

Table S1. Analysis of physicochemical properties of full-length PNT3 *wt*, PNT3 truncated and C-terminal region as provided by CIDER (<http://pappulab.wustl.edu/CIDER/>, accessed on 24 October 2022) [1].

	PNT3 <i>wt</i>	PNT3_trunc	C-terminal
N	133	77	56
f-	0.22556	0.19481	0.26786
f+	0.09774	0.09091	0.10714
FCR	0.32331	0.28571	0.37500
NCPR	-0.12782	-0.10390	-0.16071
Kappa	0.25641	0.36530	0.17898
Hydropathy	3.27594	3.25584	3.30357
Phase Plot Region	2	2	3
Phase Plot Annotation	Boundary Region	Boundary region	Coils,Hairpins and Chimeras
Net charge at pH 7.0	-16.34	-7.44	

N: Number of residues; **f-:** Fraction of negatively charged residues; **f+:** Fraction of positively charged residues; **FCR:** Fraction of charged residues; **NCPR:** Net charge per residue; **Kappa:** κ (charge patterning parameter); **Hydropathy:** The 0-9 scaled Kyte-Doolittle hydropathy score for the sequence (9 most hydrophobic, 0 least hydrophobic); **Phase Plot Region:** Location on the Das-Pappu phase plot this sequence falls; **Phase Plot Annotation:** Annotation associated with a specific region of the Das-Pappu phase plot.

Table S2. Nucleotide sequence of the primers used to generate the various constructs.

Primer Name	Sequence (5'-3')	Name of the construct	Reference
HeV PNT3-AttB1	GGGGACAAGTTTGTACAAAAAGCAGGCTTCACCCGACCGAAGAACC GCCG	PNT3-pDEST17	Salladini, et al., 2021
HeV PNT3-AttB2	GGGGACCACTTTGTACAAGAAAGCTGGGTCTTATTAGCTATCTTTACGACGCATC		
attB1	ACAAGTTTGTACAAAAAGCAGGCT		
attB2	ACCACTTTGTACAAGAAAGCTGGGT		
R_3ala-PNT3	TCGCCACGACGGCCGCTACCGGCTGCCGCTCCGGAATAACCGCGGTTCTTC	PNT3 ^{3A} -pDEST17	Salladini, et al., 2022
F_3ala-PNT3	AACCGCCGTTATTCCGGAAGCGGCAGCCGGTAGCGCCGTCGTGGCGATCTG		
F_ala1_PNT3	CGGAAGCGTATTATGGTAGCGCCGTCGTGGCGA		
R_ala1_PNT3	GCTACCATAATACGCTCCGGAATAACCGCGGTT		
F_ala2_PNT3	CGGAATATGCGTATGGTAGCGCCGTCGTGGCGA	PNT3 ^{A2} -pDEST17	This work
R_ala2_PNT3	GCTACCATACGCATATCCGGAATAACCGCGGTT		
F_ala3_PNT3	CGGAATATTATGCGGGTAGCGCCGTCGTGGCGA		
R_ala3_PNT3	GCTACCCGATAATATCCGGAATAACCGCGGTT		
NiV PNT3-AttB1	ACAAGTTTGTACAAAAAGCAGGCTCCGATCCTGCAAAAGACTCTCC	NiV_PNT3-pDEST17	This work
NiV PNT3-AttB2	ACCACTTTGTACAAGAAAGCTGGGTTTATTATGAGTCTTTGGACCGGCAC		
Trunc_PNT3_B2	ACCACTTTGTACAAGAAAGCTGGGTCTTATTAATTCATCTTCATATCCAG	PNT3_C-term_truncated - pDEST17	This work
PNT3_C255_F	CTGGAATATGAAGATGAATTTGCAAAAGCAGCAGCGAAGTGGTG	PNT3_Cys-pDEST17	This work
PNT3_C255_R	AAATTCATCTTCATATCCAG		

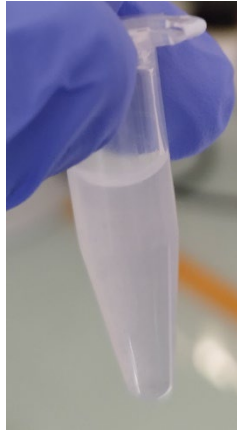


Figure S1. Cloudy solution of the HeV PNT3 protein obtained after exchanging the buffer with a Sephadex G-25 medium column previously equilibrated with sodium phosphate 50 mM buffer at pH 4.0.

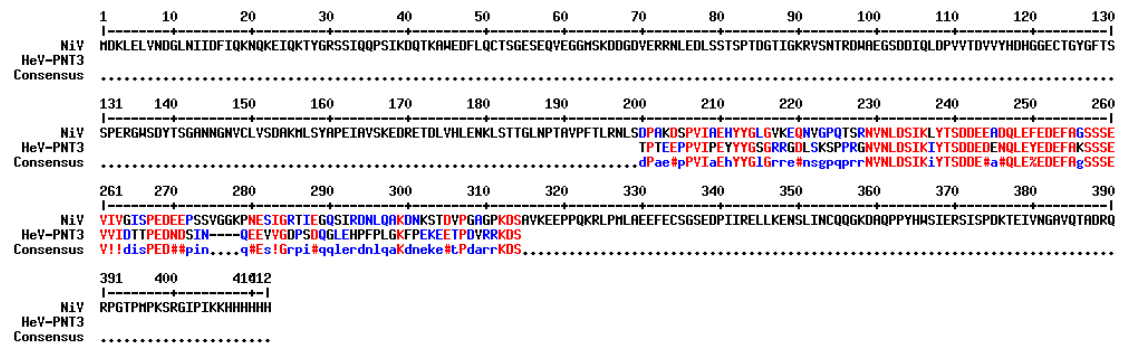


Figure S2. Alignment of NiV PNT3 and HeV PNT3 regions.

