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Supplemental Information

FEZ1 Is Recruited to a Conserved Cofactor Site on Capsid to Promote HIV-1 Trafficking

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Supplementary Data

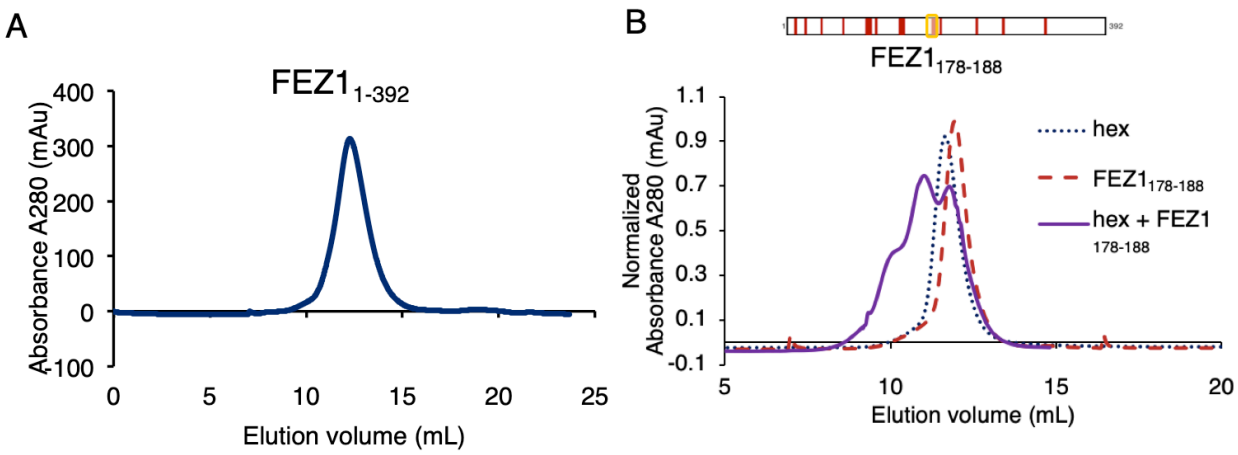

Fig. S1 Purification of full-length FEZ1$_{1-392}$ (A) and co-ellusion of FEZ1$_{178-188}$ with CA hexamer (B) in the SEC binding assay. Related to Figure 1. Schematic of the FEZ1$_{178-188}$ is boxed in yellow and shown on top of the chromatogram in (B). Red bars indicate the negatively-charged residues of FEZ1.

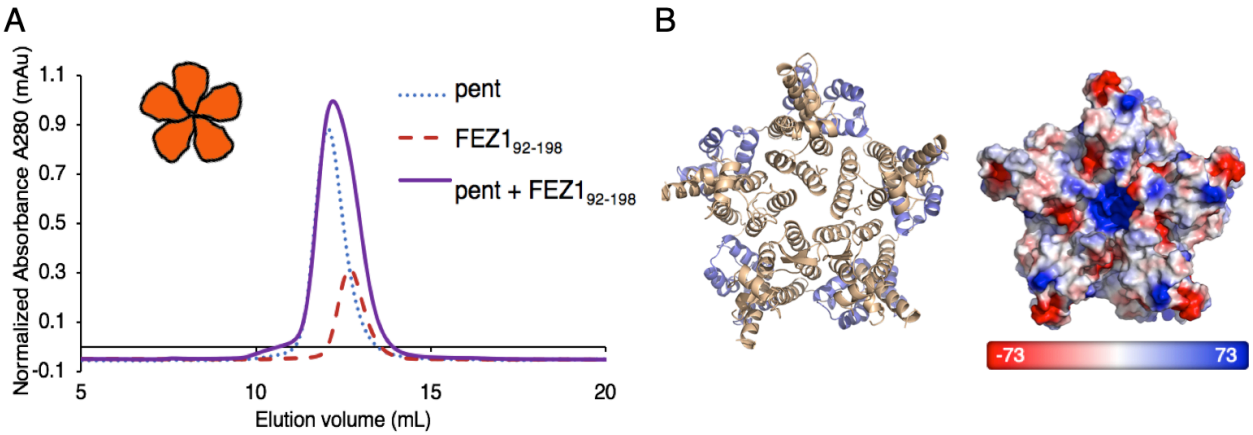

Fig. S2 (A) Size exclusion chromatography (SEC) binding assay of $FEZ1_{92-198}$ with the CA pentamer. Related to Figure 1. Schematic shown in red cartoon inset. There is no co-elution of $FEZ_{192-198}$ with the CA pentamer. (B) Crystal structure of the CA pentamer (PDBID: 3P05) (left) and its electrostatic potential surface (right). Blue, positive charge; red, negative charge. The unit of the electrostatic potential map is k_BT/e.

