Supplementary Information

S1: Peptide purification and characterization

1a: The crude peptide was purified by preparative scale RP-HPLC using a 5-95% MeCN/H₂O gradient (with 0.1% formic acid). The pure peptide was obtained in 9% overall yield based on initial resin loading. HRMS (ESI-TOF) m/z [M + H]⁺ calcd for C₇₆H₁₁₂N₁₆O₂₂S₂ 1665.7651, found 1665.76397.



1b: The crude peptide was purified by preparative scale RP-HPLC using a 20-80% MeCN/H₂O gradient (with 0.1% formic acid). The pure peptide was obtained in 7% overall yield based on initial resin loading. HRMS (ESI-TOF) m/z [M + H]⁺ calcd for C₇₆H₁₁₂N₁₆O₂₂S₂ 1665.7651, found 1665.7687.



1c: The crude peptide was purified by preparative scale RP-HPLC using a 5-95% MeCN/H₂O gradient (with 0.1% formic acid). The pure peptide was obtained in 11% overall yield based on initial resin loading. HRMS (ESI-TOF) m/z [M + H]⁺ calcd for C₇₅H₁₁₁N₁₇O₂₂S₂ 1666.7604, found 1666.7601.



1d: The crude peptide was purified by preparative scale RP-HPLC using a 20-80% MeCN/H₂O gradient (with 0.1% formic acid). The pure peptide was obtained in 8% overall yield based on initial resin loading. HRMS (ESI-TOF) m/z [M + H]⁺ calcd for C₇₂H₁₁₀N₁₆O₂₂S₂ 1615.7495, found 1615.7465.



2a: The crude peptide was purified by preparative scale RP-HPLC using a 10-60% MeCN/H₂O gradient (with 0.1% formic acid). The pure peptide was obtained in 14% overall yield based on initial resin loading. HRMS (ESI-TOF) m/z [M + H]⁺ calcd for C₇₉H₁₁₀N₁₆O₂₃S₂ 1715.7444, found 1715.7454.



2b: The crude peptide was purified by preparative scale RP-HPLC using a 10-60% MeCN/H₂O gradient (with 0.1% formic acid). The pure peptide was obtained in 4% overall yield based on initial resin loading. HRMS (ESI-TOF) m/z [M + H]⁺ calcd for C₇₉H₁₁₀N₁₆O₂₃S₂ 1715.7444, found 1715.7439.



2c: The crude peptide was purified by preparative scale RP-HPLC using a 20-80% MeCN/H₂O gradient (with 0.1% formic acid). The pure peptide was obtained in 10% overall yield based on initial resin loading. HRMS (ESI-TOF) m/z [M + H]⁺ calcd for C₇₈H₁₀₉N₁₇O₂₃S₂ 1716.7396, found 1716.7402.



2d: The crude peptide was purified by preparative scale RP-HPLC using a 10-60% MeCN/H₂O gradient (with 0.1% formic acid). The pure peptide was obtained in 11% overall yield based on initial resin loading. HRMS (ESI-TOF) m/z [M + H]⁺ calcd for C₇₅H₁₀₈N₁₆O₂₃S₂ 1665.7287, found 1665.7269.



3a: The crude peptide was purified by preparative scale RP-HPLC using a 15-50% MeCN/H₂O gradient (with 0.1% formic acid). The pure peptide was obtained in 10% overall yield based on initial resin loading. HRMS (ESI-TOF) m/z [M + H]⁺ calcd for C₇₄H₁₀₈N₁₆O₂₃S₂ 1653.7287, found 1653.7258.



3b: The crude peptide was purified by preparative scale RP-HPLC using a 15-50% MeCN/H₂O gradient (with 0.1% formic acid). The pure peptide was obtained in 5% overall yield based on initial resin loading. HRMS (ESI-TOF) m/z [M + H]⁺ calcd for C₇₄H₁₀₈N₁₆O₂₃S₂ 1653.7287, found 1653.7290.



