Supplementary information to:

The cleaved N-terminus of pVI binds peripentonal hexons in mature adenovirus.

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Supplementary Tables.

Supplementary Table S1. Peptide identifications of pVIn from LC-MS/MS analysis of mature HAdV virions. The confidence of the identification is given by the IonScore. The higher the score, the more confident the identification. Scores of >20 are considered confident.

start	end	Sequence	Modifications	IonScore
1	12	mEDINFASLAPR	N-Term(Acetyl)	105
1	12	mEDINFASLAPR	N-Term(Acetyl); M1(Oxidation)	97
13	33	HGSRPFmGNWQDIGTSNmSGG	M7(Oxidation); M18(Oxidation)	55
13	33	HGSRPFmGNWQDIGTSNMSGG	M7(Oxidation)	62
13	33	HGSRPFMGNWQDIGTSNMSGG		46

Supplementary Table S2. PSM based quantitation of pVIn release upon heating. The fraction of released pVIn is calculated as the average ± standard deviation from triplicate experiments, where for each experiment the number of PSM's, normalized to the PSM's for pVII, is divided by the average pVII-normalized number of pVIn PSM's in untreated virus.

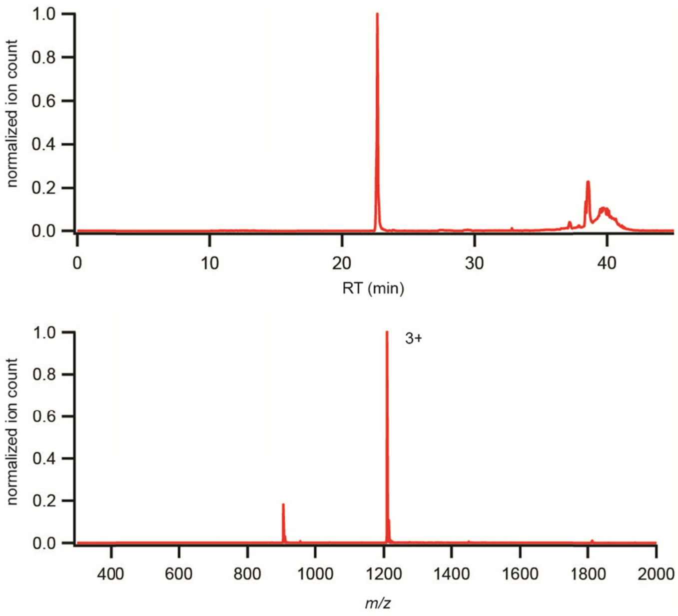
		number of PSMs		
condition	**protein**	**average**	**standard deviation**	**n**
untreated	pVII	82.3	5.0	6
	pVIn	23.5	2.7	
heated	pVII	104.0	1.7	3
	pVIn	5.7	0.6	