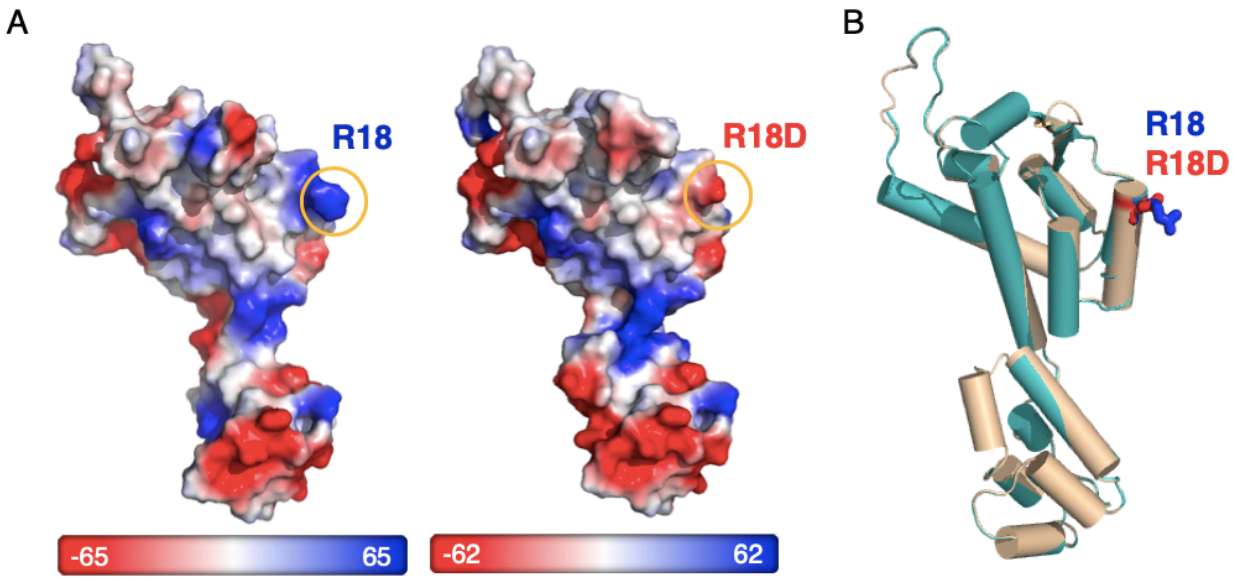

Fig. S3 Comparison of crystal structures of WT and R18D CA molecules. Related to Figure 3.

A. Electrostatic potential surface of WT (left) and R18D (right) CA. Blue, positive charge; red, negative charge. The positions of R18 and R18D are marked by orange circles. The unit of the electrostatic potential map is k_BT/e.

