Supporting information for

A de novo virus-like topology for synthetic virions

James E Noble, Emiliana De Santis, Jascindra Ravi, Baptiste Lamarre, Valeria Castelletto,

Judith Mantell, Santanu Ray, Maxim G Ryadnov

Tables and Figures

Table s1. Peptides used in the study.

Peptide	Sequence[a] *gabcdefgabcdefgabcdefgabcde*
TecVir	CGG-EIARLEQEIARLEQEIARLEYEIARLE
TecVir^{ala}	CGG-EIAALEQEIAALEQEIAALEYEIAALE
TecVir^{cap}	XGG-EIARLEQEIARLEQEIARLEYEIARLE

[a]all sequences are C-terminal amides;
[b]X is Cys with its thiol capped with iodoacetamide.

Table s2. SAXS parameters and values for TecVir.

σ/Å	Ro/Å	ν	Ri/Å	$\mu\Delta\eta$	th = R0-Ri/Å	$\Delta\eta * 10^{-8}$	R/Å	L/Å	$\Delta\eta_c * 10^{-6}$	Object
23	65	0.4	26	0	39	3.1	--	--	--	spherical shell 1
50.4	115.9	0.5	57.9	0	57.9	3.1	--	--	--	spherical shell 2
							10	40	2.2	solid cylinder

