (R)AVDNQVYVATASPARDDK
205 ----- 00495 3 0.0 1886.9 3.20 0.36 742 1 28/60 sp|q9nqr4|nit2_ (K)YFPEYAEKIPGESTQK
226 ----- 00532 3 0.0 2514.3 3.12 0.51 943 1 39/84 sp|q9nqr4|nit2_ (K)YFPEYAEKIPGESTQKLSEVAK
345 ----- 00738 3 -1.0 2000.1 3.11 0.45 1758 1 36/68 sp|q9nqr4|nit2_ (R)LALIQLQISSIKSDNVTR
213 ----- 00512 3 0.0 1751.9 3.07 0.58 594 2 28/60 sp|q9nqr4|nit2_ (K)AGTEEAIVYSDIDLKK
521 ----- 01068 2 1.0 1308.7 3.06 0.38 1572 1 18/20 sp|q9nqr4|nit2_ (R)FAELAQIYAQR
131 ----- 00357 3 0.0 1919.9 3.01 0.41 601 1 36/68 sp|q9nqr4|nit2_ (R)AVDNQVYVATASPARDDK
152 ----- 00394 2 -1.0 1920.9 2.98 0.35 1301 1 21/34 sp|q9nqr4|nit2_ (R)AVDNQVYVATASPARDDK
422 ----- 00872 4 0.0 2629.3 2.97 0.34 926 2 41/138 sp|q9nqr4|nit2_ (R)DDKASYVAWGHSTVVNPWGEVLAK
571 ----- 01170 2 0.9 1308.8 2.94 0.16 1325 1 17/20 sp|q9nqr4|nit2_ (R)FAELAQIYAQR
222 ----- 00527 3 0.0 1751.9 2.91 0.55 599 1 29/60 sp|q9nqr4|nit2_ (K)AGTEEAIVYSDIDLKK
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621 ----- 01286 3 1.0 2270.2 2.86 0.25 526 6 33/80 sp|q9nqr4|nit2_ (K)ASYVAWGHSTVVNPWGEVLAK
142 ----- 00375 2 -1.0 1487.8 2.86 0.38 1058 1 21/26 sp|q9nqr4|nit2_ (K)IPGESTQKLSEVAK
522 ----- 01069 3 0.0 1751.9 2.83 0.56 702 1 29/60 sp|q9nqr4|nit2_ (K)AGTEEAIVYSDIDLKK
615 ----- 01274 3 0.0 1751.9 2.80 0.56 683 1 30/60 sp|q9nqr4|nit2_ (K)AGTEEAIVYSDIDLKK
475 ----- 00973 3 1.0 2270.2 2.78 0.15 477 25 28/80 sp|q9nqr4|nit2_ (K)ASYVAWGHSTVVNPWGEVLAK
140 ----- 00372 2 -1.0 1487.8 2.77 0.33 1218 1 22/26 sp|q9nqr4|nit2_ (K)IPGESTQKLSEVAK
254 ----- 00580 2 -1.0 1730.8 2.77 0.52 607 1 19/28 sp|q9nqr4|nit2_ (K)IVSLPECFNSPYGAK
337 ----- 00725 4 0.0 2629.3 2.72 0.37 1761 1 52/138 sp|q9nqr4|nit2_ (R)DDKASYVAWGHSTVVNPWGEVLAK
163 ----- 00417 3 0.0 1561.8 2.69 0.31 872 1 30/56 sp|q9nqr4|nit2_ (R)AVDNQVYVATASPAR
419 ----- 00868 3 0.0 1751.9 2.68 0.54 573 1 29/60 sp|q9nqr4|nit2_ (K)AGTEEAIVYSDIDLKK
224 ----- 00529 3 -1.0 1598.9 2.62 0.24 900 4 26/48 sp|q9nqr4|nit2_ (K)KLAEIRQQIPVFR
518 ----- 01057 3 0.0 1751.9 2.60 0.48 564 1 27/60 sp|q9nqr4|nit2_ (K)AGTEEAIVYSDIDLKK
490 ----- 01000 3 0.3 1998.9 2.58 0.46 871 1 29/68 sp|q9nqr4|nit2_ (R)LALIQLQISSIKSDNVTR
