# SUPPLEMENTAL DATA

## SUPPLEMENTAL EXPERIMENTAL PROCEDURES

**Viral Encapsidation Analysis.** 293T cells were transfected with 2 µg of pDHIV3-GFP or pDHIV3-GFP/Δvif and 1 µg of EGFP-HA tagged versions of either hA3G, N1/2, CD1, NCD1, C3/4, C1/2, CD2 or NCD2 using FuGENE® 6 (Roche). Forty-eight hours after transfection cell extracts were harvested in Reporter Lysis Buffer (Promega) with Complete® Mini EDTA free protease inhibitors (Roche) and equivalent µg quantities of protein were loaded into each lane for analysis by SDS-PAGE and western blotting with HA (Convance), β-actin (Sigma), or Vif (#6459, NIH AIDS Research and Reference Reagent Program) [Simon, J. H., Southerling, T. E., Peterson, J. C., Meyer, B. E., and Malim, M. H. (1995) *J Virol* **69**(7), 4166-4172] antibodies. Viral particles were isolated by filtering the cell media through a 0.45 micron filter followed by p24 ELISA assays (Zeptometrix) to normalize viral load. p24-equivalent amounts of viral particles were pelleted through a 20% sucrose cushion at 150,000 x g for 2 hours. The viral particles were resuspended in SDS-PAGE loading buffer, boiled and analyzed by SDS-PAGE and western blotting with HA (Convance), and p24 (#3537, NIH AIDS Research and Reference Reagent Program) [Chesebro, B., Wehrly, K., Nishio, J., and Perryman, S. (1992) *J Virol* **66**(11), 6547-6554] antibodies.

**Gel Filtration Analysis.** EGFP, EGFP-C1/2 and EGFP-CD2 were transfected into 293T cells with FuGENE® 6 (Roche). Twenty-four hours after transfection cell extracts were harvested in NP-40 lysis buffer (50 mM Tris pH 7.4, 150 mM NaCl, 0.1% NP-40) with Complete® Mini EDTA free protease inhibitors (Roche). Cell extracts were treated with 40 µg/mL of RNase A for 1 hour at 37 °C. After a 5 min spin at 15,000 x g 500uL of each cell extract was loaded onto a Sephacryl S200 column (GE Healthcare) and 45, 1 mL fractions were collected. Volumes of 100 µL (EGFP) and 500 µL (EGFP-C1/2 and -CD2) from every other fraction were acetone precipitated and run on SDS-PAGE followed by western blotting with anti-GFP antibody (Roche).

**Comparison of experimental versus calculated SAXS intensity profiles.** hA3G protein purification, SAXS data collection for RNase A treated samples, and generation of the restored dummy atom model for hA3G are described in [Wedekind, J. E., Gillilan, R., Janda, A., Krucinska, J., Salter, J. D., Bennett, R. P., Raina, J., and Smith, H. C. (2006) *J Biol Chem* **281**(50), 38122-38126]. Fits of the experimental hA3G intensity profile versus those calculated from coordinate models were performed as a function of scattering vector (*q*) by use of CRYSOL [Svergun, D. I., Barberato, C., and Koch, M. H. J. (1995) *J. Appl. Cryst.* **28**, 768-773]. PDB files for tetrameric Cdd1 and APOBEC2 were retrieved from the Protein Data Bank (www.pdb.org) as entries 1r5t and 2nyt, respectively. Due to perceived increases in molecular volume associated with random coil in the lowest energy NMR coordinates of the C-terminal deaminase domain (i.e. residues 198-220 and between β2 and β2´), these structures were excluded from comparisons here, as well as Fig. 1*B* [Chen, K. M., Harjes, E., Gross, P. J., Fahmy, A., Lu, Y., Shindo, K., Harris, R. S., and Matsuo, H. (2008) *Nature* **452**(7183), 116-119].