	fraction released
average	**standard deviation**
0.81	0.02

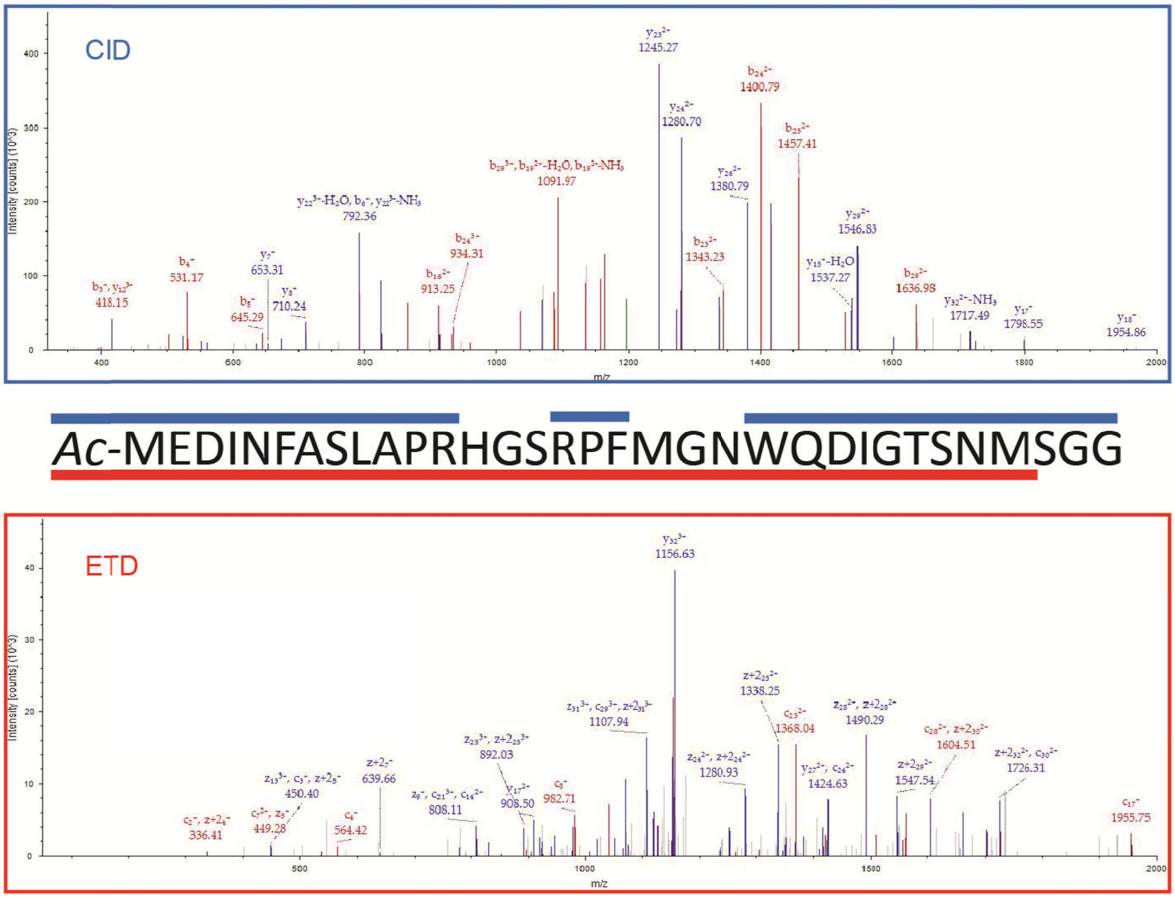
Supplementary Table S3. Hexon-pVIn masses obtained from native MS analysis.

	mass (kDa)		
	average	**standard deviation**	**error % (compared to theoretical)**
hexon_3	323.62	0.01	0.024
$\text{hexon}_3\text{pVIn}_1$	327.24	0.02	0.021
$\text{hexon}_3\text{pVIn}_2$	330.87	0.01	0.022
$\text{hexon}_3\text{pVIn}_3$	334.49	0.01	0.022
pVIn	3.624	0.00	0.002

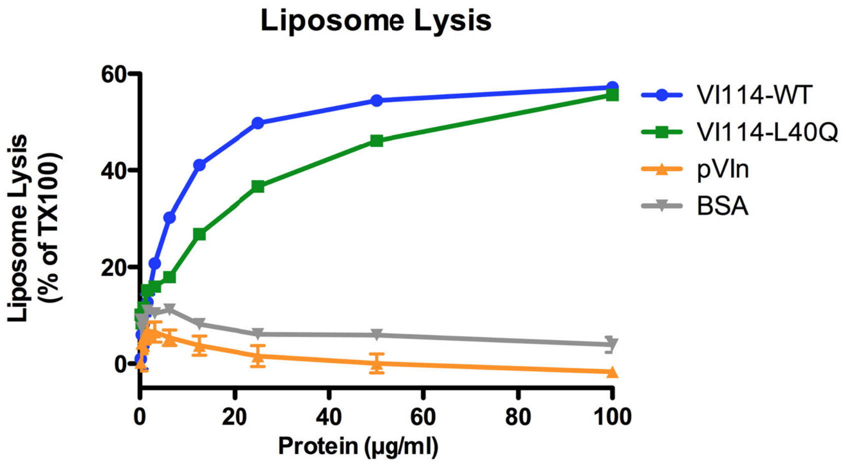
Supplementary Figures.



