



Supplementary information to:

Conformational stabilization of gp41-mimetic miniproteins opens up new ways of inhibiting HIV-1 fusion

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Figure S1. Sequences and topology of the covNHR miniproteins and peptides. Mutations in red were engineered in this work. A) covNHR-SS sequence. B) covNHR-N-dSS sequence C) CHR C34 and Y24L peptide sequences. Residues corresponding to the different binding pocket motifs are colored as follows: CTP in orange, HP in cyan, MP in blue and NTP in red.

Table S1. Description of envelope glycoproteins used in the ELISA binding experiments.

Abbreviation	Type	Description	Reference
JRFL	Gp140	A soluble uncleaved gp140 Env stabilized trimer derived from HIV-1 JRFL containing a C-terminal foldon sequence.	[1]
MN/LAI	Gp160	A hybrid oligomeric gp160 Env with gp120 derived from HIV-1 MN and gp41 derived from HIV-1 LAI.	[2]
THO23/LAI	Gp160	Recombinant Env with gp120 from HIV-1 92TH023 linked to gp41 from LAI, with a deletion in the immunodominant region.	[3]
ZM 4096	Gp140	Synthetic construct derived from gp140 sequence of the codon-optimized HIV-1 96ZM651.	[4]
CN54	Gp140	Recombinant Env trimer containing gp120+gp41 ectodomain from HIV-1 CN54.	[5]

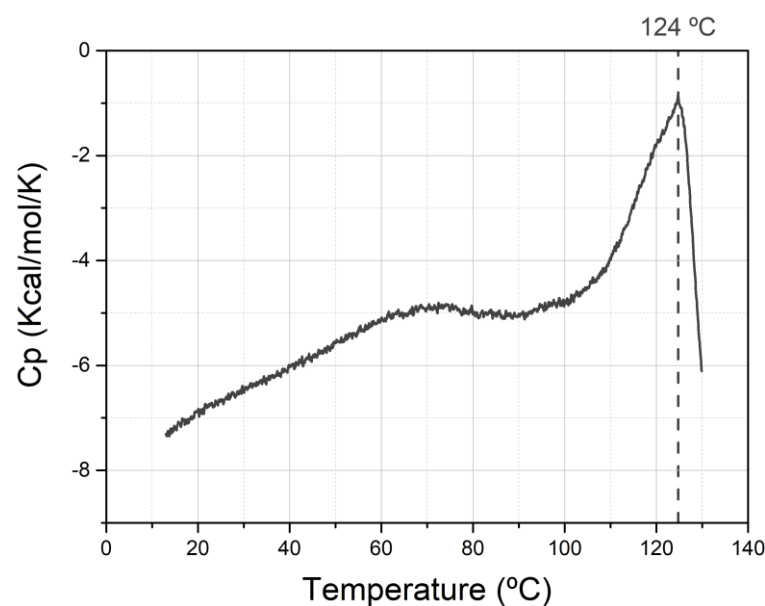


Figure S2. Differential scanning calorimetry thermogram of the thermally induced denaturation of covNHR-SS. The DSC thermogram was recorded at a scan rate of $2^{\circ}\text{C}\cdot\text{min}^{-1}$ at a concentration of $30\text{ }\mu\text{M}$.