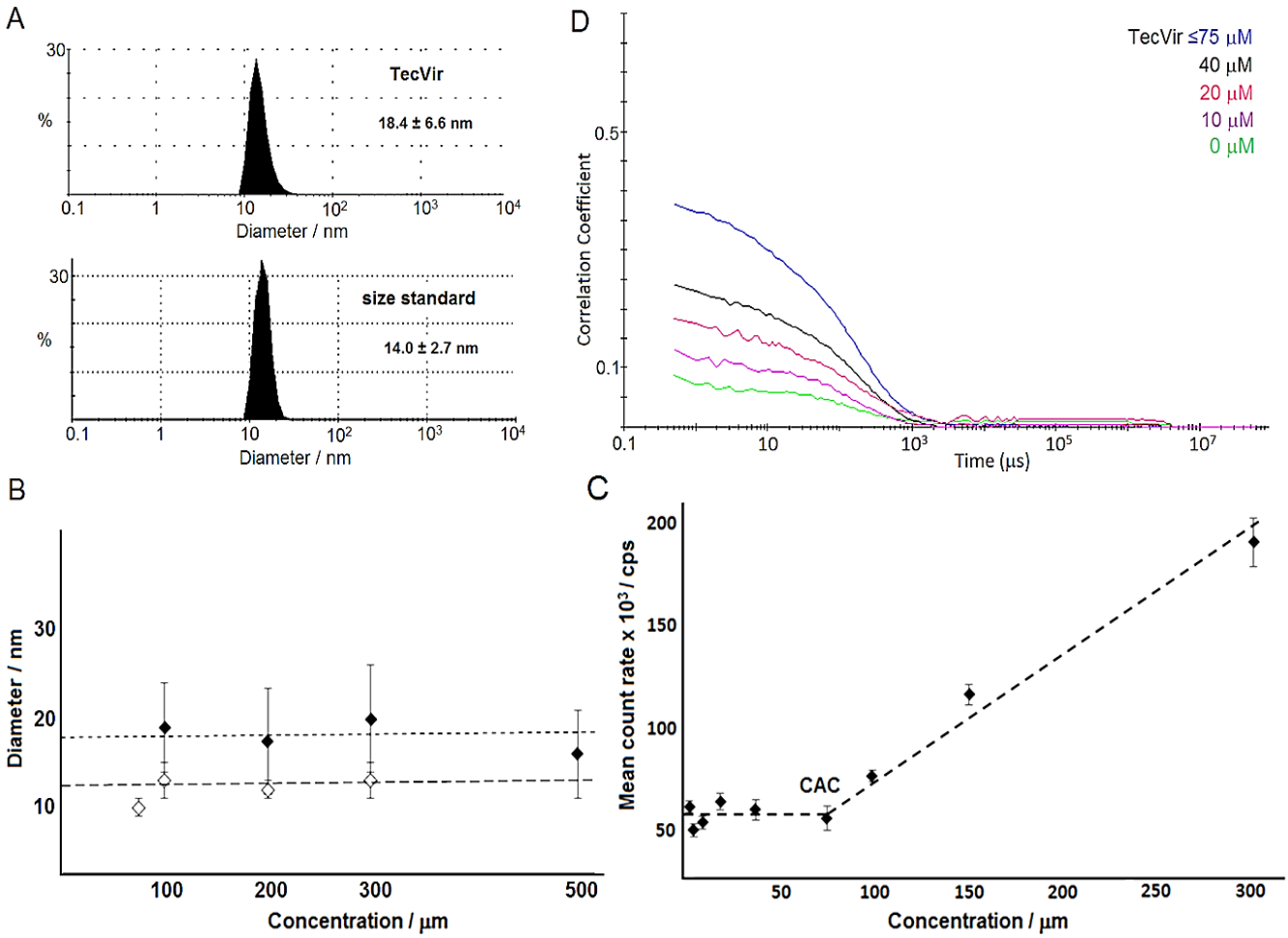

Fig s1. TecVir assembly. (A) Size distributions determined by DLS for TecVir (200 µM) in 10 mM MOPS (pH 7.4) and for a standard monoclonal IgG (0.5 mg/mL) in 10 mM phosphate buffer (pH 7.4). The antibody with the known diameter of 14.2 nm was used as a size standard for comparison (Malvern Instruments Ltd). (B) Size distributions of TecVir as a function of peptide concentration determined by cryo-TEM (white diamonds) and DLS (black diamonds). (C) Critical aggregation concentration (CAC) of TecVir in 10 mM MOPS (pH 7.4), determined by plotting mean count rates (scattering intensity) versus peptide concentrations. (D) DLS correlograms with low intercepts for TecVir at concentrations below CAC in 10 mM MOPS (pH 7.4).