B. Overlay of the two structures WT CA (cyan, PDBID: 3H47) and R18D CA (tan). R18 is in blue and R18D is in red.

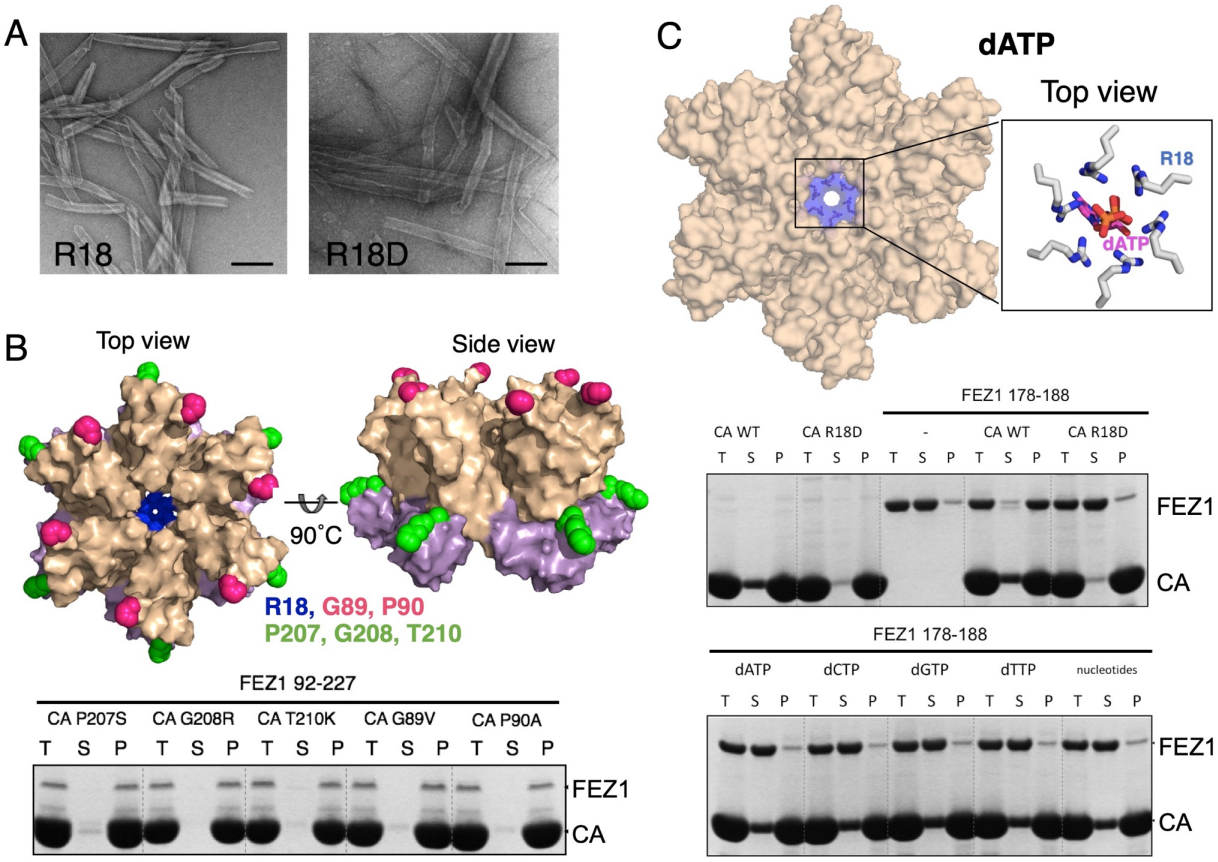

Fig. S4 Electrostatic interaction is important for FEZ1 binding with CA hexamer. Related to Figure 3.

A. Negative-stain electron microscopy micrographs of crosslinked CA tubes of WT CA (left) and R18D CA (right). Scale bar, 200 nm.

B. Top: Orthogonal views of the residues that are mutated on the CA hexamer. Green, residues implicated in escaping MxB restriction (P207 G208, T210); Yellow, residues important for CypA binding (G89, P90); R18 is in blue. Bottom: Mutations of the residues important for MxB function or CypA binding do not affect FEZ1 co-pelleting with CA tubes.

C. Top: Schematic of dATP binding with the center R18 residues of the CA hexamer (PDBID: 5HGM). Middle and bottom: Co-pelleting assay showing that the binding of $Fez1_{178-188}$ to CA tubes are abolished by R18D mutation of CA (middle) and various dNTPs (bottom), consistent with binding at the CA hexamer center.

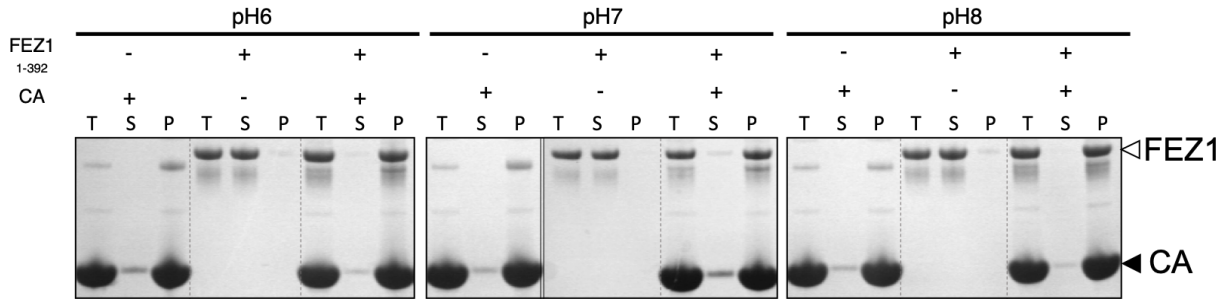

Figure S5. FEZ1 CA tubes pelleting assay at different buffer pH. Related to Figure 3.

Under different buffer pHs, pH = 6, 7, 8, FEZ1 all interacts with CA tubes.

