**Supporting Information**

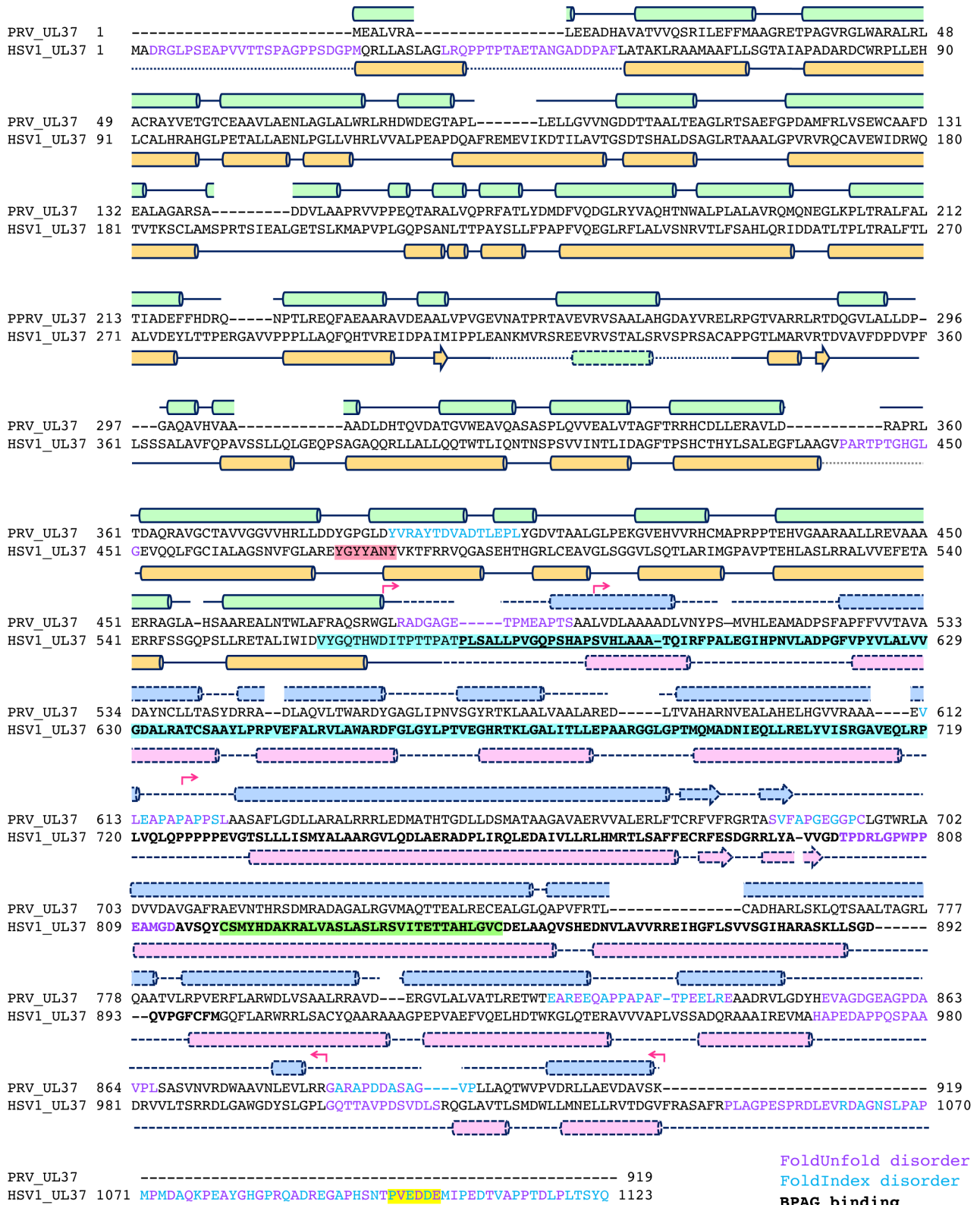**The dynamic nature of the conserved tegument protein UL37 of herpesviruses**

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Supporting information materials include
Table S1
Figures S1-S4
Supporting References

**Table S1. Thermofluor Melting Temperatures.** The melting temperature for each construct in different buffers condition is shown in degrees Celsius (°C).