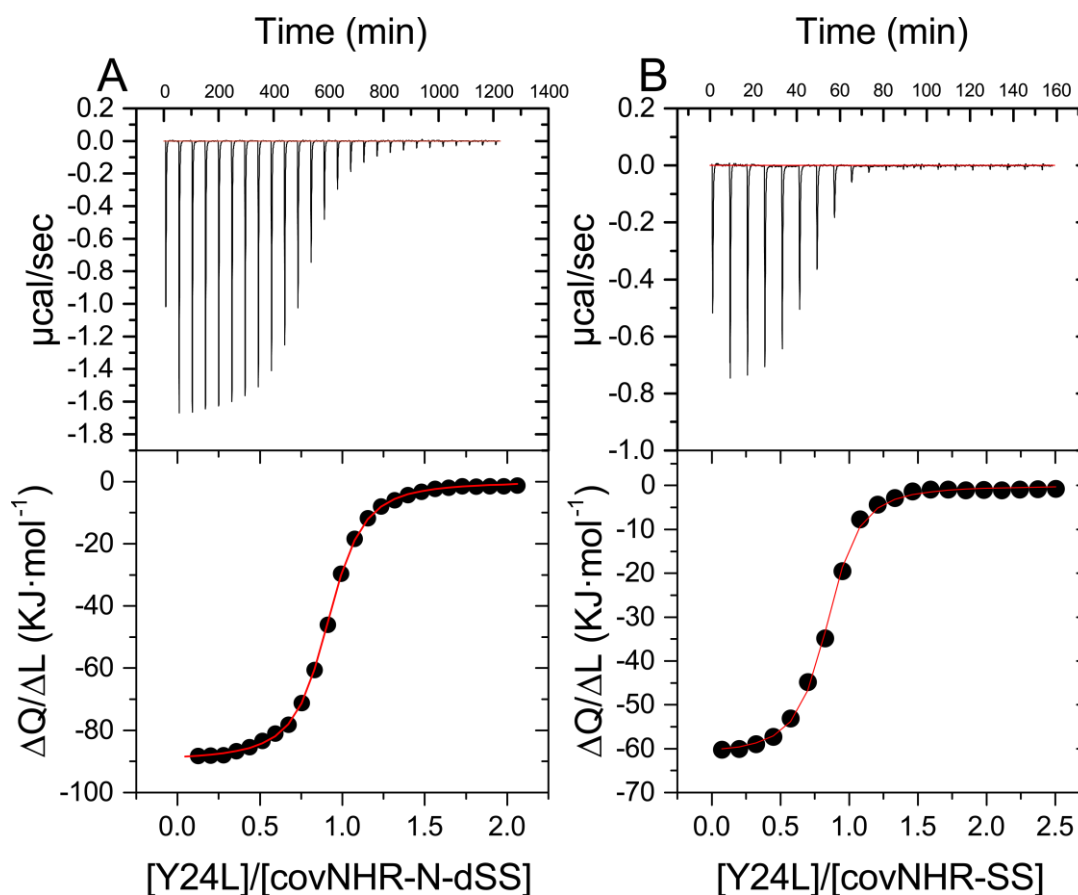


Figure S3. Isothermal titration calorimetry experiments of Y24L peptide binding to the covNHR proteins. **A)** Y24L binding to covNHR-N-dSS and **B)** to covNHR-SS. The experiments were measured at 25°C by titration of $10\text{ }\mu\text{M}$ of each miniprotein in the cell with $\sim 300\text{ }\mu\text{M}$ of Y24L peptide from the syringe. The upper panels show the experimental ITC thermograms and the lower panels the normalized binding isotherms. The symbols in the lower panels correspond to the

experimental heats normalized per mole of injected peptide and the lines represent the best fittings using a binding model of n identical and independent sites.

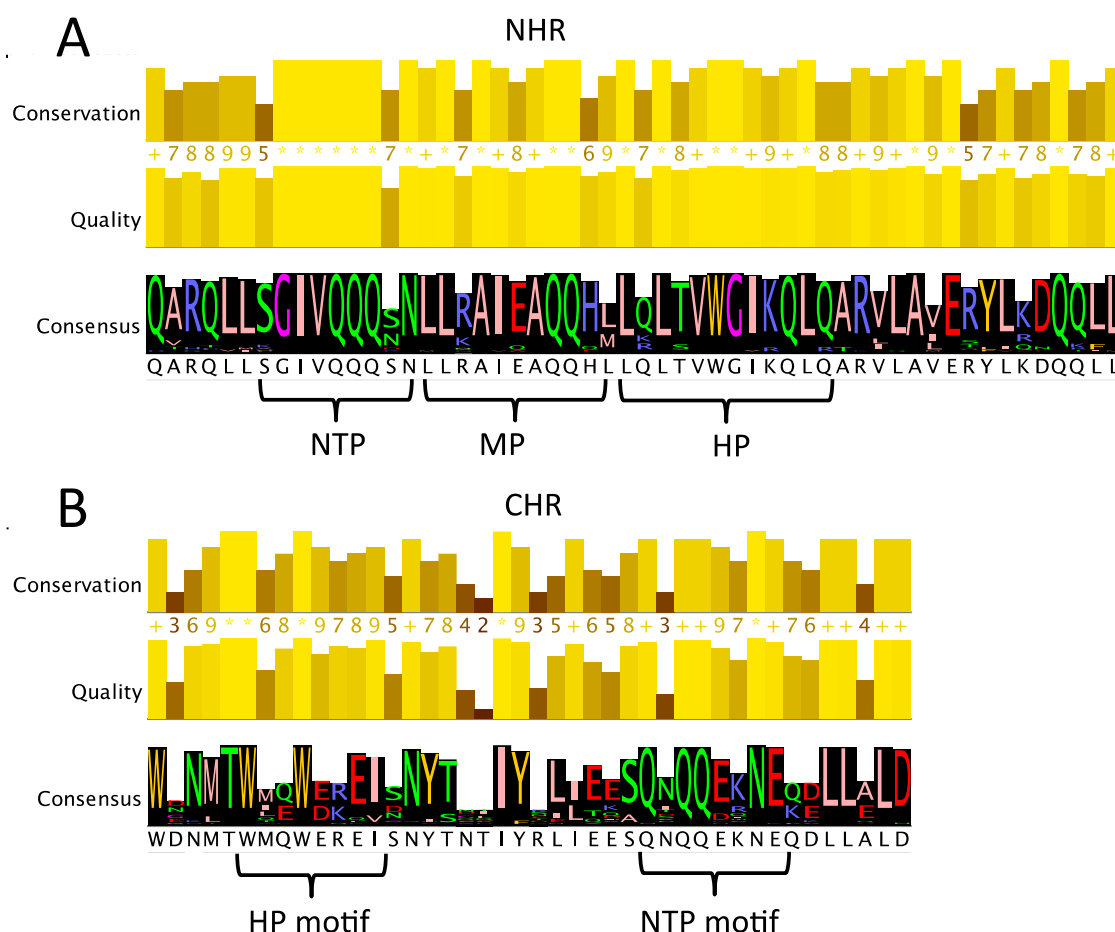


Figure S4. Sequence conservation and consensus sequence of NHR and CHR in gp41. The plots have been made with Jalview [6] using the 2018 Compendium sequence alignment [7] from Los Alamos Sequence Database (<https://www.hiv.lanl.gov>).

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