

## The p66 Immature Precursor of HIV-1 Reverse Transcriptase.

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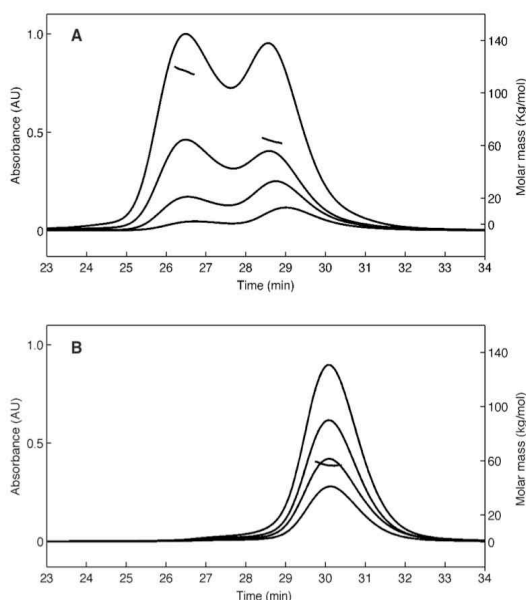
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List of the Supplementary data.

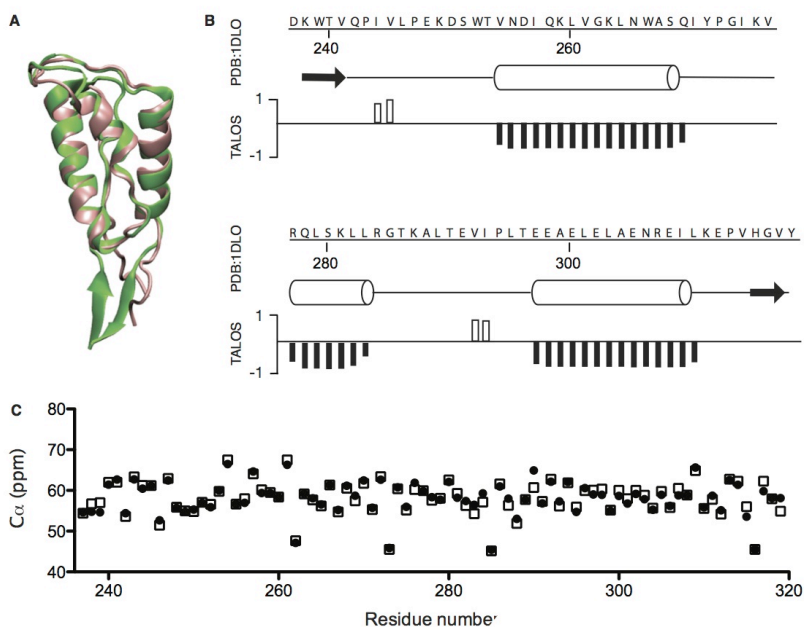
Figure S1. Multi-angle Light scattering (MALS) elution profiles of p66 and p51 protein samples.

Figure S2. Thumb domain structure.

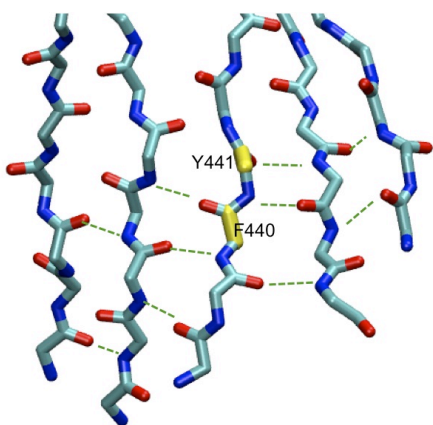
Figure S3. Region around the p51-RNH processing site in RNH structure with a subset of hydrogen bonds highlighted.



**Figure S1.** Multi-angle light scattering (MALS) elution profiles of (A) the p66 and (B) the p51 protein samples. Experiments were repeated at different protein concentrations from 10.9  $\mu\text{M}$  to 59.9  $\mu\text{M}$  and 19.3  $\mu\text{M}$  to 58.6  $\mu\text{M}$ , for p66 and p51 samples, respectively. Assuming that the samples are diluted approximately 10-fold on the gel-filtration column, homodimer dissociation constants for p66 and p51, calculated from the observed MALS results, are  $4.3 \pm 0.7 \mu\text{M}$  and  $318 \pm 116 \mu\text{M}$ , respectively, which are in excellent agreement with the published homodimer dissociation constants of  $\sim 4 \mu\text{M}$  and  $\sim 230 \mu\text{M}$  for p66 and p51, respectively, as described in the main text.



**Figure S2.** Thumb domain structure comparison: (A) Thumb domain structure in the p66 (green) and p51 (pink) subunits in the RT crystal structure (PDB: 1DLO), (B) TALOS+ derived secondary structure of the isolated Thumb domain, based on  $C_\alpha$ ,  $C_\beta$ ,  $C'$ ,  $N$ , and  $H_N$  chemical shifts. The amino acid sequence and secondary structure elements in the RT crystal structure are depicted on top. (The N-terminal methionine and the C-terminal 6 histidines are omitted), (C) Experimentally determined  $C_\alpha$  chemical shifts for the isolated Thumb domain (black circles) compared with those back-calculated from the coordinates of the Thumb domain in 1DLO, using Sparta (open square).



**Figure S3.** Region of the RNH backbone structure with a subset of hydrogen bonds highlighted (dashed lines). In the  $^1\text{H}$ - $^{15}\text{N}$  HSQC TROSY spectrum of the p66 homodimer, cross-peaks reside at similar positions, in frequencies, to these highlighted amide ones of the isolated RNH fragment ( $\pm 27$  Hz). NMR structure of the isolated RNH domain (PDB = 1O1W) was used to generate the graphics.