| Buffer Condition | $T_M$ (°C) | | | | | |
|---|---|---|---|---|---|---|
| | UL3N 1-479 | UL37C-478-919-StII | UL37C-478-884 | UL37C-618-919-StII | UL37FL (low) | UL37FL (high) |
| Na-Acetate pH 5.0 | 49.60 | 41.28 | 34.94 | 35 | 28.7 | 44.8 |
| Na-Citrate pH 5.5 | 57.38 | 41.28 | 34.94 | 39 | 41.8 | 55.8 |
| MES pH 6.0 | 55.88 | 41.28 | 34.94 | 43 | 43.1 | 59.5 |
| Na Cacodylate pH 6.5 | 59.71 | 44.98 | 41.94 | 38 | 41.2 | 53.5 |
| Bis-Tris pH 6.5 | 58.06 | 42.64 | 39.61 | 42.6 | 44.1 | 59.5 |
| Imidazole pH 7.0 | 57.57 | 42.96 | 40.95 | 41.3 | 42.1 | 58.5 |
| PIPES pH 7.0 | 59.54 | 45.32 | 41.57 | 42.6 | 42.8 | 59.5 |
| Na-Malonate pH 7.0 | 59.54 | 45.32 | 40.6 | **43.3** | 43.1 | 59.5 |
| MOPS pH 7.5 | 57.91 | 45.64 | 42.27 | 38.6 | 43.5 | 57.5 |
| HEPES pH 7.5 | 58.21 | 45.98 | 42.27 | 40.6 | 45.1 | 58.9 |
| Tris pH 8.0 | 57.19 | 45.64 | 42.64 | 39 | 43.1 | 60.1 |
| Bicine pH 9.0 | 57.57 | 45.32 | 41.94 | 41.3 | 45.8 | 59.2 |
| Glycine pH 9.0 | 57.91 | 45.32 | 43.93 | 39 | 43.5 | 58.2 |
| CHES pH 9.5 | 52.41 | 41.66 | 40.6 | 37.3 | 43.1 | 44.4 |
| Ethanolamine pH 9.5 | 56.19 | 44.63 | 42.92 | 39 | 42.1 | 43.1 |
| CAPS pH 10.0 | 54.39 | 41.66 | 39.91 | 38.6 | 44.1 | 58.2 |
| HEPES pH 7.5 | 59.37 | 43.96 | 41.57 | 38 | 43.1 | 58.9 |
| HEPES pH 7.5, 50 mM NaCl | 59.05 | 44.63 | 41.57 | 35 | 45.1 | 44.4 |
| HEPES pH 7.5, 150 mM NaCl | 58.38 | 45.98 | 42.64 | 40 | 42.1 | 55.1 |
| HEPES pH 7.5, 300 mM NaCl | 57.74 | 46.32 | 43.24 | 34.5 | 41.8 | 55.8 |
| HEPES pH 7.5, 500 mM NaCl | 57.19 | 46.99 | 41.94 | 39.3 | 43.8 | 59.9 |
| HEPES pH 7.5, 150 mM, NaCl 5% Glycerol | 59.54 | 47.32 | 43.24 | 35.7 | 44.1 | 59.9 |
| HEPES pH 7.5, 150 mM NaCl, 10% Glyerol | **60.55** | **48.64** | **44.59** | 42.3 | 45.1 | 58.9 |
| HEPES pH 7.5, 500 mM NaCl, 5% Glycerol | 58.38 | 48.31 | 44.59 | 41.3 | **45.5** | **59.9** |

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PRV_UL37   1   -------------------------MEALVRA-----------------LEEADHAVATVVQSRILEFFMAAGRETPAGVRGLWARALRL   48
HSV1_UL37  1   MADRGLPSEAPVVTTSPAGPPSDGPMQRLLASLAGLRQPPTPTAETANGADDPAFLATAKLRAAMAAFLLSGTAIAPADARDCWRPLLEH   90

PRV_UL37  49   ACRAYVETGTCEAAVLAENLAGLALWRLRHDWDEGTAPL-------LELLGVVNGDDTTAALTEAGLRTSAEFGPDAMFRLVSEWCAAFD  131
HSV1_UL37 91   LCALHRAHGLPETALLAENLPGLLVHRLVVALPEAPDQAFREMEVIKDTILAVTGSDTSHALDSAGLRTAAALGPVRVRQCAVEWIDRWQ  180