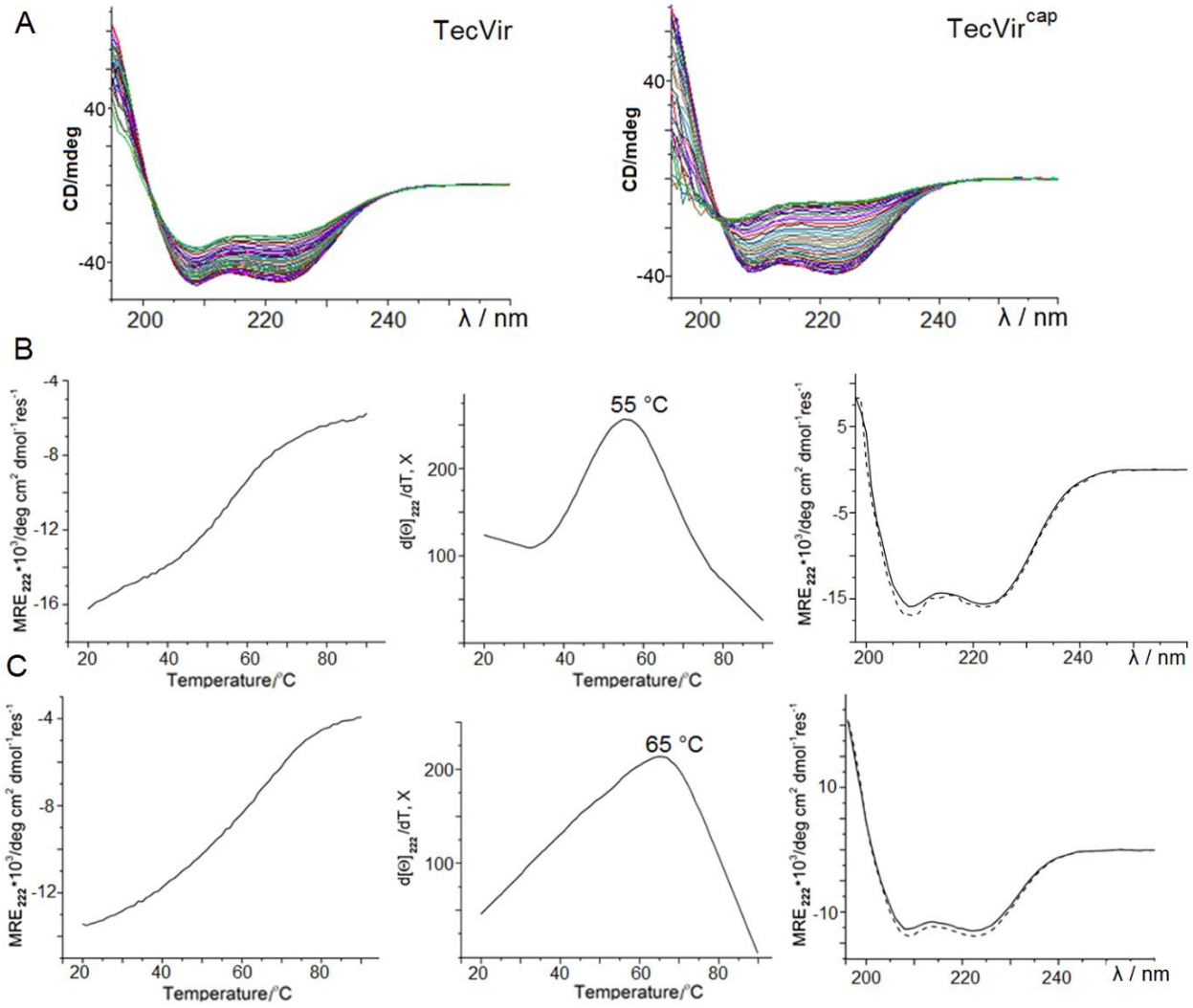

Fig s2. Peptide folding monitored by CD spectroscopy. (A) Raw CD spectra following the thermal denaturation of TecVir and TecVir^{cap}, with the first and the last spectra at 20 °C (black) and 90 °C (green), respectively. Intervening spectra were recorded every 2 °C. Note the isodichroic point at ~202 nm. (B, C) CD curves for TecVir (B) and TecVir^{cap} (C) with thermal denaturation curves (left), the first derivatives of these curves highlighting T_M values (middle) and CD spectra recorded before (solid line) and after (dashed line) thermal unfolding (right). Folding conditions: 100 µM peptide incubated overnight in 10 mM MOPS (pH 7.4) at 20 °C.