3c: The crude peptide was purified by preparative scale RP-HPLC using a 10-60% MeCN/H₂O gradient (with 0.1% formic acid). The pure peptide was obtained in 11% overall yield based on initial resin loading. HRMS (ESI-TOF) m/z [M + H]⁺ calcd for C₇₃H₁₀₇N₁₇O₂₃S₂ 1654.7230, found 1654.7240.



3d: The crude peptide was purified by preparative scale RP-HPLC using a 15-50% MeCN/H₂O gradient (with 0.1% formic acid). The pure peptide was obtained in 9% overall yield based on initial resin loading. HRMS (ESI-TOF) m/z [M + H]⁺ calcd for C₇₀H₁₀₆N₁₆O₂₃S₂ 1603.7131, found 1603.7121.



4: The crude peptide was purified by preparative scale RP-HPLC using a 5-95% MeCN/H₂O gradient (with 0.1% formic acid). The pure peptide was obtained in 2% overall yield based on initial resin loading. HRMS (ESI-TOF) m/z [M + H]⁺ calcd for C₇₁H₁₀₄N₁₆O₂₃ 1549.7533, found 1549.7545.



5: The crude peptide was purified by preparative scale RP-HPLC using a 5-95% MeCN/H₂O gradient (with 0.1% formic acid). The pure peptide was obtained in 3% overall yield based on initial resin loading. HRMS (ESI-TOF) m/z [M + H]⁺ calcd for C₆₆H₁₀₂N₁₆O₂₃ 1487.7377, found 1487.7406.



S2: Recombinant expression and purification of wild type VP40



Figure S2: Recombinant expression and purification of 6xHis-tag WT VP40 displays two peaks when subjected to size exclusion column (Superdex 200 10/300 GL). The smaller peak at elution volume \sim 50 mL corresponds to VP40 octamer while the peak at \sim 70ml elution volume corresponds to the dimer. The WT VP40 dimer and octamer were purified more than five independent times for this study.



S3: Thermal shift assay for peptides in Series 1, 2 and 3

Figure S3: Peptides which do not change the melting profiles of VP40 as compared to DMSO vehicle control are shown. Respective peptides were added to purified VP40 dimer and the melting profiles in presence of peptides were plotted. A) Series 1 of peptides with staple between positions 106-111 shown in different shades of green. B) Series 2 of peptides with staple between residues 111-115 are shown in different shades of red. C) Series 3 of peptides with staple between residues 115-120 are shown in different shades of blue. Three independent experiments were performed for each condition.

<u>S4: Thermal shift assay with Control peptides</u>



Figure S4: Linear peptides were synthesized as control peptides. Peptide with positions 115 and 120 identical to parent sequence (4) and residues 115 and 120 mutated to alanine (5) were added to VP40 dimer. A) Melting profiles of VP40 with each of these peptides was measured. B) Inflection temperature 1 (Ti₁) and inflection temperature 2 (Ti₂) were compared to vehicle control DMSO using one way ANOVA with multiple comparisons. **** indicates p<0.0001. There independent experiments were performed for each condition shown.





Figure S5: Comparison of peptides that decreased both inflection temperatures of VP40 (1c, 2b, 2c and 3b) was performed for VP40 binding ability using microscale thermophoresis (MST). Each peptide was titrated into cell lysates containing GFP-tagged VP40 and difference of fluorescence before and after irradiation of sample was calculated as the normalized fluorescence (Fnorm) and plotted versus concentration of the peptide. **3b** (D) shows the highest change in fluorescence as compared to 1c (A), 2b (B) and 2c (C).



Figure S6: Time evolution of the peptide secondary structure of the WT helix (residues 106-121) and **3b** during the 500-ns simulations for the three replica runs. The secondary structure was calculated with VMD's timeline plugin, which uses STRIDE for assigning the secondary structure. Purple color represents the helical structure, whereas cyan color represents a turn/coil structure. There independent replicates were run and shown.