Supporting information

Castellanos et al. 10.1073/pnas.1207437109

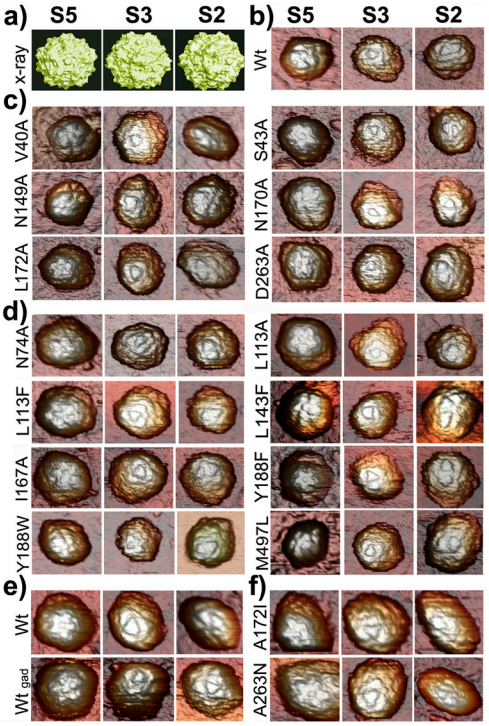

Fig. S1. MVM capsids imaged by AFM. (*A*) Molecular surface model of the MVM virion derived from crystallographic data (1). AFM images correspond to single particles of (*B*) the WT capsid (*Wt*); (*C*) mutant capsids that do not undergo the pore-associated rearrangement on heating and whose virions show drastically reduced infectivity relative to WT; (*D*) mutant capsids that show the pore-associated rearrangement and whose virions show infectivities comparable to WT; (*E*) WT capsid-like particle, either intact (*Wt*) or cross-linked with glutaraldehyde (*Wtgad*); (*F*) mutant capsid-like particles with pseudo-reversions at the base of the pores that restore the dynamics and lower S5 stiffness of the WT capsid. The mutants are labeled. For each capsid variant, three single particles are shown, viewed approximately along an S5 (*Left*), S3 (*Center*), or S2 (*Right*) axis.

1 Kontou M, et al. (2005) Structural determinants of tissue tropism and in vivo pathogenicity for the parvovirus minute virus of mice. *J Virol* 79:10931–10943.