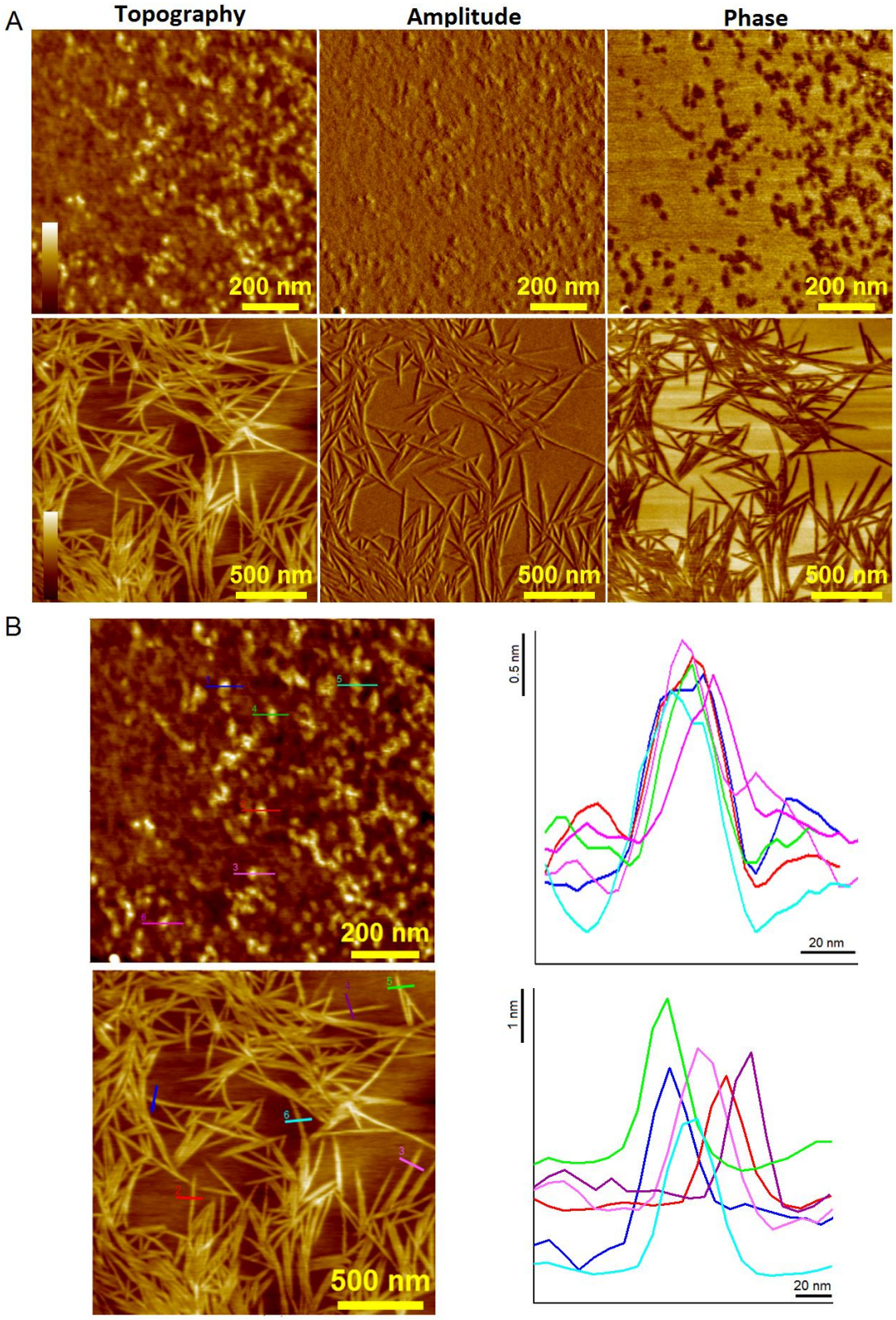

Fig s3. TecVir^{cap} heterogeneous assembly. (A) Atomic force topography, amplitude and phase images of co-existant TecVir^{cap} populations: polydisperse spheroids (upper panel) and fibers (lower panel). Color scales (height): 5 nm (upper panle) and 9 nm (lower panel), respectively. (B) Height profiles (right) taken along the highlighted lines in the topography images (left).

