Table S4: ^{13}C-NMR chemical shifts of the free peptide P-Vpu$^{41-62}$ in ppm from $TSPD_4$. Spectra were recorded at 280 K, pH = 7.2, in 20 mM sodium phosphate, H_2O;^2H_2O (9; 1 by vol). Resonances of carbons marked by – were not visible.

Residue	δC_α	δC_β	δC_γ	δC_δ
Leu 41	54.8	43.1	26.9	24.7
Ile 42	61.2	38.9	27.3	17.5;13.0
Asp 43	54.4	41.4		
Arg 44	56.0	31.0	26.8	43.5
Leu 45	55.7	42.1	27.1	24.0;23.6
ILe 46	61.1	38.9	27.3	17.5;13.0
Glu 47	56.8	30.5	36.3	
Arg 48	55.8	31.0	26.8	43.5
Ala 49	52.7	19.4		
Glu 50	56.8	30.5	36.3	
Asp 51	54.2	41.7		
pSer 52	58.0	66.2		
Gly 53	45.7			
Asn 54	–	39.5		
Glu 55	56.7	30.5	36.3	
pSer 56	57.6	66.3		
Glu 57	56.8	30.5	36.3	
Gly 58	45.4			
Glu 59	56.9	30.5	36.3	
Ile 60	61.2	38.9	27.3	17.5;13.0
Ser 61	58.3	64.1		
Ala 62	54.0	20.4		

Table S5: ^{13}C-NMR chemical shifts of the peptide P-Vpu$^{41-62}$ bound to the protein β-TrCP in ppm from $TSPD_4$. Spectra were recorded at 280 K, pH = 7.2, P-Vpu/β-TrCP = 10 in 20 mM sodium phosphate, H_2O;^2H_2O (9; 1 by vol). Resonances of carbons marked by – were not visible.

Residue	δC_α	δC_β	δC_γ	δC_δ
Leu 41	54.6	42.8	27.1	24.5
Ile 42	61.0	38.7	27.3	17.4;12.8
Asp 43	54.2	41.2		
Arg 44	55.8	30.9	27.1	43.3
Leu 45	55.5	42.0	27.1	23.8;23.5
ILe 46	60.9	38.7	27.3	17.4;12.8
Glu 47	56.6	30.3	36.1	
Arg 48	55.8	30.9	27.1	43.3
Ala 49	52.6	19.3		
Glu 50	56.6	30.3	36.1	
Asp 51	54.1	41.5		
pSer 52	58.2	65.7		
Gly 53	45.6			
Asn 54	–	39.4		
Glu 55	56.5	30.3	36.1	
pSer 56	57.6	65.8		
Glu 57	56.7	30.3	36.1	
Gly 58	45.3			
Glu 59	56.7	30.3	36.1	
Ile 60	61.0	38.7	27.3	17.4;12.8
Ser 61	58.2	64.0		
Ala 62	54.0	20.0		

Table S6: Dihedral angles of the peptide P-Vpu$^{41-62}$ bound to the protein β-TrCP assessed by MD calculations with ARIA.

Residue	ϕ	ψ	χ^1
Leu 41	–	-40.8	-57.8
Ile 42	-59.8	-40.2	-56.0
Asp 43	-58.6	-22.4	59.0
Arg 44	-71.0	-22.1	-173.9
Leu 45	-102.4	-36.4	-165.4
ILe 46	-125.5	-47.2	-57.6
Glu 47	-56.8	-20.6	162.5
Arg 48	-69.6	13.3	-170.5
Ala 49	-163.8	-25.8	-125.1
Glu 50	-122.8	39.8	-160.4
Asp 51	-53.8	147.0	63.2
pSer 52	-146.5	53.2	-16.1
Gly 53	-168.0	-40.9	–
Asn 54	-58.9	-44.3	-172.8
Glu 55	-172.0	-41.7	83.7
pSer 56	-69.6	141.1	-130.3
Glu 57	-56.6	-50.5	-179.9
Gly 58	159.0	32.1	–
Glu 59	-79.8	127.2	-58.3
Ile 60	-76.8	135.5	179.1
Ser 61	-81.2	41.8	-61.1
Ala 62	-174.9I	–	-45.2

Figure S9

(a) SDS PAGE (12% acrylamide) analysis. Line A shows the GST-β-TrCP after purification and binding to the glutathione-sepharose 4B beads, whereas line B shows the eluted protein. (b) Western blot analysis of the eluted GST-β-TrCP protein using anti β-TrCP polyclonal antibodies (C-18 from Santa Cruz). Approximate molecular weight in kDa is shown on the left.

