Supporting Information

Identification of HIV-1 Inhibitors Targeting The Nucleocapsid Protein

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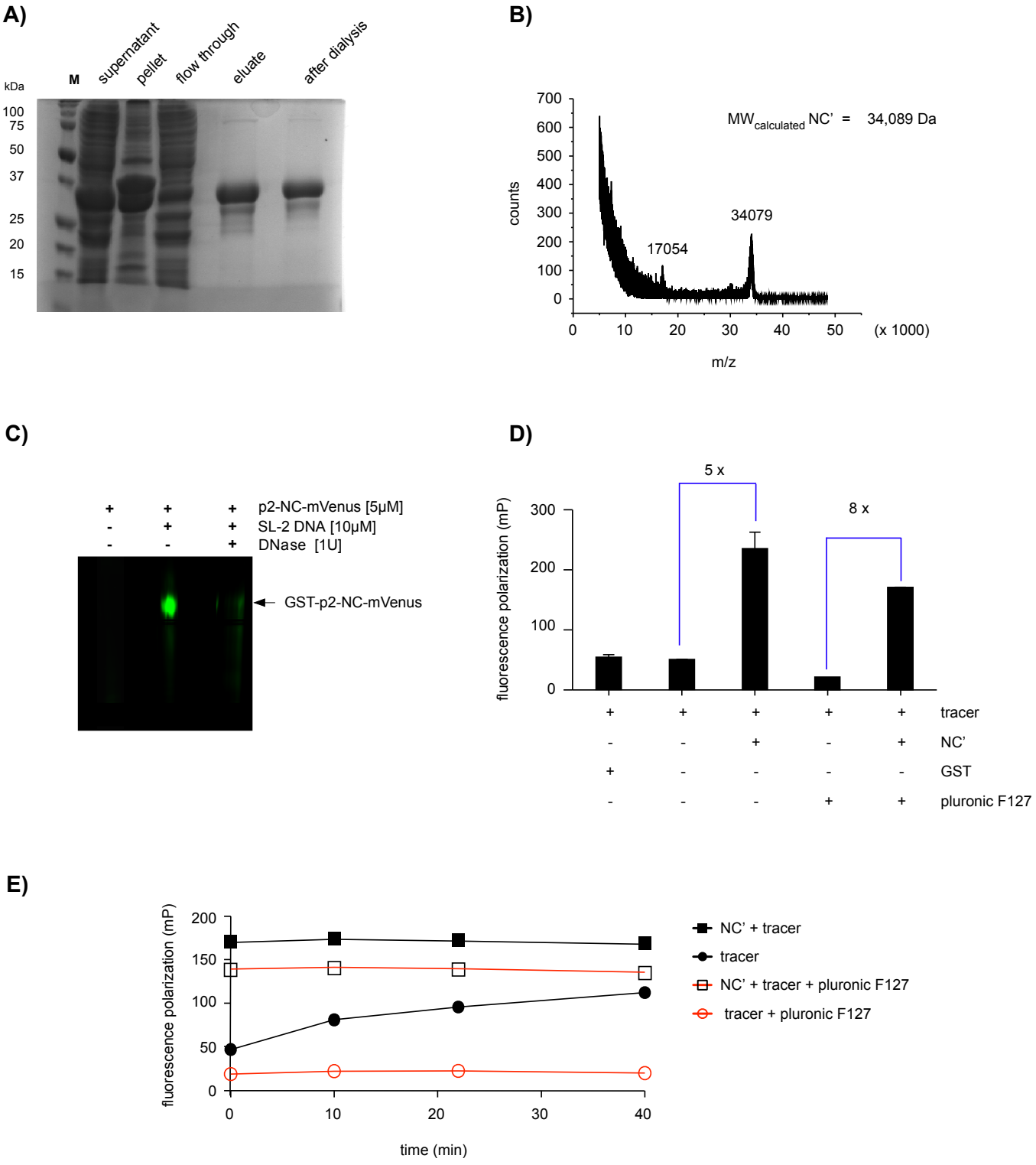
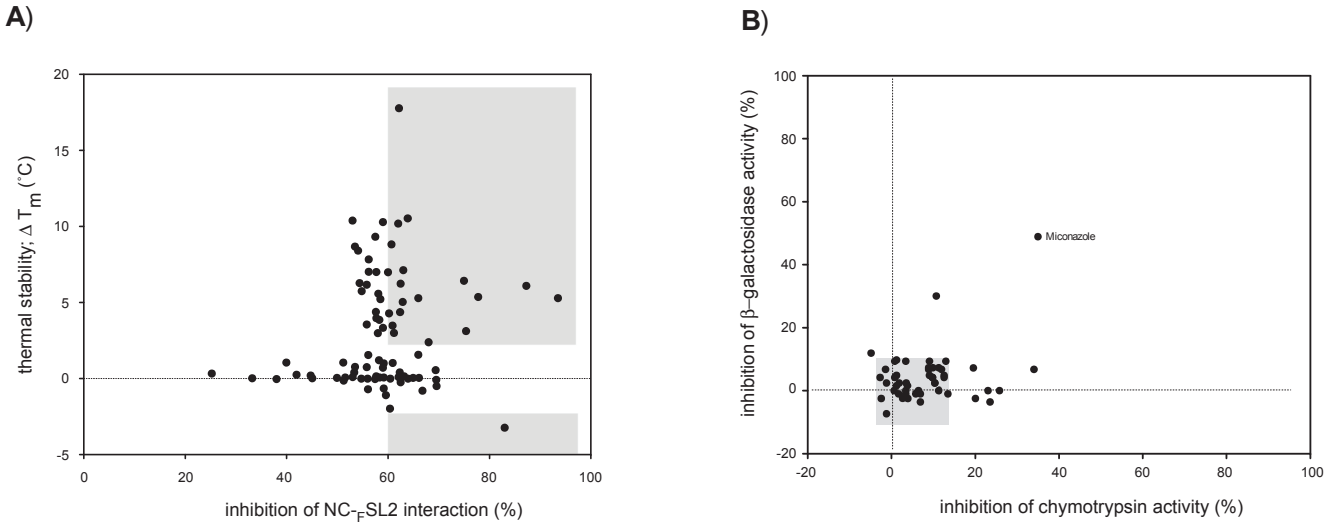