616 ----- 01275 4 0.0 2629.3 2.58 0.26 812 2 40/138 sp|q9nqr4|nit2_ (R)DDKASYVAWGHSTVVNPWGEVLAK
524 ----- 01073 4 0.0 2629.3 2.57 0.21 831 5 40/138 sp|q9nqr4|nit2_ (R)DDKASYVAWGHSTVVNPWGEVLAK
119 ----- 00333 2 0.0 2163.1 2.56 0.22 581 1 22/38 sp|q9nqr4|nit2_ (R)SRAVDNQVYVATASPARDDK
508 ----- 01034 3 0.0 1919.9 2.56 0.30 342 5 25/68 sp|q9nqr4|nit2_ (R)AVDNQVYVATASPARDDK
132 ----- 00359 3 0.0 1919.9 2.55 0.38 848 1 39/68 sp|q9nqr4|nit2_ (R)AVDNQVYVATASPARDDK
611 ----- 01263 3 0.0 1751.9 2.54 0.50 445 2 26/60 sp|q9nqr4|nit2_ (K)AGTEEAIVYSDIDLKK
431 ----- 00888 4 1.0 2629.3 2.48 0.03 655 24 37/120 sp|q6b0i6|jhd3d (R)VTFSM*DAFVRILQPERYDLWK
529 ----- 01084 4 0.0 2629.3 2.43 0.08 1108 1 46/138 sp|q9nqr4|nit2_ (R)DDKASYVAWGHSTVVNPWGEVLAK
192 ----- 00465 2 0.0 1071.5 2.42 0.34 661 2 12/16 sp|q9nqr4|nit2_ (R)SDLYAVEM*K
556 ----- 01138 3 0.3 1998.9 2.42 0.46 803 1 28/68 sp|q9nqr4|nit2_ (R)LALIQLQISSIKSDNVTR
599 ----- 01231 4 0.0 2889.5 2.39 0.12 933 5 44/144 sp|q9nqr4|nit2_ (R)GCQLLVYPGAFNLTTGPAHWELLQR
664 ----- 01392 3 0.3 1998.9 2.32 0.19 696 1 26/68 sp|q9nqr4|nit2_ (R)LALIQLQISSIKSDNVTR
660 ----- 01382 3 0.3 1998.9 2.29 0.24 795 1 29/68 sp|q9nqr4|nit2_ (R)LALIQLQISSIKSDNVTR
677 ----- 01478 2 0.0 1309.7 2.29 0.34 579 1 14/20 sp|q9nqr4|nit2_ (R)FAELAQIYAQR
618 ----- 01278 4 0.0 2629.3 2.28 0.02 647 18 36/138 sp|q9nqr4|nit2_ (R)DDKASYVAWGHSTVVNPWGEVLAK
409 ----- 00852 3 0.0 1751.9 2.25 0.36 346 31 23/60 sp|q9nqr4|nit2_ (K)AGTEEAIVYSDIDLKK
434 ----- 00899 3 0.3 1998.9 2.23 0.42 818 1 28/68 sp|q9nqr4|nit2_ (R)LALIQLQISSIKSDNVTR
534 ----- 01094 3 0.3 1998.9 2.17 0.41 500 1 26/68 sp|q9nqr4|nit2_ (R)LALIQLQISSIKSDNVTR
148 ----- 00387 2 0.0 1227.6 2.09 0.40 335 1 15/18 sp|q9nqr4|nit2_ (K)RSDLYAVEM*K
197 ----- 00480 2 0.0 1071.5 2.08 0.39 666 2 12/16 sp|q9nqr4|nit2_ (R)SDLYAVEM*K
149 ----- 00388 3 0.0 1227.6 2.01 0.17 761 28 18/36 sp|q9nqr4|nit2_ (K)RSDLYAVEM*K
251 ----- 00576 3 0.0 1998.9 2.00 0.36 557 1 29/64 sp|q9nqr4|nit2_ (K)TLSPGDSFSTFDTPYCR
384 ----- 00803 3 0.0 1326.8 1.94 0.13 500 43 21/44 sp|q9nqr4|nit2_ (R)LALIQLQISSIK
261 ----- 00592 3 -1.0 1470.8 1.94 0.07 959 1 25/44 sp|q9nqr4|nit2_ (K)LAEIRQQIPVFR