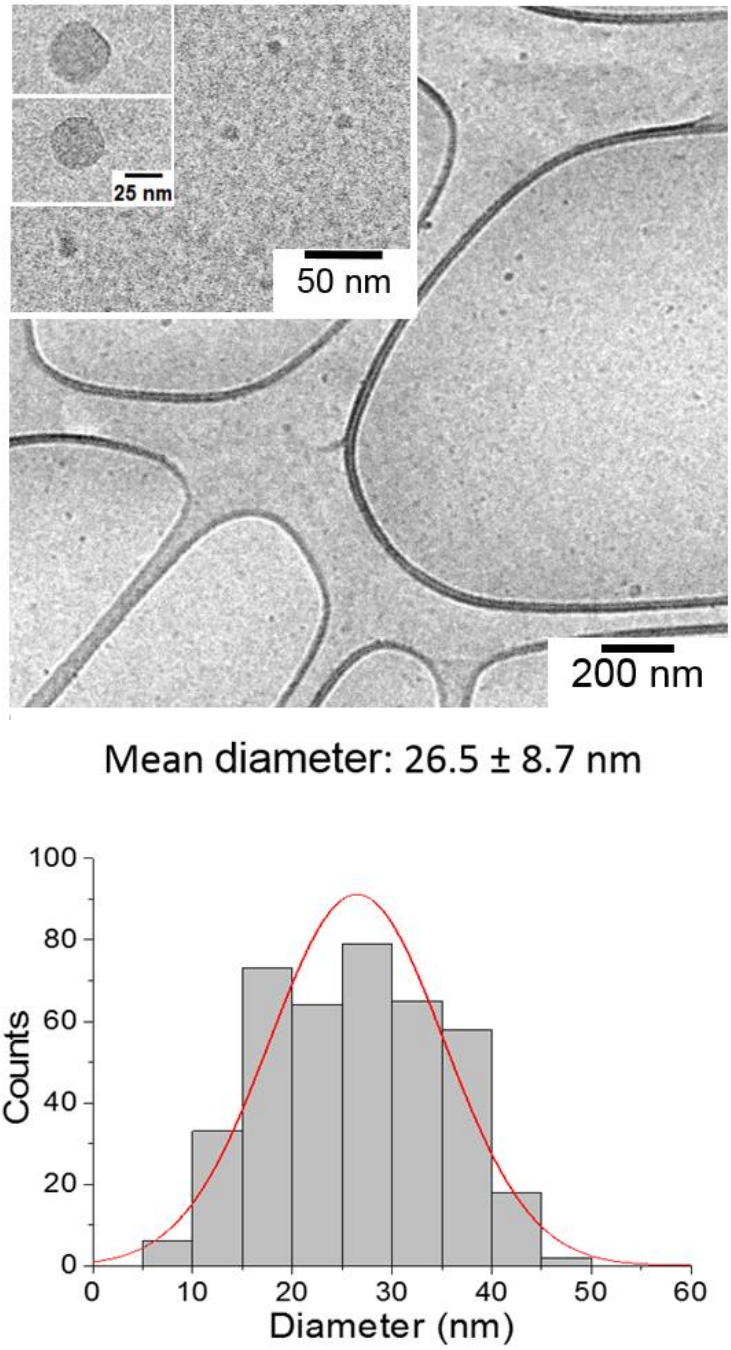

Fig s4. siRNA/TecVir assembly. Cryo-electron micrographs for siRNA/TecVir at at the N/P molar ratio of 13 x 10^{-3} at 100 µM peptide (upper) and a representative analysis of size distributions (lower).