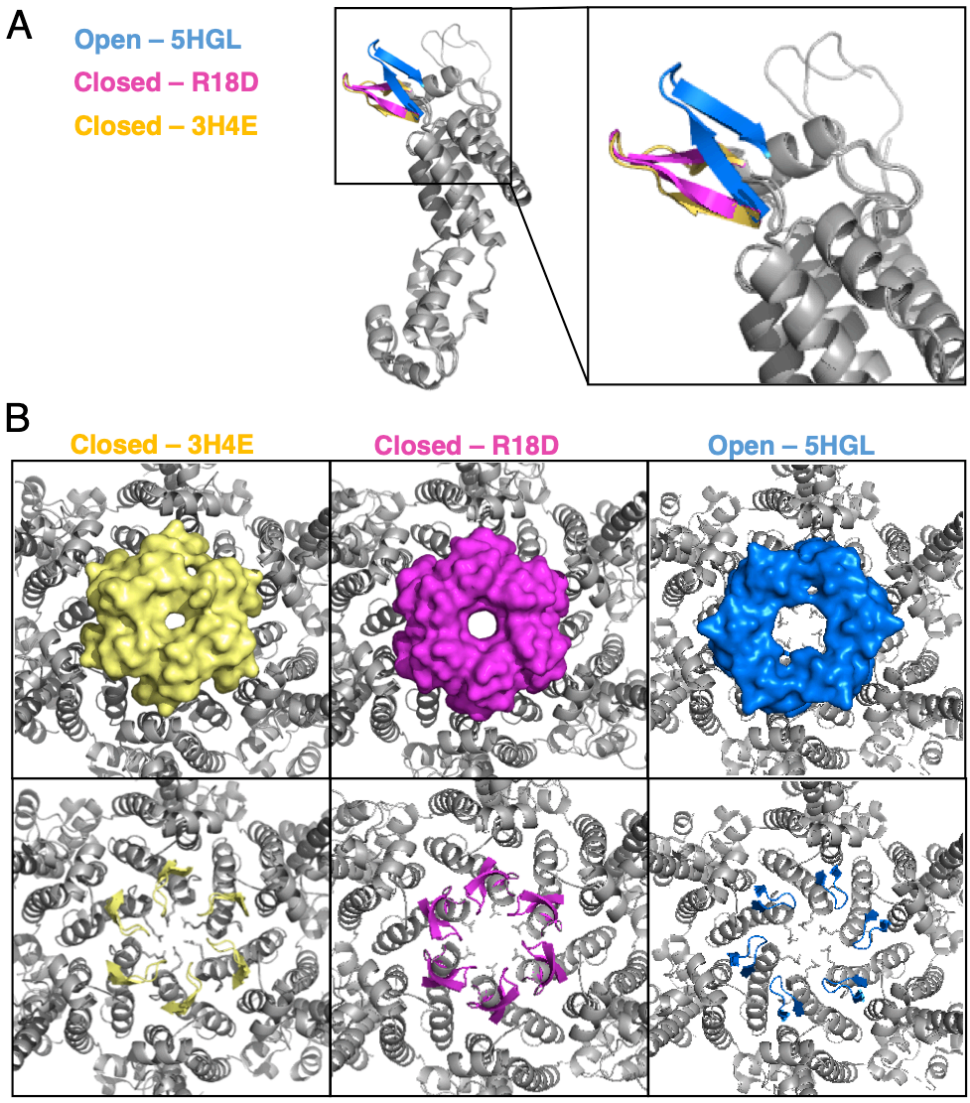

Fig. S6. Comparison of structures of the WT and R18D CA Hexamers with the open or the closed conformation of the β-hairpin. Related to Figure 3.

A. Overlay of the two WT CA structures, open (blue, PDBID: 5HGL) and closed (yellow, PDB: 3H4E) forms, and the current R18D CA structure (magenta).

B. Comparison of the open (right) and closed (left) β-hairpin conformations, showing the pore at the center of the CA hexamer. R18 or R18D is shown as stick pointing into the center. The β-hairpin is in surface representation in the top rows and in cartoon representation in the bottom rows.