105 ----- 00310 2 0.0 801.4 1.94 0.26 208 1 9/10 sp|q9nqr4|nit2_ (R)ACSFIR
 35 ----- 00145 2 0.0 859.5 1.80 0.29 514 1 13/14 sp|q9nqr4|nit2_ (K)IPGESTQK
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619 ----- 01279 3 0.0 1919.9 1.77 0.00 372 1 28/68 sp|q9nqr4|nit2_ (R)AVDNQVYVATASPARDDK
243 ----- 00561 3 3.1 1998.9 1.77 0.11 331 23 23/64 sp|q9nrw1|rab6b (K)TDLADKRQITIEEGEQR
214 ----- 00513 3 0.0 1231.6 1.71 0.30 886 1 23/36 sp|q9nqr4|nit2_ (R)VGLGICYDMR
184 ----- 00451 2 0.0 1247.6 1.67 0.45 758 1 14/18 sp|q9nqr4|nit2_ (R)VGLGICYDM*R
512 ----- 01044 3 0.0 1729.9 1.63 0.18 225 14 22/56 sp|q9nqr4|nit2_ (K)IVSLPECFNSPYGAK
551 ----- 01126 2 0.0 1247.6 1.55 0.23 454 4 12/18 sp|q9nqr4|nit2_ (R)VGLGICYDM*R
190 ----- 00463 2 0.0 1046.5 1.46 0.31 244 3 11/14 sp|q9nqr4|nit2_ (K)YFPEYAEK
183 ----- 00449 2 0.0 1046.5 1.44 0.31 297 2 12/14 sp|q9nqr4|nit2_ (K)YFPEYAEK
598 ----- 01229 2 0.0 1231.6 1.43 0.06 519 2 13/18 sp|q9nqr4|nit2_ (R)VGLGICYDMR
139 ----- 00371 3 -1.0 1487.8 1.42 0.26 423 4 28/52 sp|q9nqr4|nit2_ (K)IPGESTQKLSEVAK
603 ----- 01240 3 0.0 1729.9 1.40 0.27 474 1 27/56 sp|q9nqr4|nit2_ (K)IVSLPECFNSPYGAK
223 ----- 00528 4 0.9 2514.3 1.39 0.02 511 9 47/144 sp|q9y6j0|cabin (R)RDGEAQEAASETQPLSSPPTAASSK
391 ----- 00819 3 0.0 1729.9 1.35 0.24 485 1 27/56 sp|q9nqr4|nit2_ (K)IVSLPECFNSPYGAK
540 ----- 01109 2 0.0 1247.6 1.35 0.27 536 1 13/18 sp|q9nqr4|nit2_ (R)VGLGICYDM*R
607 ----- 01252 3 0.0 1729.9 1.34 0.07 324 1 23/56 sp|q9nqr4|nit2_ (K)IVSLPECFNSPYGAK
233 ----- 00544 4 -2.1 2515.3 1.32 0.00 525 50 40/138 sp|q9c000|nalp1 (R)KPSVMTPTEGLDTGEM*SNSTSSLK
632 ----- 01320 2 0.0 1247.6 1.31 0.19 554 1 13/18 sp|q9nqr4|nit2_ (R)VGLGICYDM*R
241 ----- 00559 3 0.0 1729.9 1.25 0.23 476 1 26/56 sp|q9nqr4|nit2_ (K)IVSLPECFNSPYGAK
505 ----- 01029 3 0.0 1729.9 1.23 0.16 387 1 24/56 sp|q9nqr4|nit2_ (K)IVSLPECFNSPYGAK
400 ----- 00836 3 0.0 1729.9 1.20 0.24 503 1 26/56 sp|q9nqr4|nit2_ (K)IVSLPECFNSPYGAK
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Fig. S6: Data obtained for the LC/MS/MS spectral analysis of (A) wild type hNit2 and (B) the Δ116-128 mutant over expressed in *E. coli*.