Figure S10

(a) Regions of a TRNOESY spectrum (t_m = 200 ms) of the P-Vpu$^{41-62}$ peptide in the presence of the GST-β-TrCP protein, showing cross peaks between the amide and the aliphatic protons. Some individual connectivities are identified with the one letter amino acid code and the sequence number. (b) Similar regions of a NOESY spectrum (t_m = 200 ms) of a control sample of Vpu$^{41-62}$ peptide (non-phosphorylated), in the presence of the GST-β-TrCP protein.

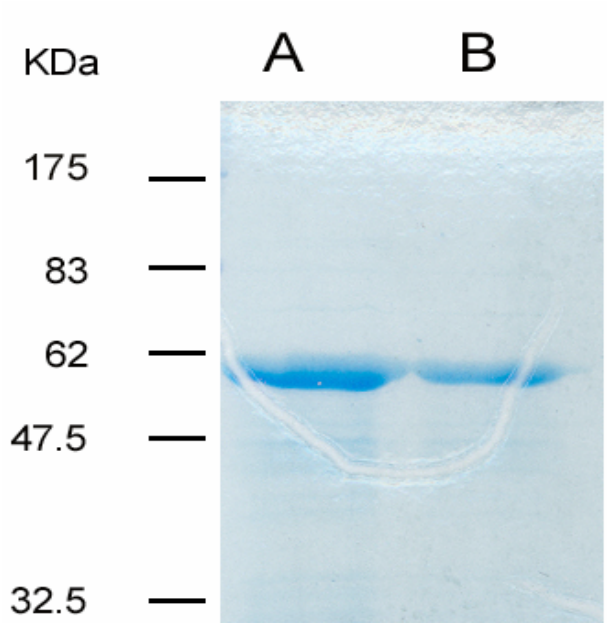
Figure S11

(a) The 1D ^{1}H NMR spectrum of P-Vpu$^{41-62}$ in the presence of GST-β-TrCP protein showed signals belonging to residual glutathione used to eluate the protein for β-TrCP protein purification. (b) These signals were not present in the corresponding 1D ^{1}H STD-NMR spectrum of the P-Vpu$^{41-62}$ peptide GST-β-TrCP protein complex, because this impurity does not bind to β-TrCP protein.

Figure S12

(a) 1D ^{1}H spectrum of Vpu$^{41-62}$ peptide in presence of GST-β-TrCP protein. (b) 1D ^{1}H STD-NMR spectrum of Vpu$^{41-62}$ peptide with GST-β-TrCP protein, showing no enhancements of resonances of any protons. Control samples were prepared with 2.0 mg of Vpu$^{41-62}$ peptide (non-phosphorylated) and 5 mg of GST-β-TrCP protein in 500 μL of buffer.

(a)



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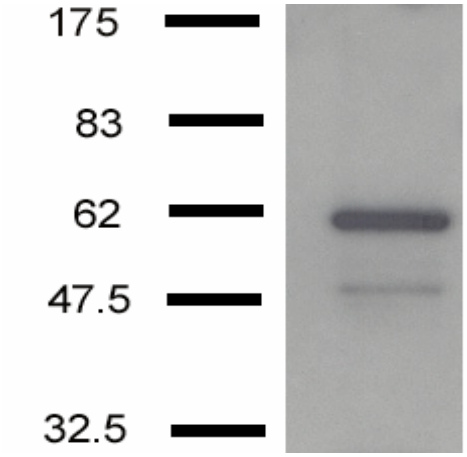