## SUPPLEMENTAL FIGURE LEGENDS

FIGURE S1 **hA3G viral encapsidation, and Gag and Vif binding** (*A*) Westerns of cell extracts and viral particles isolated from 293T cells transfected with EGFP-HA tagged hA3G domains (*indicated across top*) and co-transfected with HIV DNA (WT) or ∆vif HIV DNA (∆Vif). Antibodies used for blots are shown on the right. Blotting with antibody against β-actin or p24 demonstrates equivalent protein loading of cell extracts and viral particles, respectively. Blots show that WT virus expressed Vif but ∆Vif virus did not. HA blots show domains of different sizes expressed in cell extracts (*top*) and whether they were packaged into viral particles (*lower middle*). (*B*) Co-immunoprecipitates from 293T cell extracts co-transfected with HIV DNA and V5-hA3G or V5-N1/2. Lanes 1 and 2 are blots of whole cell extracts and V5 immunoprecipitates, respectively, of hA3G and N1/2 detected with V5 antibody. Lanes 3 and 4 are blots of whole cell extract and co-immunoprecipitates, respectively, of Gag detected with a p24 antibody. Lane 5 is a control for non-specific Gag binding to Protein A. (C) Co- immunoprecipitates from 293T cells co-transfected with V5-hA3G, V5-N1/2, V5-C1/2, or EGFP-V5-CD1 interacting with

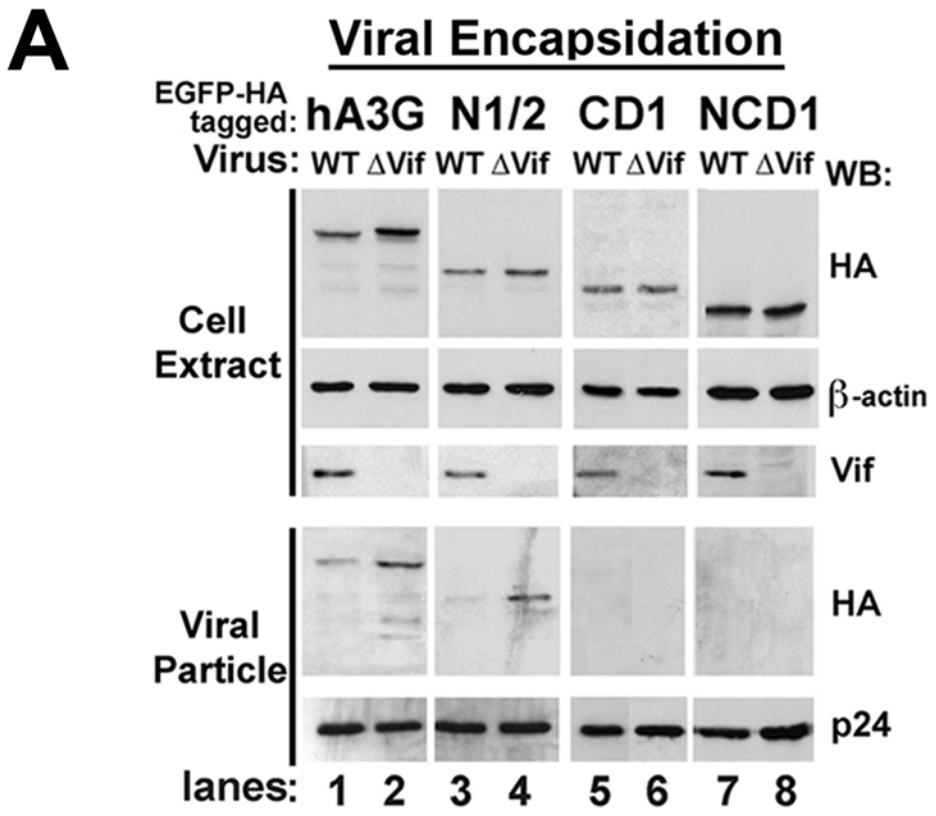
EGFP-HA-Vif. Lanes 1 and 2 are blots of whole cell extracts and GFP immunoprecipitates (*rows 1-3*) or V5 IP (*row 4*), respectively, of Vif detected with GFP (*rows 1-3*) or HA (*row 4*) antibody. Lane 3 is a control for non-specific Vif binding to Protein A. Lanes 4 and 5 are blots of whole cell extract and co- immunoprecipitates, respectively, of hA3G and hA3G domains detected with V5 antibody.