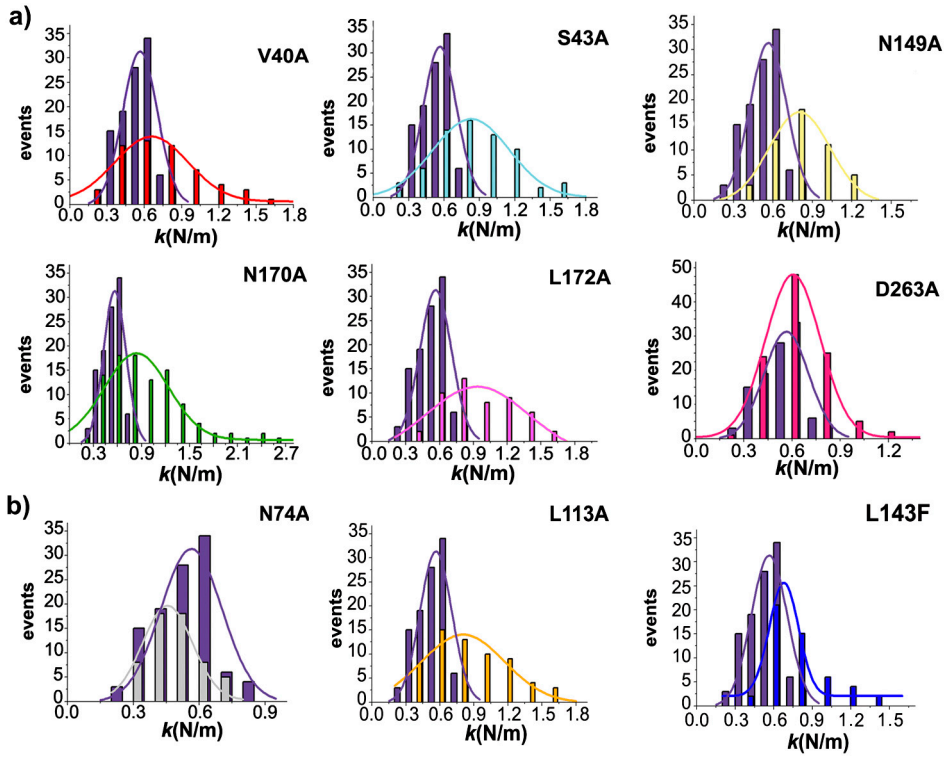

Fig. S2. Comparison of the mechanical stiffness at the S2 region between the WT and mutant MVM capsids. Each histogram depicts the number of determinations relative to the k_s obtained for a capsid variant. In each graph, the histogram in purple corresponds to the WT capsid and the one in a different color to a mutant capsid (labeled). (*A*) Capsids that carry a mutation that impairs S5 dynamics and virus infectivity relative to the WT (Table 1). (*B*) Representative examples of capsids that carry a mutation that neither impairs S5 dynamics nor virus infectivity relative to the WT (see also Table 1 and Table S1). For statistical analysis see Table S1.

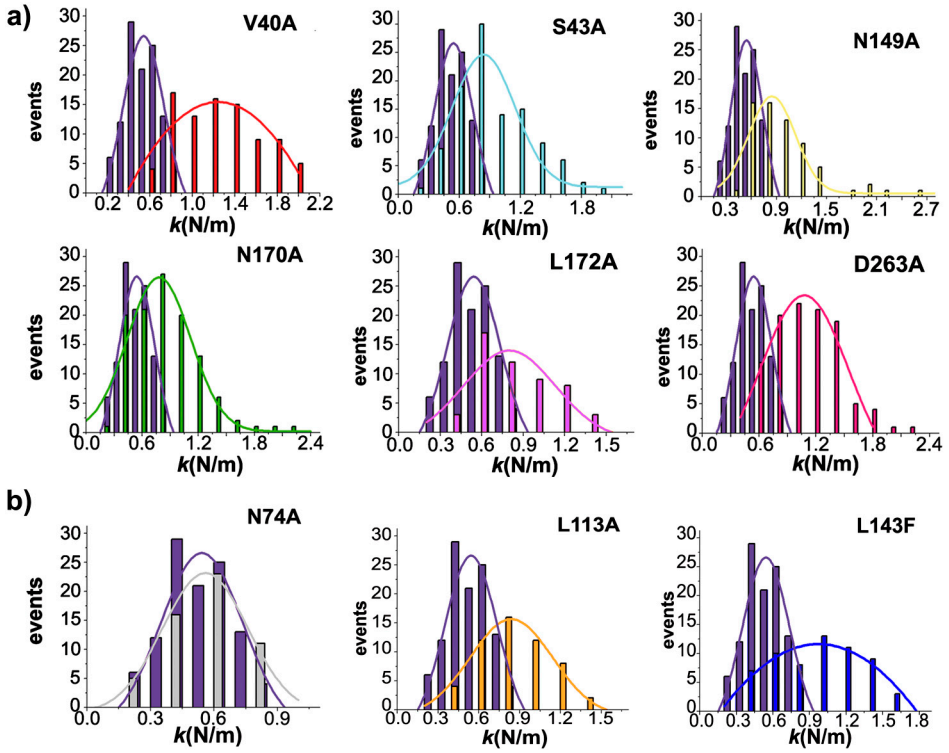

Fig. S3. Comparison of the mechanical stiffness at the S3 region between the WT and mutant MVM capsids. Each histogram depicts the number of determinations relative to the k_s obtained for a capsid variant. In each graph, the histogram in purple corresponds to the WT capsid and the one in a different color to a mutant capsid (labeled). (*A*) Capsids that carry a mutation that impairs S5 dynamics and virus infectivity relative to the WT (Table 1). (*B*) Representative examples of capsids that carry a mutation that neither impair S5 dynamics nor virus infectivity relative to the WT (see also Table 1 and Table S1). For statistical analysis see Table S1.