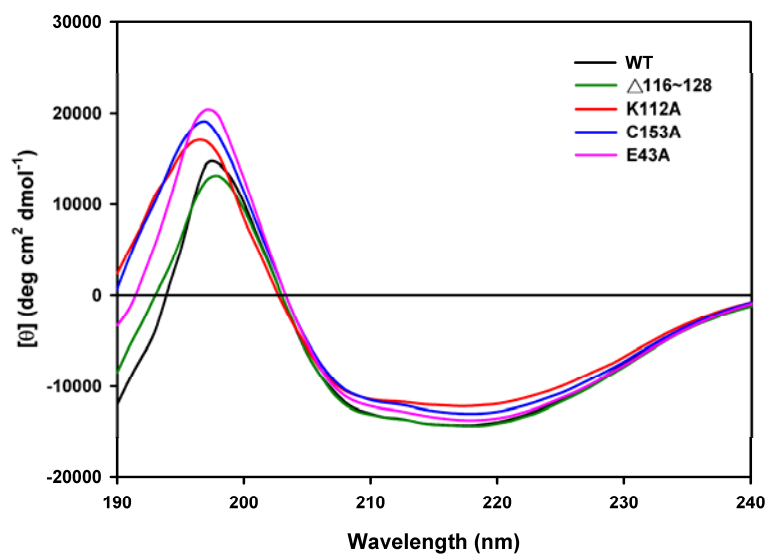
Fig. S7: CD spectra of wild type hNit2, three catalytic triad mutants and the Δ116-128 mutant. Spectra of wild type hNit2 (black), Δ116-128 (green), K112A (orange), C153A (blue) and E43A (pink) are shown. The protein concentration of each sample was 4.7 μM in 20 mM phosphate buffer, pH 7.4. All spectra were obtained at 25 °C.
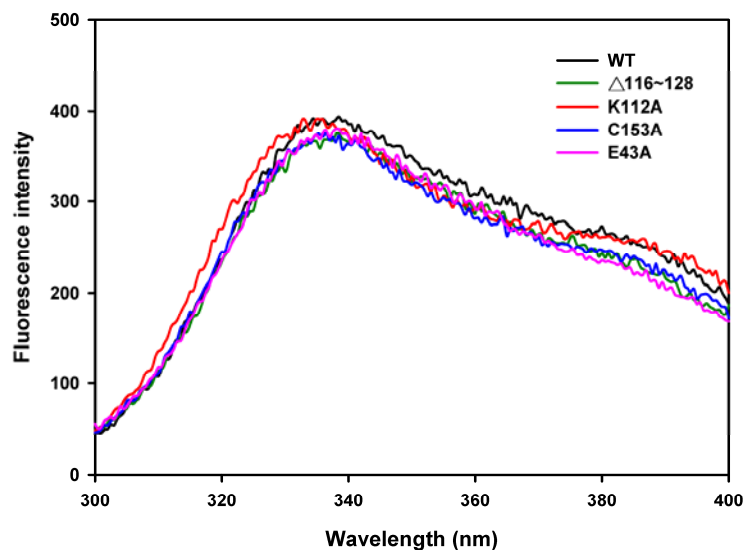

Fig. S8: Fluorescence spectra of wild type hNit2, three catalytic triad mutants and the Δ116-128 mutant. Spectra of wild type hNit2 (black), Δ116-128 (green), K112A (orange), C153A (blue) and E43A (pink) are shown. The protein concentration was 3.2 μM in 20 mM phosphate buffer, pH 7.4. The values for the relative fluorescence emission units are the average of three repeated scans. All spectra were obtained at 25 °C.