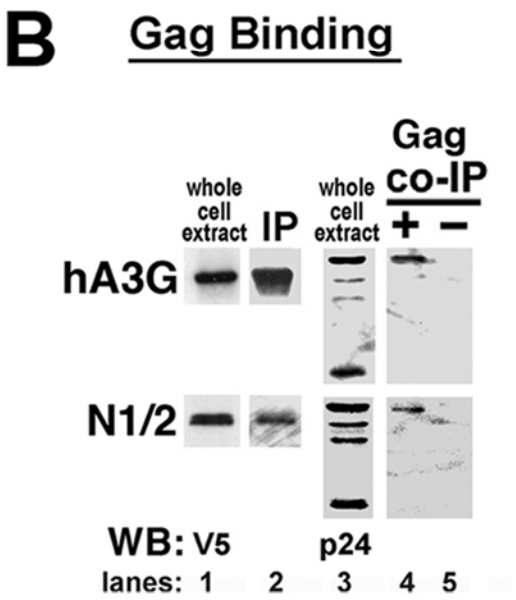
FIGURE S2 **Co-immunoprecipitates of alternatively tagged homologous N- and C-terminal domains of hA3G.** The hA3G domains co-transfected into 293T cells are represented in the left column as horizontal gray bars for CD1, NCD1, CD2 and NCD2. Tag symbols and lanes (*right*) are depicted as in Figure 3.

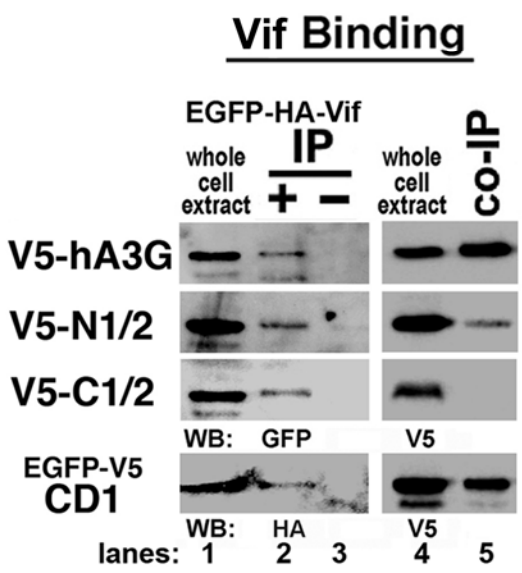
FIGURE S3 **Gel filtration chromatography on C-terminal domains of hA3G.** Cell extract of 293T cells transfected with EGFP-C1/2 (46 kDa), EGFP-CD2 (41 kDa), and EGFP (27 kDa) treated with RNase A were eluted from a gel filtration column and fractions (*indicated below each lane*) were western blotted with anti-GFP antibody. The kDa value based on molecular mass standards is indicated above the blots. Arrows indicate where a monomer of each construct are calculated to elute from the column based on calibration with protein molecular mass standards.

FIGURE S4 **Comparison of the experimental hA3G solution scattering profile versus that calculated from three respective coordinate models.** The calculated scattering profile for the 'square' cytidine deaminase, yeast Cdd1 (green curve), was generated from the crystal structure, shown as a ribbon model with a transparent surface (*right*). Each Cdd1 subunit is identical, but colored differently to emphasize the globular, domain-sequestered oligomer arrangement typical of pyrimidine metabolism enzymes. The shape agreement between the experimentally measured scattering data for dimeric hA3G (open circles) versus the scattering profile calculated from the crystallographic coordinates is indicated by the value $\chi$. Values close to 1.0 indicate outstanding agreement between the model and the structure in solution [Svergun, D. I., Barberato, C., and Koch, M. H. J. (1995) *J. Appl. Cryst.* **28**, 768-773] Similar comparisons are shown for APOBEC2 (hA2, blue line), which comprises four identical