NiV PNT3

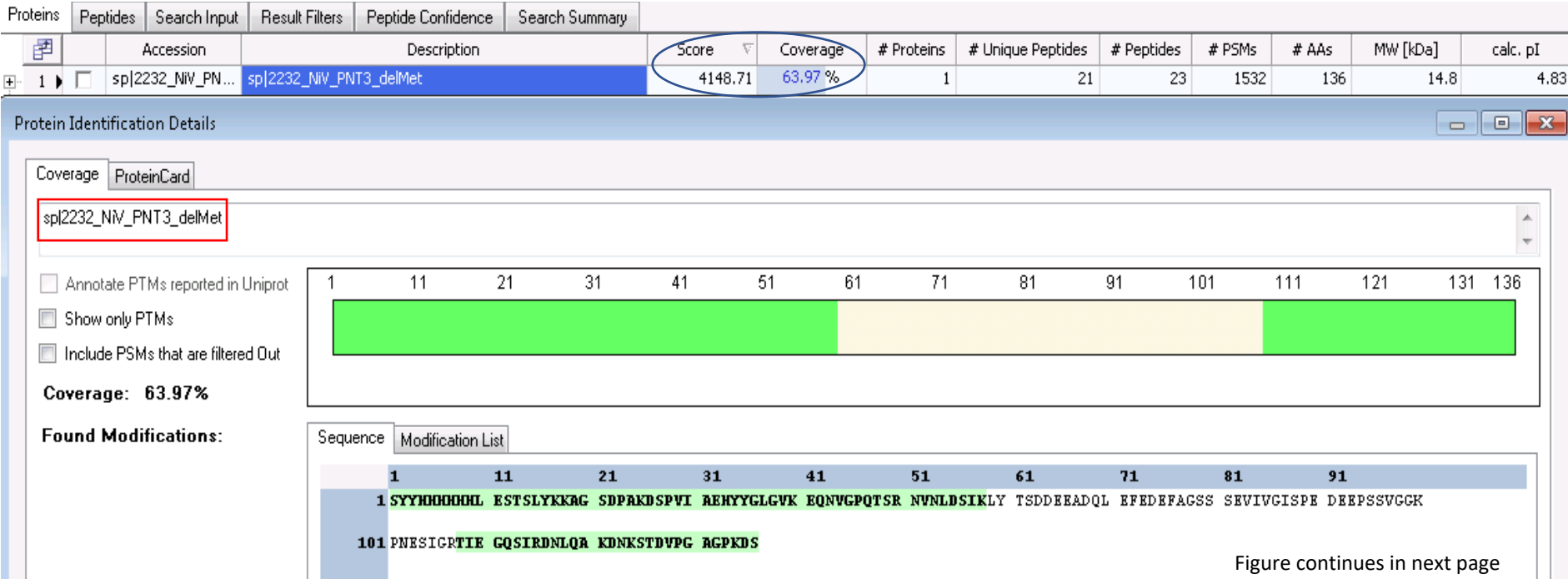


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HeV PNT3_Cys

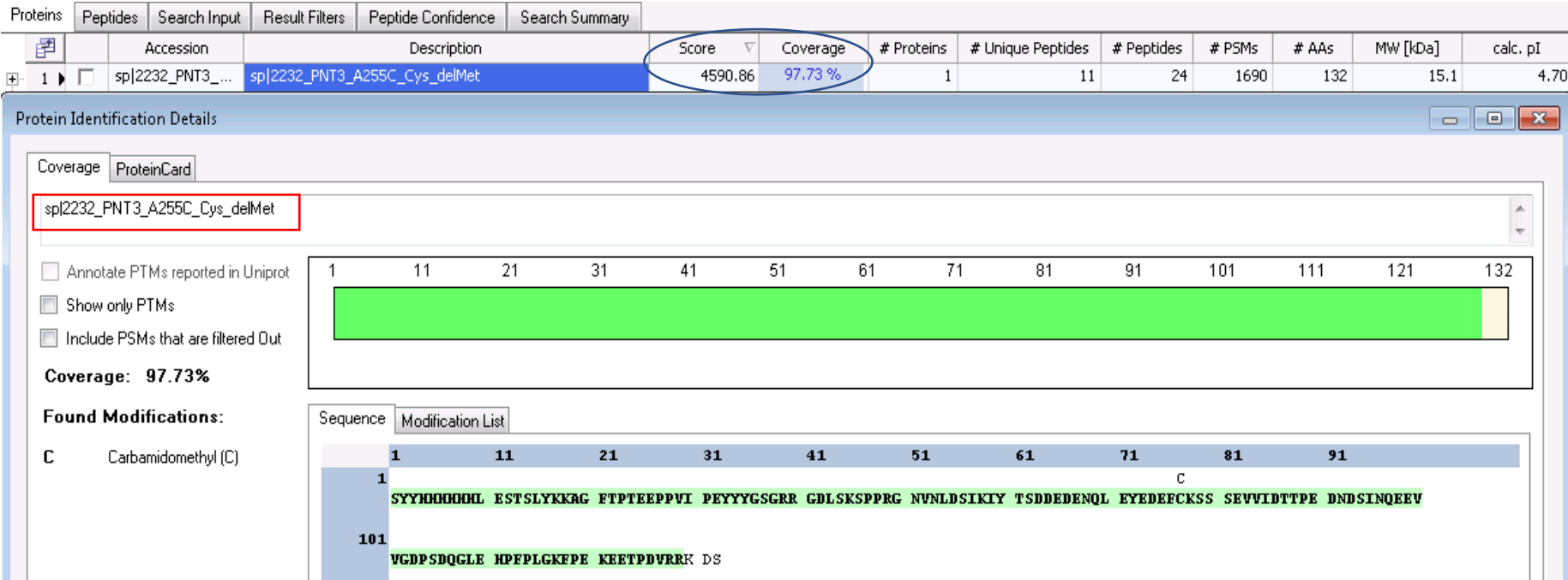


