Supplementary Information

Refined capsid structure of human adenovirus D26 at 3.4 Å resolution

Vijay S. Reddy[1*], Xiaodi Yu[1§] and Michael A. Barry[2,3,4,5]
[1]Department of Integrative Structural and Computational Biology, The Scripps Research Institute, La Jolla, CA 92037, USA
[2]Division of Infectious Diseases, [3]Department of Medicine, [4]Department of Immunology, [5]Department of Molecular Medicine, Mayo Clinic, Rochester, MN 55905, USA

[2]Department of Internal Medicine, Division of Infectious Diseases, Mayo Clinic, Rochester, MN 55902, USA

[§]Current address: SMPS, Janssen Research and Development, Spring House, PA 19044, USA

*Corresponding author:
Vijay S. Reddy, E-mail: reddyv@scripps.edu

Table S1. Sequence identity and structural similarity (RMSD) values for VIII pairs in selected HAdVs.

HAdV	Ad26	Ad5 (PDB-ID: 6B1T)	Ad41 (PDB-ID: 6Z7N)
Ad26	100 (0)	77.5 (0.61)	81.5 (0.81)
Ad5	77.5 (0.61)*	100 (0)	79.3 (0.73)
Ad41	81.5 (0.81)	79.3 (0.73)	100 (0)

*Shown in parenthesis are root mean square deviation (RMSD) values for aligned and pruned C-alpha atom pairs estimated using ChimeraX [30].

Table S2. Cryo-EM data and model quality statistics of HAdV-D26 virion.

Data collection	
Microscope	FEI Titan Krios
Camera	Gatan K2 Summit
Voltage (kV)	300
Total dose (e-/Å^2)	53
Pixel size	1.31 Å
Defocus range (mm)	0.8-3.0
Micrographs	2,017
Particles selected	37,026
Particles included in final reconstruction	30,834
Reconstruction	
Symmetry imposed	I
Resolution (Å) (FSC cut-off: 0.143)	3.38
Virion model composition and quality statistics	
Number of chains	31
Number of atoms	105,674
Number of protein residues	13,365
Clash score	15.25
Rotamer outliers	12.26
Molprobity score	3.14
EMRinger Score	2.81
Map-Model CC (main chain)	0.83
Map-Model CC (side chain)	0.81
Ramachandran statistics	
Favored	86.62 %
Allowed	13.21 %
Outliers	0.17%
R.M.S. Deviations	
Bond lengths (Å)	0.007
Bond angles (°)	0.769

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CLUSTAL 2.1 multiple sequence alignment

Ad26_Hexon|Q3S8B3      MATPSMMPQWAYMHIAGQDASEYLSPGLVQFARATDTYFSLGNKFRNPTVAPTHDVTTDR
Ad5_Hexon|P04133       MATPSMMPQWSYMHISGQDASEYLSPGLVQFARATETYFSLNNKFRNPTVAPTHDVTTDR
                       **********:****:******************:*****.******************

Ad26_Hexon|Q3S8B3      SQRLTLRFVPVDREATTYLYKARFTLAVGDNRVLDMASTYFDIRGVLDRGPSFKPYSGTA
Ad5_Hexon|P04133       SQRLTLRFIPVDREDTAYSYKARFTLAVGDNRVLDMASTYFDIRGVLDRGPTFKPYSGTA
                       ********:***** *:* ********************************:********
                                                   HVR1
Ad26_Hexon|Q3S8B3      YNSLAPKGAPNPSQWETKEKQGTTGG------------VQQEKDVTKTFGVAATGGINIT
Ad5_Hexon|P04133       YNALAPKGAPNPCEWDEAATALEINLEEEDDDNEDEVDEQAEQQKTHVFGQAPYSGINIT
                       **:*********.:*:   .    .             * *:: *:.** *. .*****

Ad26_Hexon|Q3S8B3      NQGLLLGTDETAENGKKDIYADKTFQPEPQVGEENWQENEAFYGG-RALKKDTKMKPCYG
Ad5_Hexon|P04133       KEGIQIG-----VEGQTPKYADKTFQPEPQIGESQWYETEINHAAGRVLKKTTPMKPCYG
                       ::*: :*      :*:.  ***********:**.:* *.*  :.. *.*** * ******