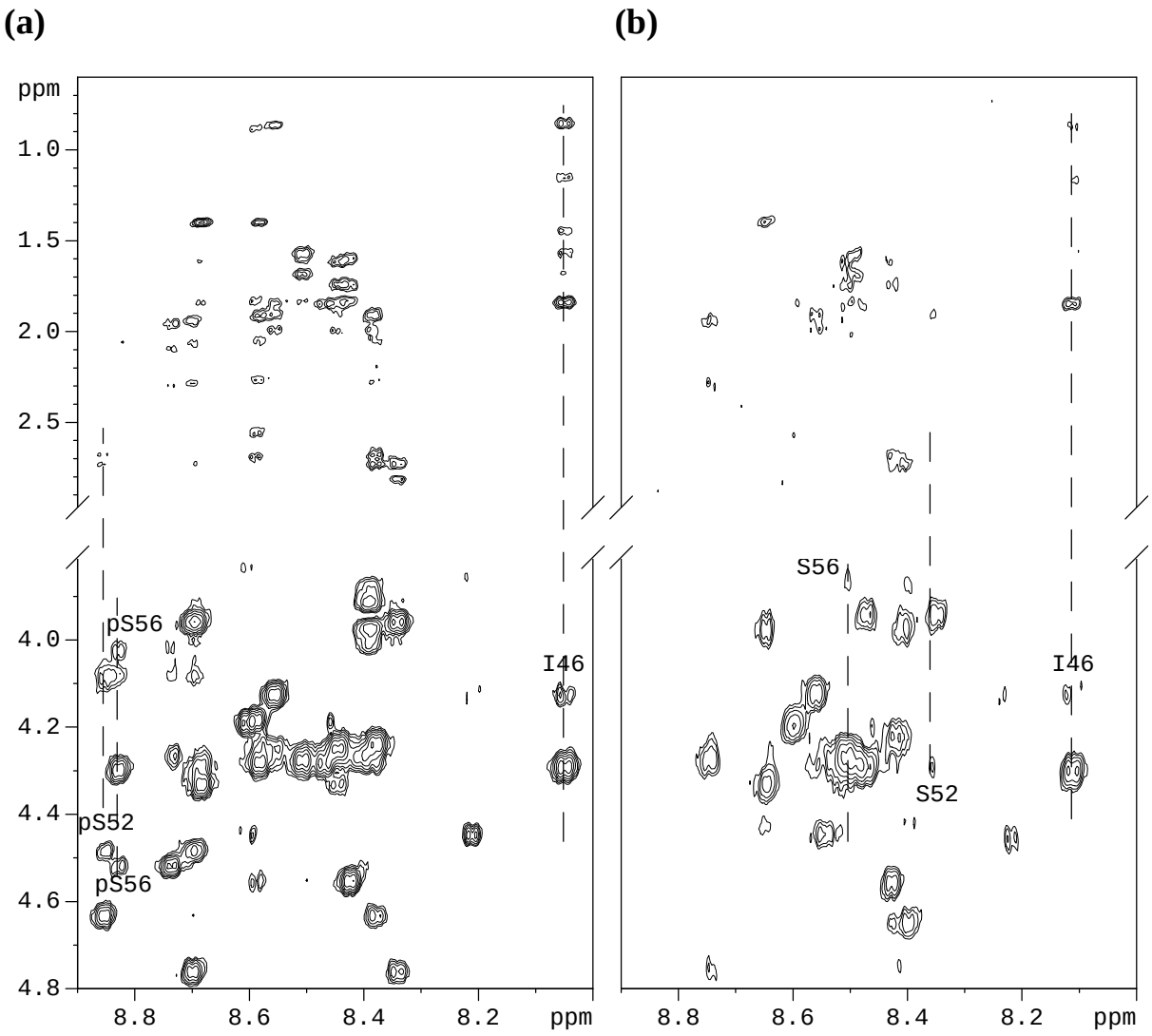
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(b)
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4.2
4.4
4.6
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pS52
pS56
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S56
S52
I46
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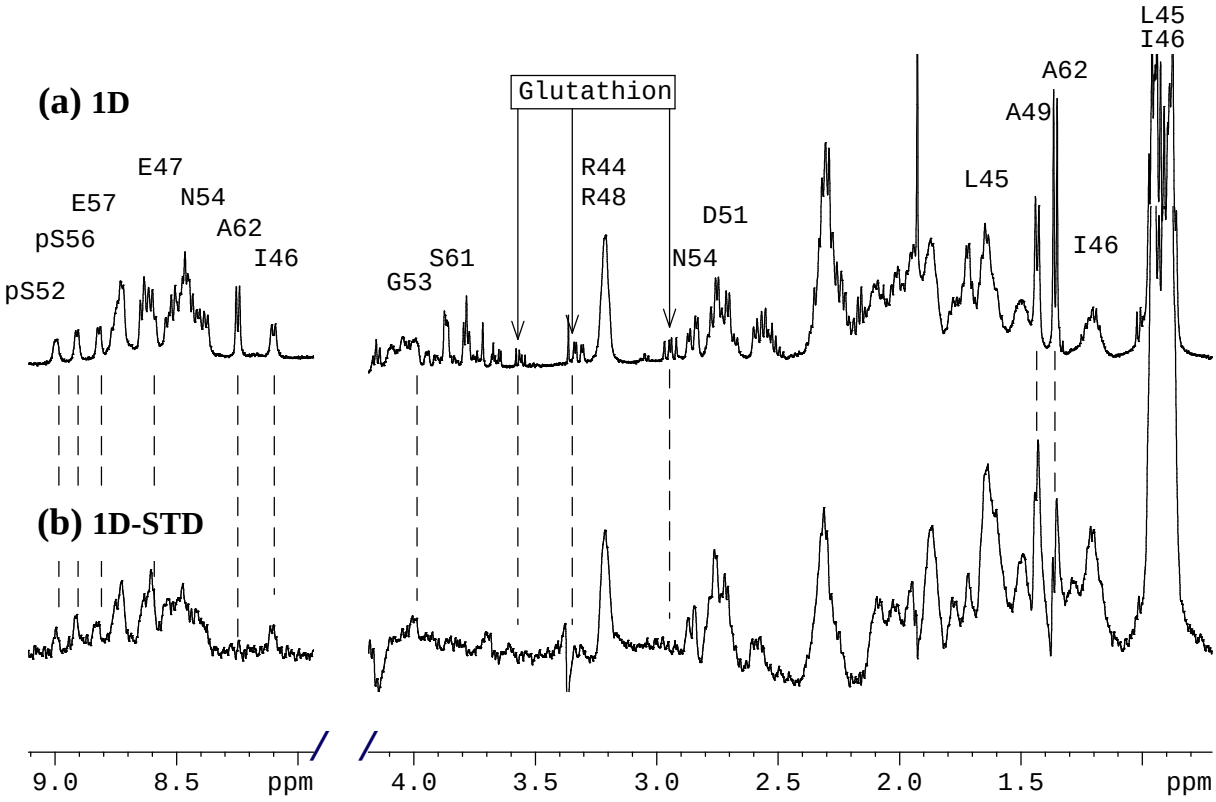
8.6
8.4
8.2
ppm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(b) 1D-STD
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pS56
E57
E47
N54
A62