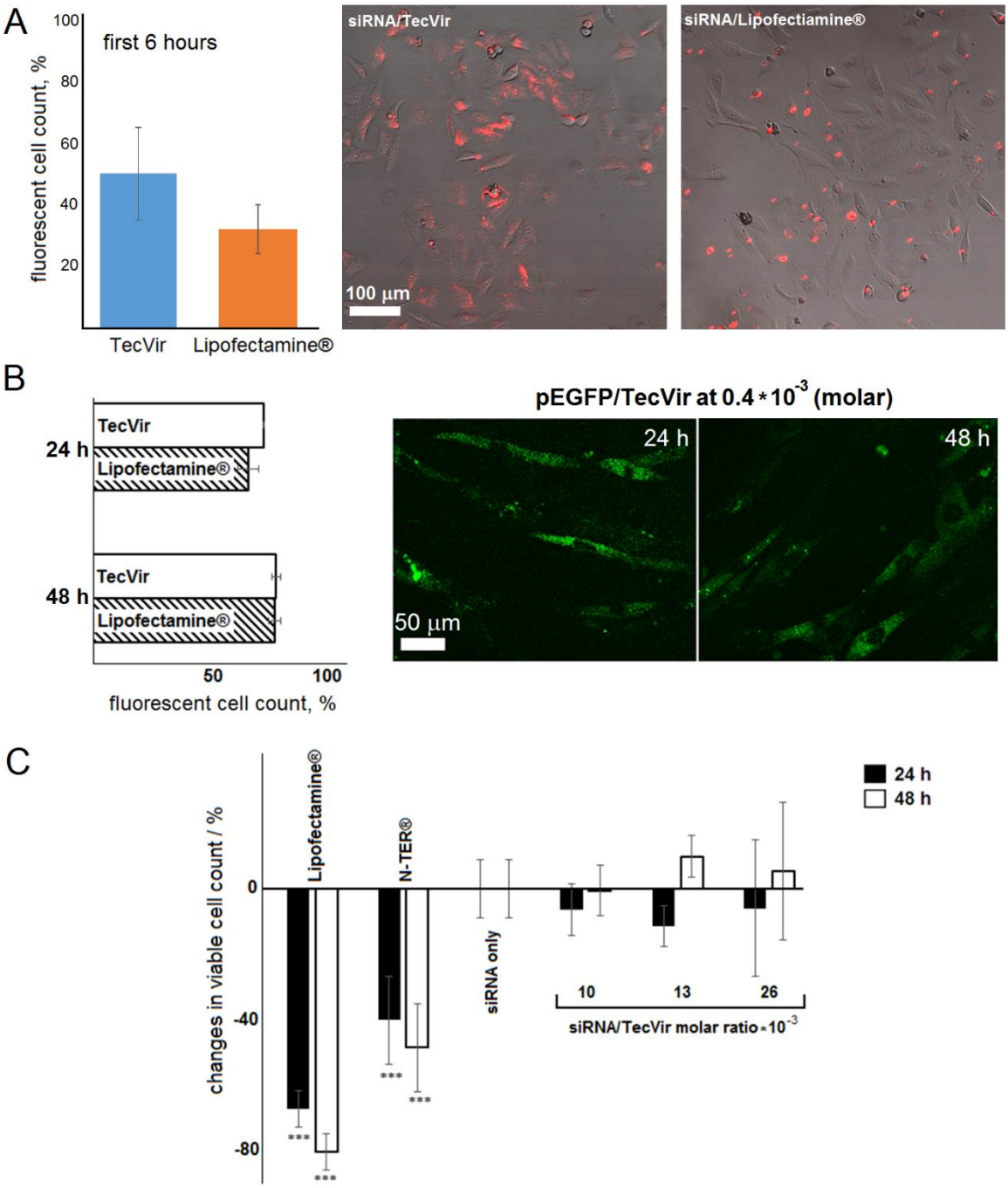

Fig s5. TecVir biological activity. (A and B) Transfections of Alexa 647-labelled siRNA (A) and pDNA-EGFP into HeLa cells over the first 6 hours (A) and human dermal fibroblasts over 24, 48 hours (B), respectively. Transfection efficiencies (left) are expressed as the total number of fluorescent cells versus the total cell count (fluorescent and non-fluorescent), which was taken as 100%. Representative fluorescent micrographs (right) are shown for each case. (C) Changes in viable cell count determined by alamarBlue® cell viability assays following 24- and 48-hour transfections. Cell viability is expressed as a total count of viable cells after subtracting total viable cell counts in the absence of transfection reagents (background viability taken as 100%). Negative values indicate decreased cell viability. According to the analysis of variance (ANOVA) followed by a Fisher post-test for three independent experiments each done in triplicate, Lipofectiamine® and N-Ter® reagents led to significantly lower numbers of metabolically active cells. Significant differences are represented with *** for $p < 0/01$.