Ad26_Hexon|Q3S8B3      SFARPTNEKGGQAKFKPVNEGEQPKDLDIDFAYFDVPGGSPPAGGSGEEYKADIILYTEN
Ad5_Hexon|P04133       SYAKPTNENGGQGILVKQQNGKLESQVEMQFFSTTEATAG-----NGDNLTPKVVLYSED
                       *:*:****:***. :   ::*:  .::::::*     . ..     .*:: ...::**:*:

Ad26_Hexon|Q3S8B3      VNLETPDTHVVYKPGTSDNSSEINLVQQSMPNRPNYIGFRDNFVGLMYYNSTGNMGVLAG
Ad5_Hexon|P04133       VDIETPDTHISYMPTIKEGNSRELMGQQSMPNRPNYIAFRDNFIGLMYYNSTGNMGVLAG
                       *::******: * *  .:..*.  : ***********.*****:****************

Ad26_Hexon|Q3S8B3      QASQLNAVVDLQDRNTELSYQLLLDSLGDRTRYFSMWNSAVDSYDPDVRIIENHGVEDEL
Ad5_Hexon|P04133       QASQLNAVVDLQDRNTELSYQLLLDSIGDRTRYFSMWNQAVDSYDPDVRIIENHGTEDEL
                       **************************:***********.****************.****

Ad26_Hexon|Q3S8B3      PNYCFPLNGTGTNSTYQGVKITNGNDGAEESEWEKDDAISRQNQICKGNVYAMEINLQAN
Ad5_Hexon|P04133       PNYCFPLGGVINTETLTKVKPKTGQ---ENGWEKDATEFSDKNEIRVGNNFAMEINLNAN
                       *******.*. ...*   ** ..*:   *:.  :.   :* :*:*  ** :******:**

Ad26_Hexon|Q3S8B3      LWKSFLYSNVALYLPDSYKYTPANVKLPANTNTYEYMNGRVVAPSLVDAYINIGARWSLD
Ad5_Hexon|P04133       LWRNFLYSNIALYLPDKLKYSPSNVKISDNPNTYDYMNKRVVAPGLVDCYINLGARWSLD
                       **:.*****:******. **:*:***:. *.***:*** *****.***.***:*******

Ad26_Hexon|Q3S8B3      PMDNVNPFNHPRNAGLRYRSMLLGNGRYVPFHIQVPQKFFAIKNLLLLPGSYTYEWNFRK
Ad5_Hexon|P04133       YMDNVNPFNHHRNAGLRYRSMLLGNGRYVPFHIQVPQKFFAIKNLLLLPGSYTYEWNFRK
                        ********* ************************************************

Ad26_Hexon|Q3S8B3      DVNMILQSSLGNDLRVDGASVRFDSVNLYATFFPMAHNTASTLEAMLRNDTHDQSFNDYL
Ad5_Hexon|P04133       DVNMVLQSSLGNDLRVDGASIKFDSICLYATFFPMAHNTASTLEAMLRNDTNDQSFNDYL
                       ****:***************::***: ************************:********

Ad26_Hexon|Q3S8B3      SAANMLYPIPAKATNVPISIPSRNWAAFRGWSFTRLKTKETPSLGSGFDPYFVYSGSIPY
Ad5_Hexon|P04133       SAANMLYPIPANATNVPISIPSRNWAAFRGWAFTRLKTKETPSLGSGYDPYYTYSGSIPY
                       ***********:******************:***************:***:.*******

Ad26_Hexon|Q3S8B3      LDGTFYLNHTFKKVSIMFDSSVSWPGNDRLLTPNEFEIKRSVDGEGYNVAQCNMTKDWFL
Ad5_Hexon|P04133       LDGTFYLNHTFKKVAITFDSSVSWPGNDRLLTPNEFEIKRSVDGEGYNVAQCNMTKDWFL
                       **************:* ******************************************

Ad26_Hexon|Q3S8B3      VQMLSHYNIGYQGFHVPEGYKDRMYSFFRNFQPMSRQVVDEINYKDYKAVTLPFQHNNSG
Ad5_Hexon|P04133       VQMLANYNIGYQGFYIPESYKDRMYSFFRNFQPMSRQVVDDTKYKDYQQVGILHQHNNSG
                       ****::********::**.*********************: :****: * : .******

Ad26_Hexon|Q3S8B3      FTGYLAPTMRQGQPYPANFPYPLIGQTAVPSVTQKKFLCDRVMWRIPFSSNFMSMGALTD
Ad5_Hexon|P04133       FVGYLAPTMREGQAYPANFPYPLIGKTAVDSITQKKFLCDRTLWRIPFSSNFMSMGALTD
                       *.********:**.***********:*** *:*********.:*****************