I46
G53
S61
Glutathion
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R48
N54
D51
L45
A49
A62
I46
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I46
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ppm
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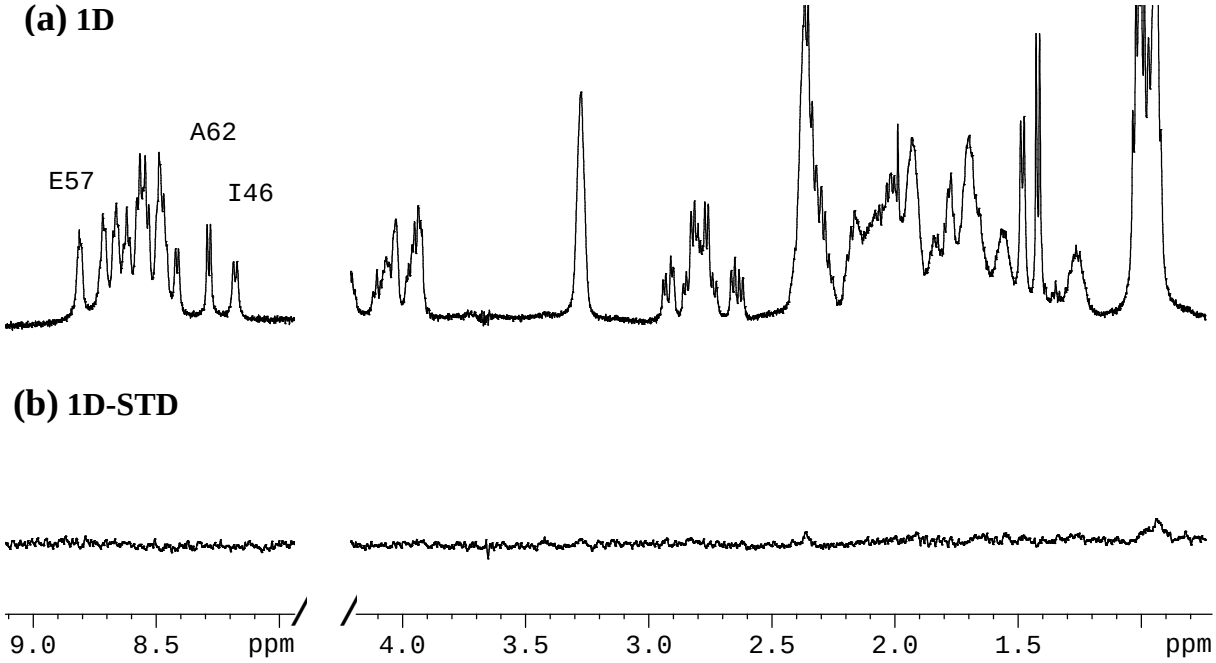
3.0
2.5
2.0
1.5
ppm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E57
I46
(b) 1D-STD
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ppm
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3.5
3.0
2.5
2.0
1.5
ppm