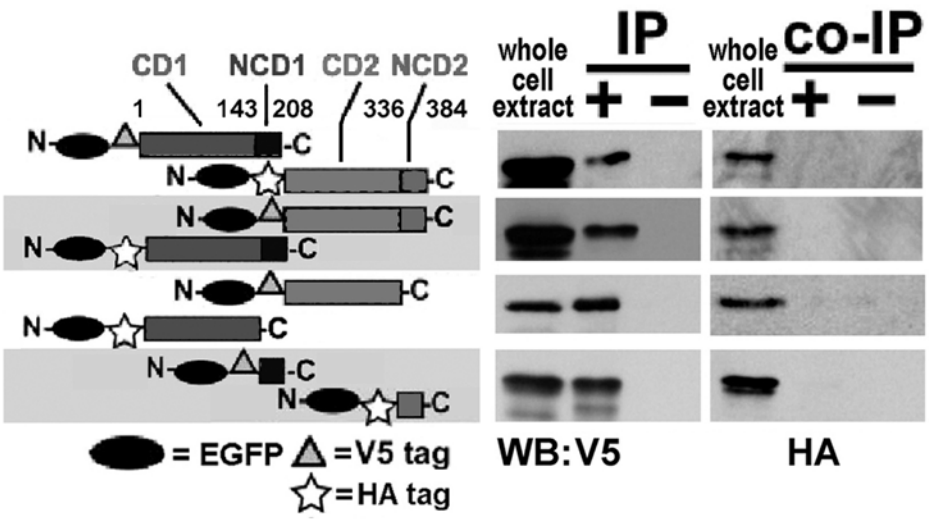
subunits, and the restored dummy atom model corresponding to hA3G (red line) as described previously (9). In the latter case, the surmised subunits are colored green and purple. Comparable comparisons at low angles of momentum transfer have been described previously [Sousa, M. C., Trame, C. B., Tsuruta, H., Wilbanks, S. M., Reddy, V. S., and McKay, D. B. (2000) *Cell* **103**(4), 633-643].


A
Viral Encapsidation
EGFP-HA tagged:
hA3G
N1/2
CD1
NCD1
Virus:
WT ΔVif WT ΔVif WT ΔVif WT ΔVif
WB:
Cell Extract
HA
β-actin
Vif
Viral Particle
HA
p24
lanes: 1 2 3 4 5 6 7 8

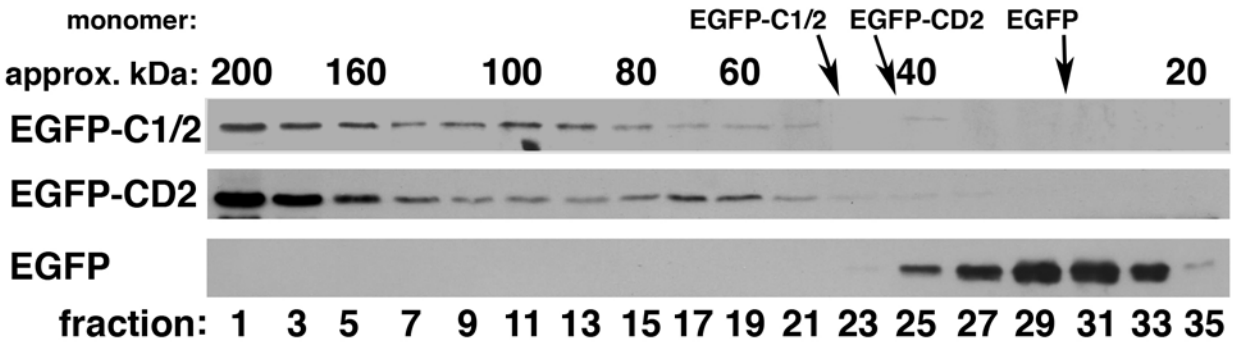

B
Gag Binding
whole cell extract
IP
whole cell extract
Gag co-IP
+ −
hA3G
N1/2
WB: V5
p24
lanes: 1 2 3 4 5


C
Vif Binding
EGFP-HA-Vif
whole cell extract
IP
+ −
whole cell extract
co-IP
V5-hA3G
V5-N1/2
V5-C1/2
WB: GFP
V5
EGFP-V5 CD1
WB: HA
V5
lanes: 1 2 3 4 5

