SUPPORTING INFORMATION

Mechanics of Viral Chromatin Reveals the Pressurization of Human Adenovirus

Alvaro Ortega-Esteban[1], Gabriela N. Condezo[2], Ana J. Pérez-Berná[2†], Miguel Chillón[3], Sarah J. Flint[4], David Reguera[5], Carmen San Martín[2]*, Pedro J. de Pablo[1]*

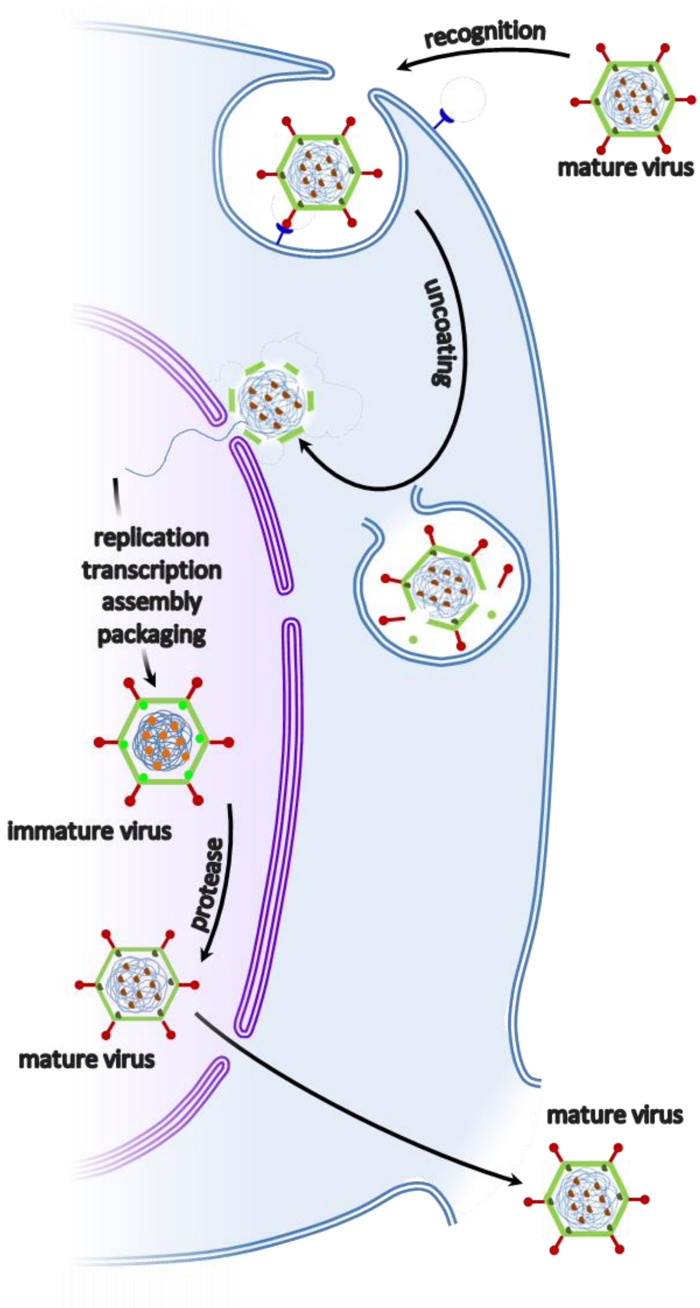


Figure S1

Cartoon summing up the adenovirus infectious cycle.