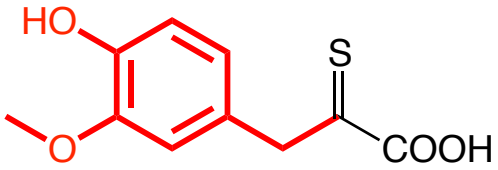
Supplementary Figure 1. Quality control and assay development of the FP-based HTS. (A) SDS-PAGE analysis of protein samples taken during the different stages of NC' purification using affinity chromatography ~ 90 % purity. (B) MALDI Mass spectrometry analysis of NC' confirmed the correct size of the fusion protein. (C) Native PAGE analysis of the NC'-SL-2 complex. Shown is the fluorescence scan of a Native Page gel. A fluorescent protein variation of NC', GST-p2-NC-mVenus[1], was incubated with or without SL-2 DNA before analysis on a Native Page gel. The presence of SL-2 DNA stabilizes GST-p2-NC-

mVenus, which is seen as a distinct protein band in the middle gel lane. In contrast, in the absence of SL-2 DNA the GST-p2-NC-mVenus is seen only as a "smear" in the first lane of the gel. When DNAse is added to the GST-p2-NC-mVenus-SL-2 complex the DNA is digested and only a smear is seen showing the same pattern as GST-p2-NC-mVenus in lane one. (D and E) Optimization of the fluorescence polarization signal for HTS use. Shown is the influence of the detergent pluronic F127 on the H/L ratio and the signal over the time.



Supplementary Figure 2. Positive and negative hit selection. (A) Correlation of DSF-based thermal stability versus FP-based inhibition of Hits identified by NC'-$_F$SL-2 FP HTS. Only compounds with a $\Delta T_m \geq 2$ ˚C and inhibition ≥ 60 % (grey shading) were selected for further evaluation. (B) Correlation of beta-galactosidase inhibition versus chymotrypsin inhibition. Compounds that showed more than 15 % inhibition (grey shading) were eliminated from further consideration. The known promiscuous inhibitor miconazole was chosen as the control for promiscuous inhibition.[2]

compounds identified by Shvadchak et al., 2009



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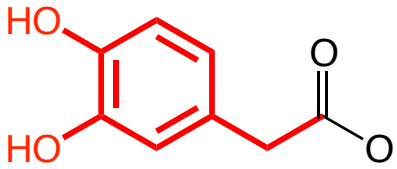
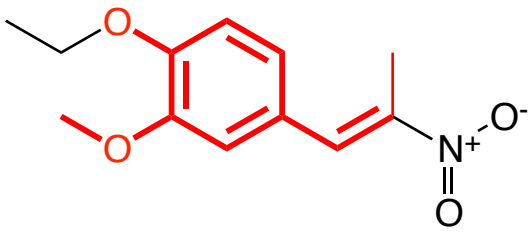