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HeV PNT3 C-terminal_truncated

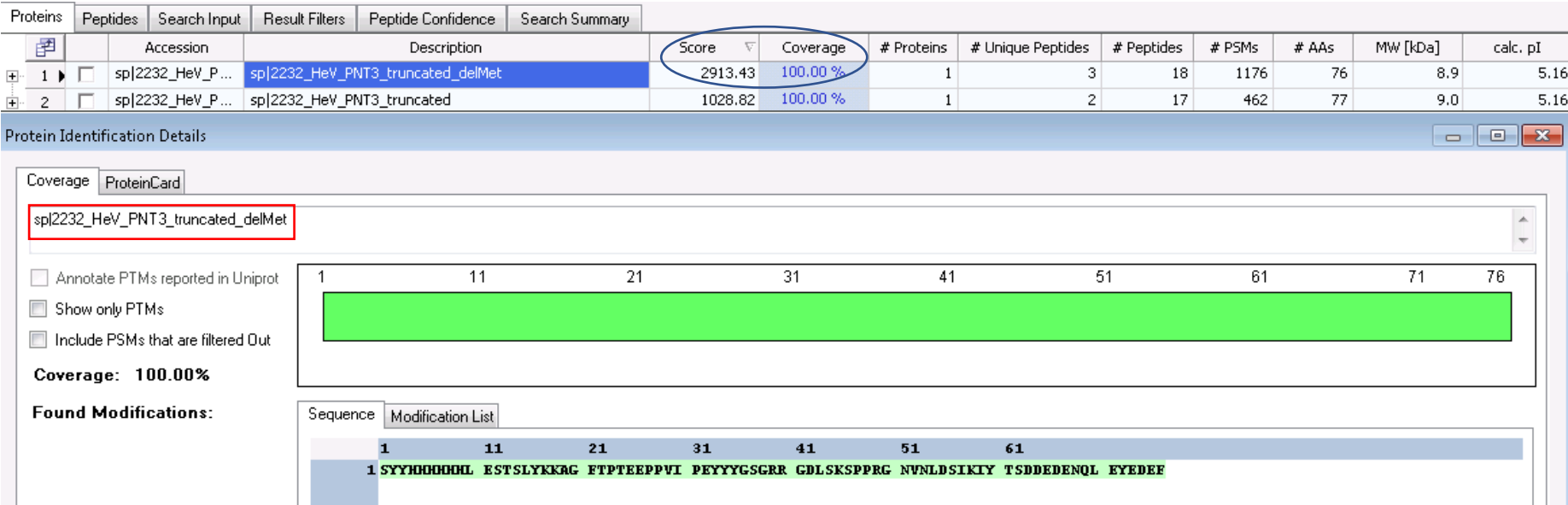


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HeV PNT3^{3A} C-terminal_truncated