Ad26_Hexon|Q3S8B3      LGQNMLYANSAHALDMTFEVDPMDEPTLLYLLFEVFDVVRVHQPHRGVIEAVYLRTPFSA
Ad5_Hexon|P04133       LGQNLLYANSAHALDMTFEVDPMDEPTLLYVLFEVFDVVRVHRPHRGVIETVYLRTPFSA
                       ****:**************************:***********:*******:*********

Ad26_Hexon|Q3S8B3      GNATT
Ad5_Hexon|P04133       GNATT
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Figure S1. Amino acid sequence alignments of hexon subunits from Ad26 and Ad5 viruses

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CLUSTAL 2.1 multiple sequence alignment


Ad26_PB|A4ZKL2      -MRRAVVSSSPPPSYESVMAQA-----------TLEVPFVPPRYMAPTEGRNSIRYSEL
Ad5_PB|P12538       MRRAAMYEEGPPPSYESVVSAAPVAAALGSPFDAPLDPPFVPPRYLRPTGGRNSIRYSEL
                      * *: ...********:: *            .*: *******: ** **********

Ad26_PB|A4ZKL2      APQYDTTRVYLVDNKSADIASLNYQNDHSNFLTTVVQNNDFTPAEASTQTINFDERSRWG
Ad5_PB|P12538       APLFDTTRVYLVDNKSTDVASLNYQNDHSNFLTTVIQNNDYSPGEASTQTINLDDRSHWG
                    ** :************:*:****************:****::*.********:*:**:**

Ad26_PB|A4ZKL2      GDLKTILHTNMPNVNEYMFTSKFKARVMVSRKHPEGVVETDLSQDKLEYEWFEFTLPEGN
Ad5_PB|P12538       GDLKTILHTNMPNVNEFMFTNKFKARVMVSRLPTK------DNQVELKYEWVEFTLPEGN
                    ****************:***.**********  .:       .* :*:***.********

Ad26_PB|A4ZKL2      FSETMTIDLMNNAILENYLQVGRQNGVLESDIGVKFDSRNFKLGWDPVTKLVMPGVYTYE
Ad5_PB|P12538       YSETMTIDLMNNAIVEHYLKVGRQNGVLESDIGVKFDTRNFRLGFDPVTGLVMPGVYTNE
                    :**************:*:**:*****************:***:**:**** ******** *

Ad26_PB|A4ZKL2      AFHPDVVLLPGCGVDFTESRLSNLLGIRKKQPFQEGFRIMYEDLEGGNIPALLDVPKYLE
Ad5_PB|P12538       AFHPDIILLPGCGVDFTHSRLSNLLGIRKRQPFQEGFRITYDDLEGGNIPALLDVDAYQA
                    *****::**********.***********:********* *:*************  *

Ad26_PB|A4ZKL2      SKKKVEDETKNAAAAT------------------------ADTTTRGDTFATPAQET---
Ad5_PB|P12538       SLKDDTEQGGGGAGGSNSSGSGAEENSNAAAAAMQPVEDMNDHAIRGDTFATRAEEKRAE
                    * *.  ::  ..*..:                        * : ******* *:*.

Ad26_PB|A4ZKL2      -----------------AADKKVEVLPIEKDESGRSYNLIQG-THDTLYRSWYLSYTYGD
Ad5_PB|P12538       AEAAAEAAAPAAQPEVEKPQKKPVIKPLTEDSKKRSYNLISNDSTFTQYRSWYLAYNYGD
                                     .:**  : *: :*.. ******.. :  * ******:*.***

Ad26_PB|A4ZKL2      PEKGVQSWTLLTTPDVTCGAEQVYWSLPDLMQDPVTFRSTQQVSNYPVVGAELMPFRAKS
Ad5_PB|P12538       PQTGIRSWTLLCTPDVTCGSEQVYWSLPDMMQDPVTFRSTRQISNFPVVGAELLPVHSKS
                    *:.*::***** *******:*********:**********:*:**:*******:*.::**

Ad26_PB|A4ZKL2      FYNDLAVYSQLIRSYTSLTHVFNRFPDNQILCRPPAPTITTVSENVPALTDHGTLPLRSS
Ad5_PB|P12538       FYNDQAVYSQLIRQFTSLTHVFNRFPENQILARPPAPTITTVSENVPALTDHGTLPLRNS
                    **** ********.:***********:****.*************************.*