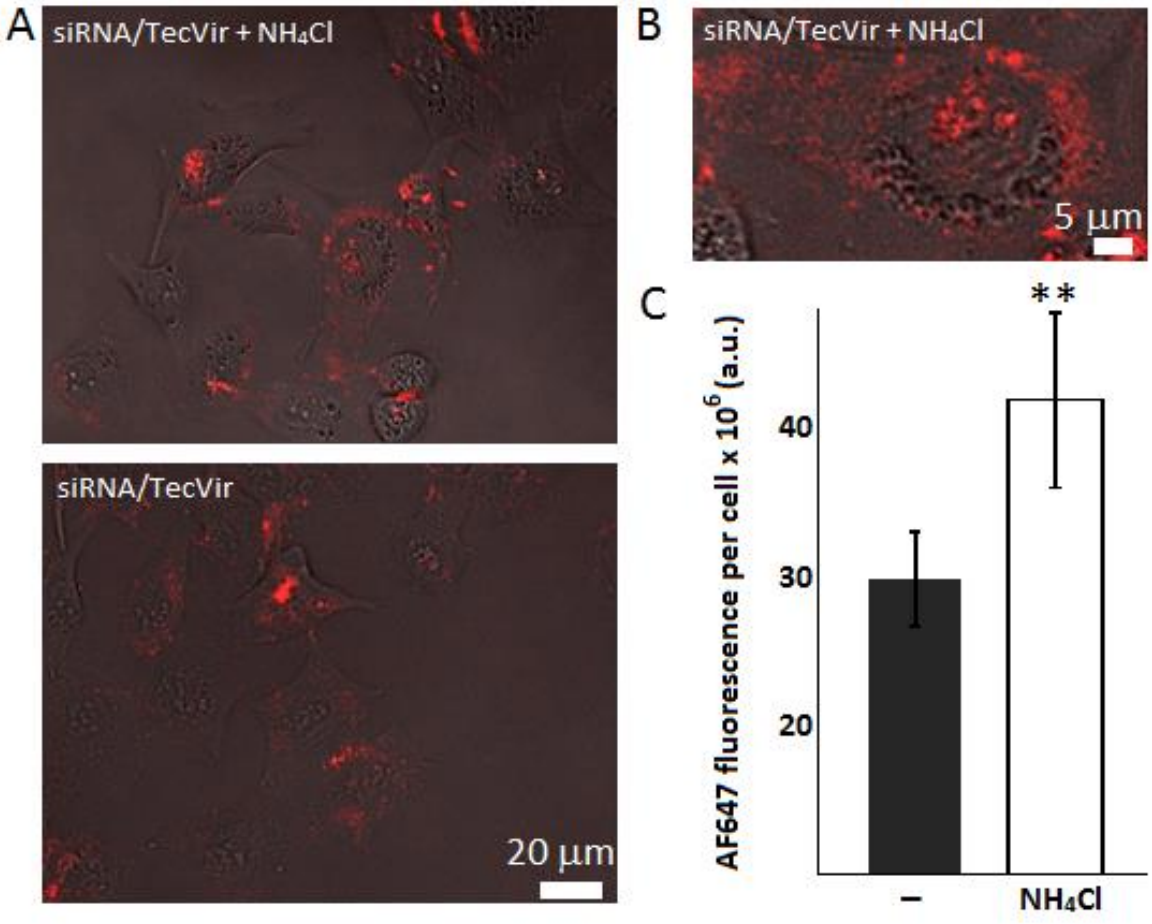

Fig s6. Endosomal siRNA delivery by TecVir. (A) Fluorescent micrographs of Alexa 647-labelled siRNA transfections over the first 3 hours into HeLa cells that were untreated (lower) and pre-treated (upper) with 50 mM NH_4Cl for 30 min. (B) A higher-magnification fluorescent micrograph of siRNA/TecVir transfections into NH_4Cl-pretreated cells showing increased endocytic uptake. (C) Median AF647 fluorescence of transfections in untreated (-) and pre-treated (NH_4Cl) cells. Error bars represent the standard deviation of three replicates. (**) indicate significantly higher fluorescence ($p < 0.05$). Results were analysed using one-way ANOVA followed by Tukey's test for multiple comparisons.

