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Supplementary information

The HIV capsid mimics karyopherin engagement of FG-nucleoporins

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The HIV capsid mimics karyopherin engagement of FG-nucleoporins

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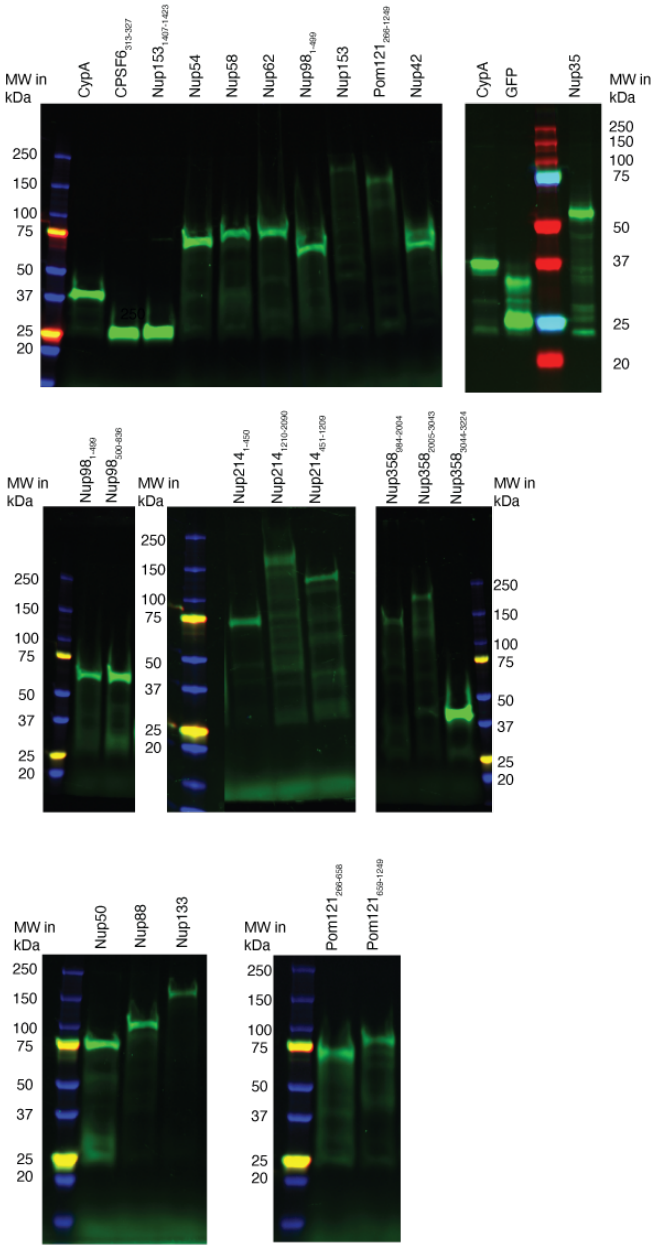
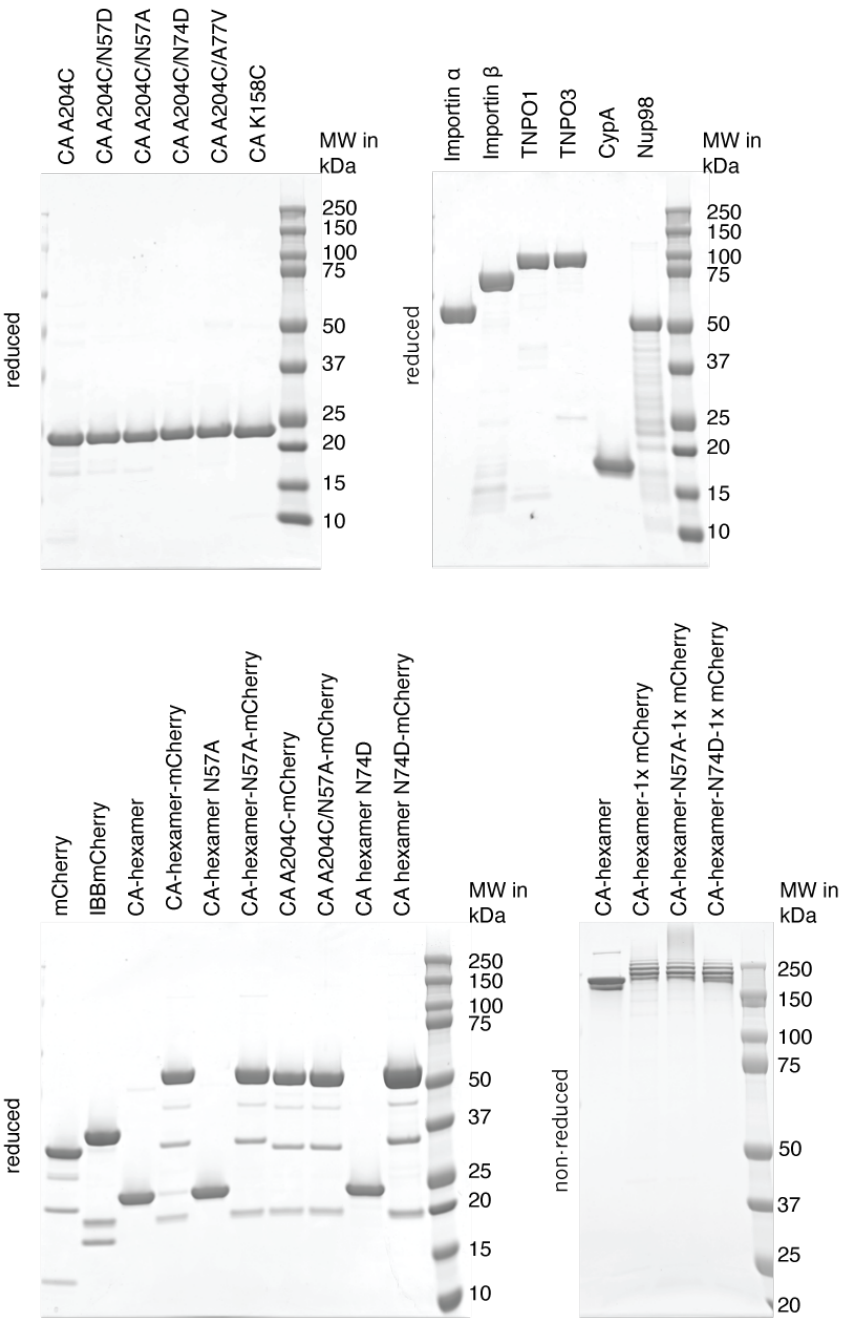