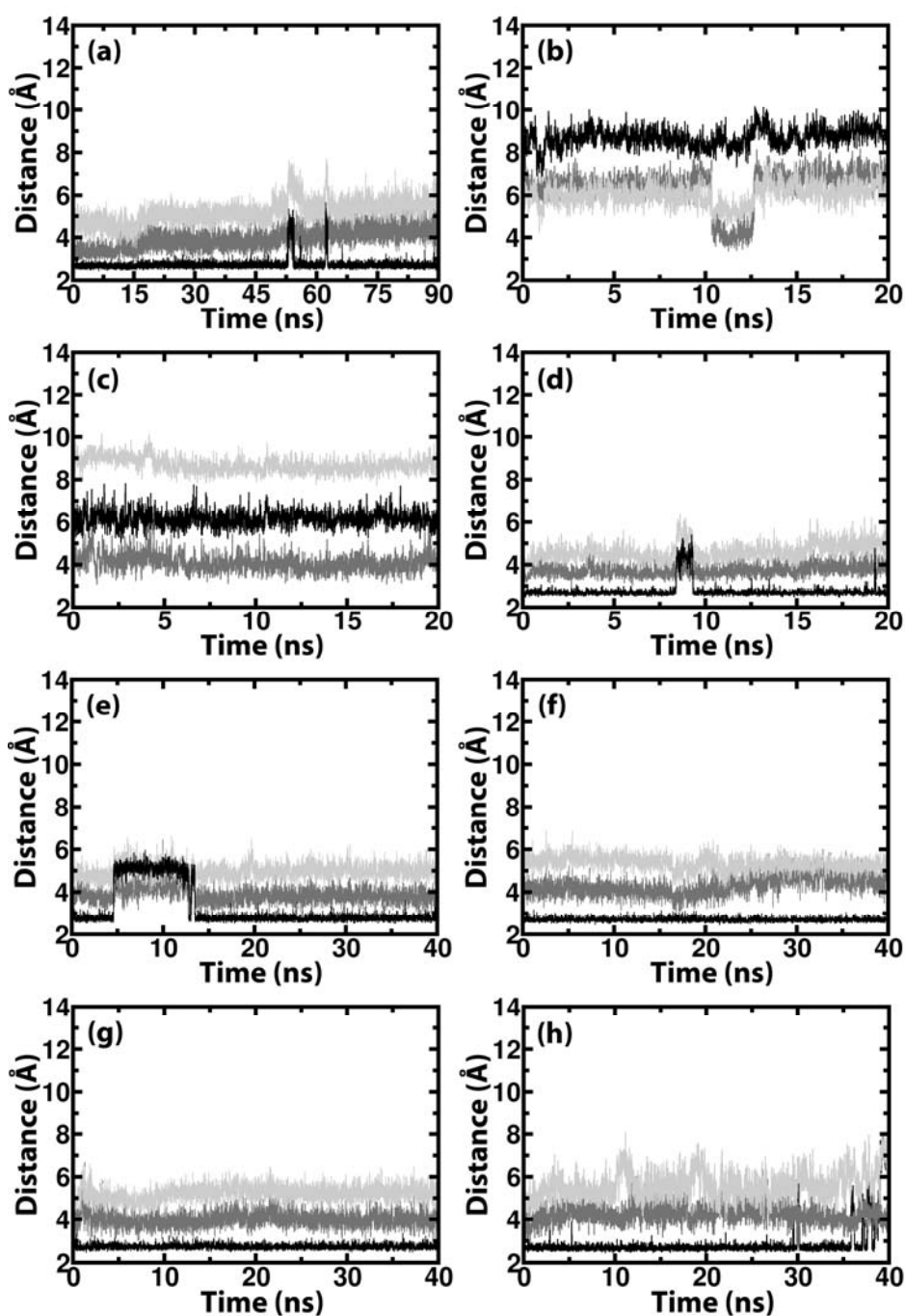
Fig. S9: Analyses of the catalytic triad cavity of wild type hNit2, four mutants and hNit2-substrate complexes. Fluctuations of the distances of OE1 (Glu43) --- NZ (Lys112) (black), OE2 (Glu43) --- S (Cys153) (dark gray), and NZ (Lys112) --- S (Cys153) (light gray) for (**a**) hNit2, (**e**) hNit2-KGM complex, (**f**) hNit2-KSM complex**, (g)** hNit2-SM complex, and (**h**) Δ116-128; Fluctuations of the distances of CB (Ala43) --- NZ (Lys112) (black), CB (Ala43) --- S (Cys153) (dark gray), and NZ (Lys112) --- S (Cys153) (light gray) for (**b**) E43A; Fluctuations of the distances of OE1 (Glu43) --- CB (Ala112) (black), OE1 (Glu43) --- S (Cys153) (dark