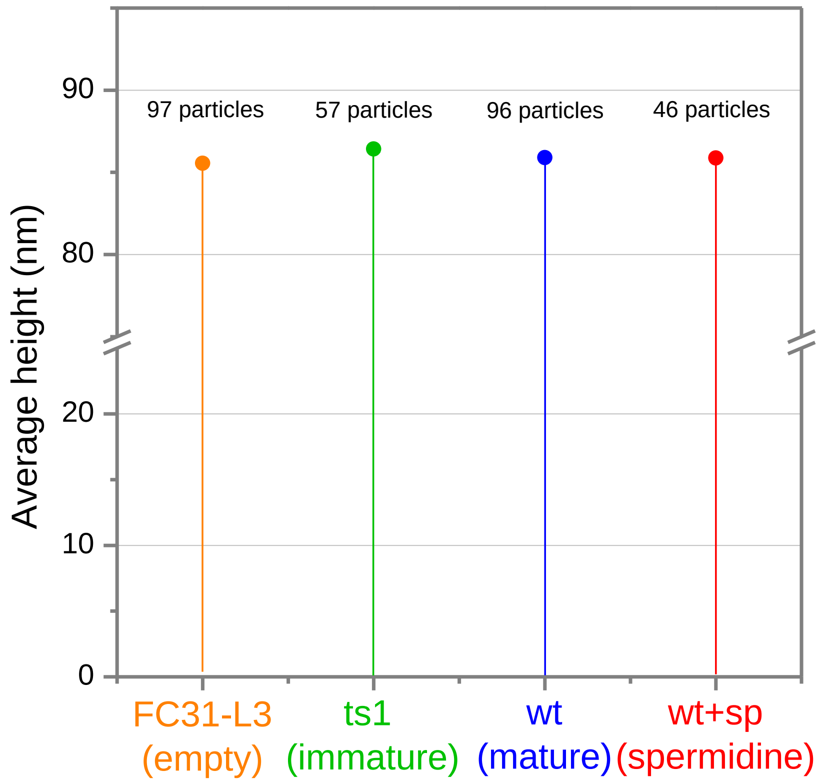
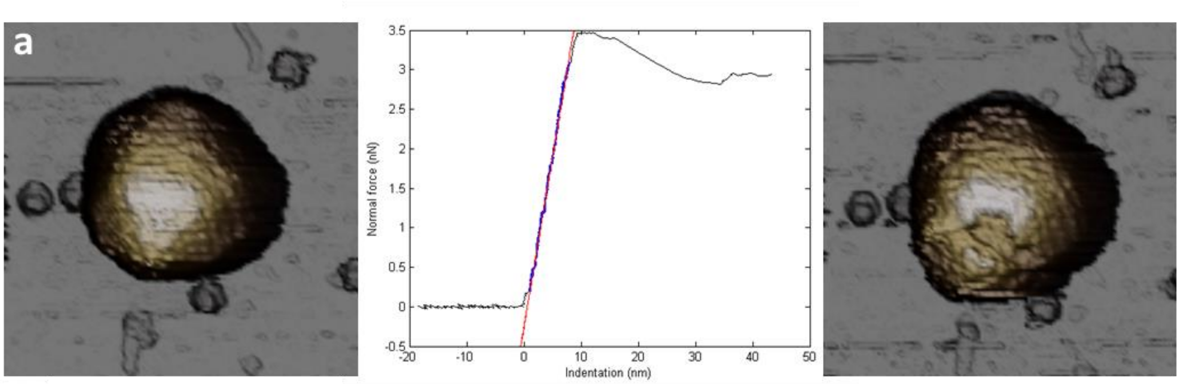


Figure S2

Average heights of the studied particles.