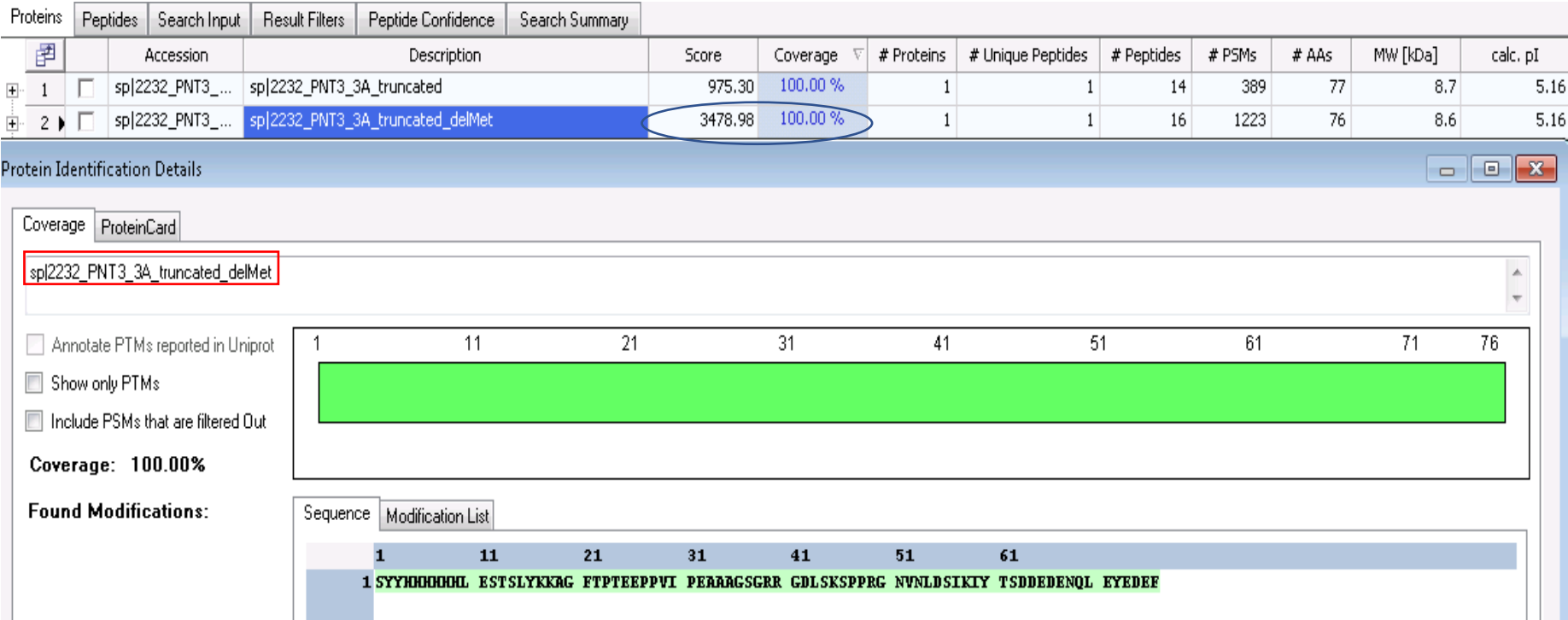
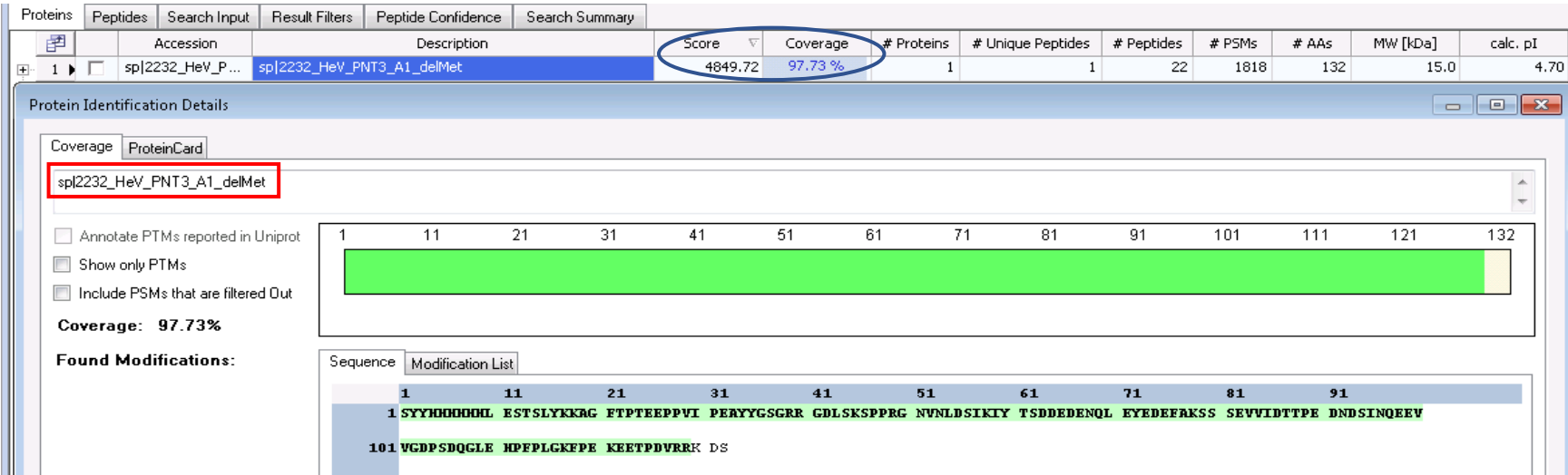


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HeV PNT3^{A1}



MSMS of the (19-39) specific peptide from the identification of the HEV_PNT3_A1 band