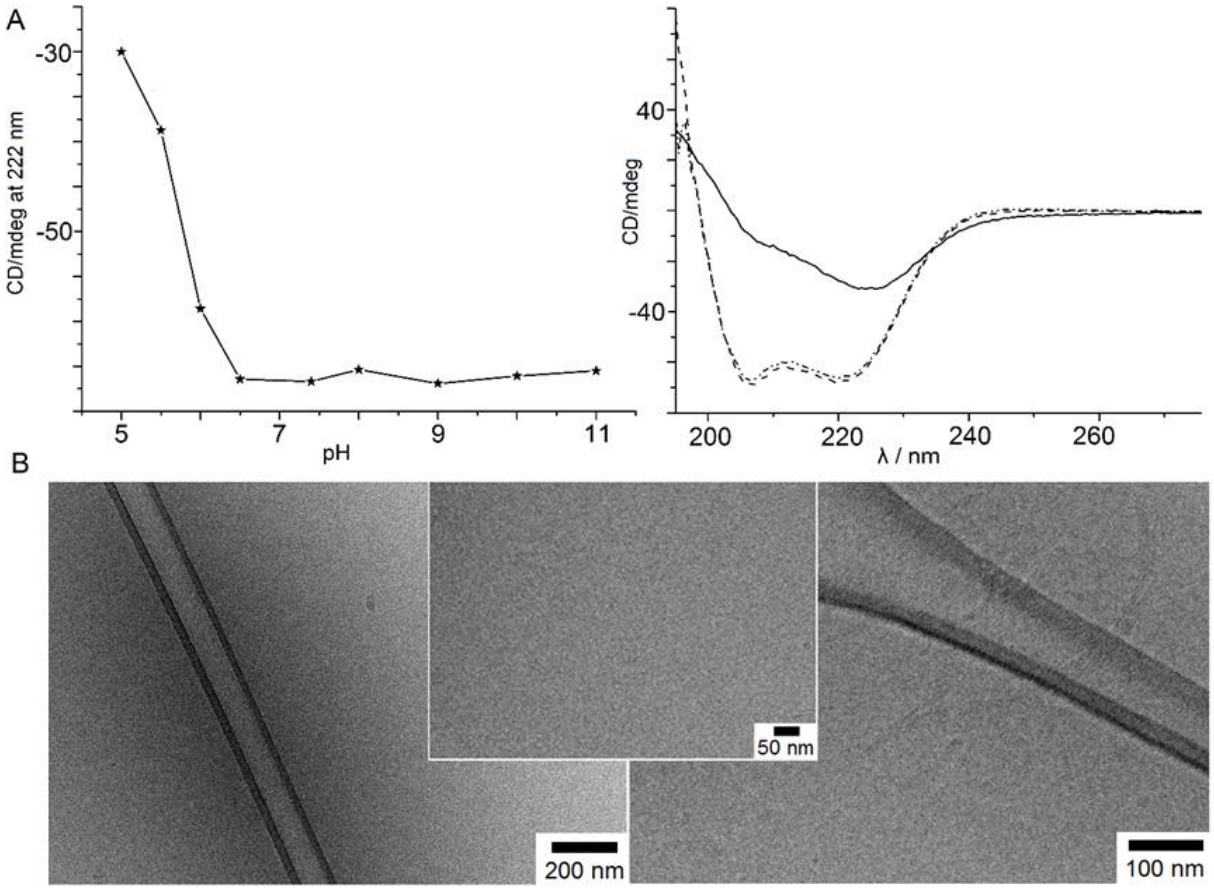

Fig s7. The pH-dependence of TecVir assembly (100 μM) monitored by CD spectroscopy. (A) Raw CD signals at 222 nm plotted versus pH (left) and raw CD spectra (right) at pH 5 (solid line), pH 7.4 (dashed line) and pH 11 (dash-dotted line). (C) Cryo-electron micrographs of TecVir (100 μM) at pH 4.