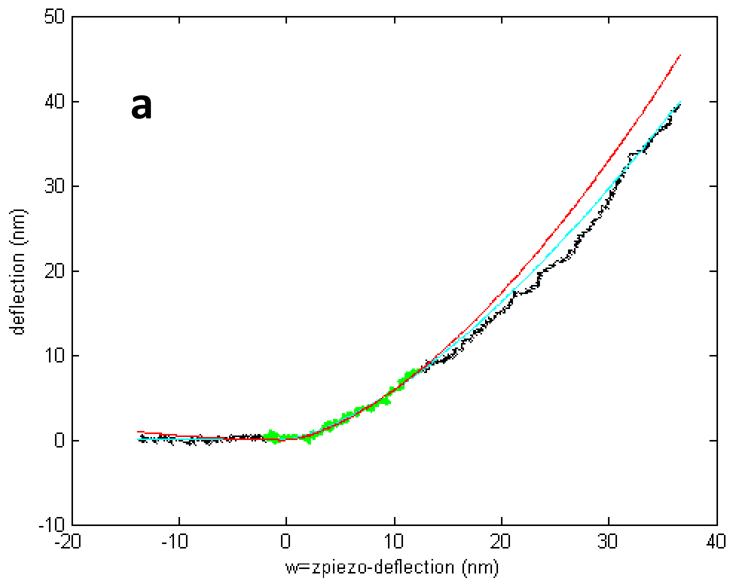


b

Particle	Spring constant (N/m)	n	SD (N/m)	SEM (N/m)	Force (nN)	n	SD (nN)	SEM (nN)
FC31-L3	0.41	61	0.11	0.01	2.6	51	1.0	0.1
ts1	0.49	20	0.16	0.04	3.0	20	1.1	0.2
wt	0.56	77	0.15	0.02	5.0	65	1.2	0.1
wt+sp	0.50	41	0.16	0.02	5.4	41	0.8	0.1

Figure S3

a) Intact wt particle, (left). Indentation curve executed on the center of a triangular facet, (middle). Broken particle, (right). b) Table summing up mechanical data of intact particles, including rigidity (spring constant) and breaking force.



b

Core	Hertz (no sample thickness) (MPa)	SEM (MPa)	Dimitriadis (considering sample thickness) (MPa)	SEM (MPa)	n
ts1	1.3	0.1	1.2	0.1	8
wt	0.35	0.05	0.30	0.04	8
wt+sp	0.81	0.09	0.71	0.07	8

Figure S4. a) Example of a typical force curve on a *ts1* core. Following the recommendations of ref. 29, we fitted the indentation curve until reaching about 10% of the sample thickness (the particle height), discarding the rest of the curve. The x and y axes represent the indentation and the cantilever bending, respectively. Green dots represent the fitted experimental data, while the red and blue curves are the fitting for the Hertzian model without considering sample thickness (Johnson, K. L. 1985. Contact mechanics. Cambridge University Press, Cambridge) and the Dimitriadis model (29), respectively. b) Estimation of Young's moduli using either the simple Hertzian or the Dimitriadis models. The relative differences between the particles are preserved. The ratio for the *ts1* and wt Young's modulus is E_{ts1}/ E_{wt} ~3.7 and ~4.0 for Hertz (28) and Dimitriadis (29) models, respectively. Likewise, the relative differences in wt core mechanics with or without addition of the condensing agent spermidine, E_{wt+SP}/ E_{wt} ~2.3 and ~2.4 for Hertz and Dimitriadis models, respectively.