Ad26_PB|A4ZKL2      IRGVQRVTVTDARRRTCPYVYKALGIVAPRVLSSRTF
Ad5_PB|P12538       IGGVQRVTITDARRRTCPYVYKALGIVSPRVLSSRTF
                    * ******:******************:*********
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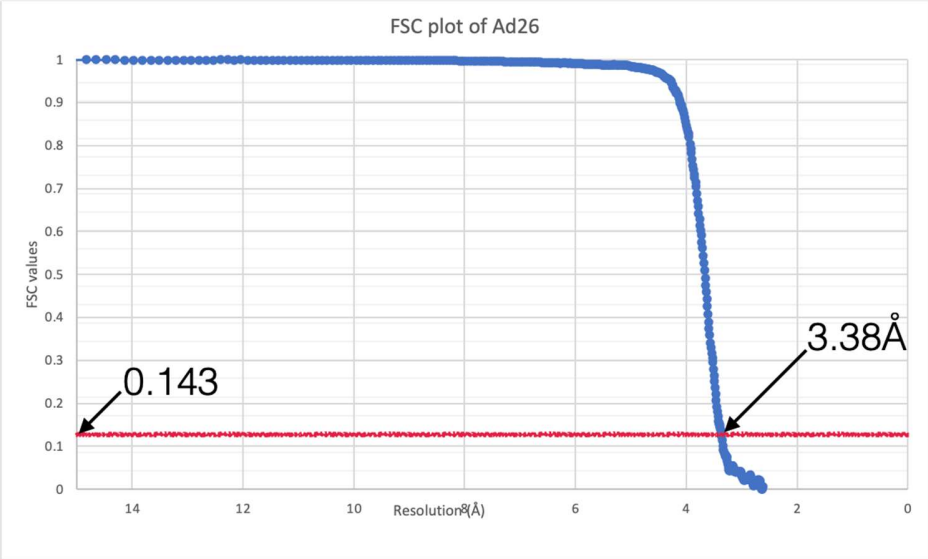
Figure S2. Amino acid sequence alignments of penton base (PB) subunits from Ad26 and Ad5 viruses

A



B

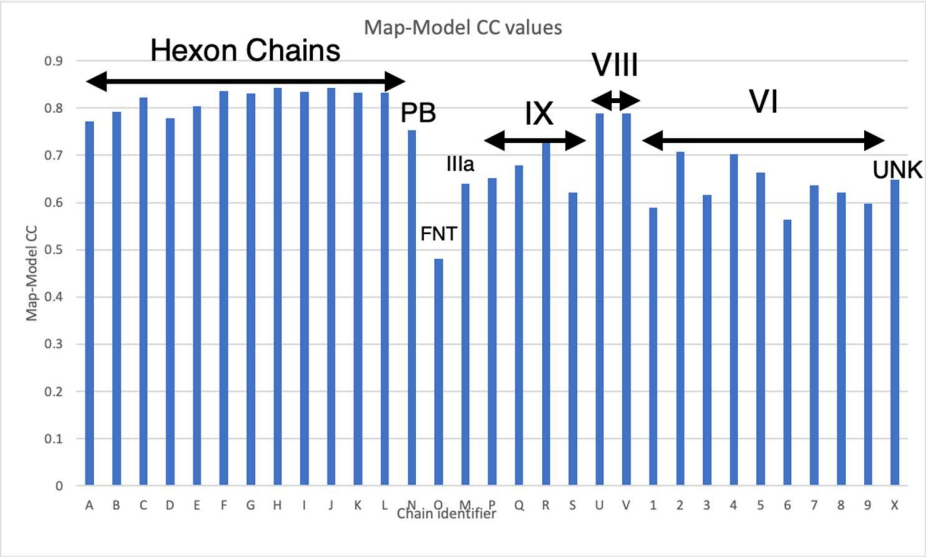


Figure S3. (**A**) FSC plot showing the resolution estimation of the cryo-EM reconstruction of HAdV-D26 (Ad26) virion. The partial FSC values used in the graph as a function of resolution were calculated in cisTEM [60]. (**B**) A bar plot of map-to-model correlation coefficient (CC) values for individual chains reported in the structure of Ad26 virion. The corresponding capsid proteins of the individual chains are identified at the top the bars. The average map-to-model CC values for all the main chain and side chain atoms are 0.83 and 0.81, respectively. The map-to-model CCs were calculated in Phenix [9].