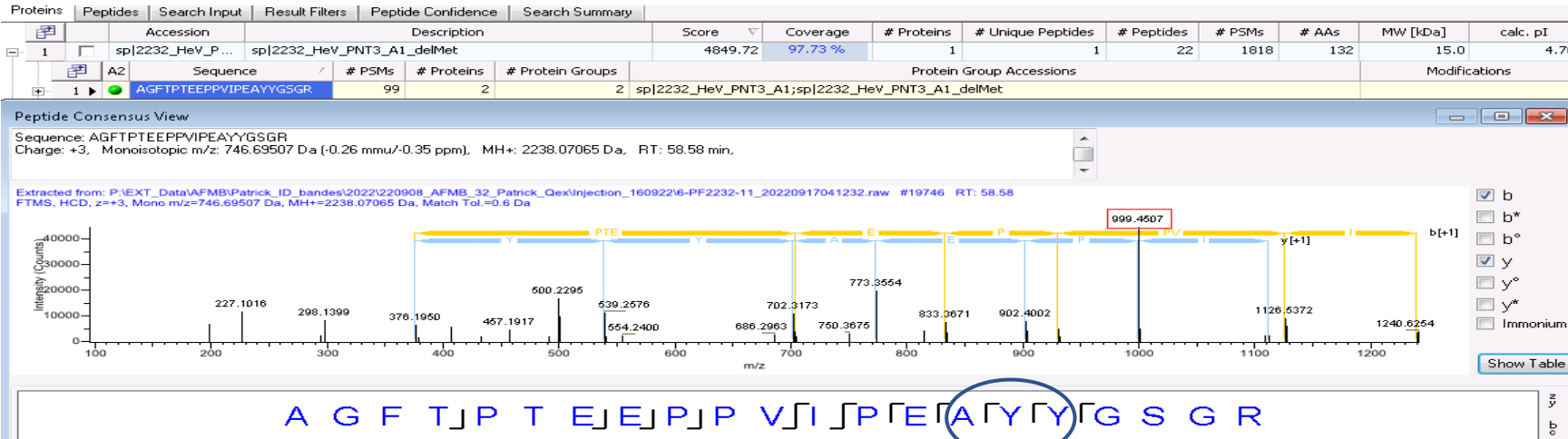
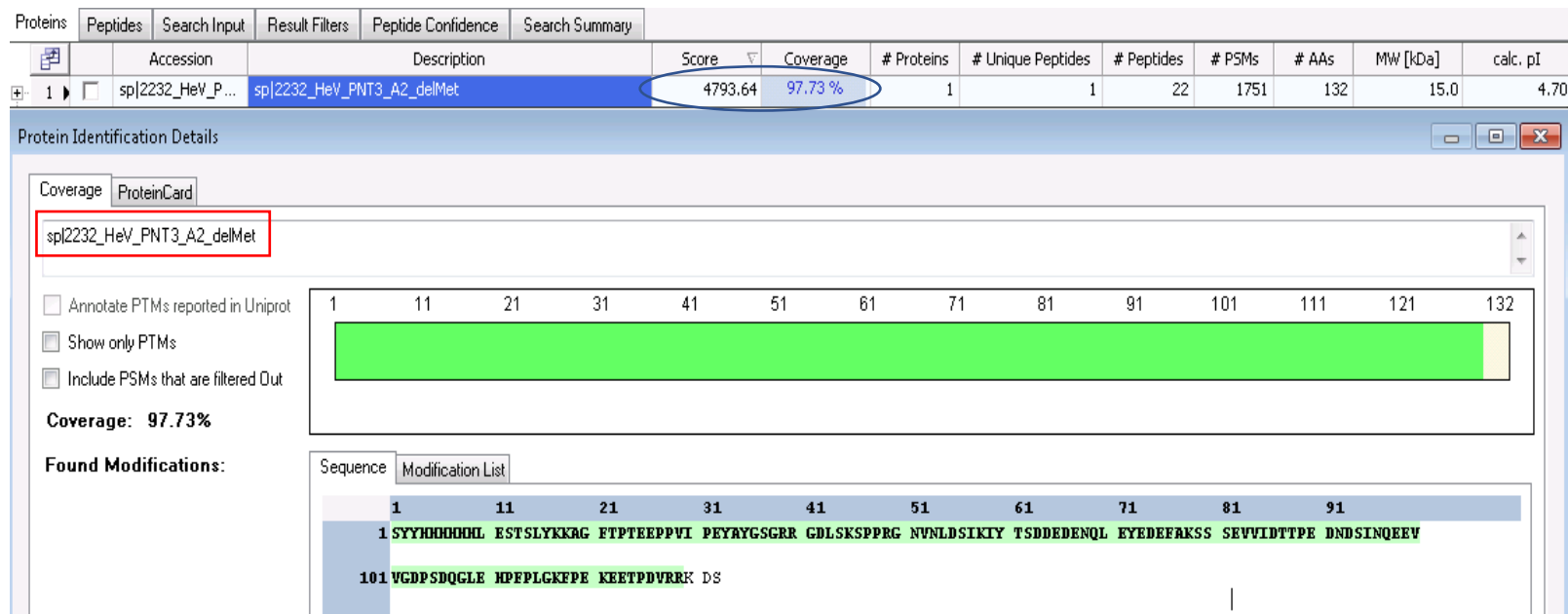


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HeV PNT3^{A2}



MSMS of the (19-39) specific peptide from the identification of the HEV_PNT3_A2 band