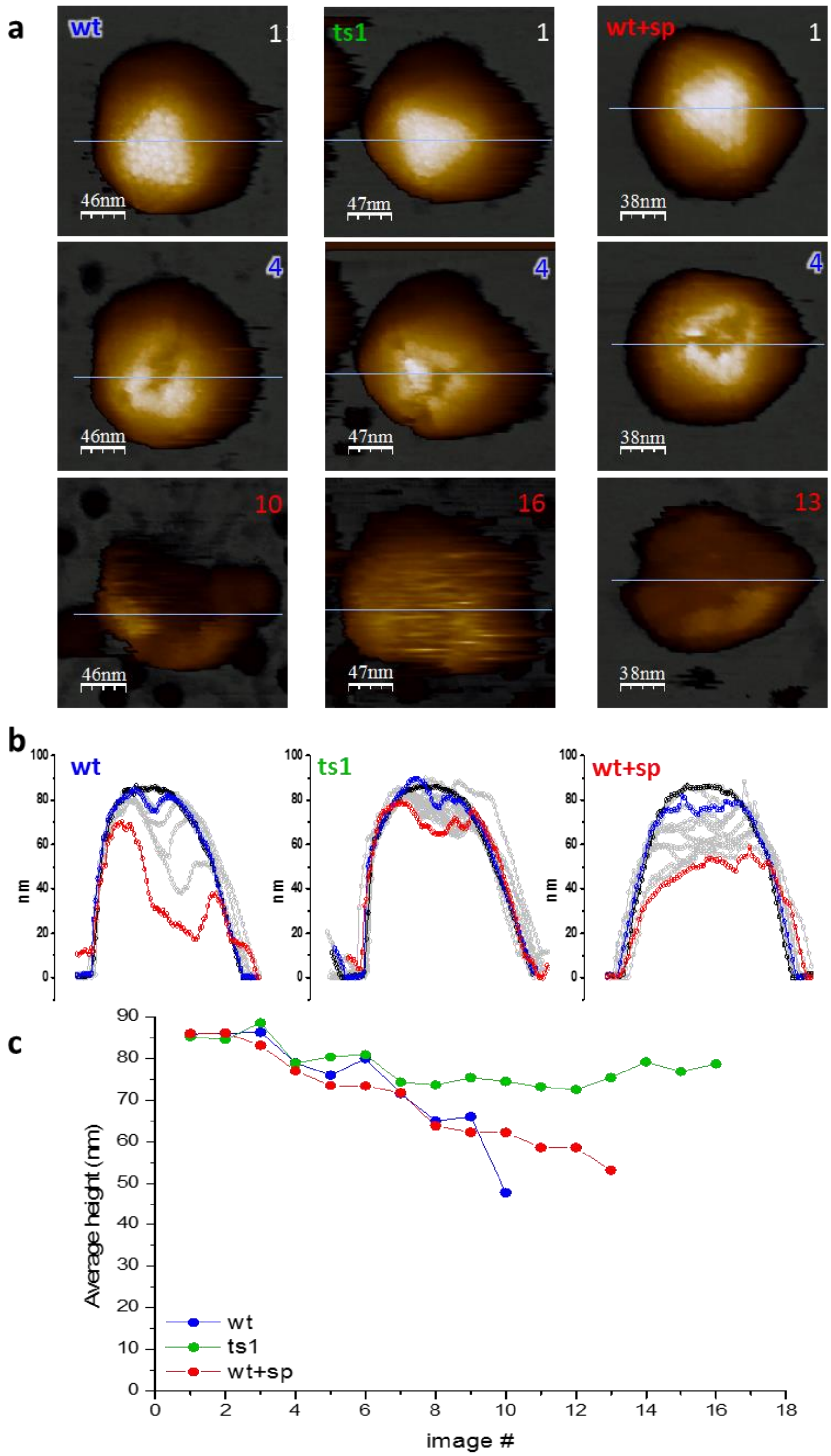


Figure S5

a) Topographical evolution of wt (left), *ts1* (middle) and wt + spermidine (right). b) Height profiles obtained at the horizontal lines of a). Black, blue and red colors correspond to the same code as the frame numbers indicated in a). c) Evolution of the average height of the center of the particles.

Finite Element (FE) simulations

Given the relatively large thickness of the adenovirus capsid wall and the finite radius of curvature of the AFM tip, finite elements simulations were performed to corroborate the estimates of the pressure based on Vella's formula (Eq. 1). Finite elements simulations of the AFM indentation of adenovirus were performed using the program COMSOL Multiphysics 4.3 (Comsol, Stockholm, Sweden). The empty capsid of wt adenovirus was modeled as a thick spherical shell with an external radius R=40.2 nm and thickness h=9.1 nm (see inset of figure S5). The capsid wall was considered as made of a homogenous material with Young Modulus E and Poisson ratio ν=0.3 (a typical value for protein-like materials). This model capsid was placed on a hard flat substrate and indented by a hard spherical object with radius R_{in}=15 nm, mimicking the AFM tip. The system was simulated using a 2D axisymmetric model that was meshed with over 3300 triangular elements. The contacts between the shell and the tip as well as the supporting surface during indentation were implemented with a contact-penalty stiffness method according to the manufacturer's manual. A parametric, non-linear solver was used to simulate the stepwise lowering of the tip onto the capsid. The spring constants were obtained from a linear fit of the force versus indentation, for small indentations between 3 and 4 nm.

Figure S6 shows the indentation curves obtained for the model adenovirus capsid with a tip of R_{in}=15nm and a value of the Young modulus E=0.28±0.02 GPa chosen to reproduce the experimental value of the *ts1* spring constant. The black line is the indentation curve for the unpressurized capsid, whereas the red line is the indentation curve when the virus has an internal pressure of 3.3 MPa, that yields a spring constant of k=0.56 N/m, identical to the experimental value measured for wt. Taking into account the experimental error bars, the estimate of the pressure in the FE simulations is 3.3±0.9 MPa, which is fully compatible with the value obtained from Vella's formula (Eq. 1).