PRV_UL37 132   EALAGARSA---------DDVLAAPRVVPPEQTARALVQPRFATLYDMDFVQDGLRYVAQHTNWALPLALAVRQMQNEGLKPLTRALFAL  212
HSV1_UL37 181  TVTKSCLAMSPRTSIEALGETSLKMAPVPLGQPSANLTTPAYSLLFPAPFVQEGLRFLALVSNRVTLFSAHLQRIDDATLTPLTRALFTL  270

PPRV_UL37 213  TIADEFFHDRQ-----NPTLREQFAEAARAVDEAALVPVGEVNATPRTAVEVRVSAALAHGDAYVRELRPGTVARRLRTDQGVLALLDP-  296
HSV1_UL37 271  ALVDEYLTTPERGAVVPPPLLAQFQHTVREIDPAIMIPPLEANKMVRSREEVRVSTALSRVSPRSACAPPGTLMARVRTDVAVFDPDVPF  360

PRV_UL37 297   ---GAQAVHVAA------------AADLDHTQVDATGVWEAVQASASPLQVVEALVTAGFTRRHCDLLERAVLD-----------RAPRL  360
HSV1_UL37 361  LSSSALAVFQPAVSSLLQLGEQPSAGAQQRLLALLQQTWTLIQNTNSPSVVINTLIDAGFTPSHCTHYLSALEGFLAAGVPARTPTGHGL  450

PRV_UL37 361   TDAQRAVGCTAVVGGVVHRLLDDYGPGLDYVRAYTDVADTLEPLYGDVTAALGLPEKGVEHVVRHCMAPRPPTEHVGAARAALLREVAAA  450
HSV1_UL37 451  GEVQQLFGCIALAGSNVFGLAREYGYYANYVKTFRRVQGASEHTHGRLCEAVGLSGGVLSQTLARIMGPAVPTEHLASLRRALVVEFETA  540

PRV_UL37 451   ERRAGLA-HSAAREALNTWLAFRAQSRWGLRADGAGE-----TPMEAPTSAALVDLAAAADLVNYPS—MVHLEAMADPSFAPFFVVTAVA  533
HSV1_UL37 541  ERRFSSGQPSLLRETALIWIDVYGQTHWDITPTTPATPLSALLPVGQPSHAPSVHLAAA-TQIRFPALEGIHPNVLADPGFVPYVLALVV  629

PRV_UL37 534   DAYNCLLTASYDRRA--DLAQVLTWARDYGAGLIPNVSGYRTKLAALVAALARED-----LTVAHARNVEALAHELHGVVRAAA----EV  612
HSV1_UL37 630  GDALRATCSAAYLPRPVEFALRVLAWARDFGLGYLPTVEGHRTKLGALITLLEPAARGGLGPTMQMADNIEQLLRELYVISRGAVEQLRP  719

PRV_UL37 613   LEAPAPAPPSLAASAFLGDLLARALRRRLEDMATHTGDLLDSMATAAGAVAERVVALERLFTCRFVFRGRTASVFAPGEGGPCLGTWRLA  702
HSV1_UL37 720  LVQLQPPPPPEVGTSLLLISMYALAARGVLQDLAERADPLIRQLEDAIVLLRLHMRTLSAFFECRFESDGRRLYA-VVGDTPDRLGPWPP  808

PRV_UL37 703   DVVDAVGAFRAEVNTHRSDMRADAGALRGVMAQTTEALRECEALGLQAPVFRTL---------------CADHARLSKLQTSAALTAGRL  777
HSV1_UL37 809  EAMGDAVSQYCSMYHDAKRALVASLASLRSVITETTAHLGVCDELAAQVSHEDNVLAVVRREIHGFLSVVSGIHARASKLLSGD------  892

PRV_UL37 778   QAATVLRPVERFLARWDLVSAALRRAVD---ERGVLALVATLRETWTEAREEQAPPAPAF-TPEELREAADRVLGDYHEVAGDGEAGPDA  863
HSV1_UL37 893  --QVPGFCFMGQFLARWRRLSACYQAARAAAGPEPVAEFVQELHDTWKGLQTERAVVVAPLVSSADQRAAAIREVMAHAPEDAPPQSPAA  980