Supplementary Figure 1: In-gel fluorescence of cell-free expressed GFP-fusion proteins used for FFS.



Supplementary Figure 2: Coomassie-stained SDS-PAGE images of recombinant proteins used in this study.

Supplementary Table 1: Summary of studies reporting effects of Nup depletion upon HIV infection, and identified Nup:Capsid binding interactions. **(1)** Reports of effects of Nup depletion on HIV infection compared to wild type cells. 'Y' indicates reported Nup depletion as having reduced infection levels (<2/3 WT), 'N' indicates no reported infection effects in genome wide studies or similar levels of infection (between 2/3 and 4/3 WT) for NPC wide studies, and 'N*' indicates infection was increased (>4/3 WT). **(2)** Reports of Nup:Capsid binding interactions. Sub-columns indicate interactions with various capsids: 'WT' without known FG-binding mutations, with a mutation at residue N57 that reduce binding to known FG-binders, or a N74 mutation that reduce binding to CPSF6. 'Y' and 'N' indicate whether or not binding was reported to WT and 'Y-' or 'Y+' indicates whether a mutation increased or decreased binding. Blank cells indicate that Nup:Capsid interaction experiment for these conditions was not performed. ^ Saito *et al.* did not detect HIV cores binding to Pom12C but found a negative impact on HIV infection with overexpression of Pom121C$_{612\text{-}987}$ in multiple cell lines.