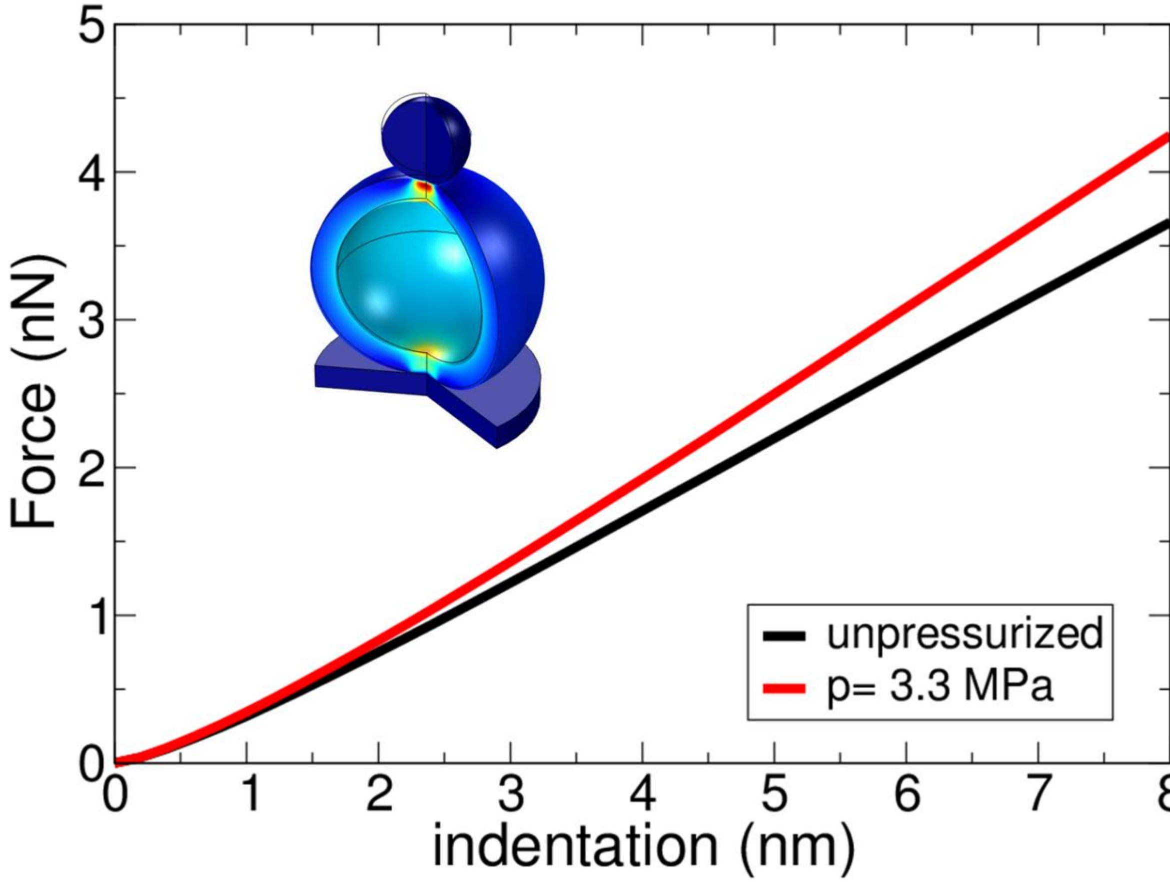


Figure S6. Force-indentation curves obtained from Finite Element simulations mimicking the AFM experiments. The black line represents the values obtained for an unpressurized capsid, and the red line corresponds to the capsid with an internal pressure of 3.3 MPa. The inset figure shows the model used in the simulations, where adenovirus is represented as a spherical shell with external radius R=40.2 nm and thickness 9.1 nm, indented on top of a hard substrate by a spherical tip with radius R_{in}=15 nm. The Young modulus of the capsid was E=0.28 GPa, chosen to yield the same value of the spring constant as the one experimentally determined for *ts1* (0.49 N/m