gray), and CB (Ala112) --- S (Cys153) (light gray) for **(c)** K112A; Fluctuations of the distances of OE1 (Glu43) --- NZ (Lys112) (black), OE1 (Glu43) --- CB (Ala153) (dark gray), and NZ (Lys112) --- CB (Ala153) (light gray) for **(d)** C153A. In each case the data were calculated from MD simulations for the wild type hNit2, all mutants and substrate-bound complexes.
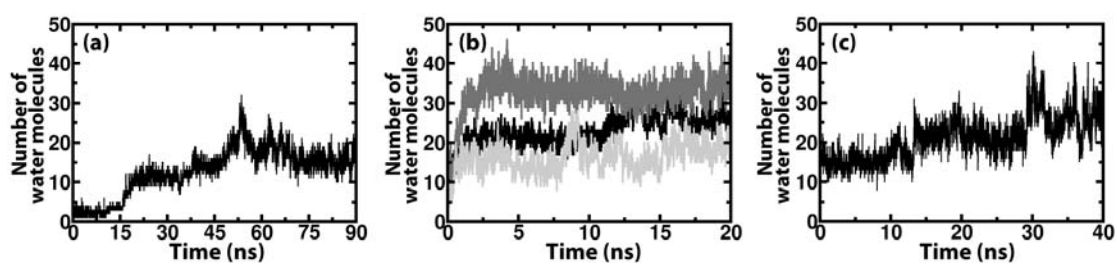
Fig. S10: Analysis of the size of the active site cavity. Profile of the number of water molecules estimated to fill the active site cavity of **(a)** the wild type hNit2, **(b)** three catalytic triad mutants: E43A (black), K112A (dark gray), and C153A (gray), and **(c)** Δ116-128.