PRV_UL37 864   VPLSASVNVRDWAAVNLEVLRRGARAPDDASAG----VPLLAQTWVPVDRLLAEVDAVSK-----------------------------  919
HSV1_UL37 981  DRVVLTSRRDLGAWGDYSLGPLGQTTAVPDSVDLSRQGLAVTLSMDWLLMNELLRVTDGVFRASAFRPLAGPESPRDLEVRDAGNSLPAP 1070

PRV_UL37       --------------------------------------------------------  919
HSV1_UL37 1071 MPMDAQKPEAYGHGPRQADREGAPHSNTPVEDDEMIPEDTVAPPTDLPLTSYQ      1123
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FoldUnfold disorder
FoldIndex disorder
**BPAG binding**
UL36 binding
gK/UL20 binding
TRAF6 binding
RIG-I binding

☐ resolved helix    ⊏⊐ predicted helix    ⤷ construct boundaries

⇨ resolved sheet    ⇨ predicted sheet

2

**Figure S1. Predicted disorder and secondary structure for PRV and HSV-1 UL37.** Secondary structure prediction for PRV (above sequence) and HSV-1 (below sequence) UL37 as calculated by PsiPred (http://bioinf.cs.ucl.ac.uk/psipred) (1). Solid cylinders and arrows represent resolved helices and sheets, respectively. Dashed cylinders and arrows represent predicted helices and sheets, respectively. Pink arrows mark construct boundaries for PRV UL37. Residues in purple are predicted to be unfolded as calculated by FoldUnfold (http://bioinfo.protres.ru/ogu/) (2). Residues in blue are predicted to be disordered as calculated by FoldIndex (https://fold.weizmann.ac.il/fldbin/findex) (3). Sequences that alternate between purple and blue indicate more than one program predicted disorder for that sequence. Residues in bold indicate the region required for BPAG binding. Residues highlighted in pink (gK/UL20), cyan (UL36), green (RIG-I) and yellow (TRAF6) are important for known binding interactions.
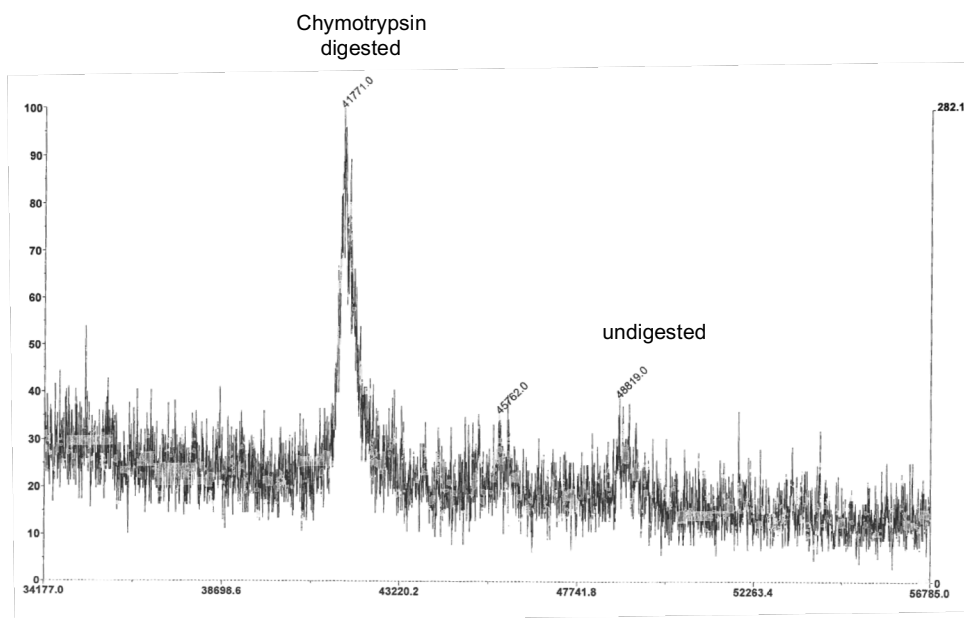