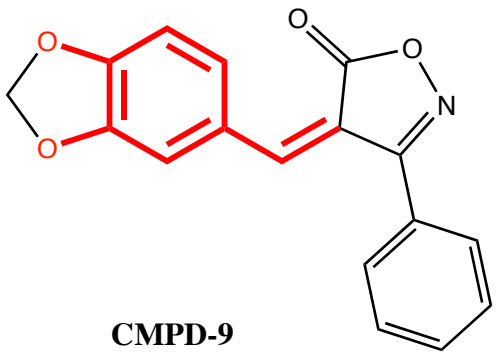
H04

compounds identified by Breuer et al., 2011

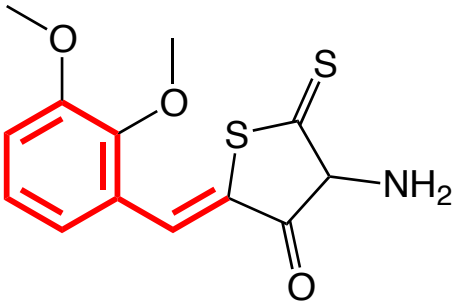
CMPD-8



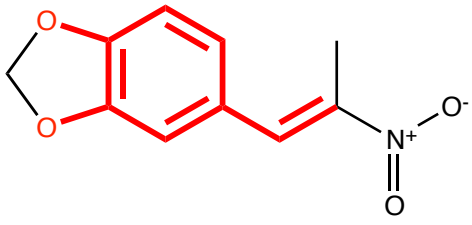
CMPD-5



CMPD-1



CMPD-9



Supplementary Figure 3. Structural comparison of small-molecule NC' inhibitor compounds. Structural comparison between previously identified inhibitors by Shvadchak and colleagues[3] and the selected 5 compounds identified herein, similarities are emphasized by red for compounds **1**, **5**, **8** and **9**.

Supplementary Table 1. Summary of the mass spectrometry analysis of the protein – compound complexes of compounds **1**, **5**, **8**, **9**, and **10** and NC'**.**

	theoretical mass of the CMPD-protein complex	analyzed mass of the CMPD-protein complex	molecular weight of the CMPD
DMSO		34056	
CMPD-1	34351.38	34117.79	295.38
CMPD-5	34349.27	34143.42	293.27
CMPD-8	34293.25	34093.29	237.25
CMPD-9	34263.19	34123.91	207.19
CMPD-10	34499.44	34140.23	443.44

Supplementary Table 2. Summary of assays results for compounds **1**, **5**, **8**, **9**, and **10** in the NCBI PubChem database[a]

Name	Compound ID	Assays tested	Assays active
CMPD-1	5712383	14	0
CMPD-5	5394836	7	0
CMPD-8	5376876	8	0
CMPD-9	5375839	78	13
CMPD-10	5704563	7	1

[a]http://pubchem.ncbi.nlm.nih.gov/

REFERENCES

(1) Breuer, S.; Sepulveda, H.; Chen, Y.; Trotter, J.; Torbett, B. E. A cleavage enzyme-cytometric bead array provides biochemical profiling of resistance mutations in HIV-1 Gag and protease. *Biochem.* **2011,** 50, 4371-4381.
(2) Seidler, J.; McGovern, S. L.; Doman, T. N.; Shoichet, B. K. Identification and prediction of promiscuous aggregating inhibitors among known drugs. *J. Med. Chem.* **2003,** 46, 4477-4486.
(3) Shvadchak, V.; Sanglier, S.; Rocle, S.; Villa, P.; Haiech, J.; Hibert, M.; Van Dorsselaer, A.; Mely, Y.; de Rocquigny, H. Identification by high throughput screening of small compounds inhibiting the nucleic acid destabilization activity of the HIV-1 nucleocapsid protein. *Biochimie.* **2009,** 91, 916-923.