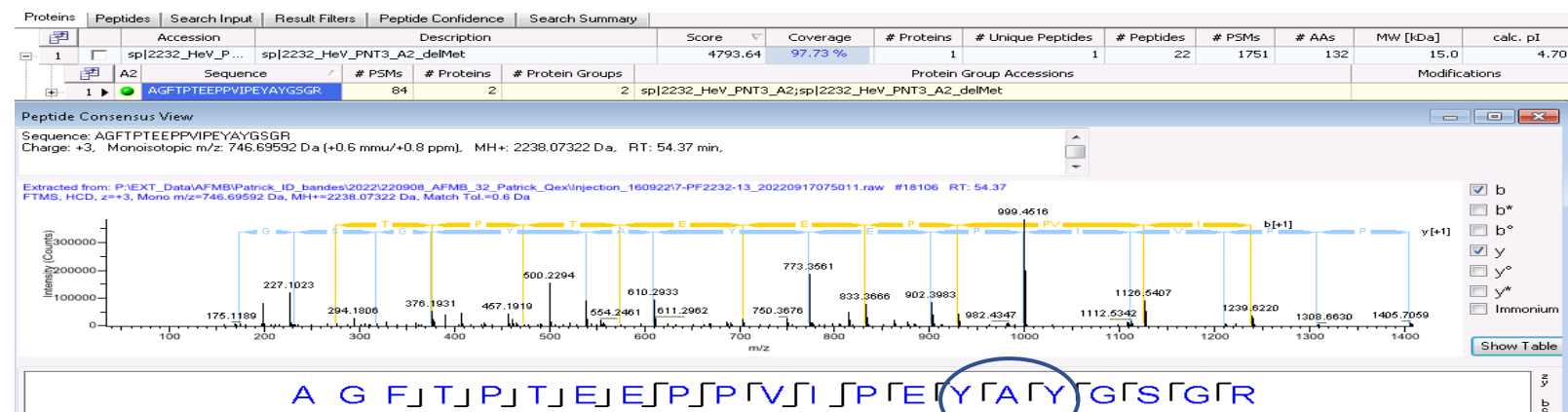


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HeV PNT3^{A3}

Proteins

Peptides

Search Input

Result Filters

Peptide Confidence

Search Summary

	Accession	Description	Score	Coverage	# Proteins	# Unique Peptides	# Peptides	# PSMs	# AAs	MW [kDa]	calc. pI
1	sp 2232_HeV_P...	sp 2232_HeV_PNT3_A3_delMet	5689.23	97.73 %	1	9	25	2010	132	15.0	4.70

Protein Identification Details

Coverage

ProteinCard

sp|2232_HeV_PNT3_A3_delMet

☐ Annotate PTMs reported in Uniprot

☐ Show only PTMs

☐ Include PSMs that are filtered Out

Coverage: 97.73%

Found Modifications:

Sequence

Modification List

1 11 21 31 41 51 61 71 81 91 101 111 121 132

1 SYHHOOOHL ESTSLYKRG FTPTEPPVI PEYYAGSGRR GDLKSPPRG NVNLDSIKY TSDDDENQL EYEDEFKSS SEVVIDTTP E DND SINQEEV

101 VGDPSDQGLE HPEPLGKPE KEETPDVRRK DS

MSMS of the (19-39) specific peptide from the identification of the HEV_PNT3_A3 band

Proteins

Peptides

Search Input

Result Filters

Peptide Confidence

Search Summary

	Accession	Description	Score	Coverage	# Proteins	# Unique Peptides	# Peptides	# PSMs	# AAs	MW [kDa]	calc. pI
1	sp 2232_HeV_P...	sp 2232_HeV_PNT3_A3_delMet	5689.23	97.73 %	1	9	25	2010	132	15.0	4.70

Peptide Consensus View

Sequence: AGFTPTTEPPVIPEYYAGSGR

Charge: +3, Monoisotopic m/z: 746.69543 Da (+0.11 mmu/+0.14 ppm), MH+: 2238.07175 Da, RT: 52.90 min.

Extracted from: P:\EXT_Data\AFMB\Patrick_ID_bandes\2022\220908_AFMB_32_Patrick_Qex\Injection_160922\8-PF2232-15_20221001162733.raw #17543 RT: 52.90

FTMS, HCD, z=+3, Mono m/z=746.69543 Da, MH+=2238.07175 Da, Match Tol.=0.6 Da

Intensity (Counts)

m/z

169.1337

227.1025

294.1809

376.1935

447.2309

500.2301

554.2462

610.2942

611.2956

703.3664

773.3672

833.3677

902.4006

982.4390

999.4520

1108.5320

1128.5427

1239.6260

1308.6599

1405.7218

b

b*

b°

y

y°

y*

Immonium

Show Table

A G F T P T E E P P V I P E Y Y A G S G R

Figure S3. Mass Spectrometry (MS) results of the PNT3 variants after digestion of the purified protein bands excised from SDS-polyacrylamide gels. Mass spectrometry analyses were carried out by LC-MSMS using an Orbitrap Qexactive spectrometer online with a nanoLC Ultimate 3000 chromatography system. The peaks obtained have been compared to the theoretical digestion peak list of the expected protein. Expected peptides found in the spectrum are represented in green. The ID score and coverage are circled. In the case of variants PNT3^{A1}, PNT3^{A2} and PNT3^{A3}, the interpretation of the results was not straightforward as the sequences are very similar and a very thorough analysis of the MSMS spectra was required. For each band, a representative MSMS spectrum of the specific (18-39) peptide is presented showing with no ambiguity the exact sequence of the protein. All the “y” fragments (in blue) containing the right amino acids in the right sequence are seen and shown squared in blue.