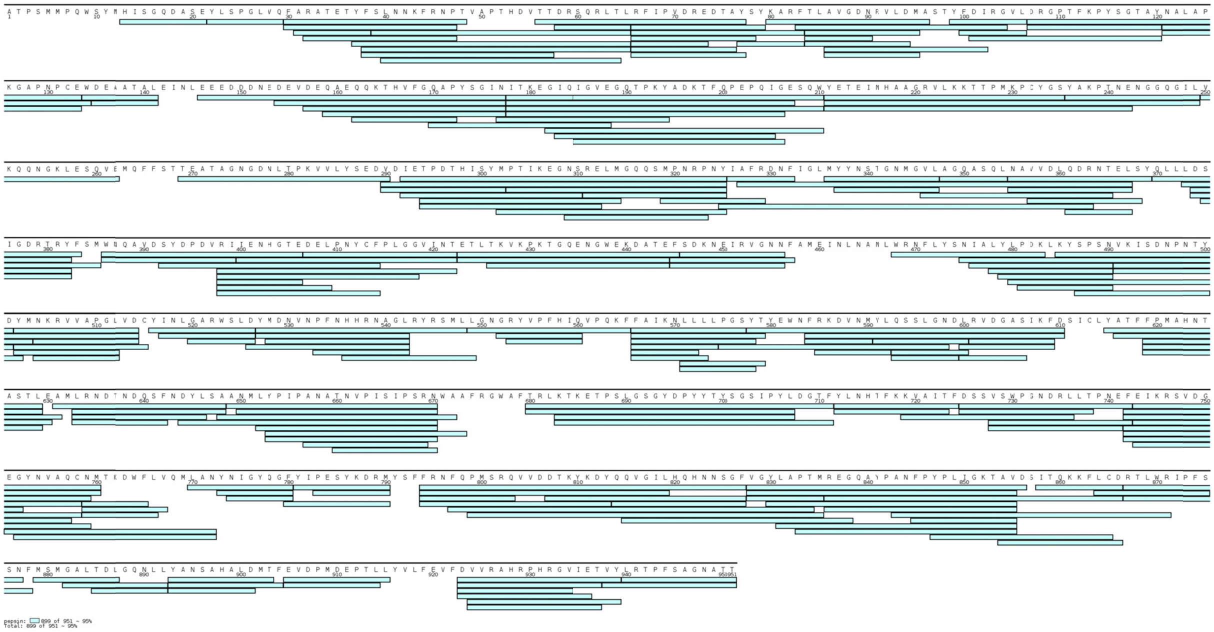
Supplementary Figure S1. LC-MS analysis of isolated peptide from heat-released hexon trimers. A single peptide is recovered from the isolation procedure as evidenced from the base-peak chromatogram (*top*). The isolated peptide has a mass of 3624.09 Da, compared to a theoretical mass of 3624.01 for pVIn.