1. HIV Infection Impaired by Nup Depletion			FG-Repeat Nups										Non-FG Nups		
First author	**Year**	**Experimental Method**	**Nup42**	**Nup50**	**Nup54**	**Nup58**	**Nup62**	**Nup98**	**Pom121**	**Nup153**	**Nup214**	**Nup358**	**Nup35**	**Nup88**	**Nup133**
Brass *et al.*[34]	2008	Genome wide knock down in Hela cells	N	N	N	N	N	N	N	Y	N	N	N	N	Y
König *et al.*[35]	2008	Genome wide knock down in 293T cells	N	Y	N	N	Y	Y	N	Y	Y	N	N	N	N
Zhou *et al*[62].	2008	Genome wide knock down in Hela cells	N	N	N	N	N	N	N	N	N	N	N	N	N
Yeung *et al*[63].	2009	Genome wide knock down in Jurkat cells	N	N	N	N	N	Y	N	N	N	N	N	N	N
Ebina *et al*[64].	2004	Targeted knock down in 293T cells						Y							
Lee *et al.*[21]	2010	Targeted knock down in Hela cells								Y					N
Matreyek *et al*[65].	2011	Targeted knock down in Hela cells								Y					
Di Nunzio *et al.*[33]	2012	Targeted knock down in Hela cells						Y		Y	Y	Y			
Ao *et al.*[66]	2012	Targeted knock down in C8166/macrophages cells					Y								
Matreyek *et al.*[15]	2013	Targeted knock down in HOS cells								Y					
Di Nunzio *et al.*[67]	2013	Targeted knock down in Jurkat cells						Y		Y					
Dharan *et al.*[68]	2016	Targeted knock down in Hela cells										Y			
Guo *et al.*[69]	2018	Targeted knock down in 293T/C8166 cells							Y						
Buffone *et al*[20].	2018	Targeted knock down in Hela cells					Y			Y	Y	Y			
Kane *et al.*[19]	2018	NPC wide knock down in Hela cells		N	N		N	Y		Y	Y	Y	N	N	N
Xue *et al.*[36]	2023	NPC wide knock down in Hela cells		Y	N*	N*	N*	Y	Y	Y	N*	Y	Y	N*	Y

2. Nup:Capsid Binding Interactions			FG-Repeat Nups										Non-FG Nups		
First author	**Year**	**Experimental Method**	**Nup42** WT N57 N74	**Nup50** WT N57 N74	**Nup54** WT N57 N74	**Nup58** WT N57 N74	**Nup62** WT N57 N74	**Nup98** WT N57 N74	**Pom121** WT N57 N74	**Nup153** WT N57 N74	**Nup214** WT N57 N74	**Nup358** WT N57 N74	**Nup35** WT N57 N74	**Nup88** WT N57 N74	**Nup133** WT N57 N74
Schaller *et al*[6].	2011	Isothermal calorimetry of Nup358CypA domain and CA NTD										Y			
Di Nunzio *et al*[33].	2012	Pull-downs from 293T cell lysate, *in vitro* HIV1 CA-NC complex										Y			
Di Nunzio *et al.*[67]	2013	Pull-downs from 293T cell lysate, *in vitro* HIV1 CA-NC complex						Y Y-		Y Y					
Matreyek *et al.*[15]	2013	Pulldown from 293T cell lysate and recombinant Nup153C, *in vitro* HIV1 CA-NC complex								Y Y- Y+					
Bhattacharya *et al.*[70]	2014	Fluorescence anisotropy of fNup153 peptide and HIV-1 CA hexamers								Y					
Saito *et al.*[37]	2017	Immunoprecipitation of HIV Cores with Pom121C							N^						
Buffone *et al.*[20]	2018	Pull-downs from 293T cell lysate, *in vitro* HIV1 CA-NC complex					Y Y- Y+	Y Y-		Y Y-	Y Y- Y+	Y			
Kane *et al.*[19]	2018	Pull-downs from HeLa cell lysate, *in vitro* CA tubes					Y			Y		Y		Y	N
Shen *et al.*[71]	2023	Co-pelleting of Recombinant Nup with *in vitro* CA tubes					Y			Y		Y			
Xue *et al.*[36]	2023	Pull-downs from 293T cell lysate, *in vitro* HIV1 CA complex							Y Y				Y Y		
This Study	**2023**	**FFS of eukaryotic cell-free Nup expression, *in vitro* A204C stabilised CLPs**	**Y Y- Y+**	**N**	**N**	**Y Y- Y+**	**Y Y- Y+**	**Y Y- Y+**	**Y Y- Y+**	**Y Y- Y+**	**Y Y- Y+**	**Y**	**N**	**N**	**N**

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