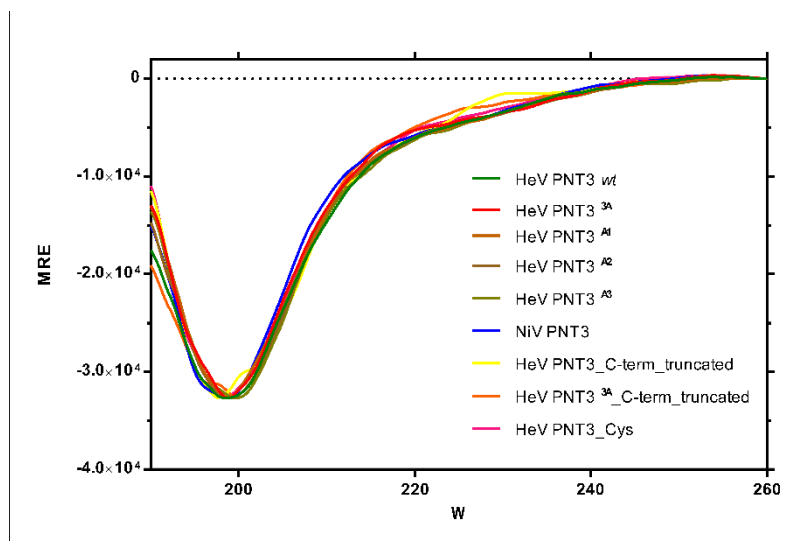


Figure S4. Far-UV circular dichroism (CD) studies of PNT3 variants. **A.** Spectra of PNT3 variants recorded in 10 mM sodium phosphate pH 7.2 at 37 °C. Protein concentration was 0.06 mg mL⁻¹ (4 μM). Spectra were recorded from a freshly purified PNT3 variant sample recorded immediately after elution from the SEC column. MRE (Θ) is expressed in deg cm² dmol⁻¹.

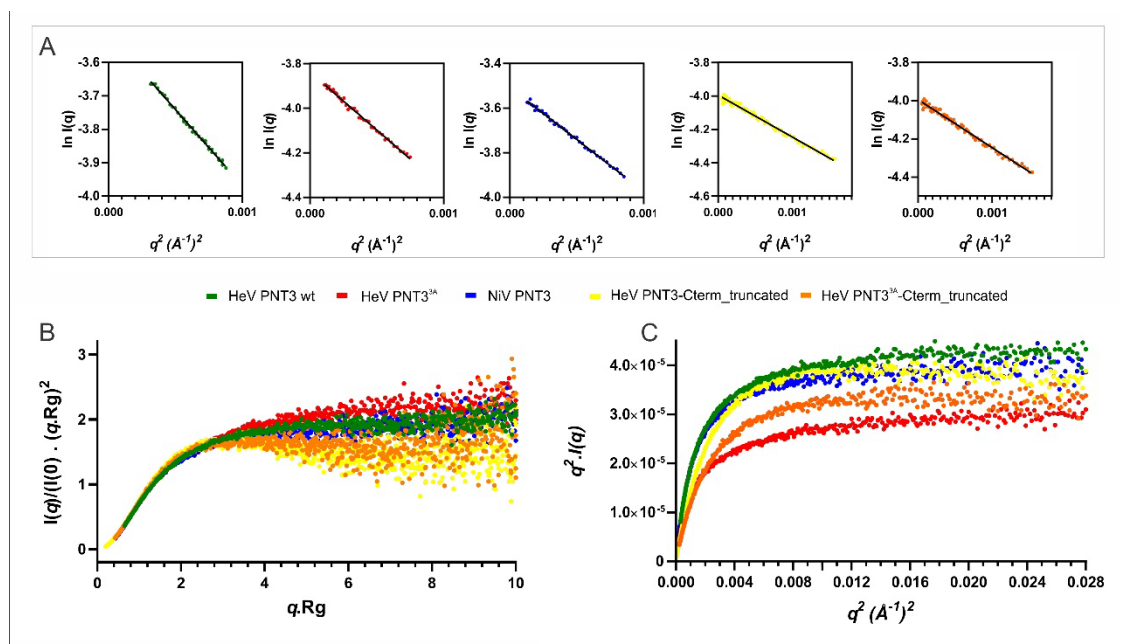


Figure S5. SEC-SAXS analysis of the monomeric form of PNT3 variants. **A.** Guinier plots of the experimental scattering curves used to determinate the radius of gyration (Guinier approximation at low angles ($q_{\text{max}} \cdot R_g < 1.1$)). **B.** Normalized Kratky plot. **C.** Kratky-Debye plot.