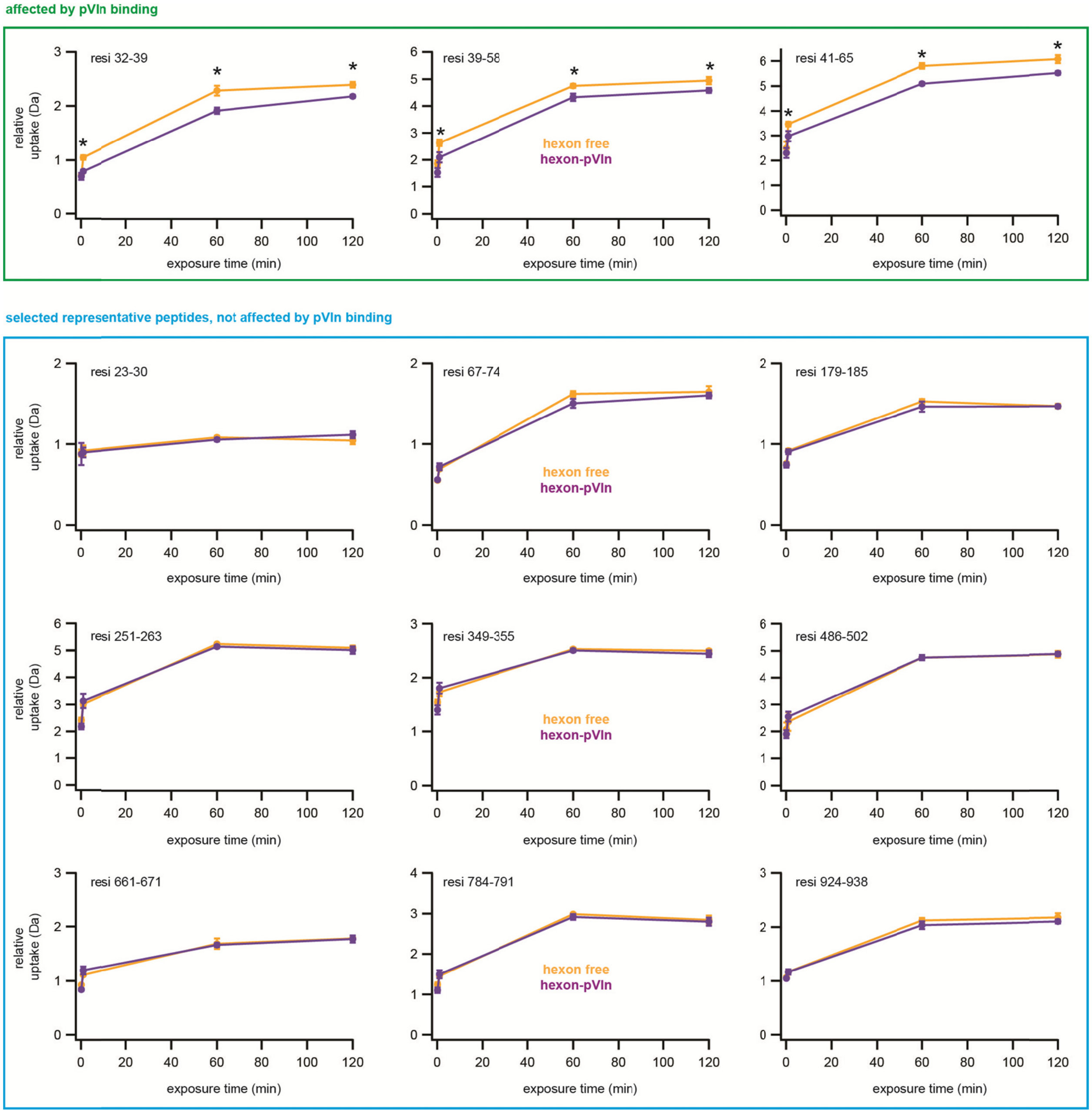
Supplementary Figure S2. MS/MS analysis of the peptide isolated from the hexon complex. A combination of CID and ETD fragmentation covers the full sequence of the peptide and confirms that the peptide is pVIn.



Supplementary Figure S3. pVIn has no membrane lytic activity. Fluorescence measurements of SulfoB release from liposomes. Lytic activity is expressed as % of total fluorescence for Triton-X100 disrupted liposomes. Points represent the average ± SEM from triplicate measurements.



Supplementary Figure S4. Sequence coverage of hexon from peptide ID's of the HDX-MS experiment.



Supplementary Figure S5. Comparison of peptides that exhibit protection for deuterium uptake upon pVIn binding (green box) with selected representative peptides that were unaffected by pVIn binding (blue box). Asterisks indicate that $p<0.05$ in unpaired, two-tailed Student's *t*-tests.