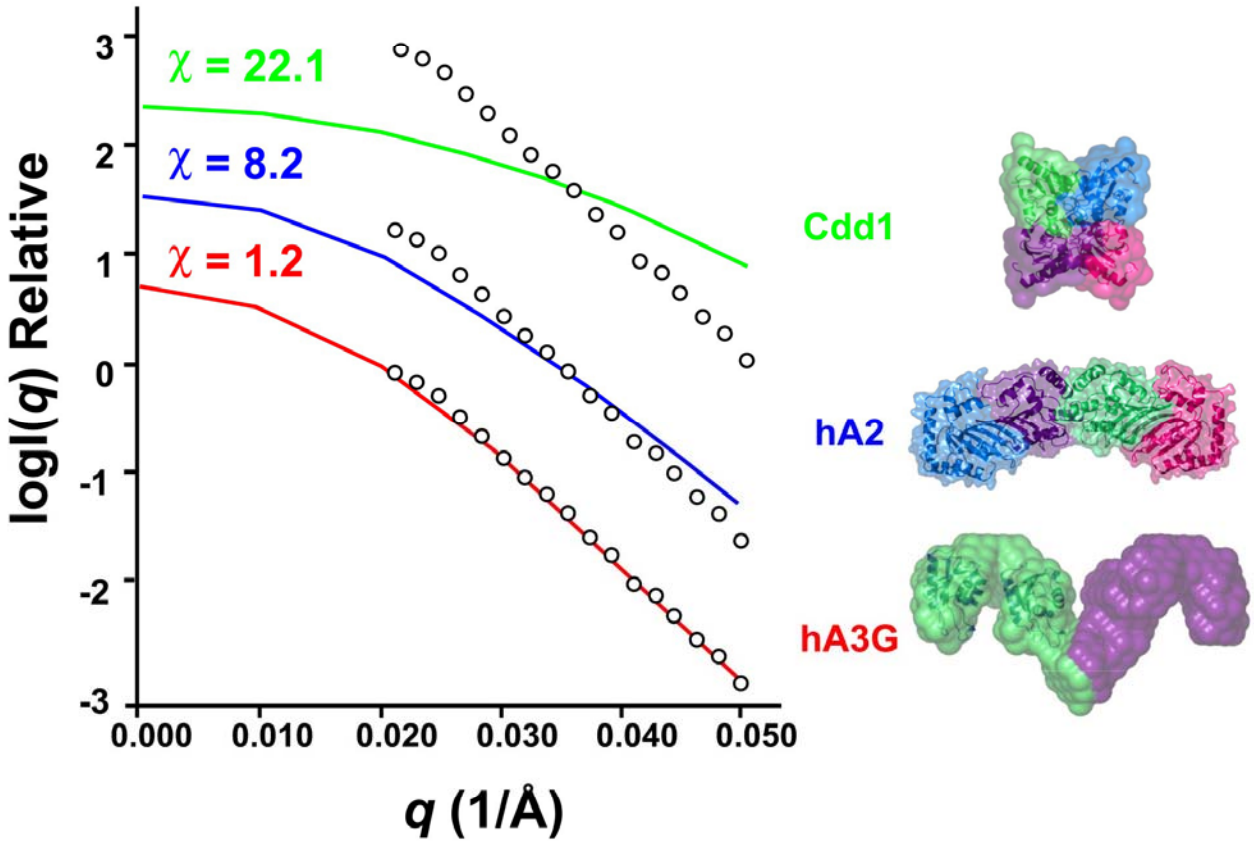
# FIGURE S1


CD1
NCD1
CD2
NCD2
1
143
208
336
384
N
C
= EGFP
=V5 tag
=HA tag
whole cell extract
IP
+
−
whole cell extract
co-IP
+
−
WB:V5
HA

# FIGURE S2


monomer:
EGFP-C1/2
EGFP-CD2
EGFP
approx. kDa: 200 160 100 80 60 40 20
EGFP-C1/2
EGFP-CD2
EGFP
fraction: 1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31 33 35

# FIGURE S3


χ = 22.1
χ = 8.2
χ = 1.2
logI(q) Relative
3
2
1
0
-1
-2
-3
0.000
0.010
0.020
0.030
0.040
0.050
q (1/Å)
Cdd1
hA2
hA3G

# FIGURE S4