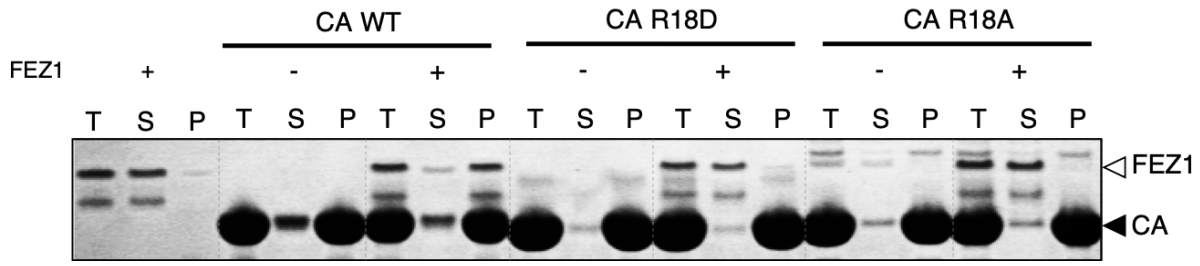

Figure S7. R18A also reduces interaction of FEZ1 with CA tubes. Related to Figure 3.
Mutation of the center ring R18 of the capsid to either aspartate or alanine reduced the interaction of $FEZ1_{92-227}$ with CA tubes, as compared with the WT CA tubes. See also Figure 3.

Table S1. Data collection and refinement statistics for the R18D CA crystal structure. Related to Figure 3.

Values shown in parentheses are for highest-resolution shell.

Data collection	
Space group	P6
Cell dimensions	
a, *b*, *c* (Å)	90.66, 90.66, 56.58
α, *β*, γ (°)	90.0, 90.0, 120.0
Resolution (Å)	45.90–2.05 (2.09-2.05)
R_{sym} or R_{merge}	0.134 (1.34)
Mean *I*/σ*I*	12.2 (1.7)
Completeness (%)	99.9
Redundancy	5.5 (5.1)
$CC^{1/2}$ (%)	39.7
Refinement	
Resolution (Å)	2.05
No. of reflections	15594
R_{work}/R_{free}	0.174/0.221 (0.241/0.296)
No. of atoms	
Protein	1692
Water	218
***B*-factors**	
All atoms ($Å^2$)	33.0
R.m.s. deviations	
Bond lengths (Å)	0.007
Bond angles (°)	0.9
Ramachandran	
Preferred	98.1%
Allowed	1.9 %
Outliers	0.0 %

Table S2. Summary of FEZ1 constructs. Related to Figures 1-7.

Construct	Figures	SEC	CA tube pelleting	ITC	Infectivity and trafficking
$FEZ1_{1-392}$	1B, 3E, 6, S5		Binds CA tubes at physiological concentrations of salt, NTPs/dNTPs and IP6, and at pH 6, 7, or 8. No binding to R18D/R18A CA tubes.		Functional for HIV-1 infectivity and virus core trafficking.
$FEZ1_{1-92}$	1B, 4A	Does not bind CA hexamer	Binds CA tubes at 50 mM but not at 150 mM NaCl. No binding to R18D CA tubes.		
$FEZ1_{92-158}$	2B	Does not bind CA hexamer	Reduced binding to CA tubes with increasing salt concentrations.		
$FEZ1_{92-198}$	1B-H, 2F, 2G, S2	Binds CA hexamer, but not CA, 1/3- or 1/2-hexamer, pentamer, or 3-fold inter-hexamer interface.	Binds CA tubes at both 50mM and 150 mM NaCl. No binding to R18D CA tubes.	Binds CA hexamer at Kd ~300±60 nM	
$FEZ1_{92-198\ 182-186EA}$	2F, 2H			Binds CA hexamer at Kd ~2.5±0.3 μM	
$FEZ1_{158-198}$	2C	Binds CA hexamer			
$FEZ1_{158-198\ 182EA}$	2E	Reduced binding to CA hexamer			
$FEZ1_{158-182}$	2D	Does not bind CA hexamer			
$FEZ1_{178-188}$	2F, 2I, 3E		Greatly reduced binding to CA tubes in the presence of 20 μM ATP, IP6, and nucleotides.	Strong binding to CA hexamer at Kd ~190±40 nM	
$FEZ1_{92-227}$	3C, 3D, S4B	Binds CA hexamer, greatly reduced binding to R18D CA hexamer	Binds CA tubes, but not with R18D tubes. Reduced binding to CA tubes with increasing salt concentrations. Binds CA tubes with CypA-and MxB-binding defective mutations.		
$FEZ1_{198-392}$	1B,	Does not bind CA hexamer	Binds CA tubes at 50 mM but not at 150 mM NaCl. No binding to R18D CA tubes.		
$FEZ1_{1-392\ 178-188EA}$	6A, 6C				Modest reduction in infectivity, no effect on trafficking
$FEZ1_{1-392\ 158-198EA}$	6B, 6D, 6E				Significant defects in infectivity and trafficking