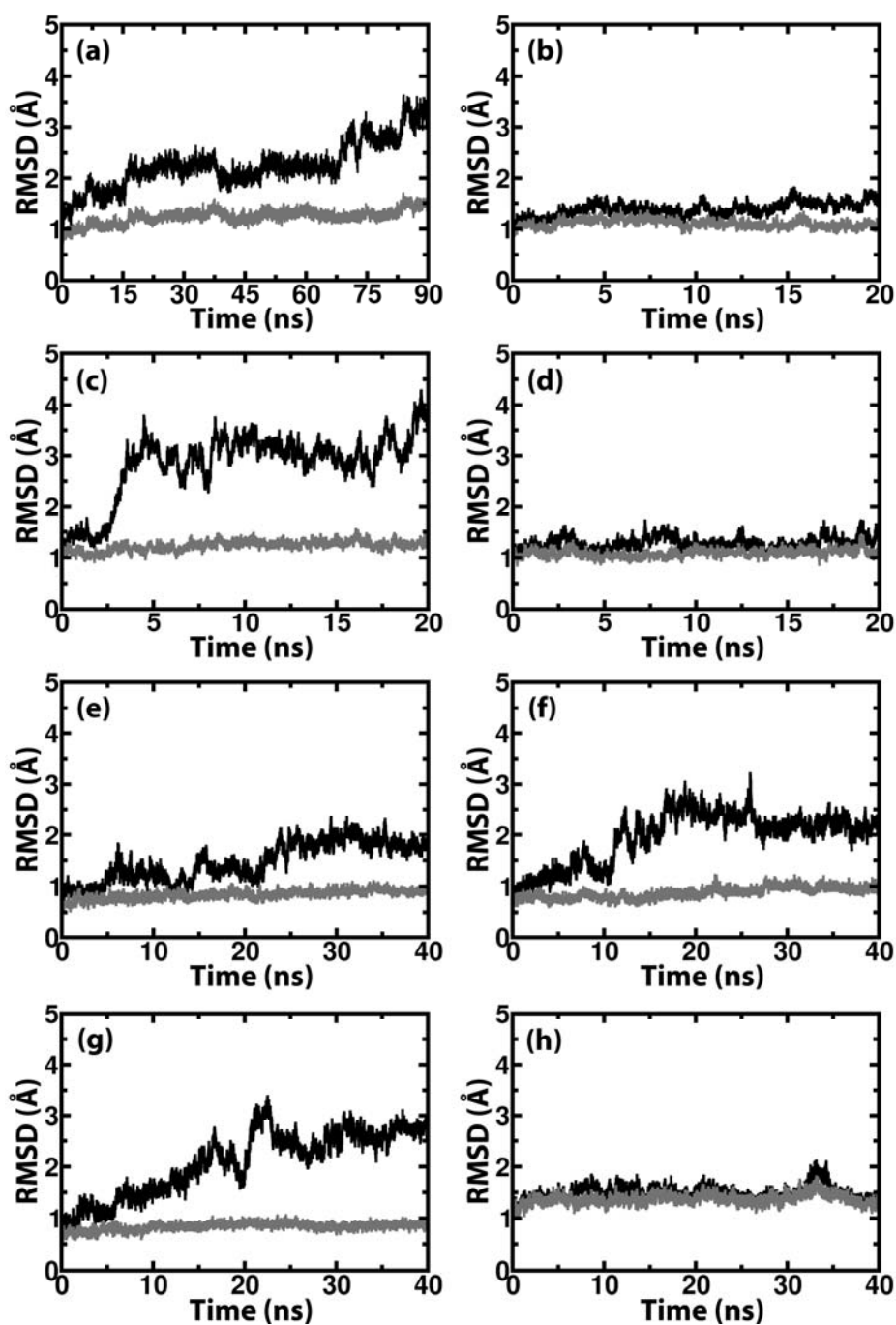
Fig. S11: Structural analysis of hNit2, four mutants and hNit2-substrate complexes. RMSD of the hNit2 backbone atoms (residues 1–260) (black) and that of the structure lacking the loop (residue 116-128), N-terminus (residue 1-4), and C-terminus (residue 245-260) (dark gray) are shown for **(a)** hNit2, **(b)** E43A, **(c)** K112A, **(d)** C153A, **(e)** hNit2-KGM complex, **(f)** hNit2-KSM complex, **(g)** hNit2-SM complex, and **(h)** Δ116-128. The C terminus (residues 261-276) of the hNit2 monomer is a random coil and not calculated. These data show that the core structure of each form remained intact during MD simulations for the wild type hNit2, all mutants, and substrate-bound complexes.

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