Table S1. Effect of mutations on capsid mechanical stiffness at S2 and S3 regions

			S2				S3			
Mutant *	Titer *	T_m (°C) †	k_s (N/m) ‡	Fz §	n §	T ¶	k_s (N/m) ‡	Fz §	n §	T ¶
WT	1	46.1 ± 0.8	0.55 ± 0.14	109	14	-	0.54 ± 0.14	110	16	-
V40A	1×10^{-4}	No	0.78 ± 0.33	55	6	8×10^{-06}	1.29 ± 0.39	88	11	2×10^{-32}
S43A	$<4 \times 10^{-5}$	No	0.89 ± 0.31	66	6	2×10^{-12}	0.99 ± 0.36	106	15	6×10^{-23}
N149A	$<8 \times 10^{-7}$	No	0.85 ± 0.21	52	5	6×10^{-14}	0.93 ± 0.25	60	9	9×10^{-18}
N170A	$<5 \times 10^{-6}$	No	1.00 ± 0.48	99	12	2×10^{-15}	0.88 ± 0.35	113	20	5×10^{-17}
L172A	No	No	0.99 ± 0.32	50	10	3×10^{-13}	0.89 ± 0.26	52	11	8×10^{-13}
D263A	$<8 \times 10^{-6}$	No	0.67 ± 0.17	104	8	1×10^{-07}	1.16 ± 0.35	103	12	7×10^{-34}
D58A	0.14	44.2 ± 0.2	0.58 ± 0.05	42	5	0.58	0.56 ± 0.03	100	12	0.08
N74A	0.93	48.5 ± 1.0	0.52 ± 0.11	57	10	0.09	0.59 ± 0.17	55	9	0.07
L113A	2.17	44.6 ± 0.3	0.91 ± 0.33	61	9	1×10^{-11}	0.89 ± 0.25	53	10	6×10^{-14}
L113F	0.88	43.3 ± 0.5	1.05 ± 0.24	71	6	2×10^{-28}	1.14 ± 0.31	70	8	2×10^{-26}
L143F	0.99	49.5 ± 0.9	0.83 ± 0.24	50	5	1×10^{-10}	1.02 ± 0.36	61	10	4×10^{-15}
I167A	0.07	45.2 ± 1.0	0.67 ± 0.18	62	6	3×10^{-5}	0.92 ± 0.28	143	17	5×10^{-32}
N183A	0.07	45.1 ± 0.2	0.57 ± 0.07	63	6	0.97	0.53 ± 0.07	59	7	0.73
Y188F	1.07	48.2 ± 0.5	0.95 ± 0.33	67	9	2×10^{-14}	1.06 ± 0.28	55	9	4×10^{-20}
Y188W	0.56	48.8 ± 2.2	0.89 ± 0.35	61	5	3×10^{-10}	0.92 ± 0.35	62	9	1×10^{-11}
M497L	1.05	48.9 ± 1.2	0.69 ± 0.24	67	8	4×10^{-05}	0.77 ± 0.29	66	12	4×10^{-08}

*Mutants are grouped in two series. The first (V40A to D263A) includes those whose virions had drastically reduced infectivity; the second (D58A to M497L), those whose virions had an infectivity comparable to WT. Mutants are listed in numerical order in each series. Titer indicates normalized virus infection titer at 37 °C, relative to WT. Mutation L172A was lethal (1).

†Transition temperature (average ± standard deviation) for the fluorescence-detected conformational transition of the capsid. No: no transition occurred.

‡Spring constant k_s (average ± standard deviation).

§Number of events (Fz) and individual particles (n) analyzed.

¶Value of T in a Student t-test (see text).

1 Farr GA, Tattersall P (2004) A conserved leucine that constricts the pore through the capsid fivefold cylinder plays a central role in parvoviral infection. *Virology* 323:243–256.