**Figure S2. Limited proteolysis MS data.** Limited proteolysis MALDI-TOF data for UL37C-478-919-StII with chymotrypsin, zoomed in to the range between 34.177 kDa and 56.785 kDa.
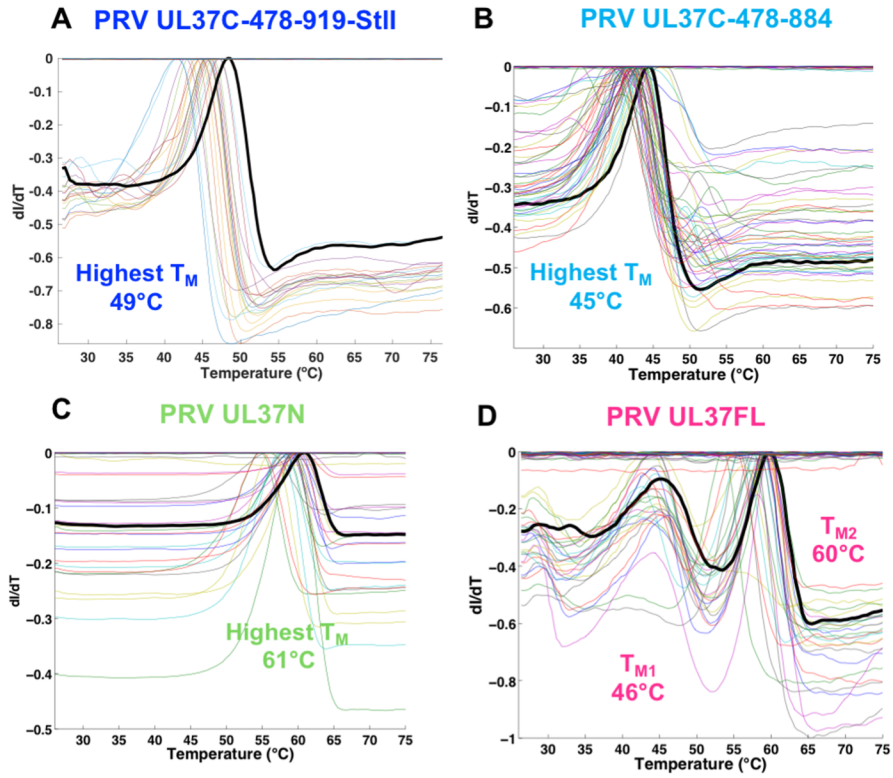
**Figure S3. Thermofluor assay of PRV UL37.** Melting temperatures as determined by the Thermofluor assay for **(a)** PRV UL37C-478-919-StII **(b)** PRV UL37C-478-884 **(c)** PRV UL37N and **(d)** PRV UL37FL.
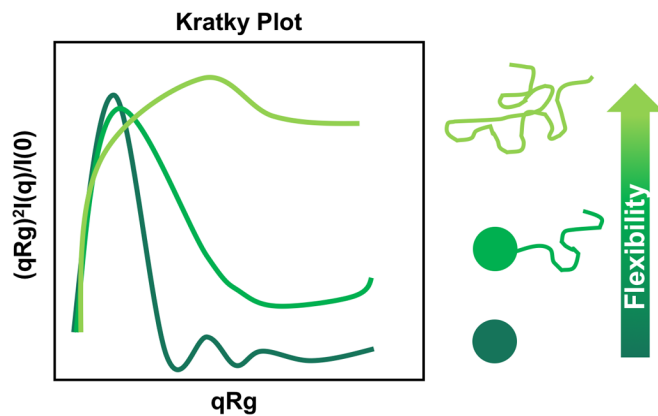
**Figure S4. Kratky Plot example.** Figure adapted from (4). Kratky Plot curves representative of varying protein flexibility shown in comparison to each other.

**Supporting References**

1. McGuffin, L. J., Bryson, K., and Jones, D. T. (2000) The PSIPRED protein structure prediction server. *Bioinformatics* **16**, 404-405
2. Galzitskaya, O. V., Garbuzynskiy, S. O., and Lobanov, M. Y. (2006) FoldUnfold: web server for the prediction of disordered regions in protein chain. *Bioinformatics* **22**, 2948-2949
3. Prilusky, J., Felder, C. E., Zeev-Ben-Mordehai, T., Rydberg, E. H., Man, O., Beckmann, J. S., Silman, I., and Sussman, J. L. (2005) FoldIndex: a simple tool to predict whether a given protein sequence is intrinsically unfolded. *Bioinformatics* **21**, 3435-3438
4. Rambo, R. P., and Tainer, J. A. (2011) Characterizing flexible and intrinsically unstructured biological macromolecules by SAS using the Porod-Debye law. *Biopolymers* **95**, 559-571