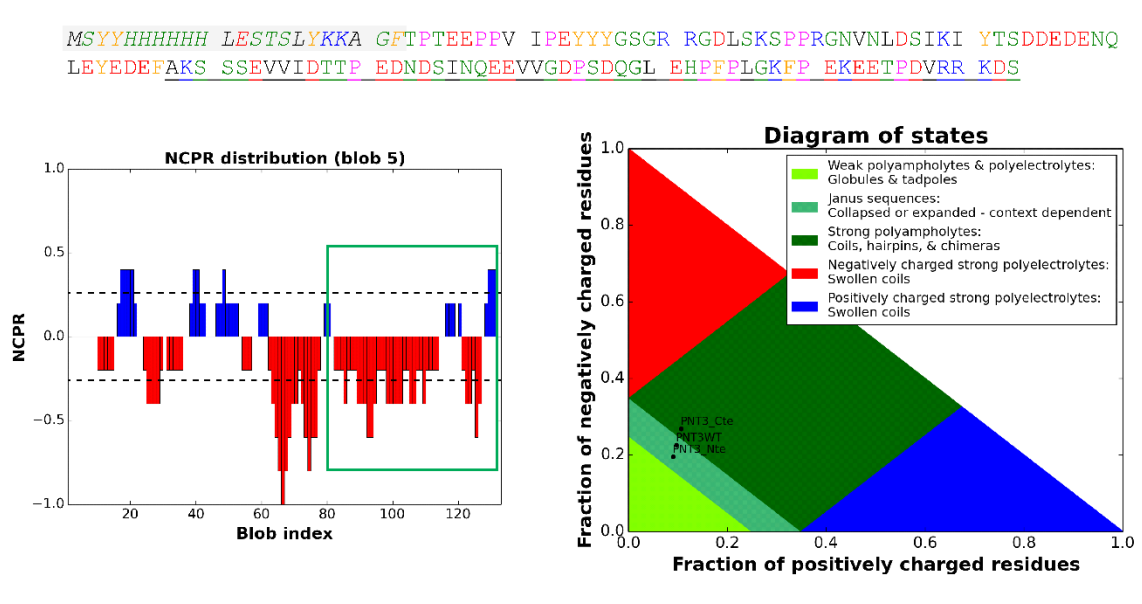


Figure S6. Analysis of charge distribution within the PNT3 sequence. The amino acid sequence of the recombinant protein is shown on the top, with vector-encoded residues shown in italic on a grey background, and residues removed in the PNT3 C-terminally truncated variant underlined. Left panel: net charge per residues (NCPR) as a function of residue number, with the C-terminal region framed. Right panel: phase diagram plot of full-length PNT3 *wt*, and of its N-terminal (Nte, aa 200-254) and C-terminal (Cte, aa 255-310) regions right panel) as provided by CIDER (<http://pappulab.wustl.edu/CIDER/>, accessed on 24 October 2022) [1].

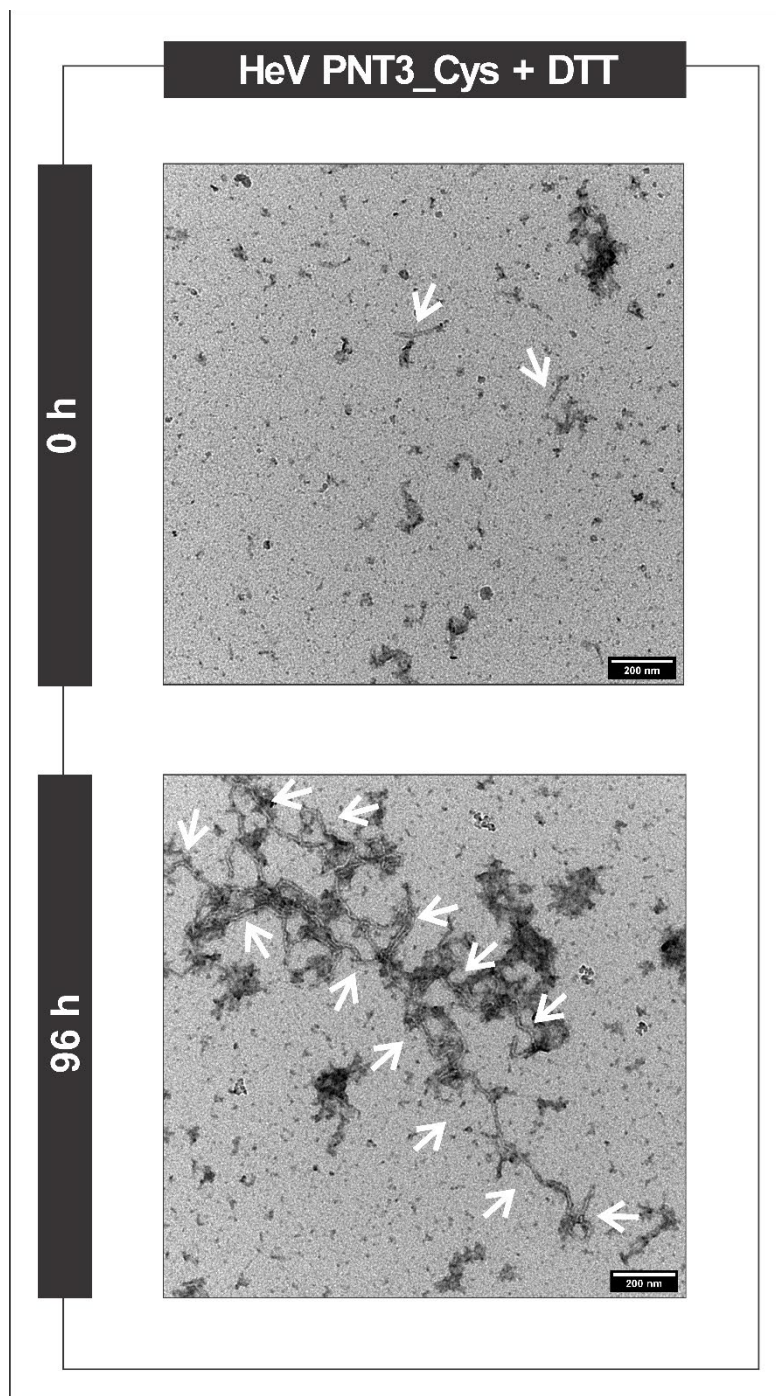


Figure S7. Ns-TEM analysis of HeV PNT3_Cys (200 μ M) in the presence of DTT (10 mM) at time zero and after 96 h of incubation at 37 $^{\circ}$ C. Note that in all cases, samples were diluted to 40 μ M prior deposition on the grid. The white arrows indicate fibrils.

References

1. Holehouse, A.S.; Das, R.K.; Ahad, J.N.; Richardson, M.O.; Pappu, R.V. CIDER: Resources to Analyze Sequence-Ensemble Relationships of Intrinsically Disordered Proteins. *Biophysical journal* 2017, 112, 16-21, doi:10.1016/j.bpj.2016.11.3200.